ELSEVIER

Chemistry and Physics of Lipids
114 (2002) 139–147

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

New modified single chained glycolipids. Part 1: synthesis of deoxy and partially *O*-methylated glycolipids with or without a sulfur containing spacer

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Received 23 April 2001; received in revised form 8 October 2001; accepted 10 October 2001

Abstract

A way to synthesize neoglycolipids with high yields and anomeric purity is described. Starting point of the synthesis strategy is the glycosylation of allyl alcohol with definite steric orientation. Introduction of the hydrophobic moiety was achieved by photoaddition of *n*-hexadecanethiol and 3-mercaptopropionic acid followed by amidation with *n*-hexadecylamine, respectively. In order to investigate the influence of different carbohydrate headgroups in the physicochemical behavior of the general glycolipid, especially the orientation of the alkyl chain, a range of neoglycolipids was synthesized. Beside the differences in the configuration between unfunctionalized glycopyranoses like D-glucose, D-galactose and D-mannose, a number of deoxy and partially *O*-methylated sugar derivatives was prepared. The divergences concerning the different carbohydrate headgroups and the hydrophobic moiety, respectively, can be compared to relatively simple structured glycolipids with hexadecyl residue and without spacer function.

Keywords: Synthesis; Neoglycolipid; Carbohydrate; Spacer

1. Introduction

Neoglycoconjugates are of growing interest in the understanding of biological phenomena as well as the physicochemical behavior of model systems. It is well known that interactions between natural carbohydrates on the surface of cells are responsible for a range of recognition processes, e.g. inflammatory response or immune response (Lee and Lee, 1995). The specificity of this ligand–receptor binding is dependent on the configurational features of the hydrophilic sugar moiety. So a complete knowledge and description of the orientation, conformation and surface density are of decisive importance for further investigations. The utilization of natural glycoconjugates, e.g. Sialyl Lewis^X (Töpfer et al., 1994) is very difficult because of the problematic isolation and the difficulties of total synthesis based on the complexity of these compounds. So

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0009-3084/02/$ - see front matter
PII: S0009-3084(01)00196-7

the interest in simple structured neoglycolipids with the possibility to vary the structure concerning the carbohydrate domain and the hydrophobic anchor is rising up. It was shown that the character of the terminal sugar headgroup determinates the efficiency of the ligand coupling to the receptor (Ladisch et al., 1992). Furthermore such an interaction can only occur when the carbohydrate domain is separated from the hydrophobic moiety ergo the size of the spacer in the molecule has to be optimized according to the specific receptor (Berthelot et al., 1998). Finally the structure of the hydrophobic lipid anchor has an influence on the properties of the general glycolipid with regard to the length of the alkyl chain and its orientation.

Main problem of conventional neoglycolipid synthesis is the relatively low overall yield of the glycosylation step between glycosyl donor and acceptor including the stereoselectivity especially in the case of long chain alcohols. A range of improvements concerning the glycosylation methods was accomplished but the low reactivity of long chain alcohols is the crucial point of the synthesis. In continuation of our efforts devoted to the synthesis of glycolipids as membrane-bound stabilizing carbohydrates (Wilhelm et al., 1995) we developed a strategy which makes use of the reactive allyloxy group as a glycosyl acceptor into the carbohydrate molecule. Photoaddition of the double bond with a long chain thiol or with 3-mercaptopropionic acid followed by amidation affords a range of new compounds which are characterized by different structured carbohydrate headgroups. The varying terminal sugar residues containing partially deoxygenated or *O*-methylated domains were synthesized to investigate biochemically the influence of the hydrogen bonding strength contingent on the stereoscopic arrangement of the hydroxy groups in the ligand receptor binding. This neoglycolipids and a number of conventionally prepared glycosides with the hexadecyloxy aglycon are destined for first physicochemical investigations to the conformational orientation of the alkyl chain by differential scanning calorimetry, X-ray diffraction and Raman spectroscopy.

2. Material and methods

NMR spectra were recorded in a Bruker AC 500 spectrometer at a frequency of 400 MHz with TMS as an internal standard. Mass spectra were obtained by using a Finnigan MAT 710 spectrometer. The ionization was effected at 4.5 kV both in negative and positive mode. Elemental analyses were performed with a CHNS-932 apparatus (LECO Corporation). Column chromatography was carried out with silica gel 60 (0.04–0.2 mm, Merck) and preparative, centrifugally accelerated, radial, thin layer chromatography with a Chromatotron (Harrison Research). All reactions were performed under argon atmosphere. Solvents were dried before use. All chemicals were purchased directly from Aldrich and Fluka.

2.1. Synthesis

2.1.1. General method for 1,2-trans-glycosylation using TMS-triflate

TMS-triflate (0.01 mol) was added to a solution of peracetylated monosaccharide **1** (0.01 mol) in dry CH_2Cl_2 (20 ml) and freshly activated molecular sieves and stirred for 30 min at 20 °C. Hexadecanol (0.01 mol) was added and the mixture stirred overnight at room temperature.

