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SYNTHESIS OF A SELECTIVELY PROTECTED TRISACCHARIDE BUILDING BLOCK OF THE CAPSULAR POLYSACCHARIDE OF *Streptococcus pneumoniae* TYPES 6A AND 6B

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ABSTRACT

4-Methoxybenzyl 2,4-di-*O*-benzyl-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**22**), a building block for the α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p* fragment of the capsular polysaccharides of *Streptococcus pneumoniae* types 6A and 6B [→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→X)-D-RibOH-(5-P→]$_n$ (6A, X = 3; 6B, X = 4) has been synthesised. Ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside was coupled with 4-methoxybenzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside in ether, using methyl triflate as promoter. The resulting α-D-Glc*p*-(1→3)-α-L-Rha*p* derivative was deallylated with KOBu^t in *N,N*-dimethylformamide followed by 0.1M HCl in 9:1 acetone–water. The product was coupled with 3,4,6-tri-*O*-acetyl-2-*O*-allyl-α,β-D-galactopyranosyl trichloroacetimidate in ether, using trimethylsilyl triflate, to yield **19**. Deacetylation, benzylation, and deallylation then gave **22**.

INTRODUCTION

Streptococcus pneumoniae is a Gram-positive bacterium, which can induce infections like pneumonia, otitis media, and meningitis in human beings[1]. The more than 82 serotypes of *S. pneumoniae* have species-specific capsular polysaccharides that are responsible for the virulence of the organisms and prevent phagocytosis in the absence of specific antibodies. The defence mechanism to *S. pneumoniae* is based on humoral immunity, whereby antibodies directed to the capsular polysaccharide protect humans against infection with viable pneumococci. A current vaccine based on isolated polysaccharides from 23 species is available for persons older than 2 years believed to be at high risk for pneumococcal diseases, including those aged over 50 years and those with chronic systemic illness. Several dis-

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advantages still remain with such a vaccine since polysaccharides are non-immunogenic in newborns and do not induce an immunological memory, whereas tolerance induction is a severe problem.

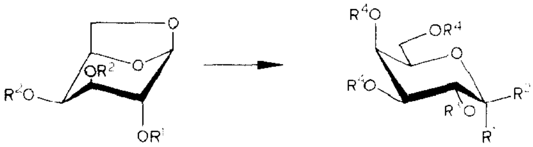
Pneumococci of group 6 are divided into two cross-reactive types (6A and 6B) and their capsular polysaccharides have the structure[2,3] [→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→X)-D-RibOH-(5-P→]$_n$ where X = 3 in 6A and 4 in 6B. These two types of capsular polysaccharides, of which 6B is one of the constituents of the current vaccine Pneumovac® 23, are cross-reactive, indicating that one capsular polysaccharide can induce immunity against both types of pneumococci.

As part of our studies concerning the development of synthetic vaccines based on oligosaccharide conjugates against infections by *S. pneumoniae* serotypes 6A and 6B, we now describe the synthesis of the selectively protected derivative 4-methoxybenzyl 2,4-di-*O*-benzyl-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-*a*-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**22**), one of the essential building blocks for the preparation of higher oligomers.

RESULTS AND DISCUSSION

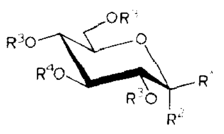
For the synthesis of **22**, the three monosaccharide synthons **5** for D-Gal, **9** for D-Glc, and **16** for L-Rha were prepared as follows.

Acetylation of 2-*O*-allyl-1,6-anhydro-β-D-galactopyranose[4,5] (**1**) gave **2** and cleavage of the 1,6-anhydro ring, using acetic anhydride and trifluoroacetic acid,

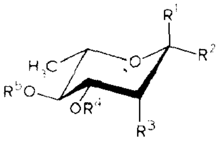


1 R^1 = allyl, R^2 = H
2 R^1 = allyl, R^2 = Ac

3 R^1, R^2 = H, OAc, R^3 = allyl, R^4 = Ac
4 R^1, R^2 = H, OH, R^3 = allyl, R^4 = Ac
5 R^1, R^2 = H, OC(=NH)CCl$_3$, R^3 = allyl, R^4 = Ac

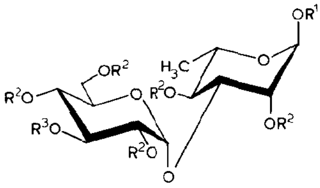


6 R^1, R^2 = H,OAc, R^3 = Ac, R^4 = allyl
7 R^1 = SEt, R^2 = H, R^3 = Ac, R^4 = allyl
8 R^1 = SEt, R^2 = R^3 = H, R^4 = allyl
9 R^1 = SEt, R^2 = H, R^3 = Bn, R^4 = allyl

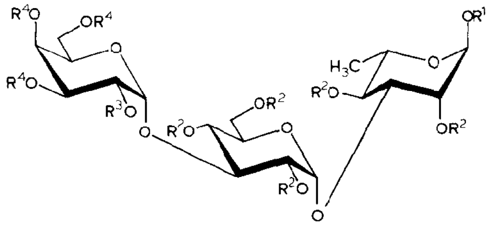


10 R^1 = Br, R^2 = H, R^3 = R^4 = R^5 = Ac
11 R^1 = OMBn, R^2 = H, R^3 = R^4 = R^5 = Ac
12 R^1 = OMBn, R^2 = H, R^3 = R^4 = R^5 = H
13 R^1 = OMBn, R^2 = R^5 = H, R^3, R^4 = C(Me)$_2$
14 R^1 = OMBn, R^2 = H, R^3, R^4 = C(Me)$_2$, R^5 = Bn
15 R^1 = OMBn, R^2 = R^3 = R^4 = H, R^5 = Bn
16 R^1 = OMBn, R^2 = R^4 = H, R^3 = R^5 = Bn

(MBn = 4-methoxybenzyl)



