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Synthesis of a Spacer-Containing Tetrasaccharide Representing a Repeating Unit of the Capsular Polysaccharide of *Streptococcus pneumoniae* Type 6B

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Abstract—The synthesis is reported of the spacer-containing tetrasaccharide α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH-(5→phosphate→$CH_2CH_2CH_2NH_2$ (**1**), using a 2+2 block synthesis approach.

Introduction

Streptococcus pneumoniae is a gram-positive bacterium that can cause pneumonia, otitis media and meningitis in humans. To date, up to 85 different serotypes of this bacterium have been identified by their capsular polysaccharide.[1] The current polyvalent vaccine Pneumovax® 23[2] is constituted of the purified capsular polysaccharides of 23 serotypes of *S. pneumoniae*. Nevertheless, this vaccine is poorly immunogenic in persons of high risk groups such as children under the age of two, old persons, chronically ill people, and splenectomised patients. Since a polysaccharide-induced immune response does not evoke an immunological memory (TI-response), only short-term protection is provided. Moreover, the induction of tolerance is a severe problem.[1] These disadvantages, especially the absence of long-term protection, may be overcome by the use of oligosaccharide-conjugate based vaccines.

In the framework of our studies towards oligosaccharide-conjugate based vaccines against *S. pneumoniae* we became interested in one of the most virulent serotypes, namely type 6, which can be divided into two cross-reactive serotypes 6A and 6B. In earlier reports[3-5] we have described the synthesis of several fragments of the capsular polysaccharides of *S. pneumoniae* types 6A and 6B, useful in immunological inhibition studies. These compounds did not contain a spacer, which is necessary to prepare neoglycoconjugates, essential for vaccination studies. Here, we report the synthesis of the spacer-containing tetrasaccharide **1** representing a repeating unit of the capsular polysaccharide of *S. pneumoniae* type 6B (Fig. 1).

Results and Discussion

To establish a convenient and systematic synthesis of several different phosphate- and spacer-containing tetrasaccharide repeating units a strategy was developed, based on block synthesis. For the synthesis of the tetrasaccharide *O*-α-D-galactopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→4)-(5-*O*-[3-aminopropyl phosphate])-D-ribitol (**1**), the building blocks 5-*O*-allyl-1-*O*-benzoyl-2,3-di-*O*-benzyl-4-*O*-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-D-ribitol (**11**) and ethyl 2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl- α- D- galactopyranosyl)-1-thio-β-D-gluco-pyranoside (**26**) were designed. In this case, the block synthesis approach is powerful because after protecting group manipulations both building blocks can easily be transformed into several larger target structures.

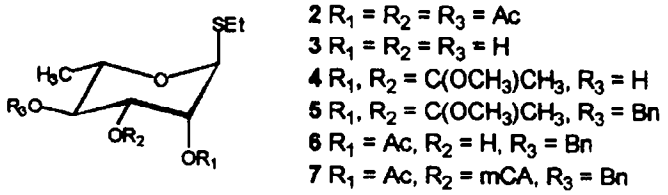
For the synthesis of disaccharide derivative **11** the rhamnose donor ethyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-monochloroacetyl-1-thio-α-L-rhamnopyranoside (**7**) and the ribitol acceptor 5-*O*-allyl-1-*O*-benzoyl-2,3-di-*O*-benzyl-D-ribitol (**9**) were prepared (Scheme I).

Ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**2**) was deacetylated (→**3**), and without purification converted in a one pot reaction,[6] via orthoacetates **4** and **5**, into **6** (80 % over 4 steps). Monochloroacetylation of HO-3, using monochloroacetyl chloride, then gave **7** (83 %). The previously described[7] 5-*O*-allyl-2,3-di-*O*-benzyl-D-ribitol (**8**) was selectively

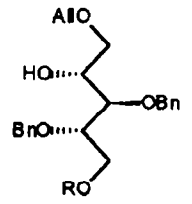
[→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH-(5→phosphate→]$_n$ (a)

α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH-(5→phosphate→$(CH_2)_3NH_2$ (b)

Figure 1. The structure of (a) the capsular polysaccharide of *S. pneumoniae* type 6B and (b) the compound synthesised in this study (**1**).



2 $R_1 = R_2 = R_3 = Ac$
3 $R_1 = R_2 = R_3 = H$
4 R_1, $R_2 = C(OCH_3)CH_3$, $R_3 = H$
5 R_1, $R_2 = C(OCH_3)CH_3$, $R_3 = Bn$
6 $R_1 = Ac$, $R_2 = H$, $R_3 = Bn$
7 $R_1 = Ac$, $R_2 = mCA$, $R_3 = Bn$



8 R=H
9 R=Bz

Scheme I. Ac = acetyl, mCA = monochloroacetyl, Bn = benzyl, Bz = benzoyl.

benzoylated[8] at HO-1 using 1-(benzoyloxy)-benzotriazole to yield **9** (91 %) (Scheme I). Condensation of **9** with **7** in CH_2Cl_2–diethyl ether in the presence of *N*-iodosuccinimide/triflic acid[9] gave disaccharide derivative **10** (85 %). De-monochloroacetylation of **10** using hydrazine dithiocarbonate[10] yielded disaccharide acceptor **11** (87 %) (Scheme II).

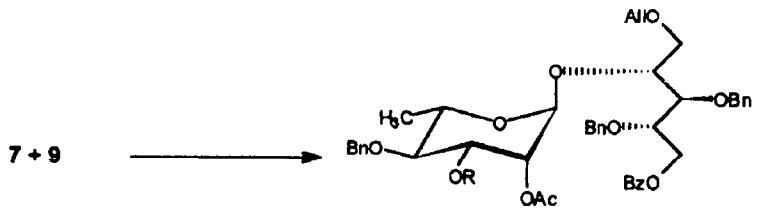
For the synthesis of disaccharide derivative **26** the galactose moieties ethyl 3,4,6-tri-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**18**) and ethyl 3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (**19**) were selected as possible donors, differing in protecting group at C-2 (Scheme III), but synthesised from the same precursor.

Bromide **12** was converted analogously to a published[11] 4-step procedure via **13**–**15** into **16** (21 % from **12**). After deacetylation (→**17**, 99 %) the HO-2 function was either *t*-butyldimethylsilylated or *p*-methoxybenzylated to give **18** and **19**, respectively, in comparable yields (87 % and 93 %).

The glucose acceptor ethyl 2,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**22**) (Scheme IV) was prepared from compound **20** by hydrogenolysis in the presence of Pd/C (→**21**, 85 %), followed by thioylation using ethanethiol and tin(IV) chloride (62 %). It should be noted that conversion of **20** into **22**, via a route comprising thioglycoside preparation followed by debenzylation, was not successful with respect to the hydrogenolysis.

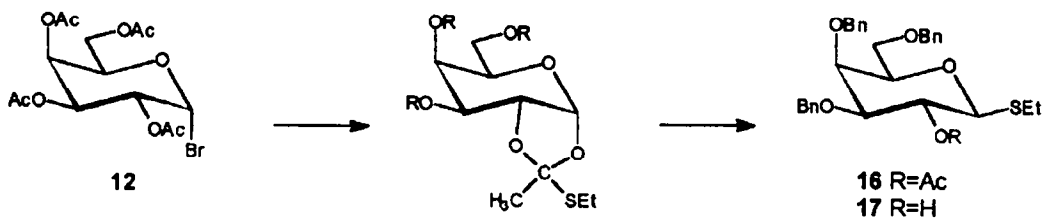
Then, the 'disarmed'[12] glucose acceptor **22** was glycosylated (Scheme V) with the 'armed' galactose donor **18** at −60 °C in 1:5 1,2-dichloroethane:diethyl ether using iodonium dicollidineperchlorate (IDCP) as a promoter, yielding disaccharide derivative **23** (49 %). Fortunately, coupling of glucose acceptor **22** with galactose donor **19** in 1:5 1,2-dichloroethane:diethyl ether using IDCP, gave **24** in a much higher yield (83 %). Deacetylation of **24** (→**25**, quantitative) and subsequent benzylation afforded disaccharide derivative **26** (83 %), which was used as a donor in the block synthesis of the target tetrasaccharide **1**.

