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Reactivity of the Hydroxy Groups in Melezitose and Their Selective Protection

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Abstract—The reactivity of melezitose hydroxy groups was studied by tritylation in pyridine with subsequent acetylation. After partial detritylation of the products, acetyl group transfer from position *4* to *6* was observed. The structure of the prepared melezitose derivatives was established on the basis of their IR, ^{1}H, ^{13}C, and ^{1}H–^{1}H COSY NMR, and mass spectra (fast atom bombardment), as well as from the results of model calculations performed with the aid of SGI Indigo Molecule-Pattern-Work-Station software package (Biosym) where the potential energy function was approximated with the CVFF potential. The reactivity of primary hydroxy groups in melezitose was found to decrease in the following order: 6′ > 6 ≈ 6″ > 1′.

Melezitose (**I**) is a natural trisaccharide, which is isolated as a white secretion from discharges of leaves of *Alhagi pseudolhagi Desv.* legumes and also from sweet discharges of a number of trees, in particular teil and poplar. This oligosaccharide exhibits a strong physiological activity and is widely used in chinese medicine, e.g., as antibacterial agent [1]. Melezitose is a nonreducing sugar, α-D-glucopyranosyl(1→3)-β-D-fructofuranosyl(2→1)-α-D-glucopyranoside. The goal of the present study was to examine the reactivity of the melezitose hydroxy groups and the possibility for selective protection of its primary hydroxy groups. For this purpose, melezitose was treated with trityl chloride, and the resulting trityl ethers were subjected to acetylation with acetic anhydride (Scheme 1).

It is known that triphenylmethyl chloride selectively reacts with primary hydroxy groups of carbohydrates and that acetic anhydride is less selective. The molecule of melezitose contains 11 hydroxy groups, four of which are primary. At a TrCl-to-melezitose molar ratio of 2:1, the major products were compounds **II** and **III**. According to the data of elemental analysis, molecule **II** contains two triphenylmethyl groups, and molecule **III**, only one. However, it was difficult to determine the position of trityl moieties in compounds **II** and **III**. As reported in [2, 3, 4], the reactivity of the fructose 1′-OH group in oligosaccharides containing a saccharose fragment (e.g., raffinose [5]) is lower than the reactivity of the other primary hydroxy groups, while the 6′-OH group is the most reactive. In order to determine the relative reactivities of primary hydroxy groups in melezitose, we made an attempt to simulate molecular models of key compounds **II** and **III**. The calculations were performed with the aid of SGI Indigo Molecule-Pattern-Work-Station software package developed by Biosym. The potential energy function was approximated with the CVFF potential [6].

Satisfactory results were obtained by calculation of compound **III**. In the tritylation of melezitose, the reaction at the 1′-OH group is ruled out for the above reason, and trityl group can be introduced to the oxygen atom in position *6*, *6′*, or *6″*. The minimal energies of the corresponding predominant conformers are 58.06, 41.17, and 61.40 kJ/mol, respectively. Therefore, the trityl group in molecule **III** is most likely to occur just at the 6′-position. This conclusion is supported by the IR, ^{1}H and ^{13}C NMR, and mass spectra of compounds **II** and **III**.

On the basis of the spectral data, compound **II** was assigned the structure of 6,6′-di-*O*-trityl-1′,2,2″,3,-3″,4,4′,4″,6″-nona-*O*-acetylmelezitose, and compound

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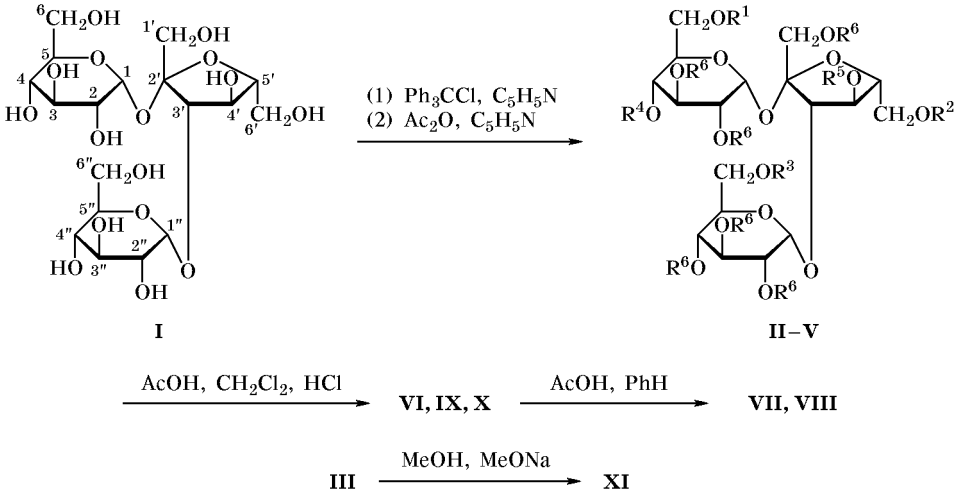
Scheme 1.



II, $R^1 = R^2 =$ Tr, $R^3 = R^4 = R^5 = R^6 =$ Ac; **III**, $R^2 =$ Tr, $R^1 = R^3 = R^4 = R^5 = R^6 =$ Ac; **IV**, $R^1 =$ Tr, $R^2 = R^3 = R^4 = R^5 = R^6 =$ Ac; **V**, $R^1 = R^2 = R^3 =$ Tr, $R^4 = R^5 = R^6 =$ Ac; **VI**, $R^1 = R^2 =$ H, $R^3 = R^4 = R^5 = R^6 =$ Ac; **VII**, $R^4 = R^5 =$ H, $R^1 = R^2 = R^3 = R^6 =$ Ac; **VIII**, $R^5 =$ H, $R^1 = R^2 = R^3 = R^4 = R^6 =$ Ac; **IX**, $R^2 =$ H, $R^1 = R^3 = R^4 = R^5 = R^6 =$ Ac; **X**, $R^1 =$ H, $R^2 = R^3 = R^4 = R^5 = R^6 =$ Ac; **XI**, $R^2 =$ Tr, $R^1 = R^3 = R^4 = R^5 = R^6 =$ H.

