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# Regio- and stereoselective glucosylation of diols by sucrose phosphorylase using sucrose or glucose 1-phosphate as glucosyl donor

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ABSTRACT

Previously it has been shown that glycerol can be regioselectively glucosylated by sucrose phosphorylase from *Leuconostoc mesenteroides* to form 2-*O*-α-D-glucopyranosyl-glycerol (Goedl et al., Angew. Chem. Int. Ed. 47 (2008) 10086–10089). A series of compounds related to glycerol were investigated by us to determine the scope of the α-glucosylation reaction of sucrose phosphorylase. Both sucrose and glucose 1-phosphate (G1P) were applied as glucosyl donor. Mono-alcohols were not accepted as substrates but several 1,2-diols were readily glucosylated, proving that the vicinal diol unit is crucial for activity. The smallest substrate that was accepted for glucosylation appeared to be ethylene glycol, which was converted to the monoglucoside for 69%. Using high acceptor and donor concentrations (up to 2.5 M), sucrose or G1P hydrolysis (with $H_2O$ being the 'acceptor') can be minimised. In the study cited above, a preference for glucosylation of glycerol on the 2-position has been observed. For 1,2-propanediol however, the regiochemistry appeared to be dependent on the configuration of the substrate. The (*R*)-enantiomer was preferentialy glucosylated on its 1-position (ratio 2.5:1), whereas the 2-glucoside is the major product for (*S*)-1,2-propanediol (1:4.1). d.e.$_p$s of 71–83% were observed with a preference for the (*S*)-enantiomer of the glucosides of 1,2-propanediol and 1,2-butanediol and the (*R*)-enantiomer of the glucoside of 3-methoxy-1,2-propanediol. This is the first example of stereoselective glucosylation of a non-natural substrate by sucrose phosphorylase. 3-Amino-1,2-propanediol, 3-chloro-1,2-propanediol, 1-thioglycerol and glyceraldehyde were not accepted as substrates. Generally, the glucoside yield is higher when sucrose is used as a donor rather than G1P, due to the fact that the released phosphate is a stronger inhibitor of the enzyme (in case of G1P) than the released fructose (in case of sucrose). Essentially the same results are obtained with sucrose phosphorylase from *Bifidobacterium adolescentis*.



## 1. Introduction

Glucosylation of alcohols and phenols is an important reaction in nature and industry. Glucosides, oligo- and polysaccharides are used by nature e.g. for energy storage, for strengthening cells and tissues, and for molecular recognition. In pharmaceutical industry, glucosylation is used to increase the solubility of drugs. Last but not least, regio- and stereoselectively formed glucosides are used as intermediates for other (bio)chemicals. Enzymatic glucosylation naturally occurs mostly by tailor-made glucosyltransferases, with a different glucosyltransferase for each glucosyl acceptor [1]. These transferases usually use UDP-glucose as glucosyl donor and therefore the equilibria of the reactions catalysed are far on the glucoside side. However, the expensive nature of this donor is one of the major bottlenecks in industrial application of these enzymes.

A class of enzymes that has been intensely studied in their ability to catalyse glucosylation with glucose as the donor are glucosidases [1–4]. The equilibrium of the reaction catalysed by these enzymes is however on the side of hydrolysis of the glucoside, leading to often low yields with these enzymes. The equilibrium can be influenced by the use of glucose derivatives with good leaving groups, like 4-nitrophenol [4], but these substrates are rather expensive. Alternative approaches involve media with minimal water content [5–9] or continuous removal of the product [10] and they sometimes give reasonable yields, but still they have inherent limitations.

More recently, phosphorylases have been extensively studied with respect to glucosylation, one of these being sucrose phosphorylase. The enzyme from *Bifidobacterium adolescentis* has been shown to catalyse the regioselective glucosylation of several sugars such as L-arabinose [11]. The natural reaction catalysed by this enzyme is the phosphorolysis of sucrose, with the concomitant formation of α-glucose 1-phosphate (G1P) and fructose:

$$\text{sucrose} + P_i \rightarrow \text{G1P} + \text{D-fructose} \quad (1)$$

The reaction proceeds through a β-glucosyl intermediate, which is formed upon reaction of the enzyme with sucrose, as shown by

*Abbreviations:* G1P, Glucose α-1-phosphate; SUC, Sucrose; Fru, Fructose; Glc, Glucose; $P_i$, Phosphate; d.e.$_p$, Diastereomeric excess of the product.