17 R^1 = MBn, R^2 = Bn, R^3 = allyl
18 R^1 = MBn, R^2 = Bn, R^3 = H



19 R^1 = MBn, R^2 = Bn, R^3 = allyl, R^4 = Ac
20 R^1 = MBn, R^2 = Bn, R^3 = allyl, R^4 = H
21 R^1 = MBn, R^2 = R^4 = Bn, R^3 = allyl
22 R^1 = MBn, R^2 = R^4 = Bn, R^3 = H

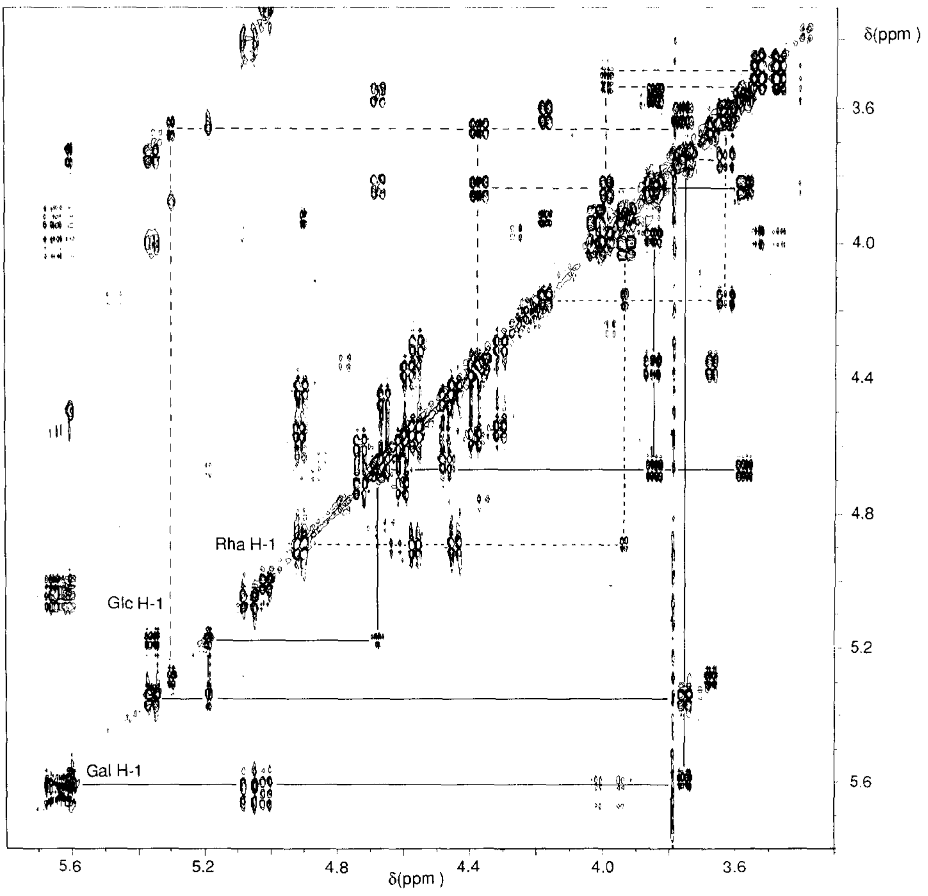


Fig. 1. 500-MHz 2D ^{1}H–^{1}H double-quantum-filtered shift-correlation spectrum (3.3–5.8 p.p.m.) of **19**. The lines in the spectrum indicate the spin connectivities: Gal, ——; Glc, – – – –; Rha, - - - - - -.

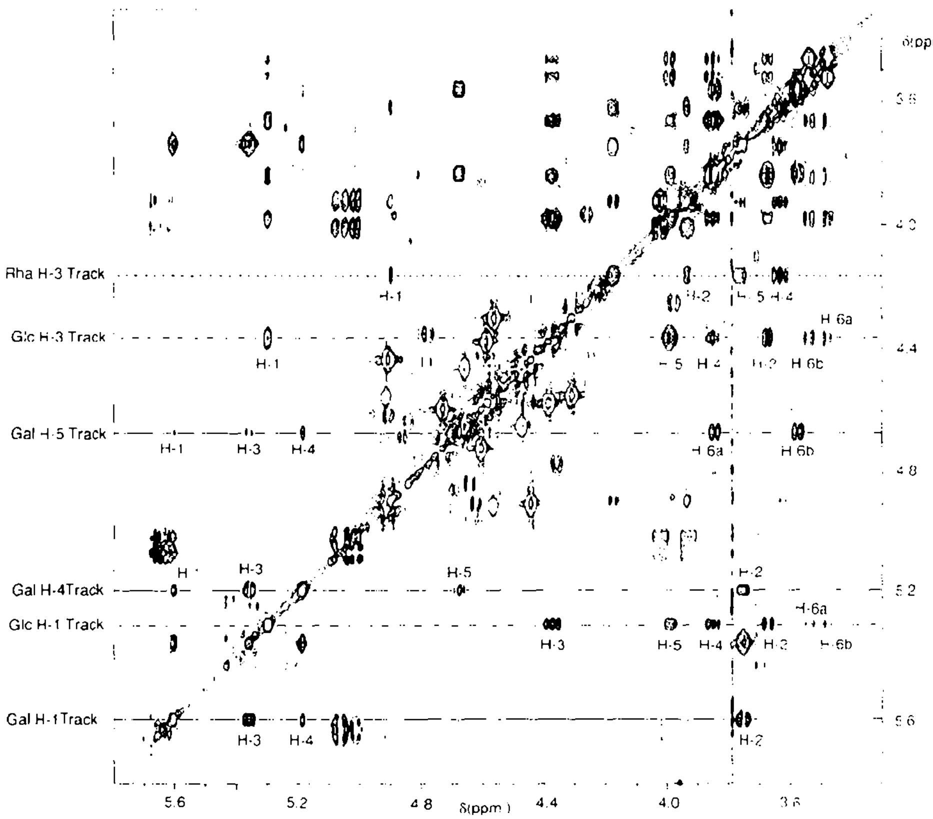


Fig. 2. 500-MHz 2D Homonuclear Hartmann–Hahn spectrum (3.3–5.8 p.p.m.) of **19**. The lines in the spectrum indicate scalar coupled networks for Gal, ——; Glc, ––––; Rha, ------.

then gave **3** (91%). Direct ring opening starting from **1** was not successful. Removal of AcO-1 in **3** with hydrazine acetate[6] (→**4**, 82%) and subsequent imidation, using potassium carbonate as a base[7], yielded 3,4,6-tri-*O*-acetyl-2-*O*-allyl-α,β-D-galactopyranosyl trichloroacetimidate (**5**, 66%).

