Alkylated Sugar Amino Acids: A New Entry towards Highly Functionalised Dipeptide Isosters

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General Experimental

(*R*)-tert-Butansulfinyl amide, 4.0 M HCl in 1,4-dioxane, *N*-(9-Fluorenylmethoxycarbonyloxy)-succinimide (FmocOSu), diisopropylethylamine (DiPEA), citric acid, methylmagnesium bromide (MeMgBr), phenylmagnesium bromide (PhMgBr), isopropylmagnesium chloride (iPrMgCl), benzylmagnesium chloride (BnMgCl), boron trifluoride etherate (BF₃·OEt₂), anhydrous zinc chloride (ZnCl₂), iodobenzene diacetate (BAIB), free radical 2,2,6,6-tetramethyl-1-piperidinyl (TEMPO), *N,N'*-diisopropylcarbodiimide (DIC), *N*-[(dimethylamino-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) were available from commercial suppliers and used without further purification. Reactions were carried out under an atmosphere of argon when anhydrous solvents were used. Column chromatography was performed using Silica gel 60 (Baker, 0.040-0.063 mm) with distilled EtOAc and petroleum ether (40-60 fraction) as eluents. Reactions were followed by TLC analysis on silicagel (Schleicher & Schuell, F 1500 LS 254) or HPTLC aluminium sheets (Merck, silicagel 60, F254), with detection by UV-absorption (254 nm) where applicable and charring at 150°C with 20% H₂SO₄ in EtOH (25 g/l), ninhydrin (3 g/l) in EtOH/AcOH (100/3, v/v), NH₄(Mo)₇O₂₄·4H₂O (25 g/l) and NH₄Ce(SO₄)₄·2H₂O (10 g/l) in 10 % aq. H₂SO₄ or KMnO₄ (2%) in 1% aq. K₂CO₃. ¹H-NMR and ¹³C-NMR spectra were recorded with a Bruker AC-200 (¹H-NMR 200 MHz, ¹³C-NMR 50 MHz), Bruker WM-300 (¹H-NMR 300 MHz; ¹³C-NMR 75 MHz), Bruker AV-400 (¹H-NMR 400 MHz, ¹³C-NMR 100 MHz), Bruker DMX-600 (¹H-NMR 600 MHz, ¹³C-NMR 150 MHz). Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. ¹H-¹H COSY, ¹H-¹³C HMQC and ¹³C-APT were used in assignment of ¹H and ¹³C data. LCMS analysis was performed on a Jasco HPLC system with detection at 214 nm and 254 nm (Alltima C-18 column, 4.0 mm x 250 mm, 5µm particle size, 1.0 ml/min flow) and a Perkin Elmer/SCIEX API 165 mass spectrometer (ESI interface). HR-MS: API QSTAR^{TM} Pulsar (Applied Biosystems).

General procedure 1: Organometallic addition, desulfinylation and N-Fmoc protection.

Compound **4** was coevaporated with anhydrous toluene and dissolved in anhydrous toluene or DCM ($c = 0.015$ M). The organic solution was cooled to -78°C and stirred for 10 min followed by slow addition of the organometallic reagent (3 equiv, 1.0-3.0 M solution in THF). The reaction mixture was stirred at -78°C until TLC indicated full conversion of all starting material (30 min-3 h) The reaction was quenched with addition of a saturated aqueous solution of NH_4Cl and the mixture was allowed to warm to rt. The reaction mixture was extracted several times with DCM and the combined organic phases were washed with a saturated aqueous solution of NaHCO_3 , H_2O , dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was dissolved in MeOH ($c = 0.01$ M) and treated with HCl in dioxane (4.0 M, 5 equiv) for 30 min. The reaction mixture was concentrated and coevaporated with MeOH. The residue was dissolved in 1,4-dioxane and DCM (1:1 v/v, $c = 0.01$ M) followed by addition of DiPEA (3 equiv) and FmocOSu (1.5 equiv) The reaction mixture was stirred for 3 h before diluting with DCM and H_2O . The organic phase was washed with an aqueous solution of citric acid (10 wt%), a saturated aqueous solution of NaHCO_3 and with H_2O . After drying (Na_2SO_4), the organic phase was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (10→20 vol% EtOAc/petroleum ether).

General procedure 2: Regioselective debenzylation, deacetylation and oxidation.

