Cholecystokinin Peptidomimetics as Selective CCK-B Antagonists: Design, Synthesis, and in Vitro and in Vivo Biochemical Properties

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Antagonists of cholecystokinin-B (CCK-B) receptors have been shown to alleviate CCK_4-induced panic attacks in humans and to potentiate opioid effects in animals. The clinical use of these compounds is critically dependent on their ability to cross the blood–brain barrier. In order to improve this property, new, peptoid-derived CCK-B antagonists, endowed with high affinity, selectivity, and increased lipophilicity have been developed. The affinity and selectivity of these compounds have been characterized in vitro and in vivo using guinea pig, rat, and mouse. Most of these compounds proved to be selective for the CCK-B receptor, the most potent analog, *N*-[*N*-[(2-adamantyloxy)carbonyl]-D-α-methyltryptophanyl]-N-[2-(4-chlorophenyl)ethyl]glycine (**26A**), having a K_i value of 6.1 nM for guinea pig cortex membranes in vitro and a good selectivity ratio (K_i CCK-A/K_i CCK-B = 174). Furthermore, the in vivo affinity of **26A** for mouse brain CCK-B receptors, following intracerebroventricular injection at different concentrations, was found to be 10 nmol. Using competition experiments with the specific CCK-B ligand [^{3}H]pBC 264, compound **26A** was shown to cross the blood–brain barrier (0.2%) after intraperitoneal administration in mice. This compound is therefore an interesting pharmacological tool to further elucidate the physiopathological role of endogenous CCK.

Introduction

The C-terminal fragment of CCK_8, ^{26}Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2, is present in large quantities in the brain where it interacts with selective binding sites.[1] One of these targets, designated the CCK-B receptor, has been recently cloned, sequenced, and shown to belong to the class of G-protein coupled receptors[2] as expected from the results of binding experiments.[3]

Considerable interest is devoted to the pharmacology of CCK-B receptors, since administration of selective agonists produces behavioral changes such as anxiety,[4] perturbation of memory,[5] and hyperalgesia,[6] and disfunctioning of CCK-B-related neural pathways could be involved in neuropsychiatric disorders.[7] Accordingly, CCK-B antagonists have been shown to block panic attacks[8] induced in humans by systemic administration of low doses of CCK_4,[9] Trp-Met-Asp-Phe-NH_2, which has a 300-fold higher affinity for the CCK-B receptor type (or subtype) than for the peripheral CCK-A receptor.[10] However, the mechanism of action of CCK_4, in particular, its primary site of action (peripheral or central) and its possible activation of a putative, selective high affinity binding subtype,[11] remains to be determined. CCK-B antagonists have also been shown to potentiate antinociceptive responses induced by exogenous substances such as morphine or endogenous opioids.[12-14] Given these findings, CCK-B antagonists have potential clinical interest.

The most potent and selective CCK-B antagonists reported to date are the benzodiazepine derivative, L-365,-260,[15] the peptoid PD 134308,[16] the ureidoacetamide RP 69758,[17] the diphenylpyrazolidinone LY 262691,[18] and the asperlicine-related quinazolinones.[19] However, one of the main problems encountered with most of these compounds is their low bioavailability.[20,21]

CCK-B receptor antagonist properties can be introduced in CCK peptides by reducing the sequence, as in Boc-Trp-Orn(Z)-Asp-NH_2,[22] or by introducing large and hindering residues as illustrated with Boc-Trp-Phg-Asp-1Nal-$N(CH_3)_2$[23] which behaves as a relatively potent (K_i = 38 nM), peptidase resistant and selective CCK-B antagonist.

A direct comparison of the structure of some of the CCK-B ligands Boc-Trp-Phe-Asp-NH_2 (compound 1) and the peptoids 2 and 3[16] showed that the size of the two latter molecules could be reduced to increase their lipophilicity as in compounds such as 4 (Figure 1). These compounds have been synthesized and some of them found to be potent and selective for CCK-B receptors. Moreover, as expected, one of them (compound **26A**) was shown more efficient in crossing the blood–brain barrier than the parent compounds.

Results

Chemistry. Schemes I–III summarize the synthetic pathways used to obtain the compounds listed in Table I. *N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-N-(2-phenylethyl)glycine (**4**) and its derivative [N-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]phenethylamine (**6**) were prepared as outlined in Scheme I, and compounds **4–14** and **20–27** were prepared in the same way using the appropriate reagents (Scheme I).

Intermediate acids **I** were prepared as described previously by Horwell et al.[16] with a slight modification of the purification procedure. Conversion of the intermediate acid **I** to compounds **6**, **9–14**, and **20–25** involved reaction with primary amines using BOP as a coupling reagent. The primary amines, if not commercially available, were prepared by quantitatively converting the cyano precursors through reduction with equimolar amounts of $LiAlH_4$ and $AlCl_3$, as reported by Nystrom.[24] The extraction procedure, however, was modified. Coupling of the acid *N*-[(2-

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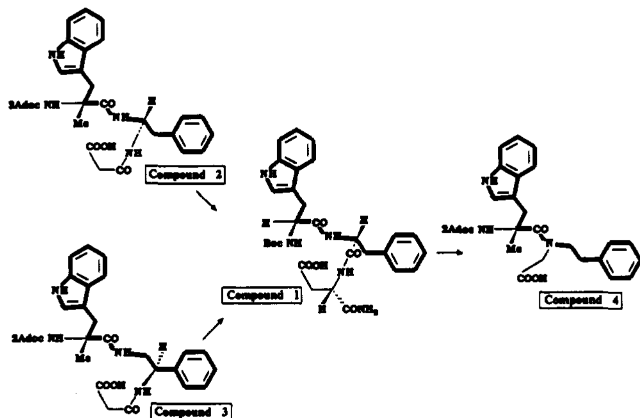
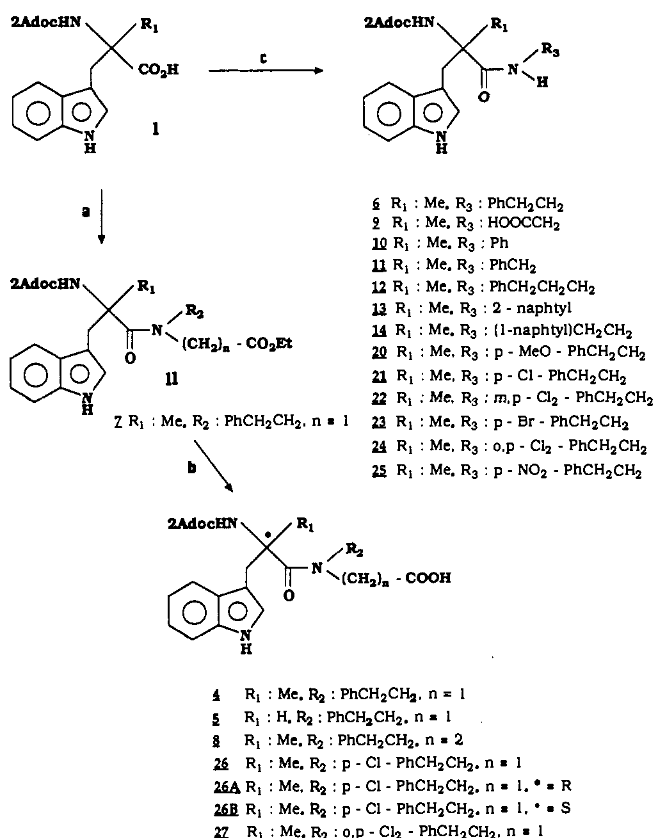


Figure 1. Rational design of compound 4 (RB 210) from compounds 1–3. The bold lines indicate homologous segments in the different structures.

Scheme I[a]

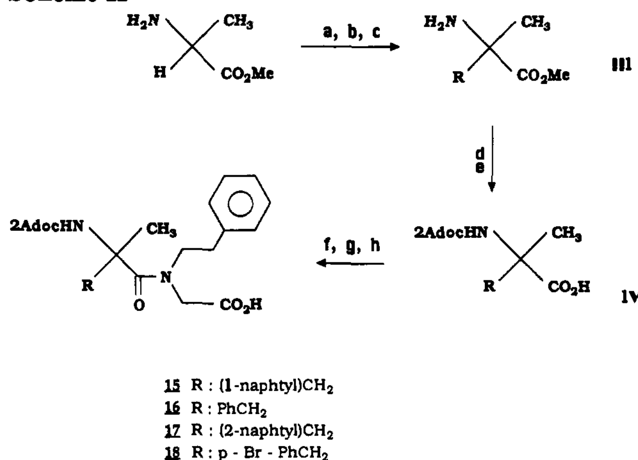


[a] Key (a) $R_2NH\ (CH_2)_nCO_2Et$, BOP, DIEA, DMF, 4–7 days; (b) $LiOH{\cdot}H_2O$, dioxane/H_2O/EtOH; (c) R_3NH_2, BOP, DIEA, DMF, 2–6 h.

adamantyloxy)carbonyl]-DL-α-methyltryptophan (I) (R_1 = CH_3) with secondary amines proved synthetically difficult as shown by a comparison of the reaction times and yields of products 4 and 6 (respectively, 7 days, 19%, and 2 h, 80%), Scheme I. An excess of secondary amine (3 equiv) was used to accelerate the product formation. The expected acid derivatives **4**, **5**, **8**, **26**, and **27** were obtained after hydrolysis of the corresponding esters **II** by treatment with LiOH in a mixture of H_2O/dioxane/EtOH. Secondary amines $RNH(CH_2)_nCO_2Et$ (n = 1 or 2) were prepared in good yields by alkylation of the corresponding primary amines with the appropriate halo ester.[25]

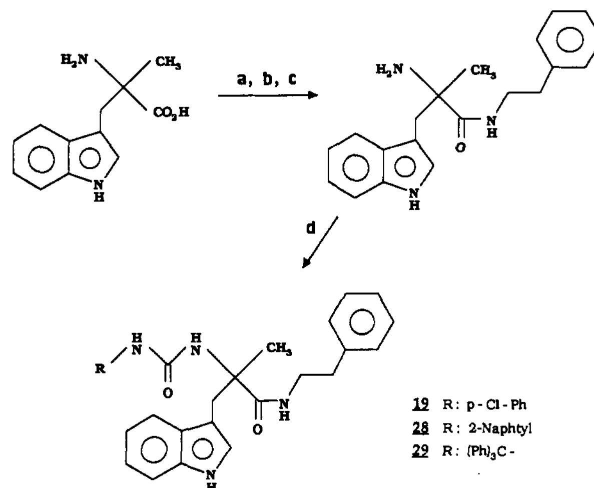
The separated enantiomers of α-methyltryptophan were used in the synthesis of compounds **26A** and **26B**, instead of the racemic mixture. Resolution of DL-α-methyltryptophan methyl ester was achieved by enzymatic hydrolysis of the L-isomer as previously described by Anantharamaiah et al.[26] HPLC with a chiral protein column, allowing the separation of the two enantiomers, was used to monitor the reaction.

