An Alternative Access to a Trisaccharide Repeating Unit of the Capsular Polysaccharide of *Streptococcus pneumoniae* Serotype 19A

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A chemical synthesis has been achieved for β-D-ManNAc-(1→4)-α-D-Glc-(1→3)-L-Rha, a trisaccharide repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* serotype 19A, by stepwise link-up of the suitably functionalized, constituent sugar units. A β-selective glycosylation of trimethylsilylethyl glucoside having free 4-OH with 2-(benzoyloxyimino)-2-deoxyglycosyl bromide, followed by *manno*-selective hydroboration, *N*-acetylation, and functionalization of the anomeric center (1-OSE→1-OH→1-F), gave a key disaccharide donor, β-D-ManNAc-(1→4)-α-D-Glc-(1→F. Ensuing glycosylation of an L-rhamnosyl acceptor with the donor substrate afforded, after deblocking, the target trisaccharide in 6.5% yield over 13 steps from D-glucose.

Key words *Streptococcus pneumoniae* type 19A; capsular polysaccharide; 2-ulose oxime; β-D-mannosaminide; glycosylation; hydroboration

Streptococcus pneumoniae serogroup 19, including two types, 19A and 19F, is of particular clinical importance, since it is known to be a major pathogenic bacterium in PRSP (penicillin-resistant *Streptococcus pneumoniae*)-provoked pneumonia.[1-3] Its immunogenic specificity depends on the cell-surface capsular polysaccharide (CPS), which incorporates the β-D-ManNAc-containing trisaccharides **1**[4] and **2**[5] (Fig. 1).

Despite the clinical utility of a multi-valent vaccine composed of natural CPS, the polysaccharide is not very immunogenic in people at high risk.[6] Accordingly, attention has been focused on a synthetic vaccine. We have investigated the practical synthetic acquisition of the trisaccharide repeating unit of the CPS from *Streptococcus pneumoniae* type 19F.[7] In this paper, we describe a synthetic access to the trisaccharide component **3** of type 19A polysaccharide.

A trisaccharide sequence, β-D-ManNAc-(1→4)-α-D-Glc-(1→3)-L-Rha (**3**) (Fig. 2) has been synthesized by other groups[8,9] by elaboration of the critical β-D-ManNAc portion from a suitably protected 2-azido-2-deoxy-α-D-mannopyranosyl bromide[8] or from a 2′-OH-selectively blocked cellobiose derivative.[9] The utility of these methods is limited by the lengthy procedures, with less than 2% yield over 16 steps from D-glucose.[8,9] Our basic approach to **3** comprises an elaboration of the β-D-ManNAc portion from 2-(benzoyloxyimino)-2-deoxyglycosyl bromide (**4**), with glycosylation of the central D-glucose unit, and consecutively the terminal L-rhamnose unit.

Although the key disaccharide donor, β-D-ManNAc-(1→4)-α-D-Glc-(1→F (**9**) has already been synthesized by us,[7] the protocol is unlikely to be of general utility, since the precursor 1-OH disaccharide **8** was generated in only 44% yield from the 4-methoxybenzyl (MBn) glycoside analogue of **7** by means of an oxidative cleavage of the O-MBn bond. We therefore selected the 2-(trimethylsilyl)ethyl (SE) group instead of the MBn group for protection of the anomeric hydroxyl group on the central glucose unit.

The 2-(trimethylsilyl)ethyl glucoside **5** having a free 4-OH group was prepared according to the reported method,[10] and was glycosylated with 2-(benzoyloxyimino)-2-deoxyglycosyl bromide **4**, a readily available indirect β-D-ManNAc donor (59% over 6 steps from D-glucose).[11] As observed previously,[7,12,13] the highly β-selective glycosidation of the bromide **4** with 4-OH of the glucoside **5** was effected by using a highly active silver zeolite (silver aluminosilicate)[14] in CH_2Cl_2 (r.t., 3 h), affording the β(1→4) disaccharide **6** in 86% yield. The β/α selectivity was estimated to be >20/1 by ^{1}H-NMR. The β-configuration of the newly formed intersaccharide linkage was confirmed by the respective $J_{3',4'}$ and $J_{4',5'}$ coupling constants of 5.2 and 5.5 Hz, indicating distortion of the pyranose ring towards the twist-boat form, as depicted in the formula **6** (Chart 1). Such a stereochemical outcome has been observed exclusively for β-anomeric 2-(benzoyloxyimino)-2-deoxyglycosides.[7,12,13,15]

