Synthesis and Biological Properties of New Constrained CCK-B Antagonists: Discrimination of Two Affinity States of the CCK-B Receptor on Transfected CHO Cells

Bruno Bellier, Isabelle McCort-Tranchepain, Bertrand Ducos, Sophie Danascimento, Hervé Meudal, Florence Noble, Christiane Garbay, and Bernard P. Roques*

Département de Pharmacochimie Moléculaire et Structurale, U266 INSERM - URA D1500, CNRS, UFR des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, 75270 Paris Cedex 06, France

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To improve our knowledge of the bioactive conformation of CCK-B antagonists, we have developed a new series of constrained dipeptoids whose synthesis and biochemical properties are reported here. These compounds, of general structure N^{α}-[(2-adamantyloxy)carbonyl]-α-methyltryptophanyl-(4-X)-proline, were designed by introducing a cyclization in the structure of the previously described CCK-B/peptoid antagonist RB 210, *N*-[*N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-(2-phenylethyl)glycine (Blommaert et al. *J. Med. Chem.* **1993**, *36*, 2868−2877), by means of a five-membered ring. Structure−affinity relationship studies showed that an *R* configuration of Trp-C^{α} and a *cis* configuration of the pyrrolidine substituents were favorable for receptor recognition. The most potent compounds of this new series had similar affinities for the CCK-B receptor as RB 210 and proved to be far more efficient in inhibiting inositol phosphate production in CHO cells stably transfected with rat brain CCK-B receptor, with IC_{50} values approaching those of the commonly used antagonists L-365,260 and PD-134,-308. Moreover, binding studies performed using transfected CHO cells showed that two affinity states of the CCK-B receptor can be discriminated by some of these compounds which also have different biological profiles and are therefore highly interesting tools for the biochemical and pharmacological characterization of CCK-B receptor heterogeneity.

Introduction

Cholecystokinin (CCK), or more precisely its C-terminal fragment CCK_8 (Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-PheNH_2), has been reported to be involved, through its interaction with the CCK-B receptor, in analgesia, anxiety, memory processes, and neuropsychiatric disorders.[1] Moreover, CCK-B receptor antagonists were also shown to suppress panic attacks induced in humans by CCK_4[2] and to potentiate antinociceptive responses induced by exogenous opiates such as morphine[3] or by endogenous opioids.[4] Such compounds also exert antidepressant effects in rodents.[5] Therefore, the search for potent and selective CCK-B antagonists, endowed with good affinities, efficacies, and bioavailabilities, remains crucial for further pharmacological and clinical investigations of such compounds. Recent binding studies using the CCK-B antagonist 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)[6] suggested the possible occurrence of two antagonist binding subtypes of the CCK-B receptor in rats and guinea pigs,[7] which do not seem to be due to receptor structural heterogeneity, as no experimental evidence has been found to date to suggest its existence.[8]

We have previously prepared and studied peptidomimetic CCK-B antagonists designated RB 210 and RB 211 (*N*-[*N*-[(2-adamantyloxy)carboxyl]-D-α-methyltryptophanyl]-*N*-[2-(4-chlorophenyl)ethyl]glycine).[9] These compounds have smaller chemical structures than the related dipeptoid PD-134,308 ([*R*-(*R**,*R**)]-4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxobutanoic acid)[10] and were shown to be resistant to peptidases and endowed with high affinity and selectivity for CCK-B receptors. In addition, RB 211 appeared to be much more efficient in crossing the blood−brain barrier than L-365,260 and PD-134,308[11] and was devoid of the weak CCK-A agonist properties of dipeptoids.[12a,b]

To improve the properties of PD-134,308, numerous conformational restrictions were introduced in its structure. Unfortunately, neither N-terminal cyclization[13] nor macrocyclization,[14a,b] or rigidification of the amide bond,[14c] led to any significant positive result. Only a C-terminal cyclization of PD-134,308 derivatives, by means of a tetrahydronaphthyl group, has been reported to increase the affinity for CCK-B receptors[15] (Figure 1).

In this study, we made the hypothesis that this latter approach would also be valid for compounds such as RB 210, in which C-terminal constraints can be easily introduced. Thus, the β carbon of the phenethyl side chain of RB 210 was linked to the α carbon bearing the carbonyl function, by means of a methylene bridge. This resulted in the formation of a proline ring (Figure 1). The corresponding peptidomimetics $_2$Adoc-αMe-Trp-Pro-(4-X) provide tools to probe the CCK-B receptor for its limits of steric tolerance. To evaluate their selectivity toward CCK-B versus CCK-A receptors, the binding properties of the new dipeptoids were determined on both guinea pig brain and pancreatic membranes. The affinities of the most promising compounds were also measured on homogenates of CHO cells transfected with the rat brain CCK-B receptor[16] and compared to those

* To whom correspondence should be addressed. Tel: (33) 01-43-25-50-45. Fax: (33) 01-43-26-69-18.

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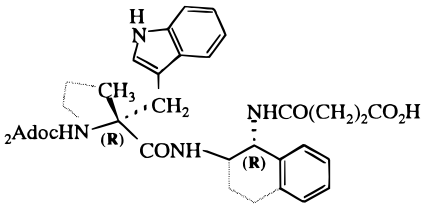
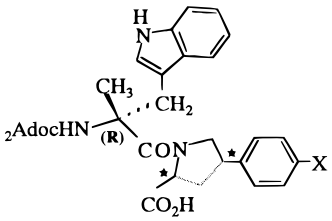


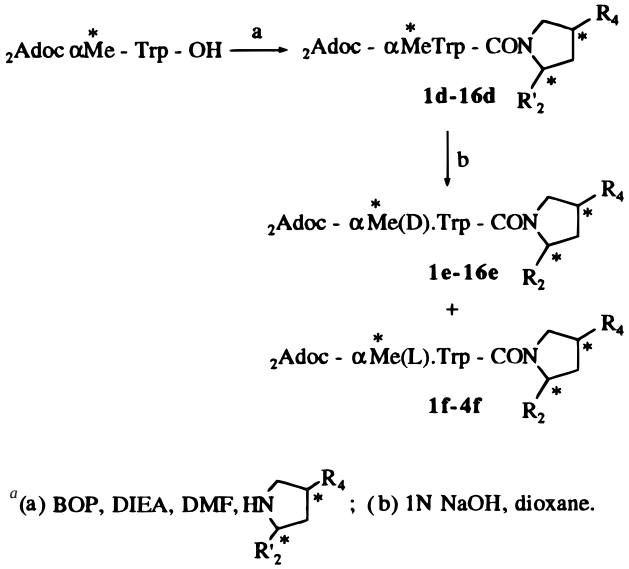
PD 134-308



RB 210 X = H
RB 211 X = Cl

Figure 1. Structures of PD-134,308, RB 210, and RB 211. Dotted lines show schematically the cyclizations done to obtain constrained derivatives of PD-134,308[13,15] and RB 210 (this study). Chiral centers created in the new series are shown by an asterisk.

Scheme 1[a]



[a] (a) BOP, DIEA, DMF, HN(R'2)(R4); (b) 1N NaOH, dioxane.

$_2$Adoc-αMe-tryptophan was used as a racemate for compounds **1-4** and as pure D-enantiomer for compounds **5-16**. For R_2, R'_2, and R_4 see Schemes 2 and 3 and Tables 1-3.

of reference antagonists. Their antagonist potency was also evaluated by an inositol phosphate assay performed using the same cells.

Chemistry

All the final compounds listed in Tables 1−3 were prepared according to Scheme 1, by coupling $_2$Adoc-αMe-Trp under racemic or pure D form with modified proline derivatives which were synthesized according to Schemes 2 and 3. The three commercially available hydroxyproline isomers provided starting compounds for the stereochemically controlled synthesis of 4-substituted prolines.[17a,b] Scheme 2 illustrates the preparation of several compounds by nucleophilic substitution of protected *cis*- and *trans*-hydroxyproline derivatives (*cis* and *trans* refer to the relative spatial disposition of the 4-substituent and the 2-carboxyl group relative to the pseudoplanar pyrrolidine ring). Compounds **13c** and **14c** were obtained by methods reported earlier.[18a,b]

Because D-*trans*-hydroxyproline is not commercially available, compounds **4c**−**11c** were obtained after two successive stereochemical inversions at the level of the C_4. Thus, a classical Mitsunobu reaction, using Boc-D-*cis*-hydroxyproline methyl ester (**3a**) yielded benzoate **3z**[19] whose controlled saponification led to the expected alcohol **4a**, without cleavage of the methyl ester.[20] Subsequent Mitsunobu reactions between **4a** and different aromatic alcohols yielded substituted prolines **4b**−**11b** with the expected configuration. Deprotection of these compounds finally provided prolylamines **4c**−**11c**. Following the same scheme, alcohols **1a**−**3a** were converted into 4-phenoxyproline derivatives **1c**−**3c**. In these reactions, the inversion of configuration from *trans*- to *cis*-substituted prolines proceeded with a better yield than the reverse (60% vs 40%) and the purification was easier.

