EurJOC
European Journal of Organic Chemistry

A Journal of

Accepted Article

Title: A novel Chemoenzymatic route to a new class of Sucrose esters

Authors: Juergen Seibel, Christian Possiel, and Alexandra Baeurle

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201701313

Link to VoR: http://dx.doi.org/10.1002/ejoc.201701313

Supported by ACES Asian Chemical Editorial Society

WILEY-VCH

10.1002/ejoc.201701313

WILEY-VCH

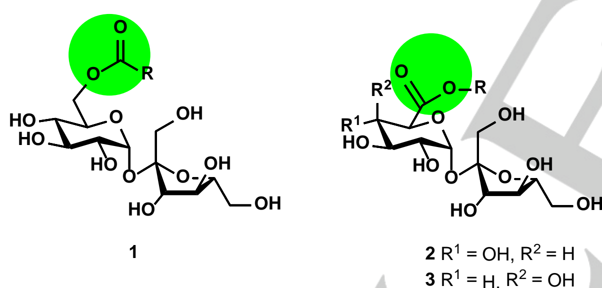
COMMUNICATION

A novel Chemoenzymatic route to a new class of Sucrose esters

Christian Possiel,[a], Alexandra Bäuerle and Jürgen Seibel*[a]

Abstract: Sucrose esters are the most developed carbohydrate esters and applied in food, cosmetic and pharmaceutical industries. Here we introduce a novel chemoenzymatic pathway for the synthesis of β-D-fructofuranosyl-(2,1)-α-D-uronic acid derivatives, a new class of sucrose esters.

Carbohydrates are an important source of renewable compounds. The disaccharide sucrose (α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside) is produced in industrial scale of 170 x 10^6 MT/y.[1] The development of chemical processes from carbohydrates instead of fossil resources became a principal of Green Chemistry. Sucrose esters are one of these examples. They have many applications in food, cosmetics and pharmaceutical industry.[2] Sucrose possesses 8 hydroxyl groups that can be esterified with fatty acids with aliphatic tails of 1 to 18 carbons.[3] The chemical production of sucrose esters has been investigated[4] but the selectivity in the synthesis of these esters remains a challenge. Lipases from i.e. *Thermomyces lanuginosus*[5], Candida *antarctica*[6] and *Rhizomucor miehei*[7] catalyze the transesterification to 2-*O*-acylsucrose or 6-*O*-acylsucrose. All these sucrose esters derive from a carboxylic acid (fatty acid) and sucrose equipped with hydroxyl-groups. We envisaged that sucrose could function as a carboxylic acid and be condensed with an alcohol to form sucrose ester of structure **2** and **3** (scheme 1).



Scheme 1. Structures of different esters with sucrose acting as an alcohol (1) and sucrose derivative acting as carboxylic acid (2, 3).

The *Bacillus subtilis* sacB gene encodes the secreted enzyme levansucrase (sucrose: 2,6-β-D-fructan 6-β-D-fructosyltransferase; EC 2.4.1.10) and belongs to the family of glycoside hydrolases 68 (GH 68). [8] It catalyzes the hydrolysis of sucrose although it also transfers fructosyl units to sucrose resulting in β(2→6)-linked fructans.[9] In previous work α(1→2)-linked sucrose analogues were synthesized enzymatically with levansucrase from *Bacillus megaterium* (SacB) in the presence of glycopyranose acceptors like D-galactose or D-xylose up to 60 % yield. [10]

We begin from the belief, supported by many experimental studies, that glucuronic acid will be tolerated and fructosylated by the levansucrase from *B. megaterium*.[10a, 11] The catalytic triad of the *B. megaterium* levansucrase (Bm-Ls) consists the nucleophile D95, the transition state stabilizer D257 and the acid/base catalyst E352 (Fig. 1). The substitution of the glucopyranoside is initiated by protonation of the glycosidic bond of sucrose with E352, followed by a nucleophilic attack of D95 to form a covalent fructosyl-enzyme intermediate (Fig. 1c) by inverting the stereogenic center of C-2 (α-configuration). The mechanism undergoes an oxocarbenium ion-like transition state (TS1, Fig. 1). Then the acceptor substrate attacks in a S_Ni/S_N2 mechanism to finally yield the fructosylated acceptor substrate. [9b, 12]