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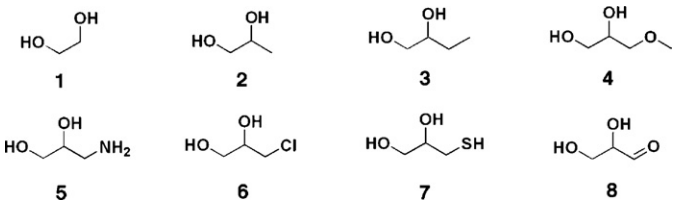


**Fig. 1.** Potential glucosyl acceptors similar to glycerol used in this study.

X-ray crystallography studies [12]. If phosphate is absent, other glucosyl acceptors can be glucosylated:

$$\text{sucrose} + \text{acceptor} \rightarrow \alpha\text{-glucoside} + \text{D-fructose} \quad (2)$$

This reaction occurs with net retention of the α-glucosidic bond. The equilibrium of this reaction is between the extreme situations of the glucosyl transferases and glucosidases; the exact equilibrium depends on the nature of the glucosyl acceptor. Both sucrose and G1P can be used to form the glucosyl–enzyme intermediate. In the latter case phosphate is released rather than fructose:

$$\text{G1P} + \text{acceptor} \rightarrow \alpha\text{-glucoside} + P_i \quad (3)$$

Recently, Nidetzky and co-workers [13] have shown that glycerol can be regioselectively converted with high yield using sucrose phosphorylase from *Leuconostoc mesenteroides*. 2-*O*-(α-D-glucopyranosyl)-*sn*-glycerol, a natural osmolyte, is formed and is currently marketed under the trade name Glycoin® [14]. To determine the scope and selectivity of sucrose phosphorylase, a series of compounds related to glycerol were investigated by us (Fig. 1), using the enzymes from *L. mesenteroides* and *B. adolescentis*. The formation of glucosylated ethylene glycol (**1**), 1,2-propanediol (**2**), 1,2-butanediol (**3**) and 3-methoxy-1,2-propanediol (**4**) is described, using sucrose or G1P as the glucosyl donor. The glucosylation products of 1,2-propanediol, 3-methoxy-1,2-propanediol and 1,2-butanediol were isolated and analysed. The glucosylation reactions of 1,2-propanediol, 1,2-butanediol and 3-methoxy-1,2-propanediol show interesting regio- and stereoselectivity.

## 2. Experimental

### 2.1. Materials

Sucrose phosphorylase from *L. mesenteroides*, sucrose, glucose 1-phosphate, glycerol, ethylene glycol, (*R*,*S*)-1,2-propanediol, (*R*)-1,2-propanediol, (*S*)-1,2-propanediol, (*R*,*S*)-1,2-butanediol, (*R*,*S*)-3-methoxy-1,2-propanediol, 3-chloro-1,2-propanediol, 1-thioglycerol, (*R*,*S*)-3-amino-1,2-propanediol, (*R*)-3-amino-1,2-propanediol, (*S*)-3-amino-1,2-propanediol, glyceraldehyde, magnesium chloride, MES buffer, disodium hydrogenphosphate, hydrogen chloride were obtained from Sigma–Aldrich (Zwijndrecht, The Netherlands). Sucrose phosphorylase from *B. adolescentis* was produced and isolated as described before [11]. Methanol (Biosolve, Valkenswaard, The Netherlands) and ethylacetate (Fischer Scientific, Loughborough, UK) were HPLC grade. NANOpure water (18.3 MΩ cm) was used during all experiments (Barnstead, Thermo Scientific). Pre-packed flash purification silica cartridges (SNAP 10 g KP-SIL) were obtained from Biotage.

### 2.2. Enzymatic glucosylation

Typical enzymatic reactions (250 μL) were done using 100 mM G1P or 100 mM sucrose. A stock solution of G1P was brought to pH 6.6 using HCl without adding additional buffer, whereas in the case of sucrose (100 mM) MES buffer pH 6.6 (50 mM) was added. Incubations at $T = 30\,^{\circ}C$ further contained glucosyl donor (see below), 10 mM $MgCl_2$ and 25 U/mL sucrose phosphorylase. The highest practical glucosyl acceptor concentration was used, namely: 2.5 M ethylene glycol, 2.5 M (*R*,*S*)-1,2-propanediol, 2.5 M (*R*)-1,2-propanediol, 2.5 M (*S*)-1,2-propanediol, 2.5 M 1,2-butanediol, 2.5 M 3-methoxy-1,2-propanediol, 0.25 M 3-chloro-1,2-propanediol, 2.5 M 1-thioglycerol, 0.25 M glyceraldehyde, 1.7 M 3-amino-1,2-propanediol. The 3-amino-1,2-propanediol stock solution was brought to pH 7 with 37% HCl prior to use. Conversions were followed in time by HPLC. The pH of the mixtures was checked after completion. In the case of G1P a maximum increase to pH 7.0 was observed. Two standard controls for all reactions were performed: (i) same conditions without enzyme; (ii) same conditions without glucosyl acceptor. For product isolation with a charcoal column, 1.0 M sucrose and 1.0 M 1,2-butanediol were used (1 mL scale); this reaction was followed using HPLC.

