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# SYNTHESIS OF FOUR SPACER-CONTAINING TRISACCHARIDES WITH THE 4-*O*-(β-L-RHAMNOPYRANOSYL)-D-GLUCOPYRANOSE UNIT IN COMMON, REPRESENTING FRAGMENTS OF CAPSULAR POLYSACCHARIDES FROM *STREPTOCOCCUS PNEUMONIAE* TYPES 2, 7F, 22F, AND 23F

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## ABSTRACT

The synthesis is reported of 3-aminopropyl 3-*O*-[4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranosyl]-α-L-rhamnopyranoside (**34**), 3-aminopropyl 2-acetamido-3-*O*-[4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranosyl]-2-deoxy-β-D-galactopyranoside (**37**), 3-aminopropyl 3-*O*-[4-*O*-(β-L-rhamnopyranosyl)-α-D-glucopyranosyl]-α-D-galactofuranoside (**41**), and 3-aminopropyl 4-*O*-[4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**45**). These are spacer-containing fragments of the capsular polysaccharides of *Streptococcus pneumoniae* type 2, 7F, 22F, and 23F, respectively, which are constituents of Pneumovax© 23. 2,3,4-Tri-*O*-benzyl-α-L-rhamnopyranosyl bromide was coupled to 1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (**3**). Opening of the anhydro ring, removal of AcO-1, and imidation of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-β-D-glucopyranose (**4β**) afforded 6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-αβ-D-glucopyranosyl trichloroacetimidate (**7αβ**). Condensation of **7αβ** with 3-*N*-benzyloxycarbonylaminopropyl 2-*O*-benzyl-5,6-*O*-isopropylidene-α-D-galactofuranoside (**26**), followed by deprotection gave **41**. Opening of the anhydro ring of **4β** followed by debenzylation, acetylation, removal of AcO-1, and imidation yielded 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)-α-D-glucopyranosyl trichloroacetimidate (**11**). Condensation of **11** with 3-*N*-benzyloxycarbonylaminopropyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**18**), with 3-*N*-ben-

zyloxycarbonylaminopropyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (**21**), or with 3-*N*-benzyloxycarbonylaminopropyl 2-*O*-acetyl-3-*O*-allyl-6-*O*-benzyl-β-D-galactopyranoside (**31**), followed by deprotection afforded **34**, **37**, and **45**, respectively.

## INTRODUCTION

Infection by *Streptococcus pneumoniae* species, leading to pneumonia, meningitis, and otitis media, is still one of the leading causes of human death. For this reason research towards the development of vaccines against these organisms is relevant. Because of the inherent problems of the current capsular polysaccharide vaccine[1] Pneumovax© 23, attention is paid to the search for synthetic vaccines based on oligosaccharide conjugates.[2]

The disaccharide 4-*O*-(β-L-rhamnopyranosyl)-D-glucopyranose is a common fragment to the capsular polysaccharides of the *S. pneumoniae* serotypes 2, 7A, 7F, 18A, 18F, 22F, 23F, and 27,[2] whilst the polysaccharides of type 2, 7F, 22F, and 23F are constituents of the current vaccine.[1,2] In the vaccine serotypes 2,[3] 7F,[4] and 23F,[5] a 4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranose unit is present, whereas in the vaccine serotype 22F[6] 4-*O*-(β-L-rhamnopyranosyl)-α-D-glucopyranose occurs.

Type 2: $[\rightarrow 3)$-β-L-Rha*p*-$(1\rightarrow 4)$-β-D-Glc*p*-$(1\rightarrow 3)$-α-L-Rha*p*-$(1\rightarrow 3)$-α-L-Rha*p*-$(1\rightarrow]_n$
α-D-Glc*p*A-$(1\rightarrow 6)$-α-D-Glc*p*-$(1\rightarrow 2)$/

Type 7F: Ac-2 \
$[\rightarrow 6)$-α-D-Gal*p*-$(1\rightarrow 3)$-β-L-Rha*p*-$(1\rightarrow 4)$-β-D-Glc*p*-$(1\rightarrow 3)$-β-D-Gal*p*NAc-$(1\rightarrow]_n$
β-D-Gal*p*-$(1\rightarrow 2)$/ α-D-Glc*p*NAc-$(1\rightarrow 2)$-α-L-Rha*p*-$(1\rightarrow 4)$/

Type 22F: Ac-2 \
$[\rightarrow 4)$-β-D-Glc*p*A-$(1\rightarrow 4)$-β-L-Rha*p*-$(1\rightarrow 4)$-α-D-Glc*p*-$(1\rightarrow 3)$-α-D-Gal*f*-$(1\rightarrow 2)$-α-L-Rha*p*-$(1\rightarrow]_n$
α-D-Glc*p*-$(1\rightarrow 3)$/

Type 23F: glycerol-(2-P$\rightarrow$3) \
$[\rightarrow 4)$-β-L-Rha*p*-$(1\rightarrow 4)$-β-D-Glc*p*-$(1\rightarrow 4)$-β-D-Gal*p*-$(1\rightarrow]_n$
α-L-Rha*p*-$(1\rightarrow 2)$/

Here we report the synthesis of 3-aminopropyl glycosides of trisaccharide fragments of the capsular polysaccharides of *S. pneumoniae* types 2, 7F, 22F, and 23F, having the 4-

*O*-(β-L-rhamnopyranosyl)-D-glucopyranose unit as the terminal element, namely 3-aminopropyl 3-*O*-[4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranosyl]-α-L-rhamnopyranoside (**34**; type 2), 3-aminopropyl 2-acetamido-3-*O*-[4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranosyl]-2-deoxy-β-D-galactopyranoside (**37**; type 7F), 3-aminopropyl 3-*O*-[4-*O*-(β-L-rhamnopyranosyl)-α-D-glucopyranosyl]-α-D-galactofuranoside (**41**; type 22F), and 3-aminopropyl 4-*O*-[4-*O*-(β-L-rhamnopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**45**; type 23F).

