5-(Tryptophylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-Based Cholecystokinin Receptor Antagonists: Reversal of CCK_1 Receptor Subtype Selectivity toward CCK_2 Receptors

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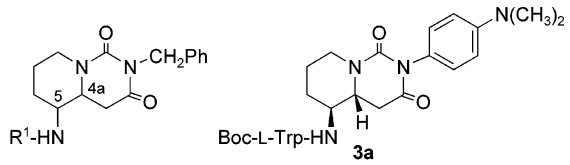
With the aim of reversing selectivity or antagonist/agonist functionality in the 5-(tryptophylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-derived potent and highly selective CCK_1 antagonists, a series of 4-benzyl and 4-methyl derivatives have been synthesized. Whereas the introduction of the benzyl group led, in all cases, to complete loss of the binding affinity, the incorporation of the methyl group gave a different result depending on the stereochemistry of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine scaffold. Thus, the introduction of the methyl group into the (4a*S*,5*R*)-diastereoisomers, giving a (4*S*)-configuration, produced a 3-fold increase in the CCK_1 binding potency and selectivity. However, the same structural manipulation in the opposite (4a*R*,5*S*)-stereochemistry, leading to a (4*R*,4a*R*,5*S*)-configuration, produced reversal of the selectivity for CCK_1 to the CCK_2 receptors. The replacement of the Boc group at the tryptophan moiety by a 2-adamantyloxycarbonyl group also contributed to that reversal. The resulting compounds displayed moderate CCK_2 antagonist activity in rat and human receptors, and a very small partial agonist effect on the production of inositol phosphate in COS-7 cells transfected with the wild-type human CCK_2 receptor.

Introduction

The cholecystokinin (CCK) family of peptides was formerly isolated and identified in the gastrointestinal tract, and later as a neurotransmitter present throughout the nervous system.[1] This family of neuropeptides include different molecular forms (e.g., CCK-58, CCK-33, CCK-8) derived from the processing of a 115-amino acid precursor protein (prepro-CCK), which have the C-terminal sequence in common,[1,2] with CCK-8 being the minimum sequence for full biological activity.[3] In the gastrointestinal tract CCK is released from endocrine cells, in response to food intake, and regulates motility, contraction of gallbladder, pancreatic enzyme secretion, gastric emptying, and gastric acid secretion.[1] In the nervous system CCK is involved in anxiogenesis,[1,4−6] satiety,[1,7−9] nociception,[1,10] thermoregulation,[1,11] and memory and learning processes.[4,12,13] Furthermore, the colocalization and interaction of CCK with other neurotransmitters in some areas of the central nervous system (CNS),[1,14] mainly with dopamine (DA),[15,16] suggests its implication in several neuropsychiatric disorders, such as schizophrenia, depression, and drug addiction.[10,15−18] These biological effects are mediated by two specific G-protein-coupled receptor subtypes, termed CCK_1 and CCK_2.[1,10]

The variety of physiological effects of CCK and its possible role in certain pathological disorders have stimulated research in this area and, over the past 15 years, a broad assortment of potent and selective nonpeptide CCK_1 and CCK_2 receptor agonists and antagonists have been reported.[10,19−25] Some of these ligands have contributed highly to the characterization and localization of CCK receptor subtypes, as well as to the study of physiological and pathological actions of CCK. However, despite the progress in this field, the complex biological effects of CCK mediated by CCK_1 and CCK_2 receptors are not yet fully established.[1,10] In this regard, we have reported the design, synthesis,[26] and pharmacological properties[27] of the 5-(tryptophylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivative **1b** (IQM-95,333, Figure 1), prototype of a family of potent and highly selective CCK_1 receptor antagonists, which include some of the most selective antagonists described to date.[25] This compound showed a CCK_1 receptor affinity in the nanomolar range, but was virtually devoid of affinity at brain CCK_2 receptors.[27] In agreement with this CCK_1 receptor affinity, compound **1b** was a potent inhibitor of the CCK-8-stimulated amylase release from isolated pancreatic acini and blocked the



1a: (4a*R*,5*S*); R^1 = Boc-L-Trp

1b: (4a*S*,5*R*); R^1 = Boc-L-Trp

2a: (4a*R*,5*S*); R^1 = 2-Adoc-L-Trp

Figure 1.

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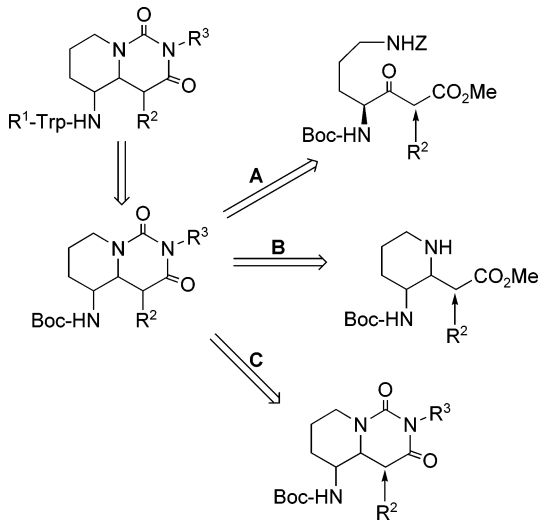
CCK-8-induced hypophagia and hypolocomotion in rats.[27] Furthermore, despite the predominant role attributed to CCK_2 receptors in the anxiogenic effects of CCK,[4,10] this CCK_1 antagonist also showed a marked anxiolytic-like activity in animal models.[27] This result supports the suggestion of some authors that CCK_1 receptors may be also involved in anxiogenesis.[28−30] Structure−activity relationship studies on these 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine-based CCK_1 receptor antagonists have shown that the Boc-L-Trp residue, the topography defined by the (4a*S*,5*R*)-1,3-dioxoperhydropyrido[1,2-*c*]-pyrimidine scaffold, and the lipophilicity and spatial orientation of the group attached to the N2 position of that skeleton are essential structural requirements for potent and selective binding to CCK_1 receptors.[26,31−33]

We were interested in expanding our assortment of CCK receptor ligands, reversing the selectivity or the functionality of our CCK_1 highly selective antagonists. Minor changes in certain groups attached to the core scaffold or in its stereochemistry have led to interconversion of the CCK_1/CCK_2 receptor subtype selectivity in most of the known families of CCK receptor ligands.[25] There are also several reports which demonstrate the feasibility of interconverting agonist/antagonist functionality of nonpeptide ligands by minor structural changes,[34] such as, for example, introducing additional alkyl groups into the structure of an antagonist,[35−37] or changes in the stereochemistry.[38] We have approached our goal of reversing the selectivity or the functionality of 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-based CCK_1 receptor antagonists by introducing additional groups (Me and CH_2Ph) into position 4 of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine skeleton and by bearing in mind those previous SAR results that pointed out a decrease in CCK_1 receptor affinity and an increase in that for the CCK_2. Taking into account the important influence of stereochemistry at the tryptophan and 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine domains upon affinity and selectivity,[26] we have attempted to search their configurational space as much as possible. Additionally, the replacements of the *N*-Boc group at the Trp moiety by the 2-adamantyloxycarbonyl group (2-Adoc) and the benzyl group at the N2 position of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine scaffold by a 4-dimethylaminophenyl group have been considered, as both modifications introduced into the diastereoisomer **1a** (Figure 1) led independently to a 1 order of magnitude increase in the CCK_2 receptor affinity of **2a** and **3a** and to a decrease of more than 1 order of magnitude[31] or the complete loss of affinity at CCK_1 receptors,[32] respectively.

Chemistry

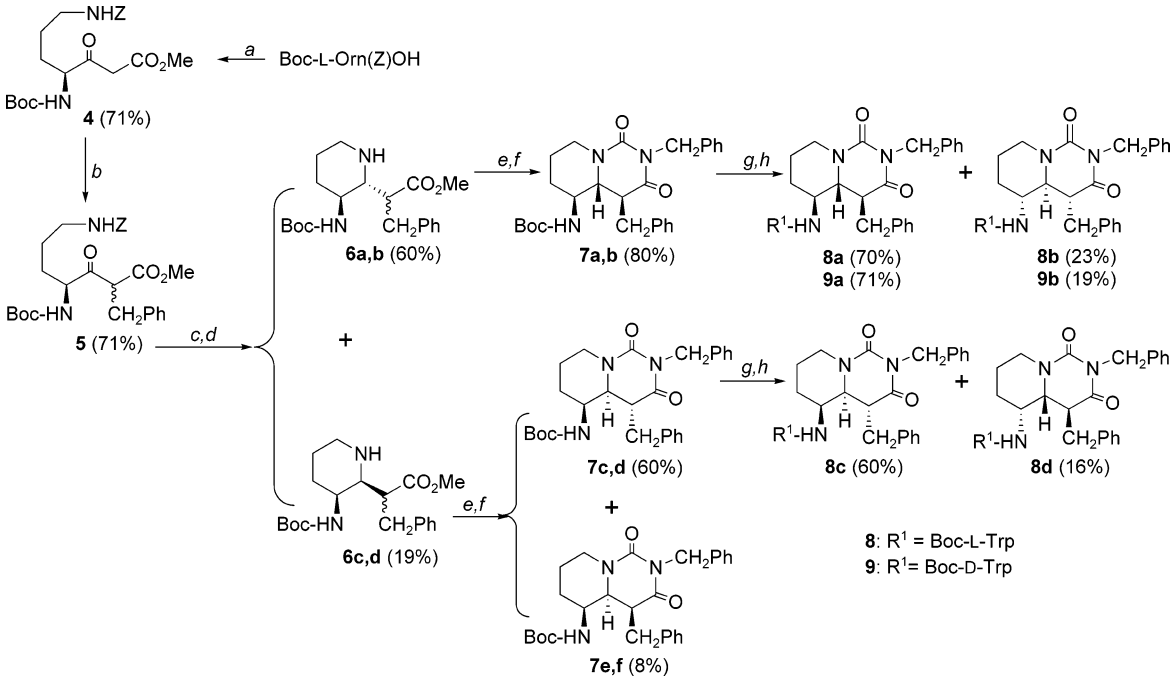
The synthesis of the target 4-substituted-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives was designed following a similar synthetic scheme to that previously used for the preparation of 4-unsubstituted analogues. This methodology involved essentially the construction of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine scaffold and subsequent coupling of the appropriate N-protected tryptophan residue. As indicated in the retrosynthetic Scheme 1, the key C-alkylation step for introducing the additional substituent at position 4 of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine core can be performed at three different stages of its elaboration. These three alternative routes were applied depending on the substitution and on the required stereochemistry. Thus, with the aim of facilitating the obtention of the highest number of stereoisomers, route A was first attempted. As shown in Scheme 2, for the synthesis of the 4-benzyl derivatives **8** and **9** from the β-keto ester **4**, this route involved alkylation with benzyl bromide, using NaH as base at 0 °C. This alkylation led to the unresolved epimeric mixture of the 2-benzyl derivatives **5** in a (≈1:1) ^1H NMR estimated ratio. β-Keto ester **4** was obtained from Boc-L-Orn(Z)-OH, applying a modified method[39] of one previously described.[40] Removal of the benzyloxycarbonyl protecting group from the 2-benzyl derivatives **5**, by catalytic hydrogenolysis, followed by intramolecular reductive amination using $NaBH_3CN$ in the presence of $ZnCl_2$, gave a (3:1) mixture of 2,3-trans- and 2,3-cis-disubstituted piperidine derivatives **6a**,**b** and **6c**,**d**, which were chromatographically separated as epimeric mixtures at the exocyclic stereogenic center in (1.2:1) and (1.4:1) ratios, respectively. Furthermore, as these reductive aminations produce racemization at the C_2 and C_3 of the piperidine ring in different extent depending on the substituents and on the reduction conditions,[26] both **6a**,**b** and **6c**,**d** included ≈22% of racemization. Treatment of each one of these mixtures with benzyl isocyanate, followed by in situ cyclization of the respective urea derivatives, provided the corresponding 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **7**. Interestingly, this cyclization took place with total or partial stereomutation at the exocyclic stereogenic center, as the (1.2:1) diastereoisomeric mixture **6a**,**b** gave exclusively the racemic mixture **7a**,**b** (80%), with a (4*R**,4aS*,5*R**)-relative configuration, while the (1.4:1) diastereoisomeric mixture **6c**,**d** led to **7c**,**d** and **7e**,**f**, which were separated in a (7.5:1) ratio. Finally, the *N*-Boc removal from the racemic mixtures **7a**,**b** and **7c**,**d**, followed by coupling with Boc-L- or D-Trp-OH, using BOP as coupling agent, provided the corresponding (≈3:1) diastereoisomeric mixtures **8a**,**b**; **8c**,**d**; and **9a**,**b**, which were chromatographically resolved.

