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Supporting Information

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A New Class of Bicyclic Proline-Based Macrocyclic Inhibitors of Hepatitis C Virus: Stereoselective Synthesis and Biological Activity

Kevin X. Chen,* F. George Njoroge, Bancha Vibulbhan, Andrew Prongay, John Pichardo, Vincent Madison, Alexei Buevich, Tze-Ming Chan

Schering-Plough Research Institute, 2015 Galloping Hill Road, K-15-3/3545, Kenilworth, New Jersey 07033 (USA)

Kevin.chen@spcorp.com

Experimental section

General Methods. Reagents and solvents, including anhydrous THF, dichloromethane and DMF, were purchased from Aldrich or other commercial sources and were used without further purification. Reactions that were moisture sensitive or using anhydrous solvents were performed under either a nitrogen or an argon atmosphere. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates obtained from Analtech. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, ethanolic H_2SO_4 or Vaughn's reagent. Compounds were purified by flash chromatography either on a glass column using Merck silica gel 60 (230-400 mesh) or on an ISCO RediSep disposable silica gel column. NMR spectra were recorded at 300, 400 or 500 MHz for ^1H and at 75, 100 or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or d_6-DMSO as solvent. The chemical shifts are given in ppm, referenced to the internal TMS or deuterated solvent signal.

Methyl *N*-*tert*-butoxycarbonyl-3, 4-dihydroxy-*L*-proline carboxylate (2) To a mixture of 3,4-dehydroproline **1** (5.32 g, 23.3 mmol), *N*-methylmorpholine-*N*-

oxide (NMO) (4.75 g, 35.1 mmol) in acetone (10.0 mL) and water (15.0 mL) was added osmium tetroxide solution in *tert*-butanol (Aldrich, 2.5% w/w, 3.5 mL, 0.344 mmol) followed by THF (10 mL). The mixture was stirred at rt overnight. Saturated $Na_2S_2O_3$ solution (30 mL) was added and stirring was continued for 10 min. EtOAc (300 mL) and brine (80 mL) were added. After the two layers were separated, the aqueous solution was extracted with EtOAc (2x100 mL). Organic solutions were combined, dried ($MgSO_4$) and concentrated under reduced pressure to give a dark liquid. Flash chromatography (4 to 8 % MeOH/CH_2Cl_2) afforded the desired 3(*R*),4(*S*)-product **2** and 3(*S*),4(*R*)-dihydroxy isomer (4.73 g, 77 %) in approximately 2 : 1 ratio as a inseparable mixture. HRMS Calcd for $C_{11}H_{20}NO_6$ $(M+H)^+$: 262.1291. Found: 262.1296.

Methyl *N*-*tert*-butoxycarbonyl-3 (*R*), 4(*S*)-(4-benzyloxybutylidene acetal) – *L*-proline carboxylate (3) To a stirred suspension of a mixture of 3(*R*),4(*S*)-dihydroxyproline **2** and 3(*S*),4(*R*)-dihydroxyproline (1.60 g, 6.12 mmol), 4-benzyloxy-1-butaraldehyde (2.32 g, 13.0 mmol) and magnesium sulfate (4.0 g, 33.2 mmol) in anhydrous CH_2Cl_2 (60 mL) at 0°C was added *p*-toluene sulfonic acid (*p*-TsOH) monohydrate (0.15 g, 1.01 mmol). The resulting mixture was vigorously stirred at 0°C and warmed to room temperature overnight along with the ice bath. Saturated $NaHCO_3$ solution (100 mL), water (50 mL) and CH_2Cl_2 (200 mL) were added and two layers were separated. The aqueous solution was extracted with CH_2Cl_2 (2 X 150 mL) and the combined organic solution was dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography (5 to 15 % EtOAc/CH_2Cl_2) afforded a mixture of **3** and its isomer (2.34 g, 91 %) as a

colorless oil. The isomers could be separated by flash chromatography (10 to 50 % EtOAc/hexane) in a 2:1 ratio with **3** as the major isomer. Two rotamers were observed in NMR spectra of **3**. ^{1}H NMR (500 MHz, d_6-DMSO) d 7.35-7.26 (m, 5 H), 4.89 (t, *J* = 4.1 Hz, 1 H), 4.70 (dd, *J* = 9.8, 6.3 Hz, 1 H), 4.64-4.62 (m, 1 H), 4.43 (s, 2 H), 4.33 & 4.23 (s, 1 H), 3.69 & 3.65 (d, *J* = 10.3 & 12.9 Hz, 4 H), 3.44-3.38 (m, 3 H), 1.68-1.60 (m, 4 H), 1.39 & 1.32 (s, 9 H); ^{13}C NMR (125 MHz, d_6-DMSO) d 170.7, 170.3, 154.3, 153.7, 139.0, 128.6, 127.7, 127.7, 105.8, 105.7, 82.9, 82.1, 80.1, 80.0, 79.9, 79.0, 72.1, 69.5, 66.2, 65.6, 55.3, 52.7, 52.7, 51.9, 51.4, 29.8, 29.7, 28.3, 28.1, 23.8, 23.5. HRMS Calcd for $C_{22}H_{32}NO_7$ $(M+H)^+$: 422.2179. Found: 422.2158.

