



(86) Date de dépôt PCT/PCT Filing Date: 1995/06/19

(87) Date publication PCT/PCT Publication Date: 1996/01/11

(45) Date de délivrance/Issue Date: 2007/01/02

(85) Entrée phase nationale/National Entry: 1996/11/27

(86) N° demande PCT/PCT Application No.: US 1995/007128

(87) N° publication PCT/PCT Publication No.: 1996/000732

(30) Priorité/Priority: 1994/06/29 (US08/267,630)

(51) Cl.Int./Int.Cl. *C07F 9/30* (2006.01),
A61K 31/66 (2006.01), *C07F 9/547* (2006.01),
C07F 9/572 (2006.01), *C07F 9/6558* (2006.01)

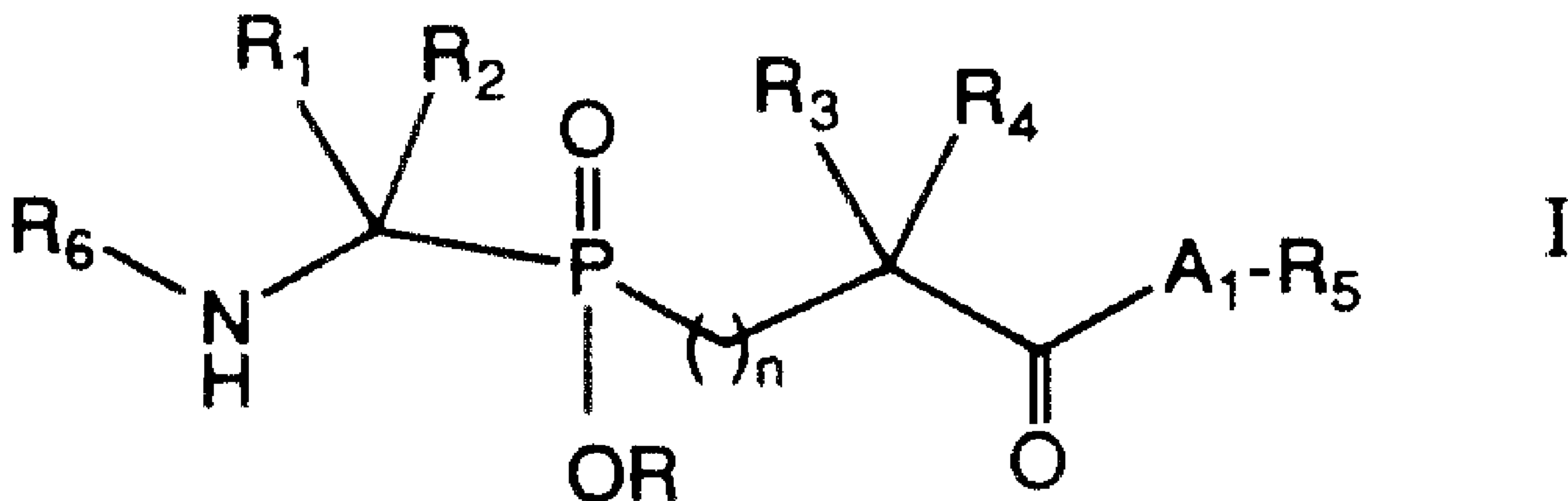
(72) Inventeurs/Inventors:
MCKITTRICK, BRIAN A., US;
CZARNIECKI, MICHAEL F., US;
CHACKALAMANNIL, SAMUEL, US;
CHUNG, SHIN, KR;
DEFREES, SHAWN, US;
STAMFORD, ANDREW W., US

(73) Propriétaire/Owner:
SCHERING CORPORATION, US

(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : DERIVES D'ACIDE PHOSPHINIQUE ET LEURS UTILISATIONS COMME INHIBITEURS D'ENZYMES DE
CONVERSION DE L'ENDOTHELINE

(54) Title: PHOSPHINIC ACID DERIVATIVES AND THEIR USE AS ENDOTHELIN CONVERTING ENZYME INHIBITORS



(57) Abrégé/Abstract:

Phosphinic acid derivatives of structural formula (I) or a pharmaceutically acceptable salt thereof, wherein R is H, alkyl or alkanoyloxymethylene; R₁, R₂, R₃ and R₄ are H, alkyl, alkenyl, alkenylalkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, hydroxyalkyl, carboxyalkyl, thioalkyl, alkoxythioalkyl, aminoalkyl, alkylaminoalkyl, cycloalkyl-substituted alkyl or heterocycloalkyl; or R₁ and R₂ form a cycloalkyl ring of 3-8 members and R₃ and R₄ are as defined; or R₃ and R₄ form a cycloalkyl ring of 3-7 members and R₁ and R₂ are as defined; or R₁ and R₂ together, and R₃ and R₄ together, each form a cycloalkyl ring; R₅ is -OR₉ or -NHR₉, wherein R₉ is hydrogen or alkyl; n is 0 or 1; A₁ is p-aminobenzoyl or p-aminobenzenesulfonyl, or A₁ and R₅ together form a radical of an α-aminoacyl derivative; and R₆ is phenylmethoxycarbonyl, arylcarbonyl, heteroarylcarbonyl or -A₂-R₇, wherein A₂ is a divalent α-iminoacyl radical, and R₇ is a substituent on the α-imino atom selected from H, R₈OCO-, R₈SO₂- and R₈NHCO-, wherein R₈ is aryl, arylmethyl or (C₁-C₈)alkyl; are disclosed for use as endothelin converting enzyme inhibitors; also disclosed are a genus of novel compounds wherein R³ and R⁴ form a cycloalkyl ring, and pharmaceutical compositions comprising said novel compounds.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07F 9/30, A61K 31/66, C07F 9/572, 9/6558	A1	(11) International Publication Number: WO 96/00732 (43) International Publication Date: 11 January 1996 (11.01.96)
---	-----------	--

(21) International Application Number: PCT/US95/07128

(22) International Filing Date: 19 June 1995 (19.06.95)

(30) Priority Data:
08/267,630 29 June 1994 (29.06.94) = US

(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

(72) Inventors: MCKITTRICK, Brian, A.; 67 Laurel Avenue, Bloomfield, NJ 07003 (US). CZARNIECKI, Michael, F.; 30 Gildersleeve Place, Watchung, NJ 07060 (US). CHACKALAMANNIL, Samuel; 79 Stratford Road, East Brunswick, NJ 08816 (US). CHUNG, Shin; Jukong Apartment #1319-1401, Chulsan 3 dong, Kwany Myong-shi, Kyongki-do (KR). DEFREES, Shawn; 540 Avenida Verde, San Marcos, CA 92069 (US). STAMFORD, Andrew, W.; 27 Overlook Road, Chatham Township, NJ 07928 (US).

(74) Agents: MAGATTI, Anita, W. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

2191454

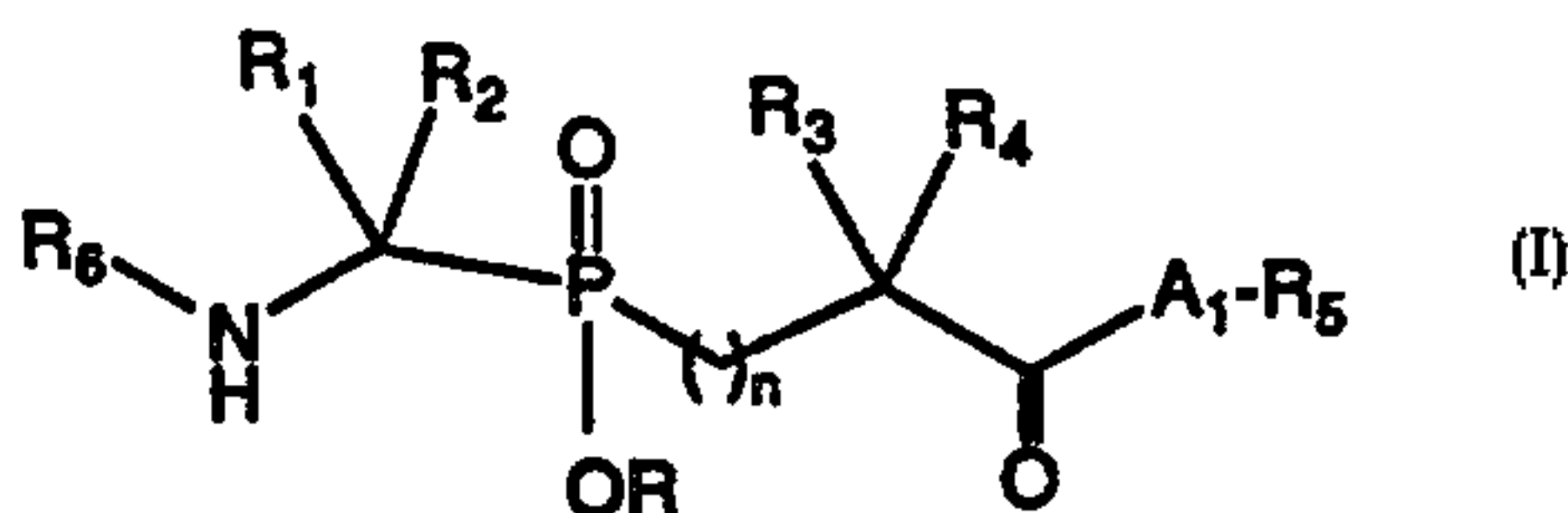
Published

With international search report.

(54) Title: PHOSPHINIC ACID DERIVATIVES

(57) Abstract

Phosphinic acid derivatives of structural formula (I) or a pharmaceutically acceptable salt thereof, wherein R is H, alkyl or alkanoyloxymethylene; R₁, R₂, R₃ and R₄ are H, alkyl, alkenyl, alkenylalkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, hydroxyalkyl, carboxyalkyl, thioalkyl, alkoxythioalkyl, aminoalkyl, alkylaminoalkyl, cycloalkyl-substituted alkyl or heterocycloalkyl; or R₁ and R₂ form a cycloalkyl ring of 3-8 members and R₃ and R₄ are as defined; or R₃ and R₄ form a cycloalkyl ring of 3-7 members and R₁ and R₂ are as defined; or R₁ and R₂ together, and R₃ and R₄ together, each form a cycloalkyl ring; R₅ is -OR₉ or -NHR₉, wherein R₉ is hydrogen or alkyl; n is 0 or 1; A₁ is p-aminobenzoyl or p-aminobenzenesulfonyl, or A₁ and R₅ together form a radical of an α-aminoacyl derivative; and R₆ is phenylmethoxycarbonyl, arylcarbonyl, heteroarylcarbonyl or -A₂-R₇, wherein A₂ is a divalent α-iminoacyl radical, and R₇ is a substituent on the α-imino atom selected from H, R₈OCO-, R₈SO₂- and R₈NHCO-, wherein R₈ is aryl, arylmethyl or (C₁-C₈)alkyl; are disclosed for use as endothelin converting enzyme inhibitors; also disclosed are a genus of novel compounds wherein R³ and R⁴ form a cycloalkyl ring, and pharmaceutical compositions comprising said novel compounds.



- 1 -

**PHOSPHINIC ACID DERIVATIVES AND THEIR
USE AS ENDOTHELIN CONVERTING ENZYME INHIBITORS**

5

10

BACKGROUND

This invention relates to the use of phosphinic acid derivatives as inhibitors of endothelin converting enzyme, to novel phosphinic acid derivatives and to pharmaceutical compositions comprising said novel compounds.

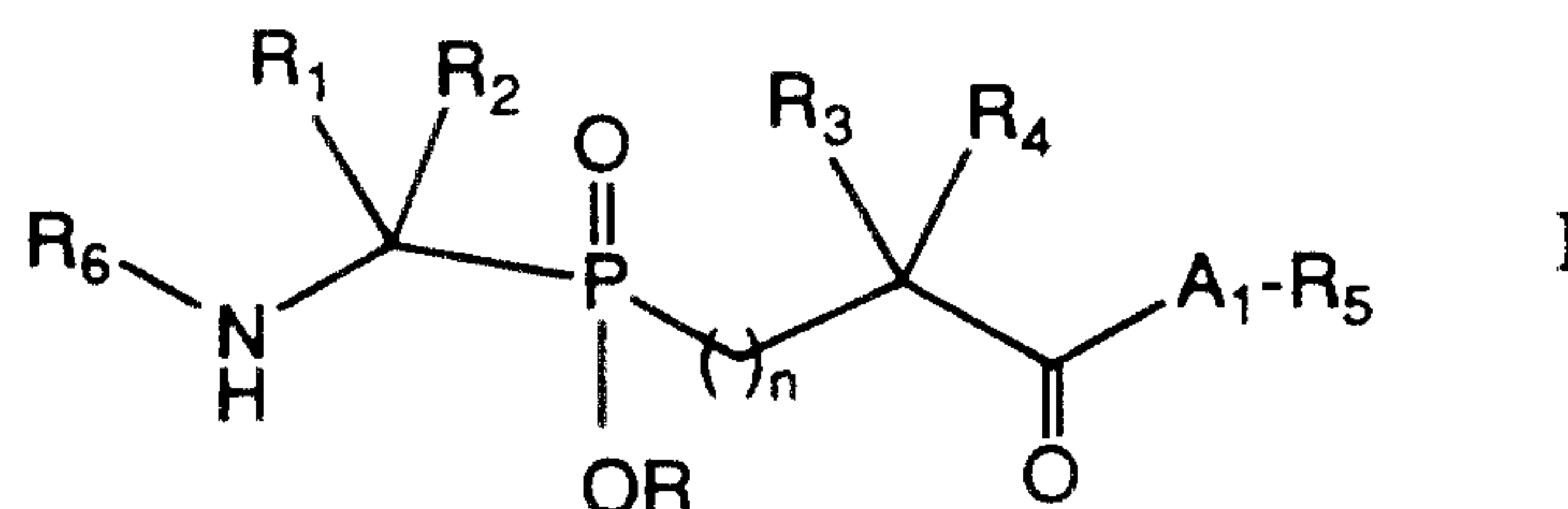
Endothelins are a family of peptides, including endothelin-1, endothelin-2 and endothelin-3 (ET-1, ET-2 and ET-3), which show potent vasoconstrictive and mitogenic activity. ET-1, approximately 100 times more potent than its precursor, big-endothelin-1 (BET-1), is believed to be liberated from BET-1 by an endothelin converting enzyme (ECE). See S. Bertenshaw, et al, *J. Med. Chem.*, 36 (1993), p. 173-176. Endothelin receptor blockers have been reported to protect against injury in renal disease progression. See A. Benigni, et al, *Kidney International*, 44 (1993), p. 440-444. Inhibition of ECE, which will also make endothelin unavailable to its receptors, can therefore treat disease states characterized by excessive production of endothelin. Therefore, phosphinic acid derivatives of this invention are useful for the treatment of myocardial ischemia, congestive heart failure, arrhythmia, hypertension, pulmonary hypertension, asthma, cerebral vasospasm, subarachnoid hemorrhage, pre-eclampsia, wound healing, control of menstruation, acute/chronic renal failure, renal ischemia, renal glomerulosclerosis, atherosclerosis, Buerger's disease, Takayasu's arteritis, complications in diabetes, pulmonary carcinoma, gastrointestinal disorders, endotoxic shock, and septicemia.

35

SUMMARY OF THE INVENTION

Compounds for use as endothelin converting enzyme inhibitors are represented by the formula I

- 2 -



or a pharmaceutically acceptable salt thereof, wherein

R is H, (C₁-C₈)alkyl or alkanoyloxymethylene;

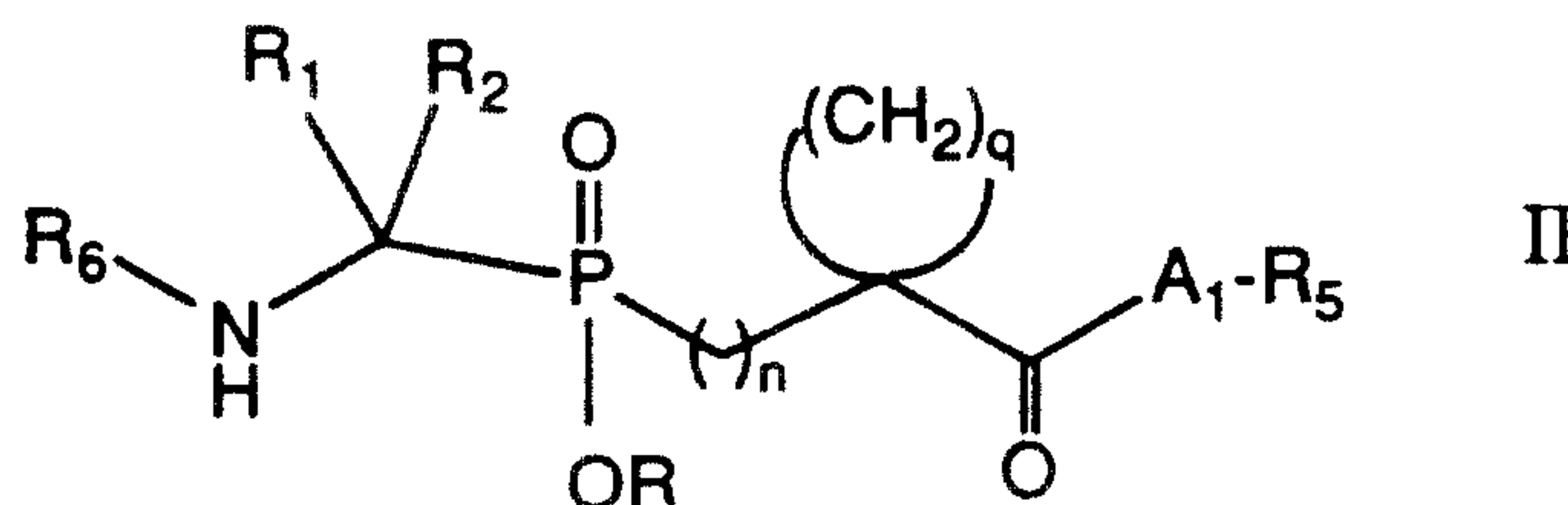
R₁, R₂, R₃ and R₄ are independently selected from the group
 5 consisting of H, (C₁-C₈)alkyl, (C₁-C₈)alkenyl, (C₁-C₈)alkenyl(C₁-C₈)alkyl,
 aryl, aryl(C₁-C₈)alkyl, heteroaryl, heteroaryl(C₁-C₈)alkyl, hydroxy(C₁-
 C₈)alkyl, carboxy(C₁-C₈)alkyl, thio(C₁-C₈)alkyl, (C₁-C₆)alkoxythio-
 (C₁-C₈)alkyl, amino(C₁-C₈)alkyl, (C₁-C₈)alkylamino(C₁-C₈)alkyl,
 10 cycloalkyl-substituted(C₁-C₈)alkyl and heterocycloalkyl; or R₁ and R₂,
 together with the carbon to which they are attached, form a cycloalkyl ring
 of 3 to 8 members and R₃ and R₄ are as defined above; or R₃ and R₄,
 together with the carbon to which they are attached, form a cycloalkyl ring
 of 3 to 7 members and R₁ and R₂ are as defined above; or R₁ and R₂
 together, and R₃ and R₄ together, each form a cycloalkyl ring as defined
 15 above;

R₅ is -OR₉ or -NHR₉, wherein R₉ is hydrogen or (C₁-C₈)alkyl;
 n is 0 or 1;

A₁ is p-aminobenzoyl or p-aminobenzenesulfonyl, or A₁ and
 R₅ together form a radical of an α-aminoacyl derivative, wherein A₁ is a
 20 divalent α-iminoacyl radical attached to the molecule at the α-imino group,
 and wherein R₅ is attached to the acyl terminus of A₁;

R₆ is phenylmethoxycarbonyl, arylcarbonyl, heteroaryl-
 carbonyl or -A₂-R₇, wherein A₂ is a divalent α-iminoacyl radical attached
 to the molecule at the acyl terminus, and wherein R₇ is a substituent on the
 25 α-imino atom selected from the group consisting of H, R₈OCO-, R₈SO₂-
 and R₈NHCO-, wherein R₈ is aryl, arylmethyl or (C₁-C₈)alkyl.

Novel compounds of the present invention are those
 represented by structural formula II:



- 3 -

or a pharmaceutically acceptable salt thereof, wherein

R is H, (C₁-C₈)alkyl or alkanoyloxymethylene;

R₁ and R₂, are independently selected from the group consisting of H, (C₁-C₈)alkyl, (C₁-C₈)alkenyl, (C₁-C₈)alkenyl(C₁-C₈)alkyl, aryl, aryl(C₁-C₈)alkyl, heteroaryl, heteroaryl(C₁-C₈)alkyl, hydroxy-(C₁-C₈)alkyl, carboxy(C₁-C₈)alkyl, thio(C₁-C₈)alkyl, (C₁-C₈)alkoxy-thio(C₁-C₈)alkyl, amino(C₁-C₈)alkyl, (C₁-C₈)alkylamino(C₁-C₈)alkyl, cycloalkyl-substituted(C₁-C₈)alkyl and heterocycloalkyl; or R₁ and R₂, together with the carbon to which they are attached, form a cycloalkyl ring of 3 to 8 members;

R₅ is -OR₉ or -NHR₉, wherein R₉ is hydrogen or (C₁-C₈)alkyl; n is 0 or 1;

q is 2 to 6, preferably 4 to 6;

A₁ is p-aminobenzoyl or p-aminobenzenesulfonyl, or A₁ and R₅ together form a radical of an α-aminoacyl derivative, wherein A₁ is a divalent α-iminoacyl radical attached to the molecule at the α-imino group, and wherein R₅ is attached to the acyl terminus of A₁;

R₆ is phenylmethoxycarbonyl, arylcarbonyl, heteroaryl-carbonyl or -A₂-R₇, wherein A₂ is a divalent α-iminoacyl radical attached to the molecule at the acyl terminus, and wherein R₇ is a substituent on the α-imino atom selected from the group consisting of H, R₈OCO-, R₈SO₂- and R₈NHCO-, wherein R₈ is aryl, arylmethyl or (C₁-C₈)alkyl.

Preferred compounds of formula I are those wherein A₁-R₅ is L-tryptophanyl, L-tyrosinyl or L-O-methyl tyrosinyl. Also preferred are compounds of formula I wherein R₁ and R₂ are independently selected from the group consisting of H, arylalkyl, branched alkyl and cycloalkyl. Especially preferred are compounds wherein one of R₁ and R₂ is hydrogen and the other is benzyl, 2-naphthylmethyl, or branched alkyl.

Another group of preferred compounds is that wherein R₃ and R₄ are independently selected from the group consisting of hydrogen and alkyl, with preferred alkyl groups being iso-propyl and iso-butyl, or R₃ and R₄, together with the carbon to which they are attached, form a cycloalkyl ring of 5 or 7 members.

In preferred compounds of formula I, n is 1. Also preferred are compounds of formula I wherein R is hydrogen and R₅ is hydroxy.

Still another group of preferred compounds of formula I is that wherein R₆ is phenylmethoxycarbonyl or A₂-R₇, wherein A₂ is an

- 4 -

α -imino acyl radical derived from a natural α -amino acid in the *R* or *S* chiral form, preferably lysine, ϵ -CBZ lysine, arginine, isoleucine, leucine, valine, phenylalanine, alanine, glycine or tyrosine, and R_7 is benzyloxy-carbonyl or methansulfonyl.

5 Similarly, preferred compounds of formula II are those wherein A_1 - R_5 is L-tryptophanyl, L-tyrosinyl or L-O-methyl tyrosinyl.

Also preferred are compounds of formula II wherein R_1 and R_2 are independently selected from the group consisting of H, arylalkyl and branched alkyl. Especially preferred are compounds
10 wherein one of R_1 and R_2 is hydrogen and the other is benzyl, 2-naphthylmethyl, or branched alkyl.

In preferred compounds of formula II, n is 1. A preferred value for q is 4. Also preferred are compounds of formula II wherein R is hydrogen and R_5 is hydroxy.

15 Still another group of preferred compounds of formula II is that wherein R_6 is phenylmethoxycarbonyl or A_2 - R_7 , wherein A_2 is an α -imino acyl radical derived from a natural α -amino acid in the *R* or *S* chiral form, preferably lysine, ϵ -CBZ lysine, arginine, isoleucine, leucine, valine, phenylalanine, alanine, glycine or tyrosine, and R_7 is benzyl-
20 oxycarbonyl or methansulfonyl.

This invention also relates to the use of the compounds of formula I as endothelin converting enzyme inhibitors, and especially to the use of compounds of formula II as endothelin converting enzyme
25 inhibitors.

In another aspect, the present invention relates to a pharmaceutical composition comprising a compound of formula II in a pharmaceutically acceptable carrier.

30 BRIEF DESCRIPTION OF THE FIGURES

Figures 1 and 2 are graphs showing the effect of two compounds of the invention (identified below) on pressor responses to BET-1 in anesthetized rats.

Figures 3, 4, 5 and 6 are graphs showing the effects of four
35 compounds of the invention (identified below) on ischemia/hypoxia-induced ET-1 release in isolated perfused guinea pig lungs.

- 5 -

DETAILED DESCRIPTION

As used herein, the term "divalent α -iminoacyl radical" refers

to a radical having the structure
$$\begin{array}{c} \text{O} \\ \parallel \\ -\text{NH}-\text{CH}-\text{C}- \\ | \\ \text{amino acid} \\ \text{side chain} \end{array}$$
. Similarly, the term

"radical of an α -aminoacyl derivative" refers to a radical of the structure

$$\begin{array}{c} \text{O} \\ \parallel \\ -\text{NH}-\text{CH}-\text{C}-\text{R}_5 \\ | \\ \text{amino acid} \\ \text{side chain} \end{array}$$
. The α -iminoacyl radicals and α -aminoacyl

derivatives are derived from natural α -amino acids or esters in the *R* or *S* chiral form.

As used herein, the term "alkyl" means straight or branched alkyl chains of 1 to 8 carbon atoms, "alkoxy" means alkoxy groups having 1 to 6 carbon atoms, and "alkenyl" means straight or branched carbon chains of 1 to 8 carbon atoms having one or more double bonds in the chain, conjugated or unconjugated.

"Cycloalkyl" means saturated carbocyclic rings of 3 to 6 carbon atoms. "Heterocycloalkyl" means saturated rings of 3 to 6 members wherein 1 to 3 ring members can be independently selected from the groups consisting of sulfur, oxygen and nitrogen, for example tetrahydrofuranyl, thiopyranyl, morpholiny, pyrrolidiny, piperidiny or tetrahydrothiophenyl

"Alkanoyloxymethylene" means a group having the formula lower alkyl-C(O)-O-CH₂-.

As used herein, "aryl" means a phenyl, indanyl, naphthyl or tetrahydronaphthyl ring with one or more substituents selected from the group consisting of H, alkyl, -OH, alkoxy, carboxy, halo, aminoalkyl, carbamyl, -NO₂, trifluoromethyl, morpholinecarbonyl, phenyl, phenoxy, benzyloxy and phenylthio, or wherein adjacent alkyl and alkoxy substituents on a phenyl ring form a five or six-membered ring, such as dihydrobenzo-furanyl.

"Heteroaryl" represents furanyl, thienyl, pyrrolyl, or their benzo-fused forms, such as indolyl and benzofuranyl, optionally substituted as described for aryl.

"Halo" refers to fluorine, chlorine, bromine or iodine radicals.

Compounds of the invention have at least one asymmetrical carbon atom and therefore all isomers, including diastereomers and

- 6 -

rotational isomers are contemplated as being part of this invention. The invention includes d and l isomers in both pure form and in admixture, including racemic mixtures. Isomers can be prepared using conventional techniques, either by reacting enantiomeric starting materials or by
5 separating isomers of a compound of formula I or II. Isomers may also include geometric isomers, e.g. when a double bond is present. All such geometric isomers are contemplated for this invention.

Those skilled in the art will appreciate that for some compounds of formula I or II, one isomer will show greater
10 pharmacological activity than another isomer.

Compounds of the invention with an amino group can form pharmaceutically acceptable salts with organic and inorganic acids. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic,
15 ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those in the art. The salt is prepared by contacting the free base form with a sufficient amount of the desired acid to produce a salt. The free base form may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous NaHCO₃. The free
20 base form differs from its respective salt form somewhat in certain physical properties, such as solubility in polar solvents, but the salt is otherwise equivalent to its respective free base forms for purposes of the invention.

Compounds of the invention which are acidic form pharmaceutically acceptable salts with inorganic and organic bases.
25 Examples of such salts are the alkali metal and alkaline earth salts, such as the lithium, sodium, potassium, calcium, aluminum, gold and silver salts. Also included are salts formed with pharmaceutically acceptable amines such as ammonia, alkyl amines, hydroxyalkylamines, N-methylglucamine and the like.

30 Compounds of formulas I and II are prepared by methods known to those skilled in the art, using starting materials which are commercially available or readily prepared using known methods. Following are descriptions which exemplify two processes for preparing the compounds of this invention.

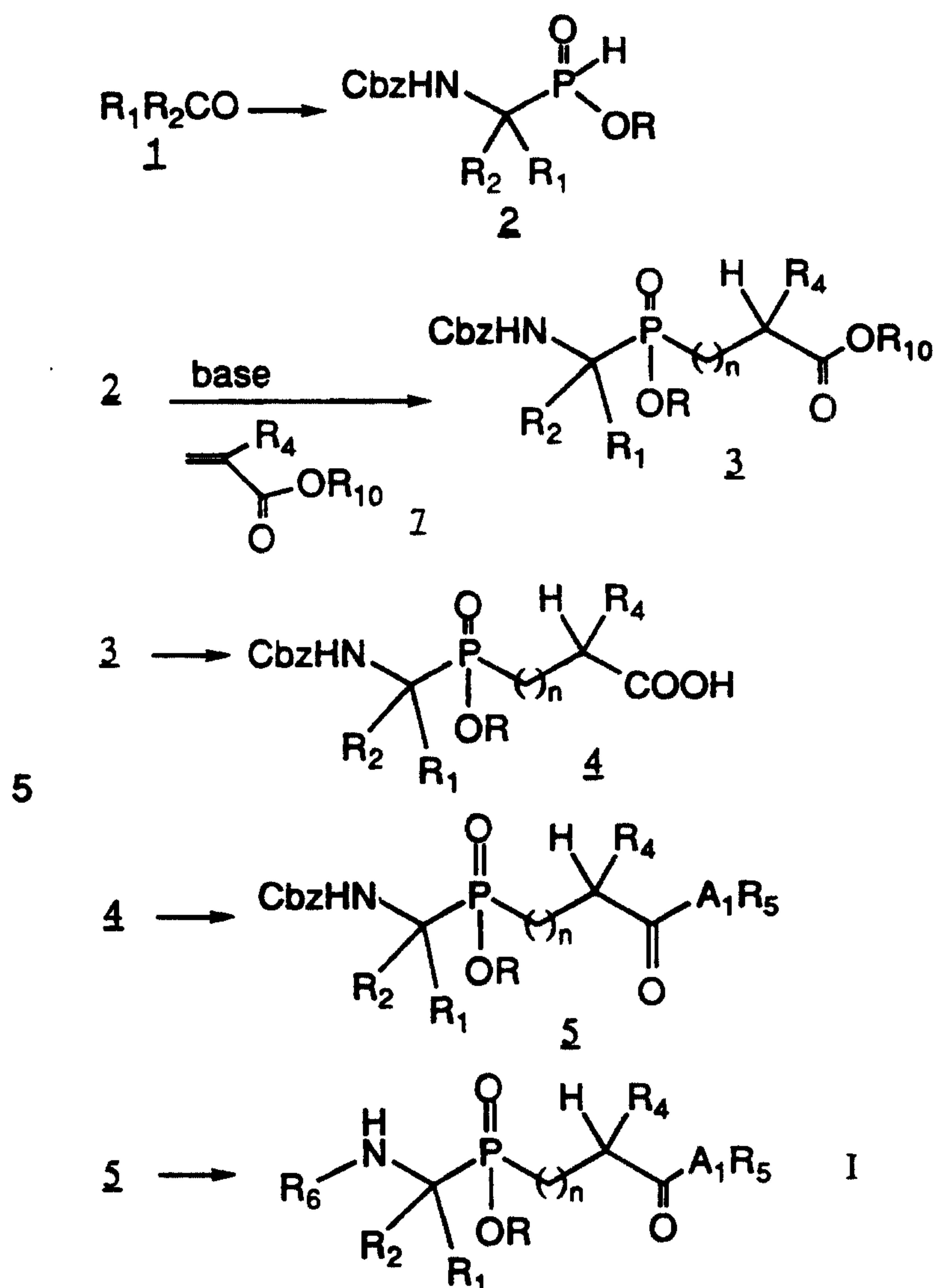
35 Process A:

Compounds of formula I wherein one of R₃ and R₄ is hydrogen and the other is as defined above, and the remaining variables

2191454

- 7 -

are as defined above, can be prepared according to the following reaction scheme, wherein R_3 is exemplified as hydrogen.



- Phosphinic acids **2**, wherein R_1 , R_2 and R are as defined above and Cbz is benzyloxycarbonyl, are known compounds and can be prepared from carbonyl compounds **1** by known methods. Alternatively, the amino group can be protected by other suitable protecting groups, e.g., tert-butoxycarbonyl. The phosphinic acids **2** are converted to the conjugate addition products **3**, wherein n is 1, by known methods or by a variation of known methods where the phosphinic acids **2** are deprotonated with one equivalent of a suitable base such as NaH and reacted in a solvent such as tetrahydrofuran (THF) with an acrylate **7**, wherein R_{10} is a carboxylic acid protecting group such as methyl, ethyl, benzyl, 2-trimethylsilylethyl or 3,4-dimethoxybenzyl. Compounds **3** are deprotected to give acids **4** using standard methodology. Acids **4** are

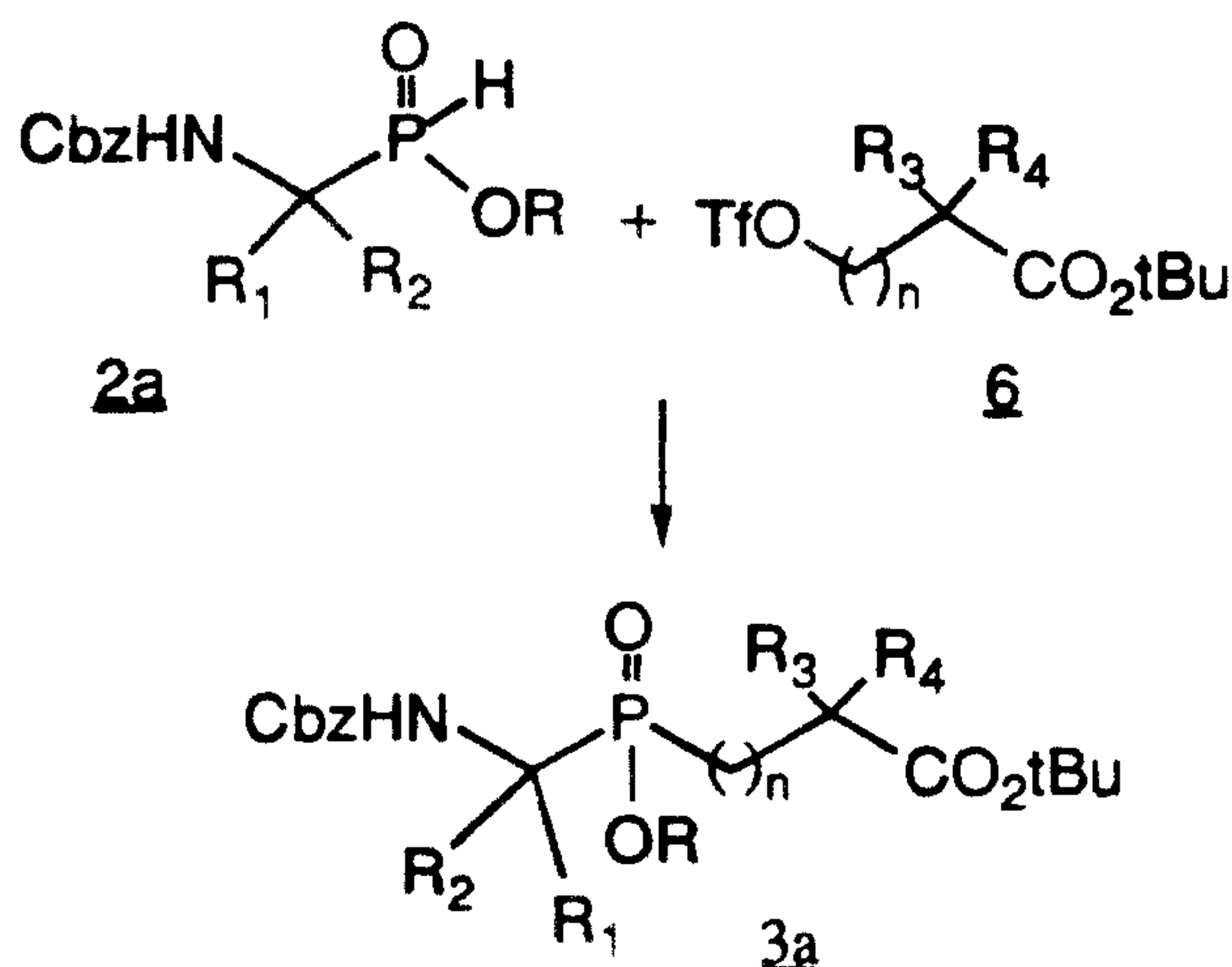
2191454

- 8 -

coupled to an amino acid ester such as tryptophan methyl ester using a suitable coupling agent such as 1,1'-carbonyldiimidazole in an organic solvent such as dimethylformamide (DMF) to give compounds 5.

Compounds 5 can be further elaborated to compounds I by standard peptide coupling procedures and protecting group manipulations where necessary.

Process B:



Phosphinic acid esters 2a, wherein R, R₁, R₂, R₃ and R₄ are as defined above, are deprotonated with a suitable base such as lithium diisopropylamide (LDA) in a suitable solvent such as THF at low temperature, then are reacted with a triflate (Tf) of formula 6 to give a compound 3a. Compound 3a can be elaborated into compounds of formula I by the methods described for Process A.

Starting materials for the above processes are known in the art or can be prepared by known methods, some of which are exemplified in the Preparations below.

The present invention relates to a method of inhibiting ECE, which method comprises administering to a mammal in need of such treatment an ECE inhibitory effective amount of a compound of formula I of this invention. The compound of formula I is preferably administered in a pharmaceutically acceptable carrier, especially a pharmaceutical carrier suitable for oral administration.

In addition to the method of treatment aspect, novel compounds of formula II are also claimed, and therefore the present invention also relates to pharmaceutical compositions comprising an ECE

- 9 -

inhibitory effective amount of a compound of formula II in a pharmaceutically acceptable carrier

The compounds of formula I or II can be administered in any conventional oral dosage form such as capsules, tablets, powders, cachets, suspensions or solutions. The formulations and pharmaceutical compositions can be prepared using conventional pharmaceutically acceptable excipients and additives and conventional techniques. Such pharmaceutically acceptable excipients and additives include non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, anti-oxidants, lubricants, flavorings, thickeners, coloring agents, emulsifiers and the like. Similar pharmaceutical compositions can also be prepared comprising compounds of formula I.

The daily ECE inhibitory dose of a compound of formula I or II is about 0.1 to about 100 mg/kg of body weight per day, preferably about 10 mg/kg. For an average body weight of 70kg, the dosage level is therefore from about 7 mg to about 7 g of drug per day, preferably about 700 mg, given in a single dose or 2-4 divided doses. The exact dose, however, is determined by the attending clinician and is dependent on the potency of the compound administered, the age, weight, condition and response of the patient.

Following are preparations of starting materials and examples of processes for obtaining compounds of formulae I and II. In the preparations and examples, abbreviations are used as follows: R.T. is room temperature, sat'd is saturated, aq. is aqueous, EtOAc is ethyl acetate, Et₂O is diethyl ether, EDC is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBt is 1-hydroxybenzotriazole hydrate, HOAc is acetic acid, Ms is methanesulfonyl, Cbz is carbobenzyloxy and Boc is t-butoxycarbonyl.

Preparation 1

4-((1,1-Dimethylethoxycarbonyl)aminomethyl)benzoic acid

To an ice-cold solution of 4-(aminomethyl)benzoic acid (7.6 g, 50 mmol) in 1M NaOH (50 ml), 1,4-dioxane (100 ml) and H₂O (50 ml) add di-t-butyl dicarbonate (12.0 g, 55 mmol) with rapid stirring. Stir the mixture for 1 h, then evaporate to low volume. Cool the resulting aq. solution in an ice-bath, cover with a layer of EtOAc (250 ml) and acidify with conc. HCl. Extract the aq. layer with EtOAc (2x100 ml), wash the combined EtOAc layers with sat'd NaCl, dry (MgSO₄), filter and evaporate to dryness. Recrystallize (EtOAc/hexanes) the residue to obtain the title

- 10 -

compound (7.78 g, 62%) as needles. Anal. calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57%. Found: C, 62.18; H, 6.82; N, 5.55%. FAB MS m/z 252 (M+H)⁺.

Preparation 2

5

(4-Methoxyphenyl)ethanal

To a stirred solution of methyl 4-methoxyphenylacetate (45.0 g) in toluene (250 ml) cooled to -78 °C, add a 1.5 M solution of DIBALH in toluene (167 ml) over 5 h, maintaining the reaction temperature at -78 °C. Stir for an additional 0.5 h, then pour into 1:1 ice/1 M HCl (1500 ml) and Et₂O (500 ml). Stir the slurry for 1 h, separate the layers and extract the aq. layer with Et₂O (2x500 ml). Wash the combined organic layers with sat'd NaHCO₃ (500 ml) and sat'd NaCl, dry (MgSO₄), filter and evaporate to give an oil. Perform bulb-to-bulb distillation (100-130 °C, 1 mmHg) to obtain the title compound (33.9 g, 90%) as a colorless liquid. ¹H Nmr (CDCl₃) δ 9.71 (1H, t, J=2.4 Hz), 7.12 (2H, m), 6.89 (2H, m), 3.79 (3H, s), 3.61 (2H, m).

