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Note

Synthesis of 4-epi-2-deoxy-2-H_{eq}-*N*-acetylneuraminic acid and 2,4-dideoxy-2-H_{eq}-*N*-acetylneuraminic acid

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We have continued our work to develop novel analogues of sialic acid [1–4] that may specifically modulate the interaction between endogenous sialic acid and influenza virus haemagglutinin [3,5,6]. Functional groups of sialic acid that have been implicated for this virus-host recongnition are the glycerol side chain, *N*-acetyl group and the axially oriented carboxylic acid function [3]. In this report we describe the synthesis of two analogues, namely, 4-epi-2-deoxy-2-H_{eq}-*N*-acetylneuraminic acid (4-epi-2-d-2-H_{eq}-Neu5Ac) and 2,4-dideoxy-2-H_{eq}-*N*-acetylneuraminic acid (2,4-d_2-2-H_{eq}-Neu5Ac).

1. Results and discussion

Synthesis of 4-epi-2-deoxy-2-H_{eq}-N-acetylneuraminic acid.—*N*-acetylneuraminic acid (Neu5Ac,**1**) was treated with CF_3COOH in methanol [7] to give its methyl ester in qunatitative yield. This methyl ester was converted into its peracelylated 2-chloro

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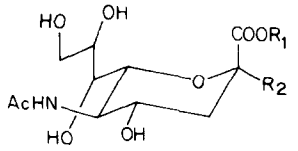
derivative by stirring with acetyl chloride [8]. Subsequent catalytic transfer hydrogenation with Pd/C in a mixture of pyridine and toluene gave 2-deoxy-$2H_{eq}$ peracetylated Neu5Ac methyl ester (95%) with 2-deoxy-2-H_{ax}-peracetylated Neu5Ac methyl ester (5%) as the by product [9]. Preparative HPLC separation [1] yielded pure 2-deoxy-2-H_{eq}-peracetylated Neu5Ac methyl ester which on saponification (aq NaOH) followed by neutralization with solid CO_2 and Amberlyst $15H^+$ yielded compound **2**. Both Zemplen saponification ($NaOCH_3/CH_3OH$) of 2-deoxy-$2H_{eq}$- peracetylated Neu5Ac methyl ester [1], or treatment of **2** with CF_3COOH in methanol, yielded compound **3**. Treatment of **3** with acetone, 2,2-dimethoxypropane in the presence of Amberlyst $15H^+$ gave the 8,9-*O*-isopropylidene derivative [1,2,10–12] **4** in 72% yield. The regioselective oxidation at the C-4 position [2,13] of **4** was carried out in a very short time with a stoichiometric amount of RuO_4, and the ketone (**6**) on reduction with borane–ammonia complex gave a mixture of 4-epi-alcohols, **7** and **4** in the ratio of 2:3. The 1,3-steric interaction between the axial OH at C-4 and bulky axial carboxylate group at C-2 in **7** may be responsible for this stereoselectivity.

Deprotection of the acetyl and ester groups of **8** by saponification followed by removal of 8,9-*O*-isopropylidene group under acidic conditions afforded 1,4-lactone (20%) along with the desired 4-epi-2d-2-H_{eq}-Neu5Ac [**10** (R1 = H) 80%, ^{1}H-NMR observation [14], Method A]. To obtain pure desired product (**11**), the lactone was cleaved under basic conditions using $Ba(OH)_2$ and purified over Florisil followed by a Dowex 50 NH_4^+ column. Since 1,4-lactone formation took place under acidic conditions were the carboxyl group and the axial C4–OH functional group were free, the deprotection of functional groups was carried out by other methods (Method B and Method C).

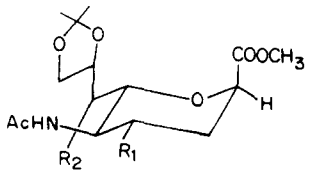
In method B, removal of the 8,9-*O*-isopropylidene group of **7** with aqueous 80% acetic acid at 60°C was carried out first, followed by saponification with aqueous NaOH. Neutralization with solid CO_2 followed by lyophilization gave the desired pure 4-epi-2-d-2-H_{eq}-Neu5Ac (**10**) in good yield (92%).

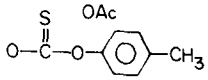
In method C, the peracetylated 4-epi-2-d-2-H_{eq}-Neu5Ac-methyl ester **9** was obtained by selective removal of the isopropylidene group with aqueous 80% acetic acid at 60°C followed by peracetylation with acetic anhydride and pyridine. Finally, compound **9** was transformed into the desired 4-epi-2-d-2-H_{eq}-Neu5Ac (**10**) by saponification.

