Synthesis of Biotinylated Multipodal Glycoclusters on a Solid Support

Marika Karskela, Miriam von Usedom, Pasi Virta and Harri Lönnberg*

Department of Chemistry, University of Turku, FIN-20014 Turku, Finland

ABSTRACT

Trivalent glycoconjugates bearing, in addition to three different sugar ligands, a biotin side-arm have been synthesized on a solid support. The conjugates were assembled on an orthogonally protected pentaerythrityl tetramine core that was anchored to the support via a backbone amide linker. Peptide coupling chemistry was applied to elongate three of the branches with β-alanine and a fully acylated glycosylacetic acid. The fourth branch was levulinoylated and oximated with aminooxy derivatized D-biotin, followed by acidolytic release into solution. The acyl protecting groups were removed by methoxide ion catalyzed transesterification in methanol.

INTRODUCTION

Carbohydrate-protein interactions play a decisive role in initiation of many human diseases. Multiantennary oligosaccharides anchored to cell membranes usually recognize the proteins of extracellular pathogens,[1-3] although the opposite is also possible: carbohydrates may become recognized by the cell membrane proteins.[4-6] The interactions between carbohydrates and carbohydrate binding proteins, called lectins, are typically multipodal; high affinity binding is realized by simultaneous binding of several sugar ligands to subunits of an oligomeric protein structure, a phenomenon known as the 'cluster glycoside effect'.[7-14] In other words, a clustered arrangement of both the sugars and their binding sites is required for high affinity and specificity. Accordingly, a plethora of multipodal glycoconjugates have been designed and synthesized as drug candidates aimed at blocking microbial infections at an early stage.[15-20]

Usually lectins are specific for only one type of sugar and, hence, homoclusters are needed for recognition. However, some recent findings lend support for the existence of a heterocluster effect,

i. e. the existence of synergic recognition of two different sugars.[21-23] Preparation of glycoclusters containing two or even three different sugars, hence, is of interest, and some examples of the syntheses of such conjugates have been reported.[24] Short linear,[25] branched[26] and cyclic[27] peptide conjugates containing up to three different sugars, lysine branched structure containing two different sugars and one nucleoside,[28] and *N*,*N*-bis(2-aminoethyl)glycine branched structure bearing two different homotrimeric sugar clusters[29] have been prepared an a solid support. Pentaerythritol-[30] and cyclic peptide-derived[31] conjugates bearing two different sugars have been obtained by modular synthesis in solution. We now supplement the available repertoire of preparation of this kind of glycoclusters by introducing a solid-supported method for synthesis of trivalent glycoconjugates bearing additionally a biotin side-arm that allow convenient immobilization of the conjugates to avidin- or streptavidin-coated surfaces. Besides biotin, the approach can be extended to conjugation of the glycoclusters with any aminooxy-functionalized compound, such as DOTA derivatives[32], oligonucleotides[33-35] and peptides[36].

EXPERIMENTAL PROCEDURES

General Remarks. 1,4-Dioxane and MeOH were dried over 3 Å molecular sieves and CH_2Cl_2, DMF, DMSO, NMP and pyridine over 4 Å sieves. Solid reagents were dried over P_2O_5 in a vacuum desiccator. For Ac_2O-capping on a solid support, a 1:1 (*v/v*) mixture of capping solutions A [acetic anhydride–(2,6-lutidine)–THF, 1:1:8, *v/v*] and B [(*N*-methyl imidazole)–THF, 4:21, *v/v*] was used. The NMR spectra were recorded at 400 or 500 MHz. The chemical shifts are given in ppm, using internal TMS or solvent residual signal as a reference. Appropriate 1D and 2D NMR methods, e.g., TOCSY, COSY, HSQC, and HMBC, were used for peak assignment. Reactions on a solid support were followed by releasing a small aliquot of the resin bound compound acidolytically and subjecting this to LC-MS(ESI) analysis on a Thermo Hypersil Hypurity C18 (150 × 4.6 mm, 5 μm) analytical column at a flow rate of 1 mL min^{-1}. A linear gradient from 0.1 % TFA in H_2O-MeCN (1:1, *v/v*) to 0.1 % TFA in MeCN in 15 min was applied. For semipreparative purifications and desalting, a Thermo Hypersil-Keystone column (250 × 10 mm, 5 μm) at a flow rate of 3 mL min^{-1} was used. An isocratic elution with 0.1 % TFA and 85 % MeCN in H_2O (protocol A) or a linear gradient from 0.1 % TFA in H_2O to 0.1 % TFA in MeCN in 30 min (protocol B) was used for purifications. UV detection at the wavelength of 215 nm was applied throughout the work. The mass spectra were recorded using ESI ionization method.

2-(2,3,4,6-Tetra-*O*-toluoyl-*β*-D-glucopyranosyloxy)acetic acid (4). Allyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside **1**[27] (2.54 g, 3.67 mmol) was dissolved in a 4:1 (*v/v*) mixture of 1,4-dioxane and water (50 mL), and OsO_4 (325 μL, 2.5 wt% in 2-methylpropanol, 25.9 μmol) was added. The solution was stirred for 1 h, after which $NaIO_4$ (1.57 g, 7.34 mmol) was added in portions over 1 h. Stirring was continued for additional 2.5 h and then the volatiles were removed under reduced pressure. The residue was dissolved in EtOAc and washed with water. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The aldehyde obtained was dissolved in acetone (100 mL) and cooled to 0 ºC. CrO_3 (367 mg, 3.67 mmol) was dissolved in the smallest possible amount of water (0.7 mL) together with H_2SO_4 (204 μL, 3.67 mmol), and added dropwise to the reaction mixture. After 5 min, the ice water bath was removed and the reaction was allowed to proceed at ambient temperature for 80 min. The reaction was quenched by addition of 2-propanol (5 mL) and water (10 mL) and the organic solvents were evaporated. The residue was dissolved in CH_2Cl_2 and washed with water and saturated aqueous NaCl. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by silica gel chromatography (2% pyridine and 10% MeOH in CH_2Cl_2) to obtain 1.93 g (74%) of **4** as white powder. The ^{1}H and ^{13}C NMR spectra were consistent with the published data.[27]

(2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyloxy)acetic acid (5). Allyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside **2**[37] (1.90 g, 4.89 mmol) was subjected to oxidation as described above for allyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside **1**. The crude product was purified by silica gel chromatography (2% pyridine and 18% MeOH in CH_2Cl_2) to obtain 1.39 g (70%) of **5** as white powder. The ^{1}H and ^{13}C NMR spectra were consistent with the published data.[38]

(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)acetic acid (6). Allyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside **3**[37] (1.56 g, 4.02 mmol) was subjected to oxidation as described above for allyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside **1**. The crude product was purified by silica gel chromatography (2% pyridine and 18% MeOH in CH_2Cl_2) to obtain 0.89 g (54%) of **6** as white powder. ^{1}H NMR (18% CD_3OD in $CDCl_3$, 400 MHz) δ 5.41 [d, 1H, J = 3.2 Hz (avg.), H-4], 5.20 [dd, 1H, J = 7.8 (avg.) and 10.4 Hz, H-2], 5.08 [dd, 1H, J = 3.2 (avg.) and 10.4 Hz, H-3], 4.65 [d, 1H, J = 7.8 Hz (avg.), H-1], 4.30 (d, 1H, J = 15.8 Hz, OC<u>H</u>H), 4.20–4.11 (m, 3H, H-6 and OCH<u>H</u>), 3.97 (m, 1H, H-5), 2.16, 2.11, 2.06 and 2.00 (4×s, 4×3H, CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.9, 170.8, 170.4, and 170.3 (CO), 100.4 (C-1), 70.7 (C-5 and C-3), 68.9 (C-2), 67.1 (C-4), 66.6 (OCH_2), 61.2 (C-6), 20.6, 20.4, and 20.3 (<u>C</u>H_3CO). HRMS (ESI): $[M + Na]^+$ $C_{16}H_{22}NaO_{12}$ requires 429.1009, found 429.0990.

***tert*-Butyl 3-[(2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranosyloxy)acetamido]propanoate (7).** Compound **4** (1.61 g, 2.27 mmol) was converted to active ester and coupled to *β*-alanine as described below for the conversion of compound **6** to **8**. The crude product was purified by silica gel chromatography (3:2 mixture of EtOAc and petroleum ether), giving **7** in 84% yield (1.60 g). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.90, 7.86, 7.78, and 7.72 (4×d, 4×2H, J = 8.2 Hz, CH Tol), 7.20 (overlapping d's, 2×2H, CH Tol), 7.14 (d, 2H, J = 8.2 Hz, CH Tol), 7.08 (d, 2H, J = 8.2 Hz, CH Tol), 6.83 (t, 1H, J = 6.0 Hz, NH), 5.90 (dd, 1H, J = 9.6 and 9.9 Hz, H-3), 5.63 (dd, 1H, J = 9.6 and 9.9 Hz, H-4), 5.50 (dd, 1H, J = 7.8 and 9.9 Hz, H-2), 4.83 (d, 1H, J = 7.8 Hz, H-1), 4.62 (dd, 1H, J = 2.9 and 12.2 Hz, H-6a), 4.44 (dd, 1H, J = 5.7 and 12.2 Hz, H-6b), 4.34 (d, 1H, J = 15.1 Hz, OC<u>H</u>H), 4.15 (ddd, 1H, J = 2.9, 5.7 and 9.9 Hz, H-5), 4.08 (d, 1H, J = 15.1 Hz, OCH<u>H</u>), 3.40 (m, 2H, NHC<u>H</u>$_2$), 2.40, 2.37, 2.34, and 2.29 (4×s, 4×3H, CH_3-Ar), 2.34 (m, 2H, CH_2COO), 1.43 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9, 168.5, 166.3, 165.8, 165.5, and 165.3 (CO), 144.5, 144.4, 144.2, and 144.0 (C Tol), 130.0, 130.0, 129.9, 129.9, 129.4, 129.3, 129.3, and 129.2 (CH Tol), 126.8, 126.2, 126.1, and 126.1 (C Tol), 101.5 (C-1), 81.0 (C_q *t*-Bu), 72.9 (C-5), 72.3 (C-3), 72.0 (C-2), 69.3 (C-4), 69.1 (OCH_2), 62.9 (C-6), 35.2 (<u>C</u>H_2COO), 34.9 ($NHCH_2$), 28.2 [C(<u>C</u>$H_3)_3$], 21.8, 21.8, 21.8, and 21.7 (CH_3-Ar). HRMS (ESI): $[M + Na]^+$ $C_{47}H_{51}NNaO_{13}$ requires 860.3258, found 860.3220.

***tert*-Butyl 3-[(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)acetamido]propanoate (8).** Compound **6** (0.870 g, 2.14 mmol) was dried over P_2O_5 overnight and dissolved in dry 1,4-dioxane (9 mL) together with *N*-hydroxysuccinimide (0.300 g, 2.61 mmol). The solution was cooled on a cold water bath, and dicyclohexylcarbodiimide (DCC; 0.536 g, 2.60 mmol) in dry 1,4-dioxane (2 mL) was added. The reaction was allowed to proceed at room temperature for 4.5 h, followed by off-filtration of the urea byproduct and washing with dry dioxane (4 mL). For the coupling reaction, K_2CO_3 (0.328 g, 2.38 mmol) and *β*-alanine *tert*-butyl ester hydrochloride (0.429 g, 2.36 mmol) were dissolved in water (0.6 mL) and added to the solution of the active ester. After overnight (18 h) reaction, the reaction mixture was filtered, the filtrate evaporated to dryness and the residue dissolved in CH_2Cl_2. The organic phase was washed with water and dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by silica gel chromatography (from 7:3 to 9:1 mixture of EtOAc and petroleum ether) to obtain 0.971 g (85%) of **8** as colorless solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.88 (t, 1H, J = 5.8 Hz, NH), 5.40 (dd, 1H, J = 0.8 and 3.4 Hz, H-4), 5.24 (dd, 1H, J = 7.9 and 10.5 Hz, H-2), 5.03 (dd, 1H, J = 3.4 and 10.5 Hz, H-3), 4.51 (d, 1H, J = 7.9 Hz, H-1), 4.30 (d, 1H, J = 15.0 Hz, OC<u>H</u>H), 4.15 (m, 2H, H-6), 4.08 (d, 1H, J = 15.0 Hz, OCH<u>H</u>), 3.94 (m, 1H, H-5), 3.52 (m, 2H, NHC<u>H</u>$_2$), 2.46 (m, 2H, CH_2COO), 2.17, 2.12, 2.05, and 2.00 (4×s,

4×3H, CH_3CO), 1.46 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 170.5, 170.2, 170.1, 169.8, and 168.4 (CO), 101.0 (C-1), 81.1 (C_q *t*-Bu), 71.2 (C-5), 70.7 (C-3), 68.9 (C-2), 68.6 (OCH_2), 67.0 (C-4), 61.3 (C-6), 35.3 ($\underline{C}H_2COO$), 34.8 ($NHCH_2$), 28.2 [$C(\underline{C}H_3)_3$], 20.9, 20.8, 20.7, and 20.7 ($\underline{C}H_3CO$). HRMS (ESI): $[M + Na]^+$ $C_{23}H_{35}NNaO_{13}$ requires 556.2006, found 556.2018.

