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Proteinase N-catalysed regioselective esterification of sucrose and other mono- and disaccharides

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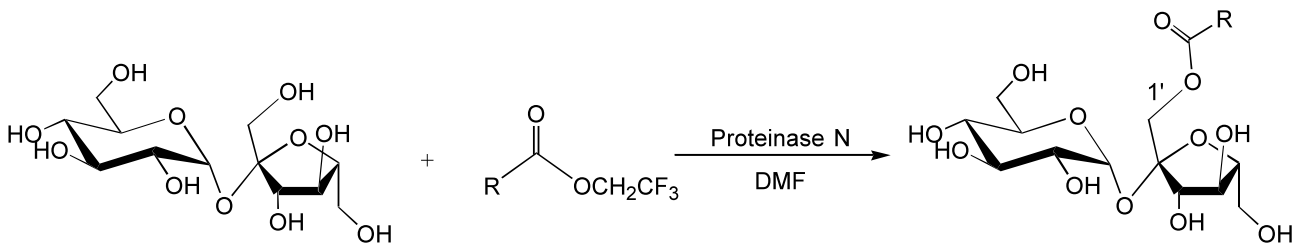
Abstract—Crude Proteinase N was used as catalyst for the synthesis of carbohydrate (in particular sucrose) esters by transesterification of activated esters in organic solvents. Polymerisable or amphiphilic sucrose esters (methacrylates and laurates) were obtained regioselectively in good yields. The influence of the reaction parameters (temperature, pH, solvent, chain length, solid support) on the reaction rate, yield and selectivity was studied.

1. Introduction

Enzymes, and notably hydrolases, are precious catalysts for the preparation of partially substituted carbohydrates since protection–deprotection sequences can thus be avoided.[1–7] The efficiency of the esterification of carbohydrates catalysed by hydrolases (proteases and lipases) depends on both the acylating agent and the sugar.[8–11] We have recently described our preliminary results on the selective preparation of sucrose monomethacrylate using Proteinase N (Fluka),[12] a crude protease isolated from *Bacillus subtilis*, which has been already used in peptide chemistry,[13] but never for carbohydrate acylation. We report herein a full study of the scope of this reaction with respect to other carbohydrates and acylating agents, as well to reaction parameters such as pH, temperature, solvent and hydration.

2. Results and discussion

Being involved in programs directed towards the preparation of carbohydrate-based polymerisable compounds, we first focussed on the synthesis of sucrose methacryloyl esters,[14–16] in comparison with other previously described enzyme-catalysed preparations using



sucrose

a : R = *n*-C_3H_7

b : R = *i*-C_3H_7

c : R = *E*-CH_3-CH=CH-

d : R = H_2C=$C(CH_3)$-

1 a-d : 1'-sucrose monoester

Scheme 1.

Abbreviations: Trehal, trehalose; MGP, methyl-α-D-glucopyranoside; MFF, methyl-α-D-fructofuranoside; TFEM, trifluoroethyl methacrylate; VL, vinyl laurate; PN, Proteinase N; PN/C, Proteinase N on Celite; SMM, sucrose monomethacrylate; TFE, trifluoroethanol; SML, sucrose monolaurate.

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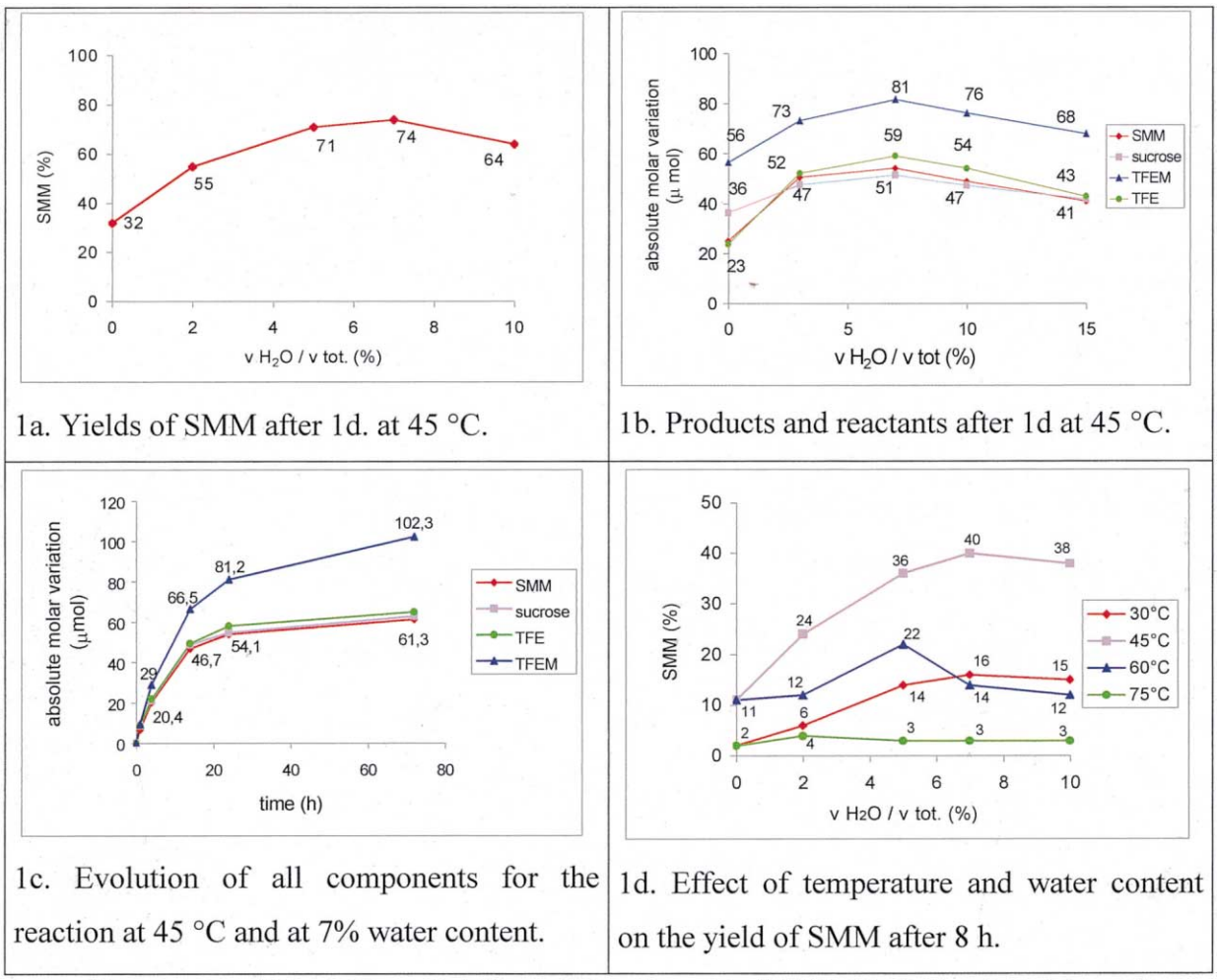
either Subtilisin Carlsberg (EC 3.4.21.62, Sigma and EC 3.4.21.14, Fluka) or Proteinase N (Amano).[17–19] Proteinase N (Fluka) was first dissolved in water and the pH of the solution was adjusted at pH 7.8 with 0.1 M KOH. If a buffer (phosphate) is used, it has to be very diluted to prevent side alkaline catalysis which would promote competitive non-regioselective esterifications. A series of experiments using C_4-acid trifluoroethyl esters as acylating agents was performed to demonstrate that in DMF, the Proteinase N-catalysed reactions lead to monoacylated derivatives of sucrose **1a–d** in a very regioselective manner, with essentially only C(1′)OH-substituted esters being formed (Scheme 1). The structure of compounds **1a–d** was established by NMR spectroscopic analyses. Notably, the position of esterification was certified (as for all compounds described in this work) by 2-D NMR spectroscopy (one-bond ^1H–^{13}C correlation (HSQC) and ^1H–^{13}C multiple-bond correlation (HMBC) showing correlation between the CO peak of the newly created ester linkage and the protons of the carbon bearing the oxygen atom of the ester function. This selectivity at the C(1′) hydroxyl group, with small amounts of C(6)- and C(6′)-monoester side products, is characteristic of protease-catalysed esterifications of sucrose. Trifluoroethyl butyrate led to the fastest reaction compared to isobutyrate. The presence of a conjugated double bond decreases the reactivity, as can be seen by the slower reaction rates for methacrylate and *trans*-crotonate esters. The influence of the leaving group was studied in the case of methacrylates by comparing trifluoroethyl and vinyl esters. When vinyl methacrylate was used, a faster initial reaction rate was observed, but lower final yields were obtained probably because of enzyme damage due to the production of acetaldehyde in the medium.

The trifluoroethyl ester was thus used for the remainder of the study, concerning notably the presence of water in the medium. Indeed, this parameter was shown to be crucial in many cases, with a partition of water between the solvent, the substrate, and the enzyme, for which



1a. Yields of SMM after 1d. at 45 °C.

