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Thermal and photochemical degradation of sodium *N*-acetylneuraminate

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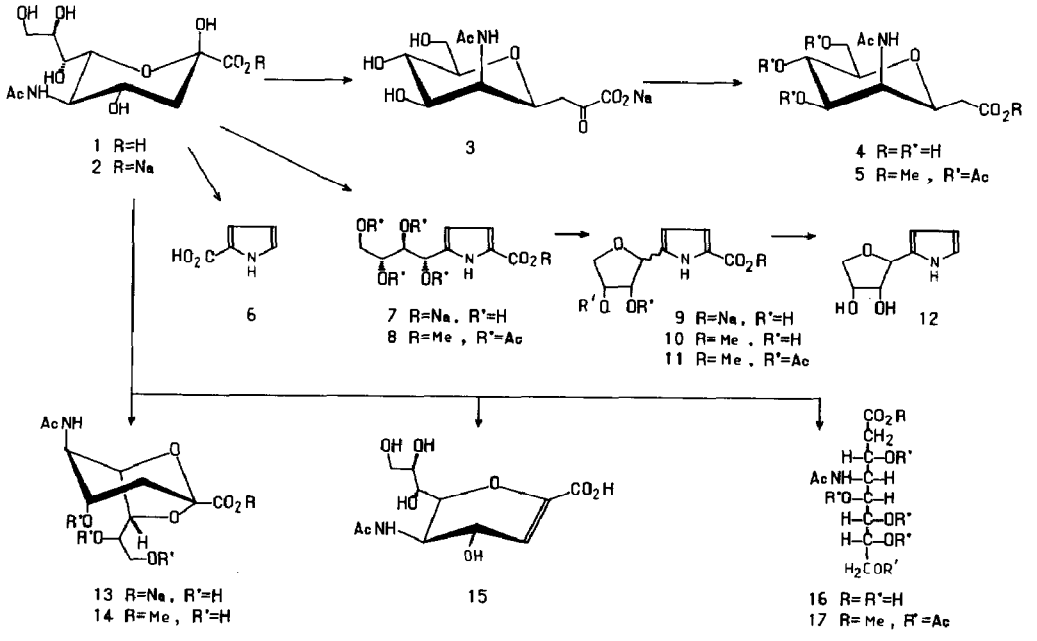
ABSTRACT

The thermal and photochemical degradation products of sodium *N*-acetylneuraminate (sodium Neu5Ac) were investigated by means of ^{1}H- and ^{13}C-n.m.r. spectroscopy, and mass spectrometry. Under all thermal conditions, sodium 5-acetamido-4,8-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonate (sodium *N*-acetyl-4,8-anhydroneuraminate) was obtained as the main product; on heating in alkaline solution, 4-acetamido-3,7-anhydro-2,4-dideoxy-D-*glycero*-D-*galacto*-octonic acid, pyrrole-2-carboxylic acid, and sodium 5-(D-*arabino*-tetrahydroxybutyl)pyrrole-2-carboxylate; on heating in acidic solution, sodium 5-(D-*erythro*-furanosyl)pyrrole-2-carboxylate and sodium 5-acetamido-2,7-anhydro-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosonate (sodium *N*-acetyl-2,7-anhydroneuraminate); on refluxing in neutral solution, 2-(β-D-*erythro*-furanosyl)pyrrole; and on heating of sodium Neu5Ac powder, 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid were obtained. Furthermore, on exposure to u.v. light (360 nm), 4-acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-octonic acid was produced.

INTRODUCTION

We recently demonstrated that sodium *N*-acetyl-4,8-anhydroneuraminate (**3**)



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was the main thermal degradation product of sodium *N*-acetylneuraminate (**2**, sodium Neu5Ac) powder and discussed a possible reaction mechanism[1]. Subsequently, we have attempted to isolate other degradation products of **2**, formed under various thermal and photochemical conditions, and we describe herein the isolation and identification of five new compounds, in addition to **3**.

RESULTS AND DISCUSSION

The degradation products of sodium Neu5Ac (**2**), which are summarized in Table I, were detected by use of t.l.c. and h.p.l.c. Compound **3**[1] was obtained as the main product under all thermal conditions tested. Compound **4** was one of the alkaline-degradation products of **2** and was isolated by Dowex 2 (HCO_2^-) ion-exchange resin column chromatography. As shown in Tables II and III, the ^1H- and ^{13}C-n.m.r. spectra of **4** resemble those of **3**. The ^{13}C-n.m.r. spectrum of **3** exhibited eleven, and that of **4** ten resonances, *i.e.*, the latter spectrum showed the disappearance of one signal of **3** in the carbonyl region, indicating that it is a decarbonyl product of **3**. In the two-dimensional (2D) long range ^{13}C–^1H correlation spectrum (Fig. 1), the ^{13}C resonance at δ 175.52 covers the peaks of H-2, H-2′, and H-3; it was therefore readily assigned to the C-1 carbonyl group, and all other resonances were also unambiguously assigned. As the positive-ion, fast-atom bombardment (f.a.b.) mass spectrum of **4** exhibited a molecular ion at *m/z* 264 $(M + H)^+$, the molecular formula, $C_{17}H_{25}NO_{10}$, was assigned to **5**, the methyl ester of the acetate of **4**. These results supported structure **4** as 4-acetamido-3,7-anhydro-2,4-dideoxy-D-*glycero*-D-*galacto*-octonic acid, lacking the carbonyl group of **3** at C-1 or C-2. Compound **4** was also obtained quantitatively from **3** on treatment with alkaline hydrogen peroxide.

