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Synthesis of Thioglycoside Analogues of Maradolipid

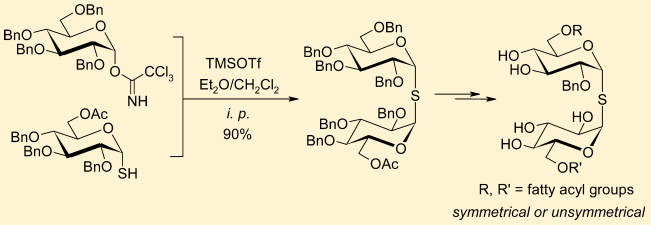
Xiaojun Zeng,[†] Raymond Smith,[‡] and Xiangming Zhu*[,†,‡]

[†] College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua 321004, China

[‡] Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland

Supporting Information

ABSTRACT: We describe here the first synthesis of thioglycoside analogues of maradolipid, based on a new procedure for the synthesis of 1-thiotrehalose developed recently in our laboratories. The challenging α,α-(1→1′) thioglycosidic linkage was constructed by Schmidt's inverse procedure in very high yield and excellent stereoselectivity. Subsequent protecting group manipulation and coupling with different fatty acids led smoothly to a group of symmetrical and unsymmetrical thiomaradolipids which would be of high value for biological studies.



There has been considerable interest in trehalose-containing glycolipids[1] since trehalose dimycolates were found to be associated with the virulence of pathogenic mycobacteria. A large number of structurally different trehalose glycolipids were isolated from bacteria in the past few decades and have been shown to have various biological activities including antitumor activity, in vivo macrophage activation, and other immunostimulatory activity. An excellent review has been published describing the synthesis and biological activities of various trehalose glycolipids.[2] Very recently, a novel class of diacyltrehaloses named maradolipids, which are similar to trehalose dimycolate in structure, were also isolated from the dauer larvae of *Caenorhabditis elegans* and represent the first group of diacyltrehaloses found in animals.[3] These glycolipids are composed of a mixture of at least 60 derivatives of trehalose, which differ only in the two fatty acid chains at the 6- and 6′-positions. The fatty acid chains can be identical; i.e., some are symmetrical maradolipids. But there are also many unsymmetrical ones in which the two fatty acids are different.[3] The major component was characterized as 6-*O*-(13-methylmyristoyl)-6′-*O*-oleoyltrehalose **1** (Figure 1), which amounts to 7−8% of the total maradolipids. Maradolipids are very likely involved in protecting dauer larvae against unfavorable environmental conditions, as the similar trehalose dimycolates and trehalose itself are known to confer on bacteria the ability to survive harsh conditions and reanimate subsequently by imparting stability to the lipid layer of the cell wall.[4] They may also possess other important immunological activities.[3]

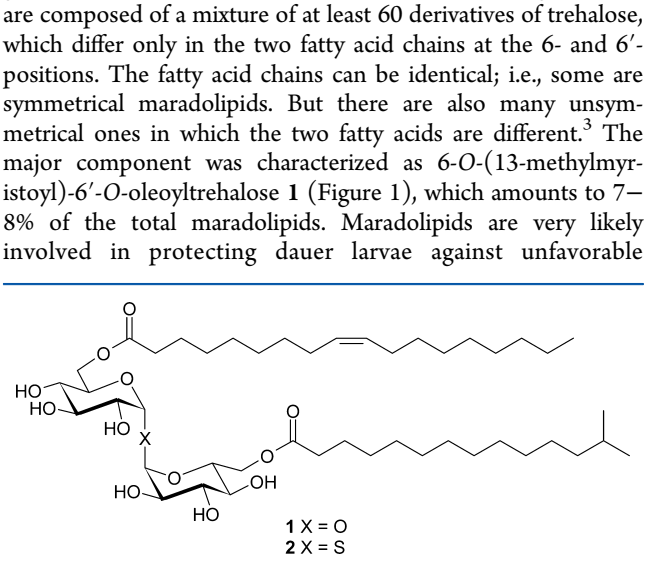


Figure 1. Structures of maradolipid **1** and *S*-maradolipid **2**.

In order to contribute to the unravelling of the biological function of maradolipids, several maradolipids containing sulfur in the glycosidic linkage were synthesized in this report as stable analogues. *S*-Glycosides are very attractive substitutes for *O*-glycosides, as it is well-known that they are much less susceptible to enzymatic cleavage as well as chemical degradation.[5] Also, they often exhibit similar solution conformation and similar or even more potent bioactivities compared to the corresponding *O*-glycosides. For example, an *S*-linked glycopeptide mimic of tyrocidine displayed a greater inhibitory activity against *B. subtilis* than the natural antibiotic.[6] Therefore, synthesis of thioglycosides has been actively pursued in many laboratories.[7] Recently we achieved the first total synthesis of the thioglycoside analogue of α-galactosylceramide KRN7000 by using a nonconventional approach,[8] and the subsequent bioassay demonstrated that this thioglycoside possessed similar potency to KRN7000 in human NKT cell activation.[9] In view of the biological importance of trehalose glycolipids,[2] investigations toward the synthesis of thioglycosidic trehalose glycolipids that may prove useful in biological studies and as potential therapeutic agents were also initiated. We wish to report here the first synthesis of thioglycoside analogues of maradolipid.[10]

Conceivably, thiomaradolipids could be assembled in two ways. One is to synthesize 1-thiotrehalose first and then introduce the fatty acid chains onto the 6- and 6′-positions, the other is a more convergent strategy in which 6-*O*-fatty acylated

