Elucidation of the topological parameters of *N*-acetylneuraminic acid and some analogues involved in their interaction with the *N*-acetylneuraminate lyase from *Clostridium perfringens*

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A series of neuraminic acid derivatives modified in the side chain or at C-3, C-4 or C-5 were tested as substrates of inhibitors of *N*-acetylneuraminate lyase (EC 4.1.3.3) from *Clostridium perfringens*. The results, together with K_m and K_i values reported previously, indicate that the region most important for the binding of sialic acids is an equatorial zone reaching from C-8 via the ring oxygen atom to C-4 of the sugar molecule, whereas the substituents at C-9 and C-5 may be varied to a higher extent without significantly disturbing enzyme action. It is shown that stereo-electronic factors are responsible for the immediate heterolytic fragmentation of the cyclic sialic acid into pyruvic acid and 2-acetamidomannose or a related C-6 sugar.

INTRODUCTION

Among the enzymes involved in the metabolism of sialic acids, *N*-acetylneuraminate lyase (*N*-acetylneuraminate pyruvate-lyase, EC 4.1.3.3) degrades these sugars into pyruvic acid and *NO*-acyl derivatives of mannosamine [1,2]. Previous studies with neuraminic acid derivatives indicated that modifications of the side chain usually lower the rate of cleavage [3–7]. In contrast, exchange of the acetamido group at C-5 by other acylamido functions influences the interaction with the lyase only slightly [5,6,8]. C-4 proved to be the most crucial area of the sialic acid molecule. Modification of the equatorial hydroxy group by acetylation or methylation renders the molecule resistant towards the lyase [5,6,9], and the 4-epi, the 4-deoxy, and the 4-oxo derivative are also not substrates. However, only the three latter ones are good inhibitors of the enzyme [7,10,11].

By using the reversibility of the lyase-catalysed reaction [12], various sialic acid derivatives have been synthesized with immobilized lyase [13–19]. These experiments have helped us to extend our knowledge of the structure–activity relationship for sialic acids and *N*-acetylneuraminate lyase, but no general hypothesis has yet been developed.

A series of publications discussed the structure–activity relationships that govern the interactions of sialic acid analogues with CMP-sialate synthase [20–22] and the inhibition of *Vibrio cholerae* sialidase by various 2,3-didehydrosialic acid derivatives [23,24]. In this connection, CPK models of sialic acid were proposed, the β- [21,22] and α-epitopes [23,24] of which enable compatible explanation for the mode of binding by enzyme. The success of this approach prompted us to correlate the equatorial zones of the aforementioned CPK models of a series of novel sialic acid analogues [25–28], with the kinetic data of the interaction with *N*-acetylneuraminate lyase and with data already published, in order to obtain some more general information on the interaction of sialic acids with lyase.

MATERIALS AND METHODS

The following substances (see Figs. 1 and 2), the syntheses of which have been described previously, were tested as substrates or inhibitors: *N*-acetyl-8-deoxyneuraminic acid (8-d-Neu5Ac, **3**) [29], *N*-acetyl-7-deoxyneuraminic acid (α-7-d-Neu5Ac, **4**) [29], 3-deoxy-D-glycero-D-galacto-2-nonulopyranosonic acid (Kdn, **8**) [28], 5-deoxy-Kdn (5-dKdn, **9**) [28], 5-deoxy-5-azido-Kdn (5-d-Kdn5N_3, **10**) [28], 2,6-anhydro-2,5-dideoxy-5-acetamido-D-erythro-L-mannononononic acid (2-d-2H_{eq}-Neu5Ac, **12**) [30], *N*-acetyl-4-deoxy-4-eq-*C*-methylneuraminic acid (4-d-Neu5Ac4Me_{eq}, **13**) [27], *N*-acetyl-4-acetamido-4-deoxyneuraminic acid (4-d-Neu4NAc_{eq}5Ac, **14**) [26], *N*-acetyl-4-epi-4-eq-methylneuraminic acid (4-epi-Neu5Ac4Me_{eq}, **16**) [31], *N*-acetyl-4-ax-methylneuraminic acid (Neu5Ac4Me_{ax}, **17**) [31], *N*-acetyl-3-eq-hydroxyneuraminic acid (Neu5Ac3OH_{eq}, **18**) [25] and 2-acetamidomannitol (Man2NAc1ol, **19**) [32]. *N*-Acetylneuraminic acid (**1**) was a gift from MECT Corporation (Tokyo, Japan). *N*-Acetylneuraminate lyase (1 unit) from *Clostridium perfringens* purchased in freeze-dried form from (Sigma, Munich, Germany) was dissolved in 0.5 ml of 0.02 mM-potassium phosphate buffer, pH 7.2, containing 1 mM-EDTA and 0.5 mg of BSA/ml and stored at 4 °C. The assay used was based on that described by Gantt *et al.* [33]. Incubation mixtures contained, in a total volume of 550 μl, 50 mM-potassium phosphate buffer, pH 7.2, 0.2 mM-NADH (Boehringer Mannheim, Mannheim, Germany), 55 units of lactate dehydrogenase from rabbit muscle (Boehringer Mannheim), 5 μl of the above mentioned lyase solution and the substrate in concentrations between 0.5 and 10 mM. The reaction mixtures without substrates (or with inhibitors in the case of inhibition studies), were allowed to equilibrate at 37 °C for 10 min while the A_{340} was recorded in a (Hitachi model 220) spectrophotometer. The reactions were started by the addition of the sialic acid, and the decrease in absorbance was recorded for periods between 5 and 10 min. Initial rates were calculated from the linear parts of the reaction curves, thereby correcting for the slow lyase-independent decrease in NADH decrease by subtracting the slope of the reaction curve before addition of the sialic acid. Experiments were performed in duplicate with four or five concentrations of substrate. The kinetic parameters K_m and $V_{max.}$ were calculated by fitting the data to the Michaelis–Menten equation with the non-linear-regression program Enzfitter (Biosoft, Cambridge, U.K.). Inhibition constants (K_i) were calculated

