Synthesis of Ten Members of the Maradolipid Family; Novel Diacyltrehalose Glycolipids from *Caenorhabditis elegans*

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Abstract: The synthesis of ten members of the maradolipid family is described using a direct route starting from trehalose.

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On exposure to difficult environmental conditions, for example overcrowding or starvation, *Caenorhabditis elegans* leaves the reproductive life cycle by generating specific dauer larvae.[1] These larvae have their own morphology and enhanced stress metabolism to sustain themselves under unfavorable conditions. The biology of dauer larva formation has been extensively investigated, however, the chemistry that supports their survival under harsh conditions is not understood. During our studies on the transition of *C. elegans* from reproductive larvae to dauer larvae,[2] we have recently identified a novel class of glycolipids called maradolipids (from maradi, Georgian for enduring or dauer).[3] These maradolipids represent a genuine lipid component that is specific for dauer larvae. Extensive mass and NMR spectroscopic studies led to the structural assignment of the maradolipids as 6,6′-di-*O*-acyltrehaloses **1** (Figure 1). It was found that the maradolipids are composed of a mixture of at least 60 derivatives of trehalose **2**, which differ in the two fatty acid acyl side chains at the 6- and 6′-positions. The fatty acid side chains can be identical ($R^1 = R^2$, symmetrical maradolipids), however, in the majority they differ ($R^1 \neq R^2$, unsymmetrical maradolipids). The structure of the maradolipids is similar to glycolipids in *Mycobacterium tuberculosis*.[4] However, to the best of our knowledge, this was the first isolation of diacyltrehaloses from animals.

About two thirds of the maradolipid mixture isolated from *C. elegans* contained at least one branched fatty acid side chain and one third of the maradolipids contained only straight and cyclopropane-containing fatty acid side chains. The maradolipid mixture found in *C. elegans* contains about 7 to 8% of 6-*O*-(13-methylmyristoyl)-6′-*O*-oleoyltrehalose (**1a**; Mar 15:0/18:1) as the major component. A selective access to the individual compounds of the maradolipid mixture was required in order to confirm the structural assignment and to identify their biological function. Considering the structural diversity of the maradolipids due to the different fatty acid side chains, we have developed a synthetic strategy that provides the individual members of this class of glycolipids in pure form. Our preliminary results are reported here.

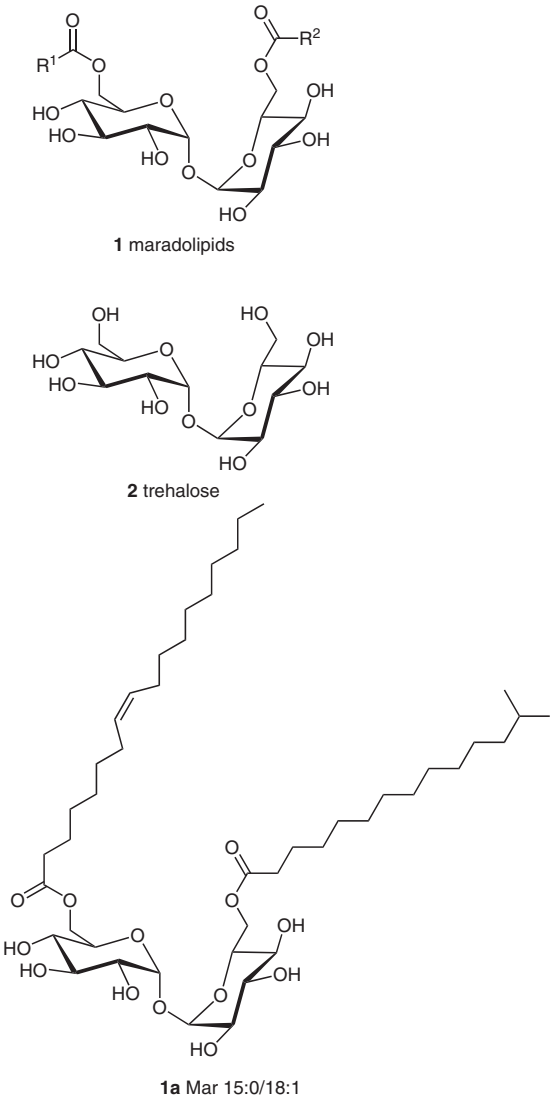

Figure 1 Structures of the maradolipids (**1**; R^1COOH, R^2COOH = fatty acids), trehalose (**2**) and the most abundant maradolipid: Mar 15:0/18:1 (**1a**).

