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RESEARCH ARTICLE

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Synthesis of specific deuterated derivatives of the long chained stratum corneum lipids [EOS] and [EOP] and characterization using neutron scattering

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The synthesis of specific deuterated derivatives of the long chained ceramides [EOS] and [EOP] is described. The structural differences with respect to the natural compounds are founded in the substitution of the 2 double bonds containing linoleic acid by a palmitic acid branched with a methyl group in 10-position. The specific deuteration is introduced both in the branched and in the terminal methyl group, which was realized by common methods of successive deuteration of carboxylic groups in 3 steps. These modified fatty acids resp. the corresponding ceramides [EOS] and [EOP] were prepared for neutron scattering investigations. First results of these investigations were presented in this manuscript showing that the deuterated compounds could be detected in the stratum corneum lipid model membranes. The deuterated ceramides [EOS] and [EOP] are valuable tools to investigate the influence of these long chained ceramide species on the nanostructure of stratum corneum lipid model membranes.

KEYWORDS
branched fatty acid, CER[EOP], CER[EOS], neutron scattering investigations, specific deuteration

1 | INTRODUCTION

The skin as largest organ of the human body consists of 3 main layers. The epidermis is the outermost of them, which is further subdivided into 4 layers. The outside part of these 4 is the stratum corneum (SC), which has to fulfill many functions. The SC is formed by cornified cells embedded in a lipid matrix[1] and represents a protective barrier against water loss and the invasion of toxic substances.[2,3] This is based on the lipids of the SC, which consist mainly of ceramides, free fatty acids, and cholesterol,[4,5] where the ceramides represent the main and also the most important compounds.[6] Ceramides are not unique in their structure.[7] The 4 amino bases sphingosine, phytosphingosine, dehydrosphingosine, and 6-hydroxysphingosine are acylated with different fatty acids at the amino group under formation of amides. Regarding the fatty acids bonded to the amino bases, the most common length is 16, 18, 22 and 24 carbon atoms. Beside these simple fatty acids also (R)-configurated α hydroxy fatty acids and a more complex, 48 carbon atoms containing a composed fatty acid was found. This last acid contains a ω-hydroxy acid with 30 carbon atoms whereas the terminal hydroxy group is esterified with linoleic acid. The long-chain acid with the linoleoyl residue is bound to sphingosine (CER[EOS]), to phytosphingosine (CER[EOP]), and 6-hydroxysphingosine (CER[EOH]). CER[EOS] appears to play a key role in one model of the nanostructure of the SC lipids because it is stated to form the long periodicity phase of the SC lipids.[8,9] This model is still an issue of scientific debate.[10-12] Therefore, additional studies are necessary concerning the impact of CER[EOS].

The research of the nanostructure of the SC lipids was focused on model systems containing the main components of skin in certain ratios. The investigative methods of choice in this direction are neutron diffraction measurements and the determination of neutron scattering density profile.[12-16] However, this method has only a benefit when deuterated or better partially deuterated ceramides are used. Main effort was par for the course in the case of partial deuteration of the linoleic acid in the molecules of CER[EOS] or CER[EOP], however, this is associated with a large effort. In former investigation, we developed an alternative to the linoleic acid part of the complex fatty acid[11] by using a palmitic acid residue with methyl branching in 10-position of the chain. This is due to very early papers in which we could show as the first that the phase transition temperatures and enthalpies were strikingly reduced through the introduction of a methyl branching in the middle position of palmitic acid chains in phosphatidylcholines.[17,18] This effect was also found in phosphatidylcholines with branched stearic acid residues.[19] We have shown the same tremendous effect of fluidization of lipids by methyl branches at definite positions in the synthesis and investigation of lipid model compounds of archaea bacteria.[20,21] In the above mentioned paper, the CER[EOS] derivative with a methyl group in 10-position of the palmitoyl residue has nearly identical physicochemical properties regarding the natural CER[EOS]. On the other hand, the branched fatty acid is stable against higher temperature and oxidative processes especially during physicochemical investigations. Another advantage of such modification of the fatty acid residue is the simpler synthesis of the complex fatty acid with 46 carbon atoms because of the complete abandonment of blocking groups with exception of the THP group (see Section 2).

Therefore, the objective of this study was to synthesize 10-methylhexadecanoic acids with deuteration in the branched methyl group and/or at the terminal methyl group of the fatty acid. These different specific deuterated fatty acids were sensitive with respect to neutron scattering experiments in the corresponding ceramides and were already published.[22] Here, we will present our results in the synthesis of CER[EOS] and CER[EOP] with the specific deuterated branched fatty acids (Figure 1). The results of neutron scattering investigation of SC lipid model membranes with the new synthesized compounds show that the deuterated long-chain ceramides are valuable tools to investigate the influence of these ceramides on the nanostructure of SC lipid model membranes.

