New Prolyl Endopeptidase Inhibitors: *In Vitro* and *in Vivo* Activities of Azabicyclo[2.2.2]octane, Azabicyclo[2.2.1]heptane, and Perhydroindole Derivatives

Bernard Portevin,[†] Alain Benoist,[†] Georges Rémond,[†] Yolande Hervé,[†] Michel Vincent,[†] Jean Lepagnol,[‡] and Guillaume De Nanteuil*[,†]

Division D of Medicinal Chemistry, Institut de Recherche Servier, 11 rue des Moulineaux, 92150 Suresnes, France, Division of Cerebral Pathology, Institut de Recherche Servier, 125 Chemin de Ronde, 78290 Croissy, France

Received November 22, 1995[⊗]

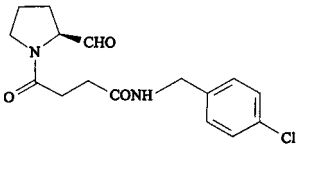
A series of potent and selective prolylendopeptidase (PEP) inhibitors of the α-keto heterocyclic type has been obtained by replacing the classical central proline of 1-[1-(4-phenylbutanoyl)-L-prolyl]pyrrolidine (SUAM 1221, **3**) by non-natural amino acids PHI, ABO, and ABH. These 4-phenylbutanoyl side-chain-containing inhibitors exhibited potent *in vitro* inhibitory potencies with IC_{50} around 30 nM (compounds **24** and **25**). Modulation of the side chain by replacement of the terminal phenyl ring by the dicyclopropyl moiety afforded derivatives **30** and **32** with improved potencies (IC_{50} between 10 and 20 nM). Furthermore, replacing the linear 4-phenylbutanoyl side chain by the (2-phenylcyclopropyl)carbonyl entity provided potent inhibitors with IC_{50} culminating at 0.9 nM on a rat cortex enzymatic preparation (compound **70**). The configuration of the cyclopropyl ring had to be *R,R* in order to obtain not only a strong PEP inhibition *in vitro* but also a good activity *in vivo*, exemplified by inhibitor **68**, which gave ID_{50} ip and po of 0.3 and 1 mg/kg, respectively. Finally, demonstration of the cognition-enhancing properties of compound **54** was given in the passive avoidance test using scopolamine-induced amnesia in the rat, where it dose dependently inhibited the scopolamine-induced decrease in avoidance response.

Introduction

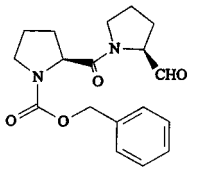
Prolyl endopeptidase (PEP, EC 3.4.21.26) is the only serine protease which is known to cleave a peptide substrate in the C terminal side of a proline residue,[1] This enzyme was first isolated in the human uterus, purified from lamb kidney, and subsequently named post proline cleaving enzyme (PPCE).[2] It is widely distributed in various mammalian tissues such as the brain, liver, and kidney.[3] In the central nervous system, PEP degrades proline-containing neuropeptides involved in the processes of learning and memory such as vasopressin, substance P (SP), and thyrotropin-releasing hormone (TRH).[4] Moreover, cognitive deficits in Alzheimer's patients is reported to show improvement with TRH, and one can postulate that PEP inhibitors could prevent memory loss and increase attention span in patients suffering from senile dementia. More recently, PEP-like immunoreactivity was detected and associated with amyloid β-peptide-like immunoreactivity, suggesting that PEP could be implicated in amyloidogenesis.[5] Yoshimoto *et al.* reported in 1987 that an *in vitro* PEP inhibitor had *in vivo* antiamnesic properties in the passive avoidance learning test using scopolamine-induced amnesia in the rat.[6]

Numerous low molecular weight inhibitors of PEP have been described previously. In general, irreversible inhibitors contain either a chloroacetyl or a diazo acetyl moiety, which reacts covalently with the enzyme.[1] Reversible inhibitors have also been extensively studied: most of the time, the chemical functionality able to react with Ser-554 in the active site of the enzyme is the formyl group.[7,8]

When we started the chemistry on this project, two prolinal derivatives were already described as PEP inhibitors: ONO 1603 (**1**)[9] and Z-Pro-prolinal (**2**).[6]

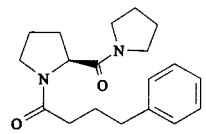


1 ONO 1603
rat cortex PEP: IC_{50} = 57 nM

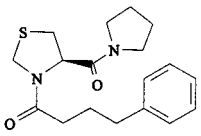


2
IC_{50} = 54 nM

Soon after, Saito et al. described a new family of inhibitors exemplified by SUAM 1221 (**3**)[10] in which a pyrrolidinylcarbonyl function at the P1 site is the crucial entity for enzyme recognition, giving rise to the transition state analog of the enzyme−substrate interaction.



3 SUAM 1221
bovine brain PEP: IC_{50} = 190 nM



4
IC_{50} = 67 nM

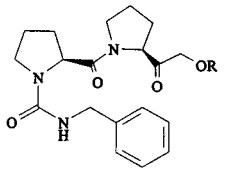
Important improvement in the *in vitro* potency was obtained when the central proline was replaced by thiaproline (thiaPRO) to give compound **4**, suggesting that modification of the central amino acid (of the proline type) could be of importance in modulating the PEP inhibitory activity. Finally, it is worth noting the presence of the lipophilic arylalkyl side chain in these two inhibitors, appended on the N-terminal side of the central amino acid.

[†] Division D of Medicinal Chemistry.
[‡] Division of Cerebral Pathology.
[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1996.

S0022-2623(95)00858-2 CCC: $12.00

This proline (or analog) seems to exert a positive hydrophobic interaction with the S2 specificity pocket in the active site of the enzyme; the second heterocycle (pyrrolidine, thiazolidine) lies in the S1 pocket, whereas the side chain accommodates another hydrophobic pocket, S3.[11]

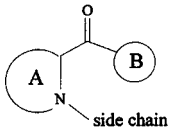
Very recently were disclosed the structures of JTP 3399 (**5**) and its metabolite JTP 4819 (**6**), currently under development by Japan Tobacco as promnesic agents.[12]



5 R = Ph

6 R = H, IC_{50} = 0.1 nM (rat brain PEP)

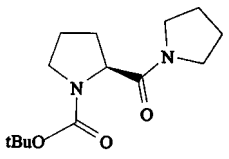
In the present paper, we disclose the synthesis as well as *in vitro* and *in vivo* activities of potent, selective, and orally active PEP inhibitors with the general formula



A = heterocycle (proline type)

B = heterocycle (pyrrolidine type)

In order to avoid stability problems often linked to the presence of an aldehyde function, we decided to limit our synthetic efforts to the class of inhibitors in which the reactive entity is the CO−pyrrolidine group, exemplified by the proline derivative **7**, previously described by Zeria.[13] Initially, our working hypothesis was (1) to

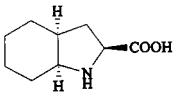


7

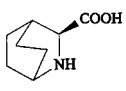
study the replacement of the central proline by non-natural proline analogs available in our laboratory (these amino acids have been already used with success by Vincent et al.[14] in order to obtain potent inhibitors of other serine proteases as well as metalloproteases) and (2) to vary the chain of the N-terminal side of the central amino acid.

Chemistry

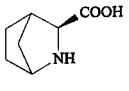
Non-natural amino acids **8** (PHI), **9** (ABO), and **10** (ABH), containing respectively a perhydroindole, an azabicyclo[2.2.2]octane, or an azabicyclo[2.2.1]heptane ring were prepared according to published procedures.[14,15]



8



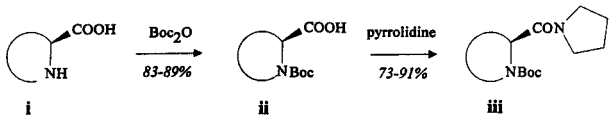
9



10

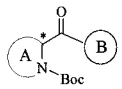
The compounds described in this paper were simply prepared starting from the free amino acid **i** (**8**, **9**, or **10**) (Scheme 1). Introduction of the *t*-Boc group on the nitrogen to give **ii**, followed by coupling with the heterocycle (most of the time pyrrolidine), afforded

Scheme 1



i **ii** **iii**

Table 1



compd no.	A	B	*	formula analysis[a]	mp (°C) recryst solv	IC_{50} (μM) [e]
7	PRO		S	$C_{14}H_{24}N_2O_3$ C, H, N	85 iPr_2O-AcOEt	32
11	ABO		S	$C_{17}H_{28}N_2O_3$ C, H, N	150 EtOAc	23
12	ABO		R	$C_{17}H_{28}N_2O_3$ C[b], H, N	150-152 CH_2Cl_2-Acetone	> 100
13	ABH		S	$C_{16}H_{26}N_2O_3$ ND[c]	142-144 CH_2Cl_2-Acetone	5.5
14	PHI		S	$C_{18}H_{30}N_2O_3$ C, H, N	149 EtOAc	5.5
15	ABO		S	$C_{20}H_{32}N_2O_3$ C, H, N	154-156 CH_2Cl_2-Acetone	> 100
16	ABO		S	$C_{16}H_{26}N_2O_3S$ C, H, N, S[d]	174 CH_2Cl_2-EtOAc	24
17	ABO		S	$C_{17}H_{26}N_2O_3$ C, H, N	190 CH_2Cl_2-EtOAc	20
18	PHI		R,S	$C_{21}H_{34}N_2O_3$ C, H, N	150-152 CH_2Cl_2-Acetone	> 100
19	PHI		R,S	$C_{17}H_{28}N_2O_3$ C, H, N	130 CH_2Cl_2-Acetone	30
20	PHI		S	$C_{19}H_{32}N_2O_3$ C, H, N	132 CH_2Cl_2-Acetone	>100
21	PHI		S	$C_{20}H_{34}N_2O_3$ C, H, N	122 CH_2Cl_2-Acetone	>100

[a] Compounds gave satisfactory analyses (±0.4%) unless otherwise indicated. [b] C: found, 66.93; calcd, 66.20. [c] ND: not determined, but satisfactory results by high resolution MS analysis were obtained. [d] S: found, 9.14; calcd, 9.82. [e] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from *Flavobacterium meningosepticum*.

compounds with the generic formula **iii**, exemplified in Table 1. The 2-azabicyclo[2.2.2]octylamine used in the preparation of compound **18** was obtained by thermal cyclization of 4-aminocyclohexane carboxylic acid followed by hydride reduction of the amide function.[16]

Compounds **iii** were also used as intermediates for the preparation of the following derivatives (Scheme 2): removal of the *t*-Boc group with gaseous HCl (to give **iv**) was followed by coupling with one of the following: (A) 4-Phenylbutyric acid or its acid chloride to give compounds **v** exemplified in Table 2. Preparation of compound **28**, in which the carbonyl function at position 2 is a ketone rather than an amide, is outlined in Scheme 3. (B) (Dicyclopropylmethyl)alkanecarboxylic acids or their acid chlorides to give compounds **vi** exemplified in Table 3. The preparation of these acids (compounds **xxi**, **xxv**, **xxvii**, **xxviii**, **xxxi**, **xxxv**, **xxxviii**) is outlined in Scheme 4 and uses classical synthetic

Scheme 2

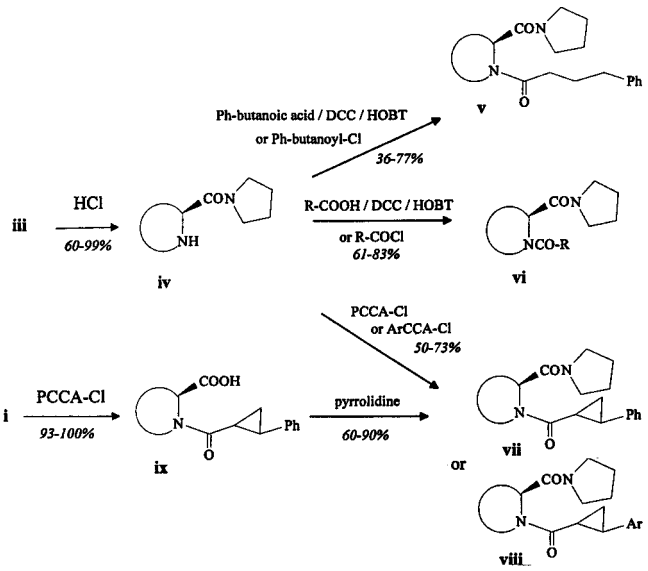
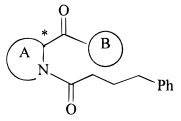


