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Synthesis of Chiral Piperazinones as Versatile Scaffolds for Peptidomimetics

Frank Rübsam, Ralph Mazitschek and Athanassios Giannis*

Institut für Organische Chemie der Universität Karlsruhe, Richard-Willstaetter-Allee 2, D-76128 Karlsruhe, Germany

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Abstract—Chiral piperazinones were synthesized as conformationally restricted peptidomimetics starting from inexpensive and readily available D-glucosamine hydrochloride and amino acid methyl esters. Different synthetic strategies are devised to allow attachment of side chains imitating the parent peptide as shown for the RGD motif. © 2000 Elsevier Science Ltd. All rights reserved.

A successful method for the development of peptidomimetics[1,2] involves synthesis of conformationally restricted compounds, i.e. locally or globally constrained peptide analogs that imitate the receptor-bound conformation of the endogenous ligands as closely as possible.[3–5] Bridging between two consecutive amino acids in a peptide leads to a dipeptide mimetic, the flexibility of which is limited compared to regular dipeptides. Piperazin-2-ones are obtained formally through $N_i \leftrightarrow C^{\alpha}_{i+1}$ cyclization of a dipeptide and can be used as an element for the generation of a local constriction. Furthermore piperazin-2-ones can serve as a rigid template exposing substituents in a conformation resembling the side chains of a parent peptide in its bioactive conformation.[6,7] Substituted piperazinones are components of a wide variety of bioactive compounds e.g. Leu-Enkephalin analogs,[8] cholecystokinin receptor antagonists,[9,10] RGD (Arg-Gly-Asp) mimetics,[11,12] neurokinin-2 receptor ligand,[13] substance P analogues,[14] inhibitors of farnesyltransferase,[15] growth hormone secretagogue,[16] and inhibitors of glycosidases.[17]

Chiral syntheses that enable an entry to all diastereomers of several 3,6-disubstituted piperazinones have recently been published by our group.[18]

Here we report a simple synthetic approach to chiral piperazinones which starts from cheap starting materials, proceeds in good yields, and allows easy scale-up to multigram scale.

As outlined in Fig. 1, the piperazinone scaffold **1** can be functionalized either on the ring nitrogens, through transformations of the hydroxymethyl side chain, or through derivatization of the amino acid residues introduced in C-3 position.

Starting from Cbz-D-glucosamine (**2**), which is easily accessible by N-protection of glucosamine hydrochloride with Cbz-Cl,[19] compound **4** is obtained by reductive amination with glycine methyl ester hydrochloride (**3**) in refluxing methanol in 80% yield (Scheme 1). Protection of the newly generated secondary amine with Boc_2O proceeds smoothly and also appears to be crucial for the final cyclization step (vide supra). Oxidative cleavage of the polyol **5** with $NaIO_4$ and subsequent rapid reduction of the aldehyde with sodium borohydride yields the amino alcohol **6** in 87% (2 steps). The piperazinone **7** is obtained through removal of the Cbz group and spontaneous cyclization in 93% yield.

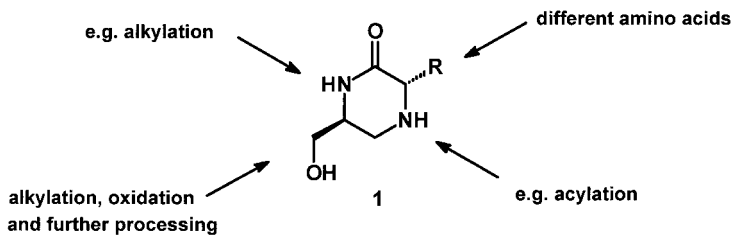

Figure 1.

Keywords: piperazinones; peptide mimetics; biologically active compounds; amino sugars.

* Corresponding author. Tel.: +49-721-608-3209, fax: +49-721-608-7652; e-mail: giannis@ochhades.chemie.uni-karlsruhe.de

