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SYNTHESIS OF 2-ACETAMIDO-6-*O*-(5-ACETAMIDO-3,5-DIDEOXY-β-D-*glycero*-D-*galacto*-2-NONULOPYRANOSYLONIC ACID)-2-DEOXY-D-GLUCOSE [2-ACETAMIDO-6-*O*-(*N*-ACETYL-β-D-NEURAMINYL)-2-DEOXY-D-GLUCOSE]

DOMINICUS J. M. VAN DER VLEUGEL, JAN W. ZWIKKER, JOHANNES F. G. VLIEGENTHART*
Department of Bio-Organic Chemistry, State University of Utrecht, Utrecht (The Netherlands)
STAN A. A. VAN BOECKEL, AND JACQUES H. VAN BOOM
Gorlaeus Laboratories, State University of Leiden, Leiden (The Netherlands)

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ABSTRACT

Silver triflate-promoted condensation of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**9**) with benzyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside, followed by removal of the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group and subsequent acetylation, afforded a 4:1 mixture of benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-α-D-glucopyranoside (**13**) and the (2→6)-α-linked isomer. After selective crystallisation, **13** was obtained in 46% yield based on the precursor of **9**, methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**8**). An additional product of the condensation reaction was methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate, which was obtained in 40% yield based on **8**. *O*-Deacetylation of **13** and subsequent saponification gave benzyl 2-acetamido-2-deoxy-6-*O*-(potassium 5-acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-α-D-glucopyranoside (**16**). Catalytic hydrogenolysis of **16** yielded the title product as its potassium salt.

INTRODUCTION

As part of our programme on the synthesis of carbohydrate chains carrying sialic acid, we demonstrated the utility of silver salicylate in the synthesis of a series of alkyl α-glycosides of *N*-acetyl-D-neuraminic acid (Neu5Ac) methyl ester[1] and of the disaccharide 6-*O*-(*N*-acetyl-α-D-neuraminyl)-D-galactose[2]. We now report on the silver trifluoromethanesulphonate (silver triflate)-promoted synthesis of 2-acetamido-6-*O*-(*N*-acetyl-β-D-neuraminyl)-2-deoxy-D-glucose (**17**).

*To whom correspondence should be addressed.

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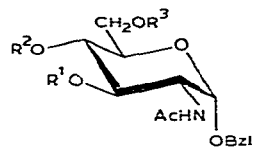
RESULTS AND DISCUSSION

For the synthesis of 2-acetamido-6-*O*-(*N*-acetyl-D-neuraminyl)-2-deoxy-D-glucose from methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate[3] (**9**), several aglycons were selected, namely, benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**2**), benzyl 2-acetamido-2-deoxy-3,4-di-*O*-(4-phenylbenzyl)-α-D-glucopyranoside (**3**), and benzyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (**6**). Compound **2**, as well as **3** (see ref. 4), turned out to be sparingly soluble in solvents commonly used in glycosylation reactions, thereby making them unsuitable for the aimed disaccharide synthesis. Compound **6**, being much more soluble, was used as the aglycon.

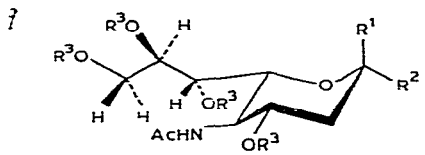
Compound **6** was synthesised along lines similar to those reported for the synthesis of methyl 3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside from methyl α-D-glucopyranoside[5]. Treatment of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[6] (**1**) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine afforded benzyl 2-acetamido-2-deoxy-4,6-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (**4**) in 55% yield. Compound **4** could not be tritylated under the usual conditions, but acetylation gave the 3-acetate **5**. The position of the *O*-acetyl group in **5** was determined by ^{1}H-n.m.r. spectroscopy. The resonance signal of H-3, assigned by selective proton–proton decoupling, appeared as a double doublet at δ 5.25.

Compound **4** was isomerised (83%) into benzyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (**6**) by using mesitylenesulphonic acid in *N*,*N*-dimethylformamide. This was shown by the relative intensities of the two NH doublets in the region δ 5.3–6.3 of the ^{1}H-n.m.r. spectrum of the



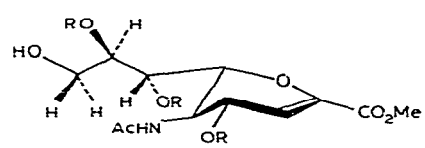
1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = Bzl$; $R^3 = H$
3 $R^1 = R^2 = 4\text{-PhBzl}$; $R^3 = H$
4 $R^1 = H$; R^2, $R^3 = TIPDSi$
5 $R^1 = Ac$; R^2, $R^3 = TIPDSi$
6 R^1, $R^2 = TIPDSi$, $R^3 = H$
7 R^1, $R^2 = TIPDSi$; $R^3 = Ac$

TIPDSi = $-Si(CHMe_2)_2OSi(CHMe_2)_2-$
4-PhBzl = 4-phenylbenzyl



8 R^1, $R^2 = OAc, CO_2Me$; $R^3 = Ac$
9 $R^1 = Cl$; $R^2 = CO_2Me$; $R^3 = Ac$
10 R^1, $R^2 = CO_2Me$, Sal; $R^3 = Ac$

Sal = salicyloyl



11 R = Ac
12 R = H

reaction mixture after removal of *N,N*-dimethylformamide and mesitylenesulphonic acid. Selective crystallisation afforded **6** in 53% yield. Tritylation of **6** occurred smoothly, indicating HO-6 to be unsubstituted. The ^{1}H-n.m.r. spectrum of the *O*-acetylated derivative (**7**) of **6** showed the presence of one *O*-acetyl group. The absence of a double doublet for H-3 or H-4 in the region δ 5.0–5.5 proved this group to be located at O-6.