Table 2



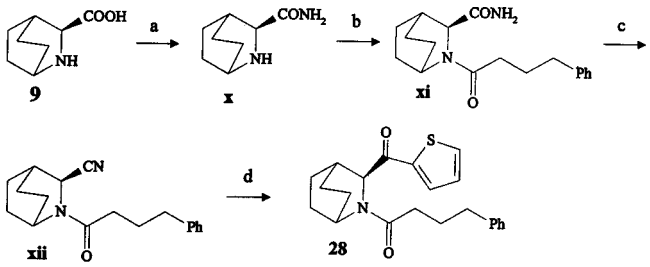
compd no.	A	B	*	formula analysis [a]	mp (°C) recryst solv	IC_{50} (nM) [c]
4	thiaPRO		S	$C_{18}H_{24}N_2O_2S$ C, H, N, S	62	540
22	ABO		S	$C_{22}H_{30}N_2O_2$ C, H, N	77 iPr_2O-Acetone	50
23	ABO		R	$C_{22}H_{30}N_2O_2$ ND[b]	70-72	2200
24	ABH		S	$C_{21}H_{28}N_2O_2$ ND	oil	27
25	PHI		S	$C_{23}H_{32}N_2O_2$ C, H, N	oil	34
26	ABO		R,S	$C_{21}H_{25}N_3O_2$ C, H, N	oil	> 100 000
27	ABO		R,S	$C_{21}H_{25}N_3O_2$ ND	oil	11 000
28	ABO		S	$C_{22}H_{25}NO_2S$ ND	oil	960

[a] Compounds gave satisfactory analyses (±0.4%) unless otherwise indicated. [b] ND: not determined, but satisfactory results by high-resolution MS analysis were obtained. [c] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from *Flavobacterium meningosepticum*.

pathways. Compounds **39**−**44** were prepared identically, starting from phenyl or cyclopentyl trifluoromethyl ketone. (C) The acid chlorides of 2-phenylcyclopropanecarboxylic acid (PCCA) or 2-arylcyclopropanecarboxylic acid (ArCCA) to give respectively compounds **vii** and **viii** described in Tables 4 and 5. For the first time, racemic PCCA was used; separation of the diastereomers by column chromatography afforded the four pairs of compounds **45**−**46**, **47**−**48**, **49**−**50**, and **51**−**52**.

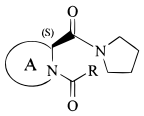
An X-ray crystallographic analysis of compound **48** (more active than its isomer **47**) (Figure 1) demonstrated that the configuration for the cyclopropane ring had to

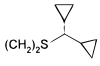
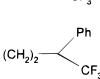
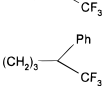
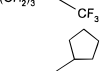
Scheme 3[a]



[a] Reagents: (a) ClCOOEt, aqueous NH_3; (b) 4-phenylbutyryl chloride; (c) pyridine, $POCl_3$; (d) thienylmagnesium bromide, THF.

Table 3

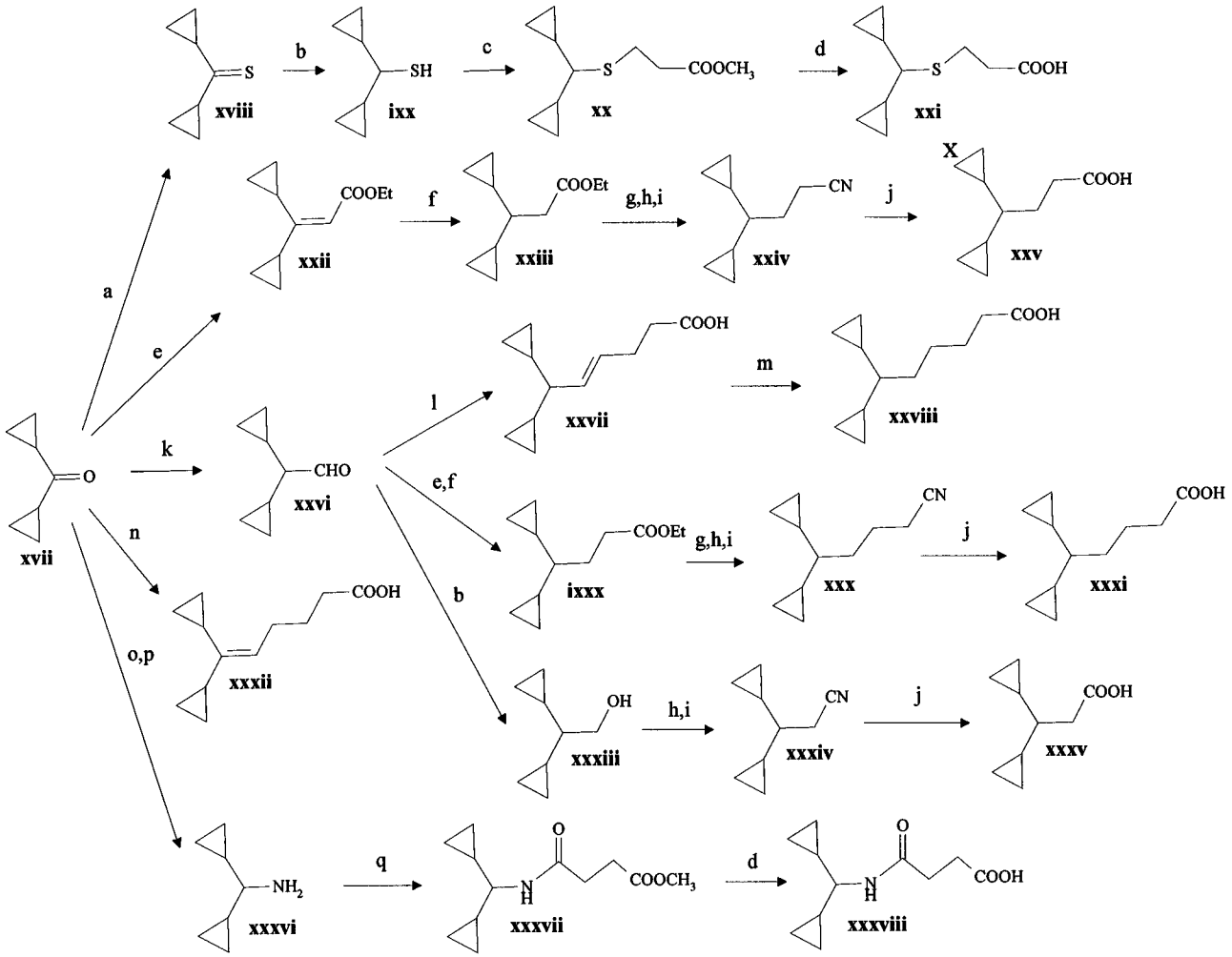


compd no.	A	R	formula analysis[a]	mp (°C) recryst solv	IC_{50} (nM) [d]
29	ABO		$C_{24}H_{38}N_2O_2$ ND[b]	86 pentane	40
30	ABO		$C_{23}H_{36}N_2O_2$ C, H, N	114 AcOEt	9
31	ABH		$C_{22}H_{34}N_2O_2$ C, H, N	oil	26
32	PHI		$C_{24}H_{38}N_2O_2$ Cc, H, N	amorphous	17
33	PHI		$C_{23}H_{36}N_2O_2$ C, H, N	amorphous	58
34	PHI		$C_{22}H_{34}N_2O_2$ C, H, N	110-112 EtOAc	8800
35	PHI		$C_{23}H_{36}N_2O_2S$ C, H, N, S	amorphous	86
36	ABO		$C_{23}H_{35}N_3O_3$ C, H, N	amorphous	2600
37	ABO		$C_{24}H_{36}N_2O_2$ ND	oil	20
38	ABO		$C_{24}H_{36}N_2O_2$ ND	oil	24
39 (α)	PHI		$C_{23}H_{29}F_3N_2O_2$ C, H, N	134 CH_2Cl_2-Acetone	36
40 (β)	PHI		$C_{23}H_{29}F_3N_2O_2$ C, H, N	98 CH_2Cl_2-Acetone	1500
41	PHI		$C_{23}H_{27}F_3N_2O_2$ C, H, N	148 CH_2Cl_2-Acetone	8000
42	PHI		$C_{24}H_{31}F_3N_2O_2$ C, H, N	amorphous	720
43	PHI		$C_{25}H_{33}F_3N_2O_2$ C, H, N	amorphous	5
44	PHI		$C_{24}H_{37}F_3N_2O_2$ C, H, N	amorphous	46

[a] Compounds gave satisfactory analyses (±0.4%) unless otherwise indicated. [b] ND: not determined, but satisfactory results by high-resolution MS analysis were obtained. [c] C: found, 74.04; calcd, 74.57. [d] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from *Flavobacterium meningosepticum*.

the *trans*-*R*,*R* in order to obtain a strong PEP inhibitory potency. Soon after, *trans*-2-PCCA was resolved with quinine,[17] and only the *trans*-*R*,*R* isomer was used in

Scheme 4[a]



[a] Reagents: (a) Lawesson reagent, toluene; (b) $NaBH_4$; (c) EtONa, methyl bromopropionate; (d) NaOH, methanol; (e) $Ph_3P^+CH_2COOEt$, Br^-, NaH; (f) H_2, 10% Pd−C; (g) LAH, Et_2O; (h) PBr_3, Et_2O; (i) KCN, EtOH; (j) KOH, then HCl; (k) $(CH_3)_3S^+$, I^-, NaH, DMSO; (l) $Ph_3P^+(CH_2)_3COOH$, I^-, tBuOK; (m) H_2, PtO_2; (n) $Ph_3P^+(CH_2)_4COOH$, I^-, tBuOK; (o) NH_2OH; (p) H_2, 10% Pd−C; (q) $HOOC(CH_2)_2COOCH_3$, DCC, HOBT, DMF.

the preparation of the following derivatives. Later on, we developed a more concise and convergent approach to these derivatives (bottom of Scheme 2): the free amino acid **i** was first acylated with the acid chloride of *trans*-(*R*,*R*)-2-PCCA; HOBT-DCC coupling of the intermediate **ix** with the corresponding heterocyclic amine (pyrrolidine, thiazolidine) gave the desired final derivative **vii**. No epimerization was detected when using this second synthetic pathway.

The substituted phenyl, naphthyl, or thienyl cyclopropanecarboxylic acids (ArCCA) were prepared using the palladium-catalyzed cyclopropanation of α,β-unsaturated carboxylic acids derivatized with Oppolzer's sultam (Scheme 5).[18] These acids **xvi** were then coupled as previously with intermediate **iv** to afford inhibitors of Table 5.

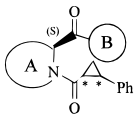
Finally two compounds, **80** and **81**, include a four-membered ring[19] in place of the usual cyclopropane moiety. Separation of *cis* and *trans* isomers was performed by medium-pressure silica gel chromatography after coupling of the side chain with ABO−pyrrolidine (Table 6).

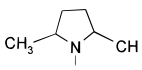
Enzyme Inhibitory Activities

To assess the validity of the replacement of the central proline by our non-natural amino acids, we prepared simple analogs of **7**, which are described in Table 1. The central proline of compound **7** was successfully replaced by ABO, ABH, and PHI to give derivatives **11**, **13**, and **14** with IC_{50} values in the same range (5−30 μM) as for the reference proline analog. Moreover, this first series of compounds gave us the opportunity to verify that, in order to obtain a strong inhibitory activity, the stereochemistry at C2 had to be *S* (for example, compare compounds **11** and **12**, with IC_{50}s respectively of 23 and >100 μM). Replacement of pyrrolidine at P1 position by larger heterocycles generally led to inactive compounds (**15**, **18**, **20**, **21**: IC_{50} > 100 μM). In contrast, activity was maintained in this first class of inhibitors when pyrrolidine was replaced by thiazolidine, 3-pyrroline, or azetidine to give respectively compounds **16**, **17**, and **19** (IC_{50} = 25 μM).

