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6-Azido D-galactose transfer to *N*-acetyl-D-glucosamine derivative using commercially available β-1,4-galactosyltransferase

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Abstract

A new strategy to tag glycoproteins carrying terminal GlcNAc was developed using commercially available bovine β-1,4-galactosyltransferase (GalT) and UDP-6-azidogalactose. The azide function was then allowed to react via a biotinylated Staudinger–Bertozzi probe demonstrating the usefulness of such a procedure to tag any glycoprotein possessing a *N*-acetylglucosamine terminal residue from any type of cell lysate.
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Many proteins are subject to post-translational glycosylation, which in turn generates structural diversity required to perform necessary physiological task.[1] Proteomic strategies typically do not distinguish among glycoforms, and simply catalog the abundance of protein, whether modified or not. Identification of glycosylated proteins requires that they could be separated from complex mixtures that contain both unglycosylated and heterogeneously glycosylated proteins.[2,3] Historically, physical separation has been accomplished by affinity chromatography using lectins or antibodies that are specific for special glycan epitopes.[4]

In newer methods, labels can be introduced either by the exploitation of glycoprotein-specific reactions, that is, the reaction of 1,2 diol with boronic acid to form boronic diesters[5,6] or enzymatical introduction of a bioorthogonal functional group on glycoprotein of interest[7–11] or by using the cell's own biosynthetic machinery.[12–16] After chemical tagging, covalent modification with a highly selective probe enables glycoprotein isolation from crude preparations. This two step labeling process can be used to outfit a target molecule for detection or for isolation, depending on the nature of the probes.

In this Letter, we report a new strategy, readily adaptable to any cell or tissue type to tag glycoprotein carrying terminal GlcNAc on their *O*- or *N*-glycans using commercially available β-1,4-galactosyltransferase (GalT). These GlcNAc-terminated proteins can be found in all higher eukaryotic organisms and are particularly abundant in N-linked glycans in plant proteins.[17] This is a post-lysis method involving biosynthetic and chemical manipulations that are completed only after cell lysis, introducing a modified carbohydrate bearing a chemical reporter. As a chemical reporter, azido group was chosen for its stability and complete lack of reactivity toward all functional groups present in biomolecules.[18] Although kinetically stable, azides are predisposed to unique modes of reactivity owing to their large intrinsic energy content. Bioorthogonal reactions have thus been developed, including the Staudinger ligation of azides with functionalized phosphines[19] and the [3+2] cycloaddition of azides with activated alkynes.[20,21]

For our purpose, we chose to introduce the azide functionality at the C-6 position of the galactose ring because

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† The two first authors participated equally to this work by being, respectively, involved in the synthesis of activated probes and their application to oligosaccharide derivation.

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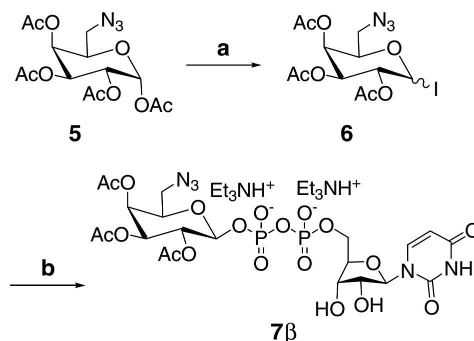
Eling and co-workers showed that a biotin moiety appended to the C-6 position of UDP-D-Gal could serve as a substrate for human GalT.[9] Thus, sugar nucleotide **1** of 6-azido-6-deoxy D-galactose was synthesized (see Fig. 1).

Two strategies could be envisioned to prepare pyrophosphate **1** (UDP-GalN_3). The first one, essentially developed by Hindsgaul's group,[22] is based on the reaction between an activated galactose moiety with uridine diphosphate.[23] In the second methodology, first developed by Moffatt and Khorana,[24] galactose-1-phosphate derivatives would react with uridine-5′-phosphoromorpholidate **10**. Even if the first methodology was often lower yielding, desired products as anomeric mixtures could be more rapidly obtained.

First, 6-azido-6-deoxygalactose **3** was prepared, starting from diisopropylidene galactose and following modified Hindsgaul and co-workers procedure (Scheme 1).[25] In our case, we could not use benzyl as hydroxyl protective group due to deprotection conditions which would affect the azide function. Hydroxyl groups were temporarily protected as their trimethylsilyl ethers. Anomeric formation of the iodide and reaction with tetrabutylammonium salt of uridine diphosphate followed by trimethylsilyl deprotection and purification[26] delivered, in our hands, GalT-substrate **1** in poor yield and as a mixture of α,β-anomers.

