Journal of Medicinal Chemistry

Freie Universität Berlin

Brief Article

Transition State-Based Sialyltransferase Inhibitors: Mimicking Oxocarbenium Ion by Simple Amide

Jian Guo, Wenming Li, Weiwei Xue, and Xin-Shan Ye

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b01644 • Publication Date (Web): 06 Feb 2017

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Transition State-Based Sialyltransferase Inhibitors: Mimicking Oxocarbenium Ion by Simple Amide

Jian Guo,[†] Wenming Li,[†] Weiwei Xue,[‡] and Xin-Shan Ye*,[†]

[†]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Road No. 38, Beijing 100191, China

[‡]School of Pharmaceutical Sciences and Innovative Drug Research Centre, Chongqing University, Daxuecheng South Road No. 55, Chongqing 401331, China

Supporting Information

ABSTRACT: In the new transition-state-based sialyltransferase inhibitors, an amide group was placed at the corresponding C-2 position of CMP-sialic acid to mimic the geometry and charge distribution in the transition-state, and simple aromatic or aliphatic rings were used instead of sialic acid moiety. All synthetic compounds exhibited excellent α(2-6)-sialyltransferase inhibition, resulting in up to a 2600-fold higher affinity for the enzyme than CMP-Neu5Ac, suggesting that amide is a key element for simulating transition-state features.

■ INTRODUCTION

Glycosidases and glycosyltransferases are involved in the biosynthesis of glycoconjugates associated with intercellular recognition, metastasis, immune response, etc., and represent viable therapeutic targets.[1-6] Of particular interest is the development of transition-state analogues as potent inhibitors for the discovery of new therapeutic agents,[7-14] as Pauling proposed in 1946 that inhibitors closely imitating the structure of the transition-state in an enzyme-catalyzed process could bind more tightly to the active site of the enzyme than ground-state substrates.[15] Many different kinds of glycosidase inhibitors based on the transition-state analogues have been reported and some of them have been developed into clinic drugs successfully. However, only very limited glycosyltransferase inhibitors based on this strategy were reported, and only a few could exhibit inhibition in the nanomolar range. Obviously, how to mimic the transition-state in the glycosyltransferase-catalyzed process more efficiently needs to be further explored.

Of all glycosyltransferases, sialyltransferase (ST) is undoubtedly one of the most important enzymes due to their biological significance.[16-19] Potent ST inhibitors could have potential applications in both glycobiology as probes and clinic treatment as anticancer, immunoregulation drugs, etc. Although current transition-state-based ST inhibitors are thought to possess poor cellular permeability because of charged phosphate groups, it is possible to solve this problem through prodrug or other drug delivery strategies. Encouraged by these facts, and as a continuation of our interest in this field,[20-22] we deemed it an important goal to search for new transition-state analogues as potent ST inhibitors. According to the proposed S_N1-like mechanism of the ST-catalyzed sialylation reactions,[23-28] the glycosyl donor in the transition state may involve the partial dissociation of the CMP moiety

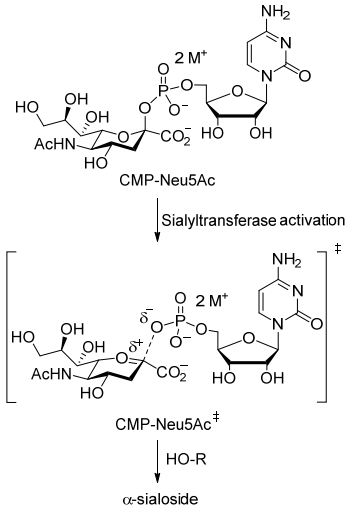


Figure 1. Mechanism of the sialylation reactions.

with increased distance from the anomeric carbon, and the formation of a trigonal planar oxocarbenium ion (Figure 1). Recognition of these salient features of the transition-state could guide the design of potential inhibitors. Compounds **1**−**4** stand for four types of successful ST transition-state analogue inhibitors (Figure 2).[12,13,21,29] In these inhibitors, functional groups with planar sp^2 carbons such as olefins and aryls, or functional groups with a planar conformation such as cyclopentane, were adopted to imitate the planar configuration of the oxocarbenium ion. It is generally believed that the separated charges of the donor in transition-state will be stabilized by the ST, though what factors are responsible for this stabilization remains an open question. There is a higher chance that

the charged sialyl oxocarbenium mutually interacts with polar amino-acid residues, the dipole of α-helix, or other factors at the active site of ST to realize the transient stability of transition-state. However, both the partially positive charge distribution between the anomeric carbon and the ring-oxygen, as well as the resulting interaction with ST have never been taken into account in the previous inhibitor design, and usual nonpolar planar functional groups could not mimic the situation here effectively.

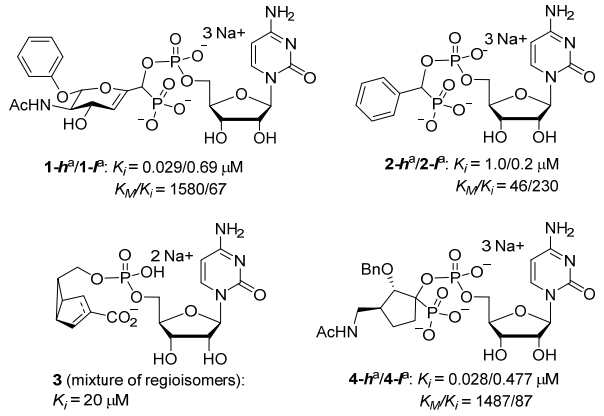


Figure 2. Typical transition-state analogue inhibitors. [a]*h*/*l* stands for higher or lower moving spots on the TLC as described in the original references.

Considering the configuration and charge distribution of the oxocarbenium, an inhibitor equipped with a both planar and highly polarized functional group to mimic the characteristics of the anomeric position of the sialyl oxocarbenium will look more like the real transition-state.[15] The amide bond is one of the most important functional groups in chemistry and biology. It is characterized as a both trigonal planar and high-polarity group due to the strong resonance interaction between the n_N and $\pi^*_{c=o}$ orbitals (Figure 3), resulting in easy formation of dipole-dipole interaction and hydrogen bond connections through both nitrogen and oxygen sites.[30] These characteristics have been utilized in the design of glycosidase inhibitors,[31-33] but no related work was reported in the design of glycosyltransferase inhibitors. If an amide bond is placed at the corresponding anomeric position of transition-state analogues of CMP-Neu5Ac, will it make the inhibitors and the interaction between the inhibitors and STs look more like the real transition-state? This similarity may lead to the discovery of potent ST inhibitors.

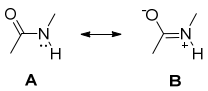


Figure 3. Resonance description of the amide bond.

