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FACILE CHEMO-ENZYMATIC ACCESS TO MONOGLUCOSYL DERIVATIVES OF 2,3-OXIRANE DIMETHANOL

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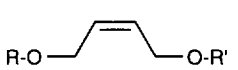
Abstract. The synthesis of glucosyl derivatives of 2,3-oxirane-dimethanol has been accomplished chemo-enzymatically by the use of glycosidases and lipases. High yields using thermophilic glycosidases coupled to the diastereoselectivity and regioselectivity of lipases lead to selectively deprotected products, useful materials for further elaboration.

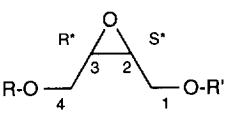
The study of the selectivity of glycosidase-catalyzed[1] reactions is of current interest in our laboratory[2]. General wide substrate specificity coupled to high yields allowed the synthesis of a series of polyol or masked polyol glycosides using thermophilic enzymes[2a]. Further enzymatic (i.e. by lipases) elaboration of peracetylated derivatives of these products allowed access to natural or unnatural polyol glycosides in greatly enriched or stereochemically pure forms[3]. The use of lipases on these products has two advantages, i) diastereoselective and ii) regioselective hydrolysis of acyl groups in specific positions thus producing specific deprotected materials useful for further elaboration.



1; R = R' = H
2; R = H, R' = α-Glu
3; R = H, R' = β-Glu
4; R = Ac, R' = α-Glu-Ac$_4$
5; R = Ac, R' = β-Glu-Ac$_4$

Table 1. Stereochemical outcome of *P. cepacia* lipase hydrolysis

Substrate	Conversion	Time	Product	Remaining substrate
6	45 %	4 h	(2R,3S)-8 90% d.e.	(2S,3R)-6 90% d.e.
7	43%	10 h	(2R,3S)-9 90% d.e.	(2S,3R)-7 70% d.e.



6; R = Ac, R' = α-Glu-Ac$_4$
7; R = Ac, R' = β-Glu-Ac$_4$
8; R = H, R' = α-Glu-Ac$_4$
9; R = H, R' = β-Glu-Ac$_4$
10; R =Bn, R' =p-NO_2Bz
11; R =Bn, R' = H
12; R =Bn, R' = α-Glu
13; R =Bn, R' = β-Glu

The preparation of glucosides[4] of **1** has been achieved by transglycosidation using mesophilic or thermophilic glycosidases from almond (50 fold molar excess of **1**, 20% yield of **3**), *Sulfolobus solfataricus* (50 or 100[2a] fold molar excess of **1**, 43 to 65% yield of **3**) and *Thermus thermophilus* (100 fold molar excess of **1**, 70% yield of **4** after acetylation). Using reverse hydrolysis approach[5] with **1** as substrate only 30% yield of **3** was recovered. Double bond epoxidations of **4** and **5** were conducted using standard literature procedures[6] (*m*-ClPBA) and obtaining in each case (83-85% yield) a 1:1 diastereomeric mixture of **6** and **7** which were in turn subjected to *P.cepacia* and *C. antarctica* lipase catalyzed[7] hydrolysis. Both enzymatic reactions are highly regioselective in that only the acetyl group of the hydroxymethylene unit of the oxirane ring was cleaved confirming previuos results obtained with *P. fluorescens* lipase on glycerol and erythritol β-glucoside.[3] The diastereoselectivity of the reaction was monitored on recovered materials, on the hydrolysis products and confirmed on re-acetylated **8** and **9** by inspection of their ^{1}H and ^{13}C NMR spectra[7,8]. Diastereoselectivity of the reaction using *C. antarctica* lipase was not practically useful for both α- and β- glucosides; recovered

materials after ca. 50% conversion were composed of a 2:1 mixture of starting materials, furthermore both diastereomers of **8** and **9** were present in the hydrolysis products at the end of reaction. The reactions performed by the enzyme from *P. cepacia* were more diastereoselective (Table 1)[7]. Enzymatic preparation of epoxy-containing compounds was achieved by hydrolysis or by transesterification of epoxyesters[9,10]; the ee's varied with experimental conditions (temperature, pH, chain length of alkyl group, etc.) from 50 to >90%. In this paper we have reported a simple procedure for the synthesis of carbohydrate containing epoxy-compounds achieving good regio- and diastereoselectivity. Further manipulations of the free primary alcohol group and oxirane ring of *P. cepacia* hydrolysis products and those of their diastereomers (after hydrolysis with *C. antarctica* lipase) could be envisaged for obtaining interesting natural products[11,12]. It was worth noting that both α- and β-glucosides gave rise to the same stereochemical outcome leading to products with the (S)-configuration at position 3 of oxirane ring as also reported for PPL-catalyzed hydrolysis[9,10] of the epoxydibutyrate derivative of **1**.

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4. Almond -β-glucosidase (5.7 U/mg) was obtained from Sigma. Crude homogenates of the thermophilic microorganisms, *Sulfolobus solfataricus* (DSM 5837) and *Thermus thermophilus* (ATCC 27634) were prepared as reported in 2b. PNP-α and β-glucoside were used as donors of carbohydrate moiety. Work-up and purification procedures are the same as reported in 2a.

