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Note

Reactivity of melezitose and raffinose under Mitsunobu reaction conditions

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Abstract—The reactivity of melezitose and raffinose under Mitsunobu conditions was studied within the scope of the use of trisaccharides for the synthesis of fatty acid esters. Melezitose led to esters with preferential substitution at primary positions following the order of reactivity 6″ > 6 > 6′. Raffinose proved to be very reluctant toward ester formation in these conditions, leading mainly to the new 3″,6″-anhydroraffinose.

Keywords: Sugar esters; Raffinose; Melezitose; Mitsunobu reaction; Anhydro sugars

Carbohydrate esters of fatty acids are well-known amphiphilic compounds used as food additives, in cosmetics or in pharmaceutical industries for their surfactant and emulsifying properties.[1] This class of surfactant is of particular interest because of its high availability, low toxicity, biocompatibility, and excellent biodegradability. They also exhibit liquid crystalline thermotropic mesophases with large dependency on the chain length and attachment position.[2] Moreover, antibacterial activity has been reported for such compounds,[3] as well as antitumor activities[4] in the case of maltotriose esters. After having studied the reactivity of sucrose under various esterification conditions,[†] we were interested in having reference samples of similar esters having larger carbohydrate moieties. Regioselective chemical acylation of free carbohydrates is a challenging task due to their multifunctionality, and we previously studied the formation of sucrose esters under Mitsunobu conditions (which differs from other ester forming reactions since the alcoholic oxygen atom is lost in the process). It was notably shown that besides the desired esters, competitive intramolecular etherifications occurred giving rise to 6-monoester-3′,6′-anhydrosucroses,[2b] together with 3′,4′-anhydro derivatives whose formation had been reported earlier.[6] Being able to attain highly pure materials allowed, for example, in the case of a disubstituted analog, the observation of a phase transition between two smectic A phases with continuous change in layer spacing.[2a,d]

The aim of the present work was to study the behavior of melezitose (**1**) and raffinose (**2**) (Chart 1), two commercially available non-reducing trisaccharides containing a sucrose scaffold, under Mitsunobu conditions thus extending the size and allowing to obtain new structures for the polar heads of the synthesized carbohydrate esters. For melezitose and raffinose, only enzyme catalyzed selective esterifications have been reported to date.[7–10] However, raffinose was used in direct fatty acid methyl esters transesterification reactions for non-selective preparation of sugars esters.[11]

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† For a recent comprehensive review of sucrose chemistry, see Ref. 5.

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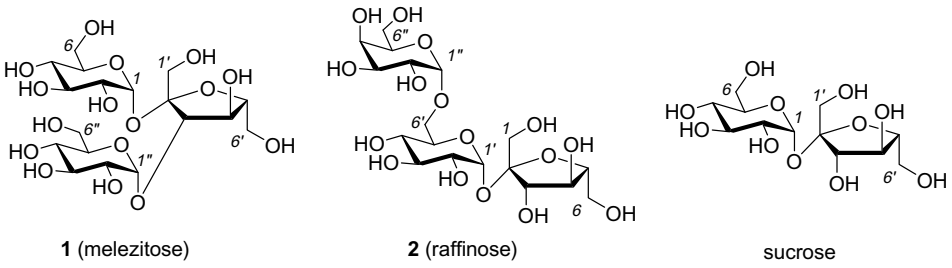


Chart 1.

Melezitose (**1**), α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl α-D-glucopyranoside[‡] is a natural non-reducing trisaccharide often present in honeydew (excretions of some insects), and has been isolated from discharges of leaves of *Alhagi pseudolhagi Desv*. legumes and a number of trees, in particular lime-tree and poplar.[12–17] It has been reported that melezitose exhibits some biological activity and is used in Chinese medicine.[16]

In the presence of diisopropyl azodicarboxylate (DIAD) and PPh_3, reaction of melezitose (**1**) with palmitic acid in DMF led to monoesters **3** and **4** corresponding to acylation at position 6 or 6″ as well as the 6,6″-diester **5** and the 6,6′,6″-triester **6** (Scheme 1). Selectivity for primary hydroxyls groups in Mitsunobu conditions was expected taking into account the sensitivity of this reaction to steric hindrance as seen in the case of sucrose.

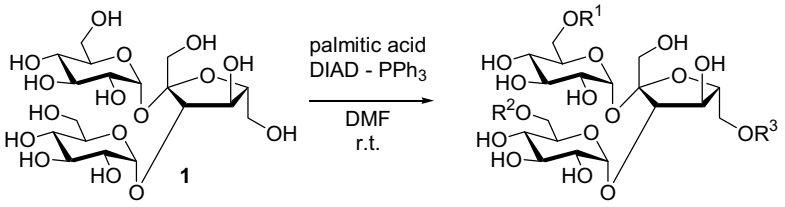
The structure of the melezitose esters was fully established using 1D and 2D NMR spectroscopy. Distinctive changes in the ^{13}C chemical shifts due to acylation were observed for carbon atoms close to esterification points (Table 1) Notably, C-6 of compound **3** is shifted downfield compared to the C-6 melezitose (64.7 vs 62.4 ppm) and C-5 of **3** is shifted upfield (71.7 vs 74.1 ppm). Also, the correlation of C=O of the ester with H-6 in the heteronuclear multiple bond correlation (HMBC) spectrum confirms the proposed structure for ester **3**. Likewise, the structure of esters **4**, **5**, and **6** was confirmed by the same methods (see Table 1).

