BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE, DELHI

IN THE MATTER OF THE PATENTS ACT, 1970 and THE PATENTS RULES 2003.

IN THE MATTER OF a pre-grant representation under Section 25(1)

AND

IN THE MATTER OF:

Indian Patent Application No. 854/DELNP/2010 filed on 08.02.2010 claiming priority of United States Patent Application No. 60/954,595 filed on 08.08.2007 by Bristol-Myers Squibb Company is National Phase of PCT Application No. PCT/US2008/071696 dated 30.07.2008.

AND

IN THE MATTER OF:

Dalbir Singh,
162, Block-F, Nihal Vihar,
Nangloi Jat, New Delhi-110041,
India   ...PETITIONER/OPPONENT

VS.

Bristol-Myers Squibb Company
Route 206 and Province Line Road,
Princeton, New Jersey 08543-4000,
USA.   ... RESPONDENT/APPLICANT

PRE-GRANT OPPOSITION BY MR. DALBIR SINGH

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5.	**Annexure 3:** Copy of article by John Hondrelis et al., One pot synthesis and conformation of N-t-butyloxycarbonyl, O-Phenacyl derivatives of proline and other secondary amino acids, Tetrahedron Letters Volume 46, Issue 2, 1990, Pages 565-576	32-43

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Dated this 6th day of May, 2016

CHITRA ARVIND
FOR RAJESHWARI & ASSOCIATES
AGENT FOR THE OPPONENT

To,
The Controller of Patents
The Patent Office, Delhi

(

IPO DELHI 06-05-2016 18:00

FORM 7A

THE PATENTS ACT,

1970 (39 OF 1970)

AND

THE PATENTS RULES, 2003

REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT

[See Rule 55]

I, Dalbir Singh, 162, Block-F, Nihal Vihar, Nangloi Jat, New Delhi-110041, India, hereby give representation by way of opposition to the grant of patent in respect of application No: **854/DELNP/2010 filed on 08.02.2010** made by Bristol-Myers Squibb Company on the grounds:

i. Section 25(1)(e): Lack of inventive step

ii. Section 25(1)(f): Subject of claims 1 to 11 is not an invention within the meaning of this Act or is not patentable under this Act

iii. Section 25(1)g: The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.

iv. Section 25(1)h: The Applicant has failed to disclose to the Controller the information required under Section 8.

(Detailed grounds are set out in the Opposition)

Our address for service in India is:

RAJESHWARI & ASSOCIATES
AMSOFT BUSINESS CENTRE
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Sector 43, Gurgaon- 122 002.
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Fax: +91-11-43851067
Mobile No. 9910048684

Dated, this 6th day of May, 2016

CHITRA ARVIND
of Rajeshwari & Associates
Agent for the Opponent

To
The Controller of Patents,
The Patent Office, Delhi

IPO DELHI 06-05-2016 18:00

BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE, DELHI

IN THE MATTER OF THE PATENTS ACT, 1970 and THE PATENTS RULES 2003.

IN THE MATTER OF a pre-grant representation under Section 25(1)

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Dalbir Singh,
162, Block-F, Nihal Vihar,
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India   ...PETITIONER/OPPONENT

VS.

Bristol-Myers Squibb Company
Route 206 and Province Line Road,
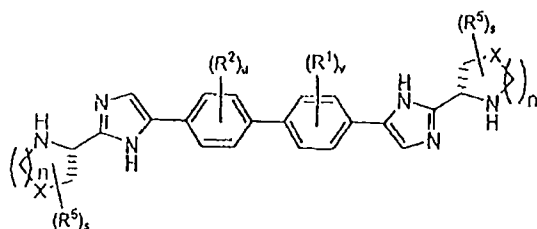Princeton, New Jersey 08543-4000,
USA.   ... RESPONDENT/APPLICANT

STATEMENT OF CASE OF OPPONENT

1.   The Opponent herein, Dalbir Singh is an individual residing in New Delhi.

2.   The Petitioner/Opponent has learnt that the Applicant has filed an Indian National Phase Application No. **854/DELNP/2010**, which is currently pending before the Patent Office, entitled "Process for synthesizing compounds useful for treating Hepatitis C", filed by Bristol-Myers Squibb Company on 08.02.2010. (Hereinafter "The Impugned Application"). The said patent Application is drawn to various claims pertaining to a process for the preparation of compound of formula (7), which IPO DELHI further reaction results in the formation of chemical moiety known as Daclatasvir.

From the publications and documents available at the Patent Office website, it appears that the application was published in the official Journal of the Patent Office dated 13.08.2010. The Indian Application was initially filed with 22 claims, which apparently appear to have been amended after objections were raised by Learned Controller in First Examination Report dated 28.01.2015, thereby amending the claims to 11 in number. The response for the First examination report was filed on 03.08.2015. The opponents by way of this present pre-grant representation submit that the claims currently pending on record are not patentable under the provisions provided in this Act. The claims on file as of 03.08.2015 are annexed herewith at Annexure 1 and reproduced herein below for ready reference:

1. A process for preparing a compound of formula (7)

(7);

or a pharmaceutically acceptable salt thereof: wherein
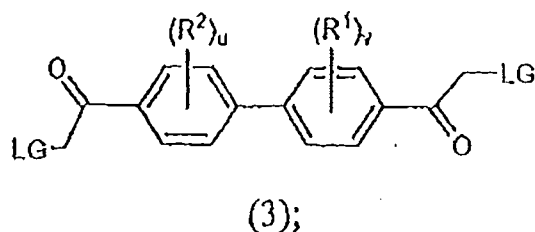
n is 0, 1, or 2;

s is 0, 1, 2, 3, or 4;

u and v are each independently selected from 0, 1, 2, or 3;

X is selected from O, S, S(O), SO_2, CH_2, CHR_5, and $C(R_5)_2$; provided that when n is 0, X is selected from CH_2, CHR_5, and $C(R_5)_2$;

R_1 and R_2 are each independently selected from alkoxy, alkyl, and halo; and

when s is 2, 3, or 4, each R5 on the ring is independently selected from alkoxy, alkyl, and aryl, wherein the alkyl can optionally form a fused three- to six- membered ring with an adjacent carbon atom, wherein the three- to six-membered ring is optionally substituted with one or two alkyl groups; provided that the two heterocyclic rings substituting the imidazole rings are identical; the process comprising:
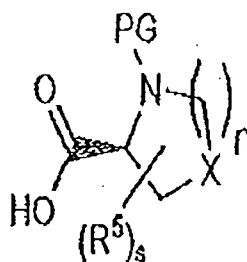
a) reacting a compound of formula (3)

(3);
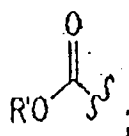
wherein

*u, v, R₁, and R₂ are as described for formula (7); and*

LG is a leaving group; with a compound of formula (4)

wherein PG is a nitrogen protecting group;

b) *treating the product of step (a) with a reagent selected from ammonium acetate, ammonium formate, ammonium sulfamate, ammonium phosphate, ammonium citrate, ammonium carbamate, and ammonia; and*

c) *treating the product of step (b) with a deprotecting agent.*

2. *The process of claim 1 wherein*

   n is 1;

   s is 0;

   u and v are each 0; and

   X is CH₂.

3. *The process of claim 1 wherein LG is a halide.*

4. *The process of claim 3 wherein the halide is a bromide.*

5. *The process of claim 1 wherein step (a) is conducted with a base.*

6. *The process of claim 5 wherein the base is diisopropylethylamine.*

7. *The process of claim 1 wherein the reagent used in step (b) is ammonium acetate.*

8. *The process of claim 1 wherein PG is represented by the formula:*

wherein

ξ denotes the point of attachment to the parent molecular moiety; and R' is selected from alkyl, aryl, and arylalkyl.

9. The process of claim 8 wherein PG is tert-butoxycarbonyl.

10. The process of claim 9 wherein the deprotecting agent of step (c) is an acid.

11. The process of claim 10 wherein the acid is hydrochloric acid.

3(a)   The claims currently on record, may be summarized as below:

Claim 1 of the present application being the impugned patent application is drawn to the process for preparation of compound of formula (7). Claims 2 to 11 are drawn to various embodiments of process claimed in claim 1.

Without acquiescing to the admissibility of such claims both in terms of technicality and procedural aspects, the opponent proceeds to submit the grounds of opposition pertaining to the said claims on record.

3(b)   As per disclosure in the impugned specification, it appears that the main object of the alleged invention is to provide an economical method, which may be applicable in the industrial scale. But, the claims are not limited to industrial-scale use, as described in the specification; nor do the claims articulate the specific conditions that render the synthetic methods set forth suitable to industrial-scale use.

3(c)   In the process as disclosed in the impugned application, it may be noted that the claim 1 of the impugned specification is drawn to a process for preparing a compound of Formula (7). The compound of Formula (7) may be prepared by coupling of compound of formula (3) and (4) to obtain a compound of Formula (5). Compound of Formula (5) then undergoes cyclization reaction in presence of ammonium acetate to form compound of Formula (6). The protected moiety of the compound of Formula (6) is deprotected using a deprotecting agent to obtain the compound of Formula (7). Claim 2 to 11 are drawn to further embodiments of the process claimed in claim 1.

3(d)   It may be noted that all disclosures at Claims 1-11 are generic, without listing any specific advantages of the reaction conditions or the reagents used, and, in particular, these disclosures do not identify the particular reaction conditions or ingredients that render the

process suitable for use on an industrial scale in a way that was not known or not obvious before from the existing knowledge.

Further, it is submitted that the use of the intermediates as set out in the impugned specification are well known and well established in prior art and such a position has been admitted by the Applicant. Hence, it is submitted that since the intermediates and the processes are already well established and well set out in prior art, such processes for the synthesis of anti-viral compounds as disclosed in the impugned application cannot be considered inventive.

The specification also discloses certain examples, which purportedly illustrate the process of the present application being the impugned application. Further the objectives of the invention as set out in the impugned Application have not been demonstrated by way of examples.

4.   The claims currently on record have been submitted by the Applicant on 03.08.2015 in response to First examination report and this representation is based on the said claims. The Opponent craves leave to add new documents or evidence as may be required in support of such amended claims.

5.   Accordingly, the Opponent submits their opposition by way of representation under Section 25(1) in respect of the said Indian Application No. **854/DELNP/2010** (Priority date: 08.08.2007) corresponding PCT Application No. PCT/US2008/071696 on the following amongst other grounds listed below, which are without prejudice and in the alternative to each other.

GROUND I

6(a)   **Section 25(1)(e): Lack of inventive step**

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 (b) or having regard to what was used in India before the priority date of the claim.

All aspects of claims 1-11 are obvious by disclosure in prior art and do not have any
inventive merit and ought to be rejected on this ground alone.

It is submitted that compounds of similar structure are already known in prior art. In addition the strategies involved in the process of preparing such compounds were known to the person skilled in the art. Hence, the process for preparing the compounds as set out in the impugned patent application are obvious and track well established procedures.

The process of the present application, hereinafter the impugned application, discloses the use of substituted bromo phenyl ethanone compound as a starting material, which further undergoes reaction and cyclization to form biphenyl diimidazole derivative.

It is submitted that preparation of biphenyl diimidazole derivatives were known as early as 1960's. Hermann Schubert and his co-workers (Schubert et al., Diimidazole, III: Synthese von Aromatisch überbrückten 4(5),4'(5')-Diimidazolen, Journal für Praktische Chemie, Volume 22, Issue 3-4, pages a140–152, November 1963) (hereinafter referred to as Schubert et al and annexed herewith as Annexure 2) published an article in year 1963 on synthesis of aromatic diimidazole derivatives. The reaction scheme disclosed in Schubert et al is reproduced herein below for ready reference at Figure 1.
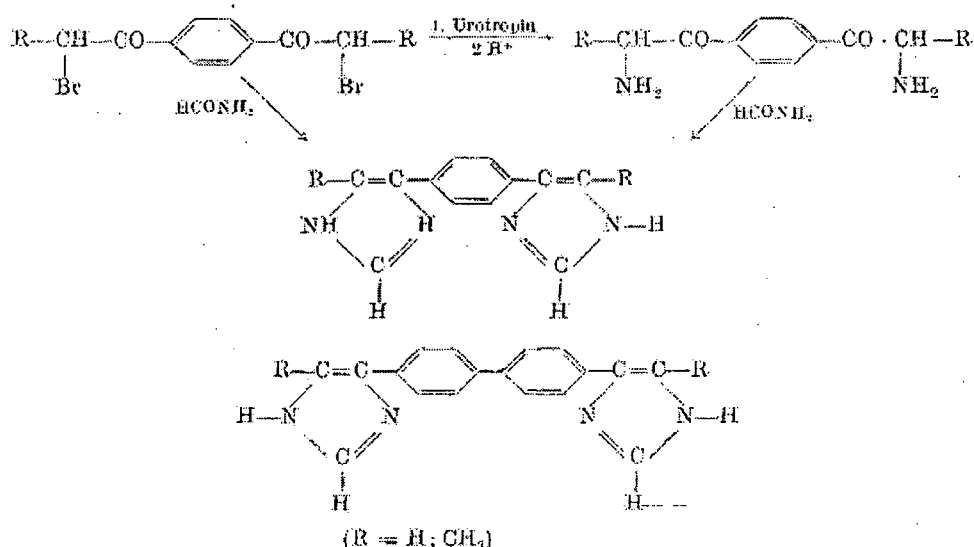

Figure 1

From the above figure it can be easily seen that the process of Schubert et al, discloses the synthesis of biphenyl diimidazole derivatives. The process starts with reacting substituted bromo phenylethanone compound, which undergoes further reaction and cyclization to form biphenyl diimidazole compound.

John Hondrelis and his co-workers (Hondrelis et al., One pot synthesis and conformation of N-t-butyloxycarbonyl, O-Phenacyl derivatives of proline and other secondary amino acids, Tetrahedron Letters Volume 46, Issue 2, 1990, Pages 565-576) (hereinafter referred to as Hondrelis et al and annexed herewith as Annexure-3) published an article in year 1990. Hondrelis et al discloses a process for the preparation of N-tert-butyloxycarbonyl, O-Phenacyl derivatives of proline. The process of Hondrelis et al is reproduced herein below for ready reference at Figure 2.
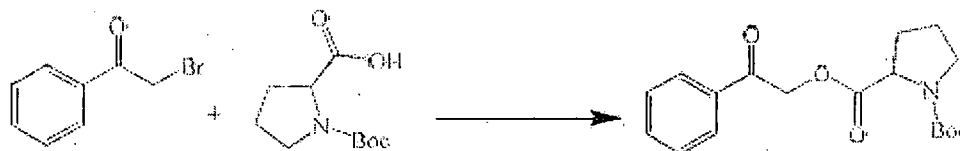

Figure 2

From the above figure, it can be easily seen that Hondrelis et al, discloses a process which starts with reacting 2-bromo-1-arylethanone with 1-(tert-butoxycarbonyl)-L-proline. It is noted that the process in Hondrelis et al is similar to the step 1 of the impugned process involving the reaction of bromo arylethanone compound of formula (3) with 1-(tert-butoxycarbonyl)-L-proline compound of formula (4) to form 1-(tert-butoxycarbonyl)-3-((3-oxo-3-phenylpropoxy)carbonyl)pyrrolidine-2-carbo-xylic acid compound of formula (5). For ease of illustration the process of Hondrelis et al is highlighted in comparison with the impugned process and set out at Figure 3.
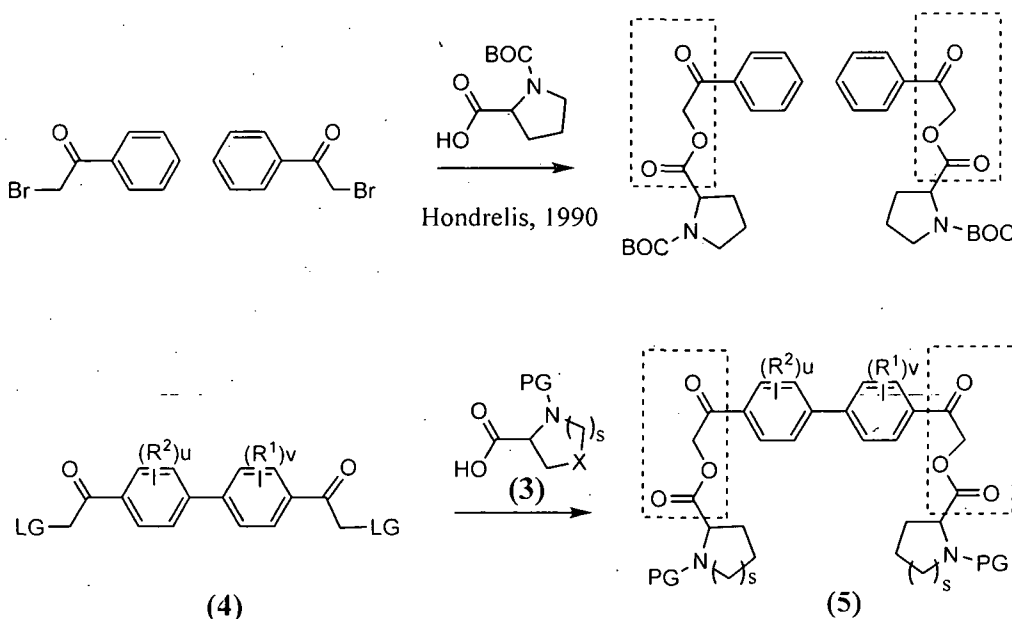
Claim 1, Step 1

Thus it may be noted that step 1 for preparing compound of formula (5) of the impugned process was already known in prior art much before the priority date of the impugned patent application. Hence, the use of bromo phenyl ethanone compound and 1-(tert-butoxycarbonyl)-L-proline as starting material was already known and well established in prior art.

Di-tert-butyl dicarbonate is a reagent widely used in organic synthesis for protection of amines. This carbonate ester reacts with amines to give N-tert-butoxycarbonyl or so-called BOC derivatives as protecting groups. Removal of the BOC can be accomplished with acids such as hydrochloric acid in methanol. One example is deprotection of a BOC protected prolinal in US 7,312,343, Example 25, published in year 2007, by R. Schmid, et al. (hereinafter referred to as Schmid et al and annexed herewith as Annexure-4). The process of Schmid et al is reproduced herein below for ready reference at Figure 4.
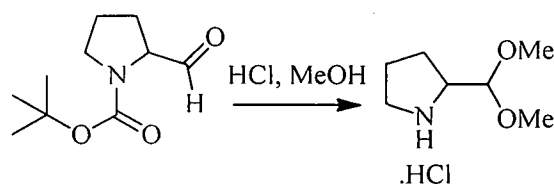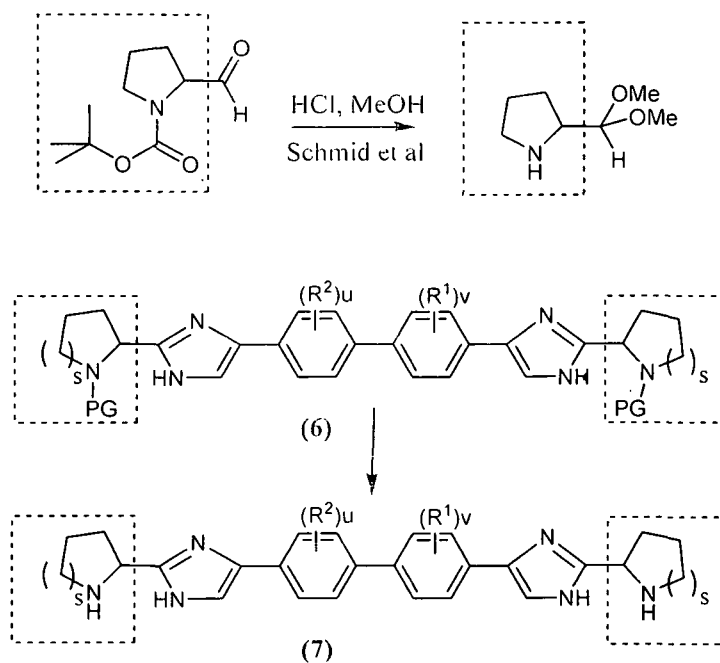

Figure 4

From the above figure it can be easily seen that the process disclosed in Schmid et al, starts with reaction of a BOC protected amine whereby reaction with hydrochloric acid form the unprotected amine. It is noted that the process disclosed in Schmid et al is similar to the impugned process involving the use of compound (6) to form unprotected compound (7). Further, the use of hydrochloric acid to deprotect a BOC protected pyrrolidine ring is the basis of dependent claims 8 through 11. For ease of illustration the process disclosed by Schmid et al is highlighted in the impugned process and set out at Figure 5.

Claim 1, Step 3

Figure 5

Thus it may be noted that the process of preparation of biphenyl imidazole compound using deprotection of protected amines as in claim 1 and more specifically deprotection of BOC protected pyrrolidine rings using hydrochloric acid as in dependent claims 8-11 were already known and well established in prior art much before the priority date of the impugned patent application.

Thus, it may be noted that the use of substituted bromo phenylethanone compounds as starting material for preparation of biphenyl diimidazole compounds was already known in prior art much before the priority date of the impugned patent application.

Anna Borioni and his co-workers (Borioni et al., Synthesis of New 4-Heteroaryl-2-Phenylquinolines and Their Pharmacological Activity as NK-2/NK-3 Receptor Ligands, Archiv der Pharmazie, Volume 340, Issue 1, pages 17–25, January 2007) (hereinafter referred to as Borioni et al and annexed herewith as Annexure-5) published an article in year 2007. Borioni et al discloses a process for the preparation of Substituted 4-heteroaryl-2-phenylquinolines. The process of Borioni et al is reproduced herein below for ready reference at Figure 6.
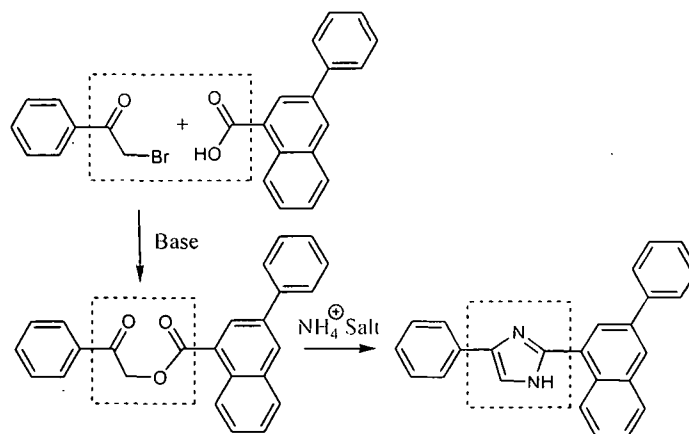
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Figure 6

From the above figure it can be easily seen that the process disclosed in Borioni et al, starts with reaction of 2-bromo-1-phenylethanone with substituted biphenyl acid derivative to form a biphenyl imidazole compound. It is noted that the process disclosed in Borioni et al is similar to the impugned process involving the use of compound (3) and compound (4) to form biphenyl imidazole compound of formula (6). Further for ease of illustration the process disclosed by Borioni et al is highlighted in the impugned process and set out at Figure 7.
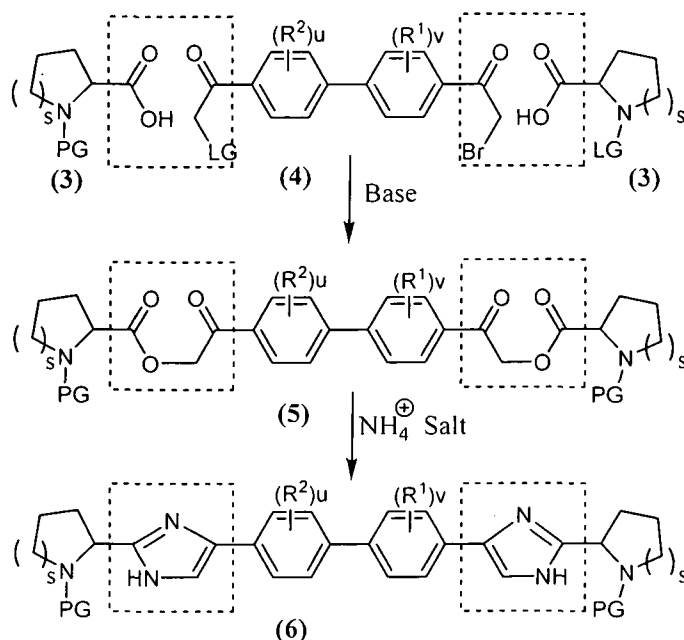
Claim 1, Steps 1 and 2

Figure 7

Thus it may be noted that the process of preparation of biphenyl imidazole compound using bromo phenylethanone compound and substituted biphenyl acid derivative as

the starting materials were already known and well established in prior art much before the priority date of the impugned patent application.

Hence, it is submitted that all components of the process of the impugned patent application including the various reaction sequences and the reagents used are known. The resulting end products are also same or similar end products and therefore the process of the impugned patent does not hold any inventive merit. Therefore, the process of the impugned patent application is obvious and ought to be rejected on this ground only.

Hence, all claims 1 to 11 ought to be rejected on the basis of obviousness.

GROUND II

6(b) Section 25(1)(f): Subject of claims 1 to 11 is not an invention within the meaning of this Act or is not patentable under this Act

The subject matter of claims 1 to 11 does not constitute an 'invention' as understood under Section 2(i)(j) of the Act:

It is submitted that since the claims 1 to 11 are not novel, are not inventive and lack industrial application, they do not constitute an 'invention' under the Act. In regard, the Petitioner craves leave to refer and rely on submission made in Grounds I above and the same are not being repeated for the sake of brevity.

The subject matter of claims 1-11 is not patentable under Section 3(d) of the Act

It is submitted that all claims 1-11 are drawn to the mere use of a known process, which does not involve a new product or employs at least one new reactant. It is submitted that the Applicants admit that the process as disclosed in the impugned application is already set out in various prior art documents as set out hereinabove and not repeated for the sake of brevity. Assuming but not conceding that there is a difference between the prior art documents and that of the present process in terms of the scale, yield and purity, such difference is a matter of routine experimentation and does not involve any inventive merit. Further, the process of the present invention

does not involve any new reactant, new starting material or new end product and therefore, squarely falls within the scope and sweep of Section 3(d).

Without prejudice to the fact that the process is anticipated by prior art and/or obvious, the process claimed is at worst, practice of prior art process with a mere optimization of the reaction conditions without any change in the reactants or the end-products obtained from such a reactant. The resultant product is the compound of Formula (7) is the same as that of prior art. Hence, the process of the impugned application falls squarely within the scope and sweep of Section 3(d) and is nothing but a mere use of a known process and is not patentable.

Hence, all claims ought to be rejected on this ground as well.

GROUND III

IV) **Section 25(1)g: The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.**

It is submitted that the impugned application 854/DELNP/2010 does not sufficiently and clearly describe the invention claimed or the method by which it is be performed.

Claims 1 to 11 of the application at hand suffer from lack of adequate description and are liable to be rejected. The Specification does not provide adequate teaching to a person skilled in the art to practice the invention. The particulars thereof are as under:

(i) As disclosed in the Impugned Application, an object of the invention is to have a process, "which are readily achievable on an industrial scale". However, there is no whisper in the impugned Application as to how the process as claimed in claim 1, may be scalable to an industrial level. The sole example remotely close to a scale beyond lab level talks of 1 kg level. In simple words, scale within range of 1 Kg level does not necessarily mean that same can be enabled at industrial scale which is sought to be covered in the impugned application.

(ii) Claim 1 is drawn to a process for preparing the compound of markush Formula (7) by reacting compound of markush formula (3) with that of markush formula

IPO DELHI (4) to form an intermediate. Claim 1 is drawn to a general chemical structure

which encompasses a wide variety of compounds of formula (7), but suffers from defect of not being enabled/described adequately. Claim 1 further state that the product of step (a) is reacted with an ammonium reagent to form a product of step (b) which is further reacted with deprotecting agent to form compound of markush formula (7). However, Claim 1 does not provide the various reaction conditions to carry out the said reaction and hence, in absence of such reaction conditions, Claim 1 is not capable of being performed by a person skilled in the art without reading other claims into Claim 1.

(iii) Claim 2 discloses further embodiments of process claimed in claim 1 but does not provide clarity with respect to the reaction product that is being formed.

(iv) Claim 3 is drawn to and dependent on claim 1, the inventive merit of which has not been demonstrated in the impugned application and there is no basis in the specification as to how the halide leaving group is relevant to the present application.

(v) Claim 4 is drawn to specific leaving group being bromide and is dependent on claim 3. But again here also the inventive merit has not been demonstrated over other halides (Fluorides, chlorides and iodides) in the impugned application and therefore this claim cannot be awarded to the applicant, in present form.

(vi) Claim 5 and 6 are drawn to a subject matter that is beyond the scope of claim 1. Claim 1 is drawn to a process starting from reactants of markush formula (3) and (4) and completes with the product of markush formula (7). Claim 5 and 6 on the contrary discloses a base. The use of said base is not disclosed anywhere in claim 1 and hence does not fall within the scope of claim 1.

(vii) Claim 7 is drawn to a specific ammonium reagent and is dependent on claim 1, the inventive merit of which reagent is not demonstrated in the impugned application and there is no basis in the specification as to how the reagent claimed in claim 7 is relevant to the present application.

(viii) Claim 8 is drawn to protecting group represented by way of a markush that potentially claims hundreds of compounds. However the only enabled compound from this markush is tert-butoxy carbonyl at Example- "Preparation of compound 5", but no other example/compound is enabled for the remaining R'-substitutions which are sought to be covered within the claim 8 markush. For instance, compounds where R' would be aryl or arylalkyl are not enabled at all. Thus the specification does not sufficiently and clearly describe the compounds covered in the entire breadth of claim 8 markush, nor does the specification give details by which such compounds may be used a protecting agent.

(ix) Claim 9 is drawn to specific protecting group, the inventive merit which is not demonstrated in the impugned application and there is no basis in the specification as to how the protecting group claimed in claim 9 is relevant to the present application.

(x) Claim 10 is drawn to a deprotecting agent and is dependent on claim 9. The said subject matter is beyond the scope of claim 9.

(xi) Claim 11 is drawn to Hydrochloric acid used as specific deprotecting agent and is dependent on claim 10. The inventive merit of this acid has not been demonstrated over other acids in the impugned application and therefore this claim cannot be awarded to the Applicant in present form.