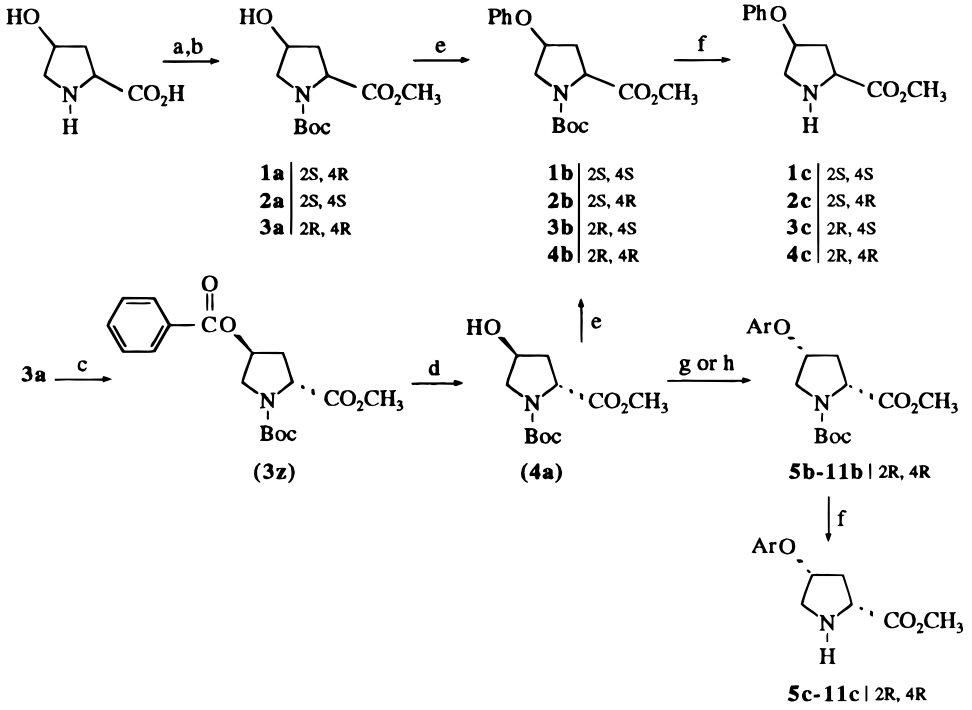
Preparation of benzyloxy derivatives **15c** and **16c** does not involve stereochemical modification and was performed by classical methods starting from Boc-D-4-*cis*-hydroxyproline (Scheme 3). Amine **12c** was prepared via homologation by an Arndt-Eistert reaction of N^{α}-Boc-protected phenoxyproline derivative **12a** obtained after saponification of the ester **6b**.

N-[(2-Adamantyloxy)carbonyl]-DL-α-methyltryptophan, prepared as described previously by Horwell et al.,[10] or its pure D isomer obtained as reported,[9,21] was then coupled to the different prolylamines **1c**−**16c** using [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as a coupling reagent to give the corresponding amides **1d**−**16d** (Scheme 1). The reaction time (1−3 days) was dependent on the nature of the 4-substituent of the proline ring. Compounds **1d**−**16d** were obtained in good yields (75−95%), in contrast to RB 210 and RB 211 resulting from similar coupling of $_2$Adoc-α-methyltryptophan with linear secondary amines (yields ∼ 16%).[9] The final dipeptoid acids **1e**−**16e** and **1f**−**4f** were obtained after saponification with 1 N NaOH in dioxane. Esters **1d**−**4d** were equimolar mixtures of diastereomeric esters, resulting from coupling of racemic $_2$Adoc-αMe-Trp with enantiomerically pure proline derivatives **1c**−**4c**. Only the corresponding acids **1e**−**4e** and **1f**−**4f** were separated by chromatography.

Conformational Analysis

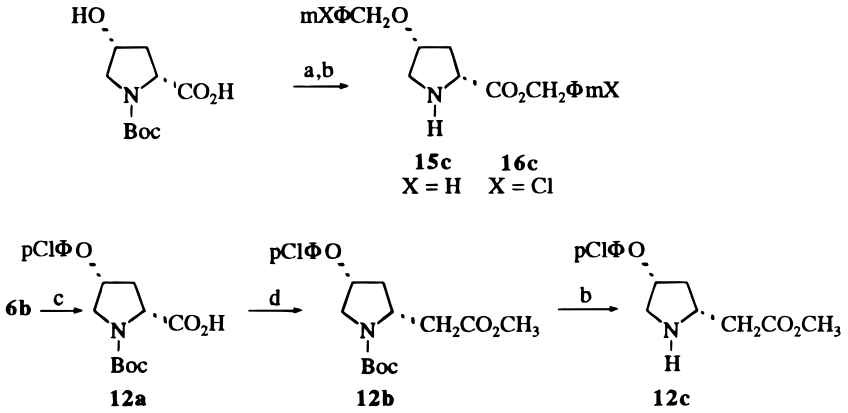
^{1}H NMR spectra of several compounds revealed the presence of two rotamers in a 1:3 ratio corresponding to a slow N-*cis*/N-*trans* isomerization around the proline amide bond (see Experimental Section for details). The major form was found to be the N-*trans* conformer based on the observation of a strong NOE cross-peak between the $CH_3\alpha$ Trp and H_5 Pro in **6e**, while the minor rotamer corresponded to the N-*cis* form, as confirmed by a NOE cross-peak between $CH_3\alpha$ Trp and H_2 Pro. Conformational analysis was also performed on compound **6e** using a high-temperature-simulated annealing protocol. Interestingly, among the 100 conformers that were generated by this method, 30 had a N-*cis* peptide bond, which is in agreement with the proportion of N-*cis*/N-*trans* rotamers observed by NMR. Finally, ^{1}H NMR spectra of **6e** in DMSO were recorded over a tempera-

Scheme 2[a]



[a] (a) Boc_2O, NaOH, dioxane; (b) Cs_2CO_3, MeI, DMF; (c) PPh_3, DEAD, $PhCO_2H$, THF; (d) NaOH, 1% MeOH; (e) PPh_3, DEAD, PhOH, THF; (f) CH_2Cl_2, TFA; (g) (*o*, *p*) (X, Y) PhOH, PPh_3, DEAD, THF; (h) 1-naphthol, PPh_3, DEAD, THF; **5**, Ar = 1-naphthyl; **6**, Ar = (*p*-Cl)Ph; **7**, Ar = (*p*-F)Ph; **8**, Ar = (*p*-I)Ph; **9**, Ar = (*p*-NO_2)Ph; **10**, Ar = (*o*,*p*-Cl_2)Ph; **11**, Ar = (*o*,*p*-F_2)Ph.

Scheme 3[a]



[a] (a) Ag_2O, mX-$PhCH_2OH$, DMF; (b) TFA, CH_2Cl_2; (c) 1 N NaOH, dioxane; (d) 1. iBuCOCl, *N*-methylmorpholine (NMM), 2. CH_2N_2, CH_2Cl_2, 3. $C_6H_5CO_2Ag$, NEt_3, MeOH.

ture range of 20−80 °C. On the basis of temperature-dependent changes in the indole NH and OCH (Adoc) signals, a $\Delta G^{\ddagger}$ of 16 kcal mol^{-1} corresponding to the *trans*-to-*cis* isomerization was obtained using the Eyring relation. The rotational barriers found in Pro-containing peptides are generally higher ($\Delta G^{\ddagger} \geq 18$ kcal mol^{-1}). This could be due to a steric hindrance in the planar N-*cis* and N-*trans* forms of **6e**.

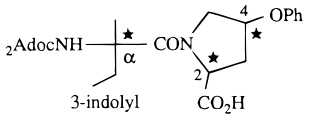
Results and Discussion

Structure−Affinity Relationships. Conformational restriction of peptides provides a useful approach to increase receptor potency and selectivity and facilitates the determination of the bioactive conformation of peptide ligands.[22] Such a strategy was undertaken in this study by synthesizing cyclic derivatives of the short peptidomimetic CCK-B antagonist RB 210. The resulting compounds **1e**−**16e** and **1f**−**4f** were evaluated for their potency in inhibiting [^{3}H]pCCK_8 binding to both guinea pig cortex and pancreatic membranes, as reported in Tables 1−3.

The ring closure introduced in the structure of RB 210 led to dipeptoids containing three chiral centers. All final compounds had lower affinities for CCK-A than for CCK-B receptors, whatever the stereochemistry of these centers. Nevertheless, the cyclic constraint introduced reduces the selectivity for CCK-B receptors. This suggests that reduction of the conformational freedom may lead to relatively more favorable conformations for CCK-A binding, as compared to the linear series.

