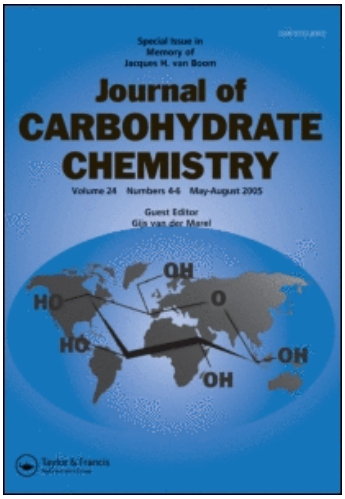

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SYNTHESIS OF CARBOHYDRATE-ANTIGENIC STRUCTURES OF *MYCOBACTERIUM TUBERCULOSIS* USING AN IODONIUM ION PROMOTED GLYCOSIDATION APPROACH[1]

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ABSTRACT

Analogues of the phenol-phthiocerol glycoside **1a** of *Mycobacterium tuberculosis* were synthesized starting from properly protected rhamnose and fucose ethyl thioglycosides. A recently developed iodonium ion promoted glycosidation procedure proved to be very efficient for the preparation of 3-aminopropyl 3-*O*-(α-L-rhamnopyranosyl)-2-*O*-methyl-α-L-rhamnopyranoside (**2**), 3-aminopropyl 3-*O*-[3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranosyl]-2-*O*-methyl-α-L-rhamnopyranoside (**3**) and 3-aminopropyl 3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranoside (**4**).

INTRODUCTION

It has been observed[3,4] that tuberculosis, caused by *Mycobacterium tuberculosis*, is an increasing health problem to man. Poor living conditions, rising bacillary resistance to anti-tuberculosis drugs and an increasing number of immunodeficient patients all contribute to

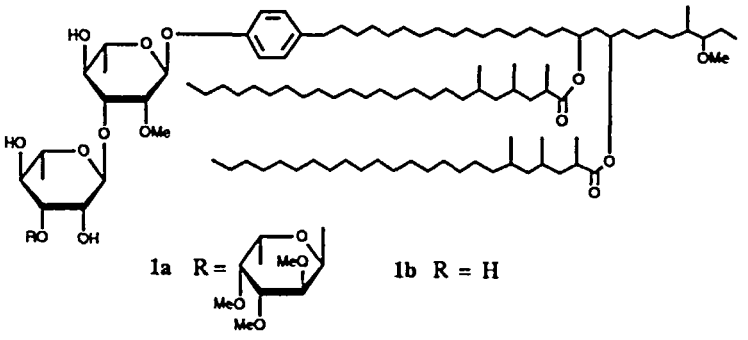


Figure 1

the exacerbation of tuberculosis. Moreover, the currently used vaccine *Mycobacterium bovis* BCG has not proved to be completely satisfactory in those countries where immunization is most needed. Hence, extensive research towards the development of more effective vaccines is of great importance.[5] It has been emphasized[6] that the immunizing epitope of tubercle bacilli resides most likely in the polysaccharide components of the tuberculin cell-wall and may induce and elicit cell-mediated immunologic responses. For this reason it is of interest to evaluate the individual immunogenicity of these carbohydrate derivatives. Inspection of the recently reported[7] structural features of trisaccharide **1a** (see *Figure 1*), which emanates as the corresponding phenol-phthiocerol glycoside from the cell wall of *M. tuberculosis* species, reveals the presence of two α-linked L-rhamnose residues, one of which is methylated at C-2, and an α-linked fully methylated L-fucose component. In addition, the structure of the analogous phenol-phthiocerol disaccharide **1b**, isolated from *M. bovis* BCG, has also been elucidated[8] and it is not excluded that **1b** may be conducive to the observed immunological cross-reactivity between *Mycobacterium* species.

As part of a programme to develop serological markers, suitable for the screening of tuberculous patients, as well as the designation of a semi-synthetic vaccine capable of inducing protective antituberculosis immunity, we now report on the synthesis of the di- and trisaccharide fragments **2**, **3** and **4** (see *Figure* 2). In our approach, an artificial 3-aminopropyl spacer was anchored to the synthetic fragments to facilitate their conjugation to protein carriers.

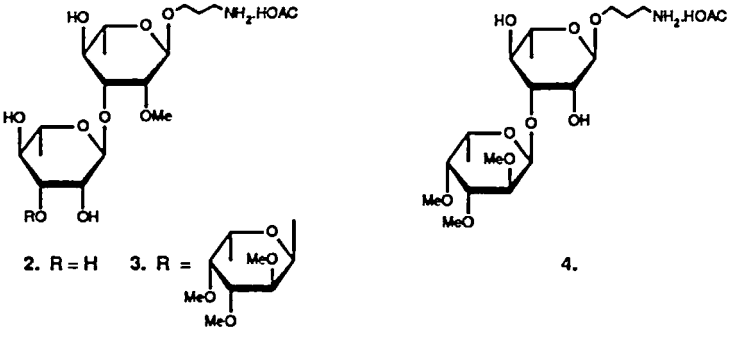


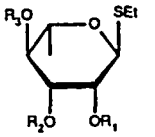
Figure 2

RESULTS AND DISCUSSION

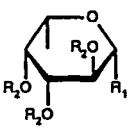
Recently,[9] we disclosed an efficient protocol towards a thioglycoside mediated formation of α-glycosidic linkages promoted by iodonium dicollidine perchlorate (IDCP). Thus it was established that C-2 alkylated ethyl thioglycosides could be condensed chemoselectively, in the presence of IDCP, with partially acylated thioglycosides to furnish predominantly α-linked disaccharides in a high yield. On the other hand, in a complementary paper[2] we reported that reaction of fully acylated thioglycosides with glycosyl acceptors and in the presence of N-iodosuccinimide (NIS) together with a catalytic amount of trifluoromethane-sulphonic acid (TfOH) proceeded extremely fast and highly stereoselective, leading to 1,2-*trans* linked di(tri)saccharides. The favorable outcome of these investigations prompted us to explore the applicability of the iodonium ion promoted glycosidation strategy towards the introduction of the required α-linkages in target fragments **2**, **3** and **4**.

