A Practical Synthesis of Mannosaminyl-β(1→4)-glucosyl-α(1→2)-rhamnose, the Trisaccharide Repeating Unit of a *Streptococcus pneumoniae* Capsular Polysaccharide

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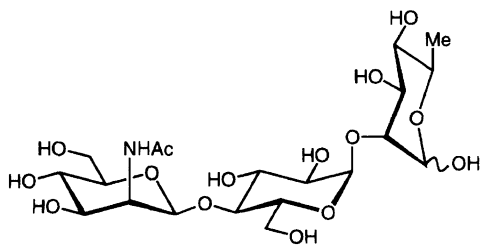
An efficient chemical synthesis is described for the title trisaccharide repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* Type 19F. The key intermediate was a well-accessible indirect β-D-mannosaminyl donor, i.e. 2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranosyl bromide, which underwent glycosidations with 20:1 β-selectivities; the benzoyloxyimino group was reduced with essential *manno* specificity. The ManNAc-β(1→4)-Glc disaccharide, thus obtained in suitably blocked form, was subsequently converted into 1-fluoride, with which a L-rhamnosyl acceptor was glycosylated by an α(1→2) linkage to yield the target trisaccharide in an altogether 6% overall yield for the 13 steps required from D-glucose.

Streptococcus pneumoniae of serotype 19F[1] is of particular clinical importance, since it is a major pathogenic bacterium responsible for acute pneumonia in infants, particularly those whose immunity against diseases is not yet fully developed.[2] The immunogenic specificity of this bacterium depends on the capsular polysaccharide, the structure of which has been elucidated[3,4] to have the *N*-acetyl-β-D-mannosamine-containing trisaccharide phosphate

→ 4)-β-D-Man*p*NAc-(1 → 4)-α-D-Glc*p*-(1 → 2)-α-L-Rha*p*-(1-PO$_4$−→

as the repeating unit. The underlying non-phosphorylated trisaccharide **1** (Fig. 1) is considered to be an epitope of the antigenic capsular polysaccharide, utilizable as a multivalent vaccine element.[1]

The biological significance of **1** has elicited substantial efforts towards its acquisition, such that three synthetic approaches have been elaborated.[5—7] Considering the practicality and overall efficiency, the critical key step in any synthesis of this trisaccharide is undoubtedly an elaboration of the *N*-acetyl-β-D-mannosamine unit. Paulsen, in his approach,[5] utilized the mannosaminyl donors, **2** and **3**, for the ManNAc-β(1→4)-Glc portion of trisaccharide **1**, whereby donor **2** gave a 5:1 β/α-anomeric mixture upon glycosylation of the 4-OH of a protected glucose, while the more reactive benzylated analog **3**, aside of its laborious preparation,[8] gave the desired disaccharide in 61% yield (Fig. 2). Sugawara and Igarashi[6] used another donor **4** for attaching the mannosamine portion to the free glucosyl 4-OH of a suitably protected glucosyl-α(1→2)-rhamnoside; yet, the obtained β-selectivities were moderate (3:1 with $AgClO_4$/2,4,6-collidine in diethyl ether as promoter, 6:1 with silver silicate in benzene/dichloromethane), and the yields (33 and 17%, respectively) were quite modest. The approach used by Ronchetti et al.[7] elaborated the β-D-ManNAc portion of **1** from a 2-*O*-acetyl-blocked cellobiose via a 5-step sequence comprising the liberation of the 2-OH, oxidation to the glycosidulose, oximation, hydride reduction, and *N*-acetylation. Although this is an established methodology for generating β-D-mannosidic linkages[9] by a direct reduction of the intermediate glycosiduloses, the efficiency of the oxime used in the Ronchetti approach[7] cannot be assessed, since no yield is given for the last two steps involving the critical oxime reduction.



→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p* (**1**)

Fig. 1.

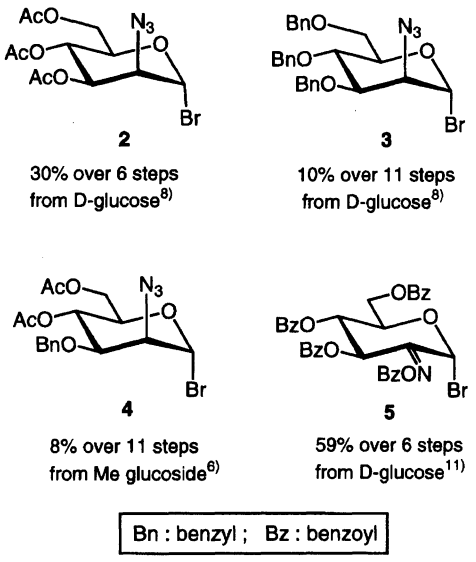


Fig. 2.

In view of this situation, a preparatively more propitious approach to the trisaccharide **1**, in general, and to the elaboration of its β-D-ManNAc portion, in particular, seemed to be desirable. We report here on our studies towards this end,[10] utilizing the exceedingly well-accessible[11] 2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranosyl bromide **5** as a highly useful indirect β-D-mannosaminyl donor.

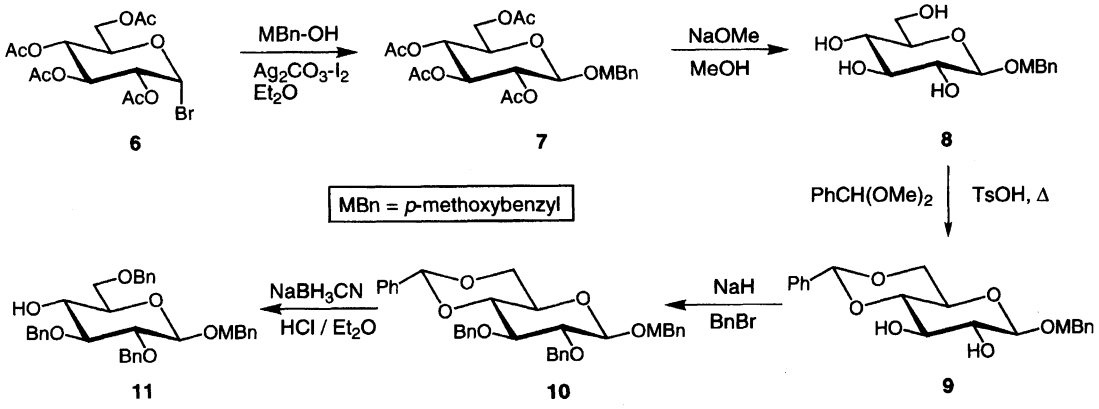
Results and Discussion

Since a well-accessible, indirect β-D-ManNAc donor, 2-(benzoyloxyimino)-2-deoxyglycosyl bromide **5** has provided β-selectivities of about 20 : 1 in silver carbonate-induced glycosidations with methanol,[12] 4-penten-1-ol,[13] di-*O*-isopropylidenegalactose,[12] and methyl 2,3,6-tri-*O*-benzyl-α-D-glucoside,[12] it appeared obvious to utilize it directly towards the synthesis of the trisaccharide **1**. Accordingly, the β-D-ManNAc donor **5** may allow glycosylations of the 4-OH of a suitably blocked glucoside or a glucosyl-α(1→2)-rhamnoside. The former possibility was realized by the generation of the ManNAc-β(1→4)-Glc portion with a subsequent introduction of the rhamnosyl unit.

