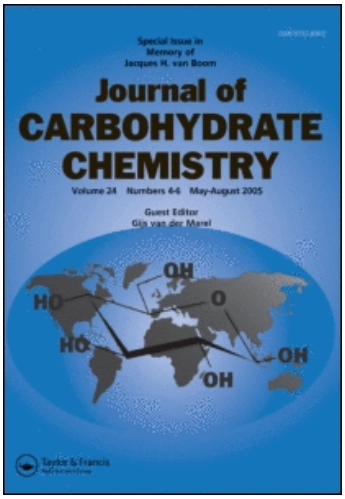

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COMMUNICATION

SYNTHESIS OF A LIBRARY OF β-GlcNAc GLYCOSIDES TO SCREEN FOR EFFICIENT IN VIVO GLYCOSYLTRANSFERASE ACCEPTORS

Yili Ding, Yoshiaki Miura, James R. Etchison, Hudson H. Freeze and Ole Hindsgaul*

The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA

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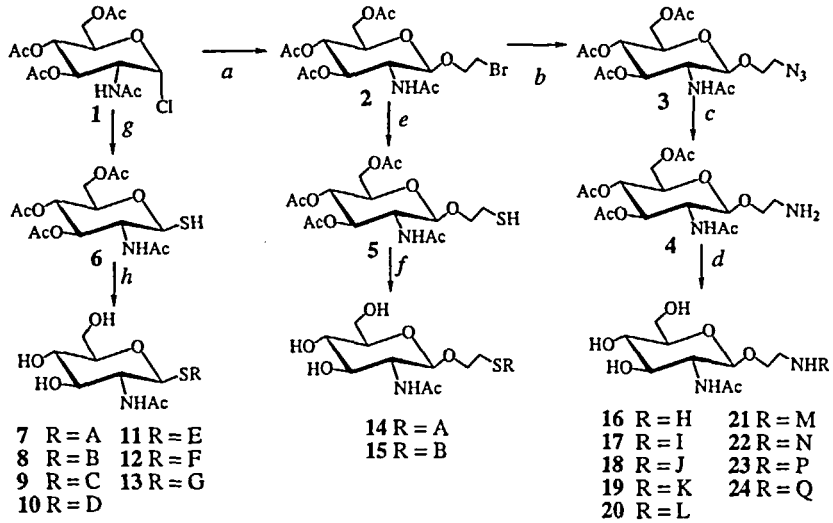
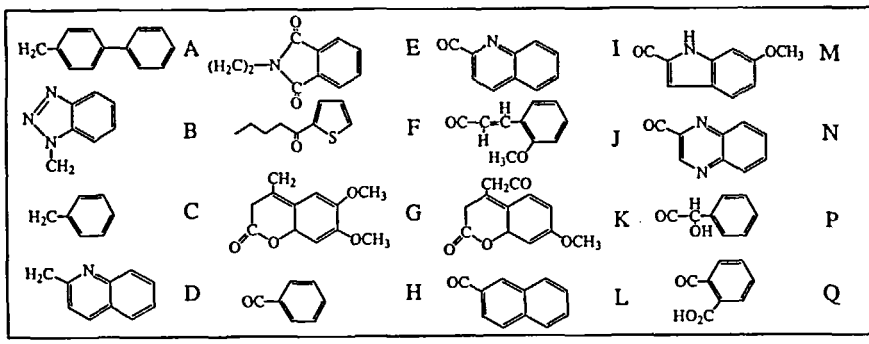
Artificial glycosides are used to prime the biosynthesis of glycosaminoglycan chains and those typical of *N*- and *O*-linked oligosaccharides, e.g., *N*-acetyllactosamine repeats.[1-8] Several studies have shown that the aglycon influences the amount and type of products assembled on such glycosides.[2-6] The amount of product made depends on two factors. The first is the ability of the glycoside to penetrate the plasma membrane and the Golgi membrane where the glycosyltransferases are located. The second is the structure of the aglycon since it can influence the addition of the first monosaccharide as well as extension with additional residues. In some instances, the aglycon is thought to mimic a portion of an underlying polypeptide chain.[2-4,6] The choice of aglycons is important, but there are no guidelines to direct the choice since many factors such as their stability to glycosidases, membrane-permeability, cellular toxicity or limitation of specificity and affinity can all be important. In the absence of such guidelines, we streamlined the process of aglycon evaluation and report here an efficient strategy to synthesize GlcNAcβ-R libraries with various aglycons.