Scheme II[a]



[a] Key (a) benzaldehyde, Et_3N, Na_2SO_4, CH_2Cl_2; (b) KOH, TEBA, RX, CH_2Cl_2; (c) HCl (1 N), Et_2O; (d) 2-Adoc-Cl, Et_3N, THF; (e) $LiOH{\cdot}H_2O$, H_2O/dioxane/EtOH; (f) DCC, THF; (g) $C_6H_5(CH_2)_2HNCH_2COOC_2H_5$, DMF, 3–5 days; (h) $LiOH{\cdot}H_2O$, dioxane/H_2O/EtOH.

Scheme III[a]



[a] Key (a) FmocOSu, DIEA, H_2O/CH_3CN; (b) $C_6H_5(CH_2)_2NH_2$, BOP, DIEA, DMF; (c) Piperidine, DMF; (d) RN=C=O, THF.

Preparation of compounds **15–18**, in which the indole moiety of compound **4** was replaced by other aromatic substituents, is outlined in Scheme II. Alanine methyl ester hydrochloride was subsequently converted to its alanine Schiff base through reaction with benzaldehyde and alkylated with 1-(bromomethyl)naphthalene, 2-(bromomethyl)naphthalene, or 4-bromobenzyl bromide under catalytic solid–liquid phase-transfer conditions.[27] α-Methyl amino acid methyl esters **III** were obtained after hydrolysis of the alkylated Schiff base product with dilute HCl.[28] Product **16** was derived from commercially available α-methylphenylalanine. The 2-Adoc N-blocked amino acids **IV**, which were obtained after classical amino protection and saponification procedures, were subsequently converted to their symmetrical anhydrides using DCC. The anhydrides were then isolated and reacted with *N*-(2-phenylethyl)glycine ethyl ester to give the desired final products **15–18** after treatment with an aqueous LiOH solution.

Table I. Potencies of CCK Ligands (4–29) in Inhibiting [^{3}H]pCCK$_8$ Specific Binding on Guinea Pig Brain and Pancreatic Membranes

compd	R_1	R_2	R_3	R_4	R_5	K_i,[a] nM brain(CCK-B)	pancreas (CCK-A)
4	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	$PhCH_2CH_2$	14 ± 0.6	1518 ± 150
5[b]	H	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	$PhCH_2CH_2$	224 ± 29	2842 ± 509
6	CH_3	2-Adoc	(3-indolyl)CH_2	H	$PhCH_2CH_2$	87 ± 16	1889 ± 690
7	CH_3	2-Adoc	(3-indolyl)CH_2	$CH_3CH_2OOCCH_2$	$PhCH_2CH_2$	543 ± 33	
8	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2CH_2$	$PhCH_2CH_2$	36 ± 8	1323 ± 63
9	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	H	986 ± 88	>4000
10	CH_3	2-Adoc	(3-indolyl)CH_2	H	Ph	407 ± 84	6082 ± 1487
11	CH_3	2-Adoc	(3-indolyl)CH_2	H	$PhCH_2$	231 ± 2	4097 ± 1188
12	CH_3	2-Adoc	(3-indolyl)CH_2	H	$PhCH_2CH_2CH_2$	182 ± 17	4249 ± 27
13	CH_3	2-Adoc	(3-indolyl)CH_2	H	2-naphthyl	402 ± 19	2356 ± 147
14	CH_3	2-Adoc	(3-indolyl)CH_2	H	(1-naphthyl)CH_2CH_2	89 ± 1.2	2367 ± 170
15	CH_3	2-Adoc	(1-naphthyl)CH_2	$HOOCCH_2$	$PhCH_2CH_2$	134 ± 19	2013 ± 164
16	CH_3	2-Adoc	$PhCH_2$	$HOOCCH_2$	$PhCH_2CH_2$	87 ± 11	3798 ± 574
17	CH_3	2-Adoc	(2-naphthyl)CH_2	$HOOCCH_2$	$PhCH_2CH_2$	70 ± 12	3006 ± 501
18	CH_3	2-Adoc	*p*-$BrPhCH_2$	$HOOCCH_2$	$PhCH_2CH_2$	50 ± 3	3414 ± 115
19	CH_3	*p*-ClPhNHCO	(3-indolyl)CH_2	H	$PhCH_2CH_2$	>10^{-5} M	6329 ± 156
20	CH_3	2-Adoc	(3-indolyl)CH_2	H	*p*-$MeOPhCH_2CH_2$	115 ± 17	3121 ± 79
21	CH_3	2-Adoc	(3-indolyl)CH_2	H	*p*-$ClPhCH_2CH_2$	39 ± 5	1614 ± 82
22	CH_3	2-Adoc	(3-indolyl)CH_2	H	*m,p*-$Cl_2PhCH_2CH_2$	184 ± 20	1540 ± 10
23	CH_3	2-Adoc	(3-indolyl)CH_2	H	*p*-$BrPhCH_2CH_2$	51 ± 3.8	2141 ± 130
24	CH_3	2-Adoc	(3-indolyl)CH_2	H	*o,p*-$Cl_2PhCH_2CH_2$	27 ± 1.5	3161 ± 158
25	CH_3	2-Adoc	(3-indolyl)CH_2	H	*p*-$NO_2PhCH_2CH_2$	79 ± 7.5	2352 ± 144
26	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	*p*-$ClPhCH_2CH_2$	6.5 ± 1.2	1129 ± 385
26A[b]	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	*p*-$ClPhCH_2CH_2$	6.1 ± 0.3	1060 ± 32
26B[c]	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	*p*-$ClPhCH_2CH_2$	36 ± 4.0	1039 ± 153
27	CH_3	2-Adoc	(3-indolyl)CH_2	$HOOCCH_2$	*o,p*-$Cl_2PhCH_2CH_2$	12.3 ± 1.4	3045 ± 155
28	CH_3	(2-naphthyl)NHCO	(3-indolyl)CH_2	H	$PhCH_2CH_2$	3478 ± 296	769 ± 173
29	CH_3	$(Ph)_3CNHCO$	(3-indolyl)CH_2	H	$PhCH_2CH_2$	20968 ± 7584	1777 ± 498

[a] The K_i values represent the means ±SEM of three separate experiments each performed in triplicate for determining CCK-B and CCK-A affinities on guinea pig cortex and pancreas membranes, respectively. [b] This compound has (*R*)-configuration at the α-carbon atom. [c] This compound has *S*-configuration at the α-carbon atom.

Ureas **19**, **28**, and **29** were obtained from DL-α-methyltryptophan as a starting compound (see Scheme III). Amino protection by Fmoc permitted coupling of the acid with 2-phenylethylamine as described by Horwell et al.[29] Deprotection of the amino group and coupling with 4-chlorophenyl isocyanate, 2-naphthyl isocyanate, or triphenylmethyl isocyanate, respectively, gave the desired final products **19**, **28**, and **29**. The isocyanates, if not commercially available, were obtained from their corresponding amines by treatment with bis(trichloromethyl) carbonate in either dichloromethane or nitrobenzene.

Receptor Binding and Structure–Activity Relationships of Compound 4 (RB 210) and Derivatives. The apparent affinities of the synthesized compounds for CCK-A and CCK-B binding sites were determined, using guinea pig pancreatic membranes and brain cortex membranes, respectively, by displacement of [^{3}H]pCCK$_8$, as described previously.[30] As shown in Table I, all the compounds exhibited a weak affinity for CCK-A sites.

We first investigated whether, in a compound as small as **4**, the α-CH_3 substituent was as important for receptor binding as in peptoids **2** and **3**, Figure 1.[16] In PD 134308, a compound closely related to **3**, the replacement of CH_3 by H was shown to induce a 245-fold reduction in CCK-B receptor binding affinity. The related decrease in binding energy ($\Delta\Delta G$ = 3.1 kcal/mol) was attributed to unfavorable entropy changes during the receptor binding process rather than to stabilization of the biologically active conformation.[31]

In our case, the loss of affinity for CCK-B receptors of **5** as compared to **4** is significantly lower (16-fold). This is probably due to the reduced number of bonds in **4** as compared to **3**, minimizing the energetically unfavorable reduction in the degree of freedom of the molecule during receptor binding.

CCK-B receptor affinity decreased 6-fold by omitting the acetic acid side chain in **4** to give **6** (respectively, 14 and 87 nM), whereas protecting the carboxylic acid moiety in **4** with an ethyl ester (**7**) dramatically reduced affinity 39 times (from 14 to 543 nM). These observations, suggesting that the acetic acid chain is an important CCK-B receptor binding component, are consistent with the literature. The presence of the carboxylic acid in peptoids was previously reported to enhance affinity (and selectivity) for CCK-B sites.[32] In order to further investigate the role of the acid group its position was changed by lengthening the aliphatic chain with one carbon, compound **8**. As this decreased the affinity 2.5-fold the original chain length was maintained for future compounds. The same functional relationship has been found previously for Asp[32] in CCK derivatives[33] and in peptoids.[16]

The importance of the 2-phenylethyl moiety is clearly illustrated by compound **9**, where its omission led to a drastic reduction in affinity for CCK-B receptors. Compounds **6** and **10–12** illustrate attempts to optimize the position of the phenyl ring in this series by changing the aliphatic chain length. Compound **10**, in which the phenyl ring was attached directly to the amide nitrogen, had poor CCK-B receptor binding affinity, whereas introducing an aliphatic chain of 1 and 2 carbons, respectively, increased affinity 2 and 4–5 times (**11**, **6**). However, compound **12**,

in which a propyl chain was introduced, had a 2-fold lower CCK-B receptor binding affinity than **6**.

