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Bisubstrate-type inhibitor of sialyltransferases

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Abstract—A convergent strategy for the construction of bisubstrate-type sialyltransferase inhibitor (**1**) was developed. It consists of consecutive coupling of three components (*N*-acetyllactosamine, sialic acid, and CMP), followed by oxidation and deprotection. As expected, compound **1** showed potent inhibitory activities toward both 2,3-(*N*)- and 2,6-(*N*)-sialyltransferase.

Structures of glycoconjugate glycan chains are principally controlled by the expression levels, activities, and substrate specificities of glycosyltransferases (GTs). Since GTs usually have narrow specificity for donor and acceptor substrates,[1] both of them are inferred to be recognized strictly in the binding pockets. Therefore, properly designed bisubstrate-like derivatives are expected to be specific and potent inhibitors.[2] Such compounds would be valuable in dissecting the active site structure of GTs and their mechanism of action, which are largely unexplored.[3]

Sialic acid (*N*-acetylneuraminic acid, NeuAc) is an important constituent of various glycoconjugates, including glycosphingolipids as well as *O*- and *N*-linked glycoproteins.[4] It typically resides at the non-reducing end of glycan chains and plays major roles in recognition events on cell surface.[5] Sialyltransferases (STs), GTs responsible for the biosynthesis of NeuAc containing glycans, use cytidine monophosphate–sialic acid (CMP–NeuAc) as the common donor substrate. Although STs have been subjects of intense research in multiple respects, their inhibitor with bisubstrate-type structure is yet to be developed.[6] We would like to report herein the first example of such compound (**1**) and preliminary results on its activities toward α-2,6-(*N*)- and α-2,3-(*N*)-STs (ST6N and ST3N, respectively).[7]

Based upon canonical transition state of ST catalyzed reaction (Fig. 1B), general structure of the inhibitor was designed as **1n** (Fig. 1A). Since NeuAc has no substituent at C-3, it was assumed that this position could be exploited as a scaffold to connect donor (CMP–NeuAc) and acceptor (*N*-acetyllactosamine, LacNAc) components, without risking functional groups responsible for enzyme–substrate interaction. In this report, synthesis of the prototypical compound **1** having single methylene group ($n=1$) is described.

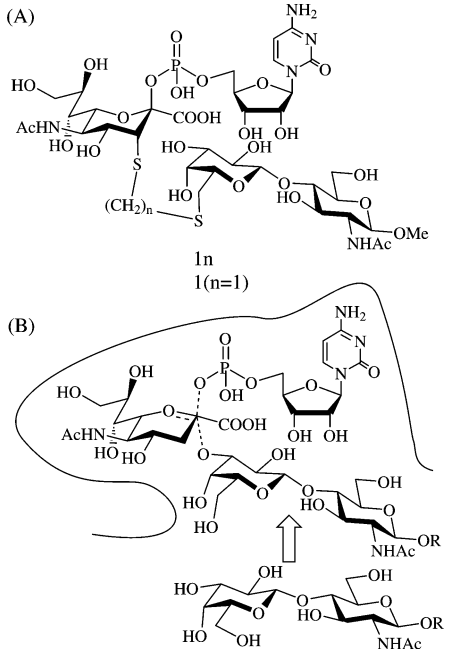

Figure 1. Bisubstrate-type inhibitors (A) and canonical transition state (B) of sialyltransferase catalyzed reaction.

Keywords: sialyltransferase; inhibitor; bisubstrate.

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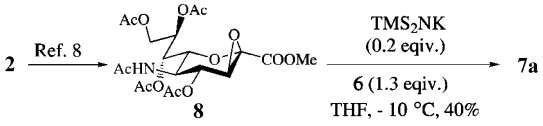
Our strategy for the convergent synthesis of **1** involves two-step assembly of three components (**2**,[8,9] **3**, and **5**[10]), followed by oxidation and deprotection as shown in Scheme 1. With this design, a series of compounds **1n** can be systematically prepared from **3n** having different length of the linker (n).

