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Facile synthesis of *N*-Boc-(2*S*,5*R*)-5-(1′-hydroxy-1′-methylethyl)proline

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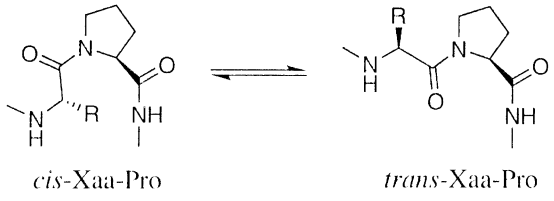
Abstract—Coupling of chiral 1-*O*-benzylglycerol-2,3-bistriflate with trilithiated chiral 2-*N*-Boc-3-phenylsulfonylpropan-1-ol derivative constitutes an efficient route to chiral C-5 substituted prolines which can potentially induce *cis* Xaa-Pro peptide bond conformation. A straightforward seven-step synthesis of the title compound is described.

1. Introduction

Unlike other peptide amide bonds which adopt predominantly more stable *trans* conformation, Xaa-Pro peptide (Xaa=any proteinogenic amino acid) bond has approximately the same energy in both *cis* and *trans* conformer. A substantial barrier for isomerization separates these two isomers. The distribution of *cis–trans*-Xaa-Pro conformers is determined by the steric interactions between the C-2 centers of the two amino acid residues in the *cis* rotamer, and between the C-2 center of Xaa and the functionality on the C-5 of proline in the *trans*-isomer (Scheme 1).

This *cis–trans*-isomerism of Xaa-Pro peptide bonds can exert a significant influence upon a number of biologically important processes, including protein folding, peptidyl-propyl isomerases (PPIases) activity and protein-ligand recognition.[1–4] In fact, the occurrence of *cis*-Xaa-Pro peptide bond has been recognized as an important structural feature in peptides and proteins.[5–8] In particular, the frequent observation of proline in β-turn,[9] arisen from a *cis*-peptidyl linkage, has evoked considerable interest in the synthesis of proline analogs that specifically favor *cis*-amide geometry for acquiring detailed knowledge of the bioactive conformation and for the rational design of therapeutics. For this purpose, two types of proline surrogates that induce *cis*-amide bond have recently been developed. The first approach is the construction of constrained *cis*-peptidyl prolinamides which are synthesized by tethering the C-2 of Xaa to the C-2 of proline,[10–13] or 4,5-fused 1,2,5-triazepine 3,6-diones,[14] as shown in Fig. 1.

The second is based on the obvious assumption that introduction of alkyl group(s) at the C-5 of proline may disfavor a *trans*-amide isomer due to the steric interactions between the C-5 substituent and Xaa residue. For example, while the *cis*-isomer population was found to be about the same with that of common proline-containing oligopeptides in most solvents for *N*-acetyl-5-methylproline *N*′-methylamide, it increased 20% for the C-5 diastereomer in nonpolar solvents.[15] In the case of 5,5-dimethyl proline, the ratio of the *cis*/*trans*-isomer was reported to be 9:1 in *N*-Boc-L-Phe-DL-5,5-dimethyl proline methyl ester.[16] More recently, an efficient methodology for the synthesis of all four



Scheme 1. *cis*- and *trans*-Xaa-Pro peptide conformers.

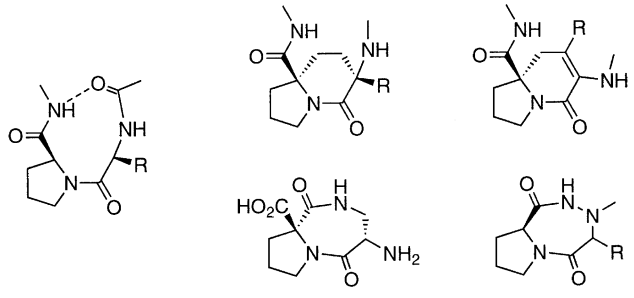


Figure 1. Conformation of type VI-β-turn and some representative *cis*-Xaa-Pro mimetics.

Keywords: *cis* conformer; C-5 substituted proline.

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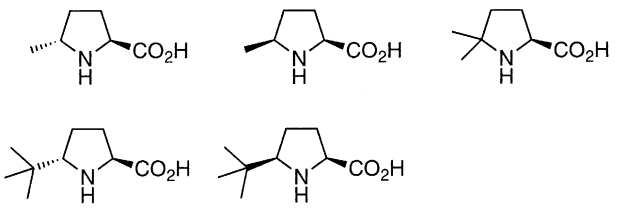


Figure 2. 5-Alkylprolines.

stereoisomers of 5-*tert*-butylproline has been reported by Lubell and coworkers (Fig. 2).[17]

When incorporated into dipeptides in the form of *N*-acetyl-L-Xaa-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamides in which Xaa represents Ala, Met, Leu, Val and Phe residues, the *cis*-isomer population in peptides has increased to 74–90% in water. Moreover, analyses by NMR, X-ray and CD have confirmed that these dipeptides adopt a type VI β-turn conformation.[18] It is reported that the Cys-Pro peptide bond *cis*-isomer population in oxytocin has increased from 10 to 35% by replacing the proline residue with (2*S*,5*R*)-*tert*-butylproline.[19]

We have been interested in developing efficient methodologies for the synthesis of conformationally constrained amino acids that can be easily incorporated into bioactive peptides starting from a common chiral synthon.[20] These conformationally constrained amino acids are illustrated in Scheme 2.[21–25]

As for a proline surrogate that can induce Xaa-Pro amide bond to adopt predominantly *cis*-amide bond conformation, it occurred to us to introduce 1-hydroxy-1-methylethyl group at the C-5 position of proline in a highly stereoselective manner. Our initial interest is to investigate whether, in addition to the steric bulkiness of this tertiary alcohol substituent, there can be any other effect such as intramolecular hydrogen bonding caused by the inherent hydroxyl group for stabilizing the *cis*-conformer and increasing the *cis*-isomer population. In this report, we describe a straightforward asymmetric synthesis of one of the four diastereoisomers of 5-(1′-hydroxy-1′-methylethyl)-proline which is selected, based on molecular calculation, as a potential *cis*-conformation inducer of the Xaa-Pro amide bond.

