β-Turned Dipeptoids as Potent and Selective CCK_1 Receptor Antagonists

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To improve our knowledge of the bioactive conformation of CCK_1 antagonists, we previously described that replacement of the α-MeTrp residue of dipeptoids with the (2*S*,5*S*,11b*R*)-2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate (IBTM) skeleton, a probed type II′ *β*-turn mimetic, led to restricted analogues (2*S*,5*S*,11b*R*,1′*S*)- and (2*S*,5*S*,11b*R*,1′*R*)-2-(benzyloxycarbonyl)amino-5-[1′-benzyl-2′-(carboxy)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole, **1a**,**b**, showing high binding affinity and selectivity for CCK_1 receptors. In this report, we describe the synthesis and binding profile of new analogues of compounds **1** designed to explore the importance of the C-terminal residue and of the type of *β*-turn on the receptor binding affinity and selectivity. Structure−affinity relationship studies show that a C-terminal free carboxylic acid and an *S* configuration of the Phe and *β*Hph residues are favorable for CCK_1 receptor recognition. Moreover, selectivity for this receptor subtype is critically affected by the *β*-turn type. Thus, while compounds **15a** and **16a**, containing the (2*S*,5*S*,11b*R*)- and (2*R*,5*R*,11b*S*)-IBTM frameworks, respectively, are both endowed with nanomolar affinity for CCK_1 receptors, restricted dipeptoid derivative **15a**, incorporating the type II′ IBTM mimetic, shows approximately 6-fold higher CCK_1 selectivity than analogue **16a**, with the type II mimetic. From these results, we propose that the presence of a *β*-turn-like conformation within the peptide backbone of dipeptoids could contribute to their bioactive conformation at the CCK_1 receptor subtype. Concerning functional activity, compounds **15a** and **16a** behave as CCK_1 receptor antagonists.

Introduction

Cholecystokinin (CCK) is a peptide hormone and neurotransmitter involved in modulating gastrointestinal and behavioral activities by interacting with specific receptors.[1] Two G-protein-coupled 7TM receptor subtypes have been characterized for CCK: CCK_1 receptors that predominate in the periphery but are also found in discrete regions of the brain, and CCK_2 receptors located mainly in the CNS.[2,3] Changes in the levels of CCK found in tissues and sera of patients with various disease states, including schizophrenia[4] and eating disorders,[5] as well as the effects of CCK derivatives in neuroprotection,[6] models of Parkinson's disease,[7] cancer,[8] anxiety,[9] and pain,[10] indicate the potential utility of CCK receptor ligands as therapeutic agents. This potentiality has prompted an intensive research in this area, and several potent and selective nonpeptide CCK_1 and CCK_2 receptor agonists and antagonists have been reported over the past decade.[11−15] Among these compounds, dipeptoids were designed from structure−activity relationship studies on the endogenous CCK_2 receptor-selective agonist CCK-4 (H-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2), which revealed the importance of the aromatic side-chains of Trp and Phe residues as key binding fragments.[16−18] Both CCK_1 and CCK_2 receptor-selective antagonists were obtained within this structural family of compounds.[19−22] Following the discovery of dipeptoids, different constrained analogues have been prepared in an attempt to establish three-dimensional structure−activity relationships and to develop pharmacological agents with improved properties. Restrictions by $N^{\alpha}-C^{\alpha}$ and $N^{\alpha}-N^{\alpha'}$ cyclization,[23,24] macrocyclization,[25,26] and amide bond rigidification[27] resulted in dipeptoid analogues with reduced affinity for CCK_2 receptors with respect to the corresponding acyclic parents. By contrast, conformational restriction of the C-terminal residue through a Pro ring[28] or by incorporation of a tetrahydronaphthyl group[29] have been reported to maintain or to increase, respectively, the affinity for CCK_2 receptors. However, in both cases the restricted derivatives showed higher affinity for CCK_1 receptors and, therefore, a lower selectivity than their parents. The enhancement of the affinity for the CCK_1 receptor subtype was especially remarkable in constrained dipeptoid analogues incorporating dehydro- and cyclopropylphenylalanine derivatives at the C-terminus,[30] for which conformational studies indicated the presence of a *β*-turn within the peptide backbone, although no preference in *β*-turn type was observed.[31] In a similar way, substitution of the α-methyltryptophan of dipeptoids by dehyrotryptophan led either to a decrease in the CCK_2 selectivity or to CCK_1-selective compounds.[32]

Taking into account that dehydro amino acids are able to stabilize *β*-turn secondary structures, when incorporated into peptide sequences,[33] we decided to investigate whether a turn-like conformation is responsible for the enhancement of CCK_1 receptor affinity showed by many

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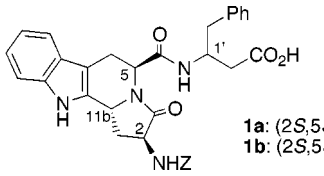
of the restricted dipeptoids. We have recently reported that replacement of the α-MeTrp residue of dipeptoids with the (2*S*,5*S*,11b*R*)-2-amino-3-oxohexahydroindolizino-[8,7-*b*]indole-5-carboxylate skeleton [(2*S*,5*S*,11b*R*)-IBTM] led to restricted analogues **1a**,**b**, showing high binding



1a: (2*S*,5*S*,11b*R*,1'*S*)
1b: (2*S*,5*S*,11b*R*,1'*R*)

