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Synthetic substrate analogues for UDP-GlcNAc: Manα1-3R β1-2-*N*-acetylglucosaminyltransferase I. Substrate specificity and inhibitors for the enzyme

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UDP-GlcNAc:Manα1-3R β1-2-*N*-acetylglucosaminyltransferase I (GlcNAc-T I; EC 2.4.1.101) catalyses the conversion of [Manα1-6(Manα1-3)Manα1-6][Manα1-3]Manβ-*O*-R to [Manα1-6(Manα1-3)Manα1-6][GlcNAcβ1-2Manα1-3]Manβ-*O*-R (R = 1-4GlcNAcβ1-4GlcNAc-Asn-X) and thereby controls the conversion of oligomannose to complex and hybrid asparagine-linked glycans (N-glycans). GlcNAc-T I also catalyses the conversion of Manα1-6(Manα1-3)Manβ-*O*-octyl to Manα1-6(GlcNAcβ1-2Manα1-3)Manβ-*O*-octyl. We have therefore tested a series of synthetic analogues of Man″α1-6(Man′α1-3)Manβ-*O*-octyl as substrates and inhibitors for rat liver GlcNAc-T I. The 2″-deoxy and the 3″-, 4″- and 6″-*O*-methyl derivatives are all good substrates confirming previous observations that the hydroxyl groups of the Man″α1-6 residue do not play major roles in the binding of substrate to enzyme. In contrast, all four hydroxyl groups on the Man′α1-3 residue are essential since the corresponding deoxy derivatives either do not bind (2′- and 3′-deoxy) or bind very poorly (4′- and 6′-deoxy) to the enzyme. The 2′- and 3′-*O*-methyl derivatives also do not bind to the enzyme. However, the 4′-*O*-methyl derivative is a substrate (K_M = 2.6 mM) and the 6′-*O*-methyl compound is a competitive inhibitor (K_i = 0.76 mM). We have therefore synthesized various 4′- and 6′-*O*-alkyl derivatives, some with reactive groups attached to an *O*-pentyl spacer, and tested these compounds as reversible and irreversible inhibitors of GlcNAc-T I. The 6′-*O*-(5-iodoacetamido-pentyl) compound is a specific time dependent inhibitor of the enzyme. Four other 6′-*O*-alkyl compounds showed competitive inhibition while the remaining compounds showed little or no binding indicating that the electronic properties of the attached *O*-pentyl groups influence binding.

Keywords: Synthetic oligosaccharides; inhibitors; N-glycans; *N*-acetylglucosaminyltransferase; biosynthesis

Abbreviations: GlcNAc-T I, UDP-GlcNAc:Manα1-3R β1-2-*N*-acetylglucosaminyltransferase I (EC 2.4.1.101); GlcNAc-T II, UDP-GlcNAc:Manα1-6R β1-2-*N*-acetylglucosaminyltransferase II (EC 2.4.1.143); MES, 2-(*N*-morpholino)ethane sulfonic acid monohydrate.

Introduction

Complex and hybrid asparagine-linked oligosaccharides (N-glycans) have many diverse biological functions [1]. UDP-GlcNAc:Manα1-3Rβ1-2-*N*-acetylglucosaminyl-trans-ferase I (GlcNAc-T I; EC 2.4.1.101) catalyses the conversion of [Manα1-6(Manα1-3)Manα1-6][Manα1-3]Manβ1-4GlcNAcβ1-4GlcNAc-Asn-X to [Manα1-6-(Manα1 - 3)Manα1 - 6][GlcNAcβ1 - 2Manα1 - 3]Manβ1 - 4GlcNAcβ1-4GlcNAc-Asn-X and thereby controls the conversion of oligomannose to complex and hybrid N-glycans [2, 3]; GlcNAc-T I can also convert **1** to **3** (Fig. 1). Chinese hamster ovary cell mutants which do not express a functional *GlcNAc-T I* gene grow normally in culture [4–6] even though they cannot synthesize hybrid or complex N-glycans. However, transgenic mice