An increase in the size of the aromatic moiety by introducing a 2-naphthyl group instead of the phenyl ring (**13**) resulted in a reduction in affinity, possibly due to an increase in unfavorable conformations for CCK-B receptor binding. The same explanation could be proposed for **10**.

Furthermore, the introduction of a 1-naphthyl moiety in **14**, in place of the phenyl ring of **6**, did not modify the affinity significantly. This suggests that the hydrophobic pocket, which binds the phenyl ring of **6**, is not limited to the size of just a phenyl ring.

To clarify the importance of the indole ring, we replaced it with 1-naphthyl (**15**), phenyl (**16**), 2-naphthyl (**17**), or 4-bromophenyl (**18**) moieties.[34] The CCK-B receptor affinities were 134, 87, 70, and 50 nM, respectively. Compound **18** was found to be only 3.5 times less active than **4**, indicating that the indole ring is not essential to obtain high potency and can be replaced by an appropriate substituent.

The (2-adamantyloxy)carbonyl group, assumed to fill the same CCK-B receptor subsite as the phenylurea moiety of L-365,260 ((3*R*)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N'*-(3-methylphenyl)urea) was replaced by a (4-chlorophenyl)urea group in **19**.

Surprisingly, this change produced a dramatic loss of affinity, as compared to **6**, since the IC_{50} value of **19** in competing with [^{3}H]pCCK$_8$ was in the order of 10^{-4} M. There are several possible explanations for this approximately 1000-fold decrease in affinity. The 2-Adoc group might fit better onto the receptor than the *p*-chlorophenyl moiety. Alternatively, there might be a reduction in the number of methylene groups in contact with the binding site, as already proposed to explain the reduced affinity of peptoids bearing a 1-Adoc in place of the 2-substituted group.[31] This proposal does not disagree with the increase in affinity observed with compounds bearing a 2-naphthylurea (**28**) or triphenylurea (**29**) when they are compared to **19** and with the good affinities of recently reported peptoids bearing 2-methylcyclohexyl substituents instead of the 2-adamantyl moieties.[35]

Another explanation could be that, due to its very large size, the 2-Adoc group eliminates a number of unfavorable conformations and promotes the biologically active conformer, thus reducing free energy expense during this receptor recognition process. Another possibility could be that the adamantyl group in the synthesized compounds and the phenylurea of the benzodiazepine L-365,260 do not fit into the same CCK-B receptor subsite.

Various substituents were introduced on the benzene ring of **6** in order to enhance biological activity. Initially, *p*-MeOPh and *p*-ClPh analogs (**20**, **21**) were synthesized as described by Horwell et al. for Amoc-α-Me-Trp-phenylethylamide derivatives.[29] As these compounds showed equal or even enhanced affinity, groups recommended by Topliss for benzene ring substitutions were introduced in compounds **22–25**.[36] This proved successful as both *p*-Cl and *o*,*p*-Cl$_2$ substituents resulted in 2–3-fold enhanced affinity as compared to compound **6**.

These latter groups were introduced into the best compound **4** to give **26** and **27**. The first had a potent affinity (K_i = 6.5 nM) and a favorable selectivity factor (174) for the CCK-B receptor of guinea pig. As expected from the literature, the *R*-isomer **26A** is more potent than the *S*-isomer **26B**.[16]

Table II. Apparent Affinities for CCK-B and CCK-A Binding Sites in Mouse and Rat

compd	K_i,^{a} nM		
	rat cortex	rat pancreas	mouse brain
RB 210 (**4**)	40.0 ± 4.7	531 ± 33	72.5 ± 12
compd **26**	18.3 ± 0.7	1359 ± 201	21.3 ± 3.3
RB 211 (**26A**)	20.9 ± 4.5	1232 ± 130	13.6 ± 1.6
compd **27**	28.0 ± 3.2	1224 ± 150	14.0 ± 1.9

^{a} Results are the mean ±SEM of three separate experiments each in triplicate. Experiments were performed with 0.2 nM [^{3}H]pBC 264 for CCK-B receptors and 0.1 nM [^{3}H]pCCK$_8$ for CCK-A receptors (pancreas).

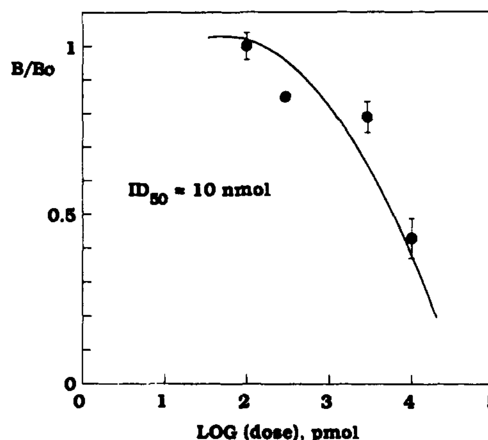


Figure 2. Inhibition of [^{3}H]pBC 264 specific binding to mouse brain after icv coinjection with RB 211 (**26A**). Each point is the mean of four determinations, and each was done in triplicate.

As differences have been reported in the pharmacological profiles of CCK-B receptors from various species,[37] binding studies were also performed on rat and mouse brain membranes using the highly potent and selective CCK-B agonist [^{3}H]pBC 264.[38]

Compounds **26**, **26A**, and **27** were shown to have better affinities and selectivities for CCK-B receptors than compound **4** in rat and mouse, as well as in guinea pig, brain (see Table II). In rat brain, these compounds showed affinities comparable with those reported for L-365,260 (11 nM) and PD 134308 (6 nM).[3]

The apparent in vivo affinity of **26A** (RB 211) in mouse brain was determined by measuring the displacement of [^{3}H]pBC 264 as previously described.[39] Inhibition of the specific binding of 10 pmol [^{3}H]pBC 264 by increasing concentrations of **26A** was studied 15 min after icv coinjection of both compounds. The apparent ID_{50} value, calculated from the competition curve (Figure 2) was 10 nmol.

Moreover, a significant inhibition (20 ± 5%) of in vivo [^{3}H]pBC 264 specific binding was also observed after ip injection of 20 mg/kg of compound **26A** (Figure 3), showing that this compound is able to enter the mouse brain and displace the selective CCK-B agonist from its binding site. The passage of **26A** across the blood–brain barrier was estimated to be around 0.2%. This was calculated, as previously described in detail,[39] by establishing the ratio of the doses administered either centrally (1.7 nmol) or peripherally (740 nmol) which produced the same percentage of inhibition (20%). Although the ID_{50} of L-365,260 could not be determined, due to its low solubility, it was estimated that 0.1% of the antagonist was present in the mouse brain 30 min after ip injection,[39] in good agreement with pharmacokinetic studies.[40] On the other hand, ip injection of 20 mg/kg of PD 134308 under the

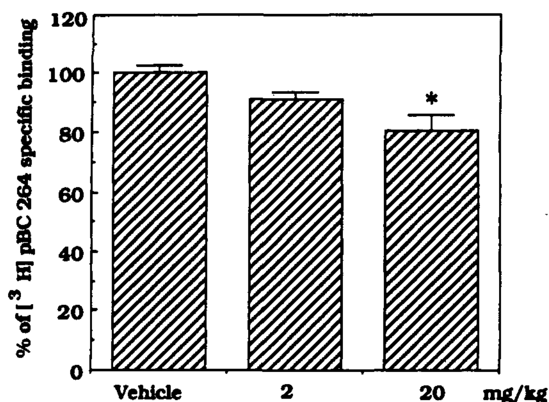


Figure 3. Inhibition of [^{3}H]pBC 264 specific binding to mouse brain after ip injection of RB 211 (**26A**). Values are the mean (±SEM) of six individual determinations, each done in triplicate. *$P < 0.05$ as compared to the controls in the student's T-test.

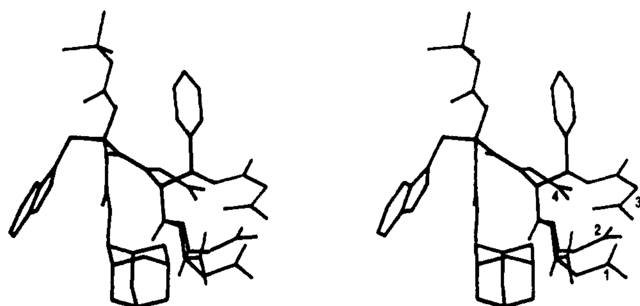


Figure 4. Stereoview of the superimposition of compounds **1–4** (minimized conformations).

same experimental conditions produced a slight but not significant reduction of in vivo [^{3}H]pBC 264 specific binding (not shown).

Discussion

The modified CCK_4 analog, Boc-Trp-Orn(Z)-Asp-NH_2, has been shown to behave as a CCK-A/CCK-B antagonist endowed with a better affinity for the peripheral receptor.[22] On the other hand, the introduction of a Phe^{31} residue was found to favor recognition of the CCK-B binding site.[41] Therefore, the modified peptide Boc-Trp-Phe-Asp-NH_2 (**1**), which contains two aromatic moieties, was selected as a starting model for comparison with the two peptoids **2** and **3** (Figure 1),[16] which have been elegantly designed by Horwell et al. by taking into account the structural characteristics of CCK peptides.[42,43]

Due to the presence of D-α-Me-Trp in the two latter compounds and a L-Trp in **1**, the alkoxycarbonyl groups did not overlap, but the superimposition of Phe and Trp aromatic rings of **1–3** showed that the free carboxyl groups, present in each compound, were spatially close (Figure 4).