Crystalline 1,2,4,6-tetra-*O*-acetyl-3-*O*-allyl-β-D-glucopyranose[8] (**6**) was treated with ethanethiol and freshly fused ZnCl$_2$[9–12] (→**7**, 70%). Deacetylation of **7** (→**8**, 97%) and subsequent benzylation gave ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**9**, 91%).

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[13] (**10**) was 4-methoxybenzylated at C-1 in the presence of Hg(CN)$_2$ (→**11**, 42%); the addition of 2,4,6-trimethylpyridine in this step caused undesirable orthoester formation (80%). Saponification of **11** with methanolic sodium methoxide gave **12** (88%) which was isopropylidenated[14] (→**13**, 99%). Subsequent benzylation (→**14**, 90%), de-isopropylidenation (→**15**, 76%), and selective benzylation at C-2 using a phase-transfer catalyst[15] gave 4-methoxybenzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**16**, 91%).

Coupling of the Glc synthon **9** and Rha synthon **16**, using methyl triflate in ether[9–12], gave **17** (59%). The allyl group at C-3 of the Glc part of **17** was removed using KOBu^t in *N,N*-dimethylformamide in combination with acid hydrolysis to yield **18** (72%). Deallylation of **17** using the Wilkinson catalyst in combination with HgO and $HgCl_2$[16,17] afforded 42% of **18**. Condensation of **18** with the Gal synthon **5** in ether, using trimethylsilyl triflate as promoter, afforded **19** (82%), the 1D ^{1}H-n.m.r. spectrum of which was assigned completely using 2D-n.m.r. techniques. Starting with the respective H-1 signals, the ^{1}H–^{1}H double-quantum-filtered COSY[18] spectrum (Fig. 1) shows complete cross-peak patterns for each of the monosaccharide residues. In the HOHAHA[19] spectrum (Fig. 2), several relevant (partial) subspectra have been indicated. Finally, **19** was deacetylated (→**20**, 98%), benzylated (→**21**, 95%), and deallylated using KOBu^t followed by acid hydrolysis to afford the target building block **22** (79%).

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra (60, 360, and 500 MHz) were recorded at 25° with a Varian 360, Bruker HX 360, or Bruker AM 500 spectrometer. The 2D double-quantum filtered ^{1}H–^{1}H correlation spectrum (COSY) was recorded in the phase-sensitive mode[18]. The 2D homonuclear Hartmann–Hahn spectrum (HOHAHA)[19] was recorded with MLEV-17 and a mixing time of 120 ms. The ^{13}C-n.m.r. spectra (50 MHz) were recorded at 25° with a Bruker WM-200 or WP-200 spectrometer. Chemical shifts (δ) are given in p.p.m. relative to the signal for internal Me_4Si.

Column chromatography was performed on Kieselgel 60 (Merck, <230 mesh) and fractions were monitored by t.l.c. on Kieselgel 60 F_{254} (Merck). Detection was effected by charring with sulfuric acid after examination under u.v. light. Optical rotations were measured at 20° with a Perkin–Elmer 241 polarimeter, using a 10-cm microcell. Melting points were determined with a Mettler FP 51 instrument. Evaporations were conducted *in vacuo* at 40° (bath). All solvents were distilled from appropriate drying agents.

3,4-Di-O-acetyl-2-O-allyl-1,6-anhydro-β-D-galactopyranose (**2**). — A solution of **1**[4,5] (6.6 g, 32.6 mmol) in dry pyridine (130 mL) and acetic anhydride (65 mL) was stirred overnight at room temperature. The reaction was then complete, as shown by t.l.c. (9:1 toluene–acetone), and the mixture was co-concentrated thrice each with toluene, ethanol, and dichloromethane. Column chromatography (15:1 toluene–acetone) of the crude product gave **2**, isolated as a syrup (8.6 g, 92%), $[\alpha]_D$ −51° (*c* 1, dichloromethane), R_F 0.49. ^{1}H-N.m.r. data ($CDCl_3$): δ 2.00 and 2.10 (2 s, each 3 H, 2 Ac), 3.41 (d, 1 H, H-2), 3.66 (dd, 1 H, H-6a), 3.90–4.53 (m, 4 H, H-5,6b, $OCH_2CH{=}CH_2$), 5.10–5.30 (m, 4 H, H-3,4, $OCH_2CH{=}CH_2$), 5.36 (s, 1 H, H-1), 5.66–6.13 (m, 1 H, $OCH_2CH{=}CH_2$).

