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THE SIDE-CHAIN CONFORMATIONS OF *N*-ACETYL-7-,8-,9-DEOXY-, AND -4,7-DIDEOXY-NEURAMINIC ACID AND THEIR EFFECT ON THE ACTIVATION OF CTP:*N*-ACYLNEURAMINIC ACID CYTIDYLYL-TRANSFERASE*

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ABSTRACT

The conformations of the deoxy side-chain analogs of *N*-acetylneuraminic acid and of *N*-acetyl-4-deoxyneuraminic acid have been studied by n.m.r. spectroscopy, which allowed us to distinguish between local minima conformations suggested by hard-sphere calculations. The conformations were correlated with the activity with CTP: *N*-acetylneuraminic acid cytidylyltransferase (CMPsialate synthase; EC 2.7.7.43).

INTRODUCTION

Sialic acids are involved in a great number of biologically important functions[1,2]. To get insight into the structure–activity relationship of these biomolecules, many analogs of sialic acid have been prepared over the past years. Structural variations at C-4 (refs. 3–5), C-7 (ref. 3), C-9 (refs. 6–9), and at the *N*-acyl group[3] have been evaluated for their effect on CMPsialate synthase. For instance, such derivatives at C-9 as azidodeoxy[6], deoxyfluoro[7], *O*-acetyl[8], aminodeoxy[9]; and acetamidodeoxy[9], 4-*O*-methyl-[4], and 4-deoxy Neu5Ac[5] could be transformed into a CMP compound. In the case of the side-chain epimers[10], the inversion of the configuration at C-8 abolished the activation, whereas 7,8-(bisepi)Neu5Ac could be activated to an extent of 50% as compared to Neu5Ac. These results incited us to determine the side-chain conformations of epi-Neu5Ac compounds[11]. As a consequence of the change of the configuration at C-8, a strongly curved β-profile

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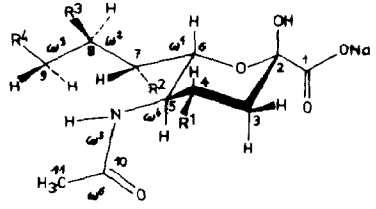
arises which cannot be recognized by the activating enzyme, whereas the surface of the 7,8-(bisepi)Neu5Ac is very similar to the nearly planar profile of Neu5Ac itself. We assumed[11] that the groups that are responsible for the recognition of Neu5Ac by the activating enzyme are OH-8, OH-2, and NH-5. The corresponding bonds are in a nearly coaxial geometry. This finding and the observation that the methyl glycoside of β-Neu5Ac is a competitive inhibitor of CMPsialate synthase[12], and the observation that both epimeric 2-deoxy analogs of Neu5Ac did not inhibit CMP-sialate synthase[12] confirmed the previously held assumption that axial O-2 is necessary for recognition[11]. To evaluate the relationship between structure and enzymic activity, it was of interest to synthesize more lipophilic *N*-acetyl-7-, 8-, and 9-deoxy-, and -4,7-dideoxy-neuraminic acid[13], and we report herein the side-chain conformation of these compounds and their β-profiles.

RESULTS AND DISCUSSION

N-Acetylneuraminic acid (**1**) and the 7-deoxy (**2**), 8-deoxy (**3**), 9-deoxy (**4**), and 4,7-dideoxy (**5**) analog[13] were transformed into the sodium salt and purified by gel chromatography. The conformation of the side chain was determined by the angles ω^1 to ω^3 and, furthermore, by the angles ω^4 to ω^6 of the acetamido group (Scheme 1). The proportions of the α anomers in **2** to **5** are less than 10% and, similar to the value observed[14] for **1**.