2. Results and discussion

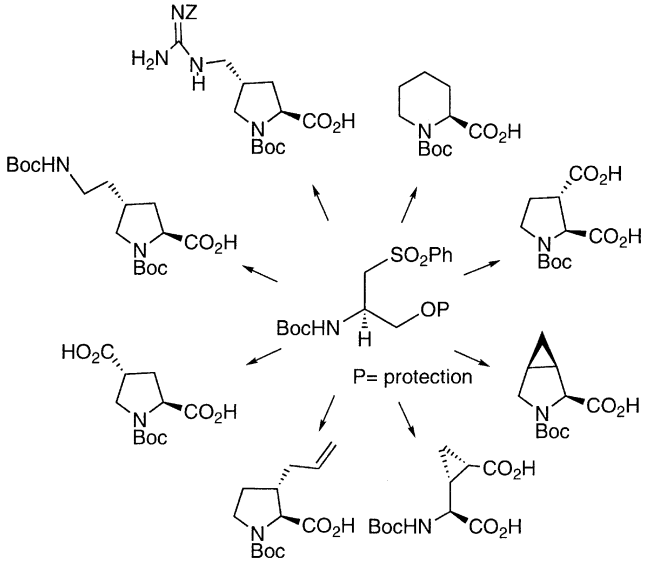
2.1. Modeling investigation

2.1.1. Computational procedure. Taking into account the number of degrees of freedom of the (2*S*,5*R*)-*N*-acetyl-5-(1′-hydroxy-1′-methylethyl)proline *N*′-methylamid (**IA**) and its (5*S*)-diastereomer (**IB**), 4000 conformations of each studied compound were generated by random search Monte Carlo method[26] and optimized by TNCG Truncated Newton minimization[27] using the Macromodel (Version 5.5) program[28] with the AMBER* force field[29] and GB/SA water solvation. Duplicate conformations as well as those that had chirality change were discarded. From these conformational searches, all the possible conformations within 3 kcal/mol from the global minimum were considered. The structures are shown in Figs. 3 and 4. The calculated steric energy differences (AMBER), as well as the most relevant geometrical parameters of these structures are summarized in Table 1.

2.1.2. Energy analysis. The calculated steric energy analysis indicates:

- that in both cases (**IA**, **IB**), the most stable conformation corresponds to a *cis*-isomer;
- that the energy differences between the most stable *cis*- and *trans*-isomers are 2.45 kcal/mol for **IA** and 3.75 kcal/mol for **IB**.

These results reveal that the relative populations of the amide *cis*- and *trans*-isomers of Xaa-Pro amide bond are 98.5/1.5 and 100/0 for **IA** and **IB**, respectively. According



Scheme 2. Conformationally constrained amino acids from the common chiral synthon.

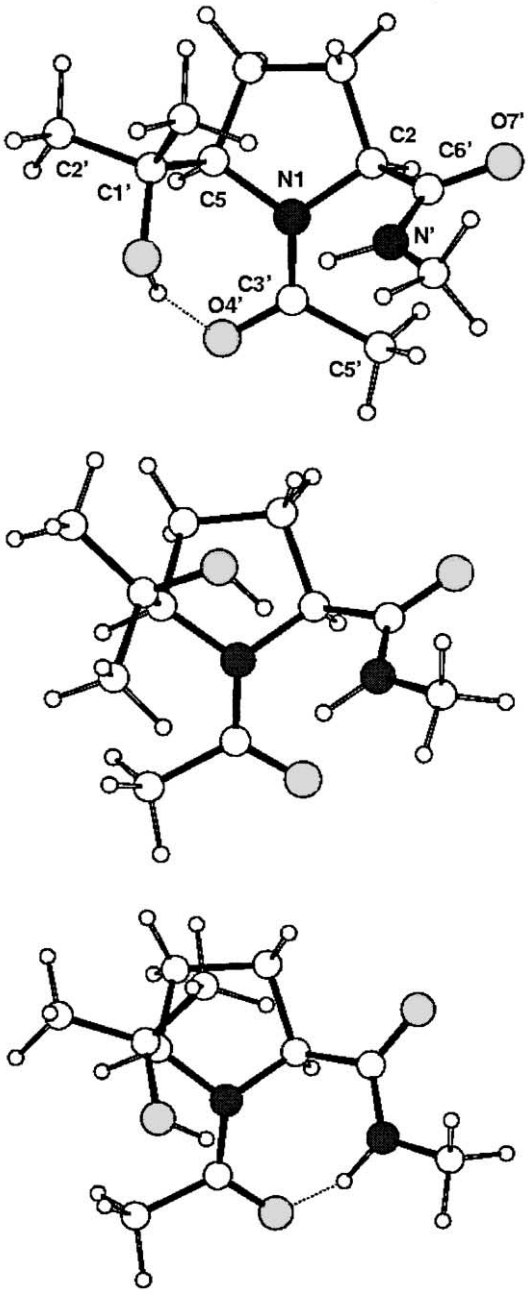


Figure 3. Conformations of the *cis*- and *trans*-isomers of **IA**.

to the calculations by Lubell and coworkers,[18a] the relative populations of the amide *cis*-isomer are 46 and 77% for (2*S*,5*R*)-*N*-acetyl-5-*tert*-butylproline *N*′-methylamide and (2*S*,5*S*)-*N*-acetyl-5-*tert*-butylproline *N*′-methylamide, respectively.

