5-(Tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-Based Potent and Selective CCK_1 Receptor Antagonists: Structure−Activity Relationship Studies on the Central 1,3-Dioxoperhydropyrido[1,2-*c*]pyrimidine Scaffold

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To further define the pharmacophore of the potent and selective 5-(tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-based CCK_1 receptor antagonists the electronic and topographic properties of the central 1,3-dioxoperhydro-pyrido[1,2-*c*]pyrimidine scaffold have been modified. With this aim, the 1- and 3-oxo groups have been replaced by the thioxo- and deoxi-analogues, and the fused piperidine ring has been contracted to the corresponding pyrrolidine moiety. The results of the evaluation of the new analogues as CCK receptor ligands, in rat pancreas and cerebral cortex preparations, showed that, whereas replacement of oxygen with sulfur is allowed, reduction of the 1- or 3-oxo groups or the contraction of the fused piperidine ring lead to the complete loss of binding affinity at CCK_1 receptors. The thioxo-analogues **5a**, **8a**, **12a**, and **12b** showed functional CCK_1 antagonist activity, inhibiting the CCK-8-stimulated amylase release from pancreatic acinar cells. The 1-thioxo analogue **5a**, with subnanomolar affinity ($IC_{50} = 0.09 \times 10^{-9}$ M), was found to be the most potent and selective compound within the family of 5-(tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-based CCK_1 antagonists.

Introduction

Cholecystokinin (CCK) is a regulatory peptide hormone, found predominantly in localized endocrine cells of the gastrointestinal tract, and a neurotransmitter present throughout the nervous system.[1] In the gastrointestinal system CCK regulates motility, pancreatic enzyme secretion, gastric emptying, and inhibition of gastric acid secretion.[1,2] In the nervous system CCK is involved in anxiogenesis,[1,3,4] satiety,[1,5] nociception,[1,6] memory and learning processes,[3,6−8] and regulation of dopamine release.[1,6] These biological effects are mediated by two specific G protein coupled receptor subtypes, termed CCK_1 and CCK_2.[6,9]

The variety of physiological effects of CCK and its possible role in some pathological disorders have stimulated research in this area, and over the past decade, a number of potent and selective non-peptide CCK_1 and CCK_2 receptor agonists and antagonists have been reported.[6,10−12] Some of these ligands have been useful tools for characterizing both CCK receptor subtypes and for gaining further insight into the functional significance of CCK in the periphery and in the central nervous system (CNS). However, the physiological effects of CCK mediated by CCK_1 or CCK_2 receptors are not completely established.[1,6] Therefore, the development of CCK receptor antagonists with higher selectivity for both receptor subtypes is of interest in order to shed further light on their functional roles. In this regard, we reported the design, synthesis,[13] and pharmacological properties[14] of the 5-(tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivative **1a** (IQM-95,333), which is one of the most selective CCK_1 receptor antagonists up to now described.[6] Thus, this compound showed a CCK_1 receptor affinity in the nanomolar range, but it was virtually devoid of affinity at brain CCK_2 receptors.[14] In accordance with this CCK_1 receptor affinity, compound **1a** was a potent inhibitor of the CCK-8-stimulated amylase release from isolated pancreatic acini, and blocked the CCK-8-induced hypophagia and hypolocomotion in rats.[14] Furthermore, despite the predominant role attributed to CCK_2 receptors in the anxiogenic effects of CCK,[3,6] this CCK_1 antagonist also showed a marked anxiolytic-like activity in animal models.[14] These results supported the suggestion of some authors that CCK_1 receptors may be involved also in anxiogenesis.[15−17] From previous structure−activity relationship studies, the Boc-L-Trp residue and the (4a*S*,5*R*)-stereochemistry at the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine skeleton emerged as essential structural requirements for potent CCK_1 binding affinity and subtype receptor selectivity.[13,18] These studies also showed the influence of the lipophilicity and spatial orientation of the moiety linked at the N2-position upon the binding profile of this family of CCK_1 antagonists.[19] Now, to further define the pharmacophore, we have studied the effect of modifying the electronic and topographic properties of the central 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine scaffold, through thionation and reduction of the 1- and/or 3-oxo groups, and the contraction of the fused piperidine to a pyrrolidine ring. Because of the lower electronegativity and higher volume of sulfur with respect to oxygen,[20,21] and to the higher polarization of the C=S bond,[22,23] thionation produces a decrease in the acceptor hydrogen-

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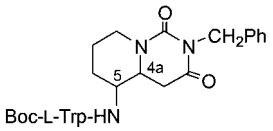
bonding properties of the 1- and 3-oxo groups. On the other hand, and differently from oxygen, sulfur could participate in additional $\pi-\pi$ interactions, using empty 3d orbitals.[24] The oxo group reduction produces important topographic changes due to the transformation of the plane sp^2 hybridization into the tetrahedral sp^3, and also to a loss in the acceptor hydrogen-bonding properties. In regard to the contraction of the fused piperidine ring to a pyrrolidine one, this structural modification affects the topography of the 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine skeleton, and, therefore, the spatial arrangement of the groups linked to positions 2 and 5, both critical for receptor recognition.[18,19] As these important topographic changes could modify the stereochemical requirements for binding at CCK_1 and CCK_2 receptors, the complete stereochemical space defined by the eight stereoisomers at the Trp residue and at positions 4a and 5 of the central skeleton was explored in this case. The present paper deals with the synthesis and CCK receptor binding profile of this new series of derivatives modified at the 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine scaffold.



1a: (4a*S*,5*R*)

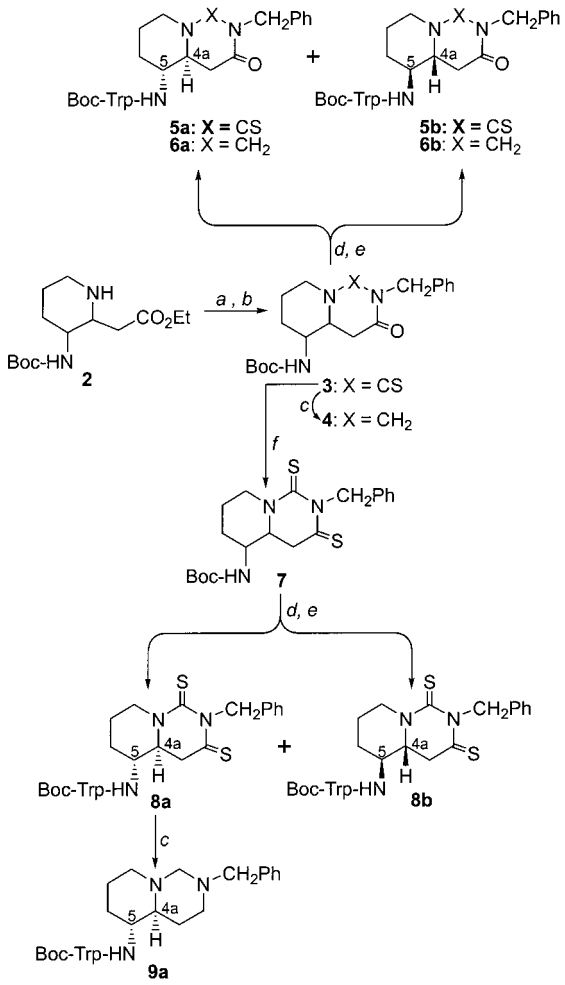
1b: (4a*R*,5*S*)

Chemistry

Similar to the lead compounds **1a** and **1b**, their analogues **5a,b** and **6a,b** (selectively modified at position 1 of the perhydropyrido[1,2-*c*]pyrimidine skeleton) were prepared from the 2,3-*trans*-3-amino-2-piperidineacetic derivative **2** [obtained as a (5:1) racemic mixture of (2*S*,3*R*)- and (2*R*,3*S*)-isomers from D-Orn(Z)-OH[13]], as shown in Scheme 1. This synthetic pathway involved reaction with benzyl isothiocyanate, followed by in situ NaH-promoted cyclization of the resulting thiourea to 5-Boc-amino-3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine (**3**). No cyclization through the sulfur was observed in this reaction.[23] Then, sequential *N*-Boc removal and coupling with Boc-L-Trp-OH provided a (5:1) diastereoisomeric mixture of the 1-thioxo analogues **5a** and **5b**, which were chromatographically resolved. Reduction of **3** with nickel boride, generated in situ from $NiCl_2 \cdot 6H_2O$ and $NaBH_4$ in a (1:1) THF-MeOH mixture,[25−28] led to the 3-oxoperhydropyrido[1,2-*c*]pyrimidine derivatives **4**, whose *N*-Boc removal followed by coupling with Boc-L-Trp-OH, and chromatographic separation yielded the 1-reduced analogues **6a** and **6b**. On the other hand, thionation of the 3-oxo-1-thioxoperhydropyrido[1,2-*c*]-pyrimidines **3** with Lawesson's reagent[29−31] led to the 1,3-dithioxo derivatives **7**, and the following *N*-Boc removal and coupling with Boc-L-Trp-OH provided the corresponding 5-(Boc-tryptophyl)amino derivatives **8a** and **8b**. Finally, the nickel boride reduction of the major diastereoisomer **8a** led to the corresponding 1,3-desulfurized analogue **9a**.

