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Synthetic substrate analogues for UDP-GlcNAc: Manα1-6R β(1-2)-*N*-acetylglucosaminyltransferase II. Substrate specificity and inhibitors for the enzyme

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UDP-GlcNAc:Manα1-6R β(1-2)-*N*-acetylglucosaminyltransferase II (GlcNAc-T II; EC 2.4.1.143) is a key enzyme in the synthesis of complex *N*-glycans. We have tested a series of synthetic analogues of the substrate Man‴α1-6(GlcNAc″β1-2Man′α1-3)Manβ-*O*-octyl as substrates and inhibitors for rat liver GlcNAc-T II. The enzyme attaches *N*-acetylglucosamine in β1-2 linkage to the 2‴-OH of the Man‴α1-6 residue. The 2‴-deoxy analogue is a competitive inhibitor (K_i = 0.13 mM). The 2‴-*O*-methyl compound does not bind to the enzyme presumably due to steric hindrance. The 3‴-, 4‴- and 6‴-OH groups are not essential for binding or catalysis since the 3‴-, 4‴- and 6‴-deoxy and -*O*-methyl derivatives are all good substrates. Increasing the size of the substituent at the 3‴-position to pentyl and substituted pentyl groups causes competitive inhibition (K_i = 1.0–2.5 mM). We have taken advantage of this effect to synthesize two potentially irreversible GlcNAc-T II inhibitors containing a photolabile 3‴-*O*-(4,4-azo)pentyl group and a 3‴-*O*-(5-iodoacetamido)pentyl group respectively. The data indicate that none of the hydroxyls of the Man‴α1-6 residue are essential for binding although the 2‴- and 3‴-OH face the catalytic site of the enzyme. The 4-OH group of the Manβ-*O*-octyl residue is not essential for binding or catalysis since the 4-deoxy derivative is a good substrate; the 4-*O*-methyl derivative does not bind. This contrasts with GlcNAc-T I which cannot bind to the 4-deoxy-Manβ- substrate analogue. The data are compatible with our previous observations that a 'bisecting' *N*-acetylglucosamine at the 4-OH position prevents both GlcNAc-T I and GlcNAc-T II catalysis. However, in the case of GlcNAc-T II, the bisecting *N*-acetylglucosamine prevents binding due to steric hindrance rather than to removal of an essential OH group. The 3′-OH of the Man′α1-3 is an essential group for GlcNAc-T II since the 3′-deoxy derivative does not bind to the enzyme. The trisaccharide GlcNAcβ1-2Manα1-3Manβ-*O*-octyl is a good inhibitor (K_i = 0.9 mM). The above data together with previous studies indicate that binding of the GlcNAcβ1-2Manα1-3Manβ- arm of the branched substrate to the enzyme is essential for catalysis.

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

Abbreviations: GlcNAc-T I, UDP-GlcNAc:Manα1-3R β(1-2)-*N*-acetylglucosaminyltransferase I (EC 2.4.1.101); GlcNAc-T II, UDP-GlcNAc:Manα1-6R β(1-2)-*N*-acetylglucosaminyltransferase II (EC 2.4.1.143); MES, 2-(*N*-morpholino)ethane sulfonic acid monohydrate.

Introduction

Complex asparagine linked oligosaccharides (*N*-glycans) have been implicated in many biological phenomena, e.g. tumour progression and metastasis, embryogenesis, cell differentation, cell-cell and receptor-ligand interactions, viral and bacterial infectivity, and the control of the immune system [1–7]. Specific inhibitors of complex *N*-glycan formation are important for studies on *N*-glycan function and are of potential therapeutic interest. The most frequently employed inhibitors of *N*-glycosylation are the antibiotic

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tunicamycin and alkaloids like swainsonine, castanospermine, nojirimycin and deoxynojirimycin [8, 9]. Tunicamycin inhibits all *N*-glycan synthesis and the alkaloid inhibitors, with the exception of swainsonine, act at an early stage of *N*-glycan processing and inhibit synthesis of both hybrid and complex *N*-glycans. Swainsonine inhibits not only α-mannosidase II but also several other mannosidases, including lysosomal mannosidase; this non-specificity may cause unwanted side-effects in the clinical use of this inhibitor.