To verify this hypothesis, compounds **5a**−**5f** with an amide bond were designed (Figure 4). According to the previous work by Schmidt *et al*., the Neu5Ac moiety is dispensable.[13] Considering the synthetic accessibility, simple aromatic or aliphatic rings were used to mimic the Neu5Ac residue, and an amide bond was placed at the corresponding C-2 position of the sialyl oxocarbenium. Moreover, other positive factors for high affinity based on Schmidt's SAR study were also introduced into the inhibitors, such as keeping CMP with another adjacent negative charge center and an increased distance between the presumptive anomeric carbon and the CMP.[13, 29, 34] In addition, the reported inhibitors **5g** and **5h** also need to be synthesized for comparison and control research. Compound **5g**, just lacking the amide bond, possesses the very similar structure to **5c**.[21] Compound **5h** is the most potent inhibitor among the simplified transition-state analogue inhibitors in which aromatic ring is used to mimic sialyl oxocarbenium.[35] After the synthetic work is completed, the inhibitory activities of these target molecules against α(2−6)-sialyltransferase will be evaluated.

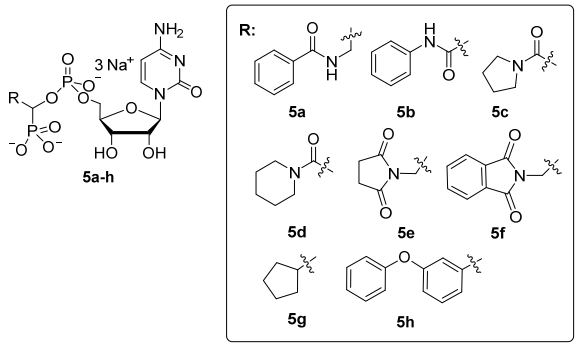


Figure 4. Designed transition-state analogues with amide bonds.

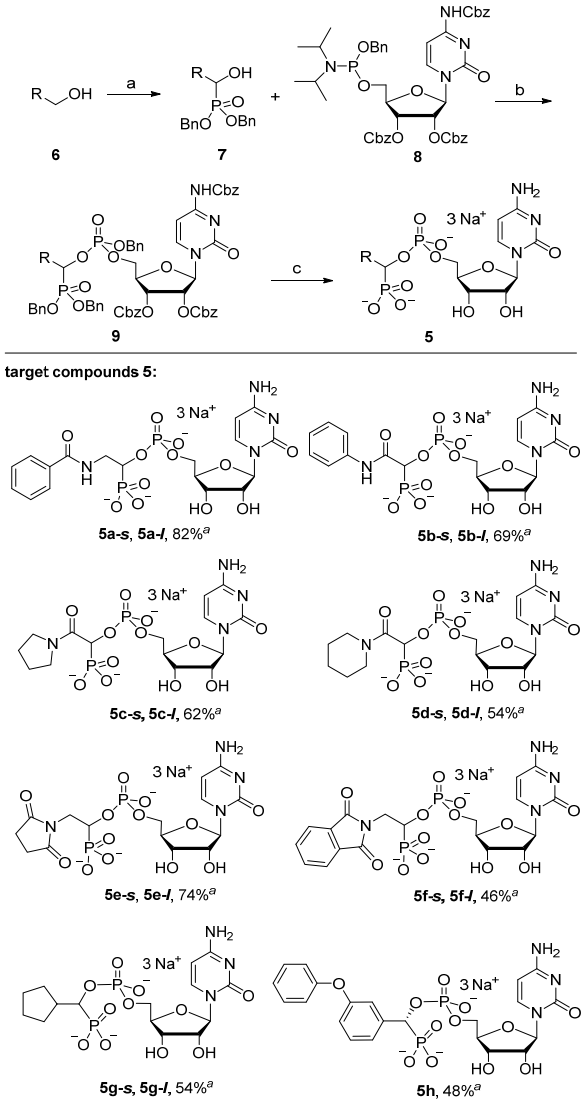
■ RESULTS AND DISCUSSION

The synthetic route of target compounds **5a**−**5h** was shown in Scheme 1. Since some final target compounds such as **5a**, **5e** and **5f**, were sensitive to strong bases and acids, the selection of proper protective groups in **7** and **8** was crucial. Through initial screening, Cbz and benzyl were prioritized as protective groups of cytidine and phosphate. They could be easily removed under neutral transfer hydrogenation, while not affecting the cytosine group.[36] Another issue that needs to be noted is the formation of a new chiral center in **7**. Since it has been confirmed that the absolute configuration of this chiral center did not show any regular influence on the final inhibitory activity,[35] and as it was not the focus of our research, no special attention was paid to the asymmetric synthesis.

With above key points in mind, the synthesis of **5** started from the known materials **6** containing the simple amides, the focus of our attention, and which are easily accessible through one step following the reported procedure. Alcohols **6** were oxidized to the corresponding aldehydes, which were reacted with dibenzyl phosphonate to afford the corresponding α-hydroxy-phosphonates **7**. The condensation of **7** with cytidine-phosphitamide **8** in the presence of *H*-tetrazole, which was followed by oxidation with *tert*-butyl hydroperoxide, afforded **9** as a mixture of four diastereoisomers. The global deprotection of **9** using 10% Pd/C and 1,4-cyclohexadiene in DMF provided the final target molecules **5**. After filtration and purification by C-18 reverse-phase column chromatography (H_2O → H_2O/MeOH 5:1), the products **5** were collected in the form of two diastereoisomers, which were further separated by preparative RP-HPLC. Finally, after converting to their corresponding sodium salts by ion-exchange (IR 120 Na^+) and lyophilization from water, the pure products **5**-***s*** and **5**-***l*** were obtained as white amorphous solids (*s* stands for the compo-

nent with a shorter retention time while *l* stands for the component with a longer retention time in preparative RP-HPLC).

Scheme 1. Synthesis of target compounds 5.



Reagents and conditions: a. i) DMSO, $(COCl)_2$, then Et_3N; ii) dibenzyl phosphonate, Et_3N; b. i) *H*-tetrazole; ii) ^{t}BuOOH; c. 1,4-cyclohexadiene, Pd/C. [a]Yield of crude **5** (2 steps from **7**) after column chromatography on RP-18 silica gel.

The inhibition rates and kinetic constants of K_i against recombinant human ST6Gal-I (aa 44-406) were carried out based on the UV/RP-HPLC method developed by Schmidt group with some modifications.[37] The potent inhibitors **5h** and **5g** with known K_i values were selected as the control. In our assay, the K_m for CMP-Neu5Ac was determined to be 41.63 ± 6.66 μM (see Supporting Information, Figure S1), comparable to the reported K_m value 46 ± 7 μM.[13] The inhibition rates of all the target compounds were obtained at 1 μM level, and subsequently the K_i values were determined for the compounds whose inhibitory rates were superior to 50% (Table 1). To our delight, all designed analogues showed potent inhibitory activity, among which **5a**–**5d** exhibited excellent α(2-6)-sialyltransferase inhibition in the nanomolar range. Compound **5a-*s*** showed the highest inhibitory activity with K_i = 0.016±0.005 μM, and its affinity to α(2-6)-sialyltransferase is 2600-fold higher than that of the natural substrate CMP-Neu5Ac, even stronger than **5h**. The 2600-fold enhancement of affinity indicates compound **5a-*s*** is the most potent sialyltransferase inhibitors up to now. Compound **5d-*s*** also showed comparable activity as **5h**. By comparing **5a** and **5b** with **2**, and **5c** with **5g**, it is obvious that the insertion of an amide group here presents a positive effect on increasing the affinity, with up to an almost 100-fold increase. This clearly emphasizes that the polar amide may be better mimic functional groups than simple nonpolar planar groups, which is consistent with our expectation. As an additional proof, in terms of **5e** and **5f**, succinimide and phthalimide are different from other amides, in which the extra carbonyl makes the charge density of nitrogen-atom in amides decrease largely, thus the charge-separated structure functions less in the resonance structure, and the dipole moment is obviously reduced,[38] therefore making **5e** and **5f** just as a configuration mimic, and exhibiting the similar inhibitory activity to compound **2**.