2.1.3. Conformational analysis of IA. The conformational analysis reveals:

- that the most stable *cis*-isomer of **IA** gives rise to hydrogen bond (1.84 Å) between the hydrogen of the hydroxyl group and the oxygen atom of the carbonyl group attached to the nitrogen atom N-1 of the pyrrolidine ring. The value of O(OH)-C-1′–C-5–N-1 dihedral angle is −44.2° indicating that the hydroxyl group is outside of the pyrrolidine ring and is pointed toward the carbonyl group of the acetyl function. Moreover, in this conformation, the hydrogen of the N′-methylamide is close to the N-1 atom (2.22 Å) by stabilizing its lone-pair electron;
- that in the most stable *trans*-isomer of **IA**, the value of O(OH)–C-1′–C-5–N-1 dihedral angle is 49.6°, that is to say, the hydroxyl group is pointed toward inside of the

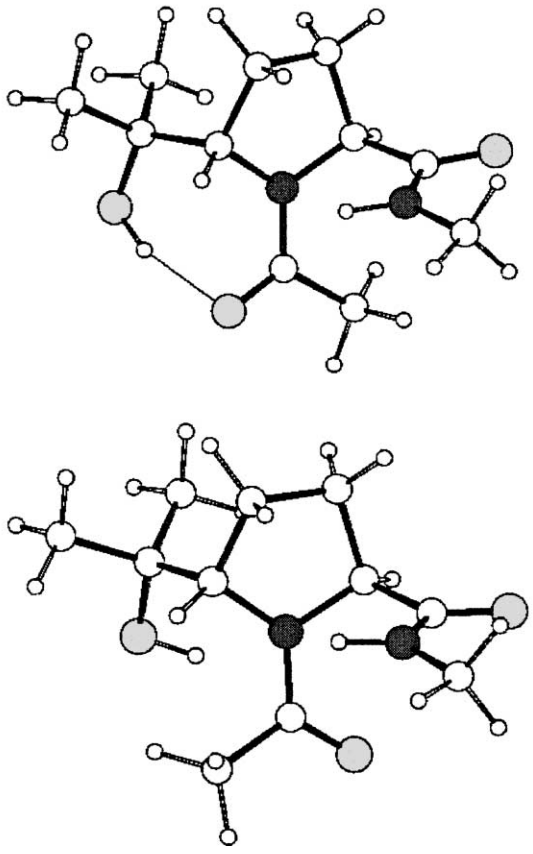


Figure 4. Conformations of the *cis*- and *trans*-isomers of **IB**.

Table 1. Energy differences and relevant parameters of the most stable *cis*- and *trans*-isomers of **IA** and **IB**

	cis		*trans*		
	IA	**IB**	**IA**	**IA**[a]	**IB**
ΔE (kJ/mol)	0	0	10.23	12.74	15.66
ΔE (kcal/mol)	0	0	2.45	3.05	3.75
Dihedral angles (degrees)					
C5′–C3′–N1–C2 (ω_1)	10.68	−17.12	−155.25	−158.91	153.95
C3′–N1–C2–C6′ (ϕ_2)	−78.19	−55.22	−87.84	−86.84	−44.33
N1–C2–C6′–N′ (ψ_2)	−12.78	−27.56	3.47	34.32	−38.32
O(OH)– C1′–C2–N1	−44.21	52.97	49.59	−40.57	43.23
Distances (Å)					
N1–H(OH)	2.42	2.50	2.31	2.11	2.23
N1–H(N′H)	2.22	2.34	2.21	2.45	2.47
O4′–H(OH)	1.84	1.78	3.99	3.33	3.17
O7′–H(OH)	5.66	6.04	3.36	4.32	5.48
O4′–H(N'H)	3.42	3.12	2.60	1.97	3.28
O(OH)– H(N'H)	2.86	4.68	2.88	3.70	4.90

[a] The *trans* isomer of **IA** presenting an hydrogen bond between the hydrogen of the *N*′-methylamide with the oxygen atom of the carbonyl group of the acetyl function. The dihedral angles are in degrees and the distances are in Å.

pyrrolidine ring. In this conformation, the hydrogen of the N'-methylamide as well as the hydrogen of the hydroxyl group are close to the N-1 atom (2.21 and 2.31 Å, respectively) by stabilizing its lone-pair electron. The calculations also indicate that this *trans*-isomer is more stable than the *trans*-isomer which gives rise to hydrogen bond (1.97 Å) between the hydrogen of the N'-methylamide and the O-4′ atom of the carbonyl group attached to the N-1 atom. Their energy difference is 0.60 kcal/mol. It means that the last one hardly exists.

2.1.4. Conformational analysis of IB. The conformational analysis reveals:

- that the most stable *cis*-isomer of **IB** gives rise to hydrogen bond (1.78 Å) between the hydrogen of the hydroxyl and the oxygen atom of the carbonyl group of the acetyl function. The value of O(OH)–C-1′–C-5–N-1 dihedral angle is 53.0°. This indicates that the hydroxyl group is outside of the pyrrolidine ring and is pointed toward the carbonyl group of the acetyl function. In this conformation, the hydrogen of the N'-methylamide is close to the N-1 atom (2.34Å) by stabilizing its lone-pair electron;
- that in the most stable *trans*-isomer of **IB**, the value of O(OH)–C-1′–C-5–N-1 dihedral angle is 43.2°, that is to say, the hydroxyl group is outside of the pyrrolidine ring and is pointed toward the carbonyl group of the acetyl function. In this conformation, the hydrogen of the hydroxyl group is close to the N-1 atom (2.23 Å) by stabilizing its lone-pair. However, the important energy difference with the most stable *cis*-isomer indicates that this *trans*-isomer does not exist. From these comparisons, it is suggested that when there is no hydrogen bond, the hydroxy group is placed inside of the pyrrolidine ring. This conformation minimizes the steric interaction and makes it energetically a more favorable one.