It is submitted that the Claims 1-11 bitterly suffer from lack of support in the specification and insufficient disclosure. Hence, on this ground alone, all claims 1-11 are liable to be rejected. Also, the invention fails to disclose the invention entirely on how it is to be practiced, therefore it is liable to be rejected under section 10(4) of the Patents Act, 1970.

GROUND IV

V) **Section 25(1)h: The Applicant has failed to disclose to the Controller the information required under Section 8.**

It is submitted that the Applicant/Respondent has failed to disclose the details of corresponding foreign applications filed, and on this ground alone the patent

application should be rejected. It is to be noted that several claims have been cancelled and/or disclaimed in the corresponding applications filed to the said PCT application during their prosecution in other countries. However, the Applicant has not disclosed any information pertaining to such claim amendments in the corresponding applications.

Hence, on this ground alone, the claims ought to be rejected.

PRAYER

In the fact and circumstances of the case, the Opponent prays as follows:

i.   that the Indian Patent Application No. 854/DELNP/2010 made by Bristol-Myers Squibb Company, be rejected under Section 25(1) of the Patents (Amendment) Act, 2005;

ii. the Opponent may be allowed to file further documents as evidence if necessary to support its averments;

iii. the Opponent may be granted an opportunity of being heard in the matter before any final orders are passed;

iv. the Opponent may be allowed to make further submissions in case the applicant makes any amendments in the claims;

v.   any other reliefs considering the facts and circumstances may be granted in favour of the Opponent in the interest of justice.
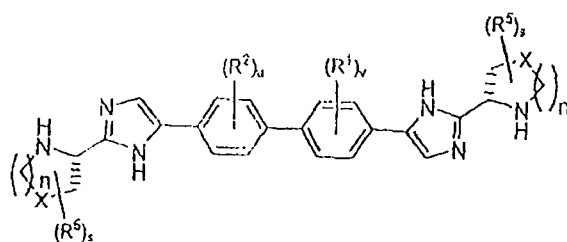
Dated this 05th day of May, 2016

CHITRA ARVIND
AGENT FOR THE OPPONENT
FOR RAJESHWARI & ASSOCIATES

To,
The Controller of Patents
The Patent Office, Delhi

IPO DELHI 06-05-2016 18:00

We claim:

1. A process for preparing a compound of formula (7)

(7);

or a pharmaceutically acceptable salt thereof;

wherein

n is 0, 1, or 2;

s is 0, 1, 2, 3, or 4;

u and v are each independently selected from 0, 1, 2, or 3;

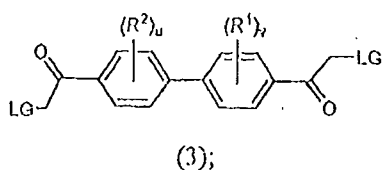X is selected from O, S, S(O), SO_2, CH_2, CHR^5, and $C(R^5)_2$;

provided that when n is 0, X is selected from CH_2, CHR^5, and $C(R^5)_2$;

R^1 and R^2 are each independently selected from alkoxy, alkyl, and halo; and when s is 2, 3, or 4, each R^5 on the ring is independently selected from alkoxy, alkyl, and aryl, wherein the alkyl can optionally form a fused three- to six-membered ring with an adjacent carbon atom, wherein the three- to six-membered ring is optionally substituted with one or two alkyl groups;

provided that the two heterocyclic rings substituting the imidazole rings are identical; the process comprising:
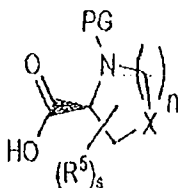
(a) reacting a compound of formula (3)

(3);

(3);

wherein

u, v, R^1, and R^2 are as described for formula (7); and

LG is a leaving group;

with a compound of formula (4)

wherein PG is a nitrogen protecting group;

(b) treating the product of step (a) with a reagent selected from ammonium acetate, ammonium formate, ammonium sulfamate, ammonium phosphate, ammonium citrate, ammonium carbamate, and ammonia; and

(c) treating the product of step (b) with a deprotecting agent.

2. The process as claimed in claim 1 wherein

   n is 1;
   s is 0;
   u and v are each 0; and
   X is CH_2.

3. The process as claimed in claim 1 wherein LG is a halide.

4. The process as claimed in claim 3 wherein the halide is a bromide.

5. The process as claimed in claim 1 wherein step (a) is conducted with a base.

6. The process as claimed in claim 5 wherein the base is diisopropylethylamine.

7. The process as claimed in claim 1 wherein the reagent used in step (b) is ammonium acetate.

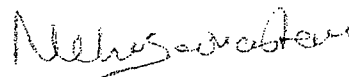8. The process as claimed in claim 1 wherein PG is represented by the formula:
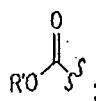
wherein

s denotes the point of attachment to the parent molecular moiety; and R' is selected from alkyl, aryl, and arylalkyl.

9. The process as claimed in claim 8 wherein PG is tert-butoxycarbonyl.

10. The process as claimed in claim 9 wherein the deprotecting agent of step (c) is an acid.

11. The process as claimed in claim 10 wherein the acid is hydrochloric acid.

Dated this 8th day of February 2010

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Diimidazole. III

Synthese von aromatisch überbrückten 4(5),4'(5')-Diimidazolen

HERMANN SCHUBERT, HANS JOACHIM LORENZ[1]) und REINER FISCHER[2])

Inhaltsübersicht

Es werden die Synthesen von alkyl- und arylsubstituierten Diimidazolen beschrieben, die in den Ringstellungen 4(5), 4'(5') durch p-Phenylen- oder p-Diphenylenreste überbrückt sind. Der Einfluß der aromatischen Substituenten auf die Assoziation wird diskutiert.

Das am längsten bekannte Diimidazol ist das in 2, 2'-Stellung verknüpfte Glykosin[3]). Während die Chemie dieser Verbindung, die allein schon wegen ihrer extremen Assoziation besonders interessant ist, recht ausgebaut ist, fehlt es an systematischen Untersuchungen über Diimidazole, die in anderen Ringstellungen miteinander verbunden sind. Im Rahmen unserer Arbeiten berichten wir zunächst über die Darstellung von aromatisch überbrückten Verbindungen dieser Stoffklasse.

Von den drei isomeren Diacetylbenzolen ist das 1,4-Derivat durch KMnO₄-Oxydation des p-Äthylacetophenons in gepufferter Lösung nach C. WEYGAND u. Mitarb.[4]) am besten zu erhalten. Sein Dibromid, das P. RUGGLI u. Mitarb.[5]) beschrieben haben, als sie das Diketon zum erstenmal über den recht langwierigen Weg einer Acetessigestersynthese erhielten, läßt sich nach H. BREDERECK u. Mitarb.[6]) in siedendem Formamid zum p-phenylenüberbrückten Diimidazol cyclisieren. Die über 300° unter Zersetzung schmelzende, extrem schwerlösliche Base wird nach diesem Verfahren nicht in so guten Ausbeuten erhalten wie die gleiche Umsetzung des ω-Bromacetophenons zum 4(5)-Phenylimidazol, dem Modellfall der Cycli-

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[1]) Aus der Dipl.-Arbeit H. J. LORENZ, Halle 1960.
[2]) Aus der Dipl.-Arbeit R. FISCHER, Halle 1961.
[3]) H. DEBUS, Liebigs Ann. Chem. 107, 199 (1858).
[4]) C. WEYGAND, Organisch-chem. Experimentierkunst, 2. Aufl., Leipzig 1948.
[5]) P. RUGGLI u. E. GASSMEYER, Helv. chim. Acta 22, 497, (1939).
[6]) H. BREDERECK u. Mitarb., Ber. dtsch. chem. Ges. 86, 88 (1953).

sierung aromatischer Halogenketone zum Imidazolsystem durch Form-
amid[6]). Das Dibromid des 1,4-Dipropionylbenzols, das auf dem oben skizzier-
ten Wege aus p-n-Propylpropiophenon in wesentlich geringerer Ausbeute zu
gewinnen ist, läßt sich nach unseren Erfahrungen viel besser mit Formamid
zweimal ringschließen. Ob dabei die beiden zusätzlichen Methylgruppen al-
lein von Einfluß sind, ist schwer zu sagen. Die Reaktionsbedingungen der
Cyclisierung in siedendem Formamid stellen gewisse Anforderungen an die
Stabilität der Bromketone, die in der aromatischen Reihe zum Unterschied
gegenüber den weniger beständigen aliphatischen Verbindungen hinreichend
erfüllt sind.

$$(R = H; CH_3)$$
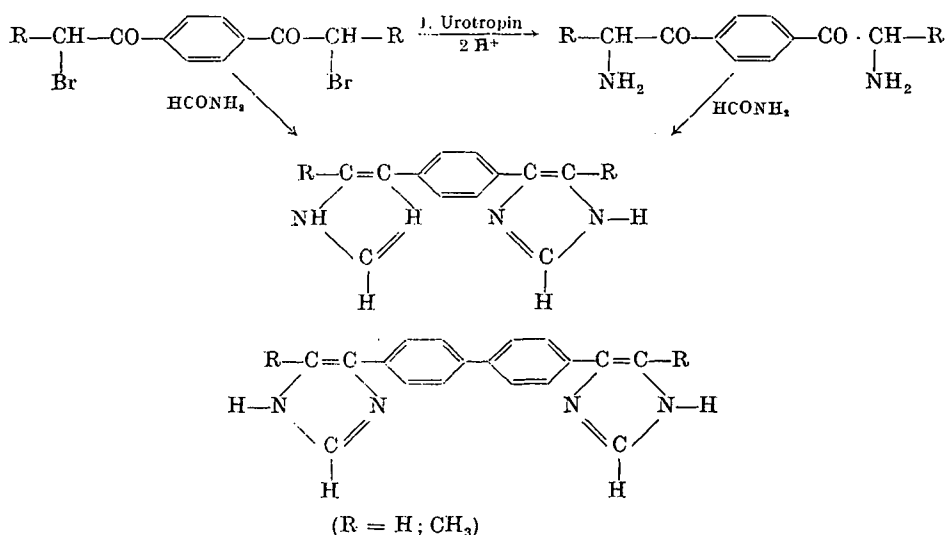
Die glatte SeO_2-Oxydation des 1,4-Diacetylbenzols zum Diglyoxal,
ebenfalls schon von P. Ruggli u. Mitarb.[5]) durchgeführt, sollte einen zwei-
ten Weg zur Darstellung der Diimidazole nach dem Verfahren von A. Pin-
ner[7]) erlauben.

P. Ruggli u. Mitarb.[5]) haben die SeO_2-Oxydation in Acetanhydrid durchgeführt. Wir
haben das für diesen Zweck gebräuchlichere Dioxan verwendet. Das von uns erhaltene Di-
hydrat des Diglyoxals schmilzt unscharf von 138—158°, der Literatur-Schmp.[5]) ist mit
110—111° angegeben. Die eigenen Analysenwerte und der Schmp. des Dichinoxalins stim-
men mit den Literaturangaben überein. Später haben P. Ruggli und W. Theilheimer[8])
nochmals versucht, das Diglyoxal auf dem von F. Kröhnke u. Mitarb.[9]) beschriebenen
Wege über die saure Hydrolyse des aus dem Pyridiniumsalz des Dibromdiacetylbenzols er-
haltenen Nitron darzustellen. Sie konnten dabei allerdings das Diglyoxal nicht in Substanz

[7]) A. Pinner, Ber. dtsch. chem. Ges. 35, 4135 (1902).

[8]) P. Ruggli u. W. Theilheimer, Helv. chim. Acta 24, 914 (1941).

[9]) F. Kröhnke u. Mitarb., Ber. dtsch. chem. Ges. 71, 2583 (1938).

fassen — bei der Nacharbeitung ist uns das gelungen — und haben es durch das Dichinoxalin charakterisiert.

Der Erfolg der Imidazolsynthese nach A. PINNER liegt in der homogenen Reaktionsführung beim Zutropfen des Gemisches aus Glyoxal und Formalin zum vorgelegten, ganz stark gekühlten konz. NH_3. Selbst bei Verwendung großer Volumina Dimethylformamid ist das nicht mehr zu erreichen. Es kommt sofort zur Abscheidung eines flockigen weißen Niederschlages, der langsam in einen cremefarbenen Ton übergeht. Beim Aufarbeiten und Reinigen erhält man ein kaum noch in organischen Lösungsmitteln umzukristallisierendes Pulver, dessen Stickstoffgehalt weit unter dem des berechneten liegt.

Siedendes Formamid cyclisiert auch die Salze von aromatischen α-Aminoketonen. Das Dibromid des 1,4-Diacetylbenzols bildet in Chloroform mit Urotropin quantitativ einen Komplex, der sich in Alkohol/HCl in vorzüglicher Ausbeute zu dem α-Aminoketon-hydrochlorid hydrolysieren läßt. Der Umsatz in Formamid ist zwar etwas besser als der des Dibromids, wiegt aber nicht den längeren Weg auf. Die α-Aminoketone lassen sich sehr gut mit Rhodanid umsetzen. Die Verwendung der dabei entstehenden 2-Mercaptodiimidazole als selektive Komplexbildner für bestimmte Metallionen wird z. Z. geprüft.

Für uns war der Ersatz der beiden Methylgruppen des aus dem 1,4-Dipropionylbenzol erhaltenen Diimidazols durch Aromaten von Interesse. Eine für die Synthese solcher Imidazolderivate geeignete Carbonylverbindung ist das 4-Phenacetyl-desoxybenzoin.

Die unterschiedliche Reaktivität der beiden Phenylkerne im Desoxybenzoin macht diese Verbindung „einseitig" der Friedel-Crafts-Reaktion zugängig. W. BORSCHE und F. SINN[10] haben diese Umsetzung auch mit Phenacetylchlorid studiert. Hier sind die Ausbeuten aber entschieden kleiner als die mit vielen anderen aliphatischen Säurechloriden erzielten. Mehr als 6% an 4-Phenacetyldesoxybenzoin wurden dabei nicht erreicht. Die möglichen Komplikationen der Friedel-Crafts-Synthese mit Phenacetylchlorid, das in Schwefelkohlenstoff leicht mit sich selbst Kondensationen eingeht, haben in einer groß angelegten Studie J. SCHMITT und Mitarb[11] untersucht. Die von ihnen erreichten Ausbeuten für das p-Phenacetylderivat des Desoxybenzoins, die frühere Mitteilung von W. BORSCHE und F. SINN[10] wird nicht zitiert, liegen kaum höher. Die französische Arbeitsvorschrift bietet dennoch mehrere Vorteile. Man braucht kein Desoxybenzoin, die Reaktion läuft über intermediär gebildetes 4-Phenacetyl-phenacetylchlorid, das sich mit Benzol „rechtzeitig" abfangen läßt. Die sehr durchgearbeitete Vorschrift ist zur Gewinnung von 30-g-Mengen des Diketons geeignet, und es werden auf gleichem Wege noch zwei weitere recht wertvolle Carbonylverbindungen mit gewonnen.

Das isomere Diketon, das 1,4-Bis-phenacetyl-benzol, kann durch eine Grignardreaktion aus Therephthalonitril gewonnen werden. Wie im Formel-

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[10] W. BORSCHE u. F. SINN, Liebigs Ann. Chem. 523, 260 (1942).
[11] J. SCHMITT u. Mitarb., Bull. Soc. chim. France 1956, 636.

schema gezeigt, entstehen durch Bromierung oder durch Oxydation mit SeO_2 und durch Zinkstaub-Reduktionen in wäßrigem Dioxan aus diesen Benzilen die Acyloine, somit liegen drei zum Ringschluß befähigte Komponenten vor.
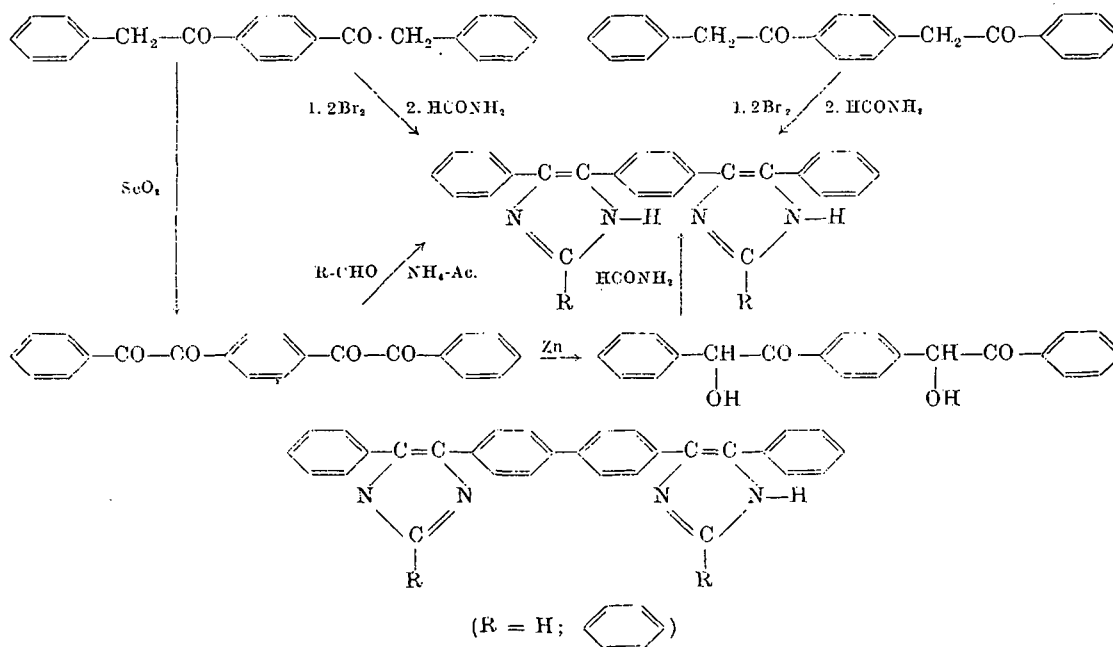
$(R = H; \langle phenyl \rangle)$

In analoger Weise lassen sich die Diimidazole mit einer p-Diphenylen-brücke aufbauen. Die p,p'-Diacylverbindungen sind durch eine Diacylierung des Diphenyls[12]) nach Friedel-Crafts in sehr guten Ausbeuten zu erhalten. Phenacetylchlorid als Diacylierungsreagens versagt dagegen. Die sehr guten Erfolge, die F. Klages u. Mitarb.[13]) damit am Dibenzyl erzielten, lassen sich nicht auf das Diphenyl übertragen. Die Darstellung des gewünschten Dike-tons aus dem 4,4'-Dicyandiphenyl mit Hilfe von Benzylmagnesiumchlorid ist ein sehr verläßlicher Weg[14]). Die Oxydation mit SeO_2 führt zu Tetrake-tonen, die einen zweimaligen Ringschluß nach D. Davidson u. Mitarb.[15]) mit der Besetzung der 2- und 2'-Stellung der Diimidazole mit weiteren Arylresten erlauben.

[12]) L. M. Long u. H. R. Henze, J. Amer. chem. Soc. 63, 1939 (1941); J. D. Ssytsch, Ukr. chem. J. (russ.) 24, 93 (1958).

[13]) F. Klages u. Mitarb., Ber. dtsch. chem. Ges. 81, 400 (1948).

[14]) C. V. Ferris u. E. E. Turner, J. chem. Soc. London 117, 1150 (1920); Cl. de Milt u. M. Sartor, J. Amer. chem. Soc. 62, 1954 (1940).

[15]) D. Davidson u. Mitarb., J. org. Chem. 2, 319 (1938).

Unsere Versuche, ausgehend vom cis- oder trans-Dibenzoyläthylen über das Dibromid[16]) zu einem brückenlosen, in 4(5),4'(5')-Stellung unmittelbar verknüpften Diimidazol zu kommen, das in den beiden anderen Ringstellungen von Aromaten flankiert ist, waren bisher erfolglos. Das Dibromid ist auch gegenüber schwach basischen Reagenzien sehr empfindlich. Die äußerst schnell einsetzende doppelte HBr-Abspaltung führt intermediär zum Dibenzoylacetylen[16]), das dann nicht mehr zu überschenden Additionsreaktionen unterliegt.

In einer folgenden Mitteilung werden wir das Assoziationsverhalten der hier beschriebenen Diimidazole mit solchen vergleichen, die in denselben Ringstellungen durch Methylenbrücken verbunden sind. Die aromatische Überbrückung der Diimidazole stellt eine ziemlich starre Verbindung zwischen den beiden heterocyclischen Kernen dar. Der Bau solcher langgestreckten und auch flachen Moleküle scheint die Ausbildung größerer, ziemlich regelmäßig angeordneter Assoziate zu begünstigen.

Beschreibung der Versuche[17])

1,4-Diacetyl-benzol: Aus p-Äthyl-acetophenon durch Oxydation mit $KMnO_4$ in stark gepufferter Lösung[4]). Bei Ansätzen mit 100 g Keton wird es notwendig, die größeren Mengen Braunstein sehr sorgfältig mit heißem Alkohol zu extrahieren. Für die Oxydationszeit genügen 3 Stunden. Aus Alkohol derbe Prismen vom Schmp. 113—114°.

1,4-Bis-bromacetyl-benzol: Aus vorst. Keton durch Bromierung in Eisessig. Die angegebene Eisessigmenge ist zu verdoppeln, um das Auskristallisieren des Bromketons noch während der Bromierung zu verhindern. Alkohol ist zum Umkristallisieren größerer Mengen wenig geeignet. In Dioxan kommt es leicht zu Rotfärbungen, am besten wird Benzol oder Chlorbenzol verwendet. Schmelzverhalten: ab 110° bilden sich Nadeln, bei 173° treten starke Umlagerungserscheinungen auf. Wird bei 175° 0,5 Stunden getempert, schmilzt die Substanz von 181—182°. Lit.-Schmp. 173°[5]).

$C_{10}H_8Br_2O_2$ (320,01)   gef. C 37,74; H 2,64;
                               ber. C 37,53; H 2,52.

2,4-Dinitrophenylhydrazon: 2 g Diketon werden mit 1 g 2,4-Dinitrophenylhydrazin in 100 ml Dioxan kurz erhitzt und dann stehen gelassen. Es fällt zuerst ein sehr feiner roter Niederschlag aus, dann kristallisieren orangefarbene Nadelrosetten. Diese werden aus sehr wenig Dioxan umkristallisiert. Schmp. 236—237° (bei 228° Umlagerung in derbe Klötzchen).

$C_{16}H_{14}N_4O_5$ (342,29)   gef. N 16,82;
                                 ber. N 16,37.

Bis-2,4-dinitrophenylhydrazon: Aus dem Diketon mit überschüssigem 2,4-Dinitrophenylhydrazin in Dioxan. Das Bis-Derivat scheidet sich beim Abkühlen langsam als roter, feiner Niederschlag ab. Aus Dimethylformamid oder α-Chlornaphthalin zarte rote Nadeln, die sich ab 310° unter Schwarzfärbung zunehmend zersetzen.

$C_{22}H_{18}N_8O_8$ (522,41)   gef. N 20,72;
                                 ber. N 21,4.

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[16]) E. Lutz u. Mitarb., J. Amer. chem. Soc. 49, 1109 (1927).
[17]) Alle Schmp. korr.

1,4-Bis-acetoxyacetyl-benzol: 5,5 g Bromketon werden mit 5 g wasserfreiem K-Acetat in 125 ml Eisessig 1 Stunde erhitzt. Nach dem Abkühlen wird in 250 ml H$_2$O eingerührt. Der voluminöse gelbe Niederschlag wird abgesaugt und aus 50proz. Alkohol mit A-Kohle umkristallisiert. Große Schuppen vom Schmp. 143—144°. Ausbeute 90% d. Th.

C$_{14}$H$_{14}$O$_6$ (278,25)   gef. C 59,81; H 4,76;
ber. C 60,4; H 5,04.

1,4-Bis-hydroxyacetyl-benzol: 5 g vorst. Diacetat werden in 100 ml Alkohol + 10 ml konz. H$_2$SO$_4$ am Rückfluß erhitzt. Nach 0,5 Stunden beginnt die Abscheidung eines gelben Niederschlages. Nach dem Abkühlen wird abgesaugt und aus Dimethylformamid umkristallisiert. Das polymere Diketol färbt sich ab 180° gelb und zersetzt sich von 220—230°.

C$_{10}$H$_{10}$O$_4$ (194,18)   gef. C 61,35; H 5,35;
ber. C 61,9; H 5,16.

1,4-Bis-(N-phthalimidoacetyl)-benzol: 12 g Bromketon werden mit 29 g Phthalimidkalium in 100 ml Dimethylformamid unter Rühren 2 Stunden im Wasserbad erhitzt. Es tritt ein Farbumschlag von rot nach gelbbraun ein. Nach dem Abkühlen wird in 300 ml Wasser eingerührt, abgesaugt und aus Dimethylformamid umkristallisiert. Man erhält 8,3 g (49% d. Th.) vom Schmp. 342—343°.

C$_{26}$H$_{16}$N$_2$O$_6$ (452,4)   gef. C 70,0; H 3,43; N 6,28;
ber. C 69,2; H 3,56; N 6,2.

Urotropinkomplex: 32 g Bromketon werden mit 28 g Urotropin in 200 ml Chloroform bei Raumtemperatur 2 Tage gerührt. Der farblose Niederschlag wird abgesaugt. Ausbeute: 60 g (100% d. Th.).

1,4-Bis-aminoacetyl-benzol-dihydrochlorid: 60 g vorst. Verbindung werden mit 200 ml konz. HCl und 450 ml Methanol bei 40° 2 Tage gerührt. Der etwas schleimige Niederschlag wird abgesaugt und aus verd. HCl mit A-Kohle umkristallisiert. Die Mutterlauge des methanolischen Filtrats wird zur Trockne eingedampft, der Rückstand wie oben umkristallisiert. Man erhält insgesamt 23,5 g (88,7% d. Th.) farblose Blättchen vom Schmp. > 260°.

C$_{10}$H$_{12}$N$_2$O$_2$ · 2 HCl (265,11)   gef. C 45,0; H 5,41; N 10,55;
ber. C 45,25; H 5,28; N 10,57.

Nitron: 12 g vorst. Bromketon werden mit 30 ml Pyridin in das Dipyridiniumsalz übergeführt. Das aus Dioxan mit sehr wenig Wasser umkristallisierte Salz sintert ab 270° und verkohlt bei weiterer Temperaturerhöhung ganz langsam. Es wird in 75proz. Alkohol gelöst und bei 0° mit Nitroso-dimethylanilin umgesetzt. Nach eintägigem Stehen im Eisschrank wird in Wasser eingerührt. Nach zweimaligem Umkristallisieren aus Dioxan/Dimethylformamid erhält man rote, verfilzte Nadeln vom Schmp. 160—161°. Ausbeute: 40—60% d. Th. Lit.-Schmp. 132—134°[8]).

C$_{26}$H$_{26}$N$_4$O$_4$ (458,49)   gef. C 66,65; H 5,86; N 12,12;
ber. C 68,12; H 5,70; N 12,22.

1,4-Diglyoxyloyl-benzol-dihydrat: a) 20,5 g Diacetylbenzol werden mit 41 g SeO$_2$ in 200 ml Dioxan + 5 ml H$_2$O 5 Stunden im Rückfluß erhitzt. Die Lösung färbt sich sehr schnell gelb. Noch heiß wird vom Se abfiltriert, dann i. Vak. eingeengt. Der rote sirupöse Rückstand wird mit 50 ml heißem Wasser behandelt, wobei er sehr schnell kristallin wird. Die Verbindung ist nur sehr schwer in siedendem Wasser löslich. Eine so bereitete Ana-

lysenprobe und die aus verd. Dioxan umkristallisierte Verbindung schmelzen von 138 bis 158°. Lit.-Schmp. 110/111°[5]). Ausbeute: 60—70% d. Th.

b) Aus dem Pyridiniumsalz ohne Isolierung des Nitrons durch dessen saure Hydrolyse mit warmer verd. H_2SO_4[9]). Aus verd. Dioxan schwach gelb gefärbter Niederschlag vom Schmp. 130—150°. Ausbeute: 25% d. Th.

$C_{10}H_6O_4 \cdot 2H_2O$ (226,15)   gef. C 53,10; H 4,49;
ber. C 53,1; H 4,42.
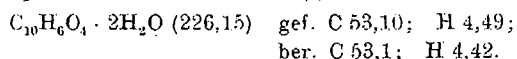
Di-chinoxalin: 1 g vorst. Verbindung wird mit 1,2 g o-Phenylendiamin in 10 ml Dioxan 1 Stunde erhitzt. Nach dem Abkühlen und Absaugen wird zweimal aus Dioxan umkristallisiert. Lange blaßgelbe Nadeln vom Schmp. 272,5—273,5°. Lit.-Schmp. 260—262°[8]).

$C_{22}H_{14}N_4$ (334,36) gef. N 16,7; ber. N 16,73.
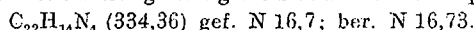
1,4-Di-[imidazolyl-4(5)]-benzol: a) 10 g Bromketon werden unter vorsichtigem Erwärmen in 100 ml Formamid gelöst und dann am Steigrohr 2,5 Stunden im schwachen Rückfluß erhitzt. Das Reaktionsgemisch färbt sich zunächst dunkelrot, hellt sich dann wieder auf und wird allmählich schwarzbraun. Beim Abkühlen fallen 7,5 g rotbrauner Niederschlag aus. Dieser wird in 300 ml verd. HCl unter Zusatz von A-Kohle 10 Minuten gekocht. Die freie Base wird mit NH_3 gefällt. Man erhält einen schwach gelb gefärbten Niederschlag, der sich schlecht absaugen läßt. Ausbeute: 4 g (60% d. Th.). Reinigung durch mehrmaliges Umkristallisieren aus viel Dimethylformamid. Das Diimidazol fällt in Form farbloser spitzer Nadeln oder Prismen an. Schmelzverhalten: ab 295° starke Sublimation zu sechseckigen Blättchen, die sich von 225—233° vollständig zersetzen.

b) 10 g Aminoketon werden in 100 ml Formamid wie vorstehend umgesetzt und aufgearbeitet. Die Ausbeute liegt bei 50—60% d. Th.