The synthetic strategy we followed comprises the following steps: *i.* preparation of a spacer containing C-2 methylated rhamnopyranoside precursor **12** and its subsequent α-rhamnosidation to give disaccharide **14** (*Scheme 1*); *ii.* assembly of key disaccharide unit **21** (*Scheme 2*) by chemospecific condensation of 1-thio-rhamnopyranoside **15** with fucosyl donor **19**; *iii.* synthesis of disaccharide **22** and trisaccharide **23** (*Scheme 2*) by glycosidation of donor **21** with the appropriate acceptor molecules **9** and **12**, respectively.

Disaccharide **14** was prepared as follows. Benzylation (BnBr/NaH) of known[10] ethyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (**5**) furnished **6** (*Figure 3*). Subsequent acid-



5. $R_1R_2 = Me_2C$, $R_3 = H$
6. $R_1R_2 = Me_2C$, $R_3 = Bn$
7. $R_1 = R_2 = H$, $R_3 = Bn$
8. $R_1 = R_2 = Ac$, $R_3 = Bn$
13. $R_1 = R_2 = R_3 = Ac$
15. $R_1 = Ac$, $R_2 = H$, $R_3 = Bn$

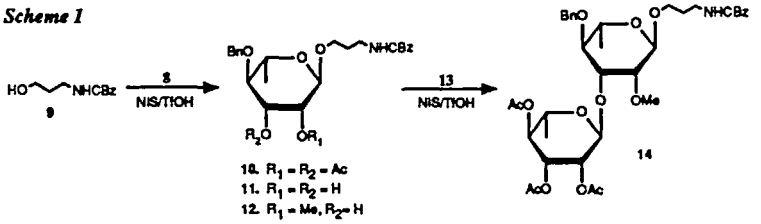


16. $R_1 = OAc$, $R_2 = Ac$
17. $R_1 = SEt$, $R_2 = Ac$
18. $R_1 = SEt$, $R_2 = H$
19. $R_1 = SEt$, $R_2 = Me$

Figure 3

hydrolysis of the acetonide function of **6** gave crystalline **7**, which, after acetylation (pyridine-acetic anhydride), afforded rhamnosyl donor **8** (82% yield, based on **5**). NIS-TfOH mediated glycosidation (*Scheme 1*) of **8** with 3-(benzyloxycarbonylamino)-1-propanol[10,12,13] (**9**) led to the isolation of **10** in a yield of 82%. Zemplén deacetylation of **10** yielded diol **11** (95%). Regioselective methylation of **11** under phase-transfer conditions[11] (MeI/ CH_2Cl_2-/5M NaOH/Bu_4NI) afforded **12** in 52% yield. Further, condensation of **12** with

Scheme 1



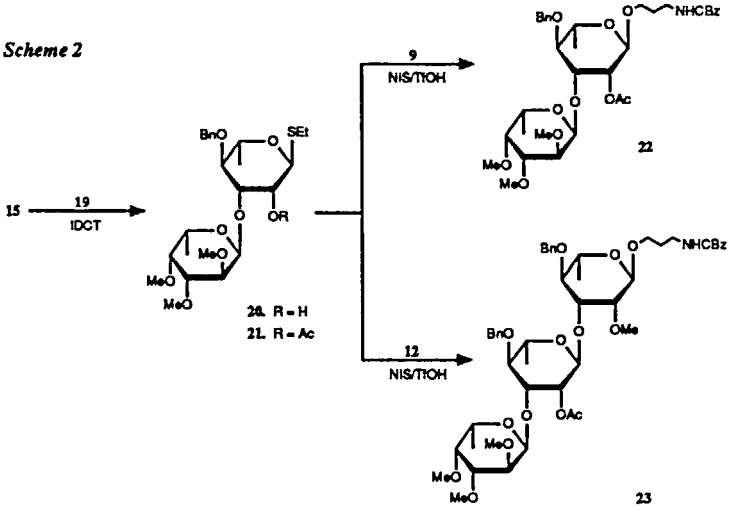
10. $R_1 = R_2 = Ac$
11. $R_1 = R_2 = H$
12. $R_1 = Me$, $R_2 = H$

known[10] ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**13**) in the presence of NIS-TfOH provided disaccharide **14** in 85% yield. Zemplén deacetylation of **14** followed by hydrogenation in the presence of Pd on charcoal produced fragment **2** (*Figure 2*). The ^1H and ^{13}C NMR data of purified **2** were in complete accordance with the proposed structure.[14]

At this stage, the feasibility to introduce the required α-fucosidic linkage *via* an IDCP-mediated thioglycoside approach was examined. First, fully methylated fucosyl donor **19** was prepared (see *Figure 3*), in an overall yield of 60%, by mercaptolysis of tetra-*O*-acetyl-α/β-L-fucopyranose (**16**) with ethanethiol in the presence of $SnCl_4$, subsequent Zemplén deacetylation of **17**, and consecutive methylation (MeI/NaH) of the thus obtained triol **18**. Further, "disarmed" C-2 acetylated 1-thio-rhamnopyranoside **15** was prepared by reacting diol **7** with trimethyl orthoacetate in the presence of camphorsulphonic acid followed by

acid hydrolysis[15] of the resulting methoxyethylidene acetal. Chemospecific IDCP-mediated condensation of fucosyl donor **19** with acceptor **15** (*Scheme 2*) resulted in the isolation of disaccharide **21** (yield 60%), the purification of which was greatly facilitated by Zemplén deacetylation of crude **21** to give **20**, followed by silica gel chromatography and reacetylation (pyridine-acetic anhydride) of **20**. In this respect it is of interest to note that the rate of fucosidation and the yield of dimer **21** could be enhanced by using the easily accessible and crystalline iodonium dicollidine *triflate* (IDCT) instead of IDCP as the thiophilic promotor. Thus the chemoselective condensation of donor **19** with acceptor **15** in the presence of IDCT was complete after 15 min at 20 °C. Further processing of crude **21** thus obtained, as mentioned earlier for the IDCP-mediated condensation of **19** with **15**, furnished **21** in a yield of 73%.

Scheme 2



In the final stage of our synthetic strategy, NIS-TfOH mediated glycosidation (*Scheme 2*) of disaccharide **21** with acceptor molecule **9** afforded fragment **22**. Disaccharide **22** was deacetylated and hydrogenated in the presence of Pd on charcoal to give fragment **4**. Similarly, condensation of donor **21** with acceptor **12** in the presence of NIS-TfOH provided trisaccharide **23**, which, after deacetylation and hydrogenation, led to the isolation of fragment **3**. ^{1}H and ^{13}C NMR data of compound **3** were in good agreement with those reported[7] for the naturally occurring glycolipid **1**.