1,3,4,6-Tetra-O-acetyl-2-O-allyl-α,β-D-galactopyranose (**3**). — To a solution of **2** (8.6 g, 30.1 mmol) in acetic anhydride (171 mL) was added trifluoroacetic acid

(14.25 mL). The mixture was stirred for 18 h at room temperature, when t.l.c. showed that reaction was complete. The crude product (R_F 0.35, 25:1 toluene–acetone), worked-up as for **2**, was purified by column chromatography (25:1 toluene–acetone) to yield **3**, isolated as a syrup (11.3 g, 97%), $[\alpha]_D$ +89° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 2.030, 2.137, and 2.146 (3 s, 6, 3, and 3 H, 4 Ac, α anomer), 3.690 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 9.7 Hz, H-2β), 3.879 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2α), 4.011–4.174 (m, 4 H, H-6a,6b, $OCH_2CH=CH_2$), 4.292 (t, 1 H, $J_{5,6}$ 6.5 Hz, H-5α), 5.002 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3β), 5.242 (dd, 1 H, H-3α), 5.197 and 5.266 (2 d, 2 H, $OCH_2CH=CH_2$, α anomer), 5.398 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4β), 5.472 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4α), 5.470 (d, 2 H, $OCH_2CH=CH_2$), 5.618 (d, 1 H, H-1β), 5.825 (m, 1 H, $OCH_2CH=CH_2$), 6.400 (d, 1 H, H-1α); ^{13}C, δ 20.3 (3 C) and 20.7 (4 $COCH_3$), 61.1 (C-6), 67.6, 68.3, 69.1, 71.9, and 72.2 ($OCH_2CH=CH_2$, C-2,3,4,5), 89.3 (C-1α), 93.7 (C-1β), 117.3 ($OCH_2CH=CH_2$), 133.9 ($OCH_2CH=CH_2$), 168.9, 169.8 (2 C), and 170.1 (4 $COCH_3$).

Anal. Calc. for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.24. Found: C, 52.51; H, 6.26.

3,4,6-Tri-O-acetyl-2-O-allyl-α,β-D-galactopyranosyl trichloroacetimidate (**5**). — To a solution of **3** (2.0 g, 5.2 mmol) in dry *N,N*-dimethylformamide (10 mL) was added hydrazine acetate (522 mg, 5.67 mmol), and the mixture was stirred until t.l.c. showed that deacetylation was complete (R_F 0.21, 9:1 chloroform–acetone). Ethyl acetate (50 mL) was added, and the organic phase was washed with aq. 5% sodium chloride (3 × 30 mL), dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (9:1 chloroform–acetone) to yield **4**, isolated as a syrup (1.5 g, 82%), $[\alpha]_D$ +40° (*c* 1, dichloromethane).

Anal. Calc. for $C_{15}H_{22}O_9$: C, 52.0; H, 6.4. Found: C, 51.6; H, 6.8.

To a solution of **4** (1.3 g, 3.7 mmol) in dry dichloromethane (9 mL) and trichloroacetonitrile (1.1 mL) was added freshly fused potassium carbonate (0.9 g). The mixture was stirred for 2 h, then diluted with dichloromethane (100 mL), filtered through silica, and concentrated. The residue was dissolved in 1:1 light petroleum (b.p. 40–60°)–ether (4 mL) and stored at −20° for 24 h when **5** crystallised as a 2:3 α/β-mixture (1.2 g, 66%), R_F 0.24 [1:1 light petroleum (b.p.40–60°)–ether], $[\alpha]_D$ +34° (*c* 1, dichloromethane). ^1H-N.m.r. data ($CDCl_3$): δ 2.06 and 2.19 (2 s, 6 and 3 H, 3 Ac), 5.83 (d, 0.6 H, $J_{1,2}$ 8.0 Hz, H-1β), 6.63 (d, 0.4 H, $J_{1,2}$ 3.9 Hz, H-1α), 8.74 (s, 0.4 H, NHα), 8.80 (s, 0.6 H, NHβ).

Anal. Calc. for $C_{17}H_{22}Cl_3NO_9$: C, 41.59; H, 4.53. Found: C, 41.09; H, 4.69.

Ethyl 2,4,6-tri-O-acetyl-3-O-allyl-1-thio-β-D-glucopyranoside (**7**). — To a solution of **6**[8] (10.0 g, 25.8 mmol) in dichloromethane (190 mL) were added molecular sieves 4 Å (30 g), ethanethiol (19.35 mL), and freshly fused $ZnCl_2$ (7 g). After 15 min, the reaction was complete (t.l.c., R_F 0.74 (α) and 0.63 (β), 9:1 dichloromethane– ethyl acetate), and the mixture was filtered through Celite, washed thrice each with M sulfuric acid and aq. saturated sodium hydrogencarbonate, and twice with water, dried ($MgSO_4$), filtered, and concentrated. After crystallisation and purification of the material in the mother liquor by column chromatography

(9:1 dichloromethane–ethyl acetate), **7** (7.05 g, 70%) was obtained with m.p. 98° (from ethanol), $[\alpha]_D$ −19° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.261 (t, 3 H, J_{CH_3,CH_2} 7.2 Hz, SCH_2CH_3), 2.069, 2.079, and 2.106 (3 s, each 3 H, 3 Ac), 2.698 (m, 2 H, SCH_2CH_3), 4.398 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.003 and 5.059 (2 d, 2 H, $OCH_2CH{=}CH_2$), 5.136 and 5.205 (2 m, 2 H, $OCH_2CH{=}CH_2$), 5.778 (m, 1 H, $OCH_2CH{=}CH_2$); ^{13}C, δ 14.4 (SCH_2CH_3), 20.3, 20.4, and 20.5 (3 CH_3CO), 23.7 (SCH_2CH_3), 62.1 (C-6), 72.8 ($OCH_2CH{=}CH_2$), 69.3, 70.9, 75.6, and 80.8 (C-2,3,4,5), 83.3 (C-1), 116.4 ($OCH_2CH{=}CH_2$), 133.9 ($OCH_2CH{=}CH_2$), 169.9–170.2 (3 CH_3CO).

Anal. Calc. for $C_{17}H_{26}O_8S$: C, 52.29; H, 6.73. Found: C, 52.31; H, 6.76.

α-Anomer, $[\alpha]_D$ +105° (*c* 1, dichloromethane).