To obtain the title tetrasaccharide **1** a series of coupling reactions were carried out between acceptor **11** and donor **26** (Scheme VI). In the presence of IDCP tetrasaccharide derivative **31** was obtained in a yield of 67 %, but with little stereoselectivity (α/β-ratio of 1:1). When methyl triflate was used as a promoter, repeated attempts in diethyl ether led to low yields (29 %) of **31**, with an unexpected α/β-ratio of 1:2, and a substantial amount of the donor-derived glycal **32**.



10 R = mCA
11 R = H

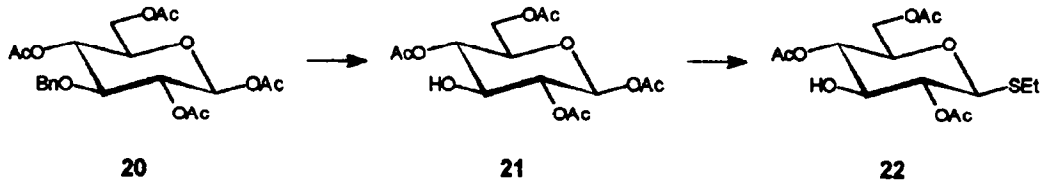
Scheme II. All = allyl.



13 R=Ac
14 R=H
15 R=Bn

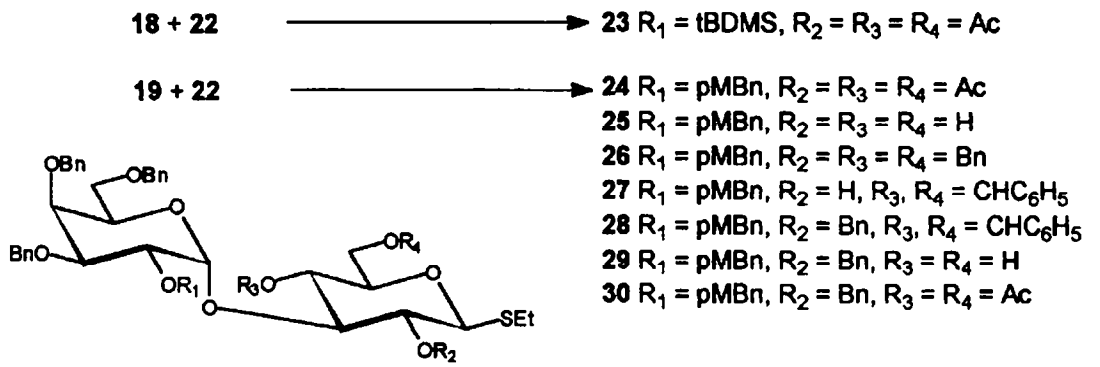
16 R=Ac
17 R=H
18 R=tBDMS
19 R=pMBn

Scheme III. tBDMS = *t*-butyldimethylsilyl, SEt = thioethyl, pMBn = *p*-methoxybenzyl.

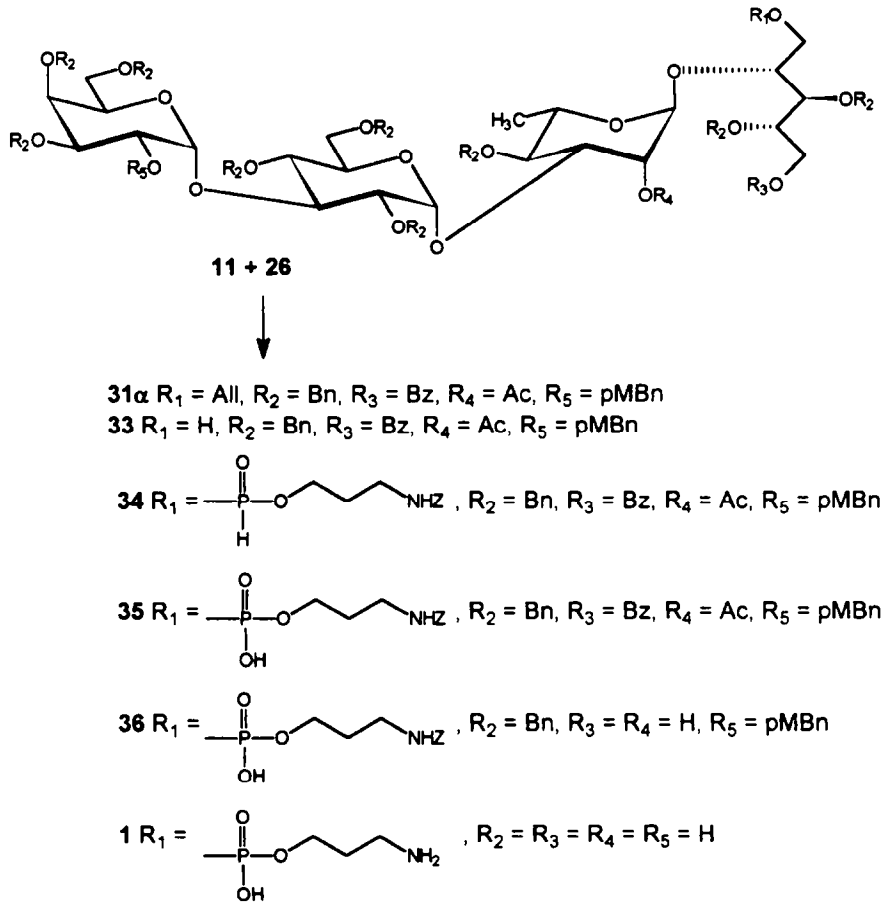


20 21 22

Scheme IV.



Scheme V.



Scheme VI. Z = benzyloxycarbonyl.

To overcome the low stereoselectivity of the glycosylation reaction between **11** and **26**, less reactive variants of **26** were prepared, namely **28** and **30** (Scheme V). To this end, compound **24** was deacetylated (→ **25**), and without purification benzylidenated using α,α-dimethoxytoluene to yield **27** (60 % over 2 steps). After benzylation of the HO-2 group (→**28**, 89 %), the benzylidene function was

removed with aq. trifluoroacetic acid in CH_2Cl_2, affording **29** (41 %) and a compound without *p*-methoxybenzyl function (9 %). Subsequent acetylation of **29** gave **30** (73 %). Test reactions with **28** as glycosyl donor using IDCP or methyl triflate as promoters were not successful, possibly due to the presence of the benzylidene protective group. It is known[13] that the reactivity of a donor molecule is reduced by the presence of a 4,6-dioxane ring. 4,6-Dioxane ring systems give a donor a more rigid structure, thereby hampering the formation of an anomeric oxonium cation in a glycosyl donor during activation. Also a test reaction with **30** as glycosyl donor using methyl triflate as a promoter in diethyl ether (–40 °C → +20 °C in 5 h) failed. A complex mixture of products was found without the desired compound.

