Synthesis and Stereochemical Structure−Activity Relationships of 1,3-Dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives: Potent and Selective Cholecystokinin-A Receptor Antagonists

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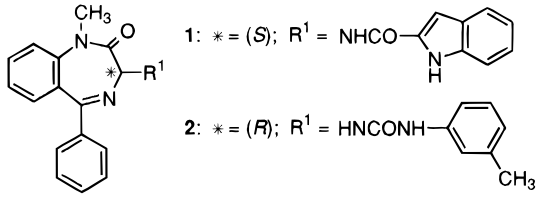
Received May 14, 1997[⊗]

The synthesis and stereochemical structure−activity relationships of a new class of potent and selective non-peptide cholecystokinin-A (CCK-A) receptor antagonists based on the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine skeleton are described. The most potent member of this series of eight diastereoisomers, (4a*S*,5*R*)-2-benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-L-tryptophyl]-amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine, displays nanomolar CCK-A receptor affinity and higher than 8000-fold potency at the CCK-A than at the CCK-B receptor. As CCK-A antagonist, this compound inhibits the CCK-8-evoked amylase release from pancreatic acinar cells at a low concentration, similar to that of the typical antagonist Devazepide. Highly strict stereochemical requirements for CCK-A receptor binding and selectivity have been found. The L-Trp and the 4a,5-*trans* disposition of the bicyclic perhydropyrido[1,2-*c*]pyrimidine are essential for binding potency and selectivity.

Introduction

Cholecystokinin (CCK) is a gastrointestinal hormone and neurotransmitter that is found in the digestive tract and in the central nervous system (CNS).[1] While several endogenous molecular forms of CCK have been isolated, the *C*-terminal octapeptide [Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2, CCK-8] appears to be the minimum sequence required for bioactivity.[2] Two CCK receptor subtypes, CCK-A and CCK-B, which mediate the diverse biological functions of CCK have been recognized so far.[3,4] CCK-A receptors are primarily found in the periphery where they mediate pancreatic enzyme secretion, gut motility, and gallbladder contraction, but they are also found in some discrete CNS areas.[4−6] CCK-A receptors have also been involved in satiety, gastrointestinal cancer, and neuroprotection.[5,7,8] CCK-B receptors are distributed throughout the CNS, and they are thought to be involved in the development of anxiety and in the control of nociception.[4,9−11]

The variety of possible therapeutic utilities for CCK receptor agonists and antagonists has prompted an intensive research in this area, and over the past decade, a number of potent and selective non-peptide CCK-A and CCK-B receptor antagonists have been reported.[12] Prominent examples are the amino acid derivatives lorglumide [DL-4-[(3,4-dichlorobenzoyl)amino]-5-(di-*n*-pentylamino)-5-oxopentanoic acid] and loxiglumide [DL-4-[(3,4-dichlorobenzoyl)amino]-5-[*N*-(3-methoxypropyl)-*N*-pentylamino]-5-oxopentanoic acid] as CCK-A antagonists[13] and the 1,4-benzodiazepine CCK-A and CCK-B antagonists Devazepide[14] (MK-329, **1**) and L-365,260[15] (**2**), respectively, both arising from the manipulation of the natural product asperlicin. All of these antagonists have served for identifying the two CCK receptor subtypes and for gaining insight into the functional significance of CCK in the periphery and in the CNS; nevertheless, the physiological effects of CCK mediated by CCK-A or CCK-B receptors remain unclear. Thus, despite the predominant role suggested for CCK-B receptors in anxiety and in the control of nociception, anxiolytic-like effects and enhancement of the morphine analgesia have been reported for the typical CCK-A antagonist Devazepide.[16,17] As has been suggested,[18] these effects could be due to the blockade of the CCK-B receptors by Devazepide at high doses, since this compound is not completely devoid of affinity for this receptor subtype (CCK-B/CCK-A, 170[19]−3000[14b]). It has also been indicated that, in general, care should be taken when attempting to distinguish effects mediated by CCK-A or CCK-B receptors with Devazepide.[20] In view of these facts, the development of CCK receptor antagonists with higher selectivity for CCK-A over CCK-B receptors is desirable in order to shed further light on the functional roles of CCK receptor subtypes. In this regard, the pharmacological characteristics of TP-680[21] [(*R*)-1-[3-[(3-carboxypyridin-2-yl)thio]-2-[(indol-2-ylcarbonyl)amino]propionyl]-4-(diphenylmethyl)piperazine] and T-0632[22] [sodium (*S*)-3-[1-(2-fluorophenyl)-2,3-dihydro-3-[(3-isoquinolinylcarbonyl)amino]-6-methoxy-2-oxo-1*H*-indole]propanoate], two novel CCK-A receptor antagonists with enhanced selectivity relative to Devazepide, have been recently reported.



