Tetrahedron Vol. 48, No. 22, pp. 4649-4658, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00

Synthesis of a Cell Wall Component of *Haemophilus (Actinobacillus) Pleuropneumoniae* Serotype 5

P.A.M. van der Klein, W. Filemon, G.J.P.H. Boons, G.H. Veeneman,
G.A. van der Marel and J.H. van Boom

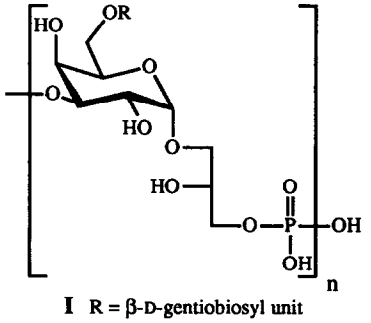
Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands

(*Received in UK* 13 *April* 1992)

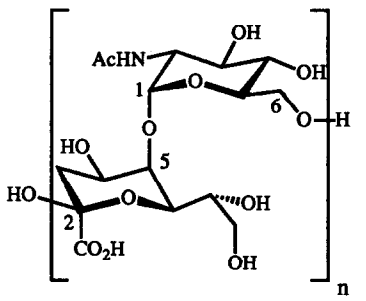
Abstract: The disaccharide α-D-GlcNAc*p*-(1-5)-β-KDO-2-$OCH_2CH_2CH_2NH_2$ containing a spacer β-linked to KDO is prepared *via* iodonium ion-assisted glycosylation of a suitable KDO acceptor with ethyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-1-thio-α/β-D-glucopyranoside. The key KDO building unit is synthesized in 7 steps starting with 2,3:5,6-di-*O*-isopropylidene-D-mannitol.

Introduction

The Gram-negative bacteria *Haemophilus (Actinobacillus) pleuropneumoniae* (HPP) is the causative agent of major respiratory disease in swine throughout the world[1,2]. It has also been established that the antigenicity of HPP serotypes is closely related to the capsular polysaccharides (CPS). Thus far, the structure of CPS and lipopolysaccharide components of several HPP serotypes have been elucidated[3].



I R = β-D-gentiobiosyl unit



II

Figure 1. Repeating unit of the CPS of *H. pleuropneumoniae* serotypes 2 (*i.e.* **I**) and 5 (*i.e.* **II**)

In order to study in detail the immunological properties of HPP serotypes, we recently embarked on a program to synthesize well defined CPS fragments of HPP serotypes. For example, fragments of the CPS

of HPP serotype 2 (see structure I) have been successfully prepared in this laboratory[4,5].

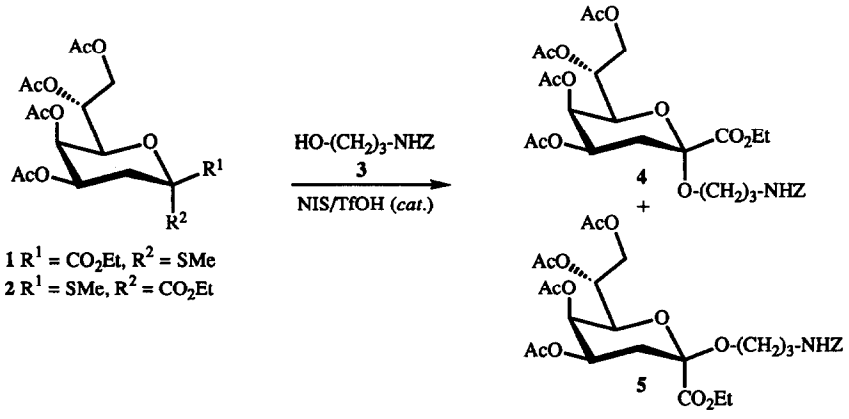
In 1987 Perry *et al.*[6] showed that the structure of the CPS of HPP serotype 5 (ATCC 33377) was a high-molecular-mass unbranched polymer of repeating disaccharide units comprising, as illustrated in structure II, 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc*p*) and 3-deoxy-D-*manno*-2-octulosonic acid (KDO) which are joined *via* α(1-5) and β(2-6) linkages.

We report here the assembly of the HPP serotype 5 fragment **23** [*i.e.* α-D-GlcNAc*p*-(1-5)-β-KDO-2-$OCH_2CH_2CH_2NH_2$] comprising one repeating unit β-*O*-linked to a spacer suitable for conjugation with macromolecular carriers.

Results and discussion

One of the key steps in the synthesis of the target dimer **23** is the stereoselective introduction of the β-linkage between the hydroxyl group of the spacer and KDO. Earlier studies[7] showed that the stereochemical outcome of the glycosylation of the spacer 3-(benzyloxycarbonylamino)-1-propanol[8] (**3**) by a fully acetylated methyl 2-thio-KDO donor, in the presence of *N*-iodosuccinimide (NIS) and catalytic trifluoromethanesulfonic acid (TfOH), strongly depended on the anomeric configuration of the methylthio

