JOC Article
pubs.acs.org/joc

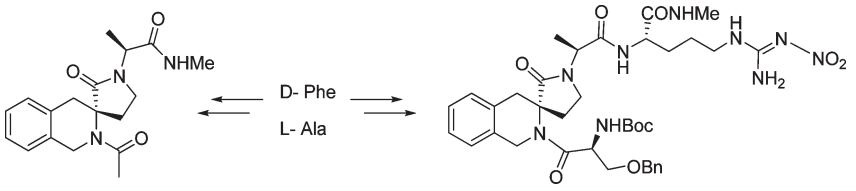
Tetrahydroisoquinoline-Based Spirocyclic Lactam as a Type II′ β-Turn Inducing Peptide Mimetic

Giordano Lesma,[†] Nicola Landoni,[†] Tullio Pilati,[‡] Alessandro Sacchetti,[§] and Alessandra Silvani*,[†]

[†]*Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, via G. Venezian 21, 20133 Milano, Italy,* [‡]*Istituto di Scienze e Tecnologie Molecolari−CNR, Via Golgi 19, 20133 Milano, Italy, and* [§]*Politecnico di Milano, Dipartimento di Chimica, Materiali ed Ingegneria Chimica 'Giulio Natta', via Mancinelli 7, 20131 Milano, Italy*

alessandra.silvani@unimi.it

Received July 10, 2009



We present here spirocyclic lactam derivatives, embodying D-Phe and L-Ala amino acids as the central core and acting as tetrapeptide and hexapeptide mimetics. An efficient route for their synthesis is demonstrated by using the strategic combination of Seebach's self-reproduction of chirality chemistry and the Pictet−Spengler condensation as key steps. The conformational behavior of peptide mimetics was investigated by molecular modeling, X-ray crystallography, NMR (solvent and temperature dependence), IR spectroscopy, and circular dichroism. All data suggest very stable and highly predictable type II′ β-turn conformations.

Introduction

Protein−protein and peptide−protein interactions play key roles in most biological processes, and therefore represent valuable targets for drug discovery. A general approach in the search of new chemical entities able to interfere with these interactions is the use of small compounds mimicking or inducing precise aspects of specific peptide secondary structures.[1] Among the secondary structure elements, reverse-turns are structural motifs commonly found in bioactive peptides that, besides being fundamental in protein folding, play a central role as molecular recognition elements.[2]

As an example, over 100 peptide-activated G protein-coupled receptors recognize ligands with turn structures.[3] These receptors represent a large, promising, and still underdeveloped source of new pharmaceutical targets for treating human deseases or medical conditions.

Therefore, drug research is focused on scaffolds that, by restricting the conformational freedom of the peptide chain, provide structural stabilization if incorporated in short linear or cyclic peptides, in order to enhance both target binding and selectivity properties. Several reverse-turn surrogates have been employed to constrain the backbone geometry and side-chain conformations, and to so develop potent and selective peptidomimetic therapeutic agents.[4]

(1) For reviews, see: (a) Ohfune, Y.; Shinada, T. *Eur. J. Org. Chem.* **2005**, *24*, 5127–5143. (b) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (c) Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585–615. (d) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789–12854.

(2) (a) Marcelino, A. M. C.; Gierasch, L. M. *Biopolymers* **2008**, *89*, 380−391 and references cited therein. (b) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467–3478.

(3) Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793–826.

(4) For selected references about reverse-turn surrogates, see: (a) Beierle, J. M.; Horne, W. S.; van Maarseveen, J. H.; Waser, B.; Reubi, J. C.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 4725–4729. (b) Molteni, M.; Bellucci, M. C.; Bigotti, S.; Mazzini, S.; Volonterio, A.; Zanda, M. *Org. Biomol. Chem.* **2009**, *7*, 2286–2296. (c) Trabocchi, A.; Sladojevich, F.; Guarna, A. *Chirality* **2009**, *21*, 584–594. (d) Arbor, S.; Kao, J.; Wu, Y.; Marshall, G. R. *Biopolymers* **2008**, *90*, 384–393. (e) Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Benito, A.; Cuerdo, L.; Fratila, R. M.; Jimenez, A.; Loinaz, I.; Miranda, J. I.; Pytlewska, K. R.; Micle, A.; Linden, A.; et al. *Letters* **2004**, *6*, 4443–4446. (f) Gutierrez-Rodriguez, M.; Garcia-Lopez, M. T.; Herranz, R. *Tetrahedron* **2004**, *60*, 5177–5183.

Published on Web 09/29/2009 DOI: 10.1021/jo901480d

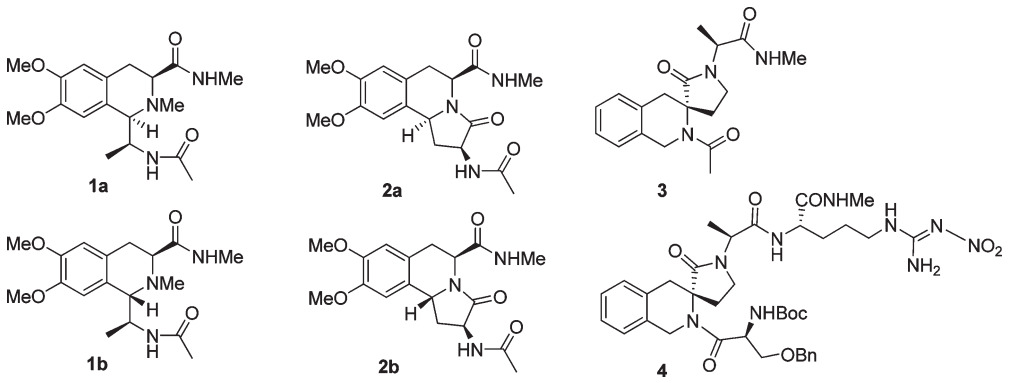


FIGURE 1. Tetrahydroisoquinoline-based reverse turn mimetics.

Conformationally constrained amino acid mimics have found widespread use in this context.[5]

In our ongoing program of identification of scaffolds of low molecular weight of potential interest in drug discovery, we recently focused on bicyclic and tricyclic frameworks based on the 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, commonly referred to as Tic, as a common privileged substructure.[6]

The Tic core, in which a carbon atom connects the aromatic ring to the backbone amino group, is a key element in several peptide-based drugs, since its incorporation not only restricts the conformational freedom of the aromatic ring but also places constraints on the peptide backbone.[7] For instance, Tic is an essential feature of numerous potent, high-affinity δ- and κ-selective opioid receptor agonists and antagonists. They have been prepared by systematic modification of endogenous GPCRs ligands, such as enkephalin, endomorphin, and dynorphin, thus allowing a better understanding of how opioid receptors function at the molecular level.[8] Also in the design and synthesis of peptidomimetics acting as antagonists of the GPCRs ligand bradykinin, SAR studies had revealed a pharmacophore containing a D-Tic residue among the key features around the backbone core.[9]

