2-Oxopiperazine-Based γ-Turn Conformationally Constrained Peptides: Synthesis of CCK-4 Analogues

Susana Herrero,[†] M. Teresa García-López,[†] Miriam Latorre,[‡] Edurne Cenarruzabeitia,[‡] Joaquín Del Río,[‡] and Rosario Herranz*,[†]

Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain, and Departamento de Farmacología, Universidad de Navarra, Irunlarrea 1, E-31080 Madrid, Spain

rosario@iqm.csic.es.

Received February 20, 2002

2-Oxopiperazine derivatives **1** have been designed as mimetics of γ-turn conformationally constrained tripeptides. The synthetic pathway devised for the preparation of both epimers of **1** at C_5 involves a reductive amination of cyanomethyleneamino pseudopeptides with amino acid derivatives, followed by regiospecific lactamization of the resulting C-backbone branched pseudopeptides. The versatility of this methodology is illustrated in the synthesis of analogues of the tetrapeptides Boc-[Nle[31]]-CCK-4 and Boc-[Lys(*o*-tolylaminocarbonyl)[31]]-CCK-4. The introduction of the new conformational restriction into these Boc-CCK-4 analogues led to a loss of 2 or 3 orders of magnitude in the affinity at CCK receptors. These results suggest the absence of a γ-turn in the bioactive conformation of the C-terminal tripeptide of CCK-4.

Introduction

Turns, defined as regions where a peptide chain reverses its overall direction, are important elements of peptide secondary structure, often located at the protein surface, where they have been implicated in molecular recognition processes, such as receptor binding, antibody recognition, and posttranslational modifications. In addition, structural studies have revealed the presence of turns in the preferred solution conformations of biological active peptides, which have been proposed to mediate their biological activity. β-Turns, which are the most frequent, involve four consecutive amino acid residues, whereas γ-turns consist of three residues, which may or may not be stabilized by an intramolecular hydrogen bond between the C=O of the first residue (*i*) and the NH of the third residue (*i*+2), forming a seven-membered ring.[1,2] γ-Turns have been classified into classic and inverse (Figure 1) on the basis of the dihedral ϕ and ψ angle values of the (*i*+1) residue. Classical γ-turns are characterized by a ϕ_{i+1} angle of 35° to 106° (mean value 75°) and a ψ_{i+1} angle of −29° to −94° (mean value −64°),[3] and the side chain of the *i*+1 residue assumes a pseudoaxial orientation, having been called also C_7 axial.[4] In contrast, inverse γ-turns are characterized by a ϕ_{i+1} angle of −9° to −110° (mean value −79°) and a ψ_{i+1} angle of 14° to 131° (mean value 69°),[5] and the side chain of the *i*+1 residue in a pseudoequatorial orientation (C_7 equatorial).[4] Although γ-turns are less frequent than β-turns, an analysis of 54 proteins from the Protein Data Bank has shown the presence of 12 classic and 117 inverse

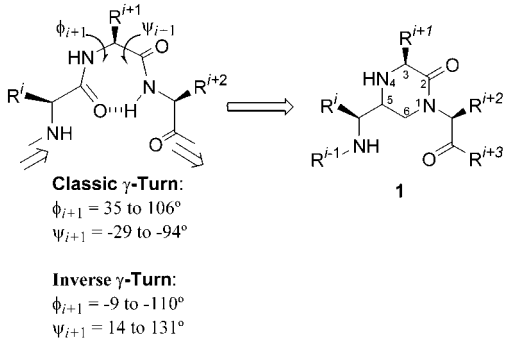


Figure 1. Schematic description of classic and inverse γ-turns and the mimetic **1**.

γ-turns, frequently forming part of higher secondary structures such as loops, β-sheets, or α-helices.[3,5] Furthermore, it has been proposed that γ-turns are present in the solution conformation of several peptides, including vasopresin,[6,7] cyclosporin,[8] angiotensin II,[9,10] bombesin,[11] endothelin antagonists,[12] bradykinin,[13] and the RGD tripeptide sequence in several fibrinogen and integrin antagonists.[14−19]

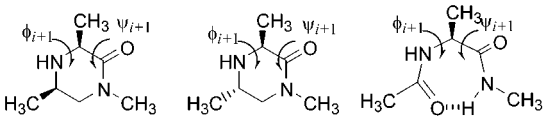
[†] Instituto de Química Médica (CSIC).
[‡] Universidad de Navarra.

(1) Smith, J. A.; Pease, L. G. *CRC Crit. Rev. Biochem*. **1980**, *8*, 315.
(2) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem*. **1985**, *37*, 1.
(3) Milner-White, E. J.; Ross, B. M.; Ismail, R.; Belhadj-Mostefa, K.; Poet, R. *J. Mol. Biol*. **1988**, *204*, 777.
(4) Rommel-Möhle, K.; Hofmann, H.-J. *J. Mol. Struct*. **1993**, *285*, 211.
(5) Milner-White, E. J. *J. Mol. Biol*. **1990**, *216*, 385.
(6) Hagler, A. T.; Osguthorpe, D. J.; Dauber-Osguthorpe, P.; Hempel, J. C. *Science* **1985**, *227*, 1309.
(7) Brickmann, K.; Yuan, Z. Q.; Sethson, I.; Somfai, P.; Kihlberg, J. *Chem. Eur. J*. **1999**, *5*, 2241.
(8) Loosli, H.-R.; Kessler, H.; Oschkinat, H.; Weber, H.-P.; Petcher, T. J.; Widmer, A. *Helv. Chim Acta* **1985**, *68*, 682.
(9) Schmidt, B.; Lindman, S.; Tong, W.; Lindeberg, G.; Gogoll, A.; Lai, Z.; Thörnwall, M.; Synnergren, B.; Nilsson, A.; Welch, C. J.; Sohtell, M.; Westerlund, C.; Nyberg, F.; Karlén, A.; Hallberg, A. *J. Med. Chem*. **1997**, *40*, 903.
(10) Lindman, S.; Lindeberg, G.; Nyberg, F.; Karlén, A.; Hallberg, A. *Bioorg. Med. Chem*. **2000**, *8*, 2375.
(11) Horwell, D. C.; Howson, W.; Naylor, D.; Osborne, S.; Pinnock, R. D.; Ratcliffe, G. S.; Suman-Chauhan, N. *Int. J. Pept. Protein Res.* **1996**, *48*, 522.
(12) Goldstein, S.; Neuwels, M.; Culot, P.; Differding, E.; Dogimont, C.; Assche, Y. Brichard, M. H.; Jaspers, D.; Van Binst, G.; Scarso, A.; Hénichart, J.-P. *Lett. Pept. Sci.* **1995**, *2*, 141.
(13) Sato, M.; Lee, J. Y. H.; Nakanishi, H.; Johnson, M. E.; Chrusciel, R. A.; Kahn, M. *Biochem. Biophys. Res. Commun*. **1992**, *187*, 999.

10.1021/jo0256336 CCC: $22.00
Published on Web 04/30/2002

Several structural motifs have been described to stabilize or mimic γ-turn conformations, including 1-aminocycloalkane carboxylic acids,[20,21] *cis*-4-amino-L-proline derivatives,[22] cyclic β-enamino nitriles,[23] tetrahydro-1*H*-azepine derivatives,[10,14,17,24] hexahydro-1,4-diazepines,[25] cyclic hydrazide derivatives,[26] morpholin-3-ones,[7] (3-aminomethyl-2-oxo-1-piperidyl)acetic acid,[13] and 1,3,5-trisubstituted-cyclohexanes.[27] However, most of the synthetic approaches described for these γ-turn mimetics do not allow to introduce diversity of side chains at appropriate positions or to control their stereochemistry. Herein we describe the design and synthesis of 2-oxopiperazine derivatives **1** (Figure 1) as new γ-turn mimetics, where a methylene bridge replaces the $i \rightarrow i+2$ hydrogen bond, locking the tripeptide into a six-membered ring. The C=O of the γ-turn *i* residue is replaced by a chiral center at C_5 in the 2-oxopiperazines **1**. Therefore, there are two possible mimetics for each tripeptide, epimers at C_5. The synthetic pathway devised for mimetics **1** allows the introduction of a wide diversity of side chains with controlled topography. To illustrate our approach several analogues of the cholecystokinin (CCK) C-terminal tetrapeptide CCK-4 (Trp-Met-Asp-Phe-NH_2) have been prepared. Although the conformational analysis of CCK-4 does not show the presence of preferred turn conformations, some authors have proposed the presence of β- or γ-turns in its bioactive conformation.[28,29] The presence of a γ-turn has also been proposed in the bioactive conformation of the CCK_1 agonist Boc-Trp-Lys-(*o*-tolylaminocarbonyl)-Asp-Phe-NH_2 at the C-terminal tripeptide.[30] On the other hand, the piperazine skeleton is considered among the privileged scaffolds for peptidomimetic search.[31] Particularly, the 2-oxopiperazine ring has been included into several ligands for receptors of neurokinins,[32−34] CCK,[34,35] and enkephalins,[36] and into fibrinogen antagonists.[37]