### 2.3. HPLC analysis

An Alltech OA-1000 column plus a wide pore C4 Security Guard (Phenomenex, Utrecht, The Netherlands) were used with 25 mM $H_2SO_4$ as eluent at a flow rate of 0.4 mL $min^{-1}$, coupled to a refractive index detector (Gilson M 131, Gilson France, Villiers le Bel, France). Samples were diluted 10-fold in water after which 20 μL was injected via a fixed volume loop. Calibration curves for phosphate, glucose, fructose, sucrose and glucosyl acceptors were linear in the concentration range measured; the same holds for G1P after correction for the overlap with the negative void peak. Concentrations of these compounds were calculated using these curves; for the glucosides the concentrations were calculated assuming that glucose production + glucoside production = glucoside-1-phosphate consumption (=phosphate production).

### 2.4. TLC analysis

Prior to product isolation reactions were checked by TLC analysis to determine optimal eluent conditions, using various ratio's of $H_2O$, methanol and ethyl acetate. Spots were colored with a molybdate-cerium based reagent (42 g $L^{-1}$ $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, 3.6 g $L^{-1}$ $Ce(NH_4)(SO_4)_4{\cdot}2H_2O$, 6.2% (v/v) $H_2SO_4$).

### 2.5. Product isolation

#### 2.5.1. Silica column

Glucoside products from (*R*,*S*)-1,2-propanediol, (*R*)-1,2-propanediol, (*S*)-1,2-propanediol, (*R*,*S*)-1,2-butanediol and (*R*,*S*)-3-methoxy-1,2-propanediol obtained with G1P as glucosyl donor were isolated using pre-packed silica cartridges using the Isolera One flash chromatography system from Biotage. This procedure led to the removal of most of the excess glucosyl acceptor, some remaining G1P, the formed phosphate and most of the formed glucose; glucoside regio- or stereoisomers could not be separated in this way. A mixture of ethyl acetate:methanol:water = 7:2:1 was used for elution. Fractions were analysed by HPLC and pooled fractions were freeze dried and taken up in $D_2O$ for NMR analysis.

#### 2.5.2. Charcoal column

Using equimolar concentrations of 1,2-butanediol and sucrose (1.0 M) resulted in 87% sucrose consumption after 96 h reaction time. The product mixture consisted of 85% glucosides (corresponding to 750 mM) and 15% glucose (120 mM). The glucoside product mix was isolated with a charcoal column based on the procedure that Goedl et al. [13,14] used for the isolation of the glucoside of glycerol. This method removed sucrose, formed fructose and glucose and remaining 1,2-butanediol, but could not separate product

**Table 1**
Details of conversion of various compounds by sucrose phosphorylase from *L. mesenteroides* after 26 h reaction time with G1P as glucosyl donor (initial concentration G1P: 100 mM). Also stereopreferences are shown, as determined by NMR.

| Aglycon | Total amount converted to glucosides (%) | Amount converted to glucose (G1P hydrolysis) (%) | Unconverted G1P (%) | Regio-/stereoisomeric composition[b] | Remarks |
|---|---|---|---|---|---|
| Ethylene glycol (**1**) | 69 | 18 | 3 | – | |
| (*R,S*)-1,2-Propanediol (**2**) | 50 | 36 | 14 | (**10**+**12**):(**11**+**13**) = 1.8:1 | |
| (*R*)-1,2-Propanediol ((*R*)-**2**) | 50 | n.d. | n.d. | **10**:**11** = 1:2.5 | |
| (*S*)-1,2-Propanediol ((*S*)-**2**) | 50 | n.d. | n.d. | **12**:**13** = 4.1:1 | |
| (*R,S*)-1,2-Butanediol (**3**) | 48 | 7 | 45 | (**14**+**16**):(**15**+**17**) = 5.6:1 | Faster conversion than 1,2-propanediol |
| (*R,S*)-3-Methoxy-1,2-propanediol (**4**) | 78 | 15 | 7 | (**18**+**20**):(**19**+**21**) = 7.4:1 | Faster conversion than 1,2-propanediol |
| (*R,S*)-3-Amino-1,2-propanediol (**5**) | 0 | n.d. | n.d. | – | No inhibition of G1P hydrolysis |
| (*R,S*)-3-Chloro-1,2-propanediol (**6**) | 0 | n.d. | n.d. | – | No inhibition of G1P hydrolysis |
| (*R,S*)-1-Thioglycerol (**7**) | 0 | n.d. | n.d. | – | No inhibition of G1P hydrolysis |
| (*R,S*)-Glyceraldehyde (**8**) | 0 | n.d. | n.d. | – | 50% inhibition of G1P hydrolysis |
| Glycerol (not shown) | Similar to ethylene glycol | Similar to ethylene glycol | Similar to ethylene glycol | **22**[a] | |

n.d.: not determined.
[a] Goedl et al. [13].
[b] See Fig. 3.

regio- or stereoisomers. A pre-packed silica cartridge (SNAP 50 g KP-SIL) was emptied and filled with a 1:1 mixture of activated charcoal Norit (type Norit SX Ultra, Sigma–Aldrich) and calcined Celite 503 filter aid (Sigma–Aldrich). Elution was done in 4 steps: 250 mL $H_2O$, 250 mL 2% EtOH, 140 mL 15% EtOH, 90 mL 25% EtOH. Fractions were dried, taken up in water and analysed by HPLC as described above. On a 1 mL scale and using a charcoal column we obtained an isolated yield of 124 mg glucoside mix (49%) with a purity >90%. The compounds eluted in the order: fructose (0–2% EtOH), glucose (2% EtOH), sucrose (15% EtOH), glucoside isomers (15–25% EtOH), 1,2-butanediol (25% EtOH).