Next day it was filtered under dry condition, filtrate was made basic with $NaHCO_3$ and extracted with CH_2Cl_2. The organic extract was washed with water and dried. The residue obtained after evaporation of the solvent was dissolved in MeOH and deacetylation was achieved with NH_3–MeOH followed by purification through chromatography over silica gel led to the desired products **2a**–**d**.

*2.1.1.1. Hexadecyl β-D-glucopyranoside (**2a**).* MS (ES, positive ions): m/z 427.58 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.58; yield 32%; Anal. Calc. for $C_{22}H_{44}O_6$: C, 65.31; H, 10.96. Found: C, 65.04; H, 10.89%.

*2.1.1.2. Hexadecyl 6-O-methyl-β-D-glucopyranoside (**2b**).* MS (ES, positive ions): m/z 441.61 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.66; yield 17%; Anal. Calc. for $C_{23}H_{46}O_6$: C, 65.99; H, 11.08. Found: C, 65.88; H, 11.15%.

*2.1.1.3. Hexadecyl 3-O-methyl-β-D-glucopyranoside (**2c**).* MS (ES, positive ions): m/z 441.61 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.65; yield 13%; Anal. Calc. for $C_{23}H_{46}O_6$: C, 65.99; H, 11.08. Found: C, 65.95; H, 11.17%.

*2.1.1.4. Hexadecyl 6-deoxy-β-D-glucopyranoside (**2d**).* MS (ES, positive ions): m/z 411.58 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.58; yield 15%; Anal. Calc. for $C_{22}H_{44}O_5$: C, 68.00; H, 11.41. Found: C, 67.92; H, 11.26%.

*2.1.2. General method for the synthesis of the peracetylated allyl glycopyranosides **4***

A solution of peracetylated monosaccharide (0.01 mol) in dry CH_2Cl_2 (15 ml) was stirred with $BF_3{\cdot}Et_2O$ (0.011 mol) at room temperature for 1 h. Allyl alcohol (0.015 mol) was then added to it and stirred at room temperature for 4 h. This was then cooled to 0 °C and the mixture stirred with saturated $NaHCO_3$ to basify and to destroy the Lewis acid it was stirred for another 30 min. The resulting mixture was then extracted with ether and washed with water. Chromatographic purification led to the allyl glycosides **4** in good yield.

*2.1.2.1. Allyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**4a**).* ^{1}H NMR ($CDCl_3$): $\delta = 1.96$, 2.01, 2.08, 2.13 (4s, 12H, 4 $COCH_3$), 3.96–4.03 (m, 2H, –OCH_2), 4.06–4.10 (dd, $J = 2.3$ and 12.1 Hz, 1H, 6-*H*), 4.14–4.19 (m, 1H, 5-*H*), 4.24–4.28 (dd, $J = 5.2$ and 12.2 Hz, 1H, 6′-*H*), 4.84 (d, $J = 1.8$ Hz, 1H, 1-*H*α), 5.20–5.37 (m, 5H, –CH=CH_2, 2-*H*, 4-*H*, 3-*H*), 5.82–5.92 (m, 1H, CH_2–C*H*=CH_2); MS (ES, positive ions): m/z 411.36 $(M+23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.44; yield 93%; Anal. Calc. for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.53; H, 6.26%.

*2.1.2.2. Allyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**4b**).* ^{1}H NMR ($CDCl_3$): $\delta = 2.00$, 2.02, 2.04, 2.08 (4s, 12H, 4 $COCH_3$), 3.67–3.71 (m, 1H, 5-*H*), 4.07–4.14 (m, 2H, –OCH_2), 4.17–4.22 (dd, $J = 2.53$ and 12.24 Hz, 1H, 6-*H*), 4.24–4.31 (dd, $J = 4.66$–12.24 Hz, 1H, 6′-*H*), 4.55 (d, $J = 7.8$ Hz, 1H, 1-*H*β), 5.00–5.12 (m, 2H, –CH=CH_2), 5.18 (dd, $J = 7.9$ and 9.5 Hz, 1H, 2-*H*), 5.21 (dd, $J = 9.4$ and 9.7 Hz, 1H, 4-*H*), 5.25 (dd, $J = 9.4$ and 9.5 Hz, 1H, 3-*H*), 5.79–5.81 (m, 1H, CH_2–C*H*=CH_2): MS (ES, positive ions): m/z 411.36 $(M+23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.42; yield 62%; Anal. Calc. for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.52; H, 6.28%.

*2.1.2.3. Allyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**4c**).* ^{1}H NMR ($CDCl_3$): $\delta = 1.96$, 2.03, 2.04, 2.14 (4s, 12H, 4 $COCH_3$), 4.08–4.19 (m, 4H, 5-*H*, –OCH_2, 6-*H*), 4.31 (dd, $J = 1.6$ and 5.9 Hz, 1H, 6′-*H*), 4.49 (d, $J = 8.3$ Hz, 1H, 1-*H*β), 4.98 (dd, $J = 7.8$ and 10.4 Hz, 1H, 2-*H*), 5.17–5.28 (m, 3H, –CH=CH_2, 4-*H*), 5.36 (dd, $J = 3.5$ and 10.4 Hz, 1H, 3-*H*), 5.79–5.88 (m, 1H, CH_2–C*H*=CH_2); MS (ES, positive ions): m/z 411.36 $(M+23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.41; yield 73%; Anal. Calc. for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.54; H, 6.27%.