Experiments including variations on the stoichiometry of the reagents showed a significant preference for position 6″ (Table 2, entry 1). Position 6′, though reported as the most reactive in a tritylation reaction,[16] appeared here to be much less reactive (Table 2, entries 1 and 2). Compared to sucrose, this selectivity order could be explained by the increased steric hindrance of the fructose moiety by the additional α-glucosyl part located at OH-3′. Starting melezitose was never entirely consumed unless large amounts of PPh_3/DIAD were used, which resulted in obtaining higher quantities of diester **5** and triester **6**.

Raffinose (**2**), α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside, is another commercially available trisaccharide reported to be the most abundant naturally occurring oligosaccharide after sucrose in plants and agricultural products, such as beet molasses, sugar cane, honey, eucalyptus manna, cottonseed meal, leaves of the yew, potatoes, grapes, and seeds of many leguminous plants.[18–20] Generally extracted from molasses (co-products of sugar production), this non-reducing trisaccharide can also be refined through chromatography and crystallization[21] as its pentahydrated form.[22] Since anhydrous conditions are required here, raffinose was dried by heating at 100 °C for 3 h under a nitrogen flux prior to the reaction. The reaction was carried out in DMF using 5.4 equiv of PPh_3 and DIAD and 5 equiv of palmitic acid and gave a complex mixture of compounds in which 6-monoacyl raffinose **7** was detected, though in a very little amount (ca. 2% yield). The main products were those arising from intramolecular cyclization, namely 3″,6″-anhydroraffinose (**8**) and its monoester **9** in 38% and 17% yields, respectively. Small amounts of 3,6–3″,6″-dianhydroraffinose (**10**) were also obtained (4% yield). Compounds **8** and **10** were identical to those obtained in the absence of palmitic acid (Scheme 2).

Identification of compounds **7**–**10** was established by NMR spectrometry experiments. ^{13}C NMR of the monoester **7** showed the typical acylation feature with a downfield chemical shift for C-6 of **7** compared to C-6 of raffinose: 66.8 versus 63.2 ppm and upfield chemical shifts for C-5 of the same compounds: 80.5 versus 83.4 ppm (Table 3). Acylation of position 6 of compound **9** was confirmed by the same shifts, downfield for C-6 and upfield for C-5. ^{1}H NMR data for the 3″,6″-anhydro moiety found in compounds **8**–**10** were consistent with those reported for 3,6–3″,6″-dianhydroraffinose hepta-acetate[23] and 3,6-anhydrogalactopyranoside derivatives[24–26] obtained as side products in some Williamson type etherification starting from chlorinated derivatives. Moreover in the ^{13}C NMR spectra, C-3″, C-5″, and C-6″ of compounds **8** and **9** were significantly shifted downfield compared to raffinose (Table 3). In

[‡] The nomenclature used herein follows the rule 2-Carb-37.1 of IUPAC for oligosaccharides without a free hemiacetal group with regard to their names and locants.



3: $R^1 = COC_{15}H_{31}$; $R^2 = R^3 = H$
4: $R^1 = R^3 = H$; $R^2 = COC_{15}H_{31}$
5: $R^1 = R^2 = COC_{15}H_{31}$; $R^3 = H$
6: $R^1 = R^2 = R^3 = COC_{15}H_{31}$

Scheme 1. Synthesis of melezitose palmitates.

Table 1. ^{13}C NMR chemical shifts (ppm) for the carbohydrate backbone of melezitose (**1**) and esters **3**–**6**

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
1[7]	93.3	73.2	75.1	71.7	74.1	62.4	64.2	105.3	85.8	74.4	83.4	63.3	101.9	73.8	74.9	72.2	74.0	62.9
3	93.1	72.9	74.5	71.7	**71.7**	**64.7**	64.5	105.2	85.6	74.6	83.2	63.8	101.7	73.4	74.5	71.9	73.9	62.7
4	93.1	72.9	74.9	71.5	74.0	62.6	64.6	105.1	85.4	74.6	83.4	63.3	101.4	73.3	74.5	71.5	**71.5**	**64.5**
5	92.2	72.0	73.9	70.7	**70.7**	**63.8**	63.0	104.4	84.9	74.0	82.7	63.5	100.8	72.6	73.7	70.7	**70.8**	**63.5**
6	91.2	71.3	73.4	70.0	**70.0**	**63.6**	63.7	103.5	84.5	74.1	**78.6**	**64.9**	99.8	71.6	72.9	70.0	**70.0**	**63.0**

All spectra were acquired in CD_3OD. Carbon atoms on which a remarkable induced shift effect due to acylation is observed are indicated in bold.