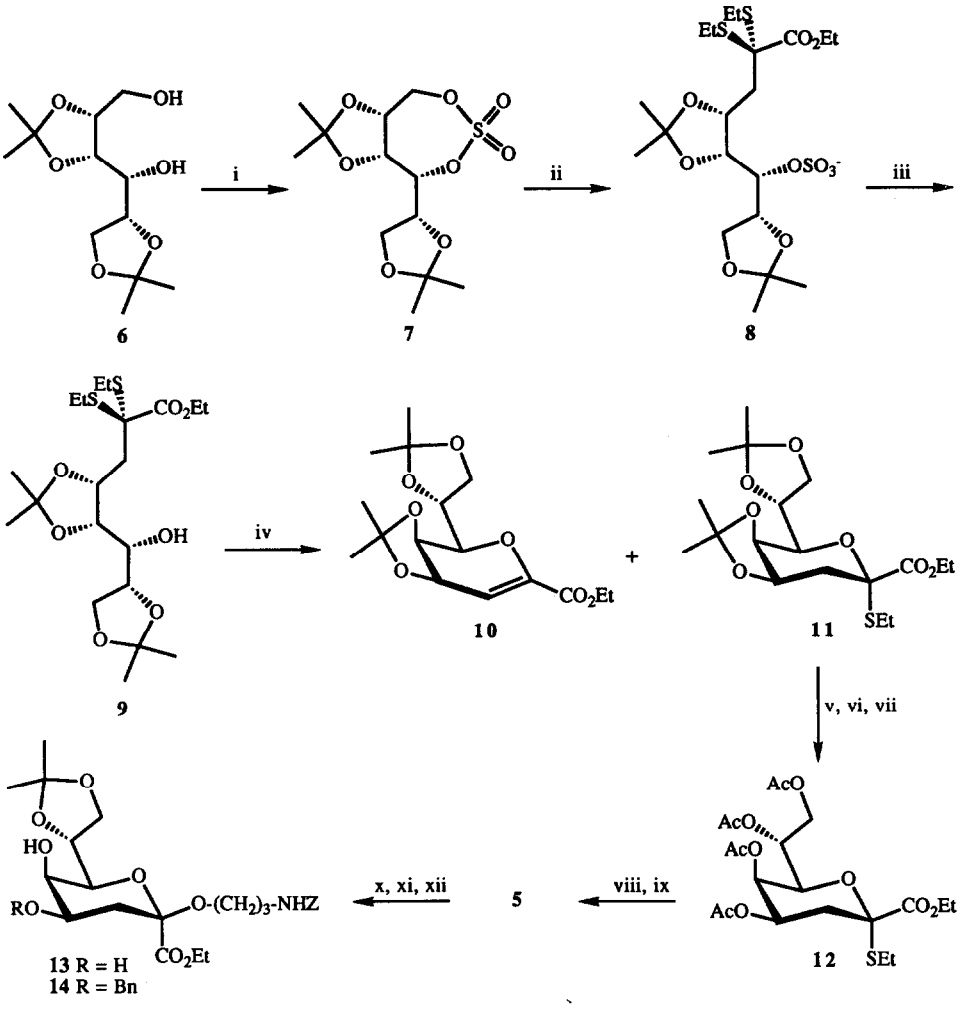
Scheme 1



1 $R^1 = CO_2Et$, $R^2 = SMe$
2 $R^1 = SMe$, $R^2 = CO_2Et$

group. Thus, glycosylation of **3** with the α-KDO donor **1** (see Scheme 1) gave an anomeric mixture of the glycosides **4** and **5**. However, condensation of the β-KDO donor **2** with **3** afforded exclusively the β-glycoside **5**. Unfortunately, the synthesis of the β-KDO donor **2**[7] is rather laborious and time-consuming. We therefore adapted a previously reported route[9] (see Scheme 2) to attain the requisite β-glycoside **5**. Thus, 2,3:5,6-di-*O*-isopropylidene-D-mannitol (**6**), obtained[10] in high yield by reduction of 2,3:5,6-di-*O*-isopropylidene-D-mannose[11] with sodium borohydride, was reacted with thionyl chloride and the resulting cyclic sulfite was subsequently oxidized[12] with sodium periodate and catalytic ruthenium(III) chloride to afford cyclic sulfate **7**[13] in a good overall yield over the two steps. Treatment of **7** with the anion of ethyl 2,2-*bis*(ethylthio)acetate in the presence of hexamethylphosphoramide in tetrahydrofuran resulted in the ring opening of the cyclic sulfate in **7** giving 6-sulfate derivative **8**. Mild acidic hydrolysis of the sulfate group in **8** furnished, after chromatography, homogeneous **9** in an overall yield of 75% (based on **7**). Cyclization of dithioketal derivative **9** with NIS gave an inseparable mixture of the minor glycal **10** and the major ethyl 2-α-thio-KDO **11**. Acidic hydrolysis of **10** and **11** followed by acetylation afforded, after purification, homogeneous **12** in 54% yield over the three chemical steps. Condensation of **12** with **3** was now executed

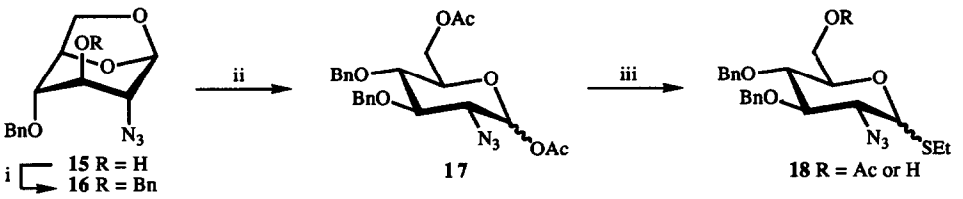
Scheme 2 [a]



[a]**Key**: (i) $SOCl_2$, C_5H_5N, EtOAc, then $RuCl_3$, $NaIO_4$, CH_2Cl_2/ CH_3CN/ H_2O [2:2:3] (85%); (ii) $(EtS)_2CHCO_2Et$, *n*-BuLi, THF, HMPA ; (iii) H_2SO_4,H_2O (75% based on **7**); (iv) NIS, $ClCH_2CH_2Cl$, 0°C; (v) HOAc/ H_2O [4:1], 50°C; (vi) Ac_2O, C_5H_5N; (vii) column chromatography (54% based on **9**); (viii) Br_2, $ClCH_2CH_2Cl$; (ix) **3**, Ag silicate aluminate (76% based on **12**); (x) KOEt, EtOH; (xi) $H_2C{=}C(OMe)CH_3$, *p*-TsOH, DMF, 0°C (64% based on **5**); (xii) Bu_2SnO, toluene, Δ, then BnBr, CsF, DMF (82%).

as follows. Treatment of donor **12** with bromine gave the expected[14] α-glycosyl bromide, which was coupled *in situ* with **3** in the presence of the insoluble catalyst silver silicate aluminate[14,15], to furnish homogeneous **5** in 76% yield (*cf.*, the fully benzoylated donor gave the corresponding β-glycoside in 60% yield[9]). Fully acetylated **5** was now transformed into the KDO acceptor **14** by a three-step protecting-group-manipulation. Thus, deacetylation followed by kinetically controlled acetonation with 2-methoxypropene[16] gave, after

Scheme 3 [a]

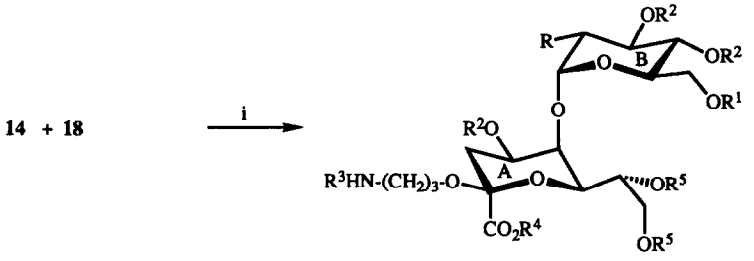


[a]**Key**: (i) BnBr, NaH, DMF (quant.); (ii) Ac_2O/TFA (95%); (iii) $BF_3{\cdot}OEt_2$, EtSH, $ClCH_2CH_2Cl$ (**18** [R = Ac], 71%).

regioselective benzylation[17,18] of **13**, homogeneous **14** in 54% overall yield (based on **5**).

On the basis of the observation that iodonium di-*sym*-collidine perchlorate (IDCP)-assisted glycosidations of ethyl 1-thio glycosides having a non-participating group at C-2 resulted in the predominant formation of 1,2-*cis* glycosides[19], we selected ethyl 2-azido-2-deoxy-1-thio-α/β-D-glucopyranoside **18** as donor for the introduction of the α(1-5) linkage in the target molecule. The preparation of donor **18** is outlined in Scheme 3 and commences with the benzylation of the 1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-D-glucopyranose (**15**), which was easily prepared by ring opening of the corresponding 2,3-cyclic sulfate[13,20] with lithium azide followed by acidic hydrolysis of the generated 3-sulfate group. Acetolysis of **16**[21] and treatment of **17** (α/β mixture) with ethanethiol under the agency of boron trifluoride etherate gave the thioglycoside **18** (R = Ac, anomeric mixture) in 67% overall yield.

