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

Eisuke KAJI,* Yumiko OSA, Mutsumi TANAIKE, Yugo HOSOKAWA, Hiroaki TAKAYANAGI, and Atsushi TAKADA

School of Pharmaceutical Sciences, Kitasato University, Shirokane 5–9–1, Minato-ku, Tokyo 108, Japan.

Received August 23, 1995; accepted September 19, 1995

A chemical synthesis has been achieved for β -D-ManNAc-(1 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 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 trimethylsilyl ethyl 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 $\rightarrow$ 1-OH $\rightarrow$ 1-F), gave a key disaccharide donor, β -D-ManNAc-(1 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 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 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 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 bromide⁸⁾ 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 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 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 CH₂Cl₂ (r.t., 3 h), affording the β (1 $\rightarrow$ 4) disaccharide **6** in 86% yield. The β/α selectivity was estimated to be >20/1 by ¹H-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 $\rightarrow$ 4) disaccharide **6** was then subjected to a *manno*-selective hydroboration, as in ref. 7, with a twelve-fold molar excess of borane-tetrahydrofuran (BH₃·THF) complex in THF to afford, on *N*-acetylation, the expected *N*-acetyl- β -D-mannosaminyl-(1 $\rightarrow$ 4)-D-glucoside **7** in 73% yield. The β -D-*manno*-configuration of the

Type 19A

Type 19F

Fig. 1

* To whom correspondence should be addressed.

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 $\text{AgClO}_4\text{-SnCl}_2$ 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** $\rightarrow$ **12**, 88% yield, 0.05 M NaOMe/MeOH) and hydrogenolysis (**12** $\rightarrow$ **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

Fig. 2

Chart 1

$\alpha/\beta = 2/1$ ($^1\text{H-NMR}$). Its ^1H - and ^{13}C -NMR spectra were identical with the reported data.⁸⁾

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 (^1H - and ^{13}C -NMR). TLC was done on Merck Silica gel 60 F₂₅₄ 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-glucopyranoside¹⁰⁾ **5** (551 mg, 1.0 mmol), molecular sieves (4 Å, 2.0 g), and silver aluminosilicate¹⁴⁾ (0.63 g, 2.0 mmol) in dry CH_2Cl_2 (20 ml) was stirred in the dark under a N_2 atmosphere at room temperature for 15 min. 2-(Benzoyloxyimino)-2-deoxyglycosyl bromide¹¹⁾ **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 $\times$ 70 ml). Drying (Na_2SO_4), evaporation to dryness, elution from a silica-gel column with $\text{MeC}_6\text{H}_5\text{-AcOEt}$ (12:1), and concentration of the major fraction furnished **6** (982 mg, 86%) as a colorless syrup. $[\alpha]_D^{25} + 7.4^\circ$ ($c = 1.0$, CHCl_3). $^1\text{H-NMR}$ (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 $\times$ 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. $^{13}\text{C-NMR}$ (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 ($4 \times \text{COPh}$). MS (FAB) m/z : 1165 ($\text{M} + \text{Na}$)⁺.

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 $\text{BH}_3\cdot\text{THF}$ complex in THF (5.76 ml) was added dropwise