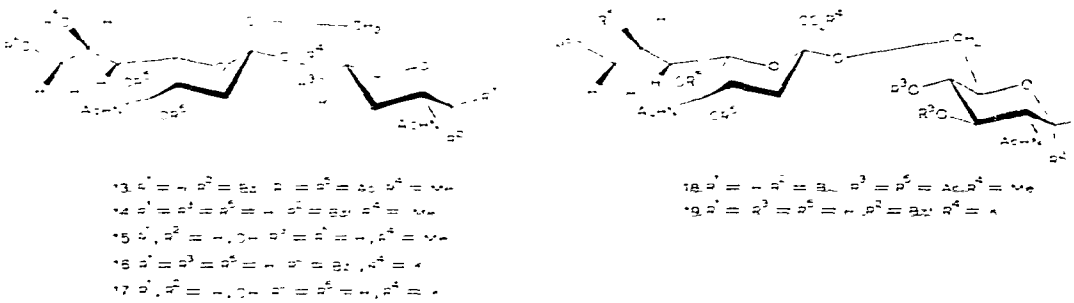
Silver salicylate-promoted condensation of **9** with **6**, under conditions similar to those applied to the synthesis of 6-*O*-(*N*-acetyl-α-D-neuraminyl)-D-galactose[2], afforded almost exclusively methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-salicyloyl-D-*glycero*-D-*galacto*-2-nonulopyranosonate[2] (**10**), accompanied by traces of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate[2] (**11**). Probably, the bulky tetraisopropyldisiloxane-1,3-diyl group of **6** lowers the rate of the silver ion-assisted nucleophilic substitution reaction of **9** and **6**, in favour of the competing reaction of **9** and silver salicylate[7]. No improvement could be obtained by using silver 2,6-dihydroxybenzoate, which has a less nucleophilic carboxylate group[8]. Alternatively, silver triflate was used as promoter of the condensation reaction.

Silver triflate-promoted glycosylations starting from glycosyl halides having participating groups vicinal to the anomeric centre have been successfully applied in stereoselective 1,2-*trans*-glycoside syntheses[9–16]. Even aglycons with relatively inaccessible hydroxyl groups have been glycosylated in good yield[10–13]. Using glycosyl halides (or preformed glycosyl triflates) with non-participating groups, the stereochemical outcome of the reaction cannot be predicted, because it depends on the reaction conditions, the structure of the glycosyl derivative, and the aglycon involved[17–21]. The use of silver triflate in the synthesis of sialodisaccharides gave anomeric mixtures, but no details have been presented[22].

The condensation of **9** with **6** was performed by adding a solution of silver triflate and 2,4,6-trimethylpyridine in 1:1 nitromethane–ethyl ether to a solution of the reactants in benzene, and yielded three products. It should be noted that the proton acceptor has no essential influence on the course of the reaction, since its replacement by 1,1,3,3-tetramethylurea or omission gave similar results. Furthermore, the absence of the nitromethane–ethyl ether mixture hardly affected the outcome of the reaction. Excess of **6** was removed by short-column chromatography. Subsequently, the mixture was treated with tetrabutylammonium fluoride, to split off the tetraisopropyldisiloxane-1,3-diyl group. One of the main components remained unaffected and was isolated by short-column chromatography, to afford **11** in 40% yield based on the precursor of **9**, methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate[3] (**8**). For ^{1}H-n.m.r. spectral data of **11**, see ref. 2. *O*-Deacetylation of **11** yielded methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate[23] (**12**).

The remaining mixture was *O*-acetylated. 360-MHz, ^{1}H-N.m.r. spectroscopy demonstrated the occurrence of the fully blocked sialodisaccharides **13** and **18** in the ratio 4:1, as deduced from the relative intensities of the H-3*eq* double-doublets

at δ 2.465 and 2.601, respectively. Selective crystallisation afforded **13** (46% yield based on **8**). The 360-MHz, ^{1}H-n.m.r. spectrum of **13** could be interpreted almost completely. Positions 3 and 4 of the 2-acetamido-2-deoxy-D-glucopyranoside (Glc*p*NAc) moiety of **13** are acetylated, as shown by the position of the double doublets of H-3 and H-4 at δ 5.239 and 5.304, respectively, confirming the presence of the (2→6) linkage in **13**. The β configuration of the interglycosidic linkage of **13** could not be deduced easily at this stage due to the lack of ^{1}H-n.m.r. reference data for chloroform-*d* solutions, but was proved after deprotection (see below). Compared to previous observations for per-*O*-acetylated, α-linked Neu5Ac derivatives in chloroform-*d* solution[1,2,24], the resonance position of H-9 (δ 5.021) in the ^{1}H-n.m.r. spectrum of this per-*O*-acetylated, β-linked Neu5Ac derivative was shifted 0.6–0.7 p.p.m. downfield. Furthermore, the value (2.5 Hz) of $J_{7,8}$ is much smaller, and the value (9.1 Hz) of $J_{8,9'}$ is larger, than the corresponding values (8–10 Hz and 6–7 Hz, respectively) reported for β-Neu5Ac[25], the methyl ester of a β-linked sialodisaccharide[2], and a number of unblocked or per-*O*-acetylated, α-linked derivatives of Neu5Ac methyl ester[1,2,24,26]. Similar effects have been observed for the methyl esters of the per-*O*-acetylated methyl β-glycoside and 6-(5-cholesten-3β-yloxy)hexyl β-thioglycoside of Neu5Ac in chloroform-*d*[24]. Probably, the influence of *O*-acetylation on the time-averaged conformation of the glycerol side-chain of the Neu5Ac moiety depends on the anomeric configuration, because it is only observed for β-linked Neu5Ac derivatives.



