Investigation of the functional roles of mammalian carbohydrates in biological processes have been an area of intense study in recent years. In contrast, much less time has been devoted to structures of glycosphingolipids (GSLs) from invertebrates that differ significantly from mammalian glycans. Due to these differences in the glycan structure we have been interested in the relationships between the structure and biological activity of GSLs from invertebrate species and have synthesized oligosaccharides found in various Protostomia phyla. In the course of our studies, we paid attention to the presence of unique GSLs found in the parasite, Schistosoma mansoni. Makaaru et al. identified a novel glycan core structure termed “schisto-core” in the GSLs isolated from S. mansoni adult worm and they can be extended to other unique glycan sequences (Fig. 1: A—F). Moreover, the parasite egg and the cercariae are a rich source of highly antigenic, multifucosylated GSLs. Studies on the glycolobiology of parasitic helminthes can contribute to a better understanding of glycolipid-mediated host–parasite interactions containing the schisto core in S. mansoni. The analyses of di- and trisaccharide derivatives were conducted by stepwise synthesis of suitably protected monosaccharide-based glycosyl donors and acceptors. The tetrasaccharide derivative was conducted by block synthesis of a disaccharide acceptor and disaccharide donor.

Results and Discussion

The synthetic scheme for the synthesis of target compounds 1—3 are shown in Charts 1—3. Initially, the per-O-acetylated 2-(trimethylsilyl)ethyl glycoside derivatives 8, 16 and 27 were prepared that serve as synthetic intermediates but also will provide useful information how changes in the glycan structure relate to different stages in the parasite development. Based on this background, we have initiated synthetic research efforts to prepare GSLs (A—F). In this paper we report on the synthesis of GSLs (A—C; 1—3) this time. Disaccharide 1 contains the schisto-core sequence GalNAcb1→4Glcb1→Cer while trisaccharide 2 and tetrasaccharide 3 are elongated glycan sequences that contain a terminal schisto-core. Oligosaccharides 1—3 serve as molecular probes to explore glycosphingolipid-mediated interactions containing the schisto core in S. mansoni. The analyses of di- and trisaccharide derivatives were conducted by stepwise synthesis of suitably protected monosaccharide-based glycosyl donors and acceptors. The tetrasaccharide derivative was conducted by block synthesis of a disaccharide acceptor and disaccharide donor.

Fig. 1. Structures of Glycosphingolipids from the Parasite S. mansoni and Target Compounds 1—3

Reagents and conditions: (a) TMSOTf, MS 4 Å, CH₂Cl₂, 97%; (b) Zn, Ac₂O, AcOH, 85%; (c) 1), Pd–C/H₂, THF–MeOH 2), Ac₂O, Pyr., 95%; (d) 1), TFA, CH₂Cl₂ 2), CCl₃CN, DBU, CH₂Cl₂, 73%; (e) TMSOTf, MS 4 Å, CH₂Cl₂, 57%; (f) MeONa, dioxane/MeOH, 67%.

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for the installation of the ceramide moiety. Glycosylation of 4 with 5 in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and 4 Å molecular sieves (MS 4 Å) in CH₂Cl₂ gave the desired disaccharide (6) after purification in 97% yield. The anomeric proton of the GalNAc unit appeared as a doublet at δ 4.30 (d, J = 8.5 Hz).