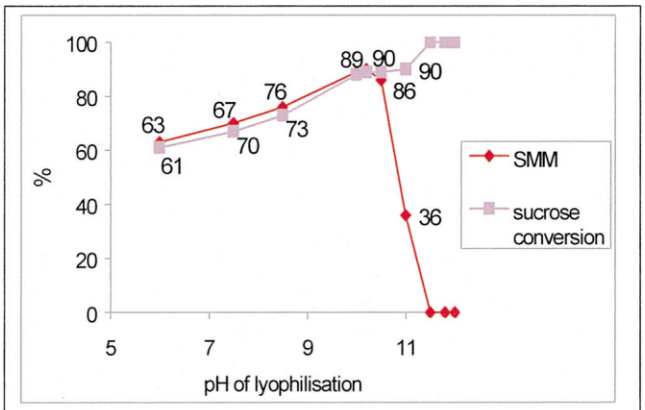
1b. Products and reactants after 1d at 45 °C.

1c. Evolution of all components for the reaction at 45 °C and at 7% water content.

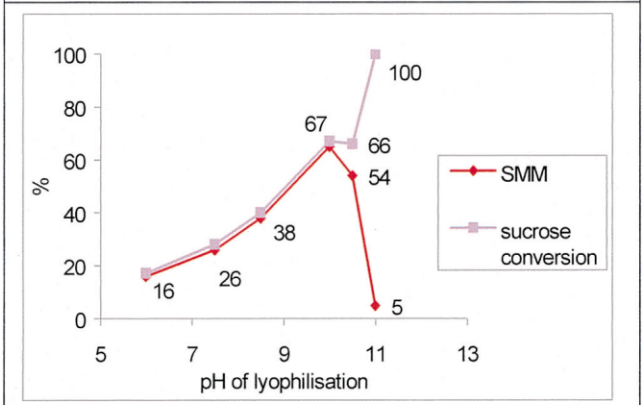
1d. Effect of temperature and water content on the yield of SMM after 8 h.

a. conditions: sucrose (25 mg, 73 µmol), trifluoroethyl methacrylate (TFEM, 52 µL, 5 eq.), Proteinase N (PN, pH 7.8, 15 mg), DMF-water (0.18 mL), stirring 250 rpm. SMM = sucrose monomethacrylate; TFE = trifluoroethanol.

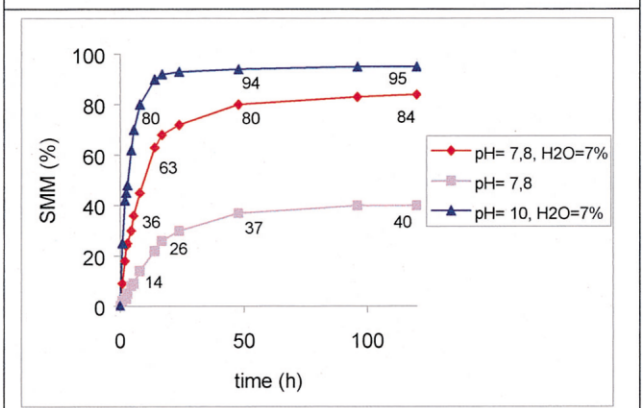
Figure 1. Influence of the amount of water on the formation of sucrose monomethacrylate by Proteinase N-catalysed transesterification reaction in DMF.[a]



2a. 7% aq. DMF, 1d.



2b. Anhydrous DMF, 1d.

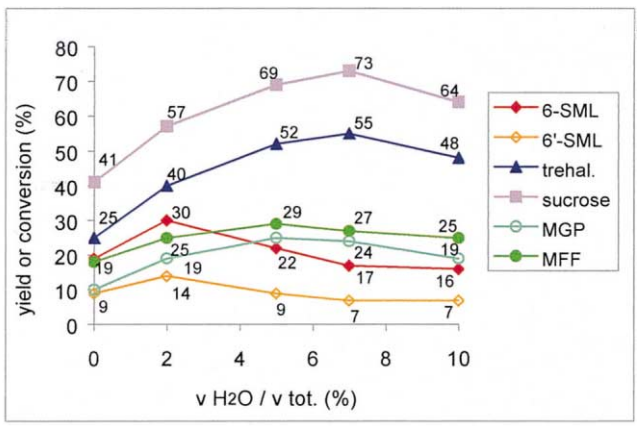


2c. Evolution of SMM yield.

a. conditions: sucrose (25 mg, 73 μmol), trifluoroethyl methacrylate (TFEM, 52 μL, 5 eq.), Proteinase N (PN,15 mg), DMF-water (0.18 mL), stirring 250 rpm, 45 °C. SMM: sucrose monomethacrylate.

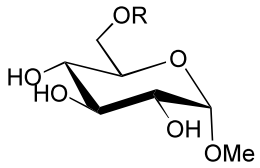
Figure 2. Influence of the lyophilisation pH of Proteinase N on the yields of sucrose monomethacrylate.[a]

the hydration is deeply connected with the overall conformation and therefore, with the catalytic activity.[20–23] The reaction of sucrose with trifluoroethyl methacrylate (5 equiv.) was achieved in the presence of various amounts of water (0–10% vol. in DMF). The addition of water led to faster reactions, providing sucrose monomethacrylate **1d** (SMM) in yields up to 74% after 1 day (86% after 3 days) without formation of disubstituted esters (Fig. 1a). In this case, 7% was the optimal amount of water. Above this amount, the reaction was slower, and the final yields were also lower. By following the outcome of the reactions with various amounts of water, it was seen that trifluoroethanol and sucrose were recovered in smaller amounts from reactions completed in the presence of more water, showing that the lower rates and yields were connected with decreased enzymatic efficiency and not with competitive hydrolysis (Fig. 1b). Moreover, no methacrylic acid was observed. Superimposition of the variations (in absolute value) of trifluoroethanol, sucrose and sucrose monomethacrylate indicates that no polysubstitution occurred, as shown by the profiles obtained at different water contents, and for those obtained at the optimal amount (7%) as a function of time. Only the variation of trifluoroethyl methacrylate is consistently higher, probably due to competitive polymerisation reaction (Fig. 1c). All these reactions were achieved at 45°C, which is usually the best balance between reaction rates and the enzyme thermal stability. As for other proteases, Proteinase N is also more active at 45°C, as seen by the yields of SMM obtained at 30°C, 45°C, 60°C and 75°C for different water contents (Fig. 1). The optimal water content was shown to depend also on the reaction temperature (7% for 30°C and 45°C, 5% at 60°C) indicating that the optimisation of hydration of the enzyme has to be reconsidered for each new set of conditions (Fig. 1d). At 30°C and 60°C, the reaction was still regioselective but slower compared to 45°C, whereas at 75°C, a mixture of regioisomers was obtained as well as polysubstituted products.

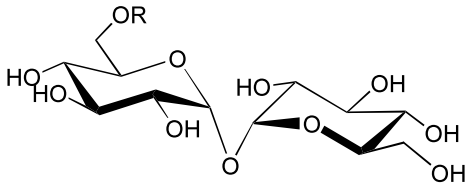


a. Conditions: sugar substrate (25 mg), trifluoroethyl methacrylate (TFEM, 5 eq.), Proteinase N (PN, pH 7.8, 0.2 mg/μmol sugar substrate), DMF-water (2.5 μL/μmol sugar substrate), stirring 250 rpm, 45 °C, 1 d. 6-SML = sucrose 6-*O*-monolaurate **4**; 6'-SML = sucrose 6'-*O*-monolaurate **5**; trehal. = trehalose **3**; MGP = methyl α-D-glucopyranoside **2**; MFF = methyl α-D-fructofuranoside **6**.

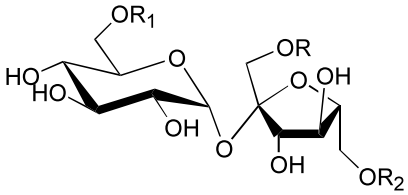
Figure 3. Comparison of the reactivity of various sugar substrates on the Proteinase N-catalysed transesterification of trifluoroethyl methacrylate.[a]



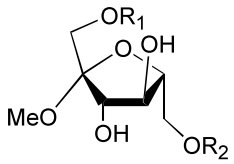
2: R = H
2a: R = COC(Me)=CH_2



3: R = H
3a: R = COC(Me)=CH_2



4: R_1 = $CO_2C_{11}H_{23}$, R_2 = R = H
4a: R_1 = $CO_2C_{11}H_{23}$, R_2 = H, R = COC(Me)=CH_2
5: R_2 = $CO_2C_{11}H_{23}$, R_1 = R = H
5a: R_2 = $CO_2C_{11}H_{23}$, R_1 = H, R = COC(Me)=CH_2



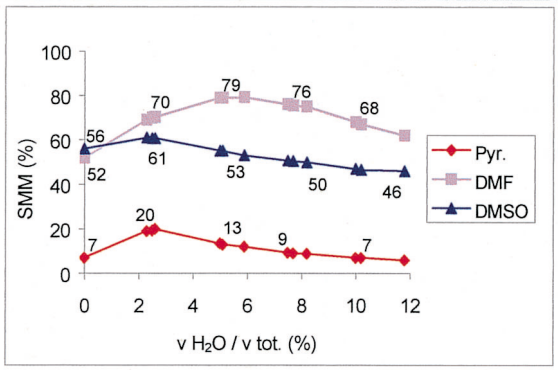
6: R_1 = R_2 = H
6a: R_1 = H, R_2 = COC(Me)=CH_2
6b: R_2 = H, R_1 = COC(Me)=CH_2

Scheme 2.