Because of the stereochemical correlations between the side chains of

ω^1 C-5—C-6—C-7—C-8
ω^2 C-6—C-7—C-8—C-9
ω^3 C-7—C-3—C-9—R-4
ω^4 H-5—H-5—H-5—HN
ω^5 NH—N-5—C-10—O-10
ω^6 O-10—C-10—C-11—H-11



	R^1	R^2	R^3	R^4
1	OH	OH	OH	OH
2	OH	H	OH	OH
3	OH	OH	H	OH
4	OH	OH	OH	H
5	H	H	OH	OH

Scheme 1

Neu5Ac and the corresponding hexitols, the spectra of the latter compounds were recorded too. The spectroscopic data of **1** and D-mannitol were used as reported (ref. 11 and literature cited therein). The first order interpretation of the ^{1}H-n.m.r. spectra of **2**, **3**, and **4** was possible without ambiguity. The values obtained were used to refine the parameters in an iterative way by use of the software delivered

TABLE I

^{1}H-N.M.R. CHEMICAL SHIFTS OF COMPOUNDS **1–5** AND RELATED ALDITOLS[a]

Hydrogen atom[b]	*Compound*								
	1[c]	D-*Mannitol*[c]	**2**	*3-Deoxy*-D-arabino-*hexitol*[d]	**3**	*2-Deoxy*-D-arabino-*hexitol*[d]	**4**	*6-Deoxy*-L-*mannitol*[e]	**5**
3*a*	1.83		1.89		1.80		1.83		1.56
3*e*	2.22		2.21		2.20		2.23		1.92
4*a* (1a)	4.03		3.96	3.72	4.00	3.80	4.05	3.82	1.78
4*e* (1b)				3.56		3.62		3.63	1.85
5 (2)	3.92		3.58	3.60	3.86	3.72	3.97	3.71	3.65
6 (3)	3.99		3.94	3.80	3.61	3.40	3.99	3.75	3.92
7a (4a)	3.52	3.79	1.65	1.50	3.82	3.98	3.35	3.56	1.66
7b (4b)			1.59	1.61					1.55
8a (5a)	3.76	3.75	3.87	3.87	1.89	1.81	3.87	3.83	3.89
8b (5b)					1.68	1.73			
9a (6a)	3.85	3.86	3.57	3.58	3.68	3.71	1.26	1.24	3.57
9b (6b)	3.62	3.67	3.46	3.47	3.68	3.71			3.45
Ac	2.06		2.06		2.05		2.08		2.06

[a]Shifts (δ) are downfield from the signal of Me_4Si at 298 K, and are iterated values. [b]Numbering of hexitol atoms is shown in parentheses. [c]From ref. 11. [d]Numbering of 3-deoxy-D-*arabino*-hexitol and 2-deoxy-D-*arabino*-hexitol is reversed (from 6a to 1b, corresponding to 4-deoxy-L-*arabino*-hexitol and to 5-deoxy-L-*arabino*-hexitol. [e]From ref. 16.

TABLE II

^{1}H-N.M.R. COUPLING CONSTANTS OF COMPOUNDS **1–5** AND RELATED ALDITOLS[a]

Coupling[b]	*Compound*								
	1[c]	D-*Mannitol*[c]	**2**	*3-Deoxy*-D-arabino-*hexitol*[d]	**3**	*2-Deoxy*-D-arabino-*hexitol*[d]	**4**	*6-Deoxy*-L-*mannitol*[e]	**5**
3*a*,3*e*	−12.9		−13.0		−13.0		−13.0		−13
3*a*,4*a*	11.4		11.6		11.3		12.0		9
3*a*,4*e*									4
3*e*,4*a*	4.9		5.0		4.9		4.5		4
3*e*,4*e*									2
4*a*,4*e* (1a,1b)				−11.7		−11.2		−11.8	−12
4*e*,5 (1a,2)	10.3		10.1	3.3	10.0	2.7	9.5	2.6	10.0
4*e*,5 (1b,2)				7.4		6.1		6.0	3.5
5,6 (2,3)	10.3		10.3	5.5	10.2	8.1	9.5	8.5	10.0
6,7a (3,4a)	1.0	0.0	2.2	2.7	1.3	2.1	0.5	1.5	2.3
6,7b (3,4b)			10.1	10.3					10.0
7a,7b (4a,4b)			−14.7	−14.6					−14.8
7a,8a (4a,5a)	8.9	9.0	9.7	10.5	8.9	9.4	8.5	7.8	9.5
7a,8b (4a,5b)					4.7	4.1			
7b,8a (4b,5a)			3.1	2.4					2.9
8a,8b (5a,5b)					−14.2	−14.2			
8a,9a (5a,6a)	2.7	3.0	3.3	3.8	6.0	5.9	6.3	6.4	4.0
8a,9b (5a,6b)	6.5	6.3	6.8	6.9	6.0	5.9			7.0
8b,9a (5b,6a)					7.2	7.2			
8b,9b (5b,6b)					7.2	7.2			
9a,9b (6a,6b)	−11.9	−11.8	−11.7	−11.7	−11.2	−11.2			−12.0

