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Synthesis of a spacer-containing repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* type 23F

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ABSTRACT

The synthesis is reported of 3-aminopropyl 4-*O*-(4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl)-β-L-rhamnopyranoside 3′-(glycer-2-yl sodium phosphate) (**25β**), which represents the repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* type 23F (American type 23) ({→4)-β-D-Glc*p*-(1→4)-[Glycerol-(2-**P**→3)][α-L-Rha*p*-(1→2)]-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→}$_n$). 2,4,6-Tri-*O*-acetyl-3-*O*-allyl-α-D-galactopyranosyl trichloroacetimidate (**5**) was coupled with ethyl 2,3-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**6**). Deacetylation of the resulting disaccharide derivative, followed by benzylidenation, and condensation with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**10**) afforded ethyl 4-*O*-[3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**11**). Deacetylation of **11**, followed by benzylation, selective benzylidene ring-opening, and coupling with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**15**) gave ethyl 4-*O*-[3-*O*-allyl-6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**16**). Deacetylation of **16** followed by benzylation, deallylation, and acetylation yielded ethyl 4-*O*-[3-*O*-acetyl-6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**20**). The glycosyl bromide derived from **20**, when coupled with 3-benzyloxycarbonylamino-1-propanol, gave the β-glycoside (**21β**) as the major product. Deacetylation of **21β** followed by condensation with 1,3-di-*O*-benzylglycerol 2-(triethylammonium phosphonate) (**27**), oxidation, and deprotection, afforded **25β**.

INTRODUCTION

Streptococcus pneumoniae can induce infections such as pneumonia, otitis media, and meningitis in human beings. A polysaccharide vaccine[1] (Pneumovax© 23) against pneumococcal diseases is available, which contains the capsular polysaccharides isolated from 23 species of *S. pneumoniae*. However, these polysaccharides are non-immunogenic in newborns and do not induce a long-lasting immunological memory (TI-response), and the induction of tolerance is a severe problem[2]. In the context of developing new vaccines, oligosaccharide conjugates (neoglycoproteins/neoglycolipids), produced from oligosaccharides obtained by synthesis or degradation of polysaccharides, are of interest. Oligosaccharides related to pneumococcal polysaccharides of types 3 (ref. 3), 6A/6B (refs. 4–6), 9V (ref. 7), 14 (refs. 8, 9), 19A (ref. 10), and 19F (ref.

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11) have been synthesised and others related to types 3, 6B, and 14 (refs. 12, 13, and 14, respectively) have been obtained by degradation of the polysaccharides. We now report on the synthesis of an oligosaccharide fragment of the capsular polysaccharide of *S. pneumoniae* type 23F (American type 23). Three different structures **1A**–**1C** have been published[15–17] for this capsular polysaccharide. At the start of our project, structure **1A** (ref. 15) and its revised form **1B** (ref. 16) were available, and phosphate-containing tri- and tetra-saccharide fragments related to structure **1B** were synthesised[18]. When this work was completed, the revised structure **1C** was published[17]. The synthesis of non-phosphorylated di- and tri-saccharides related to **1A** and **1B** has been described[19]. In order to obtain additional evidence for the structure **1C**, $^{1}H\{^{31}P\}$ relayed spin-echo difference spectroscopy (RESED)[20] was applied in order to identify the sugar residue attached to the (glycero)phosphate group. The resulting sub-spectrum (Fig. 1) of the phosphorylated sugar residues in the capsular polysaccharide type 23F proves that the phosphodiester bridge is between glycerol and galactose, in agreement with the revised structure **1C**.

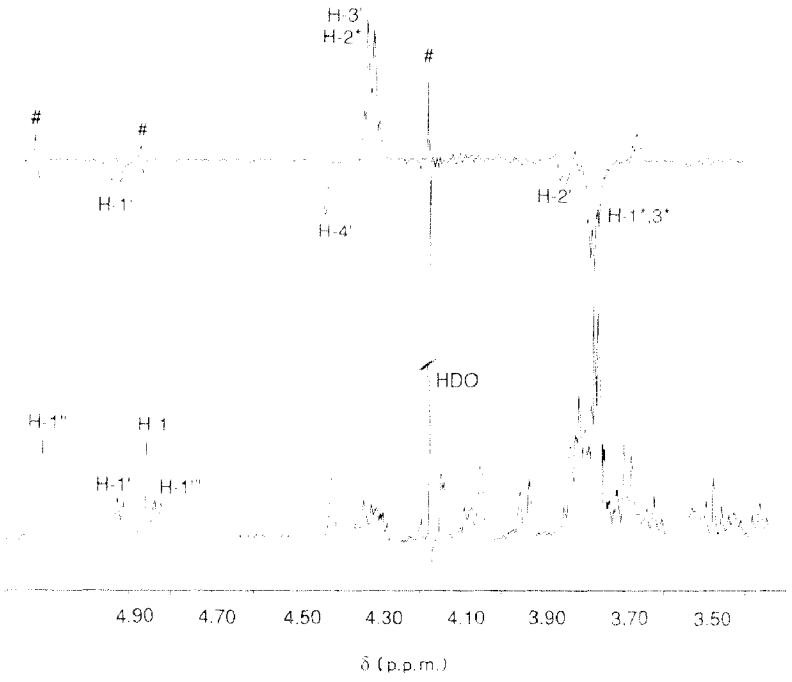
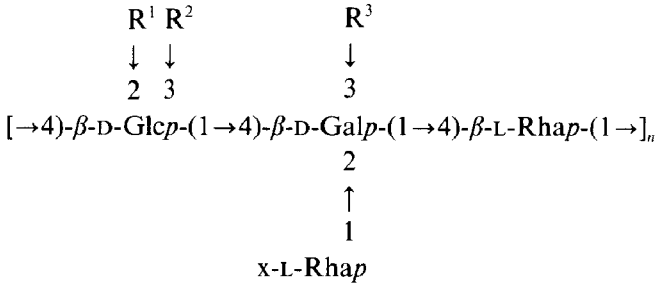


Fig. 1. $^{1}H\{^{31}P\}$-RESED and ^{1}H-n.m.r. spectra (3.30–5.20 p.p.m.) of the capsular polysaccharide of *Streptococcus pneumoniae* type 23F, recorded at 82° (single-primed numbers, galactose residue; *, glycerol residue; #, subtraction artefacts).



1A, R^1 = glycerol 1-phosphate, $R^2 = R^3 = OH$, x = β

1B, R^2 = phosphate, $R^1 = R^3 = OH$, x = α

1C, $R^1 = R^2 = OH$, R^3 = glycerol 2-phosphate, x = α

We now report the synthesis of 3-aminopropyl 4-*O*-(4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl)-β-L-rhamnopyranoside 3′-(glycer-2-yl sodium phosphate) (**25β**)*, the spacer-coupled repeating unit of polysaccharide **1C**.

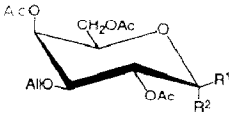
RESULTS AND DISCUSSION

In the synthesis of **25β**, the selectively protected tetrasaccharide thioglycoside **20** was the key intermediate. Thioglycosides allow coupling to different aglycons, and oligosaccharide thioglycosides can be used in the stepwise synthesis of higher oligomers. Thioglycosides[21] can be activated by thiophilic reagents or converted into the corresponding glycosyl bromides. Problems that can arise from the use of thioglycosides as acceptors have been reported, *e.g.*, formation of the thioglycoside of the donor during coupling[22]. However, the use of glycosyl trichloroacetimidates as donors with thioglycosides as acceptors has given promising results[18].