Ethyl 3-O-allyl-2,4,6-tri-O-benzyl-1-thio-β-D-glucopyranoside (**9**). — To a solution of **7** (4.75 g, 12.2 mmol) in dry methanol (50 mL) was added sodium methoxide (pH 10). After stirring for 18 h, deacetylation was complete (R_F 0.20, t.l.c., 95:5 dichloromethane–methanol), and the mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated to yield **8** (3.15 g, 97%). This compound was used without further purification. To a suspension of sodium hydride (73.2 mmol, 6 equiv.) in *N,N*-dimethylformamide (20 mL) was added a solution of **8** (1.47 g, 5.5 mmol) and benzyl bromide (3 mL) in *N,N*-dimethylformamide (30 mL) dropwise at 0°. The mixture was stirred for 2 h at room temperature [R_F 0.47, t.l.c., 9:1 light petroleum (b.p. 40–60°)–ethyl acetate], the excess of sodium hydride was destroyed with methanol, the mixture was poured onto ice and extracted with ether (3 × 50 mL), and the extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography [9:1 light petroleum (b.p. 40–60°)–ethyl acetate] to yield **9**, isolated as a syrup (2.68 g, 91%), $[\alpha]_D$ 0° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.320 (t, 3 H, J_{CH_3,CH_2} 7.5 Hz, SCH_2CH_3), 2.694–2.822 (m, 2 H, SCH_2CH_3), 3.375 (dd, 1 H, $J_{1,2}$ 9.5, $J_{2,3}$ 8.5 Hz, H-2), 3.423 (m, 1 H, H-5), 3.500–3.746 (m, 4 H, H-3,4,6a,6b), 4.418 (d, 1 H, H-1), 4.348 (m, 2 H, $OCH_2CH{=}CH_2$), 4.532–4.888 (m, 6 H, 3 $PhCH_2O$), 5.171 and 5.292 (2 m, 2 H, $OCH_2CH{=}CH_2$), 5.971 (m, 1 H, $OCH_2CH{=}CH_2$); ^{13}C, δ 15.2 (SCH_2CH_3), 24.9 (SCH_2CH_3), 69.2 (C-6), 73.4, 74.4, 75.0, and 75.5 (3 $C_6H_5CH_2$ and 1 $OCH_2CH{=}CH_2$), 77.9, 79.1, 81.7, and 85.0 (C-2,3,4,5), 86.3 (C-1), 116.7 ($OCH_2CH{=}CH_2$), 127.5–128.3 and 138.0–138.2 ($C_6H_5CH_2$), 135.0 ($OCH_2CH{=}CH_2$).

Anal. Calc. for $C_{32}H_{38}O_5S$: C, 71.88; H, 7.18. Found: C, 71.85; H, 7.23.

4-Methoxybenzyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranoside (**11**). — A mixture of dry nitromethane (40 mL), dry toluene (40 mL), 4-methoxybenzyl alcohol (8.16 mmol, 1.2 equiv.), $Hg(CN)_2$ (8.16 mmol, 1.2 equiv.), and molecular sieves 4 Å (5 g) was stirred and 40 mL of solvent was distilled off. A solution of **10**[13] (2.42 g, 6.8 mmol) in nitromethane (20 mL) and toluene (20 mL) was added dropwise, and the mixture was stirred until t.l.c. demonstrated that the reaction was complete (R_F 0.57, 9:1 dichloromethane–ethyl acetate). After filtration and concentration, the residue was dissolved in dichloromethane (200 mL), and the solution was washed with aq. 5% potassium iodide (30 mL), dried ($\mathrm{MgSO_4}$), filtered, and concentrated. The residue was purified by column chromatography (25:1 toluene–acetone) to yield **11**, isolated as a syrup (1.18 g, 42%), $[\alpha]_D$ −58° (*c* 1, chloroform). N.m.r. data ($\mathrm{CDCl_3}$): ^{1}H, δ 1.22 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.95, 2.03, and 2.10 (3 s, each 3 H, 3 Ac), 3.80 (s, 3 H, OMe), 6.90 and 7.30 (2 d, 4 H, $\mathrm{MeOC_6}H_4\mathrm{CH_2O}$); ^{13}C, δ 17.0 (C-6), 20.3 (3 $C\mathrm{H_3CO}$), 54.8 (OMe), 68.8 ($\mathrm{MeOC_6H_4}C\mathrm{H_2}$), 66.1, 68.7, 68.9, and 70.1 (C-2,3,4,5), 95.9 (C-1), 113.5, 129.4, and 159.2 ($\mathrm{MeO}C_6\mathrm{H_4CH_2}$), 169.6 ($\mathrm{CH_3}C\mathrm{O}$).

4-Methoxybenzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (**13**). — To a solution of **11** (1.8 g, 4.4 mmol) in dry methanol (9 mL) was added sodium methoxide (pH 10). After stirring overnight (R_F 0.26, t.l.c., 1:1 toluene–acetone), Dowex-50 ($\mathrm{H^+}$) resin was added (pH 7) followed by filtration, concentration, and purification of the residue by column chromatography (1:1 toluene–acetone) to yield **12**, isolated as a syrup (1.1 g, 88%), $[\alpha]_D$ −76° (*c* 1, chloroform). To a solution of **12** (2.65 g, 9.33 mmol) in 2,2-dimethoxypropane (75 mL) was added a catalytic amount of *p*TsOH. The reaction was complete after 15 min (R_F 0.47, t.l.c., 9:1 dichloromethane–acetone). Dichloromethane (100 mL) was added, and the organic phase was washed with water (50 mL), dried ($\mathrm{MgSO_4}$), filtered, and concentrated. The residue was purified by column chromatography (9:1 dichloromethane–acetone) to yield **13**, isolated as a syrup (3.00 g, 99%), $[\alpha]_D$ −43° (*c* 1, chloroform). ^{1}H-N.m.r. data ($\mathrm{CDCl_3}$): δ 1.25 (d, 3 H, H-6), 1.30 and 1.43 (2 s, each 3 H, $\mathrm{CMe_2}$), 3.80 (s, 3 H, OMe), 6.83 and 7.23 (2 d, 4 H, $\mathrm{MeOC_6}H_4\mathrm{CH_2O}$).