Table 1. Affinity of CMP-Neu5Ac (K_m) to recombinant human ST6Gal-I and inhibition data of the desired inhibitors.[a]

compound	inhibition at 1 μM (%)	K_m or K_i (μM)	Km/K_i
CMP-Neu5Ac		41.630± 6.660[d]	
5a-*s*	88.88	0.016 ± 0.005	2601.9
5a-*l*	61.68	0.174 ± 0.039	239.3
5b-*s*	69.73	0.106 ± 0.029	392.7
5b-*l*	67.70	0.122 ± 0.027	341.2
5c-*s*	76.98	0.060 ± 0.015	693.8
5c-*l*	65.70	0.165 ± 0.023	252.3
5d-*s*	86.34	0.020 ± 0.005	2081.5
5d-*l*	80.30	0.045 ± 0.022	925.1
5e-*s*	58.83	0.224 ± 0.045	185.8
5e-*l*	15.06		
5f-*s*	34.46		
5f-*l*	14.35		
5g-*s*	43.25[b,d]	5.852 ± 0.908[d]	7.1
5g-*l*	12.82[b,d]		
5h	87.58	0.019 ± 0.005[d]	2191.0
2-*h*		0.20 ± 0.05[c]	230.0
2-*l*		1.0 ± 0.2[c]	46.0

[a]For details of the procedures, see the Supporting Information. [b]Inhibition at 10 μM (%). [c]Reported data in the reference.[13] [d]Reported data in the reference.[21]

In order to explore the putative binding mode, molecular docking simulations were performed using **5a** with human ST6Gal-I crystal structure (Figure 5). The optimized docking results showed that the CMP moiety in the inhibitors **(*S*)-5a** and **(*R*)-5a** binds the similar site with CMP in the complex of ST6Gal-I and CMP, and the other moiety in the two inhibitors binds different sites (Figures 5A–5C). The phenyl in **(*S*)-5a** is

oriented to a hydrophobic region formed by Pro259, Tyr 275 and Trp257, which is similar to the recent simulation results.[39] It was suggested that the basic histidine residue at catalytic site (His370 in hST6Gal I) may help to stabilize development of the cationic transition-state.[40] Recent crystal structure research on hST6Gal-I implied the developing sialyl oxocarbenium in transition-state could mutually interact with the imidazole side chain of His370 and/or 6'-hydroxyl group of galactose acceptor through concerted mechanism.[41] In our docking simulation, the amide bond in **(*S*)-5a** was located in the proximity of residues such as His370, Asn233 and Ser323, where there is an analogous position to anomeric carbon of donor. Considering that the distance between them is within or near hydrogen-binding distance, it is possible that a slight movement of residue could result in either a strong hydrogen bond or an electrostatic interaction. We speculated one or more of above factors contributed to the increased affinity.

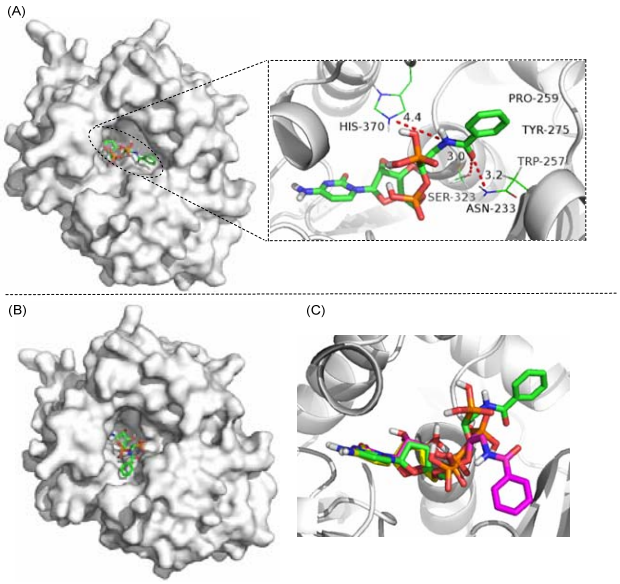


Figure 5. Molecular docking simulations for inhibitors **(*S*)-5a** and **(*R*)-5a** with hST6Gal I crystal structure (PDB code 4js2). (A) Putative binding mode of **(*S*)-5a**. (B) Putative binding mode of **(*R*)-5a**. (C) Superimposition of **(*S*)-5a** (green), **(*R*)-5a** (purple) and CMP (yellow) in the crystal structure (PDB code 4js2).

■ CONCLUSION

In summary, in an effort to explore new transition-state analogues for the development of potent ST inhibitors, an amide bond was introduced to mimic the anomeric position in the transition-state of CMP-Neu5Ac, and simple aromatic or aliphatic rings were used to replace Neu5Ac moiety, in cooperation with CMP and phosphonate, which lead to a series of potent inhibitors, especially **5a-s** as the most potent ST inhibitor up to now. Considering amide's planar configuration and strong dipolar structure, it is an excellent group for mimicking characteristics of anomeric position in sialyl oxocarbenium transition-state, and plays a key role in high affinity of ST transition-state based inhibitors. Further applicability of this strategy to other glycosyltransferases is underway.

■ EXPERIMENTAL SECTION

General Information. All chemicals purchased were reagent grade and used without further purification unless otherwise stated. All reactions were carried out under anhydrous conditions using flame-dried glassware with freshly distilled solvents, unless otherwise noted. Reactions were monitored by TLC on silica gel 60 F_{254} precoated on aluminum plates (E. Merck). Spots were detected under UV (254 nm) light and/or by staining with acidic ceric ammonium molybdate. ^{1}H NMR, and ^{13}C NMR spectra were recorded on an Avance III Bruker-600 or a Varian INOVA-500 or an Advance DRX Bruker-400 spectrometer at 25 ^{o}C. ^{31}P NMR spectra were recorded on an Advance DRX Bruker-400 or a JEOL AL-300 spectrometer at 25 ^{o}C. Chemical shifts (in ppm) were calibrated with the solvent residual peak. Mass spectra were recorded by using a Waters Xevo G2 Q-TOF or a Bruker APEX IV 70e FT-MS spectrometer. Optical rotation was determined by a Rudolph Research Analytical Autopol IV polarimeter. Preparative HPLC was performed on Agela Venusil XBP preparative C-18 reversed-phase column (10 μm, 22 ×250 mm) with Gilson HPLC by the buffer system: MeCN/TEAB (0.05 M, pH 7.2−7.5). The purity of all the final compounds was determined to be ≥95% (see Table S2) by HPLC analysis, which was performed on an Agilent 1260 Infinity system equipped with VWD detector and the data were collected at 254 nm.