[a]Iterated values. [b]Numbering of hexitols in parentheses. [c]From ref. 11. [d]Numbering of 3-deoxy-D-*arabino*-hexitol and 2-deoxy-D-*arabino*-hexitol is reversed (from 6a to 1b, corresponding to 4-deoxy-L-*arabino*-hexitol and 5-deoxy-L-*arabino*-hexitol). [e]From ref. 16.

TABLE III

^{13}C-N.M.R. CHEMICAL SHIFTS OF COMPOUNDS **1–5** AND RELATED ALDITOLS[a]

Carbon atom[b]	*Compound*								
	1[c]	D-*Mannitol*[c]	**2**[d]	*3-Deoxy*-D-arabino-*hexitol*[e]	**3**	*2-Deoxy*-D-arabino-*hexitol*[e]	**4**[f]	*6-Deoxy*-L-*mannitol*	**5**
1	177.55		177.30		177.60		177.60		178.23
2	97.33		97.00		97.22		97.29		96.02
3	40.33		40.44		40.42		40.27		31.98
4 (1)	68.21	64.04	68.14	63.31	68.14	64.00	68.18	64.04	25.98
5 (2)	53.24	71.70	57.47	75.83	53.80	72.11	53.39	72.14	50.12
6 (3)	71.19	70.13	69.70	69.20	74.46	74.05	71.09	70.49	70.74
7 (4)	69.50	70.13	35.51	35.98	66.68	67.95	73.52	74.24	35.71
8 (5)	71.31	71.70	69.07	69.22	36.07	36.19	67.68	68.19	68.99
9 (6)	64.24	64.04	66.78	66.93	59.61	59.54	20.22	19.75	66.81
10	175.71		175.66		175.84		175.60		174.61
11	23.10		23.28		23.11		23.13		23.06

[a]Shifts (δ) are downfield from the signal of Me_4Si (δ 67.40 upfield from the signal of 1,4-dioxane in D_2O at 298 K). [b]Numbering of hexitols in parentheses. [c]From ref. 11. [d]Assigned by means of ^{13}C–^{1}H-correlation. [e]Numbering of 3-deoxy-D-*arabino*-hexitol and 2-deoxy-D-*arabino*-hexitol is reversed (from 6a to 1, corresponding to 4-deoxy-L-*arabino*-hexitol and to 5-deoxy-L-*arabino*-hexitol). [f]Assigned by a Chortle[17] experiment.

TABLE IV

^{13}C-N.M.R. CHEMICAL-SHIFT DIFFERENCES BETWEEN **2–5** AND **1**, AND BETWEEN **1–4** AND RELATED ALDITOLS[a]

Compounds	*^{13}C-Shift difference between compounds at*								
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	*C-9*
2 and **1**	−0.25	−0.33	0.11	−0.07	4.25	−1.49	−33.99	−2.24	2.54
3 and **1**	0.05	−0.11	0.09	−0.07	0.56	3.27	−2.82	−35.24	−4.63
4 and **1**	0.05	−0.04	−0.06	−0.03	0.15	−0.10	4.02	−3.63	−44.02
5 and **1**	0.68	−1.31	−8.35	−42.23	−3.12	−0.45	−33.79	−2.32	2.57

Compound and corresponding alditol	*^{13}C-Shift difference between compound and corresponding alditol*				
	C-5–C-2	*C-6–C-3*	*C-7–C-4*	*C-8–C-5*	*C-9–C-6*
1 and D-mannitol	−18.46	1.06	−0.63	−0.39	0.20
2 and 3-deoxy-D-*arabino*-hexitol[a]	−18.36	0.50	−0.47	−0.15	−0.15
3 and 2-deoxy-D-*arabino*-hexitol[a]	−18.31	0.41	−1.27	−0.12	0.07
4 and 6-deoxy-L-mannitol	−18.75	0.60	−0.72	−0.51	0.47

[a]Numbering of 3-deoxy-D-*arabino*-hexitol and 2-deoxy-D-*arabino*-hexitol is reversed (from 6 to 1, corresponding to 4-deoxy-L-*arabino*-hexitol and to 5-deoxy-L-*arabino*-hexitol).