As part of our current interest in non-peptide CCK receptor selective ligands, we directed our efforts toward the incorporation of conformationally restricted structures into the sequence of the CCK-B receptor selective agonist CCK-4 (Trp[30]-Met[31]-Asp[32]-Phe[33]-NH_2) as spacers between Trp[30] and Phe[33], key amino acids for interaction

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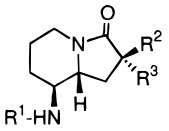
[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

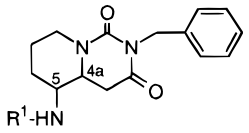
S0022-2623(97)00324-5 CCC: $14.00

with CCK receptors.[23] These two amino acids or structurally related moieties are also commonly found in various non-peptide CCK-A and CCK-B receptor antagonists as important fragments for bioactivity.[24] In contrast, a diversity of non-peptide skeletons have served as core structural motifs of ligands for CCK receptors.[24b,c,25] In spite of this diversity, the presence of a lactam function is a rather usual feature. These considerations led us to design a series of CCK-4 restricted analogues in which the Met[31]-Asp[32] fragment was replaced with a 3-oxoindolizidine ring.[26] Among these bicyclic lactams, compounds **3** and **4** were relatively modest CCK-A and CCK-B receptor antagonists, respectively. On the basis of this finding, we considered



3: R^1 = Boc-L-Trp; R^2 = CO_2Me; R^3 = CH_2Ph
4: R^1 = Boc-D-Trp; R^2 = CH_2Ph; R^3 = CO_2Me

it of interest to manipulate the 3-oxoindolizidine nucleus with the main purpose of altering the conformational properties of compounds **3** and **4**, so that the aromatic side chains could adopt more appropriate orientations to interact with the corresponding receptor site. This tactic led us to the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine **5a** (IQM-95,333), a potent and selective CCK-A receptor antagonist, both *in vitro* and *in vivo*. Very recently, we described[27] the pharmacological properties of this novel compound, which displays receptor affinity roughly similar to that of Devazepide, but it is virtually devoid of affinity at brain CCK-B receptors. Interestingly, it also shows a marked anxiolytic-like activity in animal models. Here, we report on the synthesis of **5a** and on the importance of the stereochemical structure for affinity and CCK receptor subtype selectivity of this compound. To this end, all the possible stereoisomers (**5a**−**d** and **6a**−**d**) have been prepared and evaluated.