Table 1. Relevant Topographic Parameters of the Low-Energy Conformers for 3,5-*cis*- and 3,5-*trans*-2-Oxopiperazines 2 and Optimized γ-Turns



conformer	ΔE^a (Kcal/mol)	ϕ_{i+1}	ψ_{i+1}	$C\alpha^i - C\alpha^{i+2}$ distance (Å)
		3,5-*cis*-**2**		
1	0.00	−39.66	14.28	5.01
2	1.06	−54.33	31.26	4.57
3	2.04	−45.71	16.20	5.00
		3,5-*trans*-**2**		
1	0.00	−54.22	43.49	4.69
2	0.77	13.14	33.95	5.01
3	0.97	52.41	−40.68	4.90
4	1.18	−58.56	−47.79	4.71
5	2.29	55.42	−46.70	4.84
6	2.46	19.42	29.58	5.00
classic γ-turn	1.55	68.32	−56.41	5.74
inverse γ-turn	0.72	−76.02	76.40	5.63

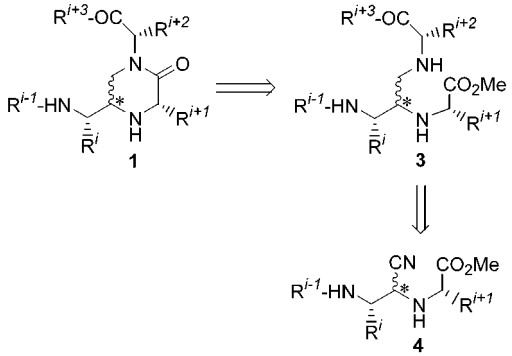
[a] Relative energy of the corresponding conformer.

Results and Discussion

Molecular Modeling. To study the ability of both epimers 3,5-*cis*- and 3,5-*trans*-**1** to mimic classic or inverse γ-turns, a conformational search of the simplified model compounds (3*S*,5*R*)- and (3*S*,5*S*)-1,3,5-trimethyl-2-oxopiperazine (3,5-*cis*- and 3,5-*trans*-**2**, respectively) was carried out by molecular dynamics at high temperature, followed by minimization in vacuo ($\epsilon = 1$) with the Insight II program.[38] The resulting unique minima were fully optimized with the semi-ab-initio method SAM1, included in the Ampac 5.0 program. The geometrical parameters of the low-energy conformers of 3,5-*cis*- and 3,5-*trans*-**2** are shown in Table 1, in comparison with those of the optimized classic and inverse γ-turn conformations for Ac-Ala-NHMe obtained with the SAM1 method, starting from the average values of the ϕ_{i+1} and ψ_{i+1} dihedral angles for these conformations.[38] The calculated values of these angles for the three low-energy conformers found for 3,5-*cis*-**2** are in the range of those reported for inverse γ-turns,[5] indicated in Figure 1. However, as it was expected, the $C_\alpha{}^i - C_\alpha{}^{i+2}$ distance was slightly shorter, as consequence of the closing of the

(14) Callahan, J. F.; Bean, J. W.; Burgess, J. L.; Eggleston, D. S.; Hwang, S. M.; Kopple, K. D.; Koster, P. F.; Nichols, A.; Peishoff, C. E.; Samanen, J. M.; Vasko, J. A.; Wong, A.; Huffman, W. F. *J. Med. Chem.* **1992**, *35*, 3970.
(15) Ku, T. W.; Ali, F. E.; Barton, L. S.; Bean, J. W.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, L.; Eggleston, D. S.; Gleason, J. G.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Karash, C. B.; Keenan, R. M.; Kopple, K. D.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Peishoff, C. E.; Samanen, J. M.; Uzinskas, I.; Venslavsky, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 8861.
(16) Haubner, R.; Schmitt, W.; Hölzemann, G.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7881.
(17) Keenan, R. M.; Miller, W. H.; Kwon, C.; Ali, F. E.; Callahan, J. F.; Calvo, R. R.; Hwang, S.-M.; Kopple, K. D.; Peishoff, C. E.; Samanen, J. M.; Wong, A. S.; Yuan, C.-K.; Huffman, W. F. *J. Med. Chem.* **1997**, *40*, 2289.
(18) Tran, T.-A.; Mattern, R.-H.; Zhu, Q.; Goodman, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 997.
(19) Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033.
(20) Balaji, V. N.; Ramnarayan, K.; Chan, M. F.; Rao, S. N. *Pept. Res.* **1994**, *7*, 60.
(21) Paradisi, M. P.; Torrini, I.; Zecchini, G. P.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G. *Tetrahedron* **1995**, *51*, 2379.
(22) Curran, T. P.; Chandler, N. M.; Kennedy, R. J.; Keaney, M. T. *Tetrahedron Lett.* **1996**, *37*, 1933.
(23) Kemp, D. S.; Carter, J. S. *J. Org. Chem.* **1989**, *54*, 109.
(24) Newlander, K. A.; Callahan, J. F.; Moore, M. L.; Tomaszek, T. A.; Huffman, W. F. *J. Med. Chem.* **1993**, *36*, 2321.
(25) Callahan, J. F.; Newlander, K. A.; Burgess, J. L.; Eggleston, D. S.; Nichols, A.; Wong, A.; Huffman, W. F. *Tetrahedron* **1993**, *49*, 3479.
(26) Ferguson, M. D.; Meara, J. P. Nakanishi, H.; Lee, M. S.; Kahn, M. *Tetrahedron Lett.* **1997**, *38*, 6961.
(27) Schmidt, B.; Kühn, C. *Synlett* **1998**, 1240.
(28) Pattou, D.; Maigret, B.; Fournie-Zaluski, M. C.; Roques, B. P. *Int. J. Pept. Protein Res.* **1991**, *37*, 440.
(29) Kolodziej, S. A.; Nikiforovich, G. V.; Skeean, R.; Lignon, M. F.; Martinez, J.; Marshall, G. R. *J. Med. Chem.* **1995**, *38*, 137.
(30) Schmitthenner, H. F.; Doring, K. G.; Downs, E. S.; Simmons, R. D.; Zongrone, J. A.; Julien, R. P.; Kaiser, F. C.; Goodman, T. D.; Rosamond, J. D. In *Peptides. Chemistry, Structure and Biology*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: Kingswinford, 1996; p 687.
(31) Patchett, A. A.; Nargund, R. P. *Annu. Rep. Med. Chem.* **2000**, *35*, 289.
(32) Logan, M. E.; Goswami, R.; Tomczuk, B. E.; Venepalli, B. R. *Annu. Rep. Med. Chem.* **1991**, *26*, 43.
(33) Tong, Y.; Fobian, Y. M.; Wu, M.; Boyd, N. D.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1679.
(34) Horwell, D. C.; Lewthwaite, R. A.; Pritchard, M. C.; Ratcliffe, G. S.; Rubin, J. R. *Tetrahedron* **1998**, *54*, 4591.
(35) Batt, A. R.; Kendrick, D. A.; Mathews, E.; Rooker, D. P.; Ryder, H.; Semple, G.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 867.
(36) Shreder, K.; Zhang, L.; Goodman, M. *Tetrahedron Lett.* **1998**, *39*, 221.
(37) Sugihara, H.; Fukushi, H.; Miyawaki, T.; Imai, Y.; Terashita, Z.; Kawamura, M.; Fujisawa, Y.; Kita, S. *J. Med. Chem.* **1998**, *41*, 489.
(38) Alkorta, I.; Suarez, M. L.; Herranz, R.; González-Muñiz, R.; García-López, M. T. *J. Mol. Model.* **1996**, *2*, 16.