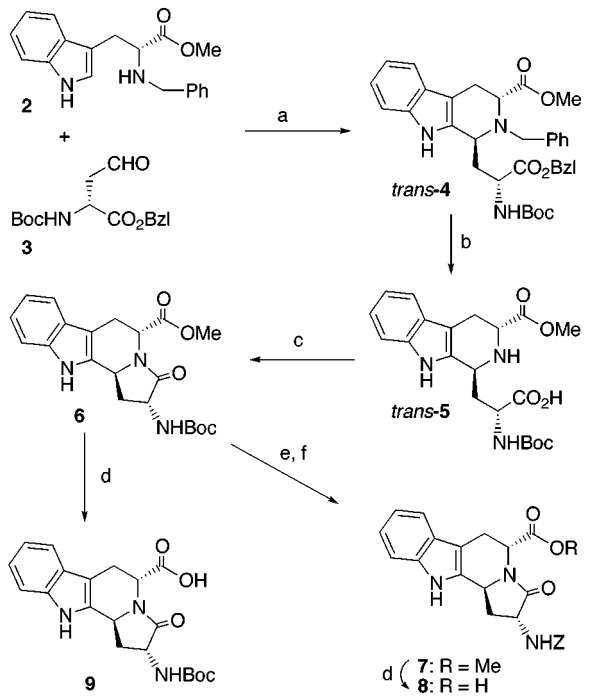
affinity and selectivity for CCK_1 receptors.[34] The (2*S*,5*S*,-11b*R*)-IBTM skeleton contains the indole side chain of the Trp residue present in dipeptoids, and as we previously demonstrated, it is able to mimic a type II′ β-turn conformation.[35,36] Therefore, it is plausible that a turn-like conformation within the peptide backbone of dipeptoids is favorable for CCK_1 receptor recognition. To optimize the structural requirements of these IBTM-containing dipeptoid analogues, we have prepared new derivatives incorporating L- and D-Phe residues instead of the corresponding β-homophenylalanine (βHph). To confirm whether the β II′ is the type of β-turn preferred for the interaction of these restricted dipeptoids with the CCK_1 receptor, a series of compounds containing the enantiomeric (2*R*,5*R*,11b*S*)-IBTM skeleton, able to stabilize a type II β-turn, have also been prepared. This paper describes the synthesis, conformational analysis by ^{1}H NMR, and CCK receptor binding affinities of this new family of β-turned restricted dipeptoid analogues.

Chemistry

The preparation of the (2*R*,5*R*,11b*S*)-2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate derivative **6** was performed by a synthetic route similar to that described for the corresponding 2*S*,5*S*,11b*R* enantiomer,[35] but some modifications were introduced in an attempt to improve overall yield (Scheme 1). The main differences with the previously described procedure concern the synthetic steps affording tetrahydro-β-carboline **4** and the intramolecular lactamization to the tetracyclic derivative **6**. Thus, condensation of the D-Trp derivative **2** with the D-Asp-derived aldehyde **3** in the presence of TFA was conducted at room temperature to afford a diastereoisomeric mixture of the corresponding *trans*- and *cis*-tetrahydro-β-carbolines **4** in a 1.5:1 ratio. This mixture was then refluxed in toluene to allow the complete epimerization of the *cis* isomer to the desired *trans* derivative **4**[37] having the 1*S*,5*R*,1′*R* configuration. Using this method, the yield of the reaction was improved by 10% with respect to the procedure involving direct refluxing of the reaction mixture of **2** and **3**. The lactamization step did not need previous esterification as it was done in the initial procedure, proceeding directly, in very good yield from the carboxylic acid derivative **5** obtained by hydrogenation of compound **4**. Removal of the Boc protecting group from **6** followed by treatment with benzyl chloroformate afforded the corresponding Z-protected derivative **7** in 52% total yield. Finally, saponification of the methyl ester groups in **6** and **7** furnished the free carboxylic acid derivatives **8** and **9**, respectively.

As indicated in Scheme 2, the Phe-containing derivatives **11a**,**b** were prepared by condensation of the

Scheme 1[a]



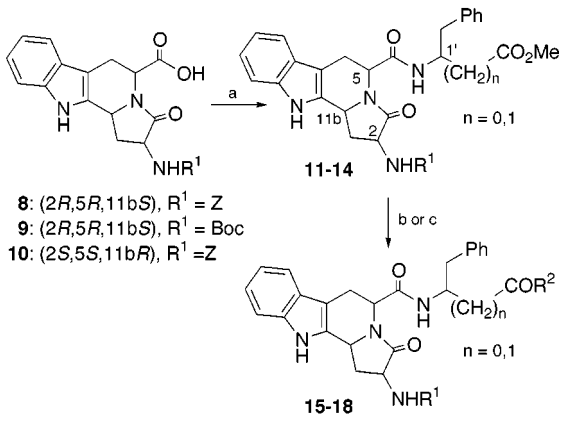
[a] Reagents and conditions: (a) TFA (1 equiv), toluene, rt to reflux; (b) H_2/Pd−C, MeOH; (c) xylene, reflux; (d) 2 N NaOH, MeOH; (e) TFA, CH_2Cl_2; (f) ZCl, CH_2Cl_2, propylene oxide.

carboxylic acid derivative **10**[35] with H-L- and H-D-Phe-OMe, respectively, using BOP as the coupling agent. To prepare compounds **13a**,**b**, containing the type II β-turn mimetic (2*R*,5*R*,11b*S*)-IBTM skeleton, two alternate procedures were undertaken. In the first one, the *N*-Boc-protected carboxylic acid derivative **9** was coupled with H-L-Phe-OMe to provide the conformationally constrained dipeptoid **12a**, which was then transformed into the corresponding Z-protected analogue **13a** upon elimination of the Boc group and treatment with benzyl chloroformate. Compound **13b** was prepared by direct coupling of Z-protected derivative **10** with H-D-Phe-OMe. Both procedures gave similar final results, and in both cases, compounds **13a**,**b** were obtained in 48% total yield from **7**. Condensation of the carboxylic acid **10** with methyl (3*S*)- and (3*R*)-amino-4-phenylbutanoate (H-L- and H-D-βHph-OMe) provided the βHph derivatives **14a**,**b**, respectively. The C-terminal free carboxylic acid derivatives **15a**,**b**, **16a**,**b**, and **17a**,**b** were prepared by saponification with NaOH of the corresponding methyl esters **11a**,**b**, **13a**,**b**, and **14a**,**b**. It is interesting to note that while the saponification of the Phe derivatives **11a**,**b** and **13a**,**b** proceeded quickly and in very good yield, a 20−30% of epimerization at C-5 was observed for the βHph analogues **17a**,**b**. This epimerization, probably due to the longer reaction times required for the completion of the reaction, led to lower yields and difficulties in the purification of the final compounds. Finally, C-terminal carboxamide derivatives **18a**,**b** were prepared by ammonolysis of compounds **11a**,**b**, respectively.