The β(1→4) disaccharide **6** was then subjected to a *manno*-selective hydroboration, as in ref. 7, with a twelve-fold molar excess of borane–tetrahydrofuran (BH_3·THF) complex in THF to afford, on *N*-acetylation, the expected *N*-acetyl-β-D-mannosaminyl-(1→4)-D-glucoside **7** in 73% yield. The β-D-*manno*-configuration of the

Type 19A

→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→PO_4^-→ (**1**)

Type 19F

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

Fig. 1

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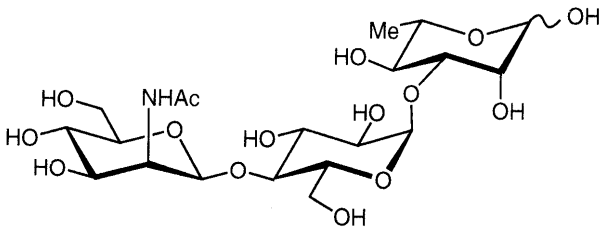
amino sugar moiety of **7** unambiguously followed from the coupling constants around the pyranose ring, notable $J_{1',2'}=1.5$, $J_{2',3'}=4.0$, and $J_{3',4'}=J_{4',5'}=9.8$ Hz.

Generation of the 1-OH-free disaccharide **8**, an important precursor of the donor substrate **9**, was remarkably improved as compared with the former case (44% yield),[7] resulting in 77% yield by means of TFA-promoted deblocking of the 1-OSE group of **7**. Ensuing fluorination of the 1-OH of **8** with diethylaminosulfur trifluoride (DAST) proceeded smoothly to provide the disaccharide fluoride **9** in 93% yield in the form of an anomeric mixture ($\alpha/\beta=ca.$ 1/3).

For attachment of the L-rhamnose unit to the glucosyl portion, the fluoride **9** was exposed to a rhamnosyl acceptor **10**[16] in the presence of $AgClO_4$–$SnCl_2$ in CH_2Cl_2, affording the desired $\alpha(1\rightarrow3)$ trisaccharide **11** (30%) along with the corresponding $\beta(1\rightarrow3)$ trisaccharide (26%). This unsatisfactory selectivity for the $\alpha(1\rightarrow3)$ glycoside **11** may be attributed to the very low reactivity of 3-OH of the L-rhamnoside acceptor **10**.

The subsequent de-*O*-benzoylation (**11**→**12**, 88% yield, 0.05 M NaOMe/MeOH) and hydrogenolysis (**12**→**3**, 93%, Pd–C/H_2/MeOH–H_2O–AcOH) proceeded smoothly to afford the target trisaccharide. The anomeric ratio of the reducing end of **3** in D_2O solution was estimated to be $\alpha/\beta=2/1$ (^{1}H-NMR). Its ^{1}H- and ^{13}C-NMR spectra were identical with the reported data.[8]



3

Fig. 2

Experimental

Melting points were measured on a Yamato MP-1 apparatus or a Yanagimoto micro melting point apparatus without correction. Spectral measurements were recorded on JASCO DIP-150 digital polarimeter ($[\alpha]_D$), JMS D-100 mass spectrometer (MS), and Varian VXR-300 or XL-400 spectrometers (^{1}H- and ^{13}C-NMR). TLC was done on Merck Silica gel 60 F_{254} with the same solvent systems as used for column chromatography. The spots were visualized under UV light (254 nm) or by charring with 10% aqueous H_2SO_4. Column chromatography was achieved on Silica gel 60 (70—230 mesh, Merck).