III, the structure of 6′-*O*-trityl-1′,2,2″,3,3″,4,4′,4″,6,6″-deca-*O*-acetylmelezitose. When the TrCl-to-melezitose was raised to 4:1, we obtained compound **V**, 6,6′,6″-tri-*O*-trityl-1′,2,2″,3,3″,4,4′,4″-octa-*O*-acetylmelezitose. This result proves that only three of the four primary hydroxy groups undergo tritylation. Mote sterically hindered 1′-OH group fails to react. However, we observed formation of compound **IV** which contains only one trityl group in a different position than in **III**, namely at position *6* of the glucose moiety. Thus, the primary hydroxy groups in melezitose exhibit different reactivities. The most active is that located at the 6′-position of the fructose ring while the 1′-OH group in the same fragment is the least reactive because of steric hindrances.

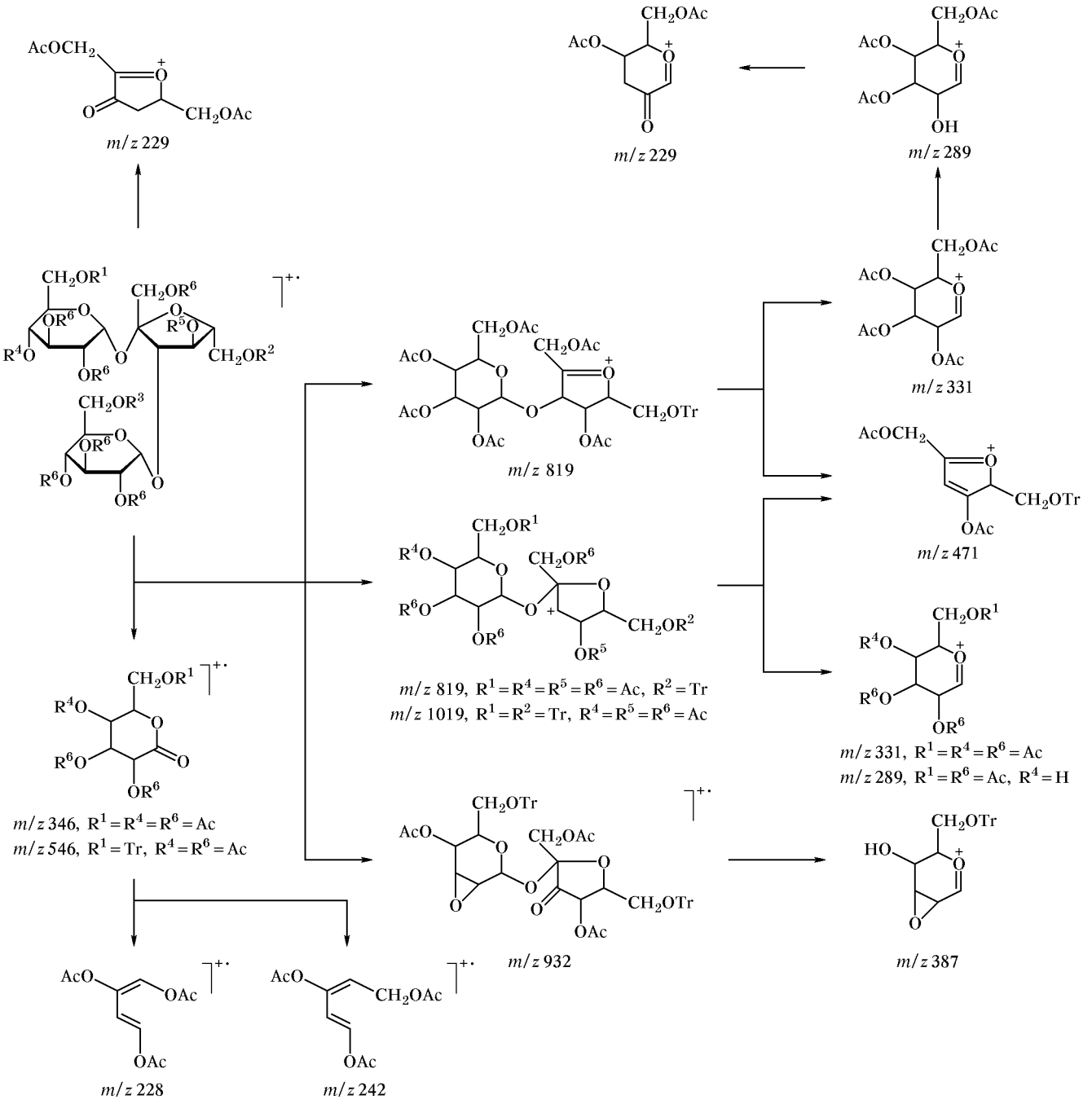
It should be noted that heating in acid medium of the product formed by removal of the trityl protection from position *6* or *6′* of acetylated melezitose leads to acetyl group migration from position *4* or *4′* to *6* or *6′*. Such migrations have been well documented [7]. Insofar as mixture **VIII**/**IX** was difficult to separate, it was heated in acid medium until the 4→6-Ac migration was complete.

In the ^{1}H NMR spectra of compounds **II** and **III**, signals from the 1′-H proton appear in a stronger field (as compared with free melezitose) due to effect of the acetyl groups, so that they fall into the region corresponding to other protons of carbohydrate rings. The chemical shifts of 1-H were determined from the ^{1}H–^{1}H COSY spectra: δ 5.85 ppm, $J_{1,2}$ = 3.78 Hz (**II**), and δ 5.84 ppm, $J_{1,2}$ = 3.93 Hz. In the ^{13}C NMR spectrum, the signal from $C^{2'}$ in the fructofuranose moiety is displaced downfield due to the presence of protective groups [8]. We also found that the trityl group exerts a stronger shielding effect on the neighboring carbon atom than does acetyl group; the corresponding upfield shift ranges from 2 to 3 ppm.