Conformational Studies

The ability of the conformationally restricted dipeptoid analogues to adopt a β-turn-like conformation in solution was evaluated by ^{1}H NMR. Independent of the

Scheme 2[a]



Compd.	R^1	R^2	n	2,5,11b,1'
11a	Z	–	0	*S,S,R,S*
11b	Z	–	0	*S,S,R,R*
12a	Boc	–	0	*R,R,S,S*
d, e → **13a**	Z	–	0	*R,R,S,S*
13b	Z	–	0	*R,R,S,R*
14a	Z	–	1	*R,R,S,S*
14b	Z	–	1	*R,R,S,R*
15a	Z	OH	0	*S,S,R,S*
15b	Z	OH	0	*S,S,R,R*
16a	Z	OH	0	*R,R,S,S*
16b	Z	OH	0	*R,R,S,R*
17a	Z	OH	1	*R,R,S,S*
17b	Z	OH	1	*R,R,S,R*
18a	Z	OH	0	*S,S,R,S*
18b	Z	NH_2	0	*S,S,R,R*
		NH_2		

[a] Reagents and conditions: (a) H-Xaa-OMe, BOP, Et_3N, CH_2Cl_2; (b) 2 N NaOH, MeOH; (c) NH_3, MeOH; (d) TFA, CH_2Cl_2; (e) ZCl, CH_2Cl_2, propylene oxide.

Table 1. Temperature Coefficients for Amide Protons

	$\Delta\delta/\Delta T$ (10^{-3} ppm/K)[a]			$\Delta\delta/\Delta T$ (10^{-3} ppm/K)[a]	
compd	2-NH	1′-NH	compd	2-NH	1′-NH
11a (**13b**)	−4.3	−2.9	**15b** (**16a**)	−5.3	−2.7
11b (**13a**)	−4.3	−2.8	**17a** (**1b**)	−5.0	−1.7
14a	−5.7	−1.9	**17b** (**1a**)	−5.3	−1.8
14b	−4.8	−1.8	**18a**	−5.3	−3.2
15a (**16b**)	−5.3	−2.8	**18b**	−5.2	−4.0

[a] Determined by least-squares linear regresion analysis from measurements over the temperature range 30−60 °C (seven points), DMSO-d_6.

solvent used, the spectra of all compounds exhibited no coupling between the downfield H-6 proton and the vicinal H-5 proton of the IBTM moiety (see Table 3, Experimental Section). This agrees with an axial disposition of the 5-carboxylate group, as required for the adoption of β-turn conformations in IBTM-containing analogues.[35,36]

It has been described that small absolute values for the temperature coefficient ($\Delta\delta/\Delta T \leq 3 \times 10^{-3}$ ppm/K) of amide protons indicate their protection from solvent exchange either by involvement in an intramolecular hydrogen bond or by inaccessibility to solvent, whereas values higher than 4×10^{-3} ppm/K indicate exposure to the solvent.[38] As can be seen in Table 1, the $\Delta\delta/\Delta T$ value for the 2-NH amide proton, in all the cases, was indicative of the accessibility of these proton to bulk solvent. Although these values for the 1′-NH proton of the Phe derivatives **11**, **13**, **15**, and **16** were higher (in absolute value) than those corresponding to this proton for the βHph analogues **14** and **17**, all of them are within the range expected for a hydrogen-bonded β-turn. However, the $\Delta\delta/\Delta T$ values for the 1′-NH amide protons of compounds **18a**,**b** were slightly higher than those required for conformational stabilization through an intramolecular hydrogen bond, suggesting that the presence of a C-terminal carboxamide function disrupts to some extent the β-turn-like conformation.

Table 2. Inhibition of the [^{3}H]pCCK-8-Specific Binding to Rat Pancreas (CCK_1) and Cerebral Cortex Membranes (CCK_2) by β-Turned Dipeptoids

				IC_{50} (nM)[a]	
compd	config at 2,5,11b,1′	*n*	R^2	CCK_1	CCK_2
11a	*S,S,R,S*	0	OCH_3	47.5 ± 11.2	>10000
11b	*S,S,R,R*	0	OCH_3	>1000	>10000
13a	*R,R,S,S*	0	OCH_3	450 ± 50	5633 ± 338
13b	*R,R,S,R*	0	OCH_3	>1000	>10000
14a	*R,R,S,S*	1	OCH_3	>1000	>10000
14b	*R,R,S,R*	1	OCH_3	>1000	4914 ± 57
15a	*S,S,R,S*	0	OH	4.7 ± 1.30	>10000
15b	*S,S,R,R*	0	OH	54.6 ± 4.8	>10000
16a	*R,R,S,S*	0	OH	1.73 ± 0.33	202.0 ± 99.0
16b	*R,R,S,R*	0	OH	>1000	3720 ± 458
17a	*R,R,S,S*	1	OH	97.8 ± 19.6	>10000
17b	*R,R,S,R*	1	OH	>1000	>10000
18a	*S,S,R,S*	0	NH_2	>1000	>10000
18b	*S,S,R,R*	0	NH_2	>1000	>10000
1a	*S,S,R,S*	1	OH	88.0 ± 26.2	>10000
1b	*S,S,R,R*	1	OH	7.4 ± 4.1	2700 ± 170
devazepide				0.71 ± 0.05	696 ± 134

[a] Values are the mean or mean± SEM of at least three experiments, performed with seven concentrations of test compounds in duplicate.

Biological Results and Discussion

The affinity of all the new dipeptoid analogues at CCK_1 and CCK_2 receptors was determined by measuring the displacement of [^{3}H]propionyl-CCK-8 binding to rat pancreatic and cerebral cortex homogenates, respectively, as previously described.[39] These data are depicted in Table 2. For comparative purposes, binding affinities of model compounds **1a**,**b**, and the well-known CCK_1 antagonist devazepide,[40] were also included in Table 2.

The results show that compounds with a free carboxylic acid at the C-terminus have better affinity for CCK_1 receptors than the corresponding methyl ester derivatives (**15a** vs **11a**, **15b** vs **11b**, **16a** vs **13a**, **17a** vs **14a**). These results are in agreement with previous findings regarding SAR of dipeptoids.[20] A more surprising result is the lack of binding affinity of the C-terminal carboxamide derivative **18a**, for which it could be expected to have binding values in the same order of magnitude than those found for the methyl ester analogue **11a**.[20] One possible explanation for this fact comes from the conformational study in solution, abovementioned, that seems to indicate that compound **18a** is not able to stabilize a β-turn conformation.