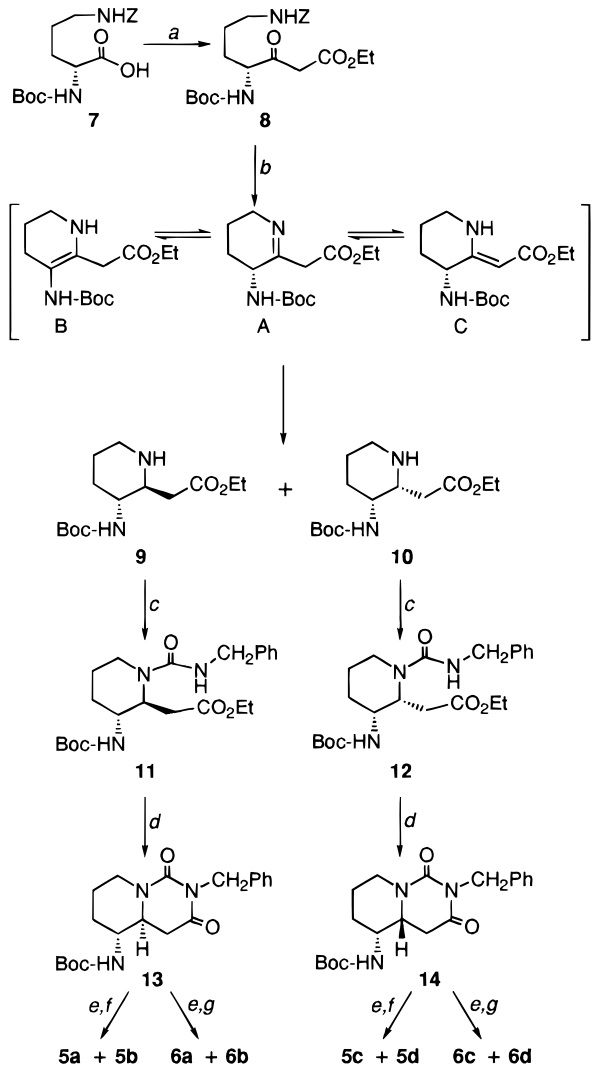


compd	R^1	C-5	C-4a
5a (IQM-95,333)	Boc-L-Trp	*R*	*S*
5b	Boc-L-Trp	*S*	*R*
5c	Boc-L-Trp	*R*	*R*
5d	Boc-L-Trp	*S*	*S*
6a	Boc-D-Trp	*R*	*S*
6b	Boc-D-Trp	*S*	*R*
6c	Boc-D-Trp	*R*	*R*
6d	Boc-D-Trp	*S*	*S*

Chemistry

As depicted in Scheme 1, the 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine skeletons of **5a**−**d** and **6a**−**d** were

Scheme 1[a]

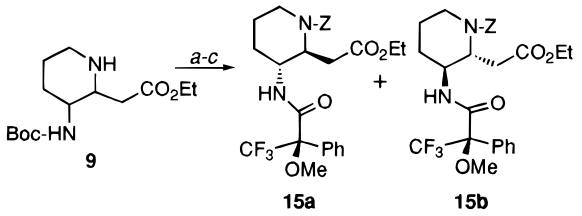


[a] Reagents: (a) 1,1′-carbonyldiimidazole, $LiCH_2CO_2Et$, THF; (b) H_2, Pd(C), EtOH or MeOH; (c) benzyl isocyanate, THF; (d) NaH, THF; (e) TFA, CH_2Cl_2; (f) Boc-L-Trp-OH, BOP, Et_3N; (g) Boc-D-Trp-OH, BOP, Et_3N.

constructed from the corresponding 3-amino-2-piperidineacetic acid derivatives **9** and **10**, which were prepared from Boc-D-Orn(Z)-OH (**7**) as previously described.[28] Reaction of *trans*-piperidine **9** with benzyl isocyanate gave the *N*-benzylcarbamoyl derivative **11**, which after treatment with NaH provided the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine **13** in 65% overall yield. Then, sequential *N*-Boc removal and coupling with Boc-L- or -D-Trp-OH, using BOP as coupling agent, led to the diasteroisomeric mixture **5a**,**b** or **6a**,**b** in a 4:1 ratio, which was chromatographically resolved to give **5a** and **5b**, or **6a** and **6b**, respectively. In a similar way, the *cis*-piperidine **10** led to the 4:1 mixtures **5c,d** and **6c,d**, which were separated by semipreparative RPHPLC. These results indicated that about 20% of racemization had occurred. As in the synthesis of 8-amino-3-oxoindolizidines **3** and **4** from 4-keto esters derived from ornithine,[29] this racemization could take place during the formation of the piperidine ring in **9** and **10**, due the existence of imine−enamine intermediates in equilibrium (structures A−C) in the intramolecular reductive amination of 3-keto esters **8**.