3-[(2,3,4,6-Tetra-*O*-toluoyl-*β*-D-glucopyranosyloxy)acetamido]propanoic acid (9). Compound **7** (1.38 g, 1.65 mmol) was dissolved in dry CH_2Cl_2 (4.5 mL) and trifluoroacetic acid (TFA; 1.5 mL) was added. After 2 h, the solution was evaporated to dryness and the residue was stored overnight in a vacuum desiccator over KOH and then dried over P_2O_5. The crude product still contained approximately 1 equiv. TFA (1.49 g of 86-percent pure product, quantitative yield). The product was used as such in the following reaction. ^1H NMR ($CDCl_3$, 500 MHz) δ 10.83 (br, 1H, OH), 7.89, 7.84, 7.78 and 7.72 (4×d, 4×2H, *J* = 8.2 Hz, CH Tol), 7.31 (br t, 1H, *J* = 6.1 Hz, NH), 7.19 (overlapping d's, 2×2H, CH Tol), 7.13 (d, 2H, *J* = 8.1 Hz, CH Tol), 7.07 (d, 2H, *J* = 8.1 Hz, CH Tol), 5.91 (dd, 1H, *J* = 9.6 and 9.8 Hz, H-3), 5.66 (dd, 1H, *J* = 9.6 and 9.9 Hz, H-4), 5.51 (dd, 1H, *J* = 7.8 and 9.8 Hz, H-2), 4.86 (d, 1H, *J* = 7.8 Hz, H-1), 4.64 (dd, 1H, *J* = 2.9 and 12.2 Hz, H-6), 4.43 (dd, 1H, *J* = 5.6 and 12.2 Hz, H-6'), 4.40 and 4.24 (2×d, 2×1H, *J* = 15.6 Hz, OCH_2), 4.16 (ddd, 1H, *J* = 2.9, 5.6 and 9.9 Hz, H-5), 3.50 (m, 2H, $NHC\underline{H}_2$), 2.55 (m, 2H, CH_2COO), 2.39, 2.35, 2.34 and 2.28 (4×s, 4×3H, CH_3-Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.1 (COOH), 170.8, 166.6, 165.9, 165.7 and 165.4 (CO), 144.8, 144.6, 144.4 and 144.3 (C Tol), 130.0, 130.0, 129.9, 129.4, 129.3, 129.3 and 129.2 (CH Tol), 126.7, 126.0, 126.0 and 125.9 (C Tol), 101.7 (C-1), 72.9 (C-5), 72.2 (C-3), 72.0 (C-2), 69.2 (C-4), 68.8 (OCH_2), 62.9 (C-6), 34.9 ($NHCH_2$), 33.4 ($\underline{C}H_2COOH$), 21.8 and 21.7 (CH_3-Ar) (peaks referring to TFA are not listed). HRMS (ESI): $[M + Na]^+$ $C_{43}H_{43}NNaO_{13}$ requires 804.2632, found 804.2622.

3-[(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)acetamido]propanoic acid (10). Compound **8** (0.948 g, 1.78 mmol) was deprotected as described above for the conversion of compound **7** to **9**. Compound **10** was obtained in quantitative yield, but only 79% pure (1.08 g) being contaminated by TFA. The product was used as such in the following reaction. ^1H NMR ($CDCl_3$, 500 MHz) δ 11.03 (br, 1H, COOH), 7.28 (br t, 1H, NH), 5.44 (dd, 1H, *J* = 0.8 and 3.4 Hz, H-4), 5.22 (dd, 1H, *J* = 7.9 and 10.5 Hz, H-2), 5.06 (dd, 1H, *J* = 3.4 and 10.5 Hz, H-3), 4.54 (d, 1H, *J* = 7.9 Hz, H-1), 4.40 and 4.22 (2×d, 2×1H, *J* = 15.5 Hz, OCH_2), 4.15 (m, 2H, H-6), 3.97 (m, 1H, H-5), 3.62 (m, 2H, $NHC\underline{H}_2$), 2.66 (m, 2H, CH_2COO), 2.18, 2.10, 2.07, and 2.01 (4×s, 4×3H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.3 (COOH), 171.1, 171.0, 170.6, 170.5, and 170.4 (CO), 101.1 (C-1), 71.2 (C-5), 70.5 (C-3), 69.1 (C-2), 68.1 (OCH_2), 67.0 (C-4), 61.4 (C-6), 34.9 ($NHCH_2$), 33.4 ($\underline{C}H_2COO$),

20.9, 20.7, 20.7, and 20.6 ($\underline{C}H_3CO$) (peaks referring to TFA are not listed). HRMS (ESI): [M + Na]$^+$ $C_{19}H_{27}NNaO_{13}$ requires 500.1380, found 500.1398.

Synthesis of acid chlorides. 3-[(2,3,4,6-Tetra-*O*-toluoyl-*β*-D-glucopyranosyloxy)acetamido]propanoyl chloride (**11**) and 3-[(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)acetamido]propanoyl chloride (**12**) were prepared by treating the corresponding acids, **9** (1.49 g, 1.64 mmol) and **10** (390 mg, 645 μmol), with $SOCl_2$ (50 equiv.). The reaction mixtures were incubated at 38º C for 1 h, after which the volatiles were evaporated. Residues were co-evaporated three times with dry CH_2Cl_2 to obtain the products in quantitative yield (1.36 g of **11** and 350 mg of **12**) as white foams. The products were used in the subsequent reactions without characterization.

(2,3,4,6-Tetra-*O*-toluoyl-*α*-D-mannopyranosyloxy)acetic acid (14). Allyl 2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside[37] (**13**; 3.2 g, 8.2 mmol) was dissolved in 0.1 mol L^{-1} NaOMe/MeOH solution and stirred for 1 h. The mixture was neutralized by addition of a strong cation-exchange resin (Dowex 50WX8-200, H$^+$-form), filtered and evaporated to dryness. The residue was dissolved in dry pyridine and toluoyl chloride (13 mL, 98 mmol) was added. The reaction was stirred at 50º C overnight and diluted with CH_2Cl_2. The organic phase was washed with water and then with saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by silica gel chromatography (CH_2Cl_2) to obtain 4.3 g (75%) of allyl 2,3,4,6-tetra-*O*-toluoyl-*α*-D-mannopyranoside as white foam. The toluoylated compound (3.7 g, 5.3 mmol) was dissolved in a 4:1 (*v/v*) mixture of 1,4-dioxane and water (40 mL) and OsO_4 (502 μL, 2.5 wt% in 2-methylpropanol, 0.040 mmol) was added. The solution was stirred for 1 h, after which $NaIO_4$ (2.3 g, 10.8 mmol) was added. Stirring was continued for additional 1.5 h and then the solution was diluted with CH_2Cl_2 and washed with water. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by silica gel chromatography (20 → 40% EtOAc in petroleum ether, then 5% MeOH in CH_2Cl_2) to obtain 1.1 g (29%) of 2,3,4,6-tetra-*O*-toluoyl-*α*-D-mannopyranosyloxy)acetaldehyde. The aldehyde (0.94 g, 1.4 mmol) was dissolved in acetone (25 mL) and cooled to 0 ºC. CrO_3 (135 mg, 1.4 mmol) was dissolved in the smallest possible amount of water (0.3 mL) together with H_2SO_4 (75 μL, 1.4 mmol) and added dropwise to the reaction mixture. After 5 min, the ice water bath was removed and the reaction was allowed to proceed at ambient temperature for 70 min. The reaction was quenched by addition of 2-propanol (5 mL) and water (10 mL) and the organic solvents were evaporated. The residue was dissolved in CH_2Cl_2 and washed with water and saturated aqueous NaCl. The combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by

silica gel chromatography (2% pyridine and 10% MeOH in CH_2Cl_2) to obtain 0.73 g (75%) of **14** as white foam. ^{1}H NMR (10% CD_3OD in $CDCl_3$, 500 MHz) δ 7.97, 7.90, 7.83, and 7.70 (4×d, 4×2H, J = 8.1 Hz, CH Tol), 7.21 (d, 2H, J = 8.1 Hz, CH Tol), 7.15 (d, 4H, J = 8.1 Hz, CH Tol), 7.05 (d, 2H, J = 8.1 Hz, CH Tol), 6.10 [dd, 1H, J = 10.0 (avg.) and 10.1 Hz, H-4], 5.93 (dd, 1H, J = 3.2 and 10.1 Hz, H-3), 5.80 (br s, 1H, H-2), 5.24 (br s, 1H, H-1), 4.68 (dd, 1H, J = 2.4 and 12.2 Hz, H-6a), 4.56 [ddd, 1H, J = 2.4, 4.0 and 10.0 (avg.) Hz, H-5], 4.43 (dd, 1H, J = 4.0 and 12.2 Hz, H-6b), 4.32 and 4.28 (2×d, 2×1H, J = 16.5 Hz, OCH_2), 2.43, 2.42, 2.34, and 2.28 (4×s, 4×3H, CH_3-Ar); ^{13}C NMR (10% CD_3OD in $CDCl_3$, 125 MHz) δ 166.5, 165.7, 165.7, and 165.6 (CO), 144.3, 144.0, and 143.7 (C Tol), 129.9, 129.8, 129.8, 129.7, 129.3, 129.2, 129.1, and 129.0 (CH Tol), 127.0, 126.4, 126.3, and 126.2 (C Tol), 97.7 (C-1), 70.3 (C-2), 69.9 (C-3), 69.3 (C-5), 66.6 (C-4), 65.3 (OCH_2), 62.6 (C-6), 21.7, 21.6, 21.5, and 21.5 (CH_3-Ar); HRMS (ESI): $[M + Na]^+$ $C_{40}H_{38}NaO_{12}$ requires 733.2261, found 733.2270.