2,4,6-Tri-*O*-acetyl-3-*O*-allyl-α-D-galactopyranosyl trichloroacetimidate (**5**) was used in the synthesis of **20** and **25β**. Deacetylation of **7**, followed by 4,6-*O*-benzylidenation allows a protected rhamnose residue to be attached at C-2 and subsequent selective reductive ring-opening exposes HO-4, to which a suitably protected glucose residue can be coupled (→→**18**). The 3-*O*-allyl group can be removed selectively from **18**, so that in a final stage the glycerol 2-phosphate unit can be introduced. For the synthesis of **5**, methyl 3-*O*-allyl-β-D-galactopyranoside[23] was acetylated (→**2**, 99%) and, after conversion of MeO-1 into AcO-1 by acetolysis (2% sulfuric acid in acetic anhydride for 1.5 h at 0°), **3** was deacetylated at C-1 using hydrazine acetate[24] (→**4**, 28%

* 3-Aminopropyl 4-*O*-(4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl)-β-L-rhamnopyranoside 3′-[2-hydroxy-1-(hydroxymethyl)ethyl sodium phosphate] (**25β**).

from **2**) and the product was treated with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene[25] to afford **5** (78%). Demethylation of **2** was more difficult than for methyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-galactopyranoside[18]. Methods applied in order to expose HO-1, such as treatment with hydrochloric acid in acetic acid[26] or boron trichloride in dichloromethane[27], yielded complex mixtures.

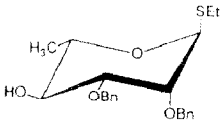


2 R^1 = OMe, R^2 = H

3 R^1, R^2 = OAc, H

4 R^1, R^2 = OH, H

5 R^1 = H, R^2 = OCNHCCl$_3$



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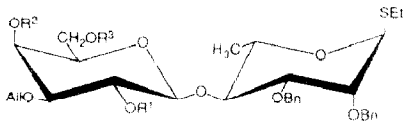
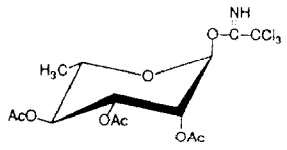


7 R^1 = R^2 = R^3 = Ac

8 R^1 = R^2 = R^3 = H

9 R^1 = H, R^2, R^3 = CHPh



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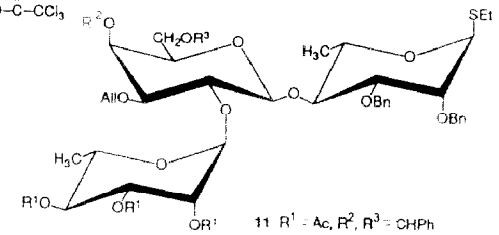
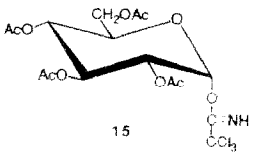


11 R^1 = Ac, R^2, R^3 = CHPh

12 R^1 = H, R^2,R^3 = CHPh

13 R^1 = Bn, R^2, R^3 = CHPh

14 R^1 = R^3 = Bn, R^2 = H



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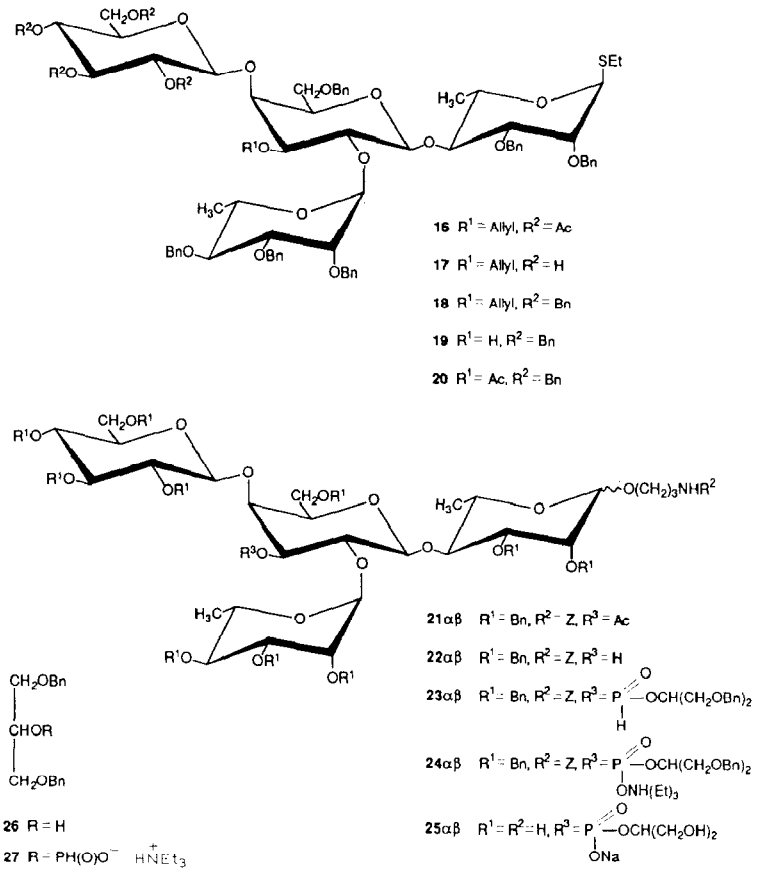
Condensation of **5** with ethyl 2,3-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside[18] (**6**) in dichloromethane at −30°, using trimethylsilyl triflate[18] as a catalyst, gave the disaccharide derivative **7**, which was easily separated from the starting compounds after deacetylation (→**8**, 70% from **6**). Compound **8** was benzylidenated (→**9**, 79%), condensed with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**10**; →**11**) under the above conditions and the product was deacetylated to afford **12** (78% from **9**). Sodium methoxide was removed by washing with water, because neutralisation with Dowex-50 (H^+) resin causes debenzylidenation[18]. Benzylation of **12** (→**13**, 93%) followed by selective opening of the 4,6-*O*-benzylidene ring with borane–trimethylamine complex and aluminium(III) chloride in tetrahydrofuran[28] yielded **14** (69%).

The tetrasaccharide derivative **16** was prepared by coupling 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[29] (**15**) with HO-4 of **14**, using trimethylsilyl triflate as a catalyst. The formation of **16** occurred within a few minutes at −10°; at −30°, only one faster-moving compound was observed in t.l.c., which was converted into **16** when the temperature was raised to −10°. Deacetylation of **16** yielded **17** (67% from **14**).



The tetrasaccharide derivative **17** was benzylated (→**18**, 68%). The allyl group was removed from **18** using the Wilkinson catalyst[30] in the presence of 1,4-diazabicyclo[2.2.2]octane followed by hydrolysis (→**19**, 73%), and acetylation then gave **20** (93%). The replacement of the 3-*O*-allyl group of the galactose moiety by an acetyl group (**18**→**20**), before coupling to the intended spacer, was necessary because of the instability of the allyl group under the conditions applied for the transformation of the ethyl thioglycoside into a glycosyl bromide[18] using bromine (see below). Deallylation in the absence of base afforded only 40% of **19**. The use of potassium *tert*-butoxide[31] to isomerise the allyl group gave complex mixtures (t.l.c.), as occurred when palladium chloride[25,32] was used to remove the allyl group.

The key intermediate **20** was coupled to the spacer 3-benzyloxycarbonylamino-1-propanol[33,34]. Test reactions on mono- and di-saccharide thioglycosides showed that direct coupling mediated by methyl triflate yielded a considerable proportion of the α-glycoside in addition to the desired β form, but reaction with the corresponding glycosyl bromides, using the insoluble silver catalyst method[35], seemed more promising. The coupling of the bromide of **20**, generated *in situ*[36] with copper(II) bromide–

tetrabutylammonium bromide, to the spacer in dichloromethane, using silver silicate[37] as a promoter, proceeded slowly and, after several days, only a small amount of product was formed. When the ethyl thioglycoside **20** was converted into the glycosyl bromide using bromine[38], subsequent condensation with 3-benzyloxycarbonylamino-1-propanol[33] in dichloromethane–toluene gave better results and yielded **21** (64%) with the β-glycoside as the major compound (^{13}C-n.m.r. data: C-1β, δ 101.4, $J_{C-1,H-1}$ 153 Hz)[39]. ^{13}C-N.m.r. spectroscopy indicated that only a small proportion of the α-glycoside was formed. Subsequent deacetylation afforded **22αβ** (98%), with HO-3 of the galactose residue available for formation of the phosphodiester bridge between the tetrasaccharide derivative and glycerol.

