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Enzymatic Glycosylation of O-Glycopeptides

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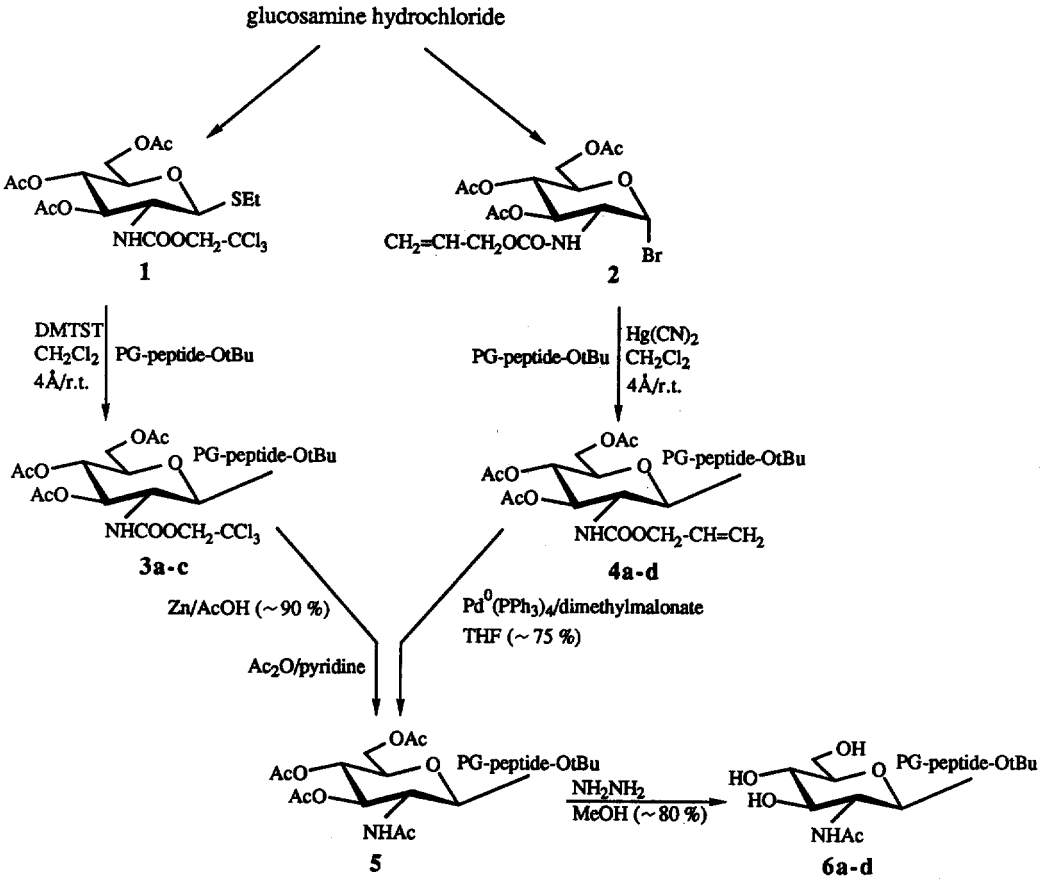
Abstract: O-Glycosylation of serine derivatives carried out with N-urethane protected glucosamine yields O-glycopeptides which are regio- and stereoselectively galactosylated with the aid of β-1,4-galactosyltransferase (EC 2.4.1.22).

Chemical syntheses of glycopeptides require a complex methodology of multi-step procedures, in order to protect and selectively deprotect the various functionalities. Especially, the regio- and stereoselective formation of the intersaccharidic bond poses great synthetic demands. In this context the use of glycosyltransferases promises a number of advantages. Characteristically, these enzymes catalyze reactions in a mild and stereoselective way and accept a broad range of substrates. Among these biocatalysts, the commercially available β-1,4-galactosyl-transferase (EC 2.4.1.22) is most thoroughly investigated. Its preparative value was successfully proven by galactosylations of oligosaccharides[1] and N-glycopeptides.[2,3] We here report on the preparative enzymatic galactosylation of O-glycopeptides derived from glucosamine. Glycoproteins which contain this linkage region widely occur in subcellular fractions of vertebrates.[4] For this reason, the chemical synthesis of the required substrates, *i.e.* β-N-acetylglucosamine serine and threonine conjugates, was another matter of interest.

The majority of O-glycopeptides of the N-acetyl-glucosamine type have been synthesized *via* haloglycosides[5,6] or oxazolines.[7] Both methods are characterized by acidic conditions and long reaction times and give only low yields of the desired product as far as tert-butyl protected amino acids are applied. Progress in this field was achieved by the use of urethane protection for the glucosamine. In this sense, by application of the known N-allyloxycarbonyl (Aloc) glucosaminyl bromide **2**[8] we achieved an efficient stereoselective glycosylation of differently protected amino acids and dipeptides.

