Expedient Synthesis of an Anomerically Modified Trisaccharide Component of the Capsular Polysaccharide from *Streptococcus pneumoniae* Type 19F

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A practical stereoselective synthesis is described for α-D-ManNAc-(1→4)-α-D-Glc-(1→2)-L-Rha, an anomerically modified trisaccharide component of the capsular polysaccharide of *Streptococcus pneumoniae* type 19F. The key intermediary disaccharide, 2-(benzoyloxyimino)-2-deoxyglycosyl-α(1→4)-glucoside, was prepared by α-selective glycosylation (AgOTf–TMU/CH_2Cl_2, 90%) of a suitably blocked *p*-methoxybenzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside with 3,4,6-tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranosyl bromide, and converted into an α-D-ManNAc-(1→4)-α-D-Glc-(1→MBn) derivative by means of a *manno*-selective reduction (BH_3·THF→Ac_2O, 55%). Consecutive anomeric activation (1-OMBn→1-OH→1-F, 45% over 2 steps), glycosylation ($SnCl_2$–$AgClO_4$/CH_2Cl_2, 71%, α:β=2:1) of 2-OH of benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside, and deblocking (NaOMe→Pd-C/H_2, 90%) gave the target trisaccharide.

Capsular polysaccharide (CPS) of *Streptococcus pneumoniae* carries immunogenic specificity corresponding to the sero-types of pneumococcal bacteria,[1] in which the type 19F strain is of particular clinical importance, since it frequently provokes acute pneumonia. The structure of the repeating unit of CPS of type 19F was elucidated[2] as being *N*-acetyl-β-D-mannosamine-containing trisaccharide **1**, of which the saccharide portion has been synthesized recently.[3—6] Other than the kinds of sugar unit, the anomeric structure of CPS is responsible for the serotypes of *Streptococcus pneumoniae*; e.g., the immunologically cross-reactive types 9A[7] and 9V[8] differ regarding the anomeric structure of the constituent sugar unit. Accordingly, our further endeavor in a quest to understand the relationships between the anomeric structure and the immunogenic activity requires chemically modified trisaccharide analogues, one of which should be an anomerically modified trisaccharide, α-D-ManNAc-(1→4)-α-D-Glc-(1→2)-L-Rha (**2**) (Fig. 1).

An assembly of the trisaccharide **2** can be executed by a stepwise linkup of the individual sugar units from the non-reducing site, where aquisition of the α-D-mannosamine unit is a crucial step. To data, a preparatively reliable synthesis of α-D-mannosamine-containing oligosaccharides is not available. Some alkyl or glycosyl α-D-mannosaminides of type **6** have been synthesized by the reduction of 2-(hydroxyimino)-2-deoxy-α-D-glycosides (**3**)[9—11] or 2-(acetoxyimino)-2-deoxy-α-D-glycoside (**4**),[11—13] the utilities of which, however, are encumbered with a very low stereoselectivity, having a gluco (**5**):manno (**6**) ratio of 1:1.5 at best[10,11] with respect to the *manno*-selectivity (Scheme 1). In contrast to the high stereoselectivity for the formation of α-D-glucosaminides (**5**) (*gluco*:*manno*=95:5)[11] by the hydroboration of 2-(acetoxyimino)-α-D-glycosides (**4**), the selectivity for the α-D-mannosaminides (**6**) remains low, as described above. Hence, a novel, expedient methodology has been requested for constructing the α-D-mannosamine unit.

Based on our previous studies[14—17] that 2-(benzoyloxyimino)-2-deoxyglycosides have been amply utilized for the synthesis of 1,2-*cis*-aminosugars, the hydroboration of 2-(benzoyloxyimino)-2-deoxy-α-D-glycosides[14,15] should give rise to the formation of α-D-glucosaminides, as similarly proposed by Lemieux et al.[11] Nevertheless, we have now found that a 2-(benzoyloxyimino)-2-deoxy-α-D-glycosyl-(1→4)-glucoside could provide, upon hydroboration, α-D-mannosaminyl-(1→4)-glucoside as a major product. Accordingly, the stereoselectivity of hydroboration of the benzoyloxyimino function to an amino group was seen to depend on the kind of aglycons. At this stage we have no decisive evidence that the *manno* selectivity is elicited from either stereochemical or electronic grounds in 2-(benzoyloxyimino)-2-deoxy-α-D-glycoside **9**. Hence, a variety of disaccharides comprising 2-(benzoyloxyimino)-2-deoxyglycosyl-α(1→6)-, α(1→4)-, α(1→3)-, and α(1→2)-glycosides are being prepared for the elucidation of the reduction mechanism of the 2-acyloxyimino group. Although the stereoselectivity mechanism still remains to be solved, this practical course of reduction of the 2-(benzoyloxyimino)-2-deoxyglycoside with sterically hindered aglycon may open a novel, facile access