Pyrrole-2-carboxylic acid[2] (**6**) and sodium 5-(D-*arabino*-tetrahydroxybutyl)pyrrole-2-carboxylate (**7**) were also alkaline-degradation products of **2**. Compound **6** was

TABLE I

Degradation products of **2** formed under various conditions

Product	*Condition*					*T.l.c.*	*H.p.l.c.*
	Alkaline	*Neutral*	*Acidic*	*Solid*	*U.v.*	(R_F)	(*Retention time*[a])
3	+	+	+	+		0.45	1.37
4	+	+					0.69
6	+	+	+	+		0.88	
7	+	+	+	+		0.71	2.43
9	+	+	+	+		0.76	5.58
12		+				0.81	
13			+	+		0.43	0.61
15	+	+	+	+		0.46	1.23
16					+	0.54	0.72

[a] Relative to that of **2**.

TABLE II

^{1}H-N.m.r. data for **2–4** and **13–16**[a]

Chemical shift (δ)	*Compound*						
	2	**3**	**4**[b]	**13**	**14**	**15**	**16**[b]
H-3a	2.252	2.983	2.604 (*H-2a*)	2.162	2.253	6.019	2.512 (*H-2a*)
H-3b	1.866	2.844	2.508 (*H-2b*)	1.998	2.085		2.512 (*H-2b*)
H-4	4.066	4.188	4.110 (*H-3*)	3.942	3.986	4.505	4.543 (*H-3*)
H-5	3.950	4.333	4.363 (*H-4*)	4.532	4.675	4.079	3.980 (*H-4*)
H-6	4.022	3.852	3.844 (*H-5*)	3.910	3.968	4.272	3.931 (*H-5*)
H-7	3.549	3.496	3.506 (*H-6*)	4.426	4.543	3.611	3.449 (*H-6*)
H-8	3.798	3.363	3.385 (*H-7*)	3.534	3.544	3.905	3.731 (*H-7*)
H-9a	3.881	3.814	3.844 (*H-8a*)	3.746	3.728	3.858	3.817 (*H-8a*)
H-9b	3.649	3.734	3.753 (*H-8b*)	3.584	3.585	3.638	3.604 (*H-8b*)
CH_3CO	2.026	2.041	2.064	2.026	2.027	2.052	2.040
OCH_3					3.915		
Coupling constant (Hz)	*Compound*						
	2	**3**	**4**[b]	**13**	**14**	**15**	**16**[b]
$J_{3a,3b}$	13.0	17.6	16.4 ($J_{2a,2b}$)	15.0	15.4		0 ($J_{2a,2b}$)
$J_{3a,4}$	4.9	8.4	8.3 ($J_{2a,3}$)	5.9	5.5	2.3	7.0 ($J_{2a,3}$)
$J_{3b,4}$	11.6	4.4	5.2 ($J_{2b,3}$)	0	0		7.0 ($J_{2b,3}$)
$J_{4,5}$	10.2	1.5	1.5 ($J_{3,4}$)	0	1.5	9.0	1.0 ($J_{3,4}$)
$J_{5,6}$	10.1	4.4	4.6 ($J_{4,5}$)	0	0	10.9	10.4 ($J_{4,5}$)
$J_{6,7}$	0.9	9.9	9.8 ($J_{5,6}$)	0.7	0	1.1	0 ($J_{5,6}$)
$J_{7,8}$	9.3	9.9	9.8 ($J_{6,7}$)	8.1	7.0	9.6	9.1 ($J_{6,7}$)
$J_{8,9a}$	2.8	4.8	2.3 ($J_{7,8a}$)	2.9	2.6	2.7	2.8 ($J_{7,8a}$)
$J_{8,9b}$	6.5	2.2	5.0 ($J_{7,8b}$)	5.9	6.0	6.3	6.3 ($J_{7,8b}$)
$J_{9a,9b}$	11.8	12.1	12.3 ($J_{8a,8b}$)	11.9	11.4	11.9	11.9 ($J_{8a,8b}$)

[a] For a solution in D_2O. [b] For compounds **4** and **16**, the exact numbering of the structures is given in parentheses. The locations in the columns is for comparison purposes.

TABLE III

^{13}C-N.m.r. data for **2–4** and **13–16**[a]

Chemical shift (δ)[b]	*Compound*				
	2	**3**	**4**[c]	**13**	**16**[c]
C-2	97.14(s)	202.25(s)	175.52(s) (*C-1*)	106.33(s)	176.37(s) (*C-1*)
C-3	40.12(t)	41.61(t)	36.81(t) (*C-2*)	36.06(t)	39.55(t) (*C-2*)
C-4	68.05(d)	73.48(d)	73.71(d) (*C-3*)	67.59(d)	66.45(d) (*C-3*)
C-5	53.02(d)	53.46(d)	53.33(d) (*C-4*)	52.73(d)	53.98(d) (*C-4*)
C-6	70.97(d)	73.70(d)	74.41(d) (*C-5*)	77.43(d)	68.35(d) (*C-5*)
C-7	69.32(d)	67.40(d)	67.49(d) (*C-6*)	72.72(d)	70.05(d) (*C-6*)
C-8	71.06(t)	80.81(d)	80.93(d) (*C-7*)	77.91(d)	71.46(d) (*C-7*)
C-9	64.04(t)	61.17(t)	61.26(t) (*C-8*)	63.04(t)	63.98(t) (*C-8*)
$COCH_3$	175.50(s)	172.06(s)	175.93(s)	174.31(s)	175.17(s)
CO_2R	177.45(s)	178.07(s)		174.80(s)	
$COCH_3$	22.84(q)	24.71(q)	22.53(q)	22.56(q)	22.60(q)

[a] For a solution in D_2O. [b] Multiplicity in parentheses. [c] See footnote [b] in Table II.

identified by comparison of its m.p. with that of an authentic sample. Compound **7** was rather unstable and its acyclic component cyclized[3] easily during purification on Dowex 2 (HCO_2^-) ion-exchange resin to give sodium 5-(D-*erythro*-furanosyl)pyrrole-2-carboxylate (**9**); it was therefore purified by neutral cellulose column chromatography and silica gel t.l.c. The ^{1}H-n.m.r. spectrum of **7** revealed heterocyclic pyrrole H-3 and H-4, which gave doublets of J 3.7 Hz at δ 6.693 and 6.190. Acetylation, followed by esterification of **7** into **8**, revealed four distinguishable *O*-acetyl groups, as indicated by the four acetate, methyl proton resonances at δ 2.090, 2.076, 2.062, and 2.043, and an *O*-methyl group, as indicated by the methoxy, methyl proton resonance at δ 3.852. These results indicated that **7** has four hydroxyl and one carbonyl groups. Assignment of the D-*arabino* configuration to **7** was based on that of its sugar precursor, **2**. Elemental analysis of **8**, the methyl ester of the acetate **7**, gave a molecular formula of $C_{18}H_{23}NO_{10}$. From these results, the structure of **7** was deduced. Gottschalk suggested[4] that **7** is an intermediate of the Ehrlich reaction of **1**, but without isolation and identification. Treatment of **3** with excess alkali also afforded **4** and **7**.

