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Synthesis of conformationally constrained analogues of RGD tripeptide

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Abstract—Convergent syntheses of *N*-functionalized (3*S*,4*S*)-3-amino-4-vinyl-piperidin-2-one **10**, *trans*-3-substituted proline **18** and (3*S*)-3-amino-piperidin-2-one **28** are developed. By incorporating these building blocks to an appropriate position, conformationally constrained analogues of RGD tripeptide (Arg-Gly-Asp) **2**, **3** and **4** are designed and synthesized.

1. Introduction

Integrin receptors consist of an α and a β subunits that are non-covalently linked and many of these receptors bind to their natural ligands through an RGD (Arg-Gly-Asp) (**1**) sequence (Fig. 1).[1–3] Among the integrin receptors discovered so far, two have emerged as being of particular interest and have been extensively studied.[4] These are the fibrinogen receptor, $\alpha_{IIb}\beta_3$, and the vitronectin receptor, $\alpha_v\beta_3$. The fibrinogen receptor $\alpha_{IIb}\beta_3$ is important for platelet aggregation, since the final common step in platelet aggregation is the binding of fibrinogen to its glycoprotein IIb/IIIa (GPIIb/IIIa, integrin $\alpha_{IIb}\beta_3$) receptor, which is located on the surface of activated platelets.[5,6] The development of $\alpha_{IIb}\beta_3$ antagonists has been one of the main focuses in antithrombotic research over the last decade.[7] The vitronectin receptor ($\alpha_v\beta_3$) is involved in a number of biological processes such as angiogenesis and adhesion of osteoclasts to the bone matrix. Antagonists to this receptor could be useful in the treatment of osteoporosis, diabetic retinopathy and cancer.[8]

The strong evidence has also been provided that $\alpha_v\beta_3$ receptor and its ligand fibronectin play critical roles in angiogenesis, resulting in tumour growth in vivo.[9] Importantly, antibodies, peptide and non-peptide antagonists of $\alpha_5\beta_1$ have been shown to block angiogenesis induced by several growth factors in both chicken embryo and murine models. Non-peptide $\alpha_5\beta_1$ antagonists could thus have a therapeutic application as either angiogenesis-blocking compounds or tumour-targeting molecules.[9] A significant number of drug candidates have been designed based on the RGD (Arg-Gly-Asp) sequence, which is a key recognition motif of integrin receptors.[9]

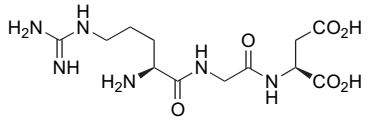

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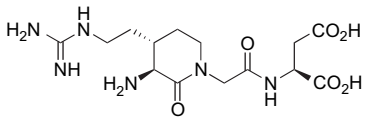
Figure 1. The RGD motif which is essential for many binding sites of naturally occurring integrin ligands.

Many groups have developed several potent compounds by mimicking the RGD sequence that possesses vectors of the pharmacophore: a basic functional group that mimics the side chain of arginine residue and a carboxylic group that represents the aspartyl side chain of C-terminal amino acids.[10–14] Recently, we have developed a method to induce structural constraint in amino acids such as, lysine, arginine, glutamic acid[15a] and aspartic acid.[15b] This was demonstrated in the synthesis of conformationally constrained analogues of the tetrapeptide AcSDKP.[15c] In this paper we wish to report further application of this method in the synthesis of three conformationally constrained analogues of RGD tripeptide **2**, **3** and **4** (Fig. 2). The purpose of our work is to investigate the effects of conformational constraint induced to



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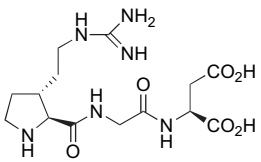

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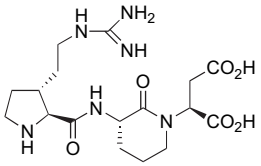

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Figure 2. Conformationally constrained RGD tripeptide analogues.

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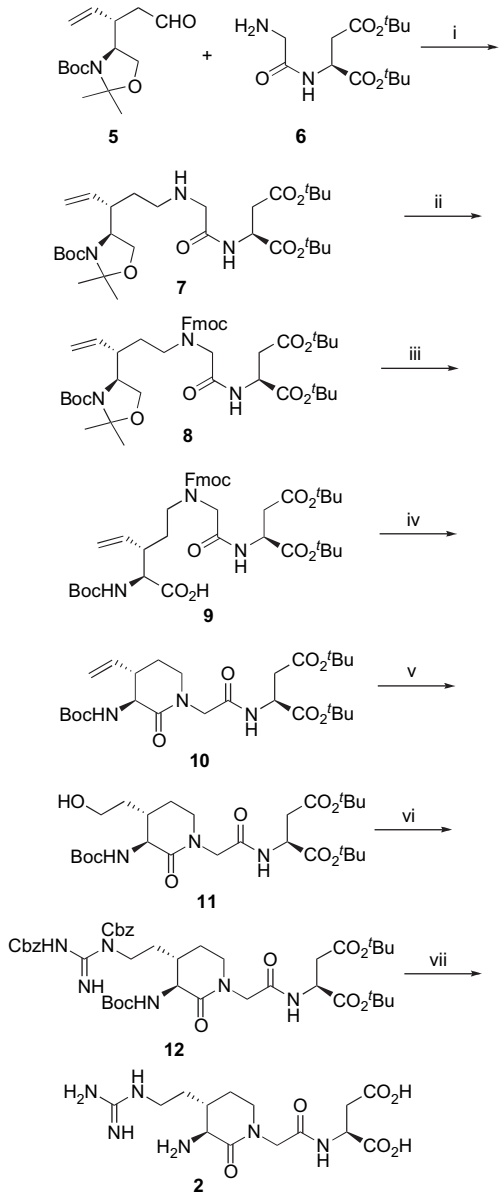
each one of the constituent amino acids with respect to biological activities of the tripeptide. We also present a new synthesis of *trans*-2,3-disubstituted pyrrolidine **18**, which constitutes key part of the constrained tripeptides.

2. Results and discussion

2.1. Synthesis of RGD tripeptide 2

Tripeptide **2** containing a 3-amino-piperidin-2-one unit is a conformationally constrained analogue of RGD. Indeed, tethering the β-carbon of the arginine and the amide nitrogen of the glycine by an ethylene unit would lead to targeted peptide **2**. The key intermediate 4-vinyl-3-amino-piperidin-2-one **10** was synthesized based on the methods developed in our laboratory (Scheme 1).[15b,c]

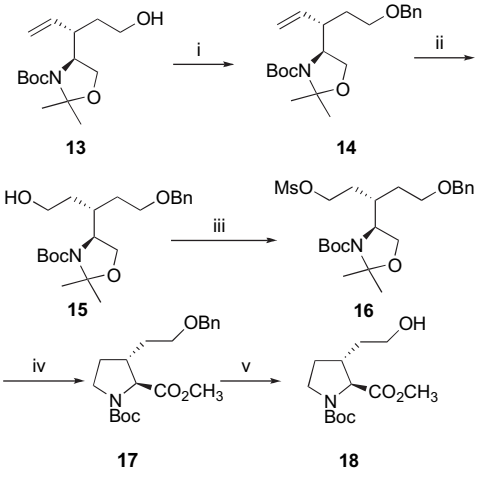


Scheme 1. Synthesis of RGD analogue **2**. *Reagents and conditions*: (i) $NaBH(OAc)_3$, THF, room temperature, 15 h, 73%; (ii) Fmoc-Cl, 0.5 M Na_2CO_3, THF/H_2O (3:1), 0 °C to room temperature, 3 h, 80%; (iii) Jones reagent, acetone, −50 °C, 15 min, room temperature, 3 h, 72%; (iv) (a) $(C_2H_5)_2NH$, THF, 0 °C, 15 min, room temperature, 6 h; (b) TBTU, HOBt, DIEA, DMF, room temperature, 24 h, 74%; (v) 9-BBN, THF, room temperature, 3 h; MeOH, H_2O, $NaBO_3\cdot 4H_2O$, 0 °C, then room temperature, 48 h, 61%; (vi) NH=C(NHCbz)$_2$, PPh_3, $(CH_3)_2CHO(C(=O)–N=N–C(=O)OCH(CH_3)_2$, THF, 0 °C, then room temperature, 48 h, 85%; (vii) (a) H_2, 10% Pd/C, MeOH, 3 h; (b) CH_2Cl_2/TFA (1:1), 0 °C, then room temperature, 4 h, 87%.