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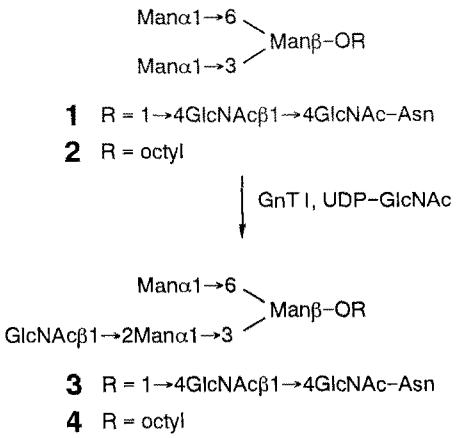


Figure 1. The reaction catalysed by GlcNAc-transferase I.

in which the *GlcNAc-T I* gene has been inactivated do not survive beyond 10.5 days of embryonic life [7, 8]. This finding is supportive of the hypothesis that complex and hybrid N-glycans are important for multi-cellular organisms in processes such as embryogenesis, morphogenesis, tumour progression and metastasis, viral and bacterial infectivity, and the control of the immune system [1, 9–12].

N-acetylglucosaminyltransferases I to VI [2, 3, 13, 14] initiate the antennae or branches characteristic of hybrid and complex N-glycans by the addition of GlcNAc residues to the $Man_3GlcNAc_2$ core. The antibiotic tunicamycin inhibits the synthesis of all N-glycans and glycosidase inhibitors such as the alkaloids swainsonine, castanospermine, nojirimicin and deoxynojirimicin are relatively non-specific [15, 16]. Specific inhibitors of the branching GlcNAc-transferases would therefore be of great interest for studies of N-glycan function and as potential therapeutic agents but no such inhibitors have as yet been isolated from natural sources. Until X-ray crystallographic studies of glycosyltransferases become available, probing the active sites of these enzymes with substrate analogues presents the only viable approach to rational inhibitor design. Active site directed inhibitors based on the highly specific oligosaccharide acceptors of the GlcNAc-transferases should be more specific than inhibitors based on the common donor substrate UDP-GlcNAc.

GlcNAc-T I controls the synthesis of complex and hybrid N-glycans and is therefore an excellent target for specific inhibition. We now report the effectiveness of a variety of synthetic substrate analogues as substrates and inhibitors of this enzyme. The compounds were obtained by chemical syntheses using interchangeable modified saccharide building units which facilitated the syntheses [17–19]. Similar procedures have been applied to other glycosyltransferases and have helped to delineate the substrate requirements of these enzymes [20–34]. The information obtained may prove of value in the development of inhibitors with therapeutic potential for treatment of diseases involving increased N-glycan branching.

Materials and methods

Materials

Rat liver GlcNAc-T I was purified as previously described [30]; the specific activity was 20 U mg^{-1} using compound **2** as acceptor (Fig. 1) and assay conditions as described below. UDP-[^{14}C]GlcNAc was synthesized [35] and diluted with non-radioactive UDP-GlcNAc (Sigma). Oligosaccharides (see Figs 1 and 2 for structures) were synthesized as follows: compound **2** [36]; **5-13** [18]; **14** and **15** [17] (**14** was obtained by hydrogenation of the olefinic precursor with Pd/C and subsequent deacetylation); **16–29** [19]. All oligosaccharide structures have been characterized by ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Kinetic experiments

GlcNAc-T I was assayed radiochemically using Pasteur pipette columns of Dowex AG1-x8 (Cl^- form, 100–200 mesh) to separate radiolabelled product from unreacted radiolabelled sugar-nucleotide donor UDP-GlcNAc [37]. Unless otherwise stated, the incubation mixture used for kinetic studies contained, in a total volume of 0.025 ml: 0.3 mU (15 ng) of enzyme (1 unit is 1 μmol min^{-1}), 5–100 nmol acceptor, 20 nmol UDP-[^{14}C]GlcNAc (2500 dpm $nmol^{-1}$), 100 mM MES (2-(*N*-morpholino)-ethane sulfonic acid monohydrate), pH 6.1, 20 mM $MnCl_2$, bovine serum albumin (25 μg), Triton X-100 (0.1%, v/v) and glycerol (4%, v/v). Samples were incubated for 20–60 min at 37 °C and the reaction was stopped by the addition of 0.4 ml ice cold water.