Anal. Calc. for $\mathrm{C_{17}H_{24}O_6}$: C, 62.94; H, 7.47. Found: C, 63.30; H, 7.52.

4-Methoxybenzyl 4-O-benzyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (**14**). — A solution of **13** (3.05 g, 9.41 mmol) and benzyl bromide (1.7 mL) in *N,N*-dimethylformamide (50 mL) was added dropwise to a stirred suspension of sodium hydride (18.8 mmol, 2 equiv.) in *N,N*-dimethylformamide (75 mL) at 0°. The stirring was continued for 2 h at room temperature, when t.l.c. demonstrated that the reaction was complete (R_F 0.75, 9:1 toluene–acetone). The excess of sodium hydride was destroyed with methanol, the mixture was poured onto ice and extracted with ether (3 × 75 mL), and the combined extracts were dried ($\mathrm{MgSO_4}$), filtered, and concentrated. The residue was purified by column chromatography (9:1 toluene–acetone) to yield **14**, isolated as a syrup (3.50 g, 90%), $[\alpha]_D$ −62° (*c* 1, chloroform). ^{1}H-N.m.r. data ($\mathrm{CDCl_3}$): δ 1.30 (d, 3 H, H-6), 1.33 and 1.46 (2 s, each 3 H, $\mathrm{CMe_2}$), 3.70 (s, 3 H, OMe), 6.60–6.80 and 7.00–7.20 (m, 9 H, Ph and $\mathrm{MeOC_6}H_4\mathrm{CH_2O}$).

4-Methoxybenzyl 4-O-benzyl-α-L-rhamnopyranoside (**15**). — A stirred suspension of **14** (3.89 g, 9.39 mmol) in 1:1 acetic acid–water (94 mL) was heated at 70° until the de-isopropylidenation was complete (2–3 h) as shown by t.l.c. (9:1 dichloromethane–acetone). The solvent was co-evaporated with toluene, and the residue was purified by column chromatography (9:1 dichloromethane–acetone) to yield **15**, isolated as a syrup (2.66 g, 76%), $[\alpha]_D$ −65° (*c* 1, chloroform), R_F 0.27 (9:1 dichloromethane–acetone). ^{1}H-N.m.r. data ($\mathrm{CDCl_3}$): δ 1.362 (d, 3 H, $J_{5,6}$ 6.0

Hz, H-6), 3.780 (s, 3 H, OMe), 4.412 and 4.617 (2 d, 2 H, $MeOC_6H_4CH_2O$), 4.726 and 4.754 (2 d, 2 H, $PhCH_2O$), 4.831 (s, 1 H, H-1), 6.868 and 7.240 (2 d, 4 H, $MeOC_6H_4CH_2O$), 7.289–7.382 (m, 5 H, Ph).

Anal. Calc. for $C_{21}H_{26}O_6$: C, 67.35; H, 7.01. Found: C, 67.37; H, 7.06.

4-Methoxybenzyl 2,4-di-O-benzyl-α-L-rhamnopyranoside (**16**). — To a solution of **15** (1.6 g, 4.27 mmol) in dichloromethane (42.8 mL) were added tetrabutylammonium bromide (344 mg), benzyl bromide (5.6 mL), and aq. 10% sodium hydroxide (4.28 mL). After stirring overnight, the reaction was complete (R_F 0.24, t.l.c., 50:1 dichloromethane–acetone). The mixture was washed with water, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (50:1 dichloromethane–acetone) to yield **16**, isolated as a syrup (1.80 g, 91%), $[\alpha]_D$ −39° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 1.339 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6), 3.334 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4), 3.789 (s, 3 H, OMe), 4.885 (s, 1 H, H-1), 4.369–4.907 (6 d, 6 H, 3 $PhCH_2O$), 6.863 and 7.216 (2 d, 4 H, $MeOC_6H_4CH_2O$), 7.231–7.371 (m, 10 H, 2 Ph); ^{13}C, δ 17.8 (C-6), 54.9 (OMe), 68.3, 72.7, and 74.7 (2 $C_6H_5CH_2$ and 1 $MeOC_6H_4CH_2$), 67.1, 71.4, 78.4, and 82.0 (C-2,3,4,5), 95.7 (C-1), 113.5, 127.4–129.2, 137.5–138.1, and 159.0 (2 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.38; H, 6.96. Found: C, 72.29; H, 7.07.

4-Methoxybenzyl 3-O-(3-O-allyl-2,4,6-tri-O-benzyl-α-D-glucopyranosyl)-2,4-di-O-benzyl-α-L-rhamnopyranoside (**17**). — A solution of **16** (1.31 g, 2.82 mmol) and **9** (2.93 g, 5.48 mmol) in dry ether (42 mL) containing powdered molecular sieves 4 Å (7.5 g) was stirred for 2 h in the dark. Methyl triflate (722 μL) was added and the mixture was stirred overnight, when t.l.c. showed that the reaction was complete [R_F 0.35 (α) and 0.43 (β), 8:2 light petroleum (b.p. 40–60°)–ethyl acetate]. Triethylamine (1.5 mL) was added, the mixture was filtered through Celite, dichloromethane (40 mL) was added, and the organic phase was washed with aq. saturated sodium hydrogencarbonate (50 mL) and water (2 × 50 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography [8:2 light petroleum (b.p. 40–60°)–ethyl acetate] to yield **17**, isolated as a syrup (1.55 g, 59%), $[\alpha]_D$ +24° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 1.342 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6), 3.789 (s, 3 H, OMe), 4.264–4.888 (12 d, 12 H, 5 $PhCH_2O$, $MeOC_6H_4CH_2O$; 2 dd, 2 H, $OCH_2CH{=}CH_2$), 4.753 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.119 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 5.119 and 5.260 (2 m, 2 H, $OCH_2CH{=}CH_2$), 5.995 (m, 1 H, $OCH_2CH{=}CH_2$); ^{13}C, δ 17.9 (C-6), 55.1 (OMe), 68.0 (C-6'), 68.2, 70.1, 74.8, 76.0, 77.6, 79.2, 80.0, and 81.8 (C-2,3,4,5,2',3',4',5'), 68.3 and 73.0–75.1 (5 $C_6H_5CH_2$, $MeOC_6H_4CH_2$, and $OCH_2CH{=}CH_2$), 94.9 (C-1), 96.9 (C-1'), 116.4 ($OCH_2CH{=}CH_2$), 113.6, 127.4–129.3, and 137.8–138.5 (5 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$), 135.2 ($OCH_2CH{=}CH_2$).