$C_{12}H_{10}N_4$ (210,24)   gef. C 67,87; H 4,91; N 26,08;
ber. C 68,55; H 4,80; N 26,65.
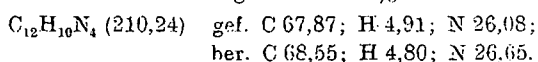
Dipikrat aus Alkohol/Dimethylformamid feiner gelber Niederschlag von ungleichmäßig ausgebildeten Kristallen, die sich ab 300° langsam zersetzen.

$C_{12}H_{10}N_4 \cdot 2 C_6H_3N_3O_7$ (668,47)   gef. N 20,68; ber. N 20,96.
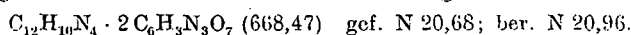
1,4-Dipropionyl-benzol: Aus p-n-Propyl-propiophenon durch Oxydation mit $KMnO_4$ in gepufferter Lösung[4]). Aus Alkohol lange farblose Nadeln vom Schmp. 103—104°. Ausbeute: 35—40% d. Th.

2,4-Dinitrophenylhydrazon: 1,9 g Keton und 2 g 2,4-Dinitrophenylhydrazin werden in Dioxan + 2 Tropfen HCl aufgekocht. Beim Abkühlen erfolgt langsam die Abscheidung eines rotgelben Niederschlages. Aus Alkohol feine orangefarbene Nadeln vom Schmp. 231—233°.
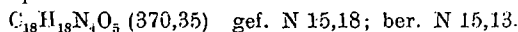
$C_{18}H_{18}N_4O_5$ (370,35) gef. N 15,18; ber. N 15,13.

Bis-2,4-dinitrophenylhydrazon: Aus dem Diketon mit überschüssigem 2,4-Dinitrophenylhydrazin in Dioxan. Aus Dimethylformamid rotgelbe Kristalle vom Schmp. 294—296° (u. Zers.).

$C_{24}H_{22}N_8O_8$ (550,47) gef. N 19,8; ber. N 20,36.
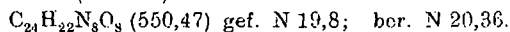
1,4-Bis-(α-brompropionyl)-benzol: 19 g vorst. Keton werden in 250 ml Eisessig mit 32 g Brom bei Zimmertemperatur unter Rühren bromiert. I. Vak. wird auf die Hälfte eingeengt, dann mit Wasser verdünnt. Aus Alkohol erhält man 30,6 g (87,7% d. Th.) Prismen oder Sägen (Stereoisomerengemisch) mit folgendem Schmelzverhalten: Bei 105° schmelzende Umlagerung, Hauptanteil von 124—127°, Rest bis 133°.

$C_{12}H_{12}Br_2O_2$ (348,06) gef. C 41,56; H 3,56;
ber. C 41,1; H 3,45.
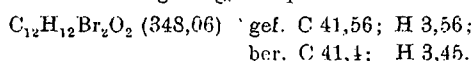
1,4-Bis-(α-acetoxypropionyl)-benzol: 5 g Bromketon werden mit 10 g wasserfreiem K-Acetat in 125 ml Eisessig 4 Stunden am Rückfluß erhitzt. Die KBr-Abscheidung beginnt erst nach 1,5 Stunden. Nach dem Abkühlen wird in Wasser eingerührt. Beim Stehen über Nacht im Eisschrank kristallisiert das ausgefallene Öl durch. Nach zweimaligem Umkristallisieren aus PAe. (40—60°) erhält man Nadeln vom Schmp. 137—138°. Ausbeute: 60—70% d. Th.

$C_{16}H_{18}O_6$ (306,3)   gef. C 62,5; H 5,90;
ber. C 62,7; H 5,84.

1,4-Bis-(x-N-phthalimidopropionyl)-benzol: 2 g vorst. Bromketon werden mit 3 g Phthalimid-K in 30 ml Dimethylformamid 1,5 Stunden im Wasserbad auf 90° unter Rühren erhitzt. Nach dem Abkühlen wird in Wasser eingerührt. Der voluminöse rosa gefärbte Niederschlag wird abgesaugt und gut getrocknet. Aus Chlorbenzol erhält man farblose verwachsene Rhomben vom Schmp. 222—238°.

$C_{28}H_{20}N_2O_6$ (480,45)   gef. C 69,45; H 4,0; N 5,77;
ber. C 70,1; H 4,17; N 5,83.

Di-pyridiniumsalz: 12 g Diketon werden mit 30 ml Pyridin umgesetzt[9]). Nach 5 Minuten steigt die Temperatur auf 75°. Hierbei hat sich alles gelöst. Dann beginnt die Abscheidung eines gelben Harzes, das beim Abkühlen zu einem spröden Kuchen erstarrt. Er wird zerrieben, abgesaugt und mit Äther gewaschen. Ausbeute: 16 g (91,6% d. Th.). Aus Alkohol kristallisieren farblose Nadeln vom Schmp. 195 bis 198° (u. Zers).

$C_{22}H_{20}Br_2N_2O_2$ (506,23)   gef. N 4,98;   ber. N 5,52.

Pyridiniumsalzzersetzung: 1 g Di-pyridiniumsalz wird in überschüssiger verd. Natronlauge gelöst. Das zunächst abgeschiedene Betain geht nach 1 Stunde wieder in Lösung. Auf Zugabe von HCl scheidet sich die Säure ab. Aus Dioxan umkristallisiert erhält man lange Nadeln, die bis 360° völlig sublimieren. Die Säure wird mit Diazomethan verestert. Der erhaltene Dimethylester der Terephthalsäure schmilzt nach dem Umkristallisieren aus Alkohol von 141—142°.

1,4-Bis-[4(5)-Methylimidazolyl-5(4)]-benzol: 10 g vorst. Bromketon werden in 100 ml Formamid 2,5 Stunden am Steigrohr erhitzt. Nach 20—30 Minuten fallen aus der orange gefärbten Lösung hellgelbe Kristalle aus. Reaktionsgemisch und Niederschlag färben sich bei weiterem Erhitzen dunkler. Man läßt erkalten und saugt ab. Das Filtrat wird in konz. NH_3 eingerührt. Nach längerem Stehen wird der graubraune Niederschlag der Rohbase abgesaugt und gemeinsam mit dem Niederschlag der Formamidlösung in 250 ml verd. HCl mit A-Kohle gereinigt. Man läßt in Eiskühlung das Hydrochlorid auskristallisieren, löst es nach dem Absaugen in heißer verd. HCl und setzt mit NH_3 das Diimidazol in Freiheit. Es wird abgesaugt und aus sehr viel heißem Dimethylformamid umkristallisiert. Ausbeute: 3 g (45% d. Th.) farblose, drusenförmig angeordnete Kristalle, die sich über 300° langsam zersetzen.

$C_{14}H_{14}N_4$ (238,33)   gef.: C 69,41; H 6,46; N 24,42;
ber.: C 70,3; H 5,87; N 23,45.

Dipikrat aus Alkohol/Dimethylformamid gelbe unregelmäßige Prismen, die sich bis 290° vollständig zersetzen.

$C_{14}H_{14}N_4 \cdot 2 C_6H_3N_3O_7$ (696,56)   gef. N 18,64;   ber. N 20,8.

Hydrochlorid aus heißer halbkonz. HCl farblose Nadeln vom Schmp. > 260°.

$C_{14}H_{14}N_4 \cdot 2 HCl$ (311,24) gef. N 18,2;   ber. N 18,1.

10*

4,4'-Diacetyl-diphenyl: 80 g Diphenyl werden in 250 ml CS$_2$ gelöst, dann in Portionen mit 480 g AlCl$_3$ versetzt. Unter Rühren werden 200 g Acetylchlorid langsam zugetropft. Nach beendeter Zugabe wird noch 3 Stunden auf dem Wasserbad unter Rühren erwärmt. Es wird wie üblich mit HCl-Eis zersetzt und aufgearbeitet. Das abgesaugte hellgelbe amorphe Keton wird zweimal als Dioxan umkristallisiert. Man erhält 145 g (71% d. Th.) farblose glänzende Blättchen vom Schmp. 195—196° [12]).

4,4'-Bis-bromacetyl-diphenyl: 23,8 g Keton werden in 250 ml Chloroform unter schwachem Erwärmen gelöst und dann mit 32 g Brom, gelöst in 50 ml Chloroform, unter Rühren bromiert. Gegen Ende der Bromierung fallen die ersten Anteile des Bromketons aus. Nach dem Stehen im Eisschrank über Nacht wird abgesaugt und mehrmals aus Chlorbenzol umkristallisiert. Man erhält 37,5 g (55% d. Th.) lange farblose Nadeln vom Schmp. 220 bis 222° [12]).

C$_{16}$H$_{12}$Br$_2$O$_2$ (396,10)   gef.: C 49,0; H 2,97;
                       ber.: C 48,52; H 3,05.

4,4'-Bis-acetoxyacetyl-diphenyl: Durch 1std. Erhitzen vorst. Bromketons mit wasserfreiem K-Acetat in Eisessig. Aus Chlorbenzol große farblose Schuppen vom Schmp. 201—202°. Ausbeute: 80—85% d. Th.

C$_{20}$H$_{18}$O$_6$ (354,35)   gef.: C 67,67; H 5,30;
                       ber.: C 67,8; H 5,10.

4,4'-Bis-hydroxyacetyl-diphenyl: Durch 2std. Erhitzen von 5 g vorst. Verbindung in 100 ml Alkohol + 5 ml konz. H$_2$SO$_4$. Beim Verdünnen mit Wasser fällt ein gelber, amorpher Niederschlag aus. Aus Dimethylformamid kristallisiert das schwach gelb gefärbte polymere Diketol vom Schmp. > 360°.

C$_{16}$H$_{14}$O$_4$ (270,27)   gef.: C 70,04; H 5,65;
                       ber.: C 71,2 ; H 5,18.

Di-pyridiniumsalz: 12 g vorst. Bromketon werden mit 30 ml Pyridin wie beschrieben [9]) in das Di-pyridiniumsalz übergeführt. Zur Reinigung wird aus Dioxan und einigen Tropfen Wasser umkristallisiert. Die Substanz hat keinen Schmp. Ausbeute: 80% d. Th.

C$_{26}$H$_{22}$Br$_2$N$_2$O$_2$ (554,27)   gef. N 4,95; ber. N 5,06.

Pyridiniumsalzzersetzung: Aus vorst. Verbindung wie oben beschrieben mit verd. Natronlauge. Verd. HCl fällt die 4,4'-Diphenyldicarbonsäure. Mit Diazomethan erhält man den Dimethylester, der aus Methanol umkristallisiert in feinen Nadeln vom Schmp. 217—218° anfällt.

Nitron: Aus vorst. Dipyridiniumsalz wie oben beschrieben. Der rote Niederschlag wird abgesaugt und mehrmals mit Dioxan ausgekocht. Die so gereinigte tiefrot gefärbte Verbindung schmilzt von 172—174° (u. Zers.).

C$_{32}$H$_{30}$N$_4$O$_4$ (534,58)   gef. C 70,2; H 5,76; N 9,80;
                       ber. C 71,9; H 5,61; N 10,44.

4,4'-Bis-glyoxyloyl-diphenyl-hydrat: a) 23,8 g vorst. Keton werden in 250 ml Dioxan + 5 ml H$_2$O mit 25 g SeO$_2$ 5 Stunden im Rückfluß erhitzt. Nach dem Abziehen des Lösungsmittels i. Vak. wird der zähe rote Rückstand mit heißem Wasser behandelt, wobei er sehr schnell durchkristallisiert. Nach dem Absaugen wird aus verd. Dioxan umkristallisiert. Man erhält 18 g eines fast farblosen Hydrats vom Schmp 136—145°. b) Das vorst. Pyridiniumsalz wird wie oben beschrieben zum Diglyoxal umgesetzt. Nach dem Umkristallisieren aus verd. Dioxan schwach gelb gefärbte Kristalle vom Schmp. 130—145°.

Ber. für ein Dihydrat $C_{16}H_{10}O_4 \cdot 2 H_2O$ (302,27)

gef.: C 61,4; H 4,03;

ber.: C 63,5 H 4,63.

Di-chinoxalin: 1 g Diglyoxal und 1 g o-Phenylendiamin werden in 20 ml Dioxan 1 Stunde erhitzt. Nach dem Abkühlen wird der Niederschlag abgesaugt und zweimal aus 75 ml Dioxan umkristallisiert. Man erhält feine blaßgelbe Nadeln vom Schmp. 266—268°.

$C_{28}H_{18}N_4$ (410,41)   gef.: C 81,64; H 4,48; N 13,5;

ber.: C 81,55; H 4,88; N 13,59.

Urotropinkomplex: 19,8 g Dibromid werden mit 14 g Urotropin in 200 ml Chloroform bei Raumtemperatur gerührt. Ausbeute: 40,9 g (93,5 d. Th.).[12]

4,4'-Bis-aminoacetyl-diphenyl-dihydrochlorid: 33 g vorst. Komplex werden bei 40° mit 45 ml konz. HCl und 225 ml Methanol 12 Stunden gerührt. Der Niederschlag (23 g) wird abgesaugt und mit Wasser gewaschen. Nach dem Umkristallisieren aus verd. HCl unter Zusatz von A-Kohle erhält man 12,6 g (49,3%, d. Th.) farblose Nadeln vom Schmp. > 360°.

$C_{16}H_{16}N_2O_2 \cdot 2 HCl$ (342,2)   gef.: C 56,85; H 5,37; N 7,63;

ber.: C 56,4; H 5,27; N 8,21.

4,4'-Di-[imidazolyl-4(5)]-diphenyl: a) 12 g vorst. Bromketon werden wie beschrieben in 100 ml Formamid umgesetzt. Während der Reaktion erfolgt ein Farbumschlag von dunkelrot über hellrot nach braun. Nach dem Abkühlen erstarrt das Reaktionsgemisch zu einem hellbraunen Brei. Nach dem Absaugen bleibt ein gallertartiger Niederschlag zurück. Er wird in heißer verd. Essigsäure gelöst und bis zur Entfärbung mit A-Kohle gekocht. Mit Ammoniak wird die freie Base gefällt. Die Aufarbeitung der Formamidmutterlauge ergibt nur geringe Mengen. Zur Reinigung wird mehrmals aus Alkohol unter Zusatz von A-Kohle umkristallisiert. Man erhält 4 g (46%, d. Th.) feine farblose, in Drusen angeordnete Nadeln, die über 300° sehr stark sublimieren und sich dann langsam zersetzen.

b) 12 g vorst. Aminoketon werden in 100 ml Formamid umgesetzt und wie vorst. aufgearbeitet. Ausbeute: 3,5 g (35% d. Th.).

$C_{18}H_{14}N_4$ (286,34)   gef.: C 74,84; H 5,51; N 18,91;

ber.: C 75,80; H 4,93; N 18,57.

Dipikrat aus Alkohol feine gelbe Kristalle, die sich ab 235° langsam zersetzen.

$C_{18}H_{14}N_4 \cdot 2 C_6H_3N_3O_7$ (744,56)   gef.: N 17,82;

ber.: N 18,81.

4,4'-Dipropionyl-diphenyl: Aus 80 g Diphenyl, 480 g $AlCl_3$, 235 g Propionylchlorid und 250 ml CS_2 wie oben beschrieben. Man erhält 118 g graugrünes Rohketon, das aus 800 ml Dioxan umkristallisiert wird. Ausbeute 86 g (62,5% d. Th.) kleine glänzende Blättchen vom Schmp. 171—174°.

$C_{18}H_{18}O_2$ (266,34)   gef.: C 81,3; H 7,03;

ber.: C 81,2; H 6,80.

4,4'-Bis-(α-brompropionyl)-diphenyl: 26,6 g vorst. Keton werden in 250 ml Chloroform bei 40° unter Rühren mit 32 g Brom bromiert. Es wird i. Vak. eingeengt, dann mit Eis gekühlt. Ausbeute: 39 g (92% d. Th.) farblose glänzende Nadeln. Die Substanz ist für die Weiterverarbeitung rein genug. Analysenprobe aus Eisessig lange farblose Nadeln vom Schmp. 174—179° (Stereoisomerengemisch).

$C_{18}H_{16}Br_2O_2$ (424,16)   gef.: C 50,67; H 4,08;

ber.: C 50,97; H 3,81.

4,4'-Bis-(α-acetoxypropionyl)-diphenyl: Aus vorst. Bromketon mit wasser-
freiem K-Acetat in Eisessig. Aus Alkohol glänzende Blättchen vom Schmp. 196—198°. Aus-
beute: 68% d. Th.

$C_{22}H_{22}O_6$ (382,42)   gef.: C 68,86; H 6,04;
                               ber.: C 69,10; H 5,00.

4,4'-Bis-(α-hydroxypropionyl)-diphenyl: 3 g vorst. Verbindung werden in
100 ml Alkohol + 5 ml konz. H_2SO_4 3 Stunden am Rückfluß erhitzt. Aus Alkohol 2,2 g
(76% d. Th.) kleine farblose Blättchen vom Schmp. 147—149°.

$C_{18}H_{18}O_4$ (290,34)   gef.: C 74,28; H 6,34;
                               ber.: C 74,47; H 6,11.

4,4'-Bis-[4(5)-methylimidazolyl-5(4)]-diphenyl: 12 g vorst. Bromketon werden
wie beschrieben mit 100 ml Formamid umgesetzt. Farbumschlag dabei von dunkel- über
hellrot nach gelb. Nach 30 Minuten beginnt die Abscheidung eines Niederschlages. Nach
dem Abkühlen wird abgesaugt. Ausbeute: 10 g (83,5% d. Th.) graubraunes Hydrobromid.
Durch Aufarbeitung des Filtrats erhält man noch 1 g Rohbase. Beide Niederschläge werden
in 300 ml siedender verd. HCl mit A-Kohle geklärt, dann mit NH_3 die Base gefällt. Zur Reini-
gung wird mehrmals aus viel Dimethylformamid umkristallisiert. Man erhält 7 g (68%
d. Th.) kräftige farblose Kristalle, die sich über 300° langsam zersetzen.

$C_{20}H_{18}N_4$ (314,40)   gef.: C 75,88; H 5,82; N 17,80;
                               ber.: C 76,41; H 5,77; N 17,82.

Dipikrat aus Alkohol/Dimethylformamid gelbe Kristalle, die sich ab 270° langsam zer-
setzen.

$C_{20}H_{18}N_4 \cdot 2 C_6H_3N_3O_7$ (772,62)   gef.: N 17,36;
                                       ber.: N 18,13.

1,4-Diphenacetyl-benzol: Zu einer Grignardlösung, bereitet aus 7 g Benzylchlorid,
1 g Mg und 50 ml abs. Äther, wird eine Lösung von 4 g Terephthalonitril in 600 ml abs.
Benzol in einem Zuge gegossen. Der rote gallertartige Niederschlag färbt sich ganz allmäh-
lich gelb. Nach diesem Farbumschlag wird der Äther abdestilliert und die benzolische Lö-
sung noch 5 Stunden auf dem Strahler erhitzt. Es wird vorsichtig mit 250 ml verd. H_2SO_4
versetzt und anschließend noch 1 Stunde erwärmt. Die gelbe Benzolschicht wird abgetrennt
und zur Trockne eingedampft. Nach zweimaligem Umkristallisieren aus Eisessig seiden-
glänzende Blättchen vom Schmp. 180—181°. Ausbeute: 40—50% d. Th.

$C_{22}H_{18}O_2$ (314,36)   gef.: C 83,5; H 5,8;
                               ber.: C 84,3; H 5,7.

Bis-2,4-dinitrophenylhydrazon: 50 mg Diketon werden mit 70 mg 2,4-Dinitro-
phenylhydrazin in 30 ml Alkohol + 2 Tropfen konz. HCl erhitzt. Nach dem Absaugen wird
der Niederschlag mit Alkohol ausgekocht, dann aus Dimethylformamid oder α-Chlor-
naphthalin umkristallisiert. Rote verfilzte Nadeln vom Schmp. 256—256,5°.

$C_{34}H_{26}N_8O_8$ (674,60)   gef.: N 16,9;   ber.: N 16,6.

1,4-Bis-phenylglyoxyloyl-benzol: Aus vorst. Diketon durch Oxydation mit
SeO_2 in Dioxan. Aus Alkohol lange gelbe Nadeln vom Schmp. 125—128°. Ausbeute: 80 bis
85% d. Th.[11]).

Di-chinoxalin: 0,1 g vorst. Verbindung werden mit 0,063 g o-Phenylendiamin in 100 ml Alkohol erhitzt. Nach 20 Min. scheiden sich lange farblose Nadeln ab. Nach 1 Stunde wird abgesaugt, dann aus Dioxan umkristallisiert. Nadeln vom Schmp. 268—268,5°.

$C_{34}H_{22}N_4$ (486,54)   gef.: N 11,2;   ber.: N 11,5.

1,4-Bis-(α-hydoxyphenacetyl)-benzol oder 1,4-Bis-hydroxyphenacyl-benzol: 3 g vorst. Tetraketon werden in 100 ml 80proz. Dioxan gelöst. Im Sieden werden im Verlauf von 7 Stunden 7 g Zn-Staub eingetragen. Es wird abgekühlt, abgesaugt und mit Wasser versetzt. Der Niederschlag wird zweimal aus sehr wenig Alkohol umkristallisiert. Man erhält 1,3 g (40% d. Th.) farbloses Pulver vom Schmp. 128—130°.

$C_{22}H_{18}O_4$ (346,36)   gef.: C 73,9; H 5,2;
                                              ber.: C 76,3; H 5,2.

1,4-Bis-(α-bromphenacetyl)-benzol: 2 g 1,4-Diphenacetyl-benzol werden in 100 ml heißem Eisessig gelöst und in der Hitze mit 2,1 g Brom unter Rühren bromiert. Beim Stehen über Nacht fällt das Bromketon in langen, glänzenden Nadeln aus. Aus Eisessig Schmp. 157—158° Ausbeute: 85% d. Th.

$C_{22}H_{16}Br_2O_2$ (472,16)   gef.: C 56,8; H 3,4;
                                              ber.: C 56,8; H 3,4.

.   1,4-Bis-[4(5)-phenylimidazolyl-5(4)]-benzol: a) 1 g vorst. Bromketon wird in 80 ml Formamid unter Erwärmen gelöst, dann wird am Steigrohr 2 Stunden im schwachen Rückfluß erhitzt. Die gelborange Lösung wird wie oben beschrieben mit verd. HCl aufgearbeitet. Aus verd. Alkohol erhält man feine Nadeln vom Schmp. 319—320°, die sich an der Luft sehr schnell rosa färben. Ausbeute: 25—30% d. Th.

b) 1 g vorst. Acyloin wird mit 20 ml Formamid umgesetzt. Nach beendeter Reaktionszeit wird in verd. NH₃ eingerührt und der Niederschlag aus verd. Alkohol umkristallisiert. Nadeln vom Schmp. 319—320°. Ausbeute: 35% d. Th.

$C_{24}H_{18}N_4$ (362,41)   gef.: C 78,1; H 5,2; N 15,1;
                                              ber.: C 79,6; H 5,0; N 15,4.

1,4-Bis-[2,4(5)-diphenylimidazolyl-5(4)]-benzol: 0,7 g vorst. Tetraketon werden mit 0,5 g Benzaldehyd und 3 g NH₄-Acetat in 10 ml Eisessig 2 Stunden am Rückfluß erhitzt[15]. Beim Einrühren in Wasser fällt ein braunes Harz aus, das sehr bald erstarrt. Es wird zerrieben und aus wenig Alkohol zweimal umkristallisiert. Man erhält blaßgelb gefärbte Nadeln vom Schmp. 338—340°, Ausbeute: 20% d. Th.

$C_{36}H_{26}N_4$ (514,59)   gef.: C 82,8; H 5,3; N 10,0;
                                              ber.: C 84,0; H 5,1; N 10,9.

4,4'-Diphenacetyl-diphenyl: Aus 4,4'-Dicyandiphenyl und Benzylmagnesiumbromid in Benzol. Aus Pyridin farblose Kristalle vom Schmp. 223—224°[14]).

Bis-2,4-dinitrophenylhydrazon: 50 mg vorst. Verbindung werden mit 51 mg 2,4-Dinitrophenylhydrazin umgesetzt. Aus Dimethylformamid oder α-Chlornaphthalin rubinrote Kristalle vom Schmp. 286—286,5°.

$C_{40}H_{30}N_8O_8$ (750,70)   gef.: N 14,3;
   ber.: N 14,9.

4,4'-Bis-phenylglyoxyloyl-diphenyl: 2 g vorst. Diketon werden in 80 ml Dioxan zum Sieden erhitzt. Im Verlauf von 10 Stunden werden in kleinen Portionen 4 g SeO₂ zugegeben. Nach üblicher Aufarbeitung wird zweimal aus viel Alkohol umkristallisiert.

Man erhält ein feinkristallines gelbes Pulver vom Schmp. 203—205° Ausbeute: 70—75% d. Th.

$C_{28}H_{18}O_4$ (418,42)   gef.: C 79,8; H 4,6;
                                       ber.: C 80,4; H 4,3.
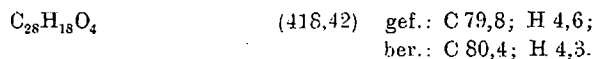
4,4′-Bis-(α-hydroxyphenacetyl)-diphenyl oder 4,4′-Bis-hydroxyphenacyl-diphenyl: 1 g vorst. Tetraketon wird wie oben beschrieben mit 6 g Zn-Staub in 80 ml siedendem Dioxan reduziert. Aus wenig Alkohol erhält man eine farblose feinkristalline Substanz vom Schmp. 130—135°. Ausbeute: 40% d. Th.

$C_{28}H_{22}O_4$ (422,45)   gef.: C 79,7; H 5,5;
                                       ber.: C 79,7; H 5,2.
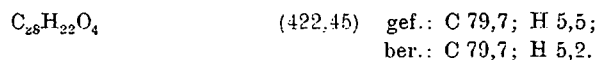
4,4′-Bis-(α-bromphenacetyl)-diphenyl: 1 g vorst. Diketon wird in 80 ml Eisessig in der Hitze gelöst und mit 0,9 g Brom unter Rühren bromiert. Nach Stehen über Nacht kristallisiert ein Teil des Bromketons aus, der Rest wird durch Einengen der Mutterlauge i. Vak. erhalten. Nach zweimaligem Umkristallisieren aus wenig Alkohol erhält man farblose feine Nadeln vom Schmp. 140—141°. Ausbeute: 60% d. Th.

$C_{28}H_{20}Br_2O_2$ (548,26)   gef.: C 61,6; H 3,9;
                                       ber.: C 61,4; H 3,6.
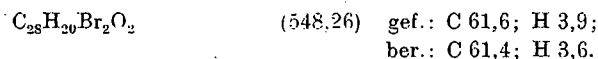
4,4′-Bis-[4(5)-phenylimidazolyl-5(4)]-diphenyl: a) 1 g vorst. Bromketon wird mit 100 ml Formamid wie oben umgesetzt. Aufarbeitung über das Hydrochlorid. Die Rohbase wird mehrmals aus Alkohol umkristallisiert. Man erhält das Diimidazol als blaßgelbe feinkristalline Substanz vom Schmp. 327—330° (u. Zers.). Ausbeute: 15% d. Th.

b) 1 g vorst. Acyloin wird in 25 ml Formamid umgesetzt. Die nach dem Einrühren in Wasser gewonnene Rohbase wird durch Umkristallisieren aus Alkohol gereinigt. Schmp. 328—330° (u. Zers.). Ausbeute 50—60% d. Th.

$C_{30}H_{22}N_4$ (438,50)   gef.: C 80,7; H 5,2; N 11,3;
                                       ber.: C 82,2; H 5,0; N 12,0.
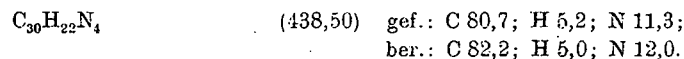
4,4′-Bis-[2,4(5)-diphenylimidazolyl-5(4)]-diphenyl: 0,7 g vorst. Tetraketon werden mit 0,8 g Benzaldehyd und 3 g NH₄-Acetat in 10 ml Eisessig 2 Stunden am Rückfluß erhitzt. Es wird heiß filtriert und in NH₃ eingerührt. Der abgesaugte gelbe Niederschlag wird gut getrocknet und zweimal aus Chlorbenzol umkristallisiert, aus dem das Diimidazol in großen farblosen Flocken ausfällt. Schmp. 343—345° (u. Zers). Ausbeute 20% d. Th.
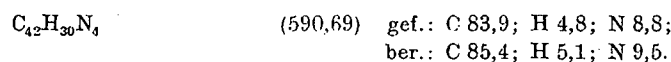
$C_{42}H_{30}N_4$ (590,69)   gef.: C 83,9; H 4,8; N 8,8;
                                       ber.: C 85,4; H 5,1; N 9,6.

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ONE POT SYNTHESIS AND CONFORMATION OF N-t-BUTYLOXYCARBONYL, O-PHENACYL DERIVATIVES OF PROLINE AND OTHER SECONDARY AMINO ACIDS.

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ABSTRACT

Proline, 4-hydroxyproline, azetidine-2-carboxylic acid, pipecolic acid and proline containing dipeptides were converted to N-t-butyloxycarbonyl, O-phenacyl derivatives in a one pot synthesis. ^{1}H- and ^{13}C-NMR spectroscopy of certain derivatives (Boc-Pro-PE, Boc-4Hyp-PE, Boc-Pro-4BrPE and Boc-Pip-PE) in CDCl$_3$ solution reveal the presence of cis-trans isomers in almost equal quantities. On the contrary, the ^{1}H- and ^{13}C-NMR spectra of the N-t-butyloxycarbonyl phenacyl ester of azetidine-2-carboxylic acid, show the presence of one isomer only. Similarly, only one urethane isomer was observed in solution for the protected C-terminal angiotensin II dipeptides, N-t-Boc-Pro-Phe-PE and N-t-Boc-Pro-Ile-PE. All phenacyl derivatives exhibited magnetic asymmetry attributed to steric factors.

