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Improved synthesis of a [4.4]-spirolactam β-turn mimetic as surrogate of the didemnin side chain dipeptide Pro-*N*-Me-D-Leu

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Abstract—An efficient synthesis of [4.4]-spirolactam restricted derivatives of the didemnin side chain dipeptide L-Pro-*N*-Me-D-Leu is described. This methodology involves: (a) peptide coupling of *N*-Boc-2-allylproline with D-Leu-OBn; (b) $OsO_4/NaIO_4$ mediated allyl oxidation and intramolecular cyclization to the corresponding cyclic hemiaminals; and (c) $NaBH_4$ mediated reduction of an intermediate *N*-acyliminium ion. This synthetic strategy gave significant better results than the previously reported strategies for the synthesis of [4.4]-spirolactam β-turn mimetics.

1. Introduction

The didemnins are a family of macrocyclic depsipeptides, isolated from several tunicates, which exhibit a wide variety of biological activities, including antitumor,[1,2] antiviral,[2] and immunosupresive properties.[2,3] These depsipeptides contain a common 23-membered macrocycle, made up of six subunits [(3*S*,4*R*,5*S*)-isostatine (Ist[1]), (2*S*,4*S*)-3-oxo-4-hydroxy-2,5-dimethylhexanoic acid (Hip[2]), (Leu[3]), (Pro[4]), [N,O-(Me)$_2$-Tyr[5]], and (Thr[6])], and differ in the side chain attached to the threonine NH, through a *N*-Me-D-leucine residue. Among these macrocyclic depsipeptides, didemnin B (Fig. 1, **1**), isolated in 1981 from the Caribbean tunicate *Tridemnum solidum*,[4] and aplidine (**2**), isolated in 1990 from the Mediterranean tunicate *Aplidium albicans*,[5] have stood out because of their potent antitumoral activity. The good antitumoral therapeutic profile of aplidine in pre-clinical and phase I clinical studies has facilitated its recent entry into phase II clinical trials.[1,2,6–9] Analysis of the X-ray crystal structure of didemnin B,[10] as well as conformational studies on didemnin B[11,12] and aplidine,[13,14] have shown that their side chain folds back toward the macrocycle into a type II β-turn conformation. With the aim of studying the contribution of this β II turn conformation to the bioactive conformation responsible for the antitumoral activity, as a first step for the design of didemnin peptidomimetics, we decided to synthesize didemnin B and aplidine analogues, where the side chain dipeptide L-Pro[8]-*N*-Me-D-Leu[7] was replaced by a β II turn mimetic.[15] As shown in Figure 1, a [4.4]-spirolactam was selected for this replacement, because

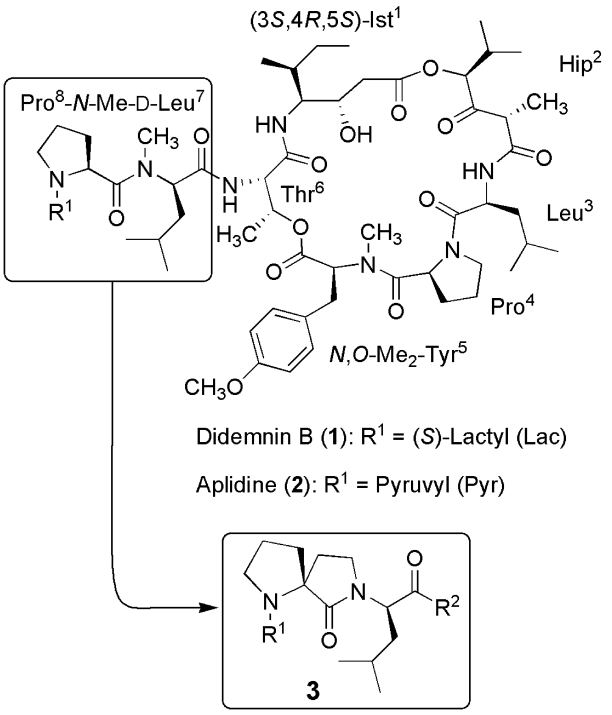

Figure 1.

it introduces only an additional methylene bridge, and allows the essential side chain and configuration of the (D-Leu[7]) residue at the $i+2$ position of the β-turn to be retained.[16,17] Furthermore, according to the described data for this type of β-turn mimetic,[18] it would fix the dipeptide backbone ϕ, ψ torsion angles into values (−50.9, 128.7; 91.1, and −5.4°) very close to those observed in the crystal