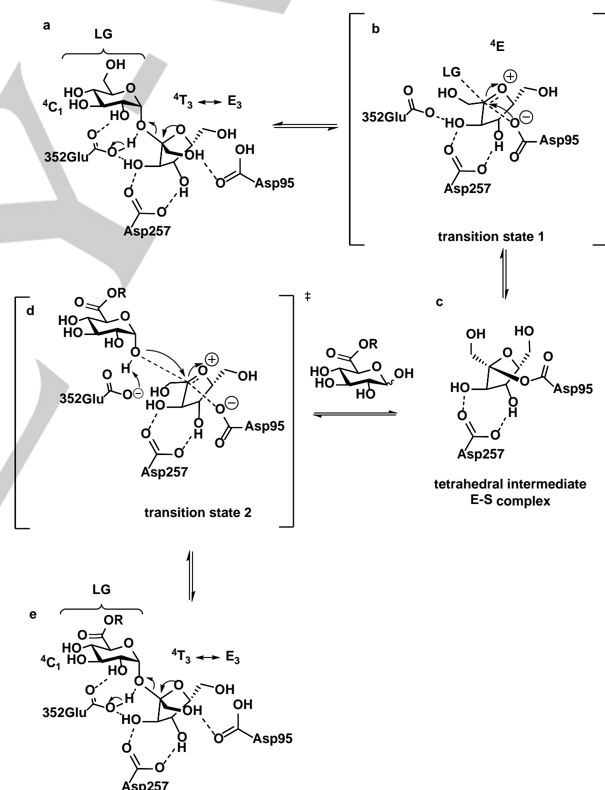


Figure 1. Proposed mechanism of the levenasucrase Bm-LS for the acceptor reaction.

Unanticipated we observed the desactivation of the enzyme by using D-glucuronic acid (500 mM) as acceptor substrate. The observation can be rationalized if a pH-shift in the catalytic pocket takes place and the nucleophile Asp95 is protonated. To avoid the pH-shift the sodium salt of the glucuronic acid (500 mM) was used in the enzymatic reaction as acceptor and indeed the desired sucrose analogue β-D-fructofuranosyl-(2,1)-α-D-glucuronic acid **2a** has been formed up to 43% (78 g/l, Fig. 2). Because galactose

[a] C. Possiel, A. Bäuerle, Prof. Dr. J. Seibel*
Institut für Organische Chemie
Julius-Maximilians Universität Würzburg
Am Hubland
E-mail: seibel@chemie.uni-wuerzburg.de

Supporting information for this article is given via a link at the end of the document.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

WILEY-VCH

has proven to be a good acceptor, [10] it was anticipated that D-galacturonic acid **6a** would lead to the similar fructosylation. β-D-fructofuranosyl-(2,1)-α-D-galacturonic acid **3a** was formed in 40% (70g/l, Fig. 2) yield.

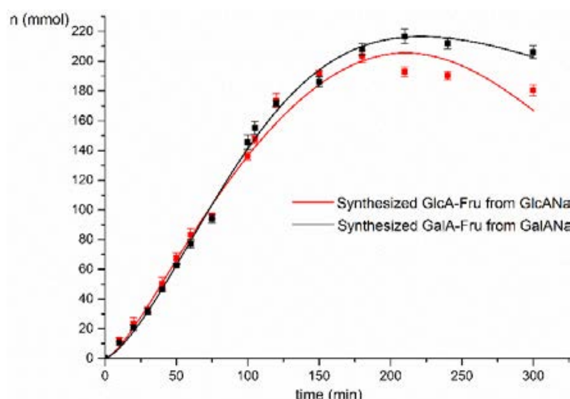


Figure 2. Determined yields of fructosylated uronic acid sodium salts (D-GlcAFruNa **2a** in red, D-GalAFruNa **3a** in black) via HPAEC. 0.7 M uronic acid sodium salt, 0.35 M sucrose, 50 mM phosphate buffer pH 6.6 and 5 % DMSO, 2 U/mL Bm-Ls.