Compound **8**, **10**, **12** or **14** was dissolved in Ac_2O and AcOH (2:1 v/v, $c = 0.02$ M) and anhydrous ZnCl_2 (10 equiv) was added in one portion. The reaction mixture was stirred for 15 h under argon at rt and quenched with H_2O . The solution was extracted several times with DCM and the combined organic fractions were washed with H_2O , several times with a saturated aqueous solution of NaHCO_3 , and finally with H_2O . After drying (Na_2SO_4), the organic fraction was concentrated *in vacuo* and coevaporated with EtOH and toluene. The residue was dissolved in MeOH ($c = 0.06$ M)

and HCl in 1,4-dioxane (4.0 M, 5 equiv) was added dropwise. The reaction mixture was stirred for 1 h at rt and concentrated *in vacuo*. The oily residue was dissolved in DCM and H₂O (1:1 v/v, *c* = 0.05 M) followed by addition of BAIB (2.2 equiv) and free radical TEMPO (0.1 equiv). The reaction mixture was stirred for 5 h and quenched by addition of a saturated aqueous solution of Na₂S₂O₃. The biphasic system was extracted several times with DCM and the combined organic phases were washed with an aqueous solution of citric acid (10 wt%) and H₂O, dried (Na₂SO₄) and concentration *in vacuo*. The residue was purified by silica gel column chromatography (0.25 vol% AcOH, 30→50 vol% EtOAc/petroleum ether).

(*R*)-2-methylpropane-2-sulfinic acid [1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-yl)methylidene]amide (4)

A solution of known formyl 2,3,4,6-tetra-*O*-benzyl-β-D-C-glucopyranoside (701 mg, 1.27 mmol) in anhydrous DCM (10 mL) was treated with anhydrous CuSO₄ (506 mg, 3.17 mmol) and (*R*)-*tert*-butanesulfinyl amide (154 mg, 1.27 mmol). The reaction mixture was stirred for 16 h at rt and filtered through a pad of celite. The celite was washed several times with DCM and the combined organic phases were concentrated. The residue was purified by silica gel column chromatography (10→20 vol% EtOAc/petroleum ether) yielding compound **4** (583 mg, 70%) as a white solid.

¹H NMR (CDCl₃) δ = 8.06 (d, *J* 4.72 Hz, 1H, CHN), 7.32-7.18 (m, 20H, 4 Ph), 4.92 (s, 2H, CH₂Ph), 4.83 (d, *J* 10.76 Hz, 1H, CH^aH^bPh'), 4.77 (d, *J* 10.56 Hz, 1H, CH^aH^bPh''), 4.63 (d, *J* 12.15 Hz, 1H, CH^aH^bPh'''), 4.61 (d, *J* 10.84 Hz, 1H, CH^aH^bPh'), 4.56 (d, *J* 10.57 Hz, 1H, CH^aH^bPh''),

4.54 (d, J 12.16 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}''''$), 4.18 (dd, J 4.72 and 9.73 Hz, 1H, H-1), 3.80 (t, J 8.86 Hz, 1H, H-3), 3.75 (m, 2H, $\text{H}^{\text{a}}\text{-6}$ and $\text{H}^{\text{b}}\text{-6}$), 3.70 (t, J 9.06 Hz, 1H, H-4), 3.60 (d, J 9.41 Hz, 1H, H-2), 3.56-3.51 (m, 1H, H-5) and 1.22 (s, 9H, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ = 165.14 (CHN), 138.15, 137.93, 137.79, 137.15, 128.33, 128.20, 128.05, 127.81 and 127.57 (Ph), 86.51 (CH), 79.63 (2x CH), 79.04 (CH), 77.59 (CH), 75.56 (CH_2), 75.01 (2x CH_2), 73.38 (CH_2), 68.37 (CH_2) 57.00 (C) and 22.25 (CH_3). $[\alpha]_{\text{D}}^{20}$ = -92.6° (c = 1.0, CHCl_3). HRMS $[\text{M}+\text{H}]^+$ m/z required 656.3045, found 656.3030.

Fmoc-N-1-(1-aminoethyl)-2,3,4,6-tetra-*O*-benzyl- β -D-glucopyranose (10)