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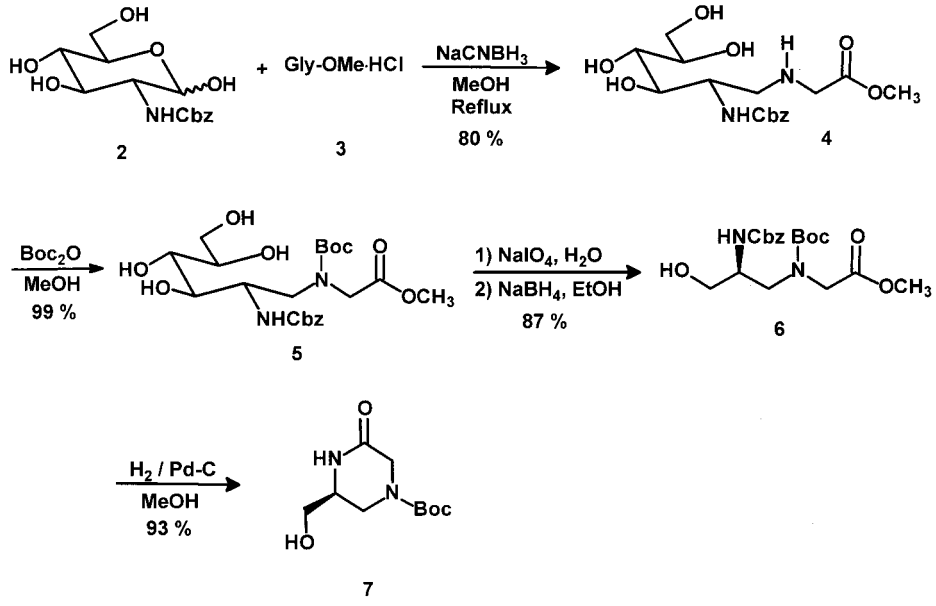
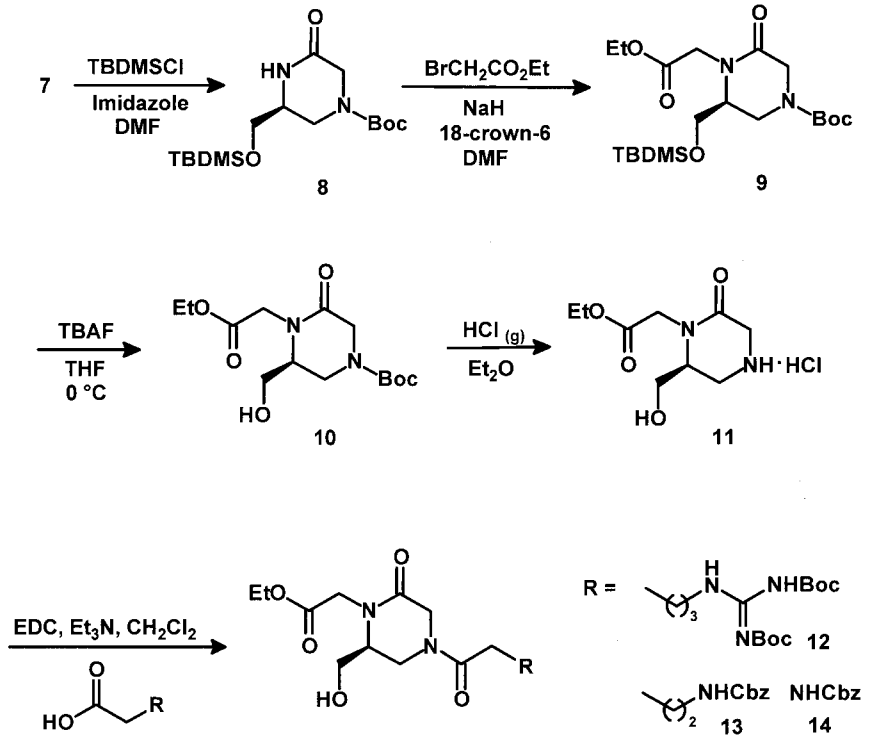


Scheme 1.

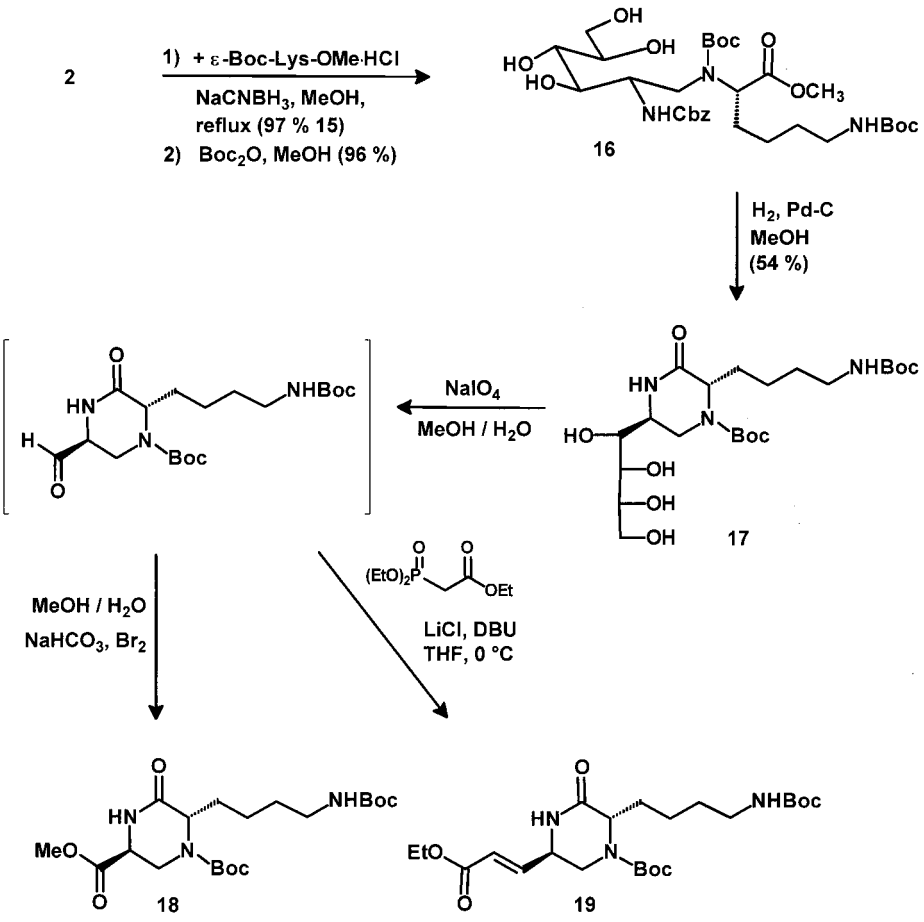
The piperazinone **7** is further elaborated into functionalized target molecules **12**–**14** which represent mimetics of the RGD peptide motif. This sequence is found in several proteins, such as fibrinogen, fibronectin, vitronectin, laminin, osteopontin, and von Willebrand factor and serves as an endogenous ligand for integrins, which in turn play a critical role in cell-to-cell adhesion, cell-to-extracellular matrix adhesion and interaction.[20,21] These interactions determine important biological phenomena: cell morphology, differentiation and viability, cellular traffic, organogenesis, angiogenesis, and blood clotting, making the RGD motif a promising target in modern drug design efforts.[22,23]

