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Synthesis of four spacer-containing 'tetrasaccharides' that represent four possible repeating units of the capsular polysaccharide of *Streptococcus pneumoniae* type 6B

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Abstract

In the framework of studies towards oligosaccharide-conjugate based vaccines against *Streptococcus pneumoniae*, the synthesis is reported of four spacer-containing 'tetrasaccharides' that each can be conceived as representing a repeating unit of the capsular polysaccharide of *S. pneumoniae* serotype 6B, namely, 3-aminopropyl D-ribityl-(5 → hydrogen phosphate → 2)-α-D-galactopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside, 3-aminopropyl α-L-rhamnopyranosyl-(1 → 4)-D-ribityl-(5 → hydrogen phosphate → 2)-α-D-galactopyranosyl-(1 → 3)-α-D-glucopyranoside, 3-aminopropyl α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-D-ribityl-(5 → hydrogen phosphate → 2)-α-D-galactopyranoside, and α-D-galactopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-5-*O*-(3-aminopropyl hydrogen phosphate)-D-ribitol. Phosphorylations were carried out using the H-phosphonate method.

Keywords: Phosphorylated oligosaccharide; Capsular polysaccharide; *Streptococcus pneumoniae* 6B

1. Introduction

Infection with the gram-positive bacterium *Streptococcus pneumoniae* is still one of the leading causes of death [1]. Early experiments [2–4] with pneumococcal capsular polysaccharide-constituted vaccines were overshadowed by the introduction of antibiotics such as penicillin in the battle against bacterial infections. However, when several bacterial strains became resistant against antibiotic treatment [5], the need for an adequate preventive vaccination program again became clear. Nowadays, 90 serotypes of *S. pneumoniae* are known [6], and can be recognised by the composition of their respective capsular polysaccharides. A 23-valent pneumococcal vaccine has been available since 1983 [7], consisting of the puri-

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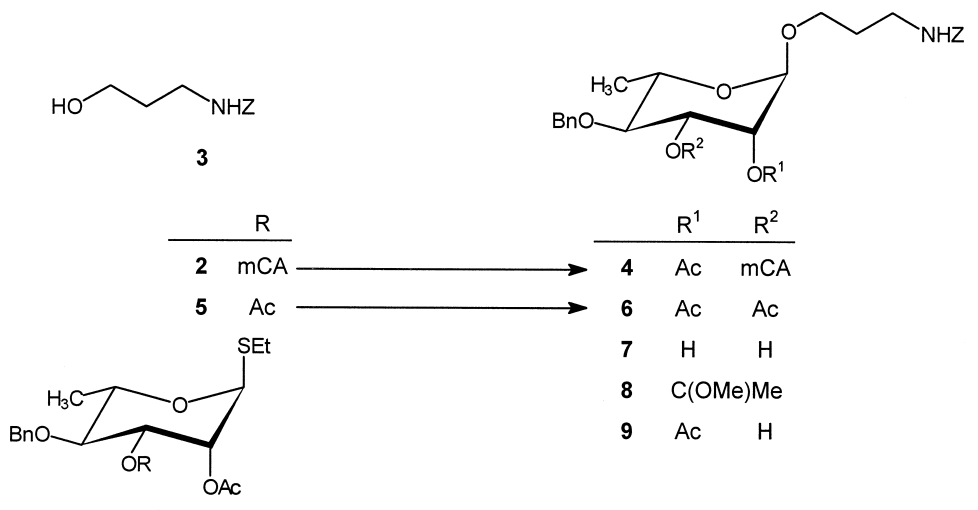
fied capsular polysaccharides of 23 serotypes of *S. pneumoniae* and accounting for 90% of bacteremic infections in the United States. However, on inoculation, this vaccine does not evoke an immunological memory because the immune system reacts towards the polysaccharide antigens via a Thymus-Independent (TI) mechanism, thereby, not creating memory cells, and furthermore, the induction of tolerance is a problem [8]. Polysaccharide–protein conjugates have shown that they can convert the TI response into a Thymus-Dependent (TD) response, with the beneficial development of immunological memory [9–16]. However, antibodies produced against this kind of conjugate are often inefficient in binding components of the immune system after opsonisation [17]. Oligosaccharide-conjugate based vaccines are under investigation [18–21] for their abilities to overcome these problems.

[→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→phosphate→] **1**
D-Rib-ol-(5→phosphate→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→$O(CH_2)_3NH_2$ **34**
α-L-Rha*p*-(1→4)-D-Rib-ol-(5→phosphate→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→$O(CH_2)_3NH_2$ **40**
α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→phosphate→2)-α-D-Gal*p*-(1→$O(CH_2)_3NH_2$ **44**
α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→phosphate→$(CH_2)_3NH_2$ **49**

In earlier reports [22,23], we have described the synthesis of several spacer-containing oligosaccharide fragments of the capsular polysaccharide of *S. pneumoniae* type 6B (**1**) for the preparation of neoglycoconjugates. The polysaccharide consists of repeating units of four monosaccharides, linked by a phosphate function. From this structure, four in chemical as well as in immunological sense different repeating units can be envisaged as synthetic targets. A requirement for the preparation of neoglycoconjugates with these repeating units is the presence of a spacer group in the molecule. Here, the synthesis of these four possible spacer-containing repeating units, namely, **34**, **40**, **44**, and **49**, is reported.

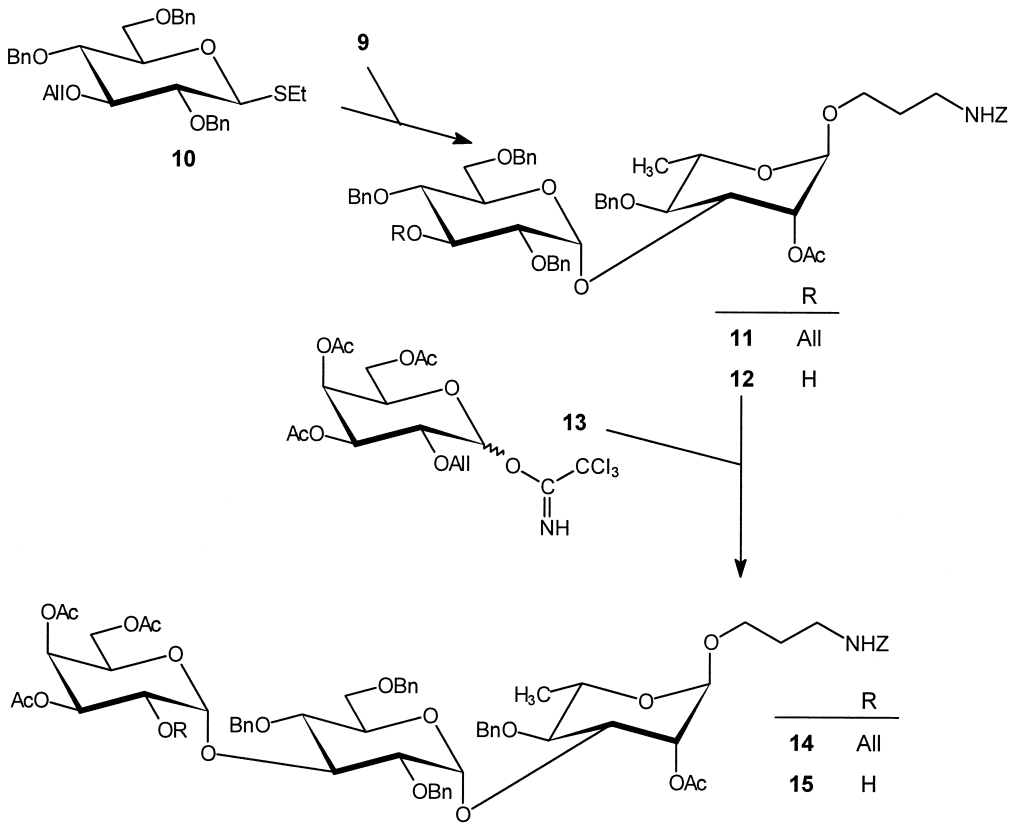
2. Results and discussion

In earlier studies, we have presented the synthesis of compound **49** in a modest yield via a blockwise coupling [22]. In this report, we describe an improved strategy for the preparation of this compound, making use of the carbohydrate moiety **29** which is synthesised in a stepwise manner, and then introducing the spacer-phosphate function by selective phosphorylation. Compound **29** is well suited for future preparation of structures larger than one repeating unit. The other spacer-containing repeating units **34**, **40**, and **44** are each prepared from two carbohydrate building blocks, which are linked through a phosphate func-



mCA = monochloroacetyl

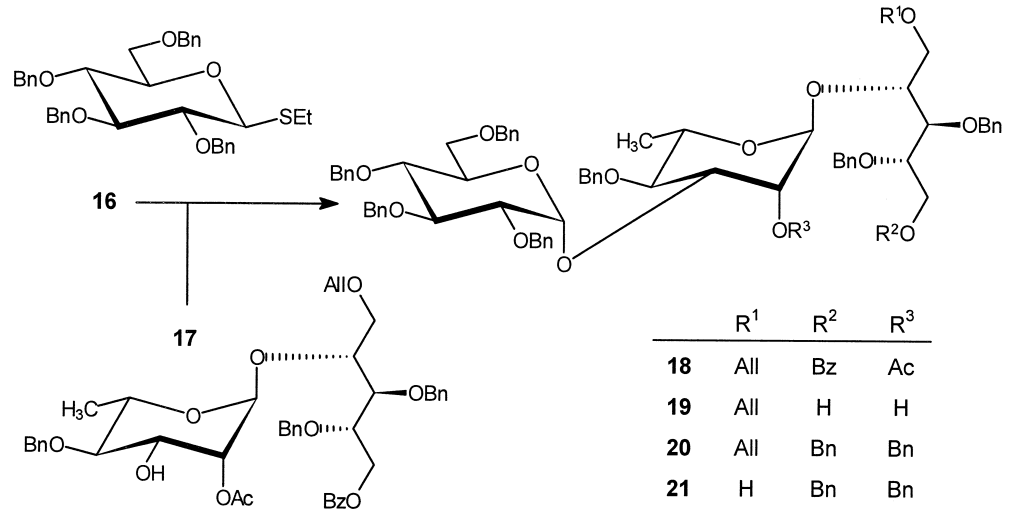
Scheme 1.



