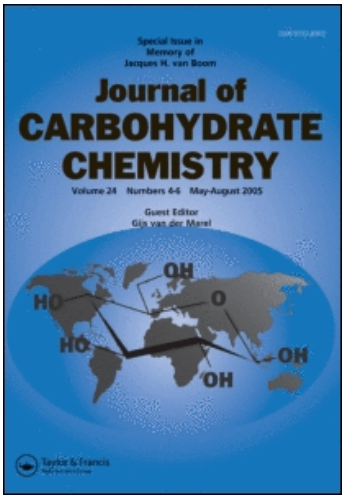

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Reductive Amination of the Lysine N^{ε}-Amino Group Leads to a Bivalent Glyco-Amino Acid Building Block Suited for SPPS

Alexander Schierholt[a]; Thisbe K. Lindhorst[a]
[a] Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Kiel, Germany

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Reductive Amination of the Lysine N^{ε}-Amino Group Leads to a Bivalent Glyco-Amino Acid Building Block Suited for SPPS

Alexander Schierholt and Thisbe K. Lindhorst

Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Otto-Hahn-Platz 4, D-24098 Kiel, Germany

Aiming at the synthesis of structurally altered glycopeptides to probe multivalency effects in carbohydrate recognition, a glyco-amino acid building block was prepared, carrying a bivalent carbohydrate branching unit. This new mannosylated lysine derivative was shown to be fully suitable for solid-phase peptide synthesis.

Keywords Glyco-amino acids, Reductive amination, Glycopeptides, Solid-phase synthesis

INTRODUCTION

Glycopeptides and glycoproteins are molecular key players in glycobiology and therefore their chemical synthesis has been intensively elaborated upon and reviewed.[1] In addition to the synthesis of glycopeptides according to the structures found in nature, it is of interest to prepare glycopeptide mimetics in order to study the biological consequences of structural alteration. In this context the investigation of multivalency is of particular interest, as multivalency effects are of prime importance in carbohydrate-protein interactions.

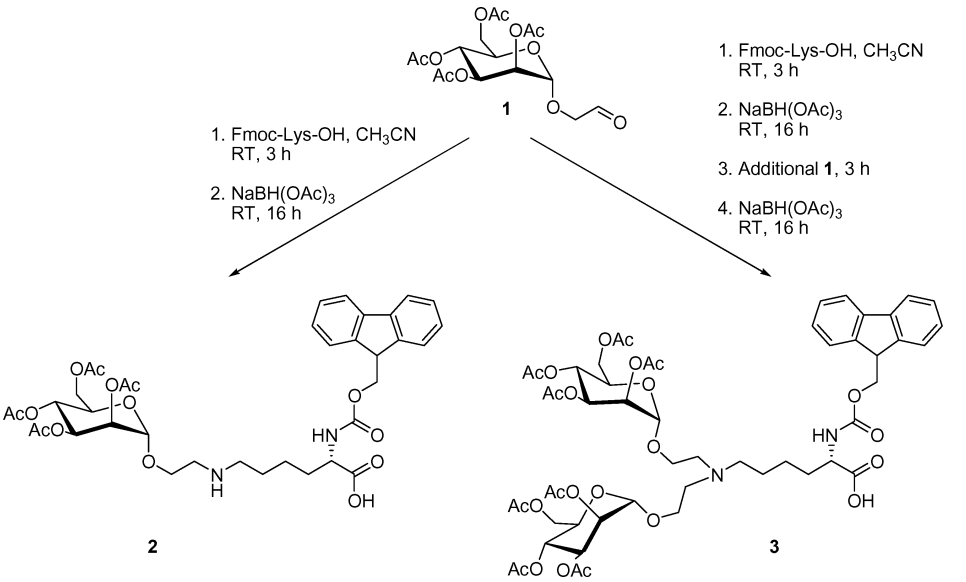
In connection with our interest in multivalent glycomimetics, we started a project utilizing peptide backbones for controlled scaffolding of multiple carbohydrate epitopes. For this work we had a need to prepare an array of relevant glyco-amino acids[2] suitable as building blocks in solid-phase peptide synthesis (SPPS). We realized that lysine bears the potential to allow the development of a branched diglyco-amino acid building block and thus we