The α and β anomers of **9** were obtained as a 1:3 mixture, after cellulose and Sephadex LH-20 column chromatography, as determined by n.m.r. spectroscopy. This mixture was then esterified with diazomethane to give the α and β anomers of **10**, which were separated by silica gel t.l.c. Acetylation of **10β** and **10α** gave **11β** and **11α**, respectively, whose ^{1}H-n.m.r. spectra showed distinguishable signals for *O*-acetyl

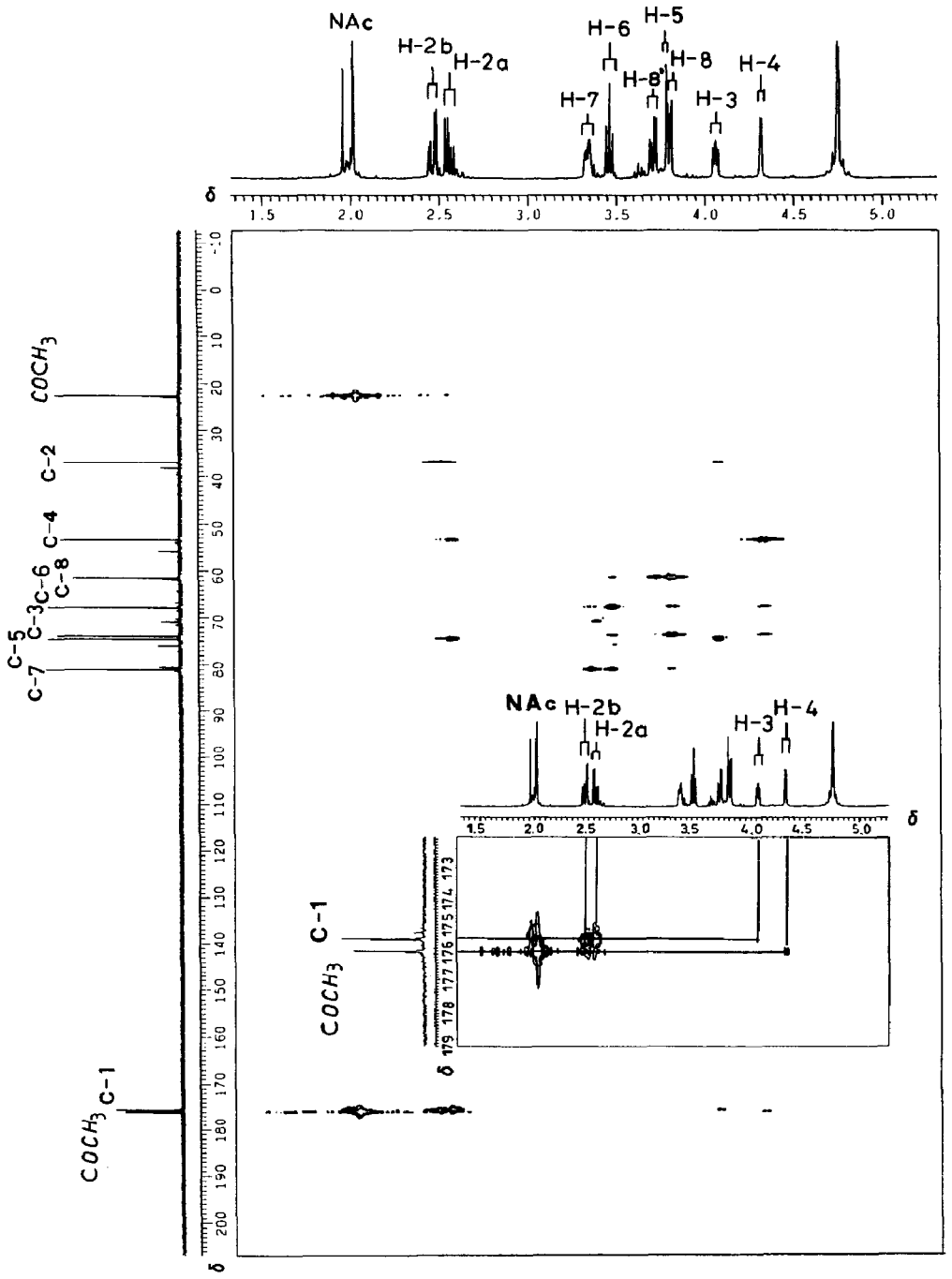

Fig. 1. 2D-Long range ^{13}C–^{1}H COSY spectrum for a solution of **4** in D_2O.

groups, *i.e.*, two acetate methyl and one methoxy methyl proton resonances at δ 2.121, 2.107, and 3.846 for **11β**, and at δ 2.181, 1.859, and 3.853 for **11α**, respectively. Both compounds **11β** and **11α** showed the same molecular ion at m/z 311 (M^+). These results indicated that **9** has two hydroxyl and one carbonyl groups. The configuration at C-1′ was determined using the empirical rule of Galbis Pérez *et al.*[5,6]; in the ^{1}H-n.m.r. spectra, the signal for the lower-shielded H-1′ doublet was observed at δ 5.232 for **11α** and at δ 5.113 for **11β**.