Scheme 1. Retrosynthesis of 2-Oxopiperazine-Based γ-Turn Mimetics



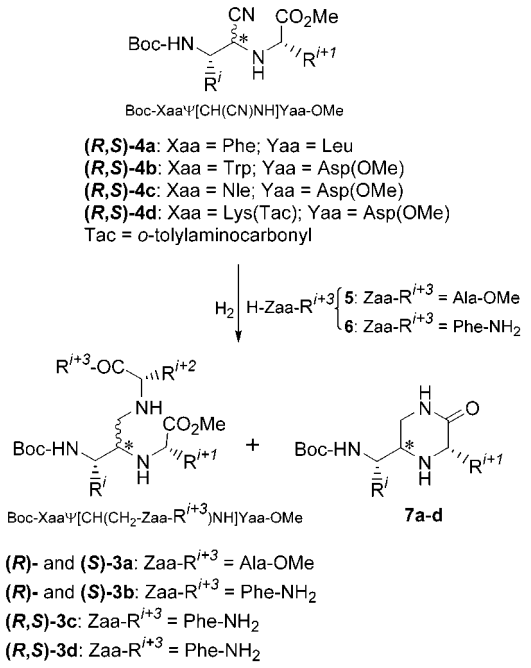
seven-membered pseudocyclic γ-turn into a six-membered ring. In the case of the epimer 3,5-*trans*-**2**, there is a higher conformational variability, since although the ϕ_{i+1} and ψ_{i+1} dihedral angles of its lowest energy conformer are also in the range of the reported values for inverse γ-turns, conformers 3 and 5 have more similarity with classic γ-turns.[3] The electronic and geometric characteristics of 3,5-*cis*- and 3,5-*trans*-**2** have been compared with those of model classic and inverse γ-turns, using the SEAL program. Conformers 1 and 2 of 3,5-*cis*-**2** and conformer 1 of 3,5-*trans*-**2** showed good similarity indices for their superposition with the model inverse γ-turn, whereas only the conformer 3 of 3,5-*trans*-**2** gave a good superposition with the classic γ-turn.[38]

Synthesis. As outlined in the retrosynthetic Scheme 1, the proposed pathway for 2-oxopiperazine derivatives **1** involves two key steps: reductive amination of cyanomethyleneamino pseudopeptides[39] **4**, with amino acid derivatives, followed by regiospecific lactamization of the resulting C-backbone branched peptides **3**. For the study of this route, we have first selected the pseudodipeptide Boc-PheΨ[CH(CN)NH]Leu-OMe[39] as starting material. Next, this synthetic methodology has been extended to the preparation of the proposed CCK-4 analogues.

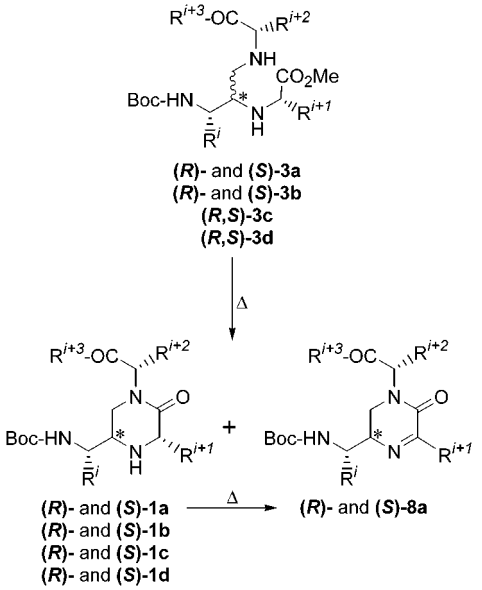
Branched peptides **3a**−**d** were prepared by applying our recently described method for the synthesis of C-backbone branched peptides, via catalytic hydrogenation of Boc-protected Ψ[CH(CN)NH] pseudopeptides in the presence of amino acid derivatives,[40] shown in Scheme 2. The starting Ψ[CH(CN)NH] pseudopeptides **4a**−**d** were used as epimeric mixtures at the stereogenic center of the peptide bond surrogate, which could not be resolved, as well as the resulting branched peptides (*R*,*S*)-**3c** and (*R*,*S*)-**3d**. Neither racemization at the inserted amino acid derivatives **5** and **6** nor epimerization at any of the stereogenic centers of the starting pseudodipeptides **4** were detected in this reductive amination. The 1-unsubstituted 2-oxopiperazines **7a**−**d**, resulting from the formation of the corresponding primary amine, followed by in situ lactamization,[40] were detected as minor products in the RP-HPLC analysis of the crude reaction mixtures.

The study of optimal reaction conditions for the lactamization of branched pseudotripeptides **3** was carried out in both resolved epimers (*R*)- and (*S*)-**3a**. Initially, this cyclization was attempted by heating in refluxing

Scheme 2. Reductive Amination of Ψ[CH(CN)NH] Pseudopeptides



Scheme 3. Lactamization of C-Backbone Branched Pseudopeptides



xylene, obtaining in each case the 2-oxopiperazine derivative (*R*)- and (*S*)-**1a**, along with their oxidation product to the 2-oxo-1,2,5,6-tetrahydropyrazine derivative (*R*)- and (*S*)-**8a**, respectively (Scheme 3). Then, with the aim of avoiding the formation of these oxidation products, we lowered the temperature, heating at 100 °C in toluene. In this way, the 2-oxopiperazines (*R*)- and (*S*)-**1a** were obtained in good yield as the only reaction products (Table 2). The study of the oxidation of these compounds in refluxing xylene showed that the 3,5-*cis* epimer (*S*)-**1a** oxidized faster (8 h for 100% oxidation) than its 3,5-*trans* epimer (*R*)-**1a** (16 h for 70% oxidation). The comparison of the ^{1}H NMR spectra of the 2-oxo-1,2,5,6-tetrahydropyrazines (*R*)- and (*S*)-**8a** with those of their respective 2-oxopiperazine analogues (*R*)- and (*S*)-**1a** showed the disappearance of the signals correspond-

(39) Herranz, R.; Suárez-Gea, M. L.; Vinuesa, S.; García-López, M. T. *J. Org. Chem.* **1993**, *58*, 5186.

(40) Herrero, S.; Suárez-Gea, M. L.; García López, M. T.; Herranz, R. *Tetrahedron Lett.* **2002**, *43*, 1421.

Table 2. Results of the Lactamization of Branched Pseudotripeptides 3

branched tripeptide	T (°C)	t	2-oxopiperazine compd	2-oxopiperazine yield (%)	2-oxo-1,2,5,6-tetrahydropyrazine compd	2-oxo-1,2,5,6-tetrahydropyrazine yield (%)
(*R*)-**3a**	135	6 h	(*R*)-**1a**	24	(*R*)-**8a**	21
(*R*)-**3a**	100	8 h	(*R*)-**1a**	81	(*R*)-**8a**	0
(*S*)-**3a**	135	6 h	(*S*)-**1a**	15	(*S*)-**8a**	58
(*S*)-**3a**	100	8 h	(*S*)-**1a**	77	(*S*)-**8a**	0
(*R*)-**3b**	100	3 days	(*R*)-**1b**	75	(*R*)-**8b**	0
(*S*)-**3b**	100	5 days	(*S*)-**1b**	62	(*S*)-**9b**	0
(*R*,*S*)-**3c** (3:1)[a]	100	5 days	(*R*)-**1c**	60	(*R*)-**8c**	0
			(*S*)-**1c**	19	(*S*)-**8c**	0
(*R*,*S*)-**3d** (2:1)[a]	100	8 days	(*R*)-**1d**	46	(*R*)-**8d**	0
			(*S*)-**1d**	21	(*S*)-**8d**	0

[a] (*R*)/(*S*)-Epimer ratio.

ing to the 3-H of the 2-oxopiperazine ring and a deshielding of 0.2−0.5 ppm for 5-H and of ≈1 ppm for the methylene attached at C_3. The ^{13}C NMR spectra showed the disappearance of the aliphatic C_3 (54−58 ppm) and its appearance in the iminic carbon range at 165−158 ppm.