Since the donor **2** is unstable and requires activation by heavy metal salts, we investigated the glucosamine thioglycoside **1** as an alternative glycosyl donor. It was synthesized stereoselectively according to known procedures [8] using the trichloroethoxycarbonyl (Teoc) group instead of Aloc in order to avoid electrophilic attack at the double bond during the glycosylation step.[9] Thiophilic activation of **1** was achieved using dimethylmethylthiosulfonyltriflate (DMTST)[10] and the desired glycopeptides **3a-c** were obtained stereoselectively in yields up to 85%. The amino group of the glucosamine part was deblocked by Pd(0)-catalyzed removal of the Aloc group[8,11] from compounds **4a-d** or reductive elimination of the Teoc group from the derivatives **3a-c**, respectively. After conventional N-acetylation, O-deacetylation of the saccharide part was carried out with hydrazine/methanol.[12] NMR-data and elemental analysis of such isolated compounds were in agreement with the assumed structures.



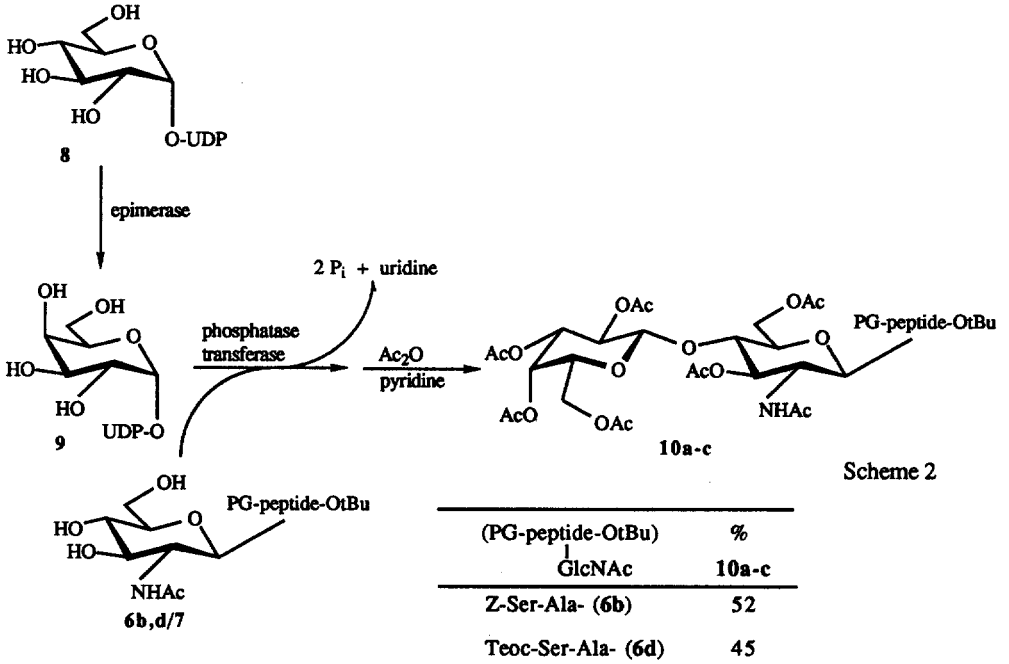
The obtained results (Table 1) prove the 2-N-Teoc/1-thioethyl derivative **1** to be the more stable and efficient glycosyl donor. The products of which are easily converted to the N-acetylated compounds by a two step-one pot reaction. Furthermore the N/O-acetylated Z-Ser-Ala derivative could readily be deprotected at the N-terminus using standard procedures and subsequently elongated with Z-Ala using carbodiimide/HOBT as activating agents to yield **7** after O-deacetylation with hydrazine/methanol.