One of the problems, in the previous synthesis, is the extreme lability of the trimethylsilylether protection, rendering impossible purification and isolation of the intermediate iodide. More stable protecting group acetate was then tried (Scheme 2). In this case, iodide **6**[27] could be isolated and reaction with tetrabutylammonium salt of uridine diphosphate afforded sugar nucleotide **7** as a unique β-anomer. This anchimeric assistance surprised us under the slightly basic reaction conditions used, but at the same time Jakeman and co-workers observed the same feature in the fucose and mannose series.[28] Even if the β stereoselectivity observed was total, it was useless for our purpose as the GalT could only use α-anomer as the substrate.



Scheme 2. Synthesis of β-anomer of UDP derivative. Reagents and conditions: (a) TMSI, BiI_3, CH_2Cl_2, 0 °C, 30 min; (b) (i) $(Bu_4N)_2$–UDP; (ii) HPLC C18, 4% (2 steps).

We thus turned our attention toward the second methodology, developed by Moffatt and Khorana and improved by Wong et al.[24] The first part of this pathway was the synthesis of the azidogalactosyl phosphate **9** (Scheme 3). Depending on the phosphorylating agent used, it would be possible to favor one of the anomers of **9**. Using 2-chloro-4*H*-benzodioxaphosphorin-4-one **8**[29] or tricoordinated phosphorous (PIII) reagent,[30,31] the configuration of the anomeric centre could be controlled and almost only α-anomer of **9** was isolated. **9α** was then reacted with uridine-5′-phosphoromorpholidate **10** to yield after purification GalT-substrate **1** exclusively under its α anomeric form.

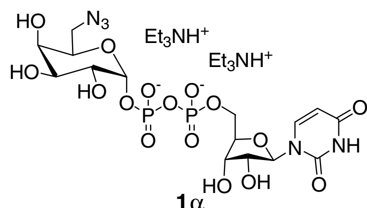
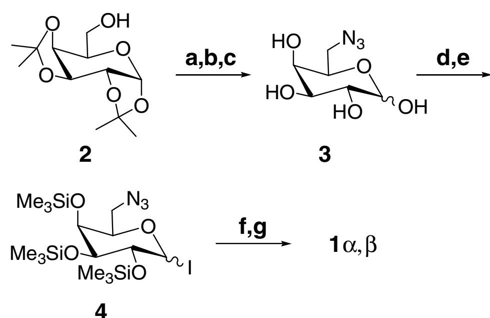
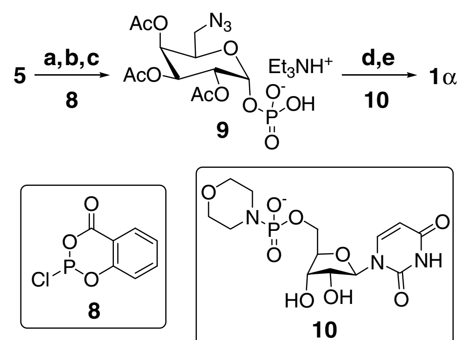


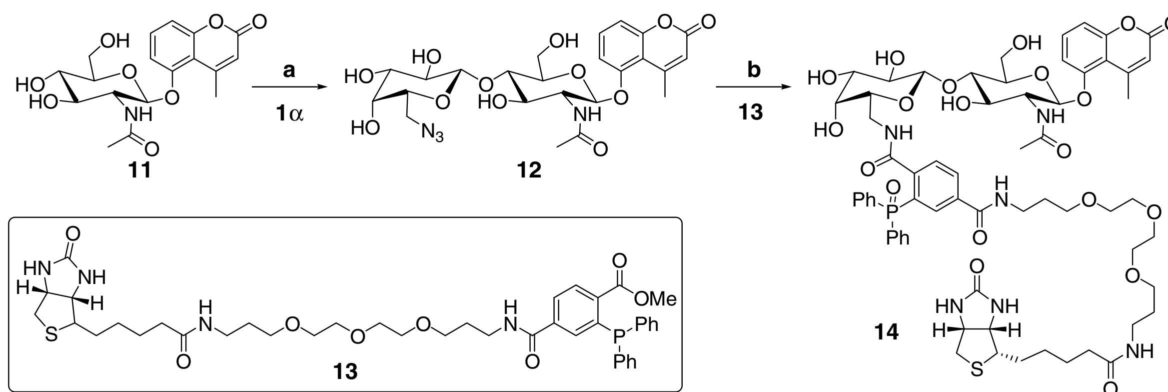
Fig. 1. Sugar nucleotide **1** of 6-azido-6-deoxy D-galactose.



Scheme 1. Synthesis of the mixture of anomers of sugar nucleotide **1**. Reagents and conditions: (a) MsCl, DMAP, CH_2Cl_2, 0 °C; (b) NaN_3, Bu_4NBr, DMF, 110 °C, 5 d, 87% (2 steps); (c) HCl, 0.06 M 80 °C, 16 h, 85%; (d) TMSCl, pyridine, 0 °C, 1 h; (e) TMSI, CH_2Cl_2, 0 °C, 30 min; (f) $(Bu_4N)_2$–UDP, overnight; (g) (i) Bu_4NF, H_2O, 90 min; (ii) alkaline phosphatase, overnight; (iii) HPLC C18, 2. 5% (4 steps).