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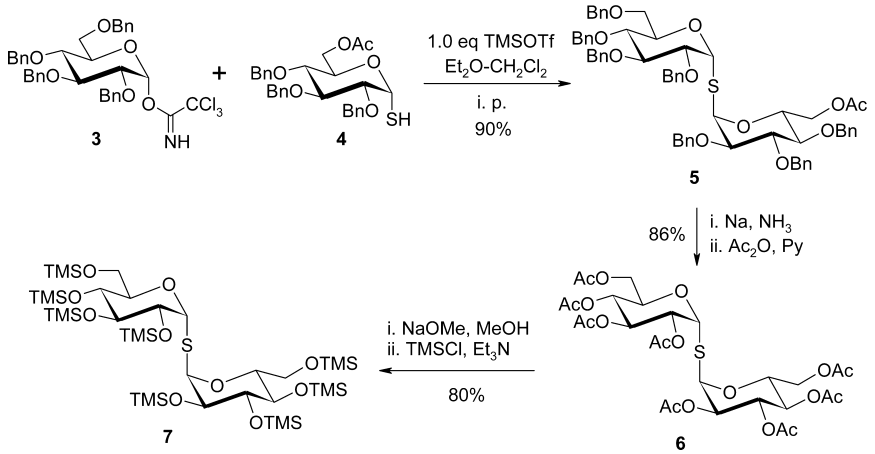
Scheme 1. Synthesis of 1-Thiotrehalose 7



sugar building blocks are preprepared and then used to build up the challenging α,α-(1→1′) thioglycosidic linkage. Apparently, the former strategy offers some advantage, as it could allow a rapid means of forming thiomaradolipid libraries through the simple attachment of different fatty acid chains. As such, this strategy was applied to our synthesis to provide more valuable thioglycosides for biological studies. Hence, 1-thiotrehalose was first synthesized based on our previous work;[11] i.e., α-thiosugar **4**[11] was glycosylated with trichloroacetimidate **3**[12] in the presence of TMSOTf to form the fully protected α,α-thiotrehalose **5** (Scheme 1). We noted previously that 1.0 equiv of TMSOTf was required for this glycosidation reaction due to the low reactivity of the α-anomeric thiol.[11] The relatively large amounts of TMSOTf very likely react with the thiol to generate *in situ* the glucosyl silyl sulfide which possessed higher reactivity than the thiol, thereby facilitating the reaction. Based on this speculation, we thought that Schmidt's inverse procedure[13] could possibly further increase the reaction yield because by the inverse procedure the thiol would first be mixed with TMSOTf prior to the coupling. Indeed, following the inverse procedure **5** was produced in a much higher yield (90%), as shown in Scheme 1. Here it should be mentioned that unlike the normal sugar hemiacetals, which could mutarotate easily under most conditions, glycosyl thiols are quite stable in terms of configuration, and their mutarotation does not occur readily.[14] The α-configuration of thiosugar **4** could thus be well maintained during the glycosidation reaction, which, together with the high α-stereoselectivity of the reaction, ensured the α,α-glycosidic linkage. The anomeric configurations of **5** were confirmed by the X-ray diffraction analysis of compound **7** (vide infra).

Subsequently, **5** was converted into the fully acetylated 1-thiotrehalose **6** as described previously in 86% overall yield.[11] To access the target glycolipids, the 6- and 6′-positions of compound **6** must be selectively exposed for the introduction of the fatty acid chains. Considering that the TMS group had already been used successfully for this purpose in the synthesis of maradolipid **1**,[10a] acetyl groups were thus replaced with TMS groups, as shown in Scheme 1, under the normal deacetylation and silylation conditions, to provide the TMS-protected 1-thiotrehalose **7** in 80% overall yield. Suitable crystals of **7** were obtained for X-ray analysis by slow crystallization from methanol at −15 °C. The crystallographic data clearly show the constructed α,α-(1→1′) glycosidic linkage (Figure 2).

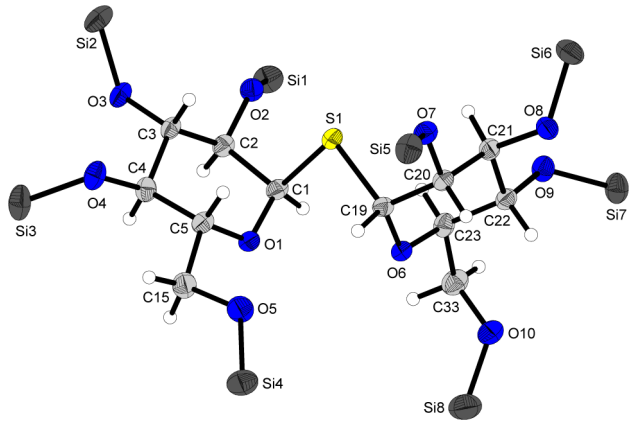


Figure 2. X-ray crystal structure of **7**; thermal ellipsoids are drawn on the 15% probability level, and TMS groups are represented by the Si atoms only.

Next, **7** was treated with K_2CO_3 in methanol and dichloromethane following Kulkarni's procedure[10a] in order to selectively remove the more labile primary TMS groups, but unfortunately the reaction did not work well leading to the formation of a mixture of several products. Some attempts to optimize the yield of the desired product, including changes in solvent ratio, the amount of K_2CO_3, and reaction temperature, were unsuccessful. Apparently, the anomeric sulfur impairs the chemoselective alkaline hydrolysis. We then turned to acidic hydrolysis, which was also known in the literature for selective removal of the TMS group on carbohydrate substrates.[15] Hence, compound **7** was subjected to acidic conditions using 50 equiv of HOAc in acetone and methanol solvent mixture, as expected, the desired product **8** was produced in a very high yield (Scheme 2). Coupling between the alcohol and fatty acids was subsequently conducted in the presence of DCC and DMAP in dichloromethane. As a test, commercially available palmitic acid was first chosen as the fatty acid and coupled with **8** under the action of DCC to furnish the maradolipid analogue **9** in 87% yield. Similarly, coupling of **8** with eicosanoic acid and docosanoic acid under the same conditions also proceeded smoothly to give the corresponding glycolipids **10** and **11** in 84% and 45% yields, respectively. Biologically more relevant fatty acids, oleic acid and 13-methylmyristic acid,[16] were also introduced onto the 6,6′-positions of thiotrehalose **8** by the same procedure to give rise to the desired maradolipid