2-(Trimethylsilyl)ethyl 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 (6) A mixture of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[10] **5** (551 mg, 1.0 mmol), molecular sieves (4 Å, 2.0 g), and silver aluminosilicate[14] (0.63 g, 2.0 mmol) in dry CH_2Cl_2 (20 ml) was stirred in the dark under a N_2 atomosphere at room temperature for 15 min. 2-(Benzoyloxyimino)-2-deoxyglycosyl bromide[11] **4** (1.01 g, 1.5 mmol) was then added, and the whole was further stirred at ambient temperature for 3 h. After dilution with CH_2Cl_2 (50 ml), the mixture was filtered through a pad of Celite, and the filtrate was washed consecutively with 5% aqueous $NaHCO_3$ (50 ml) and H_2O (3×70 ml). Drying (Na_2SO_4), evaporation to dryness, elution from a silica-gel column with MeC_6H_5–AcOEt (12:1), and concentration of the major fraction furnished **6** (982 mg, 86%) as a colorless syrup. $[\alpha]_D^{25}$ +7.4° ($c=1.0$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.02 (9H, s, $SiMe_3$), 0.99 (2H, m, $SiCH_2$), 3.36 (1H, dd, H-2), 3.4—3.7 (4H, m, H-5, 6a, 6b, OCH_2CH_2), 3.78 (1H, dd, H-3), 3.90—4.04 (CH_2Ph, OCH_2CH_2), 4.22, 4.32 (4H, d, 2×CH_2Ph), 4.29 (1H, d, H-1), 4.42 (1H, m, H-5′), 4.63 (CH_2Ph), 4.71 (1H, m, H-6′a), 4.89 (1H, m, H-6′b), 4.94 (CH_2Ph), 5.91 (1H, dd, H-4′), 6.22 (1H, d, H-3′), 6.74 (1H, s, H-1′); $J_{1,2}=8.0$, $J_{2,3}=J_{3,4}=8.8$, $J_{3',4'}=5.2$, $J_{4',5'}=5.5$ Hz. ^{13}C-NMR (75 MHz, $CDCl_3$) δ: −1.44 ($SiMe_3$), 18.48 ($SiCH_2$), 64.42 (CH_2Ph), 67.37 (OCH_2CH_2), 68.53 (C-3′), 68.74 (C-4′), 68.85 (C-6), 72.86 (C-5′), 73.24 (CH_2Ph), 73.96 (C-5), 74.30 (C-6′), 74.97 (CH_2Ph), 76.81 (C-4), 82.80 (C-3), 92.18 (C-1′), 102.76 (C-1), 156.40 (C-2′), 162.46, 164.58, 164.95, and 165.96 (4×COPh). MS (FAB) *m/z*: 1165 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7) A 1 M solution of BH_3·THF complex in THF (5.76 ml) was added dropwise