Fast atom bombardment (FAB) mass spectra turned out to be very useful in the structure determination of the compounds prepared. The application of FAB mass spectrometry to structure determination of oligosaccharides was reported in [9, 10]; in particular, the mass spectra of melezitose (**I**) obtained under bombardment by alkali metal atoms were analyzed [11, 12]. We were the first to examine the FAB mass spectra of tritylated and acetylated melezitose derivatives **II**, **III**, **V**–**VIII**, **X**, and **XI** which were isolated by column chromatography. The results are given in table. The fragmentation pattern is shown in Scheme 2. Initially, the trisaccharide readily decomposes into di- and monosaccharide fragments which then give rise to smaller species.

It is seen that the relative intensity of the $[M+\mathrm{Li}]^+$ ion is as low as 21% if the hydroxy group is located in position *4′* of the fructose moiety and that the $[M+\mathrm{Li}]^+$ ion peak is the most abundant (I_{rel} = 100%) if the hydroxy group occupies position *6* of the glucose fragment. It was impossible to determine the effect of the 6″-OH group on the bond cleavage. The observed fragment peaks are formed by cleavage of

Scheme 2.

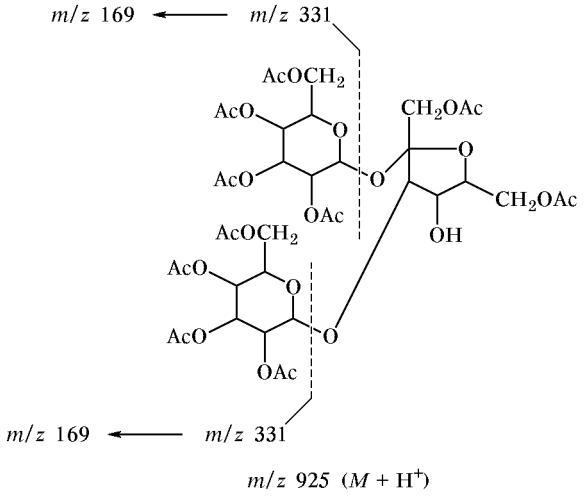


glycoside bonds in the trisaccharide, followed by successive elimination of acetyl groups.

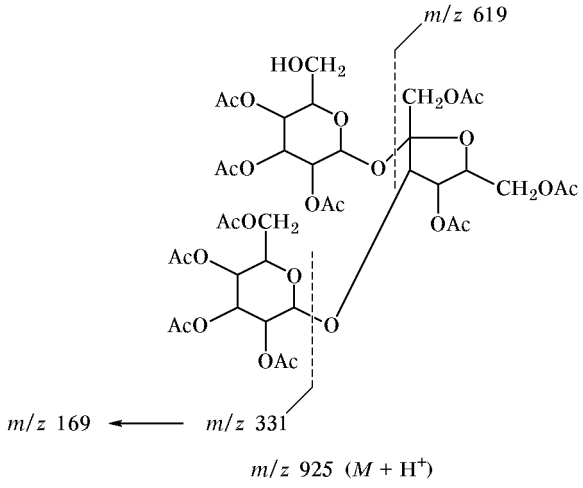
Melezitose derivatives having no trityl groups, e.g., compounds **VIII** and **X**, are characterized by a different fragmentation pattern. The mass spectra of these compounds contain fragment ion peaks with *m*/*z* 289 and 229, respectively. Presumably, the first of these originates from the glucose ring of **VIII**, and the second is formed with participation of the fructose ring in **X** (Scheme 3).

Thus our results show that the reactivity of primary hydroxy groups in melezitose molecule decreases in the following series: 6′ > 6 ≈ 6″ > 1′. On the other hand, secondary hydroxy groups in the molecule of the same trisaccharide are characterized by almost similar reactivities.

Scheme 3.



VIII



X

EXPERIMENTAL

All syntheses were carried out in pure anhydrous solvents. Thin-layer chromatography was performed on GF-254 plates (China) using the following solvent systems: A, benzene–acetone (7:1); B, diethyl ether–petroleum ether (6:1); C, diethyl ether–petroleum ether (3:1); D, ethyl acetate–diethyl ether (2:3); E, ethyl acetate–petroleum ether (2:1); F, ethyl acetate–petroleum ether (1:1.5); G, chloroform–methanol (2:1). The melting points were determined on a Yanaco MP-S3 heating device (Japan). The optical rotations were measured with a Perkin–Elmer-241 MC polarimeter. Elemental analyses were obtained with the aid of MT-3 and Perkin–Elmer 2400 automatic analyzers. The IR spectra were recorded on a Perkin–Elmer 325 spectrometer in KBr. The ^{1}H and ^{13}C NMR spectra were obtained on Bruker AX-400 (400 MHz for ^{1}H and 103.6 MHz for ^{13}C) and Bruker AC-80 instruments (80 MHz for ^{1}H and 20.1 MHz for ^{13}C) using $CDCl_3$ or D_2O as solvent and TMS as internal reference. The mass spectra were run on a VG-ZAB-HS mass spectrometer (scan range 2000–200 amu, scan rate 20 s, accelerating voltage 8 kV); bombardment by fast argon atoms; $HSCH_2CH(OH)CH_2OH$–NaCl–LiCl matrix.

