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Glycosyltransferase activity can be selectively modulated by chemical modifications of acceptor substrates

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Abstract—A range of *N*-acetyllactosamine derivatives, which are modified by a wide range of functionalities at C-2′ and C-6, have been synthesised and the kinetic parameters of transfer catalysed by recombinant α-2,6-sialyltransferase and α-1,3-fucoyltransferase VI determined. Several of the chemical modifications led to selective modulate the activity the enzymes and offer promising lead compounds for the development of oligosaccharide primers for selective metabolic inhibition of oligosaccharide biosynthesis.

Glycosyltransferases catalyse the transfer of monosaccharide residues from nucleoside mono- or diphosphate sugars to growing oligosaccharide chains.[1,2] The resulting protein- and lipid-bound oligosaccharides play critical roles in a diverse range of biological processes such as embryogenesis, fertilisation, neuronal development, hormonal activities, cell proliferation and their organisation into specific tissues.[3,4] They are also important in health science and are, for example, involved in the invasion and attachment of pathogens, inflammation, metastasis and xenotransplantation. Compounds that can inhibit the biosynthesis of oligosaccharides may find applications as novel therapeutics for a wide range of diseases as well as providing important tools for studying biological functions of glycoconjugates.[5,6] To date, only a few inhibitors of glycosyltransferases have been reported.[7–12] Most approaches have been based on analogues of donor substrates and although some potent compounds have been reported, in general these derivatives inhibited a large number of different transferases. The use of compounds that mimic donor–acceptor transition-state complexes may provide more selective inhibitors, however, the design of such derivatives has proven difficult due to the absence of structural data. Several analogues of glycosyl acceptors have been synthesised and some of these compounds displayed interesting inhibitory properties.

Metabolic inhibition by peracetylated mono- and disaccharides provides another innovative way to block glycoconjugate biosynthesis.[13,14] In this approach, acetylated saccharides are taken-up by cells by passive diffusion, which are then deacetylated by endogenous esterases. The resulting compounds resemble carbohydrate moieties of naturally occurring glycoproteins and can be utilised as substrates for glycosyltransferases diverting oligosaccharide biosynthesis from endogenous glycoconjugates. In an experimental metastasis model, it has been shown that treatment of cancer cells with a *N*-acetyllactosamine (LacNAc) primer could markedly reduce lung colonisation.[15] LacNAc is, however, a substrate for a wide range of glycosyltransferases including several sialyl-, fucosyl- and *N*-acetylglucosaminetransferases. It is important to develop oligosaccharide primers that would selective target a subset of glycosyltransferases for selective inhibition.

We report here the synthesis of a range of LacNAc derivatives, which are modified by a wide range of functionalities at C-2′ and C-6. The kinetic parameters of transfer catalysed by rat liver recombinant α-2,6-sialyltransferase and human recombinant α-1,3-fucoyltransferase VI have been determined and it was found that some compounds displayed improved catalytic efficiencies for one of the two enzymes. It is to be expected that analogues with such properties may be appropriate lead compounds for the development of

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oligosaccharide primers for selective metabolic inhibition of oligosaccharide biosynthesis.

Previous studies have established that the C-6 and C-2′ hydroxyls of LacNAc are not essential for recognition by a number of glycosyltransfereases, however, they may make direct or indirect interactions with the periphery of the binding site.[16–19] Thus, it is to be expected that these positions are ideally suited for combinatorial modification to obtain compounds that display higher catalytic efficiencies for a particular transferase. Compounds **2**–**13** (Fig. 1) are derivatives of LacNAc (**1**) and are modified at C-6 or C-2′. These compounds could be prepared from the readily available key building block **14**[19] (Scheme 1), which has orthogonal *t*-butyldimethylsilyl ether (TBDMS) and acetyl ester (Ac) protecting groups at C-6 and C-2′, respectively. Thus, saponification of the acetyl ester of **14** using NaOMe in methanol gave, after purification by silica gel column chromatography, compound **15** in a yield of 89%. Alternatively, treatment of **14** with HBF_4 in acetonitrile gave **16**, which has a free hydroxyl at C-6. The diol **17** was easily obtained by removal of the of the acetyl ester of **16** using standard conditions. O-Methylation of compounds **15**, **16** and **17** using MeI and BaO/$Ba(OH)_2$ in DMF gave the mono- and di-methylated **18**, **19** and **20**, respectively, in good yields. Next, the TBDMS ether of **18** and the acetyl ester of **19** were cleaved to give compounds **21** and **22**, respectively, and finally removal of the benzyl groups of **20**, **21** and **22** by catalytic hydrogenation over Pd/C afforded, after purification by biogel P2 size exclusion column chromatography, the target compounds **2**–**4**. Mesylation of **16** using mesyl chloride in pyridine gave compound **23**, which was deactylated (→**24**) and subjected to catalytic hydrogenation over Pd/C to afford **5**.

