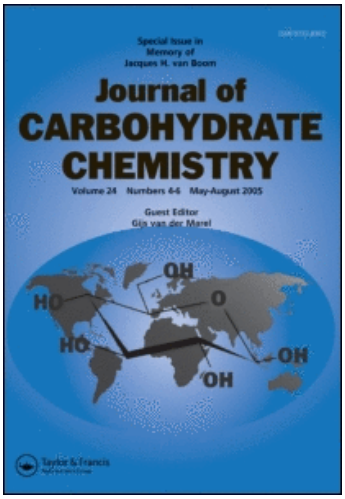

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A NEW SYNTHESIS OF *N*-ACETYL-4-DEOXY-D-NEURAMINIC ACID

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ABSTRACT

The title compound was prepared in eight steps starting from 2-acetamido-2-deoxy-D-mannose. This commercially available compound was first transformed into known 3-acetamido-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (**2**) and then, into 3-acetamido-4,5,6,7-tetra-*O*-acetyl-2,3-dideoxy-D-*manno*-heptose (**6b**). Using a Wittig-Wadsworth-Emmons reaction, aldehyde **6b** was then converted into ethyl 5-acetamido-6,7,8,9-tetra-*O*-acetyl-3,4,5-trideoxy-2-(*t*-butyldimethyl)silyloxy-D-*manno*-nonenoate (**8**). After *O*-deacetylation, saponification of the ester group and simultaneous deprotection of the silyloxy enol ether, *N*-acetyl-4-deoxy-D-neuraminic acid **1** was obtained in an overall yield of about 30% from **2**.

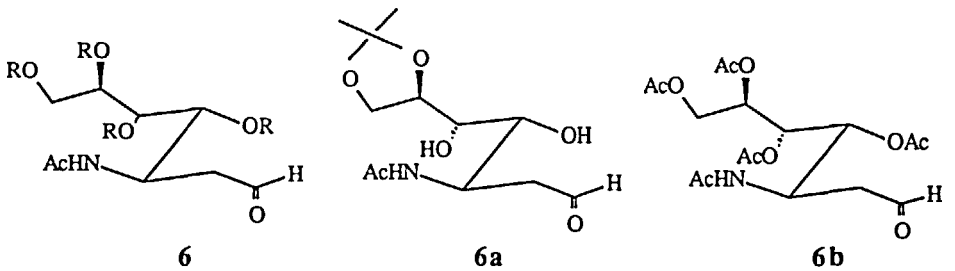
INTRODUCTION

Sialic acids, as constituents of glycoproteins and glycolipids, are of considerable importance for several biological phenomena.[1] Analogues, derivatives and their glycosides have received a great deal of attention over the past few years. In this context two syntheses of title compound **1** have recently appeared in the literature. The first, reported by Brossmer and Hagedorn,[2] consisted of a selective removal of the C_4 hydroxyl function of *N*-acetylneuraminic acid by successive transformation into mesylate, iodination

and hydrogenolysis. The second, due to Vasella's group [3] is an exploitation of the author's synthesis of *N*-acetylneuraminic acid and *N*-acetyl-4-epineuraminic acid, planned to permit modifications in (C_1) to (C_6) or (C_6) to (C_9). We wish to report here a new synthesis of compound **1** which follows a different approach.

RESULTS AND DISCUSSION

As part of a program directed toward the preparation of inhibitors of neuraminidases, we planned a synthesis of the *N*-acetylneuraminic acid backbone using the aldehyde **6**, whose hydroxylic functions would be masked, as an intermediate. Some years ago a preparation of **6a** was described.[4] This compound could have been a precursor of our target molecule **6**. However as **6a** was obtained simultaneously with its epimer in C_3, we decided to develop an access to the more convenient fully protected aldehyde **6b**.