Address correspondence to Thisbe K. Lindhorst, Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Otto-Hahn-Platz 4, D-24098 Kiel, Germany. E-mail.

aimed at the synthesis of N^{α}-(fluoren-9-ylmethoxycarbonyl)-N^{ε},N^{ε}-di-[2-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-ethyl]-L-lysine (**3**), which can be employed in SPPS to study the effects of sugar clustering in artificial glycopeptides.

RESULTS AND DISCUSSION

As reductive amination of carbonyl-functionalized glycosides was shown to be a successful method for carbohydrate conjugation[3] and clustering,[4] we envisaged this approach for addressing the ε-amino group of lysine in order to prepare N$^{\varepsilon}$-modified lysine-based glyco-amino acids. Starting with the well-known mannosidic aldehyde **1**[4] (Sch. 1), which can be easily obtained from the respective allyl mannoside by ozonolysis, we set up the first reductive amination with Fmoc-Lys-OH and sodium triacetoxyborohydride[5] in dichloromethane in order to obtain the glyco-amino acid **2**.



Scheme 1. Reductive amination of mannoside **1** leads to **2** or the branched glyco-amino acid building block **3** depending on the reaction conditions.

However, in dichloromethane as the solvent, the expected product was obtained in only minor amounts, whereas in acetonitrile[7] pure **2** was isolated in 76% yield. In this reaction a byproduct was formed, which aroused our interest. It was identified as the tertiary amine **3** carrying two carbohydrate moieties at N^{ε} of the amino acid.

Encouraged by this finding, we elaborated upon the reaction conditions in order to optimize the yield of **3**. To overcome steric hindrance, an iterative

approach of repeated application of mannoside **1** followed by addition of the reducing agent sodium triacetoxyborohydride gave the best results, furnishing the branched glyco-amino acid **3** (Sch. 1) in satisfying yield (41%).

In the next step the novel building block **3** was tested in solid-phase peptide synthesis on alanine-loaded Wang resin following the conventional Fmoc strategy (Sch. 2). A slight excess of the amino acid derivate **3** (two equivalents) was employed for coupling onto the resin, and then Fmoc deprotection with piperidine and peptide coupling with Fmoc-protected glycine, followed by removal of the Fmoc group and capping with acetic anhydride, resulted in the tripetide derivative **6**. It was cleaved from the resin using TFA and identified by MALDI-TOF mass spectrometry. Finally, careful de-*O*-acetylation according to the protocol of Zemplén and Pacsu[7] and purification by HPLC on reversed phase gave rise to pure glycopeptide **7** in 33% overall yield based on loaded resin.

Both new glyco-amino acid building blocks, **2** and bivalent **3**, are currently employed in our laboratory for the solid-phase synthesis of a variety of multivalent glycopeptide mimetics.[8]