Reductive amination of aldehyde **5**[15c] with dipeptide **6** (sodium triacetoxyborohydride, THF) proceeded smoothly to provide amine **7** in 73% yield. N-protection of the secondary amine (FmocCl, THF/H_2O, Na_2CO_3) and subsequent Jones oxidation afforded acid **9**. Removal of *N*-Fmoc function (Et_2NH, THF) followed by lactamization (TBTU/HOBt/DIEA) provided desired 4-vinyl-3-amino-piperidin-2-one **10** in 74% yield. Hydroboration of **10** (9-BBN, THF) followed by oxidation of the alkylborane intermediate with hydrogen peroxide gave alcohol **11** in moderate yield (40%). However, when $NaBO_3\cdot 4H_2O$ was used as an oxidant,[16] alcohol **11** was isolated in 61% yield. Guanylation of alcohol **11** with dibenzyloxycarbonylguanidine under Mitsunobu conditions furnished the expected dibenzyloxycarbonylguanidine **12** in 85% yield. Removal of benzyloxycarbonyl group from **12** under catalytic hydrogenolysis conditions (H_2/10% Pd/C, MeOH) followed by simultaneous deprotection of *tert*-butyl ester and *tert*-butoxycarbonyl under acidic conditions (TFA/CH_2Cl_2, 1:1, room temperature, 4 h) afforded the desired conformationally constrained RGD tripeptide **2** in 87% yield. Tripeptide **2** was thus obtained in 13% overall yield starting from aldehyde **5**.

2.2. Synthesis of RGD tripeptide analogue 3

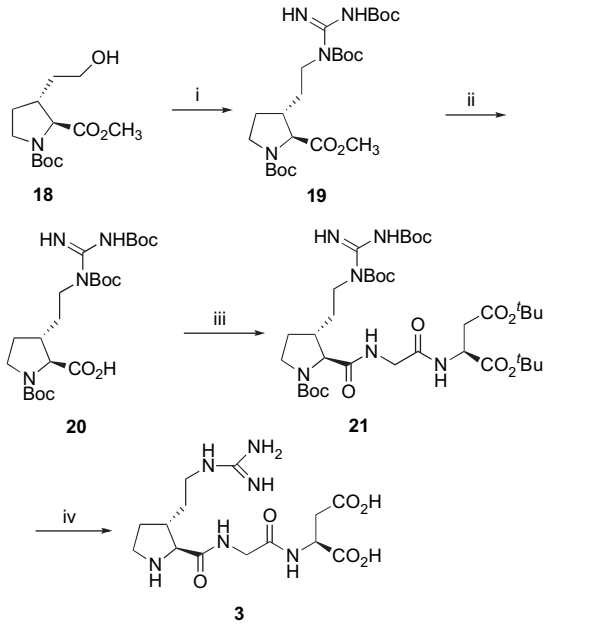
The conformationally constrained RGD tripeptide **3** was designed wherein the amide nitrogen and the β-carbon of arginine were connected via an ethylene linkage producing a more strained pyrrolidine unit. The requisite *trans*-2,3-disubstituted pyrrolidine was synthesized as shown in Scheme 2.



Scheme 2. Synthesis of 2,3 *trans*-disubstituted pyrrolidine. *Reagents and conditions*: (i) NaH, BnBr, *n*-Bu_4NI, THF, room temperature, 48 h, 75%; (ii) (a) 9-BBN, THF, room temperature, 24 h; (b) H_2O, 3 M NaOH, 30% H_2O_2, 0 °C, 5 min, then room temperature, 24 h, 90%; (iii) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min, then room temperature, 1 h; (iv) (a) Jones reagent, 0 °C, 4 h; (b) $TMSCHN_2$, toluene/MeOH (4:1), 0 °C, 30 min; (c) NaH, DMF, −25 °C, 1 h (58% from compound **15**); (v) H_2, 10% Pd/C, EtOAc, room temperature, 4 h, 86%.

Benzylation of alcohol **13**[17] (NaH, BnBr, THF, *n*-Bu_4NI) gave benzyl ether **14** in 75% yield. Hydroboration of the latter compound followed by oxidation (9-BBN, THF; 3 M NaOH, 33% H_2O_2) proceeded smoothly to afford alcohol **15** in 90% yield. Mesylation of alcohol **15** afforded mesylate in quantitative yield that was subsequently treated with Jones reagent and TMS/diazomethane to afford the corresponding amino ester. The crude methyl ester was cyclized under basic conditions (NaH, DMF, −25 °C) to provide substituted pyrrolidine **17** in 58% yield (for four steps). Debenzylation of **17** (H_2/10% Pd/C, EtOAc) provided the alcohol **18**. Thus starting from alcohol **13**, *trans*-3-substituted proline **18** was synthesized as a single isomer in six steps.

After successfully developing the methodology for 2,3-*trans*-disubstituted proline, we became interested in the incorporation of this unit in RGD framework (Scheme 3). Accordingly guanylation of alcohol **18** with di-*tert*-butoxycarbonyl guanidine under Mitsunobu conditions produced guanidine ester **19** in 85% yield. Hydrolysis of the latter (LiOH, dioxane/H_2O, 1:1, room temperature) afforded acid **20**. Coupling of acid **20** with L-Gly-Asp(O^tBu)$_2$ under standard conditions (EDC/HOBt) afforded tripeptide **21** in 86% yield. Finally simultaneous deprotection of *tert*-butyl esters and *N*-*tert*-butoxycarbonyl by TFA/CH_2Cl_2 (1:1) afforded tripeptide **3** in 83% yield. Thus tripeptide **3** was prepared in 34% yield starting from alcohol **18**.

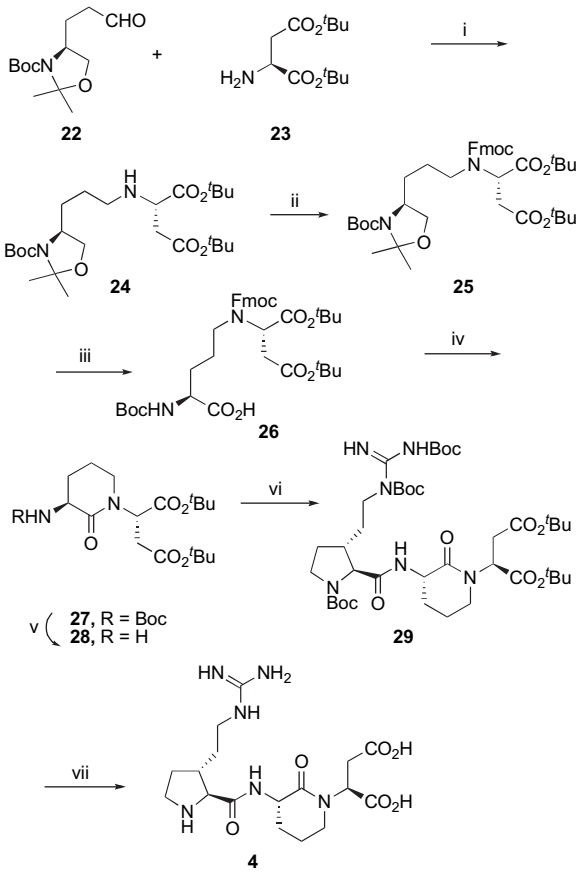


Scheme 3. Synthesis of RGD analogue **3**. *Reagents and conditions*: (i) NH=C(NHBoc)$_2$, PPh_3, $C_2H_5OC(=O)$–N=N–$C(=O)OC_2H_5$, THF, 0 °C, 5 min, then room temperature, 48 h, 64%; (ii) LiOH, dioxane/H_2O, room temperature, 7 h, 75%; (iii) L-Gly-Asp(O^tBu)$_2$, EDC, HOBt, CH_2Cl_2, room temperature, 24 h, 86%; (iv) TFA/CH_2Cl_2 (1:1), 0 °C, 5 min, then room temperature, 4 h, 83%.