The phosphonate method[40–43] chosen for the introduction of the phosphodiester bridge has the advantage that the intermediate phosphonic mono- and di-esters can be purified easily on silica gel. The phosphonic diester bridge was introduced by coupling the triethylammonium salt of the protected glycerol 2-phosphonate (**27**) with **22**. Compound **27** (89% from **26**) was prepared from 1,3-di-*O*-tritylglycerol[44] by allylation, detritylation, benzylation, deallylation using potassium *tert*-butoxide[31] (→**26**), and reaction with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one in pyridine[40,42]. Condensation of **27** with **22** in the presence of pivaloyl chloride in pyridine afforded the phosphonic diester **23αβ** (82%). The ^{1}H-n.m.r. spectrum of the product revealed equal amounts of two enantiomers of **23β** [^{1}H-n.m.r. data: δ 6.998 ($J_{P,H}$ 720 Hz) and 6.796 ($J_{P,H}$ 727 Hz), P–H][41]. Mild oxidation of **23** with iodine in water–pyridine (→**24αβ**, 91%), followed by debenzyloxycarbonylation/debenzylation and treatment with Dowex-50 (Na^+) resin, afforded **25αβ** (57%) [^{1}H-n.m.r. data: δ 4.649 ($J_{1,2}$ ~0 Hz), H-1 of **25β**; δ 5.045 ($J_{1,2}$ 1.2 Hz), H-1 of **25α**; α,β ratio 1:4). High performance anion-exchange chromatography, with pulsed amperometric detection (HPAE-PAD)[45] on a CarboPac PA1 column, was used to obtain pure **25β**. The ^{1}H-n.m.r. data of **25β**, obtained by 2D COSY[46] and 2D HOHAHA[47] measurements, are given in Table I. It may be noted that H-5 of the spacer-linked β-Rha residue resonated at 3.448 p.p.m., whereas H-5 of the terminal α-Rha unit was observed at 4.096 p.p.m. in accord with literature data[48]. The RESED[20] spectrum of **25β** showed clearly that galactose and glycerol are involved in the phosphodiester bridge (^{1}H-n.m.r. data for **25β**: galactose, H-1,2,3,4 at δ 4.94, 3.82, 4.32, and 4.43, respectively; glycerol H-1/3,2 at δ 3.77 and 4.28, respectively). For comparison, the ^{1}H-n.m.r. data of the bacterial polysaccharide have been included in Table I; there are several relatively small deviations that reflect the different microenvironments in the oligosaccharide and the polysaccharide. The reported ^{13}C-n.m.r. data of the polysaccharide [δ 103.5, 102.5, 102.1, and 101.7 (4 C-1), 63.1 (CH_2OH of glycerol), 62.5 and 62.1 (CH_2OH of galactose/glucose), 18.5 and 17.9 (CH_3 of rhamnose)][17] accord with those for **25β** (δ 103.8, 102.7, 102.1, 101.1, 62.7, 62.1, 61.9, 18.5, and 17.8).

Immunological studies of **25β** conjugated to protein will be reported elsewhere.

TABLE I

500-MHz ^{1}H-N.m.r. chemical shift data[a] for **25β** and for the polysaccharide[17] (82°) of *S. pneumoniae* type 23F.

Residue	*Proton (J)*	*δ (p.p.m.) (J in Hz)*			
		25β		*Polysaccharide*	
β-Rha	H-1 ($J_{1,2}$)	4.648	(~0)	4.851	(1.1)
	H-2 ($J_{2,3}$)	3.941	(3.0)	4.043	(2.4)
	H-3 ($J_{3,4}$)	3.80	(9.4)	3.797	(9.5)
	H-4 ($J_{4,5}$)	3.696	(9.4)	3.688	(9.1)
	H-5 ($J_{5,6}$)	3.448	(6.2)	3.418	(5.8)
	H-6	1.359		1.362	
β-Gal	H-1 ($J_{1,2}$)	4.938	(8.0)	4.935	(7.6)
	H-2 ($J_{2,3}$)	3.822	(9.6)	3.824	(9.8)
	H-3 ($J_{3,4}$)	4.325	(2.9)	4.329	(2.8)
	($J_{H,P}$)		(9.6)		(8.6)
	H-4 ($J_{4,5}$)	4.427	(<1)	4.416	(1.3)
	H-5 ($J_{5,6a}$)	n.d		3.803	(2.2)
	H-6a ($J_{6a,6b}$)	n.d.		3.787	(−11.5)
	H-6b ($J_{5,6b}$)	n.d		3.749	(6.0)
α-Rha	H-1 ($J_{1,2}$)	5.075	(1.6)	5.103	(1.0)
	H-2 ($J_{2,3}$)	4.154	(3.5)	4.141	(3.8)
	H-3 ($J_{3,4}$)	3.809	(9.8)	3.814	(9.5)
	H-4 ($J_{4,5}$)	3.470	(9.8)	3.474	(9.5)
	H-5 ($J_{5,6}$)	4.096	(6.3)	4.072	(6.0)
	H-6	1.265		1.267	
β-Glc	H-1 ($J_{1,2}$)	4.813	(8.0)	4.834	(7.4)
	H-2 ($J_{2,3}$)	3.335	(9.4)	3.357	(9.4)
	H-3 ($J_{3,4}$)	3.540	(9.1)	3.697	(9.1)
	H-4 ($J_{4,5}$)	3.404	(9.8)	3.618	(10.0)
	H-5 ($J_{5,6a}$)	3.461	(2.2)	3.519	(1.8)
	H-6a ($J_{6a,6b}$)	3.908	(−12.3)	3.939	(−11.5)
	H-6b ($J_{5,6b}$)	3.746	(n.d)	3.796	(5.7)
Glycerol	H-1a,1b ($J_{1,2}$)	3.77	(5.0)	3.78	(3.9)
	H-2 ($J_{1a,1b}=J_{3a,3b}$)	4.281	(n.d.)	4.290	(−12.2)
	H-3a,3b ($J_{2,3}$)	3.77	(5.0)	3.74	(5.2)
	($J_{H,P}$)		(8.0)		(9.8)
Spacer	H-1	3.122			
	H-2	1.968			
	H-3a	3.968			
	H-3b	3.791			

[a] Chemical shifts are relative to the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 p.p.m.) in D_2O.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra (360 and 500 MHz) were recorded at 25° with a Bruker HX 360 or AM 500 spectrometer (Bijvoet Center, Utrecht University).

2D double-quantum-filtered ^1H–^1H correlation spectra (2D DQF ^1H–^1H COSY) were recorded in the phase-sensitive mode[46], and 2D homonuclear Hartmann–Hahn spectra (2D HOHAHA) with a MLEV-17 mixing sequence of 120 ms[47]. ^{13}C-N.m.r. spectra (APT, 50 MHz) were recorded at 25° with a Bruker WP 200 spectrometer. Chemical shifts (δ) are given in p.p.m. relative to the signal for internal Me_4Si ($CDCl_3$) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225) for ^1H, and to the signal for internal Me_4Si ($CDCl_3$); indirectly to $CDCl_3$, δ 76.9) or external Me_4Si (D_2O; indirectly to internal acetone, δ 31.55) for ^{13}C.

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 1-mL cell. Melting points were determined with a Mettler FP 51 instrument. In the work-up procedures, washings were carried out three times with appropriate quantities of water or aqueous 5% sodium hydrogencarbonate unless indicated otherwise. Evaporations were conducted under reduced pressure at 40° (bath). All solvents were distilled from appropriate drying agents.

