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Note

Transglycosylation reactions with a crude culture filtrate from *Thermoascus aurantiacus*

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Abstract

Some characteristics of regioselectivity and acceptor tolerance in transglycosylation reactions, catalysed by a crude culture filtrate from *Thermoascus aurantiacus*, were examined by employing methanol and monosaccharides as acceptors. When β-D-mannopyranosyl fluoride was employed as the donor, the anomeric configuration of the newly formed bond was found to depend on the structure of the acceptor used.

Keywords: Glycosyl fluorides; Glycosidases; Transglycosylation; β-Mannosidase; *Thermoascus aurantiacus* Miehe

1. Introduction

Due to the biological significance of oligosaccharidic structures and glycoconjugates [1,2], a broad range of chemical [3,4] and enzymatic [5–7] methods for glycosidic bond formation has been developed to date. Approaches employing glycosidases as cheap and easy to handle biocatalysts are limited by the number of glycosidases available, especially when, as in an ongoing program, formation of β-D-mannosidic bonds or regiospecific transglycosylation to secondary OH-groups of the acceptor is required. In some instances, such problems have been overcome by utilisation of enzymes from new sources wherein extremophilic microorganisms are of special interest.

For this purpose a new ^{19}F NMR method [8], based on simultaneous monitoring of the fate of different glycosyl fluorides in the presence of enzyme mixtures, has been developed. By applying this technique together with the known thin-layer chromatography (TLC) method [9] in a screening program, the thermophilic fungus *Thermoascus aurantiacus* has proven to be a source of mannosidases as well as hydrolysing enzymes towards β-cellobiosyl, β-lactosyl, α-D-galactosyl, and α- and β-D-glucosyl fluorides [8].

2. Results and discussion

To roughly test the glycosyltransfer potential of these hydrolases, cellobiose **1** and β-D-mannopyranosyl fluoride **3** as donors and methanol as a simple acceptor were used (Table 1, entries 1 and 2). The methyl glycosides **2** and **4**, isolated by chromatography

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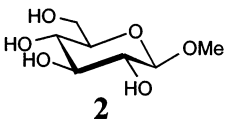
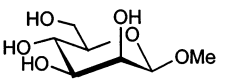
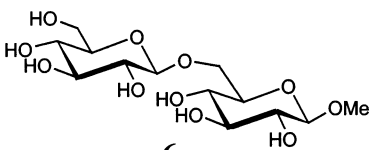
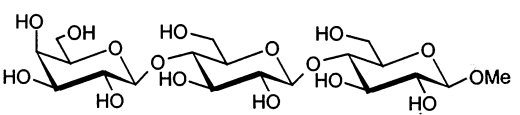
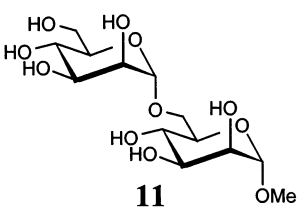
Table 1 Results of transglycosylation reactions

	donor	acceptor	product(s)	[yield][a]
1	cellobiose (**1**)	methanol	 **2**	20%[b]
2	β-D-Man-F (**3**)	methanol	 **4**	23%[b]
3	β-D-Glc-O*p*NP (**5**)	β-D-Glc-OMe (**2**)	 **6**	25%[c]
4	β-Lac-F (**7**)	β-D-Glc-OMe (**2**)	 **8** + regioisomers	Σ19%[c]
5	β-Lac-F (**7**)	α-D-Glc-OMe (**9**)	no transglycosylation products	-
6	β-D-Man-F (**3**)	α-D-Man-OMe (**10**)	 **11**	11%[c]
7	α-D-Man-F (**12**)	α-D-Man-OMe (**10**)	**11**	8%[c]
8	β-D-Man-F (**3**)	2-F-α-D-Man-F (**13**) 2-F-α-D-Glc-F (**14**) (1:1 mixture)	no transglycosylation products	-

[a] Isolated yields; [b] procedure A; [c] procedure B (isolated as peracetate).

and identified by their ^{13}C NMR spectra [10], were formed with overall retention of configuration. The β-configuration in mannopyranoside **4** was additionally proven by the magnitude of $^{1}J_{C\text{-}1,H\text{-}1}$ of 160 Hz (versus 170 Hz reported for the α anomer) [11].

