JOC*Article*

Spirolactams as Conformationally Restricted Pseudopeptides: Synthesis and Conformational Analysis

M. Montserrat Fernández, Anna Diez, Mario Rubiralta, Elvira Montenegro, and Núria Casamitjana*

Laboratori de Química Orgànica, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII, 08028 Barcelona, Spain

Marcelo J. Kogan and Ernest Giralt

Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, c/Martí i Franquès, 08028 Barcelona, Spain

casamitj@farmacia.far.ub.es

Received May 31, 2002

The synthesis of 1-(*tert*-butoxycarbonyl)-7-[1-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decanes (*S*,*S*)-**1a** and (*S*,*R*)-**1b** is described. Derivatives **17a**,**b** and **19a** are prepared for use in peptide synthesis as constrained surrogates of the Pro-Leu and Gly-Leu dipeptides. The Ac-{Gly-Leu}-Met-NH_2 derivatives (*S*,*S*,*S*)-**2a** and (*S*,*R*,*S*)-**2b**, with the tripeptidic C-terminal region present in tachykinins, are also synthesized. Conformational analyses of these tripetide analogues by NMR experiments and molecular modeling calculations show that both (*S*,*S*,*S*)-**2a** and (*S*,*R*,*S*)-**2b** epimers are γ-turn/distorted type II β-turn mimetics.

Introduction

In 1980, Freidinger used for the first time a lactam ring as a type II′ β-turn mimetic.[1] Since then, his original idea has been developed and several conformationally constrained peptidomimetics containing a lactam in their structure have been designed and synthesized.[2] Substitution and size of the lactam ring allow the restriction of up to three of the four torsion angles that define a β-turn.[3] In this context, in the past decade, different spirolactams have been reported as β-turn mimetics in which the ϕ_{i+1} and ψ_{i+1} torsion angles are restricted.[4−10] With the aim of obtaining chiral 5.6-spirolactams with the 3-amino-2-piperidone nucleus in their structure, we report herein a stereoselective synthesis of compounds (*S*,*S*)-**1a** and (*S*,*S*)-**1b**, which constitute conformationally constrained Gly-Leu and Pro-Leu surrogates (Figure 1). Our synthesis starts from chiral α-allylproline derivative **3** (Schemes 1 and 2) and builds up the piperidone ring following two strategies that allow the introduction of other amino acids in addition to leucine. Until 1998, only 5.5-spirolactams containing a pyrrolidone ring had been described as conformationally constrained peptide analogues.[4−8] More recently, a racemic synthesis of similar 5.6 spirolactams, useful as inhibitors of protein−protein interactions modulated by the SH3 domain and as β-turn

(1) (a) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656−658. (b) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104−109.

(2) For some examples, see: (a) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647−650. (b) Kahn, M.; Chen, B. *Tetrahedron Lett.* **1987**, *28*, 1623−1626. (c) Olson, G. L.; Voss, M. E.; Hill, D. E.; Kahn, M.; Madison, V.; Cook, C. M. *J. Am. Chem. Soc.* **1990**, *112*, 323−333. (d) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789−12854. (e) Wolfe, M. S.; Dutta, D.; Aubé, J. *J. Org. Chem.* **1997**, *62*, 654−663. (f) Derrer, S.; Feeder, N.; Teat, S. J.; Davies, J. E.; Holmes, A. B. *Tetrahedron Lett.* **1998**, *39*, 9309−9312. (g) Estiarte, M. A.; Rubiralta, M.; Diez, A.; Thormann, M.; Giralt, E. *J. Org. Chem.* **2000**, *65*, 6992−6999. (h) Angiolini, M.; Araneo, S.; Belvisi, L.; Cesarotti, E.; Checchia, A.; Crippa, L.; Manzoni, L.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 2571−2581. (i) Weber, K.; Ohnacht, U.; Gmeiner, P. *J. Org. Chem.* **2000**, *65*, 7406−7416. (j) Derrer, S.; Davies, J. E.; Holmes, A. B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2957−2967.

(3) (a) Rose, G. D.; Gierash, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109. (b) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467−3478. (c) Liskamp, R. M. J. *Rec. Trav. Chim. Pays Bas* **1994**, *113*, 1−63.

(4) (a) Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8778−8783. (b) Genin, M. J.; Gleason, W. B.; Johnson, R. L. *J. Org. Chem.* **1993**, *58*, 860−866. (c) Genin, M. J.; Ojala, W. H.; Gleason, W. B.; Johnson, R. L. *J. Org. Chem.* **1993**, *58*, 2334−2337. (d) Sreenivasan, U.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1993**, *36*, 256−263. (e) Genin, M. J.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1993**, *36*, 3481−3483. (f) Subasinghe, N. L.; Khalil, E. M.; Johnson, R. L. *Tetrahedron Lett.* **1997**, *38*, 1317−1320. (g) Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Nair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1999**, *42*, 628−637.

(5) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447−1449.

(6) (a) Brown, J. R.; Clegg, S. P.; Ewan, G. B.; Hagan, R. M.; Ireland, S. J.; Jordan, C. C.; Porter, B.; Ross, B. C.; Ward, P. In *Molecular Recognition*: *Chemical and Biochemical Problems*; Roberts, S. M., Ed.; Royal Society of Chemistry Special Publication No. 78; Royal Society of Chemistry: Letchworth, 1989; pp 94−111. (b) Ward, P.; Ewan, G. B. European Patent 0 360 390 Al, 1989. (c) Ward, P.; Ewan, G. B.; Jordan, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *33*, 1848−1851.

(7) (a) Moeller, K. D.; Rutledge, L. D. *J. Org. Chem.* **1992**, *57*, 6360−6363. (b) Rutledge, L. D.; Perlman, J. H.; Gershengorn, M. C.; Marshall, G. R.; Moeller, K. D. *J. Med. Chem.* **1996**, *39*, 1571−1574.

(8) Sauer, S.; Schumacher, A.; Barbosa, F.; Giese, B. *Tetrahedron Lett.* **1998**, *39*, 3685−3688.

(9) (a) Witter, D. J.; Famiglietti, S. J.; Cambier, J. C.; Castelhano, A. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3137−3142. (b) Castelhano, A. L.; Witter, D. J. PCT Int. Appl. WO 98/54208 A1, 1998.

(10) Alonso, E.; López-Ortiz, F.; Del Pozo, C.; Peralta, E.; Macías, A.; Gonzalez, J. *J. Org. Chem.* **2001**, *66*, 6333−6338.

10.1021/jo025999i CCC: $22.00
Published on Web 10/03/2002

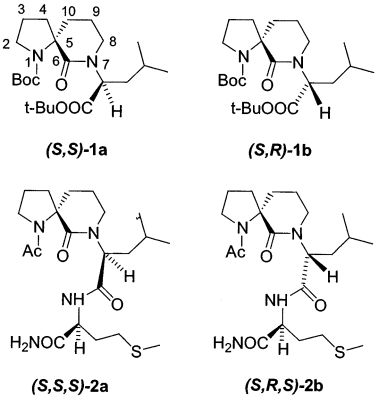


FIGURE 1. Spirolactams as conformationally restricted pseudopeptides.

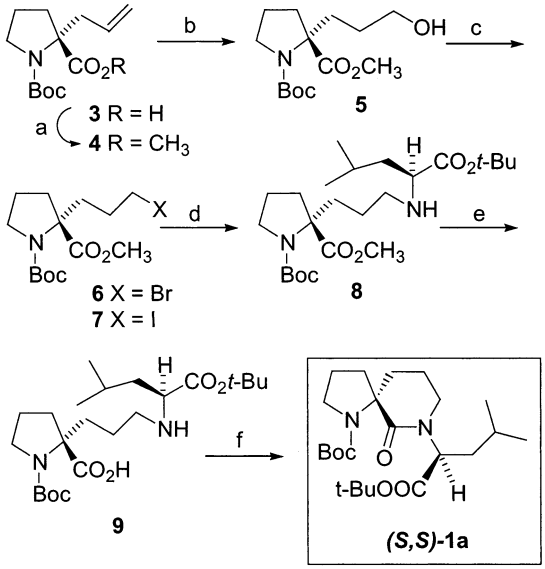
mimetics,[9] was described, as well as the synthesis of 5.5.6- and 5.6.5-spirolactamic Pro-Leu-Gly peptidomimetics, which present a thiazolidine ring condensed to the lactam ring.[4f] Lately, the synthesis of a new type of spiro β-lactams that adopt a β-turn conformation was also achieved.[10]

In connection with our studies on substance P inhibitors,[11] we chose to study compounds **1a** and **1b** as surrogates of the Gly-Leu dipeptide of the Gly-Leu-Met-NH_2 C-terminal region of tachykinins,[6c,12] which is known to be crucial for the biological activity of these neuropeptides. Previous works demonstrate that the use of (*S*)-5.5-spirolactams improves the inhibitory activity on NK_1-receptors of the native peptide, whereas the (*R*)-epimer does not.[6] It was also shown that the (*S*)-5.5-spirolactam adopts a type-II′ β-turn conformation, whereas the (*R*)-epimer does not.[6c] We were therefore interested in studying the conformational preferences of our 5.6-spirolactams for which we prepared the Ac-{Gly-Leu}-Met-NH_2 derivatives (*S*,*S*,*S*)-**2a** and (*S*,*R*,*S*)-**2b** (Figure 1).

Results and Discussion

Synthesis and Structural Assignment. The starting α-allylproline ester **4** was prepared from (*R*)-α-allylproline[13,14] as described by Johnson et al.[4a,15] with slight modifications in the *N*-protection[16] and esterification steps. Conversion of the *N*-Boc methyl ester **4** into the corresponding bromide **6**, or iodide **7**, by an anti-Markovnikov addition to the double bond using 9-BBN in the presence of Br_2[17] or ICl[18] was unsuccessful. However, hydroboration of **4** with 9-BBN followed by oxidation with H_2O_2 and NaOH[19] gave the hydroxy derivative **5** in excellent yield, which was converted into bromide **6** and iodide **7** by treatment with NBS−PPh_3[20] or I_2−PPh_3−imidazole,[21] respectively (Scheme 1).

SCHEME 1. Synthesis of Spirolactam 1a[a]

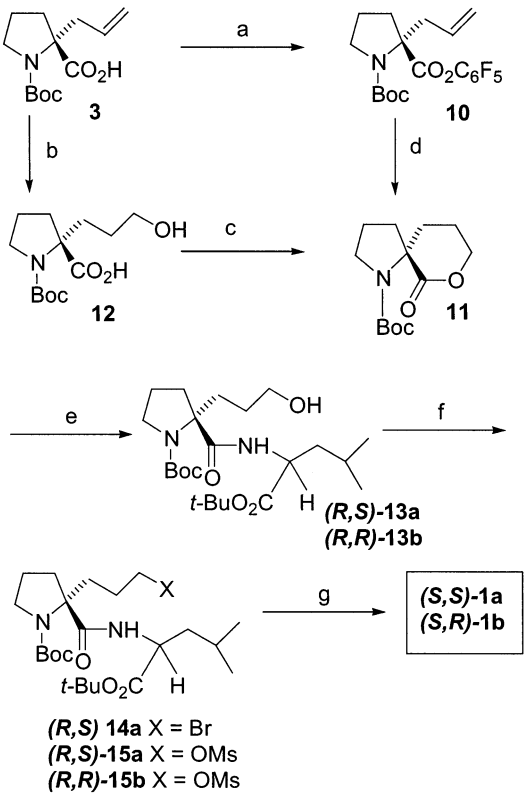


[a] Reagents and conditions: (a) CH_3I, K_2CO_3, acetone, reflux, 92%; (b) 9-BBN, THF, rt; then H_2O_2, 3 N NaOH, rt, 98%; (c) For **6**: Ph_3P, NBS, CH_2Cl_2, 0 °C; then, K_2CO_3, reflux, 71%. For **7**: Ph_3P, imidazole, I_2, CH_2Cl_2, rt, 68%; (d) H-Leu-O^{t}Bu, K_2CO_3, DMF, reflux, 61% from **6** and 81% from **7**; (e) TBAH, THF−H_2O, rt (not purified); (f) DCC, CH_2Cl_2, 0 °C, 23%.

When we tried to obtain spirolactam **1a** in a one-pot procedure, as described for the synthesis of five-membered lactams,[22] by substitution of the halide atom in **6** or **7** by Leu-O^{t}Bu, followed by intramolecular lactamization under a variety of experimental conditions, amine **8** was the only product isolated. The best results for amine **8** were obtained by treatment of iodide **7** with K_2CO_3 in DMF at 110 °C. Direct lactamization of amino ester **8** under either acidic (SiO_2)[23] or basic (Et_3N)[24] conditions failed. Cyclization of **8** was achieved by hydrolysis of the ester function with tetrabutylammonium hydroxide (TBAH)[25] and intramolecular coupling of the resulting amino acid **9** using DCC as the activating agent, which yielded the target lactam **1a**, although in a low yield.

(11) Diez, A.; Voldoire, A.; López, I.; Rubiralta, M.; Segarra, V.; Pagès, Ll.; Palacios, J. M. *Tetrahedron* **1995**, *51*, 5143−5156.
(12) (a) Deal, M. J.; Hagan, R. M.; Ireland, S. J.; Jordan, C. C.; McElroy, A. B.; Porter, B.; Ross, B. C.; Stephens-Smith, M.; Ward, P. *J. Med. Chem.* **1992**, *35*, 4195−4204 and references therein. (b) Howson, W. *DN&P* **1995**, *8*, 97−103.
(13) (a) Seebach, D.; Boes, M.; Naef, R.; Schweider, B. *J. Am. Chem. Soc.* **1983**, *105*, 5390−5398. (b) Beck, A. K.; Blank, S.; Job, K.; Seebach, D. *Org. Synth.* **1995**, *72*, 62−73. (c) Seebach, D.; Sting, A. R.; Hoffman, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2708−2748.
(14) Better results were obtained starting from *N*-trimethylsilylproline trimethylsilyl ester as described by: Annunziata, R.; Ferrari, M.; Papeo, G.; Resmini, M.; Sisti, M. *Synth. Commun.* **1997**, *27*, 23−38.
(15) Genin, M. J.; Baures, P. W.; Johnson, R. L. *Tetrahedron Lett.* **1994**, *35*, 4967−4968.
(16) Khalil, E. M.; Subasinghe, N. L.; Johnson, R. L. *Tetrahedron Lett.* **1996**, *37*, 3441−3444.
(17) Kabalka, G. W.; Sastry, K. A. R.; Hsu, H. C.; Hylarides, M. D. *J. Org. Chem.* **1981**, *46*, 3113−3115.
(18) Gooch, E. E.; Kabalka, G. W. *Synth. Commun.* **1981**, *11*, 521−525.
(19) Zydowsky, T. M.; Dellaria, J. F.; Nellans, H. N. *J. Org. Chem.* **1988**, *53*, 5607−5616.
(20) (a) Hanessian, S.; Ponpipom, M. M.; Lavallee, P. *Carbohydr. Res.* **1972**, *24*, 45. (b) Jäger, V.; Häfele, B. *Synthesis* **1987**, 801−806.
(21) (a) Garegg, J.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2866−2869. (b) Wu, I.; Ahlberg, P. *Synthesis* **1994**, 463−464. (c) Bennett, S. M.; Biboutou, R. K.; Zhou, Z.; Pion, R. *Tetrahedron* **1998**, *54*, 4761−4786.
(22) Trost, B. M.; Fleming, I. *Comprehensive Organic Synthesis*; Pergamon Press: Oxford, 1991; Vol. 6, pp 407−408.
(23) Baiocchi, L.; Picconi, G. *Tetrahedron*: *Asymmetry* **1991**, *2*, 231−234.
(24) Martín-López, M. J.; Rodríguez, R.; Bermejo, F. *Tetrahedron* **1998**, *54*, 11623−11636.
(25) Abdel-Magid, A. F.; Cohen, J. H.; Maryanoff, C. A.; Shah, R. D.; Villani, F. J.; Zhang, F. *Tetrahedron Lett.* **1998**, *39*, 3391−3394.

