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PATENT & TRADEMARK ATTORNEYS

Undertakings : Intellectual Property Laws,
Patents, Trademarks, Designs, Copyrights.
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DOMESTIC AND INTERNATIONAL

202, Elecon Chambers, Behind Saki Naka Tel. Ex., Off Kurla-Andheri Road, Saki Naka, Mumbai- 400 072, India
Tel : 91-22-2852 2901/ 2902, Fax : 91- 22- 2852 2903, e-mail : bom@patentindia.com

The Controller of Patents
The Patent Office
New Delhi

November 08, 2011

Dear Sir,

Re: Opposition under Section 25(1) against-
Patent Application No: **2933/DELNP/2009** dated May 01, 2009
Applicant: Bristol-Myers Squibb Company
Opponent: Cipla Limited
Our Ref: **PII - 0456**

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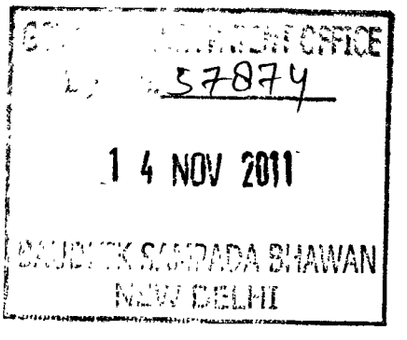
1. Sending herewith representation under Section 25(1) in duplicate. Please take the documents on record and take necessary action.
2. Please grant a hearing in due course;
3. General Power of Attorney in our favour.

We request you to kindly take the opposition on record under intimation to us.

Yours sincerely,

Mythili Venkatesh
Of S. Majumdar & Co.
Opponent's Agent

Encl: a/a.



Head office. : 5, Harish Mukherjee Road, Kolkata - 700 025, India, Tel. : 91-33-2455 7484/85/86,
Fax : 91-33-2455 7487/88, e-mail : cal@patentindia.com

BEFORE THE CONTROLLER OF PATENTS,
NEW DELHI

In the matter of section 25(1) of The Patents Act, 1970 *as amended by* The Patents (Amendment) Act 2005;

And

In the matter of The Patents (Amendment) Rules, 2006;

And

IN THE MATTER of Patents Application 2933/DELNP/2009 dated May 01, 2009 made by Bristol-Myers Squibb Company of P.O. Box 4000, Route 206 And Province Line Road, Princeton, New Jersey 08543- 4000, U.S.A.

.....Applicant

And

IN THE MATTER of opposition of the grant of a patent thereto by Cipla Limited, 289, Bellasis Road, Mumbai Central, Mumbai– 400 008;

.....Opponent

REPRESENTATION UNDER SECTION 25 (1)

We, Cipla Limited, 289, Bellasis Road, Mumbai Central, Mumbai– 400 008, India (hereinafter called 'opponent') make the following representation under Section 25(1) of the Act in opposing the grant of patent on the application indicated in the cause title.

1 OPPONENT'S BUSINESS AND ACTIVITIES

The opponent is a Company incorporated under the laws of India and having its principal office at 289, Bellasis Road, Mumbai Central, Mumbai– 400 008, India, carrying on business, *inter alia*, of manufacture of various drugs/medicine preparations. The opponent has access to the latest technologies relating to manufacture of the drugs and medicines. The opponent is the leading manufacturer of medicines in this country and the opponent's products are sold under different brands and enjoy considerable goodwill and reputation. The opponent is very well known and has been operating in this country for several decades. The opponent is also engaged in the research and development of medicines and pharmaceutical products and preparations.

2 GROUNDS OF OPPOSITION

2.1 The application is opposed on the following grounds:

a. that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in clause (a) or having regard to what was used in India before the priority date of the applicant's claim; **[Section 25(1)(e)];**

b. that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act; **[Section 25 (1) (f)]**;

c. that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed; **[Section 25 (1) (g)]**;

d. the applicant has failed to disclose to the Controller the information required by Section 8 or has furnished the information which in any material particular was false to his knowledge. **[Section 25 (1) (h)]**;

2.2 The opponent craves leave to alter, modify, add or delete the grounds in the course of the present proceedings.

3 PRELIMINARY OBJECTION

3.1 Patent Application no. 2933/DELNP/2009 entitled "Process for Preparing Atazanavir Bisulfate and Novel Forms" in the name of Bristol-Myers Squibb Company is filed in India on May 01, 2009. The application claims a priority of US 60/568,043 dated May 04, 2004. The aforesaid application is divisional to application no. 6425/DELNP/2006 filed in India on November 01, 2006.

3.2 The opponent states on preliminary analysis of claims of the parent and the divisional application it is evident that claims 18 and 21 and part of claims 8 and 9 of the impugned application are the within the scope of the parent application i.e. 6425/DELNP/2006 as amended. The claims of application under opposition are annexed and referred hereto as Annexure A1 whereas the amended claims of 6425/DELNP/2006 are annexed and referred hereto as Annexure A2.

3.3 The opponent states that this a deliberate attempt to mislead the Ld. Controller and the public at large since the claims of the parent application 6425/DELNP/2006 were amended on the same date of filing of the impugned application and the applicant was well aware that the claims of the impugned application on amendment have the same subject matter as a number of claims filed with the application i.e. 2933/DELNP/2009. It is stated that the present application was made with a malafide intention of filing multiple applications for the same invention knowing very well that the invention claimed is devoid of patentable subject matter and the further

application is merely a back-up application which could by chance get allowed if it escapes notice of the competitors. Moreover, under the guise of a divisional patent application as per Section 16 of the Act the impugned patent application claims matter already found non-patentable and thus rejected. The impugned patent application essentially reflects unhealthy attitude of the applicant towards the patent system. This is strictly against the objectives of Section 16 of Indian Patent Act. Permitting such practice means would place undue strain on the Patent Office that would have to keep re-examining the same application over and over again.

3.4 The opponent states that the final hearing in respect of opposition u/s 25(1) to 6425/DELNP/2006 had taken place before the Ld. Controller Dr. S. K. Roy on November 30TH 2009. The order dated December 20TH 2010 in respect of the aforesaid is annexed and referred hereto as Annexure A3. In the said order the Ld. Controller has held that *"On the basis of the above observation & consideration of those points I am of the considered opinion that a person skilled in the art will combine the teachings of D1, D3 and the doc'210 to obtain atazanavir crystal of larger size & narrow particle size distribution by routine experimentation with reasonable expectation of success, optimize acid addition rate profile without any extra ordinary skill.* ***In other words, choosing acid addition profile can be seen as lying with in the routine activity of the skilled person faced with the objective problem of improving particle size & narrow particle size distribution without requiring any inventive ingenuity.***

It is obvious for a skilled person to use verifying acid addition profile for timely release super saturation formation by rapid growth of crystal over the existing atazanavir bisulfate crystal rather than by secondary nucleation and optimize the best addition profile, with reasonable expectation of obtaining large crystal size with narrow particle distribution of the product. My considered view is strengthened with the EPO Board opinion that "---correct approach to inventive step is not sure predictability of success drawn from information in the prior art, but rather whether it would be obvious to try with reasonable expectation of success---" EPO Board of Appeal T 0013/93) and in EPO the Board in T 0948/01----However, the correct approach in assessing inventive step

is not whether a skilled person would drive from given information in the prior art a sure predictability of success but rather whether it would be obvious to by something with or reasonable expectation of success, which implied the ability of a skilled person to reasonably predict, on the basis of existing knowledge a successful conclusion of an experiment----"

Therefore it is my considered view that in light of the prior art teaching, the chosen acid addition profile in the present invention is with in the routine activity of the skilled person in the art, trying to improve the particle size and narrow particle size distribution without exerting any inventive ingenuity and therefore is obvious and lack in inventive step.

Regarding insufficiency of disclosure opponent argued that the applicant's claim that vide last Para page 16 to 17 the present process has resulted in four advantages i.e. relatively large particle size, narrow particle size distribution, well defined crystals and fewer fines ,leading to the advantage like less compressible filter cake and fewer hard lumps. However the singh's crystal product and the present crystals are same product, but the applicant has not provided any comparative data in the specification to highlight the increased particle size and narrow particle size distribution instead the applicant submitted during the second hearing proceedings a reference to Soojin Kim et.al. micrograph data for enhanced particle size data which was published seventeen month after the filing of the present application. ***I agree with the contention of the opponent that the comparative data should have been provided in the specification at the time of filing to justify their claim. Therefore the application lack in sufficiency of disclosure.***

As the product prepared by the process of the invention lack in inventive step and the applicant has not provided any technical data to show enhanced therapeutic efficacy of the product over the prior art compound other than the data for physical improvement leading to product crystals of large particle size with narrow particle size distribution and fewer fines to improve the processibility like effective cake deliquoring, ease in washing and drying with lesser lumps during manufacture , does not qualify the requirement u/s 3(d) of the Patent

Act and therefore product by the process as well as composition claim are also refused.

I therefore on the basis of my considered view explained in preceding Para, refuse to proceed with the application no. 6425/DELNP/2006 for grant of patent."

Evidently, the alleged invention claimed in the impugned patent application in claims *18, 21, step (a) of claim 8 and the essential part of claim 9* i.e. converting the free base to corresponding bisulfate salt in the form of Form A crystals are similar the amended claims 1 to 11 of 6425/DELNP/2006. Therefore, in view of the order refusing the claims of 6425/DELNP/2006 for obviousness and lack of inventive, lack of sufficiency of disclosure and non patentable under section 3 (d) of the Patents Act, the same would automatically apply to the patent application under opposition. The rejected matter sought to be claimed in the impugned application therefore merits refusal *in limine* with regards to Annexure A3 without any further consideration of the grounds of opposition on which the present opposition is based.

3.5 In all likelihood the wrongful act may not have come to the notice of the Ld. Controller at all in the absence of the present opposition. Such an action of the applicant besides being wholly contrary to law is an act of fraud commissioned against the Government of India and the public of India as well with the wrongful intention of creating a monopoly for the alleged invention for which no patent can be granted in India since it is obvious and lacks inventive merit, insufficient and falls under the mischief of section 3(d) of the Patents Act. Therefore, it is stated that the impugned application merits refusal *in limine* without any further consideration of the grounds of opposition on which the present opposition is based.

4 ANALYSIS OF THE CLAIMS

4.1 Without prejudice to the opponents' preliminary objection the opponent will now proceed to deal with the opposition on merits.

4.2 The impugned application for patent application 2933/DELNP/2009 was made on May 01, 2009, claiming priority of May 04, 2004 and was accompanied by a

complete specification. The impugned application entitled "Process for Preparing Atazanavir Bisulfate and Novel Forms" contains a statement of 21 claims.

4.3 Claims 1-4 of the impugned application are directed to Atazanavir bisulfate Pattern C material. Claims 5 to 8 relate to process for preparation of Atazanavir bisulfate Pattern C material. Claims 6 is redundant over claim 8.

4.4 Claim 9 to 18 relate to process for the preparation of Form A crystals.

4.5 Claims 19, 20 and another claim also numbered 20 relate to formulation comprising Pattern C material.

4.6 Claim 21 which is dependent on claim 8 relates to process for preparation of atazanavir bisulfate Pattern C material wherein the manner of addition of sulfuric acid for the preparation of Form A crystals is claimed.

5 ANALYSIS OF THE APPLICANT'S SPECIFICATION

5.1 The impugned application relates to a process for preparing HIV protease inhibitor atazanavir bisulfate of the form of Form A and novel forms thereof. The process comprises a modified cubic crystallization technique wherein sulfuric acid is added at an increasing rate according to a cubic equation and includes steps of reacting solution of atazanavir free base in an organic solvent with a first portion of sulfuric acid in an amount less than about 15% and preferably 12% by weight of the atazanavir free base; adding seeds of atazanavir bisulfate Form A crystal to reaction mixture; and as crystal of atazanavir bisulfate form, adding additional concentrated sulfuric acid in multiple stages at increasing rates according to a cubic equation for effective formation of Form A crystal.

5.2 Atazanavir free base is added in the organic solvent in which it is insoluble.

5.3 The impugned application further teaches a process for preparing a form of Atazanavir which is derived from Atazanavir bisulfate form A and the form is defined

as Pattern C material which is prepared by suspending crystals of Atazanavir bisulfate form A in water and subsequently drying. Alternatively, Pattern C material may be formed by subjecting crystals of Form A to high relative humidity of greater than about 95% RH (water vapor) for at least 24 hours. Pattern C material may also be formed by wet granulating the atazanavir bisulfate or a combination of atazanavir bisulfate and excipients and drying the wet granulation. (Page 6 paragraph 2)

5.4 Further, the specification teaches preparation of Form E3, a highly crystalline Form of triethanolate solvate of Atazanavir bisulfate.

5.5 The applicant of the impugned patent application No. 2933/DELNP/2009 aims at preparing Atazanavir bisulfate having desired substantially consistent particle size distribution and substantially consistent mean particle size which can be employed in the conversion of Form A crystals of Atazanavir bisulfate to Pattern C material which is **partial crystalline material** and can be formulated with various excipients to prepare the final drug product. [Page 5 paragraph 2 under the heading 'Brief Description of the Invention']

5.6 The applicant has specified that the process for preparing Form A crystals of Atazanavir bisulfate salts comprises cubic crystallization technique which is the modified form of conventional crystallization technique where sulfuric acid is added at an increasing rate according to cubic equation. In one embodiment of the impugned application the applicant has stated that the modified cubic crystallization technique as employed in the impugned invention provides relatively larger more, well defined Atazanavir bisulfate crystals along with narrower particle size range and fewer fines than constant addition rate crystallization. The opponents state that the applicant has itself admitted in its specification that cubic crystallization method was known from before and the crystallization method as employed in the impugned invention is a temperature controlled crystallization derived from Mullin, "Crystallization, 3rd ed.", 1993, Butterworth-Heineman, Pubs. The opponents state that such a temperature controlled method as already admitted by the applicant in its specification is known from before the priority date of the impugned application. The opponent states that what the applicant has done is that it has used a known crystallization method in its process for preparing Atazanavir bisulfate Form A.

5.7 Admittedly, *"Procedures for the preparation of crystalline forms are known in the art. The crystalline forms may be prepared by a variety of methods, including for example, crystallization or recrystallization from a suitable solvent, sublimation, growth from a melt, solid state transformation from another phase, crystallization from a supercritical fluid, and jet spraying. Techniques for crystallization or recrystallization of crystalline forms from a solvent mixture include, for example, evaporation of the solvent, decreasing the temperature of the solvent mixture, crystal seeding a supersaturated solvent mixture of the molecule and /or salt, freeze drying the solvent mixture, and addition of antisolvents (countersolvents) to the solvent mixture.....Crystals of drugs, including polymorphs, methods of preparation, an characterization of drug crystals are discussed in Solid-State Chemistry of Drugs, S.R. Byrn, R.R. Pfeiffer, and J.G. Stowell, 2nd Edition, SSCI, West Lafayette, Indiana (1999)."* [Page 12 paragraphs 2 and 3]

5.8 <u>UTILITY OF THE ALLEGED FORMS MADE BY THE PROCESS OF THE ALLEGED INVENTION.</u>

5.8.1 The specification of the alleged invention contains 55 pages including the drawing sheets showing XRD patterns, differential scanning calorimetry, thermal gravimetry analysis curve, NMR scanning calorimetry thermogram etc., of Form A , Pattern C and Form E3 being the matters forming basis of the alleged invention. Significantly, in the entire specification the applicant has disclosed various aspects of the alleged invention which involves process for making Form A, atazanavir bisulfate Pattern C material its process of preparation and composition comprising such materials as well as a further form named Form E3 of atazanavir bisulfate and pharmaceutical formulations comprising the said Forms and Pattern.

5.8.2 It is admitted that it is already disclosed in US6087383 assigned to the applicant itself that the free base form of atazanavir did not posses sufficient oral bio-availability and amongst other salts the bisulfate salt was found to show unexpected solubility behavior and therefore contributes to improved

bio-availability compared to the free base. The bisulfate salt was also found to have greater bio-availability compared to crystalline free base. It is further reported that the bisulfate salt besides improved bio-availability also showed significantly enhanced physical stability in the solid state such as physical stability under storage/stress condition. Furthermore, Form A of atazanavir bisulfate is admittedly known as is apparent from paragraph 2 under the sub-heading Brief Description of Invention in page 5. However, the applicant claims to have prepared the same Form A crystals by an alleged invented process which gives substantially consistent particle size distribution and substantially consistent mean particle size. Nowhere in the specification it is stated as to how the Form A made by the process of the alleged invention and the other converted materials such as Pattern C material and Form E3 affects the therapeutic properties or the therapeutic efficacy of the admittedly known Form A crystals. The specification is dedicated to only characterization by various techniques the Form A and its other derivative forms and materials and such characteristics have been described and brought out at great length to give a body to the specification.

5.8.3 The basic question of which no answer is found from the specification as to what was the need felt for the invention or the problem that is sought to be solved and finally solved by the applicant or what technical advance has been achieved by the alleged invention. It is stated that for the purpose of the Patent Act the mere act of making a new substance or defining a new process does not automatically entitle such new substance or new process to a patent unless the applicant convincingly demonstrates the utility of the product or the process.

6 DOCUMENTS RELIED UPON

In the present proceedings the opponent will rely upon the following documents being prior art in support of its case made out on the grounds listed above:

6.1 US 6087383 (hereinafter referred to as D1) published on July 11, 2000). D1 is annexed hereto and marked as Exhibit 1.

6.2 Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations"; Stephen Byrn et al.; Pharmaceutical Research, Vol. 12, No. 7, 1995 (hereinafter referred to as D2). D2 is annexed hereto and marked as Exhibit 2.

6.3 Solid State Characterizations of Pharmaceutical Hydrates"; D. Giron et al; Journal of Thermal Analysis and Calorimetry, Vol. 68 (2002) 453-465 (hereinafter referred to as D3). D3 is annexed hereto and marked as Exhibit 3.

6.4 US 2003/0084547 (hereinafter referred to as Hazen publication) published on May 08, 2003 entitled "Sodium carbonate recrystallization". The Hazen publication is annexed hereto and marked as Exhibit 4.

6.5 Xu, Z. et al., "Process Research and Development for an Efficient Synthesis of the HIV Protease Inhibitor BMS-232632", Organic Process Research & Development, Vol. 6, No. 3, pages 323-328 (**2002**) (hereinafter referred to as D5). D5 is annexed hereto and marked as Exhibit 5.

6.6 US 5849911 (hereinafter referred as D6) published on December 15, 1998. D4 is annexed hereto and marked as Exhibit 6.

7 OBVIOUSNESS AND LACK OF INVENTIVE STEP [Section 25(1)(e)]:

7.1 The opponent states that assessing inventive step initially requires a fact finding act so as to determine whether the invention involves any inventive step with respect to the prior art. It is stated that the impugned invention relates to a process for forming Form A crystals of Atazanavir bisulfate, formation of Pattern C. Admittedly, Form A is known and according to the applicant it has invented a new process for making Form A. The applicant allegedly has made novel forms of atazanavir bisulfate such as Pattern C material. When the applicant refers to forms it is obvious that there is no change in the molecule namely atazanavir bisulfate but only in the change of the form. It is stated that Form A is in fact a known form of a well known compound Atazanavir bisulfate for which the applicant claims a new process. It is stated that the object of the applicant is to achieve a desired substantially consistent particle size distribution and substantially

consistent mean particle size of Form A crystals of Atazanavir bisulfate. It is another object of the impugned invention to convert Form A Atazanavir bisulfate to Pattern C which is a **partially crystalline form** formulated with various excipients to prepare final drug product.

7.2 As already discussed hereinabove claim 1 of the impugned invention recites an alleged novel Pattern C material atazanavir bisulfate characterized by the powder x-ray diffraction pattern substantially in accordance with that shown in figure 6. The opponent states that claim 1 and the dependent claims 2 to 7 relate to atazanavir bisulfate partial crystalline Pattern C material. It is stated that claim 8 also relates to atazanavir bisulfate partial crystalline Pattern C material.

7.3 At the outset the opponent reiterates that the impugned patent application does not adduce any information on the utility or any advantages achieved as a result of the preparation of the new partial crystalline Pattern C material of atazanavir bisulfate prepared from Form A atazanavir bisulfate.

7.4 The opponent states that D1 entitled 'Bisulfate Salt of HIV Protease Inhibitor" discloses crystalline bisulfate salt of atazanavir which has unexpected high solubility / dissolution rate and oral bioavailability relative to the free base form.

7.5 The opponent states that D1 teaches crystalline bisulfate salt of Azapeptide HIV protease inhibitor which has superior aqueous solubility behaviour compared to other salts and significantly improve oral bioavailability compared to free base. The opponent states that Atazanavir is derived from Azapeptides and this is also admitted by the applicant of the impugned invention in its specification at page 2 lines 14 - 15. It is stated that D1 expressly teaches that bisulfate salt of Azapeptides are old in the art.

7.6 Admittedly, Form A crystals are the Type I crystals in Example 3 of U.S. Patent No. 6,087,383 to Singh et al (referred to as 'D1' herein). Thus, D1 discloses a crystalline form of atazanavir bisulfate i.e. Form A.

7.7 The opponent relies on "Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations"; Stephen Byrn et al.; Pharmaceutical Research, Vol. 12, No. 7, 1995 annexed hereto as D2 to demonstrate that identifying new forms i.e. crystalline, solvates and amorphous of a particular drug substance and characterizing them by known techniques are routine in the art.

7.8 It is submitted that D2 discloses on page 945 at column 1 that, *"interest in the subject pharmaceutical solids stems in part from the Food and Drug Administration's (FDA's) drug substance guideline that states "appropriate" analytical procedures should be used to detect polymorphic, hydrated, or amorphous forms of the drug substance."*

7.9 D2 further discloses that, *"Solid drug substances display a wide and largely unpredictable variety of solid state properties. Nevertheless, application of basic physicochemical principles combined with appropriate analytical methodology can provide a strategy for scientific and regulatory decisions related to solid state behavior in the majority of cases."*

7.10 In fact on page 946 in column 2 under the heading "A. Formation of polymorphs-Have Polymorphs Been discovered?" it is provided that, *"The first step in the polymorphs decision tree is to crystallize the substance from a number of different solvents in order to attempt to answer the question: Are polymorphs possible?* ***Solvents should include those used in the final crystallization steps and those used during formulation and processing*** *and may also include* ***water****, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, hexane and mixtures if appropriate."* It further discloses that, *"New crystal forms can often be obtained by cooling hot saturated solutions or partly evaporating clear saturated solutions. The solids produced are analyzed using X-ray diffraction and at least one of the other methods... ".*

7.11 D2 also teaches at page 951 in column 1 under the heading *"D. Determination of the Hydrate Present in the Drug Product"* that, *"Another important area is the analysis of the material which is produced after wet granulation of a substance which can form hydrates...However, upon wet granulation, there is a conversion (either partial or complete) to a hydrate.....It may also be possible to cause transformation during other processing steps. It is thus recommended that if wet granulation or processing that subjects the drug to even brief changes in temperature or pressure (e.g. milling or compression) is contemplated, then extensive studies of the ability to convert the drug substance to a new crystal form be carried out by mimicking the processing step in the laboratory."*

7.12 It is submitted that identification of Hydrates (Solvates), Desolvated solvates and amorphous forms of a drug product are also routine practices. In fact, it is evident from

D2 that different types of solids (polymorphs, solvates, desolvated solvates, and amorphous forms) of a drug product are routinely encountered as part of drug development and are characterized by routine methods well known in the art. It is submitted that this fact is admitted by the applicant itself in the impugned patent application.

7.13 The opponent thus states that stumbling upon a new form during routine experimentation which is a requirement of drug development is obvious to a person skilled in the art and the use of **water as a solvent** to convert one form to another followed by mere characterization of the form so formed by routine techniques cannot be construed as being non-obvious to a person skilled in the art.

7.14 The opponent states that demonstration of inventive step is a statutory requirement and forms the basis of an invention i.e. the need of the invention for which protection is sought.

7.15 It is evident in the present case that nowhere in the impugned patent application is there any statement let alone experimental data suggesting the need or the inventive step of the alleged invention claimed in claims 1 to 8. The opponent states that the impugned patent application is totally lacking in inventive merit and is obvious and thus warrants rejection.

7.16 The opponent relies on the article "Solid State Characterizations of Pharmaceutical Hydrates"; D. Giron et al; Journal of Thermal Analysis and Calorimetry, Vol. 68 (2002) 453-465 annexed hereto as D3 to demonstrate that manufacturing processes play a role in the conversion of one form of a drug substance to another.

7.17 It is submitted that at the paragraph bridging pages 453 and 454 of D3 under the heading 'Introduction' it is provided that, *"Manufacturing processes may involve the presence of water in the crystallization of the drug substance or in the manufacturing or in the composition of the drug product through excipients. New phases where water is a part of the crystal, called hydrates may be obtained with completely new properties in the solid state [1-8]. Dehydration steps may occur in drying, milling, mixing and tabletting processes. Some properties known to be altered by the association of solids with water, including rates of chemical degradation in the solid state, crystal growth, dissolution, dispersibility, wetting, powder flow, lubricity, compactibility, hardness. Furthermore*

drug substances and drug products are submitted to different temperatures and relative humidities, due to various climatic conditions giving rise to unexpected hydration or dehydration aging phenomena...

Several hydrates and even polymorphic forms thereof can be encountered..."

7.18 It is submitted that D3 on page 464 discloses under the heading 'Conclusion' that, *"Adequate investigations are necessary in very early stage of development for the proper choice of the candidate form, the choice of the formulation, of the process and of the packaging. A deep insight needs several analytical complementary techniques with a high level of information. Particularly informative are X-ray diffraction experiments in chambers of different humidity in parallel to sorption-desorption isotherms. Dehydration studies by TG under different temperatures and pressure simulate drying and milling processes. The crystal structure obtained by single crystal diffraction or by computational calculation from X-ray diffractometry allows to understand the type of the bonds between water and drug substance. Modelling capabilities are extremely helpful as an help for quantitative methods in drug substance and drug products."*

7.19 The opponent thus submits that it is evident that manufacturing processes or in the manufacturing or in the composition of the drug product through excipients play a role in form transition. Characterization of a form by routine and known techniques such DSC-TG, TG-MS, sorption-desorption isotherms, sub-ambient experiments, X-ray diffraction combined with temperature or moisture changes as well as crystal structure and crystal modeling in addition to solubilities and dissolution experiments make interpretation and quantitation easier.

7.20 The opponent thus submits that a mere partial crystalline Pattern C material without any evident advantages over the forms of atazanavir bisulfate already available in the art, stumbled upon during routine obvious experimentation as evident from the argument advanced hereinabove especially in view of documents D1, D2 and D3 warrants rejection under this ground itself.

7.21 The opponent moreover states that claims 2 to 4 which are dependent on claim 1 do not disclose any technical features and merely make reference to figures 6, 7 and 8 which depict XRD, DSC and thermal gravimetric analysis curve. Since claims 2 to 4 do not add any technical effects, therefore they are also devoid of any inventive step.

7.22 The opponent further submits that the step for the preparation of pattern C material as claimed in claim 7 and step (e) of claim 8 of the impugned patent application by mixing Form A crystals of atazanavir bisulfate with one or more formulating excipients and water followed by drying is well within example 4 of D1 i.e.

Example 4

Preparation of Capsule Formulations of Bisulfate Salt

A. Capsules (50 and 200 mg free base equivalent)

Capsules are provided for oral administration in which the capsule is a size #0, gray, opaque, hard gelatin capsule containing the bisulfate salt of formula II formulated as a wet granulation with lactose, crospovidone and magnesium stearate.

B. Capsules (100 mg free base equivalent)

Capsules are provided for oral administration in which the capsule is a size #0, gray, opaque, hard gelatin capsule containing the bisulfate salt of formula II suspended in Gelucire 44/14. Gelucire 44/14 is a saturated polyglycolized glyceride consisting of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethylene glycol. Capsules are prepared by melting Gelucire 44/14 at 45–70° C. followed by addition of the bisulfate salt with stirring. The molten mixture is filled into hard gelatin capsules and allowed to cool and solidify.

Thus it is evident that wet granulation of form A with excipients is disclosed in D1. It is therefore submitted that a mere characterizing of the form of the active i.e. form of atazanavir bisulfate as a result of the formulating process is routine and cannot be construed as adding any inventive merit to the alleged invention. In fact characterizing the effect of formulation on a particular form of an active is an obvious and expected step in drug development as evident from D2 and D3 and cannot be construed as non-obvious and inventive especially in view of the impugned patent application being devoid of any experimental data on the inventive merit of Pattern C material or its process of preparation. The opponent states that wet granulating of atazanavir bisulfate Form A with excipients is expected in normal course of drug formulation and subsequent characterization of the form that results in the formulation is routine. It is however pertinent to note, that the applicant is itself unsure of the process for the preparation of Pattern C material since it discloses on page 5 and page 18 of the impugned patent application that atazanavir bisulfate Form A is the source for preparation of Pattern C material while on page 6 paragraph 2, it discloses that atazanavir bisulfate in general without specifying any particular form can be used to prepare Pattern C material i.e.

"... .Pattern C material may also be formed by wet granulating ***the atazanavir bisulfate*** *or a combination* ***of atazanavir bisulfate*** *and excipients and drying the wet granulation."*

7.23 Claim 6 does not have any technical features and therefore cannot be taken to include any inventive step. Moreover, it is redundant over claim 8.

7.24 It is thus submitted that preparation of one form followed by subsequent conversion to another form during routine drug development followed by characterization as evident from D1, D2 and D3 cannot be construed as adducing any inventive merit and thus ought to be rejected. Moreso, in view of the fact that the impugned patent application is devoid of any data or even a statement on the advantages of Pattern C material.

7.25 Drawing reference to D1, column 2 lines 44 to 57, it is stated that the inventors of D1 explored use of acid addition salts to increase the bioavailability of free base form of bisulfate salts of Azapeptide. It is seen that the applicant of the impugned invention also uses concentrated sulfuric acid for increasing the bioavailability of Atazanavir bisulfate. However it is said in the impugned application that sulfuric acid is added in an increasing rate. The opponent states that addition of sulfuric acid in an increasing rate cannot also be a subject matter of inventive step of step (a) of claim 8 of the present invention. It is stated that in example 1 of D1 preparation of bisulfate from ethanol has been described. Considering example 1 it is found that a suspension of free base compound I i.e. Azapeptide in ethanol is treated by concentrated sulfuric acid and the said concentrated sulfuric acid is added in a dropwise manner. It is stated that it would be quite an obvious choice for a person skilled in the art reading the disclosure of D1 to add sulfuric acid in the free base in a dropwise way. The opponent states that a person skilled in the art can easily increase or decrease the rate of addition of sulfuric acid to the free base compound. Therefore, addition of sulfuric acid in increasing rate and in multiple stages as claimed in step (a) of claim 8 of the invention of impugned application cannot be termed as non-obvious to a person skilled in the art. It is stated that having document D1 in hand a person skilled in the art can easily reach the process as claimed in the impugned application. Therefore, claim 8 of impugned application fails the test of inventive step in respect of D1. Moreover, it is submitted that step (a) of claim 8 which was within the scope of claim 1 of the parent patent application i.e. 6425/DELNP/2006 was rejected as

being obvious. The opponent states that the applicant is attempting to claim something indirectly which could not be done directly.

7.26 The opponent now proceeds to discuss in brief the kinetics of the crystallisation process. Crystallisation is a process whereby a crystalline phase is created as a consequence of molecular aggregation in a solution, leading to the formation of nuclei and, later, crystal growth. Supersaturation, nucleation and crystal growth are the predominant physical phenomena associated with crystallisation.

- Supersaturation is the basic driving force for crystallisation and is defined as the concentration of the solute in excess of saturated concentration under given conditions of temperature.
- Nucleation is the first decisive step in crystal formation, beginning with molecules colliding with each other due to their random movement in the solution, which leads to the formation of pre-nucleating clusters. As the population of these clusters increases, they begin to associate to form an embryo. The embryos are not large enough to grow into a crystal in the existing supersaturation. Some embryos – through additional collisions – grow into nuclei (tiny crystallites of the smallest size capable of independent existence), contributing to the formation of macroscopic crystals in the process termed crystal growth.
- Crystal growth is the integration of the crystallizing components on a crystal. A higher level of supersaturation boosts the nucleation process. However, nucleation is more energetically demanding than crystal growth and there are supersaturation regions where crystal growth proceeds while nucleation is suppressed.

7.27 It is stated that the applicant being a skilled artisan in solid-state chemistry will be invariably well-versed with the principles of crystallisation and would therefore accordingly manipulate the parameters to result in crystals of the desired morphology. The applicant has emphasized on page 16 line 28 that "The crystal particle size and morphology of the atazanavir bisulfate salt formed are dependant on the addition rate of the sulfuric acid, which determines the crystallization rate." The applicant has further asserted on page 17 line 3 that, "The slow initial acid flow rate has been shown to favor crystal growth over secondary nucleation." It is stated that the aforesaid admissions of the applicant which are corollaries to the well known and established theory of crystallization

form the basis of the process for preparation of atazanavir bisulfate Form A crystals as claimed in claim 1 of the alleged invention.

7.28 The opponent relies upon US2003/0084547 published on May 08, 2003 entitled "Sodium carbonate recrystallization" by Hazen et al herein after referred to as Hazen publication teaches a process for producing sodium carbonate monohydrate crystals by introduction of anhydrous sodium carbonate into a saturated sodium carbonate brine solution under conditions in which sodium carbonate monohydrate formation is favored to highlight the lack of inventive merit in the alleged invention. The abstract of the Hazen publication clearly teaches that the said process involves controlling supersaturation and its relief to achieve growth of existing sodium carbonate monohydrate crystals rather than nucleation and formation of new sodium carbonate monohydrate crystals. The summary of the Hazen publication on page 1 paragraph [0006] *"the process of the present invention is for producing sodium carbonate monohydrate from a feedstream which includes anhydrous sodium carbonate and insoluble impurities. The process includes adding the feedstream to a saturated sodium carbonate brine solution under conditions to create supersaturation of at least about 5 g/l. The process further includes processing within parameters that preferentially relieve the supersaturation by rapid growth of existing sodium carbonate monohydrate crystals* ***rather than by nucleation.*** *In this manner, the particle size distribution of crystals is controlled to achieve a desired distribution of crystal size product."* Paragraph [0012] on page 1 teaches that, *"...Relief of supersaturation is controlled such that crystal formation primarily occurs on existing crystals, rather than occurring as nucleation or growth of newly formed crystals. In this manner, the particle size distribution of crystals is controlled* ***to achieve a desired distribution of product crystal size.***" Paragraph [0015] on page 2 further teaches *"Processes of the present invention are based on the recognition that since supersaturation is created by the introduction of anhydrous feed, the supersaturation limit can be exceeded in a localized area at the point of introduction of the feed. Therefore,* ***control of supersaturation and its relief in the local environment near where the feed is introduced is critical.***" Paragraph [0017] teaches the use of a seed crystal and reads as *"Processes of the present invention also provide a large amount of available sites for relief of supersaturation on existing crystals so that if the degree of*

supersaturation in a localized area is approaching the maximum level, i.e., the supersaturation limit, the supersaturation can be quickly relieved by sodium carbonate monohydrate formation on an existing crystal surface instead of by nucleation. Sites for crystallization are provided by the use of seed crystals and/or by maintaining a high solids content in the crystallizer. The present invention can also include pausing during the introduction of feed to allow for dispersion of local areas of very high supersaturation by agitation and/or productive relief of supersaturation on existing crystals in local areas of very high supersaturation. Control of temperature in the crystallizer is also used to control the rate of relief of supersaturation." Paragraphs [0037] and [0038] describe in detail the role of the seed crystal and read as *"supersaturation relief on existing crystals is achieved by the introduction of seed crystals of sodium carbonate monohydrate to the crystallizer 10. Thus, in contrast to other crystallization methods in which a major amount of crystal growth is by nucleation or on crystals newly formed by nucleation, processes of this particular embodiment of the present invention provide supersaturation relief primarily by growing seed crystals to crystals that are large enough to be separable from insoluble impurities on a size separation basis. Moreover, the size distribution of the product crystal population can also be controlled by adding seed crystals of a desired particle size range. By the use of seed crystals in this manner, crystal growth is productive in the sense that it occurs on crystals which will be large enough to recover on a size separation basis, rather than occurring on small particles which cannot practically be grown large enough to be separated from insoluble impurities."*

7.29 It is stated that the Hazen publication teaches the principles and the implementation of the same to prepare crystals under controlled nucleation conditions by the use of seed crystals with larger crystal sizes. The disclosure of the Hazen publication that ***"control of supersaturation and its relief in the local environment near where the feed is introduced is critical"*** is precisely the fundamental relied upon by the applicant. The alleged invention describes the dissolution of atazanavir free base in an organic solvent followed by the addition of a small and calculated amount of sulfuric acid which may be described as the reactant feed stream to induce the salt formation process at the feed inlet point wherein the supersaturation would have initiated. This is followed by seeding the

reaction mixture with Form A crystals which would provide further sites for the crystallization process to continue. The addition of the further lots of sulfuric acid in multiple stages are in accordance with the disclosure of the Hazen publication on page 6 paragraph [0061] which clearly teaches *"Crystal formation in the form of nucleation occurs when the local supersaturation level exceeds the supersaturation limit. When the rate of supersaturation generation exceeds the rate of supersaturation relief, eventually the supersaturation level somewhere in the crystallizer will exceed the supersaturation limit resulting in nucleation (sometimes referred to as "snowing-out"). Thus, to prevent the supersaturation level in a local area from exceeding the supersaturation limit, the addition of the feedstream to the saturated brine can be stopped briefly or intermittently to decrease the supersaturation level by allowing growth of existing crystals."*

7.30 Furthermore, the ***Hazen publication*** discloses at paragraphs [0084], [0085], [0086], [0087] and [0088] that, *"Physical Property of the Product... The product of the present invention also has a lower amount of dust, i.e., fines, than crystals produced by the conventional crystallization processes. .... The product of the present invention has improved flowability and decreased bridging compared to products produced by conventional methods..."*

The opponent states that the bulk of the teachings of the *Hazen publication* relate to controlled crystallization process and describes each parameter which influences the said process. The applicant has in totality implemented the Hazen publication teachings to an organic chemistry polymorph process but described the said obvious process by stating that the rate of the increased addition is based on a mathematical equation which determines the volume, time parameters etc. It is stated that the applicant has relied upon a well known principle of controlled crystallization process to avoid the formation of fines which would otherwise render the process industrially non-viable. The opponent states that atazanavir bisulfate Form A crystals were taught in D1 which the applicant has admitted in its detailed description. It is stated that a process devised to achieve a certain particle size distribution of Form A by relying upon the Hazen publication which teaches all the basic principles and methodologies does not require any extraordinary skills or knowledge. The alleged invention is therefore totally devoid of inventive merit.

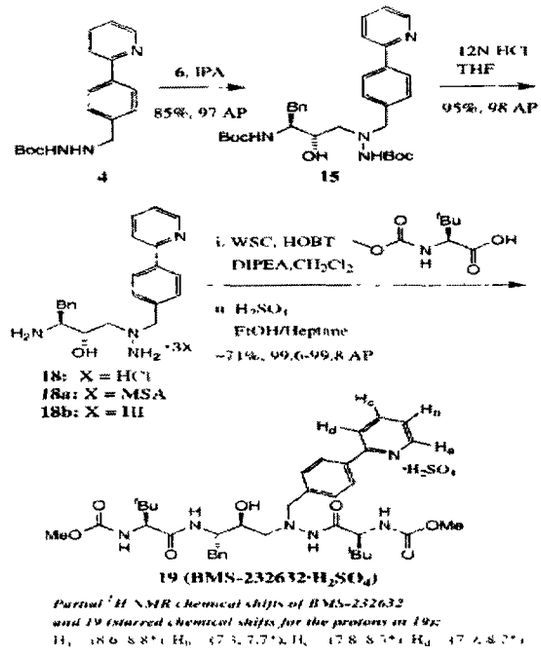
7.31 The opponent states that the applicant is merely putting together obvious steps which lack in inventive merit to present an alleged invention as non-obvious and comprising inventive merit. Thus, the alleged invention claimed in claim 8 of the impugned patent application ought to be rejected since a sum total of obvious steps each lacking in inventive merit cannot be construed as suddenly contributing inventive merit especially since the impugned patent application itself is devoid of any data or statement suggesting any inventive merit.

7.32 Claim 9 and its dependent claims 10 of the impugned invention refer to a process for preparing atazanavir bisulfate of Form A crystals. The process comprises preparing a triamine salt which is kept non-isolated while reacting with an active ester and a base in presence of an inorganic solvent to form a solution of free base. It also encompasses the conversion of the atazanavir free base to bisulfate salt by a process that was covered in claim 1 of the parent patent application i.e. 6425/DELNP/2006 which was rejected by the order dated December 20th 2010 of the Patent Office (annexed hereto as Annexure A3). It is clear that by inclusion of the process of preparing the bisulfate salt from the free base by a process that is rejected, the applicant is trying to indirectly do what he was prohibited from doing directly.

7.33 The opponent relies on D5 to demonstrate that the preparation of atazanavir bisulfate in the form of Form A crystals as claimed in claim 9 of the impugned patent application i.e. via reacting triamine salt and without isolating reacting with an active ester and a base in the presence of an organic solvent to form the atazanavir free base is very much a part of D5.

7.34 The opponent states that Scheme 5 on pages 324 column 1 of D5 discloses the conversion of a triamine salt which includes the hydrochloride salt i.e. **18** with an active ester in the presence of a base and organic solvent to form a solution of the atazanavir free base i.e. **19**. The said scheme 5 is reproduced below for ready reference:

Scheme 5. Synthesis of BMS-232632 and its bisulfate salt [9]



Partial ^{1}H NMR chemical shifts of BMS-232632 and 19 (starred chemical shifts for the protons in 19):

H_a 8.86 8.85*; H_b 7.73 7.71*; H_c 7.78 8.74*; H_d 7.74 8.27*

7.35 It is submitted that D5 covers the process for the preparation of the atazanavir free base claimed in claim 9. In fact D5 discloses that, *"..To circumvent the problematic isolation of* ***18*** *(triamine salt of claim 9 of the impugned patent application), a procedure for the deprotection and in situ coupling with N-methoxycarbonyl-L-tert-leucine (active ester of claim 9 of the impugned patent application) was developed."* Thus, it is evident that the reaction of the triamine salt with the active ester wherein the triamine salt prepared is not isolated is also taught in D5.

7.36 Moreover, D5 discloses the conversion of the atazanavir free base to a bisulfate crystalline salt which salt is a bisulfate salt due to its superior solubility. (Page 326 column 2 paragraph 2 lines 8 to 11).

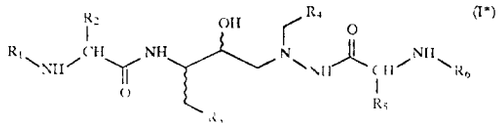
7.37 The opponent submits that preparation of atazanavir free base and its conversion to bisulfate is clearly disclosed in D5.

7.38 Furthermore, with respect to the process for the conversion of the free base to the bisulfate salt as claimed in claim 9 of the impugned patent application the opponent states that the said process claimed is the same as step (a) of claim 8 and thus suffers from the same defects and thus ought to be rejected.

7.39 The opponent submits that a mere sum total of obvious steps each lacking in inventive merit cannot be construed as to suddenly effect a non-obvious invention with inventive step especially in the absence of any statement or experimental data in the impugned patent application. The opponent thus, submits that claim 9 ought to be rejected.

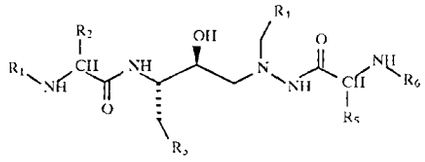
7.40 The opponent submits that claims 10 to 18 are dependent on claim 9 and derive its patentability from claim 9 and therefore are infructuous in view of the claim on which they are dependent being obvious and lacking in inventive merit.

7.41 The opponent relies on D6 to further demonstrate that alleged invention claimed in claim 9 is obvious and lacks in inventive merit. The opponent states that compound of formula I*



(I*)

especially of formula I,



(I)

wherein

R_1 is lower alkoxycarbonyl,

R_2 is secondary or tertiary lower alkyl or lower alkylthio-lower alkyl,

R_3 is phenyl that is unsubstituted or substituted by one or more lower alkoxy radicals, or C_4–C_8cycloalkyl,

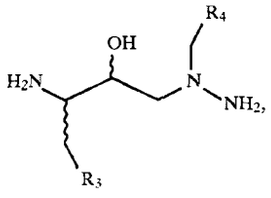
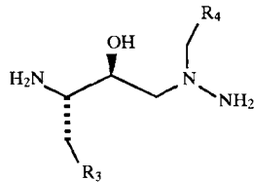
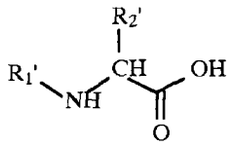
R_4 is phenyl or cyclohexyl each substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl (—SO_2—) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl,

R_5, independently of R_3, has one of the meanings mentioned for R_3, and

R_6, independently of R_1, is lower alkoxycarbonyl,

or a salt thereof, provided that at least one salt-forming group is present.

as disclosed in D6 is same as atazanavir bisulfate as described in the impugned invention which is quite apparent from the chemical structures of compound of Formula I of D6. Referring to column 14 of D6, it is stated that a diamino compound indicated as IX*/IX



in D6 is condensed with an acid indicated with formula VIIIa  . The opponent states that R_3 and R_4 as indicated in formula IX*/IX in D6 is respectively a phenyl group which is unsubstituted or substituted by one or more alkoxy radicals and phenyl or cyclohexyl each substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom and contains from 1-4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl and sulfonyl. It is stated that the triamine salt as indicated in Claim 9 of the impugned invention has similar phenyl and cyclohexyl group and specifically the structure of the triamine salt is identical to that of the diamino compound of D6. Further, from the chemical structure of the triamine salt as shown at page 19 of the impugned invention, the applicant has indicated that the triamine salt has a protecting group and during the reaction triamine acid salt remains unisolated. The opponent states that clear directions of protecting groups are found at column 15 of D6. From the above, it is crystal clear that what the applicant of the impugned invention has tried to claim in claim 9 i.e., a process for preparing atazanavir bisulfate by an alternative method from a triamine salt is already a part of a prior art, D6. Further, it is clearly disclosed in D6 in column 12, lines 64-67 that compounds of formula I i.e., derivatives of azahexane and salts thereof are prepared according to processes known in the art. Referring to claim 10, it is indicated by the applicant of the impugned invention that the triamine salt is a hydrochloride salt. The opponent states that no invention can be said to exist in claim 10 also. It is stated that for a person skilled in the art reading document D6, it would be a quite obvious choice to select hydrochloric acid so as to form hydrochloride salt of triamine. Further, referring to claim 11 the opponent states that activated esters are already disclosed at column 20, lines 25-30 in D6. Therefore, active esters as described in the impugned invention would be quite obvious for a person skilled in the field reading the disclosure of D6. As stated hereinabove, triamine compound is already taught in D6. Reaction of a triamine compound with an acid is

also found in D6. Therefore, it would be an obvious choice for a person to select hydrogen chloride reading the document D6 so as to prepare triamine hydrogen chloride salt for the preparation of atazanavir bisulfate. It is further stated that D6 describes preparation of azahexane derivatives which includes azapeptide HIV protease inhibitor in a same manner by at first preparing triamine salt. Thus reading D6 a person skilled in the art would have clear motivation to extrapolate the process for the production of the atazanavir free base as disclosed in claims 9 and 10 of the impugned invention. Moreover, the impugned patent application is entirely devoid of any alleged advantage of the process for the preparation of the atazanavir free base via the triamine salt and active ester for subsequent conversion to the atazanavir bisulfate Form A as is claimed in claim 9 of the impugned patent application.

7.42 Therefore, Claim 9 and its dependent claims 10 are devoid of any inventive step in view of D6.

7.43 Furthermore, with respect to the process for the conversion of the free base to the bisulfate salt as claimed in claim 9 of the impugned patent application the opponent states that the said process claimed is the same as step (a) of claim 8 and thus suffers from the same defects and thus ought to be rejected.

7.44 It is also submitted that the teachings D5 and D6 taken in combination also render the process for the preparation of atazanavir free base obvious to a person skilled in the art and lacking in inventive merit. Furthermore, with respect to the process for the conversion of the free base to the bisulfate salt as claimed in claim 9 of the impugned patent application the opponent states that the said process claimed is the same as step (a) of claim 8 and thus suffers from the same defects and thus ought to be rejected

7.45 It is submitted that the mere sum total of obvious steps each lacking in inventive merit cannot be construed as to suddenly effect a non-obvious invention with inventive step especially in the absence of any statement or experimental data in the impugned patent application. The opponent thus, submits that claim 9 ought to be rejected.

7.46 Furthermore, considering claims 12, 13 and 15, it is stated that the base as an organic base is already disclosed in column 23, lines 8 to 13 of D6. It is also taught in D6 that condensation of activated esters is carried out in presence of inorganic carbonates for example ammonium or alkali metal carbonates, or hydrogen carbonates such as sodium or potassium carbonate, alkali metal hydroxides such as sodium hydroxide. It is stated

that from D6 a clear idea of using an organic base or alkali metal hydroxide base is available. Referring to Claim 13, it is further specified by the applicant of the impugned invention that the base is NaOH, KOH, $Mg(OH)_2$, K_2HPO_4, $MgCO_3$, K_2CO_3, triethylamine, diisopropyl ethyl amine and organic solvent is methylene chloride, tetrahydrofuran acetonitrile or N,N dimethyl formamide. It is stated that column 23, lines 28-35 clearly teach condensation by dimethylformamide, methylene chloride, tetrahydrofuran and acetonitrile. Therefore, it is quite obvious for a skilled person to get motivation of using organic base and bases like alkali metal hydroxides as already disclosed in D6. The opponent states that by considering process of condensation of D6 and the bases used therein, one can reach the process as disclosed in claims 12 and 13 of the impugned invention easily. Therefore, Claims 12, 13 and 15 also fails the test of inventive step.

7.47 Referring to claim 14, the opponent states that the temperature as disclosed in D6 ranges from -40^0C to 100^0C and especially the temperature ranges from 10^0C to 30^0C. The temperature ranges claimed in claim 30 by the applicant of the impugned invention is from 30^0C to about 40^0C which lies well within the temperature range is taught in D6. Therefore, claim 14 also lacks inventive step.

7.48 Claims 16 and 17 describe a process where free base is converted to corresponding bisulfate salt by treating a solution of free base with methylene chloride with N-methyl pyrrolidone and acetone, heating the mixture to remove methylene chloride and treating with sulfuric acid to form bisulfate salt of the free base. Claims 16 and 17 being dependent on claim 9 draw their patentability from claim 9 and thus since claim 9 is itself obvious and lacking in inventive merit, claim 16 and 17 are infructuous. Moreover, claims 16 and 17 also do not add any inventive merit to the alleged invention claimed in claim 9 of the impugned patent application.

7.49 It is submitted that claims 19, 20 and **20** which derive their patentability from claim 9 are infructuous since the claim on which they are dependent is obvious and lacking in inventive merit.

7.50 Referring to claim 18 and claim 21 of the impugned application, it is submitted that it is an attempt by the applicant to claim indirectly what has been disallowed directly. It is submitted that the matter dependent claim 18 and claim 21 seeks to add to independent

claim 9 and claim 8 respectively is within the scope of the claims rejected by the Ld. Controller in the order dated December 20, 2010 rejecting the parent patent application No: 6425/DELNP/2006 and therefore does not add any inventive merit to the independent claims and thus ought to be rejected. Nevertheless, with respect to claim 18 and claim 21 of the impugned patent application it is submitted that the cubic crystallization which is temperature controlled crystallization is well known in the art and it is already admitted by the applicant that it is derived from known Mullin, "Crystallization, 3rd Ed.1993, Butterworth Heineman, Pubs". The opponent states that addition of sulfuric acid in an increasing rate following the cubic equation cannot be a subject matter of inventive step of claim 18 of the present invention. It is stated that in example 1 of D1 preparation of bisulfate from ethanol has been described. Considering example 1 it is found that a suspension of free base compound I i.e. Azapeptide in ethanol is treated by concentrated sulfuric acid and the said concentrated sulfuric acid is added in a dropwise manner. It is stated that it would be quite an obvious choice for a person skilled in the art reading the disclosure of D1 and Mullin, "Crystallization, 3rd Ed.1993, Butterworth Heineman, Pubs" to add sulfuric acid in the free base in a dropwise way in an increasing rate. The opponent states that therefore, having in hand D1 and knowing about Mullin, "Crystallization, 3rd Ed.1993, Butterworth Heineman, Pubs" a person skilled in the art can easily increase or decrease the rate of addition of sulfuric acid to the free base compound. Therefore, addition of sulfuric acid in increasing rate following cubic equation and in multiple stages as claimed in claims 18 and 21 of the invention of impugned application cannot be termed as non-obvious to a person skilled in the art.

Therefore, it is submitted that the mere sum total of obvious steps each lacking in inventive merit cannot be construed as to suddenly effect a non-obvious invention with inventive step especially in the absence of any statement or experimental data in the impugned patent application. The opponent thus states that the claims 18 and 21 therefore, ought to be rejected. In fact, the opponent states that the dependent claims 18 and 21 would be rendered infructuous by virtue of the independent claims from which they derive their patentability being rendered obvious and lacking in inventive merit.

7.51 The opponent thus states that it is evident that there exists no dearth of prior art documents demonstrating the obviousness and lack of inventive merit of the various alleged inventions claimed in the impugned patent application.

8 NOT INVENTION WITHIN MEANING OF ACT [Section 25 (1) (f)]:

8.1 SECTION 2(1) (ja)

The opponent states that the claimed invention falls under the mischief of Section 2(1) (ja) being devoid of inventive step. Opponent states that alleged invention is neither a technical advancement nor is it giving any economic significance. It is further stated that there is no clear problem which is solved by the specific process and product of the impugned patent. The impugned patent only has the processes and forms of atazanavir bisulfate. No technical advancement or additional advantage or surprising result is taught in the impugned patent for processes and forms of the atazanavir bisulfate and therefore devoid of inventive step. Accordingly, the impugned patent is not an invention according to Section 2(1)(ja). In order to avoid repetition the opponent relies on the arguments advanced hereinabove in paragraph 7.

8.2 SECTION 3(d)

8.2.1 The opponent states that the claimed invention falls within the mischief of Section 3 (d) which clearly states that the *mere discovery of a new form of a known substance* which does not result in the enhancement of known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the *mere use of a known processn ..... unless such known process results in a new product or employs at least one new reactant is not patentable under this Act.*

Explanation- For the purposes of this clause, salts, esters, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, *combinations* and other derivatives of known substance shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy.

8.2.2 The opponent further states that according to Section 3 (d) of the Indian Patent Act, different forms of the known substances shall be considered as the same substances unless they significantly differ in terms of properties with regard to efficacy. In other words, Section 3 (d) has set out some conditions to satisfy the criteria of patentability in order to stop the ever-greening and protection of the inventions which are already known. It should be noted that the impugned patent application is devoid of any experimental data demonstrating any advantage achieved as a result of the atazanavir bisulfate Pattern C material let alone data on efficacy to demonstrate that the Pattern C material over the compositions of atazanavir bisulfate or azapeptide HIV protease inhibitor already known in the art.

8.2.3 It is stated that the Form A atazanavir bisulfate crystals and process for its preparation claimed in the impugned patent application also falls within the mischief section 3(d) seeks to address.

8.2.4 The opponent states that the legality of this provision was challenged in the *Hon'ble* Madras High Court and such challenge was set aside. While setting aside the petition, the Ld. Judges were pleased to make *inter alia* the following observations:

"The position therefore is, if the discovery of a new form of a known substance must be treated as an invention, then the patent applicant should show that the substance so discovered has a better therapeutic affect. Darland's Medical Dictionary defines the expression "efficacy" in the field of pharmacology as "the ability of a drug to produce the desired therapeutic affect" and efficacy is independent of the potency of the drug. The dictionary meaning of the term "therapeutic" is healing of a disease – having a good effect on the body. Going by the meaning of the terms "efficacy" and "therapeutic" extracted above, what the patent applicant is expected to show is, how effective the new discovery made would be in healing a disease or having a good effect on the body? In other words, the patent applicant is definitely aware as to what is the "therapeutic effect" of the drug for which he had already got a patent and what is the difference between the therapeutic effect of the patented drug and the drug in respect of which patent is asked for. Therefore, it is a simple exercise of, though preceded by research, we state, for any patent applicant to place on record what is

the therapeutic effect/efficacy of a known substance and what is the enhancement in that known efficacy. The amended section not only covers the field of pharmacology but also the other fields. As we could see from the amended section, it is made applicable to even machine, apparatus or known process with a rider that mere use of a known process is not an invention unless such a known process results in a new product or employs atleast one new reactant. Therefore the amended Section is a comprehensive provision covering all fields of technology, including the field of pharmacology. In our opinion, the explanation would come in aid only to understand what is meant by the expression "resulting in the enhancement of a known efficacy" in the amended section and therefore we have no doubt at all that the Explanation would operate only when discovery is made in the pharmacology field"

"In our respectful opinion, when the validity of an Act is challenged on the touchstone of Article 14 of the Constitution of India, the decision has to depend upon the provisions of the concerned Statute itself, which are in challenge. Of course, law is well settled that when there is vagueness in any provision of law leading to arbitrary exercise of power / uncanalised powers, the Act should be struck down."

"We have borne in mind the object which the Amending Act wanted to achieve namely, ***to prevent evergreening****; to provide easy access to the citizens of this country to life saving drugs and to discharge their Constitutional obligation of providing good health care to its citizens."*

8.2.5 The Opponent states that all the above discussion makes it evident that applicant has failed to satisfy the conditions set out by Section 3(d) and therefore the impugned invention is liable to be rejected on the ground of inventions not patentable under the Act.

8.3 <u>SECTION 3(e)</u>

The opponent states that the alleged invention in claims 19, 20 and **20** claim a pharmaceutical formulation comprising a pharmaceutical composition of Pattern C

material suitable pharmaceutical carrier. From the text one can gather that a preferred pharmaceutical carrier is a solid carrier. It is stated that under no stretch of imagination there can be any synergy between the composition of Pattern C material and the carrier and therefore it is a simple mixture of solid carriers and Pattern C material. Furthermore, there is not even a statement let alone any demonstration of synergy. Therefore, claims 19 and the two claims number 20 and **20** cover an invention which is not a patentable subject matter in India under Section 3(e). Claims 19 and the two claims i.e. 20 and **20** ought to be rejected on this ground.

9 INSUFFICIENCY [Section 25 (1) (g)]:

9.1 The opponent states that the impugned application is liable to be rejected on the ground of insufficiency as well in that it does not describe with sufficient clarity to a general specialist in the art as to how the claimed invention may be put to practice. The opposed application is therefore liable to be rejected on the ground of insufficiency as well.

9.2 The opponent states that the impugned patent application is devoid of any experimental data demonstrating any advantages or comparative data.

9.3 The term 'Pattern C material' disclosed in the impugned patent application as **'partial crystalline'** does not provide clarity on the forms known to a person skilled in the art.

9.4 It is also submitted that the preparation of Pattern C material prepared by suspending Form A crystals of atazanavir bisulfate to a relatively high humidity of at least about 95% RH for at least 24 hours as claimed in claims 1 and 8 are not exemplified in the detailed description of the impugned patent application. It is thus evident that the applicant has failed to provide the best embodiment as required under the Patent Act and thus, the alleged invention ought to be rejected.

9.5 Furthermore, the opponent draws the attention of the Ld. Controller to the fact that while the applicant discloses and claims in the impugned patent application that the Pattern C material is derived from atazanavir bisulfate Form A crystals, it discloses at page 6 paragraph 2 that *"....Pattern C material may also be formed by wet*

granulating ***the atazanavir bisulfate*** *or a combination* ***of atazanavir bisulfate*** *and excipients and drying the wet granulation."* It is thus submitted that there exists a lack of clarity in the complete specifcation of the alleged invention claimed since on one hand the applicant claims and asserts that Pattern C material is prepared from atazanavir bisulfate Form A crystals and on the other hand it discloses that Pattern C material can be prepared from atazanavir bisulfate in general without specifying any particular form. Thus, this contradiction in the source used for the preparation of Pattern C material renders the alleged invention claimed in the impugned patent application ambiguous and unclear.

10 BREACH OF SECTION 8 [Section 25 (1) (h)]:

10.1 The applicant is required to provide all the information regarding the prosecution of his equivalent applications till the grant of his Indian application to the Controller in writing from time to time and also within the prescribed time which the applicant has failed to do.

10.2 The opponent states that the applicant is under an obligation of Section 8(2) of the Patents Act that he would furnish details to the Ld. Controller regarding the corresponding foreign applications is objected to on the ground that the invention is lacking in novelty or patentability, the amendments effected in the specifications, the claims allowed in respect thereof and other such particulars. The opponent states that the applicant has failed to fulfill the duty of disclosure imposed under Section 8(2) of the Act and therefore the impugned application is liable to be rejected on this ground alone.

11 RELIEF SOUGHT

The opponent states that it has established and made out a case on each of the aforesaid grounds of opposition and pray to the Ld. Controller for the following relief (s).

1) Take on record the present representation;
2) Leave to file evidence;
3) Forward copy of reply of applicant and evidence if any and any amendments filed;

4) Leave to file a replication to the reply of the applicant and evidence
5) Grant of hearing
6) Refusal of the application *in toto*;
7) Such other relief or reliefs as the Controller may deem appropriate.

Dated this the 08th day of November 2011

Mythili Venkatesh
Of S. Majumdar & Co.
Opponent's Agent

To
The Controller of Patents
The Patent Office
New Delhi

Annexure/Exhibits

- Annexure A1;
- Annexure A2;
- Annexure A3;
- Exhibit 1;
- Exhibit 2;
- Exhibit 3;
- Exhibit 4;
- Exhibit 5;
- Exhibit 6.

Annexure A1

WE CLAIM:

1. Atazanavir bisulfate Pattern C material.

2. The compound as defined in Claim 1 which is Pattern C material atazanavir bisulfate characterized by the powder x-ray diffraction pattern substantially in accordance with that shown in Figure 6.

3. The compound as defined in Claim 1 which is characterized by a differential scanning calorimetry thermogram substantially in accordance with that shown in Figure 7.

4. The compound as defined in Claim 1 which is characterized by a thermal gravimetric analysis curve substantially in accordance with that shown in Figure 8.

5. The compound as defined in Claim 1 prepared by suspending Form A crystals of atazanavir bisulfate in water or subjecting Form A crystals of atazanavir bisulfate to a relatively high humidity of at least about 95 % RH for at least 24 hours or wet granulating Form A crystals of atazanavir bisulfate and then drying.

6. Pattern C prepared by the process of Claim 8.

7. The compound as defined in Claim 1 in the form of a pharmaceutical composition prepared by mixing Form A crystals of atazanavir bisulfate with one or more formulating excipients and water followed by drying.

8. A process for preparing atazanavir bisulfate Pattern C material as defined in Claim 1, which comprises:

(a) preparing atazanavir bisulfate in the form of Form A crystals, which comprises reacting a solution of atazanavir free base in an organic solvent, in which the bisulfate salt of atazanavir is substantially insoluble, with a first portion of concentrated sulfuric acid in an amount to react with less than about 15% by weight of the atazanavir free base, adding seeds of Form A crystals of atazanavir bisulfate to the reaction mixture, as crystals of atazanavir bisulfate form, adding additional concentrated sulfuric acid in multiple stages at an increasing rate to effect formation

(36)

of atazanavir bisulfate crystals, and drying the atazanavir bisulfate to form Form A crystals;

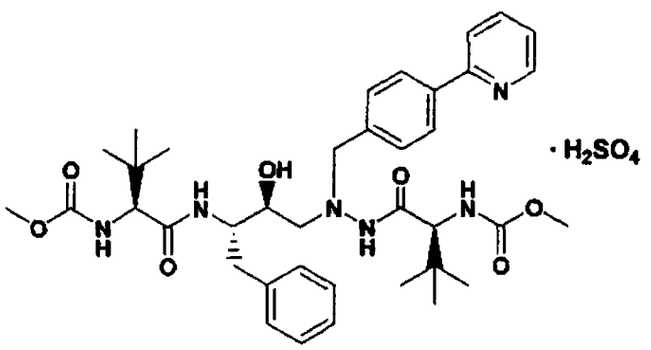
(b) suspending crystals of Form A atazanavir bisulfate in water and drying the suspension to form Pattern C material; or

(c) subjecting crystals of Form A atazanavir bisulfate to high relative humidity of greater than 95% RH for at least 24 hours to form Pattern C material; or

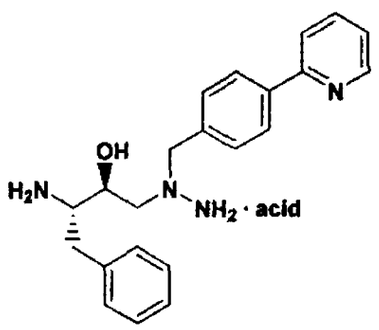
(d) wet granulating atazanavir bisulfate and drying the wet granulation to form Pattern C material; or

(e) mixing Form A crystals with one or more formulating excipients and wet granulating the resulting mixture to directly form Pattern C material in admixture with the excipients.

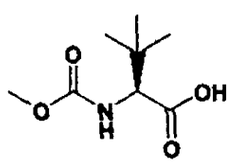
9. A process for preparing atazanavir bisulfate



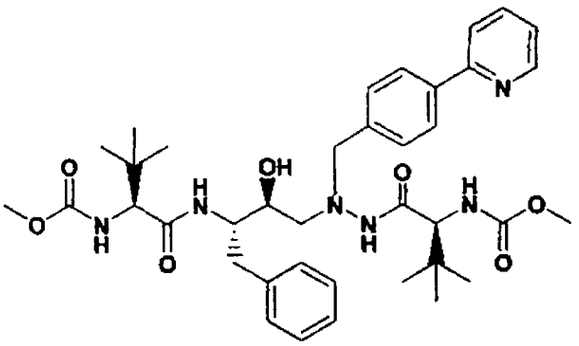
in the form of Form A crystals, which comprises preparing a triamine salt of the structure



and without isolating the triamine salt, reacting the triamine salt with an active ester of an acid of the structure

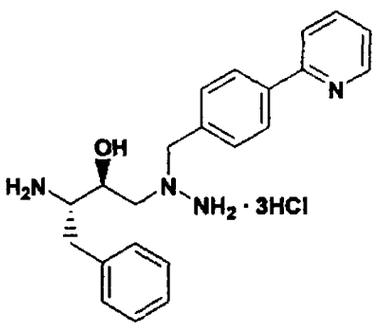


and a base in the presence of an organic solvent to form a solution of the atazanavir free base of the structure

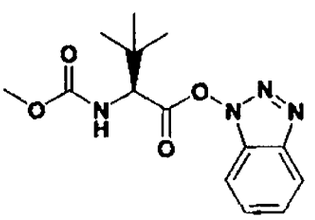


and converting the free base to the corresponding bisulfate salt in the form of Form A crystals, by the process which comprises reacting a solution of atazanavir free base in an organic solvent, in which the bisulfate salt of atazanavir is substantially insoluble, with a first portion of concentrated sulfuric acid in an amount to react with less than about 15% by weight of the atazanavir free base, adding seeds of Form A crystals of atazanavir bisulfate to the reaction mixture, as crystals of atazanavir bisulfate form, adding additional concentrated sulfuric acid in multiple stages at an increasing rate to effect formation of atazanavir bisulfate crystals, and drying the atazanavir bisulfate to form Form A crystals.

10. The process as defined in Claim 9 where the triamine salt is the hydrochloride salt



11. The process as defined in Claim 9 wherein the active ester of the acid has the structure



12. The process as defined in Claim 9 wherein the base is an alkali metal hydroxide, an alkaline earth metal hydroxide, an alkali metal carbonate, an alkaline

earth metal carbonate, an alkali metal phosphate, an alkaline earth metal phosphate or an organic base.

13. The process, as defined in Claim 12 wherein the base is NaOH, KOH, $Mg(OH)_2$, K_2HPO_4, $MgCO_3$, Na_2CO_3, K_2CO_3, triethylamine, diisopropylethylamine or N-methylmorpholine and the organic solvent is methylene chloride, ethyl acetate, dichloroethane, tetrahydrofuran, acetonitrile or N,N-dimethylformamide.

14. The process as defined in Claim 9 wherein the triamine salt and the active ester are reacted at a temperature within the range from about 30 to about 40°C.

15. The process as defined in Claim 14 wherein the triamine salt and the active ester are reacted in the presence of K_2HPO_4 as the base and methylene chloride as the solvent.

16. The process as defined in Claim 9 wherein the free base is converted to the corresponding bisulfate salt by treating a solution of free base in methylene chloride with N-methyl pyrrolidone and acetone, heating the above mixture to remove methylene chloride and treating the above mixture with sulfuric acid to form the bisulfate salt of the free base.

17. The process as defined in Claim 16 including the step of seeding the mixture of free base, acetone and N-methylpyrrolidone with crystals of atazanavir bisulfate.

18. The process as defined in Claim 9 wherein the sulfuric acid is added at an increasing rate according to the following equation

$$V_{time} = V_{total} \times \left(\frac{time}{time_{total}} \right)^3$$

where

V_{time} = Volume of sulfuric acid added during elapsed time period

V_{total} = Total volume of acid representing the 90% charge

time = Elapsed time in crystallization

$time_{total}$ = Total crystallization time or total time for acid charging

19. A pharmaceutical formulation comprising atazanavir bisulfate Pattern C material as defined in Claim 9 and a pharmaceutically acceptable carrier therefor.

20. The formulation as defined in Claim 19 comprising atazanavir bisulfate Pattern C material, one or more fillers, one or more disintegrants, optionally one or more binders, and optionally one or more glidants or lubricants.

20. The formulation as defined in Claim 19 comprising atazanavir bisulfate Pattern C material, lactose, crospovidone, and magnesium stearate.

21. The process as defined in Claim 8 wherein the sulfuric acid is added at an increasing rate according to the following equation

$$V_{time} = \frac{V_{total}}{time_{total}} \times \left(\frac{time}{\quad} \right)^3$$

where

V_{time} = Volume of sulfuric acid added during elapsed time period

V_{total} = Total volume of acid representing the 90% charge

time = Elapsed time in crystallization

$time_{total}$ = Total crystallization time or total time for acid charging.

Dated this 1st day of May, 2009.

[SHUKADEV KHURAIJAM]
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANT[S]

Annexure A2 (40)

WE CLAIM:

1. A process for preparing atazanavir bisulfate in the form of Form A crystals, which comprises reacting a solution of atazanavir free base in an organic solvent, in which the bisulfate salt of atazanavir is substantially insoluble, with a first portion of concentrated sulfuric acid in an amount to react with less than about 15% by weight of the atazanavir free base, adding seeds of Form A crystals of atazanavir bisulfate to the reaction mixture, as crystals of atazanavir bisulfate form, adding additional concentrated sulfuric acid in multiple stages at an increasing rate to effect formation of atazanavir bisulfate crystals, and drying the atazanavir bisulfate to form Form A crystals.

2. The process as claimed in claim 1, wherein the solution of atazanavir free base is initially reacted with from 5 to 15% by weight of the total amount of sulfuric acid employed.

3. The process as claimed in claim 1, wherein the solution of atazanavir free base is initially reacted with from 8 to 12% by weight of the total amount of sulfuric acid employed.

4. The process as claimed in claim 1 wherein the atazanavir free base is reacted with the first portion of sulfuric acid at a temperature within the range from 35 to 55°C.