2.1.5. Modeling conclusion. On the one hand, owing to the partial double-bond character, it is normally possible for ω to assume values only in the neighborhood of 0 or 180° which is the value generally observed (i.e. the *trans* conformation). A fully extended polypeptide is characterized by $\phi = \psi = \omega = 180°$. Moreover, in proline-containing polypeptide, the corresponding dihedral angle ϕ (C-3′–N-1–C-2–C-6′) is −71.6°. On the other hand, the values for the dihedral angles of an ideal typeVI β-turn are $\phi_1 = -60°$, $\psi_1 = 120°$, $\omega_1 = 0°$, $\phi_2 = -90°$, $\psi_2 = 0°$.

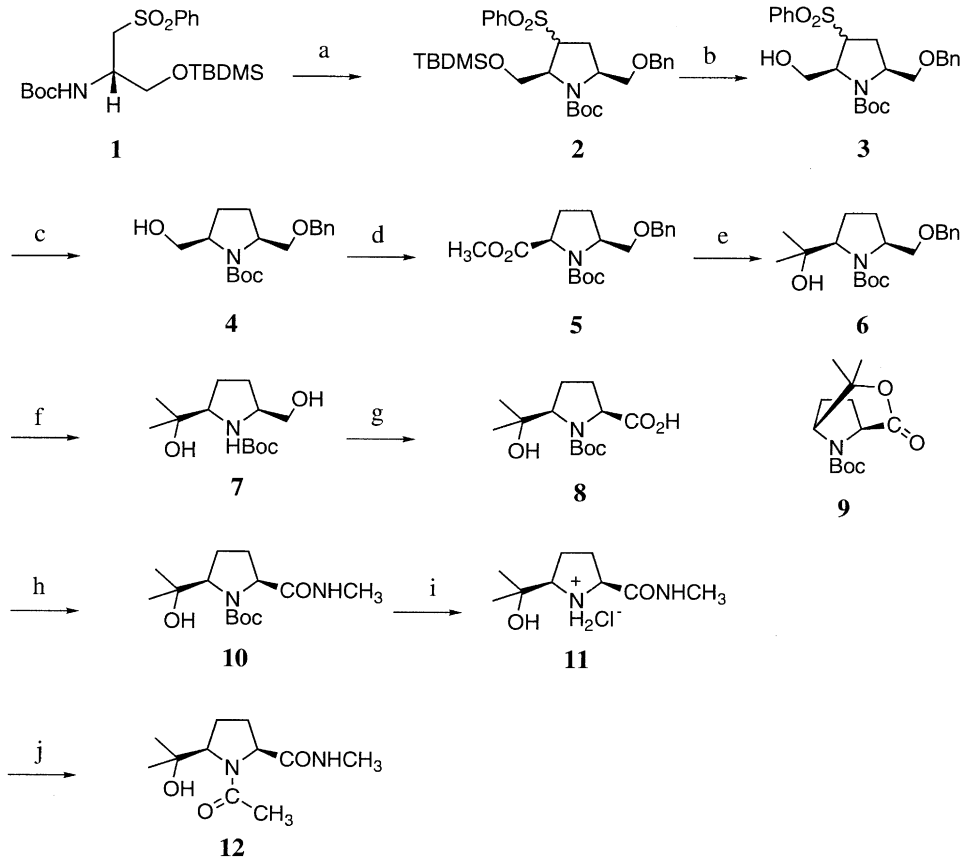
Taking into account of these ideal values ($\omega_1 = 0°$, ϕ_2 = C-3′–N-1–C-2–C-6′ = −71.6° and $\psi_2 = 0°$) and the possible hydrogen bonding, the comparison of the dihedral angles of the most stable *cis*-isomer of **IA** ($\omega_1 = 10.7°$, ϕ_2 = C-3′–N-1–C-2–C-6′ = −78.2° and $\psi_2 = -12.8°$) with those of the *trans*-isomer of **IA** ($\omega_1 = -155.3°$, ϕ_2 = C-3′–N-1–C-2–C-6′ = −87.8° and $\psi_2 = 3.5°$) allows a better understanding of their relative stability. The most stable *trans*-isomer of **IA** is less stable than its *cis*-isomer because it gives rise to no hydrogen bond. Moreover, in the most stable *cis* and *trans* conformations of **IA**, the values of the dihedral angle ψ_2 are close to 0° ($\psi_2 = -12.8$ and 3.5°, respectively) while in the most stable *cis* and *trans* conformations of **IB**, these values are $\psi_2 = -27.6$ and −38.3°, respectively.

Based on these conformational analyses, we commenced our study by synthesizing five-substituted proline derivative **8**, the pivotal precursor of **IA**, with anticipation that this amino acid can induce proline containing peptides to adopt *cis* conformation at Xaa-(1′-hydroxy-1′-methylethyl)-Pro amide bond.