Compounds in Table 2 are directly related to compounds **3** and **4**, both bearing a 4-phenylbutanoyl side chain appended on the nitrogen of the central amino acid. As previously, proline or thiazolidine were replaced by our non-natural amino acids. PEP inhibitory activity for **22**, **24**, and **25** was enhanced at least 10 times when compared to compound **4** (30−50 *vs* 540 nM). Replacement of the Boc group by the phenylbutyryl side chain appears to be of prime importance, since *in vitro* inhibition activities are dramaticaly improved

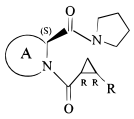
Table 4

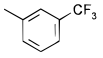
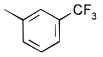
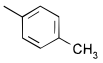
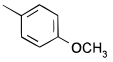
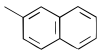


compd no.	A	B	* *	formula analysis [a]	[α]D (temp °C) solv (conc %)	mp (°C) recryst solv	IC_{50} (nM) [f] Flavobacterium meningosepticum	IC_{50} (nM) [g] Rat Cortex
45	thiaPRO		S S	$C_{18}H_{22}N_2O_2S$ ND[b]	-	134 CH_2Cl_2-EtOAc	1400	-
46	thiaPRO	"	R R	$C_{18}H_{22}N_2O_2S$ ND	-	200 CH_2Cl_2-EtOAc	150	-
47	ABO	"	S S	$C_{22}H_{28}N_2O_2$ C, H, N	+163.7 (21.5) EtOH (1)	amorphous	130	-
48	ABO	"	R R	$C_{22}H_{28}N_2O_2$ C, H, N	- 148.0 (21.5) EtOH (1)	202-204 CH_2Cl_2-EtOAc	50	5
49	ABH	"	S S	$C_{21}H_{26}N_2O_2$ C, H, N	-	175 CH_2Cl_2-EtOAc	78	-
50	ABH	"	R R	$C_{21}H_{26}N_2O_2$ C, H, N	-	153 CH_2Cl_2-EtOAc	14	1.4
51	PHI	"	S S	$C_{23}H_{30}N_2O_2$ C, H, N	-	142 iPr_2O	-	33
52	PHI	"	R R	$C_{23}H_{30}N_2O_2$ C, H, N	-	170 pentane	-	1.2
53	ABO		"	$C_{21}H_{26}N_2O_2S$ C, H, N, S	-	174 EtOAc	-	3.1
54	PHI		"	$C_{22}H_{28}N_2O_2S$ C, H, N, S	- 154.1 (24) CH_2Cl_2 (1)	amorphous	-	1.3
55	PHI [c]		"	$C_{23}H_{28}N_2O_2$ ND	-	amorphous	-	2.7
56	PHI			$C_{23}H_{28}N_2O_2$ ND	-	amorphous	-	2.4
57	PHI		"	$C_{23}H_{26}N_2O_2$ C[d], H, N	-	184 CH_2Cl_2-Acetone	-	2.3
58	PHI		"	$C_{24}H_{32}N_2O_2$ C, H, N	-	pentane	-	> 1000
59	PHI		"	$C_{24}H_{32}N_2O_2$ C, H, N	-	amorphous	-	740
60	PHI		"	$C_{25}H_{34}N_2O_2$ C, H, N	-	126 pentane	-	> 1000
61	PHI		"	$C_{23}H_{32}N_2O_2$ C, H, N	-	104 pentane	-	> 2000
62	PHI		"	$C_{23}H_{30}N_2O_3$ C, H, N	-	amorphous	-	11
63	PHI [c]		"	$C_{23}H_{28}N_2O_3$ C, H, N[e]	-	amorphous	-	290
64	PHI		"	$C_{22}H_{28}N_2O_3$ C, H, N	-	amorphous	-	2

[a] Compounds gave satisfactory analyses (±0.4%) unless otherwise indicated. [b] ND: not determined, but satisfactory results by high-resolution MS analysis were obtained. [c] *R,S* at C2. [d] C: found, 75.68; calcd, 76.21. [e] N: found, 6.88; calcd, 7.36. [f] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from *Flavobacterium meningosepticum*. [g] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from rat cortex.

Table 5



compd no.	A	R	formula analysis [a]	[α]D (temp °C) CH_2Cl_2 (1%)	mp (°C) recryst solv	IC_{50} (nM) [g] Rat Cortex
65	ABO		$C_{20}H_{26}N_2O_2S$ C[c], H, N, S	-130.5 (22)	158 pentane	4.8
66	PHI		$C_{21}H_{28}N_2O_2S$ C [b], H, N, S	-125.3 (23)	157 Et_2O	1.3
67	ABO		$C_{22}H_{27}FN_2O_2$ C[d], H, N	-132.5 (22)	195 pentane	4.0
68	PHI		$C_{23}H_{29}FNO_2$ C, H, N	-127.5 (22)	154 iPr_2O	1.1
69	ABO		$C_{23}H_{27}F_3N_2O_2$ C, H, N	-129.5 (22)	154 pentane	3.2
70	PHI		$C_{24}H_{29}F_3N_2O_2$ C, H, N	-130.6 (22)	208 CH_2Cl_2-MeOH	0.9
71	PHI		$C_{24}H_{32}N_2O_2$ C, H, N	-	142 pentane	1.4
72	ABO		$C_{23}H_{30}N_2O_3$ C[e], H, N	-	212 CH_2Cl_2-MeOH	16
73	ABO		$C_{26}H_{30}N_2O_2$ C, H, N	-	217-218 CH_2Cl_2-EtOH	5
74	PHI	 α-cis	$C_{24}H_{32}N_2O_2$ C, H, N	-47.3 (22)	145 pentane	> 1000
75	PHI	 β cis	$C_{24}H_{32}N_2O_2$ C[f], H, N	-21.6 (22)	oil	?
76	PHI	 α-trans	$C_{24}H_{32}N_2O_2$ C, H, N	+37.5 (22)	oil	120
77	PHI	 β trans	$C_{24}H_{32}N_2O_2$ C, H, N	-14.2 (22)	oil	2.5
78	ABO	 α trans	$C_{23}H_{30}N_2O_2$ C, H, N	+27.9 (22)	amorphous	140
79	ABO	 β trans	$C_{23}H_{30}N_2O_2$ C, H, N	-28.2 (22)	amorphous	4.2

[a] Compound gave satisfactory analyses (±0.4%) except otherwise indicated. [b] C: found, 67.26; calcd, 67.71. [c] C: found, 66.34; calcd, 67.01. [d] C: found, 70.45; calcd, 71.33. [e] C: found, 71.60; calcd, 72.22. [f] C: found, 75.14; calcd, 75.75. [g] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from rat cortex.

by 3 orders of magnitude. Obviously, the *S* configuration at position 2 is needed, since *R* derivative **23** was totally devoid of activity. Substitution of pyrrolidine at the P1 position by pseudoaromatic heterocycles like imidazole or pyrazole (to afford compounds **26** and **27**) resulted in a complete loss of activity. Finally, ketone **28** showed a moderate activity (around 1 μM).

Compounds in Table 3 combined the replacement of the central proline or thiazolidine by ABO, ABH, and PHI with the original substitution of the phenyl group at the end of the side chain by a dicyclopropylmethyl moiety or by a 1,1,1-trifluoro-2-phenylethyl moiety. First, it is worth note that, for this class of PEP inhibitors, the dicyclopropylmethyl entity is effectively a good substitute for a phenyl group, for compounds **30**, **31**, and **32** have similar or even better inhibitory potency (10−25 nM) than their "phenyl" analogs **22**, **24**,

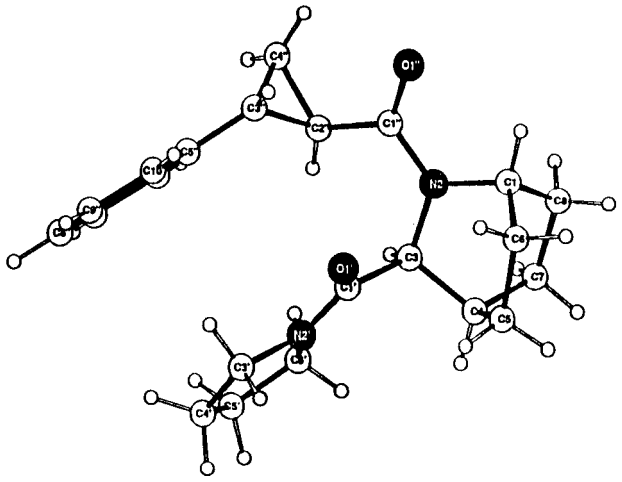
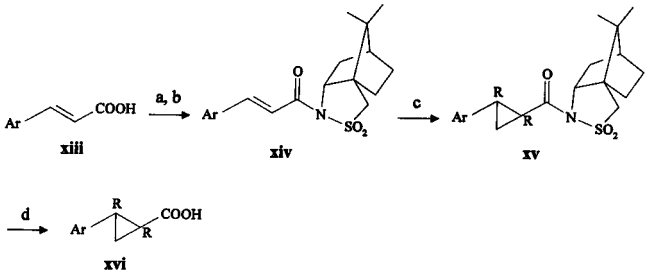


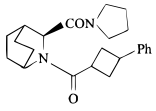
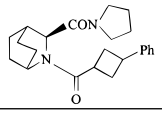
Figure 1. X-ray crystal structure of compound **48**. Assuming that configuration at C-3 was *S*, cyclopropane was found to be *R,R*.

Scheme 5[a]



[a] Reagents: (a) $(COCl)_2$; (b) (−)-bornane[10.2]sultam; (c) CH_2N_2, $Pd(OAc)_2$; (d) LiOH, aqueous THF.

Table 6

compd no		formula analysis [a]	mp (°C) recryst solv	IC_{50} (nM) [b]
80 cis		$C_{23}H_{30}N_2O_2$ C, H, N	amorphous	27
81 trans		$C_{23}H_{30}N_2O_2$ C, H, N	108 toluene-EtOH	54

[a] Compound gave satisfactory analyses (±0.4%) unless otherwise indicated. [b] IC_{50} values are concentrations of compounds required to achieve 50% inhibition against PPCE from rat cortex.

and **25**. Second, although all the possible side chains have not been prepared with the same central amino acid, it is interesting to note that $(CH_2)_3$-containing **30** was more potent than $(CH_2)_4$-containing **29**. Similarly, compound **32**, with a $(CH_2)_3$ side chain, was 3 times more potent than $(CH_2)_2$ containing **33**, itself more potent than **34** with a CH_2 side chain which was virtually inactive. Replacing a methylene group of the chain by a sulfur atom gave an approximately 4-fold decrease in potency (**35** compared to **32**); however, incorporation of an amide function within the chain, as in **37**, was quite deleterious for activity.

Interestingly, the unsaturated derivatives **37** and **38** afforded a 2-fold increase in inhibitory activity when compared to their saturated analog **29** (IC_{50} = 20−25 nM, compared to 40 nM). Similarly, we could demonstrate that the 1,1,1-trifluoro-2-phenylethyl moiety is a good mimic for the phenyl group. Examination of the

PEP IC_{50} for compounds **39** to **44** showed that a $(CH_2)_3$ side chain is again optimal for activity (compound **43**, $IC_{50} = 5$ nM). Moreover, like in the preceding group, decreasing the length of the chain was deleterious for activity (see compounds **40**, **41**, and **42**). Curiously enough, one of the isomer of the CH_2-containing derivative **39** ($IC_{50} = 36$ nM) was much more potent than its diastereomer **40** ($IC_{50} = 1500$ nM), demonstrating the importance of the chirality of the side chain for activity. This difference in activity between inhibitors **39** and **40** could also be the result of a steric hindrance close to the A ring, showing that this type of side chain can also be short, provided it is properly oriented.