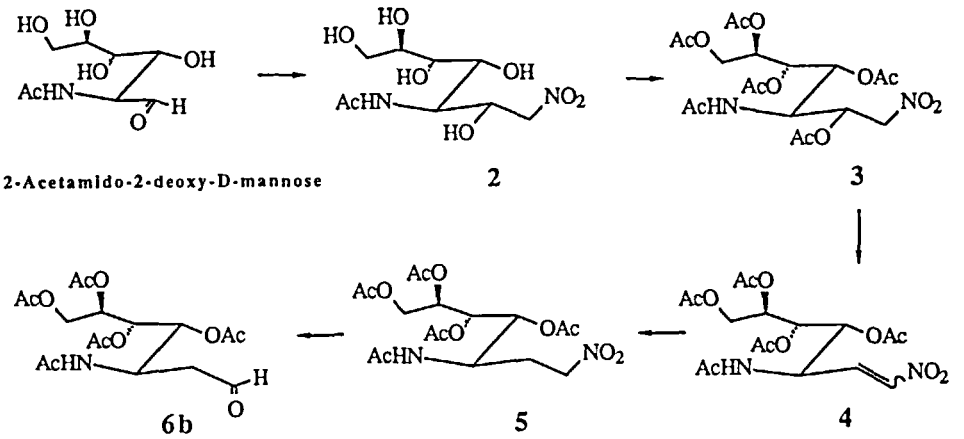


6 **6a** **6b**

R = protecting group

The commercially available 2-acetamido-2-deoxy-D-mannose was first transformed into 3-acetamido-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (**2**) according to Benzing-Nguyen and Perry [5] (Yield 55-65%) (Scheme 1). We then followed a sequence similar to that used by these authors in order to introduce the (C_2) atom into *N*-acetylneuraminic acid.[5] Thus **2** was first converted into the corresponding pentaacetate **3** (Ac_2O, cat. H^+; 86%). The β-elimination of acetic acid ($NaHCO_3$, toluene, Δ ; 91%) furnished 3-acetamido-4,5,6,7-tetra-*O*-acetyl-1,2,3-trideoxy-1-nitro-D-*manno*-1-heptenitol (**4**) and after reduction ($NaBH_4$, EtOH; 93%) 3-acetamido-4,5,6,7-tetra-*O*-acetyl-1,2,3-trideoxy-1-nitro-D-*manno*-heptitol (**5**).

To convert the nitro compound **5** into 3-acetamido-4,5,6,7-tetra-*O*-acetyl-2,3-dideoxy-D-*manno*-heptose (**6b**), we used a modified Nef reaction following the procedure of Steliou and Poupart.[6] Compound **6b** was obtained in a reproducible yield of 60-65% in addition to a small amount (10-15%) of recovered starting material **5**. Finally, the



SCHEME 1

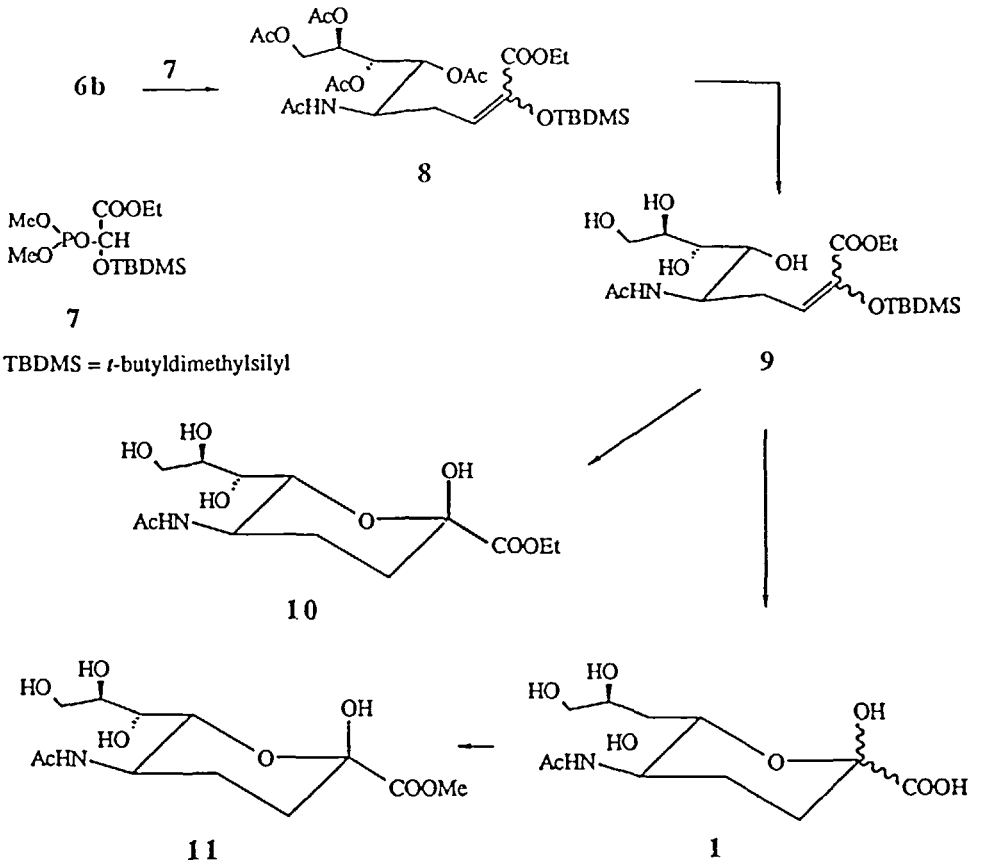
four- step sequence from **2** to **6b** could be carried out without silica-gel chromatographic purifications of intermediates in an overall yield of about 50% on a gram-scale.