SCHEME 2. Synthesis of Spirolactams 1a and 1b[a]



[a] Reagents and conditions: (a) C_6F_5OH, DCC, THF, 0 °C, 75%; (b) 9-BBN, THF, rt; then, H_2O_2, 3 N NaOH, rt; (c) C_6H_5OH, DCC, THF, 0 °C, 30% from **3**; (d) 9-BBN, THF, rt; then, H_2O_2, 3 N NaOH, rt, 54%; or BH_3, THF, rt; then TMANO, diglyme, rt and reflux; and then DIPEA, CH_2Cl_2, rt, 46%; (e) Leu-O^{t}Bu or D-Leu-O^{t}Bu, 2-hydroxypyridine, toluene, reflux, 65% for **13a** and 68% for **13b**; (f) For **14a**: NBS, Ph_3P, CH_2Cl_2, 0 °C; then, K_2CO_3, reflux, 43%. For **15a** and **15b**: MsCl, pyridine, CH_2Cl_2, rt, (not purified); (g) From **14a**: NaH, 18-crown-6, THF, rt, 48%. From **15a** and **15b**: NaH, 15-crown-5, THF, rt, 77% from **15a** and 54% from **15b**.

The lack of reactivity of amino ester **8** and the instability of compound **9** prompted us to develop a second route to obtain diazaspiro compounds **1a**,**b** on the basis of the formation of the N7−C8 bond as the key step (Scheme 2). For this route, carboxylic acid **3** was esterified with pentafluorophenol[26] to yield the corresponding activated ester **10**, which after hydroboration and oxidation, either in basic or neutral conditions, yielded spirolactone **11**. Lactone **11** was also obtained, although in a lower yield, when first performing the functionalization of the double bond to give the hydroxy acid **12**, followed by esterification with pentafluorophenol.

Conversion of spirolactone **11** into spirolactams **1a**,**b** was planned by opening of the lactone ring with the corresponding amine, followed by cyclization of the resulting hydroxy amides **13**. Thus, treatment of compound **11** with Leu-O^{t}Bu in the presence of 2-hydroxypyridine[27] yielded hydroxy amide **13a**. Cyclization of **13a** under Mitsunobu reaction conditions (DEAD and Ph_3P)[4b,c,28] gave a product resulting from substitution of the hydroxyl group by DEAD, instead of the expected lactam **1a**. Treatment of alcohol **13a** with NBS or MsCl yielded bromide **14a** or mesylate **15a**, respectively. Intramolecular alkylation[29,30] with NaH of both **14a** and **15a** gave the expected spirolactam **1a**. Similarly, spirolactam **1b** was prepared from spirolactone **11** by reaction with D-Leu-O^{t}Bu to give hydroxy amide **13b**, which was converted to its mesylate **15b** and cyclized with NaH in the presence of crown ether.

Spirolactams **1a** and **1b** were identified from their analytical data. The splitting of signals in the ^{1}H NMR and ^{13}C NMR spectra indicated the presence of slow-converting rotamers, which was confirmed for compound **1a** by a variable temperature experiment (29−65 °C) using CD_3CN as the solvent. When the temperature was raised to 65 °C, the signal patterns for the *tert*-butyl, the 2-position, and the α-position protons were considerably simplified. Thus, the *tert*-butyl protons collapsed from two singlets at 29 °C to only one singlet at 65 °C, the H-2 protons changed from one multiplet at 29 °C to a triplet at 65 °C, and the α-proton collapsed from two doublets at 29 °C to only one doublet at 65 °C. Compound **1b** also showed split signals, but their coalescence was not observed in any of the solvents used ($CDCl_3$, CD_3CN, and DMSO-d_6).

Spirolactams **1a** and **1b** were then converted into several derivatives to be used as Pro-Leu and Gly-Leu surrogates in peptide synthesis, both in Fmoc/O^{t}Bu and in Boc/OBn strategies. Thus, *N*-Boc-spirolactams **1a** and **1b** were *N*-deprotected with TMSOTf and 2,6-lutidine[31] to give **16a** and **16b** and protected again with Fmoc-Osu[32] to give the desired Fmoc/O^{t}Bu derivatives **17a** and **17b** (Scheme 3). The preparation of the Boc/OBn derivatives was not straightforward. Treatment of compound **1a** with TFA[33] furnished the trifluoroacetic salt of amino acid **18a**, which by reaction with Boc_2O and TMAH afforded the *N*-Boc-protected acid **19a** in a 33% yield. Compound **19a** was also prepared from spirolactone **11** following the same synthetic sequence used for the preparation of **1a**, using Leu-OBn to obtain amide **20a**. Interestingly, during the final cyclization step the debenzylation of the intermediate ester **21a** also took place, to give directly **19a**.

Once pseudopeptides **1a** and **1b** were obtained, we prepared Ac-{Gly-Leu}-Met-NH_2 derivatives **2a** and **2b**. The required *N*-acetylated dipeptide **23a** was prepared from spirolactam **16a** by *N*-acetylation and hydrolysis of the resulting *tert*-butyl ester **22a** (Scheme 4). Alterna-

(26) (a) Schmidt, U.; Lieberknecht, A.; Griesser, H.; Talbiersky, J. *J. Org. Chem.* **1982**, *47*, 3261−3264. (b) Kisfaludy, L.; Schon, I. *Synthesis* **1983**, 325−327. (c) Torisawa, Y.; Hosaka, T.; Tanabe, K.; Suzuki, N.; Motohashi, Y.; Hino, T.; Nakagawa, M. *Tetrahedron* **1996**, *52*, 10597−10608.

(27) (a) Strekowski, L.; Visnick, M.; Battiste, M. A. *J. Org. Chem.* **1986**, *51*, 4836−4839. (b) Kumazawa, T.; Harakawa, H.; Obase, H.; Oiji, Y.; Tanaka, H.; Shuto, K.; Ischii, A.; Oka, T.; Nakamizo, N. *J. Med. Chem.* **1988**, *31*, 770−785.

(28) Hughes, D. L. *Org. React.* **1992**, *42*, 335−376.

(29) (a) Aebi, J. D.; Guillaume, D.; Dunlap, B. E.; Rich, D. H. J. *Med. Chem.* **1988**, *31*, 1805−1815. (b) Lee, J. P.; Dunlap, B.; Rich, D. H. *Int. J. Peptide Protein Res.* **1990**, *35*, 481−494.

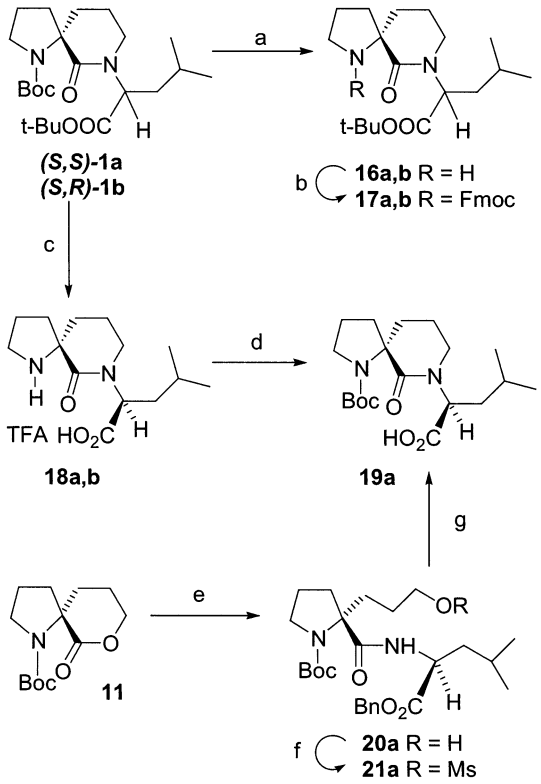
(30) (a) Bonjoch, J.; Casamitjana, N.; Quirante, J.; Rodriguez, M.; Bosch, J. *J. Org. Chem.* **1987**, *52*, 267−275. (b) Alves, J. C. F.; Simas, A. B. C.; Costa, P. R. R.; D′Angelo, J. *Tetrahedron*: *Asymmetry* **1997**, *8*, 1963−1966.

(31) (a) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870−876. (b) Zhang, A. J.; Russell, D. H.; Zhu, J.; Burgess, K. *Tetrahedron Lett.* **1998**, *39*, 7439−7442.

(32) (a) Paquet, A. *Can. J. Chem.* **1982**, *60*, 976−980. (b) Bardaji, E.; Torres, J. L.; Clapés, P.; Albericio, F.; Barany, G.; Rodríguez, R. E.; Sacristán, M. P.; Valencia, G. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1755−1759.

(33) Toshima, H.; Maru, K.; Saito, M.; Ichihara, A. *Tetrahedron* **1999**, *55*, 5793−5808.

SCHEME 3. Synthesis of Pro-Leu and Gly-Leu Surrogates[a]

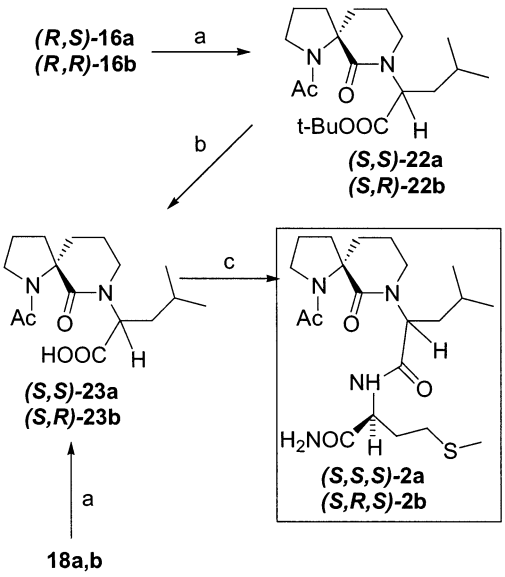


[a] Reagents and conditions: (a) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, (not purified); (b) Fmoc-Osu, $NaHCO_3$, acetone, rt, 48% for **17a** and 60% for **17b**; (c) TFA, rt, 79% for **18a** and quantitative for **18b**; (d) Boc_2O, TMAH, CH_3CN, rt, 33%; (e) H-Leu-OBn, 2-hydroxypyridine, toluene, reflux, 50%; (f) MsCl, pyridine, CH_2Cl_2, rt, (not purified); (g) NaH, 15-crown-5, THF, rt, 42%.

tively, compound **23a** was also obtained by acetylation of the amino acid salt **18a**. The coupling of **23a** with Met-NH_2 using DCC and HOBt yielded the pseudotripeptide **2a** in 70% yield. Compound **2b** was obtained from **16b** and **18b** following a similar sequence.

Conformational Studies of 2a and 2b. To determine whether compounds **2a** and **2b** can promote a β- or a γ-turn conformation, we performed conformational studies using NMR experiments and molecular modeling calculations. β- and γ-turns are nonperiodic peptide segments that reverse the orientation of the peptide chain.[34,35] The most general features of a β-turn are the distance R (<7 Å) between the Cα_i and Cα_{i+3} and the dihedral angle τ ($-90° < \tau < 90°$) formed by the four Cα atoms belonging to the tetrapeptide.[36] The major types[37]

SCHEME 4. Synthesis of Ac-{Gly-Leu}-Met-NH_2 Derivatives[a]



[a] Reagents and conditions: (a) AcCl, pyridine, CH_2Cl_2, rt, 72% for **22a**, 95% for **22b**, 59% for **23a** from **18a**, and 68% for **23b** from **18b**; (b) $H_2O-AcOH-{}^iPrOH$, 100 °C, 60% for **23a** and 88% for **23b**; (c) H-Met-NH_2·HCl, DCC,HOBt, DMF, rt, 70% for **2a** and 42% for **2b**.

of β-turns show a characteristic hydrogen bond between the oxygen atom of the carbonyl function of the first amino acid (i) and the NH amide proton of the fourth ($i + 3$). If the distance between the oxygen and the hydrogen atoms involved is less than 2.5 Å, a hydrogen bond is present,[38] while if it is between 2.5 and 4 Å,[35c,39] there is a significant interaction between them. The major β-turns are classified according to the torsion angles of the second (ϕ_1, ψ_1: $\phi_1 = -60° \pm 30°$ and $\psi_1 = 120° \pm 30°$) and third amino acids (ϕ_2, ψ_2: $\phi_2 = 80° \pm 30°$ and $\psi_2 = 0° \pm 45°$).[3] We shall maintain this classification for the β-turn mimetics of the tripeptides type **2a** and **2b**. The main features characterizing a classical γ-turn are the torsion angles of the second amino acid (ϕ_2, ψ_2: $\phi_2 = 70°/85°$ and $\psi_2 = -60°/-70°$) and the distance between the carbonyl carbon atom of the second amino acid ($i + 1$) and the nitrogen atom of the amino group of the third residue ($i + 3$) with a preferential value of 3 Å (Figures 2−4).[35a]

In our case, the first relevant observation was that in the ^{1}H NMR spectra of **2a** and **2b**, both in $CDCl_3$ and in DMSO-d_6, each of the methionine amide protons NHa showed four signals (Table 1).[40]

This fact was in accordance with the presence of several slow-converting conformations in equilibrium in solution.