The influence of the stereochemistry on CCK-B receptor recognition was investigated in detail by synthesizing the eight corresponding stereoisomers of a single molecule, N^{α}-[(2-adamantyloxy)carbonyl]-α-methyltryptophanyl-4-phenoxyproline. The results of binding studies reported in Table 1 clearly indicate that the *R*(D) configuration of tryptophan (compounds **1e**−**4e**) is

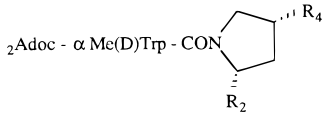
Table 1. Influence of the Configuration at the Three Chiral Centers on the Affinity and Selectivity of the New Compounds



compd[b]	α[c]	C_2[c]	C_4[c]	K_i(CCK-B)[a]	A/B[a]
1e (*c*)	*R*	*S*	*S*	63 ± 3.5	53.7
1f (*c*)	*S*	*S*	*S*	620 ± 188	10
2e (*t*)	*R*	*S*	*R*	285 ± 9	12
2f (*t*)	*S*	*S*	*R*	791 ± 31	2
3e (*t*)	*R*	*R*	*S*	98 ± 11	12.5
3f (*t*)	*S*	*R*	*S*	330 ± 67	4.3
4e (*c*)	*R*	*R*	*R*	28 ± 2	22.5
4f (*c*)	*S*	*R*	*R*	91 ± 13	26.7
RB 210				14 ± 0.6	109
RB 211				6.1 ± 0.3	173

[a] The K_i values (expressed in nM) represent the mean ± 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. K_i for CCK-A receptor is only expressed as its ratio versus K_i for CCK-B receptor. [b] (*c*) and (*t*) indicate the *cis* (*c*) or *trans* (*t*) orientation of C_2 and C_4 substituents with respect to the mean plane of the proline ring. [c] α, C_2, and C_4 refer to the configurations of the tryptophan α carbon and carbons 2 and 4 of the proline ring, respectively.

Table 2. Influence of the Prolyl C_2 and C_4 Substituents on the Affinity and Selectivity of New Compounds in the *Cis* Series



compd	R_4	R_2	K_i(CCK-B)[a]	A/B[a]
13e	Ph	CO_2H	20 ± 2	33
14e	Ph-pCl	CO_2H	32 ± 1.8	12.5
15e	OCH_2Ph	CO_2H	24 ± 2	68.6
16e	OCH_2Ph-mCl	CO_2H	56 ± 8	38
4e	OPh	CO_2H	28 ± 2	22.5
6e	OPh-pCl	CO_2H	24 ± 3	65
12e	OPh-pCl	CH_2CO_2H	122 ± 30	*b*

[a] The K_i values (expressed in nM) represent the mean ± 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. K_i for CCK-A receptor is only expressed as its ratio versus K_i for CCK-B receptor. [b] K_i(CCK-A) > 10 μM.

always preferred for CCK-B receptor binding, in agreement with reported data on dipeptoids.[10] Regarding the stereochemistry at C_2 and C_4 of the proline ring, it may be inferred that a *cis* orientation of the substituents toward the mean plane of the proline ring (both substituents located on the same side of this plane) is more favorable. Nevertheless, in compounds with a trans orientation, **3e**, but not **2e**, retains a relatively good CCK-B affinity, showing that a nonoptimal orientation of the carboxylate does not preclude receptor recognition. Compound **4e** was found to have the best affinity and gave an indication about the optimal spatial arrangement of the two side chains. This compound was therefore chosen for further investigation by introduction of different aromatic moieties in the 4 position. Modifications of the nature and the length of the chain between C_4 and the phenyl ring in **4e**, **13e**, and **15e** did not result in significant changes in affinity or selectivity. Interestingly, this was not the case in the RB 210 (Figure 1) linear series, where the aliphatic chain between the amide nitrogen and the phenyl ring was optimized with two carbons, the increase or decrease of this chain length by one carbon resulting in a more than 10-fold loss of affinity.[9] This could be related to an enhanced number of unfavorable conformations, which seem to be counteracted by the cyclization.

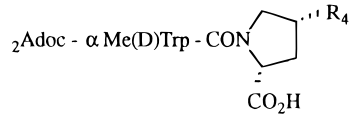
In a further attempt to modify the relative position of the aromatic ring and the carboxyl group, which is known to be essential for the activity,[9,10] a methylene was introduced between the ring and the acid group. Compound **12e** (K_i = 122 nM) was found to be 5 times less active than **6e** (K_i = 24 nM), but its selectivity for CCK-B receptors was largely improved as a result of its decreased affinity for CCK-A receptors (K_i > 10 μM), illustrating the even greater sensitivity of the latter to the position of the carboxyl group (Table 2).

A comparison of the properties of both linear (RB 210 and derivatives) and constrained (this study) series of peptoid antagonists suggests that the phenyl ring is localized in a putative hydrophobic pocket of the receptor.[23a,b] Compounds **14e**, **16e**, and **5e**−**11e** were prepared to investigate the requirements for optimal recognition of this pocket. As shown in Table 3, a naphthyl group in **5e** induced a large loss of affinity, presumably for reasons of steric hindrance. Introduction of a chlorine atom on the aromatic ring did not give any significant improvement for compounds **14e** and **16e** versus **13e** and **15e**, respectively. On the contrary, **6e** had a 3-fold higher selectivity for the CCK-B receptor than **4e** in the phenoxy series. The availability of various substituted phenols allowed variations to be developed in the aromatic ring substitution, which were more difficult to achieve in the linear series. Table 3 also reports the binding affinities of substituted 4-phenoxyproline-containing dipeptoids. Regarding affinities for the CCK-B receptor, only compound **11e**, whose aromatic ring bears a fluorine atom at both ortho and para positions, showed an improved affinity. This could result from favorable electron-withdrawing effects, which would be counteracted by steric interactions in other compounds, especially **8e**. Interestingly, substitution of the aromatic ring by a bulky substituent at the para position (**6e**, chlorine; **8e**, iodine; **9e**, NO_2) led to a 3-fold increase in selectivity for CCK-B versus CCK-A receptors, probably resulting from more important steric requirements at this level for CCK-A recognition.

In Vitro Antagonist Properties. To evaluate the pharmacological profile of the best compounds of this series (**6e**−**11e**), their ability to inhibit CCK_8-induced formation of inositol phosphates (IP) was measured in CHO cells stably transfected with the rat CCK-B receptor and compared to that of antagonists L-365,-260, PD-134,308, and RB 211. Their binding characteristics for the CCK-B receptor were also measured on membrane preparations of these cells (Table 3). The affinities determined on CHO cells were generally close to those obtained using guinea pig brain preparations, which is in agreement with literature data.[24]

All the compounds tested behaved as full antagonists, as they abolished completely IP production triggered by CCK_8 on transfected CHO cells. The inhibitory potencies of all the molecules did not strictly follow the same order as their affinities measured on the same cell homogenates. Large variations were even observed in the ratio: IC_{50} versus IP production/affinity, which

Table 3. Influence of the Aromatic Group on the Affinity, Selectivity, and Antagonist Activity of the New Compounds: Comparison with Various CCK-B Antagonists



				CHO cells		two-site model, CHO cells[d]			
compd	R_4	K_i(CCK-B)[a]	A/B[a]	K_i[b]	IC_{50}[c]	K_{i_1}	$\%_1$	K_{i_2}	$\%_2$
5e	O-(1-naphthyl)	775 ± 151	2						
6e	OPh-pCl	24 ± 3	65	24.1 ± 5.8	72 ± 9				
7e	OPh-pF	26.2 ± 9	27.4	19.9 ± 5	55.5 ± 12.4				
8e	OPh-pI	44.8 ± 5.8	96.3	74.9 ± 22	55.8 ± 30				
9e	OPh-pNO_2	26.3 ± 4.8	80	32.7 ± 21	507 ± 120	0.5	32.4	75.5	67.6
10e	OPh-o,pCl_2	45.7 ± 5.7	16.4	15.3 ± 1.7	37.4 ± 8.1	1.9	22.7	30.4	77.3
11e	OPh-o,pF_2	17.6 ± 1.4	22.4	18.6 ± 8.4	389 ± 158	6.8	76.8	324.3	23.2
RB 211		6.1 ± 0.3	173	14 ± 1	217 ± 50				
PD-134,308		0.4 ± 0.51		7.3 ± 0.7	30 ± 2				
L-365,260		2.0 ± 0.4		15 ± 1.5	39 ± 7				

[a] The K_i values (expressed in nM) represent the mean ± 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. K_i for CCK-A receptor is only expressed as its ratio on K_i for CCK-B receptor. [b] K_i values are the mean ± SEM of three separate experiments, each performed in triplicate, on the rat brain CCK-B receptor expressed in CHO cells. [c] Results (expressed in nM) are the mean ± SEM of three separate measurements of inhibition of inositol phosphate production induced by CCK_8 (0.5 nM), each in triplicate (see Experimental Section for details). [d] Results are the mean of two separate experiments, each in triplcate. K_i values are expressed in nM; $\%_1$ and $\%_2$ represent the proportions of each affinity state of the receptor.

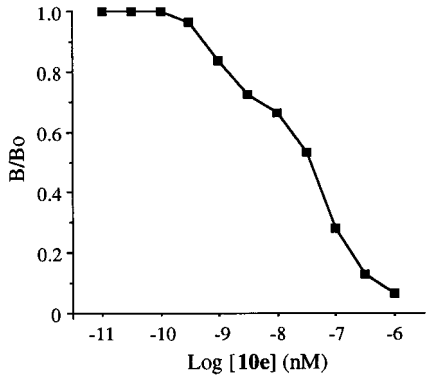


Figure 2. Inhibition of [^{3}H]pCCK_8 specific binding to a CCK-B receptor transfected CHO cell membrane preparation by compound **10e**. Each point represents the mean value of triplicate determinations.

varied from values close to unity (i.e., **8e**) to a two-figure ratio (**11e**). For example, a comparison of RB 211 with its constrained equivalent **6e** shows that the latter, although possessing a 2-fold lower affinity, was 3 times more potent in the IP assay.