An amino group at C-6 of LacNAc could easily be introduced by displacement of the mesylate of **23** with sodium azide in DMF to give **25**, which was deactylated with NaOMe (→**26**) and reduced with 1,3-propanedithiol in a mixture of pyridine/water to give amine **27**. The amino functionality of **27** was modified by acetyl and formyl function by reaction with acetic anhydride in pyridine, and formic acid and acetic anhydride in pyridine, followed by treatment of the intermediates with sodium methoxide in methanol to give compound **28** and **30**, respectively. Compound **29** was obtained by treatment with mesyl chloride and triethylamine in a mixture of methanol and dichloromethane. Compound **33**, which has a formamide at C-6 and a methyl group at C-2′ was easily obtained by methylation of **26** using MeI and BaO/$Ba(OH)_2$ in DMF followed by reduction of the azido moiety with 1,3-propanedithiol and formylation of the resulting amine **33** with formic acid and acetic anhydride. Removal of the benzyl groups of **27**–**30** and **33** by catalytic hydrogenation over Pd/C gave target compounds **6**–**10**. Finally, the ulosides **11**–**13** were prepared by selective oxidation of the C-6 hydroxyl of **17** using a catalytic amount of TEMPO and NaBr in the presence of $NaClO_2$ as the co-oxidant followed by modification of the carboxylic acid of **34** by reaction

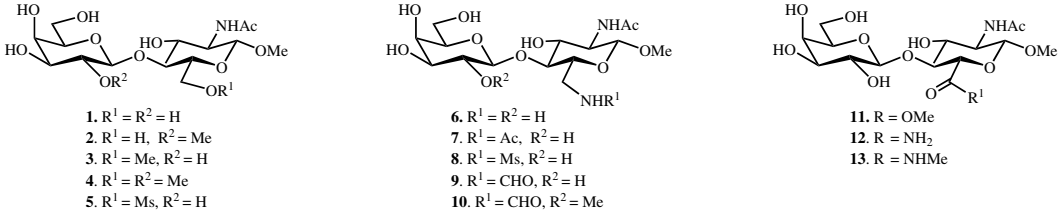
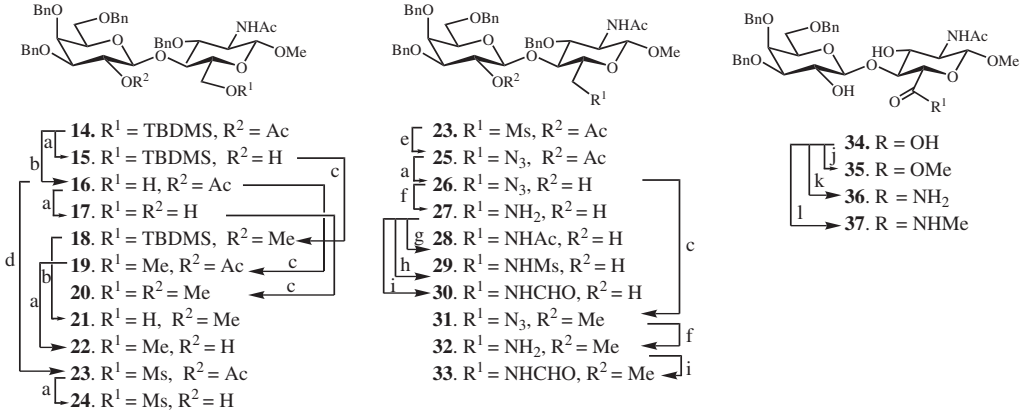


Figure 1.



Scheme 1. Reagents and conditions. (a) NaOMe, MeOH, (b) HBF_4, MeCN; (c) MeI, BaO/$Ba(OH)_2{\cdot}8H_2O$, DMF; (d) MsCl, Et_3N, MeOH/DCM; (e) NaN_3, DMF; (f) $HS(CH_2)_2SH$, Py/H_2O; (g) Ac_2O, Py; then NaOMeO, MeOH; (h) MsCl, Et_3N, MeOH/DCM; (i) Ac_2O, HCHO, Py then NaOMe, MeOH; (j) MeI, DMF; (k) DCC, pentafluorophenol, then NH_3 (g); (l) DCC, pentafluorophenol, then CH_3NH_2.

with methanol in the presence of methyl iodide in DMF to give **35**. Compounds **36** and **37** were obtained by displacement of the pentafluorophenyl ester of **34** by ammonia or methylamine, respectively. Catalytic hydrogenation over Pd/C of **35**–**37** gave the target compounds **11**, **12** and **13**, respectively.

The apparent kinetic parameters of transfer of CMP-[^{14}C]-Neu5Ac and GDP-[^{14}C]-Fucose to acceptors **1**–**13** catalysed by rat liver recombinant α-2,6-sialyltransferase (purchased from Calbiochem) and human recombinant α-1,3-fucosyltransferases VI (purchased from Calbiochem), respectively, were determined by reported assays.[16,18,20,21] In each case, the K_m for **1** was in close agreement with previous data and the V_{max} values were set at 1.

