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A Practical, Highly Stereoselective Synthesis of the Trisaccharide Repeating Unit of a *Streptococcus pneumoniae* Polysaccharide

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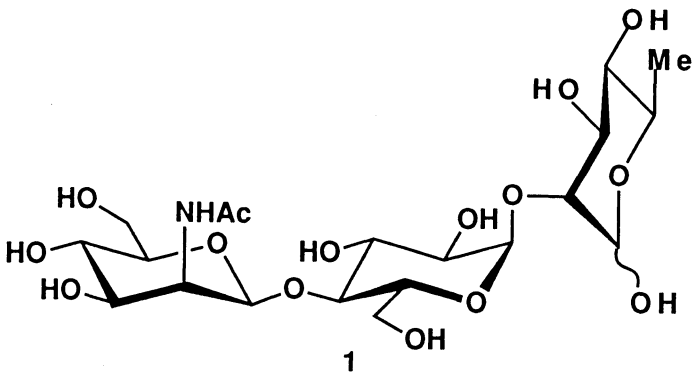
A highly stereoselective method has been developed for the synthesis of the trisaccharide component of the capsular polysaccharide of *Streptococcus pneumoniae* type 19F. The key intermediate, 2-benzoyloxyiminoglycosyl bromide was successfully utilized for the construction of the *N*-acetyl-β-D-mannosamine unit of the trisaccharide.

Streptococcus pneumoniae type 19F is a major pathogenic bacteria for serious pneumonia,[1] in particular, in infants whose immunity is still less sufficient against diseases. The structure of the repeating unit of the capsular polysaccharide of type 19F bacteria has been elucidated[2,3] as *N*-acetyl-β-D-mannosamine-containing trisaccharide:

$\rightarrow$4)-β-D-Man*p*NAc-(1$\rightarrow$4)-α-D-Glc*p*-(1$\rightarrow$2)-α-L-Rha*p*-(1-$PO_4^-\rightarrow$

The trisaccharide component **1** is to be considered an epitop of the antigenic polysaccharide and, hence, an intercellular recognition molecule for the infection. Such biological significance has elicited considerable efforts towards the synthetic acquisition of **1** in quest of artificial antigens, resulting in three different syntheses.[4-6] Since direct β-glycosylation of *N*-acetyl-D-mannosamine is not feasible — α-D-manno-



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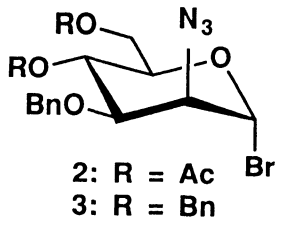
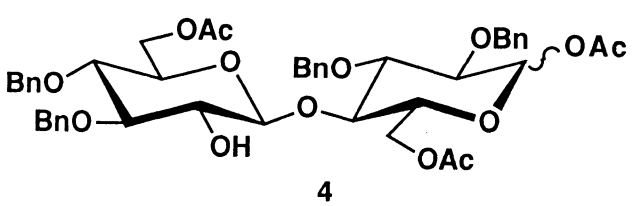


2: R = Ac
3: R = Bn



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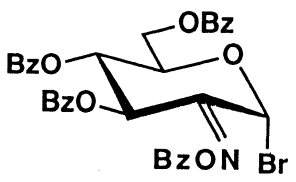