We recently reported a method for the isolation of free amino acids from natural sources and their identification as N-t-butyloxycarbonyl (Boc) amino acid benzyl esters[1,2]. This is attained in a two step synthesis. In another report we have also described the isolation of free amino acids as N-t-Boc amino acid phenacyl esters (PE)[3].This method offers the advantage of usualy affording crystalline compounds with the potential of being studied by X-ray crystallography[4,5]. Also the ^{1}H-NMR spectra of these derivatives are characterized by an AB quartet with a unique $\Delta_\nu = \nu_2-\nu_1$ value for each amino acid,due to the magnetic nonequivalence of the O-methylene phenacyl protons. Interestingly, N-t-Boc-proline phenacyl ester displayed two distinct AB quartets,revealing a simple alternate way of detecting, cis-trans isomerism about the urethane amide bond. Furthermore, in our work concerning structure-activity studies of angiotensin II analogues, proline has been found to play an important role in the overall conformation assumed by the angiotensin II peptide molecule[6-9].Therefore we thought

it of interest to synthesize a number of model N-t-butyloxycarbonyl, O-
phenacyl derivatives of proline, proline homologs and proline containing
dipeptides and study the cis-trans isomerism as revealed by ^{1}H and ^{13}C - NMR
spectroscopy (Fig. 1).These derivatives were prepared in a one pot synthe-
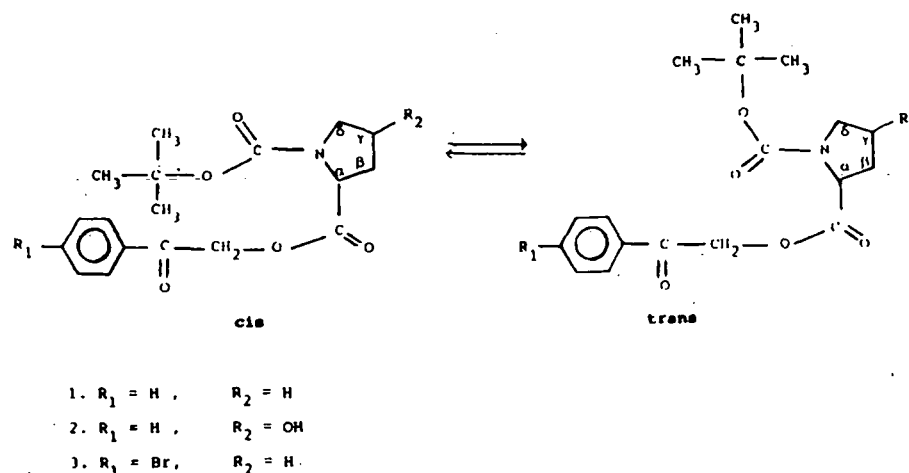sis as well as by the conventional two step derivatization.

1. R_1 = H , R_2 = H

2. R_1 = H , R_2 = OH

3. R_1 = Br, R_2 = H·

Figure 1.   Cis-trans isomerism about the urethane bond of N-t-Boc, O-phe-
            nacyl derivatives of Pro, 4Hyp.

RESULTS AND DISCUSSION

Protection of the imino group of secondary amino acids by the Boc
moiety has been achieved in good yields using t-butyl S-4, 6-dimethyl py-
rimid-2-yl thiocarbonate[10]. Subsequently,protection of the carboxyl group
was accomplished by esterification using benzyl bromide or phenacyl bro-
mide[11]. In the present study,proline and other secondary amino acids such
as 4-hydroxyproline, azetidine -2-carboxylic acid and pipecolic acid were
derivatized in a one pot synthesis using phenacyl bromide and di-tert-bu-
tyl dicarbonate which allows the clean and rapid introduction of the acid
labile Boc protecting group in amino acids, peptides and proteins[12]. One
pot amino- and carboxyl-protection of amino acids may prove to have far
reaching applications especially in the isolation of novel amino acids from
natural sources[3].

The complexity of ^{1}H-NMR spectra often hinders detection of cis-trans
isomers in proline derivatives.A reliable resonance for detecting cis-trans
isomers in the N-t-Boc amino acid derivatives is the tert-butyl group,ab-

breviated Boc.   On the other hand, the use of the phenacyl ester moiety ,
abbreviated PE, as C-terminal protecting group allows the easy crystalli-
zation of the synthesized derivatives   and provides a characteristic AB
quartet resonance for the phenacyl CH_2 methylene protons in a clean a-
rea (δ = 4.6 - 5.4 ppm)   of the NMR spectrum for distinguishing cis-trans
isomers[3-5] (Fig. 2).Thus compound 1 (Table I) in $CDCl_3$ showed an AB quar-
tet for one isomer centred at δ = 5.399 ppm with a coupling constant of
J=16 Hz and chemical shift difference Δ_ν = 0.375 ppm,while the other iso-
mer showed an AB quartet centred at δ=5.379 ppm (J=16 Hz)   but a   smaller
chemical shift difference (Δ_ν = 0.101 ppm).The presence of the two isomers
in almost equal amounts was also obvious from the two sharp singlets at
δ_1 = 4.464 and δ_2 = 1.442 ppm   attributed to the Boc groups, one for each
isomer. Similarly,compound 2 displayed an AB quartet centred at δ = 5.429
ppm (J=16 Hz, Δ_ν=0.323 ppm) for one isomer and another distinct AB quartet
centred at δ=5.395 ppm (J=16 Hz, Δ_ν=0.099 ppm) for the second isomer. Also
the Boc groups of the two isomers were identified as two sharp singlets
at δ_1 = 1.465 ppm and δ_2 = 1.442 ppm (Fig. 2B). Consequently, compound 3
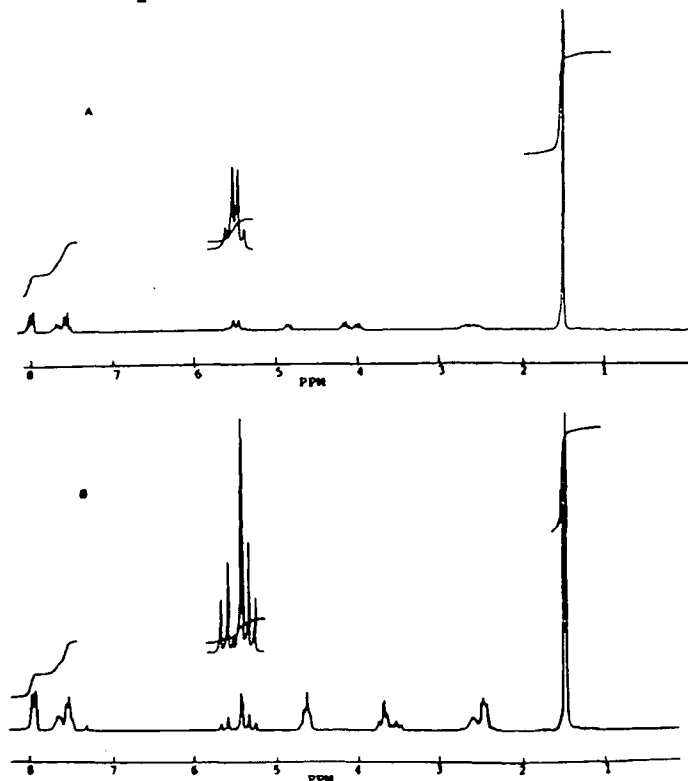
Figure 2. ^{1}H-NMR spectra of Boc-Aze-PE (A) and Boc-4Hyp-PE (B) in $CDCl_3$ +
            + TMS.

TABLE I.

Analytical ^{1}H NMR data for the AB quartet pattern of the phenacyl group distinguishing cis-trans isomers in N-t-Boc proline derivatives.[a]

Compound[b]	δ(ppm)	$\Delta_v = v_A - v_B$	v_1	v_2	v_3	v_4	J_{AB}	Boc
1. Boc-Pro-PE	5.399	0.375	5.170	5.252	5.545	5.627	16	1.464
	5.379	0.101	5.289	5.369	5.390	5.469	16	1.442
2. Boc-4HyP-PE	5.429	0.323	5.221	5.305	5.554	5.637	16	1.465
	5.395	0.099	5.304	5.388	5.403	5.487	16	1.442
3. Boc-Pro-4BrPE	5.347	0.358	5.127	5.209	5.485	5.567	16	1.464
	5.333	0.101	5.242	5.324	5.343	5.425	16	1.442
4. Boc-Pro-OBzl	5.181	0.177	5.062	5.121	5.236	5.298	16	1.464
	5.159	0.070	5.095	5.154	5.165	5.224	16	1.348
5. Trt-Pro-PE	5.345	0.269	5.170	5.251	5.438	5.520	16	–
6. Boc-Aze-PE	5.474	0.153	5.353	5.442	5.505	5.595	16	1.442
7. Boc-Pro-Phe-PE	5.423	0.189	5.288	5.371	5.477	5.559	16	1.408
8. Boc-Pro-Ile-PE	5.407	0.219	5.256	5.339	5.475	5.558	16	1.467
9. Boc-Pip-PE[c]	5.378	0.177	5.269	5.352	5.415	5.529	16	1.445
	5.411	0.065	5.350	5.420	5.415	5.485	16	1.445

[a] δ and v value decimals are given to the third decimal point as calculated by the 200 MHz NMR's computer. Two isomers were observed for compounds 1,2,3,4,9.

[b] Numbering of compounds in text as in Table I.

[c] Two AB quartets and one A_2 singlet were observed for Boc-Pip-PE. This may be due to accidental equivalence of the two Boc resonances of the two isomers.

TABLE II

^{13}C Chemical Shifts of proline derivatives[a,b]

Compound	Proline carbons					Protecting group carbons				
						Boc			Phenacyl	
	C_α	C_β	C_γ	C_δ	-COO-	C	CH_3	O-C=O	CH_2	CO
Boc-Pro-OH[c]	58.955	30.385	24.305	46.845	178.766	80.913	28.387	155.153	–	–
	58.955	29.129	23.656	46.354	176.424	80.400	28.247	153.997	–	–
Boc-Pro-PE	59.030	31.039	24.309	46.673	172.637	79.933	28.436	154.516	66.117	192.170
	58.918	30.093	23.557	46.374	172.465	79.814	28.341	153.829	65.919	195.733
Boc-Pro-4BrPE	59.009	31.039	24.325	46.677	172.622	79.998	28.442	154.526	65.952	191.349
	58.710	30.079	23.555	46.384	172.469	79.905	28.351	153.815	65.735	190.876
Boc-4Hyp-PE	57.945	39.240	69.951	54.754	172.586	80.489	28.376	154.676	66.231	192.273
	57.536	38.557	69.256	54.555	172.764	80.313	28.282	154.056	65.936	191.764
Boc-Aze-PE[d]	60.404	20.519	48.204	–	170.937	80.132	28.304	153.046	66.151	191.668

[a] δ values in ppm are given to the third decimal point as calculated by the 200 MHz NMR's computer. Assignment was also possible by DEPT experiments.

[b] CDCl$_3$ was used as solvent with TMS as internal reference.

[c] Literature chemical shifts, in DMSO-d$_6$ for this compound are slightly different[21].

[d] Assignment of the ring carbon resonances was based on previously reported ^{13}C NMR data for azetidine[22].

exhibited two AB patterns centred at δ_1=5.347 ppm (J=16 Hz, Δ_ν=0.358 ppm) and δ_2=5.333 ppm (J=16 Hz, Δ_ν=0.101 ppm) and two Boc singlets at δ_1=1.464 ppm and δ_2=1.442 ppm for the two isomers.

The nonequivalence of the CH$_2$ benzyl methylene protons in N-t-Boc a-mino acid benzyl esters, and N-t-trityl dipeptide benzyl esters, has been attributed to restricted rotations[13,14]. Accordingly, the chemical shift difference observed in the AB quartet of each proline isomer, largely re-flects the degree of free rotation of the phenacyl moeity in the phenacyl derivatives under scrutiny. We rationalized that the AB quartet in 1, 2 and 3 with the smaller chemical shift difference of the O-methylene phe-nacyl protons (Δ_ν=0.101 ppm, 0.099 ppm, 0.101 ppm respectively for 1, 2 and 3) corresponds to the trans isomer,while the AB quartet with the lar-ger chemical shift difference (Δ_ν=0.375 ppm, 0.323 ppm, 0.358 ppm) corre-sponds to the cis isomer. Rotations of the Boc moeity affect markedly the phenacyl group in the isomer assuming a cis urethane conformation. Exami-nation of molecular models indicate that the phenacyl moeity in the trans isomer is less hindered by the bulky Boc group than the phenacyl moeity in the cis isomer[14]. A trans urethane bond conformation would allow a ro-
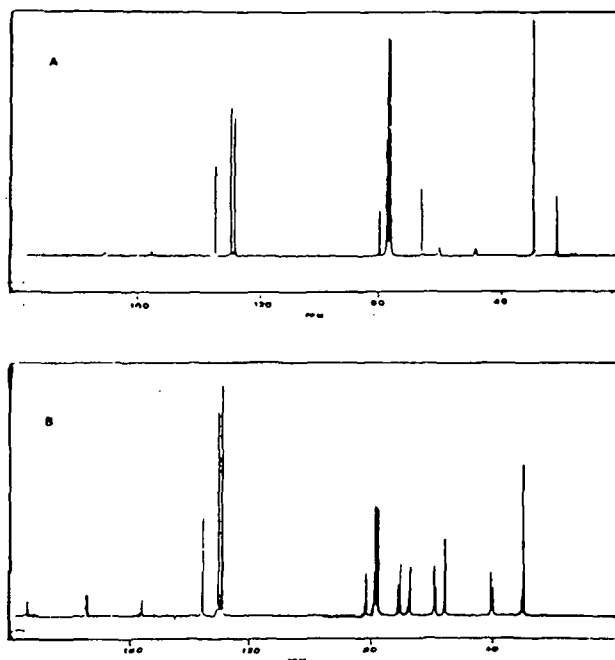
Figure 3. ^{13}C-NMR spectra of Boc-Aze-PE (A) and Boc-4Hyp-PE (B) in CDCl$_3$+ +TMS.

tational freedom for the two CH_2 phenacyl protons thus locating them in similar or perhaps identical environments. This would result in reduced or diminished chemical shift difference.

Similar considerations can be applied for N-t-Boc-Pro-OBzl (4). Two AB quartets were also observed for the cis and trans benzyl methylene protons (Table I). However, their dispersion was considerably smaller for the cis isomer (Δ_ν=0.177 ppm) and almost eliminated for the trans isomer (Δ_ν= =0.070 ppm), when compared with the dispersion of the phenacyl methylene protons (Table I).

Temperature studies in DMSO-d_6+D_2O indicated that restricted rotation was a major factor contributing to the nonequivalence of the CH_2 phenacyl protons and the spliting of the Boc resonance in phenacyl derivatives. At room temperature, both isomers of 1 showed slightly different chemical shift differences than observed in $CDCl_3$. Thus, one isomer showed Δ_ν=0.325 ppm, while the other showed Δ_ν=0.091 ppm, which could suggest a change in the population of several different conformers or a change in solvent effect on chemical shifts of the conformers. At 80° the chemical shift difference appeared to change substantially for the one isomer (Δ_ν=0.121 ppm from 0.325 ppm) (cis) but not for the other one (Δ_ν=0.089 ppm from 0.091 ppm) (trans). At 135° only one AB quartet for the phenacyl methylene protons (Δ_ν=0.070 ppm) and only one A_2 singlet for the Boc group were observed indicating a rapid cis-trans isomerization. At 160° the AB quartet was

TABLE III

[13]C Chemical shifts[a]

Boc-Pro-Ile-PE

Carbon	Pro	Ile	Boc, PE
α	61.106	59.615	-
β	30.919	37.718	-
γ	24.690	24.344	-
	-	15.445	-
δ	46.991	11.617	-
Boc(CH_3)	-	-	28.291
Boc(-C -)	-	-	80.428
PE(CH_2)	-	-	66.311

[a] Assignment of carbon resonances was based on previous ^{13}C-NMR data for Pro, Ile[16,17] and distortionless enhancement by polarization transfer (DEPT) experiments.

converted to an A_2 singlet and thus it seems reasonable to postulate that at high temperature the geminal protons become equivalent through free rotation in the molecule. These studies could suggest that the Boltzmann population of the two isomers become more equal at higher temperatures. Alternatively, the observed greater closing of the AB pattern in one of the two isomers could also suggest a steric effect of the bulky Boc group on the phenacyl CH_2 protons for this isomer, a fact which is only compatible with a cis urethane bond conformation.

Replacement of the Boc group in compound 1 by trityl, affords N-Trt-Pro-PE (5) which as expected exhibited only one AB quartet at lower field, $\delta=5.345$ ppm, suggesting a strong anisotropic effect of the trityl group on the phenacyl CH_2 methylene protons. The shielding effect suggests furthermore a close proximity of the phenacyl moeity with the N-trityl group in 5 indicating a stacking itneraction between the aromatic rings of the two groups[13].

The ^{1}H-NMR data of 1, 2 and 3 which confirm the presence of the two isomers in $CHCl_3$ solution are in agreement with the ^{13}C-NMR data. Indeed the ^{13}C-NMR of these derivatives show two resonances for each carbon (Table II). Previous studies have shown that the γ proline carbon resonances of the trans and cis conformation have distinct and non overlapping ranges of chemical shifts[15,16]. Thus the γ carbon resonance of the cis con - former in 1 was found at $\delta=23.557$ ppm and for the trans conformer at $\delta=24.309$ ppm. Similarly, the γ carbon resonance of the cis conformer in 3 was found at $\delta=23.555$ ppm and for the trans conformer at $\delta=24.325$ ppm . Replacement of the γ-methylene proton with hydroxyl, results in a downfield shift of the β, γ, δ proline ring carbons. The resonances of these carbons in compound 2 are in agreement with those of free 4-hydroxyproline[17] (Fig. 3B) (Table II). The cis and trans isomers of 1, 2 and 3 are present in almost equal amounts indicated by the same intensity resonances for each respective carbon (Fig. 1).

Interestingly, it was observed that both the ^{1}H- and ^{13}C-NMR spectra of the Boc phenacyl derivative of L-azetidine-2-carboxylic acid (6), a four membered ring proline homolog, reveal the presence of only one isomer. Thus the ^{1}H-NMR spectrum of 6 displays one distinct AB quartet for the O-methylene phenacyl protons centred at $\delta=5.474$ ppm with a chemical shift difference $\Delta_\nu=0.153$ ppm. Only one singlet was also observed at $\delta=1.445$ ppm for the Boc methyl protons (Fig. 2A). Similarly, the ^{13}C-NMR spectrum of 6 in $CHCl_3$, show only one resonance for each carbon in agreement with the presence of one isomer as suggested by the ^{1}H-NMR data (Fig. 3A). It is known that biologically active peptide analogues of angiotensin II (ANGII) , containing proline or sarcosine at position seven, exist in solution as a

mixture of conformers due to cis-trans isomerism about the X-Pro or X-Sar amide bond[8]. However, it is not yet known which conformer is the active one. Therefore, replacement of proline with azetidine in angiotensin II , and other active peptides could be of interest in terms of structure-activity relationship and could provide important information concerning the required configuration for potency. Preliminary experiments carried out by our group have shown that replacement of L-proline with L-azetidine-2--carboxylic acid in ANGII,ANGIII and [Sar[1]]ANGII produced extremely potent angiotensin II and III analogues.The biological activities of these analogues in relation to the His-Aze configuration will be published elsewhere.

Turning to the N-t-Boc, phenacyl derivatives 7 and 8 of Pro-Phe and Pro-Ile which constitute the C-terminal dipeptides for the angiotensin II super-agonist [Sar[1]]ANGII and antagonist [Sar[1], Ile[8]]ANGII, ^{1}H- and ^{13}C-NMR spectroscopy in $CDCl_3$ indicate the presence of only one urethane bond isomer.Thus the ^{1}H-NMR of 7 shows one AB quartet at δ=5.423 ppm and one Boc singlet at δ=1.408 ppm. Similarly,the spectrum of compound 8 displays one AB quartet at δ=5.407 ppm and one Boc singlet at δ=1.467 ppm. In a-greement,the ^{13}C-NMR shows a single resonance for each carbon (Table III).

EXPERIMENTAL

Capillary melting points were taken on a Buchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl Zeiss precision polarimeter (0.005°).A Varian XL 200 instrument was used to record ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$,using tetramethylsilane as internal standard. Infrared spectra were recorded with a Perkin Elmer 457 instrument. Analytical thin-layer chromatography (t.l.c.) was performed on Merck silica gel 60 F_{254} films (0.25 mm layer thickness) precoated on glass plates with the solvent systems; A, chloroform - methanol (9:1); B, hexane-ethyl acetate (6:4) and visualized by UV,iodine and ninhydrin (containing 3% trifluoroacetic acid in butanol).Elemental analyses were done by the Microanalytical Laboratory of the National Hellenic Research Foundation,and data fall within ±0.3% of the theory.Flash column chromatography was carried out on $CHCl_3$ packed silica gel column[16]. Free aminoacids (Pro, 4Hyp, Aze, Pip) and free dipeptides (Pro-Phe, Pro-Ile) were purchased from the Protein Research Foundation. Boc-Pro-OBzl and Trt-Pro-PE were prepared as previously described[1,11]. Boc-amino acid phenacyl esters 1, 2, 3, 6 and 9 and Boc-dipeptide phenacyl esters 7 and 8 were prepared from the corresponding free aminoacids or dipeptides according to the general proce -

dures for the N-t-Boc protection of amino group[10] and the phenacylation of the carboxyl group[11]. Protected dipeptides 7 and 8 were also synthesized by the mixed anhydride method,using Boc-Phe-PE and Boc-Ile-PE as starting materials. Treatment of these derivatives (0.1 mmol) with 50% solution of CF_3COOH in CH_2Cl_2 (6 ml) for 20 min at room temperature afforded the tri-fluoroacetate salts which precipitated with addition of ether (20 ml) and were collected by filtration. Boc-Pro-OH was condensed with phenylalanine phenacyl ester trifluoroacetate and isoleucine phenacyl ester trifluoro-acetate by the mixed anhydride method[19,20]. All derivatives were characte-rized by ^{1}H-, ^{13}C-NMR spectroscopy and elemental analyses. Distortionless enhancement by polarization transfer (DEPT) experiments were carried out to assign the ^{13}C chemical shifts of the proline derivatives.

TABLE IV.

Physical properties of N-t-Boc-amino acid and N-t-Boc dipeptide phenacyl esters.

Compound	Yield[a] %	Mp[b]	Crystallization[b] solvent	Rf_f[c] Rf$_A$	Rf$_B$	$[a]_D^{20}$[d]	Empirical[e] formula
Boc-Pro-PE	91	80-2°C	EtOAc/Petr.Ether	0.67	0.33	-93.73	$C_{18}H_{23}O_5N$
Boc-4Hyp-PE	87	121-2°C	EtOAc/Petr.Ether	0.43	0.07	-78.68	$C_{18}H_{23}O_6N$
Boc-Pro-4BrPE	55	80° C	Ether/Petr.Ether	0.60	0.33	-70.95	$C_{18}H_{22}O_5NBr$
Boc-Aze-PE	65	56-7°C	CHCl$_3$/Petr.Ether	0.65	0.3	-130.97	$C_{17}H_{21}O_5N$
Boc-Pro-Phe-PE	85	63-4°C	Ether/Petr.Ether	0.66	0.15	-72.66	$C_{27}H_{32}O_6N_2$
Boc-Pro-Ile-PE	82	100-1°C	EtOAC/Petr.Ether	0.68	0.19	-70.61	$C_{24}H_{34}O_6N_2$

[a]Yields for the phenacylation of N-t-Boc-aminoacids and N-t-Boc dipepti - des. Yields for the one pot synthesis varied between 35-45%. Yields of the two protected dipeptides (Pro-Phe, Pro-Ile) synthesized by the mixed anhydride method were 65% and 62% respectively.

[b]Boc-Pip-PE was crystallized from ethyl acetate/petroleum ether,m.p.=78°.

[c]Only single spots were detected for loads of at least 50 mg. Letters in-dicate solvent systems given in Experimental Section.

[d]Optical rotations were measured for 1% concentrations in CHCl$_3$.

[e]Elemental analyses:
(Found: C, 64.79; H, 6.93; N, 4.19. Calc. for $C_{18}H_{23}O_5N$: C, 64.87; H, 6.91; N, 4.20%).
(Found: C, 61.96; H, 6.57; N, 3.99. Calc. for $C_{18}H_{23}O_6N$: C, 61.89; H, 6.59; N, 4.01%).
(Found: C, 52.34; H, 5.32; N, 3.39. Calc. for $C_{18}H_{22}O_5NBr$: C,52.43; H, 5.34; N, 3.40%).
(Found: C, 63.87; H, 6.56; N, 4.40. Calc. for $C_{17}H_{21}O_5N$: C, 63.95; H, 6.58; N, 4.39%).
(Found: C, 67.35; H, 6.68; N, 5.81. Calc. for $C_{27}H_{32}O_6N_2$: C, 67.50; H, 6.67; N, 5.83%).
(Found: C, 64.43; H, 7.60; N, 6.26. Calc. for $C_{24}H_{34}O_6N_2$: C, 64.57; H, 7.62; N, 6.28%).

General procedure for a two step synthesis of N-t-Boc, O-phenacyl e-
sters of proline and secondary aminoacids. N-t-Boc proline phenacyl ester:
N-t-Boc-proline was firstly synthesized as previously reported using t-
butyl S-4,6-dimethyl pyrimid-2-yl thiocarbonate[10]. Subsequently, N-t-Boc-
proline (0.215 g, 1 mmol) was added to a solution of triethylamine (0.101
g, 1 mmol) in ethyl acetate (10 ml). Phenacyl bromide (0.199 g, 1 mmol)
was then added and the mixture was stirred at room temperature for 24 h.
The reaction mixture was then treated with a solution of 5% sodium bicar-
bonate (10 ml) followed by extraction with ethyl acetate (2X20 ml). The
ethyl acetate was washed with water (3X10 ml)and dried over anhydrous so-
dium sulfate; the solvent was removed in vacuo. The crude N-t-Boc-proline
phenacyl derivative was obtained in semi-crystallized form. Recrystalliza-
tion from ethyl acetate/pet.ether (8:2) afforded 0.305 g (91%); m.p. = 80
-82°C; $[a]_D^{25}$ = -93.73 (Cl, $CHCl_3$).

General procedure for a one pot synthesis of N-t-Boc, O-phenacyl esters
of proline and secondary amino acids. N-t-Boc-proline phenacyl ester. To
a stirred solution of proline (1.15 g, 10 mmol) and triethylamine (1.44
ml, 10 mmol) in water (5 ml), di-tert butyl dicarbonate (2.18 g, 10 mmol)
in dioxane (5 ml) was added. After three hours at room temperature, trie-
thylamine (1.44 ml, 10 mmol) was added and then a solution of phenacyl
bromide (2.00 g, 10 mmol) in dioxane (2 ml).The reaction mixture was kept
stirring at room temperature for 5 hours. Water (80 ml) and ethyl acetate
(80 ml) were then added. The ethyl acetate was then washed with 5% sodium
bicarbonate (80 ml), 5% hydrochloric acid (80 ml) and water (80 ml). The
solvent was then evaporated in vacuo.The remaining oily residue (2.030 g)
was found to be homogenious on t.l.c. and was crystallized easily from e-
thyl acetate/pentane, affording 0.960 g. A second crop of crystals (0.408
g) was obtained from the mother liquid (yield 42%).

N-t-Boc-proline-phenylalanine phenacyl ester. Mixed anhydride method[19,20].
To a chilled solution of N-t-Boc proline (1.6 mmol) in dry THF (3 ml), N-
methyl morpholine (1.6 mmol) and isobutyl-chlorocarbonate (1.6 mmol) were
added. The mixture was kept for 15 min at 0° and another 15 min at room
temperature and then mixed with 1 mmol phenylalanine phenacyl ester tri-
fluoroacetate and N-methyl morpholine (1 mmol) in THF (2 ml). After 3 h
at room temperature the solvent was evaporated in vacuo.The remaining re-
sidue was taken up with ethyl acetate (10 ml), washed with 5% $NaHCO_3$ (10
ml X 2),10% citric acid (10 ml) and water (10 ml) and dried over Na_2SO_4.
The solvent was evaporated under vacuum and the oily residue (0.330g) was
crystallized in the refrigerator.It was recrystallized from ethyl acetate
-hexane. Yields and physical properties are given in Table IV.

N-t-Boc-proline-isoleucine phenacyl ester. This compound was prepared from N-t-Boc proline (1.6 mmol) and isoleucine phenacyl ester trifluoro-acetate (1 mmol) in a similar manner to that described for N-t-Boc-proli-ne-phenylalanine phenacyl ester. The product was crystallized from ethyl acetate / hexane. Yields and physical properties are given in Table IV.

N-t-Boc-proline 4-bromophenacyl ester. This compound was prepared from N-t-Boc-proline (1 mmol) and 4-bromophenacyl bromide in a similar manner to that described for N-t-Boc-proline phenacyl ester. Yields and physical properties are given in Table IV.

Abbreviations: Standard abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature as found in Bioche-mistry 14, 449 (1975)., Biochem. J. 126, 773 (1972). All optically active amino acids used were of the L configuration. Other abbreviations used a-re: Pro, proline; 4Hyp, 4-hydroxyproline; 4BrPE, 4 bromo phenacyl; Aze, azetidine-2-carboxylic acid; Pip, pipecolic acid; Boc, t-butyloxycarbonyl; PE, phenacyl; PEBr, phenacyl bromide; Ile, isoleucine; Phe, phenylalanine; Trityl and Trt, triphenylmethyl; Bzl, benzyl; EtOAc, ethyl acetate; DCC , dicyclohexyl carbodiimide; HOBt, 1-hydroxybenzotriazole; t.l.c.; thin-la-yer chromatography; $CHCl_3$; chloroform; CF_3COOH, trifluoroacetic acid; Et_2O, diethyl ether; ANGII, angiotensin II.

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(12) **United States Patent**   (10) **Patent No.:** **US 7,312,343 B2**
Schmid et al.   (45) **Date of Patent:** **Dec. 25, 2007**

(54) **SYNTHESIS OF α-AMINO-β-ALKOXY-CARBOXYLIC ACID ESTERS**

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C07D 207/08 (2006.01)
C07C 211/27 (2006.01)

(52) **U.S. Cl.** 548/573; 564/384

(58) **Field of Classification Search** 564/384; 548/573
See application file for complete search history.