*2.1.2.4. Allyl 6-O-methyl-2,3,4-tri-O-acetyl-β-D-glucopyranoside (**4d**).* ^{1}H NMR ($CDCl_3$): $\delta = 1.93$, 1.95, 1.98 (3s, 9H, 3 $COCH_3$), 3.35 (s, 3H, –OCH_3), 3.61–3.65 (m, 1H, 5-*H*), 4.00–4.09 (m, 2H, 6-*H*, 6′-*H*), 4.18–4.30 (m, 2H, –OCH_2), 4.49 (d, $J = 7.8$ Hz, 1H, 1-*H*β), 4.93 (dd, $J = 7.6$ and 9.6 Hz, 1H, 2-*H*), 4.97 (dd, $J = 9.5$ and 9.8 Hz, 1H, 4-*H*), 5.17 (dd, $J = 9.4$ and 9.6 Hz, 1H, 3-*H*), 5.11–5.22 (m, 2H, –CH=CH_2), 5.73–5.82 (m, 1H, CH_2–C*H*=CH_2); MS (ES, positive ions): m/z 383.35 $(M+23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.45; yield 66%; Anal. Calc. for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 53.17; H, 6.82%.

*2.1.2.5. Allyl 3-O-methyl-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**4e**).* ^{1}H NMR ($CDCl_3$): $\delta = 2.04$, 2.05, 2.06 (3s, 9H, 3 $COCH_3$), 3.36 (s, 3H, –OCH_3), 3.52–3.57 (m, 1H, 5-*H*), 4.01–4.23 (m, 4H, 6-*H*, 6′-*H*, –OCH_2), 4.41 (d, $J = 8.0$ Hz, 1H, 1-*H*β), 4.94–5.14 (m, 2H, –CH=CH_2), 4.95 (dd, $J = 7.7$ and 9.6 Hz, 1H, 2-*H*), 5.13 (dd, $J = 9.5$ and 9.6 Hz, 1H, 4-*H*), 5.20 (dd, $J = 9.4$ and 9.5 Hz, 1H, 3-*H*), 5.76–5.85 (m, 1H, CH_2–C*H*=CH_2); MS (ES, positive ions): m/z 383.356 $(M+23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.36; yield 54%; Anal. Calc. for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 53.19; H, 6.81%.

*2.1.2.6. Allyl 2-deoxy-3,4,6-tri-O-acetyl-β-D-arabinohexopyranoside (**4f**).* ^{1}H NMR ($CDCl_3$): $\delta =$ 1.78–1.85 (m, 1H, 2-*H*), 1.98, 2.01, 2.07 (3s, 9H, 3 $COCH_3$), 2.21–2.26 (m, 1H, 2′-*H*), 3.92–4.14 (m, 5H, 6-*H*, 6′-*H*, $-OCH_2$, 5-*H*), 4.26 (dd, $J =$ 12.3 and 4.6 Hz, 1H, 1-*H*β), 4.94–5.17 (m, 2H, $-CH{=}CH_2$), 4.98 (t, $J = 9.9$ Hz, 1H, 4-*H*), 5.29–5.34 (m, 1H, 3-*H*), 5.83–5.93 (m, 1H, $CH_2-CH{=}CH_2$); MS (ES, positive ions): m/z 353.32 $(M + 23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.47; yield 53%; Anal. Calc. for $C_{15}H_{22}O_8$: C, 54.54; H, 6.71. Found: C, 54.47; H, 6.75%.

*2.1.2.7. Allyl 6-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranoside (**4g**).* ^{1}H NMR ($CDCl_3$): $\delta = 1.55$ (d, 3H, $-CH_3$), 1.97, 2.01, 2.02 (3s, 9H, 3 $COCH_3$), 3.51–3.55 (m, 1H, 5-*H*), 4.03–4.33 (m, 2H, $-OCH_2$), 4.49 (d, $J = 8.01$ Hz, 1H, 1-*H*β), 4.80 (dd, $J = 7.5$ and 9.5 Hz, 1H, 2-*H*), 4.95 (dd, $J = 9.5$ and 9.5, 1H, 4-*H*), 5.11–5.18 (m, 2H, $-CH{=}CH_2$), 5.22 (dd, $J = 9.6$ and 9.7 Hz, 1H, 3-*H*), 5.78–5.87 (m, 1H, $CH_2-CH{=}CH_2$); MS (ES, positive ions): m/z 453.32 $(M + 23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.5; yield 54%; Anal. Calc. for $C_{15}H_{22}O_8$: C, 54.54; H, 6.71. Found: C, 54.45; H, 6.74%.