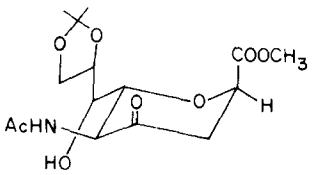
Synthesis of 2,4-dideoxy-2-H_{eq}-N-acetylneuraminic acid.—It was found by Schauer et al. [15] as well as by Ogura and co-workers [11] that the most reactive position towards acetylation is C-9 and the next most reactive position C-4. Therefore, this finding was used as the basis for selective 4-*O*-thiocarbonylation of **4** by reaction with *p*-tolyl chlorothioformate and pyridine at room temperature for only 1 h to give **12**. It is important to note that the 4-methylphenoxythiocarbonyl group of **12** remained unaffected during the hydrolysis of the isopropylidene group under acidic conditions at 60°C. Peracetylation of **12** with acetic anhydride and pyridine gave compound **14** which was used for confirmation of structure. Reaction of **12** with Bu_3SnH in the presence of AIBN gave **13** which, on deprotection, afforded the expected 2,4-d_2-2-H_{eq}-Neu5Ac (**15**) in good yield (86%).



	R_1	R_2
1	H	OH
2	H	H
3	CH_3	H



	R_1	R_2
4	OH	OH
5	OAc	OAc
12		OH
13	H	OH



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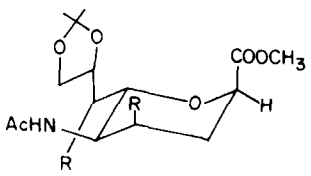
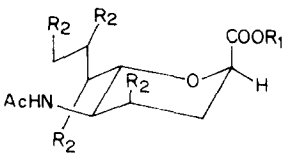
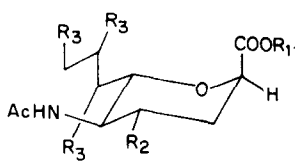
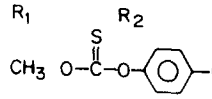


7 R = OH
8 R = OAc



	R_1	R_2
9	CH_3	OAc
10	Na	OH
11	NH_4	OH



	R_1	R_2	R_3
14	CH_3		OAc
15	Na	H	OH

2. Experimental

General methods.—Solvents were freshly distilled before use. All reactions, with the exception of those in water, were conducted in oven-dried (140°C) or flame-dried two or three necked flasks sealed by rubber septa. Addition of reagents and removal of samples for monitoring the reactions (TLC) were achieved with syringes.

Analytical thin layer chromatography (TLC) was preformed with Merck plates (silica gel $60F_{254}$, layer thickeness 0.2 mm) and compounds were visualized by spraying with a

solution of $Ce(NO_3)_4$ in 2N H_2SO_4 followed by heating at 200°C. Merck Silica gel (0.043–0.063 mm) was used for flash chromatography. ^{1}H-NMR (250 and 400 MHz) spectra were obtained on Bruker WM 250 and AM 400 WB spectrometers using $CDCl_3$ as the solvent for the protected sugars and tetramethylsilane (TMS) as the internal chemical shift standard. Reported coupling constants were obtained from a first order analysis of the spectra. In the case of solutions in D_2O, sodium 4,4-dimethyl-4-silapentanesulphate (DSS) in D_2O was used as an internal reference or spectra were referenced to HDO ($\delta = 4.80$). ^{13}C-NMR spectra were recorded at 62.9 and 100 MHz with TMS as an internal standard for solutions in $CDCl_3$ and DSS for solutions in D_2O. Mass spectra were determined using a Varian CH-7 and microanalysis was performed at the Institute of Organic Chemistry, Vienna. *N*-acetylneuraminic acid (Neu5Ac, **1**) was prepared from edible bird's nest glycoprotein. The compounds **2** and **3** were prepared according to procedures described previously [1,9].