2.3. Synthesis of RGD tripeptide analogue 4

Conformationally constrained RGD molecule **4** incorporating piperidin-2-one and pyrrolidine units in RGD framework was the next target. The design principle is as follows: connecting the nitrogen atom of the aspartic acid and the α-carbon of the glycine via a propylene linkage provides a piperidinone unit, while tethering the amide nitrogen and the β-carbon of the arginine via an ethylene linkage affords a pyrrolidine unit. The synthesis of RGD **4** required acid **20** and 3-amino-piperidin-2-one **28**. The latter compound was prepared by following the same sequence as described for 4-vinyl-3-amino-piperidin-2-one **10** (Scheme 1). The reductive amination of known aldehyde **22**[18] with L-Asp(O^tBu)$_2$ gave amine **24** in good yield. The protection of the secondary amine by Fmoc function followed by Jones oxidation gave acid **26** in good yield. Removal of Fmoc group with diethyl amine and subsequent intramolecular amidation (TBTU, HOBt, $^iPr_2NEt$) provided desired lactam **28** in 90% yield. Selective removal of the *N*-Boc function without affecting the *tert*-butyl esters was realized under mild acidic conditions (3 M HCl/EtOAc at −50 °C for 2 h and 0 °C for 1 h). Coupling reaction of resulting amine **28** with acid **20** under standard conditions (EDC, HOBt) afforded tripeptide **29** in 63% yield. Simultaneous removal of *tert*-butyl esters and *N*-*tert*-butoxycarbonyl group from **29** under acidic condition (TFA/CH_2Cl_2, 1:1) furnished tripeptide **4** in 89% yield. Tripeptide analogue **4** was thus prepared in 29% yield starting from aldehyde **22**.

Most of the synthetic intermediates described in Schemes 1–4 contain a tertiary amide unit, therefore their ^1H NMR



Scheme 4. Synthesis of RGD analogue **4**. *Reagents and conditions*: (i) $NaBH(OAc)_3$, THF, room temperature, 15 h, 85%; (ii) Fmoc-Cl, 0.5 M Na_2CO_3, THF/H_2O (3:1), room temperature, 2 h, 83%; (iii) Jones reagent, acetone, 0 °C to room temperature, 3 h, 82%; (iv) (a) $(C_2H_5)_2NH$, THF, 0 °C, 15 min, then room temperature, 6 h; (b) TBTU, HOBt, DIEA, DMF, room temperature, 24 h, 90%; (v) 3 M HCl/EtOAc, −50 °C, 2 h, then 0 °C, 1 h, 68%; (vi) acid **20**, EDC, HOBt, CH_2Cl_2, room temperature, 24 h, 63%; (viii) TFA/CH_2Cl_2, 0 °C, 5 min, then room temperature, 4 h, 89%.

spectra recorded at room temperature displayed two sets of peaks due to the presence of rotamers. However, one-single conformer was detected within NMR time scale for final products **2**, **3** and **4**.

3. Conclusion

In summary, we successfully developed the syntheses of *N*-functionalized (3*S*,4*S*)-3-amino-4-vinyl-piperidin-2-one **10**, (3*S*)-3-amino-piperidin-2-one **28** and *trans*-3-substituted proline **18**. Synthesis of conformationally constrained analogues of RGD tripeptide **2**, **3** and **4** has been realized by incorporating these building blocks at appropriate positions. It may be of great interest to furnish cyclic analogues of RGD[19] combining the present method. Biological activities of compounds **2**, **3** and **4** are currently under investigation.

4. Experimental section

4.1. General

4.1.1. (2*S*,3″*S*,4‴*S*)-2-{2′-[3″-(3‴-*tert*-Butoxycarbonyl-2‴,2‴-dimethyl-oxazolidin-4‴-yl)-pent-4″-enylamino]-acetylamino}-succinic acid di-*tert*-butyl ester (7). Aldehyde **5** (219 mg, 0.77 mmol) and amine **6** (234 mg, 0.77 mmol) were mixed in dry THF (25 mL) and then treated with sodium triacetoxyborohydride (229 mg, 1.10 mmol). The reaction mixture was stirred at room temperature under argon for 15 h. The reaction was quenched by the addition of aqueous saturated $NaHCO_3$ and the reaction mixture was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried over Na_2SO_4. The crude oil obtained was purified by flash column chromatography on silica gel with CH_2Cl_2/MeOH (20:1) to give amine **7** as a yellow oil (320 mg, 73%, two rotamers): $[\alpha]_D$ +32 (*c* 1.10, $CHCl_3$); IR (neat) ν 3330, 2977, 2933, 1730, 1691, 1513, 1478, 1455, 1388, 1365, 1250, 1148, 1087, 1050, 994, 914, 845 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J=8.2 Hz, 1H), 5.79–5.51 (m, 1H), 5.17–4.94 (m, 2H), 4.77–4.58 (m, 1H), 4.01–3.72 (m, 3H), 3.26 (s, 2H), 2.88 (dd, J=4.5, 12.2 Hz, 1H), 2.76–2.29 (m, 4H), 2.13 (br s, 1H), 1.75–1.39 (m, 8H), 1.47 (s, 9H), 1.46 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 169.9, 169.7, 152.8, 152.2, 139.1, 117.4, 116.9, 94.1, 93.6, 82.0, 81.3, 80.0, 79.7, 65.4, 65.2, 60.8, 52.3, 48.6, 48.4, 48.2, 45.8, 45.5, 37.7, 30.1, 28.4, 28.1, 27.9, 27.1, 26.4, 24.4, 22.8; MS (ESI) *m*/*z* 570 $[M+H]^+$, 592 $[M+Na]^+$; HRMS calcd for $C_{29}H_{52}N_3O_8$ (M+H) 570.3754, found 570.3740. Anal. Calcd for $C_{29}H_{51}N_3O_8$: C, 61.14; H, 9.02; N, 7.38. Found: C, 60.67; H, 9.37; N, 7.12.

4.1.2. (2*S*,3″*S*,4‴*S*)-2-{2′-[[3″-(3‴-*tert*-Butoxycarbonyl-2‴,2‴-dimethyl-oxazolidin-4‴-yl)-pent-4″-enyl]-(9*H*-fluoren-9-ylmethoxycarbonyl)-amino]-acetylamino}-succinic acid di-*tert*-butyl ester (8). To a cooled (0 °C) solution of amine **7** (300 mg, 0.53 mmol) and 0.5 M Na_2CO_3 (5 mL) in THF/water (20 mL, 3:1) was added dropwise a solution of 9-fluorenylmethyl chloroformate (150 mg, 0.58 mmol) in THF (10 mL) for 10 min and the resulting mixture was vigorously stirred at room temperature for 3 h. The reaction mixture was diluted with water, extracted with ethyl acetate and the combined organic layers were washed with brine and dried over Na_2SO_4. The residue obtained upon concentration was purified by flash column chromatography on silica gel with heptane/EtOAc (4:1) to afford compound **8** as white crystals (330 mg, 80%, two rotamers): mp 48–49 °C; $[\alpha]_D$ +35 (*c* 1.20, $CHCl_3$); IR (neat) ν 2974, 1691, 1449, 1385, 1362, 1247, 1143, 1083, 1051, 992, 911, 844 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.84–7.50 (m, 4H), 7.45–7.24 (m, 4H), 7.10–6.89 (m, 1H), 5.77–5.40 (m, 1H), 5.18–4.93 (m, 2H), 4.74–4.61 (m, 1H), 4.56–4.14 (m, 3H), 4.09–2.21 (m, 10H), 1.76–1.32 (m, 8H), 1.45 (s, 9H), 1.43 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 169.3, 168.6, 156.6, 155.6, 152.7, 152.1, 143.9, 143.8, 141.2, 138.4, 127.7, 127.1, 124.9, 120.0, 118.1, 117.4, 94.1, 93.6, 82.2, 81.5, 80.0, 79.8, 67.9, 67.8, 65.1, 65.1, 60.6, 51.3, 50.9, 48.9, 47.5, 47.2, 45.4, 45.0, 37.3, 28.4, 27.9, 27.9, 27.2, 27.1, 26.5, 24.3, 22.7; MS (ESI) *m*/*z* 814 $[M+Na]^+$; HRMS calcd for $C_{44}H_{61}N_3NaO_{10}$ (M+Na) 814.4255, found 814.4268. Anal. Calcd for $C_{44}H_{61}N_3O_{10}$: C, 66.73; H, 7.76; N, 5.31. Found: C, 66.51; H, 7.51; N, 5.11.