This approach required a central glucose unit in a form that can function, successively, as a 4-*O*-acceptor and a glycosyl donor. The *p*-methoxybenzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**11**) was selected for this purpose, since the anomeric substituent was apt to be smoothly removable by oxidative cleavage.[14] The glucose unit **11** was prepared by a conventional methodology, such that acetobromoglucose **6** was converted into the β-(*p*-methoxybenzyl) glucoside by glycosidation **6**→**7** (84%), followed by de-*O*-acetylation **7**→**8** (85%), 4,6-*O*-benzylidenation **8**→**9** (77%), 2,3-di-*O*-benzylation **9**→**10** (70%), and reductive acetal opening **10**→**11** (83%), amounting to a 32% overall yield for the five steps involved (Scheme 1).

The glycosidation of bromide **5** with the 4-OH free glucoside **11** was examined with various coupling agents (cf. Table 1). Of the insoluble silver salt-promoted glycosylations evaluated, the use of silver carbonate/silver zeolite mixtures in dichloromethane gave a β-selectivity higher than 20 : 1 (cf. Table 1, Runs 4—6); although the use of a silver aluminosilicate catalyst[15] in dichloromethane remarkably reduced the reaction time (48→2.5 h at room temperature), the yield was moderate (Run 7, 56%). If, however, the glycosidation of **5** was effected with a soluble silver catalyst, such as silver triflate in dichloromethane in the presence of 1,1,3,3-tetramethylurea (TMU) as an acid scavenger, the respective α-linked disaccharide (**12α**) was nearly exclusively obtained in 90% yield (Run 8).

Due to the availability of both anomers of **12**, their interglycosidic linkage configuration could be readily secured: the 1′-β-configuration of **12** was deduced from $J_{3',4'}$ and $J_{4',5'}$ couplings of only 5.5 Hz each, indicating a distortion of the pyranose ring towards the twist-boat form, as depicted in formula **12**. Similar observations have been made for other β-anomeric 2-(benzoyloxyimino)-2-deoxy-glycosides.[12,16—18] In contrast, the corresponding α-anomer **12α** had normal $J_{3',4'}$ and $J_{4',5'}$ values of 10 Hz each (Fig. 3),[19] as expected for the $^4C_1$ conformation of the pyranose ring.[17,18]

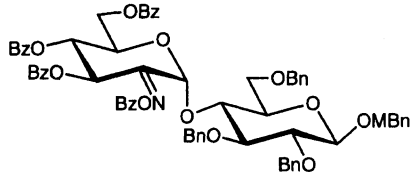


Scheme 1.

Table 1. Glycosylation of 4-Methoxybenzyl 2,3,6-Tri-*O*-benzyl-β-D-glucopyranoside (**11**) with Tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranosyl Bromide (**5**)

Run[a]	Molar equiv		Promoter	Time	Yield	α : β[b]
	5	**11**		h	**12** (%)	
1	1	2	Ag_2CO_3/I_2	48	21	1 : 20
2	1	1.1	$Ag_2CO_3/AgClO_4$	24	34	1 : 1
3	1	2	Ag_2CO_3/AgOTf	20	40	2 : 1
4	1	1.1	Ag_2CO_3/Ag-Zeolite	48	48	1 : 20
5	1.5	1	Ag_2CO_3/Ag-Zeolite	48	73	1 : 20
6	2	1	Ag_2CO_3/Ag-Zeolite	48	86	1 : 20
7	1.5	1	Ag(I)silica-alumina	2.5	56	1 : 20
8	2	1	AgOTf/TMU	20	90	10 : 1

a) All experiments were carried out in CH_2Cl_2 at room temperature. b) Estimated by ^{1}H NMR integration of the respective anomeric protons.



12α

Fig. 3.

Based on our previous observations[18] that 2-(acyloxyimino)-2-deoxy-β-D-glycosides prefered a hydride attack from the α-side, entailing the formation of 1,2-*cis*-amino sugars with a β-D-*manno* configuration, the β-disaccharide **12** was reduced with a twelve-fold molar excess of the borane-tetrahydrofuran (BH_3·THF) complex in THF (2 h, 25 °C), followed by *N*-acetylation with acetic anhydride to afford the *N*-acetyl-β-D-mannosaminyl-(1→4)-β-D-glucoside **13** in 69% yield. The β-D-*manno*-configuration of the amino sugar moiety of **13** unambiguously followed from the coupling constants around the pyranose ring, notable $J_{1',2'}$=1.2, $J_{2',3'}$=4.0, and $J_{3',4'}=J_{4',5'}$=9.8 Hz.

For the attachment of the L-rhamnose unit onto the anomeric glucosyl portion of disaccharide **13**, its conversion into a donor substrate was now required and achieved by de-*O*-methoxybenzylation (DDQ/CH_2Cl_2–H_2O, →**14**, 44%), chlorination ($SOCl_2$–DMF/$ClCH_2CH_2Cl$, →**15**, quantitative yield), and fluorination (AgF/MeCN, →**16**, 77%). Although the de-*O*-methoxybenzylation **13**→**14** is as yet unsatisfactory, the yield was improved by recycling the unreacted starting material (ca. 20% reisolable); in this way, the overall yield for the conversion **13**→**14** amounts to 55% (Scheme 2). An alternative de-*O*-methoxybenzylation using ammonium cerium (IV) nitrate,[20] propagated as being superior to DDQ, proved to be unsuited, invariably resulting in a lower yield. The direct fluorination **14**→**16** was also feasible using diethylaminosulfur trifluoride (DAST), smoothly generating the β-fluoride **16** in a yield of 72%.