General procedure for acetylation.—Approximately 100 mg of the compound to be acetylated was dissolved in 1 mL of pyridine and 1 mL of acetic anhydride, and 5 mg of 4-(dimethylaminopyridine) was added. The reaction mixture was allowed to stand at room temperature for 14 h, then solvents were removed under reduced pressure (0.001 Torr), and the crude product purified as described in each protocol.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene-*D-erythro-L-manno-*nonoate (4).*—Compound **3** (1 g, 3.26 mmol), obtained from compound **2** by treatment with CF_3COOH in methanol, and Amberlyst 15H$^+$ (1g) were dried together at 40°C (0.01 Torr) for 1 h. Anhydrous acetone (100 mL) and 2,2-dimethoxypropane (2 mL) were added and the reaction mixture was shaken until TLC (9:1, ethylacetate:methanol; R_f of **3** = 0.1 and R_f of **4** = 0.45) indicated completion of the reaction. The resin was filtered off and washed several times with acetone. The combined filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (50 g of silica gel, 9:1; ethyl acetate:methanol) yielding **4** (810 mg, 72%); ^{1}H- NMR (250 MHz, $CDCl_3$) : δ 1.33, 1.36 (2s, 2 × 3H, $C(CH_3)_2$), 1.90 (m, 1H, $J_{2,3ax}$ 4.90, $J_{3ax,4}$ 11.1, 3-H_{ax}), 2.02(s, 3H, $COCH_3$), 2.47 (ddd, 1H, $J_{2,3eq}$ 1.50, $J_{3eq,4}$ 4.00, $J_{3ax,3eq}$ 12.00, 3-H_{eq}), 3.50 (dd, 1H, $J_{6,7}$ 1.50, $J_{7,8}$ 6.00, 7-H), 3.54 (dd, 1H, $J_{5,6}$ 8.50, $J_{6,7}$ 1.5, 6-H), 3.65–3.85 (m, 4H, 4-H, $COOCH_3$), 3.97 (dd, 1H, $J_{8,9a}$ 6.00, $J_{9a,9b}$ 9.00, 9-Ha), 4.02–4.14 (m, 2H, 8-H, 9-Hb), 4.23 (ddd, 1H, $J_{4,5}$ $J_{5,NH}$ 7.00, 5-H), 4.59(dd, 1H, $J_{2,3ax}$ 4.90, $J_{2,3eq}$ 1.50, 2-H), 6.35 (d, 1H, N–H), $J_{8,9b}$ not determined; MS(70 eV, 130°C) : *m/z* (%) 332(2.78)(M^+–CH_3).

Anal. Calcd for $C_{15}H_{25}NO_8$(347.3): C, 51.80; H 7.19; N, 4.03. Found : C, 51.74; H, 7.26; N, 4.11.

*Methyl 5-acetamido-4,7-di-*O*-acetyl-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene-*D-erythro-L-manno-*nonoate(5).*—Compound **4** (400 mg, 1.15 mmol) was dissolved in anhydrous pyridine (10 mL) and acetic anhydride (10 mL) and kept at room temperature for 2 days or stirred at 60°C for 6 h. Removal of solvents led to a yellow oil which was subjected to flash chromatography (20g of silica gel, ethyl acetate:*n*-hexane, 2:1). Yield of **5** was 436 mg (88%); ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 1.37, 1.38(2s, 2 × 3H, $C(CH_3)_2$), 1.90, 2.04(2s, 2 × 3H, $COCH_3$), 2.09 (ddd, 1H, $J_{2,3ax}$ 6.80, $J_{3ax,3eq}$ 13.50, $J_{3ax,4}$ 11.20, 3-H_{ax}), 2.15(s, 3H, $COCH_3$), 2.45 (ddd, 1H, $J_{2,3eq}$ 1.50, $J_{3eq,4}$ 5.00,

3-H_{eq}), 3.80(s, 3H, $COOCH_3$) 3.93 (dd, 1H, $J_{8,9a}$ 7.00,$J_{9a,9b}$ 8.50, 9-Ha), 3.99–4.09(m, 3H, 5-H, 6-H, 9-Hb), 4.34 (ddd, 1H, $J_{7,8}$ 4.40, $J_{8,9b}$ 5.00, 8-H), 4.63 (dd, 1H, $J_{7,8}$ 1.50, 7-H), 4.98 (ddd, 1H, $J_{4,5}$ 10.00, 4-H), 5.29 (d, 1H, $J_{5,NH}$ 9.50, N—H), 5.37 (dd, 1H, 2-H), $J_{5,6}$ not determined; MS(70 eV, 140°C): m/z (%) = 416 (20.54) (M^+–CH_3).

