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Synthetic studies of new CMP–sialic acid analogues applying a novel buffer-mediated rearrangement

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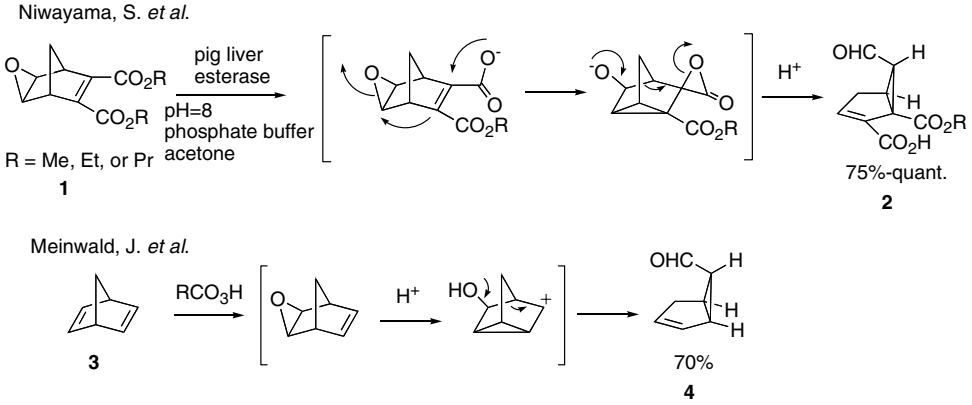
Abstract—Synthetic studies of analogues of cytidine monophosphate (CMP)–sialic acid as transition state mimics for sialylation are reported, applying selective monohydrolysis of a symmetric diester and a subsequent buffer-mediated regio- and stereospecific rearrangement we reported earlier.

Although monohydrolysis of symmetric diesters yielding half-esters is a versatile synthetic methodology, most enzyme reactions that enable this monohydrolysis induce simple chemoselective conversion of functional groups, and further skeletal conversions by the conditions of enzyme reactions are rare. However, earlier, we reported a regio- and stereospecific rearrangement initiated by enzymatic monohydrolysis of symmetric diesters, **1**, producing 1-alkoxycarbonyl-6-formylbicyclo[3.1.0]hex-2-ene-2-carboxylic acids, **2** (Scheme 1).[1] Later, we also reported that this rearrangement can occur in a racemic manner, mediated by a slightly basic buffer solution without an enzyme.[1b]

These rearranged products, **2**, possess the same skeleton as the rearranged product, **4**, initiated by peracid oxidation of norbornadiene, **3**, which was reported by Meinwald et al. (Scheme 1).[2] However, although the Meinwald rearrangement occurs catalyzed by a catalytic amount of an acid, our rearrangement takes place under the mildly basic reaction media due to the existence of the electron-withdrawing carboalkoxy groups. The product yielded by the Meinwald rearrangement, **4**, has been successfully applied to synthesis of a variety of biologically significant compounds.[3]

Sialic acids are monosaccharides distributed especially in glycoproteins and glycolipids, with the most



Scheme 1.

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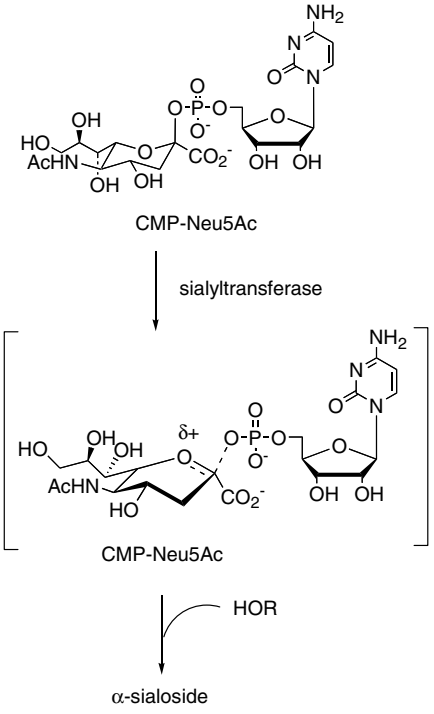
predominant one being *N*-acetylneuraminic acid (Neu5Ac). Sialyltranferases catalyze the transfer of sialic acid from cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) to a growing oligosaccharide. These processes are involved in various biological functions, thus influencing a variety of physiologically important processes, such as viral infection and inflammation.[4] Therefore, inhibitors of sialyltransferase are anticipated to serve as useful probes for dissecting the mechanisms of these biological processes (Scheme 2).