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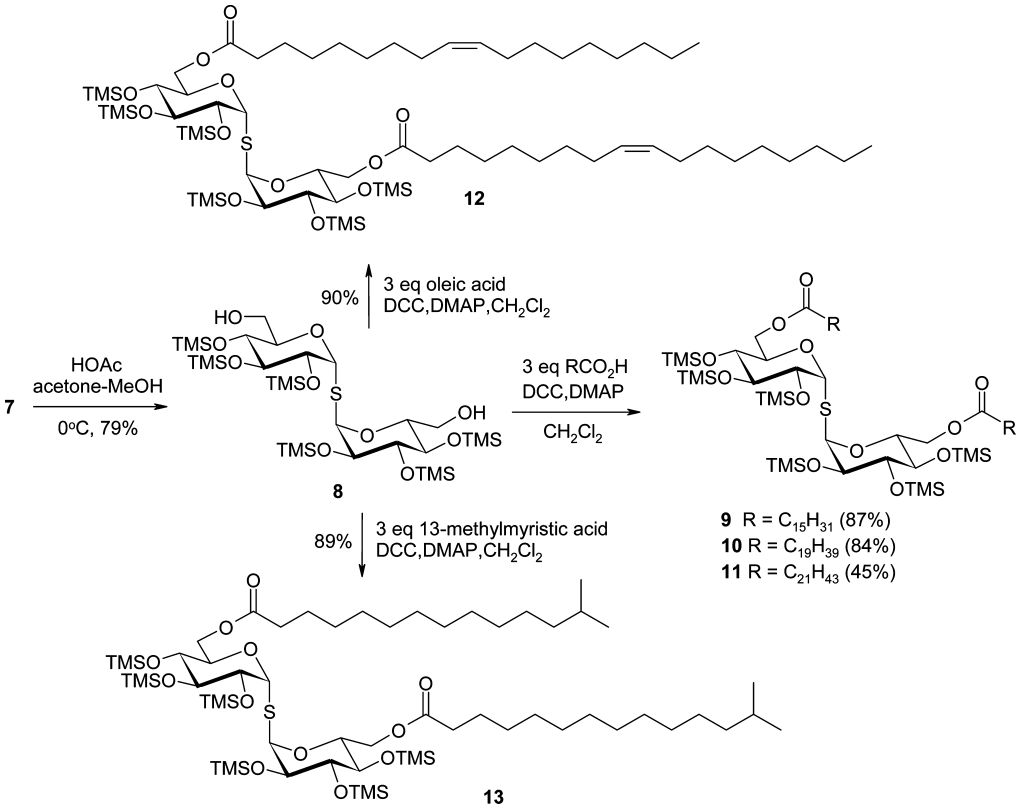
Scheme 2. Synthesis of *S*-Maradolipids 9−13



analogues **12** and **13** in 90% and 89% yields, respectively. In all these coupling reactions, an excess of fatty acids (3 equiv) was used to ensure the acylation of both 6,6′-positions to form the symmetrical thiomaradolipids.

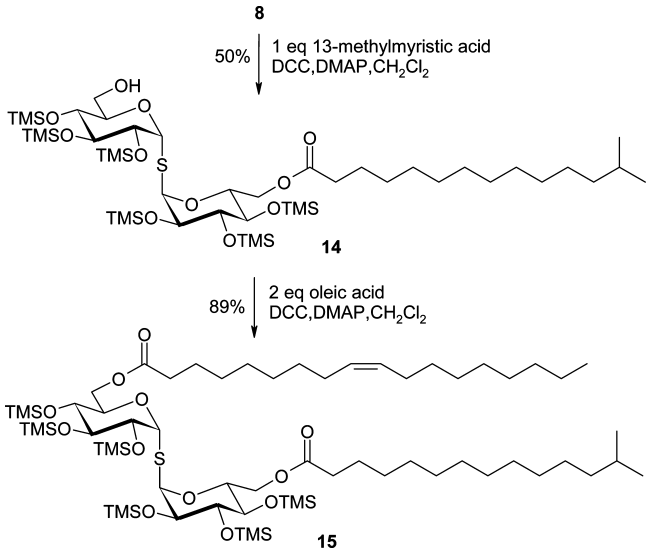
Here it is worth noting that these smooth couplings, together with the above-mentioned highly stereoselective procedure for the synthesis of 1-thiotrehalose, provided a convenient access to various symmetrical thiomaradolipids, which can be used for biological evaluation upon deprotection.

Our attention then turned toward applying the DCC coupling to the synthesis of unsymmetrical thiomaradolipids. Following Kulkarni's procedure,[10a] **8** was first treated with 1.0 equiv of 13-methylmyristic acid[16] in the presence of DCC and DMAP to afford the desired monoacylated intermediate **14** in a satisfactory yield of 50%, which was then further acylated with oleic acid under the same conditions to give the unsymmetrical thiomaradolipid **15** in very high yield (Scheme 3).

Finally, compounds **9**−**13** and **15** were all treated with acidic ion-exchange resin Amberlite IR120 in methanol to furnish the corresponding desired target molecules **16**−**20** and **2** in very high yields (80−93%), as shown in Scheme 4. The synthetic samples were purified by flash chromatography on silica gel (eluant: CH_2Cl_2/MeOH 10:1) and characterized by NMR and HR-ESIMS.

In summary, as an extension of our previous project,[8,17] in this report, a series of 1-thiotrehalose-derived glycolipids including unsymmetrical thiomaradolipid **2** were synthesized as catabolically stable analogues of maradolipid. In view of the important bioactivities of maradolipids, these compounds are of particular interest for studying their biological properties. In addition, a great advantage of the synthesis is that the key α,α-(1→1′) thioglycosidic linkage was constructed in a high-yielding and highly stereoselective manner. The synthetic

Scheme 3. Synthesis of *S*-Maradolipid 15



strategy presented here could also be applied to the preparation of other thioglycoside analogues of trehalose glycolipids. Work is in progress to test the immunological bioactivities of the synthesized glycolipids.