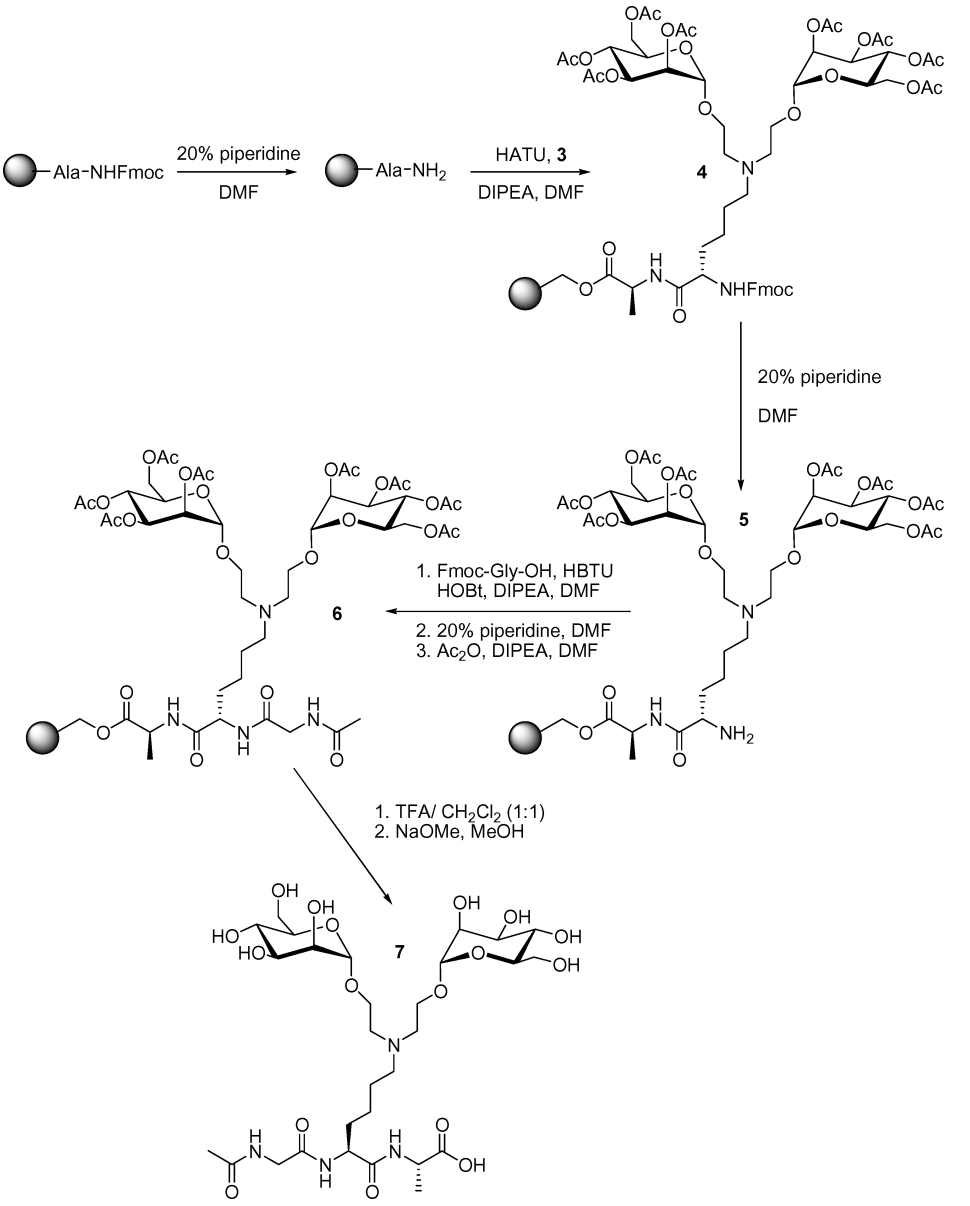
EXPERIMENTAL

General Methods

Thin layer chromatography was performed on silica gel plates (GF 254, Merck). Detection was effected by UV irradiation and subsequent charring with 10% sulfuric acid in EtOH followed by heat treatment. Flash chromatography was performed on silica gel 60 (230–400 mesh, particle size 0.040–0.063 mm, Merck) using distilled solvents. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Na-D-line: 589 nm, length of cell 1 dm). ^{1}H and ^{13}C spectra were recorded on Bruker DRX-500 and AV-600 spectrometers. Chemical shifts are reported relative to internal tetramethylsilane (δ 0.00 ppm) or D_2O (δ 4.76 ppm). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of nitrogen. Commercial reagents were used without purification unless otherwise noted.

