CHEMISTRY LETTERS, pp. 707-710, 1992. 

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

Eisuke KAJI,* Frieder W. LICHTENTHALER,+ Yumiko OSA, Keiko TAKAHASHI, Eiko MATSUI, and Shonosuke ZEN

School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108

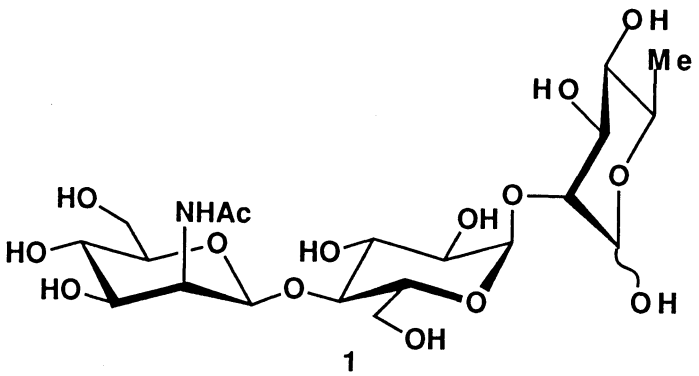
+Institut für Organische Chemie, Technische Hochschule Darmstadt, D-6100 Darmstadt, Germany

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-$\beta$-D-mannosamine unit of the trisaccharide.

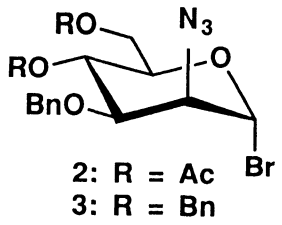
*Streptococcus pneumoniae* type 19F is a major pathogenic bacteria for serious pneumonia,[1] in particular, in infants whose immunity is still less sufficient against diseases. The structure of the repeating unit of the capsular polysaccharide of type 19F bacteria has been elucidated[2,3] as *N*-acetyl-$\beta$-D-mannosamine-containing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

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

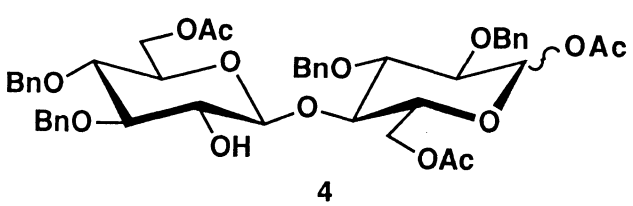
The trisaccharide component **1** is to be considered an epitop of the antigenic polysaccharide and, hence, an intercellular recognition molecule for the infection. Such biological significance has elicited considerable efforts towards the synthetic acquisition of **1** in quest of artificial antigens,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



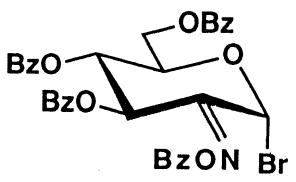

1




2: R = Ac
3: R = Bn




4




5

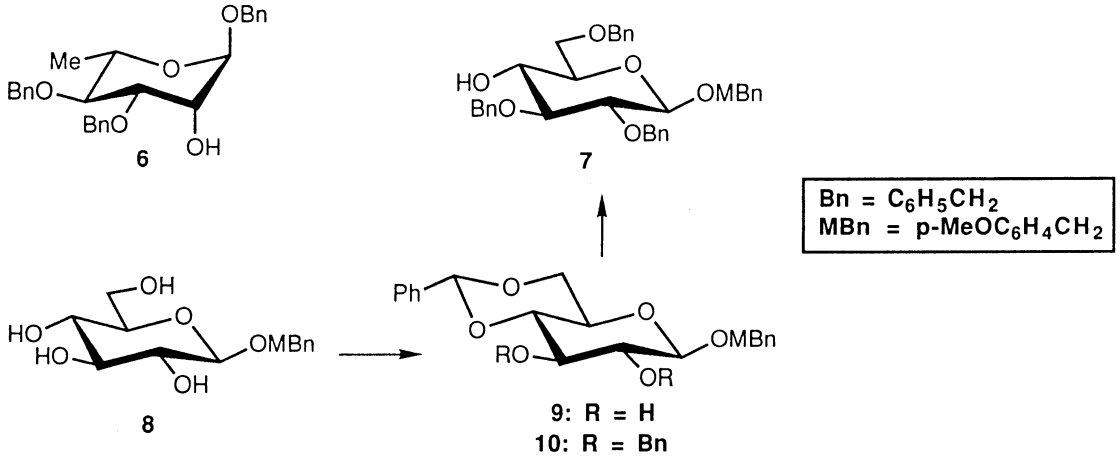
saminides will be formed exclusively —, every approach has employed designed progenitors for the generation of the *N*-acetyl-β-D-mannosamine unit of **1**. Accordingly, 2-azido-2-deoxy-mannopyranosyl halides **2**[5] and **3**[6] have been utilized as mannosaminyl donors, or alternately, the elaborately 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]