General Procedure for the Synthesis of 7. To a solution of DMSO (3.0 mmol, 3.0 equiv) in dried DCM (12 mL) at -78 °C was added oxalyl chloride (2.5 mmol, 2.5 equiv) dropwise and stirred for 15 min. Then a solution of alcohol **6** (1.0 mmol, 1.0 equiv) in DCM (2.0 mL) was added into above reaction mixture. After stirring for another 50 min at -78 °C, TEA (6.0 mmol, 6.0 equiv) was added over 5 min. Then the cooling bath was removed, and the reaction mixture was allowed to warm to r.t. and stirred for 0.5 h. The reaction mixture was diluted with DCM and washed with 1 M HCl and sat. aq. $NaHCO_3$ successively. The combined organic phases were dried with Na_2SO_4 and concentrated under reduced pressure to afford the crude intermediate aldehyde. To a solution of the intermediate aldehyde in dried DCM (10 mL) was added dibenzyl phosphonate (1.4 mmol, 1.4 equiv) and TEA (2.0 mmol, 2.0 equiv) successively. After stirring at r.t. for 2 h, the reaction mixture was concentrated under reduced pressure and purified by column chromatograph to afford **7** as colorless oil.

General Procedure for the Synthesis of 5. To a solution of **7** (0.2 mmol, 1.0 equiv) in DCM (6.0 mL) were added **8** (0.28 mmol, 1.4 equiv) and *H*-tetrazole (0.4 mmol, 2.0 equiv) under argon atmosphere. After stirring at r.t. for 1 h, TBHP (0.56 mmol, 5.5 M in decane, 2.8 equiv) was added and the reaction mixture was stirred for another 1 h. Then the reaction mixture was diluted with DCM, and washed with sat. aq. $NaHCO_3$. The resulting organic layer was dried over Na_2SO_4, filtered, concentrated to dryness to give crude product **9** as colorless oil. The crude product **9** could be used for deprotection direcly, or used after preliminary purification by column chromatography on silica gel. To the solution of crude **9** in DMF (2.0 mL) were added Pd/C (equal mass of **9**, 10%) in portions under argon atmosphere and then 1,4-cyclohexadiene (7.2 mmol, 60 equiv) was added. The reaction mixture was stirred at r.t. for 24 h. Then the Pd/C was removed by filtration and filtrate was concentrated. The residue was purified by column chromatography on RP-18 silica gel (H_2O → H_2O/MeOH 5:1) to give crude product **5**. The diastereoisomers were further separated by preparative RP-HPLC (the buffer system: MeCN/TEAB, 0.05 M, pH 7.2−7.5) and converted to the form of sodium salt

by ion-exchange resin (IR 120 Na^+) and lyophilized from water to give **5-*s*** and **5-*l*** as amorphous solids (*s* stands for the component with a shorter retention time while *l* stands for the component with a longer retention time).

Trisodium cytidin-5'-yl-(2-benzamido-1-phosphonato-ethyl)-phosphate (5a). 5a-*s*: $[\alpha]_D^{30}$ = +9.09 (c = 0.22, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.64 (d, J = 7.6 Hz, 1H), 7.59 (d, J = 7.4 Hz, 2H), 7.43 (t, J = 7.4 Hz, 1H), 7.33 (t, J = 7.6 Hz, 2H), 5.83 (d, J = 7.6 Hz, 1H), 5.59 (d, J = 3.5 Hz, 1H), 4.36 (qd, J = 10.0, 3.0 Hz, 1H), 4.10 (dd, J = 10.7, 4.5 Hz, 1H), 4.04 – 3.84 (m, 5H), 3.55 – 3.41 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 170.2, 165.4, 156.6, 141.3, 133.1, 132.4, 128.8, 127.0, 96.3, 89.6, 82.4 (d, J = 8.2 Hz), 74.4, 71.9 (dd, J = 155.0, 8.0 Hz), 68.9, 64.5 (d, J = 5.2 Hz), 41.9 (d, J = 3.8 Hz); ^{31}P NMR (121.5 MHz, D_2O) δ 14.6 (d, $J_{P,P}$ = 22.0 Hz), 1.1 (d, $J_{P,P}$ = 24.4 Hz); HRMS (ESI) calcd. for $C_{18}H_{23}N_4O_{12}P_2$ [M-H]$^-$ 549.0793, found 549.0797. **5a-*l***: $[\alpha]_D^{30}$ = -2.14 (c = 0.28, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.68 (d, J = 7.7 Hz, 1H), 7.62 (d, J = 7.6 Hz, 2H), 7.43 (t, J = 7.4 Hz, 1H), 7.34 (t, J = 7.6 Hz, 2H), 5.79 (d, J = 7.6 Hz, 1H), 5.61 (d, J = 3.1 Hz, 1H), 4.36 (qd, J = 10.0, 2.6 Hz, 1H), 4.14 (dd, J = 11.7, 4.0 Hz, 1H), 4.07 – 3.96 (m, 3H), 3.92 – 3.83 (dd, J = 13.6, 9.8 Hz, 2H), 3.58 – 3.44 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 170.1, 165.1, 156.2, 141.2, 133.0, 132.4, 128.8, 127.0, 96.1, 89.6, 82.3 (d, J = 9.0 Hz), 74.5, 72.1 (dd, J = 155.0, 6.0 Hz), 68.6, 63.8 (d, J = 5.0 Hz), 41.9 (d, J = 3.0 Hz); ^{31}P NMR (121.5 MHz, D_2O) δ 14.6 (d, $J_{P,P}$ = 22.0 Hz), 1.2 (d, $J_{P,P}$ = 24.3 Hz); HRMS (ESI) calcd. for $C_{18}H_{23}N_4O_{12}P_2$ [M-H]$^-$ 549.0793, found 549.0812.