Scheme 1. Retrosynthesis of 4-Substituted-1,3-dioxo-perhydropyrido[1,2-*c*]pyrimidine Derivatives



A similar synthetic scheme for the preparation of 4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine de-

Scheme 2[a,b]



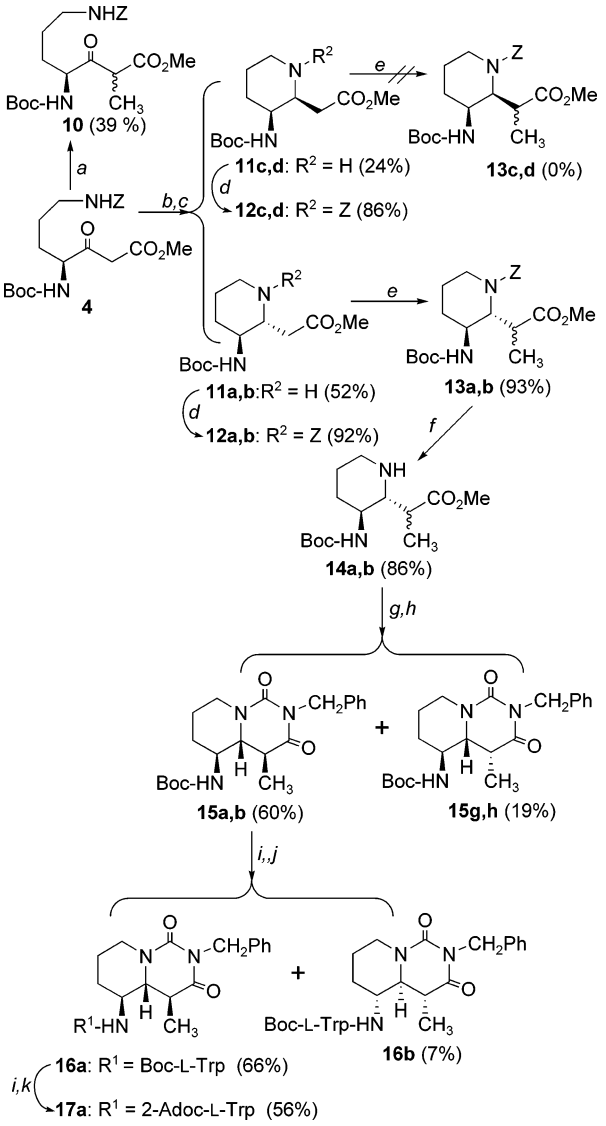
[a] Letters are used in compound numeration to indicate different stereoisomers. Thus, **a** denotes a (4*S*,4a*R*,5*S*)-configuration; **b** denotes (4*R*,4a*S*,5*R*)-configuration; **c** denotes (4*R*,4a*S*,5*S*)-configuration; **d** denotes (4*S*,4a*R*,5*R*)-configuration; **e** denotes (4*S*,4a*S*,5*S*)-configuration; **f** denotes (4*R*,4a*R*,5*R*)-configuration; **g** denotes (4*R*,4a*R*,5*S*)-configuration; **h** denotes (4*S*,4a*S*,5*R*)-configuration. [b]Reagents: (a) CDI, $MgCl_2$, $MeO_2CCH_2CO_2K$, THF; (b) NaH, $PhCH_2Br$, THF; (c) H_2, 10% Pd(C), MeOH; (d) $NaBH_3CN$, $ZnCl_2$; (e) $PhCH_2NCO$, THF; (f) NaH, THF; (g) TFA, CH_2Cl_2; (h) Boc-L- or Boc-D-Trp-OH, BOP, TEA, CH_2Cl_2.

rivatives was discarded as the starting 2-methyl-β-keto ester **10** (Scheme 3) was obtained with low yield (39%), which hampered obtaining the desired 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives in acceptable yields. Therefore, route B was applied, as shown in Scheme 3, involving akylation of the appropriate *N*-Z protected 2-piperidyl acetic acid derivatives **12** with MeI in THF at −78 °C, using lithium bis(trimethylsilyl)-amide as base, and in the presence of hexamethylphosphoric acid triamide. Under these conditions 2,3-cis-disubstituted piperidine derivatives **12c**,**d** did not react, and were recovered unchanged, while raising the reaction temperature from −78 °C to room temperature led to a complex reaction mixture. However, the (9:1) racemic mixture of 2,3-trans-disubstituted piperidines **12a**,**b** led to the methyl derivatives **13a**,**b** as a single racemic mixture, whose relative configuration at the exocyclic center could not be assigned. Removal of the *N*-Z protecting group from these 2,3-trans-disubstituted piperidine derivatives, by catalytic hydrogenolysis, followed by treatment with benzyl isocyanate, and in situ base-promoted intramolecular cyclization of the corresponding urea intermediate, yielded the mixture of 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **15a**,**b** and **15g**,**h**, which was chromatographically resolved in a (3:1) ratio. This result showed that partial stereomutation at the exocyclic stereogenic center had also occurred during the urea cyclization. Removal of the *N*-Boc protection in the major diastereoisomers **15a**,**b**, followed by coupling with Boc-L-Trp-OH and chromatographic resolution, provided the desired compounds **16a** and **16b** in a (≈9:1) ratio. *N*-Boc/*N*-(2-Adoc) exchange in the major (4*S*,4a*R*,5*S*)-diastereoisomer **16a**, by *N*-Boc removal, followed by reaction with 2-adamantyl chloroformate, gave the 2-Adoc derivative **17a**.

Finally, route C was applied for the preparation of the 4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives with a 4a,5-cis-relative configuration **16c**,**d** and **22c**,**d** (Scheme 4), that could not be obtained by the previous A or B routes, and also for the synthesis of the 2-(dimethylamino)phenyl substituted compounds **23** and **24**. This last route involved methylation of the corresponding 4-unsubstituted-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **18c**,**d**[26] and **19a**,**b**,[32] respectively, as in route B, by reaction with MeI in THF at −78 °C, using lithium bis(trimethylsilyl)amide as base, and in the presence of hexamethylphosphoric acid triamide, followed by the corresponding *N*-Boc removal and coupling with Boc-L- or Boc-D-Trp-OH. Interestingly, the methylation of the 4a,5-cis-compounds **18c**,**d** was completely stereoselective, giving rise exclusively to the 4-methyl derivatives with a (4*R**,4a*S**,5*S**) relative configuration **15c**,**d**, while in the case of the 4a,5-trans-compounds **19a**,**b** the two possible diastereoisomers **21a**,**b** and **21g**,**h** were obtained in a (≈4:1) ratio. As mentioned above, and shown in Scheme 4, the *N*-Boc/*N*-(2-Adoc) exchange in the *N*-Boc derivative **23a** provided the corresponding 2-Adoc analogue **24a**. For biological comparative purposes, the 2-Adoc derivative **20a** was similarly prepared from **3a**.

The assignment of absolute configuration to the new 4-substituted-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives was done by assuming that, despite the racemization in the intramolecular reductive amination steps, the major diastereoisomers maintain the (*S*)-configuration of the starting Boc-L-Orn(Z)-OH. Therefore, the configuration at C_5 of the major isomers is (5*S*). As shown in Figure 2, the $J_{4a,5}$ coupling constant value was used to assign the relative 4a,5-trans (10−12 Hz) or 4a,5-cis (0−2 Hz) configuration. With respect to the

Scheme 3[a]



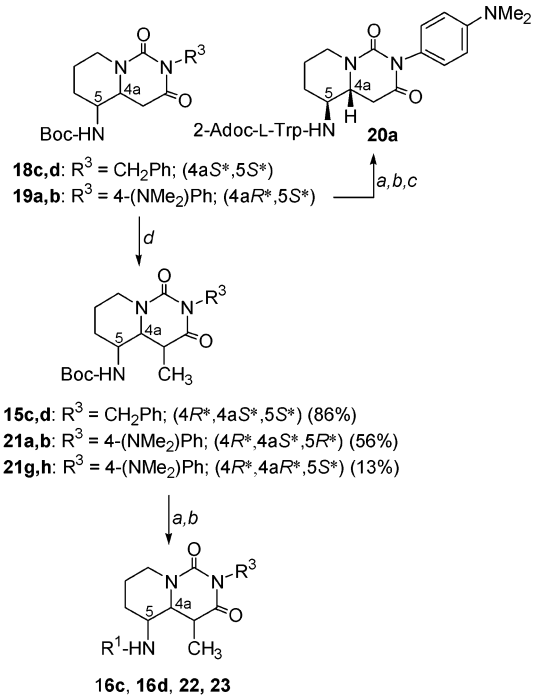
[a] Reagents: (a) NaH, MeI, THF; (b) H_2, 10% Pd(C), MeOH; (c) $NaBH_3CN$, $ZnCl_2$; (d) $PhCH_2OCOCl$, propylene oxide, CH_2Cl_2; (e) $[(CH_3)_3Si]_2NLi$, MeI, HMPA, THF; (f) H_2, 10% Pd(C), MeOH; (g) $PhCH_2NCO$, THF; (h) NaH, THF; (i) TFA, CH_2Cl_2; (j) Boc-L-Trp-OH, BOP, TEA, CH_2Cl_2; (k) 2-adamantyl chloroformate, TEA, CH_2Cl_2.

configuration at C_4, its assignment was based on the $J_{4,4a}$ values and NOE relationships observed in the DPFGSE-NOE spectra of the new 4-substituted derivatives. On the other hand, the NOE effects observed between 4a-H, 6-H_{ax}, and 8-H_{ax} protons (not shown in Figure 1) indicated that in all these derivatives the fused piperidine ring adopts a preferred chair conformation with the 4a-H in an axial disposition.