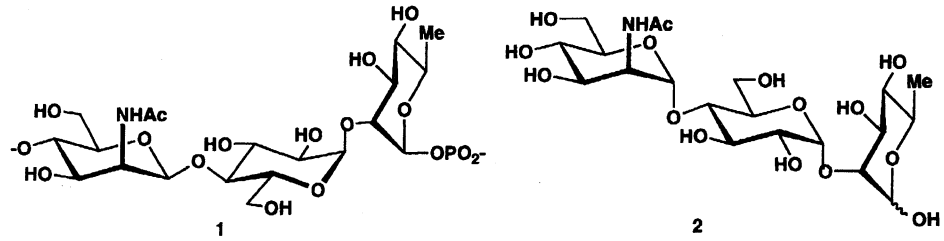
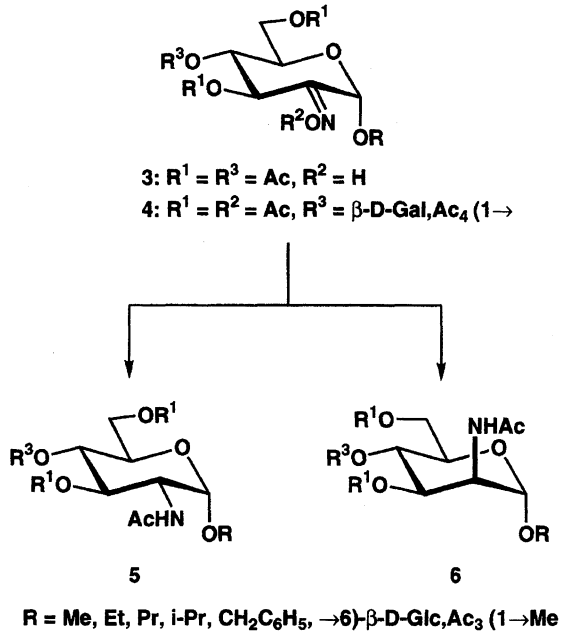


Fig. 1.



Scheme 1.

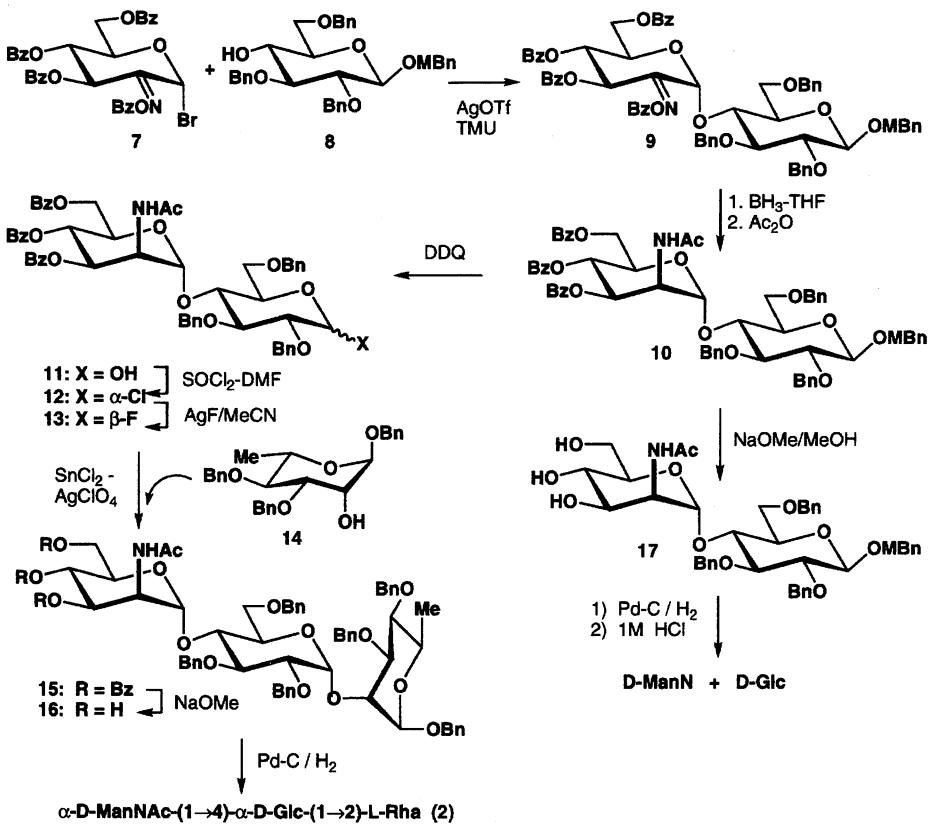
to α-D-mannosamine-containing oligosaccharides.