Scheme 2.

tion. In the following, the synthesis of these building blocks is described first.

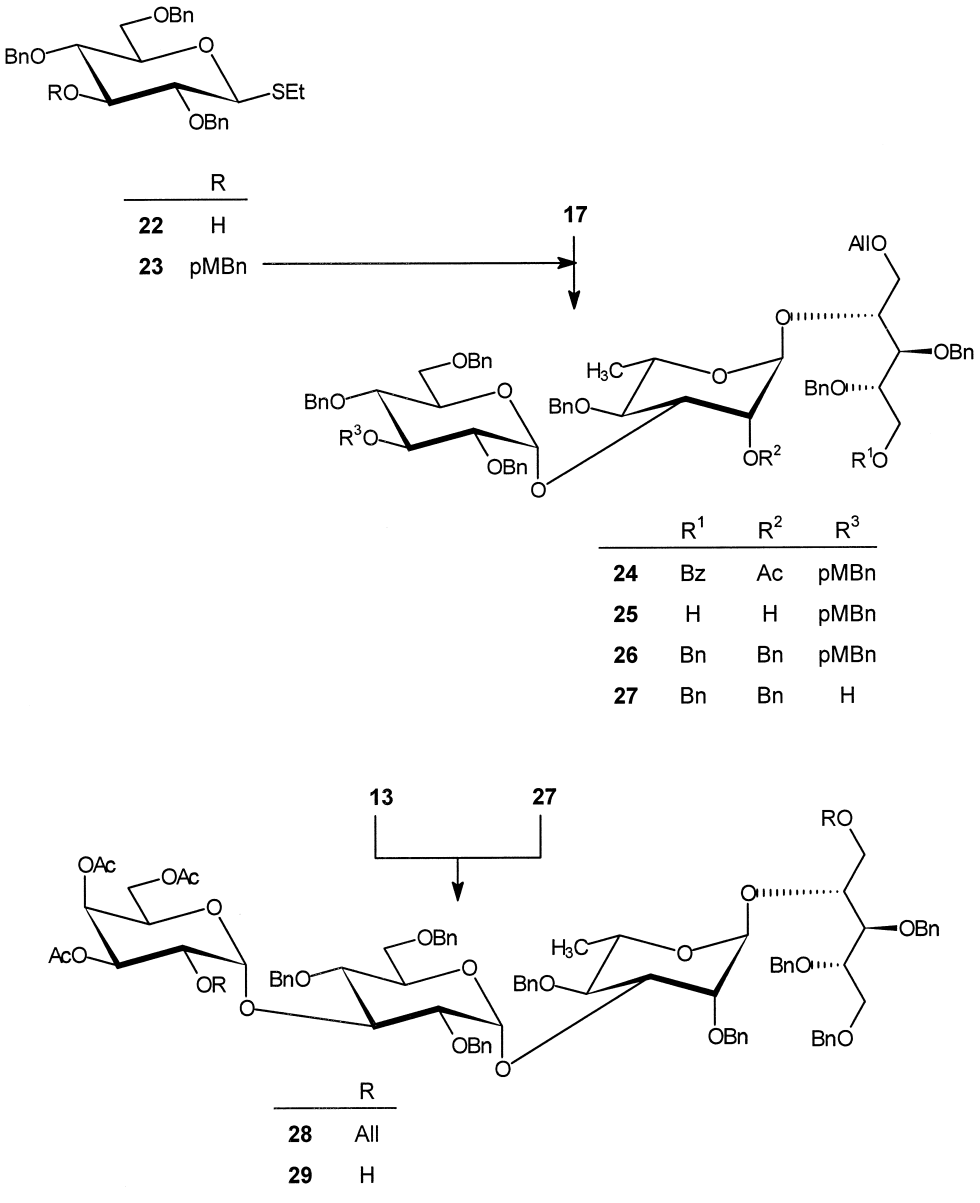
Preparation of building blocks.—In order to synthesise 3-*N*-benzyloxycarbonylaminopropyl (3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 3)-2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside (**15**), rhamnosyl acceptor **9** was prepared as follows (Scheme 1). Ethyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-monochloroacetyl-1-thio-α-L-rhamnopyranoside (**2**) [22] was linked to 3-*N*-



Scheme 3.

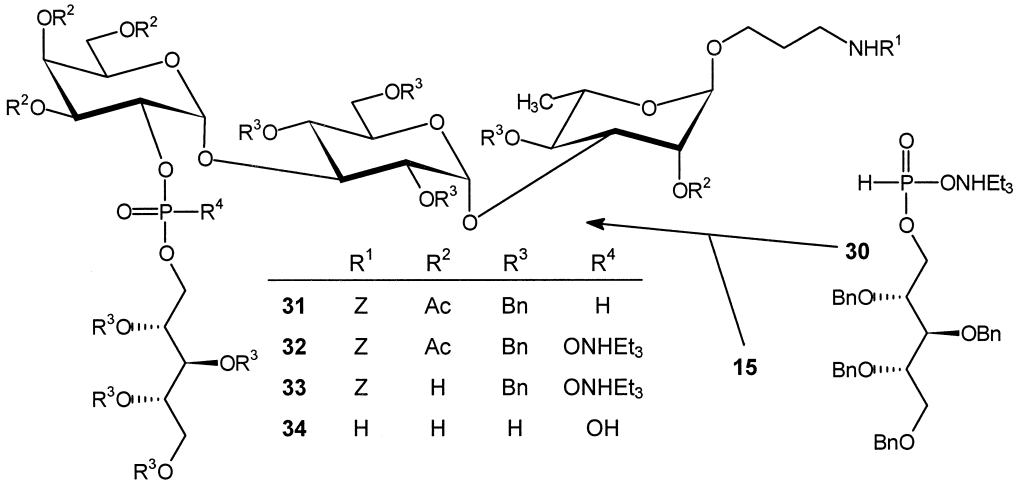
benzyloxycarbonylaminopropanol (**3**) [24] in dichloromethane using *N*-iodosuccinimide (NIS)–triflic acid (TfOH) as a promoter system, to give **4** (78%), which was demonochloroacetylated with hydrazine dithiocarbonate (HDTC) [25] in acetic acid–2,6-lutidine to give **9** in a yield of 69%. Alternatively, spacer **3** was glycosylated with ethyl 2,3-di-*O*-acetyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**5**) [26] in dichloromethane using methyl triflate as a promoter to give **6** (81%). This compound was then deacetylated ($\rightarrow$ **7**), and converted into orthoester **8** with trimethyl orthoacetate, followed by an in situ ring opening by addition of water to give **9** in a yield of 71% over 3 steps.

Glycosylation of **9** with ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**10**) [27] in dichloromethane–diethyl ether using methyl triflate as a promoter resulted in a non-stereoselective coupling reaction (α:β 1.9:1), from which the desired compound **11** could be isolated in a yield of 34%



pMBn = *p*-methoxybenzyl

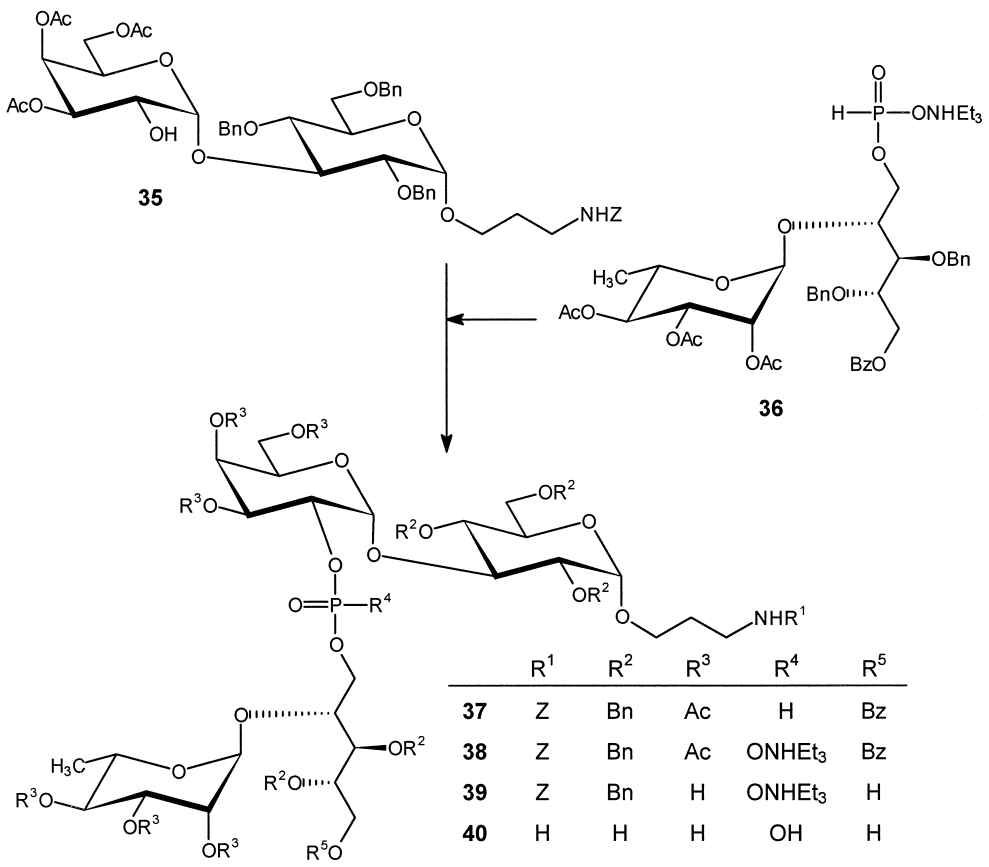
Scheme 4.



Scheme 5.

(Scheme 2 and 3). Deallylation of **11** (Wilkinson catalyst, then mercuric oxide–mercuric chloride) yielded acceptor **12** (54%).