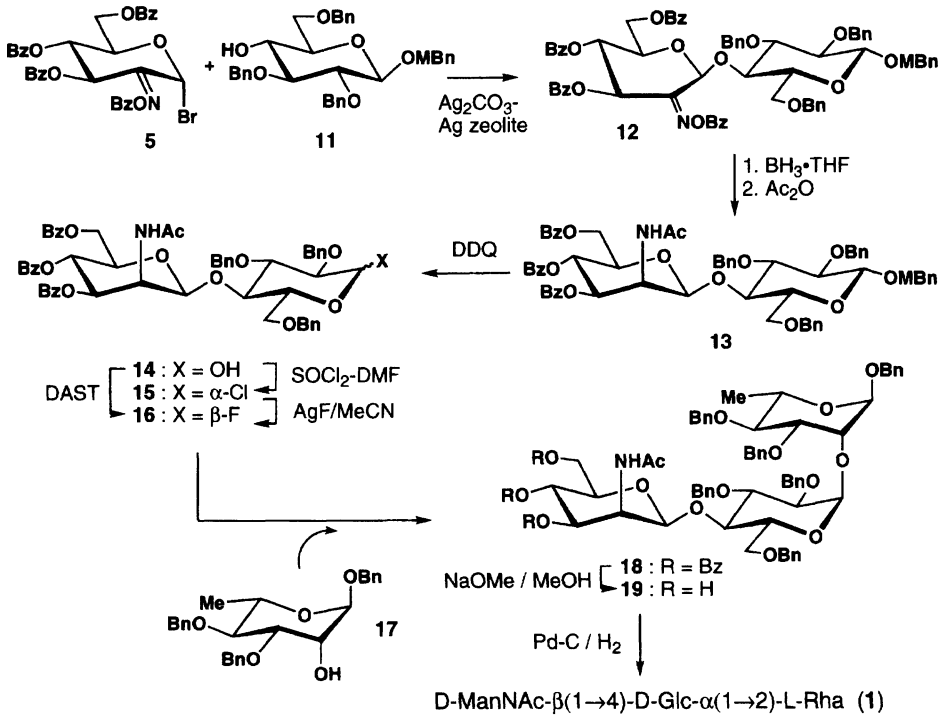
The rhamnosyl acceptor, benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**17**), was prepared by a reductive acetal cleavage of the 2,3-*O*-benzylidene moiety in benzyl 4-*O*-benzyl-2,3-*O*-benzylidene-α-L-rhamnopyranoside.[21] We first examined the use of $LiAlH_4$–$AlCl_3$ in diethyl ether as described in the literature,[22] invariably resulting in sluggish conversions, so that modified conditions ($NaBH_3CN$–HCl in diethyl ether) had to be employed, making this conversion smooth to give **17** in 72% yield.

When the fluoride **16** was exposed to the rhamnosyl acceptor **17** in the presence of $AgClO_4$–$SnCl_2$[23] in dichloromethane, α-selective glycosylation was effected to afford the desired trisaccharide **18** as a syrup in 51% yield. The coupling constants for the central glucosyl moiety, being observed as $J_{1',2'}$=3.5, $J_{2',3'}$=9 Hz, unequivocally confirmed the correct intersaccharidic linkup. Subsequent de-*O*-benzoylation **18**→**19** (89%) with 0.05 M NaOMe/MeOH and hydrogenolysis (Pd–C/H_2 in MeOH, 94%) proceeded uneventfully to the target trisaccharide **1** (1 M=1 mol dm^{-3}). The anomeric composition of **1** at the reducing end was about 2 : 1 in favor of the α-anomer (^{1}H NMR in D_2O). Its ^{1}H and ^{13}C NMR spectra were identical in all respects with those reported for the natural[3] as well as the synthetic product.[5,6]

The simple and effective synthesis of trisaccharide **1** described above makes such an oligosaccharide readily available as a biologically relevant repeating unit of an infectious *Streptococcus pneumoniae* capsular polysaccharide. This approach is decisively more efficient than the previous ones[5—7] due to an elaboration of the critical β-D-ManNAc portion from the well-accessible, indirect β-D-mannosaminyl donor **5**, whose glycosidation proceeds with the particularly propitious β-selectivity of 20 : 1. Numerous applications of this methodology can be foreseen, some of which are currently under investigation.

Experimental

General. Melting points were measured on a Yamato MP-1 apparatus and were uncorrected. Spectral data were



Scheme 2.

recorded on the following instruments: IR on a JASCO IR-810 infrared spectrophotometer; $[\alpha]_D$ on a JASCO DIP-150 digital polarimeter; MS on a JMS D-100 spectrometer; ^1H and ^{13}C NMR as well as ^1H–^1H and ^1H–^{13}C shift correlated 2D NMR on a Varian VXR-300 (300 MHz) or an XL-400 (400 MHz) spectrometer in a chloroform-*d* solution, unless otherwise noted. TLC was carried out on silica gel 60 F_{254} (Merck Art. 5735) developed with the same solvent systems for column chromatography as described individually in the experiments. The spots were visualized by UV light (254 nm) or heating with 10% aq sulfuric acid on a hot plate. Column chromatography was achieved on silica-gel 60 (70—230 mesh, Merck Art. 7734), and detected with 0.2% ninhydrin solution in 95% ethanol on a hot stage.

4-Methoxybenzyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (7). To a solution of 4-methoxybenzyl alcohol (1.73 g, 12.5 mmol) in dry diethyl ether (25 ml) was added Molecular Sives 3A (1.25 g, powder), iodine (0.64 g, 2.5 mmol), silver carbonate (2.07 g, 7.5 mmol), and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**6**, 1.03 g, 2.5 mmol). The mixture was stirred in the dark at ambient temperature for 24 h, followed by dilution with dichloromethane (50 ml), filtration through a pad of Celite, and washing with 0.1 M aq $Na_2S_2O_3$ (50 ml), water (50 ml), 5% aq $NaHCO_3$ (50 ml), and water (3×50 ml). After drying (Na_2SO_4), the solution was evaporated to dryness, and the residue was purified by being passed over a silica-gel column with chloroform–ethyl acetate (3:1). The major fraction was concentrated to give 980 mg (84%) of **7**: Colorless syrup; $[\alpha]_D^{26}$ −39.9° (*c* 1.7, MeOH); ^1H NMR (300 MHz) δ=1.99, 2.00, 2.03, 2.11 (3H each, s, OAc), 3.66 (1H, td, H-5), 3.81 (3H, s, OMe), 4.16 (1H, dd, H-6b), 4.27 (1H, dd, H-6a), 4.51 (1H, d, H-1), 4.56, 4.82 (1H each, d, CH_2), 5.35 (1H, dd, H-2), 5.12 (1H, dd, H-3), 5.16 (1H, dd, H-4); $J_{1,2}$=7.5, $J_{2,3}$=8.5, $J_{3,4}=J_{4,5}$=9.0, $J_{5,6a}$=5.0, $J_{5,6b}$=12.0, $J_{6a,6b}$=2.5 Hz.

Compound **7** was also prepared, albeit at a lower yield (63%), by a reported procedure[24] with Ag_2O as the promoter; lit,[24] $[\alpha]_D^{25}$ −43.1° (*c* 0.96, MeOH).

2-Methoxybenzyl β-D-Glucopyranoside (8). A solution of **7** (700 mg, 1.5 mmol) in 0.05 M methanolic sodium methoxide (60 ml) was first stirred at room temperature for 1 h, and subsequently neutralized with a dry acidic resin (Dowex 50W×4) and filtered. The filtrate was evaporated to dryness and the residue was partitioned between dichloromethane (50 ml) and water (50 ml). The water layer was separated in a silicone-treated phase separator (Whatman No. 2200 filter paper), and concentrated. Drying (P_2O_5) and washing the residue with diethyl ether furnished 380 mg (85%) of **8** as colorless crystals of mp 134—135 °C; $[\alpha]_D^{27}$ −61.3° (*c* 0.6, MeOH) (lit,[24] mp 139—140.5 °C); ^1H NMR (300 MHz) δ=3.17 (1H, dd, H-2), 3.23—3.38 (3H, m, H-3, 4, 5), 3.61 (1H, dd, H-6a), 3.74 (3H, s, OMe), 3.81 (1H, dd, H-6b), 4.39 (1H, d, H-1), 4.58, 4.77 (1H each, d, Ph–CH_2), 6.92, 7.31 (2H each, d, C_6H_4); $J_{1,2}$=8.0, $J_{2,3}=J_{3,4}$=9.0, $J_{5,6a}$=6.0, $J_{5,6b}$=2.0, $J_{6a,6b}$=12.5 Hz; ^{13}C NMR (75 MHz) δ=56.05 (OMe), 61.47 (C-6), 70.36 (C-5), 71.77 (Ph–CH_2), 73.79 (C-2), 76.50 (C-4), 76.62 (C-3), 101.60 (C-1); MS (EI) *m/z* 300 $[M]^+$. Found: C, 55.61; H, 6.65%. Calcd for $C_{14}H_{20}O_7$: C, 55.99; H, 6.71%.

