Development of Peptide 3D Structure Mimetics: Rational Design of Novel Peptoid Cholecystokinin Receptor Antagonists

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The two hormones cholecystokinin and gastrin share the same C-terminal sequence of amino acids, namely Gly^{29}-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2. Nevertheless, this congruence has not precluded using this structure to develop selective ligands for either CCK_1 or CCK_2 receptors. Manipulation of the hydrophobic residues at positions 31 and 33 gave a series of CCK_1 tripeptide antagonists, typified by *N*-*t*-BOC-Trp-2-Nal-Asp-2-(phenyl)ethylamide (pK_B 6.8 ± 0.3). Molecular modeling was used to identify the bioactive conformation of these CCK_1-selective compounds and prompted the design of new peptoid structures. We aimed to maintain the conformation of the parent series by exploiting patterns of hydrogen-bonding and π-stacking interactions present in the original molecule, rather than introducing additional covalent bonds. The prototype, *N*-(succinyl-D-Asp-2-phenylethylamido)-L-Trp-2-(2-naphthyl)ethylamide, was a potent and selective CCK_1 antagonist (pK_B 7.2 ± 0.3). Furthermore, the new series showed patterns of biological activity that mirrored those of the parent tripeptides. These compounds contain elements of both peptide primary and secondary structure and represent a novel approach to designing peptidomimetics. Interesting results were obtained from comparing models of a representative tripeptide CCK_1 antagonist with a conformation of CCK_{30-33} that others have proposed to be responsible for its activity at the CCK_2 receptor. The results suggest that CCK_1 and CCK_2 receptors recognize enatiomeric dispositions of the Trp^{30} indole, Asp^{32} carboxylic acid, and C-terminal phenyl groups arrayed about a common backbone configuration. This "functional chirality" may underpin the mechanism by which these closely related receptor systems bind CCK_{30-33} and explain patterns of selectivity observed with optical isomers of a number of peptoid and nonpeptide ligands.

Introduction

Cholecystokinin and gastrin are two closely related peptide hormones that mediate a range of peripheral and central biological processes. Cholecystokinin receptors are divided into two subclasses: CCK_1 and CCK_2 receptors. CCK_1 receptors occur centrally in the *nucleus tractus solitarius*[1] but are mainly located in the periphery in the pancreas,[2] gall bladder,[3] and colon. CCK_2 receptors are the predominant subtype in the brain and are widely distributed throughout the cortex.[4] The CCK_2 receptor is also located on the ECL cell of the stomach, and there is strong evidence to suggest that the central and peripheral populations of receptors are homogeneous, on the basis of their selectivity for a range of ligands and evidence from molecular hybridization studies.[5] The human CCK_1 and CCK_2 receptors have now both been cloned and shown to belong to the family of G-protein coupled receptors.[5−8]

From a chemical point of view, the two hormones need to be considered together because they share the same primary sequence of amino acids at their C-termini, namely Gly-Trp-Met-Asp-Phe-NH_2. In fact, only the last four residues, CCK_{30-33} (Trp-Met-Asp-Phe-NH_2), are required to elicit a full biological response at the CCK_2 receptors in both the periphery[9] and the brain.[10] Conversely, this fragment only has micromolar affinity for the CCK_1 receptor, which requires the sulfated octapeptide fragment (CCK-8S) for full activation. This fragment is also common to the structure of the decapeptide caerulein, found in the skin of amphibia, suggesting that the mammalian hormones cholecystokinin and gastrin may share a common evolutionary history.[11]

CCK_1 receptor antagonists have been shown to block CCK-8-induced contraction of the gallbladder and inhibit gastric emptying,[12] pancreatic secretion,[13] and satiety.[14] On the other hand CCK_2 antagonists have attracted interest on the basis of their ability to inhibit gastrin-stimulated gastric acid secretion in the periphery.[15] A number of central effects have also been noted for some of these latter compounds, such as anxiolytic behavior[16] and ability to potentiate morphine-induced analgesia.[17]

Our aim was to design novel CCK_1 and CCK_2 antagonists using the structure of the C-terminal tetrapeptide, CCK_{30-33}, as the chemical starting point. The primary requirement was that the final compounds should be selective for one receptor type over the other. A further aim was that their structures had to be sufficiently different from those of the parent peptides to try to avoid the recognized problems associated with using peptides as drugs: namely poor bioavailability, rapid proteolytic cleavage, and clearance in vivo.

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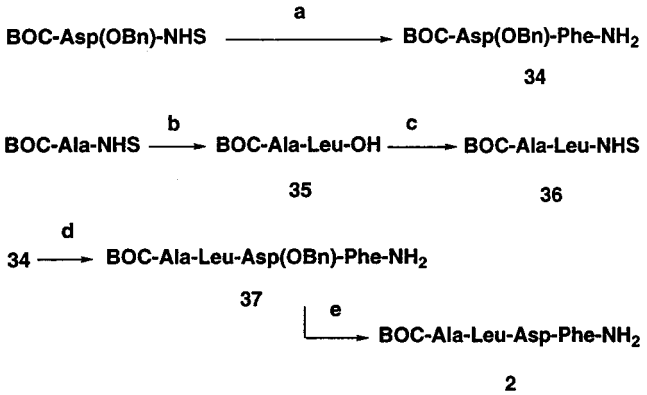
Scheme 1. Synthesis of Tetrapeptide **2**[a]



[a] (a) Phe-NH_2·NCl, $NaHCO_3$, DME, H_2O; (b) Leu, $NaHCO_3$, DME, H_2O; (c) NHS, DCCI, DME; (d) (i) TFA, (ii) **36**, $NaHCO_3$, DME; (e) H_2, Pd/C, EtOH.

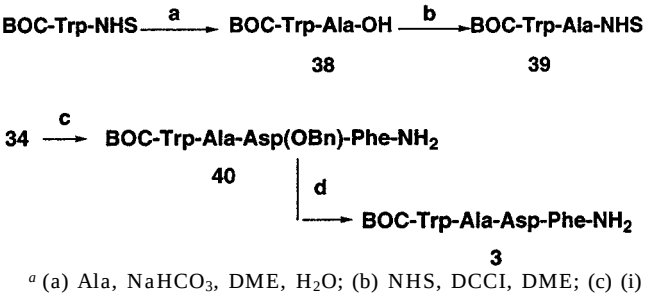
Broad cross-screening strategies have led to the discovery of a number of nonpeptide ligands for these receptors.[18−22] This work has proceeded in parallel with the alternative strategy based on using the parent hormone as the initial chemical lead. We have chosen to use this latter approach and have already reported a number of compounds designed in this way. These have included the CCK_1-selective sulfonamide 2-NAP,[23,24] as well as a range of nonpeptide CCK_2 ligands based on oxathiazinone,[25] dibenzobicyclo[2.2.2]octane (BCO),[26,27] and indole skeletons.[28] The design of effective antagonists for these receptor systems required that we tackled the twin problems of removing the ability of the hormone to activate the receptor (efficacy) and biasing the selectivity of the compounds in favor of one or other receptor system.

We have not been alone in taking the stucture of the terminal tetrapeptide as our starting point, and the Parke-Davis group has described selective CCK_1 and CCK_2 ligands designed around the structure of the dipeptide fragment Trp-Phe.[29,30] However, a number of reports suggest that several examples of these compounds retained the agonist properties of the parent hormone.[31−37] The balance between removing the ability of a molecule to cause agonism (efficacy) without disturbing its ability to bind to the receptor is clearly extremely fine and has been at the heart of our search for novel and selective antagonists of these two receptor systems. This paper will focus on our efforts to achieve this target and will describe the preparation of a number of peptoids whose structures were derived from that of BOC-CCK_{30-33}. Our efforts to interpret the patterns of activity that were obtained in terms of the 3D structure of the molecules led to the design and synthesis of a second series of peptoids. The aim of this exercise was to mimic elements of 3D structure identified in the first series, without the introduction of additional covalent links. The success of this approach is reflected in the biological activity of the new series of compounds − which is directly comparable with that of the parent series. This new series was optimized to give a number of potent and selective CCK_1 antagonists.

Chemistry

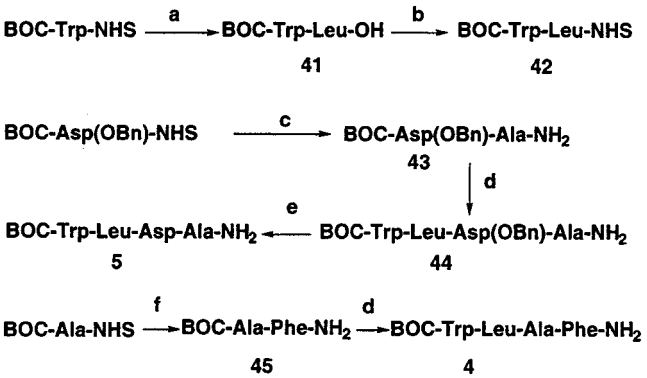
The compounds described for the first time in this paper were prepared according to Schemes 1−6. The

Scheme 2. Synthesis of Tetrapeptide **3**[a]



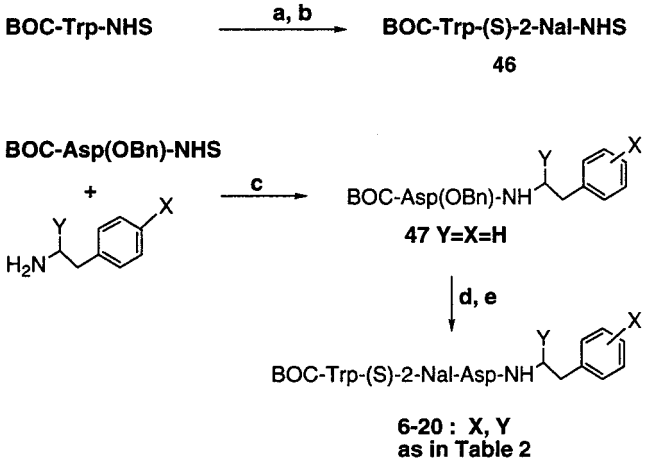
[a] (a) Ala, $NaHCO_3$, DME, H_2O; (b) NHS, DCCI, DME; (c) (i) TFA, (ii) **39**, $NaHCO_3$, DME; (d) H_2, Pd/C, EtOH.

Scheme 3. Synthesis of Compounds **4** and **5**[a]



[a] (a) Leu, $NaHCO_3$, EtOH, H_2O; (b) NHS, DCCI, DME; (c) Ala-NH_2·HCl, $NaHCO_3$, DME, H_2O; (d) (i) TFA, (ii) **42**, Et_3N, DME; (e) H_2, Pd/C, MeOH; (f) Phe-NH_2, DME.