Scheme 2. Mechanism of sialylation.

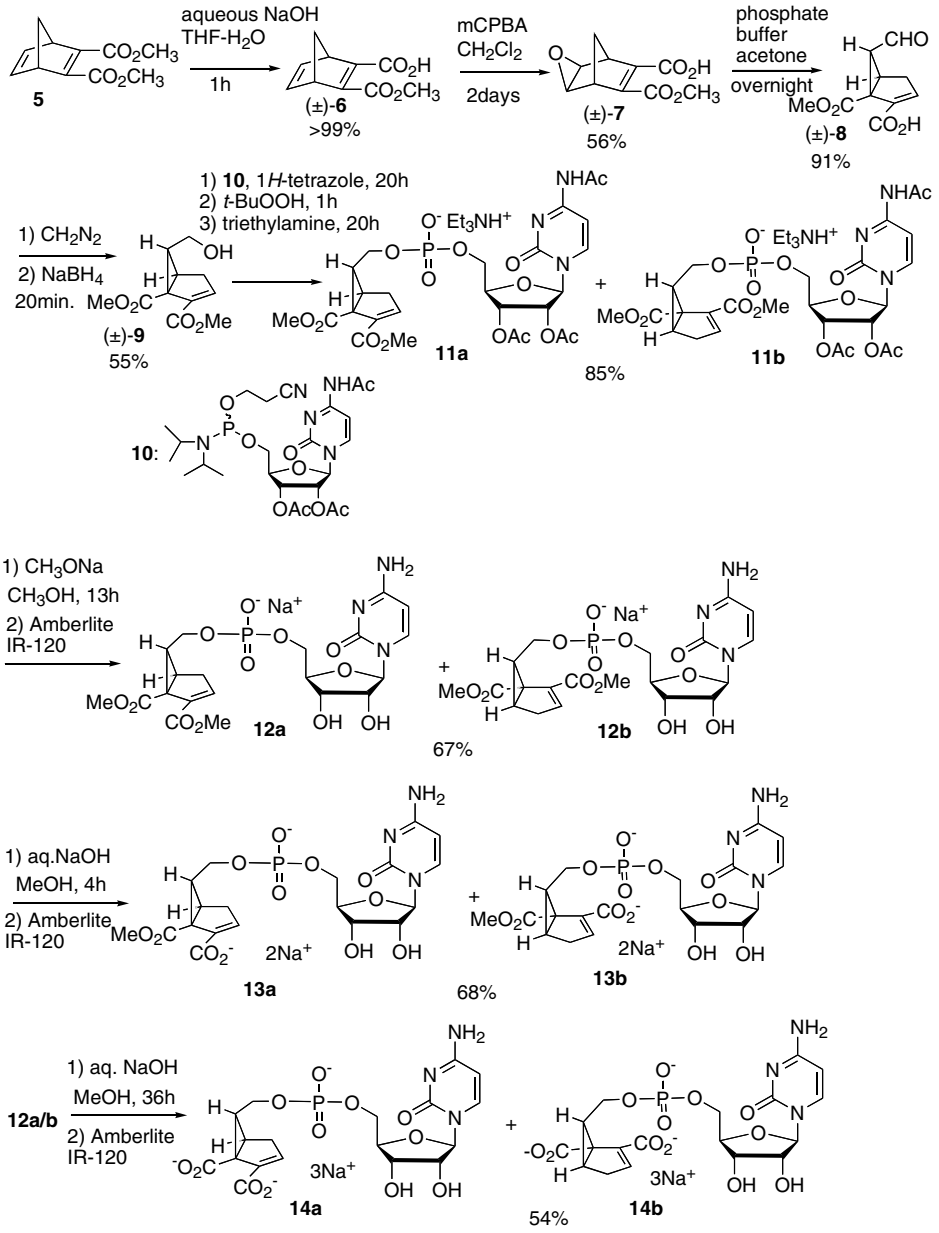
At this point, potent inhibitors of sialyltransferases are still relatively limited;[5] therefore there has been considerable interest in synthesizing various types of the inhibitors. Some examples of the common strategies are based on the electronic charge distribution, planarity having an oxocarbenium ion character, or the distance of the leaving CMP group in the proposed transition state. Inhibitors possessing the bicyclo[3.1.0]hex-2-ene structure have been reported to be effective by Horenstein et al., with the estimated K_i values 10–20 μM for 2,3- and 2,6-sialyltransferases.[5a] The structure mimics the oxocarbenium ion-like plane and the position of an aglycon above the plane, with the distance between the aglycon-mimic and the plane resembling a late transition state of sialylation. The synthesis of these CMP derivatives started with the Meinwald rearrangement product, **4**.[5a] Our rearrangement products, **2**, are thus also anticipated to serve as a useful synthetic building block, either in an optically active or racemic form.