5. The process as claimed in claim 1, wherein the solution of atazanavir free base is heated to a temperature within the range from 35 to 55°C before it is reacted with sulfuric acid.

6. The process as claimed in claim 1, wherein the reaction mixture of atazanavir free base and sulfuric acid is seeded with from 0.1 to 80 weight % of Form A crystals based on the weight of atazanavir free base.

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7. The process as claimed in claim 1, wherein the seeded reaction mixture is heated at a temperature within the range from 35 to 55°C.

8. The process as claimed in claim 1, wherein the organic solvent for the atazanavir free base is acetone, a mixture of acetone and N-methyl pyrrolidone, ethanol, or a mixture of ethanol and acetone.

9. The process as defined in claim 1, wherein the sulfuric acid is added at an increasing rate according to the following equation

$$V_{time} = V_{total} \times \left(\frac{time}{time_{total}}\right)^3$$

where

V_{time} = Volume of sulfuric acid added during elapsed time period

V_{total} = Total volume of acid representing the 90% charge

time = Elapsed time in crystallization

$time_{total}$ = Total crystallization time or total time for acid charging.

10. Form A of atazanavir bisulfate prepared by the process of claim 1.

11. A pharmaceutical formulation comprising Form A of atazanavir bisulfate as claimed in claim 10 and a pharmaceutical acceptable carrier therefor.

Dated 1/11/2006

[SHUKADEV KHURAIJAM]
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANT[S]

Annexure A3

(42)

INTELLECTUAL
PROPERTY **INDIA**
Patents/Designs/Trademark
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY
Intellectual Property Office Building
Plot No.32, Sector – 14, Dwarka
NEW DELHI - 110 075.
E-mail : delhi-patent@nic.in

http://ipindia.nic.in

✆ 28034304, 05, 06

✆ 28034322-28034320
Fax No.28034301

No. 6425 /DELNP/2006 **Dated 22/12/2010**

22 DEC 2010

TO,

REMFRY & SAGAR ATTORNEYS-
AT-LAW REMFRY HOUSE
MILLENNIUM PLAZA SECTOR-27,
GURGAON-122002, INDIA.

SUB: - DECISION U/S 25(1) IN RESPECT OF APPLICATION NO. 6425/DELNP/2006.

Sir,

Please find attached here with the copy of decision U/S 25 (1) in respect of Application No. 6425/DELNP/2006 vide opposition filed by M/S CIPLA L.T.D INDIA.

regards.

Yours Faithfully,

DEBASHISH PALIT
(Support Staff chemical)

clem/2010/5399

M/S Majumdar & Co.
5, Harish Mukherjee Road
Kolkata-700025.

RECEIVED
29 DEC 2010
S. MAJUMDAR & CO.

(13)

22 DEC 2010

The Patents Act, 1970

Section 25(1)

IN THE MATTER OF the application for patent No. 6425/delnp/2006 filed on 1st Nov.2006

And

IN THE MATTER OF representation under section 25(1) of the Patent Act, 1970 as amended by Patent (Amendment) Rules, 2005.

And

IN THE MATTER OF rule 55 of the Patent Rules, 2003, as amended by the Patent (Amendment) Rules, 2005.

M/s Bristol Myers Squibb, USA ..... The Applicant

M/s CIPLA Ltd.,India ..... The Opponent

Hearing held on November 30th 2009

Represented by

Mr S.Khuraijam — Agent for the Applicant
From M/s Remfry &Sagar

Mr S.Majumdar — Agent for the opponent
From M/s S.Majumdar & co.

A representation by way of opposition u/s 25(1) was filed by M/s S .Majumdar & company on behalf of M/s Cipla Limited on 20th January 2009,in the present case. The applicant submitted their reply statement and evidence on 1st May 2009. In between, the examination proceedings also started.The applicant submitted an amended set of claims comprising nine claims for the process of preparing of Atazanavir bisulphate form A ,one claim for product by the process and one composition claim.

The present application was filed by M/s Bristol Myers Squibb company, USA, which is a national phase application of PCT/ US05 /015333 claiming priority of US 60/568043 dated 04/05/2004 . This application was filed on 1st Nov,2006 for an invention claiming "Process for preparing ATAZANAVIR BISULPHATE and novel forms", was allotted the application number 6425/delnp/2006.

The opponent has opposed the application on these grounds i.e. lack in inventive step/obviousness u/s 25(1)(b), not an invention with in the meaning of the Act u/s 25(1)(f)

The opponent first objected to the voluntary amendment sought in claims and submitted that the claim 1 as amended includes the term "at an increasing rate" ,which was not part of the claim originally filed. It was part of claim 34 which was dependent on original claim 25 an independent claim not dependent on claim 1. Therefore it is submitted that "at an increasing rate "is now made an essential and key feature of the alleged invention although such feature was never claimed in claim 1 or any claim dependent upon claim1. Therefore amended claim1 is a new form in terms of section 59 of the Act and merit rejection..

On the ground of lack of inventive step/obviousness the opponent referred to the prior art document D1 and D3 during the hearing. The opponent argued that atazanavir bisulfate was known as atazanavir free

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base, atazanavir bisulphate salt and atazanavir form-A . According to the applicant the known atazanavir form-A was suffering from small particle size leading to handling difficulties. The opponent argued that the obvious solution lies in the enhancement of particle size, which is a known technique in the polymorph chemistry, where as applicant claimed a stage wise addition of sulphuric acid to be allegedly inventive. The opponent said that the applicant in their reply statement vide page 3,7and 9 has emphasized the addition "at an increasing rate" being key parameter to attain the desired crystal feature, though it was not appearing in claim 1 as filed.

Opponent also brought to the notice various disclosure in D1.In example -1 in which 15.013 grams of free base compound and 113ml. of 200 proof ethanol were added with stirring. To this suspension 1.28ml. concentrated sulphuric acid added drop wise over 90 seconds. In example-2 5M sulphuric acid was added drop wise to a suspension of free base (30gms) in acetone (213ml), stirred mechanically in a 50 degree oil bath. Opponent argued that teaching as a whole to be taken and applicant was incorrect in his reply Paragraph (2.21) to conclude that from a single example in D1,teaches drop wise addition in 90 seconds which implies that all the sulphuric acid was added in one lot.

The opponent's argument that the example-1 step E on page 27 of the specification describes 'total charge of the acid to be 19 grams but 10% of it added initially and rest 17.8 grams to be added in five stages as per the table-1,in accordance with the cubic equation claimed .But the aforesaid statement is contrary to the disclosure made on page22 of the reply statement of applicant which states "example -1 on page 27 is obtained by experiment and not by calculation". In D1 teaches preparation of atazanavir bisulphate form-A crystal by drop wise addition of concentrated sulphuric acid to the free base under stirring condition, whereas the impugned application carries a similar conversion involving multi stage addition of concentrated sulphuric acid. The applicant argued that in absence of essential feature "at an increasing rate" which is incorporated in claim-1 by amendment ,the

impugned application aims at achieving the same technical effect as D1 . The impugned application allegedly describe the contribution of the process claimed therein to achieve a larger crystals of Form A by addition of sulphuric acid in an increasing rate according to the cubic equation which is however defeated by the statement in the reply that "...*the data set out in example-1 is very similar to the calculated data using the cubic equation*". The opponent argued that such contradictory data should be considered carefully since the applicant contends that sulphuric acid addition at an increasing rate in accordance with the cubic equation is the crux of the invention, is the key to the determine the inventive merit which however is defeated by the applicant's reply statement .

The opponent further submitted that D3 teaches about the problem that occur with crystal forms and probable ways to overcome the same by making appropriate changes in the crystallization technique and the combined technique of D1 and D3 bring out each and every parameters the applicant followed ,in the impugned application . The opponent referred to the following para of the prior art

(1)Page 1 Para 0012 (D3)...."...*relief of super saturation is controlled such that the crystal formation primarily occurs on existing crystals, rather than occurring as nucleation or growth of newly formed crystals. In this manner the particle size distribution of crystals is controlled to achieve a desired distribution of product crystal size, "*

(2) Page 6, Para (0060) (0061) D3....."*Thus to prevent the super saturation level in a local area from exceeding the super saturation limit, the addition of the feed stream to the saturated brine can be stopped briefly and intermittently to decrease the super saturation level by allowing the growth of existing crystal "*

(3) Page 8 ,Para (0084-0086)of D3.....*The product of the present invention has a lower amount of dust i.e. fines than crystal produced by conventional process.... The product of the present invention has improved*

flow ability and decreasing bridging compound to product produced by conventional process "

The opponent argued that D1 is a document that pertains to the same technical area and D3 is a document that teaches the application of pause-break mechanism to a crystallization process . Combining the teaching found in these documents, person skilled in the art would readily arrive to the allegedly claimed invention.

The opponent also drew the attention to the Annexure (C) of the applicant's reply statement which is a paper co-authored by four of the six inventors of this impugned application, which was referred to by the applicant's expert to established the inventive merit of the present invention. The opponent drew attention to page 894 ..."*.....optimization of crystallization process for an API is important not only for the key product requirement mentioned above but also for the process efficiency* .." . On page 895 ,fig 1 depicts the uncontrolled crystallization process which is according to applicants are in accordance with disclosure in D1 and highlighted the observation of the inventors that" this process generated an exotherm of 3^0C in the lab and that the said exotherm was controlled in scale by slow addition", thereby admitting that on industrial scale the addition of sulphuric acid has to be invariably effected gradually. This statement eventually defeat the claim of the applicant that D1 teaches a constant single lot addition being a distinguishing feature between D1 and impugn application which was also acknowledged in the reply at page 9,-- "it has been found that when sulphuric acid is added in multiple stage at an increasing rate in accordance with the cubic addition equation, the process provides larger more well defined atazanavir bisulphate crystals with a narrow particle size range with lesser fines than a constant addition rate crystallization as disclosed in cited Singh et al patent D1. The opponent argued that the impugned application lack inventive merit on the face of the teaching of D1 and the combined teaching of D1 and D3.

Affidavit of Dr.Bruce Lotz dated May, 2009 who happens to be an inventor to the impugned application asserts vide paragraph 9 ,that the controlled crystallization of D1 to be disadvantageous and stated that rational control of the crystallization as in the process as defined in claim-1 is thereof is vital. The multiple stage addition preferably via the cubic crystallization as defined in claim-9, control the critical physical properties by maintaining a low super saturation of product in solution at all times". The opponent argued that the incremental addition of sulphuric acid is thus a preferred but certainly not an essential feature of the invention. Therefore in the absence of this essential feature the impugned application is merely a extrapolation of D1 since admittedly temperature control at industrial scale is effected by slow addition (Annexure –C)

The opponent further argued that in para-10 of the expert evidence that a full discussion.....by controlled crystallization...."the expert has referred to Soojan Kim et al paper (Annexure C) and stated that the paper clearly justifies three distinct process for crystallization, (1)uncontrolled crystallization (2) controlled crystallization (3)cubic addition crystallization and the expert has unequivocally stated that the advantage of atazanavir crystal of the present invention are achieved due to multiple stage addition which is merely a linear addition of sulphuric acid (Para 10 of the affidavit). The opponent submitted that D1 teaches the reactant, reagent and reaction condition to be followed for obtaining atazanavir bisulfate crystals, a person skilled in the art will combine the teaching of D1 and D3 to obtain the larger size. Therefore applicant's allegedly claiming a process which gives larger crystals and less fines may be easily designed without any extraordinary skill. The impugned application is therefore devoid of inventive merit and ought to be rejected.

The opponent argued that the applicant in their reply statement on page 21,last paragraph provided "...as seen in the table -1 page 27..... This data was obtained by experiment and not by calculation ", implying thereby that step-E which pertains to the preparation of Form-A crystal as set

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out on page 27 of the complete specification defines values which have been obtained without employing the cubic equation. The opponent argued that they clearly brought about the anomaly in the statement of the applicant in the complete specification. "the remaining sulphuric acid (17.8) was added over five hours in five stage according to the protocol defined by the cubic equation while keeping the temperature between 40-50^{0}C ", which is eventually claim 1 and claim 9 (as amended). The opponent pleaded that the applicant selecting the terminology 'at an increasing rate ' from the generic disclosure and incorporated the term in the principal claim which is out of scope of the claim originally filed which is bad in law and not allowable.

Opponent also put some case laws in favour of their arguments

(1)IN re Aller et al no. 6079 US Court of customs & patent appeal *".....normally it is to be expected that a change in temperature or it's concentration or both, would be an unpatentable modification.Under some circumstances however changes such as these may impart patentability to a process of the particular range claimed produce a new and unexpected result which is different in kind and not merely in degree in result from the result of the prior art...."*

(2) In re scherl,156F2d72, 33 C.C.P.A, patents 1193-*"however even though applicant's modification results in great improvement and utility over the prior art it may still not be patentable, if the modification was within the capabilities of one skilled in the art"* .

(3) In re irmscher 150, F2d 705, 32 C.C.P.A, patents 1259-*"more particularly where the general condition of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation."*

In the present case, the applicant has done nothing more than combining the teachings of D1 and D3 to arrive at the present invention. D1 teaches the information of Atazanavir bisulfate form –A and D3 is a document which teaches the application of pause – break mechanism to a crystallization process. It is completely within the skill of a skilled person to

arrive at the present invention by controlling the rate at which sulphuric acid is added. Thus the present invention merely optimized the rate by way of routine experimentation.

(4) EPO Board of Appeals – T0684/98 (obvious choice of process parameters) " *.....The objective problem underlying the patent in suit can only be seen in providing a purification process resulting in a caprolactam of high purity having a large crystal size and low water content and in avoiding the sticking of the caprolactam on surface coming in contact with. Finally it remains to decide whether or not the proposed solution to the objective problem underline the patent in suit is obvious in view of the state of the art..... The Board concludes from the above that the state of the art, in particular document (4)and (3) gives the person skilled in the art, concrete incentive on how to solve the objective problem underlying the patent in suit as defined in the above points (5.4) last paragraph namely by maintaining the water concentration at a level such as now claimed and by cooling by means of latent heat of evaporation at a temperature and a reduced pressure at values encompassed by the claimed ranges, thus arriving at the process of the claimed invention without involving any inventive activities".*

In the present matter D1 teaches all the reaction condition, reactants and reagents to be reacted to obtain atazanavir bisulfate crystal and D3 also teaches the control of fines and crystal size by way of optimizing the crystallization process. Thus D1 & D3 provide concrete incentive to arriving at the present invention.

(5) In the supreme court of India- Bishwanath Prasad Radhey Shyam Vs Hindustan Metal Industries "*.....It is important to bear in mind that in order to be patentable an improvement on something known before or a combination of different matters already known, should be something more than a mere workshop improvement and must independently satisfy the test of invention or an inventive step.....*"

(6)Object of invention different from what was originally defined IPAB-Novartis Vs UOI (M.P nos.1to 5/2007 in TA/1 to 5 2007/PT/CH &MP

(51)

no.33/2008.in TA/1/2007/PT/CH and TA/1 to 5/2007/PT/CH)- "*.....Thus the applicant accept that the original object of its alleged invention was not to demonstrate enhancement of efficacy of its subject compound-β crystal form of Imatinib mesylate. It carried out further detailed experiment/ study for the purpose and submitted new data which was not originally disclosed as a part of the specification. A patent is granted on the basic of its full disclosure of the invention in the specification furnished on the priority date of the application. Even an amendment is not allowed in the specification which in substance is not disclosed their in (section 59) . The patent law debars an applicant a grant of patent for belated discovery of a new thing which is not disclosed, which may or may not be pivotal in determining patentability. Thus the applicant is not entitled to make out a case for patent in its favour by importing a new matter in the specification which was later on discovered /established. The patentability, therefore if any, will have to be established on the basis of original disclosure contained in the specification .....*" it is submitted that "at an increasing rate" was not a part of originally filed claim 1. Thus the discovery that the addition if sulphuric acid at an increasing rate would provide crystals with lesser fines is a belated discovery. Dr. Lotz has also stated in his affidavit that the addition is multistaged preferably via cubic crystallization method. Which implies that even drop wise addition of sulphuric acid would provide similar result and cubic crystallization is only a preferred embodiment.

(7) Conventional trial and experimentation without employing skills beyond common general knowledge is non inventive-EPO Board of Appeals T0104/92 ".....In the Boards opinion it would be obvious for a skilled person to use varying proportion of known polymers for outer layers with reasonable expectation of obtaining better shrink and head-seal characteristic for the laminate. This a because shrink ability is due to these layers and thus the work necessary would involve only conventional trial and error experimentation without employing skill beyond common general knowledge....." The finding that the work involving mere routine

experimentation lacks inventive step is in agreement with Board of Appeals T60/80(OJIPP2 268 at point 3.2.5 and 3.2.6).

(8) EPO Board of Appeal T 0013/93 ".....the Board would emphasize that the correct approach to inventive step is not sure predictability of success drawn from given information the prior art, but rather whether it would be obvious to try with reasonable expectation of success. By way of balance the Board of Appeal have not required the patentees to show with example that there is certainty of success for everything claimed, but rather the Board are prepared to make assumptions that this is so on the basis of evidence showing that success is plausible.

(9) EPO Board of Appeals T 0712/92

".....In the present case for the reason given above, the Board is of the opinion that the skilled person was prompted by the state of the art to go into the direction of the claimed method. The so called secondary indicia relied upon by the applicant such as long felt want and commercial success can not alter the Board findings on obviousness because they are not convincing in the light of what the skilled person would have reasonably expected on the basis of the up to date knowledge at the priority date. Moreover indicia such as commercial success may depend upon factor such as market monopoly advertisement policy etc. which are unrelated to the technical feature of the invention...."

(10) EPO Board of Appeal T 0948/01

3.5.6—".... However the correct approach in assessing the inventive step is not whether a skilled person would drive the given information in the prior art a sure predictability of success, but rather whether it would be obvious to try something with reasonable expectation of success, which implies the ability of the skilled person to reasonably predict, on the basis of the existing knowledge , a successful conclusion of than experiment"

Under section 3(d) the opponent argued that claim 10 of the present invention is a product by process claim and the form has relevance

(53)

to the processing technique. The impugned application fall short of section 3(d) since the applicant has not provided data to support efficacy of form A prepared by the technique and pharmaceutical formulation comprising the form. Therefore both the claims need to rejected.

Applicant on its part first argued against the submission of opposition regarding filing of amended claims u/s 59(1). Applicant argued that this ground of opposition can not be raised as the expression "at an increasing rate" in amended claim1 and claim 9 are duly supported in the originally filed specification vide page 4 line 22-23 .Such amendments in the claims are fully with in the scope of the original disclosure. The applicant stressed that addition of acid at an increasing rate is implied with in the meaning of the originally filed claims and specification. The specification does not teach any other mode of addition of sulphuric acid. More over allowance of the amendment to the claim set, is entirely at the learned controller's discretion and opposition has no locus standii to object to the same in the present proceedings as the instant issue is not a ground of opposition.

In respect of obviousness, the applicant noted that the opponent relied upon two documents D1 & D3 and withdrawn their reliance on D2.

With respect to D1, applicant submitted that D1 discloses the bisulfate salt of atazanavir which is prepared as described in the working example 1. In example 1,as seen all the sulphuric acid required ,is added in a single addition step to a mixture of free base and ethanol. Therefore seed crystals are added and heptane to form a crystal slurry from which crystal of bisulfate salt II are obtained. Example 2 & 3 also discloses procedure similar for forming a bisulfate crystals of atazanavir by what is referred to as a uncontrolled crystallization i.e. all of the sulphuric acid is added to the free base in a single one step addition.

The intent of the applicant's invention is to provide a process for preparing form A crystals of atazanavir bisulfate salt employing multi staged addition of sulphuric acid at an increasing rate. The crystal so produced have a

desired consistent particle size and distribution. It is reiterated that the crystal particle size and morphology of the form A crystals of atazanavir bisulfate salt formed by the applicant's process as claimed in claim 1 is dependent upon the addition rate of sulphuric acid which determines the crystallization rate.It has been found that when the sulphuric acid is added at an increasing rate according to the cubic equation of claim 9, the process provides relatively large, more well defined atazanavir bisulphate crystals along with a narrow particle size range and fewer fines than obtained via a constant addition rate crystallization as desired in D1. The slow initial acid flow rate has been shown to favour crystal growth over secondary nucleation. Thus as the surface area increases with particle sign the seed bed is able to accept the increasing acid flow rate without inducing secondary nucleation. The slow initial addition rate allows time for crystal to grow larger, increasing the mean size. The multistage sulfuric acid addition crystallization as defined in claim 1 provides a less compressible filler cake, which aids in effective cake deliquoring and washing as well as more easily dried product with fewer hard lump than the constant addition rate crystallization product. Such improved properties of the crystal obtained by the application's process as claimed in claim 1 is surprising and unexpected and therefore applicant's claim process is for preparing the same is not obvious.

The applicant also refuted the opponent's contention that the teaching of D_1 may be restricted to Example 1 which describes drop wise addition of sulphuric acid in 90 second & such feature should not limit other example i.e. example 2 and 3 of the said document. The applicant submitted that the feature of addition of sulphuric acid in a drop wise manner has also been used in Ex.2 and Ex 3. The applicant also argued that the prior art D_1 does not teach or suggest the inclusion of seeding step after an addition of a portion of sulphuric acid and before multistage addition of the remainder of the sulphuric acid. Therefore there is no teaching or suggestion or incentive/motivation in the singh et al patent D_1 that would have motivated one skilled in the art to modify the proceeding of D_1 to multiple stage addition of sulphuric acid at an increasing

rate after the initial addition of sulphuric acid and to add seeds of crystal after initial sulphuric acid addition and prior to multi stage acid addition.

With regard to D_3 applicant submitted Hazen et al (D_3) discloses the procedure to prevent supersaturation level from exceeding supersaturation limit during crystallization of sodium carbonate monohydrate. There is nothing in Hazen et al (D_3) which would suggest to one skilled in the art that the pause procedure as defined in D_3 could be applied for forming crystals of of atazanavir bisulphate as taught by singh et.al. There is no reference of pause procedure in applicant's process where whole of the sulphuric acid is added at an increasing rate in various stages without any deliberate pause in between except for the initial addition of sulphuric acid followed by step of seeding.

The applicant argued that it should be noted that the Hazen et al (D_3) procedure for intermittent addition of sodium carbonate requires uniform spacing of sodium carbonate feed addition which is appreciably different from applicant's invention which involves sulphuric acid addition at an increasing rate according to the claimed cubic equation. In the present case the applicant in the initial step has reacted a solution of atazanavir bisulfate with a first portion of sulphuric acid in an amount to react with less than 15% of the atzanavir free base, seeds of atazanavir is added and the additional sulphuric acid is added in multiple stages at an increasing rate according to the cubic equation to effect the formation of Form-A crystals. The applicant argued that there is no disclosure/suggestion in Hazen et al, of the use of cubic addition of crystallization equation to calculate ,multistage or sulfuric acid or any other material for that matter. It was reiterated that even if Hazen et al (D_3) intermittent feed addition were employed in singlh et al (D1), the combined process would still not disclose or suggest applicant's process for preparing form A crystal for reason mentioned above. Applicants invention as claimed is patentable over the combination of singh et al D1 & Hazan et al D3. In view of the above the applicant pleaded that the prior art cited by the opponent, alone or in combination does not provide the

skilled person with the information to arrive at the process of the present invention of multiple stage acid addition at an increasing rate.

Applicant relied upon kim et.al paper to substantiate the inventive step of the claimed invention vide page 896 to 898, where discussion contained, ...crystal obtained from (a) uncontrolled crystallization, compared to crystal obtained from (b) liner addition of sulphuric acid where crystal are larger than obtained by uncontrolled addition and (c) crystal obtained through cubic addition (multistage crystallization) where even larger and defined crystals are obtained. The crystal particle size & morphology are dependent upon, over all addition rate of sulphuric acid. The slower the overall addition rate i.e. the longer the over all crystallization time, the larger the mean crystal size. The cubic addition protocol carried out over a relatively longer time internal provides a well defined crystal along with narrower particle size distribution with lesser fines than constant addition rate crystallization. The applicant farther submitter that the opponent's allegation that .....*'in absence of any reference of cubic crystallization reaction in the main claim, the addition of sulphuric acid in the subject invention is linier and not in accordance with cubic addition crystallization method...'* is wrong & misleading. The addition of sulphuric acid is incremental which has been clearly indicated in the main claim & the fact that the opponent's specification does not teach any other equation governing the addition of sulphuric acid, it is implied that the said incremental addition is, in accordance with cubic addition crystallization equation as mentioned in claim – 9. Therefore the particle produced by the process of the applicant's is the one as shown in -897 in fig 3(1) in kim et. al paper. In view of the above that the contention of the opponent is not maintainable & liable to be rejected.

The applicant also argued on the evidence by way of affidavit of Dr. Bruce. T.Lotz and claimed that the invention provides substantial inventive step over prior art as the process of claim 1 controls the critical physical crystal properties by maintaining a low super saturation of product in solution at all times. Following the process in affidavit emphasize the shifting of the competition between nucleation and crystal growth, in favour of crystal growth which

accounts for narrower particle size distribution and overall larger crystals. The multistage addition of sulphuric acid by cubic addition i.e. increasing the addition rate as free base concentration decreases, that maintains critically important low super saturation throughout the multistage addition process, which is deliberately designed by the applicant to produce atazanavir bisulfate form A crystal with the physical properties required to successfully manufacture safe & effective drug product.

The applicant countered some of the case laws put forward by the opponent.

Bishwanath Prasad Radhey Shyam Vs Hindustan IT Metal Industries, (Supreme Court of India, AIR 1982 SEC. 1444(1979)25CC511, (1979) 2s CR 757)

"It is important to bear in mind that in order to be patentable on improvement on something known before or a combination of different matters already known, should be something more than a mere workshop improvement and must independently satisfy the test of invention or an " inventive step."

The applicant argued that elaborate explanation about the inventive step given herein before shows that the applicant's invention is not a mere workshop improvement, rater the same involves sufficient technical advancement over the existing state of the art. This case law therefore does not further the opponent's contention of lack of inventive step of the applicant's invention.

I n Re Aller et.al No. 6079, United States Court of custom and Patent Appeal (42cc PA824).

The opponent relied upon the case to support their contention of lack of inventive step in the applicant's claim & stated that applicant's process as claimed in claim 1 which include the multiple stage addition of sulphuric acid in preparing form A crystal of atazanavir sulfate ,provide a desirable and consistent particle size and particle size distribution which is safe & efficacious. It is the multiple stage addition of sulphuric acid as claimed in claim 1 that controls critical physical properties by maintaining a low super saturation of product in solution at all times. Thus the incremental addition of sulphuric acid as per cubic addition

reaction as claimed in the applicant's process produce a new and unexpected result which is different in kind and not merely in degree, from the result of the prior art.

Mitsubishi chemical corporation vs Allied signal Inc. (Case No To 68/98-3.3.1 (EPO Broad of Appeal)

The applicant argued that the facts of the case are not relevant to the applicant's invention as none of the prior art cited by the opponent i.e singh et al (D1) & Hazen et al(D3) alone or in combination discloses a controlled multistage sulphuric acid addition process Therefore the case law does not further the opponent's contention of lack of inventive step of the applicant's invention

W.R Grace & Co. –Comm-TI0104/92-3.3.4)

(EPO Board of appeal) -----Applicant reiterates that the judgment referred to above & relied upon by the opponent could not be applied to the present case as the application's process claim does not merely involve routine experiments, rather the said process was deliberately designed by applicant after rigorous experimentation to produce atazanavir bisulfate form A crystal with the physical properties required to successfully manufacture safe and efficacious drug product. Therefore this case does not further the opponent's contention of lack of inventive step of the applicant's invention.

Novarti's AG Vs Union of (order No. 100/2009) decided by intellectual property appellate Board.

The decision of the IPAB in the matter of Novartis AG relied upon by opponent is not relevant to the present invention. However the decision is being appealed and the very finding is being challenged. The Opponent's contention that the matter disclosed in kim et. al paper is new data which was not originally disclosed as a part of the applicants specification & hence can not be relied upon. The contention of the opponent is not tenable as the applicant has relied upon to further substantiate the applicant's argument of inventive step. It may be

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noted that the specification as filed sufficiently define the invention and the inventive step. Applicant has relied up in kim et.al paper to elucidate more specifically how the present invention has clear inventive step over the prior art document cited by the opponent.

Union oil company of California (T0013/P3)

Applicant reiterates that the judgment referred to above and relied upon by the opponent can not be applied to the present case as more of the cited document D1 & D3 disclose or suggest a controlled multistage sulfuric acid addition process for preparing Form A crystal of desired particle size distribution and seeding step between the initial sulphuric acid addition & subsequent sulphric acid addition at an increasing rate. It was not obvious for a skilled person starting with D1 combining teaching of D3 to arrive at the conclusion ,that particle of atazanvir bisulfate form A crystal of desired consistent particle size and particle size distribution can be produced by multistage addition of sulphuric acid at an increasing rates. Therefore this case does not further the opponent's contention of lack of inventive step of the applicant's invention

yyrofuin oy vs Roqunette Freses (T0079/3.3.00)

Applicant submitted that the case law relied upon by the opponent is not relevant to the applicant invention as the replacement of uncontrolled crystallization (P1) by multistage addition of sulphuric acid via cubic addition crystallization equation as defined in claim 9 was not obvious to a person skilled in the arts, aware of the teachings of D1 & D3 , would not have tried this approach with any reasonable expectation, that could produce Form A crystal of desired and consistent particle size distribution as no such motivation or suggestion is being derived from the aforesaid prior art. Accordingly this case law does not further the contention of the opponent of lack of inventive step of the applicant's invention.

Allergen Inc Vs Pilkington Vision Care Inc. (T0712/92-3.3.4)

Applicant submitted that the judgment relied upon by the opponent cannot be applied to this case as none of the cited reference disclosed

or suggests a controlled multistage sulphuric acid addition process for preparing atazanavir bisulfate Form A crystal of desired consistent particles size & its distribution and a seeding between initial sulphuric acid addition & subsequent acid addition at an increasing rate. Accordingly this case law does not further the opponent's contention of lack of inventive step of the applicant's invention. The applicant submitted the opponent has failed to submit any evidence and /or persuasive and substantive arguments to question the technical advancement achieved by the subject of the invention, which proves clearly that the subject invention has inventive step.

The applicant also invited the controller's attention to the specific language of section 25(1)(e) ---" *the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve an inventive step----"* and submitted that refusal of a patent in an opposition proceedings should be only in clear case, where it is evidently clear that no inventive step is present. The applicant submitted that in view of the above submission it follows that a clear case has been established ,that the subject patent application has inventive step.

In respect of section 3(d) & 3(e) the opponent's argument that the claim 10 & 11 falls under the prohibition of section 3(d) of the act is denied by the applicant . The applicant submitted that the claim 10 is a compound produced by the claimed process i.e. a product by a process claim and claim 11 is a composition containing a novel compound and not a mere new form of a known substance as prohibited by section 3(d) of the Act. Therefore the claims *'falling within the prohibition of section 3(d)'*, does not arise.

The opponent's contention that claims 11 falls under the prohibition of section 3(e) of the Act. The objection raised by the opponent do not stand in view of the fact that is has been sufficiently established that the compound (claim 10) produced by the claimed process is novel & inventive. The pharmaceutical composition (claim 11) related to this compound is novel and inventive.

The applicant concluded that the opponent has completely failed to substantiate any of the grounds taken and therefore opposition ought to be rejected altogether.

Before arriving to the decision it is necessary here to point out that two representation were filed in the present case ,namely on behalf of (1) M/s Cipla Limited and (2) M/s Matrix Laboratories Limited. The arguments submitted by the parties are partly similar in nature and partly different from one another. A single decision could be delivered on the basis of arguments of both the oppositions and the applicant. But the second opponent requested for a separate judgment and therefore I have given my decision separately. However in arriving to the conclusion I have taken into consideration the citation and the arguments of both the opponents , the evidence and the arguments of applicant.

Having heard both the parties to the representation on the grounds of lack of inventive step, not an invention under section 3(d) & 3(e) in the first representation and on the grounds of Novelty, lack of inventive step , not an invention /not patentable and insufficiency of disclosure ,I shall now discuss each grounds of opposition on the basis of pleading of particles and the common general knowledge available on the said subject matter :

The present invention as claimed in claims is directed to a process of preparing atazanavir bisulfate in the form of Form A crystals employing multiple stage sulphuric acid addition at an increasing rate which crystals have a desired consistent particle size and particle size distribution , the process comprising (a) reacting a solution of atazanvir free base in an organic solvent in which bisulfate salt of atazanavir is substantially insoluble with a portion of concentrated sulphuric acid in an amount to react with less than 15% by wt of atazanavir free base,(b) adding atazanavir bisulfate Form A crystals to the reaction mixture (c) adding additional concentrated sulphuric acid in multiple stage at an increasing rate to effect formation of atazanavir bisulphate crystals and (d) drying the atazanavir bisulfate to form Form A crystals.

The prior art US 6087383 (D1) teaches crystalline bisulfate salt of Azapeptide HIV protease inhibitor which has superior aqueous solubility behavior

compared to other salt and significantly improves oral bio availability compared to the free base. The process utilized in the preparation of atazanavir bisulfate crystal is the free base atazanavir is suspended in ethanol. To this suspension concentrated sulphurice acid is added drop wise over 90 seconds. The clear solution formed is filtered & Atazanvir bisulfate is added followed by additional addition of heptanes. The resulting mixture is stored for six hours & the resulting crystal slurry was filtered & washed to get crystalline bisulfate salt (88.4% mole % yield).

Therefore the difference in the two process for the preparation of the atazanavir bisulfate crystal is that ,in the present invention,..... in the suspension of free base, concentrated sulphuric acid is added in an amount to react with less that 15% by wt of atazanavir fee base. Seeds of Form A crystal of atazanavir bisulfate is added & there after additional concentrated sulphuric acid is added in multistages at an increasing rate i.e. five installment of concentrate sulphuric acid is added hourly in an increasing amounts (according to the cubic addition crystallization equation), whereas in the prior art D1, the concentrated sulphuric acid is added to the suspension of the fee base by drop wise addition in 90 seconds & then seeding crystals are added after adding heptane.

So the D1 teaches preparation of atazanavir bisulfate Form A crystal by drop wise addition of concentration sulphuric acid to the free base under stirring condition whereas present application carries similar conversion involving a multistage addition of concentrate sulphuric acid according to the cubic equation allegedly preparing the Form A crystal to achieve larger crystals i.e. large particle size with narrow particle size distribution. It is my considered view that the present invention being a process patent differs from many process parameters as compared to prior art and also I agree with the argument of the applicant that the opponent has failed to provide a single document which could anticipate the invention entirely. **Therefore opponent failed to prove the ground of anticipation by way of prior publication.**

Both the opponents argued that the process adopted is nothing but an obvious modification of the process by known methods available in the prior

art & knowledge achieved through routines experimentation of said knowledge The applicant argued that the prior art cited by the opponents alone or in combination does not provide the skilled person with the information to arrive to the present process of using multiple stage addition of sulphuric acid(at an increasing rate) in the preparation of form A crystals which provides desirable particle size and consistent particle size distribution. Whether the specific crystallization method used in the present invention is inventive or not following points need to be taken into consideration, which I found from the prior arts provided by the parties to this representation ,text of the specification and the common general knowledge available on the said subject matter vis-à-vis the present invention.

(1) The object of the prior art D1 is to improve the bio availability and aqueous solubility of crystalline bisulfate salt of Azapeptides HIV protease inhibitor. The object of the present invention is to provide a process to obtain the crystalline Form A of Atazanavir bisulfate having substantially consistent particle size distribution and substantially consistent mean particle size (Page 5 of specification) for the same object i.e. to improve the bioavailability & solubility and additionally ease in the processibilty in the manufacturing process.

(2) The prior art D3 (US 2003/008 4547) relied by the opponent known as Hazen publication wherein On paragraph (0015) teaches that "the invention is for producing sodium carbonate monohydrate from a feed stream." The process includes adding the feed stream to a saturated sodium carbonate brine solution under condition to create super saturation of at least about 58/lit. The process further includes processing within parameters that preferentially relieve the super saturation by rapid growth of existing sodium carbonate monohydrate crystals rater than by nucleation. Therefore control of super saturation and its relief in the local environment near where the seed is introduced is critical." *Paragraph (0017)teaches the use of seed crystal "process of the present invention provides a large amount of available sites for relief of super saturation on existing crystals so that the degree of super saturation in a*

localized area is approaching the maximum level i.e. the super saturation limit, the super saturation can be quickly relieved by sodium carbonate monohydrate formation on an existing crystal surface instead of by nucleation sites of crystallization are provided by the use of seed crystal and or maintaining high solid content in the crystallizer". The present invention can also include pausing during the introduction of few to allow the dispersion of the local area of very high super saturation by agitating and/or productive relief of super saturation on existing crystal in a local area of very high super saturation.

(3) The prior art citation US 6429,210 raised by the second opponent, pertains to crystallization process wherein sulphuric acid is added about 10% initially and rest of the acid is added after seeding has taken place, slowly in a given time. Specifically the example 3 of the '210 document discloses slow crystallization of Clopidogrel hydrogen sulphate crystals. Here the free base clopidogrel is taken in acetone at a temperature stabilized at 20 degree centigrade under mechanical shearing and 10% of the concentrated sulphuric acid (94-96%) is added in few minutes, then calculated quantity of seeding crystals i.e. clopidogrel hydrogen sulphate crystals are added for seeding and the mixture is left for 45 minutes, then the remaining 90% of the concentrated sulphuric is added, slowly within two hours under mechanical shearing which is stopped after 30 minutes of acid addition. The mixture is then stirred for 30 minutes at 20 degree centigrade ,filtered, washed and dried in vacuo. This process for crystallization involves a small portion of acid addition before seeding and then the entire acid addition takes place slowly in two hours after seeding . In other words the sequence of addition steps are exactly the same.The only difference with the present invention is in terms of duration of acid addition after seeding ,which is 4 hours and 23 minutes in the present case whereas in the prior art it is in two hours. I do not agree with the contention of the applicant that using of mechanical shearing teaches away from the present invention. In my opinion the prior art process rather proves that the above said sequential addition leads to bigger particle size and mechanical shearing was resorted to in the prior art to break down the bigger crystals into smaller one

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(4) In the body of specification the applicant has mentioned that " *procedure for the preparation of crystalline forms are known in the art. The crystalline form may be prepared by variety of methods including for example, crystallization, recrystallization from a suitable solvent, growth from a melt. .....Technique for crystallization or recrystalisation from a crystalline form from a solvent mixture includes for example, evaporation of the solvent, decreasing the lump of the solvent mixture, crystal seeding a super saturated solvent mixture of the molecule and/or freeze drying the solvent mixture, and addition of anti solvent to the solvent mixture."* The specification clearly mention vide page 13 that "*seeding is used as a means of controlling the particles size distribution of the crystalline product*". Accordingly calculation of the amount of seed needed depends upon the size of the seed available and the desired size of an average product particle as described for example in "Programmed cooling of batch crystallizers" J.W. Mullin & J. NYURT, Chemical engineering science (1971) 26:369-377 on Page 17, the applicant mentioned the " *.....The cubic crystallization derived from Mullin "Crystallization, 3rd edition "1993, Butterworth-Heineman, Pubs....."*

".....since the crystallization atazanavir bisulfate is controlled by the addition rate of sulphuric acid the temp variable in replaced with acid volume...."

".....by controlling the crystallization rate using this expression (Cubic equation) nucleation is controlled with in acceptable limit as the system maintains low level of super saturation."

Form all the above discourse it is clear that applicant has admitted that crystallization by seeding, dependence of crystal size to the seeding crystal size, maintaining a lower super saturation for bigger crystal formation etc. are all known in the art for long.

(5) Kim, et al, paper submitted by applicant as annexure of reply statement in which, on page 895 under controlled crystallization ,has maintained that *"It is well known that a rapid change of super saturation,*

particularly in the initial stage of crystallization process, result in formation of a large number of nuclei and generally yield a non-uniform product (Mullin J.W.Nyult J Chem. Eng. Soc 1971, 26,369), while growth rate increases with higher operating level of super saturation, the increase in the nucleation rate is more sensitive to the higher super saturation level and plays a dominant role in the formation of particles especially fines. Keeping the working level of super saturation low to keep the nucleation rate low significantly improves the uniformity of product."

(6) Affidavit of Dr. Bruce Lolz (Who happens to be an inventor to the present invention) has stated that the uncontrolled crystallization of D1 to be disadvantageous and that the "*natural control of the crystallization as in the process is defined in claim 1 is vital. The multiple stage addition of sulphuric acid as claimed in claim 1 preferably via the cubic crystallization as defined in claim 34 control the critical physical crystal properties by maintaining a low super saturation of product in solution at all times. Doing so shifts the competition between nucleation and crystal growth events in favour of latter which accounts for narrow particle size distribution and over all large uniform crystals. "*

Therefore, the expert opinion emphasize on the multiple stage addition to control the supersaturation & shift the competition between nucleation & crystal growth events in favour of crystal growth and the addition could be preferably via cubic crystallization. This is clear from the last part of Para 9 that "*Thus the multiple stage crystallization of the invention (Preferably via cubic addition) was deliberalty designed to produce atazanavir sulfate with the physical properties required to successfully manufacture safe & efficacous drug product....."*

(7) On page 27 of the specification of the present invention , mentioned " .....*The rate of each addition stage was determined accordingly to*

cubic equation as is shown in the table below. The data in table 1 was obtained by experiment and not by calculation....."

Also on page 21 of the reply statement it is mentioned. *"....As seen in table 1 on page 27, reproduced below the additional five stages of sulphuric acid is added over a total period of 263 minutes or over 4.5 hours. This data was obtained by experiment and not by calculation....."*

However experimental data set out in Example 1 is very similar to the calculated data using the cubic addition equation.

Vtime = Vtotal x(time/time total)3

The above statement indicates that the quantity of addition was determined by experimentation & which was found to be very close to the data calculated on cubic addition equation. Claim originally filed does not indicate the multistage addition to effect the formation of atazanavir crystals which was later amended to add "at an increasing rate and making cubic equation of claim 9 dependent upon claim 1 by the subsequent amendment to be a preferred embodiment in multistage addition.

On summarizing all the above points it appears that the object of the present invention is to improve the particle size and to get narrow particle size distribution which will eventually provide a less compressible filter cake, during processing, which aids in effective cake deliquoring and washing as well as giving a more easily dried product with fewer hard lump. Therefore the sulfuric acid addition at an increasing rate in accordance with the cubic equation , is the crux of the invention or inventive merit of the invention. as the multiple stage addition of sulphuric acid at an increasing rate in preparing Form A crystal of atazanvir sulfate would provide product of desirable & consistent particle size distribution , controlling of crystallization through multistage addition to obtain large particle size crystal & narrow particle size distribution with lower fines.

The prior art D3 (Hazen Publication) teaches the problem that occur with crystallization process for obtaining crystal from and possible

ways to overcome these problem by making appropriate changes in crystallization techniques. The publication relates to the production of sodium carbonate monohydrate crystals from anhydrous sodium carbonate with impurities. This hazan publication vide para 17 provides to Relief of super saturation is controlled such that the crystal formation primarily occurs on existing crystals, rather than occurring at nucleation or growth of newly formed crystals. In this manner, the particle size distribution of crystal is controlled to achieve a desired distribution of product crystal size." Similarly the para 60,61 "----*Thus to prevent the super saturation level in a local area form exceeding the super saturation limit the addition of the feed stream to the saturated brine can be stopped briefly or intermittently to decrease the super saturation level by allowing growth of existing crystal. More particularly the break or pause in feed stream addition can be conducted at least about 60% time of crystallization*----" In short the prior art D3 provides the process the control of super saturation by providing large amount of available sites for relief of super saturation on existing crystal instead of by nucleation. Time of addition of seed crystal. Particle size of seed crystal and amount of seed crystal etc are also provided therein in.

Therefore to prevent the super saturation level in a local area from exceeding the super saturation limit, the addition of the feed stream to the saturated brine can be stopped briefly or intermittently to decrease the super saturation level by growth of existing crystals.....That means super saturation to be controlled by addition of feed stream to prevent secondary nucleation. The same thing have been followed in the present invention where the super saturation is controlled by the multistage addition of the feed stream.

In the present case claim 1 provided a first portion of concentrated sulpuric acid in an amount to react with less than 15% of the atazanavir free base, adding seed of form A crystal of atazanavir sulfate to the reaction mixture adding additional sulfuric acid in multiple stage to effect the formation of atazanavir bisulfate crystals and drying to obtain form A crystal. Subsequently the amendment sought in claim 1 by "adding additional

concentrated sulfuric acid in multiple stage at an increasing rate to effect the formation of atazanavir bisulfate crystal. Although opponent objected to such amendment but as the amendment makes the preferred feature to an essential feature by inserting in claim 1 and making the supporting cubic equation claim dependent upon claim 1 actually narrowed down the scope, under pressure of opposition, to justify inventive merit.

Therefore the process of controlled crystallization through controlling of feed stream for larger particle size & narrow particle size distribution is a well known process of prior art. The only point that whether the multiple stage addition of suplhuric acid at an increasing rate in accordance to the cubic crystallization equation is an un-obvious to one skilled in the art with the knowledge of D1 & D3 and prior art '210 , or not

Now it is within the common general knowledge of a person skilled in the art that the crystal growth is favoured onto the seed crystal over nucleation.Iinitial addition of the feed stream has to be minimum to control the growth over the seed crystal only and not allowing the secondary nucleation and as the crystal growth formation increases the surface area available for growth increase due to the bigger crystal with large surface area. So feed stream addition rate can be increased accordingly to the increase in surface area for crystallization without compromising the desired size. The kim et .al has mentioned in that paper ".....*as the crystallization progressed, the surface area available for growth increased at an increasing rate thereby allowing acid to be added at an increasing rate without inducing significant nucleation.....*"

The cubic addition profile which the applicant has claimed as cubic addition equation of claim 9 (after amendment) is admittedly analogous to well known cubic cooling profiles in a temperature controlled crystallization (Mullin crystallization 3rd edition1913). As in the crystallization process of the present invention, the temperature remaining consistent throughout the

crystallization, the pressure & agitation rate are kept constant during the crystallization process as implied by the body of specification. The only variable for the control of crystallization is the rate of acid addition. The temperature variable is replaced by the applicant with acid volume in the said known Cubic crystallization method (Page 17, specification). Example E of the specification clearly indicates that the data of acid addition was obtained by experiment which was formed to be very close to the amount calculated by cubic addition equation (reply statement page 21-22 and annexure D)

Therefore the addition profile is optimized by the applicant through routine experimentation keeping in mind the slow initial addition with increasing addition rate in a stage wise addition accordingly to the increasing surface area of the growing crystal during the crystallization which was found to be very close to the acid volume addition cubic equation derived by the applicant as stated above.

On the basis of the above observation & consideration of those points I am of the considered opinion that a person skilled in the art will combine the teaching of D1 , D3 and doc '210 to obtain atazanavir crystal of larger size & narrow particles size distribution by routine experimentation with reasonable expectation of success , optimize acid addition rate profile without any extra ordinary skill. In other words, choosing acid addition profile can be seen as lying with in the routine activity of the skilled person faced with the objective problem of improving particle size & narrow particle size distribution without requiring any inventive ingenuity.

It is obvious for a skilled person to use verifying acid addition profile for timely release super saturation formation by rapid growth of crystal over the existing atazanavir bisulfate crystal rather than by secondary nucleation and optimize the best addition profile ,with reasonable expectation of obtaining large crystal size with narrow particle distribution of the product. My considered view is strengthened with the EPO Board opinion that "---*correct approach to inventive step is not sure predictability of success drawn from information in the*

prior art, but rather whether it would be obvious to try with reasonable expectation of success ---" EPO Board of Appeal T 0013/93) and in EPO the Board in T 0948/01----However, the correct approach in assessing inventive step is not whether a skilled person would drive from given information in the prior art a sure predictability of success but rather whether it would be obvious to by something with or reasonable expectation of success, which implied the ability of a skilled person to reasonably predict, on the basis of existing knowledge a successful conclusion of an experiment----"

Therefore it is my considered view that in the light of the prior art teaching, the chosen acid addition profile in the present invention is with in the routine activity of the skilled person in the art, trying to improve the particle size and narrow particle size distribution without exerting any inventive ingenuity and therefore is obvious and lack in inventive step.

Regarding insufficiency of disclosure opponent argued that the applicant's claim that vide last Para page 16 to 17 the present process has resulted in four advantages i.e. relatively large particle size, narrow particle size distribution, well defined crystals and fewer fines ,leading to the advantage like less compressible filter cake and fewer hard lumps. However the singh's crystal product and the present crystals are same product, but the applicant has not provided any comparative data in the specification to highlight the increased particle size and narrow particle size distribution instead the applicant submitted during the second hearing proceedings a reference to Soojin Kim et.al. micrograph data for enhanced particle size data which was published seventeen month after the filing of the present application. I agree with the contention of the opponent that the comparative data should have been provided in the specification at the time of filing to justify their claim. Therefore the application lack in sufficiency of disclosure.

As the product prepared by the process of the present invention lack in inventive step and the applicant has not provided any technical data to show enhanced therapeutic efficacy of the product over the prior art

(72)

compound other than the data for physical quality improvement leading to product crystals of large particle size with narrow particle size distribution and fewer fines to improve the processibility like effective cake deliquoring, ease in washing and drying with lesser lumps during manufacture , does not qualify the requirement u/s 3(d) of the Patent Act and therefore product by the process as well as composition claim are also refused.

I therefore on the basis of my considered view explained in preceding Para, refuse to proceed with the application no. 6425/DELNP/2006 for grant of patent.

The applicant stand disposed, with no cost to either party to the proceedings.

Dated 20th day of December, 2010

Sd/-

(S.K.Roy)

Assistant Controller of patents and Designs

'22 DEC 2010

(1) M/s Remfry & Sagar, Remfry House,
Millennium Plaza, Sector – 27,
Gurgaon – 122002.

chem/nro/3399

(2) M/s S. Majumdar & Co.
5, Harish Mukherjee Road
Kolkata -700 025.

Exhibit 1

(73)

US006087383A

United States Patent [19]

Singh et al.

[11] Patent Number: 6,087,383

[45] Date of Patent: Jul. 11, 2000

[54] **BISULFATE SALT OF HIV PROTEASE INHIBITOR**

[75] Inventors: **Janak Singh**, Lawrenceville; **Madhusudhan Pudipeddi**, Plainsboro; **Mark D. Lindrud**, Basking Ridge, all of N.J.

[73] Assignee: **Bristol-Myers Squibb Company**, Princeton, N.J.

[21] Appl. No.: **09/217,538**

[22] Filed: **Dec. 21, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/071,968, Jan. 20, 1998.

[51] **Int. Cl.**[7] **A61K 31/44**; C07D 213/56

[52] **U.S. Cl.** **514/357**; 546/332

[58] **Field of Search** 546/332; 514/357

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,849,911 12/1999 Fassler et al. 544/335

FOREIGN PATENT DOCUMENTS

WO97/40029 10/1997 WIPO .

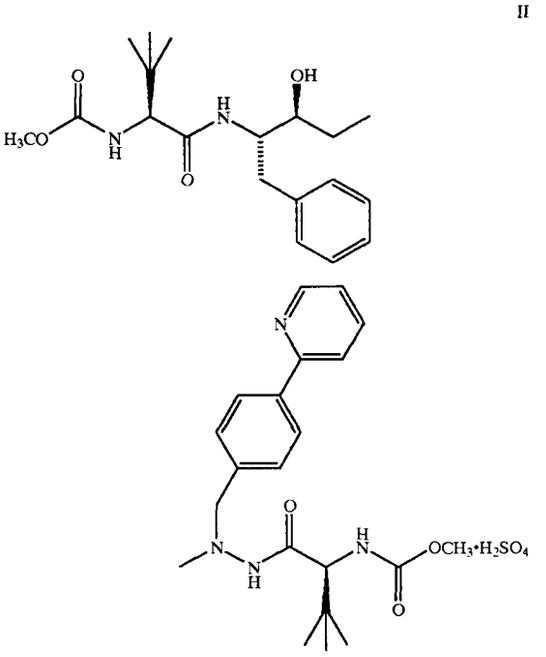
Primary Examiner—Bernard Dentz

Attorney, Agent, or Firm—David M. Morse

[57] **ABSTRACT**

The present invention provides the crystalline bisulfate salt of the formula

II



which is found to have unexpectedly high solubility/dissolution rate and oral bioavailability relative to the free base form of this azapeptide HIV protease inhibitor compound.

2 Claims, 5 Drawing Sheets

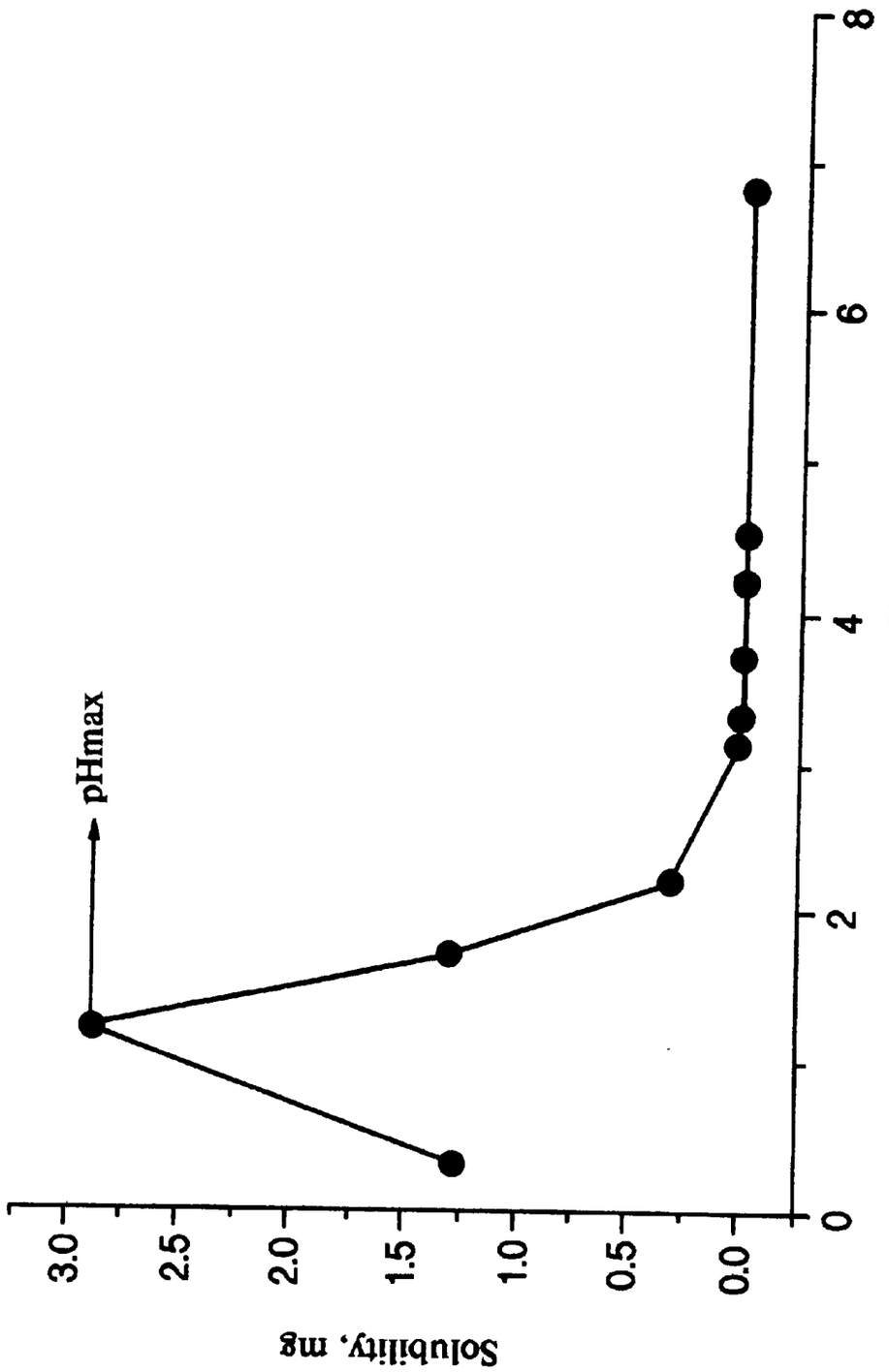


FIG. 1

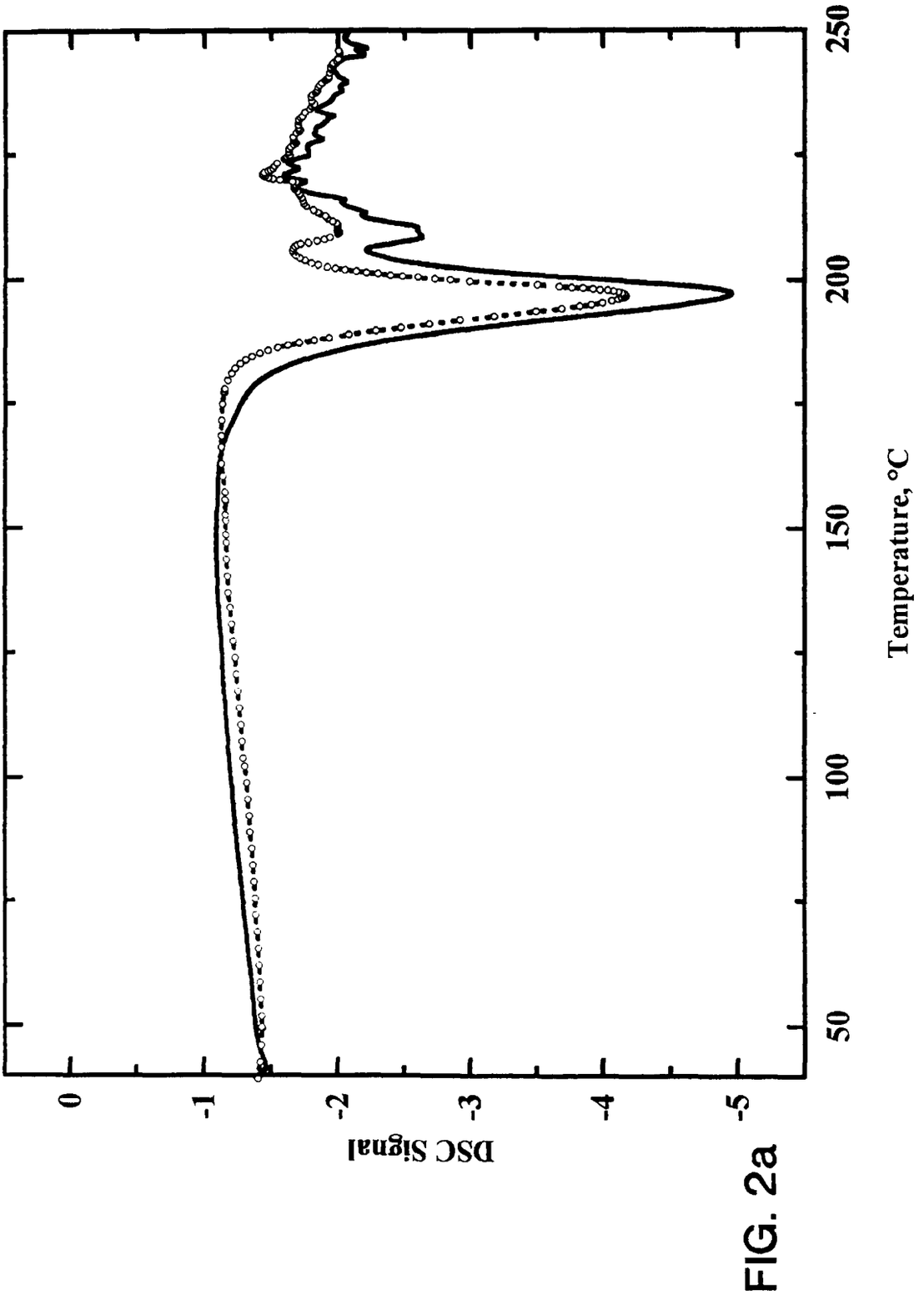
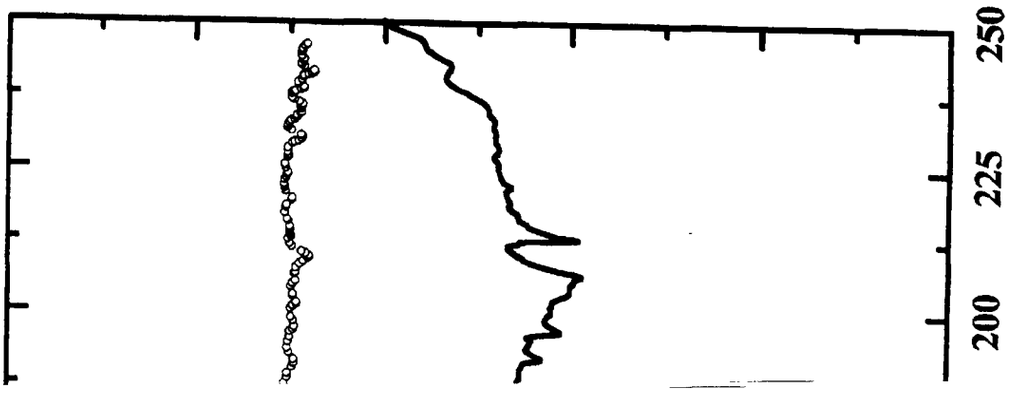


FIG. 2a



In general, conversion of salts to the unionized form or vice versa can be explained on the basis of pH-solubility theory. The solubility of the free base in water was determined as a function of pH at 24±3° C. and is shown below. The pH at which the compound exhibits the highest solubility is referred to as pH_{max} and was found to be approximately 1.2. It has been reported in the literature that at $pH>pH_{max}$ of a weakly basic organic compound, the equilibrium solid phase in an aqueous suspension of the compound is the free base. At $pH<pH_{max}$ the equilibrium solid converts to the corresponding salt form. The term "equilibrium solid phase" refers to the undissolved or excess solid in a suspension of the compound in water after sufficient equilibration time. When a salt of a weak base is equilibrated in water in an amount exceeding its solubility limit (i.e., a suspension of the salt in water), the resulting pH of the suspension may fall on either side of the pH_{max} depending on the strength of the acid among other factors. When the resulting pH is greater than the pH_{max}, the suspended solid converts to the free base.

Studies conducted with methane sulfonate and hydrochloride salts, in particular, of the free base confirmed the above described general findings reported in the literature. Amounts in excess of the solubility of these salts were equilibrated in water at 24±3° C. for at least 24 hours. The pH of the suspensions after equilibration was 2.1±0.1 which is greater than the pH_{max}. The undissolved solids from these suspensions were isolated, air-dried, and characterized. By thermal and elemental analysis the undissolved solids from these suspensions were identified as the free base. This behavior was expected based on the pH-solubility profile shown in FIG. **1** and the studies reported in the literature.

When an excess amount of the bisulfate salt was equilibrated in water a modification occurred in the solid phase in equilibrium with solution. However, the undissolved solid phase after equilibration was not the free base, although the pH (1.9±0.2) of the suspension was greater than the pH_{max} and comparable to the pH of the suspensions of methane sulfonate and hydrochloride salts described above. The solid phase after at least 24 hours of equilibration was identified by elemental analysis as a hydrated form of 2:1 salt of the free base form and sulfuric acid (referred to as the sulfate salt). This behavior of the bisulfate salt is unexpected based on pH-solubility theory.

When an excess amount of the sulfate salt, in turn, was equilibrated in water a modification occured in the solid [illegible]

mals relative to the free base. The absolute oral bioavailability of the bisulfate salt was found to be approximately 20% in dogs when administered in unformulated solid form placed in a gelatin capsule. In comparison, the crystalline free base had minimal oral bioavailability in dogs.

In addition to optimal solubility, satisfactory physical stability in the solid-state is another desirable property of pharmaceutical salt forms. The term physical stability indicates the ability of the salt form to retain its crystal structure (including solvents of crystallization, if any) under storage/stress conditions. Significant changes in the physical nature of the salt form as indicated by thermal methods such as differential scanning calorimetry are undesirable. The bisulfate salt exhibited excellent solid-state physical stability when stored at 40° C./75% relative humidity (RH) for as long as 9 months as shown in FIG. **2***a*. Differential scanning calorimetry revealed no significant changes in the thermal behavior of the stressed sample of the bisulfate salt compared to that of the unstressed sample (stored at 2–8° C. in a closed container). The methane sulfonate, hydrochloride, and the sulfate salts, on the other hand, showed significant changes in their thermal behavior when stored at 40° C./75% RH for as little as two weeks as shown in FIGS. **2***b, c,* and *d*. While differences in physical stability of salt forms is not unusual, the propensity of a particular salt to form solvates (or crystal modifications) and its ability to retain the solvent of crystallization (the physical stability of crystal modifications) under storage/stress conditions cannot be predicted apriori.

FIG. **2***a* represents Physical stability of the bisulfate salt. The solid line represents the unstressed material. The dotted line represents the material stressed at 40° C./75% RH for 9 months.

FIG. **2***b* represents Physical Stability of the hydrochloride salt. The solid line represents the unstressed material. The dotted line represents the material stressed at 40° C./75% RH for two weeks.

FIG. **2***c* represents Physical stability of the methane sulfonate salt. The solid line represents the unstressed material. The dotted line represents the material stressed at 40° C./75% RH for two weeks

FIG. **2***d* represents Physical stability of the sulfate salt. The solid line represents the unstressed material. The dotted line represents the material stressed at 40° C./75% RH for

(0.0213 mole) of free base compound I and 113 mL of 200 proof ethanol were added with stirring. To this suspension, 1.28 mL concentrated sulfuric acid was added dropwise over 90 seconds. After the addition of sulfuric acid, a clear amber-colored solution was obtained. The solution was polish filtered using #1 Whatman filter paper and washed with 5 mL of 200 proof ethanol. To this solution was added 58 mL of heptane and 37.5 mg (0.25 wt %) of seed crystals of the compound of formula II followed by 55 mL of additional heptane. The resulting mixture was stirred for 6 hours at 300 rpm. The resulting crystal slurry was filtered and washed with 50 mL ethanol/heptane (1:1) solution and dried under vacuum at 60° C. overnight to afford 15.11 g of the desired crystalline bisulfate salt (88.4 mole % yield) having formula II above.

Characterizing Properties of Bisulfate Salt

Anal. Calcd. for $C_{38}H_{52}N_6O.1.0\ H_2SO_4$. C, 56.84; H, 6.78; N, 10.37; S, 3.99. Found: C, 56.72; H, 6.65; N, 10.41; S, 3.83. m.p. 195.0°, H_2O=0.28% (KF).

Example 2

Preparation of Bisulfate Salt from Acetone

5M H_2SO_4 (8.52 mL, 42.6 mM) was added dropwise to a suspension of the free base compound of formula I (30.0 g., 42.6 mM) in acetone (213 mL) stirred mechanically in a 50° C. oil-bath. A clear solution was obtained almost immediately. The solution was seeded with crystals of the free base compound of formula II. After two minutes, a precipitate formed which became a paste. The mixture was stirred at 50° C. for one hour, at 25° C. for 30 minutes and at 0° C. for 2 hours. The solid was filtered and the first filtrate was used to transfer the remaining material in the flask to the filtration funnel. The product was washed with acetone, then heptane, and dried under vacuum overnight to give 31.48 g (corrected yield 92%) of the bisulfate salt of formula II, m.p. 198–199° C. dec.

Anal. Calcd. $C_{38}H_{52}N_6O_7.1.0\ H_2SO_4.0.2\ H_2O$: C, 56.59; H, 6.80; N, 10.42; S, 3.98; H_2O, 0.45. Found: C, 56.66; H, 6.78; N, 10.50; S, 4.20; H_2O, 0.45 (KF).

Example 3

Preparation of Bisulfate Salt from Isopropanol

Aqueous sulfuric acid (5.0 M, 0.20 mL, 1 mM) was added to a suspension of the free base compound of formula I (0.704 g, 1.00 mM) in isopropanol (4.0 mL) chilled in an ice-bath. The ice-bath was removed and the mixture stirred at room temperature. The suspension had dissolved after 15 minutes. The solution was seeded with crystals prepared as in Examples 1 or 2 above and stirred for 5 hours. The solid was filtered and the filtrate was used to transfer the solid from the flask to the funnel. The product was washed with heptane and dried under vacuum to give 0.752 g of crystalline bisulfate salt of formula II, yield 90%, m.p. 160–190° C., dec.

Anal. Calcd. for $C_{38}H_{52}N_6O_7.1.0\ H_2SO_4.2.0\ H_2O$; C, 54.40; H, 6.97; N, 10.02; S, 3.82; H_2O, 4.29. Found: C, 54.25; H, 6.73; N, 10.02; S, 3.67; H_2O, 4.53 (KF).

The crystals obtained from isopropanol showed a powder x-ray diffraction pattern different from the crystals obtained from acetonitrile, ethanol-heptane or acetone. They are now referred to as Type-II crystals. The Type-I crystals appear to be an anhydrous/desolvated crystalline material while the Type-II crystals are a hydrated, hygroscopic crystalline form.

Example 4

Preparation of Capsule Formulations of Bisulfate Salt

A. Capsules (50 and 200 mg free base equivalent)

Capsules are provided for oral administration in which the capsule is a size #0, gray, opaque, hard gelatin capsule containing the bisulfate salt of formula II formulated as a wet granulation with lactose, crospovidone and magnesium stearate.

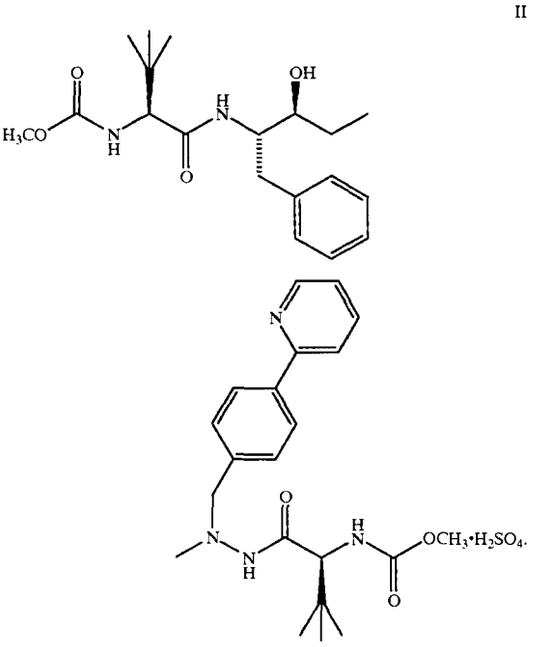
B. Capsules (100 mg free base equivalent)

Capsules are provided for oral administration in which the capsule is a size #0, gray, opaque, hard gelatin capsule containing the bisulfate salt of formula II suspended in Gelucire 44/14. Gelucire 44/14 is a saturated polyglycolized glyceride consisting of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethylene glycol. Capsules are prepared by melting Gelucire 44/14 at 45–70° C. followed by addition of the bisulfate salt with stirring. The molten mixture is filled into hard gelatin capsules and allowed to cool and solidify.

We claim:

1. The bisulfate salt having the formula

II



2. A pharmaceutical dosage form comprising the bisulfate salt of claim 1 and a pharmaceutically acceptable carrier.

* * * * *

appropriate analytical controls. One should also note that it is the responsibility of the industry to select the appropriate test or tests to identify the phase of the solid and determine its relevant pharmaceutical properties. This approach is superior to simply performing a broad range of tests without regard to their relevance.