In conclusion, the fruitful synthetic route described herein towards the preparation of cell-wall fragments of *Mycobacterium tuberculosis* indicates that a combined use of the

chemospecific thiophilic promotor IDCT and the powerful activator NIS-(*cat.*)TfOH promises to be a valuable asset to the synthesis of complex oligosaccharides.

The immunological properties of fragments **2**, **3** and **4** will be published elsewhere. At present we are exploring the thioglycoside-iodonium ion glycosidation approach towards the preparation of other naturally occurring bacterial cell-wall polysaccharides.

EXPERIMENTAL

General methods. - Pyridine was dried by refluxing with CaH_2 (5g/L) and then distilled. Dichloromethane, 1,2 dichloroethane and toluene were distilled from P_2O_5. *N,N*-dimethylformamide was stirred with CaH_2 at room temperature and distilled under reduced pressure. Diethyl ether was distilled from $LiAlH_4$. Pyridine and *N,N*-dimethylformamide were stored over molecular sieves 4Å (Aldrich), toluene and diethyl ether over sodium wire and dichloromethane and 1,2 dichloroethane were stored over alumina. Reactions were performed at ambient temperature unless noted otherwise. Column chromatography was performed on columns of silica gel 60 (Merck 70-230 mesh). Gel filtration was performed on Sephadex LH-20 (Pharmacia). TLC was conducted on DC Fertigfolien (Schleicher & Schüll F1500 LS254). Compounds were detected by charring with 20% sulfuric acid in methanol. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter, for solutions in $CHCl_3$ at 22 °C unless stated otherwise. NMR spectra were recorded with a Jeol JNM-FX200 (^{13}C, 50.1 MHz, internal Me_4Si or methanol and a Brucker WM-300 spectrometer equipped with an Aspect-2000 computer (^1H, 300 MHz, internal Me_4Si).

Ethyl 4-*O*-Benzyl-1-thio-α-L-rhamnopyranoside (7). - To a stirred solution of ethyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (**5**) (2.46 g, 10 mmol) in DMF (20 mL) was added NaH (0.29 g, 12 mmol) and benzyl bromide (2.05 g, 12 mmol). After stirring for 2 h, methanol (5 mL) was added and the reaction mixture was concentrated. The residue was redissolved in dichloromethane (50 mL) and extracted twice with water, dried ($MgSO_4$) and concentrated to give **6** (2.7 g, 90%). Compound **6** was redissolved in acetic acid-H_2O (9:1, 50 mL) and stirred for 17 h at 50 °C. The reaction mixture was concentrated followed by co-evaporation of the residual oil with toluene (2x25 mL). Addition of diethyl ether-hexane then afforded crystalline **7** (2.0 g, 80% based on **5**); m.p. 88-90°; $[\alpha]_D$ -167° (c 1). ^1H NMR data ($CDCl_3$): δ 7.34-7.31 (m, 5 H, *H*-arom.); 5.22 (s, 1 H, H-1); 4.73 (AB, 2 H, benzyl-C*H*$_2$); 4.2-3.8 (m, 3 H, H-2, H-3, H-5); 3.38 (dd, 1 H, H-4, $J_{4,3} \approx J_{4,5}$ 9.3 Hz); 2.59 (AB, 2 H, SC*H*$_2$CH$_3$); 1.33 (d, 3 H, H-6, $J_{6,5}$ 7.2 Hz) 1.28 (t, 3 H, SCH$_2$C*H*$_3$, *J*

7.2 Hz). ^{13}C NMR data ($CDCl_3$): δ 138.1-127.8 (*C*-arom.); 83.7 (C-1); 81.8 (C-4); 74.8 (benzyl-CH_2); 72.6, 71.9 (C-2, C-3); 67.7 (C-5); 25.0 (SCH_2CH_3); 17.9 (C-6); 14.9 (SCH_2CH_3).

Ethyl 2,3-Di-*O*-acetyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (8). - Compound **7** (0.89 g, 3 mmol) was dissolved in 2:1 pyridine-acetic anhydride (10 mL) and left for 4 h. Water was added followed by concentration of the resulting mixture. The residue was redissolved in dichloromethane, extracted with aq. acetic acid (20%, 20 mL), water (20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated to give **8** (1.1 g, 95%); $[\alpha]_D$ -124° (c 1). ^1H NMR data ($CDCl_3$): δ 7.38-7.26 (m, 5 H, *H*-arom.); 5.34 (dd, 1 H, H-2, $J_{2,1}$ 1.3 Hz, $J_{2,3}$ 3.5 Hz); 5.25 (dd, 1 H, H-3, $J_{3,2}$ 3.3 Hz, $J_{3,4}$ 9.5 Hz); 5.16 (d, 1 H, H-1, $J_{1,2}$ 1.3 Hz); 4.67 (AB, 2 H, benzyl-CH_2); 4.18 (m, 1 H, H-5); 3.54 (dd, 1 H, H-4, $J_{4,3} \approx J_{4,5}$ 9.5 Hz); 2.61 (AB, 2 H, SCH_2CH_3); 2.15, 1.96 (2x s, 6 H, CH_3-acetyl); 1.35 (d, 3 H, H-6, $J_{6,5}$ 6.2 Hz); 1.28 (t, 3 H, SCH_2CH_3, *J* 7.2 Hz). ^{13}C NMR data ($CDCl_3$): δ 169.9 (*C*=O); 137.9-127.5 (*C*-arom.); 81.8 (C-1); 79.0 (C-4); 74.9 (benzyl-CH_2); 72.0, 71.9 (C-2, C-3); 68.2 (C-5); 25.3 SCH_2CH_3); 20.9, 20.8 (2x CH_3-acetyl); 17.8 (C-6); 14.8 (SCH_2CH_3).

