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Synthesis of transition-state analogues as potential inhibitors of sialidase from *Influenza* virus

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Abstract

Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-*manno*-non-2-enonate (**2**) has been synthesized from *N*-acetyl-4-deoxy-neuraminic acid methyl ester (**4**). Sodium 2,6-anhydro-3-deoxy-L-*arabino*-hept-2-enonate (**3**), 4-acetamido-2,6-anhydro-3,4-dideoxy-L-*arabino*-hept-2-enonic acid (**18*e***), and 4-acetamido-2,6-anhydro-3,4-dideoxy-L-*ribo*-hept-2-enonic acid (**18*a***) have been prepared from L-arabinose. The above compounds were investigated as inhibitors of sialidase from *Influenza* virus. Only compound **2** showed a significant inhibitory activity (K_i 8×10^{-2} mM) against the enzyme.

Key words: Sialidases; *Influenza* virus; Inhibitors; Transition-state analogues; Neuraminic acid; L-Arabinose; Ritter reaction

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1. Introduction

Sialidases (EC 3.2.1.18) play an important role in splitting off the α-linked-*N*-acetylneuraminic acid (Neu5Ac) at the terminal position in glycoconjugates [1]. It has been postulated for a long time that in *Influenza*, the virus surface sialidase helps to release newly synthesized virions from infected cells. It may also assist the movement of virus through the mucus within the respiratory tract [2]. Inhibition of this enzyme might therefore restrict the establishment and progression of infection

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by the *Influenza* virus. Thus, there has been considerable interest, particularly over the last few years, in the design and synthesis of inhibitors of sialidases [3,5,7].

As part of a program aimed at the synthesis of new inhibitors of these enzymes, we have recently described the preparation of an *o*-(difluoromethyl)-phenyl-α-ketoside of *N*-acetylneuraminic acid and shown that it was a "suicide" type inhibitor of sialidase from *Clostridium perfringens* [4]. We now report the preparation of compounds belonging to another class of potential inhibitors, transition-state analogues, and their behaviour towards sialidase from the *Influenza* virus.

2. Results and discussion

Several years ago, Meindl and Tuppy reported that 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (Neu5Ac2en) (**1**) was a potent inhibitor of sialidase from *Vibrio cholerae* [5]. It has been suggested that this molecule is a transition-state analogue [6]. Since this report, several analogues of **1** have been prepared and assayed for inhibitory activity against sialidases from both microbial and viral sources [7]. Very recently, using molecular modelling, Itzstein et al. have been able to design two of them, which showed very potent inhibitory activity against the *Influenza* virus enzyme [7f].

In order to ascertain the importance of the 4-hydroxyl, as well as the replacement of both the acetamido group and the triol chain by hydroxylic functions, for *Influenza* virus sialidase binding, we decided to prepare respectively, sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-*manno*-non-2-enonate (**2**) and sodium 2,6-anhydro-3-deoxy-L-*arabino*-hept-2-enonate (**3**).

Compound **2** was obtained in four steps from methyl 5-acetamido-3,4,5-trideoxy-D-*manno*-nonulopyranosonate (**4**) (Scheme 1). The latter compound was prepared in eight steps and an overall yield of ca. 20% from commercially available 2-acetamido-2-deoxy-D-mannose. We followed the same sequence as already described for the synthesis of the corresponding ethyl ester [8] except that methyl 2-(*tert*-butyldimethyl)-silyloxy-2-(dimethylphosphono) acetate [9] was used instead of the ethyl ester for the Wadsworth–Emmons reaction [1]. Classical acetylation [11] (Ac_2O–pyridine) of **4** furnished methyl 5-acetamido-2,7,8,9-tetra-*O*-acetyl-3,4,5-trideoxy-D-*manno*-2-nonulopyranosonate (**5**). When a solution of **5** (mixture of α and β anomers) in acetonitrile was treated with trimethylsilyl trifluoromethanesulfonate (2 mol equiv) according to the modification [12] of the Claesson and Luthman procedure [13], it gave methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-D-*manno*-non-2-enonate (**6**) in 90% yield [2]. Zemplén *O*-deacetylation of **6**, followed by saponification of the methyl ester, and acidification of the resulting solution (Dowex 50 H^+) to pH 7–7.5, led to the target compound **2** in 86% yield from **6**.

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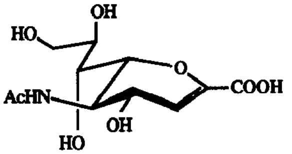
[1] The corresponding acid had previously been prepared by two different routes [10].

[2] This compound had previously been obtained from *N*-acetylneuraminic acid [14].



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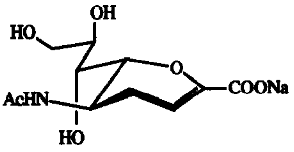

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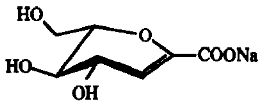

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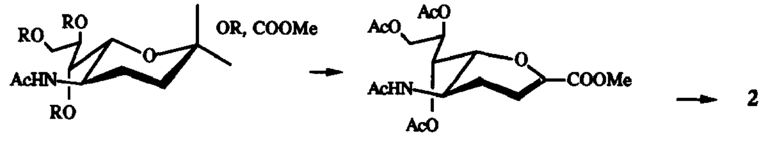