Biological Results and Discussion

The affinities of the new 5-(tryptophylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives herein described at rat CCK_1 and CCK_2 receptors were determined by measuring the displacement of [^{3}H]propionyl-CCK-8 binding to rat pancreatic and cerebral cortex homogenates, respectively, as previously described.[41] For comparative purposes, CCK-8, the CCK_2 antagonist PD-135,158,[42] and the model compounds **1a** and **1b** were also included in the assay. The results showed that,

Scheme 4[a]



compd	R^1	R^3	(4, 4a, 5) configuration	yield (%)
16c	Boc-L-Trp	CH_2Ph	(4*R*,4a*S*,5*S*)	80
16d	Boc-L-Trp	CH_2Ph	(4*S*,4a*R*,5*R*)	3
22c	Boc-D-Trp	CH_2Ph	(4*R*,4a*S*,5*S*)	81
22d	Boc-D-Trp	CH_2Ph	(4*S*,4a*R*,5*R*)	3
23a	Boc-L-Trp	4-(NMe_2)Ph	(4*S*,4a*R*,5*S*)	82
23b	Boc-L-Trp	4-(NMe_2)Ph	(4*R*,4a*S*,5*R*)	9
23g,h	Boc-L-Trp	4-(NMe_2)Ph	(4*R**,4a*R**,5*S**)	62[b]
24a	2-Adoc-L-Trp	4-(NMe_2)Ph	(4*S*,4a*R*,5*S*)	40[c]

[a] Reagents: (a) TFA, CH_2Cl_2; (b) Boc-L- or Boc-D-Trp-OH, BOP, TEA, CH_2Cl_2; (c) i. TFA, CH_2Cl_2; ii. 2-adamantyl chloroformate, TEA, CH_2Cl_2; (d) $[(CH_3)_3Si]_2NLi$, MeI, HMPA, THF. [b] Unresolved (9:1) mixture of (4*R*,4a*R*,5*S*) and (4*S*,4a*S*,5*R*) diastereoisomers. [c] Yield from **23a** after treatment with CH_2Cl_2 solution of TFA, followed by reaction with 2-adamantyl chloroformate in the presence of TEA.

independently of the stereochemistry, none of the 4-benzyl derivatives **8a**−**d** and **9a**−**b** bound at CCK_1 or CCK_2 receptors at concentrations below 10^{-5} M. The results of the 4-methyl derivatives **16**, **17**, **22**−**24** and the [4-(dimethylamino)phenyl]-4-unsubstituted analogue **20a** are shown in Table 1, along with the described affinities of the 4-unsubtituted compounds **2a**,[31] **3a**, and **3b**.[32] It is interesting to note that the introduction of a methyl group into position 4 of the 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine skeleton of the prototype **1b** led to a 3-fold improvement in the binding potency at CCK_1 receptors, providing compound **16b** with subnanomolar affinity and excellent selectivity. This modification in the model compound having (4a*R*,5*S*)-stereochemistry (**1a**) produced a significant increase in the binding affinity at CCK_2 receptors and a higher than 2 orders of magnitude decrease at CCK_1 receptors for the 4-methyl derivative **16a**. Therefore, this modification has reversed the CCK_1 selectivity of compound **1a** to the CCK_2 selectivity of compound **16a**. A moderate increase

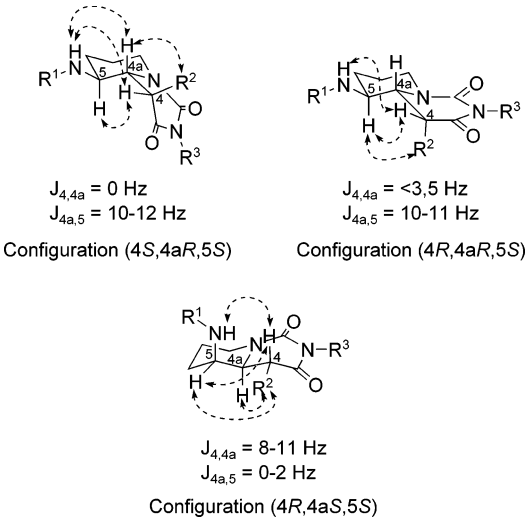
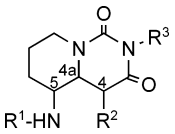


Figure 2. NOE relationships and coupling constant values used for the configuration assignment in 4-substituted-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives.

in the affinity for CCK_2 was also observed by the introduction of the 4-methyl group into the (dimethylamino)phenyl derivative **23a**. As the comparison of the NMR data of the 4-benzyl derivatives **8a**,**b** with those of their respective 4-methyl analogues **16a**,**b**, as well as with the 4-unsubstituted compounds **1a**,**b**, did not show significant conformational differences in the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine skeleton, the drastic influence of the introduction of a benzyl or a methyl group into position 4 upon the binding affinity at both CCK_1 and CCK_2 receptors seems to indicate the existence of an additional point of interaction with the receptor at that position. The complete loss of affinity resulting from the introduction of the benzyl group could be due to bad steric contacts with the receptors. Concerning the 4a,5-cis diastereoisomers **16c**, **22c**, and **22d**, the incorporation of the 4-methyl group caused the loss of the micromolar affinity shown by the 4-unsubstituted analogues at CCK_1 or CCK_2 receptors.[26] On the other hand, as in the model compound **1a**,[31] the replacement of the Boc group of **3a**, **16a**, and **23a** by the 2-adamantyloxycarbonyl group (2-Adoc) produced a significant increase in the CCK_2 binding potency of **20a**, **17a**, and **24a**, without affecting the binding at CCK_1 receptors. A CCK_2 receptor heterogeneity in the rat cerebral cortex has been previously suggested by means of the analysis of an exceptionally large number of competition curves obtained with the CCK_2 receptor antagonist, L-365,260.[43] Hill slopes were not however significantly different from unity in subsequent studies with other CCK_2 receptor ligands when using a much more reduced data set.[44] In the present study, the mean Hill slope parameter estimates, obtained from the competition curves for the more potent new CCK_2 receptor ligands **17a**, **20a**, and **24a**, were 0.91 ± 0.05, 0.82 ± 0.11 and 0.83 ± 0.10, respectively. These Hill slopes were not significantly different from unity, suggesting in principle a single binding site.

Consistent with its subnanomolar affinity at CCK_1 receptors, compound **16b** antagonized the CCK-8-stimulated amylase release from rat pancreatic acinar cells,[45] with an IC_{50} value of 0.62 ± 0.33 nM. Compounds that bound to CCK_2 receptors at concentrations below 10^{-6} M were tested for their antagonism of the CCK-4-induced contractions in isolated longitudinal muscle myenteric plexus preparations from guinea pig ileum. In this assay CCK-4 produces a contractile effect by stimulation of CCK_2 receptors.[46] As shown in Table 1, compounds **17a**, **20a**, and **24a**, with submicromolar affinities at CCK_2 receptors, inhibited the CCK-4-induced contractions with calculated pA_2 values of 5.75−

Table 1. Inhibition of the [^{3}H]pCCK-8 Specific Binding to Rat Pancreas (CCK_1) and Cerebral Cortex Homogenates (CCK_2), and Inhibition of the CCK-4-Induced Contraction of Isolated Longitudinal Muscle Myenteric Plexus from Guinea-Pig Ileum



					IC_{50} (nM)[a]		selectivity:	inhibition of CCK-4 effect[b]	
compd	R^1	R^2	R^3	stereochem.	CCK_1	CCK_2	CCK_1/CCK_2	%[c]	pA_2 (CL)[d]
CCK8					1.04 ± 0.08	5.60 ± 0.03	0.19		
PD-135,158					1123 ± 23	9.80 ± 0.40	115	83.5 ± 6.6	8.10 (7.90−8.30)
1a	Boc-L-Trp	H	CH_2Ph	(4a*R*,5*S*)	22.7 ± 4.0	6153	0.004		
1b	Boc-L-Trp	H	CH_2Ph	(4a*S*,5*R*)	1.59 ± 0.10	>10000	$<10^{-4}$		
2a[e]	2-Adoc-L-Trp	H	CH_2Ph	(4a*R*,5*S*)	340	3430	0.1		
3a[f]	Boc-L-Trp	H	4-(NMe_2)Ph	(4a*R*,5*S*)	>10000	2320			
3b[f]	Boc-L-Trp	H	4-(NMe_2)Ph	(4a*S*,5*R*)	4.30 ± 1.05	>10000	$<4 \times 10^{-4}$		
16a	Boc-L-Trp	Me	CH_2Ph	(4*S*,4a*R*,5*S*)	1350 ± 250	700 ± 220	2		
16b	Boc-L-Trp	Me	CH_2Ph	(4*R*,4a*S*,5*R*)	0.47 ± 0.25	>10000	$<5 \times 10^{-5}$		
16c	Boc-L-Trp	Me	CH_2Ph	(4*R*,4a*S*,5*S*)	>10000	>10000			
22c	Boc-D-Trp	Me	CH_2Ph	(4*R*,4a*S*,5*S*)	>10000	>10000			
22d	Boc-D-Trp	Me	CH_2Ph	(4*S*,4a*R*,5*R*)	>10000	>10000			
17a	2-Adoc-L-Trp	Me	CH_2Ph	(4*S*,4a*R*,5*S*)	>10000	181 ± 35	>55	83.0 ± 6.0	6.62 (6.39−6.81)
20a	2-Adoc-L-Trp	H	4-(NMe_2)Ph	(4a*R*,5*S*)	>10000	276 ± 36	>36	61.0 ± 2.0	5.87 (5.52−6.11)
23a	Boc-L-Trp	Me	4-(NMe_2)Ph	(4*S*,4a*R*,5*S*)	>10000	1265 ± 106	>8	15.3 ± 5.5	
23g,h	Boc-L-Trp	Me	4-(NMe_2)Ph	(4*R**,4a*R**,5*S**)	>10000	>10000			
24a	2-Adoc-L-Trp	Me	4-(NMe_2)Ph	(4*S*,4a*R*,5*S*)	>10000	121 ± 17	>83	75.0 ± 3.8	5.75 (4.97−6.22)

[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 CCK-4-induced contraction of isolated longitudinal muscle myenteric plexus preparations from guinea-pig ileum. [c] Compounds tested at a fixed 10^{-5} M concentration. Values are the mean of at least three experiments performed in triplicate. [d] Confidence limits (95%) for pA_2 values of four to six experiments. [e] Reference 31. [f] Reference 32.