A specific inhibitor of a glycosyltransferase involved in complex *N*-glycan synthesis has as yet not been isolated from natural sources. It is therefore of interest to attempt the design and *de novo* synthesis of such inhibitors. Since there have been no reported X-ray crystallography studies of glycosyltransferases and no three-dimensional structures are available, specificity studies with substrate analogues present the only source of information for a rational design of glycosyltransferase inhibitors. The *N*-acetylglucosaminyltransferases which initiate complex *N*-glycan antennae (GlcNAc-transferases I-VI) [5, 10–12] are ideal targets for inhibitors of complex *N*-glycan synthesis. Active site directed inhibitors based on the oligosaccharide acceptors of these *N*-acetylglucosaminyl transferases should be specific inhibitors because the enzymes are highly specific towards their substrates. GlcNAc-T I and II are of special interest because their prior action is required before *N*-acetylglucosaminyl transferases III, IV, V and VI, α-mannosidase II and core α6-fucosyltransferase can act to complete complex *N*-glycan synthesis.

We now report on the substrate specificity of UDP-GlcNAc:Manα1-6R β(1-2)-*N*-acetylglucosaminyltransferase II (GlcNAc-T II; EC 2.4.1.143) which converts **1** to **3** (Fig. 1).

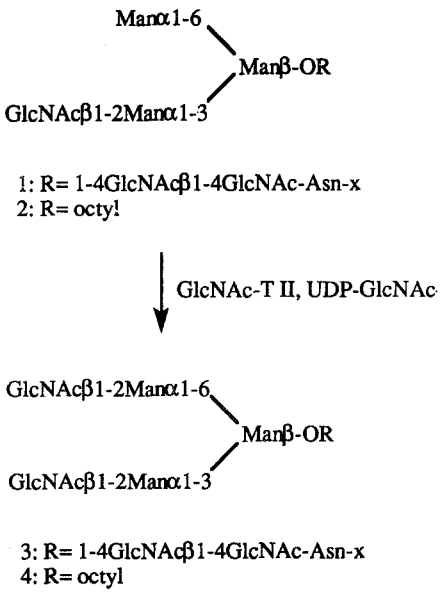


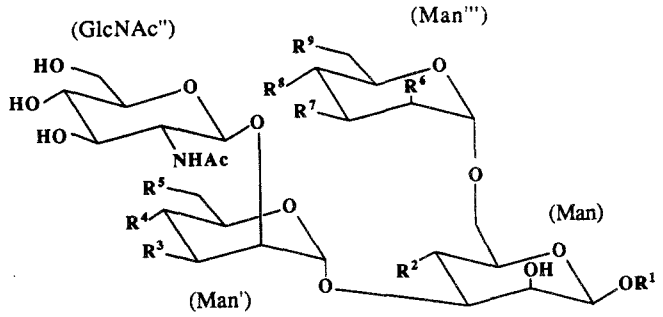
Figure 1. The reaction catalysed by GlcNAc-transferase II.

We have tested a variety of synthetic substrate analogues as substrates and inhibitors of the enzyme. These compounds were obtained by chemical and combined chemical-enzymatic synthesis using interchangeable modified saccharide building units which facilitated the synthesis; about 30 steps were required on average for every individual compound [13–17]. Similar procedures have been applied to other glycosyltransferases and have helped to delineate the substrate requirements of these enzymes [18–40]. Inhibitors obtained from this process can be used as enzyme active site probes and may prove of value in the treatment of diseases involving increased *N*-glycan branching.