***tert*-Butyl 3-[(2,3,4,6-tetra-*O*-toluoyl-α-D-mannopyranosyloxy)acetamido]propanoate (15).** Compound **14** (0.705 g, 0.992 mmol) was converted to active ester and coupled to β-alanine as described above for the conversion of compound **6** to **8**. By silica gel chromatography of the crude product (1:1 mixture of EtOAc and petroleum ether), compound **15** was obtained in 96% yield (0.797 g). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.96 and 7.92 (2×d, 2×2H, J = 8.1 Hz, CH Tol), 7.85 and 7.73 (2×d, 2×2H, J = 8.1 Hz, CH Tol), 7.21 (d, 2H, J = 8.1 Hz, CH Tol), 7.18 and 7.15 (2×d, 2×2H, J = 8.1 Hz, CH Tol), 7.17–7.14 (overlapping signals, 1H, NH), 7.07 (d, 2H, J = 8.1 Hz, CH Tol), 6.08 [dd, 1H, J = 9.9 (avg.) and 10.1 Hz, H-4], 5.88 (dd, 1H, J = 3.3 and 10.1 Hz, H-3), 5.75 (dd, 1H, J = 3.3 and 1.5 Hz, H-2), 5.14 (d, 1H, J = 1.5 Hz, H-1), 4.66 (dd, 1H, J = 2.1 and 12.2 Hz, H-6a), 4.47 (dd, 1H, J = 4.8 and 12.2 Hz, H-6b), 4.41 [ddd, 1H, J = 2.1, 4.8 and 9.9 (avg.) Hz, H-5], 4.33 and 4.14 (2×d, 2×1H, J = 15.2 Hz, OCH_2), 3.62 (m, 2H, $NHC\underline{H}_2$), 2.56 (t, 2H, J = 6.3 Hz, CH_2COO), 2.43, 2.42, 2.35, and 2.30 (4×s, 4×3H, CH_3-Ar), 1.45 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.6, 168.0, 166.3, 165.5, and 165.5 (CO), 144.4, 144.4, 144.1, and 143.8 (C Tol), 130.0, 130.0, 129.9, 129.4, 129.3, 129.2, and 129.2 (CH Tol), 127.1, 126.5, 126.4, and 126.3 (C Tol), 98.0 (C-1), 81.3 (C_q *t*-Bu), 70.0 (C-2), 69.9 (C-5), 69.7 (C-3), 67.3 (OCH_2), 66.5 (C-4), 62.7 (C-6), 35.1 ($\underline{C}H_2COO$), 34.9 ($NHCH_2$), 28.2 [$C(\underline{C}H_3)_3$], 21.9, 21.8, 21.8, and 21.7 (CH_3-Ar); HRMS (ESI): $[M + Na]^+$ $C_{47}H_{51}NNaO_{13}$ requires 860.3258, found 860.3240.

3-[(2,3,4,6-Tetra-*O*-toluoyl-α-D-mannopyranosyloxy)acetamido]propanoic acid (16). Compound **15** (0.795 g, 0.948 mmol) was deprotected as described for the conversion of compound **7** to **9** to obtain **16** in quantitative yield as an 82% pure compound (0.905 g) contaminated by TFA. The product was used as such in the next reaction. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.65 (br, 1H,

COOH), 7.95, 7.88, 7.82, and 7.71 (4×d, 4×2H, *J* = 8.1 Hz, CH Tol), 7.74 (br t, 1H, NH), 7.20 (d, 2H, *J* = 8.1 Hz, CH Tol), 7.16 (overlapping d's, 2×2H, CH Tol), 7.08 (d, 2H, *J* = 8.1 Hz, CH Tol), 6.07 (dd, 1H, *J* = 10.1 and 10.1 Hz, H-4), 5.84 (dd, 1H, *J* = 3.3 and 10.1 Hz, H-3), 5.74 [dd, 1H, *J* = 1.5 (avg.) and 3.3 Hz, H-2], 5.15 [d, 1H, *J* = 1.5 Hz (avg.), H-1], 4.70 (dd, 1H, *J* = 2.4 and 12.2 Hz, H-6a), 4.48 (dd, 1H, *J* = 4.8 and 12.2 Hz, H-6b), 4.44 (d, 1H, *J* = 15.6 Hz, OCH_2), 4.41 (m, 1H, H-5), 4.23 (d, 1H, *J* = 15.6 Hz, OCH_2), 3.64 (m, 2H, $NHC\underline{H}_2$), 2.64 (t, 2H, *J* = 6.1 Hz, CH_2COO), 2.42, 2.42, 2.34 and 2.30 (4×s, 4×3H, CH_3-Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.9 (COOH), 170.3 (CONH), 166.7, 166.5, 165.8, and 165.7 (CO), 144.8, 144.8, 144.7, and 144.1 (C Tol), 130.1, 130.0, 129.9, 129.5, 129.4, 129.3, and 129.3 (CH Tol), 126.8, 126.1, 126.0, and 125.9 (C Tol), 97.9 (C-1), 70.1 and 70.0 (C-3 and C-5), 69.8 (C-2), 66.5 (OCH_2), 66.3 (C-4), 62.7 (C-6), 34.8 ($NHCH_2$), 33.4 ($\underline{C}H_2COO$), 21.9, 21.8, and 21.8 (CH_3-Ar) (peaks referring to TFA are not listed). HRMS (ESI): $[M + Na]^+$ $C_{43}H_{43}NNaO_{13}$ requires 804.2632, found 804.2649.

***N*-[4-(4-Methoxytritylaminomethyl)benzyl]biotinylamide (18).** *N*-(4-Methoxytrityl)-1,4-phenylenedimethanamine[39] (**17**) (720 mg, 1.76 mmol) was dissolved in dry DMF (4 mL). D-Biotin (384 mg, 1.57 mmol) in dry DMF (8 mL) was added together with 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU; 607 mg, 1.60 mmol) in dry DMF (3 mL) and *N*,*N*-diisopropylethylamine (DIEA; 330 μL, 1.89 mmol). The reaction was allowed to proceed overnight (19 h) and the mixture was then evaporated to dryness. The residue was purified by silica gel chromatography (5 → 10% MeOH in CH_2Cl_2) to obtain 0.86 g (76%) of **18**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.52 (d, 4H, *J* = 7.3 Hz, MMTr), 7.43 (d, 2H, *J* = 8.9 Hz, MMTr), 7.34–7.16 (m, 10H, Ar and MMTr), 6.82 (d, 2H, *J* = 8.9 Hz, MMTr), 6.46 (t, 1H, *J* = 5.7 Hz, NH^{amide}), 6.12 and 5.25 (2×br s, 2×1H, $2\times NH^{urea}$), 4.42 (br dd, 1H, H-3') 4.37 (d, 2H, *J* = 5.7 Hz, H-6), 4.24 (br dd, 1H, H-2'), 3.77 (s, 3H, CH_3O), 3.30 (br s, 2H, H-7), 3.08 (m, 1H, H-1'), 2.83 (dd, 1H, *J* = 4.9 and 12.9 Hz, H-4a'), 2.64 (d, 1H, *J* = 12.9 Hz, H-4b'), 2.22 (br t, 2H, H-4), 1.82 (br, 1H, NH), 1.74–1.55 (m, 4H, H-1 and H-3), 1.41 (m, 2H, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3 (C-5), 163.9 (C-5'), 158.0, 140.4, 138.2, and 137.2 (MMTr), 129.9, 128.6, 128.3, 128.0, 128.0, and 126.4 (MMTr and Ar), 113.3 (MMTr), 70.6 (C_q MMTr), 61.9 (C-2'), 60.3 (C-3'), 55.7 (C-1'), 55.3 (CH_3O), 47.7 (C-7), 43.3 (C-6), 40.6 (C-4'), 36.0 (C-4), 28.3 and 28.1 (C-1 and C-2), 25.8 (C-3). HRMS (ESI): $[M + H]^+$ $C_{38}H_{43}N_4O_3S$ requires 635.3056, found 635.3024.

***tert*-Butyl 2-{[4-(biotinylaminomethyl)benzyl]amino}-2-oxoethoxycarbamate (19).** Compound **18** (1.90 g, 2.99 mmol) was dissolved in dry CH_2Cl_2 (33 mL) containing 1% TFA. After 5 min, dry MeOH (2.2 mL) was added as a scavenger and the reaction was stirred for 1.5 h, followed by evaporation to dryness. The free amine and DIEA (0.575 mL, 3.30 mmol) were dissolved in dry

DMF (8 mL). *N*-(*tert*-butoxycarbonyl)aminooxyacetic acid (0.687 g, 3.59 mmol) was dissolved in dry DMF (11 mL) together with 1-hydroxybenzotriazole monohydrate (HOBt·H_2O; 0.551 g, 3.60 mmol) and DCC (0.743 g, 3.60 mmol). After 40 min, the precipitated dicyclohexylurea (DCU) was removed and the activated acid was added to the solution containing the free amine. The reaction was allowed to proceed for 19 h, the solution was evaporated to dryness and the residue was purified by silica gel chromatography (13 → 20% MeOH in CH_2Cl_2). Despite attempted purification, approx. 1 equivalent of DCU (determined by the integral ratios of ^{1}H NMR) remained among the product, giving 1.78 g of 66% pure **19** in 73% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (t, 1H, J = 5.6 Hz, NH), 7.24 (m, 4H, Ar), 4.50 (dd, 1H, J = 4.9 and 7.8 Hz, H-3'), 4.45 and 4.36 (2×br d, 2×2H, H-6 and H-7), 4.32 (s, 2H, H-9), 4.27 (dd, 1H, J = 4.5 and 7.8 Hz, H-2'), 3.17–3.11 (m, 1H, H-1'), 2.91 (dd, 1H, J = 4.9 and 12.9 Hz, H-4a'), 2.72 (d, 1H, J = 12.9 Hz, H-4b'), 2.22 (t, 2H, J = 7.4 Hz, H-4), 1.75–1.56 (m, 4H, H-1 and H-3), 1.47 (m, 2H, H-2), 1.43 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9 (C-5), 169.7 (C-8), 164.0 (C-5'), 158.2 (CO Boc), 137.4, 136.9, 127.9, 127.8, 127.8, and 127.7 (Ar), 82.5 (C_q Boc), 75.4 (C-9), 61.8 (C-2'), 60.0 (C-3'), 55.4 (C-1'), 42.9 and 42.4 (C-6 and C-7), 40.2 (C-4'), 35.6 (C-4), 28.2 (C-1 and C-2), 27.9 [$C(CH_3)_3$], 25.4 (C-3) (peaks referring to DCU are not listed). HRMS (ESI): $[M + Na]^+$ $C_{25}H_{37}N_5NaO_6S$ requires 558.2362, found 558.2353.

Immobilization of the pentaerythrityl tetraamine scaffold to aminomethyl polystyrene support; resin 22. *N*-(Fluoren-9-ylmethoxycarbonyl)-*β*-alanine (*N*-Fmoc-*β*-Ala; 781 mg, 2.51 mmol) and HOBt·H_2O (395 mg, 2.58 mmol) were dissolved in dry DMF (3 mL) and diisopropylcarbodiimide (DIC; 388 μL, 2.52 mmol) was added. The mixture was shaken for 30 min, added onto pre-swelled aminomethyl polystyrene resin (1.00 g, loading 0.5 mmol g^{-1}) and shaken for 4 h. The resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The loading of the resin, determined by the release of benzofulvene, was 0.44 mmol g^{-1}. The unreacted amino groups were capped by a 50 min treatment with capping solutions A and B (see General Remarks). The resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The Fmoc groups were then removed with 20% piperidine in DMF in 50 min and the resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. To couple the 4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid linker to the exposed amino groups, this acid (0.485 g, 1.81 mmol) and HOBt·H_2O (0.276 g, 1.80 mmol) were dissolved in dry DMF (3mL), DIC (0.276 mL, 1.79 mmol) was added and the mixture was shaken for 20 min and then added onto a pre-swelled (7 mL of dry DMF) resin. After shaking for 6 h, the resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The pentaerythrityl tetramine scaffold was then attached to a sample of the

derivatized resin (**20**). For this purpose, 2-[*N*-(allyloxycarbonyl)aminomethyl]-2-azidomethyl-1,3-propanediamine[40] (**21**, 230 mg; 489 μmol, 5 equiv) and $NaCNBH_3$ (85%) (36 mg, 490 μmol, 5 equiv) were dissolved in a 1:4 (*v/v*) mixture of DMSO and NMP (1.0 mL) and added onto the resin (222 mg, theoretical loading of 0.44 mmol g^{-1}). After shaking for 3 h, the resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The reaction was repeated to ensure high loading. The unreacted formyl groups were capped with a mixture of $MeONH_2 \cdot HCl$ (82 mg, 977 μmol, 10 equiv) and K_2CO_3 (149 mg, 1.08 mmol, 11 equiv) in a 1:9 (*v/v*) mixture of water and THF (3.1 mL). After shaking for 1 h, the resin was filtered, washed with aq THF, THF, DMF, CH_2Cl_2 and MeOH, and dried.