The α/β-mixture of **31** could only be separated after deallylation (Wilkinson catalyst) of the ribitol unit (→**33**, 37 %) (Scheme VI). Phosphorylation of HO-5 of the ribitol unit in **33** using 3-*N*-(benzyloxycarbonyl)-aminopropyl phosphonate with pivaloyl chloride in pyridine[14] gave the spacer-tetrasaccharide-phosphonate diester **34** (53 %). Oxidation of **34** with iodine in pyridine–water, effecting the conversion of the phosphonate group into a phosphate group, gave **35** (65 %). Finally, deacetylation/debenzoylation in methanol–ammonia (→**36**) followed, without further purification, by hydrogenolysis to remove benzyl, *p*-methoxybenzyl and benzyloxycarbonyl functions, and purification on Bio-Gel P-2 gave the title oligosaccharide **1** (75 % over the last 2 steps).

The conjugation of **1** to carrier proteins and the results of immunological tests with this conjugate will be reported elsewhere.

Experimental

General methods

^1H NMR spectra (300 and 500 MHz) were recorded at 25 °C with Bruker AC 300 and Bruker AM 500 spectrometers. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si ($CDCl_3$) or internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225). Column chromatography was performed on Kieselgel 60 (Merck, < 230 mesh or 70-230 mesh) and fractions were monitored by TLC on Kieselgel 60 F_{254} (Merck), using detection with UV light and then charring with aq. 50 % sulfuric acid. Optical rotations were measured at 20 °C for solutions in $CHCl_3$ (unless stated otherwise) with a Perkin–Elmer 241 polarimeter, using a 10-cm 1-mL cell. Melting points (uncorrected) were determined with a Kofler apparatus. Evaporations were conducted under reduced pressure at 40 °C (bath). Reactions were performed under dry conditions using an atmosphere of nitrogen or argon. All solvents were distilled from appropriate drying agents.

Ethyl 2-O-acetyl-4-O-benzyl-1-thio-α-L-rhamnopyranoside (6)

To a solution of ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside[15] (**2**; 4.0 g, 12 mmol) in methanol (20 mL) was added sodium methoxide (pH 10). When TLC (9:1, CH_2Cl_2:methanol) showed the deacetylation to be completed (10 min), the mixture was concentrated and co-concentrated with CH_2Cl_2. Crude **3** was dissolved in toluene (30 mL), and CH_2Cl_2 (5 mL), *p*-toluenesulfonic acid (200 mg), and trimethyl orthoacetate (6.1 mL, 48 mmol) were added. After 45 min, TLC (9:1, CH_2Cl_2: methanol) showed a complete conversion of **3** into **4** (R_f 0.70). Then, DMF (30 mL) and sodium hydride (1.0 g, 42 mmol) were added, and the mixture was stirred until the evolution of gas stopped. Benzyl bromide was added in two portions (1.5 mL, 13 mmol; after 90 min: 2 mL, 17 mmol), and the mixture was stirred for 2.5 h, when TLC (6:4, hexane:EtOAc) showed the benzylation to be completed (**5**, R_f 0.89). After destroying the excess of sodium hydride with methanol, the mixture was diluted with EtOAc, washed with water (3 ×), and concentrated. The residue was dissolved in aq. 80 % acetic acid (20 mL), and the solution was stirred for 5 min, and co-concentrated with toluene (3 ×), ethanol (3 ×), and CH_2Cl_2 (3 ×). Column chromatography (75:25, hexane:EtOAc) of the residue yielded **6** (3.27 g, 80 %) as a syrup, $[\alpha]_D$ –120 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.37–7.26 (m, 5H, Ph), 4.822 and 4.717 (2d, each 1H, OCH_2Ph), 4.088 (m, 1H, H-5), 4.051 (dd, 1H, H-3), 3.382 (t, 1H, H-4), 2.68–2.54 (m, 2H, SCH_2CH_3), 2.160 (s, 3H, Ac), 1.356 (d, 3H, 3 H-6), 1.276 (t, 3H, SCH_2CH_3); $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4, $J_{5,6}$ = 6.2 Hz; Anal. calcd for $C_{17}H_{24}O_5S$: C, 59.98; H, 7.11 %; found: C, 59.69; H, 7.06 %.

Ethyl 2-O-acetyl-4-O-benzyl-3-O-monochloroacetyl-1-thio-α-L-rhamnopyranoside (7)

To a stirred solution of **6** (14.2 g, 41.7 mmol) in CH_2Cl_2 (125 mL) and pyridine (4.05 mL) at 0 °C was added dropwise in 1 h a solution of monochloroacetyl chloride (3.34 mL, 42 mmol) in CH_2Cl_2 (40 mL). After additional stirring for 90 min, TLC (7:3, hexane:EtOAc) showed the formation of **7** (R_f 0.55). The mixture was diluted with CH_2Cl_2, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1, toluene:EtOAc) of the residue gave **7** (14.02 g, 83 %) as a white solid, mp 61 °C; $[\alpha]_D$ –98 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.34–7.28 (m, 5H, Ph), 5.375 (dd, 1H, H-2), 5.290 (dd, 1H, H-3), 5.116 (d, 1H, H-1), 4.709 and 4.659 (2d, each 1H, OCH_2Ph), 4.199 (dq, 1H, H-5), 3.912 and 3.847 (2d, each 1H, $COCH_2Cl$), 3.569 (t, 1H, H-4), 2.69–2.55 (m, 2H, SCH_2CH_3), 2.146 (s, 3H, Ac), 1.362 (d, 3H, 3 H-6), 1.283 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 1.6 , $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.6, $J_{4,5}$ = 9.6, $J_{5,6}$ = 6.2 Hz; Anal. calcd for $C_{19}H_{25}ClO_6S$: C, 54.74; H, 6.04 %; found: C, 54.68; H, 6.16 %.

5-O-Allyl-1-O-benzoyl-2,3-di-O-benzyl-D-ribitol (9)

A solution of 5-*O*-allyl-2,3-di-*O*-benzyl-D-ribitol[7] (**8**; 2.5 g, 6.7 mmol) and 1-(benzoyloxy)-benzotriazole (1.8 g, 7.5 mmol) in CH_2Cl_2 (33.5 mL) and triethylamine (1.1 mL) was stirred overnight. Then TLC (8:2, hexane:EtOAc) showed the exclusive formation of **9** (R_f 0.38), and the mixture was diluted with CH_2Cl_2, washed with aq. 10 % $NaHCO_3$, brine and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (85:15, hexane:EtOAc) of the residue gave **9** (3.2 g, 91 %) as a syrup, $[\alpha]_D$ −39 ° (c 1). ^1H NMR ($CDCl_3$) δ 8.20–7.22 (m, 15H, 3 Ph), 5.882 (m, 1H, OCH_2CH=CH_2), 5.247 and 5.166 (2 dq, each 1H, OCH_2CH=CH_2), 4.80–4.64 (m, 4H, 2 OCH_2Ph), 4.762 (dd, 1H, H-1a), 4.514 (dd, 1H, H-1b), 4.106 (ddd, 1H, H-2), 4.036 (ddd, 1H, H-4), 3.98–3.95 (m, 2H, OCH_2CH=CH_2), 3.831 (dd, 1H, H-3), 3.597 (dd, 1H, H-5a), 3.551 (dd, 1H, H-5b); $J_{1a,1b}$ = 12.1, $J_{1a,2}$ = 3.2, $J_{1b,2}$ = 6.1, $J_{2,3}$ = 6.9, $J_{3,4}$ = 4.2, $J_{4,5a}$ = 3.5, $J_{4,5b}$ = 5.8, $J_{5a,5b}$ = 9.7 Hz.