Compounds selectively modified at position 3 were prepared from a (5:1) (4a*S*,5*R*)/(4a*R*,5*S*)-racemic mixture of the 5-Boc-amino-1,3-dioxoperhydropyrido[1,2-*c*]-pyrimidines[13] **10** (Scheme 2). First, the reaction with

Scheme 1[a]

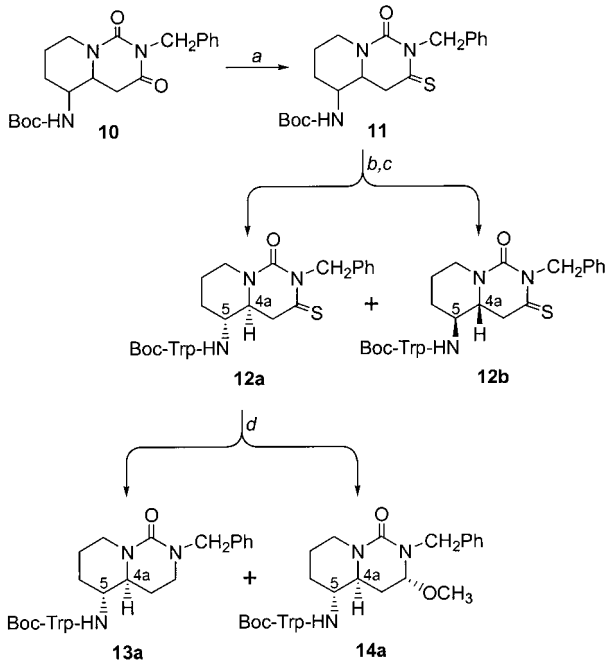


[a] Reagents: (a) Ph-CH_2-NCS, THF; (b) NaH, THF; (c) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, THF/MeOH; (d) TFA, CH_2Cl_2; (e) Boc-Trp-OH, BOP, Et_3N, CH_2Cl_2; (f) Lawesson's reagent, toluene.

the Lawesson's reagent led exclusively to the 3-thioxo analogues **11** in high yield, whose *N*-Boc removal, followed by coupling with Boc-L-Trp-OH, yielded the corresponding (5:1) diastereoisomeric mixture of the 5-(Boc-tryptophyl)amino derivatives **12a** and **12b**, which were chromatographically resolved. Then, reduction of the major diastereoisomer **12a** by treatment with nickel boride, in (1:1) THF/MeOH mixture, provided the desired 3-desulfurized analogue **13a** (30%), along with compound **14a** (36%), resulting from a stereospecific addition of MeOH to the thioxo group, followed by reductive removal of the mercapto group. A similar reaction of addition of an alcoholic solvent has been described in the reductive desulfurization of thiohydantoins and thiobarbituric acids with Raney nickel.[32] Assignment of the (*R*)-absolute configuration at position 3 in **14a** was made on the basis of the NOE effect observed between the methoxyl protons and the 4a-H proton in its ^1H NMR NOE difference spectrum, indicative of a biaxial disposition for them (Figure 1).

In comparison with the model compounds **1a** and **1b**, the ^1H- and ^{13}C NMR spectra of the thioxo analogues **5**, **8**, and **12** showed characteristic deshielding in the chemical shift of the C═S neighboring nuclei. However, no changes were observed in the ^1H coupling constants and NOE patterns, indicating that important topo-

Scheme 2[a]



[a] Reagents: (a) Lawesson's reagent, toluene; (b) TFA, CH_2Cl_2; (c) Boc-Trp-OH, BOP, Et_3N, CH_2Cl_2; (d) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, THF/MeOH.

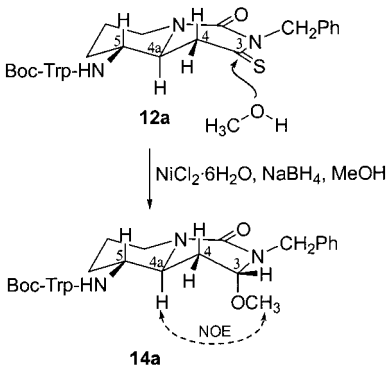
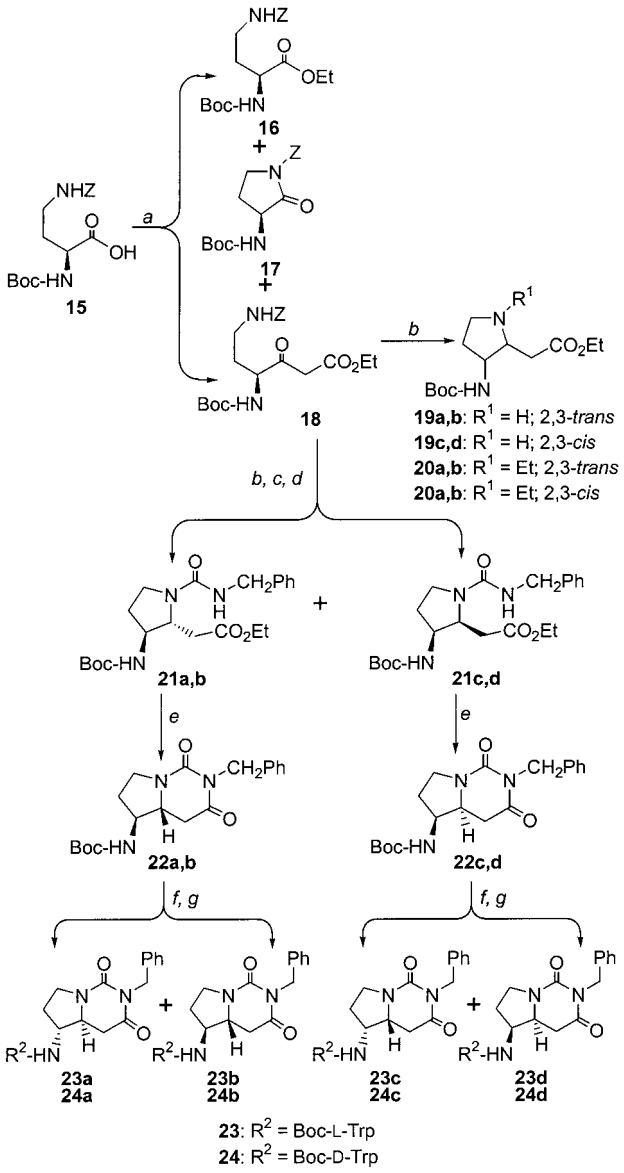


Figure 1. Synthesis and C_3 configuration assignment of the 3-methoxy derivative **14a**.

graphic changes have not taken place. Similar to these compounds, no significant changes in the coupling constants and NOE patterns for the Boc-Trp residue and the fused piperidine ring were observed in the deoxy analogues **6**, **9a**, and **13a**. However, the $N_9-C_1-N_2-C_3$ planarity in the fused pyrimidine ring is lost because of the reduction, and, therefore, the spatial disposition of the N2-benzyl group must change.

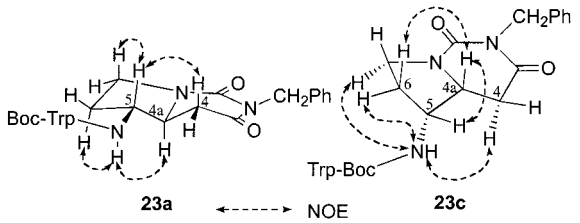
Regarding the contraction of the fused piperidine ring to the pyrrolidine analogue, the construction of the 1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine skeleton was planned using a synthetic pathway similar to that previously used for the preparation of 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives. As indicated in Scheme 3, this route involved synthesis of the appropriated pyrrolidines from 4-benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-L-butyric acid (**15**),[33] followed by reaction with benzyl isocyanate, intramolecular acylation, and finally *N*-Boc-removal and coupling with Boc-Trp-OH. Because in this case we wished to explore the complete stereochemical diversity, we did not pay special attention to stereoselectivity. Using the

Scheme 3[a]



[a] Reagents: (a) CDI, $LiCH_2CO_2Et$, THF; (b) H_2, Pd(C), EtOH; (c) $NaBH_3CN$, $ZnCl_2$, EtOH; (d) Ph-CH_2-NCO, THF; (e) NaH, THF; (f) TFA, CH_2Cl_2; (g) Boc-L-Trp-OH or Boc-D-Trp-OH, BOP, Et_3N, CH_2Cl_2.

same reaction conditions that we had used for the synthesis of Boc-Orn(Z)-OH derived β-keto esters,[13,34] the activation of the acid **15** with 1,1′-carbonyldiimidazole at room temperature for 2 h, followed by reaction with $LiCH_2CO_2Et$ [generated from ethyl acetate and lithium bis(trimethylsilyl)amide] at −78 °C for 15 min, led to the required β-keto ester **18** in only a 9% yield, along with the butyrate derivative **16** (9%), resulting from transesterification with ethyl acetate, and the γ-lactam **17** (20%). Then, with the aim of avoiding, or minimizing, the formation of this γ-lactam, the activation time and temperature were lowered to 15 min and 0 °C, respectively. In these conditions, the γ-lactam **17** was not detected in the reaction, and the β-ketoester **18** was obtained in 45% yield, along with 12% of compound **16**. The catalytic hydrogenation of **18** in EtOH, using Pd(C) as catalyst, led to a mixture of the expected 2,3-*trans*- and 2,3-*cis*-pyrrolidines **19a,b** and **19c,d** [50%, (10:1)], along with their respective *N*-ethyl



	$J_{4a,4ax}$	$J_{4a,4ec}$	$J_{4a,5}$	$J_{5,6}$	$J_{6,7}$
23a	14	4	8	8, 8	8, 2, 8, 8
23c	15	4.5	4.5	6, 0	10, 10, 2, 8

Figure 2. NOE effects and ^{1}H,^{1}H coupling constants used for the configuration assignment of 1,3-dioxoperhydropyrrolo-[1,2-*c*]pyrimidine derivatives.

