Conformationally Constrained CCK4 Analogues Incorporating IBTM and BTD β-Turn Mimetics

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To test whether a turnlike arrangement is involved in the bioactive conformation of CCK4 analogues upon CCK_1 receptor recognition, we describe the preparation of two series of CCK4 derivatives, in which the central dipeptide Met-Asp has been replaced by recognized β-turn mimetics {(2*S*,5*S*,11b*R*)- and (2*R*,5*R*,11b*S*)-2-amino-5-carboxy-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (IBTM) and β-turn dipeptide, 2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (BTD)}. The incorporation of the indolizinoindole IBTM type II β-turn mimetic is preferred over its type II′ counterpart for efficient CCK_1 receptor recognition, while BTD derivatives were completely inactive. The structure−conformation−activity relationship study in the IBTM series has shown some essential requirement of these CCK4 derivatives to favorably interact with CCK_1 receptors: (a) the adoption of turnlike conformations, (b) the presence of an L-Phe residue and a *C*-terminal carboxamide moiety, and (c) the indole ring of the IBTM skeleton. Moreover, the existence of π−π interactions between the phenyl ring of D-Phe residues and the indole ring of IBTM framework is detrimental for binding affinity. A series of potent and selective CCK_1 receptor antagonists, exemplified by compounds **8a** and **8b**, emerges among these IBTM-containing derivatives.

Introduction

Cholecystokinin (CCK) is a peptide hormone found in the periphery that plays a major role in gut function, in the digestive processes, and in the control of feeding behavior.[1,2] CCK also occurs in brain, where it acts as a neurotransmitter or neuromodulator.[1,3−7] At least two different receptors for CCK have been characterized, the CCK_1 and the CCK_2 receptors, both belonging to the class of G protein-coupled 7 TM receptors.[8,9] CCK_1 binding sites predominate in the periphery but are also found in some discrete regions of the brain, whereas CCK_2 receptors are mainly located in the central nervous system (CNS). While there are different biologically active forms of CCK, the sulfated C-terminal octapeptide (CCK8) and the C-terminal tetrapeptide (CCK4) are the minimal sequences required for a complete biological activity at CCK_1 and CCK_2 receptors, respectively. Taking into account that CCK is involved in many different and important biological processes, the therapeutic potential of the CCK receptor ligands seems to be promising. This fact drove an intensive research in this area, where several potent and selective nonpeptide CCK_1 and CCK_2 receptor agonists and antagonists have been reported.[10−13]

The *C*-terminal tetrapeptide CCK4 has previously been used as the chemical starting point for the rational design of novel ligands for CCK receptors. Modifications of this fragment showed that the *N*-terminal protection of the tetrapeptide, as in Boc-CCK4, increased CCK_2 selectivity and enzymatic stability.[14] Previous reports also pointed out the importance of Trp and Phe side chains for high CCK_2 binding affinity.[14−16] The first evidence indicating that the bioactive conformation of CCK4 is nonextended came from the work of Horwell et al., who have demonstrated that the dipeptide Boc-Trp-Phe-NH_2 was able to retain micromolar binding affinity at central CCK_2 receptors.[17] Subsequent modifications of this dipeptide derivative led to an extensive series of α-MeTrp-containing dipeptoids with nanomolar affinity at both CCK_1 and CCK_2 receptors.[18] In particular, the replacement of the α-MeTrp residue of dipeptoids by (2*S*,5*S*,11b*R*)- and (2*R*,5*R*,11b*S*)-2-amino-5-carboxy-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (IBTM) skeletons,[19,20] type II′ and II β-turn mimetics, respectively, led to derivatives **1** and **2** (Figure 1) that bound CCK_1 receptors with greater selectivity than the parent dipeptoids.[21,22] We have also introduced the IBTM scaffold in a series of dipeptoids that have been shown to behave as CCK_1 receptor agonists. As expected, the incorporation of this conformational constraint increased the selectivity for CCK_1 receptors, but it was found that the modification prevented receptor activation and the new restricted dipeptoids behaved as antagonists.[23]

Following our interest in CCK ligands, and the promising results of the IBTM skeleton in restricting the conformation of the series of dipeptoids, we decide to explore a different approximation in which the IBTM framework is used as a replacement of the Met-Asp residue of the tetrapeptide CCK4, to restrict the conformational freedom of its backbone (Figure 1). The conformational restraint of the *C*-terminal tetrapeptide has previously been explored by incorporation of im-