Materials and methods

Materials

Rat liver GlcNAc-T II (11.8 U mg^{-1}) was partially purified as previously described [41]. UDP-[^{14}C]GlcNAc was synthesized [42] and diluted with UDP-GlcNAc (Sigma). Oligosaccharides (see Fig. 2 for structures) were synthesized as follows: compounds **2**, **8**, **14**, **16**, **19**, **21** and **23** [14, 16];



5: R^1 = octyl, R^6= O-methyl
6: R^1 = octyl, R^6= deoxy
7: R^1 = octyl, R^7= O-methyl
8: R^1 = octyl, R^7= deoxy
9: R^1 = octyl, R^7= O-pentyl
10: R^1 = octyl, R^7= O-aminopentyl
11: R^1 = octyl, R^7= O-(5-iodoacetamido)pentyl
12: R^1 = octyl, R^7= O-(4,4-azo)pentyl
13: R^1 = octyl, R^8= O-methyl
14: R^1 = octyl, R^8= deoxy
15: R^1 = octyl, R^9= O-methyl
16: R^1 = octyl, R^9= deoxy
17: R^1 = octyl, R^2 = O-methyl
18: R^1 = 1-4GlcNAc, R^2 = O-methyl
19: R^1 = octyl, R^2 = O-methyl, R^9= deoxy
20: R^1 = octyl, R^2 = deoxy
21: R^1 = octyl, R^3= deoxy
22: R^1 = octyl, R^4= O-methyl
23: R^1 = octyl, R^5= deoxy

24: GlcNAcβ1-2Manα1-3Manβ-O-octyl

Figure 2. Synthetic substrate analogues for GlcANc-transferase II. R groups which are not designated in the structure descriptions are OH groups.

compounds **2**, **6**, **7**, **9**, **10**, **11**, **12**, **13**, **15** and **22** [17, 43]; compounds **5**, **6** and **24** (H. Paulsen and M. Springer, in preparation); compounds **2**, **17** and **20** [13]; compound **18** [44]. All oligosaccharide structures have been characterized by ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Kinetic experiments

GlcNAc-T II was assayed radiochemically using Pasteur pipette columns of Dowex AG1-×8 (Cl^- form, 100–200 mesh) to separate labelled product from unreacted radiolabelled sugar-nucleotide donor UDP-GlcNAc [45]. Unless otherwise stated, the incubation mixture used for kinetic studies contained, in a total volume of 0.025 ml: 0.14 mU (12 ng) of enzyme (1 U is 1 μmol min^{-1}), 2.5–100 nmol acceptor, 88 nmol UDP-[^{14}C]GlcNAc (2500 dpm nmol^{-1}), 25 mM MES [2-(*N*-morpholino)ethane sulfonic acid monohydrate], pH 6.8, 13 mM $MnCl_2$, bovine serum albumin (25 μg), Triton X-100 (0.1%, by vol) and glycerol (4%, by vol). Samples were incubated for 10–60 min at 37 °C and the reaction was stopped by the addition of 0.4 ml ice cold water.

Photoinactivation of GlcNAc-T II with compound 12

Rat liver GlcNAc-T II (0.08 mU, 1 U is 1 μmol min^{-1}) was equilibrated at room temperature for 1 h in the presence of 1.8 mM UDP-[^{14}C]GlcNAc and compound **12** (at concentrations of 0.1, 0.2, 0.4, 1.0, 2.0, 4.0 and 8.0 mM respectively) as described for GlcNAc-T II enzyme assays under kinetic experiments 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 ($T_{1/2}$ for compound **12** was about 2.5 min, data not shown). Control incubations were carried out with compound **12** which had been previously inactivated by irradiation. After irradiation, acceptor substrate **20** (25 nmol) was added to the incubation and GlcNAc-T II activity was assayed as described above.