As shown in Scheme 2, we chose to derivatize the scaffold along the N-1–N-4 axis. A strategy to provide compounds bearing substituents along the C-2–C-6 axis is described in Scheme 3.

Protection of the hydroxy function as TBDMS ether **8** proceeds smoothly in 98% yield. Subsequent coupling **9** with ethyl bromoacetate (96%) followed by removal of



Scheme 2.



Scheme 3.

the silyl protecting group with TBAF (98%) yields **10**. Removal of the Boc group was achieved with gaseous hydrochloric acid in diethyl ether since standard conditions (TFA) gave rise to side products. Furthermore **11** precipitates as the hydrochloride in cold diethyl ether and can easily be purified by simple filtration. Finally amino functionalized side chains are introduced via carbodiimide (EDC) mediated coupling with 5-(Bis-Boc-guanidino) valeric acid (27%) Cbz protected γ-amino butyric acid (GABA) (89%), or glycine (43%), respectively, to afford the protected target compounds **12**–**14**.

A slightly different synthetic approach sets the stage for a functionalization along the C-2–C-6 axis of the piperazinone scaffold (Scheme 3). Reductive amination with L-ϵ-Boc-lysine methyl ester hydrochloride in refluxing methanol in the presence of sodium borohydride afforded precursor **15** (97%). Protection of the newly generated secondary amine with Boc_2O proceeds smoothly in 96% yield to afford the polyol **16**. Removal of the Cbz group was achieved by hydrogenation in the presence of Pd-C as catalyst to yield piperazinone **17** in 54%. Oxidative cleavage of the polyol side chain with sodium periodate afforded the corresponding aldehydes, which were used without further purification, although isolation is feasible for characterization. However, to avoid racemization the aldehydes were rapidly further elaborated as shown. Oxidation according to a procedure devised by Lichtenthaler[24] with bromine in an aqueous methanol/hydrogen carbonate solution lead to the formation of methyl ester **18** (47%), whereas the vinylogous derivative **19** was obtained by reaction with triethyl phosphonoacetate (56%, 2 steps).

In summary, we have synthesized chiral piperazinones as scaffolds for the design of peptidomimetics from inexpensive and readily available starting materials. We have further shown their versatility by attaching different functionalized groups along different axis of the rigid template which are presented as to mimic the structure and the function of RGD peptides. The described strategy should enable the design and synthesis of various other small molecule peptidomimetics based on the piperazinone scaffold.

Biological properties of selected compounds obtained by this strategy will be published elsewhere.

Experimental

Solvents were purified in the usual way. Water sensitive reactions were carried out in flame dried glassware under argon. Thin layer chromatography: Merck precoated tlc-plates, silica gel 60; column chromatography: silica gel 60 (Merck, 40–63 μm). Melting points: Büchi SMP 20. Melting points are uncorrected. ^1H and ^{13}C NMR: Bruker AC-200, Bruker AC-250, Bruker AM-400. Mass

spectrometry: A. E. I. MS-30, MS-50, ion source 180°C and Finnigan MAT MS 70, FAB: Kratos Concept 1H, matrix=*m*-nitrobenzylic alcohol. Elemental analyses were performed at the Institute of Organic Chemistry and Biochemistry, Bonn, microanalytical department. Optical rotations were determined on a Perkin–Elmer 241 polarimeter.