2.2. Synthesis

Recently, we have described a concise one-step synthesis of enantiopure 2,5-disubstituted pyrrolidine derivatives.[30] The specific feature of this method is that any desired diastereomer out of the four possible isomers can be prepared from readily available chiral synthons in the same efficiency without separation of diastereoisomers. Synthesis of the title compound **8** was shown in Scheme 3. The pivotal component of our synthetic strategy is the one-step construction of enantiopure 2,5-dihydroxymethylpyrrolidine derivative in which the two hydroxymethyl groups are differentially protected. The next sequence is the transformation of one hydroxyl group to 1-hydroxy-1-methylethyl group followed by the oxidation of the other. Thus treatment of chiral synthon (*S*)-**1** with 3 equiv. of BuLi generated the corresponding trianion which was then coupled with (2*S*)-1-*O*-benzylglycerol-2,3-bistriflate[30] to give pyrrolidine **2**. The stereochemistry of C-3 was of no consequence as it will be destroyed at the later stage. Selective removal of TBDMS group under mild conditions (TBAF, THF) followed by desulfonylation (6% Na–Hg in MeOH) gave compound **4** in 62% overall yield (three steps). On the contrary, desulfonylation of comopound **2** followed by removal of TBDMS group gave inferior results. The oxoammonium salt mediated oxidation of primary alcohol using TEMPO-NaOCl gave the acid that was immediately treated with CH_2N_2 to afford the methyl ester **5** in 90% yield. Nucleophilic addition of MeMgBr to the ester function gave the tertiary alcohol **6** in 98% yield without epimerization. Hydrogenolysis followed by Jones oxidation afforded carboxylic acid **8**. It is worthy noting that TEMPO oxidation of **7** in this case gave only 18% of the acid **8**, the major product being the lactone **9** (71%). The presence of tertiary alcohol adjacent to the amino function was known to cause problem in the *N*-deprotection step under acidic conditions. Easy formation of oxazolidinone is a well-established side reaction.[31] In our hands, the desired deprotection was realized using 1 M solution of HCl in AcOEt to provide amino alcohol in 96% yield. Compound **11** was treated with Ac_2O/Et_3N in CH_2Cl_2 for 48 h to give compound **12**. NMR analysis shows that the ratio of *cis* and *trans*-isomers is about 50/50 in $CDCl_3$.

In conclusion, one of the four stereoisomers of 5-(1′-hydroxy-1′-methylethyl)proline derivative **8** has been chosen as a potential *cis* conformation inducer in the Xaa-Pro amide bond. An efficient synthesis of **8** has been effected from readily available chiral synthons using one-step construction of enantiopure 2,5-disubstituted pyrrolidine as a key component of this approach. Further applications of this methodology to the synthesis of the C-5 diastereomer of **8** and the incorporation of these C-5



Scheme 3. Reagents and conditions: (a) BuLi (3 equiv.), THF, −70°C, 30 min then (2*S*)-1-*O*-benzyglycerol-2,3-bistriflate, −70°C, 3 h; (b) Bu_4NF, THF, rt, 2 h, 64% for two steps; (c) 6% Na–Hg, Na_2HPO_4, MeOH, 0°C, 24 h, 92%; (d) (1) TEMPO, NaOCl, KBr, 5% aq. $NaHCO_3$, acetone, 0°C, 2 h; (2) CH_2N_2, CH_3OH–Et_2O, 90%; (e) CH_3MgBr, THF, 0°C, 30 min, rt, 1 h, 98%; (f) H_2 (1 atm), 10% Pd–C, EtOAc, rt, 5 h, 95%; (g) Jones reagent, acetone, 0°C, 8 h, 92%; (h) CH_3NH_2·HCl, DIEA, TBTU, CH_3CN, rt, 36 h, 75%; (i) 1 M HCl–EtOAc, rt, 2 h, 96%; (j) Ac_2O, Et_3N, CH_2Cl_2, rt, 48 h, 85%.

substituted proline analogs into peptides are currently under way in our laboratory.

3. Experimental

3.1. General

Infrared (IR) spectra were recorded on a Perkin–Elmer Spectrum BX spectrometer. ^1H and ^{13}C NMR spectra were measured on Brucker AM-300 and AC-250 (300 and 250 MHz, respectively) spectrometers with tetramethylsilane as internal standard (δ ppm). Flash chromatography was performed using Kieselgel 60 (230–400 mesh, E. Merck) and usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Solvents and reagents were purified according to standard laboratory techniques. Optical rotations were determined on a Perkin–Elmer 241 polarimeter at room temperature. Mass spectra were run on an AEI MS-9 spectrometer (CI), and Nawigator Thermo quest spectrometer (ESI), respectively. Elemental analyses were carried out at the ICSN. All reactions requiring anhydrous conditions or in an inert atmosphere were conducted under an atmosphere of Argon.

3.1.1. (2*S*,5*S*)-1-*tert*-Butoxycarbonyl-2-*tert*-butyldimethylsilyloxymethyl-3-phenylsulfonyl-5-benzyloxymethyl pyrrolidine (2). To a solution of **1** (3.35 g, 7.81 mmol) in THF (70 mL) was added dropwise BuLi (1.6 M, 14.6 mL, 23.43 mmol, 3 equiv.) at −78°C. After stirring for 30 min at the same temperature, a solution of (2*S*)-1-*O*-benzylglycerol-2,3-bistriflate[30] (4.87 g, 10.93 mmol, 1.4 equiv.) in THF (8 mL) was added dropwise, and stirring was continued for 3 h at −78°C then at rt for 1 h. The reaction was quenched by addition of satd. NH_4Cl. The reaction mixture was extracted with ether. The ether extracts were washed with brine, dried and evaporated. Column chromatography on silica gel eluting with heptane/EtOAc (10/1 then 8/1) afforded a mixture of **2** and the starting material **1** which was used directly for the next reaction. Pure compound **2** was obtained by preparative TLC (silica gel, toluene/EtOAc=6/1) as a single isomer (two rotamers): $[\alpha]_D$=−10 (*c* 4.0, $CHCl_3$); IR ($CHCl_3$) 3029, 3012, 2957, 2931, 2859, 1690, 1472, 1448, 1394, 1369, 1308, 1257 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.91–7.89 (m, 2H), 7.69–7.54 (m, 3H), 7.32–7.26 (m, 5H), 4.51–4.40 (m, 2H), 4.29 (m, 1H), 4.04 (dq, *J*=3.17, 7.19 Hz, 1H), 3.80 (quintet, *J*=4.1 Hz, 1H), 3.67–3.33 (m, 4H), 2.69–2.25 (m, 2H), 1.48, 1.41 (two s, 9H), 0.80, 0.76 (two s, 9H), −0.069, −0.10, −0.12 (three s, 6H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.0, 153.7, 138.1, 138.0, 137.6, 133.8, 129.3, 128.7, 128.6, 128.3, 127.5, 127.4, 80.1, 73.1, 70.8, 70.1, 63.5, 62.7, 62.4, 61.7, 60.2, 56.9, 28.4, 28.3, 28.0, 27.3, 25.8, 18.1, −5.7; MS (ESI) *m/z* 576 $[M+H]^+$. HRMS Calcd for $C_{30}H_{46}NO_6SSi$: 576.28149; Found: 576.28255.