Given these findings, the structures of **2** and **3** were simplified by coupling the [(2-adamantanyloxy)carbonyl]-DL-α-Me-Trp directly to the *N*-(2-phenylethyl)glycine leading to a short, relatively potent and selective compound **4** (RB 210) which retains a satisfactory spatial similarity with the peptoids **2** and **3** and compound **1** (Figure 4). It is interesting to note that the most active compounds in this new series of potent and selective, peptoid-derived CCK-B ligands, have a negatively charged carboxylate, a phenyl ring, an indole moiety, and a hydrophobic N-terminal residue, joined together on a short backbone. These features can be considered as important elements for CCK-B receptor recognition.

As compared to the parent molecules, compound **4** has an improved structural analogy with the Merck CCK-B antagonist L-365,260 (manuscript in preparation). This ligand, in which one of the chiral centers present in **2** and **3** was eliminated, has an enhanced nonpeptide character and therefore provides a useful tool to explore the structural and functional relationships between CCK antagonists belonging to three classes of molecules: pseudopeptides, peptides, and benzodiazepines.

Moreover, compound **26A** (RB 211) was shown to act as a potent and selective CCK-B ligand, capable of efficiently crossing the blood–brain barrier. This compound can therefore be considered as an interesting tool for further investigations of the physiopathological functions of brain CCK-B receptors.

Experimental Section

Synthesis. Commercial chemicals were used without further purification. Where appropriate, solvents were purified and dried by standard methods before use. All compounds in Table I were dried in vacuo over KOH and silica gel. Flash column chromatography was performed using Merck silica gel (230–400 mesh). Merck plates precoated with F254 silica gel were used for thin-layer chromatography with the following solvent systems (by volume): A, CH_2Cl_2/MeOH (50/1); B, CH_2Cl_2/MeOH (19/1); C, CH_2Cl_2/MeOH (9/1); D, CH_2Cl_2/MeOH (4/1); E, CH_2Cl_2/MeOH (2/1). Plates were developed with UV, iodine vapor, ninhydrin, or Ehrlich's reagent. Mass spectra were recorded on a quadrupole NERMAG R10-10 C apparatus. Elemental analyses, performed by Service Régional de Microanalyse (Paris, France) were within ±0.4% of the theoretical values unless noted otherwise. Melting points were performed on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brucker AC 270-MHz instrument in DMSO-d_6. Chemical shifts were measured in ppm with HMDSO as internal standard. The purity of all final compounds was checked by HPLC (Shimadzu apparatus) on a 250- × 4.6-mm Kromasil C8-5 μm column with a mixture of CH_3-CN and H_2O/$TFA_{0.05\%}$ (70/30) as eluent (flow rate 1.2 mL/min, UV detection 214 nm).

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-(2-phenylethyl)glycine (4).** *N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophan methyl ester was prepared as previously described.[16] The product was purified by flash column chromatography using a mixture of 1% MeOH in CH_2Cl_2 as the eluting solvent, yield 3.47 g (98%): R_f 0.86 (C); ^{1}H NMR (DMSO) δ 1.18–2.05 (17 H, m, adamantyl + α-CH_3), 3.07 (1 H, d, J = 14 Hz, β-CH_2), 3.33 (1 H, d, J = 14 Hz, β-CH_2), 3.52 (3 H, s, OCH_3), 4.64 (1 H, s, CHO), 6.82–7.40 (6 H, m, aro + amide NH), 10.90 (1H, s, indole NH).

The methyl ester (3.42 g, 8.33 mmol) was dissolved in a mixture of 60 mL of dioxane/H_2O/EtOH, and an aqueous solution of LiOH·H_2O (1.05 g, 25 mmol) was subsequently added. The reaction mixture was stirred overnight at room temperature. The solvents were then evaporated, and H_2O (30 mL) was added. The pH was adjusted to 3 with an aqueous 10% citric acid solution, and the product was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (pH = 3) and brine, dried with Na_2SO_4, filtered, and evaporated to yield 3.07 g of the acid (93%): R_f 0.34 (C); ^{1}H NMR (DMSO) δ 1.24 (3 H, s, α-CH_3), 1.45–2.05 (14 H, m, adamantyl), 3.11 (1 H, d, J = 14 Hz, β-CH_2), 3.31 (1 H, d, J = 14 Hz, β-CH_2), 4.65 (1 H, s, CHO), 6.65–7.45 (6 H, m, aro + amide NH), 10.89 (1H, s, indole NH), 12.32 (1 H, br s, COOH).

To a solution of *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan (400 mg, 1.01 mmol) and BOP (492 mg, 1.11 mmol) in 1 mL of dry DMF was added DIEA (261 mg, 2.02 mmol) at 0 °C. After the solution was stirred for 45 min at room temperature *N*-(2-phenylethyl)glycine ethyl ester (627 mg, 3.02 mmol), which was prepared as previously described,[25] was added to the solution. The reaction mixture was stirred for 7 days at room temperature, and then the solvent was removed in vacuo and EtOAc (10 mL) was added to the residue. The organic layer was washed with a 10% aqueous citric acid solution (3 × 10 mL)

and brine. The organic solution was dried over Na_2SO_4 and filtered. Evaporation of the solvent yielded a pale yellow oil which was chromatographed using a mixture of CH_2Cl_2/EtOAc (9/1, v/v) as the eluting solvent to give 110 mg of product (7) (19%): mp 195–204 °C; R_f 0.44 (B); MS (MH^+) 586; HPLC (t_R = 13.4 min); ^1H NMR (DMSO) δ 1.02–2.09 (20 H, m, adamantyl + α-CH_3 + CH_2CH_3), 2.40–4.49 (10 H, m, β-CH_2 + CH_2CH_2 + CH_2CO + CH_2CH_3), 4.60–4.91 (1 H, m, CHO), 6.78–7.70 (11 H, m, aro + amide NH), 10.91 (1H, s, indole NH).

The ethyl ester, *N*-[*N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-(2-phenylethyl)glycine ethyl ester (7) (107 mg, 0.183 mmol), was dissolved in a mixture of dioxane/H_2O/EtOH (15 mL), and an aqueous solution of LiOH·H_2O (15.3 mg, 0.366 mmol) was added. The reaction mixture was stirred overnight at room temperature, solvents were then evaporated, and the residue was redissolved in 10 mL of EtOAc, washed with an aqueous 10% citric acid solution and brine, dried over Na_2SO_4, and filtered. Evaporation of the organic solvent gave a white solid which was further purified by flash column chromatography (eluent, EtOAc/$CHCl_3$/MeOH/H_2O/AcOH (98/7/3/0.6/0.3, v/v/v/v/v)) to yield 95 mg of the final product (93%): mp 184–185 °C; R_f 0.53 (D); HPLC (t_R = 6.4 min); MS (MH^+) 558; ^1H NMR (DMSO) δ 1.00–2.10 (17 H, m, adamantyl + α-CH_3), 2.40–4.40 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.58–4.89 (1 H, m, CHO), 6.78–7.67 (11 H, m, aro + amide NH), 10.80–10.98 (1 H, m, indole NH). Anal. ($C_{33}H_{39}N_3O_5$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-D-tryptophanyl]-*N*-(2-phenylethyl)glycine (5).** Prepared from D-tryptophan methyl ester as in the synthesis of 4. *N*-[(2-Adamantyloxy)-carbonyl]-D-tryptophan was obtained in a 66% overall yield: R_f 0.15 (C); ^1H NMR (DMSO) δ 1.25–2.00 (14 H, m, adamantyl), 2.85–3.28 (2 H, m, β-CH_2), 4.18 (1 H, m, α-CH), 4.53 (1 H, s, CHO), 6.84–7.51 (6 H, m, aro + amide NH), 10.78 (1H, s, indole NH), 12.50 (1 H, br s, COOH).

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-D-tryptophanyl]-*N*-(2-phenylethyl)glycine ethyl ester** was subsequently prepared in 55% yield. The product was chromatographed by eluting with CH_2Cl_2/MeOH (9/1, v/v: R_f 0.67 (C); ^1H NMR (DMSO) δ 1.07–2.09 (17 H, m, adamantyl + CH_3), 2.40–4.82 (12 H, m, β-CH_2, + CH_2CH_2 + CH_2CO + CH_2CH_3 + α-CH + CHO), 6.78–7.65 (11 H, m, aro + amide NH), 10.91 (1H, s, indole NH).

The final product was obtained after saponification in 88% yield: mp 220–222 °C; R_f 0.74 (E); HPLC (t_R = 6.8 min); MS (MH^+) 544; ^1H NMR (DMSO) δ 1.03–2.00 (14 H, m, adamantyl), 2.40–3.60 (6 H, m, CH_2CH_2 + CH_2COOH), 3.74–4.00 (2 H, 2xdd, *J* = 14 Hz and *J* = 28 Hz, β-CH_2), 4.18–4.74 (2 H, m, CHO + α-CH), 6.68–7.54 (11 H, m, aro + amide NH), 10.66–10.87 (1 H, m, indole NH). Anal. ($C_{32}H_{37}N_3O_5$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-2-phenylethylamine (6).** *N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophan (65 mg, 0.16 mmol) and BOP (79 mg, 0.18 mmol) were dissolved in 1 mL of anhydrous DMF. DIEA (42 mg, 0.32 mmol) was added at 0 °C, the reaction mixture was stirred at ambient temperature for 45 min, and then 2-phenylethylamine (20 mg, 0.16 mmol) was added. The reaction mixture was treated after 2 h by evaporating the DMF and redissolving the residue in 10 mL of EtOAc. The organic phase was washed with a 10% citric acid solution (10 mL) and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residual solid was chromatographed (CH_2Cl_2/MeOH elution (100/2, v/v)) to yield 65 mg of white solid (80%): mp 98–101 °C; R_f 0.17 (A); mp 98–101 °C; HPLC (t_R = 9.3 min, CH_3CN and H_2O/$TFA_{0.05\%}$ (60/40)); MS (MH^+) 500; ^1H NMR (DMSO) δ 1.18–2.10 (17 H, m, adamantyl + α-CH_3), 2.62 (2 H, t, *J* = 7 Hz, CH_2), 3.25 (1 H, d, *J* = 14 Hz, β-CH_2), 3.42 (1 H, d, *J* = 14 Hz, β-CH_2), 4.68 (1 H, s, CHO), 6.59 (1 H, br s, urethane NH), 6.80–7.49 (10 H, m, aro), 7.74 (1 H, s, $NHCH_2$), 10.80 (1 H, s, indole NH). Anal. ($C_{31}H_{37}N_3O_3$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-(2-phenylethyl)-3-aminopropionic Acid (8).** Prepared as previously described in the synthesis of 4. Ethyl [*N*-(2-phenylethyl)-3-amino]propionate was prepared from ethyl 3-bromopropionate and 2-phenylethylamine in a 60% yield. The product was purified by vacuum distillation (bp 129 °C/1.0 mmHg) to yield a pale yield oil: ^1H NMR (DMSO) δ 1.10 (3 H, t, *J* = 7 Hz, CH_3), 2.35 (2 H, t, *J* = 7 Hz, CH_2CO), 2.55–2.82 (6 H, m, $ArCH_2CH_2$ + $NHCH_2$), 3.98 (2 H, q, *J* = 7 Hz, CH_2CH_3), 7.03–7.42 (5 H, m, aro).