Keywords: Didemnins; Peptidomimetics; β-Turn mimetics; Spirolactams; Mitsunobu type reaction; Hemiaminal reduction.
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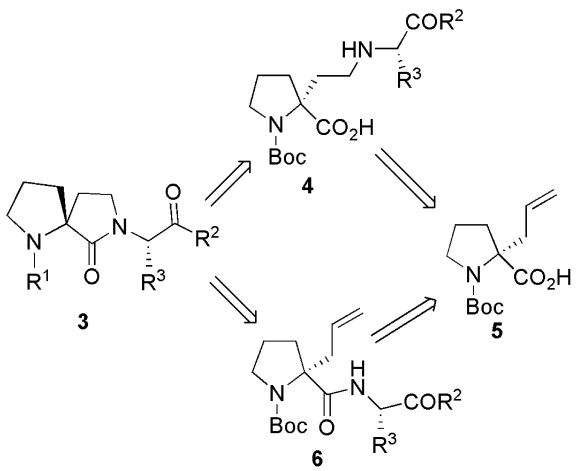
structure of didemnin B[10] (−65, 125; 103, and −29°) and those calculated for aplidine (−63, 131; 90, and −38°).[13,14] Herein, we described our efforts to develop an efficient synthesis of [4.4]-spirolactam pseudodipeptide derivatives **3**, which would give access to the proposed conformationally restricted didemnin analogues in acceptable yields.

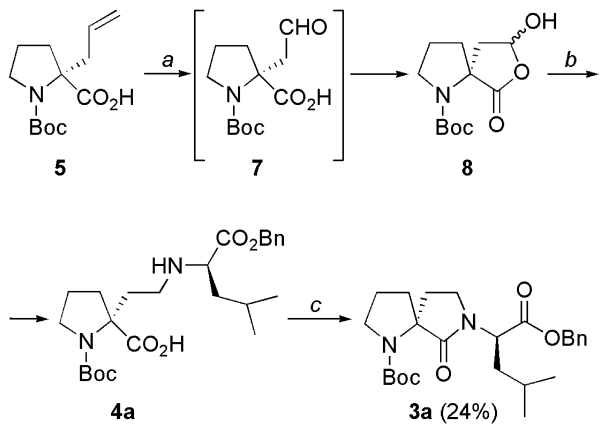
2. Results and discussion

Two alternative strategies have been described for the synthesis of [4.4]-spirolactam structures, both from 2-allylproline derivative **5** (retrosynthetic Scheme 1). The first, reported by Ward et al.[19] for the preparation of derivatives of L-Pro-L-Leu, involves the reductive *N*-alkylation of the C-terminal amino acid with *N*-protected 2-(formylmethyl)-proline, followed by lactamization of the resulting secondary amine **4**. Whereas the second, described for the synthesis of L-Pro-L-Tyr,[20,21] and L-Pro-Gly derivatives,[18,22] involves a first step of peptide coupling (**6**), followed by cyclization via allyl group oxidation–reduction and a Mitsunobu type *N*-alkylation.



Scheme 1. Retrosynthesis of spirolactam pseudodipeptides **3**.

Initially, the building of our spirolactam skeleton was tried by applying the first strategy, using *O*-Bn/*N*-Boc protecting groups. The starting material, *N*-Boc-2-allyl-L-Pro-OH (**5**), was prepared by applying the Khalil et al.[23] procedure of *N*-Boc-protection of sterically hindered amino acid to 2-allyl-L-Pro-OH, which was obtained from L-Pro-OH according to the Annunziata et al.[24] improvement to the Seebach's methodology for amino acid α-alkylation with self-reproduction of chirality.[25] As shown in Scheme 2, the oxidation of the double bond of the allyl group in (**5**), by treatment with OsO_4 and $NaIO_4$, gave a (≈1:1) mixture of epimeric hemiacetals **8** (95%), which was not resolved. The reductive amination of this mixture with H-D-Leu-OBn·PTSA, using $NaBH_3CN/ZnCl_2$ as reducing agent, led to the secondary amine **4a**, whose zwitterionic character hampered its purification. Consequently, the reaction crude was directly used in the next step of γ-lactamization. This was carried out using 2-chloro-1-methylpyridinium iodide as condensing agent,[26] obtaining the desired N- and C-protected spiropseudodipeptide **3a** in a 24% overall yield from **5**.