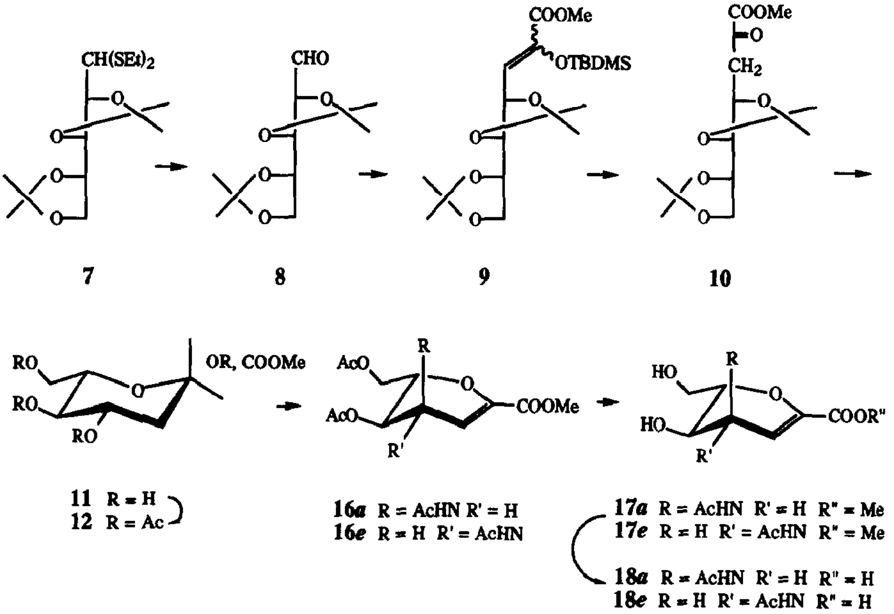
4 R = H
5 R = Ac

6

Scheme 1

In order to obtain **3** from known [15] 2,3 : 4,5-di-*O*-isopropylidene-L-arabinose diethyl dithioacetal (**7**), we first prepared 2,3 : 4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose (**8**) using the demercaptalation conditions of Just and Potvin [16] (Scheme 2). The crude aldehyde thus obtained was then submitted to the same Wittig type reaction as already used for the synthesis of compound **2** and led to 3-deoxy-4,5 : 6,7-di-*O*-isopropylidene-2-*O*-(*tert*-butyldimethyl)-silyl-L-*arabino*-hept-2-enonate (**9**) as a mixture of *Z* and *E* stereoisomers in 77% yield. Deprotection of the enol silyl ether [8,17] ($Et_3N \cdot 3HF$, MeOH) led to methyl 3-deoxy-4,5 : 6,7-di-*O*-isopropylidene-L-*arabino*-2-heptulosonate (**10**). After removal of the two isopropylidene protecting groups (MeOH, H^+), we obtained methyl 3-deoxy-L-*arabino*-2-heptulopyranosonate (**11**) in 77% yield from **9**. Compound **11** was then peracetylated (Ac_2O–pyridine, 85%) to yield methyl 2,4,5,7-tetra-*O*-acetyl-3-deoxy-L-*arabino*-heptulopyranosonate (**12**). Surprisingly, the treatment of **12** with trimethylsilyl triflate under the same conditions as used above for **5**, did not lead to the expected methyl 4,5,7-tri-*O*-acetyl-2,6-anhydro-3-deoxy-L-*arabino*-hept-2-enonate (**13**) but to a mixture of the epimeric methyl 4-acetamido-5,7-di-*O*-acetyl-2,6-anhydro-3,4-dideoxy-L-*ribo*-hept-2-enonate (**16*a***) and methyl 4-acetamido-5,7-di-*O*-acetyl-2,6-anhydro-3,4-dideoxy-L-*arabino*-hept-2-enonate (**16*e***) in 95% yield (ratio **16*a*** : **16*e*** = 79 : 21). These compounds were first identified on the basis of their ^1H NMR spectral data. In particular, a doublet corresponding to the NH acetamido group was observed in both epimers (at 7.4 for **16*e*** and 7.8 ppm for **16*a***). The orientation of H-4 was easily deduced from the value of the coupling constant $J_{4,5}$ (8.1 for **16*e*** and 4.8 Hz for **16*a***). The presence of the acetamido group at C-4 was then confirmed by the ^{13}C NMR spectra which showed a signal at 48.5 (**16*e***) and 42.9 ppm (**16*a***) for this carbon.

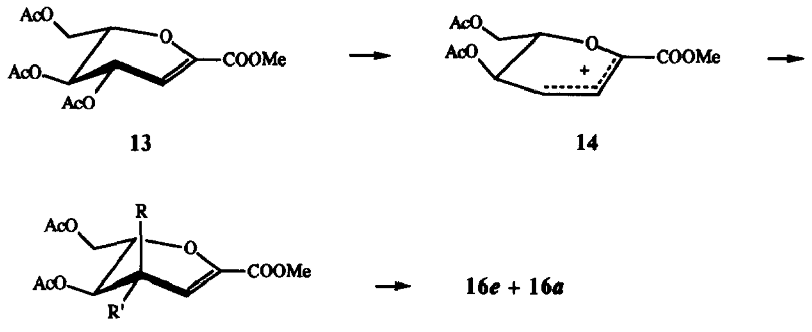
The formation of **16*a*** and **16*e*** results from a Ritter type reaction (Scheme 3). The action of trimetylsilyl triflate on **12** first led to **13**, as observed by TLC, but a



Scheme 2

further elimination of the 4-acetyl group generated the allyl carbocation **14** which, after reaction with acetonitrile used as the solvent, gave rise to epimeric cations **15*a*** and **15*e***. The reaction of water on the latter cations furnished the corresponding acetamido compounds **16*a*** and **16*e***. It worth noting that, in the literature [14,18,24b,24c], the formation of a 4,5-oxazoline derivative resulting from the intramolecular substitution of the 4-acetoxy group by the adjacent *N*-acetamido function has been observed during the treatment of peracetylated *N*-acetylneuraminic methyl ester with 2 equiv of trimethylsilyl triflate. Small quantities of the same oxazoline were formed [19] when *N*-acetylneuraminic acid methyl ester was submitted to the action of acetic anhydride in the presence of catalytic amounts of concentrated sulfuric acid at 50°C. It has been suggested that this compound derived from an allyl carbocation of type **14**.

Finally, **13** was obtained from peracetate **12** by a slightly different route (Scheme 4). Following the procedure used by Kuhn et al. [20], **12** was first transformed into methyl 4,5,7-tri-*O*-acetyl-2-chloro-2,3-dideoxy-β-L-*arabino*-2-heptulopyranosonate (**19**), which was then submitted in situ to the action of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to furnish **13** in 70% yield from **12**. A similar HCl elimination was described by Okamoto et al. using benzene as the solvent [21]. Compound **13** could be obtained in the same yield, but in a one-pot reaction, via methyl 4,5,7-tri-*O*-acetyl-2-bromo-2,3-dideoxy-β-L-*arabino*-2-heptulopyranosonate (**20**) by treating **12**, first with trimethylsilyl bromide in acetonitrile at 0°C and then with DBU [22]. The deacetylation and saponification of **13** under the conditions used for **2** led to the target compound **3** in 96% yield.



15e R = H $R' = \mathrm{N}{\equiv}\overset{+}{\mathrm{C}}{-}\mathrm{CH_3}$

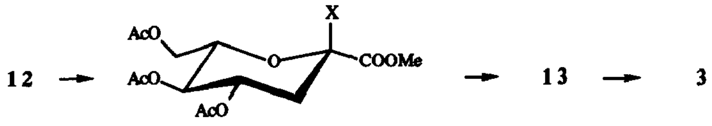
15a $R = \overset{+}{\mathrm{N}}{\equiv}\mathrm{C}{-}\mathrm{CH_3}$ R' = H

Scheme 3

Since we were interested in testing 4-acetamido-2,6-anhydro-3,4-dideoxy-L-*ribo*-hept-2-enonic acid (**18*a***) and 4-acetamido-2,6-anhydro-3,4-dideoxy-L-*arabino*-hept-2-enonic acid (**18*e***), **16*a*** and **16*e*** were then submitted to the above deacetylation and saponification conditions, except that the acidification of the aqueous solutions of the sodium salts was carried out to pH 4 furnishing the free acids (Scheme 2). During the course of deacetylation of **16*e***, the expected methyl 4-acetamido-2,6-anhydro-3,4-dideoxy-L-*arabino*-hept-2-enonate (**17e**) was obtained contaminated by ca. 30% (^{1}H NMR of the crude product) of an unidentified compound. Only the pure fraction, isolated after column chromatography, was submitted to saponification.

The behavior of compounds **2**, **3**, **18*e***, and **18*a*** towards sialidase from the *Influenza* virus was investigated and compared to that of **1** (Table 1). The K_i calculated for the latter compound is about one order of magnitude higher than the reported value [7b].