Abbreviations used: the sialic acid analogues are defined in the text; epi, epimeric; ax, axial; eq., equatorial.

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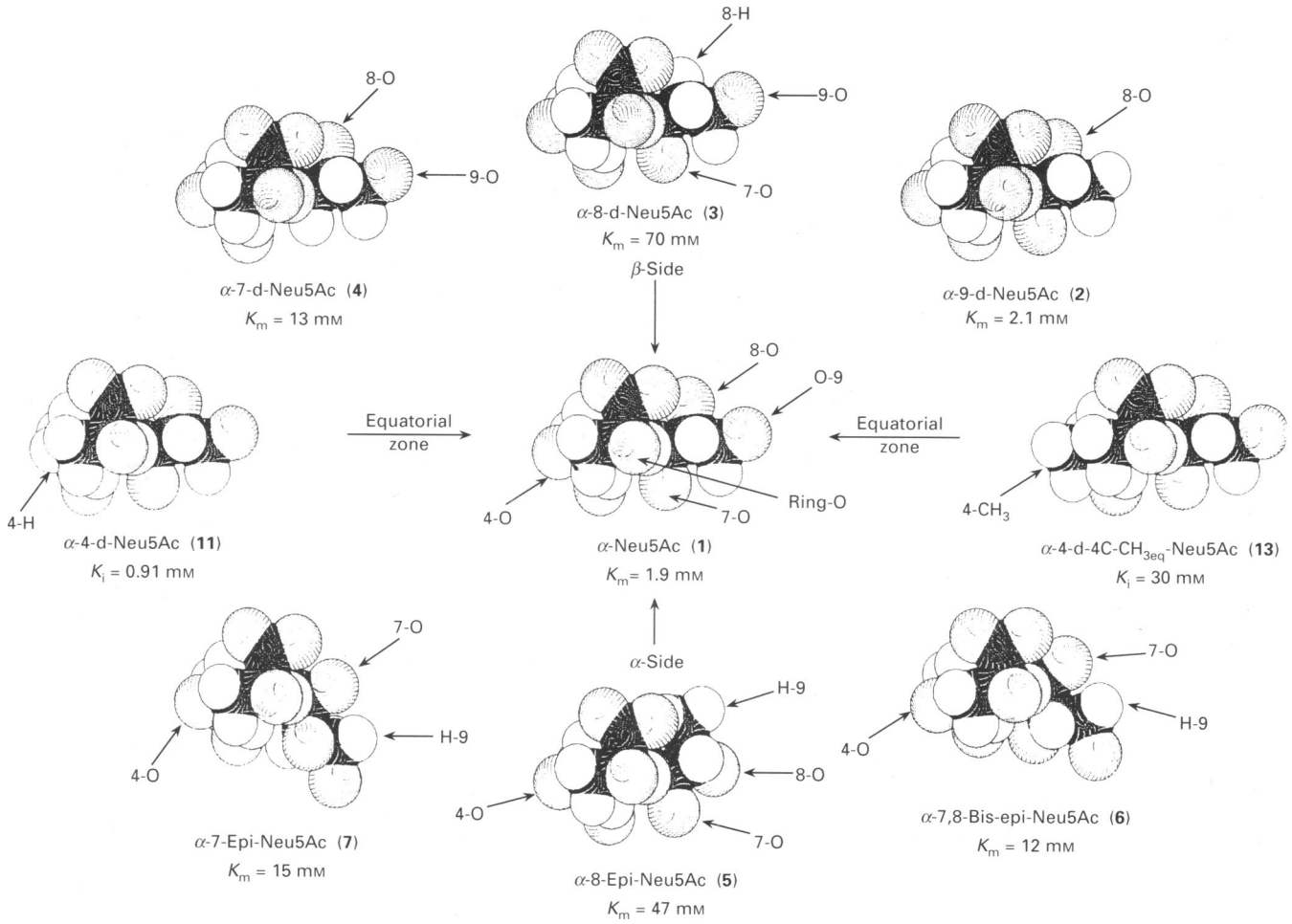