Herein we report a concise synthesis of a small library of CMP derivatives, applying the novel regio- and stereospecific rearrangement initiated by desymmetrization of a symmetric diester we reported earlier (Scheme 1).[1] These derivatives also possess the bicyclo[3.1.0]hex-2-ene skeleton; however, since this core skeleton possesses several functional groups that can be modified independently, this template allows synthesis of diverse derivatives. These derivatives are also likely to show activities as inhibitors of sialyltransferase.

Therefore, we applied our rearrangement to the synthesis of a small library of CMP derivatives. We started with the rearranged product in a racemic form in this study, as it allows large scale production at a low cost. The detailed synthetic sequence is shown in Scheme 3.

The symmetric diester, **5**,[6] was selectively monohydrolyzed to produce the corresponding half-ester, **6**, in a quantitative yield.[7] The half-ester was subjected to the epoxidation and subsequently to the rearrangement we reported earlier,[1] yielding 1-methoxycarbonyl-6-formylbicyclo[3.1.0]hex-2-ene-2-carboxylic acid, **8**, in a stereo- and regiospecific manner. After the diazomethane treatment, the formyl group of this rearranged product was reduced with $NaBH_4$ to form alcohol, **9**. This alcohol was used as a template for synthesis of various derivatives of the transition state mimics for sialyltransferase-catalyzed reactions after coupling with the CMP moiety.

For the CMP moiety, 2-cyanoethyl 2′,3′-*O*,N^4-triacetylcytidin-5′-yl *N*,*N*-diisopropylphosphoramidite, **10**, was first prepared according to the reported procedure.[8] Alcohol **9** was coupled with this phosphoramidite, **10**, in the presence of tetrazole. This coupling reaction, which is a key step in the entire synthetic scheme, took place smoothly. The product was oxidized with the use of *tert*-butyl hydroperoxide followed by the addition of triethylamine for the removal of the cyanoethyl group. These series of reactions led to the triethyl ammonium salt of diastereometric mixture, **11a/b**, in a reasonable yield after three steps (85%). Our attempts to separate these diastereomers were unsuccessful, and therefore, we decided to use these diastereomeric mixtures for further studies, as in the studies of Horenstein et al.[5a] Deacetylation of **11a/b** was achieved using sodium methoxide in methanol to yield **12a/b** in a high yield. Although there are two carbomethoxy groups that may be susceptible to hydrolysis by treatment with aqueous NaOH, the carbomethoxy group attached to the quaternary carbon clearly showed more resistance toward this hydrolysis, as has been expected from general tendencies due to the steric hindrance.[9] When the reaction time was 4 hours, it produced only monoester **13a/b**. The formation of **13a/b** was also supported by the HMBC spectrum, showing correlation between the enoic proton at around 6 ppm and the carbonyl carbon of the COO^- group at 167.5 ppm attached to the cyclopentene ring, but not showing such correlation with the carbonyl carbon of the COOMe group at 166.2 ppm. In this way, the two ester groups were successfully distinguished, potentially allowing synthesis of more diverse derivatives in the future. The remaining carbomethoxy group was hydrolyzed by the extended reaction time (36 hours). The trisodium salt was obtained in a moderate yield after the purification by column



Scheme 3. Synthetic scheme for CMP–sialic acid analogues.

chromatography, or preparative HPLC, and subsequent ion exchange with Na^+.

In summary, we synthesized several transition state mimics of sialyltransferase-catalyzed reactions, applying our regio- and stereospecific rearrangement initiated by monohydrolysis of a symmetric diester. This study is the first synthetic application of this novel rearrangement. The phosphates, **12a/b**, **13a/b**, and **14a/b**, are anticipated to serve as a useful tool to study the action of sialyltransferase.[10] In particular, phosphates **13a/b** and **14a/b** are anticipated to be more cell-permeable and expected to possess inhibitory activities of sialyltransferase. We will evaluate the inhibitory activities of these derivatives and will synthesize more derivatives based on the results. Further studies will be reported in due course.