Ethyl *N*-[*N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-(2-phenylethyl)-3-aminopropionate was obtained in a 48% yield. The product was chromatographed using CH_2Cl_2/MeOH (19/1, v/v) as eluent: R_f 0.75 (B); ^1H NMR (DMSO) δ 1.03–2.10 (20 H, m, adamantyl + α-CH_3 + CH_2CH_3), 2.39–4.08 (12 H, m, β-CH_2 + $ArCH_2CH_2$ + CH_2CH_2CO + CH_2CH_3), 4.61–4.90 (1 H, m, CHO), 6.80–7.72 (10 H, m, aro + amide NH), 10.92 (1H, s, indole NH).

Saponification gave the final product in 80% yield: mp 201–203 °C; R_f 0.73 (D); HPLC (t_R = 5.9 min); MS (MH^+) 572; ^1H NMR (DMSO) δ 1.04–2.07 (17 H, m, adamantyl + α-CH_3), 2.40–3.98 (10 H, m, β-CH_2 + CH_2CH_2 + CH_2CH_2COOH), 4.60–4.88 (1 H, m, CHO), 6.80–7.59 (11 H, m, aro + amide NH), 10.88 (1 H, s, indole NH). Anal. ($C_{33}H_{41}N_3O_5$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]glycine (9).** Prepared from *N*-[(2-adamantyloxy)-carbonyl]-DL-α-methyltryptophan and glycine as for 6 in a 98% yield using CH_2Cl_2/MeOH/AcOH (100/2/3, v/v/v) as the eluting solvent for chromatography: mp 176–179 °C; R_f 0.61 (E); MS (MH^+) 554; HPLC (t_R = 7.9 min, CH_3CN and H_2O/$TFA_{0.05\%}$ (60/40) as eluent (0.8 mL/min); ^1H NMR (DMSO) δ 1.10–2.00 (17 H, m, adamantyl + α-CH_3), 3.13 (1 H, d, *J* = 14 Hz, β-CH_2), 3.42 (1 H, d, *J* = 14 Hz, β-CH_2), 3.58–3.82 (2 H, m, CH_2), 4.62 (1 H, s, CHO), 6.68 (1 H, br s, OCONH), 6.75–7.50 (5 H, m, aro), 7.98 (1 H, s, $NHCH_2$), 10.84 (1 H, s, indole NH); Anal. ($C_{25}H_{31}N_3O_5$·0.3H_2O) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]aniline (10).** Prepared from *N*-[(2-adamantyloxy)-carbonyl]-DL-α-methyltryptophan and aniline as described for 6. The residual solid was chromatographed (CH_2Cl_2/MeOH elution (50/1, v/v)) to yield 212 mg of product (90%): mp 174–176 °C; R_f 0.78 (C); MS (MH^+) 572; HPLC (t_R = 9.0 min); ^1H NMR (DMSO) δ 1.16–2.05 (17 H, m, adamantyl + α-CH_3), 3.25 (1 H, d, *J* = 14 Hz, β-CH_2), 3.42 (1 H, d, *J* = 14 Hz, β-CH_2), 4.64 (1 H, s, CHO), 6.50–7.60 (11 H, m, aro + urethane NH), 9.55 (1 H, s, ArNH) 10.84 (1 H, s, indole NH). Anal. ($C_{29}H_{33}N_3O_3$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]benzylamine (11). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and benzylamine as for 6 in a 98% yield using CH_2Cl_2/MeOH (100/1, v/v) as the eluting solvent for chromatography: mp 180–182 °C; R_f 0.70 (C); MS (MH^+) 586; HPLC (t_R = 8.3 min); ^1H NMR (DMSO) δ 1.18–2.05 (17 H, m, adamantyl + α-CH_3), 3.19 (1 H, d, *J* = 14 Hz, β-CH_2), 3.37 (1 H, d, *J* = 14 Hz, β-CH_2), 4.14–4.34 (2 H, m, $ArCH_2$) 4.66 (1 H, s, CHO), 6.68 (1 H, s, urethane NH), 6.80–7.46 (10 H, m, aro), 8.22 (1 H, t, *J* = 7 Hz, $NHCH_2$) 10.82 (1 H, s, indole NH). Anal. ($C_{30}H_{35}N_3O_3$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl](3-phenylpropyl)amine (12). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 3-phenylpropylamine as for 6 in a 93% yield using CH_2Cl_2/MeOH (100/1, v/v) as the eluting solvent for chromatography: mp 175 °C; R_f 0.69 (C); MS (MH^+) 514; HPLC (t_R = 10.6 min); ^1H NMR (DMSO) δ 1.13–2.01 (19 H, m, adamantyl + α-CH_3 + CH_2), 2.40–2.58 (2H, CH_2), 2.86–3.49 (4 H, m, β-CH_2 + CH_2), 4.66 (1 H, s, CHO), 6.66 (1 H, br s, urethane NH), 6.80–7.46 (10 H, m, aro), 7.73 (1 H, s, $NHCH_2$) 10.81 (1 H, s, indole NH). Anal. ($C_{32}H_{39}N_3O_3$·0.3H_2O) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl](2-naphthyl)amine (13). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-naphthylamine as for 6 in a 20% yield using CH_2Cl_2/MeOH (100/1, v/v) as the eluting solvent for chromatography: mp 192–194 °C; R_f 0.79 (C); MS (MH^+) 522; HPLC (t_R = 12.3 min); ^1H NMR (DMSO) δ 1.12–2.10 (17 H, m, adamantyl + α-CH_3), 3.29 (1 H, d, *J* = 14 Hz, β-CH_2), 3.47 (1 H, d, *J* = 14 Hz, β-CH_2) 4.63 (1 H, s, CHO), 6.50–7.92 (12 H, m, aro), 8.20 (1 H, s, urethane NH), 9.78 (1 H, s, ArNH), 10.87 (1 H, s, indole NH). Anal. ($C_{33}H_{35}N_3O_3$) C, H, N. N: calcd, 8.05; found, 7.63.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(1-naphthyl)ethyl]amine (14). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(1-naphthyl)ethylamine as for 6. 2-(1-Naphthyl)ethylamine was

quantitatively prepared from 1-naphthylacetonitrile (3.00 g, 18 mmol) as described in the literature.[24] After completion of the reaction, water was added to decompose the excess hydride, and the pH was adjusted to 1 with concentrated HCl. The resulting precipitate of gray salts was filtered, washed with 25 mL of CH_2Cl_2, and resuspended in 30 mL of water and 30 mL of CH_2Cl_2. The pH was adjusted to 14 by addition of KOH, and the separated water layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, filtered, and evaporated to yield 3.06 g of a pale yellow oil (100%): ^{1}H NMR (DMSO) δ 2.80 (2 H, t, J = 7 Hz, CH_2), 3.07 (2 H, t, J = 7 Hz, CH_2), 7.28–8.13 (7 H, m, aro).

The final product (99% yield) was chromatographed using CH_2Cl_2/EtOAc (19/1 v/v) as the eluting solvent: mp 172–174 °C; R_f 0.71 (C); MS (MH^+) 550; HPLC (t_R = 12.9 min); ^{1}H NMR (DMSO) δ 1.13–2.04 (17 H, m, adamantyl + α-CH_3), 2.94–3.50 (6 H, m, β-CH_2 + CH_2CH_2), 4.66 (1 H, s, CHO), 6.68 (1 H, s, urethane NH), 6.80–8.24 (13 H, m, aro + N*H*CH_2), 10.84 (1 H, s, indole NH); Anal. ($C_{35}H_{39}N_3O_3$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyl-3-(1-naphthyl)alaninyl]-*N*-(2-phenylethyl)glycine (15).** To a suspension of alanine methyl ester hydrochloride (5.00 g, 36.0 mmol), Na_2SO_4 (3.00 g, 21.1 mmol), and Et_3N (7.29 g, 72.0 mmol) in 80 mL CH_2Cl_2 was added benzaldehyde (3.82 g, 36.0 mmol). The reaction mixture was stirred for 18 h at room temperature. The residue which was obtained after filtration and solvent removal was redissolved in 100 mL of ether, washed with water (3 × 20 mL) and brine, dried over Na_2SO_4, and filtered. Solvent removal yielded 6.68 g of benzylidene-α-methylglycine methyl ester as an oil (98%).