Fig. 1. CPK drawings of selected neuraminic acids

The Figure shows the computer-drawn projections of the proposed binding sides from selected neuraminic acids in their preferred conformations. Hydrogen atoms are drawn with no shading, oxygen atoms with light shading and carbon atoms with heavy shading.

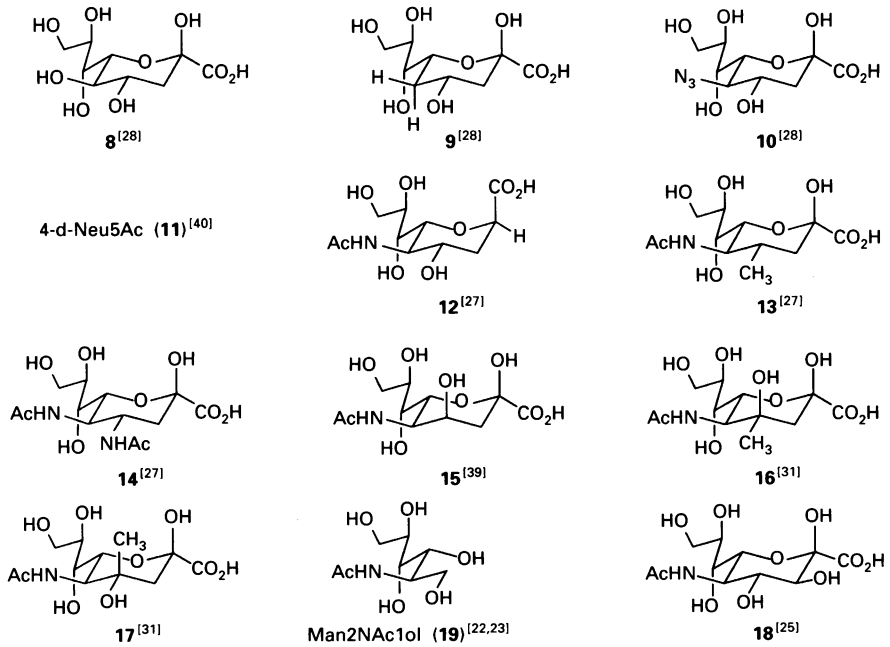


Fig. 2. Structures of the substrates measured in the present work

Relevant references are given as superscripts.

Table 1. Kinetic data for the cleavage of Neu5Ac and derivatives by *N*-acylneuraminate lyase

Substance	K_m (mM)	$V_{max.}$ (%)	K_i (mM)
Neu5Ac (**1**)	1.9	100	n.d.*
9-d-Neu5Ac (**2**)	2.1†	46†	n.d.
8-d-Neu5Ac (**3**)	70	100	n.d.
7-d-Neu5Ac (**4**)	13	25	n.d.
8-Epi-Neu5Ac (**5**)	47†	25†	n.d.
7,8-Bis-epi-Neu5Ac (**6**)	12‡	29‡	n.d.
7-Epi-Neu5Ac (**7**)	15‡	35‡	n.d.
Kdn (**8**)§	8.8	57	n.d.
5-d-Kdn (**9**)	1.8	83	n.d.
5-d-Kdn5N_3 (**10**)	3.8	38	n.d.
4-d-Neu5Ac (**11**)	–	–	0.90†
2-d-2H_{eq}-Neu5Ac (**12**)	–	–	n.i.‖
4-d-Neu5Ac4Me_{eq} (**13**)	–	–	30
4-d-Neu4NAc_{eq}5Ac (**14**)	–	–	n.i.‖
4-Epi-Neu5Ac (**15**)	–	–	0.61†
4-Epi-Neu5Ac4Me_{eq} (**16**)	–	–	150
Neu5Ac4Me_{ax} (**17**)	–	–	21
Neu5Ac3OH_{eq} (**18**)	–	–	1.2¶
Man2NAc1ol (**19**)	–	–	40‖
Neu5Ac2ol (**20,21**)	–	–	4.1**

* n.d., not determinable.
† Recalculation with ENZFITTER of the data in [7].
‡ Data from [7].
§ Kdn (or KDN in [37]), 3-deoxy-D-glycero-D-galactononulosonic acid.
‖ Measurement by the method described in [7]; n.i., no inhibition detected.
¶ K_i value for the second part of a biphasic kinetic; for details, see the text.
** Data from [42].

with the parameters obtained at two concentrations of inhibitor by using the formula:

$$K_i = K_m \times i/(K'_m - K_m)$$

where K'_m is the effective K_m in the presence of inhibitor at concentration *i*. The inhibitory effect of compounds **14** and **18** was measured as described in [7].