Moreover, spectroscopic studies have shown that the high affinity of the potent bradykinin analogue HOE 140[10] for B_2 receptors is related to its high propensity to adopt a C-terminal β-turn conformation,[11] spanning the residue Ser^6-D-Tic^7-Oic^8-Arg^9 (Oic = octahydroindole-2-carboxylic acid). Quite recently, a modest but significant increase in therapeutic index for a d-Tic containing analogue of antimicrobial peptide gramicidin S (GS), has been demonstrated generated by replacing the D-Phe residue in both the β-turn regions of the native peptide.[12]

By incorporating Tic into position $i + 2$ of model peptide surrogates, we were able to generate reverse turn mimetics, such as **1a**,**b** and **2a**,**b** (Figure 1), which can be considered Ac-Ala-Phe-NHMe constrained isosteres.[5] In particular, conformational studies performed by NMR, IR, and molecular modeling techniques demonstrated a stable type II′ β-turn conformation in solution for tetrapeptide analogue **2b**. Through a variation of our synthetic approach, the Tic-derived reverse turn nucleating moiety was now moved into position $i + 1$, enabling the formation of a spirocyclic lactam bridge to the backbone nitrogen of aminoacid at position $i + 2$.[13] The obtained spiro-pyrrolo-tetrahydroisoquinoline (SIPP) (SIPP = 2-(2′-oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanoic acid) scaffold, embodied in the tetrapeptide Ac-Phe-Ala-NHMe analogue **3**, adopted conformations almost ideally matching the prerequisites for canonical type II′ β-turn. From MM calculations, the size of the lactam ring seems to play a crucial role in determining the favorite conformations, with the structure **3**, embodying a five-membered ring, showing the best aptitude for β-turn.

In this paper, we describe an efficient synthetic approach to the model peptide surrogate **3** and a combination of

(5) (a) Fustero, S.; Mateu, N.; Albert, L.; Aceña, J. L. *J. Org. Chem.* **2009**, *74*, 4429–4432. (b) Scheffelaar, R.; Nijenhuis, R. A. K.; Paravidino, M.; Lutz, M.; Spek, A. L.; Ehlers, A. W.; de Kanter, F. J. J.; Groen, M. B.; Orru, R. V. A.; Ruijter, E. *J. Org. Chem.* **2009**, *74*, 660–668. (c) Hanessian, S.; Auzzas, L. *Acc. Chem. Res.* **2008**, *41*, 1241–1251. (d) Lesma, G.; Colombo, A.; Sacchetti, A.; Silvani, A. *Tetrahedron Lett.* **2008**, *49*, 7423–7425. (e) Bitterman, H.; Gmeiner, P. *J. Org. Chem.* **2006**, *71*, 97–102. (f) Bitterman, H.; Böckler, F.; Jürgen, E.; Gmeiner, P. *Chem.—Eur. J.* **2006**, *12*, 6315–6322. (g) Gardiner, J.; Abell, A. D. *Org. Biomol. Chem.* **2004**, *2*, 2365–2370. (h) Wang, W.; Zhang, J.; Xiong, C.; Hruby, V. J. *Tetrahedron Lett.* **2002**, *43*, 2137–2140. (i) Cativiela, C.; Díaz-de-Villegas, M. D. *Tetrahedron: Asymmetry* **2000**, *11*, 645–732.

(6) (a) Lesma, G.; Landoni, N.; Sacchetti, A.; Silvani, A. *J. Org. Chem.* **2007**, *72*, 9765–9768. (b) Lesma, G.; Meschini, E.; Recca, T.; Sacchetti, A.; Silvani, A. *Tetrahedron* **2007**, *63*, 5567–5578.

(7) (a) Kotha, S.; Khedkar, P. *Synthesis* **2008**, *18*, 2925–2928. (b) Chen, K. X.; Njoroge, F. G.; Pichardo, J.; Prongay, A.; Butkiewicz, N.; Yao, N.; Madison, V.; Girijavallabhan, V. *J. Med. Chem.* **2006**, *49*, 567–574. (c) Wang, W.; Cai, M.; Xiong, C.; Zhang, J.; Trivedi, D.; Hruby, V. J. *Tetrahedron* **2002**, *58*, 7365–7374. (d) Gibson (née Thomas), S. E.; Guillo, N.; Jones, J. O.; Buck, I. M.; Kalindjian, S. B.; Roberts, S.; Tozer, M. J. *Eur. J. Med. Chem.* **2002**, *37*, 379–389.

(8) (a) Neumeyer, J. L.; Peng, X.; Knapp, B. I.; Bidlack, J. M.; Lazarus, L. H.; Salvadori, S.; Trapella, C.; Balboni, G. *J. Med. Chem.* **2006**, *49*, 5640-5643 and references cited therein. (b) Eguchi, M. *Med. Res. Rev.* **2004**, *24*, 182–212. (c) Lazarus, L. H.; Bryant, S. D.; Cooper, P. S.; Guerrini, R.; Balboni, G.; Salvadori, S. *Drug Disc. Today* **1998**, *3*, 284–294.

(9) (a) Reissmann, S.; Imhof, D. *Curr. Med. Chem.* **2004**, *11*, 2823–2844. (b) Monteagudo, E. S.; Calvani, F.; Catrambone, F.; Fincham, C. I.; Madami, A.; Meini, S.; Terracciano, R. *J. Peptide Sci.* **2001**, *7*, 270–283.

(10) Lembeck, F.; Griesbacher, T.; Eckhardt, M.; Henke, St.; Breipohl, G.; Knolle, J. *Br. J. Pharmacol.* **1991**, *102*, 297–304.

(11) (a) Guba, W.; Haessner, R.; Breipohl, G.; Henke, S.; Knolle, J.; Santagada, V.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 7532–7540. (b) Kyle, D. J.; Blake, P. R.; Smithwick, D.; Green, L. M.; Martin, J. A.; Sinsko, J. A.; Summers, M. F. *J. Med. Chem.* **1993**, *36*, 1450–1460. (c) Kyle, D. J.; Martin, J. A.; Farmer, S. G.; Burch, R. M. *J. Med. Chem.* **1991**, *34*, 1230–1233.

(12) Solanas, C.; de la Torre, B. G.; Fernández-Reyes, M.; Santiveri, C. M.; Jiménez, M. A.; Rivas, L.; Jiménez, A. I.; Andreu, D.; Cativiela, C. *J. Med. Chem.* **2009**, *52*, 664–674.

(13) For articles dealing with the use of spirocyclic lactams, see for instance: (a) Bittermann, H.; Gmeiner, P. *J. Org. Chem.* **2006**, *71*, 97–102. (b) Gutiérrez-Rodríguez, M.; Martín-Martínez, M.; García-López, M. T.; Herranz, R.; Cuevas, F.; Polanco, C.; Rodríguez-Campos, I.; Manzanares, I.; Cárdenas, F.; Feliz, M.; Lloyd-Williams, P.; Giralt, E. *J. Med. Chem.* **2004**, *47*, 5700–5712. (c) Alonso, E.; López-Ortiz, F.; del Pozo, C.; Peralta, E.; Macías, A.; González, J. *J. Org. Chem.* **2001**, *66*, 6333–6338.