From these preliminary results, it appears than the length of the N-terminal side chain seems to be critical for activity with an optimal being between $(CH_2)_2$ and $(CH_2)_4$. The interesting results obtained with compounds **37** and **38** with an intermediate chain length between $(CH_2)_3$ and $(CH_2)_4$ prompted us to prepare compounds in which the side chain would be longer than with $(CH_2)_2$ but shorter than with $(CH_2)_3$. Rather than using an insaturation as above, our choice turned to the preparation of compounds including a 2-phenylcyclopropyl side chain, which are described in Table 4.

Compounds in Table 4 were screened first on the bacterial enzyme, but at this time, we decided to move on to a rat cortex enzymatic preparation. The IC_{50} values observed with this latter enzyme were around 10-fold weaker than with the former one. The very interesting points concerning this family of inhibitors are as follows:

(1) The analogs prepared with *cis*-PCCA are virtually inactive (data not shown).

(2) There is up to a 10-fold increase in activity between the first eluted and the second eluted diastereomer prepared with *trans*-PCCA (**45** compared to **46**, **47** compared to **48**, etc). To our knowledge, it is the first time that regio as well as stereochemistry of the side chain of PEP inhibitors are shown to be of such importance for in vitro inhibition of the enzyme. The X-ray crystallographic structural determination of compound **48** confirmed that the active stereochemical configuration of the cyclopropane ring was *R*,*R*, with stereochemistry at C2 on the central amino acid being *S*. We then hypothesized that the first isomer to be eluted was always *S*,*S*,*S* (**45**, **47**, **49**, **51**) whereas the second one was always *S*,*R*,*R* (**46**, **48**, **50**, **52**).

(3) For the first time, we noticed a slight difference in inhibitory activity, depending on the central amino acid, the rank of potency being PHI $\sim$ ABH - ABO > thiaPRO.

(4) A strong inhibition was obtained with either pyrrolidine (**52**, 1.2 nM) or thiazolidine (**53**, 3.1 nM; **54**, 1.3 nM) at P1. Nanomolar IC_{50} values (between 2 and 3 nM) were also obtained with 2-pyrroline (**55**) (although this compound was air-sensitive), 3-pyrroline (**56**), pyrrole (**57**), and isoxazolidine (**64**). In contrast, a 10-fold decrease in activity was observed with the 3-hydroxypyrrolidine-containing derivative **62** ($IC_{50} = 11$ nM). Inhibitory potency dropped seriously when the nitrogen atom was excluded from the pyrrolidine ring (**58**), when pyrrolidine was substituted by one or more methyl groups (compounds **59** and **60**), and when pyrrolidine was "opened" to the *N*-diethyl moiety (compound **61**).

As suggested by the X-ray studies, pyrrolidine and the phenyl ring on the side chain are virtually stacked; this feature seems to be of prime importance for activity since disruption of this overlapping (for example by introducing methyl groups on the pyrrolidine ring) led to inactive compounds. Modifications on the phenyl ring of the side chain lead to compounds of Table 5: replacement of the phenyl ring by thienyl or naphtyl gave compounds **65**, **66** and **73**, which presented a PEP inhibitory potency between 1 and 5 nM similar to that of the phenyl analogs **48** or **52**; simple meta or para substitution on the phenyl ring by fluorine, trifluoromethyl, methyl, or methoxy provided also highly potent inhibitors **67** to **72** with IC_{50} around 1 nM.

When benzyl was used instead of phenyl (with PHI as the central amino acid), four isomers were separated. As expected, when the two chiral centers of the cyclopropane ring presented a *cis* stereochemistry, both isomers **74** and **75**) were more or less inactive. However, when the stereochemistry of the cyclopropane ring was *trans*, the potency of the PHI- and ABO-containing derivatives **76**−**79** was higher, culminating at 2.5 nM for compound **77**. Most of the time, as previously observed, PHI containing inhibitors were more potent than their ABO counterparts. Finally, two phenylcyclobutane-containing inhibitors **80** and **81** (see Table 6) were tested against rat cortex PEP. They were found to be 20−50 times less active than the cyclopropane derivatives. Interestingly, the *cis* derivative was twice as potent as the *trans*.

Table 7

compd no	central amino acid	ID_{50} (mg/kg) ip	ID_{50} (mg/kg) po
48	ABO	5	20
52	PHI	0.3	3
53	ABO	>3	>10
54	PHI	1	8
65	ABO	≫3	≫3
66	PHI	03	≫10
67	ABO	3	7.5
68	PHI	0.3	1
69	ABO	1	8
70	PHI	1	≫3
77	PHI	>3	≫10
79	ABO	≫3	≫3

Selectivity Studies

Selectivity of inhibitor **54** was studied on a panel of 13 enzymes and 16 receptors; in each case, IC_{50}s were higher than 10^{-5} M, except for 5-lipoxygenase where an 83% of inhibition was obtained at 10^{-5} M. Moreover, no inhibition was detected at 10^{-3} M against pig pancreatic elastase and human leucocyte elastase for compounds **54** and **68**. Finally, no inhibitory activity was detected up to 33 μM on several factors of the coagulation and the fibrinolysis cascades, including thrombin, plasmin, factor Xa, kallikrein, activated protein C, urokinase, tPa, and trypsin.

In Vivo Studies

In vivo potencies as well as the pharmacological index of bioavailability were evaluated after ip and po administration in rat and *ex vivo* determination of PEP inhibition. In Table 7 are presented the respective ID_{50}s for the most active compounds selected according to their *in vitro* inhibitory potency. Most of the time, a greater activity was obtained when the central amino

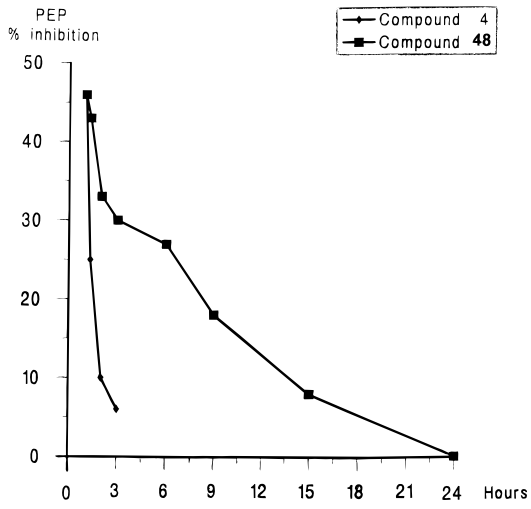


Figure 2. *Ex vivo* evaluation of PEP inhibitory potency of compound **4** (30 mg/kg ip) and compound **48** (5 mg/kg ip).

acid was PHI rather than ABO, with up to 10-fold improvement of the ID_{50}. Replacement of pyrrolidine by thiazolidine (**48** and **52** to **53** and **54**) conferred similar *in vivo* activity (**52** ID_{50} ip = 0.3 mg/kg; **54** ID_{50} ip = 1 mg/kg).

A thienyl group (**65**, **66**) or a benzyl group (**77**, **79**) on the cyclopropane ring resulted in a dramatic loss of activity. On the contrary, a strong *in vivo* potency was obtained with the 2-(4-fluorophenyl)cyclopropyl derivatives **67** and **68** (the most active compound tested: ID_{50} ip = 0.3 mg/kg; ID_{50} po = 1 mg/kg). As shown in Figure 2, compound **4** inhibited PEP activity after administration of 30 mg/kg ip. The time course of this inhibitory effect was rather a short duration, with a $t_{1/2}$ of around 1 h. Inhibitory effect of compound **48** was far more long lasting ($t_{1/2} \sim 7$ h). Furthermore, the same level of inhibition was obtained with a 6 times lower dose of **48**, as compared to **4** (5 *vs* 30 mg/kg). Identical results were obtained with PHI containing inhibitors like **52**, **54**, **68**, **70**, and **71**. A direct comparison of the ip and po inhibition curves for compound **54** gives a pharmacological index of oral bioavailability of 0.7, whatever the dose.

Although *S,S* diastereomer **47** is only 2.5-fold less active *in vitro* than its *R,R* counterpart **48**, this compound is completely inactive *in vivo* (Figure 3). As mentioned above, this is the confirmation that the side chain of our inhibitors, and more precisely its stereochemistry play a major role, not only for obtention of potent *in vitro* inhibitory potency, but also for *in vivo* pharmacological activity.

Cognition-enhancing properties of most active *in vivo* PEP inhibitors were evaluated using passive avoidance test with scopolamine-induced amnesia in the Rat (Table 8). In the present experimental conditions, the mean avoidance responses in control and scopolamine animals were 289 ± 11 and 45.9 ± 9 s, respectively. Treatment ip with compound **54** inhibited the scopolamine-induced decrease in avoidance response, and this effect was dose-related. Moreover, it appeared significantly at doses which inhibited PEP activity in the rat cortex.

Conclusion

Among the compounds previously described as PEP inhibitors, ONO 1603 (**1**) or Z-Pro-prolinal (**2**) contain

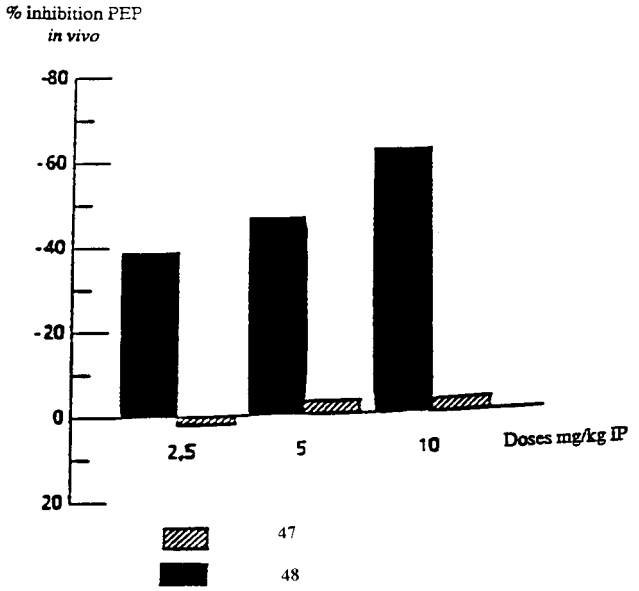


Figure 3. Dose response study for diastereomers **47** and **48**.

Table 8

dose of **54** (mg/kg ip)	avoidance response (s) (mean ± SEM)	PEP inhib (%)
0.3	129 ± 12	−12
1	203 ± 24	−27
3	225 ± 14	−42
10	283 ± 4	−58

an aldehyde function susceptible to react with Ser-554 in the active site of the enzyme. Some others, like Suam 1221 (**3**) or thiazolidine derivative **4** have a carbonyl group linking the P1 and P2 subsites. We decided to extend the field of structural variations in this latter class of inhibitors. Several conclusions can be drawn from the foregoing results:

(1) Our proline analogs amino acids have proven to be useful tools for obtaining potent inhibitors as active as the reference compounds. Most of the time, PHI provided the most potent *in vitro* inhibition; *in vivo*, PHI containing derivatives presented a good oral bioavailability and gave a strong and long-lasting PEP inhibition. In our hands, a thiazolidine ring at P1 afforded derivatives as potent *in vitro* and *in vivo* as their pyrrolidine-containing counterparts.

(2) We have shown that the phenyl ring of the side chain could be replaced by a dicyclopropylmethyl group as well as by a (trifluoromethyl)phenyl group. Optimization of the length of this side chain led to the preparation of 2-phenylcyclopropyl-containing inhibitors. Moreover, we were able to show that *R,R* configuration for the cyclopropane ring was of prime importance in order to obtain a strong enzymatic inhibitory capacity; to the best of our knowledge, this is the first time that stereochemical parameters are shown to be of importance in this S3 region of interaction between the inhibitor and the enzyme. Most important, this original side chain seems to confer a potent and long-lasting *in vivo* PEP inhibitory activity to the central amino acid it is appended to; in our case, it provided potent and long-acting PEP inhibitors, exemplified by compounds **52**, **54**, **68**, **70**, or **71**.