Assembly of the homotypic glucose cluster; resin 27. *N*-Fmoc-β-Ala (484 mg, 1.56 mmol, 40 equiv) was suspended in dry CH_2Cl_2 (3 mL) and DIC (122 μL, 0.78 mmol, 20 equiv) was added. After shaking for 40 min, the anhydride obtained was added onto the pre-swelled (1 mL of dry DMF) resin **22** (205 mg, theoretical loading of 0.19 mmol g^{-1}) to acylate the primary and secondary amino groups. The mixture was shaken for 5 h, filtered, washed with DMF, NMP, CH_2Cl_2 and MeOH, and dried. The reaction was repeated twice to ensure highest possible loading (0.15 mmol g^{-1}, determined by the release of benzofulvene) and the unreacted amino groups were acetylated with capping solutions A and B to obtain resin **23**.

The Fmoc groups were removed from resin **23** by treatment with 20% piperidine in DMF for 30 min followed by filtration, washing with DMF, CH_2Cl_2 and MeOH, and drying of the resin. To conjugate the first two glycosyl groups, 2-(2,3,4,6-tetra-*O*-toluoyl-β-D-glucopyranosyloxy)acetic acid (**4**, 220 mg, 309 μmol, 10 equiv) was dissolved in dry NMP (1 mL). 1 mol L^{-1} HOBt in NMP (308 μL, 308 μmol, 10 equiv) and 1 mol L^{-1} DCC in NMP (306 μL, 306 μmol, 10 equiv) were added and the solution was shaken for 30 min. The resin (196 mg, loading of 0.15 mmol g^{-1}) was pre-swelled in dry NMP (1 mL) and mixed with the solution of **4**. After shaking for 2 h, the resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The unreacted amino groups were acetylated with capping solutions A and B to obtain resin **24**.

Resin **24** (211 mg, theoretical loading of 0.15 mmol g^{-1}) was suspended in a 1:4 (*v/v*) mixture of water and 1,4-dioxane (3.8 mL). The azido group was reduced to amino group by adding a solution of 1 mol L^{-1} Me_3P in toluene (760 μL, 760 μmol, 24 equiv) under nitrogen and shaking for 2.5 h. The resin was then filtered, washed with dioxane, DMF, CH_2Cl_2 and MeOH, and dried. β-Alanine was coupled by dissolving Fmoc-β-Ala (202 mg, 648 μmol, 20 equiv) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 245 mg, 645 μmol, 20 equiv) in dry

DMF (1.2 mL) and mixing the solution with the pre-swelled resin in DMF (1 mL). DIEA (224 μL, 1.29 mmol, 40 equiv) was added and the mixture was shaken overnight (17 h). The resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The coupling was repeated to ensure the best possible loading. The unreacted amino groups were acetylated with capping solutions A and B to obtain resin **25**.

The Fmoc group was removed by treating the resin **25** (210 mg, theoretical loading of 0.15 mmol g^{-1}) with 20% piperidine in DMF for 30 min followed by filtration, washing with DMF, CH_2Cl_2 and MeOH, and drying of the resin. (2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)acetic acid **5** (128 mg, 316 μmol, 10 equiv) and HBTU (121 mg, 319 μmol, 10 equiv) were dissolved in dry DMF (1mL) and the solution was mixed with the pre-swelled resin in DMF (1 mL). DIEA (110 μL, 631 μmol, 20 equiv) was added and the mixture was shaken for 3 h. The resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The unreacted amino groups were acetylated with capping solutions A and B to obtain resin **26**.

Resin **26** (212 mg, theoretical loading of 0.15 mmol g^{-1}) was suspended in dry CH_2Cl_2 (2 mL) and the allyloxycarbonyl group was removed by adding $PhSiH_3$ (94 μL, 760 μmol, 24 equiv) and a catalytic amount of $Pd(Ph_3P)_4$ (approx. 0.5 equiv). The suspension was shaken for 1 h under nitrogen and then the resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The palladium treatment was repeated. The resin was pre-swelled in dry DMF (1 mL) and levulinic acid (73.0 mg, 629 μmol, 21 equiv) together with HBTU (230 mg, 606 μmol, 20 equiv) in dry DMF (1 mL) were added. DIEA (209 μL, 1.20 mmol, 40 equiv) was added and the mixture was shaken for 3 h. The resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The reaction was repeated twice. The unreacted amino groups were acetylated with capping solutions A and B to obtain resin **27**.

Assembly of the heterotypic cluster; resin 31. Compound **16** (269 mg, 282 μmol, 4 equiv) was combined with HOBt-solution (285 μL, 285 μmol, 4 equiv, 1 mol L^{-1} in NMP) and DCC was added (285 μL, 285 μmol, 4 equiv, 1 mol L^{-1} in NMP). The mixture was shaken for 30 min and added on resin **22** (185 mg, theoretical loading of 0.44 mmol g^{-1}) with dry NMP (0.55 mL) to acylate the primary amino groups. The mixture was shaken for 18 h, then filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The reaction was repeated to obtain resin **28**.

Levulinic acid (343 mg, 2.95 mmol, 40 equiv) was dissolved in dry CH_2Cl_2 (0.4 mL) and DIC (225 μL, 1.44 mmol, 20 equiv) was added. After 20 min, omitting the DCU precipitate, the mixture was added on pre-swelled (0.8 mL of dry CH_2Cl_2) resin **28** (162 mg, theoretical loading of 0.44 mmol g^{-}

[1]). The mixture was shaken for 4 h and then filtered, washed with NMP, DMF, CH_2Cl_2 and MeOH, and dried. Washing with 2% DIEA in DMF, and then with DMF, NMP, CH_2Cl_2 and MeOH, was performed to ensure the amino groups to be in unprotonated form. The reaction was repeated until no remarkable amount of free amino groups remained. The resin was then subjected to acetic anhydride treatment using the capping solutions A and B to obtain resin **29**.

PMe_3-solution (1.37 mL, 1.37 mmol, 24 equiv, 1 mol L^{-1} in toluene) was added to the resin **29** (130 mg, theoretical loading of 0.44 mmol g^{-1}) under N_2-atmosphere. 1,4-Dioxane (1.2 mL) and water (0.2 mL) were added and the mixture was shaken for 3.5 h. The resin was filtered, washed with 1,4-dioxane, MeOH, DMF, CH_2Cl_2 and MeOH, and dried. The reaction was repeated. Compound **10** (174 mg, 288 μmol, 5 equiv) and HATU (109 mg, 287 μmol, 5 equiv) were dissolved in dry DMF (680 μL) and added to the resin. DIEA (99 μL, 568 μmol, 10 equiv) was added and the mixture was shaken for 4 h. The resin was filtered, washed with DMF, CH_2Cl_2 and MeOH, and dried. The reaction was repeated twice without remarkable progress. Instead, coupling with acid chloride turned out to work well; The resin was preswelled with dry CH_2Cl_2 (0.70 mL) and DIEA (71 μL, 408 μmol) was added. Compound **12** (168 mg, 330 μmol, 7 equiv) was dissolved in CH_2Cl_2 (0.90 mL) and added to the resin. The reaction was shaken for 18 h, filtered, washed with CH_2Cl_2, DMF, CH_2Cl_2 and MeOH, and dried. The coupling was repeated and then the resin was treated with capping solutions A and B to obtain resin **30**.

Resin **30** (90 mg, theoretical loading of 0.44 mmol g^{-1}) was suspended in dry CH_2Cl_2 (1.60 mL) and the allyloxycarbonyl groups were removed by adding $PhSiH_3$ (118 μL, 956 μmol, 24 equiv) and a catalytic amount of $Pd(Ph_3P)_4$ (approx. 0.5 equiv). The suspension was shaken for 1 h under nitrogen and then the resin was filtered, washed with CH_2Cl_2, DMF, CH_2Cl_2 and MeOH, and dried. The resin was pre-swelled with dry CH_2Cl_2 (0.50 mL). Acid chloride **11** (179 mg, 220 μmol, 6 equiv) was dissolved in CH_2Cl_2 (0.60 mL) together with DIEA (47 μL, 270 μmol) and added to the resin. The reaction was shaken for 18 h, filtered, washed with CH_2Cl_2, DMF, CH_2Cl_2 and MeOH, and dried. The coupling was repeated three times and then the resin was treated with capping solutions A and B to obtain resin **31**.

Biotinylation of the homotypic cluster; conjugate 32. Compound **19** (447 mg, 0.551 mmol, 30 equiv) was dissolved in CH_2Cl_2 (1.5 mL) and MeOH (0.15 mL) and TFA (1.5 mL) were added. After stirring for 3 h, the solution was evaporated to dryness and the residue was dissolved in pyridine (2.5 mL). Half of the solution (15 equiv) was added onto pre-swelled (0.80 mL of pyridine) resin **27** (122 mg, theoretical loading of 0.15 mmol g^{-1}) and then shaken overnight (21 h).

The resin was filtered, washed with aq pyridine, pyridine, CH_2Cl_2 and MeOH, and dried. The reaction was repeated with the rest of the biotin solution (15 equiv, stored at -20º C). The product was released from the support with 50% TFA in CH_2Cl_2 (2 mL for 30 min) and the crude product mixture was filtered, evaporated to dryness and dissolved in 80% aq MeCN. RP HPLC purification (protocol A) yielded 2.5 mg of product **32**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.86 (d, 4H, J = 8.1 Hz, CH Tol), 7.83 (d, 4H, J = 8.1 Hz, CH Tol), 7.77 (d, 4H, J = 8.1 Hz, CH Tol), 7.70 (d, 4H, J = 8.1 Hz, CH Tol), 7.24–7.17 (m, 12 H, CH Tol and Ar), 7.13 (d, 4H, J = 8.1 Hz, CH Tol), 7.07 (d, 4H, J = 8.1 Hz, CH Tol), 5.88 (dd, 2H, J = 9.6 and 9.7 Hz, 2×H-3 Glc^{Tol}), 5.61 (dd, 2H, J = 9.6 and 9.8 Hz, 2×H-4 Glc^{Tol}), 5.47 (dd, 2H, J = 7.8 and 9.7 Hz, 2×H-2 Glc^{Tol}), 5.21 (dd, 1H, J = 8.7 and 10.3 Hz, H-3 Glc^{Ac}), 5.04 (dd, 1H, J = 8.7 and 10.7 Hz, H-4 Glc^{Ac}), 5.00 (dd, 1H, J = 7.2 and 10.3 Hz, H-2 Glc^{Ac}), 4.84 (d, 2H, J = 7.8 Hz, 2×H-1 Glc^{Tol}), 4.60–4.53 (m, 6 H, 2×H-6a Glc^{Tol}, 3×H-16a, and H-1 Glc^{Ac}), 4.45–4.42 (m, 5H, H-6 or 7, 2×H-6b Glc^{Tol}, and H-3'), 4.33–4.23 (m, 6H, H-6 or 7, H-9, H-6a Glc^{Ac}, and H-2'), 4.16 (m, 2H, 2×H-5 Glc^{Tol}), 4.13–4.08 (m, 4H, H-6b Glc^{Ac} and 3×H-16b), 3.72 (m, 1H, H-5 Glc^{Ac}), 3.57–3.38 (m, 6H, 3×H-15), 3.13 (m, 1H, H-1'), 2.92–2.67 (m, 10H, H-4a', H-12, 3×H-13, and H-4b'), 2.53 (m, 4H, 2×H-14), 2.45–2.35 (m, 6H, H-14, H-10, and H-11), 2.38 (s, 6H, 2×CH_3-Ar), 2.34 (s, 12H, 4×CH_3-Ar), 2.30–2.21 (m, 2H, H-4), 2.28 (s, 6H, 2×CH_3-Ar), 2.05, 2.04, 2.02, and 1.98 (4×s, 4×3H, 4×CH_3 Ac), 1.87 and 1.84 (2×s, 3H, CH_3 E/Z isomers), 1.69 (m, 2H, H-3), 1.62 (m, 2H, H-1), 1.42 (m, 2H, H-2) (peaks referring to NH are not listed). HRMS (ESI): See for Table 1.