Anal. Calcd for $C_{19}H_{29}NO_{10}$(431.3):C, 52.86;H, 6.72;N, 3.34. Found : C, 52.73; H, 6.76; N, 3.25.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidne*-D-glycero-D-galacto-*4-nonulate (6)*.—KIO_4 (1.656 g, 7.2 mmol), $RuO_2 \cdot xH_2O$(400 mg, 3 mmol) and K_2CO_3 (90 mg, 0.65 mmol) were dissolved in 20 ml H_2O, and RuO_4 was extracted five times with 25 ml $CHCl_3$. The combined organic phase was added to vigorously stirred **4** (1 g, 2.88 mmol) in $CHCl_3$ (25 mL). The progress of the reaction was monitored by TLC (ethyl acetate; R_f of **4** = 0.10, R_f of **6** = 0.36). When starting material disappeared (10 minutes), the reaction was stopped by the addition of 2-propanol (0.5ml) and stirred further for 10 minutes. The residue was removed by filtration through Celite, and washed three times with 15 mL portions of $CHCl_3$. Removal of the solvent under vacuum and flash chromatography (50 g of silica gel, ethyl acetate) yielded pure **6** (643 mg, 65%) as a colourless foam; ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 1.36, 1.37 (2s, 2 × 3H, $C(CH_3)_2$), 2.00 (dd, 1H, $J_{2,3ax}$ 6.90, $J_{3ax,3eq}$ 12.68, 3-H_{ax}), 2.10 (s, 3H, N–$COCH_3$), 2.90(dd, 1H, $J_{2,3eq}$ 2.50, 3-H_{eq}), 3.43 (ddd, 1H, $J_{6,7}$ 1.50, $J_{7,OH}$ 5.00, $J_{7,8}$ 8.00, 7-H), 3.76 (s, 3H, $COOCH_3$), 3.78 (dd, 1H, $J_{5,6}$ 11.00, 6-H), 4.05 (dd, 1H, $J_{8,9a}$ 5.20, $J_{9a,9b}$ 9.00, 9-Ha), 4.14 (dd, 1H, $J_{8,9b}$ 6.20, 9-Hb), 4.32 (ddd, 1H, 8-H), 4.69 (dd, 1H, $J_{5,NH}$ 7.00, 5-H), 4.81 (d, 1H, 7-OH), 4.98 (dd, 1H, 2-H), 6.61 (d, 1H, N–H); ^{13}C-NMR (62.9 MHz, D_2O) : 22.96 (CH_3CO), 26.62, 26.98 [$C(CH_3)_2$), 41.51 (C-3), 52.35 ($COOCH_3$), 57.39 (C-5), 67.39 (C-9) 70.64, 74.12, 74.51 (C-6, C-7, C-8), 78.34 (C-2), 170.00 (C-1) 172.69 (CH_3CO), 201.41 (C-4); MS(70 eV, 120°C: m/z (%) = 345 (0.33) = M^+ = 330 (2.81) = (M^+–CH_3).

Anal. Calcd for $C_{15}H_{24}NO_8$ (345.30): C, 52.13; H, 6.95; N, 4.05. Found : C, 52.24; H, 6.90; N, 4.01.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene*-D-glycero-D-talononate *(7)*.—Method A: Compound **6** (627 mg, 1.82 mmol) in 15 mL of absolute MeOH was cooled to 0°C for 15 min and BH_3–NH_3 (62 mg, 2 mmol) was added. The reaction mixture was stirred at the same temperature until the reaction was complete (20 min). The progress of the reaction was monitored by TLC (ethyl acetate; R_f of **4** = 0.36, R_f of **7** = 0.1). The solvent was removed under vacuum at 0°C and subsequent flash chromatography (60 g of silica gel, $CHCl_3$: acetone, 1:1) yielded **7** (250 mg, 40%) and **4** (360 mg, 57%). During the flash chromatography **7** eluted first followed later by **4**.

Method B: Compound **6** (67 mg, 0.19 mmol) in absolute MeOH (5 mL) was cooled to 0°C for 15 min, lithium tri-*tert*-butoxyaluminohydrate (0.11 ml, 0.38 mmol) was added at the same temperature, and the solution was stirred until the reaction was complete (20 min); TLC, $CHCl_3$: acetone, 1:1; R_f of **4** = 0.28, R_f of **7** = 0.41). Removal of the solvent under vacuum at 0°C and flash chromatography yielded **7** (25 mg, 37%) and **4** (38 mg, 56.4%) ; ^{1}H-NMR of **7** (400 MHz, $CDCl_3$): δ 1.37, 1.40 (2s, 2 × 3H, $C(CH_3)_2$), 2.05 (s, 3H, N–$COCH_3$), 2.21 (ddd, 1H, $J_{2,3ax}$ 3.90, $J_{3ax,3eq}$ 15.00,

$J_{3ax,4}$ 3.10, 3-H_{ax}), 2.39 (ddd, 1H, $J_{2,3eq}$ 1.00, $J_{3eq,4}$ 3.00, 3-H_{eq}), 3.49 (ddd, 1H, $J_{6,7}$ 1.50, $J_{7,OH}$ 4.50, $J_{7,8}$ 7.90, 7-H), 3.60 (m, 1H, 4-OH), 3.78 (s, 3H, $COOCH_3$), 3.79 (dd, 1H, $J_{5,6}$ 11.00, 6-H), 3.98 (ddd, 1H, $J_{4,5}$ 2.50, $J_{5,NH}$ 8.50, 5-H), 4.02 (dd, 1H, $J_{8,9a}$ 6.00, $J_{9a,9b}$ 8.50, 9-Ha), 4.09–4.14 (m, 2H, 4-H, 9-Hb), 4.30 (ddd, 1H, $J_{8,9b}$ 6.50, 8-H), 4.48 (d, 1H, 7-OH), 4.53 (dd, 1H, 2-H), 6.34 (d, 1H, N–H), $J_{4,OH}$ not determined; MS (70 eV, 130°C): m/z (%) = 332 (3.74) = (M^+-15).

Anal. Calcd for $C_{15}H_{25}NO_8$(347.3): C, 51.80; H, 7.19; N, 4.03. Found : C, 51.73; H, 7.15; N, 4.07.