4-Methoxybenzyl 4,6-*O*-Benzylidene-β-D-glucopyranoside (9). A mixture of **8** (6.3 g, 20.8 mmol), benzaldehyde dimethyl acetal (3.2 g, 20.8 mmol), and *p*-toluenesulfonic acid monohydrate (98.9 mg, 0.52 mmol) in dry *N,N*-dimethylformamide (DMF, 21 ml) was stirred at diminished pressure with a rotary evaporator for 1 h (bath temp. 60±5 °C). After the solvent was removed in vacuo, the residue was treated with saturated aq $NaHCO_3$ (30 ml) at 100 °C. Then, after cooling to 20 °C, the precipitates were collected by filtration, washed with water, and dried (P_2O_5). Recrystallization from ethanol gave 6.5 g (77%) of **9** as colorless

needles (monohydrate): Mp 127—128 °C; $[\alpha]_D^{23}$ −68.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz) δ=2.86 (1H, dd, 2-OH), 3.03 (1H, d, 3-OH), 3.42 (1H, td, H-5), 3.51 (1H, td, H-2), 3.73—3.83 (3H, m, H-3, 6), 3.80 (3H, s, OMe), 4.36 (1H, dd, H-4), 4.54 (1H, d, H-1), 4.56, 4.86 (1H each, d, Ph–CH_2), 5.52 (1H, s, Ph–CH), 6.87—7.50 (aromatic H); $J_{1,2}$=8.0, $J_{2,3}$=7.0, $J_{2,OH}$=2.0, $J_{3,OH}$=2.0, $J_{3,4}$=10.0, $J_{4,5}$=5.0 Hz; ^{13}C NMR (75 MHz) δ=55.27 (OMe), 66.40 (C-5), 68.66 (C-4, 6), 73.08 (C-3), 74.49 (C-2), 101.83 (C-1), 101.89 (Ph–CH); MS (EI) *m/z* 388 $[M]^+$. Found: C, 62.5; H, 6.89%. Calcd for $C_{21}H_{24}O_7 \cdot H_2O$: C, 62.06; H, 6.45%.

4-Methoxybenzyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (10). Sodium hydride (60% in oil, 3 g, 74 mmol) was added portionwise to a solution of **9** (monohydrate, 4 g, 9.85 mmol) in dry DMF (170 ml) under an atmosphere of nitrogen. The resulting suspension was stirred at ambient temperature for 0.5 h and cooled to 0 °C, at which temperature benzyl bromide (17.7 ml, 147.8 mmol) was added dropwise to the mixture. After stirring at room temperature overnight, dry methanol (1 ml) was added, and the solvent was evaporated to dryness. The residue was partitioned between dichloromethane (100 ml) and water (100 ml) and the organic layer was washed with water (3×100 ml), dried (Na_2SO_4), and evaporated to dryness to give a yellow syrup, which was eluted from a silica-gel column with toluene–ethyl acetate (15 : 1). The major fraction was concentrated and the residue was crystallized from methanol to provide 3.92 g (70%) of **10** as colorless needles: Mp 118—119 °C; $[\alpha]_D^{25}$ −59.0° (*c* 0.4, $CHCl_3$); ^{1}H NMR (300 MHz) δ=3.43 (1H, td, H-5), 3.52 (1H, dd, H-2), 3.71 (1H, dd, H-3), 3.75 (1H, dd, H-4), 3.78—3.88, 4.36—4.43 (1H each, m, H-6), 3.82 (3H, s, OMe), 4.62 (1H, d, H-1), 4.73—4.94 (6H, 3×Ph–CH_2), 5.58 (1H, s, Ph–CH), 6.83—7.54 (aromatic H); $J_{1,2}$=8.0, $J_{2,3}=J_{3,4}=J_{4,5}=J_{5,6a}$=9.0, $J_{5,6b}$=5.0 Hz; ^{13}C NMR (75 MHz) δ=55.26 (OMe), 66.04 (C-5), 68.82 (C-6), 80.95 (C-4), 81.51 (C-3), 82.14 (C-2), 101.14 (Ph–CH), 102.82 (C-1); MS (EI) *m/z* 567 $[M-1]^+$. Found: C, 73.72; H, 6.38%. Calcd for $C_{35}H_{36}O_7$: C, 73.92; H, 6.38%.

4-Methoxybenzyl 2,3,6-Tri-*O*-benzyl-β-D-glucopyranoside (11). An ice-cooled stirred solution of **10** (3.3 g, 5.8 mmol), MS-3A (powder, 0.9 g), and sodium cyanotrihydroborate (3.3 g, 52.2 mmol) in dry THF (87 ml) was acidified (pH<1) with HCl-saturated diethyl ether (ca. 100 ml). The resulting solution was further stirred at 0 °C for 10 min, poured into ice-water (300 ml), and filtered through silicone-treated filter paper (Whatman No. 2200). The filtrate was washed with 5% aq $NaHCO_3$ (200 ml) and water (3×200 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica-gel by elution with toluene–ethyl acetate (4 : 1). The concentration of the major fraction (R_f=0.65, toluene–ethyl acetate 4 : 1) followed by crystallization from benzene–hexane gave 2.74 g (83%) of **11**: Mp 53—54 °C; $[\alpha]_D^{22}$ −45.6° (*c* 0.9, $CHCl_3$); IR (KBr) 3340 (OH) cm^{-1}; ^{1}H NMR (300 MHz) δ=2.53—2.55 (1H, broad, 4-OH), 3.41—3.52 (3H, m, H-2, 3, 5), 3.61 (1H, td, H-4), 3.73 (1H, dd, H-6a), 3.81 (1H, dd, H-6b), 3.81 (3H, s, OMe), 4.51 (1H, d, H-1), 4.57—4.97 (8H, 4×Ph–CH_2), 6.88—7.38 (aromatic H); $J_{1,2}$=7.0, $J_{3,4}=J_{4,5}$=9.0, $J_{5,6a}$=5.0, $J_{5,6b}$=4.0, $J_{6a,6b}$=10.0 Hz; ^{13}C NMR (75 MHz) δ=55.25 (OMe), 70.29 (C-6), 71.53 (C-4), 74.09 (C-5), 81.74 (C-2), 84.10 (C-3), 102.28 (C-1); MS (FAB) *m/z* 569 $[M^+-H]$; MS (FD) *m/z* 570 $[M^+]$. Found: C, 73.72; H, 6.76%. Calcd for $C_{35}H_{38}O_7$: C, 73.66; H, 6.71%.