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

Prep. 2A: [(4-Phenylmethoxy)phenyl]ethanal

¹H Nmr (CDCl₃) δ 9.76 (1H, t, J=2.4 Hz), 7.49-7.37 (5H, m), 7.17 (2H, m), 7.02 (2H, m), 5.10 (2H, s), 3.66 (2H, m).

Preparation 3

N-(Triphenylmethyl)-(4-methoxyphenyl)acetaldimine

To a stirred slurry of (4-methoxyphenyl)ethanal (28.92 g, 0.193 mol) and MgSO₄ (20 g) in CH₂Cl₂ (100 ml), add a solution of tritylamine (50.0 g, 0.193 mol) in CH₂Cl₂ (180 ml) over 10 min. After 1 h, filter the mixture and evaporate the filtrate to dryness to obtain the title compound as a viscous oil. ¹H Nmr (CDCl₃) δ 7.36-7.15 (16H, m), 7.15 (2H, m), 6.83 (2H, m), 3.77 (3H, s), 3.73 (2H, m).

Using appropriate starting materials and essentially the same procedure, the following compounds are prepared:

Prep. 3A: N-(Triphenylmethyl)-[(4-phenylmethoxy)phenyl]acetaldimine

¹H Nmr (CDCl₃) δ 7.46-7.20 (21H, m), 7.13 (2H, m), 6.93 (2H, m), 5.08 (2H, m), 3.75 (2H, m).

35

Preparation 4

1-Amino-2-(4-methoxy)phenylethyl phosphinic acid

Prepare neat bis(trimethylsilyl)phosphonite from hexamethyl-disilazane (61 ml, 0.29 mol) and ammonium hypophosphite (24 g, 0.29

- 11 -

mol), and dilute with CH₂Cl₂ (100 ml). To a stirred ice-cold solution of Preparation 3 in CH₂Cl₂ (250 ml) under N₂, add the solution of bis(trimethylsilyl)phosphonite via canula. Allow the reaction mixture to reach R.T. After 20 h, cool the reaction mixture in an ice bath and quench with 85% EtOH (250 ml). After 4 days, collect the precipitate, wash with 85% EtOH, air-dry and dry in vacuo to obtain the title compound (23.1 g, 56%) as a white powder. FAB MS *m/z* 216 (M+H)⁺.

Using appropriate starting materials and essentially the same procedure, the following compounds are prepared:

10 **Prep. 4 A: 1-Amino-2-(4-phenylmethoxy)phenylethyl phosphinic acid**
¹H Nmr (DMSO-d₆ + TFA) δ 8.21 (3H, bs), 7.41-7.28 (5H, m), 7.21 (2H, m), 7.03 (1H, d, J=555.5 Hz), 6.95 (2H, m), 5.06 (2H, s), 3.51 (1H, bm), 3.04 (1H, m), 2.81 (1H, td, J=14.4, 8.9 Hz). FAB MS *m/z* 292.1 (M+H)⁺.

Preparation 5

15 **2-(4-Methoxy)phenyl-1(R,S)-[N-[(phenylmethoxy)carbonyl]]-aminoethyl phosphinic acid**

To a stirred solution of Preparation 4 (21.5 g, 0.10 mol) in ice-cold 1M NaOH (100 ml), separately add benzyl chloroformate (16 ml, 0.11 mol) and 1M NaOH (100 ml) and stir for 6 h. Add additional benzyl chloroformate (8 ml, 0.05 mol) and adjust the reaction mixture to pH 9 by addition of 1M NaOH. After 4 h, acidify the reaction mixture to pH 1 by addition of conc. HCl. Collect the precipitate, wash with H₂O, air-dry, then dry in vacuo to obtain the title compound (34.8 g, 100%) as a white powder. FAB MS *m/z* 350 (M+H)⁺.

25 Using essentially the same procedure the following compounds are prepared:

Prep. 5A: 1(R,S)-[N-[(Phenylmethoxy)carbonyl]]amino-2-(4-phenylmethoxy)phenylethyl phosphinic acid
¹H Nmr (DMSO-d₆) δ 7.71 (1H, d, J=9.1 Hz), 7.46-7.28 (8H, m), 7.21 (2H, m), 7.17 (2H, d, J=8.6 Hz), 6.92 (2H, d, J=8.7 Hz), 6.87 (1H, d, J=533.5 Hz), 5.07 (2H, s), 4.96 (2H, m), 3.79 (1H, m), 2.95 (1H, dt, J=14.4, 4.0 Hz), 2.69 (1H, td, J=14.4, 8.2 Hz). FAB MS *m/z* 426.1 (M+H)⁺.

Preparation 6

35 **P(R,S)-2-Phenyl-1(R)-[(phenylmethoxy)carbonyl]aminoethyl phosphinic acid methyl ester**

To a stirred solution of 2-phenyl-1(R)-[(phenylmethoxy)carbonyl]aminoethylphosphinic acid (See Baylis, et al., *J. Chem. Soc. Perkin Trans.*, 1 (1984), p. 2845)(3.12 g, 9.77 mmol) in ice-cold 10:1 1,4-

- 12 -

dioxane/MeOH (150 ml), add excess CH_2N_2 in ether. Add CH_3OH to disperse the resulting suspension and allow the reaction mixture to reach R.T. Destroy excess CH_2N_2 by addition of HOAc. Evaporate the reaction mixture to a syrup, then take up in EtOAc (100 ml), wash with sat'd

5 NaHCO_3 (50 ml) and sat'd NaCl, dry (MgSO_4), filter and evaporate to obtain the title compound (2.72 g, 84%) as a syrup which crystallizes on standing. Mixture of diastereomers at phosphorous. ^1H Nmr (CDCl_3) δ 7.32-7.17 (10 H, m), 7.01* (0.4 H, d, $J=558.8$ Hz) and 6.99* (0.6 H, d, $J=559.1$ Hz), 5.18* (0.6 H, d, $J=9.2$ Hz) and 5.09* (0.4 H, d, $J=9.0$ Hz),

10 5.00* (0.8 H, s) and 4.98* (1.2 H, s), 4.21 (1H, m), 3.73* (1.8 H, d, $J=11.4$ Hz) and 3.72* (1.2 Hd, $J=11.4$ Hz), 3.15 (1H, m), 2.91 (1H, m).

* Diastereomeric signals.

Using appropriate starting materials and essentially the same procedure the following compound is prepared:

15 **Prep. 6A:** P(R,S)-1(R,S)-[(Phenylmethoxy)carbonyl]amino-2-(4-phenylmethoxy)phenylethyl phosphinic acid methyl ester

Mixture of diastereomers at phosphorous. ^1H Nmr (CDCl_3) δ 7.44-7.21 (10 H, m), 7.15-7.06 (2H, m), 7.04* (0.5 H, d, $J=556.0$ Hz), 7.02* (0.5 H, d, $J=556.0$ Hz), 6.88 (2H, m), 5.08-4.97 (5H, m), 4.21 (1H, m), 3.76* (1.5 H, d, $J=11.9$ Hz), 3.74* (1.5 H, d, $J=11.9$ Hz), 3.12 (1H, m), 2.89 (1H, m).

20

* Diastereomeric signals.

Preparation 7

P(R,S)-2-(4-Methoxy)phenyl-1(R,S)-[(phenylmethoxy)carbonyl]aminoethyl phosphinic acid methyl ester

25 To a stirred ice-cold suspension of Preparation 5 (6.00 g, 17.2 mmol) and 4-dimethylaminopyridine (42 mg, 0.34 mmol) in 6:1 THF/ CH_3OH (60 ml), add EDC (3.62 g, 18.9 mmol). After 20 min., remove the ice bath, stir the reaction mixture for 4 h, then evaporate to a syrup. Partition the syrup between EtOAc (60 ml) and 1M HCl (60 ml). Extract the

30 aq. layer with EtOAc (2x60 ml) and wash the combined organic layers with H_2O (100 ml), 10% NaHCO_3 (100 ml) and sat'd NaCl, then dry (MgSO_4), filter and evaporate to obtain the title compound (5.40 g, 87%) as a syrup. FAB MS m/z 364.2 ($\text{M}+\text{H}$) $^+$.

Preparation 8

35 t-Butyl Cyclopentanecarboxylate

Cool a metal bomb in a CO_2 -acetone bath and charge with isobutylene (150 ml), cyclopentanecarboxylic acid (29.4 g, 0.26 mol), t-butanol (4 ml) and H_2SO_4 (1 ml). Remove the bomb from the cold-bath,

- 13 -

seal and stir for 3 days. Cool the bomb in a CO₂-acetone bath, open to the atmosphere and evaporate isobutylene under a stream of N₂ while allowing the reaction mixture to attain R.T. Add Et₂O (300 ml) and wash the mixture with sat'd NaHCO₃ (300 ml) and sat'd NaCl, then dry (MgSO₄),
5 filter and concentrate to obtain a colorless oil (41.5 g, 95%) which is used without further purification. ¹H Nmr (CDCl₃) δ 2.58 (1H, m), 1.90-1.35 (8H, m), 1.39 (9H, s).

Preparation 9

t-Butyl 1-hydroxymethylcyclopentanecarboxylate

10 To a stirred, ice-cold solution of diisopropylamine (34 ml, 0.24 mol) in THF (800 ml), add a solution of n-BuLi in hexanes (2.5 M, 100 ml, 0.25 mol). After 0.5 h, cool the mixture to -78 °C and add a solution of Preparation 8 (41 g, 0.24 mol) in THF (100 ml) via canula under N₂ over 0.5 h. After 1 h, to the stirred -78 °C solution, add paraformaldehyde (36 g,
15 5 equiv.), stir at -78 °C for 1 h, then remove the cold bath. Stir the resulting gel-like suspension for 4 h, then quench with sat'd NH₄Cl (800 ml). Extract with EtOAc (2x800 ml), wash the combined EtOAc extracts with 1 M HCl and sat'd NaCl, dry (MgSO₄), filter and concentrate. Distill the crude product to obtain the title compound (42.5 g, 89%) as a colorless liquid.
20 B.p. 100-110° (0.4 mmHg). ¹H Nmr (CDCl₃) δ 2.58 (1H, m), 1.90-1.35 (8H, m), 1.39 (9H, s).

Preparation 10

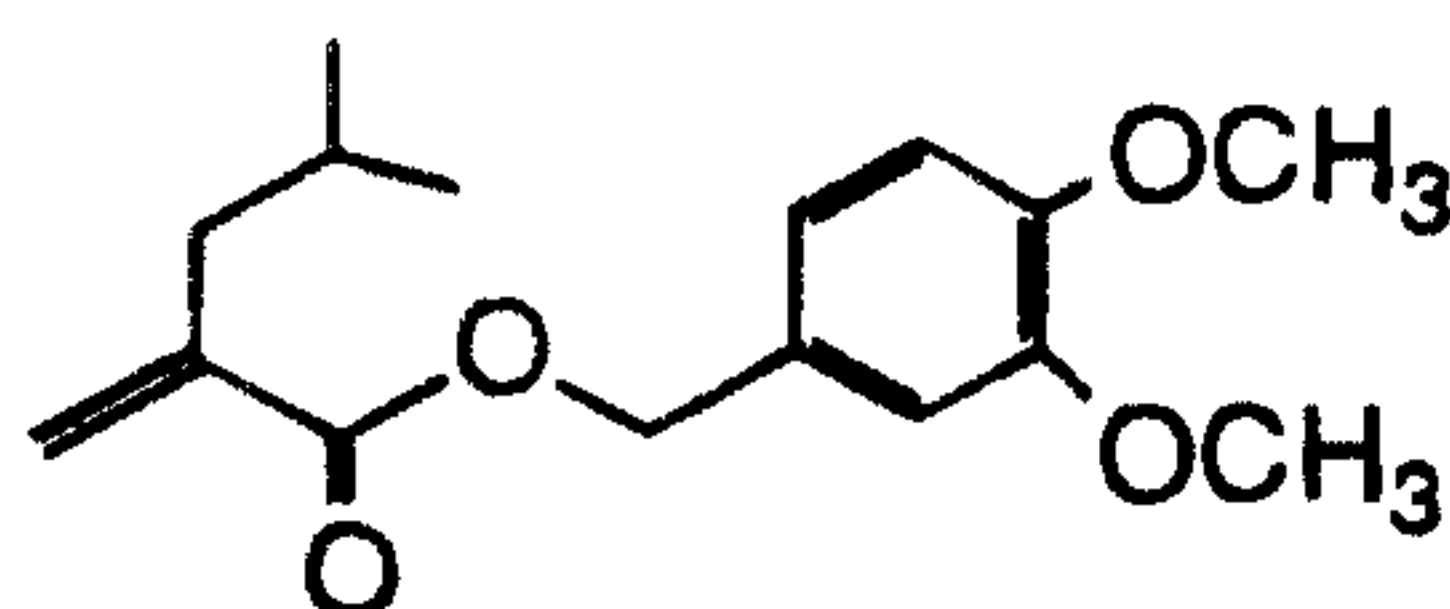
t-Butyl 1-[(trifluoromethanesulfonyl)oxy]methylcyclopentanecarboxylate

To a stirred solution of pyridine (10 ml, 124 mmol) in CH₂Cl₂
25 (75 ml) cooled to -78 °C under N₂, add a solution of triflic anhydride (10 g, 35.5 mmol) in CH₂Cl₂ (10 ml) dropwise via an addition funnel. After 10 min, add, dropwise, a solution of Preparation 9 (4.73 g, 23.7 mmol) in CH₂Cl₂ (20 ml). Stir the resulting reaction mixture at -78 °C for 0.5 h, then remove the cold-bath. When the reaction mixture reaches R.T., pour into
30 hexanes (500 ml), wash with 1 m HCl (3x200 ml), H₂O (200 ml), sat'd NaHCO₃ (200 ml) and sat'd NaCl, then dry (MgSO₄), filter and evaporate to dryness. The resulting reddish oil (7.34 g, 93%) solidifies on storage at freezer temperature, and is used without further purification. ¹H Nmr (CDCl₃) δ 4.51 (2H, s), 2.11-2.05 (2H, m), 1.79-1.54 (4H, m), 1.44 (9H, s).

35

2191454

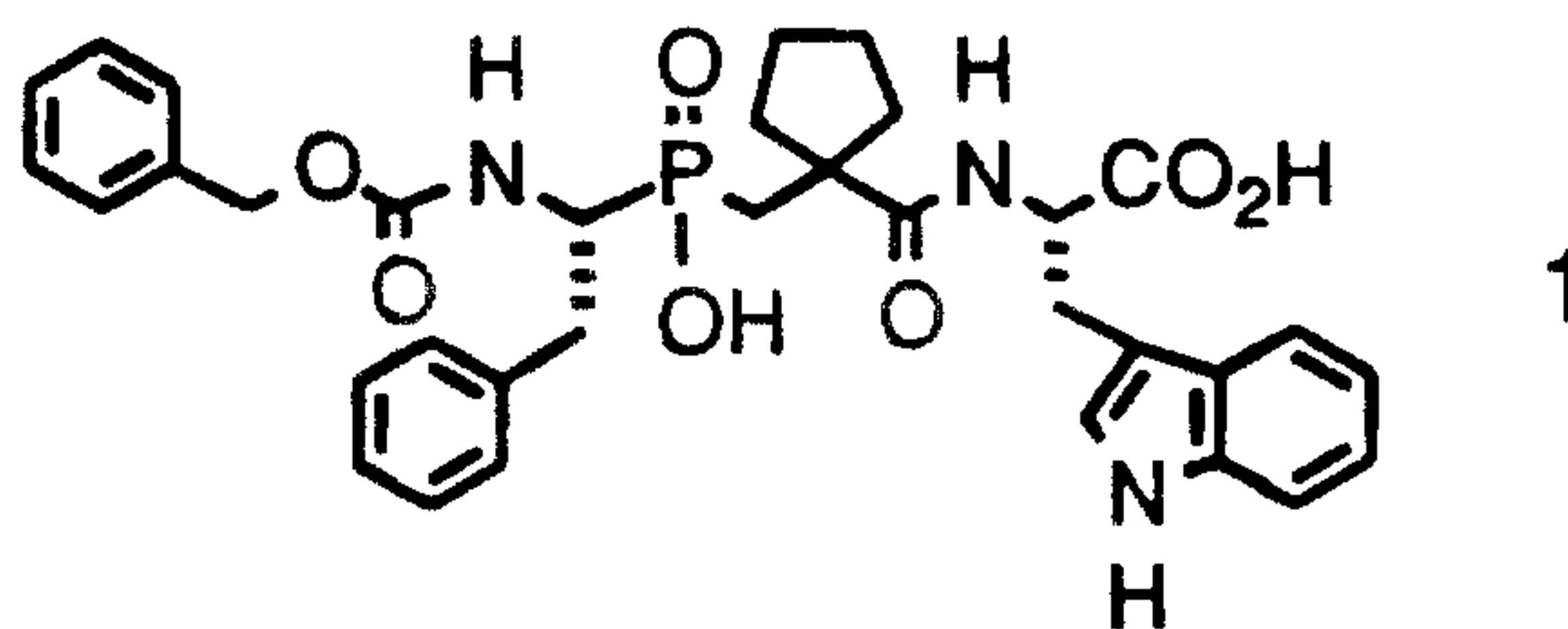
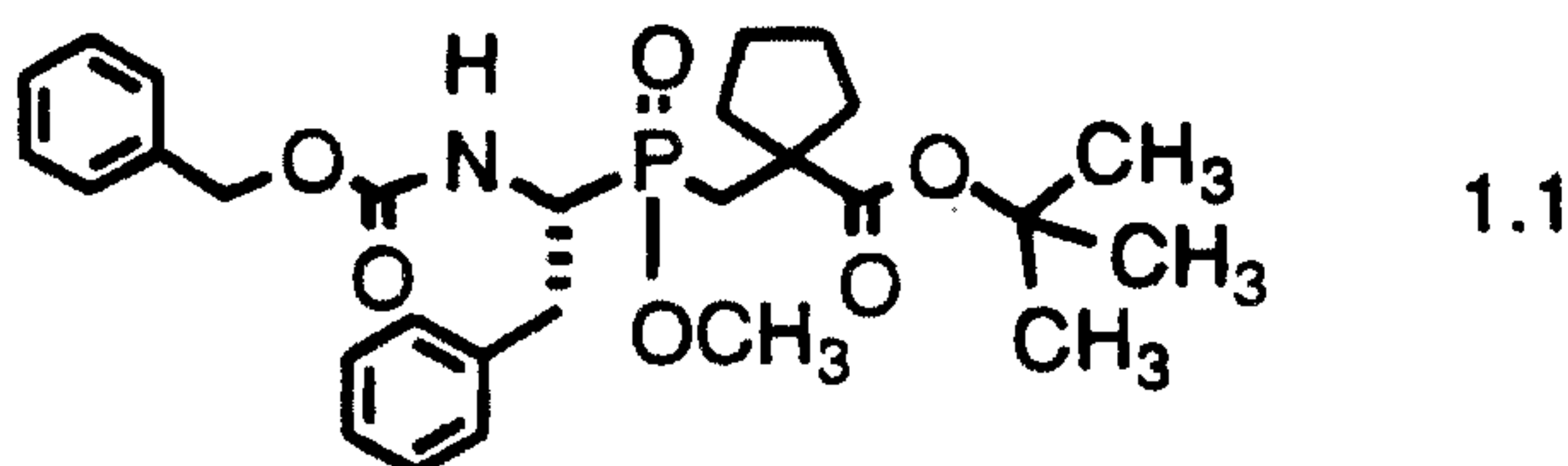
- 14 -

Preparation 11

Step 1: Add aq. formaldehyde solution (37%, 16.1 ml) to an aq. solution of $(\text{CH}_3)_2\text{NH}$ (40%, 12.5 ml) cooled in an ice bath. Stir the mixture for 10 min, then add isobutylmalonic acid (16.7g, 104 mmole). Allow the reaction mixture to warm to R.T., then heat at 90°C for 1 hr. Cool the reaction mixture to R.T., acidify with 1N HCl to pH 1 and extract with Et_2O . Dry the Et_2O layer over MgSO_4 and concentrate under reduced pressure.

Step 2: Cool a solution of the product of Step 1 in CH_2Cl_2 (90 ml) in an ice bath and add 3,4-dimethoxybenzylalcohol (29.2 g, 174 mmol), dimethylaminopyridine (750 mg, 6 mmol) and a solution of DCC (30.8 g, 150 mmol) in CH_2Cl_2 (180 ml). Stir the reaction mixture at 0°C for 1 hr, then keep in the freezer (-20°C) for 18 hrs. Filter the reaction mixture, wash the filtrate with CH_2Cl_2 , concentrate *in vacuo* and purify the crude product by chromatography on silica gel, eluting with 10% EtOAc in hexane to obtain 22 g (76% of theory) of the title compound. ^1H NMR (400 MHz, CDCl_3) δ 6.82-6.98 (m, 3H), 6.2 (s, 1H), 5.5 (s, 1H), 3.80 (s, 6H), 2.20 (d, J=6.7 Hz, 2H), 1.82 (m, 1H), 0.90 (d, J=6.7 Hz, 6H).

20

Example 1**Step 1:**

To a stirred ice-cold solution of diisopropylamine (1.93 ml, 13.8 mmol) in THF (15 ml), add a solution of n-butyllithium in hexanes (1.6 M, 8.6 ml, 13.8 mmol) under N_2 . After 15 min, cool the reaction mixture to -78 °C. To this LDA solution at -78 °C, add a solution of Preparation 6 (4.20 g, 12.5 mmol) in THF (25 ml) at such a rate that the temperature

- 15 -

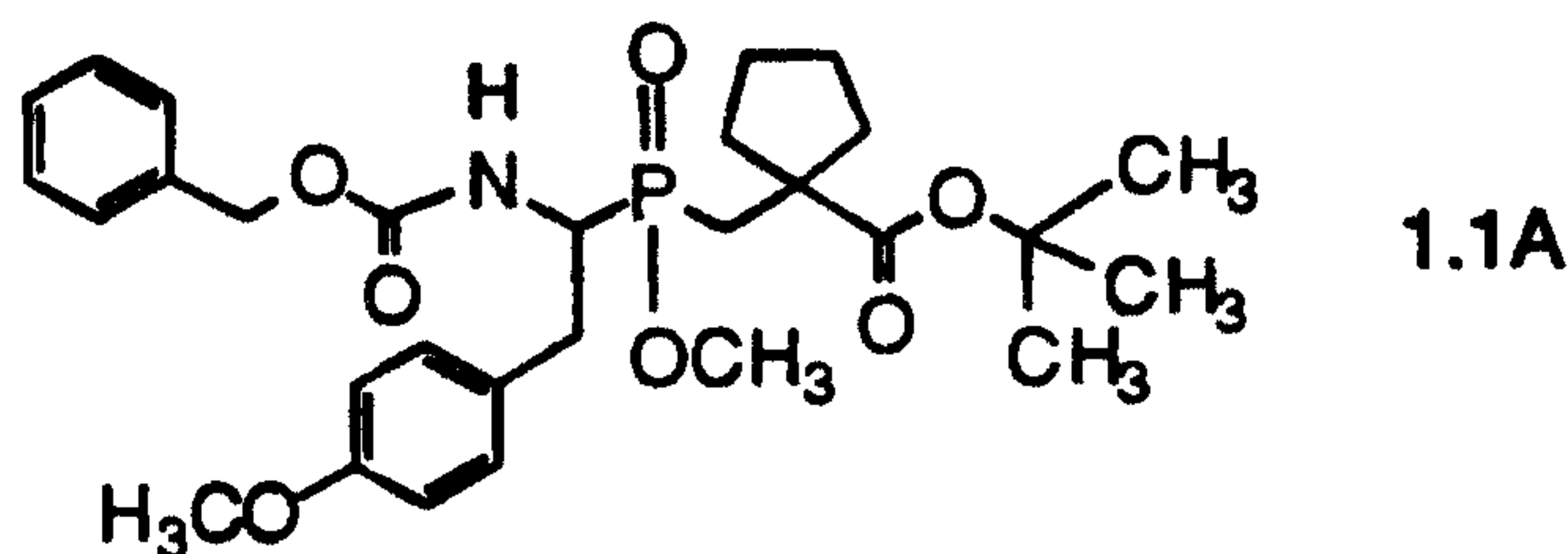
remains below -60 °C. After the addition is complete, stir the reaction mixture for 10 min., then add a solution of Preparation 10 (4.98 g, 15.0 mmol) in THF (12 ml). Stir the reaction mixture at -78 °C for 15 min, then remove the cold-bath. After 4 h, quench the reaction mixture with sat'd

5 NH₄Cl (200 ml) and extract with EtOAc (2x200 ml). Wash the combined organic extracts with 1M HCl (400 ml), sat'd NaHCO₃ (400 ml) and sat'd NaCl, then dry (MgSO₄), filter and evaporate. Flash chromatography of the residue (3:2 EtOAc/hexanes) yields compound 1.1 (3.78 g, 59 %) as a white solid. ¹H Nmr analysis shows a 2:1 mixture of diastereomers at

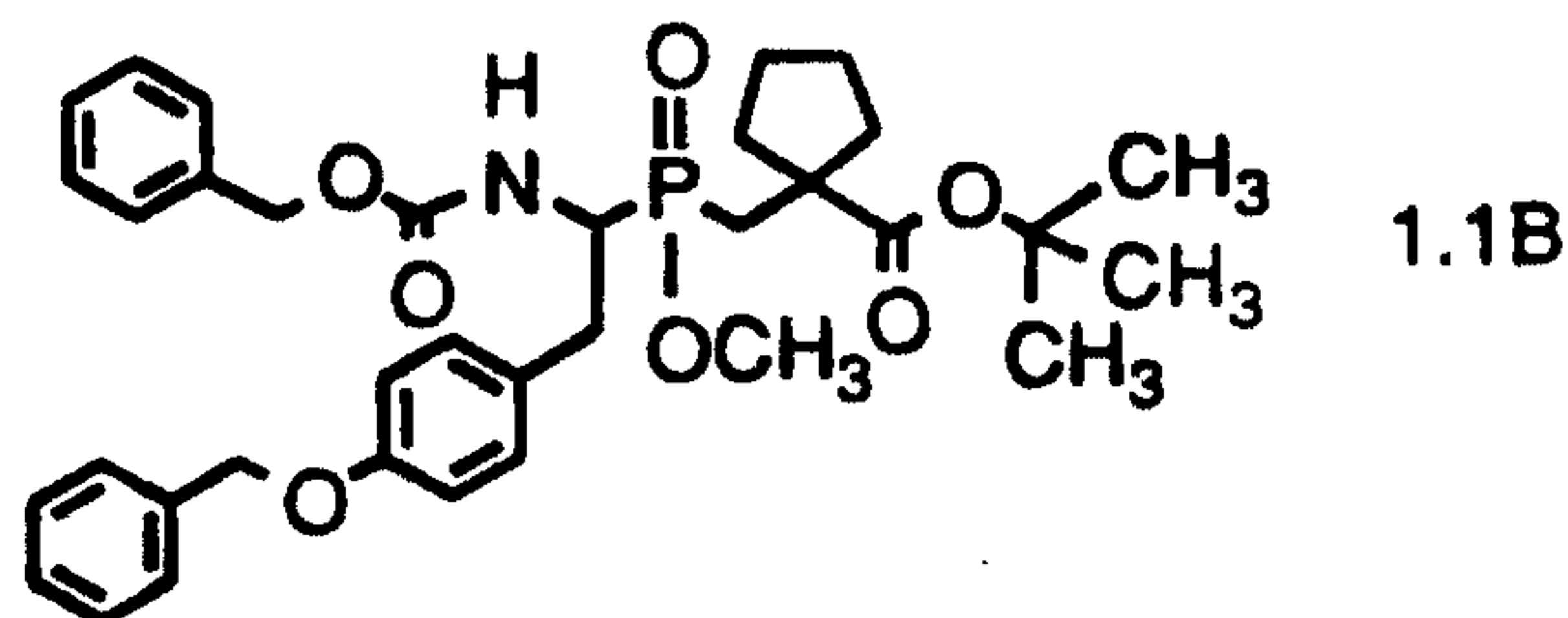
10 phosphorous. Major diastereomer; ¹H Nmr (CDCl₃) δ 7.38-7.07 (10 H, m), 5.06-4.95 (3 H, m), 4.28 (1H, m), 3.64 (3H, d, J=10.3 Hz), 3.32 (1H, m), 2.82 (1H, m), 2.31-2.03 (4H, m), 1.83-1.50 (6 H, s), 1.48 (9H, s). Minor diastereomer; ¹H Nmr (CDCl₃) δ 7.38-7.07 (10 H, m), 5.22 (1H, d, J=9.6 Hz), 5.06-4.95 (2 H, m), 4.28 (1H, m), 3.74 (3H, d, J=10.3 Hz), 3.22 (1H, m),

15 2.82 (1H, m), 2.31-2.03 (4H, m), 1.83-1.50 (6 H, m), 1.46 (9H, s). FAB HRMS calcd for C₂₈H₃₉NO₆P 516.2515 (M+H)⁺. Found 516.2532.

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

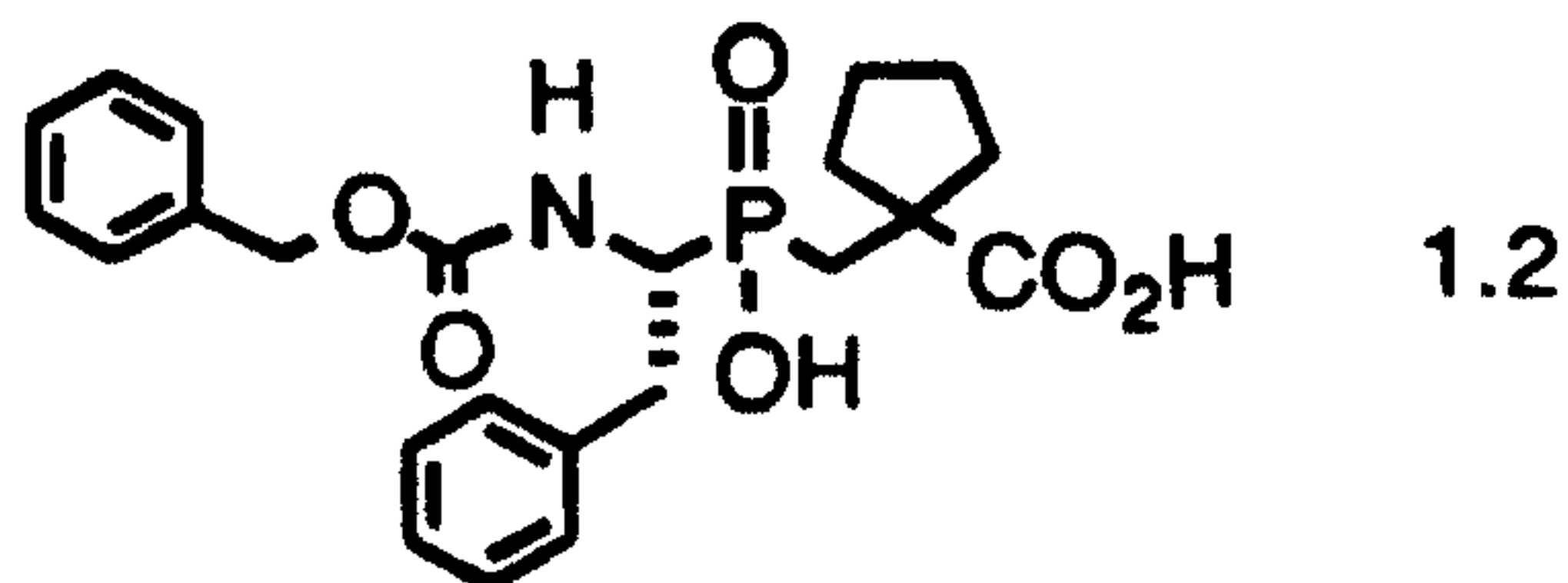


20 ¹H Nmr (CDCl₃) δ 7.36-7.08 (7 H, m), 6.84-6.76 (2 H, m), 5.21-4.92 (3H, m), 4.23 (1H, m), 3.79 (3H, s), 3.74-3.59 (3H, m), 3.28-3.09 (1H, m), 2.80 (1H, m), 2.30-1.99 (4H, m), 1.78-1.50 (6H, m), 1.46 (9H, s).



FAB MS *m/z* 622.4 (M+H)⁺.

25 Step 2:

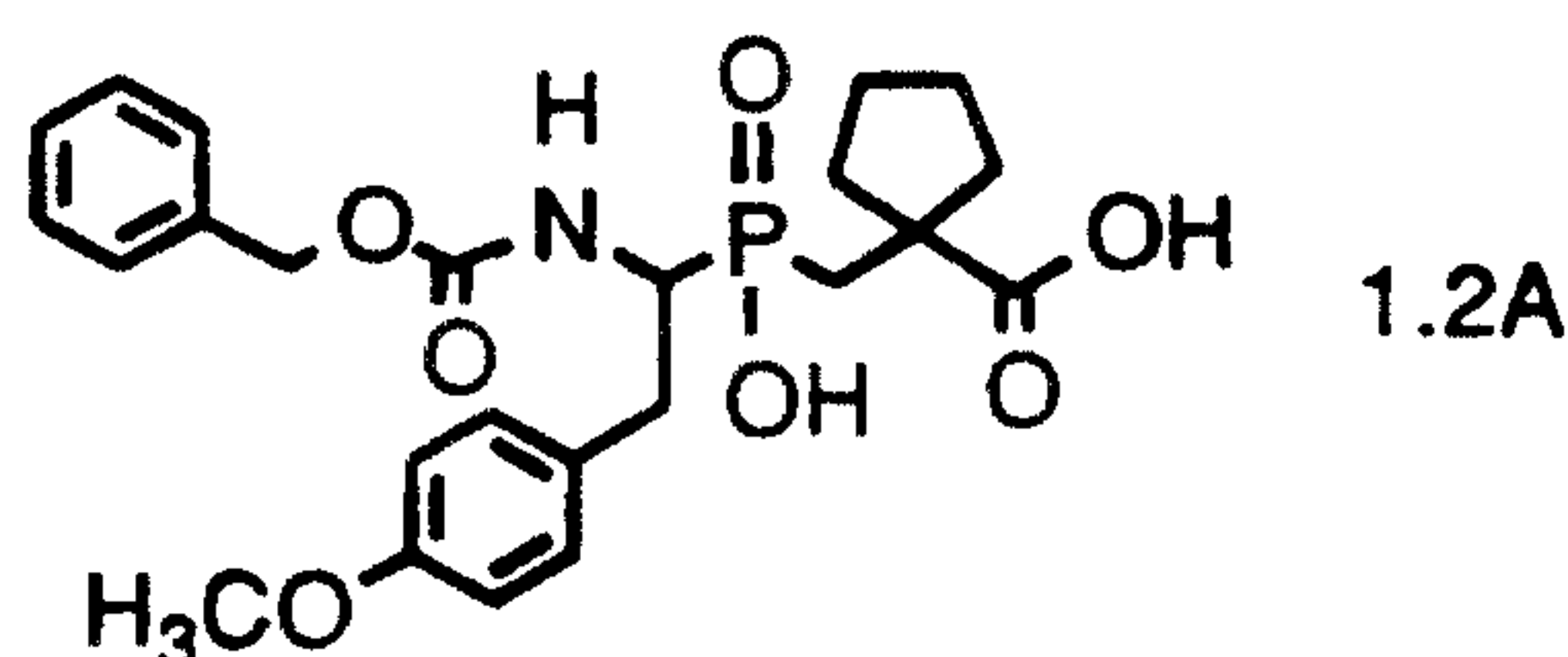


- 16 -

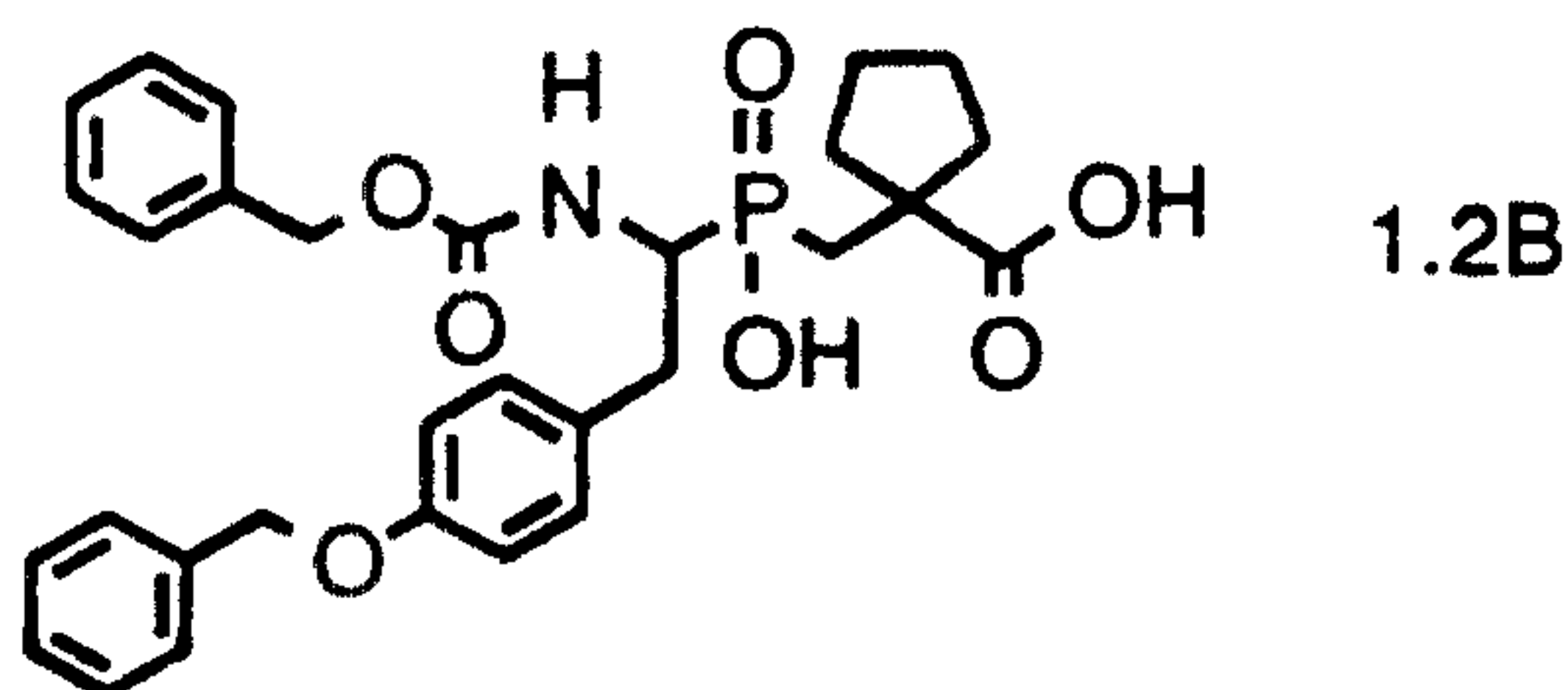
Method (i): To a stirred ice-cold solution of Example 1.1 (7.50 g, 14.6 mmol) in CH_2Cl_2 (120 ml), add $\text{CF}_3\text{CO}_2\text{H}$ (20 ml). After 1 h, add $\text{CF}_3\text{CO}_2\text{H}$ (20 ml) at hourly intervals until TLC shows no starting material (ca 4.5 h). Evaporate the reaction mixture to a syrup, then take up in CH_2Cl_2 and evaporate to dryness (2x). Crystallize the residue from EtOAc to obtain compound 1.2 (5.77 g, 89%). $[\alpha]_D -52^\circ$ (c 0.605, DMF). Anal. calcd for $\text{C}_{23}\text{H}_{28}\text{NO}_6\text{P}$: C, 62.02; H, 6.34; N, 3.14; P, 6.95%. Found: C, 62.24; H, 6.09; N, 3.25; P, 6.97%.

Method (ii): Dissolve compound 1.1 (2.144 g, 4.16 mmol) in 4 M HCl/1,4-dioxane (45 ml). Stir the reaction mixture for 4 h, then add additional 4M HCl/1,4-dioxane (45 ml). After 6 h, evaporate the reaction mixture to a syrup and take up in 1M NaOH (150 ml). Wash the aq. solution with EtOAc (2x100ml) and acidify the aq. layer to pH 1 with conc. HCl. Extract the aq. emulsion with EtOAc (150 ml), then saturate the aq. layer with NaCl and extract with EtOAc (350 ml). Wash the combined EtOAc layers with sat'd NaCl, dry (MgSO_4), filter and evaporate to dryness to obtain compound 1.2 (1.810 g, 98%) as a white solid.

Using appropriate starting materials and essentially the same procedure described in Method (ii) the following compounds are prepared:

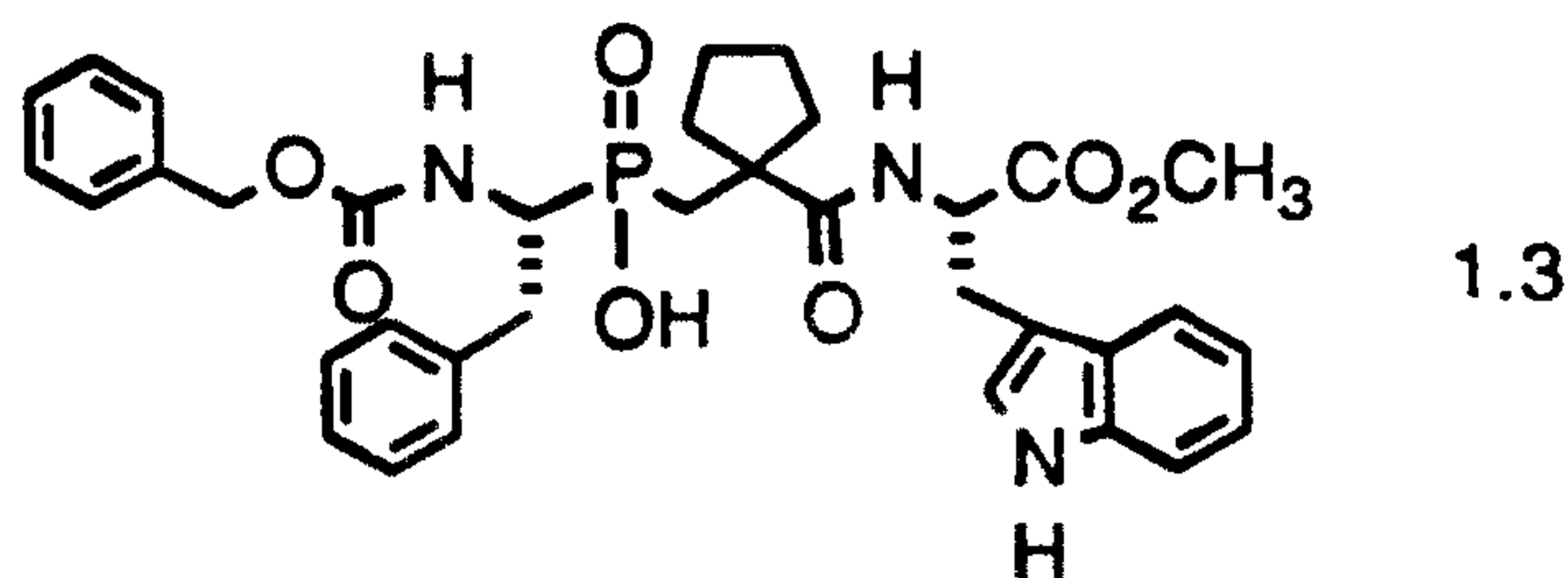


FAB HRMS Calcd for $\text{C}_{24}\text{H}_{30}\text{NO}_7\text{P}$: 476.1838; found 476.1850.