Some of these compounds are currently undergoing pharmacological studies on models of attention, learn-

ing, and memory, as well as extensive preclinical evaluation.

Experimental Section

Melting points were determined on a Tottoli apparatus and were not corrected. Elemental analyses were carried out by the analytical department of the Institut de Recherches SERVIER; results obtained for specified elements are within ±0.4% of the theoretical values. IR spectra were recorded on a Bruker IFS 28 spectrophotometer. ^{1}H NMR spectra of deuteriochloroform or DMSO-d_6 solutions were recorded on Bruker AC 200 or AM 300 spectrometers. Chemical shifts are given in ppm with TMS as the internal standard. Optical rotations were recorded with a 241 Perkin-Elmer polarimeter. Abbreviations: DCC = *N*,*N*′-dicyclohexylcarbodiimide; DCU = *N*,*N*′-dicyclohexylurea; HOBT = 1-hydroxybenzotriazole.

Group 1. Synthesis of 14: General Procedure. Step A: (2*S*,3a*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)perhydroindole-2-carboxylic Acid (Boc-PHI). To a solution of 16.9 g (0.1 mol) of PHI in 300 mL of dioxane−H_2O, 2:1, was added 100 mL of 1 N aqueous NaOH (0.1 mol). After cooling to 5 °C, a solution of 21.8 g (0.1 mol) of Boc_2O in 40 mL of dioxane was added. After stirring at room temperature overnight, the reaction was evaporated and the crude mixture was taken up with water. Acidification with citric acid, followed by EtOAc extraction, afforded 26.2 g of amorphous solid. Crystals (23 g, 85%) formed when this crude material was triturated with pentane: mp 134 °C; IR (Nujol) 3200−2400, 1750 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.4 (9H, 2s), 1.0−2.1 (10H, m), 2.2 (1H, m), 3.6 (1H, m), 4.0 (1H, t), 12.4 (1H, m).

Step B: (2*S*,3a*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (Boc-PHI-pyrrolidine, 14). To 13.5 g (0.05 mol) of Boc-PHI in DMF (100 mL) were added 3.56 g (0.05 mol) of pyrrolidine and 9.09 g (0.05 mol) of HOBT in DMF (40 mL). DCC (11.8 g, 0.05 mol) was then added, and the reaction mixture was stirred at room temperature overnight. DCU was filtered, and DMF was evaporated. The residue was taken up by EtOAc and washed with aqueous solutions of $NaHCO_3$ and citric acid and then brine. After drying on $CaSO_4$ and evaporation, the solid was recrystallized from EtOAc to yield 11.7 g (73%) of the desired compound: IR (Nujol) 1691, 1649 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.4 (15H, m), 1.4 (9H, d), 3.3−3.6 (4H, m), 3.7−3.9 (1H, m), 4.35 (1H, m).

(3*S*)-2-Aza-2-(*tert*-butyloxycarbonyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (11): IR (Nujol) 1680, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.4 (9H, 2s), 1.8 (12H, m), 2.2 (1H, m), 3.5 (4H, m), 4.2 (2H, m).

(3*R*)-2-Aza-2-(*tert*-butyloxycarbonyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (12): IR (Nujol) 1678, 1649 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.2−1.4 (9H, 2s), 1.4−2.1 (13H, m), 3.1−3.6 (4H, m), 3.9 (1H, m), 4.25 (1H, d).

(1*S*,3*S*,4*R*)-2-Aza-2-(*tert*-butyloxycarbonyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.1]heptane (13): IR (Nujol) 1699, 1643 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.38−1.45 (9H, 2s), 1.50−2.10 (10H, m), 2.66 (1H, m), 3.30−3.70 (4H, m), 4.30 (2H, m).

(3*S*)-2-Aza-2-(*tert*-butyloxycarbonyl)-3-(azabicyclo[3.3.0]-octan-3-ylcarbonyl)bicyclo[2.2.2]octane (15): IR (Nujol) 1693, 1645 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.25 (9H, 2s), 1.3−2.0 (15H, m), 2.5−2.7 (2H, m), 3.10−3.30 (2H, m), 3.4−3.7 (2H, m), 3.9 (1H, m), 4.2 (1H, d).

(3*S*)-2-Aza-2-(*tert*-butyloxycarbonyl)-3-(thiazolidin-3-ylcarbonyl)bicyclo[2.2.2]octane (16): IR (Nujol) 1680, 1689, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.4 (9H, 2s), 1.5−2.3 (9H, m), 2.9−3.2 (2H, m), 3.6−4.8 (6H, m).

(3*S*)-2-Aza-2-(*tert*-butyloxycarbonyl)-3-(pyrrolin-1-ylcarbonyl)bicyclo[2.2.2]octane (17): IR (Nujol) 1678, 1655, 1626 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.4 (9H, 2s), 1.5−2.1 (8H, m), 2.15−2.35 (1H, m), 4.0−4.6 (6H, m), 5.7−6.0 (2H, m).

(2*R*,*R*,3a*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-2-(isoquinuclidin-1-ylcarbonyl)perhydroindole (18): IR (Nujol) 1691, 1643 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.4 (20H, m), 1.4 (9H, dd), 3.3−3.95 (3H, m), 4.3−4.6 (2H, m).

(2*R*,*S*,3a*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-2-(azetidin-1-ylcarbonyl)perhydroindole (19): IR (Nujol) 1687, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.4 (13H, m), 1.45 (9H, s), 3.6−4.6 (6H, m).

(2*S*,3a*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-2-(piperidin-1-ylcarbonyl)perhydroindole (20): IR (Nujol) 1697, 1655, 1637 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.3 (9H, 2s), 0.95−1.75 (14H, m), 1.80 (1H, m), 2.05 (1H, m), 2.25 (1H, m), 3.35−3.75 (5H, m), 4.55 (1H, m).

(2*S*,3a*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-2-(homopiperidin-1-ylcarbonyl)perhydroindole (21): IR (Nujol) 1684, 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40−1.45 (9H, 2s), 1.0−2.4 (19H, m), 3.2−4.0 (5H, m), 4.55−4.60 (1H, 2t).

Group 2. Synthesis of 22: General Procedure. Step A: (3*S*)-2-Aza-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]-octane (ABO-pyrrolidine). A 11.4 g (37 mmol) sample of compound **11** was dissolved in 100 mL of EtOAc. HCl gas was bubbled through the solution, and stirring was continued at room temperature for 18 h. After evaporation *in vacuo*, the residue was taken up in 100 mL of water. After filtration, the pH was adjusted to 8 with sodium bicarbonate, and the mixture was evaporated. The residue was taken up with 100 mL of EtOH, 100 mL of CH_2Cl_2, and finally 100 mL of Et_2O. Filtration of the salts at each step and final concentration gave 6.8 g (89%) of the title compound (mp 117 °C): IR (Nujol) 3280, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.7 (13H, m), 2.3 (1H, m), 2.9 (1H, m), 3.5 (4H, m), 3.8 (1H, s).

Step B: (3*S*)-2-Aza-2-(4-phenylbutyryl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (22). The intermediate obtained in the first step (832 mg, 4 mmol) was dissolved in DMF (10 mL). To this solution were successively added HOBT (612 mg, 4 mmol) in DMF (8 mL), 4-phenylbutyric acid (657 mg, 4 mmol), and finally DCC (824 mg, 4 mmol) in DMF (10 mL). After stirring overnight at room temperature, DCU was filtered and DMF evaporated. The residue was taken up with EtOAc (200 mL) and washed with water, aqueous saturated $NaHCO_3$ solution, water, citric acid solution, water, and finally brine. The organic layer was dried over $CaSO_4$ and evaporated. The crude product was purified by chromatography (CH_2Cl_2−MeOH, 95:5) and then crystallized from an acetone−iPr_2O mixture to give 500 mg (35%) of a white solid: IR (Nujol) 1647, 1628 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.25−2.05 (15H, m), 2.05−2.4 (2H, m), 2.5−2.65 (2H, t), 3.1−3.6 (4H, m), 3.8 (1H, m), 4.3 (1H, d), 7.−7.3 (5H, m).

(3*R*)-2-Aza-2-(4-phenylbutyryl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (23): IR (Nujol) 1637 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.2−2.5 (17H, m), 2.6−2.75 (2H, t), 3.25−3.45 (2H, m), 3.6−3.85 (3H, m), 4.45 (1H, d), 7.−7.4 (5H, m).

(1*S*,3*S*,4*R*)-2-Aza-2-(4-phenylbutyryl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.1]heptane (24): IR (Nujol) 1651 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.4−2.1 (13H, m), 2.3 (2H, t), 2.7 (2H, t), 3.3−3.5 (2H, m), 3.6−3.75 (2H, m), 4.1 (1H, m), 4.45 (1H, d), 7.1−7.4 (5H, m).

(2*S*,3a*S*,7a*S*)-1-(4-Phenylbutyryl)-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (25): IR (Nujol) 1647 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.1 (17H, m), 2.3 (2H, t), 2.65 (2H, t), 3.3−3.5 (2H, m), 3.5−3.7 (2H, m), 3.85 (1H, m), 4.5 (1H, t), 7.2 (5H, m).

(3*R*,*S*)-2-Aza-2-(4-phenylbutyryl)-3-(imidazol-1-ylcarbonyl)bicyclo[2.2.2]octane (26): IR (Nujol) 1743, 1633 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.4−2.5 (13H, m), 2.65 (2H, t), 3.9 (1H, m), 4.9 (1H, m), 7.05 (1H, s), 7.1−7.3 (5H, m), 7.5 (1H, s), 8.25 (1H, s).

(3*R*,*S*)-2-Aza-2-(4-phenylbutyryl)-3-(pyrazol-1-ylcarbonyl)bicyclo[2.2.2]octane (27): IR (Nujol) 1741, 1637 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.3−2.0 (10H, m), 2.2−2.5 (3H, m), 2.6 (2H, t), 3.95 (1H, m), 5.4 (1H, m), 6.65 (1H, m), 7.1−7.4 (5H, m), 7.9 (1H, d), 8.45 (1H, d).

(3*S*)-2-Aza-2-(4-phenylbutyryl)-3-(thien-2-ylcarbonyl)-bicyclo[2.2.2]octane (28). To a THF (20 mL) solution of thien-2-ylmagnesium bromide (1.15 g, 7 mmol) was added dropwise at 0 °C 2 g (7 mmol) of (3*S*)-2-aza-3-cyano-2-(4-phenylbutanoyl)bicyclo[2.2.2]octane (obtained according Scheme 5) in 50 mL of THF (poorly soluble). After one night at room temperature, the mixture was cooled down to 0 °C, and 10 mL of a 10% aqueous NH_4Cl solution was added. The aqueous

layer was extracted with ether. The organic layers were combined, washed with water and brine, and dried over Na_2SO_4. Concentration under reduced pressure gave a crude mixture, which was purified by column chromatography (CH_2Cl_2−EtOAc, 95:5) to give 340 mg (13%) of the desired thienyl ketone; 1 g of the starting nitrile was recovered and recycled: ^{1}H NMR ($CDCl_3$) δ 1.5−2.0 (11H, m), 2.6−2.8 (4H, m), 3.5 (1H, broad s), 4.3 (1H, broad s), 6.9 (3H, t), 7.0−7.3 (5H, m).

Group 3. Synthesis of 32: General Procedure. Step A: (2*S*,3a*S*,7a*S*)-2-(Pyrrolidin-1-ylcarbonyl)perhydroindole. This compound is prepared from compound **14** according to Scheme 2: IR (Nujol) 3288, 1631 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.1−2.3 (15H, m), 2.8 (1H, broad s), 3.1 (1H, m), 3.3−3.7 (4H, m), 3.85 (1H, q).