Trisodium cytidin-5'-yl-(2-oxo-2-(phenylamino)-1-phosphonato-ethyl)-phosphate (5b). 5b-*s*: $[\alpha]_D^{30}$ = -8.00 (c = 0.35, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.65 (d, J = 8.0 Hz, 1H), 7.30 – 7.27 (m, 2H), 7.23 – 7.18 (m, 2H), 7.06 – 7.02 (m, 1H), 5.83 (d, J = 7.6 Hz, 1H), 5.66 (d, J = 3.6 Hz, 1H), 4.63 (dd, J = 10.4, 15.6 Hz, 1H), 4.13 (ddd, J = 2 .0, 4.4, 12 Hz, 1H), 4.11 – 4.00 (m, 3H), 3.92 (t, J = 4.0 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 168.8, 165.1, 156.2 (d, J = 3.0 Hz), 141.3, 136.5, 129.1, 125.6, 121.6, 96.4, 89.5, 82.5 (d, J = 8.7 Hz), 75.1 (d, J = 141, 7.8 Hz), 74.3, 69.0, 64.9 (d, J = 5.3 Hz); ^{31}P NMR (162 MHz, D_2O) δ 9.96 (d, $J_{P,P}$ = 29.2 Hz), -0.12 (d, $J_{P,P}$ = 27.5 Hz); HRMS (ESI) calcd. for $C_{17}H_{21}N_4O_{12}P_2$ [M-H]$^-$ 535.0637, found 535.0657. **5b-*l***: $[\alpha]_D^{30}$ = +24.21 (c = 0.19, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.63 (d, J = 7.6 Hz, 1H), 7.25 – 7.23 (m, 2H), 7.17 – 7.12 (m, 2H), 7.00 – 6.96 (m, 1H), 5.78 (d, J = 7.6 Hz, 1H), 5.56 (d, J = 3.2 Hz, 1H), 4.59 (dd, J = 10.4, 15.6 Hz, 1H), 4.19 (dd, J = 3.2, 11.2 Hz, 1H), 4.07 – 3.96 (m, 3H), 3.84 (t, J = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 169.7, 166.4, 157.7, 141.5, 137.3, 129.7, 126.1, 121.6, 97.1, 90.3, 82.8 (d, J = 9.4 Hz), 76.0 (dd, J = 139, 8.1 Hz), 75.1, 69.1, 64.7 (d, J = 4.4 Hz); ^{31}P NMR (162 MHz, D_2O) δ 8.75 (d, $J_{P,P}$ = 27.5 Hz), 0.31 (d, $J_{P,P}$ = 27.5 Hz); HRMS (ESI) calcd. for $C_{17}H_{21}N_4O_{12}P_2$ [M-H]$^-$ 535.0637, found 535.0649.

Trisodium cytidin-5'-yl-(2-oxo-2-(pyrrolidin-1-yl)-1-phosphonato-ethyl)-phosphate (5c). 5c-*s*: $[\alpha]_D^{30}$ = -3.39 (c = 0.35, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.80 (d, J = 7.6 Hz, 1H), 5.99 (d, J = 7.6 Hz, 1H), 5.82 (d, J = 4.3 Hz, 1H), 4.84 (dd, J = 15.5, 9.7 Hz, 1H), 4.12 – 4.03 (m, 4H), 3.93 – 3.85 (m, 1H), 3.60 – 3.52 (m, 1H), 3.51 – 3.43 (m, 1H), 3.33 – 3.18 (m, 2H), 1.86 – 1.60 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 167.9, 165.2, 156.4, 141.9, 96.4, 89.5, 82.9 (d, J = 8.8 Hz), 74.3, 72.2 (dd, J = 145, 8.0 Hz), 69.5, 64.7 (d, J = 5.1 Hz), 47.5, 46.8, 25.5, 23.6; ^{31}P (121.5 MHz, D_2O) δ 8.81 (d, $J_{P,P}$ = 31.7 Hz), -0.61 (d, $J_{P,P}$ = 31.7 Hz); HRMS (ESI) calcd. for $C_{15}H_{23}N_4O_{12}P_2$ [M-H]$^-$ 513.0793, found 513.0793. **5c-*l***: $[\alpha]_D^{30}$ = +20.65 (c = 0.31, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.81 (d, J = 7.6 Hz, 1H), 5.99 (d, J = 7.6 Hz, 1H), 5.83 (d, J = 3.6 Hz, 1H), 4.80 (dd, J = 15.3, 9.9 Hz, 1H), 4.17 – 4.09 (m, 2H), 4.08 – 4.01 (m, 2H), 4.00 – 3.94 (m, 1H), 3.61 – 3.51 (m, 1H), 3.46 – 3.36 (m, 1H), 3.33 – 3.17 (m, 2H), 1.84 – 1.53 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 168.1, 166.1, 157.6, 141.5, 96.5, 89.4, 82.4 (d, J = 9.0 Hz), 74.2, 72.3 (dd, J = 143.0, 7.0 Hz), 68.9, 64.0 (d, J = 5.0 Hz), 47.4, 46.8, 25.5, 23.5; ^{31}P (121.5 MHz, D_2O) δ 8.52 (d, $J_{P,P}$ = 31.7 Hz), -0.46 (d, $J_{P,P}$ = 29.3 Hz); HRMS (ESI) calcd. for $C_{15}H_{23}N_4O_{12}P_2$ [M-H]$^-$ 513.0793, found 513.0804.

Trisodium cytidin-5'-yl-((2-oxo-2-(piperidin-1-yl)-1-phosphonato-ethyl)-phosphate (5d). 5d-*s*: $[\alpha]_D^{30}$ = +6.40 (c = 0.25, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.81 (d, J = 7.6 Hz, 1H), 5.99 (d, J = 7.5 Hz, 1H), 5.82 (d, J = 3.9 Hz, 1H), 5.05 (dd, J = 15.9, 10.1 Hz, 1H), 4.20 – 4.01 (m, 4H), 3.95 (dd, J = 9.8, 4.4 Hz, 1H), 3.50 – 3.55 (m, 2H), 3.43 – 3.29 (m, 1H), 3.24 – 3.07 (m, 1H), 1.55 – 1.22 (m, 6H); ^{13}C NMR (125 MHz, D2O) δ 168.5, 166.7, 158.1, 142.3, 97.2, 90.2, 83.4 (d, J = 8.63 Hz), 74.9, 72.3 (d, J = 152 Hz), 70.0, 65.3, 48.6, 44.9, 26.6, 26.0, 24.5; ^{31}P NMR (121.5 MHz, D_2O) δ 9.90 (d, $J_{P,P}$ = 31.7 Hz), 0.70 (d, $J_{P,P}$ = 29.3 Hz); HRMS (ESI) calcd. for $C_{16}H_{25}N_4O_{12}P_2$ [M-H]$^-$ 527.0950, found 527.0962. **5d-*l***: $[\alpha]_D^{30}$ = +7.14 (c = 0.28, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.81 (d, J = 7.6 Hz, 1H), 5.99 (d, J = 7.4 Hz, 1H), 5.83 (d, J = 2.9 Hz, 1H), 5.01 (dd, J = 16.0, 10.1 Hz, 1H), 4.20 – 4.12 (m, 2H), 4.06 – 3.90 (m , 3H), 3.55-3.49 (m, 2H), 3.38 – 3.26 (m, 1H), 3.21 – 3.09 (m, 1H), 1.55 – 1.29 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 167.7, 164.6, 155.6, 142.2, 96.4, 89.5, 82.7 (d, J = 9.0 Hz), 74.2, 71.6 (dd, J = 144, 7.0 Hz), 69.0, 64.2 (d, J = 5.0 Hz), 47.9, 44.2, 25.8, 25.2, 23.8; ^{31}P NMR (121.5 MHz, D_2O) δ 9.82 (d, $J_{P,P}$ = 26.9 Hz), 0.80 (d, $J_{P,P}$ = 29.3 Hz); HRMS (ESI) calcd. for $C_{16}H_{25}N_4O_{12}P_2$ [M-H]$^-$ 527.0950, found 527.0972.