5-O-Allyl-1-O-benzoyl-2,3-di-O-benzyl-4-O-(2-O-acetyl-4-O-benzyl-3-O-monochloroacetyl-α-L-rhamnopyranosyl)-D-ribitol (10)

A mixture of **9** (1.04 g, 2.18 mmol), **7** (1.00 g, 2.40 mmol) and 4 Å molecular sieves (1.7 g) in CH_2Cl_2 (6.45 mL) was stirred for 30 min. Then, at 0 °C a mixture of *N*-iodosuccinimide (0.57 g, 2.4 mmol) and triflic acid (19 μL, 0.21 μmol) in 1:1 CH_2Cl_2:diethyl ether (19.5 mL) was added. After 1 min, TLC (9:1, toluene:EtOAc) showed the appearance of a single product (**10**, R_f 0.36), and the mixture was neutralised with triethylamine, filtered over Celite, diluted with CH_2Cl_2, washed with aq. 10 % sodium thiosulfate (2 ×) and water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1, toluene:EtOAc) of the residue afforded **10** (1.55 g, 85 %) as a syrup, $[\alpha]_D$ −46 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.95–7.31 (m, 20H, 4 Ph), 5.836 (m, 1H, OCH_2CH=CH_2), 5.21–5.09 (m, 2H, OCH_2CH=CH_2), 5.141 (d, 1H, H-1'), 4.759 (dd, 1H, H-1b), 4.455 (dd, 1H, H-1a), 4.313 (m, 1H, H-5'), 3.818 (dd, 1H, H-3), 3.629 (d, 2H, H-5a,5b), 3.527 (t, 1H, H-4'), 2.146 (s, 3H, Ac), 1.163 (d, 3H, 3 H-6'); $J_{1a,1b}$ = 12.0, $J_{1a,2}$ = 4.9, $J_{1b,2}$ = 6.8, $J_{2,3}$ = 7.2, $J_{3,4}$ = 2.9, $J_{4,5a}$ = 5.3, $J_{4,5b}$ = 5.3, $J_{1',2'}$ = 1.4, $J_{3',4'}$ = 10.0, $J_{4',5'}$ = 10.0, $J_{5',6'}$ = 6.2 Hz; Anal. calcd for $C_{46}H_{51}ClO_{12}$: C, 66.46; H, 6.18 %; found: C, 66.34; H, 6.08 %.

5-O-Allyl-1-O-benzoyl-2,3-di-O-benzyl-4-O-(2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-D-ribitol (11)

To a solution of **10** (705 mg, 0.82 mmol) in 1:3 acetic acid:2,6-lutidine (17 mL) was added a freshly prepared hydrazine dithiocarbonate solution[10] (4 mL). TLC (7:3, hexane:EtOAc, R_f 0.22) showed the demonochloroacetylation to be complete in 15 min. Then, the mixture was diluted with CH_2Cl_2, and washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (7:3, hexane:EtOAc) of the residue gave **11** (539 mg, 87 %) as a syrup, $[\alpha]_D$ −47 ° (c 1). ^1H NMR ($CDCl_3$) δ 8.05–7.33 (m, 20H, 4 Ph), 5.861 (m, 1H, OCH_2CH=CH_2), 5.24–5.10 (m, 2H, OCH_2CH=CH_2), 5.164 (dd, 1H, H-2'), 5.099 (d, 1H, H-1'), 4.761, 4.731, 4.714, 4.663, 4.635, and 4.585 (6d, each 1H, 3 OCH_2Ph), 4.750 (dd, 1H, H-1a), 4.449 (dd, 1H, H-1b), 4.251 (dq, 1H, H-2), 4.041 (dd, 1H, H-3'), 3.324 (t, 1H, H-4'), 1.160 (d, 3H, 3 H-6'); $J_{1a,1b}$ = 12.0, $J_{1a,2}$ = 2.5, $J_{1b,2}$ = 5.2, $J_{1',2'}$ = 1.7, $J_{2',3'}$ = 3.4, $J_{3',4'}$ = 9.5, $J_{4',5'}$ = 9.5, $J_{5',6'}$ = 6.2 Hz; Anal. calcd for $C_{44}H_{50}O_{11}\cdot{}^1/_2H_2O$: C, 69.18; H, 6.73 %; found: C, 69.29; H, 6.80 %.

Ethyl 2-O-acetyl-3,4,6-tri-O-benzyl-1-thio-α-D-galactopyranoside (16)

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**12**; 18.9 g, 46 mmol) in nitromethane (40 mL) were added tetraethylammonium bromide (0.96 g, 4.6 mmol), 2,6-lutidine (7.8 mL, 67 mmol), and ethanethiol (13.6 mL, 184 mmol). TLC (95:5, CH_2Cl_2:EtOAc) showed the conversion of **12** (R_f 0.57) into **13** (R_f 0.81) after 24 h. Then, the mixture was diluted with EtOAc, washed with aq. 10 % $NaHCO_3$ (2 ×) and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (69:30:1, hexane:EtOAc:triethylamine) of the residue yielded **13** as a syrup. To a solution of **13** in methanol (200 mL) was added sodium methoxide (pH 10), and the solution was stirred for 21 h. When TLC (85:15, CH_2Cl_2:acetone) showed the disappearance of **13** (→**14**, R_f 0.12), the solution was concentrated without neutralisation, co-concentrated twice with CH_2Cl_2, and dried *in vacuo*. A solution of crude **14** and benzyl bromide (16.6 mL, 140 mmol) in DMF (60 mL) was added dropwise to a cooled (0 °C) suspension of sodium hydride (4.0 g, 167 mmol) in DMF (40 mL). TLC (8:2, hexane:EtOAc) showed the formation of **15** (R_f 0.69) to be completed in 24 h. After destroying the excess of sodium hydride with methanol, the mixture was diluted with EtOAc, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated to yield **15** (22.3 g) as a yellow syrup.

To a solution of crude **15** (13.3 g) in CH_2Cl_2 (50 mL) was added 4 Å molecular sieves (4 g), and the mixture was stirred for 30 min. The solution was cooled (0 °C), and trimethylsilyl triflate (0.50 mL, 2.7 mmol) was added. TLC (6:4, hexane:EtOAc) showed a complete conversion of **15** into **16** (R_f 0.55) in 2.5 h. Then, the mixture was neutralised with triethylamine, diluted with CH_2Cl_2, washed with water (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (85:15, hexane:EtOAc) of the residue gave **16** (5.2 g, 21 % from **12**) as a white solid, mp 56 °C; $[\alpha]_D$ −1 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.32–7.28 (m, 15H, 3 Ph), 5.419 (t, 1H, H-2), 4.940 and 4.533 (2d, each 1H, OCH_2Ph), 4.671 and 4.574 (2d, each 1H, OCH_2Ph), 4.460 and

4.410 (2d, each 1H, OCH_2Ph), 4.330 (d, 1H, H-1), 3.990 (d, 1H, H-4), 3.537 (dd, 1H, H-3), 2.76–2.62 (m, 2H, SCH_2CH_3), 2.031 (s, 3H, Ac), 1.223 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.8, $J_{2,3}$ = 9.8, $J_{3,4}$ = 2.8, $J_{4,5}$ = 0 Hz; Anal. calcd for $C_{31}H_{36}O_6S$: C, 69.38; H, 6.76 %; found: C, 69.51; H, 6.79 %.