The results in Table 1 show that only **2** has a significant inhibitory activity against the enzyme. Its K_i value was found to be slightly lower than calculated for *Vibrio cholerae* sialidase (K_i 5×10^{-1} mM) [23], confirming the often observed differences in the recognition behavior of sialidases from different sources. The decrease of K_i by three orders of magnitude on going from **1** to **3** highlights the need for a carboxamido function at C-5 and a triol chain at C-6 for tight binding,



19 X = Cl
20 X = Br
Scheme 4

Table 1
Inhibition of sialidase from *Influenza* virus by Neu5Ac2en (**1**) and the analogues **2**, **3**, **18*e***, and **18*a***

Compound	K_i (mM)
1	4×10^{-2}
2	8×10^{-2}
3	3.5×10^{1}
18*a*	[a]
18*e*	[b]

[a] No inhibition detected up to 50 mM.
[b] Maximal inhibition (20%) observed at 50 mM.

as already observed [7c,24] for sialidases from *Vibrio cholerae*. The weak activity of compound **18*e*** is in accordance with an already observed considerable decrease in the inhibitory activity on *Vibrio cholerae* sialidase of an analogue of **1**, in which the OH-4 had been replaced by an acetamido group (K_i 3.40 mM) [14]. Finally, the observation that compound **18*a*** was devoid of any significant activity is somewhat surprising when we consider that 4-*epi*-Neu5Ac2en still shows some affinity (K_i 8.3×10^{-2} mM) [7b] as compared to Neu5Ac2en itself (K_i 2.4×10^{-3} mM) for the sialidases from the *Influenza* virus.

In summary, the above results observed with sialidase from the *Influenza* virus confirm previous observations reported in the literature with sialidases from different sources. As far as analogues of Neu5Ac2en are concerned, only compounds bearing an acetamido group at C-5 and a glycerol chain at C-6 show tight binding for the active site of the enzyme. As regards to substitution at C-4, the presence or absence of an OH group does not dramatically affect the affinity for the enzyme, but an equatorial or axial acetamido group at this position results in a considerable decrease in the inhibitory activity.

3. Experimental

General methods.—Solvents were distilled and dried before use. Reactions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) and detection was carried out by charring a 5% phosphomolybdic acid solution in EtOH containing 10% H_2SO_4 and heating. Flash column chromatography was performed on Silica Gel 60 (35–70 μm, Amicon) using the same eluent as for TLC monitoring unless stated otherwise. Melting points were determined with a Kofler hot-stage melting-point apparatus. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 25°C. IR spectra were recorded on a Perkin–Elmer 1310 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with either a Bruker AM 200 or 300 spectrometer. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were recorded on a Nermag R-1010C spectrometer. Elemental analyses were performed by the Service Central de Microanalyses du CNRS Solaize (France).

*Methyl 5-acetamido-3,4,5-trideoxy-α and β-*D-manno*-2-nonulopyranosonate* (**4**). —To a solution of methyl 2-(*tert*-butyldimethyl)-silyloxy-2-(dimethylphosphono) acetate [9] (588 mg, 1.88 mmol) in dry THF (10 mL) cooled to −40°C, was added dropwise a commercially available (Janssen) molar solution of lithium bis(trimethylsilyl)amide in THF (1.85 mL). The mixture was then warmed to 0°C and a solution of 3-acetamido-4,5,6,7-tetra-*O*-acetyl-2,3-dideoxy-D-*manno*-heptose [8] (735 mg, 1.82 mmol) in THF (10 mL) was added dropwise over 15 min and the reaction was allowed to proceed for 4 h at room temperature. After hydrolysis with satd NH_4Cl (20 mL) and extraction of the aqueous phase with Et_2O (4 × 20 mL), the combined organic extracts were dried (Na_2SO_4) and concentrated. The syrup thus obtained was purified by flash chromatography (100 g, 60:40 EtOAc–pentane) yielding methyl 5-acetamido-6,7,8,9-tetra-*O*-acetyl-3,4,5-trideoxy-(*tert*-butyldimethyl)-silyloxy-D-*manno*-non-2-enonate (904 mg, 84%) as a mixture of incompletely separated E (R_f 0.28) and Z (R_f 0.18) isomers ($E:Z = 75:25$). NMR data: ^1H (300 MHz, $CDCl_3$): E, δ 6.05 (d, 1 H, $J_{NH,5}$ 9.4 Hz, NH), 5.33 (dd, 1 H, $J_{6,7}$ 3.1, $J_{7,8}$ 8.5 Hz, H-7), 5.32 (dd, 1 H, $J_{3,4}$ 10.3, $J_{3,4'}$ 7.5 Hz, H-3), 5.13 (dd, 1 H, $J_{5,6}$ 7.4 Hz, H-6), 5.04 (ddd, 1 H, $J_{8,9}$ 6.1, $J_{8,9'}$ 3.0 Hz, H-8), 4.27–4.17 (m, 1 H, H-5), 4.20 (ABd, 1 H, $J_{9,9'}$ 12.4 Hz, H-9′), 3.99 (ABd, 1 H, H-9), 3.70 (s, 3 H, CH_3O), 2.57 (pdt, 1 H, $J_{4,4'}$ 14.5, $J_{4,5}$ 10.3 Hz, H-4), 2.31 (ddd, 1 H, $J_{4',5}$ 3.8 Hz, H-4′), 2.06, 2.03 (2 s, 2 × 3 H, 2 CH_3COO), 1.99 (s, 6 H, 2 CH_3COO), 1.81 (s, 3 H, CH_3CONH), 0.85 (s, 9 H, *tert*-Bu), 0.03, 0.02 (2 s, 2 × 3 H, $(CH_3)_2Si$). Z, δ 6.11 (d, 1 H, $J_{NH,5}$ 9.6 Hz, NH), 5.96 (pt, 1 H, $J_{3,4} = J_{3,4'} = 7.1$ Hz, H-3), 5.40 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 8.1 Hz, H-7), 5.25 (dd, 1 H, $J_{5,6}$ 8.3 Hz, H-6), 5.11 (ptd, 1 H, $J_{8,9}$ 8.9, $J_{8,9'}$ 2.8 Hz, H-8), 4.33–4.20 (m, 1 H, H-5), 4.25 (ABd, 1 H, $J_{9,9'}$ 12.4 Hz, H-9′), 4.05 (ABd, 1 H, H-9), 3.74 (s, 3 H, CH_3O), 2.48 (ddd, 1 H, $J_{4,4'}$ 15.1, $J_{4,5}$ 4.1 Hz, H-4), 2.24 (pdt, 1 H, $J_{4',5}$ 7.2 Hz, H-4′), 2.09 (s, 3 H, CH_3COO), 2.06 (s, 6 H, 2 CH_3COO), 2.05 (s, 3 H, 2 CH_3COO), 1.92 (s, 3 H, CH_3CONH), 0.95 (s, 9 H, *tert*-Bu), 0.08 (s, 6 H, $(CH_3)_2Si$). ^{13}C (75.5 MHz, $CDCl_3$): E, δ 170.5, 170.2, 170.0, 169.9, 169.8 (5 s, 5 $CH_3\mathit{C}O$), 165.6 (s, C-1), 142.9 (s, C-2), 118.7 (d, C-3), 71.7 (d, C-8), 68.6, 68.4 (2 d, C-6, C-7), 61.9 (t, C-9), 51.7 (q, CH_3O), 48.4 (d, C-5), 28.0 (t, C-4), 25.4 (q, 3 C, $(\mathit{C}H_3)_3C$), 23.2 (q, $\mathit{C}H_3CONH$), 20.9, 20.8, 20.7, 20.6 (4 q, 4 $\mathit{C}H_3CO$), 18.0 (s, $(CH_3)_3\mathit{C}$), −5.1 (2 q, $(CH_3)_2Si$). Z, δ 170.9, 170.6, 170.3, 170.2, 169.9 (5 s, 5 $CH_3\mathit{C}O$), 164.7 (s, C-1), 142.5 (s, C-2), 117.0 (d, C-3), 71.3 (d, C-8), 68.6, 68.3 (2 d, C-6, C-7), 62.2 (t, C-9), 52.0 (q, CH_3O), 47.6 (d, C-5), 27.5 (t, C-4), 25.8 (q, 3 C, $(\mathit{C}H_3)_3C$), 23.2 (q, $\mathit{C}H_3CONH$), 20.9, 20.8, 20.7, 20.6 (4 q, 4 $\mathit{C}H_3CO$), 18.5 (s, $(CH_3)_3\mathit{C}$), −4.3 (2 q, $(CH_3)_2Si$).

