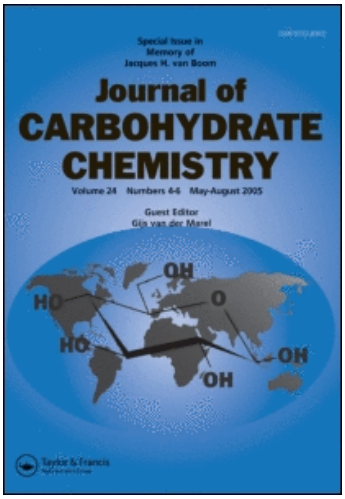

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COMMUNICATION

ENZYME-CATALYZED SYNTHESIS OF O-GLYCOPEPTIDE BUILDING BLOCKS

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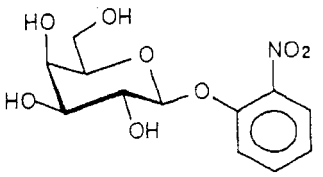
In 1988, MOSBACH *et al.*[1] reported the synthesis of α-D-mannopyranosyl-L-serine and α-D-*N*-acetylgalactosamino-L-serine by reversing the hydrolytic activity of α-mannosidase from jack beans and α-*N*-acetylgalactosaminidase from beef liver, respectively. The yields in these equilibrium-controlled syntheses[2] using high concentrations of monosaccharide and serine were highly dependent on the enzyme concentration and ranged from 5 to 10 percent in both cases.

Quite recently, CANTACUZENE *et al.*[3] reported the β-D-galactosidase-catalyzed transfer of the galactosyl moiety of lactose to some protected serine derivatives. The best yields (15 %) in these kinetically controlled syntheses[2] were observed with Boc-Ser-OMe.[4] With Z-Ser-OBz, Ac-Ser-OMe, and unprotected serine, no transglycosidation product was found.

As part of a program to develop synthetic techniques in carbohydrate protective group chemistry[5] and in glycosidic bond formation based on the use of readily available enzymes, we studied the glycosidase-catalyzed synthesis of glycopeptide building blocks.

Scheme 1. ß-galactosidase-catalyzed galactosidation of amino acid derivatives.[6]



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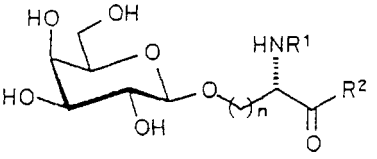

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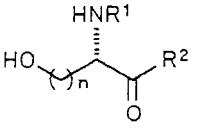
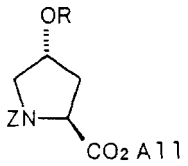
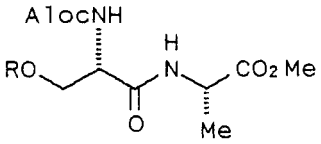


2 - 10

2,13 : R^1 = Z	R^2 = OEt	n = 1
3,14 : R^1 = Z	R^2 = OAll	n = 1
4,15 : R^1 = Aloc	R^2 = OBz	n = 1
5,16 : R^1 = Aloc	R^2 = OMe	n = 1
6,17 : R^1 = Aloc	R^2 = OAll	n = 1
7,18 : R^1 = Boc	R^2 = OAll	n = 1
8,19 : R^1 = Z	R^2 = OtBu	n = 2
9,20 : R^1 = Aloc	R^2 = OBz	n = 2
10,21 : R^1 = Aloc	R^2 = OAll	n = 2



11 : R = H
22 : R = ß-D-Gal



12 : R = H
23 : R = ß-D-Gal

At the beginning of our investigations, we examined the *E. coli* ß-galactosidase-catalyzed glycosidation of Z-Ser-OAll and Z-Ser-OEt in phosphate buffer using *o*-nitrophenyl ß-D-galactopyranoside **1** as glycosyl donor. The yields of the desired transglycosidation products ranged from 12 to 17 percent[7] (cf. Table 1).

Table 1. E. coli ß-galactosidase-catalyzed galactosidation of different amino acid derivatives.

aglycon[4,6] [acceptor]	acceptor [mmol]	donor [mmol]	enzyme [units]	buffer[a]	time [h]	yield[b] [%]
Z-Ser-OEt **2**	0.70	0.40	7.5	9 ml KP	5	17
Z-Ser-OAll **3**	0.51	0.22	30	3 ml TRIS	4	4
Aloc-Ser-OBz **4**	0.59	0.24	30	3 ml TRIS	2.5	10
Aloc-Ser-OMe **5**	0.64	0.22	15	3 ml TRIS	5	39
Aloc-Ser-OAll **6**	0.53	0.20	15	3 ml KP	2.3	28
Aloc-Ser-OAll **6**	0.53	0.20	15	3 ml TRIS	8	35
Boc-Ser-OAll **7**	0.52	0.22	10	6 ml KP	3	27
Z-hSer-OtBu **8**	0.41	0.19	30	3 ml TRIS	120	33
Aloc-hSer-OBz **9**	0.63	0.26	60	3 ml TRIS	8	23
Aloc-hSer-OAll **10**	0.80	0.23	15	3 ml TRIS	2.5	41
Z-Hyp-OAll **11**	0.49	0.23	15	3 ml TRIS	15	23
Aloc-Ser-Ala-OMe **12**	0.65	0.23	30	3 ml IMI	26	26
Aloc-Ser-Ala-OMe **12**	0.56	0.25	30	3 ml MOPS	30	20