*Methyl 5-acetamido-2,6-anyhdro-3,5-dideoxy-4,7-di-*O*-acetyl-8,9-*O*-isopropylidene-*D-glycero-D-*talononate (8)*.—Compound **7** (350 mg, 1 mmol) was acetylated with acetic anhydride (10 mL), and absolute pyridine (10 mL) by stirring at room temperature for 3 days or at 60°C for 6 h. The reaction was monitored by TLC ($CHCl_3$:acetone, 2:1, R_f of **8** = 0.36). Removal of the solvent under vacuum and co-evaporation of the residue with absolute toluene (2 × 10 mL) yielded crude product which was purified by flash chromatography ($CHCl_3$:acetone, 2:1). Yield: pure **8** (390 mg, 90%) as a colourless foam; ^{1}H-NMR (400 MHz, $CDCl_3$): δ1.35.1.38 (2s, 2 × 3H, $C(CH_3)_2$), 1.93, 2.04, 2.13 (3s, 3 × 3H, 3 × $COCH_3$), 2.18 (ddd, 1H, $J_{2,3ax}$ 4.00, $J_{3ax,4}$ 2.00, $J_{3ax,3eq}$ 14.50, 3-H_{ax}), 2.65 (ddd, 1H, $J_{2,3eq}$ 1.75, $J_{3eq,4}$ 3.50, 3-H_{eq}), 3.75 (s, 3H, $COOCH_3$), 3.94 (dd, 1H, $J_{8,9a}$ 8.00, $J_{9a,9b}$ 8.00, 9-Ha), 4.08 (dd, 1H, $J_{8,9b}$ 6.50, 9-Hb), 4.43 (m, 2H, 5-H, 6-H), 4.45 (m, 2H, 2-H, 8-H), 5.05 (ddd, 1H, $J_{4,5}$ 2.00, 4-H), 5.35 (d, 1H, $J_{5,NH}$ 9.50, N–H), 5.40 (dd, 1H, $J_{6,7}$ 2.00, $J_{7,8}$ 4.00, 7-H), $J_{5,6}$ not determined; MS (70 eV, 140°C): m/z (%) = 416 (20.54) = (M^+–CH_3).

Anal. Calcd for $C_{19}H_{29}NO_{10}$ (431.3): C, 52.86; H, 6.72; N, 3.24. Found : C, 52.77; H, 6.79; N, 3.18.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-*D-glycero-D-*talononate (9)*.—Compound **8** (25 mg, 0.06 mmol) was dissolved in 3 mL 80% CH_3COOH and the solution stirred at 60°C for 1 h. The solvent was removed under vacuum, and the residue was co-evaporated with absolute MeOH (2 × 5 mL) and dried at 40°C under vacuum for 1 h. The product was acetylated with absolute pyridine (2 mL) and acetic anhydride (2 mL) by stirring at room temperature for 3 days. The solvent was removed under vacuum, the residue was co-evaporated with absolute toluene (2 × 5 mL), and the product was purified by flash chromatography (10 g silica gel, ethyl acetate; R_f of **9** = 0.25). Yield of **9** (27 mg, 98%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.92, 2.03, 2.05, 2.12, 2.17 (5s, 5 × 3H, 5 × $COCH_3$), 2.20 (ddd, 1H, $J_{2,3ax}$ 2.50, $J_{3ax,3eq}$ 15.00, $J_{3ax,4}$ 2.80, 3-H_{ax}), 2.60 (ddd, 1H, $J_{2,3eq}$ 1.50, $J_{3eq,4}$ 3.50, 3-H_{eq}), 4.15 (ddd, 1H, $J_{8,9a}$ 5.50, $J_{9a,9b}$ 12.00, 9-Ha), 4.27(ddd, 1H, $J_{4,5}$ 3.00, $J_{5,NH}$ 10.50, 5-H), 4.41–4.46 (m, 2H, 8-H, 9-Hb), 4.49 (dd, 1H, $J_{5,6}$ 10.50 $J_{6,7}$ 2.00, 6-H), 5.05 (ddd, 1H, 4-H), 5.40 (m, 3H, 2-H, 7-H, N-H), $J_{7,8}$ and $J_{8,9b}$ not determined; ^{13}C-NMR (62.9 MHz, $CDCl_3$): δ 20.70, 20.72, 20.78, 22.14, 23.19 (5 × CH_3CO), 30.54 (C-3), 45.66 ($COOCH_3$), 51.85 (C-5), 62.33 (C-9), 67.96, 69.90, 70.10 (C-6, C-7, C-8), 77.00 (C-2), 3 × 169.53, 169.98, 170.45, 171.37 (C-1, 5 × CH_3CO); MS (70 eV, 150°C): m/z (%) = 475 (8.5) = (M^+).

Anal. Calcd for $C_{20}H_{29}NO_{12}$ (475.3): C, 50.49; H, 6.10; N, 2.92. Found : C, 50.41; H, 6.19; N, 3.02.