Subsequently, the 2,2,2-trichloroethoxycarbonyl (Troc) group was converted to an acetamido moiety by reduction and N-acetylation with Zn–Ac₂O to afford 7 in 85% yield. Removal of benzyl group from 7 by catalytic hydrogenolysis over 10% Pd/C in MeOH and O-acetylation provided 8. Disaccharide derivative 6 was selected as precursor for the synthesis of trisaccharide 16. Two methods were studied for the selective deacetylation of 6 in the presence of benzoyl- and Troc-protecting groups. Initially, we attempted to selectively saponify the acetyl groups with p-toluenesulfonic acid in CHCl₃–MeOH to provide disaccharide 12 in 60% yield. However, the yield could be significantly improved by using guanidine/guanidinium nitrate reagent that provided 12 in 89% yield. Benzylidenation of disaccharide 12 produced acceptor 13, which was subjected to glycosylation with donor 14 using TMSOTf as promoter to afford...
the desired trisaccharide 15 in 85% yield. The β-glycosidic linkage was assigned on the basis of homonuclear coupling constants (H-1 of GlcNAc, δ = 4.96 ppm, J_{H1,H2} = 8.0 Hz). Removal of the Troc-protecting group was accomplished with Zn in a mixture containing AcOH and AcOH, followed by catalytic hydrogenation over 10% Pd–C in MeOH–AcOH and acetylation to provide trisaccharide intermediate 16. Tetrasaccharide intermediate 26 was prepared by block synthesis of a disaccharide acceptor 13 and disaccharide donor 25. Glycosyl acceptor 20 was obtained from 19(21) by deacetylation, benzylidene, chloroacetylation, and reductive ring-opening of the benzylidene acetal as previously described. TMSOTf-promoted glycosylation of acceptor 20 with donor 21(22) was carried out in the presence of 4 Å MS in CHCl₃, and afforded the desired disaccharide 22 as the sole product in 86% yield. The β-glycosidic linkage in 22 was confirmed by 1H-NMR spectroscopy. The anomeric proton of the galactose moiety appeared as a doublet with a homonuclear coupling constant of 7.9 Hz. Debckling of the 3-O-chloroacetyl group in 22 was accomplished with thiourea and the resulting alcohol was converted into ester by acetylation. Selective removal of the 3-(trimethylsilyl)ethyl group in 24 was achieved with tri-fluoroacetic acid in CH₂Cl₂, and treatment with CCl₄CN in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)23) to provide the corresponding α-trichloroacetimidate 25 in 74% yield. Tetrasaccharide derivative 26 was synthesized by coupling of donor 25 with disaccharide acceptor 13 in 83% yield. Debckling of 26 was achieved using the same conditions as described for trisaccharide 16 produced the per-O-acetylated tetrasaccharide derivative 27.

Next, for the selective removal of the 2-(trimethylsilyl)ethyl group, the fully acylated oligosaccharides 8, 16 and 27 were converted to the glycosyl imidate 28). 1H- and 13C-NMR spectra were recorded with a JMN A500 FT NMR spectrometer and a JEOL JNM-ECP600 with Me₄Si as internal standard for solutions in CDCl₃. Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)-MS was recorded on an Applied Biosystems Voyager RP mass spectrometer. High-resolution mass spectra were recorded on a JEOL JMS-700 under FAB conditions. TLC was performed on Silica Gel 60 F254 (E. Merck) with detection by quenching of UV fluorescence and by charring with 10% H₂SO₄. Column chromatography was carried out on Silica Gel 60 (E. Merck). 2-(Trimethylsilyl)ethyl 2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside (4), 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylaminol-1-O-β-D-galactopyranosyl trichloroacetimidate (5), 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylaminol-1-O-β-D-galactopyranosyl trichloroacetimidate (14), 2-(trimethylsilyl)ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylaminol-β-D-glucopyranoside (19) and 2,3,4,6-tetra-O-acetyl-1-O-β-D-galactopyranosyl trichloroacetimidate (21) were prepared as reported by Benzoylceramide 10 was prepared from phytosphingosine, which was purchased from Degussa (The Netherlands) by the conventional four-step procedure. 2-(Trimethylsilyl)ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylaminol-β-D-galactopyranosyl-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside (6) A solution of 4 (1.77 g, 3.06 mmol) and 5 (2.85 g, 4.57 mmol) containing activated 4 Å MS (5.0 g) in dry CH₂Cl₂, then cooled to −40 °C. TMSOTf (62.3 μl, 0.34 mmol) was added, and the mixture was stirred for 3 h at −40 °C, then neutralized with Et₃N. The solids were filtered off and washed with CHCl₃. The combined filtrate and washings were successively washed with water, dried (<i>MgSO</i>₄) and concentrated. The residue was purified by silica gel column chromatography using 2:1 hexane–ethyl acetate as eluent to give 6 as an amorphous powder (5.72 g, 74% yield). [α]ᵢ₂₀₀° = −34.5 (c 0.10, MeOH).

Conclusion

In summary, a systematic and integrated approach for the synthesis of three glycosphingolipids 1–3 was found in the parasite, <i>Schistosoma mansoni</i> has been accomplished. The synthetic strategy described may also find use for conjugation of the glycan portion of <i>S. mansoni</i> to other non-lipid-based carrier molecules. Biological testing for schistosomiasis using GSLs 1–3 is currently in progress and results will be reported in detail elsewhere. It is expected that the prepared glycosphingolipids will find use in future studies designed to reveal the relationships between the structure and biological activity for specific antibody detection in patients with schistosomiasis.