Investigations on the Occurrence of Two Affinity States for the CCK-B Receptor. Binding studies using CHO cells showed that some compounds, such as **10e** and **11e**, which are characterized by an additional substituent at the ortho position of the phenyl ring, and also compound **9e**, bind to the receptor in a way that is significantly better described by a two-site binding model (Figure 2). It can be noticed that these three compounds are the ones bearing the most powerful electron-withdrawing substituents.

The occurrence of two affinity states of the CCK-B receptor was reported 10 years ago.[25] Several authors have described CCK-B agonists, apparently capable of discriminating two[26,27] or even three[28] binding states, but only very recently has such a result been obtained with an antagonist, namely, the benzodiazepine L-365,-260,[7] although, in this case, numerous experiments were necessary to obtain enough data to reach statistical significance.

Biological models used in these various studies are guinea pig [25−27] and rat brain membranes,[7,26] and COS cells,[28] this cell line having been transfected with a CCK-B receptor gene. In our case, stable transfection of CHO cells ensures that the receptor panel is homogeneous, indicating that the two-site binding mode must result from the coexistence of two affinity states of a single receptor, in terms of amino acid sequence. This also relies on the fact that CCK_8 neither binds nor induces IP production in nontransfected CHO cells (unpublished results). The lack of CCK_8 binding sites on untransfected CHO cells has already been observed in a previous study in which the human CCK-B receptor was transfected.[29] In this study, CCK_{33}, CCK_8, CCK_4, and gastrin were shown to interact apparently with only one class of binding sites. We obtained the same results with these molecules and the transfected rat CCK-B receptor.

To investigate this problem, the biological profiles of **10e** and **11e** are of great interest. Indeed, their antagonist potencies on IP production in CHO cells show a 10-fold difference, while their mean affinities for the CCK-B receptor (evaluated from a one-site binding model) are close to each other (Table 3). This difference in antagonist behavior is not unique, for a quick overview of the formely described CCK-B antagonists L-365,260, PD-134,308, and RB 211 suggests a possible partition of these molecules according to their biological properties. On one hand, high-affinity and high-potency antagonists of IP production would be represented by L-365,260, PD-134,308, and our new compounds **10e** or **7e**, whereas on the other hand, RB 211 and **11e** or **9e** would be high-affinity and low-potency antagonists (Table 3).

The two CCK-B receptor affinity states might furnish a convenient explanation for the discrepancy observed between the two groups of molecules. In fact, antagonist activity is linked to a disruption of the corresponding second-messenger pathway, which is, in our biological model, phospholipase C (PLC), coupled with the CCK-B receptor through a G-protein.[30] Differential coupling of

the CCK-B receptor to G-proteins illustrated by differences in the sensitivity of the binding of agonists to the addition of Gpp(NH)p, a nonhydrolyzable analogue of GTP, has been suggested by several authors to explain the presence of two affinity states.[27,31]

This hypothesis can explain the two-site binding models obtained here, provided that differential coupling of the receptor results in different conformations at the antagonist binding region of the receptor. Moreover, IP assays start with incubation with CCK_8 (0.5 nM). Therefore, if there is an equilibrium between pre- and postcoupled states of the receptor, it will be shifted in favor of the precoupled state. Thus, the inhibitory potency of antagonists in this experiment would merely result from their ability to bind to the putative precoupled state of the receptor, whatever their "mean" affinity, as determined from one-site binding models.

In any case, the hypotheses evoked by Harper et al.,[7] such as different posttranslational modifications of CCK-B receptors, remain valid here, but if they can justify two-site binding models, they fail to explain the large variations observed in antagonist activity on the PLC pathway. Be that as it may, further biological experiments are necessary to confirm any of the hypotheses described here.

Conclusion

The introduction of a bridging methylene unit in the structure of RB 211 preserved the affinity for the CCK-B receptor, indicating that unfavorable steric interaction between the receptor and the proline ring did not occur. Optimization of the configurational requirements for receptor recognition led to compounds containing two D-modified amino acids, the second being a proline bearing an aromatic substituent on C_4, with a *cis* configuration toward the carboxylate. Reduction of the conformational freedom, and therefore of the unfavorable entropy factor, unexpectedly did not improve the affinity of parent RB 211, but only its antagonist activity. Moreover, these new molecules should possess enhanced stability toward enzymatic and acid degradation and increased lipophilicity, as compared to the parent dipeptoids RB 210 and PD-134,308. This is expected to facilitate blood−brain barrier penetration, which is crucial for pharmacological studies of these new antagonists, and is currently under evaluation in our laboratory. Last, the discrimination of two affinity states of CCK-B receptors by some of the new compounds described here is suggested by biochemical studies and is now the object of further investigation.

Experimental Section

Chemistry. D-*cis*-Hyp-OH was purchased from Aldrich (France). Boc-L-*trans*-Hyp-OMe, Boc-L-*cis*-Hyp-OMe, Boc-L-*trans*-Hyp(Bzl)-OH, and DL-α-methyltryptophan were from Bachem (Switzerland). BOP was from Novabiochem. Solvents were from SDS (France). PD-134,308 was synthesized as previously described.[10]

Flash column chromatography was performed using Merck silica gel (230−400 mesh). TLC was performed on precoated silica gel plates (60F-254, 0.2 mm thick; Merck) with the following solvent systems (v/v): A, CH_2Cl_2−MeOH−AcOH (9:1:0.5); B, CH_2Cl_2−MeOH (95:5); C, CH_2Cl_2−MeOH (100:2); D, CH_2Cl_2; E, cyclohexane−EtOAc (9:1); F, cyclohexane−EtOAc (4:1); G, $CHCl_3$−MeOH−H_2O−AcOH−EtOAc (7:3:0.6:0.3:5.5); H, CH_2Cl_2−EtOAc (9:1). I, CH_2Cl_2−EtOAc (8:2).

Anhydrous solvents were dried over 4 Å molecular sieves prior to use. Plates were developed with UV, iodine vapor, ninhydrin, and/or Erlich's reagent. Melting points of the crystallized compounds were taken on an Electrothermal melting point apparatus and are uncorrected. The structure of all compounds was confirmed by ^{1}H NMR spectroscopy (Bruker WH 270 MHz) performed in DMSO-d_6 (concentrations ranging 3−5 mM). Chemical shifts were measured in ppm with HMDSO as internal standard. The purity of compounds was checked by HPLC (Shimadzu apparatus) on a Kromasil C_8 column (5 μm, 4.6 × 250 mm) or a Kromasil C_{18} column (5 μm, 4.6 × 250 mm), with a mixture of $H_2O/TFA_{0.05\%}$ (solvent A) and CH_3CN (solvent B) (flow rate 1.2 mL/min, UV detection 214 nm).

Optical rotations were measured on a Perkin-Elmer polarimeter (model 241). Mass spectra were recorded on a quadrupole NERMAG R10-10C apparatus or a double-focusing VG 70-250 SEQ instrument. Elemental analyses were performed by Service Régional de Microanalyse (Université Pierre et Marie Curie, Paris, France).

General Method A. Phenol (4.5 mmol), substituted phenol (4.5 mmol), 1-naphthol (4.5 mmol), or benzoic acid (4.5 mmol) and triphenylphosphine (4.5 mmol) were added to the alcohol (3 mmol) in THF (10 mL). To this cooled (0 °C) solution was added dropwise diethyl azodicarboxylate (4.5 mmol) over a period of 1 h. After standing overnight at room temperature, the mixture was concentrated and the residue was suspended in Et_2O (13 mL). The mixture was filtered, the filtrate was concentrated, and the residue was chromatographed.

Procedure B. Benzoate (8.3 mmol) was added to a solution of 1% NaOH in MeOH (225 mL), and the resulting mixture was stirred at room temperature for 30 min. After quenching of the reaction with AcOH (2.3 mL) to avoid saponification of the methyl ester, evaporation of the MeOH, addition of water, and workup (EtOAc), the residue was chromatographed on silica gel.

General Method C. At 0 °C the carbamate (5 mmol) in CH_2Cl_2 (10 mL) and TFA (10 mL) was stirred for 1 h and at room temperature for an additional 2 h. After evaporation of the solvents, the residue was either lyophilized and triturated from Et_2O or purified by chromatography.

General Method D. Silver(I) oxide (10 mmol) and benzyl bromide or *m*-Cl-benzyl bromide (10 mmol) were added to a solution of the hydroxy acid (3 mmol) in DMF (6 mL). After being stirred for 24 h at room temperature, the suspension was filtered, the filtrate evaporated, and the residue subjected again to the same procedure, before being purified by flash chromatography on silica gel.

General Method E. To the ester (0.5 mmol) in dioxane (6 mL) was added 1 N NaOH (0.55 mmol) at 0 °C. After 5 h at room temperature the solvent was evaporated. After addition of 1 N HCl until pH 2 and workup (EtOAc), the residue was chromatographed on silica gel (typical yield: 85%).