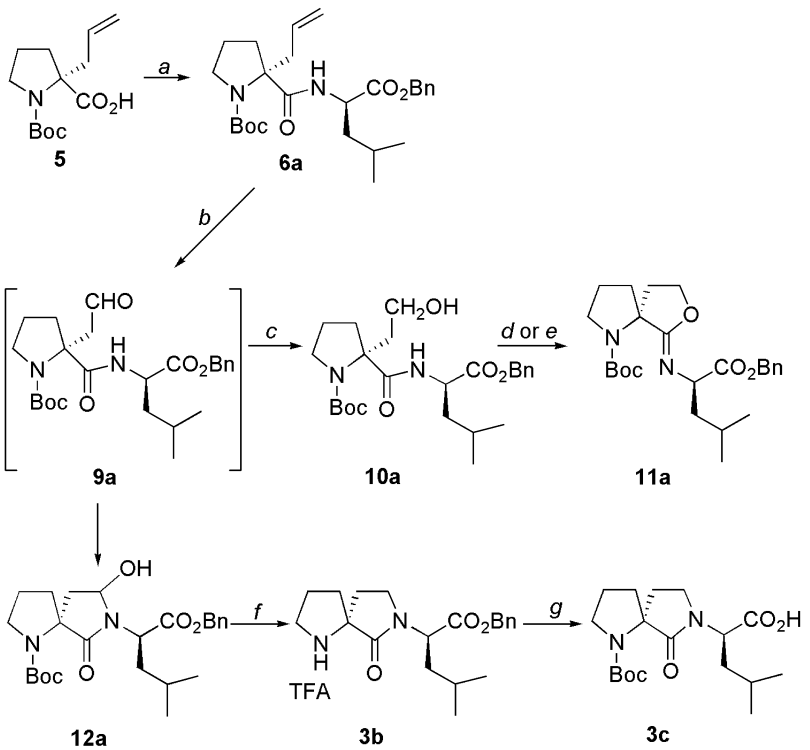


Scheme 2. Reagents: (a) OsO_4, $NaIO_4$, (2:1) MeOH/H_2O; (b) H-D-Leu-OMe·PTSA, TEA, $ZnCl_2$, $NaBH_3CN$, CH_2Cl_2; (c) 2-chloro-1-methylpyridinium iodide, TEA, CH_2Cl_2.

The low yield of the first strategy hampered the preparation of our proposed didemnin analogues in acceptable amounts. Therefore, we studied the application of the second alternative synthetic strategy shown in Scheme 3. This required, as first step, the peptide coupling of **5** with H-D-Leu-OBn·PTSA, which initially was carried out using 1-hydroxy-benzotriazole (HOBt) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) as activating reagent, in the presence of *N*-methylmorpholine (NMM).[27] These reaction conditions led to the dipeptide **6a** in a low yield (28%). Therefore, to optimize yield, we carried out a study of the coupling using other activating reagents and reaction conditions. The results are shown in Table 1. Neither the use of (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluoro-phosphate (BOP)/HOBt[27] nor *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic choride (BOP-Cl),[28,29] activating reagents for sterically hindered amino acids, improved the yield of **6a**. Moreover, when we used DCC/HOBt or BOP/HOBt, the dipeptide was obtained mixed with the 1-hydroxybenzotriazole ester derived from **5**,[†] which had identical R_f in TLC to that of **6a**. Excellent yield (>90%) of this dipeptide was obtained using 1-hydroxy-7-azabenzotriazole (HOAt)[27,30] and DCC or *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluoro-phosphate *N*-oxide (HATU),[27] and 2 equiv. of H-D-Leu-OBn·PTSA.

The oxidation of the allyl group in the dipeptide **6a** was performed with OsO_4 and $NaIO_4$, and the resulting aldehyde **9a** was reduced in situ with $NaBH_4$ to the corresponding alcohol **10a**. Unlike the previously described synthesis of other [4.4]-spirolactam dipeptide mimics,[18,20–22] the treatment of this alcohol with the Mitsunobu reagents, triphenylphosphine and diethyl azodicarboxylate (DEAD),[31] led exclusively to the product of *O*-alkylation, the cyclic imidate **11a**, and the formation of the desired γ-lactam derivative **3a** was not observed. The same result was obtained when we tried to favour the *N*-alkylation

[†] The ^1H NMR spectrum of the mixture showed the presence of ≈30% of this 1-hydroxybenzotriazole ester. The signals corresponding to this side product, as well as its R_f in TLC, were identical to those of the product resulting from the treatment of **5** with HOBt in the presence of DCC, without H-D-Leu-OBn.



Scheme 3. Reagents: (a) H-D-Leu-OMe·PTSA, HOAt, DCC, NMM, CH_2Cl_2; (b) OsO_4, $NaIO_4$, (2:1) MeOH/H_2O; (c) $NaBH_4$, EtOAc, −78 °C; (d) Ph_3P, DEAD, THF; (e) Ph_3P, NBS, CH_2Cl_2; (f) $NaBH_4$, TFA; (g) Boc_2O, $(CH_3)_4NOH·H_2O$, CH_3CN.