O-Deacetylation of **13**, followed by saponification of the methyl ester, afforded the potassium salt **16** in 88% yield. This compound was not cleavable by *Clostridium perfringens* neuraminidase. The presence of a β-linked Neu5Ac moiety was indicated by 360-MHz, ^{1}H-n.m.r. spectroscopy (deuterium oxide), which showed the Neu5Ac H-3*eq* and H-4 signals at δ 2.416 and 4.082, respectively (ranges for β-linked Neu5Ac derivatives[27]: H-3*eq*, δ 2.1–2.5; H-4, δ 3.9–4.2). In contrast to **13**, the observed values of $J_{7,8}$ (9.2 Hz) and $J_{8,9'}$ (6.5 Hz) have the usual magnitude. After *O*-deacetylation of **13**, some of the methyl ester **14** was converted into the per-*O*-trimethylsilyl derivative of **15** after catalytic hydrogenolysis. Mass-spectral data for per-*O*-trimethylsilylated **15** are summarised in Table I and are in full agreement with the anticipated structure. The intense peak at *m/z* 726, which is the analogue of the peak at *m/z*

TABLE I

INTERPRETATION OF SOME IMPORTANT FRAGMENT-IONS PRESENT IN THE MASS SPECTRUM OF THE Me_3Si DERIVATIVE OF THE METHYL ESTER OF 2-ACETAMIDO-6-*O*-(*N*-ACETYL-β-D-NEURAMINYL)-2-DEOXY-D-GLUCOSE (**15**)

m/z	*Fragment*[a]	*Intensity*[b]
1030	M	2.7
1015	M − CH_3	40.6
971	M − $COOCH_3$	29.9
940	M − Me_3SiOH	46.9
825	M − $CH(OSiMe_3)CH_2OSiMe_3$	93.8
726	Neu5Ac-$OCH_2CH{=}OSiMe_3$	32.9
624	Neu5Ac-$OSiMe_3$ − $COOCH_3$	4.5
594	[Neu5Ac]	30.1
504	594 − Me_3SiOH	45.5
420	[GlcNAc]	10.2
330	420 − Me_3SiOH	7.6
300	$AcNH{=}CHC(OSiMe_3){=}CHCH{=}CHOSiMe_3$ $AcNH{=}CHCH{=}C(OSiMe_3)CH{=}CHOSiMe_3$	100.0
298	M − HO-GlcNAc − $CH(OSiMe_3)CH_2OSiMe_3$ − Me_3SiOH	29.5
217	$Me_3SiOCH{=}CHCH{=}OSiMe_3$	21.6
204	$Me_3SiO{=}CHCHOSiMe_3$	22.2
186	$AcNHCH{=}CHCHOSiMe_3$ $AcNH{=}CHC(OSiMe_3){=}CH_2$ $NH_2{=}CHCOCH_2CH{=}CHOSiMe_3$	36.1
173	$AcNHCHCH{=}OSiMe_3$	65.7

[a]In this table, the abbreviations Neu5Ac and GlcNAc mean the derivatised units of disaccharide **15**.
[b]The intensities of the ions are given relative to that of *m/z* 300.

583 for Me_3Si-aldohexosyl-(1→6)-aldohexoses[28,29], confirms the presence of the (2→6) linkage in per-*O*-trimethylsilylated **15**.

The benzyl group was removed from **16** by palladium-catalysed hydrogenolysis, to give the amorphous, title product, in almost quantitative yield, as its stable potassium salt **17**. As shown by 360-MHz, ^{1}H-n.m.r. spectroscopy (deuterium oxide), the 2-acetamido-2-deoxy-D-glucose moiety of **17** occurs almost exclusively in the pyranoid form (H-1α: $J_{1,2}$ 3.7 Hz; H-1β: $J_{1,2}$ 8.6 Hz; αβ ratio, 2:3). The effect of anomerisation[30] of this moiety is also expressed by the doubling of the resonance signal of Neu5Ac H-3*eq* (~0.004 p.p.m.) without influencing the signal of H-3*ax*, as observed[2] for the methyl ester of 6-*O*-(*N*-acetyl-β-D-neuraminyl)-D-galactose. The resonance signals of Neu5Ac H-3*eq* and H-4 at δ 2.405 and 4.101, respectively, accord with the β configuration of the glycosidic linkage (for β ranges, see above). Also, **17** was resistant to the action of *Clostridium perfringens* neuraminidase.

360-MHz, ^{1}H-N.m.r. spectroscopy showed that the mother liquor of the crystallisation of **13** consisted of **13** and **18** in the ratio 1:6, based on the relative intensities of the Neu5Ac H-3*eq* resonances at δ 2.460 and 2.605, respectively. To determine the type and anomeric configuration of the interglycosidic linkage of **18**,

the mother liquor was subjected to *O*-deacetylation and subsequent saponification of the methyl ester. The presence of an α-linked Neu5Ac moiety in **19** was indicated by the 360-MHz, ^{1}H-n.m.r. spectrum of the resulting 1:6 mixture of **16** and **19**, which showed the Neu5Ac H-3*eq* of **19** at δ 2.751 (range for H-3*eq* in α-linked Neu5Ac derivatives[27]: δ 2.6–2.8). Furthermore, the main component **19** could be cleaved by the action of *Clostridium perfringens* neuraminidase. Methylation analysis[31,32] of the mixture of **16** and **19** proved the presence of only the (2→6) linkage. The two components could be separated by high-pressure liquid chromatography[33] (peak ratio, 1:6); the retention time of the minor compound was identical with that of pure **16** (see Experimental).