As shown in Table 2, the lactamization of branched pseudotripeptides **3b**−**d** was also carried out at 100 °C, although it required longer heating times than for compounds **1a**. We tried to decrease this lactamization time by adding 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as basic catalyst. However, this addition did not reduce the reaction time and led to dirtier reaction mixtures as a result of the formation of decomposition products. Similar unsuccessful results were obtained when the lactamization of **3b**−**d** was tried by heating in a microwave oven.

The lactamization was regiospecific between the amino group of the branching *i*+2 amino acid and the methoxycarbonyl of the *i*+1 residue. The alternative lactamization between the *i*+1 amino group and the carbonyl of the *i*+2 residue was not observed in any case. The comparison of the ^1H NMR spectra of the 2-oxopiperazines **1a**−**d** with those of their corresponding starting branched pseudotripeptide **3a**−**d** showed the disappearance of the signal corresponding to the *i*+1 methoxy group and a deshielding of 1.0−1.9 ppm for the α-H proton of the *i*+2 residue and of 0.4−1.2 ppm for the 6-H protons of the piperazine ring. The NOE effect between the piperazine 3-H and 5-H protons, observed in the DPFGSE-NOE spectra of epimers (*S*)-**1a**−**d**, indicative of a relative 3,5-*cis* diaxial disposition, allowed the assignment of the absolute configuration at C_5 in the 2-oxopiperazine derivatives **1a**−**d** and therefore at the branching center in the starting pseudotripeptides **3a**−**d**. This assignment was confirmed in compounds (*R*)- and (*S*)-**1a**, by conversion into their respective 3,6-dioxopehydroimidazo[1,5-*a*]pyrazine derivatives (*R*)- and (*S*)-**9a** (Scheme 4). The NOE effects observed in the ^1H NMR spectra of compounds **9a** allowed the unequivocal assignment of the absolute configuration at C_{8a} in each epimer, and at C_5 in (*R*)- and (*S*)-**1a**, respectively.

The above-mentioned H_3-H_5 NOE effect observed in the 3,5-*cis* epimers (*S*)-**1a**−**d**, along with their $J_{5,6}$ coupling constants (3−5 and 9−12 Hz, see Table 3), indicated the presence of a preferred chair conformation for the 2-oxopiperazine ring, (Figure 2, **A**), in which the R^{i+1} substituent (corresponding to the side chain of the *i*+1 residue of a γ-turn) would adopt an equatorial

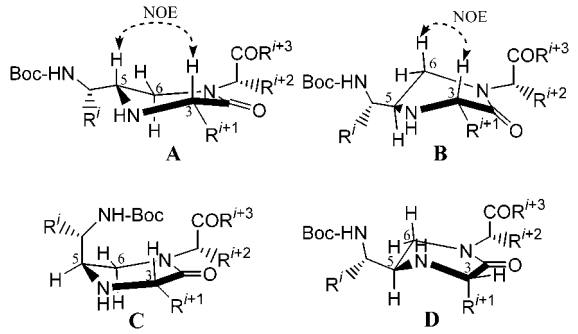
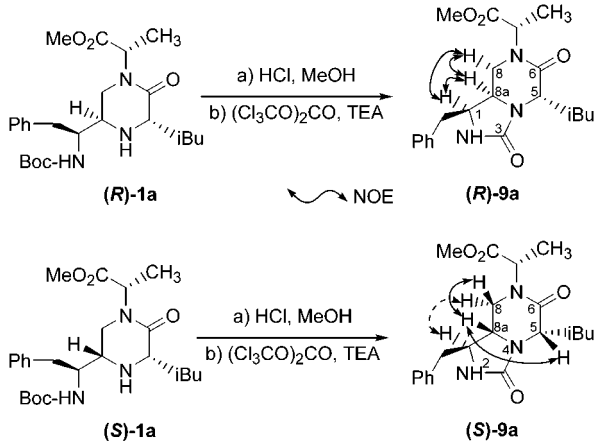


Figure 2. Preferred conformations for the 2-oxopiperazine ring in compounds **1**.

Scheme 4. Configuration Assignment at the C_5 of the 2-Oxopiperazines 1a



disposition. Therefore, these 2-oxopiperazines could mimic inverse γ-turns, in agreement with the previous molecular modeling calculations. Also according to these calculations, the ^1H NMR parameters of 3,5-*trans* epimers (*R*)-**1a**−**d** showed the existence of higher conformational variability. Thus, the $J_{5,6}$ values for (*R*)-**1a** and (*R*)-**1c** (4−5 and 9−10 Hz), and the weak NOE effect observed in their DPFGSE-NOE spectra between 3-H and one of the 6-H protons, indicative of a 1,4-cis diaxial disposition, supported the presence of a preferred boat conformation **B**, in which the R^{i+1} substituent would also adopt an equatorial disposition, as in inverse γ-turns. However, the ^1H NMR spectra of (*R*)-**1b** and (*R*)-**1d** did not show the presence of a preferred conformation between **B**, **C** or **D** (Figure 2).

Synthesis and Biological Evaluation of CCK-4 Analogues. The saponification of the methyl ester of the aspartic side chain in (*R*)- and (*S*)-**1b** led to the conformationally constrained Boc-Trp-Asp-Phe-NH_2 analogues (*R*)- and (*S*)-**10b** (Scheme 5), which contain the most significant side chains for the binding to CCK_2 receptors.[41] On the other hand, as shown in Scheme 5, Boc-removal in the tripeptide derivatives (*R*)- and (*S*)-**1c**,**d**, followed by coupling with Boc-Trp-OH, and final saponification, led to the Boc-CCK-4 analogues **12c** and **12d**. As the replacement of Met by Nle has not a significant effect on the biological activity of CCK-4 analogues,[41] we have included this replacement in the design of com-

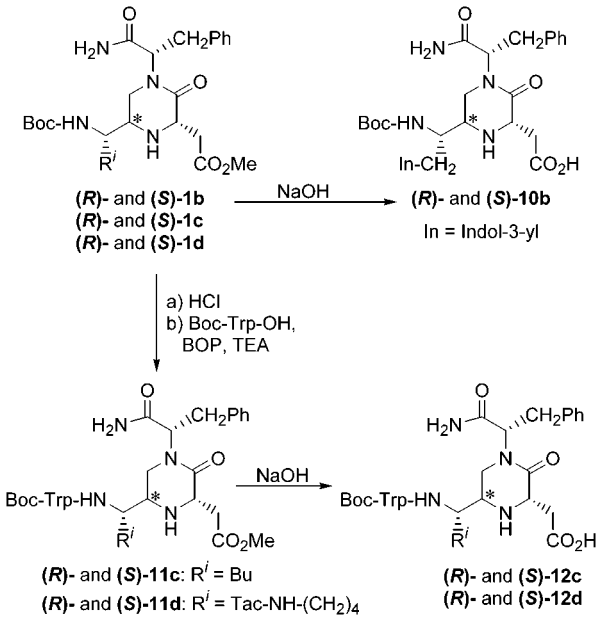
(41) Martinez, J. In *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: Oxford, 1990; Vol. 3, p 929.

Table 3. Relevant ^{1}H NMR Data for the Assignment of Configuration and Conformation in the 2-Oxopiperazines 1a−d

compd	R^i	R^{i+1}	R^{i+2}	R^{i+3}	$J_{5,6}$ (Hz)	NOE (%)	preferred conformation
(*R*)-**1a**	Ph-CH_2	^{i}Bu	Me	OMe	5, 9	H_3−H6 (4)[a]	**B**
(*S*)-**1a**	Ph-CH_2	^{i}Bu	Me	OMe	4, 11	H_3−H5 (12)[b]	**A**
(*R*)-**1b**	In-CH_2[c]	CH_2-CO_2Me	CH_2-Ph	NH_2	4, 7		**B, C, D**
(*S*)-**1b**	In-CH_2[c]	CH_2-CO_2Me	CH_2-Ph	NH_2	4, 10	H_3−H5 (7)[b]	**A**
(*R*)-**1c**	Bu	CH_2-CO_2Me	CH_2-Ph	NH_2	4, 10	H_3−H6 (1)[a]	**B**
(*S*)-**1c**	Bu	CH_2-CO_2Me	CH_2-Ph	NH_2	4, 11	H_3−H5 (5)[a]	**A**
(*R*)-**1d**	Tac-HN−$(CH_2)_4$[d]	CH_2-CO_2Me	CH_2-Ph	NH_2	4, 8		**B, C, D**
(*S*)-**1d**	Tac-HN−$(CH_2)_4$[d]	CH_2-CO_2Me	CH_2-Ph	NH_2	3, 9	H_3−H5 (2)[a]	**A**

[a] Determined in DPFGSE-NOE spectra. [b] Determined in NOE difference spectra. [c] In = Indol-3-yl. [d] Tac = *o*-tolylaminocarbonyl.