## RESULTS AND DISCUSSION

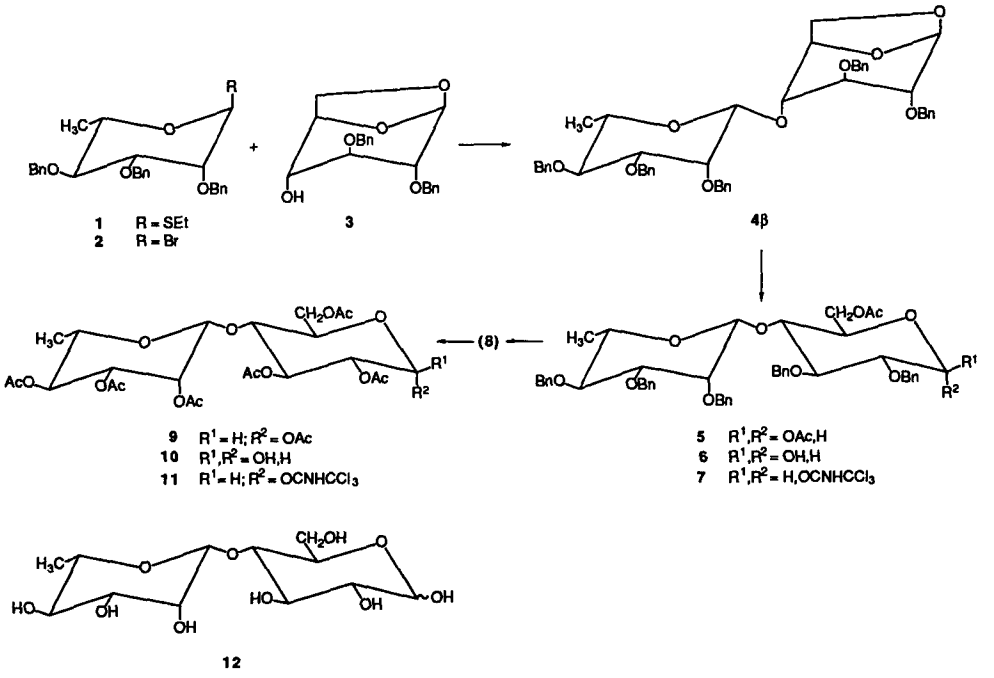
The formation of the β-(1-4)-linkage between L-rhamnose and D-glucose poses a difficult problem in oligosaccharide synthesis. In condensation reactions the β-glycosidic linkage is not favoured, whilst the HO-4 of D-glucose is relatively unreactive,[7] resulting in a strong decrease of stereoselectivity.[8] Literature data with respect to this coupling reaction are scarce. The condensation of 4-*O*-benzoyl-2,3-*O*-cyclohexylidene-α-L-rhamnopyranosyl bromide with methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside, using silver carbonate as the promoter has been described,[9] yielding 23% of the β-linked disaccharide derivative and 33% of the α-linked product. As will be shown below, the use of silver silicate[8] as a promoter, together with the 1,6-anhydro derivative of D-glucose[10] as acceptor, resulted in a higher yield of β-linked product.

In the synthesis of the four trisaccharides **32**, **35**, **38**, and **42**, the β-L-rhamnopyranosyl-(1→4)-D-glucopyranose derivative **5** is a key intermediate. After removal of AcO-1 of **5** and imidation (→**7**), a suitable donor for an α-glucose linkage is obtained. Debenzylation of **5** and acetylation, followed by removal of AcO-1 and imidation, offers a suitable synthon for the β-glucose linkage (**11**).

To prepare 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-β-D-glucopyranose (**4**β), 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl bromide (**2**), synthesized[11] from the corresponding thioglycoside **1**, was coupled with 1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose[10] (**3**) in dichloromethane using freshly prepared silver silicate[8] as the promoter. Crystalline **4**β was obtained in a yield of 43% ($^1$H NMR data: δ 3.203, H-5'),[12] whereas the α-coupled product (**4**α) was isolated in 36% ($^1$H NMR data: δ 3.751, H-5').[12] The addition of toluene to the mixture, normally used to suppress the inversion reaction of the glycosyl bromide, in this case reduced the β:α ratio. When **1** was used in the coupling reaction with methyl triflate[13] as the promoter in ether or dichloromethane as solvent, only traces of the β-disaccharide were observed on TLC. The same

holds when the copper(II) bromide/tetrabutylammonium bromide couple[14] together with silver silicate was used in the condensation reaction.