NMR Studies. 1. NOESY. In both compounds, the amide proton NHa was correlated with the 8-H proton of the piperidine ring and with the acetyl methyl group

(34) (a) Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* **1996**, *25*, 401−415. (b) Lippens, G.; Hallega, K.; Van Belle, D.; Wodak, S. J.; Nirmala, N. R.; Hill, P.; Reussell, K. C.; Smith, D. D.; Hruby, V. J. *Biochemistry* **1993**, *32*, 9423−9434.

(35) (a) Brickman, K.; Yuan, Z.; Sethon, I.; Somfai, P.; Kihlberg, J. *Chem. Eur. J.* **1999**, *5*, 2241−2253. (b) Lindvall, M. K.; Rissanen, K.; Hakala, J. M. L.; Koskinen, A. M. P. *Tetrahedron. Lett.* **1999**, *40*, 7427−7430. (c) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389−400. (d) Belvisi, L.; Gennari, C.; Madder, A.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 695−699. (e) Belvisi, L.; Bernardi, A.; Manzoni, L.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 2563−2569.

(36) Perczel, A.; McAllister, M. A.; Csaszar, P.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1993**, *115*, 4849−4858.

(37) Vencatachalam, C. M. *Biopolymers* **1968**, *6*, 1425−1436.

(38) Geffrey, G. A. *Introduction to Hydrogen Bond*; Oxford University Press: New York, 1997.

(39) Takeuchi, Y.; Marshall, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 5363−5372.

(40) To facilitate the location of the different NH amide protons, letters a, b, and c have been assigned to each of them. Different conformations are distinguished by a numerical subscript.

TABLE 1. Chemical Shifts, Δδ, upon Addition of DMSO and Temperature Coefficients for 2a and 2b

	compd **2a**					compd **2b**				
	δ($CDCl_3$) (ppm)	δ(DMSO) (ppm)	Δδ (ppm)	|Δδ/Δ*T*| (ppb/K) ($CDCl_3$)	|Δδ/Δ*T*| (ppb/K) (DMSO)	δ($CDCl_3$) (ppm)	δ(DMSO) (ppm)	Δδ (ppm)	|Δδ/Δ*T*| (ppb/K) ($CDCl_3$)	|Δδ/Δ*T*| (ppb/K) (DMSO)
Ha_1	7.90	7.62	−0.28	3.5	2.8	7.90	7.63	−0.27	3.5	3.6
Ha_2	7.76	7.61	−0.15	3.0	2.7	7.77	7.60	−0.17	3.0	2.9
Ha_3	7.67	7.49	−0.18	3.5		7.69	7.48	−0.21	4.0	
Ha_4	7.52	7.46	−0.06	2.5	3.1	7.49	7.46	−0.03	2.5	3.0
Hb_1	7.11	7.31	0.20	3.0	4.1		7.31			4.0
Hb_2	7.11	7.31	0.20	5.0	5.0	7.15	7.31	0.16	5.0	5.5
Hb_3	7.11	7.22	0.11	5.0	5.0	7.11	7.22	0.11	4.0	5.0
Hb_4	6.80	7.22	0.42	5.0		6.70	7.22	0.52		
Hc_1	5.32	7.07	1.75	4.5	6.7	5.20	7.07	1.87	3.5	8.0
Hc_2	5.29	7.04	1.75		6.0	5.18	7.04	1.86	3.0	8.0
Hc_3		6.99			9.0	5.18	7.00	1.82	3.0	
Hc_4	5.19	6.92	1.73	4.0	5.0	5.08	6.92	1.84	3.0	5.0

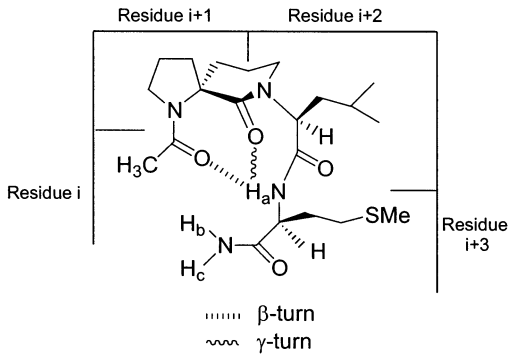


FIGURE 2. Hydrogen bonds stabilize γ- and β-turns in spirolactam **2a**.

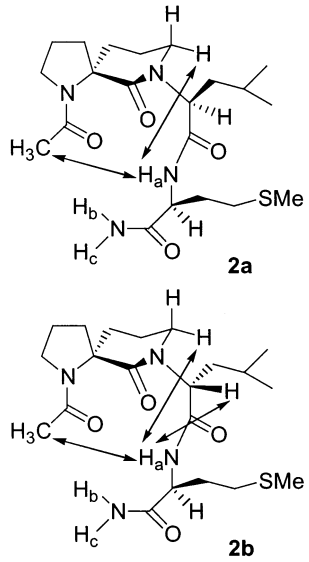


FIGURE 3. NOESY correlations in **2a** and **2b**.

(Figure 3). These correlations implied that in space the acetyl and the methionine were next to each other and that the molecule adopted folded conformations in which the pseudodihedral angle τ was probably close to 0°. Moreover, these correlations suggested proximity of proton NHa to the carbonyl residue *i*, and to the carbonyl residue $i + 1$, which was important for the adoption of a β- or a γ-turn conformation. In addition, in compound **2b** proton NHa was also correlated with the α-proton of the Leu residue. This indicated the difference in conformational space due to the leucine configuration.

NMR Studies. 2. Chemical Shift, Addition of a Competitive Solvent, and Temperature Coefficient. The temperature coefficients for proton NHc in DMSO for compounds **2a** ($|\Delta\delta/\Delta T| = 5-9$ ppb/K) and **2b** ($|\Delta\delta/\Delta T| = 5-8$ ppb/K) clearly indicated that this amide proton was exposed to the solvent[2g,4a] (Table 1).

In contrast, proton NHa was probably intramolecularly hydrogen bonded ($|\Delta\delta/\Delta T| = 2.7-3.1$ ppb/K for **2a** and $|\Delta\delta/\Delta T| = 2.9-3.6$ ppb/K for **2b**). However, temperature coefficients in DMSO for NHb amide proton ($|\Delta\delta/\Delta T| = 4-5$ ppb/K) suggested that this proton was in an equilibrium between hydrogen-bonded and non-hydrogen-bonded states.[35c] Other criteria that have been applied to study the presence of hydrogen bonds in peptide molecules are the ^{1}H NMR chemical shift of the amide protons in $CDCl_3$ and the Δδ upon addition of a good hydrogen bond forming solvent like DMSO. The ^{1}H NMR chemical shift values of protons NHa and NHb in $CDCl_3$ were similar for **2a** and **2b** around δ 7 ppm, suggesting that both protons were intramolecularly hydrogen-bonded. However, proton NHc resonated at a lower chemical shift value (δ around 5 ppm), indicating that it was non-hydrogen-bonded.[35c] Similarly, when ^{1}H NMR spectra were run in a concentration gradient of DMSO in $CDCl_3$, the chemical shift of protons NHa and NHb in both **2a** and **2b** scarcely changed ($\Delta\delta_{NHa} = 0.06-0.37$ ppm and $\Delta\delta_{NHb} = 0.11-0.52$ ppm), whereas proton NHc underwent a strong downfield shift ($\Delta\delta = 1.73-1.87$ ppm), which confirmed that protons NHa and NHb were intramolecularly hydrogen-bonded while proton NHc was non-hydrogen-bonded.[41,42] The overall NMR analysis suggested that the molecules adopted folded conformations and that, in DMSO, the amide proton NHc was non-

(41) André, F.; Vicherat, A.; Boussard, G.; Aubry, A.; Marraud, M. *J. Peptide Res.* **1997**, *50*, 372−381.

(42) In our case, the temperature coefficient values in $CDCl_3$ were less reliable. Only in some of the **2a** and **2b** conformers were the temperature coefficients low enough, according to the criteria fixed by Scolastico ($\Delta\delta/\Delta T < 2.6$ ppb/K),[35c] to be able to conclude without any doubt the existence of intramolecular hydrogen bonding between NHa and CO_{i+1}. In all the other cases, we are in the two situations described by these authors as non-hydrogen-bonded amide protons or equilibrium between hydrogen-bonded and non hydrogen-bonded states. It is worth mentioning that proton NHb can be considered non hydrogen-bonded, but surprisingly in this case proton NHc appears to be in equilibrium between the hydrogen-bonded and non hydrogen-bonded state. This could only be explained if this latter hydrogen-bond involves a third carbonyl group which can only be leucine CO_{i+2}.

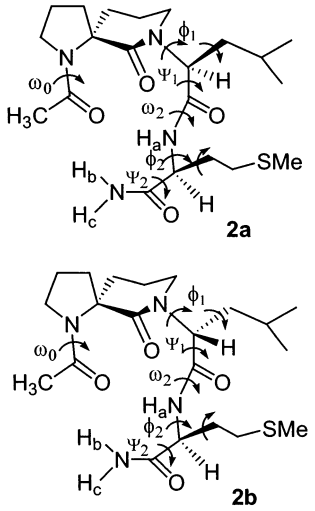


FIGURE 4. Dihedral angles for structures **2a** and **2b**.

hydrogen-bonded, proton NHa was involved in a hydrogen-bond, and proton NHb was in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state.

Molecular Modeling. 1. Monte Carlo Calculations. To determine the conformational space available to compounds **2a** and **2b**, we performed both Monte Carlo and molecular dynamics with iterative simulated annealing[43] calculations. For the Monte Carlo protocol, molecular models for compounds **2a** and **2b** were initially constructed using the model-building facility implemented in Spartan 5.0. Coordinates for these structures were used as input for the Monte Carlo protocol.[44] Rotation was allowed around the dihedral angles, which constitute the main determinants of the structure (Figure 4). A conformational search of **2a** and **2b** was carried out with Spartan version 5.0,[45a] starting from different extended conformations. Structures corresponding to the energy minima (as calculated with the MMF94 force field[45b]) within a 10 kcal/mol window above the global minimum were analyzed on the basis of all the geometric turn diagnosis parameters, which are diagnostic for the presence of γ- and β-turns. The corresponding distribution profiles are summarized in Table 2 and the Supporting Information.

A general analysis of the Monte Carlo results (Table 2) showed that two characteristic geometric features of the β-turn (R and τ) were fulfilled by most of the stable conformers of **2a** and **2b**. However, the distance constraint for NHa···CO_i was fulfilled by approximately 30% of the population of conformers of **2a** and **2b**. On the other hand, the distance between NHa and CO_{i+i} corresponded to that of a hydrogen bond, and the angle values ϕ_2 and ψ_2 agreed well with the values expected for a γ-turn in a considerable percentage of the population. Another hydrogen bond between NHb···CO_i was probably present in about half of the most stable conformers.[38]

TABLE 2. Percentages of Minima Energy Conformers of the Pseudopeptides Which Exhibit Different Features of β-II and/or γ-Turns (Monte Carlo Conformational Search)

	epimer		
	2a	**2b**	turn type
R (Cα − Cα_{i+3}) < 7 Å	81	89	β
$-90° < \tau > +90°$	83	87	
NHa···O_iC < 2.5 Å	30 (30[a])	23 (23[a])	
$+70° < \Phi_2 > +85°$	52	57	γ
$-60° < \Psi_2 > -70°$	34	35	
NHa···O_{i+1}C < 2.5 Å	65 (59[b])	63 (54[b])	
$-90° < \Phi_1 > -30°$	100	100	βII
$+90° < \Psi_1 > +150°$	54	34	
$+50° < \Phi_2 > +110°$	83	78	
$-45° < \Psi_2 > +45°$	39	37	
NHa···O_iC < 4 Å	69	81	
NHa···O_{i+1}C < 4 Å	100	100	
NHb···O_iC < 2.5 Å	41	47	
NHc···O_{i+2}C < 2.5 Å	25	15	
ω_0 trans/cis	92/5	95/8	

[a] Percentage of conformers that form hydrogen bonds: the distances NHa···O_iC are less than 2.5 Å, the NHa···O_i bond angle is larger than 120°, and the NHa···O_i=C angle is larger than 90°. [b] Percentage of conformers that form hydrogen bonds: the distances NHa···O_{i+1}C are less than 2.5 Å, the NHa···O_{i+1} bond angle is larger than 120°, and the NHa···O_{i+1}=C angle is larger than 90°.

To determine the presence of cis and trans conformers for the acetamide group, we analyzed the dihedral angle ω_0 (Figure 4). In the majority of the conformer populations of both epimers (Table 2) we found a trans disposition for the C−N bond and a small proportion of cis. This result was in agreement with the ^{1}H NMR observation of two rotamers (the acetyl methyl group was split; see the Experimental Section).

A more detailed study of the conformers obtained in the Monte Carlo search allowed us to classify the minima energy conformers of each epimer into families (Table 3, Figure 5). Epimers **2a** and **2b** yielded four families of conformers. This result agreed with the observation of four signals for the methionine NHa amide proton of **2a** and **2b** in the ^{1}H NMR spectra (in $CDCl_3$ and DMSO-d_6). All conformers met the general criteria (R and τ) for a β-turn. Compound **2a** showed distances allowing a hydrogen bond[38] between NHa and CO_{i+1} for the families of conformations **2a$_1$**, **2a$_2$**, and **2a$_4$**, which corresponded to γ-turn conformations. In contrast, family **2a$_3$** showed a hydrogen bond distance between NHa and CO_i which corresponded to a β-turn. In addition, families **2a$_1$** and **2a$_2$** had a supplementary hydrogen bond between NHb and CO_i. However, family **2a$_4$** showed a remarkably long distance between NHb and CO_i but a short distance between NHc and CO_{i+2} (1.82 Å). A closer analysis of the dihedral angles Ψ_1, Φ_1, Ψ_2, and Φ_2 confirmed that families **2a$_1$**, **2a$_2$**, and **2a$_4$** were γ-turn conformations, whereas **2a$_3$** was a β-II-type turn. A similar analysis of the data for epimer **2b** indicated that in this case all four families of conformations were γ-turns. Families **2b$_{1-3}$** had a supplementary hydrogen bond between NHb and CO_i. Family **2b$_4$** showed a long distance between NHb and CO_i, but proton NHc was close to CO_{i+2}.

(43) Corcho, F.; Filizola, M.; Peres J. J. *Chem. Phys. Lett.* **2000**, *319*, 65−70.

(44) To validate this approach on the mimetics of spiro compounds, we ran a test calculation (3000 steps) on compound **5** of ref 47 allowing rotation around all dihedral angles which constitute the main determinants of the structure. The starting conformation was totally extended. As a result we obtained similar distribution profiles to those reported by Muller et al. in ref 47 for compound **5** (data not shown).