The minor fraction (R_f=0.35, toluene–ethyl acetate 4 : 1) was concentrated and crystallized from benzene–hexane, affording 125 mg (4%) of 4-methoxybenzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside, a regioisomer of **11**; mp 110 °C; $[\alpha]_D^{29}$ −12.8° (*c* 0.5, $CHCl_3$); IR (KBr) 3360 (OH) cm^{-1}; ^{1}H NMR (300 MHz) δ=1.90 (1H, dd, 6-OH), 3.37 (1H, td, H-5), 3.48 (1H, dd, H-2), 3.58 (1H, dd, H-4), 3.67 (1H, dd, H-3), 3.72 (1H, td, H-6a), 3.81 (3H, s, OMe), 3.89 (1H, td, H-6b), 4.55 (1H, d, H-1); $J_{1,2}$=7.8, $J_{2,3}=J_{3,4}$=9.0, $J_{4,5}$=9.5, $J_{5,6a}$=5.0, $J_{5,6b}$=3.0, $J_{6a,6b}$=11.5, $J_{6a,OH}$=7.5, $J_{6b,OH}$=6.0 Hz; ^{13}C NMR (75 MHz) δ=55.27 (OMe), 62.11 (C-6), 71.36, 74.89, 75.05, and 75.68 (4×CH_2Ph), 75.01 (C-5), 77.60 (C-4), 82.32 (C-2), 84.55 (C-3), 102.53 (C-1); MS (FAB) *m/z* 593 $[M^+ + Na]$. Found: C, 73.54; H, 6.69%. Calcd for $C_{35}H_{38}O_7$: C, 73.66; H, 6.71%.

4-Methoxybenzyl 4-*O*-[3,4,6-Tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-β-D-*arabino*-hexopyranosyl]-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12). Silver carbonate (2.76 g, 10 mmol) and silver zeolite[25] (2.0 g) were added to a stirred solution of **11** (1.14 g, 2 mmol) in dry dichloromethane (30 ml) containing MS-3A (2 g, powder); the mixture was then stirred in the dark for 1 h. After 2-(benzoyloxyimino)glycosyl bromide **5**[11] (2.69 g, 4 mmol) was added, the mixture was further stirred at ambient temperature for 2 d. The mixture was diluted with dichloromethane and filtered through a pad of Celite. The filtrate was washed with saturated aq $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated to dryness. Triturating the residue with diethyl ether generated 430 mg of crystalline 3,4,6-tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranose (mp 177—179 °C),[12] a hydrolysate of **5** which was filtered off. The filtrate was concentrated and the residue was chromatographed on silica gel by elution with toluene–ethyl acetate (8 : 1), giving 1.97 g (85%) of **12** as a colorless syrup: $[\alpha]_D^{25}$ +9.8° (*c* 0.7, $CHCl_3$); ^{1}H NMR (300 MHz) δ=3.41 (2H, dd and m, H-2 and 5), 3.50 (1H, dd, H-6a), 3.65 (1H, dd, H-6b), 3.74 (1H, dd, H-3), 3.81 (3H, s, OMe), 4.03 (1H, dd, H-4), 4.35 (1H, d, H-1), 4.42 (1H, m, H-5′), 4.72 (1H, dd, H-6′a), 4.87 (1H, dd, H-6′b), 5.93 (1H, dd, H-4′), 6.24 (1H, d, H-3′), 6.74 (1H, s, H-1′); $J_{1,2}$=8.0, $J_{2,3}$=8.5, $J_{3,4}$=8.5, $J_{4,5}$=9.0, $J_{5,6a}$=5.0, $J_{5,6b}$=1.5, $J_{6a,b}$=10.5, $J_{3',4'}$=5.5, $J_{4',5'}$=5.5, $J_{5',6'a}$=5.0, $J_{5',6'b}$=6.5, $J_{6'a,b}$=11.5 Hz; ^{13}C NMR (75 MHz) δ=55.25 (OCH_3), 68.49 (C-3′), 68.62 (C-4′), 68.77 (C-6), 72.90 (C-5′), 73.94 (C-5), 74.29 (C-6′), 76.68 (C-4), 82.35 (C-2), 83.80 (C-3), 92.12 (C-1′), 101.71 (C-1), 156.42 (C-2); $J_{C1,H1}$=159.0, $J_{C1',H1'}$=174.5 Hz; MS (FAB) *m/z* 1184 $[M^+ + Na]$. Found: C, 71.45; H, 5.41; N, 1.20%. Calcd for $C_{69}H_{63}NO_{16}$: C, 71.31; H, 5.46; N, 1.21%.

4-Methoxybenzyl 4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13). A borane–THF complex (1 M solution in THF, 8.83 ml) was added dropwise to a solution of disaccharide **12** (854 mg, 0.736 mmol) in THF (9 ml) at −10 °C under a nitrogen atmosphere; the mixture was then stirred at this temperature for 0.5 h, and was subsequently allowed to warm up to room temperature. After further stirring for 2 h, excess reductant was quenched with methanol (6 ml) followed by *N*-acetylation through stirring with acetic anhydride (3 ml) for another hour at ambi-

ent temperature. Subsequent passing through a basic resin (Amberlite IR-45), washing with methanol and concentration of the eluate in vacuo gave a residue which was purified by elution from a silica-gel column with chloroform–ethyl acetate (1 : 1). The major fraction (R_f=0.70, chloroform–ethyl acetate 1 : 1) was concentrated, and the residue was dissolved in ethyl acetate–diethyl ether (1 : 4, 10 ml), followed by trituration with pentane (40 ml), providing 550 mg (69%) of **13**: Mp 129—132 °C; $[\alpha]_D^{25}$ −29.7° (*c* 0.5, $CHCl_3$); IR (KBr) 3430 (NH), 1730 (ester C=O), 1680 (NHCO), 1515 cm^{-1} (NH); ^{1}H NMR (300 MHz) δ=1.81 (NHCOCH$_3$), 3.40 (1H, m, H-5), 3.45 (1H, dd, H-2), 3.55 (1H, m, H-5′), 3.57 (1H, dd, H-3), 3.78 (1H, m, H-6a,b), 3.81 (3H, s, OMe), 4.08 (1H, dd, H-4), 4.27 (1H, dd, H-6′a), 4.41 (1H, dd, H-6′b), 4.45 (1H, d, H-1), 4.5—4.9 (8H, m, 4×CH_2Ph), ca. 4.85 (1H, m, H-2′), 5.01 (1H, d, H-1′), 5.19 (1H, dd, H-3′), 5.50 (1H, dd, H-4′), 5.79 (1H, d, NH); $J_{1,2}$=7.5, $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.0, $J_{1',2'}$=1.2, $J_{2',3'}$=4.0, $J_{2',NH}$=8.8, $J_{3',4'}$=$J_{4',5'}$=9.8, $J_{5',6'a}$=5.3, $J_{5',6'b}$=3.5, $J_{6'a,b}$=12.0 Hz; ^{13}C NMR (75 MHz) δ=22.98 (COCH$_3$), 50.94 (C-2′), 55.26 (OMe), 63.28 (C-6′), 67.26 (C-4′), 68.62 (C-6), 70.83 (CH_2Ph), 72.41 (C-3′ and 5′), 73.50 (CH_2Ph), 74.28 (C-5), 74.73 (2×CH_2Ph), 76.50 (C-4), 81.90 (C-2), 82.74 (C-3), 98.62 (C-1′), 102.26 (C-1); $J_{C1,H1}$=159.7, $J_{C1',H1'}$=162.1 Hz; MS (FAB) *m/z* 1108 [M^++Na], 1086 [M^++H]. Found: C, 70.31; H, 5.80; N, 1.34%. Calcd for $C_{64}H_{63}NO_{15}$: C, 70.77; H, 5.85; N, 1.29%.