Thus, 2-(benzoyloxyimino)-2-deoxyglycosyl bromide (**7**),[18] readily available in 59% yield over 6 steps from D-glucose, was treated with *p*-methoxybenzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**)[6] in the presence of silver triflate (AgOTf) and 1,1,3,3-tetramethylurea (TMU) in dichloromethane to afford stereoselectively ($\alpha:\beta$=10:1, ^{1}H NMR) the α(1→4)-disaccharide **9** in 90% yield. In contrast, insoluble silver salt-promoted glycosylation (Ag_2CO_3/Ag-zeolite in CH_2Cl_2) of **8** with **7** invariably resulted in the formation of the epimeric β(1→4)-disaccharide in preference to **9** ($\alpha:\beta$=1:20, ^{1}H NMR).[6] The α-configuration of **9** was confirmed on the basis of the ^{1}H NMR spectra, in which $J_{3',4'}$ and $J_{4',5'}$ values of 10 Hz each suggested a $^4C_1$ conformation characteristic for α-anomers of this type of compound,[14,15] whereas the corresponding β-glycosides exclusively showed relatively small coupling constants, being 5.5 Hz each for $J_{3',4'}$ and $J_{4',5'}$ allowing for a twist boat form resulting from a stereochemical demand of the β-anomers.[14,17]

A subsequent reduction of the benzoyloxyimino group of **9** with the borane-tetrahydrofuran (BH_3·THF) complex gave, upon *N*-acetylation, the α-D-ManNAc-(1→4)-Glc derivative **10** in 55% yield. A byproduct isolated in ca. 6% yield was characterized as being the β-D-ManNAc-(1→4)-Glc derivative, probably due to the β(1→4) isomer of **9** being a priori contaminated in the educt. The configuration of the amino sugar portion of **10** was concluded to be α-D-*manno* on the basis of its ^{1}H and ^{13}C NMR spectra, i.e., the respective couplings $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ of 1.8, 3.6, and 9.1 Hz cogently reflect the equatorial, equatorial, axial, and axial-arrangements for H-1′,2′,3′, and 4′, respectively with the $^4C_1$ conformation, as depicted in the formula (Scheme 2). Aside from the spectroscopic analyses, compound **10** was decomposed into the respective sugar units in order to confirm the kind of constituent sugars by chemical degradation. *O*-Debenzoylation (NaOMe/MeOH) of **10**, giving **17**, was followed by hydrogenolysis (Pd-C/H_2) and hydrolysis (1 M HCl, 1 M=1 mol dm^{-3}) furnished D-mannosamine hydrochloride and D-glucose, which were unequivocally identified by a comparison with authentic samples (TLC on cellulose).

In order to obtain a reactive glycosyl donor for glycosylation of suitably protected benzyl α-L-rhamnopyranoside (**14**)[19] with free 2-OH, *p*-methoxybenzyl glycoside **10** was converted into the glycosyl fluoride **13**. The oxidative deprotection of the *p*-methoxybenzyl group was carried out with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ),[20] affording the 1-OH derivative **11** (49% yield, $\alpha:\beta$=2:1, ^{1}H NMR in $CDCl_3$). Since 15% of the unreacted **10** could be recovered and recycled, the total amount of **10**→**11** conversion was estimated to be more than 56%. Ensuing chlorination ($SOCl_2$/DMF) in dichloromethane furnished α-chloride **12** (quantitatively), which was in situ fluorinated with silver fluoride to give the β-fluoride **13** in 81% yield.

Glycosylation of the rhamnoside **14**[19] with **13** in the presence of $SnCl_2$–$AgClO_4$[21] in dichloromethane readily gave the trisaccharide **15** in 71% yield ($\alpha:\beta$=2:1), Application of an etherial solvent, such as 1,2-dimethoxyethane, being propagated[21] as superior for α-glycosylation, resulted in a recovery of the educts, so that no glycosylated product was observed in the reaction mixture. The stereochemistry at the C-1′ anomeric position of the newly formed intersaccharide linkage was elucidated by its NMR spectra. An isomer having a $J_{1',2'}$ of 3.5 Hz (^{1}H NMR) and a C-1′ chemical shift of 95.57 ppm (^{13}C NMR) was deduced to be α-anomer, while



Scheme 2.

another having a $J_{1',2'}$ of 8.0 Hz and 104.79 ppm for C-1′ was deduced to be β-anomer. This C-1′ anomeric mixture of **15** was exposed to Zemplén conditions (0.05 M NaOMe/MeOH), followed by purification through a silica-gel column ($CHCl_3$–AcOEt, 8:1), giving **16** (1′-α-anomer) in 58% yield along with the corresponding 1′-β-anomer (32%). The catalytic hydrogenolysis of the 1′-α-anomer **16** proceeded uneventfully to afford the desired free trisaccharide **2** in quantitative yield.