TABLE V

RESULTS OF CALCULATIONS OF ANGLES[a] AND ENERGY[b] FOR COMPOUNDS **1** TO **5**

	Compounds							
	1		***2 and* 5**		**3**		**4**	
Conformer	*Angle* ω^1/ω^2	*E*	*Angle* ω^1/ω^2	*E*	*Angle* ω^1/ω^2	*E*	*Angle* ω^1/ω^2	*E*
1a–5a	170/180	0	170/170	0	170/190	0	170/180	0
1b–5b	170/70	3	170/70	8	170/−70	5	170/70	4
1c–5c	170/−60	9	−60/170	9	−60/190	8	170/−60	5

[a]Only the first minimum of the ω^1/ω^2 group distributed in ω^3 is given. For distribution of ω^3 to ω^6, see text. [b]Energy (E) is given in kJ above the global minimum.

TABLE VI

COMPARISONS OF CALCULTED WITH OBSERVED $^3J_{H,H}$ COUPLING CONSTANTS[a] FOR COMPOUNDS **1–5** AND THEIR CONFORMERS **a, b,** AND **c**[b]

H-Atom–H-atom	*Obs. coupling*		*Calc. coupling*			*Obs. coupling*		*Calc. coupling*			*Obs. coupling*		*Calc. coupling*	
	1	**4**	**1a,4a**	**1b,4b**	**1c,4c**	**2**	**5**	**2a,5a**	**2b,5b**	**2c,5c**	**3**	**3a**	**3b**	**3c**
6–7a	1.0	0.5	1.1	1.0	1.4	2.2	2.3	2.3	1.5	4.6	1.3	1.1	1.1	3.8
6–7b						10.1	10.0	10.3	9.8	2.6				
7a–8a	8.9	8.5	9.0	3.2	3.0	9.7	9.5	10.6	2.1	10.6	8.9	10.5	2.3	10.3
7b–8a						3.1	2.9	1.9	11.7	1.8				
7a–8b											4.7	2.5	11.2	2.3

[a]Calculated $^3J_{H,H}$ couplings[20] are the same for the conformers of **1** and **4**, and for those of **2** and **5**. [b]**1a–5a** means calculated conformer **a** (Table V) of compounds **1–5**.

with the spectrometer. In compound **5**, the assignment of both H-4 and H-7 signals was performed by double-indor experiments[15] by irradiating some frequencies of the H-5 and H-6 signals. The ^{1}H-n.m.r. spectrum of L-rhamnitol was used as published by Gillies and Lewis[16] and the other hexitol spectra were accessible to first-order interpretation (see Tables I and II).

The assignment of the carbon resonances of **2** was made in an unambiguous way by means of a carbon–proton, shift-correlated spectrum. The carbon shifts of **4** were assigned by means of a CHORTLE[17] spectrum. The shift values of **3** and **5** were assigned empirically after consideration of the shift differences to **1** on replacement of hydroxyl groups by hydrogen atoms (Tables III and IV). The assignments of the C-1 to C-3 signals of L-rhamnitol were proved by use of the (1-^{2}H)hexitol spectrum and correspond to reported values[18]. The assignment of the C-1 to C-4 signals of 3-deoxy-D-*arabino*-hexitol was proved by use of the (1-^{2}H)- and (3-^{2}H)-hexitol spectra.