The remaining two carbons of the neuraminic acid skeleton were then introduced by a Wittig-Wadsworth-Emmons reaction (Scheme 2). This approach had already been followed to prepare neuraminic acid itself, [7] analogues [8] or glycosides .[9] In our case, the utilisation of ethyl 2-(*t*-butyldimethyl)silyloxy-2-(dimethyl phosphono) acetate (**7**)[10] permitted us to carry out the deprotection of the silyloxy enol ether resulting from the Wittig reaction whether selectively or simultaneously with the ester function in a basic medium. As a result we were able to avoid acidic conditions to which α-keto-acids are rather sensitive.[11]

Thus the reaction between the aldehyde **6b** and the ylid prepared from **7** using lithium bis(trimethylsilyl)amide as a base, led to a mixture of stereoisomers **8E** and **8Z** (E/Z ratio = 76/24) in a combined yield of 84%. The de-*O*-acetylation of these compounds was carried out under the usual conditions (cat. MeONa, MeOH) and furnished the corresponding ethyl 5-acetamido-3,4,5-trideoxy-2-(*t*-butyldimethyl)silyloxy-D-*manno*-2-nonenoate (**9**) in 80% yield.

The selective deprotection of the ketonic function in **9** could be realized without difficulty [12] (Et_3N, 3HF ; MeOH, 20 °C) leading to ethyl 5-acetamido-3,4,5-trideoxy-D-*manno*-2-nonulopyranosonate (**10**) in 85% yield after silica gel chromatography.

Saponification of the ester group of **9** and simultaneous deprotection of the silyloxy enol ether, followed by neutralisation (NaOH 1N/MeOH ; Dowex 50 H^+) furnished **1**. The



SCHEME 2

crude product was observed migrating as a single spot on TLC (SiO_2 ; EtOH/AcOH/H_2O : 8:2:1 ; R_f=0.4) and gave only one signal (at 3.56 min compared to 4.0 min for *N*-acetylneuraminic acid) in HPLC (Carbopac 25 cm x 4 mm pellicular anion exchange column. Pulsed Amperometric Detector. Eluent : sodium acetate 150 mM, sodium hydroxyde 100 mM).[13] The ^1H and ^{13}C NMR characteristics of the product were in good agreement with reported data.[2,3] However the ^{13}C NMR spectrum showed the presence of a minor compound (< 10%) with chemical shifts very close to those of the main product (see Table-2). It was also found that the optical rotation was lower than reported values (see Experimental section).

Even if the presence of an impurity had not been detected in the ^{13}C NMR spectra of the intermediates, particularly in **10**, partial epimerization could have occurred during

TABLE 1 : ^1H NMR Spectral Data for compounds 3-6b, 8-11 and 1

	AcNH	H_1 $H_{1'}$	H_2 $H_{2'}$	H_3	H_4	H_5	H_6	H_7 $H_{7'}$	AcO	AcNH
3	5.98 d J=10.2	ABX 4.55 4.38 dd dd J=13.1 J=13.1 3.9 7.7	5.53 ddd J=7.8 3.9 1.4	4.55 m	5.32 dd J=10.5 1.5	5.37 dd J=7.9 1.5	5.03 ddd J=8.0 5.6 2.9	ABX 4.26 3.98 dd dd J=12.5 J=12.5 2.9 5.6	2,08 s(6H) 2.05 s(3H) 2,02 s(6H)	1,99 s
4	6.68 d J=9.0	7.1 d J=13.2	7.04 dd J=13.2 6.5	4.97 ddd J=9.0 7.1 6.5	5.30 dd J=7.1 2.5	5.44 dd J=8.1 2.5	5.13 ddd J=8.1 5.5 2.6	ABX 4.25 4.07 dd dd J=12.5 J=12.5 2.6 5.5	2.06 s(6H) 2.01 s(3H) 2.00 s(3H)	1.95 s
5	5.73 d J=9.8	ABX_2 4.42 4.37 J=14.1 6.6	2.3-2.1 m	4.26 m	5.21 dd J=8.0 2.7	5.40 dd J=8.4 2.7	5.10 ddd J=8.4 5.5 2.9	ABX 4.25 4.05 dd dd J=12.5 J=12.5 2.9 5.5	2.23(s) 2.12(s) 2.06(s) 2.04(s)	1.95 s
6b	6.27 d J=9.70	9.69 d J=1.0	ABXX' 2.70 ddd J=18.2 4.9 1.0 2.60 dd J=18.2 4.5	4.49 m	5.37 dd J=9.4 2.5	5.42 dd J=8.8 2.5	5.10 ddd J=8.8 5.7 2.9	ABX 4.24 4.02 dd dd J=12.5 J=12.5 2.9 5.7	2.16(s) 2.13(s) 2.09(s) 2.07(s)	1.96 s

TABLE 1 (continued)

