Enzymatic Synthesis of β-Galactosyloxime Derivatives using β-Galactosidase

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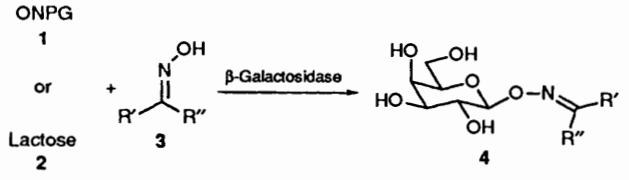
The preparation of anomerically pure oxime β-galactosides through an enzymatic reaction is described.

Glycosidases, important in catalyzing the hydrolysis of glycosidic linkages, are increasingly being used in carbohydrate synthesis.[1,2] Their major advantages are the avoidance of tedious protection/deprotection steps and high stereoselectivity at the anomeric carbon.[2]

Of two general methods for glycoside synthesis, transglycosylation[2] and reversed hydrolysis, the former is kinetically controlled. It relies on the formation of a reactive intermediate (an oxonium ion) from an activated glycosyl donor, which reacts with a nucleophile to give a new glycosidic bond, and is the most widely used method: di- or oligo-saccharides, aryl glycosides and glycosyl fluorides are suitable glycosyl donors for this reaction.[1] Glycosyl acceptors include alcohols,[3] diols,[4] steroids,[5] alkaloids[6] and serine.[7] Some of the glycosides prepared by this method, have a linkage found in *O*-glycosylated biologically active compounds.[7a] Here we describe the use of oximes as the nucleophiles in the reaction, in order to extend its scope to other glycosyl acceptors other than alcohols. The success of the reaction depends on the reactive intermediate (oxonium ion) being trapped faster by the glycosyl acceptor than by water. Certainly, good yields of products are obtained in reactions carried out in the presence of alcohols since these are better bound at the active site than water.[2] Our current work has been directed towards seeing whether oximes would give similar results.

ortho-Nitrophenyl-β-galactoside (ONPG) **1** and lactose **2** were chosen as donors with four oximes as acceptors. β-Galactosidase from *Aspergillus oryzae* was employed as catalyst (Scheme 1).



Scheme 1

The transfer reaction was initiated by using ONPG as glycosyl donor. Use of the oximes **3a** or **3b** as acceptors gave the desired glycosylic linkage products. This contrasts with the oximes **3c** and **3d** which failed to give the target galactosides. With lactose as donor the latter oximes gave the galactosyl oxime derivatives. The results are summarized in Table 1. Unidentified by-products formed in all cases, were thought to be galactose and disaccharide glycosides since it is known that the latter are obtained when ONPG is incubated in the presence of β-galactosidase.[7a] Prolonged reaction times generally resulted in decreased yields because of product hydrolysis. With use of lactose instead of ONPG longer incubation times were required to achieve similar yields (see Table 1).

The substrates were dissolved in citrate buffer, pH 5.0, in a ratio 1:10 donor:acceptor. The reactions were followed by TLC. Products were purified and isolated by flash chromatography, and characterized by ^{13}C NMR and mass spectrometry. In all cases only the β-anomer was produced, as indicated by the absence of detectable signals attributed to the α-anomer in the anomeric region of the ^{13}C NMR spectra. Reactions carried out in the absence of enzyme catalyst gave no detectable products.

Table 1 β-Galactosyloxime derivatives from **1** or **2** and oximes **3**.

Glycosidic donor	R'	R''	Product	Time (min)	Yield (%)
1	Me	Me	**4a**	5	15
1		$-(CH_2)_5-$	**4b**	10	26
2	Me	Me	**4a**	20	20
2		$-(CH_2)_5-$	**4b**	35	31
2	Me	H	**4c**	30	18
2	Me	Ac	**4d**	40	22

In conclusion, oximes are suitable glycosilic acceptors in the kinetically controlled synthesis of glycoside derivatives.

Experimental

β-Galactosidase from *Aspergillus oryzae* was purchased from Sigma (EC 3.2.1.23, grade XI). ^{13}C NMR spectroscopy was carried out using a Bruker AC-300 (75.5 MHz instrument). Mass spectra were recorded on a Hewlett-Packard 5897A spectrometer. For flash chromatography, Merck silica gel 60–230–400 mesh was used. Microanalyses were performed on a Perkin-Elmer 240B elemental analyzer. M.p.s were taken on samples in open capillary tubes using a Büchi melting-point apparatus and are uncorrected.