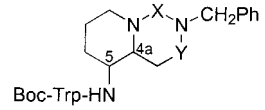
derivatives **20a,b** and **20c,d** [40%, (8:1)]. The ratio of these *N*-ethyl derivatives, which are are supposed to be formed by alkylation with acetaldehyde generated from EtOH, as described in some catalytic hydrogenations with metallic catalyst,[35−37] was not decreased by changing the time, temperature, or H_2 pressure used in the catalytic hydrogenation. To avoid the formation of *N*-ethylpyrrolidine derivatives, the Z protecting group of **18** was first removed by catalytic hydrogenolysis, and then, the intramolecular reductive amination was carried out by reduction with $NaBH_3CN$ in the presence of $ZnCl_2$, to produce a (4:3) 2,3-*trans*/-*cis* mixture of pyrrolidine derivatives **19** in 74% yield. This mixture was chromatographically resolved into the 1-(*N*-benzyl)-carbamoylpyrrolidines 2,3-*trans* **21a,b** and 2,3-*cis* **21c,d**, after its reaction with benzyl isocyanate. Then, NaH-promoted cyclization, followed by removal of the *N*-Boc protecting group, and coupling with Boc-L-Trp-OH or Boc-D-Trp-OH led, in each case, to a mixture of diastereoisomeric 5-(Boc-tryptophyl)amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine derivatives **23a,b** and **24a,b**, respectively, from the 2,3-*trans*-pyrrolidines **21a,b**, and **23c,d** and **24c,d**, from the 2,3-*trans*-isomers **21c,d**. All these diastereoisomeric mixtures, coming from racemization in the intramolecular reductive amination[13] (compounds **a** 30% of the 4a,5-*trans*-isomers **a** and **b**, and compounds **c** 37% of the 4a,5-*cis*-isomers **c** and **d**), were chromatographically resolved.

The $J_{4a,5}$ values in the ^{1}H NMR spectra of the 1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine derivatives **23** and **24** (8 Hz in the *trans*-isomers and 4−4.5 Hz in the *cis*-isomers), were not conclusive for the assignment of the C_{4a},C_5-relative configuration. This assignment was made on the basis of the NOE effects observed in ^{1}H NMR NOE difference spectra (Figure 2). Thus, the NOEs observed in 4a,5-*trans*-isomers (**23a**, **23b**, **24a**, and **24b**) between the proton 5-H and the 4-H_{axial} and 7-H protons, located in the same face of the 1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine skeleton, are indicative of a pseudoaxial disposition for these protons, as well as the NOEs observed for 5-NH with 4a-H and one 6-H. These data, along with the coupling constants for the protons of the bicyclic skeleton, suggest that the fused pyrrolidine ring adopts a preferred envelope conformation[38] with the C_6 projecting below the mean plane of the ring, and the 5-(Boc-tryptophyl)amino moiety in a pseudoequatorial disposition. In the case of the 4a,5-*cis*-isomers (**23c**, **23d**, **24c**, and **24d**), the coupling constants, and NOE effects observed for the 4a-H with the 5-H and one of the 6-H protons, and for the 5-NH with the other 6-H, one of the two 7-H and the 4-H in the same face of the bicyclic ring, suggest that the fused pyrrolidine ring also adopts a preferred envelope conformation, but in this case with the C_5 below the mean plane of the ring, and the 5-(Boc-tryptophyl)amino moiety in a pseudoaxial disposition. These conformations are similar to energy minima calculated for 5-acetylamino-2-benzyl-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine analogues by molecular dynamics, followed by energy minimization, using the Chem3D program.

Biological Results and Discussion

The affinity of the new 5-(Boc-tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine analogues herein described at CCK_1 and CCK_2 receptors was determined by measuring the displacement of [^{3}H]propionyl-CCK-8 binding to rat pancreatic and cerebral cortex homogenates, respectively, as previously described.[39] Subsequently, those compounds which showed a significant affinity at CCK_1 receptors at concentrations below 10^{-7} M were tested for their antagonism to CCK-8-stimulated amylase release from pancreatic acinar cells.[40] For comparative purposes CCK-8 and the model compounds **1a** and **1b** were also included in the assays. The results of the evaluation of the thioxo- and deoxi-analogues are shown in Table 1. Compared with the lead compound **1a**, the (4a*S*,5*R*)-thioxo-analogues **5a**, **8a**, and **12a** kept the highly potent and selective binding at the CCK_1 versus the CCK_2 receptor subtype. It is interesting to note that the thionation at position 1 led to a 1 order of magnitude increase in the binding affinity of **5a**. This compound, with a subnanomolar potency (IC_{50} = 0.09 nM) is the most potent and selective compound within the family of 5-(Boc-tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-based CCK_1 antagonists. The same thionation in the (4a*R*,5*S*)-diastereoisomers **5b** and **8b** led to a decrease of at least 1 order of magnitude in the affinity with respect to the model **1b**; the affinity of **12b**, however, was slightly increased. Compounds **5a**, **8a**, **12a**, and **12b** antagonized the CCK-8-stimulated amylase release from pancreatic acinar cells, although no linear correlation was observed between the binding affinities and the respective amylase release inhibition potency. The reason for this discrepancy, particularly marked in compounds **5a** and **8a**, is unclear. A partial agonist activity does not appear to account for the results obtained in the amylase assay, because a much higher concentration of these compounds (10 μM) did not show any intrinsic effect on amylase release. It should be noted in this regard that CCK-8 already produces a significant amylase release at a 10 pM concentration and a maximal release is generally found with a 0.5 nM concentration of the peptide.[14] In the binding assay, displacement curves with compounds **5a** and **8a** produced Hill coefficients significantly lower than unity (0.38 and 0.52 for **5a** and **8a**, respectively) suggesting the possibility of an additional low-affinity binding site. The Hill coefficient for the lead compound **1a** (0.90) was not, however, different from unity, indicating a single binding site.

Table 1. Inhibition of [^{3}H]pCCK-8 Specific Binding to CCK Receptors from Rat Pancreas (CCK_1) and Cerebral Cortex (CCK_2) Homogenates, and Inhibition of Amylase Release from Dispersed Pancreatic Acini



compd	stereochemistry	X	Y	IC_{50} (nM)[a] CCK_1	IC_{50} (nM)[a] CCK_2	amylase release[b] IC_{50} (nM)
CCK-8				1.04 ± 0.08	5.60 ± 0.30	
1a	(4a*S*,5*R*)	C=O	C=O	1.59 ± 0.10	>10000	0.64 (0.4−0.9)
1b	(4a*R*,5*S*)	C=O	C=O	22.7 ± 4.0	6153	77 (73−81)
5a	(4a*S*,5*R*)	C=S	C=O	0.09 ± 0.06	>10000	33 (16−50)
5b	(4a*R*,5*S*)	C=S	C=O	825	>10000	ND[c]
6a	(4a*S*,5*R*)	CH_2	C=O	>1000	>10000	ND[c]
6b	(4a*R*,5*S*)	CH_2	C=O	>1000	>10000	ND[c]
8a	(4a*S*,5*R*)	C=S	C=S	1.34 ± 0.40	>10000	62 (49−75)
8b	(4a*R*,5*S*)	C=S	C=S	900	>10000	ND[c]
9a	(4a*S*,5*R*)	CH_2	CH_2	>1000	>10000	ND[c]
12a	(4a*S*,5*R*)	C=O	C=S	2.83 ± 1.20	>10000	4.3 (3.6−22.6)
12b	(4a*R*,5*S*)	C=O	C=S	13.8 ± 6.6	>10000	23 (13−44)
13a	(4a*S*,5*R*)	C=O	CH_2	>1000	>10000	ND[c]
14a	(4a*S*,5*R*)	C=O	(*R*)-CH(OMe)	370	>10000	ND[c]

[a] Values are the mean or mean ± SEM of at least three experiments, performed with seven concentrations of test compounds in triplicate. [b] Inhibition of amylase release stimulated by CCK-8 (0.1 nM) in dispersed pancreatic acini. Data represent the mean of three to six independent experiments in duplicate (standard errors within ±15% of the mean). [c] ND = not determined.