Hardsphere calculations, done as previously described[11], were used to find the possible locations of the minima. The conformations found having an energy below 10 kJ above the global minimum are listed in Table V. In each row, only the first of the minima varying in ω^3 is listed. In compounds **1, 2,** and **5**, owing to the *gauche* effect, ω^3 is almost equally distributed, resulting in an OH-8–OH-9 angle of ±65°, and $^3J_{8,9}$ couplings of 3 and 7 Hz. In compound **3**, where OH-8 is replaced by a hydrogen atom, the ω^3 angle is almost equally distributed between ±65 and 180°, resulting in $^3J_{8,9}$ couplings of 6 and 7 Hz (see Table II for the observed coupling constants). For all listed minima, the angles ω^4 and ω^5 are equal to 180° and that of ω^6 to 0°. The calculated energy differences between the stretched (**2a–5a**) and the bent forms (**2b,c–5b,c**; Table V) are in the range calculated for butane (4 kJ above the stretched form[19]).

To prove that the calculated global minimum of each compound corresponds to the conformation in solution, calculated couplings[20] for each of the minima were compared to the observed ones (Table VI). For all compounds, the measured couplings are very close to that calculated for the global minimum (conformations **2a–5a**), but the presence of a minor conformation could not be excluded. Also, the observed deoxy protons H-8a and H-8b in compound **3** do not necessarily correspond to the protons named H-8a and H-8b in the computer model. Therefore, conformation **3b** would fit too. Only the content of a possible minor conformation could be restricted to <20%, if the precission of the calculation[20] is assumed to be ±1 Hz. Attempts to correlate the calculated proton–proton angles with angles obtained from DAERM-calculations[21] also failed (deviations up to 15°, data not shown) but, again, it was impossible to ascertain whether the deviations stems from an unsuited model or from a mixture of conformations. The only way to establish the presence of an extended-chain conformation in compounds **2–5** was to compare the ^{1}H- and ^{13}C-chemical shifts.

The environment of H-6 in the extended side-chain conformations **2a, 4a,** and **5a** does not differ from that of H-6 in **1**, and, as observed, the chemical shifts

are very similar (Table I). The same argument holds for H-5 of **3a** and **4a** when compared to H-5 of **1**. Comparison of the ^{13}C-chemical shift differences of the neuraminic acids and the corresponding alditols showed that all of them are very similar (Table IV). This was expected, as the chemical environment of the carbon atoms of the alditols is similar to that of the corresponding side-chain carbon atoms of **1–5**, that is in the extended form.

All these results strongly suggest that all side chains of the deoxy analog **2–5** are, in solution, mainly in the extended chain-conformation.