We should point out that, from a regulatory standpoint, if a company can establish a specification/test to ensure production of a well defined solid form of the drug substance, then it is not necessary to do all of the physical/chemical testing outlined in the decision trees. From a scientific standpoint, however, such an approach is risky since new forms may appear unpredictably during various stages of the development process. The appearance of these new forms usually slows the drug approval process and makes planning difficult.

Four decision trees are described in the sections that follow: Polymorphs; Hydrates (Solvates); Desolvated Solvates; and Amorphous Forms. Polymorphs exist when the drug substance crystallizes in different crystal packing arrangements all of which have the same elemental composition (Note that hydrates can exist in polymorphs). Hydrates exist when the drug substance incorporates water in the crystal lattice in either stoichiometric or nonstoichiometric amounts. Desolvated solvates are produced when a solvate is desolvated (either knowingly or unknowingly) and the crystal retains the structure of the solvate. Amorphous forms exist when a solid with no long range order and thus no crystallinity is produced. It is apparent that the appropriate flow chart can only be determined after the solid has been characterized using some of the tests described in the first decision point of the decision trees/flow charts (i.e. X-ray powder diffraction, elemental analysis, etc.). If there is no interest in marketing or producing an amorphous form or desolvated solvate at any stage in the process, then the corresponding flow charts do not need to be addressed. As already mentioned, it is advisable to investigate the drug substance for the existence of polymorphs and hydrates since these may be encountered at any stage of the drug manufacturing process or upon storage of the drug substance or dosage form.

All of the flow charts end (see for example Figure 1) with an indication of the types of controls which will be required based on whether a single morphic form or a mixture will be produced as the drug substance. Although this ending provides a simplistic view of a very complicated process of selecting appropriate controls, it is included to illustrate the consequence of the decisions made with regard to the drug substance. The reader should realize that the actual selection of the appropriate control could be the subject of another review which might contain another set of flow charts or decision trees.

POLYMORPHS

The flow chart/decision tree for polymorphs is shown in Figure 1. It outlines investigations of the formation of polymorphs, the analytical tests available for identifying polymorphs, studies of the physical properties of polymorphs and the controls needed to ensure the integrity of drug substance containing either a single morphic form or a mixture.

A. Formation of Polymorphs—Have Polymorphs Been Discovered?

The first step in the polymorphs decision tree is to crystallize the substance from a number of different solvents in order to attempt to answer the question: Are polymorphs possible? Solvents should include those used in the final crystallization steps and those used during formulation and processing and may also include water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, hexane and mixtures if appropriate. New crystal forms can often be obtained by cooling hot saturated solutions or partly evaporating clear saturated solutions. The solids produced are analyzed using X-ray diffraction and at least one of the other methods. In these analyses, care must be taken to show that the method of sample preparation (i.e. drying, grinding) has not affected the solid form. If the analyses show that the solids obtained are identical (e.g. have the same X-ray diffraction patterns and IR spectra) then the answer to the question "Are polymorphs possible?" is "No",

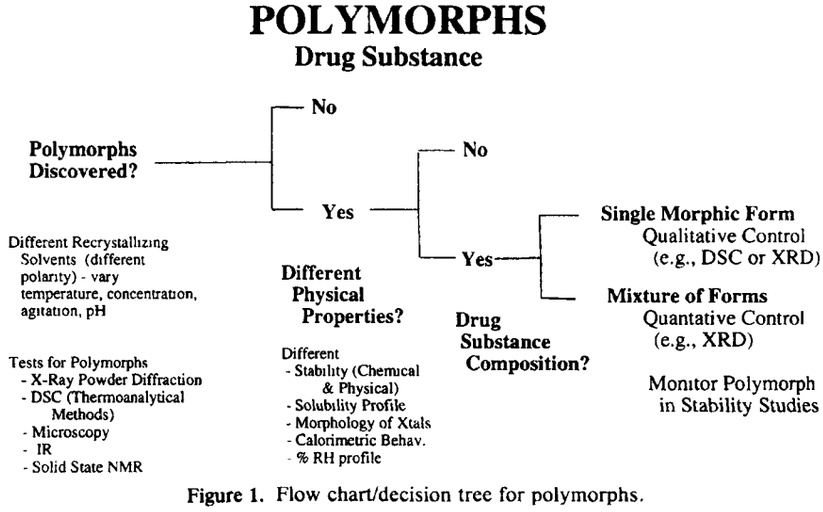


Figure 1. Flow chart/decision tree for polymorphs.

and further research is not needed. The work of Miyamae *et al.* serves as a good example of solid state studies of a drug substance which exists as polymorphs (1). Powder diffraction showed that there were two crystal forms (see Figure 2).

These workers also carried out single crystal analysis of the two crystal forms of the compound. The structures are shown in Figure 3. While such studies are not required, and indeed sometimes not possible, they provide an unequivocal confirmation of the existence of polymorphs. Moreover, once the single crystal structure of a phase has been determined, it is possible to calculate the corresponding X-ray powder pattern. This provides an irrefutable standard for identifying the phase by that method.

The DSC thermal curves of the two forms are slightly different, as shown in Figure 4 and thus may not be the preferred way of differentiating these polymorphs.

The IR spectra of the two polymorphs are quite similar(1), and IR does not appear to be a powerful method for differentiating the crystal forms in this case. Thus, for 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine, powder diffraction appears to be the best method for differentiating the two forms.

Solid-state NMR is another powerful technique for analyzing different crystal forms (2,3). Figure 5 shows the solid-state C-13 NMR spectra of Forms I and II of prednisolone. Differences in the positions of the two resonances in the 120 ppm range clearly differentiate the two forms. In principle, solid state NMR is an absolute technique in which the signal intensity is proportional to the number of nuclei provided appropriate conditions are met. In addition, solid state NMR is a bulk technique which is not very sensitive to surface changes. This method appears to be very sensitive and will undoubtedly be used more often in the future as a tool to detect different crystal forms. However, with present technology, errors in solid-state quantitative studies may be rather large.

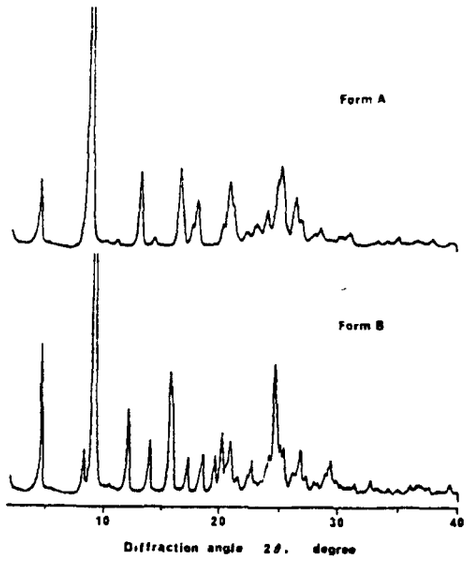


Figure 2. Powder X-ray diffraction patterns of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine (1).

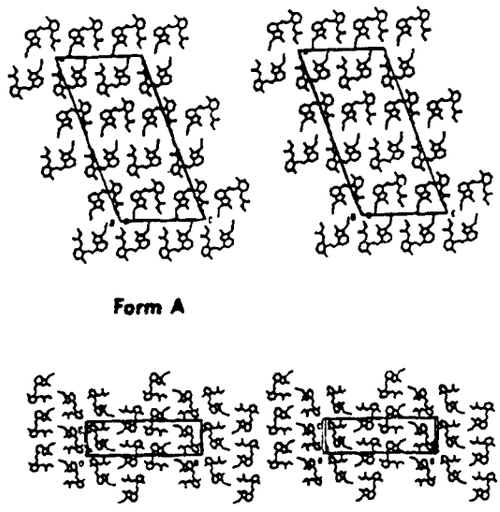


Figure 3. Stereoscopic drawings of the crystal packing of both polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine viewed along the shortest axis (Form A, b-axis; Form B, a-axis) (1).

B. Do the Polymorphs Have Different Physical Properties?

If polymorphs exist then it is necessary to examine the physical properties of the different polymorphs that can affect dosage form performance (bioavailability and stability) or manufacturing reproducibility. The properties of interest are solubility profile (intrinsic dissolution rate, equilibrium solubility), stability (chemical and physical), and crystal morphology (including both shape and particle size), calorimetric behavior, and %RH profile. If there are no discernible differences between these physico-chemical properties, then the answer to the second question in the decision tree, "Different physical properties?" is "No."

The variable physical properties of several drugs with different polymorphs are reported in the literature. For example, the dissolution profiles of the polymorphs of chloramphenicol are significantly different (4). In addition, van't Hoff solubility analysis has been used to elucidate the dif-

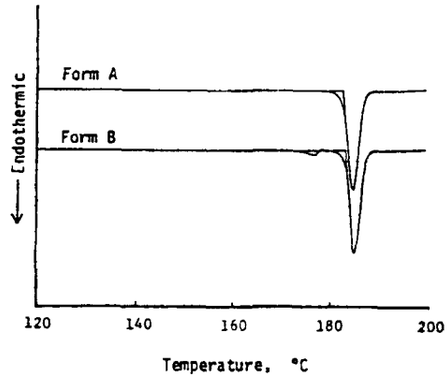


Figure 4. DSC thermal curves of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine (1). These curves show that Form A melts whereas Form B undergoes a small endothermic transition and then melts at the same temperature as Form A.

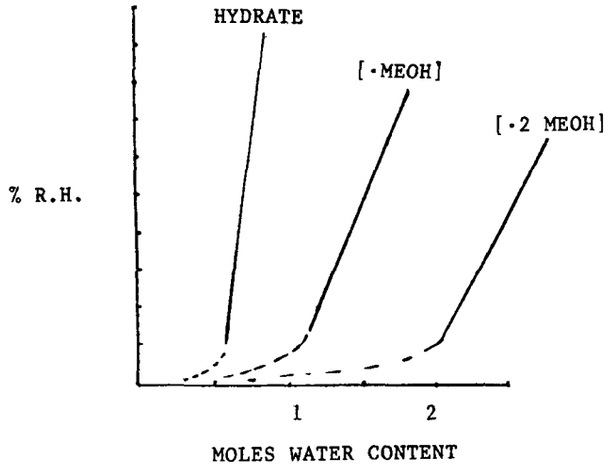


Figure 10. Water sorption by three crystal forms of cephaloridine. The brackets indicate the crystal form produced by desolvating the designated methanolate.

about one molecule of water and is the 0.75 hydrate typically obtained from water solution.

AMORPHOUS FORMS

Amorphous forms are of substantial interest because they usually are much more soluble than their crystalline counterparts. Indeed, there are cases where the amorphous forms is the only solid form that has adequate bioavailability. The initial question with this flow chart (Figure 11) is similar to the previous ones: "Are amorphous forms possible?" Amorphous forms can be prepared in different ways, for example, by spray drying or by freeze drying. One can test whether an amorphous form has been produced by using one of the methods listed. X-ray powder diffraction and microscopy are the two primary methods for determining whether an amorphous form has been produced. Powder diffraction is an excellent method for determining the existence of an amorphous form since they usually exhibit a broad hump between 2 and 20° 2θ. An amorphous form is expected to have no peaks in the powder diffraction pattern. The USP test for the presence of an amorphous form involves determining, by microscopy, whether the material lacks birefringence. IR and solid-state NMR may be useful for detecting amorphous forms since the amorphous nature of the solid sometimes results in broad lines, or in NMR, altered relaxation times. The next question on the flow chart is: "Do the amorphous forms have different physical properties?" The answer to this question will almost certainly be "Yes." Three differences from crystalline forms may generally be expected: 1) Amorphous forms would have greater solubility, 2) Amorphous forms take up water more extensively, and 3) Amorphous forms are sometimes less chemically stable. Another key question for an amorphous form is: "Does it crystallize, and how and when?" This question is very important since inadvertent crystallization can greatly affect the solubility and dissolution rate, and lead to other failures in formulation. Attempts to purposely cause amorphous forms to crystallize can provide information on the parameters involved in crystallization of amorphous forms. Specific questions include: (1) "Does the amorphous form crystallize upon exposure to heat and/or humidity?," and (2) "What other factors (e.g. mechanical pressure and seeding) can lead to the crystallization of the amorphous forms?"

The amorphous form of any substance can be partly characterized by the glass transition temperature, T_g (11). When heated to a temperature above T_g, the solid transforms from a glassy state to a more fluid-like rubbery state. The corresponding increased molecular mobility greatly raises the likelihood of two adverse events: (1) Crystallization and subsequent decreased solubility; and (2) Reduced chemical stability in the more reactive amorphous solid. Amorphous solids are also often prone to absorb moisture and this water sorption reduces the glass transition temperature further. The weight of water required to reduce the glass transition

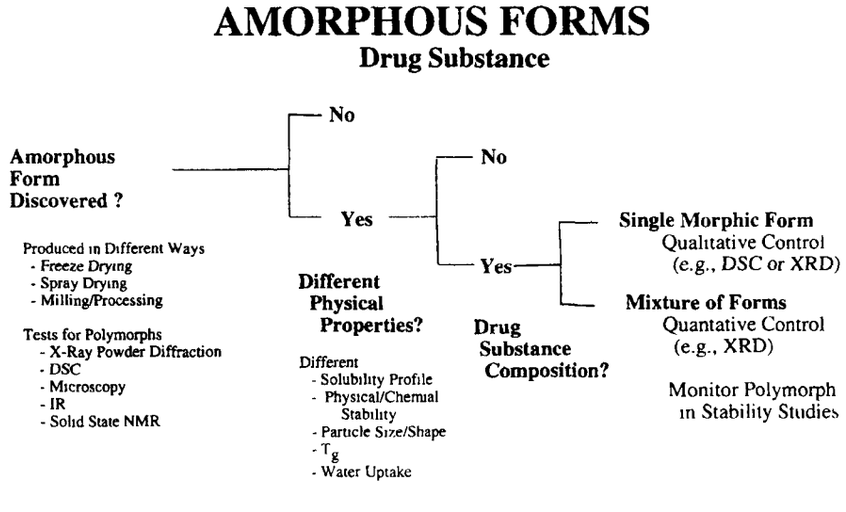


Figure 11. Flow chart for amorphous solids.

temperature to room temperature is of obvious interest and is termed W_g. Table I shows a series of interesting studies on amorphous forms of some common pharmaceuticals.

The table compares the glass transition temperatures (T_g) of a number of pharmaceutical solids with the melting temperatures (T_m). It is interesting that the average ratio of the glass transition temperature to the melting temperature is about 0.70. This table provides a simple rule of thumb which allows the prediction of the glass transition temperature of pharmaceuticals from the known melting point. Crystallization and other solid-state phenomena, such as degradation reactions, as we have said, would be more likely to occur at temperatures above the glass transition temperature. For stability, one might, therefore, wish to prepare amorphous forms only for drugs which have a T_g well above room temperature.

Amorphous indomethacin crystallizes upon standing at room temperature(Figure 12). Obviously, formulations containing amorphous indomethacin are at significant risk to crystallize and thus become less soluble. This has to some extent hampered preparing more bioavailable indomethacin dosage forms.

Quantitative analysis of mixtures of amorphous and crystalline forms provides some challenges. Cefixamine trihydrate is the subject of some early research in this area. This antibiotic, upon grinding, became a mixture of crystalline and amorphous forms. A calibration curve based upon analyzing the height of a selected powder X-ray peak was constructed and used to determine the crystallinity versus grinding time for this system. It is clear that powder diffraction provides a way to estimate the amount of amorphous cefiximine. These studies show that milling and other similar processing steps can create amorphous material and that this process may be detectable. As with wet granulation where transitions to hydrated forms can occur, processing of the drug substance can promote the formation of amorphous drug.

Pikal has compared the analysis of mixtures of crystalline and amorphous forms of several antibiotics by powder diffraction and calorimetry (20). His studies indicate that calorimetry can be a more accurate method for analysis of percent crystallinity but are complicated by water sorption. Zografi and co-workers (unpublished results) have developed a powerful method for the determination of low percentages of amorphous material based on the general propensity of amorphous materials to sorb moisture.

Table I. Pharmaceuticals Forming Glasses Above Room Temperature (21)

Pharmaceutical	T_g (K)	T_m (K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17β-Estradiol	351	445	0.80

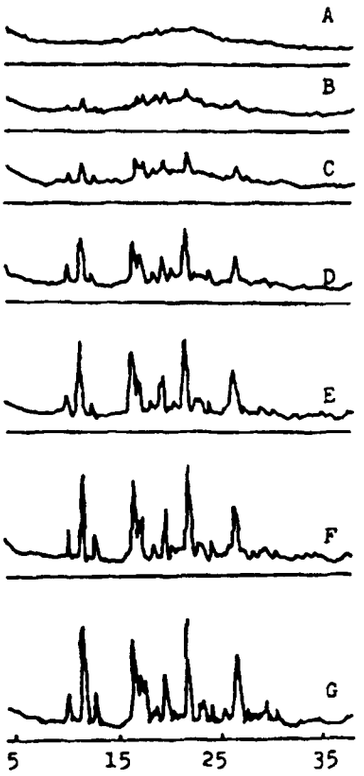


Figure 12. Behavior of amorphous indomethacin upon standing: A, at start; B 24 h; C, 48 h; D, 7d; E, 14d; F, 30d; G, 67d (22).

SUMMARY

Four flow charts which describe approaches to regulatory issues involving pharmaceutical solids have been developed. These flow charts are for the different types of solids generally encountered (polymorphs, solvates, desolvated solvates, and amorphous forms). It is hoped that these flow charts will guide the solid-state research needed to prepare a comprehensive regulatory submission on the physicochemical properties of a pharmaceutical. It is also hoped that this review has provided enough information to allow the generation of results and information necessary to prepare a drug substance submission that will be quickly approved.

ACKNOWLEDGMENTS

This review was supported in part by funds from the Purdue-Wisconsin Joint Project for the Study of the Effect of Water on the Molecular Mobility of Solid Pharmaceuticals (Members: Merck, Sandoz, Upjohn, Glaxo, Pfizer, 3M, Hoffmann-LaRoche, Syntex, Bristol-Myers Squibb). Helpful comments were provided by scientists from Merck (West Point and Montreal), Glaxo, Bristol-Myers Squibb, Syntex, Upjohn, Pfizer, Lilly, Sandoz, Pfizer, and Roche.

REFERENCES

1. A. Miyamae, S. Koda, S. Kitamura, Y. Okamoto, and Y. Morimoto. X-ray crystallographic characterization of two poly-

morphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)imidazo[1,2-a]pyridine, *J. Pharm. Sci.* 79:189–195 (1990).
2. P. A. Saindon, N. S. Cauchon, P. A. Sutton, C. J. Chang, G. Peck, and S. R. Byrn. Solid-state nuclear magnetic resonsance (NMR) spectra of pharmaceutical dosage forms, *Pharm. Res.* 10:197–203 (1993).
3. D. E. Bugay. Solid-state nuclear magnetic resonance spectroscopy: Theory and pharmaceutical applications, *Pharm. Res.* 10:317–327.
4. J. K. Haleblian and W. C. McCrone. Pharmaceutical Applications of Polymorphism, *J. Pharm. Sci.* 58:911 (1969).
5. W. I. Higuchi, P. K. Lau, T. Higuchi, and J. W. Shell. Polymorphism and Drug Availability, *J. Pharm. Sci.* 52:150–153 (1963).
6. D. P. Ip, G. S. Brenner, J. M. Stevenson, S. Lindenbaum, A. W. Douglas, S. D. Klein, and J. A. McCauley. High resolution spectroscopic evidence and solution calorimetry studies on the polymorphs of enalapril maleate, *Int. J. Pharm.* 28:183–191 (1986).
7. Y. Matsuda, E. Tatsumi, E. Chiba, and Y. Miwa. Kinetic study of the polymorphic transformations of phenylbutazone, *J. Pharm. Sci.* 73:1453–1460 (1984).
8. V. P. Tanninen and J. Yliruusi. X-ray powder diffraction profile fitting in quantitative determination of two polymorphs from their powder mixture, *Int. J. Pharm.* 81:169–177 (1992).
9. G. A. Stephenson, J. G. Stowell, P. H. Toma, D. E. Dorman, J. R. Greene, and S. R. Byrn. Solid-State Analysis of Polymorphic, Isomorphic, and Solvated Forms of Dirithromycin, *J. Am. Chem. Soc.* 116:5766–5773 (1994).
10. C. A. Hirsch, R. J. Messenger, and J. L. Brannon. Fenoprofen: drug form selection and preformulation stability studies, *J. Pharm. Sci.* 67:231–236 (1978).
11. S. Niazi. Thermodynamics of mercaptopurine dehydration, *J. Pharm. Sci.* 67:488–491 (1978).
12. T. Ishida, M. Doi, M. Shimamoto, N. Minamino, K. Nonaka, and M. Inoue. Physicochemical properties of crystalline forms of ethynylestradiol solvates: comparison of thermal behavior with x-ray crystal structure, *J. Pharm. Sci.* 78:274 (1989).
13. G. Zografi, D. Hollenbeck, S. Laughlin, M. Pikal, J. Schwartz, and L. VanCampen. Report of the advisory panel on moisture specifications, *Pharmacopeial Forum:* 1459–1474 (1991).
14. C. T.Lin, P. Perrier, G. G. Clay, P. A. Sutton, and S. R. Byrn. Solid-State Photooxidation of Cortisol-21-tert-butylacetate to Cortisone-21-tert-butylacetate, *J. Org. Chem.* 47:2978–82 (1982).
15. S. R. Byrn, P. A. Sutton, B. Tobias, J. Frye, and P. Main. Crystal structure, solid-state NMR spectra, and oxygen reactivity of five crystal forms of prednisolone tert-butylacetate, *J. Am. Chem. Soc.* 110:1609–1614 (1988).
16. Y. Matsuda and E. Tatsumi. Physicochemical characterization of furosemide modifications, *Intl. J. Pharm.* 60:11–26 (1990).
17. C. Doherty and P. York. Furosemide crystal forms; solid state and physicochemical analyses, *Int. J. Pharm.* 47:141–155 (1988).
18. E. Shefter and T. Higuchi. Dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals, *J. Pharm. Sci.* 52:781–791 (1963).
19. I. Himuro, Y. Tsuda, K. Sekiguchi, I. Horikoshi, and M. Kanke. Studies on the method of size reduction of medicinal compounds. IV. Solvate formation of chloramphenicol and its application to size reduction, *Chem. Pharm. Bull.* 19:1034–1040 (1971).
20. M. J. Pikal, A. L. Lukes, J. E. Lang, and K. Gaines. Quantitative crystallinity determinations for beta-lactam antibiotics by solution calorimetry: correlations with stability, *J. Pharm. Sci.* 67:767–769 (1978).
21. E. Fukuoka, M. Makita, and S. Yamamura. Some physicochemical properties of glassy indomethacin, *Chem. Pharm. Bull.* 34:4314–4321 (1986).
22. E. Fukuoka, M. Makita, and S. Yamamura. Glassy state of pharmaceuticals III. Thermal properties and stability of glassy pharmaceuticals and their binary glass systems, *Chem. Pharm. Bull.* 37:1047–1050 (1989).

Exhibit -3

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SOLID STATE CHARACTERIZATIONS OF PHARMACEUTICAL HYDRATES

D. Giron, Ch. Goldbronn, M. Mutz, S. Pfeffer, Ph. Piechon and Ph. Schwab

Chemical and Analytical Research and Development, Novartis Pharma, Basel, Switzerland

Abstract

Manufacturing processes may involve the presence of water in the crystallization of the drug substance or in manufacturing or in the composition of the drug product through excipients. Dehydration steps may occur in drying, milling, mixing and tabletting processes. Furthermore, drug substances and drug products are submitted to different temperatures and relative humidities, due to various climatic conditions giving rise to unexpected hydration or dehydration aging phenomena. Therefore the manufacture and the characterization of hydrates is part of the study of the physical properties of drug substances.

Several hydrates and even polymorphic forms thereof can be encountered. Upon dehydration crystal hydrates may retain more or less their original crystal structure, they can lose crystallinity and give an amorphous phase, they can transform to crystalline less hydrated forms or to crystalline anhydrous forms.

The proper understanding of the complex polyphasic system hydrates polymorphs amorphous state needs several analytical methods. The use of techniques such as DSC-TG, TG-MS, sorption-desorption isotherms, sub-ambient experiments, X-ray diffraction combined with temperature or moisture changes as well as crystal structure and crystal modelling in addition to solubilities and dissolution experiments make interpretation and quantitation easier as demonstrated with some typical examples.

Keywords: aging, DSC, freezable water, phase changes, phase transitions, pseudo-polymorphism, stability, sub-ambient DSC, TG, TG-MS, water activity

Introduction

Manufacturing processes may involve the presence of water in the crystallization of the drug substance or in the manufacturing or in the composition of the drug product through excipients. New phases where water is a part of the crystal, called hydrates may be obtained with completely new properties in the solid state [1–8]. Dehydration steps may occur in drying, milling, mixing and tabletting processes. Some properties known to be altered by the association of solids with water, including rates of chemical degradation in the solid state, crystal growth, dissolution, dispersibility, wetting, powder flow, lubricity, compactibility, hardness. Furthermore drug substances and

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drug products are submitted to different temperatures and relative humidities, due to various climatic conditions giving rise to unexpected hydration or dehydration aging phenomena. Water will be absorbed and desorbed with temperature and moisture changes. The crystallization in tablets [10, 11] of theophylline monohydrate, which has a lower dissolution rate as the two anhydrates [9] or the hydrate formation of excipients [12] such as lactose, sorbitol or magnesium stearate are among well known examples studied in the literature [3].

Several hydrates and even polymorphic forms thereof can be encountered. Upon dehydration crystal hydrates may retain more or less their original crystal structure, they can lose crystallinity and give an amorphous phase, they can transform to crystalline less hydrated forms or to crystalline anhydrous forms. In addition to physical changes, free water may react chemically [13]. Since the formation of new hydrated solid phases has the same impact as polymorphism for the bioavailability, toxicity, stability and processing as pure polymorphs, the manufacture and the characterization of hydrates is part of the study of the physical properties of drug substances according to the ICH decision tree 4 [14].

Experiments were performed with the following instruments

Perkin Elmer DSC-7 with robot system, Sub-ambient Perkin Elmer DSC-7, Perkin Elmer TGA-7, Mettler TA 850, Mettler TG-MS. X-ray diffractometer Scintag XDS 2000 with autosampler or with heating cell or with humidity cell for the X-ray diffraction experiments and DVS for the sorption-desorption curves. Crystal modelling was done using the Cerius software (MSI).

Examples

Phase diagrams considerations

In crystal hydrates, the combination of intermolecular forces (hydrogen bonding) and crystal packing can produce very strong water-solid interactions. However, they are situations where hydration and dehydration of crystals occur quite easily at low temperature. The equilibrium behaviour of the anhydrous form of a drug substance with water depends upon the phase diagrams which are drug substance specific. Figure 1 shows two typical phase diagrams for a compound formation in a binary mixture. Figure 1a deals with the case of a hydrate which has a well defined melting point and Fig. 1b corresponds to an incongruent behaviour. Such phase diagrams are responsible for the observation of the eutectic point in the thermal analysis studies as it has been described in the case of Terpin [15].

By varying the temperature and the humidity different stable hydrates may be formed. When coming back to different conditions, a reconversion may be observed upon aging. An old example is emetine dihydrochloride for which the heptahydrate (15.0–19.0% water) and the pentahydrate (11.0–15.0% water) are described in the European Pharmacopea. In 1975 we analysed a reference declared as heptahydrate (loss on

drying 18.1%, water by Karl Fisher: 17.3%). 3 months later a conversion into the pentahydrate was suggested by the values of the loss on drying in a drying oven (14.7%), the thermogravimetry (14.8%) and the Karl Fischer determination of water: 14.8%.

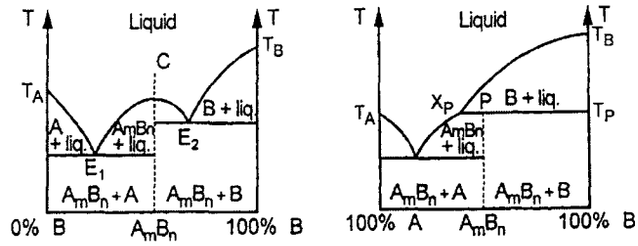

Fig. 1 Phase diagrams of binary mixtures. 1a – compound with melting, 1b – incongruent behaviour

Figure 2 examplifies the DSC behaviour of the hydrates of two different drug substances with the dehydration without melting at very different temperature. For even less stable hydrates, the endotherm of dehydration may be so broad, that it can be difficult to detect it by only DSC and TG determinations. The ultimate demonstration of the hydrate formation is the single X-ray structure.

Since it is not always available, it has been suggested [16] to use sub-ambient DSC in order to know if the water contained in the sample is tightly bound or not. The melting peak of water allows to determine the freezable water.

The example given in the Fig. 3 deals with a crystalline drug substance for which a monohydrate structure was suggested. No freezable water was found in the sample, even after exposition at 92% RH. The drug substance was slurried with water in order to maximise the uptake of water and the sample analyzed by TG and sub-ambient DSC. The TG value of the sample was 33.8%. The freezable water cal-

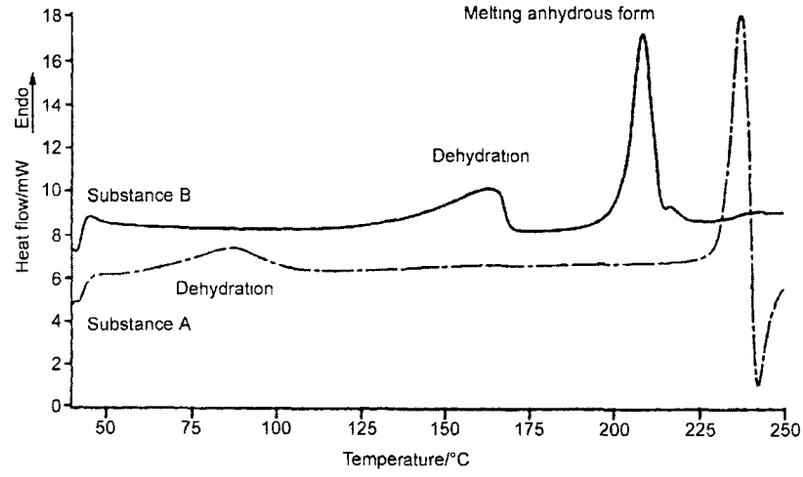

Fig. 2 DSC scans of two hydrated forms with different energies resulting in different temperatures for the dehydration endotherms

culated 31.4%. Therefore the amount of hydrated solid was 68.6% and the amount of bound water calculated 3.5%, which fit exactly with the theoretical amount of water for a monohydrate.

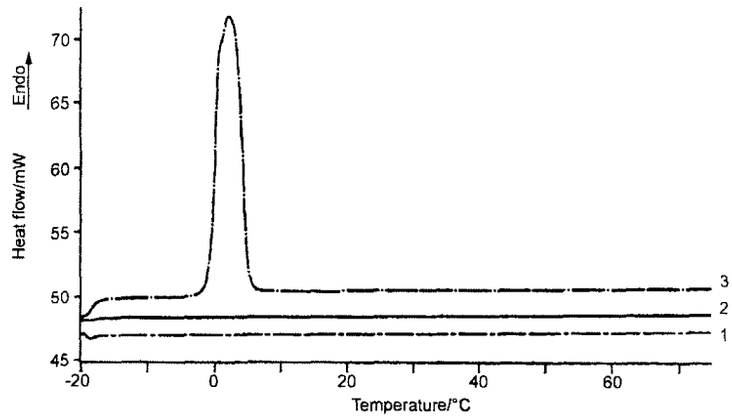

Fig. 3 Sub-ambient DSC and TG experiments for the study of bound water
1 Drug substance; 2 – drug substance stored under 92% RH; 3 – suspension of the drug substance with water. Melting peak of free water in the suspension

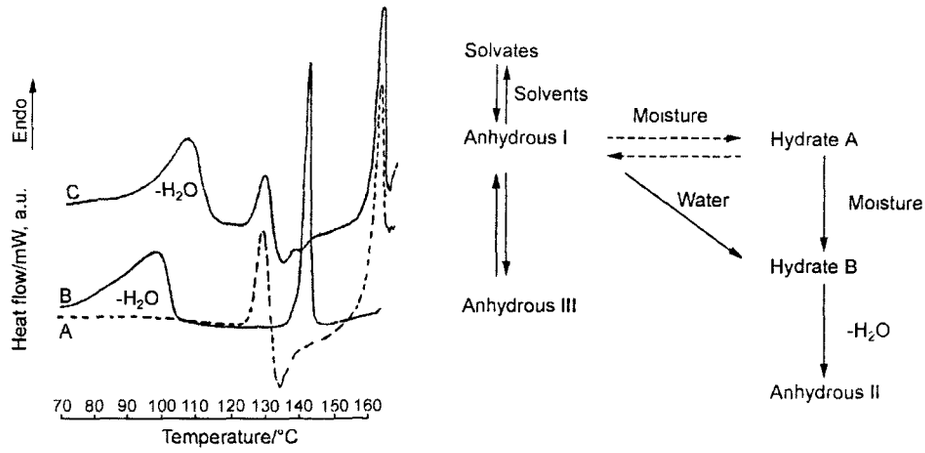

Fig. 4 Polymorphism of hydrate DSC scans of A initial anhydrate; B Hydrate A; C – Hydrate B

Polymorphism of hydrates may also occur. McCauley *et al.* [17] measured the transition temperature and the crystallization conditions as well as the dehydration of two polymorphs dihydrate of a development compound. For one form the dehydration occurs directly to the anhydrate. For the other polymorph the dehydration occurs via the monohydrate. The form A of amiloride dihydrate was found more stable than form B upon milling or compression [18]. Quite recently a new polymorphic form of

the hemihydrate of aspartame as well as a 2.5 hydrate were described [19]. In the example of Fig. 4, two polymorphs of the trihydrate were identified by their different behaviour in the DSC. The DSC curve of the anhydrous form presents a dual melting.This anhydrous form is hygroscopic and transforms reversibly into an hydrate A. But if the exposition with moisture is quite longer, a second hydrate B is observed with a dehydration into a new anhydrous form. In aqueous solutions, the hydrate B, which is less soluble is obtained.

The relevance of the polymorphism of hydrates for the chemical stability is demonstrated by the example given in the Table 1 below.

Table 1 Comparison of stability behaviour of two polymorphic forms of a monohydrate of a development new entity

Sample	Impurities by HPLC		
	Initial value/%	2 weeks at 50°C/%	Exposition 1200 klux h/%
Monohydrate A	1.4	1.4	11
Monohydrate B	1.0	12.7	24

Soustelle [20] discussed the influence of the pressure with the example of cupper sulfate. The four solid phases are the pentahydrate, the trihydrate, the monohydrate and the anhydrous form. The phase diagram P, T has different equilibrium curves: pentahydrate to trihydrate, trihydrate to monohydrate, monohydrate to anhydrous form and also pentahydrate to the monohydrate and pentahydrate to the anhydrous form. Depending on the pressure, the TG curve of the pentahydrate can be a single dehydration process with lost of 5 molecules of water, a two steps dehydration with the intermediate monohydrate or a three steps dehydration with the intermediates trihydrate and monohydrate.

Thermal dehydration of crystalline solids hydrates in view of crystallographic structures controlling conversions has been recently reviewed by Galwey [21].

The influence of the pressure in the DSC cell is a known factor of misinterpretation of DSC curves, since depending on the pan type, the melting of the hydrate or its dehydration in the solid state may occur [3, 22]. On the other hand different results will be obtained with other techniques like thermomicroscopy or temperature resolved X-ray diffraction if the atmosphere around the sample is not the same. Han *et al.* [23] applied successfully the pressure DSC (PDSC) in order to separate the dehydration and the vaporization endotherms. The quantitation of ampicilline trihydrate in mixtures was possible. Depending on the pressure, the dehydration occurs with formation of the amorphous form or of a new polymorph of the ampicilline anhydrate. The same author proposes a humidity controlled TG and X-ray powder diffractometry for the kinetic study of the dehydration of ampicillin trihydrate, the calculation of the transition vapor pressure and the knowledge of the critical water vapor pressure above it the trihydrate is the stable solid phase [24].

Sorption/desorption isotherms

Studies of hydration and dehydration are generally carried out by crystallizations in water or water/organic solvent mixtures or by the measurement of water sorption and desorption isotherms. Water can be sorbed without or with phase change. Hygroscopicity and moisture adsorption kinetics of pharmaceutical solids as well as thermodynamic background have been discussed and reviewed [25, 26].

Figures 5a and 5b show the reversible sorption-desorption curves of an amorphous substance and of a very hygroscopic crystalline anhydrate turning into a monohydrate at very low relative humidity RH.

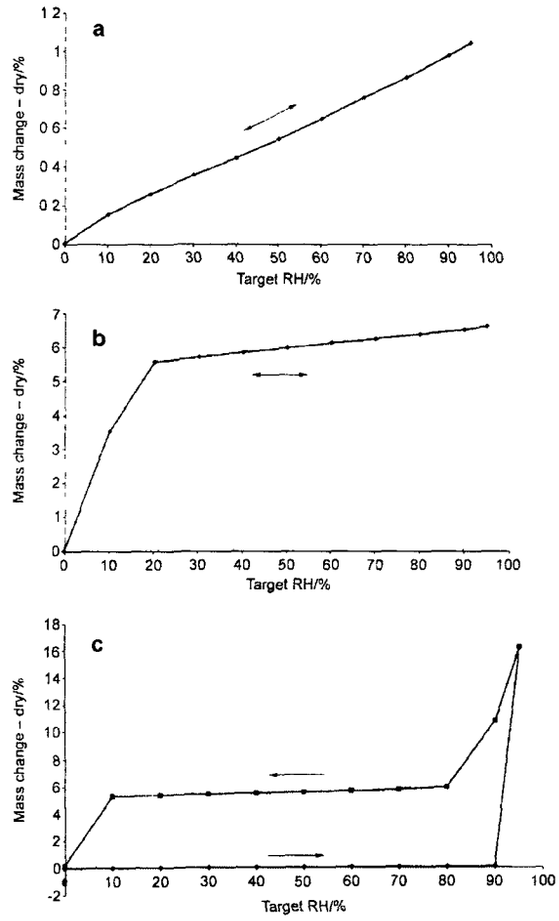

Fig. 5 Sorption-desorption types a – reversible sorption-desorption of an amorphous sample; b – reversible sorption-desorption of a strong hygroscopic anhydrate; c – sorption-desorption of the anhydrate with formation of monohydrate and strong hysteresis of the desorption into the anhydrate

When big hysteresis are found in the desorption, as it is the case of Fig. 5c which corresponds to the study of a anhydrate, the monohydrate formed is stable enough to be analyzed separately by TG, Karl Fischer, IR, Raman, X-ray. It is then very helpful from the curves to deduce the critical relative humidity (RH) at different temperatures where the anhydrate or the hydrate have their relative stabilities. However, kinetic factors may be somewhat necessary to start the solid-solid transformation. For the example above, the transformation into the monohydrate was accelerated by seeds of the monohydrate. When the desorption is reversible, deeper analysis is necessary to conclude about the hydrate formation. Combined or coupled techniques allow to add to the calorimetric signal, informations about spectral or cristallographic data [27].

Examples of investigations by combined methods

The following examples will show some successful results of such investigations.

The DSC curves of the monohydrate corresponding to Fig. 5c were different if performed in a in very tight pan or with a pierced pan. In the first case, only two endotherms were observed. In the other case, the dehydration of the sample was observed in solid state, followed by two other endotherms (Fig. 6). The temperature resolved X-ray diffractions of the monohydrate show that two other anhydrates are obtained before the last transition into the stable known anhydrate (Fig. 7). The diffractograms obtained in situ allow the characterization of these metastable forms and their detection by X-ray diffraction. More complex for this drug substance was the transformation of a methanol solvate into this monohydrate after several months. X-ray diffraction was chosen for quantitation both for the drug substance and the drug product.

This example shows how hydration and dehydration may be driven by kinetic effects. Nafragel hydrochloride can crystallise as hemihydrate or as monohydrate. The sorption of the anhydrate occurs in two steps and the dehydration only in one

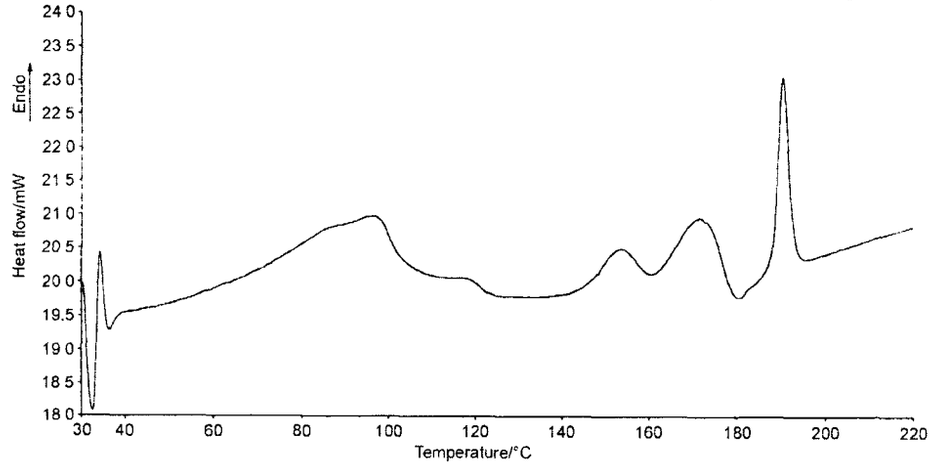

Fig. 6 DSC curve of the monohydrate corresponding to Fig. 5c in a pierced pan

step. The analysis of hydration and dehydration at different RH, revealed different kinetics, what explains the coexistence of mixtures of both hydrated forms [28].

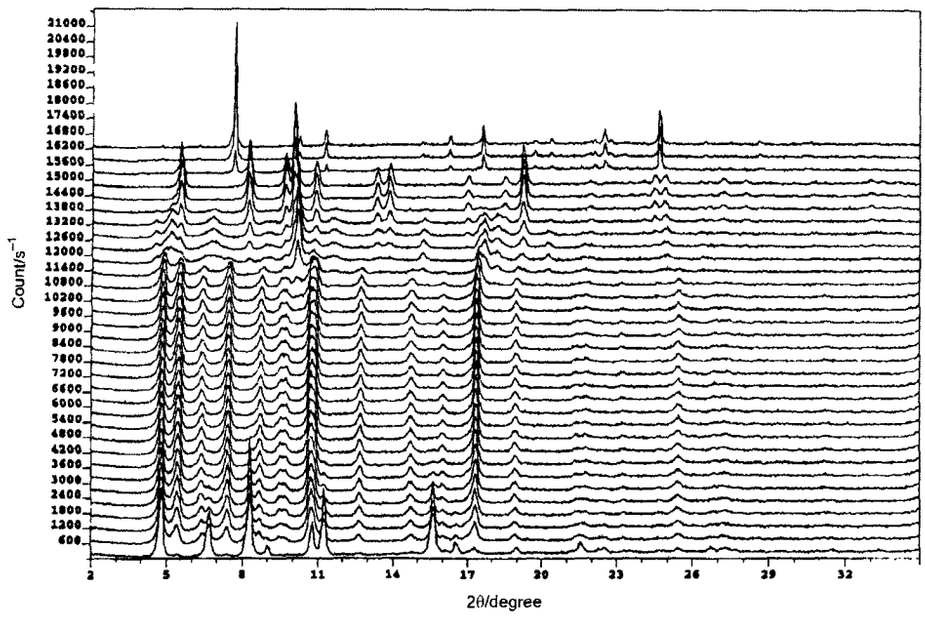

Fig. 7 Temperature resolved X-ray diffraction of the monohydrate corresponding to Fig. 6

Figure 8a shows the reversible sorption-desorption of the first batches of a drug substance. The sub-ambient DSC analyses revealed that it was a monohydrate although already at ambient temperature and low relative humidity the crystal looses already a part of water. The studies of the X-ray diffraction at different humidity levels and at different temperatures revealed the existence of a very hygroscopic intermediate anhydrate which melts in the DSC. The single crystal structure confirmed the hydrate formation. Later on

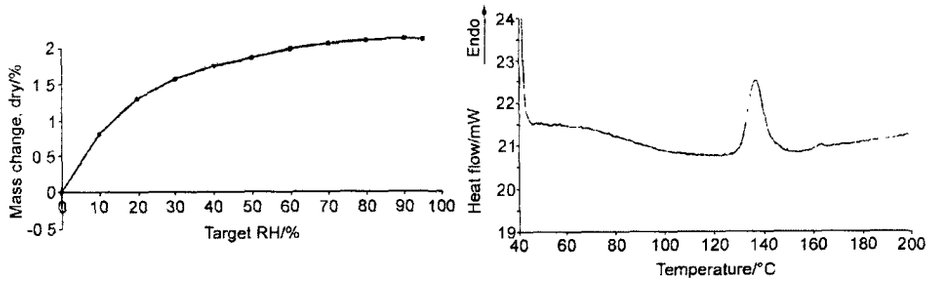

Fig. 8 Metastable anhydrous form/Monohydrate and stable new anhydrate.
a sorption-desorption of the metastable anhydrate/monohydrate pairs;
b – DSC curve of the monohydrate contamined with traces of anhydrous form B

in the development, a very stable anhydrous form B was found. This form is not hygroscopic and form A in presence of B transforms very quickly into the anhydrate B. Since the transformation into the anhydrate B from the melt was kinetically hindered, thermomicroscopy could be applied qualitatively. A very sensitive DSC method could be used with a LOD of approx. 0.5%. From the single crystal structures of A and B, the polymorphic purity of the reference samples of B or A could be demonstrated and the theoretical LOD of the X-ray diffraction evaluated. X-ray diffraction was the method of choice for quantitation (linearity, accuracy).

Figure 8b shows a DSC scan of the form A contaminated with traces of form B. Figure 9 shows the TG-MS of the form A. It was possible by using TG-MS to distinguish between the monohydrate and an acetone solvate of this molecule.

Table 2 Physico-chemical properties of the anhydrous and hydrated form of a quinoline derivative. The formation of the hydrated form (form A) is driven by the water activity of the solvents

	Method/ Conditions	Results form A, batch 1	Results form B, batch 2
Purity (eutectic)	DSC/1 K min^{-1}	99.8 mol%	99.8 mol%
DSC melting onset	DSC/10 Kmin^{-1}	208°C	195°C
DSC melting enthalpy	DSC/10 K min^{-1}	79.1 J g^{-1}	82.8 J g^{-1}
Heat of solution (in MeOH)	microcalorimetry	23.2+0.1 J g^{-1}	25.1+0.1 J g^{-1}
Thermogravimetry		0.5%	<0.05%
Crystallinity	XRPD	high	high
Dissolution rate in: a) 0.1 N HCl+0.5%Tween 20	Flow cell T=25°C		
$t_{50\%}$		5 min 22 s/ 6 min 11 s	4 min 4 s/ 5 min 17 s
$t_{80\%}$		10 min 57 s/ 12 min 53 s	10 min 36 s/ 11 min 50 s
$t_{90\%}$		16 min 20 s/ 17 min 56 s	18 min 40 s/ 19 min 10 s
b) water+0.2% LDAO			
$t_{50\%}$		7 min 09 s/ 10 min 23 s	11 min 47 s/ 9 min 19 s
$t_{80\%}$		22 min 56 s/ 24 min 44 s	28 min 35 s/ 27 min 56 s
$t_{90\%}$		42 min 09 s/ 38 min 26 s	48 min 53 s/ 50 min 21 s
Second DSC run after melting	DSC	T_g=92.5°C	T_g=93.9°C

The thermodynamic stability of hydrated forms in mixed solvents depends on water activities [29–31] as demonstrated in the figure 10a for a quinoline derivative. The modification B is anhydrous. Modification A is an hydrated form as demonstrated by the study

of the sub-ambient DSC. The corresponding sorption-desorption isotherm (Fig. 10b) is very similar to the example of Fig. 8. The formation of A or B is driven by the water activity of the solvents. Form B was selected. The detection of form A was monitored by X-ray diffraction and TG. The Table 2 below summarizes some results.

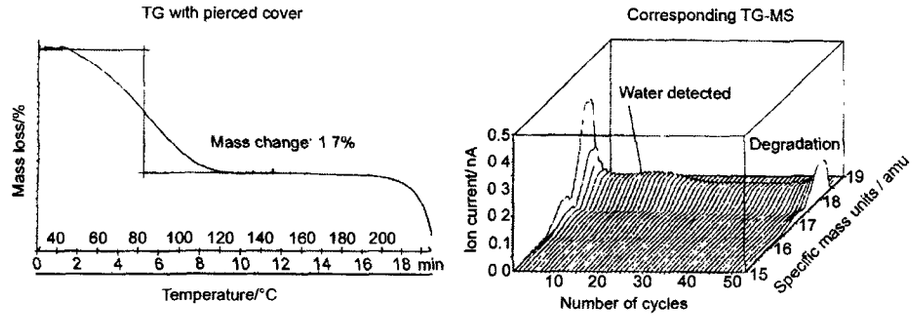

Fig. 9 TG-MS of the monohydrate of Fig. 8

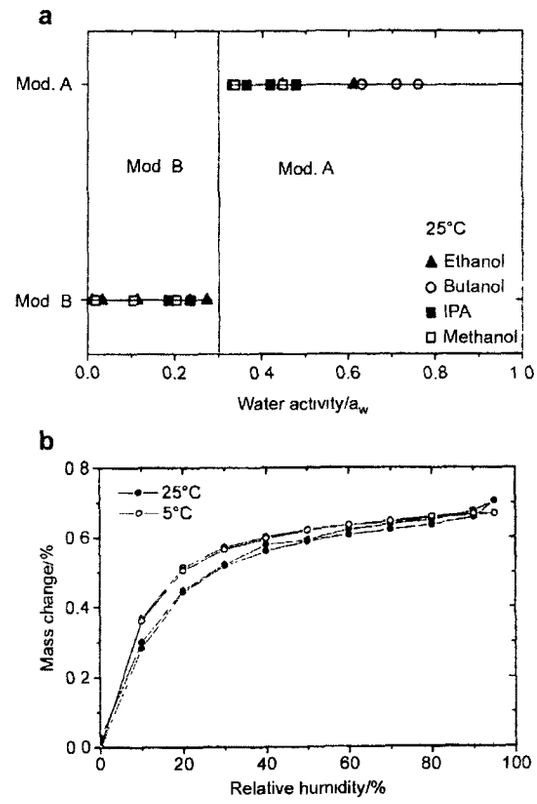

Fig. 10 Influence of water activity in solvents of crystallization a – diagram *vs.* water activity; b – sorption of form A

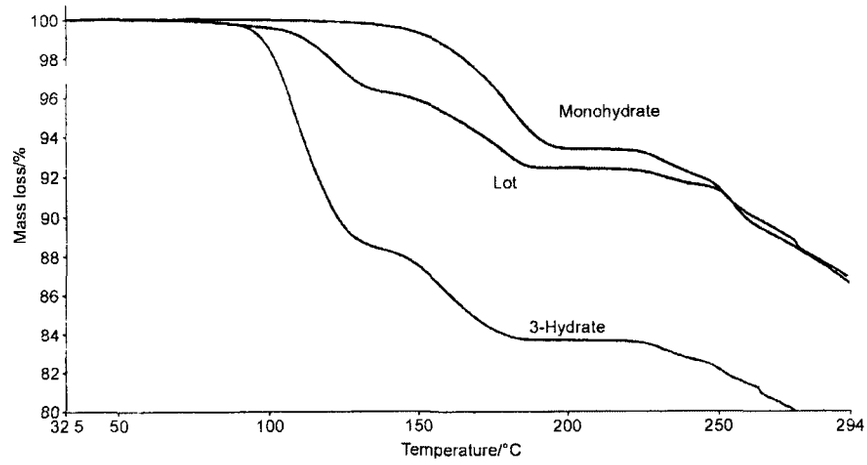

Fig. 11 TG curves of a monohydrate, of a trihydrate and of a lot containing monohydrate and trihydrate

The next example is a drug substance developed as a monohydrate. This hydrated form is manufactured in ethanol/water. The thermogravimetric analysis of a lot obtained in a scale-up revealed a significant difference (Fig. 11), although results of Karl Fischer were within requirements. Crystallization experiments, equilibration in solvent mixtures, TG, DSC, X-ray diffraction, sorption and desorption as well as combined techniques were used for a deeper investigation. Besides the anhydrate and the monohydrate, a trihydrate is obtained. The solubilities in water are summarized in Table 3.

Table 3 Solubility behaviour of a drug substance in water at different temperatures and areas corresponding to anhydrate, monohydrate and trihydrate

Temperature/°C	Solubility/mg mL^{-1}	Residual solid
10	2.1	trihydrate
25	2.8	trihydrate
40	10.9	trihydrate
60	25.3	monohydrate
80	31.6	anhydrate

The dehydration of the trihydrate occurs via the monohydrate (Fig. 11). The monohydrate is not hygroscopic. Through drying at 80°C, the anhydrate obtained is hygroscopic and transforms into the monohydrate (Fig. 12).

Manufacturing conditions of the monohydrate were optimized and a quantitative X-ray diffraction method was developped.

For the last example, laboratory batches were strongly affected by grey impurities. By using an aqueous mixture, the impurities were removed and after drying a

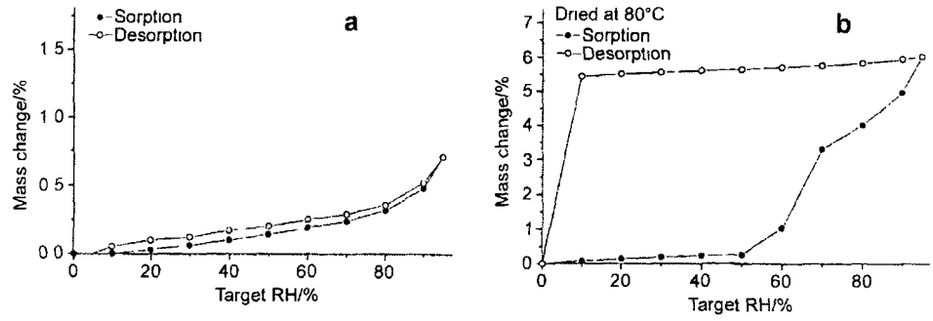

Fig. 12 Sorption-desorption of the monohydrate (left) of Fig. 11 and of the corresponding anhydrate (right) obtained by drying at 80°C

new polymorph was obtained. This form was highly hygroscopic and converted into a trihydrate. Since the drug substance was chemically very sensitive to moisture, it was decided to manufacture the anhydrous form. Slurry of the purified trihydrate in methanol allowed the transformation into the stable white anhydrous form.

Conclusions

Hydrate formation leads to complex behaviour of drug substance and drug products in every step of processing and after storage. Adequate investigations are necessary in very early stage of development for the proper choice of the candidate form, the choice of the formulation, of the process and of the packaging. A deep insight needs several analytical complementary techniques with a high level of information. Particurlarly informative are X-ray diffraction experiments in chambers of different humidity in parallel to sorption-desorption isotherms. Dehydration studies by TG under different temperatures and pressure simulate drying and milling processes. The crystal stucture obtained by single crystal diffraction or by computational calculation from X-ray diffractometry allows to understand the type of the bonds between water and drug substance. Modelling capabilities are extremely helpful as an help for quantitative methods in drug substance and drug products.

References

1 J. K. Haleblian and W. J. McCrone, Pharm. Sci., 58 (1969) 911.
2 J. K. Haleblian, Pharm. Sci., 64 (1975) 1269.
3 D. Giron, Thermochim. Acta, 248 (1995) 1.
4 D. Giron, Labo-Pharma-Probl. Techn., 307 (1981) 151.
5 D. Giron, S.T.P. Pharma, 4 (1988) 330.
6 J. Bernstein, R. J. Davey and J. O. Henck, Angew. Chem. Int. Ed., 38 (1999) 3340.
7 K. R. Morris, Structural aspects of hydrates and solvates. (Polymorphism in Pharmaceutical Solids Brittain H. G. ed., Marcel Dekker, New York), Drugs Pharm. Sci., 95 (1999)125.

8 R. K. Kankhari and D. J. W Grant, Thermochim. Acta, 248 (1995) 61.
9 E. Suzuki, Chem. Pharm. Bull., 37 (1989) 493.
10 H. Ando, Drug Dev. Ind. Pharm., 21 (1995) 2227.
11 C. M. Adeyeye, Int. J. Pharm., 116 (1995) 65.
12 D. Giron, S.T.P. Pharma (Hors serie), 6 (1990) 87.
13 J. T. Carstensen, Drug Dev. Ind. Pharm., 14 (1988) 1927.
14 International Conference on Harmonization (ICH) Guideline Specification Q6A, Decision Tree: Investigating the need to set acceptance criteria for polymorphism in drug substances and drug products, 1999.
15 P. Di Martino, F. Piva, P. Conflant and A. M. Guyot-Hermann, J. Therm. Anal. Cal., 57 (1999) 95.
16 D. Giron and C. Goldbronn, J. Thermal Anal., 49 (1997) 907 and J. Therm. Anal Cal., 51 (1998) 727
17 J. A. McCauley, R. J. Varsolona and D. A. Levorse, J. Phys. D: Appl. Phys., 26 (1993) B85.
18 M. J. Jozwiakowski, S. O. Williams and R. D. Hathaway, Int. J. Pharm., 91 (1993) 195.
19 S. S. Leung, B. E. Padden, E. J. Munson and D. J. W. Grant, J. Pharm. Sci., 87 (1998) 501.
20 M. Soustelle, Handbook of Powder Technology, J. C. Williams and T. Allen, Eds, Vol. 9, Powder Technology and Pharmaceutical Processes, D. Chulia, M. Deleuil and Y. Pourcelot, Eds, Elsevier 1994. p 27
21 A. K. Galwey, Thermochim. Acta, 355 (2000) 181.
22 D. Giron, Acta Pharm. Jugosl., 40 (1990) 95.
23 J. Han, S. Gupte and R. Suryanarayanan, Int. J. Pharm., 170 (1998) 63.
24 J. Han and R. Suryanarayanan, Thermochim. Acta, 329 (1999) 163.
25 K. Umprayn and R. W. Mendes, Drug Dev. Ind. Pharm., 13 (1987) 653.
26 M. J. Kontny and G. Zografi, Drugs Pharm. Sci., 70 (1995) 387.
27 D. Giron, J. Therm. Anal. Cal., 64 (2001) 37.
28 H. Kitaoka, Chem. Pharm. Bull., 43 (1995) 1744.
29 P. L. Gould, J. R. Howard and G. A. Oldershaw, Int. J. Pharm., 51 (1989) 195.
30 H. Zhu, C. Yuen and D. J. W. Grant, Int. J. Pharm., 135 (1996) 151.
31 H Zhu and D. J. W. Grant, Int. J. Pharm., 139 (1996) 33.

Exhibit - 4

105

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(54) **SODIUM CARBONATE RECRYSTALLIZATION**

(75) Inventors: **Wayne C. Hazen**, Denver, CO (US); **Dale Lee Denham JR.**, Arvada, CO (US); **Rudolph Pruszko**, Green River, WY (US); **David R. Baughman**, Golden, CO (US)

Correspondence Address:
SHERIDAN ROSS PC
1560 BROADWAY
SUITE 1200
DENVER, CO 80202

(73) Assignee: **Environmental Projects, Inc.**

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Related U.S. Application Data

(63) Continuation of application No. 09/239,441, filed on Jan. 28, 1999, now Pat. No. 6,284,005, which is a continuation-in-part of application No. 09/225,805, filed on Jan. 5, 1999, now abandoned.

(60) Provisional application No. 60/072,805, filed on Jan. 28, 1998.

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(51) **Int. Cl.**[7] **C01D 7/00**

(52) **U.S. Cl.** ..... **23/302 T**; 423/421; 423/206.2

(57) **ABSTRACT**

The present invention provides a process for producing sodium carbonate monohydrate crystals by introduction of anhydrous sodium carbonate into a saturated sodium carbonate brine solution under conditions in which sodium carbonate monohydrate formation is favored. As the anhydrous sodium carbonate dissolves, the brine becomes supersaturated resulting in relief of supersaturation by formation of sodium carbonate monohydrate crystals. The process includes controlling supersaturation and its relief to achieve growth of existing sodium carbonate monohydrate crystals rather than nucleation and formation of new sodium carbonate monohydrate crystals. The resulting crystals are separated from insoluble impurities on a size separation basis.

SODIUM CARBONATE RECRYSTALLIZATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. patent application Ser. No. 09/225,805, filed Jan. 5, 1999, and from U.S. Provisional Application No. 60/072,805, filed Jan. 28, 1998.

FIELD OF THE INVENTION

[0002] The present invention relates to the production of sodium carbonate monohydrate crystals from anhydrous sodium carbonate containing impurities.

BACKGROUND OF THE INVENTION

[0003] One common method of purifying a compound is to crystallize the compound in a solution. Methods of crystallization typically involve controlling macroscopic external variables such as evaporating solvent to create supersaturation or adjusting the temperature of the solvent to affect solubility. These crystallization methods are generally directed to achieving maximum solids recovery and/or purification without any regard to the size or shape of the crystals.

[0004] Therefore, there is a need for a crystallization process that can effectively control or influence the ratio of crystal growth to formation of new crystals at low energy costs.

SUMMARY OF THE INVENTION

[0005] The present invention is based on the discovery that sodium carbonate has an unexpectedly high stable supersaturation capacity under appropriate conditions that can be rapidly relieved by the introduction of sodium carbonate monohydrate crystal surfaces to produce relatively large crystals of sodium carbonate monohydrate at high rates of crystal growth. The resulting crystals can be readily separated from insoluble impurities on a size separation basis.

[0006] More particularly, the process of the present invention is for producing sodium carbonate monohydrate from a feedstream which includes anhydrous sodium carbonate and insoluble impurities. The process includes adding the feedstream to a saturated sodium carbonate brine solution under conditions to create supersaturation of at least about 5 g/l. The process further includes processing within parameters that preferentially relieve the supersaturation by rapid growth of existing sodium carbonate monohydrate crystals rather than by nucleation. In this manner, the particle size distribution of crystals is controlled to achieve a desired distribution of crystal size product. The sodium carbonate monohydrate crystals produced by the process are recovered from the saturated brine solution.

[0007] The process can include the use of a high feed rate of at least about 100 grams of feedstream per minute for each liter of solution in the crystallizer. The process can also include relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation by adding sodium carbonate monohydrate seed crystals to the saturated sodium carbonate brine solution. Such seed crystals can be produced by removing sodium carbonate monohydrate crystals from the brine solution and sizing the removed crystals to produce a seed crystal size fraction for reintroduction to the brine solution. In a preferred embodiment, the particle size of the feedstream is less than about 150 mesh and the particle size of the seed crystals is from about 100 mesh to about 150 mesh.

[0008] Relief of supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation can alternatively be achieved by a variety of methods. Such methods can include maintaining a solids content of at least about 40% in the crystallizer, agitating the brine solution at an agitation index of at least about 6, periodically lowering the temperature of the brine solution by at least about 5° C., or pausing feedstream addition at least about 60% of the time of crystallization.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 is a schematic flow diagram of one embodiment of the process of the present invention.

[0010] FIG. 2 is a phase diagram for sodium carbonate.

DETAILED DESCRIPTION OF THE INVENTION

[0011] 1.0 Introduction.

[0012] The present invention is based in part on the discovery that under certain conditions sodium carbonate has an unexpectedly high stable supersaturation capacity that can be rapidly relieved by the introduction of sodium carbonate monohydrate crystal surface to produce relatively large crystals of sodium carbonate monohydrate at high rates of crystal growth. Significant production efficiencies can be attained at high rates of crystal growth. The resulting crystals can be readily separated from insoluble impurities on a size separation basis. Relief of supersaturation is controlled such that crystal formation primarily occurs on existing crystals, rather than occurring as nucleation or growth of newly formed crystals. In this manner, the particle size distribution of crystals is controlled to achieve a desired distribution of product crystal size.

[0013] Processes of the present invention achieve supersaturation of sodium carbonate by adding an anhydrous sodium carbonate feed, e.g., calcined trona, to a saturated sodium carbonate brine solution under temperature conditions in which sodium carbonate monohydrate crystals are formed. Thus, the tendency of the anhydrous sodium carbonate feed to convert to the monohydrate form within the brine solution causes the anhydrous sodium carbonate feed to dissolve, thereby creating supersaturation, before forming sodium carbonate monohydrate. Further, it has been surprisingly found that, under appropriate conditions, sodium carbonate has a supersaturation capacity of about 30 g/l, which is about an order of magnitude higher and more stable in the absence of sodium carbonate monohydrate crystal surfaces than would be expected by one skilled in the art. Therefore, the present invention includes achieving and maintaining high levels of supersaturation near the supersaturation capacity of sodium carbonate to create a high driving force for supersaturation relief which results in rapid crystallization.

[0014] Supersaturation created in this manner is relieved by formation of sodium carbonate monohydrate. Sodium

carbonate monohydrate can form as a result of exceeding the supersaturation limit, which causes primary nucleation resulting in formation of clouds of small nuclei of sodium carbonate monohydrate. The term "supersaturation limit" is used to describe a condition where the level of supersaturation of sodium carbonate in the brine solution is unstable and results in a relatively spontaneous formation of crystals by primary and/or secondary nucleation. This type of supersaturation relief is unproductive because the small nuclei cannot easily be grown to a size large enough to be separated from insoluble impurities. Supersaturation relief can also occur by growth of existing sodium carbonate monohydrate crystals, which is desired in the present invention.

[0015] Processes of the present invention are based on the recognition that since supersaturation is created by the introduction of anhydrous feed, the supersaturation limit can be exceeded in a localized area at the point of introduction of the feed. Therefore, control of supersaturation and its relief in the local environment near where the feed is introduced is critical. The present invention provides the proper thermodynamic environment wherein it is easier to preferentially relieve supersaturation by growth of existing crystals than by nucleation.

[0016] Processes of the present invention include a multi-faceted approach to control local supersaturation and its relief to achieve the desired mechanism for supersaturation relief, preferably the growth of existing crystals. One of the elements of processes of the present invention is to use high agitation to rapidly disperse areas of local high supersaturation to avoid exceeding local supersaturation limits, and to bring the surfaces of existing crystals into contact with such areas of supersaturation. The use of high agitation is quite contrary to standard crystallization practice and technology.

[0017] Processes of the present invention also provide a large amount of available sites for relief of supersaturation on existing crystals so that if the degree of supersaturation in a localized area is approaching the maximum level, i.e., the supersaturation limit, the supersaturation can be quickly relieved by sodium carbonate monohydrate formation on an existing crystal surface instead of by nucleation. Sites for crystallization are provided by the use of seed crystals and/or by maintaining a high solids content in the crystallizer. The present invention can also include pausing during the introduction of feed to allow for dispersion of local areas of very high supersaturation by agitation and/or productive relief of supersaturation on existing crystals in local areas of very high supersaturation. Control of temperature in the crystallizer is also used to control the rate of relief of supersaturation.

[0018] The terms "recrystallization" and "crystallization" are used interchangeably herein and refer to the step of adding anhydrous sodium carbonate to a saturated sodium carbonate brine solution and crystallizing sodium carbonate monohydrate from the saturated brine solution, i.e., the anhydrous sodium carbonate dissolves in the saturated brine solution, forms a supersaturated solution which then causes growth of sodium carbonate monohydrate crystals because the temperature of the saturated brine solution is in the range of sodium carbonate monohydrate stability. A "saturated brine solution" refers to a solution which is saturated with sodium carbonate.

[0019] 2.0 Feedstream Composition and Introduction.

[0020] 2.1 Composition.

[0021] As noted above, a feedstream of the present invention comprises anhydrous sodium carbonate. For example, processes of the present invention can be used for purifying anhydrous sodium carbonate (such as calcined trona) containing impurities or for producing dense soda ash from light soda ash. Moreover, the present invention is particularly well adapted for use with feedstreams having high contents of insoluble impurities. In particular, the present invention can be used for purifying anhydrous sodium carbonate feedstreams in which impurities are included within the crystal structure even when the particles are finely ground. Thus, although the present invention can be used with a substantially pure anhydrous sodium carbonate, the present invention is particularly suitable for use with feedstreams having greater than about 15% by weight insoluble impurities, and even more particularly, having greater than about 30% by weight insoluble impurities. Although any anhydrous sodium carbonate including synthetic anhydrous sodium carbonate or calcined trona can be used, processes of the present invention will now be described in detail in reference to purification of calcined trona containing impurities and FIG. 1. And as such, the terms "calcined trona" and "anhydrous sodium carbonate" will hereinafter be used interchangeably.

[0022] 2.2 Size.

[0023] As noted, supersaturation is achieved by adding calcined trona to a saturated brine solution under temperature conditions at which sodium carbonate monohydrate forms. Thus, the calcined trona dissolves, thereby creating supersaturation and also releasing impurities, before forming sodium carbonate monohydrate. The rate and completeness of calcined trona dissolving in a saturated brine solution is determined by, among other factors, its particle size. Since the presence of undissolved hydrated calcined trona can compete with seed crystals of monohydrate as a substrate for relieving supersaturation, the calcined trona added to the saturated brine solution should dissolve substantially completely to ensure that the majority of supersaturation relief is by growth of seed crystals, not by growth on undissolved anhydrous feed, and to ensure that at least a portion of impurities present within the crystal lattice of sodium carbonate is released. If the feedstream of calcined trona dissolves only partially, the remaining particles can have undesired effects such as forming agglomerates or relieving supersaturation to form mixed particles of calcined trona and sodium carbonate monohydrate. Thus, to ensure a substantially complete dissolution of the particles the particle size of calcined trona ore in the feedstream, whether in a slurry form or a dry form, is preferably less than about 100 mesh (Tyler), more preferably less than about 150 mesh, still more preferably less than about 200 mesh, and most preferably less than about 400 mesh. It should be appreciated that when the particle size of calcined trona ore is within the above described range, any insoluble impurities present in the calcined trona ore will also be within the confines of the above described particle size.

[0024] The above particle size limitations allow calcined trona ore to dissolve relatively quickly and completely in a saturated brine solution in the crystallizer **10**.

[0025] 2.3 Feed Rate.

[0026] As noted above, it has been surprisingly found that, under appropriate conditions, sodium carbonate has a supersaturation capacity of about 30 g/l, which is about an order of magnitude higher than would be expected by one skilled in the art. Therefore, the present invention includes achieving and maintaining high levels of supersaturation near the supersaturation capacity of sodium carbonate to create a high driving force for supersaturation relief which results in rapid crystallization. For example, the process includes creating supersaturation of at least about 5 g/l, more preferably at least about 10 g/l, more preferably at least about 20 g/l and up to 30 g/l. Supersaturation can be calculated within a localized volume in a crystallizer or within the entire volume of a crystallizer. For example, supersaturation can be calculated as follows. A volume of saturated brine, which can include sodium carbonate monohydrate crystals and calcined trona, can be withdrawn from a crystallization vessel through a screen and filter to remove solid materials. Water in the withdrawn brine is then evaporated and the amount of sodium carbonate per volume of brine can be gravimetrically determined. The amount of sodium carbonate in excess of the known solubility level is the amount of supersaturation. Because of the high capacity for supersaturation and the very rapid relief of supersaturation, the rate of introduction of the feedstream or feed rate can be very high in the present invention. More particularly, the feed rate can be at least about 100 grams per minute for each liter of volume (g/l/min), preferably at least about 200 g/l/min, more preferably at least about 400 g/l/min, and even more preferably at least about 800 g/l/min. These feed rates are significantly higher than feed rates expected to be useful by one skilled in the art and those utilized by previous crystallization methods.