Methyl [2-(*S*)-benzyloxycarbonylamino-3(*R*), 4(*S*), 5(*R*), 6-tetrahydroxy-hexyl]-amino-acetate (4). Cbz-glucosamine (**2**) (12.56 g, 40 mmol), glycine methyl ester hydrochloride (**3**) (5.52 g, 44 mmol), and sodium cyanoborohydride (5.52 g, 88 mmol) were suspended in methanol (1 L) and stirred for 24 h at reflux. Evaporation and separation of the reaction mixture by column chromatography (CH_2Cl_2–MeOH–NH_3=40:10:1) afforded 12.5 g **4** (81%) as an off-white solid. R_F: 0.48 (CH_2Cl_2–MeOH–NH_3=40:10:1), mp 117–119°C (decomposition). $[\alpha]_D^{20}=-6.2$ (c=2.25, MeOH); ^{1}H NMR (250 MHz, CD_3OD): δ (ppm)=2.66–2.90 (m, 2H, CH_2), 3.34 (s, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.53–4.00 (m, 4H), 3.71 (s, 3H, CO_2CH_3), 5.09 (m, 2H, Benzyl-CH_2), 7.20–7.43 (m, 5H, Ph); ^{13}C NMR and DEPT 135 (100.63 MHz, DMSO-d_6): δ (ppm)=48.86 (CH_2), 49.32 (CH_2), 51.48 (CO_2CH_3), 53.22 (CH), 63.38 (CH_2), 65.13 (CH_2), 68.97 (CH), 70.43 (CH), 71.27 (CH), 127.26 (CH), 127.50 (CH), 128.24 (CH), 137.11 (C_q), 156.17 (CO-carbamate), 171.28 (CO_2CH_3); FAB-MS: calcd for $C_{17}H_{26}N_2O_8$: m/z=386.1689, found m/z=387.1 ($M+H^+$); $C_{17}H_{26}N_2O_8{\cdot}1H_2O$: calcd C 50.47, H 6.98, N 6.93; found C 50.12, H 6.58, N 6.83.

Methyl [2-(*S*)-benzyloxycarbonylamino-3(*R*), 4(*S*), 5(*R*), 6-tetrahydroxy-hexyl]-*tert.*-butoxy-carbonylamino-acetate (5). A solution of **4** (12.49 g, 32.3 mmol) and Boc_2O (12.54 g, 57.4 mmol, 1.5 equiv.) in methanol (100 mL) was stirred at 25°C until tlc analysis indicated completion of the reaction. After evaporation of the solvent the resulting white foamy solid was washed several times with warm petroleum ether to remove excess Boc_2O. Evaporation yielded 15.15 g (96%) **5** as a white solid. R_F: 0.95 ($CHCl_3$–MeOH=4:1), mp 92–94°C (decomposition); $[\alpha]_D^{20}=-16.0$ (c=1.05, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm)=1.32 (s, 9H, $C(CH_3)_3$), 2.76 (br, 4H), 3.20–4.15 (m, 6H), 3.64 (s, 3H, CO_2CH_3), 4.95 (d, J=12.2 Hz, 1H, benzyl-H_A), 5.13 (d, J=12.2 Hz, 1H, benzyl-H_B), 6.08 (br, 0.4H, NH), 7.13–7.37 (m, 5H, Ph); FAB-MS: $C_{22}H_{32}N_2O_{10}$ calcd m/z=486.2213, found m/z=509.2 ($M+Na^+$).

Methyl [2(*S*)-benzyloxycarbonylamino-3-hydroxy-propyl-(*tert.*-butoxycarbonyl)-amino]-acetate (6). To an ice cold suspension of **5** (2.48 g, 5.1 mmol) in water (50 mL) a solution of sodium metaperiodate (3.28 g, 19.8 mmol) in water (30 mL) was added dropwise. Since the mixture turns sticky after a few minutes, CH_2Cl_2 (20 mL) is added and the mixture is stirred for 1 h at 0°C. The aqueous layer is separated and extracted with CH_2Cl_2 (3×20 mL). The combined organic solutions are dried over $MgSO_4$ and the solvent evaporated. To avoid racemization of the α-amino aldehyde the white solid is rapidly dissolved in methanol (50 mL) and sodium borohydride (820 mg, 21.68 mmol) is added at 0°C. After stirring for 1 h at 0°C the solvent is removed in vacuo, and the residue is dissolved in water and neutralized with a sat. aqueous solution of NH_4Cl. The aqueous solution is extracted three times with CH_2Cl_2. The combined organic solutions are dried over $MgSO_4$ and the solvent evaporated to afford 1.75 g (87%) of **6**. Colorless sticky oil; R_F (aldehyde): 0.71 (CH_2Cl_2–MeOH=10:1); R_F (alcohol): 0.65 (CH_2Cl_2–MeOH=10:1); $[\alpha]_D^{20}=-14.1$ (c=1.00, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm)=1.38 (s, 9H, $C(CH_3)_3$), 3.16 (dd, J=3.7, 13.7 Hz, 0.75H, OH), 3.30–4.00 (m, 7H, 3×CH_2, CH), 3.68 (s, 3H, CO_2CH_3), 5.03 (s, 2H, benzyl-CH_2), 5.59 (d, J=7 Hz, 0.8H, NH), 7.15–7.40 (m, 5H, Ph); FAB-MS: $C_{19}H_{28}N_2O_7$ calcd m/z=396.1896 found m/z=397.2 ($M+H^+$).