Step B: (2*S*,3a*S*,7a*S*)-1-(5,5-Dicyclopropylpentanoyl)-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (32). To the intermediate obtained above (2.22 g, 0.01 mol) in DMF (40 mL) was added 1.82 g (0.01 mol) of 5,5-dicyclopropylvaleric acid in 60 mL of DMF followed by 1.55 g (0.01 mol) of HOBT in 40 mL of DMF and 2.06 g (0.01 mol) of DCC in 40 mL of DMF. The reaction was stirred at room temperature overnight, DCU was filtered, and the solvent was evaporated. After washing with aqueous saturated $NaHCO_3$ solution, 10% aqueous citric acid solution, water, and brine, the organic phase was dried over $CaSO_4$ and evaporated. Silica gel chromatography (EtOAc) gave 1.5 g (39%) of the title compound: IR (KBr) 3700−3100, 1649 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.0−0.3 (5H, m), 0.3−0.5 (4H, m), 0.5−0.7 (2H, m), 1.1−2.2 (15H, m), 2.2−2.5 (6H, m), 3.3−3.5 (2H, m), 3.5−4.0 (3H, m), 4.55 (1H, t).

(3*S*)-2-Aza-(6,6-dicyclopropylhexanoyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (29): IR (KBr) 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.0−0.7 (10H, m), 1.4−2.1 (19H, m), 2.3 (3H, m), 3.3 (2H, m), 3.7 (2H, m), 3.9 (1H, m), 4.5 (1H, m).

(3*S*)-2-Aza-(5,5-dicyclopropylpentanoyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (30): IR (Nujol) 1651, 1633 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.0−0.5 (9H, m), 0.5−0.7 (2H, m), 1.4−1.5 (19H, 2m), 3.3−3.5 (2H, m), 3.5−3.85 (2H, m), 3.9 (1H, m), 4.45 (1H, m).

(1*S*,3*S*,4*R*)-2-Aza-(5,5-dicyclopropylpentanoyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]heptane (31): IR (KBr) 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.0−0.7 (10H, m), 1.2−2.1 (15H, m), 2.3 (2H, t), 2.7 (1H, m), 3.3−3.8 (4H, m), 4.2 (1H, m), 4.5 (1H, m).

(2*S*,3a*S*,7a*S*)-1-(4,4-Dicyclopropylbutanoyl)-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (33): IR (KBr) 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.0−0.7 (11H, m), 1.0−2.2 (16H, m), 2.3 (1H, m), 2.5 (2H, m), 3.3−4.0 (5H, m), 4.5 (1H, t).

(2*S*,3a*S*,7a*S*)-1-(3,3-Dicyclopropylpropionyl)-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (34): IR (Nujol) 1655, 1618 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.0−0.5 (8H, m), 0.55−1.0 (3H, m), 1.0−2.4 (15H, m), 2.4 (2H, m), 3.3−3.65 (3H, m), 3.9 (2H, m), 4.5 (1H, t).

(2*S*,3a*S*,7a*S*)-1-[3-[(Dicyclopropylmethyl)thio]propionyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (35): IR (KBr) 1651 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.25−0.4 (4H, m), 0.5−0.7 (4H, m), 0.8−1.0 (2H, m), 1.1−2.4 (16H, m), 2.6 (2H, t), 3.0 (2H, t), 3.3−3.5 (2H, m), 3.5−3.95 (3H, m), 4.55 (1H, t).

(3*S*)-2-Aza-2-[3-[[(dicyclopropylmethyl)amino]carbonyl]-propionyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]-octane (36): IR (Nujol) 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.2−0.6 (8H, m), 0.8−1.0 (2H, m), 1.35−2.6 (15H, m), 2.6−2.8 (2H, m), 3.0−3.2 (1H, t), 3.3−3.5 (2H, m), 3.55−3.8 (2H, m), 2H, m), 3.9 (1H, m), 4.45 (1H, m), 6.1 (1H, broad s).

(3*S*)-2-Aza-2-(6,6-dicyclopropylhex-5-enoyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (37): IR (KBr) 1653, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.3−0.8 (8H, m), 0.9 (1H, m), 1.3−2.5 (20H, m), 3.4 (2H, m), 3.7 (2H, m), 3.9 (1H, m), 4.4 (1H, m), 5.1 (1H, t).

(3*S*)-2-Aza-2-(6,6-dicyclopropylhex-4-enoyl)-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (38): IR (KBr) 1639 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0−0.55 (8H, m), 0.65−0.85 (2H, m), 1.2−2.1 (13H, m), 2.1−2.5 (5H, m), 3.3−3.45 (2H, m), 3.6−3.8 (2H, m), 3.85 (1H, broad s), 4.45 (1H, broad s), 5.2−5.5 (2H, m).

(2*S*,3a*S*,7a*S*)-1-[3-Phenyl-3-(trifluoromethyl)propanoyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (isomer α) (39): IR (Nujol) 1645, 1340 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.1 (14H, m), 2.3−2.5 (1H, m), 2.6−2.7 (1H, dd), 3.0−3.2 (1H, dd), 3.3−3.7 (3H, m), 3.7−4.0 (2H, m), 4.1−4.3 (1H, m), 4.55 (1H, t), 7.3−7.4 (5H, broad s).

(2*S*,3a*S*,7a*S*)-1-[3-Phenyl-3-(trifluoromethyl)propanoyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (isomer β) (40): IR (Nujol) 1651, 1342 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.1 (15H, m), 2.9−3.0 (2H, m), 3.2−3.4 (2H, m), 3.5−3.7 (3H, m), 3.9−4.2 (1H, m), 4.4 (1H, t), 7.3 (5H, broad s).

(2*S*,3a*S*,7a*S*)-1-[3-(Trifluoromethyl)cinnamoyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (41): IR (Nujol) 1655, 1645, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.4 (15H, m), 3.2−3.65 (4H, m), 3.65−3.90 (1H, m), 3.97, 4.03, 4.34 (1H, tt), 6.57, 6.82 (1H, 2s), 703−705 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*R*,*S*)-4-Phenyl-4-(trifluoromethyl)butanoyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (42): IR (Nujol) 1649, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.9−2.3 (18H, m), 2.45 (1H, m), 3.4 (4H, m), 3.65 (1H, m), 3.85 (1H, m), 4.5 (1H, m), 7.3 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*R*,*S*)-5-Phenyl-5-(trifluoromethyl)pentanoyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (43): IR (KBr) 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.8−2.4 (19H, m), 2.3 (2H, m), 3.1−3.9 (6H, m), 4.5 (1H, m), 7.3 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*R*,*S*)-5-Cyclopentyl-5-(trifluoromethyl)pentanoyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (44): IR (KBr) 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0−2.5 (31H, m), 3.2−4.0 (5H, m), 4.5 (1H, t).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-2-Phenylcyclopropan-1-yl)carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindoles (51 and 52) (Scheme 2). To 2.2 g (0.01 mol) of PHI-pyrrolidine (obtained from **14** as described in Scheme 2) in 20 mL of CH_2Cl_2 was added at 5 °C 1.7 mL (0.012 mol) of triethylamine, followed by 1.8 g (0.01 mol) of 2-phenylcyclopropanecarbonyl chloride in 10 mL of CH_2Cl_2. After 15 h at room temperature, the reaction mixture was washed with water, aqueous $NaHCO_3$ solution, and brine. Drying over Na_2SO_4 and concentration under reduced pressure afforded 4.1 g of a brown oil. Purification on silica gel (CH_2Cl_2−EtOAc, 50:50) gave the two diastereomers **51** and **52**.

51 (isomer *S*,*S*): 1.2 g (32%); crystallization from iPr_2O; IR (Nujol) 1657, 1633 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.0−2.5 (19H, m), 3.1−3.7 (4H, m), 4.0−4.1 (1H, m), 4.45 (1H, dt), 7.0−7.3 (5H, m).

52 (isomer *R*,*R*): 900 mg (24%); crystallization from pentane; IR (Nujol) 1643 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.95−2.4 (19H, m), 3.1−3.7 (4H, m), 3.8−4.0 (1H, m), 4.45−4.8 (1H, 2t), 7.0−7.3 (5H, m).

(4*R*)-3-[(*trans*-(*S*,*S*)-2-Phenylcyclopropan-1-yl)carbonyl]-4-(pyrrolidin-1-ylcarbonyl)thiazolidine (45): IR (Nujol) 1660, 1635 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.2−1.4 (2H, 2m), 1.7 (3H, m), 1.9 (2H, m), 2.3 (2H, t), 2.9−3.6 (3H, m), 4.3, 4.9 (2H, dd), 4.6, 5.2 (2H, dd), 5.0 (1H, t), 7.1−7.4 (5H, m).

(4*R*)-3-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-4-(pyrrolidin-1-ylcarbonyl)thiazolidine (46): IR (Nujol) 1641 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.2−1.4 (2H, 2m), 1.7 (3H, m), 1.9 (2H, m), 2.3 (2H, t), 2.9−3.6 (3H, m), 4.4, 4.8 (2H, dd), 4.7, 5.0 (2H, dd), 5.25 (1H, t), 7.0−7.3 (5H, m).

(3*S*)-2-Aza-2-[(*trans*-(*S*,*S*)-2-phenylcyclopropan-1-yl)-carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]-octane (47): IR (Nujol) 1647, 1622 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.1−2.0 (15H, m), 2.1−2.4 (1H, m), 2.4−2.6 (1H, m), 3.35 (2H, m), 3.7 (2H, m), 4.1 (1H, m), 4.45 (1H, m), 7.0−7.3 (5H, m).

(3*S*)-2-Aza-2-[(*trans*-(*R*,*R*)-2-phenylcyclopropan-1-yl)-carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]-octane (48): IR (Nujol) 1643 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.1−2.1 (15H, m), 2.3−2.5 (2H, m), 3.2−3.4 (2H, m), 3.6−3.85 (2H, m), 4.1 (1H, m), 4.5−4.7 (1H, 2m), 7.1−7.4 (5H, m).

(1*S*,3*S*,4*R*)-2-Aza-2-[(*trans*-(*S*,*S*)-2-phenylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.1]-heptane (49): IR (Nujol) 1660, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.0−1.9 (12H, m), 2.1 (1H, m), 2.25 (1H, m), 2.7−2.9 (1H, m), 3.2−3.6 (4H, m), 4.35, 4.5, 4.65 (2H, 3 broad s), 7.0−7.4 (5H, m).

(1*S*,3*S*,4*R*)-2-Aza-2-[(*trans*-(*R*,*R*)-2-phenylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.1]-heptane (50): IR (Nujol) 1647 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.9−2.0 (12H, m), 2.1 (1H, m), 2.3 (1H, m), 2.7−2.9 (1H, m), 3.2−3.6 (4H, m), 4.35, 4.4, 4.75 (4H, 3 broad s), 7.0−7.4 (5H, m).

Synthesis of 54. Two-Step General Procedure. Step A: (2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]perhydroindole-2-carboxylic Acid (Scheme 2). PHI (20.3 g, 0.12 mol) was dissolved in 180 mL of water and 60 mL of 2 N NaOH. After cooling at 0−5 °C, 60 mL of 2 N NaOH and 21.7 g (0.12 mol) of *trans*-(*R*,*R*)-2-phenylcyclopropanecarbonyl chloride in 40 mL of THF were simultaneously added. The reaction mixture was stirred one night at room temperature, and then the solvent was evaporated. The aqueous layer was washed with ether and acidified with 12 mL of concentrated HCl. Extraction with CH_2Cl_2, drying over Na_2SO_4, and concentration gave an amorphous white solid (40 g, 100%): IR (Nujol) 3500−2300, 1741−1700, 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.1−1.7 (10H, m), 1.85 (1H, m), 2.1 (2H, m), 2.3 (2H, m), 3.95 (1H, m), 4.15 (1H, m), 7.1−7.35 (5H, m), 11.8 (1H, broad s).