product, via the corresponding bromo intermediate, using triphenylphosphine/*N*-bromosuccinimide. In the ^1H NMR spectrum of imidate **11a**, the 8-H proton signals appeared at 0.7 ppm lower field relative to those of γ-lactam isomer **3a**. Similarly, C_8 of **11a** appeared considerably deshielded (79.3 ppm) with respect to C_8 of **3a** (39.57). The structural assignment of imidate **11a** was supported by its HMBC spectrum which did not show correlation between 8-H and C_α(Leu), and between C_8 and α-H(Leu), observed in the γ-lactam **3a** spectrum. Interestingly, none of the previously described syntheses of spirolactams via a Mitsunobu reaction has pointed out the formation of cyclic imidates, resulting from *O*-alkylation.[18,20–22,32] In our case, the preference for the *O*-alkylation versus the *N*-alkylation could be due to a higher steric hindrance of the D-Leu side chain in comparison with those of the Gly[18,22] and Tyr[20,21] spirolactam analogues previously described. The regioselectivity of the Mitsunobu reaction in other substrates where there is also a possible competition between *N*- and *O*-alkylation products, such as in β-hydroxy-α-amino acid-derived peptides, depends on subtle steric effects.[31b,c] Thus, serine and *allo*-threonine derivatives, in general, have shown selectivity for the *O*-alkylation, leading to peptide oxazolines,[33] while threonine derivatives have shown selectivity for *N*-alkylation[31c,33b] or 1,2-dehydration.[33a]

Table 1. Study on the yield optimization for dipeptide **6a**

Activating reagent	Base	H-D-Leu-Obn (equiv.)	Solvent	**6a** (%)
DCC/HOBt	NMM	2.5	CH_2Cl_2	28[a]
BOP/HOBt	NMM	1.6	CH_2Cl_2	30[a]
BOP-Cl	NMM	2.5	CH_2Cl_2	30
HOAt/DCC	NMM	1	CH_2Cl_2	50
HOAt/DCC	NMM	2	CH_2Cl_2	96
HOAt/HATU	DIEA[b]	1	THF	55
HOAt/HATU	DIEA[b]	2	THF	90

[a] Dipeptide **6a** impure with a variable quantity of the 1-hydroxybenzotriazole ester derived from *N*-Boc-2-allylproline (**5**).
[b] *N*-Ethyldiisopropylamine.

To overcome the difficulty of preparing the γ-lactam derivative via a Mitsunobu alkylation, as an alternative, we carried out the cyclization simultaneously with the oxidation, by allowing the intermediate aldehyde **9a** to stand in the osmylation reaction mixture at room temperature for 24 h. Cyclic hemiaminals **12a** were obtained as a (1:1) mixture of epimers,[‡] that were chromatographically resolved; however, their respective ^1H NMR spectra did not show relevant NOE effects for their absolute configuration assignment. The epimeric mixture was reduced via the *N*-acyliminium ion intermediate using $NaBH_4$ in neat TFA, because both epimers showed similar reactivity. Spirolactam **3b** was isolated as its trifluoroacetate in 73% overall yield from **5**. Attempts failed to maintain the Boc protection and **12a** was recovered unchanged by carrying out the reduction in AcOH, and by using $NaBH_3CN$ in the presence of $ZnCl_2$ or AcOH.

Finally, the reaction of trifluoroacetate **3b** with di(*tert*-butyl) dicarbonate in dry acetonitrile, using tetramethylammonium hydroxide as base,[23] led directly to the *N*-Boc acylated and C-deprotected pseudodipeptide **3c** (70%). The ^1H NMR spectra of this pseudodipeptide in $CDCl_3$ and DMSO-d_6 showed the presence of two conformations in a (2:1) ratio, corresponding to the *cis* and *trans* rotamers at the

[‡] Similar hemiaminals were obtained by Genin et al.[18] as unwanted side products from the silica gel chromatography of the Pro-Gly-derived aldehyde analogue of **9a**.

Boc-spirolactam bond. Signals due to both conformations coalesced at 90 °C in DMSO-d_6. The selectively N- and C-protected [4.4]-spirolactam pseudodipeptides **3b** and **3c** give access to backbone C- and N-extensions and, therefore, to the preparation of our proposed conformationally constrained didemnin analogues.

3. Conclusion

The herein reported synthesis of [4.4]-spirolactam restricted L-Pro-D-Leu derivatives, which involves, as key steps, the cyclization of an α-(formylmethyl)-carboxamide, followed by $NaBH_4$ mediated reduction of the resulting hemiaminals, constitutes an advantageous efficient methodology to the described strategies for the synthesis of [4.4]-spirolactams, such as reductive amination of α-(formylmethyl)-proline derivatives, followed by γ-lactamization, or aldehyde reduction, followed by a Mitsunobu type alkylation.

4. Experimental

4.1. General procedures

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254}, Merck. Preparative radial chromatography was performed on 20 cm diameter glass plates coated with a 1-mm layer of silica gel PF_{254} Merck. Silica gel 60 (230–400 mesh), Merck, was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. ^{1}H NMR spectra were recorded at 300, 400 or 500 MHz, using TMS as reference, and ^{13}C NMR spectra were recorded at 50, 75 or 100 MHz. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical RP-HPLC was performed on a Waters Novapak C_{18} (3.9×150 mm, 4 μm) or a μBondapak C_{18} (3.9×300 mm, 4 μm) columns, with a flow rate of 1 mL/min, and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phases.