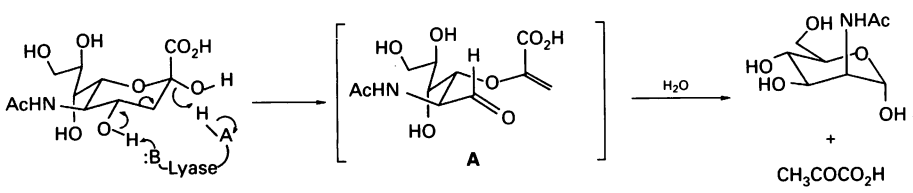
RESULTS AND DISCUSSION

The kinetic data presented in Table 1 indicate that modifications at some areas of sialic acid, for example at C-5 and C-9, are tolerated by the lyase, whereas other parts of the molecule seem to be more important for the enzyme–substrate interaction. In the following we try to correlate the K_m and K_i values of the investigated substances with their structural and topological features, which are in part revealed by the CPK models of Fig. 1. Neu5Ac (**1**) with a K_m value of 1.9 mM is chosen as reference compound. The exchange of the hydroxy group at C-9 by hydrogen (compound **2**) is accompanied by only a small decrease in affinity (K_m = 2.1 mM). This explains why the synthesis of various 9-*O*-acylsialic acids can easily be realized by the reverse enzymic reaction [13]. The importance of this region in the enzymic reaction is further shown by the ability to synthesize 9-fluoro-9-deoxy-*N*-acetylneuraminic acid [34] and 9-azido-9-deoxy-*N*-acetylneuraminic acid from pyruvic acid and 6-azido-*N*-acetylmannosamine and from 6-fluoro-*N*-acetylmannosamine respectively [50] by the reverse enzymic reaction. The obvious function of C-9 is to guarantee the conformational stability for the neighbouring bonds C-8–C-7 and C-7–C-6. The C-9–C-8 bond itself is characterized by two predominant orientations of the C-9 hydroxy group about 65° to both sides of the C-8–8-OH-bond [21]. Although the nature of the substituent at C-9 does not strongly influence the lyase reaction, the presence of this part is essential for full enzyme activity. This is shown by the removal of C-9, which shows a decrease in affinity to a K_m of 11 mM [3]; however, the reverse enzymic synthesis of the C-8 analogue of Neu5Ac is still possible [35]. Shortening of the side chain by two carbon atoms abolishes completely the recognition [4].

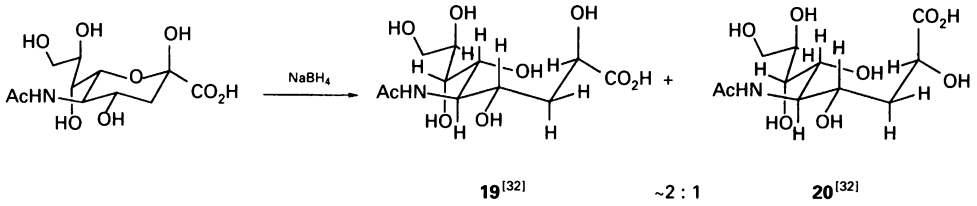
In contrast with Neu5Ac with a stretched side chain, the epimers **5–7** (Fig. 1), i.e. 7-epi-, 8-epi, and 7,8-bis-epi-*N*-acetylneuraminic acid, exhibit stable bent conformations [21]. The changes in structure are accompanied by remarkable decreases of the affinities to acylneuraminate lyase (K_m for **5**, 47 mM; for **6**, 12 mM; and for **7**, 15 mM). These results can be correlated best with a disturbed interaction of the enzyme with the hydroxy groups at C-7 and C-8 below and above the equatorial region. In the case of 7-epi- and 7,8-bis-epi-Neu5Ac, the side chain, in comparison with Neu5Ac, is bent towards the α-sector (Fig. 1), whereas for 8-epi-Neu5Ac a deviation reaching into the β-sector is observed. Thereby the interactions of the lyase with the hydroxy groups at C-7 and C-8 are impaired. For 7-epi-Neu5Ac and 7.8-bis-epi-Neu5Ac the C-7 hydroxy groups occupy areas similar to that occupied by the C-8 hydroxy group in Neu5Ac, whereas the C-8 hydroxy groups of both derivatives may function as substitutes of the C-7 hydroxy group of Neu5Ac. In 8-epi-Neu5Ac the region above C-8 is occupied by the hydroxymethyl group of C-9, cancelling a comparable interaction with the enzyme. The affinity is thus even smaller than for **6** and **7**.