Step B: (2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-(thiazolidin-3-ylcarbonyl)perhydroindole (54). To 8.7 mL (0.11 mol) of thiazolidine in 50 mL of anhydrous DMF was added at 0−5 °C the acid obtained in step A (34.5 g, 0.11 mol) in DMF (50 mL) followed by 14.9 g (0.11 mol) of HOBT in DMF (50 mL) and 22.7 g (0.11 mol) of DCC in DMF (50 mL). The reaction mixture was stirred for one night at room temperature. DCU was filtered, and the solvent was evaporated under reduced pressure. The semisolid residue was taken up with EtOAc. DCU was filtered again. The organic layer was washed with water, 10% aqueous citric acid solution, water, aqueous saturated $NaHCO_3$ solution, and brine. After drying over Na_2SO_4 and concentration, the amorphous white solid (40 g) was triturated with ether to give, after filtration, 30 g of a white solid. This solid was purified by column chromatography (CH_2Cl_2−EtOH, 99:1) to eliminate traces of the nondesired diastereomer to give 23.7 g (55%) of an amorphous white solid: IR (Nujol) 1657, 1628 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2−1.3 (2H, m), 1.2−2.0 (8H, m), 1.6−1.9 (2H, m), 1.8−2.2 (2H, m), 2.25 (1H, m), 3.1 (2H, m), 2.9−3.7 (2H, m), 3.7−3.9 (2H, m), 4.0 (1H, m), 4.6 (1H, m), 7.0−7.3 (5H, m).

(3*S*)-2-Aza-2-[(*trans*-(*R*,*R*)-2-phenylcyclopropan-1-yl)-carbonyl]-3-(thiazolidin-3-ylcarbonyl)bicyclo[2.2.2]-octane (53): IR (Nujol) 1653−1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2−1.5 (2H, m), 1.5−1.8 (8H, m), 1.7 (1H, m), 2.1 (1H, m), 2.3 (1H, m), 2.8−4.0 (4H, m), 4.2 (1H, m), 4.5 (1H, m), 4.8 (2H, m), 7.2 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-(2-pyrrolin-1-ylcarbonyl)perhydroindole (55): IR (Nujol) 1631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2 (1H, m), 1.3−1.9 (12H, m), 2.05 (1H, m), 2.25 (1H, m), 2.45 (2H, dd), 3.55 (2H, dd), 3.9 (1H, m), 4.7 (1H, m), 5.2 (1H, m), 7.0 (1H, m), 7.0−7.4 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-(3-pyrrolin-1-ylcarbonyl)perhydroindole (56): IR (Nujol) 1660, 1624 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2−2.4 (5H, m), 3.8−4.3 (5H, m), 4.5 (1H, m), 5.9 (2H, s), 7.1−7.3 (5H, 2m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-(pyrrol-1-ylcarbonyl)perhydroindoles (57): IR (Nujol) 1726, 1618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.1−1.7 (9H, m), 1.8−2.3 (5H, m), 2.45 (1H, m), 4.5 (1H, m), 5.25 (1H, m), 6.30 (2H, m), 7.15−7.3 (5H, 2m), 7.55 (2H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-[(cyclopentylamino)carbonyl]perhydroindole (58): IR (Nujol) 3700−3000, 1672−1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9−2.7 (24H, m), 3.8−4.0 (1H, m), 4.1−4.3 (1H, m), 4.4 (1H, m), 7.1−7.4 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-[[(*R*,*S*)-2-methylpyrrolidin-1-yl]carbonyl]-perhydroindole (59): IR (Nujol) 1635 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.1 (3H, d), 1.2−2.0 (12H, m), 2.1 (5H, m), 2.3 (1H, m), 2.4 (1H, m), 3.7 (1H, m), 3.4−3.9 (2H, m), 4.0 (1H, m), 4.5 (1H, m), 7.2 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-[[(*R*,*S*)-2,5-dimethyl-1-pyrrolidin-1-yl]carbonyl]perhydroindole (60): IR (Nujol) 1630, 1649 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (6H, 2d), 1.2−2.6 (19H, m), 3.8−4.7 (4H, m), 7.0−7.3 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-[(*N*,*N*-diethylamino)carbonyl]perhydroindole (61): IR (Nujol) 1631 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.1−1.3 (6H, 2t), 1.2−1.8 (10H, m), 2.1 (3H, m), 2.4−2.5 (2H, m), 3.3−3.7 (4H, m), 3.9 (1H, dd), 4.7 (1H, dd), 7.3 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-[[(*R*,*S*)-3-hydroxypyrrolidin-1-yl]carbonyl]perhydroindole (62): IR (Nujol) 3375, 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.05−2.0 (14H, m), 2.1 (1H, m), 2.25 (1H, m), 2.35 (1H, m), 3.6 (1H, m), 3.2−3.4 (2H, m), 3.9 (1H, m), 4.3 (1H, m), 4.5−4.9 (2H, m), 7.3 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-[(2-oxopyrrolidin-1-yl)carbonyl]perhydroindole (63): IR (Nujol) 1738, 1701, 1631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25 (1H, m), 1.5−2.0 (14H, m), 2.1 (1H, m), 2.35 (1H, m), 2.6 (2H, m), 3.7 (2H, m), 4.0 (1H, m), 5.35 (1H, t), 7.2 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Phenylcyclopropan-1-yl)carbonyl]-2-(isoxazolidin-2-ylcarbonyl)perhydroindole (64): IR (Nujol) 1676−1631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25 (2H, m), 1.45−2.0 (10H, m), 2.1 (1H, m), 2.25 (4H, m), 3.8 (2H, m), 2.95 (3H, m), 5.0 (1H, t), 7.2 (5H, m).

***trans*-(*R*,*R*)-2-(4-Fluorophenyl)cyclopropanecarboxylic Acid.** A 2.1 g (0.56 mmol) sample of the cyclopropanated camphorsultan derivative obtained according ref 18 was dissolved in 20 mL of THF. A solution of 940 mg of LiOH in 10 mL of water was added, and the reaction was stirred for 20 h at room temperature. THF was evaporated, 50 mL of water was added, and the aqueous layer was washed with EtOAc. The aqueous layer was then acidified with concentrated HCl and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4, and concentrated to give 750 mg (74%) of the desired acid: IR (Nujol) 3100−2400, 1691 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.3−1.5 (2H, m), 1.8 (1H, m), 2.45 (1H, m), 7.1−7.3 (4H, m); $[\alpha]^{21}_D$ −292.5° (CH_2Cl_2, 1%).

(2*S*,3a*S*,7a*S*)-1-[[*trans*-(*R*,*R*)-2-(4-Fluorophenyl)cyclopropan-1-yl]carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (68). A 431 mg (1.95 mmol) portion of PHI-pyrrolidine in DMF (50 mL) was reacted with 350 mg (1.95 mmol) of *trans*-(*R*,*R*)-2-(4-fluorophenyl)cyclopropanecarboxylic acid obtained in the previous step. HOBT (297 mg, 1.95 mmol) in DMF (10 mL) and DCC (389 mg, 1.95 mmol) in DMF (10 mL) were successively added. After 20 h at room temperature, DCU was filtered and DMF evaporated. The residue was taken up with EtOAc and washed with aqueous saturated $NaHCO_3$ solution, 10% aqueous citric acid solution, and water. After drying and evaporation, the residue was purified by column chromatography (CH_2Cl_2−MeOH, 97:3). Traces of the *S*,*S* isomer were discarded. The *R*,*R* derivative was crystallized in a minimum amount of pentane, filtered, and dried (500 mg, 67%): IR (Nujol) 1647 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.1−2.0 (15H, m), 2.05−2.4 (4H, m), 3.1−3.7 (4H, m), 3.95 (1H, m), 4.6 (1H, m), 7.0 (2H, m), 7.2 (2H, m).

(3*S*)-2-Aza-2-[(*trans*-(*R*,*R*)-2-thien-2-ylcyclopropan-1-yl]carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]-octane (65): IR (Nujol) 1639 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.0−1.7 (2H, m), 1.5−2.0 (4H, m), 1.6 (8H,m), 2.0 (1H, m), 2.1 (1H, m), 2.3 (1H, m), 3.3 (2H, m), 3.5 (2H, m), 4.4 (1H, m), 4.8 (1H, d), 7.0 (3H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-(*R*,*R*)-2-Thien-2-ylcyclopropan-1-yl)carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (66): IR (Nujol) 1732, 1641 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.0−2.3 (17H, m), 2.4 (2H, m), 3.1−3.7 (4H, m), 3.95 (1H, m), 4.45 (1H, m), 6.95 (2H, m), 7.3 (1H, d).

(3*S*)-2-Aza-2-[[*trans*-(*R*,*R*)-2-(4-Fluorophenyl)cyclopropan-1-yl]carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo-[2.2.2]octane (67): IR (Nujol) 1649 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.0−1.8 (11H, m), 1.8−2.15 (4H, m), 2.2 (1H, m), 2.4−

2.95 (1H, 2m), 3.2−3.65 (4H, m), 4.2−4.5 (1H, 2d), 4.4−4.8 (1H, 2d), 7.5−7.3 (4H, m).

(3*S*)-2-Aza-2-[[*trans*-(*R*,*R*)-2-[3-(Trifluoromethyl)phenyl]-cyclopropan-1-yl]carbonyl]-3-(pyrrolidin-1-ylcarbonyl)-bicyclo[2.2.2]octane (69): IR (Nujol) 1651 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2 (2H, m), 1.5 (4H, m), 1.7 (8H, m), 1.9 (1H, m), 2.1 (1H, m), 2.3 (1H, m), 3.4 (2H, m), 3.5 (2H, m), 4.4 (1H, m), 4.8 (1H, d), 7.4 (4H, m).

(2*S*,3a*S*,7a*S*)-1-[[*trans*-(*R*,*R*)-2-[3-(Trifluoromethyl)-phenyl]cyclopropan-1-yl]carbonyl]-2-(pyrrolidin-1-yl-carbonyl)perhydroindole (70): IR (Nujol) 1649, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0−1.5 (5H, m), 1.5 (2H, m), 1.9 (1H, m), 2.0−2.2 (8H, m), 2.25 (2H, m), 2.5 (1H, m), 3.5 (2H, m), 3.7 (2H, m), 3.9 (1H, m), 4.6 (1H, t), 7.4 (4H, m).

(2*S*,3a*S*,7a*S*)-1-[[*trans*-(*R*,*R*)-2-(4-Methylphenyl)cyclo-propan-1-yl]carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhy-droindole (71): IR (Nujol) 1645 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25 (2H, m), 1.4−2.0 (15H, m), 2.0 (1H, m), 2.2 (1H, m), 2.25 (3H, s), 3.25 (2H, m), 3.55 (2H, m), 3.9 (1H, m), 4.45 (1H, t), 7.0 (4H, m).

(3*S*)-2-Aza-2-[[*trans*-(*R*,*R*)-2-(4-methoxyphenyl)cyclo-propan-1-yl]carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo-[2.2.2]octane (72): IR (Nujol) 1653 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2 (2H, m), 1.2−2.1 (15H, m), 3.3 (2H, m), 3.4 (2H, m), 3.7 (3H, s), 4.45 (1H, s), 4.7 (1H, s), 6.7−7.1 (4H, m).

(3*S*)-2-Aza-2-[(*trans*-(*R*,*R*)-2-naphth-2-ylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]-octane (73): IR (Nujol) 1645, 1616 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.8 (1H, m), 0.9−2.2 (14H, m), 2.05 (1H, m), 2.5 (1H, m), 3.45 (2H, dd), 3.5 (2H, dd), 4.1 (1H, m), 4.45 (1H, d), 7.15 (1H, m), 7.35 (2H, m), 7.45 (1H, m), 7.7 (3H, m).

(2*S*,3a*S*,7a*S*)-1-[(*cis*-2-Benzylcyclopropan-1-yl)carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (isomer α, 74): IR (Nujol) 1626, 1646 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.7−2.1 (19H, m), 2.6 (2H, dd), 3.25 (2H, m), 3.5 (2H, m), 4.05 (1H, m), 4.45 (1H, t), 7.15 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*cis*-2-Benzylcyclopropan-1-yl)carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (isomer β, 75): IR (Nujol) 1635 cm^{-1}; ^1H NMR (DMSO-d_6) same spectrum as **76**.