Because of the successful glycosylation of a similar acceptor with 3,4,6-tri-*O*-acetyl-2-*O*-allyl-α/β-D-galactopyranosyl trichloroacetimidate (**13**) [23], this donor was also tested in the preparation of trisaccharide derivative **14**. Thus, **13** was coupled with **12** in diethyl ether using trimethylsilyl triflate as a promoter, giving the desired compound with a complete α-stereoselectivity in a yield of 52%. Finally, removal of the allyl group at C-2″ (Wilkinson catalyst, then mercuric oxide–mercuric chloride) gave the first building block **15** (37%).

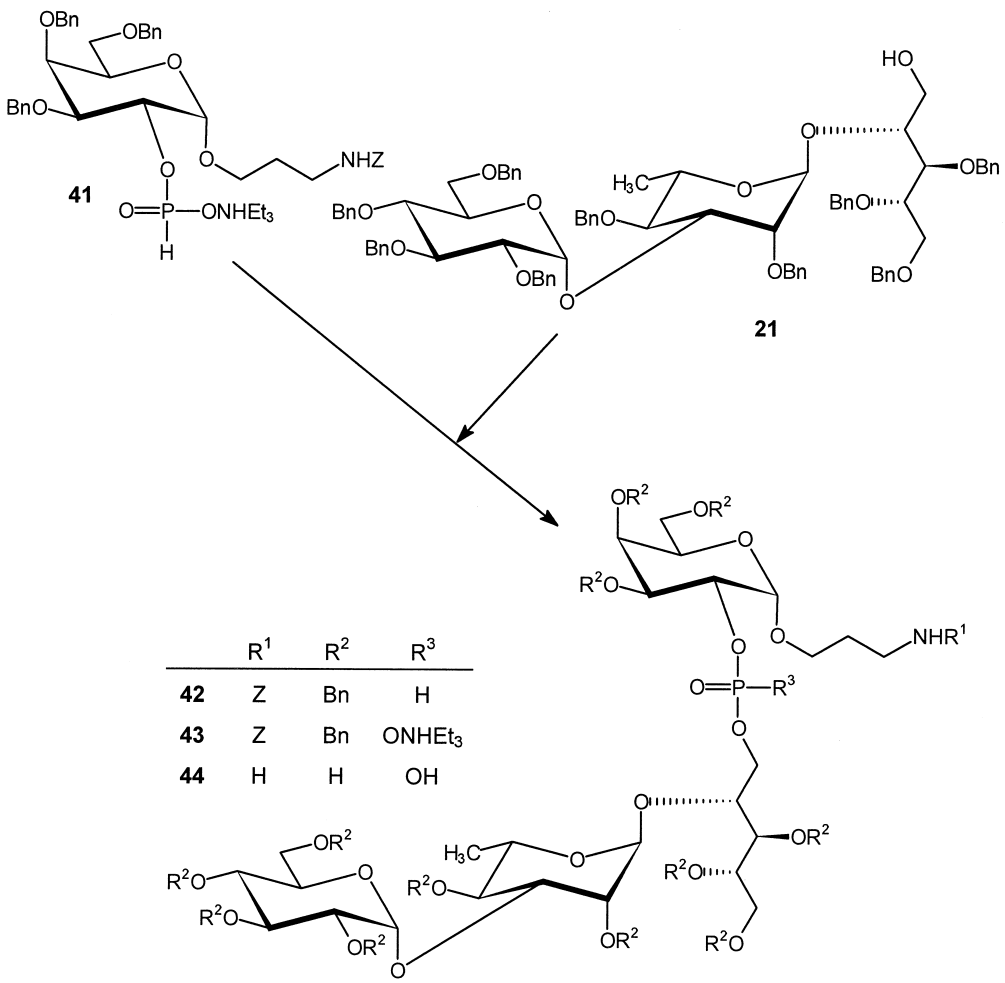


Scheme 6.

In the synthesis of (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 4)-1,2,3-tri-*O*-benzyl-D-ribitol (**21**), the previously prepared (2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 4)-5-*O*-allyl-1-*O*-benzoyl-2,3-di-*O*-benzyl-D-ribitol (**17**) [22] was glycosylated with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**16**) [28,29] in 1,2-dichloroethane–diethyl ether using iodonium dicollidineperchlorate as a promoter to yield a mixture of **18** and the β-coupled product **18**β (62%; α:β 6:1, as determined by ^{1}H and ^{13}C NMR analysis). Complete separation of the mixture could only be achieved after deacylation (→ **19**/**19**β, 82%) and subsequent benzylation of the resulting deprotected hydroxyl functions (→ **20**, 83%). Compound **20** was deallylated by treatment with potassium *tert*-butoxide in *N*, *N*-dimethylformamide at 80 °C followed by cleavage of the resulting 1-propenyl function in acetone–0.1 M hydrochloric acid, to afford building unit **21** (49%).

In the synthesis of the last building block (3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 4)-1,2,3-tri-*O*-benzyl-D-ribitol (**29**), first **23** was prepared from ethyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**22**) and *p*-methoxybenzyl chloride (81%) (Scheme 4). Then, **23** was condensed with **17** [22] in 1,2-dichloroethane–diethyl ether using iodonium dicollidineperchlorate [30], to yield a mixture of **24** and its β-analogue **24**β (62%; α:β 2:1, as established by ^{1}H and ^{13}C NMR analysis). The mixture could only be separated after deacylation (→ **25**/**25**β) and subsequent benzylation with benzyl bromide (→ **26**, 43% over 2 steps). Removal of the *p*-methoxybenzyl function using ammonium cerium(IV) nitrate gave trisaccharide **27** in a yield of 72%.

Coupling of compounds **13** and **27** in diethyl ether using trimethylsilyl triflate as a promoter yielded 'tetrasaccharide' derivative **28** with a complete α-



	R^1	R^2	R^3
42	Z	Bn	H
43	Z	Bn	$ONHEt_3$
44	H	H	OH

Scheme 7.

stereoselectivity in a yield of 81%. Removal of the allyl functions on both C-5 and C-2‴ (Wilkinson catalyst, then mercuric oxide–mercuric chloride) gave building block **29** (61%).

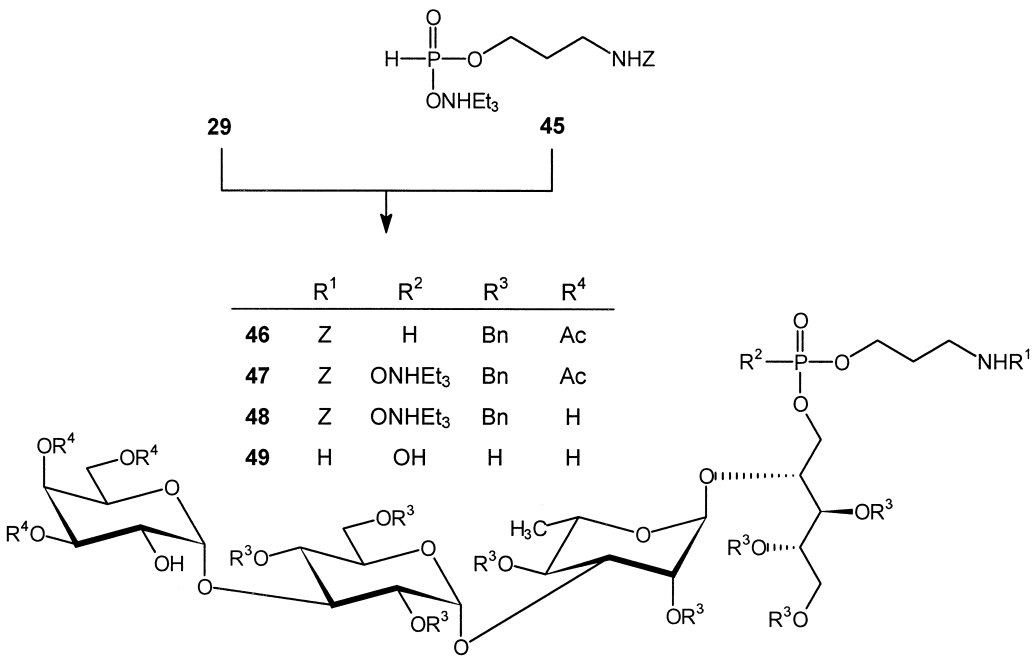
Preparation and deprotection of phosphorylated compounds.—The preparation of target compound **34** (Scheme 5) started with the coupling of 1,2,3,4-tetra-*O*-benzyl-5-*O*-(triethylammonium H-phosphonate)-D-ribitol (**30**) [23] to HO-2″ of trisaccharide derivative **15** in pyridine–acetonitrile using pivaloyl chloride [31] (→ **31**), and subsequent mild in situ oxidation of the resulting phosphonate diester using iodine in pyridine–water (→ **32**, 78% over 2 steps). Deacetylation (methanol–ammonia, → **33**) and subsequent debenzylation/debenzyloxycarbonylation (Pd–C, H_2) gave **34** after purification on Bio-Gel P-2 in a yield of 76% over 2 steps.

For the synthesis of target compound **40** (Scheme 6), as a first step 3-*N*-benzyloxycarbonylaminopropyl (3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**35**) [23] was condensed with (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1 → 4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-(triethylammonium H-phosphonate)-D-ribitol (**36**) [23] in pyridine–acetonitrile using pivaloyl chloride (→ **37**). Subsequent mild in situ oxidation using iodine in pyridine–water yielded **38** (50% over 2 steps). Treatment of **38** with methanol–ammonia (→ **39**) and subsequent debenzylation/debenzyloxycarbonylation (Pd–C, H_2) gave, after purification on Bio-Gel P-2, compound **40** (73% over 2 steps).

In the preparation of the target compound **44** (Scheme 7), trisaccharide derivative **21** was phosphonylated with 3-*N*-benzyloxycarbonylaminopropyl 3,4,6-tri-*O*-benzyl-2-*O*-(triethylammonium H-phosphonate)-α-D-galactopyranoside (**41**) [23] in pyridine–acetonitrile using pivaloyl chloride to give **42**, which was oxidised in situ with iodine in pyridine–water, yielding **43** in 49% over 2 steps. Debenzylation/debenzyloxycarbonylation of **43** (Pd–C, H_2) gave after desalting on Bio-Gel P-2, compound **44** in a yield of 72% over 2 steps.