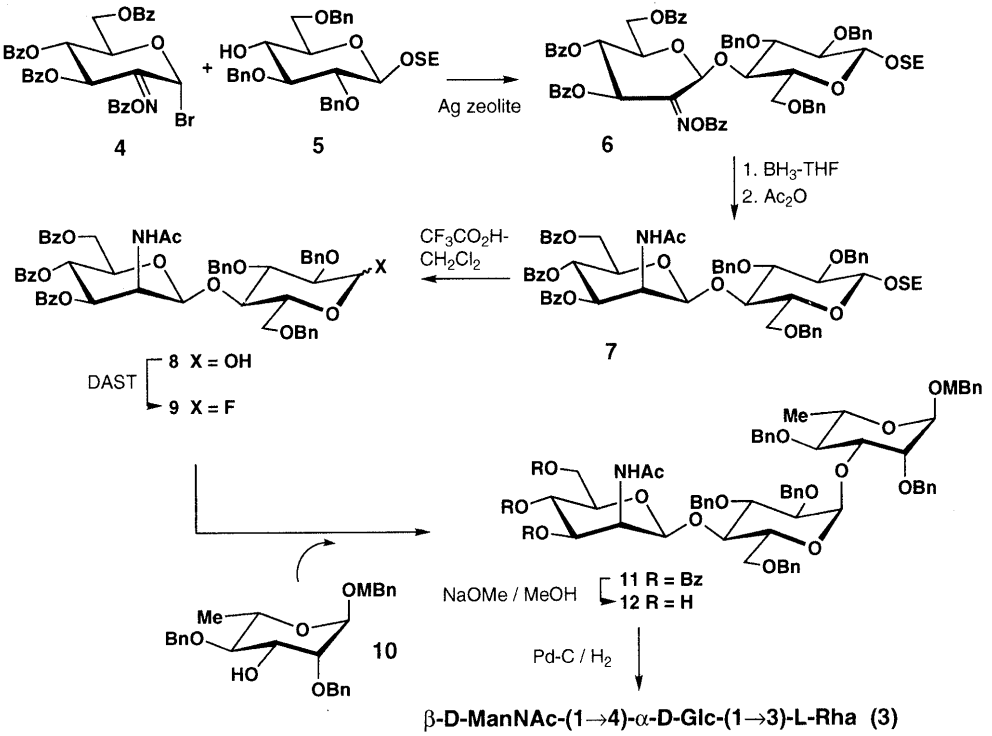


Chart 1

to a solution of the disaccharide **6** (548 mg, 0.48 mmol) in THF (11.5 ml) at −10 °C under an atmosphere of N_2. The mixture was stirred at this temperature for 0.5 h and then allowed to warm up to room temperature. After further stirring for 2 h, excess reductant was quenched with MeOH (4.0 ml) followed by *N*-acetylation through stirring with Ac_2O (2.0 ml) for another 1 h at ambient temperature. The resulting mixture was passed through a basic resin (Amberlite IR-45), and washed with MeOH. The eluate was concentrated *in vacuo* and the residue was purified by elution from a silica-gel column with MeC_6H_5–AcOEt (3 : 1). The major fraction was concentrated and the residue was crystallized from AcOEt–Et_2O and excess pentane, providing **7** (373 mg, 73%) as a colorless powder. mp 131—133 °C. $[\alpha]_D^{25}$ −19.6° (c=0.5, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.05 (9H, s, $SiMe_3$), 1.04 (2H, m, $SiCH_2$), 1.80 (3H, s, Ac-CH_3), 3.39 (1H, dd, H-2), 3.40 (1H, m, H-5), 3.50—3.64 (3H, m, H-3, 5′, OCH_2CH_2), 3.76 (2H, m, H-6a,6b), 4.0 (1H, m, OCH_2CH_2), 4.06 (1H, dd, H-4), 4.26 (1H, dd, H-6′a), 4.36 (1H, d, H-1), 4.40 (1H, dd, H-6′b), 4.57, 4.65, 4.74 (each 1H, d, CH_2Ph), 4.84 (1H, m, H-2′), 4.89 (2H, d, CH_2Ph), 5.01 (1H, d, H-1′), 5.18 (1H, dd, H-3′), 5.50 (1H, dd, H-4′), 5.75 (1H, d, NH); $J_{1,2}$=7.7, $J_{2,3}$=9.0, $J_{3,4}$=$J_{4,5}$=9.5, $J_{1',2'}$=1.5, $J_{2',3'}$=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, $J_{6'a,6'b}$=12.0 Hz. ^{13}C-NMR (75 MHz, $CDCl_3$) δ: −1.40 ($SiMe_3$), 18.49 ($SiCH_2$), 23.01 ($NHCOCH_3$), 50.92 (C-2′), 63.29 (C-6′), 67.25 (C-4′), 67.48 (OCH_2CH_2), 68.63 (C-6), 72.39 (C-3′ and 5′), 73.43 (CH_2Ph), 74.22 (C-5), 74.73 (2×CH_2Ph), 76.42 (C-4), 82.05 (C-2), 82.76 (C-3), 98.55 (C-1′), 103.23 (C-1), 165.40, 165.64, 165.93 (3×COPh), 170.32 (NHCO). MS (FAB) m/z: 1088 $(M+Na+H)^+$. *Anal.* Calcd for $C_{61}H_{65}NO_{14}Si$: C, 68.84; H, 6.16; N, 1.32. Found: C, 68.67; H, 6.39; N, 1.35.