FAB MS m/z 552.2 ($\text{M}+\text{H}$) $^+$.

25 Step 3:

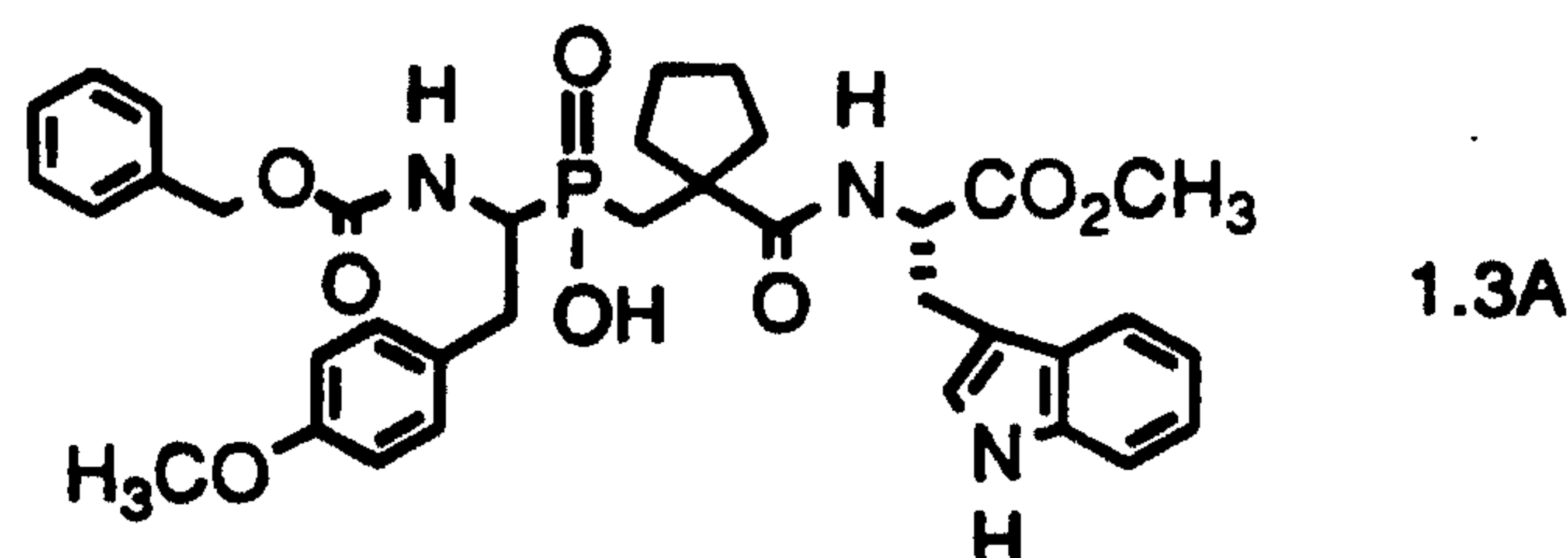


2191454

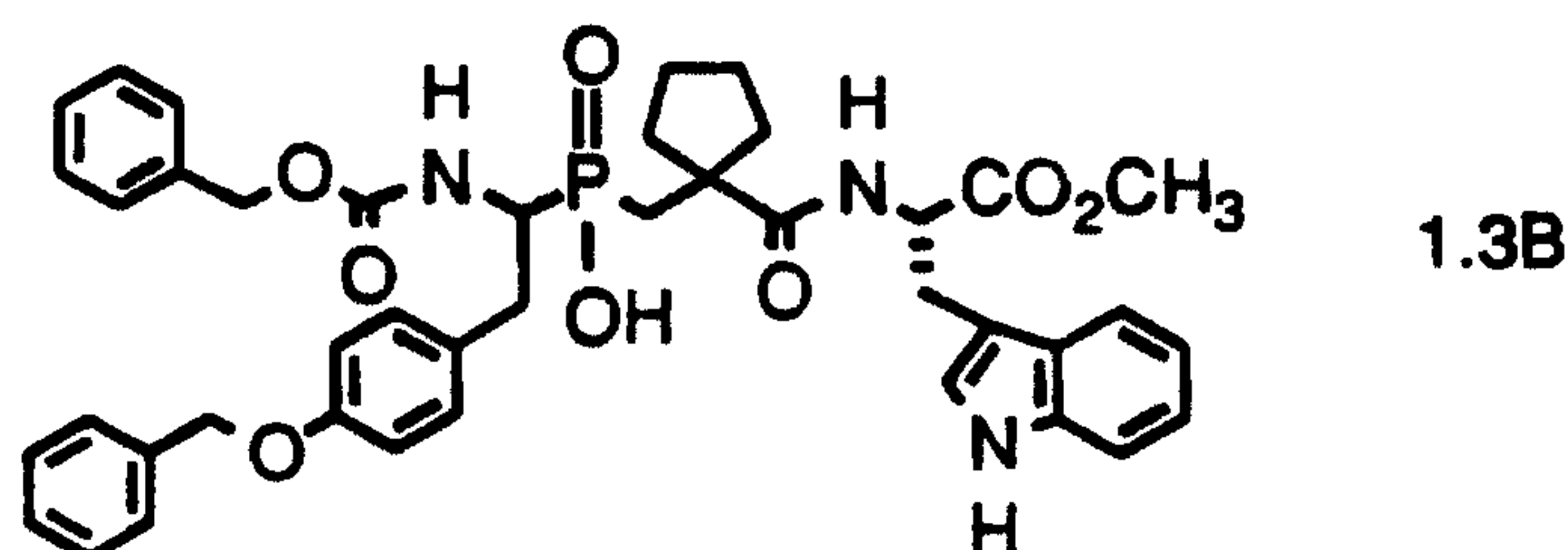
- 17 -

To a stirred ice-cold solution of Example 1.2 (1.505 g, 3.38 mmol) in DMF (18 ml), add 1,1'-carbonyldiimidazole (0.823 g, 5.07 mmol). After 0.5 h, allow the reaction mixture to warm to R.T., and after a further 1 h, add L-tryptophan methyl ester hydrochloride (1.72 g, 6.75 mmol) followed by N-methylmorpholine (0.74 ml, 6.7 mmol). Stir the reaction mixture for 48 h, then partition between EtOAc (200 ml) and 1 M HCl (200 ml). Saturate the aq. layer with NaCl and extract with EtOAc (200 ml). Wash the combined EtOAc layers several times with H₂O, once with sat'd NaCl, then dry (MgSO₄), filter and evaporate. Flash chromatography of the residue (1:24 MeOH/CH₂Cl₂ then 1:2:47 CH₃CO₂H/MeOH/CH₂Cl₂) yields compound 1.3 (1.740 g, 80%) as a beige foam. FAB HRMS calcd for C₃₅H₄₁N₃O₇P: (M+H)⁺ 646.2682; found 646.2698.

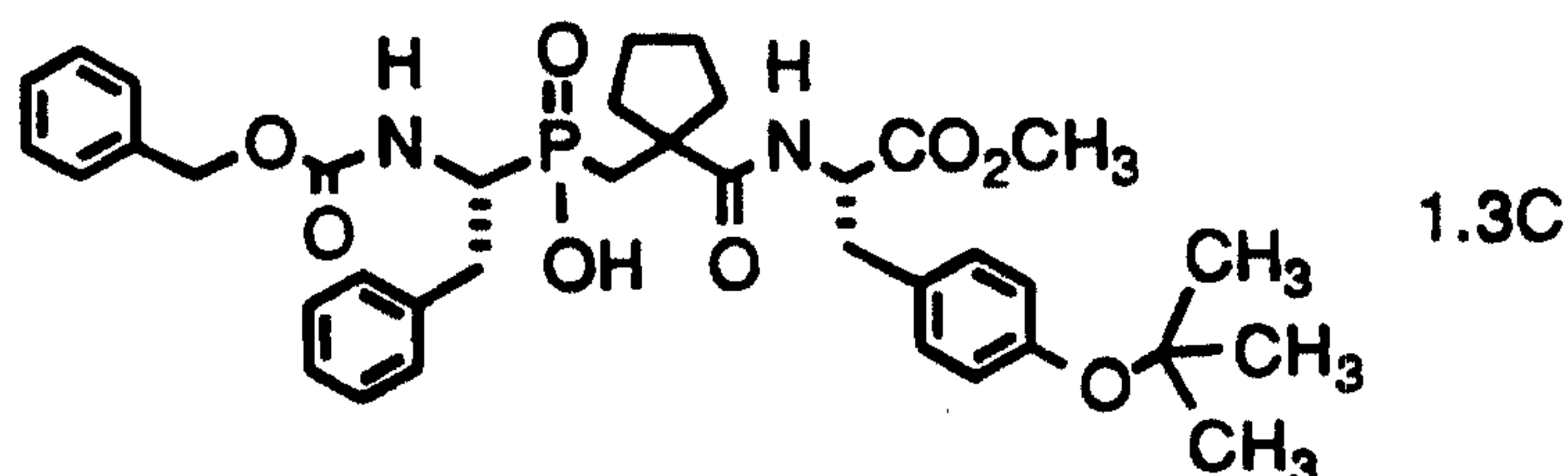
Using appropriate starting materials and essentially the same procedure the following compounds are prepared:



FAB HRMS Calcd for C₃₆H₄₃N₃O₈P: 676.2788 (M+H)⁺; found 676.2778.



FAB MS *m/z* 752.4 (M+H)⁺.



FAB MS *m/z* 679 (M+H)⁺

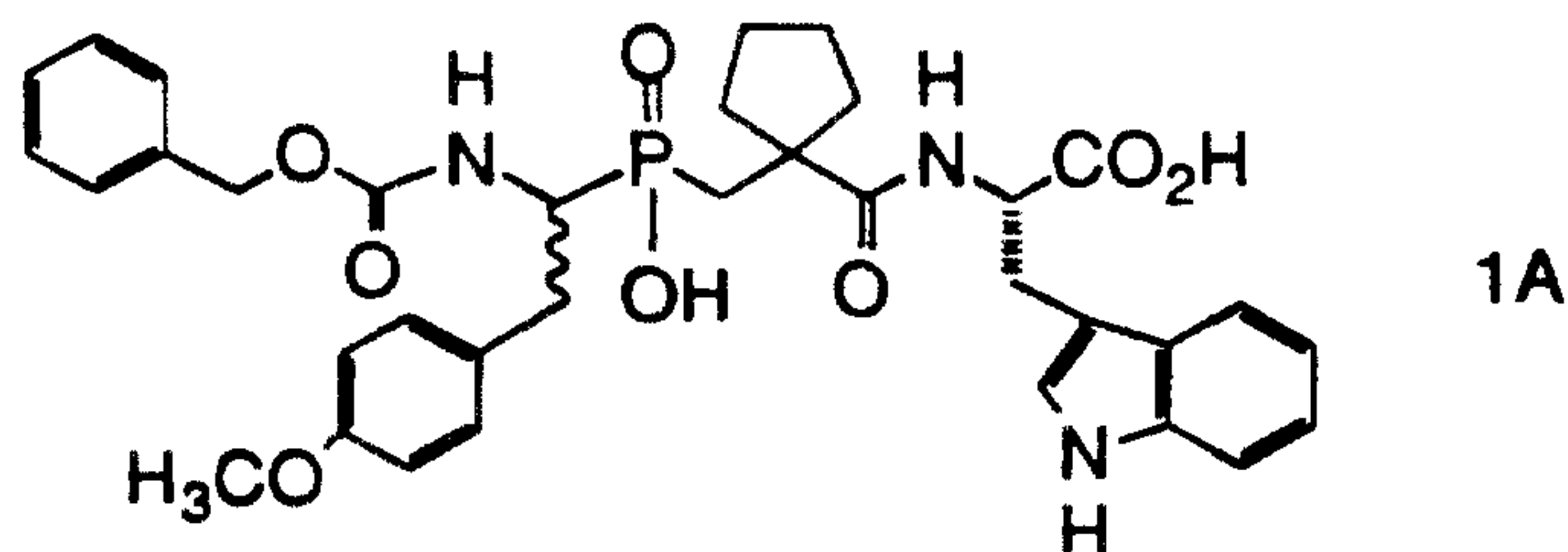
Step 4: Stir a mixture of Example 1.3 (200 mg, 0.31 mmol) and LiOH·H₂O (33 mg, 0.79 mmol) in 3:2 CH₃OH/H₂O (5 ml) at R.T. for 4 h. Concentrate the reaction mixture in vacuo and acidify the resulting aq. suspension to pH 1 with dilute HCl. Filter the precipitate, wash it with H₂O, air-dry, then dry in vacuo to give compound 1 (182 mg, 93%) as a white solid. Anal.

- 18 -

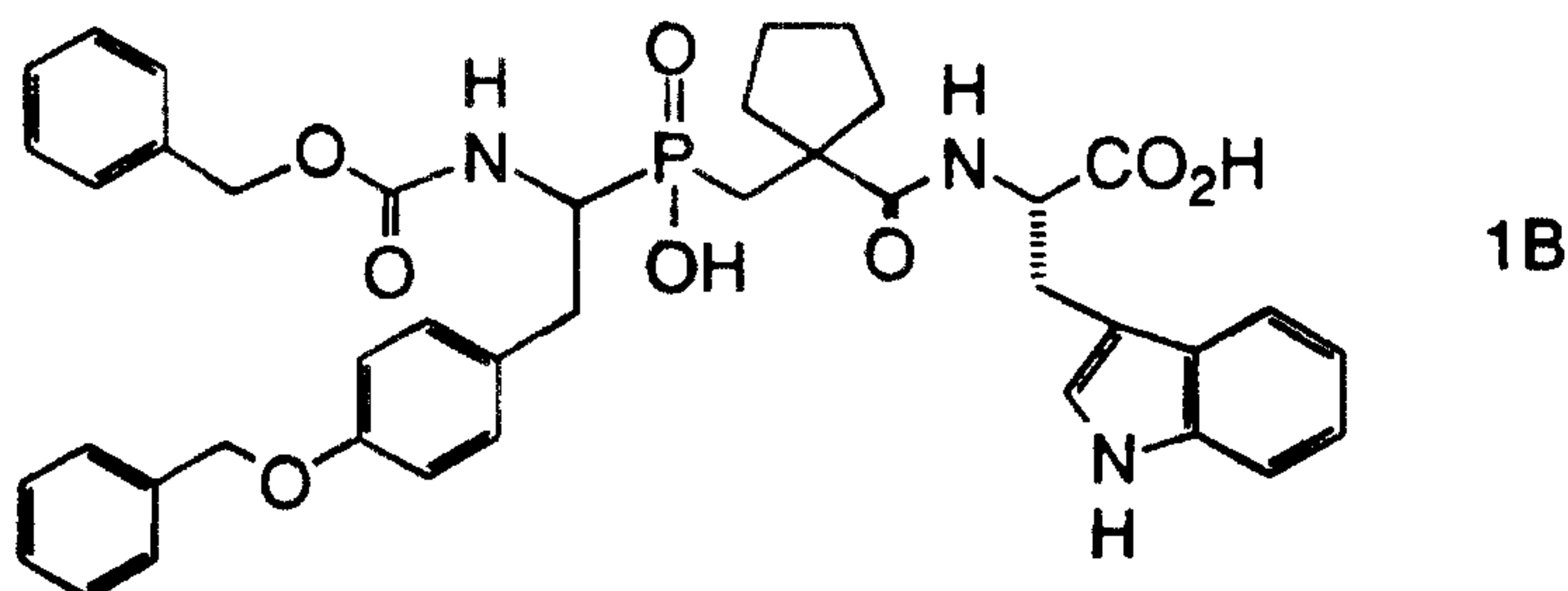
calcd for $C_{34}H_{38}N_3O_7P \cdot H_2O$: C, 62.86; H, 6.21; N, 6.47; P, 4.77%. Found: C, 63.19; H, 5.92; N, 6.40; P, 4.72%. FAB MS m/z 632 ($M+H$)⁺.

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

5



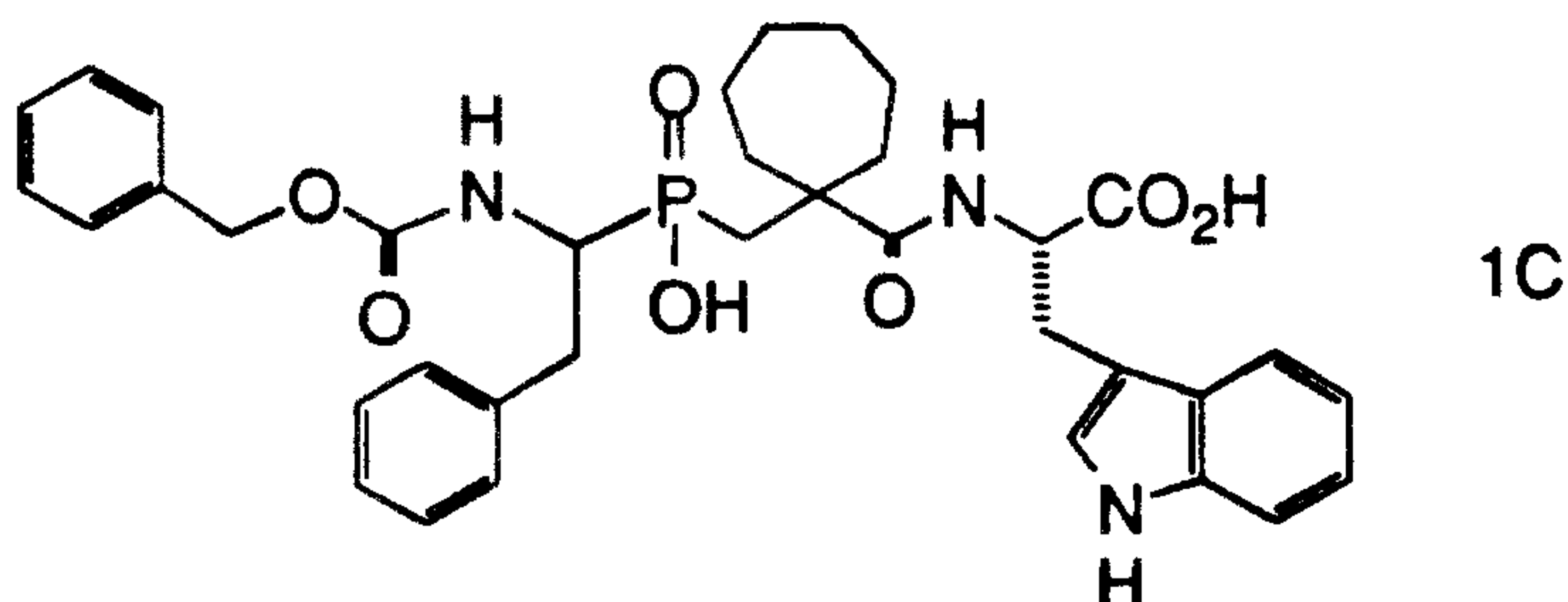
FAB HRMS Calcd for $C_{35}H_{41}N_3O_8P$: 662.2631 ($M+H$)⁺; found 662.2648.



Compound 1B is a mixture of 2 diastereomers. Anal. calcd for $C_{41}H_{44}N_3O_8P \cdot 1.2H_2O$: C, 64.84; H, 6.16; N, 5.53; P, 4.08%. Found: C, 64.61; H, 5.81; N, 5.44; P, 3.97%.

10

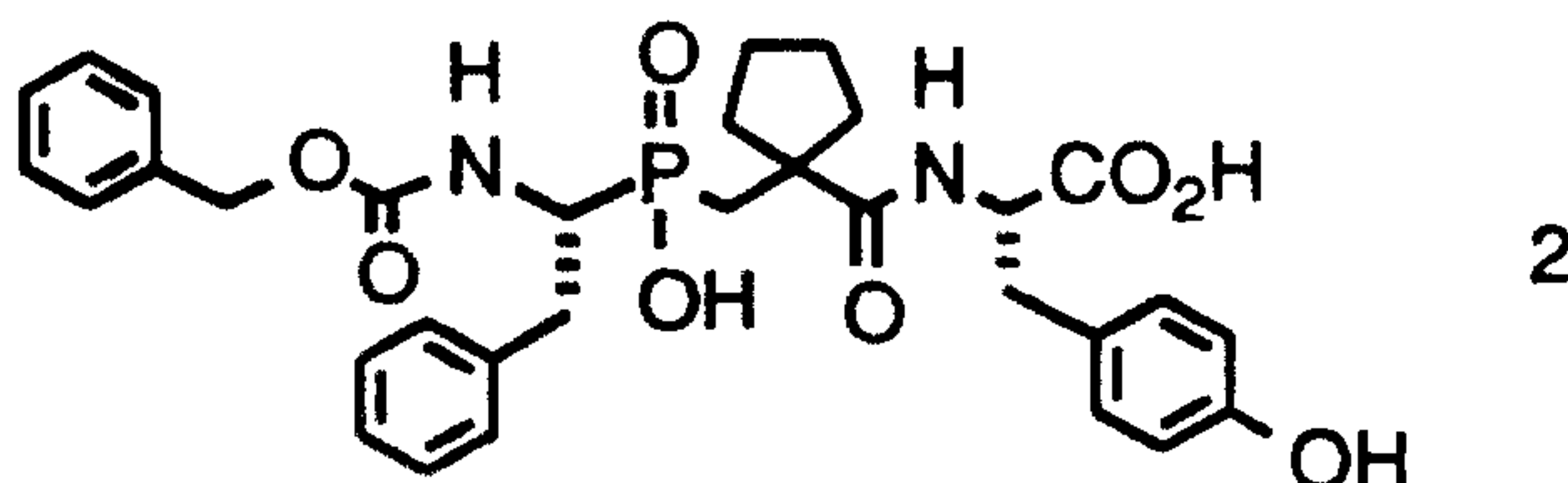
Using appropriate starting materials and following the procedures of Example 1, Steps 1-4, the following compound can also be prepared as a mixture of 2 diastereomers:



¹H Nmr (DMSO-d₆) δ 8.04-7.94 (1H, m), 7.60-7.54 (1H, m), 7.58-6.96 (15 H, m), 4.98-4.88 (2H, m), 4.58-4.42 (1H, m), 3.87-3.74 (1H, m), 3.21-3.16 (3H, m), 2.76-2.64 (1H, m), 2.16-1.13 (14H, m).

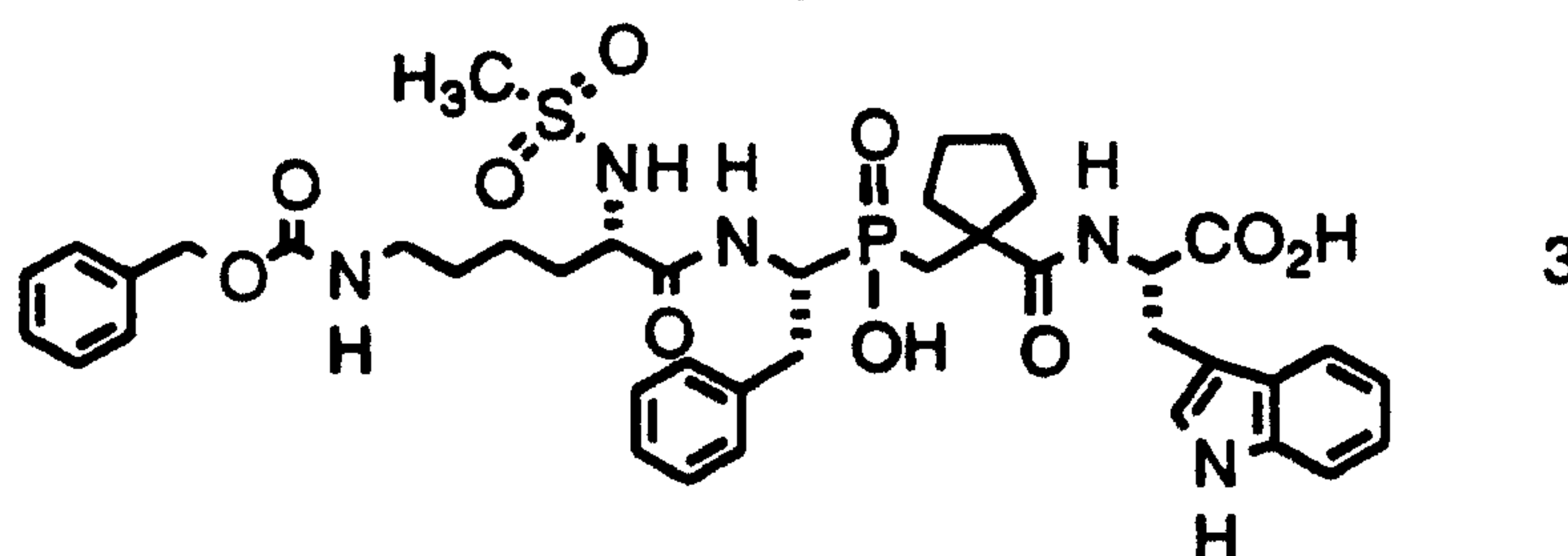
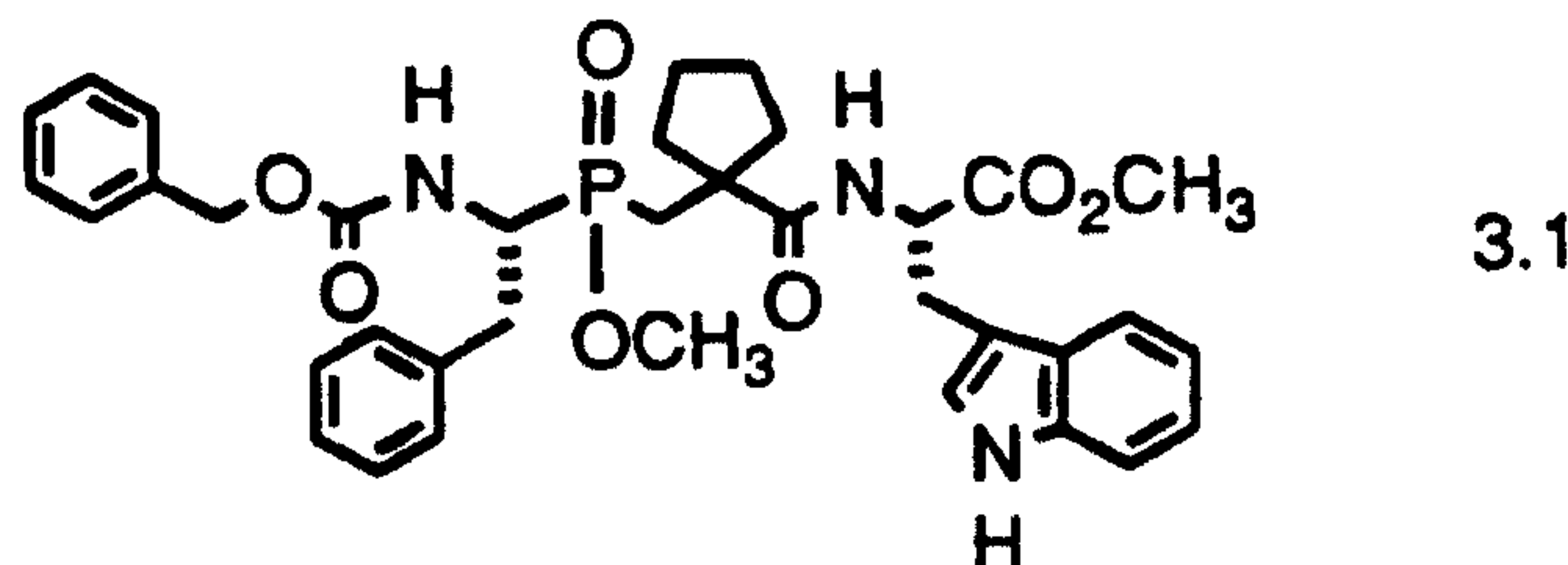
15

Example 2



- 19 -

To a stirred solution of Example 1.3C (150 mg, 0.22 mmol) in CH_2Cl_2 (4 ml), add $\text{CF}_3\text{CO}_2\text{H}$ (8 ml). After 2 h, evaporate the reaction mixture to dryness to obtain the methyl ester of the title compound as a foam (135 mg, 99%). FAB MS 623 ($\text{M}+\text{H}$)⁺. To this ester (130 mg, 0.21 mmol) in CH_3OH (3 ml), add $\text{LiOH}\cdot\text{H}_2\text{O}$ (37 mg, 0.88 mmol). Stir the reaction mixture overnight, then concentrate in vacuo to remove CH_3OH . Dilute the resulting aq. mixture with H_2O (10 ml), wash with EtOAc (10 ml), acidify to pH 1 with 1 M HCl and extract with EtOAc (50 ml). Saturate the aq. layer with NaCl and extract with EtOAc (50 ml). Wash the combined EtOAc extracts with sat'd NaCl , dry (MgSO_4), filter and evaporate to dryness to obtain compound 2 (107 mg, 84%). FAB MS m/z 609 ($\text{M}+\text{H}$)⁺.

Example 3**Step 1:**

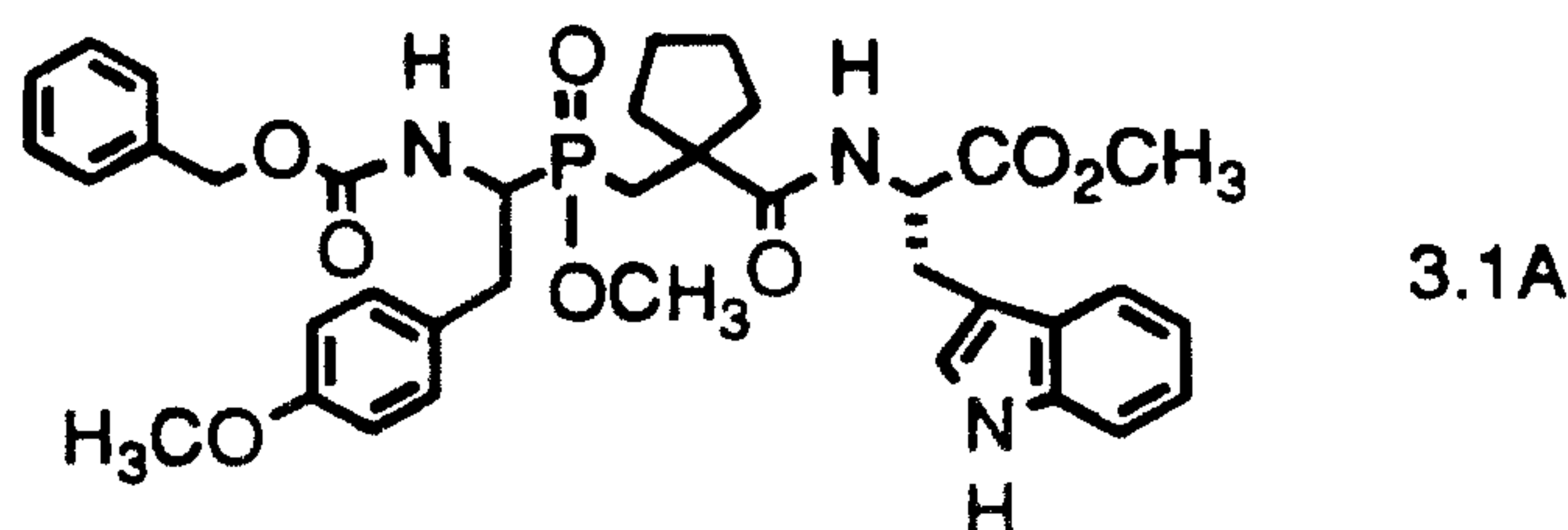
15

Treat a solution of Example 1.3 (1.80 g, 2.8 mmol) in EtOAc (50 ml) with a solution of CH_2N_2 in Et_2O until the yellow color persists. Add a few drops of $\text{CH}_3\text{CO}_2\text{H}$ to decompose the excess CH_2N_2 and wash the mixture with 1 M HCl (50 ml) and sat'd NaCl (50 ml). Treat the organic layer with excess CH_2N_2 in Et_2O . Decompose the excess CH_2N_2 by addition of $\text{CH}_3\text{CO}_2\text{H}$, wash the mixture with sat'd NaHCO_3 (50 ml) and sat'd NaCl (50 ml), dry (MgSO_4), filter and evaporate to give compound 3.1 as a foam (1.78 g, 96%). FAB HRMS calcd for $\text{C}_{36}\text{H}_{43}\text{N}_3\text{O}_7\text{P}$ ($\text{M}+\text{H}$)⁺ 660.2839. Found 660.2812.

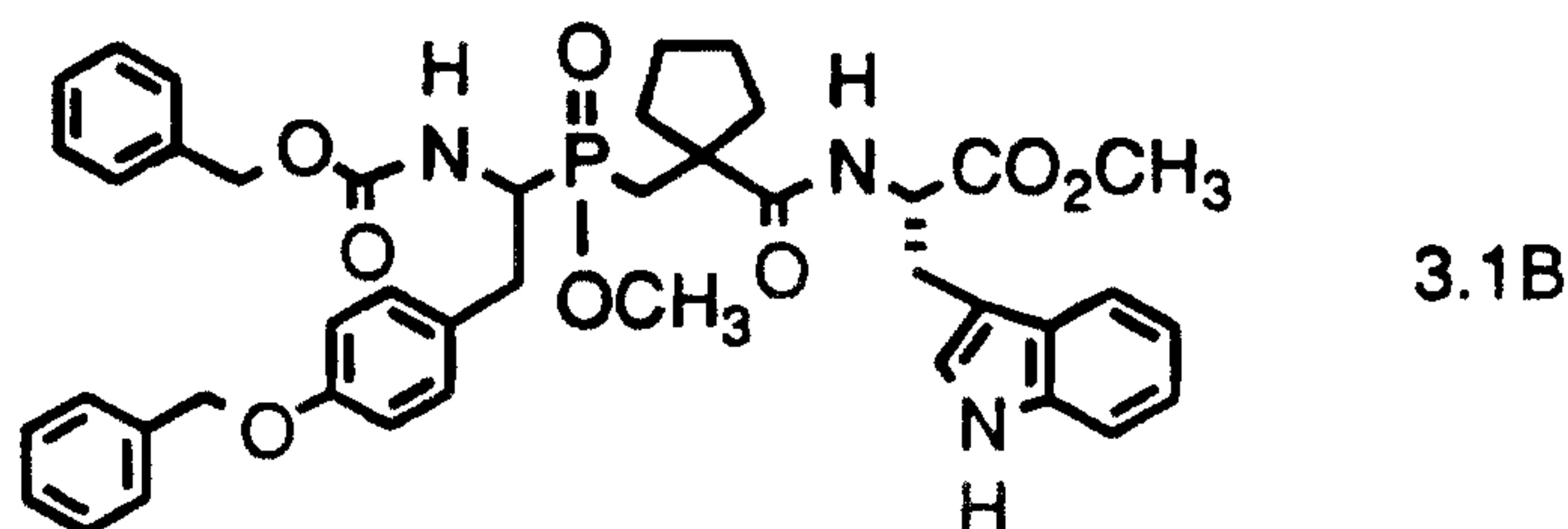
25

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

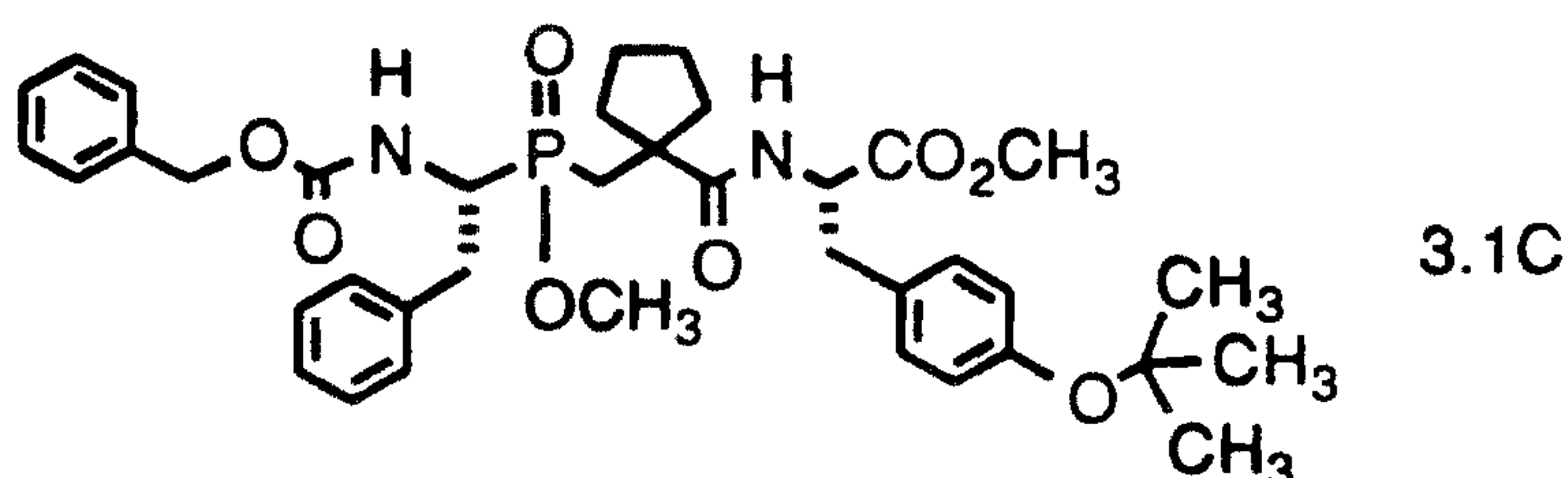
- 20 -



FAB HRMS calcd for $C_{37}H_{45}N_3O_8P$ ($M+H$)⁺ 690.2944. Found 690.2970.



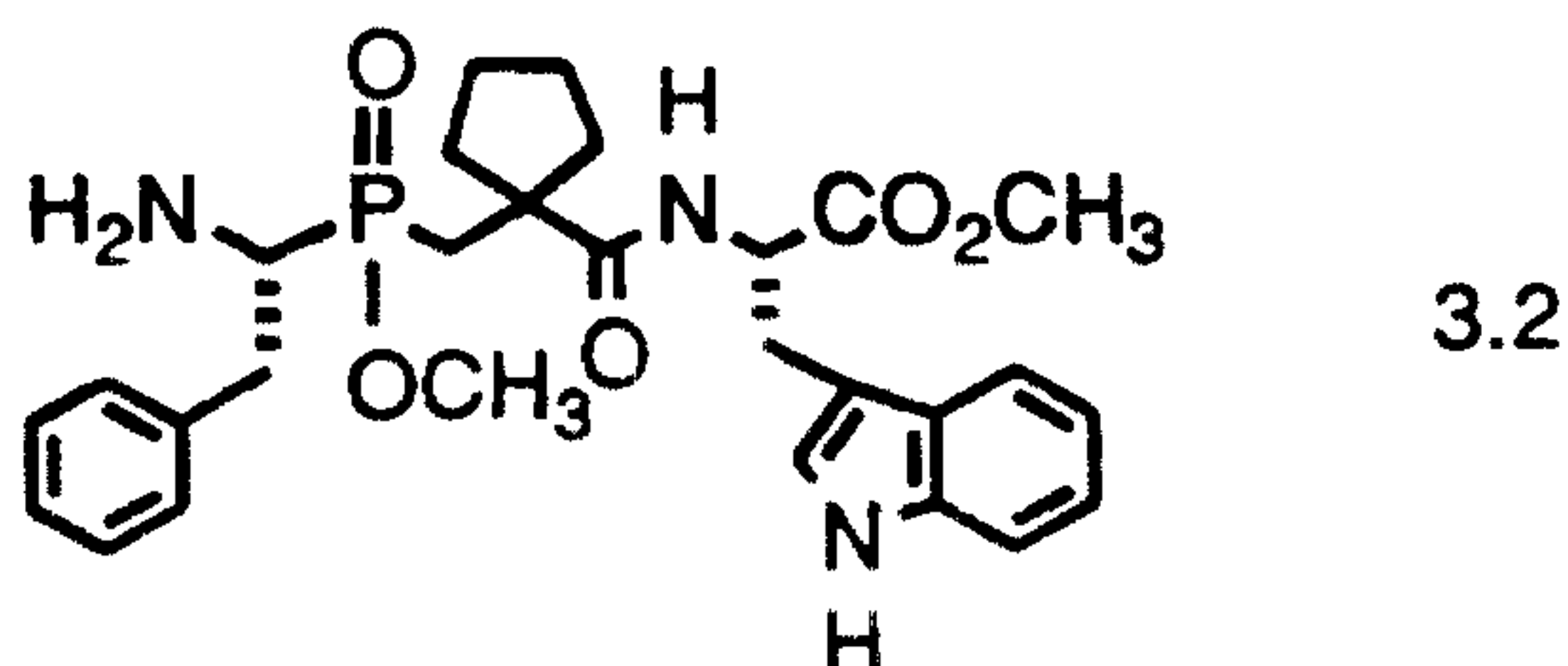
FAB MS m/z 766.7 ($M+H$)⁺.