Methyl 3(*R*), 4(*S*)-(4-benzyloxybutylidene acetal)-*L*-proline carboxylate hydrochloride (4) To the solution of **3** (0.65 g, 1.54 mmol) in EtOAc (15 mL) was added a 4 M HCl solution in 1,4-dioxane (15 mL). After stirred for about one hour, the reaction was complete as indicated by TLC. The solution was concentrated *in vacuo* to give amine hydrochloride **4** as an oil (0.66 g, quant.). ^{1}H NMR (500 MHz, D_6-DMSO) d 7.37-7.27 (m, 5 H), 4.95 (dd, *J* = 5.9, 1.2 Hz, 1 H), 4.88 (t, *J* = 5.0 Hz 1 H), 4.81-4.78 (m, 1 H), 4.59 (s, 1 H), 4.45 (s, 3 H), 3.78 (s, 3 H), 3.50 (d, *J* = 13.3 Hz, 1 H), 3.44 (t, *J* = 6.3 Hz, 1 H), 3.39-3.34 (m, 3 H), 1.79-1.75 (m, 2 H), 1.65-1.60 (m, 2 H); ^{13}C NMR (125 MHz, d_6-DMSO) d 166.9, 139.0, 128.6, 127.8, 127.7, 106.3, 81.8, 79.0, 72.2, 69.6, 66.7, 64.8, 53.7, 50.6, 29.6, 24.5. HRMS Calcd for $C_{17}H_{24}NO_5$ $(M+H)^+$: 322.1654. Found: 322.1670.

Methyl 3(*S*), 4(*R*)-(4-benzyloxybutylidene acetal)-*L*-proline carboxylate hydrochloride (5) Compound **5** was prepared from 3(*S*),4(*R*)-isomer of **3** using

the same procedures described above in the preparation of compound **4**: ^{1}H NMR (500 MHz, D_6-DMSO) d 7.36-7.27 (m, 5 H), 4.94-4.92 (m, 1 H), 4.84-4.79 (m, 2 H), 4.59-4.58 (m, 1 H), 4.44 (s, 2 H), 3.78 (s, 3 H), 3.47-3.36 (m, 4 H), 3.20 (dd, *J* = 12.6, 4.0 Hz 1 H), 1.71-1.67 (m, 2 H), 1.61-1.55 (m, 2 H); ^{13}C NMR (125 MHz, d_6-DMSO) d 165.4, 138.5, 128.1, 127.3, 127.3, 105.1, 78.5, 78.3, 71.7, 69.1, 66.2, 62.9, 52.9, 49.2, 28.9, 23.7.

Methyl *N*-*tert*-butoxycarbonyl-*L*-cyclohexylglycine-3(*R*), 4(*S*)-(4-benzyloxybutylidene acetal)-*L*-proline carboxylate (6) To a solution of **4**, *N*-Boc-cyclohexylglycine (0.40 g, 1.55 mmol) and (HATU) (0.60 g, 1.58 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0°C was added diisopropylethylamine (0.70 mL, 4.02 mmol). The reaction mixture was stirred and warmed to rt along with the ice bath overnight (18 h). Then 5% H_3PO_4 (80 mL) and CH_2Cl_2 (100 mL) were added. The separated organic solution was washed with saturated aqueous sodium bicarbonate solution (80 mL), dried (magnesium sulfate) and concentrated under reduced pressure. Flash chromatography (15 to 70 % EtOAc/hexane) afforded **6** (0.66 g, 76%). Two rotamers observed in NMR spectra of **6**. ^{1}H NMR (500 MHz, $CDCl_3$) d 7.37-7.26 (m, 5 H), 5.22 & 5.15 (d, *J* = 9.1 & 9.4 Hz, 1 H), 4.95 & 4.91 (t, *J* = 4.4 & 4.3 Hz, 1 H), 4.92 & 4.73 (s, 1 H), 4.69 (d, *J* = 2.5 Hz, 1 H), 4.65-4.63 & 4.35-4.32 (m, 1 H), 4.49 (s, 2 H), 4.20 & 4.17 (t, *J* = 11.7 & 9.6 Hz, 1 H), 3.76 & 3.75 (s, 3 H), 3.73-3.70 (m, 1 H), 3.49-3.44 (m, 2 H), 1.80-1.59 (m, 11 H), 1.42 & 1.41 (s, 9 H), 1.26-1.02 (m, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) d 172.4, 171.7, 170.0, 169.4, 155.8, 155.6, 138.8, 138.7, 128.6, 127.9, 127.8, 127.7, 107.0, 106.4, 83.6, 81.8, 80.2, 79.7, 79.5, 78.5, 77.7,

73.1, 73.1, 70.1, 70.0, 66.2, 65.2, 56.4, 56.3, 53.3, 52.8, 52.4, 51.4, 41.9, 41.0, 30.4, 30.3, 29.9, 28.6, 28.5, 27.9, 26.4, 26.3, 26.2, 24.1, 23.9. HRMS Calcd for $C_{30}H_{45}N_2O_8$ $(M+H)^+$: 561.3176. Found: 561.3182.