EXPERIMENTAL SECTION

General Remarks. Reactions were performed in oven-dried glassware. Solvents were evaporated under reduced pressure while maintaining the water bath temperature below 40 °C. All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} and the compounds visualized by UV or by treatment with 8% H_2SO_4 in methanol followed by heating. Flash chromatography was

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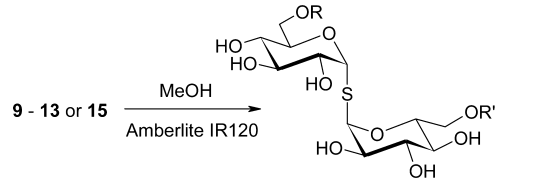
Scheme 4. Synthesis of *S*-Maradolipids 16−20 and 2



16 R = R' = Palmitoyl group (89%)
17 R = R' = Eicosanoyl group (82%)
18 R = R' = Docosanoyl group (80%)
19 R = R' = Oleoyl group (92%)
20 R = R' = 13-Methylmyristyl group (93%)
2 R = Oleoyl; R' = 13-Methylmyristyl (90%)

performed with the appropriate solvent system using 160−200 mesh silica. Optical rotations were measured at 20 °C. ^{1}H NMR spectra were obtained on a 400 or 600 MHz and reported in parts per million (δ) relative to the response of the solvent or to tetramethylsilane (0.00 ppm). Coupling constants (J) are reported in hertz (Hz). ^{13}C NMR spectra were recorded at 100 or 150 MHz and reported in δ relative to the response of the solvent. The HRMS data were recorded on an LC-time-of-flight mass spectrometer. Yields refer to chromatographically pure compounds and are calculated based on reagents consumed.

6-*O*-Acetyl-2,3,4,2′,3′,4′,6′-hepta-*O*-benzyl-1-thio-α,α-D-trehalose (5). To a stirred suspension of thiol **4** (1.62 g, 3.2 mmol) and 4 Å molecular sieves (800 mg) in dry ether (15 mL) and CH_2Cl_2 (3 mL) was added TMSOTf (580 μL, 3.2 mmol) at −78 °C under N_2. After stirring for 30 min at the low temperature, trichloroacetimidate **3** (2.40 g, 3.5 mmol) was added to the mixture. The reaction was stirred at the low temperature for 1 h and then quenched with Et_3N and filtered. The filtrates were concentrated in vacuo to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 5:1) to give the product **5** (2.96 g, 90%) as a colorless syrup: $[\alpha]_D$ +124.0 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.17 (m, 35H), 5.66 (d, J = 5.3 Hz, 1H), 5.63 (d, J = 4.1 Hz, 1H), 4.96 (d, J = 10.8 Hz, 1H), 4.94 (d, J = 10.8 Hz, 1H), 4.88 (d, J = 11.2 Hz, 1H), 4.85 (d, J = 11.2 Hz, 1H), 4.77 (d, J = 10.8 Hz, 1H), 4.76 (d, J = 11.2 Hz, 1H), 4.72−4.61 (m, 2H), 4.58 (d, J = 3.4 Hz, 1H), 4.54 (d, J = 5.08 Hz, 1H), 4.51−4.45 (m, 4H), 4.38−4.26 (m, 3H), 4.18 (dd, J = 1.8, 11.8 Hz, 1H), 3.93−3.88 (m, 3H), 3.81 (dd, J = 5.3, 9.6 Hz, 1H),3.64 (dd, J = 4.3, 10.6 Hz, 1H) 3.62−3.57 (m, 2H), 3.48 (t, J = 9.6, 9.0 Hz, 1H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 138.7, 138.5, 138.4, 138.0, 137.7, 137.6, 128.5, 128.4, 128.0, 127.8, 110.0, 82.8, 82.7, 80.6, 80.4, 79.1, 78.8, 77.6, 77.3, 77.2, 75.8, 75.0, 73.6, 72.3, 72.0, 71.7, 69.9, 63.4, 29.8, 20.9; ESI-MS m/z 1053.4 $[M + Na]^+$; ESI-HRMS calcd for $C_{63}H_{66}NaO_{11}S$ $[M + Na]^+$ 1053.4224, found 1053.4218.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-acetyl-1-thio-α,α-D-trehalose (6). To liquid NH_3 (15 mL) at −78 °C was added Na° until the solution became blue. A solution of thiotrehalose **5** (342 mg, 0.33 mmol) in THF (4 mL) was then added to the above blue solution, and the resulting mixture was stirred for 45 min at −78 °C. The reaction was quenched by the addition of ammonium chloride until the blue color disappeared. The NH_3 was allowed to evaporate slowly, and the crude residue was then poured into H_2O (30 mL) and extracted with $CHCl_3$ (20 mL). The aqueous layer was concentrated in vacuo to give the crude 1-thiotrehalose, which was directly used in the next reaction. Ac_2O (4.5 mL) was added to a solution of the crude 1-thiotrehalose in dry pyridine (10 mL), and the reaction was stirred at room temperature overnight. The mixture was then concentrated in vacuo to give a residue which was purified by flash column chromatography (petroleum ether/EtOAc, 2:1) to afford 1-thiotrehalose octaacetate **6** as a white amorphous solid (197 mg, 86% over two steps): $[\alpha]_D$ +173.8 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (d, J = 5.8 Hz, 2H), 5.35 (t, J = 9.8 Hz, 2H), 5.06 (t, J = 5.9, 4.8 Hz, 2H), 5.02 (t, J = 5.1, 9.3 Hz, 2H), 4.25 (s, 2H), 4.23 (s, 2H), 4.08 (t, J = 4.6, 9.7 Hz, 2H), 2.10 (s, 12H), 2.04 (s, 6H), 2.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 169.9, 169.6, 169.5, 78.5, 70.4, 70.2, 68.5, 68.4, 61.8, 20.6, 20.5; ESI-MS m/z 717.2 $[M + Na]^+$; ESI-HRMS calcd for $C_{28}H_{38}NaO_{18}S$ $[M + Na]^+$ 717.1677, found 717.1671.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (7). A solution of NaOMe (1.0 M) in MeOH was added to a solution of compound **6** (1.5 g, 2.16 mmol) in CH_2Cl_2 (12 mL) and MeOH (20 mL) until a pH of approximately 10 was reached. The reaction mixture was stirred at room temperature for 4 h, after which Amberlite-120 acidic resin was added to neutralize the solution. The mixture was then filtered and concentrated in vacuo to give a residue, which was azeotroped three times with toluene and directly used in the next step without purification. The crude deacetylated product was dissolved in CH_2Cl_2 (10 mL) and cooled at 0 °C. Et_3N (10 mL, 86 mmol) and TMSCl (3.2 mL, 25.9 mmol) were added to the solution, and the resulting mixture was stirred at room temperature overnight. Another portion of TMSCl (1 mL, 8.6 mmol) was added to the mixture at 0 °C, and the reaction was stirred until completion indicated by TLC and then concentrated in vacuo. The crude product was extracted with CH_2Cl_2 (25 mL × 3), and the combined organic layer was dried over Na_2SO_4, filtered, and concentrated to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 30:1) to give the title compound **7** (1.61 g, 80%) as a white amorphous solid: $[\alpha]_D$ +155.2 (c 0.48, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (d, J = 4.5 Hz, 2H), 3.91 (d, J = 8.6 Hz, 2H), 3.79 (dd, J = 11.6, 3.2, Hz, 2H), 3.68 (m, 2H), 3.63 (m, 4H), 3.56 (dd, J = 8.1, 17.1 Hz, 2H), 0.19 (s, 18H), 0.17 (s, 18H), 0.16 (s, 18H), 0.10 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.9, 75.6, 73.1, 72.8, 71.5, 61.6, 1.3, 0.9, 0.5, −0.2; ESI-MS m/z 957.1 $[M + Na]^+$; ESI-HRMS calcd for $C_{36}H_{86}NaO_{10}SSi_8$ $[M + Na]^+$ 957.3994, found 957.4022.