5

FAB HRMS Calcd for $C_{38}H_{50}N_2O_8P$ ($M+H$)⁺ 693.3305. Found 693.3319.

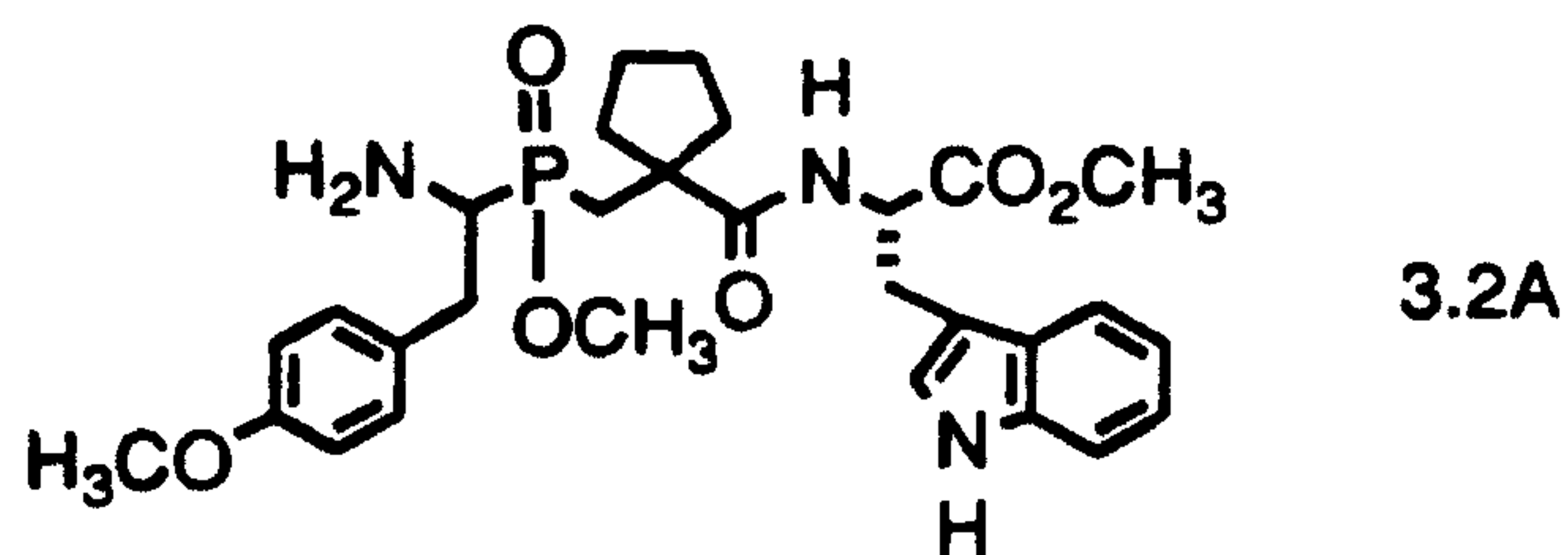
Step 2:



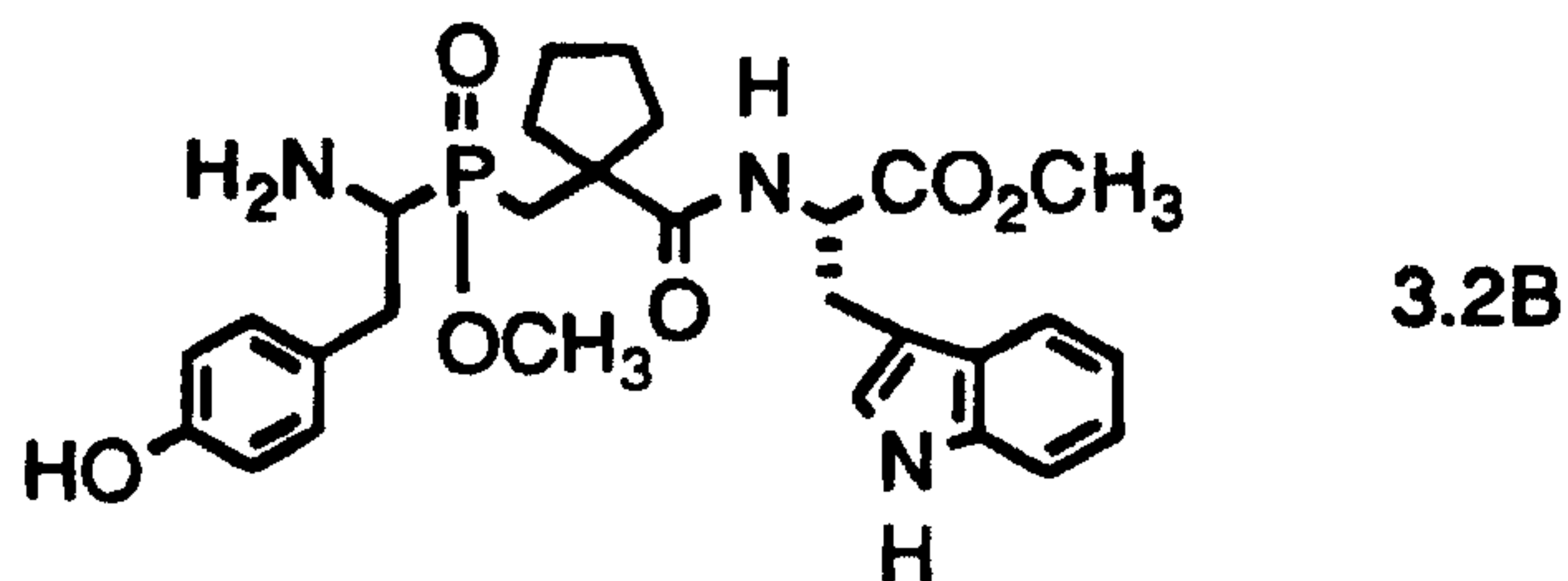
10 Stir a solution of Example 3.1 (1.75 g, 2.66 mmol) in CH_3OH (100 ml) containing 10% Pd/C (0.26 g) under a H_2 atmosphere for 5 h. Remove the catalyst by filtration through celite and wash the filter pad with CH_3OH . Evaporate the combined filtrate and washings to dryness to give compound 3.2 (1.34 g, 96%) as a colorless glass. 1H Nmr ($CDCl_3$) δ 8.31 (1H, bs), 7.66 (1H, d, J 7.5 Hz), 7.36-7.06 (10 H, m), 4.85 (1H, m), 3.59-3.41 (7H, m), 3.32-3.11 (4H, m), 2.85-2.50 (4H, bm), 2.36-1.48 (9H, m).
15 FAB MS m/z 526 ($M+H$)⁺.

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

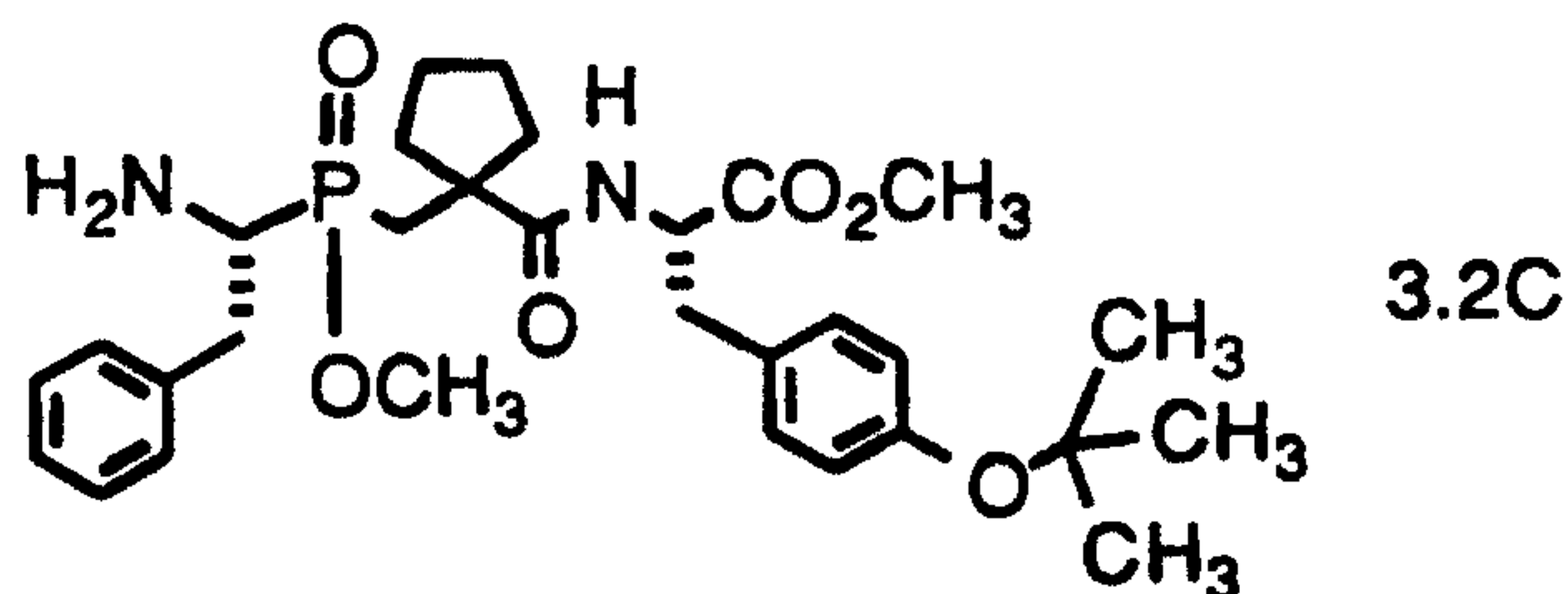
- 21 -



FAB HRMS calcd for $C_{29}H_{39}N_3O_6P$ ($M+H$)⁺ 556.2576. Found 556.2586.



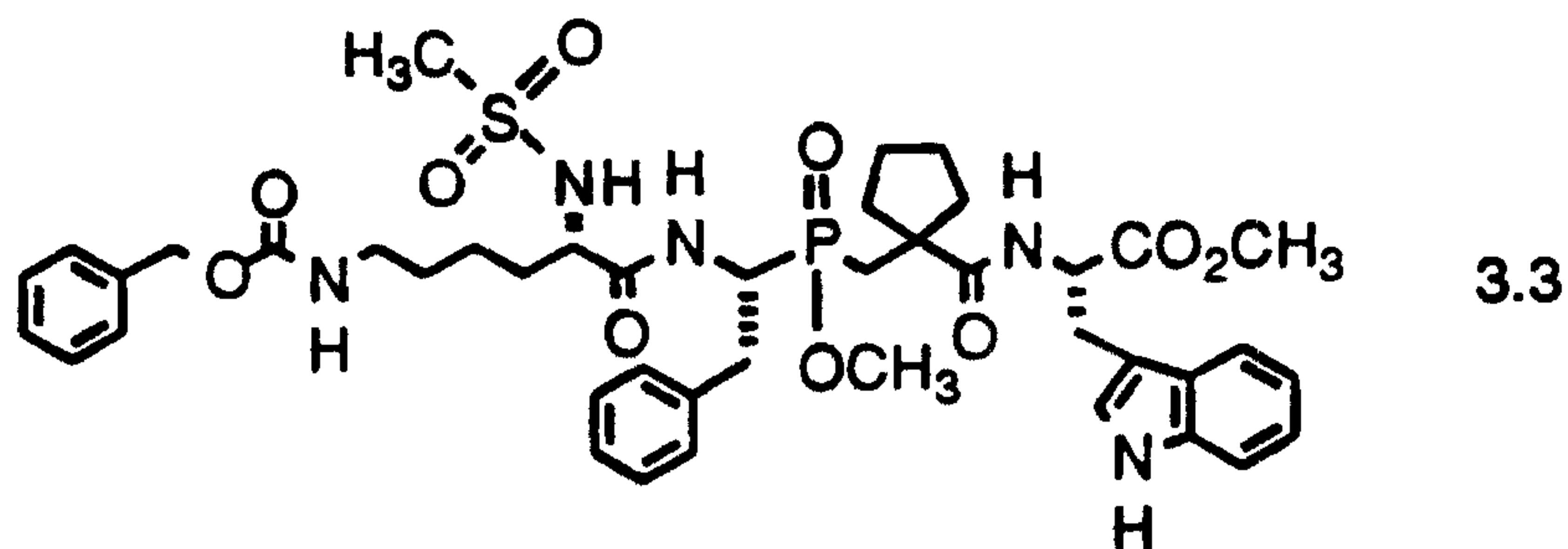
FAB MS m/z 542.2 ($M+H$)⁺.



5

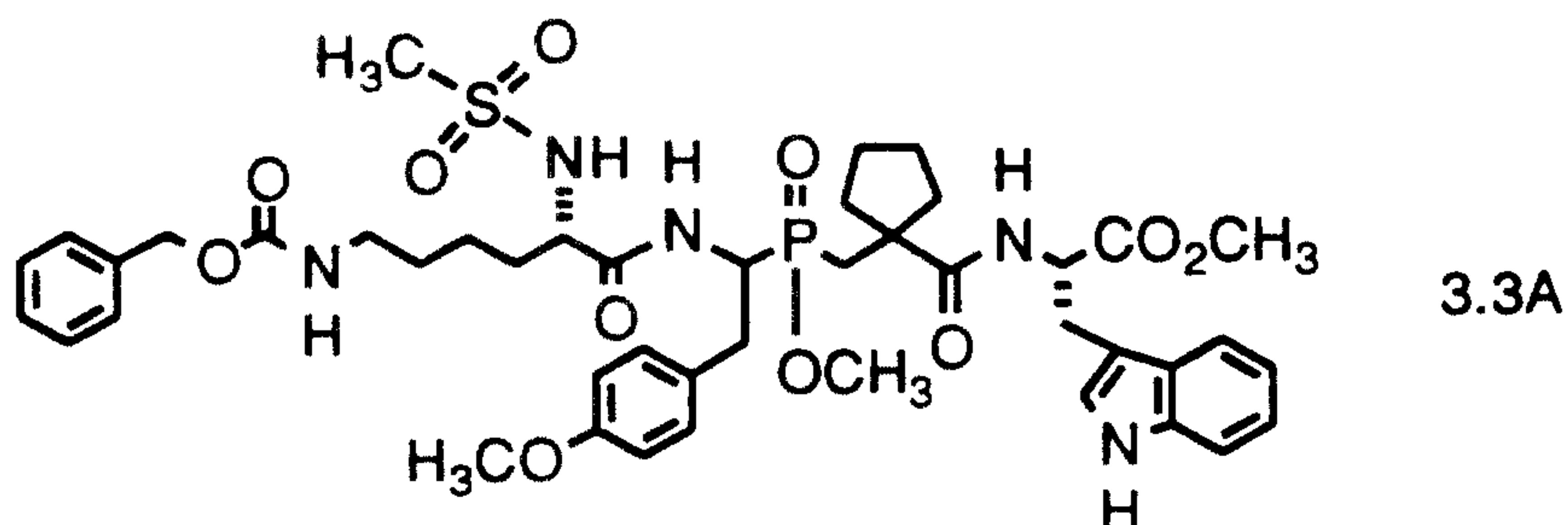
FAB MS 559.4 ($M+H$)⁺.

Step 3:

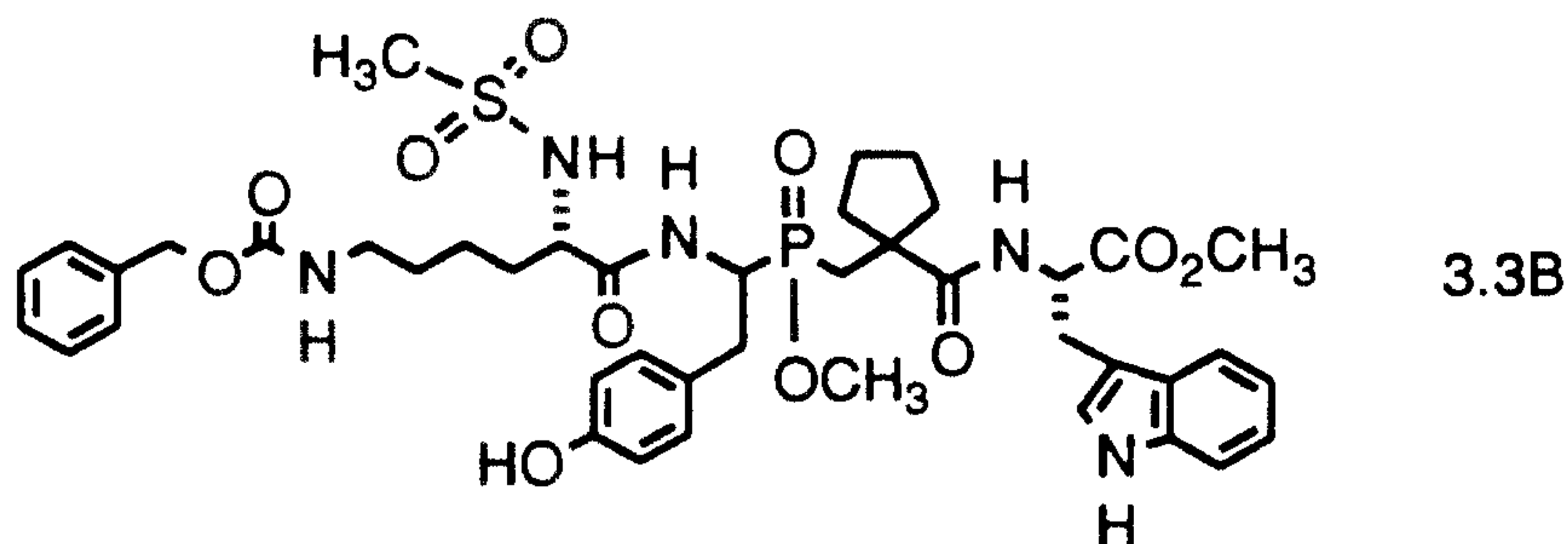


To a stirred solution of Example 3.2 (3.00 g, 5.85 mmol), N ϵ -phenylmethoxycarbonyl-N α -methylsulfonyl-(S)-lysine (ϵ -CBZ- α -Ms-Lys) (2.09 g, 5.85 mmol) and HOBT (0.87 g, 6.43 mmol) in CH_2Cl_2 (80 ml), add EDC (1.23 g, 6.43 mmol). After 48 h, pour the reaction mixture into 1 M HCl (200 ml) and extract with EtOAc (2x200 ml). Saturate the aq. layer with NaCl and extract with EtOAc (100 ml). Wash the combined organic layers with 10% $NaHCO_3$ (150 ml) and sat'd NaCl, then dry, filter and evaporate. Flash chromatography of the residue (1:10:10 CH_3OH /EtOAc/hexanes) affords compound 3.3 (4.02 g, 79%) as a pale yellow foam, FAB MS m/z 866.5 ($M+H$)⁺.

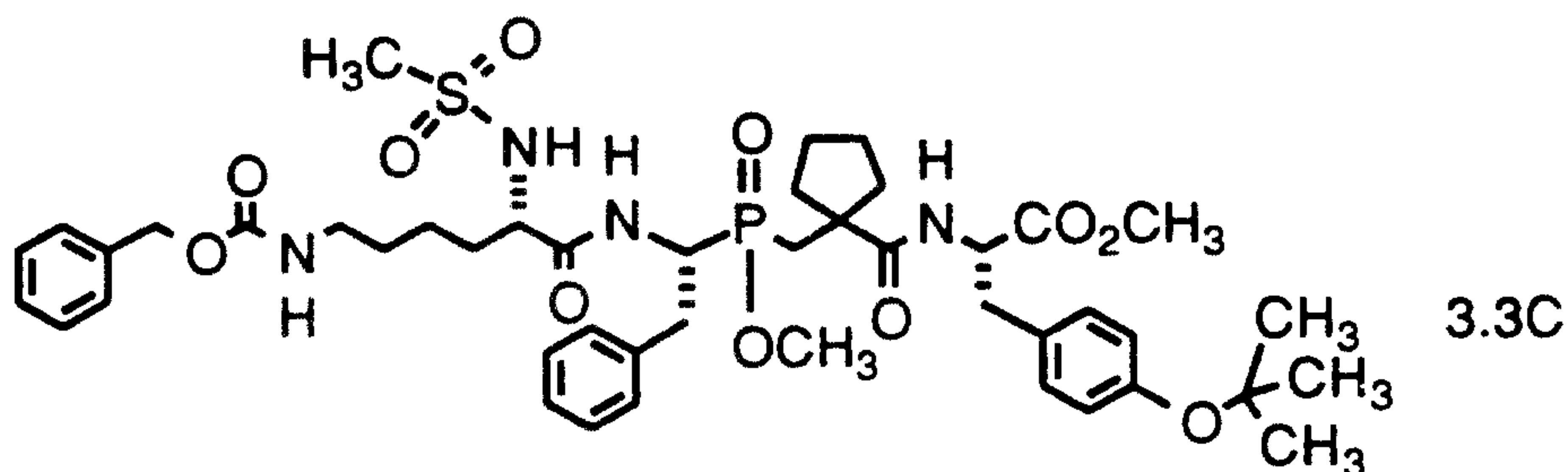
Using appropriate starting materials and essentially the same procedure the following compounds are prepared:



FAB MS m/z 896 ($M+H$)⁺.



FAB MS m/z 851.4 ($M-OCH_3+H$)⁺.



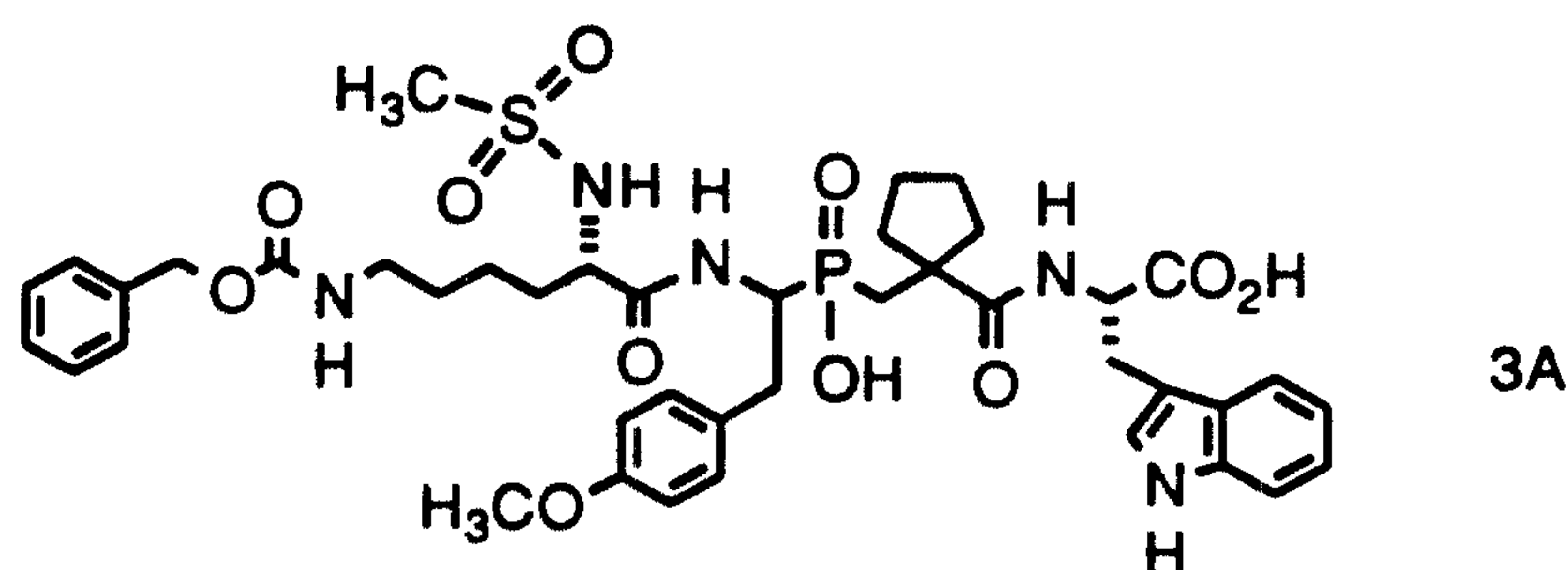
FAB HRMS calcd for $C_{45}H_{64}N_4O_{11}PS$ 899.4030 ($M+H$)⁺.

Found 899.5338.

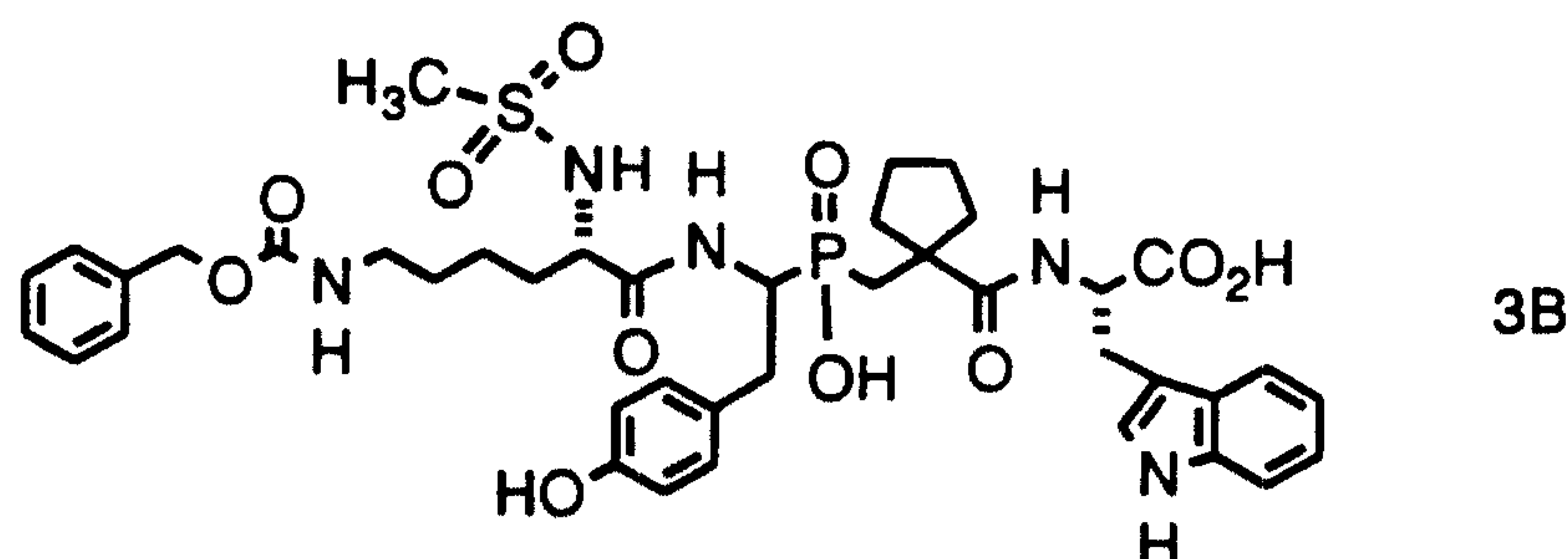
- 10 **Step 4:** Stir a solution of Example 3.3 (3.70 g, 4.27 mmol) in 96:4 CH_2Cl_2 /4M HCl-dioxane (400 ml) at R.T. overnight. Concentrate the reaction mixture in vacuo to obtain the methyl ester of compound 3 as a foam. FAB MS m/z 852.1 ($M+H$)⁺. Treat with $LiOH \cdot H_2O$, acidify and extract in a manner similar to that described in Example 2 to obtain the title
- 15 compound, 3, (3.10 g, 87%) as a white solid. Anal. calcd for $C_{41}H_{52}N_5O_{10}PS \cdot 1.1H_2O$: C, 57.41; H, 6.37; N, 8.17; P, 3.61%. Found: C, 57.06; H, 5.99; N, 8.03; P, 4.01%. FAB MS m/z 838 ($M+H$)⁺.

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

- 23 -

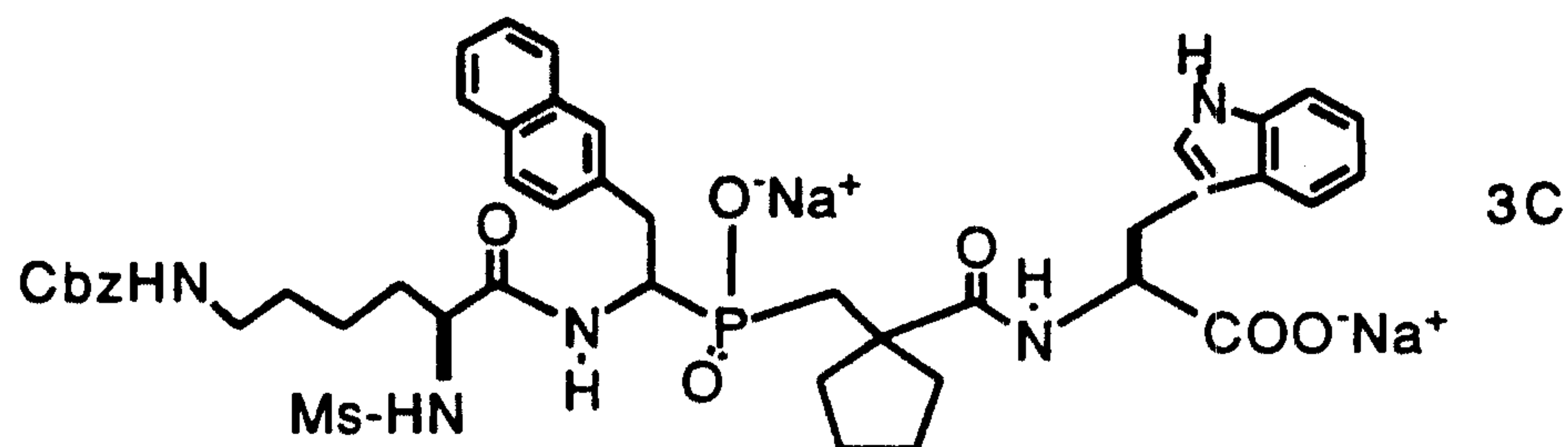


FAB HRMS calcd for $C_{42}H_{55}N_5O_{11}PS$ 868.3356 ($M+H$)⁺.
Found 868.3405



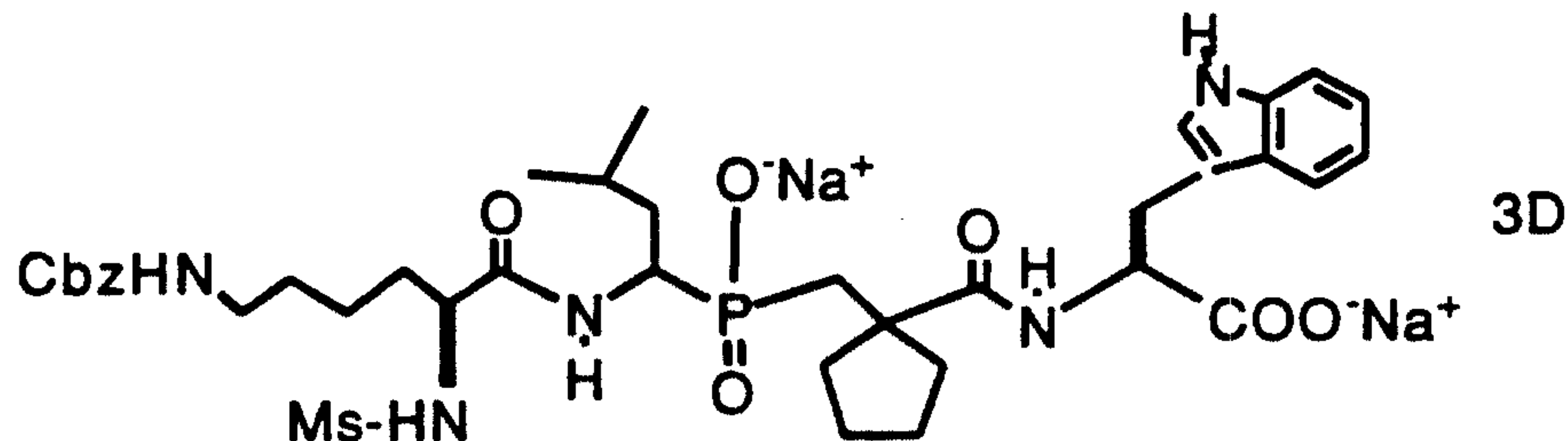
- 5 Anal. calcd for $C_{41}H_{52}N_5O_{11}PS \cdot 1.5H_2O$: C, 55.90; H, 6.29; N, 7.95; P, 3.52%. Found: C, 56.03; H, 5.95; N, 7.79; P, 3.48%.
FAB MS m/z 854.5 ($M+H$)⁺.

Using a procedure similar to that described in Example 3, Steps 1-4, the following compounds are prepared:



10

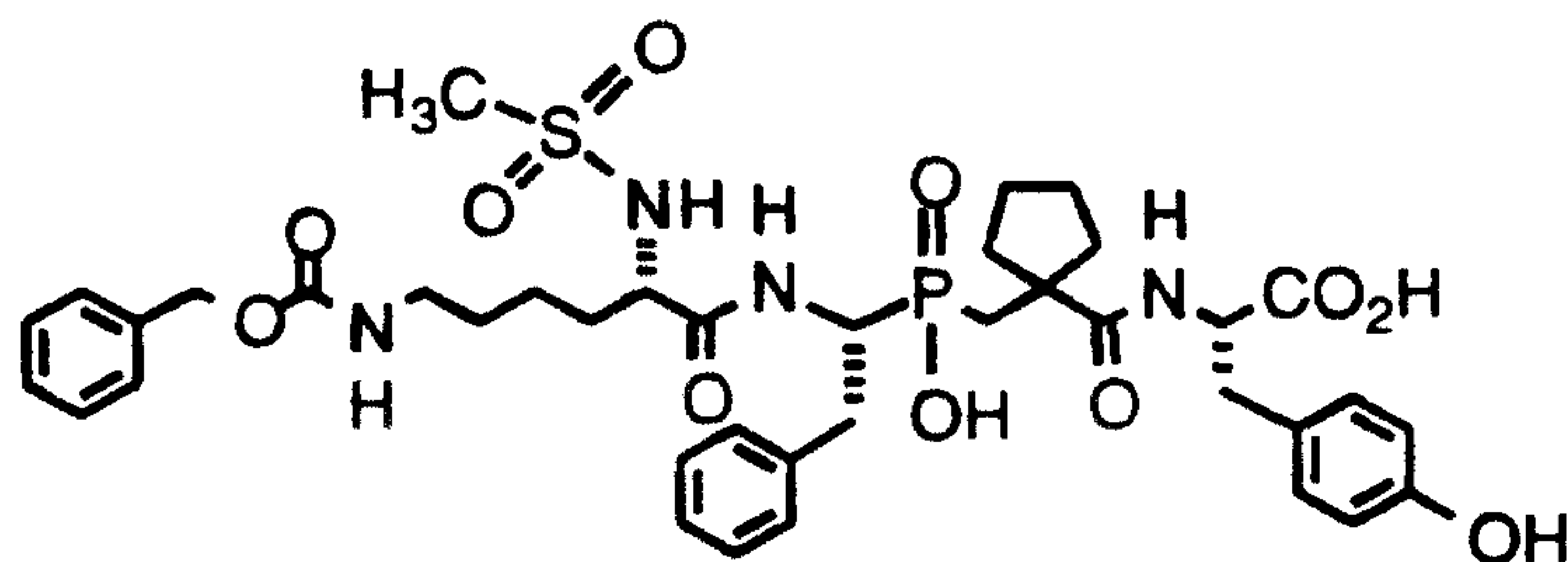
HRMS (FAB): Calcd for $C_{45}H_{52}N_5O_{10}PSNa_3$, m/e 954.2866; measured 954.2856.



15

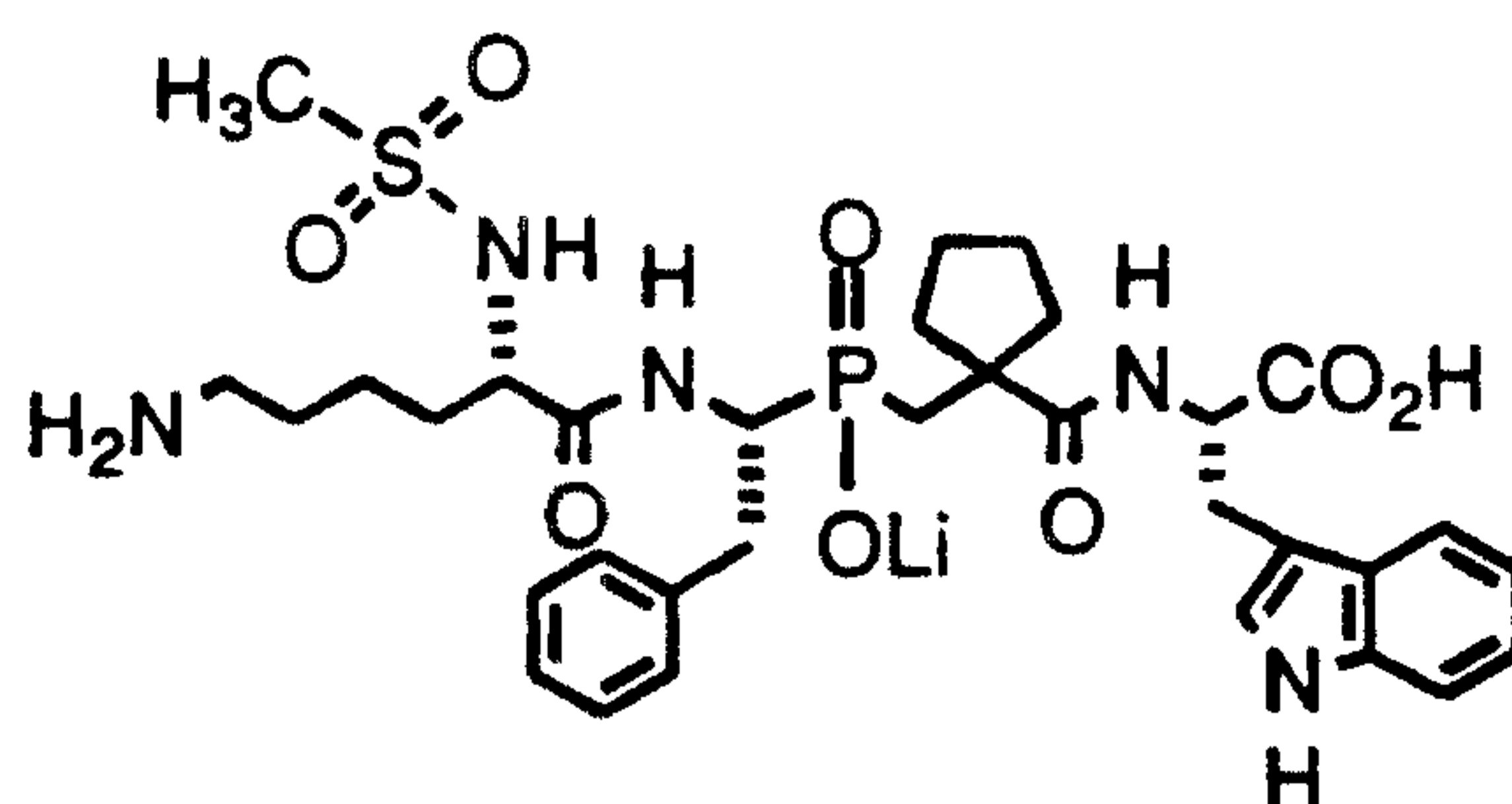
HRMS (FAB): Calcd for $C_{38}H_{54}N_5O_{10}Na$, m/e 826.3227; measured 826.3204.

- 24 -

Example 4

4

To a stirred solution of compound 3.3C (276 mg, 0.31 mmol) in CH_2Cl_2 (4 ml), add $\text{CF}_3\text{CO}_2\text{H}$ (8 ml). After 4 h, evaporate the reaction mixture to dryness. Take up the residue in CH_3OH , filter and evaporate to dryness. Dissolve the resultant foam in CH_3OH (2 ml), and add $\text{LiOH}\cdot\text{H}_2\text{O}$ (63 mg, 1.5 mmol) and H_2O (2 ml) and stir overnight. Acidify the aq. solution to pH 1 with 1M HCl, collect the precipitate, wash with H_2O , air-dry and dry in vacuo. Column chromatography of the crude product (C18, H_2O then 1:1 $\text{H}_2\text{O}/\text{CH}_3\text{OH}$) affords a mixture of the desired product and the corresponding methyl ester (142 mg), as determined by ^1H nmr spectroscopy. Treat the mixture with $\text{LiOH}\cdot\text{H}_2\text{O}$ (25 mg, 0.60 mmol) in 1:1 $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (6 ml) for 3 h. Evaporate the CH_3OH in vacuo, acidify (pH 1) the resulting aq. solution with 1 M HCl, filter the resulting precipitate, wash with H_2O , air-dry and dry in vacuo to give compound 4 (124 mg, 50%) as a white powder. Anal. calcd for $\text{C}_{39}\text{H}_{51}\text{N}_4\text{O}_{11}\text{PS}\cdot\text{H}_2\text{O}$: C, 56.24; H, 6.41; N, 6.73%. Found: C, 56.40; H, 6.11; N, 6.69%. FAB HRMS calcd for $\text{C}_{39}\text{H}_{52}\text{N}_4\text{O}_{11}\text{PS}$ ($\text{M}+\text{H}$)⁺ 815.3091. Found 815.3107.