Using an excess of *N*,*O*-bis(trimethylsilyl)acetamide and catalytic amounts of tetrabutylammonium fluoride

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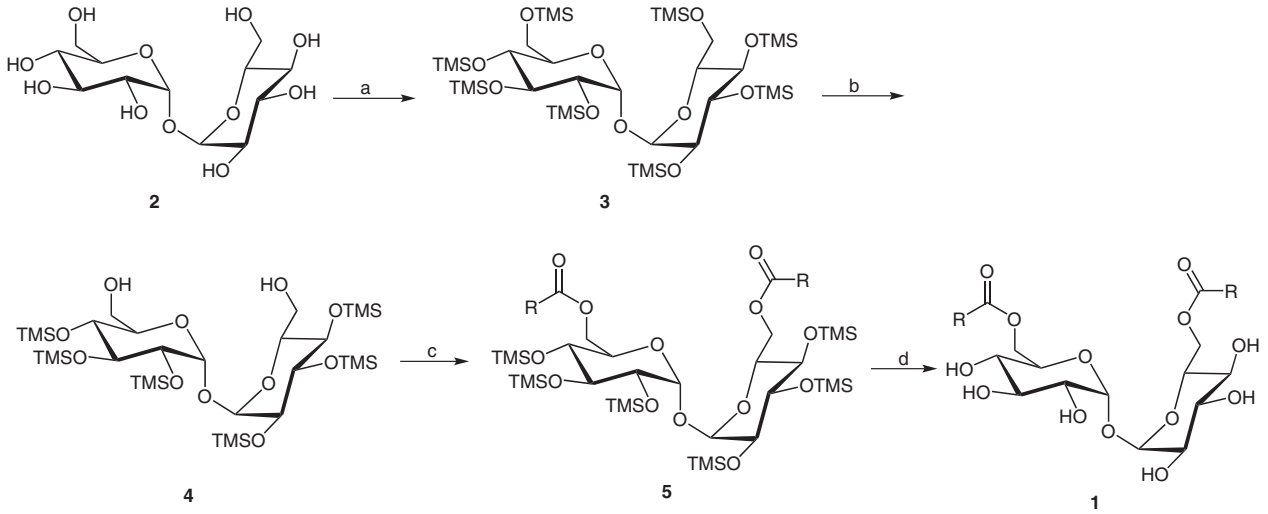
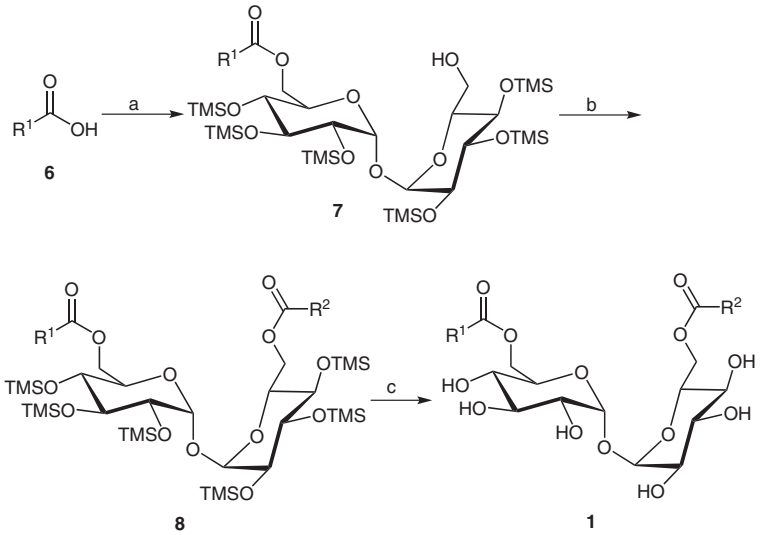


Scheme 1 Synthesis of the symmetrical maradolipids (**1**). *Reagents and conditions*: (a) *N*,*O*-bis(trimethylsilyl)acetamide (8.9 equiv), TBAF (0.06 equiv), DMF, 15 °C to r.t., 45 min; (b) K_2CO_3 (1.0 equiv), MeOH, 0 °C, 140 min, 88% over two steps; (c) RCOOH (4 equiv; fatty acid), EDC·HCl (3 equiv), cat. DMAP, toluene, 50 °C, 4 d, 73–92%; (d) 50 equiv TFA–THF–H_2O (1:4:6), 20 °C, 3 min, then evaporation, 81–90%.