4.1.1. Synthesis of (5*R*,8*RS*)-1-(*tert*-butoxycarbonyl)-8-hydroxy-6-oxo-7-oxa-1-azaspiro[4.4]nonane (8). OsO_4 (2.5%, w/w solution in *tert*-butanol, 1.9 mL, 0.15 mmol) was added to a solution of (*R*)-*N*-Boc-2-allylproline[23] (**5**) (633 mg, 2.48 mmol) in (2:1) MeOH/H_2O (45 mL), under argon, which was stirred at room temperature for 10 min. $NaIO_4$ (1.612 g, 7.43 mmol) was slowly added. After 2 h of stirring at room temperature, H_2O (30 mL) was added, and the mixture was extracted with EtOAc (3×60 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness, to yield the epimeric hemiacetals **8** as white solid (638 mg, 100%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32 [s, 9H, Me(Boc)], 1.59–1.90 (m, 4H, 3-H, 4-H), 2.35–2.50 (m, 2H, 9-H), 3.20–3.45 (m, 2H, 2-H), 6.03 (m, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.11, 28.18, 28.53, 36.22, 39.57, 41.81, 66.65, 80.24, 97.38, 161.21, 174.82. ESI-MS *m/e* 258.3 $[M+H]^+$. Anal. Calcd for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44. Found: C, 56.00; H, 7.48; N, 5.43.

4.1.2. Synthesis of (5*R*)-7-[(1*R*)-1-benzyloxycarbonyl-3-methylbutyl]-1-(*tert*-butoxy-carbonyl)-6-oxo-1,7-diaza-spiro[4.4]nonane (3a). Triethylamine (61 μL, 0.43 mmol) was added to a suspension of H-D-Leu-OBn·PTSA (95 mg, 0.43 mmol) in MeOH (10 mL), and the mixture was stirred at room temperature and under argon for 15 min. The epimeric mixture of hemiacetals **8** (470 mg, 1.83 mmol), $ZnCl_2$ (124 mg, 0.91 mmol), and $NaBH_3CN$ (126 mg, 2.01 mmol) were successively added to the mixture, and the stirring was maintained for 6 h. The solvent was removed under vacuum, and the residue was dissolved in EtOAc (20 mL). The solution was washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness. The residue (716 mg, 1.32 mmol), corresponding to the crude amino acid **4a**, was dissolved in dry CH_2Cl_2 (120 mL), and, to this solution, 2-chloro-1-methylpyridinium iodide (372 mg, 1.52 mmol) and triethylamine (0.41 mL, 2.92 mmol) dissolved in CH_2Cl_2 (3 mL) were successively added, and the mixture was refluxed for 3 days. The solution was successively washed with 10% citric acid solution (2×30 mL), saturated $NaHCO_3$ (2×30 mL), and brine (2×30 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a 10–100% gradient of EtOAc in hexane as eluant. The spirolactam pseudodipeptide **3a** was obtained as a white solid (200 mg, 24%). Mp 91 °C. $[\alpha]_D^{20}$ −1.5° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 50:50) t_R 9.47 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 and 0. 93 [2d, 6H, *J*=6 Hz, Me (Leu)], 1.32 [s, 9H, Me (Boc)], 1.40 [m, 1H, 4-H (Leu)], 1.60–2.00 [m, 7H, 3-H (Leu), 4-H, 3-H, 9-H], 2.50 [c, 1H, *J*=3, 6 Hz, 9-H], 3.10 [m, 1H, *J*=3, 9 Hz, 8-H), 3.50 (m, 2H, 1-H), 3.73 (t, 1H, *J*=9 Hz, 8-H), 4.92 [dd, 1H, *J*=5, 9 Hz, 2-H (Leu)], 5.10 [s, 2H, CH_2 (Bn)], 7.30 (m, 5H, aromatics). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.06, 22.48, 23.12, 24.91, 28.24, 30.49, 36.26, 38.28, 39.57, 47.71, 52.17, 66.62, 66.86, 80.21, 128.26, 128.39, 128.57, 135.08, 153.50, 171.24, 174.82. ESI-MS *m/e* 445.5 $[M+1]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_5$: C, 67.54; H, 8.16; N, 6.30. Found: C, 67.42; H, 8.27; N, 6.27.