Another pyrrole compound, 5-(β-D-*erythro*-furanosyl)pyrrole (**12**), was extracted from the neutral-degradation mixture of **2** with ethyl acetate and, because of its instability, it was immediately purified by silica gel column chromatography. In the ^{1}H-n.m.r. spectra, the chemical shifts and coupling constants of the furanose regions of **12** and **9β** are closely similar; the signal for H-1′ appeared as a doublet of $J_{1',2'}$ 8.1 Hz at δ 4.780 for **9β**, and of $J_{1',2'}$ 7.7 Hz at δ 4.696 for **12**. Similarly, the signals for H-2′, H-3′, and H-4′ showed the same multiplicity, and were observed at δ 4.326, 4.381, 4.285, and 3.876 for **9β**; and at δ 4.147, 4.259, 4.191, and 3.763 for **12**; respectively. These results indicated that both compounds have the same configuration at C-1′. The e.i.m.s. of **12** exhibited a molecular ion at m/z 169.

A mixture of sodium *N*-acetyl-2,7-anhydroneuraminate (**13**) and **3** was obtained from the acidic-degradation mixture of **2** by cellulose column chromatography. After **3** had been separated by recrystallization from the lyophilized mixture, **13** was purified by silica gel t.l.c. and detected by l.c. The ^{1}H-n.m.r. data for **13** and its methyl ester (**14**) are presented in Table II. Compound **14** was identified by its mass spectrum as methyl *N*-acetyl-2,7-anhydroneuraminate, previously reported by Lifely and Cottee[7] to be a byproduct of the methanolysis of **1**; thus establishing the structure of **13**. These results indicated that **2** takes on the $^5C_2$ (D) conformation under acidic conditions.

5-Acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (**15**) was isolated by heating the solid form of **2** and identified by comparison with a prepared authentic sample[8,9].

Compound **2** was rather stable under u.v. irradiation at 360 nm and gave 4-acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-octonic acid (**16**) as the sole product only after a prolonged exposure to that light for 264 d at 3.6 μW/cm^2. Compound **16** was purified by column chromatography on Dowex 2 (HCO_2^-) ion-exchange resin. The chemical shifts of **16** in the ^{1}H-n.m.r. spectrum were very close to those of **2**, and the positive-ion f.a.b.m.s. spectrum exhibited a molecular ion at m/z 282 $(M + H)^+$. Esterification, followed by acetylation of **16** to give **17**, revealed five distinguishable *O*-acetyl groups, as indicated by the five acetate methyl and the methoxy methyl proton resonances at δ 2.112, 2.100, 2.048, 2.042, 2.010, and 3.663. Compound **17** showed the molecular ion at m/z 506 $(M + H)^+$ on mass spectrometry. These results indicated that **16** was an acyclic compound derived by decarbonylation of **2**; its ^{1}H-n.m.r. spectrum was identical to that of the C_8 compound produced by the oxidative decarboxylation of **2**.

In conclusion, regardless of whether it takes place in solution or not, the intramolecular dehydration of **2** is likely to occur under thermal conditions. Successive

cyclization of **2** occurs mainly between C-4 and C-8, and also at other positions, on a change in pH. Decarbonylation is also another likely reaction for **2**, due to the presence of an α-keto carbonyl group.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes and are uncorrected. Optical rotations were measured with a Jasco DIP-4 polarimeter. I.r. spectra were recorded with a Jasco IR-810 spectrometer. N.m.r. spectra (^1H and ^{13}C) were recorded with a Jeol GX-500 spectrometer with Me_4Si as the internal standard for solutions in $CDCl_3$ and CD_3OD, and with water (at δ 4.750) for those in D_2O for ^1H-n.m.r. spectra; and with Me_4Si as the internal standard for solutions in $CDCl_3$ and CD_3OD, and with 1,4-dioxane (at δ 67.40) for those in D_2O for ^{13}C-n.m.r. spectra. Mass spectra were recorded with a Jeol D300 spectrometer using electron impact (e.i.) or f.a.b. T.l.c. was carried out on Kieselgel 60 HF-254 (Merck) with 4:2:1 (v/v) propanol–water–acetic acid, and spots were detected by spraying with H_2SO_4 in ethanol, followed by heating. H.p.l.c. of the degradation products was carried out on a column (diam., 4.6 mm; length 150 mm) of Hitachi gel 3013-N ion-exchange resin at 40°, with elution with 4:1 (v/v) 0.23% (w/v) aqueous $NH_4H_2PO_4$–acetonitrile at a flow rate of 1.6 mL/min and monitoring the effluent at 206 nm.

4-Acetamido-3,7-anhydro-2,4-dideoxy-D-glycero-D-galacto-*octonic acid* (**4**). — To a solution of **2** (30.0 g, 90.6 mmol) in water (150 mL) was added solid NaOH (360 mg, 9.0 mmol) at room temperature. The mixture was stirred for 1 h at 80°, cooled, and then lyophilized to give a brownish-yellow powder. The powder (999 mg) was fractionated on a column (diam., 20 mm; length, 180 mm) of Dowex 2 (HCO_2^-) anion-exchange resin with 0.25M formic acid. Fractions containing **4** were combined and lyophilized to yield **4** (78.3 mg, 9.9%) as a white powder, $[\alpha]_D^{20}$ −29° (*c* 1.0, water); υ_{max}^{KBr} 3378 (OH and NH), 1722 and 1646 (C=O), and 1546 cm^{-1} (NH); ^1H- and ^{13}C-n.m.r., see Tables II and III; positive-ion f.a.b.m.s.: *m/z* 264 $(M + H)^+$.

Conversion of **3** *into* **4**. — To a 10% (v/v) aqueous methanolic solution (3 mL) of **3** (60 mg, 0.19 mmol) was added 30% H_2O_2 (0.05 mL) at 0°. The mixture was stirred at 0° for 15 min and then a catalytic amount of M NaOH was added, followed by further stirring for 1 h at 0°. The mixture was slightly acidified by addition of Dowex 50 (H^+) cation-exchange resin. Lyophilization of the filtered solution gave **4** quantitatively as a white powder.