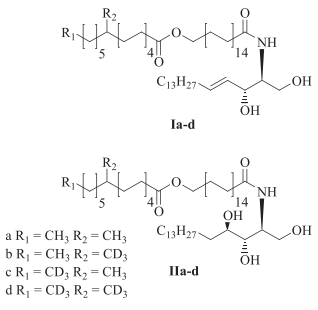
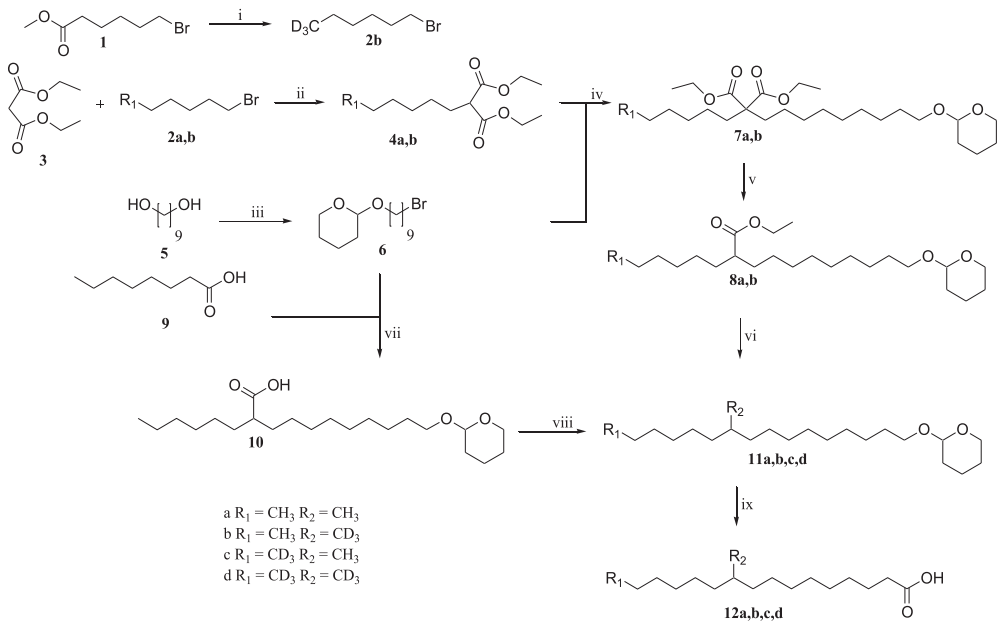


FIGURE 1 Structure of synthesized ceramides

2 | RESULTS AND DISCUSSION

2.1 | Synthesis of the branched deuterated fatty acids

For the preparation of the branched and specifically deuterated fatty acids as an alternative for the deuterated linoleic acid, we used 2 different synthetic methods. These methods are shown in Scheme 1. First, the synthetic way via a malonic acid ester dialkylation reaction is classic. However, the yields are very good especially in fatty acid chemistry. A shorter but more labour-intensive reaction pathway with respect to synthetic equipment and also for the preparation of the terminal deuterated compounds **12** is the α-alkylation of an acid dianion.[23,24] The synthetic route to the branched and deuterated compounds **12** via malonic acid ester alkylation starts with the commercially available 6-bromohexanoic acid ethyl ester (**1**). This compound was transformed in 3 steps via reduction of the ester group with lithium aluminum deuteride at 0°C to the corresponding deuterated bromo alcohol, mesylation, and repeated reduction of the corresponding mesylate with lithium aluminum deuteride to yield compound **2b.**[25,26] This compound was also commercially available, however, since we needed bigger amounts with respect to the following multistep procedure, we prepared it in this way. Malonic acid ethyl ester was deprotonated with sodium in ethanol in the case of hexylbromide **2a** as alkylating reagent but with sodium hydride in toluene in the case of 6,6,6-d_3-hexyl bromide **2b** to avoid the deuterium exchange especially in the during the alkylation step. The 2 mono alkyl malonic acid esters **4a,b** were purified using column chromatography. In a second alkylation, step **4a,b** were deprotonated using potassium hydride in dry toluene. Then the 2-(9-bromonon-1-yloxy)tetrahydro-2*H*-pyrane (**6**) reacted with the carbanion to realize the necessary chain extension to 16 carbon atoms yielding compounds **7a,b**. Compounds **6** was prepared from nonane-1,9-diol (**5**) by monobromination reaction according to Fockink et al.[27] The blocking of the free hydroxyl group that is necessary for the following step was done with 3,4-dihydro-2*H*-pyrane under the common conditions.[28] One of the 2 carboxylic ester groups is the latent methyl group with or without deuteration. Therefore, **7a,b** were monosaponified using a modified procedure of Breslow[29] including decarboxylation of the resulting free carboxyl group to the ester compounds **8a,b**. These substances were then transformed either to the normal branched methyl group or to the CD_3 group dependent on the used reagents. Following the above described reaction,