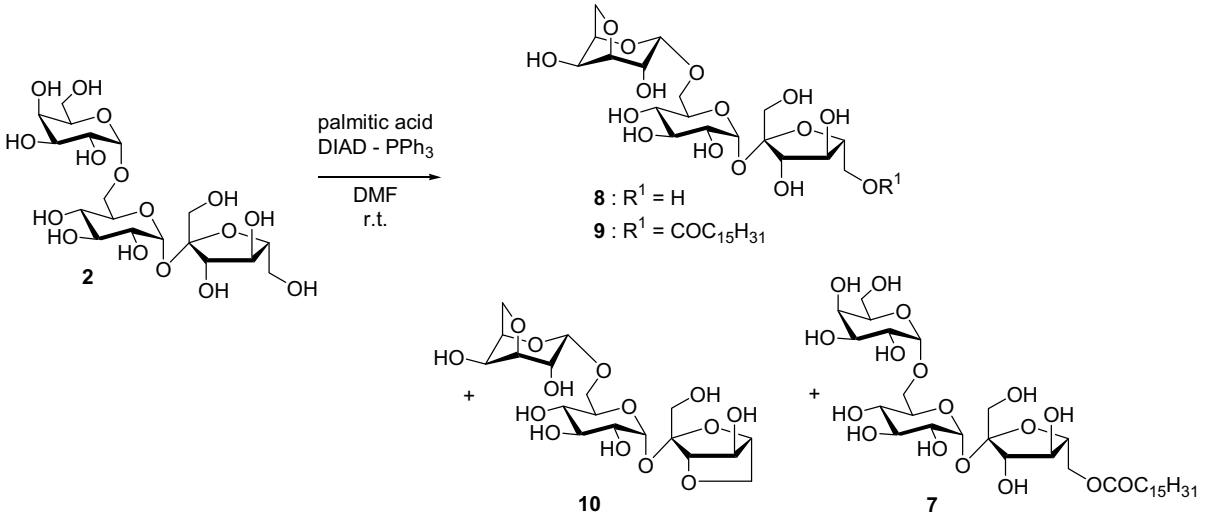
Table 2. Synthesis of melezitose esters

Entry	Reagents (equiv)		Yields[a] (%)		
	PPh_3/DIAD	Palmitic acid	**3** and **4** (ratio **3**:**4**)	**5**	**6**
1	1.6	1.5	14 (33:67)	0	0
2	2.7	2.5	44 (22:79)	11	0
3	3.2	3.0	34 (26:74)	13	2
4	5.4	5	0	22	21

[a] Isolated yields after column chromatography.

dianhydro compound **10**, the 3,6-anhydro bridge was confirmed by the downfield values observed for C-2 and C-6 compared to the one described for raffinose (110.3 vs 105.3 for C-2 and 71.9 vs 63.2 for C-6; Table 3), consistently with values in 3′,6′-anhydrosucrose. Anhydro bridges at positions 3,6 or/and a 3″,6″ were also confirmed by the HMBC 2D NMR experiment (see Fig. 1).

The proportions of the various reaction products (Table 4) show that raffinose is more prone to dehydration to form 3″,6″-anhydro derivatives under Mitsunobu conditions, and even the dianhydro compound, compared to sucrose or melezitose. No 3,4-anhydroraffinose derivative was detected here, unlike in the case of



Scheme 2. Raffinose derivatives obtained from the reaction with palmitic acid.

Table 3. ^{13}C NMR chemical shifts (ppm) for the carbohydrate backbone of raffinose (**2**) and its derivatives **7**–**10**

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
2[7]	64.2	105.3	79.2	75.3	83.4	63.2	93.4	73.0	74.4	72.0	73.3	68.3	100.5	70.5	71.4	71.0	72.4	62.8
7	64.1	105.6	78.7	76.5	**80.5**	**66.8**	93.2	73.2	74.7	72.0	73.0	68.5	100.8	70.4	71.1	71.4	72.3	62.8
8	63.2	104.4	78.2	74.4	82.4	62.1	92.3	72.0	73.3	70.4	72.5	69.2[a]	97.4	70.4	**81.5**	70.4	**77.7**	**69.0**[a]
9	64.1	104.8	79.1	76.1	**79.9**	**66.4**	92.6	72.5	74.0	70.6	72.5	69.5	97.6	70.6	**81.9**	70.6	**78.1**	**69.4**
10	62.6	**110.3**	79.2	77.9	82.9	**71.9**	94.3	73.4	74.8	71.4	73.2	69.9	98.1	71.2	**82.7**	71.3	**78.8**	**69.9**

All spectra were acquired in CD_3OD. Carbon atoms on which a remarkable induced shift effect due to acylation or cyclization is observed are indicated in bold.

[a] Assignments may be reversed.

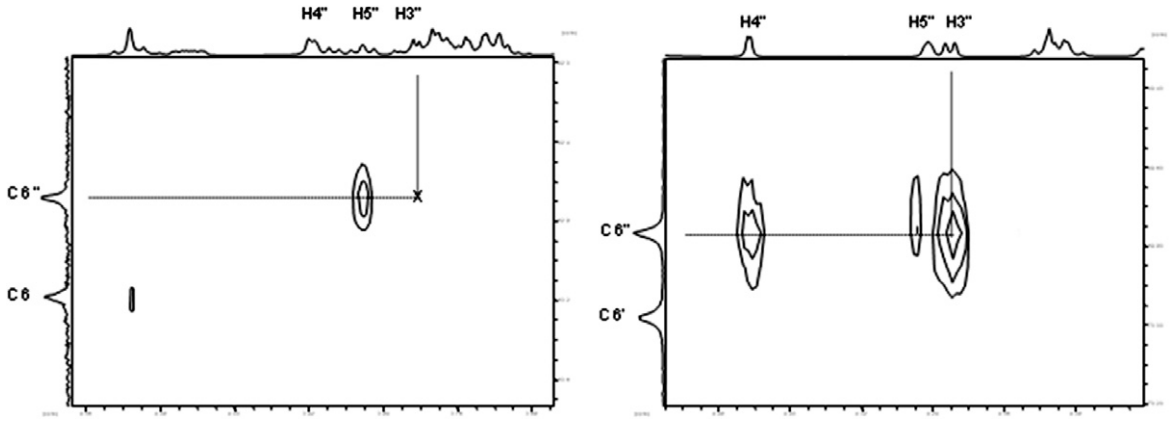


Figure 1. HMBC spectra of raffinose **2** (left) and anhydro derivative **8** (right) showing the ^3J C-6″–H-3″ correlation for **8**.