*N^{α}-(Fluoren-9-ylmethoxycarbonyl)-N^{ε}-[2-(2′,3′,4′,6′-tetra-O-acetyl-α-*D*-mannopyranosyloxy)-ethyl]-*L*-lysine (**2**)*

A solution of aldehyde **1** (260 mg, 0.66 mmol) and Fmoc-lysine-OH (243 mg, 0.66 mmol) in dry CH_3CN (7 mL) was stirred for 3 h at rt. Then sodium triacetoxyborohydride (139 mg, 0.66 mmol) was added and stirring was continued overnight. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2, dried over $MgSO_4$, filtered, and evaporated. The crude product was purified on silica gel (EtOAc/MeOH, 1:1) to yield the title glyco-amino acid (376 mg, 0.50 mmol, 76%) as a colorless foam. $\boldsymbol{R_f}$: 0.25 (EtOAc/ MeOH,



Scheme 2. Application of **3** in SPPS on alanine-loaded Wang resin: synthesis of the unprotected branched glycopeptide **7**.

1:1), $[\alpha]_D^{20} = +28.6$ (c 0.7, CH_2Cl_2); **IR**: 3345 (br), 1740, 1568, 1407, 1223, 1135, 1024,821, 730, 647 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)**: $\delta = 7.74$ (d, 2H, $^3J =$ 7.4 Hz, Ar-H), 7.62–7.57 (m, 2H, Ar-H), 7.37 (t, 2H, $^3J = 7.4$ Hz, Ar-H), 7.28 (t, 2H, $^3J = 7.7$ Hz, Ar-H), 5.32–5.21 (m, 3H, H-2′, H-3′, H-4′), 4.84 (d, 1H, $^3J_{1,2} =$

1.4 Hz, H-1′), 4.34 (dd ~ t, 1H, ^{3}J = 9.1 Hz, CHH-Fmoc), 4.27 (dd, 1H, $^{3}J_{5,6}$ = 4.9 Hz, $^{2}J_{6,6'}$ = 12.2 Hz, H-6a′), 4.25–4.20 (m, 1H, H-α), 4.18 (dd ~ t, 1H, ^{3}J = 9.1 Hz, CHH-Fmoc), 4.12–4.08 (m, 1H, CH-Fmoc), 4.07 (dd, 1H, $^{3}J_{5,6'}$ = 2.6 Hz, $^{2}J_{6,6'}$ = 12.4 Hz, H-6b′), 3.99 (m_c, 1H, H-5′), 3.97–3.92 (m, 1H, OCHHHCH$_2$), 3.77–3.71 (m, 1H, OCHHCH$_2$), 3.21–3.10 (m, 2H, OCH$_2$CH$_2$), 3.01–2.92 (m, 2H, H-ε) 2.12, 2.08, 2.02, 1.96 (s, each 3H, 4 OAc), 1.82 (bs, 2H, H-β), 1.72 (bs, 2H, H-δ), 1.49 (bs, 2H, H-γ) ppm; **^{13}C NMR (150 MHz, DMSO-d$_6$)**: δ = 173.5 (COOH), 169.9, 169.4, 169.4, 169.3 (4 COCH$_3$), 158.7 (C(O)O-Fmoc), 145.1, 142.5, 128.8, 128.5, 127.1, 121.2 (12 C-Ar), 96.7 (C-1′), 68.7 (C-2′), 68.6 (C-3′), 67.8 (C-5′), 66.3 (CH$_2$-Fmoc), 65.7 (C-4′), 65.5 (OCH$_2$CH$_2$), 61.9 (C-6′), 56.7 (C-α), 55.2 (C-ε), 53.9 (OCH$_2$CH$_2$), 47.5 (CH-Fmoc), 32.3 (C-β), 28.2 (C-δ), 24.4 (C-γ), 20.4, 20.3, 20.2, 20.2 (4 COCH$_3$) ppm; **HRESI-MS**: Calcd for [C$_{37}$H$_{46}$N$_2$O$_{14}$+ Na]$^+$: 765.2847. Found: 765.2857.