With respect to the reduced analogues **6a**, **6b**, **9a**, and **13a**, none of these compounds bound at CCK_1 or CCK_2 receptors at concentrations below 10^{-6} M. Compound **14a**, resulting from the addition of MeOH at position 3, showed a moderate affinity at CCK_1, 2 orders of magnitude lower than that of the lead compound **1a**. None of the eight diastereoisomers of the 1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine derivatives **23** and **24**, resulting from the contraction of the fused piperidine ring in compounds **1** to a pyrrolidine, showed significant affinity at CCK_1 or CCK_2 receptors at concentrations below 10^{-6} M. Therefore, whereas some electronic changes, such as oxygen replacement by sulfur, are allowed, both topographic changes produced by the reduction of the 1- and/or 3-oxo groups or by the contraction of the fused piperidine ring lead to the complete loss of the binding affinity at CCK_1 receptors.

In conclusion, the results reported herein show the crucial influence of the topography defined by the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine skeleton, with a (4a*S*,5*R*)-stereochemistry, upon the binding potency at CCK_1 receptors. This influence suggests an essential role for that skeleton in the appropriate orientation of the two moieties linked at positions 2 and 5, which, as previously demonstrated, are the key factors for a good recognition by the receptor. When these results and those previously reported[13,18,19] are considered, it is surprising that, although in other families of CCK receptor ligands it has been possible to reverse receptor selectivity from CCK_1 to CCK_2 receptors by means of small structural modifications or changes in the stereochemistry, none of the structural modifications on the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-based CCK_1 receptor antagonists has led to CCK_2 selective ligands. The high selectivity makes this family of CCK_1 receptor ligands a very specific tool for the study of this receptor subtype.

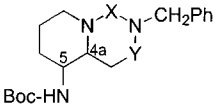
Experimental Section

Chemistry. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were obtained from Bachem Feinchemikalien AG. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254} (Merck) and preparative TLC on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254} (Merck). Silica gel 60 (230−400 mesh) (Merck) was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian Gemini-200, Varian INOVA-300, and Varian Unity-500 spectrometers, operating at 200, 300, or 500 MHz, using TMS as reference. ^{13}C NMR spectra were recorded with Varian Gemini-200 or Varian Unity-500 spectrometers, operating at 50 or 125 MHz. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical RP HPLC was performed on a Waters Nova-pak C_{18} (3.9 × 150 mm, 4 μm) column, with a flow rate of 1 mL/min, and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phases.

Synthesis of (4a*R,5*S**)-2-Benzyl-5-(*tert*-butoxycarbonyl)amino-3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine (3).** Benzyl isothiocyanate (0.64 mL, 1.99 mmol) was added to a solution of (2*R**,3*S**)-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpiperidine[41] (**2**) (500 mg, 1.66 mmol) in dry THF (13 mL). After the reaction mixture was stirred for 30 min at room temperature, it was diluted with THF (22 mL). Then, NaH (80 mg of 60% dispersion in mineral oil, 1.99 mmol) was added, and the stirring was continued for 15 additional min. Afterward, a 0 °C cooled 1 N HCl solution (50 mL) was added, and the resulting reaction mixture was extracted with EtOAc (2 × 100 mL). The organic extracts were washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 20% of EtOAc in hexane as eluant, to give the 3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine derivative **3** as a white solid (349 mg, 54%). Significant analytical and spectroscopic data are summarized in Table 2.

Synthesis of (4a*R,5*S**)-2-Benzyl-5-(*tert*-butoxycarbonyl)amino-3-oxoperhydro-pyrido[1,2-*c*]pyrimidine (4).** $NiCl_2 \cdot 6H_2O$ (323 mg, 1.36 mmol) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine (**3**) (67 mg, 0.17 mmol) in THF/MeOH mixture (1:1, 4 mL). This solution was cooled at 0 °C, then $NaBH_4$ (154 mg, 4.08 mmol) was added, and the reaction mixture was stirred for 15 min. Afterward, this mixture was filtered through Celite diatomaceous earth and evaporated to dryness. The residue was dissolved in dichloromethane (25 mL), and the solution was washed successively with a saturated solution of $NaHCO_3$ (25 mL), a saturated solution of ethylenediaminetetraacetic acid (EDTA, 2 × 25

Table 2. Significant Analytical and Spectroscopic Data of the 2-Benzyl-5-(Boc)aminoperhydropyrido[1,2-*c*]pyrimidine Derivatives **3**, **4**, **7**, and **11**



	3	**4**	**7**	**11**
X	C=S	CH_2	C=S	C=O
Y	C=O	C=O	C=S	C=S
yield (%)	54	84	63	69
mp (°C)[a]	171−173	foam	201−203[b]	139−141
formula[c]	$C_{20}H_{27}N_3O_3S$	$C_{20}H_{29}N_3O_3$	$C_{20}H_{27}N_3O_2S_2$	$C_{20}H_{27}N_3O_3S$
^{1}H-RMN[d]				
1-H	- - - -	3.78, 3.85	- - - -	- - - -
3-H	- - - -	- - - -	- - - -	- - - -
4-H	2.87	2.55	3.26−3.40	3.12, 3.59
4a-H	3.44	2.55	3.26−3.40	3.12
5-H	3.44	3.46	3.26−3.40	3.39
6-H	1.57, 2.12	1.27, 1.82	1.31, 2.07	1.34, 2.09
7-H	1.81	1.61, 1.65	1.66−1.83	1.60, 1.79
8-H	2.87, 5.31	2.06, 2.76	3.06, 5.20	2.67, 4.33
2-CH_2	5.56, 5.71	4.37, 4.73	6.23	5.56
^{13}C-RMN[e]				
C_1	181.12	69.45	177.91	155.30
C_3	164.61	167.21	197.39	201.55
C_4	32.89	28.18	43.48	44.51
C_{4a}	59.45	60.49	58.29	55.59
C_5	51.18	50.73	51.43	52.19
C_6	31.07	33.67	30.90	30.99
C_7	22.91	22.12	23.33	23.46
C_8	53.26	47.60	54.02	44.84
2-CH_2	49.59	48.66	56.89	50.51

[a] From EtOAc/hexane. [b] Sublimation. [c] Satisfactory analyses for C, H, N, and S. [d] Spectra registered at 200 MHz in $CDCl_3$, except for **7** which registered at 500 MHz. [e] Spectra registered at 50 MHz in $CDCl_3$.

mL), H_2O (25 mL), and brine (25 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography, using 33% of EtOAc in hexane as eluant, to give the 3-oxoperhydropyrido[1,2-*c*]pyrimidine derivative **4** as a foam (52 mg, 84%). Significant analytical and spectroscopic data are summarized in Table 2.

Synthesis of the 2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 5a and 5b. TFA (0.38 mL) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine (**3**) (54 mg, 0.14 mmol) in dry dichloromethane (1.5 mL). After 30 min at room temperature, the solvents were evaporated to dryness. The residue was dissolved in dry dichloromethane (1.75 mL), and Boc-L-Trp-OH (54 mg, 0.18 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 78 mg, 0.18 mmol), and TEA (45 μL, 0.32 mmol) were added successively to that solution, and stirring was continued at room temperature for 24 h. The reaction mixture was diluted with dichloromethane (25 mL), washed successively with 10% citric acid (10 mL), 10% $NaHCO_3$ (10 mL), H_2O (10 mL), and brine (10 mL), before being dried over Na_2SO_4, and evaporated. The resulting Boc-tryptophyl derivatives were purified and resolved into the diastereoisomers **5a** (lower R_f) and **5b** (higher R_f) by preparative TLC, using 3% of EtOAc in diethyl ether as eluant. Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives are summarized in Tables 3, 4, and 5.

Synthesis of the 2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-3-oxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 6a and 6b. These were prepared from (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-3-oxoperhydropyrido[1,2-*c*]pyrimidine (**4**) (50 mg, 0.14 mmol) by applying the same methodology as described above for the preparation of derivatives **5a** and **5b**. Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives **6a** (lower R_f) and **6b** (higher R_f) are summarized in Tables 3, 4, and 5.

Synthesis of (4a*R,5*S**)-2-Benzyl-5-(*tert*-butoxycarbonyl)amino-1,3-dithioxoperhydropyrido[1,2-*c*]pyrimidine (7).** Lawesson's reagent (303 mg, 0.75 mmol) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-3-oxo-1-thioxoperhydropyrido[1,2-*c*]pyrimidine (**3**) (195 mg, 0.5 mmol) in toluene (25 mL), and the reaction mixture was refluxed for 2 h. After evaporation of the solvent, the residue was purified by flash chromatography using a 20−33% gradient of EtOAc in hexane as eluant, to give the 1,3-dithioxoperhydropyrido[1,2-*c*]pyrimidine derivative **7** as a yellow solid (128 mg, 63%). Significant analytical and spectroscopic data are summarized in Table 2.