Methyl *N*-(3-hydroxyphenyl)acetyl-*L*-cyclohexylglycine-3(*R*),4(*S*)-(4-benzyloxybutylidene acetal)-*L*-proline carboxylate (7) Compound **6** was treated with 2 M hydrochloric acid in EtOAc/dioxane in a similar manner as in the preparation of compound **4** from **3** to give an amine hydrochloride intermediate (quant. yield). HRMS Calcd for $C_{25}H_{37}N_2O_6$ $(M+H)^+$: 461.2652. Found: 461.2629. The amine was coupled to 3-hydroxyphenyl acetic acid in a similar manner as in the preparation of compound **6** from **4** to give product **7** (98%, 2 steps). Two rotamers were observed in NMR spectra of **7**. ^{1}H NMR (500 MHz, $CDCl_3$) d 7.34-7.30 (m, 5 H), 7.28-7.24 (m, 1 H), 7.13-7.09 (m, 1 H), 6.80-6.69 (m, 4 H), 4.93 & 4.88 (t, *J* = 4.3 Hz, 1 H), 4.86-4.49 (m, 4 H), 4.48 (s, 2 H), 4.22 & 4.18 (d, *J* = 12.2, 13.6 Hz, 1 H), 3.71 & 3.60 (s, 3 H), 3.56-3.39 (m, 4 H), 1.76-1.57 (m, 10 H), 1.20-0.88 (m, 5 H); ^{13}C NMR (125 MHz, $CDCl_3$) d 172.2, 171.7, 171.7, 171.5, 169.9, 169.3, 157.34, 157.30, 138.63, 138.56, 136.3, 136.2, 130.2, 130.1, 128.64, 128.61, 128.0, 127.9, 127.86, 127.82, 120.9, 116.7, 116.6, 114.8, 114.7, 107.0, 106.4, 83.5, 81.7, 80.1, 78.4, 73.12, 73.08, 70.1, 69.9, 66.5, 65.4, 55.1, 55.0, 53.5, 52.9, 52.5, 51.5, 43.6, 43.5, 41.5, 40.9, 30.25, 30.17, 29.8, 28.6, 28.0, 26.3, 26.2, 26.1, 26.0, 24.2, 23.8. HRMS Calcd for $C_{33}H_{43}N_2O_8$ $(M+H)^+$: 595.3019. Found: 595.3014.

Methyl *N*-(3-hydroxyphenyl)acetyl-*L*-cyclohexylglycine-3(*R*),4(*S*)-(4-hydroxybutylidene acetal)-*L*-proline carboxylate (8) To a solution of **7** (1.90

g, 3.19 mmol) in EtOAc and ethanol (1:1, 120 mL) was added 10% palladium-carbon (0.5 g). The mixture was evacuated and refilled with hydrogen gas three times. After being vigorously stirred under a hydrogen atmosphere at rt for 16 h, it was filtered through a celite pad. Concentration of the filtrate gave phenol alcohol **8** as an oil (1.62 g, quant.). Two rotamers observed in NMR spectra. ^{1}H NMR (500 MHz, D_6-DMSO) d 7.30 & 7.18 (d, *J* = 8.5 & 9.2 Hz, 1 H), 6.31-6.27 (m, 1 H), 5.94-5.92 (m, 2 H), 5.85-5.83 (m, 1 H), 4.14 & 3.84 (t, *J* = 4.2, 5.3 Hz, 1 H), 3.78-3.74 & 3.68-3.65 (m, 1 H), 3.48 & 2.25 (d, *J* = 12.3, 13.6 Hz, 1 H), 2.77-2.55 (m, 5 H), 0.97-0.72 (m, 10 H), 0.47-0.15 (m, 5 H); ^{13}C NMR (125 MHz, D_6-DMSO) d 164.2, 163.8, 163.7, 161.2, 160.8, 149.09, 149.07, 128.5, 121.0, 120.9, 111.8, 111.7, 107.6, 107.5, 105.31, 105.27, 98.0, 97.5, 75.3, 73.3, 71.9, 70.0, 58.2, 57.1, 53.1, 53.0, 46.9, 46.5, 44.0, 43.9, 43.5, 42.6, 33.90, 33.87, 32.5, 31.7, 21.34, 21.27, 21.1, 21.0, 20.0, 19.8, 18.2, 17.7, 17.6, 17.5, 17.4. HRMS Calcd for $C_{26}H_{37}N_2O_8$ $(M+H)^+$: 505.2550. Found: 505.2553.

Methyl 8-Cyclohexyl-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy [1,3]benzenoethaniminoethano)-5H-1,3-dioxolo[4,5-c]pyrrol-4(*S*)-yl carboxylate (9) The solution of **8** (1.60 g, 3.20 mmol) and 1,1'-(azodicarbonyl) dipiperidine (ADDP) (2.50 g, 9.91 mmol) in anhydrous CH_2Cl_2 (150 mL) was bubbled with Argon gas through a frit glass bubbler for 20 min. To this solution was added triphenylphosphine (2.60 g, 9.91 mmol) and the resulting mixture was stirred at room temperature overnight (24 h) under an Argon atmosphere. After removal of solvent under reduced pressure, the residue was purified by flash chromatography (1-3 % MeOH/CH_2Cl_2) to afford the macrocycle **9** (1.09 g, 72

%). ^{1}H NMR (500 MHz, $CDCl_3$) d 7.21-7.18 (m, 1 H), 6.81-6.78 (m, 1 H), 6.65 (s, 1H), 6.17 (d, *J* = 9.13 Hz, 1 H), 5.08-5.06 (m, 1 H), 4.74 (d, *J* = 1.63 Hz, 1 H), 4.70-4.68 (m, 1 H), 4.65 (d, *J* = 9.21 Hz, 1 H), 4.61(dd, *J* = 5.97, 3.80 Hz, 1 H), 4.27 (d, *J* = 12.2 Hz, 1 H), 4.08-4.00 (m, 2 H), 3.71 (s, 3 H), 3.66 (dd, *J* = 12.6, 3.8 Hz, 1 H), 3.58 (d, *J* = 14.6 Hz, 1 H), 3.50 (d, *J* = 14.9 Hz, 1 H), 1.94-1.87 (m, 1 H), 1.80-1.57 (m, 8 H), 1.29-0.82 (m, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) d 171.2, 169.8, 158.3, 136.2, 130.1, 121.8, 117.2, 112.2, 105.5, 81.7, 80.1, 67.4, 65.1, 54.7, 52.6, 51.6, 43.9, 41.3, 29.1, 28.8, 27.9, 26.2, 25.8, 25.7, 20.0; HRMS Calcd for $C_{26}H_{35}N_2O_7$ $(M+H)^+$: 487.2444. Found: 487.2443.