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(57)   **ABSTRACT**

Compounds of formula I

I

$$R^{4'}\underset{R^4}{\overset{R^1}{\underset{|}{N}}}\overset{}{\underset{O\diagdown R^3}{\overset{}{\diagup}}}\overset{OR^2}{\underset{O}{}}$$
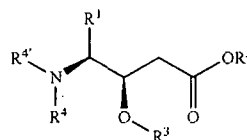
and a process for the preparation of such compounds are disclosed.

5 Claims, No Drawings

SYNTHESIS OF
α-AMINO-β-ALKOXY-CARBOXYLIC ACID ESTERS

FIELD OF THE INVENTION

The present invention relates to compounds of formula I
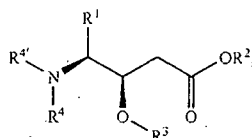
wherein R^1-R^4 are as herein defined, and to a process (Mukaiyama Aldol reaction) for the preparation of such compounds. Compounds of formula I are useful intermediates in the synthesis of other products, including, e.g. Dolastatin 10.

BACKGROUND OF THE INVENTION

Mukaiyama Aldol reactions are known. Dialkyl acetals are known to be substituted by Mukaiyama aldol reactions with silyl enol ethers or silyl ketene acetals affording β-alkoxy ketones or esters (K. Saigo, M. Osaki, T. Mukaiyama, Chem. Lett. 1976, 769-770; T. Mukaiyama, M. Murakami, Synthesis 1987, 1043-1054; S. Kano, T. Yokomatsu, H. Iwasawa, S. Shibuya, Chem. Lett. 1987, 1531-1534; M. A. Graham, A. H. Wadsworth, M. Thornten-Pett, B.Carrozzini, G. L. Cascarano, C. M. Rayner, Tetrahedron Lett. 2001, 42, 2865-2868; M. A. Graham, A. H. Wadsworth, M. Thornten-Pett, B.Carrozzini, G. L. Cascarano, C. M. Rayner, Org. Biomol. Chem. 2003, 1, 834-849).

Surprisingly, it has now been found that N-benzyl-protected dialkyl acetal derivatives undergo desired aldol reaction with high diastereoselectivity using a Mukaiyama aldol reaction.

SUMMARY OF THE INVENTION

In one embodiment, the present invention relates to a process of the preparation of compounds of formula I
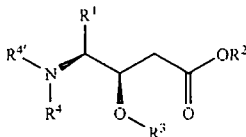
comprising reacting a compound of formula II
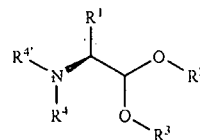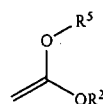
II

with a compound of formula III

III

in the presence of a Lewis acid and an organic solvent;
wherein

R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;

R^2 is C_{1-4} alkyl, benzyl, substituted benzyl or allyl;

R^3 is C_{1-4} alkyl or allyl;

R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;

$R^{4'}$ is benzyl or substituted benzyl; or alternatively

R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group; and

R^5 is a trialkylsilyl group.

In another embodiment, the invention relates to compounds of formula I as defined above.

In other embodiments, the invention relates to compounds of formula II as described above, and compounds of formula IV and VI as defined below.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms have the following meanings:

"C_{1-4} alkyl" denotes straight or branched chain hydrocarbon residues containing 1 to 4 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl or tert-butyl.

Preferably, C_{1-4} alkyl in R^1 is methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl (the R or S isomer), isobutyl or tert-butyl. More preferably, C_{1-4} alkyl in R^1 is methyl, ethyl, isopropyl or sec-butyl (the R or S isomer). Most preferably it denotes a (S)-sec-butyl group as depicted in Example 1-12.

C_{1-4} alkyl in R^2 is preferably methyl, ethyl or tert-butyl, most preferably it denotes a tert-butyl group.

C_{1-4} alkyl in R^3 is preferably methyl or ethyl; most preferably it denotes a methyl group.

C_{1-4} alkyl in R^4 is preferably methyl or ethyl; most preferably it denotes a methyl group.

The term "C_{3-6} cycloalkyl-C_{1-4} alkyl" as used herein denotes a C_{3-6} cycloalkyl (cyclopropyl, cyclobutyl, cyclo-

3

pentyl or cyclohexyl) linked to a C_{1-4} alkyl group as defined above. Preferably the term "C_{3-6} cycloalkyl-C_{1-4} alkyl" as used herein denotes a cyclohexylmethyl group.

The term "aryl-C_{1-4} alkyl" as used herein denotes a aryl group as defined below linked to a C_{1-4} alkyl group as defined above. Preferably the term "aryl-C_{1-4} alkyl" as used herein denotes a benzyl group.

The term "aryl" as used herein denotes an optionally substituted phenyl and naphthyl, both optionally benz-fused to an optionally substituted saturated, partially unsaturated or aromatic monocyclic, bicyclic or tricyclic heterocycle or carbocycle e.g. to cyclohexyl or cyclopentyl. Preferably the term "aryl" as used herein denotes a phenyl group.

The term "allyl" means a monovalent radical —C_3H_5 which contains a double bond.

The term "benzyl" means the monovalent aryl radical $PhCH_2$—.

The term "substituted benzyl" as used herein for the substituents R^2, R^4 and $R^{4'}$, denotes the following substituents attached to the benzyl group: 2,4,6-trimethyl, 3-methoxy, 4-methoxy, 2,4-dimethoxy, 3,4-dimethoxy, 3,5-dimethoxy, 2-nitro, 4-nitro, 2,4-dinitro, 4-bromo, 4-phenyl and 3,4-methylene-dioxy.

The term "trialkylsilyl group" as used herein denotes a $Si(C_{1-6}$ alkyl$)_3$, group wherein C_{1-6} alkyl denotes straight or branched chain hydrocarbon residues containing 1 to 6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, pentyl or hexyl. Preferably the term "trialkylsilyl group" denotes the following groups: dimethyl-tert-butyl-silyl, trimethyl-silyl or triethyl-silyl, most preferably it denotes a dimethyl-tert-butyl-silyl group.

The term "Lewis acid" as used herein denotes to BF_3, TMSOTf, $TiCl_4$ or the corresponding solvent complexes, such as $BF_3.OEt_2$ or $BF_3.DMF$, preferably $BF_3.DMF$.

The term "organic solvent" as used for the synthesis of compound of formula I denotes solvents such as dichloromethane MeCN, THF, DMF (N,N-dimethylformamide), $CHCl_3$, toluene or dichlorethane . Most preferably, dichloromethane may be used as the organic solvent.

The term "organic solvent" as used for the synthesis of a compound of formula II (reaction of the compound of formula IV and V) denotes alcohols, such as methanol, ethanol, propanol and butanol. In a preferred embodiment the alcohol depends on the substituent R^3. If, for example, R^3 is methyl the solvent is methanol.

The term "organic solvent" as used for the synthesis of the compound of formula II (reaction of the compound of formula VI and benzaldehyde) denotes a chlorinated solvent, such as dichloromethane, trichlormethane or dichlorethane. Most preferably, dichloromethane may be used as organic solvent.

The term "acid" as used herein denotes HCl, HBr, H_2SO_4, CF_3SO_3H or p-Toluenesulfonic acid. Most preferably the term "acid" denotes H_2SO_4 or HCl.

The term "mineral acid" as used herein means HCl, HBr, H_2SO_4 or CF_3SO_3H, preferably HCl or H_2SO_4.

The term "reducing agent" as used herein denotes $NaHB(OAc)_3$ or $NaBH_4$. Most preferably the term "reducing agent" denotes $NaHB(OAc)_3$.

The term "base" as used herein denotes typical N-containing organic bases, such as Me_3N, Et_3N, or pyridine. Most preferably, the term "base" denotes to Et_3N.

In an embodiment of the invention, a compound of formula I

4
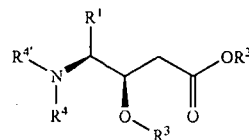
is synthesized by reacting a compound of formula II
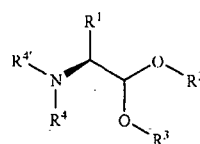
with a compound of formula III
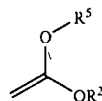
in the presence of a Lewis acid and an organic solvent;

wherein R^1-R^5 are as defined above.

The synthesis of the compound of formula I is carried out at a temperature range of -40° C. to 70° C., preferably at a temperature range of -20° C. to 30° C., and most preferably at a temperature range of -10° C. to 10° C.

In a preferred embodiment of the invention the process for the preparation of the compound of formula II comprises the reaction of a compound of formula IV
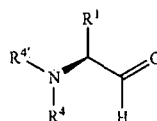
wherein R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;

R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;

$R^{4'}$ is benzyl or substituted benzyl, or

R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group;
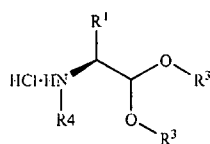
with a compound of formula V

HC(OR³)₃

V

wherein R^3 is C_{1-4} alkyl or allyl;

in the presence of an organic solvent and an acid.

In another embodiment, a compound of formula II may be obtained by contacting a compound of formula VI

wherein R^1 and R^3 are as defined above;

with benzaldehyde or substituted benzaldehyde, a reducing agent, a base and an organic solvent.

Preferably, benzaldehyde is used.

The term "substituted benzaldehyde" as used herein for the synthesis of compounds of formula IV (from compounds of formula VI), denotes to the following substituents attached to the phenyl group of the substituted benzaldehyde: 2,4,6-trimethyl, 3-methoxy, 4-methoxy, 2,4-dimethoxy, 3,4-dimethoxy, 3,5-dimethoxy, 2-nitro, 4-nitro, 2,4-dinitro, 4-bromo, 4-phenyl and 3,4-methylene-dioxy.

The substituted benzaldehyde is either commercially available or may be alternatively synthesized according to methods known from textbooks on organic chemistry (e.g. J. March (1992), "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 4th ed. John Wiley & Sons).

In a further preferred embodiment of the invention the process is carried out wherein

R^1 is methyl, ethyl, isopropyl or sec-butyl;

R^2 is methyl, ethyl or tert-butyl;

R^3 is methyl or ethyl;

R^4 is C_{1-4} alkyl, allyl or benzyl;

$R^{4'}$ is benzyl;

R^5 is dimethyl-tert-butyl-silyl, trimethyl-silyl or triethyl-silyl; and

the Lewis acid is BF_3, TMSOTf, $TiCl_4$, $BF_3.OEt_2$ or $BF_3.DMF$.

In a further preferred embodiment of the invention the process is carried out wherein

R^1 is (S)-sec-butyl;

R^2 is tert-butyl;

R^3 is methyl;

R^4 is methyl;

$R^{4'}$ is benzyl;

R^5 is dimethyl-tert-butyl-silyl; and

the Lewis acid is $BF_3.DMF$.

The compounds of formula I are new and therefore form part of the invention. Preferred are compounds of formula I
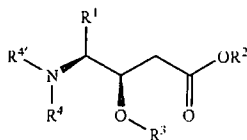
wherein

R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;

R^2 is C_{1-4} alkyl, benzyl, substituted benzyl or allyl;

R^3 is C_{1-4} alkyl or allyl;

R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;

$R^{4'}$ is benzyl or substituted benzyl; or alternatively,

R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group.

Also preferred are compounds of formula I wherein

R^1 is methyl, ethyl, isopropyl, sec-butyl, cyclohexylmethyl, and benzyl

R^2 is methyl, ethyl or tert-butyl;

R^3 is methyl or ethyl;

R^4 is methyl, ethyl, allyl or benzyl;

$R^{4'}$ is benzyl.

A further preferred embodiment of the invention is a compound of formula I wherein

R^1 is (S)-sec-butyl;

R^2 is tert-butyl;

R^3 is methyl;

R^4 is methyl;

$R^{4'}$ is benzyl.

The compounds of formula II are also novel and therefore form part of the invention. Preferred are compounds of formula II
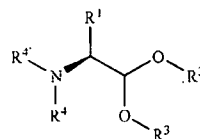
wherein

R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;

R^3 is C_{1-4} alkyl or allyl;

R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;

$R^{4'}$ is benzyl or substituted benzyl; or alternatively,

R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group.

A further preferred embodiment of the invention are compounds of formula II wherein

R^1 is methyl, ethyl, isopropyl or sec-butyl;

R^3 is methyl or ethyl;

R^4 is methyl, ethyl, allyl or benzyl; and

$R^{4'}$ is benzyl.

A further preferred embodiment of the invention are compounds of formula II wherein

R^1 is (S)-sec-butyl;

R^3 is methyl;

R^4 is methyl; and

$R^{4'}$ is benzyl.

The compounds of formula IV are new and therefore also form part of the invention. Preferred are compounds of formula IV
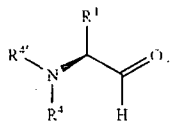
IV

wherein

R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;

R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;

$R^{4'}$ is benzyl or substituted benzyl, or alternatively,

R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group.

A further preferred embodiment of the invention are compounds of formula IV wherein

R^1 is methyl, ethyl, isopropyl or sec-butyl;

R^4 is methyl, ethyl, allyl or benzyl; and

$R^{4'}$ is benzyl.

A further preferred embodiment of the invention are compounds of formula IV wherein

R^1 is (S)-sec-butyl;

R^4 is methyl; and

$R^{4'}$ is benzyl.

General synthesis of compounds of formula IV, wherein

R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;

R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;

$R^{4'}$ is benzyl or substituted benzyl; or alternatively,

R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group, is as follows:
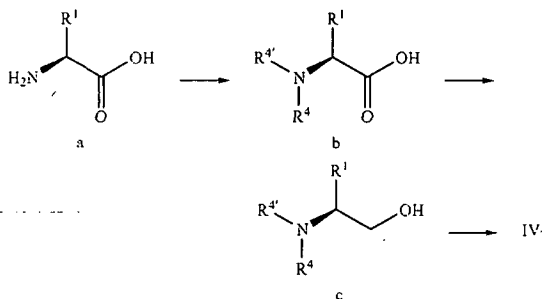
The starting material compound of formula a, wherein R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl, is either commercially available or can be synthesized according to methods well known to the skilled artisan and as for example disclosed in textbooks on organic chemistry (e.g. J. March (1992), "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 4th ed. John Wiley & Sons).

Compounds of formula VI are also new and are an embodiment of the invention.

General synthesis of compounds of formula VI, wherein

R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl; and

R^3 is C_{1-4} alkyl or allyl; is as follows:
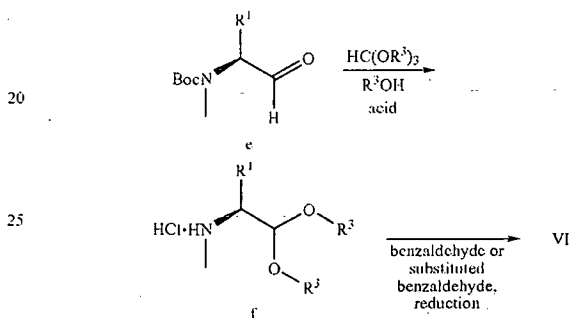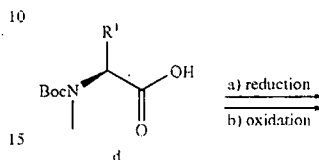
The starting material compound of formula d, wherein R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl, is either commercially available or can be synthesized according to methods well known to the skilled artisan and as for example described in textbooks on organic chemistry (e.g. J. March (1992), "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 4th ed. John Wiley & Sons).

Compound of formula e is reacted with a compound of formula V, wherein R^3 is C_{1-4}-alkyl or allyl, to obtain a compound of formula f (see also experimental part; example 3).

The compounds of formula I are important building blocks for the production of useful products in the chemical, agricultural and in the pharmaceutical industry. In particular they are useful for the production of anticancer substances as for example Dolastatin 10 or derivatives thereof as for example described in G. R. Pettit, "The Dolastatins", Progress in the Chemistry of Organic Natural Compounds, Springer, Vienna 1997, Vol. 70, 1-79, or in WO 03/008378.

Therefore, another embodiment of the present invention is the process as described above, wherein a compound of formula I is further reacted to give a compound of formula A

A
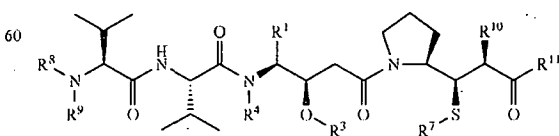
as follows:

a) the benzyl or substituted benzyl group of R^{4} in formula
I is cleaved in the presence of hydrochloric acid, hydrogen
and a palladium catalyst to give a compound of formula I-A
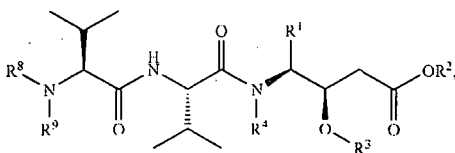
I-A
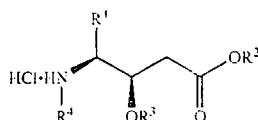
b) said compound of formula I-A is further reacted with an
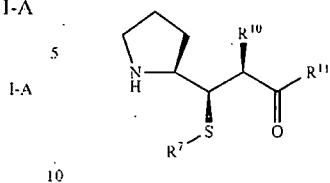N-protected valine derivative to give, after N-deprotection,
a compound of formula B;

B
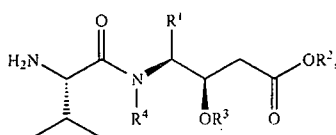
c) said compound of formula B is further reacted with a
compound of formula C

C
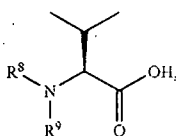
to give a compound of formula D

D

d) the compound of formula D is further reacted with a
compound of formula E

E

to give a compound of formula A; wherein

R^{1}, R^{2}, R^{3} and R^{4} are as defined herein before;

R^{7}, R^{8}, R^{9} and R^{10} independently from each other represent
alkyl; and

R^{11} is phenylalkyl-, or phenyldialkylamino or phenylalky-
loxy, having $(C_{1}-C_{4})$-alkylene and wherein the phenyl group
optionally may be substituted with one, two or three sub-
stituents selected from the group consisting of halogen,
alkoxycarbonyl, sulfamoyl, alkylcarbonyloxy, carbamoy-
loxy, cyano, mono- or di-alkylamino, alkyl, alkoxy, phenyl,
phenoxy, trifluoromethyl, trifluoromethoxy, alkylthio,
hydroxy, alkylcarbonylamino, 1,3-dioxolyl, 1,4-dioxolyl,
amino and benzyl.

In a preferred embodiment according to the present inven-
tion, the N-protected valine derivative in step b) of the
process as described above is the compound of formula F,

F
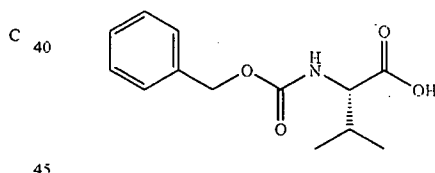
Reactions to deprotect N-protected amino acids, as for
example valine and/or derivatives thereof as mentioned
above are well known to the skilled artisan. According to the
present invention, said deprotection of the N-protected
valine derivative according to step b) of the process
described above is preferably carried out by hydrogenolysis.

Still another embodiment of the present invention is the
process as described above, wherein

R^{1} is sec-butyl;

R^{2} is tert-butyl;

R^{3}, R^{4}, R^{7}, R^{8}, R^{9}, and R^{10} are methyl; and

R^{11} is 2-(3-hydroxyphenyl) ethyl-methyl amino.

Still another embodiment of the present invention is the
process as described above for the manufacture of the
compound of formula A-1
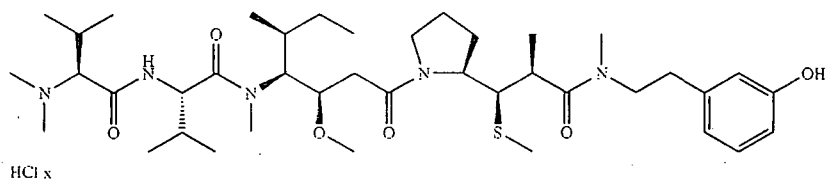
11 12

A-1

HCl x

Yet another embodiment of the present invention is the use of the process according to the present invention in the manufacture of the compounds of formula A as defined above.

Yet another embodiment of the present invention is the use of the process according to the present invention in the manufacture of the compound of formula A-1 as defined above.

In the following examples the abbreviations used have the following significations.
NMR nuclear magnetic resonance spectroscopy
IR infra red spectroscopy
HV high vacuum
min minute(s)
h hour(s)
RT room temperature
Me methyl
Et ethyl

EXAMPLE 1

(1S,2S)-(1-Hydroxymethyl-2-methyl-butyl)-methyl-carbamic Acid tert-butyl Ester
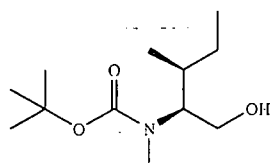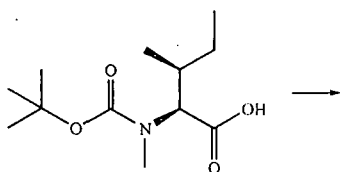
39.3 g Boc-MeIle-OH (0.160 mol; Synthetech) were dissolved in 160 ml THF and cooled to 0° C. 240 ml 1M BH₃-THF (0.24 mol; Fluka) were added at 0° C. over 1 h and the clear, colorless reaction mixture was warmed up and stirred at RT for 1 h. The reaction mixture was again cooled

to 0° C., 100 ml deionized water were carefully added at 0-5° C. over 0.5 h and after warming up to RT stirring was continued for 1 h. To the colorless solution were added 250 ml 10% Na₂CO₃ all at once and after stirring for 1 h the reaction mixture was extracted with 1000 ml and 500 ml ethyl acetate. The organic layers were washed with brine and dried (Na₂SO₄). Removal of the solvent by rotary evaporation gave 36.9 g (99.7%) product as colorless oil.

EXAMPLE 2

(1S,2S)-(1-Formyl-2-methyl-butyl)-methyl-carbamic Acid tert-butyl Ester
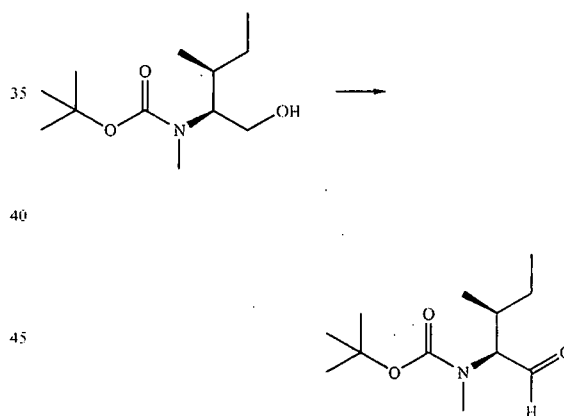
To a solution of 37.0 g Boc-N-methyl-isoleucinol (160 mmol) in 160 ml dichloromethane was added a solution of 5.4 g NaHCO₃ (64 mmol) and 1.9 g KBr (16 mmol) in 160-ml deionized water. The reaction mixture was cooled to 0° C. and after the addition of 125 mg 2,2,6,6-tetramethyl-piperidin-1-oxyl (TEMPO, 0.8 mmol), 122.6 g 10.2% aqueous sodium hypochlorite (176 mmol Cl₂) were added under stirring over 2.5 h at 0-5°. After additional stirring for 30 min the excess of NaOCl was destroyed by the addition of ca. 1 ml 38% aqueous sodium bisulfite and the reaction mixture was warmed up to 20°. The aqueous layer was extracted with 160 ml dichloromethane and the organic layers were washed with 10% brine and dried (Na₂SO₄). Removal of the solvent by rotary evaporation afforded 35.7 g (97.2%) crude product as a light orange oil.

EXAMPLE 3

(1S,2S)-(1-Dimethoxymethyl-2-methyl-butyl)-methyl-amine hydrochloride
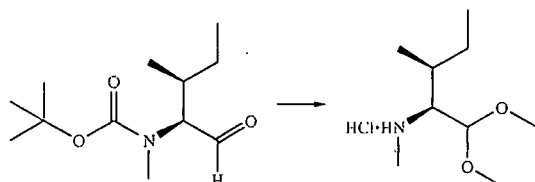
35.6 g Crude aldehyde (160 mmol) were dissolved in 200 ml methanol and cooled to ~15° C. 111 ml 2.8M HCl-MeOH (0.31 mol HCl) were added all at once and the yellowish solution was stirred at RT for 2 h. 155 ml Trimethyl orthoformate (1.42 mol; Fluka) were now added and the reaction mixture was stirred at RT over night (18 h). The solvent and the excess of the orthoester were removed by rotary evaporation (40° C./≧10 mbar) and the resulting beige, crystalline residue (33.7 g) was dissolved in ca. 310 ml isopropyl acetate at ~70° C. After cooling to RT and crystallization at 0° C. for 17 h the crystal suspension was filtered and dried (50° C./10 mbar/16 h) affording 29.6 g product as white needles, mp. 127-128° C. ¹H-NMR:

EXAMPLE 4

(1S,2S)-Benzyl-(1-dimethoxymethyl-2-methyl-butyl)-methyl-amine
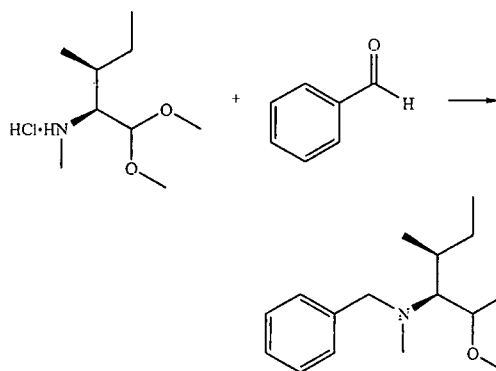
To a solution of 31.76 g the above described hydrochloride (150 mmol) in 600 ml dichloromethane were added 15.94 g triethylamine (157.5 mmol) and 17.51 g benzaldehyde (165 mmol) and the clear light orange solution was stirred at RT for 1 h. 40.16 g sodium triacetoxyborohydride (180 mmol; Aldrich) were added under ice cooling and the white suspension was stirred at RT for 24 h. The reaction mixture was washed with 600 ml 10% Na₂CO₃ and twice with 300 ml 10% brine. All three aqueous layers were extracted sequentially with 300 ml dichloromethane and the combined organic layers were dried over Na₂SO₄. Filtration and removal of the solvent by rotary evaporation (45° C./≧10 mbar) gave 41.3 g orange oily residue. Purification

by vacuum distillation gave 38.4 g (96.5%) product as colorless oil. b.p. 85-87° C./0.05 mbar. ¹H-NMR:

EXAMPLE 5

N-Benzyl-L-isoleucine (Bn-Ile-OH)
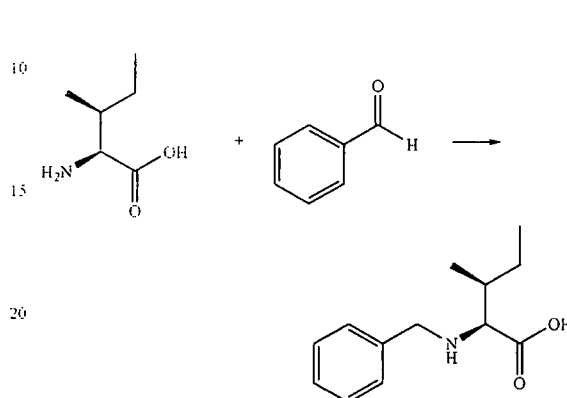
65.6 g L-Isoleucine (500 mmol; Senn Chemicals) were added in portions to 250 ml 2N NaOH (500 mmol) under stirring. After complete dissolution of the amino acid 53.1 g benzaldehyde (500 mmol) were added all at once and stirring was continued at RT for 0.5 h. 5.7 g Sodium borohydride (150 mmol) were now added under stirring in five portions at 5-15° C. and stirring at RT was continued for 3.5 h. The reaction mixture was diluted with 250 ml deionized water and extracted twice with 250 ml diethyl ether. The clear aqueous layer (pH ~14) was now slowly neutralized under vigorous stirring with ca. 400 ml 2N HCl to pH=7 and the white, viscous suspension was stirred at RT for 0.5 h. After filtration and washing with deionized water (2×250 ml), the filter cake was dried (70 h and 70° C./10 mbar/20 h) to yield 103.2 g (93.2%) product as a white powder.

EXAMPLE 6
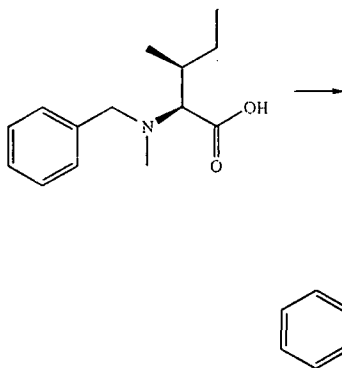
N-Benzyl-N-methyl-L-isoleucine (Bn-MeIle-OH)
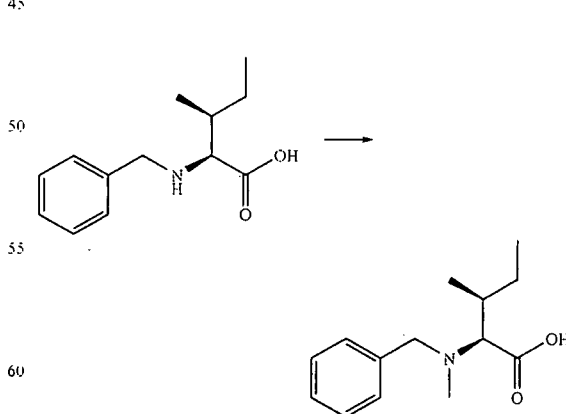
To 110.7 g of above described N-benzyl-isoleucine (0.50 mol) were added 69.0 g formic acid (1.50 mol) and 49.4 g formaldehyde 36.5% in water (0.60 mol) and the clear, colorless reaction mixture was heated under stirring to ~90°

C. for 2 h. The reaction mixture was concentrated by rotary evaporation (60° C./25 mbar) and the residue was triturated under stirring (~15 min) with 250 ml acetone. After evaporation of the solvent, triturating was repeated twice using a total of 500 ml acetone. Evaporation of the solvent (50° C./10 mbar) gave 129.9 g white, crystalline residue which was stirred with 200 ml acetone for 1 h at RT and 3 h at –20° C. The crystal suspension was filtered, washed with cold acetone and dried (16 h/50° C./10 mbar) affording 103.9 g (88.3%) product as a white, crystalline powder.