The synthetic strategy used is summarized in Scheme 1. 2-Acetamido 3,4,6-tri-*O*-acetyl 2-deoxy-α-D-glucopyranosyl chloride (**1**) was transformed to the corresponding β-linked 2-bromoethyl glycoside **2** under Koenigs-Knorr conditions[10] in 69% yield, then reacted with NaN_3 in DMF to form compound **3** in 62% yield. After reduction of the azide with H_2S/pyridine, the amine **4** was ready for acylation using structurally diverse commercial acylating agents. Replacement of the bromine in **2** with thioacetate, by reaction





Scheme 1 *a*: $HO(CH_2)_2Br$, $Hg(CN)_2$, Toluene, 69%. *b*: N_3Na, DMF, 62%. *c*: H_2S/pyridine; 80%. *d*: 1, RCOOH, DCC; 2, NaOMe/MeOH. *e*: 1, KSAc, DMSO, rt, 2 h; 2, NH_2NH_2/HOAc, DMF; 65% (two steps). *f*: 1, RX, Cs_2CO_3, DMF; 2, NaOMe/MeOH. *g*: 1, $CS(NH_2)_2$, acetone; 2, $K_2S_2O_7$, $CHCl_3/H_2O$. 52% (two steps). *h*: 1, Cs_2CO_3, RX, DMF; 2, NaOMe/MeOH.

with KSAc in DMF, followed by selective *S*-deacetylation with NH_2NH_2·AcOH, afforded thiol **5** in 65% yield, which was reacted with selected alkyl halides to afford GlcNAc derivatives. Compound **1** could also be transformed directly into the 1-thio derivative **6** in 52% yield by an established procedure,[11] then coupled with alkyl halides to form sulfur-linked GlcNAc derivatives. The aglycons selected have hydrophobic properties and UV absorption to simplify the purification and detection of glycosylation products. After deprotection, GlcNAc glycosides **7-24** were obtained in 20-30% overall yield from compound **1**. Their structures were confirmed by their NMR and MS data (Table 1).

Table 1. Spectral data for selected new compounds

Compound	^{1}H NMR (δ ppm, H-1): (d, 1H, Hz) (300MHz, CD_3OD)	^{13}C NMR (δ ppm, C-1): (300MHz, CD_3OD)	MS (*m/z*): $[M+Na]^+$
7	4.23 (J = 10.2)	84.8, 32.6 (SCH_2)	406 (100%)
8	4.45 (J = 10.8)	84.4, 47.7 (SCH_2)	391 (25%)
9	4.26 (J = 10.8)	84.2	350 (100%)
10	4.42 (J = 10.2)	84.9, 37.0 (SCH_2)	401 (70%)
11*	4.40 (J = 10.2)	84.5, 37.7 (SCH_2)	433 (100%)
12	4.50 (J = 10.5)	85.7, 38.8 (SCH_2)	412 (100%)
13	4.40 (J = 10.2)	84.5, 30.7 (SCH_2)	478 (100%)
14	4.24 (J = 9.0)	102.7	470 (30%)
15	4.40 (J = 8.7)	102.9	435 (15%)
16	4.45 (J = 8.7)	103.0	442 (100%)
17	4.44 (J = 8.7)	103.0	441 (100%)
18	4.42 (J = 8.1)	102.9	391 (100%)
19	4.32 (J = 8.1)	101.2	447 (100%)
20	4.40 (J = 7.8)	102.1, 56.7 (OCH_3)	503 (100%)
21	4.44 (J = 8.4)	102.9, 56.2 (OCH_3)	460 (30%)
22	4.47 (J = 8.4)	103.1	443 (45%)
23	4.42 (J = 8.7)	102.8	421 (50%)
24	4.42 (J = 8.4)	102.9	407 (25%)

* The NMR spectra were recorded in DMSO-D_6

Each of the β-GlcNAc glycosides was tested for its ability to serve as a biosynthetic acceptor when incubated with CHO cells in the presence of [6-^{3}H] galactose.[12] Figure 1 shows the results of labeled glycosides that were secreted into the medium. GlcNAc-β-benzyl (GnBn), GlcNAc-β-MU (GnMU) and GAP is GalNAc-α-D-phenyl (GAP) were used as positive controls, since they were previously shown to be acceptors for galactose addition, and in the case of GlcNAc-β-benzyl, for the addition of poly-*N*-acetyl lactosamine repeats.

The aglycon strongly influenced both the amount of galactosylation and the structures of the products made. The best acceptor both in terms of amount and variety of products was benzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (**9**) which produced both sialylated and non-sialyled polylactosamine chains which can be found on naturally occuring *N*-glycans made by these cells.[8] Most of the glycosides were suitable for the addition of a single Gal residue, and in some cases, 10-30% of the product was sialylated (**10, 12, 20**).