As final target structure, compound **49** (Scheme 8) was prepared along an analogous route as described for **44**. To this end, compound **29** was phosphonylated selectively at HO-5 with 3-*N*-benzyloxycarbonylaminopropyl triethylammonium H-phosphonate **45** [22] in pyridine–acetonitrile using pivaloyl chloride to give **46**, which was converted into **47** by mild in situ oxidation using iodine in pyridine–water (53% over 2 steps). Then, deacetylation (methanol–ammonia, → **48**), followed by debenzylation/debenzyloxycarbonylation (Pd–C, H_2), gave **49** after purification on Bio-Gel P-2, in a yield of 76% over 2 steps.

Conjugation of the free products to carrier proteins



Scheme 8.

and the results of immunological tests with the neoglycoconjugates will be published elsewhere.

3. Experimental

General methods.—For a survey of general methods used in this study, see Ref. [23].

3-N-*Benzyloxycarbonylaminopropyl 2*-O-*acetyl-4*-O-*benzyl-3*-O-*monochloroacetyl-α*-L-*rhamnopyranoside* (**4**).—A mixture of ethyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-monochloroacetyl-1-thio-α-L-rhamnopyranoside (**2**) [22] (2.0 g, 4.8 mmol), 3-*N*-benzyloxycarbonylaminopropanol (**3**) [24] (1.16 g, 5.54 mmol) and 4 Å molecular sieves in CH_2Cl_2 (12.5 mL) was stirred for 30 min at 0 °C. Then, a soln of NIS (1.08 g, 4.80 mmol) and TfOH (36 μL, 0.41 mmol) in 1:1 CH_2Cl_2–Et_2O (37.6 mL) was added. After 1 min, TLC (7:3 hexane–EtOAc) showed the appearance of a single product (**4**, R_f 0.25), and the mixture was neutralised with Et_3N, filtered through Celite, diluted with CH_2Cl_2, washed with aq 10% $Na_2S_2O_3$ (2×) and water (3×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4 hexane–EtOAc) of the residue afforded **4**, isolated as a syrup (2.11 g, 78%); $[\alpha]_D$ −23° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.35–7.25 (m, 10 H, 2 Ph), 5.312 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.5 Hz, H-3), 5.258 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 5.09 (bs, 2 H, COOCH_2Ph), 4.700 and 4.648 (2 d, each 1 H, OCH_2Ph), 4.683 (d, 1 H, H-1), 3.909 and 3.845 (2 d, each 1 H, 2 COCH_2Cl), 3.524 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.50–3.45 (m, 2 H, OCH_2(CH$_2$)$_2$N), 3.32–3.26 (m, 2 H, O(CH$_2$)$_2$CH_2N), 2.136 (s, 3 H, Ac), 1.84–1.77 (m, 2 H, OCH$_2$CH_2CH$_2$N), 1.347 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6). Anal. Calcd for $C_{28}H_{34}ClNO_9$: C, 59.63; H, 6.08. Found: C, 59.94; H, 5.95.

3-N-*Benzyloxycarbonylaminopropyl 2,3*-*di*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**6**).—A mixture of ethyl 2,3-di-*O*-acetyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**5**) [26] (4.29 g, 11.2 mmol), 3-*N*-benzyloxycarbonylaminopropanol (**3**; 3.29 g, 15.7 mmol) and 4 Å molecular sieves in CH_2Cl_2 (150 mL) was stirred for 30 min at room temperature. MeOTf (6.1 mL, 54 mmol) was added, and after 18 h TLC (6:4 hexane–EtOAc) showed the disappearance of **5**, and the appearance of a new product with R_f 0.27 (**6**). The mixture was neutralised with Et_3N, filtered through Celite, 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 **6**, isolated as a syrup (4.79 g, 81%); $[\alpha]_D$ −11° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.46–7.16 (m, 10 H, 2 Ph), 5.281 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3), 5.230 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 5.08 (bs, 2 H, COOCH_2Ph), 4.687 and 4.626 (2 d, each 1 H, OCH_2Ph), 4.672 (d, 1 H, H-1), 3.760 (m, 1 H, H-5), 3.490 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.47–3.42 (m, 2 H, OCH_2(CH$_2$)$_2$N), 3.34–3.25 (m, O(CH$_2$)$_2$CH_2N), 2.133 and 1.961 (2 s, each 3 H, 2 Ac), 1.84–1.76 (m, 2 H, OCH$_2$CH_2CH$_2$N), 1.332 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6). Anal. Calcd for $C_{28}H_{35}NO_9$: C, 63.50; H, 6.66. Found: C, 63.63; H, 6.69.

3-N-*Benzyloxycarbonylaminopropyl 2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**9**).—(a) To a soln of **4** (263 mg, 0.466 mmol) in 1:3 HOAC–2,6-lutidine (6.4 mL) was added a freshly prepared hydrazine dithiocarbonate (HDTC) soln [25] (4 mL). TLC (9:1 CH_2Cl_2–EtOAc, R_f 0.39) indicated the demonochloroacetylation to be completed 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 (92:7:1 CH_2Cl_2–EtOAc–HOAC) of the residue gave **9**, isolated in 69% as a foam (158 mg). (b) To a soln of **6** (1.70 g, 3.21 mmol) in MeOH (20 mL) was added NaOMe (pH 9). After 2 h, TLC (1:1 hexane–EtOAc) indicated a complete conversion into **7**. The mixture was neutralised with Dowex-50 (H^+) resin, filtered, concentrated, and co-concentrated with CH_2Cl_2. Crude **7** was dissolved in 6:1 toluene–CH_2Cl_2 (21 mL), and trimethyl orthoacetate (1.2 mL, 9.4 mmol) and a catalytic amount of *p*-toluenesulfonic acid (60 mg) were added. After 30 min, TLC (1:1 hexane–EtOAc) indicated a complete conversion of **7** into **8**. Then, water (1 mL) was added, and the soln was stirred for 20 min, when TLC (1:1 hexane–EtOAc) showed the disappearance of **7** and the appearance of a single new spot (**9**). The mixture was diluted with EtOAc, washed with water (2×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (86:13:1 CH_2Cl_2–acetone–HOAC) of the residue afforded **9**, isolated as a foam (1.11 g, 71% from **6**); $[\alpha]_D$ −30° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.34–7.32 (m, 10 H, 2 Ph), 5.080 (s, 2 H, COOCH_2Ph), 5.072 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.7 Hz, H-2), 4.825 and 4.702 (2 d, each 1 H, OCH_2Ph), 4.699 (d, 1 H, H-1), 4.073 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.76–3.64 (m, 2 H, OCH_2(CH$_2$)$_2$N), 3.441 (m, 1 H, H-5), 3.341 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.31–3.24 (m, 2 H, O(CH$_2$)$_2$CH_2N), 2.144 (s, 3 H, Ac), 1.83–1.70 (m, 2 H, OCH$_2$CH_2CH$_2$N), 1.336 (d, 3 H, $J_{5,6}$ 6.3 Hz, 3 H-6). Anal. Calcd for $C_{26}H_{33}NO_8$: C, 64.05; H, 6.82. Found: C, 63.89; H, 6.90.

3-N-*Benzyloxycarbonylaminopropyl (3*-O-*allyl-2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**11**).—A mixture of ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**10**) [27] (184 mg, 0.372 mmol), **9** (140 mg, 0.287 mmol) and 4 Å molecular sieves in 1:4 CH_2Cl_2–Et_2O (10 mL) was cooled to 0 °C, and stirred for 30 min. Then, MeOTf (162 μL, 1.43 mmol) was added. After 4 h, TLC (95:5 CH_2Cl_2–acetone) indicated the disappearance of **9** and the appearance of two spots (**11**/**11**β, R_f 0.39 and 0.46, α:β 1.9:1 as was estimated from a sample taken for ^{1}H NMR), and the mixture was neutralised with Et_3N, diluted with CH_2Cl_2, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (65:35 hexane–EtOAc) of the residue afforded **11**, isolated as a glass (93 mg, 34%); $[\alpha]_D$ +46° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.35–7.24 (m, 25 H, 5 Ph), 5.974 (m, 1 H, $OCH_2CH{=}CH_2$), 5.105 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 5.07 (bd, 2 H, $COOCH_2Ph$), 4.885, 4.826, 4.556, 4.534, 4.435, and 4.298 (6 d, each 1 H, 3 OCH_2Ph), 4.67 (bd, 2 H, OCH_2Ph), 4.644 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.137 (dd, 1 H, $J_{2',3'}$ 9.5 Hz, H-2′), 3.920 (t, 1 H, $J_{3',4'}$ 9.4 Hz, H-3′), 3.24–3.17 (m, 2 H, $O(CH_2)_2CH_2N$), 1.908 (s, 3 H, Ac), 1.79–1.66 (m, 2 H, $OCH_2CH_2CH_2N$), 1.351 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6); ^{13}C, δ 170.3 ($COCH_3$), 156.2 ($NCOOCH_2Ph$), 135.2 ($OCH_2CH{=}CH_2$), 116.3 ($OCH_2CH{=}CH_2$), 97.4 and 92.5 (C-1,1′), 81.7, 79.4, 78.9, 77.6, 71.9, 69.8, 67.9, and 67.5 (C-2,3,4,5,2′,3′,4′,5′), 76.0, 74.8, 74.1, 73.0, 72.8, 68.1, 66.4, and 65.2 (4 OCH_2Ph, $NCOOCH_2Ph$, $OCH_2(CH_2)_2N$, $OCH_2CH{=}CH_2$, and C-6′), 38.1 [$O(CH_2)_2CH_2N$], 29.4 ($OCH_2CH_2CH_2N$), 20.7 ($COCH_3$), 17.8 (C-6). Anal. Calcd for $C_{56}H_{65}NO_{13}\cdot H_2O$: C, 68.77; H, 6.90. Found: C, 69.10; H, 7.07.

