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Synthesis of a new transition-state analog of the sialyl donor. Inhibition of sialyltransferases

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Abstract—A new class of glycosyltransferase inhibitor has been designed and synthesized. The designed inhibitors **3a**/**3b** provide conformational mimicry of the transition state in sialyltransfer reactions. The key synthetic steps involve a Meinwald rearrangement and a palladium-catalyzed carbonylation reaction. The results of kinetic studies show that **3a**/**3b** exhibit significant inhibition on both 2,3- and 2,6-sialytransferases. © 2001 Published by Elsevier Science Ltd.

Sialic acids play an important role in a variety of biological processes.[1] They are usually attached to the terminal positions of glycoproteins, glycolipids and oligosaccharides. Of more than 100 different sialic acids, *N*-acetylneuraminic acid (NeuAc) is the most abundant one.[2] The sugar-nucleotide CMP-NeuAc **1** (Fig. 1) is the key substrate for biosynthesis of sialylated glycoconjugates in which **1** is transferred by sialyltransferases to an acceptor hydroxyl group in a variety of substrates including polysialic acids, glycoproteins and gangliosides.[3] These glycosylation patterns constitute important binding determinants for various cell–cell interactions which include masking of trypanosomal immunogenicity, viral infection and replication, and cell adhesion.[4] Therefore, development of potent and selective inhibitors of sialyltransferases may be useful in a variety of biochemical applications. However, only a few potent inhibitors of sialyltransferases have so far been developed.[5] We have previously reported the solvolytic behavior and enzymology of **1**, as characterized by kinetic isotope effects which strongly support an oxocarbenium ion-like transition state structure **2** for both solvolysis and the sialyltransferase catalyzed reactions.[6] According to this model, positive charge is accumulated at C-1′ and O-1′ and the carboxylate group approaches coplanarity with the oxocarbenium ion plane. A common strategy often used to design inhibitors of glycosidases and/or glycosyltransferases is to mimic the electronic charge distribution of a proposed transition state.[7] On the other hand, we noted that in addition to the positive charge and the coplanarity developed in **2**, the increased distance between the leaving CMP group and C-1′ might be another useful factor for inhibitor design. As a test for this distinct concept, we designed compound **3a**, in which the conjugated carboxylate group mimics the oxocarbenium ion coplanarity in transition state **2**, and moreover, the CMP group attached to the bicyclic structure is held at a distance to mimic a late transition state for bond cleavage. Here we wish to report the

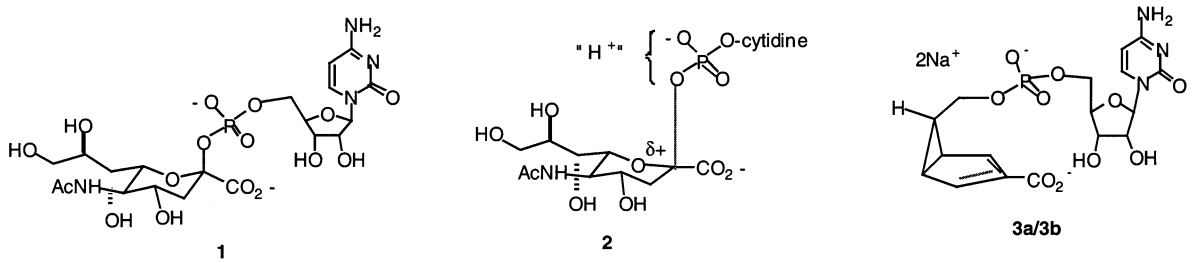

Figure 1.

Keywords: transition states; inhibitors; glycosyltransferases; sialytransferases; synthesis.

