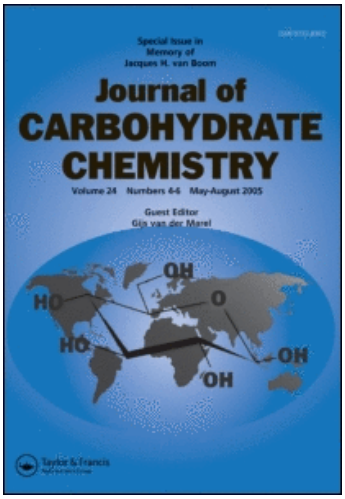

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SYNTHESIS OF A CARBOHYDRATE-CENTERED *C*-GLYCOSIDE CLUSTER[1]

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COMMUNICATION

SYNTHESIS OF A CARBOHYDRATE-CENTERED *C*-GLYCOSIDE CLUSTER[1]

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The carbohydrate moieties of glycoconjugates are involved in numerous recognition events in biological systems both in a physiological as well as a pathological context.[2,3] In these processes, multivalency of molecular interactions[4] plays an important role because certain sugar epitopes are frequently displayed in a polyvalent manner. With regard to the multiple-copy design of natural oligosaccharides, many means for clustering of carbohydrate ligands have been sought in order to provide synthetic mimetics which are more easily obtained than their natural counterparts, while possibly equally active.[5, 4c] During our attempts to inhibit the adhesion of *Escherichia coli* bacteria[6] to high mannose-type structures, we have employed different pathways for glycocluster synthesis comprising glycosidation,[7] peptide coupling,[8] thiourea bridging,[9] and photoaddition reactions.[10] In order to increase the stability of such compounds in a physiological environment, *C*-glycosides instead of *O*-glycosides can be employed in the design of carbohydrate-based antiadhesives.[11]

Here we present the synthesis of the first carbohydrate-centered[12] *C*-glycoside cluster **(5)** using two spacer-modified carbohydrate derivatives (**1** and **4**) and peptide coupling in the ligation step. Two possible pathways for the synthesis of peptide bond-ligated glycoclusters can be envisaged. Either an oligocarboxylic acid is employed as the core molecule and coated with an amino-functionalized carbohydrate derivative, or an oligoamine core is peptide-coupled to a carboxylated saccharide epitope. In either of these two pathways, according to observations

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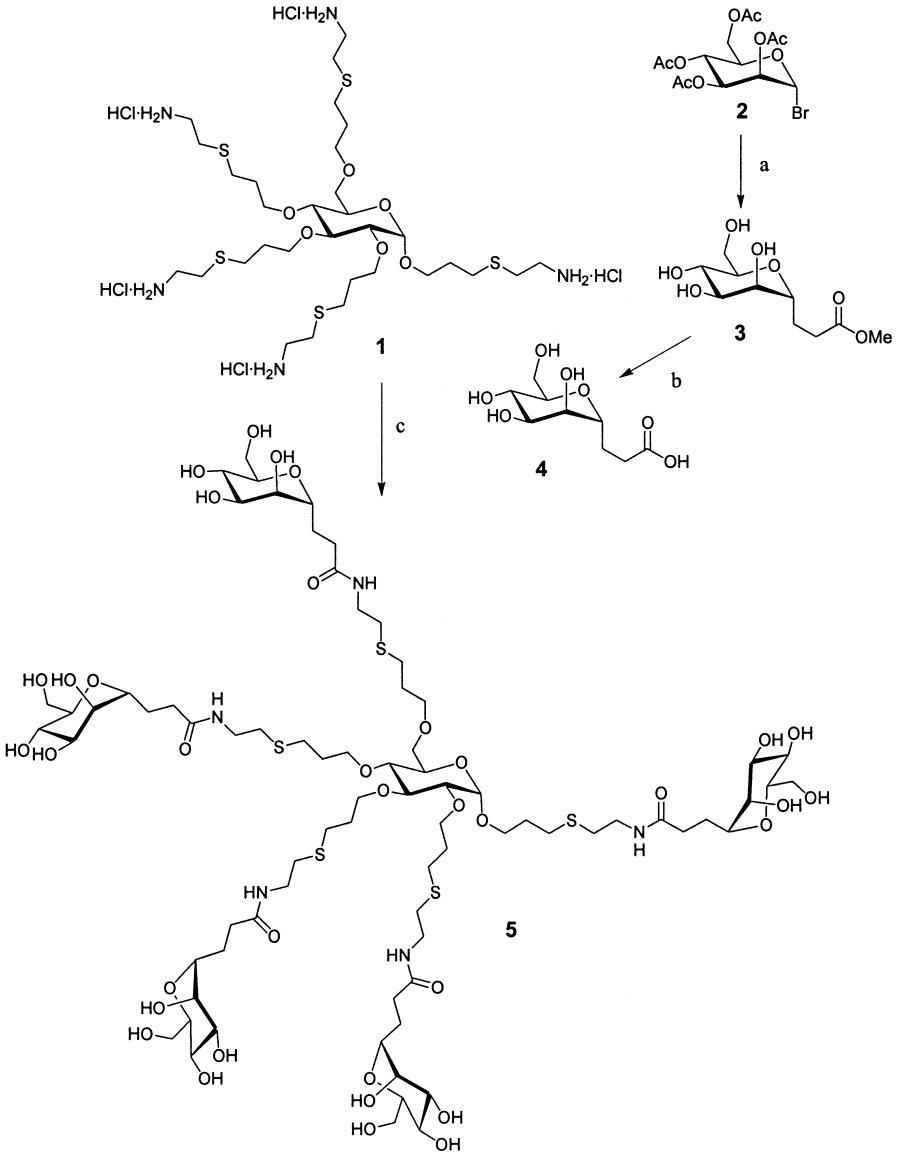
made in our laboratory, a powerful coupling reagent has to be chosen to achieve multiple peptide coupling in good yields.