Scheme 4 [a]



ii, iii, iv, v
19 R = N_3; R^1 = Ac; R^2 = Bn; R^3 = Z; R^4 = Et; R^5, R^5 = $C(Me)_2$
20 R = NHAc; R^1 = Ac; R^2 = Bn; R^3 = Z; R^4 = Et; R^5, R^5 = $C(Me)_2$
21 R = NHAc; R^1 = Ac; R^2 = Bn; R^3 = Z; R^4 = Et; R^5 = H
22 R = NHAc; R^1 = R^4 = R^5 = H; R^2 = Bn; R^3 = Z
23 R = NHAc; R^1 = R^2 = R^3 = R^4 = R^5 = H

[a]**Key**: (i) NIS/TfOH, $ClCH_2CH_2Cl/Et_2O$ [1:4] (70% based on **14**); (ii) H_2S, C_5H_5N/ Et_3N/ H_2O[3:1:1], then Ac_2O, C_5H_5N (90%); (iii) HOAc/H_2O [4:1], 50°C; (iv) 0.2 N NaOH/ dioxane/ MeOH/ H_2O [14:5:1]; (v) Pd/C, H_2, 2-propanol/H_2O/HOAc [10:5:2] (60% based on **20**).

At this stage, donor **18** (R = Ac) was coupled with acceptor **14** in the presence of the mild thiophilic promoter IDCP. In contrast with the expectation, no trace of glycosidation product **19** could be detected. The failure to glycosylate **14** with **18** (R = Ac) may be ascribed to the low reactivity of the axially orientated hydroxyl group in the glycosyl acceptor **14**. It was, however, established that glycosylation of the primary hydroxyl group in **3** with the same donor was also abortive. It may therefore be concluded that the nucleophilicity of the sulfur atom at C-1 will be decreased by the neighbouring electron-withdrawing azido group, thus making donor **18** less prone to activation by the mild thiophilic promotor IDCP. Interestingly, it was observed that the deacetylated derivative **18** (R = H) was not completely inert toward activation with IDCP. In this particular case, intramolecular cyclization of **18** (R = H) into the 1,6-anhydro derivative **16** (65% yield) occurred. On the other hand, it was to be expected that glycosylation of acceptor **14** with donor **18** (R = Ac) using the more thiophilic promotor NIS/TfOH (*cat.*)[22] proceeded smoothly. Indeed, TLC analysis of the reaction mixture, after 10 min at 0°C, revealed the presence of one glycosidation product. Workup and purification gave, as gauged by NMR spectroscopy and TLC-analysis, the homogeneous α(1-5) linked disaccharide **19** in an acceptable yield. Moreover, no trace of the isomeric β(1-5) product could be detected, indicating that the glycosylation is also a highly stereoselective process. Fully protected dimer **19** was now converted into the target molecule **23** by consecutively executing (see Scheme 4) the following four-step deblocking procedure. Reduction of the azide function[23,24] and acetylation of the free amino group gave, after purification, the *N*-acetamido derivative **20**. Surprisingly, ^{13}C NMR

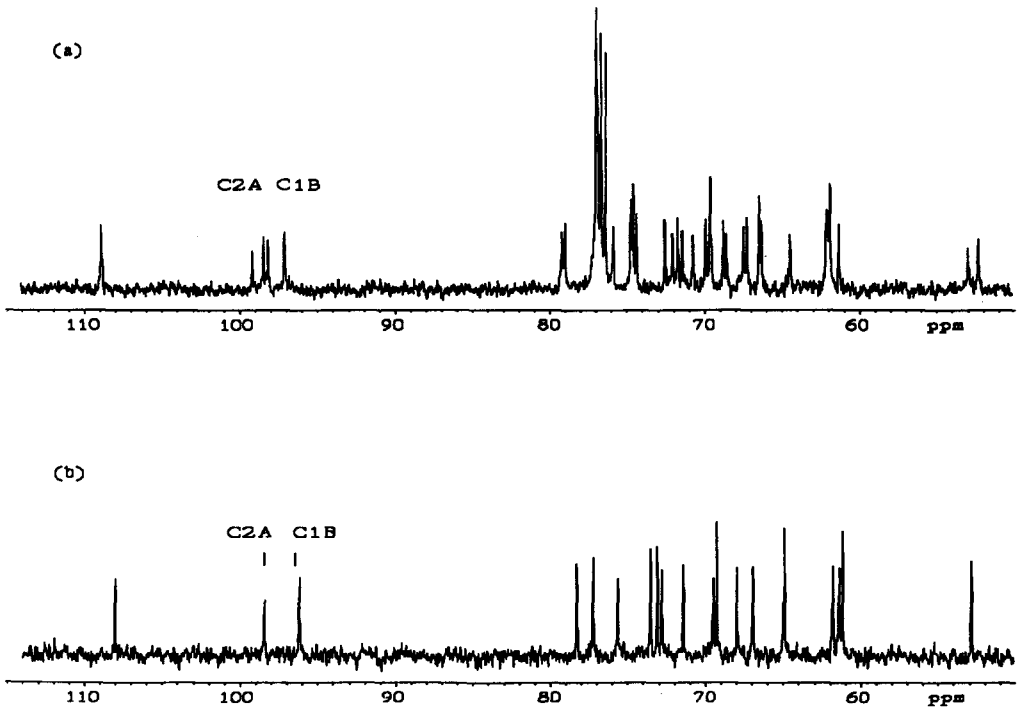


Figure 2. ^{13}C NMR spectra of dimer **20** in (a) $CDCl_3$ and (b) in $(CD_3)_2SO$, measured at 300 K.

spectroscopy of **20** in the solvent chloroform-d showed *inter alia* (see Figure 2) four resonances, instead of the expected two for the two anomeric carbon atoms. Fortunately, two distinct resonances were observed for C-2A and C-1B (see Figure 2) by recording the spectrum in the solvent dimethyl sulfoxide-d6. These results indicate that dimer **20** may exist as two distinct conformers in the non-polar solvent $CDCl_3$. Deacetonation (**20** → **21**), deesterification (**21** → **22**) and hydrogenolysis of the benzyl (Bn) and benzyloxycarbonyl (Z) groups in **22** gave, after purification, homogeneous dimer **23**, the ^1H- and ^{13}C- data of which were in good accord with those reported for the CPS of HPP serotype 5[6].