Table 2. Binding Affinities and Effects on Inositol Phosphate Production of 1,3-Dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives **17a**, **20a**, and **24a** on Wild-Type Human CCK_2 Receptors Transiently Expressed in COS-7 Cells

	binding[a]	inositol phosphate production	
compd	IC_{50} (nM)	IC_{50} (nM)[b]	EC_{50} (nM)[c]
(Thr,Nle)-CCK-9	0.96 ± 0.08		1.5 ± 0.7
17a	723 ± 73	1763 ± 1027	98 ± 11
20a	1610 ± 1135	4467 ± 2288	517 ± 88
24a	3688 ± 888	2110 ± 1050	2371 ± 1123

[a] Inhibition of specific binding of ^{125}I-BH-(Thr,Nle)-CCK-9 to COS-7 cells transfected with wild-type human CCK_2 receptors. [b] Inhibition of (Thr,Nle)-CCK-9-induced IP production. Estimated values, as stimulation could not be totally inhibited at the highest concentration used ($10^{-4.5}$ M). [c] Values were calculated from dose−response curves of total IP production stimulated by the compounds. Results are expressed as mean ± SEM of three to five separate experiments.

6.62. None of these compounds showed any intrinsic contractile effect in the ileum preparations.

The binding affinities and effects of compounds **17a**, **20a**, and **24a** were also studied in COS-7 cells transfected with wild-type human CCK_2 receptors. As shown in Table 2, the binding affinities were 1 order of magnitude lower than the respective affinities observed in rat cerebral cortex homogenates. The Hill coefficients were also close to unity. The species-specific differences in receptor structure and the use of a distinct radioligand ([^{3}H]propionyl-CCK-8 and ^{125}I-BH-(Thr,Nle)-CCK-9) may account for the discrepancies in the affinity values from the two CCK_2 receptor binding assays. The compounds inhibited the (Thr,Nle)-CCK-9-induced production of inositol phosphate with potencies in close agreement with their affinity values. However, they are not pure antagonists, as they also showed a small partial agonist activity with EC_{50} values also in the same micromolar range as the binding affinities, and efficacies in the stimulation of inositol phosphate production lower than 15% of the maximum stimulation produced by a 10^{-7} M concentration of (Thr,Nle)-CCK-9.

In conclusion, the introduction of a methyl group into position C_4 of the 5-(Boc-tryptophylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-based CCK_1 antagonists has increased the binding potency and selectivity for CCK_1 receptors in the (4a*S*,5*R*)-diastereoisomers, while in the (4a*R*,5*S*)-isomers the same structural modification, along with the replacement of the Boc group by the 2-adamantyloxycarbonyl group, has led to the reversal of the CCK_1 receptor subtype selectivity toward the CCK_2. Despite their low potency, these are the first CCK_2 selective antagonists in this series of 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives and may be good starting structures for obtaining potent and selective CCK_2 antagonists by further structure manipulation.

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). Preparative radial chromatography was performed on 20 cm diameter glass plates coated with a 1-mm layer of silica gel 60 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. NMR spectra were recorded with Varian Gemini 200, Varian INOVA-300, Varian INOVA-400, and Varian Unity-500 spectrometers, operating at 200, 300, 400, or 500 MHz for ^{1}H NMR, and at 50, 75, 100, or 125 MHz for ^{13}C NMR, and using TMS as reference. 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. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 141 polarimeter.

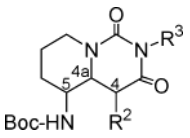
General Procedure for the Synthesis of Methyl (2*RS*, 4*S*)-2-Substituted-7-benzyloxycarbonylamino-4-(*tert*-butoxycarbonylamino)-3-oxoheptanoates 5 and 10. NaH (60% dispersion in mineral oil, 120 mg, 3 mmol) was added to a solution of methyl (4*S*)-7-benzyloxycarbonylamino-4-(*tert*-butoxycarbonylamino)-3-oxoheptanoate[40] (**4**) (1.150 g, 2.7 mmol) in dry THF (40 mL) cooled at 0 °C, and the suspension was stirred for 20 min at this temperature. Then, the corresponding alkylating agent, benzyl bromide (0.4 mL, 3.4 mmol) or methyl iodide (0.22 mL, 3.6 mmol), was added dropwise at 0 °C, and the stirring was continued at room temperature for 16 h. Afterward, water (50 mL) was added, and the resulting reaction mixture was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. Purification of the crude residue by flash chromatography, employing a (17−50%) gradient of EtOAc in hexane as eluant, yielded, in each case, the (1:1) unresolved diastereomeric mixtures **5** or **10**, which could not be resolved.

Methyl (2*RS*,4*S*)-7-Benzyloxycarbonylamino-4-(*tert*-butoxycarbonylamino)-2-phenylmethyl-3-oxoheptanoate (5). Syrup (900 mg, 71%). RP HPLC t_R = 19.24 (A:B = 45:55); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12−1.29 (m, 1 H, 6-H), 1.40 (s, 4.5 H, Boc), 1.41 (s, 4.5 H, Boc), 1.39−1.46 (m, 1 H, 5-H), 1.50−1.63 (m, 1 H, 6-H), 1.78 (m, 0.5 H, 5-H), 1.95 (m, 0.5 H, 5-H), 3.0 (m, 1 H, 7-H), 3.14 (m, 3 H, 2-CH_2, 7-H), 3.63 (s, 1.5 H, OCH_3), 3.66 (s, 1.5 H, OCH_3), 4.03 (m, 1 H, 2-H), 4.26 (m, 0.5 H, 4-H), 4.40 (m, 0.5 H, 4-H), 4.75 (m, 1 H, 7-NH), 4.93 (m, 0.5 H, 4-NH), 5.06 [s, 2 H, CH_2 (Z)], 5.15 (m, 0.5 H, 4-NH), 7.12−7.37 (m, 10 H, aromatics); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.30 and 25.62 (C_6), 27.46 and 27.57 (C_5), 28.21 [CH_3 (Boc)], 33.77 and 34.58 (2-CH_2), 40.29 (C_7), 52.47 and 52.64 (OCH_3), 56.94 and 57.04 (C_2), 58.85 (C_4), 66.56 [CH_2 (Z)], 80.04 [*C*(CH_3)$_3$], 126.70−137.98 (Ph), 155.15, 156.28 and 156.38 [CO (Boc)] and [CO (Z)], 167.33 and 168.93 (C_1), 203.29 and 203.62 (C_3). Anal. ($C_{28}H_{36}N_2O_7$) C, H, N.

Methyl (2*RS*,4*S*)-7-Benzyloxycarbonylamino-4-(*tert*-butoxycarbonylamino)-2-methyl-3-oxoheptanoate (10). Syrup (400 mg, 39%). RP HPLC t_R = 6.08 (A:B = 45:55); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (d, 1.5 H, J = 7 Hz, 2-CH_3), 1.33 (d, 1.5 H, J = 7 Hz, 2-CH_3), 1.40 (s, 4.5 H, Boc), 1.41 (s, 4.5 H, Boc), 1.46−1.55 (m, 2 H, 6-H), 1.77−1.89 (m, 2 H, 5-H), 3.19 (m, 2 H, 7-H), 3.67 (s, 1.5 H, OCH_3), 3.69 (s, 1.5 H, OCH_3), 3.74 (q, 1 H, J = 7 Hz, 2-H), 4.44 (m, 1 H, 4-H), 4.91 (m, 1 H, 7-NH), 5.06 [s, 2 H, CH_2 (Z)] 5.11 (m, 1 H, 4-NH), 7.27−7.34 (m, 5 H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.64 and 13.21 (2-CH_3), 25.80 and 25.93 (C_6), 27.91 [CH_3 (Boc)], 28.26 and 28.60 (C_5), 40.46 (C_7), 48.94 and 49.56 (C_2), 52.36 and 52.48 (OCH_3), 58.14 and 58.67 (C_4), 66.63 [CH_2 (Z)], 80.14 [*C*(CH_3)$_3$], 128.03, 128.46 and 136.60 (Ph), 155.40 and 156.45 [CO (Boc)] and [CO (Z)], 170.41 (C_1), 204.65 (C_3). Anal. ($C_{22}H_{32}N_2O_7$) C, H, N.

Synthesis of the 3-(*tert*-Butoxycarbonylamino)-2-(1-methoxycarbonyl-2-phenylethyl)piperidines 6. A solution of methyl (2*RS*,4*S*)-7-benzyloxycarbonylamino-4-(*tert*-butoxycarbonylamino)-2-phenylmethyl-3-oxoheptanoate (**5**) (769 mg, 1.5 mmol) in MeOH (100 mL) was hydrogenated, at room temperature and 1 atm of H_2 pressure, in the presence of 10% Pd (C) (80 mg) for 2 h. After filtration of the catalyst, $NaBH_3CN$ (190 mg, 3 mmol) and $ZnCl_2$ (216 mg, 1.76 mmol) were added and the resulting mixture stirred at room temperature for 1 h. Then, the solvent was evaporated, and the

Table 3. Significant Analytical and Spectroscopic Data of 5-(*tert*-Butoxycarbonyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives



	7a,**b**	**7c**,**d**	**7e**,**f**	**15a**,**b**	**15g**,**h**	**15c**,**d**	**21a**,**b**	**21g**,**h**
R^2	CH_2Ph	CH_2Ph	CH_2Ph	Me	Me	Me	Me	Me
R^3	CH_2Ph	CH_2Ph	CH_2Ph	CH_2Ph	CH_2Ph	CH_2Ph	4-$(NMe)_2$Ph	4-$(NMe)_2$Ph
stereochem	(4*R**,4a*S**,5*R**)	(4*R**,4a*S**,5*S**)	(4*R**,4a*R**,5*R**)	(4*R**,4a*S**,5*R**)	(4*R**,4a*R**,5*S**)	(4*R**,4a*S**,5*S**)	(4*R**,4a*S**,5*R**)	(4*R**,4a*R**,5*S**)
formula[a]	$C_{27}H_{33}N_3O_4$	$C_{27}H_{33}N_3O_4$	$C_{27}H_{33}N_3O_4$	$C_{21}H_{29}N_3O_4$	$C_{21}H_{29}N_3O_4$	$C_{21}H_{29}N_3O_4$	$C_{22}H_{32}N_4O_4$	$C_{22}H_{32}N_4O_4$
yield (%)	80	60	8	60	19	86	56	13
mp (°C)[b]	syrup	120−122	150−157	71−73	syrup	foam	224−225	syrup
t_R (A:B)[c]	7.81 (50:50)	6.99 (50:50)	9.55 (50:50)	4.76 (45:55)	5.82 (45:55)	6.00 (40:60)	3.17 (30:70)	4.03 (30:70)
				^{1}H NMR[d]				
4-H	3.05	3.08−3.17	3.13−3.16	2.96	2.90	2.73	3.03−3.09	3.01
4a−H	2.83	3.19	3.16	2.94−2.98	3.08	3.13	3.03−3.09	3.24
5-H	3.30	3.77	4.13	3.28	3.66	4.01	3.46	3.65−3.79
5-NH	4.04	4.61	4.44	4.53	4.33	4.69	4.50	4.45
6-H	2.00, 1.23	1.65−1.75	1.51, 1.71	1.42−1.49, 2.08	1.19, 2.05	1.56−1.70, 1.92	2.14	1.19−1.32, 2.05
7-H	1.59	1.53−1.57	1.47−1.50	1.56−1.68	1.50, 1.80	1.56−1.70	1.64−1.73	1.54−1.86
8-H	2.45−2.59, 4.38	2.53−2.68, 4.45	2.57−2.66, 4.40	2.63, 4.42	2.67, 4.17	4.46, 4.70	2.64−2.72, 4.43	2.73, 4.21
R^{2e}	3.07, 2.74	3.05, 2.91	2.95, 3.48	1.31	1.30	1.30	1.45	1.41
R^{3f}	5.05, 4.84	4.95	4.89	4.90−5.03	4.84, 4.97	4.90, 4.96	2.93	2.94
$J_{4,4a}$ (Hz)	0	2	0	0	3.5	8.0	0	3
$J_{4a,5}$ (Hz)	12	0	0	8	10	1	10	10
				^{13}C NMR[g]				
C_1	154.90	155.34	138.29	155.88	154.68	155.25	155.31	155.07
C_3	170.32	170.57	170.10	171.53	172.91	171.39	172.31	173.56
C_4	42.87	43.32	43.26	35.89	37.55	37.25	36.42	37.69
C_{4a}	58.76	57.72	57.75	63.82	57.75	60.64	63.74	57.57
C_5	49.58	48.71	45.02	50.26	47.19	46.69	50.71	47.25
C_6	32.18	29.89	30.61	32.11	30.94	29.52	32.52	30.88
C_7	24.12	20.12	20.32	24.20	22.61	19.44	24.45	22.61
C_8	46.51	45.66	46.22	46.60	44.00	45.24	46.97	43.95
R^{2e}	37.14	36.81	29.77	18.09	11.37	14.44	18.58	11.58
R^{3f}	44.04	44.11	44.40	43.85	44.15	44.40	40.86	40.47
C_1	154.90	155.34	138.29	155.88	154.68	155.25	155.31	155.07

[a] Satisfactory analyses for C, H, N. [b] From EtOAc/hexane. [c] Novapak C_{18} (3.9 × 150 mm, 4 μm), using mixtures of A = CH_3CN and B = 0.05% TFA in H_2O. [d] Measured in $CDCl_3$ at 300 MHz, except for **7a**,**b**, **15a**,**b**, and **15c**,**d** measured at 400 MHz. [e] CH_3 except for compounds **7**, where it refers to the CH_2 of CH_2Ph. [f] The CH_2 of R^3 except for compounds **21**, where it refers to NMe_2. [g] Measured in $CDCl_3$ at 75 MHz, except for **15a**,**b** and **15c**,**d**, which were measured at 100 MHz.

resulting residue was treated with water (50 mL). Afterward, 1 N NaOH solution was added dropwise until pH = 9, and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were washed with brine (100 mL) and dried over Na_2SO_4, and the solvent was evaporated to dryness. The resulting residue was purified by flash chromatography, employing a (1−9%) gradient of MeOH in CH_2Cl_2 as eluant, yielding the (1.4:1) unresolved diastereomeric mixture of the 2,3-cis piperidines **6c**,**d** (higher R_f, 19%) and the (1.2:1) unresolved diastereomeric mixture of the 2,3-trans piperidines **6a**,**b** (lower R_f, 60%).

(2*R,3*S**)-3-(*tert*-Butoxycarbonylamino)-2-(1-methoxycarbonyl-2-phenylethyl)piperidines (6a,b).** Syrup (326 mg, 60%). RP HPLC t_R = 3.59 (A:B = 40:60); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (m, 1 H, 4-H_{ax}), 1.43 (s, 6.3 H, Boc), 1.48 (s, 2.7 H, Boc), 1.43−1.50 (m, 1 H, 5-H), 1.60−1.69 (m, 1 H, 5-H), 1.89 (s, 1 H, 1-NH), 2.01 (m, 0.7 H, 4-H_{ec}), 2.09 (m, 0.3 H, 4-H_{ec}), 2.37−2.61 (m, 2 H, 2-H and 6-H_{ax}), 3.02 (m, 4 H, 2-CH, 6-H_{ec} and CH_2Ph), 3.44 (m, 0.7 H, 3-H), 3.68 (m, 0.3 H, 3-H), 3.51 (s, 2.1 H, OCH_3,) 3.62 (s, 0.9 H, OCH_3,), 4.31 (d, 0.7 H, J = 10 Hz, 3-NH), 4.42 (d, 0.3 H, J = 10 Hz, 3-NH), 7.18−7.36 (m, 5 H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.03 and 27.02 (C_5), 28.73 [CH_3 (Boc)], 32.46, 32.75, 33.53 (C_4 and CH_2Ph), 35.47 (CH_2Ph), 45.94 (C_6), 48.21 (2-CH), 50.60 (C_3), 46.50, 49.00 and 49.53 (C_6, 2-CH, C_3), 51.86 (OCH_3), 61.70 and 64.23 (C_2), 79.55 [$C(CH_3)_3$], 126.37, 128.72, 128.49, 129.41 (Ph), 140.03 [*C*(Ph)], 155.47 and 155.68 [CO (Boc)], 174.95 [CO (ester)]. Anal. ($C_{20}H_{30}N_2O_4$) C, H, N.

(2*R,3*R**)-3-(*tert*-Butoxycarbonylamino)-2-(1-methoxycarbonyl-2-phenylethyl)piperidines (6c,d).** Syrup (106 mg, 19%). RP HPLC t_R = 3.80 (A:B = 40:60); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (s, 2.97 H, Boc), 1.46 (s, 6.03 H, Boc), 1.49−1.88 (m, 4 H, 4-H and 5-H), 2.59−3.08 (m, 7 H, 2-CH, 2-H, 6-H, 1-NH and CH_2Ph), 3.45 (s, 3 H, OCH_3,), 3.69 (d, 0.33 H, J = 11 Hz, 3-H), 4.02 (d, 0.67 H, J = 11 Hz, 3-H), 5.45 (m, 1 H, 3-NH), 7.06−7.20 (m, 5 H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.86 (C_5), 28.39 [CH_3 (Boc)], 28.42 (C_4), 30.49 and 34.95 (CH_2Ph), 45.35 (C_3), 47.06 (C_3 and C_6), 50.34 and 51.18 (2-CH), 51.37 (OCH_3), 61.00 and 61.32 (C_2), 78.74 and 79.09 [$C(CH_3)_3$], 126.29−138.81 (Ph), 155.17 and 155.46 [CO (Boc)], 174.00 and 174.97 [CO (ester)]. Anal. ($C_{20}H_{30}N_2O_4$) C, H, N.

General Procedure for the Synthesis of the 2,4-Dibenzyl-5-(*tert*-butoxycarbonylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidines 7. Benzyl isocyanate (64 μL, 0.5 mmol) was slowly added to a solution of the corresponding diastereoisomeric mixture of 3-(*tert*-butoxycarbonylamino)-2-(1-methoxycarbonyl-2-phenylethyl)piperidines **6a**,**b** or **6c**,**d** (182 mg, 0.5 mmol) in dry THF (8 mL). After 1 h of stirring at room temperature, the reaction mixture was diluted with THF (8 mL). Then, NaH (60% dispersion, 24 mg, 0.6 mmol) was added, and the stirring was continued for an additional 3 h. Afterward, the reaction mixture was poured into 1 N HCl solution (25 mL) cooled to 0 °C, and the mixture was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (25 mL) and dried over Na_2SO_4, and the solvent was evaporated to dryness. The resulting residue was purified either by flash chromatography, employing 25% of EtOAc in hexane as eluant, in the case of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivative **7a**,**b** (186 mg, 80%),

Table 4. Analytical Data of the New 5-[*N*-(*tert*-Butoxycarbonyl)tryptophyl-1,3-dioxoperhydropyrido[1,2-*c*] Pyrimidine Derivatives

compd	yield (%)	mp (°C)[a]	formula[b]	t_R(min) (A:B)[c]
8a	70	118−120	$C_{38}H_{43}N_5O_5$	9.90 (50:50)
8b	23	111−113	$C_{38}H_{43}N_5O_5$	10.91 (50:50)
8c	60	100−102	$C_{38}H_{43}N_5O_5$	9.68 (50:50)
8d	16	95−97	$C_{38}H_{43}N_5O_5$	10.96 (50:50)
9a[d]	71	111−113	$C_{38}H_{43}N_5O_5$	10.39 (50:50)
9b[e]	19	118−120	$C_{38}H_{43}N_5O_5$	9.13 (50:50)
16a	66	125−127	$C_{32}H_{39}N_5O_5$	7.00 (45:55)
16b	7	108−110	$C_{32}H_{39}N_5O_5$	7.31 (45:55)
16c	80	113−116	$C_{32}H_{39}N_5O_5$	10.45 (40:60)
16d	3	foam	$C_{32}H_{39}N_5O_5$	11.89 (40:60)
17a	56	140−143	$C_{38}H_{45}N_5O_5$	20.30 (45:55)
22c[f]	81	foam	$C_{32}H_{39}N_5O_5$	10.29 (40:60)
22d[g]	3	113−116	$C_{32}H_{39}N_5O_5$	11.92 (40:60)
20a	54	170−172	$C_{38}H_{46}N_6O_5$	7.71(40:60)
23a	82	foam	$C_{33}H_{42}N_6O_5$	9.60 (30:70)
23b	9	foam	$C_{33}H_{42}N_6O_5$	10.19 (30:70)
23e,**f** (**e**:**f**, 9:1)	62	foam	$C_{33}H_{42}N_6O_5$	9.41, 7.87 (30:70)
24a	40	172−174	$C_{39}H_{48}N_6O_5$	10.05 (40:60)

[a] From EtOAc/hexane, except for **20a** and **24a** from CH_2Cl_2/MeOH. [b] Satisfactory analyses for C, H and N. [c] Novapak C_{18} (3.9 × 150 mm, 4 μm), using mixtures of, A = CH_3CN and B = 0.05% TFA in H_2O. [d] Enantiomer of **8b**. [e] Enantiomer of **8a**. [f] Enantiomer of **16d**. [g] Enantiomer of **16c**.

or by radial chromatography, using 17% of EtOAc in hexane as eluant, for **7c**,**d** (166 mg, 60%) and **7e**,**f** (22 mg, 8%), whose significant analytical and spectroscopic data are summarized in Table 3.