This anomeric mixture was used for the preparation of **4** as previously described [8].

*Methyl 5-acetamido-2,7,8,9-tetra-O-acetyl-3,4,5-trideoxy-α and β-*D-manno*-2-nonulopyranosonate* (**5**).—To a solution of **4** [8] (221 mg, 0.72 mmol) in pyridine (3,5 mL) was added, at 0°C, Ac_2O (3.5 mL). The reaction was monitored by TLC (EtOAc). After 2.5 days with magnetic stirring, the solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (20 g), giving **5** ($\alpha + \beta$) (297 mg, 87%) as a colourless foam. The ^1H NMR data were in accordance with the literature [11].

*Methyl 5-acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-3,4,5-trideoxy-*D-manno-*non-2-enonate* (**6**).—Trimethylsilyl trifluoromethane sulfonate (0.140 mL, 0.72 mmol) was added at room temperature to a solution of the peracetate **5** (169 mg, 0.36 mmol) in anhyd MeCN (5.5 mL). The reaction was complete after 5 h with magnetic stirring (TLC, EtOAc). Potassium carbonate (280 mg, 2 mmol) was then added with continuous stirring for another 15 min. After removing solids by filtration, the solvent was evaporated in vacuo, and the residue was purified by flash chromatography (10 g), yielding compound **6** (133 mg, 90%); $[\alpha]_D$) +21.7° (*c* 0.24, $CHCl_3$); lit. [11] $[\alpha]_D$) +23.6° (*c* 1.0, $CHCl_3$); ^1H NMR (80 MHz, $CDCl_3$): δ 6.09 (pt, 1 H, $J_{3,4} = J_{3,4'} = 4.0$ Hz, H-3), 5.68 (d, 1 H, $J_{NH,5}$ 8.4 Hz, NH), 5.44 (dd, 1 H, $J_{7,8}$ 3.9, $J_{6,7}$ 5.2 Hz, H-7), 5.26 (dt, 1 H, $J_{8,9}$ 3.9, $J_{8,9'}$ 7.4 Hz, H-8), 4.57 (dd, 1 H, $J_{9,9'}$ 12.2 Hz, H-9), 4.45–4.11 (m, 2 H, H-5, H-9), 4.12 (dd, H-6), 3.78 (s, 3 H, CH_3O), 2.65, 2.18 (2 pdt, 2 H, 2 $J_{4,4'}$ 18.2 Hz, H-4, H-4′), 2.11, 2.06, 2.04 (3 s, 3 × 3 H, 3 CH_3COO), 1.95 (s, 3 H, CH_3CONH) in accordance with the literature [11,14].

*Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-*D-manno-*non-2-enonate* (**2**).—Compound **6** (133 mg, 0.32 mmol) was dissolved in anhyd MeOH (7 mL) and NaOMe (35 mg, 2 mol equiv) was added. The solution was stirred at room temperature for 30 min (TLC, 80:20 EtOAc–pentane). Dowex 50W-X4 (H^+) cation-exchange resin was then added to neutralise the base. The resin was filtered off and washed twice with MeOH. The solvent was evaporated in vacuo at room temperature and the residue was dissolved in aq 0.1 M NaOH (10 mL). After 20 min with magnetic stirring at room temperature, the solution was gradually acidified with Dowex 50W-X4 (H^+) resin to pH 7–7.5 (pH meter) and freezed-dried to yield compound **2** (82 mg, 86%) as a white powder; mp 209–224°C (dec); $[\alpha]_D$ −5.2° (*c* 1.05, H_2O); ν_{max}^{KBr}: 3450 (OH), 1640 cm^{-1} (CO); NMR data: ^1H (300 MHz, CD_3OD): δ 5.71 (dd, 1 H, $J_{3,4}$ 2.6, $J_{3,4'}$ 5.8 Hz, H-3), 4.16 (ptd, 1 H, $J_{4,5} = J_{5,6} = 10.1$, $J_{4',5}$ 5.8 Hz, H-5), 3.91 (dd, 1 H, $J_{6,7}$ 1.3 Hz, H-6), 3.88 (ddd, 1 H, $J_{8,9'}$ 3.1, $J_{8,9}$ 5.5, $J_{7,8}$ 9.6 Hz, H-8), 3.82 (ABd, $J_{9',9}$ 11.3 Hz, H-9′), 3.64 (ABd, 1 H, H-9); 3.55 (dd, 1 H, H-7), 2.42 (ABpt, 1 H, $J_{4,4'}$ 17.7 Hz, H-4′), 2.18 (ABdd, 1 H, H-4), 1.98 (s, 3 H, CH_3CONH); ^{13}C (75.5 MHz, D_2O): δ 174.7 (s, CH_3CONH), 166.3 (s, C-1), 143.0 (s, C-2), 112.2 (d, C-3), 76.6 (d, C-6), 70.6, 68.7 (2 d, C-7, C-8), 63.5 (t, C-9), 43.0 (d, C-5), 27.4 (t, C-4), 22.4 (q, CH_3CONH); FAB$^+$MS: *m/z* 298 ($[M+H]^+$, 16%).