In conclusion, the highly stereoselective synthetic route reported in this paper gives access to a valuable spacer containing repeating unit of CPS of HPP serotype 5. In addition, extension of the fully protected dimers **19** and **20** may be easily accomplished after selective deesterification of the acetyl group (R^1) at C-6B of the glucopyranosyl unit.

Experimental

General methods and materials - Pyridine was dried by refluxing with CaH_2 (5 g/L) and then distilled. Dichloromethane, 1,2-dichloroethane and toluene were distilled from P_2O_5. *N,N*-dimethylformamide was stirred with CaH_2 at room temperature and distilled under reduced pressure. Ether was distilled from $LiAlH_4$. Pyridine and *N,N*-dimethylformamide were stored over molecular sieves 4Å (Aldrich), toluene and ether over sodium wire and dichloromethane and 1,2-dichloroethane were stored over alumina. Ethyl 2,2-*bis*(ethylthio)acetate was purchased from Aldrich. Reactions were performed at ambient temperature, unless noted otherwise. Column chromatography was performed on columns of silica gel 60 (Merck 230-400 mesh). TLC was conducted on DC Fertigfolien (Schleicher & Schüll F1500 LS254). Compounds were detected by charring with 20% sulfuric acid in MeOH. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter, for solutions in $CHCl_3$ unless stated otherwise. ^{13}C NMR spectra were recorded at 50.1 MHz with a Jeol JNM-FX200 spectrometer. ^1H NMR spectra were recorded on a Bruker WM-300 spectrometer equipped with an ASPECT 2000 computer. Chemical shifts are given in ppm (δ) relative to TMS as internal standard.

2,3:5,6-Di-*O*-isopropylidene-D-mannitol 1,4-sulfate (7). - To a solution of 2,3:5,6-di-*O*-isopropylidene-D-mannitol (**6**, 2.62 g, 10 mmol) and thionyl chloride (0.78 mL, 10.5 mmol) in EtOAc (50 mL) was a solution of pyridine (1.7 mL, 21 mmol) in EtOAc (10 mL). The mixture was stirred and the temperature was kept below 20°C. When TLC-analysis showed complete conversion of the starting material into cyclic sulfite, the mixture was diluted with EtOAc (100 mL), washed with water (20 mL), dried (Na_2SO_4), and concentrated. To a solution of the resulting oil in CH_2Cl_2 (20 mL) and acetonitrile (20 mL) was added water (30 mL), sodium periodate (4.28 g, 2 equiv) and ruthenium chloride (12 mg) and the mixture was stirred vigorously for 1 h at room temperature. CH_2Cl_2 (100 mL) was added and the layers were separated. The organic layer was washed with brine (25 mL), dried ($MgSO_4$) and concentrated. The residue was filtered through a pad of silica gel (97:3 CH_2Cl_2-acetone) to afford **7** (2.75 g, 85%), $[\alpha]_D^{20}$ -33.9° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.36, 1.39, 1.44, 1.52 ($C(CH_3)_2$), 4.04 (dd, 1 H, H-6, $J_{5,6}$ = 4.3 Hz, $J_{6,6}$ = 9.2 Hz), 4.14 (dd, 1 H, H-6', $J_{5,6'}$ = 6.1 Hz), 4.33-4.42 (m, 3 H, H-1, H-1', H-5), 4.45-4.50 (m, 1 H, H-2), 4.57-4.61 (m, 2 H, H-3, H-4). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 24.7, 24.8, 26.5, 26.8 ($C(CH_3)_2$), 66.2 (C-6), 68.0 (C-1), 72.9 (C-5), 73.2 (C-2), 73.8 (C-3), 79.4 (C-4), 109.6, 110.2 ($C(CH_3)_2$).

Anal. Calc. for $C_{12}H_{20}O_8S$: C, 44.44; H, 6.21. Found: C, 44.38; H, 6.18.

Ethyl 3-deoxy-4,5:7,8-di-*O*-isopropylidene-D-*manno*-2-octulonate diethyl dithioketal (9). - Ethyl 2,2-*bis*(ethylthio)acetate (271 mg, 1.3 mmol) was dissolved in dry tetrahydrofuran (2.6 mL) and hexamethylphosphoramide (0.8 mL). The temperature was lowered to -70°C and *n*-butyllithium (0.81 mL, 1.6 M) was added. After stirring for 1.5 h at -40°C, cyclic sulfate **7** (324 mg, 1 mmol in tetrahydrofuran) was added. The mixture was allowed to warm to room temperature and stirred until TLC-analysis (CH_2Cl_2-acetone 97:3), after 16 h showed complete conversion of **7**. Now sulfuric acid (50 μL) and water (18 μL) were added and

stirring was continued for 2 h at 50°C. The mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate (2 x 5 mL) and water (5 mL), dried ($MgSO_4$) and concentrated. The resulting oil was chromatographed on silica gel [1:1 light petroleum (b.p. 40-60°C)-ether] to give **9** (339 mg, 75%), $[\alpha]_D^{20}$ -67.2° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.21-1.50 (m, 18 H, SCH_2CH_3, $C(CH_3)_2$), 2.17-2.26 (m, 2 H, H-3, 6-OH), 2.51-2.81 (m, 5 H, SCH_2CH_3, H-3′), 3.53 (m, 1 H, H-6), 3.73 (s, 3 H, OCH_3), 3.98-4.15 (m, 3 H, H-7, H-8, H-8′), 4.31 (dd, 1 H, H-5, $J_{4,5}$ = 6.9 Hz, $J_{5,6}$ = 1.8 Hz), 4.63 (m, 1 H, H-4). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.3 (SCH_2CH_3), 23.5 (SCH_2CH_3), 24.5, 26.2, 26.5, 26.7 ($C(CH_3)_2$), 37.0 (C-3), 52.7 (OCH_3), 64.3 (C-2), 67.0 (C-8), 70.8, 73.8, 76.0, 76.2 (C-4,5,6,7), 107.7, 109.2 ($C(CH_3)_2$), 170.9 (C-1).