4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-D-glucopyranose (8) Trifluoroacetic acid (2.12 ml, 26.4 mmol) was added dropwise at 0 °C to a solution of the disaccharide **7** (223 mg, 0.21 mmol) in dry CH_2Cl_2 (1.0 ml), and the mixture was further stirred at 0 °C for 2 h. Acetic acid (7 ml) and toluene (14 ml) were added, and the whole was evaporated to dryness. The residue was eluted from a silica-gel column with $CHCl_3$–AcOEt (1 : 1). Concentration of the major fraction gave a syrup, which was dissolved in AcOEt–Et_2O (1 : 2) (3 ml), followed by trituration with excess pentane (15 ml) to furnish **8** (156 mg, 77%) as a colorless powder (α : β = 2 : 1) (^{1}H-NMR). mp 85—89 °C. $[\alpha]_D^{25}$ −12.4° (c=0.5, $CHCl_3$). [lit.[7] mp 87—90 °C, $[\alpha]_D^{23}$ −14.1° (c=0.33, $CHCl_3$)]. Its ^{1}H-NMR, ^{13}C-NMR, and mass spectra were identical with those of an authentic sample.[7]

4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α- and β-D-glucopyranosyl Fluoride (9) A stirred solution of **8** (492 mg, 0.51 mmol) in dry CH_2Cl_2 (13 ml) was treated with diethylaminosulfur trifluoride (DAST, 0.135 ml, 1.02 mmol) at −30 °C under an atmosphere of N_2. The mixture was further stirred for 3 h at room temperature, treated with MeOH (0.26 ml), and then evaporated to dryness. The residue was partitioned between CH_2Cl_2 (50 ml) and 5% aqueous $NaHCO_3$ (50 ml), and the organic phase was washed with H_2O (3×50 ml), and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a colorless syrup, which was eluted from a silica-gel column with $CHCl_3$–AcOEt (4 : 1) to afford an anomeric mixture of **9** (457 mg, 93%, α : β = *ca.* 1 : 3) (^{1}H-NMR). A small part of the mixture was carefully chromatographed using the above systems to give the α-anomer (**9**α) and corresponding β-anomer (**9**β). **9**α: $[\alpha]_D^{23}$ −15.5° (c=1.0, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.82 (3H, s, $COCH_3$), 3.47 (1H, ddd, H-2), 3.53 (1H, ddd, H-5′), 3.73 (2H, m, H-6), 3.89 (1H, dd, H-3), 3.91 (1H, ddd, H-5), 4.10 (1H, dd, H-4), 4.28 (1H, dd, H-6′a), 4.39 (1H, dd, H-6′b), 4.83 (1H, ddd, H-2′), 4.88 (1H, d, H-1′), 5.14 (1H, dd, H-3′), 5.50 (1H, dd, H-4′), 5.52 (1H, d, H-1), 5.70 (1H, d, NH); $J_{1,2}$=2.5, $J_{1,F}$=52.5, $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.5, $J_{5,6a}$=2.5, $J_{1',2'}$=1.3, $J_{2',3'}$=4.0, $J_{2',NH}$=8.8, $J_{3',4'}$=$J_{4',5'}$=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, $CDCl_3$) δ: 23.02 ($COCH_3$), 50.98 (C-2′), 63.24 (C-6′), 67.24 (C-4′), 67.81 (C-6), 71.97 (C-5), 72.36, 72.45 (C-3′, 5′), 75.41 (C-4), 78.90 (C-2), 79.73 (C-3), 98.81 (C-1′), 105.38 (C-1); $J_{C1,F}$=227.7, $J_{C2,F}$=24.7 Hz. MS (FAB) m/z: 990 $(M+Na)^+$.

9β: $[\alpha]_D^{24}$ −7.0° (c=1.25, $CHCl_3$). [lit.[7] $[\alpha]_D^{25}$ −7.7° (c=0.51, $CHCl_3$)]. Its ^{1}H-NMR, ^{13}C-NMR, and mass spectra were identical with the reported data.[7]