EXPERIMENTAL

Materials. — *N*-Acetyl-D-neuraminic acid (Neu5Ac) was isolated from the urine of a patient with sialuria[34]. Methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**8**) was prepared from Neu5Ac according to Kuhn *et al.*[3]. The product crystallised from chloroform–ethyl ether; m.p. 154–155°, $[\alpha]_D^{20}$ −4.6° (*c* 1.2, chloroform); lit.[3] m.p. 156–157°, $[\alpha]_D^{20}$ −3.3° (chloroform).

General methods. — Melting points were determined with a Meopta melting-point microscope and are uncorrected. Evaporations were conducted *in vacuo* at <40° (bath). Elemental analyses were carried out at the Institute for Organic Chemistry TNO, Utrecht, The Netherlands. Specific rotations were measured with a Perkin–Elmer 241 polarimeter, using a 10-cm micro-cell. I.r. spectra (KBr discs) were recorded with a Perkin–Elmer Model 457 spectrophotometer.

^{1}H-N.m.r. spectra were recorded with Varian EM-390 (90 MHz), Bruker WP-200 WB (200 MHz), and Bruker HX-360 (360 MHz) spectrometers, operating in the Fourier-transform mode at probe temperatures of 25°. Chemical shifts (δ) for solutions in chloroform-*d* are given relative to tetramethylsilane as internal standard. For solutions in deuterium oxide, sodium 4,4-dimethyl-4-silapentane-1-sulphonate was used (indirectly, acetone in deuterium oxide; δ 2.225). Prior to spectral analysis, deuterium exchange was effected by dissolution in deuterium oxide three times with intermediate lyophilisation.

Trimethylsilylation of 1-mg samples of sugars was performed with hexamethyldisilazane and chlorotrimethylsilane in pyridine[29]. Sugars were analysed by methanolysis, followed by g.l.c. of the trimethylsilylated methyl glycosides, as indicated previously[35].

G.l.c. was carried out on a Varian Aerograph 2740-30-01, equipped with a flame-ionisation detector. The injection-port temperature and the detector temperature were 210 and 230°, respectively. The nitrogen flow-rate was 35 ml/min. A glass column (2.00 m × 4.00 mm i.d.) packed with 3.8% of SE-30 on Chromosorb W HP (80–100 mesh) was used; the oven temperature was 260° for the 3-*O*-trimethylsilyl derivative of **4** and was programmed from 130→220° at 2°/min for per-*O*-trimethyl-

silylated monosaccharide methyl glycosides, and from 150→250° at 4°/min for trimethylsilylated and/or methylated monosaccharide derivatives.

G.l.c.–m.s. was performed with a combined Carlo Erba GC gas chromatograph/Kratos MS 80 mass spectrometer/Kratos DS 55 mass data analysis system. Mass spectra (70 eV) were recorded with an ion-source temperature of 225°, an accelerating voltage of 2.7 kV, and an ionising current of 100 μA. The g.l.c. conditions were similar to those described above. Direct-probe, 70-eV mass spectra of disaccharide samples were recorded on an AEI MS-902 apparatus (ion-source temperature, 140°; accelerating voltage, 6 kV; ionising current, 100 μA).

T.l.c. was performed on silica gel (Merck DC-Plastikrolle Kieselgel 60 F254) and detection was effected by u.v. light, or by spraying with 20% conc. sulphuric acid in methanol followed by charring at 130° for 5–10 min. The following solvents were used: *A*, chloroform–methanol (85:15); *B*, chloroform–methanol (25:1); *C*, ethyl acetate–2-propanol–water (2:2:1); *D*, 1-propanol–water (7:3). Short-column chromatography under medium pressure[36] was performed on silica gel (Merck Kieselgel 60, 230–400 mesh). Conventional column chromatography was performed on Merck Kieselgel 60, 70–230 mesh. In all cases the eluate was monitored by charring with 20% conc. sulphuric acid in methanol on t.l.c. plates.

Incubations with *Clostridium perfringens* neuraminidase (EC 3.2.1.18) were performed at 37° and pH 5.4 (0.1M Na/K phosphate buffer) in a total volume of 0.1 ml, containing 1.7 mU of enzyme and 0.2 μmol of substrate. Free sialic acid was determined by Warren's method[37].

Benzyl 2-acetamido-2-deoxy-4,6-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (**4**). — A solution of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[38] (23.11 g, 73.3 mmol) in dry pyridine (20 ml) was added dropwise to a stirred solution of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[6] (**1**; 20.20 g, 64.9 mmol) in dry pyridine (20 ml) at −15°. After stirring at room temperature for 1 h, 2-propanol (10 ml) was added to decompose the excess of halide and, after 30 min, the reaction mixture was concentrated. A solution of the residue in chloroform (400 ml) was washed with ice-cold, saturated, aqueous sodium hydrogencarbonate (twice) and water, dried (magnesium sulphate), and concentrated. After coevaporation with toluene to remove traces of pyridine, the residual syrup was crystallised from dry ethanol, to give **4** (19.86 g, 55%), m.p. 154–155° (with some softening at 134°). $[\alpha]_D^{20}$ +49° (*c* 1.3, chloroform); ν_{max}^{KBr} 3410 (shoulder, OH), 3305 (NH), 1640 (Amide I), 1535 (Amide II), and 699 cm^{-1} (Ph); ^{1}H-n.m.r. data (90 MHz): δ 1.09 (m, 28 H, isopropyl), 1.95 (s, 3 H, NAc), 2.93 (broad s, 1 H, OH), 4.46 and 4.72 (2 d, 2 H, J_{gem} −12.0 Hz, CH_2Ph), 4.93 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.84 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), and 7.34 (s, 5 H, Ph); m.s. data for 3-*O*-trimethylsilylated **4**: *m/z* 610 [M − CH_3], 582 [M − C_3H_7], 474 [582 − $PhCH_2OH$], 384 [474 − Me_3SiOH], 261 [M − $OSi(C_3H_7)_2OSi(C_3H_7)_2O{=}CH_2$ − NH_2Ac − CH_3], 173 [$Me_3SiO{=}CHCHNHAc$], and 170 [261 − C_7H_7].