Inactivation of GlcNAc-T II with compound 11

Rat liver GlcNAc-T II (0.64 mU) was incubated with 0.35 μmol UDP-[^{14}C]GlcNAc and 0.4 μmol compound **11** at 37 °C in 0.2 ml of buffer containing 25 mM MES, 0.1% Triton X-100 and 15 mM $MnCl_2$. Control incubations were carried out with 0.4 μmol iodoacetamide instead of compound **11**. Aliquots (0.025 ml) were withdrawn at regular intervals and acceptor **20** (10–25 nmol) was added. After 1 h incubation at 37 °C, GlcNAc-T II activity was measured as described above.

Results and discussion

Acceptor specificity and reversible inhibition of GlcNAc-T II

All *N*-acetylglucosaminyl 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. For GlcNAc-T II, which converts **1** to **3** (Fig. 1), we have previously shown that an effective acceptor molecule must have a terminal Manα1-6 residue and a terminal GlcNAcβ1-2Manα1-3 branch [41, 46]. The *N*-acetylchitobiosyl and peptide moieties of the substrate are not essential for enzyme activity and may be substituted by a β-linked octyl group [20]. Modified derivatives of the minimal acceptor structure **2** (Figs 1 and 2) were therefore synthesized and tested as substrates and inhibitors for GlcNAc-T II (Table 1). Hydroxyl groups were substituted by deoxy or *O*-methyl groups to determine whether a certain hydroxyl group in **2** is necessary for binding and catalytic activity. This enabled us to design specific inhibitors for potential use in the labelling of the active site of GlcNAc-T II.

Table 1. Substrates and inhibitors of rat liver GlcNAc-T II.

Acceptor analogue	*Enzyme activity*[a]	K_M[b] (*mM*)	K_i[b] (*mM*)	V_{max}[b] (*μmol min^{-1} mg^{-1}*)	V_{max}/K_M
2	+	0.13		7.8	60
5	−		ND[c]		
6	−		0.13		
7	+	0.26		15.5	60
8	+	3.73		71.8	19
9	−		2.40		
10	−		2.53		
11	−		...[d]		
12	−		1.0[e]		
13	+	0.26		4.2	16
14	+	0.22		10.5	48
15	+	0.20		9.7	49
16	+	0.25		13.7	55
17	−		ND[c]		
18	−		ND[c]		
19	−		ND[c]		
20	+	0.48		8.8	18
21	−		ND[c]		
22	+	0.16		11.2	70
23	+	0.55		21.4	39
24	−		0.9		

[a] GlcNAc-T II assays were carried out as described in the Materials and methods section with 10 nmol acceptor.
+ stands for an enzyme activity greater than 2 μmol min^{-1} mg^{-1}.
− stands for an enzyme activity less than 0.1 μmol min^{-1} mg^{-1}.
[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] No inhibition detected when assayed with 0.4 mM acceptor **20**.
[d] Time dependent inhibition (Fig. 6); Lineweaver Burk plots were not linear (data not shown).
[e] Without UV irradiation.

The substrate specificity results are shown in Table 1 and Fig. 3. GlcNAc-T II transfers an *N*-acetylglucosaminyl residue in β-linkage to the 2‴-OH of the Man‴α1-6 unit