Methyl 2,4,6-tri-O-acetyl-3-O-allyl-β-D-galactopyranoside (**2**). — A mixture of methyl 3-*O*-allyl-β-D-galactopyranoside[23] (9.22 g, 39.38 mmol) in pyridine (100 mL) and acetic anhydride (100 mL) was stirred for 16 h at room temperature, then co-concentrated with toluene (3 × 100 mL), ethanol (3 × 100 mL), and dichloromethane (3 × 100 mL). Column chromatography (85:15 dichloromethane–ethyl acetate) of the residue gave **2**, isolated as a syrup (14.32 g, 99%), $[\alpha]_D$ +15° (*c* 1, chloroform), R_f 0.69 (85:15 dichloromethane–ethyl acetate). N.m.r. data ($CDCl_3$): ^{13}C, δ 169.6 ($COCH_3$), 133.5 ($H_2C=CHCH_2O$), 116.6 ($H_2C=CHCH_2O$), 101.5 (C-1), 76.2, 70.3, 69.9, and 65.7 (C-2,3,4,5), 70.0 ($H_2C=CHCH_2O$), 61.3 (C-6), 56.2 (OCH_3), 20.4 and 20.1 (2 C) (3 $COCH_3$); ^1H, δ 5.781 (m, 1 H, $H_2C=CHCH_2O$), 5.415 (bd, 1 H, H-4), 5.237 and 5.163 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.085 (dd, 1 H, H-2), 4.339 (d, 1 H, H-1), 4.169 (d, 2 H, H-6a,b), 4.124 and 3.904 (2 m, each 1 H, $H_2C=CHCH_2O$), 3.813 (bt, 1 H, H-5), 3.513 (dd, 1 H, H-3), 3.499 (s, 3 H, OCH_3), 2.139, 2.088, and 2.071 (3 s, each 3 H, 3 Ac); $J_{1,2}$ 8.1, $J_{2,3}$ 10.0, $J_{3,4}$ 3.5, $J_{4,5}$ < 1, $J_{5,6}$ 6.9 Hz.

Anal. Calc. for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 53.22; H, 6.89.

2,4,6-Tri-O-acetyl-3-O-allyl-α,β-D-galactopyranose (**4**). — To a solution of **2** (6.99 g, 19.42 mmol) in acetic anhydride (73 mL) at 0° was added a solution of conc. sulfuric acid (2.9 mL) in acetic anhydride (70 mL). After 1.5 h at 0°, sodium acetate (12.1 g, 147.4 mmol) was added, the mixture was concentrated, and a solution of the residue in ethyl acetate (500 mL) was washed, dried (Na_2SO_4), filtered, and concentrated to afford **3**, which was used without further purification in the next step. To a solution of **3** (3.61 g) in dry *N,N*-dimethylformamide (10 mL) was added hydrazine acetate (901 mg, 9.77 mmol). After storage for 2 h at room temperature, the mixture was diluted with ethyl acetate (300 mL), washed with aqueous 5% sodium chloride, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (85:15 dichloromethane–ethyl acetate) of the residue afforded **4**, isolated as a syrup (1.90 g, 28%), R_f 0.45 (85:15 dichloro-

methane–ethyl acetate). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.4–170.2 ($COCH_3$), 134.0 ($H_2C=CHCH_2O$, α), 133.7 ($H_2C=CHCH_2O$, β), 117.2 ($H_2C=CHCH_2O$, β), 117.0 ($H_2C=CHCH_2O$, α), 95.6 (C-1β), 90.3 (C-1α), 70.4 ($H_2C=CHCH_2O$), 76.0, 72.5, 70.8, and 66.0 (C-2,3,4,5, β), 72.0, 70.0, 67.3, and 66.0 (C-2,3,4,5, α), 62.1 (C-6α), 61.8 (C-6β), 20.6 = 20.4 ($COCH_3$).

Anal. Calc. for $C_{15}H_{22}O_9$: C, 52.02; H, 6.40. Found: C, 51.71; H, 6.43.

*2,4,6-Tri-*O*-acetyl-3-*O*-allyl-*α*-*D*-galactopyranosyl trichloroacetimidate* (**5**). — To a solution of **4** (1.90 g, 5.49 mmol) in dry dichloromethane (70 mL) and trichloroacetonitrile (6.88 mL, 68.59 mmol) was added a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (0.82 mL, 5.49 mmol) in dichloromethane (10 mL) at 0°, and the mixture was stirred for 1 h. The reaction was then complete (t.l.c., 9:1 dichloromethane = ethyl acetate, R_F 0.57) and the mixture was concentrated. Column chromatography (9:1 dichloromethane = ethyl acetate) of the residue gave **5**, isolated as a yellow syrup (2.09 g, 78%), $[\alpha]_D$ +119° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^{13}C, δ 170.3–170.0 ($COCH_3$), 160.7 ($OCNHCCl_3$), 133.9 ($H_2C=CHCH_2O$), 117.2 ($H_2C=CHCH_2O$), 93.9 (C-1), 90.9 ($OCNHCCl_3$), 72.5, 69.4, 68.7, and 66.7 (C-2,3,4,5), 70.6 ($H_2C=CHCH_2O$), 61.8 (C-6), 20.5 (3 $COCH_3$); ^{1}H, δ 8.639 (s, 1 H, $OCNHCCl_3$), 6.570 (d, 1 H, H-1), 5.820 (m, 1 H, $H_2C=CHCH_2O$), 5.578 (bd, 1 H, H-4), 5.284 and 5.184 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.252 (dd, 1 H, H-2), 4.354 (bt, 1 H, H-5), 4.206 (dd, 1 H, H-6a), 4.052 (dd, 1 H, H-6b), 3.947 (dd, 1 H, H-3), 2.160, 2.047, and 2.039 (3 s, each 3 H, 3 Ac); $J_{1,2}$ 3.6, $J_{2,3}$ 10.4, $J_{3,4}$ 3.4, $J_{4,5}$ < 1, $J_{5,6a}$ 5.9, $J_{5,6b}$ 7.0, $J_{6a,6b}$ −11.4 Hz.

Anal. Calc. for $C_{17}H_{22}Cl_3NO_9$: C, 41.61; H, 4.52. Found: C, 41.01; H, 4.47.

*Ethyl 4-*O*-(3-*O*-allyl-β-*D*-galactopyranosyl)-2,3-di-*O*-benzyl-1-thio-α-*L*-rhamnopyranoside* (**8**). — A solution of **5** (1.50 g, 3.06 mmol) and ethyl 2,3-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside[18] (**6**; 0.93 g, 2.40 mmol) in dry dichloromethane (15 mL) containing molecular sieves (4 Å, 1 g) was stirred for 1 h under argon. A solution of trimethylsilyl triflate (28μL) in dichloromethane (0.5 mL) was added at −30°, and after 5 min, when t.l.c. showed the disappearance of **6** and a new spot **7** [R_F 0.65, 2:1 light petroleum (b.p. 40–60°)–ethyl acetate], pyridine was added, and the mixture was filtered through Celite and concentrated. To a solution of the residue in dry methanol (25 mL) was added sodium methoxide (0.99 g, 18.0 mmol). After 16 h, the solution was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography (8:2 dichloromethane–acetone) of the residue gave **8**, isolated as a white glass (0.99 g, 70%), $[\alpha]_D$ −59° (*c* 1, chloroform), R_F 0.40 (8:2 dichloromethane–acetone). N.m.r. data ($CDCl_3$): ^{13}C, δ 137.4–136.8 and 128.2–127.4 ($C_6H_5CH_2O$), 134.3 ($H_2C=CHCH_2O$), 117.4 ($H_2C=CHCH_2O$), 104.6 (C-1′), 81.1, 80.1, 79.6, 78.6, 75.6, 74.0, 71.4, 67.8, and 66.1 (C-1,2,3,4,5,2′,3′,4′,5′), 71.6 and 71.3 (2 $C_6H_5CH_2O$), 70.6 ($H_2C=CHCH_2O$), 61.3 (C-6′), 25.1 (CH_3CH_2S), 17.3 (C-6), 14.6 (CH_3CH_2S); ^{1}H, δ 7.365 = 7.219 (m, 10 H, 2 Ph), 5.936 (m, 1 H, $H_2C=CHCH_2O$), 5.313 and 5.215 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.260 (s, 1 H, H-1), 5.138 and 4.537 (2 d, each 1 H, $PhCH_2O$), 4.500 (s, 2 H, $PhCH_2O$), 4.483 (d, 1 H, H-1′), 4.015 (m, 1 H, H-5), 3.985 (bd, 1 H, H-4′), 3.710 (t, 1 H, H-4), 3.495 (bt, 1 H, H-5′), 3.347 (dd, 1 H, H-3′), 2.610 = 2.507 (m, 2 H, CH_3CH_2S), 1.368 (d, 3 H, 3 H-6), 1.229 (t, 3 H, CH_3CH_2S); $J_{1,2}$ ~0, $J_{3,4}$ = $J_{4,5}$ = 8.9, $J_{5,6}$ 6.2, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.4, $J_{3',4'}$ 3.4, $J_{4',5'}$ < 1, $J_{CH_2CH_3}$ 7.4 Hz.