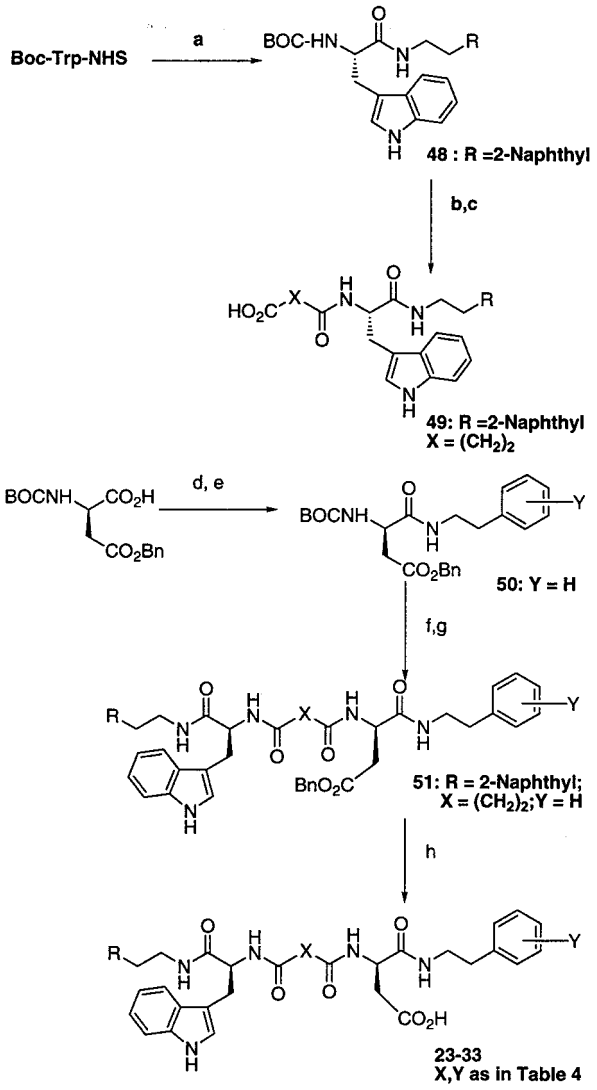
Scheme 4. Synthesis of Compounds **6**−**20**[a]



[a] (a) (*S*)-2-Naphthylalanine, $NaHCO_3$, EtOH, H_2O; (b) NHS, DCCI, DME; (c) DME; (d) (i) TFA, (ii) **46**, Et_3N, DME; (e) H_2, Pd/C, MeOH.

tetrapeptides required to evaluate the contribution of the amino acid side chains of CCK_{30-33} were synthesized using standard methods from commercially available starting materials (Schemes 1−3). The same methods were used to prepare compound **6** in which naphthylalanine (Nal) was incorporated in place of Leu[31] (Scheme 4). Modification of Phe[33] in this latter series was effected by coupling the appropriately substituted 2-phenylethylamines, or phenylalanines, to the *N*-hydroxysuccinimide ester of protected Asp (Scheme 4). The resulting fragments were then coupled to the dipeptide NHS ester **46** and the benzyl protecting group was removed by hydrogenolysis in the presence of 10% palladium on charcoal catalyst to give the peptide derivatives **6**−**20**.

Scheme 5. Synthesis of Compounds **23**−**33**[a]



(a) 2-(2-naphthyl)ethylamine , DME; (b) TFA; (c) THF, DMAP, (d) NHS, DCCI, DCM; (e) ; (f) TFA; (g) **49**, pyBOP, DCM, diisopropylethylamine; (h) H_2, Pd/C, MeOH

Similarly, convergent syntheses of compounds **23**−**33** were achieved by coupling appropriate L-Trp (**49**) and D-Asp (**50**) fragments (Scheme 5). However, we found that this reaction proceeded in higher yield if (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (pyBOP) was employed as the coupling reagent. Hydrogenolysis of the resulting adducts gave the compounds described in Table 4.

Molecular Modeling

The majority of the modeling studies described for the initial series of tripeptides **6**−**20** were carried out using molecular mechanics calculations as implemented in the commercial package SYBYL (Tripos Associates Inc.). This included the design of the prototype CCK_1 antagonist **23** described in the following section. However, compounds **17**, **7**, **10**, and **12** were also examined using a modified version of COSMIC equipped with XED (**e**xtended **e**lectronic **d**istribution) charges[38] rather than the atom-centered Gasteiger−Huckel charges used in SYBYL. We have found that these XED charge descriptions reproduce phenomena such as π-stacking of aromatic rings, anomeric effects, and polar atom interactions better than conventional atom-centered charges. The conditions used for generating sets of conformations under SYBYL and XED were the same, with the exception of the charge description used. The second series of selective CCK_1 antagonists (**23**−**33**) was modeled exclusively with XED. Full details of the XED calculations were as follows. All calculations were carried out at dielectric 4.0, allowing all rotatable bonds, including the peptide amides, to rotate. The initial set of conformations was generated using a hard spin torsional minimizer. Each input conformation was randomized 250 times (Monte Carlo), twisting each bond by 10° torsional increments, iterating through all bonds to 0.001 kcal/mol and storing all conformations within a 15.0 kcal/mol range of the lowest-energy structure found. Conformations within 15° on all torsions of any other were removed. The energies of all structures were then minimized using a combined parabolic/Fletcher−Reeves conjugate gradient technique whose accuracy was set at <0.01 cal/mol. XED charges were then added to each conformation and the energies of each structure minimized once again using the same procedure. Conformations were collected over a 15.0 kcal/mol energy range to a maximum of 1000 structures. Thus 100 conformations covering a 9.3 kcal/mol energy range were identified for the tripeptide **17**. The lowest-energy structures were compared to a model of BOC-CCK_{30-33} that was created from the published torsion angles contained in Kolodziej et al.[39] (Table 3).

Results and Discussion

The C-amidated terminal tetrapeptide fragment CCK_{30-33} (BOC-Trp-Met-Asp-Phe-NH_2) is a potent stimulant of gastric acid secretion as determined by its behavior in the isolated, lumen-perfused mouse stomach bioassay.[40] We have used this assay on a routine basis to examine CCK_2 receptor ligands as it has proven to be particularly sensitive in revealing agonist behavior that would be missed in other tissues.[41] Literature precedent states that the hydrophobic side chain of the Met[31]residue does not play an important role in determining the biological activity of CCK_{30-33}, and it is widely accepted that this chemically sensitive group can be replaced by Leu or Nle without detriment to the pharmacological properties of the molecule.[42−44] To confirm this observation and the suggested role of the amino acid side chains, we made a series of compounds in which each residue of the tetrapeptide BOC-Trp-Leu-Asp-Phe-NH_2 was sequentially exchanged with alanine. This is a recognized method of testing the contribution of side chain functional groups in a systematic manner with minimal effect on the conformation of the peptide backbone.[45] This produced an interesting variety of activities, as summarized in Table 1. The importance of the carboxylic acid side chain of the Asp residue is clearly illustrated by the lack of activity of the Ala analogue **4**, when tested at a concentration of 1×10^{-5} M. Furthermore, the bioassay results suggested that it might be possible to separate the influence of the side chain functional groups on the ability of the compound to bind to the CCK_2 receptor (affinity) and effect release

Scheme 6. Design of Prototype CCK_1 Antagonist **23** from Tripeptide **7**

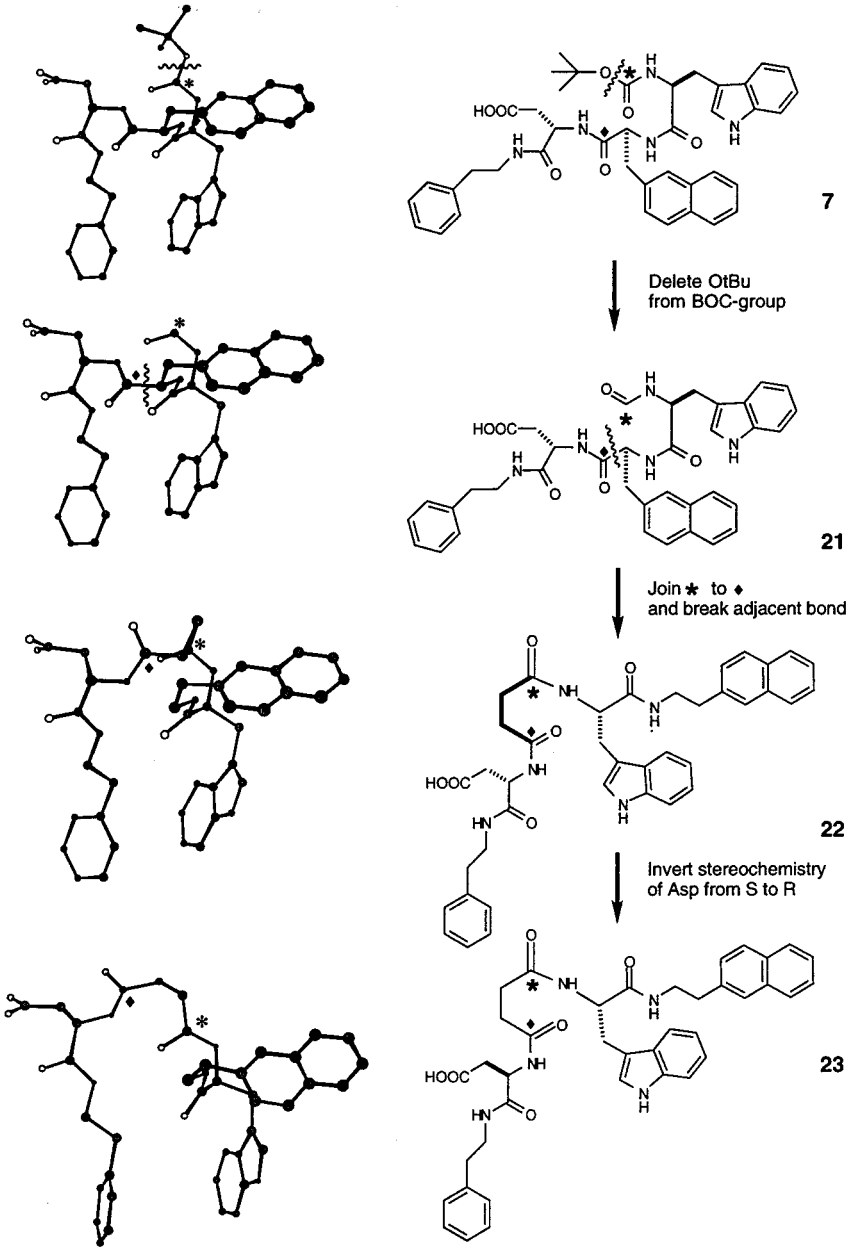


Table 1. Results of Alanine Scan on the Tetrapeptide BOC-Trp-Leu-Asp-Phe-NH_2: Activity at CCK_2 Receptors in the in Vitro Mouse Stomach Assay[40]