Scheme 5. Synthesis of CCK-4 Analogues



pounds **12c**, to avoid the chemical instability of the Met side chain.

Conformationally constrained tripeptides (*R*)- and (*S*)-**10b** and tetrapeptides (*R*)- and (*S*)-**12c** and -**12d** were evaluated as CCK_1 and CCK_2 receptor ligands, by measuring the inhibition of the specific [^{3}H]propionyl-CCK-8 binding to rat pancreas and cerebral cortex homogenates,[42] respectively. For comparative purposes CCK-8 and the CCK_1 and CCK_2 selective antagonists Devazepide[43] and PD-135,158[44] were also included in the assay. Except for the Boc-[Lys(Tac)^{31}]-CCK-4 analogue (*R*)-**12d**, which showed a modest affinity at CCK_1 receptors ($IC_{50} = 6 \times 10^{-7}$ M), none of the 2-oxopiperazine derivatives **10b**, **12c**, and **12d** showed significant affinity for CCK_1 or CCK_2 receptors at concentrations below 10^{-6} M. The important loss of affinity, higher than 2 orders of magnitude for compounds (*R*)- and (*S*)-**12c** at CCK_2 receptors, with respect to Boc-[Nle^{31}]-CCK-4,[45] and 2 or 3 orders of magnitude at CCK_1 receptors for compounds (*R*)- and (*S*)-**12d**, with respect to Boc-[Lys(Tac)^{31}]-CCK-4,[46] suggests the absence of a γ-turn, centered at the Asp residue, in the bioactive conformation of both CCK-4 analogues.

Conclusions

In conclusion, the reductive amination of cyanomethyleneamino pseudodipeptides with amino acid derivatives, followed by regiospecific lactamization affords 2-oxopiperazine derivatives, which could mimic γ-turn conformations and in particular inverse γ-turns. The application of the proposed synthetic methodology to the synthesis of several Boc-CCK-4 analogues illustrates its versatility for the preparation 2-oxopiperazine constrained tripeptide analogues with diverse amino acid side chains. Finally, the loss of affinity at CCK receptors produced by the introduction of this conformational restriction into Boc-CCK-4 analogues discards the hypothesis of the existence of a γ-turn in their bioactive conformation.

Experimental Section

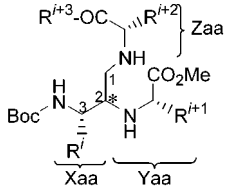
General. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254} and preparative TLC on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254}. Silica gel 60 (230−400 mesh) was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 or 500 MHz, using TMS as reference, and ^{13}C NMR spectra were recorded at 50, 75, or 125 MHz. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical RP-HPLC was performed on a C_{18} (3.9 × 300 mm, 10 mm) 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.

Molecular Modeling Methods. The conformational search was carried out using the molecular dynamics (MD) technique at high temperature and minimization in vacuo ($\epsilon = 1$) with the Insight II program.[47] The MD procedures were carried out by heating the molecules at 1500 K, increasing the temperature 10 K each 0.15 ps, and equilibrating at this temperature during 20 ps. Finally, 75 ps of simulation was carried out, storing 300 structures at equal intervals. Each structure was minimized with the cff91 force field,[48] using initially the steepest descent minimization methods, followed by the conjugate gradient until the gradient was bellow 0.0001 kcal/Å. The minima obtained were compared, and the repeated ones were eliminated. The unique minima were fully optimized with the SAM1 method.[49] Again, the new minima were compared in order to eliminate the repeated ones.

Synthesis of Branched Pseudotripeptides (*R*)- and (*S*)-3a. Et_3N (0.14 mL, 1 mmol) was added to a suspension of H-Ala-OMe·HCl (140 mg, 1 mmol) in MeOH (10 mL), and the mixture was stirred at room temperature for 15 min. Then,

(42) Daugé, V.; Bohme, G. A.; Crawley, J. N.; Durieux, C.; Stutzman, J. M.; Feger, J.; Blanchard, J. C.; Roques, B. P. *Synapse* **1990**, *6*, 73.
(43) Chang, R. S.; Lotti, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4923.
(44) Hughes, J.; Boden, P.; Costall, B.; Domeney, A.; Kelly, E.; Horwell, D. C.; Hunter, J. C.; Pinnock, R. D.; Woodruff, G. N. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6728.
(45) Holladay, M. W.; Lin, C. W.; May, C. S.; Garvey, D. S.; Witte, D. G.; Miller, T. R.; Wolfram, C. A. W.; Nadzan, A. M. *J. Med. Chem.* **1991**, *34*, 455.
(46) Shiosaki, K.; Lin, C. W.; Kopecka, H.; Craig, R.; Wagenaar, F. L.; Bianchi, B.; Miller, T.; Witte, D.; Nadzan, A. M. *J. Med. Chem.* **1990**, *33*, 2950.
(47) Biosym Technologies, Insight II (version 2.2.0), San Diego, CA.
(48) Maple, J. R.; Dinur, U.; Hagler, A. T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5350.
(49) Dewar, M. J. S.; Jie, C.; Yu, J. *Tetrahedron* **1993**, *49*, 5003.

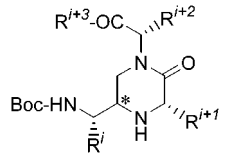
Table 4. Significant Analytical and Spectroscopic Data of Branched Pseudotripeptides 3



	(*R*)-**3a**	(*S*)-**3a**	(*R*)-**3b**	(*S*)-**3b**	(*R*,*S*)-**3c**[a]	(*R*,*S*)-**3d**[b]
R^i	Ph-CH_2	Ph-CH_2	In-CH_2	In-CH_2	Bu	Tac-HN-$(CH_2)_4$
R^{i+1}	^{i}Bu	^{i}Bu	CH_2-CO_2Me	CH_2-CO_2Me	CH_2-CO_2Me	CH_2-CO_2Me
R^{i+2}	Me	Me	Ph-CH_2	Ph-CH_2	Ph-CH_2	Ph-CH_2
R^{i+3}	OMe	OMe	NH_2	NH_2	NH_2	NH_2
config (*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*,*S*)	(*R*,*S*)
yield (%)	34	32	46	22	71	54
formula[c]	$C_{26}H_{43}N_3O_6$	$C_{26}H_{43}N_3O_6$	$C_{32}H_{43}N_5O_7$	$C_{32}H_{43}N_5O_7$	$C_{27}H_{44}N_4O_7$	$C_{35}H_{52}N_6O_8$
t_R (min) (A:B)[d]	25.41 (40:60)	23.33 (40:60)	16.00 (35:65)	14.80 (35:65)	16.90, 15.35 (35:65)	10.20, 9.65 (40:60)
			^{1}H NMR[e](δ)			
Xaa						
1-H	2.51, 2.71	2.55	2.39, 2.69	2.34, 2.52	2.30, 2.60	2.30, 2.62; 2.38, 2.52
2-H	2.36	2.55	2.66	2.52	2.60	2.50
3-H	3.87	3.80	4.15	4.07	3.60, 3.50	3.69, 3.48
4-H	2.59, 2.88	2.73, 2.81	2.78, 2.92	2.88, 2.96	1.20	1.25, 1.48
2-H (Yaa)	3.25	3.37	3.63	3.70	3.70	3.63, 3.60
2-H (Zaa)	3.25	3.21	3.13	3.20	3.30, 3.35	3.28, 3.30
			^{13}C NMR[f]			
C_1 (Xaa)	45.96	51.66	47.25	51.26	47.40	47.62, 50.98

[a] (3:1) (*R*:*S*)-Epimer ratio. [b] (2:1) (*R*:*S*)-Epimer ratio. [c] Compounds isolated as foams. Satisfactory analyses for C, H, N. [d] μBondapak C_{18}, A = CH_3CN, B = 0.05% TFA in H_2O. [e] Spectra registered at 300 or 500 MHz in $CDCl_3$. Assignment carried out with the help of DQCOSY spectra. [f] Spectra registered at 50 or 125 MHz in $CDCl_3$. Assignment carried out with the help of HMQC spectra.