(45) (a) Hehre, W. J.; Huang, W. W.; Klunzinger, P. E.; Deppmeier, B. J.; Driessen, A. J. *A Spartan Tutorial*; Wavefunction, Inc.: Irvine, CA, 1997; p 85−93. (b) (MMFF 94) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519.

TABLE 3. Characteristics of Energy Minima Conformers (Monte Carlo) for Compounds 2a and 2b

	2a				2b					
family	1	2	3	4	1	2	3	4	βII[a]	γ[a]
ΔE (kcal/mol)	0	2	2.2	4.1	0	1.3	3.2	3.7		
R (Å)	6.2	5.8	4.7	6.1	6,3	5.9	5.9	5,6	<7	
τ (deg)	11	18	5	11	22	24	7	23	0 ± 90	
NHa···O$_i$C (Å)	3.7	3.0	2.0	4.0	3,6	3.2	4.3	3.2	<2.5	
NHa···O$_{i+1}$C (Å)	1.8	1.8	2.7	1.8	2,0	1.9	1.9	1.9		<2.5
ϕ_1 (deg)	−65	−57	−58	−68	−65	−63	−70	−59	−60 ± 30	
ψ_1 (deg)	162	157	134	165	164	161	158	157	120 ± 30	
ϕ_2 (deg)	74	76	61	72	85	84	75	84	80 ± 30	70/85
ψ_2 (deg)	−68	−54	21	−78	−76	−66	−95	−70	0 ± 45	−60/−70
NHb···O$_i$C (Å)	2.0	1.8	3.1	6.4	1.9	1.8	1.9	6.0		

[a] Expected values for canonical β-II and γ turns.

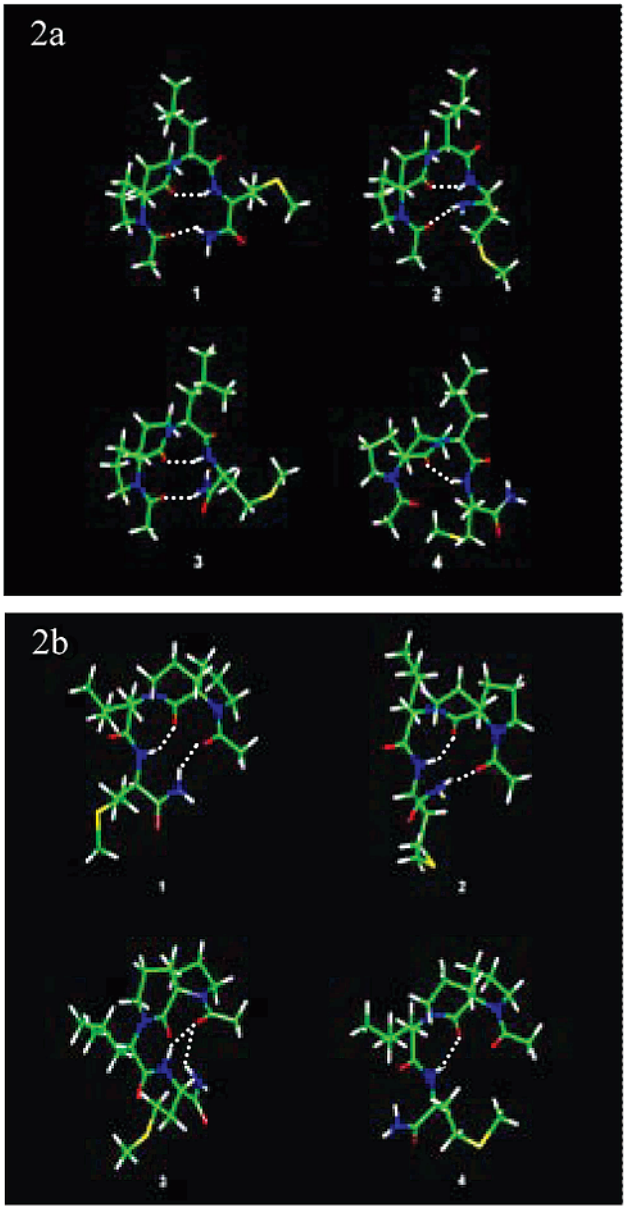


FIGURE 5. Stick conformational representation of minimum energy conformers of epimers **2a** and **2b** obtained after the Monte Carlo conformational search.

In summary, our Monte Carlo calculations allowed us to conclude that in both epimers **2a** and **2b** the adoption of γ-turn conformations was predominant (except for **2a$_3$**), even though many of the criteria for the formation of β-turn were also met, indicating that families **2a$_{1-2}$**, **2a$_4$**, and **2b$_{1-4}$** presented the so-called "γ-turn/distorted type-II β-turn" structure. In these structures, the presence of the NHa and CO$_{i+1}$ hydrogen bond defines a seven-membered pseudocycle more entropically favored than the typical 10 membered ring defined by NHa and CO$_i$ in β-turns.

Molecular Modeling. 2. Molecular Dynamics Calculations. The above results were confirmed performing a parallel conformational search using molecular dynamics with Iterative Simulated Annealing.[43] This protocol[46] was run five times, employing fully extended starting structures of both compounds (**2a** and **2b**) with AMBER (implemented in DISCOVER 3) as the force field. The profile for the main parameters characterizing γ- and β-turns were similar to those found using the Monte Carlo protocol (data not shown).

To study the dynamic behavior of the most stable family of conformations of pseudopeptides **2a** and **2b**, the global minima conformers of both compounds (**2a$_1$** and **2b$_1$**) were taken as the starting points for molecular dynamics calculations. We analyzed the geometric β- and γ-turn diagnosis parameters of the structures generated during the molecular dynamics simulation for 1 ns at 300 K in $CDCl_3$ as an explicit solvent (Table 4). The distances R found in the resulting conformers of both epimers **2a$_1$** and **2b$_1$** during the molecular dynamics remained below 7 Å. Both conformers, **2a$_1$** and **2b$_1$**, showed no major dihedral angle τ fluctuations with respect to those of the starting structures for the molecular dynamic simulations. The distances found for NHa···O$_i$C were not compatible with the presence of a hydrogen bond (which would stabilize the β-turn) in either of the conformers **2a$_1$** or **2b$_1$**. It is worth mentioning that during the molecular dynamics simulation the distances corresponding to the hydrogen bond NHb···O$_i$C were not maintained either. In contrast, the distance values corresponding to NHa and CO$_{i+1}$ of both families indicated that these atoms established a hydrogen bond and therefore that the γ-turn conformation was maintained for both epimers **2a** and **2b**. These distances remained below 2.5 Å for 32 and 63% of the population for **2a$_1$** and **2b$_1$**, respectively. The participation of proton NHa in an intramolecular

(46) To validate the Iterative Simulated Annealing method on spiro compound mimetics, we ran a test calculation on compound **5** described in ref 47. The starting conformations were totally extended. The results obtained have a distribution profile similar to those reported by Muller et al. in ref 47 for compound **5** (data not shown).

TABLE 4. Percentages of Conformers of the Pseudopeptides That Exhibit Different Features of β-II and/or γ-Turns from 1 ns, 300 K Molecular Dynamics

	conformer		
	2a$_I$	**2b$_1$**	turn type
R (Cα−Cα$_{i+3}$) < 7 Å	71	85	β
−90° < τ > +90°	90	95	
NHa···O$_i$C < 2.5 Å	16 (0[a])	2 (1[a])	
+70° < Φ$_2$ > +85°	30	71	γ
−60° < Ψ$_2$ > −70°	45	40	
NHa···O$_{i+1}$C < 2.5 Å	32 (29[b])	63 (53[b])	
−90° < Φ$_1$ > −30°	95	96	βII
+90° < Ψ$_1$ > +150°	90	88	
+50°< Φ$_2$ > +110°	50	96	
−45°< Ψ$_2$ > +45°	7	3	
NHa···O$_i$C < 4 Å	69	41	
NHa···O$_{i+1}$C < 4 Å	81	80	
NHb···O$_i$C < 2.5 Å	8	15	
NHc···O$_{i+2}$C < 2.5 Å	0	1	
w_0 trans/cis	100/0	100/0	

[a] Percentage of conformers that form hydrogen bonds: the distances NHa···O$_i$C are less than 2.5 Å, the NHa···O$_i$ bond angle is larger than 120°, and the Ha···O$_i$=C angle is larger than 90°. [b] Percentage of conformers that form hydrogen bonds: the distances NHa···O$_{i+1}$C are less than 2.5 Å, the NHa···O$_{i+1}$ bond angle is larger than 120°, and the Ha···O$_{i+1}$=C angle is larger than 90°.

hydrogen bond was evidenced by the ^{1}H NMR experiments (NOESY and temperature coefficients in $CDCl_3$ and in DMSO-d_6).

Concerning the torsion angles of compounds **2a$_1$** and **2b$_1$** during the molecular dynamics calculations, the values of Φ$_1$, Ψ$_1$, and Ψ$_2$ corresponded to a β-II-turn conformation. However, the values of Φ$_2$ were in accordance with the values expected for the two kinds of turns (β and/or γ) especially for epimer **2b$_1$** (Table 4). In conclusion, our 300 K molecular dynamics calculation showed that for both epimers the starting global minimum γ-turn conformations remained stable within a 1 ns simulation.

While NMR results showed the participation of the NHa proton in an intramolecular hydrogen bond, molecular modeling allowed us to establish that it was involved in a hydrogen bond with O$_{i+1}$C stabilizing the formation of a γ-turn. However, most of the parameters involved in the formation of β-turn were also satisfied (the distance C$_i$−C$_{i+1}$ and Φ$_1$, Φ$_2$, ψ$_1$ angles, not defined for γ-turns), indicating that pseudotripeptides **2a** and **2b** adopted a "γ-turn/distorted type-II β-turn" conformation.

In contrast with the results reported by Ward et al.,[6] in which the (*S*)-5.5-spirolactam induced a classical type-II′ β-turn conformation whereas the (*R*)-spirolactam favored an extended one, we have shown that pseudotripeptides **2a** and **2b** derived from (*S*)-5.6-spirolactams adopt "γ-turn/distorted type-II β-turn" conformations. This may alter the biological activity of pseudopeptides containing these surrogates of the native Gly-Leu dipeptide.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on a 200 or 300 MHz instrument, and 2D NMR COSY experiments were performed on a 500 MHz instrument. Unless otherwise noted, NMR spectra were registered in $CDCl_3$, and chemical shifts are expressed in parts per million (δ) relative to internal TMS. Mass spectra were determined either by chemical ionization (MS−CI) or electronic impact (MS−EI). Flash column chromatography was carried out on silica gel 60 (40−63 MM, Sds), unless otherwise indicated. Analytical TLC was performed on silica gel (F254, Macherey-Nagel) and developed with the solvent described in each case for flash chromatography. The spots were located by UV light and $KMnO_4$ reagent. Purification of reagents and solvents was effected according to standard methods.[48] All extracts were dried (Na_2SO_4) prior to concentration under reduced pressure. Elemental analyses were performed at the Serveis Cientificotecnics of Universitat de Barcelona.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(3-hydroxypropyl)proline Methyl Ester (5). To a stirred solution of carboxylic acid **3**[4a,15] (1 g, 4 mmol) in dry acetone (30 mL) at room temperature was added K_2CO_3 (595 mg, 4.4 mmol), and stirring was continued for 1 h. Next, MeI (0.318 mL, 5.2 mmol) was added, and the mixture was heated to reflux overnight. After being cooled to room temperature, the mixture was concentrated and the residue was purified by flash chromatography (hexane/AcOEt, 8/2) to afford **(*R*)-*N*-(*tert*-butoxycarbonyl)-2-allylproline methyl ester (4)** (966 mg, 92%, rotamers were observed in the NMR spectra whose data were coincidental with those described by Johnson[4a]), $[\alpha]^{22}_D = +21.9$ (*c* 0.8, $CHCl_3$). Next, to a stirred solution of alkene **4** (950 mg, 3.5 mmol) in dry THF (35 mL) at room temperature was added 9-BBN (10.6 mL, 5.25 mmol), and the mixture was stirred for 2 h. Another 10.6 mL (5.25 mmol) of 9-BBN was added, and stirring was continued overnight. Then, H_2O (0.7 mL), 3 N NaOH (10.7 mL, 31.5 mmol), and H_2O_2 (10.7 mL) were added. After 45 min, the reaction was quenched by the addition of NaCl and extracted with Et_2O. The organic extracts were dried and concentrated to afford a pale yellow oil that was chromatographed to obtain alcohol **5** (992 mg, 98%, rotamers were observed): $[\alpha]^{22}_D = +7.0$ (*c* 0.7, $CHCl_3$); IR (NaCl) 3446, 1742, 1686 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.33 and 1.37 (2s, 9 H), 1.45 (m, 1H), 1.54 (m, 1H), 1.75 (m, 2H), 1.80−2.05 (m, 3H), 2.11 (ddd, J = 13.7, 12, 5 Hz, 1H, one rotamer) and 2.24 (ddd, J = 13.7, 12, 5 Hz, 1H, one rotamer), 3.33 (m, 1H), 3.52−3.68 (m, 3H), 3.63 (s, 3H); ^{13}C NMR δ 22.6 and 23.0, 26.8, 28.2 and 28.3, 30.2 and 31.4, 36.1 and 37.3, 48.4 and 48.6, 52.0, 62.6 and 62.7, 67.2 and 67.8, 79.6 and 80.0, 153.7, 175.4; CIMS *m*/*z* 305 (M$^+$ + 18, 84), 288 (M$^+$ + 1, 100), 287 (M$^+$, 1), 272 (36), 249 (42), 188 (21), 170 (47), 128 (30). Anal. Calcd for $C_{14}H_{25}O_5N$: C, 58.52; H, 8.77; N, 4.87. Found: C, 58.60; H, 9.27; N, 4.47.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(3-bromopropyl)proline Methyl Ester (6). Triphenylphosphine (493 mg, 1.92 mmol) was added to a stirred solution of alcohol **5** (450 mg, 1.6 mmol) and NBS (335 mg, 1.92 mmol) in dry CH_2Cl_2 (30 mL). The mixture was stirred at 0 °C during 4 h. Then, solid K_2CO_3 (128 mg) was added, and the mixture was heated to reflux for 15 min. After being cooled to room temperature, the mixture was filtered, the filtrate was concentrated, and the residue was purified by flash chromatography (hexane/AcOEt, 10/0 to 8/2) to afford bromo ester **6** (388 mg, 71%, rotamers were observed): $[\alpha]^{22}_D = +10.9$ (*c* 0.5, $CHCl_3$); IR (NaCl) 1738, 1690 cm^{-1}; ^{1}H NMR δ 1.42 and 1.45 (2s, 9 H), 1.75−1.95 (m, 4H), 1.90−2.20 (m, 3H), 2.26−2.38 (m, 1H), 3.36−3.48 (m, 3H), 3.57−3.78 (m, 1H), 3.70 and 3.71 (2s, 3H); ^{13}C NMR δ 22.6 and 23.1, 27.2 and 27.8, 28.2 and 28.3, 33.2, 33.8 and 34.0, 36.2 and 37.5, 48.4 and 48.5, 52.1, 66.9 and 67.5, 79.6 and 80.2, 153.5, 175.0; CIMS *m*/*z* 368 (M$^+$ + 18, 23), 366 (24), 351 (M$^+$ + 1, 57), 350 (M$^+$, 10), 349 (59), 313 (25), 311 (28), 240 (100), 170 (39), 138 (25), 121 (58). Anal. Calcd for $C_{14}H_{24}O_4NBr$: C, 48.01; H, 6.91; N, 4.00. Found: C, 47.76; H, 6.77; N, 3.94.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(3-iodopropyl)proline Methyl Ester (7). Triphenylphosphine (611 mg, 2.25 mmol),

(47) Muller, G.; Hessler, G.; Decornes, H. Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 894−896.