Anal. Calc. for $C_{27}H_{47}NO_7Si_2$: C, 58.55; H, 8.55; N, 2.53. Found: C, 58.23; H, 8.57; N, 2.42.

When a solution of **4** (7.1 mg, 13 μmol) and chlorotriphenylmethane (11.2 mg, 40 μmol) in dry pyridine (0.3 ml) was kept at 100° for 4 h, t.l.c. (solvent *B*) revealed only **4**.

*Benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-*D*-glucopyranoside* (**5**). — A solution of **4** (300 mg, 0.54 mmol) in 1:1 acetic anhydride–pyridine (10 ml) was kept at room temperature for 29 h and then evaporated, and ethanol and then toluene were evaporated from the residue. After final purification by short-column chromatography (column: 5 × 4.5 cm) with 100:1 chloroform–methanol (100 ml) and then 96:4 chloroform–methanol, the residual syrup crystallised slowly on standing *in vacuo*, to give **5** (320 mg, 99%), m.p. 106–108°, $[\alpha]_D^{25}$ +54° (*c* 3.2, chloroform); ν_{max}^{KBr} 3300 (NH), 1755 (OAc), 1660 (Amide I), 1543 (Amide II), 1384, 1370 (i-Pr), 772, 737, and 695 cm^{-1} (Ph); ^{1}H-n.m.r. data (200 MHz): δ 1.07 (m, 28 H, isopropyl), 1.89 (s, 3 H, NAc), 2.06 (s, 3 H, OAc), 4.25 (m, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.0, $J_{2,NH}$ 10.0 Hz, H-2), 4.50 and 4.72 (2 d, 2 H, J_{gem} −12.0 Hz, CH_2Ph), 4.92 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.25 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 10.7 Hz, H-3), 5.89 (d, 1 H, $J_{2,NH}$ 10.0 Hz, NH), and 7.34 (s, 5 H, Ph).

Anal. Calc. for $C_{29}H_{49}NO_8Si_2$: C, 58.45; H, 8.29; N, 2.35. Found: C, 58.40; H, 8.19; N, 2.29.

*Benzyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-*D*-glucopyranoside* (**6**). — A solution of **4** (9.60 g, 17.3 mmol) and dry mesitylenesulphonic acid (768 mg, 3.8 mmol) in dry *N*,*N*-dimethylformamide (77 ml) was stirred overnight at room temperature. After the addition of solid sodium carbonate (500 mg), the mixture was evaporated. A solution of the residue in 1:1 ethyl ether–hexane (450 ml) was washed twice with ice-cold, saturated, aqueous sodium hydrogencarbonate and five times with saturated, aqueous sodium chloride, dried (sodium sulphate), and evaporated. After short-column chromatography (column: 9 cm × 6.5 cm) with 1:1 ethyl acetate–hexane, the syrup (8.40 g) consisted of **6** and **4** in the ratio 5:1 (deduced from the relative intensities of the NH doublets in the 90-MHz, ^{1}H-n.m.r. spectrum). A portion of **4** (766 mg) could easily be removed by selective crystallisation from dry ethanol. The mother liquor was concentrated and the syrup was crystallised from nitromethane and then ethyl ether–hexane, to give **6** (5.06 g, 53%), m.p. 126–127°, $[\alpha]_D^{20}$ +90.5° (*c* 0.9, chloroform); ν_{max}^{KBr} 3310 (NH), 1660 (Amide I), 1545 (Amide II), 730, and 700 cm^{-1} (Ph); ^{1}H-n.m.r. data (90 MHz): δ 1.04 (m, 28 H, isopropyl), 1.91 (s, 3 H, NAc), 2.67 (broad s, 1 H, OH), 4.20 (m, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6, $J_{2,NH}$ 9.6 Hz, H-2), 4.47 and 4.75 (2 d, 2 H, J_{gem} −12.0 Hz, CH_2Ph), 4.88 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.52 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH), and 7.35 (s, 5 H, Ph).

Anal. Calc. for $C_{27}H_{47}NO_7Si_2$: C, 58.55; H, 8.55; N, 2.53. Found: C, 58.17; H, 8.45; N, 2.36.

When a solution of compound **6** (8.6 mg, 16 μmol) and chlorotriphenylmethane (13.3 mg, 48 μmol) in dry pyridine (0.3 ml) was kept at 100° for 4 h, t.l.c. (chloroform) revealed a black–yellow spot originating from tritylated **6** and the almost complete disappearance of **6**.

*Benzyl 2-acetamido-6-*O*-acetyl-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside* (**7**). — A solution of **6** (298 mg, 0.54 mmol) in 2:5 acetic anhydride–pyridine (7 ml) was kept at room temperature for 22 h and then evaporated, and methanol and toluene were evaporated several times from the residue. After final purification by short-column chromatography (column: 6 × 4.5 cm) with 100:1 chloroform–methanol (100 ml) and 96:4 chloroform–methanol, the syrup crystallised on standing *in vacuo*, to give **7** (315 mg, 98%), m.p. 163–165°, $[\alpha]_D^{25}$ +88° (*c* 0.6, chloroform); ν_{max}^{KBr} 3305 (NH), 1748 (OAc), 1658 (Amide I), 1543 (Amide II), 1387, 1369 (i-Pr), 738, and 699 cm^{-1} (Ph); ^{1}H-n.m.r. data (90 MHz): δ 1.02 (m, 28 H, isopropyl), 1.91, (s, 3 H, NAc), 2.06 (s, 3 H, OAc), 4.48 and 4.74 (2 d, 2 H, J_{gem} −12.0 Hz, CH_2Ph), 4.88 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.57 (d, 1 H, $J_{2,NH}$ 9.9 Hz, NH), and 7.37 (s, 5 H, Ph).