4.1.3. (2*S*,3″*S*,2‴*S*)-2-{2′-[[3″-(*tert*-Butoxycarbonyl-amino-carboxy-methyl)-pent-4″-enyl]-(9*H*-fluoren-9-yl-methoxycarbonyl)-amino]-acetylamino}-succinic acid di-*tert*-butyl ester (9). Jones reagent (2.67 M, 8.0 mL, 21.2 mmol) was added dropwise to a solution of **8** (6.00 g, 7.60 mmol) in acetone (100 mL) at −50 °C under Ar for 15 min. After stirring at room temperature for 3 h, the reaction was quenched by the addition of 2-propanol. The reaction mixture was stirred for 15 min, neutralized with saturated aqueous $NaHCO_3$ to pH 4–5 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried and evaporated. Flash column chromatography on silica gel with $CHCl_3$/EtOAc (4:1 then 2:1) afforded **9** as white crystals (4.15 g, 72%, two rotamers): mp 78–81 °C; $[\alpha]_D$ +34 (*c* 1.10, $CHCl_3$); IR (neat) ν 2976, 1708, 1514, 1477, 1450, 1392, 1366, 1223, 1148, 1058, 924, 844 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.79 (br s, 1H), 7.87–7.22 (m, 8H), 7.15 (br d, J=8.0 Hz, 0.5H), 7.04 (br d, J=11.0 Hz, 0.5H), 5.79–5.34 (m, 1H), 5.35–4.97 (m, 3H), 4.84–2.26 (m, 12H), 1.93–1.44 (m, 2H), 1.43, 1.42, 1.38, 1.35 (4s, 27H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 170.1, 169.3, 168.9, 156.7, 155.9, 143.9, 143.8, 141.3, 135.6, 127.7, 127.2, 124.9, 120.0, 119.0, 82.4, 81.7, 80.0, 68.2, 67.8, 56.7, 56.6, 50.7, 50.5, 49.1, 47.2, 46.8, 46.5, 43.9, 37.4, 28.7, 28.3, 27.9, 27.8; MS (ESI) *m*/*z* 788 $[M+Na]^+$; HRMS calcd for $C_{41}H_{55}N_3NaO_{11}$ (M+Na) 788.3734, found 788.3741. Anal. Calcd for $C_{41}H_{55}N_3O_{11}$: C, 64.40; H, 7.24; N, 5.49. Found: C, 63.75; H, 7.29; N, 5.38.

4.1.4. (2*S*,3″*S*,4″*S*)-2-[2′-(3″-*tert*-Butoxycarbonylamino-2″-oxo-4″-vinyl-piperidin-1″-yl)-acetylamino]-succinic acid di-*tert*-butyl ester (10). To a solution of acid **9** (3.30 g, 4.30 mmol) in dry THF (100 mL) at 0 °C was added dropwise diethyl amine (30 mL) under argon for 15 min and the reaction mixture was stirred at room temperature for 6 h. The solvent was evaporated to dryness. To a solution of the oil obtained above in dry DMF (60 mL) were added TBTU (4.15 g, 12.92 mmol), HOBt (1.75 g, 12.92 mmol) and DIEA (2.23 g, 3 mL, 17.3 mmol) and the reaction mixture was stirred at room temperature for 24 h. The reaction was quenched by the addition of water, and the reaction mixture was extracted with ethyl acetate. The organic layers

were washed with 1 N $KHSO_4$, H_2O, saturated $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated. Flash column chromatography on silica gel with heptane/ethyl acetate (4:1 and then 2:1) afforded lactam **10** (1.670 g, 74%): mp 54–56 °C; $[\alpha]_D$ −16 (*c* 1.00, $CHCl_3$); IR (neat) ν 3320, 2976, 1729, 1643, 1530, 1454, 1391, 1365, 1247, 1148, 990, 916, 845 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39–7.24 (br s, 1H), 5.88–5.54 (m, 2H), 5.17–5.01 (m, 2H), 4.75–4.46 (m, 2H), 3.85–3.25 (m, 4H), 2.88–2.54 (m, 3H), 2.09–1.78 (m, 2H), 1.51–1.37 (m, 27H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 169.6, 168.2, 156.1, 138.7, 116.0, 82.0, 81.3, 79.6, 56.0, 51.3, 49.6, 47.8, 43.1, 37.0, 28.4, 28.1, 27.9, 27.4; MS (ESI) *m/z* 548 $[M+Na]^+$; HRMS calcd for $C_{26}H_{43}N_3O_8Na$ (M+Na) 548.2948, found 548.2945. Anal. Calcd for $C_{26}H_{43}N_3O_8$: C, 59.41; H, 8.25; N, 7.99. Found: C, 59.31; H, 8.19; N, 7.94.

4.1.5. (2*S*,3″*S*,4″*S*)-2-{2′-[3″-*tert*-Butoxycarbonylamino-4″-(2‴-hydroxy-ethyl)-2″-oxo-piperidin-1″-yl]-acetylamino}-succinic acid di-*tert*-butyl ester (11). To a stirred solution of lactam **10** (150 mg, 0.28 mmol) in dry THF (5 mL) was added a 0.5 M solution of 9-BBN in THF (1.75 mL, 0.87 mmol). The reaction mixture was stirred at room temperature for 3 h, then cooled to 0 °C, whereupon methanol (3.20 mL) was added dropwise. When gas evolution had ceased, water (4.2 mL) was added, followed by the addition of $NaBO_3 \cdot 4H_2O$ (1.20 g, 7.80 mmol). The ice bath was removed and the resulting reaction mixture was stirred at room temperature for 48 h. The reaction mixture was extracted with ethyl acetate and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography with CH_2Cl_2/EtOAc (9:1), then CH_2Cl_2/MeOH/AcOH (90 mL:10 mL:two drops) to yield corresponding alcohol **11** (95 mg, 61%): mp 51–52 °C; $[\alpha]_D$ −12 (*c* 0.50, $CHCl_3$); IR (neat) ν 3317, 2976, 2931, 1729, 1642, 1524, 1455, 1392, 1365, 1248, 1149, 1046, 992, 939, 888, 845 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, *J*=7.8 Hz, 1H), 5.65 (br s, 1H), 4.66 (dt, *J*=7.9, 5.2 Hz, 1H), 4.46 (d, *J*=15.8 Hz, 1H), 4.04–3.59 (m, 4H), 3.56–3.27 (m, 2H), 3.23–2.90 (m, 1H), 2.83 (dd, *J*=5.7, 11.0 Hz, 1H), 2.74 (dd, *J*=4.7, 12.0 Hz, 1H), 2.26–1.31 (m, 5H), 1.45, 1.44 (two br s, 27H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 170.2, 169.6, 168.2, 156.7, 82.3, 81.6, 79.9, 59.9, 56.5, 51.3, 49.5, 47.9, 37.1, 36.0, 35.1, 28.4, 28.1, 27.9, 26.7; MS (ESI) *m/z* 566 $[M+Na]^+$; HRMS calcd for $C_{26}H_{45}N_3O_9Na$ (M+Na) 566.3054, found 566.3046.