*N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-N$^{\varepsilon}$,N$^{\varepsilon}$-di-[2-(2′,3′,4′,6′-tetra-O-acetyl-α-*D*-mannopyranosyloxy)-ethyl]-*L*-lysine (**3**)*

A solution of the aldehyde **1** (200 mg, 0.51 mmol) and Fmoc-lysine-OH (190 mg, 0.51 mmol) in dry CH$_3$CN (10 mL) was stirred for 3 h at rt. Then sodium triacetoxyborohydride (108 mg, 0.51 mmol) was added and stirring was continued overnight. Then additional glycoside **1** (200 mg, 0.51 mmol) was added, followed by sodium triacetoxyborohydride (108 mg, 0.51 mmol) after 3 h and then stirring was continued for 1 d. The solvent was removed in vacuo and the residue dissolved in CH$_2$Cl$_2$, dried over MgSO$_4$, filtered, and evaporated. The crude product was purified on silica gel (EtOAc/MeOH, 1:1) to yield the title compound (238 mg, 0.21 mmol, 41 %) as a colorless foam. ***R$_f$***: 0.7 (EtOAc/ MeOH, 1:1), $[\alpha]_D^{20}$ = + 23.6 (*c* 1.4, CH$_2$Cl$_2$); **IR**: 3348 (br), 1738, 1560, 1366, 1216, 1040, 740 cm^{-1}; **^{1}H NMR (600 MHz, MeOH-d$_4$)**: δ = 7.82 (d, 2H, ^{3}J = 7.6 Hz, Ar-H), 7.69 (t, 2H, ^{3}J = 8.3 Hz, Ar-H), 7.41 (t, 2H, ^{3}J = 7.2 Hz, Ar-H), 7.34 (t, 2H, ^{3}J = 6.8 Hz, Ar-H), 5.33–5.26 (m, 6H, 2 H-2′, 2 H-3′, 2 H-4′), 4.94 (bs, 2H, H-1′), 4.37 (bs, 2H, CH$_2$-Fmoc), 4.29 (dd, 2H, $^{3}J_{5,6}$ = 4.7 Hz, $^{2}J_{6,6'}$ = 12.1 Hz, 2 H-6a′), 4.24 (dd ~ t, 1H, ^{3}J = 6.5 Hz, CH-Fmoc), 4.12 (dd, 2H, $^{3}J_{5,6'}$ = 1.8 Hz, $^{2}J_{6,6'}$ = 12.0 Hz, 2 H-6b′),4.12–4.09 (m, 3H, 2 H-5′, H-α), 3.98–3.88 (m, 2H, 2 OCHHHCH$_2$), 3.79–3.70 (m, 2H, 2 OCHHHCH$_2$), 3.18–3.08 (bs, 4H, 2 OCH$_2$CH$_2$), 2.88–2.80 (bs, 2H, H-ε), 2.15, 2.08, 2.05, 1.98 (s, each 6H, 8 OAc), 1.76 (bs, 2H, H-β), 1.66 (bs, 2H, H-δ), 1.49 (bs, 2H, H-γ) ppm; **^{13}C NMR (150 MHz, MeOH-d$_4$)**: δ = 172.3 (COOH), 171.6, 171.5, 171.5, 171.4 (8 COCH$_3$), 157.1 (C(O)O-Fmoc), 145.4, 142.5, 128.8, 128.1, 126.2, 120.9 (12 C-Ar), 98.9 (2 C-1′), 70.7 (2 C-2′), 70.3 (2 C-3′), 70.0 (2 C-5′), 67.7 (CH$_2$-Fmoc), 67.2 (2 C-4′), 66.1 (2 OCH$_2$CH$_2$), 63.6 (2 C-6′), 56.7 (C-α), 56.4 (C-ε), 54.4 (2 OCH$_2$CH$_2$), 48.5 (CH-Fmoc), 33.2 (C-β), 26.6 (C-δ), 24.4 (C-γ), 20.7, 20.6, 20.6, 20.6 (8 COCH$_3$) ppm; **HRESI-MS**: Calcd for [C$_{53}$H$_{68}$N$_2$O$_{24}$+ Na]$^+$: 1139.4060. Found: 1139.4093.

