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Note

Enzymic synthesis of galactopyranosyl-L-serine derivatives using galactosidases

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Although the physiological role of the carbohydrate moieties in biologically active glycoproteins is still a subject of investigation, it is known that sugars extend the biological half-life of these molecules. Furthermore, the attachment of a sugar residue to a peptide sequence could improve the affinity of the molecule for its receptor. For this reason, several glycosylated enkephalins have been synthesised[1–3].

Chemical syntheses of glycopeptides are well developed[4]. However, because carbohydrates contain multiple hydroxyl groups of similar reactivity, the chemical methods involved in regioselective synthesis require numerous protection and deprotection steps. In addition, stereospecific reactions that give the desired (α or β) anomer are often difficult.

Many enzymes are now available commercially and their routine use in synthesis[5] is becoming accepted. Most glycosidases can transfer the glycosyl moiety of a substrate to acceptors other than water and, although they are less selective than glycosyl transferases, they have been used in the synthesis of glycosides[6,7]. For example, β-D-galactosidase catalyses transgalactosidation with monosaccharides, oligosaccharides, alkanols[8–10], and phenols[11]. The reactions of *E. coli* β-D-galactosidase in the presence of various acceptors have been investigated, as have the structural requirements and reactivity[12]. Alkyl galactosides, lactose, and raffinose can be used as glycosyl donors with various primary alcohols as acceptors for the synthesis of di- or tri-saccharide glycosides[13–16].

We now report on the use of lactose and β-D-galactosidase for the formation of 3-*O*-β-D-galactopyranosyl-L-serine (β-Gal-Ser).

β-D-Galactosidase does not induce the synthesis of β-Gal-Ser from lactose and serine, and no condensation occurs with the N-urethane derivative of the amino acid or the amino acid ester. Both the amino and the carboxyl groups of the amino acid have to be protected for transgalactosidation to take place.

The choice of the amino blocking group is important. The best yields (15%) for transgalactosidation were observed with serine methyl esters N-protected by a *tert*-butoxycarbonyl group. With *N*-benzyloxycarbonylserine methyl ester, the yield was

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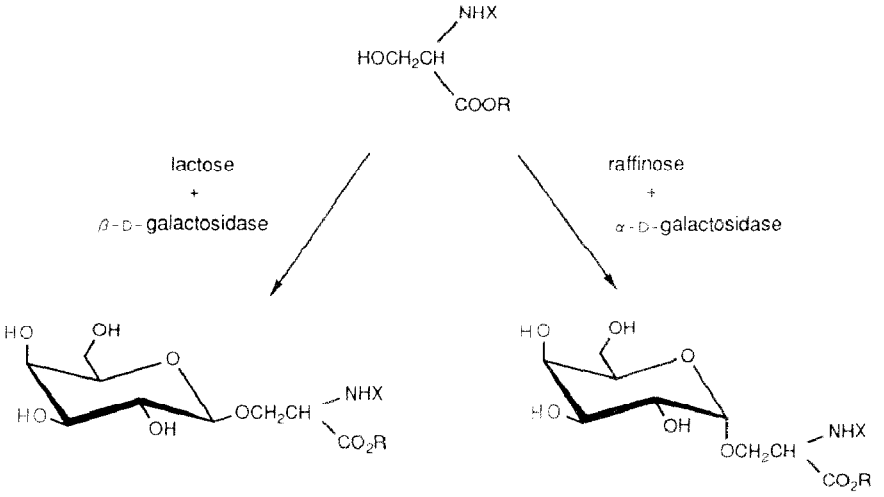
9%, possibly due to the lower solubility of Z-Ser-OMe in water since, with Z-Ser-OBzl, which is even less soluble, no condensation occurred.

The β-D-galactopyranosyl-L-serine derivative **1** is a substrate for β-D-galactosidase, so that the yield of **1** after incubation for 18 h reflected a balance between formation and degradation; the yield increased at least up to 14 h and decreased after 30 h. The condensation was not improved by the addition of such organic solvents as *N,N*-dimethylformamide, acetonitrile, or diethyleneglycol diethyl ether[17]. Reduction in yields on the addition of these solvents has been observed with glycosidases[18], which is the reverse of the effect found in protease-catalysed peptide synthesis[19].