Cleavage of the anhydro ring of **4**β with trifluoroacetic acid in acetic anhydride (→ **5**αβ, 72%), followed by the removal of AcO-1 with hydrazine acetate[15] (→**6**), and imidation using potassium carbonate[16] as base, afforded **7**αβ (84% from **5**). Debenzylation of **5** (→**8**) and subsequent acetylation gave **9** (86%), which after removal of AcO-1 (→**10**) was converted into the α-trichloroacetimidate **11** (71% from **9**) using 1,8-diazabicyclo-[5.4.0]-undec-7-ene.[17] Deacetylation of **9** afforded the deprotected disaccharide **12**.

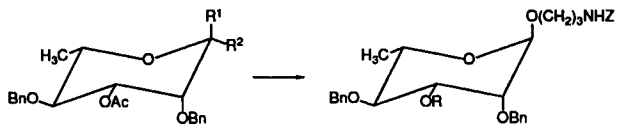



For the synthesis of the spacer-containing trisaccharide **34**, related to the capsular polysaccharide *S. pneumoniae* type 2, besides **11**, rhamnose synthon **18** was prepared. Acetylation of benzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside[18] (→**13**), conversion of BnO-1 into AcO-1 (→**14**) by acetolysis (72%), and removal of AcO-1 using hydrazine acetate[15] gave **15** (74%). To introduce the 3-*N*-benzyloxycarbonylaminopropyl group at C-1 of rhamnose as a protected spacer, **15** was treated with the Vilsmeier reagent[19] (→**16**) and condensed with 3-*N*-benzyloxycarbonylamino-1-propanol[20] in the presence of mercury cyanide to give **17** (62% from **15**). The α-configuration of **17** was proven by coupled $^{13}C$ NMR ($J_{C\text{-}1,H\text{-}1}$ = 164 Hz)[21] and $^{1}H$ NMR (δ 3.732, H-5)[12] spectroscopy, and no β-coupled product was observed. Condensation of deacetylated **17** (**18**) with **11** in dichloromethane at -30 °C using trimethylsilyl triflate as the catalyst afforded trisaccharide deriv-

**TABLE 1. 500 MHz $^1$H NMR Data for Compounds 34, 37, 41, and 45.**

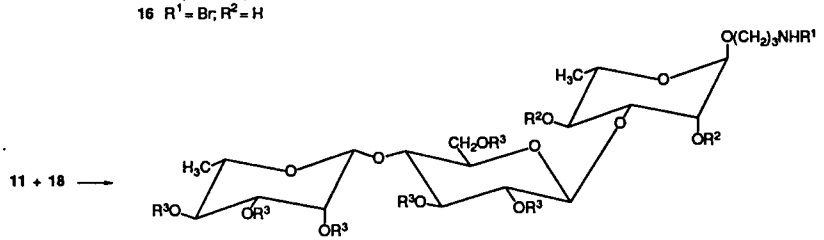
| | Proton (J) | δ (ppm) (J in Hz) | | | |
|---|---|---|---|---|---|
| | | **34** | **37** | **41** | **45** |
| | | α-Rha*p* | β-Gal*p*NAc | α-Gal*f* | β-Gal*p* |
| | H-1 ($J_{1,2}$) | 4.803 (1.9) | 4.488 (8.5) | 5.032 (4.8) | 4.438 (7.9) |
| | H-2 ($J_{2,3}$) | 4.191 (3.3) | 4.023 (10.5) | 4.363 (7.5) | 3.589 (9.9) |
| | H-3 ($J_{3,4}$) | 3.884 (9.6) | 3.84 (3.2) | 4.194 (7.0) | 3.757 (3.2) |
| | H-4 ($J_{4,5}$) | 3.610 (9.6) | 4.180 (<1) | 4.058 (5.0) | 4.165 (<1) |
| | H-5 ($J_{5,6a}$) | 3.716 (6.2) | 3.698 (4.5) | 3.779 (4.4) | 3.732 (4.9) |
| | H-6a ($J_{6a,6b}$) | 1.306 | 3.794 (-12.3) | 3.711 (-11.8) | 3.788 (-11.8) |
| | H-6b ($J_{5,6b}$) | - | 3.783 (7.6) | 3.628 (6.8) | 3.777 (6.0) |
| | -$COCH_3$ | - | 2.031 | - | - |
| Glc*p* (α or β) | H-1 ($J_{1,2}$) | 4.674 (7.9) | 4.509 (7.9) | 5.016 (3.9) | 4.657 (8.0) |
| | H-2 ($J_{2,3}$) | 3.38 (9.6) | 3.306 (9.0) | 3.591 (10.0) | 3.359 (9.2) |
| | H-3 ($J_{3,4}$) | 3.65 | 3.604 (9.1) | 3.842 (9.3) | 3.657 (9.2) |
| | H-4 ($J_{4,5}$) | 3.66 (9.5) | 3.644 (9.1) | 3.661 (9.9) | 3.630 (9.4) |
| | H-5 ($J_{5,6a}$) | 3.511 (2.5) | 3.482 (2.5) | 3.919 (2.4) | 3.496 (2.3) |
| | H-6a ($J_{6a,6b}$) | 3.899 (-12.6) | 3.883 (-12.9) | 3.88 | 3.919 (-12.7) |
| | H-6b ($J_{5,6b}$) | 3.846 (4.7) | 3.84 (4.2) | 3.88 (4.8) | 3.831 (5.0) |
| β-Rha*p* | H-1 ($J_{1,2}$) | 4.866 (~0) | 4.857 (~0) | 4.871 (~0) | 4.858 (~0) |
| | H-2 ($J_{2,3}$) | 4.077 (3.3) | 4.070 (3.4) | 4.085 (3.3) | 4.068 (3.3) |
| | H-3 ($J_{3,4}$) | 3.591 (9.4) | 3.586 (9.2) | 3.60 (9.5) | 3.584 (9.2) |
| | H-4 ($J_{4,5}$) | 3.361 (9.4) | 3.357 (9.4) | 3.368 (9.3) | 3.359 (9.1) |
| | H-5 ($J_{5,6}$) | 3.38 (5.7) | 3.38 (5.8) | 3.39 | 3.39 (5.6) |
| | H-6 | 1.313 | 1.308 | 1.31 | 1.312 |
| spacer | -$CH_2N$ | 3.17-3.08 | 3.08 | 3.17 | 3.15 |
| | C-$CH_2$-C | 2.02-1.96 | 1.94 | 2.02-1.96 | 2.03-1.98 |
| | -$OCH_a$ | 3.83 | 4.03 | 3.97 | 4.05 |
| | -$OCH_b$ | 3.59 | 3.74 | 3.62 | 3.83 |