General Procedure for the Synthesis of the 2,4-Dibenzyl-5-[*N*-(*tert*-butoxycarbonyl)tryptophylamino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 8a−d and 9a,b. TFA (0.5 mL) was added dropwise to a stirred solution of the corresponding 2,4-dibenzyl-5-(*tert*-butoxycarbonylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine **7a**,**b** and **7c**,**d** (84 mg, 0.18 mmol) in CH_2Cl_2 (2 mL), and the stirring was continued for 45 min at room temperature. Evaporation of the solvent to dryness gave a residue which was dissolved in dry CH_2Cl_2 (3 mL). Then, Boc-L- or -D-Trp-OH (66 mg, 0.26 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 95 mg, 0.26 mmol), and TEA (50 μL, 0.40 mmol) were added successively to that solution, and the stirring was continued at room temperature for 18 h. The solvent was evaporated to dryness, and the residue was dissolved in EtOAc (25 mL). The resulting solution was washed successively with 10% citric acid (10 mL), 10% $NaHCO_3$ (10 mL), water (10 mL), and brine (20 mL), dried over Na_2SO_4, and the solvent was evaporated. The resulting diastereoisomeric pairs of Boc-tryptophyl derivatives **8a**,**b**, **8c**,**d**, and **9a**,**b** were purified and resolved by flash chromatography using a (10−50%) gradient of EtOAc in hexane as eluant. Significant analytical and spectroscopic data of these compounds are summarized in Tables 4−6.

Synthesis of the 3-(*tert*-Butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidines 11. These compounds were prepared from methyl (4*S*)-7-benzyloxycarbonylamino-4-(*tert*-butoxycarbonylamino)-3-oxoheptanoate (**4**) (844 mg, 2 mmol), by applying the same methodology above mentioned for the synthesis of analogue piperidines **6**. The resulting diastereoisomeric mixture was resolved by flash chromatography, using a (1−9%) gradient of MeOH in CH_2Cl_2 as eluant, into the 2,3-cis-disubstituted-piperidine **11c**,**d** (higher R_f, 128 mg, 24%) and the 2,3-trans-disubstituted piperidine **11a**,**b** (lower R_f, 284 mg, 52%) as (≈9:1) racemic mixtures.

(2*R,3*S**)-3-(*tert*-Butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidine (11a,b).** White solid (284 mg, 52%). Mp 81−83 °C (CH_2Cl_2/MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (dq, 1 H, *J* = 11 and 4 Hz, 4-H_{ax}), 1.45 (s, 9 H, Boc), 1.56−1.76 (m, 2 H, 5-H), 1.94 (s, 1 H, 1-NH), 2.02 (m, 1 H, 4-H_{ec}), 2.38 (dd, 1 H, *J* = 17 and 9 Hz, 2-CH_2), 2.58 (dt, 1 H, *J* = 11 and 3 Hz, 6-H_{ax}), 2.70 (m, 1 H, 2-H), 2.73 (d, 1 H, *J* = 17 Hz, 2-CH_2), 3.02 (m, 1 H, *J* = 11 Hz, 6-H_{ec}) 3.31 (dq, 1 H, *J* = 10 and 4 Hz, 3-H), 3.70 (s, 3 H, OCH_3,), 4.39 (d, 1 H, *J* = 10 Hz, 3-NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.79 (C_5), 28.34 [CH_3 (Boc)], 32.44 (C_4), 37.68 (2-CH_2), 45.78 (C_6), 51.57 and 51.84 (C_2 and C_3), 58.97 (OCH_3), 79.34 [*C*(CH_3)$_3$], 155.34 [CO (Boc)], 173.20 [CO (ester)]. Anal. ($C_{13}H_{24}N_2O_4$) C, H, N.

(2*R,3*R**)-3-(*tert*-butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidine (11c,d).** White solid (128 mg, 24%). Mp 44−46 °C (CH_2Cl_2/MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.44 (s, 9 H, Boc), 1.52−1.64 (m, 3 H, 4-H and 5-H), 1.86 (m, 1 H, 4-H), 1.99 (s, 1 H, 1-NH), 2.31 (dd, 1 H, *J* = 17 and 9 Hz, 2-CH_2), 2.44 (dd, 1 H, *J* = 17 and 4 Hz, 2-CH_2), 2.67 (m, 1 H, 6-H_{ax}), 2.97 (m, 1 H, 6-H_{ec}), 3.04 (m, 1 H, 2-H), 3.69 (s, 4 H, 3-H and OCH_3,), 5.35 (d, 1 H, *J* = 9 Hz, 3-NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.60 (C_5), 28.32 [CH_3 (Boc)], 30.14 (C_4), 37.56 (2-CH_2), 40.16 (C_6), 40.73 (C_3) 51.61 (C_2), 55.98 (OCH_3), 78.89 [*C*(CH_3)$_3$], 155.54 [CO (Boc)], 172.98 [CO (ester)]. Anal. ($C_{13}H_{24}N_2O_4$) C, H, N.

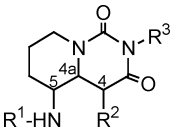
General Procedure for the Synthesis of the 1-(Benzyloxycarbonyl)-3-(*tert*-butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidines 12. Benzyl chloroformate (0.54 mL, 3.80 mmol) was slowly added to a stirred solution of the corresponding 3-(*tert*-butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidine **11a,b** or **11c,d** (523 mg, 1.90 mmol) and propylene oxide (2.02 mL, 28.80 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the stirring was continued for 20 h. Evaporation of the solvent to dryness gave a residue which was purified by flash chromatography, using a (17−50%) gradient of EtOAc in hexane, to give the 2,3-trans- and 2,3-cis-disubstituted piperidines **12a,b** and **12c,d**, respectively.

(2*R,3*S**)-1-(Benzyloxycarbonyl)-3-(*tert*-butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidines (12a,b).** Syrup (717 mg, 92%); RP HPLC t_R = 4.30 (A:B = 50:50); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9 H, Boc), 1.55−1.75 (m, 4 H, 4-H and 5-H), 2.53 (dd, 1 H, *J* = 14 and 6 Hz, 2-CH_2), 2.67 (dd, 1 H, *J* = 14 and 9 Hz, 2-CH_2), 2.91 (m, 1 H, 6-H_{ax}), 3.55 (s, 3 H, OCH_3,), 3.71 (m, 1 H, 3-H) 4.10 (m, 1 H, 6-H_{ec}), 4.72 (t, 1 H, *J* = 7 Hz, 2-H), 4.89 (d, 1 H, *J* = 7 Hz, 3-NH), 5.12 [s, 2 H, CH_2(Z)], 7.26−7.36 (m, 5 H, aromatics); ^{13}C NMR (75 MHz, $CDCl_3$) 19.59 and 23.36 (C_4 and C_5), 28.30 [CH_3 (Boc)], 34.71 (2-CH_2), 38.45 (C_6), 47.41 (C_2), 51.81 (C_3), 52.90 (OCH_3), 67.34 [CH_2(Z)], 79.54 [*C*(CH_3)$_3$], 127.69, 127.95, 128.45 and 136.41 (Ph), 154.81 [CO (Boc) and CO (Z)], 170.60 [CO (ester)]. Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

(2*R,3*R**)-1-(Benzyloxycarbonyl)-3-(*tert*-butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidines (12c,d).** White solid (681 mg, 86%). Mp 111−113 °C (hexane/EtOAc); RP HPLC t_R = 4.44 (A:B = 50:50); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9 H, Boc), 1.50−1.80 (m, 4 H, 4-H and 5-H), 2.49 (dd, 1 H, *J* = 14 and 9 Hz, 2-CH_2), 2.56 (dd, 1 H, *J* = 14 and 6 Hz, 2-CH_2), 2.79 (dt, 1 H, *J* = 14 and 2 Hz, 6-H_{ax}), 3.55 (s, 3 H, OCH_3,), 3.75 (m, 1 H, 3-H) 4.06 (d, 1 H, *J* = 14, 6-H_{ec}) 4.47 (bs, 1 H, 3-NH), 5.09 [d, 1 H, *J* = 13 Hz, CH_2(Z)], 5.18 [d, 1 H, *J* = 13 Hz, CH_2(Z)], 5.09−5.18 (m, 1 H, 2-H), 7.26−7.39 (m, 5 H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$) 24.43 and 25.51 (C_4 and C_5), 28.30 [CH_3 (Boc)], 31.18 (2-CH_2), 38.28 (C_6), 49.96 (C_2), 51.40 (OCH_3), 51.79 (C_3), 67.27 [CH_2(Z)], 79.95 [*C*(CH_3)$_3$], 127.82, 128.40, and 136.77 (Ph), 154.69 and 155.19 [CO (Boc) and CO (Z)], 171.65 [CO (ester)]. Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

Synthesis of (2*R,3*S**)-1-(Benzyloxycarbonyl)-3-(*tert*-butoxycarbonylamino)-2-[1-(methoxycarbonyl)ethyl]-piperidines 13a,b.** A solution of (2*R**,3*S**)-1-(*N*-benzyloxycarbonyl)-3-(*tert*-butoxycarbonylamino)-2-(methoxycarbonylmethyl)piperidine (**12a**,**b**) (406 mg, 1 mmol) in dry THF (7 mL) was added dropwise to a stirred solution of lithium bis-(trimethylsilyl)amide (1 M solution in THF, 2.0 mL, 2 mmol) in THF (5 mL) at − 78 °C, and the stirring was continued for 45 min at the same temperature. Afterward, a solution of methyl iodide (123 μL, 2 mmol) and hexamethylphosphoramide (100 μL, 0.58 mmol) in dry THF (5 mL) was added dropwise at − 78 °C. After the reaction mixture was stirred at this temperature for further 4 h, the resulting solution was then treated with 10% NH_4Cl solution (50 mL) and extracted with diethyl ether (2 × 50 mL). The combined organic extracts were