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 . $[\alpha]_{\text{D}}^{25} -19.6^{\circ}$ ($c=0.5$, CHCl_3). $^1\text{H-NMR}$ (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); $J_{1,2}=7.7$, $J_{2,3}=9.0$, $J_{3,4}=J_{4,5}=9.5$, $J_{1',2'}=1.5$, $J_{2',3'}=4.0$, $J_{2',\text{NH}}=8.8$, $J_{3',4'}=J_{4',5'}=9.8$, $J_{5',6'a}=5.3$, $J_{5',6'b}=3.5$, $J_{6'a,6'b}=12.0\text{ Hz}$. $^{13}\text{C-NMR}$ (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 ($2\times\text{CH}_2\text{Ph}$), 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 ($3\times\text{COPh}$), 170.32 (NHCO). MS (FAB) m/z : 1088 ($\text{M}+\text{Na}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{61}\text{H}_{65}\text{NO}_{14}\text{Si}$: 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 ($\alpha:\beta=2:1$) ($^1\text{H-NMR}$). mp 85 – 89°C . $[\alpha]_{\text{D}}^{25} -12.4^{\circ}$ ($c=0.5$, CHCl_3). [lit.⁷⁾ mp 87 – 90°C , $[\alpha]_{\text{D}}^{23} -14.1^{\circ}$ ($c=0.33$, CHCl_3)]. Its $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 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 ($3\times 50\text{ ml}$), 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%, $\alpha:\beta=ca. 1:3$) ($^1\text{H-NMR}$). 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** : $[\alpha]_{\text{D}}^{23} -15.5^{\circ}$ ($c=1.0$, CHCl_3). $^1\text{H-NMR}$ (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); $J_{1,2}=2.5$, $J_{1,\text{F}}=52.5$, $J_{2,3}=J_{3,4}=J_{4,5}=9.5$, $J_{5,6a}=2.5$, $J_{1',2'}=1.3$, $J_{2',3'}=4.0$, $J_{2',\text{NH}}=8.8$, $J_{3',4'}=J_{4',5'}=10.0$, $J_{5',6'a}=5.0$, $J_{5',6'b}=3.5$, $J_{6'a,6'b}=12.0\text{ Hz}$. $^{13}\text{C-NMR}$ (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); $J_{\text{C1,F}}=227.7$, $J_{\text{C2,F}}=24.7\text{ Hz}$. MS (FAB) m/z : 990 ($\text{M}+\text{Na}$) $^+$.

9 β : $[\alpha]_{\text{D}}^{24} -7.0^{\circ}$ ($c=1.25$, CHCl_3). [lit.⁷⁾ $[\alpha]_{\text{D}}^{25} -7.7^{\circ}$ ($c=0.51$, CHCl_3)]. Its $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 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 $\rightarrow$ 4)-*O*-(2,3,6-tri-*O*-benzyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 3)-2,4-di-*O*-benzyl- α -L-rhamnopyranoside (11**) and Its β -D-Glucopyranosyl Isomer (**11 β**)** A solution of methoxybenzyl rhamnoside **10**⁽⁶⁾ (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 R_f 0.40. $[\alpha]_{\text{D}}^{23} -3.6^{\circ}$ ($c=0.20$, CHCl_3). $^1\text{H-NMR}$ (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); $J_{1,2}=1.5$, $J_{2,3}=3.0$, $J_{3,4}=9.0$, $J_{4,5}=9.3$, $J_{5,6}=6.0$, $J_{1',2'}=3.5$, $J_{2',3'}=J_{3',4'}=9.5$, $J_{1'',2''}=1.0$, $J_{2'',3''}=3.8$, $J_{3'',4''}=J_{4'',5''}=10.0$, $J_{5'',6''a}=5.2$, $J_{5'',6''b}=3.5$, $J_{6''a,6''b}=12.0\text{ Hz}$. $^{13}\text{C-NMR}$ (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 ($\text{M}+\text{Na}$) $^+$.

11 β : TLC R_f 0.30. $[\alpha]_{\text{D}}^{23} -19.3^{\circ}$ ($c=0.85$, CHCl_3). $^1\text{H-NMR}$ (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); $J_{1,2}=1.5$, $J_{2,3}=3.0$, $J_{3,4}=9.0$, $J_{4,5}=9.5$, $J_{5,6}=6.0$, $J_{1',2'}=7.5$, $J_{2',3'}=8.8$, $J_{3',4'}=9.0$, $J_{4',5'}=9.0$, $J_{1'',2''}=1.3$, $J_{2'',3''}=3.7$, $J_{3'',4''}=9.7$, $J_{4'',5''}=9.5$, $J_{5'',6''a}=5.0$, $J_{5'',6''b}=3.5$, $J_{6''a,6''b}=12.0\text{ Hz}$. $^{13}\text{C-NMR}$ (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 ($\text{M}+\text{Na}$) $^+$.