UV irradiation of GlcNAc-T I with compound 23

Rat liver GlcNAc-T I (0.3 mU) was equilibrated at room temperature for 1 h in the presence of 1.2 mM UDP-[^{14}C]GlcNAc and 2 mM compound **23** as described for GlcNAc-T I enzyme assays above. The samples were then irradiated at 350 nm for 10 min in polystyrene test-tubes, using a Rayonet RPR 100 reactor, equipped with 16 RPR 3500 Å lamps (the half-life for compound **23** under these conditions of irradiation was about 2.5 min, data not shown). Control incubations were carried out with compound **23** which had been previously inactivated by irradiation. After irradiation, acceptor substrate **2** (25 nmol) was added to the incubation and GlcNAc-T I activity was assayed as described above.

Inactivation of GlcNAc-T I with compound 24

Rat liver GlcNAc-T I (2.4 mU) was incubated with 0.16 μmol UDP-[^{14}C]GlcNAc and 0.4 μmol compound **24**

at 37 °C in 0.2 ml of buffer containing 100 mM MES, pH 6.1, 0.1% Triton X-100, bovine serum albumin (200 μg), glycerol (4%, v/v) and 20 mM $MnCl_2$. Control incubations were carried out with 0.4 μmol iodoacetamide instead of compound **24**. Aliquots (0.025 ml) were withdrawn at regular intervals and acceptor **2** (20 nmol) was added. After 1 h incubation at 37 °C, GlcNAc-T I activity was measured as described above.

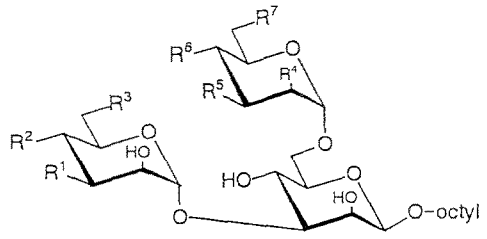
Results and discussion

Acceptor specificity and reversible inhibition of GlcNAc-T I

All GlcNAc-transferases use UDP-GlcNAc as the donor substrate but show high specificity towards different acceptor oligosaccharides. Specific inhibitors should therefore be designed on the basis of the acceptor rather than the donor substrate. Glycosyltransferase inhibitors based on the acceptor have been found using different approaches: (i) by replacing the acceptor hydroxyl group which forms the new glycosidic bond either with a hydrogen or a methoxy group [20, 26, 38], (ii) by introducing a modification, at a position removed from the site of glycosidic bond formation, which hinders catalysis but not binding to enzyme [20, 39], and (iii) by attaching a reactive group which does not interfere with binding to obtain an irreversible inhibitor [20, 40–42]. These approaches all require extensive substrate specificity studies to find a suitable substrate analogue and to define the key hydroxyl groups of this structure that are essential for binding.

We have previously shown that the trimannosyl moiety in **1** and **2** (Fig. 1) is the minimum structure required in an optimally effective acceptor for GlcNAc-T I although the linear trisaccharide Manα1-3Manβ1-4GlcNAc is also a substrate but with a 10-fold higher K_m [32]. Analogues of structure **2** were therefore synthesized and tested as substrates and inhibitors for GlcNAc-T I (Figs 2 and 3). Hydroxyl groups were substituted by deoxy groups to determine if a certain hydroxyl group in **2** is necessary for binding. Replacement of hydroxyl groups by *O*-methyl groups gave information about the nature of the interaction between a certain hydroxyl group and the enzyme and the effects of substitutions at various positions of the substrate.