(3-*tert*-Butoxycarbonylamino-2-hydroxy-hexanoyl amino)-acetic acid (11)

To a solution of carboxylic acid **10** (3.00 g, 12.0 mmol), H-*L*-glycine benzyl ester hydrochloride (2.56 g, 13.0 mmol), 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (DhBtOH) (2.16 g, 13.2 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) (2.88 g, 15.0 mmol) in DMF/CH_2Cl_2 (1:1, 150 mL) at -20°C was added *N*-Methylmorpholine (NMM) (5.40 mL, 49.1 mmol). After stirred at this temperature for 30 min, the mixture was stored in a freezer (-10°C) overnight for 16 h. Then 5% aqueous H_3PO_4 solution (100 mL), brine (100 mL) and EtOAc (300 mL) were added. After two layers were separated, the organic solution was washed with 5% H_3PO_4 (200 mL), saturated aqueous sodium bicarbonate solution (2 x 200 mL), water (200 mL) and brine (200 mL). It was then dried (magnesium sulfate), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (25-50% acetone/Hexanes) to afford the dipeptide product (4.50 g, 95%) as a mixture of

four diastereomers. ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.21 & 8.18 (t, 1 H, *J* = 6.5 Hz), 7.40-7.33 (m, 5 H), 6.39 & 5.94 (d, *J* = 9.0, 9.5 Hz, 1 H), 5.89 &5.76 (d, *J* = 6, 5.5 Hz, 1 H), 5.14 (bs, 2 H), 3.99-3.70 (m, 4 H), 1.39 &1.36 (s, 9 H), 1.48-1.07 (m, 4 H), 0.85 & 0.77 (t, *J* = 7.0, 6.5 Hz, 3 H). ^{13}C NMR (125 MHz, d_6-DMSO), δ, 173.9, 173.2, 170.5, 170.4, 156.1, 136.8, 129.3, 128.9, 128.8, 128.7, 78.5, 78.4, 74.5, 73.1, 66.6, 53.5, 41.4, 30.5, 29.1, 29.0, 19.8, 19.7, 14.7, 14.6. LRMS *m/z* MH^+= 395. HRMS calcd for $C_{20}H_{31}N_2O_6$: 395.2182 $(M+H)^+$. Found: 395.2199. To a solution of the benzyl ester (4.50 g, 11.4 mmol) in ethanol (50 mL) was added 5% Pd-C (1.0 g). The mixture was stirred vigorously under a hydrogen atmosphere at rt for 3 h before it was filtered through a celite pad. The filter cake was washed with EtOAc (2 x 30 mL). The solution was concentrated under reduced pressure to afford the product **11** (3.40 g, 98% yield) as a mixture of four diastereomers. LRMS *m/z* MH^+= 305. HRMS calcd for $C_{13}H_{25}N_2O_6$: 305.1713 $(M+H)^+$. Found: 305.1699.

[2-(3-Amino-2-hydroxy-hexanoylamino)-acetylamino]-phenyl-acetic acid *tert*-butyl ester hydrochloride (12) The coupling of carboxylic acid **11** (2.00 g, 6.57 mmol) and *L*-phenyl glycine *tert*-butyl ester hydrochloride (1.76 g, 6.57 mmol) was carried out in a manner similar to the first step that is described above for the preparation of **11**. The crude product was purified by flash chromatography (1 : 1 EtOAc /Hexanes) to afford the desired product (2.60 g, 80%) as a mixture of four diastereomers. ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.64 & 8.58 (d, 1 H, *J*=7.0 Hz), 7.91 &7.87 (m, 1 H), 7.42-7.32 (m, 5 H), 6.40 & 5.99 (dd, 1 H, *J* = 4.5 & 5.00 Hz), 5.77-5.73 (m, 1 H), 5.30-5.28 (m, 1 H), 3.97-3.70 (m, 4

H), 1.51-1.08 (m, 22 H), 0.86 & 0.79 (t, 3 H, *J* = 6.0 Hz). ^{13}C NMR (125 MHz, d_6-DMSO), δ 173.4, 172.8, 170.3, 169.4, 169.3, 155.7, 137.5, 129.5, 129.0, 128.3, 128.2, 82.2, 82.1, 78.4, 74.6, 73.2, 57.7, 53.4, 42.3, 33.7, 29.1, 29.0, 28.3, 19.7, 14.7, 14.6. HRMS calcd for: $C_{25}H_{40}N_3O_7$: 494.2866. Found: 494.2863. A solution of this product (2.6 g, 5.30 mmol) in 2 M HCl in dioxane/EtOAc (1:1) was stirred at rt for 15 min. The reaction mixture was concentrated *in vacuo* to yield **12** (2.15 g, quant.) as a pale yellow solid which was used in subsequent reactions without further purification. HRMS calcd for $C_{20}H_{32}N_3O_5$: 394.2342 $(M+H)^+$. Found: 394.2336.