Reaction of compound **4** (365 mg) in anhydrous toluene with MeMgBr (3.0 M in THF), as described in the general procedure 1, afforded compound **10** in a yield of 317 mg (72%) as a colorless oil. ^1H NMR (CDCl_3) δ = 7.76 (d, J 7.55 Hz, 2H, Ar^{Fmoc}), 7.60 (d, J 7.37 Hz, 2H, Ar^{Fmoc}), 7.39-7.25 (m, 22H, 4 Ph and Ar^{Fmoc}), 7.20 (d, J 1.55 Hz, 2H, Ar^{Fmoc}), 5.18 (d, J 10.04 Hz, 1H, CHNH), 4.95 (d, J 2.75 Hz, 2H CH_2Ph), 4.84 (d, J 10.72 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}'$), 4.78 (d, J 9.74 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}''$), 4.61 (d, J 9.43 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}'''$), 4.60 (d, J 10.84 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}'$), 4.59 (d, J 11.51 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}''''$), 4.56 (d, J 12.20 Hz, 1H, $\text{CH}^{\text{a}}\text{H}^{\text{b}}\text{Ph}''''$), 4.44 (d, J 7.02 Hz, 2H, $\text{CH}_2^{\text{Fmoc}}$), 4.29 (m, 1H, CHNH), 4.23 (t, J 7.00 Hz, CH^{Fmoc}), 3.75-3.70 (m, 3H, H-3, $\text{H}^{\text{a}}\text{-6}$ and $\text{H}^{\text{b}}\text{-6}$), 3.60 (t, J 9.55 Hz, 1H, H-4), 3.46-3.43 (m, 2H, H-2 and H-5), 3.20 (d, J 9.55 Hz, 1H, H-1) and 1.25 (d, J 6.79 Hz, 3H, CH_3). ^{13}C NMR (CDCl_3) δ = 155.93 (CO), 143.94, 143.92, 141.29, 138.52, 138.08, 137.98, 137.87, 128.64, 128.50, 128.48, 128.44, 128.43, 127.96, 127.92, 127.83, 127.71,

127.64, 127.57, 127.07, 125.05 and 119.96 (Ph and Ar^{Fmoc}), 87.09 (CH), 80.73 (CH), 78.74 (CH), 78.52 (CH), 78.25 (CH), 75.53 (CH₂), 75.50 (CH₂), 75.07 (CH₂), 73.30 (CH₂), 69.02 (CH₂), 66.51 (CH₂), 47.34 (CH), 45.42 (CH) and 19.15 (CH₃). $[\alpha]_{\text{D}}^{20} = +18.2^{\circ}$ ($c = 1.0$, CHCl₃). HRMS $[M+Na]^+$ m/z required 812.3601, found 812.3563

Fmoc-N-6-(1-aminoethyl)-3,4,5-tri-O-benzyl- β -D-glucuronic acid (**11**)

Reaction of compound **10** (259 mg), following the general procedure 2, afforded compound **11** in a yield of 150 mg (64%) as a white foam. ¹H NMR (DMSO-*d*₆) δ = 7.83 (d, J 6.79 Hz, 2H, Ar^{Fmoc}), 7.70 (d, J 6.67 Hz, 2H, Ar^{Fmoc}), 7.38-7.37 (m, 19H, 3 Ph and Ar^{Fmoc}), 6.97 (d, J 6.78 Hz, 1H, CHNH), 4.83 (d, J 10.98 Hz, 1H, CH^aH^bPh), 4.78 (d, J 11.29 Hz, 1H, CH^aH^bPh), 4.70 (d, J 10.60 Hz, 1H, CH^aH^bPh'), 4.64 (d, J 10.36 Hz, 1H, CH^aH^bPh''), 4.61 (d, J 11.08 Hz, 1H, CH^aH^bPh'), 4.49 (d, J 10.16 Hz, 1H, CH^aH^bPh''), 4.41 (m, 1H, CH^aH^b(Fmoc)), 4.31 (m, 1H, CH^aH^b(Fmoc)), 4.23 (m, 1H, CH^{Fmoc}), 4.08 (m, 1H, CHNH), 3.90 (d, J 9.02 Hz, 1H, H-2), 3.72 (m, 1H, H-4), 3.64 (t, J 8.86 Hz, 1H, H-3), 3.36 (m, 2H, H-5 and H-6) and 1.15 (d, J 5.61 Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆) δ = 169.86 (CO), 155.80 (CO), 143.79, 143.62, 140.64, 138.35, 138.11, 137.84, 128.11, 128.01, 127.85, 127.60, 127.43, 127.31, 126.92, 125.04 and 119.89 (Ph and Ar^{Fmoc}), 85.15 (CH), 79.98 (CH), 79.37 (CH), 77.61 (CH), 77.30 (CH), 74.37 (CH₂), 74.01 (CH₂), 73.75 (CH₂), 65.32 (CH₂), 46.79 (CH), 44.90 (CH) and 18.43 (CH₃). $[\alpha]_{\text{D}}^{20} = +1.6^{\circ}$ ($c = 1.0$, CHCl₃). HRMS $[M+H]^+$ m/z required 714.3066, found 714.3021.