In a second series of experiments, different donors together with monosaccharidic acceptors were used. In all cases the products were isolated and characterised as their peracetates. The respective site of glycosylation as well as the anomeric configuration of the newly formed bond was elucidated from diacritic NMR data (as acylation and glycosylation shift, $^3J_{H,H}$ and $^1J_{C\text{-}1,H\text{-}1}$) obtained from the acetylated products and verified by comparison of the NMR-data of the O-deacetylated ones with those reported for the proposed structures in the literature.

Using methyl β-D-glucopyranoside (**2**) as acceptor and *p*-nitrophenyl β-D-glucopyranoside (**5**) as donor, the disaccharide β-D-Glc*p*-(**1**→**6**)-β-D-Glc*p*-OMe (**6**) [12] was formed as the sole product (Table 1, entry 3). In the reaction with β-lactosyl fluoride (**7**) as donor, a mixture of regioisomers was obtained from which the main product could be isolated and identified as β-D-Gal*p*-(1→4)-β-D-Glc*p*-(**1**→**4**)-β-D-Glc*p*-OMe (**8**) [13] containing a new β-(1→4) linkage (Table 1, entry 4). In order to explore the influence of the anomeric configuration of the acceptor on the regioselectivity, an analogous experiment with **7** and methyl α-D-glucopyranoside (**9**) was performed, but no products of transglycosylation could be isolated (Table 1, entry 5). A similar result has been reported by Kobayashi and co-workers, when using a cellulase from *Trichoderma viride* [14].

Interestingly, transglycosylation with inversion of configuration was observed when β-D-mannopyranosyl fluoride (**3**) as donor together with methyl α-D-mannopyranoside (**10**) as acceptor was used and α-D-Man*p*-(**1**→**6**)-α-D-Man*p*-OMe (**11**) [15–18] was isolated as its peracetate [18] in low yield (Table 1, entry 6). The same α-(**1**→**6**)-linked disaccharide was formed when α-D-mannopyranosyl fluoride (**12**) was applied as donor (Table 1, entry 7). The α-configuration at both anomeric centres in disaccharide **11** was additionally proved by $^1J_{C\text{-}1,H\text{-}1}$ of 172.2 and 172.8 Hz, respectively.

No products of mannosyl transfer could be isolated when β-D-mannopyranosyl fluoride (**3**) together with an 1:1 mixture of 2-deoxy-2-fluoro-α-D-gluco- (**13**) and -α-D-mannopyranosyl fluoride (**14**) [19] was subjected to identical reaction conditions (Table 1, entry 8).

Starting from β-D-mannopyranosyl fluoride (**3**), formation of the α-configurated intersaccharidic linkage in disaccharide **11** is in contrast to own results which were obtained with methanol as simple acceptor in the presence of the same culture filtrate (see Table 1, entry 2). (Transglycosylation reactions using **3** and β-*glucosidase* from *Agrobacterium* sp. [20] also led to β-D-mannopyranosides under retention of configuration.) Therefore, control experiments under otherwise identical conditions but using D-mannose as donor were undertaken. As no disaccharide was produced, formation of **11** in the former case by (β-mannosidase catalysed) hydrolysis of the fluoride followed by (α-mannosidase catalysed) reverse hydrolysis could be excluded. Furthermore, as TLC and ^{19}F NMR monitoring disclosed, anomerisation of the β-fluoride **3** under the reaction conditions did not take place, thus excluding anomerisation prior to (α-mannosidase catalysed) transglycosylation as a possible reaction path. For further mechanistic studies, the isolation and characterisation of the individual mannosidases (either working with retention or inversion of configuration) from this multi-enzyme mixture is in progress.

3. Experimental

General procedures.—Optical rotations were measured with a Jasco DIP-360 digital polarimeter at 589 nm at ambient temperature. NMR spectra were recorded at 300.13 or 199.98 MHz (^1H) and 75.47 or 50.29 MHz (^{13}C) from solutions in $CDCl_3$ using a Bruker MSL 300 and a Varian Gemini 200 apparatus, respectively. As reference standard Me_4Si (^1H and ^{13}C NMR) was used. TLC was performed on Silica Gel 60 F254 precoated aluminium plates (E. Merck 5554) with detection by charring after spraying with vanillin/H_2SO_4 (1% w/v). For column chromatography Silica Gel 60, 230–400 mesh (E. Merck 9385) was used.