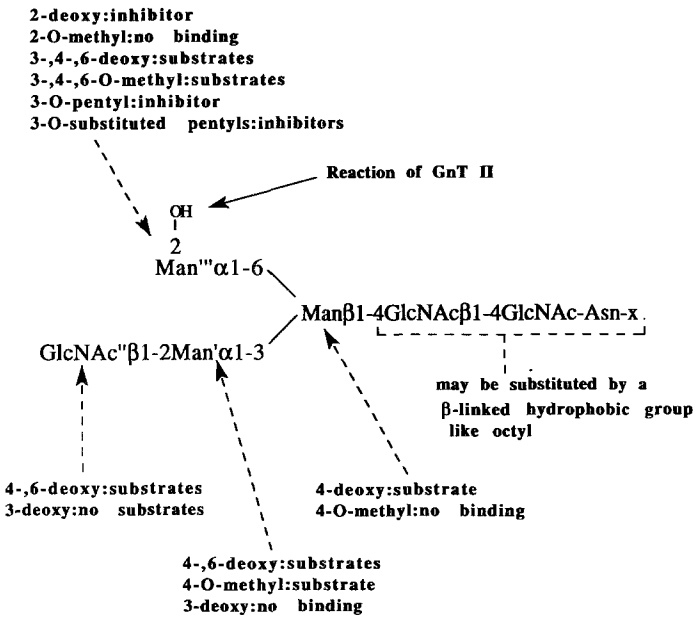


Figure 3. The substrate specificity of GlcNAc-transferase II.

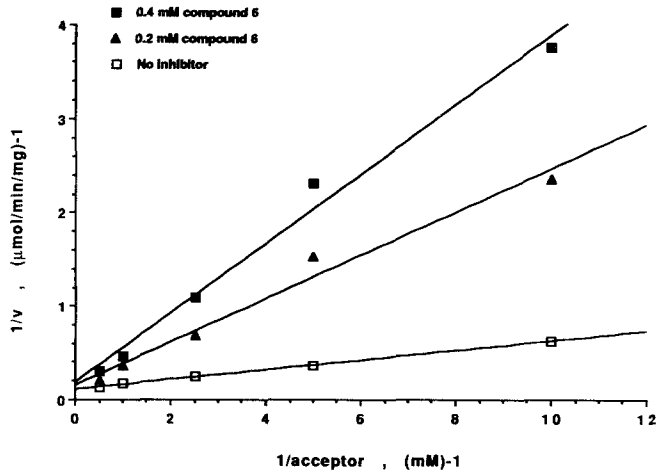


Figure 4. Effect of compound **6** on rat liver GlcNAc-T II activity. Rat liver GlcNAc-T II was assayed at several concentrations of the acceptor **20**, both in the absence of compound **6** (□) and at 0.2 mM (▲) and 0.4 mM (■) concentrations of compound **6**. Incubations were carried out with 3.5 mM UDP-[^{14}C]GlcNAc at pH 6.8, 37 °C, for 30 min. Reciprocal 1/v vs 1/S plots indicate competitive inhibition by compound **6**.

(Figs 2 and 3). The 2‴-deoxy compound **6** is a good competitive inhibitor with a binding constant similar to the substrate **2** ($K_{i6} = K_{M2} = 0.13$ mM, Fig. 4 and Table 1) showing that although the 2‴-OH is essential for catalysis, it is not required for binding. The 2‴-*O*-methyl compound **5**, however, is not an inhibitor and presumably does not bind to the enzyme because of steric hindrance.

The 3‴-, 4‴- and 6‴-deoxy analogues (**8**, **14** and **16**, respectively) are good substrates (Table 1). Compounds **14** and **16** show kinetic parameters similar to substrate **2** whereas compound **8** has higher K_M and V_{max} values (Table 1). The 3‴-, 4‴- and 6‴-*O*-methyl compounds **7**, **13** and **15** are substrates with K_M values similar to substrate **2** indicating that the relatively small methyl groups cause no steric hindrance to binding but the 4‴-*O*-methyl compound **13** does show a decreased V_{max} value. Although GlcNAc-T II transfers an *N*-acetylglucosaminyl residue to the 2‴-OH, it is surprising that neither the 2‴-OH nor the 4‴- and 6‴-OH are necessary for binding. Removal of the 3‴-OH (compound **8**) does reduce binding to the enzyme; this could be due to an involvement of the 3‴-OH in a hydrogen bond in the enzyme-substrate complex or to conformational changes of the Man‴α1-6 residue caused by deoxygenation at this position.