Procedure F. To a stirred solution of the acid (3.68 mmol) and *N*-methylmorpholine (3.68 mmol) in anhydrous THF (8 mL) at −20 °C was added dropwise isobutyl chloroformate (3.68 mmol). After 20 min, the *N*-methylmorpholine hydrochlororide was filtered off, and a solution of diazomethane (15 mmol) in CH_2Cl_2 (45 mL) was added to the filtrate at −10 °C. The cooled solution was stirred for 2 days at −4 °C. The solvents were removed in vacuo to give the intermediate diazo ketone, which was not further characterized. To a stirred solution of this pale yellow oil in dry MeOH (7 mL) was added a solution of silver benzoate (0.48 mmol) in Et_3N (7.36 mmol) causing evolution of nitrogen. When gas evolution had ceased, the resulting tan solution was stirred for a further 10 min. The solution was then treated with charcoal and filtered, and the solvents were removed in vacuo to give a residue which was dissolved in EtOAc (50 mL), worked up (water, brine), and purified by flash chromatography.

General Method G. To a solution of the amine (1.24 mmol) in dry DMF (440 μL) was added N^{α}-[(2-adamantyloxy)carbonyl]-α-methyl-(*RS*)-tryptophan (0.62 mmol), DIEA (2.48 or 3.72 mmol when the amine was used as salt), and, at 0 °C, BOP (0.69 mmol). After 1 h at this temperature, the solution was allowed to warm to room temperature and stirred further for

1−3 days. The solvent was then evaporated, and EtOAc (20 mL) was added to the residue. The organic phase was washed successively with 10% aqueous citric solution (2 × 15 mL), water (1 × 15 mL), 10% aqueous $NaHCO_3$ solution (2 × 15 mL), water (1 × 15 mL), and brine (1 × 15 mL). The organic solution was dried over Na_2SO_4 and filtered. Evaporation of the solvent gave a residue which was chromatographed on silica gel.

Boc-D-*trans*-Hyp(Bz)-OMe (3z): obtained from Boc-D-*cis*-Hyp-OMe **(3a)** according to general method A; yield 100%, after flash chromatography with CH_2Cl_2−MeOH (100:2) as eluent; R_f 0.20(C); mp 91−92 °C; $[\alpha]^{18}_D = +42.8°$ (*c* 0.5, $CHCl_3$). Anal. ($C_{18}H_{23}NO_6$) C, H, N.

Boc-L-*trans*-Hyp(Ph)-OMe (2b) and Boc-D-*trans*-Hyp-(Ph)-OMe (3b): obtained respectively from Boc-L-*cis*-Hyp-OMe **(2a)** and **3a** according to general method A; yield 40%, after flash chromatography with CH_2Cl_2 as eluent; R_f 0.16(D); mp 101 °C; **2b** $[\alpha]^{18}_D = -37.7°$ (*c* 0.5, $CHCl_3$); **3b** $[\alpha]^{18}_D = +38.3°$ (*c* 0.2, $CHCl_3$); ^{1}H NMR (DMSO) δ 1.29 and 1.32 (9H, s, $(CH_3)_3$), 2.16 (1H, m, H_3), 2.40 (1H, m, H_3), 3.44−3.67 (5H, m, OCH_3 + $2H_5$), 4.25 (1H, q, H_2), 4.98 (1H, brs, H_4), 6.90 (3H, m, Ar), 7.26 (2H, dd, Ar). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

Boc-L-*cis*-Hyp(Ph)-OMe (1b) and Boc-D-*cis*-Hyp(Ph)-OMe (4b): obtained respectively from Boc-L-*trans*-Hyp-OMe **(1a)** and Boc-D-*trans*-Hyp-OMe (**4a**), according to general method A; yield 60%, after flash chromatography with CH_2Cl_2 and then CH_2Cl_2−MeOH (100:2) as eluent; R_f 0.16(D), 0.35(C); **1b** $[\alpha]^{18}_D = -32.6°$ (*c* 0.5, $CHCl_3$); **4b** $[\alpha]^{18}_D = +33.4°$ (*c* 0.6, $CHCl_3$); ^{1}H NMR (DMSO) δ 1.30 and 1.35 (9H, s, $(CH_3)_3$), 2.13 (1H, m, H_3), 2.51 (1H, m, H_3), 3.36 (1H, m, H_5), 3.53−3.70 (4H, m, OCH_3 + H_5), 4.34 (1H, m, H_2), 4.99 (1H, m, H_4), 6.70 (2H, m, Ar), 6.80 (1H, dd, Ar), 7.24 (2H, dd, Ar). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

Boc-D-*cis*-Hyp(1-naphthyl)-OMe (5b): obtained from **4a** according to general method A; yield 52%, after flash chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.36(F); mp 103−105 °C. Anal. ($C_{21}H_{25}NO_5$) C, H, N.

Boc-D-*cis*-Hyp(Ph-pCl)-OMe (6b): obtained from **4a** according to general method A; yield 78%, after flash chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.15(E), 0.31 (F); mp 67−68 °C; $[\alpha] = +30.2°$ (*c* 0.6, $CHCl_3$). Anal. ($C_{17}H_{22}NO_5Cl$) C, H, N.

Boc-D-*cis*-Hyp(Ph-pF)-OMe (7b): obtained from **4a** according to general method A; yield 79%, after chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.22(F); mp 64−66 °C. Anal. ($C_{17}H_{22}NO_5F$) C, H, N.

Boc-D-*cis*-Hyp(Ph-pI)-OMe (8b): obtained from **4a** according to general method A; yield 70%, after chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.41(F); mp 70−72 °C. Anal. ($C_{17}H_{22}NO_5I$) C, H, N.

Boc-D-*cis*-Hyp(Ph-pNO_2)-OMe (9b): obtained from **4a** according to general method A; yield 79%, after chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.22(F); mp 64−66 °C. Anal. ($C_{17}H_{22}NO_5F$) C, H, N.

Boc-D-*cis*-Hyp(Ph-o,pCl_2)-OMe (10b): obtained from **4a** according to general method A; yield 74%, after chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.36(F); mp 52−54 °C. Anal. ($C_{17}H_{21}NO_5Cl_2$) C, H, N.

Boc-D-*cis*-Hyp(Ph-o,pF_2)-OMe (11b): obtained from **4a** according to general method A; yield 82%, after chromatography with cyclohexane−EtOAc (9:1, 8:2) as eluent; R_f 0.24(F). Anal. ($C_{17}H_{21}NO_5F_2$) C, H, N.

TFA·H-L-*trans*-Hyp(Ph)-OMe (2c) and TFA·H-D-*trans*-Hyp(Ph)-OMe (3c): obtained respectively from **2b** and **3b** according to general method C; yield 85%, after flash chromatography using CH_2Cl_2−MeOH (95:5) as eluent; R_f 0.36-(B); mp 191 °C: FAB/MS (MH^+) 222.

H-L-*cis*-Hyp(Ph)-OMe (1c) and H-D-*cis*-Hyp(Ph)-OMe (4c): obtained respectively from **1b** and **4b** according to general method C; yield 85%, after flash chromatography using CH_2Cl_2−MeOH (95:5) as eluent; R_f 0.35(B); mp 66−67 °C. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

TFA·H-D-*cis*-Hyp(1-naphthyl)-OMe (5c): obtained from **5b** according to general method C; yield 100%, without further purification; R_f 0.66(B); ^{1}H NMR (DMSO) δ 2.55 (1H, m, H_3), 2.68 (1H, m, H_3), 3.55 (3H, s, OCH_3), 3.64 (2H, m, $2H_5$), 5.32 (1H, m, H_4), 6.95−7.98 (7H, m, Ar).

TFA·H-D-*cis*-Hyp(Ph-pCl)-OMe (6c): obtained from **6b** according to general method C; yield 85%, after flash chromatography using CH_2Cl_2−MeOH (95:5) as eluent; R_f 0.37-(B); mp 198−200 °C; ^{1}H NMR (DMSO) δ 2.33 (1H, dd, H_3), 2.55 (1H, m, H_3), 3.46 (2H, m, $2H_5$), 3.66 (3H, s, OCH_3), 4.63 (1H, dd, H_2), 5.12 (1H, m, H_4), 6.86 (2H, dd, Ar), 7.31 (2H, dd, Ar); FAB/MS (MH^+) 256.

TFA·H-D-*cis*-Hyp(Ph-pF)-OMe (7c): obtained from **7b** according to general method C; yield 100%, without further purification; R_f 0.51(B); ^{1}H NMR (DMSO) δ 2.37 (1H, m, H_3), 2.57 (1H, m, H_3), 3.48 (2H, m, $2H_5$), 3.69 (3H, s, OCH_3), 4.53 (1H, m, H_2), 5.09 (1H, brs, H_4), 6.84 (2H, dd, Ar), 7.06 (2H, dd, Ar).

TFA·H-D-*cis*-Hyp(Ph-pI)-OMe (8c): obtained from **8b** according to general method C; yield 100%, without further purification; R_f 0.6(B); ^{1}H NMR (DMSO) δ 2.40 (1H, m, H_3), 2.62 (1H, m, H_3), 3.51 (2H, m, $2H_5$), 3.75 (3H, s, OCH_3), 4.69 (1H, m, H_2), 5.17 (1H, m, H_4), 6.74 (2H, dd, Ar), 7.63 (2H, dd, Ar).

TFA·H-D-*cis*-Hyp(Ph-pNO_2)-OMe (9c): obtained from **9b** according to general method C; yield 93%, after precipitation in Et_2O; R_f 0.35(B); ^{1}H NMR (DMSO) δ 2.45 (1H, m, H_3), 2.71 (1H, m, H_3), 3.59 (2H, m, $2H_5$), 3.78 (3H, s, OCH_3), 4.73 (1H, m, H_2), 5.36 (1H, brs, H_4), 7.14 (2H, dd, Ar), 8.22 (2H, dd, Ar).