Table I. Glycoside formation with donors **1** and **2**

Acceptor:		Glycosides: **3a-c** %		Glycosides: **4a-d** %
Z-Ser-OtBu	Donor **1**	85	Donor **2**	45
Z-Ser-Ala-OtBu		65		54
Z-Thr-OtBu		55		21
Teoc-Ser-Ala-OtBu		--		55

Enzymatic galactosylation of different substrates was perfomed by in situ generation of UDP-galactose **9** from UDP-glucose **8** (UDP-glucose-4´-epimerase, EC 5.1.3.2) at pH 7.4 and 37°C. Alkaline phosphatase from calf intestine (EC 3.1.3.1) was employed to destroy transferase inhibitors and, thus, facilitates the equilibrium in the product direction.[13]

In a typical procedure 36 µmol of O-deactylated substrate and 50 µmol UDP-Glucose were placed in a 2.0 ml micro test tube and dissolved in 1.1 ml of cacodylate buffer (50 mM) containing 1mg BSA, $MnCl_2$ (0.1mM), and NaN_3 (0.3 mM). After the addition of 1.5 U epimerase, 6 U phosphatase and 0.5 U galactosyltransferase the mixture was gently shaken at 37°C for 48 h at pH 7.4.(TLC: methanol/ethylacetate = 1:2; n-propanol/acetic acid/water = 30:4:1) Subsequent to centrifugation the solution was lyophilised and the resulting residue was acetylated with acetic anhydride in pyridine (1/2 (v/v)). Purification was achieved with flash chromatography (CH_2Cl_2/MeOH) and preparative HPLC (RP 18, methanol/water).[14]



Scheme 2

(PG-peptide-OtBu) GlcNAc	% 10a-c
Z-Ser-Ala- (**6b**)	52
Teoc-Ser-Ala- (**6d**)	45
Z-Ala-Ser-Ala- (**7**)	56

This method delivered different O-lactosamine derivatives of serine. It is notworthy that the O-glycosyl derivatives of threonine and serine ester (**6a**, **6c**) showed reduced reactivity. Consequently, the purification of their products revealed to be more difficult.

Although large scale enzymatic glycosylations are still limited by the availability of nucleotide sugars[15], the reported results obtained with O-glycopeptides again prove β-1,4-galactosyltransferase to be a useful tool in O-glycoconjugate synthesis which accepts a wide range of substrates. Especially the difference between the N- and O-glycosidic linkage does not seem to affect the activity of β-1,4-galactosyltransferase to a greater extent.

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14. **10a** : 100.6-MHz-^{13}C-NMR ($CDCl_3$), δ (ppm) : 18.2 (CH_3-Ala); 20.8, 20.9 ($\underline{C}H_3COO$-); 23.1 (CH_3CONH-); 27.9 (($\underline{C}H_3$)C-); 82.2 (((CH_3)$\underline{C}$-); 101.1, 101.2 (C-1, C-1)'); 128.0, 128.3, 128.5 (C-aromat.); 136.1 ((C-aromat.)$_{ipso}$); 156.5 (CO-urethane). $[\alpha]_D^{22}$=5.8 (c= 0.7, $CHCl_3$).

    10b : 400 MHz-^{1}H-NMR ($CDCl_3$), δ (ppm) : 1.36 (d, J_{vic}=7.1 Hz, 3 H, Ala-CH_3); 1.46 (s, 9 H, (CH_3)C-); 1.92, 1.95, 2.03, 2.05, 2.09, 2.13 (6s, 21 H, CH_3COO-/$C\underline{H}_3CONH$-); 4.70 (s, 2 H, CCl_3CH_2); 4.95 (dd, $J_{3/4}$=3.3 Hz; 1 H, (H-3)'); 5.34 („d", $J_{4/3}$=3.2 Hz, 1 H, (H-4)'); 5.98, 6.04 (2d, 2 H, NH-urethane/$CH_3CO\underline{N}H$-); 6.93 (d, $J_{NH\text{-}Ala/\alpha\text{-}CH}$=7.0 Hz, 1 H, NH-Ala). 100.6-MHz-^{13}C-NMR ($CDCl_3$), δ (ppm) : 18.4 (CH_3-Ala); 20.5, 20.6, 20.8 ($\underline{C}H_3COO$-); 23.3 ($\underline{C}H_3CONH$-); 28.0 (($\underline{C}H_3$)C-); 74.8 (Cl_3C-$\underline{C}H_2$); 82.4 (Cl_3C-); 101.1, 101.4 (C-1/(C-1)'); 154.2 (CO-urethane). $[\alpha]_D^{22}$=3.6 (c=0.5, $CHCl_3$).

    10c : 100.6-MHz-^{13}C-NMR ($CDCl_3$), δ (ppm) : 18.1 (2x CH_3-Ala); 20.8, 20.9 ($\underline{C}H_3COO$-); 23.2 (CH_3CONH-); 28.0 (($\underline{C}H_3$)C-); 82.1 (((CH_3)$\underline{C}$-); 101.3, 101.5 (C-1/(C-1)'); 127.7, 128.0, 128.6 (C-aromat.); 136.4 ((C-aromat.)$_{ipso}$); 156.3 (CO-urethane). $[\alpha]_D^{22}$=9.7 (c=0.6, $CHCl_3$).
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