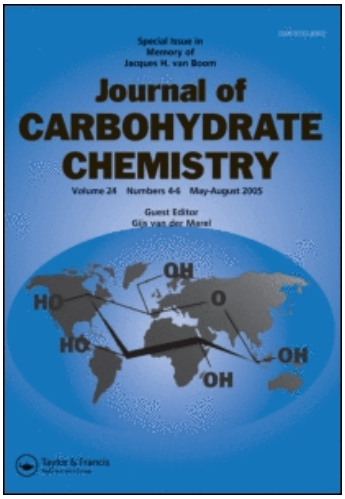

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Synthesis of Methyl 6'-Deoxy- and 6'-Thiolactosaminides and Their Inhibitory Activity Toward CMP-NeuNAc:D-galactoside-(2⟶6)-α-D-sialyltransferase

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COMMUNICATION

SYNTHESIS OF METHYL 6'-DEOXY- AND 6'-THIO-LACTOSAMINIDES AND THEIR INHIBITORY ACTIVITY TOWARD CMP-NEUNAC:D-GALACTOSIDE-(2→6)-α-D-SIALYLTRANSFERASE

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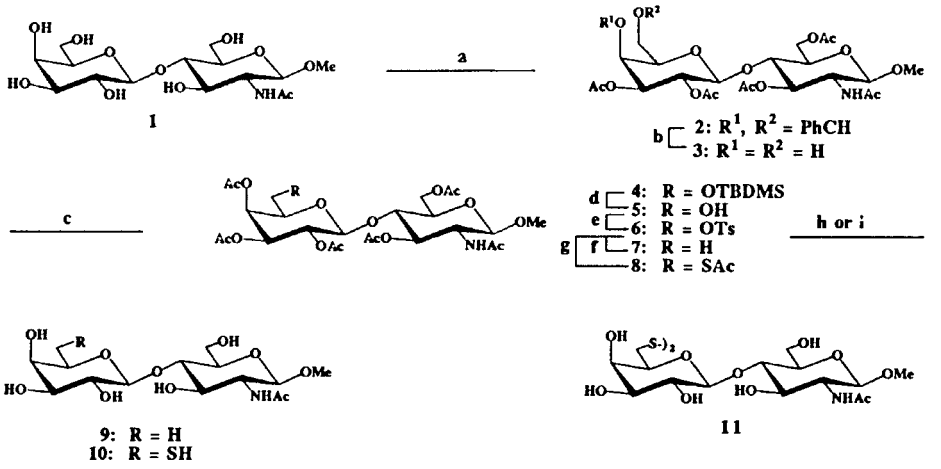
Specific inhibitors of glycosyltransferases have become of interest[1] not only for investigation of carbohydrate-participating cell-surface phenomena but also for practical use such as chemotherapeutic reagents. Glycosyltransferases catalyze the transfer of glycosyl moieties from nucleotide donors to oligosaccharide acceptors. Therefore, two kinds of substrate-analog inhibitors are possible. The donor analogs have been rather well studied, but are not specific. On the other hand, glycosyltransferases have in general strict acceptor specificity. Recently, acceptor analogs which inhibit the corresponding glycosyltransferases were reported[2-5] and as expected were acceptor-specific inhibitors. The importance of sialoside has been proved in such biological phenomena as viral adhesion,[6] cellular recognition,[7] and cell differentiation.[8] In the case of sialyltransferase

SCHEME



(a) (1) benzaldehyde dimethyl acetal, CSA, DMF, 50 °C; (2) Ac_2O, pyridine,DMAP, rt, 89% from 1; (b) 60% AcOH, 90 °C, 98%; (c) (1) TBDMSCl, imidazole, DMF, rt; (2) Ac_2O, pyridine, rt, 78% from 3; (d) 60% AcOH, 80 °C, 76%; (e) TsCl, DMAP, pyridine, 50 °C, 77%; (f) (1) NaI, dimethoxyethane, 80 °C; (2) n-Bu_3SnH, AIBN, benzene, reflux, 82% from 6; (g) KSAc, DMF, 80 °C, 84%; (h) NaOMe, MeOH, rt, 93%(9); (i) 28% NH_4OH, MeOH, DL-dithiothreitol, rt, 45% (10).