Calculation of molecular models. The calculations were performed with the use of SGI Indigo Molecule-Pattern-Work-Station software package developed by Biosym. The potential energy function was approximated by the CVFF potential. The initial conformation was optimized by molecular mechanics using the fastest descend technique. A sample was balanced in succession at 300, 500, and 800 K during 10 ps. Every picosecond, one conformer was selected. Total of 100 conformers were selected, from which one having the minimal energy was chosen by the least-squares procedure (RMSD). It was tempered at lower temperature and optimized again by molecular

Fast atom bombardment mass spectra of melezitose derivatives **II**, **III**, **V**–**VIII**, **X**, and **XI**

Comp. no.	*m/z* (I_{rel}, %)
II	1366 $[M]^{+\cdot}$ (7), 1019 $[M-C_6H_7O_2(OAc)_4]^+$ (7), 242 (100)
III	1166 $[M]^{+\cdot}$ (9), 1089 $[M-C_6H_5]^+$ (35), 819 (7), 471 (7), 331 (100)
V	1589 $[M+Na]^+$ (0.6), 1573 $[M+Li]^+$ (1.1), 932 $[M-C_6H_7O_2(OAc)_3Tr-C_2H_5OAc]+$ (9), 387 (11), 242 (47.1)
VI	905 $[M+Na]^+$ (100), 889 $[M+Li]^+$ (100), 847 $[M+Li-CH_2CO]^+$ (28), 863 $[M+Na-CH_2CO]^+$ (25)
VII	905 $[M+Na]^+$ (100), 889 $[M+Li]^+$ (91), 847 $[M+Li-CH_2CO]^+$ (30), 863 $[M+Na-CH_2CO]^+$ (23), 289 (6), 229 (12)
VIII	947 $[M+Na]^+$ (5), 931 $[M+Li]^+$ (21), 331 (7), 289 (9), 229 (18), 169 (60)
X	947 $[M+Na]^+$ (20), 931 $[M+Li]^+$ (100), 625 $[619+Li-H]^+$ (7.7), 331 (27), 311 (11), 289 (18), 229 (35)
XI	769 $[M+Na]^+$ (10), 753 $[M+Li]^+$ (9), 243 $[Tr]^+$ (100), 77 $[C_6H_5]^+$ (20)

mechanics. In such a way, the most favorable conformer was obtained.

Isolation of melezitose (I) from the natural blend *Alhagi pseudolhagi Desv.* A 200-g portion of the raw material was kept in hot water (80–90°C) for 30 min, the mixture was filtered, alcohol was added to the filtrate until it became turbid, and the mixture was left to stand for 2–3 h. The sticky material was separated, and 95% ethanol was added to the solution until a solid precipitated. The precipitate was filtered off and recrystallized from aqueous ethanol, mp 159–160°C, $[\alpha]_D^{25}$ +86.1° (*c* = 1, H_2O).

6,6′-Di-*O*-triphenylmethyl-1′,2,2″,3,3″,4,4′,4″,6″-nona-*O*-acetylmelezitose (II) and 6′-*O*-triphenylmethyl-1′,2,2″,3,3″,4,4′,4″,6,6″-deca-*O*-acetylmelezitose (III). Anhydrous melezitose, 5 g (10 mmol), and triphenylmethyl chloride, 6 g (22 mmol), were dissolved in 125 ml of dry pyridine, the mixture was stirred for 18 h at 65°C and cooled to room temperature, 100 ml of acetic anhydride was added, and the mixture was stirred for 24 h at room temperature (~25°C). The mixture was then stirred with ice water containing 1% of HCl, the precipitate was filtered off and dissolved in 150 ml of $CHCl_3$, the solution was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure, and the residue was subjected to column chromatography on silica gel using diethyl ether–petroleum ether (1:1 and 6:1) as eluent. As a result, compound **II** was isolated as a colorless powder, and compound **III**, as a colorless syrup.

Compound **II**. Colorless crystals from $CHCl_3$–MeOH. Yield 1.3 g (9.6%); mp 178–180°C; R_f 0.54 (A), 0.39 (B), 0.25 (C); $[\alpha]_D^{25}$ +118° (*c* = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 1751 (C=O); 1648, 1490 (Ar). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 7.42–7.22 m (30H, H_{arom}), 5.85 d.d (1H, 1-H, $J_{1,2}$ = 3.78), 5.77 d.d (1H, 1″-H, $J_{1″,2″}$ = 4.09), 5.45 m (1H, 3″-H), 5.39 m (1H, 4′-H), 5.36 d.d (1H, 3-H, $J_{3,4}$ = 9.46), 5.20 d.d (1H, 4-H, $J_{4,5}$ = 8.29), 4.96 d.d (1H, 4″-H, $J_{4″,5″}$ = 4.49), 4.92 d.d (1H, 2″-H, $J_{2″,3″}$ = 4.25), 4.85 d.d (1H, 2-H, $J_{2,3}$ = 7.98), 4.49 d.d (1H, 3′-H, $J_{3′,4′}$ = 5.91), 4.33 d.d (1H, 5-H, $J_{5,6A}$ = 2.0, $J_{5,6B}$ = 4.60), 4.30 m (1H, 6″-H_B), 4.28 m (1H, 5″-H), 4.16 d.d (1H, 6-H_B, $J_{6A,6B}$ = 8.03), 4.02 m (1H, 6″-H_A), 4.00 m (1H, 5′-H), 3.95 d.d (1H, 6-H_A, $J_{6A,6B}$ = 8.03), 3.83 m (1H, 6′-H_B), 3.73 m (1H, 6′-H_A), 3.30 d.d (1H, 1′-H_B, $J_{1A′,1B′}$ = 8.04), 3.13 d.d (1H, 1′-H_A, $J_{1A′,1B′}$ = 8.04), 2.15–1.90 (27H, 9CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 170.9, 170.6, 170.4, 170.3, 170.1, 169.7, 169.5, 169.3, 169.1 (C=O); 143.6, 129.8, 129.1, 128.9, 128.7, 128.2, 127.9, 127.7, 127.5, 127.1 (C_{arom}); 101.8 ($C^{2′}$), 97.5 ($C^{1″}$), 92.8 (C^1), 88.4 ($C^{3′}$), 87.4 ($C^{5′}$), 76.6 ($C^{4′}$), 75.4 ($C^{2″}$), 74.1 (C^2), 73.9 ($C^{3″}$), 73.5 (C^3), 73.4 ($C^{4″}$), 72.6 (C^4), 72.3 ($C^{5″}$), 71.8 (C^5), 70.2 ($C^{6″}$), 64.1 ($C^{1′}$), 63.4 ($C^{6′}$), 62.4 (C^6), 20.7 (**C**H_3CO). Found, %: C 64.84; H 5.77. $C_{74}H_{78}O_{25}$. Calculated, %: C 64.98; H 5.75.