4-*tert.*-Butoxycarbonyl-6(*S*)-hydroxymethyl-piperazin-2-one (7). A solution of **6** (1.74 g, 4.39 mmol) in methanol (40 mL) was hydrogenated for 24 h at 25°C and normal pressure in the presence of 10% Pd–C. After filtration of the catalyst and evaporation to dryness the crude product was further purified by column chromatography (CH_2Cl_2–MeOH=10:1) to afford 754 mg (75%) **7** as a white solid. Mp 104–105°C; R_F: 0.36 (CH_2Cl_2–MeOH=10:1); $[\alpha]_D^{20}=+31.3$ (c=1.10, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm)=1.44 (s, 9H, $C(CH_3)_3$), 2.35 (br, 0.3H, OH), 3.30–3.90 (m, 4H), 3.90–4.15 (m, 3H), 7.30 (br, 1H, NH); FAB-MS: $C_{10}H_{18}N_2O_4$ calcd m/z=230.1266 found m/z=230.1 (M^+); $C_{10}H_{18}N_2O_4$ calcd C 52.16, H 7.88, N 12.17, found (%) C 52.36H 7.88 N 12.11

4-*tert.*-Butoxycarbonyl-6-(*S*)-(*tert.*-butyldimethylsiloxymethyl)-piperazin-2-one (8). A solution of **7** (4.00 g, 17.4 mmol) in DMF (50 mL) was treated with TBDMSCl (6.55 g, 43.5 mmol) and imidazole (1.42 g, 21.0 mmol) at 25°C. The reaction mixture was stirred for 5 h before water (50 mL) was added. The aqueous layer was separated and extracted with Et_2O (3×20 mL), and the combined extracts were dried ($MgSO_4$), and concentrated in vacuo. Finally azeotropic removal of water with toluene (3×20 mL) afforded 5.87 g (98%) **8** as a white waxy solid. Mp 94–95°C; R_F: 0.89 (CH_2Cl_2–MeOH=10:1); $[\alpha]_D^{20}=-14.1$ (c=1.83, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=0.00 (s, 6H, $Si(CH_3)_2$), 0.83 (s, 9H, $SiC(CH_3)_3$), 1.38 (s, 9H, $C(CH_3)_3$), 3.00–4.30 (m, 7H, 3-CH_2, 5-CH_2, 6-CH, CH_2–O), 6.20 (br, 1H, NH); HR-MS: $C_{16}H_{32}N_2O_4Si$ calcd m/z=344.2131 found m/z=344.2138 (M^+).

Ethyl (4-*tert.*-butoxycarbonyl-6(*S*)-*tert.*-butyldimethylsiloxymethyl-2-oxo-piperazin-1-yl)-acetate (9). A solution of **8** (4.00 g, 11.6 mmol) in dry DMF was treated with sodium hydride (60% oil dispersion in mineral oil, 480 mg, 23.2 mmol) at 0°C under argon. After addition of 18-crown-6 (5 mg), ethyl bromoacetate (2.9 g, 17.4 mmol) was added dropwise under argon at 0°C. The resulting reaction mixture was stirred at 25°C for 4 h before being treated with brine (20 mL) at 0°C. The aqueous layer was separated and extracted with Et_2O (3×20 mL), and the combined extracts were dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (petroleum ether–ethyl acetate=1:1) afforded 4.8 g (96%) **9** as a pale yellow oil. R_F: 0.72 (petroleum ether–ethyl acetate=1:1); $[\alpha]_D^{20}=+56.1$ (c=1.50, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=0.00 (s, 6H, $Si(CH_3)_2$), 0.82 (s, 9H, $SiC(CH_3)_3$), 1.23 (t, J=7.6 Hz, 3H, $CO_2CH_2CH_3$), 1.40 (s, 9H, $(CH_3)_3$), 3.15–3.48 (m, 2H), 3.53–3.68 (m, 2H), 3.72–3.90 (d,

J=17 Hz, 2H), 4.05–4.40 (m, 2H), 4.15 (q, J=7.6 Hz, 2H, $CO_2CH_2CH_3$), 4.45–4.60 (d, J=17 Hz, 1H); HR-MS: $C_{20}H_{38}N_2O_6Si$ calcd m/z=430.2499 found m/z=430.2503 (M^+).

Ethyl (4-*tert*.-butoxycarbonyl-6(*S*)-hydroxymethyl-2-oxo-piperazin-1-yl)-acetate (10). A solution of **9** (921 mg, 2.1 mmol) in THF (20 mL) at 0°C was treated dropwise with a solution of TBAF (1.01 g, 3.21 mmol) in THF (5 mL) under argon. Saturated aqueous NH_4Cl (5 mL) and ethyl acetate (5 mL) were added, and the aqueous phase was extracted with ethyl acetate (3×10 mL). The combined organic phases were washed with water (3×10 mL) and brine (3×10 mL), dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (petroleum ether–ethyl acetate=1:1) afforded 662 mg (98%) **10** as a colorless oil. R_F: 0.10 (petroleum ether–ethyl acetate=1:1); $[\alpha]_D^{20}$=+73.0 (c=1.00, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=1.23 (t, J=7.2 Hz, 3H, CH_3), 1.40 (s, 9H, $C(CH_3)_3$), 3.20–4.40 (m, 11H, 5×CH_2, CH); FAB-MS: $C_{14}H_{24}N_2O_6$ calcd m/z=316.1 found m/z=316.1 (M^+).