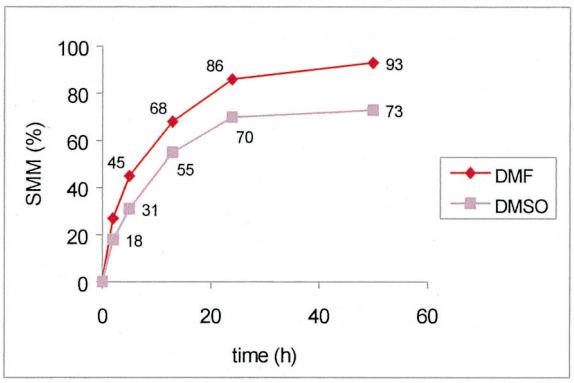
The study of the influence of the pH of the enzymatic preparation before freeze-drying indicated that the activity of the enzyme could be increased at higher pHs of up to 10 (Fig. 2). However, above this value, a dramatic loss of activity was observed. This optimisation associated with the optimal hydration of the enzyme led to very fast reactions and excellent yields of sucrose monomethacrylate. In all cases, the activity of the enzyme decreased dramatically after about 2 days, a sign of protein denaturation. In order to stay out of critical zones of enzyme stability (pH, temperature), and to get results easily comparable with literature data, the further studies were achieved at pH 7.8 and at 45°C.

In order to evaluate the influence of the geometry of the carbohydrate on the regioselectivity of the reaction, other sugars in the glucose and fructose series were then subjected to the Proteinase N-mediated esterification with trifluoromethyl methacrylate (Scheme 2).[24] All of these carbohydrates led to the corresponding methacrylic esters, but in different rates and regioselectivities. The C(6)OH of glucose was acylated in the case of methyl glucoside **2** and trehalose **3**, providing esters **2a** and **3a**, whereas no reaction occurred at this position in the case of sucrose and sucrose 6-*O*-monolaurate (**4**, 6-MLS) or 6′-*O*-monolaurate **5** (6′-MLS, purified from a regioisomeric mixture after chemical transesterification), for which only the C(1′) hydroxyl group was esterified leading to esters **4a** and **5a** (compound **5a** could also be obtained by lipase-catalysed transesterification of **1d**).[25] In the case of methyl fructofuranoside **6**, the reaction was not as selective, giving a mixture of both 1- and 6-substituted fructoside esters **6a** and **6b** in a 1/1.75 ratio. These results show that sucrose leads to highly selective reactions because of the interactions between both monosaccharidic moieties. Concerning the reaction rate, it was observed that non-substituted disaccharides were more reactive compared to monosaccharides. Fructosides are more reactive compared to glucosides, as shown by the faster reaction of methyl glucoside compared to methyl fructoside, of sucrose compared to trehalose, and of unsubstituted sucrose or 6-*O*-substituted sucrose compared to a sucrose having a substitution at the 6′-hydroxyl group. Because all of these substrates have different hydration equilibria (Fig. 3), the water content had to be optimised for each of them (7% for non-substituted disaccharides, 5% for monosaccharides, and 2% for acylated disaccharides).

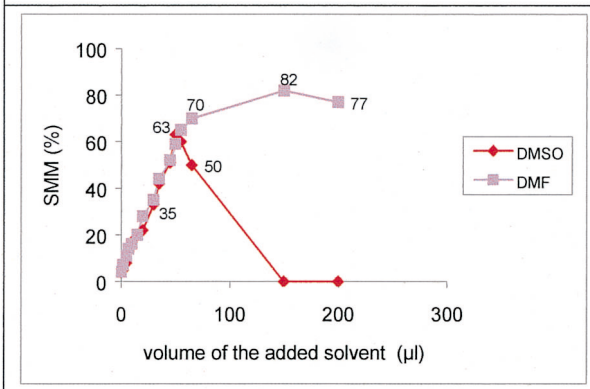
The next issue was to examine whether DMSO could be a possible solvent for this reaction, as it is indeed much less undesirable compared to DMF although belonging to the same family of aprotic dipolar solvents. However, DMSO is known to denature enzymes,[26–28] which explains why no reaction was observed when the reaction was done at concentrations similar to those used with DMF. However, the reaction could be achieved using DMSO as the solvent in the minimum amount able to provide a slurry (Fig. 4a). Although in all cases DMF was found to provide better yields, nearly as good results were obtained in DMSO slurries, with less dramatic effect of the amount of water on the reaction rates. At the optimal water content, the reaction proceeded in a similar way as for DMF suspension, with a decrease in the reaction rate after 1 day (Fig. 4b). This method allowed isolation of more than 70% yield of sucrose monomethacrylate in a selective manner and without formation of more substituted derivatives. An



4a. Effect of water addition in the organic solvent slurry on the yield after 17 h.



4b. Evolution of the yield for the reaction in slurries (DMF+H_2O 4.7% or DMSO+H_2O 2.2%).



4c. Reverse procedure: effect of the addition of the organic solvent to the sucrose aqueous syrup on the SMM yield after 17 h.

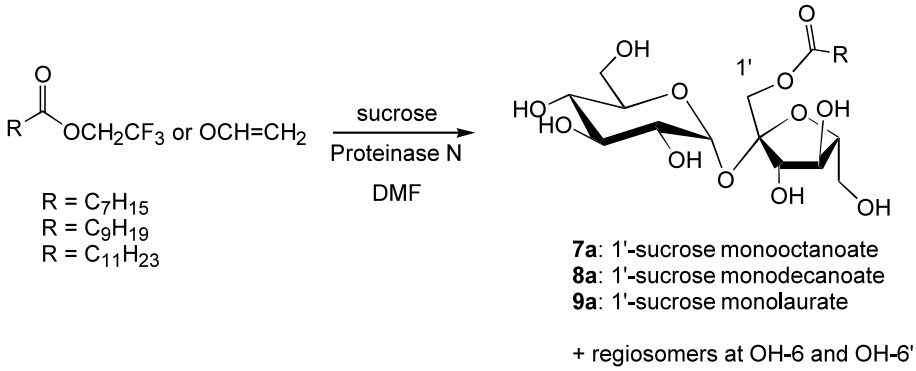
a. Conditions: sucrose (25 mg, 73 µmol), trifluoroethyl methacrylate (TFEM, 52 µL, 5 eq.), Proteinase N, (PN, pH 7.8,25 mg), solvent (DMF 150 µL, DMSO 35 µL, pyridine 730 µL), stirring 250 rpm, 45°C. SMM: sucrose monomethacrylate.

Figure 4. Effect of solvent variations on the yield of sucrose monomethacrylate from Proteinase N-catalysed transesterification.[a]

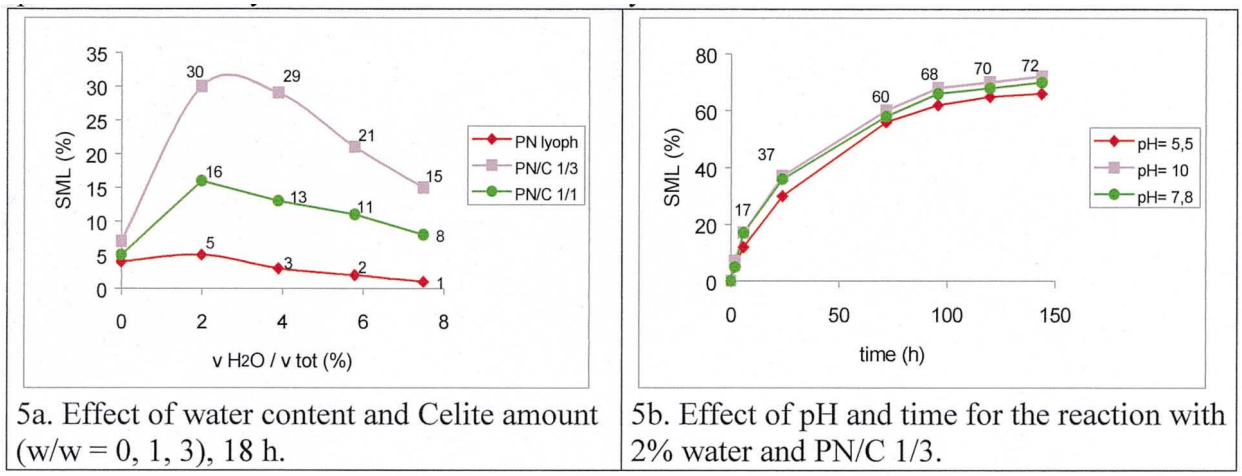
alternative was to start from a water–sucrose mixture prepared with the minimum amount of water, then adding the other component of the system, including the organic solvent (Fig. 4c). The reaction rate increased when increasing the volume of added organic solvent until a maximum. In the case of DMF, this maximum was measured for a volume corresponding to a 5% (v/v) water content, not far from the optimal water content (7%) found for the regular procedure starting from the organic solvent–sucrose solution in which water is added. However, in the case of DMSO, the maximum rate was measured at a volume corresponding to a 12% water content, much larger compared to the 2% measured when starting from the DMSO slurry. Above this amount, a dramatic change was observed indicating probably some destructive interactions between the solvent and the enzyme. It has to be noted that some enzyme-catalysed acylation could take place in DMSO–water slurries containing rather important contents of water.