Example 5

5

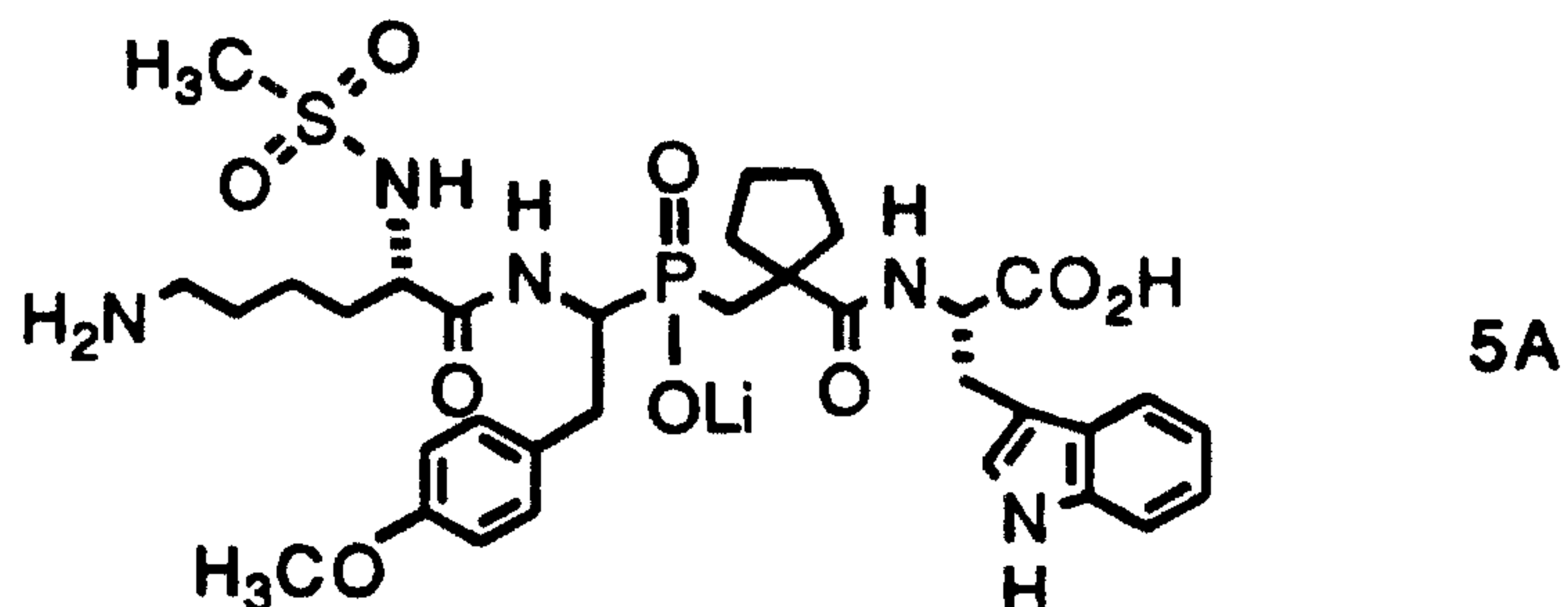
20

Stir a solution of compound 3 (106 mg, 0.127 mmol) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (5.3 mg, 0.126 mmol) in 1:1 $\text{CH}_3\text{OH}-\text{H}_2\text{O}$ (8 ml) containing 10% Pd/C (10 mg) under H_2 for 15 h. Remove the catalyst by filtration through celite and wash the filter pad with CH_3OH and H_2O . Concentrate the combined filtrate and washings in vacuo to remove CH_3OH and lyophilize the resulting aq. solution to give compound 5 (88.9 mg, 99%) as a white powder. FAB HRMS calcd for $\text{C}_{33}\text{H}_{46}\text{N}_5\text{O}_8\text{PLiS}$ ($\text{M}+\text{H}$)⁺ 710.2965. Found 710.2983. $[\alpha]_D = -18.9^\circ$ (c 0.94 H_2O).

25

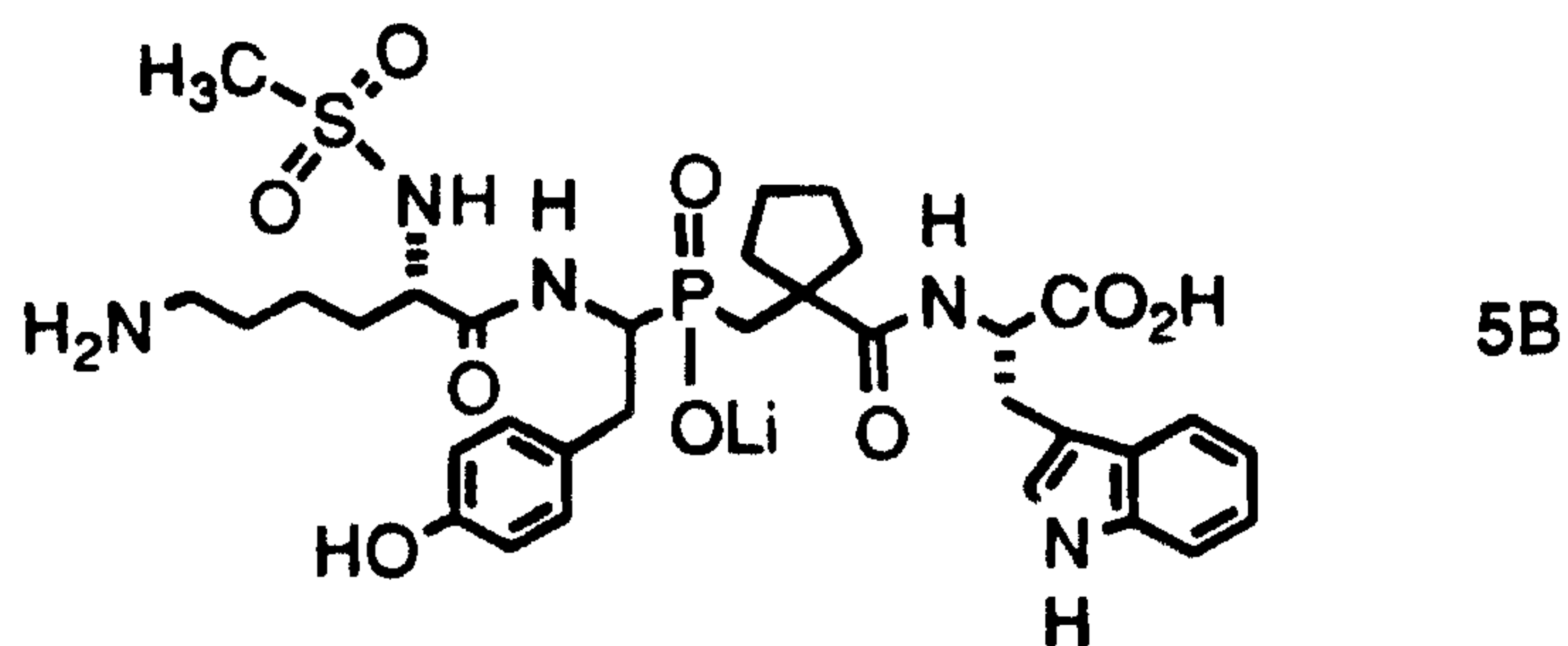
- 25 -

Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

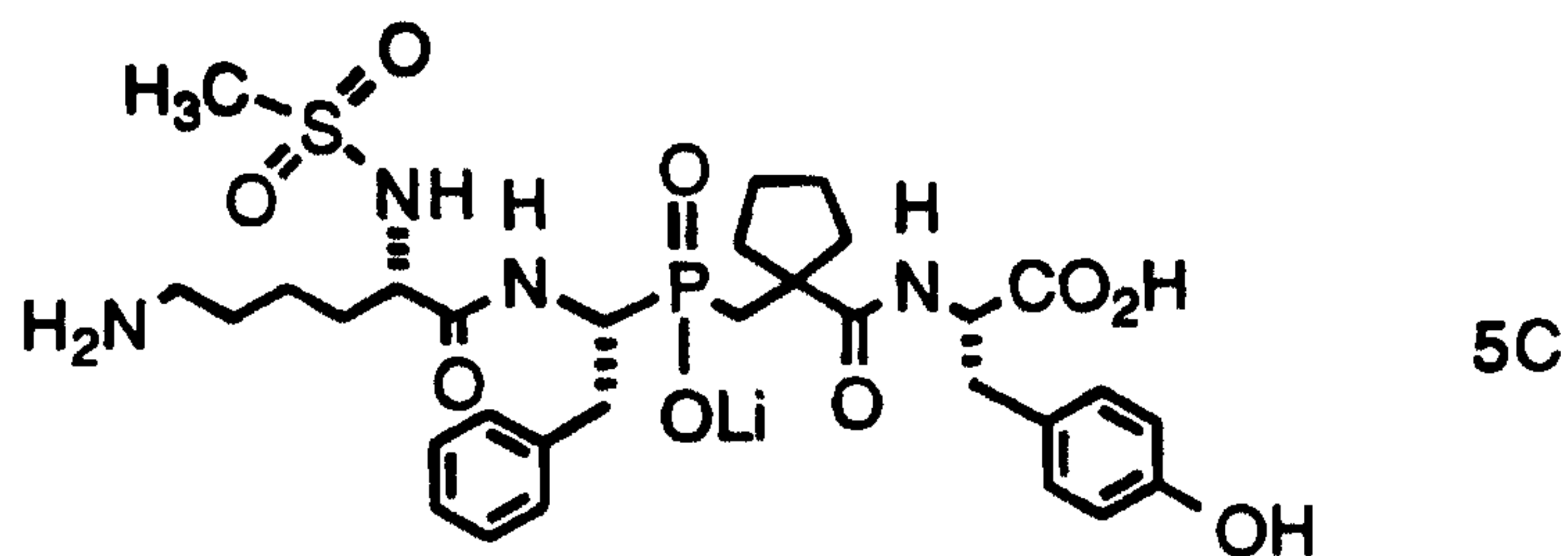


FAB HRMS calcd for $C_{34}H_{48}N_5O_9PLiS$ ($M+H$)⁺ 740.3070.
Found 740.3088.

5



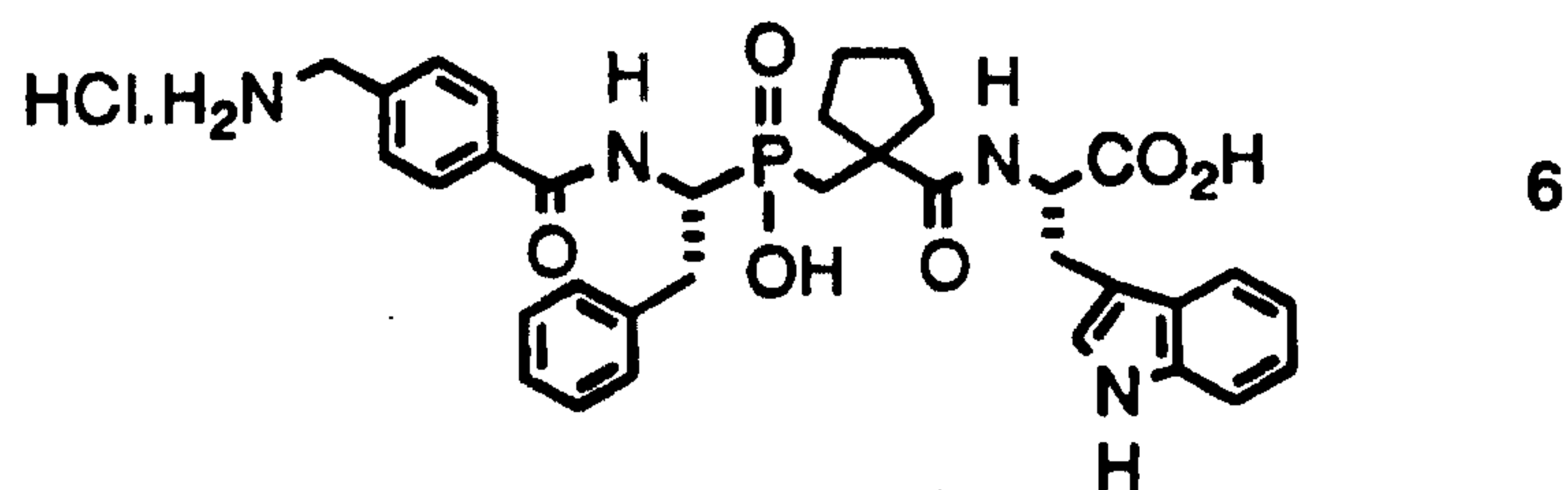
FAB MS m/z 726.5 ($M+H$)⁺.



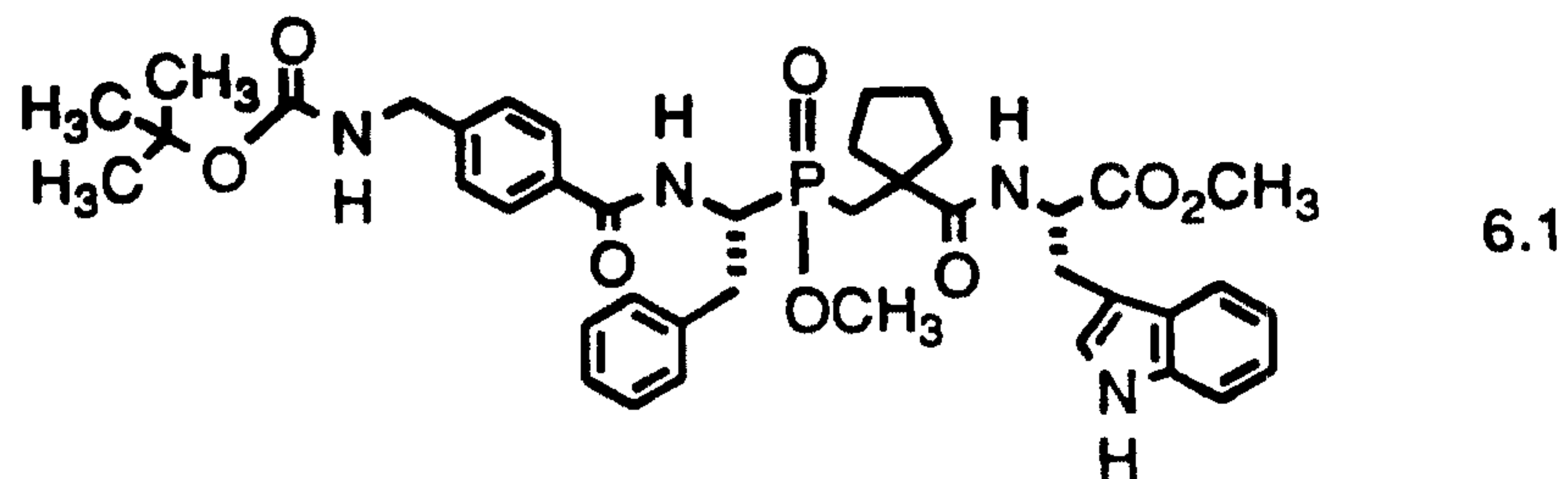
FAB MS 687 ($M+H$)⁺.

10

Example 6



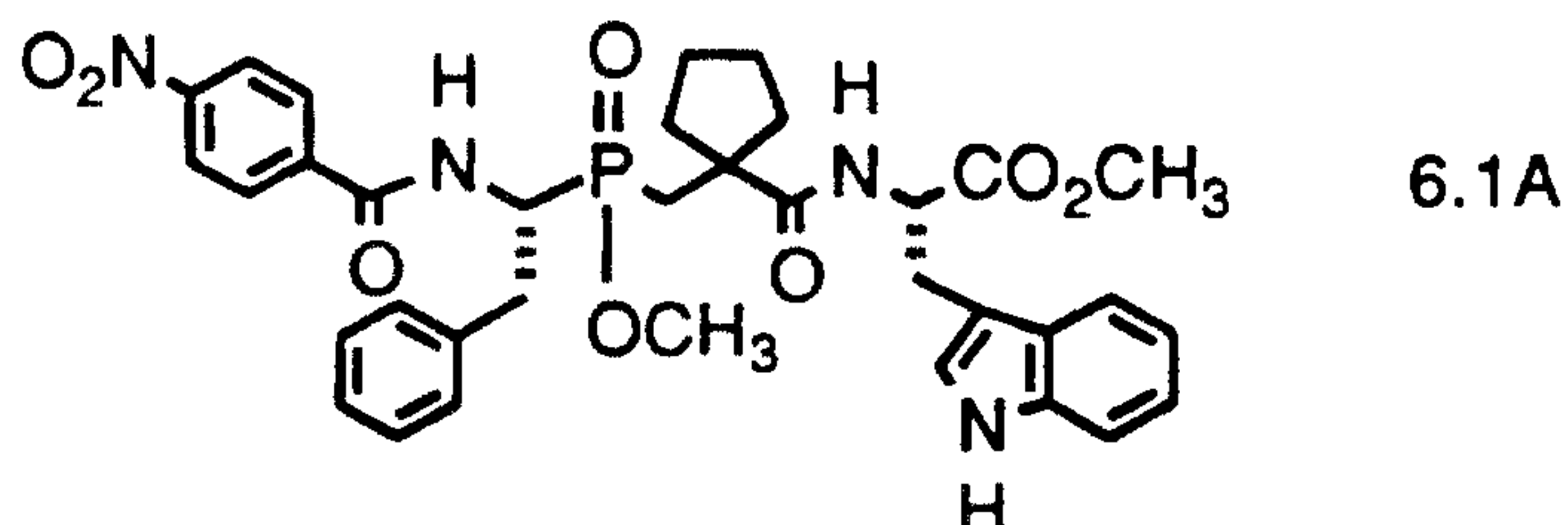
Step 1:



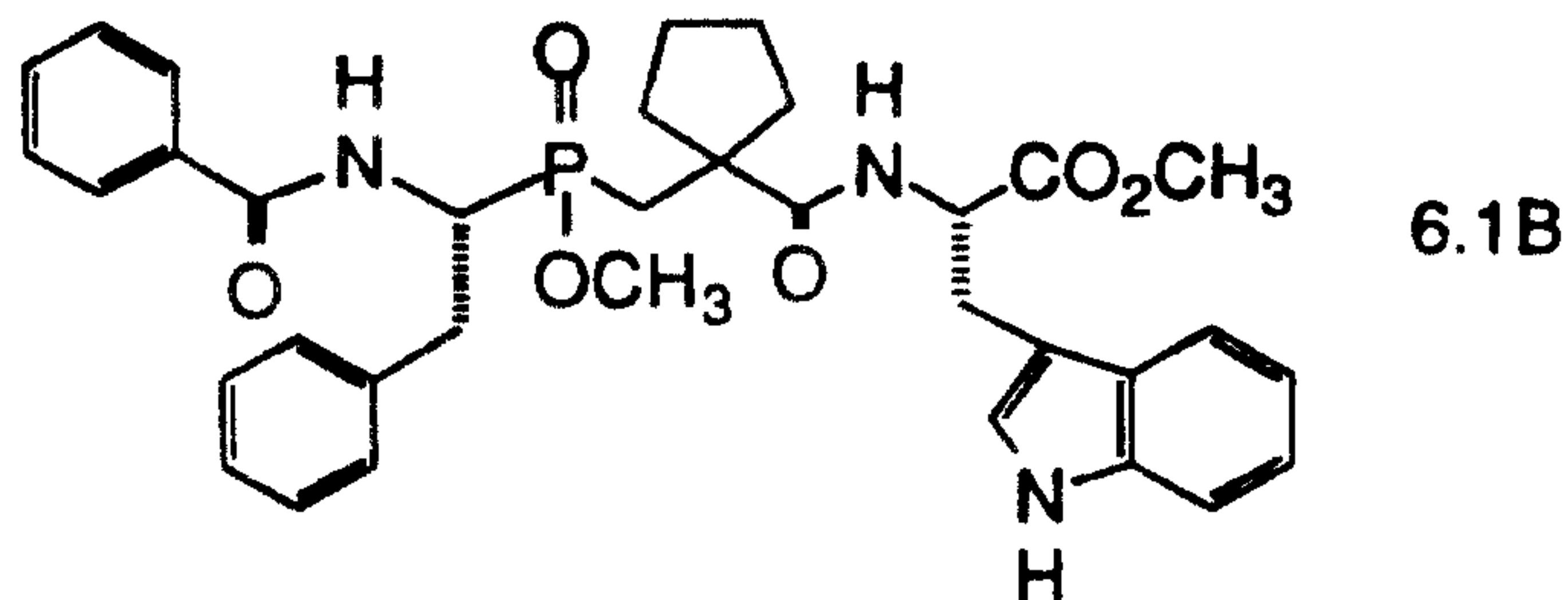
- 26 -

To a stirred ice-cold suspension of 4-((1,1-dimethylethoxycarbonyl)-amino)methylbenzoyl chloride freshly prepared from Preparation 1 (0.60 g, 2.39 mmol), (COCl)₂ (0.21 ml, 2.4 mmol) and DMF (2 drops) in CH₂Cl₂ (15 ml), add a solution of compound 3.2 (1.20 g, 2.28 mmol) and N-methylmorpholine (0.53 ml, 4.8 mmol) in CH₂Cl₂ (14 ml). Stir the reaction mixture for 3 h, then wash with 1M HCl (50 ml), sat'd NaHCO₃ and sat'd NaCl, dry (MgSO₄), filter and evaporate. Flash chromatograph the residue (3% CH₃OH/CH₂Cl₂) to obtain compound 6.1 (1.19 g, 69%) as an off-white solid. FAB MS *m/z* 759 (M+H)⁺.

10 Using appropriate starting materials and essentially the same procedure the following compounds are prepared:

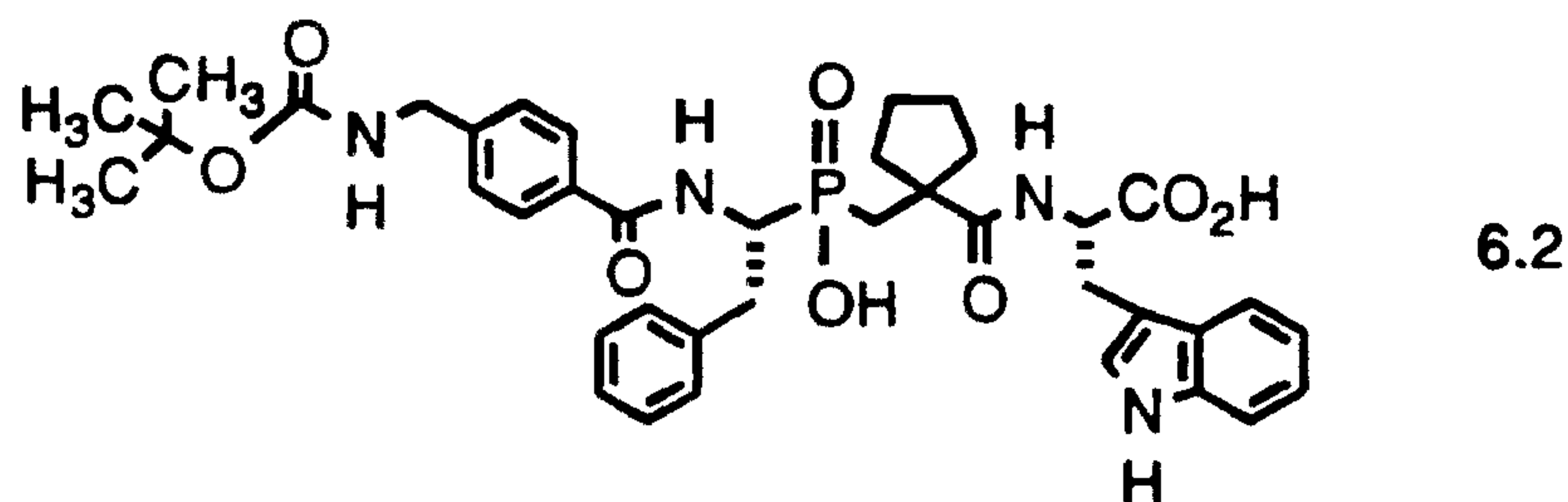


FAB HRMS calcd for C₃₅H₄₀N₄O₈P (M+H)⁺ 675.2584. Found 675.2570.



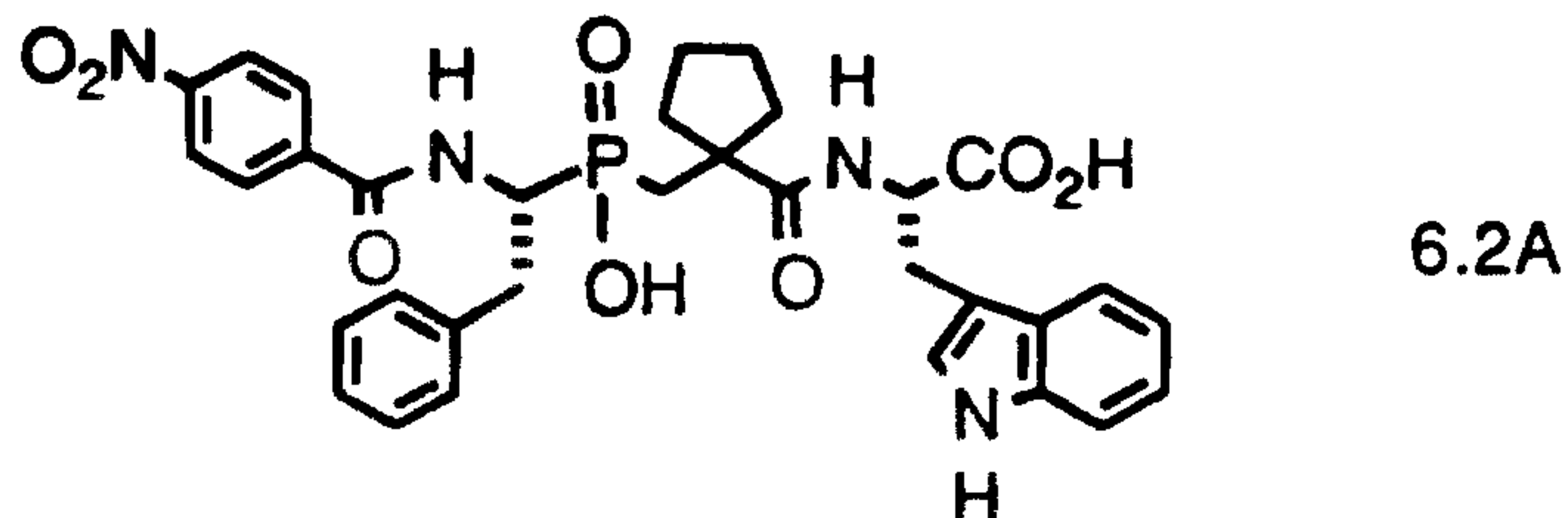
15 FAB MS *m/z* 630.4 (M+H)⁺.

Step 2: Using the procedure of Example 3, Step 4 compounds 6.1, 6.1A and 6.1B are deprotected to obtain:

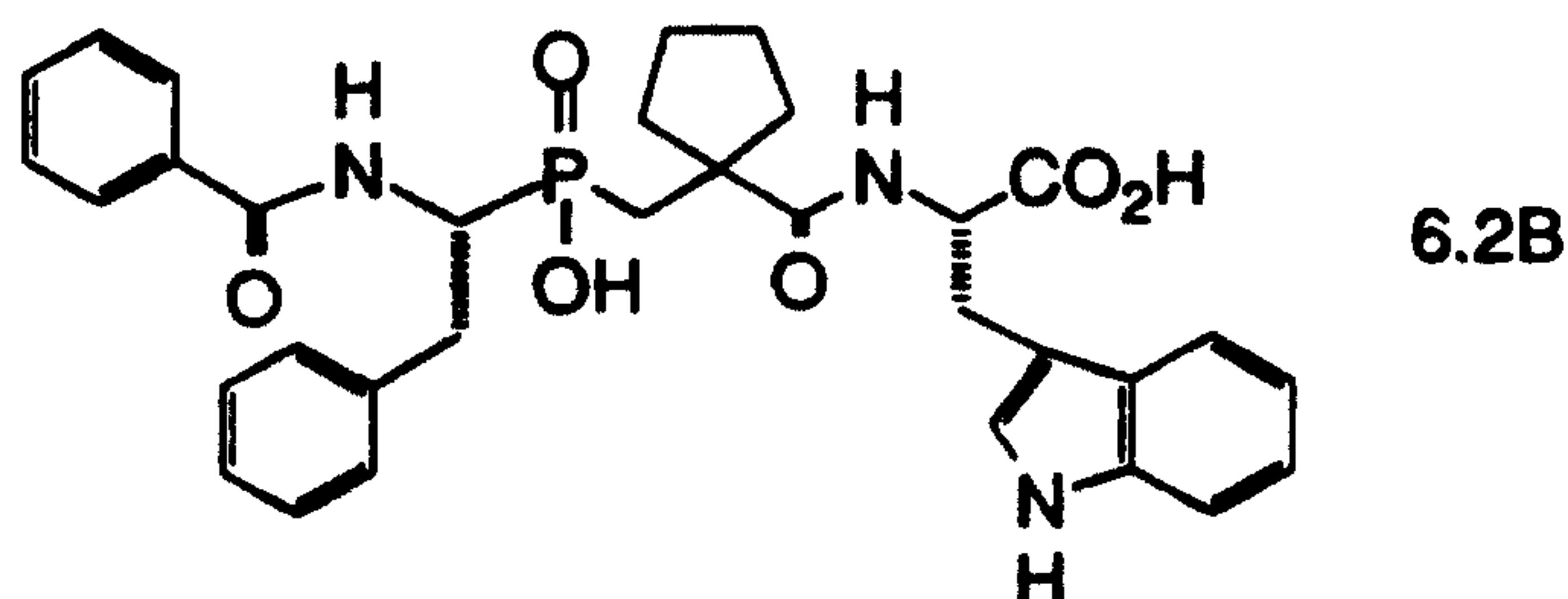


Anal. calcd for C₃₉H₄₇N₄O₈P·H₂O: C, 62.56; H, 6.61; N, 7.48; P, 4.14%.

20 Found: C, 62.84; H, 6.48; N, 7.67; P, 4.24%. FAB MS *m/z* 731.4 (M+H)⁺.



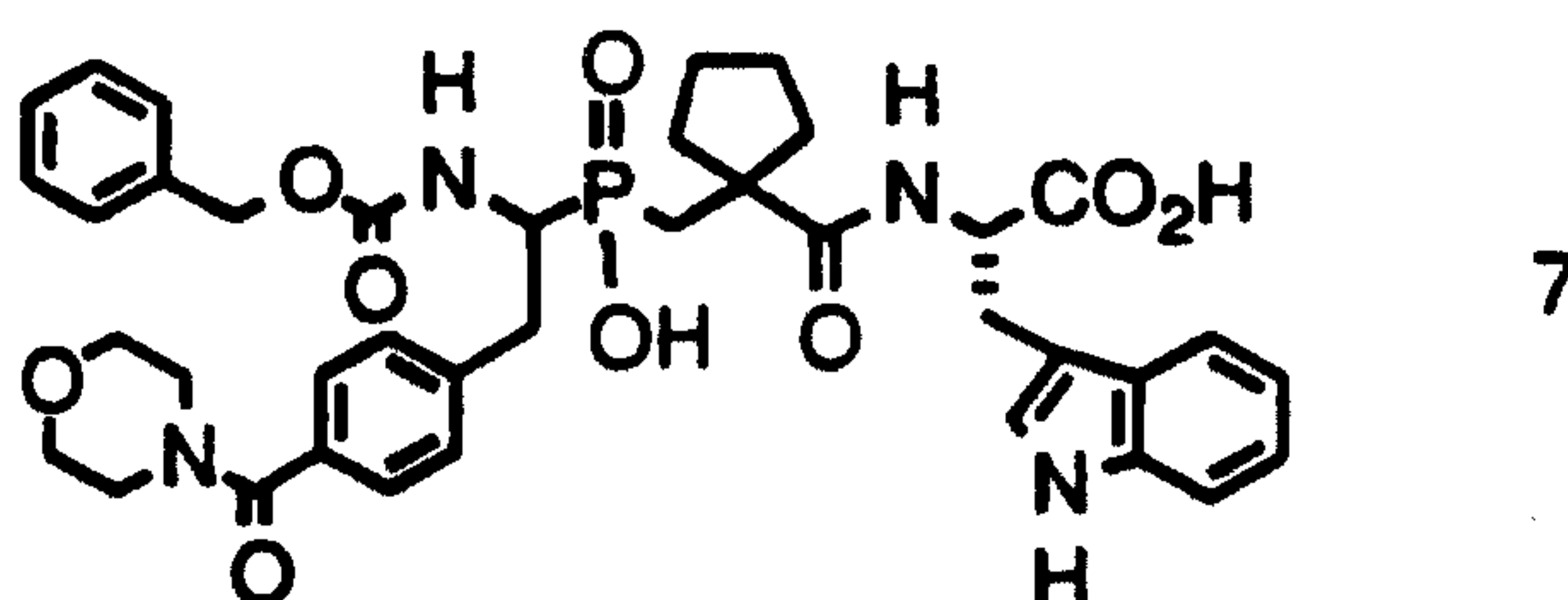
- 27 -

FAB MS m/z 647.3 (M+H)⁺.

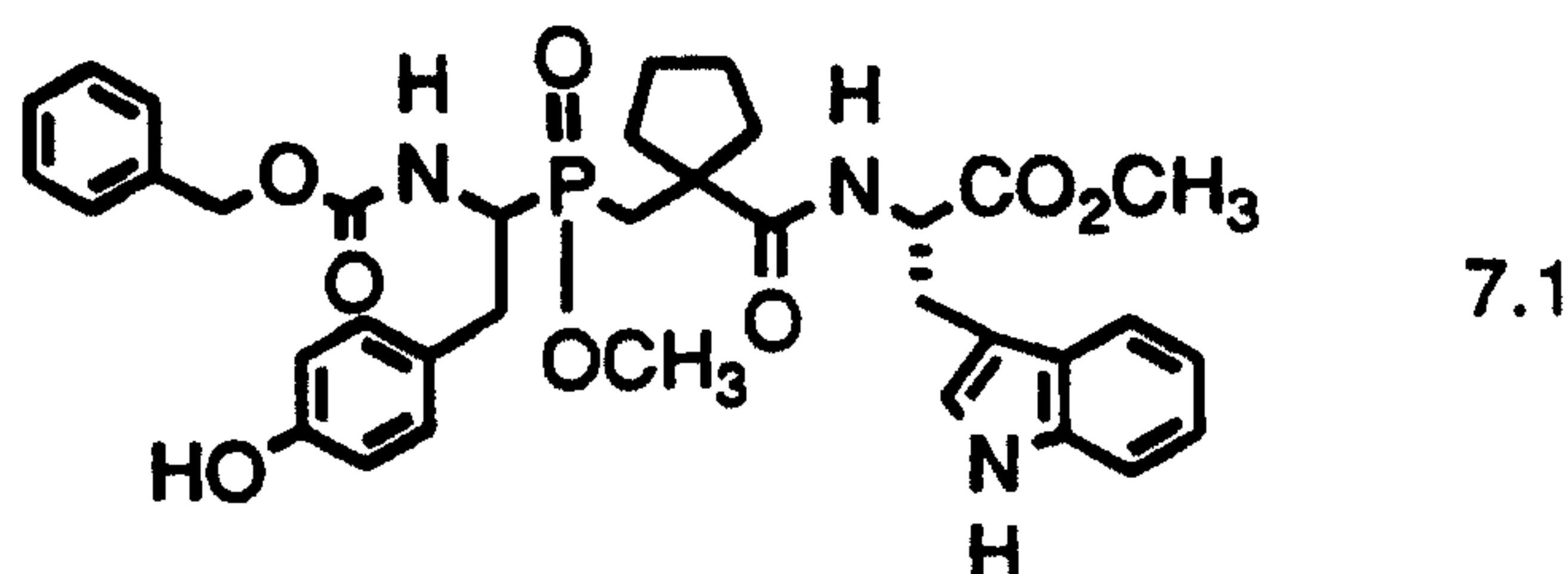
Anal. calcd for C₃₃H₃₆N₃O₆P·H₂O: C, 63.96; H, 6.18; N, 6.78; P, 4.93%.

Found: C, 63.74; H, 5.82; N, 6.72; P, 4.99%.

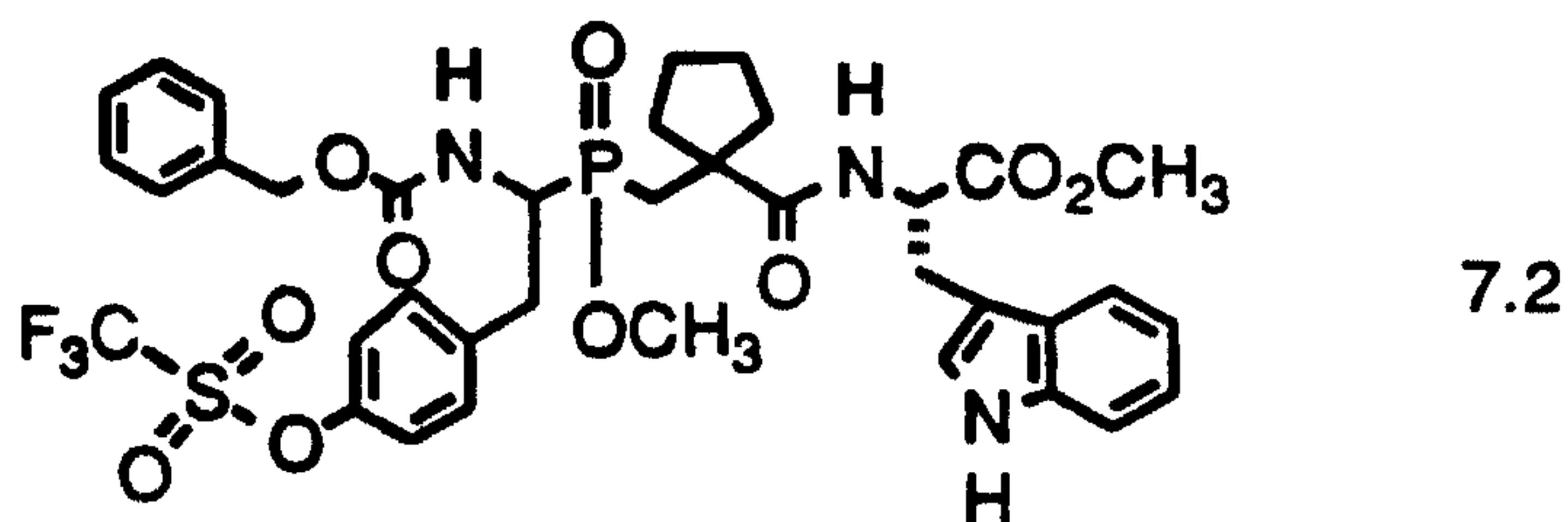
- 5 **Step 3:** Stir a mixture of compound 6.2 (300 mg, 0.41 mmol) and 4M HCl/dioxane (50 ml) for 2.5 h. Evaporate the reaction mixture to dryness to obtain the title compound, 6, (0.27 g, 100%) as a beige powder. FAB MS m/z 631 (M-HCl+H)⁺.

Example 7

10

Step 1:

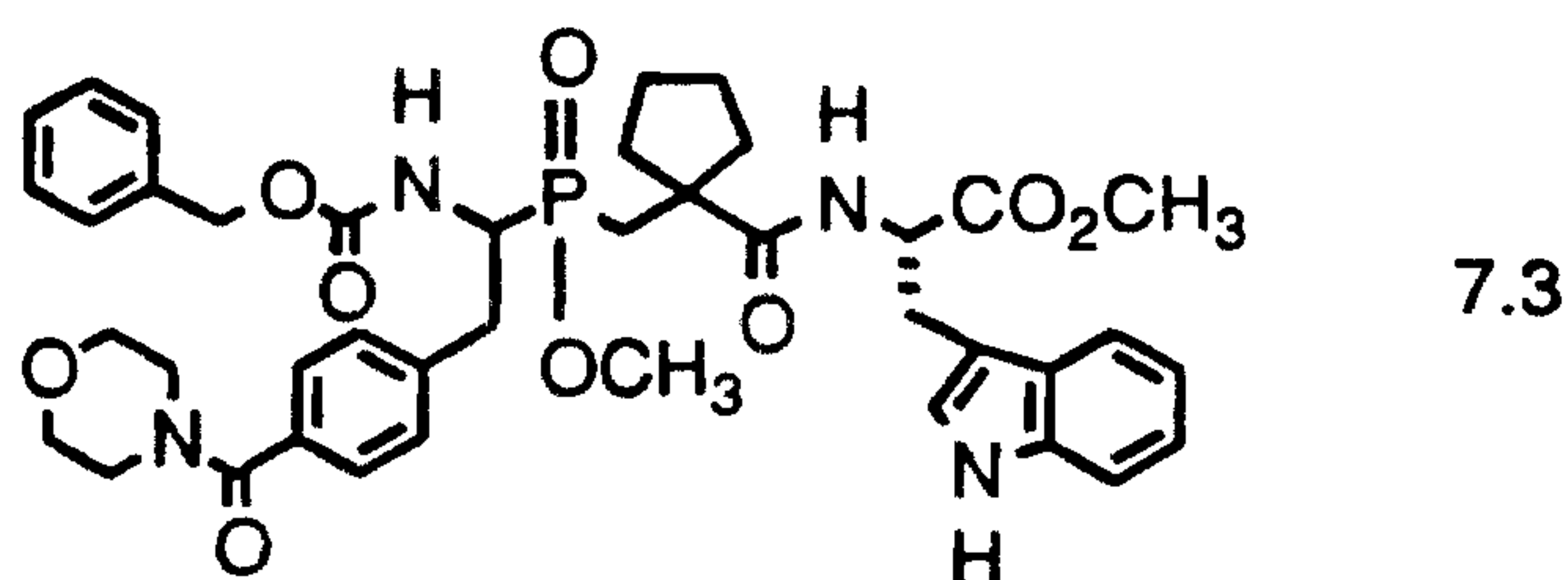
- 15 To a stirred solution of compound 3.2B (1.40 g, 2.58 mmol) in CH₂Cl₂ (25 ml), add N-(benzyloxycarbonyloxy)succinimide (0.71 g, 2.85 mmol). After 30 h, dilute the reaction mixture with EtOAc (200 ml) and wash the solution with 1 M HCl (200 ml), sat'd NaHCO₃ (200 ml) and sat'd NaCl (200 ml), then dry (MgSO₄), filter and evaporate. Flash chromatography (5% CH₃OH/CH₂Cl₂) affords compound 7.1 (1.22 g, 70%) as a white solid. FAB MS m/z 676.4 (M+H)⁺.