TFA·H-D-*cis*-Hyp(Ph-o,pCl_2)-OMe (10c): obtained from **10b** according to general method C; yield 100%, without further purification; R_f 0.55(B); ^{1}H NMR (DMSO) δ 2.50 (1H, m, H_3), 2.64 (1H, m, H_3), 3.62 (2H, m, $2H_5$), 3.77 (3H, s, OCH_3), 4.72 (1H, m, H_2), 5.22 (1H, m, H_4), 7.19 (1H, d, Ar), 7.38 (1H, d, Ar), 7.54 (1H, s, Ar).

TFA·H-D-*cis*-Hyp(Ph-o,pF_2)-OMe (11c): obtained from **11b** according to general method C; yield 100%, without further purification; R_f 0.3(B); ^{1}H NMR (DMSO) δ 2.4 (2H, m, $2H_3$), 3.48 (2H, m, $2H_5$), 3.72 (3H, s, OCH_3), 4.67 (1H, m, H_2), 5.12 (1H, brs, H_4), 6.87−7.3 (3H, m, Ar).

Boc-D-*cis*-Hyp(Ph-pCl)-OH (12a): obtained from **6b** according to general method E without further purification; yield 90%; R_f 0.10(H); ^{1}H NMR (DMSO) δ 1.29 and 1.32 (9H, s, $(CH_3)_3$), 2.09 (1H, m, H_3), 2.50 (1H, m, H_3), 3.32 (1H, d, H_5), 3.65 (1H, m, H_5), 4.20 (1H, m, H_2), 4.91 (1H, brs, H_4), 6.82 and 7.26 (4H, d, Ar). Anal. ($C_{16}H_{20}NO_5Cl$) C, H, N.

Boc-D-*cis*-*β*-homo-Hyp(Ph-pCl)-OMe (12b): obtained from **12a** according to procedure F; yield 70%, after flash chromatography with cyclohexane−EtOAc (4:1) as eluent; R_f 0.21(F); amorphous solid. Anal. ($C_{18}H_{24}NO_5Cl$) C, H, N.

TFA·H-D-*cis*-*β*-homo-Hyp(Ph-pCl)-OMe (12c): obtained from **12b** according to general method C; yield 90%, after precipitation in Et_2O; R_f 0.17(B); mp 87 °C; FAB/MS (MH^+) 270.

TFA·H-D-*cis*-Hyp(Bzl)-OBzl (15c). Boc-D-*cis*-Hyp(Bzl)-OBzl was obtained from Boc-D-*cis*-Hyp-OH according to general method D; yield 65%, after flash chromatography with CH_2Cl_2 and then CH_2Cl_2−MeOH (100:2) as eluent, as an oil; R_f 0.30(C). **15c** was obtained from the preceding compound according to general method C; yield 85%, after chromatography using CH_2Cl_2−MeOH (95:5) as eluent; R_f 0.19(B); mp 93 °C; ^{1}H NMR (DMSO + TFA) δ 2.40 (2H, m, $2H_3$), 3.30 (1H, m, H_5), 3.46 (1H, dd, H_5), 4.26 (1H, m, H_2), 4.40 (2H, dd, $OCH_2\Phi$), 4.65 (1H, brt, H_4), 5.16 (2H, s, $CO_2CH_2\Phi$), 7.10−7.40 (10H, m, Ar).

TFA·H-D-*cis*-Hyp(Bzl-mCl)-OBzl-mCl (16c). Boc-D-*cis*-Hyp(Bzl-mCl)-OBzl-mCl was obtained from Boc-D-*cis*-Hyp-OH according to general method D; yield 50%, after flash chromatography with CH_2Cl_2−MeOH (100:2) as eluent, as an oil; R_f 0.17(D); ^{1}H NMR (DMSO) δ 1.20 and 1.35 (9H, s, $(CH_3)_3$), 2.0−2.21 (1H, m, H_3), 2.38 (1H, m, H_3), 3.18−3.58 (2H, m, $2H_5$), 4.28−4.50 and 4.87−5.17 (6H, m, $2(OCH_2) + H_2 + H_4$), 7.10−7.41 (8H, m, Ar). **16c** was obtained as an oil from the preceding compound according to general method C; yield 85%; R_f 0.25(B); ^{1}H NMR (DMSO + TFA) δ 2.46 (2H, m, $2H_3$), 3.35 (1H, m, H_5), 3.51 (1H, m, H_5), 4.31 (1H, brs, H_2), 4.43 (2H, dd, $OCH_2\Phi$), 4.72 (1H, m, H_4), 5.20 (2H, s, $CO_2CH_2\Phi$), 7.12−7.56 (8H, m, Ar); FAB/MS (MH^+) 380.

$_2$Adoc-α-Me-DL-Trp-L-*cis*-Hyp(Ph)-OMe (1d), $_2$Adoc-α-Me-DL-Trp-D-*cis*-Hyp(Ph)-OMe (4d), $_2$Adoc-α-Me-D-Trp-L-*trans*-Hyp(Ph)-OMe (2d), $_2$Adoc-α-Me-DL-Trp-D-*trans*-Hyp(Ph)-OMe (3d): according to general method G (2 days), **1d**−**4d** were obtained from **1c**−**4c**, respectively; yield 85%. **1d** and **4d** FAB/MS (MH^+) 600; HPLC, t_R 9.60 and 8.45 min, 70% B. **2d** and **3d** FAB/MS (MH^+) 600; HPLC, t_R 9.25 and 10.25 min, 70% B.

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(1-naphthyl)-OMe (5d): according to general method G (3 days), **5d** was obtained from **5c**; yield 76%; R_f(H) 0.21; ^{1}H NMR (DMSO) δ 1.4−2.6 (19H, αCH_3 + adamantyl + $2H_\beta$), 3.05 and 3.12 (1H, d, H_β), 3.20−3.41 (1H, d, H_β), 3.47 and 3.49 (3H, s, OCH_3), 3.70−4.00 (2H, m, $2H_5$), 4.56 (1H, m, H_2), 4.67 and 4.82 (1H, brs, OCH), 5.12 and 5.30 (1H, brs, H_4), 6.8−8.02 (13H, m, Ar + NH), 10.88 and 10.91 (1H, brs, NH ind).

$_2$Adoc-α-Me-D-Trp-D-*cis*-Hyp(Ph-pCl)-OMe (6d): according to general method G (2 days), **6d** was obtained from **6c**; yield 85%; FAB/MS (MH^+) 634; HPLC, t_R 12.0 min, 70% B.

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-pF)-OMe (7d): according to general method G (3 days), **7d** was obtained from **7c**; yield 85%; R_f(I) 0.38; ^{1}H NMR (DMSO) δ 1.14−2.5 (19H, αCH_3 + adamantyl + $2H_\beta$), 3.05 (1H, d, H_β), 3.44 (1H, d, H_β), 3.56 and 3.58 (3H, s, OCH_3), 3.55−3.85 (2H, m, $2H_5$), 4.49 (1H, m, H_2), 4.65 and 4.82 (1H, brs, OCH), 4.84 and 5.04 (1H, brs, H_4), 6.72−7.40 (10H, Ar + NH), 10.87 (1H, brs, NH ind).

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-pI)-OMe (8d): according to general method G (3 days), **8d** was obtained from **8c**; yield 88%; R_f(H) 0.25; ^{1}H NMR (DMSO) δ 1.12−2.5 (19H, αCH_3 + adamantyl + $2H_\beta$), 3.08 (1H, d, H_β), 3.43 (1H, d, H_β), 3.52 and 3.53 (3H, s, OCH_3), 3.60−3.85 (2H, m, $2H_5$), 4.45 (1H, m, H_2), 4.65 and 4.83 (1H, brs, OCH), 4.83 and 5.03 (1H, brs, H_4), 6.60−7.55 (10H, Ar + NH), 10.87 and 10.90 (1H, brs, NH ind).

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-pNO_2)-OMe (9d): according to general method G (3 days), **9d** was obtained from **9c**; yield 95%; R_f(H) 0.21; ^{1}H NMR (DMSO) δ 1.1−2.6 (19H, m, αCH_3 + adamantyl + $2H_5$), 3.01−3.12 (2H, m, H_β), 3.5−3.8 (5H, m, OCH_3 + $2H_5$), 4.44−5.32 (3H, OCH + H_4 + H_2), 6.82−7.70 (7H, m, Ar), 8.15 (2H, m, Ar), 10.88 (1H, brs, NH ind).

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-o,pCl_2)-OMe (10d): according to general method G (3 days), **10d** was obtained from **10c**; yield 94%; R_f(H) 0.25; ^{1}H NMR (DMSO) δ 1.18−2.6 (19H, αCH_3 + adamantyl + $2H_\beta$), 3.05 and 3.12 (1H, d, H_β), 3.43 (1H, d, H_β), 3.55 and 3.57 (3H, s, OCH_3), 3.60−3.83 (2H, m, $2H_5$), 4.50 (1H, m, H_2), 4.57 and 4.84 (1H, brs, OCH), 4.84 and 5.11 (1H, brs, H_4), 6.8−7.5 (9H, Ar + NH), 10.90 (1H, brs, NH ind).