### 2.6. NMR analysis

Pooled fractions from the silica column or charcoal column were combined, freeze dried and taken up in 600 μL $D_2O$ for NMR analysis. $^1H$, $^{13}C$ (APT), COSY, HSQC and HMBC spectra were taken on a Bruker Avance III 400 MHz NMR spectrometer. Typically about 50 mg of product (purity 75–99%) was isolated for each conversion and analysed by NMR. For the purity analysis of the samples see the Supplementary file.

#### 2.6.1. (*R*)-2-Hydroxy-1-propyl α-glucopyranoside (***11***, *the major regio-isomer that is formed)*

$^1H$ NMR (400 MHz, $D_2O$) $\delta$ (ppm): 4.87 (d, $J$ = 3.3 Hz, 1H, H-1), 4.00 (1H, $CH_3CH(OH)CH_2OGlc$), 3.80 (1H, H-6), 3.69 (1H, H-6′), 3.67 (1H, H-3), 3.65 (1H, $CH_3CH(OH)CH_2OGlc$), 3.62 (1H, H-5), 3.49 (1H, H-2), 3.35 (1H, H-4), 3.31 (1H, $CH_3CH(OH)CH_2OGlc$), 1.12 (3H, $CH_3$). $^{13}C$ NMR (101 MHz, $D_2O$) $\delta$ (ppm): 98.6 (C-1), 73.1 (C-3), 72.9 ($CH_3CH(OH)CH_2OGlc$), 71.8 (C-5), 71.5 (C-2), 69.6 (C-4), 66.6 ($CH_3CH(OH)CH_2OGlc$), 60.5 (C-6), 18.1 ($CH_3$).

#### 2.6.2. (*R*)-1-Hydroxy-2-propyl α-glucopyranoside (***10***, *minor regio-isomer formed)*

$^1H$ NMR (400 MHz, $D_2O$) $\delta$ (ppm): 5.02 (1H, H-1), 3.84 (1H, $CH_3CH(OGlc)CH_2OH$), 3.84–3.65 (2H, H-6, H-6′), 3.72 (1H, H-5), 3.65 (1H, H-3), 3.59 (1H, $CH_3CH(OGlc)CH_2OH$), 3.51 (1H, $CH_3CH(OGlc)CH_2OH$), 3.47 (1H, H-2), 3.36 (1H, H-4), 1.17 (3H, $CH_3$). $^{13}C$ NMR (101 MHz, $D_2O$) $\delta$ (ppm): 98.3 (C-1), 75.8 ($CH_3CH(OGlc)CH_2OH$), 73.1 (C-3), 72.0 (C-5), 71.6 (C-2), 69.7 (C-4), 64.7 ($CH_3CH(OGlc)CH_2OH$), 60.6 (C-6), 16.9 ($CH_3$).

#### 2.6.3. (*S*)-1-Hydroxy-2-propyl α-glucopyranoside (***12***, *major regio-isomer formed)*

$^1H$ NMR (400 MHz, $D_2O$) $\delta$ (ppm): 4.99 (1H, H-1), 3.84 (1H, $CH_3CH(OGlc)CH_2OH$), 3.79 (1H, H-6), 3.74 (1H, H-5), 3.70 (1H, H-6′), 3.68 (1H, H-3), 3.59 (2H, $CH_3CH(OGlc)CH_2OH$), 3.49 (1H, H-2), 3.36 (1H, H-4), 1.08 (3H, $CH_3$). $^{13}C$ NMR (101 MHz, $D_2O$) $\delta$ (ppm): 95.6 (C-1), 73.2 ($CH_3CH(OGlc)CH_2OH$), 73.0 (C-3), 71.8 (C-5), 71.3 (C-2), 69.6 (C-4), 65.3 ($CH_3CH(OGlc)CH_2OH$), 60.5 (C-6), 14.4 ($CH_3$).