Ethyl (6(*S*)-hydroxymethyl-2-oxo-piperazin-1-yl)-acetate hydrochloride (11). A solution of **10** (300 mg, 0.95 mmol) in diethyl ether was cooled to 0°C and treated with gaseous HCl for 15 min. The precipitate was filtered, washed several times with cold diethyl ether, and dried in vacuo to afford 141 mg (59%) **11** as an off-white solid. Mp 115–118°C (decomposition); R_F: 0.59 (CH_2Cl_2–MeOH–NH_3=40:10:1); $[\alpha]_D^{20}$=+12.2 (c=2.90, DMSO); ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm)=1.18 (t, J=6.6 Hz, 3H, CH_3), 2.95–4.60 (m, 12H), 9.35 (br, 3H, NH); FAB-MS: $C_9H_{17}N_2O_4Cl$ calcd m/z=252.0877 found m/z=217.1 ($M-Cl^+$).

Ethyl [6(*S*)-hydroxymethyl-2-oxo-4-(5-(bis-*tert*.-butoxycarbonyl-guanidino)-valeriyl)-piperazin-1-yl]-acetate (12). A solution of **11** (100 mg, 0.40 mmol) and 5-(Bis-tert.-butoxycarbonyl-guanidino)-valeric acid[25] (150 mg, 0.42 mmol) in 5 mL CH_2Cl_2 was treated with triethylamine (90 mg, 0.9 mmol) at 0°C. After stirring for 10 min, EDC (96 mg, 0.5 mmol) was added and the reaction mixture was stirred for another hour at 0°C. To drive the reaction to completion, the mixture was stirred over night at room temperature. After evaporation to dryness the residue was dissolved in ethyl acetate. The organic phase was extracted with water, 5% aqueous citric acid, water saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (CH_2Cl_2–MeOH=10:1) afforded 60 mg (27%) **12** as a colorless oil. R_F: 0.80 (CH_2Cl_2–MeOH–NH_3=40:10:1); $[\alpha]_D^{20}$=+56.8 (c=1.64, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm)=1.42 (t, J=7.0 Hz, 3H, CH_3), 1.62 (s, 9H, $C(CH_3)_3$), 1.67 (s, 9H, $C(CH_3)_3$), 1.78–1.88 (br, 4H), 2.55–2.73 (br, 2H), 3.35–4.80 (m, 11H), 4.35 (q, J=7.0 Hz, 2H); FAB-MS: $C_{25}H_{43}N_5O_9$ calcd m/z=557,3 found m/z=558,2 ($M+H^+$).

Ethyl [6(*S*)-hydroxymethyl-2-oxo-4-(4-benzyloxycarbonylamino-butyryl)-piperazin-1-yl]-acetate (13). A solution of **11** (100 mg, 0.47 mmol) and Cbz-γ-butyric acid (112 mg, 0.52 mmol) in CH_2Cl_2 was treated with triethylamine (90 mg, 0.9 mmol) at 0°C. After stirring for 10 min, EDC (96 mg, 0.5 mmol) was added and the reaction mixture was stirred for another hour at 0°C. To drive the reaction to completion, the mixture was stirred for additional 12 h at 25°C. After evaporation to dryness the residue was dissolved in ethyl acetate. The organic phase was extracted with water, 5% aqueous citric acid, water, saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (CH_2Cl_2–MeOH=10:1) afforded 183 mg (89%) **13** as a colorless oil. R_F: 0.80 (CH_2Cl_2–MeOH–NH_3=40:10:1); $[\alpha]_D^{20}$=+23.4 (c=1.22, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=1.05–1.32 (m, 3H, CH_3), 1.65–2.00 (m, 2H), 2.18–2.60 (m, 2H), 3.10–3.30 (m, 2H), 3.31–4.30 (m, 11H), 4.48–5.20 (m, 1H), 5.00–5.28 (m, 3H), 7.20–7.43 (m, 5H, Ph); FAB-MS: $C_{21}H_{29}N_3O_7$ calcd m/z=435.2 found m/z=436.2 ($M+H^+$).