In the type II′ β-turned dipeptoids **1a**,**b** and **15a**,**b**, the interchange of βHph with Phe was well-tolerated at the CCK_1 receptor, but the influence of the configuration of these residues on the binding potency differed. Thus, while (*R*)-βHph derivative **1b** showed 1 order of magnitude higher affinity than its *S*-configured analogue **1a**, in the Phe analogues, this increase was shown

Table 3. Selected ^{1}H NMR (300 MHz) Chemical Shifts and Coupling Constants of β-Turned Dipeptoids

	δ (ppm)											
compd	H-1	H-2	2-NH	H-5	5-CONH	H-6	H-11b	H-1′	H-2′	R^1	R^2	$J_{5,6}$ (Hz)
11a (13b)[a]	2.15	3.87	6.16	5.21	7.60	2.85, 3.60	4.55	4.82	3.06, 3.20	5.07	3.61	7.3, 0.0
11a (13b)[b]	2.26	4.04	8.14	4.99	8.17	2.74, 3.31	4.60	4.41	2.94, 3.07	5.11	3.54	6.9, 0.0
11b (13a)[a]	2.03, 2.28	3.76	5.90	5.05	7.78	2.93, 3.68	5.35	4.71	2.93, 3.10	5.02	3.54	7.3, 0.0
11b (13a)[b]	2.34	4.04	8.19	4.97	8.21	2.78, 3.21	5.25	4.41	2.90, 3.06	5.07	3.51	7.3, 0.0
12a[a]	2.53	4.04	8.05	5.04	*d*	3.02, 3.61	5.39	4.65	2.83, 3.20	1.46	3.53	7.1, 0.0
14a[b]	2.33	3.99	8.26	4.88	7.71	2.75, 3.35	5.09	4.19	2.68	5.04	3.26	7.3, 0.0
14a[c]	2.19, 2.40	3.89	5.94	5.09	6.98	2.85, 3.68	5.09	4.39	2.40	4.95	3.14	7.7, 0.0
14b[b]	2.31	3.99	8.21	4.92	7.69	2.78, 3.35	4.85	4.24	2.78	5.13	3.48	7.1, 0.0
14b[c]	2.23, 2.36	3.93	6.08	5.15	7.42	2.91, 3.70	4.70	4.56	2.54	5.15	3.58	7.1, 0.0
15a (16b)[a]	2.31	3.95	7.72	4.99	*d*	2.70, 3.49	4.61	4.61	3.09	5.07		7.4, 0.0
15a (16b)[b]	2.25	4.03	8.10	4.95	8.00	2.71, 3.34	4.57	4.37	2.91, 3.10	5.11		7.2, 0.0
15b (16a)[a]	2.10	4.10	7.99	5.05	*d*	2.84, 3.61	5.46	4.60	3.14	5.14		7.5, 0.0
15b (16a)[b]	2.34	4.04	8.19	4.95	8.12	2.77, 3.20	5.29	4.33	2.88, 3.08	5.07		7.0, 0.0
17a[b]	2.34	4.02	8.31	4.90	7.86	2.72, 3.32	5.15	4.19	2.72	5.04		7.2, 0.0
17a[c]	2.45	4.06	5.61	4.90	*d*	2.85, 3.65	5.27	4.42	2.51	5.04		7.3, 0.0
17b[b]	2.29	3.97	8.20	4.91	7.66	2.72, 3.35	4.83	4.23	2.72	5.10		7.3, 0.0
17b[c]	2.45	4.06	6.70	5.03	*d*	2.90, 3.65	4.92	4.48	2.54	5.21		7.3, 0.0
18a[b]	2.23	4.03	8.11	5.02	7.85	2.70, 3.35	4.41	4.41	2.85, 3.07	5.15	*d*	7.0, 0.0
18a[c]	2.10, 2.26	4.09	6.54	5.20	7.56	2.85, 3.66	3.86	4.74	3.21	5.18	6.14, 6.62	6.5, 0.0
18b[b]	2.35	4.04	8.14	4.98	7.98	2.78, 3.11	5.36	4.36	2.78, 2.98	5.07	d	7.2, 0.0
18b[c]	1.72, 1.98	3.66	5.83	5.12	7.99	2.91, 3.53	4.89	4.34	2.91	4.83	5.58, 5.81	6.0, 0.0

[a] Registered in $(CD_3)_2CO$. [b] Registered in DMSO-d_6. [c] Registered in $CDCl_3$. [d] Within Ar protons.

by compound **15a**, having the 1′*S* configuration, with respect to the 1′*R* isomer **15b**. The importance of the 1′*S* configuration of the Phe moiety in these type-II′ β-turned dipeptoids for efficient interaction with CCK$_1$ receptors is also evidenced from comparing the binding affinity of methyl ester **11a** to that of **11b**. It is worth noting the high affinity and selectivity of compound **15a** for CCK$_1$ receptors. Thus, this Phe derivative displayed nanomolar CCK$_1$ receptor affinity similar to that of the best homologue **1b**, but on the contrary to this homologue, compound **15a** was unable to bind to CCK$_2$ receptors at 10^{-5} M concentrations. This result, supporting previous findings in which homologation of the phenylalanine carboxylic acid group led to a remarkable increase in CCK$_2$ affinity but not to an appreciable change in CCK$_1$ receptor binding,[20] suggests that the through-bond distance of the carboxylic acid group from the phenethyl backbone is important for the interaction of **1b** with CCK$_2$ receptors.

With regard to the type of β-turn, some interesting conclusions may be drawn from the binding affinities of dipeptoids **13**, **14**, **16**, and **17**, incorporating the type II mimetic (2*R*,5*R*,11b*S*)-IBTM. In general, the best interaction with CCK$_1$ receptors was found for those derivatives having an *S*-configured Phe or βHph residue, but none of the analogues with the *R* configuration were able to recognize this receptor subtype. While in the β II′-turned dipeptoids the Phe derivative **15a** and its βHph analogue **1b** bind to CCK$_1$ receptor with nanomolar affinity, in the type II β-turned series the βHph derivative **17a** was unable to reach the nanomolar affinity showed by the corresponding Phe analogue **16a**. Moreover, compound **16a** showed about a 20-fold decrease in CCK$_2$/CCK$_1$ selectivity when compared to the type II′-constrained dipeptoid analogue **15a**. In connection with this, many of the type II-restricted dipeptoids (**13a**, **14b**, and **16b**) were able to bind to the CCK$_2$ receptors in the micromolar range. This tendency to bind to CCK$_2$ receptors was found to be independent of the ability of these derivatives to recognize the CCK$_1$ receptors. These facts indicate that, while both the (2*S*,5*S*,11b*R*)- and (2*R*,5*R*,11b*S*)-IBTM skeletons are able to facilitate the correct orientation of the pharmacophoric side chains to interact with the CCK$_1$ receptor, the conformational constraint imposed by the type II′ β-turn mimetic is not tolerated at all by the CCK$_2$ receptor binding site. This clear difference in conformational requirements for the interaction with CCK$_1$ and CCK$_2$ receptors could be helpful to design constrained ligands with high selectivity for the CCK$_1$ receptors.