Anal. Calc. for $C_{31}H_{42}O_9S \cdot H_2O$: C, 61.17; H, 7.29. Found: C, 61.64; H, 7.08.

Ethyl 4-O-(3-O-allyl-4,6-O-benzylidene-β-D-galactopyranosyl)-2,3-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**9**). — To a solution of **8** (990 mg, 1.68 mmol) in *N,N*-dimethylformamide (4 mL) and α,α-dimethoxytoluene (2 mL) was added *p*-toluenesulfonic acid (150 mg). After 2 h, t.l.c. indicated the reaction to be complete, and solid sodium hydrogencarbonate was added. The mixture was diluted with dichloromethane (100 mL), washed with water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (95:5 dichloromethane–ethyl acetate) of the residue afforded **9**, isolated as a syrup (0.90 g, 79%), $[\alpha]_D$ −47° (*c* 1, chloroform), R_f 0.69 (9:1 dichloromethane–ethyl acetate). N.m.r. data ($CDCl_3$): ^{13}C, δ 137.8–137.0 and 128.7–126.3 ($C_6H_5CH_2O$ and C_6H_5CH), 134.9 ($H_2C=CHCH_2O$), 117.2 ($H_2C=CHCH_2O$), 104.6 (C-1′), 100.9 (PhCH), 81.4, 79.9, 78.7, 78.5, 75.9, 73.2, 70.9, 68.0, and 66.4 (C-1,2,3,4,5,2′,3′,4′,5′), 71.8 and 71.6 (2 $PhCH_2O$), 70.5 ($H_2C=CHCH_2O$), 69.1 (C-6′), 25.2 (CH_3CH_2S), 17.5 (C-6), 14.8 (CH_3CH_2S); ^1H, δ 7.499–7.252 (m, 15 H, 3 Ph), 5.959 (m, 1 H, $H_2C=CHCH_2O$), 5.501 (s, 1 H, PhC*H*), 5.308 and 4.693 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.261 (d, 1 H, H-1), 4.646, 4.562 (2 H) and 4.515 (3 d, 1, 2, and 1 H, 2 $PhCH_2O$), 4.571 (d, 1 H, H-1′), 4.266 and 4.023 (2 m, each 1 H, $H_2C=CHCH_2O$), 4.221 (d, 2 H, 2 H-6′), 4.186 (bd, 1 H, H-4′), 4.054 (m, 1 H, H-5), 3.922 (dd, 1 H, H-2′), 3.880 (t, 1 H, H-4), 3.826 (dd, 1 H, H-3), 3.789 (dd, 1 H, H-2), 3.434 (dd, 1 H, H-3′), 3.370 (bs, 1 H, H-5′), 2.607–2.501 (m, 2 H, CH_3CH_2S), 1.402 (d, 3 H, 3 H-6), 1.226 (t, 3 H, CH_3CH_2S), $J_{1,2}$ 0.9, $J_{2,3}$ 3.0, $J_{3,4} = J_{4,5}$ 9.4, $J_{5,6}$ 6.2, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.5, $J_{4',5'}$ < 1, $J_{CH_3CH_2}$ 7.4 Hz.

Anal. Calc. for $C_{38}H_{46}O_9S$: C, 67.24; H, 6.83. Found: C, 67.06; H, 6.92.

2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**10**). — To a solution of L-rhamnose tetra-acetate (19.33 g, 58.22 mmol) in dry *N,N*-dimethylformamide (50 mL) was added hydrazine acetate (5.99 g, 65.04 mmol). After 1 h, ethyl acetate (600 mL) was added, and the organic phase was washed with aqueous 5% sodium chloride and water, dried (Na_2SO_4), filtered, and concentrated to give 2,3,4-tri-*O*-acetyl-α,β-L-rhamnopyranose, isolated as a white foam (16.53 g, 98%), R_f 0.16 (9:1 dichloromethane–ethyl acetate). To a solution of part (4.00 g, 13.79 mmol) of the product in dichloromethane (170 mL) and trichloroacetonitrile (17.0 mL, 169.5 mmol) was added a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (2.06 mL, 13.79 mmol) in dichloromethane (20 mL) at 0°. The mixture was stirred for 1 h at room temperature, when the reaction was complete (t.l.c.), and then concentrated. Column chromatography (9:1 dichloromethane–ethyl acetate) of the residue gave **10**, isolated as a yellow syrup (5.36 g, 90%), $[\alpha]_D$ −52° (*c* 1, chloroform), R_f 0.73 (9:1 dichloromethane–ethyl acetate). N.m.r. data ($CDCl_3$): ^{13}C, δ 169.7 ($COCH_3$), 94.7 (C-1), 70.3, 69.2, 68.7, and 68.1 (C-2,3,4,5), 17.4 (C-6), 20.6 (2 C), and 20.5 ($COCH_3$); ^1H, 8.734 (s, 1 H, $OCNHCCl_3$), 6.204 (d, 1 H, H-1), 5.463 (dd, 1 H, H-2), 5.370 (dd, 1 H, H-3), 5.179 (t, 1 H, H-4), 4.093 (m, 1 H, H-5), 2.192, 2.075, and 2.007 (3 s, each 3 H, 3 Ac), 1.274 (d, 3 H, 3 H-6), $J_{1,2}$ 2.0, $J_{2,3}$ 3.5, $J_{3,4} = J_{4,5}$ 10.2, $J_{5,6}$ 6.3 Hz.

Ethyl 4-O-(3-O-allyl-4,6-O-benzylidene-2-O-α-L-rhamnopyranosyl-β-D-galactopyranosyl)-2,3-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**12**). — To a solution of **9** (0.90 g, 1.33 mmol) and **10** (0.85 g, 1.96 mmol) in dry dichloromethane (10 mL)

containing powdered molecular sieves (4 Å, 0.5 g) was added a solution of trimethylsilyl triflate (8 μL) in dichloromethane (0.5 mL) at −30°. After 5 min, t.l.c. (9:1 dichloromethane–ethyl acetate) showed the disappearance of **9** (R_F 0.69) and a new compound at R_F 0.84 (**11**). Pyridine (0.2 mL) was added, the mixture was filtered and concentrated, and the residue was deacetylated with sodium methoxide in methanol (pH 10) for 16 h. The solvent was evaporated, and a solution of the residue in dichloromethane (250 mL) was washed with water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (7:3 dichloromethane–acetone) of the residue gave **12**, isolated as a white glass (0.85 g, 78%), $[\alpha]_D$ −69° (*c* 1, chloroform), R_F 0.62 (7:3 dichloromethane–acetone). N.m.r. data ($CDCl_3$): ^{13}C, δ 138.3–137.7 and 128.9–126.4 ($C_6H_5CH_2O$ and C_6H_5CH), 134.6 ($H_2C=CHCH_2O$), 117.4 ($H_2C=CHCH_2O$), 101.1, 100.7, and 100.6 (C-1′,1″, and PhCH), 81.3, 80.3, 80.0, 75.5 (2 C), 75.3, 73.3, 72.9, 71.7, 70.6, 67.9, 67.7, and 65.7 (C-1,2,3,4,5,2′,3′,4′,5′,2″,3″,4″,5″), 71.6 and 71.3 (2 $PhCH_2O$), 70.3 ($H_2C=CHCH_2O$), 69.1 (C-6′), 25.3 (CH_3CH_2S), 17.9 and 17.1 (C-6,6″), 14.9 (CH_3CH_2S); ^1H, δ 7.505–7.256 (m, 15 H, 3 Ph), 5.915 (m, 1 H, $H_2C=CHCH_2O$), 5.475 (s, 1 H, PhCH), 5.301 and 5.215 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.301 (bs, 1 H, H-1), 5.148 (d, 1 H, H-1″), 4.899 (d, 1 H, H-1′), 4.657, 4.595, 4.572, and 4.442 (4 d, each 1 H, 2 $PhCH_2O$), 4.168 (bd, 1 H, H-4′), 3.912 (dd, 1 H, H-2′), 3.439 (dd, 1 H, H-3′), 3.325 (bs, 1 H, H-5′), 2.642–2.538 (m, 2 H, CH_3CH_2S), 1.420 and 1.335 (2 d, each 3 H, 3 H-6 and 3 H-6″), 1.249 (t, 3 H, CH_3CH_2S), $J_{1,2}$ ~0, $J_{5,6}/J_{5'',6''}$ 6.0/6.3, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.5, $J_{4',5'}$ <1, $J_{1'',2''}$ 1.3, $J_{3'',4''} = J_{4'',5''}$ = 9.5, $J_{CH_2CH_3}$ 7.4 Hz.

