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An efficient synthesis of CMP-3-fluoroneuraminic acid

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CMP-3-fluoroneuraminic acid, a useful mechanistic probe for sialyltransferases, has been efficiently synthesized using recent fluorination and phosphorylation techniques from a sialic acid glycal.

Sialic acids displayed on the surface of mammalian cells are involved in many cell–surface interactions including cell–cell recognition processes, cell adhesion, and viral receptor recognition.[1,2] Sialyltransferases are the enzymes responsible for transfer of sialic acid from cytidine 5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) to a growing oligosaccharide, often terminating the series at the non-reducing end.[3] We recently synthesized the nucleotide phosphate sugars of fucose and galactose fluorinated α to the anomeric carbon that demonstrated significant competitive inhibition against the respective fucosyl- and galactosyl-transferase enzymes. These results, together with isotope studies, are indicative of substantial oxocarbenium ion transition state structure.[4,5] The effect of fluorine is attributed to its strong electron-withdrawing nature which destabilizes formation of positive charge within the carbohydrate ring. Though several substrate analog inhibitors have recently been designed to effectively inhibit α(2,6)-sialyltransferase,[6–9] a cationic transition state structure has not yet been demonstrated in mechanistic studies. Only solvolysis of CMP-Neu5Ac has been shown to display a finite sialyl cation species.[10] Additionally, *N*-acetyl-3-fluoroneuraminic acid (3F-Neu5Ac) alone serves as a competitive inhibitor of bacterial and viral sialidases.[11–13] Therefore, a fluorinated CMP-Neu5Ac derivative would be expected to inhibit sialyltransferases as a non-reactive mechanism-based inhibitor if the enzyme catalyzes formation of a cationic transition state species in the donor glycon. Such inhibition by a fluorinated substrate analog would extend the cationic transition state phenomenon to sialyltransferase enzymes, as has been demonstrated with other glycosyltransferases. Here we describe the synthesis of cytidine 5′-monophospho-*N*-acetyl-3-fluoroneuraminic acid **1**, which employs a fluorine subsituent α to the anomeric carbon on the neuraminic acid, and demonstrate its competitive inhibition of α(2,6)-sialyltransferase in the presence of CMP-Neu5Ac.

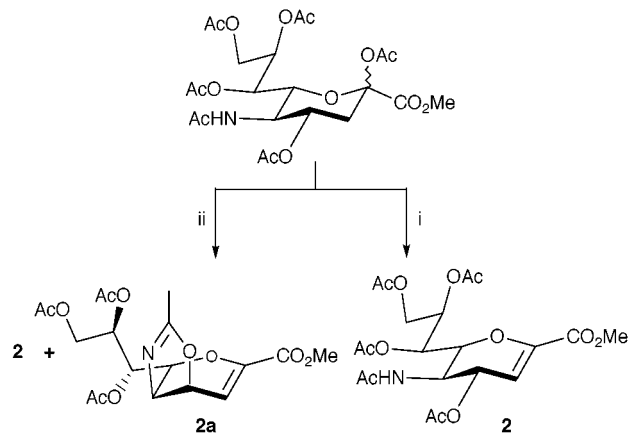
Previous syntheses of *N*-acetyl-3-fluoroneuraminic acid have relied upon fluorination of the glycal of peracetyl-*N*-acetylneuraminic acid methyl ester **2** with XeF_2–BF_3•OEt_2[12] or molecular fluorine.[13] The reported overall yield of this method gives less than 20% of the fluorinated monosaccharide in several steps from the sialic acid methyl ester. With the recent development of a one-pot, high-yielding fluorination technique using F–TEDA–CH_2Cl•$2BF_4$ (Selectfluor), the 2-hydroxy-3-fluoro protected monosaccharide **3** is available in 80% yield from the glycal.[14] Unlike previous methods, the fluorinated product retains all protecting groups from the glycal starting material, leaving a deprotected hydroxy at the anomeric position that is ready for selective chemical modification. This technique uniquely solves problems associated with the synthesis of **1**, as 3F-Neu5Ac is not a substrate of CMP-Neu5Ac synthase (EC 2.7.7.43), which converts Neu5Ac and cytidine-5′-triphosphate to CMP-Neu5Ac.[15] Hence, synthesis of the fluorinated CMP-Neu5Ac derivative must proceed *via* chemical methods. Conveniently, an improved synthesis of CMP-Neu5Ac and its conjugates was recently published enumerating a four step strategy from the 2-OH protected monosaccharide.[16] Combination of these two techniques with a new method to synthesize the glycal are reported here to synthesize **1**.