Compound **III**. Colorless syrup. Yield 3.1 g (26.8%); R_f 0.28 (A), 0.17 (B); $[\alpha]_D^{25}$ +22.6°C (*c* = 1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 1754 (C=O), 1493 (Ar). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 7.43–7.22 m (15H, H_{arom}), 5.84 d.d (1H, 1-H, $J_{1,2}$ = 3.93), 5.79 d.d (1H, 1″-H, $J_{1″,2″}$ = 3.76), 5.58 m (1H, 4′-H), 5.38 m (1H, 3-H), 5.34 m (1H, 3″-H), 5.19 m (1H, 4″-H), 5.16 m (1H, 4-H), 4.96 t (1H, 2″-H, $J_{2″,3″}$ = 4.56), 4.92 t (1H, 2-H, $J_{2,3}$ = 3.96), 4.49 d.d (1H, 3′-H, $J_{3′,4′}$ = 6.81), 4.37 m (2H, 5-H, 5″-H), 4.29 m (2H, 6-H_B, 6″-H_B), 4.09 m (1H, 5′-H), 3.99 m (1H, 6′-H_B), 3.95 m (2H, 6-H_A, 6″-H_A), 3.75 m (1H, 6′-H_A), 3.55 d.d (1H, 1′-H_B, $J_{1A′,1B′}$ = 7.72), 3.34 d.d (1H, 1′-H_A, $J_{1A′,1B′}$ = 7.72), 2.13–1.92 (30H, 10CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 170.7, 170.5, 170.3, 169.9, 169.6, 169.4, 169.2 (C=O); 143.8, 129.9, 129.3, 128.9, 128.6, 128.4, 127.9, 127.7, 127.6, 127.3 (C_{arom}); 101.7 ($C^{2′}$), 97.6 ($C^{1″}$), 92.9 (C^1), 88.4 ($C^{3′}$), 87.5 ($C^{5′}$), 76.7 ($C^{4′}$), 75.6 ($C^{2″}$), 74.2 (C^2), 73.9 ($C^{3″}$), 73.7 (C^3), 73.5 ($C^{4″}$), 72.8 (C^4), 72.6 ($C^{5″}$), 71.9 (C^5), 70.1 ($C^{6″}$), 64.1 ($C^{1′}$), 62.9 ($C^{6′}$), 61.3 (C^6), 20.6 (**C**H_3CO). Found, %: C 58.38; H 5.69. $C_{57}H_{66}O_{26}$. Calculated, %: C 58.64; H 5.70.

6-*O*-Triphenylmethyl-1′,2,2″,3,3″,4,4′,4″,6′,6″-deca-*O*-acetylmelezitose (IV) and 6,6′,6″-tri-*O*-triphenylmethyl-1′,2,2″,3,3″,4,4′,4″-octa-*O*-acetylmelezitose (V). Following an analogous procedure, compounds **IV** and **V** were obtained from 5 g (10 mmol) of anhydrous melezitose and 13 g (44 mmol) of triphenylmethyl chloride.

Compound **IV**. Colorless syrup. Yield 1.9 g (16.4%); R_f 0.27 (A), 0.16 (B). IR spectrum, ν, cm^{-1}: 1753 (C=O), 1498 (Ar). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 7.40–7.25 m (15H, H_{arom}), 5.80 d.d (1H, 1-H, $J_{1,2}$ = 3.89), 5.78 d.d (1H, 1″-H, $J_{1″,2″}$ = 3.76), 5.56 d.d (1H, 4′-H, $J_{4′,5′}$ = 7.21), 5.35 d.d (1H, 3-H, $J_{3,4}$ = 9.18), 5.31 m (1H, 3″-H), 5.15 d.d (1H, 4″-H, $J_{3″,4″}$ = 3.57), 5.12 d.d (1H, 4-H, $J_{3,4}$ = $J_{4,5}$ = 9.18), 4.95 d.d (1H, 2″-H, $J_{2″,3″}$ = 3.24), 4.91 d.d (1H, 2-H, $J_{2,3}$ = 3.78), 4.49 d.d (1H, 3″-H, $J_{3″,4″}$ = 6.78), 4.38 m (1H, 5″-H), 4.29 m (1H, 5′-H), 4.27 m (1H, 6″-H_B), 4.24 m (1H, 5-H), 4.17 m (1H, 6′-H_B), 4.00 m (1H, 6″-H_A), 3.97 m (1H, 6′-H_A), 3.77 m (1H, 6-H_B), 3.65 m (1H, 6-H_A), 3.57 d.d (1H, 1′-H_B, $J_{1A′,1B′}$ =

8.04), 3.33 d.d (1H, 1′-H_A, $J_{1A',1B'}$ = 8.04), 2.15–1.97 (30H, 10 CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 170.6, 170.4, 170.2, 169.8, 169.6, 169.3, 169.1 (C=O); 143.8, 129.9, 129.3, 128.9, 128.7, 128.5, 127.9, 127.8, 127.5, 127.2 (C_{arom}); 102.3 ($C^{2'}$), 98.1 ($C^{1''}$), 92.9 (C^1), 88.1 ($C^{3'}$), 87.3 ($C^{5'}$), 76.6 ($C^{4'}$), 75.4 ($C^{2''}$), 74.1 (C^2), 73.8 ($C^{3''}$), 73.6 (C^3), 73.3 ($C^{4''}$), 72.6 (C^4), 72.4 ($C^{5''}$), 71.8 (C^5), 70.1 ($C^{6''}$), 64.3 ($C^{1'}$), 62.8 ($C^{6'}$), 62.5 (C^6), 20.8 (**C**H_3CO). Found, %: C 58.47; H 5.57. $C_{57}H_{66}O_{26}$. Calculated, %: C 58.64; H 5.70.