	AcNH	H_3	H_4	$H_{4'}$	H_5	H_6	H_7	H_8	H_9	$H_{9'}$	AcO	AcNH	Si
(1) **8E**	6.10 d J=9.3	5.34 dd J=10.6 7.4	2.63 dt J=14.2 10.6	2.30 ddd J=14.2 7.4 3.6	4.3-4.2 m	5.18 dd J=7.6 3.1	5.38 dd J=7.8 3.1	5.08 ddd J=7.8 6.2 2.9	ABX 4.25 dd J=12.3 2.9	4,03 dd J=12,3 6,2	2.10 s(3H) 2.07 s(3H) 2.02 s(6H)	1,85 s	0.89 s(9H) 0.08 s(3H) 0.07 s(3H)
(1) **8Z**	5.69 d J=9.5	5.91 t J=7.3	2.41 ddd J=15.7 7.3 4.1	2.23 ddd J=15.6 9.4 7.3	4.3-4.2 m	5.20 dd J=8.2 3.0	5.34 dd J=8.1 3.0	5.05 ddd J=8.1 6.0 2.9	ABX 4.25 dd J=12.4 2.9	4.03 dd J=12.4 6.0	2.07(s) 2.04(s) 2.00(s) 1.99(s)	1.86 s	0.88 s(9H) 0.08 s(3H) 0.07 s(3H)
(1) **9E**	7.23 d J=7.7	5.44 dd J=9.9 7.5	2.76 ddd J=14.5 9.9 1.5	2.64 ddd J=14.5 7.5 1.5	3.60 m(1H) 3.90-3.70 m(3H)				3.58 broad d J=8.1	3.40 broad d J=8.1		1.87 s	0.88 s(9H) 0.08 s(3H) 0.07 s(3H)
(1)(2) **10**	8.26 d J=7.9		2.21-2.08 m(4H)		4.29-4.15 m(2H)		3.78 d J=8.9	3.96 ddd J=8.9 6.4 2.6	4.05 dd J=11.8 2.6	3.82 dd J=11.8 6.4		2.21 s	
(2)(3) **11**	8.04 d J=7.8		2.10-1.80 m(4H)		4.05-4.39 m(2H)		3.54 d J=9.1	3.72 ddd J=8.8 6.4 2.4	3.80 dd J=11.7 2.6	3.59 dd J=11.7 6.3		1.98 s	
(2) **1**			2.20-2.01 m(4H)		4.20-4.10 m(2H)		3.74 d J=8.6	3.96 ddd J=8.6 6.2 2.6	4.03 dd J=11.6 2.6	3.79 dd J=11.6 6.2		2.19 s	

(1) $-OCH_2CH_3$: **8, 9** : 4.14, q, J=7.1 ; 1.3,t **10** : 4.51, q ; 4.46, q,(J=7.1) ; 1.51,t . (2) D_2O (3) OCH_3 : 3.80s

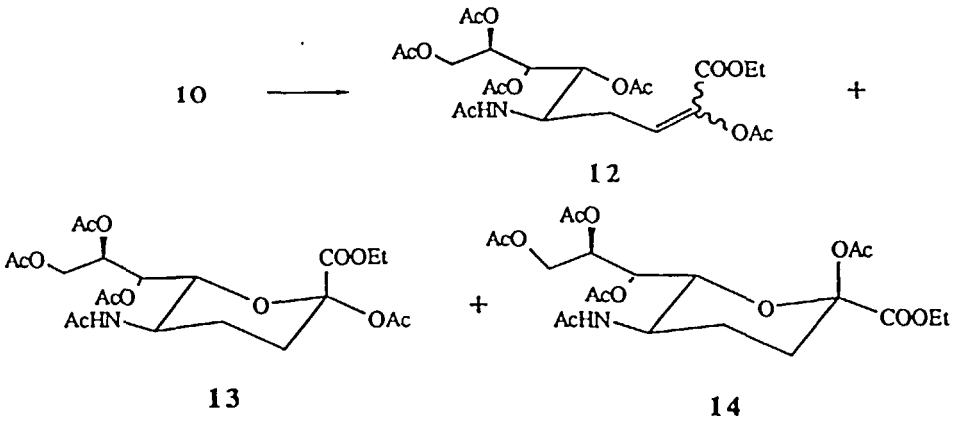
TABLE 2 : ^{13}C NMR Spectral Data for compounds 3-6b, 8-11 and 1

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	O C-CH_3	O NH-C-CH_3	O O-C-CH_3
3	75.92 (t)*	67.58 (d)	48.69 (d)	67.90 (d)	67.50 (d)	68.89 (d)	61.93 (t)	170.74 170.68 170.13 169.99 169.91 169.47 (s)	22.97 (q)	22.19 20.90 20.71 20.67 20.47 (q)
4	136.41 (d)	141.67 (d)	47.64 (d)	68.55 (d)	68.10 (d)	70.82 (d)	61.81 (t)	170.72 170.65 169.93 (2C) 169.78 (s)	23.07 (q)	20.76 20.68 20.63 20.59 (q)
5	72.17 (t)	28.67 (t)	46.25 (d)	68.52 (d)	68.05 (d)	71.38 (d)	61.91 (t)	170.59 (2C) 169.81 (3C) (s)	22.87 (q)	20.67 20.59 20.55 (2C) (q)
6b	200.74 (d)	43.87 (t)	43.92 (d)	68.35 (d)	67.85 (d)	70.49 (d)	62.10 (t)	170.65 (2C) 170.04 169.99 169.91 (s)	23.28 (q)	20.80 (2C) 20.75 (2C) (q)