The method described above comprises a novel, stereoselective construction of α-D-mannosamine-containing trisaccharide, α-D-ManNAc-(1→4)-α-D-Glc-(1→2)-L-Rha, an anomerically modified component of CPS of a *Streptococcus pneumoniae* polysaccharide in 5.6% yield over 13 steps from D-glucose. Various further ramifications of this method for the construction of biologically relevant oligosaccharides are currently under investigation.

Experimental

General. Physical and spectral data were recorded on the following instruments: Mp, Yamato MP-1 apparatus and Yanagimoto micro melting-point apparatus (uncorrected); $[\alpha]_D$ JASCO DIP-150 digital polarimeter; MS, JMS D-100 mass spectrometer; and ^1H and ^{13}C NMR, Varian VXR-300 and XL-400 spectrometers. TLC was carried out on silica gel 60 F_{254} (Merck Art. 5735) developed with the same solvent systems as used for column chromatography in the individual experimental section. The spots were made visible by UV light (254 nm) or by charring with 10% aqueous H_2SO_4. Column chromatography was achieved on silica gel 60 (Merck Art. 7734). Cellulose layer chromatography was performed on Funaseru SF (FC-0510, Funakoshi Co.) and sugars were detected with 0.2% ninhydrin in 95% EtOH solution at 100 °C for 10 min (for amino sugars), as well as 10% aqueous H_2SO_4 on a hot plate (for neutral sugars). Authentic D-mannosamine hydrochloride (D-ManN·HCl) was purchased from Aldrich Chemical Co.

4-Methoxybenzyl *O*-[3,4,6-Tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranosyl]-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9). To a stirred solution of **8**[6] (57 mg, 100 μmol) in dry dichloromethane (2 ml) with molecular sieves 3A (MS-3A) (200 mg) were added in the dark AgOTf (65 mg, 260 μmol), 2-(benzoyloxyimino)-2-deoxyglycosyl bromide **7**[18] (135 mg, 200 μmol), and TMU (24 μl, 200 μmol). The mixture was stirred at room temperature for 20 h, diluted with dichloromethane (10 ml), and filtered through a pad of Celite. The filtrate was washed with 0.05 M HCl, water, 10% aqueous $NaHCO_3$, and water, then dried (Na_2SO_4) and concentrated to dryness. The residue was eluted from a silica-gel column with toluene–ethyl acetate (10:1). The concentration of the major fraction followed by crystallization from diethyl ether–pentane afforded 105 mg (90%) of **9** as colorless crystals: Mp 60—63°C; $[\alpha]_D^{23}$ + 43.8° (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ=3.54 (1H, m, H-5), 3.57 (1H, dd, H-2), 3.75 (1H, dd, H-3), 4.18 (1H, dd, H-4), 4.55 (1H, d, H-1), 5.93 (1H, dd, H-4′), 6.42 (1H, d H-3′), 6.47 (1H, s, H-1′); $J_{1,2}=J_{2,3}=J_{3,4}=J_{4,5}=7.5$, $J_{3',4'}=J_{4',5'}=10$ Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ=55.27 (OCH_3), 68.73 (C-6′), 69.24 (C-5′), 69.66 (C-3′), 70.17 (C-4′), 73.49 (C-6). 74.29 (C-5), 78.90 (C-4), 81.90 (C-2), 83.03 (C-3), 93.23 (C-1′), 102.17 (C-1);

MS (FAB) m/z 1184 $[M+Na]^+$. Found: C, 71.03; H, 5.29; N, 1.39%. Calcd for $C_{69}H_{63}NO_{16}$: C, 71.31; H, 5.46; N, 1.29%.