Anal. Calc. for $C_{29}H_{49}NO_8Si_2$: C, 58.45; H, 8.29; N, 2.35. Found: C, 58.46; H, 8.24; N, 2.28.

*Condensation of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-β-D-glycero-D-galacto-2-nonulopyranosonate* (**9**) *and compound* **6**. — Compound **9**, freshly prepared from **8** (600 mg, 1.13 mmol) according to Kuhn *et al.*[3], was dissolved in dry benzene (7 ml). After the addition of **6** (4.68 g, 8.5 mmol) and 4 Å molecular sieves (100 mg), the mixture was stirred under dry nitrogen at room temperature. A solution of silver triflate (419 mg, 1.6 mmol) and 2,4,6-trimethylpyridine (0.17 ml, 1.3 mmol) in dry 1:1 nitromethane–ethyl ether (4 ml) was added dropwise; this was accompanied by the formation of a white turbidity. Stirring was continued in the dark for 2 h. The suspension was diluted with chloroform and then successively washed with ice-cold 5% aqueous sodium thiosulphate, ice-cold 5% aqueous sodium hydrogencarbonate, and water, dried (sodium sulphate), and evaporated. The residue was fractionated by short-column chromatography (column: 10 × 6.5 cm) with 10:3 chloroform–tetrahydrofuran. The excess of **6** (4.28 g) was eluted first, followed by a second fraction (0.98 g), which showed three components in high-performance t.l.c. (precoated Merck Kieselgel 60 F254 HPTLC plates, 15:1 chloroform–methanol) with R_F values of 0.62, 0.57, and 0.52. The mixture was subjected to desilylation conditions by treating a solution of the dry residue in dry tetrahydrofuran (4 ml) with pyridinium hydrochloride (192 mg, 1.7 mmol) and M tetrabutylammonium fluoride in tetrahydrofuran (3 ml, 3 mmol). The solution was stirred under nitrogen at room temperature until (30 min) t.l.c. (solvent *A*) revealed two spots having R_F 0.59 and 0.36. After treatment with Dowex 50W-X8 (Na^+) resin (3 g) and evaporation, a solution of the residue in chloroform was washed with water, dried (sodium sulphate), and concentrated. The residue was fractionated by short-column chromatography (column: 10 × 4.5 cm) with 10:1 chloroform–methanol.

The first fraction gave syrupy **11** (214 mg, 40% based on **8**), $[\alpha]_D^{20}$ +40° (*c* 2.3, chloroform), as identified by ^{1}H-n.m.r. spectroscopy[2]. A solution of **11** (214 mg, 0.45 mmol) in dry methanol containing a catalytic amount of potassium *tert*-butoxide was stirred at room temperature until t.l.c. (solvent *C*) showed that the

reaction was complete (4 h). After deionisation with Dowex 50W-X8 (H^+) resin and evaporation, a homogeneous residue (127 mg; t.l.c., solvent *C*) was obtained. A sample was crystallised from methanol–ethyl ether, to afford **12**, m.p. 226–228° (dec.), $[\alpha]_D^{20}$ +41° (*c* 1.0, water): lit.[23] m.p. 225–227° (dec.), $[\alpha]_D$ +41.8° (*c* 8.4, water).

The second fraction (639 mg) failed to crystallise and was treated with 1:3 acetic anhydride–pyridine (5 ml) for 18 h at room temperature. After coevaporation with dry methanol, the residue was purified by short-column chromatography (column: 6.5 × 4.5 cm) with 25:1 chloroform–methanol. The resulting syrup consisted of **13** and **18** in the ratio 4:1, as deduced from the relative intensities of the H-3*eq* signals in the ^{1}H-n.m.r. spectrum [360 MHz; δ 2.465 (dd, 0.8 H, H-3*eq*; **13**) and 2.601 (dd, 0.2 H, H-3*eq*; **18**). Crystallisation from chloroform–ethyl ether afforded **13** (452 mg, 46% based on **8**), m.p. 237–238°, $[\alpha]_D^{25}$ +67° (*c* 0.36, chloroform); ν_{max}^{KBr} 3405, 3285 (NH), 1750 (broad: OAc, $COOCH_3$), 1670 (Amide I), 1550 (Amide II), 740, and 699 cm^{-1} (Ph); ^{1}H-n.m.r. data (360 MHz): Neu5Ac unit: δ 2.465 (dd, 1 H, $J_{3ax,3eq}$ −12.6, $J_{3eq,4}$ 5.1 Hz, H-3*eq*), 4.068 (dd, 1 H, $J_{8,9}$ 9.1, $J_{9,9'}$ −12.2 Hz, H-9'), 4.152 (m, 1 H, $J_{4,5}$ 10.2, $J_{5,6}$ 10.2, $J_{5,NH}$ 10.2 Hz, H-5), 4.307 (dd, 1 H, $J_{5,6}$ 10.2, $J_{6,7}$ 2.5 Hz, H-6), 5.021 (dd, 1 H, $J_{8,9}$ 2.4, $J_{9,9'}$ −12.2 Hz, H-9), 5.171 (m, 1 H, $J_{3ax,4}$ 11.6, $J_{3eq,4}$ 5.1, $J_{4,5}$ 10.2 Hz, H-4), 5.307 (m, 1 H, $J_{7,8}$ 2.5, $J_{8,9}$ 2.4, $J_{8,9'}$ 9.1 Hz, H-8), 5.414 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 2.5 Hz, H-7), and 6.036 (d, 1 H, $J_{5,NH}$ 10.2 Hz, NH); Glc*p*NAc unit: δ 3.595 (dd, 1 H, $J_{5,6'}$ 2.8, $J_{6,6'}$ −11.1 Hz, H-6'), 3.864 (dd, 1 H, $J_{5,6}$ 2.1, $J_{6,6'}$ −11.1 Hz, H-6), 3.999 (m, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 2.1, $J_{5,6'}$ 2.8 Hz, H-5), 4.381 (m, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6, $J_{2,NH}$ 9.6 Hz, H-2), 5.097 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.239 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 5.304 (dd, 1 H, $J_{3,4}$ 9.6, $J_{4,5}$ 9.6 Hz, H-4), and 5.693 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH); other groups: δ 1.867, 1.903, 1.956, 2.008, 2.032, 2.036, 2.061, and 2.147 (8 s, 24 H, NAc and OAc), 3.787 (s, 3 H, CO_2Me), 4.465 and 4.705 (2 d, 2 H, J_{gem} −11.9 Hz, CH_2Ph), and 7.349 (m, 5 H, Ph).