Though proteases are known to be more specific for short chain acyl substrates, we finally evaluated the ability of Proteinase N to catalyse the synthesis of longer sucrose esters, which are already used as emulsifiers in the food and cosmetic fields thanks to their interesting combination of surfactant and biocompatibility properties. In a first series of experiments, applying the conditions used for methacrylates, sucrose mono-octanoate **7a**, -decanoate **8a**, and laurate **9a** were obtained in 17–40% unoptimised yields (Scheme 3). The regioisomeric distribution was 75% C(1′)OH esters, 10–13% C(6′)OH esters and 7–11% C(6)OH esters, showing lower discrimination at C(1′)OH compared to shorter esters.[29] The reaction was further studied using vinyl laurate as the acylating agent, with respect to the presence of a solid support (Celite), pH and solvent. When supported on Celite, the enzyme was better protected against denaturation, notably by action of ethanal which is produced in a stoichiometric amount by transesterification of the vinyl esters (Fig. 5). The optimal water content in DMF was 2% (v/v), much smaller than the 7% optimum for methacrylates. For the supported enzyme, there was nearly no effect of the pH (5.5, 7.8 or 10.0) of the enzyme solution containing Celite before its precipitation by addition of cold acetone, but in all cases, the enzyme remained active over a period of 5–6 days, compared to 1–2 days for the non-supported enzyme. A 70% yield of sucrose monolaurate was obtained after 5 days under these optimised conditions and this could be applied to octyl and decanoyl esters on gram scale quantities, always giving a 75% yield of esters at the C(1′) hydroxyl group.

Using the DMSO slurry method, sucrose monolaurate could also be obtained but in rather low yields (Fig. 6). It has to be noted that the reaction proceeded faster in DMSO than in DMF for the non-supported enzyme, this latter being more active in DMF than in DMSO. Further studies on the use of enzymes for the clean and direct transformation of unprotected carbohydrates are in progress in our laboratory.



Scheme 3.

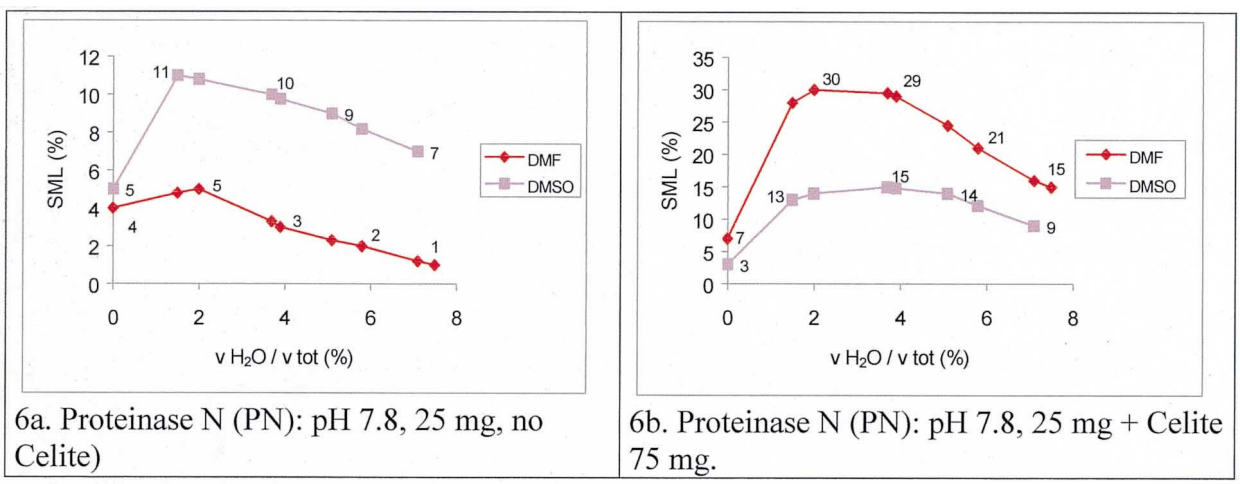


5a. Effect of water content and Celite amount (w/w = 0, 1, 3), 18 h.

5b. Effect of pH and time for the reaction with 2% water and PN/C 1/3.

a. conditions: sucrose (25 mg, 73 μmol), vinyl laurate (95 μL, 5 eq.), Proteinase N (PN, pH 7.8, 25 mg, with 0 mg, 25 mg or 75 mg of Celite), stirring 250 rpm, DMF 150 μL, 45 °C. SML = sucrose monolaurate.

Figure 5. Effect of enzyme preparation on the yield of sucrose monolaurate obtained by Proteinase N-catalysed transesterification of vinyl laurate.[a]



6a. Proteinase N (PN): pH 7.8, 25 mg, no Celite)

6b. Proteinase N (PN): pH 7.8, 25 mg + Celite 75 mg.

a. Conditions: sucrose (25 mg, 73 μmol), vinyl laurate (95 μL, 5 eq.), solvent (DMF 150 μL, DMSO 35 μL), stirring 250 rpm, 45 °C, 18 h. SML = sucrose monolaurate.

Figure 6. Effect of solvent variations and water content on the yield of sucrose monolaurate from Proteinase N-catalysed transesterification.[a]

3. Conclusion

In this study, we have shown that Proteinase N, a crude protease isolated from Bacillus subtilis (Fluka), is a versatile catalyst for carbohydrate acylation. Methyl glucoside and fructoside, trehalose and sucrose methacrylates were obtained in good yields. The importance of the presence of residual water necessary to keep the enzyme active was confirmed. A further study in the case of sucrose revealed that the reaction could proceed in a DMSO slurry instead of a DMF suspension. With the supported enzyme (on Celite) longer sucrose esters could also be prepared in good yields, however with lower regioselectivity.

4. Experimental

4.1. General procedure

The enzyme (Proteinase N, 5.6 U/mg, *Bacillus subtilis*, Fluka) was prepared according to two procedures:

Procedure 1. The lyophilised enzyme (referred to as PN) was prepared by dissolving the crude enzyme in water (5 mg/mL), adjusting at the desired pH (most often 7.8) with 0.1 M KOH and then freeze-drying the solution.

Procedure 2. The supported enzyme (referred to as PN/C) was prepared by dissolving the crude enzyme in water (5 mg/mL), adjusting at the desired pH with 0.1 M KOH, cooling at 5°C, adding Celite (1/1 or 3/1 by weight related to the enzyme) under stirring, then precipitating by addition of cold acetone (−15°C) (2.5 times the volume of the aqueous solution), filtration and washing of the solid with cold acetone and drying under vacuum.

The reactions were achieved in closed tubes at the desired temperature, under magnetic stirring at 250 rpm. For kinetic studies, each point of a curve corresponds to one experiment stopped at a precise time. The sugar and the lyophilised or supported enzyme are carefully introduced in the tube. The DMF containing the desired amount of water and the acylating agent are added. For the reactions made in DMSO, pyridine, the sugar was first dissolved in a minimum of solvent. Water and the acylating agent, and then, the enzyme, were then carefully added. In this case, the resulting mixtures are slurries. After reaction, the suspension was directly purified by flash chromatography (FC) and the product was lyophilised. For the reactions made with supported PN, the suspension was filtrated and washed with DMF before evaporation and FC. Further separation of regioisomers was achieved for some products on semi-preparative HPLC: pump LC-8A (Shimadzu), refractometer YRD-883 (Uniflows), column Nucleosil NH_2, 250×20 mm (Touzart & Matignon), eluent CH_3CN/H_2O 90/10, 20 mL/min, injected volume 2 mL, about 100 mg of product for each injection.

Typical conditions for the study of the activity of Proteinase N: *Evaluation of the optimal water content, pH, carbohydrate substrate*: Sucrose (25 mg, 73 μmol), trifluoroethyl methacrylate (52 μL, 5 equiv.), PN (15 mg), DMF+H_2O (180 μL), 250 rpm, 45°C. *Evaluation of other solvents*: Sucrose (25 mg)/trifluoroethyl methacrylate (TFEM 52 μL, 5 equiv.) or vinyl laurate (95 μL, 5 equiv.)/PN (25 mg) (pH of lyophilisation = 7.8)/DMF+H_2O (150 μL) or DMSO+H_2O (35 μL) or pyridine+H_2O (730 μL)/250 rpm/45°C. *DMSO slurry prepared from an aqueous saturated sucrose solution*: Sucrose (25 mg)/TFEM (52 μL, 5 equiv.)/PN 25 mg (pH of lyophilisation = 7.8)/H_2O (15 μL)+added DMF or DMSO. *Laurates*: Sucrose (25 mg)/vinyl laurate (95 μL, 5 equiv.)/PN/C 1/3 (100 mg)/DMF (150 μL) or DMSO (35 μL)+added water/250 rpm/45°C. The water content is calculated on the total volume liquid components (solvents+acylating agents).