3.1.2. (2*S*,5*S*)-1-*tert*-Butoxycarbonyl-2-hydroxymethyl-3-phenylsulfonyl-5-benzyloxy-methyl pyrrolidine (3). To a solution of **2** obtained above (contaminated with compound **1**) (3.88 g, 6.75 mmol) in THF (60 mL) was added tetrabutylammonium flouride (1 M solution in THF, 7.5 mL, 7.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated, and the residue was separated in EtOAc and aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc. The EtOAc extracts were washed with brine, dried and evaporated. Column chromatography on silica gel (heptane/EtOAc=2/1) gave **3** as an oil (2.31 g, 64% for two steps): $[\alpha]_D=-37$ (*c* 2.6, $CHCl_3$); IR ($CHCl_3$) 3425, 3029, 3013, 2981, 2932, 2871, 1692, 1478, 1448, 1392, 1369, 1308, 1150 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.91–7.89 (m, 2H), 7.70–7.55 (m, 3H), 7.30–7.25 (m, 5H), 4.51–4.47 (m, 3H), 3.98–3.32 (m, 7H), 2.53–2.43 (m, 2H), 1.48, 1.39 (two s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.2, 153.5, 137.0, 136.9, 133.9, 129.3, 128.5, 128.3, 127.8, 127.6, 80.4, 73.1, 70.1, 69.6, 65.0, 64.5, 60.7, 57.0, 28.6, 28.1; MS (ESI) *m/z* 462 $[M+H]^+$, 484 $[M+Na]^+$, 500 $[M+K]^+$. Anal. Calcd for $C_{24}H_{31}NO_6S$: C, 62.45; H, 6.77; N, 3.03; S, 6.95; Found: C, 62.44; H, 6.91; N, 3.01; S, 6.89.

3.1.3. (2*S*,5*R*)-1-*tert*-Butoxycarbonyl-5-hydroxymethyl-2-benzyloxymethyl pyrrolidine (4). To a solution of the sulfone **3** (990 mg, 2.15 mmol) in HPLC grade MeOH (43 mL) containing Na_2HPO_4 (3.66 g, 25.77 mmol, 12 equiv.) was added 6% Na–Hg (7.41 g, 19.33 mmol, 9 equiv.) at 0°C. The mixture was vigorously stirred at 0°C for 24 h. Mercury was removed by decanting the reaction mixture. After evaporation of MeOH in vacuo, the residue was dissolved in water and EtOAc. The aqueous layer was extracted with EtOAc. The EtOAc extracts were washed with brine, dried and evaporated. Flash chromatography on silica gel (heptane/EtOAc=4/1) afforded compound **4** as an oil (635 mg, 92%): $[\alpha]_D=-25$ (*c* 2.2, $CHCl_3$); IR ($CHCl_3$) 3415, 3030, 3010, 2980, 2871, 1683, 1477, 1455, 1402, 1368, 1167, 1117 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.58–4.48 (m, 2H), 4.00–3.46 (m, 7H), 1.98–1.68 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.3, 137.9, 128.3, 127.6, 127.6, 80.2, 73.2, 71.0, 67.2, 61.2, 58.3, 28.3, 27.2, 26.9; MS (ESI) *m/z* 322 $[M+H]^+$, 344 $[M+Na]^+$, 360 $[M+K]^+$, 665 $[2M+Na]^+$. Anal. Calcd for $C_{18}H_{27}NO_4$: C, 67.26; H, 8.47; N, 4.36; Found: C, 67.25; H, 8.55; N, 4.31.

3.1.4. (2*S*,5*R*)-1-*tert*-Butoxycarbonyl-2-benzyloxymethyl-5-methoxycarbonyl-pyrrolidine (5). To a solution of **4** (945 mg, 2.94 mmol) in acetone (22 mL) was added an aqueous 5% $NaHCO_3$ solution (7.6 mL). This heterogeneous mixture was cooled to 0°C and treated sequentially with KBr (35 mg, 0.294 mmol) and TEMPO (506 mg, 3.24 mmol). Sodium hypochlorite (5% solution in water, 5.5 mL, 3.70 mmol) was then added dropwise while the mixture was vigorously stirred and maintained at 0°C. After 1 h, additional NaOCl (5% solution in water, 4.3 mL, 2.94 mmol) was added, and stirring was continued at 0°C for another hour followed by addition of 5% $NaHCO_3$ solution. When acetone was removed on a rotary evaporator, the aqueous layer was washed with ether twice, acidified to pH 6 with 10% $KHSO_4$ and extracted with EtOAc. The combined organic extracts were dried, concentrated and treated with excess diazomethane in ether, then evaporated. The residue was purified by flash chromatography (silica gel, heptane/EtOAc=10/1 then 5/1) gave compound **5** (oil) as two rotamers (924 mg, 90%): $[\alpha]_D=-6$ (*c* 2.4, $CHCl_3$); IR ($CHCl_3$) 3024, 3013, 2982, 2955, 2868, 1749, 1691, 1478, 1455, 1438, 1396, 1368, 1167, 1096 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.34–7.33 (m, 5H), 4.62–4.49 (m, 2H), 4.33–4.17 (m, 2H), 3.86–3.76 (m, 1H), 3.70 (s, 3H), 3.49–3.36 (m, 1H), 2.25–1.91 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 173.3, 153.9, 153.4, 138.4, 138.3, 128.1, 127.3, 79.9, 73.0, 70.7, 70.3, 59.8, 59.4, 57.3, 51.8, 51.7, 28.7, 28.1, 28.1, 27.8, 27.2; MS (ESI) *m/z* 350 $[M+H]^+$, 372 $[M+Na]^+$, 388 $[M+K]^+$, 721 $[2M+Na]^+$, 737 $[2M+K]^+$. Anal. Calcd for $C_{19}H_{27}NO_5$: C, 65.31; H, 7.79; N, 4.01; Found: C, 65.25; H, 7.91; N, 3.85.