Cyclic tetramer **19**

Fmoc-Gly-Wang resin (**16**, 0.6 mmol/g) was treated with 20 vol% piperidine in DMF (twice 10 min). The resin was washed with DMF, MeOH, DCM, DMF, MeOH and DMF (twice). SAA **11** (1.0 equiv) was preactivated with HATU (0.9 equiv), HOAt (0.9 equiv) and 2,4,6-collidine (20 equiv) for 30 sec. in DMF and DCM (1:1 v/v, 1 mL) and the mixture was added to the resin (swirling 4 h). The resin was washed with DMF and the coupling with SAA **11** (1.0 equiv) was repeated. The resin was washed with DMF and capped with 5 vol% Ac₂O and 6 vol% DiPEA in DMF (10 min). The resin was washed with DMF, MeOH, DCM, DMF, MeOH and DMF (twice). The resin was treated with 20 vol% piperidine in DMF (twice 10 min). The resin was washed with DMF, MeOH, DCM, DMF, MeOH and DMF (twice). Fmoc-Gly-OH (5 equiv) was preactivated with HATU (4.95 equiv), HOAt (4.95 equiv) and 2,4,6-collidine (20 equiv) for 30 sec. in DMF and DCM (1:1 v/v, 1.5 mL) and the mixture was added to the resin (swirling 4 h). The resin was washed with DMF and capped with 5 vol% Ac₂O and 6 vol% DiPEA in DMF (10 min). The resin was washed with DMF, MeOH, DCM, DMF, MeOH and DMF (twice). The Fmoc group was removed by treatment with 20 vol% piperidine in DMF (twice 10 min) and the second SAA (**11**) and Fmoc-Gly-OH residue were coupled as described above. Subsequently, the Fmoc protective group was removed after which the resin was washed with DMF, MeOH, DCM, DMF, MeOH, DMF (twice) and finally with DCM. The resin was treated with TFA and DCM (1:1 v/v, 2 mL, 30 min). The filtrate was collected and the resin was washed with DCM (5x1 mL). The combined organic

fractions were diluted with toluene and concentrated *in vacuo*. LCMS (214 nm); R_t 13.97 min, $[M+H]^+ = 1079.7$, gradient: 0.1 vol% TFA and 50-90 vol% CH_3CN in H_2O , 26.00 min run.

The thus obtained crude linear tetramer **18** was dissolved in anhydrous DMF ($c \sim 1.0$ mM) and cooled to $0^\circ C$. HATU (2 equiv), HOAt (2 equiv) and 2,4,6-collidine were added to the solution and the reaction mixture was stirred overnight at $4^\circ C$. The solvent was concentrated *in vacuo* and cyclic tetramer **19** was purified by silica gel column chromatography (0→2 vol% MeOH in DCM). Yield: 12 mg (74%). ^1H NMR ($CDCl_3$) δ = 7.34-7.13 (m, 30H, 2x 3 Ph), 6.84 (br s, 2H, 2x NH^{Gly}), 6.54 (d, J 7.83 Hz, 2H, 2x $CHNH$), 5.01 (d, J 10.15 Hz, 2H, 2x CH^aH^bPh), 4.93 (d, J 11.06 Hz, 2H, 2x CH^aH^bPh'), 4.90 (d, J 11.07 Hz, 2H, 2x CH^aH^bPh''), 4.83 (d, J 10.01 Hz, 2H, 2x CH^aH^bPh'''), 4.80 (d, J 10.18 Hz, 2H, 2x CH^aH^bPh), 4.70 (d, J 10.02 Hz, 2H, 2x CH^aH^bPh''), 4.62 (m, 2H, 2x $CHNH$), 4.18 (dd, J 6.22 and 16.75 Hz, 2H, 2x $CH^aH^{b(Gly)}$), 3.86 (m, 4H, 2x H-1 and 2x H-2), 3.74 (m, 4H, 2x $CH^aH^{b(Gly)}$ and 2x H-4), 3.53 (t, J 9.35 Hz, 2H, 2x H-5), 3.33 (d, J 9.68 Hz, 2H, 2x H-6) and 1.25 (d, J 6.80 Hz, 6H, 2x CH_3). ^{13}C NMR ($CDCl_3$) δ = 169.18 (2x CO), 168.10 (2x CO), 138.16, 137.88, 137.78, 128.60, 128.48, 128.39, 128.36, 127.93, 127.83, 127.57 and 127.40 (Ph), 86.26 (2x CH), 81.32 (2x CH), 79.30 (2x CH), 78.06 (2x CH), 77.51 (2x CH), 75.73 (2x CH_2), 75.56 (2x CH_2), 75.45 (2x CH_2), 43.82 (2x CH_2), 43.04 (2x CH) and 19.01 (2x CH_3). LCMS (214 nm); R_t 15.89 min, $[M+H]^+ = 1061.7$, gradient: 0.1 vol% TFA and 70-90 vol% CH_3CN in H_2O , 26.00 min run. HRMS $[M+H]^+$ m/z required 1061.4882, found 1061.4912

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