Anal. Calc. for $C_{39}H_{52}N_2O_{20}$: C, 53.91; H, 6.03; N, 3.22; O, 36.83. Found: C, 54.02; H, 6.09; N, 3.17; O, 36.71.

The mother liquor (138 mg) consisted of **13** and **18** in the ratio 1:6, as shown by ^{1}H-n.m.r. spectroscopy. ^{1}H-N.m.r. data for **18** (360 MHz): Neu5Ac unit: δ 2.605 (dd, 1 H, $J_{3ax,3eq}$ −12.6, $J_{3eq,4}$ 4.5 Hz, H-3*eq*), 4.262 (dd, 1 H, $J_{8,9}$ 2.5, $J_{9,9'}$ −12.2 Hz, H-9), 4.855 (m, 1 H, $J_{3ax,4}$ ~12.5, $J_{3eq,4}$ ~4.5, $J_{4,5}$ ~9.6 Hz, H-4), 5.281 (dd, 1 H, $J_{6,7}$ <1, $J_{7,8}$ 9.3 Hz, H-7), and 5.381 (d, 1 H, $J_{5,NH}$ 9.4 Hz, NH); Glc*p*NAc unit: δ 3.385 (dd, 1 H, $J_{5,6'}$ 2.3, $J_{6,6'}$ −11.0 Hz, H-6'), 4.304 (m, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6, $J_{2,NH}$ 9.6 Hz, H-2), 4.881 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), and 5.700 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH); other groups: δ 3.770 (s, 3 H, CO_2Me), 4.440 and 4.702 (2 d, 2 H, J_{gem} −11.9 Hz, CH_2Ph), and 7.33 (m, 5 H, Ph).

To obtain further structural evidence for **18**, a portion (27 mg) of the mother liquor was *O*-deacetylated and saponified as described below for the preparation of **16**, to give a 1:6 mixture (14 mg) of **16** and **19**, as shown by ^{1}H-n.m.r. spectroscopy [360 MHz, D_2O; δ 2.419 (dd, 0.15 H, H-3*eq*; **16**) and 2.751 (dd, 0.85 H, H-3*eq*;

19)]; ^{1}H-n.m.r. data for **19** (360 MHz, D_2O): Neu5Ac unit: δ 1.756 (dd, 1 H, $J_{3ax,3eq}$ −12.5, $J_{3ax,4}$ 12.5 Hz, H-3*ax*), 2.038 (s, 3 H, NAc), and 2.751 (dd, 1 H, $J_{3ax,3eq}$ −12.5, $J_{3eq,4}$ 4.8 Hz, H-3*eq*); Glc*p*NAc unit: δ 1.957 (s, 3 H, NAc), 3.993 (dd, 1 H, $J_{5,6}$ 5.0, $J_{6,6'}$ −11.0 Hz, H-6), and 4.917 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); other groups: δ 4.559 and 4.743 (2 d, 2 H, J_{gem} −12.0 Hz, CH_2Ph), and 7.437 (m, 5 H, Ph). For ^{1}H-n.m.r. data of **16**, see below.

Sugar analysis of the mixture of **16** and **19** showed a GlcNAc/Neu5Ac *ratio* of 0.8:1.0. Methylation analysis[31,32] afforded methyl 3,5-dideoxy-2,4,7,8,9-penta-*O*-methyl-5-(*N*-methylacetamido)-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**20**), and the methyl (**21**) and benzyl (**22**) 2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-6-*O*-trimethylsilyl-α-D-glucopyranosides; m.s. data for **21**: *m/z* 334 [M − CH_3], 217 [M − Me_3SiOH − CH_2=C=O], 182 [M − 2 × CH_3OH − CH_2OSiMe_3], 160 [Me_3SiOCH_2CH=$CHOCH_3$], 142 [$AcN(CH_3)$=$CHCH$=$CHOCH_3$], 129 [$AcN(CH_3)CH$=$CHOCH_3$], and 87 [129 − CH_2=C=O]; m.s. data for **22**: *m/z* 410 [M − CH_3], 378 [410 − CH_3OH], 334 [M − C_7H_7], 205 [$AcN(CH_3)CH$=$CHOCH_2Ph$], 142 [$AcN(CH_3)$=$CHCH$=$CHOCH_3$], 129 [$AcN(CH_3)CH$=$CHOCH_3$], 91 [C_7H_7], and 87 [129 − CH_2=C=O]. For m.s. data of **20**, see ref. 32.