*Ethyl 3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (17)*

To a solution of **16** (649 mg, 1.31 mmol) in methanol (5 mL) was added sodium methoxide (pH 10). After 24 h, TLC (6:4, hexane:EtOAc) showed a complete conversion into **17** (R_f 0.49). The mixture was neutralised with Dowex-50 (H^+) resin, filtered, concentrated, and co-concentrated with CH_2Cl_2 (2 ×) and ethanol, to give **17** (593 mg, 99 %) as a white crystalline solid, mp 73 °C; $[\alpha]_D$ −7 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.31–7.26 (m, 15H, 3 Ph), 4.894, 4.593, 4.744, 4.686, 4.478, and 4.422 (6d, each 1H, 3 OCH_2Ph), 4.313 (d, 1H, H-1), 3.978 (t, 1H, H-2), 3.972 (d, 1H, H-4), 3.447 (dd, 1H, H-3), 2.76–2.67 (m, 2H, SCH_2CH_3), 1.286 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.6, $J_{2,3}$ = 9.5, $J_{3,4}$ = 2.8, $J_{4,5}$ = 0 Hz; Anal. calcd for $C_{29}H_{34}O_5S$: C, 70.42; H, 6.93 %; found: C, 70.31; H, 7.04 %.

*Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-t-butyldimethylsilyl-1-thio-β-D-galactopyranoside (18)*

To a solution of **17** (500 mg, 0.82 mmol) in acetonitrile (5 mL) were added *t*-butyldimethylsilyl chloride (0.20 g, 1.3 mmol) and diazabicyclo[2.2.2]octane (0.12 g, 1.1 mmol). After 2 h, TLC (95:5, CH_2Cl_2:acetone) showed the disappearance of **17** and appearance of **18** (R_f 0.78). The mixture was diluted with CH_2Cl_2, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (97:3, CH_2Cl_2:acetone) of the residue yielded **18** (434 mg, 87 %) as a syrup, $[\alpha]_D$ −13 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.40–7.20 (m, 15H, 3 Ph), 4.852, 4.820, 4.600, 4.516, 4.466, and 4.403 (6d, each 1H, 3 OCH_2Ph), 4.320 (d, 1H, H-1), 3.962 (t, 1H, H-2), 3.937 (d, 1H, H-4), 3.363 (dd, 1H, H-3), 2.80–2.56 (m, 2H, SCH_2CH_3), 1.274 (t, 3H, SCH_2CH_3), 0.887 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.163 and 0.023 (2s, each 3H, $Si(CH_3)_2C(CH_3)_3$); $J_{1,2}$ = 9.2, $J_{2,3}$ = 9.0, $J_{3,4}$ = 2.7, $J_{4,5}$ = 0 Hz.

*Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-p-methoxybenzyl-1-thio-β-D-galactopyranoside (19)*

A solution of **17** (4.0 g, 8.1 mmol) and *p*-methoxybenzyl chloride (1.5 mL, 10 mmol) in DMF (9.2 mL) was added dropwise to a suspension of sodium hydride (510 mg, 21 mmol) in DMF (7 mL). TLC (7:3, hexane:EtOAc) showed the formation of **19** (R_f 0.59) to be completed in 90 min. After destroying the excess of sodium hydride with methanol, the mixture was diluted with CH_2Cl_2 and washed with water (2 ×), dried ($MgSO_4$), filtered, concentrated, and co-concentrated with toluene (2 ×), ethanol (2 ×), and CH_2Cl_2 (2 ×). Column chromatography (8:2, hexane:EtOAc) of the syrup gave **19** (4.6 g, 93 %) as a white crystalline solid, mp 64 °C; $[\alpha]_D$ +2 ° (c 1). ^1H NMR ($CDCl_3$) δ 7.32–7.27 (m, 15H, 3 Ph), 6.846 (m, 4H, $C_6H_4OCH_3$), 4.943 and 4.605 (2d, each 1H, OCH_2Ph), 4.799 and 4.713 (2d, each 1H, OCH_2Ph), 4.728 (s, 2H, $OCH_2C_6H_4OCH_3$), 4.454 and 4.396 (2d, each 1H, OCH_2Ph), 4.408 (d, 1H, H-1), 3.942 (d, 1H, H-4), 3.805 (t, 1H, H-2), 3.784 (s, 3H, $C_6H_4OCH_3$), 3.544 (dd, 1H, H-3), 2.80–2.66 (m, 2H, SCH_2CH_3), 1.291 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.6, $J_{2,3}$ = 9.2, $J_{3,4}$ = 2.8, $J_{4,5}$ = 0 Hz.

*1,2,4,6-Tetra-*O*-acetyl-β-D-glucopyranose (21)*

To a solution of 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose **20**[16] (22.0 g, 50.2 mmol) in 1:1 ethanol:EtOAc (80 mL) were added 10 % Pd/C (750 mg) and acetic acid (1 mL), and the suspension was hydrogenolysed at 4 kg/cm^2 for 24 h. Because of incomplete debenzylation, the hydrogenolysis was repeated twice with intermediate filtration and addition of new catalyst. Then, TLC (1:1, hexane:EtOAc) showed the complete conversion of **20** into **21** (R_f 0.19). After final filtration, the solution was concentrated, and the residue was crystallised from ethanol to afford **21** (14.8 g, 85 %) as a white crystalline product, mp 116–121 °C; $[\alpha]_D$ +19 ° (c 1). ^1H NMR ($CDCl_3$) δ 5.667 (d, 1H, H-1), 5.001 (dd, 1H, H-2), 4.981 (t, 1H, H-4), 4.293 (dd, 1H, H-6b), 4.126 (dd, 1H, H-6a), 2.775 (brd, 1H, HO-3), 2.120, 2.111, and 2.081 (3s, 3,6,3H, 4 Ac); $J_{1,2}$ = 8.3, $J_{2,3}$ = 9.6, $J_{3,4}$ = 9.7, $J_{4,5}$ = 9.7, $J_{5,6a}$ = 2.2, $J_{5,6b}$ = 4.7, $J_{6a,6b}$ = 12.4, $J_{H\text{-}3,OH}$ = 6.8 Hz; Anal. calcd for $C_{14}H_{20}O_{10}$: C, 48.28; H, 5.79 %; found: C, 48.20; H, 5.78 %.

*Ethyl 2,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (22)*

To a solution of **21** (14.1 g, 40.4 mmol) in CH_2Cl_2 (120 mL) were added ethanethiol (3.6 mL, 50 mmol) and tin(IV) chloride (1.5 mL, 12 mmol). After stirring for 15 min, TLC (6:4, hexane:EtOAc) showed the disappearance of **21** and the formation of **22**. The mixture was diluted with CH_2Cl_2, washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4, hexane:EtOAc) of the residue yielded **22** (7.3 g, 62 %) as a syrup, $[\alpha]_D$ −25 ° (c 1). ^1H NMR ($CDCl_3$) δ 4.439 (d, 1H, H-1), 4.250 (dd, 1H, H-6a), 4.157 (dd, 1H, H-6b), 4.735 (brt, 1H, H-3), 3.643 (ddd, 1H, H-5), 2.80–2.60 (m, 2H, SCH_2CH_3), 2.137, 2.166, and 2.078 (3s, each 3H, 3 Ac), 1.275 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 10.0, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 5.1, $J_{6a,6b}$ = 12.3 Hz; Anal. calcd for $C_{14}H_{22}O_8S$: C, 47.99; H, 6.33 %; found: C, 47.84; H, 6.38 %.