2,3,4,2′,3′,4′-Hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (8). HOAc (1.8 mL) was added to a solution of compound **7** (1.5 g, 1.6 mmol) in acetone (6 mL) and MeOH (8 mL) at 0 °C. When the selective desilylation was deemed complete (TLC monitoring), the reaction was quenched with aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 4:1) to afford the title compound **8** (1.0 g, 79%) as a colorless syrup: $[\alpha]_D$ +203.4 (c 0.39, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.31 (d, J = 4.7 Hz, 2H), 3.98 (dt, J = 9.3, 2.9 Hz, 2H), 3.76 (s, 4H), 3.74−3.64 (m, 4H), 3.73−3.65 (t, J = 8.4, 2H), 1.92 (br s, 2H), 0.21 (s, 18H), 0.19 (s, 18H), 0.17 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 83.1, 75.3, 73.0, 72.7, 71.4, 61.4, 1.3, 0.9, 0.4; ESI-MS m/z 813.9 $[M + Na]^+$; ESI-HRMS calcd for $C_{30}H_{70}NaO_{10}SSi_6$ $[M + Na]^+$ 813.3203, found 813.3218.

6,6′-Di-*O*-palmitoyl-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (9). To a solution of diol **8** (79 mg, 0.1 mmol) and palmitic acid (77 mg, 0.3 mmol) in CH_2Cl_2 (2 mL) were added DCC (103 mg, 0.5 mmol) and DMAP (15.2 mg, 0.13 mmol) at 0 °C. The cooling bath was then removed, the mixture was stirred at room temperature overnight and concentranted in vacuo, and the residue was purified by flash column chromatography (petroleum ether/EtOAc, 25:1) to give the desired product **9** (110 mg, 87%) as a colorless syrup: $[\alpha]_D$ +115.9 (c 0.96, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (d, J = 4.9 Hz, 2H), 4.32 (dd, J = 11.9, 1.9 Hz, 2H), 4.15−4.10 (m, 2H), 4.07 (dd, J = 11.9, 3.3 Hz, 2H), 3.69 (dd, J = 9.1, 5.0 Hz, 2H), 3.63 (t, J = 8.6 Hz, 2H), 3.56−3.51 (t, J = 9.9, 2H), 2.34 (m, 4H), 1.67−1.58 (m, 4H), 1.27 (m, 48H), 0.88 (t, J = 6.8 Hz, 6H), 0.18 (s, 18H), 0.17 (s, 18H), 0.16 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 83.5, 75.4, 72.6, 72.0, 70.7, 63.0, 34.2, 31.9, 29.72, 29.71, 29.67, 29.65, 29.5, 29.4, 29.3, 29.2, 24.8, 22.7, 14.1, 1.3, 0.9, 0.5; ESI-MS m/z 1291.3 $[M + Na]^+$; ESI-HRMS calcd for $C_{62}H_{130}NaO_{12}SSi_6$ $[M + Na]^+$ 1289.7796, found 1289.7832.

6,6′-Di-*O*-eicosanoyl-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (10). The reaction procedure was identical to that described for **9** except that eicosanoic acid (94 mg, 0.3 mmol) was used instead of palmitic acid. Compound **10** (116 mg, 84%) was isolated as a colorless syrup: $[\alpha]_D$ +141.6 (c 0.83, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (d, J = 4.8 Hz, 2H), 4.32 (d, J = 11.0 Hz, 2H), 4.12 (d, J = 9.5 Hz, 2H), 4.07 (d, J = 12.0 Hz, 2H), 3.72−3.67

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(m, 2H), 3.63 (t, J = 8.6 Hz, 2H), 3.55 (t, J = 8.6 Hz, 2H), 2.35 (m, 4H), 1.62 (m, 4H), 1.26 (m, 64H), 0.89 (t, J = 6.6 Hz, 6H), 0.18 (s, 18H), 0.17 (s, 18H), 0.16 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 83.5, 75.4, 72.6, 72.0, 70.7, 63.0, 34.2, 32.0, 29.73, 29.69, 29.68, 29.67, 29.5, 29.39, 29.35, 29.2, 24.8, 22.7, 14.2, 1.3, 0.9, 0.5; ESI-MS m/z 1403.6 $[M + Na]^+$; ESI-HRMS calcd for $C_{70}H_{146}NaO_{12}SSi_6$ $[M + Na]^+$ 1401.9048, found 1401.9088.