#### 2.6.4. (*S*)-2-Hydroxy-1-propyl α-glucopyranoside (***13***, *minor regio-isomer formed)*

$^1H$ NMR (400 MHz, $D_2O$) $\delta$ (ppm): 4.86 (d, $J$ = 2.8 Hz, 1H, H-1), 4.02 (1H, $CH_3CH(OH)CH_2OGlc$), 3.80–3.70 (2H, H-6, H-6′), 3.66 (1H, H-3), 3.55 (1H, $CH_3CH(OH)CH_2OGlc$), 3.65 (1H, H-5), 3.48 (1H, H-2), 3.34 (1H, H-4), 3.45 (1H, $CH_3CH(OH)CH_2OGlc$), 1.13 (3H, $CH_3$). $^{13}C$ NMR (101 MHz, $D_2O$) $\delta$ (ppm): 98.1 (C-1), 73.1 (C-3), 72.4 ($CH_3CH(OH)CH_2OGlc$), 71.8 (C-5), 71.5 (C-2), 69.6 (C-4), 66.2 ($CH_3CH(OH)CH_2OGlc$), 60.6 (C-6), 18.1 ($CH_3$).

#### 2.6.5. (*S*)-1-Hydroxy-2-butyl α-glucopyranoside (***16***, *major regio- and stereoisomer formed)*

$^1H$ NMR (400 MHz, $D_2O$) $\delta$ (ppm): 4.99 (1H, H-1), 3.77 (1H, H-6), 3.74 (1H, H-5), 3.68 (1H, H-6′), 3.67 (1H, H-3), 3.62 (2H, $CH_3CH_2CH(OGlc)CH_2OH$), 3.60 (1H, $CH_3CH_2CH(OGlc)CH_2OH$), 3.48

(1H, H-2), 3.34 (1H, H-4), 1.55 (2H, $CH_3CH_2CH(OGlc)CH_2OH$), 0.86 (3H, $CH_3$). $^{13}$C NMR (101 MHz, $D_2O$) $\delta$ (ppm): 97.1 (C-1), 79.5 ($CH_3CH_2CH(OGlc)CH_2OH$), 73.0 (C-3), 71.7 (C-5), 71.6 (C-2), 69.6 (C-4), 63.2 ($CH_3CH_2CH(OGlc)CH_2OH$), 60.6 (C-6), 22.4 ($CH_3CH_2CH(OGlc)CH_2OH$), 8.8 ($CH_3$).

*2.6.6. (R)-1-Hydroxy-3-methoxy-2-propyl α-glucopyranoside (**20**, major regio- and stereoisomer formed)*

$^1$H NMR (400 MHz, $D_2O$) $\delta$ (ppm): 5.06 (d, $J$ = 3.6 Hz, 1H, H-1), 3.85 (1H, $CH_3OCH_2CH(OGlc)CH_2OH$), 3.78 (1H, H-6), 3.75 (1H, H-5), 3.69 (1H, H-6′), 3.67 (1H, H-3), 3.66 (2H, $CH_3OCH_2CH(OGlc)CH_2OH$), 3.58 (2H, $CH_3OCH_2CH(OGlc)CH_2OH$), 3.47 (1H, H-2), 3.36 (1H, H-4), 3.33 (3H, $CH_3$). $^{13}$C NMR (101 MHz, $D_2O$) $\delta$ (ppm): 97.6 (C-1), 76.4 ($CH_3OCH_2CH(OGlc)CH_2OH$), 72.9 (C-3), 72.1 (C-5), 71.6 (C-2), 71.3 ($CH_3OCH_2CH(OGlc)CH_2OH$), 69.6 (C-4), 61.5 ($CH_3OCH_2CH(OGlc)CH_2OH$), 60.6 (C-6), 58.5 ($CH_3$).

## 3. Results and discussion

### 3.1. Conversion of various alcohols with G1P as glucosyl donor

First, sucrose phosphorylase was tested for its ability to glucosylate mono-alcohols. Methanol and ethanol were not glucosylated. After this we tested various diols resembling glycerol. The enzymatic glucosylation of (*R*,*S*)-1,2-butanediol with G1P as glucosyl donor was studied by HPLC. A clear formation of a glucoside product peak at $t_R$ = 12.2 min is observed, which is absent in the control without enzyme (see Fig. S1, Supplementary material). In addition, phosphate release is observed, plus the formation of some glucose. As observed before for the 'synthesis reaction' of phosphorylases [1], water competes as a 'glycosyl acceptor', in this case forming glucose. Consequently, in the absence of (*R*,*S*)-1,2-butanediol, glucose formation is higher. While testing the mono-alcohols ethanol and methanol a similar amount of G1P hydrolysis was observed as in their absence, indication that the enzyme was not simply denatured or inhibited. At these time scales no glucose formation as a consequence of non-catalytic hydrolysis of G1P is observed (see Fig. S1, Supplementary material).

For (*R*,*S*)-1,2-butanediol, 55% of the G1P is consumed; an equilibrium is reached as can be seen in Fig. 2 (panel A), where the conversion in time is depicted. Fig. 2 also shows the conversions of ethylene glycol, (*R*,*S*)-1,2-propanediol and (*R*,*S*)-3-methoxy-1,2-propanediol (Panels B–D).