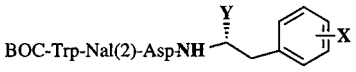
		CCK_2	
no.	compd	pA_{50}[a]	α (%)[b]
1	BOC-Trp-Leu-Asp-Phe-NH_2	8.9 ± 0.2	100 ± 4
2	BOC-**Ala**-Leu-Asp-Phe-NH_2	6.0 ± 0.2	64 ± 9
3	BOC-Trp-**Ala**-Asp-Phe-NH_2	7.0 ± 0.2	68 ± 6
4	BOC-Trp-Leu-**Ala**-Phe-NH_2	inactive[c]	
5	BOC-Trp-Leu-Asp-**Ala**-NH_2	4.0−5.0	>95

[a] The pA_{50} value is the position of half-maximal response of the agonist curve for the test compound and is the mean of at least six separate experiments. The errors quoted represent the SEM. [b] α represents the percentage maximal response for the compound relative to a pentagastrin control and is the mean of at least six separate experiments. The errors quoted represent the SEM. [c] The compound was inactive when tested at a concentration of 1×10^{-5} M.

of gastric acid (efficacy). Removing the phenylalanine aromatic ring gave a compound (**5**) with low affinity, which was nevertheless capable of eliciting almost as great a secretory response as BOC-[Leu^{31}]CCK_{30-33} (**1**) when given at a high enough concentration. This suggested that the phenyl ring made a substantial contribution to the way in which the tetrapeptide binds to the receptor but did not unduly influence the effector mechanism. Deleting the indole group from the N-terminal residue (**2**) produced a loss of both affinity and efficacy. However, the most significant result was obtained when the methionine/leucine side chain was truncated. In this case, it became apparent that compound **3** produced the same level of secretory response as the peptide in which alanine replaced tryptophan (**2**) but had retained a higher level of affinity for the receptor. This loss of efficacy was surprising in the light of the early literature precedent,[42−44] but it suggested that elaboration at this point in the peptide structure might allow us to manipulate this parameter without detriment to the overall binding.

A brief investigation was carried out in which the

Table 2. CCK_1 and CCK_2 Data Obtained as a Result of Modifying the C-Terminal Residue, Phe^{33}, of BOC-[$Nal(2)^{31}$]CCK_{30-33}



			CCK_2 [a]		CCK_1 [b]
no.	X	Y	pK_B [c]	α (%) [d]	pK_B
6	H	$CONH_2$	6.3 ± 0.2	NS	5.3 ± 0.2
7	H	H		20 ± 4	6.8 ± 0.3
8	H	CONHMe		55 ± 13	5.4 ± 0.2
9	H	$CONMe_2$		55 ± 6	6.2 ± 0.2
10	H	CH_2OH		126 ± 26	5.9 ± 0.1
11	4-OMe	$CONH_2$	5.6 ± 0.3	NS	5.8 ± 0.2
12	4-OMe	H	6.0 ± 0.2	NS	7.2 ± 0.3
13	4-Cl	$CONH_2$		74 ± 19	5.9 ± 0.3
14	4-Cl	H		60 ± 11	6.3 ± 0.4
15	2-OMe	H	5.2 ± 0.2	NS	7.1 ± 0.4
16	3-OMe	H	5.1 ± 0.3	NS	7.2 ± 0.3
17	3,4-$(OMe)_2$	H	inactive [e]		6.5 ± 0.3
18	4-F	H		29 ± 8	6.7 ± 0.3
19	4-NH_2	H	5.9 ± 0.3	NS	6.5 ± 0.2
20	3-CF_3	H	inactive [e]		6.1 ± 0.3

[a] Values determined in the in vitro isolated lumen-perfused mouse stomach.[40] [b] Values determined in the in vitro guinea-pig gallbladder with respect to the shift of the CCK-8S dose−response curve and are the mean of at least six experiments. [c] For entries in this column the values represent pK_B ± SEM determined with respect to the shift of the pentagastrin dose−response curve and are the mean of at least six separate experiments. For any compounds that show significant agonism no affinity is stated. [d] α represents the percentage maximal response for the compound relative to a pentagastrin control and is the mean of at least six separate experiments. NS, no significant secretory effect observed when the compound was tested at 1×10^{-5} M. [e] The compound was inactive when tested at a concentration of 1×10^{-5} M.

methionine residue of BOC-CCK_{30-33} was replaced with a number of other hydrophobic amino acids. This rapidly led to the identification of the *S*-3-(2-naphthyl)alanine (L-2-Nal) derivative **6** (Table 2), which was found to be a competitive antagonist with modest affinity for both CCK_1 and CCK_2 receptors − reinforcing our view that modification of the residue in position 31 was key to manipulating efficacy at the CCK_2 receptor.

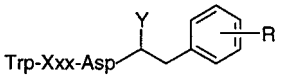
A survey of the literature also showed that the presence, or absence, of the C-terminal amide of BOC-CCK_{30-33} contributes to the observation of an agonist response. For example, a report some time ago showed that deletion of the C-terminal amide from the agonist BOC-[Leu^{31}]CCK_{30-33} converted the compound from an agonist to an antagonist, as judged by its behavior in vitro in the isolated rat stomach assay.[46] We have found that these compounds continue to behave as partial agonists in the analogous mouse stomach assay − suggesting that efficacy has been reduced but not abolished. Also, Corringer et al.[47] reported that BOC-[Phg^{31},Nal^{33}]CCK_{30-33} behaved as a full agonist in an electrophysiological assay on rat hippocampal CCK_2 receptors but that the C-terminal dimethylamide was a competitive antagonist in the same assay. We have examined the effect of similar modifications on analogues of the L-2-Nal derivative **6**.

In this series, the compound lacking the C-terminal amide showed low levels of efficacy at CCK_2 receptors but a 30-fold increase in affinity at CCK_1, relative to compound **6**. However, replacing the C-terminal amide with a hydroxymethyl group (**10**) restored the ability of the compound to cause acid secretion in the mouse stomach assay to a level equivalent to that found for the parent compound BOC-[Leu^{31}]CCK_{30-33}. Unlike Corringer et al.[47] we found that both the mono- and dimethylamides (Table 2, examples **8** and **9**, respectively) showed increased levels of agonist behavior at CCK_2 receptors. Modeling compound **7** showed that the structure of the calculated global minimum energy conformation was one in which the three aromatic rings clustered together in a 3-way π-stack. However, we cannot equate the level of efficacy observed at CCK_2 receptors to the proportion of conformations in which this arrangement of aromatic groups occurs because these were not significantly different for the antagonist **6** (30%) and the full agonist **10** (44%) over the energy range examined. Nevertheless, it is possible that this small difference is reflected in the modest increase in CCK_1 receptor affinity.

Further progress in reducing the modest levels of agonism at CCK_2 receptors within the series of phenylethylamides related to **7** was achieved by adding substituents to the phenyl ring to modify the electron distribution in this region of the molecule. In general, it appeared that electron-donating groups were best suited for this purpose. Thus the 4-methoxyphenyl analogue **12** was a competitive antagonist, but the 4-chlorophenyl derivative **14** showed increased agonist activity. The pattern was repeated for the 4-F (**18**) and 4-NH_2 (**19**) derivatives. This effect was independent of the presence of a C-terminal amide. Levels of CCK_1 affinity were unchanged throughout, and so selectivity for this class of cholecystokinin receptors began to emerge as CCK_2 activity fell.

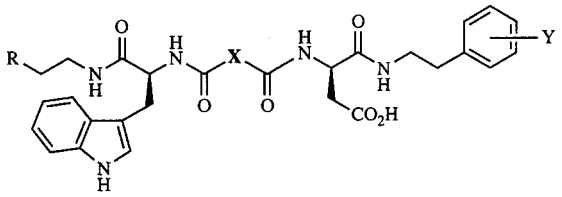
Molecular modeling showed that the driving force behind the conformational preferences observed in these CCK_1 antagonists stems from a 3-way π-stacking interaction between the indole, naphthalene, and substituted phenyl groups. We observe that these compounds bind preferentially to CCK_1 receptors and are antagonists rather than agonists at CCK_2 receptors. Compounds **7** (Y = H), **17** (Y = 3,4-$(OMe)_2$), and **12** (Y = 3-OMe) differ in the number of methoxy substituents attached to the terminal phenyl group. Careful examination of the sets of conformations generated with the XED modeling package showed that there was a steady increase in the proportion of conformations containing the 3-way π-stacking interaction (31%, 57%, and 92%, respectively, within a 3 kcal/mol range of the calculated global minimum energy conformation). However, neither the observed increase in affinity for the CCK_1 receptor nor differences in the expression of efficacy at CCK_2 receptors can be attributed to this property alone as only compound **17**, of those examined, showed a single specific biological activity. Instead, we chose to use this observation to design novel peptoid structures that maintained the conformation that we believed to be responsible for the CCK_1 activity of this series. Such compounds should be CCK_1 antagonists, as we have no evidence to suggest that the conformation we have identified is capable of evoking a response in the functional GP gallbladder assay. At this point we could have chosen to introduce covalent bonds to stabilize this structure but decided against this approach as macrocyclization had already been shown to lead to a loss of affinity for the receptor,[47] in addition to making the

Table 3. Backbone Torsion Angles (deg) for the Tetrapeptide Ac-CCK$_{30-33}$ (from ref 39) and Calculated Global Minimum of Tripeptide **17**



				Trp		Xxx		Asp		Phe
compd	Xxx	Y	R	ψ_1	ω_{12}	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4
Ac-CCK$_{30-33}$	Met	S-CONH$_2$	H	169	180	−83	93	−71	149	−118
tripeptide **17**	2-Nal	H	3,4-(OMe)$_2$	45	−179	−87	74	−88	56	

Table 4. CCK$_1$ Data for Novel Peptoids Designed from the 3D Structure of Peptoid **7**



no.	R	X	Y	CCK$_1$ activity,[a] pK_B ± SEM
23	2-naphthyl	$(CH_2)_2$	H	7.2 ± 0.3
24	2-naphthyl	$(CH_2)_2$	4-F	6.0 ± 0.3
25	2-naphthyl	$(CH_2)_2$	4-OMe	6.7 ± 0.2
26	2-naphthyl	$(CH_2)_2$	3-CF$_3$	6.0 ± 0.4
27	3,4-dichlorophenyl	$(CH_2)_2$	4-OMe	5.9 ± 0.3
28	phenyl	$(CH_2)_2$	4-OMe	5.5 ± 0.3
29	^{i}Pr	$(CH_2)_2$	4-OMe	5.4 ± 0.2 (pA_{50})[b]
30a	2-naphthyl		H	6.2 ± 0.2
30b	2-naphthyl	as above	H	6.5 ± 0.2
31	2-naphthyl	as above	4-F	6.7 ± 0.1
32	2-naphthyl	as above	4-OMe	6.7 ± 0.2
33	2-naphthyl		4-OMe	7.4 ± 0.4