Methoxybenzyl *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→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (11) and Its β-D-Glucopyranosyl Isomer (11β) A solution of methoxybenzyl rhamnoside **10**[16] (55.7 mg, 0.12 mmol) in dry CH_2Cl_2 (2 ml) with MS-4A (300 mg) was stirred in the dark for 0.5 h. Glycosyl fluoride **9** (76 mg, 0.08 mmol), $SnCl_2$ (16.7 mg, 0.088 mmol), and $AgClO_4$ (18.2 mg, 0.088 mmol) were added, and the mixture was stirred at room temperature for an additional 20 h, then diluted with CH_2Cl_2 (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 hexane–AcOEt (3 : 2) to give the desired trisaccharide **11** (33 mg, 30% yield) and its β-D-glucopyranosyl isomer **11**β (29 mg, 26% yield), each as a colorless syrup. **11**: TLC *Rf* 0.40. $[\alpha]_D^{23}$ −3.6° (c=0.20, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.34 (3H, d, H-6), 1.70 (3H, s, $COCH_3$), 3.26—3.38 (2H, m, H-6′), 3.42 (1H, m, H-5″), 3.52 (1H, dd, H-2′), 3.63 (1H, dd, H-4), 3.76 (1H, dd, H-5), 3.82 (3H, s, OCH_3), 3.84 (1H, dd, H-2), 3.92—4.04 (2H, m, H-4′, 5′), 3.96 (1H, dd, H-3′), 4.06 (1H, dd, H-3), 4.24 (1H, dd, H-6″a), 4.38 (1H, dd, H-6″b), 4.70 (1H, d, H-1″), 4.75 (1H, d, H-1), 4.78 (1H, dd, H-2″), 5.01 (1H, dd, H-3″), 5.09 (1H, d, H-1′), 5.46 (1H, dd, H-4″), 5.63 (1H, d, NH); $J_{1,2}$=1.5, $J_{2,3}$=3.0, $J_{3,4}$=9.0, $J_{4,5}$=9.3, $J_{5,6}$=6.0, $J_{1',2'}$=3.5, $J_{2',3'}$=$J_{3',4'}$=9.5, $J_{1'',2''}$=1.0, $J_{2'',3''}$=3.8, $J_{3'',4''}$=$J_{4'',5''}$=10.0, $J_{5'',6''a}$=5.2, $J_{5'',6''b}$=3.5, $J_{6''a,6''b}$=12.0 Hz. ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.10 (C-6), 22.91 ($COCH_3$), 51.12 (C-2″), 55.31 (OCH_3), 63.36 (C-6″), 67.35 (C-4″), 68.17 (C-6′), 68.53 (C-5), 69.93 (C-5′), 72.30 (C-5″), 72.70 (C-3″), 75.76 (C-4′), 76.15 (C-2), 77.55 (C-3), 79.22 (C-2′), 79.91 (C-4), 80.62 (C-3′), 96.17 (C-1′), 97.17 (C-1), 98.38 (C-1″), 170.13 ($NHCO$). MS (FAB) m/z: 1434 $(M+Na)^+$.

11β: TLC *Rf* 0.30. $[\alpha]_D^{23}$ −19.3° (c=0.85, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.32 (3H, d, H-6), 1.81 (3H, s, $COCH_3$), 3.36 (1H, m, H-5′), 3.43 (1H, dd, H-2′), 3.52—3.60 (1H, m, H-5″), 3.56 (1H, dd, H-3′), 3.64 (1H, dd, H-4), 3.68 (2H, m, H-6′), 3.74 (1H, dd, H-5), 3.80 (3H, s, OCH_3), 3.90 (1H, dd, H-2), 4.08 (1H, dd, H-4′), 4.21 (1H, dd, H-3), 4.26 (1H, dd, H-6″a), 4.38 (1H, dd, H-6″b), 4.78 (1H, d, H-1), 4.78 (1H, d, H-1′), 4.86 (1H, m, H-2″), 5.04 (1H, d, H-1″), 5.52 (1H, dd, H-4″), 5.75 (1H, d, NH); $J_{1,2}$=1.5, $J_{2,3}$=3.0, $J_{3,4}$=9.0, $J_{4,5}$=9.5, $J_{5,6}$=6.0, $J_{1',2'}$=7.5, $J_{2',3'}$=8.8, $J_{3',4'}$=9.0, $J_{4',5'}$=9.0, $J_{1'',2''}$=1.3, $J_{2'',3''}$=3.7, $J_{3'',4''}$=9.7, $J_{4'',5''}$=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, $CDCl_3$) δ: 17.94 (C-6), 23.02 ($COCH_3$), 50.88 (C-2″), 55.27 (OCH_3), 63.25 (C-6″), 67.27 (C-4″), 68.07 (C-5), 68.40 (C-6′), 72.43 (C-3″, 5″), 74.17 (C-5′), 77.20 (C-4′), 78.01 (C-3), 78.95 (C-2), 81.35 (C-4), 82.40 (C-2′), 83.10 (C-3′), 97.66 (C-1), 98.38 (C-1″), 103.60 (C-1′), 170.27 ($NHCO$). MS (FAB) m/z: 1434 $(M+Na)^+$.