3-(Benzyloxycarbonylamino)propyl 2,3-Di-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside (10) - Thioglycoside **8** (0.76 g, 2 mmol) and 3-(benzyloxycarbonylamino)propanol (**9**) (0.42 g, 2 mmol) were dissolved in 1:1 1,2-dichloroethane-diethyl ether (10 mL). Powdered molecular sieves (5Å, 1 g) were added and the mixture was stirred for 15 min at 0 °C. A solution of NIS (0.45 g, 2 mmol) and TfOH (17μL, 0.2 mmol) in 1:1 1,2-dichloroethane-diethyl ether (20 mL) was added while stirring was continued for a further 2 min. Then, the reaction mixture was filtered, diluted with dichloromethane, extracted with aq. $Na_2S_2O_3$ (1M, 20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel with 95:5 dichloromethane-acetone to furnish pure **10** (0.88 g, 84%); $[\alpha]_D$ -31.7° (c 1). ^1H NMR data ($CDCl_3$): δ 7.44-7.26 (m, 10 H, *H*-arom.); 5.28 (dd, 1 H, H-3, $J_{3,2}$ 3.3 Hz, $J_{3,4}$ 9.2 Hz); 5.24 (dd, 1 H, H-2, $J_{2,1}$ 1.3 Hz, $J_{2,1}$ 1.3 Hz, $J_{2,3}$ 3.2 Hz); 5.09 (d, 1 H, H-1, $J_{1,2}$ 1.3 Hz); 4.66 (AB, 4 H, 2x benzyl-CH_2); 3.9-3.2 (m, 6 H, H-4, H-5, H-1 spacer, H-3 spacer); 2.14, 1.97 (2x s, 6 H, 2x CH_3-acetyl); 1.81 (m, 2 H, H-2 spacer); 1.34 (d, 3 H, H-6, $J_{6,5}$ 6.2 Hz. ^{13}C NMR data ($CDCl_3$): 169.6 (*C*=O); 156.1 (*C*=O, spacer); 137.6-127.2 (*C*-arom.); 97.0 (C-1); 78.3 (C-4); 74.5 (benzyl-CH_2); 71.3, 69.8 (C-2, C-3); 67.3 (C-5); 65.9 (benzyl-CH_2); 65.1 (C-1, spacer); 37.9 (C-3, spacer); 29.1 (C-2, spacer); 20.4 (CH_3-acetyl); 17.5 (C-6).

Anal. Calcd for $C_{28}H_{35}NO_9$: C, 63.5; H, 6.7. Found: C, 63.3; H, 6.8.

3-(Benzyloxycarbonylamino)propyl 2-*O*-Methyl-4-*O*-benzyl-α-L-rhamnopyranoside (12) - Sodium methoxide (10 mg) was added to a stirred solution of compound **10** (0.84 g, 1.6 mmol) in methanol (10 mL) and left for 2 h. The reaction mixture was neutralized with Dowex (W50, H^+ form), filtered and concentrated to give diol **11** (0.71 g, 1.6 mmol) ($[\alpha]_D$ -36.5°, c 1). Compound **11** was redissolved in dichloromethane (10 mL) and aq. 10% NaOH (w/v, 4 mL), tetrabutylammonium iodide (0.12 g, 0.32 mmol) and methyl iodide (0.62 mL, 10 mmol) were added. The resulting mixture was stirred vigorously for 17 h. The organic layer was separated, extracted with water (2x 10 mL), dried ($MgSO_4$) and concentrated. The residual oil was purified by silica gel chromatography with 98:2 dichloromethane-methanol to afford **12** (0.38 g, 52%); $[\alpha]_D$ -83.0° (c 1). ^1H NMR data ($CDCl_3$): δ 7.37-7.25 (m, 10 H, *H*-arom.); 5.06, 4.73 (2x AB, 4 H, benzyl-CH_2); 4.79 (s, 1 H, H-1); 4.0-3.2 (m, 8 H, H-2, H-3, H-4, H-5, H-1 spacer, H-3 spacer); 3.45 (s, 3 H, OCH_3); 1.74 (m, 2 H, H-2 spacer); 1.28 (d, 3 H, H-6, $J_{6,5}$ 6.4 Hz). ^{13}C NMR data ($CDCl_3$): δ 156.0 (*C*=O); 138.3-127.5 (*C*-arom.); 96.1 (C-1); 81.8, 80.5 (C-2, C-4); 74.9 (benzyl-*C*H_2); 71.4 (C-3); 67.1 (C-5); 66.4 (benzyl-*C*H_2); 65.1 (C-1, spacer); 58.7 (O*C*H_3); 38.4 (C-3, spacer); 29.4 (C-2, spacer); 17.8 (C-6).

Anal. Calcd for $C_{25}H_{33}NO_7$: C, 65.3; H, 7.2. Found: C, 65.3; H, 7.4.

3-(Benzyloxycarbonylamino)propyl 2-*O*-Methyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-4-*O*-benzyl-α-L-rhamnopyranoside (14) - To a cooled (0 °C) and stirred mixture of ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**13**) (100 mg, 0.3 mmol), aglycon **12** (114 mg, 0.25 mmol) and molecular sieves (5Å, 0.5 g) in 1,2-dichloroethane (5 mL) was added a solution of NIS (72 mg, 0.32 mmol) and TfOH (2.8 μL, 32 μmol) in 1:1 1,2-dichloroethane-diethyl ether (3 mL) and stirring was continued for 2 min. The reaction mixture was filtered and washed with aq. $Na_2S_2O_3$ (1M, 10 ml), aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was chromatographed on Sephadex LH-20 with 1:1 dichloromethane-methanol to yield disaccharide **14** (160 mg, 88%); $[\alpha]_D$ -34.5° (c 1). ^{13}C NMR data ($CDCl_3$): δ 169.8, 169.6 (2x *C*=O); 156.1 (*C*=O, spacer); 136.7-127.5 (*C*-arom.); 99.3 (C-1); 96.3 (C-1′); 79.9, 79.8 (C-2, C-3, C-4); 75.4 (benzyl-*C*H_2); 70.8 (C-4′); 69.7 (C-3′); 69.0 (C-2′); 68.0 (C-5); 66.8 (C-5′); 66.3 (benzyl-*C*H_2); 65.2 (C-1, spacer); 58.4 (O*C*H_3); 38.4 (C-3, spacer); 29.5 (C-2, spacer); 20.6 (*C*H_3-acetyl); 17.8 (C-6); 17.5 (C-6′);

Anal. Calcd for $C_{37}H_{49}NO_{14}$: C, 60.7; H, 6.8. Found: C, 60.9; H, 6.7.