Ethyl 2,4,6- tri- O- acetyl- 3- O- (3,4,6- tri-O-benzyl-2-O-t-butyldimethylsilyl-α-D-galactopyranosyl)-1-thio-β-D-gluco-pyranoside (23)

A mixture of **18** (102 mg, 0.18 mmol), **22** (57 mg, 0.16 mmol) and 4 Å molecular sieves (200 mg) in 1:5 1,2-dichloroethane:diethyl ether (6 mL) was stirred for 30 min at −60 °C, then iodonium dicollidineperchlorate (0.19 g, 0.41 mmol) was added. After 30 min, TLC (6:4,

hexane:EtOAc) showed the disappearance of **18** and the appearance of a new spot (R_f 0.54). The mixture was diluted with CH_2Cl_2, filtered over Celite, washed with aq. 10 % sodium thiosulfate and water (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (Sephadex LH-20 in 1:1 CH_2Cl_2:methanol, then silica using 6:4, hexane:EtOAc) of the residue afforded **23** (70 mg, 49 %) as a syrup. ^{1}H NMR ($CDCl_3$) δ 7.33–7.24 (m, 15H, 3 Ph), 4.850 (d, 1H, H-1'), 4.803 and 4.465 (2d, each 1H, OC*H*$_2$Ph), 4.612 (2d, each 1H, OC*H*$_2$Ph), 4.526 and 4.397 (2d, each 1H, OC*H*$_2$Ph), 4.346 (d, 1H, H-1), 4.140 (dd, 1H, H-2'), 3.911 (dd, 1H, H-4'), 3.749 (t, 1H, H-3), 2.77–2.64 (m, 2H, SC*H*$_2$CH$_3$), 2.091 and 2.013 (2s, 6,3H, 3 Ac), 1.257 (t, 3H, SCH$_2$C*H*$_3$), 0.899 (s, 9H, Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.141 and 0.013 (2s, each 3H, Si(C*H*$_3$)$_2$C(CH$_3$)$_3$); $J_{1,2}$ = 10.0, $J_{1',2'}$ = 3.2 , $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 2.6, $J_{4',5'}$ = 1.3 Hz.

*Ethyl 2,4,6-tri-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-p-methoxybenzyl- α- D-galactopyranosyl)- 1- thio-β-D-gluco-pyranoside (24)*

A mixture of **19** (2.41 g, 3.92 mmol), **22** (1.25 g, 3.57 mmol) and 4 Å molecular sieves (5 g) in 1:5 1,2-dichloroethane:diethyl ether (6 mL) was stirred for 15 min at room temperature, then iodonium dicollidineperchlorate (1.84 g, 3.97 mmol) was added. After 30 min, TLC (6:4, hexane:EtOAc) showed the disappearance of the reactants and the formation of **24** (R_f 0.54). The mixture was diluted with CH_2Cl_2, filtered over Celite, washed with aq. 10 % sodium thiosulfate and water (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4, hexane: EtOAc) of the residue afforded **24** (2.68 g, 83 %) as a syrup, $[\alpha]_D$ +15 ° (c 1). ^{1}H NMR ($CDCl_3$) δ 7.50–6.75 (m, 19H, 3 Ph and C$_6$*H*$_4$OCH$_3$), 4.837 (d, 1H, H-1'), 4.886, 4.729, 4.655, 4.636, 4.557, 4.519, 4.505, and 4.382 (8d, each 1H, 3 OC*H*$_2$Ph and OC*H*$_2$C$_6$H$_4$OCH$_3$), 4.324 (d, 1H, H-1), 4.231 (dd, 1H, H-6b), 4.147 (dd, 1H, H-6a), 4.020 (m, 1H, H-5'), 3.955 (dd, 1H, H-4'), 3.913 (dd, 1H, H-2'), 3.802 (dd, 1H, H-3'), 3.704 (t, 1H, H-3), 2.80–2.60 (m, 2H, SC*H*$_2$CH$_3$), 2.079, 1.997, and 1.806 (3s, each 3H, 3 Ac), 1.255 (t, 3H, SCH$_2$C*H*$_3$); $J_{1,2}$ = 10.0, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 4.8, $J_{6a,6b}$ = 12.2, $J_{1',2'}$ = 3.2, $J_{2',3'}$ = 10.2, $J_{3',4'}$ = 2.7, $J_{4',5'}$ = 1.5 Hz; Anal. calcd for $C_{49}H_{58}O_{14}S$: C, 65.17; H, 6.47 %; found: C, 65.02; H, 6.43 %.

*Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-p-methoxybenzyl-α-D-galactopyranosyl)-1-thio-β-D-gluco-pyranoside (26)*

To a solution of **24** (400 mg, 0.44 mmol) in methanol (10 mL) was added sodium methoxide (pH 10). After refluxing overnight, TLC (95:5, CH_2Cl_2:acetone) showed the formation of **25** (R_f 0.05). The mixture was neutralised using Dowex-50 (H^+) resin, filtered, and concentrated to give **25** in a quantitative yield. A solution of **25** (300 mg, 0.41 mmol) and benzyl bromide (0.16 mL, 1.35 mmol) in DMF (4 mL) was added dropwise to a suspension of sodium hydride (0.11 g, 4.6 mmol) in DMF (2 mL). TLC (7:3, hexane:EtOAc) showed that the benzylation was completed in 90 min (**26**, R_f 0.47). After destroying the excess of sodium hydride with methanol, the mixture was diluted with CH_2Cl_2, washed with water (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2, hexane:EtOAc) of the residue gave **26** (383 mg, 83 %) as a syrup, $[\alpha]_D$ +26 ° (c 1). ^{1}H NMR ($CDCl_3$) δ 7.40–6.65 (m, 34H, 6 Ph and C$_6$*H*$_4$OCH$_3$), 5.580 (d, 1H, H-1'), 4.968, 4.863, 4.853, 4.766, 4.698, 4.673, 4.631, 4.620, 4.505, 4.494 (10d, each 1H), 4.538 (d, 2H), and 4.203 (s, 2H) (6 OC*H*$_2$Ph and OC*H*$_2$C$_6$H$_4$OCH$_3$), 4.380 (d, 1H, H-1), 4.047 (dd, 1H, H-2'), 4.000 (t, 1H, H-2), 3.969 (dd, 1H, H-3'), 3.764 (s, 3H, C$_6$H$_4$OC*H*$_3$), 2.84–2.62 (m, 2H, SC*H*$_2$CH$_3$), 1.298 (t, 3H, SCH$_2$C*H*$_3$); $J_{1,2}$ = 9.6, $J_{2,3}$ = 8.9, $J_{1',2'}$ = 3.3, $J_{2',3'}$ = 10.2, $J_{3',4'}$ = 2.6 Hz.