only donor-analog inhibitors such as CMP, CMP-NeuNAc analogs, 5-*O*-(*N*-acetylneuraminyl)-5'-fluorouridine and -inosine derivatives are known.[9] In this communication methyl 6'-deoxy- (**9**) and 6'-thio-β-lactosaminides (**10**) will be presented as the first acceptor-analog inhibitors of (2→6)-α-D-sialyltransferase (EC 2. 4. 99. 1). The former showed better and more potent activity with a Ki value of 0.76 mM. The inhibition mode was of mixed type with respect to the acceptor.

Two effective molecular modifications of acceptor-analog inhibitors toward glycosyltransferases have been recently reported. One is deoxygenation[3] of the acceptor hydroxyl group to be glycosylated and the other is thio analog of the other hydroxyl groups.[4] In the case of (2→3)-α-D-sialyltransferase the first deoxygenation strategy was found not to be effective.[3] We are interested in the (2→6)-α-D-sialyltransferase and synthesis of **9** and **10** was planned.

These two 6'-analogs of methyl *O*-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) were synthesized *via* 4', 6'-diol **3** as shown in the Scheme. The key intermediate of 6'-modification, *i.e.*, 6'-*O*-tosyl derivative **6**, was prepared *via* 6'-*O*-TBDMS ether **4**. Substitution of **6** with sodium iodide followed by reduction with tributylstannane gave the 6'-deoxy derivative **7** in 82% yield. Treatment of **6** with potassium thioacetate gave **8** in 84% yield. While de-*O*-acetylation of **7** with sodium methoxide gave desired analog **9**, de-*O*-acetylated derivative (**10**) of **8** was obtained first

by treatment with NH_4OH (28%) in the presence of DL-dithiothreitol. The 6'-SH analog **10** was further transformed to the disulfide tetrasaccharide **11** in water at 37 °C for 72 h (83%).[12] ^1H NMR{500MHz, D_2O (303 K), TPS as the external standard} data: **9**: δ 4.46 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.42(d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 3.50 (s, 3H, OMe), 2.03 (s, 3H, Ac), 1.24 (d, 3H, $J_{6',5'}$ = 6.3 Hz, Me). **10**: δ 4.48-4.45 (m, 2H, H-1', H-1), 3.50 (s, 3H, OMe), 2.80 (dd, 1H, $J_{6'a,5'}$ = 7.2 Hz, $J_{6'a,6'b}$ = 13.9 Hz, H-6'a), 2.73 (dd, 1H, $J_{6'b,5'}$ = 6.9 Hz, H-6'b), 2.03 (s, 3H, Ac). **11**: δ 4.50 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 4.46 (m, 1H, H-1), 3.50 (s, 3H, OMe), 3.00 (d, 2H, $J_{6',5'}$ = 6.5 Hz, H-6'), 2.03 (s, 3H, Ac).

The inhibitory activities of the synthetic acceptor analogs (**9-11**) toward (2→6)-α-D-sialyltransferase (rat liver, Boeringer-Mannheim) were evaluated according to the assay method of Paulson *et al.*[10] Inhibition assay was performed at 37 °C for 15-60 min in 50 mM sodium cacodylate buffer solution (pH = 6.0, 30 μL) which contained the following assay components: methyl *O* -(β-D-galactopyranosyl)- (1→4) - 2 - acetamido - 2 - deoxy - β - D - glucopyranoside **1** (0.5-3 mM), an analog of **1**, bovine serum albumin (50 μg), triton X-100 (0.5 %), CMP-[U-^{14}C] - NeuNAc (2.5 μM, 12.1 GBq/mmol) and (2→6)-α-D-sialyltransferase (2.0 x10^{-6} unit). The reaction was traced up to the 15% consumption of CMP-NeuNAc.