We further evaluated if uronic esters can be tolerated by the enzyme. For this purpose, the PDB structure of β-D-fructofuranosyl-(2,1)-α-D-glucuronic acid benzyl ester (D-GlcABn) **2b** was generated *in silico* and applied to the crystal structure of *B. subtilis* levansucrase (PDB: 1pt2)[12b] (amino acid numbering refers to the *B. megaterium*'s enzyme, Fig. 3). In this model the ester group of the D-glucuronic acid residue in 6-position can rotate out of the catalytic pocket to find enough space. We can conclude that D-GlcABn **2b** may act as an acceptor and be fructosylated. Furthermore the aromatic ring can coordinate between two tryptophan residues (Trp94 and Trp172) to create additional π-π-interactions which could lead to a better coordination within the catalytic pocket (Fig. 3).

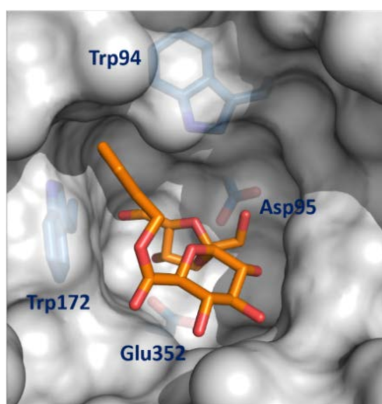
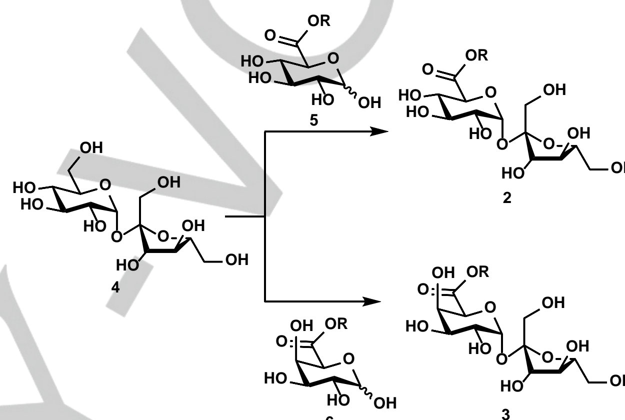


Figure 3. Crystal structure of *B. subtilis* levansucrase (PDB: 1pt2) with fructosylated GlcABn **2b** within the catalytic pocket orientated similar to crystallized sucrose.

The D-glucuronic benzyl ester **5b** and the D-galacturonic benzyl ester (D-GalABn) **6b** were prepared according to Stachulski *et al.* (SI).[13] The esters **5b** and **6b** were fructosylated by the levansucrase of *B. megaterium* (SacB) leading to the sucrose esters β-D-fructofuranosyl-(2,1)-α-D-glucuronic acid benzyl ester **2b** (45%) and β-D-fructofuranosyl-(2,1)-α-D-galacturonic acid benzyl ester **3b** (41%) (Fig. 4). The reactions were carried out at 37 °C in a 50 mM phosphate buffer with an enzyme activity of 2 U/mL. Both esters can be hydrolyzed with 1 M NaOH resulting in sucrose acids (SI).