(48) Perrin, D. D.; Armarego, W. F. L.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, 1980.

imidazole (317 mg, 4.5 mmol), and I_2 (552 mg, 2.1 mmol) were added, sequentially and in small portions, to a stirred solution of alcohol **5** (446 mg, 1.5 mmol) in dry CH_2Cl_2 (25 mL). The resulting mixture was stirred at room temperature during 2 h and then filtered over silica gel. The filtrate was concentrated and the residue purified by flash chromatography (hexane/AcOEt, 10/0 to 8/2) to afford iodo ester **7** (420 mg, 68%, rotamers were observed): IR(NaCl) 1734, 1686 cm^{-1}; ^1H NMR δ 1.43 and 1.45 (2s, 9H), 1.70−2.20 (m, 7H), 2.21−2.37 (m, 1H), 3.20 (ddd, J = 13, 6.5, 3.3 Hz, 2H), 3.41 (dt, J = 10.6, 5 Hz, 1H), 3.56−3.65 and 3.67−3.80 (2m, 1H), 3.70 and 3.72 (2s, 3H); ^{13}C NMR δ 6.61, 22.6 and 23.1, 27.9 and 28.6, 28.2, 35.4 and 36.2, 36.1 and 37.6, 48.4 and 48.5, 52.1, 66.8 and 67.5, 79.6 and 80.2, 153.5, 175.0; CIMS m/z 397 (M^+, 1), 325 (14), 170 (100), 156 (61).

Methyl (2*S*)-1-(*tert*-Butoxycarbonyl)-2-[*N*-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyll-3-amino]propyl]pyrrolidine-2-carboxylate (8). Method A. To a stirred solution of bromo ester **6** (200 mg, 0.57 mimol) in DMF (1 mL) were added H-Leu-O^{t}Bu (214 mg, 0.9 mmniol) and K_2CO_3 (95 mg, 0.684 mmol), and the resulting mixture was heated to reflux for 15 h. After being cooled to room temperature, the mixture was concentrated and the residue was purified by flash chromatography (hexane/AcOEt, 8/2 to 7/3) to afford amine **8** (160 mg, 61%, rotamers were observed). **Method B.** Operating as above, from iodo ester **7** (180 mg, 0.45 mmol) in DMF (2 mL), H-Leu-O^{t}Bu (170 mg, 1.14 mmol), and K_2CO_3 (75 mg, 0.694 mmol), a residue was obtained that was purified by flash chromatography (hexane/AcOEt, 8/2 to 6/4) to afford amine **8** (167 mg, 81%): $[\alpha]^{22}_D$ = +2.2 (c = 0.6, CH_3OH); IR (NaCl) 2956, 1740, 1728, 1699 cm^{-1}; ^1H NMR δ 0.90 and 0.93 (2d, J = 6.7 Hz, 6H), 1.40 and 1.47 (2s, 9H each), l.39−1.48 (m, 4H), 1.64−1.80 (m, 1H), 1.75−2.20 (m, 4H), 2.48 (m, 1H), 2.60 (m, 1H), 3.12 (t, J = 7.3 Hz, 1H), 3.4 (m, 1H), 3.7 (m, 1H), 3.70 (s, 3H); ^{13}C NMR δ 22.4 and 22.6; 23.1 and 24.4; 24.5 and 24.7; 24.9; 28.0, 28.2 and 28.3; 31.6 and 32.8; 36.0 and 37.3; 43.0; 48.2 and 48.3; 48.4 and 48.5; 52.0; 60.6 and 60.8; 67.3 and 67.8; 79.6, 79.7 and 80.7; 153.6; 175.4; CIMS m/z 457 (M^+ + 1, 100), 401 (83), 355 (63), 345 (29), 57 (24). Anal. Calcd for $C_{24}H_{44}O_6N_2 \cdot {}^1/_2H_2O$: C, 61.91; H, 9.74; N, 6.02. Found: C, 61.83; H, 9.98; N, 6.15.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-allylproline Pentafluorophenyl Ester (10). To an ice-cooled and stirred solution of acid **3** (1 g, 319 mmol) and pentafluorophenol (720 mg, 3.9 mmol) in THF (32 mL) was added DCC (808 mg, 3.9 mmol), and stirring was continued overnight. After gradual warming to room temperature, the reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by flash chromatography (neutral Al_2O_3, CH_2Cl_2/hexane, 9/1−10/0) to give ester **10** (1.22 g, 75%, rotamers were observed): $[\alpha]^{22}_D$ = +7.3 (c 0.7, CH_3OH); IR (NaCl) 1787, 1691 cm^{-1}; ^1H NMR δ 1.47 and 1.48 (2s, 9 H), 1.85−2.10 (m, 2H), 2.20−2.38 (m, 2H), 2.71 (dd, J = 14, 8 Hz, 1H, one rotamer), 2.70 (dd, J = 14, 8 Hz, 1H, one rotamer), 3.14 (dd, J = 14.2, 6.3 Hz, 1H, one rotamer), 3.22 (dd, J = 14, 6.7 Hz, 1H, one rotamer), 3.44 (m, 1H), 3.67 (ddd, J = 10.5, 7.6, 5.7 Hz, 1H, one rotamer), 3.79 (ddd, J = 10.6, 7.8, 4.4 Hz, 1H, one rotamer), 5.20 (m, 2H), 5.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 22.3 and 23.4, 28.1 and 28.3, 35.8 and 37.6, 38.2 and 39.1, 48.3 and 48.6, 66.9 and 67.4, 80.2 and 81.1, 119.6 and 120.1, 131.8 and 132.5, 136.0−142.9, 153.1 and 153.6, 170.5 and 170.6; CIMS m/z 439 (M^+ + 18, 21), 422 (M^+ + 1, 19), 421 (M^+, 1), 382 (21), 310 (43), 273 (100), 256 (61), 255 (26), 250 (26), 240 (20), 217 (51), 110 (35). Anal. Calcd for $C_{19}H_{20}O_4NF_5$: C, 54.16; H, 4.78; N, 3.32. Found: C, 53.75; H, 4.93; N, 3.28.

(*R*)-1-(*tert*-Butoxycarbonyl)-6-oxo-7-oxa-1-azaspiro[4.5]-decane (11). Method A. To an ice-cooled and stirred solution of alkene **3** (250 mg, 0.97 mmol) in dry THF (11 mL) was added 9-BBN (2.9 mL, 1.5 mmol), and the mixture was stirred at room temperature for 2 h. Another 2.9 mL (1.5 mmol) of 9-BBN was added, and stirring was continued for an additional 2 h. Next, 3 N NaOH (2.9 mL) and H_2O_2 (2.9 mL) were added. After 45 min, 1 equiv of oxidant was added, and the mixture was stirred overnight. The reaction was quenched with brine and extracted with Et_2O. The organic extracts were dried and concentrated to afford **(*R*)-*N*-(*tert*-butoxycarbonyl)-2-(3-hydroxypropyl)proline (12)** as a pale yellow oil. Next, to an ice-cooled and stirred solution of crude acid **12** and pentafluorophenol (181 mg, 0.98 mmol) in THF (5 mL) was added DCC (202 mg, 0.98 mmol), and stirring was continued overnight. After gradual warming to room temperature, the reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by flash chromatography (neutral Al_2O_3, CH_2Cl_2/hexane, 9/1 to 10/0) to give lactone **11** (76 mg, 30% from **3**). **Method B.** To a stirred solution of alkene **10** (500 mg, 1.2 mmol) in dry THF (10 mL) at room temperature was added BH_3 (0.475 mL, 1.44 mmol), and the resulting mixture was stirred for 3 h. Another 0.475 mL (1.44 mmol) of BH_3 was added, and stirring was continued for an additional 2 h. The mixture was concentrated, the resulting organoborane was dissolved in 2 mL of diglyme, and TMANO (160 mg, 1.44 mmol) was added all at once to this solution. The reaction mixture was gently stirred at reflux temperature for 3 h and at room temperature overnight. The reaction was quenched with brine and extracted with Et_2O. The organic extracts were dried and concentrated to afford a pale yellow oil, which was next dissolved in CH_2Cl_2 (60 mL) and DIPEA (0.23 mL, 1.32 mmol). After being stirred at room temperature overnight, the mixture was concentrated and the residue was purified by flash chromatography (neutral Al_2O_3, CH_2Cl_2/hexane, 9/1−10/0) to give lactone **11** (140 mg, 46%). **Method C.** To an ice-cooled and stirred solution of alkene **10** (1 g, 2.4 mmol) in dry THF (35 mL) was added 9-BBN (7 mL, 3.6 mmol), and the mixture was stirred at room temperature for 2 h. Another 7 mL (3.6 mmol) of 9-BBN was added, and stirring was continued for an additional 2 h. Next, 3 N NaOH (0.8 mL, 2.4 mmol) and H_2O_2 (0.8 mL, 7.2 mmol) were added. After 45 min, 1 equiv of oxidant was added, and the mixture was stirred overnight. The reaction was quenched by the addition of solid NaCl and extracted with Et_2O. The organic extracts were dried and concentrated to afford a pale yellow oil that was chromatographed (neutral Al_2O_3, CH_2Cl_2/hexane, 9/1−10/0) to obtain lactone **11** (290 mg, 54%, rotamers were observed): $[\alpha]^{22}_D$ = +10.8 (c 1, CH_3OH); IR (NaCl) 1739, 1693 cm^{-1}; ^1H NMR δ 1.46 and 1,48 (2s, 9H), 1.75 and 1.81 (2m, 1H), 1.78−2.10 (m, 4H), 2.21−2.38 (m, 2H), 2.40 (tdm, J = 12.3, 5 Hz, 1H, one rotamer), 2.55 (tdm, J = 12.3, 5 Hz, 1H, one rotamer), 3.57 (m, 2H), 4.40 (m, 2H); ^{13}C NMR 22.4 and 23.4, 28.3 and 28.4, 32.5 and 33.2, 39.1 and 40.2, 47.8 and 47.9, 64.7 and 64.8, 69.7 and 69.8, 79.9 and 80.9, 153.0 and 154.0, 173.6 and 173.8; CIMS m/z 273 (M^+ + 18, 100), 256 (M^+ + 1, 8), 255 (M^+, 1), 217 (57), 156 (30). Anal. Calcd for $C_{13}H_{21}O_4N$: C, 61.16; H, 8.26; N, 5.49. Found: C, 61.17; H, 8.78; N, 5.99.

(2*R*)-1-(*tert*-Butoxycarbonyl)-*N*-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-2-(3-hydroxypropyl)pyrrolidine-2-carboxamide (13a). A solution of lactone **11** (410 mg, 1.6 mmol), H-Leu-O^{t}Bu (900 mg, 4.8 mmol), and 2-hydroxypyridine (167 mg, 1.76 mmol) in 1 mL of toluene was heated at reflux temperature for 72 h under an argon atmosphere. After being cooled to room temperature, the mixture was concentrated and the residue was purified by flash chromatography (hexane/AcOEt, 2/1 to 1/1) to afford amide **13a** (448 mg, 65%, rotamers were present): $[\alpha]^{22}_D$ = −26.5 (c 0.6, $CHCl_3$); IR (NaCl) 3500, 1735, 1693, 1681 cm^{-1}; ^1H NMR (500 MHz, CD_3CN, 65 °C): δ 0.94 (dt, J = 10.5, 3 Hz, 6H), 1.44, 1.45 and 1.46 (4s, 18H), 1.25−1.62 (m, 5H), 1.75−1.84 (m, 2H), 1.88−1.98 (m, 2H), 2.02−2.17 (m, 2H), 2.40−2.52 (m, 1H), 3.45−3.68 (m, 2H), 3.50−3.65 (m, 1H), 4.24−4.36 (m, 1H); ^{13}C NMR (CD_3CN, 29 °C): δ 21.6 and 21.8; 23.1; 23.2 and 23.3; 25.2 and 25.6; 28.1, 28.5 and 28.6; 31.9; 33.1 and 33.7; 35.5 and 36.3; 41.1; 49.9; 52.4; 62.7; 71.3; 80.5 and 81.9; 154.5 and 156.0; 172.9; 174.8 and 175.6; CIMS m/z 460 (M^+ + 18, 9), 444 (M^+ + 2, 27), 443 (M^+ + 1, 100), 273 (39), 217 (23). Anal. Calcd for

$C_{23}H_{42}O_6N_2 \cdot {}^1/_3H_2O$: C, 61.59; H, 9.59; N, 6.25. Found: C, 61.62; H, 9.61; N. 6.29.