Analytical yields were determined by HPLC or GC. The reaction mixture was quantitatively diluted with the HPLC eluent and filtered before analysis by HPLC or GC. The HPLC system is composed of a pump LC-10AS (Shimadzu), an oven CT0-6A (Shimadzu), and a refractometer 410 (Waters). Analysis of sucrose, trehalose, methyl α-D-glucopyranoside, methyl α-D-fructofuranoside and sucrose monomethacrylates (SMM): column Lichrosorb L5OH-25F, 240×4.6 mm (Interchim), 45°C, CH_3CN/H_2O (80/20 v/v), 0.8 mL/min. Analysis of the regioisomers of sucrose monomethacrylates (SMM) and sucrose monolaurates (SML): column Spherisorb NH_2, 240×4.6 mm (Touzart & Matignon), 45°C, CH_3CN/H_2O (85/15 v/v for SMM or 90/10 v/v for SML), 0.8 mL/min. Analysis of sucrose monolaurates, sucrose monomethacrylate–monolaurate, vinyl laurate, lauric acid, sucrose dilaurates and sucrose trilaurates: column Nucleosil C8, 240×4.6 mm (Touzart & Matignon), 45°C, MeOH/H_2O (78/22), 0.8 mL/min. A gas chromatography system was used for the analysis of trifluoroethyl methacrylate and trifluoroethanol: GC-17A system (Shimadzu), injection system AOC-20i, hydrogen generator HGE-1A, column Quadrex 007 FFAP (30 m, I.D. 0.53 mm, 1.0 μm). NMR spectra were recorded at 300 or 500 MHz (^1H) and 125 or 75 (^{13}C) on Bruker DRX 300 or DRX 500 spectrometers at the University Claude Bernard Lyon 1. TLC were performed on silica gel 60 F_{254} plates (Merck) as well as flash chromatography. Optical rotations (Sodium D line) were measured at 20°C with a Perkin–Elmer 241 digital polarimeter for pure regioisomers purified from grade monoester mixtures by semi-preparative liquid chromatography. Elemental analyses and high-resolution FAB (+) mass spectra were performed by the Service central d'Analyse of the CNRS (Solaize).

4.2. Sucrose 1′-*O*-butyrate 1a

Sucrose (136 mg, 0.4 mmol), trifluoroethyl butyrate (120 μL, 2 equiv.), PN (7.2 U/mg, 57 mg), DMF (1 mL), 1 day. FC eluent: $CH_2Cl_2/Me_2CO/MeOH/H_2O$ 56/20/20/4 (R_f = 0.28). Yield: lyophilised white foam (131 mg, 80%, regioisomeric purity at OH-1′ >90%).

Data for pure **1a** after semi-preparative chromatography: ^{1}H NMR (CD_3OD, 300 MHz): δ 5.44 (d, 1H, $J_{1\text{-}2}=3.5$ Hz, H1); 4.43 (d, 1H, $J_{1'a\text{-}b}=12.1$ Hz, H1′a); 4.25 (d, 1H, $J_{1'a\text{-}b}=11.9$ Hz, H1′b); 4.15 (d, 1H, $J_{3'\text{-}4'}=8.7$ Hz, H3′); 4.09 (t, 1H, $J_{4'\text{-}5'}=8.4$, Hz, H4′); 3.9–3.6 (7H, H5, H6a, H6′, H5′, H6b, H3); 3.4 (dd, 1H, $J_{2\text{-}1}=3.5$ Hz, $J_{2\text{-}3}=9.8$ Hz, H2); 3.3 (t, 1H, $J_{3\text{-}4}=9.3$ Hz, H4); 2.4 (2H, CH_2), 1.7 (2H, CH_2), 1.0 (3H, CH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 173.6 (1C, CO); 104.5 (1C, C2′); 93.1 (1CH, C1); 82.7 (1CH, C5′); 77.7 (1CH, C3′); 73.9 (1CH, C4′); 73.5 (1CH, C5); 73.4 (1CH, C3); 72.0 (1CH, C2); 70.0 (1CH, C4); 62.7 ($1CH_2$, C1′); 62.2 ($1CH_2$, C6′); 61.2 ($1CH_2$, C6); 35.86, 18.45 ($2CH_2$), 13.0 ($1CH_3$); Anal. calcd for $C_{16}H_{28}O_{12}\cdot 1.1H_2O$: C, 44.5; H, 7.1. Found: C, 44.4; H, 7.3%; $[\alpha]_D^{20}=+62$ (*c* 1, MeOH); SM-HR (FAB+) *m*/*z* calcd (M+Na): 435.1478, found: 435.1481.

4.3. Sucrose 1′-*O*-isobutyrate 1b

Sucrose (136 mg, 0.4 mmol), trifluoroethyl isobutyrate (120 μL, 2 equiv.), PN (7.2 U/mg, 57 mg), DMF (1 mL), 1.5 day. FC eluent: $CH_2Cl_2/Me_2CO/MeOH/H_2O$ 56/20/20/4 ($R_f=0.28$). Yield: lyophilised white foam (81 mg, 50%), sucrose 1′-*O*-isobutyrate >90%. Data for pure **1b** after semi-preparative chromatography: ^{1}H NMR (CD_3OD, 300 MHz): δ 5.44 (d, 1H, $J_{1\text{-}2}=3.7$ Hz, H1); 4.35 (d, 1H, $J_{1'a\text{-}b}=12.1$ Hz, H1′a); 4.10 (d, 1H, $J_{1'a\text{-}b}=12.4$ Hz, H1′b); 4.04–3.98 (m, 2H, H3′, H4′); 3.8–3.6 (7H, H5, H6a, H6′, H5′, H6b, H3); 3.4–3.3 (2H, H2, H4); 2.6 (h, 1H, $J=6.97$ Hz, H isoprop), 1.17 (3H, CH_3), 1.15 (3H, CH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 177.8 (1C, CO); 103.9 (1C, C2′); 93.9 (1CH, C1); 83.7 (1CH, C5′); 78.5 (1CH, C3′); 74.8 (1CH, C4′); 74.4 (1CH, C5); 74.3 (1CH, C3); 72.9 (1CH, C2); 71.2 (1CH, C4); 63.5 ($1CH_2$, C1′); 63.1 ($1CH_2$, C6′); 62.0 ($1CH_2$, C6); 35.0 (1CH), 19.2 ($2CH_3$); Anal. calcd for $C_{16}H_{28}O_{12}\cdot 1.7H_2O$: C, 43.4; H, 7.2. Found: C, 43.3; H, 7.1; $[\alpha]_D^{20}=+61$ (*c* 1, MeOH); SM-HR (FAB+) *m*/*z* calcd (M+Na):435.1478, found 435.1476.

4.4. Sucrose 1′-*O*-crotonate 1c

Sucrose (136 mg, 0.4 mmol), trifluoroethyl *trans*-crotonate (120 μL, 2 equiv.), PN (7.2 U/mg, 57 mg), DMF–H_2O ($v_{H_2O}/v_{tot}=2\%$, 1 mL), 3 days. FC eluent: $CH_2Cl_2/Me_2CO/MeOH/H_2O$ 56/20/20/4 ($R_f=0.24$). Yield: lyophilised white foam (34 mg, 21%, regioisomeric purity at OH-1′ >90%).). Data for pure **1c** after semi-preparative chromatography: ^{1}H NMR (CD_3OD, 300 MHz): δ 7.1 (m, 1H, H vinyl); 5.9 (d, 1H, $J_{trans}=15.5$ Hz, H vinyl); 5.45 (d, 1H, $J_{1\text{-}2}=3.5$ Hz, H1); 4.4 (d, 1H, $J_{1'a\text{-}b}=12.1$ Hz, H1′a); 4.2 (d, 1H, $J_{1'a\text{-}b}=11.9$ Hz, H1′b); 4.15 (d, 1H, $J_{3'\text{-}4'}=8.7$ Hz, H3′); 4.09 (t, 1H, $J_{4'\text{-}5'}=8.4$, Hz, H4′); 3.8–3.5 (7H, H5, H6a, H6′, H5′, H6b, H3); 3.4 (dd, 1H, $J_{2\text{-}1}=3.5$ Hz, $J_{2\text{-}3}=9.8$ Hz, H2); 3.3 (t, 1H, $J_{3\text{-}4}=9.3$ Hz, H4); 1.9 (d, 3H, $J=7$ Hz, CH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 167.0 (1C, CO); 146.2 (1CH, -CH=); 122.1 (1CH, =CH-CH_3); 103.1 (1C, C2′); 93.1 (1CH, C1); 82.8 (1CH, C5′); 77.7 (1CH, C3′); 73.9 (1CH, C4′); 73.5 (1CH, C5); 73.4 (1CH, C3); 72.0 (1CH, C2); 70.0 (1CH, C4); 63.1 ($1CH_2$, C1′); 62.2 ($1CH_2$, C6′); 61.1 ($1CH_2$, C6); 17.1 ($1CH_3$); Anal. calcd for $C_{16}H_{26}O_{12}\cdot 1.8H_2O$: C, 43.4; H, 6.8. Found: C, 43.2; H, 6.5%; $[\alpha]_D^{20}=+59$ (*c* 1, MeOH); SM-HR (FAB+) *m*/*z* calcd for $C_{16}H_{26}O_{12}$ (M+Na): 433.1322, found: 433.1315