Ethyl (ethyl 3-deoxy-4,5:7,8-di-*O*-isopropylidene-2-thio-α-D-*manno*-octulopyranosid)onate (11). - To a solution of **9** (452 mg, 1 mmol) and molecular sieves 4Å (1 g) in 1,2-dichloroethane (10 mL) was added NIS (225 mg). After stirring for 20 min at 0°C, TLC analysis (CH_2Cl_2-acetone 97:3) showed complete conversion of **9**. The mixture was filtered, diluted with CH_2Cl_2 (30 mL), washed with M $Na_2S_2O_3$ (5 mL) and 0.9M $NaHCO_3$ (5 mL), dried ($MgSO_4$) and concentrated. Column chromatography (97:3 CH_2Cl_2-acetone) of the residue gave **11** [300 mg, 72% together with 5% of glycal **10**]. ^1H NMR ($CDCl_3$) δ 1.19 (t, 3 H, SCH_2CH_3), 1.30-1.44 (m, 15 H, OCH_2CH_3, $C(CH_3)_2$), 1.82 (dd, 1 H, H-3a, $J_{3a,3e}$ = 15.3 Hz, $J_{3a,4}$ = 2.4 Hz), 2.61 (m, 2 H, SCH_2CH_3), 3.00 (dd, 1 H, H-3e, $J_{3e,4}$ = 3.7 Hz), 3.61 (dd, 1 H, H-6, $J_{5,6}$ = 1.8 Hz, $J_{6,7}$ = 8.2 Hz), 3.93 (dd, 1 H, H-8, $J_{7,8}$ = 4.4 Hz, $J_{8,8'}$ = 8.6 Hz), 4.14 (dd, 1 H, H-8′, $J_{7,8'}$ = 6.3 Hz), 4.25 (m, 2 H, OCH_2CH_3), 4.32 (dd, 1 H, H-5, $J_{4,5}$ = 7.7 Hz), 4.35 (ddd, 1 H, H-7), 4.51 (ddd, 1 H, H-4). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.8 (OCH_2CH_3), 14.3 (SCH_2CH_3), 22.5 (SCH_2CH_3), 25.1, 25.5, 26.9 ($C(CH_3)_2$), 32.6 (C-3), 61.3 (OCH_2CH_3), 67.2 (C-8), 70.4, 71.7, 72.4, 73.3 (C-4,5,6,7), 83.5 (C-2), 109.4, 109.8 ($C(CH_3)_2$), 170.3 (C-1).

Ethyl (ethyl 4,5:7,8-tetra-*O*-acetyl-3-deoxy-2-thio-α-D-*manno*-octulopyranosid)onate (12). - A solution of compound **11** (416 mg, 1 mmol) in 4:1 acetic acid-H_2O (10 mL) was stirred for 6 h at 50°C, then concentrated and toluene (3 x 20 mL) was evaporated from the residue. A solution of the residue in pyridine (10 mL) and acetic anhydride (5 mL) was stirred for 8 h and then concentrated. The resulting residue was dissolved in toluene (20 mL) and evaporated to dryness. The residue was redissolved in CH_2Cl_2 (25 mL), washed with 0.9M $NaHCO_3$ (2 x 5 mL), dried ($MgSO_4$), and concentrated. Column chromatography (97:3 CH_2Cl_2-acetone) of the crude mixture yielded **12** (359 mg, 75%). $[\alpha]_D^{20}$ +158.0° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.21-1.39 (2xt, 6 H, OCH_2CH_3 and SCH_2CH_3), 1.98-2.75 (m, 16 H, CH_3COO, H-3a, H-3e, SCH_2CH_3), 4.11 (dd, 1 H, H-8, $J_{7,8}$ = 3.6 Hz, $J_{8,8'}$ = 12.3 Hz), 4.28 (m, 2 H, OCH_2CH_3 4.46 (d, 1 H, H-6, $J_{6,7}$ = 9.8 Hz), 4.62 (dd, 1 H, H-8′, $J_{7,8'}$ = 2.5 Hz), 5.23 (m, 1 H, H-7), 5.31-5.40 (m, 2 H, H-4,5). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.6, 13.8 (SCH_2CH_3 and OCH_2CH_3), 20.3, 20.5 (CH_3COO), 22.1 (SCH_2CH_3), 31.4 (C-3), 61.6, 61.7 (C-8, OCH_2CH_3), 64.0, 66.7, 67.3, 68.0 (C-4,5,6,7), 84.4 (C-2), 167.6 (C-1), 169.3, 169.4, 170.0, 170.1 (CH_3COO).

Anal. Calc. for $C_{20}H_{30}O_{11}S$: C, 50.20; H, 6.32. Found: C, 50.28; H, 6.35.

Ethyl (*N*-benzyloxycarbonyl-3-aminopropyl 4,5:7,8-tetra-*O*-acetyl-3-deoxy-β-D-*manno*-2-octulopyranosid)onate (5). - To a cooled (0°C) solution of compound **12** (957 mg, 2 mmol) in 1,2-dichloroethane (15 mL) was added Br_2 (1.3 eq, 0.13 mL). After 10 min at 0°C the mixture was concentrated. The resulting bromide derivative was added to a mixture of **3** (420 mg, 2 mmol), molecular sieves 4Å (1 g) and Ag silicate aluminate (2 g) in 1,2-dichloroethane (15 mL) at -40°C. The mixture was slowly warmed up to 20°C, then filtered and concentrated. Column chromatography (97:3 CH_2Cl_2-acetone) of the crude mixture gave **5** (951 mg, 76%), $[\alpha]_D^{20}$ +28.3° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.30 (t, 3 H, OCH_2CH_3), 1.78 (m, 2 H, $CH_2CH_2CH_2$), 1.98-2.13 (m, 13 H, H-3a, CH_3COO), 2.35 (dd, 1 H, H-3e, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.7 Hz), 3.30 (m, 2 H, CH_2N), 3.43 (m, 1 H, $OCHHCH_2$), 3.85 (m, 1 H, $OCHHCH_2$), 4.21 (dd, 1 H, H-6, $J_{5,6}$ = 1.3 Hz, $J_{6,7}$ = 9.6 Hz), 4.25 (q, 2 H, OCH_2CH_3), 4.35 (m, 2 H, H-8,8′), 4.90 (m, 1 H, H-4), 5.09 (m, 2 H, CH_2Ph), 5.17 (m, 1 H, H-7), 5.28 (m, 1 H, H-5), 7.23-7.38 (m, 5 $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.0 (OCH_2CH_3), 20.6 (CH_3COO), 29.1 ($CH_2CH_2CH_2$), 32.1 (C-3), 38.4 (CH_2N), 62.1, 62.2, 62.4 (C-8, OCH_2CH_3, OCH_2CH_2), 63.8, 66.9, 67.9, 70.6 (C-4,5,6,7), 66.4 (CH_2Ph), 99.1 (C-2), 127.8-128.3 ($CH_{arom.}$), 156.3 (NHCOO), 167.5 (C-1), 169.6, 169.8,

170.3, 170.6 (CH_3COO).

Anal. Calc. for $C_{29}H_{39}NO_{14}$: C, 55.68; H, 6.28. Found: C, 55.73; H, 6.32.