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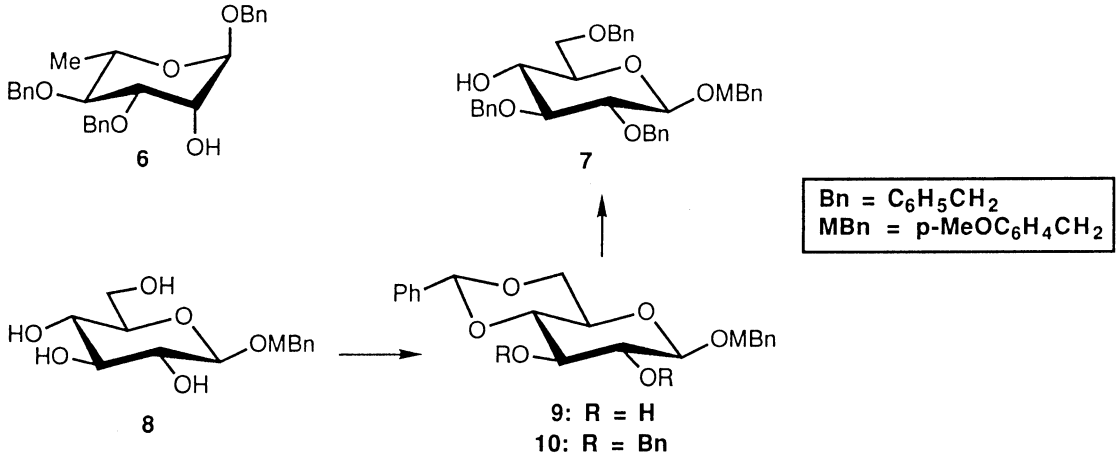
saminides will be formed exclusively —, every approach has employed designed progenitors for the generation of the *N*-acetyl-β-D-mannosamine unit of **1**. Accordingly, 2-azido-2-deoxy-mannopyranosyl halides **2**[5] and **3**[6] have been utilized as mannosaminyl donors, or alternately, the elaborately blocked cellobiose derivative **4**, generated from its monosaccharide components, in which the equatorial 2'-OH was converted into an axial 2-NHAc via oxidation, oximation, and reduction.[4] However, neither of these approaches meets practical preparative criteria, inasmuch as they entail more than 16 steps from D-glucose to **1** with overall yields of less than 1%[4,5] and 1.8%.[6]

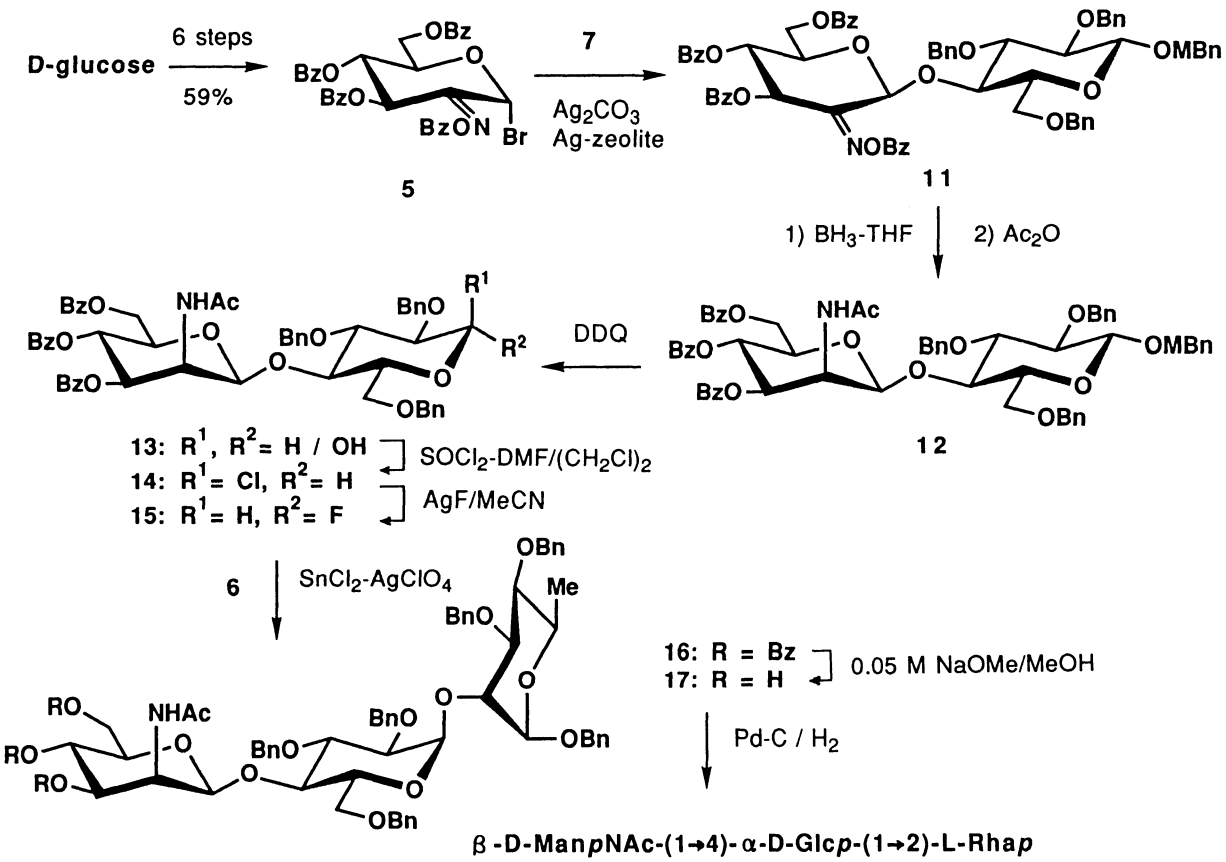
We here describe a novel, preparatively useful synthesis of the target trisaccharide **1** using as the key intermediate, the 2-benzoyloxyiminoglycosyl bromide **5**, which is readily accessible from glucose in 6-high-yielding steps (overall 59%),[7] and whose utility as a suitable glycosyl donor for the convenient successive elaboration of β-D-mannosamine units has amply been demonstrated.[8] Given the availability of L-rhamnose derivative **6**[9] as a suitable glycosyl acceptor for attachment of the rhamnose portion, an appropriately substituted central glucose derivative was required capable of functioning, successively, as a 4-*O*-acceptor and a glycosyl donor. The *p*-methoxybenzyl 2,3,6-tri-*O*-benzyl-β-D-glucoside **7** was selected for this purpose, since, upon glycosylation the anomeric substituent was apt to be removable smoothly by oxidative cleavage with DDQ.[10] The preparation of **7** followed conventional methodology, such that acetobromoglucose was converted into the β-MBn glucoside by reaction with *p*-methoxybenzyl alcohol, followed by de-*O*-acetylation, benzylidenation (**8**→**9**), benzylation (→**10**), and reductive acetal opening to provide **7** in a 32% overall yield over the 5 steps involved.