In order to clarify this point and to determine the racemization ratio in **9** and **10**, we prepared the Mosher

Scheme 2[a]



[a] Reagents: (a) benzyl chloroformate, propylene oxide, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) (*R*)-(+)-MTPA-OH, BOP, Et_3N.

Table 1. Racemization Ratio and Yield of Piperidines **9** and **10** from the 3-Keto Ester **8**

reductive amination conditions	overall yield (%)	racemization[a] (%)	**9** (%)	**10** (%)
H_2, Pd(C), EtOD, 20 °C, 4 days	90	20	79	11
H_2, Pd(C), EtOD, AcOD, 20 °C, 4 days	17	45	15	2
H_2, Pd(C), MeOD, 20 °C, 3 days	90	19	72	18
H_2, Pd(C), MeOD, 40 °C, 7 h	90	10	74	10
(1) H_2, Pd(C), EtOD, 20 °C, 1 h (2) $NaBH_3CN/ZnCl_2$, 30 min	80	4	57	23

[a] Racemization ratio determined as 50% of the deuterium incorporation into position 3 (measured by the decrease in the 3-H integral in the ^1H NMR spectra of **9** and **10**) or after the coupling of **13** and **14** with Boc-L-Trp-OH (measured by the RPHPLC analysis of the resulting diastereoisomeric mixtures **5a,b** and **6a,b**).

acid derivatives **15a,b** of the ethyl ester of 3-amino-1-[(benzyloxy)carbonyl]piperidineacetic acid derived from **9**[28] (Scheme 2). However, this diastereoisomeric mixture could not be resolved either by TLC or by RPHPLC, and its ^1H NMR analysis at room temperature in different solvents [$CDCl_3$, $(CD_3)_2CO$, C_6D_6] showed broad signals but no duplicity of any of them. It was necessary to register the spectrum at 80 °C in $(CD_3)_2SO$ to observe the 2-H, 4-H, and OMe signals of **15a** and **15b** separately. Taking into account these difficulties in the determination of the racemization ratio by the Mosher acid derivatives **15a,b**, we studied the intramolecular reductive amination of the 3-keto ester **8** in deuterated solvent (MeOD or EtOD). In this way, as the major products, the *trans*-piperidines **9** should come only from the hydrogenation of the intermediate A or C, and the incorporation of deuterium at position 3 into the enamino intermediate B should be twice the racemization at this position produced in this step. This deuterium incorporation was measured in the ^1H NMR spectrum of the mixture of piperidines **9** and **10**, previously to their separation, by the decrease in the integrals of the signals corresponding to the 3-H in both isomers. Comparison of these results with the ratios of final 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **5a**:**5b** and **5c**:**5d**, respectively, measured by RPHPLC analysis, showed that racemization ratios resulting from both analyses were similar.

Then, in order to minimize this racemization, we studied the influence of the reductive amination conditions (solvent, temperature, reducing agent) on the yield of each stereoisomeric piperidine **9** and **10** and on the racemization ratio. The results of this study are summarized in Table 1. It is interesting to note that when the hydrogenation was carried out in MeOD about 45% of transesterification was observed, but it had no influence on the yield of the final compounds, because both ethyl and methyl esters were processed together for the subsequent construction of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine nucleus in **13** and **14**. The lowest racemization occurred when the intramolecular reductive amination was carried out with $NaBH_3CN/ZnCl_2$, after the Z removal by hydrogenolysis. However, these conditions led to a considerable decrease in the selectivity for the *trans* diastereoisomers **9**. Minor isomers **5b**,**d** and **6b**,**d** were obtained as major compounds starting from Boc-L-Orn(Z)-OH.