The best compounds in this series, **15a** and **16a**, were tested for their ability to inhibit the CCK-8-stimulated amylase release from pancreatic acinar cells.[41] In accordance with the binding results, these compounds were able to antagonize the amylase release with IC$_{50}$ values of 6.0 (2.6−13.7) and 1.2 (0.6−2.2) nM for **15a** and **16a**, respectively. These compounds did not show any intrinsic effect on the amylase release at a 1 μM concentration; therefore, they behave as CCK$_1$ antagonists. The antagonist potency of compounds **15a** and **16a** was comparable to that found for the model CCK$_1$ antagonist devazepide in the same assay [IC$_{50}$ = 2.5 (2.1−2.7) nM)].

In conclusion, we have prepared compounds **15a** and **16a**, two highly constrained dipeptoid analogues containing the (2*S*,5*S*,11b*R*)- and (2*R*,5*R*,11b*S*)-IBTM frameworks, which are endowed with high binding affinity for CCK$_1$ receptors. As these frameworks are probed type II′ and type II β-turn mimetics, respectively, we propose that the presence of a β-turn-like conformation within the backbone of dipeptoids could contribute to their bioactive conformation at the CCK$_1$ receptor subtype. Moreover, we have shown that the type of β-turn is critical for maintaining good selectivity for the CCK$_1$ receptor. Thus, restricted dipeptoid analogue **15a**, containing the type II′ IBTM mimetic, is apparently devoid of affinity for CCK$_2$ receptors and is therefore a new potent and selective CCK$_1$ receptor antagonist.

Experimental Section

Chemistry. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were obtained from Bachem 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. ^{1}H NMR spectra were recorded with a Varian XL-300, operating at 300 MHz, using TMS as reference. Temperature coefficients were obtained from least-squares fits to data of 30, 35, 40, 45, 50, 55, and 60 °C in DMSO-d_6. ^{13}C NMR spectra were recorded with a Varian Gemini-200 operating at 50 MHz. Elemental analyses were obtained on a CH-O-RAPID apparatus. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Analytical HPLC was performed on a Waters Nova-pak C_{18} column (3.9 × 150 mm, 4 μm) with a flow rate of 1 mL/min, and using a tuneable 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. Compounds **2** and **10** were prepared as described.[35] H-L-βHph-OMe and H-D-βHph-OMe were synthesized from Z-L- and Z-D-Phe-OH, respectively, following a described procedure.[42]

Boc-D-Asp(H)-OBzl (3). To a solution of Boc-D-Asp-OBzl (12 g, 33.6 mmol) in dry THF (25 mL), at −15 °C, were added NMM (3.7 mL, 33.6 mmol) and isobutyl chloroformate (4.35 mL, 33.6 mmol) and stirred 10 min at that temperature. The salts were filtered and washed with THF (2 × 120 mL). To the resulting organic solution, cooled to −10 °C, was added a solution of $NaBH_4$ (1.88 g, 50.4 mmol) in H_2O (17 mL) and stirring continued until evolution of H_2 ceased. Then, H_2O (600 mL) and EtOAc (800 mL) were added and the phases separated. The organic layer was successively washed with 10% citric acid, 10% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified on a silica gel column using 20% EtOAc in hexane, to give 8.63 g (83%) of benzyl (2*R*)-(*tert*-butoxycarbonyl)amino-4-hydroxybutanoate. This compound (8.63 g, 27.9 mmol) in CH_2Cl_2 (30 mL) was slowly added to a solution previously prepared as follows: Oxalyl chloride (2.64 mL, 30.7 mmol) in CH_2Cl_2 (15 mL) was treated at −60 °C with DMSO (4.75 mL, 67 mmol) in CH_2Cl_2 (15 mL) and stirred 10 min at that temperature. The reaction mixture was stirred at −60 °C for 30 min, treated with TEA (19.8 mL, 143 mmol) and then allowed to warm to room temperature. H_2O (60 mL) was added and the phases were separated. The organic layer was dried over Na_2SO_4 and evaporated leaving a residue which was purified on a silica gel column using 15% EtOAc in hexane as eluent. The title compound (6.8 g, 84%) was obtained as a syrup. HPLC: t_R = 10.53 min (A:B = 47:35). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.71 (s, 1H, CHO), 7.35 (m, 5H, Ph), 5.42 (d, 1H, α-NH, J = 8.1), 5.17 (m, 2H, OCH_2), 4.70 (m, 1H, α-H), 3.06 (m, 2H, β-H), 1.42 [s, 9H, CH_3 (Boc)]. Anal. ($C_{16}H_{21}NO_5$) C, H, N.