Methyl 4-acetamido-5,6,7-tri-O-*acetyl-3,7-anhydro-2,4-dideoxy*-D-glycero-D-galacto-*octonate* (**5**). — To a solution of **4** (66.5 mg, 0.25 mmol) in methanol (5 mL) was added an excess amount of diazomethane in diethyl ether at 0°, followed by stirring for 1 h at room temperature, and then evaporation to dryness *in vacuo*. To the residue was added acetic anhydride (2 mL) and pyridine (1 mL), followed by stirring for 12 h at room temperature. After the reaction had been quenched with methanol, evaporation of the solvent gave a pale-yellow residue, which was purified by silica gel t.l.c. (R_F 0.59) with 20:1 (v/v) dichloromethane–methanol and then recrystallized from ethanol to give **5** as

colorless prisms (44.0 mg, 43.7%), m.p. 172–176.5°, $[\alpha]_D^{23}$ −19° (*c* 1.0, ethanol); ν_{max}^{KBr} 3378 (NH), 1751, 1737 and 1678 (C=O), 1533 (NH), and 1239 cm^{-1} (O–C–O); e.i.m.s.: *m/z* 403 (M^+), 371 ($M - OCH_3$)$^+$, 343 ($M - CH_3CO_2H$)$^+$, 329 ($M - CH_3 - COCH_3$)$^+$, 300 ($M - COCH_3 - CH_3CO_2H$)$^+$, 282 ($M - CH_3CO_2H - C_2H_5O_2$)$^+$, 240 ($M - COCH_3 - 2CH_3CO_2H$)$^+$, and 231 ($M - 4COCH_3$)$^+$.

Anal. Calc. for $C_{17}H_{25}NO_{10}$: C, 50.61; H, 6.25; N, 3.47. Found: C, 50.52; H, 6.27; N, 3.54.

Pyrrole-2-carboxylic acid (**6**). — To a solution of **2** (20.0 g, 60.4 mmol) in water (150 mL) was added solid NaOH (245 mg, 6.1 mmol) at room temperature, followed by stirring for 2.5 h at 80°, cooling, and then lyophilization to give a brownish-yellow powder. This was fractionated by gradient-elution chromatography on a column (diam., 45 mm; length, 600 mm) of Dowex 2 (HCO_2^-) anion-exchange resin with formic acid, from 0.25 to 0.5M. Fractions containing **6** were combined and extracted with diethyl ether. Evaporation of the solvent gave **6**, a white powder (140.6 mg, 2.1% from **2**), m.p. 198° (dec.); lit.[2] m.p. 201° (dec.).

*Sodium 5-(*D-arabino-*tetrahydroxybutyl)pyrrole-2-carboxylate* (**7**). — To a solution of **2** (5.0 g, 15.1 mmol) in water (100 mL) was added solid NaOH (200 mg, 5.0 mmol) at room temperature, followed by stirring for 48 h at 80°, cooling, and then lyophilization. The resultant brownish-yellow powder was extracted with methanol (250 mL). After evaporation of the solvent, the residue was fractionated by gradient-elution chromatography on a column (diam., 35 mm; length, 400 mm) of cellulose with acetonitrile–water, from 7:1 to 4:1 (v/v). Fractions containing **7** were combined and lyophilized to yield a brownish-yellow powder (crude **7**; 261 mg). An aliquot (105.3 mg) was further purified by silica gel t.l.c. (R_F 0.75) with 5:3:1 (v/v) butanol–methanol–water and cellulose t.l.c. (R_F 0.27) with 3:1 (v/v) acetonitrile–water to give **7**, a yellow powder (10.3 mg, 0.7% from **2**), m.p. 160° (dec.), $[\alpha]_D^{20}$ −12.3° (*c* 1.0, water); ν_{max}^{KBr} 3600–3240 (OH and NH) and 1563 cm^{-1} (C=O); ^{1}H-n.m.r. (D_2O): δ 6.693 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 6.190 (d, 1 H, H-4), 4.493 (d, 1 H, $J_{6,7}$ 3.7 Hz, H-6), 3.796 (dd, 1 H, $J_{7,8}$ 7.1 Hz, H-7), 3.764 (dd, 1 H, $J_{8,9}$ 3.0, $J_{9a,9b}$ 11.8 Hz, H-9a), 3.703 (ddd, 1 H, $J_{8,9b}$ 6.6 Hz, H-8), and 3.580 (dd, 1 H, H-9b); e.i.m.s. (Me_3Si deriv.): *m/z* 591 (M^+), 576 ($M - CH_3$)$^+$, 501 ($M - Me_3SiOH$)$^+$, 398 ($M - Me_3SiOH - Me_3SiOMe$)$^+$, 284 ($M - Me_3SiOMe - 2$ CHO-$SiMe_3$)$^+$ and 194 ($M - Me_3SiOH - Me_3SiOMe - 2$ $CHOSiMe_3$)$^+$.