The results of the specificity studies are shown in Table 1. The 2″-deoxy (**10**) and the 3″, 4″- and 6″-*O*-methyl derivatives (**11**, **12** and **13**) are all good substrates confirming previous observations that the hydroxyl groups of the Man″α1-6 residue do not play major roles in the binding of substrate to enzyme [30, 32]. Since GlcNAc-transferases II, V and VI cannot act on the GlcNAc-T I product of **10** [3], this compound can be used as a specific and effective acceptor in assays of



5 R^1 = H
6 R^1 = OMe
7 R^2 = OMe
8 R^3 = H
9 R^3 = OMe
10 R^4 = H
11 R^5 = OMe
12 R^6 = OMe
13 R^7 = OMe
14 R^5 = O-pentyl
15 R^5 = O-(5-aminopentyl)
16 R^3 = O-propyl
17 R^3 = O-pentyl
18 R^3 = O-(4-pentenyl)
19 R^3 = O-(4-pentanolyl,R+S)
20 R^3 = O-(5-pentanolyl)
21 R^3 = O-(4-oxo-pentyl)
22 R^3 = O-(4,5-epoxy-pentyl,R+S)
23 R^3 = O-(4,4-azo-pentyl)
24 R^3 = O-(5-iodoacetamido-pentyl)
25 R^3 = O-(5-amino-pentyl)
26 R^3 = NH_3^+
27 R^3 = N-iodoacetamido
28 R^2 = O-(5-aminopentyl)
29 R^2 = O-(5-iodoacetamido-pentyl)

Figure 2. Synthetic substrate analogues for GlcNAc-transferase I. R groups which are not designated are OH groups. Chemical formulae: Me, $-CH_3$; pentyl, $-(CH_2)_4CH_3$; 5-aminopentyl, $-(CH_2)_4CH_2NH_2$; propyl, $-(CH_2)_2CH_3$; 4-pentenyl, $-(CH_2)_3CH{=}CH_2$; 4-pentanolyl, $-(CH_2)_3CHOH.CH_3$; 5-pentanolyl, $-(CH_2)_4CH_2OH$; 4-oxo-pentyl, $-(CH_2)_3CO.CH_3$; 4,5-epoxy-pentyl, $-(CH_2)_3CH\overset{O}{\frown}CH_2$; 4,4-azo-pentyl, $-(CH_2)_3C(N{=}N)CH_3$; 5-iodoacetamido-pentyl, $-(CH_2)_4CH_2$-NH.CO.CH_2I; *N*-iodoacetamido, —NH.CO.CH_2I. R, rectus; S, sinister.

GlcNAc-T I activity. The 6″-*O*-Me compound **13** shows significantly better binding than the unmodified substrate **2** (K_m**13** = 0.30 mM, K_m**2** = 0.73 mM, Table 1). Even compounds with large substituents like a charged aminopentyl group on the 3″-position (compound **15**) are