[0027] 2.4 Method of Introduction.

[0028] The feedstream, which includes anhydrous sodium carbonate, can be introduced to the saturated brine solution using any of the known methods including by a direct injection, a screw feeder and gravity. The feedstream can be a slurry of anhydrous sodium carbonate in a saturated brine solution or dry anhydrous sodium carbonate.

[0029] A dry anhydrous sodium carbonate feedstream must be dispersed and dissolved quickly in the saturated brine solution, otherwise particles may become hydrated and form agglomerates. If the particles in the feedstream are too coarse, they will not dissolve completely, thus possibly reducing the purity of the product; therefore, the particle size of the feedstream should be within the range discussed above. On the other hand, fine particles tend to "float" on top of the saturated brine solution and become hydrated and form agglomerates. Generally, at a high feedstream addition rate discussed above, it is difficult to quickly disperse and dissolve the anhydrous sodium carbonate into the saturated brine solution. It has been found by the present inventors that these problems can be overcome by using high agitation, as discussed below.

[0030] One can also avoid these problems, such as agglomerate formation and floatation of fines, by adding a feedstream of anhydrous sodium carbonate in a slurry form. A slurry of anhydrous sodium carbonate can be prepared by mixing calcined trona ore and the saturated sodium carbonate solution at atmospheric pressure and transferring the mixture into a slurry feedstream vessel having a desired temperature at increased pressure. Alternatively, calcined trona ore and the saturated sodium carbonate solution can be fed directly into the slurry feedstream vessel at a desired temperature and pressure to form a slurry feedstream. At a temperature above the transition temperature of sodium carbonate monohydrate to anhydrous sodium carbonate (108.5° C. for a pure system of water and sodium carbonate at one atmosphere of pressure), solids in the slurry include anhydrous sodium carbonate crystals and insoluble materials originally present in the calcined trona ore. It is recognized by those skilled in the art that the transition temperature can be adjusted by various means, including by adding sodium chloride.

[0031] One method of preparing a slurry of feedstream involves mixing anhydrous sodium carbonate with a saturated sodium carbonate brine solution at a temperature at least above the transition temperature of anhydrous sodium carbonate to sodium carbonate monohydrate preferably at least about 5° C. above the transition temperature, and more preferably at least about 2° C. above the transition temperature. A "transition temperature" refers to a temperature at which stable anhydrous sodium carbonate changes its morphology to stable sodium carbonate monohydrate. See for example, line A in FIG. 2, the transition of anhydrous to sodium carbonate monohydrate. Line B in FIG. 2 represents the transition temperature between sodium carbonate heptahydrate and sodium carbonate monohydrate. It will be appreciated that this step of producing a slurry feedstream must be conducted at above atmospheric pressures and must use a feeding mechanism that maintains a continuous pressure seal between the environment of the feed slurry and of the brine solution.

[0032] It should be further appreciated that this method of introduction of anhydrous sodium carbonate can be used for processing in any aqueous solution.

[0033] 2.5 Calcination.

[0034] When trona is used as a feedstream, it must be converted into anhydrous sodium carbonate by calcination prior to being added to the saturated brine solution. Trona can be calcined using any known calcination technology. For example, calcination can be conducted with a fluidized bed calciner. When a fluidized bed calciner is used to calcine trona ore, the trona ore is comminuted and is generally separated into three size ranges: 6×20 mesh, 20×100 mesh and −100 mesh. Each size can then be separately calcined in a fluidized bed calciner. Calcined trona is then combined and comminuted to provide a feedstream having above mentioned particle size. Further, trona in the feedstream can be calcined using indirect heat calcination as disclosed in commonly assigned U.S. patent application Ser. No. 09/151,694 that was filed on Sep. 11, 1998, which is incorporated herein by reference in its entirety.

[0035] 3.0 Crystallization.

[0036] As shown in FIG. 1, a feedstream comprising calcined trona is added to a saturated sodium carbonate brine solution in a crystallizer **10** to generate supersaturation within the saturated brine solution. The feedstream and the saturated brine solution can be added simultaneously and/or sequentially. The present method controls crystallization conditions so that relief of supersaturation created by intro-

duction of the anhydrous sodium carbonate feed primarily occurs on existing sodium carbonate monohydrate crystals rather than by nucleation.

[0037] 3.1 Seed Crystals.

[0038] In one embodiment of the present invention, supersaturation relief on existing crystals is achieved by the introduction of seed crystals of sodium carbonate monohydrate to the crystallizer **10**. Thus, in contrast to other crystallization methods in which a major amount of crystal growth is by nucleation or on crystals newly formed by nucleation, processes of this particular embodiment of the present invention provide supersaturation relief primarily by growing seed crystals to crystals that are large enough to be separable from insoluble impurities on a size separation basis. Moreover, the size distribution of the product crystal population can also be controlled by adding seed crystals of a desired particle size range. By the use of seed crystals in this manner, crystal growth is productive in the sense that it occurs on crystals which will be large enough to recover on a size separation basis, rather than occurring on small particles which cannot practically be grown large enough to be separated from insoluble impurities.

[0039] Seed crystals can be prepared separately or can be prepared as a part of the process flow of the present crystallization process, as described below. For example, seed crystals can be produced by removing crystals from the crystallizer and sizing the crystals to produce a seed crystal size portion for reintroduction to the crystallizer. Furthermore, at least a portion of the product of the present process can be comminuted, e.g., ground, to a desired seed crystal size and used as a source of the seed crystals.

[0040] In a batch process, the seed crystals are typically added prior to the addition of the feedstream, whereas in a continuous process, the seed crystals are typically added continuously during the operation of the present invention. As used in this invention, a "continuous addition" can include both non-interrupted addition as well as interval addition throughout the process as needed.

[0041] The particle size of the seed crystals is selected such that a product having an acceptable particle size range is produced. For example, the seed crystals need to be large enough that, given the amount of growth achieved in a given crystallization, the resulting product crystals will be large enough to be separable from insoluble impurities on a size separation basis. Preferably, the particle size of the seed crystals is in the range from about 100 mesh (Tyler) to about 400 mesh, more preferably from about 100 mesh to about 200 mesh and most preferably from about 100 mesh to about 150 mesh. Alternatively, the range of the particle size of seed crystals is about 2 standard sieve sizes or less. A "standard sieve size" is denoted by increasing or decreasing the opening in a sieve size by the ratio of the square root of 2 or 1.414, i.e., taking a screen opening and multiplying or dividing it by the square root of 2 or 1.414. The seed crystal size range utilized is determined by the desired product particle size range. For example, a narrow seed crystal size range results in a narrow product particle size range.

[0042] The amount of seed crystals and feedstream added to the saturated brine solution depends on the volume of the saturated brine solution in the crystallizer **10**. However, as noted below, the total amount of seed crystals and feedstream added to the saturated brine solution typically results in a monohydrate slurry having a solids content in accordance with the parameters discussed below. As used herein, a "monohydrate slurry" refers to a saturated brine solution containing solid sodium carbonate monohydrate crystals. Such a high solids content ensures that sufficient surface area is available for supersaturation relief on existing crystals before any significant amount of nucleation can occur in the brine solution. In another embodiment, for the above mentioned particle sizes of seed crystals and products, the ratio of seed crystals added to the feedstream added is at least about 1:1 by weight, preferably at least about 5:1 by weight, and more preferably at least about 10:1. Generally, about an equal amount of the solids content by weight of the seed crystals and the feedstream is added to the saturated brine solution.

[0043] 3.2 Solids Content.

[0044] A further aspect of the present invention to control supersaturation relief on existing crystals of sodium carbonate monohydrate is to maintain a high solids content in the crystallizer **10**. In this manner, if the degree of supersaturation in a localized area is approaching the maximum level, supersaturation can be quickly relieved by sodium carbonate monohydrate formation on an existing crystal surface instead of by nucleation. As will be appreciated, the solids content in the crystallizer **10** depends on a variety of factors including the amount of seed crystals added and the amount and solids density of the feedstream added to the saturated brine solution, as well as the desired density for optimal crystallizer operation. These variables are controlled such that the monohydrate slurry has a solids content of at least about 17% by weight, more preferably at least about 35% by weight, even more preferably at least about 40% by weight, and most preferably at least about 60% by weight. Alternatively, the particle surface area density, i.e., the total amount of surface area of crystals present per volume, is at least about 40 cm^2/ml, preferably at least about 75 cm^2/ml, more preferably at least about 95 cm^2/ml, and most preferably at least about 125 cm^2/ml.

[0045] 3.3 Crystallizer Agitation.

[0046] As noted above, the supersaturation limit of the brine solution can be exceeded in a small localized area because supersaturation is created by the anhydrous feed dissolving to in the saturated brine solution. Therefore, control of supersaturation and its relief in the local environment, for example, by sufficiently high agitation, where the feed is introduced is critical. The term "local" refers to the immediate environment of a small portion of the brine solution in the crystallizer **10** and not the overall amount of sodium carbonate within the total volume of the crystallizer **10**.

[0047] Thus, the term "local supersaturation limit" refers to the degree of supersaturation within any volume of a crystallizer in which formation of a crystal nucleus by primary and/or secondary nucleation can occur. It will be appreciated therefore, that within the crystallizer **10**, while the average degree of supersaturation can be below the supersaturation limit, a localized region of high supersaturation can occur and thereby exceed the supersaturation limit in that localized region, resulting in undesired nucleation. To reduce or avoid this undesired nucleation, processes of the present invention can also include control of local super-

saturation by using high agitation to rapidly disperse areas of high local supersaturation. High agitation brings the surfaces of existing crystals into contact with areas of high local supersaturation and thereby, increases the effective net surface area available for supersaturation relief by increasing the probability of an existing crystal particle coming into contact with an area of local high supersaturation area. One measure of agitation is a qualitative agitation index as described below. The term "agitation index" refers to a scale of agitation in a crystallizer. An agitation index of 0 means that there is no perceptible stirring or movement within the mixture, whereas an agitation index of 10 means the mixture in the crystallizer is stirred at a very high and rapid degree of mixing and agitation such that degradation or mechanical fracturing of crystals occurs. Table 1 shows the qualitative characteristics of the 0-10 agitation index.

TABLE 1

Agitation Index	Description
1	static, no movement or mixing
2	
3	turnover of slurry, but not all solids held in suspension
4	
5	mild turnover of slurry with all solids held in suspension
6	
7	rolling surface with quick turnover and quick absorption of dry material into mass of slurry.
8	
9	violent turbulent movement of all slurry in entire vessel
10	degradation or mechanical fracturing of material

[0048] Preferably, the mixture in the crystallizer **10** is stirred at an agitation index of at least about 4, more preferably at least about 7, still more preferably at least about 8, and most preferably at least about 9.

[0049] Evidence of insufficient agitation can be readily determined by examining crystal structures of the product. The product resulting from insufficient agitation may include the presence of agglomerates, long needle-like crystals or dendrites.

[0050] In contrast to other methods, agitation in the present invention preferably does not produce a typical vortex associated with using a single propeller non-baffled agitation system. In a particular embodiment of the present invention, agitation of the monohydrate slurry is achieved by using at least two propellers having a counter pitch or other suitable agitation methods including using an attrition scrubber and any other impeller configurations which achieve the desired agitation index discussed above.

[0051] Preferably, the solution is agitated at greater than about 10 horsepower/1000 gallons (hp/1000 gal), more preferably at least about 100 hp/1000 gal, and most preferably at least about 200 hp/1000 gal. Alternatively, when a propeller system is used for agitating the monohydrate slurry, the propeller tip speed is at least about 8 feet/sec (ft/sec), preferably at least about 10 ft/sec, and more preferably at least about 22 ft/sec.

[0052] Adequate agitation can be achieved by use of any vessel providing agitation as described above. For example, such a vessel can include a one impeller system; two impellers having counter pitch, such as is used in an attrition scrubber; multiple impellers having alternating counter pitch in the crystallizer **10**, or other configurations providing the desired agitation index. Thus, in such agitation, it is important to create a rapid exchange of solid particles and the solution portion of the saturated brine solution.

[0053] It should be noted, however, that while high agitation is beneficial, it should be conducted in a manner without a significant amount of impact destruction. The term "impact destruction" refers to a process where two or more particles collide and result in a particle size reduction for one or more particles. 3.4 Temperature Control.

[0054] As discussed above, the temperature of the saturated brine solution is maintained such that the formation of sodium carbonate monohydrate is favored as determined by the phase diagram, as shown in FIG. 2. The temperature of the saturated brine solution in the crystallizer **10** is maintained at between about 40° C. and the transition temperature of anhydrous sodium carbonate to sodium carbonate monohydrate to ensure formation of sodium carbonate monohydrate, preferably between about 70° C. and the transition temperature of anhydrous sodium carbonate to sodium carbonate monohydrate, more preferably between about 90° C. and the transition temperature of anhydrous sodium carbonate to sodium carbonate monohydrate, and most preferably between about 98° C. and the transition temperature of anhydrous sodium carbonate to sodium carbonate monohydrate.

[0055] It has been discovered by the present inventors that keeping the temperature in the crystallizer as close as possible to but below the transition temperature of sodium carbonate monohydrate to anhydrous sodium carbonate reduces the "drive", i.e., the rate of conversion, of anhydrous sodium carbonate in the feedstream to change morphologically to sodium carbonate monohydrate. This discovery allows the processes of the present invention to be controlled easily and results in larger, better formed crystals as discussed in detail below.

[0056] To maintain a substantially constant temperature of the saturated brine solution within the crystallizer **10**, the temperature difference between the saturated brine solution and the feedstream should be small enough such that no significant cooling or heating of the saturated brine solution occurs during the addition of the feedstream. Preferably, the temperature difference between the feedstream and the saturated brine solution is about 20° C. or less, more preferably about 15° C. or less, and most preferably about 10° C. or less.

[0057] In another embodiment, the temperature of the dry feed particles in the feedstream is at least about 95° C., preferably at least about 120° C., and more preferably at least about 150° C.

[0058] Still in another embodiment, freshly calcined trona can be added directly to the crystallizer **10** along with a saturated brine solution to maintain the temperature of the mixture in the crystallizer **10** as disclosed above. Freshly calcined trona has a high particle temperature as it comes out of the calciner. By adding a freshly calcined, i.e., hot, trona to the saturated brine solution, the amount of energy and the cost required to maintain the mixture at the above described temperature can be significantly reduced compared to processes where calcined trona is reheated prior to being added

to the saturated brine solution or where the saturated brine solution is at a higher temperature then the feedstream.

[0059] As noted above, the present invention includes controlling supersaturation relief to achieve crystal growth on existing crystals of sodium carbonate monohydrate rather than initiating nucleation. A further aspect of the present invention is the control of supersaturation relief by modifying the temperature of the crystallization solution in a temperature cycling process to control the amount of fines as discussed in more detail below.

[0060] 3.5 Feed Addition Pause.

[0061] Crystal formation in the form of nucleation occurs when the local supersaturation level exceeds the supersaturation limit. When the rate of supersaturation generation exceeds the rate of supersaturation relief, eventually the supersaturation level somewhere in the crystallizer will exceed the supersaturation limit resulting in nucleation (sometimes referred to as "snowing-out"). Thus, to prevent the supersaturation level in a local area from exceeding the supersaturation limit, the addition of the feedstream to the saturated brine can be stopped briefly or intermittently to decrease the supersaturation level by allowing growth of existing crystals. More particularly, the break or pause in feedstream addition can be conducted at least about 60% of the time of crystallization. More preferably, the pause can be conducted at least about 30%, and most preferably, at least about 5% of the time of crystallization. For example, if the pause is 10% of the crystallization time, the feedstream would be paused 6 minutes during every hour of operation. It should be noted that when pausing is used, it is preferably conducted frequently, such as by switching between feeding and pausing every several minutes, or about every five minutes.

[0062] 3.6 Crystal Growth Rate.

[0063] It is believed that the conventional recommended crystal growth rates for good crystal quality is from about 2 microns/minute to about 5 microns/minute. A "good crystal quality" refers to crystals which are generally hexagonal, roughly equi-dimensional, slightly elongated with an aspect ratio of W×L×H of about 1:1.5:0.75. See for example, Goldschmidt, Atlas der Krystallformen, p. 128 (Carl Winters Universitatbuchhandlung, Heidelberg 1922), which is incorporated herein by reference in its entirety. The crystal growth rate of the present invention is significantly higher than the conventional recommended crystal growth rates while providing a similar crystal quality. Preferably the crystal growth rate of the present invention is at least about 5 microns/minute, more preferably at least about 10 microns/minute, and most preferably at least about 20 microns/minute. It has been found that the crystal growth rate of the present invention does not decrease significantly by having a higher solids to saturated brine solution ratio. However, it is believed the crystal growth rate does depend on the size of the seed crystals. The reason for the higher growth rate of coarser crystals is the mass transfer of sodium carbonate monohydrate crystals from finer crystals to coarser crystals. The operation of this mechanism at high crystal growth rates such as in the current invention is contrary to what would be expected by one of skill in the art.

[0064] An average crystal growth rate can be determined by a variety of methods including by a statistical analysis of a sample product crystal. For example, the average crystal growth rate can be obtained by dividing the total amount of crystal growth in the sample by the total crystallization time and the total crystal surface area.

[0065] 3.7 Nucleation Control.

[0066] Processes of the present invention involve controlling crystallization conditions as discussed in Sections 3.1-3.6 to provide conditions for relieving the supersaturation in the crystallizer **10** by growing existing crystals rather than by nucleation. If a significant amount of primary and/or secondary crystal nucleation occurs in the crystallizer **10**, then a large amount of fines is generated. Production of fines limits productive crystal growth because fines have a large ratio of surface area to volume compared to larger crystals. Since fines are small, even significant growth of them will not make them large enough to be separated from insoluble impurities on a size separation basis. Therefore, such growth is unproductive. However, it should be appreciated that some formation of new crystals by nucleation may be necessary when the process includes generating new seed crystals. Thus, processes of the present invention may be used to allow formation of new crystals by nucleation in a relatively controlled amount for this purpose.

[0067] Thus, in a further aspect of the present invention, the amount of solids in the saturated sodium carbonate brine formed by primary and/or secondary nucleation in the crystallizer **10** is maintained at about 10% by weight or less of the total sodium carbonate solids in the saturated brine, more preferably at about 5% by weight or less of the total sodium carbonate solids in the saturated brine, still more preferably at about 1% by weight or less of the total sodium carbonate solids in the saturated brine, and most preferably at about 0.5% by weight or less of the total sodium carbonate solids in the saturated brine. For example, given a defined crystal population at a point in time, one can determine whether new crystals have been formed by primary and/or secondary nucleation by determining whether the crystal population at a later point in time has smaller crystals or an increase in smaller crystals compared to the earlier point in time. One can also determine whether new crystals have been formed by primary and/or secondary nucleation by identifying whether a drop in yield of +200 mesh crystals occurs. One can also determine whether new crystals have been formed by primary and/or secondary nucleation in a continuous process by identifying fluctuations in the size distribution of crystals at a point in time at which a stable population would be expected.

[0068] In a further aspect of the invention, control of the crystallization conditions can maintain or reduce the portion of the solid material in the monohydrate slurry which has a small particle size. More particularly, the processes of the present invention can include maintaining the amount of solids in the monohydrate slurry having a particle size of less than about 400 mesh at less than about 10% by weight of the total sodium carbonate solids in the monohydrate slurry, more preferably at less than about 2% by weight of the total sodium carbonate solids in the monohydrate slurry, and most preferably at less than about 0.5% by weight of the total solids in the monohydrate slurry.

[0069] 3.8 Agglomerate/Aggregate Control.

[0070] Processes of the present invention for controlling crystallization conditions as discussed above in Sections

3.1-3.6 can also substantially avoid formation of a significant amount of agglomerates and/or aggregates. If a significant amount of agglomerates and/or aggregates are formed, the purity of any recovered product may be significantly decreased because insoluble and soluble impurities can be trapped within the agglomerates and aggregates. Thus, in one aspect of the present invention, the crystallization process is conducted by maintaining the amount of solids in the monohydrate slurry in the form of agglomerates and/or aggregates at about 10% by weight or less of the total sodium carbonate solids in the monohydrate slurry, more preferably at about 5% by weight or less of the total sodium carbonate solids in the monohydrate slurry, and most preferably at about 0.5% by weight or less of the total sodium carbonate solids in the monohydrate slurry.

[0071] As used herein, the term "aggregate" refers to a collection of particles or crystals in clusters or clumps. The particles can be held together as a result of the attraction of weak forces, such as van der Waals forces. The term "agglomerate" refers to particles or feed held together by forces stronger than van der Waals forces, which can be formed, for example, by anhydrous feed particles which are not fully dissolved acting as a site for crystallization of monohydrate crystals, or anhydrous feed that was not dispersed or dissolved absorbing water to hydrate.

[0072] 3.9 Crystallizer Pressure.

[0073] The crystallizer **10** can be equipped to be operated at a wide range of pressure. In one embodiment, the crystallizer **10** is operated at atmospheric pressure. In another embodiment, the crystallizer **10** can be operated at any desired pressure of up to about 35 pounds per square inch (psia), more preferably up to about 30 psia, and most preferably up to about 25 psia. Unless otherwise noted, the pressure refers to an absolute pressure and not a relative, i.e., gauge, pressure. Whether operated under atmospheric pressure or higher pressure, the temperature of the saturated brine solution in the crystallizer **10** is maintained to favor the formation of sodium carbonate monohydrate. When the crystallizer **10** is operated under pressure, the introduction of the feedstream is preferably at a similar pressure. A pressurized pump such as a Fuller Kinyon pump (not shown) or any other type of pump which can achieve a desired pressure can be used to introduce the dry or slurry feedstream into the crystallizer **10**. However, it should be recognized that the feedstream can be at a variety of pressures independent of the crystallization itself.

[0074] 3.10 Multiple Crystallization Vessels.

[0075] In a further embodiment, the crystallization is conducted in a series of two or more crystallizers. In this manner, the initial feedstream can be used to generate fines by nucleation in a first crystallizer. The fines are then transferred to a second crystallizer and used as seed crystals for subsequent crystallization where they are grown to a larger size. Thus, in either the second or some subsequent crystallizer, the crystals are grown large enough for a size separation from insoluble impurities. By using a multiple tank system which allows successive crystal growth conditions, the need for a separate seed crystals as discussed above in Section 3.1 can be eliminated.

[0076] 4.0 Dispersion.

[0077] Referring again to FIG. 1, at least a portion of sodium carbonate monohydrate crystals and saturated brine solution are separated from the crystallizer **10**. The sodium carbonate monohydrate product is eventually recovered in a product separator **18**, preferably on a size separation basis. However, as noted above, crystallizations are conducted at high solids content, such as at least about 17% solids content. Product separation with such a viscous mixture can be difficult. Therefore, as shown in FIG. 1, the separation process can also include transferring at least a portion of the monohydrate slurry from the crystallizer **10** to a dispersion tank **14** to decrease the solids content of the monohydrate slurry in order to, inter alia, facilitate the separation process. It should be noted that the dispersion step should not dilute the solution below saturation. Otherwise, product loss can occur by dissolution of product. Typically, a saturated brine solution having a substantially negligible solids content is added to the dispersion tank **14** to reduce the solids content of the monohydrate slurry to about 25% by weight or less, more preferably to about 15% by weight or less solids content, and most preferably to about 10% by weight or less solids content.

[0078] 5.0 Recovery.

[0079] The present invention also includes recovering product from the monohydrate slurry. The recovery process can include separating a particular particle size range of sodium carbonate monohydrate crystals from the monohydrate slurry. Size separation is conducted in a separation apparatus **18** and can be affected by any of the appropriate known methods. For example, screening, cyclones (such as hydrocyclones) or elutriation can be used.

[0080] The sodium carbonate monohydrate crystal product which is recovered typically has a particle size of greater than at least about 150 mesh. Preferably, the product has a particle size of greater than at least about 100 mesh, and more preferably greater than at least about 80 mesh. More particularly, the size cutoff for product recovery has to be at least as large as or larger than the particle size of the feed so that insoluble impurities initially in the feed are not recovered with product.

[0081] Separation of sodium carbonate monohydrate crystals is generally conducted by screening or cycloning and avoiding drying of the crystals. Drying of the crystals at this stage may result in cementing, or agglomerate formation, of crystals and/or impurities, thereby reducing the purity of the product (but not the purity of the crystals). Drying of the crystals can be avoided or reduced by reducing or eliminating evaporation of the solvent, or by covering the screen with solvent or solvent vapors to maintain solvent saturation. Alternatively, a pressurized and/or submerged size separation process can be used, which ensures that local evaporation of solvent is minimized or eliminated.

[0082] Once sodium carbonate monohydrate crystals are separated from the saturated brine solution, they can be dehydrated (i.e., dried) using known techniques to provide anhydrous sodium carbonate.

[0083] The purity of crystals produced by the processes of the present invention is at least about 99%, more preferably at least about 99.5% and most preferably at least about 99.8%. The term "purity of product" refers to the overall purity of the product and can include impurities which can be present on the surface of the crystals or which can be trapped within agglomerates. The term "purity of crystals"

refers to the presence or lack of impurities within the crystal lattice structure. In other words, the purity of product refers to the purity of a particular batch of the product produced by the process of the present invention, whereas the purity of crystals refers to the purity of crystals within the product.

[0084] 5.1 Physical Property of the Product.

[0085] Unlike some of the current crystallization processes, the process of the present invention does not utilize a crystal modifier to affect the crystal shape of the product. The majority of the product is block-like in shape, as discussed above, and is surprisingly resistant to abrasion. Preferably at least about 55% of the particles in the product is block-like in shape, more preferably at least about 75%, and most preferably at least about 95%.

[0086] It is believed that these block-like crystal are responsible for a high bulk density observed in the product of the present invention. The product of the present invention has a poured bulk density of at least about 0.95 g/ml, preferably at least about 1.0 g/ml, and more preferably at least about 1.1 g/ml. In another embodiment of the present invention, the product has a packed density of at least about 1.0 g/ml, preferably at least about 1.1 g/ml, and more preferably at least about 1.2 g/ml.

[0087] The product of the present invention also has a lower amount of dust, i.e., fines, than crystals produced by the conventional crystallization processes. Without being bound by any theory, this low amount of dust present in the product is believed to be due to a variety of novel features of the present invention including the use of seed crystals, the relief of supersaturation primarily by crystal growth rather than by formation of new crystals, and the block-like shape of the product crystals which is more resistance to abrasion than other crystal shapes.

[0088] The product of the present invention has improved flowability and decreased bridging compared to products produced by conventional methods. It is believed the block-like crystal shape and the absence of fine crystals produces higher flowability and lower bridging in storage vessels. This block-like crystal shape has smoother crystal surfaces compared to other crystal shapes such as jack-like or needle like crystal shapes. Without being bound by any theory, it is believed that the smooth surface of block-like shaped crystals has a lower frictional force than other crystal shapes. In addition, larger particles have a reduction in specific surface area, and thereby the cohesiveness between particles is reduced.

[0089] 6.0 Seed Separation.

[0090] Again referring to FIG. 1, an undersize fraction of the monohydrate slurry from the product separator **18** can be transferred to a seed crystal separation apparatus **22** to separate at least a portion of crystals from the undersize fraction for use as seed crystals. The undersize fraction will include sodium carbonate monohydrate crystals smaller than the size cutoff in the product separator **18** and insoluble impurities. To effectively produce a seed crystal population, the undersize fraction from the product separator **18** must include an upper size range which is larger than the size of the insoluble impurities. In this manner, by conducting a size separation in the seed separator **22**, seed crystals which are free of insoluble impurities can be recovered as an oversize fraction, and the insoluble impurities with small sodium carbonate monohydrate crystals are generated as the undersize fraction. The seed crystal separation can be accomplished by any of the appropriate known methods as discussed above. As discussed above, a seed crystal population produced in this manner is then used in a crystallizer.

[0091] Other methods of producing seed crystals include the following: wet comminution of monohydrate crystals; dry comminution of monohydrate crystals; dissolution of a portion of monohydrate crystals by water addition; dissolution of crystal in a slurry by cooling the slurry to increase the solubility of sodium carbonate in the brine; and controlled cooling of a slurry of anhydrous sodium carbonate.

[0092] 7.0 Thickening.

[0093] The undersize fraction from the seed separator **22**, containing saturated brine solution, insoluble impurities and/or sodium carbonate monohydrate crystals which are smaller than the desired seed crystal size is then further processed. As shown in FIG. 1, the undersized fraction from the seed separator **22** is transferred to a thickener **26** to allow for settling of insoluble impurities. The settled insoluble impurities are then purged from the system, while the clear overflow and/or the resulting clarified saturated brine solution can be recycled and reused. It should be appreciated that during the settling process, the brine solution can be diluted with water or a non-saturated brine solution to dissolve fine sodium carbonate monohydrate crystals which may be present. Furthermore, makeup water can be added as required by the overall mass balance of the system.

[0094] Prior to being purged from the system, settled insoluble impurities can be further concentrated, e.g., by filter press, to recover at least a portion of the saturated brine solution. In addition, the clear overflow and/or the clarified saturated brine solution can be further clarified by filtration to remove any fine insoluble impurities that may be present.

[0095] When the saturated brine solution is reused, it is desirable that the temperature of the saturated brine solution in the thickener is kept at no more than about 20° C. different than the temperature of the saturated brine solution in the crystallizer tank to minimize the energy cost of reheating the saturated brine solution from the thickener. Preferably, the difference in temperature between the saturated brine solution and the saturated brine solution in the crystallizer tank is about 15° C. or less, more preferably about 10° C. or less, and most preferably about 5° C. or less.

[0096] 8.0 Bicarbonate Control.

[0097] It has been found that the crystal size and/or the shape can be affected by the presence of sodium bicarbonate in the saturated brine solution. Therefore, the process of the present invention can further include maintaining the concentration of sodium bicarbonate below about 10 g/l in the saturated brine solution which is added to the crystallizer **10**, more preferably below about 5 g/l, and most preferably about 0 g/l. Larger sodium carbonate crystals can be grown in crystallization processes when the amount of bicarbonate present in the brine solution is maintained within these limits. One method of controlling the sodium bicarbonate level in the saturated brine solution is disclosed in a commonly assigned, U.S. patent application Ser. No. 09/167,627, filed on Oct. 6, 1998, which is incorporated by reference herein in its entirety.

[0098] A further advantage of the present process which has been recognized is that, in the absence of bicarbonate, crystals which are grown have a more beneficial shape, e.g., a well-formed block-like shape. In contrast, crystals grown in the presence of significant amounts of sodium bicarbonate can have a needle-like, dendritic or jack-shaped structure and/or cloudy centers. Thus, crystals produced in accordance with the present invention, having a more compact and block-like shape, produce a material having a higher bulk density and a lower friability than those produced in the presence of a relatively large amount of bicarbonate.

[0099] In a preferred embodiment of the present invention, a sufficient amount of base is used to reduce the concentration of sodium bicarbonate to within the parameters discussed above. Preferably, after neutralizing any initial sodium bicarbonate in the crystallizer, base is added to the crystallization process to maintain a concentration of at least about 0.75 mole/l of equivalent base, more preferably at least about 0.50 mole/l, and most preferably at least about 0.25 mole/l. When sodium hydroxide is used as the base, after neutralizing any initial sodium bicarbonate in the crystallizer, the amount of sodium hydroxide used is preferably at least about 6 g/l, more preferably at least about 4 g/l, and most preferably at least about 2 g/l.

[0100] 9.0 Aging.

[0101] Processes of the present invention can also include transferring at least a portion of the monohydrate slurry from the crystallizer **10** and/or at least a portion of the screened saturated brine solution into an aging apparatus (not shown). The aging apparatus allows growth of at least a portion of the crystals in the saturated brine solution by dissolving at least a portion of fines and then promoting crystal growth by relieving the supersaturation in the form of a crystal growth, i.e., some mass of fine particles is converted to coarse particles by a process of dissolving and recrystallizing.

[0102] As used in this invention, "aging" refers to a process of dissolving some of the small crystals present in the saturated brine solution and relieving at least a portion of the supersaturation by growth on existing crystals. The aging can be a natural equilibrium phenomena where crystals are constantly being dissolved and recrystallized or it can be achieved by diluting and concentrating the saturated brine solution or simply by a temperature cycling process. The aging process can be used to produce seed crystals or to increase the amount and/or the size of the product. For example, when the temperature of the saturated brine solution in the crystallizer **10** is from about 80° C. to about 90° C., it has been observed that by allowing the resulting saturated brine solution to stir or stand for an additional about 10 to about 15 minutes after the addition of the feedstream and/or the seed crystals, the amount and/or the size of larger sodium carbonate monohydrate crystals can be significantly increased. This phenomena occurs at faster rates at increased temperatures.

[0103] The temperature cycling process involves reducing the temperature of the saturated brine solution at least about 10° C., more preferably at least about 20° C., and most preferably at least about 40° C. Alternatively, the temperature of the saturated brine solution is reduced to less than about 70° C., more preferably less than about 60° C., and most preferably less than about 50° C., but always above 35° C., the top of stability range for sodium carbonate decahydrate. As FIG. 2 shows, the solubility of sodium carbonate increases as the temperature is reduced. Thus, reducing the temperature of the saturated brine solution dissolves at least a portion of the sodium carbonate monohydrate crystals. It should be appreciated that while some fines may be completely dissolved, some larger crystals may also be partially dissolved during the temperature cycling process. When the temperature of the saturated brine solution is increased, the solubility of sodium carbonate decreases as shown in FIG. 2. This reduction in solubility causes relief of supersaturation of the brine solution by growth of existing crystals or by primary and/or secondary nucleation. By maintaining a condition which limits the amount of primary and/or secondary nucleation as discussed above, the amount of fines generated can be reduced and the crystal sizes can be increased using an aging process.

[0104] As stated above, temperature cycling process can be applied to the entire monohydrate slurry in the crystallizer or to a slip stream, i.e., a portion, of the monohydrate slurry such that a portion of the monohydrate slurry is cycled through an external heat exchanger to reduce the temperature of the monohydrate slurry.

[0105] When the temperature cycling is applied to the entire monohydrate slurry as a whole, the process is typically performed by cycling the crystallizer's temperature about once an hour. If the temperature cycling is affected to a portion of the monohydrate slurry through an external heat exchanger, such temperature cycling is conducted on a continuous basis while a portion of the monohydrate slurry is continuously circulated through the heat exchanger. In one particular embodiment of a temperature cycling process, a heat exchanger is used for the temperature cycling process. In this embodiment, the temperature of the monohydrate slurry is typically lowered by at least about 5° C., more preferably at least about 10° C., and most preferably at least about 20° C.

[0106] 10.0 Fines Scavenging.

[0107] As a means for improving the product yield, the slurry of fine particles remaining after the product size monohydrate crystals have been removed can be further processed to recover the soda ash values present in the slurry of fines. The slurry of fines can also include impurities which were present in the feedstream and any fine sodium carbonate monohydrate crystals which are smaller than the product size. One technique for processing the slurry of fines to improve the product yield is to use a pressure slurry system as described below.

[0108] 10.1 Pressure Slurry System Crystallization.

[0109] In this process, the slurry of fines is thickened to a relatively high solids content, preferably to at least about 17% solids by weight, more preferably to at least about 25% solids by weight, even more preferably to at least about 40% solids by weight, and most preferably to at least about 60% solids by weight. The slurry of fines can be thickened by a conventional gravity thickener, a membrane filter, or any suitable device that permits decanting saturated brine from the slurry of fines while retaining the solids.

[0110] The thickened slurry of fines is then pumped into a pressure vessel operating above the transition temperature of monohydrate sodium carbonate to anhydrous sodium carbonate. In general, this vessel is operated at a temperature of

at least about 7° C. above the transition temperature. In the pressure vessel, the incoming slurry is heated above the transition temperature of monohydrate sodium carbonate to anhydrous sodium carbonate. This heating converts sodium carbonate monohydrate to anhydrous sodium carbonate. The resulting anhydrous sodium carbonate slurry is then added to the feedstream or to the crystallizer directly. In this manner, the slurry of sodium carbonate monohydrate fines is recycled to the crystallization process of the present invention to increase the amount of sodium carbonate recovery.

[0111] Depending on the yield of each stage of crystallization, a pressure slurry system for fines scavenging can be repeatedly used. Because the operating and capital costs in each stage of crystallization processes of the present invention are relatively low, having a multiple stage pressure crystallization process can be readily justified economically. The use of a multiple stage crystallization process increases the yield of sodium carbonate from a depletable resource such as trona.

[0112] 11.0 Product Purity Control.

[0113] Although processes of the present invention provide product crystals of a purity level as described above, in some cases, such as when soluble impurities are present in the feedstream, it may be necessary to utilize a multiple stage crystallization process to achieve the product having the above described purity level.

[0114] Crystals are produced in a first stage of crystallization. These crystals are mechanically dewatered and repulped in brine from a second stage of crystallization in the process. This repulped slurry is fed to the second stage pressure slurry crystallization system as described above. The recrystallization that takes place in this second stage will produce crystals containing less soluble impurities than were present in the product of the first stage recrystallization. This process can be repeated with as many stages as are required to get the desired purity levels.

[0115] The following example is provided for purposes of illustration and is not intended to limit the scope of the present invention.

EXAMPLE 1

[0116] This example illustrates the high capacity for supersaturation of sodium carbonate and a technique for measuring the same.

[0117] A four liter vessel with intense agitation was partially filled with a slurry of 65×100 mesh sodium carbonate monohydrate seed crystals and heated to 88° C. Minus 150 mesh calcined trona, heated to 125° C. was added rapidly to the vessel. Immediately after addition of the calcined trona was complete, the concentration of dissolved sodium carbonate in the brine was determined by withdrawing the brine through a screen and filter to exclude seed crystals and calcined trona. Water was evaporated from the withdrawn brine to produce a solid residue. The quantity of sodium carbonate per gram of withdrawn brine was gravimetrically determined. The quantity of sodium carbonate in excess of the solubility limit of sodium carbonate is the amount of supersaturation. A second sample was taken 5 minutes after feed addition was complete to evaluate the amount of supersaturation at that time and the amount of relief of supersaturation in the 5 minute interval.

[0118] Three tests were run with the amount of feed being varied. The amount of feed added, the time of addition, the percent solids, and the amount of supersaturation at 0 minutes and at 5 minutes are shown below in Table 2.

TABLE 2

	Feed	Time to Add	% Solids	Grams/liter Supersaturation	
Test #	(g/l)	Feed (seconds)	at End	0 min.	5 min.
1	30	10	12 2	15 8	7 1
2	60	10	15 7	22 5	5.9
3	120	15	25 5	26 3	1 5

[0119] The results in Table 2 illustrate that high levels of supersaturation can be obtained by practice of the present invention. For example, in Test No. 3, supersaturation of 26.3 g/l was present at the end of the feed addition. The results further illustrate that the supersaturation is rapidly relieved. For example, in Test No. 3, the amount of supersaturation at the end of feed addition went from 26.3 g/l to 1.5 g/l at 5 minutes after the end of feed addition.

[0120] The foregoing description of the present invention has been presented for purposes of illustration and description. Furthermore, the description is not intended to limit the invention to the form disclosed herein. Consequently, variations and modifications commensurate with the above teachings, and the skill or knowledge of the relevant art, are within the scope of the present invention. The embodiment described hereinabove is further intended to explain the best mode known for practicing the invention and to enable others skilled in the art to utilize the invention in such, or other, embodiments and with various modifications required by the particular applications or uses of the present invention. It is intended that the appended claims be construed to include alternative embodiments to the extent permitted by the prior art.

What is claimed is:

1. A process for producing sodium carbonate monohydrate from a feedstream comprising anhydrous sodium carbonate and impurities, the process comprising:

(a) adding the feedstream to a saturated sodium carbonate brine solution under conditions to create supersaturation of at least about 5 g/l;

(b) processing within parameters that preferentially relieve the supersaturation by rapid growth of existing sodium carbonate monohydrate crystals over nucleation; and

(c) recovering at least a portion of the sodium carbonate monohydrate crystals from the saturated brine solution.

2. The process of claim 1, wherein the supersaturation is at least about 10 g/l.

3. The process of claim 1, wherein the supersaturation is at least about 20 g/l.

4. The process of claim 1, wherein the rate of adding the feedstream is at least about 100 g/l/min.

5. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises adding sodium carbonate monohydrate seed 5 crystals to the saturated sodium carbonate brine solution.

6. The process of claim 5, wherein the seed crystals are produced by removing sodium carbonate monohydrate crystals from the brine solution and sizing the removed crystals to produce a seed crystal size fraction for reintroduction to the brine solution.

7. The process of claim 5, wherein the particle size of the feedstream is less than the particle size of the seed crystals.

8. The process of claim 5, wherein the range of the particle size of the seed crystals is not greater than about 3 standard sieve sizes.

9. The process of claim 5, wherein the particle size of the feedstream is less than about 150 mesh.

10. The process of claim 5, wherein the particle size of the seed crystals is from about 100 mesh to about 150 mesh.

11. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises maintaining a solids content of at least about 17%.

12. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises agitating the brine solution at an agitation index of at least about 4.

13. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises periodically lowering the temperature of the brine solution by at least about 5° C.

14. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises pausing feedstream addition at least about 60% of the time of crystallization.

15. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises pausing feedstream addition at least about 30% of the time of crystallization.

16. The process of claim 1, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises pausing feedstream addition at least about 5% of the time of crystallization.

17. The process of claim 1, wherein the amount of solids in the brine solution formed by primary and/or secondary nucleation in the crystallizer is maintained at about 20% by weight or less of the total sodium carbonate solids in the brine solution.

18. The process of claim 1, wherein the amount of solids in the brine solution having a particle size of less than about 400 mesh is maintained at less than about 25% by weight of the total sodium carbonate solids in the brine solution.

19. The process of claim 1, wherein the amount of solids in the brine solution in the form of agglomerates and/or aggregates is maintained at about 20% by weight or less of the total sodium carbonate solids in the brine solution.

20. The process of claim 1, wherein the step of recovering comprises removing a portion of the sodium carbonate monohydrate crystals from the brine solution, dispersing the sodium carbonate monohydrate crystals by the addition of brine solution and recovering sodium carbonate monohydrate crystals from insoluble impurities on a size separation basis.

21. The process of claim 1, wherein the temperature of the saturated brine solution is at least about 70° C.

22. The process of claim 1, wherein the saturated sodium carbonate brine solution is at a temperature above the atmospheric boiling point of the solution.

23. A process for producing sodium carbonate monohydrate from a feedstream comprising anhydrous sodium carbonate and impurities, the process comprising:

(a) adding the feedstream to a saturated sodium carbonate brine solution at a rate of at least about 100 g/l/min under a condition to create supersaturation of at least about 5 g/l;

(b) processing within a parameter that preferentially relieve the supersaturation by rapid growth of existing sodium carbonate monohydrate crystals over nucleation, wherein the step of relieving comprises adding sodium carbonate monohydrate seed crystals to the saturated sodium carbonate brine solution, maintaining a solids content of at least about 40% and agitating the brine solution at an agitation index of at least about 4; and

(c) recovering a portion of the sodium carbonate monohydrate crystals from the saturated brine solution, wherein said recovering step comprises removing a portion of the sodium carbonate monohydrate crystals from the brine solution, dispersing the sodium carbonate monohydrate crystals by the addition of brine solution and recovering sodium carbonate monohydrate crystals from insoluble impurities on a size separation basis.

24. The process of claim 23, wherein the seed crystals are produced by removing sodium carbonate monohydrate crystals from the brine solution and sizing the removed crystals to produce a seed crystal size fraction for reintroduction to the brine solution.

25. The process of claim 23, wherein the particle size of the feedstream is less than the particle size of the seed crystals.

26. The process of claim 23, wherein the range of the particle size of the seed crystals is not greater than about 3 standard sieve sizes.

27. The process of claim 23, wherein the particle size of the feedstream is less than about 150 mesh.

28. The process of claim 23, wherein the particle size of the seed crystals is from about 100 mesh to about 150 mesh.

29. The process of claim 23, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation further comprises periodically lowering the temperature of the brine solution by at least about 5° C.

30. The process of claim 23, wherein the step of relieving the supersaturation preferentially by rapid growth of existing sodium carbonate monohydrate crystals over nucleation comprises pausing feedstream addition at least about 60% of the time of crystallization.

31. The process of claim 23, wherein the amount of solids in the brine solution formed by primary and/or secondary nucleation in the crystallizer is maintained at about 20% by weight or less of the total sodium carbonate solids in the brine solution.

32. The process of claim 23, wherein the amount of solids in the brine solution having a particle size of less than about

400 mesh is maintained at less than about 25% by weight of the total sodium carbonate solids in the brine solution.

33. The process of claim 23, wherein the amount of solids in the brine solution in the form of agglomerates and/or aggregates is maintained at about 20% by weight or less of the total sodium carbonate solids in the brine solution.

34. The process of claim 23, wherein the saturated sodium brine solution is at a temperature above the atmospheric boiling point of the solution.

35. A process for producing sodium carbonate monohydrate from a feedstream comprising anhydrous sodium carbonate and impurities, the process comprising:

(a) adding a feedstream having a particle size of less than about 100 mesh to a saturated sodium carbonate brine solution at a rate of at least about 400 g/l/min under a condition to create supersaturation of at least about 5 g/l;

(b) processing within a parameter that preferentially relieve the supersaturation by rapid growth of existing sodium carbonate monohydrate crystals over nucleation, wherein the parameter comprises adding sodium carbonate monohydrate seed crystals having a particle size of from about 150 mesh to about 100 mesh to the saturated sodium carbonate brine solution, maintaining a solids content of at least about 60% and agitating the brine solution at an agitation index of at least about 4; and

(c) recovering a portion of the sodium carbonate monohydrate crystals from the saturated brine solution, wherein said recovering step comprises removing a portion of the sodium carbonate monohydrate crystals from the brine solution, dispersing the sodium carbonate monohydrate crystals to a solids content of less than about 25% by weight by the addition of brine solution and recovering sodium carbonate monohydrate crystals having a particle size of greater than at least about 100 mesh from insoluble impurities on a size separation basis.

36. The process of claim 35, wherein the particle size of said feedstream is less than about 150 mesh.

37. The process of claim 35, wherein the saturated sodium carbonate brine solution is at a temperature above the atmospheric boiling point of the solution.

38. Sodium carbonate monohydrate produced by the process of claim 1.

39. A method for processing anhydrous sodium carbonate in an aqueous solution, comprising mixing anhydrous sodium carbonate with a saturated sodium carbonate brine solution at a temperature above the transition temperature between sodium carbonate monohydrate and anhydrous sodium carbonate to form a feed slurry of dispersed anhydrous sodium carbonate; and introducing the feed slurry to the aqueous solution.

40. The method of claim 39, wherein the aqueous solution is at above atmospheric pressure and at a temperature above the atmospheric boiling point of the aqueous solution and wherein the feed slurry is introduced by a feeder that maintains a continuous pressure seal between the environment of the feed slurry and of the aqueous solution.

* * * * *

Exhibit-5

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Organic Process Research & Development **2002**, *6*, 323–328

Process Research and Development for an Efficient Synthesis of the HIV Protease Inhibitor BMS-232632

Zhongmin Xu,*,† Janak Singh,† Mark D. Schwinden,† Bin Zheng,‡ Thomas P. Kissick,† Bharat Patel,† Michael J. Humora,‡ Fernando Quiroz,‡ Lin Dong,‡ Dau-Ming Hsieh,‡ James E. Heikes,‡ Madhusudhan Pudipeddi,§ Mark D. Lindrud,‡ Sushil K. Srivastava,‡ David R. Kronenthal,‡ and Richard H. Mueller‡

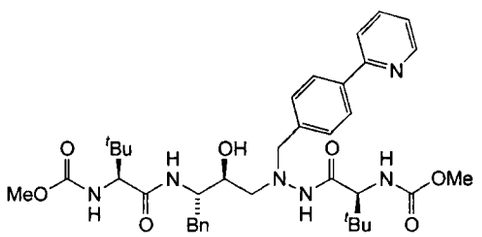
The Bristol-Myers Squibb Pharmaceutical Research Institute.
Process Research and Development, P.O. Box 4000, Princeton, New Jersey 08543, U.S.A.,
Process Research and Development, P.O. Box 191, New Brunswick, New Jersey 08903, U.S.A. and
Biopharmaceutics Research and Development, P.O. Box 191, New Brunswick, New Jersey 08903, U.S.A.

Abstract:

Development of an efficient and scalable process for the human immunodeficiency virus (HIV) protease inhibitor BMS-232632 1-[4-(pyridin-2-yl)phenyl]-5(*S*)-2,5-bis{[*N*-(methoxycarbonyl)-L-*tert*-leucinyl]-amino}-4(*S*)-hydroxy-6-phenyl-2-azahexane, is described. The key step in the synthesis of the intermediate *N*-1-(*tert*-butyloxycarbonyl)-*N*-2-[4-(pyridin-2-yl)benzylidene]hydrazone (11) was the Pd-mediated coupling of boronic acid 9 with 2-bromopyridine. An efficient procedure was developed for the chemoselective reduction of hydrazone 11 to hydrazine carbamate 4. The key intermediate *N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxy-butane (6) was prepared stereoselectively from chiral diol 10. The subsequent union of 4 and 6 followed by coupling with *N*-methoxycarbonyl-L-*tert*-leucine provided the free base BMS-232632 in high yield. Evaluation of a variety of salts and identification of bisulfate salt 19 with enhanced bioavailability are also described.

Introduction

BMS-232632 is an acyclic aza-peptidomimetic that is a potent human immunodeficiency virus (HIV) protease inhibitor.[1] Its bisulfate salt has better bioavailability than the free base, with a half-life suitable for once-daily dosing. We have developed a practical synthesis of this compound.



BMS-232632

The original process for BMS-232632 (Scheme 1) was satisfactory for preparation of material for early toxicology studies.[2] However, this route was suboptimal for the production of the larger quantities required for further toxicology studies, preclinical studies, and clinical trials. Significant modifications in the synthesis of key intermediates as well as the replacement of reagents in the subsequent steps were required. Furthermore, the crystalline free base form of BMS-232632 as a suspension in water or oil had poor oral bioavailability in animals, presumably due to its extremely low solubility in these vehicles. For development of pharmaceutical formulations, particularly oral dosage forms, a salt form of BMS-232632 with enhanced bioavailability had to be identified. We herein report on a more efficient synthesis of this compound and identification of a salt with appropriate properties.

Several limitations of the original process presented a formidable challenge for the preparation of material in large quantity. The pyridinyl benzaldehyde **3** was made in three steps involving nickel-catalyzed coupling of Grignard reagent **2** with 2-bromopyridine. Although the overall yield from **1** was high (84%), the use of a hazardous reagent diisobutylaluminum hydride and protection/deprotection of the aldehyde moiety were required.[2] Epoxide **6** was prepared from L-Boc-phenylalaninal (**5**) in two steps including a Wittig reaction with methyltriphenylphosphonium bromide. Not surprisingly, even at −78 °C, racemization of **5** occurred during the Wittig reaction, which in turn resulted in epoxide with only 80% ee. Furthermore, purification of both the Wittig reaction product and epoxide **6** proved difficult, and silica gel chromatographies were required. In the final step, coupling with *N*-methoxycarbonyl-L-*tert*-leucine, the expen-

† Process Research and Development, P.O Box 4000, Princeton, New Jersey 08543.

‡ Process Research and Development, P O. Box 191, New Brunswick, New Jersey 08903.

§ Biopharmaceutics Research and Development, P.O. Box 191, New Brunswick, New Jersey 08903.

(1) (a) Fassler, A.; Bold, G., Capraro, H., Cozens, R.; Mestan, J.; Poncioni, B.; Rosel, J.; Tintelnot-Blomley, M.; Lang, M. *J. Med. Chem* **1996**, *39*, 3203−3216. (b) Bold, G.; Faessler, A., Capraro, H., Cozens, R.; Klimkait, T., Lazdins, J.; Mestan, J.; Poncioni, B., Roesel, J.; Stover, D.; Tintelnot-Blomley, M.; Acemoglu, F.; Beck, W.; Boss, E.; Eschbach, M.; Huerlimann, T., Masso, E., Roussel, S., Ucci-Stoll, K., Wyss, D., Lang, M. *J. Med Chem* **1998**, *41*, 3387−3401 (c) Rusano, G. L.; Bilello, J. A. Preston, S. L.; O'Mara, E.; Kaul, S.; Schnittman, S.; Echols, R. *J. Infect. Dis.* **2001**, *183*, 1126−1129. (d) Gong, Y., Robinson, B. S.; Rose, R. E., Demmie, C.; Spicer, T. P.; Stock, D., Colonno, R. J., Lin, P. *Antimicrob. Agents Chemother* **2000**, *44*, 2319−2326 (e) Robinson, B. S.; Riccardi, K. A., Gong, Y.; Guo, Q., Stock, D. A., Blair, W. S.; Terry, B. J., Deminie, C. A., Djang, F.; Colonno, R. J.; Lin, P. *Antimicrob. Agents Chemother* **2000**, *44*, 2093−2099. (f) Rabasseda, X.; Silvestre, J.; Castaner, J. *Drugs Future* **1999**, *24*, 375−380

(2) (a) Giordano, C.; Pozzoli, C.; Benedetti, F. W O. Patent 012083, 2001. (b) Fassler, A.; Bold, G.; Capraro, H.; Steiner, H. W O. Patent 9746514, 1997. (c) Reference 1a.

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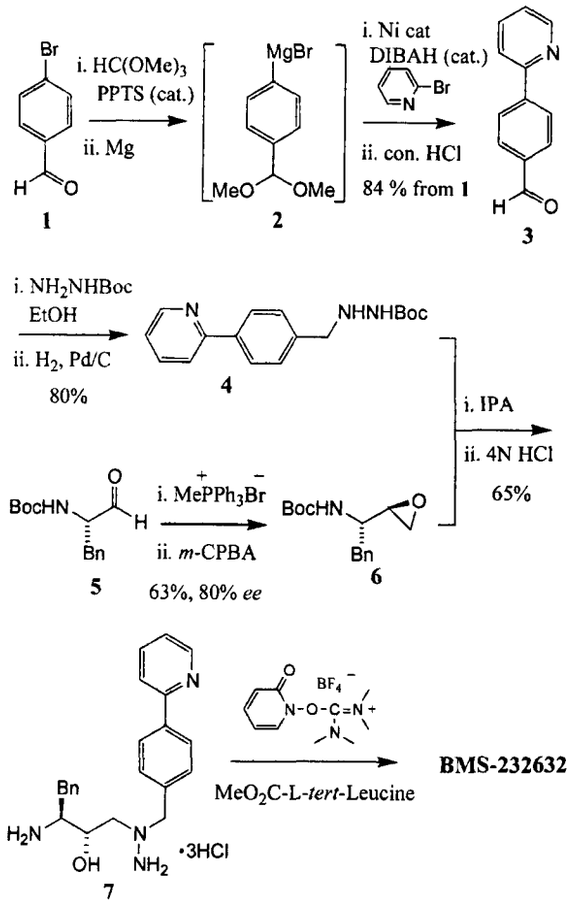
Scheme 1. **Original Synthesis of BMS-232632**



sive *O*-(1,2-dihydro-2-oxo-1-pyridyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-tetrafluoro-borate (TPTU) was used.

In addition, a mediocre yield was obtained due in part to the low enantiomeric purity of the epoxide starting material. Besides the above-mentioned issues, environmentally unfriendly solvents, such as highly flammable diethyl ether and suspected carcinogen 1,4-dioxane, were employed in this process. Therefore, a strong need for the development of a more efficient and environmentally suitable synthesis for BMS-232632 existed.

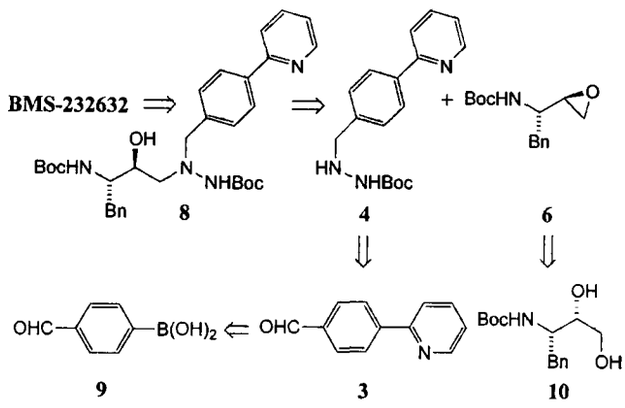
Synthesis Strategy

As depicted in the retrosynthetic analysis (Scheme 2), we envisaged new methods for preparation of hydrazinocarbamate **4** and epoxide **6**. Aldehyde **3** might be accessed through a Suzuki coupling of commercially available 4-formylbenzeneboronic acid (**9**) with 2-bromopyridine, eliminating the need to protect and deprotect the aldehyde functionality. To obtain enantiomerically pure epoxide **6**, we planned to use the commercially available diol **10**, possessing the *S*-configuration at the carbinol center. A selective formation of **6** was expected through an intramolecular S_N2 displacement reaction. The convergent formation of BMS-232632 from intermediates **4** and **6** is analogous to the original synthesis.[2]

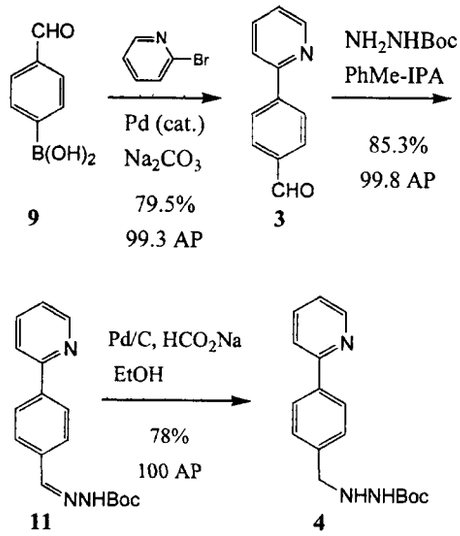
Results and Discussion

Synthesis of Hydrazinocarbamate 4. Unlike the metal-catalyzed couplings of Grignard reagents with aryl electro-

Scheme 2. **Retrosynthesis for BMS-232632**



Scheme 3. **Synthesis of hydrazino carbamate 4**



philes, the Suzuki coupling is highly tolerant of functionality.[3] The requisite aldehyde **3** (Scheme 3) was prepared through Suzuki coupling of boronic acid **9** to 2-bromopyridine. The reaction was reasonably straightforward. However, it proved critical to use a 4:3 mixture of toluene−ethanol as the solvent in order to dissolve all the reactants, thus affording a facile reaction. Also, use of a minimal amount (0.1 mol %) of Pd-$(PPh_3)_4$ catalyst was required to deliver product with low amount (5−50 ppm) of palladium. Under the optimal conditions, the Suzuki coupling afforded the desired aldehyde **3** after crystallization from toluene−*n*-heptane in 79.5% yield and with HPLC area % (AP) 94−99. It was subsequently found that the toluene solution of crude aldehyde **3** resulting from an extractive workup could be used in the next step without further purification. Curiously, wide variations in residual Pd levels (5−400 ppm) in the product were observed from batch to batch.

The subsequent condensation of aldehyde **3** with *tert*-butyl carbazate was initially carried out by heating in ethanol and then precipitating the product by the addition of water. This procedure afforded a 93% yield of **11** with less than optimal purity, ~95 AP. We found that the use of a solvent mixture

(3) For reviews, see: (a) Miyaura, N.; Suzuki, A. *Chem Rev.* **1995**, *95*, 2457−2483. (b) Suzuki, A. *J Organomet Chem* **1999**, *576* (1−2), 147−168.

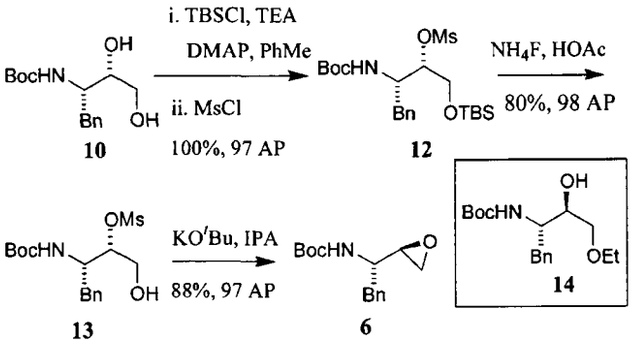
of 4:3 toluene−2-propanol was ideal for both the condensation and subsequent crystallization. Heating the crude aldehyde **3** and *tert*-butyl carbazate in this mixture of solvents for 2 h and subsequent cooling and filtration provided compound **11** in 92% yield and with ~100 AP. In addition, the residual Pd level was reduced about 10-fold compared to that in the aldehyde starting material. This procedure was scaled up to afford 5 kg of **11** in a single batch.

Our studies showed that under the original conditions (Pd/C, MeOH, and 1 atm H_2),[2] the conversion of **11** to **4** was variable, ranging from 80 to 98%. Also problematic were the side reactions involving hydrogenolysis and overreduction.[4] In contrast to the hydrogenolysis and overreduction byproducts, the removal of the unreacted hydrazone **11** through crystallization proved difficult due to its extremely low solubility compared to product **4**. Thus, a more efficient conversion was desired. Replacement of Pd/C with Pd-$(OH)_2$/C provided improved conversion (>97%) while the extent of hydrogenolysis was less than 2%. Hydrogenation of **11** using $Pd(OH)_2$ (MeOH, 1 atm H_2, 6 h) followed by crystallization provided **4** in 80−90% yields and with 94−99 AP (1−5 AP **11**). Despite these encouraging results, the required catalyst loading of 10 wt % of $Pd(OH)_2$ rendered this reduction unattractive.

Chemical reductions of hydrazone **11** using $NaBH_4$[5] (various solvents, with additives such as $NiCl_2$,[6] $ZrCl_4$,[7] and HOAc), $NaB(OAc)_3H$-HOAc,[8] $NaBH_2S_3$,[9] $LiBH_4$, and $Al(^iBu)_2H$ were sluggish; generally <20% product was observed. Reduction with Zn/HOAc[10] in refluxing methanol resulted in ~40% conversion to hydrazinocarbamate **4**.

In search for a more effective procedure, we examined catalytic phase-transfer hydrogenation.[11] Although reduction with 2 equiv of sodium formate and 1 mol % of Pd/C required an elevated temperature, complete reaction could be obtained reliably in 2 h at 56 °C, yielding crude **4** with ~98 AP. Crystallization from *tert*-butyl methyl ether and *n*-heptane furnished the desired hydrazinocarbamate in 78% isolated yield with 100 AP. This reduction procedure was successfully scaled up to afford 3.7 kg of **4** (78% yield, AP 100) in a single batch. It is noteworthy to mention that although solid hydrazinocarbamate **4** was perfectly stable in air, it readily underwent air oxidation in MeOH or MeCN solution to afford hydrazone **11**. Therefore, solutions of **4** were handled under inert gas.

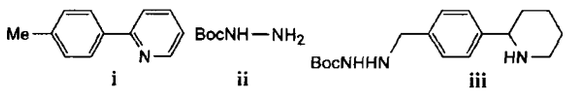
Scheme 4. **Synthesis of (2*S*,3*R*)-epoxide 6**



Synthesis of (2*S*,3*R*)-Epoxide 6. Methods for the conversion of a vicinal diol to an epoxide are well established.[12] Through modification of the reported procedures,[12d-f] a practical process for this transformation was developed. As illustrated in Scheme 4, a quantitative yield of silyl mesylate **12** was produced in one pot through selective silylation (2.2 equiv of TBSCl, DMAP (cat), 2.2 equiv of Et_3N, 50 °C, toluene) and subsequent mesylation (1.1 equiv of MsCl, 5 °C, toluene) of diol **10**. This oily intermediate was carried into the next step without further purification.

We found that desilylation of **12** could be effected using the inexpensive reagent ammonium fluoride[13] instead of the more traditional tetrabutylammonium fluoride. The resulting solid product **13** could be readily isolated and further purified through crystallization from IPA−H_2O in 80% yield with AP 98. Several bases were screened for epoxide formation from **13**. KO^{t}Bu was found to be the base of choice, giving enantiomerically pure epoxide product in 90% crystallized yield. Interestingly, use of KOH in EtOH gave an ethanolysis product **14** exclusively.[14] The reaction with KO^{t}Bu in THF−IPA was reproducible on scale-up. Following the above four-step procedure, a total of 2.7 kg of epoxide **6** was prepared in 70% average yield with 100 AP. The primary attraction of this route is that product **6** with 100% ee is obtained cleanly.

(4) Byproducts **i** and **ii** were formed by the hydrogenolysis and **iii** was formed by overreduction.



(5) (a) Karmakar, D.; Rajapati, D., Sandhu, J. S. *J Chem Res Synop.* **1996**, *10*, 464−465 (b) Wann, R. S.; Thorsen, T P.; Kreevoy, M M. *J Org Chem* **1981**, *46*, 2579−2581.

(6) Rao, H P ; Reddy, K. S.; Turnbull, K ; Borchers, V *Synth Commun* **1992**, *22*, 1339−1343

(7) Chary, K. P., Ram, S. R., Salahuddin, S., Iyengar, D. S. *Synth. Commun* **2000**, *30* (19), 3559−3563.

(8) (a) Singh, J.; Sharma, M.; Kaur, I.; Kad, L C. *Synth Commun.* **2000**, *30*, 1515−1519. (b) Baruah, B.; Dutta, P. M.; Boruah, A.; Prajapati, D.; Sandhu, J. S. *Synlett* **1999**, *4*, 409−410

(9) Ramanujam, V. S.; Trieff, M. N. *J Chem Soc , Perkin Trans. 2* **1976**, *15*, 1811−1815.

(10) Hoffmann, R W , Sieber, W. *Justus Liebigs Ann Chem* **1967**, *703*, 96−100.

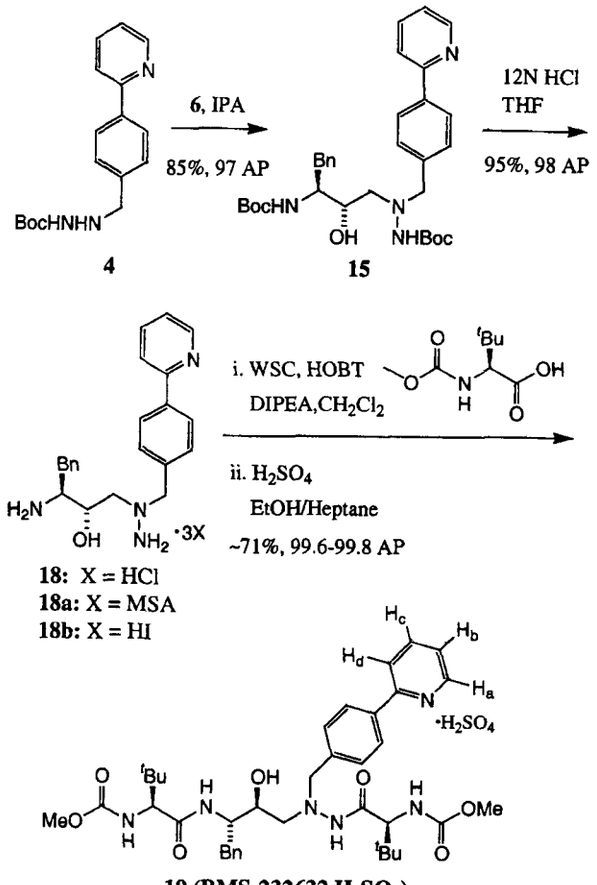
(11) (a) Watanabe, T., Nishiyama, S., Yamamura, S.; Kato, K., Nagai, M., Takita, T *Tetrahedron Lett* **1991**, *32*, 2399−2400 (b) Berlin, W K ; Zhang, W.-S.; Shen, T. Y. *Tetrahedron* **1991**, *47*, 1−20.

(12) Via conversion of 1° alcohol to a leaving group: (a) Clayden, J., McElroy, A. B.; Warren, S. *J. Chem. Soc., Perkin Trans. 1* **1995**, *15*, 1913−1934. (b) Shibuya, M.; Terauchi, H. *Tetrahedron Lett* **1987**, *28*, 2619−2623. (c) Masaki, Y.; Serizawa, Y.; Nagata, K.; Oda, H. *Tetrahedron Lett.* **1986**, *27*, 231−234. Via conversion of 2° alcohol to a leaving group: (d) Castejon, P , Pasto, M.; Moyano, A ; Pericas, M. A.; Riera, A. *Tetrahedron Lett* **1995**, *36*, 3019−3022. (e) Dondoni, A.; Fantin, G.; Fogagnolo, M., Medici, A *Angew. Chem. Int. Ed Engl* **1986**, *25*, 835−839. (f) Qian, X ; Moris-Varas, F ; Wong, C. H. *Bioorg Med Chem Lett* **1996**, *6*, 1117−1122.

(13) (a) Hassan, A. E.; Nishizono, N , Minakawa, N.; Shuto, S.; Matsuda, A. *J. Org Chem* **1996**, *61*, 6261−6267 (b) Seki, M.; Kondo, K ; Kuroda, T ; Yamanaka, T.; Iwasaki, T. *Synlett* **1995**, 609−611

(14) It is possible that the desired epoxide **6** was initially formed and that it rapidly underwent a rearrangement under the reaction conditions. This possibility was supported by the finding that subjection of **6** to excess KOH in EtOH resulted in the formation of **14**. Compound **14** was isolated and fully characterized. ^1H NMR (400 MHz, $CDCl_3$) δ 7 4−7 2 (m, 5H), 5.14 (d, J = 9.6 Hz, 1H), 3.75 (m, 1 H), 3.45 (q, J = 7.1 Hz, 2H), 3 36 (d, J = 7.0 Hz, 2H), 3.24 (s, 1H), 2.89 (m, 2H), 1.40 (s, 9H), 1.13 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14 9, 28 4, 38 3, 52 8, 66 5, 69.3, 72.4, 72 6, 76 7, 79.1, 126 1, 128.2, 129.2, 138.1, 155.6; IR (1% KBr pellet) 3440, 1713, 1695 cm^{-1}, $[\alpha]_D$ = −31 3 (c = 1, MeOH, 22 °C), Anal. Calcad for $C_{18}H_{29}NO_4$ C, 65 99; H, 8 80; N 4.53 Found· C, 65 88; H, 8 82; N 4.26.

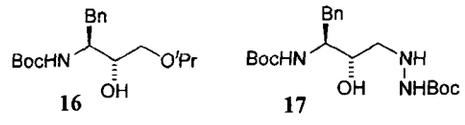
Scheme 5. **Synthesis of BMS-232632 and its bisulfate salt 19**



Partial ^{1}H NMR chemical shifts of BMS-232632 and 19 (starred chemical shifts for the protons in 19):

H_a = (8.6, 8.8*), H_b = (7.3, 7.7*), H_c = (7.8, 8.3*), H_d = (7.9, 8.2*)

Synthesis of BMS-232632. As illustrated in Scheme 5, the coupling of hydrazinocarbamate **4** and epoxide **6** was performed in refluxing IPA for 24 h, followed by the addition of water to precipitate the crude product. Subsequent recrystallization from MeCN−H_2O furnished **15** in 85% yield with AP 98. In the coupling reaction, low levels (<5%) of impurity **16**,



a consequence of epoxide opening by the solvent, were observed by in-process HPLC. Employment of *tert*-butyl alcohol produced **15** in comparable yields; however, the reaction required ~48 h for completion. Replacement of IPA with several aprotic solvents (toluene, acetonitrile) resulted in very sluggish reactions. Impurity **16** as well as **17**, resulting from epoxide opening by the hydrogenolysis product Boc-$NHNH_2$ mentioned above,[4] was purged during the initial precipitation of the product.