[a] Values determined in the in vitro guinea-pig gallbladder with respect to the shift of the CCK-8S dose−response-curve. pK_B ± SEM values quoted are the mean of at least six experiments. All compounds were inactive when tested at a concentration of 1×10^{-5} M in the functional CCK$_2$ assay. [b] See text for details.

synthesis more demanding. A prototype molecule was designed to fulfill these criteria taking the peptide **7** as the starting point. The aim was to create a structure that maintained the 3-way π-stack between the indole, naphthalene, and phenyl groups and incorporated a carboxylic acid in a position corresponding to that found in the modeled structure of **7**. The design process can formally be described in the following manner (Scheme 6): First, the *tert*-butyloxy group from the N-terminus was deleted in order to free up the valency of the sp^2 carbon (*) (**21**). This was then covalently linked to the carbonyl of the naphthylalanine residue (◆) by two methylene groups. In addition, the bond between the α-carbon of the naphthylalanine residue and its adjacent carbon was broken at this point to avoid the problems of synthesizing a highly functionalized macrocyclic system. This gave a structure (**22**) whose energy was minimized using molecular mechanics before fitting the coordinates to those of the original model of **7**. This showed that the overall spatial disposition of the side chains was retained in the new molecule, with the exception that the carboxylic acid of the aspartyl residue was pointing in a different direction. This problem was overcome by inverting the chirality at the α-carbon from *S* to *R* to give **23**. Molecular mechanics then showed that the 3-way interaction of the aromatic nuclei was retained in the low-energy conformations of the new molecule and that the carboxylic acid of the aspartic acid residue was oriented in the same region of space as that found in the parent peptoid **7**. This gave a target molecule that was readily amenable to synthesis and which appeared to maintain the disposition of the peptide side chains in space but whose backbone was substantially different from that of the parent peptide.

The new molecule **23** was a potent CCK$_1$ antagonist (pK_B 7.2 ± 0.3) when tested in vitro in the guinea-pig gallbladder strip assay. In addition, it was at least 50-fold selective for this receptor over CCK$_2$, since it did not produce any significant antagonist effect, or secretory response, when tested at 1×10^{-5} M in the mouse stomach bioassay. This result merited further investigation, and a number of analogues of **23** were prepared to examine the structure−activity relationship (SAR) between the new series of compounds and those related to **7**.

We have already demonstrated the effect of varying the nature of the substituent on the terminal phenyl ring in the parent L-2-Nal series of peptide derivatives (Table 2). In this case, introduction of electron-withdrawing groups at either the 3- or 4-position on the aromatic ring of the new series of compounds (**24**, **26**) reduced levels of CCK$_1$ activity by a factor of 10 (Table 4). However, the 4-methoxy analogue **25** was of equivalent activity to compound **23** − as observed in the parent series. The gastrin activity of all of these compounds remained low, and most were found to be inactive when tested at a concentration of 3×10^{-5} M in the in vitro CCK$_2$ bioassay.

We have postulated that the structure of this series is determined by hydrogen-bonding between elements of the amide backbone and π-stacking between the three aromatic moieties. Compounds in which the naphthalene group of compound **25** had been replaced by 3,4-dichlorophenyl (**27**) and phenyl (**28**) showed a sequential drop in activity, demonstrating the importance of this group in maintaining the overall topology of the series. This facet was explored further by introducing an isopropyl group in place of the 2-naphthalene substituent in example **29**. We were surprised to find that this compound showed a degree of agonism in the guinea-pig gall bladder assay that amounted to 44% of the control value obtained with CCK-8S. In fact, the only other compound to have shown any agonism (6% of the control) in this bioassay throughout this investigation was BOC-[Leu^{31}]CCK$_{30-33}$, which might be considered to be the closest relative of compound **29** in the parent series.

Examining a model of the lead compound **23** showed that the lowest-energy conformation was that in which

the two carbonyl groups of the succinic acid spacer were arranged *cis* to each other, relative to the ethylene unit. If this were indeed the case, then "locking" the relationship between the two carbonyl groups into this conformation might be expected to increase the affinity of the resulting compound for the CCK_1 receptor by reducing the overall entropy of the molecule. One way of achieving this aim was to tie down the conformationally mobile ethylene group by substituting 1,2-cyclohexanedicarboxyl for succinyl. This introduced two new chiral centers into the molecule, and the compounds were prepared as racemates from the respective (±)-*cis*- and (±)-*trans*-dicarboxylic acid anhydrides. However, in those cases in which the two diastereoisomers were separated (e.g. **30a**,**b**) there was little difference in the activity of the individual diastereoisomers. These two compounds were equipotent at CCK_1 receptors but also showed low levels of CCK_2 activity ($pK_B = 5.6$). Moreover, their CCK_1 activity had not increased relative to the succinyl derivative **23**. Introducing either electron-withdrawing (**31**) or electron-donating (**32**) substituents in the 4-position of the phenyl ring made no difference to levels of CCK_1 activity in this case. However, changing the configuration of the cyclohexane group from *cis* to *trans* did lead to a modest increase in activity (**33**, Y = 4-OMe: $pK_B = 7.4$) while maintaining selectivity for CCK_1 receptors. Unfortunately, the compound was tested as a racemate, and no information is available for the behavior of the individual enantiomers in this case. Nevertheless, while this does not represent an exhaustive study, the behavior of the two series of peptides/peptoids is remarkably consistent. This supports the premise that molecular modeling techniques can be used to design effective 3D mimics of bioactive conformations without the need to compromise synthetic accessibility by introducing additional covalent bonds.

Our studies in this area had led us to develop a molecular model of BOC-CCK_{30-33} that we used as the basis for development of our dibenzobicyclo[2.2.2]octane CCK_2 antagonists. This model was originally derived from fluorescence data that suggested that the indole and phenyl side chains of the two aromatic amino acids of H-CCK_{30-33} were between 5 and 7 Å apart in water.[48] The pitch of a 3_{10} helix brings the side chain of every fourth amino acid into close proximity, and with this constraint, a number of structures that fulfilled the experimental criteria were generated using molecular mechanics.[49] Moreover, this arrangement produced structures of lower energy than those obtained if the peptide backbone was allowed to adopt an alternative conformation, such as an α-helix or a simple *β*-turn. These structures were in good agreement with energy calculations on Ac-CCK_{30-33} previously published by Pincus et al.[50]

However, more recent studies have led Kolodziej et al.[39] to propose a different structure for the bioactive conformation of Ac-CCK_{30-33} at CCK_2 receptors. This research group had also recognized the importance of the Met^{31} side chain for CCK_2 activity and carried out a series of studies in which this residue was replaced with the individual *cis* and *trans* isomers of several 4-alkylthioprolines. These studies led them to propose a 3D structure for the "bioactive" conformation Ac-CCK_{30-33} in which the three peptide bonds of the Trp-Met-Asp-Phe sequence adopt a Z-like bend. The biological activities of the compounds described in these studies were assessed in membrane binding assays, and hence it is not clear whether the conformation identified is responsible for the affinity or efficacy of the peptide. Nevertheless, the authors noted that one consequence of the molecule adopting this conformation was that the hydrophobic groups clustered together on one "side" of the peptide backbone with the hydrophilic side chain of the Asp^{32} residue located on the opposite "side". In contrast, our model places the Met^{31} and Asp^{32} side chains on the same side of the peptide backbone. However, we noted that the proposed Z-bend conformation was highly reminiscent of those low-energy structures that we had identified for our CCK_1 antagonists.

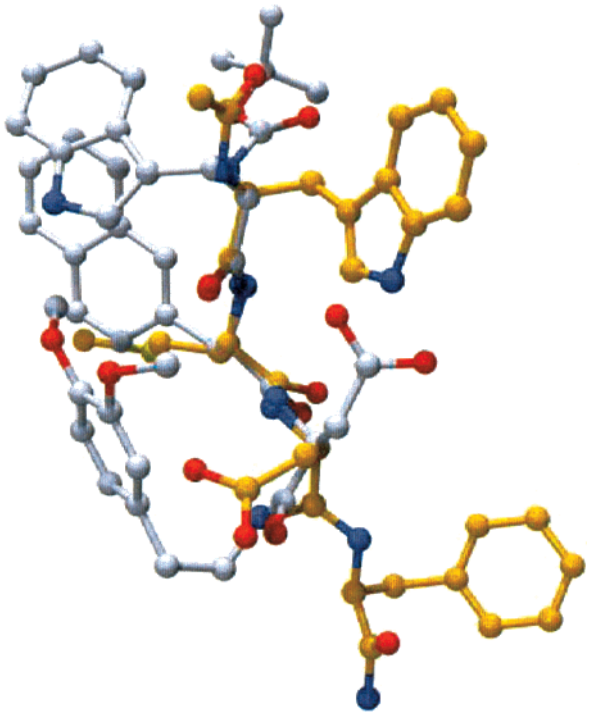


Figure 1. Conformations of the tetrapeptide Ac-CCK_{30-33} (taken from ref 39, shown in yellow) and the calculated global minimum of tripeptide **17** (shown in white). The two structures were fitted by overlaying the Cα atoms of the three amino acid residues of the peptide backbones (rms = 0.07). The backbones of the two molecules are oriented vertically with the N-termini uppermost. This shows the enantiomeric disposition of the indole, carboxylic acid, and phenyl groups relative to the backbone.