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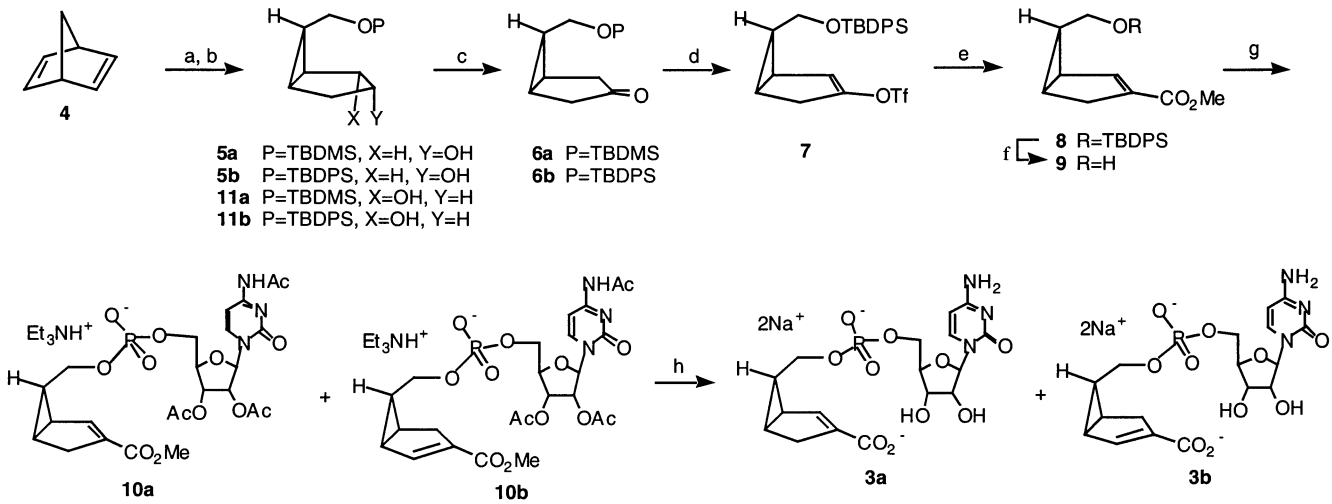
synthesis and inhibitory activities of compounds **3a** and **3b** (diastereoisomer of **3a**).

We envisioned that the bicyclic structure of **3a** could be established by Meinwald rearrangement of norbornadiene **4**.[8] With this in mind, we initiated a synthetic sequence, as shown in Scheme 1. Meinwald rearrangement of norbornadiene **4** with *m*-chloroperoxybenzoic acid and subsequent reduction with sodium borohydride gave racemic 6-*endo*-hydroxymethyl bicyclo-[3.1.0]hex-2-ene. Protection using *tert*-butyldimethylsilyl chloride or *tert*-butyldiphenylsilyl chloride in the presence of imidazole proceeded in 85–90% yields. Hydroboration–oxidation furnished the desired alcohol **5a** and its regioisomer **11a** in high yields (**5a**:**11a** ≈ 5:2, by NMR spectroscopy).[9] The ratio of diastereomers produced by hydroboration was insensitive to the *O*-protecting group. Stereoisomers **5a**/**11a** or **5b**/**11b** could be separated by careful column chromatography. Oxidation of **5a** or **5b** with chromium trioxide-pyridine gave ketone **6a** (78%) or ketone **6b** (86%), respectively. Alternatively, a mixture of **5b**/**11b** was used for the oxidation reaction to give the desired ketone **6b**, which could be easily separated from its regioisomer by flash column chromatography. Reaction of ketone **6b** with trifluoromethanesulfonic anhydride using 2,6-di-*tert*-butyl-4-methylpyridine as a base afforded vinyl triflate **7** in 69% yield. Palladium-catalyzed carbonylation of **7** in methanol proceeded smoothly to form the unsaturated ester **8** in 80% yield.[10] Deprotection of **8** with tetrabutylammonium fluoride (TBAF) gave the key intermediate **9** in 85% yield.

Cytidine-2′,3′-di-*O*-acetyl-4-*N*-acetyl-5′-(*N*,*N*-diisopropyl-2-cyanoethyl)-phosphoramidite was prepared from the triacetyl cytidine[11a] by the literature method.[11c] We found that a more facile synthesis of the triacetyl cytidine could be carried out with the following one-pot sequence. Selective protection of the 5′-hydroxyl group of *N*-acetyl-cytidine[11b] with 4,4′-dimethoxytrityl chloride in pyridine, followed by acetylation of the 2′,3′-hydroxyl groups with acetic anhydride in pyridine and deprotection of the 5′-hydroxyl group with 90% aqueous acetic acid, gave the required triacetate in 41% overall yield. The coupling of **9** with the cytidine 5′-phosphoramidite in the presence of tetrazole, followed by oxidation with *tert*-butyl hydroperoxide and base-catalyzed removal of the cyanoethyl group gave a diastereomeric mixture of **10a** and **10b**. Saponification of **10a**/**10b** with sodium methoxide in methanol–water afforded a mixture of **3a** and **3b** (1:1 by NMR), which was purified by anion-exchange HPLC (NH_4HCO_3–MeOH–H_2O buffer as eluent).[12] Our efforts to separate the diastereoisomers **3a** and **3b** were unsuccessful, so the mixture of **3a**/**3b** was used for the inhibition studies.