6,6′-Di-*O*-docosanoyl-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (11). The reaction procedure was identical to that described for **9** except that docosanoic acid (102 mg, 0.3 mmol) was used instead of palmitic acid. Compound **11** (65 mg, 45%) was isolated as a colorless syrup: $[\alpha]_D$ +90.0 (c 1.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.29 (d, J = 5.1 Hz, 2H), 4.30 (dd, J = 12.0, 2.1 Hz, 2H), 4.13−4.08 (m, 2H), 4.05 (dd, J = 12.1, 3.4 Hz, 2H), 3.67 (dd, J = 9.2, 5.1 Hz, 2H), 3.62 (t, J = 8.8 Hz, 2H), 3.53 (t, J = 8.9 Hz, 2H), 2.40−2.26 (m, 4H), 1.65−1.52 (m, 4H), 1.35−1.19 (m, 72H), 0.87 (t, J = 7.0 Hz, 6H), 0.16 (s, 18H), 0.15 (s, 18H), 0.14 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 83.5, 75.4, 72.6, 72.0, 70.6, 63.0, 34.9, 32.0, 29.73, 29.70, 29.69, 29.67, 29.5, 29.40, 29.35, 29.2, 25.5, 22.7, 14.2, 1.3, 0.9, 0.5; ESI-MS m/z 1459.6 $[M + Na]^+$; ESI-HRMS calcd for $C_{74}H_{154}NaO_{12}SSi_6$ $[M + Na]^+$ 1457.9674, found 1457.9724.

6,6′-Di-*O*-oleoyl-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (12). The reaction procedure was identical to that described for **9** except that oleic acid (97 μL, 0.3 mmol) was used instead of palmitic acid. Compound **12** (121 mg, 90%) was isolated as a colorless syrup: $[\alpha]_D$ +125.0 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (m, 4H), 5.30 (d, J = 4.8 Hz, 2H), 4.32 (d, J = 11.6 Hz, 2H), 4.11 (d, J = 9.6 Hz, 2H), 4.06 (dd, J = 12.0, 3.1 Hz, 2H), 3.68 (dd, J = 9.1, 5.1 Hz, 2H), 3.63 (t, J = 8.6 Hz, 2H), 3.54 (t, J = 8.7 Hz, 2H), 2.34 (m, 4H), 2.01 (m, 8H), 1.62 (m, 4H), 1.29 (m, 40H), 0.88 (m, 6H), 0.17 (s, 18H), 0.16 (s, 18H), 0.15 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 130.0, 129.7, 83.6, 75.4, 72.6, 72.0, 70.7, 63.0, 34.2, 31.9, 29.8, 29.7, 29.5, 29.3, 29.24, 29.15, 29.1, 27.22, 27.18, 24.8, 22.7, 14.1, 1.3, 0.9, 0.5; ESI-MS m/z 1343.5 $[M + Na]^+$; ESI-HRMS calcd for $C_{66}H_{134}NaO_{12}SSi_6$ $[M + Na]^+$ 1341.8109, found 1341.8177.

6,6′-Di-*O*-(13-methylmyristyl)-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (13). The reaction procedure was identical to that described for **9** except that 13-methylmyristic acid (73 mg, 0.3 mmol) was used instead of palmitic acid. Compound **13** (110 mg, 89%) was isolated as a colorless syrup: $[\alpha]_D$ +101.4 (c 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.32 (d, J = 5.1 Hz, 2H), 4.33 (dd, J = 12.0, 2.0 Hz, 2H), 4.13 (m, 2H), 4.09 (dd, J = 12.1, 3.3 Hz, 2H), 3.71 (dd, J = 9.2, 5.1 Hz, 2H), 3.65 (t, J = 5.6 Hz, 2H), 3.56 (t, J = 5.8 Hz, 2H), 2.41−2.31 (m, 4H), 1.65−1.63 (m, 4H), 1.56−1.50 (m, 2H), 1.31−1.27 (m, 32H), 1.17−1.15 (m, 4H), 0.89 (d, J = 6.6 Hz, 12H), 0.19 (s, 18H), 0.18 (s, 18H), 0.17 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 83.5, 75.4, 72.6, 72.0, 70.7, 63.0, 39.1, 34.2 30.0, 29.74, 29.68, 29.66, 29.5, 29.3, 29.2, 28.0, 27.4, 24.8, 22.7, 1.3, 0.9, 0.5; ESI-MS m/z 1263.2 $[M + Na]^+$; ESI-HRMS calcd for $C_{60}H_{126}NaO_{12}SSi_6$ $[M + Na]^+$ 1261.7483, found 1261.7480.

6-*O*-(13-Methylmyristyl)-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (14). To a solution of diol **8** (158 mg, 0.2 mmol) and 13-methylmyristic acid (48 mg, 0.2 mmol) in CH_2Cl_2 (3 mL) were added DCC (82 mg, 0.4 mmol) and DMAP (12.6 mg, 0.1 mmol) at 0 °C. The resulting mixture was allowed to warm up to room temperature, stirred for 5 h, and then concentranted in vacuo, and the residue was purified by flash column chromatography (petroleum ether/EtOAc, 8:1) to give the product **14** (101 mg, 50%) as a colorless syrup: $[\alpha]_D$ +131.3 (c 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (d, J = 3.4 Hz, 1H), 5.31 (d, J = 3.8 Hz, 1H), 4.36 (d, J = 11.5 Hz, 1H), 4.12 (m, 2H), 3.95 (m, 1H), 3.72−3.74 (m, 2H), 3.70−3.68 (m, 4H), 3.56−3.51 (m, 2H), 2.32−2.36 (m, 2H), 1.70 (t, J = 6.5 Hz, 1H), 1.67−1.62 (m, 2H), 1.57−1.50 (m, 1H), 1.28 (m, 16H), 1.18 (m, 2H), 0.88 (d, J = 6.6 Hz, 6H), 0.19 (s, 18H), 0.18 (s, 18H), 0.17 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 83.3, 83.2, 75.5, 75.4, 73.1, 73.0, 72.8, 72.4, 71.7, 71.0, 63.2, 61.6, 39.0, 34.2, 29.9, 29.61, 29.57, 29.55, 29.4, 29.2, 29.1, 27.9, 27.3, 24.8, 22.5, 1.21, 1.18, 0.9, 0.8, 0.4, 0.3; ESI-MS m/z 1038.9 $[M + Na]^+$; ESI-HRMS calcd for $C_{45}H_{98}NaO_{11}SSi_6$ $[M + Na]^+$ 1037.5343, found 1037.5371.