*2.1.3. General method for the photoaddition reaction of hexadecyl thiol to the peracetylated allyl glycopyranosides **4***

To a solution of **4** (0.01 mol) in 5 ml $CHCl_3$ hexadecyl thiol (0.05 mol) was added. The mixture was stirred in a quartz flask under argon atmosphere at room temperature and irradiated at 254 nm for 2 h.

Detection of the quantitative reaction of double bond was achieved by a solution of bromine in MeOH. The mixture was diluted with water and $CHCl_3$, the organic phase was separated and dried over Na_2SO_4. The residue obtained after filtration and evaporation of the solvent was purified by flash chromatography (petroleum ether/EtOAc 8:2 v/v) to give **5** in good yield.

*2.1.3.1. (4-Thiaicosyl) 2′,3′,4′,6′-tetra-O-acetyl-α-D-mannopyranoside (**5a**).* ^{1}H NMR ($CDCl_3$): $\delta =$ 0.82–0.86 (t, 3H, $-CH_3$), 1.23–1.28 (s, 26H, $-CH_2-$), 1.51–1.57 (m, 4H, $-SCH_2CH_2-$), 1.99, 2.01, 2.02, 2.04 (4s, 12H, $COCH_3$), 2.42 (t, 2H, $-SCH_2(CH_2)_{14}-$), 2.51 (t, 2H, $-SCH_2(CH_2)_2O-$), 3.73–3.88 (m, 2H, $-OCH_2-$), 4.02–4.07 (dd, $J =$ 2.3 and 12.3 Hz, 1H, 6-*H*), 4.12–4.17 (m, 1H, 5-*H*), 4.25–4.29 (dd, $J = 5.2$ and 12.2 Hz, 1H, 6′-*H*), 4.87 (d, $J = 1.7$ Hz, 1H, 1-*H*α), 5.17–5.42 (m, 3H, 2-*H*, 4-*H*); MS (ES, positive ions): m/z 685.88 $(M + 39)^+$; R_f ($CHCl_3/Et_2O$ 8:2) 0.54; yield 95%; Anal. Calc. for $C_{33}H_{58}O_{10}S$: C, 61.27; H, 9.04; S, 4.96. Found: C, 61.23; H, 9.11; S, 5.00%.

*2.1.3.2. (4-Thiaicosyl) 2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranoside (**5b**).* ^{1}H NMR ($CDCl_3$): $\delta =$ 0.84–0.87 (t, 3H, $-CH_3$), 1.22–1.28 (s, 26H, $-CH_2-$), 1.50–1.56 (m, 4H, $-SCH_2CH_2-$), 1.98, 2.00, 2.02, 2.06 (4s, 12H, $COCH_3$), 2.44 (t, 2H, $-SCH_2(CH_2)_{14}-$), 2.50 (t, 2H, $-SCH_2(CH_2)_2O-$), 3.61–3.69 (m, 2H, $-OCH_2-$), 3.89–3.95 (m, 1H, 5-*H*), 4.18–4.26 (m, 2H, 6-*H*, 6′-*H*), 4.47 (d, $J = 7.8$ Hz, 1H, 1-*H*β), 4.94 (dd, $J = 7.8$ and 9.5 Hz, 1H, 2-*H*), 5.03 (dd, $J = 9.4$ and 9.6 Hz, 1H, 4-*H*), 5.16 (dd, $J = 9.5$ and 9.8, 1H, 3-*H*); MS (ES, positive ions): m/z 685.88 $(M + 39)^+$; R_f ($CHCl_3/Et_2O$ 8:2) 0.53; yield 95%; Anal. Calc. for $C_{33}H_{58}O_{10}S$: C, 61.27; H, 9.04; S, 4.96. Found: C, 61.21; H, 9.13; S, 5.01%.

*2.1.3.3. (4-Thiaicosyl) 2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranoside (**5c**).* ^{1}H NMR ($CDCl_3$): $\delta =$ 0.83–0.88 (t, 3H, $-CH_3$), 1.23–1.27 (s, 26H, $-CH_2-$), 1.49–1.53 (m, 4H, $-SCH_2CH_2-$), 1.97, 2.04, 2.05, 2.17 (4s, 12H, 4 $COCH_3$), 2.46 (t, 2H, $-SCH_2(CH_2)_{14}-$), 2.51 (t, 2H, $-SCH_2(CH_2)_2O-$), 3.63–3.69 (m, 2H, $-OCH_2-$), 4.09–4.22 (m, 4H, 5-*H*, $-OCH_2$, 6-*H*), 4.34 (dd, $J = 1.8$ and 5.8 Hz, 1H, 6′-*H*), 4.53 (d, $J = 8.5$ Hz, 1H, 1-*H*β), 4.99 (dd, $J = 7.8$ and 10.5 Hz, 1H, 2-*H*), 5.16–5.23 (m, 1H, 4-*H*), 5.42 (dd, $J = 3.3$ and 10.4 Hz, 1H, 3-*H*); MS (ES, positive ions): m/z 669.87 $(M + 23)^+$; R_f ($CHCl_3/Et_2O$ 8:2) 0.48; yield 95%; Anal. Calc. for $C_{33}H_{58}O_{10}S$: C, 61.27; H, 9.04; S, 4.96. Found: C, 61.18; H, 9.12; S, 4.99%.