For the synthesis of glycodendrimers and glycoclusters using amide connectivities, HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate N-oxide) was shown to be superior to other onium salts.[13] In a peptide coupling reaction, HATU has to



Scheme 1. a. Bu_3SnH, AIBN, methyl acrylate, toluene; NaOMe, MeOH, 24%. b. LiOH, H_2O. c. HATU, DIPEA, DMF, 68% (two steps).

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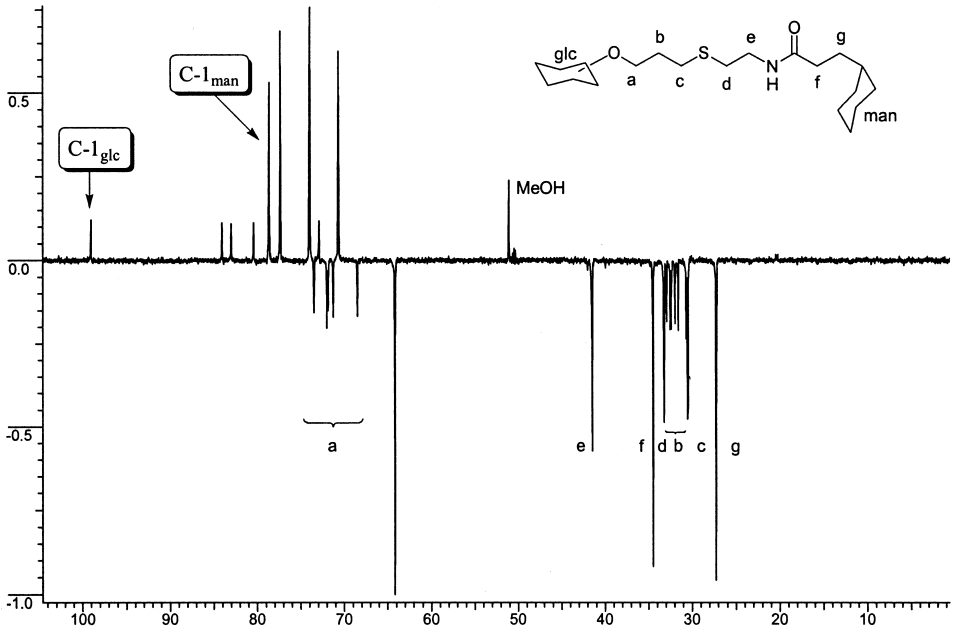
react first with the carboxylic acid component. This has to be a fast reaction in order to avoid decomposition of the expensive reagent.[14] In order to prepare a cluster glycoside by peptide coupling, it is therefore not advisable to use an oligocarboxylic acid but rather a polyamine as the core molecule. The latter was reacted with a carboxy-functionalized *C*-glycoside **(4)** as the acid component (Scheme 1). *C*-Mannoside **4** (Scheme 1) was synthesized, starting from the glycosyl bromide **2** in a classical radical reaction using methyl acrylate.[15] This reaction furnished methyl ester **3**, which in turn was saponified[16] to give the corresponding acid **4** in situ. This is a known compound,[11] which has, however, never been used in glycocluster synthesis. Here, it could be readily ligated to the glucose-centered pentaamino hydrochloride **1**,[10] which was selected as the core pentaamine in the peptide coupling reaction from a spectrum of different possibilities. 1.5 Equivalents of the acid **4** were used per amino group to obtain the *C*-glycosidically linked mannose cluster **5** in good yield. It is worthwhile to point out that due to the chiral core unit, NMR spectra of this type of molecules can be unequivocally solved (Scheme 2).