*2,3:4,5-Di-O-isopropylidene-*aldehydo-L-*arabinose* (**8**).—To a well stirred solution of the dithioacetal **7** [15] (8.74 g, 26 mmol) in acetone (150 mL) at 0°C, was added water (10 mL) and $NaHCO_3$ (4.8 g, 57 mmol) and then, in small portions, iodine (7.5 g, 29.5 mmol). The mixture was allowed to warm to room temperature and was stirred for 6 h. Iodine (7.5 g) and $NaHCO_3$ (4.8 g) were then added portionwise over a period of ca. 10 h, until total disappearance of the starting material at which time the excess iodine was destroyed by adding satd sodium thiosulfate solution. After partial evaporation of the acetone, the product was extracted with EtOAc (3 × 100 mL) and the combined extracts were dried (Na_2SO_4). After solvent evaporation, the crude product (quantitative yield) was used in the next step without purification; ν_{max}^{film}: 1730 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$): d 9.76 (d, 1 H, $J_{1,2}$ 1 Hz, H-1), 4.41 (dd, 1 H, $J_{2,3}$ 6 Hz, H-2), 4.2–3.8 (m, 4 H, H-3, H-4, H-5, H-5′), 1.48 (s, 3 H, $-C(CH_3)_2$), 1.43 (s, 3 H, $-C(CH_3)_2-$), 1.38 (s, 3 H, -C$(CH_3)_2$-), 1.35 (s, 3 H, -C$(CH_3)_2$-), in agreement with the literature [15] for the D isomer.

*Methyl 3-deoxy-4,5 : 6,7-di-O-isopropylidene-2-O-(*tert*-butyldimethyl)silyl-*L-*arabino*-hept-2-enonate* (**9**).—To a stirred solution of methyl (2-*tert*-butyldimethyl)-silyloxy-2-(dimethylphosphono)-acetate (4.13 g, 13.25 mmol) in THF (60 mL) was added, at −40°C, a commercially available solution of lithium bis(trimethylsilyl)-amide 1 M (12 mL, 0.9 mol equiv). To this solution was added at 0°C a solution of aldehyde **8** (2.54 g, 11.04 mmol) in THF (60 mL), and the mixture was stirred for 4 h at room temperature. Water (100 mL) was then added, and the solution was extracted with ether (4 × 100 mL), the combined organic layers were washed with satd NaCl (1 × 60 mL), dried (Na_2SO_4), concentrated, and the resulting residue was purified by flash chromatography (450 g, 20 : 80 diethyl ether–pentane) to give the two isomers $\mathbf{9}_E$ (3.17 g, 69%) and $\mathbf{9}_Z$ (0.35 g, 8%) as colourless syrups; R_f (*E*) 0.25, R_f (*Z*) 0.31; $\mathbf{9}_E$ $[\alpha]_D$ −5.5° (*c* 0.80, $CHCl_3$); ν_{max}^{film}: 1720 cm^{-1} (COO); NMR data: ^1H (300 MHz, $CDCl_3$): δ 5.34 (d, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 5.16 (dd, 1 H, $J_{4,5}$ 8.0 Hz, H-4), 4.11–4.07 (m, 2 H, H-6, H-7), 3.90 (dd, 1 H, $J_{7,7'}$ 10.6, $J_{6,7'}$ 9.1 Hz, H-7′), 3.73 (s, 3 H, CH_3O), 3.68 (dd, 1 H, $J_{5,6}$ 5.3 Hz, H-5), 1.38, 1.37, 1.35, 1.28 (4 s, 4 × 3 H, 2 $(CH_3)_2C$), 0.90 (s, 12 H, *tert*Bu), 0.12 (s, 6 H, $(CH_3)_2Si$); ^{13}C (75.5 MHz, $CDCl_3$): δ 159.5 (s, C-1), 138.8 (s, C-2), 114.7 (d, C-3), 104.4 (s, 2 C, 2 $(CH_3)_2C$), 76.4 (d, C-4); 70.9, 69.7 (2 d, C-5, C-6), 61.3 (t, C-7), 46.8 (q, CH_3O), 22.2, 21.9, 21.5, 20.5 (4 q, 2 $(CH_3)_2C$), 20.4 (q, 3 C, $(CH_3)_3C$), 13.2 (s, $(CH_3)_3C$), 0 (q, 2 C, Me_2Si). Anal. Calcd for $C_{20}H_{36}O_7Si$: C, 57.66; H, 8.71; Si, 6.74. Found: C, 57.41; H, 8.79; Si, 6.47.

*Methyl 3-deoxy-4,5 : 6,7-di-O-isopropylidene-*L-arabino*-2-heptulosonate* (**10**).—To a solution of compound $\mathbf{9}_{E+Z}$ (889 mg, 2.06 mmol) in MeOH (9 mL) was added $Et_3N \cdot 3HF$ (0.15 mL). The mixture was stirred for 30 min until no trace of **9** was visible any longer by TLC, concentrated, and then purified by flash chromatography (35 g, 35 : 65 diethyl ether–pentane) to give compound **10** (516 mg, 83%) as a white solid; R_f 0.18; mp 32–34°C; $[\alpha]_D$ −26.4° (*c* 0.74, $CHCl_3$); ν_{max}^{film}: 1740 (COO), 1720 cm^{-1} (CO); NMR data: ^1H (300 MHz, $CDCl_3$): δ 4.43 (ptd, 1 H, $J_{3,4}$ 4.5, $J_{3',4} = J_{4,5} = 7.7$ Hz, H-4), 4.14 (ABd, 1 H, $J_{6,7}$ 5.9, $J_{7,7'}$ 8.2 Hz, H-7), 4.02 (ddd, 1 H, $J_{6,7'}$ 5.0, $J_{5,6}$ 8.4 Hz, H-6), 3.93 (ABd, 1 H, H-7′), 3.72 (s, 3 H, CH_3O), 3.60 (dd, 1 H, H-5), 3.25 (ABd, 1 H, $J_{3,3'}$ 16.7 Hz, H-3), 3.17 (ABd, 1 H, H-3′), 1.40, 1.39, 1.37, 1.32 (4 s, 4 × 3 H, 2 $(CH_3)_2C$); ^{13}C (75.5 MHz, $CDCl_3$): δ 191.3 (s, C-2), 160.7 (s, C-1), 109.8, 109.7 (2 s, 2 $(CH_3)_2C$), 80.7, 76.9, 75.6 (3 d, C-4, C-5, C-6), 67.9 (t, C-7), 48.1 (q, CH_3O), 42.8 (t, C-3), 27.0, 26.9, 26.7, 25.2 (4 q, 2 $(CH_3)_2C$). Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.62; H, 7.33. Found: C, 55.51; H, 7.04.