(1*S*,3*R*,2′*R*)-2-Benzyl-1-[2′-benzyloxycarbonyl-2′-(*tert*-butoxycarbonyl)aminoethyl]-3-methoxycarbonyl-1,2,3,4-tetrahydro-*β*-carboline (4). To a solution of aldehyde **3** (3.42 g, 11.09 mmol) and Bzl-D-Trp-OMe (**2**; 3.42 g, 11.09 mmol) in toluene (34 mL) was added TFA (0.85 mL, 11.09 mmol) at room temperature. After stirring overnight at that temperature, the mixture was refluxed for 1 h. The reaction mixture was allowed to warm to room temperature, neutralized with 10% $NaHCO_3$ and evaporated to dryness. The resulting residue was extracted with EtOAc, washed with H_2O and the organic phase was separated, dried over Na_2SO_4 and evaporated. The residue was purified on a silica gel column, using 15% of EtOAc in hexane as eluent, to give 4.54 g (68%) of the title compound as a syrup. HPLC: t_R = 7.19 min (A:B = 70:30). $[\alpha]_D$ −23.0° (c 0.4 in $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.35 (s, 1H, NH^i), 7.58−7.08 [m, 14H, In and C_6H_5 (Bzl)], 5.24 (d, 1H, 2′-NH, J = 7.3), 5.10 [m, 1H, CH_2 (Bzl)], 4.41 (m, 1H, H-2′), 4.10 (dd, 1H, H-3, J = 10.1, 5.9), 3.82 (m, 5H, CO_2CH_3, 2-CH_2, H-1), 3.44 (d, 1H, 2-CH_2, J = 13.5), 3.08 (m, 2H, H-4), 2.19 (m, 2H, H-1′), 1.35 [s, 9H, CH_3 (Boc)]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.06, 171.71, 156.12 (CO), 138.89−106.85 (20C, Ar), 80.32 [C (Boc)], 67.37 [CH_2 (Bzl)], 57.12 (OCH_3), 52.93 (2-CH_2), 52.75 (C-2′), 52.69 (C-3), 52.25 (C-1), 38.64 (C-1′), 28.17 [CH_3 (Boc)], 20.19 (C-4). Anal. ($C_{35}H_{39}N_3O_6$) C, H, N.

(1*S*,3*R*,2′*R*)-1-[2′-(*tert*-Butoxycarbonyl)amino-2′-carboxyethyl]-3-methoxycarbonyl-1,2,3,4-tertahydro-*β*-carboline (5). Compound **4** (3 g, 5.02 mmol) was dissolved in MeOH (100 mL) and hydrogenated at room temperature and 30 psi of pressure for 2h in the presence of 10% Pd−C (0.6 g). After filtration of the catalyst the solvent was evaporated to provide 2.05 g (98%) of the title compound as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.17 (s, 1H, NH^i), 7.49−7.02 (m, 5H, In and 2′-NH), 4.80 (m, 1H, H-2′), 3.79 (s, 3H, CO_2CH_3), 3.60 (m, 2H, H-1 and H-3), 3.33 (dd, 1H, H-4, J = 15.2, 5.1), 3.04 (dd, 1H, H-4, J = 15.2, 9.5), 2.39 (m, 2H, H-1′), 1.40 [s, 9H, CH_3 (Boc)]. Anal. ($C_{21}H_{27}N_3O_6$) C, H, N.

(2*R*,5*R*,11b*S*)-2-(*tert*-Butoxycarbonyl)amino-5-methoxycarbonyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole (6). A solution of tetrahydro-β-carboline **5** (1.5 g, 3.58 mmol) in xylene (50 mL) was refluxed for 2 h. After evaporation of the solvent the resulting residue was purified on a silica gel column using 30% of EtOAc in hexane as eluent, to provide 1.34 g (93%) of the title compound as a white solid. mp 118−120 °C (EtOAc/hexane). HPLC: t_R = 34.76 min (A:B = 28:72). $[\alpha]_D$ −73.0° (c 4 in $CHCl_3$). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.08 (s, 1H, NH^i), 7.56 (d, 1H, 2-NH, J = 8.4), 7.43−6.93 (m, 4H, In), 5.18 (m, 2H, H-5 and H-11b), 4.08 (m, 1H, H-2), 3.62 (s, 3H, CO_2CH_3), 3.21 (d, 1H, H-6, J = 15.8), 3.00 (ddd, 1H, H-6, J = 15.8, 7.1, 2.0), 2.34 (m, 2H, H-1), 1.40 [s, 9H, CH_3 (Boc)]. ^{13}C NMR (50 MHz, DMSO-d_6): δ 171.80, 170.75, 155.17 (CO), 136.11, 133.03, 126.25, 121.33, 118.79, 117.94, 111.26, 103.99 (Ar), 78.27 [C (Boc)], 52.42 (OCH_3), 50.91 (C-2), 49.92 (C-5), 49.60 (C-11b), 31.73 (C-1), 28.20 [CH_3 (Boc)], 22.85 (C-6). Anal. ($C_{21}H_{25}N_3O_5$) C, H, N.

(2*R*,5*R*,11b*S*)-2-(Benzyloxycarbonyl)amino-5-methoxycarbonyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole (7). A solution of compound **6** (1.33 g, 3.32 mmol) in CH_2Cl_2 (4 mL) was treated at room temperature with TFA (2 mL, 26 mmol). After stirring at that temperature for 1h the reaction mixture was evaporated to dryness. The resulting residue was dissolved in CH_2Cl_2 (10 mL), cooled to 0 °C, and treated successively with TEA (1.4 mL, 6.64 mmol) and benzyl chloroformate (0.95 mL, 6.64 mmol). After stirring overnight at room temperature the solvent was evaporated, the residue was extracted with EtOAc and washed with H_2O and brine. The organic extract was separated, dried over Na_2SO_4 and evaporated. The resulting residue was purified on a silica gel column using 30% of EtOAc in hexane as eluent, to provide 0.63 g (52%) of the title compound as a white solid. Mp: 109−111 °C (EtOAc). HPLC: t_R = 10.49 min (A:B = 40:60). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.11 (s, 1H, NH^i), 8.05 (d, 1H, 2-NH, J = 8.4), 7.42−6.95 [m, 9H, In and C_6H_5 (Z)], 5.21 (m, 2H, H-5 and H-11b), 5.05 [s, 2H, CH_2 (Z)], 4.16 (m, 1H, H-2), 3.61 (s, 3H, CO_2CH_3), 3.23 (d, 1H, H-6, J = 15.6), 2.97 (ddd, 1H, H-6, J = 15.6, 7.1, 2.0), 2.38 (m, 2H, H-1). ^{13}C NMR (50 MHz, DMSO-d_6): δ 171.38, 170.75, 156.20 (CO), 136.26−105.79 (14 C, Ar), 67.22 [CH_2 (Z)], 52.42 (OCH_3), 51.57 (C-2), 50.11 (C-5), 49.91 (C-11b), 31.80 (C-1), 23.05 (C-6). Anal. ($C_{24}H_{23}N_3O_5$) C, H, N.