Scheme 3. Synthesis of α-anomer of UDP analogue **1**. Reagents and conditions: (a) CH_3COONH_4, DIEA, DMF, 2 d, 85%; (b) (i) 2-chloro-4*H*-benzodioxaphosphorin-4-one **8**, Et_3N, dioxanne, THF, 0 °C, 20 min; (ii) H_2O, 74% (2 steps); (c) *t*-BuOOH (2 equiv), I_2 cat., THF, 2 d, 74%; (d) **10**, 1*H*-tetrazole, pyridine, 5 d, 46%; (e) (i) Et_3N, NH_4HCO_3, CH_3OH, 1 d; (ii) HPLC C18, 53%.



Scheme 4. Coupling of 6-azido-6-deoxy D-galactose to a GlcNAc-terminated substrate **11** and activation through a Staudinger ligation. Reagents and conditions: (a) **1α**, **11**, β-1,4-galactosyltransferase from bovine milk, alkaline phosphatase, $MnCl_2$, glycine buffer, 37 °C, 2 d; (b) **13**, THF/H_2O (5/95, v/v), rt, overnight.

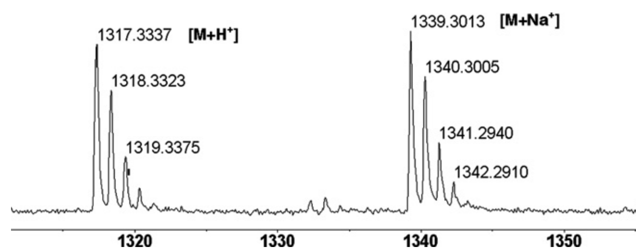


Fig. 2. MALDI-TOF spectrum of compound **14**.

Having UDP-$GalN_3$ **1α** in hands, we examined the ability of GalT to transfer this activated sugar onto a GlcNAc substrate such as 4-methyl umbelliferyl *N*-acetyl β-D-glucosaminide **11** (MU-GlcNAc).[32] Using wild-type bovine GalT, transfer of $GalN_3$ could be observed by MALDI-TOF MS analysis of the resulting product.[33] The resulting azido-labeled disacccharide **12** was then submitted to a Staudinger ligation with a biotinylated phosphine[34] **13**. MALDI-TOF MS analysis indicated that the oligosaccharide was quantitatively converted into its biotin-labeled derivative **14** (Scheme 4 and Fig. 2) in mild conditions as required for in vitro or in vivo applications to biological molecules.

To conclude, we have developed a new strategy, to tag glycoprotein carrying terminal GlcNAc. Using commercially available bovine β-1,4-galactosyltransferase (GalT), we could transfer 6-azidogalactose onto the *N*-acetylglucosamine residue of MU-GlcNAc. The azide function was then allowed to react via a Staudinger–Bertozzi rearrangement with a biotinylated probe demonstrating the usefulness of such a procedure to tag any glycoprotein possessing a *N*-acetylglucosamine terminal residue from any type of cell lysate.

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Supplementary data

Supplementary data (experimental part and spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.018.

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32. In vitro labelling of MU-GlcNAc with 6-azido D-galactose: 200 μg of UDP 6-azido D-galactose **1α** was incubated in 100 mM glycine buffer, pH 8.4, containing 10 mM $MnCl_2$ with 10 μg of MU-GlcNAc **11** (Aldrich chemicals) and 50 mU of β-1,4-galactosyltransferase from bovine milk (Aldrich chemicals) for 48 h at 37 °C. Alkaline phosphatase (50 U) (Aldrich chemicals) was added to hydrolyse UDP. Disaccharide **12** was purified on a carbograph LudgerClean™ EB10 cartridge by elution with a 1/1 H_2O/CH_3CN mixture (Ludger).
33. MALDI mass spectrometric analysis: Labelling and coupling reactions were monitored on an Applied Biosystems Voyager-DE Pro time-of-flight mass spectrometer. The accelerating voltage was 20 kV, guide wire 0.002%, delay time 100 ns and grid voltage 80%. The instrument was operated in reflector mode under positive ion conditions. A nitrogen laser was used at 337 nm with 300 laser shots averaged per spectrum. 2,5-dihydroxybenzoic acid (Aldrich chemicals) was used as the matrix for all experiments. The matrix concentration was 5 mg/mL in acetonitrile/trifluoroacetic acid 0.1% (3/7). Sample (1 μL) was spotted onto the MALDI target followed by 1 μL of matrix. The mixture was then allowed to air dry prior to analysis. Calibration was performed using instrument default settings and data analysis was carried out using Data Explorer software.
34. Staudinger ligation between azido-labelled disaccharide and biotinylated phosphine: 200 μg of biotinylated phosphine **13** was incubated overnight at rt with azido-labelled disaccharide **12** in a water/THF mixture (95/5, v/v).