Biological Results and Discussion

The affinity of the 1,3-dioxoperhydropyrido[1,2-*c*]-pyrimidine derivatives **5a**−**d** and **6a**−**d** at CCK-A and CCK-B receptors was determined by measuring the displacement of the [^3H]propionyl-CCK-8 binding to rat pancreatic and brain cortex homogenates, respectively, as previously described.[30] These data are depicted in Table 2. For comparative purposes the 3-oxoindolizidine derivatives **3** and **4**, CCK-8, and Devazepide were also included in the assay. In general, these pyrido[1,2-*c*]-pyrimidine derivatives showed preference for the CCK-A versus the CCK-B receptor subtype. The most remarkable result is that obtained with compound **5a**, the best compound of this series, with a CCK-A receptor affinity similar to that of Devazepide but with a much higher selectivity at CCK-A than at CCK-B receptors. Unlike various precedent series of ligands for CCK receptors,[15a,31] stereochemical changes in **5a** did not reverse the sense of selectivity, except for **6d** with a very modest affinity

Table 2. Inhibition of [^3H]pCCK-8 Specific Binding to Rat Pancreas (CCK-A) and Cerebral Cortex Membranes (CCK-B) and Inhibition of Amylase Release from Dispersed Pancreatic Acini

	stereochemistry			K_i (nM)[a]			amylase release[b]
compd	Trp	4a	5	CCK-A	CCK-B	B/A	IC_{50} (nM) (*n*)
CCK-8				0.52 ± 0.04	2.8 ± 0.15	5	
Devazepide (**1**)				0.30 ± 0.03	342 ± 67	1140	25.4 (5)
3	L			1030	>5000	>4	ND[c]
4	D			>5000	1560	<0.3	ND
5a	L	*S*	*R*	0.62 ± 0.05	>5000	>8000	21.3 (9)
5b	L	*R*	*S*	10.6 ± 2	2730 ± 273	257	201 (3)
5c	L	*R*	*R*	572	>5000	>8	ND
5d	L	*S*	*S*	2890	2910	1	ND
6a	D	*S*	*R*	298	>5000	>16	ND
6b	D	*R*	*S*	813	>5000	>6	ND
6c	D	*R*	*R*	>5000	>5000		ND
6d	D	*S*	*S*	>5000	1890	<0.4	ND

[a] Values are the mean or mean ± SEM of at least three experiments, performed with seven concentrations of test compounds in triplicate (SEM within ±15% of the mean). [b] Inhibition of amylase release stimulated by CCK-8 (0.5 nM) in dispersed pancreatic acini. Data represent the mean of *n* independent experiments in duplicate (standard errors within ±15% of the mean). [c] ND = not determined.

for CCK-B receptors. However, a dramatic influence of the stereochemical structure on the binding potency and selectivity is observed. Thus, the replacement of the L-Trp residue of compounds **5a**−**d** by D-Trp in **6a**−**d** led to a 2−500-fold decrease in the CCK-A binding affinity. Concerning the stereochemistry of the bicyclic skeleton, compounds **5a**,**b**, having a 4a,5-*trans* disposition, showed from 2 to 3 orders of magnitude higher CCK-A binding potency than their corresponding 4a,5-*cis* diastereoisomers **5c**,**d**. What is clear is that the 5*R*-configured diastereoisomers **5a**,**c** have higher affinity and selectivity for CCK-A receptors than their 4*S*-configured counterpart **5b**,**d**, respectively. It is also clear that replacement of the 3-oxoindolizidine skeleton in compound **3** with a 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine template, having the same stereochemistry, produced the analogue **5b** and an important increase in CCK-A affinity (≈200-fold). This considerable improvement in affinity could be attributed to a better spatial disposition of the pharmacophoric 2-benzyl and L-Trp groups. However, additional interactions of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine ring with the CK-A binding sites can not be discarded.