Biotinylation of the heterotypic cluster; conjugate 33. Compound **19** (851 mg, 1.05 mmol, 30 equiv) was dissolved in CH_2Cl_2 (3.0 mL) and MeOH (0.30 mL) and TFA (3.0 mL) were added. After stirring for 3 h the solution was evaporated to dryness and the residue was dissolved in pyridine (3.0 mL). Half of the solution (15 equiv) was added onto pre-swelled (1.0 mL of pyridine) resin **31** (79 mg, theoretical loading of 0.44 mmol g^{-1}) and then shaken overnight (21 h). The resin was filtered, washed with aq pyridine, pyridine, CH_2Cl_2 and MeOH, and dried. The reaction was repeated with the rest of the biotin solution (15 equiv, stored at -20º C). The product was released from the support with 50% TFA in CH_2Cl_2 (2 mL for 30 min) and the crude product mixture was filtered, the filtrate was evaporated to dryness and dissolved in 80% aq MeCN. RP HPLC purification (protocol A) yielded 5.5 mg of product **33**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99 (d, 2H, J = 8.1 Hz, CH Tol), 7.90 (d, 2H, J = 8.1 Hz, CH Tol), 7.88 (d, 2H, J = 8.1 Hz, CH Tol), 7.84 (d, 4H, J = 8.1 Hz, CH Tol), 7.79 (d, 2H, J = 8.1 Hz, CH Tol), 7.72 (d, 2H, J = 8.1 Hz, CH Tol), 7.69 (d, 2H, J = 8.1 Hz, CH Tol), 7.25–7.14 (m, 16 H, CH Tol and Ar), 7.10 (d, 2H, J = 8.1 Hz, CH Tol), 7.07 (d, 2H, J = 8.1 Hz, CH Tol), 6.08 (m, 1H, H-4 Man), 5.91–5.85 (m, 2H, H-3 Glc and H-3

Man), 5.79 (bs s, 1H, H-2 Man), 5.58 (br dd, 1H, H-4 Glc), 5.44 (m, 1H, H-2 Glc), 5.42 (d, 1H, *J* = 2.8 Hz, H-4 Gal), 5.21 (dd, 1H, *J* = 7.9 and 10.3 Hz, H-2 Gal), 5.12 (br s, 1H, H-1 Man), 5.06 (dd, 1H, *J* = 2.8 and 10.3 Hz, H-3 Gal), 4.85 (d, 1H, *J* = 7.7 Hz, H-1 Glc), 4.70 (br d, 1H, H-6a Man), 4.61–4.52 (m, 5H, H–6a Glc, H-1 Gal, and 3×H-16a), 4.48–4.42 (m, 5H, H-6b Glc, H-3', H-6 or 7, and H-5 Man), 4.37–4.30 (m, 6H, H-6 or 7, H-9, H-6b Man, and H-2'), 4.18–4.07 (m, 6H, H-6 Gal, H-5 Glc, and 3×H-16b), 3.96 (br dd, 1H, H-5 Gal), 3.72–3.44 (m, 6H, 3×H-15), 3.18 (m, 1H, H-1'), 2.94–2.76 (m, 10H, H-4a', H-12, 3×H-13, and H-4b'), 2.63 (m, 2H, H-14), 2.51 (m, 6H, H-10, H-11, and H-14), 2.46, 2.45 (2×s, 2×3H, 2×CH_3-Ar), 2.40 (s, 5H, H-14 and CH_3-Ar), 2.36 (s, 9H, 3×CH_3-Ar), 2.31–2.30 (m, 8H, 2×CH_3-Ar and H-4), 2.15, 2.08, 2.05, and 2.01 (4×s, 4×3H, 4×CH_3 Ac), 1.86 and 1.83 (2×s, 3H, CH_3 E/Z isomers), 1.72 (m, 2H, H-3), 1.65 (m, 2H, H-1), 1.44 (m, 2H, H-2) (peaks referring to NH are not listed). HRMS (ESI): See for Table 1.

Deprotected conjugates 34 and 35. Protected conjugates (2.5 mg of **32** and 5.5 mg of **33**) were treated with a solution of 20 mmol L^{-1} NaOMe in MeOH (0.5 mL) for 18 h, and then strong cation-exhange resin (Dowex 50WX8-200) was added to neutralize the mixture. The mixture was filtered, the filtrate was evaporated to dryness and the resulted residue was dissolved in a biphasic mixture of Et_2O and water (1:1, *v/v*, 1.0 mL). The organic layer was removed, the water phase was concentrated to remove the remaining Et_2O, and the crude mixture was desalted and purified by RP HPLC (protocol B) to give the globally deprotected conjugates **34** (0.5 mg) and **35** (2.6 mg).
34: ^{1}H NMR (D_2O, 500 MHz) δ 7.21 (m, 4H, CH Ar), 4.51–4.46 (m, 3H, H-3' and H-9), 4.38 (d, 3H, *J* = 7.9 Hz, 3×H-1 Glc), 4.35 (br s, 2H, H-6 or 7), 4.30–4.22 (m, 6H, 3×H-16a, H-6 or 7, and H-2'), 4.13 (d, 3H, *J* = 15.7 Hz, 3×H-16b), 3.81 (dd, 3H, *J* = 2.2 and 12.3 Hz, 3×H-6a Glc), 3.62 (dd, 3H, *J* = 5.6 and 12.3 Hz, 3×H-6b Glc), 3.51–3.40 (m, 6H, 3×H-15), 3.40 [dd, 3H, *J* = 8.8 and 9.4 (avg.) Hz, 3×H-3 Glc], 3.37–3.30 (m, 6H, 3×H-5 Glc and 3×H-4 Glc), 3.25 [dd, 3H, *J* = 7.9 and 9.4 (avg.) Hz, 3×H-2 Glc], 3.18 (m, 1H, H-1'), 2.98–2.82 (m, 9H, H-12, 3×H-13, and H-4a'), 2.69 (d, 1H, *J* = 13.0 Hz, H-4b'), 2.48–2.43 (m, 10H, 3×H-14, H-10, and H-11), 2.22 (br t, 2H, H-4), 1.87 and 1.83 (2×s, 3H, CH_3 E/Z isomers), 1.61–1.43 (m, 4H, H-3 and H-1), 1.27 (m, 2H, H-2) (peaks referring to NH are not listed). HRMS (ESI): See for Table 1.
35: ^{1}H NMR (D_2O, 500 MHz) δ 7.21 (m, 4H, CH Ar), 4.78 (br d, 1H, H-1 Man), 4.51–4.45 (m, 3H, H-3' and H-9), 4.38 (d, 1H, *J* = 8.0 Hz, H-1 Glc), 4.34 (br s, 2H, H-6 or 7), 4.32 (d, 1H, *J* = 7.7 Hz, H-1 Gal), 4.29–4.22 (m, 6H, 3×H-16a, H-6 or 7, and H-2'), 4.16–4.10 (m, 4H, 3×H-16b and H-6a Gal), 3.98 (dd, 1H, *J* = 3.1 and 15.3 Hz, H-6b Gal), 3.92 (br dd, 1H, H-2 Man), 3.83 (br d, 1H, H-4 Gal), 3.81 (dd, 1H, *J* = 2.0 and 12.3 Hz, H-6a Glc), 3.79–3.74 (m, 2H, H-6a Man and H-3 Man), 3.71–3.54 (m, 5H, H-6b Man, H-6b Glc, H-5 Gal, H-4 Man, and H-3 Gal), 3.51–3.39 (m, 9H, H-2

Gal, H-5 Man, 3×H-15, and H-3 Glc), 3.37–3.30 [m, 2H, H-5 Glc and H-4 Glc), 3.25 (dd, 1H, *J* = 8.0 and 9.3 Hz, H-2 Glc), 3.18 (m, 1H, H-1'), 2.96–2.84 (m, 9H, H-12, 3×H-13, and H-4a'), 2.68 (d, 1H, *J* = 13.0 Hz, H-4b'), 2.48–2.44 (m, 10H, 3×H-14, H-10, and H-11), 2.22 (br t, 2H, H-4), 1.87 and 1.83 (2×s, 3H, CH_3 E/Z isomers), 1.61–1.43 (m, 4H, H-3 and H-1), 1.27 (m, 2H, H-2) (peaks referring to NH are not listed). HRMS (ESI): See for Table 1.

RESULTS AND DISCUSSION

Synthesis of Glycosyl Ligands. Scheme 1 outlines the preparation of fully acylated 1-*O*-functionalized glycopyranosides used for the assembly of glycoclusters. Allyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside (**1**)[27], allyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (**2**)[37], allyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside (**3**)[37] and allyl 2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside (**13**)[37] used as starting materials were prepared as described in literature. Osmium tetroxide hydroxylation of the double bond followed by oxidative cleavage of the diol and Jones oxidation of the aldehyde obtained gave the desired carboxymethyl derivatives (**4**, **5**, **6** and **14**) in satisfactory yield (74% for **4**). The Ru(III)-catalyzed oxidation of the glycosidic allyl group to carboxymethyl group[27,41] suffered from cleavage of the toluoyl groups and, hence, gave rather modest yields (53% for **4**). It should also be noted that the acetylated allyl *α*-D-mannopyranoside **13**[37] was converted to its toluoylated counterpart before the oxidation, since toluoylation was known to improve the chromatographic behavior of glycoclusters.

To couple a *β*-alanine tether to the carboxy function of **4**, **6** and **14**, these compounds were converted to active esters with *N*-hydroxysuccinimide (HOSu) and dicyclohexylcarbodiimide (DCC). Acyl substitution with *β*-alanine *tert*-butyl ester then afforded **7**, **8** and **15**, and removal of *tert*-butyl group with trifluoroacetic acid (TFA) gave the free acids **9**, **10** and **16**. Despite thorough evaporation after the acid treatment, the products contained approximately 1 equiv. TFA. The TFA containing products could, however, be successfully used in the subsequent reactions. Compounds **9** and **10** were further converted to acid chlorides (**11** and **12**) with thionyl chloride to ensure efficient coupling on assembling the sterically somewhat crowded clusters on a solid-support.

Synthesis of the Biotin Ligand (19). The biotin-derived ligand aimed at allowing the attachment of glycoclusters to streptavidin coated surfaces was prepared as depicted in Scheme 2. Besides biotin, the ligand contained a 1,4-phenylene chromophore and a Boc-protected aminooxy function. The chromophore was aimed at keeping the cluster UV-active upon removal of the toluoyl protections, while the aminooxy functionality allowed conjugation to the keto group on the levulinoyl branch by

solid-supported oximation. In principle, the carboxy function of biotin could be coupled directly, instead of levulinic acid, to the amino group of the tetramine scaffold. Conjugation to a sterically less hindered site, viz. to keto group of a levulinoyl side arm, by oximation, however, ensures higher efficiency of conjugation. It should be noted that the high efficiency of solid-supported oximation has been demonstrated by synthesis of numerous structurally complicated bioconjugates.[33-35,42,43] Accordingly, D-biotin and *N*-(4-methoxytrityl)-1,4-phenylenedimethanamine[39] (**17**) were first coupled by 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) activation to obtain **18**. Ligand **19** was then obtained by removing the 4-methoxytrityl protection with TFA and coupling *N*-Boc-aminooxyacetic acid to the exposed amino group by DCC activation using 1-hydroxybenzotriazole (HOBt) as an auxiliary nucleophile. A related tag bearing a primary amino function has previously been used to facilitate isolation and mass spectrometric characterization of oligosaccharides.[44]

Assembly of a Homotypic Gluco Cluster (27). The applicability of the previously[40] reported pentaerythrityl tetramine core (**21**) to the synthesis of sugar clusters was first tested with toluoyl and acetyl protected glucopyranosides **4** and **5**. 4-(4-Formyl-3,5-dimethoxyphenoxy)butyric acid was first attached by HOBt/DIC-activated coupling to an aminomethyl polystyrene resin derivatized with a β-alanine spacer to obtain support **20** (Scheme 3). Pentaerythrityl tetramine **21** was then attached by reductive amination to this support. The support (**22**) bearing both an unprotected primary and secondary amino group underwent selective acylation of the primary function when coupled with β-alanine, consistent with previously[40] reported acylation with α-amino acids. However, this was not the case when a closely related core structure bearing a second azido group in place of the Alloc-amino group[40] was used (data not shown); no selectivity between the acylation of the primary and secondary amino groups with β-alanine could be observed. The selectivity introduced by the Alloc group on **22** was later exploited in the synthesis of a heterotypic cluster, but not in the assembly of the gluco cluster (**27**). Consequently, both of the unprotected amino functions were acylated at a single step with Fmoc-β-alanine by the anhydride method to obtain **23**. The Fmoc protections were removed with piperidine and toluoylated glucose ligand **4** was coupled to the exposed amino groups using HOBt/DCC activation. Coupling to β-alanine branches proceeded smoothly and no unreacted amino groups could be detected. The azido group was reduced to amino group with trimethylphosphine and the third branch was elongated with Fmoc-β-alanine by HBTU-promoted coupling to obtain **25**. After removal of the Fmoc group with piperidine, the third sugar ligand (**5**) was coupled by HBTU activation. The Alloc protection was removed from the resulted support (**26**) by Pd^0 treatment, and levulinic acid was coupled to the exposed branch by HBTU

activation to conclude the synthesis of the ketone bearing glucose cluster **27**. Although not performed here, the method allows the branches to be further elongated with additional *β*-alanine units before attachment of the sugar residues.