4.5. Sucrose 1′-*O*-methacrylate 1d

Sucrose (500 mg, 1.46 mmol), trifluoroethyl methacrylate (1.05 mL, 5 equiv.), PN (7.2 U/mg, 220 mg), DMF–H_2O ($v_{H_2O}/v_{tot}=7\%$, 3.7 mL), 3 days. FC eluent: $CH_2Cl_2/Me_2CO/MeOH/H_2O$ 56/20/20/4 ($R_f=0.24$). Yield: lyophilised white foam (515 mg, 86%, regioisomeric purity at OH-1′ >90%). Data for pure **1d** after semi-preparative chromatography: ^{1}H NMR (CD_3OD, 500 MHz): δ 6.19, 5.70 (2s, 2H, H vinyl); 5.45 (d, 1H, $J_{1\text{-}2}=3.5$ Hz, H1); 4.45 (d, 1H, $J_{1'a\text{-}b}=12.1$ Hz, H1′a); 4.25 (d, 1H, $J_{1'a\text{-}b}=11.9$ Hz, H1′b); 4.15 (d, 1H, $J_{3'\text{-}4'}=8.7$ Hz, H3′); 4.09 (t, 1H, $J_{4'\text{-}5'}=8.4$, Hz, H4′); 3.9–3.6 (7H, H5, H6a, H6′, H5′, H6b, H3); 3.4 (dd, 1H, $J_{2\text{-}1}=3.5$ Hz, $J_{2\text{-}3}=9.8$ Hz, H2); 3.3 (t, 1H, $J_{3\text{-}4}=9.3$ Hz, H4); 2.0 (3H, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): δ 167.0 (1C, CO); 136.4 (1CH, -CH=); 125.8 ($1CH_2$, $=CH_2$); 103.1 (1C, C2′); 93.1 (1CH, C1); 82.8 (1CH, C5′); 77.7 (1CH, C3′); 73.9 (1CH, C4′); 73.5 (1CH, C5); 73.4 (1CH, C3); 72.0 (1CH, C2); 70.0 (1CH, C4); 63.1 ($1CH_2$, C1′); 62.2 ($1CH_2$, C6′); 61.1 ($1CH_2$, C6), 17.5 ($1CH_3$); Anal. calcd for $C_{16}H_{26}O_{12}\cdot 1.5H_2O$: C, 43.9; H, 6.7. Found: C, 43.9; H, 6.6%; $[\alpha]_D^{20}=+60$ (*c* 1, MeOH); SM-HR (FAB+) *m*/*z* calcd for (M+Na): 433.1322, found: 433.1322.

4.6. Methyl α-D-glucopyranoside 6-*O*-methacrylate 2a

Methyl α-D-glucopyranoside (190 mg, 0.98 mmol), trifluoroethyl methacrylate (0.7 mL, 5 equiv.), PN (5.6 u/mg, 190 mg), DMF–H_2O ($v_{H_2O}/v_{tot}=2\%$, 2.5 mL), 7 days. FC eluent: CH_2Cl_2 then $CH_2Cl_2/Me_2CO/MeOH/H_2O$ 78/10/10/2 ($R_f=0.33$). Yield: yellow oil (86 mg, 33%). ^{1}H NMR (CD_3OD, 500 MHz): δ 6.07, 5.58 (2s, 2H, H vinyl); 4.61 (d, 1H, $J_{1\text{-}2}=3.75$ Hz, H1); 4.47 (dd, 1H, $J_{6a\text{-}6b}=11.78$ Hz, $J_{6a\text{-}5}=2.15$ Hz, H6a); 4.27 (dd, 1H, $J_{6a\text{-}6b}=11.82$ Hz, $J_{5\text{-}6b}=6.25$ Hz, H6b); 3.70 (m, 1H, H5); 3.64 (t, 1H, $J_{2\text{-}3}=J_{3\text{-}4}=9.27$ Hz, H3); 3.37–3.32 (m, 4H, OCH_3+H2); 3.26 (t, 1H, $J_{4\text{-}5}=J_{3\text{-}4}=9.37$ Hz, H4); 1.89 (s, 3H, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): δ 167.7 (1C, CO); 136.7 (1C, C=); 125.8 ($1CH_2$, H_2C=); 100.02 (1CH, C1); 74.2 (1CH, C3); 72.5 (1CH, C2); 70.9 (1CH, C4); 70.1 (1CH, C5); 64.6 ($1CH_2$, C6); 54.5 ($1CH_3$, OCH_3); 7.4 ($1CH_3$); Anal. calcd for $C_{11}H_{18}O_7$: C, 50.4; H, 7.0. Found: C, 50.1; H, 7.1%; $[\alpha]_D^{20}=+124$ (*c* 1, MeOH).

4.7. Trehalose 6-*O*-methacrylate 3a

Trehalose (408 mg, 1.2 mmol), trifluoroethyl methacrylate (0.85 mL, 5 equiv.), PN (5.6 u/mg, 228 mg), DMF–H_2O ($v_{H_2O}/v_{tot}=2\%$, 3 mL), 3 days. FC eluent: CH_2Cl_2 then $CH_2Cl_2/Me_2CO/MeOH/H_2O$ 78/10/10/2 ($R_f=0.27$). Yield: lyophilised foam (122 mg, 25%). ^{1}H NMR (CD_3OD, 500 MHz): δ 6.12, 5.63 (2s, 2H, H vinyl); 5.13 (d, 1H, $J_{1\text{-}2}=3.76$ Hz, H1); 5.11 (d, 1H, $J_{1'\text{-}2'}=3.74$ Hz, H1′); 4.42 (dd, 1H, $J_{6a\text{-}6b}=11.91$ Hz,

$J_{6a\text{-}5}=2.11$ Hz, H6a); 4.28 (dd, 1H, $J_{6a\text{-}6b}=11.88$ Hz, $J_{5\text{-}6b}=5.43$ Hz, H6b); 4.08 (m, 1H, H5); 3.86–3.81 (m, 4H, H5′, H3′, H3, H6′a); 3.47 (dd, 1H, $J_{6a'\text{-}6b'}=11.90$ Hz, $J_{6b'\text{-}5'}=5.4$ Hz, H6′b); 3.52 (dd, 1H, $J_{1\text{-}2}=3.80$ Hz, $J_{2\text{-}3}=9.76$ Hz, H2); 3.50 (dd, 1H, $J_{1'\text{-}2'}=3.80$ Hz, $J_{2'\text{-}3'}=9.76$ Hz, H2′); 3.41 (t, 1H, $J_{4\text{-}3}=J_{5\text{-}4}=9.05$ Hz, H4); 3.35 (t, 1H, $J_{4'\text{-}3'}=J_{5'\text{-}4'}=9.08$ Hz, H4′); 1.94 (s, 3H, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): δ 167.8 (1C, CO); 136.7 (1C, C=); 125.5 ($1CH_2$, H2C=); 94.2 (1CH, C1′); 94.0 (1CH, C1); 73.6 (1CH, C3′); 73.5 (1CH, C3); 72.9 (1CH, C5′); 72.2 (2CH, C2, C2′); 71.0 (1CH, C4); 70.9 (1CH, C4′); 70.4 (1CH, C5); 63.9 ($1CH_2$, C6); 61.6 ($1CH_2$, C6′); 17.5 ($1CH_3$); Anal. calcd for $C_{16}H_{26}O_{12}\cdot 1H_2O$: C, 44.9; H, 6.6. Found: C, 44.9; H, 6.5%; $[\alpha]_D^{20}=+155$ (c 1, MeOH); SM-HR (FAB+) m/z calcd (M+Li): 417.1584, found 417.1590.

4.8. Sucrose 6-*O*-monolaurate 1′-*O*-monomethacrylate 4a

Sucrose 6-*O*-monolaurate (200 mg, 381 μmol), trifluoroethyl methacrylate (272 μL, 5 equiv.), PN (5.6 u/mg, 80 mg), DMF–H_2O ($v_{H_2O}/v_{tot}=2\%$, 0.96 mL), 3 days. FC eluent: CH_2Cl_2/MeOH 9/1 ($R_f=0.33$). Yield: yellow oil (102 mg, 45%). ^{1}H NMR (CD_3OD, 500 MHz): δ 6.17, 5.69 (2s, 2H, H vinyl); 5.42 (d, 1H, $J_{1\text{-}2}=3.77$ Hz, H1); 4.43 (dd, 1H, $J_{6a\text{-}6b}=11.91$ Hz, $J_{6a\text{-}5}=2.01$ Hz, H6a); 4.41 (d, 1H, $J_{1'a\text{-}b}=12.15$ Hz, H1′a); 4.22 (d, 1H, $J_{1'a\text{-}b}=12.05$ Hz, H1′b); 4.18 (dd, 1H, $J_{6a\text{-}6b}=11.96$ Hz, $J_{6b\text{-}5}=5.58$ Hz, H6b); 4.13 (d, 1H, $J_{4'\text{-}3'}=8.68$ Hz, H3′); 4.07 (m, 1H, H5); 4.04 (t, 1H, $J_{4'\text{-}3'}=J_{4'\text{-}5'}=8.38$ Hz, H4′); 3.85–3.72 (m, 3H, H6′a,b, H5′); 3.68 (t, 1H, $J_{4\text{-}3}=J_{3\text{-}2}=9.35$ Hz, H3); 3.44 (dd, 1H, $J_{2\text{-}1}=3.79$ Hz, $J_{2\text{-}3}=9.82$ Hz, H2); 3.32 (t, 1H, $J_{4\text{-}3}=J_{4\text{-}5}=9.59$ Hz, H4); 2.39 (t, 2H, $J=7.47$ Hz, CH_2CO); 1.98 (s, 3H, CH_3 methacrylic); 1.63 (m, 2H, CH_2CH_2CO); 1.32 (m, 16H, $8CH_2$); 0.92 (t, 1H, $J=6.9$ Hz, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): δ 174.5 (1C, CO on 6); 167.0 (1C, CO on 1′); 136.5 (1C, C=); 125.7 ($1CH_2$, $=CH_2$); 103.1 (1C, C2′); 93.0 (1CH, C1); 83.0 (1CH, C5′); 77.6 (1CH, C3′); 74.2 (1CH, C4′); 73.4 (1CH, C3); 71.9 (1CH, C2); 71.0 (1CH, C5), 70.7 (1CH, C4); 63.7 ($1CH_2$, C6); 63.1 ($1CH_2$, C1′); 62.8 ($1CH_2$, C6′); 33.9 ($1CH_2$, CH_2CO); 32.1, 29.8–29.3, 22.8 ($8CH_2$); 25.0 ($1CH_2$, CH_2CH_2CO); 17.5 ($1CH_3$, CH_3 methacrylic); 13.5 ($1CH_3$, CH_3); Anal. calcd for $C_{28}H_{48}O_{13}$: C, 56.7; H, 8.2. Found: C, 56.6; H, 8.3%; $[\alpha]_D^{20}=+48$ (c 1, MeOH).