4.1.3. Synthesis of *N*-[2-allyl-*N*-(*tert*-butoxycarbonyl)-L-prolyl]-D-leucine benzyl ester (6a). HOAt (980 mg, 7.2 mmol), H-D-Leu-OBn·PTSA (3.983 g, 18 mmol), NMM (1.820 g, 18 mmol), and DCC (1.485 g, 7.2 mmol) were successively added under argon to a 0 °C cooled solution of (*R*)-*N*-Boc-2-allylproline[23] (**5**) (1.530 g, 6 mmol) in CH_2Cl_2 (33 mL). The stirring was maintained at 0 °C for 2 h, and subsequently at room temperature for 15 h. The precipitated *N*,*N*′-dicyclohexylurea was filtered off, and the solution was evaporated to dryness. The residue was dissolved in EtOAc (30 mL), and the resulting solution was successively washed with 10% citric acid solution (2×25 mL), saturated $NaHCO_3$ (2×25 mL), and brine (25 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a (6:1) hexane/EtOAc mixture as eluant, to give the dipeptide **6a** as a syrup (2.638 g, 96%). $[\alpha]_D^{20}$ +12.4° (*c*, 1 in MeOH). HPLC [μBondapack C_{18}] (A/B, 40:60) t_R 9.08 min. ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C): δ 0.85 and 0.88 [2d, 6H, *J*=6, 7 Hz, Me (Leu)], 1.37 [s, 9H, Me (Boc)], 1.55–1.72

[m, 5H, 3-H (Leu), 2-H (Leu), 4-H (Pro)], 2.00 [m, 2H, 3-H (Pro)], 2.64 [m, 1H, 1-H (allyl)], 2.86 [m, 1H, 1-H (allyl)], 3.15 [m, 1H, 5-H (Pro)], 3.55 [m, 1H, 5-H (Pro)], 4.41 [m, 1H, 2-H (Leu)], 5.05 [s, 2H, CH_2 (Bn)], 5.10 [dd, 2H, J=14, 6 Hz, 3-H (allyl)], 5.63–5.72 [m, 1H, 2-H (allyl)], 7.28–7.35 (m, 5H, aromatics), 7.46 (bs, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.65, 22.30, 22.87, 24.67, 28.32, 34.71, 38.26, 41.15, 49.41, 50.98, 66.76, 69.73, 80.14, 119.37, 128.34, 128.54, 132.77, 135.63, 153.90, 172.79, 173.92. ESI-MS *m/e* 459.3 $[M+H]^+$. Anal. Calcd for $C_{26}H_{38}N_2O_5$: C, 68.10; H, 8.35; N, 6.11. Found: C, 68.02; H, 8.47; N, 6.08.

4.1.4. Synthesis of *N*-[*N*-(*tert*-butoxycarbonyl)-2-(2-hydroxyethyl)-L-prolyl]-D-leucine benzyl ester (10a). OsO_4 (2.5%, w/w solution in *tert*-butanol, 4.81 mL, 0.38 mmol) was added to a solution of the dipeptide **6a** (2.603 g, 5.68 mmol) in (2:1) MeOH/H_2O (180 mL), under argon, which was stirred at room temperature for 10 min. $NaIO_4$ (3.645 g, 17.11 mmol) was slowly added. After 2 h of stirring at room temperature, H_2O (40 mL) was added, and the mixture was extracted with EtOAc (3×80 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved into dry EtOAc (180 mL), and the solution was cooled to −78 °C. To this solution, $NaBH_4$ (215 mg, 5.68 mmol) dissolved into MeOH (13 mL) was added, the mixture was stirred at −78 °C for 1 h, and then 30 min at room temperature. H_2O (50 mL) was added, and the organic phase was washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a 20–100% gradient of EtOAc in hexane as eluant, to give the alcohol **10a** as a syrup (1.200 g, 50%). $[\alpha]_D^{20}$ −1.8° (*c*, 1.5 in MeOH). HPLC [μBondapack C_{18}] (A/B, 37:63) t_R 9.98 min. ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C): δ 0.89 and 0.86 [2d, 6H, J=7, 6 Hz, Me (Leu)], 1.38 [s, 9H, Me (Boc)], 1.55–1.73 [m, 5H, 3-H (Leu), 4-H (Leu), 4-H (Pro)], 1.90–2.08 [m, 4H, 3-H (Pro), 1-H (hydroxyethyl)], 3.13–3.63 [m, 4H, 5-H (Pro), 2-H (hydroxyethyl)], 4.35 [m, 1H, 2-H (Leu)], 5.11 [s, 2H, CH_2 (Bn)], 7.34 (m, 5H, aromatics), 7.80 (bs, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.58, 22.77, 24.59, 28.27, 36.44, 38.22, 41.13, 48.57, 51.01, 58.78, 60.29, 66.87, 80.10, 128.21, 128.32, 128.51, 135.01, 153.51, 172.51, 174.54. ESI-MS *m/e* 463.3 $[M+H]^+$. Anal. Calcd for $C_{25}H_{38}N_2O_6$: C, 64.91; H, 8.28; N, 6.06. Found: C, 64.79; H, 8.45; N, 6.03.