Table 4. Synthesis of raffinose esters

Entry	Reagents (equiv)		Yields[a] (%)			
	PPh_3/DIAD	Palmitic acid	**7**	**8**	**9**	**10**
1[b]	2.7 * 3	2.5 * 3	0	32	16	6
2[b]	2	0	—	48	—	8
3[b]	5.4	5.0	2	38	17	4
4[c]	5.4	5	—	—	10	4

[a] Isolated yields after column chromatography.

[b] Starting material is raffinose.

[c] Starting material is anhydroraffinose (**8**).

sucrose for which the 6-*O*-ester of 3′,4′-anhydrosucrose was often found in a larger amount than the 3′,6′-anhydro derivative.[2b] This could be explained by an effect of the galactosyl residue on the overall conformation of the molecule and consequently on the equilibrium between a cyclic pentavalent phosphorous intermediate and non-cyclic oxyphosphonium species at OH-4 and OH-6 which would be less favorable to the cyclic system in the case of raffinose than that of sucrose, even though these changes did not affect the hydrogen bond network which was shown to be the same for sucrose and raffinose, involving O-2 and either OH-1′ or OH-3′ (O-2′, OH-1, and OH-3 in raffinose numbering).[27]

To summarize, melezitose esters are easily formed under Mitsunobu conditions with the following selectivity order: $6'' > 6 > 6'$. No reaction at OH-1′ of the fructosyl moiety was observed during the course of this study. Raffinose led essentially to anhydro derivatives: on the galactose moiety, or in a less reactive manner on the fructose ring. When compared to sucrose, the reactivity of the fructosyl moiety was affected in both cases, with decreased reactivity of OH-6′ in melezitose, and there was no 3,4-anhydro formation for raffinose. Compounds prepared here will be used as reference compounds for further studies on trisaccharide derivatives. This work is also the first synthesis and full structural study of 3″,6″-mono-anhydroraffinose.

1. Experimental

1.1. General methods

Palmitic acid and trisaccharides were obtained from Aldrich. Chromatography solvents were purchased from SDS and Carlo Erba. Reactions were monitored by TLC using Silica Gel plates (Merck 60 F_{254}). The plates were developed using UV light and vaporization with a solution of 10% H_2SO_4 in EtOH (v/v). Flash-chromatography separations were performed using Merck Gerudan Silica Gel Si 60 (40–63 μm). NMR spectra were recorded on Bruker AC spectrometers at 75.47 MHz (or 125.77 MHz) for ^{13}C NMR and 300.13 MHz (or 500.13 MHz) for ^1H NMR. SI-mass spectra were recorded by the Centre de Spectrométrie

de Masse of the Université Claude Bernard (Villeurbanne). Microanalyses were performed by the Service Central d'Analyse of the CNRS (Vernaison). Optical rotations were measured with a Perkin Elmer 241 polarimeter.

1.2. Acylation of melezitose

Melezitose (1.97 g, 1.98 mmol) was dissolved in anhyd DMF (20 mL) by stirring under N_2 at 60 °C. The mixture was cooled to room temperature before the addition of triphenylphosphine (2.80 g, 2.7 equiv), palmitic acid (2.50 g, 2.5 equiv), and DMF (6 mL). After complete dissolution, the medium was cooled to 0 °C and DIAD (2.09 mL, 2.7 equiv) was added. The obtained mixture was stirred for 24 h at room temperature and DMF was removed under diminished pressure at $T = 40$–42 °C. The crude residue was then purified by silica gel chromatography (elution gradient: 78:10:10:1.5 (A) to 14:5:5:1 (B) v/v CH_2Cl_2–acetone–MeOH–water). Successively were collected a fraction containing the 6,6″-*O*-dipalmitate **5** ($R_f = 0.78$ in mixture B, 0.32 g, 8%) then a mixture of 6-*O*-monopalmitate **3** and 6″-*O*-monopalmitate **4** in a 11:39 ratio ($R_f = 0.31$ in mixture B, 1.27 g, 44%). All compounds were obtained as white powders.

1.3. α-D-Glucopyranosyl-(1→3)-β-D-fructofuranosyl 6-*O*-hexadecanoyl-α-D-glucopyranoside (6-*O*-palmitoyl melezitose) (3) and 6-*O*-hexadecanoyl-α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl α-D-glucopyranoside (6″-*O*-palmitoyl melezitose) (4)

Characteristic signals for **3**: ^{1}H NMR (CD_3OD, 500 MHz): δ 5.42 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.02–4.05 (m, 1H, H-5), 3.94–4.00 (m, 1H, H-5″); ^{13}C NMR (CD_3OD, 125 MHz): see Table 2, δ 174.6 (CO), 34.9 ($CH_{2\alpha}$), 23.6–33.0 ($(CH_2)_{13}$), 14.5 (CH_3). Characteristic signals for **4**: ^{1}H NMR (CD_3OD, 500 MHz): δ 5.48 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.07–4.11 (m, 1H, H-5″), 3.90–3.94 (m, 1H, H-5); ^{13}C NMR (CD_3OD, 500 MHz): see Table 2, δ 174.8 (CO), 34.9 ($CH_{2\alpha}$), 23.6–33.0 ($(CH_2)_{13}$), 14.5 (CH_3). HRSIMS *m*/*z*: calcd for $C_{34}H_{62}O_{17}Na$ $[M+Na]^+$: 765.3877. Found: 765.3885. Anal. Calcd for $C_{34}H_{62}O_{17}\cdot 2H_2O$: C, 52.42; H, 8.48; O, 39.10. Found: C, 52.46; H, 8.33; O, 39.21.