In a preliminary inhibition assay with 0.5 mM of **1** and 0.5 mM of **9**, the fractional inhibition was 38%, whereas in the case of **10** the same fractional inhibition rate was observed at 2 mM. The kinetic parameters, *i.e.*, Ki values of **9-11** as well as Km of **1** (0.90 mM), were obtained as previously described.[4] As shown in the Table, the Ki value of **9** is 0.76 mM and its relative activity is five times as high as that of **10**. Furthermore, the disulfide **11** has a stronger activity than the thiol **10**.

The reported Ki values[3] of acceptor-analog inhibitors of four glycosyltransferases are higher than the Km values of the corresponding unmodified acceptors (Ki / Km[11] > 1.8~7). Thus 6'-deoxy analog **9** is the first acceptor-analog inhibitor having remarkable, albeit not strong enough for universal practical use, inhibitory activity (Ki / Km = 0.8) toward (2→6)-α-D-sialyltransferase. In addition, the observed moderate activity of the disulfide **11** suggested the 6'-position of the lactosaminide-analog acceptors in the binding site of the (2→6)-α-D-sialyltransferase faced an open space.

It is noteworthy that the inhibition modes of the 6'-modified analogs **9-11** showed a mixed type for the glycosyl acceptor as shown in the Fig. (the case of **9**), and a noncompetitive one for the donor, CMP-NeuNAc (data not shown). In contrast to this, the inhibition mode of *N*-acetyllactosamine for (2→6)-α-D-sialyltransferase from bovine colostrum was reported as competitive, when asialo α_1-acid glycoprotein was used as the

TABLE. Inhibitory Activities[a] of Methyl *O*-(β–D-Galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β–D-glucopyranoside Analogs toward (2→6)-α-D-Sialyltransferase

Compound	Ki (mM) (Inhibition mode)	Relative activity
6'-deoxy analog **9**	0.76 (mixed)	5.0
6'-SH analog **10**	3.78 (mixed)	1.0
$(6'\text{-S})_2$ analog **11**	2.00 (mixed)	1.9

a. Determination of Ki values : see Fig. in the case of 6'-deoxy analog **9** as an example.

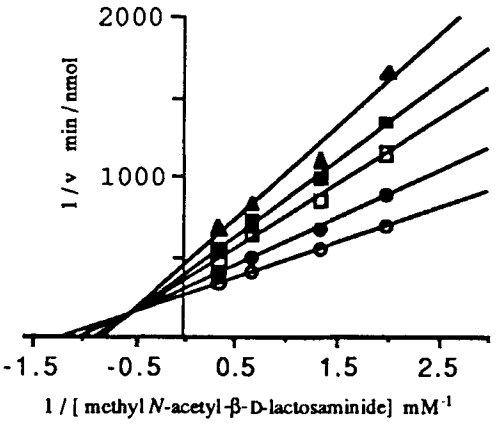


FIG. Double reciprocal plot of (2→6)-α-D-sialyltransferase reaction.The concentration of 6'-deoxy analog **9** was changed together with that of Methyl *O*-(β–D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β–D-glucopyranoside **1**. **9**: 0 (○), 250 (●), 500 (□), 750 (■), 1000 (▲) μM. Ki value was obtained from replot of slopes vs concentration of **9** using computer program as previously described[4]: Ki = 760 ± 63 μM.

acceptor.[10] Furthermore, the other acceptor-analog inhibitors towards four glycosyltransferases, that is, (1→2)-α-L- and (1→4)-α-L-fucosyltransferases, two kinds of (1→6)-β-D-glucosaminyltransferase (V and mucin core-2), interfere with the corresponding glycosyl transfers in competitive mode.[3] Thus, the (2→6)-α-D-sialyltransferase showed a characteristic difference in its acceptor binding mode compared to other glycosyltransferases.

In summary, we have synthesized three functionally 6'-modified analogs of **1** and found that they behave as inhibitors of (2→6)-α-D-sialyltransferase. The 6'-deoxy analog **9** is found especially to have remarkable inhibitory activity. A further study in order to explain the characteristic difference in the inhibition mode is going on.

ACKNOWLEDGMENT

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12. This oxidative dimerization could be monitored by ^{1}H NMR signals of 6'-methylene protons at 500 MHz. At the concentration of 3 mM the thiol **10** changed by 20% to the disulfide **11** after 1 h (37 ℃).