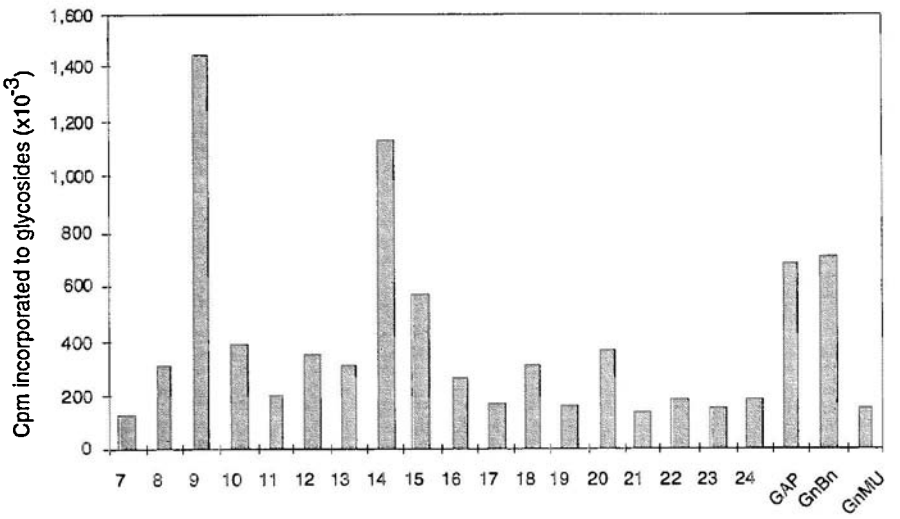


Figure 1. Biosynthesis of [6-^{3}H]-galactose-labeled glycoside products by CHO cells. The bars indicate the amount of radiolabelled products.

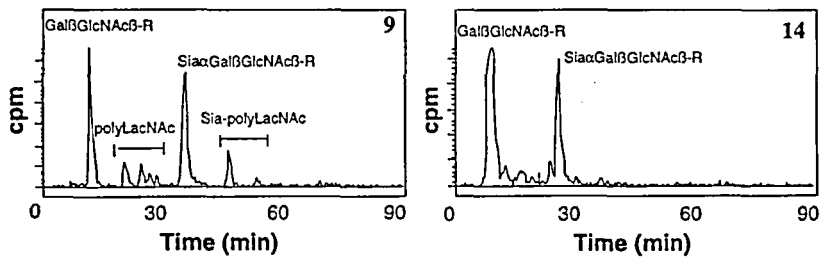


Figure 2. The elution position of most of the glycoside products biosynthesized in CHO cells was typical of the standard Galβ1,4GlcNAc-MU indicating the addition of a single β-D-Gal. However, **9** clearly formed both and non-sialylated and sialylated polylactosamine (polyLacNAc) chains based on sequential enzymatic digestions with sialidase, β-galactosidase and β-hexosaminidase (or endo-β-galactosidase). In addition, in some cases their LacNAc products were also sialylated based on sialidase digestions (such as **14**).

In summary, the screening of a synthetic GlcNAc library containing various aglycons showed that thio-linked benzyl-β-D-GlcNAc was the most efficient biosynthetic acceptor and produced the greatest variety of galactosylated products when incubated with CHO cells.

ACKNOWLEDGEMENTS

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12. Each of the compounds was tested for its ability to serve as a primer for oligosaccharide biosynthesis in Chinese hamster ovary cells transfected with Core 2 GlcNAc transferase[13]. Cells were incubated in 2 mL α-MEM medium containing 20 Ci/mL [6-^{3}H] galactose for 18 h along with each compound at 0.4 mM. During the incubation, the glycosides diffuse into the cells and the Golgi apparatus where they can serve as acceptors for one or more enzymes. Galactose is the first sugar added to an available GlcNAcβ-R. The ^{3}H-labeled glycosides are rapidly secreted into the medium and were isolated after first passing the medium through a 3×0.5 cm column of QAE-Sephadex. This step removes the phenol red indicator dye and allows molecules with up to 3 negative charges to pass through. The runthrough material was then bound to a C-18 Sep Pak cartridge washed with 3×2 mL of ammonium formate and 1 mL of water. The cartridge was washed with 50, 70 and 100% MeOH and aliquots were monitored for radioactivity using a scintillation counter. Nearly all glycosides eluted with 50% MeOH. Each of the products was analyzed by combined amine-adsorption anion exchange HPLC.[1,9]
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