TABLE 2 (continued)

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	$\underset{\underset{O}{\|}}{C}\text{-}CH_3$	$NH\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH_3$	$O\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH_3$	$Si\begin{matrix}\diagup\\ \\ \diagdown\end{matrix}$
(1) 8E	165.34 (s)	143.32 (s)	118.55 (d)	27.93 (t)	48.53 (q)	68.66 (q)	68.55 (d)	71.83 (d)	62.02 (t)	170.59 170.30 170.08 170.03 169.99 (s)	23.25 (q)	20.86 20.84 20.73 20.69 (q)	25.45(q)(3C) 18.07(s) -5.01(q)(2C)
(1)(2) 9E	165.79 (s)	142.81 (s)	120.62 (d)	28.57 (t)	52.40 (d)	70.92 (d)	70.51 (d)	72.44 (d)	64.72 (t)	172.80 (s)	22.99 (q)		25.48(q)(3C) 18.07(s) -4.98(q) -4.96(q)
(1)(2) 10	170.71 (s)	93.64 (s)	30.61 (t)	24.72 (t)	44.54 (d)	69.64 (d)	69.01 (d)	71.38 (d)	63.60 (t)	170.31 (s)	22.45 (q)		
(2)(3) 11	170.71 (s)	93.64 (s)	30.57 (t)	24.65 (t)	44.51 (d)	69.51 (d)	68.94 (d)	71.33 (d)	63.54 (t)	170.54 (s)	22.38 (q)		
(4) 1	174.66 (s) 173.46 (5)	94.40 (s)	30.81 (t) 31.60	24.37 (t) 26.90	44.52 (d) 44.40	70.65 (d) 70.84	68.43 (d) 68.18	71.06 (d) 71.18	63.21 (t) 62.77	173.93 (s)	21.92 (q) 20.45		

* Mutliplicity : DEPT (1) O-CH_2-CH_3 **8E** 61.12(t) ; 14.03 (q) **9E** 61.25 (t) ; 14.02(q) **10** 60.87 (t) 13.98 (q). (2) (D_6) DMSO (3) OCH_3 52.04 (q) (4) D_2O. (5) Minor component.



SCHEME 3

the nitromethane condensation,[5] or later during the basic elimination of acetic acid. Unfortunately we were not able to determine the relative configurations of carbons C_5 and C_6 from the ^1H NMR data of **1**. The keto-ester **10** was then peracetylated using the procedure of Baumberger and al.[14] Treatment of **10** (Ac_2O, Pyr, 20 °C, 3 days) led to a mixture of only three detectable compounds on TLC (SiO_2 ; ethyl acetate), i.e. : **12** (R_f = 0.47), **13** (R_f = 0.29) and **14** (R_f = 0.21) (Scheme 3). Purification by silica gel chromatography permitted us to obtain a pure sample of **12** and a mixture of **13** and **14** in an overall yield of 87 %. The spectrum of **12** was in full accord with literature data for the corresponding methyl ester.[14] The spectrum of the mixture **13** + **14** was rather complicated but, fortunately, the signal for H_6 in the minor (~30 %) compound **13** was nicely resolved (4.67 dd J=10.5, 2.5 Hz) in agreement with literature reports[14] (4.71 dd J=10.5, 2.5 Hz) ensuring an axial-axial relationship between this proton and H_5.

On the basis of the results described above, in which no evidence was obtained for the presence of an impurity in appreciable quantity in **1**, we formulated the hypothesis that the minor compound detected in the ^{13}C NMR spectrum of this compound could be the α epimer. Indeed, although Vasella[3] did not mention its presence for **1**, this epimer (about 9 %) had already been reported for *N*-acetylneuraminic acid itself.[1]

One argument in agreement with this hypothesis comes from the fact that when **1** was transformed into its methyl ester **11** (abs MeOH, Dowex 50 H^+, 20 °C, 1 day)[15](Scheme 2), the ^{13}C NMR spectrum of the crude product was in perfect agreement with reported data[3] and no longer showed the presence of an impurity. This ester **11** was

obtained in an overall yield of 77 % from 9 after silica gel chromatography. The ^1H NMR characteristics of **11** and molecular rotation were also in accordance with reported literature values and its melting point was sensibly higher than the reported value.[3]

In conclusion, the sequence that we describe here, permitted us to prepare 4-deoxy-D-neuraminic acid and its ethyl ester in about 15-20% from a commercially available starting material. As part of our research program toward the preparation of neuraminidase inhibitors, we are presently introducing functional modifications in C_4 (from the aldehyde **6b**) or in C_3 (from the silyl enol ether **8**).