Subsequent removal of the two Boc groups in **15** was performed with concentrated hydrochloric acid in THF at 50 °C. Although a clean deprotection product **18** was generated, the isolation of this trihydrochloride salt proved difficult due to its hygroscopic nature. Attempted crystallization or precipitation from several solvent systems afforded material which was still extremely hygroscopic. The corresponding MsOH and HI salts **18a** and **18b** were isolated by deprotection using MsOH and TMSI, respectively. These materials were foamy solids and also very hygroscopic. To circumvent the problematic isolation of **18**, a procedure for the deprotection and in situ coupling with *N*-methoxycarbonyl-L-*tert*-leucine was developed. After removal of the Boc groups from **15** with HCl, the wet THF layer was decanted. The residual oily product was then washed with THF and taken up in dichloromethane. The resulting solution was subjected to the original coupling conditions (3 equiv of *N*-methoxycarbonyl-L-*tert*-leucine, 1 equiv of TPTU, 6 equiv of EtN^iPr_2, CH_2Cl_2),[2] affording BMS-232632 in ~80% yield with high purity. To avoid use of the expensive reagent TPTU, the coupling step was reevaluated. Replacement of TPTU by a combination of water-soluble carbodiimide [WSC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] and 1-hydroxybenzotrizaole (HOBT) formed the coupling product in a similar yield. The solution of crude trihydrochloride salt **18** was treated with diisopropylethylamine and added to a mixture of *N*-methoxycarbonyl-L-*tert*-leucine, WSC and HOBT in dichloromethane. A minimum of 2.6 equiv each of WSC and HOBT, and 2.5 equiv of *N*-methoxycarbonyl-L-*tert*-leucine were required for complete reaction.[15] Using this process we prepared 3.5 kg of BMS-232632 (yield 82% from **15**, AP 98.8).

Formation of the Bisulfate Salt 19.[16] Free base BMS-232632 is highly insoluble in water (<1 μg/mL at 24 °C) and has poor oral bioavailability in animals. Therefore, several acid salts were explored. A number of commonly used acid salts such as the hydrochloride, benzenesulfonate, methanesulfonate, *p*-toluenesulfonate, phosphate, nitrate, 1,2-ethanedisulfonate, 2-hydroxyethanesulfonate, sulfate, and bisulfate were evaluated. While these salts in their crystalline form exhibited higher aqueous solubility (0.5−5 mg/mL) than the free base, bisulfate salt **19** was chosen as the final form for development due to its superior solubility (4−5 mg/mL).

Bisulfate salt **19** was obtained in two crystalline forms, as determined by powder X-ray diffraction patterns. Type-I crystals were formed in acetone, MeCN, or EtOH; the Type-II crystals were obtained from IPA. Due to its reproducibility, the Type-I form was selected for further development. The Type-I form **19** was initially prepared in MeCN with 9 M H_2SO_4 and in the presence of seed crystals. Although a very easily filterable, dense, sandy solid was obtained, this procedure was abandoned due to concerns of possible toxicity associated with residual trace amounts of MeCN. Alternatively, the formation of **19** was performed with concentrated H_2SO_4 in EtOH at ambient temperature. Direct crystallization

(15) In-process HPLC analysis indicated that the amide bond on the amine was formed prior to that on the hydrazine. Fewer equivalents of WSC, HOBT and *N*-methoxycarbonyl-L-*tert*-leucine resulted in an incomplete reaction, evidenced by a high ratio of monocoupling product to BMS-232632

(16) Singh, J.; Pudipeddi, M., Lindrud, D. M. W.O. Patent 9936404, 1999

by addition of *n*-heptane provided the bisulfate salt **19** as an easily filterable solid in 85% yield with >99.6 AP on 3.6 kg input scale. ^{1}H NMR analyses of the free base BMS-232632 and bisulfate salt **19** indicated that the pyridine ring in **19** was protonated, as evidenced by the downfield chemical shifts of protons H_{a-d} compared to those of the free base (Scheme 5, starred chemical shifts are for the protons in the salt **19**).

Summary

A short and efficient synthesis of hydrazinocarbamate **4** has been developed. A novel process for selective formation of epoxide **6** from diol **10** has been established. The convergent formation of BMS-232632 from **4** and **6** has been significantly improved, providing the free base BMS-232632 in good yield. Finally, the bisulfate salt **19** with enhanced bioavailability was identified and obtained in excellent purity.

Experimental Section

All new compounds described in the Experimental Section were fully characterized. Analytical and spectral data for these compounds are for lab batches. HPLC analysis results are described as area % (AP).

4-Pyridin-2-yl-benzaldehyde (3). A mixture of 4-formyl-phenyl-boronic acid (**9**) (200 g, 666.9 mmol) and 2-bromopyridine (110.6 g, 700.3 mmol) in 700 mL of 4:3 toluene/95% ethanol was purged with N_2 for 15 min and then heated under a N_2 atmosphere, resulting in a clear solution. A slurry of $Pd(PPh_3)_4$ (1 g, 0.86 mmol) in 10 mL of a 4:3 mixture of toluene and 95% ethanol was added, followed by 1.16 L of 3 M aqueous Na_2CO_3. The resulting mixture was gently refluxed at ~76 °C for 13 h, at which time an additional 10-mL slurry of $Pd(PPh_3)_4$ (540 mg, 0.467 mmol) was added. The reaction was continued for another 7 h and then cooled to ambient temperature. The solid was removed by filtration, and the filtrate was poured into a separatory funnel. The layers were separated, and the aqueous layer was washed with toluene (2 × 250 mL). The combined organic layers were stirred over charcoal (10 g) for 5 h, filtered, and partially concentrated in vacuo to provide a toluene solution of aldehyde **3**, which contained approximately 150 mL of toluene. This unpurified product was submitted directly to the next reaction. Alternatively, the crude **3** could be purified through crystallization from a mixture of 1:1 toluene and *n*-hexane. Typically, a 80% crystallized yield was obtained.

***N*-1-(*tert*-Butyloxycarbonyl)-*N*-2-[4-(pyridin-2yl)benzylidene]-hydrazone (11).** To a 100-L glass plant reactor was added 4-pyridin-2-yl-benzaldehyde (**3**) (4.131 kg, 22.25 mol), *tert*-butyl carbazate (2.967 kg, 22.23 mol), 6.05 L of 2-propanol, and 8.01 L of toluene. The mixture was heated to reflux (80−85 °C) under inert atmosphere for 2 h, cooled to 22 °C over 4 h, and stirred at that temperature for about 14 h. The resulting mixture was filtered, and the filter cake was washed with a cold mixture of toluene and heptane (1:3, 0−5 °C, 4 × 3.56 L). The cake was air-dried under vacuum to afford 5.644 kg of **11** (85.3% yield, 99.8 AP).

***N*-1-(*tert*-Butyloxycarbonyl)*N*-2-[4-(pyridin-2-yl)benzylidene]-hydrazine (4).** In to a mixture of hydrazone **11** (113.4 g, 0.382 mol), and 10% palladium on activated carbon (4.05 g) in 382 mL of ethanol, a solution of sodium formate (46.7 g, 0.687 mol) in water (70 mL) was added. The resulting mixture was heated to 57 °C, and slight gas evolution was observed within 5 min. After 1.5 h, the reaction was cooled to about 40 °C, and MTBE (380 mL) was added. The solid was removed through filtration. The filtrate was washed with 10% sodium chloride solution (550 mL), dried over anhydrous sodium sulfate (100 g), filtered, and concentrated to provide a colorless gel which was dissolved in MTBE (110 mL) under argon atmosphere at 50 °C. *n*-Heptane (350 mL) was added dropwise to the warm solution, and the resulting mixture was slowly cooled to 23 °C and stirred for 16 h. Filtration and washing with heptane (2 × 300 mL) afforded **4** as a white solid (89.1 g, 78% yield, AP 100).

[3-*tert*-Butyl-dimethylsilanyloxy-2(*S*)-[(methylsulfonyl)-oxy]-1(*S*)-(phenylmethyl)propyl]-carbamic Acid, 1,1-Dimethylethyl Ester (12). A solution of diol **10** (544 g, 1.034 mol) in 1.2 L of toluene was heated to 88 °C, and a clear solution was obtained. The solution was then cooled to 50 °C. Dimethylamino pyridine (23.6 g, 0.195 mol) and triethylamine (325 mL, 2.32 mol) were charged followed by the slow addition of *tert*-butyl-dimethylsilyl chloride (350 g, 2.32 mol) while keeping the internal temperature around 50 °C. The reaction mixture was cooled to 0 °C over 3 h. Triethylamine (417 mL) was added followed by the slow addition of trifluoromethanesulfonyl chloride (198 mL), keeping the internal temperature under 5 °C. The resulting mixture was stirred at 0 °C for about 3 h. The solid was filtered through Celite and washed with toluene (2 × 700 mL). The filtrate was washed with water (4 L), 1 N HCl (4 L), and brine (4 L), in that order, and then concentrated in a vacuum to afford 1.04 kg of product **12** as a yellow oil. This product was subjected to the next step without further purification.

[3-Hydroxy-2(*S*)-[(methylsulfonyl)oxy]-1(*S*)-(phenylmethyl)propyl]-carbamic Acid, 1,1-Dimethylethyl Ester (13). Into a reactor was charged ammonium fluoride (358 g, 9.67 mol), a solution of the crude mesylate **12** (1.04 kg, 1.034 mol) in methanol (5.6 L), and acetic acid (550 mL). The mixture was stirred at ambient temperature for 11 h. The reaction mixture was concentrated to dryness to afford a solid, which was dissolved in 11 L of methyl *tert*-butyl ether. The resulting solution was washed with water (5 L), 5% sodium bicarbonate (3 × 4 L), and brine (4 L) and then dried over $MgSO_4$ (300 g). Filtration and partial concentration afforded 5 L of solution. The concentrated solution was then cooled to 4 °C and stirred at this temperature for 18 h to give a slurry. The solid was filtered, washed with cold MTBE (200 mL) and dried under partial pressure to afford 489.1 g of **13**. The filtrate was concentrated to 1 L, cooled to 4 °C, and stirred at this temperature for 18 h to give a slurry. Another 61.7 g of solid was obtained after filtration and drying. Thus, a total of 550.8 g of product **13** was obtained as a white solid (80% yield, AP 98).

***N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxy-butane (6).** To a clear solution of hydroxy

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mesylate **13** (629.9 g, 1.75 mol) in a mixture of IPA (6.3 L) and THF (1.8 L) at 17 °C, was added KO'Bu (207 g, 95%, 1.75 mol) over 20 min. The mixture was stirred for 1.5 h followed by addition of 30 mL of acetic acid over 15 min. The resulting solution was concentrated under vacuum to dryness to afford a white solid. The solid was dissolved in MTBE (9.0 L), and the resulting solution was washed with water (4.5 L), saturated sodium bicarbonate solution (4.5 L), and brine (4.5 L), dried over anhydrous Na_2SO_4, filtered, and concentrated to give an oil (455.2 g). The oil was diluted with hexane (1.3 L) followed by addition of water (200 mL). The mixture was cooled to −4 °C, and solid was observed. The solid was collected by filtration, washed with 700 mL of cold hexane (0 °C), and dried under vacuum for 18 h to give epoxide **6** as a white solid (400.5 g, 88% yield, AP 97).

1-[4-(Pyridin-2-yl)phenyl]-5(*S*)-2,5-bis[(*tert*-butyloxycarbonyl)-amino]-4(*S*)-hydroxy-6-phenyl-2-azahexane (15). To a mixture of compounds **4** (1.68 kg, 5.58 mol) and **6** (1.735 kg, 6.56 mol) was added 23.83 L of 2-propanol. The solution was refluxed for 24 h under an inert atmosphere and then cooled to 22 °C. Water (28.9 L) was added, and the resulting mixture was stirred at 22 °C for 16 h to afford a slurry. The solid was collected by filtration and washed with water (2 × 11 L) and cold MTBE (~0 °C, 2 × 4 L). This wet cake was dissolved in acetonitrile (44 L), and water (44 L). The crystallized material was filtered and dried to give 2.681 kg (85.4% yield, AP 99) of **15**.

***N*-Methoxycarbonyl-L-*tert*-leucine.** To a solution of L-*tert*-leucine (750 g, 5.7 mol) was added 9.42 L of 2 N aqueous sodium hydroxide and methylchloroformate (882 mL, 11.34 mol). The resulting mixture was heated at 60 °C for 18 h and then cooled to 22 °C. The reaction mixture was acidified by addition of 4 N HCl (4.8 L) to pH ~2 followed by an extractive workup with ethyl acetate (3 × 6 L). The combined extracts were washed with brine and concentrated under vacuum to afford an oil. To the oil 7.7 L hexane was added to produce a large solid mass. The solid was collected by filtration and air-dried to afford 883 g of *N*-methoxycarbonyl-L-*tert*-leucine (82% yield).

1-[4-(Pyridin-2-yl)phenyl]-5(*S*)-2,5-bis{[*N*-(methoxycarbonyl)-L-*tert*-leucinyl]amino}-4(*S*)-hydroxy-6-phenyl-2-azahexane (BMS-232632). To a stirred solution of **15** (3.5 kg, 6.22 mol) in 17.5 L of THF, concentrated HCl (2.38 L, 12 N) was added dropwise at 22 °C to give an amber-colored solution. The mixture was stirred for 3 h at 45−55 °C to produce a biphasic mixture. The mixture was cooled to 22−25 °C over 3 h, and the wet THF layer was decanted away from a brown oil. The oil was rinsed with 7 L of THF, which was decanted. The brown oil was dissolved in a mixture of 14 L of CH_2Cl_2 and 6.65 L of DIPEA. The resulting mixture was slowly transferred by pump at 22 °C into a premixed solution of *N*-methoxycarbonyl-L-*tert*-leucine (2.94 kg, 15.5 mol), HOBT (2.32 kg, 17.2 mol), and WSC (3.13 kg, 16.3 mol) in 4.4 L of CH_2Cl_2. The new reaction mixture was stirred for 3−4 h at 25 °C. The reaction mixture was then washed with water (1 × 13.1 L), $NaHCO_3$ (1 × 13.1 L), and brine (1 × 14 L). The organic layer was concentrated to a viscous oil and treated with 42 L of a 98:2 isopropyl ether−EtOH solution at 55−70 °C until a mild reflux was observed. The slurry was cooled to 25−30 °C, and the solid was collected by filtration, washed with 13.3 L of a 98:2 mixture of isopropyl ether−EtOH, and dried at 35−40 °C under vacuum to give 3.86 kg of crude free base. The crude product was crystallized from 36.4 L of a mixture of ethanol−water (45/55), filtered, washed, and dried to afford 3.64 kg of BMS-232632 in 82.1% yield and with AP 98.8.

1-[4-(Pyridin-2-yl)phenyl]-5(*S*)-2,5-bis{[*N*-(methoxycarbonyl)-L-*tert*-leucinyl]amino}-4(*S*)-hydroxy-6-phenyl-2-azahexane, Bisulfate Salt (19). To a solution of BMS-232632 (3.6 kg, 5.1 mol) in 27 L of ethanol, concentrated H_2SO_4 (309 mL, 5.65 mol) was added dropwise at 22 °C to give a yellow-orange colored solution. After complete dissolution, the solution was filtered and transferred into another vessel. Then 18 L of *n*-heptane was added followed by seeding with 10 g of pure **19**. After seeding, an additional 12 L of *n*-heptane was added. The mixture was stirred for 15 h. The solid was collected through filtration and washed with heptane−ethanol (1:1) solution and dried to afford 3.49 kg of **19** as a white solid (85.2% yield, AP 99.8). ^{1}H NMR (270 MHz DMSO-d_6) δ 9.35 (s, 1H), 9.0 (d, J = 4.1 Hz, 1H), 8.64 (t, J = 8.2 Hz, 1 H), 8.43 (d, J = 8.2 Hz, 1H), 8.07 (m, 2H), 8.32 (t, J = 8.2 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 8.73 (d, J = 8.2 Hz, 1H), 7.67 (m, 1H), 7.32 (d, J = 4.7 Hz, 1H), 7.27 (m, 3H), 7.09 (d, J = 9.4 Hz, 1H), 7.03 (d, J = 9.3 Hz, 1H), 4.20 (m, 1H), 3.97 (m, 2H), 3.76 (d, J = 9.4 Hz, 1H), 3.62 (m, 2H), 3.64 (s, 3H), 3.60 (s, 3H), 2.90−2.63 (m, 2H), 0.89 (s, 9H), 0.75 (s, 9H); ^{13}C NMR (68 MHz DMSO-d_6) δ 26.3, 26.7, 33.4, 33.6, 37.7, 51.4, 51.6, 60.7, 61.0, 61.3, 63.2, 68.3, 124.2, 124.8, 125.9, 127.5, 128.0, 129.1, 129.3, 131.3, 139.0, 141.6, 144.2, 144.7, 152.3, 156.5, 170.1; IR (1% KBr pellet) 3426, 2959, 1701, 1676, 1653, 1514, 1370, 1244, 1065, 777; $[\alpha]_D$ = −46.1 (c = 1, 1:1 MeOH/H_2O, pH = 2.6, 22 °C); Anal. Calcd for $C_{38}H_{52}N_6O_7 \cdot H_2SO_4 \cdot 0.35\ H_2O$: C, 56.40; H, 6.81; N 10.39; S, 3.96; H_2O, 0.78. Found: C, 56.54; H, 6.81; N 10.35; S, 3.98; H_2O, 0.74.

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Exhibit-6

(124)

US005849911A

United States Patent [19]

Fässler et al.

[11] Patent Number: 5,849,911

[45] Date of Patent: Dec. 15, 1998

[54] ANTIVIRALLY ACTIVE HETEROCYCLIC AZAHEXANE DERIVATIVES

[75] Inventors: **Alexander Fässler**, Macclesfield, Great Britain; **Guido Bold**, Gipf-Oberfrick; **Hans-Georg Capraro**, Rheinfelden, both of Switzerland; **Marc Lang**, Mulhouse, France; **Satish Chandra Khanna**, Bottmingen, Switzerland

[73] Assignee: **Novartis Finance Corporation**, Summit, N.J.

[21] Appl. No.: **831,630**

[22] Filed: **Apr. 9, 1997**

[30] **Foreign Application Priority Data**

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Jan. 31, 1997 [CH] Switzerland 0223/97

[51] **Int. Cl.**[6] **C07D 241/02**; C07D 263/34; A61K 31/42; A61K 277/54

[52] **U.S. Cl.** **544/335**; 544/406; 546/332; 548/204; 548/338.1; 548/335; 548/267.6; 548/247; 548/236; 549/76; 549/77; 514/365; 514/357; 514/438; 514/381; 514/255; 514/399; 514/400; 514/256; 514/383; 514/374; 514/378

[58] **Field of Search** 544/335, 406; 546/332; 548/204, 338.1, 335, 267.6, 247, 236; 549/76, 77; 514/365, 357, 438, 381, 255, 399, 400, 256, 383, 374, 378

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,556,654	12/1985	Showalter et al.	514/222
5,461,067	10/1995	Norbeck et al.	514/333
5,621,109	4/1997	Norbeck et al.	548/182

FOREIGN PATENT DOCUMENTS

0486948	5/1992	European Pat. Off.	C07D 213/26
9318006	9/1993	WIPO	C07D 243/08
9414436	7/1994	WIPO	A61K 31/425
9419332	9/1994	WIPO .	
9422840	10/1994	WIPO .	
9502582	1/1995	WIPO	C07D 255/02

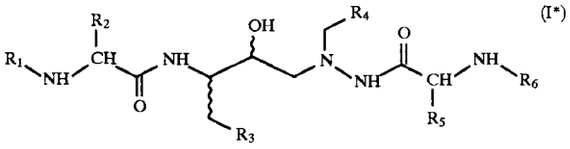
OTHER PUBLICATIONS

Sham, et al., J. Chem. Soc. Chem. Commun., 1993, p. 1052.
Fässler, et al., J. Med. Chem. vol. 39, 1996, pp. 3203–3216.
Fässler, et al., Bioorganic & Medicinal Chemistry Letters, vol. 3, No. 12, pp. 2837–2842.

Primary Examiner—S. Mark Clardy
Assistant Examiner—Sabiha N. Qazi
Attorney, Agent, or Firm—Hesna J. Pfeiffer

[57] **ABSTRACT**

There are described compounds of formula I*,



(I*)

wherein

R_1 is lower alkoxycarbonyl,

R_2 is secondary or tertiary lower alkyl or lower alkylthio-lower alkyl,

R_3 is phenyl that is unsubstituted or substituted by one or more lower alkoxy radicals, or C_4–C_8cycloalkyl,

R_4 is phenyl or cyclohexyl each substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl (—SO_2—) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl,

R_5, independently of R_2, has one of the meanings mentioned for R_2, and

R_6, independently of R_1, is lower alkoxycarbonyl,

or salts thereof, provided that at least one salt-forming group is present.

The compounds are inhibitors of retroviral aspartate protease and can be used, for example, in the treatment of AIDS. They exhibit outstanding pharmacodynamic properties.

11 Claims, No Drawings

ANTIVIRALLY ACTIVE HETEROCYCLIC AZAHEXANE DERIVATIVES

The invention relates to heterocyclic azahexane derivatives that can be employed as substrate isosteres of retroviral aspartate proteases, to salts thereof, to processes for the preparation of those compounds and their salts, to pharmaceutical compositions comprising those compounds or their salts, and to the use of those compounds or their salts (alone or in combination with other antiretrovirally active compounds) in the therapeutic or diagnostic treatment of the human or animal body or in the preparation of pharmaceutical compositions.

BACKGROUND TO THE INVENTION

According to WHO estimates there are clearly more than 20 million people infected by the "Human Immuno Deficiency Virus", HIV-1 or HIV-2. With very few exceptions, in infected subjects the disease results, by way of preliminary stages, such as ARDS, in a manifest disease of the immune system which is known as "Acquired Immunodeficiency Syndrome" or AIDS. In the overwhelming number of cases the disease sooner or later leads to the death of the infected patients.

Hitherto, the treatment of retroviral diseases, such as AIDS, has involved principally the use of inhibitors of reverse transcriptase, an enzyme effective in the conversion of retroviral RNA into DNA, such as 3'-azido-3'-deoxythymidine (AZT) or dideoxyinosine (DDI), and also trisodium phosphonoformate, ammonium-21-tungstenato-9-antimonate, 1-β-D-ribofuranoxyl-1,2,4-triazole-3-carboxamide and dideoxycytidine and also adriamycin. Attempts have also been made to introduce into the body, for example in the form of a recombinant molecule or molecule fragment, the T4-cell receptor which is present on certain cells of the defence system of the human body and is responsible for the anchoring and introduction of infectious virus particles into those cells and thus for their infection, the objective being that binding sites for the virus will be blocked so that the virions will no longer be able to bind to the cells. Compounds that prevent the virus penetrating the cell membrane in some other way, such as polymannoacetate, are also used.

The first inhibitor of so-called retroviral aspartate protease to be approved for combatting the infection was saquinavir, [N-tert-butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-2-quinolyl-carbonyl-L-asparaginyl]amino]butyl]-(4aS, 8aS)-isoquinoline-3(S)-carboxamide (Ro 31-8959)]. Since then others have followed (indinavir (Merck) and ritonavir (Abbott)).

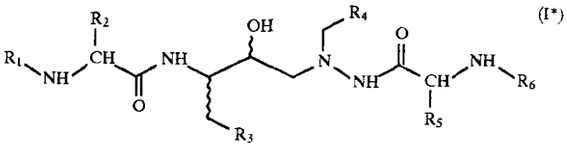
Also under development are a number of further inhibitors of retroviral aspartate protease, an enzyme the function of which can be characterised as follows:

In the AIDS viruses, HIV-1 and HIV-2, and other retroviruses, for example corresponding viruses in cats (FIV) and apes (SIV), the proteolytic maturation of, for example, the core proteins of the virus is brought about by an aspartate protease, such as HIV-protease. Without that proteolytic maturation, infectious virus particles cannot be formed. Owing to the central role of the said aspartate proteases, such as HIV-1- or HIV-2-protease, in the maturation of viruses and on the basis of experimental results, for example on infected cell cultures, it has become plausible that effective suppression of the maturation step brought about by that protease will suppress the assembly of mature virions in vivo. Inhibitors of that protease can therefore be used therapeutically.

The aim of the present invention is to provide a novel type of compound that is equipped, especially, with a high degree of inhibitory activity against virus replication in cells, high anti-viral activity against numerous virus strains, including those which are resistant to known compounds, such as saquinavir, ritonavir and indinavir, and especially advantageous pharmacological properties, for example good pharmacokinetics, such as high bioavailability and high blood levels, and/or high selectivity.

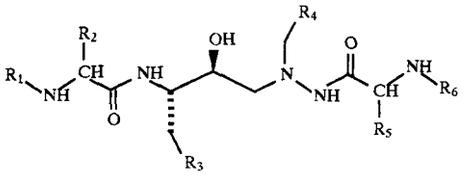
FULL DESCRIPTION OF THE INVENTION

The azahexane derivatives according to the invention are compounds of formula I*,



(I*)

especially of formula I,



(I)

wherein

R_1 is lower alkoxycarbonyl,

R_2 is secondary or tertiary lower alkyl or lower alkylthio-lower alkyl,

R_3 is phenyl that is unsubstituted or substituted by one or more lower alkoxy radicals, or C_4–C_8cycloalkyl,

R_4 is phenyl or cyclohexyl each substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl (—SO_2—) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl,

R_5, independently of R_2, has one of the meanings mentioned for R_2, and

R_6, independently of R_1, is lower alkoxycarbonyl,

or a salt thereof, provided that at least one salt-forming group is present.

Those compounds exhibit unexpectedly good and surprisingly positive pharmacological properties, as indicated in detail below, and are relatively simple to synthesise.

Unless indicated to the contrary, the general terms used hereinabove and hereinbelow preferably have the following meanings within the scope of this disclosure:

The term "lower" indicates a radical having up to and including a maximum of 7 carbon atoms, preferably up to and including a maximum of 4 carbon atoms, the radicals in question being unbranched or branched one or more times.

Lower alkyl and C_1–C_4alkyl are especially tert-butyl, sec-butyl, isobutyl, n-butyl, isopropyl, n-propyl, ethyl and methyl.

Any reference to compounds, salts and the like in the plural also includes a compound, a salt and the like.

Any asymmetric carbon atoms present, for example the carbon atoms bonded to the radicals R_2 and R_5, may be in the (R)—, (S)— or (R,S)-configuration, preferably in the

(R)— or (S)-configuration, the (S)-configuration being especially preferred in the case of the carbon atoms carrying the radical R_2 and/or R_5 in compounds of formula I. Accordingly, the compounds in question may be in the form of isomeric mixtures or in the form of pure isomers, preferably in the form of enantiomerically pure diastereoisomers.

Lower alkoxycarbonyl is preferably C_1–C_4alkoxycarbonyl wherein the alkyl radical may be branched or unbranched, and is especially ethoxycarbonyl or methoxycarbonyl.

Secondary or tertiary lower alkyl is especially sec-butyl, tert-butyl or isopropyl.

Lower alkylthio-lower alkyl is especially methylthiomethyl.

Phenyl that is unsubstituted or substituted by one or more lower alkoxy radicals is especially phenyl that is unsubstituted or substituted by from one to three lower alkoxy radicals, especially methoxy. In the case when there are three methoxy substituents, these are especially in the 2,3,4-positions of the phenyl ring and in the case when there is one methoxy substituent, that substituent is especially in the 2-, 3- or, more especially, in the 4-position. Unsubstituted phenyl is preferred.

C_4–C_8cycloalkyl is especially cyclopentyl or, more especially, cyclohexyl.

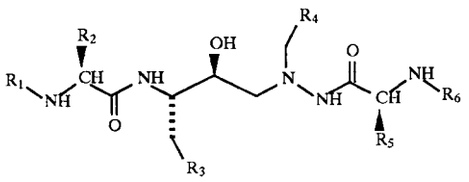
As R_3 phenyl is preferred to cyclohexyl.

In phenyl or cyclohexyl substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl ($—SO_2—$) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl, the corresponding heterocyclyl has especially the following meanings:

Unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl ($—SO_2—$) and is unsubstituted or substituted by lower alkyl, especially by methyl, or by phenyl-lower alkyl wherein the lower alkyl radical is unbranched or branched, especially by 1-methyl-1-phenylethyl, is especially one of the following radicals bonded by way of a ring carbon atom: thienyl (=thiophenyl); oxazolyl; thiazolyl; imidazolyl; 1,4-thiazinyl; triazolyl that is unsubstituted or, especially, substituted by 1-methyl-1-phenyl-ethyl or preferably by tert-butyl or especially by methyl, such as 1-, 2- or 4-(methyl or tert-butyl)triazol-3-yl; tetrazolyl that is unsubstituted or, especially, substituted by 1-methyl-1-phenylethyl or preferably by lower alkyl, such as by tert-butyl or especially by methyl, such as 2H-tetrazol-5-yl substituted by 1-methyl-1-phenyl-ethyl or preferably by lower alkyl, such as by tert-butyl or especially by methyl, or 1H-tetrazol-5-yl substituted by tert-butyl or especially by methyl; pyridinyl; pyrazinyl; and pyrimidinyl; more especially 2- or 3-thienyl (=thiophen-2-yl or thiophen-3-yl); thiazol-5-yl; thiazol-2-yl; 2H-tetrazol-5-yl that is unsubstituted or, especially, substituted in the 2-position by 1-methyl-1-phenyl-ethyl or preferably by tert-butyl or especially by methyl; 1H-tetrazol-5-yl substituted in the 1-position by methyl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; or pyrazin-2-yl.

R_4 is preferably phenyl substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl ($—SO_2—$) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl, wherein heterocyclyl preferably has the meanings defined above as being preferred.

The compounds of formula I preferably have the formula Ia,



(Ia)

wherein the radicals are as defined.

Salts are especially the pharmaceutically acceptable salts of compounds of formula I.

Such salts are formed, for example, by compounds of formula I having a basic R_4–CH_2-carrying nitrogen atom as acid addition salts, preferably with inorganic acids, for example hydrohalic acid, such as hydrochloric acid, sulfuric acid or phosphoric acid, or with strong organic sulfonic, sulfo or phosphoric acids or N-substituted sulfamic acids (preferably: pKa<1). Other salts may be present when basic heterocyclyl radicals, such as pyridyl, are present in R_4. Those salts includes especially acid addition salts with organic or inorganic acids, especially the pharmaceutically acceptable salts. Suitable inorganic acids are, for example, hydrohalic acids, such as hydrochloric acid, sulfuric acid and phosphoric acid. Suitable organic acids are, for example, carboxylic, phosphonic, sulfonic or sulfamic acids, for example acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, 2-hydroxybutyric acid, gluconic acid, glucosemonocarboxylic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, glucaric acid, galactaric acid, amino acids, such as glutamic acid, aspartic acid, N-methylglycine, acetylaminoacetic acid, N-acetylasparagine or N-acetylcysteine, pyruvic acid, acetoacetic acid, phosphoserine, 2- or 3-glycerophosphoric acid, glucose-6-phosphoric acid, glucose-1-phosphoric acid, fructose-1,6-bisphosphoric acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexanecarboxylic acid, adamantanecarboxylic acid, benzoic acid, salicylic acid, 1- or 3-hydroxynaphthyl-2-carboxylic acid, 3,4,5-trimethoxybenzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, mandelic acid, cinnamic acid, glucuronic acid, galacturonic acid, methanesulfonic or ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 1,5-naphthalenedisulfonic acid, 2-, 3- or 4-methylbenzenesulfonic acid, methylsulfuric acid, ethylsulfuric acid, dodecylsulfuric acid, N-cyclohexylsulfamic acid, N-methyl-, N-ethyl- or N-propyl-sulfamic acid, or other organic protonic acids, such as ascorbic acid.

When negatively charged radicals are present, such as tetrazolyl in R_4, salts may also be formed with bases, e.g. metal or ammonium salts, such as alkali metal or alkaline earth metal salts, e.g. sodium, potassium, magnesium or calcium salts or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, e.g. triethylamine or tri(2-hydroxyethyl)-amine, or heterocyclic bases e.g. N-ethyl-piperidine or N,N'-dimethylpiperazine.

For the purposes of isolation or purification it is also possible to use pharmaceutically unsuitable salts, for example picrates or perchlorates. Only the pharmaceutically acceptable salts or the free compounds (optionally in the

form of pharmaceutically compositions) are used therapeutically and they are therefore preferred.

In view of the close relationship between the novel compounds in free form and in the form of their salts, including those salts that can be used as intermediates, for example in the purification of the novel compounds or for identifying them, hereinbefore and hereinafter any reference to the free compounds should be understood as including the corresponding salts as appropriate and expedient.

The compounds of formula I have valuable pharmacological properties. They have antiretroviral activity, especially against the viruses HIV-1 and HIV-2 which are regarded as causes of AIDS, and may surprisingly exhibit synergistic effects in combination with other compounds that are active against retroviral aspartate proteases. The compounds of formula I are inhibitors of retroviral aspartate proteases, especially inhibitors of the aspartate protease of HIV-1 or also HIV-2 and are therefore suitable for the treatment of retroviral diseases, such as AIDS or its preliminary stages (e.g. ARDS). Compounds of formula I also exhibit activity against corresponding animal retroviruses, such as SIV (in apes) or FIV (in cats).

Compounds of formula I exhibit, surprisingly, especially advantageous and important pharmacological properties, for example a very high antiviral activity in cell tests against various virus strains, including those which are resistant to other protease inhibitors, for example in MT2-cells, good pharmacokinetics, such as high bioavailability, high selectivity and, especially, high blood levels (even in the case of oral administration).

The inhibitory action of the compounds of formula I on the proteolytic activity of HIV-1-protease can be shown, for example, according to known procedures (see A. D. Richards et al., J. Biol. Chem. 265(14), 7733–7736 (1990)). In that method the inhibition of the action of HIV-1-protease (preparation: see S. Billich et al., J. Biol. Chem. 263(34), 17905–17908 (1990)) is measured in the presence of the icosapeptide RRSNQVSQNYPIVQNIQGRR (a synthetic substrate of HIV-1-protease, prepared by peptide synthesis in accordance with known procedures (see J. Schneider et al., Cell 54, 363–368 (1988)), which contains as substrate analogue one of the cleavage sites of the gag-precursor protein (natural substrate of HIV-1-protease). That substrate and its cleavage products are analysed by high performance liquid chromatography (HPLC).

The test compound is dissolved in dimethyl sulfoxide. The enzymatic test is carried out by adding suitable dilutions of the inhibitor in 20 mM β-morpholinoethanesulfonic acid (MES) buffer pH 6.0 to the test mixture. That mixture consists of the above-mentioned icosapeptide (122 μM) in 20 mM MES-buffer pH 6.0. 100 μl are used per test batch. The reaction is started by the addition of 10 ml of HIV-1-protease solution and is stopped after one hour's incubation at 37° C. by the addition of 10 μl of 0.3M $HClO_4$. After centrifugation of the sample at 10 000×g for 5 minutes, 20 μl of the resulting supernatant are applied to a 125×4.6 mm Nucleosil® C18-5m-HPLC column (reversed-phase material supplied by Macherey & Nagel, Duren, FRG, based on silica gel that has been charged with C_{18}alkyl chains). The uncleaved icosapeptide and its cleavage products are eluted from the column by means of the following gradient: 100 % eluant 1→50% eluant 1+50% eluant 2 (eluant 1:10% acetonitrile, 90% H_2O, 0.1% trifluoroacetic acid (TFA); eluant 2:75% acetonitrile, 25% H_2O, 0.08% TFA) for 15 minutes, throughflow rate 1 ml/min. The quantification of the eluted peptide fragments is carried out by measuring the peak height of the cleavage product at 215 nm.

Compounds of formula I exhibit inhibitory actions in the nanomolar range; they preferably exhibit IC_{50} values (IC_{50}= that concentration which brings about a 50% reduction in the activity of HIV-1-protease in comparison with a control without inhibitor) of approximately 2×10^{-7} to 5×10^{-9}M, preferably 5×10^{-8} to 5×10^{-9}M.

An alternative method (see Matayoshi et al., Science 247, 954–958 (1990), here modified) of determining the inhibitory action against HIV-1-protease may be described briefly as follows: the protease (purification: see Leuthardt et al., FEBS Lett. 326, 275–80 (1993)) is incubated at room temperature in 100 μl of assay buffer (20 mM MES pH 6.0; 200 mM NaCl; 1 mM dithiothreitol; 0.01% polyethylene glycol (average molecular weight 6000 to 8000 da) with 10 μM fluorogenic substrate SC4400 (4-(4-dimethylaminophenylazo)benzoyl-γ-aminobutyryl-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS (EDANS=5-(2-aminoethylamino)-1-naphthalenesulfonic acid); Neosystem Laboratoire, France). The reaction is discontinued by the addition of 900 μl of 0.03M $HClO_4$. The HIV-1-protease activity is determined by measuring the increase in fluorescence at λex=336, λem=485 nm. The IC_{50} values of compounds of formula I are determined as the concentration of the compound that is necessary to inhibit the protease activity in the assay by 50%. The numerical values are obtained from computer-generated graphs from data relating to at least 5 concentrations of the compound of formula I in question with threefold determination per concentration.

In a further test it can be shown that compounds of formula I protect cells normally infected by HIV from such an infection or at least slow down such an infection. For this test, MT-2-cells infected with HIV-1/MN are used. MT-2-cells have been transformed with HTLV-1 (a virus causing leukaemia) and a continuous producer thereof; they are therefore especially sensitive to the cytopathogenic effect of HIV. MT-2-cells can be obtained via the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH from Dr. Douglas Richman (see J. Biol. Chem. 263, 5870–5875 (1988) and also Science 229, 563–566 1985)). The MT-2-cells are cultured in RPMI 1640-medium (Gibco, Scotland; RPMI comprises an amino acid mixture without glutamine) supplemented with 10% heat-inactivated foetal calf serum, glutamine and standard antibiotics. In all cases the cells, and also the virus stock solution used for the infection (HIV-1/MN), are free of mycoplasms. The virus stock solution is prepared as a cell culture supernatant of the permanently infected cell line H9/HIV-1/MN, which can likewise be obtained via the AIDS Research and Reference Program, Division of AIDS, NIAID, NIH from Dr. Robert Gallo (see also Science 224, 500–503 (1984) and Science 226, 1165–1170 (1984)). The titre of the HIV-1/MN virus stock solution (determined by titration onto MT-2-cells) is 4.2×10^5 TCID50/ml (TCID50=Tissue Culture Infective Dose=dose that infects 50% of the MT-2-cells). In order to measure the infection-inhibiting action of the compounds of formula I, 50 μl of the test compound in question in culture medium and 2800 TCID50 of HIV-1/MN in 100 μl of culture medium are added to 2×10^4 exponentially growing MT-2-cells which have been applied in 50 μl of culture medium to 96-well microtitre plates (having a round base). After 4 days' incubation (at 37° C., 5% CO_2) a 10 μl sample of the supernatant is taken from each well, transferred to a further 96-well microtitre plate and (if necessary) stored at −20° C. In order to measure the activity of the virus-associated reverse transcriptase, 30 μl of reverse transcriptase (RT) cocktail are added to each sample. The reverse transcriptase cocktail consists of 50 mM Tris (α,α,α-tris(hydroxymethyl)

methylamine, Ultra pur, Merck, Germany) pH 7.8; 75 mM KCl, 2 mM dithiothreitol, 5 mM $MgCl_2$; 0.1% Nonidet P-40 (detergent; Sigma, Switzerland), 0.8 mM EDTA, 10 μg/ml Poly-A (Pharmacia, Uppsala, Sweden) and 0.16 μg/ml oligo (T) (=pdT(12–18), Pharmacia, Uppsala, Sweden) as "template primer"—if desired, the mixture is filtered through a 0.45 mm Acrodisc filter (Gelman Sciences Inc., Ann Arbor, USA). It is stored at −20° C. Prior to the test, 0.1% (v/v) [alpha-^{32}P]dTTP is added to aliquots of the solution in order to establish a radioactivity of 10 μCi/ml.

After mixing, the plate is incubated for 2 hours at 37° C. 5 μl of the reaction mixture are transferred to DE81 paper (Whatman, one filter per well). The dried filters are washed three times for 5 minutes with 300 mM NaCl/25 mM trisodium citrate and then once with ethanol and again dried in the air. The radioactivity on the filters is measured in a Matrix Packard 96-well counter (Packard, Zürich, Switzerland). The ED_{90} values are calculated and are defined as the concentration of the test compound that reduces the RT activity by 90% in comparison with a control without test compound.

The preferred compounds of formula I here exhibit an ED_{90}, that is to say a 90% inhibition of virus replication, at concentrations of from 10^{-7} to 10^{-9}M, especially from 5×10^{-9} to 10^{-9}M.

Accordingly, the compounds of formula I are suitable for the highly effective retardation of the replication of HIV-1 in cell cultures.

In order to determine their pharmacokinetics, the compounds of formula I are dissolved in dimethyl sulfoxide (DMSO) in a concentration of 240 mg/ml. The resulting solutions are diluted 1:20 (v/v) with 20% (w/v) aqueous hydroxypropyl-β-cyclodextrin solution in order to obtain a concentration of the test compound in question of 12 mg/ml. The resulting solution is treated briefly with ultrasound and administered orally to female BALB/c mice (Bomholtgarden, Copenhagen, Denmark) by artificial tube feeding at a dose of 120 mg/kg. At fixed times (for example 30, 60, 90, 120 min) after administration, mice are sacrificed and the plasma stored in heparinised test tubes. The blood is centrifuged (12 000×g, 5 min) and the plasma removed. The plasma is deproteinised by the addition of an equal volume of acetonitrile. The mixture is mixed using a vortex mixer and and left to stand at room temperature for 20 to 30 minutes. The precipitate is pelleted by centrifugation (12 000×g, 5 min), and the concentration of the test compound is determined by reversed phase high performance liquid chromatography (HPLC).

The HPLC analysis of the samples obtained in accordance with the method described above is carried out on a 125×4.6 mm Nucleosil® C_{18}-column (reversed-phase material supplied by Macherey & Nagel, Düren, Germany, based on silica gel derivatised with carbon radicals having 18 carbon atoms), using a 2 cm long preliminary column of the same column material. The test is carried out with the following linear acetonitrile/water gradient (in each case in the presence of 0.05% trifluoroacetic acid): 20% acetonitrile to 100% acetonitrile for 20 min; then 5 min 100% acetonitrile; then returning to the initial conditions for 1 min and 4 min reequilibration. The flow rate is 1 ml/min. Under those conditions the compound of formula I from Example 1, for example, has a retention time of about 15.5 minutes, and its detection limit is 0.1–0.2 μM. The test compound is detected by UV absorption measurement at 255 nm. Peaks are identified by the retention time and the UV spectrum between 205 and 400 nm. The concentrations are determined by the external standard method; the peak heights are obtained for determining the concentrations by comparison with standard curves. The standard curves are obtained by analogous HPLC analysis of mouse plasma that contains known concentrations of the test compound in question and that has been worked up in accordance with the method described above.

In that experiment compounds of formula I produce plasma concentrations far above the ED_{90} determined above in the cell experiment, for example up to 8000 times greater than the ED_{90} after 30 minutes and up to 10 500 times greater than the ED_{90} after 90 minutes, preferably plasma concentrations of from 0.1 μM to 25 μM, especially from 1 to 25 μM, 30 minutes after oral administration, and plasma concentrations of from 0.5 to 35 μM, especially from 1 to 35 μM, 90 minutes after oral administration.

Analogously, in dogs, the blood level of the compounds of formula I, for example the title compound of Example 46, can be measured, for example, using the formulations according to either Example 63 or Example 64, there being used, for example, from 92 to 100 mg/kg of the compound which is administered by stomach tube, the blood levels then being measured, e.g. 1, 2, 3, 4, 6, 8 and 24 hours after administration. Here, also, blood levels in the micromolar range can be found.

In particular, the combination of high bioavailability (high plasma levels), which is surprising in itself, and unexpectedly excellent ED_{90} in the cell experiment renders the compounds of the present invention valuable in an unforeseen way. Activity against inhibitors of retroviral aspartate proteases to which resistance has already developed is also still possible and is a further important advantage of the compounds according to the invention.

That can be demonstrated, for example, by the following or analogous tests: Inhibitor-resistant HIV-1 protease variants are cloned as follows: By way of PCR-supported mutagenesis and cloning, HIV-1 protease mutants are generated that are based on the infectious clone pNL4-3 (freely available via the "NIH AIDS reference and reagent program", the original reference is A. Adachi et al. J. Virol (1986) 59, 284–91—but it can, of course, be any other HIV clone, or even clinical material, provided that comparability is ensured). Those otherwise isogenic point mutants contain only those changes which have been described in publications in connection with viral resistance to various protease inhibitors. The cloned fragments are, for example, only 500 base pairs in length, all of the remainder being unchanged. By using mutations in always the same clone, direct comparability is ensured, which would not be the case in a direct comparison of clinical samples or of different HIV clones. In the transient DNA transfection assay in human T4-positive cells (HeLaT4), the resulting proviruses also demonstrate the finding of reduced inhibitor activity in comparison with the wild type virus, that is to say increased resistance. That system is used as a transient DNA transfection system for tests:

1) in order to identify possible cross-resistances of protease variants to several protease inhibitors; and
2) in order to establish the potency and resistance profile of novel inhibitor candidates.

For example, in the said transfection system 1-[4-(pyridin-2-yl)phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane (Example 46) has an in vitro potency that, with an IC90 of <30 nM, is in practical terms better than that of saquinavir (Hoffmann-LaRoche, see below) and the activity against a resistant variant (45I/76F strain) which has been established against 5(S)-(tert-butoxycarbonylamino)-4(S)-

hydroxy-6-phenyl-2(R)-(2,3,4-trimethoxyphenylmethyl)-hexanoyl-(L)-valyl-N-(2-methoxyethyl)-amide (=Lasinavir, see EP 0 708 085, published on 24, Apr. 1996; Novartis AG, originally Ciba-Geigy AG), is comparable with saquinavir and better than that of indinavir (Merck & Co., Inc., see below) or ritonavir (Abbott, see below). Compared with other strains (e.g. 46I/47V/50V (VX478)), 10 nM produced an activity that was more potent (not quantified) than that of saquinavir, ritonavir and indinavir. Instead of the strains mentioned, there may be used any human T4-positive cells, such as the HeLa T4 cells, deposited under that name by Richard Axel and Paul Maddon in "NIH AIDS reference and reagent program" and obtainable via that source.

In principle, the relevant mutations for the above test systems for resistances are known (see e.g. relating to the 48V/90M strain (saquinavir resistance): Jacobsen, H., Yasargil, K., Winslow, D. L., Craig, J. C., Krohn, A., Duncan, I. B., & Mous, J. Virology 206, 527 (1995); Merck Mutationen (several, e.g. 71V/82T/84V): Condra, J. H., Schleif, W. A., Blahy, O. M., Gabryelski, L. J., Graham, D. J., Quintero, J. C., Rhodes, A., Robbins, H. L., Roth, E., Shivaprakash, M., & et al Nature 374, 569 (1995); Abbott 82V/84A strain: Markowitz, M., Mo, H., Kempf, D. J., Norbeck, D. W., Bhat, T. N., Erickson, J. W., & Ho, D. D. J. Virol. 69, 701 (1995).

In the determination of the anti-enzymatic activity against numerous human aspartate proteases in accordance with known methods (see, for example, Biochem. J. 265, 871–878 (1990)), compounds of formula I exhibit a high selectivity towards the retroviral aspartate protease of HIV, especially HIV-1. For example, the inhibition constant (IC_{50}) for compounds of formula I in the test against cathepsin D is more than 10 μM, especially more than 25 μM. The IC_{50} against human cathepsin D in that test is measured at pH 3.1. The test is carried out in accordance with known procedures using the substrate KPIQF*NphRL (see Jupp, R. A., Dunn, B. M., Jacobs, J. W., Vlasuk, G., Arcuri, K. E., Veber, D. F., S. Perow, D. S., Payne, L. S., Boger, J., DeLazlo, S., Chakrabarty, P. K., TenBroeke, J., Hangauer, D. G., Ondeyka, D., Greenlee, W. J. and Kay, J.: The selectivity of statine-based inhibitors against various human aspartic proteases. Biochem. J. 265: 871–878 (1990)).

The compounds of formula I can be used alone or in combination (as a set combination of corresponding compositions or as a combination of individual compounds or individual compositions in a time-staggered sequence) with one or more other pharmaceutically active substances (or salts thereof provided that at least one salt-forming group is present) that are effective against retroviruses, especially HIV, such as HIV-1 or HIV-2; especially with inhibitors of reverse transcriptase, more especially nucleoside analogues, especially 3'-azido-3'-deoxypyrimidine (=zidovudine=®RETROVIR, Burroughs-Wellcome), 2',3'-dideoxycytidine (=zalcitabine=®HIVID, Hoffmann-LaRoche), 2',3'-dideoxyinosine (=didanosine=®VIDEX, Bristol-Myers-Squibb) or (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (=lamivudine, Glaxo); especially d4C=2',3'-didehydro-2',3'-dideoxycytidine, d4T=2',3'-didehydro-2',3'-dideoxythymidine (=stavudine =®ZERIT) or 2',3'-dideoxyinosine (=ddlno =DZI=®didanosine=®VIDEX); or non-nucleoside analogues, such as 11-cyclopropyl-5,11-dihydro-4-methyl-(6H)-dipyrido[3,2-b;2',3'-e]-[1,4]-diazepin-6-one; or with one or more (especially one or also two) other inhibitors of retroviral aspartate proteases, especially aspartate proteases of HIV, such as HIV-1 and HIV-2, especially

a) one of the inhibitors mentioned in EP 0 346 847 (published on 20, Dec. 1989) and EP 0 432 695 (published on 19, Jun. 1991; corresponds to U.S. Pat. No. 5,196,438, published on 23, Mar. 1993), especially the compound designated Ro 31-8959 (=saquinavir; Hoffmann-LaRoche);

b) one of the inhibitors mentioned in EP 0 541 168 (published on 12, May 1993; corresponds to U.S. Pat. No. 5,413,999), especially the compound designated L-735,524 (=indinavir=®CRIXIVAN; Merck & Co., Inc.);

c) one of the inhibitors mentioned in EP 0 486 948 (published on 27, May 1992; corresponds to U.S. Pat. No. 5,354,866), especially the compound designated ABT-538 (=ritonavir; Abbott);

d) the compound designated KVX-478 (or VX-478 or 141W94; GlaxoWellcome, Vertex and Kissei Pharmaceuticals)

e) the compound designated AG-1343 (Agouron);

f) the compound designated KNI-272 (Nippon Mining);

g) the compound designated U-96988 (Upjohn);

h) the compound designated BILA-2011 BS (=palinavir; Boehringer-Ingelheim), and/or

I) the compound 5(S)-(tert-butoxycarbonylamino)-4(S)-hydroxy-6-phenyl-2(R)-(2,3,4-trimethoxyphenylmethyl)-hexanoyl-(L)-valyl-N-(2-methoxy-ethyl)-amide (=lasinavir, see EP 0 708 085, published on 24, Apr. 1996; Novartis AG, originally Ciba-Geigy AG),

or in each case a salt thereof, provided that salt-forming groups are present.

The compounds of formula I can also be used in the prevention, control and treatment of retrovirus infections, especially HIV, such as HIV-1 or HIV-2, in cell cultures, especially cell cultures of lymphocyte cell lines, from warm-blooded animals, which is advantageous especially in the case of very valuable cell cultures that produce, for example, specific antibodies, vaccines or messenger substances, such as interleukins and the like, and are therefore of great commercial value.

Finally, the compounds of formula I can be used as standards in experiments, for example as HPLC standards or as standards for the comparison of animal models in respect of different aspartate protease inhibitors, for example in respect of the blood levels achievable.

In the groups of preferred compounds of formula I mentioned below, it is possible where expedient (for example in order to replace more general definitions by more specific definitions or, especially, by definitions described as being preferred) to use definitions of substituents from the general definitions given above; in each case preference is given to the definitions described above as being preferred or given as examples.

Preference is given to a compound of formula I, especially of formula Ia, wherein

R_1 is lower alkoxycarbonyl, especially methoxycarbonyl or ethoxycarbonyl,

R_2 is isopropyl, sec-butyl (preferably in the (S)-configuration), or tert-butyl,

R_3 is phenyl or also cyclohexyl,

R_4 is phenyl substituted in the 4-position by one of the following radicals bonded by way of a ring carbon atom: thienyl (=thiophenyl); oxazolyl; thiazolyl; imidazolyl; 1,4-thiazinyl; triazolyl that is unsubstituted or, especially, substituted by 1-methyl-1-phenyl-ethyl or

preferably by tert-butyl or especially by methyl, such as 1-, 2- or 4-(methyl or tert-butyl)triazol-3-yl; tetrazolyl that is unsubstituted or, especially, substituted by 1-methyl-1-phenylethyl or preferably by lower alkyl, such as by tert-butyl or especially by methyl, such as 2H-tetrazol-5-yl substituted by 1-methyl-1-phenyl-ethyl or preferably by lower alkyl, such as by tert-butyl or especially by methyl, or 1H-tetrazol-5-yl substituted by methyl; pyridinyl; pyrazinyl; and pyrimidinyl; especially 2- or 3-thienyl (=thiophen-2-yl or thiophen-3-yl); thiazol-5-yl; thiazol-2-yl; 2H-tetrazol-5-yl that is unsubstituted or, especially, substituted in the 2-position by 1-methyl-1-phenyl-ethyl or preferably by tert-butyl or especially by methyl; 1H-tetrazol-5-yl substituted in the 1-position by methyl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; or pyrazin-2-yl;

R_5 is isopropyl, sec-butyl (preferably in the (S)-configuration), tert-butyl or methylthiomethyl, and

R_6 is lower alkoxycarbonyl, especially methoxycarbonyl or ethoxycarbonyl,

or a salt thereof (especially a pharmaceutically acceptable salt thereof, provided that at least one salt-forming group is present.

Greater preference is given to a compound of formula I, wherein

R_1 is methoxycarbonyl or ethoxycarbonyl,

R_2 is isopropyl, sec-butyl or tert-butyl,

R_3 is phenyl,

R_4 is phenyl substituted in the 4-position of the phenyl ring by 2- or 3-thienyl (=thiophen-2-yl or thiophen-3-yl); thiazol-5-yl; thiazol-2-yl; 2H-tetrazol-5-yl that is unsubstituted or, especially, substituted in the 2-position by 1-methyl-1-phenyl-ethyl or preferably by tert-butyl or especially by methyl; 1H-tetrazol-5-yl substituted in the 1-position by methyl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; or by pyrazin-2-yl; and is especially 4-(thiazol-2-yl)-phenyl; 4-(thiazol-5-yl)-phenyl; 4-(pyridin-2-yl)-phenyl; or 4-(2-methyl-tetrazol-5-yl)-phenyl;

R_5 is isopropyl, sec-butyl, tert-butyl or methylthiomethyl; and

R_6 is methoxycarbonyl or ethoxycarbonyl;

with the proviso that at least one of the two radicals R_2 and R_5 is tert-butyl, provided that R_4 is phenyl substituted in the 4-position of the phenyl ring by 2- or 3-hienyl (=thiophen-2-yl or thiophen-3-yl), thiazol-5-yl; thiazol-2-yl; 2H-tetrazol-5-yl that is unsubstituted or, especially, substituted in the 2-position by 1-methyl-1-phenyl-ethyl or preferably by tert-butyl or especially by methyl; 1H-tetrazol-5-yl substituted in the 1-position by methyl; pyridin-3-yl; pyridin-4-yl; or by pyrazin-2-yl;

or a (preferably pharmaceutically acceptable) salt thereof, provided that at least one salt-forming group is present.

Special preference is given to a compound of formula I, wherein

R_1 is methoxycarbonyl or ethoxycarbonyl,

R_2 is isopropyl, sec-butyl or tert-butyl,

R_3 is phenyl,

R_4 is 4-(thiazol-2-yl)-phenyl, 4-(thiazol-5-yl)-phenyl, 4-(pyridin-2-yl)-phenyl or 4-(2-methyltetrazol-5-yl)-phenyl;

R_5 is isopropyl, sec-butyl, tert-butyl or methylthiomethyl; and

R_6 is methoxycarbonyl or ethoxycarbonyl;

or a (preferably pharmaceutically acceptable) salt thereof, provided that at least one salt-forming group is present.

Each of the compounds of formula I mentioned below, or a (preferably pharmaceutically acceptable) salt thereof, is highly preferred:

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-valyl)amino-5(S)-N(-N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-S-methylcysteinyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane;

1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(2-methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane;

1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane;

1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane;

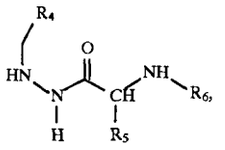
1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane; or

1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane.

Special preference is given to the compounds of formula I mentioned in the Examples, or to pharmaceutically acceptable salts thereof provided that at least one salt-forming group is present.

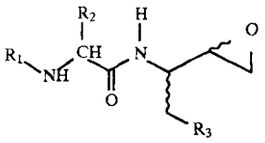
The compounds of formula I and salts of those compounds having at least one salt-forming group are prepared according to Processes known per se, for example as follows:

a) a hydrazine derivative of formula



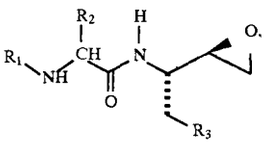
(III)

wherein the radicals R_4, R_5 and R_6 are as defined for compounds of formula I, is added to an epoxide of formula IV*,



(IV*)

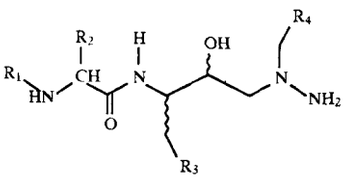
especially of formula IV,



(IV)

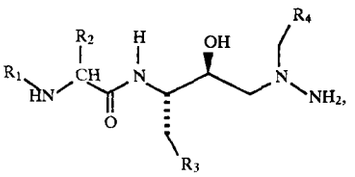
wherein the radicals R_1, R_2 and R_3 are as defined for compounds of formula I, free functional groups with the exception of those participating in the reaction being, if necessary, in protected form, and any protecting groups are removed, or

b) an amino compound of formula V*,



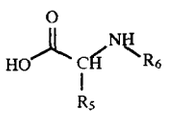
(V*)

especially of formula V



(V)

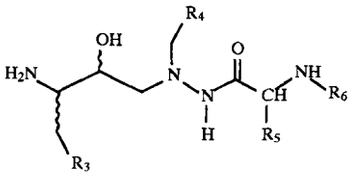
wherein the radicals R_1, R_2, R_3 and R_4 are as defined for compounds of formula I, is condensed with an acid of formula



(VI)

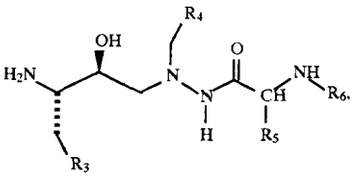
or with a reactive acid derivative thereof, wherein the radicals R_5 and R_6 are as defined for compounds of formula I, free functional groups with the exception of those participating in the reaction being, if necessary, in protected form, and any protecting groups are removed, or

c) an amino compound of formula VII*,



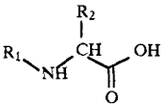
(VII*)

especially of formula VII



(VII)

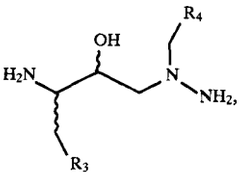
wherein the radicals R_3, R_4, R_5 and R_6 are as defined for compounds of formula I, is condensed with an acid of formula



(VIII)

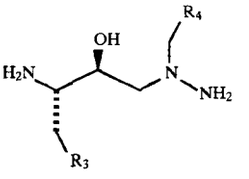
or with a reactive acid derivative thereof, wherein R_1 and R_2 are as defined for compounds of formula I, free functional groups with the exception of those participating in the reaction being, if necessary, in protected form, and any protecting groups are removed, or

d) to prepare a compound of formula I wherein the pairs of substituents R_1 and R_6 and R_2 and R_5 are in each case two indentical radicals, as defined for compounds of formula I, and R_3 and R_4 are as defined for compounds of formula I, a diamino compound of formula IX*



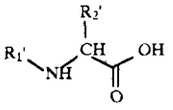
(IX*)

especially of formula IX,



(IX)

wherein the radicals are as just defined, is condensed with an acid of formula

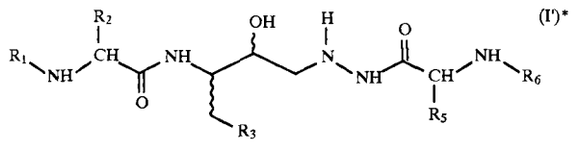


(VIIIa)

or with a reactive acid derivative thereof, wherein R_1' and R_2' are as defined for R_1 and R_6 and for R_2 and R_5, respectively, in formula I, the pairs R_1 and R_6 and R_2

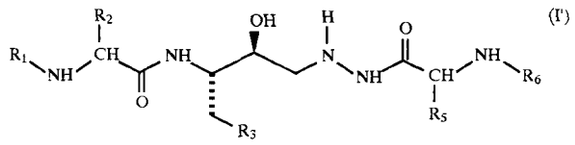
and R_5 being in each case two identical radicals, free functional groups with the exception of those participating in the reaction being, if necessary, in protected form, and any protecting groups are removed, or

e) an imino compound of formula (I')*,



(I')*

especially of formula I'



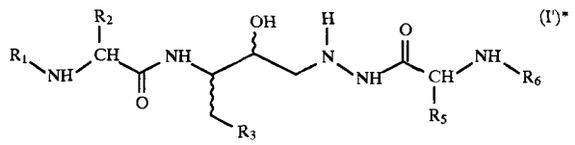
(I')

wherein the radicals R_1, R_2, R_3, R_5 and R_6 are as defined for compounds of formula I, is reacted with a compound of formula X,

(X)

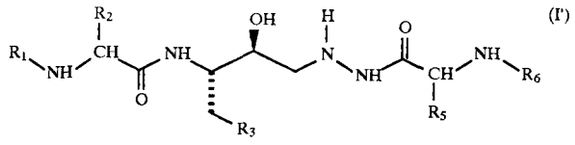
wherein X is a leaving group and R_4 is as defined for compounds of formula I, free functional groups with the exception of those participating in the reaction being, if necessary, in protected form, and any protecting groups are removed, or

f) an imino compound of formula (I')*,



(I')*

especially of formula I'



(I')

wherein the radicals R_1, R_2, R_3, R_5 and R_6 are as defined for compounds of formula I, is reacted with an aldehyde of formula X*,

(X*)

wherein R_4 is as defined for compounds of formula I, or with a reactive derivative thereof, with reductive alkylation, free functional groups with the exception of those participating in the reaction being, if necessary, in protected form, and any protecting groups are removed,

and, if desired, a compound of formula I having at least one salt-forming group obtainable in accordance with any one of processes a) to f) above is converted into its salt or an obtainable salt is converted into the free compound or into a different salt and/or isomeric mixtures which may be obtainable are separated and/or a compound of formula I according to the invention is converted into a different compound of formula I according to the invention.

The above processes are described in more detail below with reference to preferred embodiments.

In the following description of the individual processes and the preparation of the starting materials, unless otherwise indicated the radicals R_1, R_2, R_3, R_4, R_5 and R_6 are as defined for compounds of formula I, preference being given in each case to the definitions given as being preferred.

Process a) (Addition of an Amine to an Epoxide):

In the hydrazine derivatives of formula III, the amino group participating in the reaction preferably has a free hydrogen atom; it may, however, itself have been derivatised in order to increase the reactivity of the hydrazine derivative.

The epoxide of formula IV enables the terminal addition of the hydrazine derivative to proceed preferentially.

In starting materials, functional groups the reaction of which is to be avoided, especially carboxy, amino and hydroxy groups, can be protected by suitable protecting groups (conventional protecting groups) which are customarily used in the synthesis of peptide compounds, and also in the synthesis of cephalosporins and penicillins as well as nucleic acid derivatives and sugars. Those protecting groups may already be present in the precursors and are intended to protect the functional groups in question against undesired secondary reactions, such as acylation, etherification, esterification, oxidation, solvolysis and the like. In certain cases the protecting groups can additionally cause reactions to proceed selectively, for example stereoselectively. It is characteristic of protecting groups that they can be removed easily, i.e. without undesired secondary reactions taking place, for example by solvolysis, reduction, photolysis, and also enzymatically, for example also under physiological conditions. Radicals analogous to protecting groups may also be present in the end products, however. Compounds of formula I having protected functional groups may have greater metabolic stability or pharmacodynamic properties that are better in some other way than the corresponding compounds having free functional groups. Hereinabove and hereinbelow, protecting groups are referred to in their true sense when the radicals in question are not present in the end products.

The protection of functional groups by such protecting groups, the protecting groups themselves and the reactions for their removal are described, for example, in standard works such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in Th. W. Greene, "Protective Groups in Organic Synthesis", Wiley, New York 1981, in "The Peptides", Volume 3 (E. Gross and J. Meienhofer, eds.), Academic Press, London and New York 1981, in "Methoden der organischen Chemie" ("Methods of Organic Chemistry"), Houben-Weyl, 4th edition, Volume 15/l, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, "Aminosäuren, Peptide, Proteine" ("Amino acids, peptides, proteins"), Verlag Chemie, Weinheim, Deerfield Beach and Basle 1982, and in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide und Derivate" ("The Chemistry of Carbohydrates: monosaccharides and derivatives"), Georg Thieme Verlag, Stuttgart 1974.

A carboxy group is protected, for example, in the form of an ester group which can be cleaved selectively under mild conditions. A carboxy group protected in esterified form is esterified especially by a lower alkyl group that is preferably branched in the 1-position of the lower alkyl group or substituted in the 1- or 2-position of the lower alkyl group by suitable substituents.

A protected carboxy group esterified by a lower alkyl group is, for example, methoxycarbonyl or ethoxycarbonyl.

A protected carboxy group esterified by a lower alkyl group that is branched in the 1-position of the lower alkyl group is, for example, tert-lower alkoxycarbonyl, for example tert-butoxycarbonyl.

A protected carboxy group esterified by a lower alkyl group that is substituted in the 1- or 2-position of the lower alkyl group by suitable substituents is, for example, arylmethoxycarbonyl having one or two aryl radicals, wherein aryl is phenyl that is unsubstituted or mono-, di- or trisubstituted, for example, by lower alkyl, for example tert-lower alkyl, such as tert-butyl, lower alkoxy, for example methoxy, hydroxy, halogen, for example chlorine, and/or by nitro, for example benzyloxycarbonyl, benzyloxycarbonyl substituted by the mentioned substituents, for example 4-nitrobenzyloxycarbonyl or 4-methoxybenzyloxycarbonyl, diphenylmethoxycarbonyl or diphenylmethoxycarbonyl substituted by the mentioned substituents, for example di(4-methoxyphenyl)methoxycarbonyl, and also carboxy esterified by a lower alkyl group, the lower alkyl group being substituted in the 1- or 2-position by suitable substituents, such as 1-lower alkoxy-lower alkoxycarbonyl, for example methoxymethoxycarbonyl, 1-methoxyethoxycarbonyl or 1-ethoxyethoxycarbonyl, 1-lower alkylthio-lower alkoxycarbonyl, for example 1-methylthiomethoxycarbonyl or 1-ethylthioethoxycarbonyl, aroylmethoxycarbonyl wherein the aroyl group is benzoyl that is unsubstituted or substituted, for example , by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromoethoxycarbonyl or 2-iodoethoxycarbonyl, as well as 2-(tri-substituted silyl)-lower alkoxycarbonyl wherein the substituents are each independently of the others an aliphatic, araliphatic, cycloaliphatic or aromatic hydrocarbon radical that is unsubstituted or substituted, for example, by lower alkyl, lower alkoxy, aryl, halogen and/or by nitro, for example lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl each of which is unsubstituted or substituted as above, for example 2-tri-lower alkylsilyl-lower alkoxycarbonyl, such as 2-tri-lower alkylsilylethoxycarbonyl, for example 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methylsilyl)-ethoxycarbonyl, or 2-triarylsilylethoxycarbonyl, such as triphenyisilylethoxycarbonyl.

A carboxy group may also be protected in the form of an organic silyloxycarbonyl group. An organic silyloxycarbonyl group is, for example, a tri-lower alkylsilyloxycarbonyl group, for example trimethylsilyloxycarbonyl.

A protected carboxy group is preferably tert-lower alkoxycarbonyl, for example tert-butoxycarbonyl, benzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 9-fluorenylmethoxycarbonyl or diphenylmethoxycarbonyl.

A protected amino group may be protected by an amino-protecting group, for example in the form of an acylamino, arylmethylamino, etherified mercaptoamino, 2-acyl-lower alk-1-enylamino or silylamino group , or in the form of an azido group.

In a corresponding acylamino group, acyl is, for example, the acyl radical of an organic carboxylic acid having, for example, up to 18 carbon atoms, especially an unsubstituted or substituted, for example halo- or aryl-substituted, lower alkanecarboxylic acid or an unsubstituted or substituted, for example halo-, lower alkoxy- or nitro-substituted, benzoic acid, or, preferably, of a carbonic acid semiester. Such acyl groups are, for example, lower alkanoyl, such as formyl, acetyl, propionyl or pivaloyl, halo-lower alkanoyl, for example 2-haloacetyl, such as 2-chloro-, 2-bromo-, 2-iodo-, 2,2,2-trifluoro- or 2,2,2-trichloro-acetyl, unsubstituted or substituted, for example halo-, lower alkoxy- or nitro-substituted, benzoyl, such as benzoyl, 4-chlorobenzoyl, 4-methoxybenzoyl or 4-nitrobenzoyl, lower alkoxycarbonyl, preferably lower alkoxycarbonyl that is branched in the 1-position of the lower alkyl radical or suitably substituted in the 1- or 2-position, for example tert-lower alkoxycarbonyl, such as tert-butoxycarbonyl, arylmethoxycarbonyl having one, two or three aryl radicals which are phenyl that is unsubstituted or mono- or polysubstituted, for example, by lower alkyl, especially tert-lower alkyl, such as tert-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, such as chlorine, and/or by nitro, for example benzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, diphenylmethoxycarbonyl, 9-fluorenylmethoxycarbonyl or di(4-methoxyphenyl) methoxycarbonyl, aroylmethoxycarbonyl wherein the aroyl group is preferably benzoyl that is unsubstituted or substituted, for example, by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromoethoxycarbonyl or 2-iodoethoxycarbonyl, 2-(trisubstituted silyl)-lower alkoxycarbonyl, for example 2-tri-lower alkylsilyl-lower alkoxycarbonyl, such as 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methylsilyl)-ethoxycarbonyl, or triarylsilyl-lower alkoxycarbonyl, for example 2-triphenylsilylethoxycarbonyl.

In an arylmethylamino group, for example a mono-, di- or especially tri-arylmethylamino group, the aryl radicals are especially unsubstituted or substituted phenyl radicals. Such groups are, for example, benzyl-, diphenylmethyl- or especially trityl-amino, or very especially 1-aryl-lower alkylmethylamino wherein the lower alkyl radical is preferably branched in the 1-position, such as in 1-methyl-1-phenylethylamino. In an etherified mercaptoamino group the mercapto group is especially in the form of substituted arylthio or aryl-lower alkylthio wherein aryl is, for example, phenyl that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro, for example 4-nitrophenylthio.