Comparing a model of the 3,4-dimethoxy analogue **17**, a selective CCK_1 antagonist, with the structure proposed for the bioactive conformation of Ac-CCK_{30-33}[39] allows us to speculate about the origins of this reversal of selectivity. The overlay, shown in Figure 1, was generated by fitting the α-carbons of the calculated global minimum of compound **17** to the equivalent centers of a model of Ac-CCK_{30-33}, (rms = 0.07), which had been generated using the published torsion angles. In fact, the configuration of the central portion of the backbone of tripeptide **17** is remarkably similar to the Z-shaped arrangement proposed for the bioactive conformation of Ac-CCK_{30-33} at CCK_2 receptors (Table 3). In addition, overlaying the two molecules in this way shows that the side chains of methionine in Ac-CCK_{30-33} and the (2-naphthyl)alanine side chains of **17** are also

Chart 1. Stereoisomeric Cholecystokinin Ligands

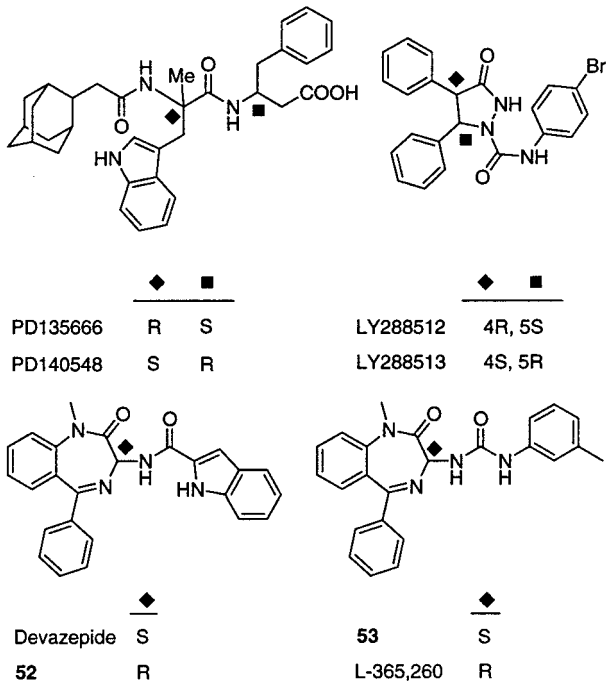


Table 5. CCK_1/CCK_2 Selectivities of Stereoisomeric Cholecystokinin Receptor Ligands Shown in Chart 1

compd	IC_{50} (nM)[a] CCK_1	CCK_2	CCK_1/CCK_2 selectivity	ref
PD 135666	25.5	0.15	0.005	30
PD 140458	2.8	259	100	30
LY288512	6400	370	0.05	21
LY288513	20500	19	0.0009	21
devazepide	0.08	270	3375	51
52	8.3	3700	445	51
53	3	150	50	19
L-365,260	280	2	0.007	19

[a] IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I]Bolton-Hunter CCK-8 to CCK receptors in the rat pancreas (CCK_1) or mouse cerebral cortex (CCK_2). Data taken from the reference specified.

located in similar regions of space relative to the backbone. However, the other functional groups are effectively distributed on opposite sides of the peptide backbones, as if they had been reflected through a mirror plane running along the plane of the backbone. Further investigations showed that the same pattern of behavior was also observed for compounds **7** and **12**, which were examined using the same protocol. In addition, we have shown that mimicking the arrangement of functional groups, described for the calculated global minima of these tripeptides, produced the new series of peptidomimetic CCK_1 antagonists related to **23**.

A number of independent reports have described opposite CCK_1/CCK_2 selectivities for enantiomeric pairs of peptoid,[30] benzodiazepine,[19,51] and diphenylpyrazolidinone[21] cholecystokinin ligands (Chart 1; Table 5). The structures of these compounds are highly diverse, and while they cannot be assumed to act at identical sites on the receptor, there is also no current evidence to the contrary. Nevertheless, we were intrigued by the possibility that this stereoselective behavior was a common theme that also applied to the peptide hormone itself. The absolute configurations of Ac-CCK_{30-33} and the tripeptide **17** are identical. However, the picture obtained from overlaying these two peptides (Figure 1) strongly suggests that the CCK_1 and CCK_2 receptors recognize enantiomeric dispositions of the tryptophan indole, aspartic acid carboxylate, and terminal phenyl groups. These results lead us to propose that this "functional chirality" forms the basis of the mechanism by which these two closely related receptor systems select between different conformations of this common fragment of their parent hormones.

Conclusion

We have speculated that the CCK_1 selectivity of simple peptide derivatives of CCK_{30-33} arises from the disposition of the side chain functional groups and noted that these mirror the arrangement proposed by Kolodziej et al.[39] for the bioactive conformation of Ac-CCK_{30-33} at CCK_2 receptors. In practice, there appear to be two dominant factors controlling the conformation of these molecules: namely hydrogen-bonding through the amide backbone and interaction of the aromatic rings as a result of hydrophobic collapse. The "functional chirality" inherent in the arrangement of the amino acid side chains may underpin the mechanism by which these closely related receptor systems bind CCK_{30-33}. It may also explain patterns of selectivity observed with optical isomers of several series of peptoid and nonpeptide ligands. We have exploited these features in the design of a novel series of selective CCK_1 antagonists. This led to the creation of peptidomimetics such as **33** which are potent CCK_1 antagonists ($pK_B = 7.4$) and are at least 250-fold selective for this receptor over the closely related CCK_2 receptor. Furthermore, no residual efficacy at CCK_2 receptors is evident as we have moved away from the "bioactive" conformation of CCK_{30-33} proposed by Kolodziej et al.[39] This result was achieved without introducing additional covalent bonds or macrocyclization. In general, there is a high level of consistency between the behavior of the original series of peptides and the new series of compounds. This suggests that our original proposal concerning the bioactive conformation of the former was reasonable. In addition, it should be possible to extend this principle to the design of novel CCK_2 receptor antagonists, using the literature model of Ac-CCK_{30-33}[39] as a template, although this corollary remains untested to date.

Experimental Section

General. Nuclear magnetic resonance spectra were recorded on either a Nicolet GE300 or Bruker DRX 300 machine. Elemental analyses were carried out at the London School of Pharmacy and all compounds gave analytical results within ±0.4% of the theoretical values. Flash column chromatography was performed using Merck Kieselgel 60 silica grade 9385.

BOC-*β*-benzyl-Asp-Phe-NH_2 (34). To a solution of phenylalaninamide hydrochloride (0.70 g, 3.5 mmol) and $NaHCO_3$ (0.29 g, 3.5 mmol) in water (5 mL) was added a solution of BOC-Asp(OBn)-NHS (1.47 g, 3.5 mmol) in DME (10 mL). The mixture was stirred at room temperature overnight, then acidified to pH 2 with 1 M HCl. Water (5 mL) was added and the mixture maintained at 0 °C for 2 h. The resultant white precipitate was filtered, washed with water, and dried to yield the title compound (1.43 g, 88%): ^{1}H NMR (DMSO-d_6) δ 7.7 (1H, d), 7.4 (1H,s), 7.35 (5H, s), 7.2 (7H, m), 5.0 (2H, s), 4.4 (1H, m), 4.3 (1H, m), 3.0 (1H, m), 2.8 (1H, m), 2.7 (1H, m), 2.5 (1H, m), 1.3 (9H, s).

BOC-Ala-Leu (35). To a solution of leucine (0.66 g, 5 mmol) and $NaHCO_3$ (0.42 g. 5 mmol) in water (10 mL) was added a solution of BOC-Ala-NHS (1.14 g, 4 mmol) in DME and the solution was stirred at room temperature for 3 h. The mixture was acidified to pH 2 with 1 M HCl and a further 10 mL water was added. On evaporation of the DME, an oil separated which was extracted into ethyl acetate, dried and evaporated to give the title compound (1.16 g, 96%): ^{1}H NMR (DMSO-d_6) δ 7.9 (1H, d), 6.8 (1H, d), 4.2 (1H, m), 4.0 (1H, m), 1.6 (1H, m), 1.5 (2H, m), 1.3 (9H, s), 1.1 (3H, d), 0.9 (3H, d), 0.8 (3H, d).

BOC-Ala-Leu *N*-Hydroxysuccinimide Ester (36). To a solution of **35** (1.14 g, 3.8 mmol) in dry DME (20 mL) were added *N*-hydroxysuccinimide (0.44 g, 3.8 mmol) and DCCI (0.78 g, 3.8 mmol) and the mixture stirred at 5 °C overnight. The precipitated dicyclohexylurea was removed by filtration and the filtrate evaporated to give 1.59 g of crude product, which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 8.4 (1H, d), 6.9 (1H, d), 4.6 (1H, m), 4.0 (1h, m), 2.8 (4H, s), 1.8−1.5 (3H, m), 1.3 (9H, s), 0.9 (3H, d), 0.8 (3H, d).

BOC-Ala-Leu-Asp(OBn)-Phe-NH_2 (37). Benzyl ester **34** (0.35 g, 0.75 mmol) was deprotected on stirring in trifluoroacetic acid (3 mL) for 1 h. The solvent was evaporated and the residue dissolved in DME (6 mL). NEt_3 (0.3 mL, 2.2 mmol) and NHS ester **36** (0.33 g, 0.75 mmol) were added and the mixture stirred at room temperature overnight. Water (10 mL) was added and the mixture stirred at 0 °C for a further 1 h. The resultant precipitate was filtered, washed with cold water, and dried. Recrystallization from ethanol/water gave 0.17 g (35%) of product: ^{1}H NMR (DMSO-d_6) δ 8.2 (1H, d), 7.8 (2H, m), 7.3 (5H, s), 7.1 (5H, m), 7.0 (1H, d), 5.0 (2H, s), 4.5 (1H, m), 4.3 (1H, m), 4.2 (1H, m), 3.9 (1H, m), 3.0 (2H, m), 2.8 (2H, m), 1.6 (1H, m), 1.3 (11H, s), 1.1 (3H, m), 0.8 (6H, m).

BOC-Ala-Leu-Asp-Phe-NH_2 (2). The benzyl ester **37** (0.24 g, 0.37 mmol) was dissolved in MeOH (30 mL) and stirred with 10% palladium-on-carbon (20 mg) under an atmosphere of hydrogen for 4 h. The catalyst was removed by filtration through a pad of Celite and the solvent evaporated. The residue was recrystallized from aqueous ethanol to yield the title compound (0.14 g, 68%): $[\alpha]^{20}_D = -46.5°$ (*c* 0.86, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.2 (1H, d), 7.8 (2H, m), 7.2 (7H, m), 7.0 (1H, t), 4.4 (1H, m), 4.3 (2H, m), 4.0 (1H, m), 3.0 (1H, m), 2.8 (1H, m), 2.6 (1H, m), 2.4 (1H, m), 1.6 (1H, m), 1.4 (11H, m), 1.1 (3H, m), 0.8 (6H, m). Anal. ($C_{27}H_{41}N_5O_8$) C, H, N.

BOC-Trp-Ala-H (38). To a solution of alanine (0.18 g, 2 mmol) and $NaHCO_3$ (0.34 g, 4 mmol) in water (5 mL) was added a suspension of BOC-Trp-NHS (0.8 g, 2 mmol) in ethanol (6 mL). The mixture was stirred at room-temperature overnight, the ethanol was evaporated and the residue was acidified to pH 2 with 1 M HCl. The separated oil was extracted into ethyl acetate, dried and evaporated to give 0.76 g crude product that was used without further purification: ^{1}H NMR (DMSO-d_6) δ 8.2 (1H, d), 7.6 (1H, d), 7.3 (1H, d), 7.1 (1H, s), 7.0 (2H, m), 6.7 (1H, d), 4.2 (2H, m), 3.1 (1H, m), 2.8 (1H, m), 1.25 (9H, s), 1.1 (3H, d).

BOC-Trp-Ala-NHS (39). To a solution of dipeptide **38** (0.75 g, 2 mmol) in dry DME (10 mL) were added *N*-hydroxysuccinimide (0.23 g, 2 mmol) and DCCI (0.41 g, 2 mmol), and the mixture stirred at 5 °C overnight. The precipitated dicyclohexylurea was removed by filtration and the filtrate evaporated. The crude product (0.98 g) was dissolved in a 1:1 mixture of CH_2Cl_2 and EtOAc (20 mL) and filtered through silica to afford 0.61 g (67%) of the title compound: ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.7 (1H, d), 7.6 (1H, d), 7.3 (1H, d), 7.1 (1H, s), 7.0 (2H, m), 6.7 (1H, d), 4.7 (1H, m), 4.2 (1H, m), 3.1 (1H, m), 2.8 (5H, m), 1.5 (3H, d), 1.25 (9H, s).