Ethyl (*N*-benzyloxycarbonyl-3-aminopropyl 3-deoxy-7,8-*O*-isopropylidene-β-D-*manno*-2-octulopyranosid)onate (13). - Compound **5** (940 mg, 1.5 mmol) was dissolved in dry ethanol (20 mL) and potassium *tert*-butoxide (50 mg) was added. After stirring for 3 h, the reaction mixture was neutralized with Dowex W50 (H^+-form), filtered and concentrated. DMF was evaporated (2x10 mL) from the residue and the remaining oil was dissolved in DMF (10 mL). The mixture was cooled (0°C) and 2-methoxypropene (151 µL, 1.05 eq) and a catalytic amount of *p*-toluenesulfonic acid were added. After stirring for 0.5 h, an extra amount of 2-methoxypropene (75 µL) was added and stirring was continued for 1 h. The solution was neutralized (Et_3N) and concentrated. The residue was chromatographed on silica gel (97:3 CH_2Cl_2-MeOH) to give **13** (R = H, 478 mg, 64%), $[\alpha]_D^{20}$ +19.8° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.25-1.38 (m, 9 H, OCH_2CH_3, $C(CH_3)_2$), 1.74 (m, 2 H, $CH_2CH_2CH_2$), 1.90 (t, 1 H, H-3a, $J_{3a,3e} \approx J_{3a,4} \approx 12.5$ Hz), 2.40 (dd, 1 H, H-3e, $J_{3e,4} = 4.6$ Hz), 3.24 (m, 2 H, CH_2NH), 3.38-4.37 (m, 10 H, H-4,5,6,7,8,8', OCH_2CH_2, OCH_2CH_3), 5.08 (s, 2 H, CH_2Ph), 5.22 (t, 1 H, NH), 7.31-7.38 (m, 5 $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.9 (OCH_2CH_3), 24.9, 26.6 ($C(CH_3)_2$), 28.9 ($CH_2CH_2CH_2$), 34.4 (C-3), 37.9 (CH_2N), 61.2, 61.6 (OCH_2CH_2, OCH_2CH_3), 65.9, 66.9, 73.0, 75.5 (C-4,5,6,7), 66.4 (CH_2Ph), 67.0 (C-8), 99.0 (C-2), 109.2 ($C(CH_3)_2$), 127.8-128.1 ($CH_{arom.}$), 136.3 ($C_{arom.}$), 156.3 (NHCOO), 168.3 (C-1).

Ethyl (*N*-benzyloxycarbonyl-3-aminopropyl 4-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-β-D-*manno*-2-octulopyranosid)onate (14). - A mixture of compound **13** (R = H, 450 mg, 0.9 mmol), dibutyltin oxide (270 mg, 1.1 mmol) and molecular sieves 4Å (150 mg) in toluene was heated under reflux. After 2 h, the mixture was filtered and the filtrate was concentrated. The residue was dissolved in DMF (8 mL) and benzyl bromide (0.13 mL, 1.1 mmol) and CsF (164 mg, 1.1 mmol) were added. After stirring for 16 h, the reaction mixture was concentrated. The residue was dissolved in CH_2Cl_2 (30 mL), washed with water (5 mL), dried ($MgSO_4$) and concentrated. Column chromatography (97:3 CH_2Cl_2-acetone) of the crude product afforded **14** (R = Bn, 435 mg, 82%), $[\alpha]_D^{20}$ +25.2° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.18-1.48 (m, 9 H, OCH_2CH_3, $C(CH_3)_2$), 1.77 (m, 2 H, $CH_2CH_2CH_2$), 2.03 (t, 1 H, H-3a, $J_{3a,3e} \approx J_{3a,4} \approx 12.5$ Hz), 2.44 (dd, 1 H, H-3e, $J_{3e,4} = 4.6$ Hz), 3.18-4.43 (m, 14 H, OCH_2CH_3, $OCH_2CH_2CH_2$, H-4,5,6,7,8,8'), 4.61, 5.09 (2xm, 4 H, CH_2Ph), 5.30 (t, 1 H, NH) 7.21-7.42 (m, 10 $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.8 (OCH_2CH_3), 24.9, 26.6 ($C(CH_3)_2$), 28.9 ($CH_2CH_2CH_2$), 32.1 (C-3), 38.1 (CH_2N), 61.4, 61.6 (OCH_2CH_2, OCH_2CH_3), 63.5, 72.9, 73.6, 75.5 (C-4,5,6,7), 66.3, 67.1, 69.9 (CH_2Ph, C-8), 99.1 (C-2), 109.2 ($C(CH_3)_2$), 127.5-128.3 ($CH_{arom.}$), 137.2 ($C_{arom.}$), 156.2 (NHCOO), 168.2 (C-1).

Anal. Calc. for $C_{31}H_{41}NO_{10}$: C, 63.36; H, 7.03. Found: C, 63.29; H, 7.06.

1,6-Anhydro-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranose (16). - To a solution of **15** (1.1 g, 3.6 mmol) in DMF (15 mL) was added NaH (1 g, 4.2 mmol) and benzyl bromide (0.5 mL, 4.2 mmol). After stirring for 1 h, excess NaH was destroyed with methanol and the reaction mixture concentrated. The residue was redissolved in CH_2Cl_2 (100 mL), extracted with water, dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel [1:1 light petroleum (b.p. 40-60°)-ether] to give **16** (1.32 g, quant.), $[\alpha]_D^{20}$ +34.0° (*c* 1). ^1H NMR ($CDCl_3$) δ 3.26 (bs, 1 H, H-2), 3.37 (bs, 1 H, H-4), 3.66 (bs, 1 H, H-3), 3.69 (dd, 1 H, H-6, $J_{5,6}$ = 6,1 Hz, $J_{6,6'}$ = 7.3 Hz), 3.99 (dd, 1 H, H-6', $J_{5,6'} < 1.0$ Hz), 4.45-4.61 (m, 5 H, CH_2Ph, H-5), 5.47 (bs, 1 H, H-1), 7.30-7.33 (m, 5 $H_{arom.}$).
$^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 59.5 (C-2), 65.0 (C-6), 71.0, 72.0 (CH_2Ph), 74.0, 75.6, 75.9 (C-3,4,5), 100.2 (C-1), 127.3-128.2 ($CH_{arom.}$), 137.0, 137.2 ($C_{arom.}$).

1,6-Di-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranose (17). - Compound **16** (1.3 g, 3.5 mmol) was dissolved in acetic anhydride (10 mL) and trifluoroacetic acid (1 mL) and stirred for 4 h at 50°C. The mixture was concentrated and toluene (5x30 mL) was evaporated from the remaining residue. Purification of the residue with column chromatography on silica gel with 97:3 CH_2Cl_2-acetone afforded **17** (1.56 g, 95%). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 20.6, 20.7 (CH_3COO), 62.3 (C-6), 62.6 (C-2α), 65.0 (C-2β), 71.3, 77.2, 80.5 (C-

3,4,5α), 73.7, 76.8, 83.0 (C-3,4,5β), 90.3 (C-1α), 92.5 (C-1β), 128.0-129.0 ($CH_{arom.}$), 137.4, 137.6 ($C_{arom.}$), 168.5, 170.3 (CH_3COO).

Ethyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-α/β-D-glucopyranoside (18). -To a solution of compound **17** (1.5 g, 3.2 mmol) in dichloroethane (15 mL) and ethanethiol (0.26 mL, 3.5 mmol) was added $BF_3{\cdot}OEt_2$ (0.79 mL, 6.4 mmol). After stirring for 1 h at 35°C the reaction mixture was dissolved in CH_2Cl_2 (50 mL), washed with 0.9M $NaHCO_3$ (15 mL) and water (15 mL). The organic layer was dried ($MgSO_4$) and concentrated to give, after column chromatography on silica gel (97:3 CH_2Cl_2-acetone) **18** [0.95 g, 71% based on recovered **16** (130 mg)]. $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.7 (α-SCH_2CH_3), 15.0 (β-SCH_2CH_3), 20.7 (CH_3COO), 24.5 (α-SCH_2CH_3), 24.7 (β-SCH_2CH_3), 62.7 (C-6α), 62.9 (C-6β), 63.8 (C-2α), 66.1 (C-2β), 69.4, 77.9, 81.7 (C-3,4,5α), 74.9, 75.6, 75.7 (CH_2Ph), 77.0, 77.3, 84.3 (C-3,4,5β), 83.1 (C-1α), 85.0 (C-1β), 127.9-128.5 ($CH_{arom.}$), 137.3, 137.4, 137.5 ($C_{arom.}$), 170.4 (CH_3COO).

Ethyl (*N*-benzyloxycarbonyl-3-aminopropyl 5-*O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-4-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-β-D-*manno*-2-octulopyranosid)onate (19). - To a solution of **14** (R = H, 120 mg, 0.2 mmol) and **18** (115 mg, 0.24 mmol) in 1:4 1,2-dichloroethane-ether (3 mL), powdered molecular sieves (4 Å, 100 mg) were added, and the mixture was stirred for 10 min at 0°C. A solution of *N*-iodosuccinimide (54 mg, 0.24 mmol) and triflic acid (2.6 μL, 0.03 mmol) in 1:1 1,2-dichloroethane-ether (3 mL) was added, the mixture was stirred for 15 min, then filtered, diluted with CH_2Cl_2 (30 mL), washed with M $Na_2S_2O_3$ (5 mL) and 0.9M $NaHCO_3$ (5 mL), dried ($MgSO_4$), and concentrated. Column chromatography (94:6 CH_2Cl_2-acetone) of the residue gave **19** (140 mg, 70%), $[\alpha]_D^{20}$ +60.2° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.20 (t, 3 H, OCH_2CH_3), 1.26, 1.34 (2xs, 6 H, $C(CH_3)_2$), 1.72 (m, 2 H, $CH_2CH_2CH_2$), 1.97 (s, 3 H, CH_3COO), 2.08 (t, 1 H, H-3Aa, $J_{3a,3e} \approx J_{3a,4} \approx$ 12.3 Hz), 2.49 (dd, 1 H, H-3Ae, $J_{3e,4}$ = 3.8 Hz), 3.22 (m, 2 H, CH_2N), 3.31-3.43 (m, 3 H, H-2B,4A,6A), 3.52 (m, 1 H, $OCHHCH_2$), 3.59 (dd, H-4B, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 10.2 Hz), 3.78-3.86 (m, 2 H, H-6B, $OCHHCH_2$), 3.92 (dd, 1 H, H-6B′, $J_{6,6'}$ = 12.5 Hz, $J_{5,6'}$ = 2.4 Hz), 3.96-4.42 (m, 5 H, H-8A, H-8A′, H-3B, OCH_2CH_3), 4.38 (m, 1 H, H-7A, $J_{6,7}$ = 10.2 Hz), 4.45 (m, 1 H, H-5B), 4.47-5.12 (m, 8 H, CH_2Ph), 5.07 (m, 2 H, H-5A, H-1B, $J_{1,2}$ = 4.6 Hz), 7.15-7.42 (m, 20 $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.0 (OCH_2CH_3), 20.7 (CH_3COO), 24.9, 26.8 ($C(CH_3)_2$), 28.9 ($CH_2CH_2CH_2$), 32.1 (C-3), 37.8 (CH_2N), 60.9, 61.7, 62.2 (OCH_2CH_2, OCH_2CH_3, C-6B), 64.0 (C-2B), 66.5, 67.7, 70.2, 74.9, 75.2 (C-8, CH_2Ph), 68.7, 71.1, 72.0, 73.3, 76.5, 77.7, 79.9 (C-3B,4B,5B,4A,5A,6A,7A), 97.8 (C-1B), 99.1 (C-2A), 109.3 ($C(CH_3)_2$), 127.7-128.9 ($CH_{arom.}$), 137.2, 137.5, 137.7 ($C_{arom.}$), 156.3 (NHCOO), 168.3 (C-1A), 170.3 (CH_3COO).

Ethyl (*N*-benzyloxycarbonyl-3-aminopropyl 5-*O*-(2-acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-4-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-β-D-*manno*-2-octulopyranosid)onate (20). - Compound **19** (100 mg, 0.1 mmol) was dissolved in a mixture of 3:1:1 pyridine-water-Et_3N (5 mL) and H_2S was led through the solution for 2 h. After stirring for 1 h, the mixture was concentrated and the residue was evaporated twice from pyridine (5 mL) and dissolved in pyridine (2 mL) and acetic anhydride (2 mL). After standing for 4 h, the mixture was concentrated and the residue was dissolved in CH_2Cl_2 (20 mL), washed with water (5 mL), dried ($MgSO_4$) and concentrated. Purification of the residue with column chromatography on silica gel with 97:3 CH_2Cl_2-MeOH) yielded **20** (91 mg, 90%), $[\alpha]_D^{20}$ +97.3° (*c* 1). ^1H NMR (DMSO-d6) δ 1.15 (t, 3 H, OCH_2CH_3), 1.24, 1.31 (2xs, 6 H, $C(CH_3)_2$), 1.62 (m, 2 H, $CH_2CH_2CH_2$), 1.84 (s, 3 H, CH_3COO), 1.96 (s, 3 H, CH_3CON), 2.02 (t, 1 H, H-3Aa, $J_{3a,3e} \approx J_{3a,4} \approx$ 12.4 Hz), 2.34 (dd, 1 H, H-3Ae, $J_{3e,4}$ = 4.0 Hz), 3.03 (m, 2 H, CH_2N), 3.34-3.46 (m, 3 H, $OCHHCH_2$, H-4A, H-6A), 3.49 (dd, 1 H, H-4B, $J_{3,4}$ = 8.9 Hz, $J_{4,5}$ = 9.9 Hz), 3.62-3.67 (m, 1 H, $OCHHCH_2$), 3.81 (dd, 1 H, H-3B, $J_{2,3}$ = 10.7 Hz), 3.86-3.97 (m, 5 H, H-5A,8A,2B,6B,6B′), 4.04-4.16 (m, 4 H, H-7A,8A′, OCH_2CH_3), 4.22 (dt, 1 H, H-5B), 4.46-4.97 (m, 8 H, CH_2Ph), 5.01 (d, 1 H, H-1B), 7.04-7.41 (m, 20 $H_{arom.}$). $^{13}C\{^1H\}$ NMR (DMSO-d6) δ 13.3 (OCH_2CH_3), 19.9 (CH_3COO), 21.9 (CH_3CON), 24.6, 26.1 ($C(CH_3)_2$), 29.4 ($CH_2CH_2CH_2$), 31.9 (C-3A), 37.4 (CH_2N), 52.5 (C-2B), 60.8, 61.1, 61.9, 64.8, 66.8, 69.4, 72.8, 73.2 (OCH_2CH_2, OCH_2CH_3, C-6B,8A, CH_2Ph), 68.1, 69.6, 71.5, 73.3, 75.8, 77.4, 78.5 (C-3B,4B,5B,4A,5A, 6A,7A), 96.6 (C-1B), 98.9 (C-2A), 108.5 ($C(CH_3)_2$), 126.8-127.6 ($CH_{arom.}$), 137.6,