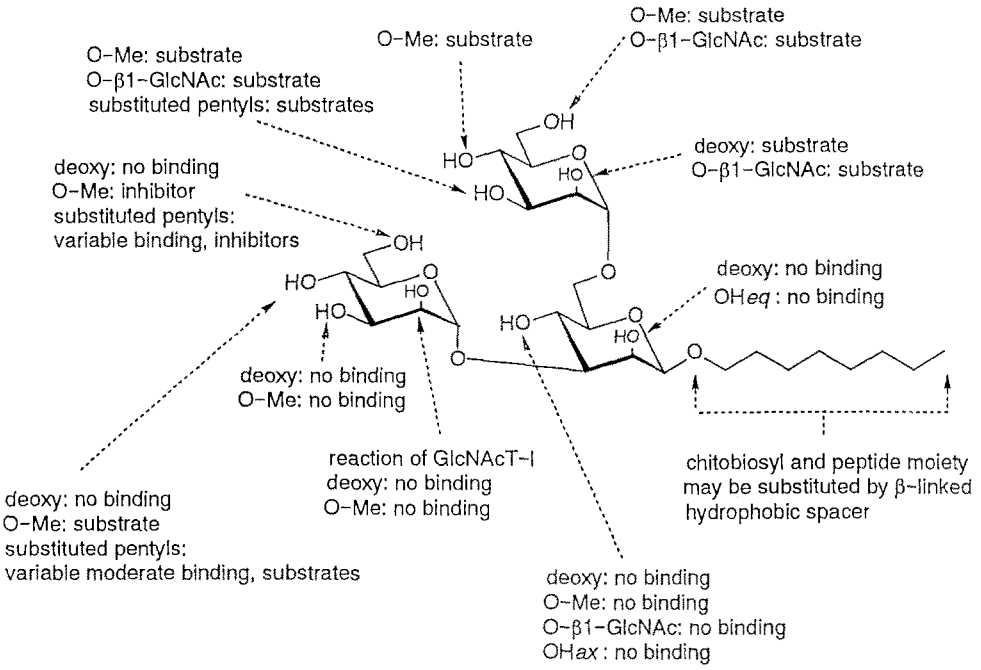


Figure 3. The substrate specificity of GlcNAc-transferase I. The data summarized in this Figure are derived from the work presented in this paper and from previous work by our group [30–32] and by Kaur and Hindsgaul [23].

very good substrates indicating that the 3″-OH of the acceptor is probably oriented away from the enzyme during catalysis. We did not test the 3″-, 4″- and 6″-deoxy compounds and we have therefore not ruled out the possibility that the corresponding hydroxyl groups may be involved in binding as hydrogen bond acceptors.

In contrast to the Man″α1-6 arm, all four hydroxyl groups on the Man′α1-3 residue are essential; the corresponding 2′-deoxy [30] and 3′-deoxy (compound **5**, Table 1) derivatives do not bind to the enzyme and the 4′-deoxy [30] and 6′-deoxy (compound **8**, Table 1) derivatives are poor substrates. The 6′,6″-dideoxy derivative has also been shown to be a poor substrate for GlcNAc-T I (K_m = 2.2 to 2.8 mM) [43]. The 2′-, 3′-, 4′- and 6′-*O*-methyl compounds, however, show interesting differences. Neither the 2′-*O*-methyl derivative [30] nor the 3′-*O*-methyl compound **6** bind to GlcNAcT-I. This suggests that either the 2′-OH and the 3′-OH are essential as hydrogen bond donors or they are required as hydrogen bond acceptors and methylation at these positions prevents binding by unfavourable steric interactions. Methylation of the 4′-position (compound **7**) results in a good substrate. This indicates that the 4′-OH is essential because it is a hydrogen bond acceptor in the enzyme substrate complex. The 6′-*O*-methyl compound **9** is a competitive inhibitor (Fig. 4) with a K_i (0.76 mM) comparable to the K_m (0.73 mM) of the unmodified substrate **2** (Table 1). Presumably the 6′-OH is also an essential hydrogen bond acceptor but methylation of this position prevents the transfer of GlcNAc during catalysis probably by steric hindrance. The principle that *O*-alkyl substitution of an essential hydroxyl group of the acceptor molecule may prevent catalysis but not binding, resulting in reversible inhibition, has been called by Khan *et al.* [39] "steric exclusion" and examples have been reported for GlcNAc-transferase II [20] and GlcNAc-transferase V [39].

The finding that compounds **7** and **10–15** are good substrates for GlcNAc-T I is of special interest because these compounds can be converted on a preparative scale, using GlcNAc-T I and UDP-GlcNAc, to give modified tetrasaccharides [21] which are specific substrates and inhibitors for GlcNAc-T II [20].