1.4. 6-*O*-Hexadecanoyl-α-D-glucopyranosyl-(1→3)-β-D-fructofuranoside 6-*O*-hexadecanoyl-α-D-glucopyranoside (di-6,6″-*O*-palmitoyl melezitose) (5)

$[\alpha]_D$ +44 (*c* 0.5, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 5.45 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.10 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 4.43 (dd, 2H, $J_{5'',6b''} = J_{5,6b}$ 1.7 Hz, H-6b″, H-6b), 4.27 (m, 4H, H-6a″, H-6a, H-3′, H-4′), 4.12 (ddd, 1H, $J_{5'',6b''}$ 1.7 Hz, $J_{5'',6a''}$ 4.5 Hz, $J_{4'',5''}$ 10.2, H-5″), 4.07 (ddd, 1H, $J_{5,6b}$ 1.7 Hz, $J_{5,6a}$ 5.7 Hz, $J_{4,5}$ 9.7 Hz, H-5), 3.81–3.85 (m, 2H, H-5′, H-6b′), 3.76–3.77 (m, 1H, H-6a′), 3.73 (d, 1H, $J_{1a',1b'}$ 12.3 Hz, H-1a′), 3.70 (dd, 1H, $J_{2'',3''} = J_{3'',4''}$ 9.3 Hz, H-3″), 3.66 (d, 1H, $J_{1a',1b'}$ 12.3 Hz, H-1b′), 3.61 (dd, 1H, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 3.42–3.45 (m, 2H, H-2, H-2″), 3.35–3.39 (m, 2H, H-4, H-4″), 2.38 (t, 4H, $J_{CH_{2\alpha},CH_{2\beta}}$ 7.5 Hz, $CH_{2\alpha}$), 1.59–1.64 (m, 4H, $CH_{2\beta}$), 1.25–1.33 (m, 48H, $(CH_2)_{24}$), 0.9 (t, 6H, J_{CH_2,CH_3} 7.0 Hz, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): see Table 2, δ 176.9 (CO), 174.5 (CO), 34.0 ($2 \times CH_{2\alpha}$), 25.0–29.8 ($(CH_2)_{26}$), 13.4 ($2 \times CH_3$). HRSIMS *m*/*z*: calcd for $C_{50}H_{92}O_{18}Na$ $[M+Na]^+$: 1003.6181. Found: 1003.6188. Anal. Calcd for $C_{50}H_{92}O_{18}\cdot 1.5H_2O$: C, 59.58; H, 9.43; O, 30.99. Found: C, 59.62; H, 9.34; O, 31.04.

1.5. 6-*O*-Hexadecanoyl-α-D-glucopyranosyl-(1→3)-6-*O*-hexadecanoyl-β-D-fructofuranosyl 6-*O*-hexadecanoyl-α-D-glucopyranoside (tri-6,6′,6″-*O*-palmitoyl melezitose) (6)

$[\alpha]_D$ +35 (*c* 0.8, 9:1 CH_2Cl_2–MeOH). ^{1}H NMR ($CD_3OD/CDCl_3$, 5/5, 500 MHz): δ 5.17 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.83 (d, 1H, $J_{1'',2''}$ 3.8 Hz, H-1″), 3.90–4.16 (m, 7H, H-6a, H-6b, H-4′, H-6a′, H-6b′, H-6a″, H-6b″), 3.81 (d, 1H, $J_{3',4'}$ 7.9 Hz, H-3′), 3.77–3.82 (m, 2H, H-5, H-5″), 3.70–3.74 (m, 1H, H-5″), 3.44 (d, 1H, $J_{1a',1b'}$ 12.5 Hz, H-1a′), 3.42 (dd, 1H, $J_{2'',3''}$ 9.3 Hz, $J_{3'',4''}$ 9.5 Hz, H-3″), 3.38 (d, 1H, $J_{1a',1b'}$ 12.5 Hz, H-1b′), 3.37 (dd, 1H, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.5 Hz, H-3), 3.19–3.23 (m, 2H, H-2, H-2″), 3.09–3.11 (m, 1H, H-4″), 3.02 (dd, 1H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 2.04–2.12 (m, 6H, $CH_{2\alpha}$), 1.32–1.35 (m, 6H, $CH_{2\beta}$), 0.96–1.03 (m, 72H, $(CH_2)_{36}$), 0.6 (t, 9H, J_{CH_2,CH_3} 7.0 Hz, CH_3); ^{13}C NMR ($CD_3OD/CDCl_3$, 5/5, 125 MHz): see Table 2, δ 174.4 (CO), 174.3 (CO), 173.8 (CO), 33.7 ($3 \times CH_{2\alpha}$), 22.3–31.5 ($(CH_2)_{39}$), 13.5 ($3 \times CH_3$). Anal. Calcd for $C_{66}H_{122}O_{19}\cdot H_2O$: C, 64.05; H, 10.10; O, 25.85. Found: C, 64.16; H, 10.03; O, 25.81.