When the substituent at the 3‴-OH is further increased in size to a pentyl or substituted pentyl group (compounds **9**, **10**, or **12**), the increased steric hindrance prevents catalytic activity but does not prevent binding to the enzyme since these three compounds are all competitive inhibitors with K_i values from 1.0–2.5 mM (Table 1). This indicates that the 3‴-OH position on Manα1-6 is facing the binding site of the enzyme. This is an example of what Khan *et al.* [26] have called 'steric exclusion'; they obtained a competitive inhibitor for GlcNAc-T V by substitution of a non-essential hydroxyl group with *O*-methyl. Similarly, the 3‴-OH of GlcNAc-T II substrate is not essential for enzyme-mediated transfer of *N*-acetylglucosamine to acceptor since it can be removed or methylated without loss of enzyme activity. Larger substituents at the 3‴-OH position, however, prevent catalysis but not enzyme binding either by steric hindrance of substrate movements at the active site of the enzyme during catalysis or by preventing essential conformational changes of the enzyme. Methyl substitution at other OH positions can cause steric hindrance to binding, e.g. methyl groups at 2‴-OH (compound **5**) and at the 4-OH of the β-linked mannose residue (compounds **17**, **18** and **19**) prevent binding.

The 4-OH position of the β-linked mannose residue is of special interest because it has been suggested that substitution of this residue with *N*-acetylglucosamine by GlcNAc-T III may play an important control function as a stop signal in the synthesis of complex *N*-glycans [5, 10–12, 46]. Bisected structures with a GlcNAcβ1-4Manβ- branch are not substrates for GlcNAc-T II, IV and V, α-mannosidase II and core α6-fucosyltransferase and therefore cannot be further branched. The 4-OH group of the β-linked mannose is essential for GlcNAc-T I since the enzyme does not work on bisected structures nor on acceptors with a 4-deoxy-, 4-*O*-methyl- or 4-OH$_{ax}$ (talose) group at this position [29, 31]. This hydroxyl group is probably needed for a hydrogen bond in the binding site of GlcNAc-T I. The situation is different for GlcNAc-T II. The 4-deoxy compound **20** is a substrate whereas the 4-*O*-methyl compounds **17**, **18** and **19** show no binding (Table 1). These findings suggest that steric factors are responsible for the lack of activity of GlcNAc-T II towards bisected structures.

The 4-deoxy compound **20** is of interest as a specific substrate for the assay of GlcNAc-T II in the presence of GlcNAc-T III. GlcNAc-T III cannot act on **20** because the target acceptor hydroxyl group is not present.

The importance of the 2-OH group of the β-linked mannose residue for GlcNAc-T II binding has not as yet been investigated. This OH is essential for binding to GlcNAc-T I [29].

Removal of the 3′-hydroxyl group of the Man′α1-3 residue has a strong effect on substrate binding to GlcNAc-T II since the 3′-deoxy compound **21** is neither a substrate nor an inhibitor. The 4′- and 6′-hydroxyl groups of Man′α1-3 are, however, not required for binding, since the 4′-*O*-methyl compound **22** and the 6′-deoxy compound **23** are good substrates (Table 1). A 4′-deoxy compound has been synthesized [20] and found to be a substrate. Srivastava *et al.* [23] investigated the GlcNAc″β1-2 residue, which we did not study, and found that both the 4″- and 6″-deoxy compounds were substrates, but a 3″-deoxy compound was not a substrate.