Anal. Calc. for $C_{44}H_{56}O_{13}S·H_2O$: C, 62.69; H, 6.93. Found: C, 63.10; H, 6.97.

Ethyl 4-O-[3-O-allyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**13**). — A solution of **12** (0.43 g, 0.52 mmol) and benzyl bromide (0.4 mL, 3.3 mmol) in dry *N,N*-dimethylformamide (7 mL) was added to a stirred suspension of sodium hydride (0.15 g, 6.0 mmol) in *N,N*-dimethylformamide (2.5 mL) at 0°. After 2 h, t.l.c. indicated the disappearance of **12**, and methanol was added to destroy the excess of sodium hydride. The mixture was poured into ice–water (100 mL) and extracted with ether (3 × 30 mL), and the combined extracts were dried (Na_2SO_4), filtered, and concentrated. Column chromatography (95:5 dichloromethane–ethyl acetate) of the residue gave **13**, isolated as a syrup (0.53 g, 93%), $[\alpha]_D$ −40° (*c* 1, chloroform), R_F 0.62 (95:5 dichloromethane–ethyl acetate). N.m.r. data ($CDCl_3$): ^{13}C, δ 139.2–137.8 and 128.9–126.5 ($C_6H_5CH_2O$ and C_6H_5CH), 134.8 ($H_2C=CHCH_2O$), 117.2 ($H_2C=CHCH_2O$), 101.2 and 100.7 (C-1′ and PhCH), 98.8 (C-1″), 81.3, 80.5, 80.4, 80.3, 79.9, 75.6, 75.5, 75.0, 74.6, 72.9, 67.9, 67.7, and 65.7 (C-1,2,3,4,5,2′,3′,4′,5′,2″,3″,4″,5″), 74.7, 72.0 (2 C), 71.6, and 71.0 (5 $PhCH_2O$), 70.1 ($H_2C=CHCH_2O$), 69.2 (C-6′), 25.3 (CH_3CH_2S), 18.0 and 17.6 (C-6,6″), 15.0 (CH_3CH_2S); ^1H, δ 7.363–7.178 (m, 30 H, 6 Ph), 5.765 (m, 1 H, $H_2C=CHCH_2O$), 5.473 (s, 1 H, PhCH), 5.283 (bs, 1 H, H-1), 5.262 and 5.150 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.217 (d, 1 H, H-1″), 4.905 (d, 1 H, H-1′), 4.383 (m, 1 H, H-5″), 3.587 (t, 1 H, H-4″), 3.431 (dd, 1 H, H-3′), 3.336 (bs, 1 H, H-5′), 2.658–2.525 (m, 2 H, CH_3CH_2S), 1.423 (d, 6 H, 3 H-6 and 3 H-6″), 1.259 (t, 3 H, CH_3CH_2S), $J_{1,2}$ ~0, $J_{5,6}/J_{5'',6''}$ 6.2/6.2, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.5, $J_{4',5'}$ <1, $J_{1'',2''}$ 1.4, $J_{3'',4''} = J_{4'',5''}$ = 9.5, $J_{CH_2CH_3}$ 7.4 Hz.

Anal. Calc. for $C_{65}H_{74}O_{13}S$: C, 71.28; H, 6.81. Found: C, 71.17; H, 6.99.

Ethyl 4-O-[3-O-allyl-6-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**14**). — Borane–trimethylamine complex[28] (0.36 g, 4.93 mmol), powdered molecular sieves (4 Å, 2 g), and **13** (0.47 g, 0.43 mmol), in tetrahydrofuran (20 mL) were stirred for 1 h. Aluminium(III) chloride (0.79 g, 5.94 mmol) was added at 0° and the mixture was stirred overnight at room temperature. T.l.c. (9:1 dichloromethane–ethyl acetate) then showed the conversion of **13** (R_t 0.82) into **14** (R_t 0.43). The mixture was filtered, washed with cold 0.5M sulfuric acid, water, aqueous 5% sodium hydrogencarbonate, and water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (9:1 dichloromethane–ethyl acetate) of the residue afforded **14**, isolated as a syrup (0.32 g, 69%), $[\alpha]_D$ −54° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^{13}C, δ 139.1–137.7 and 128.2–127.0 ($C_6H_5CH_2O$), 134.2 ($H_2C=CHCH_2O$), 117.4 ($H_2C=CHCH_2O$), 101.0 (C-1′), 98.8 (C-1″), 25.2 (CH_3CH_2S), 17.8 and 17.5 (C-6,6″), 14.9 (CH_3CH_2S); ^1H, 7.343–7.064 (m, 30 H, 6 Ph), 5.727 (m, 1 H, $H_2C=CHCH_2O$), 5.250 (d, 1 H, H-1), 5.205 (d, 1 H, H-1′), 5.263 and 5.164 (2 m, each 1 H, $H_2C=CHCH_2O$), 4.856 (d, 1 H, H-1′), 4.343 (m, 1 H, H-5″), 4.006 (m, 1 H, H-5), 3.370 (dd, 1 H, H-3′), 2.623–2.530 (m, 2 H, CH_3CH_2S), 1.386 and 1.338 (2 d, each 3 H, 3 H-6 and 3 H-6″), 1.244 (t, 3 H, CH_3CH_2S); $J_{1,2}$ 1.2, $J_{4,5}$ 9.6, $J_{5,6}/J_{5'',6''}$ 6.3/6.1, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.6, $J_{3',4'}$ 3.3, $J_{1'',2''}$ 1.5, $J_{4'',5''}$ 9.6, $J_{CH_3CH_2}$ 7.4 Hz.

Anal. Calc. for $C_{65}H_{76}O_{13}S$: C, 71.14; H, 6.98. Found: C, 71.01; H, 7.05.

Ethyl 4-O-[3-O-allyl-6-O-benzyl-4-O-β-D-glucopyranosyl-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**17**). — To a solution of **14** (0.65 g, 0.59 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[29] (**15**; 0.40 g, 0.81 mmol) in dry dichloromethane (10 mL) containing powdered molecular sieves (4 Å, 0.50 g) was added a solution of trimethylsilyl triflate (14 μL) in dichloromethane (1 mL) at −10°. As shown by t.l.c. (9:1 dichloromethane–ethyl acetate), **16** (R_t 0.55) was formed in 5 min. Pyridine was added, and the mixture was filtered and concentrated. Conventional deacetylation and column chromatography (65:35 dichloromethane–acetone) of the product afforded **17**, isolated as a syrup (0.50 g, 67%), $[\alpha]_D$ −39° (*c* 1, chloroform), R_t 0.50 (65:35 dichloromethane–acetone). N.m.r. data ($CDCl_3$): ^{13}C, δ 139.0–137.7 and 128.4–127.0 ($C_6H_5CH_2O$), 133.4 ($H_2C=CHCH_2O$), 118.6 ($H_2C=CHCH_2O$), 105.5 (C-1‴), 101.0 (C-1′), 98.9 (C-1″), 25.3 (CH_3CH_2S), 17.9 and 17.4 (C-6,6″), 14.9 (CH_3CH_2S); ^1H, 7.389–7.066 (m, 30 H, 6 Ph), 5.701 (m, 1 H, $H_2C=CHCH_2O$), 5.261 and 5.196 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.256 (d, 1 H, H-1), 5.131 (d, 1 H, H-1″), 4.821 (d, 1 H, H-1′), 4.311 (d, 1 H, H-1‴), 2.627–2.526 (m, 2 H, CH_3CH_2S), 1.386 and 1.305 (2 d, each 3 H, 3 H-6 and 3 H-6″), 1.248 (t, 3 H, CH_3CH_2S); $J_{1,2}$ 0.9, $J_{5,6}/J_{5'',6''}$ 6.3/6.2, $J_{1',2'}$ 7.8, $J_{1'',2''}$ 1.6, $J_{1''',2'''}$ 7.5, $J_{CH_3CH_2}$ 7.4 Hz.