EXAMPLE 7

N-Benyl-N-methyl-L-isoleucinol

To a grey suspension of 22.8 g lithium aluminum hydride (0.60 mol) in 600 ml tetrahydrofuran were added at 0-10C. 94.1 g N-benzyl-N-methyl-L-isoleucine (0.40 mol) in 6 portions over 20 min. The ice-methanol bath was removed and the reaction mixture was heated up and refluxed for 1.5 h. The reaction mixture was again cooled to 0° C., diluted with 450 ml diethyl ether and then hydrolyzed by the slow addition of 23 ml deionized water. After the addition of 23 ml 15% aqueous NaOH at 0° C. a thick grey suspension was formed. 70 ml Deionized water were added over 0.5 h and the color of the suspension turned from grey to white. Stirring was continued at 0° C. for 15 min and at RT for 0.5 h. The white precipitate was filtered off, washed with 270 ml diethyl ether and the filtrate was evaporated (35° C./≧10 mbar) affording 85.1 g (96.1%) of the product as colorless oil.

EXAMPLE 8

(2S,3S)-2-(Benzyl-methyl-amino)-3-methyl-pentanal
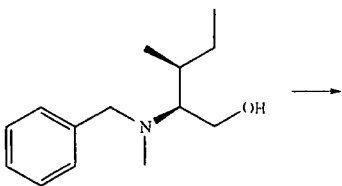

-continued
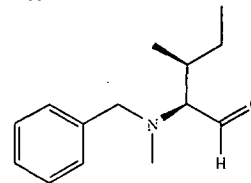
To a stirred solution of 36 ml oxalyl chloride (0.42 mol; Fluka) in 1100 ml dichloromethane were added at –70° C. a solution of 44 ml dimethyl sulfoxide (0.62 mol) in 800 ml dichloromethane over 0.5 h. After stirring at –700 for 15 min a solution of 77.5 g N-benzyl-N-methyl-isoleucinol (0.35 mol) in 700 ml dichloromethane was added at –70° C. over ~0.5 h and stirring was continued for 15 min. After the addition of 234 ml triethylamine (1.68 mol) at –65° C. stirring was continued for 5 min and the white suspension stirred at –50° C. for 1 h. The dry ice-bath was removed and 3000 ml deionized water was added drop wise and under stirring while the reaction mixture was warmed up to RT. The organic layer was washed twice with 2000 ml 10% brine and the organic layer was dried (Na₂SO₄). Removal of the solvent by rotary evaporation (40° C./≧10 mbar/0.1 mbar, 2 h) yielded 76.9 g (100.2%) yellow oily product which was used without purification in the next step.

EXAMPLE 9

### Benzyl-((1S,2S)-1-dimethoxymethyl-2-methyl-butyl)-methyl-amine
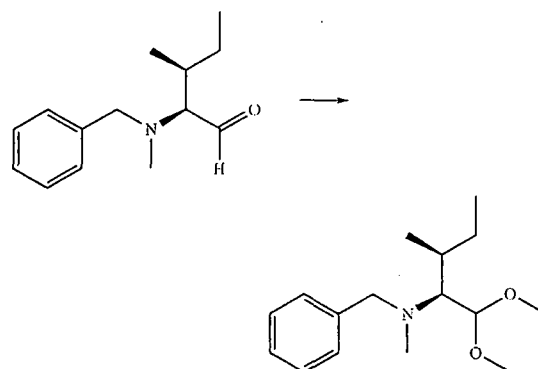
To 76.9 g of the above described aldehyde (0.35 mol) dissolved in 350 ml methanol were added drop wise and under cooling 70 ml conc. H₂SO₄ (~1.30 mol). After stirring for 0.5 h 350 ml trimethyl orthoformate (3.20 mol; Fluka) were added all at once and stirring at RT was continued for 3 h. The reaction mixture was diluted with 2000 ml ethyl acetate and washed with 2000 ml 10% Na₂CO₃ and 2000 ml 10% brine. The organic layer was dried (Na₂SO₄) and evaporated by rotary evaporation (45° C./≧10 mbar) affording 91.9 g yellow oil. Purification by a vacuum distillation gave 86.0 g (92.6%) product as a light yellow oil, b.p. 106° C./0.1 mbar. ¹H-NMR:

EXAMPLE 10

(1-tert-Butoxy-vinyloxy)-tert-butyl-dimethyl-silane
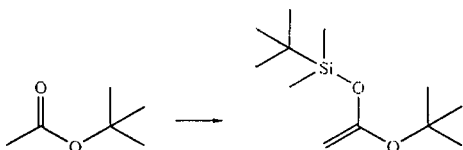
To a stirred solution of 58.2 g diisopropylamine (575 mmol) in 500 ml tetrahydrofuran were added at 0° C. 344 ml 1.6M BuLi in hexane (550 mmol) over 20 min. After stirring for 15 min the solution was cooled to −70° C. and 58.1 g tert-butyl acetate (500 mmol; Fluka) were added at −70° C. over 15 min and stirring continued for 15 min. After the addition of 75 ml HMPA (Fluka) a solution of 81.6 g tert-butyldimethylchlorosilane (525 mmol; Fluka) in 100 ml tetrahydrofuran was added at −70° C. over 15 min and the reaction mixture was warmed to RT over ~1 h. The reaction mixture was concentrated by rotary evaporation (40° C./≧10 mbar) and the honey-oily residue was partitioned between 1000 ml hexane and 1000 ml deionized water. The organic layer was washed with 10% brine (2×500 ml) and dried (Na$_2$SO$_4$) affording after removal of the solvent by rotary evaporation (40° C./≧10 mbar) 116.1 g bright yellow oil. Purification by vacuum distillation gave 106.0 g (92.0%) product as a colorless oil, b.p. 53-54° C./2.0 mbar. ^{1}H-NMR:

EXAMPLE 11

(3R,4S,5S)-4-(Benzyl-methyl-amino)-3-methoxy-5-methyl-heptanoic acid tert-butyl ester (Bn-Dil-OtBu)
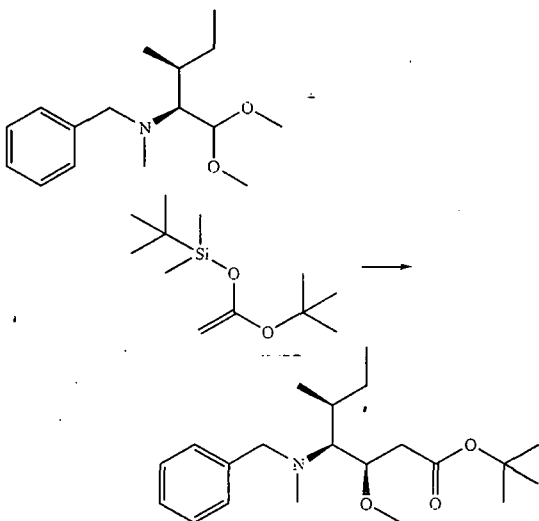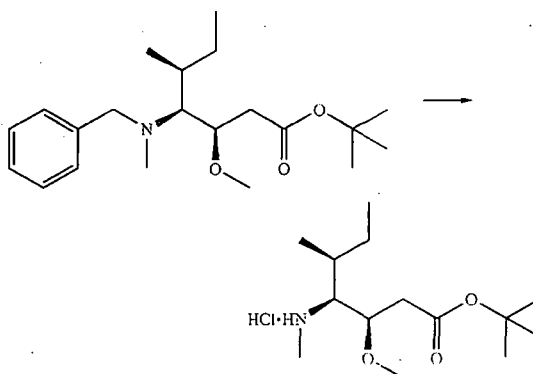

To a stirred solution of the above described 13.27 g dimethylacetal (50 mmol) and the above described 17.28 g TBS-silylketene acetal (75 mmol) in 200 ml dichloromethane was added at 0° C. a solution of 8.04 g N,N-

dimethylformamide and 14.19 g boron trifluoride ethyl etherate (100 mmol=12.56 ml; Fluka) in 50 ml dichloromethane over 15 min. After stirring at 0° C. for 24 h the reaction mixture was washed with 250 ml 10% Na$_2$CO$_3$ and with 10% brine (2×125 ml). The organic layer was (Na$_2$SO$_4$) and the solvent was removed by rotary evaporation (40° C./≧10 mbar/0.1 mbar, 2 h) yielding 17.5 g (100.1%) crude product as a yellow oil which was used without purification in the next step. OD [α]$_D$=−18.0° (CHCl$_3$; c=1). ^{1}H-NMR:

EXAMPLE 12

(3R,4S,5S)-3-Methoxy-5-methyl-4-methylamino-heptanoic acid tert-butyl ester hydrochloride (H-Dil-OtBu.HCl)

To a stirred solution of 17.5 g of the above described ester (50 mmol) in 250 ml ethanol were added 0.87 g Pd-C 10% (Degussa) and 4.59 ml 37% HCl (55 mmol). The black suspension was hydrogenated under vigorous stirring at RT for 18 h. The flask was flashed with Ar and the black suspension was filtered. After removal of the solvent by rotary evaporation (40° C./≧10 mbar) the white crystalline residue (14.54 g) was dissolved in 350 ml ethyl acetate at ~80° C. After cooling to RT and crystallization under stirring at 0° C. for 17 h the crystal suspension was filtered, washed with −20° cold ethyl acetate and dried (50° C./10 mbar/16 h) affording 12.87 g (87.0% over two steps) white crystalline product, m.p. 153-154° C. (dec.). OD [α]$_D$=+6.71° (CHCl$_3$; c=1). MS ([M+1]$^+$ of the free base). ^{1}H-NMR:
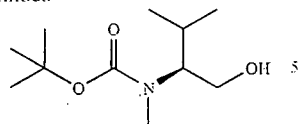
EXAMPLE 13

((S)-1-Hydroxymethyl-2-methyl-propyl)-methyl-carbamic acid tert-butyl ester

19   **20**

EXAMPLE 15

-continued

((S)-1-Dimethoxymethyl-2-methyl-propyl)-methyl-amine hydrochloride
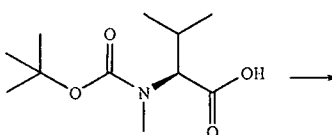
20.0 g Boc-McVal-OH (86.5 mmol; Fluka) were dissolved in 80 ml THF and cooled to 0° C. 130 ml 1M BH₃-THF (0.13 mol; Fluka) were added at 0° C. over 1 h and the clear, colo reaction mixture was warmed up and stirred at RT for 1 h. The reaction mixture was again cooled to 0° C., 75 ml deionized water were carefully added at 0-5° C. over 0.5 h and after warming up to RT stirring was continued for 1 h. To the colorless solution were added 150 ml 10% Na₂CO₃ all at once and after stirring for 1 h the reaction mixture was extracted with 600 ml and 300 ml ethyl acetate. The organic layers were washed with brine and dried (Na₂SO₄). Removal of the solvent by rotary evaporation gave 17.4 g (94%) product as colorless oil. ¹H-NMR:

5.0 g Crude Boc-N-methyl-valinal (23.2 mmol) were dissolved in 40 ml methanol and cooled to ~15° C. A solution of 3.3 ml (46.4 mmol) acetyl chloride in 10 ml methanol was added all at once and the yellowish solution was stirred at RT for 1 h (gas evolution). 22.2 g Trimethyl orthoformate (209 mmol) were now added and the reaction mixture was stirred at RT over night (18 h). The solvent and the excess of the orthoester were removed by rotary evaporation (40° C./≧10 mbar) and the resulting beige, crystalline residue (4.6 g) was dissolved in ca. 45 ml isopropyl acetate at ~70° C. After cooling to RT and crystallization at -20° C. for 17 h the crystal suspension was filtered and dried (50° C./10 mbar/16 h) affording 3.2 g (69%) product as greenish needles, [α]_D=40.3 (CHCl₃; c=1). ¹H-NMR:

EXAMPLE 14

((S)-1-Formyl-2-methyl-propyl)-methyl-carbamic acid tert-butyl ester

EXAMPLE 16

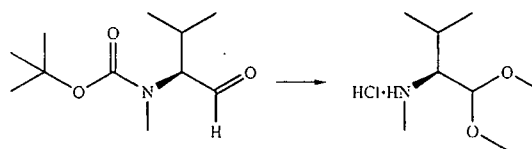Benzyl-((S)-1-dimethoxymethyl-2-methyl-propyl)-methyl-amine
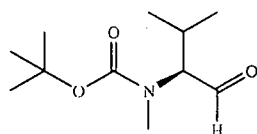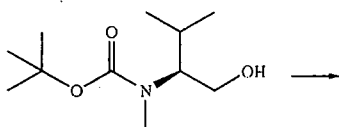
To a solution of 10.5 g Boc-N-methyl-valinol (48.3 mmol) in 50 ml dichloromethane was added a solution of 1.63 g NaHCO₃ (19.3 mmol) and 0.58 g KBr (4.8 mmol) in 50 ml deionized water. The reaction mixture was cooled to 0° C. and after the addition of 77 mg 2,2,6,6-tetramethyl-piperidin-1-oxyl (TEMPO, 0.48 mmol), 36.6 g 10.3% aqueous sodium hypochlorite (53.1 mmol Cl₂) were added under stirring over 2.5 h at 0-5°. After additional stirring for 30 min the excess of NaOCl was destroyed by the addition 38% aqueous sodium bisulfite and the reaction mixture was warmed up to 20°. The aqueous layer was extracted with 50 ml dichloromethane and the organic layers were washed with 50 ml 10% brine and dried (Na₂SO₄). Removal of the solvent by rotary evaporation afforded 9.9 g (95%) crude product as a light orange oil, [α]_D=-130 (CHCl₃; c=1). ¹H-NMR:
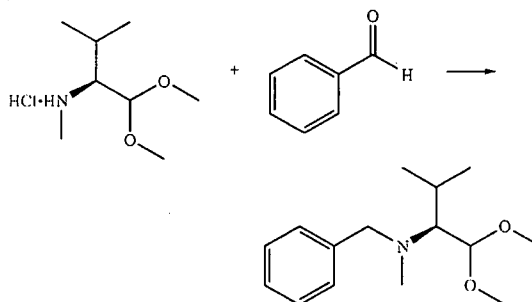
To a solution of 7.13 g the above N-Me-Valinal dimethylacetal hydrochloride (36.1 mmol) in 140 ml dichloromethane were added 3.83 g triethylamine (37.9 mmol) and 4.21 g benzaldehyde (39.7 mmol) and the solution was stirred at RT for 0.5 h. 10.2 g sodium triacetoxyborohydride (43.3 mmol; Aldrich) were added under ice cooling and the white suspension was stirred at RT for 22 h. The reaction mixture was washed with 100 ml 10% Na₂CO₃ and twice with 100 ml 10% brine. All three aqueous layers were extracted sequentially with 200 ml dichloromethane and the combined organic layers were dried over Na₂SO₄. Filtration and removal of the solvent by rotary evaporation (45° C./≧10 mbar) gave 9.54 g orange oily residue. Purification by vacuum distillation gave 8.34 g (92%) product, as light yellow oil, b.p. 86° C./0.2 mbar, [α]_D=-21.8 (CHCl₃; c=1). ¹H-NMR:

EXAMPLE 17

(3R,4S)-4-(Benzyl-methyl-amino)-3-methoxy-5-methyl-hexanoic acid tert-butyl ester
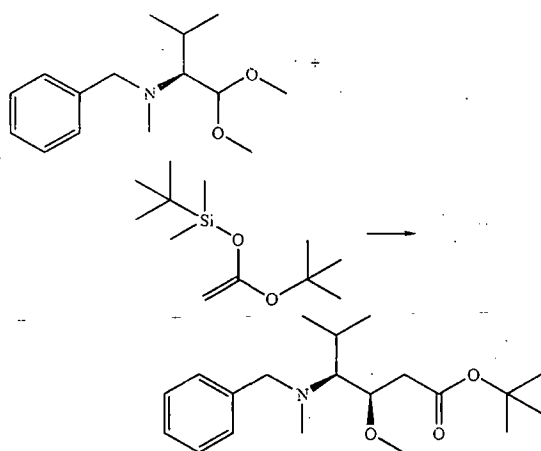
To a stirred solution of the above described dimethylacetal 7.54 g (30 mmol) and the above described TBS-silylketene acetal 10.4 g (45 mmol) in 110 ml dichloromethane was added at 0° C. a solution of 4.83 g (66 mmol) N,N-dimethylformamide and 8.52 g boron trifluoride ethyl etherate (60 mmol=12.56 ml; Fluka) in 30 ml dichloromethane over 15 min. After stirring at 0° C. for 69 h the reaction mixture was washed with 150 ml 10% Na_2CO_3 and with 10% brine (2×80 ml). The organic layer was dried (Na_2SO_4) and the solvent was removed by rotary evaporation (40° C./≧20 mbar/0.1 mbar, 2 h) yielding 10.1 g (100%) crude product as a yellow oil which was used without purification in the next step, $[\alpha]_D$=−20.2 (CHCl₃; c=1). ¹H-NMR:

EXAMPLE 18

(3R,4S)-3-Methoxy-5-methyl-4-methylamino-hexanoic acid tert-butyl ester hydrochloride
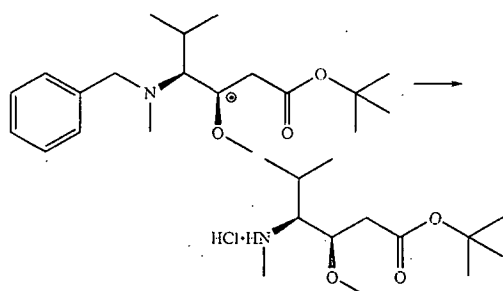

To a stirred solution of 10.0 g of the above described ester (30 mmol) in 150 ml ethanol were added 1.0 g Pd-C 10% (Degussa) and 2.6 ml 37% HCl (31.2 mmol). The black suspension was hydrogenated under vigorous stirring at RT for 18 h. The flask was flashed with Ar and the black

suspension was filtered. After removal of the solvent by rotary evaporation (40° C./≧10 mbar) the white crystalline residue (8.76 g) was dissolved in 25 ml hot isopropyl acetate at −80° C. After cooling to RT and crystallization under stirring at −15° C. for 17 h the crystal suspension was filtered, washed with −20° cold isopropyl acetate and dried (50° C./10 mbar/16 h) affording 4.84 g (57.0% over two steps) white crystalline product, $[\alpha]_D$32 7.9 (CHCl₃; c=1). ¹H-NMR:

EXAMPLE 19

((S)-2-Hydroxy-1-methyl-ethyl)-methyl-carbamic acid tert-butyl ester
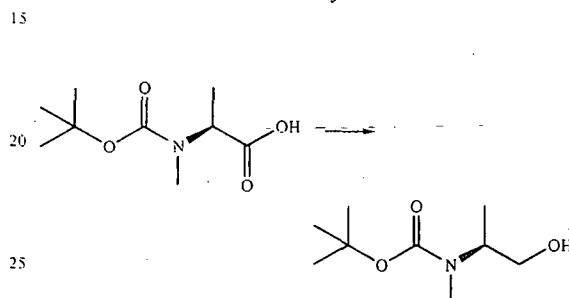
20.3 g Boc-MeAla-OH (100 mmol; Fluka) were dissolved in 80 ml THF and cooled to 0° C. 150 ml 1M BH₃-THF (150 mmol; Fluka) were added at 0° C. over 1 h and after additional stirring at 0° C. for 1 h, 65 ml deionized water were added carefully at 0-5° C. After warming up to RT 160 ml 10% Na_2CO_3 were added and stirring continued for 1 h. The reaction mixture was extracted with 500 ml and 400 ml ethyl acetate and the organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent by rotary evaporation gave 18.5 g (98%) product as a colorless oil, $[\alpha]_D$=−6.1 (CHCl₃; c=1). ¹H-NMR:

EXAMPLE 20

### Methyl-((S)-1-methyl-2-oxo-ethyl)-carbamic acid tert-butyl ester
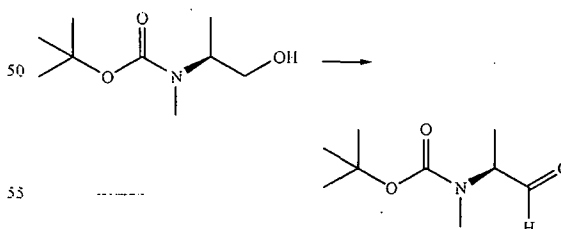
To a solution of 18.0 g Boc-N-methyl-alaninol (95 mmol) in 95 ml dichloromethane was added a solution of 3.2 g NaHCO₃ (38 mmol) and 1.14 g KBr (9.5 mmol) in 95 ml deionized water. The reaction mixture was cooled to 0° C. and after the addition of 152 mg 2,2,6,6-tetramethyl-piperidin-1-oxyl (TEMPO, 0.95 mmol), 72 g 10.3% aqueous sodium hypochlorite (105 mmol Cl₂) were added under stirring over 2.5 h at 0-5°. After additional stirring for 30 min the excess of NaOCl was destroyed by the addition 38%

23   24

aqueous sodium bisulfite (1 ml) and the reaction mixture was warmed up to 20°. The aqueous layer was extracted with 100 ml dichloromethane and the organic layers were washed with 100 ml 10% brine and dried (Na$_2$SO$_4$). Removal of the solvent by rotary evaporation afforded 14.4 g (80%) crude product as a light orange oil, [α]$_D$=-82 (CHCl$_3$; c=1). ^{1}H-NMR:

EXAMPLE 21

((S)-2,2-Dimethoxy-1-methyl-ethyl)-methyl-amine hydrochloride
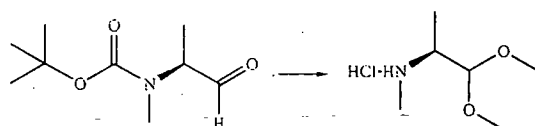
13.1 g Crude Boc-N-methyl-alaninal (70 mmol) were dissolved in 100 ml methanol and cooled to ~15° C. A solution of 11 g (140 mmol) acetyl chloride in 30 ml methanol was added all at once and the yellowish solution was stirred at RT for 1 h (gas evolution). 67.52 g Trimethyl orthoformate (630 mmol) were now added and the reaction mixture was stirred at RT over night (18 h). The solvent and the excess of the orthoester were removed by rotary evaporation (40° C./≧10 mbar) and the resulting 11.9 (100%) beige, crystalline residue was crystallized from 120 ml hot isopropyl acetate yielding after stirring at -20° C. for 17 h 8.4 g (71%) beige product, [α]$_D$=10.8 (CHCl$_3$; c=1). ^{1}H-NMR:

EXAMPLE 22

Benzyl-((S)-2,2-dimethoxy-1-methyl-ethyl)-methyl-amine
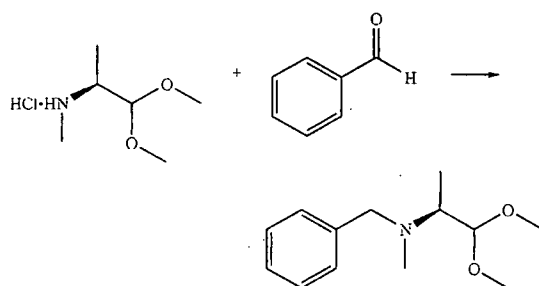
To a solution of 7.63 g the above N-Methyl-alaninal dimethylacetal hydrochloride (45 mmol) in 180 ml dichloromethane were added 4.78 g triethylamine (47.2 mmol) and 5.25 g benzaldehyde (49.5 mmol) and the solution was stirred at RT for 0.5 h. 12.7 g sodium triacetoxyborohydride (54 mmol; Aldrich) were added under ice cooling and the white suspension was stirred at RT for 22 h. The reaction mixture was washed with 180 ml 10% Na$_2$CO$_3$ and twice with 90 ml 10% brine. All three aqueous layers were extracted sequentially with 100 ml dichloromethane and the combined organic layers were dried over Na$_2$SO$_4$. Filtration

and removal of the solvent by rotary evaporation (45° C./≧10 mbar) gave 10.0 g orange oily residue. Purification by vacuum distillation gave 8.1 g (80%) product, as light yellow oil, b.p. 119° C./0.8 mbar, [α]$_D$=-2.2 (CHCl$_3$;c=1). ^{1}H-NMR:

EXAMPLE 23

(3R,4S)-4-(Benzyl-methyl-amino)-3-methoxy-pentanoic acid tert-butyl ester
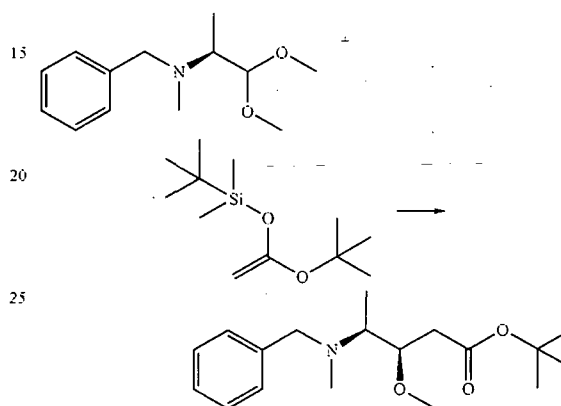
To a stirred solution of the above described 2.90 g dimethylacetal (13 mmol) and the above described 8.99 g TBS-silylketene acetal (39 mmol) in 50 ml dichloromethane was added at 0° C. a solution of 5.70 g N,N-dimethylformamide (78 mmol) and 5.53 g boron trifluoride ethyl etherate (39 mmol=12.56 ml; Fluka) in 13 ml dichloromethane. After stirring at 0° C. for 24 h the reaction mixture was washed with 65 ml 10% Na$_2$CO$_3$ and with 10% brine (2×30 ml). The organic layer was dried (Na$_2$SO$_4$) and the solvent was removed by rotary evaporation (40° C./≧10 mbar/0.1 mbar, 2 h) yielding 4.66 g crude product as a yellow oil which was chromatographed over silica (200 g) with toluene-ethyl acetate 19:1 (30 ml fractions). Evaporation of fraction 21-40 gave 2.97 g (74%) yellow oil. ^{1}H-NMR:

EXAMPLE 24

(3R,4S)-3-Methoxy-4-methylamino-pentanoic acid tert-butyl ester hydrochloride
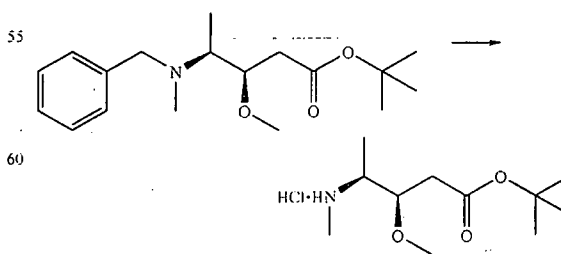
To a stirred solution of 1.54 g of the above described ester (5 mmol) in 25 ml ethanol were added 0.15 g Pd-C 10%

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(Degussa) and 0.52 g 37% HCl (5.2 mmol). The black suspension was hydrogenated under vigorous stirring at RT for 2 h. The flask was flashed with Ar and the black suspension was filtered. After removal of the solvent by rotary evaporation (40° C./≧10 mbar) the white crystalline residue (1.19 g) was dissolved in hot acetonitrile (8 ml) and crystallized at 0° C. yielding 1.02 g (80%) beige product. [α]$_D$=6.2 (CHCl$_3$; c=1) ^{1}H-NMR:

EXAMPLE 25

(S)-2-Dimethoxymethyl-pyrrolidine hydrochloride
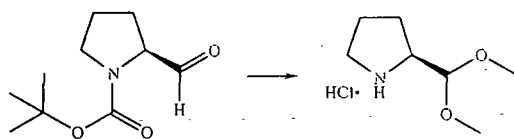
13.9 g Crude Boc-prolinal (70 mmol; Omega Chem, freshly distilled) were dissolved in 110 ml methanol and cooled to −15° C. A solution of 11.0 g (140 mmol) acetyl chloride in 30 ml methanol was added all at once and the yellowish solution was stirred at RT for 1 h (gas evolution). 66.9 g Trimethyl orthoformate (630 mmol) were now added and the reaction mixture was stirred at RT for 42 h. The solvent and the excess of the orthoester were removed by rotary evaporation (40° C./≧10 mbar) and the resulting 14.0 g beige, crystalline residue was crystallized from 300 ml hot ethyl acetate yielding after stirring at 0° C. for 17 h 8.9 g (70%) white needles. [α]$_D$=−15.4 (CHCl$_3$; c=1). ^{1}H-NMR:

EXAMPLE 26

(S)-1-Benzyl-2-dimethoxymethyl-pyrrolidine
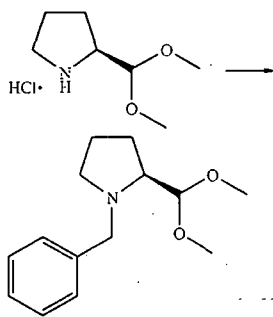
To a solution of 8.2 g of the above described prolinal dimethylacetal hydrochloride (45 mmol) in 150 ml dichloromethane were added 4.78 g triethylamine (47.2 mmol) and 5.25 g benzaldehyde (49.5 mmol) and the solution was stirred at RT for 0.5 h. 12.7 g sodium triacetoxyborohydride (54 mmol; Aldrich) were added under ice cooling and the white suspension was stirred at RT for 26 h. The reaction mixture was washed with 180 ml 10% Na$_2$CO$_3$ and twice with 90 ml 10% brine. All three aqueous layers were extracted sequentially with 90 ml dichloromethane and the

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combined organic layers were dried over Na$_2$SO$_4$. Filtration and removal of the solvent by rotary evaporation (45° C./≧10 mbar) gave 10.5 g yellow oily residue. Purification by vacuum distillation gave 8.93 g (84%) product, as a colorless oil, b.p. 120° C./0.4 mbar. [α]$_D$=−73.8 (CHCl$_3$; c=1). ^{1}H-NMR:

EXAMPLE 27

(R)-3-((S)-1-Benzyl-pyrrolidin-2-yl)-3-methoxy-propionic acid tert-butyl ester
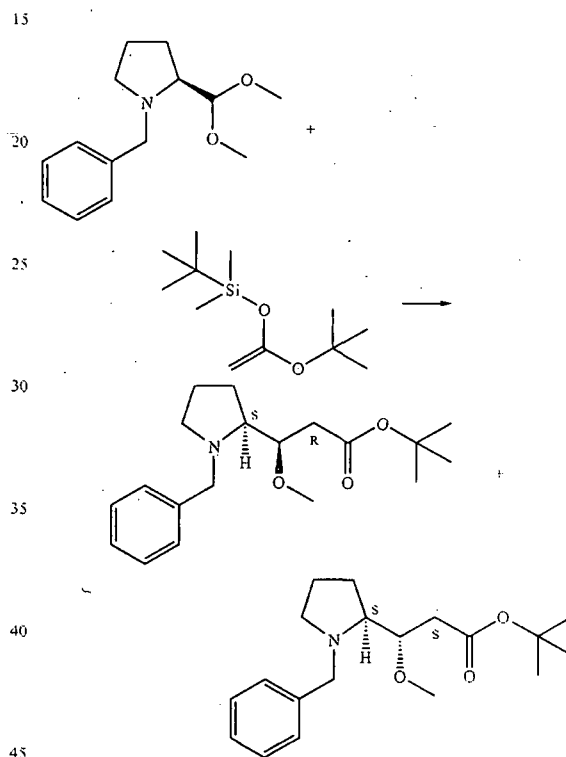
To a stirred solution of the above described dimethylacetal 4.71 g (20 mmol) and the above described TBS-silylketene acetal 13.83 g (60 mmol) in 75 ml dichloromethane was added at 0° C. a solution of 6.43 g (88 mmol) N,N-dimethylformamide and 11.35 g boron trifluoride ethyl etherate (80 mmol) in 25 ml dichloromethane. After stirring at 0° C. for 24 h the reaction mixture was washed with 100 ml 10% Na$_2$CO$_3$ and with 10% brine (50 ml). The organic layer was dried (Na$_2$SO$_4$) and the solvent was removed by rotary evaporation (40° C./≧10 mbar/0.1 mbar, 2 h) yielding 10.2 g crude oily product as a ca. 2:1 mixture of two diastereoisomers. Chromatography over silica (700 g) with hexane-ethyl acetate 14:1 (200 ml fractions) gave 2.49 g oily (3R,4S)-diastereomer (39%; fractions 14-30) and 1.23 g (3S,4S)-diastereomer (19%; fractions 35-44). (3R,4S)-diastereomer: [α]$_D$=−88.7 (CHCl$_3$; c=1) (3R,4S)-diastereomer: ^{1}H-NMR:
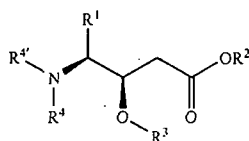
(3S,4S)-diastereomer: 1H-NMR:

EXAMPLE 28

(R)-3-Methoxy-3-(S)-pyrrolidin-2-yl-propionic acid
tert-butyl ester hydrochloride


absolute configuration
confirmed by X-ray
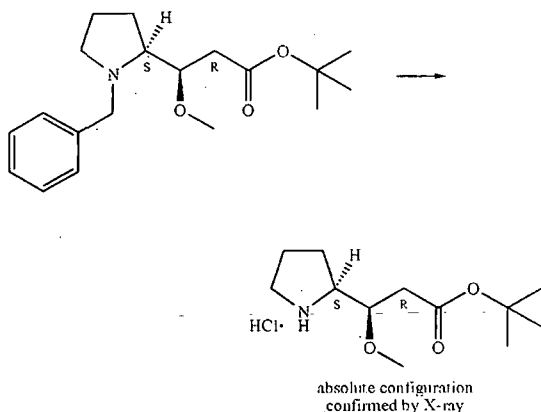
To a stirred solution of 1.92 g of the above described ester (6 mmol) in 30 ml ethanol were added 0.20 g Pd-C 10% (Degussa) and 0.62 g 37% HCl (6.3 mmol). The black suspension was hydrogenated under vigorous stirring at RT for 2 h. The flask was flashed with Ar and the black suspension was filtered. After removal of the solvent by rotary evaporation (40° C./≧10 mbar) the white crystalline residue (1.57 g) was dissolved in 7.5 ml hot isopropyl acetate at ~80° C. Crystallization at –20° C. yielding 1.37 g (86%) white crystalline product, $[\alpha]_D$=–36.4 (CHCl₃; c=1). ¹H-NMR:

What is claimed is:

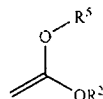1. A process for the preparation of a compound of formula I

comprising reacting a compound of formula II
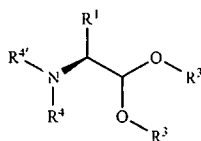

with a compound of formula III

in the presence of a Lewis acid and an organic solvent;
wherein
R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;
R^2 is C_{1-4} alkyl, benzyl, substituted benzyl or allyl;
R^3 is C_{1-4} alkyl or allyl;
R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl;
$R^{4'}$ is benzyl or substituted benzyl; or alternatively,
R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group; and
R^5 is a trialkylsilyl group.