Table 5. Analytical Data of 2-Oxopiperazines 1a−d



compd[a]	R^i	R^{i+1}	R^{i+2}	R^{i+3}	config (*)	yield (%)	formula[b]	t_R (A:B)[c]
(*R*)-**1a**	Ph-CH_2	^{i}Bu	Me	OMe	(5*R*)	81	$C_{25}H_{39}N_3O_5$	6.91 (50:50)
(*S*)-**1a**	Ph-CH_2	^{i}Bu	Me	OMe	(5*S*)	77	$C_{25}H_{39}N_3O_5$	4.80 (50:50)
(*R*)-**1b**	In-CH_2	CH_2-CO_2Me	CH_2-Ph	NH_2	(5*R*)	75	$C_{31}H_{39}N_5O_6$	20.40 (30:70)
(*S*)-**1b**	In-CH_2	CH_2-CO_2Me	CH_2-Ph	NH_2	(5*S*)	62	$C_{31}H_{39}N_5O_6$	18.51 (30:70)
(*R*)-**1c**	Bu	CH_2-CO_2Me	CH_2-Ph	NH_2	(5*R*)	60	$C_{26}H_{40}N_4O_6$	20.53 (30:70)
(*S*)-**1c**	Bu	CH_2-CO_2Me	CH_2-Ph	NH_2	(5*S*)	19	$C_{26}H_{40}N_4O_6$	18.26 (30:70)
(*R*)-**1d**	Tac-HN-$(CH_2)_4$	CH_2-CO_2Me	CH_2-Ph	NH_2	(5*R*)	46	$C_{34}H_{48}N_6O_7$	9.70 (40:60)
(*S*)-**1d**	Tac-HN-$(CH_2)_4$	CH_2-CO_2Me	CH_2-Ph	NH_2	(5*S*)	21	$C_{34}H_{48}N_6O_7$	6.11 (40:60)

[a] Foams, except for (*R*)-**1a**, white solid, mp 142−144 °C (EtOAc−hexane). [b] Satisfactory analyses for C, H, N. [c] μBondapak C_{18}, A = CH_3CN, B = 0.05% TFA in H_2O.

the pseudodipeptide Boc-PheΨ[CH(CN)NH]Leu-OMe [(*R*,*S*)-**4a**] (200 mg, 0.5 mmol) and 10% Pd(C) (300 mg) were added, and the mixture was hydrogenated at 1 atm of H_2 and room temperature for 8 h. Afterward, the catalyst was filtered off and washed with MeOH, the solvent was evaporated, and the reaction crude was dissolved in EtOAc (20 mL). This solution was washed successively with H_2O and brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography using 5−30% EtOAc in hexane gradient as mobile phase. Significant analytical and spectroscopic data of (*R*)- and (*S*)-**3a** are summarized in Table 4.

General Procedure for the Preparation of Branched Pseudotripeptides 3b−d. Pd(C) (10%, 300 mg) was added to a solution of the corresponding Ψ[CH(CN)NH] pseudodipeptide (*R*,*S*)-**4b**−**d** (0.5 mmol) and H-Phe-NH_2 (**6**) (164 mg, 1 mmol) in MeOH (10 mL), and the mixture was hydrogenated at 1 atm of H_2 and room temperature for 5 days. Afterward, the reaction mixture was worked up as above indicated for the synthesis of **3a**. The resulting branched pseudotripeptides **3b**−**d** were purified by flash chromatography, using 0−6% MeOH in CH_2Cl_2 gradient as mobile phase. Significant analytical and spectroscopic data of these compounds are summarized in Table 4.

General Procedure for the Synthesis of 2-Oxopiperazines 1a−d. A solution of the corresponding branched pseudotripeptide **3a**−**d** (0.3 mmol) in toluene (50 mL) was stirred at 100 °C for 6 h to 8 days, depending on the starting pseudotripeptide. Then, the solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography, using 10−50% EtOAc in hexane (**1a** and **1c**) or 1−5% MeOH in CH_2Cl_2 (**1b** and **1d**) gradients as mobile phases. (*R*)-Epimers and (*S*)-epimers of **1c** and **1d** were resolved in this chromatographic purification. The significant analytical and spectroscopic data of these conformationally constrained tripeptide analogues are summarized in Tables 5 and 6.

Synthesis of the 2-Oxo-1,2,5,6-tetrahydropyrazines (*R*)- and (*S*)-8a. A solution of the 2-oxopiperazines (*R*)- and (*S*)-**1a** (69 mg, 0.15 mmol) in xylene (25 mL) was heated at 135 °C for 8 and 16 h, respectively. Afterward, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using 10−50% EtOAc in hexane gradient as mobile phase.

Table 6. Relevant ^{1}H NMR[a] Data of 2-Oxopiperazines 1a−d

compd	3-H	5-H	6-H^{ax}	6-H^{ec}	$J_{5,6}$ (Hz)	3-CH$_2$	5-CH	1-CH
(*R*)-**1a**	3.53	3.20	3.26	3.35	5, 9	1.55, 1.70	3.80	5.15
(*S*)-**1a**	3.60	3.35	3.21	3.10	4, 11	1.40, 1.86	3.88	5.06
(*R*)-**1b**	3.84	2.76	3.12	3.36	4, 7	2.43, 2.72	3.80	5.24
(*S*)-**1b**	3.58	3.40	2.74	3.22	4, 10	2.63, 2.86	3.80	4.60
(*R*)-**1c**	3.75	2.78	3.20	3.44	4, 10	2.20, 2.47	3.43	5.40
(*S*)-**1c**	3.70	2.92	3.23	3.17	4, 11	2.37, 2.76	3.51	5.10
(*R*)-**1d**	3.84	2.79	3.16	3.37	4, 8	2.40, 2.71	3.50	4.82
(*S*)-**1d**	3.70	2.73	3.20	3.04	3, 9	2.67, 2.85	3.50	4.90

[a] Spectra registered at 300 or 500 MHz in $CDCl_3$, except for (*R*)- and (*S*)-**1c**, registered in $(CD_3)_2CO$. Assignment carried out with the help of DQCOSY spectra.

(5*R*)-3-Isobutyl-5-[(1*S*)-1-(*tert*-butyloxycarbonylamino)-2-phenylethyl]-1-[(1*S*)-1-methoxycarbonylethyl]-2-oxo-1,2,5,6-tetrahydropyrazine [(*R*)-8a]: white solid (35 mg, 51%); mp 42−45 °C (EtOAc−hexane); t_R 12.13 (A:B = 50:50); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.94 and 0.97 [2d, 6H, J = 7 Hz, CH_3 (^{i}Bu)],1.36 (s, 9H, Boc), 1.44 [d, 3H, J = 7 Hz, CH_3 (Ala)], 2.10 [m, 1H, CH (^{i}Bu)], 2.40 [dd, 1H, J = 6 and 14 Hz, CH_2 (^{i}Bu)], 2.66 [dd, 1H, J = 5 and 14 Hz, CH_2 (^{i}Bu)], 2.98 (d, 2H, J = 8 Hz, *CH*$_2$Ph), 3.34 (dd, 1H, J = 9.5 and 13 Hz, 6-H), 3.70 (m, 2H, 5-H and 6-H), 3.72 (s, 3H, OMe), 4.02 (m, 1H, 5-CH), 5.04 (d, 1H, J = 9 Hz, NH), 5.16 [q, 1H, J = 7 Hz, 1-H (Ala)], 7.08−7.20 (m, 5H, aromatics); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.21 (CH_3), 23.43 (CH_3), 23.21 (CH_3), 27.00 (CH), 29.03 (CH_3), 30.46 (CH_2), 39.99 (CH_2), 42.34 (CH_2), 52.40 (CH), 53.25 (CH_3), 53.64 (CH), 59.81 (CH), 80.00 (C), 126.41, 128.81, 129.44 (CH), 137.82 (C), 155.30, 158.00, 165.22, 171.57 (C). Anal. Calcd for $C_{25}H_{37}N_3O_5$: C, 65.34; H, 8.11; N, 9.14. Found: C, 65.30; H, 8.38; N, 8.98.