Figure 3 summarizes present knowledge of GlcNAc-T I specificity from this and previous work. All the hydroxyl groups of Man′α1-3 are involved in binding. The 4′- and 6′-hydroxyl groups serve as hydrogen bond acceptors. Both the 2-OH and 4-OH groups of the β-linked mannose are essential [30, 31]. The Man″α1-6 residue does not play a major role in binding to enzyme but we have not ruled out the possibility that the 3″-OH, 4″-OH and 6″-OH may serve as hydrogen bond acceptors. The chitobiosyl and peptide moieties of the substrate are not essential for enzyme activity and may be substituted by a β-linked hydrophobic group like octyl [23] but not by an α-methyl group [32].

Table 1. Substrates and inhibitors of rat liver β2-GlcNAc-transferase I.

Acceptor analogue (Fig. 2)	*Enzyme activity*[a] *(%)*	K_m^b *(mM)*	K_i^b *(mM)*	V_{max}^b *(μmol min^{-1} mg^{-1})*	$\frac{V_{max}}{K_m}$
2	100	0.73		20	27
5	<2		NI[c]		
6	<2		NI[c]		
7	70	2.6		13	5
8	2	ND[d]			
9	<2		0.76		
10	107	0.60		19	32
11	81	ND[d]			
12	48	0.91		15	16
13	60	0.30		12	40
14	50	3.5		6.2	1.8
15	103	ND[d]			
16	<2		NI[c]		
17	<2		NI[c]		
18	<2		NI[c]		
19	2	ND[d]			
20	<2		10.2		
21	<2		8.8		
22	<2		2.6		
23	<2		1.1[e]		
24	<2		TDI[f]		
25	<2		NI[c]		
26	<2				
	pH8.5: 3	ND[d]			
27	<2		NI[c]		
28	<2				
29	5	5.0		1.2	0.24

[a]Relative incorporation of radioactivity into acceptor, using GlcNAc-T I and [^{14}C]-UDP-GlcNAc. GlcNAc-T I assays were carried out as described under Experimental procedures with 10 nmol acceptor, 60 min incubation.
[b]K_m, V_{max} and K_i values were determined from at least four acceptor concentrations by linear double reciprocal Lineweaver Burk plots. The standard deviations of the slopes and intercepts ranged from 2 to 6% and 6 to 20% respectively. The R-squared values of the regressions ranged from 0.98 to 1.00.
[c]NI, no inhibition detected when assayed with 0.4 mM acceptor **2**.
[d]ND, not determined.
[e]Without UV irradiation.
[f]TDI time-dependent inhibition (Fig. 5).

Inhibition of GlcNAcT-I by compounds with reactive groups

We have shown (Table 1) that the 4′- and the 6′-hydoxyl groups of the Man′α1-3 residue may be substituted by *O*-methyl without interfering with binding. We therefore decided to introduce reactive groups in these positions in the hope of obtaining irreversible inhibitors. An electrophilic epoxy group, a photoreactive diazirino group and an electrophilic iodoacetamido group were attached via a pentyl spacer (compounds **22**, **23**, **24** and **29**, respectively). An iodoacetamido group was also directly linked to the 6′-amino compound **26** to give the iodoacetamide **27**. The iodoacetamido group has been found to react readily with the nucleophilic side chains of cysteine and methionine, and more slowly with other nucleophilic amino acid side chains like histidine [44]. The diazirino group is photoactivatable and may react with any neighbouring group of the protein, but also with water [45].