Table 3. Analytical Data of the 2-Benzyl-5-(Boc-Trp)aminoperhydropyrido[1,2-*c*]pyrimidine Derivatives **5**, **6**, **8**, **9**, and **12**−**14**

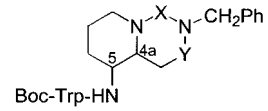
compd	yield (%)	mp (°C)[a]	t_R (min) (A:B)[b]	formula[c]
5a	73	112−114	39.80 (40:60)	$C_{31}H_{37}N_5O_4S$
5b	9	119−121	37.07 (40:60)	$C_{31}H_{37}N_5O_4S$
6a	73	99−101	11.67 (33:67)	$C_{31}H_{39}N_5O_4$
6b	8	91−92	12.00 (45:55)	$C_{31}H_{39}N_5O_4$
8a	39	218−220	17.33 (50:50)	$C_{31}H_{37}N_5O_3S_2$
8b	5	107−109	16.87 (50:50)	$C_{31}H_{37}N_5O_3S_2$
9a	38	169−171	7.87 (45:55)	$C_{31}H_{41}N_5O_3$
12a	52	109−111	43.40 (33:67)	$C_{31}H_{37}N_5O_4S$
12b	8	113−115	45.00 (33:67)	$C_{31}H_{37}N_5O_4S$
13a	33	95−97	9.80 (40:60)	$C_{31}H_{39}N_5O_4$
14a	36	111−113	5.47 (40:60)	$C_{32}H_{41}N_5O_5$

[a] From EtOAc/ethyl ether. [b] Nova-pak C_{18}, A = CH_3CN, B = 0.05% TFA in H_2O. [c] Satisfactory analyses for C, H, N, and S.

Synthesis of the 2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-1,3-dithioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 8a and 8b. These were prepared from (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-1,3-dithioxoperhydropyrido[1,2-*c*]pyrimidine (**7**) (57 mg, 0.14 mmol) by applying the same methodology as described above for the preparation of derivatives **5a** and **5b**. Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives **8a** (lower R_f) and **8b** (higher R_f) are summarized in Tables 3, 4, and 5.

Synthesis of (4a*S*,5*R*)-2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]-aminoperhydropyrido[1,2-*c*]pyrimidine (9a). $NiCl_2 \cdot 6H_2O$ (148 mg, 0.62 mmol) was added to

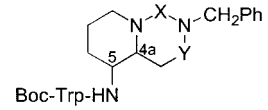
Table 4. Significant ^{1}H-NMR[a] Spectroscopic Data of the 2-Benzyl-5-(Boc-Trp)aminoperhydropyrido[1,2-*c*]pyrimidine Derivatives **5**, **6**, **8**, **9**, and **12**−**14**



compd	(4a,5) config.	X	Y	1-H	3-H	4-H	4a-H	5-H	6-H	7-H	8-H	2-CH_2	(Trp) α-H
5a	(*S*,*R*)	C=S	C=O	----	----	2.56, 2.66	3.18	3.69	1.18, 1.64	1.64	2.90, 5,22	5.52, 5.71	4.42
5b	(*R*,*S*)	C=S	C=O	----	----	2.34	2.85	3.67	1.27, 1.84	1.72	2.85, 5.16	5.51, 5.73	4.40
6a	(*S*,*R*)	CH_2	C=O	3.61, 3.75	----	2.16, 2.39	2.16	3.73	1.00, 1.57	1.30−1.76	1.91, 2.67	4.36, 4.69	4.44
6b	(*R*,*S*)	CH_2	C=O	3.43, 3.63	----	1.71, 2.17	1.59	3.56	1.14, 1.59	1.31−1.50	1.87, 2.59	4.37, 4.56	4.29
8a	(*S*,*R*)	C=S	C=S	----	----	2.63, 3.02	2.73	3.76	1.27, 1.64−1.73	1.64−1.73	2.98, 5.17	6.23, 6.32	4.46
8b	(*R*,*S*)	C=S	C=S	----	----	2.71, 3.01	2.64	3.61	1.15−1.84	1.46, 1.65	2.59, 4.26	5.54, 5.60	4.38
9a	(*S*,*R*)	CH_2	CH_2	2.49, 3.57	1.82, 2.62	1.37−1.64	1.37−1.64	3.67	1.37−1.64	1.37, 1.64	1.90, 2.84	3.45, 3.55	4.36
12a	(*S*,*R*)	C=O	C=S	----	----	2.90, 3.25	2.90	3.63	1.06, 1.70	1.55−1.70	2.56, 4.28	5.55, 5.59	4.45
12b	(*R*,*S*)	C=O	C=S	----	----	2.92	3.07	3.71	1.27, 1.83	1.55−1.74	2.92, 5.11	6.21, 6.31	4.38
13a	(*S*,*R*)	C=O	CH_2	----	2.92, 3.19	1.64	2.74	3.16	0.87, 1.44−1.58	1.44−1.58	2.37, 4.54	4.46, 4.54	4.33
14a	(*S*,*R*)	C=O	(*R*)-CH(OMe)	----	4.20 (3.18[b])	1.60, 1.94	2.85	3.56	1.47−1.53	1.47−1.53	2.85, 4.47	4.00, 5.25	4.34

[a] Spectra registered in $CDCl_3$ at 300 MHz, except for **6a**, **9a**, **12a**, and **14a** which registered at 500 MHz. [b] OMe.

Table 5. Significant ^{13}C-NMR[a] Spectroscopic Data of the 2-Benzyl-5-(Boc-Trp)aminoperhydropyrido[1,2-*c*]pyrimidine Derivatives **5**, **6**, **8**, **9**, and **12**−**14**



compd	(4a,5) config.	X	Y	C_1	C_3	C_4	C_{4a}	C_5	C_6	C_7	C_8	2-CH_2	Trp C_α
5a	(*S*,*R*)	C=S	C=O	180.87	164.63	32.03	59.24	49.24	30.69	22.73	53.46	49.61	56.08
5b	(*R*,*S*)	C=S	C=O	181.94	165.44	33.03	59.13	50.10	31.15	23.28	53.59	50.23	56.03
6a	(*S*,*R*)	CH_2	C=O	63.39	167.24	28.01	59.53	49.09	29.69	21.90	49.05	47.65	55.93
6b	(*R*,*S*)	CH_2	C=O	69.33	167.24	32.46	58.98	49.04	29.68	21.80	48.07	47.71	55.81
8a	(*S*,*R*)	C=S	C=S	177.70	197.30	42.66	57.30	49.63	30.19	22.83	53.50	56.37	56.19
8b	(*R*,*S*)	C=S	C=S	177.25	197.67	43.00	57.16	49.82	30.15	22.85	53.33	56.61	53.33
9a	(*S*,*R*)	CH_2	CH_2	77.16	51.46	26.92	65.78	50.38	30.99	23.39	51.99	59.45	55.61
12a	(*S*,*R*)	C=O	C=S	150.77	201.32	43.68	54.65	50.22	30.20	23.03	44.49	50.22	56.28
12b	(*R*,*S*)	C=O	C=S	150.65	201.52	43.57	54.47	50.42	30.29	22.93	44.41	50.27	55.66
13a	(*S*,*R*)	C=O	CH_2	155.97	44.03	24.51	59.57	49.83	31.49	24.51	42.35	51.48	55.73
14a	(*S*,*R*)	C=O	(*R*)-CH(OMe)	149.89	83.78 (55.57[b])	30.66	54.45	40.99	30.10	23.68	42.77	50.44	55.48

[a] Spectra registered in $CDCl_3$ at 50 MHz. [b] OMe.

a solution of (4a*S*,5*R*)-2-benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-1,3-dithioxoperhydropyrido[1,2-*c*]pyrimidine (**8a**) (23 mg, 0.04 mmol) in a THF/MeOH mixture (1:1, 3 mL). This solution was cooled at 0 °C, then $NaBH_4$ (73 mg, 1.92 mmol) was added, and the reaction mixture was stirred for 15 min at this temperature. Afterward, this mixture was worked up as indicated for the synthesis of compound **4**, yielding the perhydropyrido[1,2-*c*]pyrimidine derivative **9a** (8 mg, 38%) as a yellow solid, whose more significant analytical and spectroscopic data are summarized in Tables 3, 4, and 5.

Synthesis of (4a*R,5*S**)-2-Benzyl-5-(*tert*-butoxycarbonyl)amino-1-oxo-3-thioxoperhydropyrido[1,2-*c*]pyrimidine (11).** 2,4-Bis(4-methoxyphenyl)-1,3,2,4-dithiaphosphetane (Lawesson's reagent, 303 mg, 0.75 mmol) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine[13] (**10**) (188 mg, 0.5 mmol) in toluene (25 mL), and the reaction mixture was refluxed for 2 h. After evaporation of the solvent, the residue was purified by flash chromatography, using a 20−33% gradient of EtOAc in hexane as eluant, to give the 1-oxo-3-thioxoperhydropyrido[1,2-*c*]pyrimidine derivative **11** as a pale yellow solid (134 mg, 69%). Significant analytical and spectroscopic data are summarized in Table 2.