*Methyl 3-deoxy-*L-arabino*-2-heptulopyranosonate* (**11**).—To a solution of the ketoester **10** (494 mg, 1.64 mmol) in MeOH (25 mL) was added Dowex H^+ resin (8 g). The reaction was monitored by TLC (80 : 20 EtOAc–MeOH). No traces of starting material were detectable after 2.5 h of stirring. After filtration and solvent evaporation in vacuo, compound **11** was obtained as a very hygroscopic white solid (338 mg, 93%) but was pure enough for further reaction. Column chromatography of an aliquot afforded an analytical sample; R_f 0.33; $[\alpha]_D$ −41.7° (*c* 0.86, MeOH); ν_{max}^{film}: 1745 cm^{-1} (COO); NMR data: ^1H (300 MHz, CD_3OD): δ 3.90–3.80 (m, 2 H, H-5, H-7), 3.88 (ptd, 1 H, $J_{3e,4}$ 5.1, $J_{3a,4} = J_{4,5} = 11.7$ Hz, H-4), 3.77 (s, 3 H, CH_3O), 3.76 (ddd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 1.7, $J_{6,7'}$ 5.0 Hz, H-6), 3.70 (ABd, 1 H, $J_{7,7'}$ 10.7 Hz, H-7'), 2.15 (dd, 1 H, $J_{3e,3a}$ 12.8 Hz, H-3*e*), 1.78 (dd, 1 H, H-3*a*); ^{13}C (75.5 MHz, D_2O): δ 174.2 (s, C-1), 98.0 (s, C-2), 76.8 (d, C-6), 73.3, 71.1 (2 d, C-4, C-5), 63.4 (t, C-7), 56.3 (q, CH_3O), 41.3 (t, C-3); CIMS (NH_3 + isobutane): *m/z* 240 ($[M + NH_3 + H]^+$, 100%).

*Methyl 2,4,5,7-tetra-O-acetyl-3-deoxy-*L-arabino*-heptulopyranosonate* (**12**).—To a solution of polyol **11** (904 mg, 4.07 mmol) in pyridine (17 mL), Ac_2O (17 mL) was slowly added at 0°C. The formation of anomeric peracetates **12** was controlled by TLC (35:65 EtOAc–pentane). After 2.5 days with magnetic stirring, the starting material was undetectable. The mixture was concentrated in vacuo and the solvent was co-evaporated with toluene (3 × 10 mL). Compound **12** was then purified by flash chromatography (100 g) to yield a mixture [R_f (α) 0.27, R_f (β) 0.21] of white solids (1.35 g, 85%). A sample of the major isomer β was recrystallized (80:20 diethyl ether–pentane); mp 114–115°C; $[\alpha]_D$ $-60.9°$ (*c* 1.0, $CHCl_3$); ν_{max}^{film}: 1775, 1735 cm^{-1} (CO); NMR data: ^1H (300 MHz, $CDCl_3$): δ 5.30 (ddd, 1 H, $J_{3e,4}$ 5.2, $J_{3a,4}$ 11.4, $J_{4,5}$ 9.4 Hz, H-4), 5.12 (dd, 1 H, $J_{5,6}$ 9.9 Hz, H-5), 4.35 (dd, 1 H, $J_{6,7}$ 4.5, $J_{7,7'}$ 12.7 Hz, H-7), 4.10 (dd, 1 H, $J_{6,7'}$ 2.2 Hz, H-7'), 4.06 (ddd, 1 H, H-6), 3.80 (s, 3 H, CH_3O), 2.66 (dd, 1 H, $J_{3e,3a}$ 8.4 Hz, H-3*e*), 2.17 (s, 3 H, CH_3CO), 2.12–2.04 (m, 1 H, H-3*a*), 2.08, 2.05, 2.03 (3 s, 3 × 3 H, 3 CH_3CO); ^{13}C (75.5 MHz, $CDCl_3$): δ 170.7, 170.1, 169.6, 168.4, 166.4 (5s, 4CH_3CO, C-1), 97.3 (s, C-2), 71.5, 68.4, 68.2 (3 d, C-4, C-5, C-6), 61.7 (t, C-7), 53.3 (q, CH_3O), 35.6 (t, C-3), 20.9, 20.8, 20.7, 20.6 (4 q, 4 $\mathit{C}H_3CO$). Anal. Calcd for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.68. Found: C, 49.13; H, 5.62.

*Methyl 4,5,7-tri-O-acetyl-2,6-anhydro-3-deoxy-*L-arabino*-hept-2-enonate* (**13**).—*Via* **19**. A solution of a mixture of α/β **12** (287 mg, 0.74 mmol) in freshly distilled AcCl (15 mL) was cooled to −40°C, and saturated with dry HCl. The sealed flask was left at +4°C for 15 h. The solvent was then evaporated in vacuo to furnish **19** as a colourless foam. To a solution of crude **19** in anhyd CH_2Cl_2 (10 mL) was added DBU (0.8 mL, 7 equiv) dropwise. The reaction was complete after 15 min with magnetic stirring (TLC, 35:65 EtOAc–pentane). The solution was then acidified with 33% HCl (pH 3), washed twice (2 × 10 mL), and dried (Na_2SO_4). The residue was then purified by flash chromatography (20 g) to yield **13** (171 mg, 70% from **12**) as a colourless syrup.

Via **20**. To a stirred solution of **12** (30 mg, 0.08 mmol) in anhyd MeCN (2 mL) at 0°C was added trimethylsilyl bromide (20 mL, 2 mol equiv). After 2 h, DBU (30 mL, 2.5 mol equiv) was added and stirring continued for 5 min. The mixture was then extracted with CH_2Cl_2 (5 mL), the organic phase washed with HCl 10% (5 mL) and dried (Na_2SO_4). The residue was purified by flash chromatography (35:65 EtOAc–pentane) to afford **13** (18 mg, 70%); R_f 0.16; $[\alpha]_D$ $+10.6°$ (*c* 1.15, $CHCl_3$); ν_{max}^{film}: 1740, 1720 cm^{-1} (CO); NMR data: ^1H (300 MHz, $CDCl_3$): δ 6.44 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.44 (dd, 1 H, $J_{4,5}$ 5.0 Hz, H-4), 5.25 (dd, 1 H, $J_{5,6}$ 6.8 Hz, H-5), 4.47 (dd, 1 H, $J_{6,7}$ 5.8, $J_{7,7'}$ 11.6 Hz, H-7), 4.46–4.41 (m, 1 H, H-6), 4.21 (dd, 1 H, $J_{6,7'}$ 2.4 Hz, H-7'), 3.83 (s, 3 H, CH_3O), 2.10 (s, 3 H, CH_3CO), 2.08 (s, 6 H, 2 CH_3CO); ^{13}C (75.5 MHz, $CDCl_3$): δ 170.6, 170.0, 169.5 (3 s, 3 $CH_3\mathit{C}O$), 161.8 (s, C-1), 145.0 (s, C-2), 106.6 (d, C-3), 75.0 (d, C-6), 66.9, 66.4 (2 d, C-4, C-5), 60.7 (t, C-7), 52.7 (q, CH_3O), 20.8, 20.7, 20.6 (3 q, 3 CH_3CO); Anal. Calcd for $C_{14}H_{18}O_9$: C, 50.91; H, 5.49. Found C, 50.77; H, 5.49.