4.1.5. Synthesis of (5*R*)-6-[(1*R*)-1-benzyloxycarbonyl-3-methylbutyl]imino-1-(*tert*-butoxycarbonyl)-7-oxa-1-azaspiro-[4.4]nonane (11a). Triphenylphosphine (1.348 g, 5.35 mmol) and DEAD (588 mg, 3.38 mmol) were added to a solution of the alcohol **10a** (1.200 g, 2.6 mmol) in dry THF (20 mL), and the mixture was stirred at room temperature for 14 h. The solvent was evaporated, and the residue was treated with ethyl ether (20 mL), to precipitate the formed triphenylphosphine oxide, which was filtered off. The solution was evaporated to dryness, and the residue was purified by flash chromatography, using a 15–50% gradient of EtOAc in hexane as eluant, to give the cyclic imidate **11a** as a syrup (700 mg, 61%). $[\alpha]_D^{20}$ −38.3° (*c*, 1.4 in MeOH). HPLC [μBondapack C_{18}] (A/B, 37:63) t_R 9.51 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 and 0.87 [2d, 6H, J=6 Hz, Me (Leu)], 1.41 [s, 9H, Me (Boc)], 1.45–1.78 [m, 5H, 3-H (Leu), 4-H (Leu), 4-H, 3-H), 1.84–1.91 (m, 2H, 9-H), 2.10 (m, 1H, 3-H), 2.20 (m, 1H, 4-H), 2.80 (m, 1H, 9-H), 3.38–3.61 (m, 2H, 2-H), 3.92 (m, 1H, 8-H), 4.23 [m, 1H, 2-H (Leu)], 4.41 (m, 1H, 8-H), 5.10 [m, 2H, CH_2 (Bn)], 7.34 (m, 5H, aromatics). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.15, 23.32, 23.42, 24.20, 28.40, 34.31, 39.42, 41.01, 42.39, 47.75, 57.58, 65.96, 66.34, 67.08, 79.32, 127.92, 128.00, 128.22, 128.35, 136.41, 153.14, 165.99, 173.80. ESI-MS *m/e* 445.3 $[M+H]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_5$: C, 67.54; H, 8.16; N, 6.30. Found: C, 67.40; H, 8.36; N, 6.27.

4.1.6. Synthesis of (5*R*,8*RS*)-7-[(1*R*)-1-benzyloxy-carbonyl-3-methylbutyl]-1-(*tert*-butoxycarbonyl)-8-hydroxy-6-oxo-1,7-diazaspiro-[4.4]nonane (12a). OsO_4 (2.5%, w/w solution in *tert*-butanol, 2.9 mL, 0.23 mmol) was added to a solution of the dipeptide **6a** (1.567 g, 3.42 mmol) in (2:1) MeOH/H_2O (108 mL), under argon, which was stirred at room temperature for 10 min. $NaIO_4$ (2.195 g, 10.3 mmol) was slowly added. After 24 h of stirring at room temperature, H_2O (40 mL) was added, and the mixture was extracted with EtOAc (3×60 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a 20–100% gradient of EtOAc in hexane as eluant, to give the two epimeric hemiaminals **12a**, as white solids, whose absolute configuration at C_8 could not be assigned. *Epimer* **A**. (584 mg, 38%). Mp 140–141 °C. $[\alpha]_D^{20}$ −4° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 40:60) t_R 14.45 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.88 and 0.91 [2d, 6H, J=7, 6 Hz, Me (Leu)], 1.29 [s, 9H, Me (Boc)], 1.64–2.30 [m, 9H, 3-H (Leu), 4-H (Leu), 3-H, 4-H, 9-H], 2.68 (dd, 1H, J=6, 13 Hz, OH), 3.37 (m, 2H, 2-H), 4.51 [dd, 1H, J=5, 11 Hz, 2-H (Leu)], 5.13 [d, 2H, J=15 Hz, CH_2 (Bn)], 5.79 (t, 2H, J=5 Hz, 8-H), 7.40 (m, 5H, aromatics). ^{13}C NMR (75 MHz, acetone-d_6): δ 21.80, 23.10, 24.58, 24.87, 28.59, 39.57, 40.79, 41.65, 48.48, 53.42, 66.89, 67.06, 79.41, 81.24, 128.64, 128.99, 129.08, 129.29, 135.26, 153.74, 171.67, 172.01. ESI-MS *m/e* 483.4 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_6$: C, 65.20; H: 7.88; N, 6.08. Found: C, 65.18; H, 7.92; N, 6.06. *Epimer* **B**. (584 mg, 38%). Mp 134–135 °C. $[\alpha]_D^{20}$ +26° (*c*, 1.2 in MeOH). HPLC [Novapack C_{18}] (A/B, 40:60) t_R 15.97 and 18.75 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 and 0.89 [2d, 6H, J=7, 6 Hz, Me (Leu)], 1.40 [s, 9H, Me (Boc)], 1.50–2.60 [m, 9H, 3-H (Leu), 4-H (Leu), 3-H, 4-H, 9-H], 3.40 (m, 2H, 2-H), 4.20–5.40 [m, 5H, 2-H (Leu), CH_2 (Bn), 8-H], 7.40 (m, 5H, aromatics). ^{13}C NMR (75 MHz, acetone-d_6): δ 21.30, 23.47, 24.14, 24.93, 28.28, 38.93, 40.25, 42.55, 47.73, 53.21, 66.82, 67.74, 77.58, 80.66, 128.11, 128.38, 128.54, 128.64, 135.83, 154.14, 171.24, 174.16. ESI-MS *m/e*483.4 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_6$: C, 65.20; H, 7.88; N, 6.08.