Synthesis of the 2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-1-oxo-3-thioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 12a and 12b. These were prepared from (4a*R**,5*S**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-1-oxo-3-thioxoperhydropyrido[1,2-*c*]pyrimidine (**11**) (54 mg, 0.14 mmol) by applying the same methodology described above for the preparation of derivatives **5a** and **5b**. Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives **12a** (lower R_f) and **12b** (higher R_f) are summarized in Tables 3, 4, and 5.

Synthesis of (4a*S*,5*R*)-2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-1-oxoperhydropyrido[1,2-*c*]-pyrimidine (13a) and (3*R*,4a*S*,5*R*)-2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-3-methoxy-1-oxo-perhydropyrido[1,2-*c*]pyrimidine (14a). $NiCl_2 \cdot 6H_2O$ (83 mg, 0.35 mmol) was added to a solution of (4a*S*,5*R*)-2-benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-1-oxo-3-thioxo-perhydropyrido[1,2-*c*]pyrimidine (**12a**) (23 mg, 0.04 mmol) in THF/MeOH mixture (1:1, 1.5 mL). This solution was cooled at 0 °C, then $NaBH_4$ (40 mg, 1.04 mmol) was added, and the reaction mixture was stirred for 15 min at this temperature. This mixture was filtered through Celite diatomaceous earth and evaporated to dryness. The residue was dissolved in dichloromethane (25 mL), and the solution was washed successively with a saturated solution of $NaHCO_3$ (15 mL), a saturated solution of EDTA (2 × 15 mL), H_2O (15 mL), and brine (15 mL), then dried over Na_2SO_4, and evaporated. The residue was purified by preparative TLC, using 33% of EtOAc in hexane as eluant, yielding the 1-oxoperhydropyrido[1,2-*c*]-pyrimidine derivative **13a** (lower R_f, 8 mg, 33%) and the 3-methoxy-1-oxoperhydropyrido[1,2-*c*]pyrimidine derivative **14a** (higher R_f, 9 mg, 36%). Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives are summarized in Tables 3, 4, and 5.

Synthesis of Ethyl (4*S*)-6-(Benzyloxycarbonyl)amino-4-(*tert*-butoxycarbonyl)amino-3-oxohexanoate (18). METHOD A: 1,1′-Carbonyldiimidazole (CDI, 1.40 g, 8.62 mmol) was added to a solution of (2*S*)-4-(benzyloxycarbonyl)-amino-2-(*tert*-butoxycarbonyl)aminobutyric acid[33] (**15**) (2.53 g,

7.18 mmol) in dry THF (20 mL), and the solution was stirred at room temperature for 2 h. Then this solution was added, under argon atmosphere, to a −78 °C cooled solution of $LiCH_2$-CO_2Et [obtained by slow addition of dry EtOAc (3.1 mL, 31.6 mmol) to 1 M solution of lithium bis(trimethylsilyl)amide in hexane (31.6 mL, 31.6 mmol)], and stirring was continued for 20 min at this temperature. Afterward, 1 N HCl was slowly added until pH 7 was reached, and the mixture was extracted with EtOAc (2 × 100 mL). The organic extracts were washed successively with saturated $NaHCO_3$ (150 mL), H_2O (150 mL), and brine (150 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 33% of ethyl ether in hexane as eluant, to give, from higher to lower R_f, ethyl (2*S*)-4-(benzyloxycarbonyl)amino-2-(*tert*-butoxycarbonyl)aminobutyrate (**16**) (265 mg, 10%), the *β*-ketoester **18** (265 mg, 9%), and the 2-oxopyrrolidine **17** (480 mg, 20%). METHOD B: Similar to method A, but the initial activation with CDI was carried out at 0 °C for 15 min. In this way, only ethyl (2*S*)-4-(benzyloxycarbonyl)amino-2-(*tert*-butoxycarbonyl)aminobutyrate (**16**) (322 mg, 12%) and the *β*-ketoester **18** (1.34 g, 45%) were obtained.

Ethyl (2*S*)-4-(Benzyloxycarbonyl)amino-2-(*tert*-butoxycarbonyl)aminobutyrate (16). Syrup. ^{1}H NMR (200 MHz, $CDCl_3$) *δ* 1.27 [t, 3H, *J* = 7 Hz, CH_3 (Et)], 1.45 (s, 9H, Boc), 1.79 and 2.22 (2m, 2H, 3-H), 3.30 and 3.65 (2m, 2H, 4-H), 4.18 [c, 2H, *J* = 7 Hz, CH_2 (Et)], 4.29 (m, 1H, 2-H), 5.11 [s, 2H, CH_2(Z)], 5.57 (m, 1H, 2-NH), 5.91 (s, 1H, 4-NH), 7.34 [m, 5H, Ph (Z)]. Anal. ($C_{19}H_{28}N_20_6$) C, H, N.

(3*S*)-1-Benzyloxycarbonyl-3-(*tert*-butoxycarbonyl)amino-2-oxopyrrolidine (17). White solid. Mp 112−114 °C (EtOAc/hexane). ^{1}H NMR (200 MHz, $CDCl_3$) *δ* 1.45 (s, 9H, Boc), 1.89 and 2.63 (2m, 2H, 4-H), 3.60 and 3.91 (2m, 2H, 5-H), 4.27 (m, 1H, 2-H), 5.08 (m, 1H, 3-NH), 5.29 [s, 2H, CH_2 (Z)], 7.32−7.50 [m, 5H, Ph (Z)]. Anal. ($C_{17}H_{22}N_20_5$) C, H, N.

Ethyl (4*S*)-6-(Benzyloxycarbonyl)amino-4-(*tert*-butoxycarbonyl)amino-3-oxohexanoate (18). White solid. Mp 69−71 °C (ethyl ether). ^{1}H NMR (200 MHz, $CDCl_3$) *δ* 1.27 [t, 3H, *J* = 7 Hz, CH_3 (Et)], 1.44 (s, 9H, Boc), 1.61 and 2.10 (2m, 2H, 5-H), 3.07 and 3.47 (2m, 2H, 6-H), 3.53 (s, 2H, 2-H), 4.17 [c, 2H, *J* = 7 Hz, CH_2 (Et)], 4.33 (m, 1H, 4-H), 5.11 [s, 2H, CH_2 (Z)], 5.47 (m, 2H, 3-NH and 5-NH), 7.25−7.38 [m, 5H, Ph (Z)]. Anal. ($C_{21}H_{30}N_20_7$) C, H, N.

Synthesis of the 3-(*tert*-Butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines 19 and Their 1-Ethyl Derivatives 20. METHOD A: A solution of ethyl (4*S*)-6-(benzyloxycarbonyl)amino-4-(*tert*-butoxycarbonyl)amino-3-oxohexanoate (**18**) (256 mg, 0.62 mmol) in EtOH (25 mL) was hydrogenated, at room temperature and 3 atm of H_2 pressure, in the presence of 10% Pd(C) (100 mg) for 24 h. Afterward, the catalyst was filtered off, washed with EtOH (10 mL), and the solution was evaporated to dryness. The residue was purified by flash chromatography, using 3% of MeOH in CH_2-Cl_2 as eluant. In this way, the (8:1) mixture of 2,3-*trans*- and 2,3-*cis*-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methyl-1-ethylpyrrolidines **20** (higher R_f, 87 mg, 44%) was separated from the (10:1) mixture of 2,3-*trans*- and 2,3-*cis*-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines **19** (lower R_f, 84 mg, 50%). Neither of these two mixtures could be resolved.

2,3-*trans*- and 2,3-*cis*-3-(*tert*-Butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines 19. Syrup. ^{1}H NMR (500 MHz, $CDCl_3$) 2,3-*Trans*-**19a,b**: *δ* 1.26 [t, 3H, CH_3 (Et)], 1.44 (s, 9H, Boc), 1.61 (ddd, 1H, *J* = 7, 12.5 and 13.5 Hz, 4-H), 2.24 (ddd, 1H, *J* = 7, 8 and 13.5 Hz, 4-H), 2.36 (dd, 1H, *J* = 9.5 and 16 Hz, 2-CH_2), 2.58 (dd, 1H, *J* = 3.5 and 16 Hz, 2-CH_2), 2.89 (s, 1H, 1-H), 3.06 (m, 2H, 5-H), 3.17 (m, 1H, 2-H), 3.41 (m, 1H, 3-H), 4.14 [c, 2H, CH_2 (Et)], 4.16 (d, 1H, *J* = 7 Hz, 3-NH). 2,3-*Cis*-**19c,d**: *δ* 1.19 [t, 3H, CH_3 (Et)], 1.44 (s, 9H, Boc), 1.61 (m, 1H, 4-H) 2.24 (m, 1H, 4-H), 2.32 (m, 1H, 2-CH_2), 2.69 (dd, 1H, *J* = 2.5 and 16 Hz, 2-CH_2), 2.81 and 3.06 (2m, 2H, 5-H), 3.11 (m, 1H, 2-H), 3.34 (m, 1H, 3-H), 4.07 (c, 2H, CH_2 (Et)], 4.94 (d, 1H, *J* = 8 Hz, 3-NH). Anal. ($C_{13}H_{24}N_20_4$) C, H, N.

