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Stereospecific Chemoenzymatic Synthesis of Galactopyranosyl-L-Serine

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Abstract : The transglycosylation from raffinose and lactose to Aloc-Ser-OMe is catalyzed respectively by α and β galactosidases. The simplicity of the enzymatic synthesis, the stereospecificity of the condensations in one-pot reactions and the ease of purification give the method value for large scale preparation of β-linked derivatives. The protective groups of the serine residue can be cleaved under mild conditions: the ester group has been removed quantitatively by papain catalyzed hydrolysis and the Aloc group by a Pd (0) hydrostannolytic cleavage.

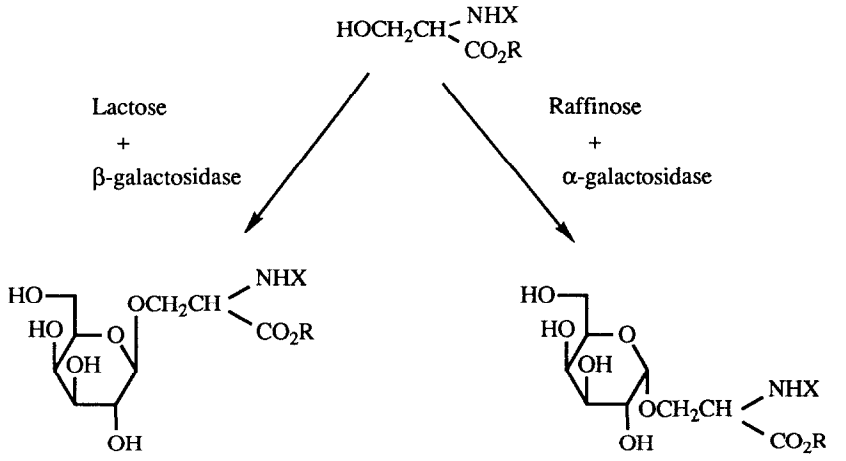
The importance of glycoproteins in control of biological processes has been recognized for a long time [1]. They play a central role in oncogenesis [2], bacterial and viral infection [3], and uptake of various macromolecular substances. Furthermore they determine blood-group type [4], and are responsible for cell-cell recognition, cell growth and cell differentiation. The attachement of a sugar residue to a peptide sequence offers protection against proteolytic enzymes [5] and should improve the affinity of the molecule for its receptor. For this reason, several glycosylated enkephalins have been synthetized [6-8].

Glycopeptide synthesis requires the protection of many more functional groups than peptide (or carbohydrate) synthesis. The presence of the glycosidic linkage with a definite configuration renders the chemical synthesis often cumbersome and mild conditions for the removal of appropriate protective groups are necessary [9]. An alternative to the chemical synthesis of glycopeptides is the use of enzymes. The galactosyl-serine linkage being found in some glycoproteins [10], we chose the galactosyl-L-serine derivative as a model compound for an enzymatic synthesis with galactosidases.

In a previous paper, we showed that the stereospecific formation of a glycosidic bond between galactose and protected serine using galactosidases was possible [11]. The simplicity of the method and the easy purification gave the enzymic synthesis value for large scale preparation of β-linked galactosyl-L-serine derivatives. In this paper, we describe an improvement in the procedure as well as the selective enzymatic removal of the protective ester group of the aminoacid.

As reported previously, β-galactosidase induces a highly stereospecific synthesis of the expected β-

galactosyl-L-serine derivative as long as both the amino and the carboxyl groups of serine are protected. The amino group of serine was protected by a t-butyloxycarbonyl (Boc) group and the acid as a methyl ester (scheme 1, R = Me, X = Boc). However the glycosidic bond is more or less acid sensitive and the N-Boc protective group is not the most appropriate for serine since anomerization often occurs during the removal of the Boc group by means of trifluoroacetic acid. For this reason, we chose the allyloxycarbonyl (Aloc) protective group developed by Kunz [12] for the protection of the amine. The benzyloxycarbonyl (Cbz) group could also be a candidate since it is cleaved by hydrogenolysis in mild conditions but the yields of the condensation of lactose and N-benzyloxycarbonyl serine methyl ester yielding **3** are somewhat lower (8% as compared to 15% with the Boc-serine ester). An other possibility for the N-protection of serine methyl ester was the 2-(2-pyridyl)ethoxycarbonyl-(Pyoc) residue since it is easily removable [13]. Unfortunately no condensation took place with this derivative.