(2*R*)-1-(*tert*-Butoxycarbonyl)-*N*-[(1*R*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-2-(3-hydroxypropyl)pyrrolidine-2-carboxamide (13b). Operating as above in the preparation of **13a**, from lactone **11** (770 mg, 3 mmol), H-D-Leu-O^{t}Bu (1.69 g, 9 mmol) and 2-hydroxypyridine (291 mg, 3.3 mmol) in 3 mL of toluene/Et_2O (1:1), amide **13b** (900 mg, 68%, rotamers were present) was obtained after flash chromatography (hexane/AcOEt, 2/1−1/1): $[\alpha]^{22}_D = -27$ (*c* 1.1, $CHCl_3$); IR (NaCl) 3580, 1734, 1679 cm^{-1}; ^{1}H NMR (CD_3OD): δ 0.88−0.96 (m, 6H), 1.45 and 1.47 (2s, 18H), 1.52−1.64 (m, 3H), 1.65−1.76 (m, 2H), 1.78−1.88 (m, 1H), 1.90−2.04 (br s, 2H), 2.08−2.30 (br s, 3H), 3.26−3.36 (m, 1H), 3.46−3.64 (m, 2H), 3.68−3.82 (m, 1H), 4.28−4.42 (m, 1H); ^{13}C NMR (CD_3OD) δ 21.7, 23.0, 23,5, 25.8, 27.7, 28.3 and 28.7, 31.5 and 32.0, 39.1, 41.1 and 41.3, 50.4, 52.9, 62.9, 69.7 and 70.5, 81.2, 81.8, 155.7, 173.3, 177.5; CIMS *m*/*z* 460 ($M^+ + 18$, 11), 445 (28), 444 ($M^+ + 2$, 100), 442 (M^+, 5). Anal. Calcd for $C_{23}H_{42}O_6N_2 \cdot {}^1/_3H_2O$: C, 61.59; H, 9.59; N, 6.25. Found: C, 61.57; H, 9.61; N, 6.15.

(2*R*)-1-(*tert*-Butoxycarbonyl)-*N*-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-2-(3-bromopropyl)pyrrolidine-2-carboxamide (14a). Triphenylphosphine (43 mg, 0.17 mmol) was added to a stirred solution of amide **13a** (60 mg, 0.14 mmol) and NBS (29 mg, 0.17 mmol) in dry CH_2Cl_2 (5 mL). The mixture was stirred at 0 °C during 4 h, solid K_2CO_3 (114 mg, 0.82 mmol) was added, and stirring was continued at reflux temperature for 15 min. After being cooled to room temperature, the mixture was filtered, the filtrate was concentrated, and the residue was purified by flash chromatography (hexane/AcOEt,10/0−1/1) to afford bromo amide **14a** (27 mg, 43%, rotamers were observed): $[\alpha]^{22}_D = -11.2$ (*c* 0.4, CH_3OH); IR (NaCl) 1736, 1681 cm^{-1}; ^{1}H NMR (500 MHz, CD_3CN, 65 °C) δ 0.95 (d, $J = 6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 0.97 (d, $J = 6.3$ Hz, 3H), 0.98 (d, $J = 6.9$ Hz, 3H), 1.48 (s, 9H), 1.49 (s, 9H), 1.59−1.65 (m, 2H), 1.66−1.87 (m, 3H), 1.96−2.00 (m, 2H), 2.17−2.54 (br s, 4H), 3.33−3.41 (m, 1H), 3.52 (d, $J = 6.6$ Hz, 2H), 3.58−3.72 (m, 1H), 4.27−4.38 (m, 1H); ^{13}C NMR (CD_3CN, 29 °C) δ 21.9 and 22.2, 23.0 and 23.4, 23.5, 25.6 and 25.9, 28.5 and 28.9, 34.6, 35.4 and 35.6, 35.7 and 36.1, 36.9 and 37.2, 41.5 and 41.9, 50.2, 52.8, 69.5 and 70.8, 81.1 and 82.1, 154.2 and 154.8, 173.2, 175.1; ElMS *m*/*z* 236 (21), 234 (21), 190 (15), 57 (100). Anal. Calcd for $C_{23}H_{41}O_5N_2Br$: C, 54.64; H, 8.43; N, 5.94. Found: C, 54.64; H, 8.30; N, 5.84.

(5*S*)-1-(*tert*-Butoxycarbonyl)-7-[(1*S*)-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (1a). Method A. To a solution of amine **8** (100 mg, 0.22 mmol) in THF−H_2O (15:1) was added TBAH (142 mg, 0.22 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 48 h. The reaction was quenched by the addition of water and extracted with AcOEt. The aqueous layer was acidified with citric acid (pH 3−4) and extracted with AcOEt. The organic extracts were dried and concentrated to afford **(2*S*)-1-(*tert*-butoxycarbonyl)-2-[*N*-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-3-aminopropyl]pyrrolidine-2-carboxylic acid (9).** To a stirred solution of the above crude acid **9** in CH_2Cl_2 (1 mL) was added DCC (40 mg, 0.22 mmol) at 0 °C, and the mixture was stirred for 6 h. The reaction mixture was quenched by addition of water and extracted with AcOEt. The organic extracts were dried and concentrated to afford a residue that was purified by flash chromatography (hexane/AcOEt, 10/0−8/2) to give spirolactam **1a** (21 mg, 23%, rotamers were observed). **Method B.** To a stirred solution of NaH (60% in mineral oil, 3 mg, 0.122 mmol) was added the bromo amide **14a** (27 mg, 0.06 mmol) in THF (2 mL) followed by 18-crown-6 (15 mg, 0.058 mmol), and stirring was continued at room temperature for 3 h. Another 3 mg of NaH (60% in mineral oil, 0.122 mmol) was added, and stirring was continued for 4 h. The reaction was quenched by the addition of H_2O and extracted with AcOEt. The organic extracts were dried and concentrated to afford a pale yellow oil that was chromatographed (hexane/AcOEt, 10/0 to 8/2) to yield spirolactam **1a** (12 mg, 48%). **Method C.** To an ice-cooled solution of amide **13a** (180 mg, 0.4 mmol) in dry CH_2Cl_2 (2 mL), under argon atmosphere, were added pyridine (0.036 mL, 0.44 mmol) and MsCl (0.065 mL, 1.2 mmol). The resulting mixture was stirred at room temperature for 6 h. The reaction was quenched by the addition of 4% aqueous citric acid, and the organic layer was successively washed with 4% aqueous citric acid and 5% aqueous $NaHCO_3$ solution. The organic extracts were dried and concentrated to afford **(2*R*)-1-(*tert*-butoxycarbonyl)-*N*-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-2-[3-(methanesulfonyl)propyl]pyrrolidine-2-carboxamide (15a)** as a pale yellow oil (190 mg). Next, to a stirred solution of NaH (60% in mineral oil, 35 mg, 0.79 mmol) in dry THF (2 mL) was added the crude mesylate **15a** (190 mg, 0.36 mmol) in THF (2 mL) followed by 15-crown-5 (0.08 mL, 0.4 mmol). The resulting mixture was stirred at room temperature for 3 h. Another 35 mg of NaH (60% in mineral oil, 0.4 mmol) was added, and stirring was continued for 4 h. The reaction was quenched by the addition of H_2O and extracted with AcOEt. The organic extracts were dried and concentrated to afford a pale yellow oil that was chromatographed (hexane/AcOEt, 10/0−8/2) to yield spirolactam **1a** (120 mg, 77% from **13a**): $[\alpha]^{22}_D = -23.2$ (*c* 0.4, CH_3OH); IR (NaCl) 1735, 1696, 1689 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.88 (dd, $J = 9$, 6 Hz, 3H), 0.94 (t, $J = 6.6$ Hz, 3H), 1.38 and 1.40 (2s, 18H), 1.42−2.04 (m, 9H), 2.18−2.32 (m, 1H), 2.51 (td, $J = 12$, 6 Hz, 1H), 3.06−3.14 (m, 1H), 3.42−3.53 (m, 2H), 3.66 (dt, $J = 12$, 6 Hz, 1H, one rotamer), 5.22−5.27 (m, 1H, one rotamer), 5.32 (dd, $J = 11.5$, 4.5 Hz, 1H, one rotamer); ^{13}C MNR δ 21.1 and 22.0, 21.5 and 21.6, 22.2 and 23.3, 22.7 and 23.7, 23.9 and 24.9, 27.9 and 28.4, 32.7 and 33.4, 37.0 and 37.2, 38.9 and 41.2, 43.7 and 43.8, 48.6, 54.6 and 55.1, 65.4 and 65.6, 78.9 and 79.6, 80.8 and 81.2, 153.7 and 153.8, 171.0 and 171.4, 172.6 and 172.8; EIMS *m*/*z* 424 (M^+, 3), 267 (21), 110 (28), 57 (100). Anal. Calcd for $C_{23}H_{40}O_5N_2 \cdot {}^3/_2H_2O$: C, 61.05; H, 9.60; N, 6.19. Found: C, 61.05; H, 9.12; N, 6.00.

(5*S*)-1-(*tert*-Butoxycarbonyl)-7-[(1*R*)-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (1b). Operating as for the preparation of **1a** (method C), from amide **13b** (900 mg, 2.05 mmol), pyridine (0.18 mL, 2.3 mmol), and MsCl (0.53 mL, 6.15 mmol) in dry CH_2Cl_2 (10 mL) was obtained **(2*R*)-1-(*tert*-butoxycarbonyl)-*N*-[(1*R*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-2-[3-(methanesulfonyl)-propyl]pyrrolidine-2-carboxamide (15b)** (938 mg) as a pale yellow oil. Next, from crude mesylate **15b** (938 mg, 1.8 mmol), NaH (60% in mineral oil, two times 82 mg, 0.18 mmol)), and 15-crown-5 (0.04 mL, 0.22 mmol) in dry THF (20 mL) was obtained a pale yellow oil that was purified by flash chromatography (hexane/AcOEt, 10/0−8/2) to give spirolactam **1b** (462 mg, 54% from **13b**): $[\alpha]^{22}_D = +8.7$ (*c* 0.5, CH_3OH); IR (NaCl) 1730, 1693, 1648 cm^{-1}; ^{1}H NMR δ 0.85−0.96 (m, 6H), 1.42 and 1.44 (2s, 9H), 1.44 and 1.46 (2s, 9H), 1.53−2.02 (m, 9H), 2.10−2.32 (m, 1H), 2.50−2.67 (m, 1H), 3.47−3.55 (m, 2H), 3.58−3.66 (m, 2H), 4.55 (dd, $J = 10$, 4.5 Hz, 1H, one rotamer), 5.05 (t, $J = 7.5$ Hz, 1H, one rotamer); ^{13}C NMR δ 21.4 and 21.5, 22.0, 22.2 and 22.5, 23.2 and 23.3, 23.5, 24.7 and 25.1, 32.6 and 33.7, 36.9 and 38.2, 39.0 and 41.1, 43.6 and 45.9, 48.6, 55.2 and 57.7, 65.6 and 65.8, 78.9 and 79.9, 80.9 and 81.1, 153.7 and 154.1, 170.5 and 171.1, 172.7 and 173.5; CIMS *m*/*z* 442 ($M^+ + 18$, 44), 425 ($M^+ + 1$, 100), 424 (M^+, 1), 283 (23). Anal. Calcd for $C_{23}H_{40}O_5N_2$: C, 65.07; H, 9.50; N, 6.60. Found: C, 65.17; H, 9.64; N, 6.53.

(5*S*)-7-[(1*S*)-1-(*tert*-Butoxycarbonyl)-3-methylbutyl]-1-[(fluoren-9-yl)methoxycarbonyl]-6-oxo-1,7-diazaspiro-[4.5]decane (17a). To a solution of spirolactam **1a** (190 mg, 0.45 mmol) in dry CH_2Cl_2 (3 mL) were added 2,6-lutidine (0.11 mL, 0.9 mmol) and TMSOTf (0.12 mL, 0.68 mmol) at room temperature, and the resulting mixture was stirred under argon atmosphere for 15 min. The reaction was quenched by the addition of two drops of saturated aqueous NH_4Cl solution. Next, saturated aqueous Na_2CO_3 solution (10 mL) was added, and the mixture was extracted with Et_2O. The organic extracts

were dried and concentrated to afford **(5*R*)-7-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro-[4.5]decane (16a)** (145 mg) as a pale yellow oil. To a stirred solution of the above crude amine **16a** (110 mg, 0.36 mmol) in acetone (1 mL) were added solid $NaHCO_3$ (46 mg, 0.54 mmol) and Fmoc-OSu (183 mg, 0.54 mmol), and the resulting mixture was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by flash chromatography (hexane/AcOEt 1/0−l/1) to afford Fmoc-spirolactam **17a** (88 mg, 48% from **Ia**, rotamers were observed): $[\alpha]^{22}_D$ = −27.6 (*c* 0.5, CH_3OH); IR (NaCl) 1728, 1702, 1648 cm^{-1}; ^1H NMR δ 0.88 and 0.94 (2d, J = 6.3 Hz, 6H), 1.46 and 1.45 (2s, 9H), 1.62−1.68 (dd, J = 10, 4 Hz, 2H), 1.66 (td, J = 10, 6 Hz, 2H), 1.72−1.78 (m, 3H), 1.82−1.96 (m, 1H), 1.98−2.14 (m, 1H), 2.31−2.42 (m, 1H), 2.51−2.63 (m, 1H), 3.14−3.22 (m, 1H), 3.29−3.40 (m, 1H), 3.64−3.81(m, 2H), 4.10 (td, J = 8, 2 Hz, 1H), 4.27 (t, J = 7 Hz, 1H), 4.44 (dd, J = 10, 7 Hz, 1H), 5.34 (dd, J = 10, 5.7 Hz, 1H), 7.32 (ddd, J = 7.5, 2.3, 1.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.60−7.66 (2d, J = 7.5 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H); ^{13}C NMR δ 21.2, 21.3, 22.4 and 23.0, 23.4, 23.5, 27.9, 32.4, 37.0, 38.9, 43.9, 47.0, 48.3, 54.8, 66.1, 66.9 and 67.0, 81.0, 119.7, 125.0 and 125.4, 126.8 and 126.9, 127.4, 141.0 and 141.1, 143.8 and 144.4, 154.0, 171.3, 172.3; CIMS *m*/*z* 564 (M^+ + 18, 22), 563 (57), 547 (M^+ + 1, 37), 546 (M^+, 100), 491 (18), 473 (20), 325 (35), 178 (23); Anal. Calcd for $C_{33}H_{42}O_5N_2 \cdot {}^1/_4H_2O$: C, 71.92; H, 7.77; N, 5.08. Found: C, 71.92; H, 7.65; N, 4.86.