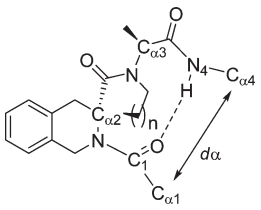


FIGURE 2. Parameters for the characterization of β-turn propensity of tetrahydroisoquinoline-based spirocyclic lactams.

experimental and molecular mechanics investigations of its conformational behavior. To demonstrate the general applicability of the strategy, we also elaborated a synthetic pathway incorporating protected serine and arginine at the i and $i+3$ positions, to build the hexapeptide **4**, analogous of the β-turn terminal portion of HOE 140. To simplify the solution NMR analysis, Boc and NHMe groups were chosen respectively as the (i) and (i + 5) residues, so as to provide methyl signals in the NMR high-field region. In particular, the Boc group has been proposed as an N-terminal residue mimic that does not affect the conformational behavior of short peptides.[14]

Results and Discussion

Modeling Studies. First of all, a computer-assisted conformational analysis, as a function of the dimension of the lactam ring, was performed on Tic-based spirocyclic lactams such as **3**. The reverse turn mimicry ability was evaluated by computing[15] the main geometric features of β-turns. The interatomic distance d_α, here represented by the ($C\alpha_1-C\alpha_4$) distance, should be less than 7 Å, and the virtual torsion angle β, defined by $C_1-C\alpha_2-C\alpha_3-N_4$, should be $|\beta| < 30°$ (Figure 2).

Another important feature in stabilizing the reverse turn conformation is the presence of the characteristic hydrogen bond $C_1O\text{---}HN_4$, that was estimated by means of the "hydrogen bonds" function implemented in the software.[16] The computational procedure consisted of an unconstrained Monte Carlo/energy minimization conformational search using the molecular mechanics MMFF94 force field[17] in vacuo. For each compound only conformations within 6 kcal/mol of the global minimum were kept. Results are reported in Table 1 as percentage of conformers which meet the requirements for a generic β-turn.

The best performance is achieved with compound **3** (n = 1), with 100% of conformers satisfying all the β-turn requirements. The low number of conformers within 6 kcal/mol of the global minimum (3 conformers) is a consequence of the high rigidity of this scaffold. The increase of the lactam ring size produces more flexible structures with a rise of conformers within 6 kcal/mol of the global minimum (9 conformers for n = 2 and 19 for n = 3). The percentages of conformers

TABLE 1. MC/EM Conformational Analysis for Tic-Based Spirocyclic Lactams[a]

n	no. of conf. <6 kcal/mol	% d_α < 7 Å	% $\|\beta\|$ < 30°	% H bond
0	8	37.5	37.5	25
1	3	100	100	100
2	9	89	89	0
3	19	26	21	0

[a]Results are reported as percentage of conformers which meet the indicated requirement.

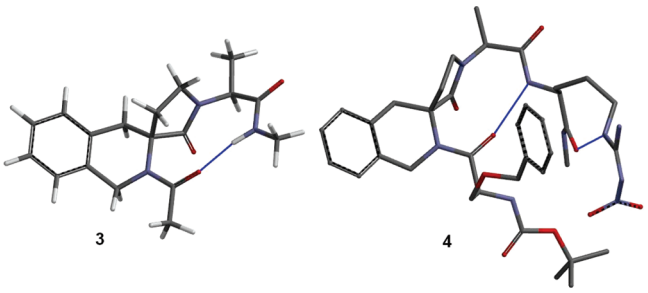


FIGURE 3. Low-energy conformers for **3** and **4**. For **4** hydrogen atoms are omitted for clarity.

meeting the β-turn requirements decrease and intramolecular hydrogen bonds are absent. The spiro β-lactam ring (n = 0) also shows a poor aptitude to mimic a β-turn conformation. So we focused our attention on the spirocyclic γ-lactam **3**. For **3**, the same global minimum was found, both in vacuo and in water, the latter being implicitly represented by a solvation model.[18] According to the definition of β-turn types, the inspection of the dihedral angle values of the amide backbone for the global minimum of **3** (Figure 3) ascribes this compound to a β-turn of type II′ (see Table 2).

Subsequently, the serine and arginine fragments were added to obtain the desired hexapeptide mimic **4**. Also in this case a conformational search was performed with the same protocol. In vacuo, six conformations were found within 6 kcal/mol of the global minimum, and in water 14. The global minimum is the same both in vacuo and in water (Figure 3) and its properties (Table 2) are still consistent with a β-turn of type II′.

Synthesis. The target compounds were synthesized from the key precursor aldehyde **5** (Scheme 1). Preparation of **5** was accomplished following the protocol described by Scheidt et al., starting in our case from D-Phe.[19] Using chemistry introduced by Seebach,[20] aldehyde **5** was obtained in 41% overall yield as a single diastereoisomer, starting from D-Phe.

Reductive amination of **5** with the HCl salt of L-alanine methyl ester, followed by heating in toluene in the presence of HOBt, afforded pyrrolidinone **6**, from which amine **7** was derived, by removal of the Cbz protecting group. Pictet–Spengler condensation (20% TFA, CH_2Cl_2) of amine **7** with formaldehyde afforded the tetrahydroisoquinoline **8** in moderate yield.

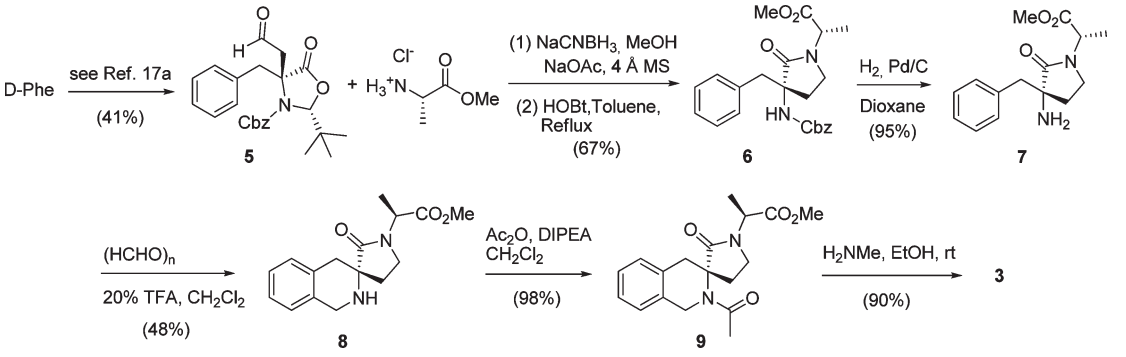
(14) Shaw, R.; Perczel, A.; Fasman, G. D.; Mantsch, H. H. *Int. J. Peptide Res.* **1996**, *48*, 71–78.
(15) *Spartan'08*; Wavefunction, Inc., Irvine, CA.
(16) Hydrogen bonds are defined as nonbonded contacts between a nitrogen or oxygen and a hydrogen attached to nitrogen or oxygen, separated by a distance ranging from 1.6 to 2.1 Å and making an X–H-Y (X, Y = N, O) angle > 120°.
(17) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490–519.
(18) The empirical solvation model SM.4 implemented in *Spartan '08* was applied. This model estimates the aqueous solvation energy. See also: Chambers, C. C.; Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. A* **1996**, *100*, 16385–16398.
(19) (a) Scheidt, K. A.; Roush, W. R.; McKerrow, J. H.; Selzer, P. M.; Hansell, E.; Rosenthal, P. J. *Bioorg. Med. Chem.* **1998**, *6*, 2477–2494. (b) Zydowsky, T. M.; Dellaria, J. F. Jr.; Nellans, H. N. *J. Org. Chem.* **1988**, *53*, 5607–5616.
(20) Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, *68*, 1243.