In a 2-acyl-lower alk-1-enyl radical that can be used as an amino-protecting group, acyl is, for example, the corresponding radical of a lower alkanecarboxylic acid, of a benzoic acid that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro, or especially of a carbonic acid semiester, such as a carbonic acid lower alkyl semiester. Corresponding protecting groups are especially 1-lower alkanoyl-lower alk-1-en-2-yl, for example 1-lower alkanoyl-prop-1-en-2-yl, such as 1-acetyl-prop-1-en-2-yl, or lower alkoxycarbonyl-lower alk-1-en-2-yl, for example lower alkoxycarbonyl-prop-1-en-2-yl, such as 1-ethoxycarbonyl-prop-1-en-2-yl.

A silylamino group is, for example, a tri-lower alkylsilylamino group, for example trimethylsilylamino or tert-butyldimethylsilylamino. The silicon atom of the silylamino group can also be substituted by only two lower alkyl groups, for example methyl groups, and by the amino group or carboxy group of a second molecule of formula I. Compounds having such protecting groups can be prepared, for example, using the corresponding chlorosilanes, such as dimethylchlorosilane, as silylating agents.

An amino group can also be protected by conversion into the protonated form; suitable corresponding anions are especially those of strong inorganic acids, such as sulfuric acid,

phosphoric acid or hydrohalic acids, for example the chlorine or bromine anion, or of organic sulfonic acids, such as p-toluenesulfonic acid.

Preferred amino-protecting groups are lower alkoxycarbonyl, phenyl-lower alkoxycarbonyl, fluorenyl-lower alkoxycarbonyl, 2-lower alkanoyl-lower alk-1-en-2-yl, 1-methyl-1-phenyl-ethyl and lower alkoxycarbonyl-lower alk-1-en-2-yl.

A hydroxy group can be protected, for example, by an acyl group, for example lower alkanoyl that is substituted by halogen, such as chlorine, such as 2,2-dichloroacetyl, or especially by an acyl radical of a carbonic acid semiester mentioned for protected amino groups. A preferred hydroxy-protecting group is, for example, 2,2,2-trichloroethoxycarbonyl, 4-nitrobenzyloxycarbonyl, diphenylmethoxycarbonyl or trityl. A hydroxy group can also be protected by tri-lower alkylsilyl, for example trimethylsilyl, triisopropylsilyl or tert-butyl-dimethylsilyl, a readily removable etherifying group, for example an alkyl group, such as tert-lower alkyl, for example tert-butyl, an oxa- or a thia-aliphatic or -cycloaliphatic, especially 2-oxa- or 2-thia-aliphatic or -cycloaliphatic, hydrocarbon radical, for example 1-lower alkoxy-lower alkyl or 1-lower alkylthio-lower alkyl, such as methoxymethyl, 1-methoxyethyl, 1-ethoxyethyl, methylthiomethyl, 1-methylthioethyl or 1-ethylthioethyl, or 2-oxa- or 2-thia-cycloalkyl having from 5 to 7 ring atoms, such as 2-tetrahydrofuryl or 2-tetrahydropyranyl, or a corresponding thia analogue, and also by 1-phenyl-lower alkyl, such as benzyl, diphenylmethyl or trityl, wherein the phenyl radicals can be substituted, for example, by halogen, for example chlorine, lower alkoxy, for example methoxy, and/or by nitro.

A hydroxy group and an amino group that are adjacent to one another in a molecule can be protected, for example, by bivalent protecting groups, such as a methylene group that is preferably substituted, for example by one or two lower alkyl radicals or by oxo, for example unsubstituted or substituted alkylidene, for example lower alkylidene, such as isopropylidene, cycloalkylidene, such as cyclohexylidene, a carbonyl group or benzylidene.

In the context of this disclosure, a protecting group, for example a carboxy-protecting group, is to be understood as being expressly also a polymeric carrier that is bonded in a readily removable manner to the functional group, for example the carboxy group, to be protected, for example a carrier suitable for the Merrifield synthesis. Such a suitable polymeric carrier is, for example, a polystyrene resin weakly cross-linked by copolymerisation with divinylbenzene and carrying bridge members suitable for reversible bonding.

The addition of the compounds of formula III to the epoxides of formula IV is carried out preferably under the reaction conditions customarily used for the addition of nucleophiles to epoxides.

The addition is carried out especially in aqueous solution and/or in the presence of polar solvents, such as alcohols, for example methanol, ethanol, isopropanol or ethylene glycol, ethers, such as dioxane, amides, such as dimethylformamide, or phenols, such as phenol, and also under anhydrous conditions, in non-polar solvents, such as benzene or toluene, or in benzene/water emulsions, optionally in the presence of acidic or basic catalysts, for example alkali hydroxide solutions, such as sodium hydroxide solution, or in the presence of solid phase catalysts doped with the hydrazine, such as aluminium oxide, in ethers, for example diethyl ether, generally at temperatures of from approximately 0° C. to the boiling temperature of the reaction mixture in question, preferably from 20° C. to reflux temperature, optionally under elevated pressure, for example in a bomb tube, in which case it is also possible to exceed the boiling temperature measurable at normal pressure, and/or under an inert gas, such as nitrogen or argon, it being possible for each of the two compounds of formulae III and IV to be present in excess, for example in a molar ratio of from 1:1 to 1:100, especially in a molar ratio of from 1:1 to 1:10, more especially in a ratio of from 1:1 to 1:3.

The freeing of protected groups may be effected in accordance with the methods described below under the heading "Removal of protecting groups".

Process b) (Formation of an amide bond)

In starting materials of formulae V and VI, functional groups, with the exception of groups that are to participate in the reaction or that do not react under the reaction conditions, are protected independently of one another by one of the protecting groups mentioned under Process a).

The compounds of formula VI either contain a free carboxy group or are in the form of a reactive acid derivative thereof, for example in the form of a derived activated ester or reactive anhydride, or in the form of a reactive cyclic amide. The reactive acid derivatives may also be formed in situ.

Activated esters of compounds of formula VI having a terminal carboxy group are especially esters unsaturated at the carbon atom linking the radical to be esterified, for example esters of the vinyl ester type, such as vinyl esters (obtainable, for example, by transesterification of a corresponding ester with vinyl acetate; activated vinyl ester method), carbamoyl esters (obtainable, for example, by treatment of the corresponding acid with an isoxazolium reagent; 1,2-oxazolium or Woodward method), or 1-lower alkoxyvinyl esters (obtainable, for example, by treatment of the corresponding acid with a lower alkoxyacetylene; ethoxyacetylene method), or esters of the amidino type, such as N,N'-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with a suitable N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide or especially N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide; carbodiimide method), or N,N-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with an N,N-disubstituted cyanamide; cyanamide method), suitable aryl esters, especially phenyl esters suitably substituted by electron-attracting substituents (obtainable, for example, by treatment of the corresponding acid with a suitably substituted phenol, for example 4-nitro- phenol, 4-methylsulfonylphenol, 2,4,5-trichlorophenol, 2,3,4,5,6-pentachlorophenol or 4-phenyldiazophenol, in the presence of a condensation agent, such as N,N'-dicyclohexylcarbodiimide; activated aryl esters method), cyanomethyl esters (obtainable, for example, by treatment of the corresponding acid with chloroacetonitrile in the presence of a base; cyanomethyl esters method), thio esters, especially unsubstituted or substituted, for example nitro-substituted, phenylthio esters (obtainable, for example, by treatment of the corresponding acid with unsubstituted or substituted, for example nitro-substituted, thiophenols, inter alia by the anhydride or carbodiimide method; activated thiol esters method), or especially amino or amido esters (obtainable, for example, by treatment of the corresponding acid with an N-hydroxyamino or N-hydroxyamido compound, for example N-hydroxysuccinimide, N-hydroxypiperidine, N-hydroxyphthalimide, N-hydroxy-5-norbornene-2,3-dicarboxylic acid imide, 1-hydroxybenzotriazole or 3-hydroxy-3,4-dihydro-1,2,3-

benzotriazin-4-one, for example by the anhydride or carbodiimide method; activated N-hydroxy esters method). Internal esters, for example γ-lactones, can also be used.

Anhydrides of acids may be symmetric or preferably mixed anhydrides of those acids, for example anhydrides with inorganic acids, such as acid halides, especially acid chlorides (obtainable, for example, by treatment of the corresponding acid with thionyl chloride, phosphorus pentachloride, phosgene or oxalyl chloride; acid chloride method), azides (obtainable, for example, from a corresponding acid ester via the corresponding hydrazide and treatment thereof with nitrous acid; azide method), anhydrides with carbonic acid semiesters, for example carbonic acid lower alkyl semiesters (especially chloroformic acid methyl esters) (obtainable, for example, by treatment of the corresponding acid with chloroformic acid lower alkyl esters or with a 1-lower alkoxycarbonyl-2-lower alkoxy-1,2-dihydroquinoline; mixed O-alkylcarbonic acid anhydrides method), or anhydrides with dihalogenated, especially dichlorinated, phosphoric acid (obtainable, for example, by treatment of the corresponding acid with phosphorus oxychloride; phosphorus oxychloride method), anhydrides with other phosphoric acid derivatives (for example those obtainable with phenyl-N-phenylphosphoramidochloridate or by reaction of alkylphosphoric acid amides in the presence of sulfonic acid anhydrides and/or racemisation-reducing additives, such as N-hydroxybenzotriazole, or in the presence of cyanophosphonic acid diethyl ester) or with phosphorous acid derivatives, or anhydrides with organic acids, such as mixed anhydrides with organic carboxylic acids (obtainable, for example, by treatment of the corresponding acid with an unsubstituted or substituted lower alkane- or phenyl-lower alkane-carboxylic acid halide, for example phenylacetic acid chloride, pivalic acid chloride or trifluoroacetic acid chloride; mixed carboxylic acid anhydrides method) or with organic sulfonic acids (obtainable, for example, by treatment of a salt, such as an alkali metal salt, of the corresponding acid with a suitable organic sulfonic acid halide, such as a lower alkane- or aryl-, for example methane- or p-toluene-sulfonic acid chloride; mixed sulfonic acid anhydrides method) and symmetric anhydrides (obtainable, for example, by condensation of the corresponding acid in the presence of a carbodiimide or 1-diethylaminopropyne; symmetric anhydrides method).

Suitable cyclic amides are especially amides with five-membered diazacycles of aromatic character, such as amides with imidazoles, for example imidazole (obtainable, for example, by treatment of the corresponding acid with N,N'-carbonyldiimidazole; imidazole method), or pyrazole, for example 3,5-dimethylpyrazole (obtainable, for example, via the acid hydrazide by treatment with acetylacetone; pyrazolide method).

As mentioned, derivatives of carboxylic acids used as acylating agents may also be formed in situ. For example, N,N'-disubstituted amidino esters may be formed in situ by reacting a mixture of the starting material of formula V and the acid used as acylating agent in the presence of a suitable N,N'-disubstituted carbodiimide, for example N,N'-cyclohexylcarbodiimide or especially N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide. In addition, amino or amido esters of the acids used as acylating agents may be formed in the presence of the starting material of formula V to be acylated, by reacting a mixture of the corresponding acid and amino starting materials in the presence of an N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide, and of an N-hydroxyamine or N-hydroxyamide, for example N-hydroxysuccinimide, where appropriate in the presence of a suitable base, for example 4-dimethylamino-pyridine. Furthermore, activation in situ can be achieved by reaction with N,N,N',N'-tetraalkyluronium compounds, such as O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate, O-(1,2-dihydro-2-oxo-1-pyridyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (in the presence or absence of 1,8-diazabicyclo[5.4.0]undec-7-ene(1,5-5)) or O-(3,4-dihydro-4-oxo-1,2,3-benzotriazolin-3-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate. Finally, phosphoric acid anhydrides of the carboxylic acids of formula VI can be prepared in situ by reacting an alkylphosphoric acid amide, such as hexamethylphosphoric acid triamide, in the presence of a sulfonic acid anhydride, such as 4-toluenesulfonic acid anhydride, with a salt, such as a tetrafluoroborate, for example sodium tetrafluoroborate, or with another derivative of hexamethylphosphoric acid triamide, such as benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluoride, preferably in the presence of a racemisation-reducing additive, such as N-hydroxybenzotriazole.

The amino group of compounds of formula V that participates in the reaction preferably carries at least one reactive hydrogen atom, especially when the carboxy, sulfonyl or phosphoryl group reacting therewith is present in reactive form; it may, however, itself have been derivatised, for example by reaction with a phosphite, such as diethylchlorophosphite, 1,2-phenylene chlorophosphite, ethyldichlorophosphite, ethylene chlorophosphite or tetraethylpyrophosphite. A derivative of such a compound having an amino group is, for example, also a carbamic acid halide or an isocyanate, the amino group that participates in the reaction being substituted by halocarbonyl, for example chlorocarbonyl, or modified in the form of an isocyanate group, respectively.

Condensation to form an amide bond can be carried out in a manner known per se, for example as described in standard works, such as Houben-Weyl, "Methoden der organischen Chemie", 4th edition, Volume 15/II (1974), Volume IX (1955), Volume E11 (1985), Georg Thieme Verlag, Stuttgart, "The Peptides" (E. Gross and J. Meienhofer, eds.), Volumes 1 and 2, Academic Press, London and New York, 1979/1980, or M. Bodansky, "Principles of Peptide Synthesis", Springer-Verlag, Berlin 1984.

The condensation of a free carboxylic acid with the appropriate amine can be carried out preferably in the presence of one of the customary condensation agents, or using carboxylic acid anhydrides or carboxylic acid halides, such as chlorides, or activated carboxylic acid esters, such as p-nitrophenyl esters. Customary condensation agents are, for example, carbodiimides, for example diethyl-, dipropyl- or dicyclohexyl-carbodiimide or especially N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, also suitable carbonyl compounds, for example carbonylimidazole, 1,2-oxazolium compounds, for example 2-ethyl-5-phenyl-1,2-oxazolium-3'-sulfonate and 2-tert-butyl-5-methylisoxazolium perchlorate, or a suitable acylamino compound, for example 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, N,N,N',N'-tetraalkyluronium compounds, such as O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate or especially O-(1,2-dihydro-2-oxo-1-pyridyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (in the presence or absence of 1,8-diazabicyclo[5.4.0]undec-7-ene-(1,5-5)), also activated phosphoric acid derivatives, for example diphenylphosphorylazide, diethylphosphorylcyanide, phenyl-N-phenylphosphoroamidochloridate, bis(2-oxo-3-oxazolidinyl)phosphinic acid chloride or

1-benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate.

If desired, an organic base is added, preferably a tertiary amine, for example a tri-lower alkylamine, especially ethyidiisopropylamine or more especially triethylamine, and/or a heterocyclic base, for example 4-dimethylaminopyridine or preferably N-methylmorpholine or pyridine.

The condensation of activated esters, reactive anhydrides or reactive cyclic amides with the corresponding amines is customarily carried out in the presence of an organic base, for example simple tri-lower alkylamines, for example triethylamine or tributylamine, or one of the above-mentioned organic bases. If desired, a condensation agent is additionally used, for example as described for free carboxylic acids.

The condensation of acid anhydrides with amines can be effected, for example, in the presence of inorganic carbonates, for example ammonium or alkali metal carbonates or hydrogen carbonates, such as sodium or potassium carbonate or hydrogen carbonate (if desired together with a sulfate).

Carboxylic acid chlorides, for example the chlorocarbonic acid derivatives derived from the acid of formula VI, are condensed with the corresponding amines preferably in the presence of an organic amine, for example the above-mentioned tri-lower alkylamines or heterocyclic bases, where appropriate in the presence of a hydrogen sulfate or a hydroxide, preferably an alkali metal hydroxide, such as sodium hydroxide.

The condensation is preferably carried out in an inert, aprotic, preferably anhydrous, solvent or solvent mixture, for example in a carboxylic acid amide, for example formamide or dimethylformamide, a halogenated hydrocarbon, for example methylene chloride, carbon tetrachloride or chlorobenzene, a ketone, for example acetone, a cyclic ether, for example tetrahydrofuran or dioxane, an ester, for example ethyl acetate, or a nitrile, for example acetonitrile, or in a mixture thereof, as appropriate at reduced or elevated temperature, for example in a temperature range of from approximately −40° to approximately +100° C., preferably from approximately −10° to approximately +70° C., and when arylsulfonyl esters are used also at approximately from +100° to +200° C., especially at temperatures of from 10° to 30° C., and if necessary under an inert gas atmosphere, for example a nitrogen or argon atmosphere.

Aqueous, for example alcoholic, solvents, for example ethanol, or aromatic solvents, for example benzene or toluene, may also be used. When alkali metal hydroxides are present as bases, acetone may also be added where appropriate.

The condensation can also be carried out in accordance with the technique known as solid-phase synthesis which originates from R. Merrifield and is described, for example, in Angew. Chem. 97, 801–812 (1985), Naturwissenschaften 71, 252–258 (1984) or in R. A. Houghten, Proc. Natl. Acad. Sci. USA 82, 5131–5135 (1985).

The freeing of protected groups may be effected in accordance with the methods described below under the heading "Removal of protecting groups."

Process c) (Formation of an amide bond)

In starting materials of formulae VII and VIII, functional groups, with the exception of groups that are to participate in the reaction or that do not react under the reaction conditions, are protected independently of one another by one of the protecting groups mentioned under Process a).

The process is entirely analogous to that given under Process b) but compounds of formula VII are used instead of those of formula V and compounds of formula VIII are used instead of those of formula VI.

The freeing of protected groups may be effected in accordance with the methods described below under the heading "Removal of protecting groups".

Process d) (Formation of an amide bond)

In starting materials of formula IX and in the acid of formula VIIIa suitable for the introduction of the identical acyl radicals, or in reactive derivatives thereof, functional groups that are not to participate in the reaction or that do not react under the reaction conditions, are protected independently of one another by one of the protecting groups mentioned under Process a).

Preferred starting compounds of formula IX, which may be protected by protecting groups, are those of formula II described below in the section relating to starting compounds.

The process is entirely analogous to that given under Process b) but compounds of formula IX are used instead of those of formula V and compounds of formula VIIIa are used instead of those of formula VI.

The freeing of protected groups may be effected in accordance with the methods described below under the heading "Removal of protecting groups".

Process e) (Alkylation of a secondary nitrogen atom)

In starting materials of formula I' and formula X or in reactive derivatives thereof, functional groups that are not to participate in the reaction or that do not react under the reaction conditions, are protected independently of one another by one of the protecting groups mentioned under Process a).

A leaving group X is especially a nucleofugal leaving group selected from hydroxy esterified by a strong inorganic or organic acid, such as hydroxy esterified by a mineral acid, for example a hydrohalic acid, such as hydrochloric, hydrobromic or hydriodic acid, hydroxy esterified by a strong organic sulfonic acid, such as a lower alkanesulfonic acid that is unsubstituted or substituted, for example, by halogen, such as fluorine, or by an aromatic sulfonic acid, for example benzenesulfonic acid that is unsubstituted or substituted by lower alkyl, such as methyl, halogen, such as bromine, and/or by nitro, for example a methanesulfonic, p-bromotoluenesulfonic or p-toluenesulfonic acid, and hydroxy esterified by hydrazoic acid.

The substitution can take place under the conditions of a first or second order nucleophilic substitution.

For example, one of the compounds of formula X wherein X is a leaving group having high polarisability of the electron shell, for example iodine, can be used in a polar aprotic solvent, for example acetone, acetonitrile, nitromethane, dimethyl sulfoxide or dimethylformamide. The reaction can also be carried out in water, optionally in admixture with an organic solvent, for example ethanol, tetrahydrofuran or acetone, as solubiliser. The substitution reaction is carried out, as appropriate, at reduced or elevated temperature, for example in a temperature range of from approximately −40° to approximately 100° C., preferably from approximately −10° to approximately 50° C., and if necessary under an inert gas, for example under a nitrogen or argon atmosphere.

Process e) is not successful in all cases, is often possible only under special conditions and is therefore a less preferred process.

The freeing of protected groups may be effected in accordance with the methods described below under the heading "Removal of protecting groups".

Process f) (Reductive alkylation of a secondary amino group)

In starting materials.of formula I' and formula X* or in reactive derivatives thereof, functional groups that are not to

participate in the reaction or that do not react under the reaction conditions, are protected independently of one another by one of the protecting groups mentioned under Process a).

Reactive derivatives of the compounds of formula I are, for example, corresponding bisulfite adducts or especially semiacetals or ketals of compounds of formula X* with alcohols, for example lower alkanols; or thioacetals of compounds of formula X* with mercaptans, for example lower alkanesulfides. The free aldehydes of formula X* are preferred.

The reductive alkylation is preferably carried out with hydrogenation in the presence of a catalyst, especially a noble metal catalyst, such as platinum or especially palladium, which is preferably bonded to a carrier material, such as carbon, or a heavy metal catalyst, such as Raney nickel, at normal pressure or at pressures of from 0.1 to 10 MegaPascal (MPa), or with reduction by means of complex hydrides, such as borohydrides, especially alkali metal cyanoborohydrides, for example sodium cyanoborohydride, in the presence of a suitable acid, preferably relatively weak acids, such as lower alkanecarboxylic acids or especially a sulfonic acid, such as p-toluenesulfonic acid; in customary solvents, for example alcohols, such as methanol or ethanol, or ethers, for example cyclic ethers, such as tetrahydrofuran, in the presence or absence of water.

The freeing of protected groups may be effected in accordance with the methods described below under the heading "Removal of protecting groups".

Removal of protecting groups

The removal of protecting groups that are not constituents of the desired end product of formula I, for example carboxy-, amino- and hydroxy-protecting groups, is effected in a manner known per se, for example by means of solvolysis, especially hydrolysis, alcoholysis or acidolysis, or by means of reduction, especially hydrogenolysis or chemical reduction, and also photolysis, stepwise or simultaneously as appropriate, it being possible also to use enzymatic methods. The removal of the protecting groups is described, for example, in the standard works mentioned hereinabove in the section relating to protecting groups.

For example, protected carboxy, for example tert-lower alkoxycarbonyl, lower alkoxycarbonyl substituted in the 2-position by a trisubstituted silyl group or in the 1-position by lower alkoxy or by lower alkylthio, or unsubstituted or substituted diphenylmethoxycarbonyl can be converted into free carboxy by treatment with a suitable acid, such as formic acid, hydrogen chloride or trifluoroacetic acid, where appropriate with the addition of a nucleophilic compound, such as phenol or anisole. Carboxy can be freed from lower alkoxycarbonyl also by bases, such as hydroxides, for example alkali metal hydroxides, such as NaOH or KOH. Unsubstituted or substituted benzyloxycarbonyl can be cleaved, for example, by means of hydrogenolysis, i.e. by treatment with hydrogen in the presence of a metal hydrogenation catalyst, such as a palladium catalyst. In addition, suitably substituted benzyloxycarbonyl, such as 4-nitrobenzyloxycarbonyl, can be converted into free carboxy also by reduction, for example by treatment with an alkali metal dithionite, such as sodium dithionite, or with a reducing metal, for example zinc, or a reducing metal salt, such as a chromium(II) salt, for example chromium(II) chloride, customarily in the presence of a hydrogen-yielding agent that, together with the metal, is capable of producing nascent hydrogen, such as an acid, especially a suitable carboxylic acid, such as an unsubstituted or substituted, for example hydroxy-substituted, lower alkanecarboxylic acid, for example acetic acid, formic acid, glycolic acid, diphenylglycolic acid, lactic acid, mandelic acid, 4-chloromandelic acid or tartaric acid, or in the presence of an alcohol or thiol, water preferably being added. By treatment with a reducing metal or metal salt, as described above, 2-halo-lower alkoxycarbonyl (where appropriate after conversion of a 2-bromo-lower alkoxycarbonyl group into a corresponding 2-iodo-lower alkoxycarbonyl group) or aroylmethoxycarbonyl can also be converted into free carboxy. Aroylmethoxycarbonyl can be cleaved also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate or sodium iodide. 2-(Trisubstituted silyl)-lower alkoxycarbonyl, such as 2-tri-lower alkylsilyl-lower alkoxycarbonyl, can also be converted into free carboxy by treatment with a salt of hydrofluoric acid that yields the fluoride anion, such as an alkali metal fluoride, for example sodium or potassium fluoride, where appropriate in the presence of a macrocyclic polyether ("crown ether"), or with a fluoride of an organic quaternary base, such as tetra-lower alkylammonium fluoride or tri-lower alkylaryl-lower alkylammonium fluoride, for example tetraethylammonium fluoride or tetrabutylammonium fluoride, in the presence of an aprotic, polar solvent, such as dimethyl sulfoxide or N,N-dimethylacetamide. Carboxy protected in the form of organic silyloxycarbonyl, such as tri-lower alkylsilyloxycarbonyl, for example trimethylsilyloxycarbonyl, can be freed in customary manner by solvolysis, for example by treatment with water, an alcohol or an acid, or, furthermore, a fluoride, as described above. Esterified carboxy can also be cleaved enzymatically, for example by means of esterases or suitable peptidases, for example using trypsin.

A protected amino group is freed in a manner known per se and, according to the nature of the protecting groups, in various ways, preferably by solvolysis or reduction. Lower alkoxycarbonylamino, such as tert-butoxycarbonylamino, can be cleaved in the presence of acids, for example mineral acids, for example a hydrogen halide, such as hydrogen chloride or hydrogen bromide, or sulfuric or phosphoric acid, but preferably hydrogen chloride, or in the presence of strong organic acids, such as a trihaloacetic acid, for example trifluoroacetic acid, or formic acid, in the presence or absence of polar solvents, such as water, or ethers, preferably cyclic ethers, such as dioxane; or nitriles, such as acetonitrile, 2-halo-lower alkoxycarbonylamino (where appropriate after conversion of a 2-bromo-lower alkoxycarbonylamino group into a 2-iodo-lower alkoxycarbonylamino group), or, dissolved directly in a liquid organic carboxylic acid, such as formic acid, aroylmethoxycarbonylamino or 4-nitrobenzyloxycarbonylamino can be cleaved, for example, by treatment with a suitable reducing agent, such as zinc in the presence of a suitable carboxylic acid, such as aqueous acetic acid. Aroylmethoxycarbonylamino can be cleaved also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate, and 4-nitrobenzyloxycarbonylamino also by treatment with an alkali metal dithionite, for example sodium dithionite. Unsubstituted or substituted diphenylmethoxycarbonylamino, tert-lower alkoxycarbonylamino or 2-(tri-substituted silyl)-lower alkoxycarbonylamino, such as 2-tri-lower alkylsilyl-lower alkoxycarbonylamino, can be cleaved by treatment with a suitable acid, for example formic acid or trifluoroacetic acid; unsubstituted or substituted benzyloxycarbonylamino can be cleaved, for example, by means of hydrogenolysis, i.e. by treatment with hydrogen in the presence of a suitable

hydrogenation catalyst, such as a platinum or palladium catalyst; unsubstituted or substituted triarylmethylamino or formylamino can be cleaved, for example, by treatment with an acid, such as a mineral acid, for example hydrochloric acid, or an organic acid, for example formic, acetic or trifluoroacetic acid, where appropriate in the presence of water, and an amino group protected in the form of silylamino can be freed, for example, by means of hydrolysis or alcoholysis. An amino group protected by 2-haloacetyl, for example 2-chloroacetyl, can be freed by treatment with thiourea in the presence of a base, or with a thiolate salt, such as an alkali metal thiolate of thiourea, and subsequent solvolysis, such as alcoholysis or hydrolysis, of the resulting substitution product. Amino is freed from trifluoroacetylamino, for example, by hydrogenolysis with bases, such as alkali metal hydroxides or carbonates, such as Na_2CO_3 or K_2CO_3, in polar solvents, for example alcohols, such as methanol, in the presence or absence of water, at temperatures of from 0° to 100° C., especially at reflux temperature. An amino group protected by 2-(tri-substituted silyl)-lower alkoxycarbonyl, such as 2-tri-lower alkylsilyl-lower alkoxycarbonyl, can be converted into the free amino group also by treatment with a salt of hydrofluoric acid that yields fluoride anions, as indicated above in connection with the freeing of a correspondingly protected carboxy group. A 1-aryl-lower alkylmethyl protecting group wherein the lower alkyl radical is preferably branched in the 1-position, such as 1-methyl-1-phenyl-ethyl, can be removed especially in the presence of a strong acid, such as sulfuric acid (e.g. 80% sulfuric acid) in aqueous solution, at preferred temperatures of from −10° to 30° C., especially at approximately 0° C.

Likewise, silyl, such as trimethylsilyl, bonded directly to a hetero atom, such as nitrogen, can be removed using fluoride ions.

Amino protected in the form of an azido group is converted into free amino, for example, by reduction, for example by catalytic hydrogenation with hydrogen in the presence of a hydrogenation catalyst, such as platinum oxide, palladium or Raney nickel, by reduction using mercapto compounds, such as dithiothreitol or mercaptoethanol, or by treatment with zinc in the presence of an acid, such as acetic acid. The catalytic hydrogenation is preferably carried out in an inert solvent, such as a halogenated hydrocarbon, for example methylene chloride, or in water or in a mixture of water and an organic solvent, such as an alcohol or dioxane, at approximately from 20° C. to 25° C., or with cooling or heating.

A hydroxy group protected by a suitable acyl group, by a tri-lower alkylsilyl group or by unsubstituted or substituted 1-phenyl-lower alkyl is freed analogously to a correspondingly protected amino group. A hydroxy group protected by 2,2-dichloroacetyl is freed, for example, by basic hydrolysis, and a hydroxy group protected by tert-lower alkyl or by a 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radical is freed by acidolysis, for example by treatment with a mineral acid or a strong carboxylic acid, for example trifluoroacetic acid. Adjacent hydroxy and amino groups that are protected together by a bivalent protecting group, preferably, for example, by a methylene group mono- or di-substituted by lower alkyl, such as by lower alkylidene, for example isopropylidene, cycloalkylidene, for example cyclohexylidene, or benzylidene, can be freed by acid solvolysis, especially in the presence of a mineral acid or a strong organic acid. A tri-lower alkylsilyl group is likewise removed by acidolysis, for example by a mineral acid, preferably hydrofluoric acid, or a strong carboxylic acid. 2-Halo-lower alkoxycarbonyl is removed using the above-mentioned reducing agents, for example a reducing metal, such as zinc, reducing metal salts, such as chromium(II) salts, or using sulfur compounds, for example sodium dithionite or especially sodium sulfide and carbon disulfide.

When several protected functional groups are present, if desired the protecting groups can be so selected that more than one such group can be removed simultaneously, for example by removal of trifluoroacetyl as amino-protecting group by base catalysis, for example with K_2CO_3 in methanol/water, and later removal of tert-butoxycarbonyl as amino-protecting group, for example with HCl in dioxane or acetonitrile (in the presence or absence of water) or with formic acid, or selective removal of 1-methyl-1-phenyl-ethyl as amino-protecting group using sulfuric acid; or generally by acidolysis, such as by treatment with trifluoroacetic acid, or with hydrogen and a hydrogenation catalyst, such as a palladium-on-carbon catalyst. Conversely, the groups can also be so selected that they cannot all be removed simultaneously, but rather in a desired sequence, the corresponding intermediates being obtained.

Additional Process Steps

In the additional process steps, which are optional, functional groups of the starting compounds that are not to participate in the reaction may be unprotected or may be in protected form, for example they may be protected by one or more of the protecting groups mentioned above under Process a). The protecting groups may be retained in the end products or some or all of them may be removed in accordance with one of the methods mentioned under the heading "Removal of protecting groups".

Salts of compounds of formula I having a salt-forming group can be prepared in a manner known per se. For example, acid addition salts of compounds of formula I can be obtained, for example, by treatment with an acid or a suitable anion exchange reagent.

Salts can be converted into the free compounds in customary manner, for example by treatment with a suitable basic agent.

Stereoisomeric mixtures, for example mixtures of diastereoisomers, can be separated into the corresponding isomers in a manner known per se by suitable separating procedures. For example, mixtures of diastereoisomers can be separated into the individual diastereoisomers by fractional crystallisation, chromatography, solvent partitioning and the like. Such separation can be carried out either at the stage of one of the starting materials or with the compounds of formula I themselves.

In a compound of formula I wherein R_2 is phenyl, that phenyl radical can be hydrogenated, for example by catalytic hydrogenation, especially in the presence of heavy metal oxides, such as rhodium/platinum mixed oxides, for example with the Nishimura catalyst, preferably in a polar solvent, such as an alcohol, for example methanol or ethanol, at temperatures of from 0° to 80° C., especially from 10° to 40° C., and at a preferred hydrogen pressure of from 1 to 10 atm, preferably at about normal pressure.

In a compound of formula I wherein R_4 is 4-tetrazol-5-ylphenyl, a lower alkyl group, for example methyl, can be converted by reaction with a lower alkyl halide or a lower alkylarylsulfonate, such as a lower alkyl iodide or a lower alkyltoluenesulfonate, for example methyl iodide or tert-butyl iodide, preferably in the presence of caesium carbonate in a mixture of a cyclic ether, such as dioxane, and an N,N-di-lower alkyl-lower alkanecarboxylic acid amide,

such as dimethylformamide, at preferred temperatures of from −10° to 40° C., especially from 0° to about 30° C.

In a compound of formula I wherein R_4 is 4-(1- or 2-phenyl-lower alkyl, such as 1- or 2-(1-methyl-1-phenylethyl)-tetrazol-5-yl)phenyl, the phenyl-lower alkyl radical (preferably 1-methyl-1-phenylethyl) can be removed by treatment with a strong mineral acid, such as sulfuric acid, in aqueous solution, preferably at temperatures of from −20° to 30° C., for example at 0° C.

General Process Conditions

All the process steps given in this text can be carried out under reaction conditions known per se, but preferably under those specifically mentioned, in the absence or usually in the presence of solvents or diluents, preferably those solvents or diluents that are inert towards the reagents used and are solvents therefor, in the absence or presence of catalysts, condensation agents or neutralising agents, for example ion exchangers, such as cation exchangers, for example in the H^+ form, depending upon the nature of the reaction and/or the reactants at reduced, normal or elevated temperature, for example in a temperature range of from approximately −100° to approximately 190° C., preferably from approximately −80° to approximately 150° C., for example from −80° to −60° C., at room temperature, at from −20° to 40° C. or at the boiling point of the solvent used, under atmospheric pressure or in a closed vessel, optionally under pressure, and/or in an inert atmosphere, for example under an argon or nitrogen atmosphere.

In the case of all starting materials and intermediates, salts may be present when salt-forming groups are present. Salts may also be present during the reaction of such compounds, provided that the reaction will not be affected.

In all reaction steps, any isomeric mixtures that are formed can be separated into the individual isomers, for example diastereoisomers or enantiomers, or into any desired mixtures of isomers, for example racemates or diastereoisomeric mixtures, for example analogously to the methods described under the heading "Additional process steps".

In certain cases, for example in the case of hydrogenation, it is possible to carry out stereo-selective reactions so that, for example, individual isomers may be obtained more easily.

The solvents from which those suitable for a particular reaction can be selected include, for example, water, esters, such as lower alkyl-lower alkanoates, for example diethyl acetate, ethers, such as aliphatic ethers, for example diethyl ether, or cyclic ethers, for example tetrahydrofuran, liquid aromatic hydrocarbons, such as benzene or toluene, alcohols, such as methanol, ethanol or 1- or 2-propanol, nitrites, such as acetonitrile, halogenated hydrocarbons, such as methylene chloride, acid amides, such as dimethylformamide, bases, such as heterocyclic nitrogen bases, for example pyridine, carboxylic acid anhydrides, such as lower alkanoic acid anhydrides, for example acetic anhydride, cyclic, linear or branched hydrocarbons, such as cyclohexane, hexane or isopentane, or mixtures of those solvents, for example aqueous solutions, unless the description of the processes indicates otherwise. Such solvent mixtures can also be used in working-up, for example by chromatography or partitioning.

The invention relates also to those forms of the process in which a compound obtainable as intermediate at any stage is used as starting material and the remaining steps are carried out or the process is interrupted at any stage or a starting material is formed under the reaction conditions or is used in the form of a reactive derivative or salt, or a compound obtainable in accordance with the process of the invention is produced under the process conditions and further processed in situ, it being preferable to use those starting materials which result in the compounds described above as being preferred, especially those described as being especially preferred, more especially preferred and/or very especially preferred.

The preparation of compounds of formula I is preferably carried out analogously to the processes and process steps given in the Examples.

The compounds of formula I, including their salts, may also be obtained in the form of hydrates, or their crystals may include, for example, the solvent used for crystallisation.

Pharmaceutical Compositions

The invention relates also to pharmaceutical compositions comprising compounds of formula I*, which means especially a compound of the formula I, and most especially of formula Ia.

The pharmacologically acceptable compounds of the present invention may be used, for example, in the preparation of pharmaceutical compositions that comprise an effective amount of the active ingredient together or in admixture with a significant amount of inorganic or organic, solid or liquid, pharmaceutically acceptable carriers.

The invention relates also to a pharmaceutical composition suitable for administration to a warm-blooded animal, especially a human being, for the treatment or prevention of a disease that is responsive to inhibition of a retroviral protease, especially a retroviral aspartate protease, such as HIV-1 or HIV-II gag protease, for example a retroviral disease, such as AIDS or its preliminary stages, comprising a compound of formula I*, or a pharmaceutically acceptable salt thereof, in an amount effective in the inhibition of the retroviral protease, together with at least one pharmaceutically acceptable carrier.

The pharmaceutical compositions according to the invention are compositions for enteral, such as nasal, rectal or oral, or parenteral, such as intramuscular or intravenous, administration to warm-blooded animals (human beings and animals) that comprise an effective dose of the pharmacological active ingredient alone or together with a significant amount of a pharmaceutically acceptable carrier. The dose of the active ingredient depends on the species of warm-blooded animal, body weight, age and individual condition, individual pharmacokinetic data, the disease to be treated and the mode of administration.

The invention relates also to a method of treating diseases caused by viruses, especially by retroviruses, especially AIDS or its preliminary stages, wherein a therapeutically effective amount of a compound of formula I* or a pharmaceutically acceptable salt thereof is administered in a dose that is effective in the treatment of said disease especially to a warm-blooded animal, for example a human being, who on account of one of the mentioned diseases, especially AIDS or its preliminary stages, requires such treatment. The preferred dose to be administered to warm-blooded animals, for example human beings of approximately 70 kg body weight, is from approximately 3 mg to approximately 3 g, preferably from approximately 10 mg to approximately 1.5 g, for example approximately from 50 mg to 1000 mg per person per day, divided preferably into 1 to 3 single doses which may, for example, be of the same size. Usually, children receive half of the adult dose.

The pharmaceutical compositions comprise from approximately 1% to approximately 95%, preferably from approximately 20% to approximately 90%, active ingredient. Pharmaceutical compositions according to the invention may be, for example, in unit dose form, such as in the form of ampoules, vials, suppositories, dragées, tablets or capsules.

The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of conventional dissolving, lyophilising, mixing, granulating or confectioning processes.

Solutions of the active ingredient, and also suspensions, and especially isotonic aqueous solutions or suspensions, are preferably used, it being possible, for example in the case of lyophilised compositions that comprise the active ingredient alone or together with a carrier, for example mannitol, for such solutions or suspensions to be made up prior to use. The pharmaceutical compositions may be sterilised and/or may comprise excipients, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers, or acids, for example citric acid, and are prepared in a manner known per se, for example by means of conventional dissolving or lyophilising processes. The said solutions or suspensions may comprise viscosity-increasing substances, such as sodium carboxymethylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose (e.g. cellulose HPM603), silica gel, dextran, polyvinylpyrrolidone or gelatin.

Suspensions in oil comprise as the oil component the vegetable, synthetic or semi-synthetic oils customary for injection purposes. There may be mentioned as such especially liquid fatty acid esters that contain as acid component a long-chained fatty acid having from 8 to 22, especially from 12 to 22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brassidic acid or linoleic acid, if desired with the addition of antioxidants, for example vitamin E, β-carotene or 3,5-di-tert-butyl-4-hydroxytoluene. The alcohol component of those fatty acid esters has a maximum of 6 carbon atoms and is a mono- or poly-hydric, for example a mono-, di- or tri-hydric, alcohol, for example methanol, ethanol, propanol, butanol or pentanol or the isomers thereof, but especially glycol and glycerol. The following examples of fatty acid esters are therefore to be mentioned: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (polyoxyethylene glycerol trioleate, Gattefosse, Paris), "Miglyol 812" (triglyceride of saturated fatty acids with a chain length of C_8 to C_{12}, H üls AG, Germany), but especially vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, soybean oil and more especially groundnut oil and sesame oil.

The injection compositions are prepared in customary manner under sterile conditions; the same applies also to introducing the compositions into ampoules or vials and sealing the containers.

Pharmaceutical compositions for oral administration can be obtained by combining the active ingredient with solid carriers, if desired granulating a resulting mixture, and processing the mixture, if desired or necessary, after the addition of appropriate excipients, into tablets, dragée cores or capsules. It is also possible for the active ingredients to be incorporated into plastics carriers that allow the active ingredients to diffuse or be released in measured amounts.

Suitable carriers are especially fillers, such as sugars, for example lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, and also binders, such as starch pastes using, for example, corn, wheat, rice or potato starch, gelatin, tragacanth, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, such as the above-mentioned starches, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof, such as sodium alginate. Excipients are especially flow conditioners and lubricants, for example silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable, optionally enteric, coatings, there being used inter alia concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as ethylcellulose phthalate or hydroxypropylmethylcellulose phthalate.

Capsules are hard gelatin capsules and also soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The hard gelatin capsules may comprise the active ingredient in the form of granules, for example with fillers, such as lactose, binders, such as starches, and/or glidants, such as talc or magnesium stearate, and if desired with stabilisers. In capsules the active ingredient is preferably dissolved or suspended in suitable oily excipients, such as fatty oils, paraffin oil or liquid polyethylene glycols, it likewise being possible for stabilisers and/or antibacterial agents to be added. There may be mentioned as such oils especially liquid fatty acid esters that contain as acid component a long-chained fatty acid, for example having from 8 to 22, especially from 12 to 22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brassidic acid or linoleic acid, if desired with the addition of antioxidants, for example vitamin E, β-carotene or 3,5-di-tert-butyl-4-hydroxytoluene. The alcohol component of those fatty acid esters has a maximum of 6 carbon atoms and is a mono- or poly-hydric, for example a mono-, di- or tri-hydric, alcohol, for example methanol, ethanol, propanol, butanol or pentanol or the isomers thereof, but especially ethylene or propylene glycol and glycerol. The following examples of fatty acid esters are therefore to be mentioned: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (polyoxyethylene glycerol trioleate, Gattefossé, Paris), "Miglyol 812" (triglyceride of saturated fatty acids with a chain length of C_8 to C_{12}, Hüls AG, Germany), but especially vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, groundnut oil, soybean oil and more especially sesame oil. Paraffin oil is also possible. Stabilisers, such as emulsifiers, wetting agents or surfactants, binders, such as starch pastes using, for example, corn, wheat, rice or potato starch, gelatin, tragacanth, methylcellulose, hydroxypropylmethylcellulose or hydroxypropylcellulose (preferred), sodium carboxymethylcellulose, cyclodextrin(s) and/or polyvinylpyrrolidone, and/or antibacterial agents may be added. Suitable emulsifiers are especially oleic acid, nonionic surfactants of the fatty acid polyhydroxy alcohol ester type, such as sorbitan monolaurate, monooleate, monostearate or monopaimitate, sorbitan tristearate or trio leate, polyoxyethylene add ucts of fatty acidpolyhydroxy alcohol

esters, such as polyoxyethylene sorbitan monolaurate, mono-oleate, monostearate, monopalmitate, tristearate or trioleate, polyethylene glycol fatty acid esters, such as polyoxyethyl stearate, polyoxyethylene glycol (300 or 400) stearate, poly-ethylene glycol 2000 stearate, especially ethylene oxide/propylene oxide block polymers of the ®Pluronic type (Wyandotte Chem. Corp.; trade mark of BASF, FRG) or ®Synperonic type (ICI). For example, if the active ingredient is not soluble in the mentioned oils it is present in the form of a suspension, for example having a particle size of approximately from 1 to 100 mm. Such suspensions may also be used as such, that is to say without capsules.

Colourings or pigments may be added to the tablets or drage coatings or to capsule walls, for example for identification purposes or to indicate different doses of active ingredient.

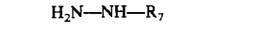
Starting materials

The present invention relates also to novel starting materials and/or intermediates and to processes for their preparation. The starting materials used and the reaction conditions selected are preferably those which result in the compounds described as being preferred.

In the preparation of all starting materials, free functional groups that are not to participate in the reaction in question may be unprotected or may be in protected form, for example they may be protected by the protecting groups mentioned above under Process a). Those protecting groups can be removed at suitable times by the reactions described under the heading "Removal of protecting groups".

The starting materials of Process a) are known or, if novel, can be prepared in accordance with processes known per se; for example the compounds of formula III can be prepared from hydrazine or suitable derivatives thereof, and the compounds of formula IV can be prepared from suitable amino acids or analogues thereof, for example having one of the mentioned side chains R_3.

The compounds of formula III can be obtained, for example, from compounds of formula

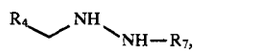


(XI),

which are known per se or can be prepared from hydrazine by the introduction of protecting groups as described under Process a) and in which R_7 is hydrogen or an amino-protecting group as described above under Process b), especially tert-lower alkoxycarbonyl, such as tert-butoxycarbonyl, aryl-lower alkoxycarbonyl, such as benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl, or one of the above-mentioned acylamino-protecting groups, especially trifluoroacetyl, by alkylation with a compound of formula X as described above under Process e), or by reaction of the radical of sub-formula

(A)

wherein R_4 is as defined or compounds of formula I, by reaction of a suitable carbonyl compound of formula X*, or a reactive derivative thereof, both as defined under Process f), with the free amino group of the compound of formula XI or or an acylated derivative thereof and subsequent reduction of the resulting hydrazone to form a hydrazine derivative of formula



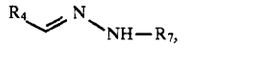
(XII)

the radicals in all the mentioned compounds being as defined above and functional groups in the reagents involved that are not to participate in the reaction being protected as necessary, and removal of the protecting group R_7 as necessary and by condensation under the conditions mentioned above under Process b) with an acid of formula VI, or an acid derivative thereof mentioned under Process b).

The carbonyl compounds of formula X*, or reactive derivatives thereof, suitable for the introduction of the radical of sub-formula A that are used for the preparation of the compounds of formula XII, as defined above under Process f), are aldehydes or reactive derivatives thereof, the reactive carbonyl group of which, after the reaction with compounds of formula XI and the subsequent reduction, is a constituent of one of the mentioned radicals of sub-formula A.

The reaction of the carbonyl compounds with the compounds of formula XI to form the corresponding hydrazones is carried out under the conditions customarily used for the reaction of carbonyl compounds with amines, preferably in polar organic solvents, for example ethers, such as tetrahydrofuran or diethyl ether, alcohols, such as methanol or ethanol, carboxylic acid amides, such as dimethylformamide, or esters, such as ethyl acetate, or in aqueous solution, preferably in methanol, and also in the presence or absence of acid catalysts, for example carboxylic acids, such as formic acid or acetic acid, or sulfonic acids, such as p-toluenesulfonic acid, at temperatures of from 0° C. to the reflux temperature of the reaction mixture, preferably at temperatures of from 20° C. to the reflux temperature of the reaction mixture.

Compounds of formula



(XII*)

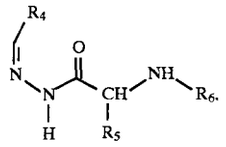
wherein R_4 and R_7 are as defined for compounds of formula XII are obtained.

The reduction of the resulting hydrazones of formula XII* is preferably carried out by hydrogenation in the presence of a suitable catalyst or with complex hydrides in the presence of acids. As catalysts suitable for hydrogenation there are used metals, such as nickel, iron, cobalt or ruthenium, or noble metals or oxides thereof, such as palladium or rhodium or oxides thereof, optionally, for example, applied to a suitable carrier, such as barium sulfate, aluminium oxide or carbon (active carbon) or in the form of skeleton catalysts, such as Raney nickel. Solvents customarily used for the catalytic hydrogenation are, for example, water, alcohols, such as methanol or ethanol, esters, such as ethyl acetate, ethers, such as dioxane, chlorinated hydrocarbons, such as dichloromethane, carboxylic acid amides, such as dimethylformamide, or carboxylic acids, such as glacial acetic acid, or mixtures of those solvents. The hydrogenation is carried out preferably at temperatures of from 10° to 250° C., especially from room temperature to 100° C., and preferably at hydrogen pressures of from 1 to 200 bar, especially from 1 to 10 bar, in the customary apparatus. For the reduction with complex hydrides, especially borohydrides, such as alkali metal cyanoborohydrides, for example sodium cyanoborohydride, it is preferable to add weak acids, such as sulfonic acids, for example p-toluenesulfonic acid, or carboxylic acids, such as acetic acid, preferably in alcohols, such as methanol or ethanol, or mixtures thereof with water (see, for example, Tetrahedron 49, 8605–8628 (1993)).

It is also possible for compounds of formula XI to be alkylated by reduction directly with compounds of formula X*, or reactive derivatives thereof, as defined under Process f), under conditions analogous to those mentioned in Process f).

Also especially preferred for the preparation of compounds of formula XI are reaction conditions analogous to those described in J. Chem. Soc. Perkin I, 1712 (1975).

Compounds of formula III can also be obtained, for example, by reacting a compound of formula XII*, as defined above, wherein R_7 is hydrogen (obtainable, for example, by the removal of protecting groups when R_7 is a protecting group), directly, with condensation under the conditions mentioned above under Process b) with acids of formula VI, or the acid derivatives thereof mentioned under Process b), to form compounds of formula

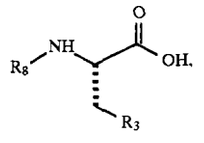


(III*)

wherein the radicals are as defined for compounds of formula I, which are then converted into compounds of formula III by reduction under conditions analogous to the conditions mentioned for the reduction of hydrazones of formula XII*.

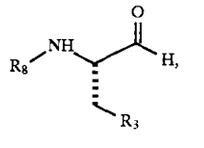
Compounds of formula III* can also be obtained from the corresponding compounds of formula III', which are as defined as described below, by reacting the latter with compounds of formula X*, as defined above, to form the hydrazones of formula III* under conditions analogous to those described above for the reaction of carbonyl compounds of formula X* with hydrazines of formula XI.

A compound of formula IV can be obtained, for example, by reduction of an amino acid of formula



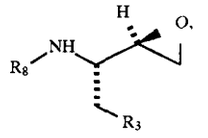
(XIII)

wherein R_8 is hydrogen or especially one of the amino-protecting groups mentioned under Process a), especially tert-lower alkoxycarbonyl, such as tert-butoxycarbonyl, aryl-lower alkoxycarbonyl, such as benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl, or one of the acylamino-protecting groups mentioned under Process a), especially trifluoroacetyl, and R_3 is as defined for compounds of formula I, to form an aldehyde of formula



(XIV)

wherein the radicals are as last defined, subsequent reaction of that aidehyde with a ylid compound, preferably a sulfur ylid compound, to form an epoxide of formula

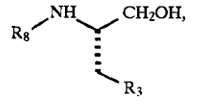


(XV)

wherein the radicals are as last defined, removal of the protecting group R_8 (the resulting free amino compound wherein R_8=hydrogen may be stable, for example in the form of an acid addition salt) and finally acylation of the amino group of the resulting compound with an acid of formula VIII, wherein the radicals are as defined for formula VIII, under suitable conditions analogous to the conditions described for Process b).

The reduction of amino acids of formula XIII to the corresponding aldehydes of formula XIV is carried out, for example, by reduction to the corresponding alcohols and subsequent oxidation to the mentioned aldehydes.

The reduction to the alcohols (a free compound or (if necessary after the introduction of protecting groups, as described under Process a)) a compound N-protected by R_8, having the formula



(XIII*)

wherein the radicals are as defined for compounds of formula XIII) is carried out, for example, by hydrogenation of the acid halides or other activated carboxylic acid derivatives mentioned under Process b) under the conditions mentioned for the hydrogenation of hydrazones obtained from compounds of formula XII, with diborane or with complex hydrides, such as sodium borohydride. The subsequent oxidation of the resulting alcohols is possible, for example, by oxidation of the hydroxy group with a sulfoxide, such as dimethyl sulfoxide, in the presence of a reagent that activates the hydroxy group, such as a carboxylic acid chloride, for example oxalyl chloride, in inert solvents, for example a halogenated hydrocarbon, such as dichloromethane, and/or an acyclic or cyclic ether, such as tetrahydrofuran, at from −80° to 0° C., for example from −78° to −50° C., or by oxidation, for example, with chromic acid or a derivative thereof, such as pyridinium chromate or tert-butyl chromate, dichromate/sulfuric acid, sulfur trioxide in the presence of heterocyclic bases, such as pyridine/SO_3, and also nitric acid, pyrolusite or selenium dioxide, in water, organic solvents, such as halogenated solvents, for example methylene chloride, carboxylic acid amides, such as dimethylformamide, or di-lower alkylsulfoxides, such as dimethyl sulfoxide, in the presence or absence of basic amines, for example tri-lower alkylamines, such as triethylamine, at temperatures of from −50° to 100° C., preferably at from −10° to 50° C., or by catalytic dehydrogenation, for example in the presence of metallic silver, copper, copper chromium oxide or zinc oxide at approximately from 200° to 400° C. (in the contact tube) with subsequent rapid cooling. Oxidation with 2,2,6,6-tetramethyl-piperidin-1-oxyl in the presence of NaOCl is also possible (see Anelli et al., Org. Synth. 69, 212 (1990)).

The direct reduction of the amino acids to the aldehydes is also possible, for example by hydrogenation in the presence of a partially poisoned palladium catalyst or by reduction of the corresponding amino acid esters, for example the lower alkyl esters, such as the ethyl ester, with complex hydrides, for example borohydrides, such as sodium borohydride, or preferably aluminium hydrides, for example

lithium aluminium hydride, lithium tri(tert-butoxy) aluminium hydride or especially diisobutylaluminium hydride, in non-polar solvents, for example in hydrocarbons or aromatic solvents, such as toluene, at from −100° to 0° C., preferably from −70° to −30° C., and subsequent reaction to form the corresponding semicarbazones, for example with the corresponding acid salts of semicarbazones, such as semicarbazide hydrochloride, in aqueous solvent systems, such as alcohol/water, for example ethanol/water, at temperatures of from −20° to 60° C., preferably from 10° to 30° C., and reaction of the resulting semicarbazone with a reactive aldehyde, for example formaldehyde, in an inert solvent, for example a polar organic solvent, for example a carboxylic acid amide, such as dimethylformamide, at temperatures of from −30° to 60° C., preferably from 0° to 30° C., and then with an acid, for example a strong mineral acid, such as a hydrogen halide, in aqueous solution, optionally in the presence of the solvent used previously, at temperatures of from −40° to 50° C., preferably from −10° to 30° C. The corresponding esters are obtained by reaction of the amino acids with the corresponding carboxylic acids, for example ethanol, analogously to the conditions employed in the condensation under Process b), for example by reaction with inorganic acid halides, such as thionyl chloride, in organic solvent mixtures, such as mixtures of aromatic and alcoholic solvents, for example toluene and ethanol, at temperatures of from −50° to 50° C., preferably from −10° to 20° C.

The preparation of the compounds of formula XIV is carried out in an especially preferred manner under conditions analogous to the reaction conditions mentioned in J. Org. Chem. 47, 3016 (1982) or J. Org. Chem. 43, 3624 (1978).

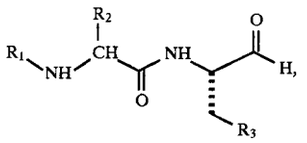
A sulfur ylid suitable for the conversion of compounds of formula XIV into the epoxides of formula XV is, for example, a dialkylsulfonium methylide, for example dimethylsulfonium methylide, an alkyl- or phenyl-dialkylaminosulfoxonium methylide, for example methyl- or phenyl-dimethylaminosulfoxonium methylide, or a dialkylsulfoxonium methylide, for example dimethyl- or diethyl-sulfoxonium methylide.

The sulfur ylid compound in question is advantageously prepared in situ from the corresponding sulfonium or sulfoxonium salt and a base, for example sodium hydride, in a dipolar aprotic solvent, for example dimethyl sulfoxide, or an ether, for example tetrahydrofuran or 1,2-dimethoxyethane, and is then reacted with the compound of formula XIV. The reaction is normally carried out at room temperature, with cooling, for example down to −20° C., or with gentle heating, for example up to 40° C. The sulfide, sulfinamide or sulfoxide formed at the same time is removed in the subsequent aqueous working-up.

The reaction with a sulfur ylid is effected in an especially preferred manner analogously to the conditions mentioned in J. Org. Chem. 50, 4615 (1985).

A compound of formula XV can also be obtained from a compound of formula XIV, as defined above, by reaction thereof with a tri-lower alkylsilylmethyl Grignard compound, for example prepared from the corresponding halomethylsilane, such as chloromethyl-trimethylsilane, in an inert solvent, for example an ether, such as dioxane or diethyl ether, at temperatures of from 0° to 50° C., for example from room temperature to approximately 40° C., subsequent elimination with removal of the silyl radical and formation of a double bond, for example by means of a Lewis acid, such as BF_3, any amino-protecting group R_8 preferably also being removed, in an inert solvent, for example an ether, such as diethyl ether, or a halogenated hydrocarbon, such as dichloromethane, or a mixture thereof, at temperatures of from −50° C. to the reflux temperature, especially from 0° to 30° C., if necessary acylation again with the introduction of an amino-protecting group R_{12}, as defined above, and oxidation of the resulting double bond to form the oxirane, preferably with a percarboxylic acid, for example m-chloroperbenzoic acid or monoperphthalic acid (for example in magnesium salt form), in an inert solvent, for example a halogenated hydrocarbon, such as dichloromethane, or alcohols, such as methanol, lower alkanoyinitriles, such as acetonitrile, water or mixtures thereof, at temperatures of from −20° C. to the reflux temperature of the mixture, for example at from 10° to 50° C.

Compounds of formula IV are preferably prepared by starting directly with an alcohol of formula XIII*, as defined above, which is also commercially available, reacting that alcohol with an acid of formula VIII, or with a reactive derivative thereof, as defined for Process c), under the conditions mentioned therein, with, if necessary, protecting groups being introduced, as described under Process a), and removed at suitable times, as described under the heading "Removal of protecting groups", there being obtained a compound analogous to the compound of formula XIII* wherein the place of R_8 is taken by the corresponding acyl radical from the acid of formula VIII; the resulting compound is oxidised under conditions analogous to those mentioned for the oxidation of alcohols of formula XIII* to form the corresponding aldehyde of formula



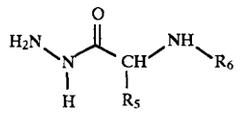
(XIV*)

wherein the radicals are as defined, and that aldehyde is then converted, for example with an ylid compound, as described for the conversion of compounds of formula XIV into compounds of formula XV, into the compound of formula IV.

The starting materials of Processes b), c) and d) are known or, if novel, can be prepared in accordance with processes known per se: for example a compound of formula V can be prepared from a suitable hydrazine derivative of formula XII wherein R_7 is a protecting group and the remaining radicals are as defined for compounds of formula V and a suitable epoxide of formula IV wherein the radicals are as defined for compounds of formula I (Process b); a compound of formula VII can be prepared from a suitable hydrazine derivative of formula III wherein the radicals are as defined for compounds of formula I and a suitable epoxide of formula XV wherein R_8 is a protecting group and the remaining radicals are as defined for compounds of formula I (Process c); and the compound of formula IX can be prepared from a suitable hydrazine derivative of formula XII wherein R_7 is hydrogen and the remaining radicals are as defined for compounds of formula I and a suitable epoxide of formula XV wherein R_8 is a protecting group and the remaining radicals are as defined for compounds of formula I (Process d), analogously to Process a), optionally using and removing protecting groups, as described under Process a) and under the heading "Removal of protecting groups", the protecting groups R_7 and R_8 preferably being as

defined above in the definition of compounds of formula XI and XIII, respectively.

Compounds of formula I', wherein the substituents are as defined above, can be prepared, for example, from compounds of formula III',



(III')

wherein the radicals are as defined for compounds of formula I, in a manner analogous to that described in Process b), by reaction with a compound of formula IV, wherein any functional groups present that are not to participate in the reaction may be protected as described in Process b) and freed again after the reaction.

Compounds of formula III' can be obtained from compounds of formula XI, as defined above, by reaction with an acid of formula VI, or a reactive acid derivative thereof, wherein the radicals are as defined above, in a manner analogous to that described for the reaction of compounds of formula XII with an acid of formula VI, and, as necessary, subsequent removal of the protecting group R_7 in accordance with one of the methods described under the heading "Removal of protecting groups".

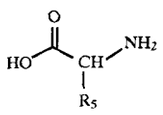
Where two amino-protecting groups are present they may be identical or different.

The amino-protecting groups used are, for example, the amino-protecting groups mentioned above under Process a). Preference is given to the corresponding compounds wherein the protecting groups are selected from those described as being preferred for R_7 and R_8 in compounds of formulae XI and XIII, respectively.

The preparation of the protected compounds of formula I is carried out, for example, in accordance with any one of the processes mentioned hereinbefore, especially from compounds of formulae III and IV wherein functional groups may be protected by protecting groups, as described under Process a).

The acids of formulae VI, VIII and VIIIa and the compounds of formula X, and the aldehydes suitable for the introduction of the radical of sub-formula A that are used for the preparation of the compounds of formula XII can be prepared in accordance with processes known per se if they are not already known.

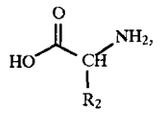
The preparation of the acids of formula VI is effected by reaction of derivatives of a lower alkoxycarboxylic acid that are suitable for the introduction of lower alkoxycarbonyl radicals, for example by reaction of the corresponding pyrocarbonic acid di-lower alkyl esters (especially pyrocarbonic acid dimethyl ester; Aldrich, Buchs, Switzerland) or preferably haloformic acid lower alkyl esters, such as chloroformic acid lower alkyl esters (especially chloroformic acid methyl ester, Fluka, Buchs, Switzerland), with amino acids of the formula



(XVI)

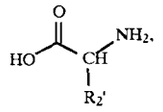
wherein R_5 is as defined for compounds of formula VI, under conditions analogous to those described for acylation under Process b), especially in an aqueous alkali metal hydroxide solution, for example aqueous sodium hydroxide solution, in the presence of dioxane at temperatures of from 20° to 100° C., especially from 50° to 70° C.

Correspondingly, the compounds of formula VIII can be obtained from amino acids of formula



(XVII)

wherein R_2 is as defined for compounds of formula I, and the compounds of formula VIIIa can be obtained from amino acids of formula

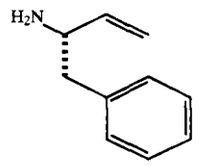


(XVIII)

wherein R_2' is as defined for compounds of formula VIII', by reaction with derivatives of a lower alkoxycarboxylic acid that are suitable for the introduction of lower alkoxycarbonyl radicals.

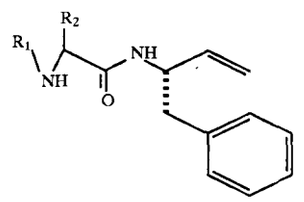
The amino acids of formulae XVI, XVII and XVIII are known or can be prepared in accordance with processes known per se. They are preferably in the (S)-form (in respect of the α-carbon atom).

Compounds of formula IV can also be prepared by condensing a compound of formula XIX



(XIX)

with a compound of formula XVIII, as defined above. The condensation with an acid of formula VIII, or an acid derivative thereof, is carried out under conditions analogous to those mentioned above under Process e). A compound of formula XX,



(XX)

wherein R_1 and R_2 are as defined for compounds of formula I, is obtained.

Epoxidation with oxygen, or preferably chemically bonded oxygen, for example in hydroperoxides, hydrogen peroxides or peroxy acids, such as perbenzoic acid, performic acid, peracetic acid, monoperoxyphthalic acid, pertungstic acid or especially m-chloroperbenzoic acid, in inert solvents, such as ethers, for example diethyl ether, or chlorinated hydrocarbons, such as chloroform or dichloromethane, at preferred temperatures of from -20° to 50° C., yields a compound of formula IV, as defined above.

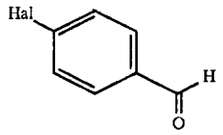
The starting material of formula XIX is obtained preferably by reaction of a compound of formula XIV wherein R_3 is phenyl and R_8 is a protecting group with a Grignard reagent that introduces the methylidene group, especially with the trimethylsilylmethyl Grignard reagent ($ClMgCH_2Si(CH_3)_3$—which can be prepared from chloromethyltrimethylsilane (Fluka, Buchs, Switzerland) under conditions customary for the preparation of Grignard compounds) in an

inert solvent, such as an ether, for example diethyl ether, at a preferred temperature of from −65° to 0° C. and subsequent removal of the hydroxy group and the trimethylsilyl group, for example with boron trifluoride in an ether, such as diethyl ether, at preferred temperatures of from −20° to 30° C., with simultaneous removal of the protecting group R_8 (especially in the case of removal of the tert-butoxycarbonyl protecting group) or with subsequent removal of the protecting group, as described under the heading "Removal of protecting groups".

Also possible is synthesis starting with a compound of formula XIV wherein R_3 is phenyl and R_8 is a protecting group using a suitable Wittig reagent, such as methyltriphenylphosphonium bromide or iodide in the presence of a strong base, such as sodium amide, at temperatures of from −90° to 0° C., followed by removal of the protecting group R_8 in accordance with the conditions mentioned under the heading "Removal of protecting groups".

Compounds of formula X* are known, can be prepared in accordance with processes known per se or can be prepared, for example, as follows:

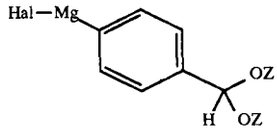
Using a compound of formula XXI,



(XXI)

wherein Hal is halogen, especially bromine or chlorine, and reacting it with an unsaturated heterocycle that has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl (—SO_2—) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl, especially with thiazole or thiophene, in the presence of tetrakis(triphenylphosphine) palladium as catalyst and in the presence of an alkali metal lower alkanoate, such as potassium acetate, in a suitable solvent, especially a N,N-di-lower alkyl-lower alkanoyl-amide, such as dimethyl acetamide, at preferred temperatures of from 80° C. to the boiling temperature of the mixture, for example at approximately 150° C., the corresponding compound of formula X*, especially 4-(thiazol-5-yl)-benzaldehyde or 4-(thiopen-2-yl)-benzaldehyde, can be obtained.

Alternatively, it is possible, starting with a compound of formula XXI, as last defined, to obtain the corresponding di-lower alkylacetal (see for example J. Org. Chem. 56, 4280 (1991)), for example the bromobenzaldehyde dimethylacetal (obtainable, for example, by reaction of 4-bromobenzaldehyde with orthoformic acid trimethyl ester in an alcohol, such as methanol, in the presence of an acid, such as p-toluenesulfonic acid (can also be used in the form of the hydrate)). The resulting 4-halo-benzaldehyde di-lower alkylacetal is then converted, by reaction with magnesium in the presence of a catalytic amount of iodine in a suitable solvent, such as an ether, for example tetrahydrofuran, at preferred temperatures of from 0° to 70° C., into the corresponding Grignard reagent of formula XXII,



(XXII)

wherein Hal is halogen, especially chlorine or bromine, and Z is lower alkyl, which is then reacted, in the presence of 1,3-bis(diphenylphosphino)propane nickel(II) chloride as catalyst in a suitable solvent, such as ether, for example tetrahydrofuran, there being added in an especially preferred process variant a suitable complex hydride, especially diisobutyl-aluminium hydride, (for example dissolved in a hydrocarbon, such as hexane), at preferred temperatures of from 0° to 60° C., with a compound of formula XXIII,

$$R_9—Hal' \qquad (XXIII)$$

wherein R_9 is an unsaturated heterocycle that has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl (—SO—) and sulfonyl (—SO_2—) and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl, and wherein Hal' is chlorine or especially bromine, with subsequent acid hydrolysis of the acetal (for example with hydrogen chloride in water), to form the corresponding aldehyde compound of formula X*. Especially preferred as compounds of formula XXIII are 2-bromothiazole, 2- or 3-bromopyridine or 2-chloropyrazine in the preparation of the following compounds of formula X*: 4-(thiazol-2-yl)-benzaldehyde, 4-(pyridin-2-yl or -3-yl) -benzaldehyde or 4-(pyrazin-2-yl)-benzaldehyde.

Compounds of formula X* wherein R_4 is 4-(tetrazolyl-5-yl)-phenyl, are obtainable by reaction of 4-cyanobenzaldehyde with an alkali metal azide, such as sodium azide, in the presence of a suitable alkali metal halide, such as lithium chloride, in a suitable solvent, such as 2-methoxyethanol, preferably at boiling temperature. By reaction with phenyl-lower alkyl halides or preferably with phenyl-lower alkenes, such as 2-phenylpropene, in a suitable solvent, such as toluene, and a suitable acid, such as methanesulfonic acid, preferably under reflux, the corresponding 1- or 2-phenyl-lower alkyl compounds of formula X* are obtained. By reaction with a lower alkyl halide, such as the iodide or bromide, for example methyl iodide, in the presence of alkali metal carbonates, such as potassium or especially caesium carbonate, and suitable solvents, such as dioxane, at preferred temperatures of from approximately 0° to approximately 30° C., compounds of formula X* substituted in the tetrazolyl ring by lower alkyl or by phenyl-lower alkyl, especially 4-(1-methyl-tetrazol-5-yl)-benzaldehyde, are obtained.

Compounds of formula X may be obtained from the corresponding compounds of formula X* by reduction of the aldehyde function to a hydroxymethyl group (for example with complex hydrides, such as lithium aluminium hydride in ethanol, disiamylborane in tetrahydrofuran, sodium borohydride in the presence of lithium chloride in diglycol or sodium borohydride in ethanol) and subsequent introduction of the radical X by esterification by a strong inorganic or organic acid, such as by a mineral acid, for example a hydrohalic acid, such as hydrochloric, hydrobromic or hydriodic acid, or by a strong organic sulfonic acid, such as an unsubstituted or substituted, for example halo-substituted, for example fluoro-substituted, lower alkanesulfonic acid or an aromatic sulfonic acid, for example a benzenesulfonic acid that is unsubstituted or substituted by lower alkyl, such as methyl, halogen, such as bromine, and/or by nitro, for example methanesulfonic acid, p-bromotoluenesulfonic acid or p-toluenesulfonic acid, or hydrazoic acid, in accordance with standard methods. For example, by reaction with inorganic acid halides, such as thionyl or phosphoryl halides (for example the chlorides, bromides or iodides), halogen radicals X can be introduced, or the remaining compounds of formula X can be obtained by reaction with other suitable organic or inorganic acids, such as strong organic sulfonic acids (used for example as acid chlorides).

Starting materials (especially those of formulae IV*, V*, VII*, IX* and (I')*) can also be prepared analogously to the processes mentioned in EP 0 521 827 or EP 0 672 448 or are obtainable from the reference sources mentioned therein, or they are known, can be prepared according to processes known per se or are commercially available.

The preparation of starting materials for the preparation of compounds of formula I is preferably carried out analogously to the processes and process steps mentioned in the Examples.