The apparent kinetic parameters of the sialylation of compounds **1**–**4** by α-2,6-sialyltransferase showed that the methyl groups at C-6 and C-2′ hydroxyls significantly lowered the catalytic efficiency (V_{max}/K_m) of the transfer due to a notable increased K_m and a somewhat smaller V_{max} (Table 1). Furthermore, the data shows that the both methyl groups induce similar and additive effects. Interestingly, compound **5**, which has a more bulky methanesulfonyl group at C-6, displayed a slightly improved catalytic efficiency indicating that electronic and not steric effects are probably important for favourable interactions with the periphery of the enzymes' binding pocket.

The apparent kinetic data for α-1,3-fucosyltransferases VI (Table 2) demonstrate that this enzyme responds differently to the introduced chemical modifications and for compounds **2**–**5** slightly improved catalytic efficiencies were measured. In particular, the effect of di-O-methylation was dramatic and in the case of the α-2,6-sialyltransferase a 15-fold loss of catalytic efficiency was observed whereas a 2.5-fold increase was measured for the α-1,3-fucosyltransferases VI. It can be concluded that the architecture of the binding sites of the two enzymes differ significantly, which can be exploited for the preparation of selective substrates.

Table 1. Apparent kinetic parameters for the transfer of *N*-acetylneuraminic acid to glycosyl acceptors **1**–**13** by α-2,6-sialyltransferase

Acceptor	K_m (mM)	Rel (V_{max})	Rel (V_{max}/K_m)
1	1.7 ± 0.2	1.0	0.6
2	4.1 ± 0.8	0.8	0.2
3	4.3 ± 0.4	0.8	0.2
4	11.2 ± 0.8	0.5	0.04
5	0.7 ± 0.1	0.9	1.3
6	5.8 ± 0.2	0.5	0.08
7	1.4 ± 0.2	0.6	0.4
8	0.8 ± 0.1	0.7	0.9
9	1.1 ± 0.3	1.6	1.5
10	3.1 ± 0.6	1.0	0.3
11	N.d.	N.d.	0.2
12	>10	<0.2	0.02
13	2.4 ± 0.2	0.2	0.08

N.d. not determined.

Table 2. Apparent kinetic parameters for the transfer of *N*-acetylneuraminic acid to glycosyl acceptors **1**–**13** by α-1,3-fucosyltransferase VI

Acceptor	K_m (μM)	Rel (V_{max})	Rel (V_{max}/K_m)
1	350 ± 50	1.0	2.8
2	390 ± 50	1.2	3.1
3	290 ± 50	1.3	4.5
4	120 ± 30	0.8	6.7
5	250 ± 30	0.9	3.6
6	400 ± 50	0.6	1.5
7	190 ± 20	1.3	6.8
8	115 ± 10	1.0	8.7
9	140 ± 10	1.0	7.3
10	75 ± 7	0.7	9.3
11	450 ± 70	0.6	1.2
12	N.a	N.a.	N.a.
13	1540 ± 50	0.6	0.4

N.a. not active.

The fact that different enzymes respond differently to chemical modification of LacNAc was also born out by the observation that the sialyltransferase did not tolerate the introduction of a free amine at C-6 (compound **6**) whereas this modification had only minor effect on the fucosyltransferase. Interestingly, for both enzymes acylation of the amine by an acetyl (**7**), sulfonyl (**8**) or formyl group (**9**) gave compounds that were equally or slightly better acceptors than LacNAc and the data of compounds **6**–**10** indicate that electronic and not steric factors are important determinants for the observed modulation of catalytic efficiencies. The introduction of a formamide at C-6 and methyl ether at C-2′ gave compound **10**, which was a remarkable good substrate for the fucosyltransferase and displayed the lowest K_m of all compounds tested. On the other hand, these modifications resulted in a small loss of catalytic efficiency for the sialyltransferase. For both enzymes, methyl ester **11** was a slightly poorer substrate than LacNAc, whereas introduction of an amide (**12**) and methyl amide (**13**) resulted in large reductions in catalytic efficiency. Comparing the data of compound **7**, which is modified by a $NHC(O)CH_3$ function and compound **13** which has a $C(O)NHCH_3$ group, shows that the arrangement of atoms has a dramatic effect on catalytic efficiencies and this observation indicates that these functional groups make direct interactions with the binding site of the enzymes.

Previous studies have identified key polar functionalities of a glycosyl acceptor by selective methylation or deoxygenation of hydroxyls.[16,18] For some other hydroxyls, these modifications lead to a reduction of acceptor activity and it has been proposed that these functionalities interact with the periphery of the binding site of the enzyme. The results described in this communication show that by employing a wider range of functionalities, a more detailed picture of the architecture of a binding site can be obtained. Furthermore, it is shown that the introduction of these functionalities may lead to compounds that display high selectivity for particular transferases. Such analogues will be developed as primers for selective metabolic inhibition of

oligosaccharide biosynthesis and the results of these studies will be reported in due course.

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