Typical Procedure for the Synthesis of Galactosidases from ONPG **1**.—PNPG **1** (2 mmol) was dissolved in citrate/NaOH buffer (pH 5.00; 4 cm^3) with acetone oxime **3a** (20 mmol). β-Galactosidase (200 mg) was then added and the resulting mixture was allowed to react at 30 °C for 5 min with shaking (it was monitored by TLC). The enzyme was denatured by boiling. The solution was then centrifuged and the concentrated supernatant was subjected to flash chromatography on silica gel (AcOEt–MeOH–H_2O, 16:2:1) to give the pure product (71 mg, 15%). Compounds **4c** and **4d** were treated as follows. Lactose (2.5 mmol) and acetaldehyde oxime **3c** (25 mmol) was dissolved in the same buffer solution (20 cm^3) and then the enzyme (400 mg) was added. The mixture was shaken for 30 min at 30 °C after which the product was isolated and purified in a similar way to that described earlier. It was obtained in 18% yield (99.4 mg).

4a: Syrup (Found: C, 46.1; H, 7.3; N, 6.0. $C_9H_{17}NO_6$ requires C, 45.94, H, 7.29, N, 5.96%); δ_C (D_2O) 163.22 (C=N), 103.92 (C-1), 75.90 (C-5), 73.31 (C-3), 69.74 (C-2), 69.08 (C-4), 61.51 (C-6), 21.38 (CH_3) and 16.26 (CH_3); *m/z* (EI, 70 eV), 235 (M^+ < 1), 218 (< 1), 186 (< 1), 163 (22.23), 144 (27.84), 127 (10.03), 91 (49.95), 73 (44.87) and 56 (100).

4b: Syrup (Found: C, 52.4; H, 7.7); N, 5.1. $C_{12}H_{21}HO_6$ requires C, 52.34; H, 7.69; N, 5.09%); δ_C(D_2O) 168.12 (C=N),

103.54 (C-1), 75.53 (C-5), 72.93 (C-3), 69.36 (C-2), 68.71 (C-4), 61.16 (C-6), 31.73 (CH_2), 26.84 (CH_2), 25.95 (CH_2), 25.62 (CH_2) and 25.10 (CH_2); *m/z* (EI, 70 eV), 275 (M^+ < 1), 258 (< 1), 186 (< 1), 163 (9.72), 144 (18.82), 127 (19.97), 96 (100), 91 (60.25) and 73 (49.75).

4c: White solid; m.p. 145–146 °C (Found: C, 43.3; H, 6.85; N, 6.35. $C_8H_{15}NO_6$ requires C, 43.42; H, 6.84; N, 6.33%); δ(CD_3OD) 151.30 (C=N), 105.58 (C-1), 76.48 (C-5), 74.70 (C-3), 70.60 (C-2), 69.82 (C-4), 62.04 (C-6) and 14.91 (CH_3); *m/z* (EI, 70 eV) 221 (M^+ < 1), 204 (< 1), 163 (10.96), 144 (33.87), 127 (16.99); 91 (41.34), 73 (83.26) and 42 (88.43).

4d: Syrup (Found: C, 45.5; H, 6.5; N, 5.3. $C_{10}H_{17}NO_7$ requires C, 45.61; H, 6.51; N, 5.32%); δ(D_2O) 201.64 (C=O), 158.32 (C=N), 105.48 (C-1), 76.34 (C-5), 73.31 (C-3), 69.69 (C-2), 69.03 (C-4), 61.41 (C-6), 25.29 (CH_3) and 8.39 (CH_3); (EI, 70 eV) 163 (7.11), 144 (18.20), 127 (7.41), 91 (37.24), 85 (16.72), 73 (45.16) and 43 (100).

Acknowledgements

Financial support of this work by CICYT (project BIO92-0751) is gratefully acknowledged. M.P. is the recipient of a pre-doctoral fellowship from the Ministerio de Educación y Ciencia which is acknowledged. We also thank Prof. Pablo Bernad for mass spectra.

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Paper 3/01277H
Received 4*th March* 1993
Accepted 9*th March* 1993