ative **32** (69%). Deacetylation of **32** (→**33**) followed by hydrogenolysis to remove benzyl and benzyloxycarbonyl groups gave **34** (94%). The $^1$H NMR data of **34**, obtained by 2D HOHAHA and 2D COSY measurements, are given in Table 1.




**13** $R^1$ = OBn; $R^2$ = H
**14** $R^1$ = OAc; $R^2$ = H
**15** $R^1$,$R^2$ = OH,H
**16** $R^1$ = Br; $R^2$ = H

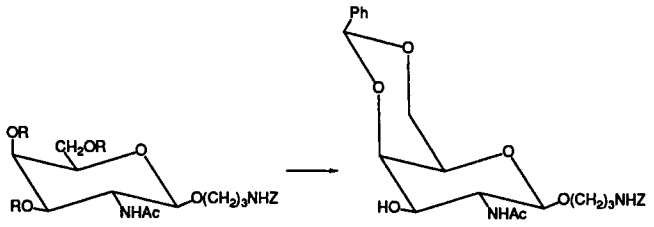
**17** R = Ac
**18** R = H




**32** $R^1$ = Z; $R^2$ = Bn; $R^3$ = Ac
**33** $R^1$ = Z; $R^2$ = Bn; $R^3$ = H
**34** $R^1$ = $R^2$ = $R^3$ = H

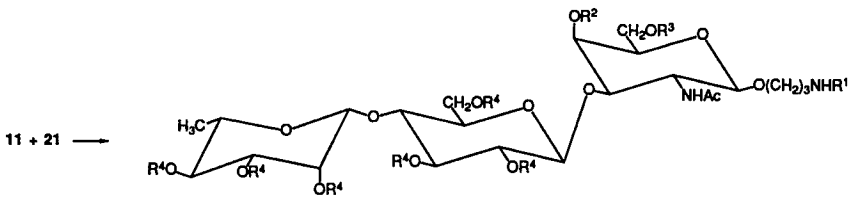
The synthesis of the spacer-containing trisaccharide **37**, related to the capsular polysaccharide of *S. pneumoniae* type 7F, involves firstly the condensation of **11** with *N*-acetylgalactosamine synthon **21**. To this end 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl bromide[22] was coupled to 3-*N*-benzyloxycarbonylamino-1-propanol[20] in dichloromethane, with mercury cyanide as the promoter, affording **19** (72%). Deacetylation (→**20**) and subsequent benzylidenation with benzaldehyde and formic acid[23] gave crystalline **21** (67%). Condensation of **21** with **11** in dichloromethane at -30 °C with trimethylsilyl triflate as a catalyst afforded **35** (29%). When trifluoroborane-etherate was used as a catalyst, no product formation was observed on TLC. Also well-established methods[24] to couple to HO-3 of a protected *N*-acetylgalactosamine residue failed, *i.e.* using a glycosyl bromide as donor with mercury cyanide/mercury bromide as promoters in nitromethane-toluene mixtures or dichloromethane. Under those conditions, the glycosyl bromide prepared from **10** with Vilsmeier reagent,[19] was not reactive enough to couple to **21**. Silver triflate was also tried as a promoter, but without success. Compound **35** was deacetylated (→**36**) and hydrogenolysed, affording a product with the 4,6-*O*-benzylidene group still present. Subsequent mild acid hydrolysis gave **37** (19%, not optimised). The $^1$H NMR data of **37** are presented in Table 1.

For the synthesis of the spacer-containing trisaccharide **41**, related to the capsular polysaccharide of *S. pneumoniae* type 22F, besides **7**, galactose synthon **26** was synthe-