3.1.5. (2*S*,5*R*)-1-*tert*-Butoxycarbonyl-2-benzyloxymethyl-5-(1′-hydroxy-1′-methylethyl)-pyrrolidine (6). To a solution of **5** (665 mg, 1.90 mmol) in THF (19 mL) at 0°C was added a solution of MeMgBr in ether (3.0 M, 1.5 mL, 4.56 mmol). The reaction mixture was stirred at 0°C for 30 min, then at room temperature for 1 h. The reaction was quenched by addition of saturated aqueous NH_4Cl at 0°C, and the reaction mixture was extracted with EtOAc. The organic layers were washed with brine, dried and evaporated. Column chromatography on silica gel (heptane/EtOAc=5/1) afforded compound **6** (650 mg, 98%): $[\alpha]_D=-12$ (*c* 4.1, $CHCl_3$); IR ($CHCl_3$) 3410, 2982, 2869, 1674, 1455, 1393, 1368, 1171, 1111 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.37–7.32 (m, 5H), 4.54 (s, 2H), 4.00 (m, 1H), 3.82 (m, 1H), 3.60 (m, 1H), 3.47–3.43 (m, 1H), 2.12–1.85 (m, 4H), 1.42 (s, 9H), 1.19 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.5, 137.2, 128.1, 127.6, 127.5, 79.6, 72.9 72.5, 70.3, 67.7, 58.6, 28.0, 27.8, 26.6, 26.4, 24.5; MS (ESI) *m/z* 372 $[M+Na]^+$, 388 $[M+K]^+$. Anal. Calcd for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94; N, 4.01; Found: C, 68.86; H, 9.03; N, 3.91.

3.1.6. (2*S*,5*R*)-1-*tert*-Butoxycarbonyl-2-hydroxymethyl-5-(1′-hydroxy-1′-methylethyl)-pyrrolidine (7). A suspension of **6** (864 mg, 2.48 mmol) and 10% Pd/C (132 mg) in EtOAc (25 mL) was hydrogenated at 1 atm for 5 h. The reaction mixture was filtered through a short pad of Celite, the filtrate was evaporated in vacuo and purified by flash chromatography on silica gel (heptane/EtOAc=1/1) to afford compound **7** (608 mg, 95%): $[\alpha]_D=+3.4$ (*c* 4.4, $CHCl_3$); IR ($CHCl_3$) 3376, 2981, 1676, 1393, 1368, 1255, 1169, 1112 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.35 (br s, 2H), 3.97–3.84 (m, 3H), 3.58 (dd, *J*=10.6, 3.2 Hz), 2.07–1.87 (m, 4H), 1.47 (s, 9H), 1.22 (s, 3H), 1.13 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.6, 80.0, 73.2, 67.2, 62.7, 60.4, 28.2, 27.9, 26.4, 26.1, 25.1; MS (ESI) *m/z* 260 $[M+H]^+$, 282 $[M+Na]^+$, 298 $[M+K]^+$, 519 $[2M+H]^+$, 541 $[2M+Na]^+$, 557 $[2M+K]^+$. Anal. Calcd for $C_{13}H_{25}NO_4$: C, 60.21; H, 9.72; N, 5.40; Found: C, 60.29; H, 9.96; N, 5.12.

3.1.7. (2*S*,5*R*)-1-*tert*-Butoxycarbonyl-2-hydroxycarbonyl-5-(1′-hydroxy-1′-methylethyl)-pyrrolidine (8). Jones reagent (2.67 M, 2.5 mL, 6.56 mmol) was added dropwise

to a solution of **7** (425 mg, 1.64 mmol) in acetone (16 mL) at 0°C. The mixture was stirred at 0°C for 8 h. Acetone was evaporated, and the residue was dissolved in aq. $NaHCO_3$, extracted with ether (2×), acidified with 10% $KHSO_4$ to pH=6, extracted with EtOAc. The EtOAc extracts were dried and evaporated to give the carboxylic acid **8** (410 mg, 92%), which was purified by column chromatography (silica gel, CH_2Cl_2/MeOH=95/5): $[\alpha]_D$=+18 (*c* 2.5, $CHCl_3$); IR ($CHCl_3$) 3343, 3175, 3026, 2983, 2937, 1701, 1682, 1476, 1455, 1369, 1168, 1145, 1116 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.33 (br s, 1H), 4.41–4.38 (m, 1H), 3.93 (m, 1H), 2.37–1.88 (m, 4H), 1.45 (s, 9H), 1.30 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 177.4, 155.9, 81.2, 74.5, 67.0, 61.0, 29.2, 28.5, 28.1, 27.0, 25.0; MS (ESI) *m/z* 274 $[M+H]^+$, 296 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12; Found: C, 57.25; H, 8.69; N, 4.91.