With the 7-d- and 8-d-Neu5Ac (**4** and **3**, Fig. 1), which have a stretched [22] side chain, a significant decrease in affinity (K_m of **4**, 13 mM; of **3**, 70 mM) was observed in both cases [7]. These results may be explained by the role the hydroxy groups of the sialic acids are assumed to play in the interactions with the enzyme mentioned above. In 7-d-Neu5Ac only the interaction with the C-8 hydroxy group above the equatorial plane is possible, whereas in 8-d-neu5Ac only the C-7 hydroxy group below the equatorial plane is available for interaction. That the K_m for 8-d-Neu5Ac is 5.4-fold higher than that for 7-d-Neu5Ac indicates that the hydroxy group above the plane is the more important for recognition from the equatorial region. The necessity for recognition of the ring oxygen atom at C-6 is highlighted by the fact that the thio analogue is neither a substrate nor an inhibitor of the lyase [36].

Some interesting observations were made by structural variations at C-5. Faillard *et al.* [8] reported a scarcely diminished



Scheme 1. Proposed heterolytic fragmentation scheme according to Grob [41]



Scheme 2. Epimeric pair of reduced *N*-acetylneuraminic acid

Projections of the β-like (**19**) and α-like (**20**) reduction products of *N*-acetylneuraminic acid. The most stable conformation of **20** in aqueous solution has a complete zig-zag form reaching from C-8 to C-2, whereas the zig-zag form in **19** is situated between C-8 and C-4. Relevant references are shown as superscripts.

rate of cleavage when the *N*-acetyl group was exchanged by an *N*-carbobenzoxy group. Similar observations were made by Schauer *et al.* [5] when they substituted the *N*-acetyl group by *N*-formyl (K_m 1.75 mM), *N*-glycoloyl (K_m 2.20 mM), *N*-monochloracetyl (K_m 2.70 mM), *N*-monofluoracetyl (K_m 10.0 mM) and *N*-succinyl groups (K_m 1.93 mM). It is noticeable that the exchange of the acetamido group by hydrogen in **9**, as reported recently by Schreiner & Zbiral [28], does not significantly influence the affinity (K_m 1.8 mM). The K_m value for the 5-azidosialic acid analogue **10** (K_m 3.8 mM) is only about twice that of Neu5Ac. Furthermore, the 5-hydroxy analogue of sialic acid, Kdn (**8**), first described by Nadano *et al.* [37] as a terminal unit of polysialoglycoproteins of the membranes of unfertilized eggs of the rainbow trout (*Salmo gairdnerii*), and later on prepared enzymically by Augé *et al.* [13], exhibits a K_m of 8.8 mM. Augé's group reported the synthesis of even a 5-phenyl$_{eq}$ analogue of sialic acid [38] from 2-phenylmannose and pyruvic acid, showing that this hydrophobic substituent does not prevent the reaction of the lyase. As in these cases the C-5 substituents are in equatorial position, residues in this position of the sialic acid molecule seem to interact with the active pocket of the enzyme only weakly. The interaction is influenced rather less by sterical factors than by electronic effects, as is indicated by the *N*-monofluoracetylneuraminic acid and Kdn. If, however, the substituent at C-5 occupies an axial position, the recognition is severely hampered by the size of the group because, in contrast with 5-epi-Kdn [13,17], an enzymic formation of 5-epi-acetamidoneuraminic acid is not possible [17].