The known protected sialic acid glycal **2** was prepared directly from the peracetylated neuraminic acid (Scheme 1).[17] The described procedure utilizing TMSOTf in MeCN[18] led generally to a mixture of glycal **2** (65%) along with the 2,3-dehydro-4,5-oxazoline **2a**.[19,20] We found that the use of a catalytic amount of PPh_3HBr[21] in place of TMSOTf gave **2** in nearly quantitative yield (96%) without detectable cyclized product.

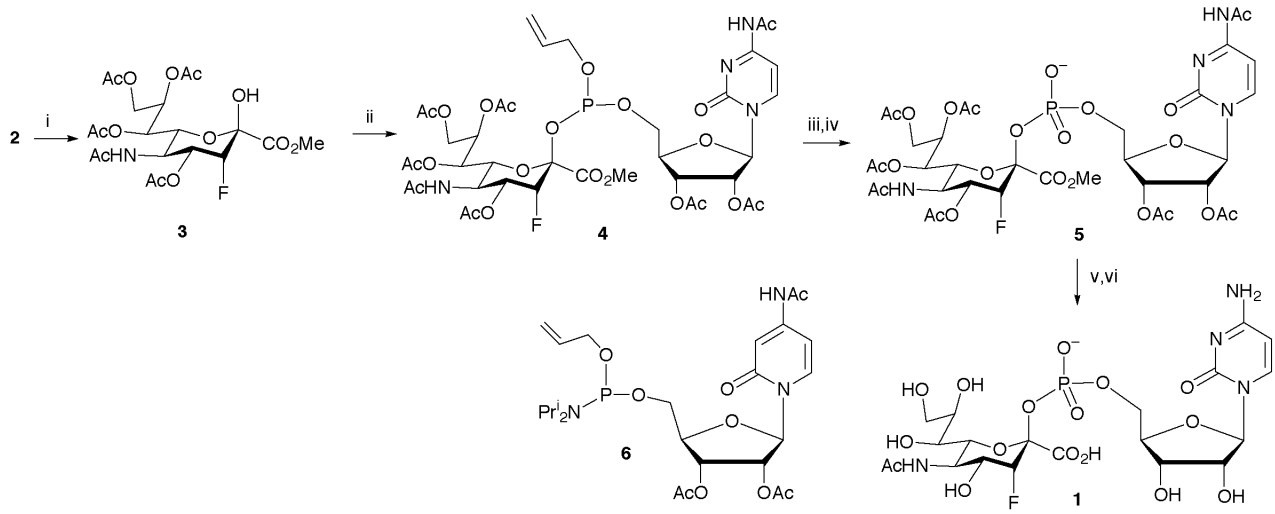
The glycal **2** was fluorinated with F–TEDA–BF_4 following the general glycal fluorination procedure in DMF and H_2O (Scheme 2).[14] Purification by silica gel chromatography yields **3** in 80% yield (3 : 1 F_{ax}/F_{eq}). The 2-*O*-acetylated derivative of this molecule and the fully deacetylated 3-fluoro-Neu5Ac have been previously characterized and were demonstrated to contain β anomeric configuration based upon no ^{13}C–^{19}F coupling between C1 and F3.[13] For this reason as well as an established anomeric effect for sialic acid[22] and the yield of only one stereoisomer from this reaction, we concluded **3** to be in the pure β form.

Compound **3** (F_{ax}) was condensed with phosphoramidite **6**[16] in MeCN followed by quench with Et_3N. The product was purified by Dowex LH-20 size-exclusion chromatography in MeCN to give the protected phosphite **4** in 54% yield.[23] Anomeric stereochemistry was determined to retain β configuration by ^{13}C–^{19}F coupling between C1 and F3 (0 Hz) and by the chemical shift of H3 deviating less than 0.1 ppm from **3**. Anomerization is well known to give rise to significant chemical shift at H3.[24] Furthermore, only one stereochemical isomer was isolated.