In conclusion, it was shown that a novel carbohydrate-centered *C*-mannoside glycocluster could be readily obtained by the peptide-coupling reaction of a branched oligoamine represented by **1** and a carboxylated *C*-mannoside such as **4**, involving a number of one-pot procedures en route to the product **5.** The method can be recommended for the multi-100 mg synthesis of the respective glycoclusters and will be further explored in our group.



Scheme 2.

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EXPERIMENTAL

General methods. TLC was performed on silica gel plates (GF_{254}, Merck). Detection was effected by UV irradiation and subsequent charring with 10% sulphuric acid in ethanol followed by heat treatment. Flash chromatography was performed on silica gel 60 (230–400 mesh, particle size 0.040–0.063 mm, Merck). Optical rotations were measured on a Perkin-Elmer 241 polarimeter (sodium-D-line: 589 nm, length of cell 1 dm) in water. NMR spectra were recorded on Bruker AMX 400 (400.13 MHz for ^{1}H, 100.61 MHz for ^{13}C) and DRX 500 (500.13 MHz for ^{1}H, 125.76 MHz for ^{13}C). Assignment of the peaks was achieved with the aid of 2D-NMR techniques (^{1}H-^{1}H-COSY and HMQC). MALDI-TOF-mass spectra were recorded on a Bruker Biflex III with 19 kV acceleration voltage and DHB (2,4-dihydroxy benzoic acid) as matrix (c = 10 μg/μL in 40% acetonitrile/water). Ionisation was effected with a nitrogen laser at 337 nm. Elemental analyses were carried out in the Institute of Inorganic Chemistry, Christian-Albrechts-University Kiel.

Methyl 3-(α-D-mannopyranosyl)propanoate (3). A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **(2)** (17.72 g, 43.1 mmol) and methyl acrylate (19.5 mL, 215.2 mmol) in degassed toluene (50 mL) was stirred at 85 °C and a solution of Bu_3SnH (22.8 mL, 86.2 mmol) and AIBN (400 mg) in toluene (30 mL) was added dropwise over 1 h. Then stirring was continued for 0.5 h at 85 °C. The solution was concentrated and the residue dried in high vacuum. The residual syrup was dissolved in acetonitrile, washed with hexane, concentrated and purified by column chromatography (1:1 toluene-EtOAc; R_f 0.42). The crude acetylated product was dissolved in dry MeOH and stirred for 2 h with 1M NaOMe in MeOH. The solution was neutralized with Dowex-H^+, filtered and purified by column chromatography (9:1 EtOAc-MeOH) to yield 1,5-anhydro-D-mannitol (470 mg, 7 %, R_f 0.06) as side product and the title compound **2** (2.62 g, 24 %, R_f 0.15) as a white solid: $[\alpha]_D$ +18.7° (*c* 1.4, H_2O); mp 77.7 °C; ^{1}H NMR (400 MHz, D_2O) δ 3.80-3.95 (m, 2H, H-1, H-2), 3.80–3.85 (m, 2H, H-3, H-6), 3.74 (dd≈t, 1H, *J* 6.1 Hz, H-4), 3.71 (s, 3H, OMe), 3.65 (dd≈t, 1H, *J* 9.4 Hz, H-6′), 3.51 (ddd, 1H, *J* 2.5, 6.1, 9.2 Hz, H-5), 2.43–2.58 (m, 2H, $OCH_2CH_2CO_2Me$), 2.11 (m, 1H, $OCHHCH_2CO_2Me$), 1.83 (m, 1H, $OCHHCH_2CO_2Me$) ppm; ^{13}C-NMR (100.67 MHz, D_2O) δ 176.9 (CO_2Me), 78.0 (CH, C-1), 74.1 (C-5), 71.7 (C-2), 71.3 (C-3), 67.7 (C-4), 61.6 (C-6), 52.7 (OMe), 30.6 ($OCH_2CH_2CO_2Me$), 23.3 ($OCH_2CH_2CO_2Me$) ppm.