Table 1 summarises the amounts of glucosylated products formed for all glycerol analogues that were investigated. For (*R*,*S*)-1,2-propanediol, ethylene glycol and (*R*,*S*)-3-methoxy-1,2-propanediol, >85% of G1P is consumed. The rate of product formation increases in the order (*R*,*S*)-1,2-propanediol → (*R*,*S*)-3-methoxy-1,2-propanediol → ethylene glycol → (*R*,*S*)-1,2-butanediol, but differences are not large. With (*R*,*S*)-1,2-propanediol a relatively large amount of G1P hydrolysis is observed. For (*R*,*S*)-3-amino-1,2-propanediol (**5**), (*R*,*S*)-3-chloro-1,2-propanediol (**6**), (*R*,*S*)-1-thioglycerol (**7**) and (*R*,*S*)-glyceraldehyde (**8**) no glucoside product formation was observed. For (*R*,*S*)-1-thioglycerol a product was observed which was also formed in the absence of enzyme, presumably due to non-enzymatic disulfide formation. The presence of (*R*,*S*)-glyceraldehyde decreased glucose formation by 50%, indicating binding in the active site without conversion. For 3-amino-1,2-propanediol both stereoisomers were also tested separately, both showing no conversion.

During the conversions a decrease in rate of product formation in time is observed for all four products. To test if this is due to inhibition by released phosphate, the experiment with ethylene glycol was repeated with an initial phosphate concentration of 300 mM. The result was immediate inhibition for more than 95% (not shown). This could also be an explanation for the previously higher observed yield of glucosylation of glycerol using sucrose as glucosyl donor compared to G1P [13].

We also tested glucosylation of all eight diols mentioned in this study with sucrose phosphorylase from *B. adolescentis* [11,12], giving essentially the same results during TLC and HPLC analysis.

### 3.2. Isolation and NMR analysis of product structure, regio- and stereoselectivity

In Fig. 3 the possible products of all conversions in this study are shown, plus product 22 that was found for the glucosylation of glycerol [13]. The product of ethylene glycol was not analysed by NMR; only one peak was observed during HPLC analysis so we assume it is the monoglucoside 9.

For glucosylation of (*R*,*S*)-1,2-propanediol there is an overall preference for the 2-position, as earlier observed for glucosylation of glycerol [13], but the ratio 2-glucoside:1-glucoside ((**10** + **12**):(**11** + **13**)) is less marked (1.8:1). Interestingly, when we analysed glucosylation of the individual enantiomers we observed the 1-regioisomer to be the major product for the (*R*)-1,2-propanediol (**10**:**11** = 1:2.5), whereas the 2-regioisomer is the major product for (*S*)-1,2-propanediol (**12**:**13** = 4.1:1). Overall this means that the stereoselectivity *R*:*S* is 1:6 (d.e.$_p$ = 71%), assuming that in the racemate the preference of the enzyme for the 1- or 2-position of either of the enantiomers is the same as that for the individual enantiomers.

Also for glucosylation of (*R*,*S*)-1,2-butanediol and (*R*,*S*)-3-methoxy-1,2-propanediol there is an overall preference for the 2-position. The ratio 2-glucoside (**14** + **16**):1-glucoside (**15** + **17**) is 5.6:1 for (*R*,*S*)-1,2-butanediol (**3**). Likewise, the ratio (**18** + **20**):(**19** + **21**) is 7.4:1 for (*R*,*S*)-3-methoxy-1,2-propanediol (**4**). Both ratios are larger than for (*R*,*S*)-1,2-propanediol (**2**). For (*R*,*S*)-1,2-butanediol (**3**) the stereoselectivity is 1:6.6 (d.e.$_p$ = 74%) in benefit of one of the enantiomers. If the analogy with propanediol can be applied, the preference would be for the *S*-enantiomer as well. For (*R*,*S*)-3-methoxy-1,2-propanediol (**4**) the stereoselectivity is even higher (1:11, d.e.$_p$ = 83%). If the analogy with (*R*,*S*)-1,2-propanediol can be applied, the preference would be for the *R*-enantiomer (which has the same three-dimensional structure as (*S*)-1,2-propanediol).

Very recently it was shown by Schwartz et al. [15] that also the β-glucoside of glycerol can be formed enzymatically using a thermostable cellobiose phosphorylase from *Pyrococcus furiosus* and cellobiose as the glucosyl donor. It is conceivable that also interesting enantioselectivity will be observed with this enzyme in combination with the compounds studied by us.

### 3.3. Conversion of 1,2-butanediol: G1P vs. sucrose as glucosyl donor

Having identified four new substrates for sucrose phosphorylase, we subsequently compared the use of (cheap) sucrose and G1P as glucosyl donors for this enzyme. As expected, all glucosides were also formed using sucrose as a donor. Fig. 4 shows the glucosylation of 1,2-butanediol (**3**) as a function of time.