Experimental

General Methods

Optical rotations were measured with a Jasco P-1020 digital polarimeter. 1H- and 13C-NMR spectra were recorded with a JMN A500 FT NMR spectrometer and a JEOL JNM-ECP600 with Me₄Si as internal standard for solutions in CDCl₃. Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)-MS was recorded on an Applied Biosystems Voyager RP mass spectrometer. High-resolution mass spectra were recorded on a JEOL JMS-700 under FAB conditions. TLC was performed on Silica Gel 60 F254 (E. Merck) with detection by quenching of UV fluorescence and by charring with 10% H₂SO₄. Column chromatography was carried out on Silica Gel 60 (E. Merck).
As an amorphous powder (1.30 g, 1.04 mmol) was added guanidinium nitrate as eluent to give as white solid (14.5 mmol, 67%). 

The mixture was filtered and concentrated. The product was purified by Sephadex LH-20 column chromatography in MeOH to give as white solid (14.5 mmol, 67%). 

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eluent to give 15 as an amorphous powder (1.75 g, 85%). $[\alpha]_{D}^{20} +35.7$ (c=4.4, CHCl₃). $1^H$-NMR (500 MHz, CDCl₃): δ 4.96 (1H, d, J = 8.0 Hz, H-1 of GlcN), 4.80 (1H, d, J = 9.3 Hz, H-1 of GalN), 4.72 (1H, d, J = 9.3 Hz, H-1 of Glc). $1^3$C-NMR (125 MHz, CDCl₃): δ 170.4, 170.2, 169.3, 165.4, 165.2, 153.8, 138.7, 133.7, 134.3, 134.8, 132.76, 129.77x2, 129.6x2, 128.94, 128.87, 128.6, 128.5x2, 128.4x2, 128x2, 127.9x2, 127.8, 127.7, 126.3, 100.7, 100.4 (C-1 of Galc), 100.4 (C-1 of GalN), 99.1 (C-1 of GlcN), 95.5, 95.4, 75.5, 75.10, 74.99, 74.8, 74.4, 73.2, 73.1, 72.8, 71.8, 68.4, 68.3, 67.3, 66.2, 61.9, 60.6, 53.8, 20.72, 20.52, 20.48, 17.92x2, 1.68. MALDI-TOF-MS: Caled for C₅₃H₇₀N₂O₂₄SiNa: [(M+Na)⁺] m/z 1485.2. Found 1486.3.

2-(Trimethylsilyl)ethyl 2-Acetamido-O-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-di-O-acetyl-2-deoxy-β-D-galactopyranosyl (1→4)-6-O-acetyl-2,3-di-O-benzoyl-β-D-glucopyranoside (16) To a solution of 15 (1.75 g, 0.13 mmol) in Ac₂O (10.0 ml) and AcOH (20.0 ml) was added zinc powder (3.0 g). The reaction mixture was stirred for 16 h at 45 °C. After completion of the reaction, the mixture was filtered and the filtrate was diluted with CHCl₃, washed with water, dried (MgSO₄) and concentrated to give the intermediate. To a solution of the residue in MeOH (10.0 ml) was added AcOH (5.0 ml) and hydrogenolyzed under hydrolysis in the presence of 10% Pd/C (500 mg) for 18 h at room temperature, then filtered and concentrated. The residue was acetylated with acetic anhydride (20.0 ml) in pyridine (30.0 ml). The reaction mixture was added to 20% NaOH (10.0 ml). The residue was extracted with dichloromethane using 3:1 toluene-acetone as eluent to give 16 as syrup (855 mg, 62%). $[\alpha]_{D}^{20} +31.3$ (c = 2.5, CHCl₃). $1^H$-NMR (500 MHz, CDCl₃): δ 4.91 (1H, d, J = 8.2 Hz, H-1 of GlcN), 4.88 (1H, d, J = 8.2 Hz, H-1 of GalN), 4.79 (1H, d, J = 7.7 Hz, H-1 of Glc). $1^3$C-NMR (150 MHz, CDCl₃): δ 100.4 (C-1 of Gal), 100.3 (C-1 of Glc). MALDI-TOF-MS: Caled for C₉₆H₁₃₃N₃O₂₉Na: [(M+Na)⁺] m/z 1617.2. Found 1617.7.