O-(3,4,6-Tri-O-benzyl-2-O-p-methoxybenzyl-α-D-galacto-pyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-α/β-D-glucopyr-anosyl)-(1→3)-O-(2-O-acetyl-4-O-benzyl-α-L-rhamnopyr-anosyl)- (1→4)- 5- O- allyl- 1-O-benzoyl-2,3-di-O-benzyl-D-ribitol (31αβ)

A mixture of **26** (121 mg, 0.12 mmol), **11** (78 mg, 0.095 mmol) and 4 Å molecular sieves (0.3 g) in 1:5 1,2-dichloroethane:diethyl ether (3 mL) was stirred for 30 min, after which iodonium dicollidineperchlorate (107 mg, 0.23 mmol) was added. TLC (8:2, hexane:EtOAc) showed the disappearance of the reactants and the formation of **31** (R_f 0.25) in 90 min. Then, the mixture was diluted with CH_2Cl_2, filtered, and washed with aq. 10 % sodium thiosulfate and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (Sephadex LH-20 in 1:1 CH_2Cl_2:methanol, then silica using 4:1 hexane: EtOAc) of the residue afforded **31**αβ (115 mg, 67 %, α:β ratio 1:1) as a syrup. ^{1}H NMR ($CDCl_3$) δ 7.95–6.50 (m, 54H, 10 Ph and C$_6$*H*$_4$OCH$_3$), 5.858 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.591 (d, 1H, H-1''', **31**α), 5.504 (d, 1H, H-1''', **31**β), 5.310 (d, 1H, H-1'', **31**α), 5.414 (d, 1H, H-1', **31**β), 5.116 (d, 1H, H-1', **31**α), 3.671 (s, 3H, C$_6$H$_4$OC*H*$_3$, **31**β), 3.664 (s, 3H, C$_6$H$_4$OC*H*$_3$, **31**α), 2.125 (s, 3H, Ac, **31**β), 1.857 (s, 3H, Ac, **31**α), 1.192 (d, 3H, 3 H-6', **31**α), 1.116 (d, 3H, 3 H-6', **31**β); **31**α: $J_{1',2'}$ = 1.6, $J_{1'',2''}$ = 3.6, $J_{1''',2'''}$ = 3.3, $J_{5',6'}$ = 6.1 Hz; **31**β: $J_{1',2'}$ = 1.7, $J_{1''',2'''}$ = 3.5, $J_{5',6'}$ = 6.1 Hz.

*Ethyl 4,6-*O*-benzylidene-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-p-methoxybenzyl-α-D-galactopyranosyl)-1-thio-β-D-gluco-pyranoside (27)*

Compound **24** (3.28 g, 3.63 mmol) was converted into **25** as described above. Crude **25** was dissolved in DMF (10 mL) and α,α-dimethoxytoluene (0.65 mL), and *p*-toluenesulfonic acid (pH 2) was added. The mixture was stirred for 5 h at 50 °C, when TLC (95:5, CH_2Cl_2: acetone) showed the formation of **27** (R_f 0.56). Solid $NaHCO_3$ was added, and the mixture was diluted with EtOAc, washed with aq. 10 % $NaHCO_3$ and water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4, hexane:EtOAc) of the residue afforded **27** (1.87 g, 60 % from **24**) as a syrup, $[\alpha]_D$ +8 °

(c 1). ^{1}H NMR ($CDCl_3$) δ 7.45–7.25 (m, 20H, 4 Ph), 7.00–6.55 (m, 4H, $C_6H_4OCH_3$), 5.498 (s, 1H, C*H*Ph), 5.422 (d, 1H, H-1'), 4.280 (d, 1H, H-1), 3.734 (s, 3H, $C_6H_4OCH_3$), 2.76–2.63 (m, 2H, SCH_2CH_3), 1.292 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.7, $J_{1',2'}$ = 3.5 Hz.

Ethyl 2- O*-benzyl-4,6-*O*-benzylidene-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (28)*

A solution of **27** (330 mg, 0.38 mmol) and benzyl bromide (54 μL, 0.45 mmol) in DMF (3 mL) was added dropwise to sodium hydride (30 mg, 1.3 mmol). TLC (98:2, CH_2Cl_2:acetone, R_f 0.30) showed the benzylation to be completed in 15 min. After destroying the excess of sodium hydride with methanol, the mixture was diluted with EtOAc, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (98:2, CH_2Cl_2:acetone) of the residue gave **28** (322 mg, 89 %) as a syrup, $[\alpha]_D$ +24 ° (c 1). ^{1}H NMR ($CDCl_3$) δ 7.35–7.20 (m, 25H, 5 Ph), 7.00–6.60 (m, 4H, $C_6H_4OCH_3$), 5.668 (d, 1H, H-1'), 5.469 (s, 1H, C*H*Ph), 4.940, 4.856, 4.715, 4.602, 4.573, 4.478, and 4.456 (7d, 1,2,1,1,1,1,1H, 4 OCH_2Ph), 4.501 (d, 1H, H-1), 3.963 (dd, 1H, H-2'), 3.891 (dd, 1H, H-3'), 3.727 (s, 3H, $C_6H_4OCH_3$), 3.443 (dt, 1H, H-5), 3.417 (dd, 1H, H-6a), 3.260 (dd, 1H, H-6b), 2.84–2.66 (m, 2H, SCH_2CH_3), 1.312 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.7, $J_{4,5}$ = 10.5, $J_{5,6a}$ = 6.5, $J_{5,6b}$ = 7.0, $J_{6a,6b}$ = 9.4, $J_{1',2'}$ = 3.4, $J_{2',3'}$ = 10.2, $J_{3',4'}$ = 2.6 Hz; Anal. calcd for $C_{57}H_{62}O_{11}S$: C, 71.68; H, 6.54 %; found: C, 71.48; H, 6.48 %.

*Ethyl 2-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (29)*

A solution of **28** (51 mg, 53 μmol) in CH_2Cl_2 (2 mL) was mixed with 1:2 water:trifluoroacetic acid (6 μL) under vigorous stirring. After 45 h, TLC (6:4, hexane:EtOAc) showed the disappearance of **28** (R_f 0.59) and the appearance of two new spots (R_f 0.19 and R_f 0.09). Solid $NaHCO_3$ was added, and the mixture was diluted with CH_2Cl_2, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4, hexane: EtOAc) of the residue afforded **29** (R_f 0.19, 19 mg, 41 %) as a syrup, $[\alpha]_D$ +9 ° (c 0.25), and part of the lower moving component (R_f 0.09, 4 mg, 9 %). ^{1}H NMR ($CDCl_3$) δ 7.45–6.70 (m, 24H, 4 Ph and $C_6H_4OCH_3$), 5.022 (d, 1H, H-1'), 4.899, 4.805, 4.783, 4.705, 4.665, 4.543, 4.254, and 4.199 (8d, each 1H, 3 OCH_2Ph and $OCH_2C_6H_4OCH_3$), 4.758 (s, 2H, OCH_2Ph), 4.443 (d, 1H, H-1), 4.155 (brdd, 1H, H-4), 4.082 (dd, 1H, H-2'), 4.000 (dd, 1H, H-3'), 3.775 (s, 3H, $C_6H_4OCH_3$), 2.80–2.76 (m, 2H, SCH_2CH_3), 1.299 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.8, $J_{1',2'}$ = 3.3, $J_{2',3'}$ = 9.8, $J_{3',4'}$ = 2.6 Hz; Anal. calcd for $C_{50}H_{58}O_{11}S$: C, 69.26; H, 6.74 %; found: C, 69.38; H, 6.77 %.