The best compounds of this series, **5a**,**b,** were tested for their antagonism of the CCK-8-stimulated amylase release from pancreatic acinar cells.[32] In accordance with the binding results, compound **5a** showed an antagonist potency similar to that of the well-known CCK-A antagonist Devazepide (Table 2), while the diastereoisomer **5b** was approximately 1 order of magnitude less potent. Compounds **5a**,**b** did not show any intrinsic effect on amylase release at a 1 μM concentration and, like all other compounds of the series, were not able to induce at a 10 μM concentration a contractile response in the isolated longitudinal muscle-myenteric plexus preparation from guinea pig ileum, which is known to be sensitive to both CCK-A and CCK-B receptor agonists.[33]

In conclusion, the use of the 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine as template for appending the aromatic side chains of CCK-4 has led to potent and highly selective CCK-A receptor ligands with antagonist activity. Compound **5a** is one of the most selective CCK-A antagonists reported to date and remains the best compound of this series. Besides its selectivity, the most important feature of this potent and novel CCK-A antagonist is its lack of structural resemblance to those previously reported and its independence in genesis. Therefore, this compound may be a useful pharmacological tool to investigate the functional role of CCK-A receptors. Modifications of the two structural domains on the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine core of **5a**, namely, Boc-L-Trp and the 2-benzyl group, which could lead to improve oral bioavailability and duration of action, are in progress.

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. 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. Optical rotations were determined with a Perkin-Elmer-241 MC polarimeter. ^{1}H NMR spectra were recorded with a Varian Gemini-200 or Unity-500 spectrometer, operating at 200 or 500 MHz, using TMS as reference. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical RPHPLC was performed on a Waters Nova-pak C_{18} (3.9 × 150 mm, 4 μm) column, with a flow rate of 1 mL/min, 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 phase. Semipreparative RPHPLC was performed on a Waters Nova-pak C_{18} (25 × 100 mm, 6 μm) cartridge, with a flow rate of 6.5 mL/min of 40:60 CH_3CN and 0.05% TFA in H_2O.

(2*R,3*S**)-1-(*N*-Benzylcarbamoyl)-3-[(*tert*-butoxycarbonyl)amino]-2-[(ethoxycarbonyl)methyl]piperidine (11).** Benzyl isocyanate (0.62 mL, 5 mmol) was added to a solution of (2*R**,3*S**)-3-[(*tert*-butoxycarbonyl)amino]-2-[(ethoxycarbonyl)methyl]piperidine (**9**) [obtained as a 4:1 mixture of 2*S*,3*R*- and 2*R*,3*S*-stereoisomers from Boc-D-Orn(Z)-OH as previously described[28]] (1.145 g, 4 mmol) in dry THF (30 mL). After 1 h of stirring at room temperature, the solvent was evaporated, and the residue was purified by flash chromatography, using a 17−25% gradient of AcOEt in hexane as eluant, to give **11**, with 20% of its enantiomer, as a foam (1.443 g, 86%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12 [t, 3H, CH_3(Et)], 1.25 (m, 1H, 5-H), 1.34 (s, 9H, Boc), 1.56 (m, 3H, 4-H, 5-H), 2.36 (dd, 1H, J = 10.3, 16.5 Hz, 2-CH_2), 2.64 (m, 1H, 6-H), 2.81 (dd, 1H, J = 3.7, 16.5 Hz, 2-CH_2), 3.52 (m, 1H, 3-H), 4.00 [q, 2H, CH_2 (Et)], 4.12 (m, 1H, 6-H), 4.26 [m, 2H, CH_2 (Bzl)], 4.39 (m, 1H, 2-H), 5.06 (d, 1H, J = 7.3 Hz, 3-NH), 5.89 (br s, 1H, 1-CONH), 7.14−7.22 (m, 5H, aromatics). Anal. ($C_{22}H_{33}N_3O_5$) C, H, N.