3-Aminopropyl 2-*O*-Methyl-3-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (2) - Sodium methoxide (10 mg) was added to a solution of compound **14** (100 mg, 0.14 mmol) in methanol (10 mL) and the mixture was stirred for 2 h. The reaction mixture was neutral-

ized with Dowex (W50, H^+ form), filtered and concentrated to dryness. The residue was redissolved in 7:3:0.5 2-propanol-water-acetic acid (8 mL) and hydrogenated in the presence of 10% Pd on charcoal for 24 h. The catalyst was removed by filtration and the filtrate was concentrated to give a colourless oil which was chromatographed on Sephadex LH-20 with methanol to provide fragment **2** as the corresponding acetate (55 mg, 90%); $[\alpha]_D$ -51.4 (c 1, MeOH). ^{1}H NMR data (CD_3OD): δ 4.98 (d, 1 H, H-1′, $J_{1,2}$ 1.54 Hz); 4.83 (d, 1 H, H-1, $J_{1,2}$ 1.54 Hz); 4.0-3.2 (m, 10 H, H-2, H-3, H-4, H-5, H-2′, H-3′, H-4′, H-5′, H-1, spacer); 3.47 (s, 3 H, OCH_3); 3.04 (t, 2 H, H-3 spacer, *J* 7.2 Hz); 1.96 (m, 2 H, H-2 spacer); 1.28 (d, 3 H, $J_{6,5}$ 5.9 Hz), 1.24 (d, 3 H, $J_{6,5}$ 5.4 Hz), H-6 and H-6′). ^{13}C NMR data (CD_3OD): δ 104.1 (C-1′, J_{CH} 170 Hz); 98.3 (C-1, J_{CH} 169 Hz); 81.6 (C-2); 79.5 (C-3); 73.9 (C-4′); 73.4 (C-4); 72.2, 72.1 (C-2′), C-3′); 70.3, 70.2 (C-5, C-5′); 65.5 (C-1, spacer); 59.2 (OCH_3); 38.5 (C-3, spacer); 28.6 (C-2, spacer); 25.3 (CH_3-acetate); 18.2, 18.1 (C-6, C-6′).

Anal. Calcd for $C_{18}H_{35}NO_{11}$: C, 49.0; H, 8.0. Found: C, 48.9; H, 8.2.

Ethyl 2-*O*-Acetyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (15) - Diol **7** (0.6 g, 2 mmol) was dissolved in acetonitrile (8 mL) followed by addition of trimethyl orthoacetate (0.5 mL, 4 mmol) and camphorsulphonic acid (5 mg). The resulting mixture was stirred for 30 min when 4:1 acetic acid-water (8 mL) was added. Stirring was continued for an additional 15 min. The reaction mixture was diluted with dichloromethane and the organic layer was separated, extracted with water (10 mL), aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The remainder was applied onto a column of silica gel and eluted with 97:3 dichloromethane-acetone. Concentration of the appropriate fractions gave pure **15** (0.56 g, 83%); $[\alpha]_D$ -117.6° (c 1). ^{1}H NMR data ($CDCl_3$): δ 5.18 (s, 1 H, H-1); 5.16 (dd, 1 H, H-2, $J_{2,1}$ 1.5 Hz, $J_{2,3}$ 3.1 Hz); 4.75 (AB, 2 H, benzyl-CH_2); 4.06 (m, 1 H, H-5); 4.02 (dd, 1 H, H-3, $J_{3,2}$ 3.1 Hz, $J_{3,4}$ 9.0 Hz); 3.38 (dd, 1 H, H-4, $J_{4,3}\approx J_{4,5}$ 9.2 Hz); 2.60 (AB, 2 H, SCH_2CH_3); 2.15 (s, 3 H, CH_3-acetyl); 1.34 (d, 3 H, H-6, $J_{6,5}$ 6.4 Hz); 1.26 (t, 3 H, SCH_2CH_3). ^{13}C NMR data ($CDCl_3$): δ 170.7 (*C*=O); 138.1-127.7 *C*-arom.); 82.0, 81.7 (C-1, C-4); 75.0 (benzyl-CH_2); 74.5 (C-2); 70.5 (C-3); 67.9 (C-5); 25.4 (SCH_2CH_3); 21.0 (CH_3-acetyl); 17.8 (C-6); 14.8 SCH_2CH_3).

Ethyl 2,3,4-Tri-*O*-acetyl-1-thio-β-L-fucopyranoside (17) - To a cooled (0 °C) solution of 1,2,3,4-tetra-*O*-acetyl-α/β-L-fucopyranoside (**16**) (3.32 g, 10 mmol) in dichloromethane (20 mL) was added ethanethiol (0.89 mL, 12 mmol) and tin(IV) chloride (0.35 mL, 3 mmol) and the resulting mixture was stirred for 2 h. Then, the cooling bath was removed and stirring was continued for 4 h at room temperature. The reaction mixture was extracted with aq. KF (1M, 2x50 mL), aq. $NaHCO_3$ (0.9M, 20 mL), dried ($MgSO_4$) and concentrated.

The residue was redissolved in ethanol from which pure **17** crystallized (2.3 g, 70%); m.p 78-79 °C; $[\alpha]_D$ -3.0° (c 1). ^{1}H NMR data ($CDCl_3$): 5.27 (d, 1 H, H-4, $J_{4,3}$ 3.3 Hz); 5.22 (dd, 1 H, H-2, $J_{2,1} \approx J_{2,3}$ 9.8 Hz); 5.05 (dd, 1 H, H-3, $J_{3,2}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz); 4.48 (d, 1 H, H-1 $J_{1,2}$ 9.8 Hz); 3.85 (q, 1 H, H-5, $J_{5,6}$ 6.4 Hz); 2.71 (AB, 2 H, SCH_2CH_3); 2.18, 2.07, 1.98 (3x s, 9 H, 3x CH_3-acetyl); 1.28 (t, 3 H, SCH_2CH_3, *J* 7.5 Hz); 1.22 (d, 3 H, H-6, $J_{6,5}$ 6.6 Hz). ^{13}C NMR data ($CDCl_3$): δ 170.2, 169.7, 169.3 (3x *C*=O); 83.1 (C-1, J_{CH} 154 Hz); 72.8, 72.0, 70.1 (C-2, C-3, C-4); 67.0 (C-5); 23.7 (SCH_2CH_3); 20.5, 20.3 (CH_3-acetyl); 16.1 (C-6); 14.4 (SCH_2CH_3).