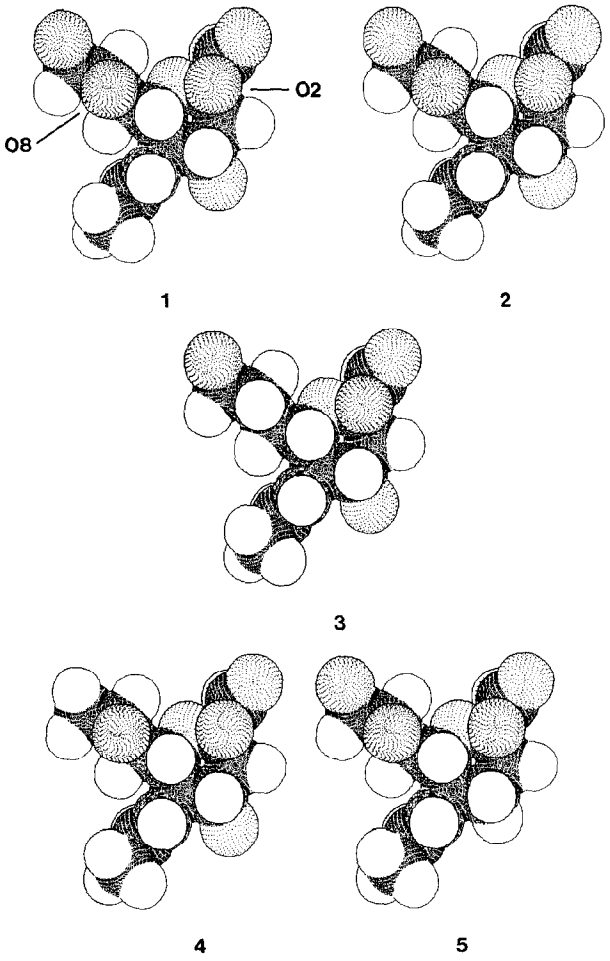


Fig. 1. Computer-drawn projections of CPK-models for compounds **1–5** in their prefered conformation, looking in the direction O-2→C-2, thus showing the polar binding site (β-side) consisting of O-2, O-8, and the amido group for **1, 2, 4,** and **5**. On replacement of OH-8 by H (in compound **3**), the ability to bind to CMPsialate synthase is nearly lost, and on replacement of OH-2 no binding occurs[12]. Hydrogen atoms are drawn blank, oxygen atoms are dotted, carbon atoms are marked with dashed lines, and nitrogen atoms with dashed, dotted lines.

The enzyme kinetics were determined as previously described[10]. The conversion of the substrates was monitored by both the thiobarbituric acid[22] and the orcinol test[23]. Surprisingly, all deoxy derivatives gave a chromophore in the thiobarbituric acid test. In the case of the 7-deoxy derivatives, one possible explanation for this behavior in the thiobarbituric acid test is that the open-chain forms are cleaved between C-5 and C-6 with sodium periodate to give either β-formylpyruvic acid from **2**, or a β-formylpyruvic acid homolog from **5**. 5-Deoxyneuraminic acid yielded no color under these conditions[24].

The K_m values determined under CTP-saturating conditions (10mM) were 0.7mM for **1, 2,** and **5**; 0.4mM for **4**; and for **3** no K_m could be calculated. Since the K_m values determined are largely affected by the experimental conditions, and our goal was a comparison with results previously obtained[10], the rate of conversion is reported relative to that of **1**, *i.e.*, **1**, 100; **2**, 97 ±16; **3**, 4 ±4; **4**, 106 ±18; and **5**, 67 ±18%. To demonstrate the large dependence of determined K_m values from the experimental conditions, the K_m of **1** was measured at various CTP concentrations. It was 0.7mM at CTP-saturation conditions, which is identical to the K_m reported[4] for the enzyme from a different source, and 2.1mM at CTP-limiting conditions. Additional reported K_m values for **1** are 1.8 (ref. 7) and 1.4mM (ref. 9). Although the source of the enzymes used[4,7,9] was different from ours, there seems to be a correlation between the CTP concentrations used and the K_m values reported.

As suggested earlier[11], the relative rates of conversion were easily correlated with the β-profiles of **1–5** (Fig. 1). Both the profile of **2** and that of **5** are very similar to that of **1**, thus explaining the ease of activation by the enzyme, whereas the profile of **3**, where OH-8 is missing, explains the low activation. The large influence of the absent hydroxyl group may be explained by the availability of only three polar groups (OH-8, NHAc-5, and OH-2) for recognition and conversion of the substrate into the CMP-derivative. By observing the β-surface of Neu5Ac (**1**) (Fig. 1), it becomes evident that the derivatives at C-4 (refs. 3–5) or the 7,8-(bisepi)Neu5Ac[11], where OH-7 occupies the position of OH-8, are also recognized by the enzyme. Of interest is that 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid, where the *N*-acyl group of neuraminic acid is replaced by a hydroxyl group, is recognized by the activating enzyme[25].

EXPERIMENTAL

General. — Compounds **2–5** were prepared as described[13]. L-Rhamnose and 2-deoxy-D-*arabino*-hexose were commercial samples. The sodium salts of **2–5** were prepared by neutralization of the corresponding acids with NaOH solution in slight excess. The compounds were freed from impurities and the excess of base by passage through a Bio-Gel P-2 (mesh size −400) column. 3-Deoxy-D-*arabino*-hexose was prepared by a variation of the method of Rembarz[26], and 3-deoxy-D-(3-^{2}H)*arabino*-hexose was prepared with $LiAl(^2H)_4$ instead of $LiAlH_4$. 2-Deoxy-D-*arabino*-hexitol, 3-deoxy-D-*arabino*-hexitol, 3-deoxy-D-(3-^{2}H)*arabino*-hexitol, 6-

deoxy-L-mannitol, and the (1-^{2}H) derivatives of 3-deoxy-D-*arabino*-hexitol and 6-deoxy-L-mannitol were prepared by sodium borohydride or deuteride reduction of the hexoses for 2 h at 4°. The alditols were freed from salts by gel-permeation chromatography on Bio-Gel P-2 (−400 mesh).