*Methyl 5-(*D-arabino-*tetraacetoxybutyl)pyrrole* (**8**). — A solution of **7** (165.8 mg, 0.65 mmol) in acetic anhydride (3 mL)–pyridine (2 mL) was stirred for 12 h at room temperature. After the reaction had been quenched with methanol, evaporation of the solvent gave a pale-yellow residue. To a solution of the residue in methanol (10 mL) was added an excess amount of diazomethane in diethyl ether at 0°, followed by stirring for 1 h at room temperature. Evaporation of the mixture gave a pale-yellow residue, which was purified on a column of silica gel with dichloromethane and by silica gel t.l.c. (R_F 0.40) with 15:1 (v/v) dichloromethane–ethanol to give **8**, colorless oil (19.0 mg, 7.8%), $[\alpha]_D^{23}$ −18.4° (*c* 1.0, chloroform); $\nu_{max}^{CHCl_3}$ 3436 (NH) and 1743 cm^{-1} (C=O); ^{1}H-n.m.r. ($CDCl_3$): δ 9.392 (br, 1 H, NH), 6.816 (dd, 1 H, $J_{3,4}$ 3.8, $J_{3,NH}$ 2.7 Hz, H-3), 6.285 (dd, 1 H, $J_{4,NH}$ 2.7 Hz, H-4), 6.105 (d, 1 H, $J_{6,7}$ 5.2 Hz, H-6), 5.565 (dd, 1 H, $J_{7,8}$ 7.1 Hz, H-7), 5.183

(ddd, 1 H, $J_{8,9a}$ 3.2, $J_{8,9b}$ 5.5 Hz, H-8), 4.235 (dd, 1 H, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.109 (dd, 1 H, H-9b), 3.852 (s, 3 H, OCH_3), 2.090, 2.076, 2.062, and 2.043 (4 s, each 3 H, 4 $COCH_3$); e.i.m.s.: *m/z* 413 (M^+), 382 ($M - OCH_3$)$^+$, 353 ($M - CH_3CO_2H$)$^+$, 311 ($M - CH_3CO_2H - CH_3CO$)$^+$, 293 ($M - 2\ CH_3CO_2H$)$^+$, 251 ($M - 2\ CH_3CO_2H - CH_3CO$)$^+$, and 209 ($M - 2\ CH_3CO_2H - 2\ CH_3CO$)$^+$.

Anal. Calc. for $C_{18}H_{23}NO_{10}$: C, 52.30; H, 5.61; N, 3.39. Found: C, 52.30; H, 5.75; N, 3.27.

*Sodium 5-(*D*-erythrofuranosyl)pyrrole-2-carboxylate* (**9**). — A solution of **2** (20.0 g, 60.4 mmol) in Clark–Lubs buffer [200 mL; 0.2M KCl–0.2M HCl (pH 2.0)] was stirred for 33 h at 80°, cooled, and then evaporated *in vacuo* to give a brownish-yellow residue, which was extracted with methanol (700 mL). Evaporation of the solvent gave a brownish-yellow residue (12.4 g). This was fractionated by gradient-elution chromatography on a column (diam., 35 mm; length, 600 mm) of cellulose with acetonitrile–water, from 1:0 to 5:1 (v/v). Fractions containing **9** were combined and lyophilized to yield a pale-yellow powder (crude **9**; 538.9 mg). An aliquot (243.1 mg) was further purified on a column (diam., 20 mm; length, 330 mm) of Sephadex LH-20 with methanol to give **9**, pale-yellow powder (52.5 mg, 0.7% from **2**), $[\alpha]_D^{20}$ −68° (*c* 1.1, water; β:α = 33:10); ν_{max}^{KBr} 3600–3000 cm^{-1} (OH and NH); ^{1}H-n.m.r. (D_2O) for **9**β: δ 6.913 (d, 1 H, $J_{3,4}$ 4.0 Hz, H-3), 6.341 (d, 1 H, H-4), 4.780 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.381 (ddd, 1 H, $J_{2',3'}$ 4.8, $J_{3',4'}$ 4.4, $J_{3',4'}$ 2.2 Hz, H-3'), 4.326 (dd, 1 H, H-2'), 4.285 (dd, 1 H, J_{gem} 10.3 Hz, H-4'), and 3.876 (dd, 1 H, H-4'); e.i.m.s. (Me_3Si deriv.): *m/z* 501 (M^+), 486 ($M - CH_3$)$^+$, 429 ($M - Me_3Si + H$)$^+$, 414 ($M - Me_3Si + H - Me_3SiOH$)$^+$, and 296 ($M - Me_3Si + H - CH_3CHO_2SiMe_3$)$^+$.

*Methyl 5-(2',3'-di-*O*-acetyl-*β*-*D*-erythrofuranosyl)pyrrole-2-carboxylate* (**11**β) *and methyl 5-(2',3'-di-*O*-acetyl-*α*-*D*-erythrofuranosyl)pyrrole-2-carboxylate* (**11**α). — To a solution of **9** (40 mg, 0.19 mmol) in water (10 mL) was added a catalytic amount of Dowex 50 (H^+) cation-exchange resin, followed by stirring for 2 h at room temperature, filtration, and lyophilization. To a solution of the residue in methanol (5 mL) was added an excess amount of diazomethane in diethyl ether, followed by stirring for 1 h at room temperature. Evaporation of the mixture gave a pale-yellow residue, which was fractionated by silica gel t.l.c. with 4:1 (v/v) chloroform–methanol. Fractions containing **10**β (R_F 0.64) and **10**α (R_F 0.74) were separately combined and evaporated. Compounds **10**β and **10**α were separately acetylated with acetic anhydride and pyridine and, after the addition of water, the reaction mixtures were extracted with chloroform (15 mL). The extracts were dried ($MgSO_4$) and evaporation of the solvent gave pale-yellow residues. Each residue was purified on a column (diam., 10 mm; length, 150 mm) of silica gel with 9:1 (v/v) chloroform–ethyl acetate to give **11**β (22 mg, 55%) and **11**α (6.3 mg, 12%) as pale-yellow powders, respectively; $[\alpha]_D^{23}$ −127° (*c* 1.0, chloroform) for **11**β, +167° (*c* 0.6, chloroform) for **11**α; $\nu_{max}^{CHCl_3}$ 3422 (NH), and 1747 and 1703 cm^{-1} (C=O) for **11**β; ^{1}H-n.m.r. ($CDCl_3$) for **11**β: δ 9.540 (br., 1 H, NH), 6.850 (dd, 1 H, $J_{NH,3}$ 2.6, $J_{3,4}$ 3.7 Hz, H-3), 6.183 (dd, 1 H, H-4), 5.397 (ddd, 1 H, $J_{2',3'} = J_{3',4'}$ 5.5, $J_{3',4'}$ 4.0 Hz, H-3'), 5.223 (dd, 1 H, $J_{1',2'}$ 6.2 Hz, H-2'), 5.113 (d, 1 H, H-1'), 4.359 (dd, 1 H, J_{gem} 10.3 Hz, H-4'), 3.975 (dd, 1 H, H-4'), 3.846 (s, 3 H, OCH_3), 2.121 and 2.107 (2 s, each 3 H, 2 $COCH_3$); ^{1}H-n.m.r.