To a suspension of powdered KOH (2.05 g, 36.6 mmol) and TEBA (417 mg, 3.66 mmol) in 20 mL of CH_2Cl_2 at 0 °C was added dropwise a solution of the imine (3.50 g, 18.3 mmol) and 1-(bromomethyl)naphthalene (4.05 g, 18.3 mmol) in 20 mL of CH_2Cl_2. The mixture was stirred at 10–15 °C for 3 h, after which 70 mL of HCl (1 N) and 20 mL of ether were added, and stirring was continued at 0 °C for 1 h. Organic solvents were evaporated, and the aqueous layer was washed with ether (3 × 20 mL). The pH was adjusted to 9 with a cold 10% $NaHCO_3$ solution, and the product was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to yield 3.18 g of DL-α-methyl-3-(1-naphthyl)alanine methyl ester as an oil (75%): R_f 0.60 (C); ^{1}H NMR (DMSO) δ 1.25 (3 H, s, α-CH_3), 1.80 (2 H, br s, NH_2), 3.24 (1 H, d, J = 14 Hz, β-CH_2), 3.35 (1 H, d, J = 14 Hz, β-CH_2), 3.45 (3 H, s, OCH_3), 7.20–8.15 (7 H, m, aro).

DL-α-Methyl-3-(1-naphthyl)alanine methyl ester (3.10 g, 13.4 mmol) was reacted with (2-adamantyloxy)carbonyl chloride in THF in the presence of Et_3N and subsequently treated with an aqueous LiOH solution as described above in the synthesis of 4 to give *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyl-3-(1-naphthyl)alanine, overall yield 4.22 g (73%): R_f 0.47 (C); ^{1}H NMR (DMSO) δ 1.17 (3 H, s, α-CH_3), 1.36–2.12 (14 H, m, adamantyl), 3.46 (1 H, d, J = 14 Hz, β-CH_2), 3.78 (1 H, d, J = 14 Hz, β-CH_2), 4.65 (1 H, s, CHO), 6.95 (1 H, s, amide NH), 7.18–8.10 (7 H, m, aro), 12.40 (1H, br s, COOH).

To an anhydrous 40-mL THF solution of *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyl-3-(1-naphthyl)alanine (4.22 g, 10.7 mmol) was added DCC (1.21 g, 5.86 mmol). The reaction mixture was stirred for 2 h at room temperature and filtered and the solvent evaporated to yield 3.96 g of symmetrical anhydride (96%).

To a solution of the symmetrical anhydride (1.00 g, 1.29 mmol) in 1 mL of dry DMF was added *N*-(2-phenylethyl)glycine ethyl ester (805 mg, 3.90 mmol). The reaction mixture was stirred at room temperature for 72 h, the solvent was removed in vacuo, and EtOAc (10 mL) was added to the residue. The organic layer was washed with a 10% aqueous citric acid solution (3 × 10 mL) and brine, and the organic solution was dried over Na_2SO_4 and filtered. Evaporation of the solvent yielded a pale yellow oil which was chromatographed using 100% $CHCl_3$ as the eluting solvent to give 635 mg of product (84%) which was subsequently treated with an aqueous LiOH solution as described above in the synthesis of 4, yield 370 mg (53%): mp 150–152 °C; R_f 0.69 (D); MS (MH^+) 569; HPLC (t_R = 11.1 min); ^{1}H NMR (DMSO): δ 1.00–2.16 (17 H, m, adamantyl + α-CH_3), 2.40–4.50 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.58–5.00 (1 H, m, CHO), 6.69–8.14 (13 H, m, aro + amide NH). Anal. ($C_{35}H_{40}N_2O_5$·0.6H_2O) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methylphenylalaninyl]-*N*-(2-phenylethyl)glycine (16).** *N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyl-3-phenylalanine was prepared from DL-α-methylphenylalanine (1.79 g, 10 mmol) by aminoprotection with (2-adamantyloxy)carbonyl chloride (2.57, 12 mmol) as for 4 to yield 1.30 g (38%) of a white solid after flash column chromatography using CH_2Cl_2/MeOH (100/2) as the eluting solvent: R_f 0.28 (A); ^{1}H NMR (DMSO) δ 1.18 (3 H, s, α-CH_3) 1.30–2.10 (14 H, m, adamantyl), 2.28 (1 H, d, J = 14 Hz, β-CH_2), 3.27 (1 H, d, J = 14 Hz, β-CH_2), 4.58 (1 H, s, CHO), 6.70–7.30 (6 H, m, aro + urethane NH).

The final product was obtained as in the synthesis of 15 with a 28% overall yield (251 mg): mp 123–125 °C; R_f 0.40 ($CHCl_3$/MeOH/H_2O/AcOH/EtOAc = 7/3/0.6/0.3/98); MS (MH^+) 519; HPLC (t_R = 8.0 min); ^{1}H NMR (DMSO): δ 1.20 (3 H, s, α-CH_3) 1.30–2.20 (14 H, m, adamantyl), 2.40–4.20 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.60–4.90 (1 H, m, CHO), 6.95–7.35 (6 H, m, aro + urethane NH). Anal. ($C_{31}H_{33}N_2O_5$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyl-3-(2-naphthyl)alaninyl]-*N*-(2-phenylethyl)glycine (17).** Prepared as in the synthesis of 15. DL-α-Methyl-3-(2-naphthyl)alanine methyl ester was prepared from the alanine Schiff base and 2-(bromomethyl)naphthalene with a 78% yield as for 15: R_f 0.63 (C); ^{1}H NMR (DMSO) δ 1.22 (3 H, s, α-CH_3), 1.89 (2 H, br s, NH_2), 2.88 (1 H, d, J = 14 Hz, β-CH_2), 3.02 (1 H, d, J = 14 Hz, β-CH_2), 3.56 (3 H, s, OCH_3), 7.18–7.87 (7 H, m, aro).

N-[(2-Adamantyloxy)carbonyl]-DL-α-methyl-3-(2-naphthyl)alanine was prepared as for 4 to yield 490 mg of a white solid (71%): R_f 0.45 (C); ^{1}H NMR (DMSO) δ 1.22 (3 H, s, α-CH_3), 1.39–2.09 (14 H, m, adamantyl), 3.10 (1 H, d, J = 14 Hz, β-CH_2), 3.45 (1 H, d, J = 14 Hz, β-CH_2), 4.68 (1 H, s, CHO), 6.93 (1 H, s, amide NH), 7.15–7.90 (7 H, m, aro), 12.55 (1H, br s, COOH).

The final product was subsequently obtained in a 19% yield (140 mg): mp 163–164 °C; R_f 0.68 (D); HPLC (t_R = 11.7 min); MS (MH^+) 569; ^{1}H NMR (DMSO): δ 1.00–2.20 (17 H, m, adamantyl + α-CH_3), 2.40–4.48 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.61–4.96 (1 H, m, CHO), 6.84–7.90 (13 H, m, aro + amide NH). Anal. ($C_{35}H_{40}N_2O_5$·1H_2O) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyl-3-(4-bromophenyl)alaninyl]-*N*-(2-phenylethyl)glycine (18).** Prepared as in the synthesis of 15. DL-α-Methyl-3-(4-bromophenyl)alanine methyl ester was prepared from the alanine Schiff base and 4-bromobenzyl bromide in a 65% yield as for 15: R_f 0.63 (C); ^{1}H NMR (DMSO) δ 1.27 (3 H, s, α-CH_3), 2.69 (1 H, d, J = 14 Hz, β-CH_2), 2.72 (1 H, d, J = 14 Hz, β-CH_2), 3.54 (3 H, s, OCH_3), 7.05 (2 H, d, J = 7 Hz, aro), 7.41 (2 H, d, J = 7 Hz, aro).

N-[(2-Adamantyloxy)carbonyl]-DL-α-methyl-3-(4-bromophenyl)alanine was prepared as for 4 to yield 4.49 g (90%): ^{1}H NMR (DMSO) δ 1.17 (3 H, s, α-CH_3), 1.34–2.05 (14 H, m, adamantyl), 2.90 (1 H, d, J = 14 Hz, β-CH_2), 2.90 (1 H, d, J = 14 Hz, β-CH_2), 4.60 (1 H, s, CHO), 6.87–7.08 (3 H, m, aro + amide NH), 7.40 (2 H, d, J = 7 Hz, aro).

The final product was subsequently obtained as in the synthesis of 15 in 7% yield (47 mg): mp 180–183 °C; R_f 0.65 (D); HPLC (t_R = 12.0 min); MS (MH^+) 597; ^{1}H NMR (DMSO) δ 1.00–2.09 (17 H, m, adamantyl + α-CH_3), 2.40–4.41 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.55–4.94 (1 H, m, CHO), 6.80–7.70 (10 H, m, aro + amide NH). Anal. ($C_{31}H_{37}N_2O_5Br$) C, H, N.

[*N*-(4-Chlorophenyl)-DL-α-methyltryptophanyl][(2-phenylethyl)amino]urea (19). To a solution of α-methyltryotphan (2.36 g, 10 mmol) in a mixture of 50 mL of H_2O/CH_3CN (1/1 v/v) was added FmocOSu (3.21 g, 9.5 mmol), and the pH was adjusted to 8.5 with DIEA. After 4.5 h at room temperature, the organic solvent was evaporated, and 25 mL of H_2O and 75 mL of EtOAc were added. The pH was adjusted to 3 with a $KHSO_4$ (1 M) solution, and the organic layer was washed with $KHSO_4$ solution (4 × 30 mL), water (2 × 30 mL), and brine, dried over Na_2SO_4, and filtered to yield 4.05 g of *N*-(Fmoc)-DL-α-methyltryptophan as a white solid (92%): mp 176–178 °C; R_f 0.35 (C); ^{1}H NMR (DMSO) δ 1.23 (3 H, s, α-CH_3), 3.09 (1 H, d, J = 14 Hz, β-CH_2), 3.40 (1 H, d, J= 14 Hz, β-CH_2), 4.11–4.40 (3 H, m, $CHCH_2O$), 6.80–7.90 (14 H, m, aro + amide NH), 10.84 (1 H, s, indole NH).