3-N-*Benzyloxycarbonylaminopropyl (2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**12**).—To a soln of **11** (41 mg, 43 μmol) and 1,4-diazabicyclo[2.2.2]octane (DABCO) (15 mg, 0.13 mmol) in 8:3:1 EtOH–toluene–water (5 mL) was added tris(triphenylphosphine)rhodium(I) chloride (10 mg). After boiling under reflux for 3 h, TLC (95:5 CH_2Cl_2–acetone) showed the reaction to be completed. The mixture was cooled, diluted with CH_2Cl_2, washed with 0.1 M HCl and water (2 ×), and concentrated. To a soln of the residue in 9:1 acetone–water (5 mL) were added $HgCl_2$ (60 mg, 0.22 mmol) and a catalytic amount of HgO (1.1 mg). After stirring the mixture for 1 h, TLC (6:4 hexane–EtOAc) showed the conversion into **12** to be completed, and the mixture was diluted with CH_2Cl_2, filtered, washed with water, aq 5% Kl, water, aq 10% $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4 hexane–EtOAc) of the residue afforded **12**, isolated in 54% as a glass (21 mg); $[\alpha]_D$ +49° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.37–7.24 (m, 25 H, 5 Ph), 5.319 (dd, 1 H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.3 Hz, H-2), 5.179 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.08 (bd, 2 H, $COOCH_2Ph$), 4.848, 4.812, 4.720, 4.543, 4.498, 4.460, 4.303, and 4.196 (8 d, each 1 H, 4 OCH_2Ph), 4.673 (d, 1 H, H-1), 3.504 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.421 (dd, 1 H, H-3), 3.26–3.19 (m, 2 H, $O(CH_2)_2CH_2N$), 2.38 (bs, 1 H, HO-3′), 1.964 (s, 3 H, Ac), 1.81–1.68 (m, 2 H, $OCH_2CH_2CH_2N$), 1.352 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6).

3-N-*Benzyloxycarbonylaminopropyl (3,4,6-tri*-O-*acetyl-2*-O-*allyl-α*-D-*galactopyranosyl)-(1 → 3)-(2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**14**).—A mixture of 3,4,6-tri-*O*-acetyl-2-*O*-allyl-α/β-D-galactopyranosyl trichloroacetimidate (**13**) [23] (98 mg, 0.20 mmol), **12** (107 mg, 0.116 mmol) and 4 Å molecular sieves in Et_2O (11 mL) was cooled to 0 °C, and stirred for 1 h. Then, TMSOTf (45 μL, 0.23 mmol) was added. After 1 h, TLC (92.5:7.5 CH_2Cl_2–acetone) indicated the disappearance of **12** and the appearance of a single product (**14**, R_f 0.51), and the mixture was neutralised with Et_3N, diluted with CH_2Cl_2, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (93:7 CH_2Cl_2–acetone) of the residue afforded **14**, isolated as a syrup (76 mg, 52%); $[\alpha]_D$ −36° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.40–7.04 (m, 25 H, 5 Ph), 3.26–3.19 (m, 2 H, $O(CH_2)_2CH_2N$), 2.059, 2.015, and 1.934 (3 s, 3,6,3 H, 4 Ac), 1.373 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6). Anal. Calcd. for $C_{68}H_{81}NO_{21}$: C, 65.42; H, 6.54. Found: C, 65.30; H, 6.46.

3-N-*Benzyloxycarbonylaminopropyl (3,4,6-tri*-O-*acetyl-α*-D-*galactopyranosyl)-(1 → 3)-(2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**15**).—To a soln of **14** (70 mg, 56 μmol) and DABCO (15 mg, 0.13 mmol) in 8:3:1 EtOH–toluene–water (6 mL) was added tris(triphenylphosphine)rhodium(I) chloride (10 mg). After boiling under reflux for 90 min, TLC (9:1 CH_2Cl_2–EtOAc) indicated the reaction to be completed. The mixture was cooled, diluted with CH_2Cl_2, washed with 0.1 M HCl and water (2 ×), and concentrated. To a soln of the residue in 9:1 acetone–water (5 mL) were added $HgCl_2$ (200 mg, 0.737 mmol) and a catalytic amount of HgO (1.0 mg). After

stirring for 3 h, the mixture was diluted with CH_2Cl_2, filtered, washed with water, aq 5% Kl, water, aq 10% $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–EtOAc) of the residue afforded **15**, isolated in 37% as a glass (25 mg); $[\alpha]_D$ −12° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.70–7.03 (m, 25 H, 5 Ph), 5.571 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1″), 5.306 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.1 Hz, H-2′), 5.270 (dd, 1 H, $J_{3'',4''}$ 3.1, $J_{4'',5''} < 1$ Hz, H-4″), 5.215 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.134 (dd, 1 H, $J_{2'',3''}$ 10.5 Hz, H-3″), 5.07 (bs, 2 H, COOCH_2Ph), 4.693 (d, 1 H, H-1), 4.160 (dd, 1 H, $J_{2',3'}$ 9.6 Hz, H-2′), 3.27–3.21 (m, 2 H, O(CH_2)$_2$CH_2N), 2.619 (d, 1 H, HO-2″), 2.070, 2.027, 1.955, and 1.931 (4 s, each 3 H, 4 Ac), 1.372 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6).

(2,3,4,6-Tetra-O-*benzyl*-α/β-D-*glucopyranosyl)-(1 → 3)-(2*-O-*acetyl-4*-O-*benzyl*-α-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-1*-O-*benzoyl-2,3-di*-O-*benzyl*-D-*ribitol* (**18**/**18**β), (2,3,4,6-tetra-O-benzyl-α/β-D-*glucopyranosyl)-(1 → 3)-(4*-O-*benzyl*-α-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-2,3-di*-O-*benzyl*-D-*ribitol* (**19**/**19**β), (2,3,4,6-tetra-O-benzyl-α-D-*glucopyranosyl)-(1 → 3)-(2,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-1,2,3-tri*-O-*benzyl*-D-*ribitol* (**20**), and (2,3,4,6-tetra-O-benzyl-α-D-*glucopyranosyl)-(1 → 3)-(2,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl)-(1 → 4)-1,2,3-tri*-O-*benzyl*-D-*ribitol* (**21**).—A mixture of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**16**) [28,29] (266 mg, 0.455 mmol), (2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 4)-5-*O*-allyl-1-*O*-benzoyl-2,3-di-*O*-benzyl-D-ribitol (**17**) [22] (232 mg, 0.307 mmol) and 4 Å molecular sieves in 1:5 1,2-dichloroethane–Et_2O (10.2 mL) was stirred for 30 min, then IDCP (426 mg, 0.909 mmol) was added. After 1 h, TLC (7:3 hexane–EtOAc) indicated the disappearance of **17** and the appearance of a new spot (R_f 0.43). The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with aq 10% $Na_2S_2O_3$ (2 ×) and water (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2 hexane–EtOAc) of the residue afforded **18**/**18**β, isolated as a glass (243 mg, 62%, α:β 6:1); NMR ($CDCl_3$): ^{1}H, δ 7.95–7.05 (m, 40 H, 8 Ph), 5.865 (m, 1 H, $OCH_2CH{=}CH_2$), 5.486 (dd, 1 H, $J_{1',2'}$ 2.0, $J_{2',3'}$ 2.6 Hz, H-2′, **18**), 5.342 (dd, 1 H, $J_{1',2'}$ 1.8, $J_{2',3'}$ 3.4 Hz, H-2′, **18**β), 5.243 (d, 1 H, $J_{1'',2''}$ 3.4 Hz, H-1″, **18**), 5.240 (d, 1 H, H-1′, **18**), 5.20–5.11 (m, 2 H, $OCH_2CH{=}CH_2$), 5.135 (d, 1 H, H-1′, **18**β), 2.144 (s, 3 H, Ac, **18**β), 1.935 (s, 3 H, Ac, **18**), 1.240 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′, **18**), 1.156 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′, **18**β); ^{13}C, δ 170.2 ($COCH_3$, **18**), 169.9 ($COCH_3$, **18**β), 166.2 (*C*OPh), 134.6 ($OCH_2CH{=}CH_2$), 116.7 ($OCH_2CH{=}CH_2$), 103.1 (C-1″, **18**β), 97.3 (C-1″, **18**), 92.2 (C-1′), 21.0 ($COCH_3$, **18**β), 20.7 ($COCH_3$, **18**), 17.7 (C-6′, **18**), 17.6 (C-6′, **18**β).

To a soln of **18**/**18**β (202 mg, 0.158 mmol) in 1:5 CH_2Cl_2–MeOH (5 mL) was added NaOMe (pH 11). After 1 h, TLC (8:2 toluene–EtOAc) showed a complete conversion into **19**/**19**β (R_f 0.26). The mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography (8:2 toluene–EtOAc) of the residue afforded **19**/**19**β, isolated in 82% as a glass (146 mg); ^{1}H NMR ($CDCl_3$): δ 7.33–7.11 (m, 35 H, 7 Ph), 5.865 (m, 1 H, $OCH_2CH{=}CH_2$), 5.30–5.10 (m, 2 H, $OCH_2CH{=}CH_2$), 5.170 (d, 1 H, $J_{1',2'} < 1$ Hz, H-1′, **19**), 5.128 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′, **19**β), 4.919 (d, 1 H, $J_{1'',2''}$ 2.1 Hz, H-1″, **19**), 1.242 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′, **19**), 1.191 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′, **19**β).

A soln of **19**/**19**β (207 mg, 0.183 mmol) and benzyl bromide (75 μL, 0.63 mmol) in DMF (3.5 mL) was added dropwise to a stirred, cooled (0 °C) suspension of NaH (50 mg, 2.1 mmol) in DMF (1 mL). After 3 h, TLC [8:1 toluene–EtOAc, R_f 0.59 (**20**), 0.66 (**20**β)] showed the benzylation to be completed. The excess of NaH was destroyed with MeOH, the mixtur e was diluted with EtOAc, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:1 toluene–EtOAc) of the residue gave **20** (199 mg, 83%) and **20**β (37 mg, 15%), both isolated as a syrup; for **20**: $[\alpha]_D$ +5° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.28–7.18 (m, 45 H, 9 Ph), 5.775 (m, 1 H, $OCH_2CH{=}CH_2$), 5.21–5.06 (m, 2 H, $OCH_2CH{=}CH_2$), 5.189 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1″), 5.152 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 1.188 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′); ^{13}C, δ 134.7 ($OCH_2CH{=}CH_2$), 116.5 ($OCH_2CH{=}CH_2$), 97.6 (C-1″), 94.0 (C-1′), 82.1, 80.1, 79.4 (2 C), 78.3, 77.7, 75.7, 75.2, 75.1, 70.2, and 68.7 (C-2,3,4,2′,3′,4′,5′,2″,3″,4″,5″), 75.4, 74.7, 73.5, 73.2, 73.0, 72.8, 72.7, 72.3, 71.9, 70.3, 70.1 (2 C), and 68.2 (C-1,5,6″, 9 OCH_2Ph, and O$CH_2CH{=}CH_2$), 18.0 (C-6″).