(2*R,3*R**)-1-(*N*-Benzylcarbamoyl)-3-[(*tert*-butoxycarbonyl)amino]-2-[(ethoxycarbonyl)methyl]piperidine (12).** As above indicated for the synthesis of **11**, this compound was obtained from (2*R**,3*R**)-3-[(*tert*-butoxycarbonyl)amino]-2-[(ethoxycarbonyl)methyl]piperidine (**10**),[28] as a foam (1.175 g, 70%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12 [t, 3H, CH_3 (Et)], 1.24 (m, 1H, 4-H), 1.34 (s, 9H, Boc), 1.59 (m, 3H, 4-H, 5-H), 2.50 (m, 3H, 6-H, 2-CH_2), 3.58 (m, 1H, 3-H), 4.00 [q, 2H, CH_2 (Et)], 4.18 (m, 1H, 6-H), 4.29 [m, 2H, CH_2 (Bzl)], 4.50 (d, 1H, J = 6.5 Hz, 3-NH), 4.61 (m, 1H, 2-H), 5.83 (br s, 1H, 1-CONH), 7.15−7.25 (m, 5H, aromatics). Anal. ($C_{22}H_{33}N_3O_5$) C, H, N.

(4a*R,5*S**)-2-Benzyl-5-[(*tert*-butoxycarbonyl)amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (13).** NaH (108 mg of dispersion in mineral oil, 2.7 mmol) was added to a solution of the piperidine **11** (1.133 g, 2.7 mmol) in dry THF (50 mL). After the mixture stirred for 3 h at room temperature, H_2O (100 mL) was added, and the reaction mixture was extracted with AcOEt (2 × 200 mL). Then, the organic phase was washed with 0.1 N HCl (100 mL) and brine (100 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography, using a 0.1−1% gradient of acetone in CH_2Cl_2 as eluant, to give **13** as a foam (766 mg, 76%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.33 (m, 1H, 6-H), 1.44 (s, 9H, Boc), 1.55 and 1.78 (2m, 2H, 7-H), 2.08 (m, 1H, 6-H), 2.64 (m, 1H, 8-H), 2.76 (dd, 1H, J = 9, 17 Hz, 4-H), 2.96 (dd, 1H, J = 5, 17 Hz, 4-H), 3.07 (m, 1H, 4a-H), 3.41 (m, 1H, 5-H), 4.38 (m, 2H, 8-H, 5-NH), 4.98 (s, 2H, 2-CH_2), 7.14−7.23 (m, 5H, aromatics). Anal. ($C_{20}H_{27}N_3O_4$) C, H, N.

(4a*R,5*R**)-2-Benzyl-5-[(*tert*-butoxycarbonyl)amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (14).** This compound was obtained as a foam in 48% yield, from the piperidine **12** as above indicated for the synthesis of **13**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.44 (s, 9H, Boc), 1.62 (m, 3H, 6-H, 7-H), 1.84 (m, 1H, 6-H), 2.64 (m, 1H, 8-H), 2.73 (m, 2H, 4-H), 3.45 (m, 1H, 4a-H), 3.78 (m, 1H, 5-H), 4.33 (m, 1H, 8-H), 4.68 (d, 1H, J = 10 Hz, 5-NH), 4.91 and 4.82 (2d, 2H, J = 15 Hz, 2-CH_2), 7.17−7.33 (m, 5H, aromatics). Anal. ($C_{20}H_{27}N_3O_4$) C, H, N.

General Procedure for the Synthesis of the 5-[*N*-[(*tert*-Butoxycarbonyl)tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 5a−d and 6a−d. TFA (3 mL) was added to a solution of the corresponding *N*-Boc-protected 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivative **13** or **14** (373 mg, 1 mmol) in CH_2Cl_2 (6 mL); after 4 h at room temperature, the solvents were evaporated to dryness. The residue was dissolved in dry CH_2Cl_2 (25 mL), and Boc-L- or -D-Trp-OH (365 mg, 1.2 mmol), (benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP; 549 mg, 1.2 mmol), and triethylamine (32 mL, 2.2 mmol)