The minor fractions from the column separation were also collected, one of which was identified by MS and ^{1}H NMR spectroscopy as the α-D-mannosaminyl isomer, 4-methoxybenzyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (32 mg, 4%): ^{1}H NMR (300 MHz) δ=1.79 (3H, s, NHAc), 4.55 (1H, d, H-1), 4.86 (1H, td, H-2′), 5.46 (1H, d, H-1′), 5.68 (1H, dd, H-3′); $J_{1',2'}$=1.8, $J_{2',3'}$=3.6, $J_{3',4'}$=9.1 Hz; ^{13}C NMR (75 MHz) δ=50.75 (C-2′), 82.27 (C-2), 99.42 (C-1′), 102.13 (C-1); MS (FAB) *m/z* 1109 [M+Na+H]$^+$, 1089 [M+2H]$^+$. The other fractions (ca. 90 mg) were not identified.

4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-D-glucopyranose (14). A mixture of **13** (107 mg, 0.1 mmol) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (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% aq $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to dryness. The residue was eluted from a column of silica gel with chloroform–ethyl acetate (4 : 1→1 : 2 gradient) to afford 42 mg (44%) of **14**, comprising α : β=2 : 1 (^{1}H NMR) and 21 mg (20% recovery) of unreacted educt **13**. Compound **14** was dissolved in ethyl acetate–diethyl ether (1 : 2, 1 ml), followed by trituration with pentane (3 ml), providing an amorphous powder: mp 87—90 °C; $[\alpha]_D^{23}$ −14.1° (*c* 0.33, $CHCl_3$); ^{1}H NMR (300 MHz) α-anomer: δ=1.81 (3H, s, NHCOCH$_3$), 3.02 (1H, d, 1-OH), 3.51 (1H, dd, H-2), ca. 3.5 (1H, m, H-5′), 3.7—3.8 (2H, m, H-5, 6), 3.87 (1H, dd, H-3), 4.06 (1H, dd, H-4), 4.2—4.5 (2H, m, H-6′), 4.83 (1H, td, H-2′), 4.95 (1H, d, H-1′), 5.13 (1H, dd, H-3′), 5.20 (1H, dd, H-1), 5.50 (1H, dd, H-4′), 5.71 (1H, d, 2′-NH); $J_{1,2}$=3.5, $J_{1,OH}$=2.5, $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.0, $J_{1',2'}$=1.5, $J_{2',NH}$=9.0, $J_{2',3'}$=4.0, $J_{3',4'}$=$J_{4',5'}$=9.8 Hz; β-anomer: δ=1.81 (3H, s, NHCOCH$_3$), 3.33 (1H, dd, H-2), 3.45—3.55 (2H, m, H-4, 5′), 3.59 (1H, dd, H-3), 3.7—3.8 (3H, m, H-5, 6), 4.25—4.45 (2H, m, H-6′), ca. 4.7 (H-1), ca. 4.8 (H-2′), 4.95 (1H, d, H-1′), 5.16 (1H, dd, H-3′), 5.49 (1H, dd, H-4′), 5.71 (NH); $J_{1,2}$=7.8, $J_{2,3}$=$J_{3,4}$=9.0, $J_{1',2'}$=1.5, $J_{2',3'}$=4.0, $J_{3',4'}$=9.5 Hz; ^{13}C NMR (75 MHz) α-anomer: δ=23.03 (COCH$_3$), 51.02 (C-2′), 63.29 (C-6′), 67.29 (C-4′), 68.52 (C-6), 69.71 (C-5), 72.38 and 72.43 (C-3′,5′), 76.31 (C-4), 79.56 (C-2), 80.02 (C-3), 91.29 (C-1), 98.76 (C-1′); β-anomer: 23.03 (COCH$_3$), 50.93 (C-2′), 63.24 (C-6′), 67.22 (C-4′), 68.65 (C-6), 69.72 (C-5), 72.38 and 72.43 (C-3′,5′), 74.28 (C-4), 82.61 (C-3), 82.82 (C-2), 97.49 (C-1), 98.64 (C-1′); MS (FAB) *m/z* 988 [M^++Na], 966 [M^+]. Found: C, 68.74; H, 5.68; N, 1.54%. Calcd for $C_{56}H_{55}NO_{14}$·0.5 H_2O: C, 68.98; H, 5.79; N, 1.44%.

4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl Fluoride (16). A. Fluorination via Glycosyl Chloride (15): An ice-cooled solution of **14** (235 mg, 0.24 mmol) in dry dichloroethane (2 ml) was treated with dry DMF (0.016 ml, 0.21 mmol) and thionyl chloride (0.087 ml, 1.215 mmol). The mixture was stirred overnight at ambient temperature, then filtered through a pad of silica gel (100 mg), and co-evaporated in vacuo with toluene. The brownish residue (250 mg of chloride **15**, quant.) was used for fluorination without further purification, and thus dissolved in acetonitrile (3 ml) and stirred overnight with silver fluoride (77 mg, 0.61 mmol) in the dark at room temperature. The mixture was diluted with ethyl acetate (10 ml) and filtered through a pad of Celite. The filtrate was washed with 10% aq NaCl (20 ml) and water (20 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was passed through a column of silica gel with chloroform–ethyl acetate (4 : 1); the major fraction was concentrated and solidified with AcOEt–Et_2O–pentane: 180 mg (77%) of **16** as a white powder; mp 65—68 °C; $[\alpha]_D^{25}$ −7.7° (*c* 0.51, $CHCl_3$); ^{1}H NMR (300 MHz) δ=1.79 (3H, s, NHCOCH$_3$), 3.55 (1H, dd, H-2), 3.54—3.60 (2H, m, H-5, 5′), 3.61 (1H, dd, H-3), 3.78 (2H, m, H-6), 4.18 (1H, dd, H-4), 4.30 (1H, dd, H-6′a), 4.41 (1H, dd, H-6′b), 4.5—4.8 (6H, 3×$PhCH_2$), 4.85 (1H, td, H-2′), 4.97 (1H, d, H-1′), 5.19 (1H, dd, H-3′), 5.25 (1H, dd, H-1), 5.50 (1H, dd, H-4′), 9.0 (1H, d, NH); $J_{1,2}$=6.0, J_{HF}=53, $J_{2,3}$=8.5, $J_{3,4}$=$J_{4,5}$=9.0, $J_{1',2'}$=1.0, $J_{2'NH}$=9.0, $J_{2',3'}$=4.0, $J_{3',4'}$=10.0, $J_{4',5'}$=9.5, $J_{5',6'a}$=5.0, $J_{5',6'b}$=3.5, $J_{6'a,6'b}$=12.0 Hz; ^{13}C NMR (75 MHz) δ=23.01 (NHCOCH$_3$), 50.85 (C-2′), 63.20 (C-6′), 67.23 (C-4′), 68.27 (C-6), 72.28 (C-3), 72.46 (C-2), 76.31 (C-3′), 73.70, 74.23, and 74.50 (3×$PhCH_2$), 75.77 (C-4), 80.64 and 80.96 (C-5, 5′), 98.47 (C-1′), 109.56 (C-1); MS (FAB) *m/z* 990 [M^++Na], 968 [M^++1].