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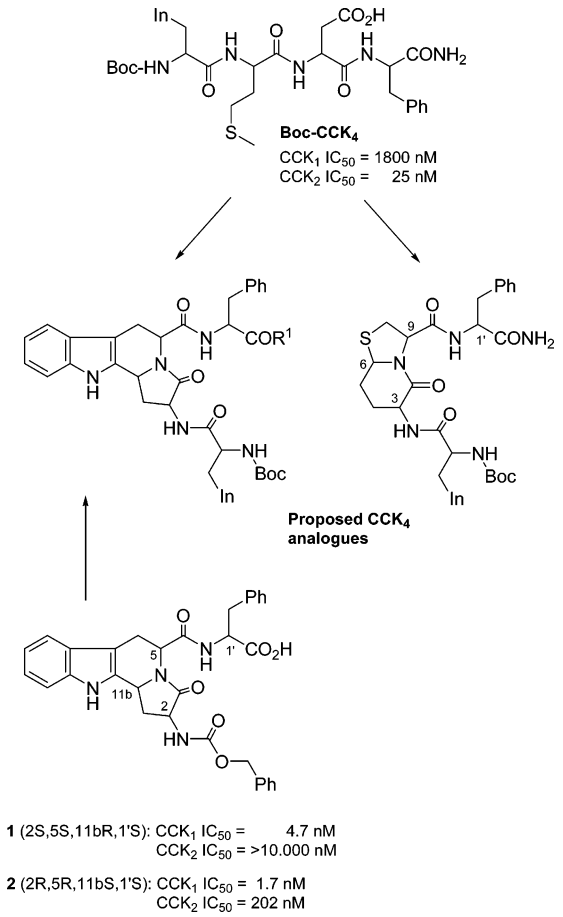
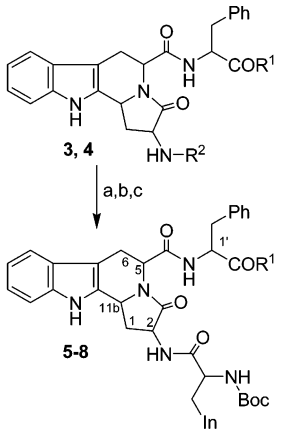


Figure 1. Proposed conformationally restricted CCK4 analogues.

portant amino acid side chains of CCK4 into rigid structures, some of them designed on the basis of previous conformational studies.[24−29] Therefore, the knowledge of the three-dimensional conformation of CCK fragments could be an important aid for the design of nonpeptide CCK receptor ligands. NMR and molecular modeling studies on CCK8 and CCK4 showed that, despite its intrinsic flexibility, they exist preferentially in folded forms, although a certain controversy persists. It has been shown that the tetrapeptide CCK4 adopts a Z-shaped conformation with a relatively well-defined orientation of side chains, in which the hydrophobic groups (Trp, Met, Phe) clustered together on one side of the peptide backbone.[30] However, an alternative study placed the Met and Asp side chains on the same side of a similar Z-bend arrangement.[16] On the other hand, conformational studies on the octapeptide CCK8 and analogues evidenced the presence of differently positioned γ- and β-turns.[31,32] In particular, an NMR conformational study on [Nle^{31}]CCK7, which shows nanomolar affinity at CCK_1 and CCK_2 receptors, suggested a β-turn centered on the Nle-Asp dipeptide fragment.[33]

On the basis of all the above literature precedents, it is plausible that a turnlike conformation within the peptide backbone of the CCK4 would be favorable for CCK_1 receptor recognition, as has previously been demonstrated for the dipeptoid derivatives. Thus, to test whether a turnlike arrangement is adopted at the CCK_1 receptor site, we describe here the preparation, NMR, and molecular modeling conformational analysis, as well as the binding affinity, of a series of conformationally restricted CCK4 analogues in which the Met-Asp dipeptide fragment has been replaced by different β-turn mimetics, namely the IBTM and BTD (β-turn dipeptide, 2-oxo-7-thio-1-azabicyclo[4.3.0]nonane)[34−36] scaffolds.

Scheme 1[a]



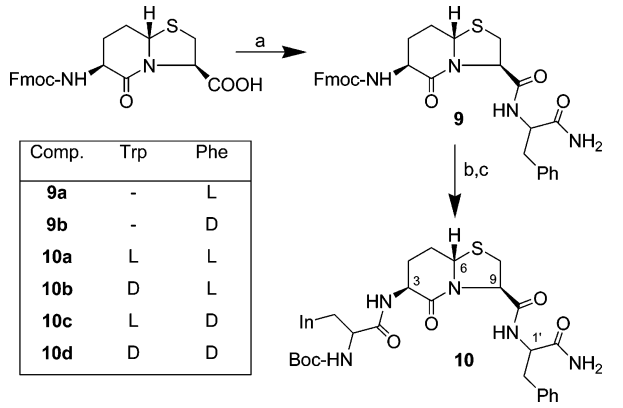
Comp.	Config. 2,5,11b	Trp	Phe	R^1	R^2
3a	SSR	-	L	OMe	Z
3b	SSR	-	D	NH_2	Z
4a	RRS	-	L	OMe	Boc
4b	RRS	-	D	NH_2	Boc
5a	SSR	L	L	OMe	-
5b	SSR	D	L	OMe	-
6a	SSR	L	L	NH_2	-
6b	SSR	D	L	NH_2	-
6c	SSR	L	D	NH_2	-
6d	SSR	D	D	NH_2	-
7a	RRS	L	L	OMe	-
7b	RRS	D	L	OMe	-
8a	RRS	L	L	NH_2	-
8b	RRS	D	L	NH_2	-
8c	RRS	L	D	NH_2	-
8d	RRS	D	D	NH_2	-

[a] Reagents and conditions: (a) H_2/Pd−C, MeOH or TFA, CH_2Cl_2; (b) Boc-Trp-OH, BOP, Et_3N, CH_2Cl_2; (c) NH_3,MeOH.