($CDCl_3$) for **11α**: δ 9.490 (br. s, 1 H, NH), 6.798 (dd, 1 H, $J_{NH,3}$ 2.6, $J_{3,4}$ 3.7 Hz, H-3), 6.107 (dd, 1 H, H-4), 5.555 (ddd, 1 H, $J_{2',3'}$ 4.8, $J_{3',4'}$ 4.0, $J_{3',4'}$ 4.0 Hz, H-3'), 5.517 (dd, 1 H, $J_{1',2'}$ 6.2 Hz, H-2'), 5.232 (d, 1 H, H-1'), 4.100 (d, 2 H, H_2-4'), 3.853 (s, 3 H, OCH_3), 2.181 and 1.859 (2 s, each 3 H, 2 $COCH_3$); e.i.m.s. for **11β**: *m/z* 311 (M^+), 280 ($M - OCH_3$)$^+$, 251 ($M - CH_3CO_2H$)$^+$, and 192 ($M - CH_3CO_2H - CH_3CO_2$)$^+$.

Anal. (high resolution e.i.m.s.): Calc. for $C_{14}H_{17}NO_7$:M^+, 311.1005. Found for **11β**: M^+, 311.1008.

*2-(β-*D*-Erythrofuranosyl)pyrrole* (**12**). — A solution of **2** (10 g, 30.4 mmol) in water (100 mL) was refluxed for 18.5 h, cooled, and then extracted with ethyl acetate (200 mL × 3). After evaporation of the solvent, the residue was fractionated on a column (diam., 18 mm; length, 250 mm) of silica gel with 7:1 (v/v) chloroform–methanol. Fractions containing **12** were combined and evaporated to yield a pale-yellow oil (5.6 mg, 0.1%); ν_{max}^{KBr} 3348 (OH and NH) and 1125 cm^{-1} (C–O); ^{1}H-n.m.r. (CD_3OD): δ 6.714 (dd, 1 H, $J_{2,3}$ 2.9, $J_{2,4}$ 1.5 Hz, H-2), 6.100 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 6.020 (dd, 1 H, H-3), 4.696 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.259 (ddd, 1 H, $J_{2',3'}$ 5.1, $J_{3',4'\beta}$ 4.8, $J_{3',4'\alpha}$ 2.9 Hz, H-3'), 4.191 (dd, 1 H, J_{gem} 9.5 Hz, H-4'β), 4.147 (dd, 1 H, H-2'), and 3.763 (dd, 1 H, H-4'α); e.i.m.s.: *m/z* 169 (M^+) and 96 ($M - CHCHOHCHOH$)$^+$.

*Sodium 5-acetamido-2,7-anhydro-3,5-dideoxy-*D*-glycero-α-*D*-galacto-nonulopyranosonate* (**13**). — A solution of **2** (20.0 g, 60.4 mmol) in Clark–Lubs buffer (200 mL; pH 2.0) was stirred for 40 h at 80°, cooled, and concentrated. The residue was purified in the same manner as described for the isolation of **9**. Fractions containing **3** and **13** were combined and lyophilized to yield **3**, a pale-yellow powder (6.16 g); it crystallized from methanol and was filtered off. Evaporation of the mother liquor gave crude **13** (1.93 g) as a yellow powder. An aliquot (200 mg) was purified by silica gel t.l.c. with 3:1 (v/v) methanol–chloroform to give **13** (R_F 0.62), a white powder (89 mg, 4.3%), m.p. 205–210° (dec.), $[\alpha]_D^{20}$ +55.5° (*c* 0.73, water); ν_{max}^{KBr} 3650–3000 (OH and NH), 1628 (C=O), and 1550 cm^{-1} (NH); ^{1}H- and ^{13}C-n.m.r., see Tables II and III; positive ion f.a.b.m.s.: *m/z* 336 ($M + Na$)$^+$ and 314 ($M + H$)$^+$.

*Methyl 5-acetamido-2,7-anhydro-3,5-dideoxy-*D*-glycero-α-*D*-galacto-nonulopyranosonate* (**14**). — To a solution of **13** (5 mg, 0.016 mmol) in dimethyl sulfoxide (100 μL) was added methyl iodide (100 μL) at room temperature. The mixture was kept for 30 min and evaporation of the solvent gave **14** quantitatively as a pale-yellow residue; ^{1}H-n.m.r., see Table II; e.i.m.s. (Me_3Si deriv.): *m/z* 506 ($M - CH_3$)$^+$, 462 ($M - CO_2CH_3$)$^+$, 418 ($M - CH_2OSiMe_3$)$^+$, 416 ($M - CH_3 - Me_3SiOH$)$^+$, 328 ($M - CH_2OSiMe_3 - Me_3SiOH$)$^+$, 316 ($M - CH_2OSiMe_3$-$CHOSiMe_3$)$^+$, 238 ($M - CH_2O$-$SiMe_3 - 2\,Me_3SiOH$)$^+$, and 228 ($M - CH_2SiMe_3$-$CHOSiMe_3$-$CHO - NH_2COCH_3$)$^+$; lit.[7]: *m/z* 506, 462, 418, 416, 328, 316, 238, and 228.