(TBAF),[5] trehalose (**2**) was transformed into the corresponding octakis(trimethylsilyl)trehalose (**3**), which was converted into the hexakis(trimethylsilyl)trehalose **4** in situ by chemoselective cleavage of the silyl ethers at the primary alcohol functions (Scheme 1).[6] For the synthesis of the symmetrical maradolipids, compound **4** was treated with an excess of the corresponding fatty acid and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride in the presence of catalytic amounts of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) to afford the diacyl derivative **5**. Cleavage of the silyl ether protecting groups using a large excess of trifluoroacetic acid (TFA) and subsequent purification by HPLC led to the symmetrical maradolipids **1b–e** in four steps and 55–73% overall yield based on trehalose (**2**) (Figure 2).

For the synthesis of the unsymmetrical maradolipids, a differentiation between the two primary hydroxy groups of trehalose (**2**) had to be achieved. Thus, a modified approach that could be used for selective monoacylation was required (Scheme 2). The fatty acids were commercially available except for the cyclopropano-fatty acid for the synthesis of the maradolipid **1j**, which was easily prepared by cyclopropanation of oleic acid using Charette's procedure.[7] The first fatty acid **6** (oleic acid or 13-methylmyristic acid) was treated with two equivalents of hexakis(trimethylsilyl)trehalose **4** in the presence of 2.5 equivalents of EDC hydrochloride and catalytic amounts of DMAP to provide the monoacyl derivative **7**. The second acylation was achieved by reaction with an excess of the second fatty acid (R^2COOH) and EDC hydrochlo-



Scheme 2 Synthesis of the unsymmetrical maradolipids (**1**). *Reagents and conditions*: (a) **4** (2 equiv), EDC·HCl (2.5 equiv), cat. DMAP, toluene, 50 °C, 4 d, 54%; (b) R^2COOH (3 equiv), EDC·HCl (2.0 equiv), cat. DMAP, toluene, 50 °C, 4 d, 97–100%; (c) 50 equiv TFA–THF–H_2O (1:4:6), 20 °C, 3 min, then evaporation, 73–84%.