1.6. Acylation of raffinose

Raffinose pentahydrate (1.16 g, 2.22 mmol) was dried under N_2 and stirring at 100 °C for 3 h. Anhyd raffinose thus obtained was dissolved in anhyd DMF (11 mL) by stirring under N_2 at room temperature before the addition of triphenylphosphine (3.13 g, 5.4 equiv), and palmitic acid (2.84 g, 5.0 equiv). After complete dissolution, the medium was cooled to 0 °C and DIAD (2.37 mL, 5.4 equiv) was introduced. After 20 h at room temperature, DMF was removed under diminished pressure at $T = 40$–42 °C, and the crude residue was purified by silica gel chromatography (elution gradient: 78:10:10:1.5 (A) to 14:5:5:1 (B) v/v CH_2Cl_2–acetone–MeOH–water). Successively were collected fractions containing 6-*O*-palmitoyl-3″,6″-anhydroraffinose (**9**) ($R_f = 0.42$ in mixture B, 0.28 g, 17%) then 6-*O*-palmitoyl

raffinose (**7**) ($R_f = 0.34$ in mixture B, 0.03 g, 2%), then 3,6–3″,6″-dianhydroraffinose (**10**) ($R_f = 0.19$ in mixture B, 0.04 g, 4%), and then 3″,6″-anhydroraffinose (**8**) ($R_f = 0.11$ in mixture B, 0.41 g, 38%). All compounds were obtained as white powders.

1.7. α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl 6-*O*-hexadecanoyl-β-D-fructofuranoside (6-*O*-palmitoyl raffinose) (7)

$[\alpha]_D$ +77 (*c* 0.4, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 5.37 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′), 4.90 (d, 1H, $J_{1'',2''}$ 3.2 Hz, H-1″), 4.35 (m, 2H, H-6a, H-6b), 4.05–4.10 (m, 3H, H-3, H-4, H-5′), 3.87–3.96 (m, 4H, H-5, H-6a′, H-3″, H-5″), 3.68–3.81 (m, 6H, H-3′, H-6b′, H-2″, H-4″, H6a″, H-6b″), 3.62 (m, 2H, H-1a, H-1b), 3.44 (dd, 1H, $J_{1',2'}$ 3.8 Hz, $J_{2',3'}$ 9.5 Hz, H-2′), 3.33–3.36 (m, 1H, H-4′), 2.35 (t, 2H, $J_{CH_{2\alpha},CH_{2\beta}}$ 7.6 Hz, $CH_{2\alpha}$), 1.60–1.63 (m, 2H, $CH_{2\beta}$), 1.28–1.32 (m, 24H, $(CH_2)_{12}$), 0.90 (t, 3H, J_{CH_2,CH_3} 7.0 Hz, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): see Table 3, δ 175.5 (CO), 34.9 ($CH_{2\alpha}$), 23.7–33.0 ($(CH_2)_{13}$), 14.4 (CH_3). HRSIMS *m*/*z*: calcd for $C_{34}H_{62}O_{17}Na$ $[M+Na]^+$: 765.3885. Found: 765.3883. Anal. Calcd for $C_{34}H_{62}O_{17}\cdot 3H_2O$: C, 51.23; H, 8.53; O, 40.24. Found: C, 51.03; H, 8.30; O, 40.57.

1.8. 3,6-Anhydro-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside (3″,6″-anhydro-raffinose) (8)

$[\alpha]_D$ +69 (*c* 0.5, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 5.43 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.91 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1″), 4.49 (d, 1H, $J_{4'',5''}$ 1.3 Hz, H-4″), 4.29 (m, 1H, H-5″), 4.26 (d, 1H, $J_{2'',3''}$ 5.3 Hz, H-3″), 4.11–4.80 (m, 3H, H-3, H-4, H-6a″), 4.00–4.05 (m, 3H, H-5′, H-6b′, H-6b″), 3.97 (dd, 1H, $J_{1'',2''}$ 2.0 Hz, $J_{2'',3''}$ 5.3 Hz, H-2″), 3.82–3.90 (m, 3H, H-5, H-6b, H-6a′), 3.76–3.79 (m, 2H, H-6a, H-3′), 3.67 (d, 1H, $J_{1a,1b}$ 12.4 Hz, H-1b), 3.63 (d, 1H, $J_{1a,1b}$ 12.4 Hz, H-1a), 3.52 (dd, 1H, $J_{1',2'}$ 3.7 Hz, $J_{2',3'}$ 9.8 Hz, H-2′), 3.39 (dd, 1H, $J_{3',4'} = J_{4',5'}$ 9.4 Hz, H-4′); ^{13}C NMR (CD_3OD, 125 MHz): see Table 3. HRSIMS *m*/*z*: calcd for $C_{18}H_{30}O_{15}Na$ $[M+Na]^+$: 509.1482. Found: 509.1482. Anal. Calcd for $C_{18}H_{30}O_{15}\cdot 2H_2O$: C, 41.35; H, 6.51; O, 52.14. Found: C, 41.28; H, 6.21; O, 52.51.