4.1.7. Synthesis of (5*R*)-7-[(1*R*)-1-benzyloxycarbonyl-3-methylbutyl]-6-oxo-1,7-diaza-spiro[4.4]nonane trifluoroacetate (3b). $NaBH_4$ (106 mg, 2.8 mmol) was added to a solution of the epimeric mixture of hemiaminals **12a** (430 mg, 0.93 mmol) in neat TFA (10 mL), and the mixture was stirred at room temperature for 2 h. The solvent was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (20 mL). This solution was washed with H_2O

(5 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in H_2O (3 mL), and the resulting solution was lyophilized, to give the title compound as a yellow syrup (414 mg, 100%). $[\alpha]_D^{20}$ +15° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 60:40) t_R 1.45 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.91 and 0.92 [2d, 6H, *J*=6 Hz, Me (Leu)]; 1.50 [m, 1H, 4-H (Leu)]; 1.66–1.94 [m, 2H, 3-H (Leu)]; 2.11–2.72 (m, 6H, 3-H, 4-H, 9-H); 3.35–3.72 (m, 4H, 2-H, 8-H); 4.74 [dd, 1H, *J*=6, 15 Hz, 2-H (Leu)]; 5.18 [s, 2H, CH_2 (Bn)]; 7.37 (m, 5H, aromatics). ^{13}C NMR (75 MHz, acetone-d_6): δ 21.21, 23.26, 23.89, 30.24, 34.61, 30.01, 42.24, 46.65, 53.93, 67.59, 67.99, 69.58, 129.07, 129.35, 129.45, 136.77, 161.12, 161.57, 170.95, 172.65. ESI-MS *m/e* 443.2 $[M+1]^+$. Anal. Calcd for $C_{22}H_{29}F_3N_2O_4$: C, 59.72; H, 6.61; N, 6.33. Found: C, 59.86; H, 6.65; N, 6.29.

4.1.8. Synthesis of (5*R*)-1-(*tert*-butoxycarbonyl)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]-nonane (3c). Tetramethylammonium hydroxide pentahydrate (158 mg, 0.87 mmol) and di(*tert*-butyl) dicarbonate (144 mg, 0.66 mmol) were added to a solution of the trifluoroacetate **3b** (150 mg, 0.44 mmol) in acetonitrile (5 mL). After 48 h of stirring at room temperature, the solvent was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (3 mL). The solution was extracted with H_2O (5 mL), and the extracts were lyophilized. The resulting residue was purified by flash chromatography, using 8–40% gradient of MeOH in CH_2Cl_2 as eluant, to give the title compound **3c** as a white solid (110 mg, 70%). Mp 192–194 °C. $[\alpha]_D^{20}$ −3° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 50:50) t_R 13.41 min. ^{1}H NMR (300 MHz, DMSO-d_6, *T*=90 °C): δ 0.87 and 0.91 [2d, 6H, *J*=7 Hz, Me (Leu)], 1.36 [s, 9H, Me (Boc)], 1.40 [m, 1H, 4-H (Leu)], 1.64 [t, 2H, *J*=8 Hz, 3-H (Leu)], 1.70–1.90 (m, 5H, 3-H, 4-H, 9-H), 2.50 (m, 1H, 9-H), 3.10 (c, 1H, *J*=9 Hz, 8-H), 3.30 (m, 2H, 2-H), 3.70 (t, 1H, *J*=10 Hz, 8-H), 4.39 [t, 1H, *J*=8 Hz, 2-H (Leu)]. ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.05, 22.60, 23.40, 25.24, 28.54, 30.44, 36.87, 38.20, 40.59, 48.19, 54.32, 67.29, 81.37, 154.09, 174.97, 176.01. ESI-MS *m/e* 353.4 $[M-1]^-$. Anal. Calcd for $C_{18}H_{30}N_2O_5$: C, 61.00; H, 8.53; N, 7.90. Found: C, 59.93; H, 8.59; N,7.88.

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