[2-(3-Amino-2-hydroxy-hexanoylamino)-acetylamino]-phenyl-acetic acid dimethyl amide hydrochloride (13) The coupling of carboxylic acid **11** (2.50 g, 8.21 mmol) and *L*-phenyl glycine dimethyl amide hydrochloride (1.85 g, 8.62 mmol) was carried out in a manner similar to the first step that is described above for the preparation of **11**. The crude product was purified by flash chromatography (1 : 1 EtOAc /Hexanes) to afford the product (3.13 g, 82%) as a mixture of four diastereomers. ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.51-8.43 (m, 1 H), 7.93-7.88 (m, 1 H), 7.37-7.28 (m, 5 H), 6.41 & 5.98 (d, *J* = 9.4 & 9.1 Hz, 1 H), 5.81 (d, *J* = 7.5 Hz, 1 H), 5.77-5.72 (m, 1 H), 3.94 & 3.85 (dd, *J* = 5.4, 3.1 & 6.0, 3.4 Hz, 1 H), 3.80-3.66 (m, 3 H), 2.92 & 2.91 (s, 3 H), 2.831 & 2.827 (s, 3 H), 1.37, 1.34 & 1.31 (s, 9 H), 1.30-1.10 (m, 4 H), 0.84 & 0.78 (t, *J* = 7.0 Hz, 3 H), ^{13}C NMR (125 MHz, d_6-DMSO) δ 171.9, 169.23, 169.21, 167.91, 167.86, 167.8, 155.2, 137.63, 137.56, 137.53, 128.6, 127.83, 127.77, 77.54, 77.49, 73.7, 72.34, 72.29, 53.0, 52.9, 52.53, 52.50, 41.6, 36.6, 35.3, 32.7, 29.8, 28.2, 28.1, 18.9,

13.8, 13.7. HRMS calcd for $C_{23}H_{37}N_4O_6$ $(M+H)^+$: 465.2713. Found: 465.2712. The product was treated with 2 M HCl in 1,4-dioxane/EtOAc (1 : 1) at rt for 2 h before it was concentrated under reduced pressure to give **13** (2.81 g, quant.) as an off-white solid. HRMS calcd for $C_{18}H_{29}N_4O_4$ $(M+H)^+$: 365.2189. Found: 365.2187.

3-Amino-2-hydroxy-hexanoic acid allylamide hydrochloride (14) The coupling of carboxylic acid **10** and allyl amine was carried out in a manner similar to the first step that is described above for the preparation of **11**. The crude product was purified by flash chromatography (1 : 1 EtOAc /Hexanes) to afford the product as a mixture of four diastereomers. The product was treated with 2 M HCl in 1,4-dioxane/EtOAc (1 : 1) at rt for 2 h before it was concentrated under reduced pressure to give **14** as a mixture of four diastereomers.. ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.27 & 8.21 (t, *J* = 5.9 & 6.2, 1 H), 8.16 (bs, 1 H), 7.93 (bs, 1 H), 5.85-5.75 (m, 1 H), 5.16-5.03 (m, 1 H), 4.29 & 4.10 (d, *J* = 2.8 & 4.1 Hz, 1 H), 3.79-3.68 (m, 2 H), 3.39 & 3.24 (bs, 2 H), 1.57-1.21 (m, 4 H), 0.87-0.80 (m, 3 H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 170.8, 170.4, 135.0, 115.2, 115.1, 70.7, 69.5, 54.9, 52.7, 52.5, 40.7, 40.6, 30.7, 29.1, 18.1, 18.0, 13.7, 13.6. HRMS calcd for $C_9H_{19}N_2O_2$ $(M+H)^+$: 187.1447. Found: 187.1437.

3-Amino-2-hydroxy-heptanoic acid {[(dimethylcarbamoyl-phenyl-methyl)-carbamoyl]-methyl}-amide hydrochloride (16) Amine intermediate **16** was prepared in a manner similar to that is described above for the preparation of **13** starting with norleucine carboxylic acid **15**. The product was obtained as a mixture of four diastereomers. ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.68 (t, *J* = 7.4 Hz) & 8.51 (d, *J* = 7.4 Hz) & 8.28-8.22 (m, 1 H), 8.21-8.16 (m, 1 H), 7.95 (bs, 1

H), 7.40-7.26 (m, 5 H), 6.68 (*J* = 5.2 Hz) & 6.54 (*J* = 3.8 Hz) & 6.31 (*J* = 4.3 Hz, 1 H), 5.83-5.80 (m, 1 H), 4.53 (s) & 4.38 (d, *J* = 2.9 Hz) & 4.31 & 4.12 (s, 1 H), 3.88-3.71 (m, 2 H), 3.36 & 3.22 (bs, 2 H), 2.92 (s, 3 H), 2.84 (s, 3 H), 1.67-1.16 (m, 6 H), 0.88-0.79 (m, 3 H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 173.7, 173.5, 172.5, 172.4, 171.8, 170.12, 170.11, 168.99, 168.96, 168.58, 168.56, 138.42, 138.38, 129.52, 129.47, 128.81, 128.80, 128.72, 128.69, 128.66, 128.65, 128.63, 71.7, 70.64, 70.61, 70.20, 70.18, 54.1, 54.03, 54.01, 54.00, 53.91, 53.86, 53.83, 53.5, 53.4, 52.9, 42.6, 42.52, 42.49, 37.47, 37.46, 36.24, 36.22, 29.6, 29.1, 27.9, 27.81, 27.79, 27.76, 27.71, 27.66, 27.6, 27.4, 22.8, 22.75, 22.73, 22.68, 14.59, 14.55, 14.51, 14.48. HRMS calcd for $C_{19}H_{31}N_4O_4$ $(M+H)^+$: 379.2345. Found: 379.2334.