Trisodium cytidin-5'-yl-(2-(2,5-dioxopyrrolidin-1-yl)-1-phosphonato-ethyl)-phosphate (5e). 5e-*s*: $[\alpha]_D^{30}$ = +18.93 (c = 0.34, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.82 (d, J = 8.0 Hz ,1H), 6.02 (d, J = 7.6 Hz, 1H), 5.80 (d, J = 3.1 Hz, 1H), 4.36 (qd, J = 10.9, 2.6 Hz, 1H), 4.18 – 4.11 (m, 2H), 4.07 (td, J = 12.3, 7.3, 2.7 Hz, 2H), 3.93 (ddd, J = 12.3, 5.7, 3.7 Hz, 1H), 3.82 (ddd, J = 14.3, 11.2, 6.5 Hz, 1H), 3.59 (d, J = 14.2 Hz, 1H), 2.62 (s, 4H); ^{13}C NMR (100 MHz, D_2O) δ 181.5, 164.9, 156.0, 142.1, 96.3, 89.5, 83.0 (d, J = 9.0 Hz), 74.2, 70.7 (dd, J = 155.0, 8.0 Hz), 69.3, 64.5 (d, J = 5.0 Hz), 40.3 (d, J = 11.0 Hz), 28.1; ^{31}P NMR (121.5 MHz, D_2O) δ 13.94 (d, $J_{P,P}$ = 17.0 Hz), 1.27 (d, $J_{P,P}$ = 17.1 Hz); HRMS (ESI) calcd. for $C_{15}H_{21}N_4O_{13}P_2$ [M-H]$^-$ 527.0586, found 527.0588. **5e-*l***: $[\alpha]_D^{30}$ = -2.05 (c = 0.29, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.84 (d, J = 7.7 Hz, 1H), 6.06 (d, 1H), 5.81 (d, J = 3.6 Hz, 1H), 4.33 (qd, J = 10.9, 2.5 Hz, 1H), 4.20 – 4.12 (m, 2H), 4.07 (td, J = 4.7, 2.3 Hz, 1H), 4.05 – 3.95 (m, 2H), 3.81 (ddd, J = 14.3, 11.1, 6.2 Hz, 1H), 3.59 (d, J = 14.1 Hz, 1H), 2.61 (s, 4H); ^{13}C NMR (100 MHz, D_2O) δ 181.5, 163.8, 154.6, 142.4, 96.2, 89.4, 83.0 (d, J = 9.0 Hz), 74.3, 70.2 (dd, J = 154.0, 7.0 Hz), 69.1, 64.2 (d, J = 5.0 Hz), 40.4 (d, J = 10.0 Hz), 28.1; ^{31}P NMR (121.5 MHz, D_2O) δ 13.87 (d, $J_{P,P}$ = 17.0 Hz), 1.18 (d, $J_{P,P}$ = 19.6 Hz); HRMS (ESI) calcd. for $C_{15}H_{21}N_4O_{13}P_2$ [M-H]$^-$ 527.0586, found 527.0598.

Trisodium cytidin-5'-yl-(2-(1,3-dioxoisoindolin-2-yl)-1-phosphonato-ethyl)-phosphate (5f). 5f-*s*: $[\alpha]_D^{30}$ = -3.09 (c = 0.30, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.69 – 7.60 (m, 5H), 5.77 (d, J = 7.6 Hz, 1H), 5.59 (d, J = 3.0 Hz, 1H), 4.51 (dd, J = 19.5, 8.7 Hz, 1H), 4.06 – 3.72 (m, 7H); ^{13}C NMR (100 MHz, D_2O) δ 171.1, 165.8, 156.9, 142.2, 135.3, 132.2, 124.1, 96.8, 90.3, 83.1 (d, J = 7.6 Hz), 75.1, 72.2 (dd, J = 152.1, 7.1 Hz),

69.4, 65.1 (d, J = 4.8 Hz), 40.2; ^{31}P NMR (162 MHz, D_2O) δ 13.36 (d, $J_{P,P}$ = 14.5 Hz), 0.21 (d, $J_{P,P}$ = 14.9 Hz); HRMS (ESI) calcd. for $C_{19}H_{21}N_4O_{13}P_2$ $[M-H]^-$ 575.0586, found 575.0599. **5f-*l***: $[\alpha]_D^{30}$ = +8.00 (c = 0.35, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.74 – 7.53 (m, 4H), 7.48 (d, J = 7.6 Hz, 1H), 5.64 (d, J = 7.5 Hz, 1H), 5.42 (d, J = 2.3 Hz, 1H), 4.53 (dd, J = 19.6, 9.6 Hz, 1H), 4.08 – 3.74 (m, 7H); ^{13}C NMR (100 MHz, D_2O) δ 171.2, 166.5, 157.6, 141.4, 135.2, 132.1, 124.1, 96.9, 90.1, 82.2 (d, J = 9.1 Hz), 75.2, 73.0 (d, J = 159.8 Hz), 68.6, 63.9, 40.7; ^{31}P NMR (162 MHz, D_2O) δ 13.40 (d, $J_{P,P}$ = 12.0 Hz), -0.61 (d, $J_{P,P}$ = 12.5 Hz); HRMS (ESI) calcd. for $C_{19}H_{21}N_4O_{13}P_2$ $[M-H]^-$ 575.0586, found 575.0603.

RP-HPLC Based Sialyltransferase Inhibition Assay. The assay was performed according to the UV/RP-HPLC method developed by Schmidt group.[35] Recombinant hST6Gal-I was purchased from R&D (aa 44-406, 7620-GT), CMP-Neu5Ac was available from Sigma and *p*-nitrophenyl LacNAc was purchased from Toronto Research Chemicals Inc. Each vial of enzyme was diluted with the storage buffer (25 mM Tris, 150 mM NaCl and 50% (v/v) Glycerol, pH 7.5) into 20 ng/μL, split into small portions and stored at -80 °C before use. The enzyme-catalyzed reaction was performed in the assay buffer (0.5% (w/v) Triton X-100, 2 mM $MnCl_2$, 25 mM Mes buffer, pH 6.5).[42] RP-HPLC analysis was performed with Agilent 1260 Infinity system and data were collected at 300 nm. Column: Agilent ZORBAX SB-C18 Stable Bond Analytical 4.6 × 250 mm, 5-Micron. Eluent: 0.1% FA (pH = 2.66): CH_3CN = 85/15 at 1.0 mL/min. Acetonitrile was HPLC grade, water was Milli Q quality, solutions were filtered through 0.45 μm membranes. In all assays, the consumption of CMP-Neu5Ac was limited less than 15% in order to get reliable initial rates.