Compound **V**. Colorless syrup. Yield 5.3 g (34.2%); R_f 0.65 (A); $[\alpha]_D^{25}$ +91.3°C (c = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 1745 (C=O); 1600, 1490 (Ar). ^1H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 7.43–7.20 m (45H, H_{arom}), 5.66 d.d (1H, 1-H, $J_{1,2}$ = 3.67), 5.65 d.d (1H, 1″-H, $J_{1'',2''}$ = 3.62), 5.41 d.d (1H, 3′-H, $J_{3',4'}$ = 5.94), 5.38 m (2H, 3-H, 3″-H), 4.99 m (2H, 4-H, 4″-H), 4.86 m (2H, 2-H, 2″-H), 4.51 d.d (1H, 4′-H, $J_{4',5'}$ = 6.34), 4.29 m (2H, 5-H, 5″-H), 4.01 m (1H, 6′-H_B), 3.86 m (4H, 6-H, 6″-H), 3.82 m (1H, 5′-H), 3.74 m (1H, 6′-H_A), 3.32 d.d (1H, 1′-H_B, $J_{1A',1B'}$ = 8.04), 3.15 d.d (1H, 1′-H_A, $J_{1A',1B'}$ = 8.04), 2.15–1.90 (24H, 8 CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 171.1, 170.9, 170.6, 170.3, 170.2, 169.8, 169.6, 169.2, 168.9 (C=O); 143.7, 129.9, 129.2, 128.9, 128.8, 128.3, 127.9, 127.7, 127.3, 126.7 (C_{arom}); 101.9 ($C^{2'}$), 95.6 ($C^{1''}$), 92.8 (C^1), 89.0 ($C^{3'}$), 87.1 ($C^{5'}$), 76.4 ($C^{4'}$), 75.3 ($C^{2''}$), 74.1 (C^2), 73.8 ($C^{3''}$), 73.5 (C^3), 73.3 ($C^{4''}$), 72.4 (C^4), 72.1 ($C^{5''}$), 71.5 (C^5), 70.1 ($C^{1'}$), 63.4 ($C^{6''}$), 63.0 ($C^{6'}$), 62.2 (C^6), 20.7 (**C**H_3CO). Found, %: C 70.50; H 6.02. $C_{91}H_{90}O_{24}$. Calculated, %: C 69.72; H 5.79.

1′,2,2″,3,3″,4,4′,4″,6″-Nona-*O*-acetylmelezitose (VI). Compound **II**, 4.5 g (3.3 mmol), was dissolved in 40 ml of methylene chloride, 40 ml of glacial acetic acid and 0.8 ml of concentrated hydrochloric acid were added to the solution, and the mixture was stirred for 1.5 h on cooling with ice water. The progress of the reaction was monitored by TLC (solvent system D). The mixture was neutralized with a saturated solution of $NaHCO_3$, washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to column chromatography on silica gel using ethyl acetate–petroleum ether (1 : 1 and 2 : 1) to isolate compound **VI** as a colorless syrupy substance. Yield 1.9 g (73%); R_f 0.29 (D), 0.11 (E); $[\alpha]_D^{25}$ +101° (c = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 3524 (6,6′-OH), 1743 (C=O). ^1H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 5.76 d.d (1H, 1-H, $J_{1,2}$ = 5.68), 5.67 d.d (1H, 1″-H, $J_{1'',2''}$ = 3.34), 5.57 d.d (1H, 3″-H, $J_{3'',4''}$ = 12.7), 5.39 d.d (1H, 4′-H, $J_{4',5'}$ = 10.35), 5.37 m (1H, 3-H), 5.29 d.d (1H, 4-H, $J_{4,5}$ = 8.36), 4.98 d.d (1H, 4″-H, $J_{4'',5''}$ = 5.34), 4.75 d.d (1H, 2″-H, $J_{2'',3''}$ = 5.68), 4.45 d.d (1H, 2-H, $J_{2,3}$ = 4.10), 4.49 d.d (1H, 3′-H, $J_{3',4'}$ = 8.21), 4.34 m (1H, 5-H), 4.31 m (1H, 6″-H_B), 4.29 m (1H, 5″-H), 4.09 m (1H, 6″-H_A), 4.06 d.d (1H, 6-H_B, $J_{6A,6B}$ = 10.0), 3.91 m (1H, 5′-H), 3.82 m (1H, 6′-H_B), 3.78 d.d (1H, 6-H_A, $J_{6A,6B}$ = 10.0), 3.71 m (1H, 6′-H_A), 3.65 d.d (1H, 1′-H_B, $J_{1A',1B'}$ = 6.01), 3.61 d.d (1H, 1′-H_A, $J_{1A',1B'}$ = 6.01), 2.15–1.90 (27H, 9 CH_3CO). Found, %: C 49.46; H 5.70. $C_{36}H_{50}O_{25}$. Calculated, %: C 48.96; H 5.71.