2. The process according to claim 1, wherein
R^1 is methyl, ethyl, isopropyl or sec-butyl;
R^2 is methyl, ethyl or tert-butyl;
R^3 is methyl or ethyl;
R^4 is C_{1-4} alkyl, allyl or benzyl;
$R^{4'}$ is benzyl;
R^5 is dimethyl-tert-butyl-silyl, trimethyl-silyl or triethyl-silyl; and
the Lewis acid is BF₃, TMSOTf, TiCl₄, BF₃.OEt₂ or BF₃.DMF.

3. The process according to claim 1, wherein
R^1 is (S)-sec-butyl;
R^2 is tert-butyl;
R^3 and R^4 are methyl;
$R^{4'}$ is benzyl;
R^5 is dimethyl-tert-butyl-silyl; and
the Lewis acid is BF₃.DMF.

4. A compound of formula I
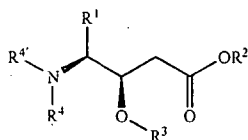
wherein
R^1 is C_{1-4} alkyl, C_{3-6} cycloalkyl-C_{1-4} alkyl or aryl-C_{1-4} alkyl;
R^2 is C_{1-4} alkyl, benzyl, substituted benzyl or allyl;
R^3 is C_{1-4} alkyl or allyl;
R^4 is C_{1-4} alkyl, allyl, benzyl or substituted benzyl; and
$R^{4'}$ benzyl or substituted benzyl; or alternatively,
R^1 and R^4 together with the nitrogen to which they are bound form a pyrrolidine group;
and wherein "substituted benzyl" denotes a benzyl group substituted with a group selected from 2,4,6-trimethyl, 3-methoxy, 4-methoxy, 2,4-dimethoxy, 3,4-dimethoxy, 3,5-dimethoxy, 2-nitro, 4-nitro, 2,4-dinitro, 4-bromo, 4-phenyl or 3,4-methylene-dioxy.

5. The compound according to claim 4, wherein
R^1 is methyl, ethyl, isopropyl or sec-butyl;
R^2 is methyl, ethyl or tert-butyl;
R^3 methyl or ethyl;
R^4 is C_{1-4} alkyl, allyl or benzyl; and
R^4 is benzyl.

*　*　*　*　*

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Full Paper

Synthesis of New 4-Heteroaryl-2-Phenylquinolines and Their Pharmacological Activity as NK-2/NK-3 Receptor Ligands

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Dipartimento del Farmaco, Istituto Superiore di Sanità, Roma, Italy

Substituted 4-heteroaryl-2-phenylquinolines were synthesized and tested on NK-2 and NK-3 receptors in order to get a better insight in the structure-activity relationship. On the whole, these molecules, which can be regarded as bioisosters of the NK-3 antagonist SB 218795, displayed a lower activity than the template. Ring electronic distribution and H-bond donor and acceptor positions played some role in selectivity, 2-imidazolyl substituted 2a showing affinity mainly towards NK-3 while 3-pyrazolyl substituted 4 displayed a preferential interaction with NK-2 receptor. Structural characterization of the synthesized compounds was achieved by NMR and mass techniques. Bidimensional 'H-NOESY experiments were a helpful tool for the assignment of the isomeric structures of compounds 9 and 11b–c.

Introduction

Tachykinins are peptidic neurotransmitters characterized by the common C-terminal sequence Phe-X-Gly-Leu-MetNH$_2$; they include substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), along with the recently discovered hemokinin-1 (HK-1). The physiological role of tachykinins is exerted through the transmembrane G-protein coupled receptors designated neurokinin-1 (NK-1), NK-2, and NK-3. Although tachykinins do not show complete selectivity to their receptors, SP binds mainly to NK-1, NKA to NK-2, and NKB to NK-3. These receptors are widely distributed within both the central and peripheral nervous system, and also in non-neural tissues, and are involved in many physiological and pathological processes including pain, neurogenic inflammation, smooth muscle contraction, neuro immunomodulation, blood pressure control, anxiety, and depression [1]. The NK-3 receptor is mainly expressed in the central nervous system and is involved in the modulation of the central monoaminergic system [2]. NK-3 antagonists have been proposed for the treatment of irritable bowel syndrome [3], asthma and chronic obstructive pulmonary disease [4], urinary incontinence [5], anxiety and depression [6], hypertension and tachycardia [7], psychotic symptoms of schizophrenia [8], and panic disorders [9].

At present, only few chemical classes of selective non-peptidic NK-3 receptor antagonists have been developed: 2-arylalkylcarbamates as PD 161182 [10], 1-acylpiperidines like osanetant [11] and its analogues [12], and 2-aryl-quinoline-4-carboxamides including the potent and selective antagonist SB 218795 (Fig. 1) which led to the development of talnetant [13–17].

The importance of the arylquinoline class of ligands also lies in the fact that these molecules were rather simple to synthesize so that many different substituents could be introduced in the basic skeleton. Hence, a good and rather complete study on the structural key points for receptor binding and selectivity was achieved and information was obtained on the binding site of the NK-3 receptor [14].

The purpose of this publication is to extend the structure-activity studies of the 2-phenylquinolines on NK-3

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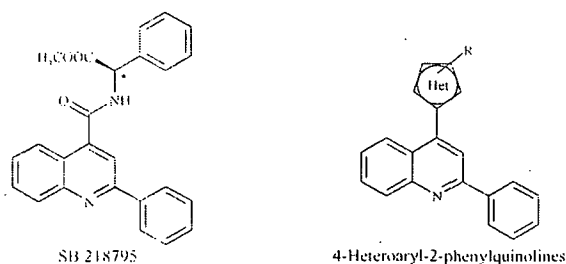
Arch. Pharm. Chem. Life Sci. 2007, *340*, 17–25

Figure 1. Chemical structure of SB 218795 and the bioisosters 4-heteroaryl-2-phenylquinolines.

and NK-2 receptors by the synthesis and the pharmacological characterization of new substituted 4-heteroaryl-2-phenylquinolines where the amidic group of the template (Fig. 1) has been replaced by a series of substituted heterocyclic rings such as imidazole, pyrazole, triazole, oxazole, some of which can be regarded as amide bioisosters.

A docking study of the 2-phenylcarboxamides in interaction with NK-3 receptor [14] evidenced that the amide group is surrounded by aromatic residues. In our compounds more π-interaction with the aromatic gorge could be provided by the heteroaryl moiety and its different aromatic substituents.

Results and discussion

Chemistry

2-Phenyl-4-(4-phenylimidazol-2-yl)quinoline **2a**, along with 2-phenyl-4-(4-phenyloxazol-2-yl)quinoline **2b** as secondary product, was obtained by converting 2-phenyl-4-quinolinecarboxylic acid into its phenacyl ester **1** and by treating the latter with ammonium acetate in refluxing acetic acid. The synthesis of compound **4** was accomplished in two steps: first ethyl 2-phenyl-4-quinolinecarboxylate and acetophenone were condensated in the presence of sodium hydride to afford the intermediate 4-(benzoylacetyl)-2-phenylquinoline **3**. Subsequent reaction of **3** with hydrazine afforded 2-phenyl-4-(5-phenyl-pyrazol-3-yl)quinoline **4**. Ethyl 2-phenyl-4-quinolinecarboxylate gave also condensation with acetonitrile to form 4-(cyanacetyl)-2-phenylquinoline **5**, which afforded 4-(3-aminopyrazol-5-yl)-2-phenylquinoline **6a** by hydrazine addiction; introduction of a benzyl, 3-thienylmethyl or α-phenylpropyl substituent on the aminic group to give **6b–d** was performed via azomethinic intermediates; acylation with benzoyl chloride in pyridine gave **6e**.

Condensation of ethyl 2-phenyl-4-quinolinecarboxylate with aminoguanidine gave 4-(5-amino-[1,2,4]triazol-

3-yl)-2-phenylquinoline **7a** which afforded **7b** by reaction with benzaldehyde and subsequent reduction with sodium borohydride (Scheme 1).

2-Phenyl-4-(3-phenyl-[1,2,4]triazol-5-yl)quinoline **8** was obtained from 2-phenyl-4-quinolinecarbonitrile and benzhydrazide in diphenyl ether at 220°C. Condensation of 2-phenyl-4-quinolinecarboxamide with dimethylformamide dimethyl acetal and subsequent reaction of the resulting amidine with phenylhydrazine afforded 2-phenyl-4-(1-phenyl-[1,2,4]triazol-5-yl)quinoline **9** whose structure was elucidated by ¹H-NOE experiments. The study was aimed to ascertain if an 1,3- or 1,5-disubstitution occurred on triazole ring. ¹H-NOESY spectrum showed consistent NOEs between the protons of the phenylic substituent of triazole and the quinoline H-3 and H-5 while no interaction between the triazole C–H and the same phenyl protons was observed. These data indicate the proximity of the phenylic substituent of the triazole ring to the quinoline moiety confirming the structure of compound **9**. Reaction between 4-(bromoacetyl)-2-phenylquinoline and benzamidine in absolute ethanol gave 2-phenyl-4-(2-phenylimidazol-4-yl)quinoline **10**; the condensation of 4-acetyl-2-phenylquinoline with dimethylformamide dimethyl acetal and the reaction of the resulting enamine with hydrazine gave 2-phenyl-4-(pyrazol-3-yl)quinoline **11a**, which by a Mannich reaction with formaldehyde and anilines gave products **11b–c** (Scheme 2). NOE experiments indicated that the aminomethyl group was selectively directed to the pyrazole nitrogen away from the quinoline ring. ¹H NOESY spectrum showed NOEs between the CH₂ group and the pyrazole H-4 and H-5, no NOE with the quinoline protons being observed.

Pharmacology and structure-activity studies

The binding affinities of the synthesized compounds for the human cloned neurokinin receptors subtype 2 and 3 expressed in CHO cells (Perkin Elmer Life Sciences) were measured employing [¹²⁵I]-neurokinin A and B as radiotracers. Concentration-response curves for the compounds were first run in duplicate; for compounds which displayed $IC_{50} < 10$ μM tests were repeated in three independent experiments. IC_{50} values were obtained by fitting the competition curves according to a 4-parameter logistic model (ALLFIT program) [18]. The apparent inhibition constants (K_i) were calculated fitting the competition curves according to LIGAND program [19] and are reported in Table 1.

The synthesized structures resulted to be less potent than the lead compounds; their activity has been found in the micromolar concentration range (Table 1).

The carbamide of the template (Fig. 1) was reported to form a hydrogen bond with a tyrosine or a histidine in

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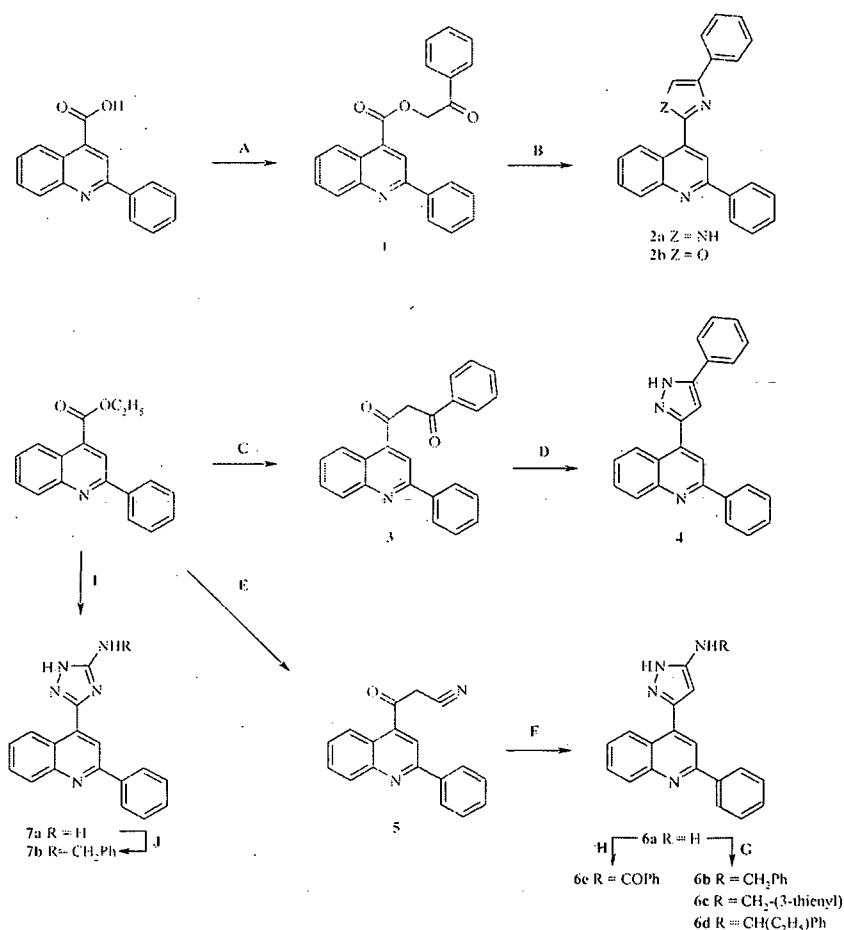
Reagents: A:a) KOH, PhCOCH₂Br, DMF; B: NH₄OAc, HOAc, reflux; C: PhCOCH₃, NaH, THF; D: N₂H₄.H₂O, EtOH, HOAc, 80°C; E: CH₃CN, NaH, DMF; F: N₂H₄.H₂O, EtOH, HOAc, reflux; G: PhCHO for 6b, 3-thiophenecarboxaldehyde for 6c, PhCOCH₂CH₃ for 6d; b) NaBH₄, EtOH. H: a) PhCOCl pyridine; I: H₂NC(=NH)NHNH₂, H₂CO₃, NaOCH₃, MeOH, reflux; J: a) PhCHO, EtOH; b) NaBH₄, EtOH.

Scheme 1. Synthesis route of compounds 1–7.

the TM6 section of the NK-3 receptor and with the same residues in the NK-2 receptor [14]. Our synthesized structures maintained the possibility of acting as hydrogen-bond donor or acceptor although the strength and the direction of the hydrogen bond could be altered because of the delocalization of the hydrogen on different nitrogen atoms. On the whole, the heterocyclic structure seemed not to have the proper shape for affinity, however, some structural aspects could be emphasized.

We considered compound 2a bearing the isosteric 2-imidazolyl group as structural reference (Fig. 2). 2a displayed a $K_i = 1.1$ µM for NK-3 and a threefold lower activity for NK-2 receptor. The *a* and *b* positions, where the

nitrogen atoms could act as H-bond donors and acceptors, were confirmed to be peculiar for NK-3 affinity and the introduction of an additional nitrogen in the *c* position (triazole 8) did not substantially alter binding features. Shifting of the phenyl ring onto the triazole nitrogen (compound 9) was more detrimental for NK-2 than for NK-3 affinity and led to an enhancement of NK-3 vs. NK-2 selectivity. Lack of the N_a–C(–Q)–N_b sequence resulted in a loss of NK-3 affinity: going deep, the shift of the *a* nitrogen atom to the *c* position led to an inversion of selectivity (compounds 4 and 6b–d). The sequence N_b–N_c working as H-bond donors and acceptors seemed to be more suitable for NK-2 interaction. Absence of N_b (imida-
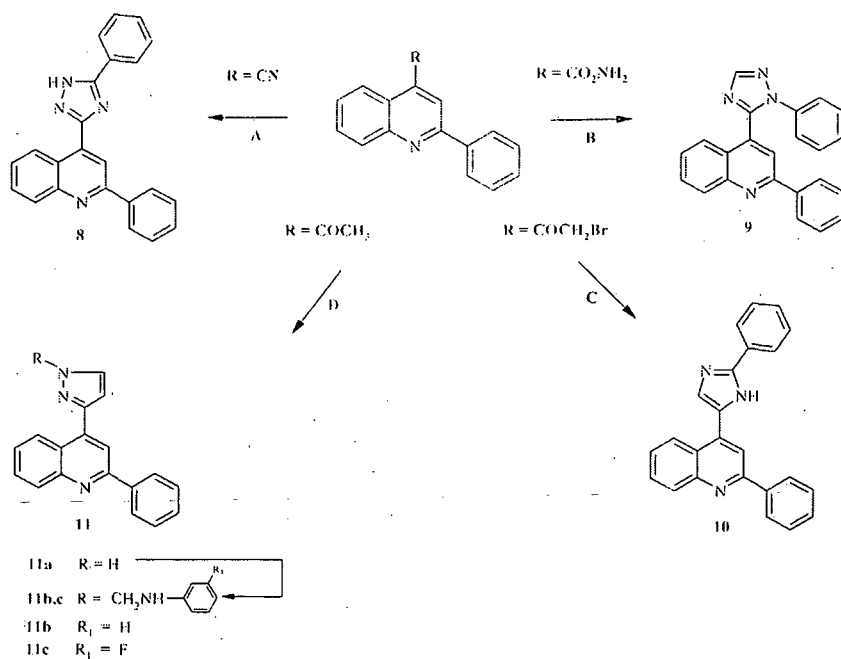
Reagents: **A:** PhCONHNH₂, (Ph)₂O, 220°C; **B:** a) (CH₃)₂NCH(OCH₃)₂, reflux; b) Ph NHNH₂, AcOH, 90°C; **C:** PhC(=NH)NH₂, Na₂CO₃, EtOH; **D:** a) (CH₃)₂NCH(OCH₃)₂, reflux; b) N₂H₄.H₂O, AcOH. 90°C; **E:** RNH₂, HCHO, toluene, 80°C.

Scheme 2. Synthesis route of compounds 8–11.
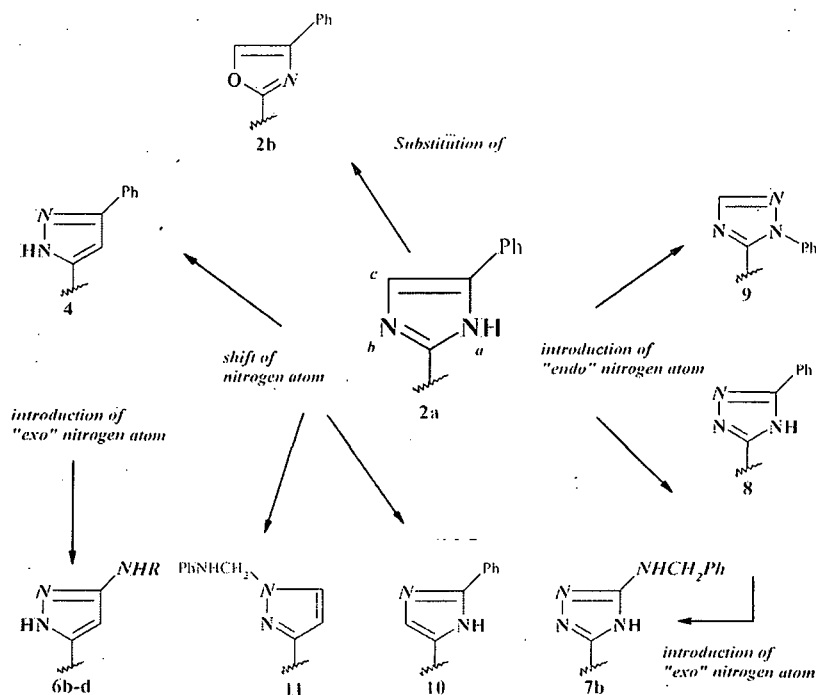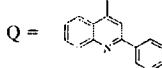
**Figure 2.** Structure-activity relationship of synthesized compounds.

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NK-2/NK-3 Receptor Ligands   21

Table 1. *In vitro* affinity of compounds **2, 4,** and **6–11.**

Structure		K_i [µM]	
		NK-2	NK-3
Q =			
	2a Z = NH	3.6 ± 0.9	1.1 ± 0.5
	2b Z = O	7.2 ± 1.4	7.7 ± 1.3
	4	0.47 ± 0.03	2.9 ± 0.3
	6b R = CH₂Ph	9.4 ± 1.5	>10
	6c R = CH₂(thienyl)	4.8 ± 1.2	>10
	6d R = CH(C₂H₅)Ph	8.2 ± 2.0	>10
	6e R = COPh	10.5 ± 0.6	>10
	7b	6.1 ± 0.1	>10
	8	3.4 ± 0.5	1.6 ± 0.3
	9	>10	2.4 ± 0.2
	10	>10	>10
	11b R₁ = H	>10	>10
	11c R₁ = F	>10	>10
R =			
	SB-218795	1.2 ± 0.2	0.013 ± 0.003

Data are given as mean ± SD (n = 3).

zole **10**) as well as N$_c$-substitution (pyrazole **11**) led to a loss of affinity. Considering the nitrogen atom in the *b* position as an indispensable requirement for some affinity, the selectivity ratio NK-3/NK-2 seemed to be modulated by the key positions *a* and *c*.

The addition of an *exo* nitrogen atom directly bound to the pyrazolyl moiety (derivatives **6**), aimed to provide an addictive hydrogen bond to the receptor and to build a more extended and flexible aromatic substituent, maintained a preferential although lower affinity for NK-2 binding. The most active compound in this series was **6c** which displayed NK-2 K_i = 4.8 µM. The presence of this aminic side chain in the triazole moiety (compound **7b**), changed the selectivity of the related compound **8**, the

former showing NK-2 K_i = 6.1 µM, NK-3 K_i > 10 µM, the latter NK-2 K_i = 3.4 µM, NK-3 K_i = 1.6 µM.

More distance of the *exo* nitrogen atom from the heteroaryl ring gave rise to the inactive compounds **11**. Substitution of a nitrogen with an oxygen atom (oxazole **2b**) led to a weakening of the biological interaction.

In conclusion, the pharmacological study of the moderate NK-2/NK-3 ligands 4-heteroaryl-2-phenylquinolines suggested that the interaction of small molecules with the neurokinin receptors is very strictly ruled by structural and electronic requirements. Isosteric replacement of the amide group by the slightly more restricted heteroaryl rings did not assure an effective contact with the receptor. Different ring electronic distribution joined to H-bond donor and acceptor positions seemed to play some role in selectivity and might be an intriguing subject to elucidate differences and requirements for selective neurokinins ligands. The interest of this study lies also in the feasible synthesis and in the structural characterization of these new heterocyclic molecules. Intermediate compounds **1, 3,** and **5** which have never been synthesized previously were characterized and represent potential starting material for the preparation of substituted quinolinic structures.

Experimental

Chemistry

General

Melting points were determined on a Kofler hot stage apparatus (C. Reichert, Vienna, Austria) and are uncorrected. Column chromatographic separations were accomplished on Merck silica gel (70–230 mesh) or on Merck aluminium oxide 90 (Merck, Darmstadt, Germany). The purity of each compound was checked on silica gel C. Erba 60 F₂₅₄ (Carlo Erba, Milan, Italy) or Merck aluminium oxide 60 F₂₅₄ (type E; Merck) plates and spots were located by UV light. Sodium sulfate was used to dry organic solutions. Elemental analyses were within ± 0.4% of the theoretical values.

¹H-NMR spectra and 2D-COSY and NOESY experiments were performed on Bruker Avance 700 and 400 instruments (Bruker) in CDCl₃ or DMSO-d₆ solution; all values are reported in ppm (δ) and standard abbreviations were used (a = apparent; b = broad; d = doublet; dd = doublet of doublets; dt = doublet of triplets; m = multiplet; q = quadruplet; s = singlet). Electron Ionisation Mass spectra were recorded on a HP 59980 B spectrometer operating at 70 eV (Hewlett-Packard, Palo Alto, CA, USA).

The 2-phenyl-4-quinolinecarboxylic acid [20], the corresponding amide [21] and nitrile [20], 4-acetyl-2-phenylquinoline [22], and 4-(bromoacetyl)-2-phenylquinoline hydrobromide [23] were prepared according to literature methods; ethyl 2-phenyl-4-qui-

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nolinecarboxylate was obtained from the acid by a conventional esterification with absolute ethanol and sulphuric acid.

Phenacyl 2-phenyl-4-quinolinecarboxylate 1

Phenacyl bromide (2.0 g, 0.010 mol) was added portion-wise to a solution of potassium 2-phenyl-4-quinolinecarboxylate (3.0 g, 0.010 mol) at 0–5°C in anhydrous dimethylformamide (20 mL) and the mixture was kept overnight at room temperature. After addition of water (3 mL), the mixture was vigorously stirred at 5–10°C for 1 h; a solid separated which was collected by filtration and washed with water and diethyl ether; m. p. 120–122°C (ethyl acetate/hexane), yield 76%. ^{1}H-NMR (DMSO-d_6): δ 8.68 (d, 1H, H-8, $J_{8,7}$ = 8.4 Hz), 8.55 (s, 1H, H-3), 8.30 (d, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 8.0 Hz), 8.26 (d, 1H, H-5, $J_{5,6}$ = 8.6 Hz), 8.08 (d, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 7.4 Hz), 7.90 (dd, 1H, H-6, $J_{6,5}$ = 8.6 Hz, $J_{6,7}$ = 7.2 Hz), 7.75 (dd, 1H, H-7, $J_{7,8}$ = 8.4 Hz, $J_{7,6}$ = 7.1 Hz), 7.70-7.57 (m, 6H, H-3′, H-4′, H-5′, H-3′, H-4″, H-5″), 5.96 (s, 2H, OCH$_2$CO). MS: (m/z) 367 [M$^+$], 309, 232, 204. Anal. C$_{24}$H$_{17}$NO$_3$.

2-Phenyl-4-(4-phenylimidazol-2-yl)quinoline and 2-Phenyl-4-(4-phenyloxazol-2-yl)quinoline 2a and 2b

A solution of 1 (0.8 g, 2.2 mmol) and ammonium acetate (1.5 g, 19.5 mmol) was refluxed in acetic acid (12.5 mL) for 3 h. Most of the acetic acid was removed in a rotary evaporator and the residue was diluted with water and alkalinized with aqueous ammonium hydroxide. The solid which separated was collected by filtration, dried under vacuum and was chromatographed on a silica gel column, eluent 1 : 3 ethyl acetate/hexane; 2b eluted first followed by 2a.