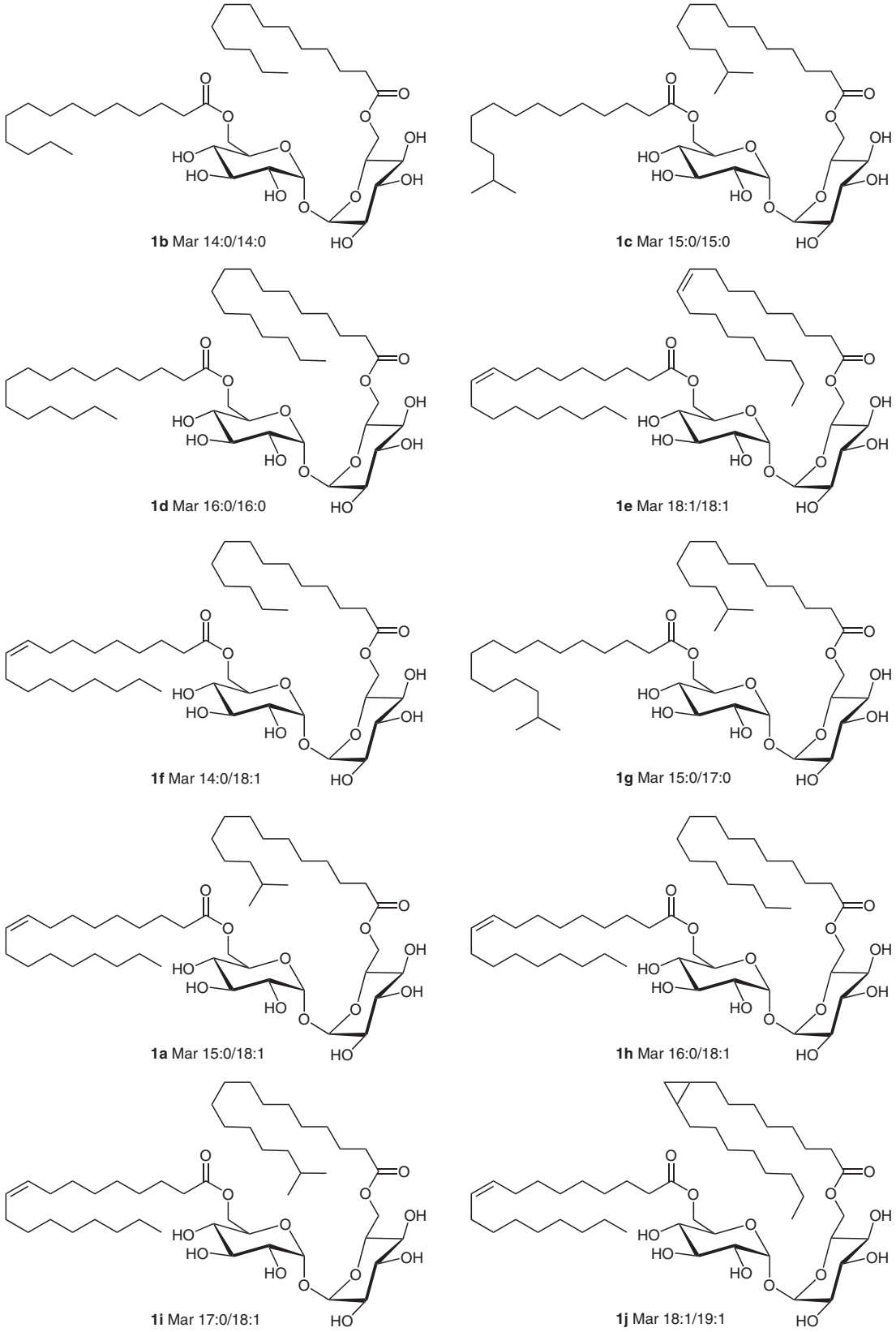


Figure 2 Maradolipids **1a–j** obtained by synthesis from trehalose (**2**). The symmetrical maradolipids **1b–e** were synthesized following the route shown in Scheme 1; the unsymmetrical maradolipids **1a** and **1f–j** were synthesized following the route shown in Scheme 2.

ride in the presence of DMAP to provide the diacyl derivative **8** almost quantitatively. Finally, deprotection with TFA and purification by HPLC afforded the unsymmetrical maradolipids **1a** and **1f–j** in five steps and 34–40% overall yield based on trehalose (**2**) (Figure 2).