Ethyl [6(*S*)-hydroxymethyl-2-oxo-4-(2-benzyloxycarbonylamino-acetyl)-piperazin-1-yl]-acetate (14). A solution of **11** (100 mg, 0.47 mmol) and Cbz-glycine (109 mg, 0.52 mmol) in CH_2Cl_2 was treated with triethylamine (90 mg, 0.9 mmol) at 0°C. After stirring for 10 min, EDC (96 mg, 0.5 mmol) was added and the reaction mixture was stirred for another hour at 0°C. To drive the reaction to completion, the mixture was stirred for additional 12 h at 25°C. After evaporation to dryness the residue was dissolved in ethyl acetate. The organic phase was extracted with water, 5% aqueous citric acid, water, saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (CH_2Cl_2–MeOH=10:1) afforded 82 mg (43%) **12** as a colorless oil. R_F: 0.81 (CH_2Cl_2–MeOH–NH_3=40:10:1)); $[\alpha]_D^{20}$=+16.4 (c=1.10, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=0.70–1.00 (m, 2H), 1.00–1.70 (m, 3H, CH_3), 3.14–4.85 (m, 12H), 5.00–5.15 (m, 2H, benzylic-CH_2), 5.60–5.82 (m, 1H), 7.20–7.50 (m, 5H, Ph); HR-MS: $C_{19}H_{25}N_3O_7$ calcd m/z=407.1693 found m/z=407.1696 (M^+).

Methyl 2-(*S*)-[(2-(*S*)-benzyloxycarbonylamino-3(*R*), 4(*S*), 5(*R*), 6-tetrahydroxy-hexyl)-amino]-6-*tert*.-butoxycarbonylamino-hexanoate (15). A suspension of Cbz-glucosamine **2** (1.66 g, 5.29 mmol) and ε-Boc-LysOMe·HCl (1.73 g, 5.79 mmol) in methanol (130 mL) was treated with sodium cyanoborohydride (0.73 g, 11.64 mmol). The mixture was stirred for 8 h under reflux before the volatiles were removed in vacuo. Flash chromatography (CH_2Cl_2–MeOH–NH_3=40:10:1) of the resulting white solid afforded 2.85 g (97%) **15** as a white solid. Mp 102–104°C; R_F=0.67 (CH_2Cl_2–MeOH–NH_3=40:10:1); $[\alpha]_D^{20}$=−5 (c=1.00, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=1.40 (s, 9H, $(CH_3)_3$), 1.20–1.70 (m, 6H, 3×CH_2), 1.80–2.13 (m, 2H, CH_2), 2.75–4.16 (m, 12H), 3.69 (s, 3H, CO_2CH_3), 4.65–4.90 (br, 2H), 4.97–5.20 (m, 2H, Benzyl-CH_2), 7.20–7.45 (m, 5H, Ph); FAB-MS: $C_{26}H_{43}N_3O_{10}$ calcd m/z=557.2948 found m/z=558.3 ($M+H^+$); $C_{26}H_{43}N_3O_{10}$·1/2H_2O, calcd C 55.09, H 7.83, N 7.42; found C 55.29, H 7.79, N 7.74.

Methyl 2-(*S*)-[(2-(*S*)-benzyloxycarbonylamino-3(*R*), 4(*S*), 5(*R*), 6-tetrahydroxy-hexyl)-*tert*.-butoxycarbonylamino]-6-*tert*.-butoxycarbonylamino-hexanoate (16). A solution of **15** (1.00 g, 1.79 mmol) in methanol (20 mL) was treated with Boc_2O (5.86 g, 2.69 mmol). After stirring for 12 h at 25°C the solvent was removed in vacuo, and the residue was

washed several times with warm petroleum ether to remove residual Boc_2O. Removal of the volatiles in vacuo afforded 1.13 g (96%) **16** as a white solid. Mp >240°C; R_F=0.77 (CH_2Cl_2–MeOH–NH_3=40:10:1); $[\alpha]_D^{20}$=−36.9 (*c*=0.98, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm)=1.40 (s, 9H, $(CH_3)_3$), 1.46 (s, 9H, $(CH_3)_3$), 1.20–1.70 (m, 6H, 3×CH_2), 1.80–2.13 (m, 2H, CH_2), 2.75–4.16 (m, 12H), 3.69 (s, 3H, CO_2CH_3), 4.65–4.90 (br, 2H), 4.97–5.20 (m, 2H, Benzyl-CH_2), 5.58–5.97 (br, 1H, NH), 7.20–7.45 (m, 5H, Ph); FAB-MS: $C_{31}H_{51}N_3O_{12}$ calcd *m*/*z*=657.3473 found *m*/*z*=658.3 ($M+H^+$).