Determination of the CMP-Neu5Ac K_m. The donor CMP-Neu5Ac was diluted to 1000, 500, 250, 125, 50 μM. The enzyme was diluted to 13.6 ng/12 μL with the assay buffer. To a 0.6 mL microtube were added 6.0 μL of donor, 6.0 μL of 5 mM acceptor, 12.0 μL of enzyme and 6.0 μL of assay buffer (total volume: 30 μL). Then the mixtures were mixed and incubated in water bath at 37 °C for 30 min. The reactions were quenched by adding 200 μL of deionized water and being heated at 95 °C for 2 min. Then the samples were centrifuged at 4 °C with 14000 rpm for 20 min and analyzed with RP-HPLC. The K_m was obtained with non-linear regression analysis computed by using GraphPad Prism 6.

Determination of the inhibition percent at 1 μM. The enzyme was diluted to 80 ng/12 μL with the assay buffer. To a 0.6 mL microtube were added 6.0 μL of 5 μM inhibitors, 6.0 μL of 250 μM donor, 6.0 μL of 5 mM acceptor and 12.0 μL of enzyme (total volume: 30 μL). Then the mixtures were mixed and incubated in water bath at 37 °C for 30 min. The reactions were quenched by adding 200 μL of deionized water and being heated at 95 °C for 2 min. Then the samples were centrifuged at 4 °C with 14000 rpm for 20 min and analyzed with RP-HPLC. The inhibition data were average from a duplicated enzyme inhibition assay.

Determination of the inhibitor K_i. The donor CMP-Neu5Ac was diluted to 1000, 500, 250, 125, 50 μM. The inhibitors were diluted to three different concentrations (Best: 4 × K_i to 0.4 × K_i). The enzyme was diluted to 20 ng/12 μL with the assay buffer. To a 0.6 mL microtube were added 6.0 μL of inhibitor, 6.0 μL of donor, 6.0 μL of 5 mM acceptor and 12.0 μL of enzyme (total volume: 30 μL). Then the mixtures were mixed and incubated in water bath at 37 °C for 30 min. The reactions were quenched by adding 200 μL of deionized water and being heated at 95 °C for 2 min. Then the samples were centrifuged at 4 °C with 14000 rpm for 20 min and analyzed with RP-HPLC. The K_i was obtained with non-linear regression analysis computed by using GraphPad Prism 6.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Procedures for preparing compounds **6**, **7**, and **8**, HPLC purity of the target compounds, details of biological assay, and NMR spectral data (PDF)

Molecular formula strings (CSV)

AUTHOR INFORMATION

Corresponding Author

*Phone: +86-10-82805736; e-mail: xinshan@bjmu.edu.cn.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the grants (2012CB822100, 2013CB910700) from the Ministry of Science and Technology of China, and the National Natural Science Foundation of China (21232002, 81473084).

ABBREVIATIONS USED

CMP, cytidine monophosphate; hST6Gal-I, human β-galactoside α-2,6-sialyltransferase I; Neu5Ac, *N*-acetylneuraminic acid; RP, reversed phase; ST, sialyltransferase; TEAB, triethylammonium bicarbonate.

REFERENCES

(1) Glavey, S. V.; Huynh, D.; Reagan, M. R.; Manier, S.; Moschetta, M.; Kawano, Y.; Roccaro, A. M.; Ghobrial, I. M.; Joshi, L.; O' Dwyer, M. E. The cancer glycome: carbohydrates as mediators of metastasis. *Blood Rev.* **2015**, *29*, 269-279.

(2) Vasconcelos-dos-santos, A.; Oliveira, I. A.; Lucena, M. C.; Mantuano, N. R.; Whelan, S. A.; Dias, W. B.; Todeschini, A. R. Biosynthetic machinery involved in aberrant glycosylation: promising targets for developing of drugs against cancer. *Front. Oncol.* **2015**, *5*, 138.

(3) Sun, X. L. Glycosyltransferases as potential drug targets. *Med. Chem.* **2013**, *3*, e106.

(4) Lairson, L. L.; Henrissat, B.; Davies, G. J.; Withers, S. G. Glycosyltransferases: structures, functions, and mechanisms. *Annu. Rev. Biochem.* **2008**, *77*, 521-555.

(5) Dwek, R. A.; Butters, T. D.; Platt, F. M.; Zitzmann, N. Targeting glycosylation as a therapeutic approach. *Nat. Rev. Drug Discovery* **2002**, *1*, 65-75.

(6) Fuster, M. M.; Esko, J. D. The sweet and sour of cancer: glycans as novel therapeutic targets. *Nat. Rev. Cancer* **2005**, *5*, 526-542.

(7) Gloster, T. M.; David, J. V. Developing inhibitors of glycan processing enzymes as tools for enabling glycobiology. *Nat. Chem. Biol.* **2012**, *8*, 683-694.

(8) Whitworth, G. E.; Macauley, M. S.; Stubbs, K. A.; Dennis, R. J.; Taylor, E. J.; Davies, G. J.; Greig, I. R.; Vocadlo, D. J. Analysis of PUGNAc and NAG-thiazoline as transition state analogues for human O-GlcNAcase: mechanistic and structural insights into inhibitor selectivity and transition state poise. *J. Am. Chem. Soc.* **2007**, *129*, 635-644.

(9) Schramm, V. L. Transition states, analogues, and drug development. *ACS Chem. Biol.* **2013**, *8*, 71-81.

(10) Adamson, C.; Pengelly, R. J.; Abadi, S. S. K.; Chakladar, S., Draper, J., Britton, R., Gloster, T. M.; Bennet, A. J. Structural snapshots for mechanism - based inactivation of a glycoside hydrolase by

cyclopropyl carbasugars. *Angew. Chem. Int. Ed.* **2016**, *55*, 14978-14982.

(11) Gloster, T. M.; Davies, G. J. Glycosidase inhibition: assessing mimicry of the transition state. *Org. Biomol. Chem.* **2010**, *8*, 305-320.

(12) Sun, H.; Yang, J.; Amaral, K. E.; Horenstein, B. A. Synthesis of a new transition-state analog of the sialyl donor. Inhibition of sialyltransferases. *Tetrahedron Lett.* **2001**, *42*, 2451-2453.

(13) Müller, B.; Schaub, C.; Schmidt, R. R. Efficient sialyltransferase inhibitors based on transition-state analogues of the sialyl donor. *Angew. Chem. Int. Ed.* **1998**, *37*, 2893-2897.

(14) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. Recent developments of transition-state analogue glycosidase inhibitors of non-natural product origin. *Chem. Rev.* **2002**, *102*, 515-554.

(15) Pauling, L. Molecular architecture and biological reactions. *Chem. Eng. News* **1946**, *24*, 1375-1377.

(16) Dall' Olio, F.; Malagolini, N.; Trinchera, M.; Chiricolo, M. Sialosignaling: sialyltransferases as engines of self-fueling loops in cancer progression. *Biochim. Biophys. Acta, Gen. Subj.* **2014**, *1840*, 2752-2764.