Ethyl 2,3,4-Tri-*O*-methyl-1-thio-β-L-fucopyranoside (19) - Thioglycoside **17** (1.33 g, 4 mmol) was dissolved in methanol (20 mL) followed by addition of sodium methoxide (10 mg). After stirring for 2 h the mixture was neutralized with Dowex (W50, H^+ form), filtered and concentrated. The residue was co-evaporated with toluene (2x25 mL) to give **18** (0.82 g) which was redissolved in DMF (10 mL) followed by addition of sodium hydride (0.43 g, 18 mmol). After stirring for 10 min methyl iodide (1 mL, 16 mmol) was added and stirring was continued for 2 h. Excess NaH was destroyed by addition of methanol (5 mL) and the resulting mixture was concentrated. The residue was redissolved in dichloromethane (20 mL), extracted with water (2x50 mL), aq. NaCl (2.5M, 50 mL), dried ($MgSO_4$) and concentrated once more. The remaining oil was purified by chromatography on silica gel with 98:2 dichloromethane-acetone which led to the isolation of solid **19** (0.81 g, 82%); $[\alpha]_D$ +27.9 (c 1). ^{1}H NMR data ($CDCl_3$): δ 4.26 (d, 1H, H-1, $J_{1,2}$ 9.0 Hz); 3.59, 3.58, 3.54 (3x s, 3x OCH_3); 3.46 (q, 1 H- H-5, $J_{6,5}$ 6.4 Hz); 3.42 (d, 1H, H-4, $J_{2,1} \approx J_{2,3}$ 9.0 Hz); 3.18 (dd, 1 H, H-3, $J_{3,4}$ 2.8 Hz, $J_{3,2}$ 9.0 Hz); 2.71 (AB, 2 H, SCH_2CH_3); 1.31 (d, 3 H, H-6, $J_{6,5}$ 6.3 Hz); 1.28 (t, 3 H, SCH_2CH_3, *J* 7.5 Hz). ^{13}C NMR data ($CDCl_3$): δ 85.7 (C-3); 84.1 (C-1); 79.4, 78.3 (C-2, C-3); 73.9 (C-5); 61.3, 60.6, 57.8 (3x OCH_3); 24.1 SCH_2CH_3); 16.6 (C-6); 14.5 (SCH_2CH_3).

Anal. Calcd for $C_{11}H_{22}O_4S$: C, 52.8; H, 8.9. Found: C, 52.7; H, 8.9.

Iodonium Dicollidine Triflate (IDCT) - Silver triflate (2.57 g, 10 mmol) was suspended in dichloromethane (20 mL) and *sym*-collidine (3.2 mL, 24 mmol) was introduced slowly with stirring. To the resulting clear solution was added iodine (2.54 g, 10 mmol) and stirring was continued for 15 min. The mixture was filtered over Celite in order to remove precipitated AgI, followed by addition of diethyl ether (60 mL) to the filtrate. IDCT crystallized rapidly and was collected by filtration, washed with diethyl ether and dried *in vacuo* (4.2 g, 81%).

Ethyl 2-*O*-Acetyl-3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (21) - IDCT (0.52 g, 1 mmol) was added to a stirred mixture of

glycosyl donor **19** (0.23 g, 0.93 mmol), aglycon **12** (0.27 g, 0.8 mmol) and powdered molecular sieves (5Å, 0.5 g) in 1:5 1,2-dichloroethane-diethyl ether (7 mL). After 15 min, TLC analysis showed the absence of **19** and **12** and the reaction mixture was filtered. The filtrate was diluted with dichloromethane, extracted with aq. $Na_2S_2O_3$ (1M, 10 mL), aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was dissolved in methanol (10 mL) and sodium methoxide (10 mg) was added. After stirring for 2 h, the mixture was neutralized with Dowex (W50, H^+ form), filtered and concentrated. The residue was chromatographed on silica gel with 95:5 dichloromethane-acetone to furnish **20** (0.29 g, 0.6 mmol). Compound **20** was redissolved in 2:1 pyridine-acetic anhydride (6 mL) and stirred for 3 h at 60 °C. Water (4 mL) was added and the resulting mixture was concentrated, redissolved in dichloromethane, extracted succesively with aq. acetic acid (20%, 20 mL), water (2x 10 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated once more to yield disaccharide **21** (0.30 g, 73%); $[\alpha]_D$ -157.8° (c 1). ^1H NMR data ($CDCl_3$): δ 5.22 (dd, 1 H, H-2, $J_{2,1}$ 1.8 Hz, $J_{2,3}$ 3.3 Hz); 5.19 (d, 1 H, H-1′, $J_{1,2}$ 3.6 Hz); 5.17 (d, 1 H, H-1, $J_{1,2}$ 1.8 Hz); 4.87 (AB, 2 H, benzyl-C*H*$_2$); 4.07 (dd, 1 H, H-2′, $J_{2,1}$ 3.3 Hz, $J_{2,3}$ 9.5 Hz); 4.06 (m, 1 H, H-5); 3.91 (q, 1 H, H-5′, $J_{5,6}$ 6.9 Hz); 3.6-3.4 (m, 4 H, H-3, H-4, H-3′, H-4′); 3.58, 3.50, 3.33 (3x s, 9 H, 3x OC*H*$_3$); 2.61 (AB, 2 H, SC*H*$_2$-CH$_3$); 2.18 (s, 3 H, C*H*$_3$-acetyl); 1.32 (d, 3 H, H-6, $J_{6,5}$ 6.2 Hz); 1.28 (t, 3 H, SCH$_2$C*H*$_3$); 1.22 (d, 3 H, H-6′, $J_{6,5}$ 6.7 Hz). ^{13}C NMR data ($CDCl_3$): δ 170.4 (*C*=O); 138.6-127.2 (*C*-arom.); 99.4 (C-1′); 82.1 (C-1); 80.4, 80.3 (C-4, C-2′); 79.1 (C-4′); 77.6 (C-3′); 77.1 (C-3); 74.7 (benzyl-*C*H$_2$); 74.4 (C-2); 68.5 (C-5); 66.8 (C-5′); 61.6, 59.4, 57.8 (3x O*C*H$_3$); 25.5 (S*C*H$_2$CH$_3$); 21.2 (*C*H$_3$-acetyl); 17.7 (C-6); 16.3 (C-6′); 14.9 (SCH$_2$*C*H$_3$).