Step 2:

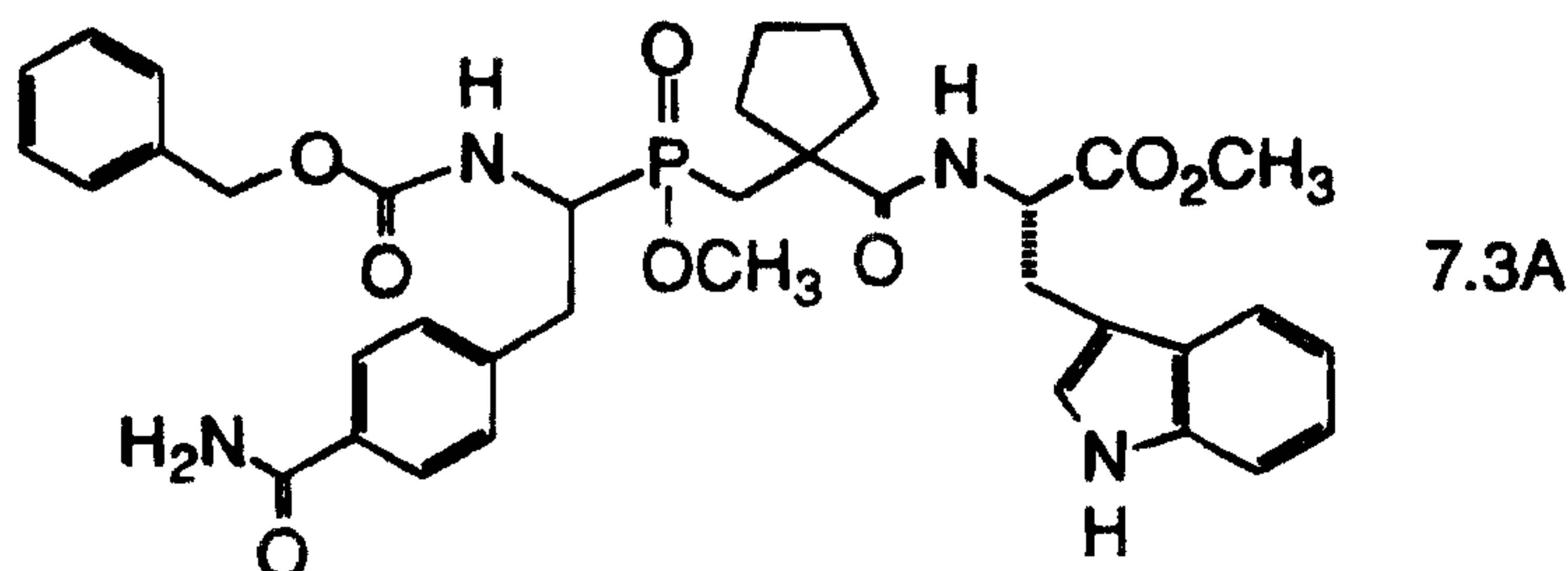
- 28 -

To a stirred ice-cold solution of compound 7.1 (1.090 g, 1.61 mmol) and triethylamine (0.27 ml, 1.9 mmol), add N-phenyl triflimide. Allow the reaction mixture to warm to R.T. and stir for 20 h, then partition between EtOAc (150 ml) and 1 M HCl (150 ml). Wash the organic layer with sat'd NaHCO₃ (150 ml) and sat'd NaCl (150 ml), then dry (MgSO₄), filter and evaporate to dryness. Flash chromatography (20% hexanes/EtOAc then EtOAc) affords compound 7.2 (1.070 g, 82%) as a foam. Anal. calcd for C₃₇H₄₁N₃O₁₀PF₃S.0.5H₂O: C, 54.41; H, 5.18; N, 5.14%. Found C, 54.21; H, 5.07; N, 5.05%. FAB MS *m/z* 808.6 (M+H)⁺.

10 Step 3:



To a solution of compound 7.2 (150 mg, 0.186 mmol) and morpholine (0.50 ml, 5.7 mmol) in DMF (1 ml), add 1,3-bis(diphenylphosphino)propane (16 mg, 0.039 mmol) and Pd(OAc)₂ (5 mg, 0.02 mmol), then bubble CO through the mixture for 3 min. Heat the mixture at 70 °C under a CO atmosphere for 6 h and allow to cool to R.T. Add EtOAc (15 ml) and wash the solution with 1 M HCl (2x15 ml), H₂O (4x15 ml) and sat'd NaCl. Treat the organic layer with a solution of CH₂N₂ in Et₂O until the yellow color persists, and decompose the excess CH₂N₂ by adding CH₃CO₂H. Dry (MgSO₄), filter and concentrate. Flash chromatography (4% CH₃OH/CH₂Cl₂) of the residue affords compound 7.3 (99 mg, 69%) as a colorless glass. FAB MS *m/z* 773.5 (M+H)⁺.

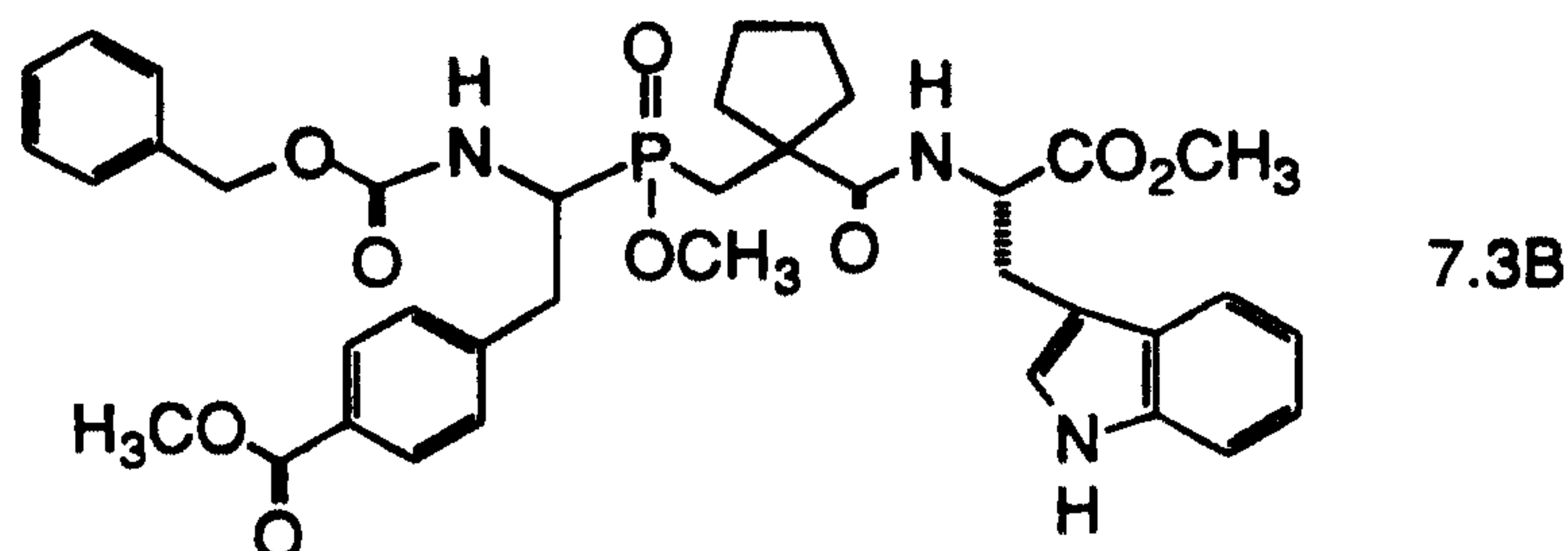


To a solution of compound 7.2 (200 mg, 0.25 mmol) and triethylamine (77 µl, 0.55 mmol) in DMF (1 ml) add 1,3-bis(diphenylphosphino)propane (20 mg, 0.048 mmol) and Pd(OAc)₂. Bubble NH₃ through the mixture for 2 min., then bubble CO through the mixture for 2 min. Place the mixture under a balloon containing a mixture of CO and NH₃ and heat to 70 °C for

2191454

- 29 -

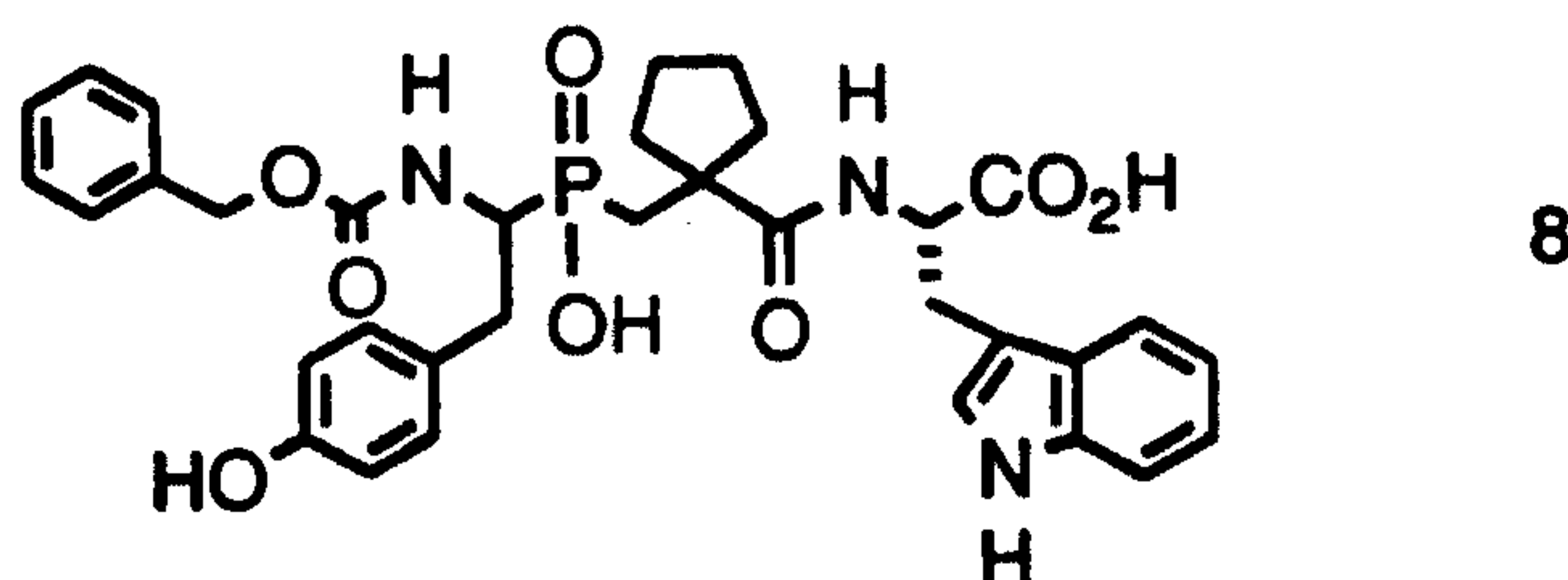
- 6 h. Allow the reaction mixture to cool, dilute with EtOAc (15 ml), then wash with H₂O (6x15 ml) and sat'd NaCl. Dry (MgSO₄), filter and concentrate. Flash chromatography (5% MeOH/CH₂Cl₂) of the residue affords compound 7.3A (63 mg, 36%) as a colorless glass. ¹H Nmr (CD₃OD) δ 7.62-7.49 (3H, m), 7.42-7.29 (2H, m), 7.20-7.01 (6H, m), 6.99-6.79 (5H, m), 4.88-4.63 (3H, m), 4.62-4.53 (1H, m), 4.13-3.92 (1H, m), 3.50-3.32 (6H, m), 3.19-2.38 (4H, m), 2.20-1.72 (4H, m), 1.55-1.22 (6H, m).



- Use a procedure similar to that described above, but omit NH₃ and include CH₃OH in the reaction mixture to obtain compound 7.3B. FAB MS *m/z* 718.5 (M+H)⁺.

- Step 4:** Sequentially deprotect compound 7.3 (93 mg, 0.12 mmol) with HCl/1,4-dioxane/CH₂Cl₂ and LiOH.H₂O as described in Example 3, Step 4 to afford the title compound (75 mg, 83%) as an off-white powder. Anal. calcd for C₃₉H₄₅N₄O₉P.2.5H₂O: C, 59.31; H, 6.38; N, 7.09; P, 3.92%. Found C, 59.12; H, 6.03; N, 7.02; P, 4.37%. FAB MS *m/z* 745.5 (M+H)⁺.

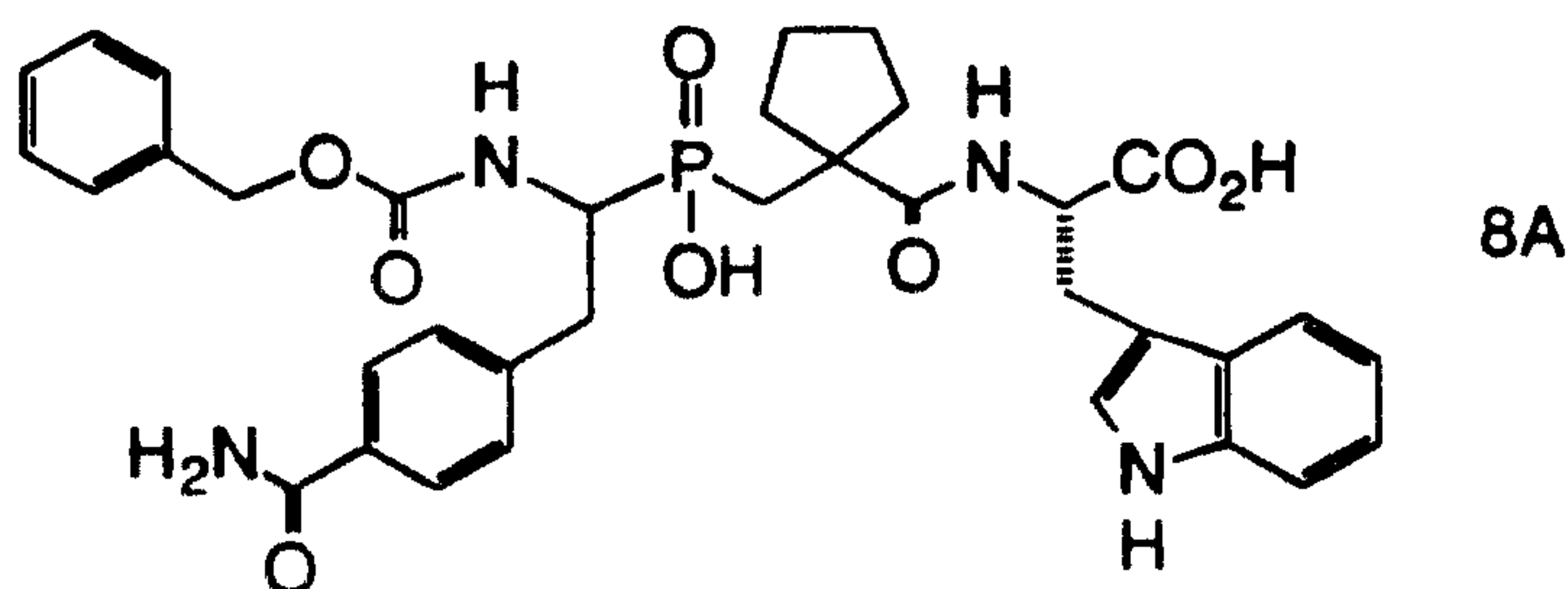
Example 8



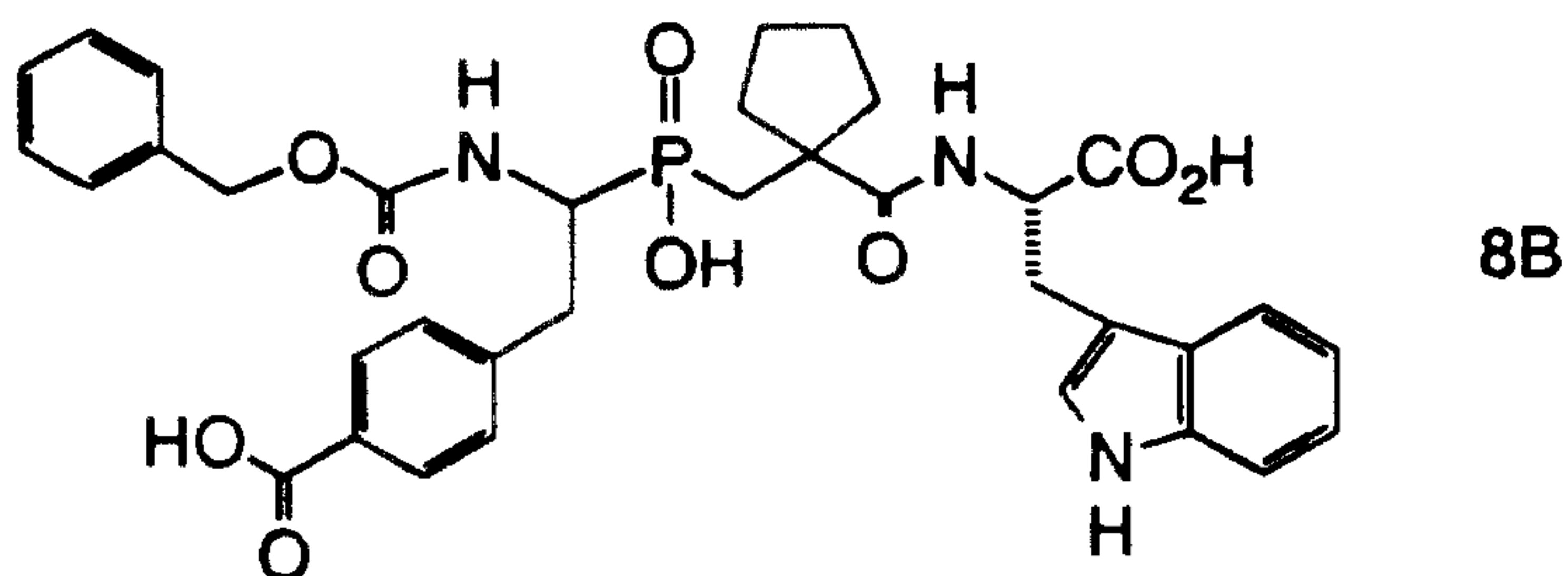
- Sequentially deprotect compound 7.1 (46 mg, 0.068 mmol) with HCl/1,4-dioxane/CH₂Cl₂ and LiOH.H₂O as described in Example 3, Step 4, to obtain compound 8 (41 mg, 92%). Anal. calcd for C₃₄H₃₈N₃O₈P.2H₂O: C, 59.73; H, 6.19; N, 6.15%. Found C, 59.96; H, 5.83; N, 6.14%. FAB MS *m/z* 648.3 (M+H)⁺.

- In a similar manner, deprotect compounds 7.3A and 7.3B to obtain the following compounds 8A and 8B, each of which are mixtures of 2 diastereomers:

- 30 -

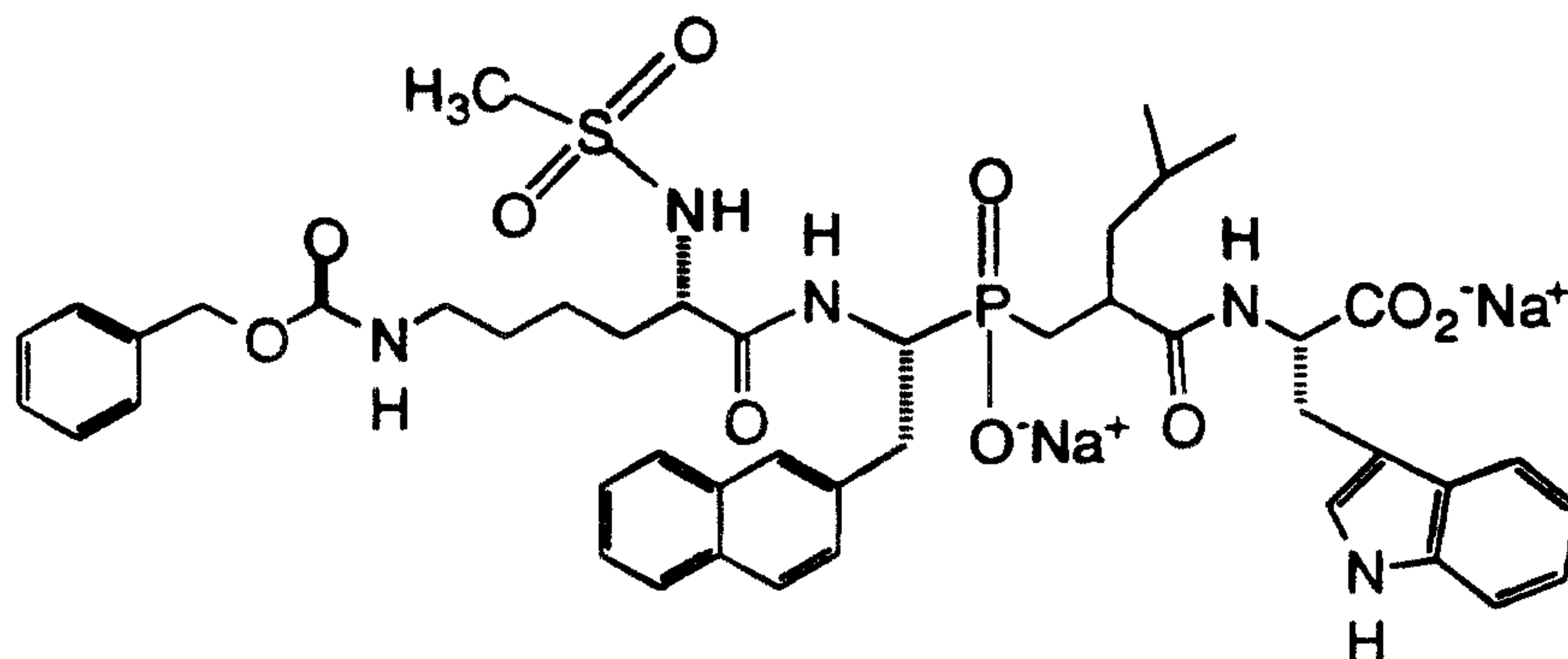


Anal. calcd for $C_{35}H_{39}N_4O_8P \cdot 2H_2O$: C, 59.15; H, 6.10; N, 7.88%. Found: C, 59.31; H, 5.74; N, 7.15%. FAB MS m/z 675 ($M+H$)⁺.



- 5 Anal. calcd for $C_{35}H_{38}N_3O_9P \cdot 1.5H_2O$: C, 59.82; H, 5.88; N, 5.98; P, 4.41%. Found: C, 59.69; H, 5.64; N, 5.89; P, 4.62%.

Example 9



- 10 **Step 1:** To a solution of 2-naphthyl triflate (90 g, 326 mmol) in DMF (240 ml), add triphenylphosphine (9.6 g, 36.6 mmol), vinyl acetate (149 g, 1862 mmol) and palladium acetate (3.8 g, 17 mmol). Reflux the reaction mixture for 1 h, then quench by addition of sat'd $NaHCO_3$ solution. Extract the mixture with Et_2O , dry and concentrate. Purify the residue by chromatography on silica gel (5% $EtOAc$ in hexane) to obtain 35 g (165 mmol, 51%) 2-(2-naphthyl)vinyl acetate.

- 20 To a warm solution of aminodiphenylmethane (106 g, 578 mmol) and dry H_3PO_2 (95 g, 1376 mmol) in dioxane (200 ml), under vigorous stirring, add a solution of 2-(2-naphthyl)-vinyl acetate (70 g, 330 mmol) in dioxane (200 ml). To this mixture add conc. HCl (30 ml), reflux for 3 h, and then allow to cool to R.T. Collect the white precipitate by filtration, wash with CH_3OH and dry to obtain the hypophosphorous acid (120 g,

91% of theory). ^1H NMR (200 MHz, Acetic acid- d_4) δ 3.5-3.75 (m, 1H), 3.85-4.00 (m, 1H), 4.10-4.25 (m, 1H), 5.92 (s, 1H), 6.15-9.00 (d, $J = 570$ Hz, 1H), 7.55-8.25 (m, 17H).

Step 2: Stir a mixture of the product of Step 1 (80 g, 200 mmol), $\text{CF}_3\text{CO}_2\text{H}$ (150 ml) and anisole (150 ml) at 85-90°C for 3 h. Remove the volatiles under high vacuum. Triturate the residue with CH_2Cl_2 . Collect the precipitate by filtration.

Dissolve the resultant residue in 10% aq. NaOH (140 ml) and dilute to 550 ml with H_2O . Cool the solution in an ice bath and add benzylchloroformate (83.65 g, 490 mmole) dropwise, while maintaining the pH of the solution between 10-12 by addition of 10% aq. NaOH. After the addition of benzylchloroformate, acidify the reaction mixture to pH 1 using conc. HCl. Add EtOAc (500 ml) to the reaction mixture to precipitate the product and collect by filtration (55 g, 75%).

To a solution of the resultant residue (7.5 g, 20.3 mmol) in a mixture of dioxane (300 ml) and CH_3OH (40 ml) cooled to 0°C, add a solution of trimethylsilyldiazomethane in hexane (Aldrich) dropwise until a yellow color persists. Stir the reaction mixture at 0°C for 30 min and concentrate *in vacuo*. Purify the product by chromatography on silica gel, eluting with 3% CH_3OH in CH_2Cl_2 to obtain 7.2 g of product (93 %). MS (FAB) m/e 384 ($\text{M}+\text{H}$) $^+$.

Step 3: To a solution of the product of Step 2 (7.2 g, 18.8 mmol) and Preparation 11 (8.5 g, 30.5 mmol) in deoxygenated, dry THF (100 ml) cooled to -78°C under argon, add a dispersion of NaH in oil (70 mg, 60%, 1.8 mmol). Discontinue cooling and allow the reaction mixture to warm to R.T. After 1 h, quench the reaction by addition of sat'd. aq. NH_4Cl (200 ml). Extract the organic phase with Et_2O (3 X 200 ml) and wash with brine. Dry the combined organic phase over MgSO_4 and concentrate under vacuum. Purify the crude product by chromatography on silica gel, eluting with 30% EtOAc in hexanes to obtain 11.0 g of product (88 %). ^1H NMR (300 MHz, CDCl_3) δ 0.85-1.00 (m, 6H), 1.27-1.40 (m, 1H), 1.45-1.70 (m, 2H), 1.80-2.00 (m, 1H), 2.27-2.40 (m, 1H), 2.90-3.55 (m, 3H), 3.62-3.76 (m, 3H), 4.87-4.95 (m, 6H), 4.5 (m, 1H), 4.90-5.23 (m, 5H), 6.85-7.90 (m, 15H).

Step 4: Mix the product of Step 3 (11.0 g, 16.6 mmol) with a solution of $\text{CF}_3\text{CO}_2\text{H}$ (50 ml) in anisole (20 ml) and cool to 0°C. Stir the reaction mixture for 40 min and remove the volatiles under kugel rotor distillation at R.T. Dissolve the resultant residue in DMF (100 ml) and add tryptophan methyl ester hydrochloride (8.45 g, 33.17 mmol), HOBt (3.9 g, 29 mmol),

- 32 -

- EDCI (14.5 g, 48.8 mmol) and N-methylmorpholine (15.6 g, 154 mmol). Stir the reaction mixture at R.T. for 18 hrs under argon. Add dilute HCl (1N, 200 ml), then extract with Et₂O (3 X 150 ml). Wash the combined organic layer with brine and sat'd NaHCO₃ solution. Purify the crude
- 5 product by chromatography on silica gel (5% CH₃OH in EtOAc) to obtain 9.5 g (71 %) of the desired product. ¹H NMR (300 MHz, CDCl₃) δ 0.70-1.50 (m, 6H), 1.05-2.10 (m, 4H), 2.20-2.40 (m, 1H), 2.70-3.50 (m, 5H), 3.55-3.90 (m, 6H), 4.35-4.55 (m, 1H), 7.00-8.60 (m, 17H). MS (FAB) m/e calcd for 712.3164, found 712.3152 (M+H)⁺.
- 10 Step 5: To a solution of the product of Step 4 (300 mg, 0.42 mmol) in ethyl alcohol (100 ml), add palladium on carbon (10%, 100 mg). Hydrogenate the suspension at 60 psi for 2 hrs. Remove the catalyst by filtration and concentrate the filtrate *in vacuo*.
- Using a procedure similar to that described in Step 4, couple the
- 15 resultant amine (780 mg, 1.31 mmol) with ε-CBZ-α-Ms-Lys (1.00g, 2.8 mmol) to obtain 660 mg (72 % theory) of the product as a mixture of two sets of diastereomers in 1:2 ratio. Less polar diastereomer: ¹H NMR (300 MHz, CD₃OD) δ 0.95 (d, J=7.0 Hz, 3H), 1.03 (d, J=7.0 Hz, 3H), 1.20-1.75 (m, 9H), 1.83 (s, 3H), 1.87-2.02 (m, 1H), 2.13-2.30 (m, 1H), 2.80-3.10 (m,
- 20 5H), 3.15-3.80 (m, 8H), 4.65-4.80 (m, 2H), 5.11 (s, 2H), 7.0-7.9 (m, 17H). MS (FAB) m/e 918.8 (M+H)⁺. More polar distereomer: ¹H NMR (300 MHz, CD₃OD) δ 0.7-1.1 (m, 12H), 1.2-1.45 (m, 2H), 1.55-1.77 (m, 1H), 1.80- 2.05 (m, 1H), 2.10-2.32 (m, 1H), 2.40-3.50 (m, 9H), 3.55-3.85 (m, 7H), 4.70-4.90 (m, 2H), 5.10-5.15 (m, 2H), 7.0-7.9 (m, 17H). MS (FAB) m/e 918.8 (M+H)⁺.
- 25 Step 6: To a solution of 2.7 g of the product of Step 6 in THF (100 ml), add 0.5 m aq NaOH (100 ml). Stir the reaction mixture at R.T. for 15 h and adjust the pH to 8 by addition of dilute HCl. Remove the solvents under high vacuum and subject the residue to reverse phase HPLC (C-18
- column, elution with CH₃OH/H₂O) to obtain three diastereomers: **A** (400
- 30 mg, 14.5 % theory); **B** (720 mg, 26 % theory); and **C** (350 mg, 12.7 % theory).
- A**: ¹H NMR (400 MHz, CD₃OD) δ 0.95 (d, J=6.5 Hz, 6H) 1.78 (s, 3H), 1.15-1.80 (m, 11H) 2.87-3.02 (m, 4H), 3.22 (m, 1H), 3.53 (d, J=12 Hz, 1H), 3.70 (m, 1H), 4.45 (m, 1H), 4.63 (m, 1H), 5.10 (s, 2H), 6.90-7.87 (m,
- 35 17H). MS (FAB) m/e calcd for 956.3016, found 956.3022 (M+Na)⁺.
- B**: ¹H NMR (400 MHz, CD₃OD) δ 0.63 (d, J=6.5 Hz, 3H), 0.86 (d, J=6.5 Hz, 1.5H), 0.94 (d, J=6.5 Hz, 1.5H), 0.90-2.05 (m, 14H), 2.78 (s, 1.5H), 2.65-2.95 (m, 3H), 3.02 (m, 1H), 3.25 (m, 1H), 3.37-3.52 (m, 2H), 3.7

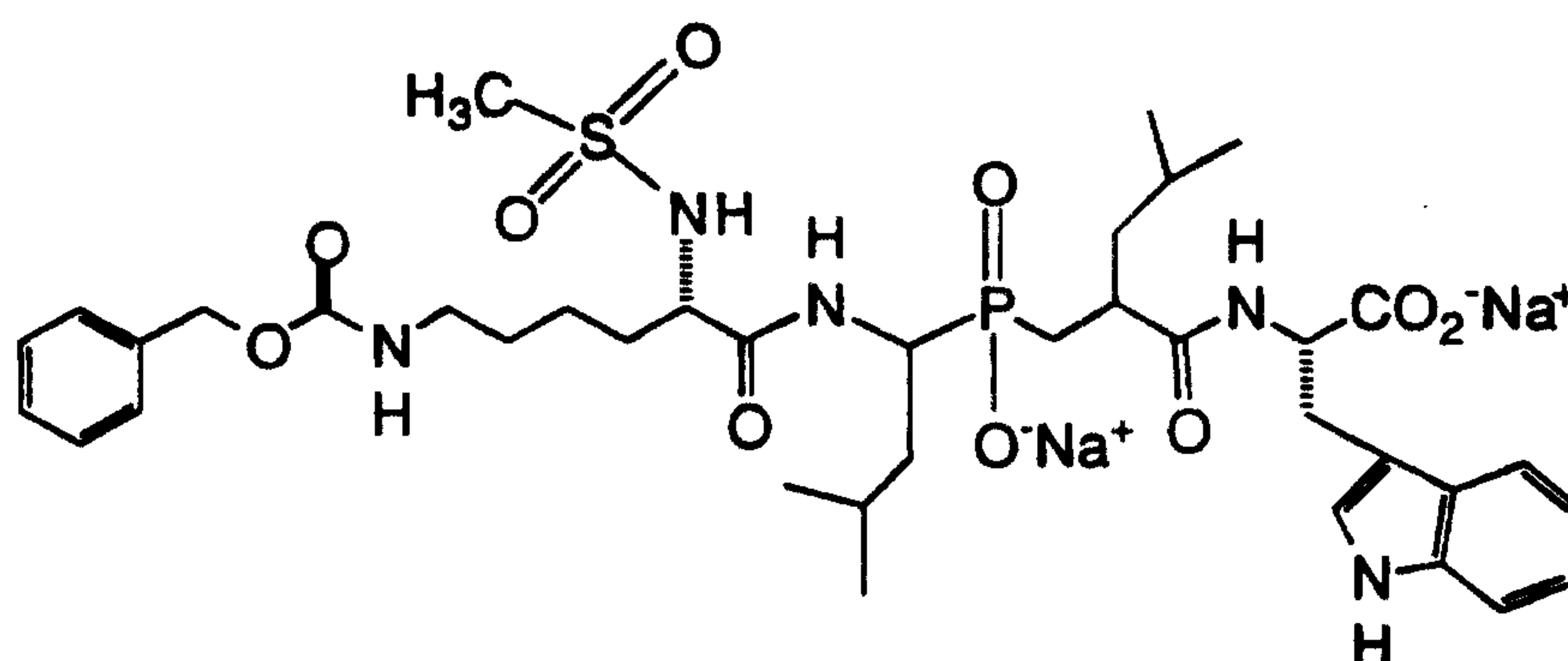
2191454

- 33 -

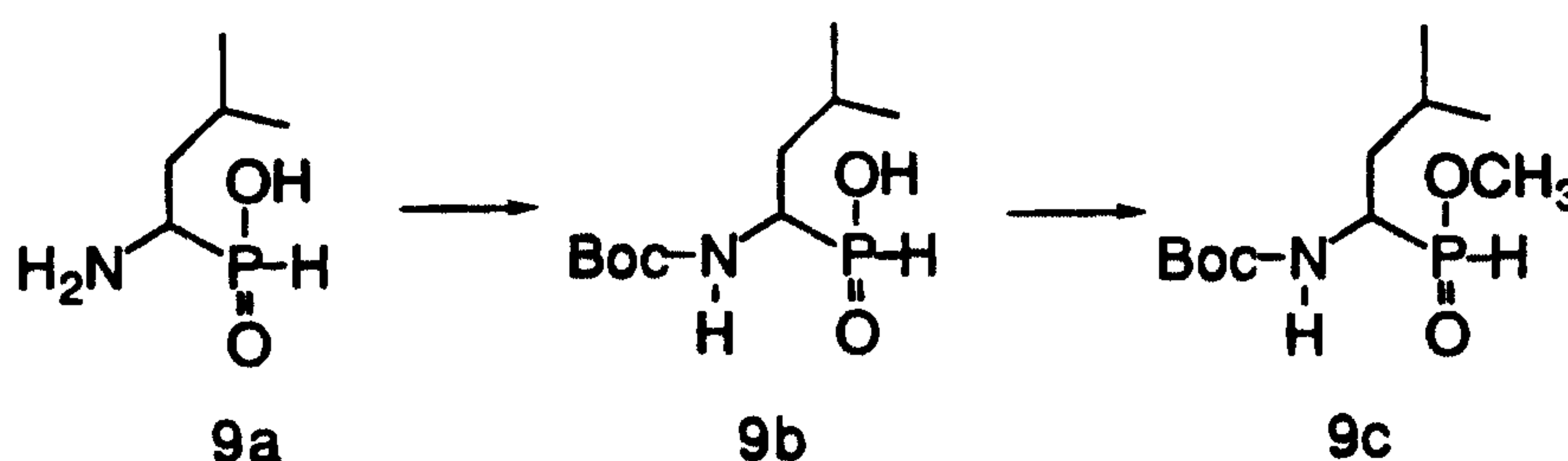
(m, 1H), 4.30-4.47 (m, 1H), 4.63 (m, 0.5H), 4.79 (m, 0.5H), 5.07-5.15 (m, 2H), 6.98-7.85 (m, 17H). MS (FAB) m/e 912 ($M + 2H - Na$)⁺ 912.

C: ¹H NMR (400 MHz, CDCl₃) δ 0.70 (m, 6H), 0.80-1.18, m, 7H), 1.50 (m, 2H), 1.67 (m, 1H), 1.88 (m, 1H), 2.67-2.87 (m, 3H), 2.90 (s, 3H), 2.97 (m, 1H), 3.22 (m, 1H), 3.51 (m, 2H), 3.79 (m, 1H), 4.47 (m, 1H), 4.82 (m, 1H), 5.15 (s, 2H), 7.07 (m, 1H), 7.14 (m, 1H), 7.22 (s, 1H), 7.33-7.52 (m, 9H), 7.68-7.90 (m, 5H).

Example 10



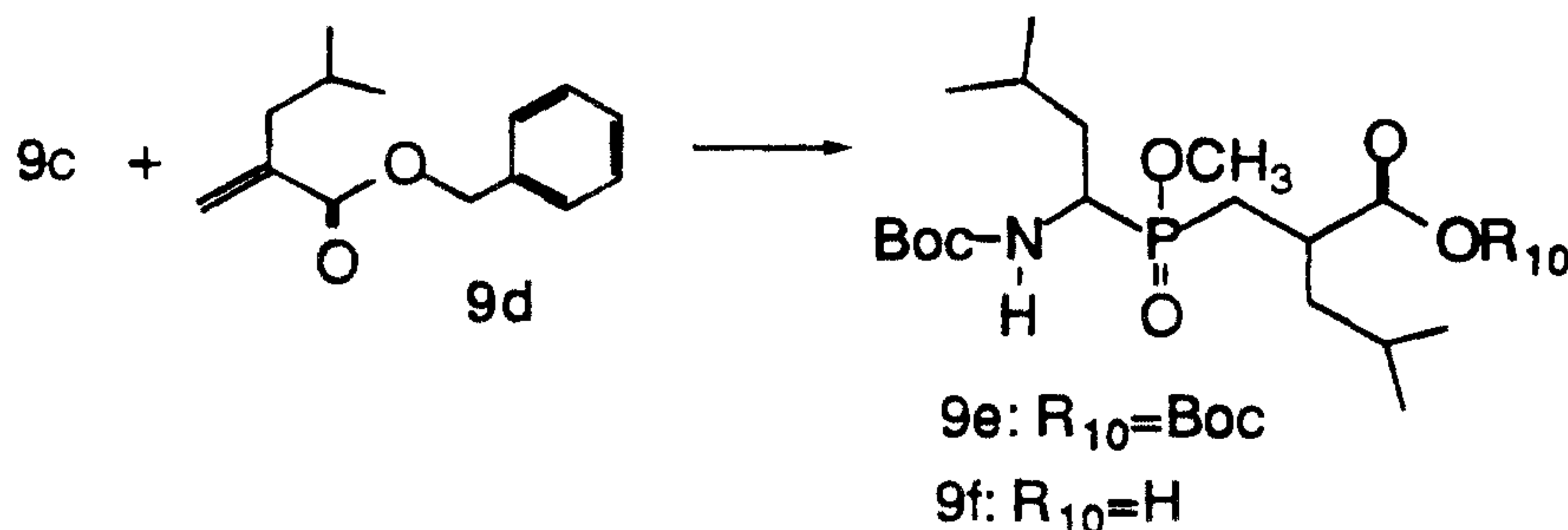
10 Step 1:



To a solution of compound 9a (4.80 g, 31 mmol) in aq. NaOH (22 ml, 1M) at R.T., add di-tert-butylcarbonate (11 g, 40 mmol) and stir at R.T. for 1 h, keeping the pH at 8-9. Extract the reaction mixture with Et₂O. Acidify the aqueous phase to pH 2 using 6 N HCl and extract with EtOAc. Dry the organic layer and concentrate *in vacuo* to yield compound 9b (6.0 g, 86%). ¹H NMR (acetone-d₆, 300 MHz) δ 7.85 (s, 0.5H), 5.80 (s, 0.50H), 6.25 (s, 1H), 3.80 (s, 1H), 1.10-1.90 (m, 12H), 0.85 (m, 6H).

To a solution of phosphinic acid 9b (6.0 g, 27 mmol) in dioxane (20 ml) and CH₃OH (1 ml), add a solution of trimethylsilyldiazomethane in hexane (Aldrich) until a yellow color persists. Stir the reaction mixture at R.T. overnight. Remove solvents *in vacuo* and the chromatograph the residue on silica gel, eluting with 2% CH₃OH in CH₂Cl₂ to afford compound 9c (4.4 g, 61%). ¹H NMR (CDCl₃, 300 MHz) δ 8.90 (s, 0.5H), 6.10 (s, 0.5H), 4.55-4.70 (m, 1H), 3.95-4.20 (m, 1H), 3.85 (m, 3H), 1.45 (s, 9H) 1.40-1.90 (m, 3H), 0.95-1.05 (m, 6H).

- 34 -

Step 2:

Treat a solution of compound 9c (1.65 g, 6.2 mmol) and acrylate 9d (1.3 g, 6.0 mmol) in a manner similar to Example 9, Step 3, to obtain compound 9e (1.91 g, 3.9 mmol, 63%). ¹H NMR (CDCl₃) δ 7.30-7.42 (m, 5H), 5.12 (m, 2H), 3.45-3.80 (m, 3H), 2.80-3.15 (m, 1H), 1.40 (br. s, 9H), 1.10-2.20 (m, 9H), 0.80-1.00 (m, 12H).