4.1.6. (2*S*,3″*S*,4″*S*)-2-{2′-[3″-*tert*-Butoxycarbonylamino-4″-(2‴-*N*,*N*′-bis(benzyloxycarbonyl)-guanidino-ethyl)-2″-oxo-piperidin-1″-yl]-acetylamino}-succinic acid di-*tert*-butyl ester (12). To a solution of *N*-*N*′-bis(benzyloxycarbonyl)guanidine (156 mg, 0.48 mmol) and PPh_3 (93 mg, 0.36 mmol) in dry THF (10 mL) under argon was added alcohol **11** (130 mg, 0.24 mmol). The reaction mixture was cooled to 0 °C, and diisopropyl azodicarboxylate (72 mg, 0.07 mL, 0.36 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 48 h. Several drops of water was added and the solvent was evaporated under reduced pressure. Flash column chromatography of the crude residue on silica gel with heptane/EtOAc (2:1), then toluene/EtOAc (1:1) to give compound **12** as white crystals (170 mg, 85%): mp 68–69 °C; $[\alpha]_D$ −7 (*c* 0.50, $CHCl_3$); IR (neat) ν 3383, 2976, 2930, 1716, 1649, 1608, 1510, 1453, 1408, 1366, 1241, 1199, 1150, 1099, 1005, 938, 909, 846, 807 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$,) δ 9.36 (br s, 1H), 9.22 (br s, 1H), 7.44–7.13 (m, 11H), 5.61 (d, *J*=6.8 Hz, 1H), 5.34–5.02 (m, 4H), 4.73–4.58 (m, 1H), 4.43 (d, *J*=15.9 Hz, 1H), 4.14–3.86 (m, 2H), 3.74–3.59 (m, 2H), 3.43–3.08 (m, 2H), 2.87–2.68 (m, 2H), 2.33–1.82 (m, 3H), 1.69–1.48 (m, 2H), 1.44 (s, 9H), 1.41 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 169.5, 168.1, 163.7, 160.3, 156.4, 155.7, 136.8, 134.6, 128.9, 128.9, 128.5, 128.4, 127.9, 82.0, 81.3, 79.7, 69.0, 66.9, 56.3, 51.3, 49.5, 47.8, 42.2, 37.1, 36.2, 31.8, 28.3, 28.0, 27.9, 26.8; MS (ESI) *m/z* 875 $[M+Na]^+$; HRMS calcd for $C_{43}H_{60}N_6O_{12}Na$ (M+Na) 875.4167, found 875.4163.

4.1.7. (2*S*,3″*S*,4″*R*)-2-{2′-[3″-Amino-4″-(2‴-guanidino-ethyl)-2″-oxo-piperidin-1″-yl]-acetylamino}-succinic acid (2). A suspension of bis-benzyloxy carbonyl tripeptide **12** (100 mg, 0.11 mmol) and 10% Pd/C (50 mg) in methanol was hydrogenated at room temperature for 3 h. The catalyst was removed by filtration through a pad of Celite and washed with methanol. The filtrate was concentrated under reduced pressure. To the crude product obtained after hydrogenation was added TFA/CH_2Cl_2 (10 mL, 1:1) dropwise at 0 °C and then the reaction mixture was stirred at room temperature for 4 h. The volatile substance was removed to give compound **2** as a white solid (38 mg, 87%): mp 93–96 °C; $[\alpha]_D$ +27 (*c* 0.25, MeOH); IR (neat) ν 3345, 3185, 2930, 1658, 1650, 1633, 1537, 1504, 1427, 1349, 1178, 1127, 986, 886, 836 cm^{-1}; ^1H NMR (300 MHz, CD_3OD,) δ 4.78 (t, *J*=5.8 Hz, 1H), 4.16 (s, 2H), 3.74 (d, *J*=10.8 Hz, 1H), 3.55–3.14 (m, 4H), 2.89–2.82 (m, 2H), 2.28–1.90 (m, 3H), 1.42–1.13 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.0, 173.8, 170.1, 167.6, 158.8, 55.9, 50.8, 50.4, 48.7, 39.4, 36.9, 35.5, 32.0, 26.6; MS (ESI) *m/z* 373 $[M+H]^+$; HRMS calcd for $C_{14}H_{25}N_6O_6$ (M+H) 373.1836, found 373.1845.

4.1.8. (4*S*,1′*S*)-4-[1′-(2″-Benzyloxy-ethyl)-allyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (14). To a suspension of NaH (55–65% in mineral oil, 490 mg, 12.3 mmol, washed with pentane) in THF (25 mL) at 0 °C was added alcohol **13** (1.52 g, 5.33 mmol) in THF (20 mL). After being stirred for 30 min, benzyl bromide (1.46 mL, 12.6 mmol) and catalytic amount of nBu_4NI (20 mg) were added. The reaction mixture was stirred at room temperature under argon for 48 h. The reaction was quenched by the addition of water and the reaction mixture was extracted with ether. The combined organic extracts were washed with brine, dried and evaporated. Flash column chromatography on silica gel with heptane/ethyl acetate (4:1) afforded compound **14** (1.50 g, 75%, two rotamers): $[\alpha]_D$ +32 (*c* 0.50, $CHCl_3$); IR (neat) ν 2975, 2932, 2867, 1692, 1477, 1453, 1384, 1362, 1311, 1252, 1203, 1172, 1086, 1027, 994, 914, 847 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5H), 5.75–5.44 (m, 1H), 5.04–4.99 (m, 2H), 4.48 (d, *J*=12.0 Hz, 1H), 4.42 (d, *J*=12.0 Hz, 1H), 3.94–3.81 (m, 3H), 3.53–3.36 (m, 2H), 2.68–2.50 (m, 1H), 1.84–1.82 (m, 2H), 1.62, 1.56 (2S, 6H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.7, 152.2, 138.6, 138.4, 128.3, 127.6, 127.4, 117.8, 116.7, 94.1, 93.8, 79.8, 79.6, 72.8, 68.4, 68.3, 65.3, 60.8, 60.7, 44.5, 44.0, 30.2, 29.5, 28.5, 28.2, 27.1, 26.3, 24.5, 22.9;

MS (ESI) *m/z* 398 $[M+Na]^+$; HRMS calcd for $C_{22}H_{33}NO_4Na$ (M+Na) 398.2307, found 398.2307.

4.1.9. (4*S*,1′*R*)-4-[3′-Benzyloxy-1‴-(2″-hydroxy-ethyl)-propyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (15). To a stirred solution of **14** (1.50 g, 4.00 mmol) in dry THF (30 mL) was added a 0.5 M solution of 9-BBN in THF (23.40 mL, 12.30 mmol). The reaction mixture was stirred at room temperature for 24 h, then cooled to 0 °C, water (30 ml), 3 M NaOH (10.50 mL) and 33% H_2O_2 (4.00 mL) were added dropwise and the resulting reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then extracted with ethyl acetate and the combined organic extracts were washed with brine, dried and evaporated. Flash column chromatography with heptane/ethyl acetate (2:1) gave alcohol **15** (1.42 g, 90%, two rotamers): $[\alpha]_D$ +24 (*c* 0.60, $CHCl_3$); IR (neat) *ν* 3431, 2974, 2929, 2868, 1693, 1477, 1453, 1391, 1364, 1255, 1204, 1171, 1148, 1084, 1058, 1025, 947, 853, 807 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 7.40–7.20 (m, 5H), 4.51 (d, *J*=11.9 Hz, 1H), 4.45 (d, *J*=11.9 Hz, 1H), 3.95–3.77 (m, 3H), 3.74–3.59 (m, 2H), 3.51 (t, *J*=6.2 Hz, 2H), 2.27–2.13 (m, 1H), 1.98–1.85 (m, 1H), 1.75–1.11 (m, 8H), 1.45 (s, 9H), 0.94–0.77 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 153.1, 152.5, 138.4, 128.4, 127.6, 94.4, 93.7, 80.4, 79.8, 72.9, 69.3, 64.2, 60.9, 60.7, 60.1, 34.8, 34.1, 29.0, 27.8, 27.0, 26.2, 24.2, 22.7; MS (ESI) *m/z* 416 $[M+Na]^+$; HRMS calcd for $C_{22}H_{35}NO_5$ (M+Na) 416.2413, found 416.2403.