*2.1.3.4. (4-Thiaicosyl) 6′-O-methyl-2′,3′,4′-tri-O-acetyl-β-D-glucopyranoside (**5d**).* ^{1}H NMR ($CDCl_3$): $\delta = 0.84–0.91$ (t, 3H, $-CH_3$), 1.18–1.39 (s, 26H, $-CH_2-$), 1.50–1.55 (m, 4H, $-SCH_2CH_2-$),1.98, 2.00, 2.02 (3s, 9H, 3 $COCH_3$),

2.44 (t, 2H, –SCH_2(CH$_2$)$_{14}$–), 2.50 (t, 2H, –SCH_2(CH$_2$)$_2$O–), 3.39 (s, 3H, –OCH_3), 3.89–3.95 (m, 1H, 5-*H*), 4.07–4.09 (m, 2H, 6-*H*, 6′-*H*), 4.13–4.21 (m, 2H, –OCH_2), 4,47 (d, $J = 8.0$ Hz, 1H, 1-*H*β), 4.94 (dd, $J = 7.6$ and 9.7 Hz, 1H, 2-*H*), 5.06 (dd, $J = 9.3$ and 9.7 Hz, 1H, 4-*H*), 5.13 (dd, $J = 9.5$ and 9.7 Hz, 1H, 3-*H*); MS (ES, positive ions): m/z 657.86 $(M + 39)^+$; R_f ($CHCl_3$/Et_2O 8:2) 0.59; yield 95%; Anal. Calc. for $C_{32}H_{58}O_9S$: C, 62.11; H, 9.45; S, 5.18. Found: C, 62.05; H, 9.54; S, 5.13%.

2.1.3.5. (4-Thiaicosyl) 3′-O-methyl-2′,4′,6′-tri-O-acetyl-β-D-glucopyranoside ***(5e)***. ^{1}H NMR ($CDCl_3$): $\delta = 0.84$–0.89 (t, 3 H, –CH_3), 1.18–1.42 (s, 26H, –CH_2–), 1.50–1.57 (m, 4H, –SCH$_2$CH_2–), 2.04, 2.05, 2.06 (3s, 9H, 3 COCH_3), 2.44 (t, 2H, –SCH_2(CH$_2$)$_{14}$–), 2.51 (t, 2H, –SCH_2(CH$_2$)$_2$O–), 3.37 (s, 3H, –OCH_3), 3.43–3.48 (m, 1H, 5-*H*), 4.07–4.21 (m, 4H, 6-*H*, 6′-*H*, –OCH_2), 4.37 (d, $J = 8.0$ Hz, 1H, 1-*H*β), 4.95 (dd, $J = 7.8$ and 9.5 Hz, 1H, 2-*H*), 5.00 (dd, $J = 9.4$ and 9.6 Hz, 1H, 4-*H*), 5.05 (dd, $J = 9.5$ and 9.7 Hz, 1H, 3-*H*); MS (ES, positive ions): m/z 657.86 $(M + 39)^+$; R_f ($CHCl_3$/Et_2O 8:2) 0.41; yield 95%; Anal. Calc. for $C_{32}H_{58}O_9S$: C, 62.11; H, 9.45; S, 5.18. Found: C, 62.03; H, 9.52; S, 5.23%.

2.1.3.6. (4-Thiaicosyl) 2′-deoxy-3′,4′,6′-tri-O-acetyl-β-D-arabinohexopyranoside ***(5f)***. ^{1}H NMR ($CDCl_3$): $\delta = 0.86$–0.89 (t, 3H, –CH_3), 1.24–1.27 (s, 26H, –CH_2–), 1.54–1.59 (m, 4H, –SCH$_2$CH_2–), 1.78–1.88 (m, 1H, 2-*H*), 2.01, 2.03, 2.09 (3s, 9H, 3 COCH_3), 2.21–2.26 (m, 1H, 2′-*H*), 2.48 (t, 2H, –SCH_2(CH$_2$)$_{14}$–), 2.58 (t, 2H, –SCH_2(CH$_2$)$_2$O–), 3.44–3.50 (m, 1H, 6-*H*), 3.72–3.78 (m, 1H, 6′-*H*), 4.03–4.07 (m, 1H, 5-*H*), 4.08–4.14 (m, 2H, –OCH_2), 4.28 (dd, $J = 12.3$ and 4.7 Hz, 1H, 1-*H*β), 4.94 (t, $J = 9.8$ Hz, 4-*H*), 5.27–5.33 (m, 1H, 3-*H*); MS (ES, positive ions): m/z 611.84 $(M + 23)^+$; R_f ($CHCl_3$/Et_2O 8:2) 0.55; yield 95%; Anal. Calc. for $C_{31}H_{56}O_8S$: C, 62.23; H, 9.59; S, 5.44. Found: C, 62.18; H, 9.62; S, 5.41%.