Results

1. Chemistry. The preparation of the IBTM-containing analogues **3** and **4** was performed by coupling of Z- and Boc-IBTM-OH derivatives with H-Phe-OMe or H-Phe-NH_2, following the previously described synthetic route.[21,22] As indicated in Scheme 1, final CCK4 constrained analogues **5**−**8** were prepared by condensation of derivatives **3** and **4** with Boc-L- and Boc-D-Trp-R^1, prior to elimination of the Z- and Boc protecting groups of the IBTM moiety by catalytic hydrogenation or by treatment with TFA, respectively. The *C*-terminal carbamoyl derivatives were obtained either by direct coupling of Phe-NH_2 residues to the IBTM skeletons or by ammonolysis of the corresponding methyl ester analogues. Standard BOP-mediated peptide couplings were used in all cases. In general, the formation of the amide bonds proceeded in good yields, except for the synthesis of compounds **7b** and **8d**, which incorporate the Boc-D-Trp-OH residue at the 2-amino group of the

Scheme 2



Comp.	Trp	Phe
9a	-	L
9b	-	D
10a	L	L
10b	D	L
10c	L	D
10d	D	D

[a] Reagents and conditions: (a) H-Phe-NH_2, BOP, Et_3N, CH_2Cl_2; (b) piperidine; (c) Boc-Trp-OH, BOP, Et_3N, CH_2Cl_2.

Table 1. Temperature Coefficients for Amide Protons in CCK4 Restricted Analogues [$\Delta\delta/\Delta T$ (10^{-3} ppm/K)][a]

compd	2-NH	1′-NH	compd	3-NH	1′-NH
6a, **8d**	−3.9	−3.6	**10a**	−3.7	−3.1
6b, **8c**	−5.3	−2.9	**10b**	−5.5	−2.4
6c, **8b**	−4.6	−4.1	**10c**	−3.2	−4.8
6d, **8a**	−5.7	−4.2	**10d**	−4.6	−5.0

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

corresponding (2*R*,5*R*,11b*S*)-IBTM derivative. These products were obtained in moderate yield (41 and 35%, respectively).

As depicted in Scheme 2, BTD-containing CCK4 restricted analogues **9** and **10** were prepared by a similar synthetic route to that described above for IBTM derivatives. Thus, the commercially available Fmoc-BTD-OH was coupled with H-L- or H-D-Phe-NH_2, followed by removal of the Fmoc protecting group with piperidine, and subsequent amide bond formation between the free 3-NH_2 group and Boc-L- or Boc-D-Trp-OH.

2. Conformational Studies. 2.1. NMR Studies. To evaluate the ability of the conformationally restricted CCK4 analogues **6**, **8**, and **10** to adopt β-turnlike conformations in solution, the ^{1}H NMR $J_{5,6}$ coupling constants (for IBTM derivatives) and the temperature coefficients of the amide protons were measured. As required for the adoption of β-turn conformations in IBTM-containing analogues,[19,20] the spectra of compounds **6** and **8** exhibited no coupling between the downfield H-6 proton and the vicinal H-5 proton, which agrees with an axial disposition of the 5-carboxylate group. On the other hand, 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.[37] As shown in Table 1, the $\Delta\delta/\Delta T$ values found for the 2-NH amide proton in all compounds, for the 3-NH proton in derivative **10b**, and for the 1′-NH or 3-NH protons in compounds **6c**,**d**, **8a**,**b**, and **10c**,**d** are indicative of the accessibility of these protons to bulk solvent. In contrast, the values obtained for the 1′-NH proton in derivatives **6b**, **8c** and **10b** indicated protection from the solvent, whereas for compounds **6a**, **8d**, and **10a** and for the 3-NH amide proton of derivative **10a**,**c** the respective temperature coefficients are not conclusive in this regard. Although the $\Delta\delta/\Delta T$ values of some derivatives are within the range required for a hydrogen-bonded inverse turn, molecular modeling studies have shown that the protection from the solvent is not due to the adoption of this kind of turn, as discussed later. On the whole, these data indicate that the protection of the 1′-NH proton from the solvent is mainly dependent on the type of β-turn mimetic and on the configuration of the Phe residue. Thus, derivatives incorporating type II′ β-turn mimetics [either BTD or (2*S*,5*S*,11b*R*)-IBTM frameworks] require an L-Phe residue at *C*-terminus, while a D-Phe amino acid is preferred for derivatives with the type II mimetic [(2*R*,5*R*,11b*S*)-IBTM skeleton]. In contrast, little influence of the configuration of Trp residue on the 1′-NH temperature coefficients was observed. Although both frameworks, IBTM and BTD, have shown good ability to induce β-turn secondary structures in other peptides,[20,35,36] we have previously observed that the presence of a *C*-terminal carboxamide function in the closely related dipeptoids, which incorporate the IBTM scaffold, contribute to disrupt the β-turnlike conformation to some extent.[22] The need for minute conformational changes to accommodate the appended hydrophobic residues (Phe and Boc-Trp) could also be responsible for the β-turn destabilization.

2.2. Molecular Modeling Studies. To get further insights into the 3D structure of this family of CCK4 analogues, we analyzed, by simulated annealing molecular dynamics, the conformational space of IBTM derivatives **8a**−**d**, which incorporate the type II β-turn mimetic, some of which show high affinity at CCK_1 receptors. The obtained minima, within 3 kcal/mol window above the global minimum, were compared and were clustered into families according to their heavy atom root mean square values and to the peptide backbone torsion angle values. On the whole, and based on the N−H orientations, we could make a broad division of the different conformers into two families. Each of these families could subsequently be subdivided in clusters depending on the Phe and Trp torsion angles. Figure 2 shows one representative conformer for each of these two broad families in derivatives **8a**−**d**.