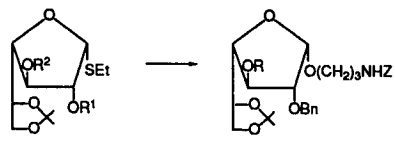

19 R = Ac
20 R = H

21



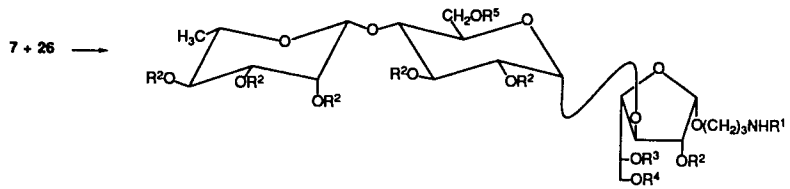

35 $R^1$ = Z; $R^2$,$R^3$ = CHPh; $R^4$ = Ac
36 $R^1$ = Z; $R^2$,$R^3$ = CHPh; $R^4$ = H
37 $R^1$ = $R^2$ = $R^3$ = H

sised. Isopropylidenation of ethyl 1-thio-α-D-galactofuranoside[25] with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide yielded **22** (54%), which was regioselectively benzylated at HO-2. Treatment of **22** in dichloromethane and aqueous 10% potassium hydroxide with benzyl bromide in the presence of tetrabutylammonium bromide gave **23**, which was isolated in 20% yield from a mixture of 2,3-di-*O*-benzylated **22** (3%) and 3-*O*-benzylated **22** (27%). Thioglycoside **23** was acetylated (→**24**), treated with copper(II) bromide/tetrabutylammonium bromide,[14] to effect *in situ* formation of the glycosyl bromide, and condensed with 3-*N*-benzyloxycarbonylamino-1-propanol[20] in ether, using silver silicate as a promoter, to yield **25**α (63%) and **25**β (9%). Coupling of deacetylated **25**α (**26**) with **7**αβ in ether in the presence of trimethylsilyl triflate gave **38** (45%), which was deisopropylidenated with trifluoroacetic acid in dichloromethane (→**39**), deacetylated (→**40**), and debenzylated/debenzyloxycarbonylated to afford **41** (58%). The $^1$H NMR data of **41** are given in Table 1.




22 $R^1$ = $R^2$ = H
23 $R^1$ = Bn; $R^2$ = H
24 $R^1$ = Bn; $R^2$ = Ac

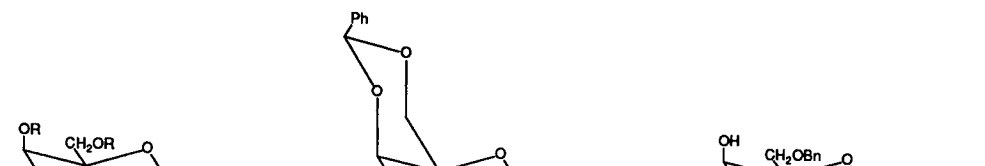
25 R = Ac
26 R = H



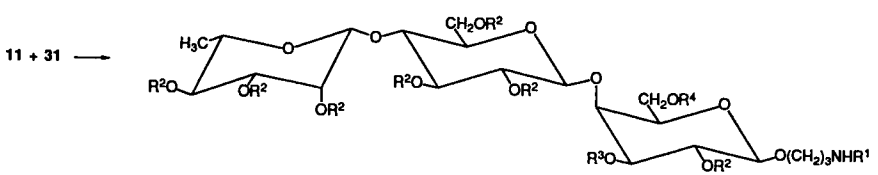

**38** $R^1$ = Z; $R^2$ = Bn; $R^3$,$R^4$ = $C(CH_3)_2$; $R^5$ = Ac
**39** $R^1$ = Z; $R^2$ = Bn; $R^3$ = $R^4$ = H; $R^5$ = Ac
**40** $R^1$ = Z; $R^2$ = Bn; $R^3$ = $R^4$ = $R^5$ = H
**41** $R^1$ = $R^2$ = $R^3$ = $R^4$ = $R^5$ = H

To obtain the spacer-containing trisaccharide **45**, related to the capsular polysaccharide of *S. pneumoniae* type 23F, besides **11**, the galactose synthon **31** was synthesised. 2,4,6-Tri-*O*-acetyl-3-*O*-allyl-α-D-galactopyranosyl trichloroacetimidate[26] was coupled to 3-*N*-benzyloxycarbonylamino-1-propanol[20] (→**27**, 83%), and subsequent deacetylation (→**28**), benzylidenation (→**29**, 68% from **27**), acetylation (→**30**), and selective opening of the 4,6-*O*-benzylidene ring with borane-trimethylamine complex and aluminium(III) chloride in tetrahydrofuran[27] afforded **31** (67% from **29**). The disaccharide donor **11** was coupled to **31** in dichloromethane with trimethylsilyl triflate as the catalyst (→**42**, 71%). Deallylation using the Wilkinson[28] catalyst in the presence of diazobicyclo[2.2.2]-octane (→**43**, 69%) and subsequent deacetylation (→**44**), followed by debenzylation/debenzyloxycarbonylation, afforded **45**. In principle, the chosen reaction pathway includes the possibility of attaching a glycerol phosphate at C-3 of galactose. The $^1$H NMR data of **45** are presented in Table 1.

Immunological studies on **34**, **37**, **41**, and **45** conjugated to protein will be reported elsewhere.