The mixture of **16** and **19** (30 μg) could be separated by high-pressure liquid chromatography under conditions described recently[33]. Isocratic elution with 4:1 acetonitrile–15mM potassium phosphate (pH 5.2) gave two peaks (ratio, 1:6) having retention times of 0.45 and 0.25, relative to Neu5Ac. Under similar conditions, **16** (see below) had a relative retention time of 0.45.

*Benzyl 2-acetamido-2-deoxy-6-*O*-(potassium 5-acetamido-3,5-dideoxy-β-*D*-glycero-*D*-galacto-2-nonulopyranosylonate)-α-D-glucopyranoside* (**16**). — A solution of **13** (199 mg, 0.23 mmol) in dry methanol (20 ml) containing a catalytic amount of potassium *tert*-butoxide was stirred at room temperature until t.l.c. (solvent *C*) showed that *O*-deacetylation was complete (3 h), yielding **14**.

An aliquot of **14** (2 mg) was used for mass-spectral analysis. A solution of the material in dry methanol (1 ml) was deionised with Dowex 50W-X8 (H^+) resin at 0° and evaporated, and the residue was hydrogenolysed over Pd/C (10%, 5 mg) in dry methanol (1 ml) for 1 day. The mixture was filtered and evaporated, to give **15** (1 mg). The m.s. data for per-*O*-trimethylsilylated **15** are given in Table I.

The remainder of **14** was treated with 0.02M potassium hydroxide (12 ml) at 5° for 22 h, and, after concentration by lyophilisation, the solution was freed from alkali on a column (33 × 3 cm) of Bio-Gel P-2 (200–400 mesh) by elution with water. The product was further purified on a column (66 × 2.5 cm) of Dowex 50W-X8 (K^+) resin (200–400 mesh) by elution with water, and lyophilised, to give amorphous **16** (133 mg, 88%). The product was homogeneous in t.l.c. (solvents *C* and *D*), and had $[\alpha]_D^{20}$ +89° (*c* 0.3, water); ν_{max}^{KBr} 3360 (broad; NH, OH), 1630 (broad; Amide I, COOK), 1550 (Amide II), 740, and 700 cm^{-1} (Ph); ^{1}H-n.m.r. data (360 MHz, D_2O): Neu5Ac unit: δ 1.673 (dd, 1 H, $J_{3ax,3eq}$ −13.3, $J_{3ax,4}$ 11.7 Hz, H-3*ax*), 2.040 (s, 3 H, NAc), 2.416 (dd, 1 H, $J_{3ax,3eq}$ −13.3, $J_{3eq,4}$ 4.9 Hz, H-3*eq*), 3.550

(dd, 1 H, $J_{6,7}$ ~1.0, $J_{7,8}$ 9.2 Hz, H-7), 3.674 (dd, 1 H, $J_{8,9'}$ 6.5, $J_{9,9'}$ −12.5 Hz, H-9'), and 4.082 (m, 1 H, $J_{3ax,4}$ 11.7, $J_{3eq,4}$ 4.9, $J_{4,5}$ 9.5 Hz, H-4); Glc*p*NAc unit: δ 1.956 (s, 3 H, NAc), 3.589 (dd, 1 H, $J_{5,6'}$ 4.4, $J_{6,6'}$ −11.4 Hz, H-6'), and 4.921 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1); other groups: δ 4.527 and 4.774 (2 d, 2 H, J_{gem} −12.0 Hz, CH_2Ph), and 7.441 (s, 5 H, Ph); sugar analysis for **16**: GlcNAc/Neu5Ac ratio, 0.9:1.0.

Anal. Calc. for $C_{26}H_{37}KN_2O_{14} \cdot H_2O$: C, 47.41; H, 5.97; K, 5.94; N, 4.25. Found: C, 47.45; H, 5.57; K, 6.04; N, 4.19.

*2-Acetamido-2-deoxy-6-O-(potassium 5-acetamido-3,5-dideoxy-β-*D-glycero-D-galacto-*2-nonulopyranosylonate)-*D-*glucose* (**17**). — A solution of **16** (51.5 mg, 0.08 mmol) in 90% aqueous methanol (15 ml) was hydrogenolysed over Pd/C (10%, 200 mg) for 1 day. The reaction mixture was filtered, concentrated, and finally lyophilised from an aqueous solution, to give amorphous **17** (44 mg, 99%). The product was homogeneous in t.l.c. (solvents *C* and *D*), and had $[\alpha]_D^{25}$ +19° (*c* 0.58, water); ^{1}H-n.m.r. data (360 MHz, D_2O): Neu5Ac unit: δ 1.665 (dd, 1 H, $J_{3ax,3eq}$ −13.1, $J_{3ax,4}$ 11.5 Hz, H-3*ax*), 2.048 (s, 3 H, NAc), 2.403 and 2.407 (2 dd, 1 H, $J_{3ax,3eq}$ −13.1, $J_{3eq,4}$ 4.9 Hz, H-3*eq* [α and β anomer of Glc*p*NAc]), and 4.101 (m, 1 H, $J_{3ax,4}$ 11.5, $J_{3eq,4}$ 4.9, $J_{4,5}$ ~10 Hz, H-4); Glc*p*NAc unit: δ 2.048 (s, 3 H, NAc), 4.704 (d, 0.6 H, $J_{1,2}$ 8.6 Hz, H-1β), and 5.186 (d, 0.4 H, $J_{1,2}$ 3.7 Hz, H-1α); sugar analysis for **17**: GlcNAc/Neu5Ac ratio, 1.0:1.0.

Anal. Calc. for $C_{19}H_{31}KN_2O_{14} \cdot H_2O$: C, 40.14; H, 5.85; K, 6.88; N, 4.93. Found: C, 39.97; H, 5.68; K, 6.93; N, 4.85.

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