Of the starting materials according to the invention the following are especially preferred (when radicals are not specifically defined, the meanings mentioned in the definition for compounds of formula I apply in each case):

(1) compounds of formula XX wherein R_1 is methoxycarbonyl or ethoxycarbonyl and R_2 is tert-butyl;

(2) compounds of formula IV wherein R_1 is methoxycarbonyl or ethoxycarbonyl and R_2 is tert-butyl;

(3) compounds of formula III*, especially those wherein R_5 is tert-butyl and R_6 is methoxy- or ethoxycarbonyl;

(4) compounds of formula XII;

(5) compounds of formula XII*;

(6) compounds of formula III;

(7) compounds of formula V;

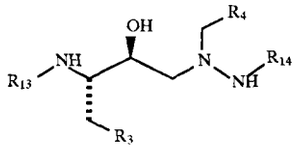
(8) compounds of formula VII;

(9) compounds of formula IX;

(10) compounds of formula X;

(11) a compound of formula X* selected from 4-(1-methyl-tetrazol-5-yl)-benzaldehyde, 4-(thiazol-2-yl)-benzaldehyde, 4-(pyridin-2-yl or -3-yl)-benzaldehyde, 4-(pyrazin-2-yl)-benzaldehyde, 4-(thiazol-5-yl)-benzaldehyde and 4-(thiophen-2-yl)-benzaldehyde;

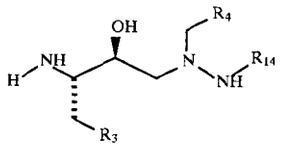
(12) compounds of formula XXIV



(XXIV)

wherein R_{13} and R_{14} are amino-protecting groups, which are different from one another, selected from those mentioned under Process a), especially tert-lower alkoxycabonyl, such as tert-butoxycarbonyl, or an acylamino-protecting group, especially trifluoroacetyl; preferably R_{13} is trifluoroacetyl and R_{14} is tert-butoxycarbonyl; (those compounds are compounds of formula IX that are protected at both amino groups);

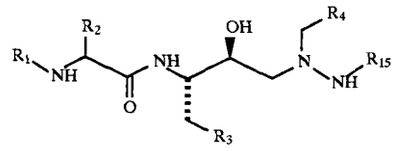
(13) compounds of formula XXV,



(XXV)

wherein R_{14} is an amino-protecting group, as defined for compounds of formula XXIV, especially tert-butoxycarbonyl;

(14) compounds of formula XXVI,



(XXVI)

wherein R_{15} is an amino-protecting group, especially tert-butoxycarbonyl, and the remaining radicals are as defined for compounds of formula I;

(15) 1-[4-(2-tert-butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-N-(tert-butyloxycarbonyl)amino-2-N-[N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane (as intermediate, but also pharmaceutically active).

When salt-forming groups are present, the compounds mentioned above under (1) to (15) as starting materials may also be in the form of a salt.

EXAMPLES

The following Examples serve to illustrate the invention without limiting the scope thereof:

Temperatures are indicated in degrees Celsius (° C.). Where no temperature is indicated, the reactions that follow are carried out at room temperature. The R_f values, which indicate the ratio of the seepage propagation of the substance in question to the seepage propagation of the eluant front, are determined on silica gel thin-layer plates (Merck, Darmstadt, Germany) by thin-layer chromatography (TLC) using the solvent systems mentioned in each case.

HPLC gradients used:

$HPLC_{20-100}$	20% → 100% a) in b) for 20 min.
$HPLC_{20-100(12')}$	20% → 100% a) in b) for 12 min., then 8 min 100% a)
$HPLC_{5-60}$	5% → 60% a) in b) for 15 min

Eluant a): acetonitrile+0.05% TFA; eluant b): water+0.05% TFA. Column (250×4.6 mm) packed with "reversed-phase" material C18-Nucleosil (5 μm mean particle size, silica gel covalently derivatised with octadecylsilanes, Macherey & Nagel, Düren, Germany). Detection by UV-absorption at 254 nm. Retention times (t_{Ret}) are given in minutes. Flow rate 1 ml/min.

The other abbreviations used have the following meanings:

abs.	absolute (indicates that solvent is anhydrous)
anal.	elemental analysis
Boc	tert-butoxycarbonyl
calc.	calculated
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene-(1,5-5)
TLC	thin-layer chromatography
DIPE	diisopropyl ether
DMF	dimethylformamide
DPPP	[1,3-bis(diphenylphosphino)propane]nickel(II) chloride (Aldrich, Milwaukee, USA)
EDC	N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride
ether	diethyl ether
FAB-MS	fast atom bombardment mass spectroscopy
sat	saturated
HOAc	acetic acid
HOBT	1-hydroxy-benzotriazole
HPLC	High Performance Liquid Chromatography
Hunig base	N-ethyldiisopropylamine

-continued

MeOH	methanol
min	minute(s)
NMM	N-methylmorpholine
Pd/C	palladium on charcoal
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)palladium
iso-PrOH	isopropanol
R_f	ratio of seepage propagation to the eluant front in TLC
SiO_2	silica gel
m.p.	melting point
brine	saturated sodium chloride solution
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran (dist. over sodium/benzophenone)
TPTU	O-(1,2-dihydro-2-oxo-1-pyridyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
p-TSA	p-toluenesulfonic acid

Source of some amino acid derivatives used as starting materials:

(2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane J. Org. Chem. 50, 4615 (1985)

(2R)-[(1'S)-(trifluoroacetyl)amino-2'-phenylethyl]oxirane (European Patent Application EP 0 521 827, page 78, Ex. 16d))

N-methoxycarbonyl-(L)-valine (Preparation see *Chem. Lett.* 705 (1980))

N-ethoxycarbonyl-(L)-valine (Preparation see *J. Org. Chem.* 60, 7256 (1995))

N-methoxycarbonyl-(L)-iso-leucine (Preparation see *Chem. Lett.* 705 (1980))

Example 1

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

With the exclusion of moisture, 735 mg (4.20 mmol) of N-methoxycarbonyl-(L)-valine (see EP 0 604 368, Example 2b)), 1548 mg (8.07 mmol) of EDC and 654 mg (4.844 mmol) of HOBT are placed in 10 ml of DMF. 1.13 ml (8.07 mmol) of TEA are added to the white suspension and the mixture is stirred at room temperature for 30 min. Then 595 mg (1.62 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-hexane dissolved in 1 ml of DMF are added and the mixture is stirred ovenight to complete the reaction. The reaction mixture is concentrated by evaporation; the resulting oil is dissolved in methylene chloride and washed with 10% citric acid solution, sat. $NaHCO_3$ solution and brine. The aqueous phases are extracted 2× with methylene chloride; the combined organic phases are filtered through cotton wadding and concentrated by evaporation. Column chromatography (SiO_2; CH_2Cl_2/MeOH/H_2O/HOAc 85:13:1.5:0.5) and precipitation with DIPE from a concentrated solution in methylene chloride yield the title compound: TLC: R_f=0.57 (CH_2Cl_2/MeOH/H_2O/HOAc 85:13:1.5:0.5); $HPLC_{20\text{-}100}$: t_{Ret}=13.0; FAB MS $(M+H)^+$=683.

The starting material is prepared as follows:

1a) 4-(Thiazol-5-yl)-benzaldehyde

In a bomb tube, a mixture of 3.7 g (20 mmol) of 4-bromobenzaldehyde (Fluka, Buchs, Switzerland), 6.64 ml (93 mmol) of thiazole, 2.94 g of potassium acetate and 1.16 g (1 mmol) of $Pd(PPh_3)_4$ in 50 ml of dimethylacetamide is stirred at 150° C. for 12 hours. The reaction mixture is concentrated by evaporation. Water is added to the residue and the mixture is extracted 3× with methylene chloride. The organic phases are filtered through cotton wadding, concentrated by evaporation and chromatographed (SiO_2; hexane/ethyl acetate 1:2), yielding the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=11.4; ^{1}H-NMR (CD_3OD) δ 9.98 (s, HCO), 9.03 (s, $H(2)^{thiazole}$), 8.32 (s, $H(4)^{thiazole}$), 7.95 and 7.85 (2d, J=8, each 2H; additionally also signals of the hydrate (≈12%): 8.92 (s, $H(2)^{thiazole}$), 8.15 (s, $H(4)^{thiazole}$)7.62 and 7.53 (2d, J=8, each 2H), 5.54 (s, $HC(OH)_2$).

1b) N-1-(tert-Butoxycarbonyl)-N-2-{[4-(thiazol-5-yl)-phenyl]-methylidene}-hydrazone

A solution of 1.22 g (6.45 mmol) of 4-(thiazol-5-yl)-benzaldehyde and 1.12 g (6.14 mmol) of tert-butyl carbazate (Fluka, Buchs, Switzerland) in 40 ml of ethanol is stirred at 80° C. for 12 hours. Cooling and crystallisation by the addition of 60 ml of water at 0° C. yield the title compound: m.p: 170°–171° C.; $HPLC_{20\text{-}100}$: t_{Ret}=13.5.

1c) N-1-(tert-Butoxycarbonyl)-N-2-[4-(thiazol-5-yl)-benzyl]-hydrazone

Under a nitrogen atmosphere, 20.4 g (67.2 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{[4-(thiazol-5-yl)-phenyl]-methylidene}hydrazone are placed in 120 ml of THF, and 4.67 g (70.7 mmol; 95%) of sodium cyanoborohydride are added. A solution of 12.8 g (67.2 mmol) of p-toluenesulfonic acid monohydrate in 120 ml of THF (pH 3–4) is then added dropwise thereto. After 7 hours, water and ethyl acetate are added and the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed with brine, sat. $NaHCO_3$ solution and brine, dried (Na_2SO_4) and concentrated by evaporation. To the resulting viscous oil there are added 80 ml of dichloroethane and 80 ml of 1N NaOH solution (foams) and the mixture is boiled under reflux for 7 hours. The reaction mixture is cooled and diluted with methylene chloride and water; the aqueous phase is separated off and extracted 2× with methylene chloride. The organic phases are dried (Na_2SO_4), concentrated by evaporation and chromatographed (SiO_2; hexane/ethyl acetate 2:1). Stirring in hexane yields the title compound: m.p: 93°–95° C.; TLC: R_f=0.12 (hexane/ethyl acetate 2:1); Anal. ($C_{15}H_{19}N_3O_2S$) calc. C 58.99, H 6.27, N 13.76, S 10.5; found C 58.98, H 6.34, N 13.64, S 10.66; $HPLC_{20\text{-}100}$: t_{Ret}=10.1.

1d) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane

A suspension of 1.21 g (4.6 mmol) of (2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane and 1.4 g (4.6 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(thiazol-5-yl)-benzyl]-hydrazine in 25 ml of iso-PrOH is heated at boiling overnight. The reaction mixture is cooled and water is added. The supernatant phase is decanted off from the oil that has separated out; the oil is dried in vacuo and chromatographed (SiO_2; methylene chloride/methanol 30:1), yielding the title compound: TLC: R_f=0.2 (methylene chloride/methanol 30:1); $HPLC_{20\text{-}100}$: t_{Ret}=17.2.

1e) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-S(S)-2,5-diamino-6-phenyl-2-azahexane

A solution of 1.14 g (2.0 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane in 100 ml of formic acid is stirred at room temperature for 3 hours and then concentrated by evaporation. Sat. $NaHCO_3$ solution and methylene chloride are added to the residue; the aqueous phase is separated off and extracted 2× with methylene chloride. The organic phases are treated with brine, filtered through cotton wadding and concentrated by evaporation to form the title compound which is used further directly.

Example 2

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 344 mg of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane and 191 μl (1.74 mmol) of NMM in 5.6 ml of DMF are added to 122 mg (0.696 mmol) of N-methoxycarbonyl-(L)-valine and 173 mg (0.58 mmol) of TPTU in 2.9 ml of DMF and the mixture is stirred at room temperature for 16 hours. The reaction mixture is poured into ice-water, stirred for 30 min and filtered. Column chromatography of the residue (SiO_2; methylene chloride/THF 4:1) and stirring in ether yield the title compound: m.p: 134°–135° C.; $HPLC_{20\text{-}100}$: t_{Ret}=14.0; FAB MS $(M+H)^+$= 697.

The starting material is prepared as follows:

2a) 1-[4-(Th iazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxVcarbonyl)amino-5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane

A suspension of 5.32 g (20.5 mmol) of (2R)-[(1'S)-(trifluoroacetyl)amino-2'-phenylethyl]-oxirane and 5.7 g (18.6 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(thiazol-5-yl)-benzyl]hydrazine (Example 1c) in 95 ml of iso-PrOH is heated at boiling for 8 hours. After cooling, the reaction mixture is partially concentrated by evaporation and left to stand at 0° C., resulting in the crystallisation of the title compound which is filtered off with suction and dried. TLC: R_f=0.39 (methylene chloride/THF 10:1); $HPLC_{20\text{-}100}$: t_{Ret}= 16.5; FAB MS $(M+H)^+$=565. Further product can be obtained from the mother liquor by boiling again with (2R)-[(1'S)-(trifluoroacetyl)amino-2'-phenylethyl]oxirane in iso-PrOH and column chromatography (SiO_2; methylene chloride /THF 15:1).

2b) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane

100 ml of a 1N K_2CO_3 solution are added dropwise to a solution of 5.646 g (10.0 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane in 100 ml of methanol and the mixture is stirred at 70° C. for 15 hours. Methylene chloride and water are added; the aqueous phase is separated off and extracted 2× with methylene chloride. The organic phases are washed 2× with water, dried (Na_2SO_4) and concentrated by evaporation, yielding the title compound: Anal. ($C_{25}H_{32}N_4O_3S$ (0.53 H_2O)) calc. C 62.80, H 6.97, N 11.72, S 6.71, H_2O 2.00: found C 63.2, H 7.01, N 11.57, S 6.49, H_2O 1.98; $HPLC_{20\text{-}100}$: t_{Ret}=11.5.

2c) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

Under an nitrogen atmosphere, 1.36 g (7.2 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e), 2.59 g (13.5 mmol) of EDC and 1.22 g (9.0 mmol) of HOBT are dissolved in 20 ml of DMF. After 15 min, 3.79 ml (27 mmol) of TEA are added and then a solution of 2.11 g (4.5 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane in 41 ml of DMF is added dropwise. After 3 hours the reaction mixture is concentrated by evaporation. The resulting oil is dissolved in ethyl acetate and a small amount of THF and washed with 2× water, sat. $NaHCO_3$ solution, 2× water and brine. The aqueous phases are extracted with ethyl acetate; the combined organic phases are dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/THF 5:1) and crystallisation from ethyl acetate/DIPE yield the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=16.0; FAB MS $(M+H)^+$=640.

2d) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

742 mg (1.16 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane and 12 ml of formic acid are stirred at room temperature for 7 hours and then concentrated by evaporation. Sat. $NaHCO_3$ solution and ethyl acetate are added to the residue; the aqueous phase is separated off and extracted with ethyl acetate. The organic phases are treated with water and brine, dried (Na_2SO_4) and concentrated by evaporation, yielding the title compound which is used further directly.

2e) N-(Methoxycarbonyl)-(L)-tert-leucine

23.5 ml (305 mmol) of methyl chloroformate are added over a period of 20 min to a solution of 20 g (152 mmol) of (L)-tert-leucine (=2(S)-amino-3,3-dimethyl-butyric acid= (L)-α-tert-butylglycine; Fluka, Buchs/Switzerland) in a mixture of 252 ml (504 mmol) of 2N aqueous sodium hydroxide solution and 80 ml of dioxane and the reaction solution is heated at 60° C. for 14 hours. After cooling to room temperature, the reaction solution is washed 2× with methylene chloride. The aqueous phase is acidified to pH 2 with 4N aqueous hydrochloric acid and extracted three times with ethyl acetate. The organic extracts are combined, dried (Na_2SO_4) and concentrated by evaporation, the product beginning to solidify. Digestion of the solidified solid with hexane yields the title compound in the form of a white powder.

M.p. 106°–108° C.

Example 3

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane

Under an argon atmosphere, 292 mg of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane (Example 2d) and 165 μl (1.5 mmol) of NMM in 4.8 ml of DMF are added to 113.5 mg of N-methoxycarbonyl-(L)-tert-leucine (Example 2e) and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF and the mixture is stirred at room temperature for 14 hours. The reaction mixture is poured into 0.2 litre of ice-water, stirred for 45 min and filtered. Column chromatography of the residue (SiO_2; methylene chloride/ethanol 20:1) and crystallisation from ethyl acetate/ether/hexane yield the title compound: m.p: 207°–209° C.; TLC: R_f=0.25 (methylene chloride/ethanol 20:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.7; FAB MS $(M+H)^+$= 711.

Example 4

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonl-(L)-tert-leucyl)amino-6-phehyl-2-azahexane

Under an argon atmosphere, 292 mg of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane (Example 2d) and 165 μl (1.5 mmol) of NMM in 4.8 ml of DMF are added to 113 mg of N-methoxycarbonyl-(L)-iso-leucine and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF, and the mixture is stirred at room temperature for 14 hours and worked up analogously to Example 3, yielding the title compound: m.p: 139°–141° C.; TLC: R_f=0.7 (methylene chloride/methanol 10:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.6; FAB MS $(M+H)^+$=711.

Example 5

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-S-methylcysteinyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenvi-2-azahexane

Under an argon atmosphere, 292 mg of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane (Example 2d) and 165 μl (1.5 mmol) of NMM in 4.8 ml of DMF are added to 116 mg (0.60 mmol) of N-methoxycarbonyl-(L)-S-methylcysteine and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF, and the mixture is stirred at room temperature for 5 hours and worked up analogously to Example 3, yielding the title compound: TLC: R_f=0.4 (methylene chloride/methanol 10:1); $HPLC_{20-100}$: t_{Ret}=13.6; FAB MS $(M+H)^+$=715.

The starting material is prepared as follows:

5a) N-methoxycarbonyl-(L)-S-methylcysteine

With ice-cooling, 16.8 g (177.5 mmol) of chloroformic acid methyl ester are added dropwise to a solution of 12.0 g (88.8 mmol) of S-methyl-(L)-cysteine ((S)-2-amino-3-methylmercaptopropionic acid: Fluka; Buchs/Switzerland) in 150 ml of 2N sodium hydroxide solution and 18 ml of dioxane and the mixture is stirred at 70° C. overnight to complete the reaction. The reaction mixture is diluted with 150 ml of methylene chloride; the aqueous phase is separated off, acidified with 1N HCl and extracted 3× with ethyl acetate. Drying (Na_2SO_4) and concentration of the ethyl acetate phases by evaporation yield the title compound: FAB MS $(M+H)^+$=194.

Example 6

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hvdroxy-2-N-(N-ethoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 344 mg of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane (Example 2d) and 191 μl (1.74 mmol) of NMM in 5.6 ml of DMF are added to 132 mg (0.7 mmol) of N-ethoxycarbonyl-(L)-valine (EP 0 604 368, Example 9a) and 173 mg (0.58 mmol) of TPTU in 2.9 ml of DMF, and the mixture is stirred at room temperature overnight and worked up analogously to Example 3, yielding the title compound: TLC: R_f=0.45 (methylene chloride/THF 4:1); $HPLC_{20-100}$: t_{Ret}=14.7; FAB MS $(M+H)^+$=711.

Example 7

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxcarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valylamino-6-phenyl-2-azahexane

Under argon, 213 mg (1.13 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e), 431 mg (2.25 mmol) of EDC and 304 mg (2.25 mmol) of HOBT are placed in 18 ml of DMF. After 15 min, 627 μl (4.5 mmol) of TEA and 0.75 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl) amino-6-phenyl-2-azahexane are added. After 2 hours, water and ethyl acetate are added; the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed 2× with water, sat. $NaHCO_3$ solution, 2× water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/THF 5:1) and crystallisation from ether yield the title compound: m.p: 200°–201° C.; $HPLC_{20-100}$: t_{Ret}=14.0; FAB MS $(M+H)^+$=697.

The starting material is prepared as follows:

7a) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 2.66 g (15.2 mmol) of N-methoxycarbonyl-(L)-valine, 5.46 g (28.5 mmol) of EDC and 2.57 g (19 mmol) of HOBT are dissolved in 42 ml of DMF. 7.9 ml (57 mmol) of TEA are added and after 20 min a solution of 4.46 g (9.5 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane (Example 2b) in 85 ml of DMF is added dropwise. After 1.5 hours, the reaction mixture is worked up analogously to Example 2c. Crystallisation from THF/ether yields the title compound: m.p: 114°–115° C.; $HPLC_{20-100}$: t_{Ret}=15.1; FAB MS $(M+H)^+$=626.

7b) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

1.25 g (2.0 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane and 18 ml of formic acid are reacted analogously to Example 2d to form the title compound: $HPLC_{20-100}$: t_{Ret}=10.0.

Example 8

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxycarbonyl-(L)-valyl)-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 7, 213 mg (1.13 mmol) of N-ethoxycarbonyl-(L)-valine, 431 mg (2.25 mmol) of EDC and 304 mg (2.25 mmol) of HOBT in 18 ml of DMF and 627 μl (4.5 mmol) of TEA are reacted with 0.75 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane (Example 7b) to form the title compound: m.p: 243°–244° C; $HPLC_{20-100}$: t_{Ret}=14.0; FAB MS $(M+H)^+$=697.

Example 9

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-íso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 0.6 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane and 198 μl (1.8 mmol) of NMM in 5.8 ml of DMF are added to 136 mg (0.72 nmol) of N-methoxycarbonyl-(L)-iso-leucine and 179 mg (0.60 mmol) of TPTU in 3 ml of DMF and the mixture is stirred at room temperature for 14 hours and worked up analogously to Example 3, yielding the title compound: TLC: R_f=0.59 (methylene chloride/THF 3:1); $HPLC_{20-100}$: t_{Ret}=14.0; FAB MS $(M+H)^+$=697.

Example 10

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-S-methylcysteinyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 0.58 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane and 191 μl (1.74 mmol) of NMM in 5.6 ml of DMF are added to 134 mg (0.696 mmol) of N-methoxycarbonyl-(L)-S-methylcysteine (Example 5a) and 173 mg (0.58 mmol) of TPTU in 2.9 ml of DMF and the mixture is stirred at room temperature for 15 hours and worked up analogously to Example 3, yielding the title compound: TLC: R_f=0.17 (methylene chloride/THF 4:1); $HPLC_{20-100}$: t_{Ret}=13.0; FAB MS $(M+H)^+$=701.

Example 11

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under argon, 0.5 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane and 165 µl (1.5 mmol) of NMM in 4.8 ml of DMF are added to 113.5 mg (0.60 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e) and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF and the mixture is stirred at room temperature for 14 hours. Ice-water and and ethyl acetate are added; the aqueous phase is separated off and extracted with ethyl acetate. The organic phases are washed 2× with water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; ethyl acetate) and crystallisation from ethyl acetate/ether/hexane yield the title compound: TLC: R_f=0.42 (methylene chloride/ethanol 10:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.8; FAB MS $(M+H)^+$=711.

The starting material is prepared as follows:

11a) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 1.36 g (7.2 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 2.59 g (13.5 mmol) of EDC and 1.22 g (9 mmol) of HOBT are dissolved in 20 ml of DMF. After 30 min, 3.79 ml (27 mmol) of TEA are added and a solution of 2.11 g (4.5 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane (Example 2b) in 40 ml of DMF are added dropwise. After 3 hours, the reaction mixture is worked up analogously to Example 2c to form the title compound: m.p: 163°–166° C.; Anal. ($C_{33}H_{45}N_5O_6S$ (0.14 H_2O)) calc. C 61.71, H 7.11, N 10.90, S 4.99, H_2O 0.39: found C 61.61, H 7.10, N 10.79, S 4.76, H_2O 0.4; $HPLC_{20\text{-}100}$: t_{Ret}=16.0; FAB MS $(M+H)^+$=640.

11b) 1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

320 mg (0.50 mmol) of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane and 6 ml of formic acid are reacted analogously to Example 2d to form the title compound which is used further directly.

Example 12

1-[4(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 7, 140 mg (0.80 mmol) of N-methoxycarbonyl-(L)-valine, 288 mg (1.5 mmol) of EDC and 135 mg (1.0 mmol) of HOBT in 2 ml of DMF and 418 µl of TEA are reacted with 0.5 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane in 5 ml of DMF to form the title compound: m.p: 202°–204° C.; $HPLC_{20\text{-}100}$: t_{Ret}=14.0; FAB MS $(M+H)^+$= 697.

Example 13

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-iso-leucyl)amino]-6-phenyl-2-azahexane

Analogously to Example 7, 175 mg (0.92 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 332 mg (1.7 mmol) of EDC and 156 mg (1.15 mmol) of HOBT in 2.5 ml of DMF and 483 µl (3.47 mmol) of TEA are reacted with 0.578 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5 (S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane (Example 11b) in 5.2 ml of DMF to form the title compound: m.p: 213°–216° C.; $HPLC_{20\text{-}100}$: t_{ret}=14.7; FAB MS $(M+H)^+$=711.

Example 14

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxycarbonyl-(L)-valyl)-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 7, 175 mg (0.92 mmol) of N-ethoxycarbonyl-(L)-valine, 332 mg (1.7 mmol) of EDC and 156 mg (1.15 mmol) of HOBT in 2.5 ml of DMF and 483 µl (3.47 mmol) of TEA are reacted with 0.578 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane (Example 11b) in 5.2 ml of DMF to form the title compound: m.p: 200°–203° C.; $HPLC_{20\text{-}100}$: t_{Ret}=14.6; FAB MS $(M+H)^+$=711.

Example 15

1-[4-(Thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-S-methylcysteinyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 0.5 mmol of 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane (Example 11b) and 165 µl (1.5 mmol) of NMM in 4.8 ml of DMF are added with ice cooling to 116 mg (0.60 mmol) of N-methoxycarbonyl-(L)-S-methylcysteine (Example 5a) and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF and the mixture is stirred at room temperature for 12 hours. Water and ethyl acetate are added; the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed 2× with water and brine, dried (Na_2SO_4) and partially concentrated by evaporation. The addition of ether causes the title compound to crystallise: m.p: 179°–181° C.; TLC: R_f=0.67 (methylene chloride/ethanol 10:1); $HPLC_{20\text{-}100}$: t_{Ret}=13.6; FAB MS $(M+H)^+$=715.

Example 16

1-[4-Thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane

Under an argon atmosphere, 2.58 g (13.7 mmol) of N-methoxycarbonyl-(L)-tert-leucine and 4.09 g (13.7 mmol) of TPTU are dissolved in 15.5 ml of DMF; 5.7 ml (24.8 mmol) of Hünig base are added with cooling and the mixture is stirred for 10 min. Then a solution of 2.29 g (6.20 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane in 15.5 ml of DMF is added and the mixture is stirred at room temperature for 16 hours. The light-yellow reaction solution is poured into ice-water; ethyl acetate is added and the mixture is stirred for 30 min. The aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are extracted 2× with water, sat. $NaHCO_3$ solution and 2× with brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; hexane/ethyl acetate 1:3) and crystallisation from methylene chloride/DIPE yield the title compound: TLC: R_f=0.18 (hexane/ethyl acetate 1:3); $HPLC_{20\text{-}100(12')}$: t_{Ret}=11.0; FAB MS $(M+H)^+$711; $[\alpha]^\circ$.(c=0.6, ethanol)=−46°.

The starting material is prepared as follows:

16a) 4-(Thiazol-2-yl)-benzaldehyde

Under argon, 9.2 g (379 mmol) of magnesium are placed in 84 ml of THF and heated to 60° C. A solution of 82.6 g (357 mmol) of 4-bromobenzaldehyde dimethyl acetal (for

preparation see *J. Org. Chem.* 56, 4280 (1991)) in 677 ml of THF is added dropwise thereto within a period of 30 min and the mixture is stirred at boiling temperature for a further 40 min. The Grignard solution is cooled, decanted into a dropping funnel and added dropwise within a period of 30 min to a reddish suspension of 31.7 ml (338 mmol) of 2-bromothiazole (Fluka, Buchs, Switzerland) and 5.39 g (9.95 mmol) of DPPP in 1.68 litres of THF. The mixture is stirred at room temperature for 12 hours; a further 5.39 g of DPPP are added and the mixture is stirred for a further 7 hours. 840 ml of water are added and the mixture is stirred for 10 min; the THF is evaporated off using a rotary evaporator and the residue is stirred for 1.5 hours in 1.0 litre of ether and 340 ml of 2N HCl. The aqueous phase is separated off and extracted 2× with ethyl acetate. The organic phases are washed 2× with 0.5N HCl, water, sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation. Chromatography (SiO_2; hexane/ethyl acetate 4:1) and digestion in hexane yield the title compound: TLC: R_f=0.21 (hexane/ethyl acetate 3:1); m.p: 91°–92° C.; Anal. ($C_{10}H_7NOS$) calc. C 63.47, H 3.73, N 7.40, S 16.94: found C 63.14, H 3.79, N 7.27, S 17.08; ^{1}H-NMR ($CDCl_3$) δ 10.05 (s, HCO), 8.15 (d, J=8, 2H), 7.95 (m, 3H), 7.45 (d, J=3, 1H).

16b) N-1-(tert-Butoxycarbonyl)-N-2-{[4-(thiazol-2-yl)-phenyl]-methylidene}-hydrazone

A solution of 27.6 g (145 mmol) of 4-(thiazol-2-yl)-benzaldehyde and 19.7 g (149 mmol) of tert-butyl carbazate in 920 ml of ethanol is stirred at 80° C. for 18 hours. Cooling, concentration by evaporation and stirring from DIPE yield the title compound: TLC: R_f=0.31 (toluene/ethyl acetate 3:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.5.

16c) N-1-(tert-Butoxycarbonyl)-N-2-[4-(thiazol-2-yl)-benzyl]-hydrazine

Under a nitrogen atmosphere, 77.6 g (256 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{[4-(thiazol-2-yl)-phenyl]-methylidene}-hydrazone are placed in 450 ml THF, and 16.9 g (257 mmol; 95%) of sodium cyanoborohydride are added. A solution of 49.6 g (261 mmol) of p-toluenesulfonic acid monohydrate in 450 ml of THF (pH 3–4) is added dropwise thereto.

After 17 hours, a further 3.38 g of sodium cyanoborohydride are added; the mixture is adjusted to pH 3–4 with p-toluenesulfonic acid monohydrate solution and stirred for 3 hours to complete the reaction. Water and ethyl acetate are added; the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed with brine, sat. $NaHCO_3$ solution and 2× brine, dried (Na_2SO_4) and concentrated by evaporation. The resulting viscous oil is taken up with 300 ml of 1,2-dichloroethane; 300 ml of 1N NaOH solution are slowly added (foams) and the mixture is boiled under reflux for 3.5 hours. The mixture is cooled and diluted with methylene chloride and water; the aqueous phase is separated off and extracted 2× with methylene chloride. The organic phases are dried (Na_2SO_4), concentrated by evaporation and chromatographed (SiO_2; toluene/acetone 9:1→6:1). Stirring in hexane yields the title compound: TLC: R_f=0.3 (hexane/ethyl acetate 3:2); $HPLC_{20\text{-}100}$: t_{Ret}=11.1.

16d) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane

A solution of 6.00 g (22.8 mmol) of (2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane and 5.37 g (17.6 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(thiazol-2-yl)-benzyl]-hydrazine in 550 ml of iso-PrOH is heated at boiling overnight. The reaction mixture is cooled to room temperature, poured into 0.2 litre of water, with stirring, and cooled with ice. Filtration with suction, washing with water and ether and drying yield the title compound: TLC: R_f=0.36 (hexane/acetone 3:2); $HPLC_{20\text{-}100(12')}$: t_{Ret}=12.7. Further product can be isolated from the mother liquor by chromatography (SiO_2; hexane/acetone 3:2).

16e) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane

A solution of 4.3 g (7.56 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane in 378 ml of formic acid is stirred at room temperature for 3.5 hours (argon) and then concentrated by evaporation. Sat. $NaHCO_3$ solution and methylene chloride are added to the residue; the aqueous phase is separated off and extracted 2× with methylene chloride. The organic phases are treated with brine, dried (Na_2SO_4) and concentrated by evaporation to form the title compound: $HPLC_{20\text{-}100(12')}$: t_{Ret}=6.8.

Example 17

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 294 mg of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane and 165 μl (1.5 mmol) of NMM in 4.8 ml of DMF are added to 113.5 mg (0.60 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e) and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF at 0° C. and the mixture is stirred at room temperature for 18 hours. Water and ethyl acetate are added; the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed 2× with water, sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/THF 4:1) and precipitation with hexane from a concentrated solution in methylene chloride yield the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=14.5; FAB MS $(M+H)^+$=697.

The starting material is prepared as follows:

17a) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane

With the exclusion of air, 4.8 g (18.5 mmol) of (2R)-[(1'S)-(trifluoroacetyl)amino-2'-phenylethyl]oxirane and 3.78 g (12.4 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(thiazol-2-yl)-benzyl]-hydrazine (Example 16c) in 62 ml of iso-PrOH are heated at boiling for 10 hours. Cooling the reaction mixture, filtration and washing with ether yield the title compound: Anal. ($C_{27}H_{31}N_4F_3O_4S$) calc. C 57.44, H 5.53, N 9.92, F 10.09, S 5.68: found C 57.27, H 5.49, N 9.91, F 9.94, S 5.70; $HPLC_{20\text{-}100}$: t_{Ret}=16.9; FAB MS $(M+H)^+$=565. Further product can be isolated from the filtrate by concentration by evaporation, column chromatography (SiO_2; methylene chloride/THF 25:1) and stirring from ether/ethyl acetate.

17b) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane

55 ml of a 1N K_2CO_3 solution are added dropwise to 3.12 9 (5.5 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S) -(trifluoroacetyl)amino-6-phenyl-2-azahexane in 55 ml of methanol and the mixture is stirred at 70° C. for 9 hours. The mixture is cooled and ≈30 ml of methanol are evaporated off; methylene chloride and water are added and the aqueous phase is separated off and extracted with methylene chloride; the organic phases are washed with water, dried (Na_2SO_4) and concentrated by evaporation, yielding the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=11.9; FAB MS $(M+H)^+$=469.

17c) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 1.4 g (8.0 mmol) of N-methoxycarbonyl-(L)-valine, 2.87 g (15 mmol) of EDC and 1.35 g (10 mmol) of HOBT are dissolved in 22 ml of DMF. After 45 min, 4.2 ml (30 mmol) of TEA are added and then a solution of 2.34 g (5.0 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane in 45 ml of DMF is added dropwise. After 1.5 hours, the reaction mixture is concentrated by evaporation; the residue is taken up in methylene chloride and washed with water, sat. $NaHCO_3$ solution, water and brine. The aqueous phases are extracted 2× with methylene chloride; the combined organic phases are dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/ethyl acetate 2:1) and crystallisation from ethyl acetate/ether yield the title compound: m.p: 178°–179° C.; $HPLC_{20\text{-}100}$: t_{Ret}=15.8.

17d) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino6-phenyl-2-azahexane

0.94 9 (1.5 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane and 18 ml of formic acid are stirred at room temperature for 6 hours and worked up analogously to Example 2d to form the title compound: FAB MS $(M+H)^+$=526.

Example 18

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 7, 106 mg (0.56 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 201 mg (1.05 mmol) of EDC and 95 mg (0.7 mmol) of HOBT in 4.6 ml of DMF and 293 µl (2.1 mmol) of TEA are reacted with 0.35 mmol of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5 (S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane to form the title compound: m.p: 227°–229° C.; $HPLC_{20\text{-}100}$: t_{Ret}=14.5; FAB MS $(M+H)^+$=697.

Example 19

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 7, 106 mg (0.56 mmol) of N-ethoxycarbonyl-(L)-valine, 201 mg (1.05 mmol) of EDC and 95 mg (0.7 mmol) of HOBT in 4.6 ml of DMF and 293 µl (2.1 mmol) of TEA are reacted with 0.35 mmol of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane to form the title compound: Anal. ($C_{35}H_{48}N_6O_7S$ (0.20 H_2O)) calc. C 60.01, H 6.96, N 12.00, S 4.58, H_2O 0.51: found C 60.07, H 6.78, N 11.93, S 4.70, H_2O 0.52; $HPLC_{20\text{-}100}$: t_{Ret}=14.6; FAB MS $(M+H)^+$=697.

Example 20

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

Analogously to Example 7, 140 mg (0.80 mmol) of N-methoxycarbonyl-(L)-valine, 288 mg (1.5 mmol) of EDC and 135 mg (1.0 mmol) of HOBT in 2.2 ml of DMF and 418 µl (3.0 mmol) of TEA are reacted with 0.5 mmol of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane in 4.5 ml of DMF to form the title compound: m.p: 207°–210° C.; $HPLC_{20\text{-}100}$: t_{Ret}=13.8; FAB MS $(M+H)^+$=683.

Example 21

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 294 mg of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane and 165 µl (1.5 mmol) of NMM in 4.8 ml of DMF are added to 113.5 mg (0.60 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e) and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF at 0° C. and the mixture is stirred at room temperature for 16 hours. Ice-water and ethyl acetate are added; the aqueous phase is separated off and extracted with ethyl acetate. The organic phases are washed 2× with water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; ethyl acetate) and crystallisation from ethyl acetate/ether/hexane yield the title compound: Anal. ($C_{36}H_{50}N_6O_7S$ (1.4% H_2O)) calc. C 59.97, H 7.15, N 11.66, S 4.45: found C 59.99, H 7.18, N 11.35, S 4.59; TLC: R_f=0.51 (methylene chloride/THF 3:1); $HPLC_{20\text{-}100}$: t_{Ret}=15.2; FAB MS $(M+H)^+$=711.

The starting material is prepared as follows:

21a) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 938 mg (4.96 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 1.78 g (9.3 mmol) of EDC and 838 mg (6.2 mmol) of HOBT are dissolved in 13.7 ml of DMF. After 30 min, 2.6 ml (18.6 mmol) of TEA are added and then a solution of 1.45 g (3.1 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane (Example 17b) in 28 ml of DMF is added dropwise thereto. After 3 hours the reaction mixture is concentrated by evaporation; the residue is taken up in ethyl acetate and a small amount of THF and washed with water, sat. $NaHCO_3$ solution, water and brine. The aqueous phases are extracted with ethyl acetate; the combined organic phases are dried over Na_2SO_4 and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/THF 5:1) and stirring from ethyl acetate/DIPE yield the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=16.3; FAB MS $(M+H)^+$=640.

21b) 1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

761 mg (1.2 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane and 12 ml of formic acid are stirred at room temperature for 7 hours and worked up analogously to Example 2d to form the title compound.

Example 22

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 321 mg (0.60 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane (Example 21b) and 182 mg (1.8 mmol) of NMM in 5.8 ml of DMF are added to 136 mg (0.72 mmol) of N-ethoxycarbonyl-(L)-valine and 178 mg (0.60 mmol) of TPTU in 3 ml of DMF and the mixture is stirred at room temperature for 15 hours. The reaction mixture is poured into ice-water, stirred for 30 min and filtered. Crystallisation from THF with DIPE and hexane yields the title compound: m.p.: 209°–211° C.; $HPLC_{20\text{-}100}$: t_{Ret}=15.2; FAB MS $(M+H)^+$=711.

Example 23

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 321 mg (0.60 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane (Example 21b) and 182 mg (1.8 mmol) of NMM in 5.8 ml of DMF are added to 126 mg (0.72 mmol) of N-methoxycarbonyl-(L)-valine and 178 mg (0.60 mmol) of TPTU in 3 ml of DMF; the mixture is stirred at room temperature for 15 hours and worked up analogously to Example 3. TLC: R_f=0.15 (methylene chloride/THF 4:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.5; FAB MS $(M+H)^+$=697.

Example 24

1-[4-(Thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-S-methylcysteinyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Under an argon atmosphere, 303 mg (0.50 mmol) of 1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane (Example 21 b) and 165 µl (1.5 mmol) of NMM in 5 ml of DMF are added to 116 mg (0.60 mmol) of N-methoxycarbonyl-(L)-S-methylcysteine (Example 5a) and 149 mg (0.50 mmol) of TPTU in 2.5 ml of DMF with ice-cooling and the mixture is stirred at room temperature for 4 hours. The mixture is poured into ice-water, stirred for 30 min and extracted 2× with ethyl acetate. The organic phases are washed 2× with water, sat. $NaHCO_3$ solution, 2× with water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/ethanol 20:1) and stirring from DIPE yield the title compound: TLC: R_f=0.39 (methylene chloride/methanol 10:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.0; FAB MS $(M+H)^+$=715.

Example 25

1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane

With the exclusion of air, 261 mg (1.38 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e), 496 mg (2.58 mmol) of EDC and 232 mg (1.72 mmol) of HOBT are dissolved in 7.5 ml of DMF. After 15 min, 0.72 ml (5.17 mmol) of TEA and 585 mg (0.86 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane hydrochloride in 3.5 ml of DMF are added. After 20 hours, the mixture is concentrated by evaporation and water and methylene chloride are added to the residue; the aqueous phase is separated off and extracted 2× more with methylene chloride. The organic phases are washed with 10% citric acid solution, sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation. Precipitation from a concentrated solution in ethyl acetate with DIPE/hexane yields the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=17.5; FAB MS $(M+H)^+$= 814.

The starting material is prepared as follows:

25a) 4-(Tetrazol-5-yl)-benzaldehyde

20.0 g (0.47 mol) of lithium chloride and 20.5 g (0.315 mol) of sodium azide are added to 41.2 g (0.315 mol) of 4-cyano-benzaldehyde (Fluka, Buchs, Switzerland) in 310 ml of methoxyethanol (Fluka, Buchs, Switzerland) and the mixture is heated at boiling for 6 hours (argon atmosphere). The cooled reaction mixture is poured into 1 litre of ice/37% HCl 10:1 and stirred thoroughly to complete the reaction. Filtration and washing with water yield the title compound: m.p.: 180°–182° C.; ^{1}H-NMR (DMSO-d_6) δ 10.11 (s, HCO), 8.29 and 8.14 (2d, J=8, each 2H).

25b) 4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-benzaldehyde

Under a nitrogen atmosphere, a solution of 6.9 g (58 mmol) of 2-phenyl-propene (Fluka, Buchs, Switzerland) and 22 ml of toluene is added dropwise to 10 g (57 mmol) of 4-(tetrazol-5-yl)-benzaldehyde and 1 g (5.7 mmol) of methanesulfonic acid in 44 ml of boiling toluene and the mixture is then stirred under reflux conditions for 1 hour. The cooled reaction mixture is washed 2× with sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation to form the title compound: ^{1}H-NMR (DMSO-d_6) δ 10.09 (s, HCO), 8.29 and 8.08 (2d, J=8, each 2H), 7.33 and 7.17 (2m, 5H), 2.17 (s, 6H).

25c) N-1-(tert-Butoxycarbonyl)-N-2-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl-methylidene}-hydrazone

13.0 g (42 mmol) of 4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-benzaldehyde and 5.98 g (45.2 mmol) of tert-butyl carbazate in 300 ml of ethanol are stirred at 80° C. for 20 hours. The reaction mixture is then concentrated to half by evaporation; 420 ml of water are added and the mixture is extracted 3× with ethyl acetate. The organic phases are washed 2× with sat. $NaHCO_3$ solution and brine, dried (Na_2SO_4) and concentrated by evaporation to form the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=17.7.

25d) N-1-(tert-Butoxycarbonyl)-N-2-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-benzyl}-hydrazine

Under a nitrogen atmosphere, 11.6 g (28.5 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl-methylidene}-hydrazone are placed in 140 ml of THF, and 2.32 g (31.3 mmol; 85%) of sodium cyanoborohydride are added. A solution of 5.42 g (28.5 mmol) of p-toluenesulfonic acid monohydrate in 90 ml of THF is added dropwise thereto. After 4 hours, the mixture is concentrated by evaporation; the residue is taken up in ethyl acetate and washed with sat. $NaHCO_3$ solution and brine. The aqueous phases are extracted 2× with ethyl acetate; the organic phases are dried (Na_2SO_4) and concentrated by evaporation. The residue is taken up in 250 ml of methanol and 125 ml of THF, 37 g of $K_2B_4O_7 \times H_2O$ in 125 ml of water are added, with cooling, and the mixture is stirred overnight. The mixture is partially concentrated by evaporation using a rotary evaporator and is diluted with methylene chloride and water; the aqueous phase is separated off and extracted 2× with methylene chloride. The organic phases are dried (Na_2SO_4) and concentrated by evaporation to form the title compound: $HPLC_{20\text{-}100}$: t_{Ret}= 16.4.

25e) 1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane

A mixture of 6.05 g (23.4 mmol) of (2R)-[(1'S)-(trifluoroacetyl)amino-2'-phenylethyl]oxirane and 9.54 g (23.4 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-benzyl}-hydrazine in 200 ml of iso-PrOH is heated at 90° C. for 24 hours. Concentration by evaporation, chromatography (SiO_2; methylene chloride/ether 20:1) and crystallisation from MeOH yield the title compound: Anal. ($C_{34}H_{40}N_7O_4F_3$) calc. C 61.16, H 6.04, N 14.68: found C 61.37, H 6.02, N 14.80.

25f) 1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane

28 ml of a 1N K_2CO_3 solution are added dropwise, at 70° C., to 1.9 g (2.8 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)-amino- 5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane in 29 ml of methanol and the mixture is stirred for 15 hours. After cooling and concentration by evaporation, methylene chloride and water are added; the aqueous phase is separated off and extracted with methylene chloride. The organic phases are washed with water, dried (Na_2SO_4) and concentrated by evaporation to yield the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=5.1; FAB MS $(M+H)^+$=469.

25g) 1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

With the exclusion of air, 868 mg (4.59 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e), 1.64 g (8.58 mmol) of EDC and 773 mg (5.72 mmol) of HOBT are dissolved in 24.5 ml of DMF. After 15 min, 2.39 ml (17.2 mmol) of TEA and 1.64 g (2.86 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane in 12 ml of DMF are added. After 20 hours, the mixture is concentrated by evaporation, and water and methylene chloride are added to the residue; the aqueous phase is separated off and extracted 2× more with methylene chloride. The organic phases are washed with 10% citric acid solution, sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation.

Digestion from DIPE yields the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=18.6; FAB MS $(M+H)^+$=743.

25h) 1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane hydrochloride

Under a nitrogen atmosphere, 1.37 g (1.84 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S) -hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane are stirred in 64 ml of acetonitrile and 64 ml of aqueous 2N HCl at room temperature for 6 days. The reaction mixture is filtered, and the filtrate is concentrated by evaporation under a high vacuum at room temperature and is finally lyophilised from dioxane to yield the title compound: $HPLC_{20\text{-}100}$: t_{ret}=14.2; ^{1}H-NMR (CD_3OD) inter alia δ 8.10 (d, J=8, $2H^{arom}$), 7.8 (m, $1H^{arom}$), 7.53 (m, $2H^{arom}$), 7.32 (m, $3H^{arom}$), 7.17 (m, $6H^{arom}$), 2.23 (s, 2 $H_3C^{tetrazole\text{-}protecting\ group}$).

Example 26

1-[4-(Tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane

34.5 ml of an 80% aqueous H_2SO_4 solution are added to 345.6 mg (0.424 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-5(S)-2,5-bis [N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane, with ice-cooling. After stirring for 75 min, the mixture is poured into 800 ml of ice-water and extracted 3× with ethyl acetate. The organic phases are washed 3× with water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; ethyl acetate/ethanol 8:1→2:1) yields the title compound: TLC: R_f=0.38 (ethyl acetate/ethanol 2:1); $HPLC_{20\text{-}100}$: t_{Ret}=12.5; FAB MS $(M+H)^+$=696.

Example 27

1-[4-(2-Methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane (and 1-methyl-1H-tetrazolyl isomer)

Under a nitrogen atmosphere, 100 mg (0.144 mmol) of 1-[4-(tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane are dissolved in 1 ml of DMF/dioxane 1:1 and, at 0° C., 73.2 mg (0.224 mmol) of Cs_2CO_3 and 6.9 μl (0.111 mmol) of methyl iodide in 1 ml of dioxane are added. The mixture is allowed to warm up slowly to room temperature overnight and a further 1 equivalent of Cs_2CO_3 and of methyl iodide are added. After stirring for a further 4 hours at room temperature, the mixture is diluted with ethyl acetate and 1N sodium hydroxide solution. The aqueous phase is separated off and extracted 2× with ethyl acetate. The organic phases are washed 2× with water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; methylene chloride/ethyl acetate 1:1→1:2) yields the pure title compound A (≈3 parts), followed by 1-[4-(1-methyl-1H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane (B) (≈1 part): A: TLC: R_f=0.26 (methylene chloride/ethyl acetate 1:1); $HPLC_{20\text{-}100}$: t_{Ret}=14.2; FAB MS $(M+H)^+$=710. B: TLC: R_f=0.09 (methylene chloride/ethyl acetate 1:1); $HPLC_{20\text{-}100}$: t_{Ret}= 13.3; FAB MS $(M+H)^+$=710.

Alternative synthesis of the title compound:

Under a nitrogen atmosphere, 14.56 g (77 mmol) of N-methoxycarbonyl-(L)-tert-leucine and 22.87 g (77 mmol) of TPTU are stirred in 77 ml of DMF and 37.3 ml (218 mmol) of Hünig base at room temperature for 30 min. The reaction mixture is then added dropwise to an ice-cooled solution of 35.2 mmol of 1-[4-(2-methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane dihydrochloride in 77 ml of DMF. After stirring at room temperature for 15 hours, the reaction mixture is partially concentrated by evaporation and the residue (≈80 ml) is poured into 5 litres of water; the mixture is stirred for 30 min and the crude product is filtered off. Dissolution in 90 ml of boiling ethanol, addition of 600 ml of DIPE and cooling yield the title compound: m.p.: 191°–192° C.; $[\alpha]_D$=–46° (c=0.5; ethanol).

The starting material is prepared as follows:

27a) 4-(2-Methyl-2H-tetrazol-5-yl)-benzaldehyde

With ice-cooling, a solution of 75.5 g (0.434 mol) of 4-(tetrazol-5-yl)-benzaldehyde (Example 25a) in 550 ml of DMF/dioxane 1:1 is added dropwise to 179.7 g (1.30 mol) of K_2CO_3 in 200 ml of DMF/dioxane 1:1; the mixture is stirred for 30 min and then 40 ml (0.64 mol) of methyl iodide are added. The mixture is stirred in an ice bath for 3 hours and, finally, at room temperature for 15 hours; the reaction mixture is poured into 2.8 litres of ice-water and stirred for 10 min; the title compound is filtered off and washed with water: m.p.: 137°–139° C.; $_1$H-NMR ($CD_3OD/CDCl_3$) d 10.05 (s, HCO), 8.29 and 8.03 (2d, J=8, each 2H), 4.43 (s, 3H).

27b) N-1-(tert-Butyloxycarbonyl)-N-2-[4-(2-methyl-2H-tetrazol-5-yl)-phenylmethylidene]-hydrazone

75.0 g (0.40 mol) of 4-(2-methyl-2H-tetrazol-5-yl)-benzaldehyde and 56.4 g (0.426 mol) of tert-butyl carbazate in 1400 ml of iso-PrOH are stirred at 90° C. for 24 hours. 2.2 litres of water are added to the cooled reaction mixture and the mixture is stirred thoroughly to complete the reaction; the title compound is filtered off and washed with water: m.p.: 195°–197° C.; Anal. ($C_{14}H_{18}N_6O_2$) calc. C 55.62, H 6.00, N 27.80: found C 55.50, H 5.93, N 27.61.

27c) N-1-(tert-Butyloxycarbonyl)-N-2-[4-(2-methyl-2H-tetrazol-5-yl)-benzyl]-hydrazine

Under a nitrogen atmosphere, 30.0 g (99.2 mmol) of N-1-(tert-butyloxycarbonyl)-N-2-[4-(2-methyl-2H-tetrazol-

5-yl)-phenyl-methylidene]-hydrazone are placed in 350 ml of THF, and 8.79 g (119 mmol; 85%) of $NaCNBH_3$ are added. A solution of 22.6 g (119 mmol) of p-toluenesulfonic acid monohydrate in 175 ml of THF is added dropwise thereto (→precipitation). After 2 hours, the solid is filtered off, washed thoroughly with ethyl acetate and discarded. Water and ethyl acetate are added to the filtrate; the aqueous phase is separated off and extracted 2× more with ethyl acetate. The organic phases are washed with sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation. The resulting crystals are taken up in 417 ml of methanol and 208 ml of THF, and a solution of 127 g (415 mmol) of $K_2B_4O_7.4H_2O$ in 417 ml of H_2O is added dropwise (→production of foam). The mixture is stirred at room temperature overnight, poured into 2.2 litres of water and extracted 3× with ethyl acetate. The organic phases are washed with sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated by evaporation. The crude product is combined with material from a second, identical batch and filtered through silica gel using methylene chloride/THF 10:1 as the eluant. Concentration by evaporation to a residual volume of about 0.1 litres and addition of 150 ml of DIPE yield the crystalline title compound (which, alternatively, may also be obtained by catalytic hydrogenation of N-1-(Boc)-N-2-[4-(2-methyl-2H-tetrazol-5-yl)-phenyl-methylidene]-hydrazone with Lindlar catalyst in methanol): m.p.: 100°–102° C.; TLC: R_f=0.47 (methylene chloride/THF 10:1); ^{1}H-NMR (CD_3OD) d 8.06 and 7.52 (2d, J=8, each 2H), 4.42 (s, 3H); 4.00 (s, 2H); 1.44 (s, 9H); $HPLC_{20\text{-}100}$: t_{Ret}=10.2.

27d) 1-[4-(2-Methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butyloxycarbonyl)amino]-6-phenyl-2-azahexane

36.33 g (138 mmol) of (2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane and 38.17 g (125 mmol) of N-1-(tert-butyloxycarbonyl)-N-2-[4-(2-methyl-2H-tetrazol-5-yl)-benzyl]-hydrazine are heated in 964 ml of iso-PrOH at 90° C. for 20 hours. The crystallised title compound can be separated from the cooled reaction mixture by filtration. Further product crystallises out of the filtrate after the addition of 1.2 litres of water: m.p.: 175°–178° C.; TLC: R_f=0.22 (methylene chloride/ethyl acetate 6:1); $HPLC_{20\text{-}100}$: t_{Ret}=16.9.

27e) 1-[4-(2-Methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-phenyl-2-azahexane dihydrochloride

93 ml of 4N aqueous hydrochloric acid solution are added to a solution of 20.0 g (35.2 mmol) of 1-[4-(2-methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butyloxycarbonyl)amino]-6-phenyl-2-azahexane in 279 ml of THF. The mixture is stirred at 50° C. for 8 hours and then concentrated gently by evaporation (room temperature; high vacuum). The oily residue is taken up 3× more in ethanol and again concentrated by evaporation, yielding the crystalline title compound. In order to determine the analytical data, 1 g of the crude product was stirred in 6 ml of hot iso-PrOH, 6 ml of DIPE was added, and cooling and separation by filtration were carried out: m.p.: 227°–230° C.; $HPLC_{20\text{-}100}$: t_{Ret}=7.4; Anal. ($C_{19}H_{25}N_7O$.2 HCl (+0.20 H_2O)) calc. C 51.40, H 6.22, N 22.08, Cl 15.97, H_2O 0.81: found C 51.50, H 6.33, N 22.28, Cl 15.88, H_2O 0.80.

Example 28

1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

With the exclusion of air, 261 mg (1.38 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 496 mg (2.58 mmol) of EDC and 232 mg (1.72 mmol) of HOBT are dissolved in 7.5 ml of DMF. After 15 min, 0.72 ml (5.17 mmol) of TEA and 585 mg (0.86 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane hydrochloride (Example 25h) in 3.5 ml of DMF are added. After 20 hours, the mixture is worked up as described under Example 25i. Precipitation with DIPE from a concentrated solution in methylene chloride yields the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=17.5; FAB MS $(M+H)^+$=814.

Example 29

1-[4-(Tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

35 ml of an 80% aqueous H_2SO_4 solution are added to 354 mg (0.435 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-isleucyl)amino- 5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane, with ice-cooling. After stirring for 75 min, the mixture is worked up analogously to Example 26 to yield the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=12.6; FAB MS $(M+H)^+$= 696.

Example 30

1-[4-(2-Methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 72 mg (0.103 mmol) of 1-[4-(tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)-amino]-6-phenyl-2-azahexane are dissolved in 0.5 ml of DMF and, at 0° C., 71 mg (0.217 mmol) of Cs_2CO_3 and 6.9 μl (0.111 mmol) of methyl iodide in 1 ml of dioxane are added. The mixture is allowed to warm up slowly to room temperature overnight and is then diluted with ethyl acetate and 1N sodium hydroxide solution. The aqueous phase is separated off and extracted 2× with ethyl acetate. The organic phases are washed 2× with water and brine, dried (Na_2SO_4) and concentrated by evaporation to yield title compound A, which additionally contains ≈20% 1-[4-(1-methyl-1H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane (B): $HPLC_{20\text{-}100}$A: t_{Ret}=14.3; $HPLC_{20\text{-}100}$B: t_{Ret}=13.3; FAB MS $(M+H)^+$=710.

Example 31

1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl) amino-6-phenyl-2-azahexane

With the exclusion of air, 128 mg (0.67 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e), 243 mg (1.27 mmol) of EDC and 114 mg (0.84 mmol) of HOBT are dissolved in 2 ml of DMF. After 15 min, 0.35 ml (2.5 mmol) of TEA and 286 mg (0.42 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane hydrochloride in 1.5 ml of DMF are added. After 20 hours, the mixture is worked up as described under Example 25. Chromatography (SiO_2; ethyl acetate/toluene/methylene chloride 2:1:1) yields the title compound: TLC: R_f=0.22 (methylene chloride/ethyl acetate 1:1); $HPLC_{20\text{-}100}$: t_{Ret}=17.3, FAB MS $(M+H)^+$=814.

The starting materials are prepared as follows:

31a) 1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

With the exclusion of air, 270 mg (1.43 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 513 mg (2.67 mmol) of EDC and 241 mg (1.78 mmol) of HOBT are dissolved in 7.8 ml of DMF. After stirring for 15 min, 0.75 ml (5.4 mmol) of TEA and 510 mg (0.89 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane (Example 25f) in 3.7 ml of DMF are added. After 20 hours, the mixture is worked up analogously to Example 25g to yield the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=18.5; FAB MS $(M+H)^+$=743.

31b) 1-{4-[2-(1-Methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane hydrochloride

Under a nitrogen atmosphere, 317 mg (0.43 mmol) of 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane in 15 ml of acetonitrile and 15 ml of 2N HCl are stirred at 50° C. for 20 hours and worked up analogously to Example 25h to form the title compound: $HPLC_{20\text{-}100}$: t_{Ret}=14.4.

Example 32

1-[4-(Tetrazol-5-yl)-phenyl-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino]-6-phenyl-2-azahexane

Analogously to Example 26, 1-{4-[2-(1-methyl-1-phenyl-ethyl)-2H-tetrazol-5-yl]-phenyl}-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane is deprotected with 80% sulfuric acid to form the title compound.

Example 33

1-[4-(2-Methyl-2H-tetrazol-5-yl)-pheny]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 30, 1-[4-(tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl) amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino]-6-phenyl-2-azahexane in DMF/dioxane is methylated with Cs_2CO_3 and methyl iodide.

Example 34

1-[4-(2-tert-Butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl) amino]-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 54 mg (0.28 mmol) of N-methoxycarbonyl-(L)-tert-leucine and 84 mg (0.28 mmol) of TPTU in 1 ml of DMF and 94 µl (0.85 mmol) of NMM are stirred at room temperature for 10 min. 175 mg (0.283 mmol) of 1-[4-(2-tert-butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-amino-2-N-[N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane hydrochloride in 2 ml of DMF are then added thereto and the mixture is stirred at room temperature overnight to complete the reaction. The reaction mixture is poured into 40 ml of water and extracted 3× with methylene chloride. The organic phases are filtered through cotton wadding, concentrated by evaporation and chromatographed (SiO_2; methylene chloride/methanol 25:1): TLC: R_f=0.48 (methylene chloride/methanol 19:1); $HPLC_{20\text{-}100(12')}$: t_{Ret}= 11.8; FAB MS $(M+H)^+$=752.

The starting material is prepared as follows:

34a) N-1-(tert-Butyloxycarbonyl)-N-2-[N-methoxycarbonyl-(L)-tert-leucyl]-hydrazine

With the exclusion of air, 10.0 g (52.8 mmol) of N-methoxycarbonyl-(L)-tert-leucine, 11.1 g (58 mmol) of EDC and 7.85 g (58 mmol) of HOBT are placed in 130 ml of ethyl acetate, and 7.0 ml (63 mmol) of NMM are added. After 30 min, 7.69 g (58 mmol) of tert-butyl carbazate are added and the mixture is then stirred at room temperature for 16 hours. The reaction mixture is diluted with 300 ml of ethyl acetate and washed with sat. $NaHCO_3$ solution, water and brine. The aqueous phases are back-extracted 2× with ethyl acetate. The organic phases are dried (Na_2SO_4) and concentrated by evaporation to form the title compound: ^{1}H-NMR (CD_3OD) d 3.98 (s, 1H), 3.66 (s, 3H), 1.47 and 1.03 (2s, 2× 9H).

34b) [N-Methoxycarbonyl-(L)-tert-leucyl]-hydrazine

52.8 mmol of N-1-(tert-butyloxycarbonyl)-N-2-[N-methoxycarbonyl-(L)-tert-leucyl]-hydrazine are dissolved in 100 ml of 4N HCl/dioxane and stirred at room temperature for 18 hours. The suspension is concentrated by evaporation; the residue is taken up in sat. $NaHCO_3$ solution and extracted 4× with large amounts of methylene chloride. Filtration of the organic phases through cotton wadding and concentration by evaporation yield the title compound: ^{1}H-NMR (CD_3OD) d 3.89 (s, 1H), 3.66 (s, 3H), 0.99 (s, 9H).

34c) N-1-[N-Methoxycarbonyl-(L)-tert-leucyl]-N-2-[4-(tetrazol-5-yl)-phenyl-methylidene]-hydrazone

A solution of 3.0 g (14.8 mmol) of [N-methoxycarbonyl-(L)-tert-leucyl]-hydrazine and 2.57 g (14.8 mmol) of 4-(tetrazol-5-yl)-benzaldehyde (Example 25a) in 30 ml of isoPrOH is heated at boiling for 18 hours. The mixture is cooled; 100 ml of water are added and the precipitated title compound is filtered off: ^{1}H-NMR (CD_3OD) d 8.23 (s, 1H), 8.15–7.9 (m, 4H), 4.08 (s, 1H), 3.67 (s, 3H), 1.06 (s, 9H).

34d) N-1-[N-Methoxycarbonyl-(L)-tert-leucyl]-N-2-[4-(2-tert-butyl-2H-tetrazol-5-yl)-phenyl-methylidene]-hydrazone

In an autoclave, 3.0 g (8.3 mmol) of N-1-[N-methoxycarbonyl-(L)-tert-leucyl]-N-2-[4-(tetrazol-5-yl)-phenyl-methylidene]-hydrazone, 1.2 g of isobutene and 54 µl of methanesulfonic acid in 25 ml of toluene are heated at 110° C. for 1 hour. The reaction mixture is diluted with ethyl acetate and washed with sat. $NaHCO_3$ solution and brine. The aqueous phases are back-extracted 2× with ethyl acetate; the organic phases are dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; hexane/ethyl acetate 1:1) yields the title compound: TLC: R_f=0.22 (hexane/ethyl acetate 1:1); $HPLC_{20\text{-}100(12')}$: t_{Ret}= 11.1; FAB MS $(M+H)^+$=416.

34e) N-1-[N-Methoxycarbonyl-(L)-tert-leucyl]-N-2-[4-(2-tert-butyl-2H-tetrazol-5-yl)-benzyl]-hydrazine

Under a nitrogen atmosphere, 2.00 g (4.81 mmol) of N-1-[N-methoxycarbonyl-(L)-tert-leucyl]-N-2-[4-(2-tert-butyl-2H-tetrazol-5-yl)-phenyl-methylidene]-hydrazone are dissolved in 9 ml of THF, and 317 mg (4.8 mmol; 95%) of $NaCNBH_3$ are added. A solution of 915 mg (4.8 mmol) of p-toluenesulfonic acid monohydrate in 9 ml of THF is added dropwise thereto. After 18 hours, ethyl acetate is added and the mixture is washed with sat. $NaHCO_3$ solution and brine. The aqueous phases are extracted a further 2× with ethyl acetate. The organic phases are dried (Na_2SO_4) and concentrated by evaporation. The residue is taken up in 20 ml of

THF and 20 ml of water; 6.18 g (20 mmol) of $K_2B_4O_7.4H_2O$ are added and the mixture is stirred at room temperature overnight. The reaction mixture is diluted with ethyl acetate and washed with sat. $NaHCO_3$ solution and brine. The aqueous phases are extracted 2× with ethyl acetate; the organic phases are dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; hexane/ethyl acetate 1:2) yields the title compound: TLC: R_f=0.28 (hexane/ethyl acetate 1:2); ^{1}H-NMR (CD_3OD) d 8.07 and 7.53 (2d, J=8, each 2H), 4.03 (s, 2H); 3.84 (s, 1H); 3.64 (s, 3H); 1.81 and 0.92 (2s, each 9H).

34f) 1-[4-(2-tert-Butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-N-(tert-butyloxycarbonyl)-amino-2-N-[N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane

737 mg (2.80 mmol) of (2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane and 1.17 g (2.80 mmol) of N-1-[N-methoxycarbonyl-(L)-tert-leucyl]-N-2-[4-(2-tert-butyl-2H-tetrazol-5-yl)-benzyl]-hydrazine are heated in 15 ml of iso-PrOH at 90° C. for 16 hours. On the addition of 100 ml of water the product crystallises and can be filtered off. Recrystallisation by the addition of DIPE/hexane to a concentrated solution in methylene. chloride at 0° C. yields the title compound: TLC: R_f=0.34 (CH_2Cl_2/MeOH 30:1); $HPLC_{20\text{-}100(12')}$: t_{Ret}=12.5.

34q) 1-[4-(2-tert-Butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-amino-2-N-[N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane hydrochloride

Under a nitrogen atmosphere, 200 mg (0.293 mmol) of 1-[4-(2-tert-butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-N-(tert-butyloxycarbonyl)amino-2-N-[N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane are dissolved in 2.3 ml of THF; 1.6 ml of aqueous 2N HCl are added and the mixture is stirred at 50° C. for 8 hours. The reaction solution is concentrated by evaporation; the residue is taken up several times in ethanol and concentrated by evaporation again (→title compound): TLC: R_f=0.08 (CH_2Cl_2/MeOH 30:1); $HPLC_{20\text{-}100(12')}$: t_{Ret}=9.9; ^{1}H-NMR (CD_3OD) d 8.03 and 7.50 (2d, J=8, each 2H), 7.32 (m, 5H), 4.18 and 3.91 (2d, J=4, 2H), 3.80 (m, 1H), 3.68 (s, 1H), 3.58 (s, 3H), 3.57 (m, 1H), 3.3–2.9 (m, 4H), 1.81 and 0.75 (2s, each 9H).

Example 35

1-[4-(2-tert-Butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 54 mg (0.308 mmol) of N-methoxycarbonyl-(L)-valine and 92 mg (0.308 mmol) of TPTU in 1 ml of DMF and 101 µl (0.91 mmol) of NMM are stirred at room temperature for 10 min. 190 mg (0.308 mmol) of 1-[4-(2-tert-butyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-amino-2-N-[N-methoxycarbonyl-(L)-tert-leucyl]amino-6-phenyl-2-azahexane hydrochloride (Example 34g) in 2 ml of DMF are added thereto and the mixture is stirred at room temperature overnight to complete the reaction. The reaction mixture is diluted with methylene chloride and washed with brine. The aqueous phases are extracted 2× with methylene chloride; the organic phases are filtered through cotton wadding, concentrated by evaporation and chromatographed (SiO_2; methylene chloride/methanol 30:1): TLC: R_f=0.21 (methylene chloride/methanol 19:1); FAB MS $(M+H)^+$=738.

Example 36

1-[4-(2-Methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

The title compound may be prepared analogously to one of the Examples mentioned hereinabove and hereinbelow.

Example 37

1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

With the exclusion of moisture, 455 mg (2.6 mmol) of N-methoxycarbonyl-(L)-valine, 940 mg (4.9 mmol) of EDC and 405 mg (3 mmol) of HOBT are placed in 10 ml of DMF and heated at 40° C. 1.1 ml (7.9 mmol) of TEA are added and the mixture is stirred for a further 15 min. 500 mg (0.98 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane hydrochloride are added thereto and the mixture is stirred at room temperature overnight. The reaction mixture is extensively concentrated by evaporation under a high vacuum; the residue is dissolved in methylene chloride and washed in succession with sodium carbonate solution (1×), phosphate buffer pH=7 (2×) and brine. After removal of the solvent, the residue is chromatographed on silica gel (eluant: methylene chloride/methanol 15:1). The product-containing fractions are concentrated and the title compound is precipitated with DIPE. The product can be lyophilised from dioxane. $HPLC_{20\text{-}100}$: t_{Ret}=10.06; FAB MS $(M+H)^+$=677. 1H-NMR (CD_3OD; 200 MHz) i.a.: 8.58/m (1H); 7.78 and 7.50/each d, J=5 (2×2H); 8.0–7.73/m (2H); 7.33/m (1H); 7.30–7.05/m (5H); 3.62 and 3.60/each s (2×2H); 1.85 and 1.68/each m (2×1H); 0.76/'T', J=4 (6H); 0.65 and 0.58/each d, J=4 (2×3H).

The starting material is prepared as follows:

37a) 4-Bromobenzaldehyde dimethyl acetal

21.1 g (114 mmol) of 4-bromobenzaldehyde and 20 ml (182 mmol) of trimethyl orthoformate (both Fluka, Buchs, Switzerland) are dissolved in 35 ml of methanol, and 0.65 g (3.4 mmol) of p-toluenesulfonic acid monohydrate is added at room temperature (exothermic reaction). The reaction mixture is stirred at room temperature under nitrogen for 20 hours. The acid is then neutralised with 0.62 ml of 30% sodium methanolate solution in methanol (3.4 mmol); the reaction mixture is concentrated using a rotary evaporator and the residue is distilled. The title compound is obtained in the form of a colourless liquid. TLC: R_f=0.58 (hexane/ethyl acetate 2:1). B.p.: 90°–92° C. (4 mbar). 1H-NMR ($CDCl_3$; 200 MHz): 7.50 and 7.32/each d, J=9 (2×2H); 5.36/s (1H); 3.31/s (6H).

37b) 4-(Pyridin-2-yl)-benzaldehyde

6.93 g (29.9 mmol) of 4-bromobenzaldehyde dimethyl acetal in 40 ml of THF are added dropwise to a warm (from 40° C. to 50° C.) suspension of 0.8 g (31.6 mmol) of magnesium turnings and a small amount of iodine in 10 ml of THF. The reaction mixture is heated to 65° C. and stirred at that temperature for about 30 min. The mixture is allowed to cool to room temperature and the Grignard reagent is added dropwise to a solution of 4.46 g (28.2 mmol) of 2-bromopyridine (Fluka, Buchs, Switzerland) and 0.4 g (0.74 mmol) of DPPP (Fluka, Buchs, Switzerland) in 100 ml of THF (slightly exothermic reaction). After the dropwise addition is complete, the reaction mixture is boiled under reflux for 4 hours and is then allowed to cool; 100 ml of water are added. The mixture is concentrated to about 50 ml using a rotary evaporator, diluted with ethyl acetate and extracted with 0.1N hydrochloric acid (3×). The combined HCl extracts are stirred at room temperature for 20 min, rendered basic with concentrated ammonia solution and extracted with methylene chloride. After removal of the solvent, the residue is chromatographed on silica gel (hexane/ethyl acetate 2:1). The product-containing fractions are concentrated, with the desired title compound crystallising out spontaneously.