1 R = Me, X = Boc (15%)
2 R = Me, X = Aloc (13%)
3 R = Me, X = Cbz (8%)

4 R = Me, X = Boc (3%)
5 R = Me, X = Aloc (8%)

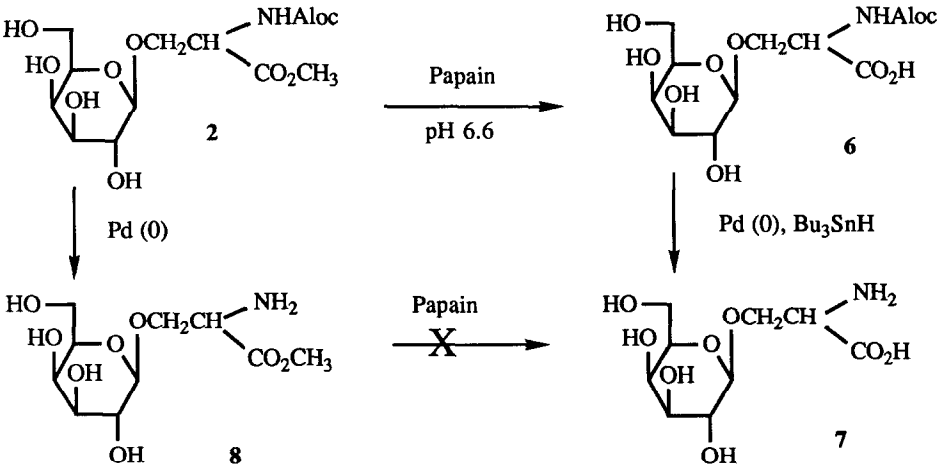
Scheme 1

On the other hand, the β-O-glycosidic linkage is base labile and in sodium bicarbonate solution the protected galactosyl-serine methyl esters **1-3** are cleaved rapidly and quantitatively before the methylester is hydrolysed. However this methyl ester group could not be replaced since no condensation was observed with β-galactosidase and lactose if N-protected serine allyl or benzyl ester was used.

The β–galactosidase (*E.coli*) catalyzed condensation of lactose with allyloxycarbonylserine methyl ester was performed as described before [11]. The yield in the β–galactosyl derivative **2** (13%) obtained after purification by chromatography on silicagel [20], were about the same as in the condensations with Boc-serine methyl ester. However an improvement was observed in the condensation of raffinose with N-Aloc-serine methyl ester using α-galactosidase *(coffee bean)* as the catalyst (yield of α-galactosylserine derivative **5** : 8% as compared to 3% obtained for the homologous Boc derivative **4**).

Deprotection of the galactosyl-serine derivative **2** was undertaken. Due to the extreme lability of the glycosidic bond hydrolysis of the methyl ester under the usual basic conditions was excluded. An enzymatic deprotection by proteolytic enzymes, under neutral conditions, was considered. Papain was choosen since it is known to be poorly specific [14] and we showed that esterifications could be accomplished with Boc-SerOH [15] (the reverse normal hydrolysis of the ester should therefore be possible).

In fact the papain catalyzed hydrolysis of the galactosyl-L-serine derivative **2** (scheme 2) yields **6** quantitatively at pH 6.6 : no cleavage of the glycosidic bond was observed [21]. Subtilisin can also be used as a catalyst : in that case hydrolysis was performed at pH 7.5. This method of ester deprotection is very attractive since no by-products were observed and the configuration of the galactosyl-serine bond was maintained. Recently the hydrolysis of aminoacids heptyl esters by lipase has been used, aiming also at the hydrolysis of the ester function in glycopeptides [16]. Similarly, papain-catalyzed hydrolysis has been shown to occur on peracetylated N-glycosylated aminoacids [17].



Scheme 2

The next step is the removal of the allyloxycarbonyl (Aloc) group of **6** which is accomplished by palladium catalyzed hydrostannolytic cleavage with tributyltin hydride in the presence of acetic acid in dimethylformamide as the solvent according to a published procedure [18]. The fully deprotected β-galactosyl-serine **7** is obtained (yield 68%) and its characteristics are similar to the ones reported in the litterature [19]. However, for unknown reasons a sixteen-fold excess of tributytin hydride is necessary to complete the reaction [22] as compared to the 10% excess usually needed. This is surprising, since if the deprotection of the amino group is performed before the deprotection of the ester, the cleavage of the Aloc residue is obtained, as described [18], with only a 10% excess of the allyl acceptor, to give the expected derivative **8**. Unfortunately the cleavage of the ester by papain has to be performed before the cleavage of the Aloc group since the enzymatic hydrolysis is not observed if the amino group of the serine residue is unprotected.

The papain-catalyzed hydrolysis of the methyl ester proceeds also well for the α-galactosyl-serine derivative **5** without anomerization about the glycosidic bond. However α-galactosidase is expensive and we could not

obtain enough material to achieve full deprotection of the product.