4.1.10. (2*S*,3*R*)-3-(2′-Benzyloxy-ethyl)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (17). To a solution of alcohol **15** (700 mg, 1.78 mmol) in CH_2Cl_2 (25 mL) at 0 °C were added Et_3N (0.32 mL, 2.31 mmol) and MsCl (0.16 mL, 2.13 mmol). After stirring at room temperature for 1 h, the reaction was quenched with water and the reaction mixture was extracted with CH_2Cl_2. The organic extracts were washed with 1 N $KHSO_4$, water, saturated $NaHCO_3$ solution and brine, then dried over Na_2SO_4 and evaporated under vacuum to give mesylate **16** (800 mg, 95%). Jones reagent (2.67 M, 2.5 mL, 6.54 mmol) was added to a solution of the crude mesylate **16** in acetone (20 mL) at 0 °C. After stirring at 0 °C for 4 h, the reaction was quenched by the addition of 2-propanol. The reaction mixture was then stirred at room temperature for 30 min and neutralized with saturated aqueous $NaHCO_3$ to pH 4–5 and extracted with ethyl acetate. The organic layers were washed with brine, dried and evaporated. The residue was dissolved in toluene/MeOH (20 mL, 4:1) and to this solution was added dropwise at 0 °C TMS/diazomethane (1.41 mL, 3.24 mmol) and the reaction mixture was stirred at that temperature for an additional 30 min. The solvents were evaporated under reduced pressure and the residue was dried under vacuum for several hours. To a suspension of NaH (55–65% in mineral oil, 178 mg, 7.12 mmol, washed with pentane) in DMF (10 mL) at −25 °C was added the crude methyl ester obtained above in dry DMF (15 mL). After being stirred at −25 °C for 1 h, the reaction was quenched by the addition of water and the reaction mixture was extracted with ether. The combined ether layers were washed with water and brine, dried and evaporated. Flash column chromatography on silica gel with heptane/ethyl acetate (4:1, then 2:1) afforded ester **17** (375 mg, 58% for four steps, two rotamers): $[\alpha]_D$ −18 (*c* 0.65, $CHCl_3$); IR (neat) *ν* 2972, 2929, 2866, 1745, 1702, 1697, 1693, 1477, 1453, 1434, 1392, 1364, 1272, 1253, 1225, 1196, 1163, 1132, 1103, 1026, 899, 862 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 7.41–7.23 (m, 5H), 4.52 (d, *J*=12.0 Hz, 1H), 4.46 (d, *J*=12.0 Hz, 1H), 3.99 (d, *J*=5.4 Hz, 0.4H), 3.88 (d, *J*=6.4 Hz, 0.6H), 3.72, 3.70 (2s, 3H), 3.63–3.38 (m, 4H), 2.48–2.32 (m, 1H), 2.13–1.82 (m, 2H), 1.76–1.51 (m, 2H), 1.45, 1.40 (2s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 173.5, 173.3, 154.4, 153.7, 138.3, 128.4, 127.6, 80.0, 79.9, 73.0, 68.2, 64.8, 64.2, 52.1, 51.9, 45.9, 45.7, 41.9, 40.7, 33.4, 33.3, 30.6, 30.1, 28.4, 28.3; MS (ESI) *m/z* 386 $[M+Na]^+$; HRMS calcd for $C_{20}H_{29}NO_5Na$ (M+Na) 386.1943, found 386.1984.

4.1.11. (2*S*,3*R*)-3-(2′-Hydroxy-ethyl)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (18). A suspension of **17** (2.50 g, 6.88 mmol) and 10% Pd/C (500 mg) in ethyl acetate (75 mL) was hydrogenated at 1 atm for 4 h. The reaction mixture was filtered through a short pad of Celite, the filtrate was evaporated and purified by flash column chromatography on silica gel with heptane/ethyl acetate (4:1 then 1:1) to give alcohol **18** (1.61 g, 86%, two rotamers): $[\alpha]_D$ −27 (*c* 0.50, $CHCl_3$); IR (neat) *ν* 3440, 2973, 2934, 2879, 1744, 1701, 1697, 1692, 1686, 1680, 1477, 1396, 1365, 1248, 1161, 1196, 1130, 1048, 1016, 968, 895, 857 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 3.99 (d, *J*=5.5 Hz, 0.5H), 3.89 (d, *J*=6.4 Hz, 0.5H), 3.73 (s, 3H), 3.71–3.37 (m, 4H), 2.90 (br s, 1H), 2.47–2.30 (m, 1H), 2.20–2.02 (m, 1H), 1.93–1.76 (m, 1H), 1.73–1.51 (m, 2H), 1.45, 1.40 (2s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 173.8, 173.5, 154.5, 153.8, 80.2, 80.0, 64.8, 64.3, 60.4, 52.2, 52.0, 45.9, 45.7, 41.7, 40.5, 36.1, 30.6, 30.2, 28.4, 28.3; MS (ESI) *m/z* 296 $[M+Na]^+$, 569 $[2M+Na]^+$; HRMS calcd for $C_{13}H_{23}NO_5Na$ (M+Na) 296.1474, found 296.1471.

4.1.12. (2*S*,3*R*)-3-(2′-*N*,*N*′-Bis(*tert*-butoxycarbonyl)-guanidino-ethyl)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (19). This compound was prepared according to the same procedure described for **12** starting from alcohol **18** (100 mg, 0.36 mmol), 1,3-bis(*tert*-butoxycarbonyl)guanidine (189 mg, 0.73 mmol) and diethyl azodicarboxylate (0.20 mL, 1.09 mmol). Flash column chromatography with heptane/EtOAc (4:1, then 1:1) gave **19** as an oil (120 mg, 64% yield, two rotamers): $[\alpha]_D$ −3 (*c* 0.57, $CHCl_3$); IR (neat) *ν* 3382, 2975, 1745, 1702, 1697, 1642, 1606, 1504, 1454, 1391, 1365, 1271, 1246, 1139, 1096, 980, 887, 852, 811 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 9.54–9.11 (br s, 2H), 4.05–3.85 (m, 3H), 3.73 (s, 3H), 3.68–3.40 (m, 2H), 2.27–2.10 (m, 2H), 1.95–1.81 (m, 1H), 1.77–1.60 (m, 2H), 1.53, 1.48, 1.46, 1.40 (4s, 27H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 173.4, 173.1, 163.7, 160.4, 154.8, 154.3, 153.7, 84.0, 79.9, 79.8, 78.7, 64.7, 64.3, 52.1, 51.9, 45.9, 45.7, 43.1, 42.4, 41.3, 32.5, 32.4, 30.1, 29.8, 28.4, 28.3, 28.1; MS (ESI) *m/z* 537 $[M+Na]^+$; HRMS calcd for $C_{24}H_{42}N_4O_8Na$(M+Na) 537.2900, found 537.2910.

4.1.13. (2*S*,3*R*)-3-(2′-*N*,*N*′-Bis(*tert*-butoxycarbonyl)-guanidino-ethyl)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester (20). A solution of **19** (100 mg, 0.19 mmol) in H_2O/dioxane (4 mL, 1:1) was treated with $LiOH\cdot H_2O$ (20 mg, 0.48 mmol), and stirred at room temperature for 7 h. The reaction mixture was evaporated to dryness and

the residue was dissolved in H_2O (10 mL), acidified with 1 N $KHSO_4$ to pH 4 and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. Column chromatography with CH_2Cl_2/EtOAc (1:1), then CH_2Cl_2/MeOH (50:1) afforded acid **20** (73 mg, 75%, two rotamers): mp 76–79 °C; $[\alpha]_D$ −16 (*c* 0.50, $CHCl_3$); IR (neat) ν 3381, 2976, 1706, 1606, 1507, 1390, 1365, 1272, 1246, 1139, 1098, 980, 887, 851, 811 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 5.25–4.88 (m, 2H), 4.10–3.90 (m, 2H), 3.86 (d, *J*=5.9 Hz, 1H), 3.62–3.52 (m, 1H), 3.50–3.38 (m, 1H), 2.31–2.16 (m, 2H), 1.98–1.88 (m, 1H), 1.78–1.63 (m, 2H), 1.58, 1.57 (2s, 9H), 1.49 (s, 9H), 1.48 (s, 3H), 1.44 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.5, 176.1, 164.4, 161.6, 156.2, 156.0, 155.9, 85.4, 81.6, 81.2, 79.9, 66.2, 65.6, 47.1, 46.9, 44.5, 44.4, 43.8, 42.9, 33.7, 31.2, 30.9, 28.9, 28.8, 28.7, 28.5; MS (ESI) *m*/*z* 523 $[M+Na]^+$; HRMS calcd for $C_{23}H_{40}N_4O_8Na$(M+Na) 523.2744, found 523.2740.