A soln of **20** (85 mg, 65 μmol) in DMF (5 mL) was heated at 80 °C, and KO*t*Bu (120 mg, 1.07 mmol) was added, giving the solution a deep black colour. After 30 min, TLC (30:1 toluene–acetone) indicated a complete conversion of the allyl (R_f 0.46) into the 1-propenyl function (R_f 0.66). The mixture was cooled, diluted with CH_2Cl_2, washed with aq 5% NaCl and water, and concentrated. The

residue was dissolved in 9:1 acetone–0.1 M HCl (10 mL) and boiled under reflux for 40 min, when TLC (30:1 toluene–acetone) indicated a complete conversion of the 1-propenyl-containing compound into a lower moving spot (R_f 0.09). The mixture was neutralised with aq 25% NH_4OH, concentrated, diluted with CH_2Cl_2, washed with aq 10% $NaHCO_3$ and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (7:3 hexane–EtOAc) of the residue afforded **21**, isolated in 49% as a glass (40 mg); $[\alpha]_D$ +2° (*c* 0.25); ^{1}H NMR ($CDCl_3$): δ 7.34–7.09 (m, 45 H, 9 Ph), 5.207 (d, 1 H, $J_{1'',2''}$ 3.4 Hz, H-1″), 4.964 (d, 1 H, $J_{1',2'} < 1$ Hz, H-1′), 2.43 (m, 1 H, HO-5), 1.245 (d, 3 H, $J_{5'',6''}$ 6.1 Hz, 3 H-6′).

Ethyl 2,4,6-tri-O-*benzyl-3*-O-p-*methoxybenzyl-1-thio-β*-D-*glucopyranoside* (**23**).—A soln of ethyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**22**) (1.01 g, 2.04 mmol) and *p*-methoxybenzyl chloride (0.35 mL, 2.6 mmol) in DMF (5 mL) was added dropwise to a stirred, cooled (0 °C) suspension of NaH (150 mg, 6.25 mmol) in DMF (5 mL). TLC (7:3 hexane–EtOAc) showed the formation of **23** (R_f 0.56) to be completed in 2 h. After destroying the excess of NaH with MeOH, the mixture was diluted with CH_2Cl_2, washed with water (3×), dried ($MgSO_4$), filtered, concentrated, and co-concentrated with toluene (2×), EtOH (2×), and CH_2Cl_2 (2×) to give a white solid. Crystallisation of the solid from EtOH gave **23** (1.02 g, 81%) as white crystals; ^{1}H NMR ($CDCl_3$): δ 7.33–6.81 (m, 19 H, 3 Ph and $C_6H_4OCH_3$), 4.918, 4.841, 4.824, 4.765, 4.748, 4.595, 4.562, and 4.533 (8 d, each 1 H, 3 OCH_2Ph and $OCH_2C_6H_4OCH_3$), 4.455 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 3.781 (s, 3 H, $C_6H_4OCH_3$), 3.740 (dd, 1 H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.664 (dd, 1 H, $J_{5,6b}$ 4.8 Hz, H-6b), 3.665 (t, 1 H, $J_{2,3} = J_{3,4} = 8.7$ Hz, H-3), 3.581 (t, 1 H, $J_{4,5}$ 8.9 Hz, H-4), 3.457 (m, 1 H, H-5), 3.421 (dd, 1 H, H-2), 2.88–2.64 (m, 2 H, SCH_2CH_3), 1.323 (t, 3 H, SCH_2CH_3).

(2,4,6-Tri-O-*benzyl-3*-O-p-*methoxybenzyl-α/β*-D-*glucopyranosyl)-(1 → 3)-(2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-1*-O-*benzoyl-2,3-di*-O-*benzyl*-D-*ribitol* (**24**/**24**β), (2,4,6-tri-O-benzyl-3-O-p-methoxybenzyl-α/β-D-*glucopyranosyl)-(1 → 3)-(4*-O-*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-2,3-di*-O-*benzyl*-D-*ribitol* (**25**/**25**β), (2,4,6-tri-O-benzyl-3-O-p-methoxybenzyl-α-D-*glucopyranosyl)-(1 → 3)-(2,4-di*−O−*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-1,2,3-tri*-O-*benzyl*-D-*ribitol* (**26**), and (2,4,6-tri-O-benzyl-α-D-*glucopyranosyl)-(1 → 3)-(2,4-di*-O-*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-1,2,3-tri*-O-*benzyl*-D-*ribitol* (**27**).—A mixture of **23** (100 mg, 0.163 mmol), (2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 4)-5-*O*-allyl-1-*O*-benzoyl-2,3-di-*O*-benzyl-D-ribitol (**17**) [22] (98 mg, 0.13 mmol), and 4 Å molecular sieves in 1:5 1,2-dichloroethane–Et_2O (3.8 mL) was stirred for 30 min, then IDCP (152 mg, 0.324 mmol) was added. After 1 h, TLC (6:4 hexane–EtOAc) indicated the disappearance of **17** and the appearance of a new spot (**24**/**24**β, R_f 0.34). The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with aq 10% $Na_2S_2O_3$, water, aq 10% $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2 hexane–EtOAc) of the residue afforded **24**/**24**β, isolated as a syrup (106 mg, 62%, α:β 2:1); NMR ($CDCl_3$): ^{1}H, δ 7.95–6.68 (m, 39 H, 7 Ph and $C_6H_4OCH_3$), 5.871 (m, 1 H, $OCH_2CH=CH_2$), 5.478 (dd, 1 H, $J_{1',2'}$ 2.0, $J_{2',3'}$ 2.9 Hz, H-2′, **24**), 5.328 (dd, 1 H, $J_{1',2'}$ 1.8, $J_{2',3'}$ 3.4 Hz, H-2′, **24**β), 5.234 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″, **24**), 5.159 (d, 1 H, H-1, **24**β), 5.126 (d, 1 H, H-1′, **24**), 3.746 (s, 3 H, $C_6H_4OCH_3$, **24**β), 3.704 (s, 3 H, $C_6H_4OCH_3$, **24**), 2.141 (s, 3 H, Ac, **24**β), 1.928 (s, 3 H, Ac, **24**), 1.234 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′, **24**), 1.150 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′, **24**β); ^{13}C, δ 170.2 ($COCH_3$, **24**), 169.9 ($COCH_3$, **24**β), 166.2 (*C*OPh), 134.6 ($OCH_2CH=CH_2$), 116.8 ($OCH_2CH=CH_2$), 103.2 (C-1″, **24**β), 97.3 (C-1′, **24**β), 96.5 (C-1″, **24**), 92.4 (C-1′, **24**), 55.1 ($C_6H_4OCH_3$), 21.0 ($COCH_3$, **24**β), 20.7 ($COCH_3$, **24**), 17.8 (C-6′, **24**), 17.7 (C-6′, **24**β).

To a soln of **24**/**24**β (361 mg, 0.276 mmol) in 1:3 CH_2Cl_2–MeOH (8 mL) was added NaOMe (pH 12). After 18 h, the pH of the mixture had decreased, and an extra amount of NaOMe was added (pH 12). After another 18 h, TLC (3:1 toluene–EtOAc) indicated a complete conversion of **24**/**24**β into **25**/**25**β (R_f 0.34). The mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography (65:35 hexane–EtOAc) of the residue afforded **25**/**25**β, isolated in 85% as a glass (272 mg). A soln of **25**/**25**β (73 mg, 63 μmol) and benzyl bromide (25 μL, 0.21 mmol) in DMF (1 mL) was added dropwise to a stirred, cooled (0 °C) suspension of NaH (13 mg, 0.54 mmol) in DMF (1 mL). TLC [8:2 hexane–EtOAc, R_f 0.59 (**26**), 0.66 (**26**β)] showed the benzylation to be completed in 1 h. After destroying the excess of NaH with MeOH, the mixture was diluted with EtOAc, washed with water (3×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (24:1 toluene–EtOAc) of the residue gave **26** (43 mg, 51%) and **26**β (22 mg, 26%), both isolated as a syrup; for **26**: $[\alpha]_D$ +3° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.32–6.73 (m, 44 H, 8 Ph

and $C_6H_4OCH_3$), 5.801 (m, 1 H, $OCH_2CH{=}CH_2$), 5.24–5.08 (m, 2 H, $OCH_2CH{=}CH_2$), 5.20–5.18 (m, 2 H, H-1′,1″), 3.714 (s, 3 H, $C_6H_4OCH_3$), 1.241 (d, 3 H, $J_{5',6'}$ 6.1 Hz, 3 H-6′); ^{13}C, δ 134.7 ($OCH_2CH{=}CH_2$), 116.5 ($OCH_2CH{=}CH_2$), 97.7 and 94.0 (C-1′, 1″), 81.7, 80.1, 79.5, 79.4, 78.3 (2 C), 77.7, 75.7, 75.2, 75.1, and 68.7 (C-2,3,4,2′,3′,4′,5′,2″,3″,4″,5″), 75.4, 75.0, 74.7, 73.6, 73.2 (2 C), 73.0, 72.9, 72.7, 72.3, 71.9, 70.1, and 68.2 (8 OCH_2Ph, $OCH_2C_6H_4OCH_3$, $OCH_2CH{=}CH_2$, and C-1,5,6″), 55.1 ($C_6H_4OCH_3$), 18.0 (C-6′).