It is interesting to note that, independent of the stereochemistry at the Phe and Trp residue, there is a preferred geometry that involves an interaction of the 1′-NH group with the indole ring of the central scaffold (Figure 2, conformers I). Moreover, this tendency is almost exclusive for derivatives that support a D-Phe residue, **8c** (100%) and **8d** (94%) (Table 2). The distances from the hydrogen of the 1′-NH to the centroid of the aromatic ring range from 3.4 to 3.8 Å, which are within the mean distances for described N−H/π interactions.[38] Another feature of compounds with a D-Phe residue is that the distance between the centroids of the aromatic rings of Phe and IBTM residues is less than 5 Å (Table 2). This could be indicative of the existence of $\pi-\pi$ interactions that are likely contributing to stabilize this spatial disposition. In contrast, only a few conformers of compounds with an L-Phe residue display this particularly short distance (4 and 19%). The conformational analysis of these latter compounds, **8a**,**b**, showed the

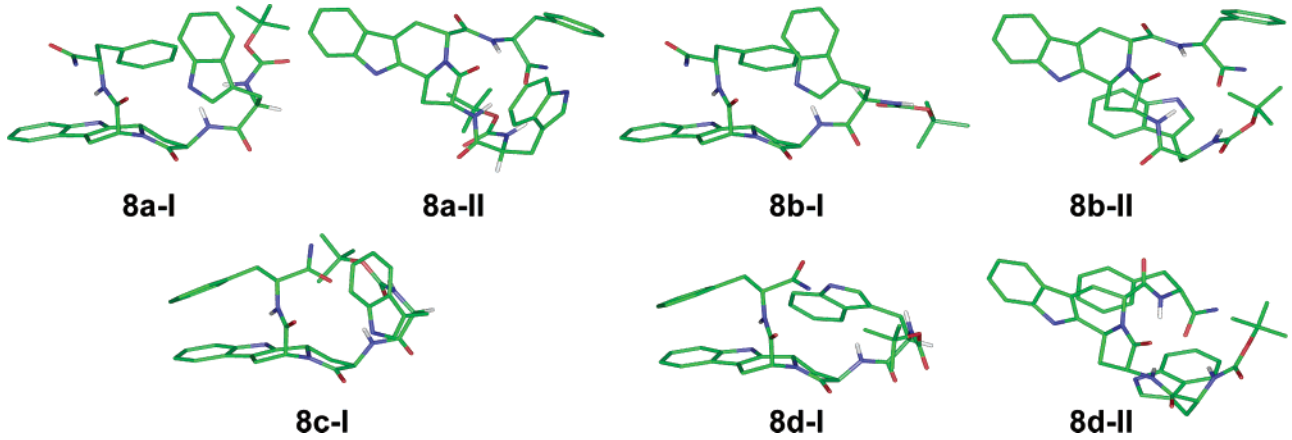


Figure 2. Representative conformers of derivatives **8a**−**d**. For clarity, only the NH amide hydrogens are shown.

Table 2. Statistical Data Obtained for the Conformers within a 3 kcal/mol Window above the Global Minimum

compd	IC_{50} CCK_1 (nM)	$D_{1'-NH-In}$[a] < 4 Å	$D_{1'-NH-CO}$[b] < 4 Å	d_{Ph-In}[c] < 5 Å
8a	54.9	76	24	19
8b	25.9	66	34	4
8c	>1000	100	0	96
8d	582	94	5	100

[a] *d*[1′-NH−In(IBTM)] (%). The distance refers to the centroid of the aromatic moiety, and to the hydrogen atom of the NH(Phe). [b] *d*[1′-NH−CO(3)] (%). [c] *d*[Ph(Phe)−In(IBTM)] (%).The distance refers to the centroids of the aromatic moieties.

presence of another family of conformers (Figure 2, conformers II), in which this amino group is pointing toward the CO(3). This spatial disposition is also present in derivative **8d**, albeit in very low percentage (Table 2). In conformers II, the distance between the hydrogen atom of the 1′-NH group and the oxygen of CO(3) is not within that required for a hydrogen bond. However, for these conformers in the most active derivatives **8a**,**b**, the values of the ϕ and ψ dihedral angles of the $i + 2$ residue are within the standard values (±30°) that define γ-turns. The molecular modeling conformational studies have shown that none of the studied derivatives have intramolecular H-bonds. However, the ^{1}H NMR study has shown that the 1′-NH is protected from the solvent in derivatives **8c** and **8d**. A possible explanation for this fact is the existence of the above-mentioned N−H/π contacts, which together with the close interaction between the two aromatic moieties that surround this NH make it inaccessible to the solvent.

Although for compounds **8a**,**b** there is a small percentage of conformers (<10%) that show the existence of the H-bond characteristic of a β-turn, none of them have values of the torsion angles within that required for any type of β-turn.

3. Biological Results and Discussion. The binding affinities of all conformationally constrained CCK4 analogues at CCK_1 and CCK_2 receptors were determined by measuring the displacement of [^{3}H]propionyl-CCK8 binding to rat pancreatic and cerebral cortex homogenates, respectively, as previously described (Table 3).[39] For comparatives purposes, the described affinities for Boc-CCK4 and the representative CCK_1 dipeptoid antagonists **1** and **2**[22] were also included.