4.1.14. (2*S*,2″*S*,3″*S*)-2-(2′-{[1″-*tert*-Butoxycarbonyl-3″-(2‴-*N*,*N*′-bis(*tert*-butoxycarbonyl)-guanidino-ethyl)-pyrrolidine-2″-carbonyl]-amino}-acetylamino)-succinic acid di-*tert*-butyl ester (21). To a solution of acid **20** (115 mg, 0.23 mmol) and L-Gly–Asp ($O^tBu)_2$ (76 mg, 0.25 mmol) in CH_2Cl_2 (10 mL) were added EDC (52 mg, 0.27 mmol) and HOBt (36 mg, 0.27 mmol) and the reaction mixture was stirred at room temperature under Ar for 24 h. Water was added and the reaction mixture was extracted with CH_2Cl_2. The organic extracts were washed with 1 N $KHSO_4$, H_2O, saturated $NaHCO_3$, brine, successively, dried over Na_2SO_4 and evaporated under reduced pressure. Flash column chromatography with CH_2Cl_2/ethyl acetate (1:1), then CH_2Cl_2/MeOH (50:1) gave 155 mg of **21** (86%): mp 68–70 °C; $[\alpha]_D$ +32 (*c* 0.25, $CHCl_3$); IR (neat) ν 2976, 1672, 1608, 1392, 1365, 1248, 1141, 846 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 4.51–4.44 (m, 1H), 3.86–3.61 (m, 5H), 3.46–3.35 (m, 2H), 2.63 (dd, *J*=6.8, 9.6 Hz, 0.5H), 2.54 (d, *J*=5.2 Hz, 1H), 2.45 (dd, *J*=5.9, 10.4 Hz, 0.5H), 2.20–1.95 (m, 2H), 1.79–1.62 (m, 1H), 1.60–1.46 (m, 2H), 1.37, 1.30, 1.28, 1.24 (4s, 45H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.4, 175.1, 171.2, 171.0, 170.8, 170.3, 164.4, 161.6, 156.1, 155.9, 155.8, 85.2, 83.1, 83.0, 82.4, 82.2, 81.5, 81.3, 79.8, 67.3, 51.1, 50.9, 47.6, 47.0, 44.3, 43.9, 43.5, 43.3, 42.8, 38.4, 38.3, 33.1, 31.4, 30.3, 28.9, 28.7, 28.4, 28.3, 28.2; MS (ESI) *m*/*z* 807 $[M+Na]^+$; HRMS calcd for $C_{37}H_{64}N_6O_{12}Na$ (M+Na) 807.4480, found 807.4449.

4.1.15. (2*S*,2″*S*,3″*R*)-2-(2′-{[3″-(2‴-Guanidino-ethyl)-pyrrolidine-2″-carbonyl]-amino}-acetylamino)-succinic acid (3). To a solution of **21** (100 mg, 0.12 mmol) in CH_2Cl_2 (3 mL) was added TFA (3 mL) at 0 °C, then the reaction mixture was stirred at room temperature for 4 h. The solvents were evaporated and the residue was dried under vacuum to give compound **3** (52 mg, 83%): mp 84–86 °C; $[\alpha]_D$ +51 (*c* 0.26, MeOH); IR (neat) ν 3209, 3084, 1658, 1650, 1633, 1555, 1422, 1179, 1128, 1042, 837 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 4.73 (t, *J*=5.6 Hz, 1H), 4.13 (d, *J*=16.6 Hz, 1H), 3.93 (d, *J*=9.3 Hz, 1H), 3.85 (d, *J*=16.6 Hz, 1H), 3.47–3.27 (m, 4H), 2.88–2.77 (m, 2H), 2.46–2.35 (m, 1H), 2.34–2.20 (m, 1H), 2.13–2.02 (m, 1H), 1.84–1.66 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.2, 174.0, 171.0, 170.0, 158.7, 65.4, 50.4, 47.0, 43.3, 42.9, 40.8, 36.9, 32.3, 31.3; MS (ESI) *m*/*z* 373 $[M+H]^+$; HRMS calcd for $C_{14}H_{25}N_6O_6$ (M+H) 373.1836, found 373.1805.

4.1.16. (2*S*,4″*S*)-2-[3′-(3″-*tert*-Butoxycarbonyl-2″,2″-dimethyl-oxazolidin-4″-yl)-propylamino]-succinic acid di-*tert*-butyl ester (24). This compound was prepared according to the same procedure described for **7** starting from L-Asp($O^tBu)_2$ **23** (3.51 g, 14.33 mmol), aldehyde **22** (3.34 g, 13.03 mmol) and sodium triacetoxyborohydride (3.85 g, 18.24 mmol). Flash column chromatography with heptane/ethyl acetate (4:1 then 2:1) gave 5.37 g (85%, two rotamers) of **24** as a yellow oil: $[\alpha]_D$ +16 (*c* 0.50, $CHCl_3$); IR (neat) ν 2976, 1727, 1692, 1454, 1386, 1364, 1255, 1144, 1086, 942, 845, 805 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.97–3.68 (m, 3H), 3.48–3.39 (m, 1H), 2.79–2.39 (m, 4H), 1.47, 1.45 (2s, 27H), 1.89–1.24 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 170.1, 152.1, 151.8, 93.6, 93.1, 81.2, 80.6, 79.8, 79.3, 67.1, 66.7, 58.6, 57.6, 57.3, 48.0, 39.4, 31.5, 30.7, 28.5, 28.1, 27.6, 27.0, 26.8, 24.6, 23.3; MS (ESI) *m*/*z* 487 $[M+H]^+$, 509 $[M+Na]^+$; HRMS calcd for $C_{25}H_{47}N_2O_7$ (M+H) 487.3383, found 487.3395.

4.1.17. (2*S*,4″*S*)-2-[[3′-(3″-*tert*-Butoxycarbonyl-2″,2″-dimethyl-oxazolidin-4″-yl)-propyl]-(9*H*-fluoren-9-yl-methoxycarbonyl)-amino]-succinic acid di-*tert*-butyl ester (25). This compound was prepared according to the same procedure described for **8** starting from **24** (4.77 g, 9.81 mmol). Flash column chromatography with CH_2Cl_2/ethyl acetate (4:1 then 2:1) gave 5.80 g (83%, two rotamers) of **25** as white foam: $[\alpha]_D$ −14 (*c* 0.62, $CHCl_3$); IR (neat) ν 2976, 2932, 1727, 1692, 1477, 1451, 1421, 1388, 1364, 1246, 1208, 1146, 1081, 1013, 943, 919, 844, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J*=7.1 Hz, 2H), 7.67–7.51 (m, 2H), 7.45–7.23 (m, 4H), 4.81–4.15 (m, 4H), 3.98–3.62 (m, 3H), 3.54–2.21 (m, 4H), 1.47, 1.43, 1.40 (3s, 27H), 1.75–1.16 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.1, 169.1, 155.7, 155.5, 152.1, 151.7, 143.9, 141.3, 127.7, 127.1, 125.1, 125.0, 124.7, 120.0, 93.7, 93.2, 82.0, 81.7, 81.0, 80.8, 80.0, 79.5, 67.5, 67.1, 66.8, 66.7, 66.5, 58.8, 57.8, 57.5, 57.1, 48.9, 47.4, 47.3, 37.0, 36.5, 31.0, 30.1, 28.5, 28.1, 27.9, 27.7, 26.9, 25.9, 25.3, 24.6, 23.2; MS (ESI) *m*/*z* 731 $[M+Na]^+$; HRMS calcd for $C_{40}H_{56}N_2NaO_9$ (M+Na) 731.3884, found 731.3896.

4.1.18. (2*S*,4′*S*)-2-[(4′-*tert*-Butoxycarbonylamino-4′-carboxy-butyl)-(9*H*-fluoren-9-ylmethoxycarbonyl)-amino]-succinic acid di-*tert*-butyl ester acid (26). This compound was prepared according to the same procedure described for **9** starting from **25** (5.10 g, 7.20 mmol). Flash column chromatography with heptane/ethyl acetate (4:1 then 2:1) gave 4.03 g (82%, two rotamers) of **26** as white crystals: mp 56–57 °C; $[\alpha]_D$ −28 (*c* 0.55, $CHCl_3$); IR (neat) ν 2975, 1698, 1478, 1450, 1422, 1392, 1366, 1246, 1148, 1014, 844 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.05 (br s, 1H), 7.81–7.69 (m, 2H), 7.64–7.50 (m, 2H), 7.43–7.25 (m, 4H), 5.40 (d, *J*=6.6 Hz, 0.5H), 5.24 (d, *J*=7.5 Hz, 0.5H), 4.80–4.65 (m, 0.5H), 4.63–4.40 (m, 1.5H), 4.38–4.01 (m, 3H), 3.54–2.21 (m, 4H), 1.47, 1.44, 1.41, 1.39 (4s, 27H), 1.95–1.13 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 176.1, 170.8, 170.3, 169.1, 156.1, 155.8, 155.6, 143.9, 143.7, 141.4, 127.7, 127.1, 125.1, 124.9, 124.6, 120.0, 82.2, 82.0, 81.2, 81.0, 79.9, 67.7, 66.9, 58.6, 57.8, 53.2, 48.3, 47.4,

47.2, 36.8, 36.3, 29.5, 29.1, 28.3, 28.1, 27.9, 24.9, 24.7; MS (ESI) *m/z* 705 $[M+Na]^+$; HRMS calcd for $C_{37}H_{50}N_2NaO_{10}$ (M+Na) 705.3363, found 705.3375.