The highly β-selective glycosidation of bromide **5** with the 4-OH-free glucoside **7** was effected by using Ag_2CO_3/Ag-zeolite as the catalyst in dichloromethane (48 h, 25 °C), affording an approximate 10:1 (^{1}H-NMR)[11] β/α anomeric mixture of disaccharides from which **11**[11] was isolated in 85% yield. If, however, glycosidation of **5** with **7** was induced by silver triflate, the respective α-linked disaccharide[11] was obtained in a yield of 90%.

Reduction of the β-disaccharide **11** with borane-THF complex (2 h, 25 °C) proceeded with high preference for hydride attack from the α-side (12:1 based on ^{1}H-NMR) and subsequent *N*-acetylation with acetic anhydride afforded the *N*-acetyl-β-D-mannosaminyl-(1→4)-β-D-glucoside **12**[14] in 69% yield.

In order to convert the disaccharide **12** into a glycosyl donor sufficiently reactive to glycosylate rhamnoside **6**, the glycosyl fluoride **15** was generated by way of de-*O*-methoxybenzylation (DDQ/CH_2Cl_2-H_2O, →**13**, 43%),[15] chlorination ($SOCl_2$-DMF/$(CH_2Cl)_2$, →**14**, quant.), and fluorination (AgF/MeCN, 77%).