BOC-Trp-Ala-Asp(OBn)-Phe-NH_2 (40). Benzyl ester **34** (0.47 g, 1.0 mmol) was deprotected by stirring in trifluoroacetic acid (3 mL) for 1h. The solvent was evaporated and the residue was dissolved in DME (6 mL). NEt_3 (0.4 mL, 3 mmol) followed by **39** were added and the mixture stirred at room temperature overnight. Water (10 mL) was added and the mixture was stirred at 0 °C for a further 1 h. The resultant precipitate was filtered, washed with cold water, and dried. Recrystallization from ethanol/water gave 0.40 g (55%) of product: ^{1}H NMR (DMSO-d_6) δ 8.25 (1H, d), 8.0 (1H, d), 7.8 (1H, d), 7.6 (1H, d), 7.4−6.9 (12H, m), 6.85 (1H, d), 5.0 (2H, s), 4.6 (1H, m), 4.3 (1H, m), 4.2 (2H, m), 3.2−2.6 (6H, m), 1.3 (9H, s), 1.1 (3H, m).

BOC-Trp-Ala-Asp-Phe-NH_2 (3). The benzyl ester **40** (0.30 g, 0.4 mmol) was dissolved in MeOH (30 mL) and stirred with 10% palladium-on-carbon (30 mg) under an atmosphere of hydrogen for 4 h. The catalyst was removed by filtration and washed with hot MeOH. The filtrate was evaporated and the residue crystallized from aqueous MeOH to afford 0.16 g (64%) white solid: mp 213−215 °C; $[\alpha]^{20}_D = -21.2°$ (*c* 0.85, DMSO); ^{1}H NMR (DMSO-d_6) δ 8.2 (1H, d), 8.0 (1H, d), 7.8 (1H, d), 7.6 (1H, d), 7.3−6.9 (11H, m), 6.8 (1H, d), 4.5−4.0 (4H, m), 3.0 (2H, m), 2.8 (2H, m), 2.7−2.3 (2H, m), 1.2 (9H, s), 1.1 (3H, m). Anal. ($C_{32}H_{40}N_6O_8 \cdot 0.5H_2O$) C, H, N.

BOC-Trp-Leu-H (41). BOC-Trp-NHS was coupled to leucine using the method described for **38**: ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 7.05 (1H, d), 7.6 (1H, d), 7.3 (1H, d), 7.1−6.9 (3H, m), 6.7 (1H, d), 4.2 (2H, m), 3.0 (2H, m), 1.6 (1H, br s), 1.5 (2H, br s), 1.3 (9H, s), 0.8 (6H, m).

BOC-Trp-Leu-NHS (42). Prepared from **41** using the method described for **39**: ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.6 (1H, d), 6.9−7.1 (3H, m), 6.8 (1H, d), 4.7 (1H, m), 4.2 (1H, m), 3.0 (2H, m), 2.8 (4H, s), 1.6 (1H, m), 1.5 (2H, m), 1.3 (9H, s), 0.9 (6H, dd).

BOC-Asp(OBn)-Ala-NH_2 (43). Prepared according to the method given for **34** except that Ala-NH_2 hydrochloride was used in place of Phe-NH_2 hydrochloride: yield (65%); ^{1}H NMR (DMSO-d_6) δ 7.8 (1H, s), 7.3 (6H, s), 7.2 (1H, d), 7.0 (1H, s), 5.0 (2H, s), 4.3 (1H, m), 4.1 (1H, m), 2.3 (2H, m), 1.3 (9H, s), 1.1 (3H, d).

BOC-Trp-Leu-Asp(OBn)-Ala-NH_2 (44). Benzyl ester **43** was deprotected and coupled to NHS ester **42** following the method described for **40**. The product was recrystallized from aqueous MeOH: yield 61%; ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 7.9 (1H, d), 7.8 (1H, d), 7.6 (1H, d), 7.3 (6H, m), 7.0 (5H, m), 5.0 (2H, s), 4.6 (1H, m), 4.3 (1H, m), 4.1 (2H, m), 2.8 (4H, m), 1.6 (1H, br s), 1.65 (2H, m), 1.6 (2H, m), 1.3 (9H, s), 1.2 (3H, d), 0.8 (6H, m).

BOC-Trp-Leu-Asp-Ala-NH_2 (5). Using the method described for compound **2**, benzyl ester **44** was hydrogenolysed to give the title compound: yield 96%; $[\alpha]^{20}_D = -50.9°$ (*c* 0.31, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.3 (1H, d), 7.9 (1H, d), 7.8 (1H, d), 7.6 (1H, d), 7.3 (1H, d), 7.2 (1H, s), 7.0 (4H, m), 6.8 (1H, d), 4.5 (1H, m), 4.3 (1H, m), 4.1 (2H, m), 3.1−2.6 (4H, m), 1.6 (1H, m), 1.4 (2H, m), 1.25 (9H, s), 1.2 (3H, d), 0.8 (6H, m). Anal. ($C_{29}H_{42}N_6O_8$) C, H, N.

BOC-Trp-Leu-Ala-Phe-NH_2 (4). Dipeptide **45** was prepared from BOC-Ala-NHS and Phe-NH_2·hydrochloride, according to the method given for **34**, and then coupled to **42**: yield 72%; ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.0 (1H, d), 7.9 (1H, d), 7.8 (1H, d), 7.6 (1H, d), 7.2 (11H, m), 6.8 (1H, d), 4.3 (2H, m), 4.2 (2H, m), 3.1−2.7 (4H, m), 1.6−1.0 (15H, m), 0.8 (6H, m). Anal. ($C_{34}H_{46}N_6O_6 \cdot 0.5H_2O$) C, H, N.

BOC-Trp-3-(2-naphthyl)alanine-NHS (46). Using the method described for **35**, BOC-Trp-NHS was coupled to L-3-(2-naphthyl)alanine. The NHS ester **46** was then prepared using the method described for **36**: yield 98%; ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.8 (1H, d), 7.8 (4H, m), 7.5 (4H, m), 7.3 (1H, m), 7.0 (3H, m), 6.7 (1H, d), 5.1 (1H, m), 4.2 (1H, m), 2.8 (4H, s), 2.6−3.3 (4H, m), 1.2 (9H, s).

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-Phe-NH_2 (6). Benzyl ester **34** was deprotected using trifluoroacetic acid and coupled to NHS ester **46** following the method described for **37**. The product was recrystallized from EtOH−H_2O: yield 65%; ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.5 (1H, d), 8.0 (2H, d), 7.8 (4H, m), 7.5−6.8 (20H, m), 5.0 (2H, s), 4.6 (2H, m), 4.4 (1H, m), 4.1 (1H, m), 3.2−2.5 (8H, m), 1.2 (9H, s).

The resulting benzyl ester (0.35 g, 0.4 mmol) was then dissolved in MeOH (40 mL) and AcOH (0.5 mL) and a catalytic amount of 10% palladium-on-carbon added. The mixture was stirred under an atmosphere of hydrogen overnight. The catalyst was removed by filtration and the filtrate evaporated to give the title compound (0.28 g, 90%). An analytically pure sample of **6** was obtained by recrystallization from EtOH−

H_2O: $[\alpha]^{20}_D = -21.0°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.5 (1H, d), 8.0 (2H, m), 7.7 (4H, m), 7.5−6.9 (15H, m), 6.8 (1H, d), 4.7 (1H, m), 4.5 (1H, m), 4.4 (1H, m) 4.1 (1H, m), 3.2−2.6 (8H, m) 1.2 (9H, s). Anal. ($C_{42}H_{46}N_6O_8 \cdot 2.8H_2O \cdot 1.0$EtOH) C, H, N.

BOC-Asp(OBn)-2-phenylethylamide (47). A solution of BOC-Asp(OBn)-NHS (1.0 g, 2.4 mmol) and 2-phenylethylamine (0.3 mL, 2.4 mmol) in dry DME (15 mL) was stirred at room temperature overnight. The mixture was poured into water (50 mL) and the resultant precipitate was filtered, washed with water and dried to yield the title compound (0.88 g, 84%): ^{1}H NMR (DMSO-d_6) δ 7.9 (1H, t), 7.4−7.2 (10H, m), 7.1 (1H, d), 5.1 (2H, s), 4.3 (1H, m), 3.3 (2H, m), 2.7 (2H, m), 2.6 (2H, m), 1.4 (9H, s).