8-Cyclohexyl-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy[1,3]-benzenoethaniminoethano)-5H-1,3-dioxolo[4,5-c]pyrrol-4(*S*)-yl carboxylic acid (17) To the solution of methyl ester **9** (1.05 g, 2.16 mmol) in THF (20 mL) and methanol (20 mL) at 0 °C was added an aqueous lithium hydroxide solution (0.200 g, 8.33 mmol). The resulting mixture was vigorously stirred and allowed to warm to rt. After 4 h, it was concentrated *in vacuo* to a third of its original volume. The aqueous residue was acidified to pH ~ 2 by addition of 1 N HCl. EtOAc (100 mL) was added and layers were separated. The aqueous solution was extracted with EtOAc (2 x 60 mL). The combined organic solution was dried ($MgSO_4$), filtered and concentrated to give compound **17** (0.95 g, 93%), which was used without further purification. ^{1}H NMR (500 MHz, d_6-DMSO) δ 7.63 (d, *J* = 9.0 Hz, 1 H), 6.37-6.34 (m, 1 H), 6.02 (d, *J* = 7.3 Hz, 1 H), 5.97-5.93 (m, 2 H), 4.24 (dd, *J* =

3.8, 1.9 Hz, 1 H), 3.95 (d, *J* = 6.0 Hz, 1 H), 3.86-3.80 (m, 3 H), 3.49 (d, *J* = 12.3 Hz, 1 H), 3.36-3.31 (m, 1 H), 3.28-3.23 (m, 1 H), 2.90 (dd, *J* = 12.3, 4.1 Hz, 1 H), 2.85 (d, *J* = 13.4 Hz, 1 H), 2.55 (d, *J* = 13.4 Hz, 1 H), 1.16-0.75 (m, 9 H), 0.59-0.06 (m, 6 H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 164.4, 163.4, 150.0, 128.7, 121.2, 113.6, 108.0, 104.2, 97.2, 73.9, 72.1, 58.9, 46.6, 43.6, 34.0, 32.3, 20.8, 20.5, 19.7, 17.8, 17.3, 17.2, 12.0. HRMS Calcd for $C_{25}H_{33}N_2O_7$ $(M+H)^+$: 473.2288. Found: 473.2294.

1,1-Dimethylethyl-α-(*S*)-[[[[3-[[[8(*S*)-cyclohexyl-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy[1,3]benzenoethaniminoethano) -5H-1,3-dioxolo-[4,5-c]pyrrol-4(*S*)-yl]carbonyl]amino]-1,2-dioxohexyl] amino]acetyl]amino] benzeneacetate (18 & 21). The acid **17** (0.200 g, 0.423 mmol), amine **12** (0.190 g, 0.442 mmol), DhBtOH (0.072 g, 0.441 mmol) and EDC (0.100 g, 0.522 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 60 mL). *N*-Methylmorpholine (0.16 mL, 1.45 mmol) was added to this solution at -20°C. After stirred at this temperature for 30 min, the mixture was stored in a freezer (-10°C) overnight. Then 5% aqueous H_3PO_4 solution (30 mL), brine (30 mL) and EtOAc (100 mL) were added. After the layers were separated, the organic solution was washed with 5% H_3PO_4 (80 mL), saturated aqueous sodium bicarbonate solution (2 x 80 mL) and water (80 mL), dried (magnesium sulfate), filtered and concentrated *in vacuo* to give the α-hydroxy amide as a mixture of diastereomers (0.180 g, 0.212 mmol, 50 %). HRMS Calcd for $C_{45}H_{62}N_5O_{11}$ $(M+H)^+$: 848.4446. Found: 848.4434. To this compound in dichloromethane (60 mL) at 0°C was added Dess-Martin periodinane (0.20 g, 0.471 mmol). The mixture was stirred at rt for 3 h before

saturated $Na_2S_2O_3$ and $NaHCO_3$ solutions (30 mL each) were added. After stirred for 5 min, the layers were separated. The aqueous solution was extracted with CH_2Cl_2 (2 X 60 mL) and the combined organic solution was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The two isomers were partially separated by flash chromatography (2 to 5 % MeOH/CH_2Cl_2) to afford less polar product **18** (35 mg), more polar product **21** (41 mg) and a mixture (55 mg) of **18** & **21** (73% total yield). Data for compound **18**: ^{1}H NMR (300 MHz, $CDCl_3$) d 7.48-7.42 (m, 1 H), 7.39-7.32 (m, 5 H), 7.23-7.10 (m, 6 H), 7.01 (d, *J* = 7.1 Hz, 1 H), 6.81-6.66 (m, 3 H), 6.27 (d, *J* = 9.5 Hz, 1 H), 5.45 (d, *J* = 7.1 Hz, 1 H), 5.30-5.21 (m, 1 H), 5.11-4.97 (m, 2 H), 4.79-4.60 (m, 3 H), 4.27 (d, *J* = 12.6 Hz, 1 H), 4.13-3.86 (m, 4 H), 3.78-3.47 (m, 3 H), 2.08-1.51 (m, 14 H), 1.38 (s, 9 H), 1.49-0.83 (m, 8 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d 193.9, 171.8, 171.3, 169.6, 168.4, 166.5, 159.2, 158.3, 136.8, 136.7, 130.1, 128.8, 128.4, 127.0, 121.7, 117.2, 112.0, 104.8, 83.1, 80.4, 80.0, 67.4, 65.1, 56.9, 54.7, 53.9, 51.9, 43.9, 42.4, 41.7, 33.1, 29.3, 28.7, 28.0, 27.8, 26.0, 25.8. 19.9, 18.9, 13.7. HRMS Calcd for $C_{45}H_{60}N_5O_{11}$ $(M+H)^+$: 846.4289. Found: 846.4296. Data for compound **21**: ^{1}H NMR (300 MHz, $CDCl_3$) d 7.58-7.40 (m, 1 H), 7.38-7.26 (m, 7 H), 7.20-7.14 (m, 1 H), 6.89-6.65 (m, 3 H), 6.49-6.27 (m, 1 H), 5.51-5.47 (m, 1 H), 5.25-5.20 (m, 1 H), 4.93-4.63 (m, 4 H), 4.43-4.20 (m, 2 H), 4.19-3.82 (m, 4 H), 3.79-3.52 (m, 3 H), 1.93-1.50 (m, 13 H), 1.39 (s, 9 H), 1.35-0.76 (m, 9 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d 194.7, 171.3, 171.0, 168.9, 166.7, 159.2, 158.2, 136.7, 136.3, 130.4, 129.9, 128.8, 128.3, 126.9, 122.2, 116.8, 111.8, 106.7, 105.0, 83.1, 81.0, 80.2, 67.4, 56.8, 56.5, 54.8, 54.3, 52.3, 43.7, 42.3, 41.3, 39.5, 33.3, 32.9, 29.4, 29.2, 28.9, 27.7, 26.0, 25.7,