10-α-D-Mannopyranosyl-8-oxo-4-thia-7-azadecyl-2,3,4,6-tetra-*O*-[10-α-D-mannopyranosyl-8-oxo-4-thia-7-azadecyl]-α-D-glucopyranoside (5). The octopus glycoside 3-(2-aminoethylthio)propyl-2,3,4,6-tetra-*O*-3(2-aminoethylthio)propyl-α-D-glucopyranoside **(1)**[9] (54 mg, 0.057 mmol) and diisopropylethyl amine (DIPEA, 0.1 mL, 0.580 mmol) were dissolved in dry DMF (4 mL) and stirred for 1 d. In the meantime **3** (110 mg, 0.44 mmol) was dissolved in methanol (6 mL) and water (4 mL) and LiOH × H_2O (102 mg, 2.4 mmol) was added. The solution was stirred at 0 °C until ester cleavage was completed (TLC, MeOH). Additional water was added (20 mL) and the solution was neutralized at 0 °C with 2M hydrochloric acid and freeze dried to yield **4**, which was used as a crude product.

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Then, a solution of **3** in DMF (4 mL) was added to the earlier prepared solution of **1.** The coupling reagent HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, 167 mg, 0.44 mmol) and DIPEA (0.15 ml, 0.88 mmol) were added and the solution was stirred for 36 h at rt. For work-up, the solution was passed over a Sephadex LH-20 column with methanol as the eluent, to yield **5** (82 mg, 68 %) as a colorless syrup: $[\alpha]_D$ +22.0° (*c* 1.4, H_2O); ^{1}H NMR (D_2O) δ 4.95 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1_{glc}), 3.93–3.48 (m, 49H, 5 (glc)OCH_2, H-3_{glc}, H-5_{glc}, H-6_{glc}, H-$6'_{glc}$, 5 H-1_{man}, 5 H-2_{man}, 5 H-3_{man}, 5 H-4_{man}, 5 H-5_{man}, 5 H-6_{man}, 5 H-$6'_{man}$), 3.43–3.35 (m, 10H+MeOH, SCH_2CH_2N), 3.31–3.25 (m, 2H, H-2_{glc}, H-4_{glc}), 2.75–2.64 (m, 20H, 5 (glc) $OCH_2CH_2CH_2SCH_2$), 2.45–2.29 (m, 10H, 5 (man)CH_2CH_2), 2.13–2.01 (m, 5H, 5 (man)C*H*H), 2.00–1.80 (m, 15H, 5 (man)C*H*H, 5 (glc)OCH_2CH_2) ppm; ^{13}C-NMR (125.77 MHz, D_2O): δ 99.1 (C-1_{glc}), 84.0 (C-3_{glc}), 83.0 (C-2_{glc}), 80.4 (C-4_{glc}), 78.7 (5×) (5 C-1_{man}), 77.4 (5×) (5 C-5_{man}), 74.0 (5×), 74.0 (5×) (5 C-2_{man}, 5 C-3_{man}), 72.9 (C-5_{glc}), 73.5, 72.0 (2×), 71.9, 71.3, 68.5 (C-6_{glc}, (glc)CH_2), 70.7 (5×) (5 C-4_{man}), 64.2 (5×) (5 C-6_{man}), 41.5 (5×) (5 SCH_2CH_2N), 34.5 (5×) (5 (man)CH_2CH_2), 33.3–33.2 (5×) (5 SCH_2CH_2N), 32.9, 32.5, 32.4, 32.0, 31.6 (5 (glc)OCH_2CH_2), 30.7, 30.6 (2×), 30.5 (2×) (5 (glc)$OCH_2CH_2CH_2S$), 27.3 (5×) (5(man)CH_2) ppm; MALDI-TOF-MS *m/z* 1879.4 ($(M+Na)^+$ calcd 1855.8).

Anal.Calcd for $C_{76}H_{137}N_5O_{36}S_5$ (1857.24): C 49.15, H 7.44, N 3.77; Found C 48.61, H 7.24, N 3.36%.

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