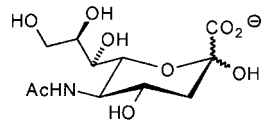
From Substrate to Transition State Analogues: The First Potent Inhibitor of Sialyltransferases

Peer Nils Schröder and Athanassios Giannis*

Sialic acid containing glycoconjugates are cell-type specific markers that are widely distributed throughout the organism. They play a vital role in a variety of fundamental physiologically and pathologically important processes.[1–3] These include embryogenesis, organ development, immune defense, migration and homing of leukocytes, metastasis of neoplastic cells, as well as inflammation and the infection by a variety of pathogens. Up to now more than 40 different sialic acids have been discovered, of which *N*-acetylneuraminic acid (**1**) is the most abundant one. They are often attached to the exposed terminal position of the oligosaccharide moiety of glycoconjugates and determine many of the biological properties of these structures. The influenza infection (common flu) is a well-known example of a cell/virus interaction mediated by sialic acid. Both adhesion to the cell prior to infection as well as the release of newly formed viruses from the infected cells are accomplished by structures containing sialic acid. Thus, the virus-releasing enzyme influenza sialidase has been an early target for pharmaceutical research.[3] Inhibitors of the biosynthesis of sialic acids on the other hand might prove to be useful as antiinflammatory, immunosuppressive, and antimetastatic agents. Although the biosynthetic pathway that leads to *N*-acetylneuraminic acid was discovered some time ago,[4] only a few such inhibitors have so far been developed.[5]

Another issue of research is the inhibition of sialyltransferases, a class of enzymes that catalyze the transfer of sialic acid moieties to their specific acceptor molecules. Nineteen members of this enzyme class have been identified so far.[6] Until a recent report by R. R. Schmidt et al., no potent and specific inhibitors of these enzymes had been described. The authors present the synthesis of compound **4**, which exhibits an inhibition constant of K_i of 40 nM to rat liver α(2-6)-sialyltransferase [EC2.4.99.1], and thus has a 1000-fold higher affinity than the natural substrate.[7] Originally, the rational design of this inhibitor was based on cytidine monophosphate-*N*-acetylneuraminic acid (CMP-Neu5Ac, **2**), which is the common donor substrate of all known sialyltransferases. One example of such inhibitors of substrate analogues is the CMP-Neu5Ac-derived compound **3**, which reduces the transfer of



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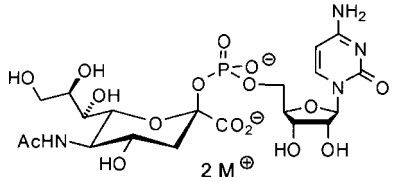

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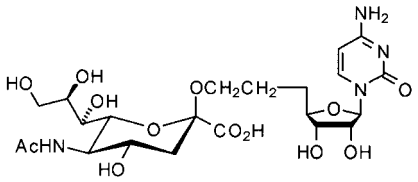

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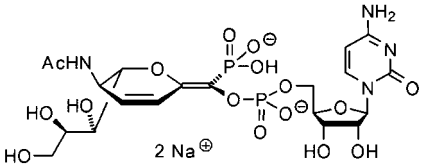

4

N-acetylneuraminic acid to lactosyl ceramide by 38%, and to the ganglioside GM_3 by 63%.[8] The concept of transition state analogues gained more and more attention during the development of compound **4**. Back in 1946 Linus Pauling stated that inhibitors closely related structurally to the transition state of an enzyme-catalyzed transformation should bind more tightly to the active site of the enzyme than analogues of the substrate in its ground state.[9] Kinetic studies indicated that for a one-substrate reaction the transition-state analogue is bound 10^7 to 10^{15} times tighter than the substrate in its ground state.[10] At the very beginning of the project, potent transition state analogues for the sialidases related to the replication of the influenza virus had already been developed. These compounds have been derived from the dehydroacetylneuraminic acid (Neu5Ac2en, **5**) and imitate the geometry of the proposed carboxonium intermediate **6** during the release of the *N*-acetylneuraminic acid moiety. Two of these drugs, derivative **7** with the guanidino substituent (Zanamivir, Glaxo Wellcome) and the cyclohexene derivative