This acid (1.16 g, 2.63 mmol) was reacted with 2-phenylethylamine (319 mg, 2.63 mmol) as described above for compound 6 to yield 881 mg of [*N*-(Fmoc)-α-methyltryptophanyl](2-phenylethyl)amine (62%). Flash column chromatography was performed using CH_2Cl_2/MeOH (100/1, v/v, and 100/2, v/v, respectively) as the eluting solvent: mp 90–92 °C; R_f 0.22 (A); ^{1}H NMR (DMSO) δ 1.06 (3 H, s, α-CH_3), 2.59 (2 H, m, CH_2), 3.00–3.50 (4 H, m, β-CH_2 + NHCH_2), 4.06–4.37 (3 H, m, CHCH_2O), 6.73–7.99 (15 H, m, aro + amide NH), 10.78 (1 H, s, indole NH).

[DL-α-Methyltryptophanyl](2-phenylethyl)amine was obtained from [*N*-(Fmoc)-α-methyltryptophanyl](2-phenylethyl)amine (868 mg, 1.59 mmol) as described by Horwell et al.[29] after flash column chromatography with a mixture of CH_2Cl_2/MeOH (100/2, v/v) to yield 451 mg of product (88%): mp 119–121 °C; R_f 0.33 (A); ^{1}H NMR (DMSO) δ 1.16 (3 H, s, α-CH_3), 1.63 (2 H, br s, NH_2), 2.47 (2 H, m, CH_2), 2.68 (1 H, d, *J* = 14 Hz, β-CH_2), 3.13 (1 H, d, *J* = 14 Hz, β-CH_2), 3.29 (2 H, m, NHCH_2), 6.84–7.56 (11 H, m, aro), 7.70 (1 H, t, amide NH), 10.81 (1 H, s, indole NH).

A solution of this amine (118 mg, 0.368 mmol) in 2 mL of THF was treated with 4-chlorophenyl isocyanate (57 mg, 0.368 mmol) at 0 °C. The organic solvent was removed after being stirred for 1 h at room temperature and the residue chromatographed with a mixture of CH_2Cl_2/MeOH (50/1, v/v) to yield 173 mg of the final product (99%): mp 111–113 °C; R_f 0.66 (C); HPLC (t_R = 9.2 min, CH_3CN and $H_2O/TFA_{0.05\%}$ (60/40), 1 mL/min); MS (MH^+) 575; ^{1}H NMR (DMSO) δ 1.40 (3 H, s, α-CH_3), 2.62 (2 H, t, *J* = 7 Hz, ArCH_2), 3.10–3.48 (4 H, m, β-CH_2 + CH_2), 6.30 (1 H, s, ArNHCON*H*), 6.72–7.50 (14 H, m, aro), 7.93 (1 H, t, *J* = 7 Hz, N*H*CH_2), 8.87 (1 H, s, ArNH), 10.79 (1 H, s, indole NH). Anal. ($C_{27}H_{27}N_4O_2Cl{\cdot}0.3H_2O$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(4-methoxyphenyl)ethyl]amine (20). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(4-methoxyphenyl)ethylamine as for 6 in 91% yield using CH_2Cl_2/MeOH (50/1, v/v) as the eluting solvent for chromatography: mp 184–187 °C; R_f 0.69 (A); HPLC (t_R = 8.2 min); MS (MH^+) 530; ^{1}H NMR (DMSO) δ 1.25 (3 H, α-CH_3), 1.34–2.00 (14 H, m, adamantyl), 2.50–2.62 (2 H, m, CH_2), 3.00–3.48 (4 H, m, β-CH_2 + CH_2), 3.65 (3 H, s, OCH_3), 4.64 (1 H, s, CHO), 6.58 (1 H, s, urethane NH), 6.70–7.46 (9 H, m, aro), 7.72 (1 H, s, N*H*CH_2), 10.82 (1 H, s, indole NH). Anal. ($C_{32}H_{39}N_3O_4{\cdot}0.3H_2O$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(4-chlorophenyl)ethyl]amine (21). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(4-chlorophenyl)ethylamine as for 6 in a 60% yield using CH_2Cl_2/MeOH (50/1, v/v) as the eluting solvent for chromatography: mp 188–191 °C; R_f 0.73 (C); HPLC (t_R = 11.7 min); MS (MH^+) 534; ^{1}H NMR (DMSO) δ 1.21 (3 H, s, α-CH_3), 1.34–2.05 (14 H, m, adamantyl), 2.50–2.80 (2 H, m, CH_2) 3.05–3.40 (4 H, m, β-CH_2 + CH_2), 4.65 (1 H, s, CHO), 6.52 (1 H, s, urethane NH), 6.80–7.46 (9 H, m, aro), 7.75 (1 H, s, N*H*CH_2), 10.84 (1 H, s, indole NH). Anal. ($C_{31}H_{36}N_3O_3Cl$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(3,4-dichlorophenyl)ethyl]amine (22). The reduction of 3,4-dichlorobenzyl cyanide (4.00 g. 21.5 mmol) with $AlCl_3$ (3.15 g, 23.7 mmol) and $LiAlH_4$ (900 mg, 23.7 mmol) was effected as described above in the synthesis of 14 to yield 4.00 g of a pale yellow oil (98%): ^{1}H NMR (DMSO) δ 2.58 (2 H, t, *J* = 7 Hz, CH_2), 2.65 (2 H, br s, NH_2), 2.72 (2 H, t, *J* = 7 Hz, CH_2), 7.15 (1 H, d, *J* = 7 Hz, aro), 7.36–7.58 (2 H, m, aro).

The final product was subsequently prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(3,4-dichlorophenyl)ethylamine as in the synthesis of 6 in 100% yield using CH_2Cl_2/EtOAc (19/1, v/v) as the eluting solvent for chromatography: mp 113–116 °C; R_f 0.73 (C); HPLC (t_R = 14.9 min); MS (MH^+) 568; ^{1}H NMR (DMSO) δ 1.21 (3 H, s, α-CH_3), 1.34–2.02 (14 H, m, adamantyl), 2.54–2.72 (2 H, m, CH_2), 3.00–3.46 (4 H, m, β-CH_2 + CH_2), 4.62 (1 H, s, CHO), 6.61 (1 H, s, urethane NH), 6.78–7.50 (8 H, m, aro), 7.74 (1 H, s, N*H*CH_2) 10.84 (1 H, s, indole NH). Anal. ($C_{31}H_{35}N_3O_3Cl_2{\cdot}0.3H_2O$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(4-bromophenyl)ethyl]amine (23). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(4-bromophenyl)ethylamine as for 6 in 84% yield using CH_2Cl_2/EtOAc (19/1, v/v) as the eluting solvent for chromatography: mp 181–182 °C; R_f 0.71 (C); HPLC (t_R = 12.6 min); MS (MH^+) 578; ^{1}H NMR (DMSO) δ 1.21 (3 H, s, α-CH_3), 1.34–2.07 (14 H, m, adamantyl), 2.59 (2 H, t, *J* = 7 Hz, CH_2), 3.02–3.45 (4 H, m, β-CH_2 + CH_2), 4.62 (1 H, s, CHO), 6.63 (1 H, s, OCONH), 6.72–7.50 (8 H, m, aro), 7.75 (1 H, s, N*H*CH_2), 10.86 (1 H, s, indole NH). Anal. ($C_{31}H_{36}N_3O_3Br$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(2,4-dichlorophenyl)ethyl]amine (24). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(2,4-dichlorophenyl)ethylamine as for 6 with a 80% yield using CH_2Cl_2/EtOAc (14/1, v/v) as the eluting solvent for chromatography: mp 113–116 °C; R_f 0.75 (C); HPLC (t_R = 16.3 min); MS (MH^+) 568; ^{1}H NMR (DMSO) δ 1.24 (3 H, s, α-CH_3), 1.35–2.05 (14 H, m, adamantyl), 2.74 (2 H, t, *J* = 7 Hz CH_2), 3.03–3.44 (4 H, m, β-CH_2 + CH_2), 4.61 (1 H, s, CHO), 6.62 (1 H, s, urethane NH), 6.76–7.62 (8 H, m, aro), 7.82 (1 H, s, N*H*CH_2), 10.84 (1 H, s, indole NH). Anal. ($C_{31}H_{35}N_3O_3Cl_2$) C, H, N.

[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl][2-(4-nitrophenyl)ethyl]amine (25). Prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and 2-(4-nitrophenyl)ethylamine as in the synthesis of 6 with a 72% yield using CH_2Cl_2/EtOAc (19/1, v/v) as the eluting solvent for chromatography: mp 174–176 °C; R_f 0.76 (C); HPLC (t_R = 7.8 min); MS (MH^+) 545; ^{1}H NMR (DMSO) δ 1.00–2.00 (17 H, m, adamantyl + α-CH_3), 2.83 (2 H, t, *J* = 7 Hz, CH_2), 3.02–3.40 (4 H, m, β-CH_2 + CH_2), 4.62 (1 H, s, CHO), 6.64 (1 H, s, urethane NH), 6.76–7.49 (7 H, m, aro), 7.79 (1 H, s, N*H*CH_2), 8.00–8.15 (2 H, d, *J* = 8 Hz, aro), 10.84 (1 H, s, indole NH). Anal. ($C_{31}H_{36}N_4O_5$) C, H, N; N: calcd, 10.29; found, 9.74.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-[2-(4-chlorophenyl)ethyl]glycine (26).** *N*-[2-(4-Chlorophenyl)ethyl]glycine ethyl ester was prepared from ethyl bromoacetate and 2-(4-chlorophenyl)ethylamine with a 42% yield as for 4. The product was purified by flash column chromatography using CH_2Cl_2/MeOH (19/1, v/v) as eluent to yield a pale yellow oil (3.40 g): R_f 0.47 (B); ^{1}H NMR (DMSO) δ 1.15 (3 H, t, *J* = 7 Hz, CH_3), 2.60–2.78 (4 H, m, CH_2CH_2), 3.33 (2 H, s, CH_2CO), 4.03 (2 H, q, *J* = 7 Hz, C$H_2$$CH_3$), 7.15–7.35 (4 H, m, aro).