2,3-*Trans*- and 2,3-*Cis*-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methyl-1-ethylpyrrolidines 20. Foam. ^{1}H NMR (500 MHz, $CDCl_3$) 2,3-*trans*-**20a,b**: *δ* 1.01 and 1.20 [2t, 3H, CH_3 (Et)], 1.37 (s, 9H, Boc), 1.54 (m, 1H, 4-H), 2.11−2.21 [m, 2H, 4-H and CH_2 (1-Et)], 2.30 (dd, 1H, *J* = 9 and 17.5 Hz, 5-H), 2.41 and 2.49 (2m, 2H, 2-CH_2), 2.57 (m, 1H, 2-H), 2.71 [m, 1H, CH_2 (1-Et)], 3.00 (m, 1H, 5-H), 3.82 (m, 1H, 3-H), 4.08 [c, 2H, CH_2 (OEt)], 4.74 (s, 1H, 3-NH). 2,3-*cis*-**20c,d**: *δ* 0.99 and 1.20 [2t, 3H, CH_3 (Et)], 1.37 (s, 9H, Boc), 1.54 (m, 1H, 4-H), 2.11−2.21[m, 2H, 4-H and CH_2 (1-Et)], 2.30 (m, 1H, 5-H), 2.41 and 2.49 (2m, 2H, 2-CH_2), 2.57 (m, 1H, 2-H), 2.71 [m, 1H, CH_2 (1-Et)], 3.11 (m, 1H, 5-H), 3.82 (m, 1H, 3-H), 4.08 [c, 2H, CH_2 (OEt)], 5.09 (d, 1H, *J* = 8 Hz, 3-NH). Anal. ($C_{15}H_{28}N_20_4$) C, H, N.

Synthesis of the 3-(*tert*-Butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines 19. METHOD B: A solution of ethyl (4*S*)-6-(benzyloxycarbonyl)amino-4-(*tert*-butoxycarbonyl)amino-3-oxo-hexanoate (**18**) (475 mg, 1.16 mmol) in EtOH (50 mL) was hydrogenated, at room temperature and 1 atm of H_2 pressure, in the presence of 10% Pd(C) (50 mg) for 1 h. The catalyst was filtered off, washed with EtOH (5 mL), and $NaBH_3CN$ (602 mg, 9.28 mmol) and $ZnCl_2$ (76 mg, 0.58 mmol) were added to the solution. After the solution was stirred at room temperature for 48 h, the solvent was evaporated to dryness, and the residue was dissolved in EtOAc (50 mL). The resulting solution was successively washed with H_2O (25 mL), 0.1 N HCl (25 mL), saturated $NaHCO_3$ (25 mL), and brine (25 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 4% of MeOH in CH_2Cl_2 as eluant, to yield a 4:3 mixture of 2,3-*trans*- and 2,3-*cis*-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines **19** (236 mg, 74%).

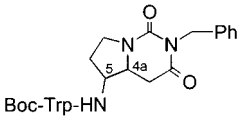
Synthesis of the 1-(Benzylcarbamoyl)-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines 21. Benzyl isocyanate (90 *μ*L, 0.86 mmol) was added to a solution of the 4:3 mixture of 2,3-*trans*- and 2,3-*cis*-3-(*tert*-butoxycarbonyl)amino-2-(ethoxycarbonyl)methylpyrrolidines **19** (154 mg, 0.56) in dry THF (3 mL), and this reaction mixture was stirred at room temperature for 4 h. EtOAc (25 mL) was added, and the resulting solution was successively washed with a 10% solution of citric acid (15 mL), saturated $NaHCO_3$ (15 mL), H_2O (15 mL), and brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by preparative TLC, using 2% of MeOH in CH_2Cl_2 as eluant, to yield the 2,3-*trans*-pyrrolidines **21a,b** (higher R_f, 100 mg, 43%) and the 2,3-*cis*-pyrrolidines **21c,d** (lower R_f, 70 mg, 31%).

2,3-*trans*-1-(Benzylcarbamoyl)-3-(*tert*-butoxycarbonyl)-amino-2-(ethoxycarbonyl)methylpyrrolidines (21a,b). Foam. ^{1}H NMR (200 MHz, $CDCl_3$) *δ* 1.23 [t, 3H, *J* = 7 Hz, CH_3 (Et)], 1.43 (s, 9H, Boc), 1.85 (m, 1H, 4-H), 2.16 (dddd, 1H, *J* = 3.5, 6, 9, and 13 Hz, 4-H), 2.51 (dd, 1H, *J* = 6 and 16 Hz, 2-CH_2), 2.70 (dd, 1H, *J* = 6.5 and 16 Hz, 2-CH_2), 3.35 (ddd, 1H, *J* = 3.5, 9.5, and 10 Hz, 5-H), 3.62 (dd, 1H, *J* = 9 and 10 Hz, 5-H), 3.97 (m, 1H, 3-H), 4.10 [c, 2H, *J* = 7 Hz, CH_2 (Et)], 4.12 (m, 1H, 2-H), 4.40 and 4.42 (2s, 2H, CH_2-Ph), 4.74 (d, 1H, *J* = 5.5 Hz, 3-NH), 5.47 (br s, 1H, 1-CONH), 7.30 (m, 5H, Ph). Anal. ($C_{21}H_{31}N_30_5$) C, H, N.

2,3-*cis*-1-(Benzylcarbamoyl)-3-(*tert*-butoxycarbonyl)-amino-2-(ethoxycarbonyl)methylpyrrolidines (21c,d). Foam. ^{1}H NMR (200 MHz, $CDCl_3$) *δ* 1.21 [t, 3H, *J* = 7 Hz, CH_3 (Et)], 1.44 (s, 9H, Boc), 1.75 (ddd, 1H, *J* = 9, 9.5, and 12 Hz, 4-H), 2.21 (dddd, 1H, *J* = 2, 7, 7.5, and 12 Hz, 4-H), 2.46 (dd, 1H, *J* = 4 and 15.5 Hz, 2-CH_2), 2.64 (dd, 1H, *J* = 6 and 15.5 Hz, 2-CH_2), 3.35 (ddd, 1H, *J* = 2, 9, and 10 Hz, 5-H), 3.47 (ddd, 1H, *J* = 7.5, 9.5, and 10 Hz, 5-H), 4.08 [c, 2H, *J* = 7 Hz, CH_2 (Et)], 4.22 (m, 1H, 3-H), 4.40 and 4.42 (2s, 2H, CH_2-Ph), 4.43 (m, 1H, 2-H), 4.93 (br s, 1H, 3-NH), 5.23 (br s, 1H, 1-CONH), 7.28 (m, 5H, Ph). Anal. ($C_{21}H_{31}N_30_5$) C, H, N.

Synthesis of the 2-Benzyl-5-(*tert*-butoxycarbonyl)-amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine Derivatives 22. NaH (14 mg of 60% dispersed in mineral oil, 0.36 mmol) was added to a solution of the corresponding 2,3-*trans*- and 2,3-*cis*-1-(benzylcarbamoyl)-3-(*tert*-butoxycarbonyl)-amino-2-(ethoxycarbonyl)methylpyrrolidines **21a,b** or **21c,d** (114 mg, 0.24 mmol) in dry THF (6 mL), and the mixture was stirred at room temperature for 15 min. The mixture was

Table 6. Significant Analytical and Spectroscopic Data of the 2-Benzyl-5-(Boc-Trp)amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine Derivatives **23** and **24**



	23a (24b)[a]	**23b (24a)**[a]	**23c (24d)**[a]	**23d (24c)**[a]
(4a,5) config.	*S*,*R* (*R*,*S*)	*R*,*S* (*S*,*R*)	*R*,*R* (*S*,*S*)	*S*,*S* (*R*,*R*)
Trp	L (D)	L (D)	L (D)	L (D)
yield (%)	12 (28)	26 (18)	27 (50)	48 (17)
formula[b]	$C_{30}H_{35}N_5O_5$	$C_{30}H_{35}N_5O_5$	$C_{30}H_{35}N_5O_5$	$C_{30}H_{35}N_5O_5$
t_R[c]	19.20	16.53	17.80	14.53
^1H-RMN[d]				
4-H	2.41, 2.68	2.23, 2.37	2.09, 2.53	1.60, 2.30
4a-H [J_{4a-5} (Hz)]	3.08 (8)	2.39 (8)	3.68 (4.5)	3.47 (4.5)
5-H	4.03	3.94	4.42	4.33
6-H	1.33, 1.95	1.38, 2.06	1.35, 1.94	1.56, 1.93
7-H	3.45, 3.53	3.37	3.18, 3.48	2.76, 3.37
2-CH_2	4.85, 4.91	4.88	4.82, 4.88	4.75, 4.95
5-NH	5.85	5.69	5.92	5.56
(Trp) α-H	4.40	4.46	4.42	4.64
$^{13}C-RMN$[e]				
C_1	151.46	151.31	151.74	151.37
C_3	168.53	168.70	169.15	169.36
C_4	36.50	36.37	32.86	32.86
C_{4a}	55.52	56.04	54.74	54.74
C_5	54.89	54.37	51.22	51.38
C_6	28.63	28.72	28.93	28.87
C_7	43.32	43.24	43.46	42.92
2-CH_2	43.59	43.47	43.64	43.68
(Trp) C_α	55.85	54.91	55.60	54.49