B. Direct Fluorination of 14 with DAST: To a stirred solution of **14** (26.4 mg, 27.3 mmol) in dry dichloromethane (1 ml) was added diethylaminosulfur trifluoride (DAST, 7.2 ml, 54.6 mmol) at −30 °C under an atmosphere of nitrogen. The mixture was further stirred for 4 h at room temperature, treated with methanol (10 ml) at −30 °C, and then evaporated to dryness. The residue was partitioned between dichloromethane (10 ml) and 5% aq $NaHCO_3$ (10 ml), and the organic phase was washed with water (3×10 ml), and dried (Na_2SO_4). Removal of the solvent gave a colorless syrup, which was purified through a silica-gel column to afford 19 mg (72%) of **16**, being fully identified with the authentic sample obtained by method A.

Benzyl 3,4-Di-*O*-benzyl-α-L-rhamnopyranoside (17). A mixture of benzyl 4-*O*-benzyl-2,3-*O*-benzylidene-α-L-rhamnopyranoside[21] (130 mg, 0.3 mmol) in THF (4.5 ml) containing MS-3A (45 mg) was treated with sodium cyanotrihydroborate (170 mg, 2.7 mmol) and HCl-saturated diethyl ether (4 ml) as described for **11**. Workup and purification through silica-gel column (toluene–ethyl acetate, 3:1) afforded 92.3 mg (71%) of **17** as a colorless syrup: $[\alpha]_D^{24}$ −50.5° (*c* 0.4, $CHCl_3$) [lit,[22] 69%, $[\alpha]_D$ −58° (*c* 0.6 $CHCl_3$)]; IR (firm) 3440 cm^{-1} (OH); ^1H NMR (300 MHz) δ=1.34 (3H, d, H-6), 2.46—2.58 (1H, 2-OH), 3.59 (1H, td, H-4), 3.82 (1H, dd, H-5), 3.91 (1H, dd, H-3), 4.09 (1H, dd, H-2), 4.49 and 4.71 (1H each, d, $C\underline{H}_2Ph$), 4.66 and 4.81 (1H each, d, $C\underline{H}_2Ph$), 4.70 (2H, s, $C\underline{H}_2Ph$), 4.91 (1H, s, H-1), 7.24—7.41 (3×C_6H_5); $J_{1,2}$=0, $J_{2,3}$=3.5, $J_{3,4}$=$J_{4,5}$=9.5, $J_{5,6}$=6.5 Hz; ^{13}C NMR (75 MHz) δ=17.91 (C-6), 67.53 (C-5), 68.62 (C-2), 69.04, 72.08 and 75.46 (3×$C\underline{H}_2Ph$), 80.00 (C-4), 80.13 (C-3), 98.21 (C-1); MS (FAB) *m/z* 457 [M^++Na], 433 [M^+−H].

Benzyl *O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (18). A solution of benzyl rhamnoside **17** (23.6 mg, 54.3 mmol) in dry dichloromethane (1 ml) with MS-4A (200 mg) was stirred in the dark for 0.5 h. Silver perchlorate (11.3 mg, 54.3 mmol), $SnCl_2$ (10.3 mg, 54.3 mmol) and a solution of the fluoride **16** (52.6 mg, 54.3 mmol) in dry dichloromethane (1 ml) was added; the mixture was stirred at room temperature for an additional 20 h. The resulting mixture was diluted with dichloromethane (10 ml) and filtered through a pad of Celite. The filtrate was washed with 5% aq $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to give a residue, which was purified through a silica-gel column by elution with hexane–ethyl acetate (2:1). The major fraction was concentrated to afford 38 mg (51%) of **18** as a yellowish syrup: ^1H NMR (300 MHz) δ=1.33 (3H, d, H-6), 3.20 (2H, m, H-6′), 3.41 (1H, td, H-5″), 3.47 (1H, dd, H-2′), 3.61 (1H, dd, H-4), 3.81 (1H, dd, H-5), 3.90 (2H, m, H-3, 3′), 4.00—4.06 (2H, m, H-4′, 5′), 4.08 (1H, dd, H-2′), 4.23 (1H, dd, H-6″a), 4.37 (1H, dd, H-6″b), 4.74 (1H, d, H-1″), 4.77 (1H, td, H-2″), 4.83 (1H, d, H-1′), 4.86 (1H, d, H-1), 5.03 (1H, dd, H-3″), 5.49 (1H, dd, H-4″), 5.70 (1H, d, NH); $J_{1,2}$=3.5, $J_{2,3}$=1.5, $J_{3,4}$=9.0, $J_{4,5}$=9.5, $J_{5,6}$=6.0, $J_{1',2'}$=3.5, $J_{2',3'}$=9.0, $J_{1'',2''}$=1.0, $J_{2'',NH}$=8.5, $J_{2'',3''}$=3.5, $J_{3'',4''}$=$J_{4'',5''}$=10.0, $J_{5'',6''a}$=5.0, $J_{5'',6''b}$=3.5, $J_{6''a,6''b}$=12.0 Hz; ^{13}C NMR (75 MHz) δ=17.98 (C-6), 22.93 ($CO\underline{C}H_3$), 51.12 (C-2″), 63.34, (C-6″), 67.28 (C-4″), 67.52 (C-5), 68.07 (C-6′), 68.47 (C-2), 72.32 (C-5″), 72.70 (C-3″), 75.17 and 75.43 (C-4′, 5′), 78.94 (C-3), 79.23 (C-2′), 80.18 and 80.23 (C-3′, 4).