2a: This compound was obtained from 1 in 59% yield, m. p. 114–116°C (ethyl acetate/hexane). ^{1}H-NMR (CDCl$_3$): δ 8.72 (d, 1H, H-8, $J_{8,7}$ = 8.4 Hz), 8.15 (d, 1H, H-5, $J_{5,6}$ = 8.4 Hz), 8.08 (d, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 7.9 Hz), 7.94 (s, 1H, H-3), 7.81 (bm, 2H, H-6, NH), 7.70 (t, 1H, H-7, $J_{7,8}$ = $J_{7,6}$ = 8.4 Hz), 7.54 (overlapped d and s, 3H, H-2″, H-6″, imidazole H-5), 7.50–7.29 (m, 6H, H-3′, H-4′, H-5′, H-3′, H-4″, H-5″). MS: (m/z) 347 [M$^+$], 270, 229, 174. Anal. C$_{24}$H$_{17}$N$_3$.

2b: This compound was obtained from 1 in 6% yield, m. p. 127–129°C (ethyl acetate / hexane). ^{1}H-NMR (CDCl$_3$): d 9.43 (d, 1H, H-8, $J_{8,7}$ = 8.4 Hz), 8.58 (s, 1H, oxazole H-5), 8.24 (two overlapped d, 3H, H-5, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 8.2 Hz), 8.15 (s, 1H, H-3), 7.92 (d, 2H, H-2″, H-6″, $J_{2'',3''}$ = $J_{6'',5''}$ = 8.4 Hz), 7.80 (t, 1H, H-6, $J_{6,5}$ = 8.2 Hz, $J_{6,7}$ = 8.2 Hz), 7.69 (t, 1H, H-7, $J_{7,8}$ = 8.4 Hz, $J_{7,6}$ = 8.2 Hz), 7.60–7.34 (m, 6H, H-3′, H-4′, H-5′, H-3′, H-4″, H-5″). MS: (m/z) 348 [M$^+$], 319, 204. Anal. C$_{24}$H$_{16}$N$_2$O.

4-(Benzoylacetyl)-2-phenylquinoline 3

Acetophenone (5.5 mL, 0.047 mol) was added drop-wise to a suspension of ethyl 2-phenyl-4-quinolinecarboxylate (13.0 g, 0.047 mol) and sodium hydride (1.9 g, 60% in mineral oil, 0.047 mol) in anhydrous tetrahydrofuran (100 mL) at 0°C. The mixture was kept overnight at room temperature under stirring, then concentrated *in vacuo*, diluted with water, and extracted with ethyl acetate; the solvent was evaporated and the residue purified by crystallization, m. p. 193–195°C (ethyl acetate/hexane), yield 92%. ^{1}H-NMR (DMSO-d_6): δ 8.46 (d, 1H, H-8, $J_{8,7}$ = 8.2 Hz), 8.25 (dd, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 8.2 Hz), 8.05 (d, 1H, H-5, $J_{5,6}$ = 8.2 Hz), 8.00 (s, 1H, H-3), 7.83 (m, 2H, H-2″, H-6″), 7.72 (t, 1H, H-6, $J_{6,5}$ = $J_{6,7}$ = 8.2 Hz), 7.60–7.40 (m, 4H, H-7, H-3′, H-4′, H-5′), 7.34 (m, 3H, H-3″, H-4″, H-5″), 6.06 (s, 2H, COCH$_2$CO). MS: (m/z) 351 [M$^+$], 263, 205, 176 Anal. C$_{24}$H$_{17}$NO$_2$.

2-Phenyl-4-(5-phenylpyrazol-3-yl)quinoline 4

A solution of 3 (0.5 g, 1.4 mmol) and hydrazine hydrate (0.15 mL, 3.1 mmol) in absolute ethanol (5 mL) and acetic acid (1 mL) was heated at 80°C for 1.5 h, cooled to room temperature, diluted with water, and extracted with ethyl acetate. Compound 4 was obtained by evaporation of the organic layer and was purified by crystallization, m. p. 112–115°C (ethyl acetate/hexane), yield 59%. ^{1}H-NMR (DMSO-d_6): δ 8.45 (d, 1H, H-8, $J_{8,7}$ = 8.2 Hz), 8.25 (d, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 7.5 Hz), 8.06 (d, 1H, H-5, $J_{5,6}$ = 8.4 Hz), 8.00 (s, 1H, H-3), 7.84 (m, 2H, H-3′, H-5′), 7.73 (dd, 1H, H-6, $J_{6,5}$ = 8.4 Hz, $J_{6,7}$ = 7.8 Hz), 7.52 (m, 4H, H-7, H-4′, H-2″, H-6″), 7.35 (m, 3H, H-3″, H-4″, H-5″), 6.07 (s, 1H, pyrazole H-4). MS: (m/z) 347 [M$^+$], 271, 229, 174. Anal. C$_{24}$H$_{17}$N$_3$.

4-(Cyanacetyl)-2-phenylquinoline 5

Ethyl 2-phenyl-4-quinolinecarboxylate (5.54 g, 0.020 mol) and then acetonitrile (1.9 mL, 0.036 mol) were added drop-wise to a suspension of sodium hydride (0.96 g, 60% in mineral oil, 0.024 mol) in anhydrous dimethylformamide (50 mL) at 0°C. The mixture was kept overnight at room temperature, then concentrated *in vacuo*, diluted with water, and extracted with ethyl acetate; the aqueous phase was neutralized with diluted hydrochloric acid and extracted again, the combined organic layers were evaporated and the residue purified by chromatography on a silica gel column, by eluting with an ethyl acetate/hexane mixture (1 :8), m. p. 142–144°C (methanol), yield 81%. ^{1}H-NMR (DMSO-d_6): δ 8.32 (two overlapped doublets, 3H, H-8, H-2′, H-6′), 8.16 (d, 1H, H-5, $J_{5,6}$ = 8.4 Hz), 8.14 (s, 1H, H-3), 7.86 (dd, 1H, H-6, $J_{6,5}$ = 8.4 Hz, $J_{6,7}$ = 7.0 Hz), 7.69 (dt, 1H, H-7, $J_{7,8}$ =8.4 Hz, $J_{7,6}$ = 7.0 Hz), 7.60–7.50 (m, 3H, H-3′, H-4′, H-5′), 5.10 (broad, 2H, COCH$_2$CN). MS: (m/z) 272 [M$^+$], 232, 204, 176. Anal. C$_{18}$H$_{12}$N$_2$O.

4-(3-Aminopyrazol-5-yl)-2-phenylquinoline 6a

A solution of 5 (2.0 g, 7.4 mmol) and hydrazine hydrate (0.8 mL, 16.5 mmol) in ethanol (10 mL) and acetic acid (0.8 mL) was refluxed for 6 h; then cooled to room temperature, diluted with water and alkalinized with aqueous ammonia. The resulting precipitate was collected by filtration and chromatographed on a silica gel column by eluting with 5% methanol in ethyl acetate, m. p. 130–132°C (ethyl acetate), yield 17%. ^{1}H-NMR (CDCl$_3$): δ 8.24 (dd, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 8.4 Hz), 8.16 (d, 1H, H-8, $J_{8,7}$ = 8.0 Hz), 8.15 (d, 1H, H-5, $J_{5,6}$ = 7.2 Hz), 7.89 (s, 1H, H-3), 7.77 (dt, 1H, H-6, $J_{6,7}$ = 8.2 Hz, $J_{6,5}$ = 7.2 Hz, $J_{6,8}$ = 1.5 Hz), 7.56-7.50 (m, 4H, H-7, H-3′, H-4′, H-5′), 6.11 (s, 1H, pyrazole H-4), 3.05 (broad, 2H, NH$_2$). MS: (m/z) 286 [M$^+$], 257, 244, 230, 152. Anal. C$_{18}$H$_{14}$N$_4$.

4-(3-Benzylaminopyrazol-5-yl)-2-phenylquinoline 6b

Benzaldehyde (0.17 mL, 1.7 mmol) was added to a solution of 6a (0.5 g, 1.7 mmol) in absolute ethanol (5 mL) and the mixture was kept at 45°C for 2 h. After cooling to room temperature sodium borohydride (0.1 g, 2.6 mmol) was slowly added and after 1 h of additional stirring, the mixture was concentrated *in vacuo*, diluted with water, and extracted with ethyl acetate. Evaporation of the organic layer gave 8c, m. p. 158–160°C (ethyl acetate/hexane), yield 91%. ^{1}H-NMR (CDCl$_3$): δ 8.24 (d, 1H, H-8, $J_{8,7}$ = 8.0 Hz), 8.20 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 8.10 (dd, 2H, H-2′, H-6′, $J_{2',3'}$ = $J_{6',5'}$ = 7.9 Hz), 7.86 (s, 1H, H-3), 7.72 (dt, 1H, H-6, $J_{6,5}$ = 8.0 Hz, $J_{6,7}$ = 7.0 Hz, $J_{6,8}$ = 1.4 Hz), 7.55–7.30 (m, 10H, H-7, H-3′, H-4′, H-5′, benzyl aromatics, NH), 5.99 (s, 1H, pyrazole H-4), 4.41 (s, 2H, benzyl CH$_2$). MS: (m/z) 376 [M$^+$], 348, 299, 230. Anal. C$_{25}$H$_{20}$N$_4$.

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4-[3-(3-Thienylmethyl)aminopyrazol-5-yl]-2-phenylquinoline 6c

This compound was obtained from 6a and 3-thiophenecarboxaldehyde analogously to the above compound. m. p. 133 – 135°C (ethyl acetate/hexane); yield 67%. ^{1}H-NMR (CDCl$_3$): δ 8.23 (d, 1H, H-8, J$_{8,7}$ = 7.5 Hz). 8.17 (d, 1H, H-5. J$_{5,6}$ = 7.8 Hz), 8.09 (dd, 2H, H-2', H-6', J$_{2',3'}$ = J$_{6',5'}$ = 8.2 Hz), 7.86 (s, 1H, H-3), 7.73 (dt, 1H, H-6. J$_{6,5}$ = 7.8 Hz, J$_{6,7}$ = 7.1 Hz, J$_{6,8}$ = 1.4 Hz), 7.55 – 7.40 (m, 4H, H-7, H-3', H-4', H-5' Hz), 7.28 (q, 1H, thiophene H-5), 7.22 (bs, 1H, thiophene H-2), 7.11 (d, 1H, thiophene H-4), 6.01 (s, 1H, pyrazole H-4), 4.42 (s, 2H, thienylmethyl CH$_2$). MS: (m/z) 382 [M$^+$]. 381. 380. 286. 271. Anal. C$_{23}$H$_{18}$N$_4$S.

4-[3-(1-Phenylpropyl)aminopyrazol-5-yl]-2-phenylquinoline 6d

A mixture of 6a (0.5 g, 1.7 mmol) and propiophenone (0.23 mL, 1.7 mmol) in toluene (20 mL) was refluxed for two days; then toluene was removed *in vacuo* and the residue dissolved in absolute ethanol (20 mL); sodium borohydride (0.15 g, 4.0 mmol) was portion-wise added under stirring at room temperature. After 3 h stirring, the mixture was concentrated *in vacuo*, added with water, and extracted with ethyl acetate; the organic layer was evaporated *in vacuo* and the residue chromatographed on an aluminium oxide column, by eluting with a 1 : 1 ethyl acetate/hexane mixture, resinous solid, yield 28%. ^{1}H-NMR (CDCl$_3$): δ 8.15 (two overlapped d, 2H, H-8, H-5, J$_{8,7}$ = 8.4 Hz, J$_{5,6}$ = 8.4 Hz), 8.08 (d, 2H, H-2', H-6', J$_{2',3'}$ = J$_{6',5'}$ = 8.1 Hz), 7.80 (s, 1H, H-3), 7.68 (dt, 1H, H-6, J$_{6,5}$ = 8.4 Hz, J$_{6,7}$ = 7.2 Hz, J$_{6,8}$ = 1.3 Hz), 7.50 – 7.25, (m, 9H, H-7, H-3', H-4', H-5', H-2'', H-3'', H-4'', H-5'', H-6''), 5.76 (s, 1H, pyrazole H-4), 4.26 (t, 1H, CH$_3$CH$_2$CH), 1.81 (m, 2H, CH$_3$CH$_2$CH), 0.91 (t, 3H, CH$_3$CH$_2$CH). MS: (m/z) 404 [M$^+$], 376. 375. 286. Anal. C$_{27}$H$_{24}$N$_4$.

4-(3-Benzoylaminopyrazol-5-yl)-2-phenylquinoline 6e

A solution of 6a (0.5 g, 1.7 mmol) and benzoyl chloride (0.39 mL, 3.4 mmol) in pyridine (7 mL) was stirred overnight at room temperature; pyridine was then evaporated *in vacuo* and the residue dispended in water and extracted with ethyl acetate. After evaporation, the organic layer gave a solid which was dissolved in ethanol (5 mL) and added with the same volume of 40% aqueous sodium hydroxide. The resulting slurry was kept under stirring for 1 h and after addition of water a solid separated which was collected by filtration, washed with water, and dried *in vacuo*, m. p. 140 – 142°C (ethyl acetate/hexane). yield 91%. ^{1}H-NMR (DMSO-d$_6$): δ 8.53 (d, 1H, H-8, J$_{8,7}$ = 8.4 Hz), 8.34 (d, 2H, H-2', H-6', J$_{2',3'}$ = J$_{6',5'}$ = 8.0 Hz), 8.25 (s, 1H, H-3), 8.16 (d, 1H, H-5, J$_{5,6}$ = 8.4 Hz), 8.07 (d, 2H, H-2'', H-6'', J$_{2'',3''}$ = J$_{6'',5''}$ = 7.2 Hz), 7.84 (at, 1H, H-6, J$_{6,5}$ = 8.4 Hz, J$_{6,7}$ = 7.4 Hz), 7.69 (at, 1H, H-7, J$_{7,8}$ = 8.4 Hz, J$_{7,6}$ = 7.4 Hz), 7.60-7.45 (m, 6H, H-3', H-4', H-5', H-3', H-4'', H-5''), 7.17 (s, 1H, pyrazole H-4). MS: (m/z) 390 [M$^+$], 363, 362, 361, 230. Anal. C$_{25}$H$_{18}$N$_4$O.

4-(3-Amino-[1,2,4]triazol-5-yl)-2-phenylquinoline 7a

Aminoguanidine bicarbonate (2.5 g, 18.4 mmol) was added to a solution of sodium methoxide (17.4 mmol) in methanol (25 mL) at 0°C, ethyl 2-phenyl-4-quinolinecarboxylate (1.3g, 4.7 mmol) was then portion-wise added at the same temperature and the mixture was refluxed for 18 h. After cooling to room temperature, the reaction was poured onto crushed ice and neutralized with diluted hydrochloric acid; about 1 h later, the solid product was collected by filtration, washed with water and diethyl ether, and dried *in vacuo*; m. p. 190 - 192°C (ethyl acetate), yield 72%. ^{1}H-NMR (DMSO-d$_6$): δ 9.67 (bs, 1H, triazole NH), 8.73 (d, 1H,

H-8, J$_{8,7}$ = 8.4 Hz), 8.23 (d, 2H, H-2', H-6', J$_{2',3'}$ = J$_{6',5'}$ = 8.0 Hz), 8.13 (s, 1H, H-3), 8.03 (d, 1H, H-5, J$_{5,6}$ = 8.4 Hz). 7.72 (dt, 1H, H-6, J$_{6,5}$ = 8.4 Hz, J$_{6,7}$ = 7.2 Hz, J$_{6,8}$ = 1.4 Hz), 7.60 – 7.49 (m, 4H, H-7, H-3', H-4', H-5'), 4.69 (s, 2H, NH$_2$). MS: (m/z) 287. Anal. C$_{17}$H$_{13}$N$_5$.

4-(3-Benzylamino-[1,2,4]triazol-5-yl)-2-phenylquinoline 7b

Benzaldehyde (0.17mL, 1.7 mmol) was added to a solution of 7a (0.5 g, 1.7 mmol) in absolute ethanol (5 mL) and the mixture was refluxed for 2 h. After cooling to room temperature, sodium borohydride (0.1 g, 2.6 mmol) was slowly added and stirring was continued for 1 h. Then water was added and the resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give 7b, m. p. 130 – 132°C (ethyl acetate/hexane). yield 76%. ^{1}H-NMR (CDCl$_3$): δ 8.19 (d, 1H, H-8, J$_{8,7}$ = 8.2 Hz). 8.15 (d, 1H, H-5, J$_{5,6}$ = 8.2 Hz), 8.06 (dd, 2H, H-2', H-6', J$_{2',3'}$ = J$_{6',5'}$ = 8.0 Hz), 7.81 (s, 1H, H-3), 7.67 (dt, 1H, H-6, J$_{6,5}$ = 8.2 Hz, J$_{6,7}$ = 7.0 Hz, J$_{6,8}$ = 1.5 Hz), 7.50 – 7.27 (m, 9H, H-7, H-3', H-4', H-5', benzyl aromatics), 5.95 (s, 1H, NH), 4.67 (s, 1H, NH), 4.37 (s, 2H, benzyl CH$_2$). MS: (m/z) 377 [M$^+$]. Anal. C$_{24}$H$_{19}$N$_5$.

2-Phenyl-4-(5-phenyl-[1,2,4]triazol-3-yl)quinoline 8

Benzhydrazide (2.5 g, 18.4 mmol) was added portion-wise to a solution of 2-phenyl-4-quinolinecarbonitrile (2.0 g, 8.7 mmol) in diphenyl ether (50 g) at 220°C. The mixture was maintained at the same temperature for 4 h and, after cooling to room temperature, hexane (200 mL) was added. The solid which separated was collected by filtration and chromatographed on an aluminium oxide column, by eluting with an ethyl acetate/hexane mixture (1 : 3), m. p. 180 – 182°C (ethyl acetate / hexane), yield 13%. ^{1}H-NMR (DMSO-d$_6$): δ 9.26 (d, 1H, H-8, J$_{8,7}$ = 8.2 Hz), 8.71 (s, 1H, H-3), 8.33 (d, 2H, H-2'', H-6'', J$_{2'',3''}$ = J$_{6'',5''}$ = 6.6 Hz), 8.18 (two overlapped d, 3H, H-5, H-2', H-6'), 7.86 (dt, 1 H, H-6, J$_{6,5}$ = 8.2 Hz, J$_{6,7}$ = 6.7 Hz, J$_{6,8}$ = 1.4 Hz), 7.73 (dt, 1H, H-7, J$_{7,8}$ = 8.2 Hz, J$_{7,6}$ = 6.7 Hz, J$_{7,5}$ = 1.2 Hz), 7.60 – 7.45 (m, 7H, H-3', H-4', H-5', H-3'', H-4'', H-5'', NH). MS: (m/z) 348 [M$^+$], 320, 245, 229, 217, 174. Anal. C$_{23}$H$_{16}$N$_4$.

2-Phenyl-4-(1-phenyl-[1,2,4]triazol-5-yl)quinoline 9

A mixture of 2-phenyl-4-quinolinecarboxamide (5.5 g, 0.022 mol) in dimethylformamide dimethyl acetal (20 mL) was refluxed for 1 h; the acetal was then removed *in vacuo* and the residue dissolved in acetic acid (7 mL), added with phenylhydrazine (2.36 mL, 0.024 mol) and heated at 90°C for 2 h. The mixture was dried *in vacuo* and chromatographed on a silica gel column, by eluting with an ethyl acetate/hexane mixture (9 : 1), m. p. 125 – 127°C (methanol), yield 26%. ^{1}H-NMR (DMSO-d$_6$): δ 8.53 (s, 1H, triazole H-3), 8.15 (s, 1H, H-3), 8.14 (d, 1H, H-8, J$_{8,7}$ = 7.7 Hz), 8.09 (dd, 2H, H-2', H-6', J$_{2',3'}$ = J$_{6',3'}$ = 7.8 Hz), 7.83 (d, 1H, H-5, J$_{5,6}$ = 7.6 Hz), 7.82 (dt, 1H, H-7, J$_{7,8}$ = 7.7 Hz, J$_{7,6}$ = 7.6 Hz, J$_{7,5}$ = 1.5 Hz), 7.57 (dt, 1H, H-6, J$_{6,5}$ = J$_{6,7}$ = 7.6 Hz, J$_{6,8}$ = 1.2 Hz), 7.50 (m, 3H, H-3', H-5', H-4'), 7.42 – 7.32 (m, 5H, H-2'', H-3'', H-4'', H-5'', H6''). MS: (m/z) 348 [M$^+$], 271, 229, 174. Anal. C$_{23}$H$_{16}$N$_4$.

2-Phenyl-4-(2-phenylimidazol-4-yl)quinoline 10

A mixture of 4-(bromoacetyl)-2-phenylquinoline hydrobromide (1.0 g, 2.5 mmol), benzamidine (0.5 g, 4.2 mmol) and sodium carbonate (0.4 g, 3.8 mmol) in absolute ethanol (20 mL) was kept at room temperature for 24 h under stirring. After the solvent evaporation, water was added to the mixture and stirring was continued for 2 h. The solid which separated was filtered, washed

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with water, and dried *in vacuo*, m. p. 200–202°C (ethyl acetate/hexane), yield 70%. ^{1}H-NMR (DMSO-d$_6$): δ 9.90 (broad, 1H, imidazole N*H*), 8.72 (d, 1H, H-8, J$_{8,7}$= 8.4 Hz), 8.24 (d, 2H, H-2′, H-6′, J$_{2′3′}$ = J$_{6′5′}$ = 8.2 Hz), 8.12 (s, 1H, H-3), 8.03 (d, 1H, H-5, J$_{5,6}$ = 8.3 Hz), 7.83 (d, 2H, H-2″, H-6″, J$_{2″3″}$ = J$_{6″5″}$ = 6.7 Hz), 7.70 (m, 2H, H-6, H-4″), 7.60–7.45 (m, 7H, H-7, triazole H-5, H-3′, H-4′, H-5′, H-3′, H-5″). MS: (m/z) 347. Anal. C$_{24}$H$_{17}$N$_3$.

2-Phenyl-4-(pyrazol-3-yl)quinoline 11a

A solution of 4-acetyl-2-phenylquinoline (1.6 g, 6.5 mmol) in dimethylformamide dimethyl acetal (6 mL) was refluxed for 1.5 h; afterwards the acetal was evaporated and the residue dissolved in acetic acid (2 mL), added with hydrazine hydrate (0.35 mL, 7.1 mmol) and heated at 90°C for 2 h. The mixture was then cooled, diluted with water, and alkalinized with sodium carbonate. A precipitate formed which was collected by filtration, and washed with water and diethyl ether, m. p. 145–146°C (ethyl acetate/hexane), yield 91%. ^{1}H-NMR (CDCl$_3$): δ 8.86 (d, 1H, H-8, J$_{8,7}$= 8.4 Hz), 8.33 (d, 2H, H-2′, H-6′, J$_{2′3′}$ = J$_{6′5′}$ = 8.1 Hz), 8.30 (s, 1H, H-3), 8.13 (d, 1H, H-5, J$_{5,6}$ = 8.3 Hz), 7.97 (d, 1H, pyrazole H-5, J = 2.0 Hz), 7.79 (t, 1H, H-6, J$_{6,5}$ = J$_{6,7}$ = 8.3 Hz), 7.62 (t, 1H, H-7, J$_{7,6}$ = J$_{7,8}$ = 8.4 Hz), 7.53 (m, 3H, H-3′, H-4′, H-5′), 7.08 (d, 1H, pyrazole H-4, J = 2.0 Hz). MS: (m/z) 271 [M$^+$], 270, 244. Anal. C$_{18}$H$_{13}$N$_3$.

General method for the preparation of the 2-phenyl-4-[1-(arylaminomethyl)pyrazol-3-yl]quinolines 11b–c

A mixture of 11a (1.0 g, 3.7 mmol), the appropriate aniline (3.7 mmol) and 37% aqueous formaldehyde (0.28 mL, 3.7 mmol) in toluene (7 mL) was heated at 80°C for 4 h. The mixture was then evaporated to dryness and chromatographed on an aluminium oxide column, by eluting with an ethyl acetate/hexane mixture.

2-Phenyl-4-[1-(phenylaminomethyl)pyrazol-3-yl]quinoline 11b

This compound was obtained from 11a and aniline, m. p. 146–147°C (ethyl acetate/hexane, 1:1), yield 25%. ^{1}H-NMR (DMSO-d$_6$): δ 8.93 (d, 1H, H-8, J$_{8,7}$ = 8.4 Hz), 8.31 (d, 2H, H-2′, H-6′, J$_{2′3′}$ = J$_{6′5′}$ = 8.2 Hz), 8.24 (s, 1H, H-3), 8.11 (d, 1H, H-5, J$_{5,6}$ = 8.2 Hz), 8.04 (d, 1H, pyrazole H-5, J = 2.3 Hz), 7.79 (dt, 1H, H-6, J$_{6,5}$ = J$_{6,7}$ = 8.2 Hz, J$_{6,8}$ = 1.2 Hz), 7.56 (m, 4H, H-7, H-3′, H-4′, H-5′), 7.20–7.00 (m, 4H, H-3′, H-4″, H-5″, pyrazole H-4), 6.89 (d, 2H, H-2″, H-6″, J$_{2′,3}$″ = J$_{6″,5″}$ = 7.8 Hz), 6.64 (t, 1H, CH$_2$NHC$_6$H$_5$, J = 7.2 Hz), 5.65 (d, 2H, C*H$_2$* NH C$_6$H$_5$, J = 7.2 Hz). MS (m/z) 376 [M$^+$], 375, 299. Anal. C$_{25}$H$_{20}$N$_4$.

4-[1-(3-Fluorophenylaminomethyl)pyrazol-3 -yl]-2-phenylquinoline 11c

This compound was obtained from 11a and 3-fluoroaniline, m. p. 132–133°C (ethyl acetate/hexane, 1:1), yield 28%. ^{1}H-NMR (DMSO-d$_6$): δ 8.93 (d, 1H, H-8, J$_{8,7}$ = 8.3 Hz), 8.32 (d, 2H, H-2′, H-6′, J$_{2′3′}$ = J$_{6′5′}$ = 7.1 Hz), 8.25 (s, 1H, H-3), 8.11 (d, 1H, H-5, J$_{5,6}$ = 8.4 Hz), 8.07 (d, 1H, pyrazole H-5, J = 2.3 Hz), 7.80 (at, 1H, H-6, J$_{6,5}$ = 8.4 Hz, J$_{6,7}$ = 7.2 Hz), 7.62–7.48 (m, 4H, H-7, H-3′, H-4′, H-5′), 7.41 (t, 1H, CH$_2$NHC$_6$H$_5$, J = 7.1 Hz), 7.14 (m, 1H, H-5″), 7.07 (d, 1H, pyrazole H-4, J = 2.3 Hz), 6.78 (dt, 1H, H-4″), 6.71 (dd, 1H, H-6″), 6.43 (dt, 1H, H-2″), 5.67 (d, 2H, C*H$_2$* NH C$_6$H$_5$, J = 7.1 Hz). MS: (m/z) 394 [M$^+$], 271, 244, 165. Anal. C$_{25}$H$_{19}$FN$_4$.

Pharmacology

[^{125}I]NKB (specific activity, 2200 Ci/mmol) and [^{125}I]NKA (specific activity, 2200 Ci/mmol) were obtained from Perkin Elmer Life Sciences (USA). The tachykinin peptides NKA and NKB were purchased from Bachem AG (Weil am Rhein, Germany).

The binding of [^{125}I]NKB and [^{125}I]NKA (Perkin Elmer Life Sciences) was measured in 1 mL reaction mixture containing 50 mM Hepes-Tris pH 7.4, 1 mM MnCl$_2$, 0.1% (w/v) bovine serum albumin (BSA) and 5 or 10 µg of membrane proteins from CHO cells, expressing the hNK-3 and the hNK-2 receptors, respectively. The concentration of radiotracer was maintained constant at ~50000 cpm in the presence of increasing concentrations of compounds to be tested. Reactions lasted 90 min at room temperature and were terminated by rapid filtration onto GF/B glass fiber filtering microplates pretreated with 0.1% Ethylene Imine Polymer (PEI) (Filtermate 196; Packard Instruments, Meriden, CT, USA). Filters were washed three times with 1 mL of ice-cold 50 mM Tris-HCl pH 7.4 and allowed to dry for 30 min at 37°C. The plates were counted in a Top Count (Packard Instruments) after the addition (50 µL) of Microscint 20 (Packard) to each well.

Concentration-response curves for each compound were run using duplicate samples in at least three independent experiments. The reported values are the apparent inhibition constants (K$_i$) which were obtained by fitting the competition curves according to Ligand Programm [19].

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[See sections 127 and 132; rule 135]

I, **DALBIR SINGH**, an individual, residing at 162, Block-F, Nihal Vihar, Nangloi Jat, New Delhi – 110041; hereby authorize Rajeshwari H and Chitra Arvind, all citizens of India, Advocates /

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I, **DALBIR SINGH**, an individual, residing at 162, Block-F, Nihal Vihar, Nangloi Jat, New Delhi – 110041; hereby authorize Rajeshwari H and Chitra Arvind, all citizens of India, Advocates / Patent Agents, of RAJESHWARI & ASSOCIATES, Trademark & Patent Attorneys, AMSOFT BUSINESS CENTRE, UNITECH TRADE CENTRE, Sector 43, Gurgaon- 122 002, Haryana, India; jointly or severally to act on our behalf for filing an opposition and/or representation by way of opposition against an invention entitled: "Process for synthesizing compounds useful for treating Hepatitis C", Indian Patent Application No: 854/DELNP/2010 filed on 08.02.2010 claiming priority of United States Patent Application No. 60/954,595 filed on 08.08.2007 by Bristol-Myers Squibb Company is National Phase of PCT Application No. PCT/US2008/071696 dated 30.07.2008 under the above mentioned Act and in all matters and proceedings relating to the patent applications before the Controller of Patents or the Government of India in connection therewith or incidental thereto and in general do all act or things, including filing of any representations, statements, replies, extensions, fees, evidence and any or all documents or pleadings, attending hearings and appointments of a substitute or substitutes as the said agent(s) may deem necessary or expedient and request that all notices, requisitions and communication relating thereto be sent to such agent(s) at Rajeshwari & Associates, India.

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