Ethyl 4-O-[3-O-allyl-6-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]-2,3-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**18**). — A solution of **17** (0.50 g, 0.40 mmol) and benzyl bromide (0.38 mL, 3.2 mmol) in *N,N*-dimethylformamide (5 mL) was added to a stirred suspension of sodium hydride (0.1 g, 4.2 mmol) in dry *N,N*-dimethylformamide (3 mL) at 0°. After 2 h at room temperature, t.l.c. indicated the complete disappearance

of **17**. Methanol was added to destroy the excess of sodium hydride, the mixture was poured into ice–water (75 mL) and extracted with ether (3 × 25 mL), and the combined extracts were dried (Na_2SO_4), filtered, and concentrated. Column chromatography (97:3 dichloromethane–ethyl acetate) of the residue yielded **18**, isolated as a syrup (0.44 g, 68%), $[\alpha]_D$ −31° (*c* 1, chloroform), R_F 0.80 (95:5 dichloromethane–ethyl acetate). ^{13}C-N.m.r. data ($CDCl_3$): δ 139.3–137.9 and 128.9–127.0 ($C_6H_5CH_2O$), 134.3 ($H_2C=CHCH_2O$), 118.6 ($H_2C=CHCH_2O$), 101.6 (C-1‴), 101.0 (C-1′), 98.6 (C-1″), 25.3 (CH_3CH_2S), 17.7 (C-6,6″), 15.0 (CH_3CH_2S).

Anal. Calc. for $C_{99}H_{110}O_{18}S$: C, 73.40; H, 6.84. Found: C, 73.20; H, 6.99.

*Ethyl 2,3-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranosyl]-1-thio-α-*L*-rhamnopyranoside* (**19**). — To a mixture of **18** (0.20 g, 0.12 mmol) and 1,4-diazabicyclo[2.2.2]octane (103 mg) in 8:3:1 ethanol–toluene–water (6 mL) was added tris(triphenylphosphine)rhodium(I) chloride (25 mg), and the mixture was boiled under reflux for 16 h, then cooled, and concentrated. The residue was diluted with dichloromethane (200 mL), washed with water, cold M hydrochloric acid (40 mL), aqueous 5% sodium hydrogencarbonate, and water, dried (Na_2SO_4), filtered, and concentrated. A solution of the residue in acetone (4.5 mL) and M hydrochloric acid (0.5 mL) was boiled under reflux for 2 h, when t.l.c. (95:5 dichloromethane–ethyl acetate) showed the formation of **19** (R_F 0.35). The mixture was neutralised with aqueous 5% sodium hydrogencarbonate, concentrated, and diluted with dichloromethane (200 mL), washed with water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (92:8 dichloromethane–ethyl acetate) of the residue gave pure **19**, isolated as a syrup (0.14 g, 73%), $[\alpha]_D$ −29° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$): ^{13}C, δ 139.3–137.0 and 128.5–126.9 ($C_6H_5CH_2O$), 104.4 (C-1‴), 100.9 (C-1′), 97.8 (C-1″), 25.3 (CH_3CH_2S), 18.0 and 17.7 (C-6,6″), 15.0 (CH_3CH_2S); ^{1}H, δ 7.375–7.021 (m, 50 H, 10 Ph), 5.248 (d, 1 H, H-1), 5.115 (d, 1 H, H-1″), 2.625–2.516 (m, 2 H, CH_3CH_2S), 1.395 and 1.371 (2 d, each 3 H, 3 H-6 and 3 H-6″), 1.255 (t, 3 H, CH_3CH_2S); $J_{1,2} < 1$, $J_{5,6}/J_{5'',6''}$ 6.3/5.9, $J_{1'',2''} < 1$, $J_{CH_2CH_3}$ 7.4 Hz.

*Ethyl 4-*O*-[3-*O*-acetyl-6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranosyl]-2,3-di-*O*-benzyl-1-thio-α-*L*-rhamnopyranoside* (**20**). — A solution of **19** (0.13 g, 0.08 mmol) in pyridine (2 mL) and acetic anhydride (2 mL) was stirred overnight, then concentrated, and co-concentrated with toluene (3 × 20 mL), ethanol (3 × 20 mL), and dichloromethane (3 × 20 mL). Column chromatography (95:5 dichloromethane–ethyl acetate) of the residue yielded **20**, isolated as a syrup (0.13 g, 93%), $[\alpha]_D$ −24° (*c* 1, chloroform), R_F 0.69 (95:5 dichloromethane–ethyl acetate). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.1 ($COCH_3$), 138.6–137.9 and 128.2–127.0 ($C_6H_5CH_2O$), 103.3 (C-1‴), 100.9 (C-1′), 98.7 (C-1″), 25.3 (CH_3CH_2S), 20.7 ($COCH_3$), 17.8 (C-6,6″), 15.0 (CH_3CH_2S).

Anal. Calc. for $C_{98}H_{108}O_{19}S$: C, 72.57; H, 6.71. Found: C, 72.48; H, 6.79.

*3-Benzyloxycarbonylaminopropyl 4-*O*-[3-*O*-acetyl-6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranosyl]-2,3-di-*O*-benzyl-α,β-*L*-rhamnopyranoside* (**21αβ**). — To a solution of

20 (60 mg, 37 μmol) in dry dichloromethane (5 mL) was added a solution of bromine (20 μL, 0.4 mmol) in dry dichloromethane (0.5 mL). After 10 min, the mixture was co-concentrated with dry toluene (3 × 5 mL). A solution of the resulting glycosyl bromide in toluene (1 mL) was added to a stirred mixture of 3-benzyloxycarbonylamino-1-propanol[33] (80 mg, 0.41 mmol), molecular sieves (4 Å, 100 mg), and silver silicate (90 mg) in dry dichloromethane (5 mL). After 2 h, t.l.c. (95:5 dichloromethane–ethyl acetate) indicated a new slower-moving spot (R_t 0.15). The mixture was diluted with dichloromethane (75 mL), filtered through Celite, and concentrated. Column chromatography (92:8 dichloromethane–ethyl acetate) of the residue yielded a mixture of **21αβ** and its analogue with HO-1 unsubstituted. After conventional acetylation and column chromatography (92:8 dichloromethane–ethyl acetate), **21αβ** (40 mg, 64%) was obtained as a syrup. N.m.r. data ($CDCl_3$): ^{13}C, δ 170.0 ($COCH_3$), 156.3 ($NHCOOCH_2C_6H_5$), 139.1–136.6 and 128.2–126.9 ($C_6H_5CH_2O$), 103.4 (C-1″), 101.4 (C-1β), 100.7 (C-1′), 98.7 (C-1α), 98.2 (C-1‴), 66.4, 65.3, 38.2, and 29.6 ($OCH_2CH_2CH_2NHCOOCH_2C_6H_5$), 20.7 ($COCH_3$), 17.8 (C-6,6′), $J_{C-1,H-1}$ 153 (β), $J_{C-1,H-1}$ 161, $J_{C-1',H-1'}$ 170, $J_{C-1'',H-1''}$ 159 Hz; ^1H, δ 1.879 (m, 2 H, $OCH_2CH_2CH_2NH$), 1.830 (s, 3 H, Ac), 1.361 and 1.277 (2 d, each 3 H, 3 H-6 and 3 H-6′), $J_{5,6}$, $J_{5',6'}$ 6.1, 6.2 Hz.