Iodoacetamide **24** shows time dependent inhibition of GlcNAc-T I (Fig. 5). After 5 h incubation of GlcNAc-T I with **24** and UDP-GlcNAc, the enzyme activity is reduced by about 50% relative to the controls. The inhibition is specific because iodoacetamide and the iodoacetamides **27** and **29** do not show this effect at the same concentrations. Iodoacetamide **27** does not bind to the enzyme and no inhibition is detectable. Iodoacetamide **29** is a poor substrate (K_m = 5.0 mM) and does not show time dependent inhibition (data not shown). Pre-

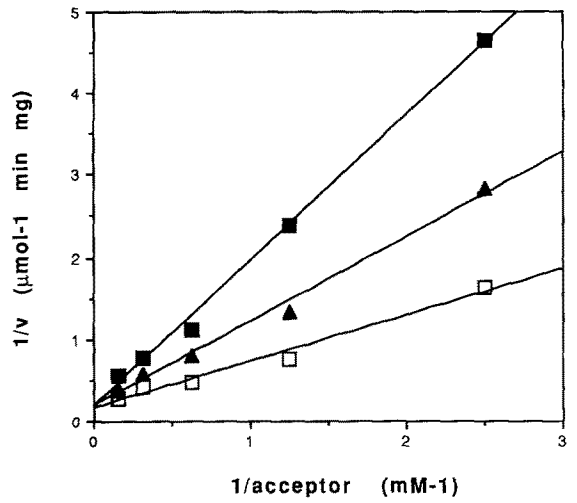


Figure 4. Effect of compound **9** on rat liver GlcNAc-T I activity. Rat liver GlcNAc-T I was assayed at several concentrations of the acceptor **2**, both in the absence of compound **9** (□) and at 2 mM (▲) and 4 mM (■) concentrations of compound **9**. Incubations were carried out with 1.2 mM UDP-[^{14}C]GlcNAc at pH 6.1, 37 °C, for 60 min. Reciprocal 1/v vs 1/S plots indicate competitive inhibition by compound **9**.

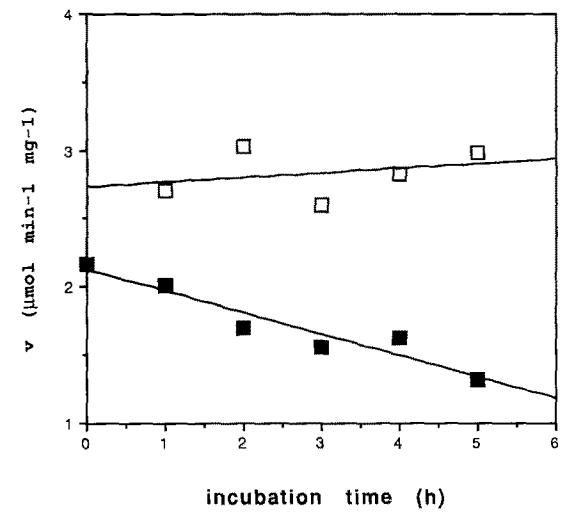


Figure 5. Effect of compound **24** on rat liver GlcNAc-T I activity. Rat liver GlcNAc-T I (2.4 mU) was incubated with 0.16 µmol UDP-[^{14}C]GlcNAc and 0.4 µmol **24** at 37 °C in 0.2 ml of buffer containing 100 mM MES, pH 6.1, 0.1% Triton X-100, glycerol (4%, v/v), bovine serum albumin (200 µg) and 20 mM $MnCl_2$. Control incubations were carried out with 0.4 µmol iodoacetamide instead of **24**. Aliquots (0.025 ml) were withdrawn at regular intervals and acceptor **2** (20 nmol) was added. After 1 h incubation at 37 °C, GlcNAc-T I activity was measured as described under Materials and methods. GlcNAc-T I activity is plotted against time of incubation with iodoacetamide (□) and with compound **24** (■).

sumably the iodoacetamido group in **29** is not in the right position relative to a nucleophilic group of the enzyme for bond formation. The epoxide **22** (which consists of a 1:1 mixture of the diastereomeric epoxides which could not be separated) is less reactive towards nucleophiles than the iodoacetamide **24** and no time dependent inhibition is observed, but **22** shows reversible inhibition (K_i = 2.6 mM).