25.6, 23.8, 18.9, 18.8, 13.6, 13.5. HRMS m/z (M^+) Calcd for $C_{45}H_{60}N_5O_{11}$: 846.4289. Found: 846.4288

a **-(S)-[[[[3-[[[8(*S*)-Cyclohexyl-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy[1,3]benzenoethaniminoethano)-5H-1,3-dioxolo[4,5-c]pyrrol-4(*S*)-yl]carbonyl]amino]-1,2-dioxohexyl]amino]acetyl]-amino]benzeneacetic acid (19 & 22)** To the solution of **18** (6.0 mg, 0.0071 mmol) in dichloromethane (8 mL) at rt was added trifluoroacetic acid (TFA) (2 mL). After stirred at rt for 5 h, it was concentrated in vacuo to give the acid **19** (6.0 mg, quant.). HRMS m/z (M^+) Calcd for $C_{41}H_{52}N_5O_{11}$ $(M+H)^+$: 790.3663. Found: 790.3665. Compound **22** was prepared from **21** under the same conditions as described above. HRMS m/z (M^+) Calcd for **22** $C_{41}H_{52}N_5O_{11}$ $(M+H)^+$: 790.3663. Found: 790.3696.

8(S)-Cyclohexyl-N-[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenyl-ethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy[1,3]benzeno-ethanimino-ethano)-5H-1,3-dioxolo[4,5-c]pyrrole-4(*S*)-carboxamide (20 & 23) Compounds **20** (less polar) and **23** (more polar) were prepared from carboxylic acid **17** and amine **13** according to the procedures described above for the preparation of compounds **18** and **21**. Data for compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$) d 7.80-7.67 (m, 1 H), 7.56-7.42 (m, 1 H), 7.36-7.25 (m, 6 H), 7.23-7.14 (m, 1 H), 6.98-6.67 (m, 3 H), 6.46-6.36 (m, 1 H), 5.91-5.84 (m, 1 H), 5.24-5.17 (m, 1 H), 5.04-4.97 (m, 1 H), 4.93-4.78 (m, 2 H), 4.76-4.62 (m, 2 H), 4.45-3.88 (m, 5 H), 3.76-3.51 (m, 3 H), 2.99 (s, 3 H), 2.91 (s, 3 H), 2.10 (bs, 1 H), 2.00-1.41 (m, 11 H), 1.39-0.75 (m, 11 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d 194.8, 171.6, 171.3, 168.8, 166.4, 159.1, 136.7,

130.4, 130.0, 129.1, 128.5, 127.8, 122.2, 121.7, 116.8, 112.1, 104.9, 81.0, 80.2, 67.4, 65.6, 54.8, 54.3, 54.0, 52.4, 43.7, 42.3, 41.4, 36.9, 36.1, 33.2, 31.8, 29.2, 29.0, 28.9, 28.0, 26.1, 25.7, 22.6, 20.1, 18.8, 14.1, 13.5; HRMS Calcd for $C_{43}H_{57}N_6O_{10}$ $(M+H)^+$: 817.4136. Found: 817.4139. Data for compound **23**: ^{1}H NMR (300 MHz, $CDCl_3$) d 7.70 (bs, 1 H), 7.62 (bs, 1 H), 7.52-7.30 (m, 5 H), 7.22-7.16 (m, 2 H), 6.77-6.76 (m, 2 H), 6.69 (s, 1 H), 6.53-6.51 (m, 1 H), 5.84 (d, *J* = 7.0 Hz, 1 H), 5.28-5.22 (m, 1 H), 5.06-4.99 (m, 2 H), 4.86-4.61 (m, 3 H), 4.26 (d, *J* = 12.6 Hz, 1 H), 4.12-3.84 (m, 4 H), 3.62-3.47 (m, 3 H), 2.99 (s, 3 H), 2.91 (s, 3 H), 2.05-0.80 (m, 22 H); ^{13}C NMR (75 MHz, $CDCl_3$) d 194.6, 171.7, 171.3, 169.3, 168.5, 166.2, 159.0, 158.2, 136.7, 136.4, 130.0, 129.1, 128.9, 128.5, 127.8, 121.7, 117.0, 112.2, 104.9, 80.6, 80.0, 67.4, 65.1, 54.7, 54.0, 52.0, 43.8, 42.2, 41.6, 36.9, 36.1, 33.1, 31.9, 29.3, 29.0, 28.7, 28.0, 26.1, 25.8, 22.7, 20.0, 18.9, 14.1, 13.6. HRMS m/z (M^+) Calcd for $C_{43}H_{57}N_6O_{10}$ $(M+H)^+$: 817.4136. Found: 817.4139.