Assembly of a Heterotypic Cluster (31). To obtain a cluster with three different sugar ligands a slightly modified protocol was used. Sugar ligands **9**, **10** and **16**, bearing a *β*-alanine elongated side-arm, were used to diminish the number of coupling steps on-support. In addition, selective acylation of the primary amino group of **22** was exploited to achieve the desired versatility. Hence, toluoylated mannose ligand **16** was first coupled to the resin by selective HOBt/DCC-activation to obtain support **28** (Scheme 4). The acylation of the secondary amino group turned out to be very difficult with *β*-alanine tethered sugars, although several coupling reagents were tested (data not shown). Therefore, the coupling order was altered and the secondary amino group was acylated with levulinic acid applying repeated anhydride couplings to obtain **29** in a satisfactory yield. The azido group of **29** was then reduced to amino group and the third branch was subjected to HATU-promoted coupling with acetylated galactose ligand **10**. Despite repeated couplings, only modest progress was obtained. The presence of one *β*-alanine sugar ligand seemed to markedly retard the subsequent couplings. Nevertheless, usage of acid chlorides in these difficult couplings solved the problem. Accordingly, galactose ligand **12** was reacted with the support to obtain **30**. The Alloc group was then removed from the last branch, and the exposed amino group was reacted with glucose ligand **11** to obtain the desired heterotypic cluster **31**.

Synthesis of the Biotin Conjugates (32–35). The biotin-derived ligand was attached to the support-bound clusters by oximation. The Boc-protection of **19** was thus removed with TFA to expose the aminooxy group and oximation of supports **27** and **31** was carried out in pyridine. The biotinylated clusters were acidolytically released from the support and purified by RP HPLC to afford the protected clusters **32** and **33** (Figure 1). Global deprotection with NaOMe/MeOH was used to remove the acetyl and toluoyl groups, followed by RP HPLC purification (Figure 1), to conclude the synthesis of the homotypic (**34**) and heterotypic (**35**) conjugates. The authenticity of the conjugates (**32**–**35**) was verified by ESI-MS (Table 1) and the conjugates were characterized by ^{1}H NMR spectroscopy.

CONCLUSION

A method for the synthesis of trivalent homo- and heterotypic glycoclusters on a solid support has been developed. The branches of the glycoclusters can be selectively constructed through elongation with β-alanine and conjugation with various carbohydrates bearing a carboxy-functionalized side-arm. Conjugation with carbohydrates bearing a β-alanine elongated sidearm was also applied in order to diminish the number of coupling steps on-support. However, this led to sterically more demanding couplings, where carbohydrates with acid chloride -functionalized side-arms were needed to ensure efficient coupling. For conjugation of the clusters with D-biotin, an aminooxy derivatized biotin ligand was attached by on-support oximation. Removal of the acyl protections in solution gave the fully deprotected conjugates. Most likely, these clusters can be utilized in the preparation of various other glycoconjugates by oximation as well.

ASSOCIATED CONTENT

Supporting Information. NMR spectral data for **6–10**, **14–16**, **18**, **19**, and **32–35**, and MS spectral data for **6–10**, **14–16**, **18**, **19**, **34** and **35**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding author.
*(M.K.) Tel +358-2-333 6780, Fax +358-2-333 6700, E-mail: marika.karskela@utu.fi

ACKNOWLEDGMENT

The authors thank Dr. Johanna Katajisto and M.Sc. Emilia Kiuru for preparing some of the starting materials.

REFERENCES

(1) Dwek, R. A. (1996) Glycobiology: Toward understanding the function of sugars. *Chem. Rev. 96*, 683–720.

(2) Gabius, H.-J., Siebert, H.-C., André, S., Jiménez-Barbero, J., and Rüdiger, H. (2004) Chemical biology of the sugar code. *ChemBioChem 5*, 740–764.

(3) Ernst, B., and Magnani, J. L. (2009) From carbohydrate leads to glycomimetic drugs. *Nat. Rev. Drug Discovery 8*, 661–677.

(4) Srinivas, O., Larrieu, P., Duverger, E., Boccaccio, C., Bousser, M.-T., Monsigny, M., Fonteneau, J.-F., Jotereau, F., and Roche, A.-C. (2007) Synthesis of glycocluster-tumor antigenic peptide conjugates for dendritic cell targeting. *Bioconjugate Chem. 18*, 1547–1554.

(5) Yan, H., and Tram, K. (2007) Glycotargeting to improve cellular delivery efficiency of nucleic acids. *Glycoconj. J. 24*, 107–123.

(6) Zhu, L., Ye, Z., Cheng, K., Miller, D. D., and Mahato, R. I. (2008) Site-specific delivery of oligonucleotides to hepatocytes after systemic administration. *Bioconjugate Chem. 19*, 290–298.

(7) Lee, Y. C., and Lee, R. T. (1995) Carbohydrate–protein interactions: basis of glycobiology. *Acc. Chem. Res. 28*, 321–327.

(8) Mammen, M., Choi, S.-K., and Whitesides, G. M. (1998) Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors *Angew. Chem. Int. Ed. 37*, 2754–2794.

(9) Lundquist, J. J., and Toone, E. J. (2002) The cluster glycoside effect. *Chem. Rev. 102*, 555–578.

(10) Collins, B. E., and Paulson, J. C. (2004) Cell surface biology mediated by low affinity multivalent protein–glycan interactions. *Curr. Opin. Chem. Biol. 8*, 617–625.

(11) Munoz, E. M., Correa, J., Fernandez-Megia, E., and Riguera, R. (2009) Probing the relevance of lectin clustering for the reliable evaluation of multivalent carbohydrate recognition. *J. Am. Chem. Soc. 131*, 17765–17767.

(12) Pieters, R. J. (2009) Maximising multivalency effects in protein–carbohydrate interactions. *Org. Biomol. Chem. 7*, 2013–2025.

(13) Dam, T. K., and Brewer, C. F. (2010) Multivalent Lectin-Carbohydrate Interactions: Energetics and Mechanisms of Binding. *Adv. Carbohydr. Chem. Biochem. 63*, 139–164.

(14) Cecioni, S., Faure, S., Darbost, U., Bonnamour, I., Parrot-Lopez, H., Roy, O., Taillefumier,

C., Wimmerov, M., Praly, J.-P., Imberty, A., and Vidal, S. (2011) Selectivity among two lectins: probing the effect of topology, multivalency and flexibility of "clicked" multivalent glycoclusters. *Chem. Eur. J. 17*, 2146–2159.

(15) Imberty, A., Chabre, Y. M., and Roy, R. (2008) Glycomimetics and glycodendrimers as high affinity microbial anti-adhesins. *Chem. Eur. J. 14*, 7490–7499.

(16) Ernst, B., and Magnani, J. L. (2009) From carbohydrate leads to glycomimetic drugs. *Nat. Rev. Drug Discovery 8*, 661–677.

(17) Chabre, Y. M., Giguere, D., Blanchard, B., Rodrigue, J., Rocheleau, S., Neault, M., Rauthu, S., Papadopoulos, A., Arnold, A. A., Imberty, A., and Roy, R. (2011) Combining glycomimetic and multivalent strategies toward designing potent bacterial lectin inhibitors. *Chem. Eur. J. 17*, 6545–6562.

(18) Almant, M., Moreau, V., Kovensky, J., Bouckaert, J., and Gouin, S. G. (2011) Clustering of Escherichia coli type-1 fimbrial adhesins by using multimeric heptyl α-D-mannoside probes with a carbohydrate core. *Chem. Eur. J. 17*, 10029–10038.

(19) Soomro, Z. H., Cecioni, S., Blanchard, H., Praly, J.-P., Imberty, A., Vidal, S., and Matthews, S. E. (2011) CuAAC synthesis of resorcin[4]arene-based glycoclusters as multivalent ligands of lectins. *Org. Biomol. Chem. 9*, 6587–6597.

(20) Galante, E., Geraci, C., Sciuto, S., Campo, V. L., Carvalho, I., Sesti-Costa, R., Guedes P. M. M., Silva, J. S., Hill, L., Nepogodiev, S. A., and Field, R. A. (2011) Glycoclusters presenting lactose on calix[4]arene cores display trypanocidal activity. *Tetrahedron 67*, 5902–5912.

(21) Ambrosi, M., Cameron, N. R., and Davis, B. G. (2005) Lectins: tools for the molecular understanding of the glycocode. *Org. Biomol. Chem. 3*, 1593–1608.

(22) Gomez-Garcia, M., Benito, J. M., Rodriguez-Lucena, D., Yu, J.-X., Chmurski, K., Ortiz Mellet, C., Gutierrez Gallego, R., Maestre, A., Defaye, J. and Garcia Fernandez, J. M. (2005) Probing secondary carbohydrate−protein interactions with highly dense cyclodextrin-centered heteroglycoclusters: the heterocluster effect. *J. Am. Chem. Soc. 127*, 7970–7971.

(23) Gomez-Garcia, M., Benito, J. M., Butera, A. P., Ortiz Mellet, C., Garcia Fernandez, J. M., and Jimenez Blanco, J. L. (2012) Probing carbohydrate-lectin recognition in heterogeneous environments with monodisperse cyclodextrin-based glycoclusters *J. Org. Chem. 77*, 1273–1288.

(24) Lönnberg, H. (2009) Solid-supported synthesis of glycoconjugates. *Curr. Org. Synth. 6*, 400–425.

(25) Arya, P., Barkley, A., and Randell, K. D. (2002) Automated high-throughput synthesis of artificial glycopeptides. Small-molecule probes for chemical glycobiology. *J. Comb. Chem. 4*, 193–198.

(26) Katajisto, J., Karskela, T., Heinonen, P., and Lönnberg, H. (2002) An orthogonally protected *α*,*α*-bis(aminomethyl)-*β*-alanine building block for the construction of glycoconjugates on a solid support. *J. Org. Chem. 67*, 7995–8001.

(27) Virta, P., Karskela, M., and Lönnberg, H. (2006) Orthogonally protected cyclo-*β*-tetrapeptides as solid-supported scaffolds for the synthesis of glycoclusters. *J. Org. Chem. 71*, 1989–1999.

(28) Filippov, D. V., van den Elst, H., Tromp, C. M., van der Marel, G. A., van Boeckel, C. A. A., Overkleeft, H. S., and van Boom, J. H. (2004) Parallel solid phase synthesis of tricomponent bisubstrate analogues as potential fucosyltransferase inhibitors. *Synlett* 773–778

(29) Diaz-Moscoso, A., Benito, J. M., Ortiz Mellet, C., and Garcia Fernandez, J. M. (2007) Efficient use of Ellman safety-catch linker for solid-phase assisted synthesis of multivalent glycoconjugates. *J. Comb. Chem. 9*, 339–342.