EXPERIMENTAL

General Methods and Material. All solvents were distilled before use : THF from Na-benzophenone, Et_2O from KOH, petroleum-ether from P_2O_5, AcOEt from K_2CO_3, pyridine from CaH_2, MeOH from Mg. All reactions were performed under a constant flow of dry nitrogen. Solutions in organic solvents were dried with anhydrous sodium sulfate and concentrated on a rotary evaporator at 40 °C/15 mm Hg (unless otherwise stated). Merck Silica-Gel 60 F_{254} (0.2 mm) was used for TLC, detection being carried out by spraying with an alcoholic solution (3%) of phosphomolybdic acid, followed by heating. Flash column chromatography [16] was performed on silica-gel Amicon 35-70 µ. Melting points were determined on a Kofler block apparatus. IR spectra were recorded with a Perkin-Elmer Model 1310 spectrophotometer (calibration : polystyrene film) and are expressed in cm^{-1}. NMR spectra were recorded in $CDCl_3$ (unless otherwise specified) on a Brüker AM 300 (300 MHz for ^1H and 75.47 MHz for ^{13}C). Chemical shifts are expressed in parts per million downfield from TMS. Coupling constants are in Hz and splitting pattern abbreviations are : s, singlet ; d, doublet ; q, quartet ; m, multiplet. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter at 20 ± 2 °C. Elemental analyses were performed by "Service Central de Microanalyses du CNRS" 69 Solaize (France).

3-Acetamido-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (2). This compound was prepared [5] from commercially available (SIGMA) 2-acetamido-2-deoxy-D-mannose (*N*-acetyl-D-mannosamine). The crude product (55-65 %) could be recrystallized from methanol : mp 202-204 °C, $[\alpha]_D$=-61° (*c* 0.75, water) (lit.[5] mp 200-202 °C; $[\alpha]_D$= -59° (*c* 0.75, water)).

3-Acetamido-2,4,5,6,7-penta-O-acetyl-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (3). To a suspension of **2** (2.51 g, 8.9 mmol) in acetic anhydride (40 mL) was added three drops of concd sulfuric acid (36 N) and the mixture

was magnetically stirred for 1 h at 80 °C, then cooled at room temperature and poured into ice-water (400 mL). Following extraction with chloroform (2x200 mL), the chloroform solution was washed successively with a saturated sodium bicarbonate solution (100 mL) and water until neutrality. Concentration of the dried extract gave a crystalline crude product which was washed with cold petroleum ether (100 mL) filtered off and dried under reduced pressure. We thus obtained 3.74 g (86 %) of **3** which could be used directly in the next step. An analytical sample of **3** was obtained after recrystallization from petroleum ether : mp 157-159 °C ; $[\alpha]_D$ = - 3.2° (*c* 0.4, chloroform); IR ($CHCl_3$) 3430, 2970, 1750, 1690, 1560, 1490, 1370, 1240, 1190, 1030.

Anal. Calcd for $C_{19}H_{28}N_2O_3$: C, 46.34 ; H, 5.73 ; O, 5.69 ; N, 42.23. Found : C, 46.28 ; H, 5.67 ; O, 5.63 ; N, 42.69.

3-Acetamido-4,5,6,7-tetra-O-acetyl-1,2,3-trideoxy-1-nitro-D-*manno*-1-heptenitol (4). A solution of **3** (8.21 g, 16.7 mmol) in toluene (300 mL) was refluxed for 2 h with sodium bicarbonate (9 g, 106 mmol). The cooled reaction mixture was filtered and the filtrate was concentrated. The crude product was pure enough for the next step. An analytical sample of **4** was obtained after recrystallisation from ethyl acetate-petroleum ether (7:3) : mp 136-138 °C $[\alpha]_D$ = - 10.4° (*c* 0.75, chloroform); IR ($CHCl_3$) 3430, 2970, 1750, 1680, 1660, 1560, 1460, 1430, 1240, 1190, 1030.

Anal. Calcd for $C_{17}H_{24}N_2O_{11}$: C, 47.22 ; H, 5.59 ; N, 6.47 ; O, 40.7. Found : C, 47.21 ; H, 5.50 ; N, 6.40 ; O, 40.62.

3-Acetamido-4,5,6,7-tetra-O-acetyl-1,2,3-trideoxy-1-nitro-D-*manno*-1-heptitol (5). A solution of **4** (1.75 g, 4 mmol) in ethanol (60 mL) was treated at -5 °C with sodium borohydride (0.6 g, 15.9 mmol), stirred during 25 min and then acidified with acetic acid (0.93 g, 14.4 mmol). When no more hydrogen was evolved, the reaction mixture was concentrated and the residue extracted with ethyl acetate (150 mL). Concentration of the filtered ethyl acetate extract afforded a crude product which could be used directly in the next synthetic step. An analytical sample of **5** was obtained after recrystallisation from ethyl acetate-petroleum ether (7:3) : mp 146-148 °C. $[\alpha]_D$ = + 31.8° (*c* 0.42, chloroform); IR ($CHCl_3$) 3430, 2960, 1750, 1680, 1560, 1500, 1460, 1430, 1370, 1250-1190, 1030.