To a solution of 16 (297 mg, 0.13 mmol) in MeOH (5.0 ml) was added EtOH (5.0 ml) and the filtrate was diluted with CHCl₃, washed with water, dried (MgSO₄) and concentrated to give the intermediate. To a solution of the residue in MeOH (10.0 ml) was added MeOH (5.0 ml) and hydrogenolyzed under hydrolysis in the presence of 10% Pd/C (500 mg) for 18 h at room temperature, then filtered and concentrated. The residue was acetylated with acetic anhydride (20.0 ml) in pyridine (30.0 ml). The reaction mixture was added 20% NaOH (10.0 ml). The residue was extracted with dichloromethane using 3:1 toluene-acetone as eluent to give 16 as syrup (855 mg, 62%). $[\alpha]_{D}^{20} +31.3$ (c = 2.5, CHCl₃). $1^H$-NMR (500 MHz, CDCl₃): δ 4.91 (1H, d, J = 8.2 Hz, H-1 of GlcN), 4.88 (1H, d, J = 8.2 Hz, H-1 of GalN), 4.79 (1H, d, J = 7.7 Hz, H-1 of Glc). $1^3$C-NMR (150 MHz, CDCl₃): δ 100.4 (C-1 of Gal), 100.3 (C-1 of Glc). MALDI-TOF-MS: Caled for C₉₆H₁₃₃N₃O₂₉Na: [(M+Na)⁺] m/z 1617.2. Found 1617.7.

2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-2-deoxy-2,2,2-trichloroethoxy-carbonyl-β-D-glucopyranoside (20) To a solution of compound 19 (3.0 g, 0.0158 mol) in CH₃Cl (60.0 ml) was added pyridine (6.0 ml) then cooled to 0 °C. CICCl₄ (40.8 ml, 597 mmol, 2.0 eq) was added and the mixture was stirred for 10 h at room temperature. The mixture was diluted with CHCl₃, washed with 5% HCl, 1% sodium bicarbonate (aq.), dried (MgSO₄) and concentrated under reduced pressure. MALDI-TOF-MS: Caled for C₅₃H₇₀N₂O₂₄SiNa: [(M+Na)⁺] m/z 1485.2. Found 1486.3.
and concentrated. The product was purified by silica gel column chromatography using 3:2 hexane-ethyl acetate as eluent to give 23 as a white solid (624 mg, 85%). \([\text{C} 37\text{H}52\text{Cl}3\text{NO}17\text{SiNa}]: (M^+ + 9.5 = 0.08, \text{CHCl}_3).\)

\[\text{FAB-MS: Calcd for C}_{37}\text{H}_{52}\text{Cl}_3\text{NO}_{17}\text{SiNa}: (\text{[M} + \\]Na\text{]}^+) = m/z 896. 896.862.

98.

- D-glucopyranosyl-(1→3)-O-benzyl-2-deoxy-2,2-(2,2-trichloroethoxy)carbonylaminio)-O-\[\text{glucopyranosyl-(1→4)}\)-O-benzyl-2-deoxy-2,2-(2,2-trichloroethoxy)carbonylaminio)-O-glucopyranosyl-(1→4)-O-benzyl-2-deoxy-2,2-(2,2-trichloroethoxy)carbonylaminio) was prepared from 35 \(\text{mmol} \) by the same method described for preparation of 23. The product was purified by silica gel column chromatography using 3:2 hexane-ethyl acetate as eluent to give 23 as a white solid (624 mg, 85%). \([\text{C} 37\text{H}52\text{Cl}3\text{NO}17\text{SiNa}]: (M^+ + 9.5 = 0.08, \text{CHCl}_3).\)

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- D-glucopyranosyl-(1→4)-O-benzyl-2-deoxy-2,2-(2,2-trichloroethoxy)carbonylaminio)-O-glucopyranosyl-(1→4)-O-benzyl-2-deoxy-2,2-(2,2-trichloroethoxy)carbonylaminio) was prepared from 35 \(\text{mmol} \) by the same method described for preparation of 23. The product was purified by silica gel column chromatography using 3:2 hexane-ethyl acetate as eluent to give 23 as a white solid (624 mg, 85%). \([\text{C} 37\text{H}52\text{Cl}3\text{NO}17\text{SiNa}]: (M^+ + 9.5 = 0.08, \text{CHCl}_3).\)
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