[*] Prof. Dr. A. Giannis, Dipl.-Chem. P. N. Schröder
Institut für Organische Chemie der Universität
Richard-Willstätter-Allee 2, D-76128 Karlsruhe (Germany)
Fax: (+49) 721-608-7652
E-mail: giannis@ochhades.chemie.uni-karlsruhe.de

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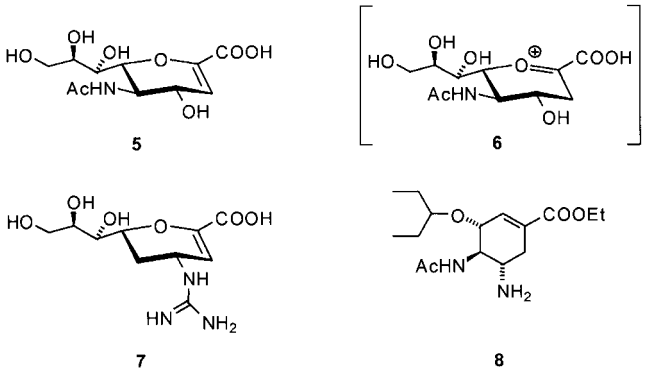
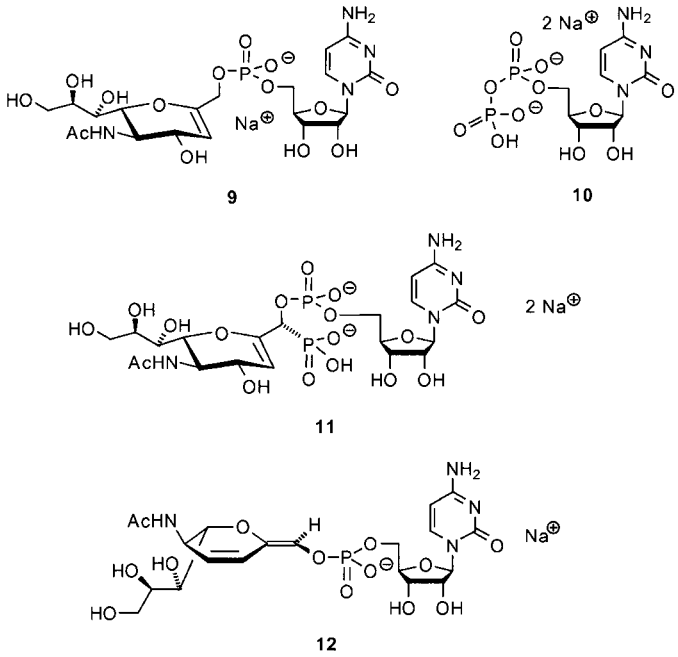


8 (GS4104, Hofmann-La Roche) successfully passed clinical testing phases I–III and are both believed to become approved by the Drug Administration authorities.[3, 11] It was questionable whether this concept would be directly transferable to the development of sialytransferase inhibitors because of its different mode of action: The transferase reaction proceeds under inversion at the anomeric carbon center while sialidases act under retention of configuration. Thus, it was not surprising that early attempts failed. Compound **9** has only a low affinity ($K_i = 2$ mM) to the α(2-6)-sialyltransferase from rat liver [EC 2.4.99.1],[12] compared to the natural substrate, CMP-Neu5Ac, which has a Michaelis–Menten constant K_M of 46 μM. On the other hand, cytidine diphosphate (CDP, **10**) was known to inhibit this specific sialyltransferase with a K_i value of 10 μM. The combination of the Neu5Ac2en moiety and the dianionic structure of CDP led to compound **11**, which is indeed a potent inhibitor with a K_i value of 350 nM.[12] Replacement of the Neu5Ac2en moiety by **12**, an elimination by-product of the synthetic pathway that leads to **11**, even outperformed this result. Compound **4** is the most potent inhibitor of the α(2-6)-sialyltransferase known today. Subsequent studies revealed that it also strongly inhibits α(2-3)-sialyltransferases.



The work of Schmidt et al. is a convincing example of rational inhibitor design in the challenging area of glycosyl transferases. The transition state analogue **4** is an important tool that may serve to further elucidate the function of sialic acid containing glycoconjugates. To reach this goal it will nevertheless be necessary to develop cell-permeable analogues, for example, prodrugs.

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