Anal. Calcd for $C_{17}H_{26}N_2O_{11}$: C, 47.00 ; H, 6.03 ; N, 6.45 ; O, 40.51. Found : C, 46.95 ; H, 6.25 ; N, 6.32 ; O, 40.87.

3-Acetamido-4,5,6,7-tetra-O-acetyl-2,3-dideoxy-D-*manno*-heptose (6b). To a solution of **5** (1.12 g, 2.62 mmol) in methanol (25 mL) kept at - 5 °C, was added dropwise an aqueous solution of potassium hydroxide (25 mL, 0.01 M) and then an aqueous solution of potassium permanganate (35 mL, 0.05 M) containing magnesium sulfate (0.47 g, 1.94 mmol). The reaction mixture was stirred 15 min after the end of the

addition and then filtered on celite. The filtrate was saturated with sodium chloride and extracted with ethyl acetate (5 x 80 mL). Concentration of the dried extracts gave a crude product which was purified by chromatography on a column of silica gel (100 g) (eluent : ethyl acetate). We thus obtained 0.133 g (12 %) of recovered starting material **5** and 0.680 g (65 %) of **6b**. (A more polar minor compound was not identified). Aldehyde **6b** was a slightly yellow foam $[\alpha]_D$ = + 19.8° (*c* 0.57, chloroform); IR ($CHCl_3$) 3440, 2960, 2850, 2740, 1760, 1690, 1500, 1460, 1240-1200, 1030.

Anal. Calcd for : $C_{17}H_{25}NO_{10}$; C, 50.62 ; H, 6.20 ; N, 3.47 ; O, 39.70. Found : C, 50.66; H, 6.49 ; N, 3.38 ; O, 39.42.

Ethyl 5-acetamido-6,7,8,9-tetra-O-acetyl-3,4,5-trideoxy-2-(*t*-butyl dimethyl)silyloxy-D-*manno*-2-nonenoate (8). To a solution of ethyl 2-(*t*-butyldimethyl)silyloxy-2-(dimethyl phosphono) acetate (**7**) (0.6 g, 1.76 mmol) in dry tetrahydrofuran (10 mL) cooled at - 40 °C, was added dropwise a commercially available (JANSSEN) molar solution of lithium bis(trimethylsilyl)amide in tetrahydrofuran (1.75 mL). The mixture was then warmed to 0 °C and a solution of the aldehyde **6b** (0.645 g, 1.6 mmol) in tetrahydrofuran (6 mL) was added in 15 min and the reaction was allowed to proceed for 2 h at room temperature. After hydrolysis with a saturated aqueous solution of ammonium chloride (12 mL) and extraction of the aqueous phase with diethyl ether (3 x 15 mL), the combined organic extracts were dried and concentrated. The syrup thus obtained was chromatographed on silica gel (70 g) using a mixture of ethyl acetate-hexane (6:4) as the eluent, furnishing **8E** (0.623 g, 76 %) and **8Z** (0.192 g, 24 %) in a combined yield of 84 %. **8E** $[\alpha]_D$ - 11.2° (*c* 1.05, chloroform); IR ($CHCl_3$) 3410, 3370, 2960, 2900, 2860, 1750, 1700, 1680, 1640, 1500, 1460, 1440, 1260-1240, 1040.

Anal. Calcd for $C_{27}H_{45}NO_{12}Si$: C, 53.73 ; H, 7.46 ; N, 2.32. Found : C, 53.20 ; H, 7.45 ; N, 2.31.

Ethyl 5-acetamido-3,4,5-trideoxy-2-(*t*-butyldimethyl)silyloxy-D-*manno*-2-nonenoate (9). To a stirred solution of **8** (0.5 g, 0.8 mmol) in dry methanol (5 mL) was added, at room temperature, sodium methoxide (10 mg) and the reaction was monitored by TLC (ethyl acetate-methanol, 3:1). After complete disappearance of the starting material (about 15-20 min) the mixture was acidified with Dowex 50 (H^+) resin. The resin was then filtered off and washed with methanol. The filtrate and washings were combined and concentrated. The crude product was purified by column chromatography on silica gel (40 g) using a mixture of ethyl acetate-methanol (3:1) as the eluent. We thus isolated 0.280 g (80 %) of **9** (foam). $[\alpha]_D$ = - 82.4° (*c* 1.2, chloroform); IR ($CHCl_3$) 3340, 2940, 2920, 2860, 1700, 1640, 1500, 1370, 1260, 1090, 1030, 1010.

Anal. Calcd for $C_{19}H_{37}NO_8Si$: C, 52.41 ; H, 8.50 ; N, 3.21. Found : C, 51.74 ; H, 8.67 ; N, 3.14.