6-*O*-(13-Methylmyristyl)-6′-*O*-oleoyl-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-1-thio-α,α-D-trehalose (15). To a solution of the monoacylated maradolipid **14** (80 mg, 79 μmol) and oleic acid (51 μL, 0.16 mmol) in CH_2Cl_2 (3 mL) were added DCC (65 mg, 0.32 mmol) and DMAP (10 mg, 79 μmol) at 0 °C. The cooling bath was then removed, the mixture was stirred at room temperature overnight and concentranted in vacuo, and the residue was purified by flash column chromatography (petroleum ether/EtOAc, 25:1) to give the title compound **15** (90 mg, 89%) as a colorless syrup: $[\alpha]_D$ +116.9 (c 1.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.37−5.36 (m, 2H), 5.32 (d, J = 5.5 Hz, 2H), 4.33 (d, J = 11.8 Hz, 2H), 4.14−4.12 (m, 2H), 4.09 (dd, J = 12.1, 3.3 Hz, 2H), 3.71 (dd, J = 9.2, 5.1 Hz, 2H), 3.65 (t, J = 8.8 Hz, 2H), 3.61−3.47 (t, J = 8.9 Hz, 2H), 2.38−2.32 (m, 4H), 2.10−2.00 (m, 4H), 1.64−1.63 (m, 4H), 1.55−1.50 (m, 1H), 1.30 (m, 36H), 1.17−1.15 (m, 2H), 0.91−0.87 (m, 9H), 0.19 (s, 18H), 0.181 (s, 18H), 0.176 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 173.5, 130.0, 129.7, 83.5, 75.4, 72.5, 72.0, 70.7, 63.0, 39.1, 34.2, 34.1, 31.9, 30.0, 29.8, 29.74, 29.73, 29.67, 29.65, 29.54, 29.50, 29.34, 29.25, 29.18, 29.16, 29.15, 28.0, 27.4, 27.22, 27.19, 24.79, 24.77, 22.70, 22.68, 14.1, 1.3, 0.9, 0.5; ESI-MS m/z 1303.4 $[M+Na]^+$; ESI-HRMS calcd for $C_{63}H_{130}NaO_{12}SSi_6$ $[M + Na]^+$ 1301.7796, found 1301.7836.

6,6′-Di-*O*-palmitoyl-1-thio-α,α-D-trehalose (16). A solution of compound **9** (66 mg, 52 μmol) in CH_2Cl_2/MeOH (4:1, 10 mL) was treated with Amberlite IR120 (H^+) resin (360 mg) at room temperature for 1 h. The resin was removed and washed with MeOH, and the solution was evaporated under vacuum to give a residue, which was then purified by flash column chromatography (CH_2Cl_2/MeOH, 10:1) to give the maradolipid **16** (39 mg, 89%) as a white amorphous solid: $[\alpha]_D$ +186.0 (c 0.3, Py); ^{1}H NMR (600 MHz, C_5D_5N) δ 6.27 (d, J = 4.7 Hz, 2H), 5.06 (t, J = 7.2 Hz, 2H), 4.90 (d, J = 11.0 Hz, 2H), 4.81 (dd, J = 11.7, 5.7 Hz, 2H), 4.60−4.55 (m, 4H), 4.17 (m, t, J = 8.9 Hz, 2H), 2.33−2.23 (m, 4H), 1.59−1.57 (m, 4H), 1.16−1.24 (m, 48H), 0.84 (t, J = 6.8 Hz, 6H); ^{13}C NMR (150 MHz, C_5D_5N) δ 174.9, 84.6 77.7, 74.3, 73.3, 73.0, 65.7, 35.6 33.4, 31.29, 31.26, 31.22, 31.21, 31.06, 30.91, 30.88, 30.7, 26.5, 24.2, 15.6; ESI-MS m/z 858.3 $[M + Na]^+$; ESI-HRMS calcd for $C_{44}H_{82}NaO_{12}S$ $[M + Na]^+$ 857.5425, found 857.5425.

6,6′-Di-*O*-eicosanoyl-1-thio-α,α-D-trehalose (17). The reaction procedure was identical to that described for **16**. Compound **10** (60 mg, 43 μmol) yielded the title compound **17** (34 mg, 82%) as a white amorphous solid: $[\alpha]_D$ +210.0 (c 0.3, Py); ^{1}H NMR (600 MHz, C_5D_5N) δ 6.29 (d, J = 4.7 Hz, 2H), 5.08−5.09 (m, 2H), 4.91 (dd, J = 11.54 1.58 Hz, 2H), 4.82 (dd, J = 11.7, 5.8 Hz, 2H), 4.63−4.56 (m, 4H), 4.19 (dd, J = 9.7, 8.3 Hz, 2H), 2.33−2.23 (m, 4H), 1.62−1.55 (m, 4H), 1.27−1.15 (m, 64H), 0.84 (t, J = 7.0 Hz, 6H); ^{13}C NMR (150 MHz, C_5D_5N) δ 173.4, 83.1, 76.2, 72.8, 71.8, 71.5, 64.2, 34.1, 31.9, 29.83, 29.82, 29.81, 29.79, 29.78, 29.74, 29.72, 29.6, 29.41, 29.40, 29.2, 25.0, 22.7, 14.1; ESI-MS m/z 970.4 $[M + Na]^+$; ESI-HRMS calcd for $C_{52}H_{97}O_{12}S$ $[M - H]^+$ 945.6701, found 945.6734.