*Sodium 2,6-anhydro-3-deoxy-*L-arabino*-hept-2-enonate* (**3**).—Deacetylation of **13** (285 mg, 0.86 mmol), followed by saponification, as described for **2**, gave **3** (175 mg, 96%) as a white powder; mp 127–130°C (dec); $[\alpha]_D$ −14.5° (*c* 1.46, MeOH); ν_{max}^{KBr}: 3425 (OH), 1600 (CO); NMR data: ^1H (300 MHz, D_2O): δ 5.64 (d, 1 H, $J_{3,4}$ 2.3 Hz, H-3), 4.33 (dd, 1 H, $J_{4,5}$ 7.4 Hz, H-4), 3.97–3.93 (m, 2 H, H-6, H-7), 3.85 (dd, 1 H, $J_{6,7'}$ 6.0, $J_{7',7}$ 12.7 Hz, H-7'), 3.65 (dd, $J_{5,6}$ 9.3 Hz, H-5); ^{13}C (75.5 MHz, D_2O): δ 170.0 (s, C-1), 148.0 (s, C-2), 107.9 (d, C-3), 79.2 (d, C-6), 69.6, 69.1 (2 d, C-4, C-5), 61.0 (t, C-7); FAB$^-$MS: *m/z* 211 ([M − H]$^-$, 38%), 189 ([M − Na]$^-$, 100%).

*Methyl 4-acetamido-5,7-di-O-acetyl-2,6-anhydro-3,4-dideoxy-*L-arabino*-hept-2-enonate* (**16*e***) *and methyl 4-acetamido-5,7-di-O-acetyl-2,6-anhydro-3,4-dideoxy-*L-ribo*-hept-2-enonate* (**16*a***).–To a solution of peracetate **12** (288 mg, 0.74 mmol) in MeCN (2 mL) at 0°C, was added a solution of trimethylsilyl triflate (0.29 mL, 1.49 mmol.) in the same solvent (1 mL). After 3 h stirring, no more starting material was detected (TLC, EtOAc). Potassium carbonate (204 mg, 2 mol equiv) was then added and the mixture was stirred for a further 15 min. After removing solids by filtration and concentration under reduced pressure, the residue was purified by flash chromatography (20 g) yielding a total of 281 mg (95%) of a mixture of incompletely separated **16*e*** (R_f 0.27) and **16*a*** (R_f 0.22) as colourless hygroscopic solids; **16e** mp 90–92°C; $[\alpha]_D$ −3.3° (*c* 1.06, MeOH); ν_{max}^{KBr} 1740 (COO), 1655 cm^{-1} (CON); NMR data: ^1H (300 MHz, acetone-d_6): δ 7.40 (d, 1 H, $J_{NH,4}$ 8.2 Hz, NH), 5.85 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-3), 5.13 (dd,1 H, $J_{4,5}$ 8.1, $J_{5,6}$ 9.2 Hz, H-5), 4.83 (td, 1 H, H-4), 4.46 (ABd, 1 H, $J_{6,7}$ 4.5, $J_{7,7'}$ 12.3 Hz, H-7), 4.38 (ddd, 1 H, $J_{6,7'}$ 2.2 Hz, H-6), 4.18 (ABd, 1 H, H-7'), 3.76 (s, 3 H, CH_3O), 2.02, 2.01 (2 s, 2 × 3 H, 2 CH_3COO), 1.90 (s, 3 H, CH_3CONH); ^{13}C (75.5 MHz): δ 170.7 (s, 2C, 2 CH_3CO), 170.4 (s, CH_3CO), 162.5 (s, C-1), 145.1 (s, C-2), 111.6 (d, C-3), 76.7 (d, C-6), 68.0 (d, C-5), 62.1 (t, C-7), 52.5 (q, CH_3O), 48.5 (d, C-4); 22.9, 20.8, 20.6 (3 q, 3 CH_3CO); CIMS (NH_3 + isobutane): *m/z* 347 ([M + NH_3 + H]$^+$, 9%), 331 ([M + 2H]$^+$, 23%), 330 ([M + H]$^+$, 100%), 329 ([M]$^+$, 8%); **16*a*** mp 44–46°C; $[\alpha]_D$ −117.2° (*c* 1.02, MeOH); ν_{max}^{KBr} 1740 (COO), 1660 cm^{-1} (CON); NMR data: ^1H (200 MHz, acetone-d_6): δ 7.80 (d, 1 H, $J_{NH,4}$ 8.2 Hz, NH), 5.95 (d, 1 H, $J_{3,4}$ 4.9 Hz, H-3), 5.11 (dd,1 H, $J_{4,5}$ 4.8, $J_{6,5}$ 8.2 Hz, H-5), 4.92 (ddd, 1 H, H-4), 4.46–4.24 (m, 1 H, H-6), 4.42 (ABd, 1 H, $J_{6,7}$ 4.5, $J_{7,7'}$ 10.7 Hz, H-7), 4.35 (ABd, 1 H, $J_{6,7'}$ 3.1 Hz, H-7'), 3.77 (s, 3 H, CH_3O), 2.02 (s, 6 H, 2 CH_3COO), 1.97 (s, 3 H, CH_3CONH); ^{13}C (75.5 MHz): δ 172.8, 171.3, 170.8 (3s, 3CH_3CO), 163.0 (s, C-1), 145.5 (s, C-2), 108.3 (d, C-3), 76.6 (d, C-6), 66.5 (d, C-5), 62.4 (t, C-7), 52.9 (q, CH_3O), 42.9 (d, C-4), 20.9, 20.8, 20.7 (3 q, 3 CH_3CO); CIMS (NH_3 + isobutane): *m/z* 347 ([M + NH_3 + H]$^+$, 12%), 330 ([M + H]$^+$, 23%).

*4-Acetamido-2,6-anhydro-3,4-dideoxy-*L-arabino*-hept-2-enonic acid* (**18*a***).— Deacetylation of **16*a*** (195 mg, 0.59 mmol) and saponification of the resulting ester **17*a*** were carried out as described for compounds **2** and **3**, except that the acidification of the aqueous solution of the sodium salt was carried to pH 4 (pH meter). We thus obtained the free acid **18*a*** (121 mg, 81%) as a white powder; mp

212–214°C (dec); $[\alpha]_D$ −147.8° (*c* 1.01, MeOH); ν_{max}^{KBr} 3420 (OH), 1715 (COO) 1655 cm^{-1} (CON); NMR data: ^1H (300 MHz, CD_3OD): δ 8.04 (d, 1 H, $J_{NH,4}$ 8.3 Hz, NH), 5.74 (d, 1 H, $J_{3,4}$ 5.1 Hz, H-3), 4.62 (pdt, 1 H, $J_{4,5}$ 5.1 Hz, H-4), 3.87 (ABd, 1 H, $J_{6,7}$ 1.7 $J_{7,7'}$ 11.4 Hz, H-7), 3.85–3.75 (m, 2 H, H-5, H-6), 3.73 (ABd, 1 H, $J_{6,7'}$ 5.7 Hz, H-7'), 1.99 (s, 3 H, CH_3CONH); ^{13}C (75.5 MHz, D_2O): δ 174.8 (s, $CH_3\mathit{C}ONH$), 170.0 (s, C-1), 148.8 (s, C-2), 103.6 (d, C-3), 77.0 (d, C-6), 64.9 (d, C-5), 61.0 (t, C-7), 44.9 (d, C-4), 22.6 (q, $\mathit{C}H_3CONH$); CIMS (NH_3 + isobutane): *m/z* 249 ($[M + NH_3 + H]^+$, 16%), 232 ($[M + H]^+$, 21%), 214 ($[M + H - H_2O]^+$, 100%).