Ethyl 5-acetamido-3,4,5-trideoxy-β-D-*manno*-nonulopyranosonate (10). To a solution of **9** (0.1 g, 0.23 mmol) in dry methanol (1 mL) was added two drops of triethylamine tris-hydrofluoride (Et_3N-3HF purchased from FLUKA). After about 15 min of stirring at room temperature, a white crystalline product precipitated which was filtered off furnishing pure **10** (0.05 g) mp 198-200 °C. An additional quantity of **10** (0.025 g), somewhat less pure (TLC), was obtained after concentration of the filtrate (combined yield : 85 %); $[\alpha]_D$ = - 34.2° (*c* 0.5, methanol); IR (KBr) 3340, 2940, 1740, 1650, 1530, 1435, 1375, 1315, 1290, 1150, 1120, 1070, 1020.

Anal. Calcd for $C_{13}H_{23}NO_8$: C, 48.59 ; H, 7.16 ; N, 4.36 ; O, 39.87. Found : C, 48.48 ; H, 7.28 ; N, 4.36 ; O, 39.85.

N-Acetyl-4-deoxy-D-neuraminic acid (1). To a solution of **9** (0.440 g, 1.01 mmol) in dry methanol (1 mL) was added a 1N methanolic solution of sodium hydroxyde (4.1 mL). After 3 h of stirring at room temperature the methanol was removed under reduced pressure (10^{-1} mm Hg) and the residue was dissolved in water (12 mL) and extracted with ethyl acetate (3 x 10 mL). The aqueous phase was then acidified with Dowex 50 (H^+) resin. The resin was filtered off and washed with methanol. The filtrate and washings were combined and concentrated (10^{-1} mm Hg) furnishing quantitatively crude **1**; mp 160 °C (dec) $[\alpha]_D$=-31° (*c* 0.5, water) (lit.[3] mp 162 °C $[\alpha]_D$=-47.6° (*c* 1.1, water); lit. [4] $[\alpha]_D$=-38° (*c* 0.4, water)); IR (KBr) 3700-2300, 2940, 1725, 1660, 1530, 1440.

Peracetylation of (10). To a solution of **10** (0.1 g, 0.31 mmol) in dry pyridine (2 mL) was added acetic anhydride (2.25 mL) and the mixture was stirred at room temperature for 3 days. After concentration and co-evaporation with toluene (3 x 20 mL), the residue was purified by column chromatography on silica gel (10 g) using ethyl acetate as the eluent. We first eluted the enolacetate **12** (0.011 g, 7 %) and then a mixture of pyranosonates **13** and **14** (0.128 g, 80 %).

12 : IR ($CHCl_3$) 3030, 2995, 1745, 1680, 1500, 1435, 1370, 1305, 1210, 1130, 1050; ^{1}H-NMR δ 6.50 (dd, J=8.47, 7.65 -H-3), 5.48 (d, J=9.84 -NH-), 5.33 (dd, J=8.15, 3.05 -H-7), 5.18 (dd, J=3.10, 8.34 -H-6), 5.05 (m -H-8), 4.19 (q, J=7.12 -O-CH_2-CH_3), 4.30 (m -H-5), 4.21 (dd, J=3.10, 12.40 -H-9), 4.00 (dd, J=12.40, 5.61 -H-9'), 2.40 -2.20 (m, 2H -H-4), 2.22, 2.08, 2.06, 2.02, 2.01, 1.86 (6 CH_3 -CO-), 1.25 (t, O-CH_2-CH_3)

13 + 14 : IR ($CHCl_3$) 2990, 1745, 1680, 1500, 1435, 1370, 1290, 1240, 1220, 1080, 1050; ^{1}H-NMR (selected signals) δ 5.2 (td, J=6.0, 3.5 -H-3 in **13** 30 %), 5.00 (td, J=4.0, 2.0 -H-8 in **14** 70 %), 4.67 (dd, J=10.5, 2.5 -H-6 in **13**), 4.45 (dd, J=12.3, 2.2 -H-9 in **14**),4.32 (dd, J=12.5, 2.4 -H-9 in **13**).

Methyl 5-acetamido-3,4,5-trideoxy-β-D-*manno*-2-nonulopyranosonate (11). To a solution of **1** (0.3 g, 0.1 mmol) in dry methanol (1 mL) was added Dowex 50 (H^+) resin (0.2 g) and the mixture was stirred at room temperature for 24 h. After removal of the methanol, crude ester was purified by column chromatography on silica gel (3 g) using a mixture of dichloromethane-methanol (3:1) as the eluent. We thus obtained 0.024 g (77 % from **9**) of crystalline **11** (mp 188-190 °C); $[\alpha]_D$ = - 39° (*c* 0.62, methanol) (lit.[3] mp 163-164 °C ; $[\alpha]_D$ = - 38° (*c* 1.0, methanol); IR ($CHCl_3$) 3335, 2935, 1740, 1650, 1540, 1440, 1370, 1320, 1310, 1285, 1155, 1125, 1075, 1050, 1020.

Anal. Calcd for $C_{12}H_{21}O_8N$; C, 46.90 ; H, 6.84 ; N, 4.56. Found : C, 46.30 ; H, 6.78 ; N, 4.39.

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