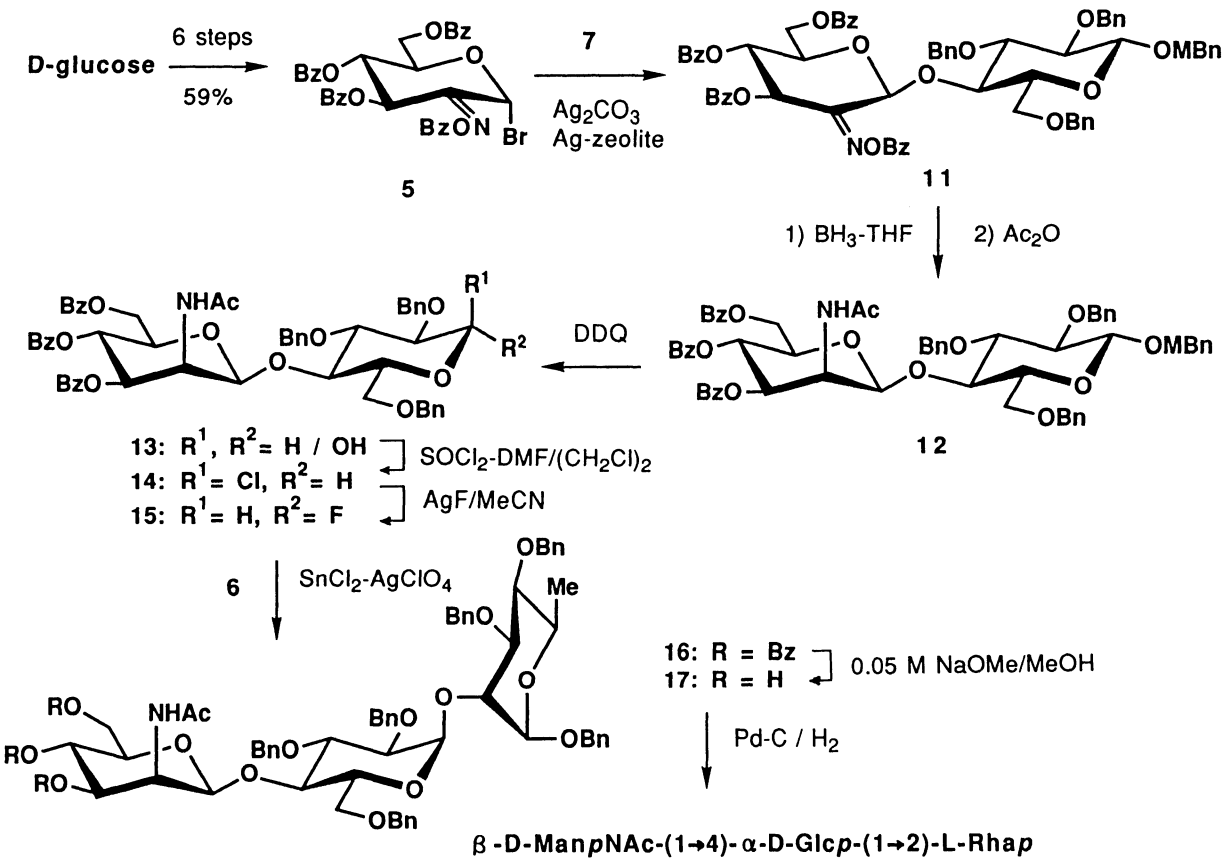
We here describe a novel, preparatively useful synthesis of the target trisaccharide **1** using as the key intermediate, the 2-benzoyloxyiminoglycosyl bromide **5**, which is readily accessible from glucose in 6-high-yielding steps (overall 59%),[7] and whose utility as a suitable glycosyl donor for 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 $\alpha$-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 $\beta$-D-mannosaminyl portion. The construction of analogs of **1** as well as other, more complex oligosaccharides with a $\beta$-D-Man*p*NAc unit is currently under investigation.