(5*S*)-7-[(1*R*)-1-(*tert*-Butoxycarbonyl)-3-methylbutyl]-1-[(fluoren-9-yl)methoxycarbonyl]-6-oxo-1,7-diazaspiro-[4.5]decane (17b). Operating as above for the preparation of **17a**, from spirolactam **1b** (60 mg, 0.14 mmol), 2,6-lutidine (0.033 mL, 0.28 mmol), and TMSOTf (0.04 mL, 0.21 mmol) in 1 mL of dry CH_2Cl_2 was obtained **(5*R*)-7-[(1*R*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]-decane (16b)** (34 mg). Next, from crude amine **16b** (30 mg, 0.09 mmol), solid $NaHCO_3$ (12 mg, 0.14 mmol), and Fmoc-OSu (47 mg, 0.135 mmol) in 1 mL of dry acetone was obtained Fmoc-spirolactam **17b** (30 mg, 60% from **1b**, rotamers were observed): $[\alpha]^{22}_D$ = +32.3 (*c* 0.7, $CHCl_3$); IR (NaCl) 1732, 1696, 1647 cm^{-1}; ^1H NMR δ 0.89 and 0.98 (m, 6H), 1.46 and 1.48 (2s, 9H), 1.64−2.10 (m, 9H), 2.20−2.32 (m, 1H), 2.62 (td, J = 13.5, 3 Hz, 1H), 3.14−3.25 (m, 1H), 3.52 (td, J = 12, 4 Hz, 1H), 3.68−3.80 (m, 2H), 3.98 (td, J = 9.5, 5.5 Hz, 1H), 4.09 (dd, J = 10, 2 Hz, 1H), 4.13−4.29 (m, 1H), 4.39 (dd, J = 7.2, 4.2 Hz, 1H, one rotamer), 4.72−4.85 (m, 1H), 7.31 (dt, J = 7.5, 1.5 Hz, 2H), 7:39 (t, J = 7.5 Hz, 2H), 7.62 (dd, J = 15, 7.5 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); ^{13}C NMR δ 21.5 and 21.7, 21.9, 22.8 and 23.3, 23.5, 24.5 and 25.0, 28.0, 32.5, 37.1, 38.8, 44.9, 47.3, 48.4, 55.2 and 56.8, 66.1, 67.0, 81.2 and 82.7, 119.8, 125.1 and 125.3, 126.9, 127.5, 141.0 and 141.1, 143.8 and 144.4, 153.9, 170.2, 172.4; CIMS *m*/*z* 564 (M^+ + 18, 52), 547 (M^+ + 1, 21), 326 (21), 325 (100), 284 (13), 283 (94), 179 (18). Anal. Calcd for $C_{33}H_{42}O_5N_2 \cdot {}^7/_4H_2O$: C, 68.55; H, 7.93; N, 4.84. Found: C, 68.22; H, 7.75; N, 5.00.

(5*R*)-7-[(1*S*)-1-Carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (18a). A solution of spirolactam **1a** (200 mg, 0.47 mmol) and TFA (1.4 mL) was stirred at room temperature for 30 min. The reaction was quenched by the addition of water (3 mL) and the mixture was lyophilized to afford spirolactam **18a** trifluoroacetate (141 mg, 79%): IR (NaCl) 2961, 1726, 1670 cm^{-1}; ^1H NMR (CD_3OD) δ 1.05 (d, J = 6.3 Hz, 3H), 1.07 (d, J = 6.6 Hz, 3H), 1.61−1.70 (m, 1H), 1.89 (dd, J = 9, 5.4 Hz, 1H), 1.94 (dd, J = 10.5, 4.5 Hz, 1H), 2.12−2.27 (m, 6H), 2.28−2.45 (m, 2H), 3.44−3.53 (m, 3H), 3.63 (dt, J = 11.5, 6.5 Hz, 1H), 5.07 (dd, J = 10.5, 5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 20.6, 21.9, 23.6, 24.7, 26.2, 31.3, 37.3, 38.2, 45.8, 47.6, 57.3, 69.1, 170.0, 173.9; EIMS *m*/*z* 268 (M^+, 4), 226 (11), 212 (14), 153 (46), 110 (60), 96 (100), 83 (46).

(5*R*)-7-[(1*R*)-1-Carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (18b). Operating as above for the preparation of **18a**, from spirolactam **1b** (181 mg, 0.42 mmol) and TFA (1.1 mL) was obtained spirolactam **18b** trifluoroacetate (162 mg, quantitative): IR (NaCl) 2960, 1726, 1666 cm^{-1}; ^1H NMR (CD_3OD) δ 0.99 (d, J = 6.3 Hz, 3H), 1.07 (d, J = 7 Hz, 3H), 1.56−1.66 (m, 1H), 1.80−2.01 (m, 2H), 1.88 (dd, J = 10.2, 5.4 Hz, 1H), 1.94 (dd, J = 10.5, 4.5 Hz, 1H), 2.16−2.33 (m, 6H), 3.43−3.56 (m, 2H), 3.58−3.67 (m, 2H), 5.09 (dd, J = 10.5, 5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 20.7, 21.3, 23.6, 24.7, 26.2, 31.5, 37.6, 38.4, 44.3, 47.5, 56.3, 69.2, 171.2, 173.8; ElMS *m*/*z* 268 (M^+, 3), 226 (11), 153 (35), 110 (39), 96 (100), 83 (69), 69 (20).

(2*R*)-*N*-[(1*S*)-1-(Benzyloxycarbonyl)-3-methylbutyl]-1-(*tert*-butoxycarbonyl)-2-(3-hydroxypropyl)pyrrolidine-2-carboxamide (20a). Operating as above in the preparation of **13a**, from lactone **11** (350 mg, 1.4 mmol), H-Leu-OBzl (905 mg, 4.1 mmol), and 2-hydroxypyridine (167 mg, 1.76 mmol) in 5 mL of toluene was obtained a residue that was purified by flash chromatography (hexane/AcOEt, 2/1−1/1) to yield amide **20a** (314 mg, 50%, rotamers were observed): $[\alpha]^{22}_D$ = −19.9 (*c* 0.51, $CHCl_3$); IR (film) 1739, 1675, 1649 cm^{-1}; ^1H NMR (CD_3CN, 65 °C) δ 0.91 and 0.92 (2d, J = 6.6 Hz, 3H each), 1.44 and 1.46 (2s, 9H), 1.60−1.68 (m, 2H), 1.69−1.77 (m, 3H), 2.01−2.15 (br s, 3H), 2.39−2.49 (br s, 1H), 3.3 (td, J = 10.5, 6.3 Hz, 1H), 3.46−3.52 (br s, 2H), 3.53−3.55 (m, 1H), 4.43−4.49 (m, 1H), 5.16 (s, 2H), 7.39 (m, 5H); ^{13}C NMR (CD_3CN (65 °C)) δ 21.6, 22.7, 23.2, 25.5, 28.1 and 28.5, 31.9, 35.5, 38.5, 41.2, 49.9 and 50.2, 51.8 and 51.9, 62.6, 67.3, 69.4 and 71.2, 80.4 and 81.7, 129.1, 129.2, 129.5, 137.1, 156.3 and 157.0, 173.5, 175.2 and 175.9; EIMS *m*/*z* 494 (M^+ + 18, 8), 478 (M^+ + 2, 100), 477 (M^+ + 1, 1). Anal. Calcd for $C_{26}H_{40}O_6N_2$: C, 65.54; H, 8.40; N, 5.88; Found: C, 65.36; H, 8.60; N, 5.80.

(5*S*)-1-(*tert*-Butoxycarbonyl)-7-[(1*S*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (19a). Method A. To a suspension of spirolactam **18a** (53 g, 0.17 mmol) in dry acetonitrile (2 mL) was added TMAH (33 mg, 0.17 mmol), and the mixture was stirred at room temperature for 30 min. Then, Boc_2O (54 mg, 0.26 mmol) was added, and the mixture was stirred at room temperature for 2 days. Afterward, another 18 mg of Boc_2O was added, and stirring was continued for 1 day. The mixture was concentrated and extracted with water and Et_2O. The aqueous layer was acidified to pH 3−4 with citric acid and extracted with AcOEt. The organic extracts were dried and concentrated to afford acid **19a** (20 mg, 33%, rotamers were observed). **Method B.** Operating as in the preparation of **1a** (method C), from amide **20a** (105 mg, 0.22 mmol), pyridine (0.02 mL, 0.24 mmol), and MsCl (0.05 mL, 0.66 mmol) in dry CH_2Cl_2 (1 mL) was obtained **(2*R*)-1-(*tert*-butoxycarbonyl)-*N*-[(1*S*)-1-(benzyloxycarbonyl)-3-methylbutyl]-2-[3-(methanesulfonyl)propyl]pyrrolidine-2-carboxamide (21a)** (110 mg) as a pale yellow oil. Next, from crude mesylate **21a** (110 mg, 0.20 mmol), NaH (60% in mineral oil, two times 19 mg, 0.44 mmol), and 15-crown-5 (0.04 mL, 0.22 mmol) in THF (20 mL) was obtained a pale yellow oil that was chromatographed (hexane/acetate, 10/0−8/2) to afford spirolactam **19a** (31 mg, 42%): $[\alpha]^{22}_D$ = −8 (*c* 0.45, CH_3OH); IR (NaCl) 1683, 1646 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.90 and 0.95 (2d, J = 6.5, 3H), 0.97 and 0.98 (2d, J = 6 Hz, 3H), 1.42, 1.43 and 1.44 (3s, 9H), 1.68−1.86 (m, 2H), 1.64 (ddd, J = 14.5, 11, 5 Hz, 2H), 1.87−1.96 (m, 3H), 1.99−2.03 (m, 1H, one rotamer), 2.17 (dddd, J = 12, 10.5, 7, 1 Hz, 1H, one rotamer), 2.10 (td, J = 12, 6.5 Hz, 2H, one rotamer), 2.25 (dt, J = 12.5, 7.5 Hz, 1H), 2.41 (td, J = 12, 6.5 Hz, 1H, one rotamer), 2.55 (td, J = 13, 4.5 Hz, 1H, one rotamer), 3.22−3.55 (m, 2H), 3.46−3.49 (m, 2H, one rotamer), 3.58 (dt, J = 10.5, 7 Hz, 2H, one rotamer), 5.12 (dd, J = 9, 6.5 Hz, 1H, one rotamer), 5.37 (dd, J = 11.5, 4.5 Hz, 1H, one rotamer); ^{13}C NMR (CD_3OD) δ 21.8, 22.2, 23.2, 23.9 and 24.3, 25.2, 28.8, 33.3 and 33.9, 38.3 and 39.8, 42.0, 45.1 and 45.8, 49.3, 55.6 and 55.7, 67.2, 80.6 and 81.7, 155.2, 174.6 and 175.2, 182.0; CIMS *m*/*z* 386 (M^+ + 18, 20), 369 (M^+ + 1, 23), 368 (M^+, 100), 269 (32). Anal. Calcd for $C_{19}H_{32}O_5N_2 \cdot {}^1/_2H_2O$: C, 60.46; H, 8.81; N, 7.42. Found: C, 60.41; H, 8.68; N, 7.03.

(5*S*)-1-Acetyl-7-[(1*S*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5] decane (22a). To a solution

of amine **16a** (77 mg, 0.24 mmol) in dry CH_2Cl_2 (0.5 mL) were added pyridine (38 mL, 0.48 mmol) and acetyl chloride (34 mL, 0.48 nmmol). The mixture was stirred at room temperature for 6 h under argon atmosphere. The reaction was quenched by the addition of 0.1 N HCl and extracted with CH_2Cl_2. The organic extracts were washed with saturated aqueous $NaHCO_3$ solution and brine, dried, and concentrated to afford acetyl spirolactam **22a** (62 mg, 72%, rotamers were observed) as a pale yellow oil: $[\alpha]^{22}_D = -21$ (*c* 0.51, $CHCl_3$); IR (NaCl) 1727, 1648 cm^{-1}; ^1H NMR δ 0.93 and 0.96 (2d, $J = 6.6$ Hz, 3H each one), 1.44 and 1.47 (2s, 9H), 1.60−1.69 (m, 1H), 1.70−2.00 (m, 7H), 2.03 (s, 3H), 2.19−2.37 (m, 1H), 2.47−2.58 (m, 1H), 3.11−3.23 (m, 1H), 3.29−3.38 (m, 1H), 3.50−3.70 (m, 2H), 4.70 (t, $J = 8$ Hz, 1H, one rotamer), 5.34 (dd, $J = 9.5$, 7 Hz, 1H, one rotamer); ^{13}C NMR δ 20.4, 20.5, 22.3, 22.5, 22.9, 23.2, 27.0, 30.9, 36.3, 37.4, 42.8, 48.4, 53.8, 65.0, 80.0, 167.0, 170.4, 170.9; ElMS *m*/*z* 366 (M^+, 9), 310 (29), 265 (100), 237 (68), 110 (84), 96 (46), 83 (39). Anal. Calcd for $C_{20}H_{34}O_4N_2$: C, 65.54; H, 9.35; N, 7.64. Found: C, 65.88; H, 9.50; N, 7.49.

(5*S*)-1-Acetyl-7-[(1*R*)-1-(*tert*-butoxycarbonyl)-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (22b). Operating as above for the preparation of **22a**, from amine **16b** (82 mg, 0.24 mmol) in dry CH_2Cl_2 (0.5 mL), pyridine (38 mL, 0.48 mmol), and acetyl chloride (34 mL, 0.48 mmnol) was obtained acetyl spirolactam **22b** (82 mg, 95%, rotamers were observed) as a pale yellow oil: $[\alpha]^{22}_D = +32$ (*c* 1, $CHCl_3$); IR (NaCl) 1726, 1646, 1605 cm^{-1}; ^1H NMR δ 0.90 and 0.93 (2d, $J = 6$ Hz, 3H each), 1.45 and 1.47 (2s, 9H), 1.52−1.74 (m, 1H), 1.75−2.10 (m, 8H), 2.03 (s, 3H), 2.10−2.36 (m, 1H), 2.47−2.62 (m, 1H), 3.11−3.23 (m, 1H), 3.26−3.40 (m, 1H), 3.50−3.70 (m, 2H), 4.70 (t, $J = 8$ Hz, 1H, one rotamer), 5.35 (dd, $J = 10$, 6 Hz, 1H, one rotamer); ^{13}C NMR δ 21.5 and 21.8, 21.6 and 22.0, 23.3, 23.9, 24.0 and 24.3, 25.0, 28.1, 31.9 and 32.0, 37.3 and 37.4, 38.3 and 38.5, 43.9 and 45.3, 49.5, 54.9 and 57.3, 66.2, 81.1, 168.1 and 168.3, 170.2, 171.5 and 171.8; ElMS *m*/*z* 366 (M^+, 12), 310 (31), 266 (26), 265 (100), 238 (15), 237 (87), 226 (29), 195 (26), 110 (80), 96 (31), 83 (29). Anal. Calcd for $C_{20}H_{34}O_4N_2 \cdot 1/2H_2O$: C, 63.97; H, 9.39; N, 7.46. Found: C, 63.77; H, 9.31; N, 7.17.