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-o,pF_2)-OMe (11d): according to general method G (3 days), **11d** was obtained from **11c**; yield 93%; R_f(H) 0.13; ^{1}H NMR (DMSO) δ 1.1−2.6 (19H, αCH_3 + adamantyl + $2H_\beta$), 3.0−3.5 (1H, d, H_β), 3.55 and 3.56 (3H, s, OCH_3), 3.6−3.85 (2H, m, $2H_5$), 4.5 (1H, m, H_2), 4.62 and 4.85 (1H, brs, OCH), 4.7 and 5.04 (1H, brs, H_4), 6.7−7.45 (9H, Ar + NH), 10.88 (1H, brs, NH ind).

$_2$Adoc-α-Me-D-Trp-D-*cis*-β-homo-Hyp(Ph-pCl)-OMe (12d): according to general method G (2 days), **12d** was obtained from **12c**; yield 82%; FAB/MS (MH^+) 648; t_R 26.50 min, 60% B.

$_2$Adoc-α-Me-D-Trp-D-*cis*-Pro(4-Ph)-OMe (13d): according to general method G (3 days), **13d** was obtained from H-D-*cis*-Pro(4-Ph-pCl)-OMe **(13c)**; yield 78%; FAB/MS (MH^+) 584; HPLC, t_R 9.35, min, 70% B.

$_2$Adoc-α-Me-D-Trp-D-*cis*-Pro(4-Ph-pCl)-OMe (14d): according to general method G (3 days), **14d** was obtained from **14c**; yield 85%; FAB/MS (MH^+) 618; HPLC t_R 9.50 min, 70% B.

$_2$Adoc-α-Me-DL-Trp-D-*cis*-Hyp(Bzl)-OBzl (15d) and $_2$Adoc-α-Me-DL-Trp-D-*cis*-Hyp(Bzl-mCl)-OBzl-mCl (16d): according to general method G (1 day), **15d** and **16d** were obtained from **15c** and **16c** with 85% and 80% yields, respectively. **15d** FAB/MS (MH^+) 690; HPLC t_R 14.80 and 12.05 min, 80% B. **16d** FAB/MS (MH^+) 758; HPLC t_R 11.2 and 9.2 min, 80% B.

$_2$Adoc-α-Me-D-Trp-L-*cis*-Hyp(Ph)-OH (1e), $_2$Adoc-α-Me-L-Trp-L-*cis*-Hyp(Ph)-OH (1f), $_2$Adoc-α-Me-D-Trp-D-*cis*-Hyp(Ph)-OH (4e), $_2$Adoc-α-Me-L-Trp-D-*cis*-Hyp(Ph)-OH (4f), $_2$Adoc-α-Me-D-Trp-L-*trans*-Hyp(Ph)-OH (2e), $_2$Adoc-α-Me-L-Trp-L-*trans*-Hyp(Ph)-OH (2f), $_2$Adoc-α-Me-L-Trp-D-*trans*-Hyp(Ph)-OH (3e), $_2$Adoc-α-Me-D-Trp-D-*trans*-Hyp(Ph)-OH (3f): according to general method E, **1e**,**f**−**4e**,**f** were obtained from **1d**−**4d**, respectively. **1e** and **4f** mp 180−182 °C; ^{1}H NMR (DMSO + TFA) δ 1.0−2.50 (19H, m, adamantyl + αCH_3 + $2H_\beta$), 2.80−3.60 (2H, m, $2H_\beta$), 3.70−4.20 (2H, m, $2H_5$), 4.35−4.72 (2H, m, H_2 + OCH), 4.96 and 5.07 (1H, brs, H_4), 6.60−7.42 (11H, m, Ar + NH), 10.85 (1H, brs, NH_{ind}). **1f** and **4e** mp 161−164 °C; ^{1}H NMR (DMSO + TFA) δ 1.0−2.50 (19H, m, adamantyl + αCH_3 + $2H_\beta$), 2.90−3.90 (4H, m, $2H_\beta$ + $2H_5$), 4.38 (1H, m, H_2), 4.64 and 4.84 (1H, brs, OCH), 4.73 and 4.95 (1H, brs, H_4), 6.70−7.40 (11H, m, Ar + NH), 10.84 (1H, brs, NH_{ind}). **2e** and **3f** mp 240−244 °C; ^{1}H NMR (DMSO) δ 1.04−2.60 (19H, m, adamantyl + αCH_3 + $2H_\beta$), 2.90−3.60 (2H, m, $2H_\beta$), 3.65−5.10 (5H, m, $2H_5$ + H_2 + OCH + H_4), 6.70−7.50 (11H, m, Ar + NH), 10.88 (1H, brs, NH_{ind}). **2f** and **3e** mp 140−141 °C; ^{1}H NMR (DMSO + TFA) δ 1.10−2.45 (19H, m, adamantyl + αCH_3 + $2H_\beta$), 3.03 and 3.13 (1H, d, H_β), 3.50 and 3.55 (1H, d, H_β), 3.84−4.18 (2H, m, $2H_5$), 4.37 (1H, m, H_2), 4.62 and 4.83 (1H, brs, OCH), 5.08 (1H, brs, H_4), 6.80−7.50 (11H, m, Ar + NH), 10.90 (1H, s, NH_{ind}).

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(1-naphthyl)-OH (5e): according to general method E, **5e** was obtained from **5d**; ^{1}H NMR (DMSO + TFA) δ 1.18 and 1.22 (3H, s, αCH_3), 1.25−2.6 (16H, m, adamantyl + $2H_\beta$), 3.05 and 3.12 (1H, d, H_β), 3.45 (1H, d, H_β), 3.70−4.10 (2H, m, H_5), 4.45 (1H, m, H_2), 4.61 and 4.85 (1H, brs, OCH), 5.04 and 5.23 (1H, brs, H_4), 6.78−8.12 (13H, m, Ar + NH), 10.84 and 10.87 (1H, brs, NH ind); ES/MS ($(M + Na)^+$) 658.3.

$_2$Adoc-α-Me-D-Trp-D-*cis*-Hyp(Ph-pCl)-OH (6e): according to general method E, **6e** was obtained from **6d**; mp 167−169 °C; ^{1}H NMR (DMSO + TFA) δ 1.08−2.45 (19H, m, adamantyl + αCH_3 + $2H_\beta$), 3.10 (1H, m, H_β), 3.45 (1H, m, H_β), 3.60−3.90 (2H, m, $2H_5$), 4.41 (1H, m, H_2), 4.66 and 4.86 (1H, brs, OCH), 4.86 and 5.01 (1H, brs, H_4), 6.72−7.44 (10H, m, Ar + NH), 10.88 (1H, brs, NH_{ind}).

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-pF)-OH (7e): according to general method E, **7e** was obtained from **7d**; ^{1}H NMR (DMSO + TFA) δ 1.13 and 1.22 (3H, s, αCH_3), 1.3−2.5 (16H, m, adamantyl + $2H_\beta$), 3.05 (1H, d, H_β), 3.42 (1H, d, H_β), 3.58−3.82 (2H, m, H_5), 4.37 (1H, m, H_2), 4.65 and 4.90 (1H, brs, H_4), 4.67 and 4.79 (1H, brs, OCH), 6.72−7.40 (10H, m, Ar + NH), 10.78 (1H, brs, NH ind); ES/MS ($(M + Na)^+$) 626.3.

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-pI)-OH (8e): according to general method E, **8e** was obtained from **8d**; ^{1}H NMR (DMSO + TFA) δ 1.12 and 1.17 (3H, s, αCH_3), 1.35−2.6 (16H, adamantyl + $2H_\beta$), 3.02 and 3.09 (1H, d, H_β), 3.40 (1H, d, H_β), 3.55−3.80 (2H, m, $2H_5$), 4.63 and 4.82 (1H, brs, OCH), 4.68 and 4.96 (1H, brs, H_4), 6.55−7.55 (10H, m, Ar + NH), 10.85 and 10.87 (1H, brs, NH ind); ES/MS ($(M + Na)^+$) 734.2.

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-pNO_2)-OH (9e): according to general method E, **9e** was obtained from **9d**; ^{1}H NMR (DMSO + TFA) δ 1.08−2.6 (19H, m, αCH_3 + adamantyl + $2H_\beta$), 3.03 and 3.11 (1H, d, H_β), 3.41 (1H, d, H_β), 3.72 (2H, m, $2H_5$), 4.36 (1H, m, H_2), 4.63 and 4.83 (1H, brs, OCH), 4.95 and 5.20 (1H, brs, H_4), 6.7−8.2 (10H, m, Ar + NH), 10.83 and 10.87 (1H, brs, NH ind); ES/MS ($(M + Na)^+$) 653.2.

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-o,pCl_2)-OH (10e): according to general method E, **10e** was obtained from **10d**; ^{1}H NMR (DMSO + TFA) δ 1.18 and 1.73 (3H, s, αCH_3), 1.3−2.6 (16H, m, adamantyl + $2H_\beta$), 3.00 and 3.08 (1H, d, H_β), 3.39 (1H, d, H_β), 3.55 (1H, m, H_5), 3.8 (1H, m, H_5), 4.35 and 4.40 (1H, m, H_2), 4.60 and 4.82 (1H, brs, OCH), 4.64 and 4.97 (1H, brs, H_4), 6.78−7.50 (10H, m, Ar + NH), 10.83 and 10.86 (1H, brs, NH ind); ES/MS (^{35}Cl, ^{35}Cl − $(M + Na)^+$, and ^{35}Cl,^{37}Cl − $(M + Na)^+$) 676.2 and 678.2.