References

1) H. J. Jennings, *Adv. Carbohydr. Chem. Biochem.*, **41**, 155 (1983).
2) N. Ohno, T. Yadomae, and T. Miyazaki, *Carbohydr. Res.*, **80**, 297 (1980).
3) H. J. Jennings, K. G. Rossel, and D. J. Carlo, *Can. J. Chem.*, **58**, 1069 (1980).
4) L. Panza, F. Ronchetti, G. Russo, and L. Toma, *J. Chem. Soc., Perkin Trans. 1*, **1987**, 2745.

5) T. Sugawara and K. Igarashi, *Carbohydr. Res.*, **172**, 195 (1988).
6) H. Paulsen, B. Helpap, and J. P. Lorentzen, *Carbohydr. Res.*, **179**, 173 (1988).
7) F. W. Lichtenthaler, P. Jarglis, and W. Hempe, *Liebigs Ann. Chem.*, **1983**, 1959.
8) E. Kaji, F. W. Lichtenthaler, T. Nishino, A. Yamane, and S. Zen, *Bull. Chem. Soc. Jpn.*, **61**, 1291 (1988).
9) A. Liptak, P. Fugedi, and P. Nanasi, *Carbohydr. Res.*, **65**, 209 (1978).
10) Y. Oikawa, T. Yoshida, and O. Yonemitsu, *Tetrahedron Lett.*, **23**, 885 (1982).
11) Disaccharide **11** was obtained as a colorless syrup of $[\alpha]_D^{25}$ + 9.8° (*c* 0.7, $CHCl_3$), its $\beta$-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 $\beta$-anomeric 2-benzoyloxyiminoglycosides (Refs. 8, 12, and 13). In contrast, the corresponding $\alpha$-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.
12) F. W. Lichtenthaler, E. Kaji, and S. Weprek, *J. Org. Chem.*, **50**, 3505 (1985).
13) F. W. Lichtenthaler and E. Kaji, *Liebigs Ann. Chem.*, **1985**, 1659.
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 $\alpha$-anomer. $^1$H-NMR (300 MHz, $D_2O$): $\delta$= 1.21 (3H, d, $\alpha$-H-6), 1.22 (3/2H, d, $\beta$-H-6), 1.99 (3H, s, NHAc), 3.92 (1/2H, dd, $\beta$-H-2), 4.01 (1H, td, $\alpha$-H-5'), 4.47 (1H, dd, H-2"), 4.81 (1H, d, H-1"), 4.86 (1/2H, broad s, $\beta$-H-1), 4.92 (1H, d, $\alpha$-H-1'), 5.00 (1/2H, d, $\beta$-H-1'), 5.14 (1H, d, $\alpha$-H-1); $J_{1,2}$ = 1.5 ($\alpha$), 1.0 ($\beta$), $J_{5,6}$ = 6.3 ($\alpha$), 5.5 ($\beta$), $J_{1',2'}$ = 3.8 ($\alpha$), 3.7 ($\beta$), $J_{1'',2''}$ = 1.3, $J_{2'',3''}$ = 4.2 Hz. $^{13}$C-NMR (75 MHz, $D_2O$): d = 17.5 ($\alpha$- and $\beta$-C-6), 22.9 (NHCOMe), 54.1 (C-2"), 60.6 ($\alpha$- and $\beta$-C-6'), 61.2 (C-6"), 67.5 (C-4"), 69.5 ($\alpha$-C-5), 70.1 ($\alpha$-C-3), 71.1 ($\alpha$-C-5'), 71.4 ($\beta$-C-5'), 72.0 ($\alpha$-C-2'), 72.1 ($\alpha$-C-3'), 72.4 ($\beta$-C-4), 72.7 ($\beta$-C-2',3'), 72.8 ($\beta$-C-3, $\alpha$-C-4), 72.9 (C-3"), 73.1 ($\beta$-C-5), 77.4 (C-5"), 78.2 ($\alpha$-C-2), 79.3 ($\beta$-C-4'), 79.5 ($\alpha$-C-4'), 81.8 ($\beta$-C-2), 92.4 ($\alpha$-C-1), 94.6 ($\beta$-C-1), 98.5 ($\alpha$-C-1'), 100.2 (C-1"), 101.8 ($\beta$-C-1'), 176.3 (NHCO).

(Received December 19, 1991)