***O*-(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-L-rhamnopyranose (3)** A solution of **11** (44 mg, 0.031 mmol) in 0.05 M MeONa in MeOH was stirred at ambient temperature for 20 h. Subsequent neutralization (Dowex 50×8), filtration, and evaporation to dryness gave a residue, which was eluted from a silica-gel column with $CHCl_3$–MeOH (8 : 1). Concentration of the major fraction gave the de-*O*-benzoylated trisaccharide **12** as a colorless syrup (30 mg, 88% yield), which was subjected to de-*O*-benzylation without further purification. **12**: MS (FAB) m/z: 1122 $(M+Na)^+$.

A solution of **12** (29 mg, 26.4 μmol) in MeOH–H_2O (4 : 1 v/v, 30 ml) with AcOH (1 ml) was hydrogenated in the presence of 10% Pd on carbon (70 mg) under an atmosphere of H_2 (3.10×10^5 Pa) for 2 d. The mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* to give a syrup, which was purified by elution from a silica-gel column with $CHCl_3$–MeOH (1 : 2). The major fraction was concentrated to afford **3** (13 mg, 93% yield) as a colorless syrup. The α/β-anomeric ratio of the reducing end was estimated as 2 : 1 by ^{1}H-NMR. $[\alpha]_D^{25}$ +39.8° (c=0.60, H_2O). [lit. $[\alpha]_D^{20}$ +49° (c=1.0, MeOH),[8] +31° (c=0.75, H_2O)[9]]. ^{1}H-NMR (300 MHz, D_2O) δ: 1.28 (2H, d, H-6-α), 1.30 (1H, d, H-6-β), 2.06 (3H, s, $COCH_3$), 3.40—3.47 (5/3H, m, H-4-β, 5-β, 5″), 3.52 (5/3H, dd, H-4-α, 4″), 3.60 (1H, dd, H-2′), 4.02 (1H, m, H-5′), 4.10 (2/3H, dd, H-2-α), 4.12 (1/3H, dd, H-2-β), 4.54 (1H, dd, H-2″), 4.85 (1/3H, d, H-1-β), 4.88 (1H, d, H-1″), 5.05 (2/3H, d, H-1′-α), 5.08 (1/3H, d, H-1′-β), 5.14 (2/3H, d, H-1-α), 3.63—3.95 (other protons); $J_{1,2}$=2.0 (α), $J_{1,2}$=1.0 (β), $J_{2,3}$=3.0 (α), $J_{2,3}$=3.0 (β), $J_{3,4}$=$J_{4,5}$=9.5 (α), $J_{5,6}$=6.2 (α), $J_{5,6}$=5.0 (β), $J_{1',2'}$=4.0, $J_{2',3'}$=10.0, $J_{1'',2''}$=1.2, $J_{2'',3''}$=4.3, $J_{3'',4''}$=$J_{4'',5''}$=9.5 Hz. ^{13}C-NMR (75 MHz, D_2O) δ: 19.49 (C-6-β), 19.52 (C-6-α), 24.62 ($COCH_3$), 55.95 (C-2″), 62.34 (C-6′), 63.03 (C-6″), 69.27 (C-4″), 70.19 (C-2-α), 70.53 (C-2-β), 71.00 (C-5-α), 72.76 (C-5-β, 5′), 73.05 (C-4-α), 73.79 (C-2′), 74.13 (C-3′), 74.58 (C-4-β, 3″), 78.30 (C-3-α), 79.13 (C-5″), 80.41 (C-3-β), 81.14 (C-4′), 96.05 (C-1-β), 96.22 (C-1-α), 97.83 (C-1′-β), 98.14 (C-1′-α), 101.93 (C-1″), 178.06 ($NHCO$). MS (FAB) m/z: 552 $(M+Na)^+$.

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