TABLE 2. Calculated Values of Selected Geometrical Parameters for the Lowest Energy Conformers of 3 and 4 and X-ray Derived Geometrical Parameters for 3 in the Solid State

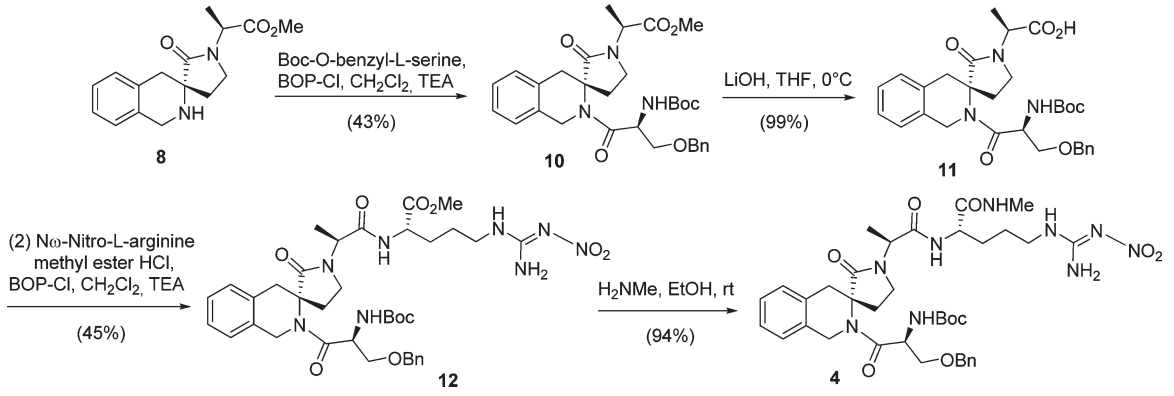
compd	$_{(i)}C=O\cdots HN_{(i+3)}$ (Å)	$\Phi_{(i+1)}$; $\psi_{(i+1)}$ (deg)	$\Phi_{(i+2)}$; $\psi_{(i+2)}$ (deg)	$d(C\alpha_{(i)}\cdots C\alpha_{(i+3)})$ (Å)	β (deg)	$_{(i)}O\cdots H-N_{(i+3)}$ (deg)
3 (MM/MC low energy conformer[a])	1.957	51.7; −136.4	−103.8; 41.1	5.59	−19.5	156.63
3 (solid state)	2.103	48.9; −133.7	−89.4; 10.8	5.47	−11.9	168.9
4 (MM/MC low energy conformer[a])	1.856	53.3; −137.5	−96.6; 43.4	5.27	−12.6	147.98
II′ β-turn (ideal)	1.8−2.5	60; −120	−80; 0	< 7	0	160

[a]The global minimum is the same both in vacuo and in water.

SCHEME 1



SCHEME 2



In the end, compound **8** was subjected to N-acetylation to afford acetamide **9**, which was reacted with H_2NMe, allowing the target tetrapeptide analogue **3** to be obtained.

Starting from tetrahydroisoquinoline **8** (Scheme 2), BOP-Cl mediated condensation with Boc-*O*-benzyl-L-serine afforded **10** in moderate yield. Hydrolysis of methyl ester with LiOH to give **11**, followed by BOP-Cl mediated condensation with N_ω-nitro-L-arginine methyl ester hydrochloride, gave compound **12**, which was converted by treatment with H_2NMe to the desired hexapeptide **4**.

Conformational Analysis. The results from the computational modeling studies showed that the SIPP core in both peptide analogues **3** and **4** could serve as a turn-inducing element and that for both model systems a β-turn (type II′) is a viable conformation. With **3** and **4** in hand, we now undertook a detailed conformational investigation on the secondary structure of these model systems, by means of X-ray crystallography and spectroscopic techniques.

X-ray Crystal Structure Determination of 3. We were able to obtain single crystals for crystallographic analysis from a 2-propanol solution of the tetrapeptide mimic **3**. Figure 4 shows the molecular structure of **3** in the crystal, where it adopts a turn conformation, with an 2.103 Å intramolecular hydrogen bond between the CO_i oxygen atom acceptor and the NH_{i+3} amide donor (see Table 2). The hydrogen bond directionality defined by the $_{(i)}O\cdots H-N_{(i+3)}$ angle of 168.9° is almost ideal.

For β-turns, an additional important criterion is that the $C\alpha_{(i)}\cdots C\alpha_{(i+3)}$ distance should be smaller than 7 Å. The X-ray crystal structure of **3** shows a $C\alpha_{(i)}\cdots C\alpha_{(i+3)}$ distance of 5.467 Å, which nicely complies with a β-turn arrangement. A more detailed analysis of the structural data revealed the presence of a well-defined β-turn conformation of type II′. In fact, in addition to the typical intramolecular hydrogen bond, all structural criteria used to define canonical type II′ β-turns are also satisfactory fulfilled by the peptidomimetic in the solid state. The X-ray outcome is remarkable, since it highlights that an intramolecular hydrogen bond persists also when the intermolecular packing forces have become important, that is to say in crystal formation and,

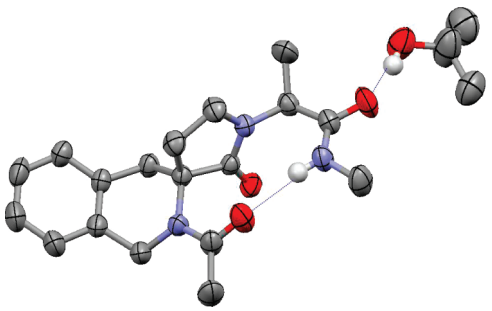


FIGURE 4. Plot of the molecular structure of compound **3**, as determined by X-ray crystallography. Atomic displacement parameters at the 50% probability level (hydrogen atoms omitted: see the Experimental Section for details). β-Turned conformation and intramolecular 10-membered H bond are shown. A coordinated molecule of 2-propanol is also present.

even more, in the presence of a highly coordinating protic solvent such as *i*PrOH.

Unfortunately, we did not succeed in obtaining crystals of mimic **4** that were of sufficient quality for an X-ray diffraction analysis.

Since intermolecular forces in the solid phase may lead to a change in secondary structure, solution phase studies were also required in order to give a complete representation of the conformation for both **3** and **4**.