A higher conversion of aglycon is obtained with sucrose as a donor; in contrast to G1P (see Fig. 2) sucrose is almost completely consumed, which is most likely due to a more favorable equilibrium. However, the disadvantage of using sucrose is the release of fructose. This byproduct is more cumbersome to remove compared to phosphate in the case of G1P, which is also reflected in the HPLC chromatograms (see Fig. S2, Supplementary material). Thus for product identification via NMR we used isolated glucosides made with G1P as a donor. Obviously, for industrial application sucrose is much cheaper, but it might be conceivable that when

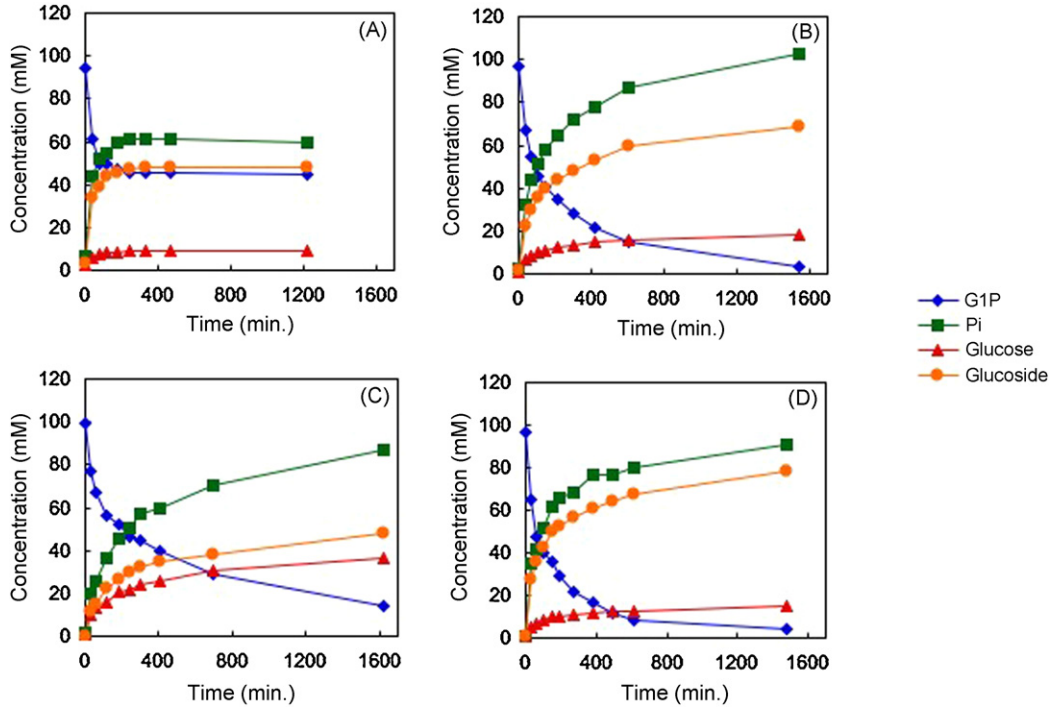


**Fig. 2.** Glucosylation of (*R*,*S*)-1,2-butanediol (A), ethylene glycol (B), (*R*,*S*)-1,2-propanediol (C), and (*R*,*S*)-3-methoxy-1,2-propanediol (D) by sucrose phosphorylase from *L. mesenteroides* as a function of time with G1P as glucosyl donor.