Anal. Calc. for $C_{58}H_{64}O_{11}$: C, 74.32; H, 6.90. Found: C, 74.35; H, 6.90.

4-Methoxybenzyl 2,4-di-O-benzyl-3-O-(2,4,6-tri-O-benzyl-α-D-glucopyranosyl)-α-L-rhamnopyranoside (**18**). — To a solution of **17** (329 mg, 0.351 mmol) in 7:3:1 ethanol–toluene–water (10.2 mL) was added tris(triphenylphosphine)rhodium(I) chloride (102 mg); the mixture was stirred and boiled under reflux overnight, and became dark brown. After concentration at low temperature, the residue was dissolved in 10:1 acetone–water (6 mL), and HgO (215 mg) and $HgCl_2$ (193 mg) were added. The removal of the propenyl group occurred after 30–45 min, as shown by t.l.c. (R_F 0.18, 25:1 toluene–acetone). The mixture was filtered and concentrated, and a solution of the residue in dichloromethane (50 mL) was washed with aq. 5% potassium iodide (2 × 20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (25:1 toluene–acetone) to yield **18**, isolated as a syrup (132 mg, 42%), $[\alpha]_D$ +30° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.346 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6), 3.800 (s, 3 H, OMe), 4.819 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1), 5.208 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 6.841, 7.378–7.064 (m, 29 H, 5 Ph and $MeOC_6H_4CH_2O$); ^{13}C, δ 18.0 (C-6), 55.2 (OMe), 68.3, 68.4, 68.5, 69.9, 72.2, 72.9, 73.3, 73.9, 75.3, 74.5, 75.4, 75.7, 77.7, 79.2, and 80.2 (C-2,3,4,5,2',3',4',5',6', 5 $C_6H_5CH_2$, and $MeOC_6H_4CH_2$), 94.0 (C-1), 96.7 (C-1'), 113.7, 127.4–130.8 and 137.8–138.7 (5 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$).

Anal. Calc. for $C_{55}H_{60}O_{11}$: C, 73.63; H, 6.75. Found: C, 73.44; H, 6.81.

A solution of **17** (1.47 g, 1.57 mmol) in *N*,*N*-dimethylformamide (28 mL) was heated at 80°, and $KOBu^t$ (750 mg) was added. After 2.5 h, the reaction was complete (t.l.c., 25:1 toluene–acetone). The mixture was cooled, dichloromethane (30 mL) was added, and the organic phase was washed with water (10 mL), dried ($MgSO_4$), filtered, and concentrated. The dark-coloured syrup was dissolved in 9:1 acetone–0.1M hydrochloric acid (10 mL) and boiled under reflux for 30 min, when t.l.c. (25:1 toluene–acetone) showed that the reaction was complete. The mixture was extracted with dichloromethane, and the extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (25:1 toluene–acetone) to yield **18**, isolated as a syrup (1.0 g, 72%).

*4-Methoxybenzyl 2,4-di-O-benzyl-3-O-[2,4,6-tri-O-benzyl-3-O-(3,4,6-tri-O-acetyl-2-O-allyl-α-*D*-galactopyranosyl)-α-*D*-glucopyranosyl]-α-*L*-rhamnopyranoside* (**19**). — A suspension of **18** (0.91 g, 1.01 mmol), **5** (0.90 g, 1.83 mmol), and powdered molecular sieves 3 Å (15 g) in dry ether (50 mL) was stirred for 2 h under nitrogen at room temperature. Then, at −30°, trimethylsilyl triflate (200 μL, 1.10 mmol) was added and the mixture was stirred for 30 min, when t.l.c. showed the reaction to be complete (R_F 0.55, 96:4 dichloromethane–ethyl acetate). Pyridine (500 μL) was added, the mixture was dissolved in dichloromethane (50 mL), and the solution was filtered through Celite, washed with water (20 mL), aq. saturated sodium hydrogencarbonate (20 mL), and water (20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (96:4 dichloromethane–ethyl acetate) to yield **19**, isolated as a syrup (1.00 g, 82%). N.m.r. data ($CDCl_3$): ^1H, δ 1.362 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.853, 2.003, and 2.034 (3 s, each 3 H, 3 Ac), 3.476 (dd, 1 H, H-6a'), 3.543 (dd, 1 H, H-6b'), 3.578 (dd, 1 H, H-6a"), 3.644 (t, 1 H, $J_{4,5} = J_{3,4}$ 9.3 Hz, H-4), 3.673 (dd, 1 H, $J_{2',3'}$ 9.9, $J_{1',2'}$ 3.4 Hz, H-2'), 3.753 (dd, 1 H, $J_{2'',3''}$ 10.7, $J_{1'',2''}$ 3.6 Hz, H-2"), 3.78 (m, 1 H, H-5), 3.790 (s, 3 H, OMe), 3.847 (dd, 1 H, H-6b"), 3.852 (dd, 1 H, $J_{4',5'} = J_{3',4'}$ 9.8 Hz, H-4'), 3.940 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.00 (m, 1 H, H-5'), 4.176 (dd, 1 H, H-3), 4.375