^{1}H NMR Studies. Spin system identification and assignment of individual resonances for peptidomimetic model **3** were straightforward with ^{1}H-COSY. The study of conformational behavior was conducted primarily in $CDCl_3$, to identify intramolecular hydrogen bonding.[21] The involvement of the NH amide proton in intramolecular hydrogen bonding was first estimated from evaluation of its chemical shift value and of the temperature coefficient ($CDCl_3$), by recording 12 spectra from 218 to 328 K with increments of approximately 10 deg (Table 3). All data were measured at 2.0 mM concentration, that is in the absence of any noticeable intermolecular aggregation. The downfield chemical shift value (7.79 ppm) and the significant small temperature coefficient (−2.75 ppb/deg) were found for the NH proton of **3**, thus confirming its participation in intramolecular hydrogen bonding.

A supplementary indication of intramolecular hydrogen bonding was obtained from DMSO titration studies in $CDCl_3$,[22] indicating that the chemical shift of NH in **3** is almost constant up to 30% of DMSO (chemical shift difference of 0.11 ppm in absolute value[23]). Also by switching from the noncoordinating ($CDCl_3$) solvent to the hydrogen bond-acceptor solvent DMSO-d_6 a negligible chemical shift difference (0.15 ppm in absolute value) was found, which indicates a strongly hydrogen-bonded state for the amide proton, not accessible to the solvent.

Finally, since NH in compound **3** has two potential modes of intramolecular hydrogen bond, that is to say 7-membered (γ-turn) and 10-membered ring (β-turn), we evaluated the stability of the 10-membered hydrogen bond structure by the parameter r ($1 - \Delta\delta/\Delta\delta_{ref}$),[24] as a further probe of β-turn conformation. $\Delta\delta$ (δ(in DMSO-d_6) − δ(in $CDCl_3$)) is the chemical shift difference for NH between solutions in two neat solvents referred to compound **3**, while $\Delta\delta_{ref}$ is for a reference proton with no possibility of a 10-membered intramolecular hydrogen bond. As a reference, we have used the NHMe amide proton in compound **13** (Scheme 3). We observed an r value of 0.90, which is a clear evidence of stability for the 10-membered hydrogen bond in **3**.

The study of the conformational behavior of the hexapeptide analogue **4** was conducted in DMSO-d_6, due to the difficulty in assigning the NH signals in $CDCl_3$. The spin system identification and assignment of individual resonances were performed by means of bidimensional NMR techniques, and fully accomplished also in the region of NH signals, except for NH^3 (see Figure 5), whose signal overlaps in the aromatic zone. 2D NOESY NMR data did not suggest the close proximity of the two peptide chains, since no interstrand correlations, but only intrastrand contacts, were observed. As usual, the presence of intramolecular hydrogen bonding was determined by evaluation of the chemical shift values of the amide NH protons and their temperature coefficients ($\Delta\delta$H/ΔT). The chemical shift of 7.90 ppm and the temperature coefficient of −2.69 ppb/deg for NH^2 support a 10-membered hydrogen-bonded state also for **4**, as already predicted by the computational studies (see Figure 3). On the other hand, the chemical shift of 6.85 ppm and the large temperature dependence (−8.03 ppb/deg) for NH^1 would suggest a weak 14-membered hydrogen-bonded state in equilibrium with a non-hydrogen-bonded conformations. In our opinion, this behavior is due to the engagement of the H-bond acceptor CONH^{3}Me in an intramolecular hydrogen bond with NH^4, as strongly suggested by the chemical shift (7.75 ppm) and by the low (−1.69 ppb/deg) temperature coefficient of NH^4. Evidently, the peculiar NH containing side chain of arginine hampers the two peptide chains of hexapeptide **4** to take an antiparallel alignment in a 2:2 type β-hairpin[25] arrangement, as, also in this case, neatly predicted by MM calculations, for the minimum energy conformer (see Figure 3).

FT-IR Studies.[26] The IR spectrum of a 2.0 mM solution of **3** in $CHCl_3$ showed a unique, extensive absorption band at 3352 cm^{-1} for a hydrogen-bonded NH stretch, thus further attesting the presence of a stable secondary structure for **3**.

The IR spectrum of **4** exhibited an extensive absorption band at 3322 cm^{-1} for the hydrogen-bonded NH stretch and a broad band at 3438 cm^{-1} for the non-hydrogen-bonded NH stretch, in accordance with the presence of both states in this molecule.

CD Studies.[27] The ability of peptidomimetic **4** to adopt a secondary structure in solution was evaluated by CD spectroscopy (Figure 6). The spectrum was measured in methanol (0.2 mM) and displayed two negative minima, one at 203 nm and a second one at 218 nm, and a negative maximum at 209 nm. To our delight, this behavior follows completely the same pattern with respect to well-defined type II′ β-turn peptide backbone geometries. In fact, it has

(21) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389–400.
(22) For all data, see the Supporting Information.
(23) For DMSO-d_6/$CDCl_3$ solutions, chemical shifts were standardized by reference to residual proton resonance for CHD_2CD_3SO (2.49 ppm).
(24) Tonan, K.; Ikawa, S. *Spectrochim. Acta A* **2003**, *59*, 111–120.
(25) de Alba, E.; Jiménez, M. A.; Rico, M. *J. Am. Chem. Soc.* **1997**, *119*, 175–183.
(26) Vass, E.; Hollòsi, M.; Besson, F.; Buchet, R. *Chem. Rev.* **2003**, *103*, 1917–1954.
(27) Cai, X.; Dass, C. *Curr. Org. Chem.* **2003**, *7*, 1841–1854.

TABLE 3. Chemical Shifts and Temperature Coefficients of Amide Protons in 3 and 4[a]

compd	δNH (ppm)	δNH^1 (ppm)	δNH^2 (ppm)	δNH^4 (ppm)	$\Delta\delta NH/\Delta T$ (ppb/deg)	$\Delta\delta NH^1/\Delta T$ (ppb/deg)	$\Delta\delta NH^2/\Delta T$ (ppb/deg)	$\Delta\delta NH^4/\Delta T$ (ppb/deg)
3	7.79				−2.75			
4		6.85	7.90	7.75		−8.03	−2.69	−1.69

[a]All NMR experiments were performed with 2.0 mM solutions in $CDCl_3$ (for 3) or DMSO-d_6 (for 4). Chemical shift values (δ) reported are the values at 298 K. Temperature coefficients were determined between 218 and 328 K for **3** and between 303 and 353 K for **4**. See the Supporting Information for all experimental data.

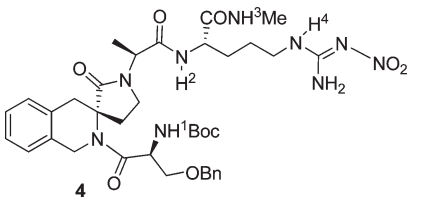


FIGURE 5. Compound **4** including amide proton numbering.