N-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-[2-(4-chlorophenyl)ethyl]glycine ethyl ester was prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and *N*-[2-(4-chlorophenyl)ethyl]glycine ethyl ester as for 4 with a 16% yield. The product was chromatographed using CH_2Cl_2/EtOAc (24/1, v/v) as eluent: R_f 0.50 (B); ^{1}H NMR (DMSO) δ 1.04–2.08 (20 H, m, adamantyl + α-CH_3 + CH_2CH_3), 2.40–4.49 (10 H, m, β-CH_2 + CH_2CH_2 + CH_2CO + C$H_2$$CH_3$), 4.60–4.89 (1 H, m, CHO), 6.73–7.59 (11 H, m, aro + amide NH), 10.87 (1H, s, indole NH).

The final product was obtained from its ester as for 4 with a 50% yield: mp 193–196 °C; R_f 0.53 (D); HPLC (t_R = 8.5 min); MS (MH^+) 592; ^{1}H NMR (DMSO) δ 1.03–2.10 (17 H, m, adamantyl + α-CH_3), 2.40–4.42 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.53–4.91 (1 H, m, CHO), 6.72–7.70 (10 H, m, aro + amide NH), 10.78–10.98 (1 H, m, indole NH). Anal. ($C_{33}H_{38}N_3O_5Cl$) C, H, N.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-D-α-methyltryptophanyl]-*N*-[2-(4-chlorophenyl)ethyl]glycine (26A).** Method and data exactly as for 26, except for $[\alpha]_D$ = +22.6° (c 0.130; MeOH). The product was prepared from D-α-MeTrpOMe which was obtained as previously described,[26] $[\alpha]_D$ = −22.6° (c 4.21; MeOH) (lit.[26] $[\alpha]_D$ = −28.3° (c 9.9; MeOH). The enzymatic reaction was monitored by means of HPLC using a chiral column based on BSA (250 × 0.25 mm) and 0.1 M K_2HPO_4 (pH 8.7) as eluent (flow = 1 mL/min): t_R (L-α-MeTrp) = 6.3 min; t_R (D-α-MeTrp) = 10.7 min; t_R (DL-α-MeTrpOMe) = 12.8 min.

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-L-α-methyltryptophanyl]-*N*-[2-(4-chlorophenyl)ethyl]glycine (26B).** Method and data exactly as for 26, except for $[\alpha]_D$ = −24.1° (c 0.207; MeOH). The product was prepared from L-α-MeTrpOMe which was obtained as previously described,[26] $[\alpha]_D$ = +21.13° (c 5.74; MeOH) (lit.[26] $[\alpha]_D$ = +27.8° (c 9.9; MeOH).

***N*-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-[2-(2,4-dichlorophenyl)ethyl]glycine (27).** Prepared as in the synthesis of 4. *N*-[2-(2,4-Dichlorophenyl)ethyl]glycine ethyl ester was prepared from ethyl (bromoethyl)acetate and 2-(2,4-dichlorophenyl)ethylamine with a 55% (4.40 g) yield as described in the synthesis of 4. The product was chromato-

graphed using CH_2Cl_2/MeOH (19/1, v/v) as eluent to give an oil: ^{1}H NMR (DMSO) δ 1.15 (3 H, t, J = 7 Hz, CH_3), 2.61–2.82 (4 H, m, CH_2CH_2), 3.28 (2 H, s, CH_2CO), 4.01 (2 H, q, J = 7 Hz, CH_2-CH_3), 7.25–7.55 (3 H, m, aro).

N-[*N*-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-[2-(2,4-dichlorophenyl)ethyl]glycine ethyl ester was prepared from *N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophan and *N*-[2-(2,4-dichlorophenyl)ethyl]glycine ethyl ester as for 4 in 8% yield. The product was chromatographed with CH_2Cl_2/EtOAc (19/1, v/v): R_f 0.63 (B); ^{1}H NMR (DMSO) δ 1.00–2.09 (20 H, m, adamantyl + α-CH_3 + CH_2CH_3), 2.40–4.50 (10 H, m, β-CH_2 + CH_2CH_2 + CH_2CO + CH_2CH_3), 4.60–4.90 (1 H, m, CHO) 6.80–7.59 (9 H, m, aro + amide NH), 10.89 (1H, s, indole NH).

The final product was obtained from its ester in 92% yield: mp 201–202 °C; R_f 0.58 (D); HPLC (t_R = 10.9 min); MS (MH$^+$) 626; ^{1}H NMR (DMSO) δ 0.94–2.10 (17 H, m, adamantyl + α-CH_3), 2.40–4.40 (8 H, m, β-CH_2 + CH_2CH_2 + CH_2COOH), 4.50–4.91 (1 H, m, CHO), 6.78–7.63 (9 H, m, aro + amide NH), 10.76–10.86 (1 H, m, indole NH); Anal. ($C_{33}H_{37}N_3O_5Cl_2$) C, H, N.

[*N*-(2-Naphthyl)-DL-α-methyltryptophanyl][(2-phenylethyl)amino]urea (28): To 2-naphthylamine (716 mg, 5.0 mmol) in 6 mL of nitrobenzene was added a solution of bis(trichloromethyl) carbonate (1.48 g, 4.99 mmol) in 3.3 mL of toluene at 0 °C. The mixture was allowed to warm to room temperature and was heated afterwards to 90–95 °C for 4 h. Then the solvent was evaporated, and ether was added to the residue which was subsequently filtered and used without any further purification and characterization.

A solution of (DL-α-methyltryptophanyl)(2-phenylethyl)amine (64.1 mg, 0.2 mmol) was treated with the crude naphthyl isocyanate as described above in the synthesis of 19, yield 95 mg (97%): mp 128–131 °C; R_f 0.37 (B); HPLC (t_R = 8.2 min, CH_3CN and $H_2O/TFA_{0.05\%}$ (60/40)); MS (MH$^+$) 491; ^{1}H NMR (DMSO) δ 1.44 (3 H, s, α-CH_3) 2.65 (2 H, t, CH_2), 3.12–3.51 (4 H, m, β-CH_2 + NH_2CH_2), 6.36 (1 H, s, ArNHCON*H*), 6.70–8.10 (18 H, m, aro + amide NH), 8.92 (1 H, s, ArNH), 10.78 (1 H, m, indole NH). Anal. ($C_{31}H_{30}N_4O_2 \cdot 0.2H_2O$) C, H, N.

[*N*-(Triphenylmethyl)-DL-α-methyltryptophanyl][(2-phenylethyl)amino]urea (29). To a solution of bis(trichloromethyl) carbonate (1.10 g, 3.70 mmol) in 15 mL of dry CH_2Cl_2 was added triphenylmethylamine (2.50 g, 10.0 mmol) and Et_3N (3.84 g, 37.9 mmol) in 10 mL of CH_2Cl_2 at 0 °C. The organic solvent was removed after stirring at ambient temperature for 2 h, EtOAc was added to the residue, and the mixture was filtered. Evaporation of the organic solvent yielded the isocyanate quantitatively (2.85 g).

A solution of (DL-α-methyltryptophanyl)(2-phenylethyl)amine (64 mg, 0.2 mmol) was treated with the isocyanate (57 mg, 0.2 mmol) as described above for 19 using toluene instead of THF and stirring at reflux temperature for 48 h to yield 50 mg of product (41%): mp 140–142 °C; R_f 0.20 (A); HPLC (t_R = 7.6 min); MS (MH$^+$) 607; ^{1}H NMR (DMSO) δ 1.11 (3 H, s, α-CH_3), 2.54 (2 H, t, CH_2), 2.99–3.21 (4 H, m, β-CH_2 + NH_2CH_2), 6.42–7.49 (28 H, m, aro + urea + amide NH), 10.79 (1 H, m, indole NH). Anal. ($C_{40}H_{36}N_4O_2 \cdot 0.2H_2O$) C, H, N.

In Vitro Binding Assays. [^{3}H]pCCK$_8$ (specific activity 60 Ci/mmol) was purchased from Amersham, and [^{3}H]pBC 264 was synthesized as previously described.[38]

Incubations (final volume 1 mL) were carried out at 25 °C in 50 mM Tris–HCl buffer (pH 7.4), 5 mM $MgCl_2$, and 0.2 mg/mL of bacitracin for 60 min in the presence of brain membranes (0.6 mg of protein per tube) or in 10 mM Pipes–HCl buffer (pH 6.5), 30 mM $MgCl_2$, 0.2 mg/mL of bacitracin, and 0.2 mg/mL of soybean trypsin inhibitor for 120 min in the presence of pancreatic membranes (0.2 mg of protein per tube).

With brain membranes, [^{3}H]pCCK$_8$ or [^{3}H]pBC 264 was incubated at 0.2 nM, and with pancreatic membranes [^{3}H]pCCK$_8$ was used at 0.1 nM, in the presence of varying concentrations of the competitor. Nonspecific binding was determined in the presence of 1 μM CCK$_8$. Incubation was terminated by filtration through Whatman GF/B glass-fiber filters precoated with buffer containing 0.1% bovine serum albumin. The filters were rinsed with 2 × 5 mL of ice-cold buffer and dried and the radioactivity counted. K_i values were calculated using the Cheng–Prusoff equation. Hill coefficients in all experiments were close to 1.

In Vivo Binding Assays. The experiments with icv and ip injections were performed as described previously.[39] Briefly, mice were killed by cervical dislocation 15 min after icv injection of [^{3}H]pBC 264, and their brains were quickly removed. A delay of 15 min was chosen, as previous studies showed that specific binding of [^{3}H]pBC 264 is maximum at this time.[39] The brain (minus cerebellum) was homogenized in 10 mL of cold 50 mM Tris–HCl buffer containing 0.02% bacitracin. Aliquots (0.15 mL) of the homogenate were immediately filtered through Whatman GF/B glass filters and rinsed twice with 5 mL of cold buffer. Free radioactivity (F) was calculated as the difference between total radioactivity and radioactivity retained on the filters, which was considered as bound radioactivity. [^{3}H]pBC 264 (10 pmol, 1 μCi) was injected icv.

Compound **26A** was prepared as a stock solution in ethanol/Chremophor EL/H_2O (1/1/8) and diluted in saline before icv coinjection with [^{3}H]pBC 264 or ip administration at different concentrations 5 min prior to [^{3}H]pBC 264.

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