As indicated in Table 3, replacement of Met-Asp dipeptide fragment of CCK4 with IBTM skeletons led

Table 3. Inhibition of the [^{3}H]pCCK8-specific Binding to Rat Pancreas (CCK_1) and Cerebral Cortex Membranes (CCK_2) by CCK4 Restricted Analogues

					IC_{50} (nM)[a]	
compd	config 2,5,11[b]	Phe	Trp	R_1	CCK_1	CCK_2
5a	*SSR*	L	L	OMe	>10000	>10000
5b	*SSR*	L	D	OMe	>10000	>10000
6a	*SSR*	L	L	NH_2	625 ± 88.3	>10000
6b	*SSR*	L	D	NH_2	817 ± 92.5	>10000
6c	*SSR*	D	L	NH_2	>1000	524 ± 150
6d	*SSR*	D	D	NH_2	>1000	938 ± 133
8a	*RRS*	L	L	NH_2	54.9 ± 13.3	>10000
8b	*RRS*	L	D	NH_2	25.9 ± 1.6	>10000
8c	*RRS*	D	L	NH_2	>1000	>10000
8d	*RRS*	D	D	NH_2	582.0 ± 59.2	>10000
10a	*SSR*[b]	L	L		>1000	>10000
10b	*SSR*[b]	L	D		>1000	>10000
10c	*SSR*[b]	D	L		>1000	>10000
10d	*SSR*[b]	D	D		>1000	>10000
1	*SSR*	L		OH	4.7 ± 1.30	>10000
2	*RRS*	L		OH	1.7 ± 0.30	202 ± 99
Boc-CCK4[c]					1800	25

[a] Values are the mean or mean ± SEM of at least three experiments, performed with seven concentrations of test compounds in duplicate. [b] Configuration at positions 3, 6, and 9 in the BTD skeleton. [c] From ref 22.

to a series of compounds with modest to good affinity for either CCK_1 or CCK_2 receptors. In agreement with the described importance of the carbamoyl moiety of CCK4 for binding and activation of CCK receptors,[14] the *C*-terminal group of the restricted CCK4 analogues had a significant effect on the affinity. Thus, while derivatives **5a**,**b**, with a methoxycarbonyl group, were not recognized by either of the CCK receptors subtypes, the corresponding analogues **6a**,**b**, bearing a carboxamide group at the *C*-terminus, moderately bound to CCK_1 receptors.

With regard to the type of IBTM turn mimetic, some interesting conclusions can be drawn from the binding affinities of compounds **6** and **8**. CCK4 analogues **6a**−**d**, incorporating the type II′ (2*S*,5*S*,11b*R*)-IBTM skeleton, bound CCK_1 or CCK_2 receptor subtype, depending on the configuration of the Phe residue. Thus, L-Phe-containing derivatives **6a**,**b** bound selectively to CCK_1 receptors, whereas their D-Phe counterparts **6c**,**d** preferred the CCK_2 receptor subtype. Related stereochemical changes within single structural types of other nonpeptide CCK ligands give rise to interconversions of CCK_1/CCK_2 or CCK_2/CCK_1 selectivity.[13,18a] In con-

trast, derivatives **8a**,**b**,**d**, that incorporated the type II β-turn IBTM mimetic displayed selective CCK_1 receptor affinities and were unable to bind to CCK_2 receptors at 10^{-5} M concentrations. In compounds **8**, and in terms of CCK_1 binding potency, the presence of an L-Phe residue was again preferred (**8a**,**b** versus **8c**,**d**), and the configuration of the appending Trp residue has almost no influence (**8a** vs **8b**).

Compared to Boc-CCK4, some of the IBTM-containing analogues showed a significant increase of the affinity at CCK_1 receptors, while none of these derivatives were able to reach the nanomolar affinity showed by the CCK4 at the CCK_2 receptors. In contrast, none of the CCK4 analogues that incorporate the well-known type II′ mimetic BTD were able to bind to either CCK_1 or CCK_2 receptors at all (Table 3). This points to the importance of the indole group of the IBTM framework for binding to CCK receptors. Thus, the IBTM skeleton appeared not to be just a scaffold for the proper disposition of the aromatic side chains of Phe and Trp residues but a part of the pharmacophore itself.

On the whole, the study of the binding data shows that the main factors that governs the affinity are, first, the stereochemistry of the central scaffold, where a type II β-turn mimetic is preferred, and, second, the configuration of the Phe residue, where an L-Phe residue is favored. In general, changes in the stereochemistry of the Trp residue lead to insignificant affinity variation.

To try to get deeper insights into the structure−conformation−activity relationships (SAR) of this series of restricted CCK4 derivatives, we proceeded to correlate low-energy conformers with CCK_1 receptor affinities. It is interesting to note that an increase in the conformers with Ph(Phe)−In(IBTM) stacking turns out to be detrimental for affinity, as observed by comparison of L-Phe and D-Phe derivatives (Table 2). In contrast, the increase in the percentage of conformers with a distance between 1′-NH and CO(3) of less than 4 Å correlates directly with an increase in the CCK_1 receptor binding potency. Although the latter distance is not within the value required for a H-bonded γ-turn, we have shown that derivatives **8a**,**b**, with higher CCK_1 receptor affinities, have a higher population of conformers with γ-turnlike conformations. This might suggest the existence of an inverse turnlike arrangement in the bioactive conformation recognized by CCK_1 receptors.