No reaction occurred when *N*-acetylserine methyl ester was used. Furthermore,



1 R = Me, X = Boc
2 R = Me, X = Z

3 R = Me, X = Boc

when allyl alcohol was added, the well known transgalactosidation was not observed[14]. The enzyme is probably inhibited, since with a mixture that contained lactose, Boc-Ser-OMe, and allyl alcohol, allyl β-D-galactopyranoside was formed.

The condensations with β-D-galactosidase were highly regioselective, which, together with the stereospecificity, makes the method attractive. Purification of the products and recovery of the excess of reagent by column chromatography was straightforward. Although not optimised, the yields of these reactions were in the range 10–15% and were comparable to those usually obtained in transgalactosidation reactions[16,20–21]. Only traces of unidentified material were detected (t.l.c.).

When compared with the β-D-galactosidases from *Saccharomyces* or *Aspergillus oryzae*, the best results were obtained with the enzyme from *E. coli*.

The transglycosylation from raffinose to Boc-Ser-OMe was also catalysed by α-D-galactosidase, to give the expected α-D-galactopyranosyl-L-serine derivative **3**, characterised as the tetra-acetate. Although **3** was a substrate for α-D-galactosidase, it continued to accumulate for at least four days. However, the yields were low (2–3%)

and **3** was difficult to purify. Raffinose is not a good donor[14] and alkyl α-D-galactopyranosides should give better results.

The simplicity of the above enzymic syntheses, the stereospecificity of the condensations in one-pot reactions, and the easy purification give the method value for the large-scale preparation of β-linked derivatives.

EXPERIMENTAL

General. — α-D-Galactosidase (coffee bean) and β-D-galactosidase (*E. coli*) were obtained from Sigma. A solution of β-D-galactosidase was prepared by dissolving the enzyme powder (0.5 mg) in 0.03M sodium phosphate buffer (pH 7.8, 5 mL) that contained mM $MgCl_2$ and 5mM dithiothreitol. Boc Ser-OMe was prepared from serine methyl ester by a standard procedure[22]. Lactose hydrolysis and galactosyl transfer were monitored by t.l.c. on Silica Gel F_{254} (Merck) with 2-propanol–aqueous 20% NH_3–water (7:1:2) and detection by charring with sulfuric acid. C.i.-mass spectra (NH_3, 90 eV) were obtained with a Nermag R 10-10C instrument. ^{1}H-N.m.r. spectra [300 MHz, internal Me_4Si or 3-(trimethylsilyl)propionic acid, sodium salt] were recorded with a Bruker instrument. Chemical shifts for ^{13}C-n.m.r. data are given relative to that for 1,4-dioxane (67.86 p.p.m. downfield from the signal for Me_4Si).

Enzymic synthesis with β-D-galactosidase. — (*a*) N-tert-*Butoxycarbonyl-3*-O-β-D-*galactopyranosyl*-L-*serine methyl ester* (**1**). — To a solution of lactose (850 mg, 2.5 mmol) in 0.03M sodium phosphate buffer (pH 7.8, 2 mL) that contained mM $MgCl_2$ and 5mM dithiothreitol were added Boc-Ser-OMe (1.6 g, 7.3 mmol) and β-D-galactosidase (600 μL, 40 U). The mixture was stirred for 18 h at 30° and extracted with dichloromethane; the material in the aqueous phase was then subjected to column chromatography on silica gel (Merck, 0.040–0.063 mm). Elution with dichloromethane–methanol (97:3) removed small amounts of Boc-Ser-OMe. Elution with dichloromethane–methanol–ethanol–water (60:35:10:8) gave **1** (132 mg, 15%), m.p. 72°, $[\alpha]_D$ −11.5° (*c* 1, water). N.m.r. data (D_2O): ^{1}H, δ 1.43 (s, 9 H, ^{t}Bu), 3.5 (m, 1 H, H-2), 3.6–3.9 (H-3,4,5,6), 3.78 (s, OMe), 3.80–4.25 (m, 2 H, Ser CH_2O), 4.38 (d, 1 H, *J* 7.5 Hz, H-1), 4.48 (m, 1 H, Ser CHα); ^{13}C, δ 173,52 (*C*OOMe), 158.45 (CONH), 103.74 (C-β), 82.52 [*C*$(CH_3)_3$], 75.91, 73.33, 71.38, 69.29 (C-2,3,4,5), 69.64 (Ser CH_2O), 61.65 (C-6), 54.78 (CHα), 53.9 (CO_2CH_3), 28.32 [C(*C*$H_3)_3$]; the n.m.r. data accorded with those for similar derivatives[23,24]. Mass spectrum: *m/z* 382 ($M^+ + H$).