Indeed, when the fluoride **15** was exposed to the rhamnosyl acceptor **6**[9] in the presence of $AgClO_4$–$SnCl_2$ in dichloromethane, an essentially α-selective glycosylation was effected to afford the desired trisaccharide **16** as a syrup in a 51% yield. The coupling constants for the central glucosyl moiety ($J_{1',2'}$ = 3.5, $J_{2',3'}$ = 9.0 Hz) unequivocally proved the correct intersaccharidic link-up. Subsequent de-*O*-benzoylation of **16** with 0.05 M NaOMe/MeOH (→**17**, 89%), and hydrogenolysis for removing the benzyl protecting groups (Pd-C/H_2, MeOH, 94%) proceeded smoothly to the target trisaccharide **1**, which was isolated as an amorphous product of $[\alpha]_D^{22}$ + 28° (*c* 0.5, MeOH). Its ^{1}H- and ^{13}C-NMR data[16] were identical in all respects with those reported for the natural[2] as well as the synthetic product.[5,6]

In summation, a straightforward, highly stereoselective synthesis of the repeating unit of an infectious capsular polysaccharide has been achieved. Our method is not only more efficient — 5% yield over 14 steps from D-glucose — than the previous ones,[4–6] but also practical in elaboration of the critical β-D-mannosaminyl portion. The construction of analogs of **1** as well as other, more complex oligosaccharides with a β-D-Man*p*NAc unit is currently under investigation.

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11) Disaccharide **11** was obtained as a colorless syrup of $[\alpha]_D^{25}$ + 9.8° (*c* 0.7, $CHCl_3$), its β-configuration followed from $J_{3',4'}$ and $J_{4',5'}$ couplings of only 5.5 Hz each, indicating distortion of the pyranoid ring towards the twist-boat form as depicted in formula **11**. Similar observations have been made for other β-anomeric 2-benzoyloxyiminoglycosides (Refs. 8, 12, and 13). In contrast, the corresponding α-anomer of **11**, of mp 60-63 °C and $[\alpha]_D^{23}$ + 43.8°(*c* 0.5, $CHCl_3$), had the normal $J_{3',4'}$ and $J_{4',5'}$ values of 10 Hz each.
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14) Relevant physical data for **12**: mp 130-132 °C, $[\alpha]_D^{23}$ - 29.7°(*c* 0.5, $CHCl_3$); $J_{1',2'}$ = 1.2, $J_{2',3'}$ = 4.0 Hz.
15) Application of another de-*O*-methoxybenzylating agent, ammonium cerium(IV) nitrate was found to be less effective than DDQ.
16) The anomeric composition of trisaccharide **1** at the reducing end was about 2:1 in favor of the α-anomer. ^{1}H-NMR (300 MHz, D_2O): δ= 1.21 (3H, d, α-H-6), 1.22 (3/2H, d, β-H-6), 1.99 (3H, s, NHAc), 3.92 (1/2H, dd, β-H-2), 4.01 (1H, td, α-H-5'), 4.47 (1H, dd, H-2"), 4.81 (1H, d, H-1"), 4.86 (1/2H, broad s, β-H-1), 4.92 (1H, d, α-H-1'), 5.00 (1/2H, d, β-H-1'), 5.14 (1H, d, α-H-1); $J_{1,2}$ = 1.5 (α), 1.0 (β), $J_{5,6}$ = 6.3 (α), 5.5 (β), $J_{1',2'}$ = 3.8 (α), 3.7 (β), $J_{1'',2''}$ = 1.3, $J_{2'',3''}$ = 4.2 Hz. ^{13}C-NMR (75 MHz, D_2O): d = 17.5 (α- and β-C-6), 22.9 (NHCOMe), 54.1 (C-2"), 60.6 (α- and β-C-6'), 61.2 (C-6"), 67.5 (C-4"), 69.5 (α-C-5), 70.1 (α-C-3), 71.1 (α-C-5'), 71.4 (β-C-5'), 72.0 (α-C-2'), 72.1 (α-C-3'), 72.4 (β-C-4), 72.7 (β-C-2',3'), 72.8 (β-C-3, α-C-4), 72.9 (C-3"), 73.1 (β-C-5), 77.4 (C-5"), 78.2 (α-C-2), 79.3 (β-C-4'), 79.5 (α-C-4'), 81.8 (β-C-2), 92.4 (α-C-1), 94.6 (β-C-1), 98.5 (α-C-1'), 100.2 (C-1"), 101.8 (β-C-1'), 176.3 (NHCO).

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