Compounds **2**, **5**, **9** and **10** were purchased from Fluka; donors **3**, **7** and **12** were prepared according to literature procedures [8,12].

The crude culture filtrate used in this study was from the strain *Thermoascus aurantiacus* Miehe, and was prepared according to Ref. [8].

Procedure A.—To a soln of the donor (0.20 M) in acetate buffer (pH 5.0, 50 mM) containing 20 vol% MeOH, the lyophilised culture filtrate (0.5–1 mg per mg donor) was added and the mixture was shaken at room temperature (rt) until all the donor was consumed (typically overnight). After addition of toluene and evaporation of the solvent under diminished pressure, the products were isolated by column chromatography (5:1 $CHCl_3$–MeOH).

Procedure B.—To a soln of the donor (0.50 M) and acceptor (1.5 M) in acetate buffer (pH 5.0, 50 mM), the lyophilised culture filtrate (1–2 mg per mg donor) was added and the reaction mixture was shaken at rt until all the donor was consumed. After addition of toluene and evaporation of the solvent under diminished pressure, the crude product mixture was acetylated in Ac_2O–pyridine. After extractive work-up, the peracetates were isolated by column chromatography (2:1 cyclohexane–EtOAc).

Methyl 2,3,4-tri-O-*acetyl-6*-O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl)*-β-D-*glucopyranoside* (*peracetate of* **6**).—*p*-Nitrophenyl β-D-glucopyranoside (**5**, 176 mg, 0.58 mmol) and methyl β-D-glucopyranoside (**2**, 340 mg, 1.75 mmol) were brought to reaction as described in procedure B yielding the title compound (95 mg, 25%) as a colourless oil. $[\alpha]_D^{20}$ −20° (*c* 1.35, $CHCl_3$); R_f 0.42 (1:1 cyclohexane–EtOAc); ^{13}C NMR (50.29 MHz, $CDCl_3$): δ 170.7–169.3 (OAc), 101.5 and 100.8 (C-1, 1′), 73.3 (C-5), 72.8 and 72.8 (C-3, 3′), 72.0 (C-5′), 71.3 and 71.1 (C-2, 2′), 69.2, 68.3 and 68.3 (C-4, 6, 4′), 61.8 (C-6′), 57.1 (OMe), 20.7–20.6 (OAc); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 5.13 (t, 2 H, $J_{2,3} = J_{3,4} = J_{2',3'} = J_{3',4'}$ 9.5 Hz, H-3, 3′), 4.92 and 4.85 (dd each, 1 H each, $J_{1,2} = J_{1',2'}$ 8.0 Hz, H-2, 2′), 5.00 and 4.82 (t each, 1 H each, $J_{4,5} = J_{4',5'}$ 9.5 Hz, H-4, 4′), 4.54 and 4.33 (d each, 1 H each, H-1, 1′), 4.20 (dd, 1 H, $J_{5',6'a}$ 4.8 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6′a), 4.05 (bd, 1 H, H-6′b), 3.81 (bd, 1 H, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.67–3.60 (m, 2 H, H-5, 5′), 3.55 (dd, 1 H, $J_{5,6b}$ 7.4 Hz, H-6b), 3.44 (s, 3 H, OMe), 2.1–1.9 (m, 21 H, 7 OAc).