4.1.19. (2*S*,3′*S*)-2-(3′-*tert*-Butoxycarbonylamino-2′-oxo-piperidin-1-yl)-succinic acid di-*tert*-butyl ester (27). This compound was prepared according to the same procedure described for **10** starting from acid **26** (3.76 g, 5.52 mmol). Flash column chromatography with CH_2Cl_2 then CH_2Cl_2/ethyl acetate (4:1) gave **27** as an oil (2.24 g, 90%): $[\alpha]_D$ +1.8 (*c* 0.54, $CHCl_3$); IR (neat) *ν* 2975, 2932, 1720, 1714, 1658, 1650, 1483, 1391, 1364, 1327, 1285, 1247, 1147, 1050, 946, 873, 845 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 5.53 (br s, 1H), 5.01 (t, *J*=6.2 Hz, 1H), 4.10–3.96 (m, 1H), 3.43–3.29 (m, 2H), 2.95 (dd, *J*=5.8, 10.3 Hz, 1H), 2.65 (dd, *J*=8.9, 7.1 Hz, 1H), 2.56–2.34 (m, 1H), 2.04–1.84 (m, 2H), 1.69–1.51 (m, 1H), 1.46, 1.44, 1.44 (3s, 27H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 170.2, 169.8, 168.6, 155.8, 82.2, 81.1, 79.4, 56.2, 51.6, 45.0, 35.7, 28.4, 28.0, 27.1, 20.5; MS (ESI) *m/z* 465 $[M+Na]^+$; HRMS calcd for $C_{22}H_{38}N_2O_7Na$ (M+Na) 465.2577, found 465.2565. Anal. Calcd for $C_{22}H_{38}N_2O_7$: C, 59.71; H, 8.65; N, 6.33. Found: C, 59.55; H, 8.91; N, 6.06.

4.1.20. (2*S*,3′*S*)-2-(3′-Amino-2′-oxo-piperidin-1-yl)-succinic acid di-*tert*-butyl ester (28). To a solution of **27** (600 mg, 1.75 mmol) in ethyl acetate (9 mL) concentrated HCl (3 mL) was added dropwise at −50 °C, and the reaction mixture was stirred at that temperature for 2 h, then at 0 °C for 1 h. The reaction mixture was neutralized with saturated solution of $NaHCO_3$ and extracted with ethyl acetate. The organic layers were combined and washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. Flash column chromatography with CH_2Cl_2, then CH_2Cl_2/MeOH (20:1) gave 320 mg (68%) of **28** as an oil: $[\alpha]_D$ −38 (*c* 0.50, $CHCl_3$); IR (neat) *ν* 2975, 1725, 1643, 1455, 1392, 1366, 1288, 1249, 1147, 947, 845 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 4.95–4.87 (m, 1H), 3.49–3.25 (m, 3H), 3.04–2.93 (m, 1H), 2.69 (dd, *J*=2.3, 6.2 Hz, 0.5H), 2.62 (dd, *J*=2.4, 6.2 Hz, 0.5H), 2.52 (br s, 2H), 2.30–2.13 (m, 1H), 2.06–1.73 (m, 2H), 1.71–1.52 (m, 1H), 1.45 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 173.0, 170.0, 168.7, 81.9, 81.0, 56.5, 52.0, 46.5, 35.5, 29.2, 28.0, 28.0, 21.2; MS (ESI) *m/z* 342 $[M+H]^+$, 365 $[M+Na]^+$; HRMS calcd for $C_{17}H_{30}N_2O_5Na$ (M+Na) 365.2052, found 365.2043.

4.1.21. (2*S*,3′*S*,2″*S*,3″*R*)-2-(3′-{[1″-*tert*-Butoxycarbonyl-3″-(2‴-*N*,*N*′-bis(*tert*-butoxycarbonyl)-guanidino-ethyl)-pyrrolidine-2″-carbonyl]-amino}-2′-oxo-piperidin-1′-yl)-succinic acid di-*tert*-butyl ester (29). This compound was prepared according to the same procedure described for **21** starting from acid **20** (168 mg, 0.34 mmol) and amine **28** (138 mg, 0.40 mmol). Flash column chromatography with CH_2Cl_2/EtOAc (2:1, then 1:1) gave 175 mg (63%) of **30** as white crystals: mp 77–79 °C; $[\alpha]_D$ −12 (*c* 0.25, $CHCl_3$); IR (neat) *ν* 3380, 2974, 1700, 1653, 1608, 1506, 1435, 1390, 1365, 1274, 1248, 1143, 1099, 981, 887, 810, 846 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) *δ* 4.87–4.84 (m, 1H), 4.46–4.33 (m, 1H), 4.00–3.86 (m, 3H), 3.57–3.44 (m, 4H), 3.00 (dd, *J*=6.6, 9.5 Hz, 1H), 2.66 (dd, *J*=7.5, 8.7 Hz, 1H), 2.39–2.29 (m, 1H), 2.22–2.19 (m, 2H), 2.06–1.94 (m, 3H), 1.82–1.67 (m, 3H), 1.58 (s, 9H), 1.50, 1.47, 1.45 (3s, 36H); ^{13}C NMR (125 MHz, CD_3OD) *δ* 174.9, 174.7, 171.6, 171.2, 170.8, 170.2, 164.7, 161.8, 156.1, 156.0, 85.3, 83.3, 82.2, 81.6, 81.1, 79.8, 67.5, 67.2, 58.3, 58.2, 51.5, 51.4, 47.4, 47.3, 47.1, 44.5, 44.2, 42.7, 36.4, 33.5, 30.9, 30.2, 28.9, 28.6, 28.5, 28.1, 22.3, 22.1; MS (ESI) *m/z* 847 $[M+Na]^+$; HRMS calcd for $C_{40}H_{68}N_6NaO_{12}$ (M+Na) 847.4793, found 847.4833.

4.1.22. (2*S*,3′*S*,2″*S*,3″*R*)-2-(3′-{[3″-(2‴-Guanidino-ethyl)-pyrrolidine-2″-carbonyl]-amino}-2′-oxo-piperidin-1′-yl)-succinic acid (4). This compound was prepared according to the same procedure as described for **3** starting from compound **29** (130 mg, 0.15 mmol) to give compound **4** (58 mg, 89%) as a white solid: mp 95–97 °C; $[\alpha]_D$ −28 (*c* 0.25, MeOH); IR (neat) *ν* 3194, 3078, 2952, 1659, 1650, 1643, 1632, 1495, 1469, 1427, 1360, 1330, 1271, 1177, 1127, 985, 910, 835 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) *δ* 4.87 (t, *J*=6.9 Hz, 1H), 4.58 (dd, *J*=6.2 Hz, 1H), 3.90 (d, *J*=8.5 Hz, 1H), 3.59–3.41 (m, 4H), 3.37–3.33 (m, 2H), 3.12 (dd, *J*=6.4, 10.2 Hz, 1H), 2.82 (dd, *J*=7.7, 8.9 Hz, 1H), 2.48–2.39 (m, 1H), 2.40–2.33 (m, 1H), 2.19–1.95 (m, 4H), 1.87–1.77 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) *δ* 174.4, 172.7, 171.4, 169.3, 158.8, 65.5, 58.3, 51.5, 48.3, 47.0, 43.1, 40.9, 34.7, 32.2, 31.6, 28.0, 22.2; MS (ESI) *m/z* 413 $[M+H]^+$; HRMS calcd for $C_{17}H_{29}N_6O_6$ (M+H) 413.2149, found 413.2123.

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