Sodium or ammonium 5-acetamido-2,6-anhydro-3,5-dideoxy-D-glycero-D-*talo-nonate (10 or 11).*—Method A: Compound **8** (60 mg, 0.14 mmol) was co-evaporated with absolute MeOH (3 mL) and dried under vacuum at 40°C for 1 h. The residue it was dissolved in absolute MeOH (2 mL) and 0.28 mmol of freshly prepared CH_3ONa in CH_3OH was added. The reaction mixture was kept at 4°C for 14 h. The extent of acetyl deblocking was monitored by TLC (ethyl acetate). After removal of the solvent under vacuum, the residue was co-evaporated with absolute MeOH (3 × 5 mL), and dissolved in water (5 mL), and stirred at room temperature until the deblocking of ester was complete (4 h). TLC (isopropanol:water:acetic acid, 15:4:0.5). The reaction mixture was diluted with water (10 mL) and stirred further with 500 mg Amberlyst $15H^+$ (pH 3) for 2 h. Filtration of the resin followed by evaporation of the solvent under vacuum yielded a mixture of **10** (80%) and 1,4-lactone (20%) (observed from ^{1}H-NMR [14]). The 1,4-lactone was cleaved by treating the crude product mixture (25 mg) in 5 mL water with barium hydroxide (94.5 mg, 0.3 mmol) and stirring at room temperature for 0.5 h. Barium was removed in the form of $BaSO_4$ by treating the above reaction mixture with 0.05 M $H_2SO_4 \cdot BaSO_4$ was removed by centrifugation and filtration. The product was purified by chromatography on a Flosisil (200–300 mesh) column (5 g) eluted with CH_2Cl_2: MeOH: 0.2 N NH_3 in water, 4:4:1, followed by chromatography on 5 g Dowex 50 NH_4^+ form (50–100 mesh) using water as an eluant. Removal of water under vacuum and lyophilization yielded pure **11** (15 mg, 35%).

Method B: Compound **9** (60 mg, 0.126 mmol) in absolute MeOH (3 mL) and 0.143 mmol freshly prepared CH_3ONa in absolute MeOH was kept at 4°C for 14 h. Deblocking of the acetyl groups was confirmed by TLC (ethyl acetate: MeOH, 9:1; $R_f = 0.1$ or isopropanol : water : acetic acid, 15:4:0.5; $R_f = 0.51$). Solvent was removed under vacuum and the residue was co-evaporated with absolute MeOH (2 × 5 mL). The residue was dissolved in 5 mL of water and the wolution was stirred at room temperature until the reaction was complete; TLC (isopropanol : water : acetic acid, 15:4:0.5, R_f of **10** = 0.2). The reaction mixture was neutralised by addition of solid CO_2. Removal of water under vacuum and lyophilization yielded **10** (25 mg, 92%).

Method C: Compound **8** (35 mg, 0.1 mmol) in 80% CH_3COOH (2 mL) was stirred at 60°C for 1 h. The solvent was removed under vacuum and the residue was co-evaporated with absolute MeOH (2 × 5 mL). The residue was dissolved in 1 N NaOH (2 mL) and the solution was stirred at room temperature until the reaction was complete (4 h); TLC (ethyl acetate: MeOH, 9:1). The reaction mixture was neutralised with solid CO_2 and purified by chromatography over 5g Dowex Na^+ form (50–100 mesh). Removal of water under vacuum and lyophilization yielded **11** (18 mg, 83%), ^{1}H-NMR (400 MHz, D_2O): δ 2.00 (s, 3H, N–$COCH_3$), 2.23 (ddd, 1H, $J_{2,3ax}$ 6.10, $J_{3ax,3eq}$ 15.40, $J_{3ax,4}$ 2.75, 3-H_{ax}), 2.46 (ddd, 1H, $J_{2,3eq}$ 1.75, $J_{3eq,4}$ 4.00, 3-H_{eq}), 3.66 (dd, 1H, $J_{5,6}$ 10.00, $J_{6,7}$ 2.00, 6-H), 3.71 (dd, 1H, $J_{8,9a}$ 6.00 $J_{9a,9b}$ 12.00, 9-Ha), 3.90 (ddd, 1H, $J_{7,8}$ 6.20, $J_{8,9b}$ 2.50, 8-H), 3.94 (dd, 1H, 9-Hb), 4.10 (dd, 1H, $J_{4,5}$ 3.00, 5-H), 4.20 (ddd, 1H, 4-H), 4.29 (dd, 1H, 2-H), 4.31 (dd, 1H, 7-H); ^{13}C-NMR (100.6 MHz, D_2O) : 26.23 (CH_3CO), 37.09 (C-3), 53.21 (C-5), 67.15 (C-9), 69.77, 72.90, 73.15, 75.53 (C-4, C-6 C-7, C-8), 76.01 (C-2), 178.62 (CH_3CO). MS(70 eV, 180°C); $m/z(\%) = 310\ (27.4) = M^+$.