(5*S*)-3-Isobutyl-5-[(1*S*)-1-(*tert*-butyloxycarbonylamino)-2-phenylethyl]-1-[(1*S*)-1-methoxycarbonylethyl]-2-oxo-1,2,5,6-tetrahydropyrazine [(*S*)-8a]: foam (46 mg, 67%); t_R 14.30 (A:B = 50:50); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.98 and 0.99 [2d, 6H, J = 6 Hz, CH_3 (^{i}Bu)],1.40 (s, 9H, Boc), 1.40 [d, 3H, J = 8 Hz, CH_3 (Ala)], 2.20 [m, 1H, CH (^{i}Bu)], 2.55 [m, 2H, CH_2 (^{i}Bu)], 3.07 (d, 2H, J = 7 Hz, *CH*$_2$Ph), 3.15 (dd, 1H, J = 4 and 12.5 Hz, 6-H), 3.28 (t, 1H, J = 12.5 Hz, 6-H), 3.60 (m, 1H, 5-H), 3.57 (s, 3H, OMe), 4.00 (m, 1H, 5-CH), 4.71 (d, 1H, J = 10 Hz, NH), 5.20 [q, 1H, J = 7 Hz, 1-H (Ala)], 7.10−7.40 (m, 5H, aromatics); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.18 (CH_3), 22.37 (CH_3), 22.80 (CH_3), 25.96 (CH), 28.29 (CH_3), 29.71 (CH_2), 39.29 (CH_2), 42.59 (CH_2), 51.92 (CH), 52.36 (CH_3), 52.93 (CH), 56.93 (CH), 79.50 (C), 126.49, 128.55, 129.51 (CH), 136.00 (C), 152.41, 155.69, 166.00, 169.50 (C). Anal. Calcd for $C_{25}H_{37}N_3O_5$: C, 65.34; H, 8.11; N, 9.14. Found: C, 65.07; H, 8.41; N, 8.85.

Synthesis of the 3,6-Dioxoperhydroimidazo[1,5-*a*]pyrazines (*R*)- and (*S*)-9a. A solution of the corresponding 2-oxopiperazine (*R*)- and (*S*)-**1a** (25 mg, 54 mmol) in saturated solution of HCl in MeOH (5 mL) was stirred at room temperature for 5 h. After the solvent was removed under reduced pressure, the residue was dissolved in water and lyophilized. The residue was dissolved in dry CH_2Cl_2 (5 mL), and the solution was cooled to 0 °C. Then, TEA (15 μL, 108 μmol), bis(trichloromethyl) carbonate (8 mg, 27 μmol), and TEA (22 μL, 162 μmol) were added successively, and the reaction mixture was stirred at 0 °C for 4 h. Afterward, this reaction mixture was diluted with CH_2Cl_2 (5 mL), washed with water and brine, dried over Na_2SO_4, and avaporated. The residue was purified by preparative TLC using (1:1) EtOAc−hexane as eluant.

(1*S*,5*S*,8a*R*)-5-Isobutyl-7-[(1*S*)-1-methoxycarbonylethyl]-1-phenylmethyl-3,6-dioxoperhydroimidazo[1,5-*a*]pyrazine [(*R*)-9a]: foam (13 mg, 52%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86 and 0.93 [2d, 6H, J = 6 Hz, 3-H (^{i}Bu)], 1.32 [d, 3H, J = 7 Hz, 2-H (ethyl)], 1.53 [m, 1H, 1-H (^{i}Bu)], 1.69 [m, 1H, 2-H (^{i}Bu)], 1.73 [m, 1H, 1-H (^{i}Bu)], 2.71 (dd, 1H, J = 10 and 13.5 Hz, CH_2-Ph), 2.82 (dd, 1H, J = 5 and 13.5 Hz, CH_2-Ph), 3.10 (dd, 1H, J = 4 and 11 Hz, 8-H), 3.68 (s, 3H, OCH_3), 3.69 (t, 1H, J = 11 Hz, 8-H), 3.86 (m, 1H, 8a−H), 4.08 (m, 1H, 1-H), 4.39 (dd, 1H, J = 4 and 11 Hz, 5-H), 4.50 (s, 1H, 2-H), 5.08 [q, 1H, J = 7 Hz, 1-H (ethyl)], 7.21 (m, 5H, aromatics). Anal. Calcd for $C_{21}H_{29}N_3O_4$: C, 65.09; H, 7.54; N, 10.84. Found: C, 65.42; H, 7.87; N, 11.01.

(1*S*,5*S*,8a*S*)-5-Isobutyl-7-[(1*S*)-1-methoxycarbonylethyl]-1-phenylmethyl-3,6-dioxoperhydro-imidazo[1,5-*a*]pyrazine [(*S*)-9a]: foam (11 mg, 53%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90 and 0.92 [2d, 6H, J = 6 Hz, 3-H (^{i}Bu)], 1.28 [d, 3H, J = 7 Hz, 2-H (ethyl)], 1.80 [m, 1H, 1-H (^{i}Bu)], 2.00 and 2.08 [2m, 2H, 2-H (^{i}Bu)], 2.80 (dd, 1H, J = 3 and 11 Hz, 8-H), 2.86 (dd, 1H, J = 7 and 13 Hz, CH_2-Ph), 3.00 (dd, 1H, J = 4 and 13 Hz, CH_2-Ph), 3.15 (dd, 1H, J = 10 and 11 Hz, 8-H), 3.68 (m, 2H, 1-H and 8a−H), 3.73 (s, 3H, OCH_3), 4.19 (dd, 1H, J = 4 and 7 Hz, 5-H), 4.86 (s, 1H, 2-H), 5.18 [q, 1H, J = 7 Hz, 1-H (ethyl)], 7.21 (m, 5H, aromatics). Anal. Calcd for $C_{21}H_{29}N_3O_4$: C, 65.09; H, 7.54; N, 10.84. Found: C, 65.31; H, 7.65; N, 10.48.

Synthesis of the 2-Oxopiperazine Derivatives (*R*)- and (*S*)-10b. NaOH (1 N, 0.5 mL, 50 mmol) was added to a solution of the corresponding methyl ester (*R*)- and (*S*)-**10b** (23 mg, 40 μmol) in 10% H_2O in MeOH (10 mL), and the mixture was stirred at room temperature for 3 h. Afterward, the solvent was removed under reduced pressure, the residue was dissolved in H_2O, and the solution was washed with CH_2Cl_2. The aqueous phase was acidified to pH 5−6, adding DOWEX 50×4 acid resin. After adding EtOAc, the resin was filtered off, and the two phases were separated. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was triturated with diethyl ether, to yield the corresponding carboxylic acid (*R*)- or (*S*)-**10b** quantitatively.

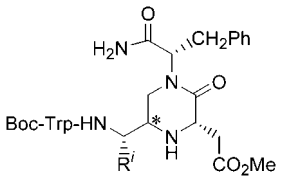
(3*S*,5*R*)-5-[(1*S*)-1-(*tert*-Butyloxycarbonylamino)-2-(indol-3-yl)ethyl]-1-[(1*S*)-1-carbamoyl-2-phenylethyl]-3-carboxymethyl-2-oxopiperazine [(*R*)-10b]: white solid (22 mg, 100%); mp 159−161 °C (CH_2Cl_2−MeOH); t_R 7.73 (A:B = 50:50); ^{1}H NMR [$(CD_3)_2CO$ + D_2O, 300 MHz] δ 1.30 [s, 9H, CH_3 (Boc)], 2.10−2.40 [m, 2H, 2-H (Asp)], 2.80−3.50 [m, 8H, 5-H, 6-H, 2-H and 3-H (Trp), and 3-H (Phe)], 3.80−4.00 [m, 2H, 3-H and 2-H (Phe)], 5.30 (bs, 1H, N*H*-Boc), 6.90−7.30 [m, 8H, Ph, and 2-, 5- and 6-H (In)], 7.35 [d, 1H, J = 7 Hz, 7-H (In)], 7.53 [d, 1H, J = 7 Hz, 4-H (In)], 10.15 [bs,1H, NH (In)]. Anal. Calcd for $C_{30}H_{37}N_5O_6$: C, 63.93; H, 6.62; N, 12.43. Found: C, 63.84; H, 6.73; N, 12.16.