1.9. 3,6-Anhydro-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl 6-*O*-hexadecanoyl-β-D-fructofuranoside (6-*O*-palmitoyl-3″,6″-anhydroraffinose) (9)

$[\alpha]_D$ +55 (*c* 0.6, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 5.35 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1′), 4.84 (d, 1H, $J_{1'',2''}$ 2.4 Hz, H-1″), 4.45 (d, 1H, $J_{4'',5''}$ 1.6 Hz, H-4″), 4.26–4.33 (m, 2H, H-6a, H-6b), 4.24 (m, 1H, H-5″), 4.23 (d, 1H, $J_{2'',3''}$ 5.5 Hz, H-3″), 4.09 (d, 1H, $J_{6a'',6b''}$ 9.8 Hz, H-6a″), 3.98–4.02 (m, 4H, H-3, H-4, H-6a′, H-6b″), 3.90–3.95 (m, 2H, H-5, H-5′), 3.92 (dd, 1H, $J_{1'',2''}$ 2.4 Hz, $J_{2'',3''}$ 5.5 Hz, H-2″), 3.86 (dd, 1H, $J_{6a',6b'}$ 11.4 Hz, $J_{5',6b'}$ 1.9 Hz, H-6b′), 3.70 (dd, 1H, $J_{2',3'}$ 9.8 Hz, $J_{3',4'}$ 9.5 Hz, H-3′), 3.64 (d, 1H, $J_{1a,1b}$ 12.3 Hz, H-1a), 3.59 (d, 1H, $J_{1a,1b}$ 12.3 Hz, H-1b), 3.45 (dd, 1H, $J_{1',2'}$ 4.0 Hz, $J_{2',3'}$ 9.8 Hz, H-2′), 3.36 (dd, 1H, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, H-4′), 2.33 (t, 2H, $J_{CH_{2\alpha},CH_{2\beta}}$ 7.6 Hz, $CH_{2\alpha}$), 1.57–1.61 (m, 2H, $CH_{2\beta}$), 1.22–1.28 (m, 24H, $(CH_2)_{12}$), 0.86 (t, 3H, J_{CH_2,CH_3} 7.0 Hz, CH_3); ^{13}C NMR (CD_3OD, 125 MHz): see Table 3, δ 175.1 (CO), 34.6 ($CH_{2\alpha}$), 29.8–32.5 ($(CH_2)_{13}$), 14.3 (CH_3). HRSIMS *m*/*z*: calcd for $C_{34}H_{60}O_{16}Na$ $[M+Na]^+$: 747.3779. Found: 747.3778. Anal. Calcd for $C_{34}H_{60}O_{16}\cdot 1.5H_2O$: C, 54.26; H, 8.38; O, 37.36. Found: C, 54.41; H, 8.33; O, 37.26.

1.10. 3,6-Anhydro-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl 3,6-anhydro-β-D-fructofuranoside (3,6–3″,6″-dianhydroraffinose) (10)

$[\alpha]_D$ +82 (*c* 0.3, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 5.38 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′), 4.88 (d, 1H, $J_{1'',2''}$ 2.7 Hz, H-1″), 4.44 (d, 1H, $J_{4'',5''}$ 1.9 Hz, H-4″), 4.39 (d, 1H, $J_{3,4}$ 2.1 Hz, H-4), 4.28 (m, 1H, H-5), 4.21 (m, 1H, H5″), 4.17 (d, 1H, $J_{2'',3''}$ 5.3 Hz, H-3″), 4.11 (d, 1H, $J_{3,4}$ 2.1 Hz, H-3), 3.97–4.10 (m, 6H, H-6a, H-1a, H-5′, H-6a′, H-6a″, H-6b″), 3.86 (dd, 1H, $J_{1'',2''}$ 2.7 Hz, $J_{2'',3''}$ 5.3 Hz, H-2″), 3.82–3.91 (m, 2H, H-6b, H-6b′), 3.70 (dd, 1H, $J_{2',3'} = J_{3',4'}$ 9.5 Hz, H-3′), 3.62 (d, 1H, $J_{1a,1b}$ 12.8 Hz, H-1b), 3.49 (dd, 1H, $J_{1',2'}$ 3.8 Hz, $J_{2',3'}$ 9.5 Hz, H-2′), 3.37 (dd, 1H, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, H-4′); ^{13}C NMR (CD_3OD, 125 MHz): see Table 3. HRSIMS *m*/*z*: calcd for $C_{18}H_{28}O_{14}Na$ $[M+Na]^+$: 491.1377. Found: 491.1378.

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References

1. For examples, see: (a) Baker, I. J. A.; Matthews, B.; Suares, H.; Krodkiewska, I.; Furlong, D. N.; Grieser, F.; Drummond, C. J. *J. Surfactants Deterg.* **2000**, *3*, 1–11; (b) Kabir, M. H.; Ishitobi, M.; Kunieda, H. *Colloid. Polym. Sci.* **2002**, *280*, 841–847; (c) Ebeler, S. E.; Breyer, L. M.; Walker, C. E. *J. Food Sci.* **1986**, *51*, 1276–1278; (d) Nelen, B. A. P.; Cooper, J. M. In *Emulsifiers in Food Technology*; Whitehurst, R. J., Ed.; Blackwell Publishing Ltd: Oxford, 2004; pp 131–161; (e) Hill, K.; Rhode, O. *Fett/Lipid* **1999**, *101*, 25–33; (f) Muller, A.-S.; Gagnaire, J.; Queneau, Y.; Maitre, J.-P.; Bouchu, A. *Colloids Surf., A* **2002**, *203*, 55–66.