$_2$Adoc-αMe-D-Trp-D-*cis*-Hyp(Ph-o,pF_2)-OH (11e): according to general method E, **11e** was obtained from **11d**; ^{1}H NMR (DMSO + TFA) δ 1.15 and 1.21 (3H, s, αCH_3), 1.3−2.6 (16H, m, adamantyl + $2H_3$), 3.03 and 3.12 (1H, d, H_β), 3.45 (1H, d, H_β), 3.72 (1H, m, $2H_5$), 4.40 (1H, m, H_2), 4.63 and 4.84 (1H, brs, OCH), 4.67 and 4.93 (1H, m, H_4), 6.8−7.4 (m, Ar + NH), 10.88 (1H, brs, NH ind); ES/MS ($(M + Na)^+$) 644.3.

$_2$Adoc-α-Me-D-Trp-D-*cis*-β-homo-Hyp(Ph-pCl)-OH (12e): according to general method E, **12e** was obtained from **12d**; mp 149−151 °C; FAB/MS (MH^+) 634; HPLC t_R 17.00, min, 60% B; ^{1}H NMR (DMSO) δ 1.0−2.62 (19H, m, adamantyl + αCH_3

+ $2H_3$), 2.75−3.90 (9H, m, $2H_{\beta Pro}$ + $2H_{\beta Trp}$ + OCH_3 + $2H_5$), 4.03 and 4.13 (1H, m, H_2), 4.53 and 4.60 (1H, s, OCH), 4.78 (1H, brs, NH_{ind}).

$_2$Adoc-α-Me-D-Trp-D-*cis*-Pro(4-Ph)-OH (13e): according to general method E, **13e** was obtained from **13d**; mp 171−174 °C; ^{1}H NMR (DMSO + TFA) δ 1.10−2.12 (17H, m, adamantyl + αCH_3), 2.38 (1H, m, H_3), 2.61 (1H, m, H_3), 2.85−3.51 (4H, m, $2H_\beta$ + $2H_5$), 4.01−4.40 (2H, m, H_4 + H_2), 4.68 and 4.82 (1H, brs, OCH), 6.80−7.44 (11H, m, Ar + NH), 10.86 (1H, brs, NH_{ind}).

$_2$Adoc-α-Me-D-Trp-D-*cis*-Pro(4-Ph-pCl)-OH (14e): according to general method E, **14e** was obtained from **14d**; mp 165−168 °C; ^{1}H NMR (DMSO + TFA) δ 1.20−2.31 (17H, m, adamantyl + αCH_3), 2.34−2.80 (2H, m, $2H_3$), 3.0−3.60 (4H, m, $2H_\beta$ + $2H_5$), 4.07−4.40 (2H, m, H_4 + H_2), 4.73 and 4.89 (1H, brs, OCH), 6.88−7.50 (10H, m, Ar + NH), 10.92 (1H, s, NH_{ind}).

$_2$Adoc-α-Me-D-Trp-D-*cis*-Hyp(Bzl)-OH (15e) and $_2$Adoc-α-Me-D-Trp-D-*cis*-Hyp(Bzl-mCl)-OH (16e): according to general method E, **15e** was obtained from **15d** and **16e** from **16d. 15e** mp 229−231 °C; ^{1}H NMR (DMSO + TFA) δ 1.05−2.35 (19H, m, adamantyl + αCH_3 + $2H_3$), 3.0 and 3.10 (1H, d, H_β), 3.36−3.58 (2H, m, H_β + H_5), 3.71 (1H, d, H_5), 3.86 and 4.13 (1H, s, H_4), 4.32 (3H, dd, H_2 + OCH_2), 4.62 and 4.81 (1H, brs, OCH), 6.78−7.40 (11H, m, Ar + NH), 10.83 (1H, brs, NH_{ind}). **16e** mp 154−157 °C; ^{1}H NMR (DMSO + TFA) δ 1.05−2.35 (19H, m, adamantyl + αCH_3 + $2H_3$), 3.06 and 3.14 (1H, d, H_β), 3.40−3.80 (3H, m, H_β + $2H_5$), 3.94 and 4.12 (1H, brs, H_4), 4.40 (3H, m, H_2 + OCH_2), 4.69 and 4.89 (1H, brs, OCH), 6.83−7.46 (10H, m, Ar + NH), 10.90 (1H, s, NH_{ind}).

Binding Assays. [^{3}H]pCCK$_8$ (specific activity 60 Ci/mmol) was purchased from Amersham. 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 bacitracin for 60 min in the presence of brain membranes (0.6 mg of protein/tube) or in 10 mM Pipes-HCl buffer (pH 6.5), 30 mM $MgCl_2$, 0.2 mg/mL bacitracin, and 0.2 mg/mL soybean trypsin inhibitor for 120 min in the presence of pancreatic membranes (0.2 mg of protein/tube).

[^{3}H]pCCK$_8$ was incubated at a concentration of 0.2 nM with brain membranes and 0.1 nM with pancreatic membranes, 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 rapid 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 was counted. K_i values were calculated using the Cheng−Prusoff equation. Moreover, when the Hill slope values of the curves was significantly less than unity, the data were reanalyzed with a two-site model. The displacement curves better fitted ($p < 0.05$) by a two-site model than a one-site model were considered significant.

Cell Growth, Transfection, and Transformant Cell Selection. Chinese hamster ovary (CHO) cells were grown in HAM-F12 medium containing 10% fetal calf serum, 50 μg/mL gentamycin, and 1 mM sodium pyruvate, in 5% CO_2 at 37 °C. One day before transfection, cells were plated at a density of 3×10^5 cells/9-cm diameter tissue culture dish. Cells were transfected with 15 μg of the pcDNA3/RKB vector using the calcium phosphate method;[32] 2 days after the transfection, cells were grown in the presence of 0.4 mg/mL G 418. After 3 weeks, growing clones of cells resistant to G 418 were observed. A pure cell line was obtained by cloning by the limit dilution method. CHO cells transfected with the human CCK-B receptor have already been described.[29]

Preparation of CHO Membranes and Ligand Binding Assays. Cells were plated at a density of 1×10^6 cells/15-cm diameter tissue culture dish in the presence of 0.4 mg/mL G 418. At confluency, cells were rinsed with cold phosphate-buffered saline (PBS), scraped from the tissue culture dish, and resuspended in PBS. The cells were centrifuged at 4 °C for 5 min at 2000 rpm. The pellet was homogenized at 4 °C in 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM $MgCl_2$ and centrifuged at 4 °C for 35 min at 100000*g*. The resulting pellet was rehomogenized in a large excess of ice-cold buffer and centrifuged under the same conditions. The final pellet was homogenized at 4 °C in 5 mL of Tris-HCl buffer (pH 7.4) with 5 mM $MgCl_2$ and bacitracin. The membranes were aliquoted and frozen at −80 °C. Protein concentration was estimated using the Pierce bicinchoninic acid protein assay reagent with bovine serum albumin as a standard. The binding assays were performed in 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, and 0.2 mg/mL bacitracin as previously described.[23b] Each assay contained the membrane preparation (around 40 μg of protein) and [^{3}H]pCCK$_8$ (0.4 nM) in a final volume of 1 mL. The nonspecific binding was determined in the presence of 1 μM CCK$_8$.

Inositol Phosphate Assays. CHO cells stably expressing wild-type CCK-B rat receptor were assayed for agonist (and/or antagonist)-stimulated IP hydrolysis essentially as previously described.[29,33] Cells were plated in 24-well microtiter plates. Before confluency cells were grown in the presence of 1 μCi/mL *myo*-[2-^{3}H]inositol for 16 h, at 37 °C. Cells were treated with 10 mM LiCl for 30 min at 37 °C and with 0.5 nM CCK$_8$ for 5 min, and various concentrations of antagonists were then added to the cells. After 45 min at 37 °C, the incubation medium was removed and the cells were washed twice with 1 mL of PBS. The reaction was stopped by adding 400 μL of ice-cold 67% methanol and 300 μL of 0.125% Triton. The cells were scraped, and the suspension was subjected to chloroform extraction; 0.5 mL of the aqueous phase was added to 4.5 mL of water. The solution was then loaded onto a 0.5-mL column of AG1-X8 Dowex anion-exchange resin. The column was washed with 1 mL of distilled water followed by 5 mL of 5 mM sodium borate/60 mM sodium formate. Total [^{3}H]inositol phosphates were then eluted into scintillation vials with 5 mL of 1 M ammonium formate/0.1 M formic acid. Scintillation mixture was then added and the radioactivity counted. The data are expressed as the percentage of the maximal response induced by 0.5 nM of CCK$_8$.

Structural Analyses by NMR. The N-*cis* and N-*trans* isomers of the proline compounds were established by measuring the relative intensities of at least three different signals. Assignment of the peaks to each rotamer was established by NOESY experiments (200 ms of mixing time) using a Brüker AMX600 spectrometer operating at 600 MHz for proton. The rotational barrier ($\Delta G^{\ddagger}$) around the proline bond was measured by evaluation of the line widths of three signals at various temperatures.

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