(2*R*,5*R*,11b*S*)-2-(Benzyloxycarbonyl)amino-5-carboxy-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (8). A solution of compound **6** (0.6 g, 1.38 mmol) in MeOH (15 mL) was treated with 2 N NaOH (1.05 mL, 2.1 mmol) and the mixture was stirred at room temperature for 18 h. After evaporation of the MeOH the remaining aqueous mixture was diluted with H_2O (10 mL), acidified with 1 N HCl to pH 3, and extracted with EtOAc. The extract was dried (Na_2SO_4) and evaporated. The residue was purified on a silica gel column using 15% of MeOH in CH_2Cl_2 to provide 0.55 g (96%) of the title compound as a white solid. Mp: 135−137 °C. HPLC: t_R = 16.23 min (A:B = 30:70). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.08 (s, 1H, NH^i), 8.03 (d, 1H, 2-NH, J = 8.4), 7.41−6.92 [m, 9H, In and C_6H_5 (Z)], 5.25 (m, 1H, H-11b), 5.08 (d, 1H, H-5, J = 7.2), 5.04 [s, 2H, CH_2 (Z)], 4.15 (m, 1H, H-2), 3.23 (d, 1H, H-6, J = 15.8), 2.92 (ddd, 1H, H-6, J = 15.8, 7.2, 1.6), 2.37 (m, 2H, H-1). ^{13}C NMR (50 MHz, DMSO-d_6): δ 172.12, 171.64, 156.02 (CO), 137.05−104.38 (14 C, Ar), 65.75 [CH_2 (Z)],

51.60 (C-2), 50.16 (C-5), 49.89 (C-11b), 31.76 (C-1), 23.29 (C-6). Anal. ($C_{23}H_{21}N_3O_5$) C, H, N.

(2*R*,5*R*,11b*S*)-2-(*tert*-Butoxycarbonyl)amino-5-carboxy-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (9). This compound 0.62 g (93%) was obtained from **6** (0.67 g, 1.7 mmol) following the above-described procedure for the preparation of compound **8**. White solid. Mp: 141−144 °C (EtOAc). HPLC: t_R = 10.39 min (A:B = 30:70). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.97 (s, 1H, NH^i), 7.40−6.93 (m, 5H, 2-NH and In), 5.34 (m, 1H, H-11b), 4.80 (d, 1H, H-5, J = 7.4), 4.03 (m, 1H, H-2), 3.30 (d, 1H, H-6, J = 15.2), 2.82 (dd, 1H, H-6, J = 15.2, 7.4), 2.32 (m, 2H, H-1), 1.41 [s, 9H, CH_3 (Boc)]. ^{13}C NMR (50 MHz, DMSO-d_6): δ 172.56, 172.38, 155.75 (CO), 136.66, 133.73, 126.87, 121.83, 119.34, 118.42, 111.80, 104.85 (Ar), 78.83 [C (Boc)], 51.60 (C-2), 50.64 (C-5), 50.18 (C-11b), 32.22 (C-1), 28.73 [CH_3 (Boc)], 23.66 (C-6). Anal. ($C_{20}H_{23}N_3O_5$) C, H, N.

General Procedure for Coupling of 5-Carboxyhexahydroindolizino[8,7-*b*]indoles with Phe Derivatives. A solution of the 5-carboxyhexahydroindolizino[8,7-*b*]indole **8**, **9** or **10** (0.54 mmol) and the corresponding Phe or βHph derivative (0.54 mmol) in dry CH_2Cl_2 (14 mL) was successively treated with BOP (0.24 g, 0.54 mmol) and TEA (0.15 mL, 1.08 mmol) at room temperature. After stirring overnight the solvent was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10%), $NaHCO_3$ (10%)and brine. The organic layer was dried (Na_2SO_4) and evaporated leaving a residue which was purified on a silica gel column as specified in each case.

(2*S*,5*S*,11b*R*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-(1′-methoxycarbonyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,-11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (11a). Yield: 73% (from **10** and H-L-Phe-OMe). Eluent: 40% of EtOAc in hexane. White foam. $[\alpha]_D$ −2.5° (*c* 0.01 in MeOH). HPLC: t_R = 18.73 min (A:B = 40:60). Anal. ($C_{33}H_{32}N_4O_6$) C, H, N.

(2*S*,5*S*,11b*R*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-(1′-methoxycarbonyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,-11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (11b). Yield: 63% (from **10** and H-D-Phe-OMe). Eluent: 40% of EtOAc in hexane. White foam. HPLC: t_R = 21.80 min (A:B = 40:60). Anal. ($C_{33}H_{32}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-(*tert*-Butoxycarbonyl)amino-5-(1′-methoxycarbonyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,-11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (12a). Yield: 84% (from **9** and H-L-Phe-OMe). Eluent: 50% of EtOAc in hexane. White foam. HPLC: t_R = 13.20 min (A:B = 40:60). Anal. ($C_{30}H_{34}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-(1′-methoxycarbonyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,-11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (13b). Yield: 94% (from **8** and H-D-Phe-OMe). Eluent: 40% of EtOAc in hexane. White foam. $[\alpha]_D$ 2.4° (*c* 0.01 in MeOH). HPLC: t_R = 18.73 min (A:B = 40:60). Anal. ($C_{33}H_{32}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-[1′-benzyl-2′-(methoxycarbonyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (14a). Yield: 65% (from **8** and H-L-βHph-OMe). Eluent: 40% of EtOAc in hexane. White foam. HPLC: t_R = 9.93 min (A:B = 45:55). Anal. ($C_{34}H_{34}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-[1′-benzyl-2′-(methoxycarbonyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (14b). Yield: 87% (from **8** and H-D-βHph-OMe). Eluent: 60% of EtOAc in hexane. White foam. HPLC: t_R = 15.95 min (A:B = 40:60). Anal. ($C_{34}H_{34}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-(1′-methoxycarbonyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,-11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (13a). A solution of compound **12a** (0.29 g, 0.54 mmol) in CH_2Cl_2 (1 mL) was treated with TFA (0.33 mL, 4.3 mmol) and stirred at room temperature for 1 h. After evaporation to dryness, the residue was dissolved in CH_2Cl_2 (5 mL), cooled to 0 °C, and treated successively with TEA (0.19 mL, 1.35 mmol) and benzyl chloroformate (0.12 mL, 0.81 mmol). After stirring overnight at room temperature the solvent was evaporated, the residue was extracted with EtOAc and washed with H_2O and brine. The organic extract was separated, dried over Na_2SO_4 and evaporated. The resulting residue was purified on a silica gel column using 60% of EtOAc in hexane as eluent, to provide 0.19 g (62%) of the title compound as a foam. HPLC: t_R = 21.82 min (A:B = 40:60). Anal. ($C_{33}H_{32}N_4O_6$) C, H, N.