2. (a) Molinier, V.; Kouwer, P. J. J.; Queneau, Y.; Fitremann, J.; MacKenzie, G.; Goodby, J. W. *Chem. Commun.* **2003**, 2860–2861; (b) Molinier, V.; Fitremann, J.; Bouchu, A.; Queneau, Y. *Tetrahedron: Asymmetry* **2004**, *15*, 1753–1762; (c) Molinier, V.; Kouwer, P. J. J.; Fitremann, J.; Bouchu, A.; MacKenzie, G.; Queneau, Y.; Goodby, J. W. *Chem. Eur. J.* **2006**, *12*, 3547–3557; (d) Molinier, V.; Kouwer, P. J. J.; Fitremann, J.; Bouchu, A.; MacKenzie, G.; Queneau, Y.; Goodby, J. W. *Chem. Eur. J.* **2007**, *13*, 1763–1775; (e) Goodby, J. W.; Görtz, V.; Cowling, S. J.; MacKenzie, G.; Martin, P.; Plusquellec, D.; Benvegnu, T.; Boullanger, P.; Lafont, D.; Queneau, Y.; Chambert, S.; Fitremann, J. *Chem. Soc. Rev.* **2007**, *36*, 1971–2032.
3. Devulapalle, K. S.; Gómez de Segura, A.; Ferrer, M.; Alcalde, M.; Mooser, G.; Plou, F. J. *Carbohydr. Res.* **2004**, *339*, 1029–1034.
4. Ferrer, M.; Perez, G.; Plou, F. J.; Castell, J. V.; Ballesteros, A. *Biotechnol. Appl. Biochem.* **2005**, *42*, 35–39.
5. Queneau, Y.; Jarosz, S.; Lewandowski, B.; Fitremann, J. *Adv. Carbohydr. Chem. Biochem.* **2007**, *61*, 217–292.
6. (a) Abouhilale, S.; Greiner, J.; Riess, J. G. *Carbohydr. Res.* **1991**, *212*, 55–64; (b) Guthrie, R. D.; Jenkins, I. D.; Thang, S.; Yamasaki, R. *Carbohydr. Res.* **1983**, *121*, 109–117.
7. Pérez-Victoria, I.; Morales, J. C. *Tetrahedron* **2006**, *62*, 878–886.
8. Pérez-Victoria, I.; Morales, J. C. *Tetrahedron* **2006**, *62*, 2369–2631.
9. Pérez-Victoria, I.; Zafra, A.; Morales, J. C. *Carbohydr. Res.* **2007**, *342*, 236–242.
10. Pérez-Victoria, I.; Morales, J. C. *J. Mol. Catal. B* **2007**, *48*, 8–15.
11. Hass, H. B.; Snell, F. D., York, W. C.; Osipow, L. I. German Patent DE 1262988, 1968; *Chem. Abstr.* **1968**, *68*, 106248.
12. Gorbach, G. *Forschungsdienst* **1941**, *11*, 426–438.
13. Bacon, J. S. D.; Dickenson, B. *Biochem. J.* **1957**, *66*, 289–297.
14. Astwood, K.; Lee, B.; Manley-Harris, M. *Agric. Food. Chem.* **1998**, *46*, 4958–4962.
15. Fischer, M. K.; Völkl, W.; Schopf, R.; Hofmann, K. H. *J. Insect Physiol.* **2002**, *48*, 319–326.
16. Tsao, L.; Dou, K.; Sun, G.; Lyu, Y.; Koroteev, A. M.; Krasnov, G. B. *Russ. J. Org. Chem.* **2003**, *39*, 384–391.
17. Cotte, J. F.; Casabianca, H.; Chardon, S.; Lheritier, J.; Grenier-Loustalot, M. F. *Anal. Bioanal. Chem.* **2004**, *380*, 698–705.
18. Hough, L.; Salam, M. A.; Tarelli, E. *Carbohydr. Res.* **1977**, *57*, 97–101.
19. Hudson, C. S.; Harding, T. D. *J. Am. Chem. Soc.* **1914**, *36*, 2110–2114.
20. Englis, D. T.; Decker, R. T.; Adams, A. B. *J. Am. Chem. Soc.* **1925**, *47*, 2724–2726.
21. Hiroshi, I.; Yoshihiro, S., Osamu, S.; Yuichi, O. European Patent EP 1035128, 2000; *Chem. Abstr.* **2000**, *133*, 239627; Japanese Patent JP 11075900; *Chem. Abstr.* **1999**, *130*, 253962.
22. Jeffrey, G. A.; Huang, D. *Carbohydr. Res.* **1990**, *206*, 173–182.
23. Hough, L.; Richardson, A. C.; Salam, M. *Carbohydr. Res.* **1980**, *80*, 117–122.
24. Aspinall, G. O.; Carpenter, R. C.; Khondo, L. *Carbohydr. Res.* **1987**, *165*, 281–298.
25. France, C. J.; McFarlane, I. M.; Newton, C. G.; Pitchen, P.; Barton, D. H. R. *Tetrahedron* **1991**, *47*, 6381–6388.
26. Vogel, C.; Morales Torres, G.; Reinke, H.; Michalik, D.; Voss, A. *Carbohydr. Res.* **2007**, *342*, 520–528.
27. Davies, D. B.; Christofides, J. C. *Carbohydr. Res.* **1987**, *163*, 269–274.