(30) Ortega-Muñoz, M., Perez-Balderas, F., Morales-Sanfrutos, J., Hernandez-Mateo, F., Isac-Garcia, J, and Santoyo-Gonzalez, F. (2009) Click multivalent heterogeneous neoglycoconjugates – modular synthesis and evaluation of their binding affinities. *Eur. J. Org. Chem. 15*, 2454–2473.

(31) Thomas, B., Fiore, M., Bossu, I., Dumy, P., and Renaudet, O. (2012) Synthesis of heteroglycoclusters by using orthogonal chemoselective ligations. *Beilstein J. Org. Chem. 8*, 421–427.

(32) Hovinen, J. (2006) Synthesis of aminooxy-functionalized lanthanide(III) chelates for carbonyl-group conjugation. *Chem. Biodiversity 3*, 296–303.

(33) Katajisto, J., Virta, P., and Lönnberg, H. (2004) Solid-phase synthesis of multiantennary oligonucleotide glycoconjugates utilizing on-support oximation. *Bioconjugate Chem. 15*, 890–896.

(34) Karskela, M., Virta, P., Malinen, M., Urtti, A., and Lönnberg, H. (2008) Synthesis and cellular uptake of fluorescently labeled multivalent hyaluronan disaccharide conjugates of oligonucleotide phosphorothioates. *Bioconjugate Chem. 19*, 2549–2558.

(35) Karskela, M., Helkearo, M., Virta, P., and Lönnberg, H. (2010) Synthesis of oligonucleotide glycoconjugates using sequential click and oximation ligations. *Bioconjugate Chem. 21*, 748–755.

(36) Forget, D., Boturyn, D., Defrancq, E., Lhomme, J. and Dumy, P. (2001) Highly efficient synthesis of peptide-oligonucleotide conjugates: chemoselective oxime and thiazolidine formation. *Chem. Eur. J. 7*, 3976–3984.

(37) Schmidt, M., Chatterjee, S.K., Dobner, B., and Nuhn, P. (2002) New modified single chained glycolipids. Part 1: synthesis of deoxy and partially *O*-methylated glycolipids with or without a sulfur containing spacer. *Chemistry and Physics of Lipids 114*, 139–147.

(38) Binder, W. H., and Schmid, W. (1995) Synthesis of a symmetric multivalent molecule containing four carbohydrate substituents. *Monatsh. Chem., 126*, 923–931.

(39) Kiviniemi, A., Virta, P., and Lönnberg, H. (2010) Solid-supported synthesis and click conjugation of 4´-C-alkyne functionalized oligodeoxyribonucleotides. *Bioconjugate Chem. 21*, 1890–1901.

(40) Virta, P., Leppänen, M., and Lönnberg, H. (2004) Pentaerythrityltetramine scaffolds for solid-phase combinatorial chemistry. *J. Org. chem. 69*, 2008–2016.

(41) Ghosh, M., Dulina, R. G., Kakarla, R., and Sofia, M. J. (2000) Efficient synthesis of a stereochemically defined carbohydrate scaffold: carboxymethyl 2-acetamido-6-azido-4-*O*-benzyl-2-deoxy-*α*-D-glucopyranoside. *J. Org. Chem. 65*, 8387–8390.

(42) Edupuganti, O. P., Singh, Y., Defrancq, E., and Dumy, P. (2004) New strategy for the synthesis of 3′,5′-bifunctionalized oligonucleotide conjugates through sequential formation of chemoselective oxime bonds. *Chem. Eur. J. 10*, 5988–5995.

(43) Meyer, A., Spinelli, N., Dumy, P., Vasseur, J.-J., Morvan, F., and Defrancq, E. (2010) Oligonucleotide sequential bis-conjugation via click-oxime and click-Huisgen procedures. *J. Org. Chem. 75*, 3927–3930.

(44) Hsu, J., Chang, S. J., and Franz, A. H. (2006) MALDI-TOF and ESI-MS analysis of oligosaccharides labeled with a new multifunctional oligosaccharide tag. *J. Am. Soc. Mass Spectrom. 17*, 194–204.

TABLES

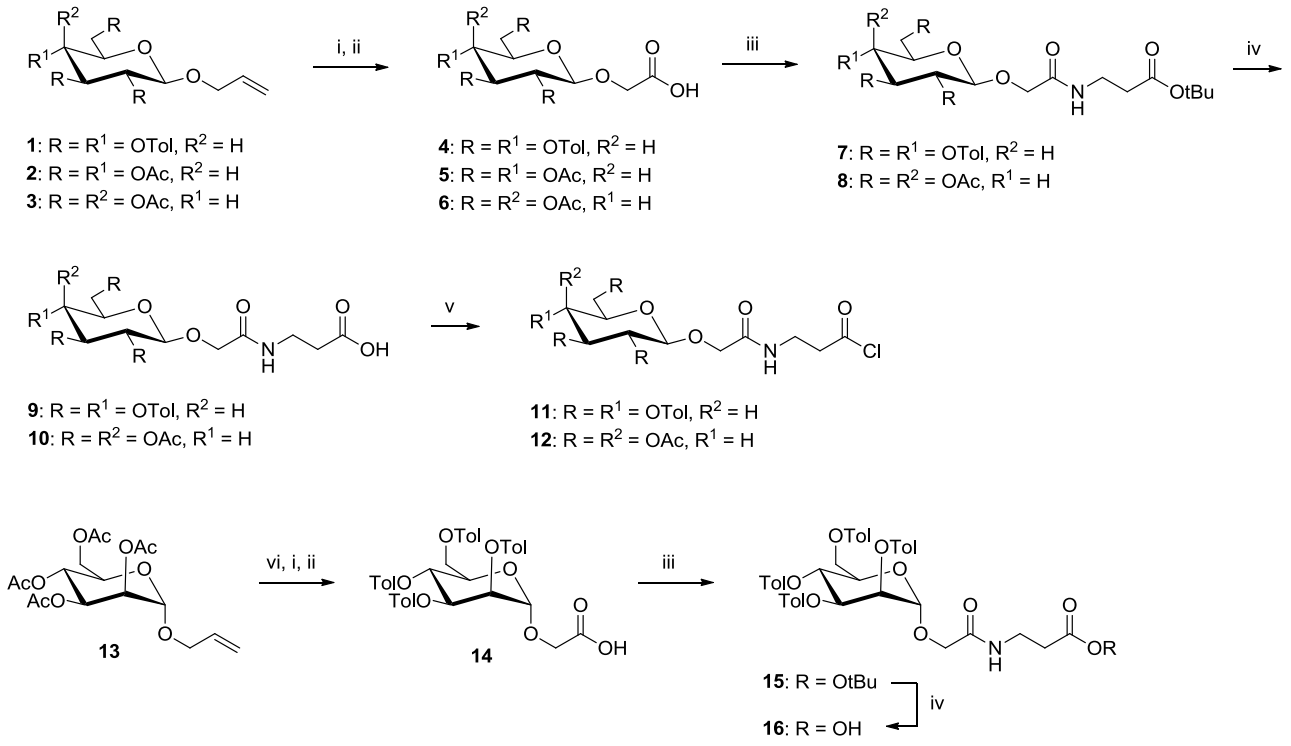
Table 1. ESI-MS Data of Biotinylated Glycoclusters **32**–**35**

Conjugate	$[(M + 2H^+) / 2]_{found}$	$[(M + 2H^+) / 2]_{req}$
32	1317.5209	1317.5184
33	1317.5212	1317.5184
34	761.3320[a]	761.3298
35	761.3337[a]	761.3298

[a] E/Z isomers, both peaks exhibited same molecular mass (Figure 1).

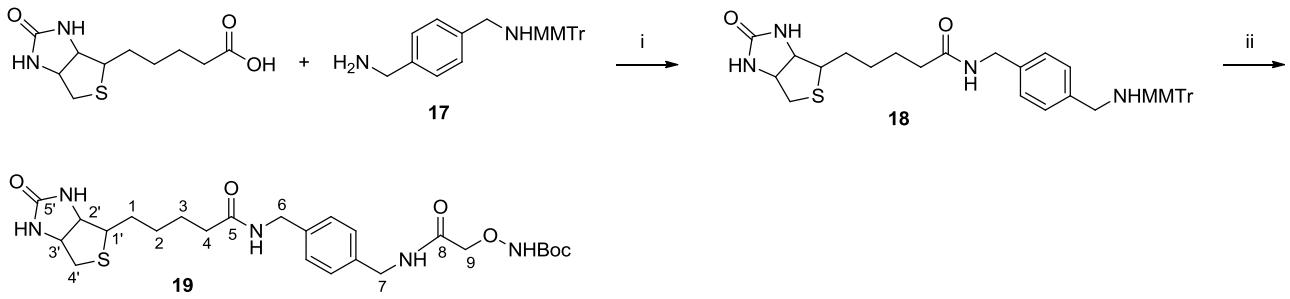
SCHEMES

Scheme 1. Synthesis of the Sugar Units[a]



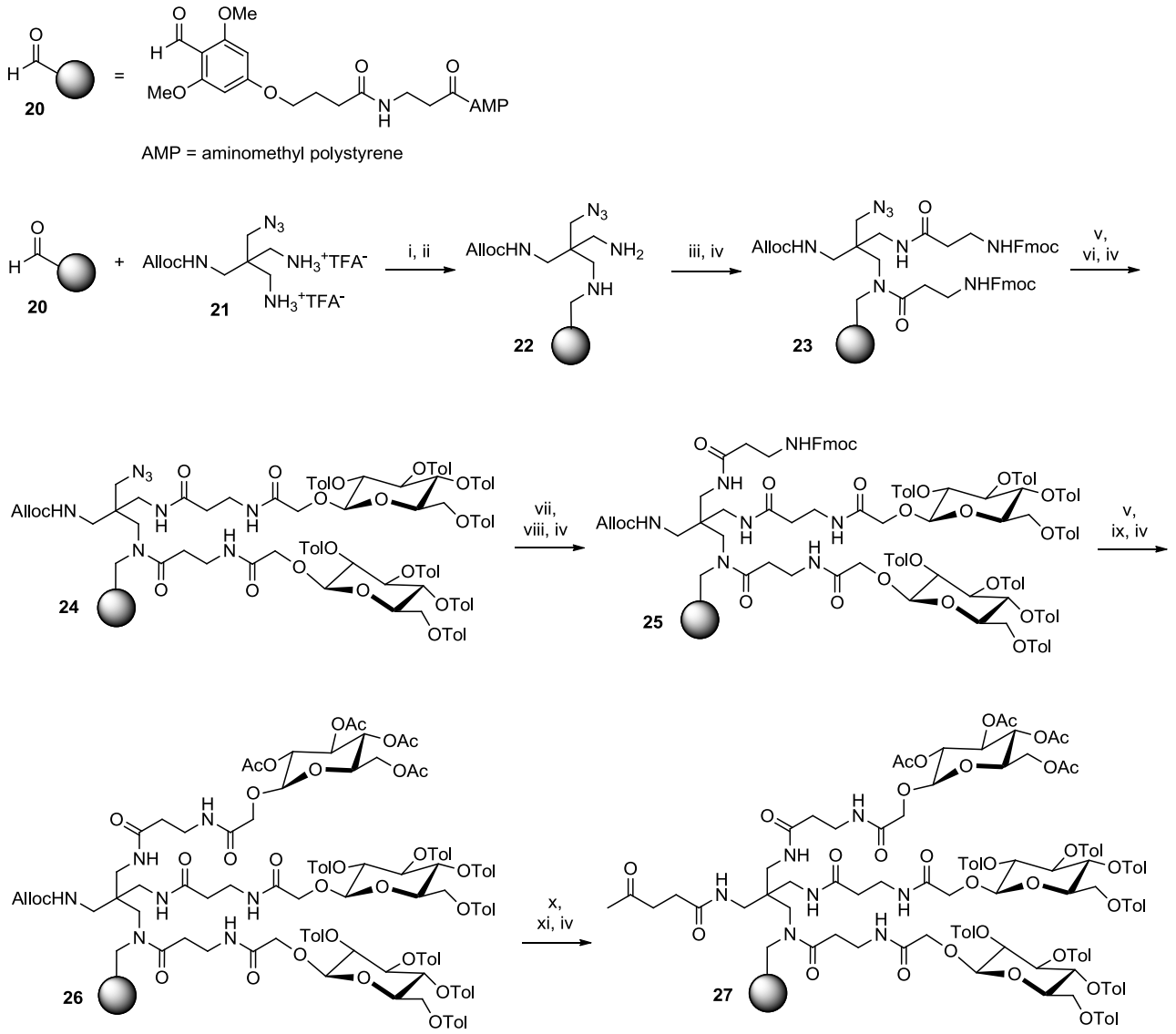
[a] Reagents and conditions: (i) OsO_4, $NaIO_4$, aq 1,4-dioxane; (ii) CrO_3, H_2SO_4, aq acetone; (iii) (1) HOSu, DCC, 1,4-dioxane, (2) H-*β*-Ala-O*t*Bu·HCl, K_2CO_3, H_2O; (iv) TFA, CH_2Cl_2; (v) $SOCl_2$; (vi) (1) NaOMe, MeOH, (2) 4-toluoyl chloride, Py.