N.m.r. spectroscopy. — ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Bruker WM-250 instrument at 298 K, with a deuterium lock on the water signal, and an external reference of sodium 4,4-dimethyl-4-silapentanoate in D_2O (δ 0) for the proton spectra, and 1,4-dioxane in D_2O (δ 67.40) for the carbon spectra. The proton–carbon shift-correlated spectrum was recorded by use of the spectrometer software, the INDOR-spectrum using the method of Kessler *et al.*[15], and the CHORTLE-spectrum that of Pearson[17].

Computations. — All calculations were done as described earlier[11]. The starting coordinates for **2–5** were prepared by means of bond modification of the reported[27] X-ray structure of **1**.

Enzyme kinetics. — The enzyme kinetics were performed as already described[10] (0.05–1mM substrate; 10mM CTP; and ~10 mU.mL^{-1} of rat CMPNeu5Ac synthase, EC 2.7.7.43). Relative rates were measured at 0.5mM substrate concentration and under CTP saturation conditions (10mM) with ~70 mU.mL^{-1} of enzyme. The product formation was monitored with both the thiobarbituric acid[22] and the orcinol test[23].

*Methyl 3-deoxy-α-*D-arabino-*hexopyranoside.* — This compound was prepared by hydrogenation of methyl 4,6-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside[26] (600 mg) in methanol (60 mL) with PdO (20 mg) as catalyst. On evaporation of the solvent, the product was obtained in crystalline form (400 mg), and recrystallized from ethanol, m.p. 123–124°; lit.[28] m.p. 123–124°; ^{1}H-n.m.r. (D_2O; 298 K): δ 4.60 (dd, 1 H, $J_{1,2}$ 1.5, $J_{1,3e}$ 1 Hz, H-1), 3.92 (ddd, 1 H, $J_{2,3e}$ 3.5, $J_{2,3a}$ 3 Hz, H-2), 3.88 (dd, 1 H, $J_{6a,6b}$ −12 Hz, H-6a), 3.81 (ddd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.71 (dd, 1 H, H-6b), 3.60 (ddd, 1 H, $J_{5,6a}$ 2.5, $J_{5,6b}$ 6.5 Hz, H-5), 3.42 (s, 3 H, CH_3O), 2.08 (dddd, 1 H, $J_{3e,3a}$ −13.5, $J_{3e,4}$ 5 Hz, H-3*e*), and 1.97 (ddd, $J_{3a,4}$ 11 Hz, H-3*a*); ^{13}C-n.m.r. (D_2O, 298 K): 100.50 (C-1), 74.50 (C-5), 68.00 (C-2), 62.38 (C-4), 62.06 (C-6), 55.49 (CH_3O), and 34.49 (C-3); recording the spectrum of a 1:1 mixture of the title compound with the corresponding (3-^{2}H) derivative showed the splitting of the signals for C-2 and C-4.

*3-Deoxy-*D-arabino-*hexose.* — Methyl 3-deoxy-α-D-*arabino*-hexopyranoside (200 mg) was treated with acetic anhydride (2 mL) containing H_2SO_4 (6%) for 2 h at 4°. The solution was poured into a cold, saturated solution of Na_2CO_3 (50 mL) and extracted with chloroform after CO_2 formation ceased. The organic solution was dried ($MgSO_4$) and evaporation of the solvent gave 1,2,4,6-tetra-*O*-acetyl-3-deoxy-D-*arabino*-hexopyranose (mainly the α-D anomer; 95% by ^{1}H-n.m.r.) as a colorless syrup (330 mg, 88%); ^{1}H-n.m.r. ($CDCl_3$, 298 K): δ 6.01 (dd, 1 H, $J_{1,2}$ 1, $J_{1,3e}$ 1 Hz, H-1), 5.07 (ddd, 1 H, $J_{4,5}$ 10 Hz, H-4), 4.94 (dddd, 1 H, $J_{2,3e}$ 3.5, $J_{2,3a}$ 3, $J_{2,5}$ 0.5 Hz, H-2), 4.26 (dd, 1 H, $J_{6a,6b}$ −12.5 Hz, H-6a), 4.14 (dd, 1 H, H-6b), 4.01 (dddd, 1 H, $J_{5,6a}$ 2.5, $J_{5,6b}$ 5 Hz, H-5), 2.32 (dddd, 1 H, $J_{3e,3a}$ −13.5, $J_{3e,4}$ 5 Hz, H-3*e*), and 2.00