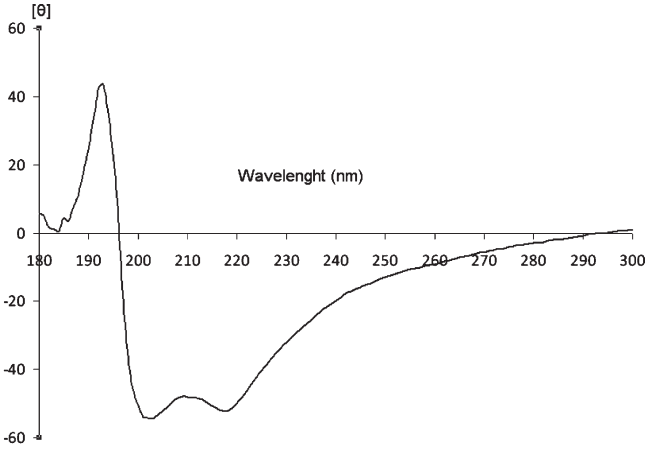
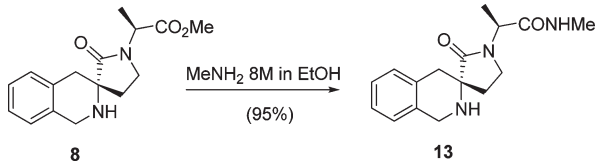


FIGURE 6. CD spectrum of peptidomimetic **4** (MeOH, 0.2 mM solution).

SCHEME 3



been demonstrated that two distinct types of CD spectra are associated with two of the many possible turn geometries, that is to say the type II and II′ β-turn.[28] Since the phenylalanyl side-chain chromophore makes a negligible contribution to the CD spectra of peptidomimetics in the 195−240-nm range, we can conclude that also hexapeptide analogue **4** adopts a type II′ β-turn conformation.

Conclusions

In this paper, we were able to establish an efficient approach to a novel type of tetrapeptide and hexapeptide mimics, embodying a constrained Phe-Ala dipeptide analogue as the central core. Both MM calculations and spectroscopic NMR, IR, and X-ray investigations support the conclusion that the SIPP scaffold embodied in the spirocyclic lactam **3** is a potent and very stable type II′ β-turn inducer, with highly predictable stereostructural properties. The turn mimic properties of **3** are efficiently transferred to the hexapeptide mimic **4**, for which a type II′ β-turn conformation was also predicted by MM calculations and strongly assessed with NMR, IR, and CD experiments.

Work to illustrate the application of these novel spirocyclic lactam peptidomimetics to the synthesis of β-turned ligands for G-protein-coupled receptors is in progress in our laboratory.

Experimental Section

General Methods. All solvents were distilled and properly dried, when necessary, prior to use. All chemicals were purchased from commercial sources and used directly, unless indicated otherwise. All reactions were run under N_2, unless otherwise indicated. All reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254; spots were visualized with UV light or by treatment with 1% aqueous $KMnO_4$ solution. Products were purified by flash chromatography on silica gel 60 (230−400 mesh). ^1H and ^{13}C NMR spectra were recorded with 300 and 400 MHz spectrometers. Chemical shifts (δ) are expressed in ppm relative to TMS at $\delta = 0$ ppm for ^1H NMR and relative to $CDCl_3$ at $\delta = 77.16$ ppm for ^{13}C NMR. High-resolution MS spectra were recorded with a FT-ICR (Fourier Transform Ion Ciclotron Resonance) instrument, equipped with an ESI source, or a standard MS instrument, equipped with an EI source.

(2*R*,4*S*)-Benzyl 4-Benzyl-2-*tert*-butyl-5-oxo-4-(2-oxoethyl)-oxazolidine-3-carboxylate, 6. To a suspension of **5**[29] (954 mg, 2.33 mmol), NaOAc (400 mg, 4.88 mmol), and 4 Å molecular sieves (2.60 g) in MeOH (50 mL) was added the hydrochloride salt of alanine methyl ester (374 mg, 2.69 mmol). After 45 min, $NaCNBH_3$ (305 mg, 4.85 mmol) was added and the reaction mixture was stirred at 25 °C overnight. Then, the suspension was filtered and the filtrate was acidified to pH 1 with 1 N aq HCl and stirred for 20 min. The solution was basified to pH 9 with saturated aq $NaHCO_3$ and then extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to yield a yellow oil. This oil was dissolved in toluene (30 mL), and HOBt (310 mg, 2.30 mmol) was added. The mixture was then heated at reflux for 16 h then cooled, and the toluene was removed in vacuo. The residue was taken up in EtOAc (50 mL) and washed with saturated aq $NaHCO_3$ (3 × 50 mL) and brine, then dried over Na_2SO_4, filtered, and concentrated to dryness to yield a crude yellow oil. Purification of the crude product by flash chromatography (hexane/EtOAc 1:3) afforded lactam **6** (646 mg, 67%) as a clear oil. R_f 0.39 (hexane/EtOAc 1:1). $[\alpha]^{25}_D$ +13.3 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, δ)

(28) Bush, C. A.; Sarkar, S. K.; Kopple, K. D. *Biochemistry* **1978**, *17*, 4951–4954.

(29) Compound **5** was prepared according to ref 17. ^1H and ^{13}C NMR spectra are in accordance with literature. $[\alpha]^{25}_D$ +19.6 (*c* 1.0, $CHCl_3$).

7.40−7.19 (m, 10H), 5.65 (br, s, 1H), 5.17 (d, AB system, J = 12.2 Hz, 1H), 5.12 (d, AB system, J = 12.2 Hz, 1H), 4.78 (q, J = 7.4 Hz, 1H), 3.71 (s, 3H), 3.28 (d, AB system, J = 12.6 Hz, 1H), 3.15 (t, J = 9.4 Hz, 1H), 3.07 (d, AB system, J = 12.6 Hz, 1H), 2.75−2.68 (m, 1H), 2.53−2.46 (m, 1H), 2.28 (dd, J = 16.6, 8.6 Hz, 1H), 1.07 (d, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 173.3, 171.4, 155.0, 136.4, 135.2, 130.1−127.2 (10C), 66.6, 61.5, 52.4, 49.4, 42.5, 41.0, 31.2, 14.1. HRMS (EI) calcd for $C_{23}H_{26}N_2O_5$ 410.1842, found 410.1837.

(*S*)-Methyl 2-((*S*)-3-Amino-3-benzyl-2-oxopyrrolidin-1-yl)-propanoate, 7. To a solution of **6** (300 mg, 0.73 mmol) in 1,4-dioxane (15 mL) was added 10% Pd/C (30 mg). After thoroughly flushing the flask with N_2, a hydrogen atmosphere was introduced. After 17 h, the reaction was filtered through Celite, then concentrated in vacuo to yield a light-yellow oil. Amine **7** (191 mg, 95%) was used without further purification. R_f 0.35 (hexane/EtOAc 1:2). $[\alpha]^{25}_D$ −11.1 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.32−7.20 (m, 5H), 4.80 (q, J = 7.5 Hz, 1H), 3.70 (s, 3H), 3.16 (td, J = 9.2, 2.8 Hz, 1H), 2.99 (d, AB system, J = 13.0 Hz, 1H), 2.81 (d, AB system, J = 13.0 Hz, 1H), 2.48 (dd, J = 16.9, 7.8 Hz, 1H), 2.32−2.24 (m, 1H), 1.90 (m, 3H), 1.13 (d, 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 177.4, 171.8, 136.2, 130.1 (2C), 128.3 (2C), 127.0, 60.5, 52.3, 49.4, 44.7, 39.7, 31.5, 14.3. HRMS (EI) calcd for $C_{15}H_{20}N_2O_3$ 276.1474, found 276.1481.