Scheme 2. Synthesis of the Biotin Moiety[a]



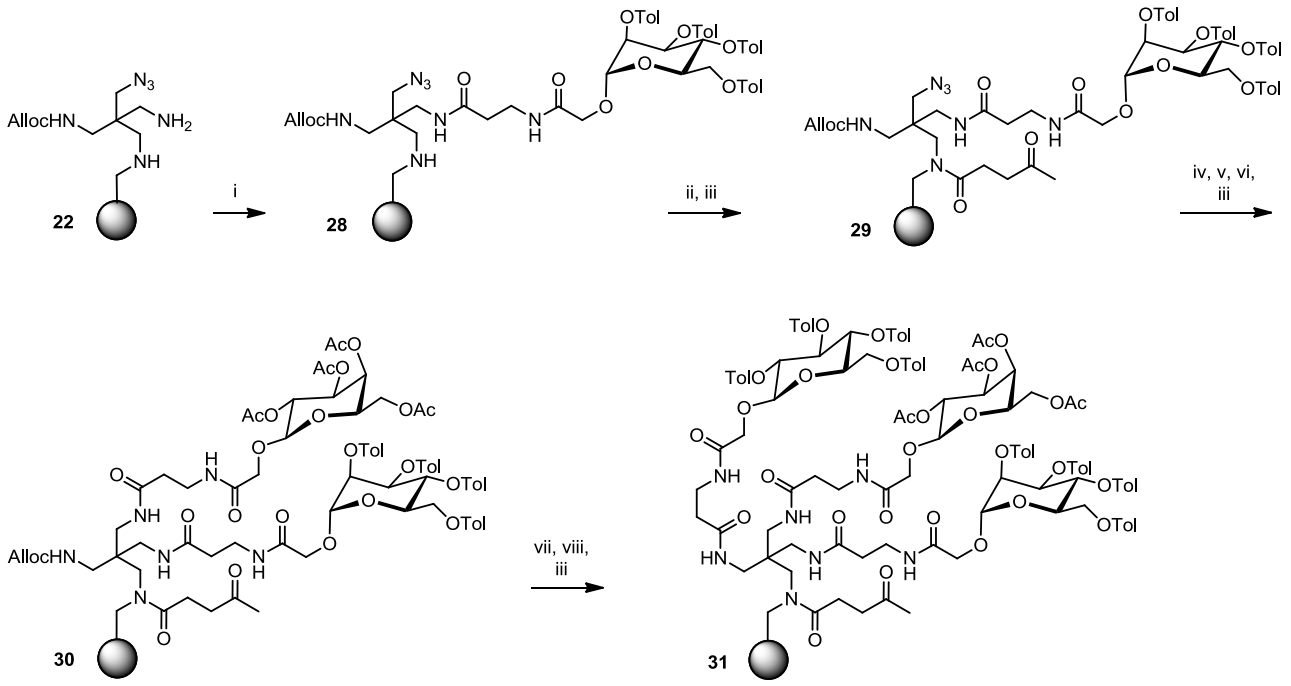
[a] Reagents and conditions: (i) HATU, DIEA, DMF; (ii) (1) TFA, CH_2Cl_2, MeOH, (2) BocAOAOH, HOBt·H_2O, DCC, DIEA, DMF.

Scheme 3. Synthesis of the Glucose Cluster[a]



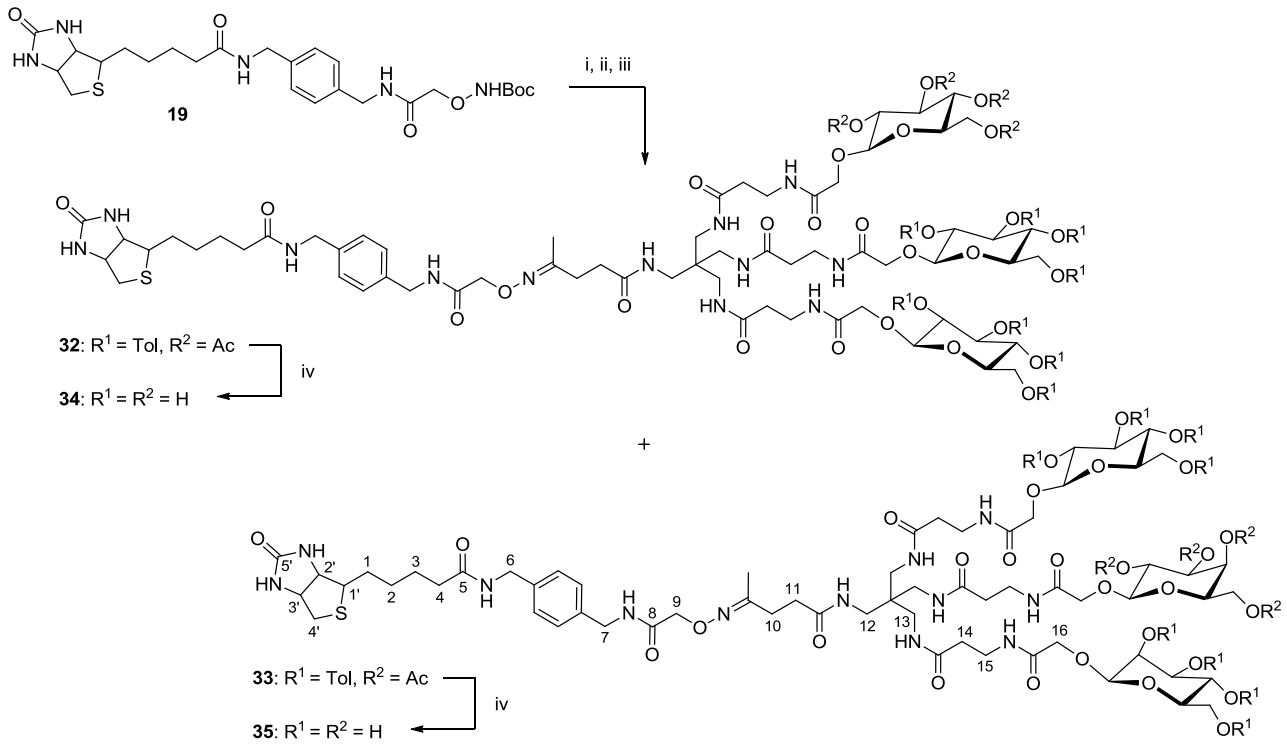
[a] Reagents and conditions: (i) $NaCNBH_3$, DMSO, NMP; (ii) $MeONH_2 \cdot HCl$, K_2CO_3, aq THF; (iii) $(Fmoc\text{-}\beta\text{-}Ala)_2O$, CH_2Cl_2, DMF; (iv) Ac_2O-capping; (v) piperidine, DMF; (vi) **4**, HOBt, DCC, NMP; (vii) Me_3P, toluene, aq 1,4-dioxane; (viii) Fmoc-β-Ala-OH, HBTU, DIEA, DMF; (ix) **5**, HBTU, DIEA, DMF; (x) $Pd(Ph_3P)_4$, $PhSiH_3$, CH_2Cl_2; (xi) $[CH_3CO(CH_2)_2CO]_2O$, CH_2Cl_2.

Scheme 4. Synthesis of the Heterotypic Cluster[a]



[a] Reagents and conditions: (i) **16**, HOBt, DCC, NMP; (ii) $[CH_3CO(CH_2)_2CO]_2O$, CH_2Cl_2; (iii) Ac_2O-capping; (iv) Me_3P, toluene, aq 1,4-dioxane; (v) **10**, HATU, DIEA, DMF; (vi) **12**, DIEA, CH_2Cl_2; (vii) $Pd(Ph_3P)_4$, $PhSiH_3$, CH_2Cl_2; (viii) **11**, DIEA, CH_2Cl_2.

Scheme 5. Synthesis of the Biotin Conjugates[a]



[a] Reagents and conditions: (i) TFA, CH_2Cl_2, MeOH; (ii) resin **27** or **31**, Py; (iii) TFA, CH_2Cl_2; (iv) NaOMe, MeOH.

FIGURES

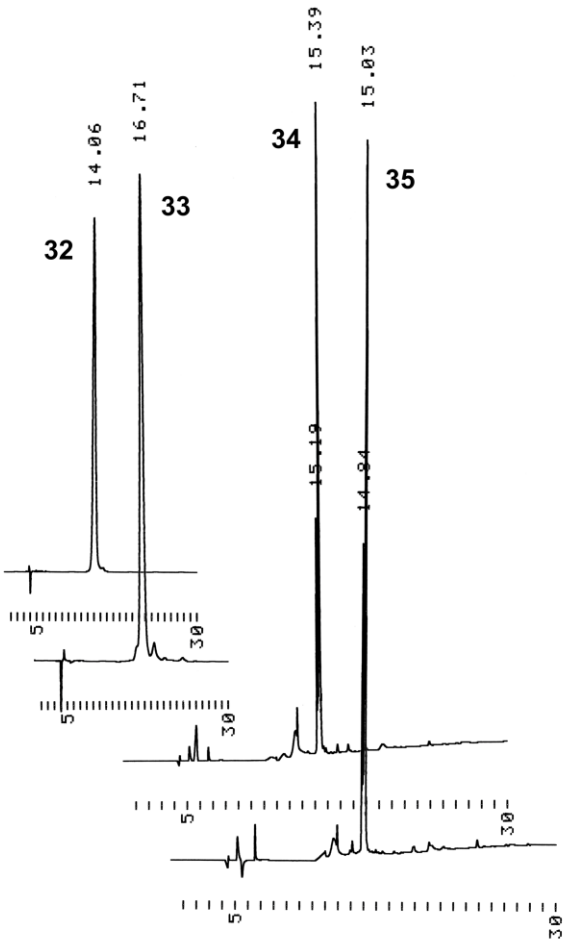


Figure 1. RP HPLC profiles of the purified biotin conjugates in protected (**32** and **33**, protocol A) and deprotected (**34** and **35**, protocol B) form. The deprotected conjugates show two peaks referring to the E/Z isomers formed upon oximation.

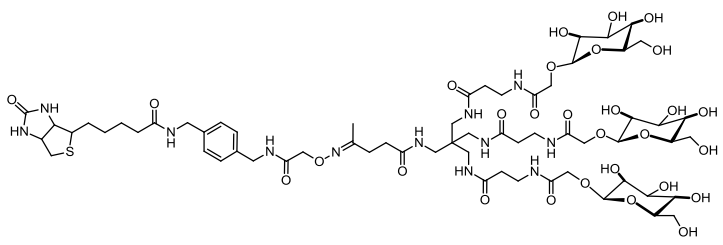


Table of Contents Graphic