TLC: R_f=0.22 (hexane/ethyl acetate 2:1). $HPLC_{20-100}$: t_{Ret}=6.08. 1H-NMR ($CDCl_3$; 200 MHz): 8.73/d, J=5 (2H); 8.16 and 7.97/each d (2×2H); 7.80/d, J=4 (2H); 7.3/m (1H).

37c) N-1-(tert-Butoxycarbonyl)-N-2-{4-[(pyridin-2-yl)-phenyl]-methylidene}-hydrazone

A solution of 2 g (1.05 mmol) of 4-(pyridin-2-yl)-benzaldehyde and 1.37 g (1 mmol) of tert-butyl carbazate (Fluka, Buchs, Switzerland) in 30 ml of ethanol is stirred at 80° C. for 5 hours (after 4 hours, a further 0.05 equivalent of tert-butyl carbazate is added). The reaction mixture is allowed to cool and diluted with water, with the desired title compound crystallising out. TLC: R_f=0.51 (methylene chloride/methanol 15:1). $HPLC_{20-100}$: tRet=8.92. 1H-NMR ($CDCl_3$; 200 MHz): 8.68/m (1H); 8.21/s (1H); 7.98/d, J=9 (2H, portion A of aromatic AB system); 7.85/s (1H); 7.8–7.6/m (4H); 7.22/m (1H); 1.53/s (9H).

37d) N-1-(tert-Butoxycarbonyl)-N-2-[4-(pyridin-2-yl)-benzyl]-hydrazine

2 g (6.7 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{4-[(pyridin-2-yl)-phenyl]-methylidene}-hydrazone and 0.2 g of 5% Pd/C in 30 ml of methanol are hydrogenated under normal pressure at room temperature for 8 hours. The catalyst is filtered off and washed with methanol; the solvent is removed. The title compound is obtained in the form of a colourless, viscous oil, which solidifies on drying under a high vacuum. TLC: R_f=0.46 (methylene chloride/methanol 15:1). $HPLC_{20-100}$: t_{Ret}=6.71. 1H-NMR ($CDCl_3$; 200 MHz) i.a.: 8.69/m (1H); 7.96 and 7.45/each d, J=2 (2×2H); 7.8–7.65/m (2H); 7.22/m (1H); 4.06/s (2H); 1.47/s (9H).

37e) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane

A solution of 1.06 g (4 mmol) of (2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane and 1.2 g (4 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(pyridin-2-yl)-benzyl]-hydrazine in 20 ml of iso-PrOH is stirred at 80° C. for 16 hours. After cooling, the reaction solution is concentrated using a rotary evaporator, with the title compound precipitating out as a colourless precipitate. Further product can be precipitated out by adding water to the mother liquor. TLC: R_f=0.53 (methylene chloride/methanol 15:1). $HPLC_{20-100}$: t_{Ret}=13.15. 1H-NMR (CD_3OD; 200 MHz) ia.: 8.57/s (1H); 7.85 and 7.48/each d, J=9 (2×2H); 8.0–7.7/m)2H); 7.33/m (1H); 7.3–7.0/m (6H); 3.91/s (2H); 3.82–3.55/m (2H); 3.05–2.45/m (4H); 1.31/s (18H).

37f) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane hydrochloride

10 ml of DMF are added to a mixture consisting of 1.43 g (2.54 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane in 30 ml of 4N hydrogen chloride in dioxane (Aldrich) (exothermic reaction) and the mixture is stirred at room temperature for 2 hours. The solvent is then removed; toluene is added to the residue three times and the mixture is concentrated by evaporation. The residue is dissolved in hot methanol and the title compound is precipitated in the form of a resinous precipitate with DIPE/hexane. On drying under a high vacuum, a voluminous foam is obtained.

$HPLC_{5-60}$: t_{Ret}=9.87. 1H-NMR (CD_3OD; 200 MHz) i.a.: 8.78/d, J=5 (1H); 8.72/dxt, J=2.5 and 7.5 (1H); 8.35/d, J=7.5 (1H); 8.1/dxd, J=each 7.5 (1H); 8.02 and 7.72/each d, J=9 (2×2H); 7.45–7.15/m (5H); 4.27 and 4.15/each d, J=12.5 (2×2H).

Example 38

1-l [4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-ethoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

Analogously to Example 37, after working up the title compound is obtained from 300 mg (0.59 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane hydrochloride (Example 37f), 446 mg (2.36 mmol) of N-ethoxycarbonyl-(L)-valine, 679 mg (3.54 mmol) of EDC, 398 mg (2.95 mmol) of HOBT and 0.82 ml (5.9 mmol) of TEA in 10 ml of DMF. TLC: R_f=0.19 (methylene chloride/methanol 15:1). $HPLC_{20-100}$: t_{Ret}=11.68. FAB MS $(M+H)^+$=705.

Example 39

1-[4-(Pyridin-3-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

Analogously to Example 37, the title compound is obtained from 550 mg (1.52 mmol) of 1-[4-(pyridin-3-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane, 691 mg (3.94 mmol) of N-methoxycarbonyl-(L)-valine, 1.45 g (7.59 mmol) of EDC, 614 mg (4.55 mmol) of HOBT and 1.06 ml (7.59 mmol) of TEA in 10 ml of DMF. (Contrary to the description in Example 37, the organic phase is washed with sat. sodium hydrogen carbonate solution, 10% citric acid and brine.) TLC: R_f=0.4 (methylene chloride/methanol 15:1). $HPLC_{20-100}$: t_{Ret}=9.91; FAB MS $(M+H)^+$=677.

The starting material is prepared as follows:

39a) 4-(Pyridin-3-yl)-benzaldehyde

Analogously to Example 37b, the title compound is obtained from 6.39 g (29.9 mmol) of 4-bromobenzaldehyde dimethyl acetal (prepared in accordance with Example 37a), 0.8 g (31.6 mmol) of magnesium turnings, 2.77 ml (28.2 mmol) of 3-bromopyridine (Fluka, Buchs, Switzerland) and 0.4 g (0.74 mmol) of DPPP in 150 ml of THF. $HPLC_{20-100}$: t_{Ret}=5.50. 1H NMR (CD_3OD; 200 MHz): 10.04/s (1H); 8.87/d, J=2.5 (1H); 8.58/dxd, J=about 1.5 and 5 (1H); 8.17/m ia. J=7.5 (1H); 8.05 and 7.88/each d, J=9 (2×2H); 7.56/dxd, J=7.5 and 5 (1H).

39b) N-1-(tert-Butoxycarbonyl)-N-2-{4-[(Pyridin-3-yl)-phenyl]-methylidene}-hydrazone

Analogously to Example 37c, the title compound is obtained from 4.11 g (22.4 mmol) of 4-(pyridin-3-yl)-benzaldehyde and 2.82 g (21.3 mmol) of tert-butyl carbazate (Fluka, Buchs, Switzerland) in 60 ml of ethanol. $HPLC_{20-100}$: t_{Ret}=8.88. 1H-NMR (CD_3OD; 200 MHz): 8.83/d, J=2.5 (1H); 8.53/d, J=5 (1H); 8.14/m i.a. J=7.5 (1H); 7.97/s (1H); 7.85 and 7.71/each d, J=9 (2×2H); 7.53/dxd, J=7.5 and 5 (1H).

39c) N-1-(tert-Butoxycarbonyl)-N-2-[4-(pyridin-3-yl)-benzyl]-hydrazine

Analogously to Example 37d, the title compound is obtained from 5.03 g (16.9 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{4-[(pyridin-3-yl)-phenyl]methylidene}-hydrazone and 0.5 g of 5% Pd/C in 120 ml of methanol, the title compound being processed further in unpurified form. $HPLC_{20-100}$: t_{Ret}=6.36. 1H-NMR (CD_3OD; 200 MHz) ia.: 7.63 and 7.51/each d, J=9 (2×2H); 3.97/s (2H); 1.43/s (9H).

39d) 1-[4-(Pyridin-3-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane

Analogously to Example 37e, the title compound is obtained from 3.82 g (12.8 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(pyridin-3-yl)-benzyl]-hydrazine and 3.36 g (12.8 mmol) of (2R)-[(1'S)-Boc-amino-2'-phenylethyl]oxirane after 14 hours at 80° C. Purification is carried out by chromatography on silica gel (hexane/ethyl acetate 1:2). TLC: R_f=0.27 (hexane/ethyl acetate 1:2). $HPLC_{20-100}$: t_{Ret}=13.0. 1H-NMR (CD_3OD; 200 MHz) ia.: 7.62 and 7.52/each d, J=9 (2×2H); 7.4–7.0/m (5H); 3.93/s (2H); 1.33 and 1.31/each s (2×9H).

39e) 1-[4-(Pyridin-3-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane

1 g (1.88 mmol) of 1-[4-(pyridin-3-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane is dissolved in 10 ml of formic acid and the solution is stirred at room temperature for 5 hours. The reaction mixture is concentrated by evaporation, the residue dissolved in methylene chloride and the organic phase washed with sat. sodium hydrogen carbonate solution and brine. After removal of the solvent, the title compound is obtained in the form of a brown oil, which is processed further without purification.

Example 40

1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

Analogously to Example 37, the title compound is obtained from 473 mg (0.75 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride, 263 mg (1.5 mmol) of N-methoxycarbonyl-(L)-valine, 575 mg (3 mmol) of EDC (Fluka, Buchs, Switzerland), 405 mg (3 mmol) of HOBT (Fluka) and 1.7 ml (12 mmol) of TEA in 10 ml of DMF. Working up is performed analogously to Example 40f, using ethyl acetate instead of methylene chloride. The compound can be lyophilised from dioxane. TLC: R_f=0.28 (ethyl acetate). $HPLC_{20\text{-}100}$: t_{Ret}=13.11; FAB MS $(M+H)^+$=678.

The starting material is prepared as follows:

40a) 4-(Pyrazin-2-yl)-benzaldehyde

[see EP 0 344 577]

50 ml of THF are poured over 2.72 g (112 mmol) of magnesium turnings, which have been de-greased with hexane and activated with a small amount of iodine, and the mixture is heated at 50° C. A solution of 4-bromobenzaldehyde dimethyl acetal (prepared in accordance with Example 37a) in 200 ml of THF is added dropwise to the mixture within a period of about 30 min. Initially, the reaction is exothermic; towards the end of the dropwise addition the reaction mixture is heated to about 60° C. After stirring at 60° C. for a further 30 min, the mixture is allowed to cool to room temperature and decanted off from the unreacted magnesium; the resulting solution containing the Grignard reagent is added dropwise at room temperature over a period of 20 min to a suspension of 11.45 g (100 mmol) of 2-chloropyrazine (Fluka, Buchs, Switzerland) and 1.6 g of DPPP (Aldrich, Buchs, Switzerland) in 500 ml of THF (slightly exothermic reaction). The mixture is then stirred at room temperature for 19 hours. Then 250 ml of water are added to the reaction mixture and the mixture is stirred for 10 min. The THF is removed in vacuo; 300 ml of ethyl acetate and 100 ml of 2N hydrochloric acid are added to the emulsion that remains and the mixture is stirred for 5 min. After separation of the organic phase, that phase is stirred twice more with 100 ml, in each case, of 0.5N hydrochloric acid for 5 min. The ethyl acetate phase is washed in succession with sat. sodium hydrogen carbonate solution, water and brine and is concentrated. The title compound is obtained in the form of light-brown crystals. Recrystallisation from methylene chloride/hexane is carried out. M.p.: 86°–88° C. TLC: R_f=17 (hexane/ethyl acetate 2:1). $HPLC_{20\text{-}100}$: t_{Ret}=11.06. 1H-NMR ($CDCl_3$; 200 MHz): 10.12/s (1H); 9.14/d, J≦1 (1H); 8.70/d, J≦1 (1H); 8.60/t, J≦1 (1H); 8.22 and 8.03/each d, J=9 (2×2H).

40b) N-1-(tert-Butoxycarbonyl)-N-2-{4-[(pyrazin-2-yl)-phenyl]-methylidene}-hydrazone

Analogously to Example 37c, the title compound is obtained from 12.4 g (67.3 mmol) of 4-(pyrazin-2-yl)-benzaldehyde and 8.5 g (64 mmol) of tert-butyl carbazate (Fluka, Buchs, Switzerland) in 170 ml of ethanol after 5 hours at 80° C., with the title compound crystallising out spontaneously. M.p: 190°–198° C. TLC: R_f=0.47 (ethyl acetate). $HPLC_{20\text{-}100}$: t_{Ret}=13.41.

40c) N-1-(tert-Butoxycarbonyl)-N-2-[4-(pyrazin-2-yl)-benzyl]-hydrazine

Analogously to Example 37d, the title compound is obtained in the form of an oil from 0.6 g (2 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{4-[(pyrazin-2-yl)-phenyl]-methylidene}hydrazone and 0.15 g of 5% Pd/C in 15 ml of THF after hydrogenation for 13 hours at room temperature. The title compound crystallises out on trituration with ether. Recrystallisation from ethyl acetate/petroleum ether is carried out. M.p.: 110°–111° C. $HPLC_{20\text{-}100}$: t_{Ret}=9.62. 1H-NMR (CD_3OD; 200 MHz): 9.09/s (1H); 8.65/t, J≦1 (1H); 8.51/t, J≦1 (1H); 8.05 and 7.53/each d, J=5 (2×2H); 4.00/s (2H); 1.43/s (9H).

40d) 1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane

Analogously to Example 37e, the title compound is obtained in the form of beige crystals from 10.5 g (35 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(pyrazin-2-yl)-benzyl]- hydrazine and 11.7 g (45 mmol) of (2R)-[(1'S)-(trifluoroacetyl)amino-2'-phenylethyl]oxirane (EP 0 521 827, Example 16d) in 150 ml of isopropanol. M.p.: 194°–196° C. TLC: R_f=0.38 (hexane/ethyl acetate 1:2). $HPLC_{20\text{-}100}$: t_{Ret}=16.27.

40e) 1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane

11.75 g (21 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-(trifluoroacetyl)amino-6-phenyl-2-azahexane are suspended in 500 ml of methanol and, at 60° C., 105 ml of a 1M K_2CO_3 solution in water are added. The mixture is stirred at 75° C. for about 3 hours; the methanol is evaporated off and the residue is extracted with ethyl acetate. The organic phase is washed once each with water and brine and concentrated. The title compound is obtained in the form of orange-brown crystals, which can be recrystallised from ethyl acetate/petroleum ether. M.p.: 146°–148° C. TLC: R_f=0.08 (methylene chloride/methanol 10:1). $HPLC_{20\text{-}100}$: t_{Ret}=11.23.

40f) 1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 37, the title compound is obtained from 3.2 g (7 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]- 4(S)-hydroxy-2-(tert-butoxycar oonyl)amino-5(S)-amino-6-phenyl-2-azahexane, 2.54 g (14 mmol) of N-methoxycarbonyl-(L)-valine, 5.4 g (28 mmol) of EDC (Fluka, Buchs, Switzerland), 3.8 g (28 mmol) of HOBT (Fluka, Buchs, Switzerland) and 7.1 g (70 mmol) of TEA in 130 ml of DMF. The reaction mixture is worked up by removing the DMF, taking up the residue in methylene chloride and washing the organic phase in succession with water, sat. sodium hydrogen carbonate solution/water 1:1, 10% citric acid, water and brine. The compound crystallises out on concentration. M.p.: 218°–220° C. TLC: R_f=0.29 (methylene chloride/methanol 10:1). $HPLC_{20\text{-}100}$: t_{Ret}=15.11.

40g) 1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride

3.4 g (5.5 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-

methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane in 100 ml of 4N hydrogen chloride in dioxane (Aldrich) and 10 ml of methanol are stirred at room temperature for 2 hours. The solvents are removed; dioxane is added twice to the residue and evaporated off. The title compound is obtained in the form of a viscous oil, with the compound crystallising out on trituration with ether. M.p.: 194°–198° C. TLC: R_f=0.35 (methylene chloride/methanol 10:1). $HPLC_{20\text{-}100}$: t_{Ret}=9.77.

Example 41

1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-[N-(N-methoxycarbonyl-(L)-iso-leucyl)amino]-5(S)-[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-Phenyl-2-azahexane

142 mg (0.75 mmol) of N-methoxycarbonyl-(L)-iso-leucine and 223 mg (0.75 mmol) of TPTU in 3 ml of DMF are stirred at room temperature for 10 min and then a solution of 473 mg (0.75 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxy-carbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride (Example 40g) and 0.33 ml of NMM in 3 ml of DMF is added. The mixture is stirred at room temperature overnight. Working up is carried out by the slow, dropwise addition of the reaction mixture to 100 ml of water, stirring at room temperature for 20 min and isolation of the resulting precipitate by filtration. The precipitate is washed with water and taken up in methylene chloride; the organic phase is washed in succession with water, sat. sodium hydrogen carbonate solution/water 1:1, water and brine. After removal of the solvent, the residue is digested in ether, with the title compound being obtained in the form of a colourless powder. The compound can be lyophilised from dioxane. TLC: R_f=0.28 (ethyl acetate). $HPLC_{20\text{-}100}$: t_{Ret}=13.78. FAB MS(M+H)$^+$=692.

Example 42

1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-5(S)-[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane

Analogously to Example 41, after working up the title compound is obtained from 142 mg (0.75 mmol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e), 223 mg (0.75 mmol) of TPTU in 3 ml of DMF (solution A) and 435 mg (0.75 mmol) of 1-[4-(pyrazin- 2-yl)-phenyl]-4(S)-hydroxy-2-amino-5-(S)-N-(N-methoxycarbonyl-(L)-valyl) amino-6-phenyl-2-azahexane hydrochloride (Example 40g) and 0.33 ml of NMM in 3 ml of DMF (solution B), the title compound crystallising out spontaneously on evaporation of the solvent. The compound can be lyophilised from dioxane. TLC: R_f=0.46 (methylene chloride/methanol 10:1). $HPLC_{20\text{-}100}$: t_{Ret}=13.85. FAB MS(M+H)$^+$=692.

Example 43

1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxy-carbonyl-(L)-iso-leucyl)amino]-6-phenyl-2-azahexane

Analogously to Example 41, after working up the title compound is obtained from 132 mg (0.7 mmol) of N-methoxycarbonyl-(L)-iso-leucine and 208 mg (0.7 mmol) of TPTU in 3 ml of DMF (solution A) and 400 mg (0.7 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane hydrochloride (Example 44b) and 0.31 ml (2.8 mmol) of NMM in 3 ml of DMF, the title compound being obtained in crystalline form by digestion with ether. M.p.: 211°–217° C. TLC: R_f=0.41 (methylene chloride/methanol 10:1). $HPLC_{20\text{-}100}$: t_{Ret}=14.49. FAB MS(M+H)$^+$=706.

Example 44

1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-[N-(N-methoxycarbonyl-(L)-valyl)aminol-5(S)-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane

Analogously to Example 41, after working up the title compound is obtained from 175 mg (1 mmol) of N-methoxycarbonyl-(L)-valine, 297 mg (1 mmol) of TPTU (Fluka, Buchs, Switzerland) in 4 ml of DMF (solution A) and 571 mg (1 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane hydrochloride and 0.44 ml (4 mmol) of NMM in 4 ml of DMF (solution B); the title compound can be obtained in crystalline form by digestion with ether. M.p.: 205°–208° C. $HPLC_{20\text{-}100}$: t_{Ret}=13.87. FAB MS(M+H)$^+$=692.

The starting material is prepared as follows:

44a) 1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 37, the title compound is obtained from 2.3 g (5 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-amino-6-phenyl-2-azahexane (Example 40e), 1.9 g (10 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 3.8 g (20 mmol) of EDC, 2.7 g (20 mmol) of HOBT and 5.1 g (50 mmol) of TEA in 90 ml of DMF. Working up is carried out as described in Example 40f. The compound can be recrystallised from ethyl acetate. TLC: R_f=0.58 (methylene chloride/methanol 10:1). $HPLC_{20\ 100}$: t_{Ret}=15.68. ^{1}H-NMR (CD_3OD; 200 MHz) i.a.: 9.08/s (1H); 8.65/bs (1H); 8.51/t, J≦1 (1H); 8.02 and 7.52/each d, J=5 (2×2H); 7.3–7.1/m (5H); 3.92/s (2H); 3.62/s (3H); 1.28/s (9H); 0.8/t, J=5 (3H); 0.73/d, J=4 (3H).

44b) 1-[4-(Pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methaxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane hydrochloride

Analogously to Example 40g, the title compound is obtained from 2.1 g (3.3 mmol) of 1-[4-(pyrazin-2-yl)-phenyl]-4(S)-hydroxy-2-(tert-butoxycarbonyl)amino-5(S)-N-(N-methoxy-carbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane in 60 ml of 4N hydrogen chloride in dioxane and 10 ml of methanol, with the title compound being caused to crystallise with ether. M.p.: 200°–201° C. $HPLC_{20\text{-}100}$: t_{Ret}=10.52.

Example 45

1-[4-(Thiophen-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxy-carbonyl-(L)-valyl)amino]-phenyl-2-azahexane

Analogously to Example 37, the title compound is obtained from 500 mg (1.36 mmol) of 1-[4-(thiophen-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane, 620 mg (3.54 mmol) of N-methoxycarbonyl-(L)-valine, 1.3 (6.8 mmol) of EDC, 551 mg (4.08 mmol) of HOBT and 0.95 ml (6.8 mmol) of TEA in 10 ml of DMF, the title compound being lyophilised from dioxane. TLC: R_f0.51 (methylene chloride/methanol 15:1). $HPLC_{20\text{-}100}$: t_{Ret}= 15.30. FAB MS(M+H)$^+$=682.

The starting material is prepared as follows:

45a) 4-(Thiophen-2-yl)-benzaldehyde

[see Heterocycles 31, 1951 (1990)]

3.7 g (20 mmol) of 4-bromobenzaldehyde, 9.5 ml (120 mmol) of thiophene, 2.94 g (30 mmol) of potassium acetate and 1.16 g (1 mmol) of tetrakis(triphenylphosphine)-palladium (Fluka, Buchs, Switzerland) in 50 ml of dimethylacetamide are placed in a pressure reactor and stirred at

150° C. under nitrogen for 16 hours. The reaction mixture is concentrated by evaporation; the residue is taken up in water and extracted three times with methylene chloride. After removal of the solvent, the residue is chromatographed on silica gel (hexane/ethyl acetate 4:1). The title compound is obtained in the form of a yellow solid. TLC: R_f0.36 (hexane/ethyl acetate 4:1). $HPLC_{20\text{-}100}$: t_{Ret}=15.26. 1H-NMR (CD_3OD; 200 MHz): 9.98/s (1H); 7.93 and 7.85/each d, J=9.5 (2×2H); 7.60/d, J=2.5 (1H); 7.52/d, J=5 (1H); 7.17/dxd, J=2.5 and 5 (1H).

45b) N-1-(tert-Butoxycarbonyl)-N-2-{4-[(thiophen-2-yl)-phenyl]-methylidene}-hydrazone

Analogously to Example 37c, the title compound is obtained in the form of yellow crystals from 2.47 g (13.1 mmol) of 4-(thiophen-2-yl)-benzaldehyde and 1.65 g (12.49 mmol) of tert-butyl carbazate (Fluka, Buchs, Switzerland) in 30 ml of ethanol (4.5 hours at 90° C.). M.p.: 162°–165° C. $HPLC_{20\text{-}100}$: t_{Ret}=16.08. 1H-NMR (CD_3OD; 200 MHz) i.a.: 7.91/s (1H) 1.53/s (9H).

45c) N-1-(tert-Butoxycarbonyl)-N-2-[4-(thiophen-2-yl)-benzyl]-hydrazine

3.35 g (11.1 mmol) of N-1-(tert-butoxycarbonyl)-N-2-{4-[(thiophen-2-yl)-phenyl]-methylidene}-hydrazone and 0.819 g (11.1 mmol) of sodium cyanoborohydride (Fluka, Buchs, Switzerland) are dissolved in 11 ml of THF (black solution) and added dropwise over a period of 5 hours to 2.11 g (11.1 mmol) of p-toluenesulfonic acid monohydrate dissolved in 11 ml of THF. The mixture is stirred overnight at room temperature and under nitrogen (pH=about from 3 to 4) and then diluted with ethyl acetate; the organic phase is washed in succession with brine, sat. sodium hydrogen carbonate solution and again brine. The organic phase is concentrated by evaporation and the residue is taken up in 13.3 ml of 1 N sodium hydroxide solution; 15 ml of methylene chloride are added and the mixture is boiled under reflux for 3 hours at a bath temperature of 60° C. After separation of the organic phase, that phase is concentrated to dryness by evaporation. The title compound is obtained in the form of a slightly yellowish oil. $HPLC_{20\text{-}100}$: t_{Ret}=12.36. 1H-NMR (CD_3OD; 200 MHz) i.a.: 3.91/s (2H); 1.42/s (9H).

45d) 1-[4-(Thiophen-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)-amino]-6-phenyl-2-azahexane

Analogously to Example 37e, the title compound is obtained from 3.39 g (11.1 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(thiophen-2-yl)-benzyl]-hydrazine and 2.93 g (11.1 mmol) of (2R)-[(2'S)-Boc-amino-2'-phenylethyl]oxirane (*J. Org. Chem.* 50, 4615 (1985)) in 50 ml of isopropanol, with the title compound crystallising out spontaneously on cooling of the reaction solution. M.p.: 165°–168° C. $HPLC_{20\text{-}100}$: t_{Ret}=18.84. 1H-NMR (CD_3OD; 200 MHz) i.a.: 7.56/d, J=9 (2H); 7.5–7.3/m (4H); 7.3–7.1/m (5H); 7.08/dxd, J=2 and 5 (1H); 3.85/s (2H) 1.33 and 1.32/each s (2×9H).

45e) 1-[4-(Thiophen-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane

Analogously to Example 39e, the title compound is obtained in the form of a slightly yellowish oil from 3.16 g (5.57 mmol) of 1-[4-(thiophen-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane in 30 ml of formic acid after stirring at room temperature for 6 hours, that oil being processed further without purification. 1H-NMR (CD_3OD; 200 MHz) i.a.: 7.62/d, J=9 (2H); 7.5–7.1/several m's, superimposed (9H); 7.09/dxd, J=2 and 5 (1H); 3.72/s (2H).

Example 46

1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxy-carbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane

Process A:

With the exclusion of moisture, 10.85 g of N-methoxycarbonyl-(L)-tert-leucine (Example 2e) and 17.1 g of TPTU are placed in 65 ml of DMF. 35.1 ml of Hünig base are added to the white suspension and the mixture is stirred at room temperature for 20 min. Then 13.2 g (26 mmol) of 1-[4-(pyridin-2-yl) -phenyl]-4(S)-hydroxy-5(S) -2,5-diamino-6-phenyl-2-azahexane hydrochloride (Example 37f) dissolved in 65 ml of DMF are added and the mixture is stirred for 24 hours to complete the reaction (after 20 hours, a further 5 ml of Hünig base are added). The reaction mixture is poured into 600 ml of water and the resulting precipitate is filtered off and washed with water. The filter residue is then dissolved in methylene chloride and washed 2× with sat. $NaHCO_3$ solution, water and brine. After drying over sodium sulfate and concentration, the resulting foam is digested with DIPE; the solid is filtered off and dried. The resulting crude product is dissolved again in methylene chloride, treated with active carbon and, after filtration, precipitated with ether. The resulting title compound is dried in a heated desiccator at 40° C. under a high vacuum: m.p.: 202°–204° C.; TLC: R_f=0.38 (ethyl acetate); $HPLC_{20\text{-}100}$: t_{Ret}=11.81; FAB MS $(M+H)^+$=705. Further product can be obtained from the mother liquor after chromatography (SiO_2, hexane/ethyl acetate, then ethyl acetate) and after crystallisation from ether (m.p. 206°–207° C.).

Process B:

Analogously to Example 4, 1.32 g of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane in 5 ml of DMF are added to 0.42 g (2.2 mmol) of (N-methoxycarbonyl-(L)-tert-leucine, 0.654 g (2.2 mmol) of TPTU and 840 µl (5 mmol) of Hünig Base in 5 ml of DMF, and the mixture is stirred at room temperature for 22 hours and worked up analogously to Example 3 to yield the title compound.

The starting compounds are prepared as follows:

46a) 1 –4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-Boc-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 1, a solution of 3.93 g (8.5 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-(N-Boc-amino)-5(S)-amino-6-phenyl-2-azahexane hydrochloride (Example 47b) in 50 ml of DMF is added dropwise to a mixture of 2.58 g (13.6 mmol) of N-methoxycarbonyl-(L) -tert-leucine, 4.88 g (25.5 mmol) of EDC and 2.3 g (17 mmol) of HOBT in 50 ml of DMF. After working up, the crude product is digested in methylene chloride/DIPE, filtered off and dried to yield the title compound. TLC: R_f=0.5 (ethyl acetate); $HPLC_{20\text{-}100}$: t_{Ret}=12.32; FAB MS $(M+H)^+$= 634.

46b) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane hydrochloride

Analogously to Example 37f), 130 ml of 4M HCl in dioxane are added to 4.4 g (6.94 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-Boc-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane and the mixture is diluted with 7 ml of DMF. After 2.75 hours, the mixture is worked up. The title compound is obtained: TLC: R_f=0.44 (methylene chloride/methanol: 9/1); $HPLC_{20\text{-}100}$: t_{Ret}=8.47; FAB MS $(M+H)^+$= 534.

An alternative procedure for the preparation of the title compound from Example 46 is as follows:

Example 46*

1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane

With the exclusion of moisture, 567 g.(3.0 mol) of N-methoxycarbonyl-(L)-tert-leucine (Example 2e) and 891 g (3.0 mol) of TPTU are placed in 3 litres of methylene chloride; with ice-cooling, 775 g (6 mol) of Hünig base are added dropwise and the mixture is stirred for 20 min. A suspension of 432 g (1.0 mol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane trihydrochloride in 3 itres of methylene chloride is then added to the solution and the mixture is stirred at room temperature overnight to complete the reaction. The reaction mixture is washed with 10 litres of water, 10 litres of sat. $NaHCO_3$ solution and 5 litres of brine. The aqueous phases are extracted a further 2× with 5 litres of methylene chloride; the organic phases are dried (Na_2SO_4) and concentrated by evaporation. The residue is dissolved in 6 litres of ethyl acetate and filtered through 500 g of silica gel; the column is rinsed with 6 litres of ethyl acetate and the product-containing fractions are concentrated by evaporation. Stirring in boiling DIPE/ethanol 49:1 (9 litres; 1 hour), cooling and filtration yield the title compound, which can be further purified by recrystallisation from ethanol/water (m.p. 207°–209° C.).

The starting compounds are prepared as follows:

*a) 4-(Pyridin-2-yl)-benzaldehyde

11 g of iodine, followed by 200 g of 4-bromobenzaldehyde dimethyl acetal (Example 37a), are added to 317 g (13.0 mol) of magnesium in 3.5 litres of THF (nitrogen atmosphere). Once the reaction has started (heating if necessary), 2540 g (in total 2740 g; 11.8 mol) of 4-bromobenzaldehyde dimethyl acetal in 3.5 litres of toluene are added dropwise (from 25° to 30° C., 1 hour) and the mixture is then stirred at room temperature for 1 hour. The Grignard reagent is then transferred to the dropping funnel of a second apparatus containing 1750 g (11.0 mol) of 2-bromopyridine (Fluka, Buchs, Switzerland) in 3.3 litres of THF, 38 g (70 mmol) of DPPP and 330 ml of diisobutylaluminium hydride (20% in hexane). At from 15° to 20° C., the Grignard reagent is added dropwise (45 min). After being stirred at room temperature for 90 min, the reaction mixture is poured into 10 kg of ice, 1.5 litres of concentrated hydrochloric acid and 1.5 kg of citric acid. 1 kg of Hyflo Super Cel is added, and the mixture is stirred for 1 hour and then filtered; the residue is washed with 2 litres of water, 2×2 litres of toluene and, finally, 2×2 litres of 1 N HCl solution. The first filtrate and the washing water are combined; the aqueous phase is separated off and extracted 2× with the two toluene filtrates. The resulting organic phases are washed with the two hydrochloric-acid-containing filtrates. The aqueous phases are combined; 6 litres of toluene are added and the mixture is adjusted to a pH of from 8 to 9 with 4.6 litres of sodium hydroxide solution (30% in water). The mixture is filtered through Hyflo (filtration aid based on kieselguhr, Fluka, Buchs, Switzerland); the aqueous phase is separated off and extracted 2× with 2 litres of toluene. The organic phases are washed 2× with water, dried (Na_2SO_4) and treated with active carbon. Addition of 0.5 kg of silica gel, stirring, filtration and concentration by evaporation yield the title compound (physical data as Example 37b).

*b) N-1-(tert-Butoxycarbonyl)-N-2-{4-[(pyridin-2-yl)-phenyl]-methylidene}-hydrazone

A solution of 1770 g (9.67 mol) of 4-(pyridin-2-yl)-benzaldehyde and 1220 g (9.2 mol) of tert-butyl carbazate (Fluka, Buchs, Switzerland) in 12.5 litres of ethanol is heated at boiling for 4 hours. The mixture is cooled to 40° C. and 6 kg of ice are added; the mixture is filtered off and the title compound is washed with 6 litres of water, that compound then being obtained in pure form (physical data as in Example 37c).

*c) N-1-(tert-Butoxycarbonyl)-N-2-[4-(pyridin-2-yl)-benzyl]-hydrazine

A suspension of 1655 g (5.57 mol) of N-1-(tert-butoxycarbonyl)-N-2{4-[(pyridin-2-yl)-phenyl]-methylidene}-hydrazone in 12 litres of methanol is hydrogenated in the presence of 166 g of 10% Pd/C under normal pressure at room temperature. The catalyst is filtered off and washed thoroughly with methanol; the solvent is removed. Crystallisation from hexane yields the title compound: m.p.: 74°–77° C.

*d) 1-[4-(Pyridin-2-yl)-phenyl]-4(S) -hydroxy-5 (S)-2,5-bis [(tert-butoxycarbonyl)amino]-6-phenyl-2-azahexane

A solution of 1185 g (4.5 mol) of (2R)-[(1'S)-(tert-butoxycarbonyl)-amino-2'-phenylethyl]-oxirane and 1230 g (4.1 mol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(pyridin-2-yl)-benzyl]-hydrazine in 14 litres of iso-propanol are heated at boiling for 16 hours. After cooling, 15 kg of ice and 10 litres of water are added; the mixture is stirred for 2 hours; the crystals are filtered off and washed with 6 litres of water. Stirring twice in 5 litres of ether in each case, filtration, washing with 2 litres of ether and, finally, 2 litres of ether/tert-butyl methyl ether 1:1 yield the title compound: m.p.: 183°–188° C.

*e) 1-[4-(Pyridin-2-yl)-phenyl]-4 (S)-hydroxy-5(S)-2,5-diamino-6-phenyl-2-azahexane trihydrochloride

A solution of 1465 g (2.6 mol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[tert-butoxycarbonyl) amino]-6-phenyl-2-azahexane in 12 litres of THF and 4 litres of hydrochloric acid (4N in water) is stirred at 50° C. for 4 hours. The aqueous phase is separated from the resulting two-phase mixture and concentrated by evaporation in vacuo. The residue is diluted with 4 litres of ethanol, concentrated by evaporation, diluted with 4 litres of ethanol/-toluene 1:1, concentrated by evaporation, diluted with 4 litres of ethanol and concentrated by evaporation again. Stirring in 9 litres of DIPE and filtration yield the title compound (physical data as Example 37f).

*e(i): Alternatively, 1-(4-(pyridin-2-yl)-phenyl)-4(S)-hydroxy-5(S)-2,5-di[(tert-butoxycarbonyl)-amino]-6-phenyl-2-azahexane is prepared as follows:

Under a nitrogen atmosphere, 2.1 ml (2.1 mmol) of a 1.00M solution of diisobutylaluminium hydride in methylene chioride are slowly added dropwise to an ice-cooled solution of 200 mg (0.347 mmol) of 1-[4-(pyridin-2-yl) phenyl]-1-oxo-5-(S)-2,5-di[(tert-butoxycarbonyl)-amino]-4 (S)-hydroxy-6-phenyl-2-azahexane in 5 ml of THF (foams). After 2 hours, 7 ml of ethyl acetate are added and, after a further 30 min, 70 ml of methanol. The reaction mixture is warmed to room temperature and stirred for 2 hours; 0.5 ml of water and 5 g of sodium sulfate are added and the mixture is stirred again for 1 hour to complete the reaction. The salts are filtered off and the filtrate is concentrated by evaporation. Medium-pressure chromatography (SiO_2, hexane/ethyl acetate 3:2→ethyl acetate) yields the title compound: m.p. 184° C.; TLC (hexane/ethyl acetate 1:1): R_f=0.26; FAB MS $(M+H)^+$=563.

The synthesis of the starting material, 1-[4-(pyridin-2-yl) phenyl]-1-oxo-5-(S)-2,5-di[(tert-butoxycarbonyl)amino]-4 (S)-hydroxy-6-phenyl-2-azahexane, is carried out via the following steps:

Step (1) 4-(Pyridin-2-yl)-benzoic acid methyl ester:

24.0 g (150 mmol) of 4-cyanobenzoic acid methyl ester (Fluka, Buchs, Switzerland) in 150 ml of toluene are placed under an acetylene atmosphere in an autoclave and 0.30 g (1.6 mmol) of cobaltocene (=dicyclopentadienylcobalt; Aldrich, Milwaukee, USA) is added. The mixture is then subjected to an acetylene pressure of 15 atm, heated at 180° C. and stirred for 12 hours. After cooling and release of the

pressure, 9.5 g of active carbon are added to the black suspension; the mixture is diluted with 250 ml of toluene, stirred for 30 min, filtered and concentrated by evaporation. Crystallisation from warm ether by the addition of hexane yields the title compound: m.p. 96° C.; TLC (hexane/ethyl acetate 4:1): R_f=0.37; FAB MS $(M+H)^+$=214. Further product can be obtained from the mother liquor by column chromatography (SiO_2, hexane/ethyl acetate 19:1→4:1).

Step (2) 4-(Pyridin-2-yl)-benzoic acid:

12.85 g (60.2 mmol) of 4-(pyridin-2-yl)-benzoic acid methyl ester in 125 ml of methanol and 67 ml of 1N sodium hydroxide solution are stirred at room temperature for 6 hours. The resulting solution is partially concentrated by evaporation; the aqueous residue is extracted with ethyl acetate and acidified to pH≈1.5 with 2N HCl solution. The title compound precipitates out and can be filtered off and washed with water: TLC (ethyl acetate): R_f≈0.35; FAB MS $(M+H)^+$≈200.

Step (3) 4-(Pyridin-2-yl)-benzoic acid iso-butyloxyformic acid anhydride:

With the exclusion of air, 6.0 g (30 mmol) of 4-(pyridin-2-yl)-benzoic acid are suspended at −20° C. in 90 ml of THF, and 9.90 ml (90 mmol) of N-methyl-morpholine and 4.32 ml (33 mmol) of isobutyl chloroformate are added. After 30 min, the mixture is filtered, washed with a small amount of cold THF, and the filtrate is partially concentrated by evaporation; the residue is diluted with methylene chloride, washed with ice-water and cold brine, dried (Na_2SO_4) and concentrated by evaporation to form the title compound: ^{1}H-NMR ($CDCl_3$) i.a. 8.75 (m, 1H), 8.16 (AB, J=8, 4H), 7.81 (m, 2H), 7.32 (4-line system, J=5, 1 H), 4.16 (d, J=7, 2H), 2.10 (9-line system, J=7, 1 H), 1.02 (d, J=7, 6H).

Step (4) 1-(R)-Cyano-2(S)-(N-tert-butoxycarbonylamino)-3-phenylpropyl [4-(2-pyridyl)]-benzoate:

At 0° C., 250 mg (0.9 mmol) of benzyltriethylammonium chloride are added to 2.0 g (30 mmol) of potassium cyanide in 7.5 ml of water and 7.5 ml of methylene chloride. Then a solution of 6.21 g (24.9 mmol) of Boc-(L)-phenylalaninal in 10 ml of methylene chloride and a solution of ≈30 mmol of 4-(pyridin-2-yl)-benzoic acid-iso-butyloxyformic acid anhydride in 10 ml of methylene chloride are simultaneously added dropwise. After 20 min at 0° C., stirring is carried out at room temperature for a further 4 hours and the reaction mixture is finally diluted with methylene chloride/water. The aqueous phase is separated off and extracted 2× with methylene chloride; the organic phase is washed 3× with water and brine, dried (Na_2SO_4) and concentrated by evaporation. Column chromatography (SiO_2; hexane/ethyl acetate 4:1→2:1) yields a ≈5:1 mixture of 1-(R)-cyano-2(S)-(N-tert-butoxycarbonylamino)-3-phenylpropyl [4-(2-pyridyl)]-benzoate and 1-(S)-cyano-2(S)-(N-tert-butoxycarbonylamino)-3-phenylpropyl [4-(2-pyridyl)]-benzoate: TLC (hexane/ethyl acetate 4:1): R_f=0.11; FAB MS $(M+H)^+$=458; ^{1}H-NMR ($CDCl_3$) i.a. 5.66 (d, J=6, 5/6H, 1-(R) epimer), 5.53 (m, 1/6H, 1-(S) epimer). Digestion in DIPE results in diastereoisomerically pure 1-(R)-cyano-2(S)-(N-tert-butoxy-carbonylamino)-3-phenylpropyl [4-(2-pyridyl)]-benzoate: m.p. 140°–141° C.

Step (5) 4-(S)-1,4-Di[(tert-butoxycarbonyl)amino]-3(R)-[4-(pyridin-2-yl)phenyl]-carbonyloxy-5-phenyl-1-azapent-1-ene:

2.29 g (5.0 mmol) of 1-(R)-cyano-2(S)-(N-tert-butoxcarbonylamino)-3-phenylpropyl [4-(2-pyridyl)]-benzoate are dissolved in 80 ml of methanol, and 900 mg (15 mmol) of acetic acid and 661.5 mg (5 mmol) of tert-butyl carbazate are added; after the addition of 2.3 g of Raney nickel, the mixture is hydrogenated. The partially precipitated product is dissolved by the addition of methanol and gentle heating; the catalyst is filtered off and the filtrate is concentrated by evaporation. The residue is taken up in ethyl acetate/sat. $NaHCO_3$ solution; the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed with brine, dried (Na_2SO_4) and concentrated by evaporation. Medium-pressure chromatography (SiO_2; hexane/ethyl acetate 4:1→ethyl acetate) yields the title compound: m.p. 195°–196° C.; TLC (hexane/ethyl acetate 1:1): R_f=0.39; FAB MS $(M+H)^+$=575.

Step (6) 1-[4-(Pyridin-2-yl)phenyl]-1-oxo-5-(S)-2,5-di[(tert-butoxycarbonyl)amino]-4(S)-hydroxy-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 111 mg (85%; 1.5 mmol) of $NaCNBH_3$ are added to a solution of 862 mg (1.5 mmol) of 4-(S)-1,4-di[(tert-butoxycarbonyl)amino]-3(R)-[4-(pyridin-2-yl)phenyl]-carbonyloxy-5-phenyl-1-azapent-1-ene in 10 ml of THF. A solution of 290 mg (1.5 mmol) of p-toluenesulfonic acid in 4 ml of THF is added dropwise thereto. After stirring for 2.5 hours, a further 55 mg of $NaCNBH_3$ and 145 mg of p-toluenesulfonic acid in 2 ml of THF are added and the mixture is stirred again for 2.5 hours. The reaction mixture is then poured into 230 ml of a 1% solution of $K_2B_4O_7.4H_2O$ in water, stirred overnight to complete the reaction, filtered and washed with water. The residue is taken up in ethyl acetate; the solution is washed with brine, dried (Na_2SO_4) and concentrated by evaporation {→4-(S)-1,4-di[(tert-butoxycarbonyl)amino]-3(S)-[4-(pyridin-2-yl)phenyl]-carbonyloxy-5-phenyl-1-azapentane: TLC (hexane/ethyl acetate 1:1): R_f=0.45}. The resulting foam is dissolved in 25 ml of diethylene glycol dimethyl ether; 250 μl of 7-methyl-1,5,7-triaza-bicyclo[4.4.0]dec-5-ene (Fluka; Buchs, Switzerland) are added and the mixture is heated at 80° C. for 1.5 hours. The mixture is concentrated by evaporation under a high vacuum and the residue is taken up in ethyl acetate/water; the aqueous phase is separated off and extracted a further 2× with ethyl acetate. The organic phases are washed with brine, dried (Na_2SO_4) and concentrated by evaporation. Crystallisation from DIPE/hexane yields the title compound: m.p. 104°–105° C.; TLC (hexane/ethyl acetate 1:1): R_f=0.20; FAB MS $(M+H)^+$=577.

Example 47

[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-iso-leucyl)amino]-6-phenyl-2-azahexane

Under a nitrogen atmosphere, 0.45 g (1.5 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 0.85 g (4.5 mmol) of EDC and 0.4 g (3 mmol) of HOBT are dissolved in 10 ml of DMF. After the addition of 1.26 ml of TEA and stirring for 10 min, a solution of 0.96 g (1.5 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)-amino-6-phenyl-2-azahexane hydrochloride in 10 ml of DMF is then added dropwise. After 2 hours, the reaction mixture is concentrated by evaporation. The resulting oil is taken up in methylene chloride and washed with water, 2× sat. $NaHCO_3$ solution, water and brine. The aqueous phases are extracted with methylene chloride; the combined organic phases are dried (Na_2SO_4) and concentrated by evaporation. The residue is digested first in DIPE and then in methylene chloride/ether, then filtered off and dried to yield the title compound: TLC: R_f=0.45 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=11.71; FAB MS $(M+H)^+$=705.

The starting material is prepared as follows:

47*a*) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-(N-Boc-amino)-5(S)-trifluoroacetyl-amino-6-phenyl-2-azahexane

Analogously to Example 37*e*), 7 g (23 mmol) of N-1-(tert-butoxycarbonyl)-N-2-[4-(pyridin-2-yl)-benzyl]

hydrazine are reacted with 6 g (23 mmol) of (2R)-[(1'S)-trifluoroacetyl-amino-2'-phenylethyl]oxirane in 125 ml of isopropanol at 80° C. to form the title compound. TLC: R_f=0.33 (methylene chloride/methanol: 1/1); $HPLC_{20-100}$: t_{Ret}=12.76; FAB MS $(M+H)^+$=559.

47*b*) 1-[4(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-(N-Boc-amino)-5(S)-amino6-phenyl-2-azahexane

Analogously to Example 40*e*, 5.6 g (10 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2-(N-Boc-amino)-5-(trifluoroacetyl-amino)-6-phenyl-2-azahexane are dissolved in 130 ml of methanol, heated to 65° C. and converted into the title compound by the dropwise addition of 50 ml of a 1M aqueous potassium carbonate solution. TLC: R_f=0.17 (methylene chloride/methanol: 9/1); $HPLC_{20-100}$: t_{Ref}=8.50; FAB MS $(M+H)^+$=463.

47*c*) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-Boc-amino-5(S)-N-(N-methoxy-carbonyl-(L)-isoeucyl)amino-6-phenyl-2-azahexane

Analogously to Example 1, a solution of 1.62 g (3.5 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-(N-Boc-amino)-5(S)-amino-6-phenyl-2-azahexane in 25 ml of DMF is added dropwise to a mixture of 1.06 g (5.6 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 2.01 g (10.5 mmol) of EDC and 0.95 g (7 mmol) of HOBT in 20 ml of DMF. After working up, the crude product is digested in DIPE, filtered off and dried. TLC: R_f=0.59 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=12.52. FAB MS $(M+H)^+$=634.

47*d*) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane hydrochloride

Analogously to Example 40g, 40 ml of 4M HCl in dioxane are added to 1.9 g (3 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-Boc-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane and the mixture is diluted with 3 ml of DMF. After 2.5 hours, the mixture is worked up. The title compound is obtained: TLC: R_f=0.55 (methylene chloride/methanol: 9/1); $HPLC_{20-100}$: t_{Ret}=8.74; FAB MS $(M+H)^+$=534.

Example 48

1-[4-(Pyridin-2-yl)-phenyl]4(S)-hydroxy-2-N-(N-methoxy-carbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 1, a solution of 0.964 g (1.5 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)- tert-leucyl)amino-6-phenyl-2-aza-hexane hydrochloride in 10 ml of DMF is added dropwvise to a mixture of 0.42 g (2.4 mmol) of N-methoxycarbonyl-(L)-valine, 0.862 g (4.5 mmol) of EDC, 0.405 g (3 mmol) of HOBT and 1.26 ml of TEA in 10 ml of DMF. After working up, the crude product is digested in DIPE, filtered off and dried. Subsequent column chromatography (SiO_2; hexane/ethyl acetate: 1/1 to 3/1) yields the pure title compound (TLC: R_f=0.35 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=10.9. FAB MS $(M+H)^+$=691.

Example 49

1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxy-carbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 1, a solution of 0.315 g (0.5 mmol) of 1-[4-(pyridin-2-yl)-phenyl]- 4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride in 3 ml of DMF is added dropwise to a mixture of 0.152 g (0.8 mmol) of N-methoxycarbonyl-(L)-tert-leucine, 0.287 g (1.5 mmol) of EDC, 0.135 g (1 mmol) of HOBT and 0.49 ml of TEA in 3 ml of DMF. After working up, the crude product is purified by subsequent medium-pressure column chromatography (SiO_2; hexane/ethyl acetate) to yield the title compound. TLC: R_f=0.35 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=11.05. FAB MS $(M+H)^+$=691.

The starting compounds are prepared as follows:

49*a*) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-Boc-amino-5(S)-N-(N-methoxy-carbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 1, a solution of 4.1 g (8.87 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-(N-Boc-amino)-5(S)-amino-6-phenyl-2-azahexane (Example 47 *b*) in 50 ml of DMF is added dropwise to a mixture of 2.49 g (14.2 mmol) of N-methoxycarbonyl-(L)-valine, 5.1 g (26.6 mmol) of EDC, 2.4 g (17.7 mmol) of HOBT and 7.45 ml of TEA in 50 ml of DMF. After working up, the crude product is digested 2× in DIPE, filtered off and dried to yield the title compound. TLC: R_f=0.42 (ethyl acetate); $HPLC_{20-100}$: t_{Ref}=11.92. FAB MS $(M+H)^+$=620.

49*b*) 1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride

Analogously to Example 37*f*), 30 ml of 4M HCl in dioxane are added to 3.5 g (5.65 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-Boc-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane and the mixture is diluted with 5 ml of DMF. After 3.5 hours, the mixture is worked up. The title compound is obtained: TLC: R_f=0.53 (methylene chloride/methanol: 9/1); $HPLC_{20-100}$: t_{Ret}=8.00; FAB MS $(M+H)^+$=520.

Example 50

1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxy-carbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane

Analogously to Example 46, 0.96 g (1.5 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane 3HCl (Example 47 *d*) in 10 ml of DMF are reacted with 0.263 g (1.5 mmol) of N-methoxycarbonyl-(L)-valine, 0.446 g (1.5 mmol) of TPTU and 0.78 ml (4.5 mmol) of DBU in 7 ml of DMF. After working up, the title compound is obtained: TLC: R_f=0.4 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=11.23. FAB MS $(M+H)^+$=691.

Example 51

1-(Pyridin-2-yl)-phenyl 4(S)-hydroxy-2-N-(N-methoxy-carbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl) amino6-phenyl-2-azahexane

Analogously to Example 1, a solution of 1.26 g (2 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride (Example 49*b*) in 12 ml of DMF is added dropwise to a mixture of 0.6 g (3.2 mmol) of N-methoxycarbonyl-(L)-iso-leucine, 1.14 g (6 mmol) of EDC, 0.54 g (4 mmol) of HOBT and 1.68 ml of TEA in 13 ml of DMF. After working up, the crude product is digested in DIPE and purified by subsequent medium-pressure column chromatography (SiO_2; hexane/ethyl acetate) to yield the title compound. TLC: R_f=0.32 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=11.04. FAB MS $(M+H)^+$=691.

Example 52

1-[4-(Pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxy-carbonyl-(L)-valyl)-amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane

Analogously to Example 1, a solution of 0.629 g (1 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-amino-5(S)-

N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane hydrochloride (Example 49 *b*) in 5 ml of DMF is added dropwise to a mixture of 0.303 g (1.6 mmol) of N-ethoxycarbonyl-(L)-valine, 0.575 g (3 mmol) of EDC, 0.27 g (2 mmol) of HOBT and 0.98 ml of TEA in 7 ml of DMF. After working up, the crude product is digested in DIPE and purified by subsequent medium-pressure column chromatography (SiO_2; hexane/ethyl acetate) to yield the title compound. TLC: R_f=0.33 (ethyl acetate); $HPLC_{20-100}$: t_{Ret}=11.13. FAB MS $(M+H)^+$=691.

Example 53

1-[4-(Pyrid-2-yl)-phenyl-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane methanesulfonate salt 210 mg (0.28 mmol) of 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxy-carbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane (Example 46) are dissolved in 10 ml of methylene chloride with heating and 19.5 µl (0.3 mmol) of methanesulfonic acid are added. The title compound is precipitated with ether, filtered off and dried under reduced pressure at 50° C. FAB MS $(M+H)^+$=705. ^{1} H-NMR (CD_3OD) (chemical shifts of the pyridine protons of the free base in brackets); δ: 8.81 (8.6), 8.65 (7.9), 8.36 (7.8), 8.05 (7.35) and also, in addition, signals of the methyl group of the salt: δ: 2.7 ppm.

Example 54

1-[4-(Pyrid-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane hydrochloride salt

70 mg (0.094 mmol) of 1-[4-(pyrid-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxy-carbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane (Example 46) are dissolved in 6 ml of dioxane, and 25 µl of a 4M HCl solution in dioxane are added. The resulting precipitate is filtered off and dried. FAB MS $(M+H)^+$=705. ^{1} H-NMR (CD_3OD) (chemical shifts of the pyridine protons of the free base in brackets); δ: 8.81 (8.6), 8.65 (7.9), 8.36 (7.8), 8.05 (7.35). Elemental analysis of the hydrate of the title compound: Cl found: 4.6%; calc.: 4.63%.

Example 55

Gelatin solution:

A sterile-filtered aqueous solution, containing 20% cyclodextrins as solubiliser, of one of the compounds of formula I mentioned in the preceding Examples (e.g. the title compound from Example 2) as active ingredient, is so mixed, with heating and under aseptic conditions, with a sterile gelatin solution containing phenol as preservative that 1.0 ml of solution has the following composition:

active ingredient	3 mg
gelatin	150 mg
phenol	4.7 mg
dist. water containing 20% cyclodextrins as solubiliser	1.0 ml

Example 56

Sterile dry substance for injection:

5 mg of one of the compounds of formula I mentioned in the preceding Examples (for example the title compound from Example 3) as active ingredient are dissolved in 1 ml of an aqueous solution containing 20 mg of mannitol and 20% cyclodextrins as solubiliser. The solution is sterile-filtered and, under aseptic conditions, introduced into a 2 ml ampoule, deep-frozen and lyophilised. Before use, the lyophilisate is dissolved in 1 ml of distilled water or 1 ml of physiological saline. The solution is administered intramuscularly or intravenously. The formulation can also be introduced into double-chamber disposable syringes.

Example 57

Nasal spray:

500 mg of finely ground (<5.0 mm) powder of one of the compounds of formula I mentioned in the preceding Examples (for example the compound from Example 4) are suspended as active ingredient in a mixture of 3.5 ml of Myglyol 812® and 0.08 g of benzyl alcohol. The suspension is introduced into a container having a metering valve. 5.0 g of Freon 12® (dichlorodifluoromethane; trade mark of DuPont) are introduced under pressure through the valve into the container. The "Freon" is dissolved in the Myglyol/benzyl alcohol mixture by shaking. The spray container contains approximately 100 single doses which can be administered individually.

Example 58

Film-coated tablets

The following constituents are processed for the preparation of 10 000 tablets each comprising 100 mg of active ingredient:

active ingredient	1000 g
corn starch	680 g
colloidal silicic acid	200 g
magnesium stearate	20 g
stearic acid	50 g
sodium carboxymethyl starch	250 g
water	quantum satis

A mixture of one of the compounds of formula I mentioned in the preceding Examples (for example the compound from Example 5) as active ingredient, 50 g of corn starch and the colloidal silicic acid is processed with a starch paste made from 250 g of corn starch and 2.2 kg of demineralised water to form a moist mass. That mass is forced through a sieve of 3 mm mesh size and dried in a fluidised bed dryer at 450 for 30 min. The dried granules are pressed through a sieve of 1 mm mesh size, mixed with a previously sieved mixture (1 mm sieve) of 330 g of corn starch, the magnesium stearate, the stearic acid and the sodium carboxymethyl starch and compressed to form slightly convex tablets.

Example 59

Capsules (I)

A compound from one of the afore-mentioned Examples (e.g. the title compound from Example 6) is micronised (particle size about 1 to 100 µm) using a customary knife mixer (e.g. Turmix). ®Pluronic F 68 (block polymer of polyethylene and polypropylene glycols; Wyandotte Chem. Corp., Michigan, USA; also obtainable from Emkalyx, France; trade mark of BASF) is likewise micronised using a customary mixer and the fines content is removed using a sieve (0.5 mm) and used further as below. 16.00 g of sesame oil are placed in a glass beaker and 1.20 g of the micronised active ingredient, 1.20 g of the fines content of ®Pluronic F 68 and 1.20 g of hydroxypropylmethylcellulose (Cellulose HP-M-603 from Shin-Etsu Chemicals Ltd., Tokyo, JP) are added with stirring using a stirring device (IKA-Werk, FRG) combined with a toothed stirrer (diameter: 46 mm) (stirring speed: 2000 rev/min). Twenty minutes' stirring at the speed indicated produces a suspension of pasty consistency which is introduced into hard gelatin capsules (20×40 mm; R. P. Scherer AG, Eberbach, FRG).

Example 60

Capsules (II):

For the preparation of 10 000 capsules comprising 100 mg of active ingredient (from one of the afore-mentioned Examples, for example the title compound from Example 7) per capsule, the following constituents are processed as follows:

active ingredient	1000 g
® Pluronic F 68	1000 g
hydroxypropylmethylcellulose	1000 g
sesame oil	1000 g
(for origin of constituents see Example 10)	

The sesame oil is placed in a heatable vessel (Fryma) and the ®Pluronic F 68 is scattered in. The vessel is heated at 60° C. and the ®Pluronic F 68 is distributed with stirring (duration about 2 hours). With stirring and homogenisation, the mixture is cooled to about 30° C. The hydroxypropylmethylcellulose and the active ingredient are scattered in and, with stirring and homogenisation (about 1 hour), distributed in the oil mass. The suspension of pasty consistency is introduced into hard gelatin capsules (size 0; obtainable, for example, from Elanco or Parke-Davies (Caprogel)) or soft gelatin capsules (20 mm oblong; R. P. Scherer AG, Eberbach, FRG) using customary apparatus.

Example 61

Dispersion:

For the preparation of a dispersion comprising 120.0 mg of active ingredient 10 ml (preferably the title compound from Example 46), the following constituents are processed as follows:

active ingredient	120.0 mg
® Klucel HF (hydroxypropylcellulose; Hercules, Germany)	50.0 mg
® Tween 20 (polyoxyethylene sorbitan monolaurate; Fluka, Buchs, Switzerland)	100.0 mg
demineralised water	10.0 ml

The demineralised water is placed in a container; the hydroxypropylcellulose is scattered in slowly with stirring using a magnetic stirrer and allowed to swell for 1 hour. The polyoxyethylene sorbitan monolaurate is then added and the mixture is stirred for 5 min using the magnetic stirrer. Finally, the active ingredient is added and the mixture is stirred for 15 min using the magnetic stirrer.

Example 62

Inhibitory activity in respect of HIV-1-protease

Using the test system described above with the icosapeptide RRSNQVSQNYPIVQNIQGRR, the IC_{50} values given below are obtained for the following Examples:

Example	IC_{50} (μM)
1	0 032
2	0.014
3	0 041
4	0.038
5	0.04
6	0.022
7	0.013
8	0 01
9	0.019
10	0.02
11	0 037
12	0.02
13	0.032
14	0.031
15	0.05
16	0.033
17	0.018
18	0.025
19	0 022
20	0 015
21	0 043
22	0.04
23	0.034
24	0.05
25	0.1
26	0.021
27	0.027
27 (1-methyl-1H-tetrazolyl isomer)	0.051
28	0 083
29	0.014
30	0.054
31	0.171
34	0 072
35	0 058
37	0.029
38	0.085
39	0.012
40	0.021
41	0.032
42	0.015
43	0.037
44	0.029
45	0.012
46	0.026
47	0.04
48	0.031
49	0.02
50	0.028
51	0.034
52	0 034

Example 63

Protection of MT-2 cells against HIV infection

Using the afore-mentioned test system, in inhibiting the infection of MT-2 cells by the virus syrain HIV-1/MN the title compound from Example 46, 1-[4-(pyridin-2-yl) phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane, has the following ED_{90} value: ED_{90}=0.003 μM.

Example 64

Blood levels in mice:

Using the afore-mentioned test system for the determination of the pharmacokinetics of compounds of formula I, the title compound from Example 46, 1-[4-(pyridin-2-yl) phenyl]-4(S)-hydroxy-5(S)-2,5-bis[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane, exhibits in mice the following blood levels after oral administration of 120 mg/kg:

Plasma level (μM) of title compound of Example 46	
30 min	90 min after administration
21.83	31.76

Example 65

Formulation as solution (I):

The formulation comprises 100 mg of the title compound from Example 46 as active ingredient, 100 mg of racemic

lactic acid (90%), Celluliose-HP-M-603, silica gel (Aerosil 200) and deionised water (2 g).

Example 66

Formulation as solution (II):

The formulation comprises 18.4 mg of the title compound from Example 46 as active ingredient, 5 mg of Celluliose-HPM-603, 40 mg of N-melthylpyrrolidone and double-distilled water ad 1 ml.

Example 67

Analogously to one of the afore-mentioned processes, there are prepared:

A) 1-[4-(pyridin-2-yl)phenyl]-4(R)-hydroxy-5(S)-2,5-bis [N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane;

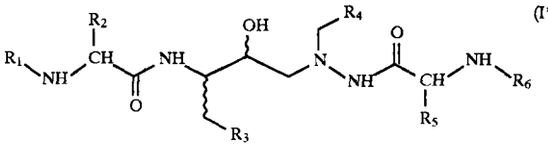
B) 1-[4-(pyridin-2-yl)phenyl]-4(R)-hydroxy-5(R)-2,5-bis [N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane;

C) 1-[4-(pyridin-2-yl)phenyl]-4(S)-hydroxy-5(S)-2-[N-(N-methoxycarbonyl-(L)-tert-leucyl)-amino-5-[N-(N-methoxycarbonyl-(D)-tert-leucyl)amino]-6-phenyl-2-azahexane; or

D) 1-[4-(pyridin-2-yl)phenyl]-4(S)-hydroxy-5(S)-2-[N-(N-methoxycarbonyl-(D)-tert-leucyl)-amino]-5-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane.

What is claimed is:

1. A compound of the formula I*,



(I*)

wherein

R_1 is lower alkoxycarbonyl,

R_2 is secondary or tertiary lower alkyl or lower alkylthio-lower alkyl,

R_3 is phenyl that is unsubstituted or substituted by one or more lower alkoxy radicals, or

C_4–C_8cycloalkyl,

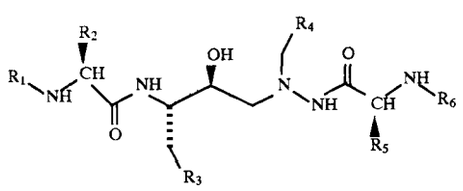
R_4 is phenyl or cyclohexyl each substituted in the 4-position by unsaturated heterocyclyl that is bonded by way of a ring carbon atom, has from 5 to 8 ring atoms, contains from 1 to 4 hetero atoms selected from nitrogen, oxygen, sulfur, sulfinyl and sulfonyl and is unsubstituted or substituted by lower alkyl or by phenyl-lower alkyl,

R_5, independently of R_2, has one of the meanings mentioned for R_2, and

R_6, independently of R_1, is lower alkoxycarbonyl,

or a salt thereof.

2. A compound according to claim 1 of formula Ia,



(Ia)

wherein the radicals are as defined in claim 1, or a salt thereof.

3. A compound of formula Ia according to claim 2, wherein

R_1 is lower alkoxycarbonyl,

R_2 is isopropyl, sec-butyl or tert-butyl,

R_3 is phenyl or cyclohexyl,

R_4 is phenyl substituted in the 4-position by one of the following radicals bonded by way of a ring carbon atom: thienyl; oxazolyl; thiazolyl; imidazolyl; 1,4-thiazinyl; triazolyl that is unsubstituted or substituted by 1-methyl-1-phenyl-ethyl, tert-butyl or by methyl; tetrazolyl that is unsubstituted or substituted by 1-methyl-1-phenyl-ethyl, tert-butyl or by methyl; pyridinyl; pyrazinyl; and pyrimidinyl;

R_5 is isopropyl, sec-butyl, tert-butyl or methylthiomethyl, and

R_6 is lower alkoxycarbonyl,

or a salt thereof.

4. A compound of formula Ia according to claim 2, wherein

R_1 is methoxycarbonyl or ethoxycarbonyl,

R_2 is isopropyl, sec-butyl or tert-butyl,

R_3 is phenyl,

R_4 is phenyl substituted in the 4-position of the phenyl ring by 2- or 3-thienyl; thiazol-5-yl; thiazol-2-yl; 2H-tetrazol-5-yl that is unsubstituted or substituted in the 2-position by 1-methyl-1-phenyl-ethyl, tert-butyl or by methyl; 1H-tetrazol-5-yl substituted in the 1-position by methyl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; or by pyrazin-2-yl;

R_5 is isopropyl, sec-butyl, tert-butyl or methylthiomethyl; and

R_6 is methoxycarbonyl or ethoxycarbonyl;

with the proviso that at least one of the two radicals R_2 and R_5 is tert-butyl, provided that R_4 is phenyl substituted in the 4-position of the phenyl ring by 2- or 3-thienyl; thiazol-5-yl; thiazol-2-yl; 2H-tetrazol-5-yl that is unsubstituted or substituted in the 2-position by 1-methyl-1-phenyl-ethyl, tert-butyl or by methyl; 1H-tetrazol-5-yl substituted in the 1-position by methyl; pyridin-3-yl; pyridin-4-yl; or by pyrazin-2-yl;

or a salt thereof.

5. A compound of formula Ia according to claim 2, wherein

R_1 is methoxycarbonyl or ethoxycarbonyl,

R_2 is isopropyl, sec-butyl or tert-butyl,

R_3 is phenyl,

R_4 is 4-(thiazol-2-yl)-phenyl, 4-(thiazol-5-yl)-phenyl, 4-(pyridin-2-yl)-phenyl or 4-(2-methyl-tetrazol-5-yl)-phenyl;

R_5 is isopropyl, sec-butyl, tert-butyl or methylthiomethyl; and

R_6 is methoxycarbonyl or ethoxycarbonyl;

or a pharmaceutically acceptable salt thereof.

6. A compound of formula Ia according to claim 2, selected from the following compounds:

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycartionyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexare;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycartonyl-(L)-S-methylcysteinyl)-amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-ethoxycarboiyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)-amino]-6-phenyl-2-azahexane;

1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane;

1-[4-(thiazol-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-iso-leucyl)amino-6-phenyl-2-azahexane;

1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-valyl)amino]-6-phenyl-2-azahexane;

1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-valyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-6-phenyl-2-azahexane; and

1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-2-N-(N-methoxycarbonyl-(L)-tert-leucyl)amino-5(S)-N-(N-methoxycarbonyl-(L)-valyl)amino-6-phenyl-2-azahexane;

or in each case a pharmaceutically acceptable salt thereof.

7. A compound of formula Ia according to claim **2** named 1-[4-(thiazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)-amino]-6-phenyl-2-azahexane, or a salt thereof.

8. A compound of formula Ia according to claim **2** named 1-[4-(2-methyl-2H-tetrazol-5-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)amino]-6-phenyl-2-azahexane, or a salt thereof.

9. A compound of formula Ia according to claim **2** named 1-[4-(pyridin-2-yl)-phenyl]-4(S)-hydroxy-5(S)-2,5-bis-[N-(N-methoxycarbonyl-(L)-tert-leucyl)-amino]-6-phenyl-2-azahexane, or a salt thereof.

10. A pharmaceutical composition suitable for administration to a warm-blooded animal for the treatment of AIDS or its preliminary stages, comprising a compound of formula I* according to claim **1**, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutically acceptable carrier.

11. A method for the treatment of AIDS or its preliminary stages, wherein a therapeutically effective amount of a compound of formula I* according to claim **1**, or a pharmaceutically acceptable salt thereof, is administered to a human, who on account of said disease requires such treatment, in a dose that is effective in the treatment of said disease.

* * * * *

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GENERAL POWER OF ATTORNEY

In the matter of The Patents Act, 1970 *as amended by* The Patents (Amendment) Act of 1999 and 2002, and The Patents Amendment Act, 2005,

And

In the matter of The Patents Rules, 2003 *as amended by* The Patents (Amendment) Rules of 2006,

And

In the matter of CIPLA LIMITED, Mumbai Central, Mumbai 400 008, India

TRUE COPY
08/11/11.

Original filed with opposition U/S 25(1) against 2221/Del NP/2008. dated. 14/03/2008

Q-

We, the above named **CIPLA LIMITED** do hereby retain, constitute and appoint **S. MAJUMDAR, M. MAJUMDAR, DR. SANCHITA GANGULI, ABHISHEK SEN, AMIT CHAKRABORTY, A. MUKHERJEE, MYTHILI VENKATESH, N. R. SETH, MRIGANKI DUTTA, SULTANA SHAIKH, AMRITA MAJUMDAR** representatives of the Firm of S. MAJUMDAR & CO., 5, Harish Mukherjee Road, Calcutta – 700 025, India, all of Indian nationality, jointly and severally to be our Agents and Attorneys for the purpose of all acts under the Patents Act, 1970 (as amended by the Patents (Amendment) Act, 2005 or as may be amended hereafter) for all matters in which the name of the said firm of S. MAJUMDAR & CO., appears in the address for service in the respective matters and we authorize any of them to sign our name and on our behalf on all applications and other papers and writings and do such acts, as may be necessary or expedient and lastly we request that all official communications now or hereafter relating to the same may be addressed to them at their office in Calcutta and that they be recognized as our authorized Agents in all proceedings incidental thereto. Cipla Limited retains the power to revoke this Power of Attorney at any time at its own discretion.

We authorize them to appoint agents, advocates and attorneys on Cipla's expressed consent. We hereby confirm all actions, if any, already taken by them in this matter. This Power of Attorney supercedes all previous Powers of Attorney given in favour of said firm of S. MAJUMDAR & CO.

Dated this 29th day of January 2010

Name: Mr. Amar Lulla

Status: Joint Managing Director