4-*tert.*-Butoxycarbonyl-3(*S*)-(4-*tert.*-butoxycarbonylamino-butyl)-6(*S*)-[1′(*R*), 2′(*S*), 3′(*R*), 4′-tetrahydroxybutyl]-piperazin-2-one (17). A solution of **16** (800 mg, 1.22 mmol) in dry methanol (20 mL) was hydrogenated at 25°C and 3 bar pressure in the presence of 10% Pd-C. Upon completion the catalyst is filtered off over celite and the solvent was removed in vacuo. Column chromatography (CH_2Cl_2–MeOH=2:1) afforded 324.8 mg (54%) **17** as a white solid. Mp 148°C; R_F=0.72 (CH_2Cl_2–MeOH=2:1 +1% NH_3); $[\alpha]_D^{20}$=+76.1 (*c*=2.39, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$–D_2O): δ (ppm)=1.10–2.00 (m, 24H, 2×$(CH_3)_3$ and 3×CH_2), 2.92–3.12 (m, 2H), 3.14–3.30 (d, *J*=12 Hz, 1H), 3.40–3.90 (m, 5H), 3.95–4.10 (d, *J*=13.6 Hz, 1H), 4.30–4.50 (br, 1H), 4.90–5.10 (br, 1H); FAB-MS: $C_{22}H_{41}N_3O_9$ calcd *m*/*z*=491.2843 found *m*/*z*=492.3 ($M+H^+$) and 514.3 ($M+Na^+$).

Methyl 3-[4-*tert.*-butoxycarbonyl-3(*S*)-(4-*tert.*-butoxycarbonyl-amino-butyl)-2-oxo-piperazin-6(*S*)-yl]-carboxylate (18). A solution of **17** (150 mg, 0.31 mmol) in H_2O–MeOH=2:1 (20 mL) was cooled to 0°C and treated with sodium metaperiodate (196 mg, 0.92 mmol). After stirring for 30 min the reaction mixture was treated with solid $NaHCO_3$ (126 mg, 1.5 mmol) and bromine (160 mg, 1 mmol) was added dropwise over 30 min. After stirring for additional 45 min the reaction was quenched with sodium thiosulfate, filtered, extracted with chloroform (3×10 mL), dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (CH_2Cl_2–MeOH=10:1) afforded 62 mg (47%) **18** as a white solid. Mp 76–78°C; R_F=0.7 (CH_2Cl_2–MeOH=10:1); $[\alpha]_D^{20}$=+72.3 (*c*=1.56, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=1.10–2.00 (m, 24H, 2×$(CH_3)_3$ and 3×CH_2), 3.03–3.17 (m, 2H), 3.29–3.52 (m, 1H), 3.65 (s, 3H, CO_2CH_3), 3.98–4.06 (m, 1H), 4.30–4.70 (m, 3H), 6.20 (br, 1H, NH); FAB-MS: $C_{20}H_{35}N_3O_7$ calcd *m*/*z*=429.2475 found *m*/*z*=430.2 ($M+H^+$).

Ethyl 3-[4-*tert.*-butoxycarbonyl-3(*S*)-(4-*tert.*-butoxycarbonylamino-butyl)-2-oxo-piperazin-6(*S*)-yl]-acrylate (19). A solution of **17** (250 mg, 0.51 mmol) in MeOH–H_2O=2:1 (25 mL) was cooled to 0°C and treated with sodium metaperiodate (327 mg, 1.53 mmol). After stirring for 30 min the reaction mixture was extracted with CH_2Cl_2 (3×10 mL). The combined organic phases were dried ($MgSO_4$), and concentrated in vacuo. The crude aldehyde was kept at 0°C and used without further purification. Anhydrous lithium chloride (45 mg, 1.018 mmol) was suspended under argon atmosphere in absolute THF (25 mL) and triethyl phosphonoacetate (225 mg, 1.02 mmol) was added. The mixture was stirred for 15 min at r.t. and then cooled to 0°C. After addition of DBU (155 mg, 1.02 mmol), the mixture was stirred for another 30 min before the aldehyde component (dissolved in 10 mL THF) was added dropwise through a septum. After 0.5–1 h the reaction was complete according to tlc and was quenched with aqueous saturated NH_4Cl and then extracted with diethyl ether (3×50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. Column chromatography (CH_2Cl_2–MeOH=10:1) afforded 119 mg (56% for 2 steps) **19** as a white solid. Mp 135–138°C; R_F=0.46 (CH_2Cl_2–MeOH=10:1); $[\alpha]_D^{20}$=+84.1 (*c*=1.55, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm)=1.20 (t, *J*=7 Hz, 3H, CH_3), 1.32 (s, 9H, $(CH_3)_3$), 1.37 (s, 9H, $(CH_3)_3$), 1.25–1.55 (m, 4H, CH_2), 1.65–1.78 (m, 1H), 1.80–1.96 (m, 1H), 3.05 (br, 2H), 3.12–3.40 (m, 1H), 3.95–4.20 (m, 1H), 4.10 (q, *J*=7 Hz, 2H, $CO_2CH_2CH_3$), 4.30–4.70 (m, 2H), 5.90 (d, *J*=15.3 Hz, 1H, CO_2CH=CH), 6.15 (br, 1H, amide-NH), 6.85 (dd, *J*=15.5, 4.8 Hz, 1H, CH=C*H*–CH); HR-MS: $C_{23}H_{39}N_3O_7$ calcd *m*/*z*=469.2799 found *m*/*z*=469.2794 (M^+); $C_{23}H_{39}N_3O_7 \cdot 1/3H_2O$, calcd C 58.09, H 8.41, N 8.84, found C 58.05, H 8.17, N 8.78.

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