(*S*)-Methyl 2-((*S*)-2′-Oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanoate, 8. To a solution of **7** (700 mg, 2.53 mmol) in CH_2Cl_2 (14 mL) were added $(CH_2O)_n$ (137 mg, 4.56 mmol) and TFA (6 mL). After 20 h at room temperature, saturated aq $NaHCO_3$ was slowly added until pH 8. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/MeOH 19:1) to give 399 mg (48%) of **8**, as an oil. R_f 0.58 (EtOAc/MeOH 4:1). $[\alpha]^{25}_D$ −13.8 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.15−7.02 (m, 4H), 4.85 (q, J = 7.4 Hz, 1H), 4.13 (d, AB system, J = 17.3 Hz, 1H), 4.04 (d, AB system, J = 17.3 Hz, 1H), 3.72 (s, 3H), 3.56 (td, J = 14.0, 3.4 Hz, 1H), 3.48 (dt, J = 14.0, 7.5 Hz, 1H), 2.99 (d, AB system, J = 16.2 Hz, 1H), 2.61 (d, AB system, J = 16.2 Hz, 1H), 2.23−2.10 (m, 1H), 2.03−1.93 (m, 2H), 1.46 (d, 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 175.9, 171.7, 134.6, 132.6, 129.7, 128.3, 126.3, 125.8, 59.2, 52.4, 49.8, 44.2, 40.8, 34.1, 30.4, 14.9. HRMS (EI) calcd for $C_{16}H_{20}N_2O_3$ 288.1474, found 288.1466.

(*S*)-Methyl 2-((*S*)-2-Acetyl-2′-oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanoate, 9. Compound **8** (50 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (2 mL) and DIPEA (45 μL, 0.26 mmol) was added. The solution was then cooled to 0 °C and acetic anhydride (24 μL, 0.26 mmol) was slowly added. After the addition, the mixture was stirred at room temperature for 12 h. Then, the reaction mixture was poured into ice water and extracted with CH_2Cl_2 and the organic solution was washed with 5% aq $NaHCO_3$. The organic phase was then dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford **9** (56 mg, 98% yield) as an oil. The compound was used without further purification. R_f 0.56 (EtOAc/MeOH 8:1). $[\alpha]^{25}_D$ −20.5 (c 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.34−7.22 (m, 4H), 4.76 (q, J = 7.4 Hz, 1H), 4.65 (d, AB system, J = 14.2 Hz, 1H), 4.57 (d, AB system, J = 14.2 Hz, 1H), 3.79 (s, 3H), 3.58 (td, J = 9.9, 2.2 Hz, 1H), 3.35 (q, J = 8.5 Hz, 1H), 3.19 (d, AB system, J = 14.6 Hz, 1H), 2.69 (d, AB system, J = 14.6 Hz, 1H), 2.31−2.23 (m, 1H), 2.25 (s, 3H), 1.68−1.62 (m, 1H), 1.50 (d, 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 174.3, 172.1, 170.0, 135.4, 135.0, 128.7, 128.6, 127.8, 126.6, 64.2, 52.9, 51.3, 48.9, 41.4, 37.9, 30.0, 23.7, 15.3. HRMS (EI) calcd for $C_{18}H_{22}N_2O_4$ 330.1580, found 330.1586.

(*S*)-Methyl 2-((*S*)-2-((*S*)-3-(Benzyloxy)-2-(*tert*-butoxycarbonylamino)propanoyl)-2′-oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanoate, 10. Boc-*O*-benzyl serine (77 mg, 0.26 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) at 0 °C. Et_3N (40 μL, 0.29 mmol) and BOPCl (1.5 equiv, 90 mg, 0.35 mmol) were added and the reaction mixture was stirred for 40 min. Keeping the mixture at 0 °C, a solution of compound **8** (68 mg, 1.26 mmol) in anhydrous CH_2Cl_2 (2 mL) and Et_3N (40 μL, 0.29 mmol) was slowly added. The reaction mixture was allowed to go up to room temperature and stirred for 24 h. The reaction was quenched with H_2O (4 mL) and the organic layer was washed with 5% aq H_3PO_4 (2 × 4 mL), 5% aq $NaHCO_3$ (2 × 4 mL), and water (4 mL), dried over Na_2SO_4, and filtered, then the solvent was removed in vacuo. Purification by flash chromatography (hexane/EtOAc 1:1) gave compound **10** (57 mg, 43%) as a colorless oil. R_f 0.70 (EtOAc/MeOH 9:1). $[\alpha]^{25}_D$ −8.5 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO, 100 °C, 1:1 mixture of two conformers, δ) 7.40−7.18 (m, 9H), 6.49 (br, d, J = 7.1 Hz, 0.5H), 6.16 (br, d, J = 7.1 Hz, 0.5H), 4.93−4.77 (m, 2H), 4.61−4.44 (m, 4H), 3.75−3.58 (m, 2H), 3.69 (s, 3H), 3.51−3.34 (m, 2H), 3.03−2.98 (m, 1H), 2.83 (dd, J = 14.6, 1.6 Hz, 1H), 2.23−2.06 (m, 1H), 1.61−1.54 (m, 1H), 1.44 (s, 4.5H), 1.42−1.39 (m, 7.5H). ^{13}C NMR (100 MHz, $CDCl_3$, 1:1 mixture of two conformers, δ) 174.1 and 173.8, 172.1, 170.3 and 170.1, 155.7, 138.8 and 138.3, 135.2 and 135.1, 134.8, 129.2−126.9 (9C), 80.4, 74.0, 72.4, 71.7, 70.5 and 70.4, 64.9 and 64.8, 52.9, 51.4 and 51.2, 48.1 and 47.7, 41.4 and 41.1, 37.7 and 37.5, 48.9, 29.7 and 29.5, 29.0, 15.3. HRMS (EI) calcd for $C_{31}H_{39}N_3O_7$ 565.2788, found 565.2779.