Methyl 2,3,6-tri-O-*acetyl-4*-O-*[2,3,6-tri*-O-*acetyl*-4-O-*(2,3,4,6*-tetra-O-*acetyl*-β-D-*galactopyranosyl)*-β-D-*glucopyranosyl]*-β-D-*glucopyranoside* (*peracetate of* **8**).—β-Lactosyl fluoride (**7**, 200 mg, 0.58 mmol) and methyl β-D-glucopyranoside (**2**, 338 mg, 1.74 mmol) were treated according to procedure B yielding a mixture of regioisomeric trisaccharides (105 mg, Σ 19%, colourless oil). By repeated column chromatography (1:1 cyclohexane–EtOAc), a clean fraction of the title compound was isolated and characterised; $[\alpha]_D^{20}$ −11° (*c* 0.15, CH_2Cl_2); R_f 0.33 (1:2 cyclohexane–EtOAc); ^{13}C NMR (50.29 MHz, $CDCl_3$): δ 170.4–169.2 (OAc), 101.5, 101.2 and 100.6 (C-1, 1′, 1″), 76.6 and 76.1 (C-4, 4′), 73.1, 72.8, 72.8, 72.6, 72.0 and 71.7 (C-2, 3, 5, 2′, 3′, 5′), 71.1 and 70.9 (C-3″, 5″), 66.7 (C-4″), 62.4, 61.9 and 60.9 (C-6, 6′, 6″), 57.1 (OMe), 21.0–20.6 (OAc); ^{1}H NMR (199.98 MHz, $CDCl_3$): δ 5.34 (bd, 1 H, $J_{3'',4''}$ 3.3 Hz, H-4″), 5.16 and 5.13 (t each, 1 H each, $J_{2,3} = J_{3,4} = J_{2',3'} = J_{3',4'}$ 9.2 Hz, H-3, 3′), 5.09 (dd, 1 H, $J_{1'',2''}$ 8.1 Hz, $J_{2'',3''}$ 10.3 Hz, H-2″), 4.93 (dd, 1 H, H-3″), 4.88 and 4.83 (dd each, 1 H each, $J_{1,2} = J_{1',2'}$ 7.8 Hz, H-2, 2′), 4.49 and 4.43 (d each, 1 H each, H-1, 1′), 4.37 (d, 1 H, H-1″), 4.0–4.6 (m, 6 H, H-6, 6′, 6″), 3.85 (bd, 1 H, $J_{5'',6''}$ 6.6 Hz, H-5″), 3.78 and 3.76 (t each, 1 H each, $J_{4,5} = J_{4',5'}$ 9.2 Hz, H-4, 4′), 3.5–3.65 (m, 2 H, H-5, 5′), 3.47 (s, 3 H, OMe), 2.20–1.95 (m, 30 H, 10 OAc).

Methyl 2,3,4-tri-O-*acetyl-6*-O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (*peracetate of* **11**).—Application of procedure B to a mixture of α- or β-D-mannopyranosyl fluoride (**12** or **3**, 150 mg, 0.823 mmol) and methyl α-D-mannopyranoside (**10**, 480 mg, 2.47 mmol) led to the title compound as a colourless oil (43 or 59 mg, 8 or 11%, respectively). $[\alpha]_D^{20}$ +72° (*c* 1.10, $CHCl_3$); R_f 0.26 (1:1 cyclohexane–EtOAc); ^{13}C NMR (50.29 MHz, $CDCl_3$): δ 170.6–169.8 (OAc), 98.4 ($J_{C\text{-}1,H\text{-}1}$ 172.2 Hz, C-1), 97.5 ($J_{C\text{-}1,H\text{-}1}$ 172.8 Hz, C-1′), 69.5, 69.4, 69.2, 69.1, 69.0, 68.7, 66.6 and 66.0 (C-2, 3, 4, 5, 2′, 3′, 4′, 5′), 66.7 (C-6), 62.4 (C-6′), 55.3 (OMe), 20.9–20.7 (OAc); ^{1}H NMR (199.98 MHz, $CDCl_3$): δ 5.40–5.15 (m, 6 H, H-2, 3, 4, 2′, 3′, 4′), 4.83 and 4.67 (d each, 1 H each, $J_{1,2} = J_{1',2'}$ 1.3 Hz, H-1, 1′), 4.22 (dd, 1 H, $J_{5',6'a}$ 5.4 Hz, $J_{6'a,6'b}$

12.2 Hz, H-6′a), 4.15–4.0 (m, 2 H, H-5, 6′b), 3.90 (ddd, 1 H, $J_{4,5}$ 8.5 Hz, $J_{5,6a}$ 5.8 Hz, $J_{5,6b}$ 2.4 Hz, H-5), 3.75 (dd, 1 H, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.54 (dd, 1 H, H-6b), 3.38 (s, 3 H, OMe), 2.15–1.9 (m, 21 H, 7 OAc).

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