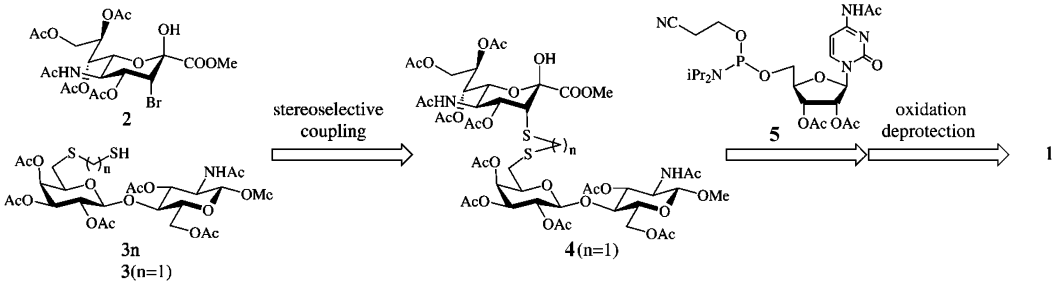
The most crucial in this strategy is the thioether linkage formation between **2** and **3** with thermodynamically unfavorable axial orientation.[9] Conditions for this transformation was first optimized using **6**[11] as a model substrate. (Table 1) Somewhat unexpectedly, the stereochemical outcome of the reaction was sensitive to the nature of the base employed and the temperature. As shown in Table 1, the best result was obtained with TMS_2NK in THF.[12] Exclusive formation of the α-isomer **7a** was observed, when the reaction was conducted below −10°C. This stereoselectivity may well be rationalized by the intermediacy of 2,3-anhydro derivative **8**[8]. As a matter of fact, treatment of separately prepared **8** afforded **7a** under identical conditions. (Scheme 2)

These conditions were applied to the preparation of **4**, through coupling of **2** with **3**. As a precursor of **3**, thioacetate **9** was prepared from **10**[13], via *S*-chloromethylation followed by substitution with KSAc. Since thiol **3** turned out to be labile, corresponding thiolate was generated from **9** with KOMe and immediately quenched with **2** to afford **4**. Resultant **4** was transformed to **1** according to Kajihara's protocol developed for the preparation of CMP–NeuAc.[10] Namely, the incorporation of CMP to the C-2 hemiketal was effected by phosphoramidite **5**. Subsequent oxidation with *t*-butyl hydroperoxide (TBHP) and removal of the cyanoethyl group was followed by deacetylation to afford **11**. Finally, alkaline hydrolysis under careful monitoring with NMR afforded **1**[14], having C_1 linker between C-6′ of LacNAc and C-3 of NeuAc (Scheme 3). Noticeably, the hydrolytic stability of the phosphodiester linkage of methyl ester **11** was much higher than that of free acid **1**,[15a] underscoring the proposed oxocarbenium ion stabilizing effect of sialic acid carboxylate anion.[15b]

The inhibitory activities of **1** toward ST6N and ST3N (rat recombinant)[16] were then evaluated. Assays were performed using CMP-NeuAc (**12**, 200 μM) and *p*-nitrophenyl LacNAc (**13**, 1.00 mM) as a donor and an acceptor substrate, respectively,[17] in the presence of

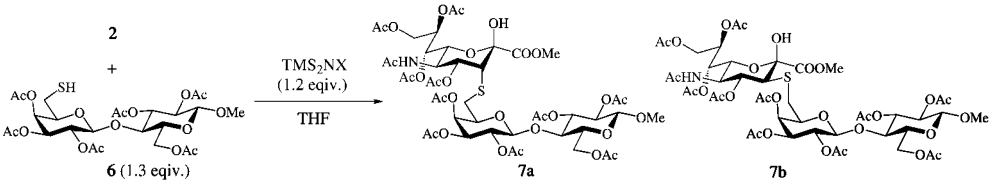


Scheme 2.



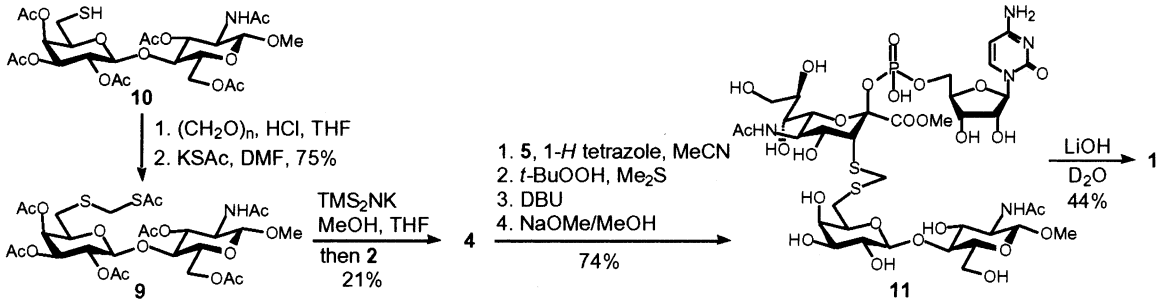
Scheme 1.