4-Methoxybenzyl *O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10). A 1 M solution of BH_3·THF complex in THF (4.92 ml) was added to a solution of the disaccharide **9** (480 mg, 0.41 mmol) in THF (5 ml) at −10 °C under a nitrogen atmosphere. The mixture was stirred for 0.5 h, and then allowed to warm up to room temperature. After further stirring for 2 h, any excess reductant was quenched with methanol (4 ml). Acetic anhydride (2 ml) was added to the solution, which was stirred at ambient temperature for 1 h; the mixture was then passed through a basic resin (Amberlite IR-45), and washed with methanol. The eluate was concentrated in vacuo, and the residue was purified by elution from a silica-gel column with chloroform–ethyl acetate (5:1). The major fraction was concentrated and the residue crystallized from ethyl acetate–diethyl ether (1:2) and excess pentane to give 244 mg (55%) of **10** as colorless crystals: Mp 184—185 °C; $[\alpha]_D^{22}$ +16.4° (c 0.7, $CHCl_3$); IR (KBr) 3410 (NH), 1670 cm^{-1} (CONH); ^1H NMR (300 MHz, $CDCl_3$) δ=1.79 (3H, s, Ac-CH_3), 3.52 (1H, dd, H-2), 3.60 (1H, ddd, H-5), 3.74 (1H, dd, H-3), 3.80—3.91 (2H, m, H-6), 3.81 (3H, s, OMe), 3.92 (1H, dd, H-4), 4.24—4.41 (3H, m, H-5′,6′), 4.55 (1H, d, H-1), 4.86 (1H, ddd, H-2′), 5.46 (1H, d, H-1′), 5.50 (1H, d, NH), 5.60 (1H, dd, H-4′), 5.68 (1H, d, H-3′); $J_{1,2}$=7.6, $J_{2,3}=J_{3,4}$=9.1, $J_{1',2'}$=1.8, $J_{2',NH}$=9.1, $J_{2',3'}$=3.6, $J_{3',4'}=J_{4',5'}$=9.1 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ=23.06 (Ac-CH_3), 50.75 (C-2′), 55.28 (OCH_3), 63.10 (C-6′), 67.17 (C-4′), 69.15 (C-5′), 69.35 (C-6), 70.14 (C-3′), 74.24 (C-5), 74.78 (C-4), 82.27 (C-2), 84.58 (C-3), 99.42 (C-1′), 102.13 (C-1); MS (FAB) m/z 1089 $[M+2H]^+$, 1109 $[M+Na+H]^+$. Found: C, 70.53; H, 5.86; N, 1.21%. Calcd for $C_{64}H_{63}NO_{15}$: C, 70.77; H, 5.85; N, 1.29%.

The minor fractions collected from the column comprised 27 mg (6%) of β-D-mannosaminyl analogue of **10** identified with the authentic sample[6] and ca. 30 mg of a mixture of products containing conceivably α-D-glucosaminyl analogue of **10**.

***O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranose (11).** A mixture of **10** (107 mg, 0.1 mmol) and DDQ (96% purity, 26.0 mg, 0.11 mmol) in dichloromethane (2 ml) and water (0.1 ml) was stirred at room temperature overnight. The resulting orange-colored suspension was diluted with dichloromethane, washed with 5% aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to dryness. The residue was eluted from a silica-gel column with chloroform–ethyl acetate (3:1) to give 47 mg (49%) of **11** and 16 mg (15% recovey) of unreacted **10**. Crystallization of **11** from ethyl acetate–diethyl ether (1:2) and excess pentane provided colorless crystals ($\alpha:\beta$=2:1, ^1H NMR): Mp 139—140 °C; $[\alpha]_D^{23}$ +35.6° (c 0.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) α-anomer: δ=1.82 (3H, s, Ac-CH_3), 3.25 (1H, d, 1-OH), 3.58 (1H, dd, H-2), 3.88 (1H, dd, H-4), 4.70 (1H, dd, H-3), 4.89 (1H, ddd, H-2′), 5.24 (1H, d, H-1), 5.41 (1H, d, H-1′), 5.54 (1H, d, NH), 5.61 (1H, dd, H-4′), 5.71 (1H, dd, H-3′); $J_{1,2}$=3.0, $J_{1,OH}$=2.5, $J_{2,3}=J_{3,4}=J_{4,5}$=9.0, $J_{1',2'}$=2.0, $J_{2',NH}$=9.0, $J_{2',3'}$=3.0, $J_{3',4'}=J_{4',5'}$=9.0 Hz; β-anomer: δ=1.78 (3H, s, Ac-CH_3), 2.38 (1H, d, 1-OH), 3.43 (1H, dd, H-2), 3.86 (1H dd, H-4), 4.75 (1H, d, H-1), 4.85 (1H, ddd, H-2′), 5.43 (1H, d, H-1′), 5.54 (1H, d, NH), 5.61 (1H, dd, H-4′), 5.68 (1H, dd, H-3′): $J_{1,OH}$=2.5, $J_{1,2}=J_{2,3}=J_{3,4}=J_{4,5}$=9.0, $J_{1',2'}$=2.0, $J_{2',NH}$=9.0, $J_{2',3'}$=3.0, $J_{3',4'}=J_{4',5'}$=9.0 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) α-anomer: δ=23.04 (Ac-CH_3), 50.78 (C-2′), 63.00 (C-6′), 67.07 (C-4′), 70.03 (C-3′), 75.41 (C-4), 80.28 (C-2), 81.14 (C-3), 90.84 (C-1), 99.83 (C-1′); β-anomer: δ=23.04 (Ac-CH_3), 50.78 (C-2′), 67.07 (C-4′), 70.03 (C-3′), 75.04 (C-4), 83.00 (C-2), 84.23 (C-3), 97.42 (C-1), 99.51 (C-1′); MS (FD) m/z 966 $[M]^+$. Found: C, 68.93; H, 5.71; N, 1.40%. Calcd for $C_{56}H_{55}NO_{14}$·0.5 H_2O: C, 68.92; H, 5.74; N, 1.43%.