To a soln of **26** (180 mg, 0.134 mmol) in 3:6:1 toluene–acetonitrile–water (10 mL) was added ammonium cerium(IV) nitrate (CAN; 300 mg, 0.547 mmol). After 1 h, TLC (8:2 hexane–EtOAc) showed the conversion of the starting compound into one new spot (**27**, R_f 0.36). The reaction mixture was diluted with CH_2Cl_2, washed with water, aq 5% $NaHSO_3$, aq 10% $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2 hexane–EtOAc) of the residue afforded **27**, isolated as a syrup (118 mg, 72%); $[\alpha]_D$ +8° (*c* 1); ^1H NMR ($CDCl_3$): δ 7.32–7.09 (m, 40 H, 8 Ph), 5.809 (m, 1 H, $OCH_2CH{=}CH_2$), 5.25–5.22 (m, 2 H, H-1′,1″), 1.223 (d, 3 H, $J_{5',6'}$ 6.1 Hz, 3 H-6′). Anal. Calcd for $C_{76}H_{84}O_{14}$: C, 74.73; H, 6.93. Found: C, 74.55; H, 6.84.

(3,4,6-Tri-O-*acetyl-2*-O-*allyl-α*-D-*galactopyranosyl)-(1 → 3)-(2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-(2,4-di*-O-*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-5*-O-*allyl-1,2,3-tri*-O-*benzyl*-D-*ribitol* (**28**).—A mixture of **13** (110 mg, 0.224 mmol), **27** (123 mg, 0.101 mmol) and 4 Å molecular sieves in Et_2O (10 mL) was cooled to 0 °C, and stirred for 1 h. Then, TMSOTf (42 μL, 0.22 mmol) was added. After 20 min, TLC (8:2 toluene–EtOAc) showed the disappearance of acceptor **27** and the appearance of a single product (**28**, R_f 0.48), and the mixture was neutralised with Et_3N, diluted with CH_2Cl_2, washed with water (3 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:1 toluene–EtOAc) of the residue afforded **28**, isolated as a syrup (126 mg, 81%); $[\alpha]_D$ +66° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.33–7.11 (m, 40 H, 8 Ph), 5.820 and 5.613 (2 m, each 1 H, 2 $OCH_2CH{=}CH_2$), 5.596 (d, 1 H, $J_{1''',2'''}$ 3.5 Hz, H-1‴), 5.33–5.31 (m, 2 H, H-1′,1″), 5.166 (dd, 1 H, $J_{3''',4'''}$ 3.2, $J_{4''',5'''}$ 1.1 Hz, H-4‴), 2.026, 1.997, and 1.845 (3 s, each 3 H, 3 Ac), 1.265 (d, 3 H, $J_{5',6'}$ 6.1 Hz, 3 H-6″); ^{13}C, δ 170.3, 170.0, and 169.6 (3 $COCH_3$), 134.7 and 134.3 (2 $OCH_2CH{=}CH_2$), 117.5 and 116.6 (2 $OCH_2CH{=}CH_2$), 97.7, 97.0, and 91.9 (C-1′,1″,1‴), 20.7 (2 C) and 20.5 (3 $COCH_3$), 18.0 (C-6′). Anal. Calcd for $C_{91}H_{104}O_{22}$: C, 70.52; H, 6.76. Found: C, 70.48; H, 6.65.

(3,4,6-Tri-O-*acetyl-α*-D-*galactopyranosyl)-(1 → 3)-(2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-(2,4-di*-O-*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-1,2,3-tri*-O-*benzyl*-D-*ribitol* (**29**).—To a solution of **28** (76 mg, 49 μmol) and DABCO (50 mg, 0.44 mmol) in 8:3:1 EtOH–toluene–water (12 mL) was added tris(triphenylphosphine)rhodium(I) chloride (29 mg). After boiling under reflux for 3 h, TLC (6:4 hexane–EtOAc) showed the reaction to be completed. The mixture was cooled, diluted with CH_2Cl_2, washed with 0.1 M HCl and water (2 ×), and concentrated. To a solution of the residue in 9:1 acetone–water (6 mL) were added $HgCl_2$ (96 mg, 0.35 mmol) and a catalytic amount of HgO (4.8 mg). After stirring the mixture for 2 h, TLC (6:4 hexane–EtOAc) showed the conversion of **28** into **29** (R_f 0.16) to be completed. The mixture was diluted with CH_2Cl_2, filtered, washed with water, aq 5% Kl, water, aq 10% $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (6:4 hexane–EtOAc) of the residue afforded **29**, isolated in 61% as a glass (44 mg); $[\alpha]_D$ +58° (*c* 1); ^1H NMR ($CDCl_3$): δ 7.29–7.05 (m, 40 H, 8 Ph), 5.533 (d, 1 H, $J_{1''',2'''}$ 3.8 Hz, H-1‴), 5.286 (d, 1 H, $J_{1'',2''}$ 3.4 Hz, H-1″), 5.204 (dd, 1 H, $J_{3''',4'''}$ 3.3, $J_{4''',5'''} \approx 1$ Hz, H-4‴), 5.118 (dd, 1 H, $J_{2''',3'''}$ 10.5 Hz, H-3‴), 5.065 (d, 1 H, $J_{1',2'}$ 2.3 Hz, H-1′), 2.059, 2.035, and 1.860 (3 s, each 3 H, 3 Ac), 1.271 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6″). Anal. Calcd for $C_{85}H_{96}O_{22}$: C, 69.47; H, 6.58. Found: C, 69.40; H, 6.48.

3-N-*Benzyloxycarbonylaminopropyl (1,2,3,4-tetra*-O-*benzyl*-D-*ribityl)-(5 → triethylammonium phosphate → 2)-(3,4,6-tri*-O-*acetyl-α*-D-*galactopyranosyl)-(1 → 3)-(2,4,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-2*-O-*acetyl-4*-O-*benzyl-α*-L-*rhamnopyranoside* (**32**) and *3-aminopropyl*-D-*ribityl-(5 → hydrogen phosphate → 2)-α*-D-*galactopyranosyl-(1 → 3)-α*-D-*glucopyranosyl-(1 → 3)-α*-L-*rhamnopyranoside* (**34**).—A mixture of 1,2,3,4-tetra-*O*-benzyl-5-*O*-(triethylammonium H-phosphonate)-D-ribitol (**30**) [23] (41 mg, 61 μmol) and pivaloyl chloride (60 μL, 0.49 mmol) in 2:5 pyridine–acetonitrile (0.7 mL) was stirred for 60 min, then a soln of **15** (20 mg, 17 μmol) in acetonitrile (1.0 mL) was added. After 90 min, TLC (9:1 CH_2Cl_2–acetone) revealed the disappearance of **15** and the appearance of a new spot (**31**). A 0.5 M soln of iodine in 95:5 pyridine–water (150 μL) was added, and after 18 h, TLC (9:1

CH_2Cl_2–acetone) showed the disappearance of **31** and the appearance of a new spot on the baseline. The mixture was diluted with CH_2Cl_2, washed with aq 5% $Na_2S_2O_3$ and 1 M triethylammonium bicarbonate (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (90:9:1 CH_2Cl_2–acetone–Et_3N, then 90:9:1 CH_2Cl_2–MeOH–Et_3N) of the residue and subsequent purification on Sephadex LH-20 (50:50:1 CH_2Cl_2–MeOH–Et_3N) gave **32**, isolated as a glass (32 mg, 78%). A soln of **32** (16 mg, 8.5 μmol) in 2:1 MeOH–aq 25% NH_4OH (3 mL) was heated for 48 h at 50 °C, then concentrated to yield crude **33**, which was purified on Sephadex LH-20 (50:50:1 CH_2Cl_2–MeOH–Et_3N). To a soln of **33** in 1:2:2:2 water–EtOAc–2-propanol–EtOH (3 mL) was added 10% Pd–C (10 mg), and the mixture was hydrogenolysed at atmospheric pressure for 24 h. After filtration, the hydrogenolysis procedure was repeated. Then, the mixture was concentrated, and purified by Bio-Gel P-2 gel-permeation chromatography using water as an eluent, affording **34**, isolated as a white powder (4.9 mg, 76%); NMR (D_2O): ^{1}H, δ 5.643 (d, 1 H, $J_{1'',2''}$ 4.2 Hz, H-1″), 5.409 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.091 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 3.465 (t, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 2.03–1.96 (m, 2 H, $OCH_2CH_2CH_2N$), 1.317 (d, 3 H, $J_{5,6}$ 5.9 Hz, 3 H-6); ^{31}P, δ 1.65 (PO_4). FABMS$^+$ Calcd for $C_{26}H_{50}NO_{22}P$: *m/z* 760.6 $[M + H]^+$. Found: *m/z* 760.6 $[M + H]^+$.