The phosphite **4** was oxidized to the phosphate with *tert*-butyl hydroperoxide and deprotected with $Pd(PPh_3)_4$ and Pr^i_2NH. Silica gel chromatography gave **5** in 72% yield.[25] Deprotection of the methyl ester with NaOMe in MeOH was followed by immediate size-exclusion chromatography in water. The product was deacetylated with 1 M NaOH and subsequently purified



Scheme 1 *Reagents and conditions*: i, PPh_3HBr, MeCN, 96%; ii, TMSOTf, MeCN.



Scheme 2 *Reagents and conditions*: i, Selectfluor, DMF–H_2O (3 : 1), 60 °C, 80%; ii, **6**, 1*H*-tetrazole, MeCN, −78 °C→room temp., 60%; iii, Bu^{t}OOH, Et_3N; iv, $Pd(PPh_3)_4$, Pr^i_2NH, 72% for 2 steps; v, NaOMe, MeOH; vi, NaOH, 80% for 2 steps.

by aqueous size-exclusion chromatography to provide **1** in 80% yield over two steps.[26]

Compound **1** was assayed as an inhibitor of α(2,6)-sialyltransferase (Calbiochem, San Diego, CA) using standard radioisotopic assay procedures. These results show that **1** competes with CMP-Neu5Ac for the enzyme with K_i = 5.7 ± 1.2 μM.[27] Compared to the K_m for CMP-Neu5Ac of 15 μM, this result is consistent with a transition state structure containing considerable oxocarbenium ion characteristic.[5]

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23 *Selected data* for **4**: δ_H(400 MHz, $CDCl_3$) 8.95 (s, 1H), 7.66 (*J* 7.2, 1H), 7.60 (d, *J* 10, 1H), 7.45 (*J* 7.6, 1H), 5.95–5.84 (m, 1H), 5.73 (*J* 3.4, 1H), 5.67-5.62 (m, 3H), 5.52–5.55 (m,, 1H), 5.30 (dd, *J* 1.5, 17.1, 1H), 5.20–5.16 (m, 2H), 5.06 (d, *J* 2.2, 51.6, 1H), 4.67 (d, *J* 2.6, 12.0, 1H), 4.41–4.33 (m, 6H), 4.22–4.18 (m, 2H), 4.09 (dd, *J* 8.5, 12.1, 1H), 3.81 (s, 3H), 2.22 (s, 3H), 2.19 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.85 (s, 3H); δ_F (376 MHz, $CDCl_3$) −180.40 (dd).
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25 *Selected data* for **5**: δ_H(400 MHz, $CDCl_3$) 8.33 (d, *J* 6.0, 1H), 7.38 (d, *J* 5.8, 1H), 6.33 (d, *J* 4.9, 1H), 5.53–5.43 (m, 3H), 5.29 (dd, *J* 9.3, 11.5, 1H), 5.03 (d, *J* 50.2, 1H), 4.80 (dd, *J* 2.6, 12.1, 1H), 4.53 (dd, *J* 10.5, 21.0, 1H), 4.48–4.43 (m, 1H), 4.29–4.21 (m, 3H), 3.82 (s, 3H), 2.22 (s, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.86 (s, 3H); δ_F(376 MHz, $CDCl_3$) −179.80 (dd); LRMS (M + Cs) calc. for $C_{35}H_{45}FN_4O_{23}PCs$, 1073; found 1073.
26 *Selected data* for **1**: δ_H(400 MHz, $CDCl_3$) 7.96 (d, *J* 7.7, 1H), 6.06 (d, *J* 7.7, 1H), 5.83 (d, *J* 4.4, 1H), 4.23 (t, *J* 5.0, 1 H), 4.19 (t, *J* 4.7, 1H), 4.16 (m, 2H), 4.13 (m, 1H), 4.08–4.01 (m, 2H), 3.97 (dd, *J* 9, 11.7, 1H), 3.76 (m, 2H), 3.72 (dd, *J* 2.5, 11.9, 1H), 3.47 (dd, *J* 6.44, 1.7, 1H), 3.29 (d, *J* 9.1, 1H), 1.90 (s, 3H), 1.90 (d, *J* 40, 1H); δ_F(376 MHz, $CDCl_3$) −194.02 (dd, *J* 11.0, 49.0); LRMS (M + Na) calc. for $C_{20}H_{30}FN_4O_{16}PNa$, 655.1276; found 655.
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