Anal. Calcd for $C_{11}H_{22}N_2O_8$ (310.3): C, 42.54; H, 7.09; N, 9.02. Found : C, 42.71; H, 7.15; N, 9.05.

Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-4-O-((4-methyl phenoxy) thiocarbonyl)-8,9-O-isopropylidene-D-erythro-L-*mannononononate (12)*.—Compound **4** (347 mg, 1 mmol) in absolute pyridine (10 mL) and O-*p*-tolyl chlorothioformate (0.2 mL, 1.3 mmol) was stirred at room temperature for 1 h. The progress of the reaction was monitored by TLC (ethyl acetate, R_f of **12** = 0.68). Removal of the solvent under vacuum and co-evaporation of the residue with absolute toluene (2 × 10 mL) followed by flash chromatography (50 g silica gel, ethyl acetate) yielded pure **12** (320 mg, 64%) ; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.38, 1.40 (2s, 2 × 3H, $C(CH_3)_2$), 2.02 (s, 3H, N–$COCH_3$), 2.20 (ddd, 1H, $J_{2,3x}$ 6.25, $J_{3ax,3eq}$ 13.50, $J_{3ax,4}$ 10.50, 3-H_{ax}), 2.30 (s, 3H, Ar–CH_3), 2.70 (ddd, 1H, $J_{2,3eq}$ 1.50, $J_{3eq,4}$ 5.50, 3-H_{eq}), 3.48 (m, 1H, 7-H), 3.65 (dd, 1H, $J_{5,6}$ 10.00, $J_{6,7}$ 2.00, 6-H), 3.80 (s, 3H, $COOCH_3$), 4.04 (dd, 1H, $J_{8,9a}$ 6.00, $J_{9a,9b}$ 9.00, 9-Ha), 4.10 (dd, 1H, $J_{8,9b}$ 6.60, 9-Hb), 4.20 (ddd, 1H, $J_{4,5}$ 10.50, $J_{5,NH}$ 8.50, 5-H), 4.30 (ddd, 1H, $J_{7,8}$ 6.50, 8-H), 4.49(d, 1H, $J_{7,OH}$ 5.00, 7-OH), 4.73 (dd, 1H, 2-H), 5.81 (ddd, 1H, 4-H), 6.28 (d, 1H, N–H), 6.94, 6.96, 7.21, 7.23, (4s, 4H, Ar–H); MS (70 eV, 170°C): m/z (%) = 466 (14.74) = $M^+ - 29$.

Anal. Calcd for $C_{23}H_{31}NO_9S$ (497.3): C, 55.50; H, 6.23; N, 2.81; S, 6.43. Found : C, 55.41; H, 6.12; N, 2.79; S, 6.35.

Methyl 5-acetamido-2,6-anhydro-3,4,5-trideoxy-8,9-O-isopropylidene-D-erythro-L-*mannononononate (13)*.—Compound **12** (185 mg, 0.37 mmol) was dissolved in absolute toluene (50 mL), Bu_3SnH (0.294 mL, 1.11 mmol, 3 equivalent), and AIBN (10 mg) were added and reaction mixture was stirred at 80–90°C for 4 h. The progresss of the reaction was monitored by TLC (ethyl acetate, R_f of **13** = 0.25, R_f of **12** = 0.68). The solvent was removed under vacuum and the product was purified by flash chromatography (20 g silica gel, ethyl acetate). Yield of **13** = 65 mg (53%); ^{1}H-NMR (400 MHz, $CDCl_3$:δ1.31, 1.33 (2s, 2 × 3H, $C(CH_3)_2$), 1.45(q, 1H, $J_{3ax,4a} = J_{4a,4b} = J_{4a,5}$ = 12.80, $J_{3eq,4a}$ 5.00, 4-Ha), 1.86–1.98 (m, 5H, 3-H_{ax}, 4-Hb, N–$COCH_3$), 2.16 (m, 1H,$J_{2,3eq}$ 1.50, $J_{3ax,3eq}$ 15.10, $J_{3eq,4b}$ 4.40, 3-H_{eq}), 3.45 (dd, 1H, $J_{5,6}$ 10.50, $J_{6,7}$ 1.50, 6-H), 3.50 (ddd, 1H, $J_{7,8}$ 6.50, $J_{7,OH}$ 2.30, 7-H), 3.88 (q, 1H, $J_{4b,5}$ 3.40, $J_{5,NH}$ 8.50, 5-H), 3.97 (dd, 1H, $J_{8,9a}$ 6.20, $J_{9a,9b}$ 9.00, 9-Ha), 4.04 (dd, 1H, $J_{8,9b}$ 6.80, 9-Hb), 4.16 (d, 1H, 7-OH). 4.23 (ddd, 1H, 8-H), 4.46 (dd, 1H, $J_{2,3ax}$ 5.10, 2-H), 5.42 (d, 1H, N–H), $J_{3ax,4b}$ not determined.