Acknowledgments

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10. Selected data for these phosphates are as follows:
Phosphate (**11a/b**, 1:1 diastereomeric mixture); ^{1}H NMR (CD_3OD, 300 MHz): 1.15 (t, $J = 7.2$ Hz), 2.06 (s), 2.10 (s), 2.12 (s), 2.24–2.26 (m), 2.48–2.51 (m), 2.81–2.84 (m), 3.02 (q, $J = 7.2$ Hz), 3.28–3.31 (m), 3.63 (s), 3.66 (s), 3.78–3.82 (m), 4.09–4.11 (m), 4.38 (br s), 5.41–5.44 (m), 6.18–6.21 (m), 6.52 (m), 7.45 (m), 8.4 (m); ^{13}C NMR (CD_3OD, 75 MHz): 8.1, 18.2, 19.3/19.2, 23.5, 27.4/27.4, 27.5/27.4, 31.6, 32.4/32.3, 41.3, 51.1/51.4, 60.1, 64.1, 70.9/70.8, 74.4/74.3, 82.2/82.1, 88.3/88.2, 97.8, 133.0, 144.5, 144.6, 145.2, 156.9, 163.5, 164.9, 169.8, 170.1, 171.7, 172.0; HRMS calcd for $C_{32}H_{48}N_4O_{15}P$ $(M+H)^+$: 759.2854; found, 759.2851.
Phosphate (**12a/b**, 1:1 diastereomeric mixture); ^{1}H NMR ($CDCl_3$, 300 MHz): 0.98–1.12 (m), 2.21–2.41 (m), 2.71–2.75 (m), 3.12–3.20 (m), 3.38–3.41 (m), 3.52 (s), 3.60–3.61 (m), 3.65 (s), 3.88–3.93 (m), 4.0–4.1 (m), 4.14–4.16 (m), 5.87 (m), 5.96 (m), 6.53 (br s), 6.65 (br s), 7.74 (m); ^{13}C NMR ($CDCl_3$, 75 MHz): 18.5, 32.1/32.0, 33.2/33.0, 41.3, 52.9/52.6, 60.6/60.5, 64.1, 69.3/69.2, 74.5/74.3, 82.5/82.4, 89.7/89.4, 96.6/96.5, 131.4, 141.6/141.5, 147.6, 147.9, 157.7, 166.2, 166.6, 174.1; HRMS calcd for $C_{20}H_{26}N_3NaO_{12}P$ $(M+H)^+$: 554.1152; found, 554.1160.
Phosphate (**13a/b**, 1:1 diastereomeric mixture); ^{1}H NMR (D_2O, 300 MHz): 1.81–2.24 (m), 2.62–2.67 (m), 3.01–3.04 (m), 3.34–3.38 (m), 3.61 (s), 3.92–3.99 (m), 4.06–4.08 (m), 4.16–4.19 (m), 5.86–6.02 (m), 6.46 (br s), 6.51 (br s), 6.78 (m), 7.63 (m), 7.78–7.80 (m); ^{13}C NMR (75 MHz, D_2O): 25.8/25.7, 30.4/30.2, 32.2/32.0, 41.6, 52.3, 64.0, 69.3/69.1, 74.5/74.4, 82.6/82.5, 89.6/89.4, 96.9/96.6, 125.3/124.9, 131.8, 133.1, 141.6/141.4, 145.7/145.5, 157.9, 166.2, 167.5; HRMS calcd for $C_{19}H_{22}N_3Na_3O_{12}P$ $(M+Na)^+$: 584.0634; found, 584.0639.
Phosphate (**14a/b**, 1:1 diastereomeric mixture); ^{1}H NMR (D_2O, 300 MHz): 1.92–2.07 (m), 2.10–2.13 (m), 2.48–2.59 (m), 3.28–3.38 (m), 3.58–3.62 (m), 3.88–3.96 (m), 4.10–4.16 (m), 5.84–6.12 (m), 6.69 (m), 7.78 (m), 8.43 (br s); ^{13}C NMR (D_2O, 75 MHz): 26.0, 29.6, 31.2, 43.3, 64.0, 69.3/69.1, 74.5/74.3, 83.0, 89.5, 96.9, 129.4, 125.1, 134.4, 141.7/141.5, 145.9/145.6, 158.0, 166.5, 167.5; HRMS calcd for $C_{18}H_{20}N_3Na_3O_{12}P$ $(M+H^+)$: 570.0477; found, 570.0479.