1′,2,2″,3,3″,4″,6,6′,6″-Nona-*O*-acetylmelezitose (VII). Compound **VI**, 0.5 g (0.6 mmol), was dissolved in 10 ml of benzene, and 3 ml of glacial acetic acid was added to the solution. The mixture was heated to the boiling point on an oil bath and was stirred for 16 h. The progress of the reaction was monitored by TLC using solvent system D. When the reaction was complete, the mixture was evaporated under reduced pressure, and the residue was subjected to preparative thin-layer chromatography on silica gel using solvent system D. Product **VII** was isolated as a colorless syrupy substance. Yield 0.38 g (76%); R_f 0.41 (D), 0.19 (E); $[\alpha]_D^{25}$ +71° (c = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 3422 (4,4′-OH), 1750.6 (C=O). ^1H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 5.74 d.d (1H, 1-H, $J_{1,2}$ = 4.58), 5.61 d.d (1H, 1″-H, $J_{1'',2''}$ = 3.32), 5.47 m (1H, 3″-H), 5.36 m (1H, 3-H), 4.96 d.d (1H, 4″-H, $J_{4'',5''}$ = 4.49), 4.92 d.d (1H, 2″-H, $J_{2'',3''}$ = 5.25), 4.85 d.d (1H, 2-H, $J_{2,3}$ = 3.78), 4.52 d.d (1H, 3′-H, $J_{3',4'}$ = 5.91), 4.40 m (1H, 4′-H), 4.32 m (1H, 5-H), 4.30 m (1H, 6″-H_B), 4.28 m (1H, 5″-H), 4.21 m (1H, 6-H_B), 4.05 m (1H, 6′-H_B), 4.02 m (1H, 6″-H_A), 4.00 m (1H, 6-H_A), 3.96 m (1H, 6′-H_A), 3.83 m (1H, 5′-H), 3.58 m (1H, 4-H), 3.33 d.d (1H, 1′-H_B, $J_{1A',1B'}$ = 6.50), 3.18 d.d (1H, 1′-H_A, $J_{1A',1B'}$ = 6.50), 2.32–2.02 (27H, 9 CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 170.9, 170.6, 170.4, 170.2, 170.1, 169.6, 169.3, 169.1, 168.9 (C=O); 102.3 ($C^{2'}$), 97.3 ($C^{1''}$), 92.8 (C^1), 89.9 ($C^{3'}$), 82.3 ($C^{5'}$), 76.2 ($C^{4'}$), 75.3 ($C^{2''}$), 74.1 (C^2), 73.6 ($C^{3''}$), 73.4 (C^3), 73.2 ($C^{4''}$), 72.4 (C^4), 72.2 ($C^{5''}$), 71.7 (C^5), 70.0 ($C^{6''}$), 63.9 ($C^{1'}$), 62.3 ($C^{6'}$), 61.2 (C^6), 20.6 (**C**H_3CO). Found, %: C 49.80; H 5.70. $C_{36}H_{50}O_{25}$. Calculated, %: C 48.96; H 5.71.

1′,2,2″,3,3″,4,4″,6,6′,6″-Deca-*O*-acetylmelezitose (VIII). Following the procedure described above for the synthesis of compound **VI**, from 5 g (4.3 mmol) of compound **III** we obtained 2.8 g of a mixture of products **VIII** and **IX** [**IX**: R_f 0.49 (D), 0.17 (F)]. A 0.5-g portion of mixture **VIII**/**IX** was dissolved

in 10 ml of benzene, 3 ml of glacial acetic acid was added, and the mixture was heated to the boiling point and was stirred for 36 h under reflux (TLC). The mixture was evaporated under reduced pressure, and the residue was separated by thin-layer chromatography on silica gel using solvent system D. Product **VIII** was isolated as a colorless syrupy substance. Yield 0.34 g (68%); R_f 0.57 (D), 0.25 (F); $[\alpha]_D^{25}$ +106° (c = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 3528 (OH), 1751 (C=O). ^1H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 5.71 d.d (1H, 1-H, $J_{1,2}$ = 3.55), 5.63 d.d (1H, 1″-H, $J_{1'',2''}$ = 3.80), 5.57 m (1H, 3″-H), 5.39 d.d (1H, 3-H, $J_{3,4}$ = 7.48), 5.28 m (1H, 4-H), 5.26 m (1H, 4″-H), 4.98 m (1H, 2″-H), 4.94 m (1H, 2-H), 4.74 d.d (1H, 3′-H, $J_{3',4'}$ = 3.87), 4.43 m (1H, 5-H), 4.41 m (1H, 6″-H_B), 4.40 m (1H, 4′-H), 4.37 m (1H, 5″-H), 4.34 m (1H, 6″-H_A), 4.28 m (1H, 6-H_B), 4.05 m (1H, 5′-H), 4.11 d.d (1H, 6′-H_B, $J_{6A',6B'}$ = 11.87), 4.04 m (1H, 6-H_A), 3.92 d.d (1H, 6′-H_A, $J_{6A',6B'}$ = 11.87), 3.57 m (1H, 1′-H_B), 3.43 m (1′-H_A), 2.13–1.92 (30H, 10CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 171.9, 171.5, 170.7, 170.2, 170.1, 169.9, 169.7 (C=O); 102.4 ($C^{2'}$), 97.8 ($C^{1''}$), 93.0 (C^1), 88.5 ($C^{3'}$), 87.7 ($C^{5'}$), 75.7 ($C^{2''}$), 74.6 (C^2), 74.3 ($C^{4'}$), 73.9 ($C^{3''}$), 73.7 (C^3), 73.5 ($C^{4''}$), 72.8 (C^4), 72.6 ($C^{5''}$), 71.9 (C^5), 70.5 ($C^{6''}$), 64.2 ($C^{1'}$), 63.0 ($C^{6'}$), 61.5 (C^6), 20.6 ($\mathbf{C}H_3CO$). Found, %: C 49.13; H 5.62. $C_{38}H_{52}O_{26}$. Calculated, %: C 49.34; H 5.67.