a. abbreviation of buffers (pH-range 7-7.5/0.1 M each): KP - potassium phosphate; TRIS - Tris-(hydroxymethyl)-methylamine; IMI - imidazole; MOPS - morpholinopropanesulfonic acid;
b. isolated yields after flash chromatography; all products were characterized by ^{1}H and ^{13}C NMR; correct FAB mass spectra were obtained in all cases.

We now wish to report on further studies including the β-galactosidase-catalyzed galactosidation of serine, homoserine (hSer), and hydroxyproline (Hyp) derivatives.[8] The use of this enzymatic reaction for the glycosidation of a dipeptide was demonstrated for the first time.

In a typical run, 60 mg (0.2 mmol) *ortho*-nitrophenyl β-D-galactopyranoside **1**, 121 mg (0.53 mmol) allyloxycarbonyl serine allylester (Aloc-Ser-OAll) **6**, and 15 units *E. coli* β-galactosidase [10 μL $(NH_4)_2SO_4$-suspension, from Boehringer Mannheim] were stirred in 3 mL of potassium phosphate buffer (0.1 M, pH 7.0) at room temperature (25 °C). The reaction was monitored by TLC and/or HPLC.

After complete disappearance of **1** (after about 2.3 h), the reaction mixture was lyophilized and subjected to flash chromatography on silica gel ($CHCl_3$/MeOH/hexane 5/1/1) to afford 22 mg (28 %) of the desired anomerically pure β-configurated glycoconjugate **17**.

Our enzymatic synthesis affords the acid and base labile glycoconjugates in anomerically pure form and in only one step without tedious protecting group transformations. Compared to this, chemical syntheses often suffer from poor stereoselectivities and make time consuming protection, deprotection, and purification steps necessary.

As can be seen from the summary of our results in Table 1, the enzymatic method of hydroxy amino acid glycosidation based on the use of nitrophenyl galactoside **1** as an activated glycosyl donor is a considerable improvement over the recently published procedure.[3]

In the course of optimization of the reaction conditions, it became clear that the correct choice of buffer and protective groups is important (cf. Table 1). However, the influence of the buffer is not yet clearly understood. The choice of the protecting groups seems to be crucial. Here, steric interactions or solubility properties might play an essential role. To increase the substrate solubility, organic cosolvents were added to the aqueous reaction medium. DMSO, DMF, DMPU, DMEU,[4] and acetonitrile were tolerated up to about 50 % (v/v). Nevertheless, upon addition of these solvents the transglycosidation yield decreased dramatically.

In summary, scope as well as yields and simplicity of the enzyme-catalyzed transglycosidation of *ortho*-nitrophenyl β-D-galactopyranoside **1** makes this new method a valuable tool for the stereospecific synthesis of various partially protected glycopeptide building blocks which can then be used in chemoenzymatic syntheses of glycopeptides.

Acknowledgement

We wish to thank Dr. Becker and Dr. Vogel for the measurement of NMR and mass spectra.

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4. Abbreviations used in the text: Ac, acetyl; All, allyl; Aloc, allyloxycarbonyl; Boc, *t*-butyloxycarbonyl; Bz, Benzyl; Z, benzyloxycarbonyl; DMEU, "dimethylethylene urea", 1,3-dimethyl imidazolidine-2-one; DMF, dimethyl formamide; DMPU, "dimethylpropylene urea", 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone; DMSO, dimethyl sulfoxide.

5. a) E. W. Holla, *J. Carbohydr. Chem.* **9**, 113 (1990); b) E. W. Holla, *Angew. Chem.* **101**, 222 (1989); *Angew. Chem. Int. Ed. Engl.* **28**, 220 (1989).

6. The *N*-protection with Boc, Aloc, and Z residues as well as the synthesis of benzyl, allyl, methyl, and *t*-butyl esters was achieved according to standard procedures.

7. European patent application 0 455 101 A2, priority 25.04.90.

8. During the course of the preparation of this manuscript, the stereospecific attachment of carbohydrates to amino acid derivatives using β-glucosidase and β-xylosidase was reported: N. J. Turner, M. C. Webberley, *J. Chem. Soc. Chem. Commun.* 1349 (1991).