(5*S*)-1-Acetyl-7-[(1*S*)-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (23a). Method A. Pyridine (38 mL, 0.46 mmol) and acetyl chloride (25 mL, 0.35 mmol) were added to a solution of spirolactam **18a** trifluoroacetate (88.6 mg, 0.23 mmol) in dry CH_2Cl_2 (0.4 mL), and the resulting mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of 0.1 N HCl and extracted with CH_2Cl_2. The organic extracts were washed with more 0.1 N HCl and saturated aqueous Na_2CO_3 solution, dried, and concentrated to furnish acid **23a** (43 mg, 59%) as a yellow oil. **Method B.** A solution of spirolactam **22a** (70 mg, 0.19 mmol) in 10.5 mL of H_2O−AcOH−^{i}PrOH (1:2.5:1) was heated at 100 °C for 48 h. After being cooled to room temperature, the mixture was concentrated, and the residue was washed with aqueous 0.1 N HCl (3 mL) and extracted with AcOEt. The organic extracts were dried and concentrated, and the residue was purified by flash chromatography (hexane/AcOEt, 8/2−6/4) to afford acid **23a** (35 mg, 60%, rotamers were observed): $[\alpha]^{22}_D = -25$ (*c* 1, $CHCl_3$); IR (NaCl) 3357, 1624 cm^{-1}; ^1H NMR δ 0.91 and 0.95 (2d, $J = 6.6$, 3H each), 1.40 (m, 1H), 1.60−2.50 (m, 8H) 1.84−1.89 (m, 1H), 1.94−2.00 (m, 1H), 2.05 and 2.08 (2s, 6H), 3.14−3.32 (m, 1H), 3.20−3.33 (m, 1H), 3.56−3.70 (m, 1H), 3.63 (dd, $J = 8.5$, 4.5 Hz, 1H), 5.43 (dd, $J = 11$, 5 Hz, 1H); ^{13}C NMR δ 21.3 and 21.4, 22.8, 23.3, 23.4, 23.8, 24.4 and 24.8, 31.6 and 31.7, 35.8, 37.1 and 37.3, 44.7, 49.4, 54.8 and 55.4, 66.3 and 66.5, 168.1 and 169.8, 172.3, 174.7; EIMS *m*/*z* 310 (M^+, 39), 266 (34), 226 (70), 180 (50), 153 (45), 138 (37), 110 (100), 96 (73), 83 (53). Anal. Calcd for $C_{16}H_{26}O_4N_2 \cdot {}^1/_5TFA$: C, 58.96; H, 7.90; N, 8.37. Found: C, 58.97; H, 8.07; N, 8.04.

(5*S*)-1-Acetyl-7-[(1*R*)-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.5]decane (23b). Method A. Operating as above for the preparation of **23a** (method A), from spirolactamn **18b** trifluoroacetate (96 mg, 0.25 mmol) in CH_2Cl_2 (0.4 mL), pyridine (40 mL, 0.5 mrnol), and acetyl chloride (27 mL, 0.25 mmol) was obtained acid **23b** (53 mg, 68%) as a yellow oil. **Method B.** Operating as above for the preparation of **23a** (method B), from spirolactam **22b** (80 mg, 0.22 mmol) in 10.5 mL of H_2O−AcOH−^{i}PrOH (1:2:1) was obtained acid **23b** (37 mg, 88%, rotamers were observed): $[\alpha]^{22}_D = +79$ (*c* 1, $CHCl_3$); IR (NaCl) 3319, 1625 cm^{-1}; ^1H NMR δ 0.88 and 0.92 (2d, $J = 6.3$ Hz, 3H each), 1.34−1.48 (m, 1H), 1.60−2.10 (m, 10H), 2.00 and 2.02 (2s, 3H), 3.24 (tdm, $J = 11.7$, 3 Hz, 2H), 3.63 (dd, $J = 8.5$, 4.5 Hz, 2H), 5.44 (dd, $J = 10.6$, 5 Hz, 1H); ^{13}C NMR δ 21.1, 21.4 and 21.6, 22.8, 23.4 and 23.5, 23.8 and 23.9, 24.9, 31.8, 35.9, 37.1, 42.5, 49.5, 54.8, 66.5, 170.0, 172.2; EIMS *m*/*z* 310 (M^+, 12), 266 (24), 221 (40), 195 (25), 180 (36), 138 (38), 110 (85), 96 (100), 83 (73). Anal. Calcd for $C_{16}H_{26}O_4N_2 \cdot {}^2/_3H_2O$: C, 59.60; H, 8.55; N, 8.69. Found: C, 59.55; H, 8.21; N, 8.33.

Tripeptide Analogue {Ac-Gly-Leu-Met-NH_2} 2a. To a solution of acid **23a** (35 mg, 0.11 mmol) in DMF (1 mL) were added DCC (26 mg, 0.13 mmol) and HOBt (18 mg, 0.13 mmol), and the resulting mixture was stirred at room temperature for 10 min. Next, H-Met-NH_2·HCl (21 mg, 0.11 mmol) and TEA (0.03 mL, 0.17 mmol) were added, and stirring was continued at room temperature for 16 h. The mixture was concentrated, and the residue was purified by flash chromatography (hexane/AcOEt, 70/30−0/100) to obtain compound **2a** (37 mg, 70%, rotamers were observed): $[\alpha]^{22}_D = +23$ (*c* 1.2, CH_3OH); IR (NaCl) 3316, 1680, 1667, 1649 and 1623 cm^{-1}; ^1H NMR (500 MHz) δ 0.86 (d, $J = 6.5$ Hz, 3H, one rotamer), 0.90 (d, $J = 6.5$ Hz, 3H, one rotamer), 0.91 (d, $J = 7$ Hz, 3H, one rotamer), 0.94 (d, $J = 6.5$ Hz, 3H, one rotamer), 1.50−1.56 (m, 1H), 1.57−1.74 (m, 3H), 1.83−1.91 (m, 2H), 1.93−1.98 (m, 3H), 1.99 and 2.01 (2s, 3H), 2.07 and 2.08 (2s, 3H), 2.09−2.14 (m, 2H), 2.17−2.28 (m, 2H), 2.38 (td, $J = 13$, 3.5 Hz, 1H, one rotamer), 2.45 (dd, $J = 8.5$, 3.5 Hz, 1H, one rotamer), 2.48 (dd, $J = 9$, 3.5 Hz, 1H, one rotamer) and 2.53−2.59 (m, 1H, one rotamer), 3.15−3.23 (m, 2H), 3.68−3.77 (m, 2H), 4.36 (dd, $J = 9.5$, 5.5 Hz, 1H, one rotamer), 4.37 (dd, $J = 8.5$, 5 Hz, 1H, one rotamer), 4.49−4.63 (m, 1H, one rotamer), 5.17 and 5.26 (2 br s, 1H), 5.36 (dd, $J = 12$, 4 Hz, 1H), 6.70 and 7.15 (2 br s, 1H) and 7.69 (d, $J = 7.5$ Hz, 1H, one rotamer), 7.76 (d, $J = 7.5$ Hz, 1H, one rotamer), 7.77 (d, $J = 7$ Hz, 1H, one rotamer) and 7.90 (d, $J = 8.5$ Hz, 1H, one rotamer); ^{13}C NMR δ 15.3 and 15.4; 20.9, 21.1, 21.2 and 21.3; 21.9 and 21.8; 22.8, 22.9 and 23.1; 23.3, 23.4 and 23.5; 24.0, 24.8, 25.3 and 25.4; 30.3, 30.4, 30.5 and 30.6; 30.8, 31.0, 31.3 and 31.4; 31.5 and 32.0; 37.4 and 37.2; 37.9 and 37.8; 41.5 and 41.8; 49.3, 49.4, 51.2 and 52.2; 53.2, 53.3, 53.4, 53.6, and 54.9; 66.4, 66.5 and 66.6; 169.3, 169.4 and 169.5; 170.5, 170.6, 170.9 and 171.0; 172.4, 172.7, 173.1 and 173.3; 174.3, 174.6 and 175.1; HRMS calcd for $C_{21}H_{36}O_4N_4S$ 440.2457, found 440.2454.

Tripeptide Analogue {Ac-Gly-Leu-Met-NH_2} 2b. Operating as above for the preparation of **2a**, from acid **23b** (46 mg, 0.15 mmol) in 1 mL of dry DMF, DCC (34 mg, 0.17 mmol), HOBt (30 mg, 0.17 mmol), and H-Met-NH_2·HCl (22 mg, 0.15 mmol) was obtained tripeptide analogue **2b** (38 mg, 42%, rotamers were observed) after purification by flash chromatography (hexane/AcOEt, 70/30−0/100): $[\alpha]^{22}_D = -44$ (*c* 1.1, CD_3OD); IR (NaCl) 3316, 1681, 1667, 1649 and 1621 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.88 (d, $J = 7$ Hz, 3H), 0.96 (d, $J = 6.5$ Hz, 3H), 1.39−1.44 (m, 1H), 1.72 (dd, $J = 5.5$, 2.5 Hz, 1H), 1.74 (dd, $J = 6.5$, 2 Hz, 1H), 1.76−1.86 (m, 1H), 1.88−1.97 (m, 2H), 2.00−2.19 (m, 6H), 2.01 and 2.03 (2s, 3H), 2.07 and 2.09 (2s, 3H), 2.26 and 2.37 (m, 1H), 2.44−2.53 (m, 1H), 2.55−2.62 (m, 1H), 3.23 (dt, $J = 12$, 2 Hz, 1H), 3.41 (td, $J = 12$, 3.5 Hz, 1H), 3.59−3.64 (m, 1H), 3.68−3.73 (m, 1H), 4.29 (dd, $J = 10$, 5.5 Hz, 1H, one rotamer), 4.44 (dd, $J = 10.5$, 5 Hz, 1H, one rotamer), 4.46 (dd, $J = 9.5$, 5 Hz, 1H, one rotamer), 5.38 (dd, $J = 9.5$, 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 15.3, 21.7, 22.0, 22.9 and 23.0, 23.8, 24.7 and 24.8, 25.9 and 26.2, 31.1 and 31.6, 32.2 and 32.4, 32.5 and 32.7, 36.2, 38.1 and 38.2, 43.2, 50.2 and 50.4, 54.6, 55.3, 67.8 and 68.0, 170.7, 172.5, 174.9 and 177.0; HRMS calcd for $C_{21}H_{36}O_4N_4S$ 440.2457, found 440.2466.

Molecular Modeling Studies. Conformational Search. All calculations were run on an SGI workstation (R4000, 128 MB RAM, 19 GB hard disk) under an Irix 5.3 operating system. Molecular mechanics calculations were carried out with Spartan v. 5.0 and InsightII discover 3.0 v.1997.

Optimized Monte Carlo Search and Energy Minimization. In this paper, we used the MMFF94 force field implemented in Spartan v 5.0. By default, atomic partial charges were calculated from data in the molecular mechanics force field chosen. The conformational search of epimers **2a** and **2b** was carried out with an optimized Monte Carlo method employing the MMFF94 force field as implemented in Spartan 5.0. Energy minimization used a conjugated gradient method, with a final gradient of 0.0001 kcal/Å mol as the convergence criteria. All conformers within a 10 kcal/mol window above the global minimum were used to determine the profiles. To determine the conformer families, atoms belonging to the spirolactam skeleton and peptide backbones were considered. We employed a similar protocol for the conformational search of compounds **2a** and **2b** starting from extended structures. Eight degrees of freedom were considered here (see Figure 4). Each run included 3000 steps. The structures obtained were clustered by conformation and energy. The resulting lowest energy conformations (**2a$_1$** and **2b$_1$**) were the starting points for molecular dynamics studies.

Molecular Dynamics. The resulting lowest energy conformers of compounds **2a** and **2b** (resulting from the Monte Carlo search and Iterative simulated annealing) constituted the starting points for molecular dynamics studies using a distant dependent dielectric (4*r*) constant and an explicit solvent model in chloroform. NVT calculations at 300 K were performed using cubic boxes of 48 Å side length and 433 chloroform molecules. Periodical bounded conditions were applied. After adequate heating and equilibration of the system for 50 ps, evolution times were 1 ns. 1000 structures were saved periodically from each profile distribution for further analyses. Profile distributions were analyzed for conformational preferences measuring the γ and/or β-turn descriptors. For the β-turn, these are the distance R (R < 7 Å) between the Cα_i, and the Cα_{i+1}, the dihedral angle τ ($-90 < \tau < 90$) formed by the four Cα atoms, and the distance between the carbonyl function of the first amino acid (i) and the amide group of the fourth ($i + 3$). The major β-turns are classified according to the torsion angles of the second (ϕ_1, ψ_1: $\phi_1 = -60 \pm 30$ and $\psi_1 = 120 \pm 30$) and third amino acids (ϕ_2, ψ_2: $\phi_1 = 80 \pm 30$ and $\psi_1 = 0 \pm 45$). For the γ-turn, they are the distance between the carbonyl function of the second amino acid ($i + 1$) and the amide group of the fourth amino acid (i+3), and the torsion angles of the second amino acid (ϕ_2, ψ_2: $\phi_1 = 70/85$ and $\psi_1 = -60/-70$).

Iterative Simulated Annealing. The calculations were carried out within the molecular mechanics using the AMBER force field implemented in DISCOVER v. 97. They were conducted under vacuum with a distant dependent dielectric constant (4*r*) and a cutoff of 13 Å. Starting from extended structures of the epimers, the structure is minimized and subsequently heated to 900 K in a very short period of time. The structure is then cooled slowly to 100 K and minimized. In our case the heating was carried out in steps. At each step the temperature was raised by 100 K in 0.1 ps, and then the system was allowed to stay 1 ps at the new temperature. The system was allowed to stand for 10 ps at 900 K and then cooled in steps. At each step the temperature was lowered by 100 K in 0.1 ps, and after cooling, the system was allowed to stay 10 ps at the new temperature. This structure is the starting conformation for another cycle, creating a library of conformations that are rank ordered by energy every 150 cycles. The procedure is repeated until no new conformations appear after a predetermined number of cycles (in our case 5 times) within a 5 kcal/mol energy range with respect to the lowest energy structure already found. Heating has to be carried out rapidly in order to make the molecule jump to a different region in the space. In contrast, cooling is slow in order to obtain the lowest energy minimum of the region. This protocol was run five times, employing fully extended starting structures of both compounds (**2a** and **2b**).

Acknowledgment. Support for this research has been provided by the CIRIT (Generalitat de Catalunya) through Grant Nos. QFN95-4703, 1995SGR-000429, 1997SGR-00075, and 1999SGR-00077 and by the DGYCIT (Ministerio de Educación y Cultura, Spain) through Grant Nos. PB97-0976 and 2FD97-0293. We also thank the Universitat de Barcelona (División de Ciencies de la Salut) for a fellowship given to M.M.F.

Supporting Information Available: Distribution profiles for Monte Carlo Calculations of compounds **2a** and **2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO025999I