A more sterically demanding molecule D-glucuronic acid *iso*-propyl ester (D-GlcA*i*Pr) **5c** was successfully recognized as acceptor by the levansucrase. Its fructosylation was performed in the same way as for D-GlcABn **5b** leading to the formation of β-D-fructofuranosyl-(2,1)-α-D-glucuronic acid isopropyl ester **2c** in 52% yield, respectively.



	acceptor	product	yield
R=H	**5a**	**2a**	43%
R=Bn	**5b**	**2b**	45%
R=*i*Pr	**5c**	**2c**	52%
R=H	**6a**	**3a**	40%
R=Bn	**6b**	**3b**	41%

Figure 4. 1 M of **5** or **6**, 0.5 M sucrose, 50 mM phosphate buffer pH 6.6, levansucrase SacB 2 U/mL, 2 h.

The presented approach departs from existing methods of sucrose ester synthesis. While other methods yield mixtures of sucrose esters with different acylation pattern this method allows the selective mono esterification of position 6 in sucrose. In this approach the carboxylic acid is embedded in sucrose as glucose residue was substituted against D-glucuronic acid or D-galacturonic acid/esters, resulting in β-D-fructofuranosyl-(2,1)-α-D-glucuronic acid and β-D-fructofuranosyl-(2,1)-α-D-galacturonic acid acids/esters, a new class of sucrose analogue esters.

Acknowledgements

Financial support from the 7th Framework Programme for Research and Technological Development (project "SuSy": Sucrose synthase as effective mediator of glycosylation) is acknowledged.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

WILEY-VCH

COMMUNICATION

Keywords: sucrose esters • sucrose analogs • levansucrase • biocatalysis • substrate engineering

[1] *United States Department of Agriculture* https://apps.fas.usda.gov/psdonline/circulars/sugar.pdf **2017**.
[2] X. Pan, P. Sengupta, D. C. Webster, *Green Chem* **2011**, *13*, 965-975.
[3] L. Osipow, F. D. Snell, W. C. York, A. Finchler, *Industr Eng Chem* **1956**, *48*, 1459-1462.
[4] L. I. Osipow, W. Rosenblatt, *J Am Oil Chem Soc* **1967**, *44*, 307-309.
[5] M. Ferrer, M. A. Cruces, M. Bernabe, A. Ballesteros, F. J. Plou, *Biotechnol Bioeng* **1999**, *65*, 10-16.
[6] R. Ye, D. G. Hayes, R. Burton, A. J. Liu, F. M. Harte, Y. M. Wang, *Catalysts* **2016**, *6*.
[7] R. Ye, D. G. Hayes, *J Am Oil Chem Soc* **2012**, *89*, 455-463.
[8] V. Lombard, H. G. Ramulu, E. Drula, P. M. Coutinho, B. Henrissat, *Nucleic Acids Res* **2014**, *42*, D490-D495.
[9] a) M. Elena Ortiz-Soto, C. Possiel, J. Gorl, A. Vogel, R. Schmiedel, J. Seibel, *Glycobiology* **2017**; b) A. Homann, R. Biedendieck, S. Gotze, D. Jahn, J. Seibel, *Biochem J* **2007**, *407*, 189-198.
[10] a) C. P. Strube, A. Homann, M. Gamer, D. Jahn, J. Seibel, D. W. Heinz, *J Biol Chem* **2011**, *286*, 17593-17600; b) M. E. Ortiz-Soto, M. Rivera, E. Rudino-Pinera, C. Olvera, A. Lopez-Munguia, *PEDS* **2008**, *21*, 589-595.
[11] J. Seibel, R. Moraru, S. Gotze, K. Buchholz, S. Na'amnieh, A. Pawlowski, H. J. Hecht, *Carbohyd Res* **2006**, *341*, 2335-2349.
[12] a) G. Meng, K. Futterer, *Nat Struct Biol* **2003**, *10*, 935-941; b) G. Meng, K. Futterer, *Bmc Struct Biol* **2008**, *8*.
[13] E. R. Bowkett, J. R. Harding, J. L. Maggs, B. K. Park, J. A. Perrie, A. V. Stachulski, *Tetrahedron* **2007**, *63*, 7596-7605.

WILEY-VCH

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201701313

WILEY-VCH
Accepted Manuscript
This article is protected by copyright. All rights reserved.