*4-Acetamido-2,6-anhydro-3,4-dideoxy-*L*-*ribo*-hept-2-enonic acid* (**18*e***).—Under the usual deacetylation conditions, **16*e*** (76 mg, 0.23 mmol) gave a mixture of **17*e*** and an unidentified by-product incompletely separated after flash chromatography (5 g, 95:5 EtOAc–MeOH). Only the pure fraction of **17*e*** (16.6 mg, 30%) was submitted to saponification as described for **18*a***, yielding **18*e*** (16.2 mg, 96%) as a colourless solid; mp 204–210°C (dec); $[\alpha]_D$ +46.7° (*c* 0.99, MeOH); ν_{max}^{KBr} 3360 (OH), 1715 (COO), 1655 cm^{-1} (CON); NMR data ^1H (300 MHz, CD_3OD): δ 5.52 (d, 1 H, $J_{3,4}$ 2.4 Hz, H-3), 4.55 (dd, 1 H, $J_{4,5}$ 8.5 Hz, H-4), 3,99 (ABd, 1 H, $J_{6,7}$ 2.2, $J_{7,7'}$ 11.6 Hz, H-7), 3.84–3.80 (m, 1 H, H-6), 3.73 (ABd, 1 H, $J_{6,7'}$ 7.3 Hz, H-7'), 3.51 (dd, 1 H, $J_{5,6}$ 9.7 Hz, H-5), 1.98 (s, 3 H, CH_3CONH); ^{13}C (50 MHz, D_2O): δ 177.3 (s, $CH_3\mathit{C}ONH$), 172.0 (s, C-1), 150.8 (s, C-2), 108.1 (d, C-3), 81.6 (d, C-6), 69.1 (d, C-5), 63.0 (t, C-7), 52.9 (d, C-4), 24.7 (q, $\mathit{C}H_3CONH$); CIMS (NH_3 + isobutane): *m/z* 249 ($[M + NH_3 + H]^+$, 41%), 232 ($[M + H]^+$, 76%), 161 (100%), 110 (72%).

Inhibition experiments.—2,3-Didehydro-2-deoxy-*N*-acetylneuraminic acid (**1**) was purchased from Boehringer. *Influenza* virus A/HK/1/68 was a kind gift from Mrs. Joly and Mrs. Aymard (Laboratoire de Virologie, Université de Lyon, France). Viruses were grown in the allantoic cavity of 11-day-old embryonated eggs for 48 h at 33°C. The allantoic fluid was harvested, centrifuged at 30000 rpm for 20 min, and the resulting pellet was resuspended in phosphate buffered saline. Aliquots were stored in small portions at −80°C. The titre of virus in the allantoic fluid was 7×10^8 infectious doses/mL (ID/mL). Compounds **1**, **2**, **3**, **18*e***, and **18*a*** were tested for their capacity to inhibit neuraminidase activity of A/HK/1/68 using *p*-nitrophenyl-*N*-acetyl-α-D-neuraminate [25] as substrate. All assays were carried out in duplicate on microtitre plates. Virus (1.4×10^6 ID/mL) containing 0.36 U sialidase/mL [3], substrate and inhibitor at the concentrations of 0.015, 0.03, 0.06, 0.125, 0.250, 0.50, 1, and 2.5 mM (compound **2**) or 3, 6, 12.5, 25, and 50 mM (compounds **3**, **18*e***, and **18*a***) were incubated for 45 minutes at 37°C in 50 mM NaCl, 0.1 M sodium acetate buffer, pH 5.5, in a final volume of 100 mL. The reaction was stopped by adding 10 mL of diethanolamine. Neuraminidase activity was measured by monitoring the increase in absorbance at 405 nm due to the release of *p*-nitrophenol. Optical densities were read on a plate reader (Titertek MCC/340). Inhibition constants were determined by measuring residual activity at

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[3] One unit of sialidase is defined as the quantity of enzyme capable of liberating 1 mmol of *p*-nitrophenol/min at 37°C from *p*-nitrophenyl-*N*-acetyl-α-D-neuraminate used as substrate.

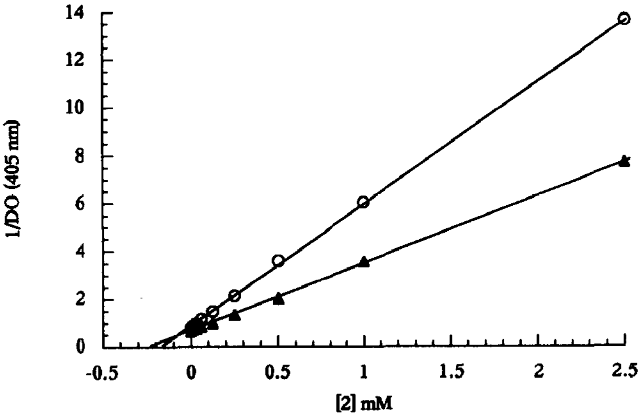

Fig. 1. Effect of **2** on *Influenza* virus sialidase activity. Substrate (*p*-nitrophenyl-*N*-acetyl-α-D-neuraminate) concentrations were 0.5 (○) and 1 mM (△) and inhibitor concentrations as shown. The reaction was initiated by adding *Influenza* virus sialidase (1.4 10^6 ID/mL final concentration) to 0.1 M acetate buffer pH 5.5 containing 50 mM NaCl, **2**, and substrate. The reaction was allowed to proceed for 45 min at 37°C. At the end of this period, samples were processed as described in the text.

two concentrations of substrate (0.5 and 1 mM). For inhibition assays with compound **2**, linearity was always seen over the concentration range used. It was already maximal for a concentration of inhibitor of 1.0 mM. The K_i values were obtained from Dixon plots of the data using linear regression analysis for curve fitting (Fig. 1). For compound **3**, attempts to increase inhibition by increasing the concentration of inhibitor resulted in nonlinear kinetics from 25 mM upwards. Therefore, we calculated the K_i for three different experiments using concentrations of inhibitor up to 25 mM, and obtained the values of 33, 36, and 38 mM with an average of 35 mM. A typical inhibition plot is shown in Fig. 2.

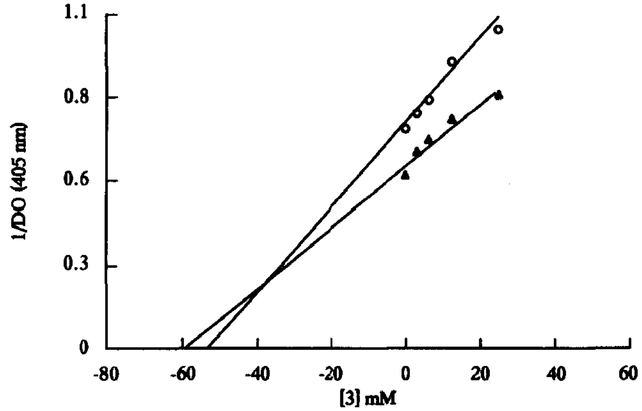

Fig. 2. Effect of **3** on *Influenza* virus sialidase activity.

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