**27** R = Ac
**28** R = H
**29** R = H
**30** R = Ac
**31**




**42** $R^1$ = Z; $R^2$ = Ac; $R^3$ = Allyl; $R^4$ = Bn
**43** $R^1$ = Z; $R^2$ = Ac; $R^3$ = H; $R^4$ = Bn
**44** $R^1$ = Z; $R^2$ = $R^3$ = H; $R^4$ = Bn
**45** $R^1$ = $R^2$ = $R^3$ = $R^4$ = H

## EXPERIMENTAL

**General methods.** $^1H$ NMR spectra (360 and 500 MHz) were recorded at 25 °C with Bruker HX 360 or AM 500 spectrometers (Bijvoet Center, Department of NMR Spectroscopy, Utrecht University). 2D Double-quantum-filtered $^1H$-$^1H$ correlation spectra (2D DQF $^1H$-$^1H$ COSY) were recorded in the phase-sensitive mode,[29] and 2D homonuclear Hartmann-Hahn spectra (2D HOHAHA) with a MLEV-17 mixing sequence of 120 ms.[30] $^{13}C$ NMR spectra (APT, 50 MHz) were recorded at 25 °C with a Bruker WP 200 spectrometer. Chemical shifts (δ) are given in ppm 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 TLC on Kieselgel 60 $F_{254}$ (Merck). Detection was effected by charring with sulfuric acid after examination under UV light. Melting points were determined with a Mettler FP 51 instrument. Optical rotations were measured at 20 °C with a Perkin-Elmer 241 polarimeter, using a 10-cm 1-mL cell. All solvents were distilled from appropriate drying agents. In the work-up procedures, washings were carried out three times with appropriate quantities of water or aqueous 5% sodium hydrogencarbonate unless indicated otherwise, and drying of organic solutions was performed with $Na_2SO_4$. The boiling point of the light petroleum used was 40-60 °C. Evaporations were conducted under reduced pressure at 40 °C (bath).

**Ethyl 2,3,4-Tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (1).** A solution of ethyl thio-α-L-rhamnopyranoside[31] (9.70 g, 46.57 mmol) and benzyl bromide (25.4 mL, 210 mmol) in dry *N*,*N*-dimethylformamide (60 mL) was added to a suspension of sodium hydride (7.9 g, 328 mmol) in *N*,*N*-dimethylformamide (50 mL) at 0 °C. After 16 h, methanol was added to destroy the excess of sodium hydride, and the mixture was poured into ice-water (1 L), extracted with ether (3 x 200 mL); the combined extracts were washed with water, dried, filtered, and concentrated. Column chromatography (8:2 light petroleum-ethyl acetate) of the residue afforded **1** (17.61 g, 79%) as a syrup: $[\alpha]_D$ -64° (*c* 1, chloroform); $R_F$ 0.48 (8:2 light petroleum-ethyl acetate); $^1H$ NMR ($CDCl_3$) δ 7.390-7.255 (m, 15H, 3Ph), 5.257 (d, 1H, H-1), 4.940, 4.720, 4.682, 4.632, 4.587, and 4.555 (6d, each 1H, 3PhC$H_2$O), 4.025 (m, 1H, H-5), 3.830 (dd, 1H, H-2), 3.789 (dd, 1H, H-3), 3.634 (t, 1H, H-4), 2.627-2.491 (m, 2H, $CH_3CH_2S$), 1.325 (d, 3H, H-6,6,6), 1.227 (t, 3H, $CH_3CH_2S$), $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.2 Hz.

**1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-αβ-L-rhamnopyranosyl)-β-D-glucopyranose (4αβ).** To a solution of **1** (1.61 g, 3.36 mmol) in dry dichloromethane (47 mL) was added a solution of bromine (0.34 mL, 6.63 mmol) in dichloromethane (13 mL). When TLC (5:3 light petroleum-ethyl acetate) showed the absence of **1** ($R_F$ 0.45), the mixture was concentrated and co-concentrated with toluene (3 x 40 mL), to yield 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl bromide (**2**), which was directly used in the next step. A solution of 1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose[7] (**3**) (0.77 g, 2.24 mmol) in dry dichloromethane (16 mL) containing silver silicate[8] (1.5 g) and powdered molecular sieves (4Å, 0.76 g) was stirred for 1 h under argon. A solution of freshly prepared **2** (3.36 mmol) in dichloromethane (8 mL) was added dropwise in 27 h, and the mixture was stirred for an additional period of 19 h. TLC (5:3 light petroleum-ethyl acetate) showed two new compounds with $R_F$ 0.55 (**4β**) and 0.50 (**4α**), respectively. The mixture was diluted with dichloromethane, filtered through Celite, and concentrated. Column chromatography (4:1 light petroleum-ethyl acetate) of the residue gave **4β** (0.74 g, 43%) as white crystals: mp 105-106 °C (from ethanol); $[\alpha]_D$ +22° (*c* 1, chloroform); and **4α** (0.61 g, 36%) as a syrup: $[\alpha]_D$ -65° (*c* 1, chloroform). $^{13}$C NMR **4β** ($CDCl_3$) δ 138.5-137.7 and 128.4-127.4 ($C_6H_5CH_2O$), 100.9 (C-1,1'), 66.9 (C-6), 17.8 (C-6'); $^1$H NMR **4β** ($CDCl_3$) δ 7.476-7.212 (m, 25H, 5Ph), 5.457 (s, 1H, H-1), 4.953, 4.938, 4.853, 4.676, 4.636, 4.622, 4.568, 4.468, 4.453, and 4.383 (10d, each 1H, 5PhC$H_2$O), 4.324 (s, 1H, H-1'), 3.866 (d, 1H, H-6a), 3.721 (d, 1H, H-2'), 3.647 (dd, 1H, H-6b), 3.572 (t, 1H, H-4'), 3.832 (d, 1H, H-2), 3.297 (dd, 1H, H-3'), 3.203 (m, 1H, H-5'), 1.323 (d, 3H, H-6',6',6'), $J_{1,2} \approx 0$ Hz, $J_{2,3} = 4.4$ Hz, $J_{5,6a} \approx 0$ Hz, $J_{5,6b} = 5.3$ Hz, $J_{6a,6b} = -7.2$ Hz, $J_{1',2'} \approx 0$ Hz, $J_{2',3'} = 3.0$ Hz, $J_{3',4'} = J_{4',5'} = 9.4$ Hz, $J_{5',6'} = 6.2$ Hz; $^{13}$C NMR **4α** ($CDCl_3$) δ 138.5-137.4 and 128.2-127.4 ($C_6H_5CH_2O$), 100.5 (C-1), 96.3 (C-1'), 65.1 (C-6), 17.7 (C-6'), $J_{H-1,C-1} = 168$ Hz, $J_{H-1',C-1'} = 166$ Hz; $^1$H NMR **4α** ($CDCl_3$) δ 7.337-7.264 (m, 25H, 5Ph), 5.437 (bs, 1H, H-1), 3.751 (m, 1H, H-5'), 3.543 (bs, 1H, H-3), 3.337 (bs, 1H, H-2), 1.277 (d, 3H, H-6',6',6'), $J_{1,2} \approx 0$ Hz, $J_{4',5'} = 9.4$ Hz, $J_{5',6'} = 6.1$ Hz.