Benzyl *O*-(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (19). A solution of **18** (37.3 mg, 27 mmol) in 0.05 M methanolic sodium methoxide (1.4 ml) was stirred at ambient temperature for 20 h. Subsequent neutralization (Dowex 50W×4), filtration through Celite, and evaporation to dryness gave a residue, which was eluted from a silica-gel column with chloroform–methanol (8:1). Concentration of the major fraction gave **19** as a colorless syrup (25.7 mg, 89%): $[\alpha]_D^{26}$ +29.5° (*c* 0.1, $CHCl_3$); ^1H NMR (300 MHz) δ=1.37 (3H, d, H-6), 1.80 (3H, s, $COC\underline{H}_3$), 2.98 (1H, td, H-5″), 3.15 (1H, broad, H-6′), 3.20 (1H, dd, H-3″), 3.43 (1H, dd, H-4″), 3.53 (1H, dd, H-2′), 3.54 (1H, dd, H-6″a), 3.61 (1H, dd, H-4), 3.69 (1H, dd, H-6″b), 3.80 (1H, dd, H-5), 3.88—3.92 (3H, m, H-3, 3′, 4′), ca. 3.98 (2H, m, H-2″, 5′), 4.07 (1H, dd, H-2), 4.50 (1H, d, H-1″), 4.86 (1H, d, H-1), 4.87 (1H, d, H-1′), 5.99 (1H, d, NH); $J_{1,2}$=1.5, $J_{2,3}$=3.0, $J_{3,4}$=9.5, $J_{4,5}$=9.0, $J_{5,6}$=6.0, $J_{1',2'}$=3.5, $J_{2',3'}$=9.0, $J_{1'',2''}$=1.0, $J_{2'',NH}$=5.5, $J_{2'',3''}$=3.5, $J_{3'',4''}$=9.0, $J_{4'',5''}$=9.5, $J_{5'',6''a}$=5.0, $J_{5'',6''b}$=3.0, $J_{6''a,6''b}$=11.5 Hz; ^{13}C NMR (75 MHz) δ=18.01 (C-6), 22.76 ($CO\underline{C}H_3$), 55.14 (C-2″), 62.00 (C-6″), 67.85 (C-6′), 68.42 (C-5), 68.58 (C-4″), 69.66 (C-2), 74.51 and 75.24 (C-4′, 5′), 75.08 (C-3″), 76.19 (C- 5″), 78.99 and 79.78 (C-3, 3′), 79.36 (C-2′), 80.23 (C-4), 96.27 and 96.37 (C-1, 1′), 98.08 (C-1″), 173.69 ($\underline{C}OCH_3$); MS (FAB) *m/z* 1092 [M^++Na].

***O*-(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-L-rhamnopyranose (1).** A solution of **19** (22.6 mg, 21.1 mmol) in methanol–water (4:1 v/v, 20 ml) with acetic acid (1 ml) was hydrogenated in the presence of 10% palladium on carbon (50 mg) under an atmosphere of hydrogen (3.10×10^5 Pa) for 2 d. The mixture was filtered through a pad of Celite and a short column of IR-45 resin. The filtrate was concentrated in vacuo to give a syrup, which was purified by elution from a silica-gel column with chloroform–methanol–water (5:4:1). The major fraction was concentrated and the residual syrup was lyophilized to give 10.5 mg (94%) of **1** as an amorphous colorless powder. The α/β-anomeric ratio of the reducing end was estimated as 2:1 by ^1H NMR; $[\alpha]_D^{22}$ +27.6° (*c* 0.53, MeOH); ^1H 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, $COC\underline{H}_3$), 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) δ=17.5 (α- and β-C-6), 22.9 ($CO\underline{C}H_3$), 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 ($COCH_3$).

References

1) H. J. Jennings, *Adv. Carbohydr. Chem. Biochem.*, **41**, 155 (1983).

2) C.-J. Lee and B. A. Fraser, *J. Biol. Chem.*, **255**, 6847 (1980).

3) N. Ohno, T. Yadomae, and T. Miyazaki, *Carbohydr. Res.*, **80**, 297 (1980).

4) H. J. Jennings, K. G. Rossel, and D. J. Carlo, *Can. J. Chem.*, **58**, 1069 (1980).

5) H. Paulsen, B. Helpap, and J. P. Lorentzen, *Carbohydr. Res.*, **179**, 173 (1988).

6) T. Sugawara and K. Igarashi, *Carbohydr. Res.*, **172**, 195 (1988).

7) E. Micheli, F. Nicotra, L. Panza, F. Ronchetti, and L. Toma, *Carbohydr. Res.*, **139**, C1 (1985); L. Panza, F. Ronchetti, G. Russo, and L. Toma, *J. Chem. Soc., Perkin Trans. 1*, **1987**, 2745.

8) H. Paulsen, J. P. Lorentzen, and W. Kutschker, *Car-*

bohydr. Res., **136**, 153 (1985).
9) Recent examples: J. Kerekgyarto, J. G. M. van der Ven, J. P. Kamerling, A. Liptak, and J. F. G. Vliegenthart, *Carbohydr. Res.*, **238**, 135 (1993); F. W. Lichtenthaler and T. Schneider-Adams, *J. Org. Chem.*, **59**, 6728 (1994); F. W. Lichtenthaler, T. Schneider-Adams, and S. Immel, *J. Org. Chem.*, **59**, 6735 (1994).
10) Preliminary communication: E. Kaji, F. W. Lichtenthaler, Y. Osa, K. Takahashi, E. Matsui, and S. Zen, *Chem. Lett.*, **1992**, 707.
11) F. W. Lichtenthaler, P. Jarglis, and W. Hempe, *Liebigs Ann. Chem.*, **1983**, 1959.
12) E. Kaji, F. W. Lichtenthaler, T. Nishino, A. Yamane, and S. Zen, *Bull. Chem. Soc. Jpn.*, **61**, 1291 (1988).
13) F. W. Lichtenthaler, U. Kläres, M. Lergenmüller, and S. Schwidetzky, *Synthesis*, **1992**, 179.
14) Y. Oikawa, T. Yoshida, and O. Yonemitsu, *Tetrahedron Lett.*, **23**, 885 (1982).
15) C. A. A. van Boeckel, T. Beetz, and S. F. van Aelst, *Tetrahedron*, **40**, 4097 (1984).
16) F. W. Lichtenthaler, E. Kaji, and S. Weprek, *J. Org. Chem.*, **50**, 3505 (1985).
17) F. W. Lichtenthaler and E. Kaji, *Liebigs Ann. Chem.*, **1985**, 1659.
18) E. Kaji and F. W. Lichtenthaler, *Trends Glycosci. Glycotechnol.*, **5**, 121 (1993).
19) E. Kaji, E. Matsui, M. Kobayashi, and S. Zen, *Bull. Chem. Soc. Jpn.*, **68**, 1449 (1995).
20) R. Johansson and B. Samuelsson, *J. Chem. Soc., Perkin Trans. 1*, **1984**, 2371.
21) J. S. Brimacombe, B. D. Jones, M. Stacey, and J. J. Willard, *Carbohydr. Res.*, **2**, 167 (1966).
22) A. Liptak, P. Fügedi, and P. Nanasi, *Carbohydr. Res.*, **65**, 209 (1978).
23) T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, **1981**, 431; T. Mukaiyama, Y. Hashimoto, and S. Shoda, *Chem. Lett.*, **1983**, 935.
24) H. Itokawa, Y. Oshida, A. Ikuta, H. Inatomi, and T. Adachi, *Phytochemistry*, **21**, 1935 (1982).
25) P. J. Garegg and P. Ossowski, *Acta Chem. Scand., Ser. B*, **B37**, 249 (1983).