(3*S*,5*S*)-5-[(1*S*)-1-(*tert*-Butyloxycarbonylamino)-2-(indol-3-yl)ethyl]-1-[(1*S*)-1-carbamoyl-2-phenylethyl]-3-carboxymethyl-2-oxopiperazine [(*S*)-10b]: white solid (21 mg, 100%); mp 165−168 °C (CH_2Cl_2−MeOH); t_R 9.33 (A:B = 50:50); ^{1}H NMR [$(CD_3)_2CO$ + D_2O, 300 MHz] δ 1.30 [s, 9H, CH_3 (Boc)], 2.40 [dd, 1H, J = 8 and 16 Hz, 2-H (Asp)], 2.60−2.90 [m, 3H, 5-H and 3-H (Phe)], 2.80 [dd, 1H, J = 5 and 16 Hz, 2-H (Asp)], 3.00−3.40 [m, 4H, 6-H, and 3-H (Trp)], 3.60 [m, 1H, 2-H (Trp)], 4.00 [m, 2H, 3-H, and 2-H (Phe)], 6.25 (d, 1H, J = 8 Hz, N*H*-Boc), 6.70−7.10 [m, 8H, Ph, and 2-, 5- and 6-H (In)], 7.40 [d, 1H, J = 7 Hz, 7-H (In)], 7.50 [d, 1H, J = 7 Hz, 4-H (In)], 10.30 [bs,1H, NH (In)]. Anal. Calcd for $C_{30}H_{37}N_5O_6$: C, 63.93; H, 6.62; N, 12.43. Found: C, 63.83; H, 6.72; N, 12.36.

General Procedure for the Synthesis the 2-Oxopiperazine-Based Tetrapeptide Analogues 11c and 11d. A solution of the corresponding 2-oxopiperazine derivative (*R*)- and (*S*)-**1c,d** (50 mmol) in saturated solution of HCl in MeOH (5 mL) was stirred at room temperature for 5 h. After the solvent was removed under reduced pressure, the residue was dissolved in water and lyophilized. The resulting residue was dissolved in dry CH_2Cl_2 (5 mL); Boc-L-Trp-OH (22 mg, 75 μmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 32 mg, 75 μmol), and TEA (13 μL, 100 μmol) were added successively to that solution; and stirring was continued at room temperature for 16 h. The reaction mixture was diluted with CH_2Cl_2, washed successively with 10% citric acid, 10% $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and evaporated. The residue was purified by preparative TLC using 2% MeOH in CH_2Cl_2 as eluant. The relevant analytical and spectroscopic data of compounds (*R*)- and (*S*)-**11c**,**d** are summarized in Table 7.

Synthesis of the Boc-CCK-4 Analogues (*R*)- and (*S*)-12c,d. These compounds were prepared by saponification of the methyl esters (*R*)- and (*S*)-**11c**,**d** (40 μmol), by applying the methodology described above for the preparation of the

Table 7. Relevant Analytical and Spectroscopic Data of 2-Oxopiperazine-Based Tetrapeptide Analogues 11c,d[a]



	(*R*)-**11c**	(*S*)-**11c**	(*R*)-**11d**	(*S*)-**11d**
R^i	$CH_3(CH_2)_3$	$CH_3(CH_2)_3$	Tac-NH-$(CH_2)_4$	Tac-NH-$(CH_2)_4$
config (*)	(*R*)	(*S*)	(*R*)	(*S*)
yield (%)	84	76	79	73
t_R (min) (A:B)[b]	9.90 (40:60)	9.98 (40:60)	33.55 (32:68)	37.41 (32:68)
formula[c]	$C_{37}H_{50}N_6O_7$	$C_{37}H_{50}N_6O_7$	$C_{45}H_{59}N_8O$	$C_{45}H_{59}N_8O$
		^{1}H NMR[d] (δ)		
2-oxopiperazine				
3-H	3.50	3.46	3.52	3.57
5-H	2.10	2.25	2.32	2.46
6-H^{ax}	2.65	2.55	2.77	2.88
6-H^{ec}	2.85	2.35	3.00	2.64
$J_{5,6}^{ax}$	9	10	8.5	11
$J_{5,6}^{ec}$	5	2	4	3
3-CH_2	2.20, 2.50	2.38, 2.70	2.28, 2.58	2.54, 2.76
5-CH	3.65	3.46	3.58	3.63
1-CH	5.22	4.17	5.18	4.94
Trp				
2-H	4.27	4.30	4.44	4.46
3-H	3.05, 3.13	3.05, 3.12	3.17, 3.20	3.14, 3.30

[a] Foams, except for (*R*)-**11d**, mp 115−118 °C (EtOAc−hexane). [b] μBondapak C_{18}, A = CH_3CN, B = 0.05% TFA in H_2O. [c] Satisfactory analyses for C, H, N. [c] Spectra registered at 500 MHz in $CDCl_3$. Assignment carried out with the help of DQCOSY spectra.

Table 8. Relevant Analytical and Spectroscopic Data of the Boc-CCK-4 Analogues 12c,d[a]

	(*R*)-**12c**	(*S*)-**12c**	(*R*)-**12d**	(*S*)-**12d**
R^i	$CH_3(CH_2)_3$	$CH_3(CH_2)_3$	Tac-NH−$(CH_2)_4$	Tac-NH−$(CH_2)_4$
config (*)	(*R*)	(*S*)	(*R*)	(*S*)
mp (°C)	151−154	110−113	120−123	130−132
t_R (min) (A:B)[b]	13.65 (35:65)	15.60 (35:65)	15.23 (35:65)	16.61 (35:65)
formula[c]	$C_{36}H_{48}N_6O_7$	$C_{36}H_{48}N_6O_7$	$C_{44}H_{57}N_8O_8$	$C_{44}H_{57}N_8O_8$
		^{1}H NMR[d] (δ)		
2-oxopiperazine				
3-H	3.60	3.24	3.67	3.34
5-H	2.65	2.24	2.74	2.37
6-H^{ax}	2.85	2.88	3.42	3.04
6-H^{ec}	3.20	2.12	3.48	3.07
$J_{5,6}^{ax}$	10	11	14	14.5
$J_{5,6}^{ec}$	3	1	9	7
3-CH_2	2.08, 2.30	2.42, 2.73	2.07, 2.36	2.50, 2.76
5-CH	3.84	3.67	3.84	3.40
1-CH	5.24	4.30	5.35	4.40
Trp				
2-H	4.42	3.75	4.47	3.99
3-H	3.04, 3.28	3.24	3.13, 3.27	3.27

[a] Obtained in quantitative yields. [b] μBondapak C_{18}, A = CH_3CN, B = 0.05% TFA in H_2O. [c] Satisfactory analyses for C, H, N. [c] Spectra registered at 500 MHz in $(CD_3)_2CO$ + D_2O. Assignment carried out with the help of DQCOSY spectra.

carboxylic acids **10b**. The relevant analytical and spectroscopic data of compounds (*R*)- and (*S*)-**12c**,**d** are summarized in Table 8.

CCK_1 and CCK_2 Receptor Binding Assays. Binding assays were performed using rat pancreas and cerebral cortex homogenates, respectively, according to the method described by Daugé et al.[42] 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]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 100 000g. 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.

Acknowledgment. This work has been supported by the CICYT (SAF00-0147), and Consejeria de Educación y Cultura de la Comunidad de Madrid (CAM-08.5/0006/1998).

Supporting Information Available: Complete combustion elemental analytical date for the new described compounds, and significant ^{13}C NMR data for the 2-oxopiperazine derivatives **1a**−**d**, **11c**,**d**, and **12c**,**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0256336