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-phenylethylamide (7). Benzyl ester **47** was deprotected using trifluoroacetic acid and coupled to NHS ester **46** in DME containing NEt_3 following the method described for **37**. The crude product was purified by column chromatography then hydrogenolysed following the procedure used for the preparation of example **2**: $[\alpha]^{20}_D = -8.9°$ (*c* 0.9, DMSO); ^{1}H NMR (DMSO-d_6) δ 10.9 (1H, s), 8.4 (1H, m), 8.1 (2H, m), 7.9−6.8 (17H, m), 6.7 (1H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.5−2.3 (10H, m), 1.2 (9H, s). Anal. ($C_{41}H_{45}N_5O_7$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-Phe-NHMe (8). Prepared following the methods described for **7**, except that Phe-NHMe was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -70.0°$ (*c* 0.21, EtOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (2H, m), 7.7 (4H, m), 7.4 (4H, m), 7.2 (7H, m), 6.9 (4H, m), 4.6 (1H, m), 4.5 (1H, m), 4.3 (1H, m), 4.0 (1H, m), 2.9 (8H, m), 2.5 (3H, d), 1.2 (9H, s). Anal. ($C_{43}H_{47}N_6O_8$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-Phe-NMe$_2$ (9). Prepared following the methods described for **7**, except that Phe-NMe$_2$was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -37.7°$ (*c* 0.37, EtOH); ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.9 (1H, d), 7.7 (4H, m), 7.4 (3H, m), 7.2 (7H, m), 6.9 (4H, m), 4.8 (1H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.0 (6H, m), 2.7 (6H, d), 2.6 (2H, m), 1.2 (9H, s). Anal. ($C_{44}H_{45}N_6O_8 \cdot 2.5H_2O$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-phenylalaninol (10). Prepared following the methods described for **7**, except that L-phenylalaninol was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -60.0°$ (*c* 0.20, EtOH); ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.4 (1H, d), 7.9 (1H, d), 7.7 (5H, m), 7.2 (10H, m), 7.0 (2H, m), 6.9 (2H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.8 (1H, m), 3.2 (2H, m), 2.8 (8H, m), 1.2 (9H, s). Anal. ($C_{42}H_{47}N_5O_8$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-(4-methoxy)Phe-NH$_2$ (11). Prepared following the methods described for **7**, except that L-(4-methoxy)Phe-NH$_2$ was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -45.7°$ (*c* 0.33, EtOH); ^{1}H NMR (DMSO-d_6) δ 10.7 (1H, s), 8.5 (1H, d), 7.9 (2H, m), 7.8 (4H, m), 7.4 (6H, m), 7.0 (6H, m), 6.8 (3H, m), 4.6 (1H, m), 4.5 (1H, m), 4.3 (1H, m), 4.1 (1H, m), 3.5 (3H, m), 2.8 (8H, m), 1.2 (9H, s). Anal. ($C_{43}H_{48}N_6O_9 \cdot 1.0H_2O$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(4-methoxyphenyl)ethylamide (12). Prepared following the methods described for **7**, except that 2-(4-methoxyphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -30.6°$ (*c* 0.46, EtOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.7 (5H, m), 7.4 (5H, m), 7.0 (4H, m), 6.9 (2H, m), 6.8 (2H, m), 4.6 (1H, m), 4.4 (1H, m), 4.1 (1H, m), 3.6 (3H, s), 3.1 (2H, m), 2.8 (6H, m), 2.6 (2H, m), 1.2 (9H, s). Anal. ($C_{42}H_{47}N_5O_8 \cdot 0.5H_2O$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-L-(4-chloro)Phe-NH$_2$ (13). Prepared following the methods described for **7**, except that L-(4-chloro)Phe-NH$_2$ was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -7.8°$ (*c* 0.13, DMF); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.5 (1H, d), 8.0 (2H, m), 7.8 (4H, m), 7.3 (11H, m), 7.0 (2H, m), 6.9 (1H, m), 6.8 (1H, d), 4.7 (1H, m), 4.6 (1H, m), 4.4 (1H, m), 4.1 (1H, m), 2.8 (8H, m), 1.2 (9H, s). Anal. ($C_{42}H_{45}ClN_6O_8$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(4-chlorophenyl)-ethylamide (14). Prepared following the methods described for **7**, except that 2-(4-chlorophenyl)ethylamine was used in place of 2-phenylethylamine: ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.8 (5H, m), 7.3 (7H, m), 7.2 (2H, m), 7.0 (2H, m), 6.9 (2H, m), 4.6 (1H, m), 4.5 (1H, m), 4.2 (1H, m), 3.2 (4H, m), 2.8 (6H, m), 1.2 (9H, s). Anal. ($C_{41}H_{44}ClN_5O_7$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(2-methoxyphenyl)ethylamide (15). Prepared following the methods described for **7**, except that 2-(2-methoxyphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -35.5°$ (*c* 0.45, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.8−6.7 (18H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.7 (3H, s), 3.1 (2H, m), 3.1−2.4 (8H, m), 1.2 (9H, s). Anal. ($C_{42}H_{47}N_5O_8 \cdot 0.8H_2O$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(3-methoxyphenyl)ethylamide (16). Prepared following the methods described for **7**, except that 2-(3-methoxyphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -30.6°$ (*c* 0.49, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.9−6.7 (18H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.7 (3H, s), 3.5−2.6 (10H, m), 1.2 (9H, s). Anal. ($C_{42}H_{47}N_5O_8 \cdot 0.8H_2O$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(3,4-dimethoxyphenyl)ethylamide (17). Prepared following the methods described for **7**, except that 2-(3,4-dimethoxyphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -42.0°$ (*c* 0.45, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.9−6.5 (17H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.7 (3H, s), 3.6 (3H, s), 3.5−2.4 (10H, m), 1.2 (9H, s). Anal. ($C_{43}H_{49}N_5O_9$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(4-fluorophenyl)-ethylamide (18). Prepared following the methods described for **7**, except that 2-(4-fluorophenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -38.7°$ (*c* 0.98, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.9−6.7 (18H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.2−2.5 (10H, m), 1.2 (9H, s). Anal. ($C_{41}H_{44}FN_5O_7$) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(4-aminophenyl)-ethylamide (19). Prepared following the methods described for **7**, except that 2-(4-aminophenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -20.4°$ (*c* 0.49, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.9−6.8 (14H, m), 6.8 (2H, m), 6.4 (2H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.3−2.3 (10H, m), 1.2 (9H, s). Anal. ($C_{41}H_{46}N_6O_7 \cdot 0.7$EtOAc) C, H, N.

BOC-Trp-L-3-(2-naphthyl)alanyl-Asp-2-(3-trifluoromethylphenyl)ethylamide (20). Prepared following the methods described for **7**, except that 2-(3-trifluoromethylphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = -40.3°$ (*c* 0.77, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4 (1H, d), 8.0 (1H, d), 7.9−6.8 (20H, m), 4.6 (1H, m), 4.5 (1H, m), 4.1 (1H, m), 3.4−2.5 (10H, m), 1.2 (9H, s). Anal. ($C_{42}H_{44}F_3N_5O_7 \cdot 0.5H_2O$) C, H, N.

BOC-Trp-2-(2-naphthyl)ethylamide (48). BOC-Trp-NHS (1.20 g, 3.0 mmol) was added to a solution of 2-(2-naphthyl)-ethylamine (0.51 g, 3.0 mmol) in dry DME. The mixture was stirred at room temperature for 2 h and partitioned between 1 M HCl (100 mL) and EtOAc (50 mL). The aqueous phase was then washed with a further portion of EtOAc and the combined organic layers washed with H_2O (3 × 50 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash column chromatography, on silica gel with 2% MeOH−CH_2Cl_2, to give the title compound as a white foam (1.30 g 95%): ^{1}H NMR ($CDCl_3$) δ 7.95 (1H, s), 6.9−7.8 (12H, m), 5.7 (1H, bs), 5.15 (1H, bs), 4.4 (1H, m, CH), 3.45 (2H, m), 3.3 (1H, m), 2.7 (1H, m), 2.7 (2H, m), 1.4 (9H, s).

***N*-Succinyl-Trp-2-(2-naphthyl)ethylamide (49).** Compound **48** (0.5 g, 1.1 mmol) was treated with trifluoroacetic acid (5 mL) and the mixture stirred for 40 min at room temperature. The solvent was then removed in vacuo and the crude salt taken up in dry THF. The solution was then treated with NEt_3 (0.4 mL) followed by succinic anhydride (0.12 g, 1.2 mmol) and a catalytic amount of 4-(dimethylamino)pyridine. The reaction mixture was stirred at room temperature for 18

h before removal of the solvent in vacuo. The residue was then dissolved in CH_2Cl_2 (40 mL), washed with 1 M HCl (2 × 30 mL) and H_2O (30 mL) and the organic phase dried ($MgSO_4$), and concentrated to give the crude product which was used directly in the next stage (0.47 g, 94%): ^{1}H NMR (DMSO-d_6) δ 12.1 (1H, bs), 10.8 (1H, s), 8.0−6.9 (14H, m), 4.4 (1H, m), 3.6−2.2 (10H, m).

BOC-D-Asp(OBn)-2-phenylethylamide (50). 2-Phenylethylamine (0.89 mL, 7.1 mmol) was added to BOC-D-Asp(OBn)-H (2.3 g, 7.0 mmol) and DCCI (1.5 g, 7.5 mmol) in anhydrous CH_2Cl_2 (50 mL) at −10 °C under argon. The reaction mixture was stirred at −10 °C for 45 min, than at 4 °C for 64 h, then filtered and evaporated to dryness. The residue was dissolved in EtOAc, filtered and evaporated to dryness yielding a yellow oil. Trituration with ether gave a white amorphous solid (1.2 g). The concentrated liquors were chromatographed on silica gel with acetone−toluene eluant yielding a further 0.6 g of product (total yield 61%): ^{1}H NMR (DMSO-d_6) δ 7.85 (1H, t), 7.3 (5H, s), 7.2 (5H, m), 7.1 (1H, t), 5.05 (2H, s), 4.25 (1H, q), 3.2 (2H,m), 2.6 (4H, m), 1.3 (9H, s).

***N*-(Succinyl-D-Asp(OBn)-2-phenylethylamido)-Trp-2-(2-naphthyl)ethylamide (51).** Benzyl ester **50** (0.25 g, 0.6 mmol) was treated with trifluoroacetic acid (3 mL) and the mixture stirred at room temperature for 1 h before removal of the solvent in vacuo. The residue was then dissolved in CH_2Cl_2 (5 mL) and treated sequentially with $^iPr_2NEt$ (0.3 mL, 1.7 mmol), **49** (0.27 g, 0.6 mmol) and (benzotriazol-1-yloxy)-trispyrrolidinophosphonium hexafluorophosphate (pyBOP; 0.31 g, 0.6 mmol). The mixture was stirred for 1 h at room temperature and the solvent removed in vacuo. The residue was then taken up in EtOAc (30 mL) and washed with 5% $KHSO_4$ (3 × 20 mL), $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was then dried ($MgSO_4$) and evaporated to give the crude product which was purified by flash column chromatography on silica gel in 5% MeOH−CH_2Cl_2 to give the title compound as a white solid (0.16 g, 37%): ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (1H, d), 8.1 (1H, d), 8.0 (1H, t), 7.9 (1H, t), 7.8 (2H, m), 7.6 (1H, s), 7.5−6.8 (19H, m), 5.0 (2H, s), 4.6 (1H, m), 4.4 (1H, m), 3.4−2.2 (16H, m).

***N*-(Succinyl-D-Asp-2-phenylethylamido)-Trp-2-(2-naphthyl)ethylamide (23). 51** (0.23 g, 0.3 mmol) was dissolved in MeOH (50 mL) with warming. The solution was then cooled to room temperature and stirred under 1 atm of hydrogen in the presence of a catalytic amount of 10% palladium on charcoal for 18 h. The mixture was then filtered through Celite, and the filtrate concentrated in vacuo to give the title compound as a white solid (0.15 g, 74%): $[\alpha]^{20}_D = +19.0°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (2H, m), 8.05 (1H, t), 7.9 (1H, t), 7.8 (3H, m), 7.6 (1H, s), 7.4 (3H, m), 7.35−6.9 (10H, m); 4.45 (2H, m), 3.4−2.2 (16H, m). Anal. ($C_{39}H_{41}N_5O_6 \cdot 0.65H_2O$) C, H, N.

***N*-[Succinyl-D-Asp-2-(4-fluorophenyl)ethylamido]-Trp-2-(2-naphthyl)ethylamide (24)**. The compound was prepared as in example **23**, except that 2-(4-fluorophenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = +15.0°$ (*c* 0.83, DMSO); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (2H, m), 8.1 (1H, t), 8.0 (1H, t), 7.8 (3H, m), 7.6 (1H, s), 7.5 (3H, m), 7.3 (2H, m), 7.1 (2H, m), 7.0 (4H, m), 6.9 (1H, m), 4.45 (2H, m), 3.4−2.3 (16H, m). Anal. ($C_{39}H_{40}FN_5O_6$) C, H, N.