Our present knowledge on the specificity of GlcNAc-T II towards acceptor oligosaccharide is summarized in Fig. 3. The 2‴-OH and 3‴-OH, and probably also the 4‴-OH, of the Man‴α1-6 residue face the active site of the enzyme since binding and catalysis are affected by substitutions of these hydroxyl groups. However, strong binding to the enzyme depends on the GlcNAc″β1-2Man′α1-3Manβ- moiety and requires the 3′-OH of the Man′α1-3 residue as well as the 3″-OH of the GlcNAc″β1-2 residue [23]. This view is supported by the finding that the trisaccharide **24**, which lacks the Man″α1-6 residue, nevertheless shows relatively strong binding to the enzyme with $K_i = 0.9$ mM whereas the trisaccharide Manα1-6(Manα1-3)Manβ-octyl and the disaccharide Manα1-6Manβ-octyl show no binding (data not shown). The GlcNAcβ1-2Manα1-3Manβ- moiety is an essential substrate requirement not only for GlcNAc-T II but also for GlcNAc-transferases III and IV, α-mannosidase II and core α6-fucosyltransferase [5, 10–12]. Substitution of the 3′-OH, 3″-OH and 3‴-OH by deoxy groups has a strong effect on binding to GlcNAc-T II, either preventing binding totally (3′-deoxy, 3″-deoxy) or reducing binding significantly (3‴-deoxy). It remains to be determined whether these hydroxyl groups are directly involved in binding to the enzyme or whether conformational changes in the deoxygenated oligosaccharides prevent binding.

Inhibition of GlcNAc-TII with compounds 11 and 12

The results from the specificity studies were used to design specific inhibitors for potential active site labelling of GlcNAc-T II. Reactive groups were attached to **2** to obtain active site-specific reagents. We decided to introduce reactive groups at the 3‴-position of the Man‴α1-6 residue. This position has several advantages over the other hydroxyl groups: a) it is near the active site of the enzyme during catalysis, b) binding is not prevented by a larger group like pentyl at this position, and c) a larger substituent like pentyl sterically blocks enzyme-mediated glycosylation of the compound in the presence of UDP-GlcNAc. The latter point is especially important in the case of relatively slow-reacting compounds like iodoacetamides because UDP-GlcNAc must be present for the compound to bind. We chose iodoacetamido and a photoreactive diazirino as reactive groups. Iodoacetamides tend to react with nucleophilic amino acids of the protein [47]. The diazirino group is an efficient photoreactive group and may react with any neighbouring group of the protein. Neither the diazirino derivative **12** nor the iodoacetamido derivative **11** was a substrate for GlcNAc-T II but both compounds showed specific inhibition of the enzyme.

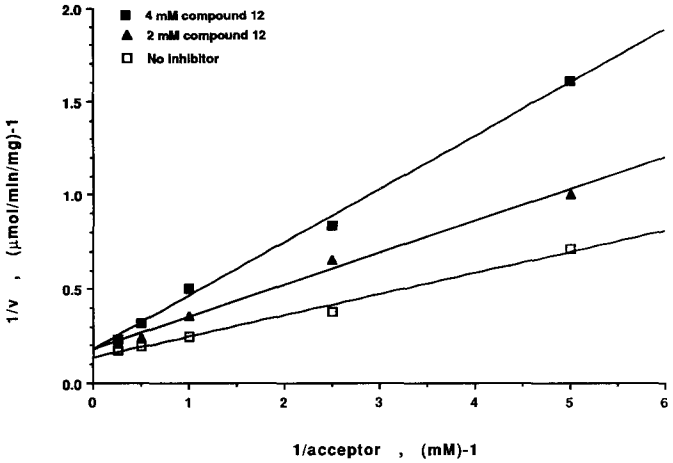


Figure 5. Effect of compound **12** on rat liver GlcNAc-T II activity in the absence of irradiation. Rat liver GlcNAc-T II was assayed at several concentrations of the acceptor **20**, both in the absence of compound **12** (□) and at 2.0 mM (▲) and at 4.0 mM (■) concentrations of compound **12**. Incubations were carried out with 3.5 mM UDP-[^{14}C]GlcNAc at pH 6.8, 37 °C, for 60 min. Reciprocal 1/v vs 1/S plots indicate competitive inhibition by compound **12**.