3.1.8. Lactone (9). $[\alpha]_D$=+14 (*c* 3.0, $CHCl_3$); IR ($CHCl_3$) 3027, 2985, 1740, 1698, 1423, 1392, 1370, 1332, 1310, 1282, 1153, 1125 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.70 (br s, 1H), 4.20 (br s, 1H), 2.16–1.89 (m, 4H), 1.47 (s, 12H), 1.39 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 168.7, 153.0, 85.6, 81.0, 58.7, 57.1, 29.2, 28.2, 27.2, 24.6, 22.4; MS (ESI) *m/z* 256 $[M+H]^+$, 278 $[M+Na]^+$, 533 $[2M+Na]^+$. Anal. Calcd for $C_{13}H_{21}NO_4$: C, 61.16; H, 8.29; N, 5.49; Found: C, 61.56; H, 8.42; N, 5.21.

3.1.9. (2*S*,5*R*)-1-*tert*-Butoxycarbonyl-2-methylaminocarbonyl-5-(1′-hydroxy-1′-methyl-ethyl)-pyrrolidine (10). To a solution of **8** (330 mg, 1.20 mmol) in acetonitrile (12 mL) was added DIEA (1.24 g, 1.7 mL, 9.6 mmol), $MeNH_2$·HCl (194 mg, 2.88 mmol) and TBTU (694 mg, 2.16 mmol). The reaction mixture was stirred at room temperature for 36 h, and partitioned between brine and EtOAc. The aqueous layer was extracted with EtOAc (3×). The organic extracts were washed with 0.1 M HCl, water, 5% $NaHCO_3$ and brine, dried and evaporated. Flash column chromatography on silica gel (heptane/EtOAc=1/3 then EtOAc) afforded compound **10** (257 mg, 75%): $[\alpha]_D$=+26 (*c* 3.5, $CHCl_3$); IR ($CHCl_3$) 3453, 3344, 2982, 2936, 1673, 1539, 1393, 1378, 1369, 1169, 1149 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.25 (t, *J*=8.3 Hz, 1H), 3.89, (d, *J*=7.8 Hz, 1H), 2.82 (d, *J*=4.8 Hz, 3H), 2.29–1.81 (m, 4H), 1.45 (s, 9H), 1.29 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 176.0, 155.6, 80.5, 73.0, 66.8, 61.7, 29.6, 28.8, 28.2, 26.9, 26.5, 26.2; MS (ESI) *m/z* 287 $[M+H]^+$. Anal. Calcd for $C_{14}H_{26}N_2O_4$: C, 58.72; H, 9.15; N, 9.78; Found: C, 58.31; H, 9.32; N, 9.63.

3.1.10. (2*S*,5*R*)-2-Methylaminocarbonyl-5-(1′-hydroxy-1′-methylethyl)-pyrrolidine hydrochloride (11). A solution of **10** (149 mg, 0.52 mmol) in 1 M HCl–EtOAc was stirred at room temperature for 2 h. The solvent was removed in vacuo to give compound **11** (110 mg, 95%): $[\alpha]_D$=−39 (*c* 2.1, MeOH); IR (nujol) 3236, 2923, 1660, 1576, 1461, 1378, 1284 $cm^{-1;}$ ^1H NMR (300 MHz, CD_3OD) δ 4.34 (dd, *J*=10.0, 3.8 Hz, 1H), 3.63 (dd, *J*=11.0, 6.2 Hz, 1H), 2.81 (s, 3H), 2.51–2.37 (m, 1H), 2.16–1.99 (m, 2H), 1.94–1.79 (m, 1H), 1.35 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 170.4, 70.7, 69.0, 59.9, 31.1, 28.8, 26.8, 25.7; MS (ESI) *m/z* 187 $[M+H]^+$. HRMS Calcd for $C_9H_{19}N_2O_2$: 187.1446°; Found: 187.1459.

3.1.11. (2*S*,5*R*)-1-Acetyl-2-methylaminocarbonyl-5-(1′-hydroxy-1′-methylethyl)-pyrrolidine (12). A mixture of **11** (110 mg, 0.50 mmol), Et_3N (152 mg, 210 μL, 1.5 mmol), AC_2O (102 mg, 95 μL, 1.0 mmol) in dichloromethane was stirred at rt for 48 h, then evaporated to dryness. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH=95/5) to give **12** (97 mg, 85%) as white solid: $[\alpha]_D$=+50.6 (*c* 2.0, MeOH); IR (nujol) 3452, 3331, 3062, 3015, 1667, 1632, 1538, 1403, 1207, 1046 $cm^{-1;}$ ^1H NMR (250 MHz, $CDCl_3$) δ 7.78 (br q, *J*=4.3 Hz, 0.5 H), 7.48 (br q, *J*=4.7 Hz, 0.5 H), 5.70 (br s, 0.5 H), 5.28 (br s, 0.5 H), 4.50 (t, *J*=8.8 Hz, 0.5 H), 4.36 (t, *J*=8.1 Hz, 0.5 H), 4.28 (br d, *J*=6.4 Hz, 0.5 H), 3.83 (d, *J*=7.7 Hz, 0.5 H), 2.82 (d, *J*=4.7 Hz, 0.5 H), 2.78 (d, *J*=4.7 Hz, 0.5 H), 2.42–1.78 (m, 4 H), 2.14 (s, 0.5 H), 2.04 (s, 0.5 H), 1.32, 1.28, 1.23, 1.21 (4S, 6H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.4, 174.8, 172.3, 171.6, 73.5, 72.7, 68.5, 66.7, 63.0, 61.1, 31.0, 29.2, 28.6, 28.3, 27.6, 27.1, 26.4, 26.3, 22.8, 22.7; MS (CI) *m/z* 229 $[M+H]^+$. HRMS Calcd for $C_{11}H_{21}N_2O_3$: 229.1552; Found: 229.1525.

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