Following these results, it might be thought that the IBTM-containing CCK4 analogues described here could be conceptually related to the IBTM-restricted dipeptoids **1** and **2**,[22] which differ only because the benzyloxycarbonyl group at position 2 has been replaced by Boc-Trp moieties in **5**−**8**. Thus, in both series the ability to adopt an inverse turn conformation seems to be directly related to the affinity at CCK_1 receptors. The decrease in affinity of compounds **8a**,**b** compared with the restricted dipeptoid **2**, might be due to the fact that the former is able to adopt γ-turnlike arrangements, whereas the latter prefers β-turn conformations. Moreover, in both series of IBTM-containing compounds the configuration of the Phe residue is determinant for high affinity, the optimal being an L-Phe residue. However, some divergence can be found between the CCK4 restricted analogues and the dipeptoid series. In the new CCK4 analogues described here, there are more restricted stereochemistry requirements for the central scaffold, where only the incorporation of a type II β-turn mimetic leads to derivatives able to interact with CCK_1 receptors in the nanomolar range. Another point of discrepancy between the two series comes from the need for a carboxamide moiety in the CCK4 analogues, similarly to the endogenous peptide. In the dipeptoid series, a *C*-terminal methoxycarbonyl group or a free carboxylic acid is required in order to get high CCK_1 affinity, while a carboxamide moiety led to inactive compounds.[22] These differences and similarities in the SAR of the two series might be explained through the interaction with distinct groups within a common binding site or, alternatively, they might only be sharing part of the binding site. Directed mutagenesis studies will be required to explore deeper the binding pockets of these compounds.

The functional activity of the best compounds in this series, **8a** and **8b**, was evaluated in the amylase release assay.[40,41] These compounds were able to antagonize the CCK8-induced amylase secretion from pancreatic acini with IC_{50} values of 130 ± 18 and 829 ± 402 nM, respectively, while they did not show any intrinsic effect on the amylase release at a 1 μM concentration. Therefore, compounds **8a** and **8b** are representative members of this family of selective CCK_1 receptor antagonists.

Conclusions

In the present study, we have demonstrated that the incorporation of the β-turn inductor frameworks IBTM, as replacement for the Met-Asp central dipeptide fragment of CCK4, led to active CCK_1 compounds, whereas the related BTD-containing derivatives turn out to be inactive. The affinity of the IBTM derivatives was mainly dependent on the configuration of the Phe residue (L much better than D) and on the type of β-turn mimetic (type II preferred over type II′). A careful comparison of the conformational studies and the biological results revealed that the IBTM framework has a substantial contribution to the way in which these derivatives bind to the CCK receptors, suggesting that it is directly involved in the interaction with them. Although in this series of CCK4 derivatives the IBTM framework is not able to induce any typical β-turn conformation, molecular modeling studies have revealed the existence of γ-turnlike arrangements that are favorable for high binding affinity to CCK_1 receptors. On the whole, the introduction of conformational constraints into the CCK4 sequence by means of the IBTM skeleton resulted in a powerful way to obtain CCK_1 selective antagonists, illustrated by **8a** and **8b**. Further studies on these compounds, which proved to be relatively easy to synthesize, could serve to achieve a better definition of the structural and conformational requirements for CCK_1 receptor recognition.

Experimental Section

Chemistry. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254}. Silica gel 60 (230−400 mesh) was used for flash chromatography. ^{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. Elemental analyses were obtained on a CH-O-RAPID apparatus. 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 tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phase. Compounds **3a**,**b** and **4a** were prepared as described.[20]

(2*R*,5*R*,11b*S*,1′*R*)-2-(*tert*-Butoxycarbonyl)amino-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (4b). A solution of (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[19] (262 mg, 0.68 mmol) in dry CH_2Cl_2 (14 mL) was successively treated with H-D-Phe-NH_2 (146.2 mg, 0.89 mmol), BOP (393.6 mg, 0.89 mmol), and TEA (0.124 mL, 0.89 mmol). After stirring overnight, the solvent was evaporated, and 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 that was purified on a silica gel column using a gradient from 15 to 67% acetone in hexane. The title compound (374.6 mg, 95%) was obtained as a syrup. Anal. ($C_{41}H_{44}N_6O_7$) C, H, N.

General Procedures for the Coupling of 2-Aminohexahydroindolizino[8,7-*b*]indole Derivatives with Boc-Tryphophans. Method A. A solution of the corresponding 2-benzyloxycarbonyl-substituted hexahydroindolizinoindole **3** (139 mg, 0.25 mmol) in MeOH (25 mL) was hydrogenated at room temperature and 30 psi of pressure for 5 h, using 10% Pd−C as catalyst (20% w/w). After filtration of the catalyst, the solvent was evaporated. The resulting residue was dissolved in dry CH_2Cl_2 (6 mL), and Boc-L- or Boc-D-Trp-OH (97.4 mg, 0.32 mmol), BOP (141.5 mg, 0.32 mmol), and TEA (0.08 mL, 0.32 mmol) were successively added. 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 that was purified on a silica gel column as specified in each case.