The structural variations at C-4 provide further information about the structure–activity relationship. Not all these sialic acids are cleaved by lyase. The 4-*O*-methyl-*N*-acetylneuraminic acid [9] and the *N*-acetyl-4-eq-acetamido-4-deoxyneuraminic acid (**14**, Fig. 2) prepared recently [26] do not behave as competitive inhibitors, probably because of sterical hindrance by the equatorial substituents at C-4. The exchange of the natural 4-hydroxy group by the more hydrophobic hydrogen atom transforms Neu5Ac into the inhibitor **11** with a K_i of 0.90 mM as measured by us or 1.23 mM as determined by Gross & Brossmer [10]. The transformation of Neu5Ac into 4-d-Neu5Ac4Me (**13**) is accompanied by a strong decrease in inhibitory potency (K_i = 30 mM) when compared with **1**. Insertion of an axial methyl group into Neu5Ac, resulting in Neu5AcMe$_{ax}$ (**17**, [31]), enables a slightly better interaction with the enzyme (K_i 21 mM). It is noticeable that the sialic acid analogue **17** is recognized by the enzyme, but not cleaved, although it fits the stereo-electronic requirements for a heterolytic fragmentation. The inhibition by its epimer **16** [31] with an equatorial methyl group as in **13**, however, was almost negligible (K_i 150 mM). The 4-epi-*N*-acetylneuraminic acid (**15**) [39], containing an axially orientated hydroxy group as in **16**, strongly interacts with the enzyme (K_i 0.6 mM measured by us; 2.3 mM by Gross & Brossmer [10]). The fact that it is not cleaved into pyruvic acid and 2-acetamidomannose can be explained by stereo-electronic factors governing the heterolytic fragmentation as formulated by Grob & Schiess [40] and Grob [41]. It probably goes via the putative pyruvate enol ether intermediate, **A**, to 2-acetamidomannose [42,43] (Scheme 1). The amino acid of the lyase involved in abstracting the proton from the 4-hydroxy group is probably histidine, as discussed previously [44]. Summarizing the effects of substitutions at C-4 on the recognition of the sialic acid by the lyase, it becomes obvious that a methyl group in the equatorial position impairs the binding interaction more than it does in the axial position. Changing the configuration at C-4 does not result in significant effects.

A remarkable strong enzyme–substrate interaction was found by Gross & Brossmer for 4-oxo-*N*-acetylneuraminic acid with a K_i of 0.025 mM [10]. Probably this value reflects a reversible addition of a histidine residue to the carbonyl group of 4-oxo-Neu5Ac. Another interesting aspect should be discussed. Deijl & Vliegenthart [42], as well as Baumann *et al.* [43], observed that the *N*-acetylmannosamine is formed immediately in its α-form by lyase action on the α-anomer of Neu5Ac. Considering Scheme 1, it becomes clear that the aldehyde group and the acetamido group of the putative intermediate **A** are situated in a *trans* position to each other, especially immediately after the fragmentation. The cyclization of this open chain of *N*-acetylmannosamine between the 5-hydroxy group (corresponding to the 8-OH of Neu5Ac) and the aldehyde group can be realized preferentially as a process giving the α-anomer. With regard to the C-4–C-9 part of *N*-acetylneuraminic acid one can determine that its distance is exactly the same as in the corresponding 2-acetamido-mannitol **19** (Fig. 2) in its predominantly zig-zag conformation [22] reaching from C-6 to C-1. Therefore it was not surprising to find not a strong, but rather a significant, binding interaction with acylneuraminate lyase (K_i 40 mM), in contrast with that of acetamidomannose (K_m 700 mM) [17].

The 3-eq-hydroxy-*N*-acetylneuraminic acid (**18**) [25] gives a biphasic reaction curve when it is used as an inhibitor of Neu5Ac cleavage. After a first phase with a higher reaction rate lasting 2–3 min, a slower and constant rate is reached, resulting in a competitive inhibition (K_i 1.2 mM) that is of the same order of magnitude as the K_i values of 4-epi- and 4-d-Neu5Ac (Table 1). This means that the transformation of the hydrophobic C-3 region into a hydrophilic one does not counteract the binding to the enzyme. On the other hand, a cleavage to 3-hydroxypyruvate and 2-acetamidomannose is not observed when the assay mixture is analysed by h.p.l.c. as described by Shukla & Schauer [6]. In agreement with this, the enzymic formation of **18** from the components could not be realized [17]. We suggest that the best explanation for this behaviour is the disturbance of the heterolytic fragmentation, described by Grob [41] (Scheme 1) if an electron-donating group, such as OH, is present at this position, which corresponds to the number 3 of the common sequence of five

atoms formulated in [40,41]. It is well known that a hydroxy group destabilizes a carbanion and does not favour its formation, whereas an electron-accepting group at position 3 would significantly enhance the rate of heterolytic fragmentation [45]. The irreversible inhibition of the lyase by Neu5Ac3OH$_{ax}$ observed by Basabe *et al.* [46] does not interfere with our proposal about the reversible enzyme substrate interactions.

With regard to C-2, the necessity of the equatorial α-oxygen for binding and cleavage of sialic acids should be recalled. Deijl & Vliegenthart observed that the α-methyl glycoside of Neu5Ac, in contrast with the β-methyl glycoside, is an inhibitor of the lyase (K_i 8.1 mM) [42]. Furthermore, 2-deoxy-2-H$_{eq}$-*N*-acetylneuraminic acid (**12**) [30] did not exhibit any inhibition, thereby underlining the importance of the 2-α-hydroxy group. The relative abundance of the α-anomeric form of all derivatives tested is in the range of 8–12% (results not shown). The binding data presented are not corrected for these minor differences in the availability of the substrates.