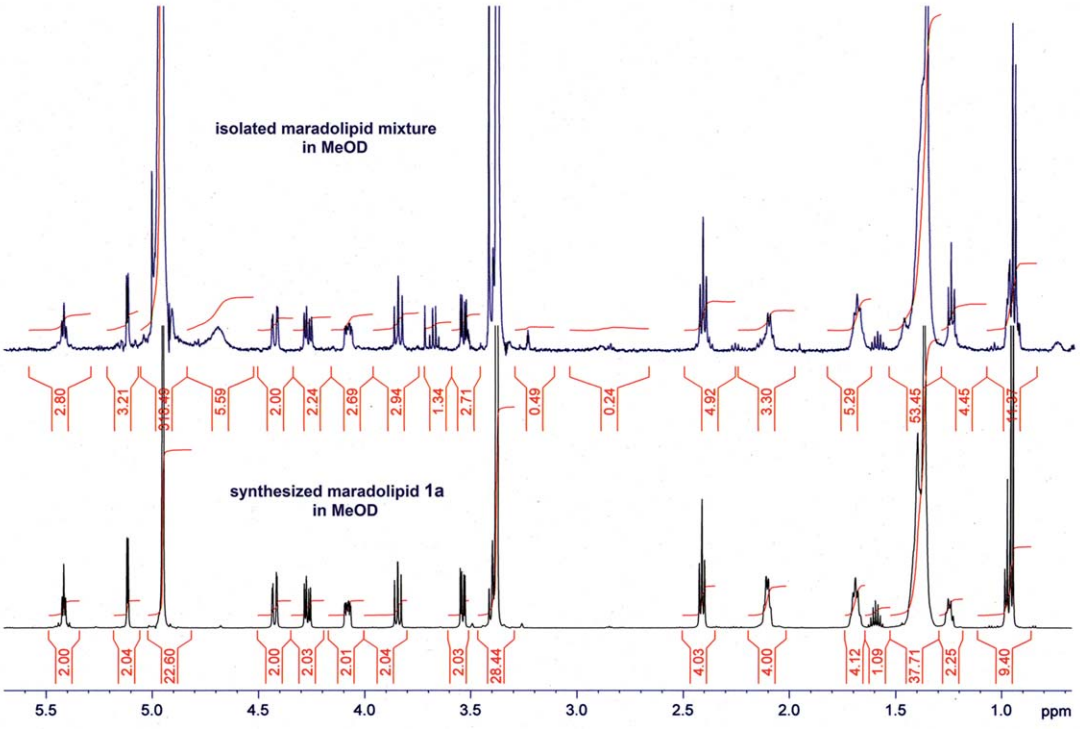


Figure 3 Comparison of the 500 MHz ^{1}H NMR spectrum (CD_3OD) of the natural maradolipid mixture (top spectrum) with that of 6-*O*-(13-methylmyristoyl)-6′-*O*-oleoyltrehalose (**1a**; Mar 15:0/18:1; bottom spectrum).

The maradolipids **1a**–**j** that have been synthesized in this study were fully characterized from their spectroscopic data (see the Supporting Information for complete ^{1}H and ^{13}C NMR spectra of **1a**–**j**). A comparison of the original ^{1}H NMR spectrum of the maradolipid mixture obtained from *C. elegans* with that of 6-*O*-(13-methylmyristoyl)-6′-*O*-oleoyltrehalose (**1a**), obtained by the synthesis described above, confirmed the assignment of the signals as reported in our preceding paper (Figure 3).[3]

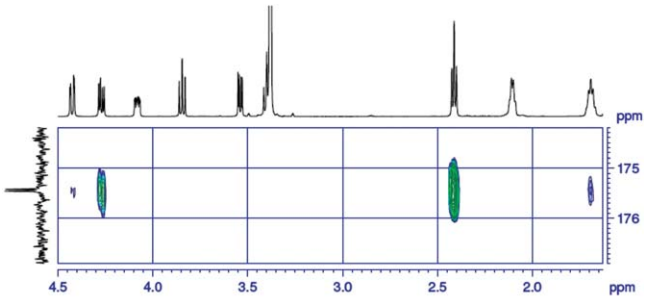


Figure 4 HMBC spectrum (150/600 MHz, CD_3OD) of 6-*O*-(13-methylmyristoyl)-6′-*O*-oleoyltrehalose (**1a**; Mar 15:0/18:1).

The HMBC spectrum of pure synthetic **1a** (150/600 MHz), clearly shows the difference between the chemical shifts of the two fatty acid carbonyl groups at the 6-*O*- and 6′-*O*-positions of trehalose (Figure 4; compare with Figure 3b of our previous paper[3]).

When worms were fed with the synthetic maradolipid **1b** (Mar 14:0/14:0) to form dauer larvae at 25 °C, it was found that dauer larvae of the type *daf-2*;Δ*tps*, a double-mutant strain that cannot produce maradolipids due to inhibition of trehalose biosynthesis,[3] took up and accumulated **1b** (Figure 5).

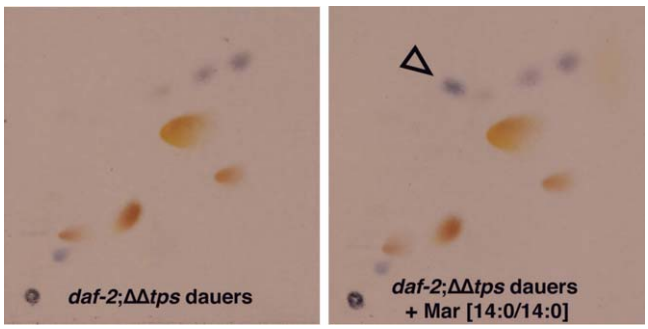


Figure 5 Left: 2D-TLC plate of the lipid extracts of the double-mutant strain dauer larvae *daf-2*;Δ*tps*; right: 2D-TLC plate of the lipid extracts of the double-mutant strain dauer larvae *daf-2*;Δ*tps* fed with the synthetic maradolipid **1b** (Mar 14:0/14:0); the arrowhead indicates the band of the maradolipid.

In conclusion, we have developed a highly efficient synthetic route to symmetrical as well as to unsymmetrical maradolipids **1**. Our present work provides broad access to these novel glycolipids for the study of their biological function as well as their biophysical properties.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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