To a solution of compound 9e (1.9 g, 3.9 mmol) in EtOAc (20 ml), add Pd-C (10%, 300 mg). Hydrogenate the suspension at R.T. under 60 psi for 2h. Filter the mixture through a celite pad and concentrate the filtrate *in vacuo* to afford carboxylic acid 9f (1.4 g, 92%). ¹H NMR (acetone-d₆, 300 MHz) δ 6.00-6.30 (m, 1H), 3.80-4.10 (m, 1H), 3.55-3.65 (m, 3H), 2.70-2.90 (m, 1H), 1.30-1.40 (br. s, 9H), 1.30-2.20 (m, 9H), 0.75-0.95 (m, 12H).

Step 3: Couple 9f (0.60 g, 1.5 mmol) and tryptophan methyl ester hydrochloride (48 g, 1.9 mmol) in a manner similar to Example 9, Step 4, eluting the column with 5% EtOAc in hexanes to obtain 0.540 g (63%) of desired product. ¹H NMR (acetone-d₆, 300 MHz) δ 6.90-7.65 (m, 6H), 5.95-6.20 (m, 1H), 4.65-4.75 (m, 1H), 3.75-4.05 (m, 1H), 3.35-3.65 (m, 6H), 3.05-3.30 (m, 2H), 2.70-2.90 (m, 1H), 1.10-2.20 (m, 9H), 1.30-1.40 (br. s, 9H), 0.65-0.95 (m, 12H).

To a solution of the resultant compound (0.40 g, 0.71 mmol) in CH₂Cl₂, stirred under argon, add CF₃CO₂H. Stir the reaction mixture at R.T. for 1 h, remove solvents *in vacuo*, dissolve the residue in CH₂Cl₂ and wash with aq. NaHCO₃. Remove solvents to obtain the desired crude amine (0.250 g, 76%).

Step 4: Couple the product of Step 3 (0.100 g, 0.21 mmol) and ε-CBZ-α-Ms-Lys (.119 g, 0.33 mmol) using the procedure of Example 9, Step 4, eluting the column with 2% CH₃OH in CH₂Cl₂ to obtain 0.110 g (61%) of product. ¹H NMR (CD₃OD): δ 7.50-7.60 (m, 1H), 7.20-7.40 (m, 6H), 6.95-7.20 (m, 3H), 5.00-5.15 (br. s, 2H), 4.60-4.80 (m, 1H), 4.25-4.45 (m, 1H),

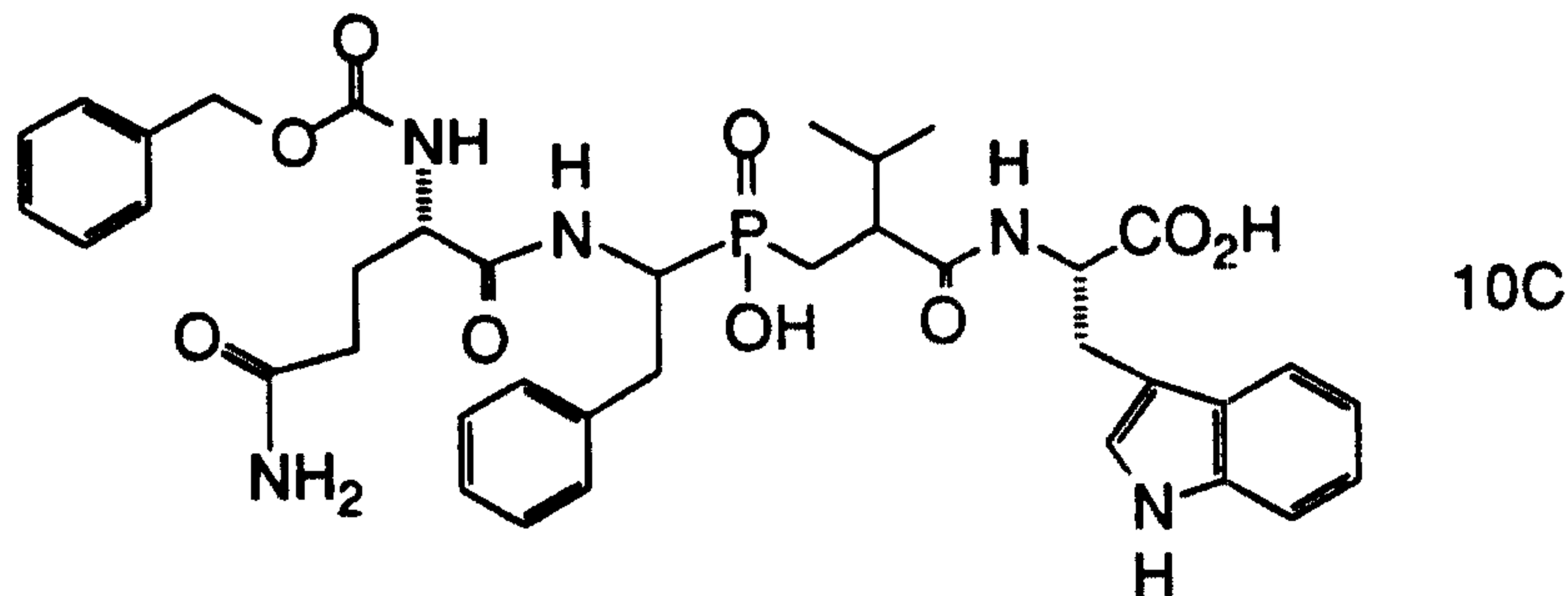
3.85-3.95 (m, 1H), 3.45-3.80 (m, 6H), 3.05-3.45 (m, 5H), 2.70-3.00 (m, 6H), 1.10-2.20 (m, 11H), 0.60-1.10 (m, 12H).

Step 5: Dissolve the ester from Step 4 in THF (30 ml) and add a solution of NaOH (0.5 N, 30 ml). Stir the reaction mixture at R.T. overnight.

- 5 Remove the solvents *in vacuo* and subject the residue to reverse phase column chromatography on silica gel (C-18), eluting with CH₃OH-H₂O (75% CH₃OH to 100% CH₃OH) to obtain the title compound as two fractions:

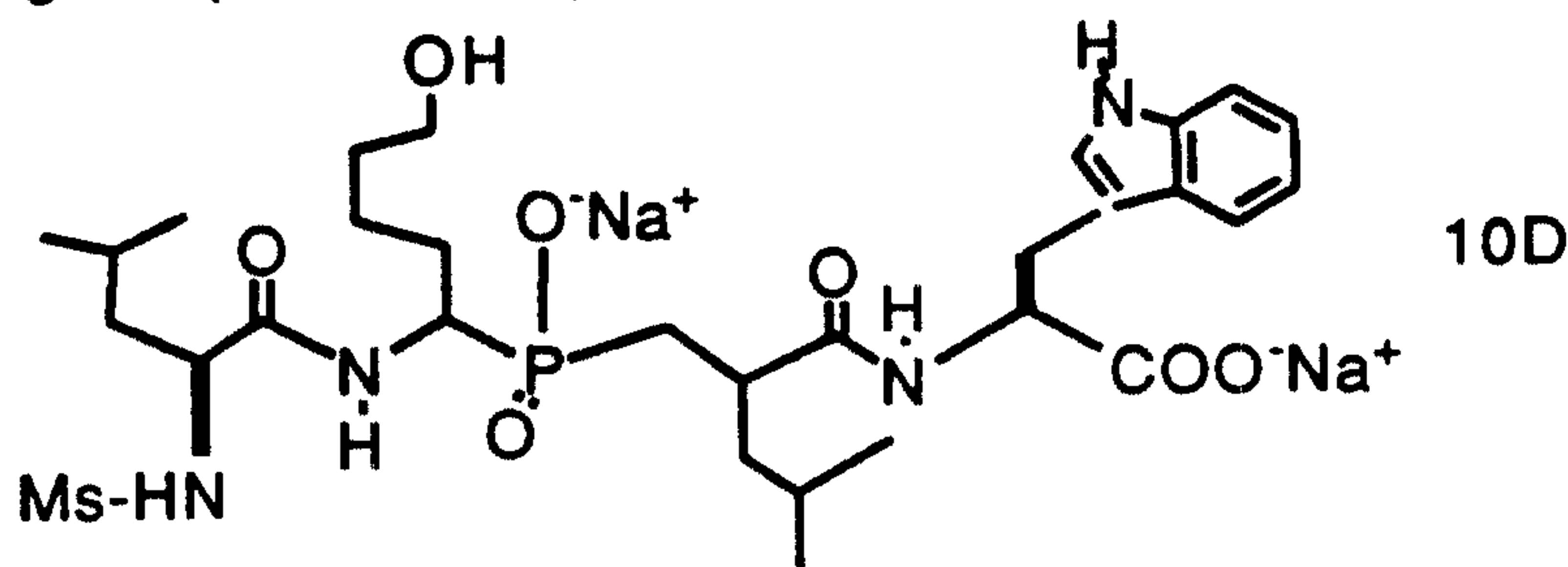
- 10 **10A** (0.045 g, 25 %), a single diastereomer: ¹H NMR (CD₃OD): δ 7.40-7.60 (m, 2H), 6.90-7.40 (m, 10H), 5.50 (s, 0.5H), 5.00-5.05 (br. s, 1.5H), 4.50-4.60 (m, 2H), 4.25-4.35 (m, 1H), 4.05-4.15 (m, 1H), 3.85-3.95 (m, 1H), 3.65-3.75 (m, 1H), 2.90 (s, 2H), 2.92-3.20 (m, 4H), 2.50-2.95 (m, 5H), 1.00-1.90 (m, 15H). 0.75-1.00 (m, 8H). MS (FAB) 850.5 (M+H)⁺, 872.5 (M+Na)⁺
- 15 **10B** (0.120g, 65 %), a mixture of three diastereomers. ¹H NMR (CD₃OD): δ 7.40-7.70 (m, 2H), 6.90-7.40 (m, 10H), 5.01-5.10 (br. s, 2H), 4.50-4.70 (m, 2H), 3.80-4.50 (m, 2H), 3.30-3.45 (m, 2H), 3.05-3.20 (m, 3H), 2.95 (s, 1H), 2.90 (s, 2H), 2.55-2.75 (m, 2H), 1.10-1.90 (m, 14H), 0.70-1.00 (m, 9H), 0.55-0.60 (m, 3H). MS (FAB) 850.5 (M+H)⁺, 872.5 (M+Na)⁺.

- 20 Using appropriate starting materials and following a similar procedure to that outlined in Steps 2-5 of Example 10, the following compound can also be prepared as a mixture of 4 diastereomers:



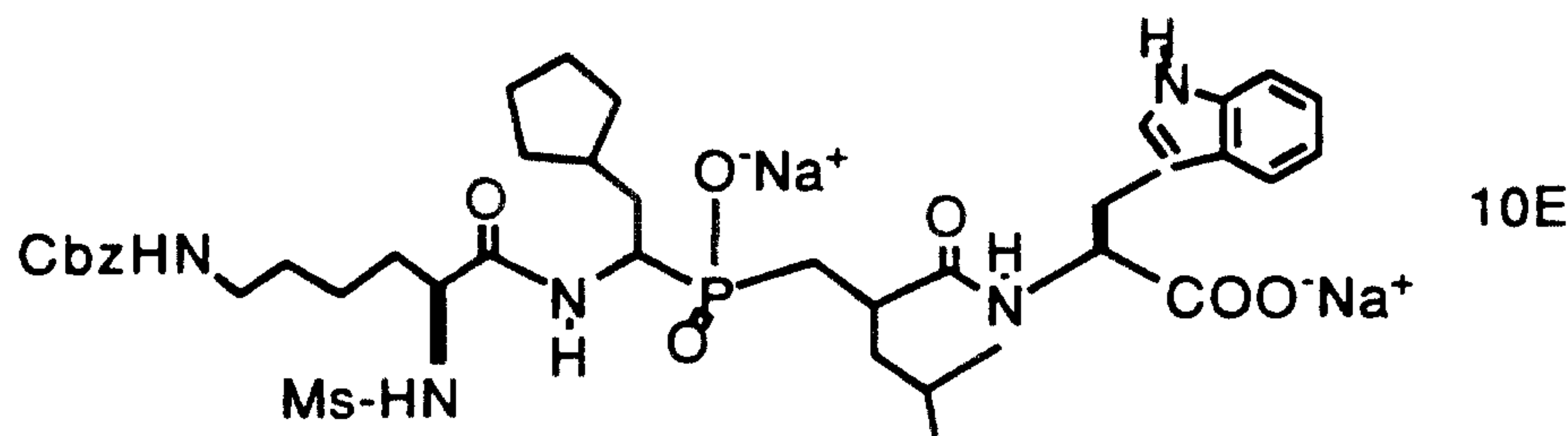
FAB MS *m/z* 748.3 (M+H)⁺.

- 25 Using a procedure similar to that described in Example 10, the following compounds are prepared:



- 36 -

MS (FAB) m/e 739 ((M+Na)⁺, monosodium salt). ¹H NMR (400 MHz, CD₃OD) δ 0.57-0.70, 0.97-1.07 (12H, m, CH₃), 0.80-1.80 (10.5H, m), 1.80-2.10 (3.5H, m), 2.55-2.75 (1H, m), 2.92 (1.8H, s), 3.01 (1.2H, s), 3.13-3.25 (1H, m), 3.35-3.50 (1H, m), 3.50-3.70 (2H, m), 3.85-4.10 (2H, m), 3.65 (1H, m), 6.97-7.40 (4H, m), 7.65-7.72 (1H, m).



MS (FAB) m/e 854 ((M+2Na)⁺, disodium salt). ¹H NMR (400 MHz, CD₃OD) δ 0.86 (3H, d, J=7.0 Hz), 0.95 (3H, d, J=7.0 Hz), 1.10-2.00 (16H, m), 2.89 (1H, m), 2.93 (3H, s), 3.02-3.22 (4H, m), 3.96 (1H, m), 4.08 (1H, m), 4.59 (1H, m), 5.08 (2H, s), 7.00-7.43 (9H, m), 7.70 (1H, d, J=8.0 Hz).

The following formulations exemplify some of the dosage forms of this invention. In each, the term "active compound" designates a compound of formula I or II.

15

EXAMPLE A**Tablets**

<u>No.</u>	<u>Ingredient</u>	<u>mg/tablet</u>	<u>mg/tablet</u>
1	Active Compound	100	500
2	Lactose USP	122	113
3	Corn Starch, Food Grade, as a 10% paste in Purified Water	30	40
4	Corn Starch, Food Grade	45	40
5	Magnesium Stearate	3	7
Total		300	700

Method of Manufacture

Mix Item Nos. 1 and 2 in suitable mixer for 10-15 minutes. Granulate the mixture with Item No. 3. Mill the damp granules through a coarse screen (e.g., 1/4", 0.63 cm) if necessary. Dry the damp granules. Screen the dried granules if necessary and mix with Item No. 4 and mix for 10-15 minutes. Add Item No. 5 and mix for 1-3 minutes. Compress the mixture to appropriate size and weight on a suitable tablet machine.

- 37 -

EXAMPLE B**Capsules**

<u>No.</u>	<u>Ingredient</u>	<u>mg/tablet</u>	<u>mg/tablet</u>
1	Active Compound	100	500
2	Lactose USP	106	123
3	Corn Starch, Food Grade	40	70
4	Magnesium Stearate NF	4	7
	Total	250	700

Method of Manufacture

- 5 Mix Item Nos. 1, 2 and 3 in a suitable blender for 10-15 minutes. Add Item No. 4 and mix for 1-3 minutes. Fill the mixture into suitable two-piece hard gelatin capsules on a suitable encapsulating machine.

Example C**10 Parenteral Preparation**

<u>Ingredient</u>	<u>mg/vial</u>	<u>mg/vial</u>
Active Compound Sterile Powder	100	500

Method of Manufacture

- 15 For reconstitution, add sterile water for injection or bacteriostatic water for injection.

The in vitro and in vivo ECE inhibitory activity of the compounds of formulae I and II can be determined by the following procedures.

- 20 **BET Challenge Method:** ECE in vivo activity is determined as described by Vemulapalli et al., in *Life Sciences*, 53 (1993), pp. 783-793. Briefly, male Charles River CD rats (Wilmington, MA) weighing 250-275 g are anesthetized with Inactin*, 100 mg/kg, ip. The trachea is catheterized. The right carotid artery and the right jugular vein are cannulated to record
- 25 blood pressure (BP) and to infuse drugs, respectively. The BP is recorded by connecting the arterial catheter to Statham* pressure transducers and displayed on a Grass* polygraph. The rectal temperature is kept at 37°C. After an equilibration period of 15-30 min, the rats are infused with saline vehicle (20 µl/min) or test compounds (0.01-0.25 mg/kg/min) throughout
- 30 the study. Thirty minutes after the start of infusion, the rats are challenged

* trade-mark

- 38 -

with BET-1 (5 µg/kg) via the venous catheter, and the BP is monitored for another 15 min.

Figures 1 and 2 show the results of the BET challenge for two compounds of the invention:

5 Compound A : N-[[1-[[hydroxy-[1(R)-[[6-amino-2(S)-
[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-phenylethyl]-
phosphinyl)methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;

 Compound B: N-[2-[[hydroxy[1-[[2(S)-[(methylsulfonyl)amino]-1-
10 oxo-6-[[phenylmethoxy)-carbonyl]amino]hexyl]amino]-2-(2-
naphthalenyl)ethyl]phosphinyl)methyl]-4-methyl-1-oxopentyl]-L-tryptophan
disodium salt.

Figure 1 shows that compared to the vehicle, Compound A, administered at a dose of 0.1 mg/kg/min, shows an approximate 15 mmHg lower blood pressure in response to the BET-1 challenge. Figure 2 shows that
15 compared to the vehicle, Compound B administered at a dose of 0.1 mg/kg/min does not have an effect, while administration of a dose of 0.3 mg/kg/min shows an approximate 22 mmHg lower blood pressure in response to the BET-1 challenge.

20 Isolated Perfused Guinea Pig Lung Method: The method is as described by Vemulapalli et al., in *J. Pharmacol. Exp. Therap.*, 262, 3 (1992), pp. 1062-1069. Briefly, male Dunkin-Hartley guinea pigs (400-600 g) are anesthetized with pentobarbital 30 mg/kg, ip. The trachea is cannulated to facilitate respiration. The right jugular vein is cannulated to administer
25 saline vehicle or test compound, or compounds are administered orally or subcutaneously. Ten minutes after the administration of saline vehicle or test compound, a thoracotomy is performed and the trachea, lungs and heart are removed intact and placed in warmed Tyrode's buffer (34-35°C) of the following composition in mM: NaCl 120, KCl 4.8, MgSO₄·7H₂O 1.2,
30 KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂·2H₂O 11.25, glucose 11 and BSA 0.5%. The pulmonary artery and trachea are cannulated and the lungs are mounted on a perfusion apparatus. Lungs are perfused at a rate of 3.2 ml/min via the pulmonary artery with oxygenated (95% O₂ and 5% CO₂) Tyrode's solution maintained at 34-35°C. The lungs are mechanically
35 ventilated with room air with a volume of 2.5 ml/stroke and 50 strokes/min. Pulmonary insufflation pressure (PIP) is monitored through a side arm of the perfusion apparatus with a Statham pressure transducer and recorded on a Grass polygraph. Lungs are allowed to stabilize for 15 min. Lung

- 39 -

effluents (50 ml) are collected every 15 min into chilled polypropylene centrifuge tubes (Corning) containing 100 µl each of aprotinin (200 kallikrein inhibitory units/ml) and soybean trypsin inhibitor (20 N- α -benzoyl-L-arginine ethyl ester units/ml). Ischemia-hypoxia (I/H) is produced by cessation of ventilation and perfusion for 15 min followed by reperfusion and reventilation for 60 min. At the end of the experiment, the lungs are tested for cardiopulmonary reactivity by challenging with 5-hydroxy-tryptamine (0.5 mg) directly into the lungs via a pulmonary artery cannula.

10 In a separate study, anesthetized guinea pigs are administered saline vehicle or test compound. Ten minutes later, the lungs are removed and mounted on a perfusion apparatus as described above. After an equilibration period of 15 min, BET-1 (30 µg over 15 min) or ET-1 (0.6 µg over 15 min) is infused directly into the perfusion medium and the effects on PIP in vehicle and test compound-treated guinea pig lungs are assessed.

Figures 3 to 6 show the results of four compounds of the invention on I/H-induced ET-1 release in isolated perfused guinea pig lungs. The compounds include Compounds A and B, identified above, and:

Compound C: N-(2-((1-((6-amino-2-(methylsulfonyl)amino)-1-oxohexyl)amino)-2-phenylethyl)hydroxyphosphinyl)methyl)-3-methyl-1-oxobutyl)-L-tryptophan; and

Compound D: N-[2-[[hydroxy[2-[1-[4-methyl-1-oxo-2-[[[(phenyl-methoxy)carbonyl]amino]pentyl]amino]-2-phenylethyl]phosphinyl]methyl]-4-methyl-1-oxopentyl]-tryptophan.

Figure 3 shows that the elevation in PIP produced by I/H (see the results of administering vehicle only) is attenuated at 1 hour and at 3 hours by oral administration of Compound A at a dose of 30 mg/kg. The asterisks indicate statistical significance ($P < 0.05$) compared to the vehicle.

Figure 4 shows that the elevation in PIP produced by I/H is attenuated at 1 hour by a subcutaneous (sc) administration of 3 mg/kg and at 6 hours by sc administration of 10 and 30 mg/kg of Compound B.

Figure 5 shows that the elevation in PIP produced by I/H is attenuated by a intravenous (iv) administration of 10 mg/kg of Compound C. Similarly, Figure 6 shows the attenuation of ET-1 release following I/H by iv administration of 10 mg/kg of Compound D.

- 40 -

ECE Assay Method (in vitro): The ECE assay is based on the quantitative determination of [125 I]ET-1 released from [125 I]BET-1 labeled on Tyrosine-13 by binding to the membrane-bound ET receptor. Guinea pig lung membrane (GPLGM) was selected as a suitable tissue source of phosphoramidon-sensitive ECE. GPLGM was also found to contain a high density of ET receptors. Thus, this preparation was utilized as a source of both ECE and a saturating concentration of ET receptors for the quantitative determination of [125 I]ET-1 from the conversion of [125 I]BET-1. Under selected experimental conditions of the ECE assay, specific [125 I]ET-1 bound was directly related to ECE activity. Assays were carried out in the presence of varying concentrations of test compounds to evaluate their efficacy in inhibiting ECE activity.

Preparation of Guinea Pig Lung Membrane (GPLGM): Frozen guinea pig lungs (Keystone Biologicals, Cleveland, OH) are weighed and homogenized in 10 times gram tissue weight of solution A (50 mM Tris-HCl, pH 7.4, 0.25 M sucrose, and 2 mM EDTA) using a Polytron* tissue homogenizer (probe PTA-20S). Homogenization is repeated four times at speed setting seven each for 10 s and with 5-8 min intervals on ice in between homogenization. Homogenates are spun for 30 min at 2000g (4100 rpm in an SS34 rotor or 3800 rpm in an SA-600 rotor). Supernatants containing membranes are carefully decanted and saved. Pellets are rehomogenized in solution A as described, and homogenates are spun at 2000g for 30 min. Supernatants are removed, mixed with the supernatants from the first spin, and spun at 37000 rpm in a 45 Ti rotor (107022g) for 60 min. Pellets containing membranes are suspended in solution B (10 mM Tris-HCl, pH 7.4, and 0.125 M sucrose) using a Dounce* homogenizer. Samples are divided into 1 ml fractions, rapidly frozen in a dry icemethanol bath, and stored at -80°C. All steps are carried out at 4°C.

Preparation of Lubrol-Treated GPLGM (L-GPLGM): GPLGM fractions prepared as described above are thawed and resuspended at a concentration of 1 mg membrane protein/mL in a solution containing 0.06% lubrol PX™, 50 mM Tris-HCl (pH 7.4), and 100 mM NaCl. Stir the mixture for 1 h and then spin for 1 h at 37000 rpm in a 45 Ti rotor (107022g). Discard the supernatants and resuspend the pellets in a solution containing 50 mM Tris-HCl (pH 7.4), and 100 mM NaCl using a Dounce* homogenizer, and adjust the final volume to half the original volume. Spin the homogenates for 1 h at 37000 rpm in a 45 Ti rotor

* trade-mark

- 41 -

(107022g). Suspend the membrane pellets in solution B and treat as described above. All steps are performed at 4°C.

ECE Assay: Samples of L-GPLGM containing 20 µg protein are incubated in a solution containing 100 mM Tris-HCl (pH 7), 100 mM NaCl, and 5 mg/ml BSA (Fraction V, 98-99% albumin) (Sigma Chemical Co.) at a final reaction volume of 100 µL. The ECE assay is initiated by the addition of [¹²⁵I]BET-1 to obtain a final concentration of 500 pM. Reactions are carried out for 30 min at 37°C. Reactions are terminated by addition of 4 mL of solution C (10 mM Tris-HCl, pH 7.4, and 150 mM NaCl) at 4°C followed by rapid filtration on Whatman* GF/B glass microfiber filters. Filters are presoaked for 1 h at 4°C in a solution containing 50 mM Tris-HCl (pH 7.4), 10 mg/mL BSA, and 0.1% sodium azide. Wash test tubes and filters four times with 4 mL of solution C at 4°C, and count radioactivity retained on the filters in a γ counter. Non-specific binding is determined in the presence of 1 µM of unlabeled ET-1 (Peninsula Laboratories, Inc., Belmont, CA) in the reaction mixture. All reactions are performed in duplicate.

Assay of ECE Inhibition: To test the effect of test compounds on ECE activity, ECE assays are carried out in the presence of varying concentrations of the compounds. Assays are initiated following a 2 h incubation of L-GPLGM with the compound at 4°C. ECE activity in the presence of compounds is expressed as a percentage of control ECE activity of the membrane preparation, which is determined simultaneously. The concentration of compounds producing a 50% inhibition of ECE activity (IC₅₀ values) is determined from a plot of the percentage of control ECE activity versus log concentration of compounds.

Evaluation of the ECE Assays: To evaluate the selectivity of ECE inhibition by the compounds, simultaneous experiments identical to the ECE assays are carried out using 250 pM [¹²⁵I]ET-1 instead of [¹²⁵I]BET-1. The amount of specific [¹²⁵I]ET-1 binding is expressed as a percentage of that in controls containing no compound. These results provide information about the direct effect of compounds on ET-1 binding to the receptor. Such experiments are carried out routinely throughout the investigation and results are used as an index of integrity of the ECE assay and validity of values of ECE inhibitory activity.

Protein Assay. Protein concentrations are determined by an amido black binding assay using BSA as a standard.

* trade-mark

- 42 -

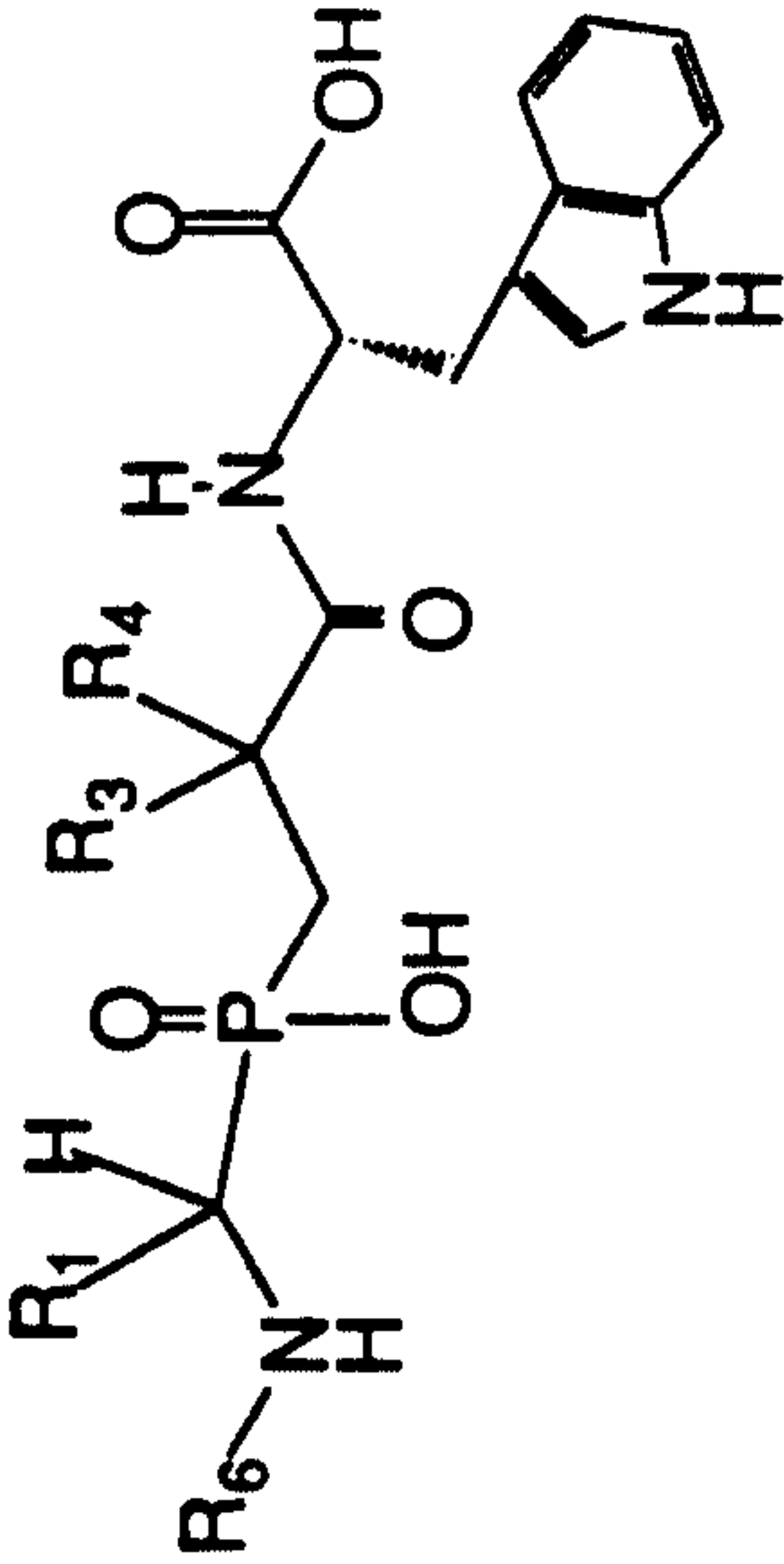
Using the in vitro ECE assay described above, ECE IC₅₀ (nM) data was obtained for many of the compounds shown in the examples:

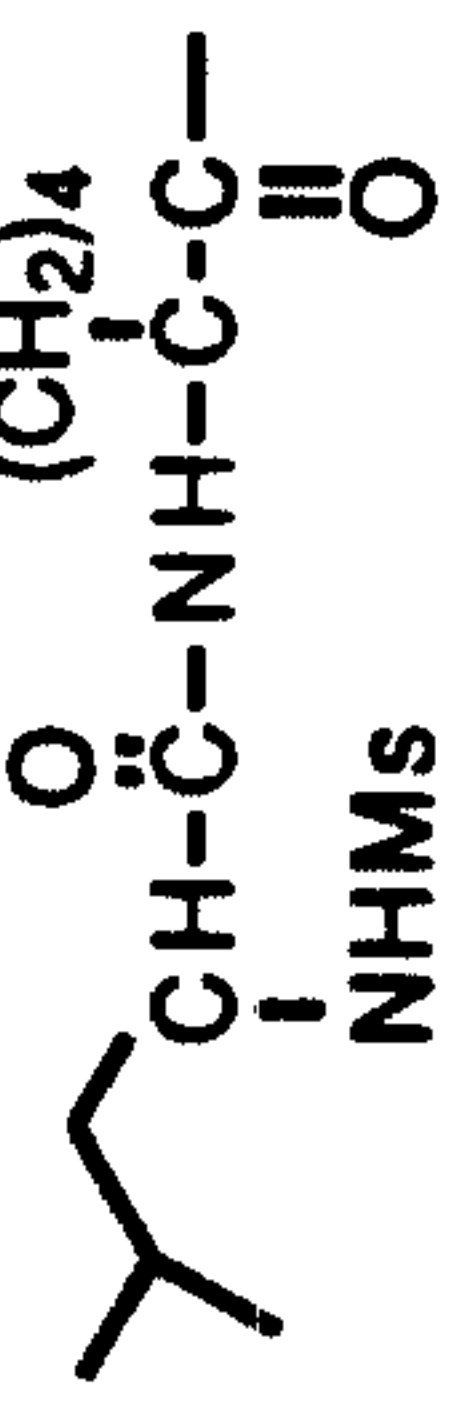
Ex. No.	IC ₅₀ (nM)
1	190
1A	115
1B	400
1C	800
2	130
3	60
3A	50
3B	55
3C	55
3D	55
4	119
5	70
5A	40
5B	100
5C	100

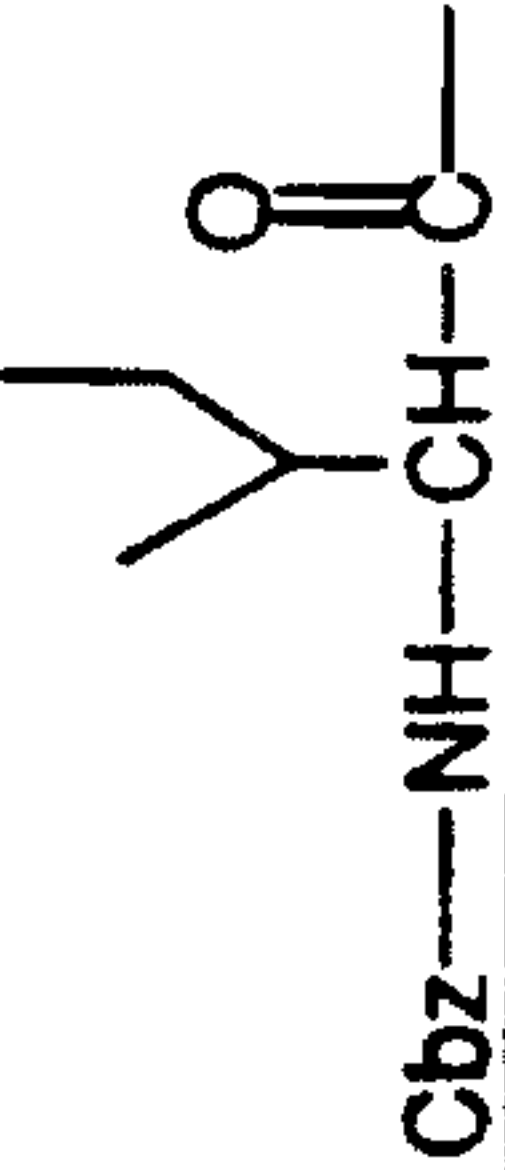
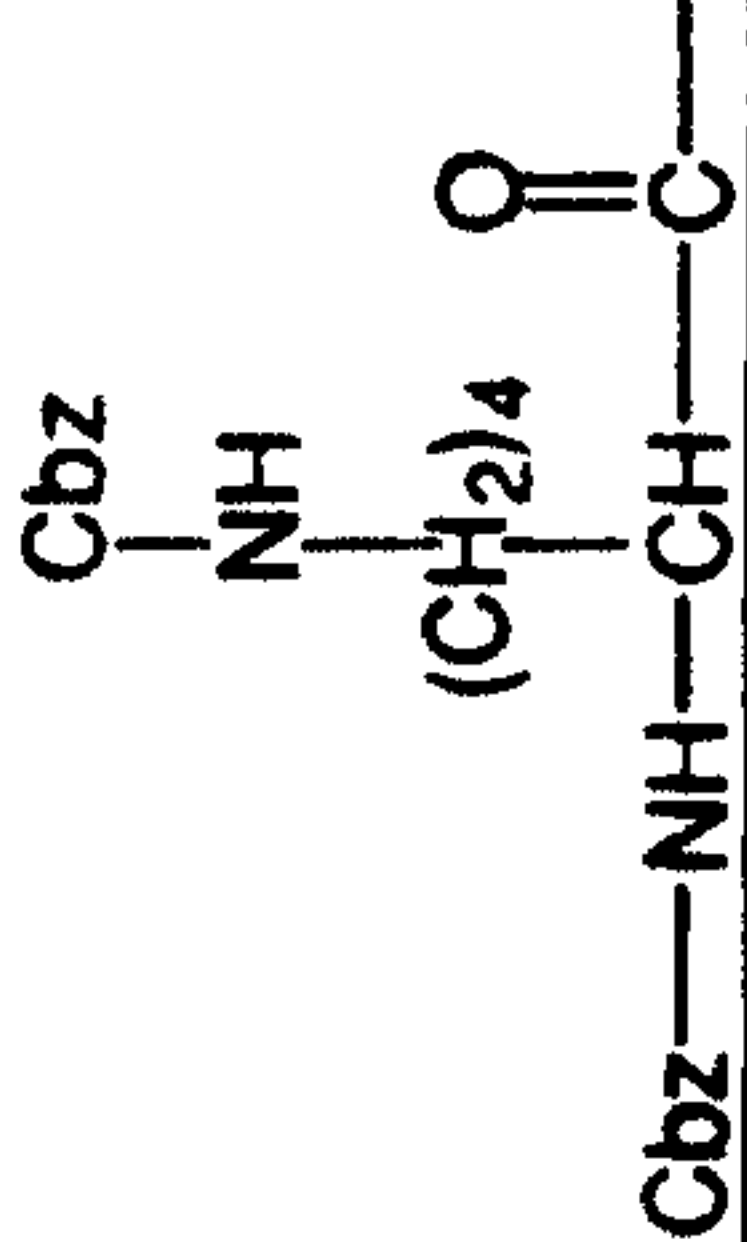
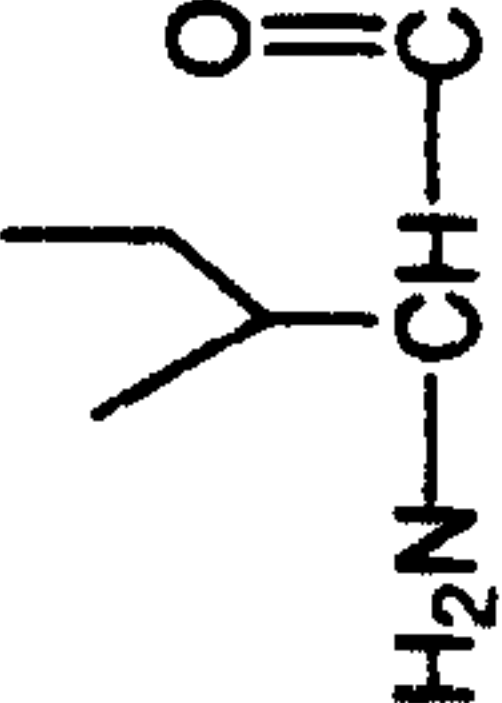
Ex. No.	IC ₅₀ (nM)
6	90
6.2A	50
6.2B	250
7	50
8	85
8A	290
8B	490
9A	65
9B	4500
9C	4500
10A	40
10B	1400
10C	200
10D	150
10E	55

- 5 Additional compounds within the scope of the present invention were prepared by methods similar to those described above and were found to have the in vitro ECE inhibition activity shown in the following table:

2191454



Isomer	R ₆	R ₁	R ₃	R ₄	IC ₅₀ (nM)
A	H ₂ N(CH ₂) ₄ CH(NHMs)C(O)-	2-naphthylmethyl	H	iso-butyl	27
B	H ₂ N(CH ₂) ₄ CH(NHMs)C(O)-	2-naphthylmethyl	H	iso-butyl	60
C	H ₂ N(CH ₂) ₄ CH(NHMs)C(O)-	2-naphthylmethyl	H	iso-butyl	68
--	Cbz-NH-CH(((CH ₃) ₂ CHCH ₂))C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-butyl	160
--	H ₃ CO-C ₆ H ₄ -C(O)-	C ₆ H ₅ -CH ₂ -	R ₃ +R ₄ =-(CH ₂) ₄ -		160
--	Cbz	C ₆ H ₅ -CH ₂ -	R ₃ +R ₄ =-(CH ₂) ₄ -		190
--	Cbz	HO-(CH ₂) ₄ -	H	iso-butyl	200
--		2-naphthylmethyl	H	iso-butyl	230
--	H ₂ N(CH ₂) ₄ CH(NHCbz)C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-propyl	250
--	Cbz-NH(CH ₂) ₄ CH(NHMs)-C(O)-	iso-butyl	R ₃ +R ₄ =-(CH ₂) ₄ -		260
--	Cbz	2-naphthylmethyl	H	iso-butyl	300
A	Cbz-NH(CH ₂) ₄ CH(NHMs)-C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-propyl	390