Table 5. Significant ^{1}H NMR[a] Spectroscopic Data of 5-(Boc- and 2-Adoc-Trp)-Amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives



compd	4-H	4a-H	5-H	6-H	7-H	8-H	R^{2b}	R^{3c}	α-H (Trp)	$J_{4,4a}$	$J_{4a,5}$
8a[d]	2.33	2.43	3.60	1.89 1.01−1.05	1.51	2.04−2.36 4.32	2.51, 2.79	5.05, 4.98	4.09	0	11.5
8b[e]	2.74	2.35−2.43	3.71	0.94, 1.81	1.52	2.35−2.43 4.36	2.51, 2.85	5.00, 5.08	4.48	0	11
8c	2.37	2.95	4.16	1.37−1.41 1.63	1.37−1.41	2.37−2.46 4.19	2.81, 3.17	4.93	4.36	8	2
8d	2.78	3.06	4.02	1.25 1.30−1.41	0.86 1.22−1.27	2.44, 4.25	2.90, 3.00	4.84, 4.88	4.31	5	2
16a	2.10	2.53	3.56	1.21, 1.85	1.51	2.43−2.54 4.32	1.02	4.91, 4.98	4.34	0	11
16b	2.49−2.57	2.70	3.55−3.61	1.14−1.54	1.46	2.49−2.57 4.35	1.17	4.90, 5.00	4.42	0	11
16c[f]	1.68−1.76	2.84	4.19	1.34−1.41 1.72	1.04 1.34−1.41	2.46, 4.12	1.07	4.85, 4.97	4.54	11	2
16d[g]	2.41	2.96	4.17−4.22	1.22−1.42	1.22−1.40	2.47 4.17−4.22	1.18	4.82, 4.92	4.34	10	2
17a	1.95	2.50	3.50	1.16, 1.95	1.59	2.41, 4.27	0.96	4.85, 4.92	4.36	0	11
20a	2.14−2.24	2.57	3.64	1.20,1.82	154−1.67	2.47 4.22−4.12	-	2.93	4.43	9.5	9
23a	1.98	2.62	3.69	1.20−1.27 1.90	1.62	2.-8−2.59 4.31	1.08	2.93	4.26	0	11
23b	2.56	2.82	3.84	1.03−1−87	1.63−1.68	2.61, 4.39	1.28	2.97	4.49	0	11
23g,h	1.96	2.57	3.90	1.08, 1.87	1.46, 1.81	2.61, 4.12	1.28	2.95, 2.94	4.47	*h*	11
24a	1.97−2.01	2.62	3.67	1.24 1.97−2.01	1.56	2.51, 4.30	1.08	2.94	4.37	0	10

[a] Spectra registered in $CDCl_3$ at 400 MHz except for **8a**−**c**, **16a**−**d**, and **24g**,**h** registered at 500 MHz. [b] The CH_3 of R^2, except for compounds **8**, where it is the CH_2. [c] The CH_2 of R^3, except for **20a**, **23**, and **23a**, where it refers to NMe_2. [d] The same data for its enantiomer **9b**. [e] The same data for its enantiomer **9a**. [f] The same data for its enantiomer **22d**. [g] The same data for its enantiomer **22c**. [h] The 4-H and 4a-H signals did not have enough resolution to measure this coupling constant.

Table 6. Significant ^{13}C NMR[a] Spectroscopic Data of 5-(Boc- and 2-Adoc-Trp)-Amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives

compd	C_1	C_3	C_4	C_{4a}	C_5	C_6	C_7	C_8	R$^{2\,b}$	R$^{3\,c}$	C_α (Trp)
8a[d]	155.49	171.45	41.61	58.22	47.46	31.40	23.72	46.56	36.66	44.06	55.82
8b[e]	155.78	171.17	42.16	58.59	47.38	32.23	24.03	46.82	37.18	44.38	56.82
8c	153.37	171.47	42.88	56.00	45.18	28.94	18.95	45.04	33.47	44.28	54.68
8d	152.66	171.84	43.81	56.71	46.42	29.13	19.29	45.41	35.51	44.10	55.78
16a	151.97	171.65	34.50	34.50	63.07	31.63	23.83	46.54	17.94	43.78	55.80
16b	152.29	171.94	35.63	63.83	48.60	31.94	24.17	46.85	18.19	44.08	56.24
16c[f]	151.98	171.74	32.09	63.07	48.40	31.70	23.86	46.63	17.97	43.83	55.81
16d[g]	154.59	170.97	36.30	59.01	43.83	28.80	18.27	44.56	12.02	44.42	54.71
17a	154.35	171.61	36.77	59.39	44.24	28.62	18.47	44.79	12.96	44.39	55.98
20a	150.23	171.44	33.46	54.28	50.55	30.63	23.06	44.48	-	40.51	55.82
23a	152.30	171.79	35.09	62.84	48.47	31.75	23.88	46.66	18.15	40.54	55.63
23b	150.25	171.44	35.30	63.24	48.44	29.67	23.94	46.72	18.22	40.59	56.02
23g,h	154.96	173.92	36.57	56.92	45.62	30.28	22.42	43.77	11.53	40.53	55.40
24a	152.35	171.43	32.40	62.82	48.63	31.95	23.86	46.71	18.21	40.53	55.99

[a] Spectra registered in $CDCl_3$ at 75 MHz except for **8c**, **20a**, and **23g**,**h**, which were registered at 100 MHz. [b] CH_3 except for compounds **8** and **9**, where it is the CH_2 of CH_2Ph. [c] The CH_2 of CH_2Ph, except for compounds **22** and **23a** where it refers to NMe_2. [d] The same data for its enantiomer **9b**. [e] The same data for its enantiomer **9a**. [f] The same data for its enantiomer **22d**. [g] The same data for its enantiomer **22c**.

washed successively with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. Evaporation of the solvent to dryness gave a residue which was purified by flash chromatography, using a (10−50%) gradient of EtOAc in hexane as eluant, to give the title compounds **13a**,**b** (syrup, 391 mg, 93%) as a single racemic mixture. RP HPLC t_R = 8.27 (A:B = 45:55); ^{1}H NMR (300 MHz, DMSO, 80 °C) δ 0.95 (d, 1 H, J = 7 Hz, CH_3), 1.37 (s, 9 H, Boc), 1.37−1.57 (m, 2 H, 4-H) 1.82 (m, 2 H, 5-H), 2.73 (m, 1 H, 6-H_{ax}), 2.92 (q, 0.5 H, J = 7 Hz, 2-CH), 2.95 (q, 0.5 H, J = 7 Hz, 2-CH), 3.48 (m, 1 H, 3-H), 3.66 (s, 3 H, OCH_3,), 3.95 (m, 1 H, J = 13 Hz, 6-H_{ec}), 4.30 (d, 1 H, J = 11 Hz, 2-H), 5.10 [s, 2 H, CH_2(Z)], 6.47 (ws, 1 H, 3-NH), 7.30−7.37 (m, 5 H, aromatics). ^{13}C NMR (75 MHz, $CDCl_3$) 14.16 (Me) 19.40 and 19.68 (C_5) 23.42 (C_4), 28.32 [CH_3 (Boc)], 38.52 (C_6), 39.11 and 39.35 (2-CH), 46.28 and 46.86 (C_3), 52.13 (OCH_3), 57.80 and 57.73 (C_2), 67.37 [CH_2(Z)], 79.98 [$C(CH_3)_3$], 127.73, 127.98, 128.49 and 136.40 (Ph), 154.61 and 156.28 [CO (Boc) and CO (Z)], 174.49 [CO (ester)]. Anal. ($C_{22}H_{32}N_2O_6$) C, H, N.

Synthesis of (2*R,3*S**)-3-(*tert*-Butoxycarbonylamino)-2-[1-(methoxycarbonyl)ethyl]piperidines 14a,b.** A solution of (2*R**,3*S**)-1-benzyloxycarbonyl-3-(*tert*-butoxycarbonylamino)-2-(1-(methoxycarbonyl)ethyl)piperidine (**13a**,**b**) (350 mg, 0.83 mmol) in MeOH (30 mL) was hydrogenated at room temperature and 1 atm of H_2 pressure in the presence of 10% Pd(C) (35 mg) for 30 min. Afterward, the catalyst was filtered off and washed with MeOH (2 × 3 mL), and the filtrate was

evaporated to dryness. The resulting residue was purified by flash chromatography, using a (1−4%) gradient of MeOH in CH_2Cl_2 as eluant The title compound **14a**,**b** was obtained as a syrup which solidified on standing as a white solid (197 mg, 83%). Mp 61−62 °C (CH_2Cl_2/MeOH); ^{1}H NMR δ 1.07−1.27 (m, 3 H, 4-H_{ax}), 1.18 (d, 3 H, J = 7 Hz, CH_3), 1.40 (s, 9 H, Boc), 1.48−1.64 (m, 2 H, 5-H), 1.67 (s, 1 H, 1-NH), 2.03 (m, 1 H, 4-H_{ec}), 2.50 (dq, 1 H, J = 12 and 3 Hz, 6-H_{ax}), 2.64 (dd, 1 H, J = 10 and 3 Hz, 2-H), 2.76 (m, 1 H, 2-CH), 2.98 (m, 1 H, J = 12 Hz, 6-H_{ec}) 3.38 (dq, 1 H, J = 10 and 4 Hz, 3-H), 3.61 (s, 3 H, OCH_3,), 4.30 (d, 1 H, J = 10 Hz, 3-NH). ^{13}C NMR (50 MHz, $CDCl_3$) 10.76 (Me) 25.91 (C_5) 28.34 [CH_3 (Boc)], 33.00 (C_4), 39.92 (2-CH), 46.02 (C_6), 49.45 (C_3), 51.80 (OCH_3), 62.90 (C_2), 79.30 [$C(CH_3)_3$], 155.24 [CO (Boc)], 176.72 [CO (ester)]. Anal. ($C_{14}H_{26}N_2O_4$) C, H, N.

Synthesis of the 2-Benzyl-5-(*tert*-butoxycarbonylamino)-4-methyl-1,3-dioxo-perhydropyrido[1,2-*c*]pyrimidine Derivatives 15. These compounds were prepared from the (2*R**,3*S**)-3-(*tert*-butoxycarbonylamino)-2-(1-(methoxycarbonyl)ethyl)piperidines **14a**,**b** (150 mg, 0.52 mmol), by applying the same methodology as described above for the preparation of analogues **7**. The resulting diastereoisomeric mixture of **15a**,**b** and **15g**,**h** was purified and resolved by flash chromatography, employing 25% of EtOAc in hexane as eluant, followed by preparative radial chromatography, employing a (0.2−1%) gradient of MeOH in CH_2Cl_2 as eluant, to give the racemic mixtures **15g**,**h** (higher R_f, 38 mg, 19%) and **15a**,**b** (lower R_f, 120 mg, 60%). Significant analytical and spectroscopic data of these compounds are summarized in Table 3.