Method B. To a solution of the corresponding derivative **4** (139 mg, 0.25 mmol) in CH_2Cl_2 (2.5 mL) was added TFA (1.25 mL). The solution was stirred at room temperature for 1 h, and the solvent was removed by repeated coevaporations with CH_2Cl_2. The residue was dissolved in dry CH_2Cl_2 (6 mL); successively treated with Boc-L- or Boc-D-Trp-OH (97.4 mg, 0.32 mmol), BOP (141.5 mg, 0.32 mmol), and TEA (0.08 mL, 0.57 mmol); and stirred overnight. The workup was continued as above.

(2*S*,5*S*,11b*R*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-5-[(1′-methoxycarbonyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole (5a). Yield: 71% (from **3a** and Boc-L-Trp-OH, method A) as a foam. Eluent: 3% of MeOH in CH_2Cl_2. HPLC: t_R = 18.33 min (A:B = 45/55). Anal. ($C_{41}H_{44}N_6O_7$) C, H, N.

(2*S*,5*S*,11b*R*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-5-[(1′-methoxycarbonyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole (5b). Yield: 76% (from **3a** and Boc-D-Trp-OH, method A) as a foam. Eluent: 3% of MeOH in CH_2Cl_2. HPLC: t_R = 16.20 min (A:B = 45/55). Anal. ($C_{41}H_{44}N_6O_7$) C, H, N.

(2*S*,5*S*,11b*R*,1′*R*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (6c). Yield: 70% (from **3b** and Boc-L-Trp-OH, method A) as a foam. Eluent: gradient from 10 to 50% of acetone in hexane. HPLC: t_R = 5.08 min (A:B = 45/55). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

(2*S*,5*S*,11b*R*,1′*R*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (6d). Yield: 70% (from **3b** and Boc-D-Trp-OH, method A) as a foam. Eluent: gradient from 10 to 50% of acetone in hexane. HPLC: t_R = 5.10 min (A:B = 45/55). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-5-[(1′-methoxycarbonyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole (7a). Yield: 71% (from **4a** and Boc-L-Trp-OH, method B) as a foam. Eluent: 2% of CH_2Cl_2 in MeOH. HPLC: t_R = 16.16 min (A:B = 45/55). Anal. ($C_{41}H_{44}N_6O_7$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-5-[(1′-methoxycarbonyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino-[8,7-*b*]indole (7b). Yield: 41% (from **4a** and Boc-D-Trp-OH, method B) as a foam. Eluent: 2% of CH_2Cl_2 in MeOH. HPLC: t_R = 14.76 min (A:B = 45/55). Anal. ($C_{41}H_{44}N_6O_7$) C, H, N.

(2*R*,5*R*,11b*S*,1′*R*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (8c). Yield: 72% (from **4b** and Boc-L-Trp, method B). Eluent: gradient from 15 to 50% of acetone in hexane. HPLC: t_R = 10.33 min (A:B = 40/60). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*R*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-5-[(1′-carbamoyl-2′phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (8d). Yield: 35% (from **4b** and Boc-D-Trp, method B). Eluent: gradient from 15 to 67% of acetone in hexane. HPLC: t_R = 11.60 min (A:B = 40/60). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

Preparation of *C*-Terminal Carbamoyl Derivatives. A solution of the corresponding 1′-methoxycarbonyl derivative (0.04 mmol) in MeOH saturated with NH_3 (10 mL) was stirred at room temperature for 2 days. After evaporation to dryness, the resulting residue was purified on a silica gel column using a gradient from 1 to 4%of MeOH in CH_2Cl_2.

(2*S*,5*S*,11b*R*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (6a). Yield: 97% (from **5a**) as a foam. HPLC: t_R = 11.60 min (A:B = 40/60). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

(2*S*,5*S*,11b*R*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (6b). Yield: 65% (from **5b**) as a foam. HPLC: t_R = 10.33 min (A:B = 40/60). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (8a). Yield: 95% (from **7a**) as a foam. HPLC: t_R = 9.52 min (A:B = 40/60). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

(2*R*,5*R*,11b*S*,1′*S*)-2-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-5-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (8b). Yield: 72% (from **7b**) as a foam. HPLC: t_R = 9.80 min (A:B = 40/60). Anal. ($C_{40}H_{43}N_7O_6$) C, H, N.

Coupling of Fmoc-BTD-OH with H-Phe-NH_2. To a solution of Fmoc-BTD-OH (300 mg, 0.68 mmol) in dry CH_2Cl_2 (18 mL) were added successively H-L-Phe-NH_2 or H-D-Phe-NH_2 (146 mg, 0.89 mmol), BOP (393.3 mg, 0.89 mmol), and TEA (0.12 mL, 0.89 mmol). After stirring overnight the solvent was evaporated and the workup continues as in the coupling of IBTM derivatives (method A).

(3*S*,6*S*,9*R*,1′*S*)-3-[9′-Fluoromethyloxycarbonyl]-9-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (9a). Yield: 95% as a foam. Eluent: 3% of CH_2Cl_2 in MeOH. HPLC: t_R = 3.26 min (A:B = 30/70). Anal. ($C_{32}H_{32}N_4O_5S$) C, H, N, S.

(3*S*,6*S*,9*R*,1′*R*)-3-[9′-Fluoromethyloxycarbonyl]-9-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (9b). Yield: 90% as a foam. Eluent: 2% of CH_2Cl_2 in MeOH. HPLC: t_R = 11.20 min (A:B = 40/60). Anal. ($C_{32}H_{32}N_4O_5S$) C, H, N, S.