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7. *Candida antarctica* (3.3 U/mg; 62299) and *Pseudomonas cepacia* (609 U/mg; 62309) lipases were obtained from Fluka. The substrates (40mg/ml) were dissolved in acetone/phosphate buffer (as reported in 3) and enzyme (25 mg/ml) was added. At ca. 50% of conversion the products were purified by silica-gel chromatography. **(2S,3R)-6**: selected ^{13}C NMR signals, δ ($CDCl_3$): aglycon (66.39; 61.99; 52.92; 54.23); glucose (96.23; 70.52; 69.91; 68.41; 67.58; 61.86); selected ^{1}H NMR signals, δ ($CDCl_3$): 3.92-3.64 (H1a-H1b); 4.21-4.06 (H-4a-H4b) 3.27 (2H, H2-H3); 5.48 (H3'); 5.15 (H-1'); 5.05 (H-4'); 4.89 (H-2'); 4.22-4.08 (H-6'a-H-6'b); 4.05 (H-5'). $[\alpha]_D^{20}$ = 85.2 (c = 0.9, $CHCl_3$). **(2R,3S)-6**: selected ^{13}C NMR signals, δ ($CDCl_3$): aglycon (66.36; 61.97; 53.21; 53.61); glucose (95.90; 70.62; 69.69; 68.35; 67.49; 61.83) selected ^{1}H NMR signals, δ ($CDCl_3$): 3.77 (H1); 4.26-4.08 (H-4a-H4b) 3.29 (2H, H2-H3); 5.50 (H3'); 5.10 (H-1'); 5.07 (H-4'); 4.88 (H-2'); 4.26-4.08 (H-6'a-H-6'b); 4.06 (H-5'). $[\alpha]_D^{20}$ = 40.9 (c = 0.7, $CHCl_3$). **(2R,3S)-8**: selected ^{13}C NMR signals, δ ($CDCl_3$): aglycon (66.24; 60.23; 55.74; 53.94); glucose (95.74; 70.61; 69.92; 68.42; 67.51; 61.92) selected ^{1}H NMR signals, δ ($CDCl_3$): 3.92-3.68 (H1a-H1b); 3.79 (H-4) 3.27 (2H, H2-H3); 5.50 (H3'); 5.09 (H-1'); 5.07 (H-4'); 4.88 (H-2'); 4.25-4.11 (H-6'a-H-6'b); 4.06 (H-5'). $[\alpha]_D^{20}$ = 119.1 (c = 1.3, $CHCl_3$). **(2S,3R)-7**: selected ^{13}C NMR signals, δ ($CDCl_3$): aglycon (67.50; 61.71; 53.60; 53.26); glucose (100.57; 72.66; 71.90; 71.03; 68.16; 62.26) selected ^{1}H NMR signals, δ ($CDCl_3$): 3.93-3.75 (H1a-H1b); 3.98-4.25 (H4a-H4b); 3.23 (2H, H2-H3); 5.17 (H3') 5.06 (H4') 4.98 (H2') 4.53 (H1') 4.27-4.11 (H6'a-H6'b) 3.70 (H5'). $[\alpha]_D^{20}$ = -7.69 (c = 1.8, $CHCl_3$). **(2R,3S)-7**: selected ^{13}C NMR signals, δ ($CDCl_3$): aglycon (67.17; 61.69; 54.44; 52.64; glucose (100.30; 72.64; 71.66; 71.02; 68.15; 62.27) selected ^{1}H NMR signals, δ ($CDCl_3$): 3.65-4.01 (H1a-H1b); 4.02-4.20 (H4a-H4b); 3.21 (2H, H2-H3); 5.18 (H3') 5.06 (H4') 4.98 (H2') 4.60 (H1') 4.26-4.13 (H6'a-H6'b) 3.70 (H5'). $[\alpha]_D^{20}$ = -16.1 (c = 2.1, $CHCl_3$). **(2R,3S)-9**: selected ^{13}C NMR signals, δ ($CDCl_3$): aglycon (67.21; 60.16; 55.45; 54.55); glucose (100.33; 72.58; 71.66; 71.10; 68.25; 61.74) selected ^{1}H NMR signals, δ ($CDCl_3$): 3.94-3.82 (H1a H1b); 3.21 (2H, H2-H3); 3.76 (H-4); 5.21 (H-3'); 5.08 (H-4'); 5.00 (H-2'); 4.60 (H-1'); 4.23-4.15 (H-6'a-H-6'b); 3.71 (H-5'). $[\alpha]_D^{20}$ = -13.3 (c = 2.4, $CHCl_3$).

8. The stereochemical assignments of hydrolysis products were accomplished by synthesizing authentic materials starting from commercially available pure diastereomers of **10** with cleavage of the ester group (MeOH, K_2CO_3), enzymatic transglycosydation (almond β-glucosidase or *Thermus thermophilus* α-glucosidase), and hydrogenolysis obtaining **8** or **9** and acetylation for the synthesis of **6** and **7**.

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