Anal. Calcd for $C_{47}H_{50}O_9$: C, 74.39; H, 6.64. Found **4β**: C, 74.43; H, 6.97.

**1,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-αβ-D-glucopyranose (5αβ).** To a solution of **4β** (199 mg, 0.26 mmol) in acetic anhydride (3.0 mL), was added trifluoroacetic acid (0.14 mL), and the mixture was kept at room temperature for 16 h. Then TLC (5:2 light petroleum-ethyl acetate) showed the disappearance of the starting material, and the mixture was concentrated and co-concentrated with toluene, ethanol, and dichloromethane (each 3 x 25 mL). Column chromatography (5:2 light petroleum-ethyl acetate) of the residue gave **5αβ** as a syrup, the

α-anomer as the major product (163 mg, 72%): $R_F$ 0.31 (5:2 light petroleum-ethyl acetate); $^{13}C$ NMR ($CDCl_3$) δ 170.7 and 168.9 (2*C*OCH$_3$), 138.7-137.2 and 128.3-127.0 (*C*$_6$H$_5$CH$_2$O), 101.9 (C-1'), 93.9 (C-1β), 89.4 (C-1α), 82.5, 81.2, 79.7, 78.4, 75.8, 74.0, 71.6, and 70.7 (C-2,3,4,5,2',3',4',5'), 75.4, 75.3, 73.9, 72.8, and 71.5 (5Ph*C*H$_2$O), 63.1 (C-6), 21.0 and 20.7 (2CO*C*H$_3$), 17.6 (C-6').

Anal. Calcd for $C_{51}H_{56}O_{12}$: C, 71.15; H, 6.56. Found: C, 70.71; H, 6.89.

**6-*O*-Acetyl-2,3-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-αβ-D-glucopyranosyl Trichloroacetimidate (7αβ).** To a solution of **5** (65 mg, 0.07 mmol) in dry *N*,*N*-dimethylformamide (1.5 mL), was added hydrazine acetate (15 mg, 0.16 mmol). After 2 h (TLC 2:1 light petroleum-ethyl acetate; **6** $R_F$ 0.14) the mixture was diluted with ethyl acetate (50 mL), washed with aqueous 5% sodium chloride, dried, filtered, and concentrated. To a solution of the residue in dry dichloromethane (3 mL) and trichloroacetonitrile (75 μL, 0.75 mmol), was added freshly fused potassium carbonate (75 mg), and the mixture was stirred for 5 h. When TLC (2:1 light petroleum-ethyl acetate) showed the conversion of **6** into two new compounds **7**αβ with $R_F$ 0.60 and 0.54, the mixture was filtered through silica gel (9:1 dichloromethane-ethyl acetate), and concentrated to give **7**αβ (61 mg, 84%) as a syrup. The compound was used directly in the next step.

**1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)-α-D-glucopyranose (9).** A solution of **5** (390 mg, 0.45 mmol) in 2-propanol (10 mL) and ethyl acetate (2 mL) was hydrogenolysed using 10% Pd/C (78 mg) at 4 kg/cm$^2$ for 48 h, filtered through Celite, and concentrated to yield **8**. A solution of **8** in pyridine (5 mL) and acetic anhydride (5 mL) was stirred for 16 h, and then the mixture was concentrated and co-concentrated with toluene, ethanol and dichloromethane (each 3 x 20 mL). Column chromatography (93:7 dichloromethane-acetone) of the residue afforded **9** (242 mg, 86%) as white crystals: mp 178 °C (from ethanol); $[\alpha]_D$ +74° (*c* 1, chloroform); $R_F$ 0.42 (9:1 dichloromethane-acetone); $^{13}C$ NMR ($CDCl_3$) δ 170.1-168.8 (*C*OCH$_3$), 99.2 (C-1'), 89.0 (C-1), 62.2 (C-6), 20.6-20.5 (CO*C*H$_3$), 17.1 (C-6'), $J_{C\text{-}1',H\text{-}1'}$ = 159 Hz, $J_{C\text{-}1,H\text{-}1}$ = 177 Hz; $^1H$ NMR ($CDCl_3$) δ 6.250 (d, 1H, H-1), 5.444 (dd, 1H, H-3), 5.336 (d, 1H, H-2'), 5.005 (t, 1H, H-4'), 4.992 (dd, 1H, H-2), 4.908 (dd, 1H, H-3'), 4.672 (bs, 1H, H-1'), 3.998 (m, 1H, H-5), 3.920 (t, 1H, H-4), 3.458 (m, 1H, H-5'), 2.172, 2.159, 2.098 (2x), 2.047, 1.999, and 1.978 (6s, 3,3,6,3,3,3H, 7Ac), 1.259 (d, 3H, H-6',6',6'), $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 2.9 Hz, $J_{5,6b}$ = 3.4 Hz, $J_{1',2'} \approx 0$ Hz, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 10.2 Hz, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.2 Hz.