*3-Benzyloxycarbonylaminopropyl 2,3-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranosyl]-α,β-*L*-rhamnopyranoside* (**22αβ**). — To a solution of **21αβ** (40 mg, 23 μmol) in dichloromethane (2 mL) and methanol (3 mL) was added sodium methoxide to pH 10. After stirring for 48 h, the mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography (95:5 dichloromethane–ethyl acetate) of the residue gave **22αβ**, isolated as a syrup (38 mg, 98%), R_t 0.48 (92:8 dichloromethane–ethyl acetate). ^{13}C-N.m.r. data ($CDCl_3$): δ 156.3 ($NHCOOCH_2C_6H_5$), 139.3–137.0 and 128.5–126.7 ($C_6H_5CH_2O$), 104.4 (C-1″), 101.3 (C-1β), 100.7 (C-1′), 97.2 (C-1‴), 38.2 and 29.6 ($OCH_2CH_2CH_2NH$), 18.0 and 17.8 (C-6,6″).

Anal. Calc. for $C_{105}H_{115}NO_{21}$: C, 73.02; H, 6.71. Found: C, 72.58; H, 7.14.

*3-Benzyloxycarbonylaminopropyl 2,3-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranosyl]-α,β-*L*-rhamnopyranoside 3′-(1,3-di-*O*-benzylglycer-2-yl phosphonate)* (**23αβ**). — Compound **27** (20 mg, 46 μmol) and **22αβ** (38 mg, 22 μmol) were co-concentrated in pyridine (2 × 5 mL), and the residue was dissolved in dry pyridine (4 mL). Pivaloyl chloride (9 μL, 73 μmol) was added, the mixture was stirred for 16 h at room temperature, water was added, and the mixture was diluted with dichloromethane (75 mL), washed with M triethylammonium hydrogencarbonate (2 × 15 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (92:8 dichloromethane–ethyl acetate) of the residue yielded **23αβ**, isolated as a syrup (37 mg, 82%), R_t 0.48 (92:8 dichloromethane–ethyl acetate). ^1H-N.m.r. data for **23β** ($CDCl_3$): δ 6.998 (d, 0.5 H, $J_{H,P}$ 720 Hz, P–H) and 6.796 (d, 0.5 H, $J_{H,P}$ 727 Hz, P–H) of two enantiomers.

*3-Benzyloxycarbonylaminopropyl 2,3-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-*

β-D-galactopyranosyl]-α,β-L-rhamnopyranoside 3'-(1,3-di-O-benzylglycer-2-yl triethylammonium phosphate) (**24αβ**). — To a solution of **23** (37 mg, 18 μmol) in tetrahydrofuran (2 mL) was added 0.35M iodine in 2:1 water–pyridine (115 μL). After 3 h, t.l.c. (92:8 dichloromethane–ethyl acetate) indicated the total disappearance of phosphonate **23**, and a new product with R_f 0 was observed. The excess of iodine was destroyed with aqueous 5% sodium hydrogensulfite (10 mL), and the mixture was diluted with dichloromethane (50 mL), washed with M triethylammonium hydrogencarbonate (2 × 15 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (2:1 dichloromethane–methanol) of the residue on Sephadex LH-20 gave **24αβ**, isolated as a syrup (35 mg, 91%). N.m.r. data ($CDCl_3$): ^{13}C, δ 156.3 ($NHCOOCH_2C_6H_5$), 139.6 138.1 and 129.0–126.8 ($C_6H_5CH_2O$), 102.5 (C-1‴), 101.3 (C-1β), 100.4 (C-1'), 97.7 (C-1″), 44.8 [$NH(CH_2CH_3)_3$], 38.1 and 29.6 ($OCH_2CH_2CH_2NH$), 17.8 (C-6,6″), 8.1 [$NH(CH_2CH_3)_3$]; ^{31}P, δ −0.06.

3-Aminopropyl 4-O-(4-O-β-D-glucopyranosyl-2-O-α-L-rhamnopyranosyl-β-D-galactopyranosyl)-β-L-rhamnopyranoside 3'-(glycer-2-yl sodium phosphate) (**25β**). — To a solution of **24** (35 mg, 16 μmol) in 1:2:2 ethyl acetate–2-propanol–methanol (5 mL) was added 10% Pd–C (40 mg). Hydrogenolysis was performed at 4 kg/m^2 for 48 h, the mixture was filtered and concentrated, and a solution of the residue in water was passed through a column of Dowex-50 (Na^+) resin, then lyophilised to yield **25**, isolated as a white powder (8 mg, 57%). The ^1H-n.m.r. spectrum of **25** indicated mainly the β anomer together with α anomer and degradation products (totalling ~30%). Final purification to yield **25β** was performed by HPAE-PAD chromatography using CarboPac PA1 on a Dionex-LC system[45] by elution with 0.1M NaOH for 5 min, followed by a linear gradient of 0→600mM NaOAc in 0.1M NaOH for 30 min, and 600mM NaOAc for 5 min. The major fraction, eluted at 9 min, was collected, neutralised with 0.1M HCl, and desalted. N.m.r. data (D_2O): ^{13}C, δ 103.8, 102.7, 102.1, and 101.1 (C-1,1',1″,1‴), 62.7 (C-1,3 glycerol), 62.1 and 61.9 (C-6',6‴), 68.6, 39.0, and 28.2 ($OCH_2CH_2CH_2NH_2$), 18.5 and 17.8 (C-6,6″); ^{31}P, δ 0.47; ^1H, see Table I.

1,3-Di-O-Benzylglycerol 2-(triethylammonium phosphonate) (**27**). — To a solution of 1,3-di-*O*-benzylglycerol (**26**; 0.96 g, 3.52 mmol) in acetonitrile (40 mL) was added pyridine (12.5 mL) and 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (1.0 g, 4.9 mmol). After 1 h, t.l.c. (9:1 dichloromethane–ethyl acetate) showed the disappearance of **26** (R_f 0.9) and a new spot at R_f 0. Water (3 mL) was added, and the solution was diluted with 99:1 dichloromethane–triethylamine (200 mL), washed with M triethylammonium hydrogencarbonate (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (80:20:0.1 dichloromethane–acetone-triethylamine, followed by 92:8:0.1 dichloromethane–methanol–triethylamine) of the residue yielded **27**, isolated as a syrup (1.38 g, 89%). N.m.r. data ($CDCl_3$): ^{13}C, δ 138.0 and 127.9–127.1 ($C_6H_5CH_2O$), 72.8 ($PhCH_2O$), 71.8 (d, C-2), 70.3 (d, C-1,3), 45.0 [$N(CH_2CH_3)_3$], 8.0 [$N(CH_2CH_3)_3$], $^2J_{C\text{-}2,P}$ 5.3, $^3J_{C\text{-}1,P} = {}^3J_{C\text{-}3,P}$ = 3.8 Hz; ^1H, δ 7.293–7.222 (m, 10 H, 2 Ph), 6.977 (d, 1 H, P–H), 4.595 (m, 1 H, H-2), 4.539 and 4.503 (2 d, each 2 H, 2 $PhCH_2O$), 3.660 (m, 4 H, H-1,1,3,3), 2.959 [q, 6 H, $N(CH_2CH_3)_3$], 1.219 [t, 9 H, $N(CH_2CH_3)_3$], $^1J_{H,P}$ 635, $J_{1,2} = J_{2,3}$ = 5.3, $^3J_{H\text{-}2,P}$ 10.6, $J_{CH_2CH_3}$ 7.3 Hz; ^{31}P, δ 4.46 (dd), $^1J_{H,P}$ 635, $^3J_{H\text{-}2,P}$ 10.6 Hz.

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