4.9. Methyl α-D-fructofuranoside monomethacrylates 6a and 6b

Methyl-α-D-fructofuranoside (250 mg, 1.29 mmol), trifluoroethyl methacrylate (0.92 mL, 5 equiv.), PN (5.6 u/mg, 250 mg), DMF–H_2O ($v_{H_2O}/v_{tot}=5\%$, 3.25 mL), 3 days. FC eluent: CH_2Cl_2 then CH_2Cl_2/MeOH 9/1 ($R_f=0.34$). Yield: yellow oil (110 mg, 33%), 6/1 regioisomers (1.75/1); Anal. calcd for $C_{11}H_{18}O_7\cdot 0.2H_2O$: C, 49.7; H, 7.0. Found: C, 49.5; H, 7.1%; SM-HR (FAB+) m/z calcd (M+Li): 269.1212, found: 269.1197. Partial separation allowed to differentiate NMR signals of both compounds. Methyl α-D-fructofuranoside 6-*O*-monomethacrylate **6a**: ^{1}H NMR (CD_3OD, 125 MHz): δ 6.17, 5.66 (2s, 2H, H vinyl); 4.39 (1H, dd, $J_{6a\text{-}b}=11.86$ Hz, $J_{6a\text{-}5}=3.41$ Hz, H6a); 4.25 (1H, dd, $J_{6a\text{-}b}=11.79$ Hz, $J_{6b\text{-}5}=6.22$ Hz, H6b); 4.07 (1H, d, $J_{3\text{-}2}=4.17$ Hz, H3); 4.01 (1H, m, H5); 3.91 (m, 1H, H4); 3.72 (1H, d, $J_{1a\text{-}b}=12.03$ Hz, H1a); 3.64 (1H, d, $J_{1a\text{-}b}=12.0$ Hz, H1b); 3.33 ($1CH_3$, OCH_3); 2.0 (3H, s, CH_3 methacrylic); ^{13}C NMR (CD_3OD, 125 MHz): δ 167.6 (1C, CO methacrylic coupled with H6); 136.5 (1C, =**C**(CH_3)); 125.6 ($1CH_2$, $=CH_2$); 108.3 (1C, C2); 81.9 (1CH, C3); 80.6 (1CH, C5); 78.7 (1CH, C4); 64.5 ($1CH_2$, C6); 59.2 ($1CH_2$, C1); 48.1 ($1CH_3$, CH_3O); 17.4 ($1CH_3$). Methyl α-D-fructofuranoside 1-*O*-monomethacrylate **6b**: ^{1}H NMR (CD_3OD, 125 MHz): δ 6.17, 5.66 (2s, 2H, H vinyl); 4.44 (1H, d, $J_{1'a\text{-}b}=11.83$ Hz, H1′a); 4.21 (1H, d, $J_{1'a\text{-}b}=11.83$ Hz, H1′b); 4.01 (1H, m, H3′); 3.91 (m, 2H, H4′, H5′); 3.75, 3.64 (2H, m, H6′a, H6′b); 3.33 ($1CH_3$, OCH_3); 2.0 (3H, s, CH_3 methacrylic); ^{13}C NMR (CD_3OD, 125 MHz): δ 167.3 (1C, CO methacrylic coupled with H1′); 136.5 (1C, =**C**(CH_3)); 125.6 ($1CH_2$, $=CH_2$); 107.5 (1C, C2′); 84.8 (1CH, C5′); 81.3 (1CH, C3′); 78.4 (1CH, C4′); 62.2 ($1CH_2$, C6′); 60.3 ($1CH_2$, C1′); 48.1 ($1CH_3$, CH_3O); 17.4 ($1CH_3$).

4.10. Sucrose monooctanoate 7a

Sucrose (1.36 g, 3.98 mmol), trifluoroethyl octanoate (4.4 g, 5 equiv.), PN/C (1/3 m/m) (7.2 U/mg, 3 g), DMF–H_2O ($v_{H_2O}/v_{tot}=4\%$, 10 mL), 7 days. FC eluent: CH_2Cl_2/Me_2CO/MeOH/H_2O 56/20/20/4 ($R_f=0.42$). Yield: lyophilised white foam (325 mg, 17%); regioisomers proportions 3/3′, 4/2/4′/6/1′/6′ = 2/2/2/ε/7/75/12, separated on semi-preparative HPLC. Pure sucrose 1′-*O*-monooctanoate **7a**: ^{1}H NMR (D_2O, 300 MHz): δ 5.42 (d, 1H, $J_{1\text{-}2}=3.44$ Hz, H1); 4.35 (d, 1H, $J_{1'a\text{-}b}=12.14$ Hz, H1′a); 4.16–4.10 (3H, H1′b, H3′, H4′); 3.85–3.72 (m, 7H, H5, H6′a,b, H6a,b, H5′, H3); 3.56 (dd, 1H, $J_{1\text{-}2}=3.55$, $J_{2\text{-}3}=9.92$ Hz, H2); 3.47 (t, 1H, $J_{3\text{-}4}=J_{4\text{-}5}=9.34$ Hz, H4); 2.41 (t, 2H, $J=7.30$ Hz, CO-CH_2); 1.62 (m, 2H, CO-CH_2CH_2); 1.29 (m, 8H, 4 CH_2); 0.86 (t, 3H, $J=6.63$ Hz, CH_3); ^{13}C NMR (D_2O, 75 MHz): δ 175.17 (1C, CO); 102.85 (1C, C2′); 93.09 (1CH, C1); 82.14 (1CH, C5′); 77.11 (1CH, C3′); 73.44 (1CH, C4′); 72.99 (2CH, C5, C3); 71.39 (1CH, C2); 69.54 (1CH, C4); 62.71 ($1CH_2$, C1′); 62.04 ($1CH_2$, C6′); 60.57 ($1CH_2$, C6); 34.51 ($1CH_2$, **C**H_2-CO); 32.03, 29.33, 29.24, 22.88 ($4CH_2$); 25.04 ($1CH_2$, **C**H_2CH_2-CO); 14.18 ($1CH_3$); Anal. calcd for $C_{20}H_{36}O_{12}\cdot 1.55H_2O$: C, 48.4; H, 7.9. Found: C, 48.7; H, 7.9%; $[\alpha]_D^{20}=+54$ (c 1, MeOH); SMHR (FAB+) m/z calcd (M+Na) 491.2104, found 491.2096.

4.11. Sucrose monodecanoate 8a

Sucrose (1.1 g, 3 mmol), vinyl dodecanoate (3.6 mL, 5 equiv.), PN/C (1/3 m/m) (7.2 U/mg, 2.5 g), DMF–H_2O ($v_{H_2O}/v_{tot}=4\%$, 8 mL), 3 days. FC eluent: CH_2Cl_2/Me_2CO/MeOH/H_2O 56/20/20/4 ($R_f=0.44$). Yield: lyophilised white foam (638 mg, 40%); regioisomers proportion 6/1′/6′ = 11/76/10+3% on secondary hydroxyl groups, separated by semi-preparative HPLC.