(ddd, $J_{3a,4}$ 11 Hz, H-3*a*); ^{13}C-n.m.r. ($CDCl_3$, 298 K): 89.81 (C-1), 70.60 (C-5), 68.14 (C-2), 63.94 (C-4), 62.68 (C-6), and 29.18 (C-3); recording the spectrum of a 1:1 mixture of the title compound with the corresponding (3-^{2}H) derivative showed the splitting of the signals for C-2 and C-4.

Alkali hydrolysis of 1,2,4,6-tetra-*O*-acetyl-3-deoxy-D-*arabino*-hexopyranose with methanolic sodium methoxide gave, in quantitative yield, 3-deoxy-D-*arabino*-hexose; the ^{1}H- and the ^{13}C-n.m.r. data were identical to that published[29] in part; the ratio of α-pyranose:β-pyranose:α-furanose:β-furanose was 12:5:6:2; lit.[29] 10:5:3:0; ^{1}H-n.m.r. (D_2O, 298 K): δ 5.27 (ddd, $J_{1,2}$ 1, $J_{1,3a}$ 0.5, $J_{1,3b}$ 0.5 Hz, H-1αf), 5.20 (d, $J_{1,2}$ 4.5 Hz, H-1βf), 5.01 (dd, $J_{1,2}$ 2, $J_{2,3e}$ 1 Hz, H-1αp), 4.88 (d, $J_{1,2}$ 1.5 Hz, H-1βp), 4.26 (ddd, $J_{2,3a}$ 7.5, $J_{2,3b}$ 4.5 Hz, H-2βf), 4.21 (ddd, $J_{2,3a}$ 6, $J_{2,3b}$ 2.7 Hz, H-2αf), 3.91 (ddd, $J_{2,3e}$ 3.7, $J_{2,3a}$ 3 Hz, H-2αp), 3.91 (ddd, $J_{2,3e}$ 3.5, $J_{2,3a}$ 3 Hz, H-2βp), 2.39 (dddd, $J_{3a,3b}$ −14, $J_{3a,4}$ 8.3 Hz, H-3$a\alpha f$), 2.31 (m, H-3$a\beta f$), 2.24 (ddd, $J_{3e,3a}$ −13.7, $J_{3e,4}$ 5 Hz, H-3$e\beta p$), 2.07 (dddd, $J_{3e,3a}$ −13.7, $J_{3e,4}$ 4.3 Hz, H-3$e\alpha p$), 1.90 (m, H-3$b\beta f$), 1.87 (ddd, $J_{3a,4}$ 11 Hz, H-3$a\alpha p$), 1.87 (dddd, $J_{3b,4}$ 5.5 Hz, H-3$b\alpha f$), and 1.74 (ddd, $J_{3a,4}$ 11.5, H-3$a\beta p$); ^{13}C-n.m.r. (D_2O, 298 K): 102.84 (C-1αf), 96.01 (C-1βf), 95.44 (C-1βp), 93.49 (C-1αp), 80.69 (C-5βp), 78.99 (C-4αf), 77.51 (C-4βf), 76.03 (C-2αf), 74.59 (C-5βf), 74.34 (C-5αp), 73.60 (C-5αf), 71.78 (C-2βf), 68.68 (C-2αp), 68.47 (C-2βp), 63.56 (C-6αf), 63.42 (C-6βf), 62.45 (C-4αp), 62.33 (C-4βp), 62.12 (C-6βp), 61.91 (C-6αp), 37.60 (C-3βp), 33.81 (C-3αp), 33.00 (C-3αf), and 31.89 (C-3βp); recording of the spectrum of a 1:1 mixture of the title compound with the corresponding (3-^{2}H) derivative showed the splitting of all C-2 and C-4 signals.

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