***O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl Fluoride (13).** Thionyl chloride (73 μl, 1.0 mmol) and DMF (13 μl, 0.20 mmol) were added to an ice-cooled solution of **11** (193 mg, 0.20 mmol) in dry dichloromethane (1.2 ml). The mixture was stirred at room temperature overnight, filtered through a pad of silica gel, and evaporated to dryness. The residue containing the chloride **12** was used for fluorination without further purification. A mixture of the chloride (213 mg) and silver fluoride (62.5 mg, 0.49 mmol) in acetonitrile (3 ml) was stirred in the dark at room temperature overnight. The resulting mixture was diluted with ethyl acetate (10 ml) and filtered through a pad of Celite. The filtrate was washed with 5% aqueous NaCl and water, dried (Na_2SO_4), and evaporated to dryness. The residue was eluted from a silica-gel column with chloroform–ethyl acetate (4:1). From the major fraction 157 mg (81%) of **13** was obtained as colorless crystals which was recrystallized from ethyl acetate–hexane; Mp 205—206 °C; $[\alpha]_D^{21}$ +45.2° (c 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ=1.83 (3H, s, Ac-CH_3), 3.59—3.68 (1H, m, H-2), 3.77—3.90 (4H, m, H-3,5,6), 4.07 (1H, dd, H-4), 4.21—4.36 (3H, m, H-5′,6′), 4.55—4.70 (4H, 2×Bn-CH_2), 4.86 (1H, ddd, H-2′), 4.84, 4.93 (each 1H, d, Bn-CH_2), 5.38 (1H, dd, H-1), 5.40 (1H, d, H-1′), 5.52 (1H, d, NH), 5.60 (1H, dd, H-4′), 5.67 (1H, d, H-3′); $J_{1,2}$=6.5, $J_{3,4}$=9.0, $J_{1',2'}$=2.0, $J_{2',NH}$=9.0, $J_{2',3'}$=4.0, $J_{3',4'}=J_{4',5'}$=10.0, $J_{1,F}$=53.0 Hz, ^{13}C NMR (100 MHz, $CDCl_3$) δ=23.10 (Ac-CH_3), 50.63 (C-2′), 62.97 (C-6′), 66.96 (C-4′), 68.89 (C-6), 69.16 (C-5′), 69.96 (C-3′), 73.62, 73.98, 74.54 (3×Bn-CH_2), 73.76 (C-4), 74.27 (d, C-5), 80.90 (d, C-2), 83.04 (d, C-3), 99.23 (C-1′), 109.41 (d, C-1); $J_{C1,H1}$=220, $J_{C1',H1'}$=175.5, $J_{C1,F}$=217.6, $J_{C2,F}$=23.3, $J_{C3,F}$=9.7, $J_{C5,F}$=4.4 Hz; MS (FAB) m/z 968 $[M+H]^+$, 990 $[M+Na]^+$. Found: C, 69.45; H, 5.63; N, 1.46%. Calcd for $C_{56}H_{54}NO_{13}F$: C, 69.48; H, 5.62; N, 1.45%.

Benzyl *O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-mannopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (15) and Its β-D-Glucopyranosyl Isomer (15β). To a stirred solution of benzyl rhamnoside **14**[19] (20 mg, 45 μmol) in dry dichloromethane (1 ml) with MS-4A (200 mg) were added $AgClO_4$ (9.4 mg, 45 μmol), $Sn(II)Cl_2$ (8.5 mg, 45 μmol), and a solution of the fluoride **13** (48 mg, 50 μmol) in dry dichloromethane (1 ml). The mixture was stirred at room temperature for 20 h, diluted with dichloromethane (10 ml) and filtered through a pad of Celite. The filtrate was washed with 5% aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to dryness. The residue was eluted from a silica-gel column