Anal. Calcd for $C_{26}H_{40}O_9S$: C, 59.1; H, 7.7. Found: C, 59.0; H, 7.6.

3-(Benzyloxycarbonylamino)propyl 2-*O*-Acetyl-3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-4-*O*-benzyl-α-L-rhamnopyranoside (22) - To a cooled (-30 °C) mixture of thioglycoside **21** (130 mg, 0.24 mmol), aglycon **9** (50 mg, 0.25 mmol) and molecular sieves (5Å, 0.5 g) in 1:1 1,2-dichloroethane-diethyl ether (6 mL) was added a solution of NIS (61 mg, 0.27 mmol) and TfOH (2.4 µL, 27 µmol) in 1:1 1,2-dichloromethane-diethyl ether (2.7 mL). After stirring for 10 min the reaction mixture was filtered and the filtrate was diluted with dichloromethane, extracted with aq. $Na_2S_2O_3$ (1M, 10 mL), aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. Purification of the residual oil on silica gel with 95:5 dichloromethane-acetone furnished compound **22** (120 mg, 75%); $[\alpha]_D$ -73.4° (c 1). ^1H NMR data ($CDCl_3$): δ 7.33-7.28 (m, 10 H, *H*-arom.); 5.16 (dd, 1 H, H-2, $J_{2,1}$ 1.8 Hz, $J_{2,3}$ 3.1 Hz); 5.11 (d, 1 H, H-1′, $J_{1,2}$ 3.1 Hz); 5.09 (d, 1 H, H-1, $J_{1,2}$ 1.8 Hz); 4.85, 4,67 (2x AB, 4 H, benzyl-C*H*$_2$); 4.08 (dd, 1 H, H-2′, $J_{2,1}$ 3.1 Hz, $J_{2,3}$ 9.4 Hz); 3.92 (q, 1 H, H-5′,

$J_{5,6}$ 6.4 Hz); 3.8-3.2 (m, 9 H, H-3, H-4, H-5, H-3′, H-4′, H-1 spacer, H-3 spacer); 3.57, 3.50, 3.32 (3x s, 3x OCH_3); 2.16 (s, 3 H, CH_3-acetyl); 1.79 (m, 2 H, H-2 spacer); 1.30 (d, 1 H, H-6, $J_{6,5}$ 5.9 Hz); 1.20 (d, 3 H, H-6′, $J_{6,5}$ 6.4 Hz). ^{13}C NMR data ($CDCl_3$): δ 170.4 (*C*=O); 156.1 (*C*=O); 138.5-127.3 (*C*-arom.); 99.4 (C-1′); 97.0 (C-1); 80.3 (C-2′); 79.9 (C-4); 79.1 (C-4′); 77.5 (C-3′); 76.9 (C-3); 74.7 (benzyl-CH_2); 72.5 (C-2); 67.8 (C-5); 66.7 (C-5′); 66.4 (benzyl-CH_2); 65.4 (C-1, spacer); 61.5, 59.2, 57.7 (3x OCH_3); 38.4 (C-3, spacer); 29.4 (C-2, spacer); 21.1 (CH_3-acetyl); 17.7 (C-6); 16.2 (C-6′).

Anal. Calcd for $C_{35}H_{49}NO_{12}$: C, 62.2; H, 7.3. Found: C, 62.3; H, 7.4.

3-Aminopropyl 3-*O*-(2,3,4-Tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranoside (4). - Sodium methoxide (10 mg) was added to a solution of compound **22** (100 mg, 0.15 mmol) in methanol (8 mL). The resulting mixture was stirred for 2 h at 50 °C, neutralized with Dowex (H^+ form), filtered and concentrated. The residue was redissolved in 2-propanol-water-acetic acid 7:3:0.5 (8 mL) and hydrogenated in the presence of palladium (10% on charcoal) for 24 h. The reaction mixture was filtered and concentrated. The residual oil was chromatographed on Sephadex LH-20 with methanol to give fragment **4** as the corresponding acetate (65 mg, 93%); $[\alpha]_D$ -95.8 (c 1, methanol). ^{1}H NMR data (CD_3OD): δ 5.20 (d, 1 H, H-1′, $J_{1,2}$ 3.6 Hz); 4.68 (s, 1 H, H-1); 4.09 (q, 1 H, H-5′, $J_{5,6}$ 6.2 Hz); 3.9-3.4 (m, 9 H, H-2, H-3, H-4, H-5, H-2′, H-3′, H-4′, H-1 spacer); 3.55, 3.51, 3.50 (3x s, 3x OCH_3); 3.03 (m, 2 H, H-3 spacer); 1.94 (s, 3 H, CH_3-acetate); 1.93 (m, 2 H, H-2 spacer); 1.26 (d, 3 H, H-6, $J_{6,5}$ 5.2 Hz); 1.16 (d, 3 H, H-6′, $J_{6,5}$ 6.2 Hz). ^{13}C NMR data (CD_3OD): δ 101.5 (C-1, J_{CH} 168 Hz); 100.2 (C-1′, J_{CH} 168 Hz); 81.2 (C-2′); 80.5 (C-3); 80.3 (C-4′); 79.4 (C-3′); 72.8 (C-4); 72.0 (C-2); 70.2 (C-5); 67.8 (C-5′); 65.4 (C-1, spacer); 62.0, 58.8, 58.1 (3x OCH_3); 38.4 (C-3, spacer); 28.6 (C-2, spacer); 18.0 (C-6); 16.7 (C-6′).

Anal. Calcd for $C_{28}H_{39}NO_{11}$: C, 51.2; H, 8.4. Found: C, 51.1; H, 8.6.