6,6′-Di-*O*-docosanoyl-1-thio-α,α-D-trehalose (18). The reaction procedure was identical to that described for **16**. Compound **11** (50 mg, 35 μmol) yielded the title compound **18** (28 mg, 80%) as a white amorphous solid: $[\alpha]_D$ +105.0 (c 0.13, Py); ^{1}H NMR (600 MHz, C_5D_5N) δ 6.28 (d, J = 4.7 Hz, 2H), 5.09−5.06 (m, 2H), 4.91 (dd, J = 11.6, 1.45 Hz, 2H), 4.81 (dd, J = 11.7, 5.8 Hz, 2H), 4.61−4.56 (m, 4H), 4.18 (dd, J = 9.7, 8.3 Hz, 2H), 2.31−2.22 (m, 4H), 1.55−1.59 (m, 4H), 1.23−1.28 (m, 72H), 0.84 (t, J = 7.0 Hz, 6H); ^{13}C NMR (150 MHz, C_5D_5N) δ 174.9, 84.6, 77.7, 74.3, 73.3, 73.1, 65.7, 35.6, 33.4, 31.33, 31.32, 31.31, 31.30, 31.29, 31.25, 31.23, 31.1, 30.9, 30.7, 27.2, 24.2, 15.6; ESI-MS m/z 1026.3 $[M + Na]^+$; ESI-HRMS calcd for $C_{56}H_{105}O_{12}S$ $[M - H]^+$ 1001.7327, found 1001.7296.

6,6′-Di-*O*-oleoyl-1-thio-α,α-D-trehalose (19). The reaction procedure was identical to that described for **16**. Compound **12** (62 mg, 47 μmol) yielded the title compound **19** (38 mg, 92%) as a white amorphous solid: $[\alpha]_D$ +114.9 (c 1.2, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 5.46 (d, J = 5.5 Hz, 2H), 5.39−5.34 (m, 4H), 4.38 (dd, J = 11.6, 1.6 Hz, 2H), 4.28 (dd, J = 11.6, 5.7 Hz, 2H), 4.24 (m, 2H), 3.76 (dd, J = 9.6, 5.5 Hz, 2H), 3.59 (t, J = 9.2 Hz, 2H), 3.36 (t, J = 9.3 Hz, 2H), 2.40−2.35 (m, 4H), 2.10−2.05 (m, 8H), 1.65−1.62 (m, 4H), 1.35−1.32 (m, 44H), 0.95−0.90 (m, 6H); ^{13}C NMR (150 MHz,

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CD_3OD) δ 174.0, 129.5, 129.4, 81.9, 74.5, 71.4, 70.6, 70.4, 63.2, 33.7, 31.7, 29.53, 29.50, 29.3, 29.1, 29.04, 29.01, 28.9, 26.8, 24.7, 22.4, 13.2; ESI-MS m/z 910.2 $[M + Na]^+$; ESI-HRMS calcd for $C_{48}H_{86}NaO_{12}S$ $[M + Na]^+$ 909.5738, found 909.5765.

6,6′-Di-*O*-(13-methylmyristyl)-1-thio-α,α-D-trehalose (20). The reaction procedure was identical to that described for **16**. Compound **13** (60 mg, 48 μmol) yielded the title compound **20** (36 mg, 93%) as a white amorphous solid: $[\alpha]_D$ +291.0 (*c* 0.7, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 5.45 (d, J = 5.5 Hz, 2H), 4.38 (dd, J = 11.6, 1.7 Hz, 2H), 4.28 (dd, J = 11.6, 5.8 Hz, 2H), 4.25−4.22 (m, 2H), 3.76 (dd, J = 9.6, 5.5 Hz, 2H), 3.60 (t, J = 9.2 Hz, 2H), 3.36 (t, J = 9.3 Hz, 2H), 2.37 (t, J = 7.5 Hz, 4H), 1.65−1.62 (m, 4H), 1.58−1.51 (m, 2H), 1.33 (m, 32H), 1.22−1.18 (m, 4H), 0.91 (d, J = 6.6 Hz, 12H); ^{13}C NMR (150 MHz, CD_3OD) δ 174.0, 81.8, 74.5, 71.4, 70.5, 70.4, 63.2, 38.9, 33.7, 29.7, 29.51, 29.47, 29.4, 29.3, 29.1, 28.9, 27.8, 27.2, 24.7, 21.7; ESI-MS m/z 830.2 $[M + Na]^+$; ESI-HRMS calcd for $C_{42}H_{78}NaO_{12}S$ $[M + Na]^+$ 829.5112, found 829.5145.

6-*O*-(13-Methylmyristyl)-6′-*O*-oleoyl-1-thio-α,α-D-trehalose (2). The reaction procedure was identical to that described for **16**. Compound **15** (58 mg, 45 μmol) yielded the maradolipid **2** (34 mg, 90%) as a white amorphous solid: $[\alpha]_D$ +128.0 (*c* 2.0, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 5.46 (d, J = 5.5 Hz, 2H), 5.40−5.35 (m, 2H), 4.38 (dd, J = 11.6, 1.6 Hz, 2H), 4.28 (dd, J = 11.7, 5.7 Hz, 2H), 4.25−4.22 (m, 2H), 3.77 (dd, J = 9.6, 5.5 Hz, 2H), 3.60 (t, J = 9.3 Hz, 2H), 3.36 (t, J = 9.4 Hz, 2H), 2.37 (t, J = 7.5 Hz, 4H), 2.10−2.04 (m, 4H), 1.66−1.63 (m, 4H), 1.58−1.21 (m, 1H), 1.36 (m, 36H), 1.21−1.18 (m, 2H), 1.00−0.84 (m, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 174.0, 173.9, 129.5, 129.4, 81.9, 74.5, 71.4, 70.5, 70.4, 63.2, 38.9, 33.71, 33.69, 31.8, 29.8, 29.53, 29.50, 29.46, 29.4, 29.3, 29.2, 29.1, 29.0, 28.93, 28.90, 27.8, 27.3, 26.8, 24.69, 24.68, 22.4, 21.8, 13.2; ESI-MS m/z 870.2 $[M + Na]^+$; ESI-HRMS calcd for $C_{45}H_{81}O_{12}S$ $[M - H]^+$ 845.5449, found 845.5414.

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data for **7**, ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Xiangming.Zhu@ucd.ie.

Notes

The authors declare no competing financial interest.

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