3-N-*Benzyloxycarbonylaminopropyl (2,3,4-tri*-O-*acetyl-α*-L-*rhamnopyranosyl)-(1 → 4)-(1*-O-*benzoyl-2,3-di*-O-*benzyl*-D-*ribityl)-(5 → triethylammonium phosphate → 2)-(3,4,6-tri*-O-*acetyl-α*-D-*galactopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α*-D-*glucopyranoside* (**38**) and *3-aminopropyl* α-L-*rhamnopyranosyl-(1 → 4)*-D-*ribityl-(5 → hydrogen phosphate → 2)-α*-D-*galactopyranosyl-(1 → 3)-α*-D-*glucopyranoside* (**40**). —A mixture of (2,3,4-tri-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1 → 4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-(triethylammonium H-phosphonate)-D-ribitol [23] (**36**; 13 mg, 15 μmol) and pivaloyl chloride (60 μL, 0.49 mmol) in 2:5 pyridine–acetonitrile (1.2 mL) was stirred for 30 min, then 3-*N*-benzyloxycarbonylaminopropyl (3,4,6-tri-*O*-acetyl-*α*-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-*α*-D-glucopyranoside [23] (**35**; 10 mg, 11 μmol) in acetonitrile (0.5 mL) was added. After 24 h, TLC (8:2 toluene–acetone) revealed the disappearance of **35** and the appearance of a new spot (**37**). To this mixture, a 0.5 M solution of iodine in 95:5 pyridine–water (300 μL) was added. After 1 h, TLC (8:2 toluene–acetone) indicated the disappearance of **37** and the appearance of a new spot on the baseline. The mixture was diluted with CH_2Cl_2, washed with aq 5% $Na_2S_2O_3$ and 1 M triethylammonium bicarbonate (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (80:19:1 toluene–acetone–Et_3N, then 80:19:1 toluene–MeOH–Et_3N) of the residue and subsequent purification on Sephadex LH-20 (50:49:1 CH_2Cl_2–MeOH–Et_3N) gave **38**, isolated as a glass (9.7 mg, 50%); NMR ($CDCl_3$): ^{1}H, δ 7.98–7.15 (m, 35 H, 7 Ph), 5.855 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 5.437 (dd, 1 H, $J_{2',3'}$ 10.7, $J_{3',4'}$ 3.3 Hz, H-3′), 5.368 (dd, 1 H, $J_{4',5'}$ 1.7 Hz, H-4′), 5.24–5.23 (m, 2 H, H-1,1‴), 2.726 (q, 6 H, $N(CH_2CH_3)_3$), 2.097, 2.017, 1.978, 1.961, 1.926, and 1.807 (6 s, each 3 H, 6 Ac), 1.129 (t, 9 H, $N(CH_2CH_3)_3$), 0.920 (d, 3 H, $J_{5''',6'''}$ 6.2 Hz, 3 H-6‴); ^{13}C, δ 170.2–169.6 (*C*OCH$_3$), 166.1 (*C*OPh), 156.2 (N*C*OOCH$_2$Ph), 97.1, 96.4, and 96.0 (C-1,1′,1‴), 61.9 [O*C*H$_2$(CH$_2$)$_2$N], 45.7 [N(*C*H$_2$CH$_3$)$_3$], 38.6 [O(CH$_2$)$_2$*C*H$_2$N], 29.5 (OCH$_2$*C*H$_2$CH$_2$N), 17.0 (C-6‴), 9.8 [N(CH$_2$*C*H$_3$)$_3$]; ^{31}P, δ −2.39 (PO_4).

A soln of **38** (25 mg, 14 μmol) in 2:1 MeOH–aq 25% NH_4OH (6 mL) was heated for 48 h at 50 °C, then concentrated to yield crude **39**, which was purified on Sephadex LH-20 (50:50:1 CH_2Cl_2–MeOH–Et_3N). To a soln of **39** in 1:2:2:2 water–EtOAc–2-propanol–EtOH (5 mL) was added 10% Pd–C (20 mg), and the mixture was hydrogenolysed at 4 kg/cm^2 for 24 h. After filtration, the hydrogenolysis procedure was repeated. Then, the mixture was concentrated, and purified by Bio-Gel P-2 gel-permeation chromatography using water as an eluent, affording **40**, isolated as a white powder (7.7 mg, 73%); NMR (D_2O): ^{1}H, δ 5.618 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1′), 5.086 (d, 1 H, $J_{1''',2'''}$ 1.5 Hz, H-1‴), 4.943 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1′), 3.462 (t, 1 H, $J_{3''',4'''} = J_{4''',5'''} = 9.7$ Hz, H-4‴), 3.21–3.10 (m, 2 H, O(CH$_2$)$_2$C*H*$_2$N), 2.05–1.96 (m, 2 H, OCH$_2$C*H*$_2$CH$_2$N), 1.293 (d, 3 H, $J_{5''',6'''}$ 6.3 Hz, 3 H-6‴); ^{13}C, δ 101.4, 99.8, and 98.6 (C-1,1′,1‴), 67.3, 65.6, 63.9, 62.4, and 61.8 (O*C*H$_2$(CH$_2$)$_2$N and C-6,6′,1″,5‴), 39.3 [O(CH$_2$)$_2$*C*H$_2$N], 28.2 (OCH$_2$*C*H$_2$CH$_2$N), 17.9 (C-6‴); ^{31}P: δ 0.23 (PO_4). FABMS$^+$ Calcd for $C_{26}H_{50}NO_{22}P$: *m/z* 760.6 $[M + H]^+$. Found: *m/z* 760.6 $[M + H]^+$.

3-N-*Benzyloxycarbonylaminopropyl (2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-(2,4-di*-O-*benzyl-α*-L-*rhamnopyranosyl)-(1 → 4)-(1,2,3-tri*-O-*benzyl*-D-*ribityl)-(5 → triethylammonium phosphate → 2)-3,4,6-tri*-O-*benzyl-α*-D-*galactopyrano-side* (**43**) and *3-aminopropyl* α-D-*glucopyranosyl-(1 → 3)-α*-L-*rhamnopyranosyl-(1 → 4)*-D-*ribityl-(5 → hydrogen*

phosphate → 2) - α - D - galactopyranoside (**44**).—A mixture of 3-*N*-benzyloxycarbonylaminopropyl 3,4,6-tri-*O*-benzyl-2-*O*-(triethylammonium H-phosphonate)-α-D-galactopyranoside (**41**) [23] (14 mg, 17 μmol) and pivaloyl chloride (30 μL, 0.24 mmol) in 2:5 pyridine–acetonitrile (0.7 mL) was stirred for 30 min, then **21** (20 mg, 16 μmol) in acetonitrile (1.5 mL) was added. After 2 h, TLC (8:2 toluene–acetone) revealed the disappearance of **21** and the appearance of a new spot (**42**). To this mixture, a 0.5 M soln of iodine in 95:5 pyridine–water (500 μL) was added, and after 24 h, TLC (8:2 toluene–acetone) indicated the disappearance of **42** and the appearance of a new spot on the baseline. The mixture was diluted with CH_2Cl_2, washed with aq 5% $Na_2S_2O_3$ and 1 M triethylammonium bicarbonate (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (80:19:1 toluene–acetone–Et_3N, then 80:19:1 toluene–MeOH–Et_3N) of the residue and subsequent purification on Sephadex LH-20 (50:49:1 CH_2Cl_2–MeOH–Et_3N) gave **43**, isolated as a glass (16 mg, 49%). To a solution of **43** (16 mg, 7.7 μmol) in 1:2:2:2 water–EtOAc–2-propanol–EtOH (3 mL) was added 10% Pd–C (10 mg), and the mixture was hydrogenolysed at atmospheric pressure for 8 h. After filtration, the hydrogenolysis procedure was repeated. Then, the mixture was concentrated and purified by Bio-Gel P-2 gel-permeation chromatography using water as an eluent, affording **44**, isolated as a white powder (4.2 mg, 72%); NMR (D_2O): ^{1}H, δ 5.164 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.147 (bs, 1 H, $J_{1'',2''} < 1$ Hz, H-1″), 5.124 (d, 1 H, $J_{1''',2'''}$ 4.2 Hz, H-1‴), 3.467 (t, 1 H, $J_{3'',4''} = J_{4'',5''} = 9.6$ Hz, H-4″), 3.21–3.13 (m, 2 H, $O(CH_2)_2CH_2N$), 2.22–2.15 (m, 2 H, $OCH_2CH_2CH_2N$), 1.315 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, 3 H-6″); ^{31}P, δ 2.93 (PO_4). FABMS$^+$ Calcd for $C_{26}H_{50}NO_{22}P$: *m/z* 760.6 $[M + H]^+$. Found: *m/z* 760.6 $[M + H]^+$.

*(3,4,6-Tri-*O*-acetyl-α-D-galactopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 4)-1,2,3-tri-*O*-benzyl-5-*O*-(3-*N*-benzyloxycarbonylaminopropyl triethylammonium phosphate)-D-ribitol* (**47**) and α-D-*galactopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-5-*O*-(3-aminopropyl hydrogen phosphate)-D-ribitol* (**49**).—To a solution of **29** (10 mg, 6.8 μmol) in 2:5 pyridine–acetonitrile (1 mL) was added dropwise a solution of 3-*N*-benzyloxycarbonylaminopropyl triethylammonium H-phosphonate [22] (**45**; 10 mg, 37 μmol) and pivaloyl chloride (3.3 μL, 27 μmol) in 2:5 pyridine–acetonitrile (1.0 mL). When 300 μL of the mixture was added, TLC (9:1 CH_2Cl_2–acetone) revealed the disappearance of **29** and the appearance of a major product (**46**) and some side-products. To the mixture, a 0.5 M solution of iodine in 95:5 pyridine–water (250 μL) was added, and after 18 h, TLC (9:1 CH_2Cl_2–acetone) showed the disappearance of **46** and the appearance of a new spot on the baseline. The mixture was diluted with CH_2Cl_2, washed with aq 5% $Na_2S_2O_3$ and 1 M triethylammonium bicarbonate (2 ×), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (80:19:1 CH_2Cl_2–MeOH–Et_3N) of the residue gave **47**, isolated as a glass (6.6 mg, 53%). A soln of **47** (6.1 mg, 3.3 μmol) in 2:1 MeOH–aq 25% NH_4OH (2 mL) was heated for 24 h at 50 °C, and concentrated, to yield crude **48**, which was purified on Sephadex LH-20 (50:50:1 CH_2Cl_2–MeOH–Et_3N). To a soln of **48** in 1:2:2:2 water–EtOAc–2-propanol–EtOH (3 mL) was added 10% Pd–C (7 mg), and the mixture was hydrogenolysed at atmospheric pressure for 8 h. After filtration, the hydrogenolysis procedure was repeated. Then, the mixture was concentrated, and purified by Bio-Gel P-2 gel-permeation chromatography using water as an eluent, affording **49**, isolated as a white powder (1.9 mg, 76%); NMR (D_2O): ^{1}H, δ 5.396 (d, 1 H, $J_{1''',2'''}$ 4.1 Hz, H-1‴), 5.134 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1′), 5.125 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″), 3.589 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.8$ Hz, H-4′), 3.161 (t, 2 H, $O(CH_2)_2CH_2N$), 2.05–2.00 (m, 2 H, $OCH_2CH_2CH_2N$), 1.318 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′); ^{31}P, δ 1.67 (PO_4). FABMS$^+$ Calcd for $C_{26}H_{50}NO_{22}P$: *m/z* 760.6 $[M + H]^+$. Found: *m/z* 760.6 $[M + H]^+$.

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