with chloroform–ethyl acetate (2:1) to give 44 mg (71%) of **15** as a mixture of C-1′ anomers ($\alpha:\beta$=2:1, ^{1}H NMR), a colorless syrup: ^{1}H NMR (300 MHz, $CDCl_3$) α-anomer: δ=1.45 (3H, d, H-6), 1.84 (3H, s, Ac-CH_3), 3.32 (1H, dd, H-6″a), 3.42 (1H, dd, H-6″b), 3.56 (1H, dd, H-2′), ca. 3.55 (1H, m, H-5′), 3.74 (1H, dd, H-4), 3.84 (1H, m, H-5), 3.91 (1H, m, H-6′a), 4.02 (1H, dd, H-3), 4.14 (1H, m, H-2), 4.18 (1H, m, H-5″), 4.89 (1H, d, H-1), 4.92 (1H, d, H-1′), 4.96 (1H, ddd, H-2″), 5.44 (1H, d, H-1″), 5.51 (1H, d, NH), 5.65 (1H, dd, H-4″), 5.75 (1H, dd, H-3″); $J_{1,2}$=1.0, $J_{2,3}$=3.0, $J_{3,4}=J_{4,5}$=9.0, $J_{5,6}$=6.0, $J_{1',2'}$=3.5, $J_{2',3'}$=9.0, $J_{1'',2''}$=2.0, $J_{2'',3''}$=4.0, $J_{2'',NH}$=9.5, $J_{3'',4''}=J_{4'',5''}$=10.0, $J_{5'',6''a}$=2.0, $J_{5'',6''b}$=3.0, $J_{6''a,b}$=11.0 Hz; β-anomer: δ=1.32 (3H, d, H-6), 1.81 (3H, s, Ac-CH_3), 3.80 (1H, m, H-5), ca. 4.14 (1H, m, H-2), 4.70 (1H, d, H-1′), ca. 4.85 (1H, m, H-2″); 5.09 (1H, d, H-1), 5.48 (1H, d, H-1″), 5.61 (1H, dd, H-4″), 5.69 (1H, dd, H-3″); $J_{1,2}$=1.5, $J_{5,6}$=6.0, $J_{1',2'}$=8.0, $J_{1'',2''}$=2.0, $J_{2'',3''}$=4.0, $J_{3'',4''}=J_{4'',5''}$=9.5 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) α-anomer: δ=95.57 (C-1′), 96.21 (C-1), 99.82 (C-1″); β-anomer: δ=99.00 (C-1), 99.21 (C-1″), 104.79 (C-1′).

***O*-(2-Acetamido-2-deoxy-α-D-mannopyranosyl)-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-L-rhamnopyranose (2).** The solution of a 2:1 mixture of **15** and **15β** (43 mg, 31 μmol) in 0.05 M NaOMe in dry methanol (3 ml) was stirred at room temperature overnight. Consecutive neutralization (Dowex 50W X8), filtration, and evaporation to dryness gave a residue, which was eluted from a silica-gel column with chloroform–methanol (8:1) to afford **16** (19 mg, 58%) and the corresponding β-D-glucopyranosyl isomer **16β** (11 mg, 32%) both as a syrup. A solution of **16** (19 mg, 18 μmol) in methanol–water (4:1, 20 ml) containing acetic acid (1 ml) was hydrogenolyzed in the presence of 10% Pd-C (50 mg) under an atmosphere of hydrogen (3.1×10^5 Pa) for 2 d. The resulting mixture was filtered through a pad of Celite and a short column of a basic resin (Amberlite IR-45). The filtrate was concentrated in vacuo to give a syrup, which was eluted from a silica-gel column with chloroform–methanol–water (5:4:1). The major fraction was concentrated to give 9.5 mg (quant.) of **2** as a colorless syrup ($\alpha:\beta$=2:1, ^{1}H NMR in D_2O): $[\alpha]_D^{20}$ +51.7° (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) α-anomer: δ=1.18 (3H, d, H-6), 1.93 (3H, s, Ac-CH_3), 3.38 (1H, dd, H-3′), 3.42 (1H, dd, H-2′), 3.51—3.52 (2H, dd, H-4′,4″), 3.63 (1H, m, H-5″), 3.69—3.74 (4H, m, H-6′,6″), 3.75—3.90 (3H, m, H-2,3,4), 3.90 (1H, dd, H-3″), 4.02 (1H, m, H-5′), 4.36 (1H, dd, H-2″), 4.89 (1H, d, H-1′), 5.11 (1H, s, H-1), 5.15 (1H, s with fine splittings, H-1″); $J_{1,2}$=2.0, $J_{1',2'}$=3.5, $J_{2',3'}=J_{3',4'}=J_{4',5'}$=10.0, $J_{1'',2''}$=1.5, $J_{2'',3''}$=4.5, $J_{3'',4''}=J_{4'',5''}$=10.0 Hz; β-anomer: δ=1.19 (3H, d, H-6), 1.93 (3H, s, Ac-CH_3), 3.25 (1H, dd, H-3′), 3.32 (1H, dd, H-5), 3.46 (1H, dd, H-2′), 3.51—3.52 (each 1H, dd, H-4′,4″), 3.63 (1H, m, H-5″), 3.69—3.74 (4H, m, H-6′,6″), 3.75—3.90 (3H, m, H-2,3,4), 3.90 (1H, dd, H-3″), 4.02 (2H, m, H-5′), 4.36 (1H, dd, H-2″), 4.83 (1H, s, H-1), 4.98 (1H, d, H-1′), 5.15 (1H, s with fine splittings, H-1″); $J_{1,2}$<1.0, $J_{4,5}$=9.5, $J_{5,6}$=6.0, $J_{1',2'}$=4.0, $J_{2',3'}=J_{3',4'}$=10.0, $J_{1'',2''}$=1.5, $J_{2'',3''}$=4.5, $J_{3'',4''}$=10.0 Hz; ^{13}C NMR (100 MHz, D_2O) α-anomer: δ=17.53 (C-6), 22.76 (Ac-CH_3), 53.68 (C-2″), 61.07, 61.13 (C-6′,6″), 67.34 (C-4″), 69.46 (C-5), 69.85 (C-3″), 70.14 (C-3), 71.25 (C-5′), 72.21 (C-2′), 72.87 (C-3′), 73.90 (C-4), 74.09 (C-5″), 76.69 (C-4′), 78.05 (C-2), 92.39 (C-1), 98.49 (C-1′), 101.09 (C-1″), 175.52 (CONH); β-anomer: δ=17.53 (C-6), 22.76 (Ac-CH_3), 53.68 (C-2″), 61.07, 61.13 (C-6′,6″), 67.34 (C-4″), 69.85 (C-3″), 71.49, 72.67, 72.87 (C-2′,3′,5′), 72.76 (C-4), 73.12 (C-5), 74.09 (C-5″), 74.18 (C-3), 76.36 (C-4′), 81.66 (C-2), 94.65 (C-1), 101.09 (C-1″), 101.86 (C-1′), 175.5 (CONH).