2.1.3.7. (4-Thiaicosyl) 6′-deoxy-2′,3′,4′-tri-O-acetyl-β-D-glucopyranoside ***(5g)***. ^{1}H NMR ($CDCl_3$): $\delta = 0.83$–0.92 (t, 3H, –CH_3), 1.21–1.37 (s, 26H, –CH_2–), 1.53–1.57 (m, 7H, –SCH$_2$CH_2–, –CH_3), 1.98, 2.0, 2.04 (3s, 9H, 3 COCH_3), 2.49 (t, 2H, –SCH_2(CH$_2$)$_{14}$–), 2.53 (t, 2H, –SCH_2(CH$_2$)$_2$O–), 3.55–3.58 (m, 1H, 5-*H*), 4.08–4.39 (m, 2H, –OCH_2), 4.53 (d, $J = 8.2$ Hz, 1H, 1-*H*β), 4.86 (dd, $J = 7.7$ and 9.6 Hz, 1H, 2-*H*), 4.99 (dd, $J = 9.5$ and 9.7 Hz, 1H, 4-*H*), 5.27 (dd, $J = 9.6$ and 9.8 Hz, 1H, 3-*H*); MS (ES, positive ions): m/z 611.84 $(M + 23)^+$; R_f (petroleum ether$_3$/EtOAc 6:4) 0.32; yield 95%; Anal. Calc. for $C_{31}H_{56}O_8S$: C, 62.23; H, 9.59; S, 5.44. Found: C, 62.17; H, 9.65; S, 5.42%.

After deacetylation of **5** with NH_3–MeOH finally the glycolipids **6** were obtained in good yield.

2.1.3.8. (4-Thiaicosyl) α-D-mannopyranoside ***(6a)***. MS (ES, positive ions): m/z 501.73 $(M + 23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.56; yield 73%; Anal. Calc. for $C_{25}H_{50}O_6S$: C, 62.72; H, 10.53; S, 6.70. Found: C, 62.67; H, 10.57; S, 6.74%.

2.1.3.9. (4-Thiaicosyl) β-D-glucopyranoside ***(6b)***. MS (ES, positive ions): m/z 501.73 $(M + 23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.54; yield 82%; Anal. Calc. for $C_{25}H_{50}O_6S$: C, 62.72; H, 10.53; S, 6.70. Found: C, 62.65; H, 10.58; S, 6.72%.

2.1.3.10. (4-Thiaicosyl) β-D-galactopyranoside ***(6c)***. MS (ES, positive ions): m/z 501.73 $(M + 23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.66; yield 78%; Anal. Calc. for $C_{25}H_{50}O_6S$: C, 62.72; H, 10.53; S, 6.70. Found: C, 62.67; H, 10.61; S, 6.74%.

2.1.3.11. (4-Thiaicosyl) 6′-O-methyl-β-D-glucopyranoside ***(6d)***. MS (ES, positive ions): m/z 531.75 $(M + 39)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.78; yield 73%; Anal. Calc. for $C_{26}H_{52}O_6S$: C, 63.38; H, 10.64; S, 6.51. Found: C, 63.33; H, 10.71; S, 6.57%.

2.1.3.12. (4-Thiaicosyl) 3′-O-methyl-β-D-glucopyranoside ***(6e)***. MS (ES, positive ions): m/z 531.75 $(M + 39)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.76; yield 85%; Anal. Calc. for $C_{26}H_{52}O_6S$: C, 63.38; H, 10.64; S, 6.51. Found: C, 63.32; H, 10.72; S, 6.54%.

*2.1.3.13. (4-Thiaicosyl) 2′-deoxy-β-D-glucopyranoside (**6f**).* MS (ES, positive ions): m/z 485.73 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.67; yield 76%; Anal. Calc. for $C_{25}H_{50}O_5S$: C, 64.89; H, 10.89; S, 6.93. Found: C, 64.78; H, 10.94; S, 6.87%.

*2.1.3.14. (4-Thiaicosyl) 6′-deoxy-β-D-glucopyranoside (**6g**).* MS (ES, positive ions): m/z 485.73 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.72; yield 16%; Anal. Calc. for $C_{25}H_{50}O_5S$: C, 64.89; H, 10.89; S, 6.93. Found: C, 64.81; H, 10.92; S, 6.87%.

*2.1.3.15. Hexadecyl 7-(β-D-galactopyranosyloxy)-4-thiaheptanoic amide (**7**).* To a solution of **4c** (0.01 mol) in 5 ml $CHCl_3$ mercaptocarboxylic acid (0.05 mol) was added. The mixture was stirred in a quartz flask under argon atmosphere at room temperature and irradiated at 254 nm for 2 h.

Detection of the quantitative reaction of double bond was achieved by a solution of bromine in MeOH. The mixture was diluted with water and $CHCl_3$, the organic phase was separated and dried over Na_2SO_4. The filtered and evaporated residue was purified by flash chromatography (petroleum ether/EtOAc 8:2 v/v) to give **7a** in good yield.