1′,4′,2,2″,3,3″,4,4″,6′,6″-Deca-*O*-acetylmelezitose (X) was synthesized in a similar way from compound **IV**. Colorless syrupy substance. Yield 1.8 g (69%); R_f 0.41 (D); $[\alpha]_D^{25}$ +134° (c = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 3450 (OH), 1735 (C=O). ^1H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 5.81 d.d (1H, 1-H, $J_{1,2}$ = 3.89), 5.79 d.d (1H, 1″-H, $J_{1'',2''}$ = 3.78), 5.56 d.d (1H, 4′-H, $J_{4',5'}$ = 7.17), 5.33 m (1H, 3-H), 5.30 m (1H, 3″-H), 5.16 d.d (1H, 4″-H, $J_{4'',5''}$ = 3.57), 5.11 d.d (1H, 4-H, $J_{4,5}$ = 9.18), 4.94 d.d (1H, 2″-H, $J_{2'',3''}$ = 3.24), 4.90 d.d (1H, 2-H, $J_{2,3}$ = 3.78), 4.49 d.d (1H, 3′-H, $J_{3',4'}$ = 6.12), 4.39 m (1H, 5″-H), 4.28 m (1H, 5′-H), 4.26 m (1H, 6″-H_B), 4.24 m (1H, 5-H), 4.18 m (1H, 6′-H_B), 4.08 m (1H, 6-H_B), 3.99 m (1H, 6″-H_A), 3.97 m (1H, 6′-H_A), 3.76 m (1H, 6-H_A), 3.60 d.d (1H, 1′-H_B, $J_{1A',1B'}$ = 9.82), 3.36 d.d (1H, 1′-H_A, $J_{1A',1B'}$ = 9.82), 2.13–2.04 (30H, 10CH_3CO). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 171.8, 171.5, 170.6, 170.2, 170.1, 169.8, 169.4 (C=O); 101.1 ($C^{2'}$), 97.4 ($C^{1''}$), 92.6 (C^1), 88.1 ($C^{3'}$), 87.4 ($C^{5'}$), 75.4 ($C^{2''}$), 74.4 (C^2), 74.2 ($C^{4'}$), 73.7 ($C^{3''}$), 73.5 (C^3), 73.3 ($C^{4''}$), 72.6 (C^4), 72.3 ($C^{5''}$), 71.5 (C^5), 70.2 ($C^{6''}$), 64.0 ($C^{1'}$), 63.1 ($C^{6'}$), 61.0 (C^6), 20.7 ($\mathbf{C}H_3CO$). Found, %: C 49.55; H 5.65. $C_{38}H_{52}O_{26}$. Calculated, %: C 49.34; H 5.67.

6′-*O*-Triphenylmethylmelezitose (XI). Metallic sodium was added to 15 ml of anhydrous methanol to attain pH 9, and 0.5 g of compound **III** was added. The mixture was stirred for 2 h at room temperature, neutralized with glacial acetic acid, and evaporated under reduced pressure, and the residue was subjected to column chromatography on silica gel using chloroform–methanol (4 : 1) as eluent. Product **XI** was isolated as a colorless syrupy substance. Yield 0.26 g (82%); R_f 0.25 (G), 0.36 (F); $[\alpha]_D^{25}$ +65° (c = 0.1, H_2O). IR spectrum, ν, cm^{-1}: 3425 (OH); 1633, 1491 (Ar). ^1H NMR spectrum (D_2O), δ, ppm (J, Hz): 5.45 d.d (1H, 1-H, $J_{1,2}$ = 3.80), 5.18 d.d (1H, 1″-H, $J_{1'',2''}$ = 3.80), 4.32 d.d (1H, 3′-H, $J_{3',4'}$ = 7.60), 4.30 d.d (1H, 4′-H, $J_{3',4'}$ = 7.60, $J_{4',5'}$ = 8.0), 3.94 d.d (1H, 5-H, $J_{5,6A}$ = 2.20, $J_{5,6B}$ = 4.80), 3.93 d.d (1H, 5″-H, $J_{5'',6A''}$ = 2.20, $J_{5'',6B''}$ = 4.80), 3.91 d.d (1H, 5′-H, $J_{4',5'}$ = 8.0), 3.90 d.d, (1H, 6-H_B, $J_{5,6B}$ = 4.80), 3.86 d.d (1H, 6″-H_B, $J_{5'',6B''}$ = 4.80), 3.81 d.d (1H, 1′-H_B, $J_{1A',1B'}$ = 12.0), 3.79 d.d (1H, 6″-H_A, $J_{6A'',6B''}$ = 12.2), 3.78 d.d (1H, 6-H_A, $J_{6A,6B}$ = 12.2), 3.75 d.d (1H, 3″-H, $J_{2'',3''}$ = 10.0, $J_{3'',4''}$ = 9.0), 3.67 d.d (1H, 3-H, $J_{2,3}$ = 10.0, $J_{3,4}$ = 8.80), 3.65 d.d (1H, 1′-H_A, $J_{1A',1B'}$ = 12.0), 3.58 d.d (1H, 2″-H, $J_{1'',2''}$ = 3.80, $J_{2'',3''}$ = 10.0), 3.56 d.d (1H, 2-H, $J_{1,2}$ = 3.80, $J_{2,3}$ = 10.0), 3.53 m (2H, 6′-H_A, 6′-H_B), 3.45 d.d (1H, 4″-H, $J_{3'',4''}$ = 9.0, $J_{4'',5''}$ = 10.2), 3.44 d.d (1H, 4-H, $J_{3,4}$ = 8.80, $J_{4,5}$ = 10.2). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 104.8 ($C^{2'}$), 101.0 ($C^{1''}$), 92.7 (C^1), 84.3 ($C^{3'}$), 78.4 ($C^{5'}$), 74.6 ($C^{4'}$), 74.1 ($C^{2''}$), 73.8 (C^2), 73.2 ($C^{3''}$), 72.5 (C^3), 71.9 ($C^{4''}$), 70.6 (C^4), 70.3 ($C^{5''}$), 70.0 (C^5), 63.2 ($C^{6''}$), 62.4 ($C^{1'}$), 61.4 (C^6), 60.4 ($C^{6'}$). Found, %: C 59.48; H 6.22. $C_{37}H_{46}O_{16}$. Calculated, %: C 59.51; H 6.21.

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