Table 3. Significant ^{1}H NMR Spectroscopic Data[a] of the 1,3-Dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives **5a**−**d** and **6a**−**d**

proton	**5a** or **6b**	**5b** or **6a**	**5c** or **6d**	**5d** or **6c**
α-Trp	4.43	4.81	4.39	4.59
2-CH_2	4.91 4.97	4.91 4.96	4.82 4.90	4.79 5.01
4-H	2.53	2.18	2.39 2.53	1.92 2.35
4a-H	2.83	2.48	3.67	3.20
5-NH	5.67	5.45	5.98	5.62
5-H	3.62	3.58	4.03	3.94
6-H	1.01 1.66	1.06 1.82	1.39	1.40 1.64
7-H	1.55	1.50 1.67	0.93 1.39	0.45 1.40
8-H	2.53 4.27	2.48 4.24	2.53 4.18	2.44 3.94

[a] Registered in $CDCl_3$ at 500 MHz.

were added successively to that solution; stirring was continued at room temperature for 12 h. Afterwards, the solvent was evaporated, the residue was dissolved in AcOEt (25 mL), and the resulting solution was washed successively with 10% citric acid (10 mL), 10% $NaHCO_3$ (10 mL), H_2O (10 mL), and brine (10 mL), dried over Na_2SO_4, and evaporated. Flash chromatography of the residue with a 20−50% gradient of AcOEt in hexane yielded, in each case, the isolated *trans*-(4a*S*,5*R*)- and (4a*R*,5*S*)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **5a**, **5b** and **6a**, **6b**, respectively, and the diastereoisomeric *cis* mixtures **5c**,**d** and **6c**,**d**. These mixtures were resolved by semipreparative HPLC as above mentioned. The ^{1}H NMR spectroscopic data of all these compounds are summarized in Table 3.

(4a*S*,5*R*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-L-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (5a): white solid (70% from **13**); mp 119−121 °C; $[\alpha]^{26}_D = -31.8°$ (*c* 1.00, $CHCl_3$); RPHPLC t_R = 50.87 min (33:67). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*R*,5*S*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-L-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (5b): white solid (17% from **13**); mp 174−176 °C; $[\alpha]^{26}_D = +52.6°$ (*c* 1.00, $CHCl_3$); RPHPLC t_R = 46.87 min (33:67). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*R*,5*R*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-L-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (5c): white solid (60% from **14**); mp 116−118 °C; HPLC t_R = 41.93 min (35:65). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*S*,5*S*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-L-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (5d): white solid (14% from **14**); mp 112−114 °C; HPLC t_R = 37.07 min (35:65). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*S*,5*R*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-D-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (6a): enantiomer of **5b** (74% from **13**); $[\alpha]^{26}_D = -51.8°$ (*c* 1.00, $CHCl_3$). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*R*,5*S*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-D-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (6b): enantiomer of **5a** (18% from **13**); $[\alpha]^{26}_D = +31.4°$ (*c* 1.00, $CHCl_3$). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*R*,5*R*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-D-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (6c): enantiomer of **5d** (54% from **14**). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

(4a*S*,5*S*)-2-Benzyl-5-[*N*-[(*tert*-butoxycarbonyl)-D-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (6d): enantiomer of **5c** (14% from **14**). Anal. ($C_{31}H_{37}N_5O_5$) C, H, N.

Binding Assays. CCK-A and CCK-B receptor binding assays were performed using rat pancreas and cerebral cortex homogenates, respectively, according to the method described by Daugé *et al.*,[30] 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 of 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 of 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 1 μM CCK-8 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.*[32] 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 in two 6 min incubations at 37 °C and washing three times the tissue 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.*[34] Samples (2 mL) of acini suspension were placed in plastic tubes and incubated for 30 min at 37 °C in an atmosphere of pure O_2 with agitation (70 cycles/min). Amylase activity was determined using the Amyl Kit Reagent (Boeringher 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 (0.5 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 control release (vehicle), S_{CCK} is release elicited by CCK-8, and S_T is 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.

Acknowledgment. This work has been supported by the CICYT (SAF 94-0705) and Comunidad de Madrid (AE 0004/95).

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JM9703247