To a solution of **7a** (0.01 mol) in 100 ml CCl_4 *N*-hydroxysuccinimide (0.012 mol) was added and the mixture was cooled at 0 °C. Then DCC (0.014 mol) was added and stirred for 12 h. The solid (dicyclohexyl urea) was filtered and hexadecylamine (0.01 mol) in 50 ml CH_2Cl_2 was added to the solution at room temperature which was stirred for 4 h

The organic phase was washed with water, separated and dried over Na_2SO_4. The residue obtained after evaporation was deacetylated as described earlier and then purified by flash chromatography (petroleum ether/EtOAc 8:2 v/v) to give **7c** in good yield.

*2.1.3.16. 7-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactopyranosyloxy)-4-thiaheptanoic acid (**7a**).* MS (ES, positive ions): m/z 517.51 $(M+23)^+$; R_f (petroleum ether/EtOAc 6:4) 0.18; yield 43%; Anal. Calc. for $C_{20}H_{30}O_{12}S$: C, 48.58; H, 6.11; S, 6.48. Found: C, 48.46; H, 6.18; S, 6.52%.

*2.1.3.17. Hexadecyl 7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyloxy)-4-thiaheptanoic amide (**7b**).* MS (ES, positive ions): m/z 740.95 $(M+23)^+$; R_f ($CHCl_3$/EtO_2 8:2) 0.46; yield 80%; Anal. Calc. for $C_{36}H_{63}NO_{11}S$: C, 60.23; H, 8.84; N, 1.95; S, 4.47. Found: C, 60.12; H, 9.01; N, 1.87; S, 4.32%.

*2.1.3.18. Hexadecyl 7-(β-D-galactopyranosyloxy)-4-thiaheptanoic amide (**7c**).* MS (ES, positive ions): m/z 572.80 $(M+23)^+$; R_f ($CHCl_3$/MeOH 8:2) 0.69; yield 76%; Anal. Calc. for $C_{28}H_{55}NO_7S$: C, 61.17; H, 10.08; N, 2.55; S, 5.83. Found: C, 60.98; H, 10.15; N, 2.72; S, 5.69%.

3. Results and discussion

*3.1. Synthesis of hexadecyl β-D-glycopyranosides **2***

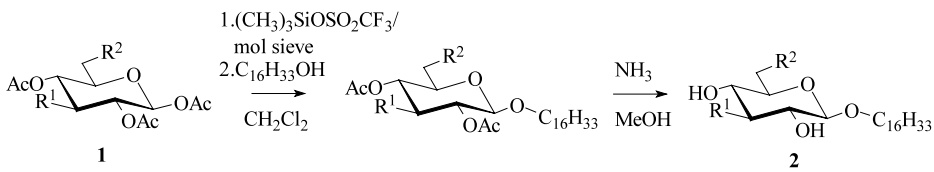
A range of hexadecyl β-D-glycopyranosides **2** was synthesized by conventional 1,2-*trans*-glycosylation of hexadecyl alcohol using the TMS-triflate method (Wilhelm et al., 1995) with the corresponding peracetylated glycopyranose derivatives **1** which either are commercially available or were obtained according to the literature (Koizumi and Utamura, 1981). All of the glycolipids were obtained in ordinary satisfying yield after deacetylation of the corresponding peracetylated lipid with NH_3–MeOH (Fig. 1).

*3.2. Synthesis of (4-thiaicosyl) β-D-glycopyranosides **6***

The motive for these investigations is the relatively low yields of conventional glycosylation methods for the synthesis of glycolipids thus far reported. Starting points were the good results of perallylation (Kieburg et al., 1997) at the synthesis of glycodendrimers (Roy et al., 2001). According to the literature (Takano et al., 1990; Ewstigneewa et al., 1993) the preparation of allyl glycopyranoside derivatives starting from the peracetylated sugar compounds by the use of catalytic amounts of BF_3–etherate-complex as Lewis acid afforded very good results concerning to the

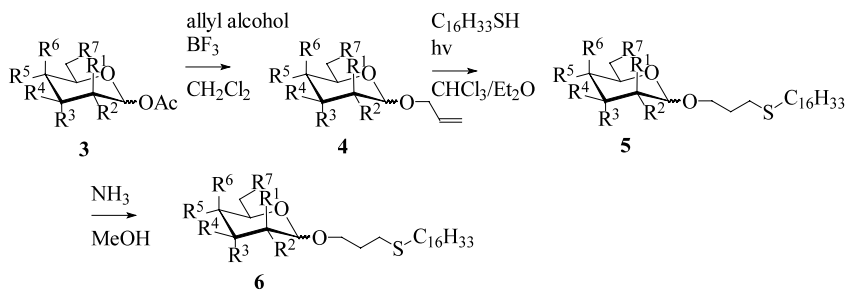
overall yield and the stereoselectivity with an anomeric ratio of about 95:5. The divergent bibliography concerning the reactant ratios (Takano et al., 1990; Ewstigneewa et al., 1993) sets us to optimize the reaction conditions with the result of an optimal reactant ratio glycosyl acceptor/glycosyl donor/BF_3–etherate-complex 1.5:1:1.1 and a reaction time of 4 h.