(2*S*,3a*S*,7a*S*)-1-[(*trans*-2-Benzylcyclopropan-1-yl)carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (isomer α, 76): IR (Nujol) 1631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.7 (1H, m), 0.8−2.4 (18H, m), 2.6 (2H, m), 3.2 (2H, m), 3.45 (1H, m), 3.5 (3H, m), 4.4 (1H, m), 7.2 (5H, m).

(2*S*,3a*S*,7a*S*)-1-[(*trans*-2-Benzylcyclopropan-1-yl)carbonyl]-2-(pyrrolidin-1-ylcarbonyl)perhydroindole (isomer β, 77): IR (Nujol) 1631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.75 (1H, m), 0.8−2.2 (18H, m), 2.65 (2H, dd), 3.2 (2H, m), 3.45 (2H, dd), 3.6 (1H, m), 4.35 (1H, t), 7.0−7.5 (5H, m).

(3*S*)-2-Aza-2-[(*trans*-2-Benzylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (isomer α, 78): IR (Nujol) 1657, 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.7−1.0 (2H, m), 1.35 (1H, m), 1.5−1.7 (8H, m), 1.7−1.85 (5H, m), 2.0 (1H, m), 2.65 (2H, dd), 3.3−3.5 (4H, m), 4.2 (1H, m), 4.35 (1H, m), 7.3 (5H, m).

(3*S*)-2-Aza-2-[(*trans*-2-benzylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (isomer β, 79): IR (Nujol) 1651, 1614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.75−1.15 (2H, m), 1.2 (1H, m), 1.4−1.7 (8H, m), 1.7−2.0 (5Hm, m), 2.1 (1H, m), 2.6 (2H, dd), 3.3 (4H, m), 4.3 (1H, m), 4.45 (1H, m), 7.3 (5H, m).

(3*S*)-2-Aza-2-[(*cis*-3-phenylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (80): IR (Nujol) 1664 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.3−1.8 (8H, m), 1.8−1.9 (4H, m), 2.05 (1H, m), 2.3−2.7 (4H, m), 3.3 (1H, m), 3.4 (3H, m), 3.6 (2H, m), 3.7 (1H, broad s), 4.4 (1H, broad s), 7.3 (5H, m). The *cis* configuration was determined by NOE experiments.

(3*S*)-2-Aza-2-[(*trans*-3-phenylcyclopropan-1-yl)carbonyl]-3-(pyrrolidin-1-ylcarbonyl)bicyclo[2.2.2]octane (81): IR (Nujol) 1649, 1616 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.3−1.7 (8H, m), 1.75−1.9 (4H, m), 2.5 (4H, m), 3.25−3.5 (4H, m), 3.3 (1H, m), 3.35 (1H, m), 3.8 (1H, broad s), 4.3 (1H, broad s), 7.2−7.3 (5H, 2m). The *trans* configuration was determined by NOE experiments.

Assay of PEP Activity. Proline-specific endopeptiase activity was measured by a modification of the method of Yoshimoto and Tsuru.[20] PEP inhibitory effects of the tested compounds were estimated by using either purified enzyme isolated from Flavobacterium meningosepticum or tissue extract from rat brain. In this latest case, brain cortex was rapidly removed and homogenized in 25 mM phosphate buffer, pH 7.0 (5 mL/g tissue). After centrifugation at 10000*g* for 20 min, the supernatant was used for the enzyme assay. PEP activity was measured using Z-Gly-Pro-pNa or PyroGlu-His-Pro-pNA as substrates (bacterial and brain PEP, respectively). The amount of released pNA was estimated by measuring the absorbance at 410 nm.

(1) *In Vitro* Studies on Bacterial PEP Inhibitory Potency. A test compound was dissolved in DMSO at various concentrations. The solution (10 μL) or vehicle was preincubated with 990 μL of 0.1 M phosphate buffer, pH 7.0, and 250 μL of 2 M substrate solution at 37 °C for 5 min. The enzymatic reaction was initiated by adding 100 μL of PEP solution (0.5 unit/mL) and incubation at 37 °C continued for 10 min. The reaction was stopped by adding 2 mL of Triton X-100 solution containing 1 M acetate buffer (pH 4.0). The potency of inhibitory activity was represented by the IC_{50} value, which was defined as the concentration of the test compound that resulted in 50% inhibition of the absorbance with respect to the DMSO control.

(2) *In Vitro* Studies on Cerebral PEP Inhibitory Potency. Ten microliters of test compound solution was preincubated with 1890 μL of 25 mM phosphate buffer and 200 μL of brain extract at 37 °C for 15 min. The reaction was initiated by adding 250 μL of 9.4 mM substrate solution. Twenty minutes after, the amount of released pNA was immediately determined and inhibitory potency was represented as for bacterial PEP experiments.

(3) *Ex Vivo* Studies of Cerebral PEP Inhibitory Potency. At defined times after *in vivo* administration of tested compounds (inqueous solution with tween 80), the brain of each rat was rapidly removed and the cortex was dissected. A tissue extract was prepared as for *in vitro* experiments, and the supernatant was frozen at −20 °C until assay of PEP by the *in vitro* method described above. The potency of inhibitory activity was represented by the ID_{50} value, i.e. the dose which inhibited by 50% the PEP activity with respect to that the control animals. PEP activity was expressed as nmol of pNA/min per mg of protein.

Passive Avoidance Test. The one-trial step-through passive avoidance test was performed using a two-compartments apparatus, one dark and one bright and illuminated from above by a 6 W daylight lamp. The two chambers were separated by a guillotine type door, and the grid floor of the dark compartment was connected to an electric shock generator. The acquisition trial consisted of placing the rat in the bright compartment, opening the door after 60 s and shutting the door as soon as all four legs of the rat had entered the dark compartment. The animal was then subjected to 0.6 mA foot shock during 3 s, and 30 s after, it returned to its home cage. After 24 h, the retention test consisted of placing the rat in the bright compartment and measuring the time before the animal entered the dark compartment, up to a maximum of 300 s, i.e. the duration of the passive avoidance response (control value is 288.6 ± 11.4 sec). Avoidance response exceeding 300 s was recorded as 300 s. Scopolamine HBr was administered ip. 30 min before the acquisition trial at a dose of 1 mg/kg. Tested compound was administered ip 30 min before scopolamine, i.e. 60 min before acquisition trial.

Acknowledgment. The authors are greatful to Prof. Claudine Pascard (Gif sur Yvette, France) for X-ray structural determination, to Dr. Jean-Paul Volland and his group for analytical and spectral studies, and finally to Mrs. Danielle Pommier for expert technical assistance.

References

(1) Yoshimoto, T.; Orlowski, R. C.; Walter, R. Post proline Cleaving Enzyme: Identification as Serine Protease using Active Site Specific Inhibitors. *Biochemistry* **1977**, *16*, 2942−2948.

(2) Koida, M.; Walter, R. Post proline Cleaving Enzyme. *J. Biol. Chem*. **1976**, *251*, 7593−7599.

(3) Yoshimoto, T.; Ogita, K.; Walter, R.; Koida, M.; Tsuru, D. Post proline Cleaving Enzyme. *Biochem. Biophys. Acta* **1979**, *569*, 184−192.

(4) Kovacs, G. L.; Bohus, B.; Versteeg, D. H. G.; De Kloet, R.; De Wied, D. Effect of Oxytocin and Vasopressin on Memory Consolidation: Sites of Action and Catecholaminergic Limbic-Midbrain Structures. *Brain Res*. **1975**, *175*, 303−314.

(5) Fukunari, A.; Kato, A.; Sakai, Y.; Yoshimoto, T.; Ishiura, S.; Suzuki, K.; Nakajima, T. Colocalization of Prolyl Endopeptidase and Amyloid β-peptide in Brains of Senescence-accelerated mouse. *Neurosci. Lett*. **1994**, *176*, 201−204.

(6) Yoshimoto, T.; Kado, K.; Matsubara, F.; Koriyama, N.; Kaneto, H.; Tsuru, D. Specific Inhibitors for Prolyl Endopeptidase and their Anti-amnesic Effect. *J. Pharmacobio-Dyn*. **1987**, *10*, 730−735.

(7) Rennex, D.; Hemmings, B. A.; Hofsteenge, J.; Stone, S. R. cDNA Cloning of Porcine Brain Prolyl Endopeptidase and Identification of the Active-Site Seryl Residue. *Biochemistry* **1991**, *30*, 2195−2203.

(8) Higuchi, N. Inhibitors of Prolyl Endopeptidase. *Des. Enzyme Inhib. Drugs* **1994**, *2*, 766−779.

(9) Toda, M.; Ochuchida, S.; Ohno, H. Anti-amnesic Pyrrolidine or Thiazolidine Derivatives. European Patent EP 0345 428 A1, 1989.

(10) Saito, M.; Hashimoto, M.; Kawaguchi, N.; Shibata, H.; Fukami, H.; Tanaka, T.; Higuchi, N. Synthesis and inhibitory Activity of Acyl-peptidyl-pyrrolidine Derivatives toward post-proline cleaving enzyme; a study of subsite specificity. *J. Enzyme Inhib*. **1991**, *5*, 51.

(11) Yoshimoto, T.; Tsuru, D.; Yamamoto, N.; Ikezawa, R.; Furukawa, S. Structure Activity Relationship of Inhibitors specific for Prolyl Endopeptidase. *Agric. Biol. Chem*. **1991**, *1*, 37−43.

(12) Kobayashi, K.; Nishii, K.; Iwata, K.; Uchida, I. New proline derivatives inhibit prolyl endo peptidase for treatment and prevention of amnesia. European Patent EP 0468 469 A2.

(13) Arai, H.; Nishioka, H.; Niwa, S.; Yamanaka, T.; Tanaka, Y.; Yoshinaga, K.; Kobayashi, N.; Miura, N.; Ikeda, Y. Synthesis of Prolyl Endopeptidase Inhibitors and Evaluation of their Structure-Activity Relationship: in vitro Inhibition of Prolyl Endopeptidase from Canine Brain. *Chem. Pharm. Bull*. **1993**, *41*, 1583−1588.

(14) Vincent, M.; Rémond, G.; Portevin, B.; Serkiz, B.; Laubie, M. Stereoselective Synthesis of new Perhydroindole Derivative of Chiral Iminoacid, a potent Inhibitor of Angiotensin Converting Enzyme. *Tetrahedron Lett*. **1982**, *23*, 1677−1680.

(15) Vincent, M.; Pascard, C.; Césario, M.; Rémond, G.; Bouchet, J. P.; Charton, Y.; Laubie, M. Synthesis and Conformational Studies of Zabicipril (S 9650-3)a, potent Inhibitor of Angiotensin Converting Enzyme. *Tetrahedron Lett*. **1992**, *33*, 7369−7372. Vincent, M.; Rémond, G.; Laubie, M. Acides Aza Bicyclo Heptane Carboxyliques substitués, leur Préparation et leur Application en Thérapeutique. (Preparation and Therapeutic use of Substituted Aza bicycloheptane Carboxylic acids.) French Patent FR 2525604, 1983.

(16) Pearlman, W. M. 3-Isoquinuclidone. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 670−672.

(17) Newman, P. *Optical Resolution Procedures for chemical Compounds, Vol. 2, acids, Part 1*; Optical resolution Information Center: Riverdale, 1981; pp 458.

(18) Vallgarda, J.; Hacksell, U. Stereoselective Palladium-catalyzed Cyclopropanation of α,β-unsaturated Carboxylic Acids derivatized with Oppolzer's Sultam. *Tetrahedron Lett*. **1991**, *32*, 5625−5628.

(19) Beard, C.; Burger, A. 3-phenylcyclobutylamine. II. *J. Am. Chem. Soc*. **1962**, *27*, 1647−1650.

(20) Yoshimoto, T.; Tsuru, D. Proline Specific Endopeptidase EC-3.4.21 from *Flavobacterium*. *Agric. Biol. Chem*. **1978**, *42*, 2417−19.

JM950858C