*N-Acetyl-glycyl-N$^{\varepsilon}$,N$^{\varepsilon}$-di-[2-(α-*D*-mannopyranosyloxy)-ethyl]-*L*-lysyl-*L*-alanine (**7**)*

The glycopeptide was assembled manually using a fritted glass reaction vessel according to a standard Fmoc SPPS protocol. Fmoc-Ala-Wang resin (110 mg, 0.088 mmol) was swollen in DMF (4 mL) for 2 h prior to the synthesis and then deprotected with 20% piperidine solution in DMF (2 × 15 min). The branched glyco-amino acid **3** (196 mg, 0.176 mmol) and HATU (67 mg, 0.176 mmol) were dissolved in DMF (3 mL) and shaken for 5 min, and then DIPEA (30 μL, 0.176 mmol) was added and this mixture was kept for another 2 min at rt before it was transferred to the reaction vessel that contained the resin. The reaction mixture was shaken overnight at rt, filtered, and washed with DMF (5 × 5 mL). Any unreacted amino groups were capped as acetamides by treatment of the resin with a solution of Ac_2O (160 μL) and DIPEA (280 μL) in 3 mL DMF (1 × 60 min, 1 × 30 min). For the next peptide coupling step Fmoc protection was removed with piperidine and was washed with DMF, and a solution of Fmoc-Gly-OH (105 mg, 0.352 mmol), HOBt (47 mg, 0.352 mmol), HBTU (120 mg, 0.316 mmol), and DIPEA (60 μL, 0.352 mmol) in DMF, which was prepared as for the initial coupling step, was added. The mixture was shaken for 4 h, filtered, washed with DMF, treated with piperidine, and capped. Cleavage of the glycopeptide from the resin was achieved with TFA/CH_2Cl_2 (1:1) (1 × 15 min, 1 × 10 min) and washing with EtOH. The solvent was removed in vacuo and the crude product was lyophilized from water and analyzed by MALDI-TOF mass spectrometry. Then it was dissolved in dry MeOH and sodium methoxide solution (27 mg NaOMe in 5 mL MeOH) was added. The mixture was stirred overnight, neutralized with Amberlite IR120 (H), filtered, and concentrated. The unprotected glycopeptide was purified by RP HPLC on a LiChrosorb RP-8 column at a flow rate of 10 mL/min using a linear gradient of 10% B to 100% B over 80 min to yield the title glycopeptide (21 mg, 28.8 μmol, 33%) as a white amorphous solid.

HPLC: t_R = 9.3 min (A = H_2O, B = acetonitrile + 1% TFA, 20% B → 100% B, 80 min, 10 mL/min); $[\alpha]_D^{20}$ = + 21.6 (*c* 0.9, MeOH); **^{1}H NMR (600 MHz, H_2O/D_2O 11:1)**: δ = 8.44 (d, 1H, $^3J_{H-\alpha,H-NH}$ = 6.4 Hz, NH_{Ala}), 8.21 (d, 1H, $^3J_{H\text{-}\alpha,H\text{-}NH}$ = 6.1 Hz, NH_{Gly}), 8.18 (d, 1H, $^3J_{H\text{-}\alpha,H\text{-}NH}$ = 7.2 Hz, NH_{Lys}), ~ 4.80 (2 H-1′, below H_2O peak, HSQC cross peak), 4.29 (m, 2H, H-α_{Ala}, H-α_{Lys}), 4.01–3.95 (m, 2H, 2 OC<u>H</u>HCH_2), 3.87 (bs, 2H, 2 H-2′), 3.82–3.79 (m, 2H, 2 OCH<u>H</u>CH_2), 3.81–3.78 (m, 2H, H-α_{Gly}), 3.78–3.73 (m, 2H, 2 H-6a′), 3.70–3.62 (m, 4H, 2 H-3′, 2 H-6b′), 3.55 (dd~t, 2H, ^3J = 9.9 Hz, 2 H-4′), 3.51–3.48 (m, 2H, 2 H-5′), 3.48–3.41 (m, 4H, 2 CH_2C<u>H</u>$_2$N), 3.22–3.16 (m, 2H, H-ε), 1.95 (s, 3H, NHAc), 1.80–1.72 (m, 1H, H-β_{Lys}), 1.72–1.62 (m, 3H, H-β'_{Lys}, H-δ), 1.38–1.34 (m, 2H, H-γ), 1.32 (d, 3H, $^3J_{H\text{-}\alpha Ala,H\text{-}\beta Ala}$ = 7.2 Hz, 3 H-β_{Ala}) ppm; **^{13}C NMR (150 MHz, D_2O)**: δ = 178.1–166.8 (<u>C</u>OOH, 2 <u>C</u>ONH, <u>C</u>OCH_3), 99.9 (2 C-1′), 73.2 (2 C-5′), 70.5 (2 C-3′), 69.8 (2 C-2′), 66.6 (2 C-4′), 61.1, 60.9 (2 C-6, 2 O<u>C</u>H_2CH_2), 53.9 (C-ε), 53.1 (C-α), 52.3 (2 CH_2<u>C</u>H_2N), 48.8 (C-α), 42.6 (C-α_{Gly}), 30.6