Synthesis of (4*S*,4a*R*,5*S*)- and (4*R*,4a*S*,5*R*)-2-Benzyl-5-[[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino]-4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidines 16a and 16b. These compounds were prepared from the (≈9:1) racemic mixture of (4*R**,4a*S**,5*R**)-2-benzyl-5-(*tert*-butoxycarbonylamino)-4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidines **15a**,**b** (97 mg, 0.25 mmol), applying the same procedure as described for the preparation of analogues **8**. Significant analytical and spectroscopic data of these Boc-tryptophyl derivatives **16a** (higher R_f, 94 mg, 66%) and **16b** (lower R_f, 10 mg, 7%) are summarized in Tables 4−6.

General Procedure for the Synthesis of (4*S*,4a*R*,5*S*)-5-[[*N*-(2-Adamantyloxycarbonyl)-L-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 17a, **20a**, **and 24a.** TFA (0.2 mL) was added to a stirred solution of the corresponding (4*S*,4a*R*,5*S*)-5-[[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino]-1,3-dioxoperhydropyrido[1,2-*c*]-pyrimidine **16a**, **3a**, or **23a** (0.10 mmol) in CH_2Cl_2 (2 mL). After 4 h of stirring at room temperature, the solvent was evaporated to dryness, and the resulting residue was dissolved in dry THF (2 mL). Then, dry triethylamine (28 μL, 0.20 mmol) was added to the resulting solution, and the reaction mixture was stirred for 10 min. Afterward, a solution of 2-adamantyl chloroformate [0.30 mmol, prepared from 2-adamantanol (50 mg, 0.33 mmol) as previously described[47] in THF (2 mL) was added, and the stirring was continued for further 18 h. After removal of the solvent to dryness under reduced pressure, water (5 mL) was added, and the suspension was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with brine (10 mL) and dried over Na_2SO_4, and the solvent was evaporated to dryness. The crude residue was purified by preparative radial chromatography, using a (25−50%) gradient of EtOAc in hexane (**17a**) or (1−10%) gradient of MeOH in CH_2Cl_2 (**20a** and **24a**) as eluants, yielding the corresponding 5-*N*-[(2-adamantyloxycarbonyl)-L-tryptophyl]amino derivatives **17a**, **20a**, and **24a**, whose analytical and spectroscopic data are summarized in Tables 4−6.

General Procedure for the Synthesis of the 5-(*tert*-Butoxycarbonylamino)-4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 15c,d, 21a,b, and 21g,h. A solution of the corresponding 5-(*tert*-butoxycarbonylamino)-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivative **18c**,**d**[27] or **19a**,**b**[32] (0.75 mmol) in dry THF (5 mL) was added dropwise to a stirred solution of lithium bis(trimethylsilyl)-amide (1 M solution in THF, 1.5 mL, 1.5 mmol) in THF (5 mL) at − 78 °C, and the stirring was continued for 45 min at the same temperature. Afterward, a solution of methyl iodide (92 μL, 1.5 mmol) and hexamethylphosphoramide (75 μL, 0.44 mmol) in dry THF (4 mL) was added dropwise at −78 °C. After the reaction mixture was stirred at this temperature for further 4 h, the resulting solution was then treated with 10% NH_4Cl solution (50 mL) and extracted with diethyl ether (2 × 50 mL). The combined organic extracts were washed successively with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. Evaporation of the solvent to dryness gave a residue which was purified by flash chromatography, using a (20−50%) gradient of EtOAc in hexane as eluant, to give the title compounds **15c**,**d**, **21a**,**b**, and **21g**,**h** as racemic mixtures, whose significant analytical and spectroscopic data are summarized in Table 3.

General Procedure for the Synthesis of the 5-[[*N*-(*tert*-butoxycarbonyl)-L- and D-tryptophyl]amino]-4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 16c,d 22c,d, and 23. These compounds were prepared from the appropriate 5-(*tert*-butoxycarbonylamino)-4-methyl-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivative **15c**,**d**, **21a**,**b**, or **21g**,**h** (0.2 mmol), by applying the same procedure as above indicated for the synthesis of the analogues **8** and **9**. The (9:1) diastereoisomeric mixture **23g**,**h**, resulting from **21g**,**h**, could not be resolved. Significant analytical and spectroscopic data of the title compounds are summarized in Tables 4−6.

Biological Methods. Materials. [^{3}H]Propionyl-CCK-8 (specific activity, 60−80 mCi mmol^{-1}) was from Amersham Biosciences. CCK-8 and CCK-4 were from Sigma-Aldrich. PD-135,158 was a gift from Parke Davis. Amylase kit reagent was from Boeringher Mannheim. (Thr, Nle)-CCK-9 was synthesized by Luis Moroder (Max Planck Institut fur Biochimie, Munchen, Germany). ^{125}INa was from Amersham Biosciences. (Thr, Nle)CCK-9 was conjugated with Bolton-Hunter reagent, purified and radioiodinated as described previously by Fourmy et al.[48] The specific activity of radioiodinated peptide was 1600−2000 Ci/mmol. Myo-2-[^{3}H]inositol was from Amersham Biosciences.

Rat CCK_1 and CCK_2 Receptor 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.,[41] 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 50 000*g*. For displacement assays, pancreatic membranes (0.2 mg protein/tube) were incubated with 0.5 nM [^{3}H]propionyl-CCK-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 100 000*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. IC_{50} values were calculated from the displacement curves analyzed with GraphPad Prism software.[49]

Amylase Release. Dispersed rat pancreatic acini were prepared by using a modification of the technique of Jensen et al.[45] 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.[50] 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 (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 (0.1 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 was control release (vehicle), S_{CCK} the release elicited by CCK-8 and S_T 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.

Isolated Longitudinal Muscle−Myenteric Plexus (LMMP) Preparation from Guinea-Pig Ileum. Guinea-pigs were killed and bled. The ileum was excised approximately 10 cm from ileo-caecal junction, and longitudinal muscle strips with the myenteric plexus (LMMP) attached were prepared.[51] LMMP strips were suspended in a 10 mL organ bath containing Krebs bicarbonate solution (composition in mM: NaCl 118.2, KCl 4.6, $CaCl_2 \cdot 2H_2O$ 1.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.8 and glucose 1.0) maintained at 37 °C and aerated with 95% O_2/5% CO_2. Tissues were equilibrated for 30 min at 0.5 mg applied force and then field-stimulated (1 Hz, 1 ms, 10−15 V) for 30 min. The strips were subsequently stimulated by KCl (40 mM) to obtain a maximal contractile response. The preparation was then washed with Krebs bicarbonate solution and equilibrated for 20 min period before performing the different experiments. After control responses to KCl had been obtained, noncumulative concentration−response curves (CRC) to CCK_4 were obtained by stepwise increases in concentration every 10 min; the preceding concentration was washed out, and the tissue was exposed to the peptide for a period of 2 min. CRC for each peptide were calculated as percentages of the initial KCl contraction, and EC_{50} values were determined. In studies with drugs, each strip was used to record two CRC: the first for the agonist alone and the second for the agonist in the presence of the antagonist, each strip serving as its own control. Antagonists were allowed to preequilibrate for 30 min prior to addition of the agonist. The effect of antagonists was expressed as percentage of inhibition of maximal response obtained with the agonist alone in the same tissue, and pA_2 values were calculated according to the following equation,[52]

$$pA_2 = -\log([B]/(DR - 1))$$

where [B] is the concentration of the antagonist and DR (dose ratio) is the quotient between EC_{50} of the agonist in the presence of the antagonist and control EC_{50}.

Transient Transfection of COS-7 Cells. COS-7 cells (1.5×10^6) were plated onto 10-cm culture dishes and grown in Dulbelcco's Modified Eagle's Medium containing 5% fetal calf serum (complete medium) in a 5% CO_2 atmosphere at 37 °C. After overnight incubation, cells were transfected with 2.5 μg/plate of pRFENeo vectors containing the cDNA coding for the human CCK_2 receptor, using a modified DEAE-dextran method. Approximately 24 h posttransfection, the cells were washed twice with phosphate-buffered saline pH 6.95 and then seeded onto 24-well dishes in complete medium at a density of approximatively 1×10^5 cells/well. For inositol phosphates assay, the cells were resuspended in complete medium in the presence of 2 μCi/ml myo-2-[^{3}H] inositol and incubated overnight in 24-well dishes.

Wild-Type Human CCK_2 Receptor Binding Assay. Approximately 24 h after the transfer of transfected cells to 24-well plates, the cells were washed with phosphate-buffered saline pH 6.95, 0.1% BSA and then incubated for 60 min at 37 °C in 0.5 mL Dulbelcco's Modified Eagle's Medium, 0.1% BSA with either 71 pM ^{125}I-BH-(Thr, Nle)CCK-9 in the presence or the absence of competing compound. The cells were washed twice with phosphate-buffered saline pH 6.95 containing 2% BSA, and cell-associated radioligand was collected with 0.1 N NaOH added to each well. The radioactivity was directly counted in a gamma counter (Auto-Gamma, Packard, Downers Grove, IL) or added to scintillant and counted for the tritiated radioligand. Nonspecific binding was always less than 10% of total binding.

Inositol Phosphate Assays. Approximately 24 h after the transfer to 24-well plates and following overnight incubation in complete medium containing 2 μCi/mL of myo-2-[3H]inositol, the transfected cells were washed with Dubelcco's Modified Eagle's Medium and then incubated for 30 min in 2 mL/well Dubelcco's Modified Eagle's Medium containing 20 mM LiCl at 37 °C. The cells were washed with PI buffer at pH 7.45: phosphate-buffered saline containing 135 mM NaCl, 20 mM HEPES, 2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1 mM EGTA, 10 mM LiCl, 11.1 mM glucose, and 0.5% BSA. The cells were then incubated for 60 min at 37 °C in 0.5 mL PI buffer with or without ligands at various concentrations. The reaction was stopped by adding 1 mL methanol/chlorhydric acid to each well, and the content was transferred to a column (Dowex AG 1-X8, formate form, Bio-Rad, Hercules, CA) for the extraction of inositol phosphates. The columns were washed twice with 5 mL of distilled water and twice more with 2 mL of 5 mM sodium tetraborate/60 mM sodium formate. The content of each column was eluted by addition of 2.5 mL of 1 M ammonium formate/100 mM formic acid. Samples of the eluted fraction (0.5 mL) were added to scintillant, and β-radioactivity was counted.

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Supporting Information Available: Table of combustion analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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