Similarly in the absence of UV irradiation the diazirino compound **23** is a reversible inhibitor (K_i = 1.1 mM, similar to K_m = 0.73 mM for the non-modified substrate **2**). Upon UV irradiation at 350 nm the diazirine **23** generates a highly reactive carbene which reacts instantly under insertion with water to form the secondary alcohol **19** (approximately 40%) and intramolecularly to form the olefin **18** (approximately 60%); the reaction was observed by thin layer chromatography using the standards **18** and **19** for identification of reaction products (data not shown). However, when **23** is irradiated together with GlcNAc-T I and UDP-GlcNAc, no additional photoinhibition is detectable compared to the inhibition observed when **23** and GlcNAc-T I are UV irradiated separately, indicating that insertion of the carbene derived from **23** into enzyme is probably less than 5%. Low yields in photolabelling with diazirines have been reported in the literature in many cases and are a drawback of the high reactivity of these compounds.

Since compounds **22** and **23** showed competitive inhibition with significantly different K_i values (Table 1), we were interested in testing other similar 6′-modified compounds. Such compounds were readily available by deprotection of the precursors of the chemical syntheses of compounds **22**, **23** and **24**, giving compounds with many different functional groups in the 6′-*O*-R group (compounds **16–27**, Fig. 2). Surprisingly, the K_i values for these compounds vary from K_i > 15 mM (no binding detected) to K_i = 1.1 mM (Table 1), a finding that cannot be explained by steric factors alone. The alcohols **19** and **20** and the ketone **21** show no or very poor binding, compared to the epoxide **22** and diazirin **23** (K_i = 2.6 mM and 1.1 mM respectively). The 6′-*O*-alkyl (other than *O*-methyl) compounds **16**, **17** and **18** also show no binding. The observed differences in the K_i and K_m values (a 10- to 20-fold variation) correspond to differences in free energy of approximately 1–2 kcal and could be caused by different van der Waal's interactions or different hydrogen bonding of the *O*-alkyl group with the protein. Obviously steric factors do not alone determine if a substituent is tolerated in the binding site but the enthalpic and entropic contributions of even a relatively small substituent on an oligosaccharide ligand can have a considerable effect on the binding constants. This is important in the interpretation of results from specificity studies using substrate derivatives with substituents larger than *O*-methyl and has to be considered

in the design of inhibitors based on substrates with relatively high K_m values.

The amino compounds **25** and **28** show no binding probably due to the positive charge present at the assay pH. The 6′-amino compound **26** is not a substrate at pH 6.1, which is the normal pH optimum for GlcNAc-T I, but is a weak substrate at the higher pH of 8.5, indicating that the enzyme prefers the amino group in the unprotonated form. Compound **26** was converted on a preparative scale to the corresponding enzyme product which was characterized by ^{1}H-NMR spectroscopy (data not shown).

In summary, we have studied the substrate specificity of GlcNAc-T I and shown that the enzyme can be used to synthesize modified oligosaccharide products. We describe for the first time competitive inhibitors of the enzyme, the 6′-*O*-methyl compound **9** and the 6′-*O*-(4,4-azo-pentyl) diazirino compound **23**, with K_i values similar to the K_m value of the unmodified substrate **2**. The iodoacetamide **24** is a specific time dependent and possibly irreversible inhibitor and may be useful in attempts to label active site residues in GlcNAc-T I. The results of inhibitor design are similar to those found previously for GlcNAc-T II [20] and GlcNAc-T V [26, 39], i.e. the best inhibitors have K_i values similar to the K_m values of the substrate. Unfortunately the K_m values for oligosaccharide acceptors are relatively high for many glycosyltransferases (GlcNAc-T I, ~1 mM; GlcNAc-T II, ~0.15 mM; GlcNAc-T V, ~0.05 mM). In order to obtain a better inhibitor, its design should probably be based on a transition state analogue rather than on a substrate analogue [46] because enzymes have evolved, according to Pauling [47], towards maximal stabilization of the transition state rather than of the substrate. Unfortunately very little is known about the transition state of glycosyltransferase-substrate complexes and models are speculative. Attempts in this direction have shown little success so far [48–50].

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