***N*-[Succinyl-D-Asp-2-(4-methoxyphenyl)ethylamido]-Trp-2-(2-naphthyl)ethylamide (25).** The compound was prepared as in example **23**, except that 2-(4-methoxyphenyl)-ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = +15°$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H,s), 8.2−7.9 (4H, m), 7.8−6.7 (16H, m), 4.5 (2H, m), 3.7 (3H, s), 3.4−2.2 (16H, m). Anal. ($C_{40}H_{43}N_5O_7$) C, H, N.

***N*-[Succinyl-D-Asp-2-(3-trifluoromethylphenyl)ethylamido]-Trp-2-(2-naphthyl)ethylamide (26).** The compound was prepared as in example **23**, except that 2-(3-trifluoromethylphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = +13.0°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.1−7.95 (4H, m), 7.9 (3H, m), 7.6 (1H, s), 7.5−7.3 (9H, m), 7.1−6.9 (3H, m), 4.45 (2H, m), 3.4−2.2 (16H, m). Anal. ($C_{40}H_{40}F_3N_5O_6 \cdot 1.5H_2O$) C, H, N.

***N*-[Succinyl-D-Asp-2-(3,4-dichlorophenyl)ethylamido]-Trp-2-phenylethylamide (27).** The compound was prepared as in example **23**, except that 2-(3,4-dichlorophenyl)ethylamine was used in place of 2-(2-naphthyl)ethylamine and 2-(4-methoxyphenyl)ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = +18.9°$ (*c* 0.74, DMF); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (2H, dd), 7.95 (2H, dt), 7.5 (1H, d), 7.3 (3H, m), 7.1 (4H, m), 7.0 (1H, t), 6.8 (2H, d), 4.4 (2H, m), 3.7 (3H, s), 3.2 (4H, m), 3.1 (2H, m), 2.8 (2H, m), 2.6 (4H, m), 2.4 (4H, m). Anal. ($C_{36}H_{39}Cl_2N_5O_7 \cdot 4.0H_2O$) C, H, N.

***N*-[Succinyl-D-Asp-2-(4-methoxyphenyl)ethylamido]-Trp-2-phenylethylamide (28).** The compound was prepared as in example **23**, except that 2-phenylethylamine was used in place of 2-(2-naphthyl)ethylamine and 2-(4-methoxyphenyl)-ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = 17.7°$ (*c* 0.8, DMF); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (2H, dd), 8.0 (2H, dt), 7.55 (1H, s), 7.3 (1H, d), 7.2 (2H, d), 7.1 (7H, m), 7.0 (1H, t), 6.8 (2H, d), 4.45 (2H, m), 3.7 (3H, s), 3.2 (8H, m), 2.6 (4H, t), 2.4 (4H, m). Anal. ($C_{36}H_{41}N_5O_7 \cdot 0.6CH_2Cl_2$) C, H, N.

***N*-(Succinyl-D-Asp-2-phenylethylamido)-Trp-(2-methyl)propylamide (29).** The compound was prepared as in example **23**, except that 2-methylpropylamine was used in place of 2-(2-naphthyl)ethylamine and 2-(4-methoxyphenyl)-ethylamine was used in place of 2-phenylethylamine: $[\alpha]^{20}_D = +21.4°$ (*c* 0.7, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (2H, dd), 7.9 (2H, q), 7.6 (1H, d), 7.3 (1H, d), 7.1 (5H, m), 6.8 (2H, d), 4.4 (2H, t), 3.7 (3H, s), 3.2 (4H, m), 2.9 (2H, m), 2.5 (4H, m), 2.4 (2H, m), 1.6 (1H, m), 0.8 (6H, dd). Anal. ($C_{32}H_{41}N_5O_7$) C, H, N.

***N*-(1,2-*cis*-Cyclohexanedicarboxyl-D-Asp-2-phenylethylamido)-Trp-2-(2-naphthyl)ethylamide (30a,b)**. The compounds were prepared as in example **23**, except that (±)-1,2-*cis*-cyclohexanedicarboxylic anhydride was used in place of succinic anhydride. The two diastereoisomeric benzyl esters were separated by flash column chromatography on silica gel, using toluene/EtOAc (1:1), before being hydrogenated separately to give examples **30a**,**b**. **30a**: $[\alpha]^{20}_D = +8.2°$ (*c* 0.73, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H s), 8.1 (1H, t), 8.0 (3H, m), 7.8 (3H, m), 7.6 (1H. s), 7.5−6.9 (13H, m), 4.5 (1H, m), 4.4 (1H, m), 3.4−2.5 (12H, m), 1.8−1.2 (10H, m). Anal. ($C_{43}H_{47}N_5O_6$) C, H, N. **30b:** $[\alpha]^{20}_D = +5.3°$ (*c* 0.76, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.1−7.7 (6H, m), 7.6 (1H, s), 7.45 (4H, m), 7.4−6.9 (10H, m), 4.4 (2H, m), 3.5−2.4 (12H, m), 2.2−1.2 (10H, m). Anal. ($C_{43}H_{47}N_5O_6 \cdot 3.5H_2O$) C, H, N.

***N*-[(±)-1,2-*cis*-Cyclohexanedicarboxyl-D-Asp-2-(4-fluorophenyl)ethylamido]-Trp-2-(2-naphthyl)ethylamide (31).** The compound was prepared as in example **23**, except that (±)-1,2-*cis*-cyclohexanedicarboxylic anhydride was used in place of succinic anhydride and 2-(4-fluorophenyl)ethylamine was used in place of 2-phenylethylamine: ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.4−6.9 (20H, m), 4.4 (2H, m), 3.5−2.2 (12H, m), 2.0−1.2 (10H, m). Anal. ($C_{43}H_{46}FN_5O_6 \cdot 2.2H_2O$) C, H, N.

***N*-[(±)-1,2-*cis*-Cyclohexanedicarboxyl-D-Asp-2-(4-methoxyphenyl)ethylamido]-Trp-2-(2-naphthyl)ethylamide (32)**. The compound was prepared as in example **23**, except that (±)-1,2-*cis*-cyclohexanedicarboxylic anhydride was used in place of succinic anhydride and 4-methoxyphenylethylamine was used in place of 2-phenylethylamine: ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (3H, m), 7.8 (3H, m) 7.7 (1H, s), 7.4 (5H, m), 7.1 (5H, m), 6.8 (2H, m), 4.5 (1H, m), 4.4 (1H, m), 4.1 (1H, m), 3.7 (3H, s), 3.2−2.3 (11H, m), 1.9−1.1 (11H, m). Anal. ($C_{44}H_{49}N_5O_7 \cdot 2.0H_2O \cdot 0.8CH_2Cl_2$) C, H, N.

***N*-[(±)-1,2-*trans*-Cyclohexanedicarboxyl-D-Asp-2-(4-methoxyphenyl)ethylamido]-Trp-2-(2-naphthyl)ethylamide (33).** The compound was prepared as in example **23**, except that (±)-1,2-*trans*-cyclohexanedicarboxylic anhydride was used in place of succinic anhydride and 4-methoxyphenylethylamine was used in place of 2-phenylethylamine: ^{1}H NMR (DMSO-d_6) δ 10.8 (1H, s), 8.2 (2H, m), 7.8−6.7 (18H, m), 4.5−4.35 (2H, m), 3.7 (3H, s), 3.2−2.4 (12H, m), 1.9−1.3 (10H, m). Anal. ($C_{44}H_{49}N_5O_7 \cdot 4.0H_2O \cdot 0.3CH_2Cl_2$) C, H, N.

Biological Methods. Materials: BOC-pentagastrin (Sigma) was dissolved in DMF to a concentration of 40 mM serially

diluted to 4 mM in DMF and subsequently in water to 0.4 μM. CCK-8S (Genosys) was diluted in water to a concentration of 2 mM and subsequently serially diluted in water to 20 nM.

Protocols: At least eight preparations were used simultaneously in each assay and randomized block designs were used throughout for the allocation of experimental treatments to each organ bath. Test compound or vehicle was incubated for 1 h before a single cumulative *E*/[*A*] curve was obtained. In all experiments the total volume of drug added to the organ baths did not exceed 700 μL in a single experiment.

Guinea-pig gall bladder muscle strip CCK_1 bioassay: The CCK_1 receptor gall bladder assay was performed as described previously.[51] In brief, four longitudinal strips of smooth muscle were dissected from each gall bladder taken from male Dunkin-Hartley guinea-pigs (250−500 g). The strips were suspended in 20 mL organ baths maintained at 29 °C in a low Ca^{2+} Krebs−Henseleit solution and gassed with 95% O_2/5% CO_2. Following the application of a single 1 g load, the tissues were allowed to relax until a stable baseline was produced and the preparations were washed twice during this period. Force was continuously recorded with an isometric transducer and responses expressed as changes in mm chart record, where 100 mm is equal to 1 g force in all experiments.

Isolated, lumen-perfused mouse stomach CCK_2 assay: Gastric acid secretion was measured in isolated, lumen-perfused mouse stomachs, prepared essentially as described previously.[40] Male mice (Charles River CD1, 22−26 g, 18 h fasted, water ad libitum) were used. After sacrifice, the stomach was cannulated via the duodenal sphincter. The esophagus was ligated at the level of the cardiac sphincter and the stomach excised. A small incision was made in the fundic region, a cannula ligated tightly into the incision, and the contents of the stomach flushed through with mucosal solution to remove any remaining food. The stomach was placed in an organ bath containing 40 mL of buffered serosal solution. The serosal solution was maintained at 37 ± 1 °C and gassed vigorously with 95% O_2 and 5% CO_2. The stomachs were perfused with mucosal solution gassed with 100% O_2 at a rate of 1 mL min^{-1} and the perfusate passed over an internally referenced pH electrode which was placed 12 cm above the stomach to provide a back pressure to distend the stomach. 3-Isobutylmethylxanthine (0.3 mM) was added to the serosal solution because it appeared that phosphodiesterase inhibition improved the signal-to-noise ratio in preliminary experiments when BOC-pentagastrin was used as the agonist. The preparations were allowed to stabilize for 90 min before the addition of drugs to the serosal solution in the organ bath. Agonist-stimulated responses were expressed as the change in the pH of the lumen-perfusate from the basal pH immediately prior to the first addition of agonist. A continuous record of pH was obtained from chart recorders coupled to a pH electrode amplifier (Fylde Scientific). Individual agonist concentration−effect curves were fitted to the Hill equation, as described previously.[40] The effect of drug treatment was assessed by one-way analysis of variance (ANOVA) and the Bonferroni modified *t*-test for multiple comparisons.[52] *P*-values of less than 0.05 were considered to be significant.

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