Pure sucrose 1′-*O*-monodecanoate **8a**: ^{1}H NMR (D_2O, 500 MHz): δ 5.42 (d, 1H, $J_{1\text{-}2}=2.94$ Hz, H1); 4.34 (d, 1H, $J_{1'a\text{-}b}=12.07$ Hz, H1′a); 4.15–4.12 (3H, H1′b, H3′, H4′); 3.85–3.72 (7H, H5, H6′a,b, H6a,b, H5′, H3); 3.54 (dd, 1H, $J_{1\text{-}2}=3.17$, $J_{2\text{-}3}=9.97$ Hz, H2); 3.47 (t, 1H, $J_{3\text{-}4}=J_{4\text{-}5}=9.49$ Hz, H4); 2.39 (t, 2H, $J=7.04$ Hz, CO-CH_2); 1.61 (m, 2H, CO-CH$_2$CH_2); 1.28 (m, 12H, 6 CH$_2$); 0.86 (t, 3H, $J=6.34$ Hz, CH$_3$); ^{13}C NMR (D_2O, 125 MHz): δ 174.73 (1C, CO); 102.79 (1C, C2′); 93.04 (1CH, C1); 82.11 (1CH, C5′); 77.0 (1CH, C3′); 73.17 (1CH, C4′); 72.95 (1CH, C5); 72.91 (1CH, C3); 71.3 (1CH, C2); 69.50 (1CH, C4); 62.60 (1CH$_2$, C1′); 61.80 (1CH$_2$, C6′); 60.50 (1CH$_2$, C6); 34.26 (1CH$_2$, **C**H$_2$-CO); 32.36, 30.07, 29.85, 29.61, 23.04 (6CH$_2$); 25.10 (1CH$_2$, **C**H$_2$CH$_2$-CO); 14.24 (1CH$_3$); Anal. calcd for $C_{22}H_{40}O_{12}\cdot 1.4H_2O$: C, 50.6; H, 8.3. Found: C, 50.9; H, 8.1%; $[\alpha]_D^{20}=+52$ (*c* 1, MeOH); SMHR (FAB+) *m*/*z* calcd (M+Na) 519.2417, found 519.2419.

4.12. Sucrose monolaurates 4, 5 and 9a

Sucrose (1.1 g, 3 mmol), vinyl laurate (4.2 mL, 5 equiv.), PN/C (1/3 m/m) (7.2 U/mg, 2.5 g), DMF–H_2O ($v_{H_2O}/v_{tot}=4\%$, 8 mL), 3 days. FC eluent: CH_2Cl_2/Me_2CO/MeOH/H_2O 56/20/20/4 ($R_f=0.45$). Yield: lyophilised white foam (360 mg, 21%); regioisomers proportion 6/1′/6′=9/75/13+3% on secondary hydroxyl groups, separated by semi-preparative HPLC. Sucrose 1′-*O*-monolaurate **9a**. ^{1}H NMR (D_2O, 300 MHz): δ 5.37 (1H, d, $J_{1\text{-}2}=2.81$ Hz, H1); 4.33 (1H, d, $J_{1'a\text{-}b}=12.18$ Hz, H1′a); 4.13–4.12 (3H, H1′b, H3′, H4′); 3.85–3.72 (7H, H5, H6′a,b, H6a,b, H5′, H3); 3.56 (1H, dd, $J_{1\text{-}2}=3.04$, $J_{2\text{-}3}=9.74$ Hz, H2); 3.47 (1H, t, $J_{3\text{-}4}=J_{4\text{-}5}=9.34$ Hz, H4); 2.39 (2H, m, CO-CH_2); 1.61 (2H, m, CO-CH$_2$CH_2); 1.28 (16H, m, 8 CH$_2$); 0.86 (3H, t, $J=6.41$ Hz, CH$_3$); ^{13}C NMR (D_2O, 75 MHz): δ 174.61 (1C, CO); 102.85 (1C, C2′); 93.1 (1CH, C1); 82.16 (1CH, C5′); 77.10 (1CH, C3′); 73.44 (1CH, C4′); 72.99 (2CH, C5, C3); 71.39 (1CH, C2); 69.51 (1CH, C4); 62.70 (1CH$_2$, C1′); 61.74 (1CH$_2$, C6′); 60.63 (1CH$_2$, C6); 34.32 (1CH$_2$, **C**H$_2$-CO); 32.49, 30.37, 30.03, 29.78, 23.12 (8CH$_2$); 25.19 (1CH$_2$, **C**H$_2$CH$_2$-CO); 14.28 (1CH$_3$); Anal. calcd for $C_{24}H_{44}O_{12}\cdot 0.9H_2O$: C, 53.2; H, 8.6. Found: C, 52.9; H, 8.5%; $[\alpha]_D^{20}=+50$ (*c* 1, MeOH); SMHR (FAB+) *m*/*z* calcd (M+Na) 547.2730, found 547.2718. Sucrose 6-*O*-monolaurate **4**: ^{1}H NMR (D_2O, 500 MHz): δ 5.39 (d, 1H, $J_{1\text{-}2}=3.81$ Hz, H1); 4.42 (dd, 1H, $J_{6a\text{-}b}=11.92$ Hz, $J_{6a\text{-}5}=2.12$ Hz, H6a); 4.19 (dd, 1H, $J_{6a\text{-}b}=11.97$ Hz, $J_{6b\text{-}5}=5.37$ Hz, H6b); 4.12 (d, 1H, $J_{3'\text{-}4'}=8.07$ Hz, H3′); 4.05 (m, 1H, H5); 4.00 (1H, t, $J_{4'\text{-}3'}=J_{4'\text{-}5'}=8.02$ Hz, H4′); 3.85–3.73 (m, 4H, H5′, H6′a,b, H3); 3.65 d, (1H, $J_{1'a\text{-}b}=12.36$ Hz, H1′a); 3.61 (d, 1H, $J_{1'a\text{-}b}=12.27$ Hz, H1′b); 3.45 (dd, 1H, $J_{1\text{-}2}=3.80$, $J_{2\text{-}3}=9.76$ Hz, H2); 3.31 (t, 1H, $J_{3\text{-}4}=J_{4\text{-}5}=9.12$ Hz, H4); 2.35 (t, 2H, CO-CH_2); 1.60 (m, 2H, CO-CH$_2$CH_2); 1.41 (m, 16H, 8 CH$_2$); 0.96 (t, 3H, CH$_3$); ^{13}C NMR (D_2O, 75 MHz): δ 174.5 (1C, CO on 6); 104.2 (1C, C2′); 92.4 (1CH, C1); 82.9 (1CH, C5′); 78.3 (1CH, C3′); 74.4 (1CH, C4′); 73.5 (1CH, C3); 72.2 (1CH, C2); 71.0 (1CH, C5); 70.6 (1CH, C4); 63.7 (1CH$_2$, C6); 63.1 (1CH$_2$, C1′); 63.0 (1CH$_2$, C6′); 34.0 (1CH$_2$, **C**H$_2$-CO); 32.1, 29.7–29.2, 27.7 (8CH$_2$); 25.0 (1CH$_2$, **C**H$_2$CH$_2$-CO); 13.5 (1CH$_3$); Anal. calcd for $C_{24}H_{44}O_{12}\cdot H_2O$: C, 53.1; H, 8.6. Found: C, 53.2; H, 8.7%; $[\alpha]_D^{20}=+51$ (*c* 1, MeOH); SMHR (FAB+) *m*/*z* calcd (M+Na) 547.2730, found 547.2718. Sucrose 6′-*O*-monolaurate **5**: ^{1}H NMR (D_2O, 500 MHz): δ 5.37 (d, 1H, $J_{1\text{-}2}=3.81$ Hz, H1); 4.40 (dd, 1H, $J_{6'a\text{-}b}=11.69$ Hz, $J_{6'a\text{-}5}=7.79$ Hz, H6′a); 4.34 (d, 1H, $J_{6'a\text{-}b}=11.71$ Hz, $J_{6'b\text{-}5}=7.79$ Hz, H6′b); 4.12 (d, 1H, $J_{3'\text{-}4'}=8.20$ Hz, H3′); 4.03 (t, 1H, $J_{4'\text{-}3'}=8.08$ Hz, H4′); 3.95 (m, 1H, H5′); 3.84 (m, 2H, H6a, H5); 3.72 (m, 2H, H6b, H3); 3.66 (d, 1H, $J_{1'a\text{-}b}=12.30$ Hz, H1′a); 3.63 (d, 1H, $J_{1'a\text{-}b}=12.26$ Hz, H1′b); 3.44 (dd, 1H, $J_{1\text{-}2}=3.82$, $J_{2\text{-}3}=9.77$ Hz, H2); 3.35 (t, 1H, $J_{3\text{-}4}=J_{4\text{-}5}=9.61$ Hz, H4); 2.36 (t, 2H, $J=7.37$, CO-CH_2); 1.63 (m, 2H, CO-CH$_2$CH_2); 1.39 (m, 16H, 8 CH$_2$); 0.92 (t, 3H, $J=7.04$ Hz, CH$_3$); ^{13}C NMR (D_2O, 75 MHz): δ 174.5 (1C, CO on 6′); 104.6 (1C, C2′); 92.5 (1CH, C1); 79.7 (1CH, C5′); 78.1 (1CH, C3′); 76.0 (1CH, C4′); 73.8 (1CH, C3); 73.2 (1CH, C5); 72.3 (1CH, C2); 70.6 (1CH, C4); 65.9 (1CH$_2$, C6′); 62.9 (1CH$_2$, C1′); 61.6 (1CH$_2$, C6); 34.0 (1CH$_2$, **C**H$_2$-CO); 32.0, 29.7–29.2, 27.7 (8CH$_2$); 25.0 (1CH$_2$, **C**H$_2$CH$_2$-CO); 13.4 (1CH$_3$); Anal. calcd for $C_{24}H_{44}O_{12}\cdot H_2O$: C, 53.1; H, 8.6. Found: C, 52.9; H, 8.6%; $[\alpha]_D^{20}=+52$ (*c* 1, MeOH); SMHR (FAB+) *m*/*z* calcd (M+Na) 547.2730, found 547.2718.

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