The procedure described herein for the synthesis of β-galactosyl-L-serine **7** using a β–galactosidase catalyzed transglycosylation from lactose to Aloc-Ser-OMe is very simple and can be of value for large scale preparations. The condensations are highly stereospecific and the products easily purified. As compared to the chemical methods, no protection of the sugar residue is necessary and the reactions are performed in water, at room temperature. The protective groups of the serine residue are removed under mild conditions by protease catalyzed hydrolysis of the ester and Pd (0) catalyzed cleavage of the amino protective group.

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20. In a typical experiment 3.4 g of lactose (10 mmoles) were incubated with 4 g of Aloc-Ser-OMe (19 mmoles) in 16 ml of buffer (0.03 M sodium phosphate buffer, pH 7.8, containing mM $MgCl_2$ and 5 mM dithiothreitol) 3.2 ml of β-galactosidase (0.1 mg per ml) for 18 h. The mixture was extracted with dichloromethane, the organic phase washed with water and the combined aqueous phases subjected to column chromatography on silicagel. Elution with dichloromethane-methanol-ethanol-water (60: 35: 10: 8) gave **2** (450 mg, 13%), m.p. 89°C, $[\alpha]_D$ - 10 (c 1, water). N.m.r. data (CD_3OD): ^1H, δ 3.5 (m, 3H), 3.7-3.8 (m, 8H), 3.7 (s, OCH_3), 4.22 (d, 1H, J 7.5 Hz, H-1), 3.8-4.38 (2H, ser), 4.45 (m, 1H, CHα), 4.58 (2H, allyl), 5.22-5.32 (m, 2H), 5.95 (m, 1H, allyl). ^{13}C, δ 172.4 (COOMe), 158.6 (CONH), 132.4 (CH= allyl), 117.7 (CH_2 allyl), 105.2 (C-β), 76.8 (C-5), 74.8 (C-3), 72.4 (C-2), 70.5 (CH_2 ser), 70.2 (C-4), 66.7 (CH_2 allyl), 62.4 (C-6), 55.7 (CHα) 52.9 (CO_2CH_3). Mass spectrum: m/z 366 (M^+ + H).
21. Product **2** (250 mg, 0.68 mmole is incubated with 20 mg of papain (Sigma) and 1 ml of 0.05 M cysteine at pH 6.6. The pH is maintained constant by addition of 0.5N NaOH with a pH stat. The end of the reaction is controlled by t.l.c. The aqueous phase is lyophylized, product **6** treated by a Dowex 50w-X4 resin and purified on a silicagel column with the same solvent as above. m.p. 123°C , $[\alpha]_D$ + 5.8 (c 1, water). N.m.r. data (D_2O): ^1H, δ 3.38-3.9 (m, 7H), 4.15 (1H, CHα), 3.9-4.15 (2H, CH_2 ser), 4.35 (d, 1H, J 7.2 Hz, H-1) 4.55 (2H, allyl), 5.22-5.32 (m, 2H, allyl); ^{13}C δ 177.35 (CO_2H), 158.53 (CONH), 133.38 (CH= allyl), 117.98 (CH_2 allyl), 103.6 (C-β), 75.81 (C-5), 73.19 (C-3), 71.33 (C-2), 70.92 (CH_2 ser), 69.29 (C-4), 66.57 (CH_2 allyl), 61.66 (C-6), 57.09 (CHα). Mass spectrum: m/z 352 (M^+ + H).
22. 100 mg of product **6** (0.28 mmole), 8 mg of $PdCl_2(PPh_3)_2$ and 34 µl of acetic acid were dissolved in 2 ml of DMF under argon. 91 µl of Bu_3SnH was then quickly added in one portion to the reaction mixture. This is repeated several times (about sixteen times) until no more gas evolution appears (crude **6** can also be used directly after the hydrolysis by papain and lyophilization). The completely deprotected β-galactosyl-serine **7** precipitated from the medium. DMF was evaporated and the reaction mixture was extracted with ether. Product **7** was then purified by column chromatograhy as above (51 mg, 68%), m.p. 165°C, $[\alpha]_D$ - 5 (c 1, water). N.m.r. data (D_2O):^1H δ 3.48 (m, 1H, H-2), 3.82-3.58 (5H), 3.88-4.0 (m, 2H, CH_2 ser), 4.2 (m, 1H, CHα), 4.35 (d,1H, J 7.2 Hz); ^{13}C, δ 172.87 (COOH), 103.42 (C-β), 76.12 (C-5), 73.34 (C-3), 71.50 (C-2), 69.46 (C-4), 68.78 (CH_2 ser), 61.91 (C-6), 55.60 (CHα). Mass spectrum: m/z 268 (M^+ + H).