*Ethyl 4,6-di-*O*-acetyl-2-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (30)*

A solution of **29** (40 mg, 46 μmol) in 2:1 pyridine:acetic anhydride (3 mL) was stirred for 24 h, when TLC (6:4, hexane:EtOAc) showed the formation of **30**. The mixture was co-concentrated with toluene (3 ×), ethanol (3 ×), and CH_2Cl_2 (3 ×). Column chromatography (7:3, hexane: EtOAc) of the residue gave **30** (32 mg, 73 %) as a white solid, mp 104 °C; $[\alpha]_D$ +29 ° (c 1). ^{1}H NMR ($CDCl_3$) δ 7.45–6.80 (m, 24H, 4 Ph and $C_6H_4OCH_3$), 5.211 (d, 1H, H-1'), 5.142 (t, 1H, H-4), 4.897, 4.845, 4.788, 4.763, 4.674, and 4.518 (6d, each 1H, 3 OCH_2Ph), 4.605 (s, 2H, OCH_2Ph), 4.392 (d, 1H, H-1), 4.284 (s, 2H, $OCH_2C_6H_4OCH_3$), 4.198 (dd, 1H, H-6b), 4.069 (dd, 1H, H-6a), 3.779 (s, 3H, $C_6H_4OCH_3$), 3.737 (dd, 1H, H-4'), 2.85–2.65 (m, 2H, SCH_2CH_3), 2.073 and 1.825 (2s, each 3H, 2 Ac), 1.320 (t, 3H, SCH_2CH_3); $J_{1,2}$ = 9.8, $J_{3,4}$ = 9.5, $J_{4,5}$ = 9.5, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 5.2, $J_{6a,6b}$ = 12.2, $J_{1',2'}$ = 3.3, $J_{3',4'}$ = 1.2, $J_{4',5'}$ = 2.6 Hz; Anal. calcd for $C_{54}H_{62}O_{13}S$: C, 68.19; H, 6.57 %; found: C, 68.40; H, 6.50 %.

*O-(3,4,6-Tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-D-ribitol (33)*

To a solution of **31**αβ (105 mg, 0.057 mmol) and diazabicyclo[2.2.2]octane (38 mg, 0.34 mmol) in 8:3:1 ethanol:toluene:water (9 mL) was added tris(triphenylphosphine)rhodium(I) chloride (20 mg). After refluxing for 90 min, TLC (3:1, hexane:EtOAc) showed a complete conversion of the allyl into the propenyl function (R_f 0.43). The mixture was concentrated, dissolved in acetone (4 mL), and to the solution were added mercuric oxide (30 mg, 0.14 mmol) and mercuric chloride (26 mg, 0.11 mmol). After stirring the mixture for 30 min, TLC (8:2, hexane:EtOAc) showed the depropenylation to be completed. Then, the mixture was diluted with CH_2Cl_2, filtered, washed with aq. 5 % KI and water, dried ($MgSO_4$), filtered, and concentrated. Preparative TLC (8:2 hexane: EtOAc) made it possible to separate the α/β coupling mixture, yielding **33** (36 mg, 37 %) as a syrup. ^{1}H NMR ($CDCl_3$) δ 7.95–6.50 (m, 54H, 10 Ph and $C_6H_4OCH_3$), 5.579 (d, 1H, H-1'''), 5.289 (d, 1H, H-1''), 5.005 (d, 1H, H-1'), 3.668 (s, 3H, $C_6H_4OCH_3$), 1.858 (s, 3H, Ac), 1.220 (d, 3H, 3 H-6'); $J_{1',2'}$ = 1.8, $J_{1'',2''}$ = 3.5, $J_{1''',2'''}$ = 3.4, $J_{5',6'}$ = 6.2 Hz; Anal. calcd for $C_{103}H_{110}O_{22}$: C, 72.77; H, 6.52 %; found: C, 72.61; H, 6.45 %.

*O-(3,4,6-Tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyrano-syl)-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-(3-*N*-[benzyl-oxycarbonyl]-aminopropyl phosphate)-D-ribitol (35)*

A mixture of **33** (29 mg, 16 μmol) and 3-*N*-(benzyloxycarbonyl)-aminopropyl phosphonate (20 mg, 78 μmol; prepared by phosphonylation of 3-*N*-(benzyloxycarbonyl)-aminopropanol with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-2-one[14]), were co-

concentrated with pyridine (2 ×). Then, pyridine (0.5 mL) was added, and 0.5 mL of a stock solution of pivaloyl chloride (0.18 mL) in pyridine (10 mL) was injected into the solution. After 20 min, TLC (9:1, CH_2Cl_2:acetone) showed the formation of **34** (R_f 0.45). The mixture was diluted with CH_2Cl_2, washed with saline and water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (9:1, CH_2Cl_2:acetone) of the residue afforded **34** (16 mg, 53 %).

A 0.2 M solution of I_2 in 95:5 pyridine:water (2 mL) was added to **34** (16 mg, 8.5 μmol). After 20 min, TLC (9:1, CH_2Cl_2:acetone) showed the disappearance of **34** and a new spot on the baseline. The mixture was diluted with CH_2Cl_2, washed with aq. 5 % sodium thiosulfate, aq. 10 % $NaHCO_3$, and water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2:acetone, then 9:1 CH_2Cl_2:methanol) of the residue gave **35** (11 mg, 65 %). ^1H NMR ($CDCl_3$) δ 7.90–6.45 (m, 54H, 10 Ph and $C_6H_4OCH_3$), 5.575 (d, 1H, H-1'''), 5.433 (d, 1H, H-2'), 5.253 (d, 1H, H-1''), 5.050 (d, 1H, H-1'), 3.666 (s, 3H, $C_6H_4OCH_3$), 3.424 (m, 2H, $O(CH_2)_2CH_2NH$), 3.12 (m, 2H, $OCH_2(CH_2)_2NH$), 1.756 (s, 3H, Ac), 1.127 (d, 3H, 3 H-6'); $J_{1',2'}$ <1, $J_{1'',2''}$ = 3.3, $J_{1''',2'''}$ = 3.3, $J_{5',6'}$ = 5.7 Hz; Anal. calcd for $C_{114}H_{124}NO_{27}P$: C, 69.46; H, 6.34 %; found: C, 69.31; H, 6.44 %.

O-α-D-Galactopyranosyl-(1→3)-O-α-D-glucopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→4)-(5-O-[3-aminopropyl phosphate])-D-ribitol (1)

A solution of **35** (7.3 mg, 2.7 μmol) in 2:1 methanol:aq. 25 % ammonia (1.5 mL) was heated for 24 h at 50 °C, and concentrated. This deprotection procedure was repeated twice, and crude **36** was purified by column chromatography (8:2 CH_2Cl_2:acetone, then 8:2 CH_2Cl_2:methanol, both containing 1 % triethylamine). To a solution of **36** in 1:2:2 EtOAc:2-propanol:methanol (2.5 mL) was added Pd/C (10 mg), and the mixture was hydrogenolysed at 4 kg/cm^2 for 24 h. After filtration, the mixture was concentrated, and purified by Bio-Gel P-2 gel-permeation chromatography, affording **1** (2.1 mg, 75 %) as a white powder. ^1H NMR (D_2O) δ 5.396 (d, 1H, H-1'''), 5.134 (d, 1H, H-1'), 5.125 (d, 1H, H-1''), 3.40 (m, 2H, $O(CH_2)_2CH_2ND_2$), 2.82 (brd, 2H, $OCH_2(CH_2)_2ND_2$), 2.027 (m, 2H, $OCH_2CH_2CH_2ND_2$), 1.273 (d, 3H, 3 H-6'); $J_{1',2'}$ = 1.8, $J_{1'',2''}$ = 3.7, $J_{1''',2'''}$ = 3.7, $J_{5',6'}$ = 4.4 Hz; Anal. MS (FAB) calcd for $C_{26}H_{50}NO_{22}P$: 760.6 ($M + H^+$); found m/z: 760.6 ($M + H^+$).

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