Anal. Calcd for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.25; H, 5.85.

**2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)-α-D-glucopyranosyl Trichloroacetimidate (11).** To a solution of **9** (0.95 g, 1.54 mmol) in dry *N*,*N*-dimethylformamide (3 mL), was added hydrazine acetate (0.25 g, 2.7 mmol). The mixture was stirred for 1 h at 50 °C, then diluted with ethyl acetate (150 mL), washed with aqueous 5% sodium chloride, dried, filtered, and concentrated, yielding **10**. To a solution of **10** in dichloromethane (15 mL) and trichloroacetonitrile (0.77 mL, 7.7 mmol), a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (0.24 mL, 1.62 mmol) in dichloromethane (2 mL) was added at 0 °C. After 45 min TLC (9:1 dichloromethane-acetone) indicated an almost complete conversion of **10** into **11** ($R_F$ 0.57), and the mixture was concentrated. Column chromatography (9:1 dichloromethane-acetone) of the residue gave **11** (0.78 g, 71%) as a syrup: $[\alpha]_D$ +82° (*c* 1, chloroform); $^{13}C$ NMR ($CDCl_3$) δ 170.2-169.1 ($COCH_3$), 160.8 ($OCNHCCl_3$), 99.4 (C-1'), 92.9 (C-1), 90.6 ($OCNHCCl_3$), 62.2 (C-6), 20.6-20.3 ($COCH_3$), 17.1 (C-6'); $^1H$ NMR ($CDCl_3$) δ 8.645 (s, 1H, $OCNHCCl_3$), 6.485 (d, 1H, H-1), 5.537 (t, 1H, H-3), 5.343 (bd, 1H, H-2'), 5.040 (dd, 1H, H-2), 5.004 (t, 1H, H-4'), 4.909 (dd, 1H, H-3'), 4.681 (bs, 1H, H-1'), 4.121 (m, 1H, H-5), 3.959 (t, 1H, H-4), 3.450 (m, 1H, H-5'), 2.169, 2.093, 2.086, 2.046, 1.994, and 1.977 (6s, each 3H, 6Ac), 1.265 (d, 3H, H-6',6',6'), $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 9.8 Hz, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 3.1 Hz, $J_{5,6b}$ = 3.3 Hz, $J_{1',2'} \approx 0$ Hz, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, $J_{5',6'}$ = 6.2 Hz.

**4-*O*-(β-L-Rhamnopyranosyl)-D-glucopyranose (12).** To a solution of **9** (12 mg, 24 μmol) in methanol (5 mL), sodium methoxide was added to pH 9. The mixture was stirred overnight, neutralised with Dowex-50 ($H^+$) resin, filtered, and concentrated, to give **12** (7.6 mg, 96%) as a white powder: $[\alpha]_D$ +70° (*c* 0.7, water); $^{13}C$ NMR ($D_2O$) δ 102.1 (C-1', α-anomer), 102.0 (C-1', β-anomer), 97.2 (C-1β), 93.3 (C-1α), 62.1 (C-6, β-anomer), 61.0 (C-6, α-anomer), 18.0 (C-6'); $^1H$ NMR ($D_2O$) δ 5.224 (d, 0.4H, H-1α), 4.868 (bs, 0.4H, H-1', α-anomer), 4.862 (bs, 0.6H, H-1', β-anomer), 4.631 (d, 0.6H, H-1β), $J_{1,2}$ = 3.8 Hz (α), $J_{1,2}$ = 8.0 Hz (β), $J_{1',2'} \approx 0$ Hz.

**1,3-Di-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranose (14).** A solution of benzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside[18] (2.71 g, 6.34 mmol) in pyridine (15 mL) and acetic anhydride (15 mL) was stirred overnight, concentrated and co-concentrated using toluene, ethanol and dichloromethane (each 3 x 60 mL), yielding benzyl 3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**13**). To a solution of **13** (2.96 g, 6.21 mmol) in acetic anhydride (18 mL) and acetic acid (10 mL) was added a solution of 2% concd sulfuric acid in acetic anhydride (2 mL). After 4 h, TLC (98:5 dichloromethane-ethyl acetate) indicated the conversion of **13** ($R_F$ 0.73) into a lower moving spot **14** ($R_F$ 0.63). The mixture was poured into cold aqueous saturated sodium hydrogencarbonate (750 mL), stirred overnight, extracted with dichloromethane (3 x 150 mL); the combined extracts