The photolabile diazirine **12** was found to be a competitive inhibitor of GlcNAc-T II ($K_i = 1.0$ mM, Fig. 5) in the absence of irradiation. On irradiation of **12** in the presence of GlcNAc-T II and UDP-GlcNAc, an additional 25% photoinhibition (at an inhibitor concentration of 3 mM or higher) could be observed (data not shown). The amount of photoinhibition could not be raised above 25% probably due to competition for binding sites on the protein by non-reactive photolytic decomposition products of **12**. The possible use of compound **12** for specific labelling of the active site is presently under investigation.

The iodoacetamide **11** shows time-dependent inactivation of GlcNAc-T II (Fig. 6). It is possible that this is due to a relatively slow formation of a covalent bond between inhibitor and enzyme but further work is required to establish this. After 6 h incubation of GlcNAc-T II with compound **11** in the presence of UDP-GlcNAc at 37 °C and pH 6.8, the enzymatic activity is decreased by 30% relative

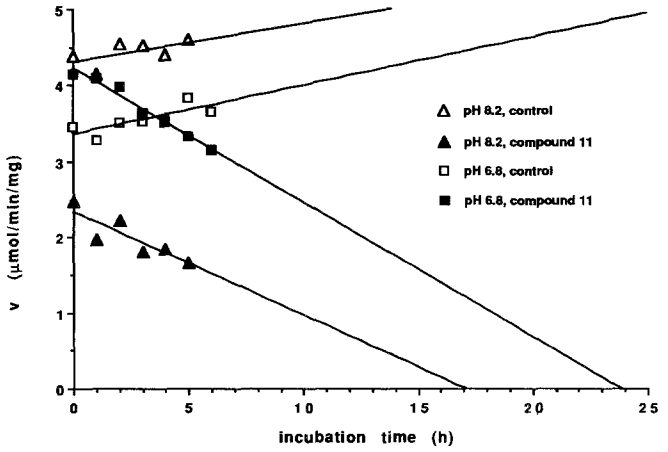


Figure 6. Effect of compound **11** on rat liver GlcNAc-T II activity. Rat liver GlcNAc-T II (0.64 mU) was incubated with 0.35 µmol UDP-[^{14}C]GlcNAc and 0.4 µmol **11** at 37 °C in 0.2 ml of buffer containing 25 mM MES, 0.1% Triton X-100 and 15 mM $MnCl_2$. Control incubations were carried out with 0.4 µmol iodoacetamide instead of **11**. Aliquots (0.025 ml) were withdrawn at regular intervals and acceptor **20** (10 or 25 nmol) was added. After 1 h incubation at 37 °C, GlcNAc-T II activity was measured as described in the Materials and methods section. GlcNAc-T II activity is plotted against time of incubation with iodoacetamide at pH 6.8 (□) and pH 8.2 (△), or with compound **11** at pH 6.8 (■) and pH 8.2 (▲). Concentration of compound **20**: 0.4 mM (▲); 1.0 mM (□, △, ■). Inactivation is slower at pH 6.8 (24 h) than at pH 8.2 (17 h).

to controls (Fig. 6); 80% inhibition is observed after 24 h incubation (data not shown). The inhibition is pH dependent; the relatively more rapid inactivation at pH 8.2 suggests that a nucleophilic group other than the -SCH_3 group of methionine is reacting with the iodoacetamido group [48]. Iodoacetamide, however, shows no effect on enzyme activity at the same concentration thus ruling out non-specific inactivation by compound **11**. Compound **11** may permit covalent labelling of the enzyme active site. Both compounds **11** and **12** can be readily prepared as radioactive derivatives by using UDP-[^{14}C]GlcNAc in the enzymatic glycosylation step used for their synthesis [17].

In summary, we have tested a series of synthetic substrate analogues of GlcNAc-T II as substrates and inhibitors of the enzyme. This information will be useful in the design of more effective GlcNAc-T II inhibitors. Such compounds should be useful in the study of complex *N*-glycan function and are potential therapeutic agents.

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