To investigate whether an open-chain form of sialic acid can interact with the lyase, Deijl & Vliegenthart [42] simulated this structure by reduction of Neu5Ac with $NaBH_4$ to the corresponding alditol. They found that the reduction product is a competitive inhibitor (K_i 4.09 mM). Therefore they concluded that the alditol has a conformation similar to that of α-Neu5Ac. We repeated the $NaBH_4$-reduction experiment and found that the two epimeric alditol species, **20** and **21**, (Scheme 2) are formed in a ratio of 1:2. Only compound **20** exhibits a configuration at C-2 comparable with that of α-Neu5Ac and therefore should be the better inhibitor. As is reported in a separate contribution [32], n.m.r. studies have shown that compound **20** exists predominantly in a completely stretched (zig-zag) conformation in aqueous solution, whereas compound **21** is only stretched from C-8 to C-5. The calculated energy content [47] of the zig-zag form of **20** is only 2.93 kJ (0.7 kcal)/mol lower than that of its folded α-sialic acid-like conformation.

The importance of the free carboxy function at C-1 is highlighted by the fact that its methyl ester is neither a substrate nor an inhibitor of the lyase [5].

Comparing the interactions of lyase and CMP-sialate synthase (acylneuraminate cytidylyltransferase, EC 2.7.7.43) with sialic acids as well as of *Vibrio cholerae* sialidase (EC 3.2.1.18) with 2,3-didehydrosialic acids, some remarkable differences, but also some similarities, are observable. Whereas for the activities of the synthase and of the lyase the C-8 hydroxy group situated at the β-side is more important than the hydroxy group at C-7, the reverse is observed with the 2,3-didehydrosialic acids and sialidase. The large decrease in binding caused by the more extended substituents at C-4 is seen both with the lyase described here and with the sialidase [48]. The strongly reduced binding to sialidases of sialic acids modified at C-5 with substituents other than small acylamido groups [49] is contrasted by the minor effects these changes have on the interaction with the lyase. Summarizing these results it may be concluded that the active sites of these enzymes are not much related, although they bind the same substrate.

The data presented here may be used for a prognosis about the possibility of lyase-catalysed syntheses of C-9 and C-8 sugars, which are analogues of sialic acid and Kdo, by using the appropriate C-6 and C-5 sugars and pyruvate as starting materials.