*5-Acetamido-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enonic acid* (**15**). — Compound **2** in powder form (20 g, 60.4 mmol) was heated for 9 h at 140°. The still friable product (8 g) was dissolved in methanol and then fractionated by gradient-elution chromatography on a column (diam., 45 mm; length, 540 mm) of cellulose with acetonitrile–water, from 8:1 to 5:1 (v/v). Fractions containing **15** were combined and lyophilized to yield a pale-yellow powder (mixture of **2**, **3**, and **15**; 450 mg). To an aliquot

(98 mg), dissolved in water (5 mL), was added 30% H_2O_2 (1 mL) at 0°, followed by stirring for 5 min. After the addition of a catalytic amount of M NaOH, the base was neutralized with Dowex 50 (H^+) cation-exchange resin and the solution was lyophilized to give a pale-yellow powder (90 mg; mixture of **2**, **4**, and **15**). The powder was fractionated on a column (diam., 22 mm; length, 100 mm) of Dowex 1 (HCO_2^-) anion-exchange resin with 0.3M formic acid. Fractions containing **15** were combined and lyophilized to give **15**, a white powder (5.1 mg, 0.3%); ν_{max}^{KBr} 3325 (OH and NH), 1718 and 1648 (C=O), and 1556 cm^{-1} (NH); ^{1}H-n.m.r., see Table II; e.i.m.s. (Me ester, Me_3Si deriv.): *m/z* 593 (M^+), 578 ($M - CH_3$)$^+$, 388 ($M - CHOSiMe_3$-CH_2OSiMe_3)$^+$, 341 ($M - Me_3SiOH - CH_2OSiMe_3 - NH_2COCH_3$)$^+$, 307 ($CH_2OSiMe_3$-$CHOSiMe_3$-$CH=OSiMe_3$)$^+$, 298 ($M - CHOSiMe_3$-$CH_2OSiMe_3 - Me_3SiOH$)$^+$, 227 ($M - CHOSiMe_3$-$CHOSiMe_3$-$CHOSiMe_3$-$CH_2OSiMe_3 - NH_2COCH_3$)$^+$, 217 ($CH_2=COSiMe_3$-$CH=OSiMe_3$)$^+$, 205 ($CH_2OSiMe_3$-$CH=OSiMe_3$)$^+$, and 186 ($CH_3CONH=CH$-$CH=CHOSiMe_3$)$^+$; lit.[9]: *m/z* 593, 578, 388, 341, 307, 298, 227, 217, 205, and 186.

4-Acetamido-2,4-dideoxy-D-glycero-D-galacto-*octonic acid* (**16**). — A solution (5 mL) of **2** (500 mg, 1.51 mmol) was exposed to u.v. light at 360 nm at room temperature for 264 d at 3.6 $\mu W/cm^2$ and then lyophilized. The residue (150 mg) was purified on a column (diam., 18 mm; length, 200 mm) of Dowex 2 (HCO_2^-) anion-exchange resin with 0.25M formic acid. Fractions containing **16**, were combined and lyophilized to yield **16**, a white powder (6.6 mg, 5.2% from **2**), $[\alpha]_D^{23}$ −43° (*c* 1.0, water): ν_{max}^{KBr} 3700–3000 (OH and NH), 1722 and 1618 (C=O), and 1540 cm^{-1} (NH); ^{1}H- and ^{13}C-n.m.r., see Tables II and III; positive-ion f.a.b.m.s.: *m/z* 304 ($M + Na$)$^+$, 282 ($M + H$)$^+$, and 264 ($M + H - H_2O$)$^+$.

Methyl 4-acetamido-3,5,6,7,8-penta-O-*acetyl-2,4-dideoxy*-D-glycero-D-galacto-*octonate* (**17**). — To a solution of **16** (160 mg, 0.57 mmol) in methanol (8 mL) was added an excess amount of diazomethane in ether at room temperature, followed by stirring for 30 min. After evaporation of the solvent, dry pyridine (3 mL) and acetic anhydride (5 mL) were added to the residue, followed by stirring for 2 h at room temperature. After addition of water (8 mL), the mixture was extracted with chloroform (30 mL × 2). Evaporation of the solvent gave a pale-yellow oil, which was purified by column (diam., 14 mm; length, 130 mm) chromatography of silica gel with 1:4 (v/v) hexane–ethyl acetate to give **17** as colorless needles (28.5 mg, 9.9%), m.p. 146–147°, $[\alpha]_D^{23}$ +2.3° (*c* 1.0, ethanol); ν_{max}^{KBr} 1749 and 1653 (C=O) cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 5.623 (d, 1 H, $J_{NH,4}$ 10.4 Hz, NH), 5.372 (dd, 1 H, $J_{6,7}$ 8.2, $J_{5,6}$ 2.1 Hz, H-6), 5.281 (dd, 1 H, $J_{4,5}$ 10.4 Hz, H-5), 5.164 (ddd, 1 H, $J_{2,3}$ 6.5, $J_{2',3}$ 6.5, $J_{3,4}$ 1.6 Hz, H-3), 5.043 (ddd, 1 H, $J_{7,8'}$ 5.5, $J_{7,8}$ 3.0 Hz, H-7), 4.543 (ddd, 1 H, H-4), 4.257 (dd, 1 H, $J_{8,8'}$ 12.5 Hz, H-8a), 3.992 (dd, 1 H, H-8b), 3.663 (s, 3 H, OCH_3), 2.498 (d, 2 H, H-2,2'), 2.112, 2.100, 2.048 and 2.010 (4 s, each 3 H, 4 $COCH_3$), and 2.042 (s, 6 H, 2 $COCH_3$); positive-ion f.a.b.m.s.: *m/z* 506 ($M + H$)$^+$.

Anal. Calc. for $C_{21}H_{31}NO_{13}$: C, 49.90; H, 6.18; N, 2.77. Found: C, 49.92; H, 6.02; N, 2.59.

Conversion of **2** *into* **16**. — To a solution of **2** (500 mg, 1.72 mmol) in water (10 mL) was added 35% H_2O_2 (5 mL) at 0° and a catalytic amount of M NaOH, followed by stirring at room temperature until the starting material had completely disappeared on t.l.c. (30 min). Evaporation of the solvent yielded quantitatively **16** as a white powder.

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