(t, 1 H, H-3′), 4.678 (m, 1 H, H-5″), 4.902 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.014 and 5.066 (2 m, 2 H, $OCH_2CH{=}CH_2$), 5.188 (dd, 1 H, $J_{3'',4''}$ 3.3, $J_{4'',5''}$ 1.3 Hz, H-4″), 5.300 (d, 1 H, H-1′), 5.361 (dd, 1 H, H-3″), 5.605 (d, 1 H, H-1″), 5.640 (m, 1 H, $OCH_2CH{=}CH_2$), 6.835–6.852 and 7.063–7.357 (m, 29 H, 5 Ph and $MeOC_6H_4CH_2O$); ^{13}C, δ 17.8 (C-6), 20.4 and 20.6 (2 C) (3 CH_3CO), 55.0 (OMe), 61.4 (C-6″), 92.8 (C-1), 96.1 (C-1′), 97.6 (C-1″), 117.5 ($OCH_2CH{=}CH_2$), 113.6, 126.4, and 159.0 ($MeOC_6H_4CH_2$), 127.0–129.3 and 136.9–138.3 (5 $C_6H_5CH_2$), 169.6, 169.9, and 170.2 (3 CH_3CO).

Anal. Calc. for $C_{70}H_{80}O_{19}$: C, 68.60; H, 6.59. Found: C, 68.20; H, 6.70.

*4-Methoxybenzyl 2,4-di-O-benzyl-3-O-[2,4,6-tri-O-benzyl-3-O-(3,4,6-tri-O-benzyl-α-*D*-galactopyranosyl)-α-*D*-glucopyranosyl]-α-*L*-rhamnopyranoside* (**22**). — To a solution of **19** (1.00 g, 0.82 mmol) in dry methanol (15 mL) was added sodium methoxide to pH 10. After stirring overnight at room temperature, deacetylation was complete as shown by t.l.c. (R_F 0.31, 3:1 toluene–acetone). The mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. The residue was purified by column chromatography (3:1 toluene–acetone) to yield **20**, isolated as a syrup (870 mg, 98%). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.9 (C-6), 55.1 (OMe), 93.3 (C-1), 96.1 (C-1′), 97.1 (C-1″), 113.7, 126.3–129.4, 137.1–138.2 and 159.1 (5 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$), 117.9 ($OCH_2CH{=}CH_2$), 134.2 ($OCH_2CH{=}CH_2$).

Anal. Calc. for $C_{64}H_{74}O_{16}$: C, 69.91; H, 6.80. Found: C, 69.65; H, 6.85.

To a suspension of sodium hydride (39 mg, 6 equiv.) in *N,N*-dimethylformamide (2 mL) was added a mixture of **20** (304 mg, 0.28 mmol) and benzyl bromide (0.12 mL) in *N,N*-dimethylformamide (1.5 mL) at 0°. After 2 h, the benzylation was complete (R_F 0.36, t.l.c., 25:1 toluene–acetone). The excess of sodium hydride was destroyed with methanol, the mixture was poured onto ice and extracted with ether (3 × 10 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (25:1 toluene–acetone) to afford **21**, isolated as a syrup (338 mg, 95%), $[\alpha]_D$ +42° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.340 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 3.722 (s, 3 H, OMe), 4.059 and 4.262 (2 m, 2 H, $OCH_2CH{=}CH_2$), 4.896 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.074 and 5.109 (2 m, 2 H, $OCH_2CH{=}CH_2$), 5.215 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 5.628 (d, 1 H, $J_{1'',2''}$ 3.3 Hz, H-1″), 5.738 (m, 1 H, $OCH_2CH{=}CH_2$), 6.816 and 7.060–7.349 (m, 44 H, 8 Ph and $MeOC_6H_4CH_2$); ^{13}C, δ 17.8 (C-6), 55.1 (OMe), 93.7 (C-1), 96.6 (C-1′), 97.8 (C-1″), 113.6, 126.7–129.3, 137.6–138.7, and 159.1 (8 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$), 117.4 ($OCH_2CH{=}CH_2$), 134.8 ($OCH_2CH{=}CH_2$).

Anal. Calc. for $C_{85}H_{91}O_{16}$: C, 74.58; H, 6.71. Found: C, 74.14; H, 6.81.

A solution of **21** (277 mg, 0.22 mmol) in *N,N*-dimethylformamide (4 mL) was heated at 80°, and $KOBu^t$ (110 mg) was added. After 2.5 h, the isomerisation was complete, as shown by t.l.c. (R_F 0.48, 20:1 toluene–acetone). The mixture was cooled and, after the addition of dichloromethane (20 mL), washed with water (10 mL), aq. saturated sodium hydrogencarbonate (10 mL), and water (10 mL), dried ($MgSO_4$), filtered, and concentrated. A solution of the residue, dissolved in 9:1 acetone–0.1M hydrochloride acid (5 mL), was boiled under reflux until t.l.c. (15:1

toluene–acetone) showed complete conversion into **22** (R_F 0.35). The mixture was taken up in dichloromethane (20 mL), washed with water (10 mL), aq. saturated sodium hydrogencarbonate (10 mL), and water (10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (25:1 toluene–acetone) to yield **22**, isolated as a syrup (213 mg, 79%). N.m.r. data ($CDCl_3$): ^1H, δ 1.335 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 3.772 (s, 3 H, OMe), 4.831 (bs, 1 H, H-1), 5.137 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 5.602 (d, 1 H, $J_{1'',2''}$ 4.0 Hz, H-1″), 6.839 and 7.022–7.324 (m, 44 H, 8 Ph and $MeOC_6H_4CH_2$); ^{13}C, δ 17.8 (C-6), 55.1 (OMe), 94.2 (C-1), 96.7 (C-1′), 98.7 (C-1″), 113.6, 127.5–129.4, 137.5–138.5, and 159.1 (8 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$).

Anal. Calc. for $C_{82}H_{88}O_{16}$: C, 74.06; H, 6.68. Found: C, 74.04; H, 6.89.

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