(*S*)-2-((*S*)-2-((*S*)-3-(Benzyloxy)-2-(*tert*-butoxycarbonylamino)-propanoyl)-2′-oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanoic Acid, 11. To a solution of **10** (50 mg, 0.09 mmol) in THF (1 mL) at 0 °C was added 1 M LiOH (100 μL, 0.10 mmol) slowly. After 3 h, the solvent was concentrated in vacuo and 5% H_3PO_4 aq solution was added until the solution was at pH 3. The aqueous phase was extracted with ethyl acetate (2 × 30 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure affording pure acid **11** (48 mg, 99%) as a foam. The compound was used without purification. R_f 0.09 (hexane/EtOAc 1:2). $[\alpha]^{25}_D$ −40.9 (c 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 2:1 mixture of two conformers, δ) 7.42−6.91 (m, 9H), 5.62 (d, J = 8.6 Hz, 0.33H), 5.51 (d, J = 8.6 Hz, 0.67H), 5.18−5.05 (m, 1.33H), 5.02−4.92 (m, 1.33H), 4.65−4.53 (m, 2H), 4.48 (d, AB system, J = 11.7 Hz, 0.67H), 4.39 (d, AB system, J = 11.7 Hz, 0.67H), 3.82−3.71 (m, 1.67H), 3.55−3.31 (m, 3.33H), 3.28 (d, AB system, J = 14.6 Hz, 0.67H), 3.26 (d, AB system, J = 14.6 Hz, 0.33H), 2.74 (d, AB system, J = 14.6 Hz, 0.33H), 2.72 (d, AB system, J = 14.6 Hz, 0.67H), 2.21−1.99 (m, 1H), 1.90−1.72 (m, 1H), 1.52 (d, J = 7.3 Hz, 2H), 1.51 (d, J = 7.3 Hz, 1H), 1.47 (s, 6H), 1.44 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 2:1 mixture of two conformers, δ) 173.3 (major) and 173.1 (minor), 171.9 (minor) and 171.8 (major), 170.7, 155.0, 137.8 (minor) and 137.3 (major), 133.9 (minor) and 133.7 (major), 133.5, 128.3−126.4 (9C), 80.2 (major) and 80.1 (minor), 73.5, 71.5, 70.6, 64.4 (minor) and 64.3 (major), 51.6, 50.6, 47.7, 47.3, 40.2, 37.4 (minor) and 37.2 (major), 30.0 (minor) and 29.8 (major), 28.4, 14.2. HRMS (EI) calcd for $C_{30}H_{37}N_3O_7$ 551.2632, found 551.2647.

(*S*)-Methyl 2-((*S*)-2-((*S*)-2-((*S*)-3-(Benzyloxy)-2-(*tert*-butoxycarbonylamino)propanoyl)-2′-oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanamido)-5-((*E*)-2-nitroguanidino)-pentanoate, 12. The acid **11** (28 mg, 0.05 mmol) was dissolved in anhydrous CH_2Cl_2 (1 mL) at 0 °C. Et_3N (10 μL, 0.07 mmol) and BOPCl (1.5 equiv, 20 mg, 0.08 mmol) were added and the reaction mixture was stirred for 40 min. Keeping the mixture at 0 °C, N_ω-nitro-L-arginine methyl ester HCl (15 mg, 0.06 mmol) previously dissolved in anhydrous CH_2Cl_2 (1 mL) and Et_3N (10 μL, 0.07 mmol) was slowly added. The reaction mixture was allowed to go up to room temperature and stirred for 24 h. The reaction was quenched with H_2O (4 mL) and the organic layer was washed with 5% aq H_3PO_4 (2 × 4 mL), saturated aqueous solution of $NaHCO_3$

(2 × 4 mL), and water (4 mL), dried over Na_2SO_4, and filtered, then the solvent was removed in vacuo. Purification by column chromatography (EtOAc) gave compound **12** (17 mg, 45%) as a foamy oil. R_f 0.64 (EtOAc/MeOH 9:1). $[\alpha]^{25}_D$ −42.8 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 3:2 mixture of two conformers, δ) 8.30 (br, s, 1H), 8.13 (br, s, 0.6H), 8.04 (br, s, 0.4H), 7.45−6.91 (m, 9H), 5.93 (br, d, J = 7.2 Hz, 0.6H), 5.64 (br, d, J = 7.2 Hz, 0.4H), 5.18−4.95 (m, 2H), 4.94−4.79 (m, 2H), 4.74−4.53 (m, 3H), 4.50 (d, AB system, J = 11.8 Hz, 1H), 4.41 (d, AB system, J = 11.8 Hz, 1H), 3.76 (s, 1.8H), 3.80−3.71 (m, 1H), 3.69 (s, 1.2H), 3.53 (t, J = 8.8 Hz, 1H), 3.48−3.37 (m, 1H), 3.36−3.18 (m, 2H), 3.28 (d, AB system, J = 14.6 Hz, 1H), 2.77−2.71 (m, 1H), 2.72 (d, AB system, J = 14.6 Hz, 1H), 2.21−1.99 (m, 1H), 1.90−1.72 (m, 1H), 1.52 (d, J = 7.3 Hz, 3H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, 3:2 mixture of two conformers, δ) 173.5, 171.8, 171.5, 170.4, 159.4, 155.6 (minor) and 155.3 (major), 137.4, 134.0, 133.5, 128.3−127.2 (8C), 126.3, 79.9, 73.4 (2C), 71.8, 70.2, 64.2, 52.6 (major) and 52.4 (minor), 52.1, 50.7, 47.4 (minor) and 47.2 (major), 40.3 (minor) and 39.9 (major), 37.8, 29.6, 29.2, 28.4 (3C), 24.8, 13.8. HRMS (EI) calcd for $C_{37}H_{50}N_8O_{10}$ 766.3650, found 766.3671.

(*S*)-*N*-Methyl-2-((*S*)-2′-oxo-2,4-dihydro-1*H*-spiro[isoquinoline-3,3′-pyrrolidine]-1′-yl)propanamide, 13. Compound **8** (22 mg, 0.08 mmol) was dissolved at room temperature in a solution of methyl amine in EtOH (5 mL, 8.03 M). The reaction mixture was stirred for 12 h and evaporated to give **13** (21 mg, 95%) as a foam. R_f 0.63 (EtOAc/MeOH 4:1). $[\alpha]^{25}_D$ −26.9 (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.28−7.06 (m, 4H), 6.45 (br, s, 1H), 4.73 (q, J = 7.2 Hz, 1H), 4.14 (d, AB system, J = 17.5 Hz, 1H), 4.06 (d, AB system, J = 17.5 Hz, 1H), 3.47−3.37 (m, 2H), 3.06 (d, AB system, J = 16.3 Hz, 1H), 2.80 (d, J = 4.7 Hz, 3H), 2.64 (d, AB system, J = 16.3 Hz, 1H), 2.19−2.10 (m, 1H), 2.10−1.96 (m, 2H), 1.45 (d, 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 176.9, 171.3, 135.1, 133.1, 130.4, 127.0 (2C), 126.4, 60.0, 51.3, 44.8, 41.1, 34.6, 30.6, 26.9, 14.3. HRMS (EI) calcd for $C_{16}H_{21}N_3O_2$ 287.1634, found 287.1628.

Supporting Information Available: Detailed experimental methods for compound **3** (from **9**) and for compound **4** (from **12**), crystal data for compound **3** (also in CIF format), NMR spectra of compounds **3**−**4** and **6**−**13**, VT ^{1}H NMR data for **3** and **4**, DMSO-d_6 titration ^{1}H NMR data for **3**, IR spectra for **3** and **4**, and computational data from conformational analysis for **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.