Anal. Calc. for $C_{15}H_{27}O_{10}N{\cdot}H_2O$: C, 45.10; H, 7.32; N, 3.51. Found: C, 45.23; H, 7.23; N, 3.51.

(*b*) N-*Benzyloxycarbonyl-3*-O-β-D-*galactopyranosyl*-L-*serine methyl ester* (**2**). — To a solution of lactose (850 mg, 2.5 mmol) in the phosphate buffer (3 mL) were added Z-Ser-OMe (900 mg, 8 mmol) and β-D-galactosidase (600 μL, 40 U). The mixture was stirred for 18 h at 30°, then worked-up as in (*a*). However, the dichloromethane extract contained also some **2**. Z-Ser-OMe was removed by chromatography with a linear gradient of 0–2% of methanol in dichloromethane. Elution as in (*a*) for **1** then gave **2**

[93 mg (9%) recovered from the aqueous and organic phases], m.p. 82°, $[\alpha]_D$ −4.5° (*c* 1, methanol). N.m.r. data (CD_3OD): ^1H, δ 3.45 (m, 3 H), 3.75 (s, OMe), 3.65–3.80 (m), 4.25 (d, 1 H, *J* 7.1 Hz, H-1), 4.4–3.72 (m, Ser CH_2O), 4.55 (m, 1 H, CHα), 5.1 (s, 2 H), 7.35 (m, 5 H); ^{13}C, δ 170.159 (CO_2CH_3), 156.146 (CONH), 136.769 (C aromatic), 128.338, 127.843, 127.770 (CH aromatic), 104.040 (C-β), 75.266, 72.916, 70.462, 67.905 (C-2,3,4,5), 68.941 (Ser CH_2O), 65.629 (CH_2-Ph), 60.255 (C-6), 54.083 (CHα), 52.155 (CO_2CH_3). Mass spectrum: *m/z* 416 (M^+ + H).

Anal. Calc. for $C_{18}H_{25}O_{10}N{\cdot}H_2O$: C, 49.88; H, 6.28; N, 3.23. Found: C, 49.94; H, 6.17; N, 3.29.

(*c*) N-tert-*Butoxycarbonyl-3*-O-α-D-*galactopyranosyl*-L-*serine methyl ester* (**3**). — To a solution of raffinose (1.5 g, 2.5 mmol) in 0.03M sodium phosphate buffer (pH 6.5, 8 mL) were added Boc-Ser-OMe (2.8 g, 12.7 mmol) and α-D-galactosidase (0.5 mL, 25 U). The mixture was stirred for 5 days at 30°, then extracted with dichloromethane, and **3** was isolated, as in (*a*), as a hygroscopic oil (31 mg, 3%). Compound **3** was acetylated conventionally with acetic anhydride–sodium acetate and the product purified by column chromatography (8:2 hexane–ethyl acetate). The n.m.r. spectrum of the tetra-acetate corresponded to those reported for similar galactopyranosides[25]. N.m.r. data ($CDCl_3$): ^1H, δ 1.5 (s, 9 H, ^{t}Bu), 2, 2.88, 3.02, 3.5 (4 s, 12 H, 4 Ac), 3.78 (s, 3 H, OMe), 3.9, 4.2 (m, 5 H, H-5,6,6′ and Ser CH_2), 4.48 (m, 1 H, CHα), 5.08 (2 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.04 Hz, H-1,2), 5.3 (dd, 1 H, $J_{2,3}$ 10.04, $J_{3,4}$ 3.3 Hz, H-3), 5.48 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4); ^{13}C, δ 20.57, 20.63, 20.67 ($OCOCH_3$), 28.29 [$C(CH_3)_3$], 52.48 (CO_2CH_3), 53.89 (CHα Ser), 61.61 (C-6), 66.71, 67.40, 67.83, 67.98 (C-2,3,4,5), 69.39 (Ser CH_2O), 80.35 [$C(CH_3)_3$], 97.01 (C-1α), 155.34 (CONH), 170.02, 170.13, 170.36, 170.47 ($COCH_3$ + CO_2CH_3). Mass spectrum: *m/z* 550 (M^+ + H).

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