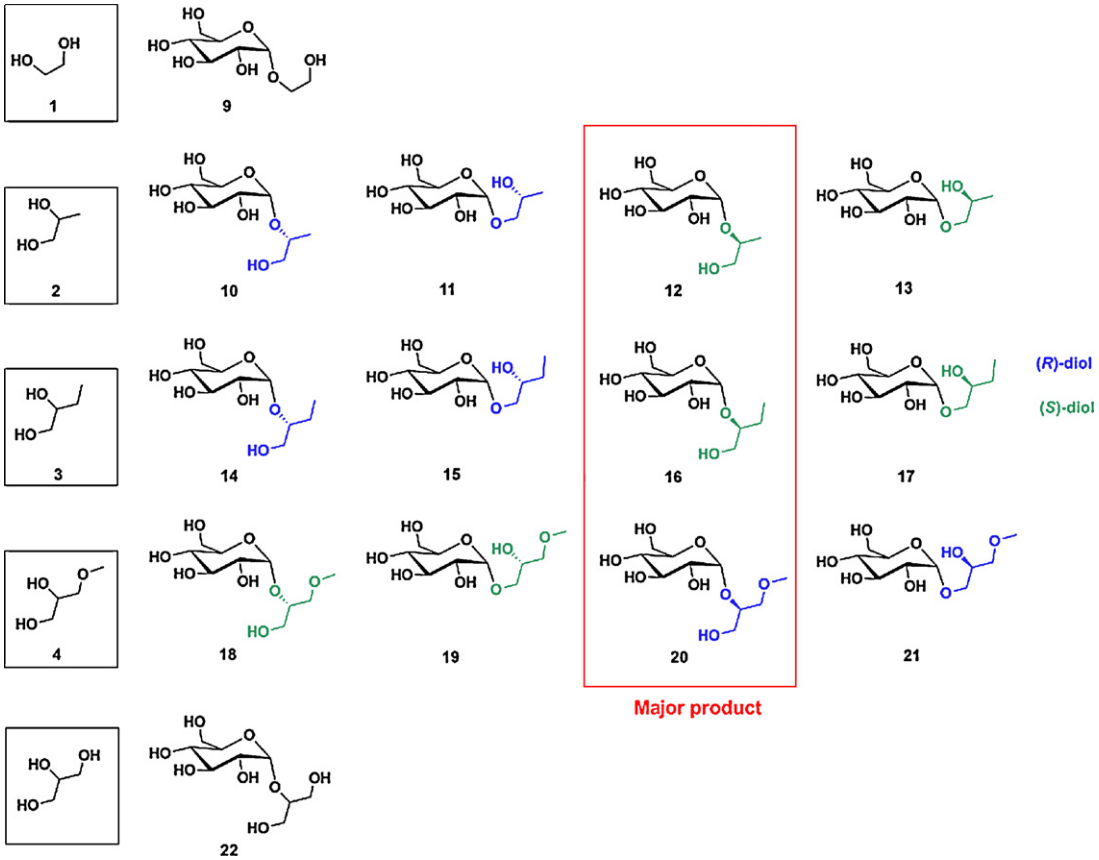


**Fig. 3.** Possible mono-glucosides of ethylene glycol (**9**), 1,2-propanediol (**10**–**13**), 1,2-butanediol (**14**–**17**) and 3-methoxy-1,2-propanediol (**18**–**21**) plus the observed single glucosylation product of glycerol (**22**) [13]. (*R*)-Glucosyl acceptor units are indicated in blue and (*S*)-glucosyl acceptor units in green. (For the mentioned colors in this legend the reader is referred to the web version of this article).

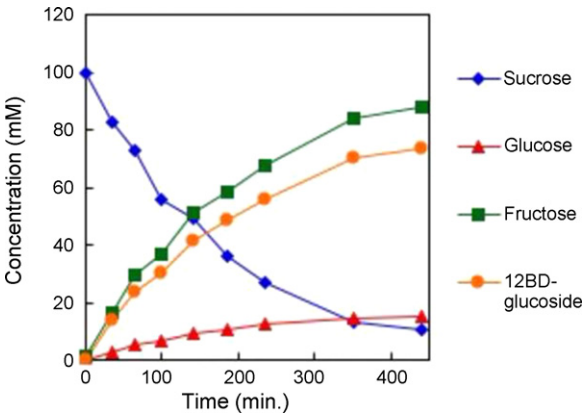


**Fig. 4.** Glucosylation of 1,2-butanediol (**3**) by sucrose phosphorylase from *L. mesenteroides* as a function of time with sucrose as glucosyl donor.

G1P is generated enzymatically this route become cost-effective as well. We have preliminary results that by starting from glycogen and using a cascade of glycogen phosphorylase and sucrose phosphorylase it is possible to form the glucoside of ethylene glycol (not shown).

With both glucosyl donors no side product formation apart from glucose was observed; we anticipated some formation of maltose, as a consequence of α-glucosylation of the formed glucose; however, at the retention time of maltose (8.40 min) no peak was observed in any of our experiments.

## 4. Conclusions

Four new compounds resembling glycerol were found to be glucosylated by sucrose phosphorylase from *L. mesenteroides* and *B. adolescentis*, namely ethylene glycol, 1,2-propanediol, 1,2-butanediol and 3-methoxy-1,2-propanediol. Since mono-alcohols are not converted, the minimal unit necessary for these compounds to be accepted as substrates appears to be ethylene glycol. Both α-glucose 1-phosphate and sucrose can be used as the glucosyl donor. If one terminal hydroxyl group of glycerol is substituted, regioselective α-glucosylation on the 2-position (overall) is observed for the four compounds mentioned above, whereas for other compounds no glucosylation is observed. We show that glucosylation of the individual enantiomers of 1,2-propanediol results in a different regioselectivity for each enantiomer; the 1-regioisomer (2-hydroxy-1-propyl α-glucopyranoside, **11**) is the major product for the reaction of (*R*)-1,2-propanediol, whereas the 2-regioisomer (1-hydroxy-2-propyl α-glucopyranoside, **14**) is the major product for (*S*)-1,2-propanediol. For the compounds tested also enantioselectivity is observed with ee's up to 83%. This is the first time that this stereoselectivity is observed for sucrose phosphorylase. It is conceivable that this type of stereoselectivity will also be found for other phosphorylases converting glycerol-like compounds.

## Note added in proof

The Nidetzki group has also very recently published on the sucrose phosphorylase mediated glucosylation of 1,2-propanediol, see [16].

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2010.08.009.

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