***O*-(2-Acetamido-2-deoxy- β -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*- α -D-glucopyranosyl-(1 $\rightarrow$ 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 ($\text{M}+\text{Na}$) $^+$.

A solution of **12** (29 mg, 26.4 μmol) in $\text{MeOH-H}_2\text{O}$ (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 ($3.10\times 10^5\text{ Pa}$) 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 $^1\text{H-NMR}$. $[\alpha]_{\text{D}}^{25} +39.8^{\circ}$ ($c=0.60$, H_2O). [lit. $[\alpha]_{\text{D}}^{20} +49^{\circ}$ ($c=1.0$, MeOH),⁸⁾ $+31^{\circ}$ ($c=0.75$, H_2O)⁹⁾]. $^1\text{H-NMR}$ (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), $J_{1',2'}=4.0$, $J_{2',3'}=10.0$, $J_{1'',2''}=1.2$, $J_{2'',3''}=4.3$, $J_{3'',4''}=J_{4'',5''}=9.5\text{ Hz}$. $^{13}\text{C-NMR}$ (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 ($\text{M}+\text{Na}$) $^+$.

References

- 1) Appelbaum P. C., *Clin. Infect. Dis.*, **15**, 77—83 (1992).
- 2) Munoz R., Musser J. M., Crain M., Briles D. E., Marton A., Parkinson A. J., Sorensen U., Tomasz A., *Clin. Infect. Dis.*, **15**, 112—118 (1992).
- 3) Ubukata K., *Rinsho To Biseibutsu (Clinical Microbiology)*, **22**, 137—144 (1995).
- 4) Katzenellenbogen E., Jennings H. J., *Carbohydr. Res.*, **124**, 235—245 (1983).
- 5) Jennings H. J., Rosell K.-G., Carlo D. J., *Can. J. Chem.*, **58**, 1069—1074 (1980); Ohno N., Yadomae T., Miyazaki T., *Carbohydr. Res.*, **80**, 297—304 (1980).
- 6) van Dam J. E. G., Fleer A., Snippe H., *Antonie van Leeuwenhoek*, **58**, 1—47 (1990).
- 7) Kaji E., Lichtenthaler F. W., Osa Y., Takahashi K., Matsui E., Zen S., *Chem. Lett.*, **1992**, 707—710; Kaji E., Lichtenthaler F. W., Osa Y., Takahashi K., Zen S., *Bull. Chem. Soc. Jpn.*, **68**, 2401—2408 (1995).
- 8) Paulsen H., Helpap B., Lorentzen J. P., *Carbohydr. Res.*, **179**, 173—197 (1988).
- 9) Panza L., Ronchetti F., Toma L., *Carbohydr. Res.*, **180**, 242—245 (1988).
- 10) Jansson K., Ahlfors S., Frejd T., Kihlberg J., Magnusson G., *J. Org. Chem.*, **53**, 5629—5647 (1988).
- 11) Lichtenthaler F. W., Jarglis P., Hempe W., *Liebigs Ann. Chem.*, **1983**, 1959—1972.
- 12) Kaji E., Lichtenthaler F. W., *Trends Glycosci. Glycotechnol.*, **5**, 121—142 (1993).
- 13) Kaji E., Anabuki N., Zen S., *Chem. Pharm. Bull.*, **43**, 1441—1447 (1995).
- 14) van Boeckel C. A. A., Beetz T., van Aelst S. F., *Tetrahedron*, **40**, 4097—4107 (1984).
- 15) Kaji E., Lichtenthaler F. W., Nishino T., Yamane A., Zen S., *Bull. Chem. Soc. Jpn.*, **61**, 1291—1297 (1988).
- 16) Slaghek T. M., van Vliet M. J., Maas A. A. M., Kamerling J. P., Vliegthart J. F. G., *Carbohydr. Res.*, **195**, 75—86 (1989).