The following photoaddition was achieved by the exposure of the allyl glycosides **4** and hexadecyl thiol to radiation at 254 nm in a quartz flask with almost quantitative yields ($>95\%$). After deacetylation a range of glycolipids **6** was obtained which are characterized by a long alkyl chain as hydrophobic anchor, a sulfur-linked propyl spacer and a terminal carbohydrate headgroup. The differences between the unfunctionalized glycopyranoses D-glucose, D-galactose, D-mannose and the number of deoxy and partially *O*-methylated sugar derivatives seem to have an influence in the aggregation, e.g. the *gauche*-conformation of the alkyl chain which can be determined by means of C–C vibration measured by comparative Raman spectral study (Fig. 2).



2a: R^1=OH; R^2=OH **2b**: R^1=OH; R^2=OCH_3
2c: R^1=OCH_3; R^2=OH **2d**: R^1=OH; R^2=H

Fig. 1. Reaction pathway to the synthesis of compounds **2**.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
3 - 5a:	OAc	H	H	OAc	OAc	H	OAc
3 - 5b:	H	OAc	H	OAc	OAc	H	OAc
3 - 5c:	H	OAc	H	OAc	H	OAc	OAc
3 - 5d:	H	OAc	H	OAc	OAc	H	OCH_3
3 - 5e:	H	OAc	H	OCH_3	OAc	H	OAc
3 - 5f:	H	H	H	OAc	OAc	H	OAc
3 - 5g:	H	OAc	H	OAc	OAc	H	H

Fig. 2. Reaction pathway to the synthesis of compounds **3**–**5**.

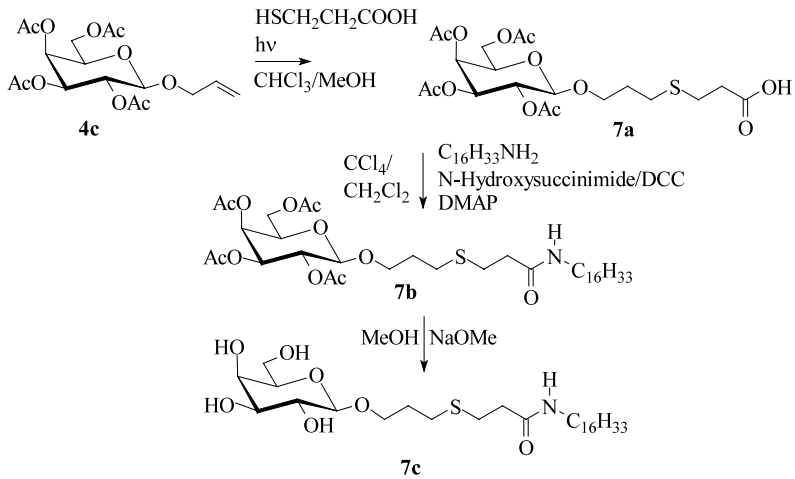


Fig. 3. Reaction pathway to the synthesis of compound **7c**.

3.3. Synthesis of hexadecyl 7-(β-D-galacto-pyranosyloxy)-4-thiaheptanoic amide **7**

Based on the excellent results of the addition of the thiol functionality onto the alkenyl moiety of **4** using photochemical conditions an alternative method for the preparation of spacered neoglycolipids was searched for. According to the experience of other authors (van Seeventer et al., 1997; Peerlings et al., 1998; Roy et al., 2001) on the synthesis of glycodendrimeres the allyl derivative **4c** was converted with 3-mercaptopropionic acid into the free acid derivative **7a**. Introduction of the hydrophobic moiety on the terminal of hexadecyl amine with *N*-hydroxysuccinimide/DCC to **7b** followed by deacetylation using Zemplen conditions afforded **7c** in very good yields. This method for the preparation of spacered neoglycolipids is a good alternative to, e.g. ethyleneoxy groups because the size of spacer can be varied by the use of different mercaptocarboxylic acids (Fig. 3).

4. Conclusions

With these methods a range of neoglycolipids was synthesized which allows the stereoselective preparation of amphiphilic molecules in excellent yield. On the one hand a comparison between glycolipids with identical carbohydrate headgroups concerning the influence of the three different hydrophobic anchors in the physicochemical behavior of the general lipid is under progress. On the other hand the differences between the unfunctionalized glycopyranoses D-glucose, D-galactose, D-mannose and the number of deoxy and partially *O*-methylated sugar derivatives have an influence on the aggregation, e.g. the *gauche*-conformation of the alkyl chain. The polymorphism of the lipids synthesized, the chain packing and the order/disorder of the hydrocarbon chains are going to be studied by differential scanning calorimetry, X-ray diffraction and Raman spectroscopy. Results of these physicochemical investigations and further biochemical recognition studies will be published soon.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 197).

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