The inhibitory effects of **3a**/**3b** on rat 2,3- and 2,6-sialytransferases were investigated with the use of radiolabeled [9-^{3}H] CMP-NeuAc as a donor substrate. The acceptor sugars employed were lactose (for 2,3-sialytransferase) and lacNac (for 2,6-sialytransferase). The results show that **3a**/**3b** are competitive inhibitors of both 2,3- and 2,6-sialyltransferases, and the K_is were estimated to be 10 and 20 μM for 2,3- and 2,6-sialytransferases, respectively. These results provide further impetus for development of new analogs that incorporate additional features of the enzyme-bound transition state.

In summary, we report the design and synthesis of a new class of sialyltransferase inhibitors which mimic transition state geometry. The key synthetic steps involve a Meinwald rearrangement and a palladium-catalyzed carbonylation reaction. Despite lacking some of the structural features (e.g. C6–C9 glycerol side chain) of the natural sialyltransferase substrate, CMP-NeuAc, the designed inhibitors **3a**/**3b** showed significant inhibition on both 2,3- and 2,6-sialytransferases.



Scheme 1. *Reagents and conditions*: (a) (i) *m*-CPBA; (ii) $NaBH_4$; (iii) TBDMSCl or TBDPSCl, imidazole. (b) (i) $BH_3{\cdot}SMe_2$; (ii) H_2O_2, NaOH, 90–95%. (c) CrO_3, pyridine, 78% for **6a**; 86% for **6b**. (d) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, 69%. (e) $Pd(OAc)_2$, PPh_3, Et_3N, MeOH, CO, DMF, rt, 80%. (f) TBAF, THF, 85%. (g) (i) Cytidine-2′,3′-di-*O*-acetyl-4-*N*-acetyl-5′-(*N*,*N*-diisopropyl-2-cyanoethyl)-phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) *t*-BuOOH; (iii) Et_3N, CH_2Cl_2, 51% for three steps. (h) (i) NaOMe, MeOH–H_2O; (ii) HPLC (MonoQ, NH_4HCO_3–MeOH–H_2O); (iii) desalting with Amberlite IR-120, 30%.

We expect that analogs of **3a**/**3b** will exhibit more potent and selective inhibition on sialyltransferases, and may be extended to other glycosyltransferases.

Acknowledgements

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12. Selected data for **9**: ^{1}H NMR (300 MHz, CD_3Cl) δ 6.75 (m, 1H), 3.71 (s, 3H), 3.53 (m, 1H), 3.36 (m, 1H), 2.76 (m, 1H), 2.45 (m, 1H), 2.20 (m, 1H), 1.89 (m, 1H), 1.48 (m, 1H), 1.38 (bs, 1H); ^{13}C NMR (75 MHz, CD_3Cl) δ 164.87, 141.61, 135.68, 56.31, 51.39, 31.14, 28.88, 24.10, 21.38; HRMS calcd for $C_9H_{13}O_3$ (M^++H): 169.0865; found: 169.090. For **3a**/**3b**: ^{1}H NMR (300 MHz, D_2O) δ 7.75 (d, J=7.62 Hz, 1H), 6.29 (bs, 1H), 5.88 (d, J=7.62 Hz, 1H), 5.75 (m, 1H), 4.45 (m, 3H), 3.93 (m, 1H), 3.83 (m, 1H), 3.58 (m, 1H), 3.44 (m, 1H), 2.45 (m, 1H), 2.20–2.18 (m, 2H), 1.69 (m, 1H), 1.28 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 181.87, 173.91, 166.47, 158.12, 142.10, 141.61, 137.61, 96.82, 89.63, 89.52, 83.01, 74.46, 69.70, 64.46, 61.68, 31.96, 28.83, 23.65, 21.01; HRMS calcd for $C_{17}H_{21}N_3O_{10}$ P (M^+–2Na^++H): 458.0965; found: 458.087.