(17) Varki, A.; Gagneux, P. Multifarious roles of sialic acids in immunity. *Ann. N.Y. Acad. Sci.* **2012**, *1253*, 16-36.

(18) Harduin-Lepers, A.; Vallejo-Ruiz, V.; Krzewinski-Recchi, M.; Samyn-Petit, B.; Julien, S.; Delannoy, P. The human sialyltransferase family. *Biochimie* **2001**, *83*, 727-737.

(19) Chiang, C.; Wang, C.; Chang, H.; More, S. V.; Li, W.; Hung, W. A novel sialyltransferase inhibitor AL10 suppresses invasion and metastasis of lung cancer cells by inhibiting integrin-mediated signaling. *J. Cell. Physiol*. **2010**, *223*, 492-499.

(20) Wang, X.; Zhang, L.-H.; Ye, X.-S. Recent development in the design of sialyltransferase inhibitors. *Med. Res. Rev.* **2003**, *23*, 32-47.

(21) Li, W.; Niu, Y.; Xiong, D.-C.; Cao, X.; Ye, X.-S. Highly substituted cyclopentane-CMP conjugates as potent sialyltransferase inhibitors. *J. Med. Chem.* **2015**, *58*, 7972-7990.

(22) Guo, J.; He, Y.; Ye, X.-S. Design and discovery of sialyltransferase inhibitors. *Prog. Chem.* **2016**, *28*, 1712-1720.

(23) Bruner, M.; Horenstein, B. A. Isotope trapping and kinetic isotope effect studies of rat liver α-(2->6)-sialyltransferase. *Biochemistry* **1998**, *37*, 289-297.

(24) Horenstein, B. A. Quantum mechanical analysis of an α-carboxylate-substituted oxocarbenium ion. isotope effects for formation of the sialyl cation and the origin of an unusually large secondary ^{14}C isotope effect. *J. Am. Chem. Soc.* **1997**, *119*, 1101-1107.

(25) Audry, M.; Jeanneau, C.; Imberty, A.; Harduin-Lepers, A.; Delannoy, P.; Breton, C. Current trends in the structure-activity relationships of sialyltransferases. *Glycobiology* **2011**, *21*, 716-726.

(26) Hamada, Y.; Yusuke, K.; Masanori, T. Quantum mechanics/molecular mechanics study of the sialyltransferase reaction mechanism. *Biochemistry* **2016**, *55*, 5764-5771.

(27) Schmölzer, K.; Luley-Goedl, C.; Czabany, T.; Ribitsch, D.; Schwab, H.; Weber, H.; Nidetzky, B. Mechanistic study of CMP-Neu5Ac hydrolysis by α2,3-sialyltransferase from *Pasteurella dagmatis*. *FEBS Lett.* **2014**, *588*, 2978-2984.

(28) Ni, L.; Chokhawala, H. A.; Cao, H.; Henning, R.; Ng, L.; Huang, S.; Yu, H.; Chen, X.; Fisher, A. J. Crystal structures of *Pasteurella multocida* sialyltransferase complexes with acceptor and donor analogues reveal substrate binding sites and catalytic mechanism. *Biochemistry* **2007**, *46*, 6288-6298.

(29) Schwörer, R.; Schmidt, R. R. Efficient sialyltransferase inhibitors based on glycosides of *N*-acetylglucosamine. *J. Am. Chem. Soc.* **2002**, *124*, 1632-1637.

(30) Popov, E. M.; Zheltova, V. N. Electronic structure and properties of the peptide group. *J. Mol. Struct.* **1971**, *10*, 221-230.

(31) Nishimura, Y.; Adachi, H.; Satoh, T.; Shitara, E.; Nakamura, H.; Kojima, F.; Takeuchi, T. All eight stereoisomeric D-glyconic-δ-lactams: synthesis, conformational analysis, and evaluation as glycosidase inhibitors. *J. Org. Chem.* **2000**, *65*, 4871-4882.

(32) Panday, N.; Meyyappan, M.; Vasella, A. A comparison of glucose- and glucosamine-related inhibitors: probing the interaction of the 2-hydroxy group with retaining β-glucosidases. *Helv. Chim. Acta* **2000**, *83*, 513-538.

(33) Williams, S. J.; Notenboom, V.; Wicki, J.; Rose, D. R.; Withers, S. G. A new, simple, high-affinity glycosidase inhibitor: analysis of binding through X-ray crystallography, mutagenesis, and kinetic analysis. *J. Am. Chem. Soc.* **2000**, *122*, 4229-4230.

(34) Amann, F.; Schaub, C.; Müller, B.; Schmidt, R. R. New potent sialyltransferase inhibitors-synthesis of donor and of transition-state analogues of sialyl donor CMP-Neu5Ac. *Chem. Eur. J.* **1998**, *4*, 1106-1115.

(35) Skropeta, D.; Schworer, R.; Haag, T.; Schmidt, R. R. Asymmetric synthesis and affinity of potent sialyltransferase inhibitors based on transition-state analogues. *Glycoconjugate J.* **2004**, *21*, 205-219.

(36) Johnson, D. C.; Widlanski, T. S. Facile deprotection of *O*-Cbz-protected nucleosides by hydrogenolysis: an alternative to *O*-benzyl ether-protected nucleosides. *Org. Lett.* **2004**, *6*, 4643-4646.

(37) Schaub, C.; Müller, B.; Schmidt, R. R. New sialyltransferase inhibitors based on CMP-quinic acid: development of a new sialyltransferase assay. *Glycoconjugate J.* **1998**, *15*, 345-354.

(38) Nasyrov, D. M.; Vereshchagin, A. N. Polarity and polarizability of the amide group. *Russ. Chem. Bull.* **1981**, *30*, 414-417.

(39) Montgomery, A.; Szabo, R.; Skropeta, D.; Yu, H. Computational characterisation of the interactions between human ST6Gal I and transition-state analogue inhibitors: insights for inhibitor design. *J. Mol. Recognit.* **2016**, *29*, 210-222.

(40) Bruner, M.; Horenstein, B. A. Use of an altered sugar-nucleotide to unmask the transition state for α(2->6) sialyltransferase. *Biochemistry* **2000**, *39*, 2261-2268.

(41) Kuhn, B.; Benz, J.; Greif, M.; Engel, A. M.; Sobek, H.; Rudolph, M. G. The structure of human α-2,6-sialyltransferase reveals the binding mode of complex glycans. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2013**, *69*, 1826-1838.

(42) Hidari, K. I.; Oyama, K.; Ito, G.; Nakayama, M.; Inai, M.; Goto, S.; Kanai, Y.; Watanabe, K.; Yoshida, K.; Furuta, T.; Kan, T.; Suzuki, T. Identification and characterization of flavonoids as sialyltransferase inhibitors. *Biochem. Biophys. Res. Commun.* **2009**, *382*, 609-613.

Table of Contents (TOC)