(C-β_{Lys}), 22.4 (C-δ), 22.0 (C-γ), 21.7 ($CO\underline{C}H_3$), 16.1 (C-β_{Ala}) ppm; **MALDI-TOF-MS**: Calcd for $[C_{29}H_{52}N_4O_{17}+ Na]^+$: 751.32. Found: 751.74.

REFERENCES

1. Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. Synthesis of Glycopeptides Containing Carbohydrate and Peptide Recognition Motifs. *Chem. Rev.* **2000**, *100*, 4495–4537; Pratt, M.R.; Bertozzi, C.R. Synthetic glycopeptides and glycoproteins as tools for biology. *Chem. Soc. Rev.* **2005**, *34*, 58–68; Becker, T.; Dziadek, S.; Wittrock, S.; Kunz, H. Synthetic Glycopeptides from the Mucin Family as Potential Tools in Cancer Immunotherapy. *Curr. Cancer Drug Targets* **2006**, *6*, 491–517; Buskas, T.; Ingale, S.; Boons, G.-J. Glycopeptides as versatile tools for glycobiology. *Glycobiology* **2006**, *16*, 113R–136R; Hojo, H.; Nakahara, Y. Recent Progress in the Field of Glycopeptide Synthesis. *Biopolymers (Peptide Science)* **2007**, *88*, 308–324.

2. Elizabeth, C.; Maljaars, P.; Halkes, K.M.; de Oude, W.L.; van der Poel, S.; Pijnenburg, N.J.M.; Kamerling, J.P. Preparation of S- and N-Linked Glycosylated Amino Acid Building Blocks for Solid-phase Glycopeptide Library Synthesis. *J. Carbohydr. Chem.* **2005**, *24*, 353–367.

3. Turnbull, W.B.; Pease, A.R.; Stoddart, J.F. Toward the synthesis of large oligosaccharide-based dendrimers. *Chem. Bio. Chem.* **2000**, *1*, 70–74.

4. Dubber, M.; Lindhorst, Th.K. Exploration of Reductive Amination for the Synthesis of Cluster Glycosides. *Synthesis* **2001**, 327–330.

5. Abdel-Magid, A.F.; Carson, K.G.; Harris, B.D.; Maryanoff, C.A.; Shah, R.D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem.* **1996**, *61*, 3849–3862.

6. Beshore, D.C.; Dinsmore, C.J. Preparation of Substituted Piperazinones via Tandem Reductive Amination–(*N*, *N*′-Acyl Transfer)-Cyclization. *Org. Lett.* **2002**, *4*, 1201–1204.

7. Zemplén, G.; Pacsu, E. Saponification of acetylated sugars and related substances. *Ber. Dtsch. Chem. Ges.* **1929**, *62*, 1613–1614.

8. Shaikh, H.; Sönnichsen, F.; Lindhorst, Th.K. Synthesis of glycocluster peptides. *Carbohydr. Res.* **2008**, *343*, 1665–1674.