[a] Foam. [b] Satisfactory analyses for C, H, and N. [c] Nova-pak C_{18}, using (35:65) mixture of CH_3CN and 0.05% TFA in H_2O as mobile phase. [d] Registered in $CDCl_3$ at 500 MHz. [e] Registered in $CDCl_3$ at 125 MHz.

added to a 0 °C cooled 1 N solution of HCl (50 mL), and the resulting solution was extracted with EtOAc (2 × 25 mL). The organic extracts were washed with brine (15 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 50% of EtOAc in hexane as eluant, to yield, in each case, the (4a*R**,5*S**)- and (4a*R**,5*R**)-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine derivatives **22a,b** and **22c,d**, respectively.

(4a*R,5*S**)-2-Benzyl-5-(*tert*-butoxycarbonyl)amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidines (22a,b).** Foam (100 mg, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H, Boc), 1.73 (ddd, 1H, J = 9.5, 10, and 12.5 Hz, 6-H), 2.32 (dddd, 1H, J = 2, 7, 7.5, and 12.5 Hz, 6-H), 2.57 (dd, 1H, J = 13 and 16 Hz, 4-H), 3.02 (dd, 1H, J = 4 and 16 Hz, 4-H), 3.37 (ddd, 1H, J = 4, 8.5, and 13 Hz, 4a-H), 3.54 (ddd, 1H, J = 7.5, 10, and 11.5 Hz, 7-H), 3.67 (ddd, 1H, J = 3, 5 and 11.5 Hz, 7-H), 3.94 (m, 1H, 5-H), 4.66 (br s, 1H, 5-NH), 4.89 and 4.94 (2d, 2H, J = 15 Hz, 2-CH_2), 7.19−7.39 (m, 5H, Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.23 [CH_3 (Boc)], 29.06 (C_6), 36.84 (C_4), 43.20 and 43.55 (C_7 and 2-CH_2), 56.40 and 56.45 (C_{4a} and C_5), 80.28 [*C*(CH_3)$_3$], 127.27, 128.30, 128.56, 137.67 (Ph), 151.57 (C_1), 155.12 [CO(Boc)], 168.65 (C_3). Anal. ($C_{19}H_{25}N_3O_4$) C, H, N.

(4a*R,5*R**)-2-Benzyl-5-(*tert*-butoxycarbonyl)amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidines (22a,b).** Foam (42 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (s, 9H, Boc), 1.92 (dddd, 1H, J = 2, 2.5, 7, and 13.5 Hz, 6-H), 2.19 (dddd, 1H, J = 6, 9.5, 10, and 13.5 Hz, 6-H), 2.49 (dd, 1H, J = 14 and 16 Hz, 4-H), 2.78 (dd, 1H, J = 4 and 16 Hz, 4-H), 3.56 (ddd, 1H, J = 7, 9.5, and 10 Hz, 7-H), 3.66 (dt, 1H, J = 2.5 and 9.5 Hz, 7-H), 3.78 (dt, 1H, J = 4 and 14 Hz, 4a-H), 4.33 (ddd, 1H, J = 2, 4, and 6 Hz, 5-H), 4.74 (d, 1H, J = 8 Hz, 5-NH), 4.89 and 4.94 (2d, 2H, J = 14 Hz, 2-CH_2), 7.21−7.40 (m, 5H, Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.25 [CH_3 (Boc)], 29.61 (C_6), 33.04 (C_4), 43.55 and 43.71 (C_7 and 2-CH_2), 52.71 and 55.30 (C_{4a} and C_5), 80.48 [*C*(CH_3)$_3$], 127.33, 128.31, 128.80, 137.69 (Ph), 151.90 (C_1), 155.123 [CO(Boc)], 169.31 (C_3). Anal. ($C_{19}H_{25}N_3O_4$) C, H, N.

General Procedure for the Synthesis of the 2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)-L- and -D-tryptophyl]amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidine Derivatives 23 and 24. From the (4a*R**,5*S**)- and (4a*R**,5*R**)-2-benzyl-5-(*tert*-butoxycarbonyl)amino-1,3-dioxoperhydropyrrolo[1,2-*c*]pyrimidines **22a,b** and **22c,d** (50 mg, 0.14 mmol), applying the same methodology as described for the synthesis of the Boc-tryptophyl derivatives **5a** and **5b**. Each resulting diastereoisomeric mixture **23a,b**, **23c,d**, **24a,b**, and **24c,d**, was resolved by preparative TLC, using 2% of MeOH in CH_2Cl_2 as eluant. Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives are summarized in Table 6.

Binding Assays. CCK_1 and CCK_2 receptor binding assays were performed using rat pancreas and cerebral cortex homogenates respectively, according to the method described by Daugé et al[39] with minor modifications. Briefly, rat pancreas tissue was carefully cleaned and homogenized in Pipes HCl buffer, pH 6.5, containing 30 mM $MgCl_2$ (15 mL/g of wet tissue) and the homogenate was then centrifuged twice at 4 °C for 10 min at 50000*g*. For displacement assays, pancreatic membranes (0.2 mg protein/tube) were incubated with 0.5 nM [^{3}H]-pCCK-8 in Pipes HCl buffer, pH 6.5, containing $MgCl_2$ (30 mM), bacitracin (0.2 mg/mL), and soybean trypsin inhibitor (SBTI, 0.2 mg/mL), for 120 min at 25 °C. Rat brain cortex was homogenized in 50 mM Tris−HCl buffer pH 7.4 containing 5 mM $MgCl_2$ (20 mL/g of wet tissue), and the homogenate was centrifuged twice at 4 °C for 35 min at 100000*g*. Brain membranes (0.45 mg protein/tube) were incubated with 1 nM [^{3}H]pCCK-8 in 50 mM Tris−HCl buffer, pH 7.4, containing $MgCl_2$ (5 mM) and bacitracin (0.2 mg/mL) for 60 min at 25 °C. Final incubation volume was 0.5 mL in both cases. Nonspecific binding was determined using CCK-8 1 μM as the cold displacer. The inhibition constants (K_i) were calculated, using the equation of Cheng and Prusoff, from the displacement curves analyzed with the receptor fit competition LUNDON program.

Amylase Release. Dispersed rat pancreatic acini were prepared by using a modification of the technique of Jensen et al.[40] The rat was decapitated and the pancreas was carefully cleaned. Tissue was injected with 1 mL of a solution of collagenase (type V, Sigma) at a concentration of 1 mg/mL (in distilled water) and subjected to the digestion step consisting of two 6-min incubations at 37 °C, and washing the tissue three

times in buffer A (composition in mM: NaCl 140, KCl 4.87. $MgCl_2$ 1.13, $CaCl_2$ 1.10, Glucose 10, and Hepes 10, pH = 7.4) after each incubation. The tissue obtained after the last incubation was dispersed with the aid of a Pasteur pipet, and the homogenate was centrifuged twice (100*g*, 1 min, 4 °C). The final pellet was resuspended in 100 mL of buffer B (NaCl 98 mM, KCl 6 mM, NaH_2PO_4 2.5 mM, $CaCl_2$ 0.5 mM, theophylline 5 mM, glucose 11.4 mM, L-glutamine 2 mM, L-glutaric acid 5 mM, fumaric acid 5 mM, pyruvic acid 5 mM, SBTI 0.01%, bovine serum albumin 1%, essential amino acid mixture 1%, and essential vitamin mixture 1%). Amylase release was measured using the procedure of Peikin et al.[42] Samples (2 mL) of acini suspension were placed in plastic tubes and incubated for 30 min at 37 °C in atmosphere of pure O_2 with agitation (70 cycles/min). Amylase activity was determined using the Amyl Kit Reagent (Boehringer Mannheim). Release (*S*) was calculated as the percentage of the amylase activity in the acini that was released into extracellular medium during the incubation period. The percentage of inhibition of amylase release elicited by a fixed CCK-8 concentration (0.1 nM) produced by the assayed compounds was calculated according to the formula

$$\% I = [(S_{CCK} - S_C) - (S_T - S_C)/(S_{CCK} - S_C)] \times 100$$

where S_C is the control release (vehicle), S_{CCK} is the release elicited by CCK-8, and S_T is the release elicited by CCK-8 in the presence of increasing drug concentrations. Linear regression analysis was used in order to estimate the IC_{50} values of the compounds on the dose response curves.

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