General Procedure for Removal of the C-Terminal Protecting Group. A solution of the corresponding 1′-methoxycarbonyl derivative (0.17 mmol) in MeOH (3 mL) was treated with 2 N NaOH (0.13 mL, 0.25 mmol) and the mixture was stirred at room temperature for 5−18 h. After evaporation of the MeOH the remaining aqueous mixture was diluted with H_2O (5 mL), acidified with 1 N HCl to pH 3, and extracted with EtOAc. The extract was dried (Na_2SO_4) and evaporated. The residue was purified on a silica gel column using a 15% of MeOH in CH_2Cl_2.

(2*S*,5*S*,11b*R*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-(1′-carboxy-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (15a). Yield: 83% (from **11a**). White foam. $[\alpha]_D$ 20.9° (*c* 0.01 in MeOH). HPLC: t_R = 7.73 min (A:B = 40:60). Anal. ($C_{32}H_{30}N_4O_6$) C, H, N.

(2*S*,5*S*,11b*R*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-(1′-carboxy-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (15b). Yield: 75% (from **11b**). White foam. HPLC: t_R = 8.73 min (A:B = 40:60). Anal. ($C_{32}H_{30}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-(1′-carboxy-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (16a). Yield: 93% (from **13a**). White foam. HPLC: t_R = 8.70 min (A:B = 40:60). Anal. ($C_{32}H_{30}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-(1′-carboxy-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (16b). Yield: 87% (from **13b**). White foam. $[\alpha]_D$ −20.1° (*c* 0.01 in MeOH). HPLC: t_R = 7.71 min (A:B = 40:60). Anal. ($C_{32}H_{30}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-(1′-benzyl-2′-carboxyethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (17a). Yield: 60% (from **14a**). White solid. Mp: 210−212 °C. HPLC: t_R = 9.73 min (A:B = 40:60). Anal. ($C_{33}H_{32}N_4O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-(1′-benzyl-2′-carboxyethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (17b). Yield: 40% (from **14b**). White foam. HPLC: t_R = 8.95 min (A:B = 40:60). Anal. ($C_{33}H_{32}N_4O_6$) C, H, N.

General Procedure for Preparation of C-Terminal Carbamoyl Derivatives. A solution of the corresponding 1′-methoxycarbonyl derivative (0.1 g, 0.17 mmol) in saturated NH_3 in MeOH was stirred overnight at room temperature. After evaporation to dryness, the resulting residue was purified on a silica gel column using a 3% MeOH in CH_2Cl_2.

(2*S*,5*S*,11b*R*,1′*S*)-2-(Benzyloxycarbonyl)amino-5-(1′-carbamoyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (18a). Yield: 89% (from **11a**). White foam. HPLC: t_R = 5.27 min (A:B = 40:60). Anal. ($C_{32}H_{31}N_5O_5$) C, H, N.

(2*S*,5*S*,11b*R*,1′*R*)-2-(Benzyloxycarbonyl)amino-5-(1′-carbamoyl-2′-phenylethyl)carbamoyl-3-oxo-2,3,5,6,11,-11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (18b). Yield: 92% (from **11b**). White solid. Mp: 139−141 °C. HPLC: t_R = 6.20 min (A:B = 40:60). Anal. ($C_{32}H_{31}N_5O_5$) C, H, N.

Binding Assays. CCK$_1$ and CCK$_2$ receptor binding assays were performed using rat pancreas and cerebral cortex homogenates, respectively, according to the method previously described.[39] Briefly, rat pancreas tissue was carefully cleaned and homogenized in Pipes HCl buffer, pH 6.5, containing 30 mM $MgCl_2$ (15 mL/g of wet tissue) and the homogenate was then centrifuged twice at 4 °C for 10 min at 50000*g*. For displacement assays, pancreatic membranes (0.2 mg protein/tube) were incubated with 0.5 nM [^{3}H]pCCK-8 in Pipes HCl buffer, pH 6.5, containing $MgCl_2$ (30 mM), bacitracin (0.2 mg/mL) and soybean trypsin inhibitor (SBTI, 0.2 mg/mL), for 120

min at 25 °C. Rat brain cortex was homogenized in 50 mM Tris-HCl buffer pH 7.4 containing 5 mM $MgCl_2$ (20 mL/g of wet tissue) and the homogenate was centrifuged twice at 4 °C for 35 min at 100000*g*. Brain membranes (0.45 mg protein/tube) were incubated with 1nM [^{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. Non specific binding was determined using CCK-8 (1 μM) as the cold displacer. The inhibition constants (K_i) were calculated using the equation of Cheng−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.[41] The rat was decapitated and the pancreas was carefully cleaned. Tissue was injected three times with 5 mL of a solution of collagenase (Worthinton) at a concentration of 70 U/mL (in mix buffer) and subjected to the digestion step consisting in three 10-min incubations at 37 °C in atmosphere of pure O_2 and agitation (200 cycles/min). The tissue was washed two times tin mix buffer (composition: 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-glutamic acid 5 mM, fumaric acid 5 mM, piruvic acid 5 mM, SBTI 0.01%, essential amino acid mixture 1%, essential vitamin mixture 1%, pH = 7.4) after each incubation. The tissue obtained after the last incubation was transferred to stop buffer (4% BSA) and shook vigorously for 10 min. The homogenate was centrifuged twice (100*g*, 1 min, 4 °C); the pellet obtained in the first centrifugation was resuspended in wash buffer (0.2% BSA) and the final pellet was resuspended in incubation buffer (1% BSA). Cells were allowed to rest for 30 min and then they were centrifuged (100*g*, 1 min, 4 °C) and resuspended again in incubation buffer.

Amylase release was measured using the procedure of Peikin et al.[43] Samples (2 mL) of the 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 by drugs of amylase release elicited by a fixed CCK-8 concentration (0.1 nM) 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 in the presence of increasing drug concentrations. IC_{50} values were calculated for the drug tested.

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