Coupling of H-BTD-Phe-NH_2 with Boc-Trp-OH. To a solution of the corresponding Fmoc derivative **9** (111 mg, 0.19 mmol) in THF (10 mL) was added piperidine (0.037 mL, 0.38 mmol). After stirring for 2 h, the solvent was evaporated to dryness and the residue precipitated from Et_2O. The resulting

residue was dissolved in dry CH_2Cl_2 (6 mL), and Boc-L- or Boc-D-Trp-OH (76.5 mg, 0.25 mmol), BOP (111 mg, 0.25 mmol), and TEA (0.034 mL, 0.25 mmol) were successively added. After stirring overnight, the solvent was evaporated, and the residue was treated as in the coupling of IBTM derivatives with Trp (method A).

(3*S*,6*S*,9*R*,1′*S*)-3-[(*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-9-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10a). Yield: 74% (from **9a**) as a foam. Eluent: gradient from 1 to 10% of CH_2Cl_2 in MeOH. HPLC: t_R = 8.88 min (A:B = 40/60). Anal. ($C_{33}H_{40}N_6O_6S$) C, H, N, S.

(3*S*,6*S*,9*R*,1′*S*)-3-[(*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-9-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10b). Yield: 86% (from **9a**) as a foam. Eluent: 1 to 5% of CH_2Cl_2 in MeOH. HPLC: t_R = 6.96 min (A:B = 40/60). Anal. ($C_{33}H_{40}N_6O_6S$) C, H, N, S.

(3*S*,6*S*,9*R*,1′*R*)-3-[(*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]-9-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10c). Yield: 82% (from **9b**) as a foam. Eluent: gradient from 10 to 50% of CH_2Cl_2 in MeOH. HPLC: t_R = 4.86 min (A:B = 40/60). Anal. ($C_{33}H_{40}N_6O_6S$) C, H, N, S.

(3*S*,6*S*,9*R*,1′*R*)-3-[(*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]-9-[(1′-carbamoyl-2′-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10d). Yield: 75% (from **9b**) as a foam. Eluent: gradient from 10 to 50% of CH_2Cl_2 in MeOH. HPLC: t_R = 4.16 min (A:B = 40/60). Anal. ($C_{33}H_{40}N_6O_6S$) C, H, N, S.

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]

Amylase Release. Dispersed rat pancreatic acini were prepared by using a modification of the technique of Jensen et al.[40] A 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 of three 10-min incubations at 37 °C in an atmosphere of pure O_2 and with agitation (200 cycles/min). The tissue was washed twice in mix buffer (composition: 98 mM NaCl, 6 mM KCl, 2.5 mM NaH_2PO_4, 0.5 mM $CaCl_2$, 5 mM theophylline, 11.4 mM glucose, 2 mM L-glutamine, 5 mM L-glutamic acid, 5 mM fumaric acid, 5 mM piruvic acid, 0.01% SBTI, 1% essential amino acid mixture, 1% essential vitamin mixture, pH 7.4) after each incubation. The tissue obtained after the last incubation was transferred to stop buffer (4% BSA) and shaken 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.[41] Samples (2 mL) of the acini suspension were placed in plastic tubes and incubated for 30 min at 37 °C in an atmosphere of pure O_2 with agitation (70 cycles/min). Amylase activity was determined using the amyl kit reagent (Boeringher Mannheim). Release (S) was calculated as the percentage of amylase in the acini that was released into extracellular medium during the incubation period. The percentage of inhibition of amylase release by drugs elicited by a fixed CCK8 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 the control release (vehicle). S_{CCK} is release elicited by CCK8, and S_T is release in the presence of increasing drug concentrations. IC_{50} values were calculated for the drug tested.

Molecular Modeling Studies. Conformational Search. All calculations were run on an SGI workstation (Fuel, RP14000, 500 MB RAM) under an Irix 6.5 operating system. The initial conformation of the IBTM derivatives **8a**−**d** were built using the library of fragments available in the molecular modeling program Insight II (version 2000.1, Biosym Technologies, San Diego, CA). The calculations were carried out within the molecular mechanics using the AMBER force field implemented in DISCOVER. They were conducted under vacuum with a distance dependent dielectric constant (4*r*) and a cutoff of 14 Å. In the simulations, a force constant of 500 kcal/mol/Å was used to restrain the amide plane in a trans configuration. The structure is heated to 1000 K and equilibrated during 10 ps. The structure is then cooled slowly to 300 K in steps; in each step the temperature was lowered by 100 K, and the system was allowed to stay at the new temperature for 100 ps. After cooling to 300 K, the final conformation obtained is energy-refined using a conjugated gradient algorithm with a final gradient of 0.001 kcal/mol as the convergence criteria. The conformer is stored and used to start a new simulation at high temperature. This procedure produced samples of 100 energy-minimized conformations, which were compared with each other to eliminate the identical ones. The protocol was run three times, employing different starting structures for derivatives **8a**−**d**. Independent of the starting structure, the percentage of the different families of conformers where equivalent.

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Supporting Information Available: ^{1}H NMR data and elemental analysis for all new compounds (**4**−**10**). This material is available free of charge via the Internet at http://pubs.acs.org.

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