3-(Benzyloxycarbonylamino)propyl 3-*O*-[3-*O*-(2,3,4-Tri-*O*-methyl-α-L-fucopyranosyl)-2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl]-2-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (23) - A solution of NIS (50 mg, 0.22 mmol) and TfOH (2 µL, 22 µmol) in 1:1 1,2-dichloroethane-diethyl ether (2.2 mL) was added dropwise to a cooled (-30 °C) and stirred mixture of donor **21** (105 mg, 0,2 mmol), aglycon **12** (69 mg, 0.15 mmol) and molecular sieves (5Å, 0.5 g) in 1:1 1,2-dichloroethane-diethyl ether (6 mL). Processing of the reaction mixture, as described for the preparation of compound **22**, led to the isolation of trimer **23** (115 mg, 83%); $[\alpha]_D$ -77.3 (c 1). ^{1}H NMR data ($CDCl_3$): δ 5.31 (dd, 1 H, H-2′, $J_{2,1}$ 1.6 Hz, $J_{2,3}$ 3.1 Hz); 5.22 (d, 1 H, H-1″, $J_{1,2}$ 3.6 Hz); 5.07, 5.02, 4.58 (3x AB, 6 H, benzyl-CH_2); 5.00 (d, 1 H, H-1′, $J_{1,2}$ 1.6 Hz); 4.77 (d, 1 H, H-1, $J_{1,2}$<1Hz); 4.23 (dd,

1 H, H-3', $J_{3,2}$ 3.3 Hz, $J_{3,4}$ 9.5 Hz); 3.99 (m, 1 H, H-5'); 3.96 (dd, 1 H, H-3, $J_{3,2}$ 2.9 Hz, $J_{3,4}$ 9.3 Hz); 3.93 (q, 1 H, H-5", $J_{5,6}$ 6.7 Hz); 3.7-3.2 (m, 11 H, H-2, H-4, H-5, H-4', H-2", H-3", H-4", H-1 spacer, H-3 spacer); 3.54, 3.49, 3.46, 3.31 (4x s, 12 H, 4x OCH_3); 2.12 (s, 3 H, CH_3-acetyl); 1.75 (m, 2 H, H-2 spacer); 1.32 (d, 3 H, H-6', $J_{6,5}$ 6.2 Hz); 1.23 (d, 3 H, H-6, $J_{6,5}$ 6.2 Hz); 1.07 (d, 3 H, H-6", $J_{6,5}$ 6.5 Hz). ^{13}C NMR data ($CDCl_3$): δ 170.2 (*C*=O); 156.3 (*C*=O, spacer); 138.9-127.2 (*C*-arom.); 99.6 (C-1'); 99.5 (C-1"); 96.3 (C-1); 80.4 (C-2"); 80.1 (bs, C-2, C-3, C-4); 79.8 (C-4'); 79.1 (C-4"); 77.6 (C-3"); 76.5 (C-3'); 75.5, 74.6 (2x benzyl-*C*H_2); 72.8 (C-2'); 68.4 (C-5'); 68.0 (C-5); 66.7 (C-5"); 66.4 (benzyl-*C*H_2); 65.3 (C-1, spacer); 61.6, 59.5, 58.4, 57.7 (4x O*C*H_3); 38.5 (C-3, spacer); 29.5 (C-2, spacer); 21.1 (*C*H_3-acetyl); 18.0 (C-6'); 17.9 (C-6); 16.2 (C-6").

Anal. Calcd for $C_{49}H_{67}NO_{16}$: C, 63.6; H, 7.3. Found: C, 63.5; H, 7.1.

3-Aminopropyl 3-*O*-[3-*O*-(2,3,4-Tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranosyl]-2-*O*-methyl-α-L-rhamnopyranoside (3). - Compound **23** (92 mg, 0.1 mmol) was deacetylated and hydrogenated, as described for the preparation of **4**, to furnish trisaccharide **3** as the corresponding acetate (55 mg, 88%); $[\alpha]_D$ -89.1 (c 1, MeOH). ^{1}H NMR data (CD_3OD): δ 5.23 (d, 1 H, H-1", $J_{1,2}$ 3.8 Hz); 4.95 (d, 1 H, H-1', $J_{1,2}$ 1.8 Hz); 4.82 (d, 1 H, H-1, $J_{1,2}$ 1.8 Hz); 4.11 (q, 1 H, H-5", $J_{5,6}$ 6.6 Hz); 4.02 (1 H, H-2', $J_{2,1}$ 1.8 Hz, $J_{2,3}$ 3.3 Hz); 3.79, 3.52 (2x m, H-1 spacer); 3.77 (m, 2 H, H-3, H-5'); 3.75 (dd, 1 H, H-3', $J_{3,2}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz); 3.68 (dd, 1 H, H-3", $J_{3,4}$ 3.0 Hz, $J_{3,2}$ 10.3 Hz); 3.58 (dd, 1 H, H-4', $J_{4,3}\approx J_{4,5}$ 9.5 Hz); 3.57 (d, 1 H, H-4"); 3.55, 3.50, 3.49, 3.47 (4x s, 12 H, 4x O*C*H_3); 3.54 (m, 1 H, H-5); 3.53 (dd, 1 H, H-2"); 3.48 (dd, 1 H, H-2); 3.44 (dd, 1 H, H-3, $J_{3,2}\approx J_{3,4}$ 9.5 Hz); 3.00 (m, 2 H, H-3 spacer); 1.93 (m, 2 H, H-2 spacer); 1.89 (s, 3 H, CH_3-acetate); 1.27 (d, 3 H, H-6, $J_{6,5}$ 6.2 Hz); 1.24 (d, 3 H, H-6', $J_{6,5}$ 6.1 Hz); 1.20 (d, 3 H, H-6", $J_{6,5}$ 6.6 Hz). ^{13}C NMR data (CD_3OD): δ 103.9 (C-1', J_{CH} 172 Hz); 100.1 (C-1", J_{CH} 169 Hz); 98.3 (C-1, J_{CH} 169 Hz); 81.6 (C-2); 81.3 (C-2"); 80.5 (C-3); 80.4 (C-4"); 79.5 (bs, C-3, C-3"); 73.3 (C-4); 73.0 (C-4'); 72.0 (C-2'); 70.4 (bs, C-5, C-5'); 67.9 (C-5"); 65.7 (C-1, spacer); 62.0, 59.3, 58.9, 58.1 (4x O*C*H_3); 38.7 (C-3, spacer); 29.2 (C-2, spacer); 24.2 (*C*H_3-acetate); 18.1 (C-6); 18.0 (C-6'); 16.7 (C-6").

Anal. Calcd for $C_{27}H_{51}NO_{15}$: C, 51.5; H, 8.4. Found: C, 51.3; H, 8.6.

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