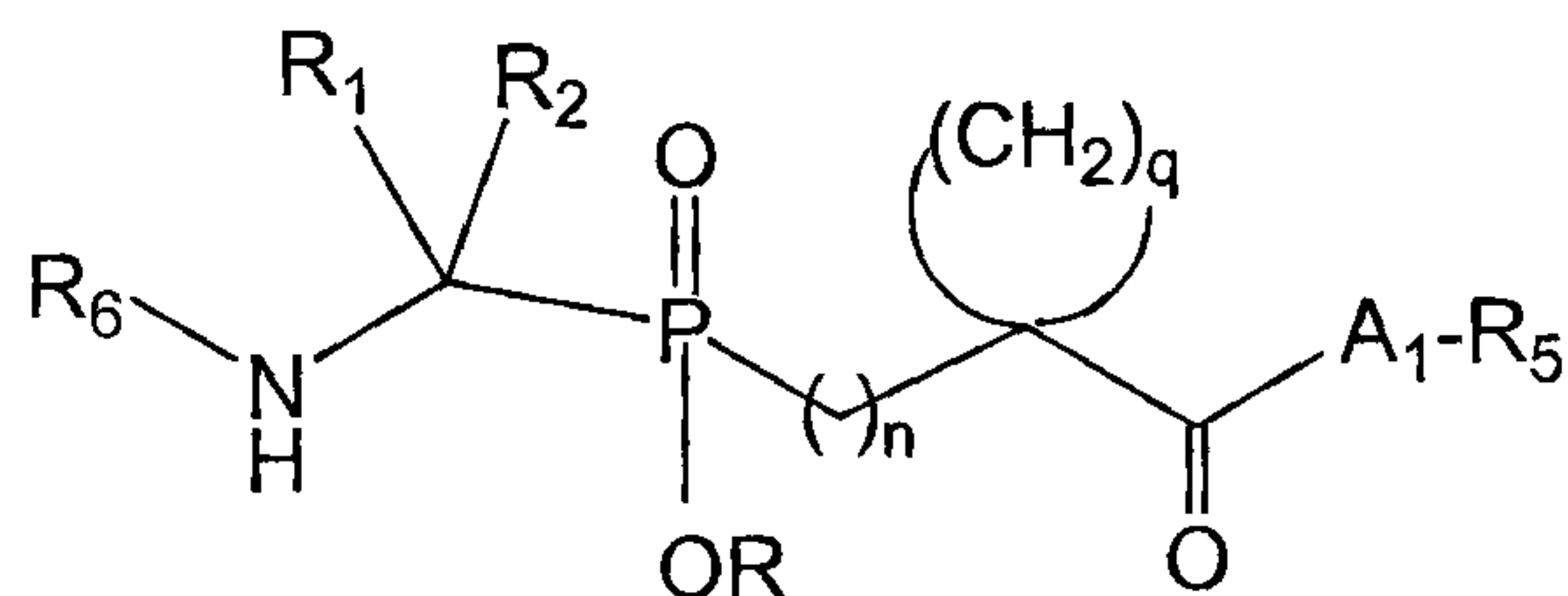
Isomer	R ₆	R ₁	R ₃	R ₄	IC ₅₀ (nM)
B	Cbz-NH(CH ₂) ₄ CH(NHMs)-C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-propyl	1500
--	Cbz	NH ₂ -(CH ₂) ₄ -	H	iso-butyl	400
--		C ₆ H ₅ -CH ₂ -	H	iso-propyl	400
--	Cbz	H	R ₃ +R ₄ = -(CH ₂) ₄ -		420
--	H ₂ N(CH ₂) ₄ CH(NHMs)C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-propyl	500
--	Cbz-NH(CH ₂) ₄ CH(NHMs)-C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-butyl	500
--	Cbz	2-naphthylmethyl	R ₃ +R ₄ = -(CH ₂) ₄ -		500
--	H ₂ N(CH ₂) ₃ CH(NHMs)C(O)-	C ₆ H ₅ -CH ₂ -	H	iso-propyl	550
--	Cbz-NH(CH ₂) ₄ CH(NHMs)-C(O)-	2-naphthylmethyl	H	iso-propyl	800
--	Cbz	cyclopentylmethyl	H	iso-butyl	1000
--		C ₆ H ₅ -CH ₂ -	H	iso-propyl	1100
--	Cbz	C ₆ H ₅ -CH ₂ -	H	iso-propyl	1300
--	Cbz-NH(CH ₂) ₄ CH(NHMs)-C(O)-	iso-butyl	H	iso-butyl	1400
--		C ₆ H ₅ -CH ₂ -	H	iso-propyl	1600

Cbz=carbobenzyloxy; Ms=methanesulfonyl

- 45 -

CLAIMS:

1. A compound represented by the structural formula



or a pharmaceutically acceptable salt thereof, wherein

R is H, (C₁-C₈)alkyl or (C₁-C₈)alkanoyloxymethylene;

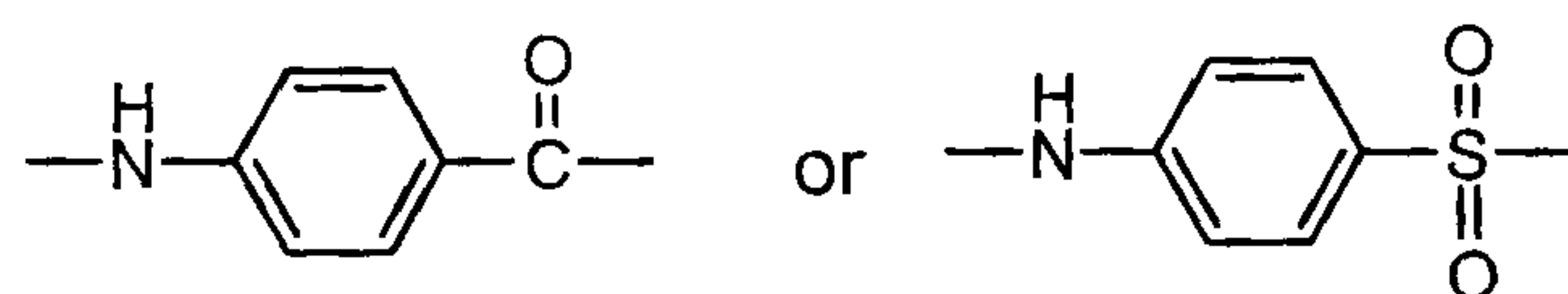
R₁ and R₂ are independently selected from the group consisting of H, (C₁-C₈)alkyl, (C₂-C₈)alkenyl, (C₂-C₈)alkenyl(C₁-C₈)alkyl, aryl, aryl(C₁-C₈)alkyl, heteroaryl, heteroaryl(C₁-C₈)alkyl, hydroxy-(C₁-C₈)alkyl, carboxy(C₁-C₈)alkyl, thio(C₁-C₈)alkyl, (C₁-C₆)alkoxy-thio(C₁-C₈)alkyl, amino(C₁-C₈)alkyl, (C₁-C₈)alkylamino(C₁-C₈)alkyl, cycloalkyl-substituted(C₁-C₈)alkyl and heterocycloalkyl; or R₁ and R₂, together with the carbon to which they are attached, form a cycloalkyl ring of 3 to 8 members;

R₅ is -OR₉ or -NHR₉, wherein R₉ is hydrogen or (C₁-C₈)alkyl;

n is 0 or 1;

q is 2 to 6;

A₁ is p-aminobenzoyl or p-aminobenzenesulfonyl of formula:



or A₁ and R₅ together form an α -aminoacyl radical, wherein A₁ is a divalent α -iminoacyl radical attached to the molecule at the α -imino group, and wherein R₅ is attached to the acyl terminus of A₁, said α -aminoacyl radical and divalent α -iminoacyl radical being derived from natural amino acids or esters in the R or S chiral form;

R₆ is phenylmethoxycarbonyl, arylcarbonyl, heteroaryl-carbonyl or

- 46 -

-A₂-R₇, wherein A₂ is a divalent α -iminoacyl radical attached to the molecule at the acyl terminus, and wherein R₇ is a substituent on the α -imino atom selected from the group consisting of H, R₈OCO-, R₈SO₂- and R₈NHCO-, wherein R₈ is aryl, arylmethyl or (C₁-C₈)alkyl.

2. A compound of claim 1, wherein n is 1; -A₁-R₅ is L-tryptophanyl, L-tyrosinyl or L-O-methyl tyrosinyl; and R is hydrogen.

3. A compound of claim 1 or 2, wherein R₁ and R₂ are independently selected from the group consisting of H, aryl(C₁-C₈)alkyl and branched (C₃-C₈)alkyl.

4. A compound of claim 1, 2 or 3, wherein R₆ is phenyl-methoxycarbonyl or -A₂-R₇, wherein A₂ is an α -iminoacyl radical derived from lysine, ϵ -CBZ lysine, arginine, isoleucine, leucine, valine, phenylalanine, alanine, glycine or tyrosine, and R₇ is benzyloxycarbonyl or methanesulfonyl.

5. A compound of claim 1 selected from the group consisting of:
 N-[[1-[[hydroxy[2-phenyl-1(R)-[[phenylmethoxy]carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-(4-methoxyphenyl)-1(R,S)-[[phenylmethoxy]carbonyl]amino]ethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-phenyl-1(R)-[[phenylmethoxy]carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tyrosine;
 N-[[1-[[hydroxy[2-phenyl-1(R)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-(4-methoxyphenyl)-1(R,S)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-(4-hydroxyphenyl)-1(R,S)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;

- 47 -

N-[[1-[[hydroxy[2-phenyl-1(R)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[[(phenylmethoxy)carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tyrosine;

N-[[1-[[hydroxy-[1(R)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;

N-[[1-[[hydroxy-[1(R,S)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-(4-methoxyphenyl)ethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;

N-[[1-[[hydroxy-[1(R,S)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-(4-hydroxyphenyl)ethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;

N-[[1-[[hydroxy-[1(R)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tyrosine lithium salt;

N-[[1-[[hydroxy-[1(R)-[[4-(aminomethyl)benzoyl]amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;

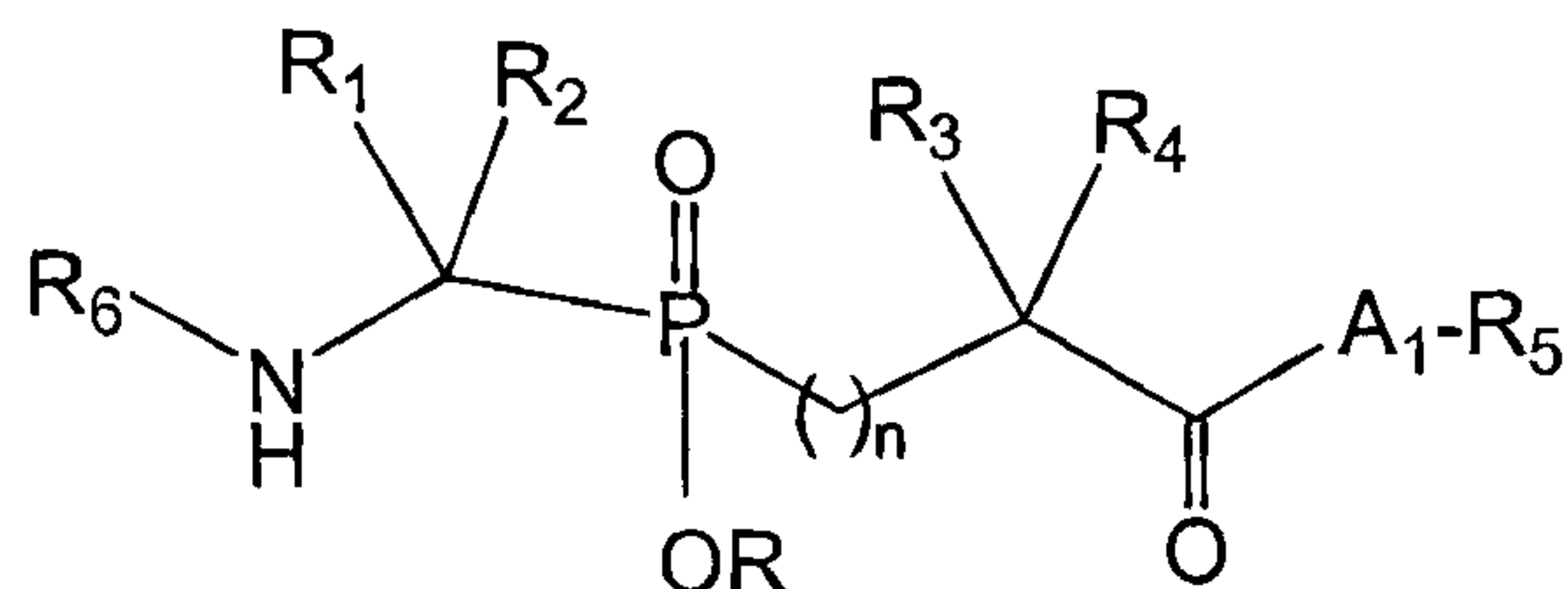
N-[[1-[[hydroxy-[1(R)-[[4-nitrobenzoyl]amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;

N-[[1-[[hydroxy[2-[4-(4-morpholinecarbonyl)phenyl]-1(R,S)-[[[(phenylmethoxy)carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan; and

N-[[1-[[hydroxy[2-(4-hydroxyphenyl)-1(R,S)-[[[(phenylmethoxy)carbonyl]-amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan.

6. A pharmaceutical composition comprising a compound or salt of claim 1, 2, 3, 4 or 5, in a pharmaceutically acceptable carrier.

7. The use of a compound represented by the formula



- 48 -

or a pharmaceutically acceptable salt thereof, wherein

R is H;

R₁ and R₂ are independently selected from the group consisting of H, (C₁-C₈)alkyl, (C₂-C₈)alkenyl, (C₂-C₈)alkenyl(C₁-C₈)alkyl, aryl, aryl(C₁-C₈)alkyl, heteroaryl, heteroaryl(C₁-C₈)alkyl, hydroxy-(C₁-C₈)alkyl, carboxy(C₁-C₈)alkyl, thio(C₁-C₈)alkyl, (C₁-C₆)alkoxy-thio(C₁-C₈)alkyl, amino(C₁-C₈)alkyl, (C₁-C₈)alkylamino(C₁-C₈)alkyl, cycloalkyl-substituted(C₁-C₈)alkyl and heterocycloalkyl; or R₁ and R₂, together with the carbon to which they are attached, form a cycloalkyl ring of 3 to 8 members;

R₃ and R₄ are independently selected from the group consisting of hydrogen and (C₁-C₈)alkyl, or R₃ and R₄, together with the carbon to which they are attached, form a cycloalkyl ring of 5 or 7 members;

A₁-R₅ is L-tryptophanyl, L-tyrosinyl or L-O-methyl tyrosinyl;

R₆ is phenylmethoxycarbonyl, arylcarbonyl, heteroarylcarbonyl or -A₂-R₇, wherein A₂ is a divalent .alpha.-iminoacyl radical attached to the molecule at the acyl terminus, and wherein R₇ is a substituent on the .alpha.-imino atom selected from the group consisting of H, R₈OCO-, R₈SO₂ and R₈NHCO-, wherein R₈ is aryl, arylmethyl or (C₁-C₈)alkyl; and

n is 0 or 1;

for the preparation of a medicament for inhibiting endothelin converting enzyme.

8. The use of claim 7, wherein R₁ and R₂ are independently selected from the group consisting of H, aryl(C₁-C₈)alkyl, branched (C₃-C₈)alkyl and cycloalkyl; R₆ is phenylmethoxy-carbonyl or -Ar₂-R₇, wherein A₂ is an α -iminoacyl radical derived from lysine, ϵ -CBZ lysine, arginine, isoleucine, leucine, valine, phenylalanine, alanine, glycine or tyrosine and R₇ is benzyloxycarbonyl or methansulfonyl.

9. The use of a compound is selected from the group consisting of: N-[[1-[[hydroxy[2-phenyl-1(R)-[[[(phenylmethoxy)carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;

N-[[1-[[hydroxy[2-(4-methoxyphenyl)-1(R,S)-[[phenylmethoxy]carbonyl]-amino]ethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-phenyl-1(R)-[[phenylmethoxy]carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tyrosine;
 N-[[1-[[hydroxy[2-phenyl-1(R)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-(4-methoxyphenyl)-1(R,S)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-(4-hydroxyphenyl)-1(R,S)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy[2-phenyl-1(R)-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy]carbonyl]amino]hexyl]amino]-ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tyrosine;
 N-[[1-[[hydroxy-[1(R)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;
 N-[[1-[[hydroxy-[1(R,S)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-(4-methoxyphenyl)ethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;
 N-[[1-[[hydroxy-[1(R,S)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-(4-hydroxyphenyl)ethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan lithium salt;
 N-[[1-[[hydroxy-[1(R)-[[6-amino-2(S)-[(methylsulfonyl)amino]-1-oxohexyl]-amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tyrosine lithium salt;
 N-[[1-[[hydroxy-[1(R)-[[4-(aminomethyl)benzoyl]amino]-2-phenylethyl]phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;
 N-[[1-[[hydroxy-[1(R)-[[4-nitrobenzoyl]amino]-2-phenylethyl]phosphinyl]-methyl]cyclopentyl]carbonyl]-L-tryptophan;

- 50 -

N-[[1-[[hydroxy[2-[4-(4-morpholinecarbonyl)phenyl]-1(R,S)-[[[(phenylmethoxy)carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan;

N-[[1-[[hydroxy[2-(4-hydroxyphenyl)-1(R,S)-[[[(phenylmethoxy)carbonyl]amino]ethyl]-phosphinyl]methyl]cyclopentyl]carbonyl]-L-tryptophan,

N-[2-[[hydroxy[1-[[2(S)-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy)carbonyl]amino]hexyl]amino]-2-(2-naphthalenyl)ethyl]phosphinyl]methyl]-4-methyl-1-oxopentyl]-L-tryptophan disodium salt, and

N-[2-[[hydroxy [3-methyl-1-[[2-[(methylsulfonyl)amino]-1-oxo-6-[[phenylmethoxy)-carbonyl]amino]hexyl]amino]butyl]phosphinyl]methyl]-4-methyl-1-oxopentyl]-L-tryptophan disodium salt,

for the preparation of a medicament for inhibiting endothelin converting enzyme or a pharmaceutically acceptable salt thereof for inhibiting endothelin converting enzyme.

10. The use of a compound as defined in any one of claims 1 to 5, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inhibiting endothelin converting enzyme.

11. A process for the preparation of a pharmaceutical composition or medicament as defined in claim 6 comprising admixing a compound or salt as defined in any one of claims 1 to 5 with a pharmaceutically acceptable carrier.

12. A compound or salt of claim 1, wherein n is 1.

13. A compound or salt of claim 1, wherein -A₁-R₅ is L-tryptophanyl, L-tyrosinyl or L-O-methyl tyrosinyl.

14. A compound or salt of claim 1, wherein R₁ and R₂ are independently selected from the group consisting of H, aryl(C₁-C₈)alkyl and branched (C₁-C₈) alkyl.

15. A compound or salt of claim 14, wherein one of R₁ and R₂ is hydrogen and the other is benzyl, 2-naphthylmethyl, or branched (C₁-C₈) alkyl.

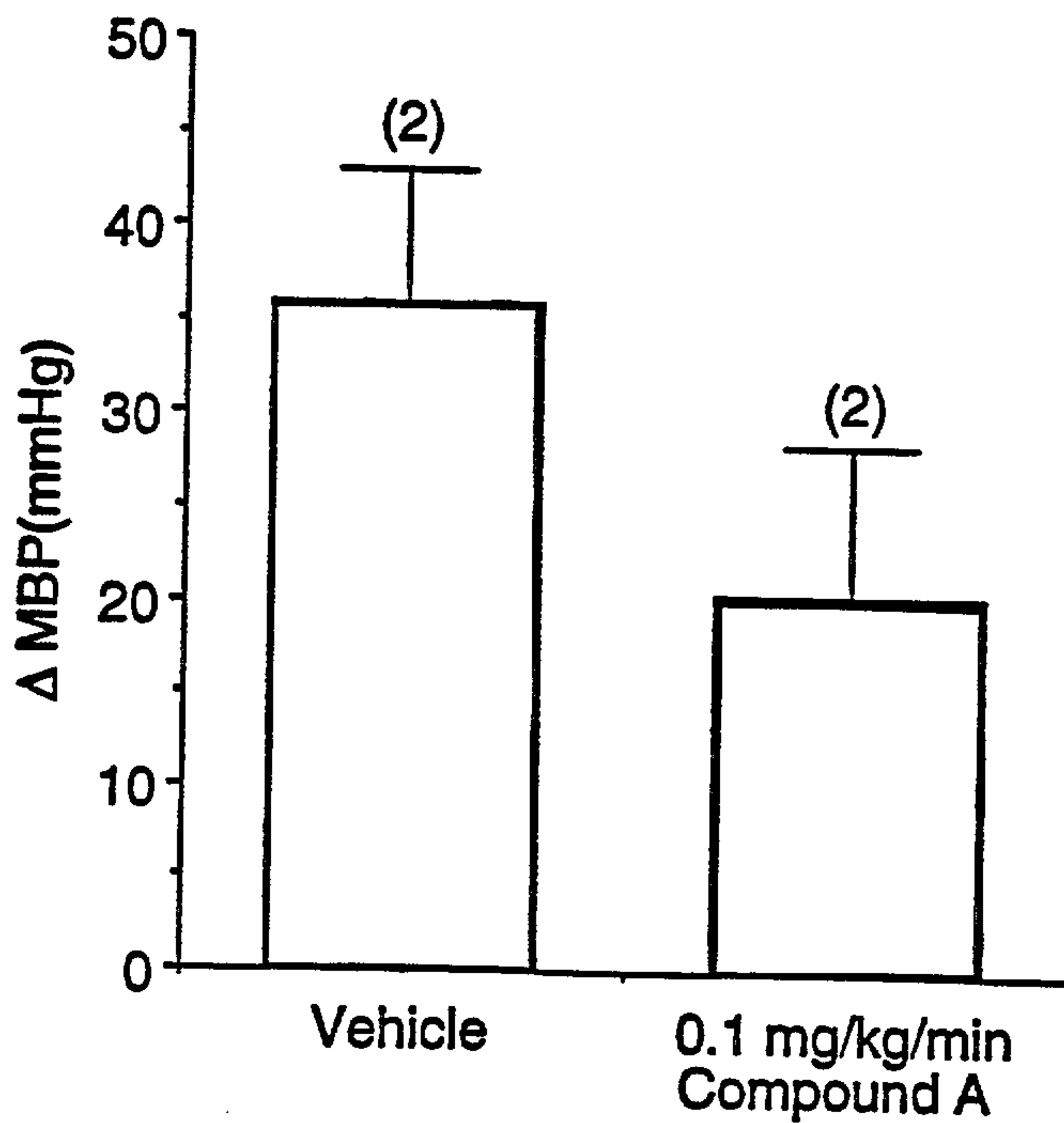
- 51 -

16. A compound or salt of claim 1, wherein R is hydrogen and R₅ is hydroxy.
17. A compound or salt of claim 1, wherein R₆ is -A₂-R₇ or phenyl-methoxycarbonyl.
18. A compound or salt of claim 1, wherein A₂ is an α -aminoacyl radical derived from lysine, ϵ -CBZ lysine, arginine, isoleucine, leucine, valine, phenylalanine, alanine, glycine or tyrosine, and R₇ is benzyloxycarbonyl or methansulfonyl.
19. A compound or salt of claim 1, wherein q is 4.
20. A compound or salt of claim 1, wherein n is 1; -A₁-R₅ is L-tryptophanyl, L-tyrosinyl or L-O-methyl tyrosinyl; R₁ and R₂ are independently selected from the group consisting of H, aryl(C₁-C₈)alkyl and branched (C₁-C₈) alkyl; and R₆ is -A₂-R₇ or phenyl-methoxycarbonyl.
21. A compound or salt of claim 20, wherein one of R₁ and R₂ is hydrogen and the other is benzyl, 2-naphthylmethyl, or branched (C₁-C₈)alkyl; A₂ is an α -aminoacyl radical derived from lysine, ϵ -CBZ lysine, arginine, isoleucine, leucine, valine, phenylalanine, alanine, glycine or tyrosine, and R₇ is benzyloxycarbonyl or methansulfonyl.
22. A compound or salt of claim 21, wherein R is hydrogen, R₅ is hydroxy and q is 4.
23. A compound or salt of any one of claims 1 to 5 or 12 to 22, for use in inhibiting endothelin converting enzyme.
24. An endothelin converting enzyme inhibitor pharmaceutical composition comprising an acceptable endothelin converting enzyme inhibiting amount of a compound of the formula defined in any one of claims 1 to 5 or 12 to

- 52 -

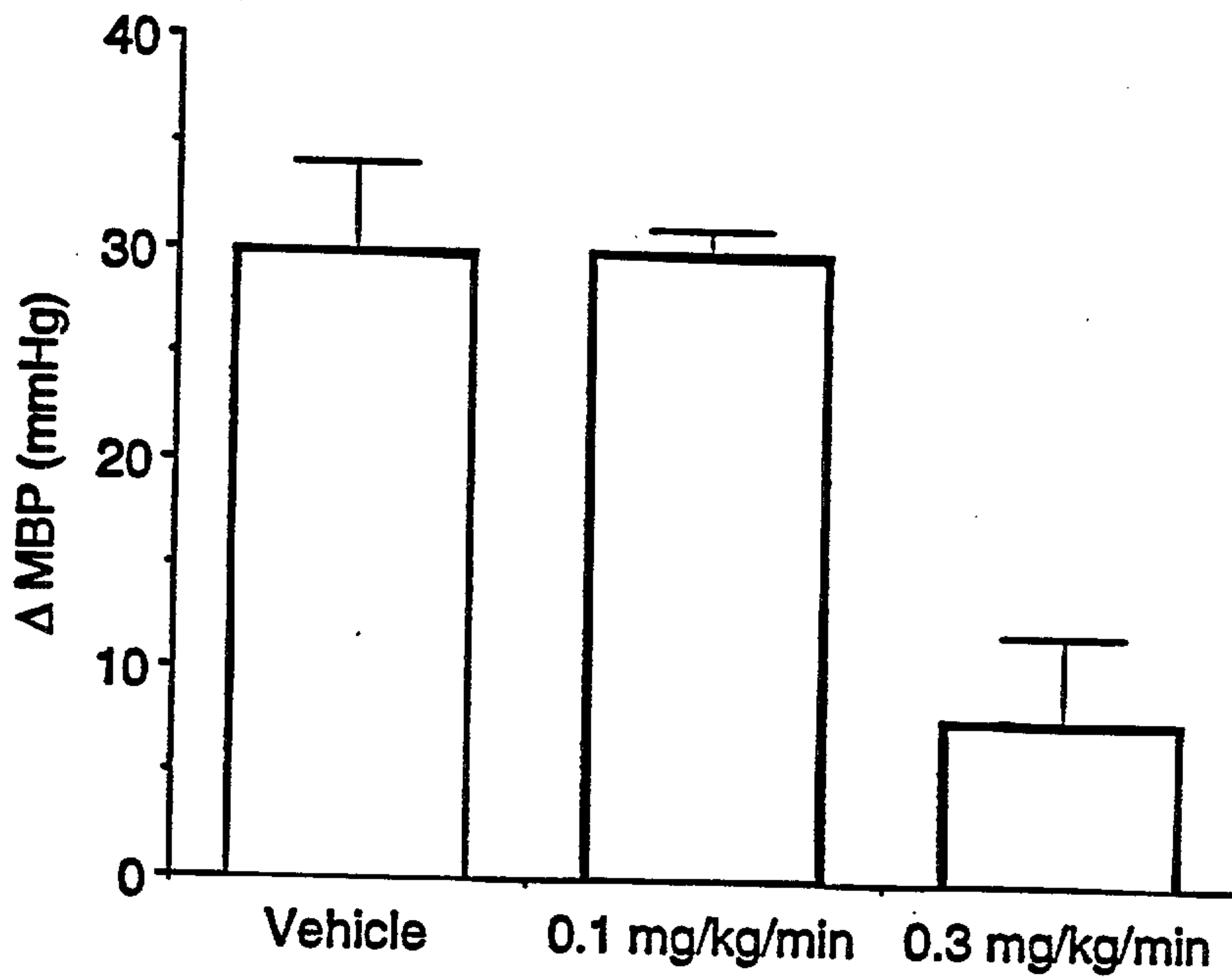
22, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier.

1/6

**Fig. 1**

2191454

2/6

**Fig. 2**

2191454

3/6

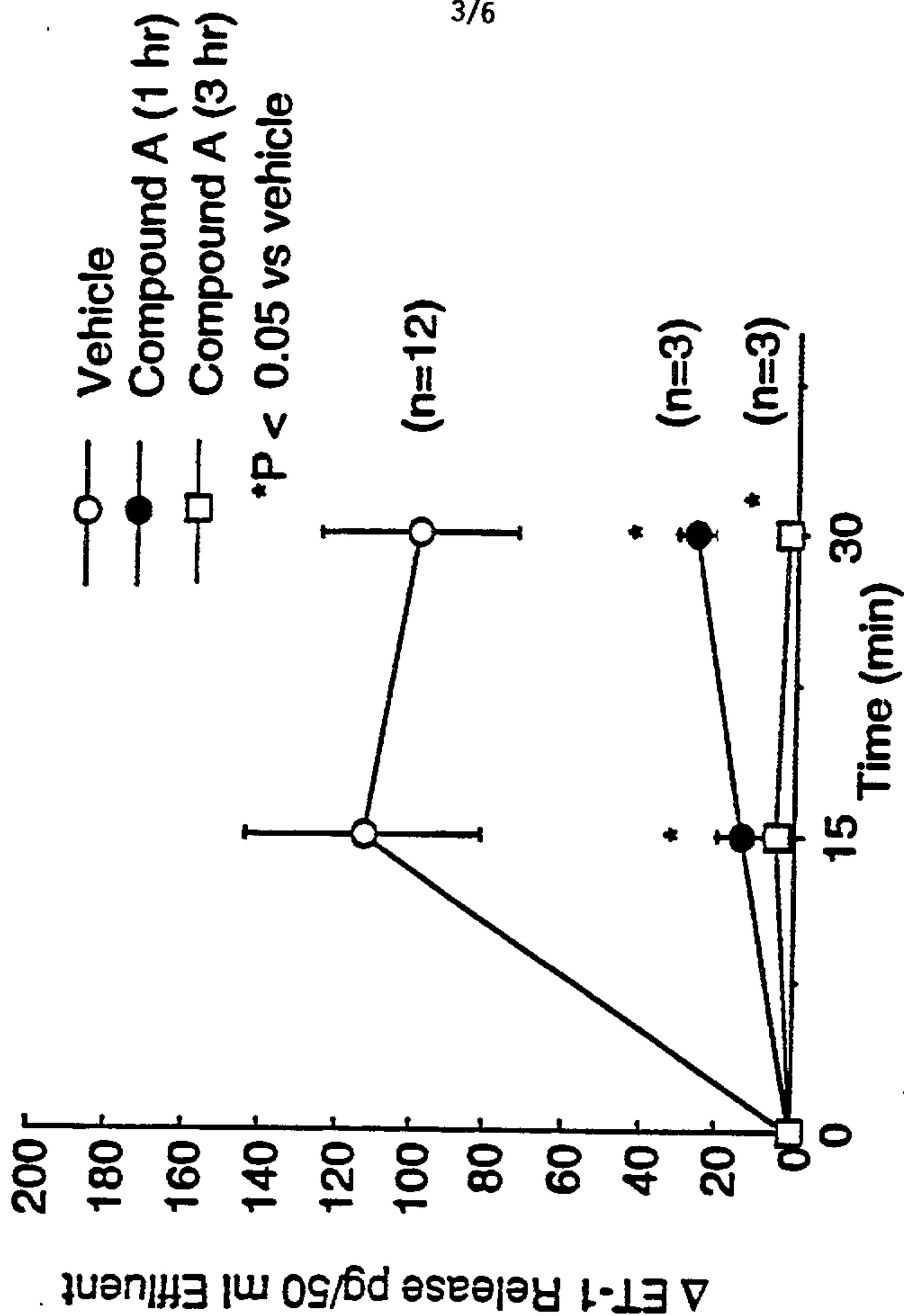


Fig. 3

4/6

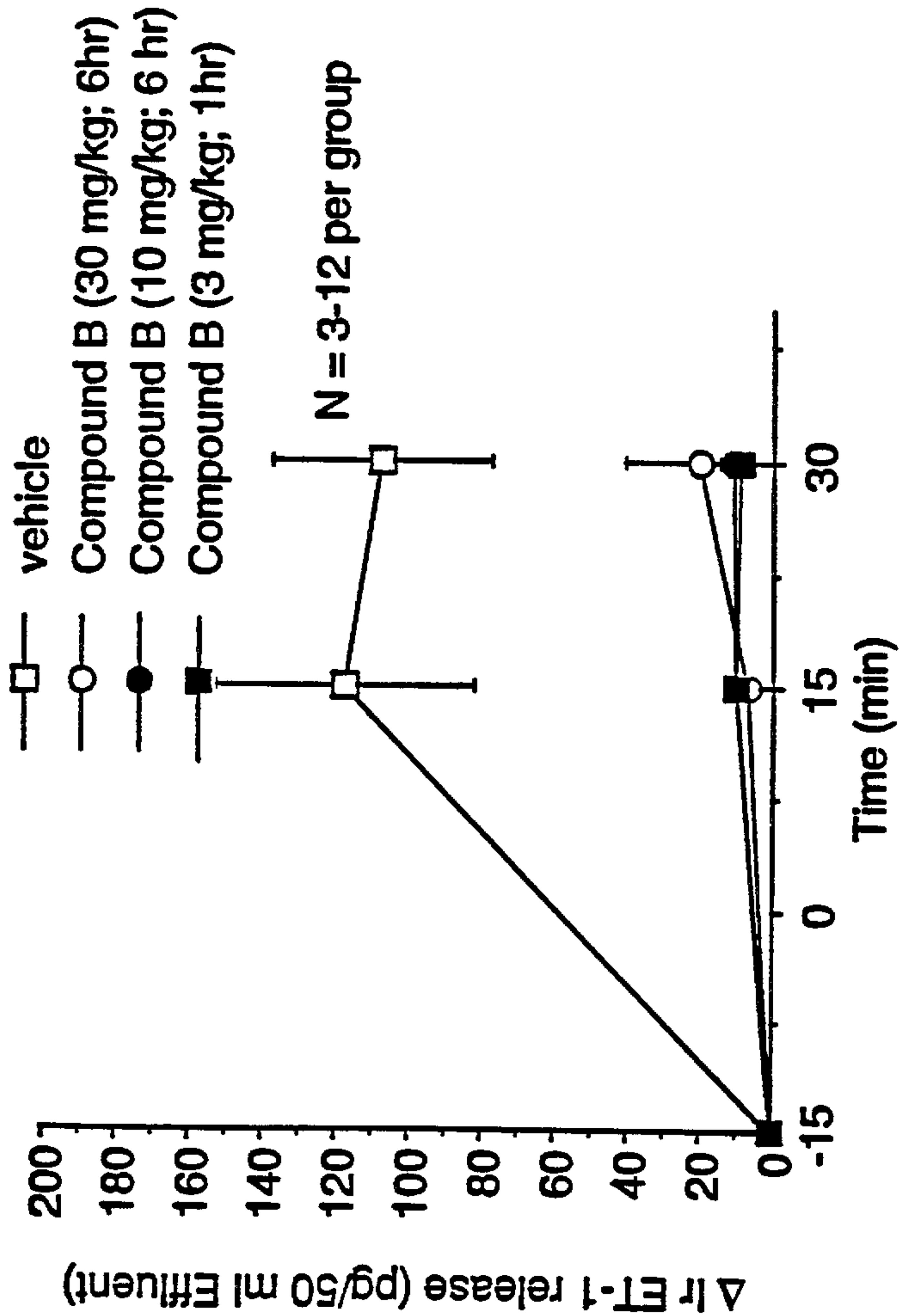


Fig. 4

5/6

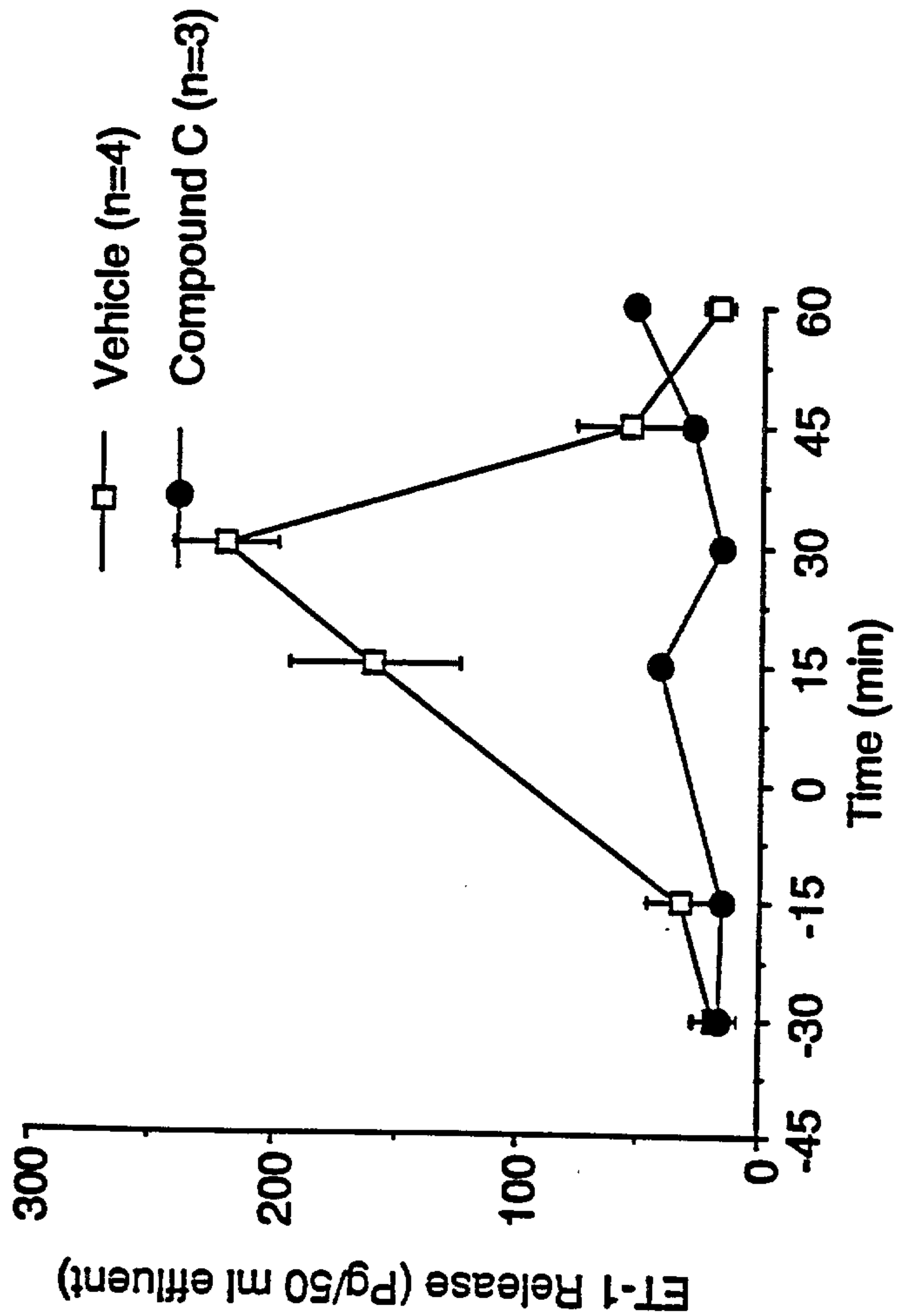


Fig. 5

2191454

6/6

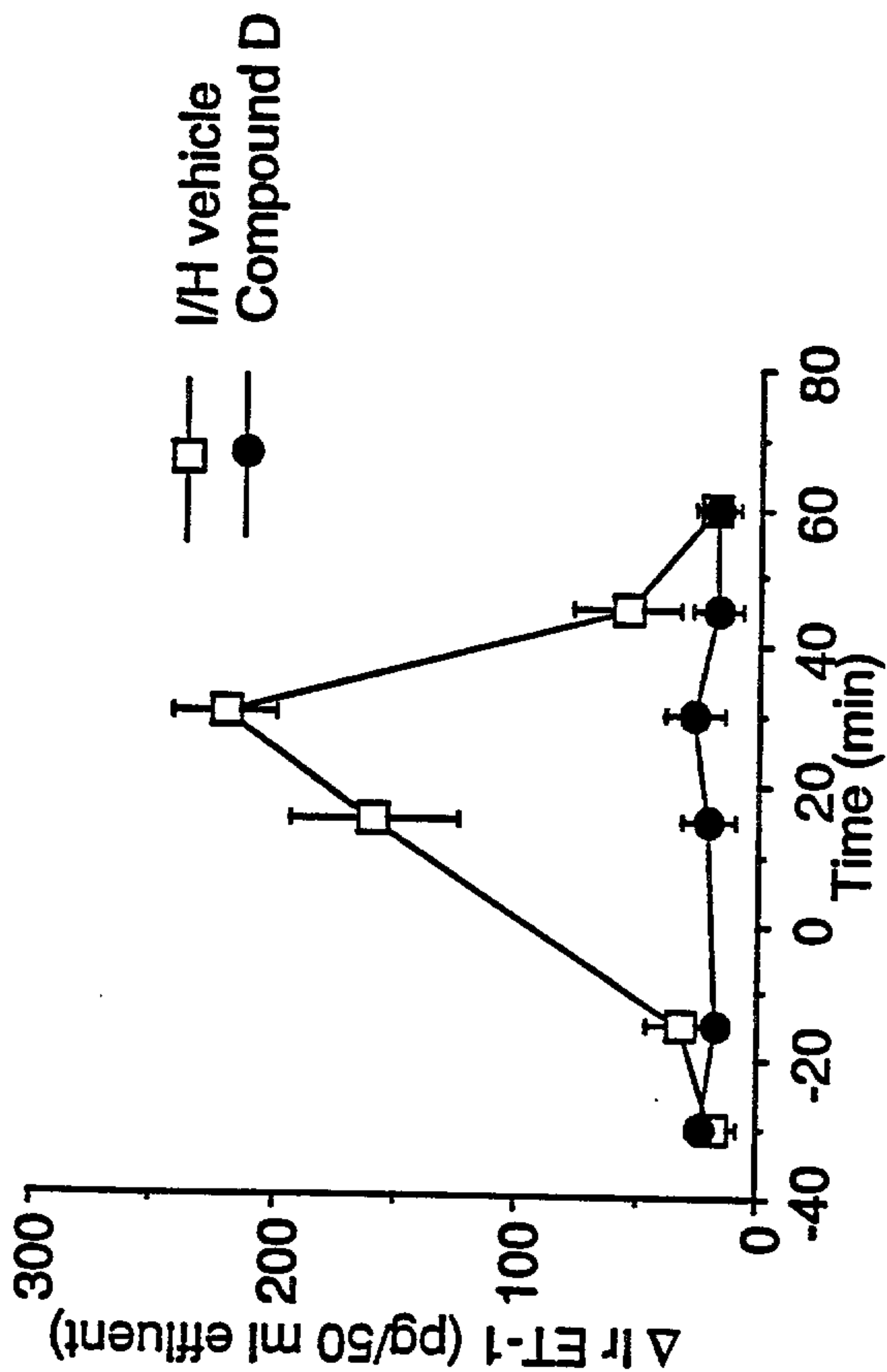


Fig. 6