137.8, 138.3 ($C_{arom.}$), 156.2 (NHCOO), 167.4 (C-1A), 168.5, 169.3 (CH_3COO).

Sodium (aminopropyl 5-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-3-deoxy-β-D-*manno*-2-octulopyranosid)onate (23). - Compound **20** (81 mg, 0.08 mmol) was dissolved in 4:1 acetic acid-water (3 mL) and stirred for 4 h. The mixture was concentrated and toluene (3 x 5 mL) was evaporated from the residue. Remaining compound **21** was dissolved in a 0.2 N NaOH in a 14:5:1 dioxane/MeOH/H_2O mixture (2 mL) and left for 3 h at room temperature. The reaction mixture was neutralized with Dowex W50 (H^+-form), filtered and concentrated. A solution of residue **22** in 10:5:2 2-propanol-water-acetic acid (5 mL) was hydrogenated in the presence of 10% Pd/C for 24 h, then filtered and concentrated. The residue was purified by hiload Sephadex S100 HR 26/60 column chromatography (eluent, 2 M TEAB). The appropriate fraction were collected, concentrated and converted into the Na^+ salt by passing through a cation-exchanger column (Dowex W50) with water to give **23** (24 mg, 60%). $[\alpha]_D^{20}$ +17.3° (*c* 1, H_2O). ^1H NMR (D_2O) δ 1.82 (t, 1 H, H-3Aa, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz), 1.88 (m, 2 H, $CH_2CH_2CH_2$), 2.03 (s, 3 H, CH_3CON), 2.37 (dd, 1 H, H-3Ae, $J_{3e,4}$ = 4.4 Hz), 3.01-3.05 (m, 2 H, CH_2N), 3.45 (m, 1 H, $OCHHCH_2$), 3.55 (dd, 1 H, H-4B), 3.68-3.86 (m, 11 H, $OCHHCH_2$, H-3B,6B,6B',4A,7A,8A,8A'), 3.93-3.98 (m, 2 H, H-2B,5A), 4.16 (m, 1 H, H-5B), 4.99 (d, 1 H, H-1B, $J_{1,2}$ = 3.6 Hz). $^{13}C\{^1H\}$ NMR (D_2O) δ 22.6 (CH_3CON), 28.2 ($CH_2CH_2CH_2$), 36.2 (C-3A), 38.5 (CH_2NH_2), 54.6 (C-2B), 60.6 (C-6B), 63.4 (OCH_2CH_2), 64.7 (C-8), 67.8 (C-4A), 69.4 (C-7), 70.3 (C-4B), 71.6 (C-3B), 72.5 (C-5B), 74.6 (C-5A), 74.7 (C-6A), 99.3 (C-1B), 102.2 (C-2A), 174.3 (C-1A), 174.9 (CH_3CON).

References

1. Jensen, A.E.; Bertram, T.A. *Infect. Immun.* **1986**, *51*, 419.
2. Hani, H.; Koning, H.; Nicolet, J. *Schweiz. Arch. Tierheilkd.* **1973**, *115*, 191.
3. Beynon, L.M.; Richards, J.C.; Perry, M.B. *Carbohydr. Res.* **1991**, *220*, 185 and references cited therein.
4. Veeneman, G.H.; Brugghe, H.F.; Hoogerhout, P.; Van der Marel, G.A.; Van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1988**, *107*, 610.
5. Veeneman, G.H.; Brugghe, H.F.; Van den Elst, H.; Van Boom, J.H. *Carbohydr. Res.* **1990**, *195*, C1.
6. Altman, E.; Brisson, J.-R.; Perry, M.B. *Eur. J. Biochem.* **1987**, *170*, 185.
7. Boons, G.J.P.H.; Van Delft, F.L.; Van der Klein, P.A.M.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron* **1992**, *48*, 885.
8. Berntsson, P.; Brändström, A.; Junggren, H.; Palme, L.; Sjöstrand, S.E.; Sundell, G. *Acta. Pharm. Suec.* **1977**, *14*, 229.
9. Van der Klein, P.A.M.; Boons, G.J.P.H.; Veeneman, G.H.; Van der Marel, G.A.; Van Boom, J.H. *Synlett*, **1990**, 311.
10. Austin, P.W.; Hardy, F.E.; Buchanan, J.G.; Baddiley, J. *J. Chem. Soc.* **1964**, 2128.
11. Kartha, K.P.R. *Tetrahedron Lett.* **1986**, *27*, 3415.
12. Gao, Y.; Sharpless, K.B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.
13. Van der Klein, P.A.M.; Boons, G.J.P.H.; Veeneman, G.H.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron Lett.* **1989**, *30*, 5477.
14. Van Boeckel, C.A.A.; Beetz, T.; Kock-Van Dalen, A.; Van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 596.
15. Paulsen, H.; Hayauchi, Y.; Unger, F.M. *Liebigs Ann. Chem.* **1984**, 1270.
16. Paulsen, H.; Stiem, M.; Unger, F.M. *Liebigs Ann. Chem.* **1987**, 273.
17. David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.
18. Nagashima, N.; Ohno, M. *Chem. Lett.* **1987**, 141.
19. Veeneman, G.H.; Van Boom, J.H. *Tetrahedron Lett.* **1990**, *31*, 275.
20. Van der Klein, P.A.M.; Filemon, W.; Veeneman, G.H.; Van der Marel, G.A.; Van Boom, J.H. *J. Carbohydr. Chem.*, submitted for publication.
21. Paulsen, H.; Schüller, M. *Liebigs Ann. Chem.* **1987**, 249.
22. Veeneman, G.H.; Van Leeuwen, S.H.; Van Boom, J.H. *Tetrahedron Lett.* **1990**, *31*, 1331.
23. Adachi, T.; Yamada, Y.; Inoue, I.; Saneyoshi, M. *Synthesis* **1977**, 45.
24. Garegg, P.J.; Haraldsson, M.; Lönn, H.; Norberg, T. *Glycoconjugate. J.* **1987**, *4*, 231.