Table 1. Optimize the reaction condition of the coupling **2** with thioalcoxide of **6**



Entry	X[a]	Temperature[a] (°C)	Time (h)	Ratio **7a**:**7b**	Yield[b,c] (%)
1	Li	−78 to rt	24	0:1	21
2	Na	−78 to 12	3	7:1	62
3	K	−78 to 5	2	7:1	61
4	K	−78 to −10	3	1:0	75

[a] Base was added to the THF solution of **2** and **6** at −78°C, then the mixture was warmed up gradually.
[b] Total yield of **7a** and **7b** insulated by column chromatography.
[c] In entry 1 and 2, *S*-acetylated and de-*O*-acetylated byproducts were identified.



Scheme 3.

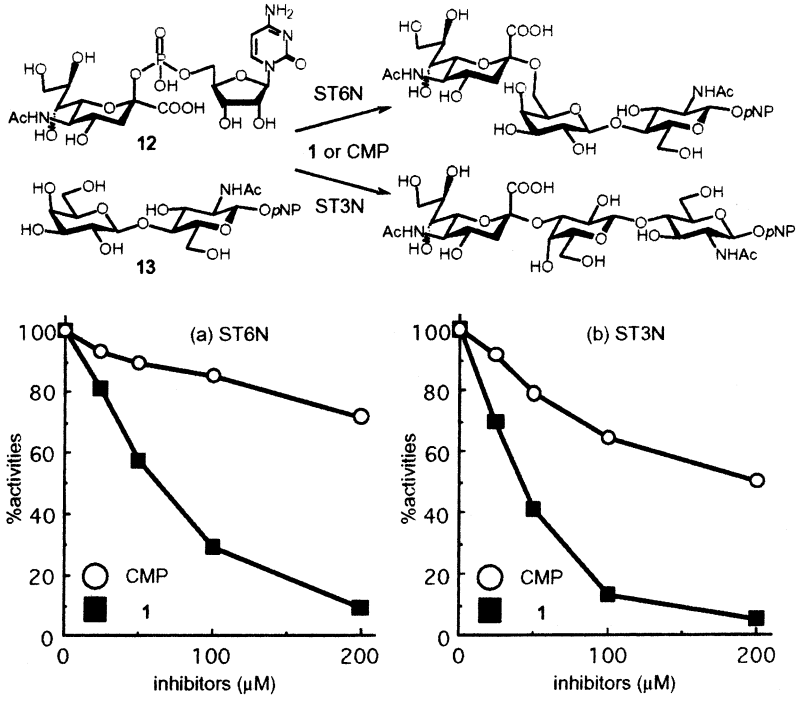


Figure 2. Inhibition of (a) 2,6-sialyltransferase and (b) 2,3-sialyltransferase by **1** and CMP, in the presence of inhibitors (0, 25, 50, 100, 200 μM), CMP-NeuAc **12** (200 μM), and *p*NP-LacNAc **13** (1.0 mM).

varying concentration (0–200 μM) of inhibitors (**1** and CMP) and the results are summarized in Fig. 2.

For both ST6N and ST3N, K_i values of **1** were 10 μM against donor and 13 μM against acceptor.[18] Referring reported K_m values[7b] of CMP–NeuAc and LacNAc (42.7 μM and 2.38 mM for ST6N, and 74.1 μM and 2.63 mM for ST3N), affinities of **1** toward enzyme binding pockets were estimated to be 4- and 130-fold higher for ST6N, and 7- and 200-fold higher for ST3N, compared to donor and acceptor substrates, respectively.

In summary, we have developed a synthetic strategy to bisubstrate-type STs inhibitor **1**. Since it employs technically facile bromide–thiolate coupling as the key reaction, systematic preparation of bisubstrate analogues **1n** with variable linker length should be possible. Since crystal structure of STs has not been reported, these compounds would be valuable exploring the active site structure and mechanism of action[19] of this class of enzymes. Further study along this line will be reported in due course.

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