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REFERENCES

1. Corfield, A. P. & Schauer, R. (1982) in Sialic Acids (Cell Biol. Monogr. **10**, Schauer, R., ed.), pp. 195–261, Springer, Vienna
2. Schauer, R. (1982) Adv. Carbohydr. Chem. Biochem. **40**, 131–234
3. Suttajit, M., Urban, C. & McLean, R. L. (1971) J. Biol. Chem. **246**, 810–814
4. Suttajit, M. & Winzler, R. (1971) J. Biol. Chem. **246**, 3398–3403
5. Schauer, R., Wember, M., Wirtz-Peitz, F. & Ferreira do Amaral, C. (1971) Hoppe-Seyler's Z. Physiol. Chem. **352**, 1073–1080
6. Shukla, A. K. & Schauer, R. (1986) Anal. Biochem. **158**, 158–164
7. Schauer, R., Stoll, S., Zbiral, E., Schreiner, E., Brandstetter, H. H., Vasella, A. & Baumberger, F. (1987) Glycoconjugate J. **4**, 361–369
8. Faillard, H., Ferreira do Amaral, C. & Blohm, M. (1969) Hoppe-Seyler's Z. Physiol. Chem. **350**, 798–802
9. Beau, J. M. & Schauer, R. (1980) Eur. J. Biochem. **106**, 531–540
10. Gross, H. J. & Brossmer, R. (1988) FEBS Lett. **232**, 145–147
11. Hagedorn, H. W. & Brossmer, R. (1986) Helv. Chim. Acta **69**, 2127–2132
12. Comb, D. G. & Roseman, S. (1960) J. Biol. Chem. **235**, 2529–2537
13. Augé, Cl., David, S., Gautheron, Ch., Malleron, A. & Cavayé, B. (1988) New J. Chem. **12**, 733–744
14. Augé, Cl., David, S. & Malleron, A. (1989) Carbohydr. Res. **188**, 201–205
15. Augé, Cl., David, S. & Gautheron, Ch. (1984) Tetrahedron Lett. **25**, 4663
16. Augé, Cl., David, S., Gautheron, Ch. & Veyrieres, A. (1985) Tetrahedron Lett. **26**, 2439–2440
17. Kim, M. J., Hennen, W. J., Sweers, H. M. & Wong, C. H. (1988) J. Am. Chem. Soc. **10**, 6481–6482
18. Simon, E. S., Bednarski, M. D. & Whitesides, G. M. (1988) J. Am. Chem. Soc. **110**, 7159–7163
19. Bednarski, M. D., Chenault, H. R., Simon, E. S. & Whitesides, G. M. (1987) J. Chem. Soc. **109**, 1283–1285
20. Zbiral, E. & Brandstetter, H. H. (1985) Monatsh. Chem. **116**, 87–98
21. Christian, R., Schulz, G., Brandstetter, H. H. & Zbiral, E. (1987) Carbohydr. Res. **162**, 1–11
22. Christian, R., Schreiner, E., Zbiral, E. & Schulz, G. (1989) Carbohydr. Res. **194**, 49–61
23. Zbiral, E., Brandstetter, H. H., Christian, R. & Schauer, R. (1987) Liebigs Ann. Chem. 781–786
24. Zbiral, E., Schreiner, E., Christian, R., Kleineidam, R. G. & Schauer, R. (1989) Liebigs Ann. Chem. 159–165
25. Paulsen, H., Krogmann, C. & von Deesen, U. (1988) Liebigs Ann. Chem. 277–278
26. Zbiral, E., Schreiner, E. & Christian, R. (1989) Carbohydr. Res. **194**, c15–c18
27. Hartmann, M. & Zbiral, E. (1990) Tetrahedron Lett. **31**, 2875–2878
28. Schreiner, E. & Zbiral, E. (1990) Liebigs Ann. Chem. 581–586
29. Zbiral, E., Brandstetter, H. H. & Schreiner, E. (1988) Monatsh. Chem. **119**, 127–141
30. Schmid, W., Christian, R. & Zbiral, E. (1988) Tetrahedron Lett. **29**, 3643–3647
31. Hartmann, M. & Zbiral, E. (1991) Monatsh. Chem. **122**, 111–126
32. Christian, R., Schulz, G. & Zbiral, E. (1991) Monatsh. Chem. **122**, 521–528
33. Gantt, R., Millner, S. & Binkley, S. B. (1964) Biochemistry **3**, 1952–1960
34. Conradt, H. S., Bünsch, A. & Brossmer, R. (1984) FEBS Lett. **170**, 295–300
35. Augé, Cl., Bouxom, B., Cavayé, B. & Gautheron, Ch. (1989) Tetrahedron Lett. **30**, 2217–2220
36. Hagedorn, H., Mack, H., Gross, H. J. & Brossmer, R. (1986) Proc. Int. Carbohydr. Symp. 13th, Cornell University, Ithaca, 13 (abstr. A4)
37. Nadano, D., Iwasaki, M., Endo, S., Kitajima, K., Inoue, S. & Inoue, Y. (1986) J. Biol. Chem. **261**, 11550–11557
38. Augé, Cl., Gautheron, Ch., David, S., Malleron, A., Cavayé, B. & Bouxom, B. (1990) Tetrahedron **46**, 201–214
39. Hartmann, M. & Zbiral, E. (1989) Monatsh. Chem. **120**, 899–908
40. Grob, C. A. & Schiess, P. W. (1967) Angew Chem. **79**, 1–14
41. Grob, C. A. (1969) Angew Chem. **81**, 543–554
42. Deijl, C. M. & Vliegenthart, J. F. G. (1983) Biochem. Biophys. Res. Commun. **111**, 668–674
43. Baumann, W., Freidenreich, J., Weisshaar, G., Brossmer, R. & Fribolin, H. (1989) Biol. Chem. Hoppe-Seyler **370**, 141–149

44. Nees, S., Schauer, R., Mayer, F. & Ehrlich, K. (1976) Hoppe-Seyler's Z. Physiol. Chem. **357**, 839–853
45. Grob, C. A., Unger, F. M., Weiler, E. D. & Weiss, A. (1972) Helv. Chim. Acta **55**, 501–509
46. Villalva Basabe, J., Gross, H. J. & Brossmer, R. (1985) Jt. Meet. Biochem. Soc. Fr. Germ. Switz. 3rd, Basle (Biol. Chem. Hoppe-Seyler **366**, 863)
47. Allinger, N. L. (1977) J. Am. Chem. Soc. **99**, 8127–8134
48. Schreiner, E., Zbiral, E., Kleineidam, R. G. & Schauer, R. (1991) Liebigs Ann. Chem. 129–134
49. Schreiner, E., Zbiral, E., Kleineidam, R. G. & Schauer, R. (1991) Carbohydr. Res. **216**, 61–66
50. Brossmer, R., Rose, U., Kasper, D., Smith, T. L., Grasmuk, H. & Unger, F. M. (1980) Biochem. Biophys. Res. Commun. **96**, 1282–1289

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