Anal. Calcd for $C_{15}H_{25}NO_7$ (331.3): C, 54.33; H, 7.55; N, 4.23. Found : C, 54.37; H, 7.44; N, 4.29.

Methyl 5-acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-4-O-[(4-methyl phenoxy) thiocarbonyl]-D-erythro-L-*mannononoate (14)*.—Compound **12** (20 mg, 0.04 mmol) in 80% CH_3COOH (3 ml) was stirred at 60°C for 1 h to cleave the isopropylidene group; TLC (ethyl acetate), R_f of **12** = 0.68 and after cleavage of isopropylidene, R_f = 0.1. Solvent was removed under vacuum, and the residue was co-evaporated with absolute MeOH (2 × 5 mL) and dried at 40°C under vacuum for 30 min. The residue was acetylated with absolute pyridine (1 mL), acetic anhydride (1 mL) at 60°C for 6 h. Removal of the solvent under vacuum, co-evaporation of the residue with absolute toluene (2 × 5 mL) and purification by flash chromatography (5g of silica gel, ethyl acetate) yielded pure **14** (15 mg, 63%); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 1.92, 2.05, 2.14, 2.16 (4s, 4 × $COCH_3$), 2.23 (m, 1H, 3-H_{ax}), 2.62 (ddd, 1H, $J_{2,3eq}$ 1.30, $J_{3ax,3eq}$

13.00, $J_{3eq,4}$ 4.90, 3-H_{eq}), 3.76 (s, 3H, $COOCH_3$), 4.09 (ddd, 1H, $J_{4,5} = J_{5,NH} = J_{5,6}$ = 10.00, 5-H), 4.11 (dd, 1H, $J_{8,9a}$ 6.00, $J_{9a,9b}$ 12.00 9-Ha), 4.15 (ddd, 1H, $J_{7,8}$ 6.00, $J_{8,9b}$ 6.80, 8-H), 4.32 (dd, 1H, 9-Hb), 4.65 (dd, 1H, $J_{6,7}$ 1.00, 7-H), 4.86 (ddd, 1H, $J_{3ax,4}$ 11.60, 4-H), 5.30–5.42 (m, 3H, 2-H, 6-H, N–H), 6.97, 7.00, 7.15, 7.18 (4s, 4H, Ar–H), $J_{2,3ax}$ not determined.

Anal. Calcd for $C_{25}H_{33}NO_{12}S$ (571.3): C, 52.51; H, 5.78; N, 2.45; S, 5.60. Found : C, 52.40; H, 5.71; N, 2.41; S, 5.53.

Sodium methyl-5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-erythro-L-manno-*nonoate (15)*.—Compound **13** (45 mg, 0.14 mmol) in 80% CH_3COOH (4 mL) was stirred at 60°C for 1 h. The solvent was removed under vacuum and the residue was co-evaporated with absolute MeOH (3 × 5 mL). NaOH (1N, 2 mL) was added and the solution was stirred at room temperature for 4 h until the reaction was complete; TLC (ethyl acetate, R_f of **15** = 0.01). The reaction mixture was neutralised by addition of solid CO_2. The product was purified by chromatography over 5g Dowex Na^+ form packed in a column and eluted with water (50 mL). Removal of water under vacuum and lyophilization yielded pure **15** (35 mg, 86%) as a white solid; ^{1}H-NMR (250 MHz, D_2O): δ 1.45 (m, 1H, 4-Ha), 1.85–2.10 (m, 5H, 3-H_{ax}, 4-Hb, N–$COCH_3$), 2.21 (m, 1H, 3-H_{eq}), 3.58 (dd, 1H, $J_{8,9a}$ 6.75, $J_{9a,9b}$ 11.50, 9-Ha), 3.65 (ddd, 1H, $J_{4a,5}$ 9.50, $J_{4b,5}$ 2.20, $J_{5,6}$ 9.50, 5-H), 3.74–3.94 (m, 4H, 6-H, 7-H, 8-H, 9-Hb), 4.48 (dd, 1H, $J_{2,3ax}$ 5.00, $J_{2,3eq}$ 1.50, 2-H); ^{13}C-NMR (62.9 MHz, D_20) : 24.60 ($COCH_3$), 27.1, 29.07 (C-3, C-4), 47.84 (C-5), 65.64 (C-9), 70.99, 73.77, 75.72 (C-6, C-7, C-8), 77.52 (C-2), 176.78, 179.70 (C-1, CH_3CO); MS(70 eV, 170°C) : $m/z(\%) = 299\ (31.7) = M^+$.

Anal. Calcd for $C_{11}H_{18}NO_7Na$ (299.3); C, 44.10; H, 6.01; N, 4.68. Found : C, 43.96; H, 6.08; N, 4.72.

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