8-Cyclohexyl-*N*-[1-[1,2-dioxo-2-(2-propenylamino)ethyl]butyl]-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy[1,3]benzenoethaniminoethano)-5H-1,3-dioxolo[4,5-c]pyrrole-4(*S*)-carboxamide (24) Compound **24** was prepared from carboxylic acid **17** and amine **14** according to the procedures described above for the preparation of compounds **18**. The two P1 diastereomers were not separable by flash chromatography. It was tested as a mixture of two diastereomers. The ratio of the two isomers was in the range of 1:1.5 to 1: 0.67 depending on reaction conditions. ^{1}H NMR (500 MHz, d_6-DMSO) d 8.89 (t, *J* = 6.0 Hz) & 8.71 (dd, *J* = 9.9, 7.0 Hz, 1 H), 8.82 (t, *J* = 6.1 Hz) & 8.42 (dd, *J* = 9.1,

2.5 Hz, 1 H), 7.76-7.55 (m, 1 H), 7.17-7.14 (m, 1 H), 6.76 (d, *J* = 7.5 Hz, 1 H), 6.74-6.72 (m, 2 H), 5.83-5.75 (m, 1 H), 5.13-4.87 (m, 4 H), 4.74 (d, *J* = 6.3 Hz) & 4.64 (td, *J* = 6.7, 4.3 Hz, 1 H), 4.61 (dd, *J* = 5.3, 1.2 Hz, 1 H), 4.54-4.50 (m, 1 H), 4.46 (t, *J* = 9.6 Hz, 1 H), 4.19-3.98 (m, 3 H), 3.80-3.62 (m, 4 H), 3.21 (dd, *J* = 13.4, 4.6 Hz, 1 H), 1.92-1.31 (m, 12 H), 1.11-1.02 (m, 4 H), 0.93-0.80 (m, 4 H); ^{13}C NMR (125 MHz, d_6-DMSO) d 197.7, 197.3, 171.3, 171.2, 170.93, 170.91, 170.6, 170.3, 161.8, 161.5, 158.6, 158.5, 139.0, 138.9, 135.1, 135.0, 130.1, 122.74, 122.71, 116.51, 116.48, 116.3, 113.4, 113.1, 105.34, 105.31, 83.1, 82.9, 80.69, 80.66, 67.9, 67.8, 66.1, 55.8, 54.71, 54.66, 54.5, 54.4, 53.3, 53.2, 42.92, 42.88, 41.73, 41.68, 32.35, 32.28, 29.5, 29.3, 28.71, 28.67, 26.82, 16.76, 26.75, 26.67, 26.65, 26.23, 26.21, 21.12, 21.07, 19.64, 19.55, 19.51, 19.45, 14.4, 14.3. HRMS m/z (M+H$^+$) Calcd for $C_{34}H_{47}N_4O_8$ $(M+H)^+$: 639.3394. Found: 639.3393.

8-Cyclohexyl-N-[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(S)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoxethyl]pentyl]-3a(*R*),4,6,6a(*S*)-tetrahydro-7,10-dioxo-2,5-(propanoxy[1,3]benzenoethaniminoethano)-5H-1,3-dioxolo[4,5-c]pyrrole-4(*S*)-carboxamide (25) Compound **25** was prepared from carboxylic acid **17** and amine **16** according to the procedures described above for the preparation of compounds **18**. The two P1 diastereomers were not separable by flash chromatography. It was tested as a mixture of two diastereomers. The ratio of two isomers was in the range of 1:1.5 to 1: 0.67 depending on reaction conditions. ^{1}H NMR (500 MHz, d_6-DMSO) d 8.77-8.31 (m, 3H), 7.69-7.66 (m, 1 H), 7.40-7.30 & 7.18-7.09 (m, 5 H), 6.80-6.63 (m, 4 H), 5.83-5.79 (m, 1 H), 5.07-4.87 (m, 2 H), 4.77-4.37 (m, 4 H), 4.21-4.01 (m, 4 H), 3.84-3.54 (m, 3 H), 3.44-

3.31 (m, 1 H), 3.24-3.19 (m, 1 H), 3.09 & 2.92 & 2.83 & 2.82 (s, 6 H), 1.88-1.56 (m, 13 H), 1.31-1.01 (m, 7 H), 0.97-0.81 (m, 4 H); ^{13}C NMR (125 MHz, d_6-DMSO) d 197.3, 195.5, 170.5, 170.4, 170.3, 169.9, 169.6, 169.52, 169.47, 169.1, 167.7, 167.6, 159.1, 158.4, 157.6, 154.6, 138.2, 138.0, 137.75, 137.71, 137.5, 137.4, 129.24, 129.18, 129.0, 128.8, 128.54, 128.50, 127.8, 127.7, 121.8, 121.5, 115.5, 115.3, 114.6, 113.3, 110.6, 106.3, 81.1, 79.9, 79.7, 74.0, 67.1, 66.8, 57.1, 55.3, 54.8, 52.9, 42.7, 42.3, 41.92, 41.91, 41.50, 41.45, 41.2, 40.7, 36.5, 36.4, 35.27, 35.25, 35.24, 35.1, 30.4, 30.3, 29.0, 28.9, 28.6, 28.4, 28.33, 28.32, 27.77, 27.75, 27.4, 27.3, 25.9, 25.8, 25.65, 25.62, 25.4, 25.3, 25.2, 24.8, 22.9, 21.6, 13.6, 13.4. HRMS m/z ($M+H^+$) Calcd for $C_{44}H_{59}N_6O_{10}$ $(M+H)^+$: 831.4293. Found: 831.4307.