4-Methoxybenzyl *O*-(2-Acetamido-2-deoxy-α-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (17). The disaccharide **10** (63 mg, 0.058 mmol) was dissolved in a 0.05 M solution of sodium methoxide in dry methanol (3 ml). After stirring at room temperature for 20 h, the resulting solution was neutralized with acidic resin (Dowex 50W X8), and filtered. The filtrated was concentrated in vacuo to give a residue, which was eluted from a silica-gel column with chloroform–methanol (8:1). Concentration of the major fraction gave 24 mg (53%) of **17** as a colorless syrup; $[\alpha]_D^{25}$ +29.7° (*c* 1.05, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=1.82 (3H, s, Ac-CH_3), 3.41 (1H, m, H-5), 3.51 (1H, dd, H-2), 3.5—3.6 (4H, m, H-4′,5′,6′), 3.61 (1H, dd, H-3), 3.72 (2H, m, H-6), 3.79 (3H, s, OMe), 3.80 (1H, dd, H-4), 3.88 (1H, dd, H-3′), 4.31 (1H, m, H-2′), 4.48 (1H, d, H-1), 5.27 (1H, broad s, H-1′), 6.25 (1H, broad, NH); $J_{1,2}$=7.5, $J_{2,3}=J_{3,4}$=8.8, $J_{1',2'}$<1.0, $J_{2',3'}$=3.5, $J_{3',4'}$=8.0 Hz; ^{13}C NMR (75 MHz, $CDCl_3$), δ=22.89 (Ac-CH_3), 53.71 (C-2′), 55.26 (OCH_3), 61.44 (C-6′), 67.42 (C-4′), 68.72 (C-6), 71.26 (C-3′), 72.84 (C-5′), 74.33 (C-4,5), 82.25 (C-2), 84.80 (C-3), 99.70 (C-1′), 102.08 (C-1), 172.38 (NHCO); MS (FAB) *m/z* 774 $[M+H]^+$, 796 $[M+Na]^+$.

Hydrogenolysis and Hydrolysis of 17. A solution of **17** (20 mg, 25.8 μmol) in methanol–water (4:1, 19 ml) containing acetic acid (1 ml) was hydrogenolyzed in the presence of 10% Pd-C (50 mg) under an atmosphere of hydrogen (3.45×10^5 Pa) for 2 d. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to dryness. The residue was dissolved in 1 M HCl (10 ml) and heated at 95 °C for 2.5 h. After concentration of the solution, the residue was identified by TLC on cellulose powder to be D-ManN·HCl [R_f 0.41, AcOEt–pyridine–AcOH–H_2O (5:5:1:3); cf. authentic D-ManN·HCl: R_f 0.41], and D-Glc [R_f 0.23, $CHCl_3$–MeOH (3:2); authentic D-Glc: R_f 0.23].

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