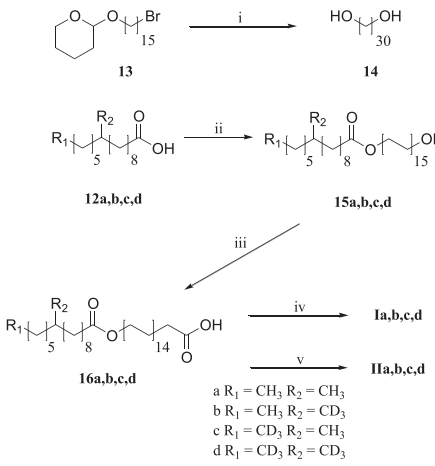
SCHEME 1 i: a) $LiAlD_4$, Et_2O, 0°C, CH_3SO_2Cl, TEA, $CHCl_3$, 0°C, c) $LiAlD_4$, Et_2O, 0°C; ii: NaH, toluene, reflux; iii: a) 48% HBr, b) DHP, CH_2Cl_2, PPTS; iv: KH, toluene, reflux; v: a) KOH, ethanol, 65°C, b) TEA, cumene, pyridine, reflux; vi: $LiAlD_4/LiAlH_4$, Et_2O, reflux, CH_3SO_2Cl, TEA, $CHCl_3$, 0°C, c) $LiAlD_4/LiAlH_4$, Et_2O, reflux; vii: NaH, DIPA, n-BuLi, THF; viii: $LiAlD_4/LiAlH_4$, Et_2O, reflux, CH_3SO_2Cl, TEA, $CHCl_3$, 0°C, c) $LiAlD_4/LiAlH_4$, Et_2O, reflux; ix: a) MeOH, PPTS, reflux, b) CrO_3, H_2SO_4, acetone, $CHCl_3$, 0°C

sequence **8a,b** were reduced with lithium aluminum hydride in the case of the "normal" methyl group or with lithium aluminum deuteride in the case of the deuterated methyl branching. The resulting alcohols were transformed into the methanesulfonic acid esters, which were reduced once more with the corresponding hydride reduction reagents to compounds **11a-d**.

According to reaction pathway B, starting material was octanoic acid (**9**). First, **9** was transformed into the dianion by double deprotonation in a modified way according to Creger.[24] This was realized by preparing the sodium salt of the acid with sodium hydride in tetrahydrofuran/diisopropylamine. After the hydrogen evolution was complete at 60°C, the mixture was treated with the appropriate amount of BuLi and the double deprotonation proceeds at room temperature. This process is sensible with respect to reaction time and the reaction temperature. The advantage of dianions towards enolates in alkylation reactions is due to the higher reaction temperatures. The dianion was then reacted with the bromide **6**. From resulting acid **10**, compounds **11a,b** were prepared in the same way described already in pathway A with the difference that the acid was reduced without transformation into the corresponding ester. However, synthetic success of the alkylation reaction is connected with the full formation of the dianion as mentioned already above. The final branched fatty acids **12a-d** were received by elimination of the tetrahydropyranyl residue with methanol and PPTS and oxidation of the resulting branched fatty alcohol with Jones reagent.

2.2 | Synthesis of the branched and deuterated CER[EOS] and CER[EOP]

In Scheme 2, the syntheses of the 10-methylhexadecanoyloxytriacontanoic acids (**15a-c**) and the final ceramides **I** and **II** are shown. First, the preparation of the ω-acyl fatty acids **15a-c** starts with the acylation of triacontan-1,30-diol (**14**), which was prepared by Grignard coupling of suitable bromides.[30] Therefore, the selective deuterated 10-methylpalmitic acids **12a-c** were transformed into the corresponding acid chlorides using thionyl chloride.



SCHEME 2 i: a) Mg, THF; b) MeOH, PPTS, reflux; ii: a) $SOCl_2$; b) $CHCl_3$, reflux; iii: CrO_3, H_2SO_4, acetone, $CHCl_3$, 0°C; iii: KOH, D_2O, Pd/C (10%), 195°C, 15 bar, 100 h; iv: PyBOP, DIPEA, CH_2Cl_2, sphingosine, RT; v: PyBOP, DIPEA, CH_2Cl_2, phytosphingosine, RT

Regarding to the normal acylation procedure using acid chlorides in our case, we dispense with a base and dropped the acid chloride very slowly to the diol in boiling chloroform. The procedure enables us to avoid to a great extent the double acylation of the diol. While working below 5°C and using a helper base, we isolated only the diester.[11] This is due to the bad solubility of the diol at that temperature. The acylated alcohols **15a-c** were then transformed to the corresponding acids **16a-c** by a modified Jones oxidation in good yields. This is the advantage of the use of a branched fatty acid instead of linoleic acid in the natural ceramides because of the instability of the double bonds against the harsh oxidation conditions. This means also a reduction of synthetic steps and a reaction pathway without any blocking groups already mentioned above. The fatty acids **16a-c** were finally converted into the corresponding ceramides. For the *N*-acylation of sphingobases like sphingosine or phytosphingosine PyBOP was the reagent of choice.[31] Other authors used various forms of activated esters.[32,33] We have studied this procedure with all these activators. However, we find that the use of PyBOP is the best reagent in this direction. To our knowledge, we are the first to use this substance. The yield of the ceramides is very high (>77%), and the workup is very simple especially in the case of the ceramides containing phytosphingosine.

2.3 | Neutron scattering measurements

Neutron scattering can be used as a very efficient tool to determine the nanostructure of multilamellar CER-based mixtures. These models are used to model the properties of the SC, the uppermost layer of the skin. To be able to access specific properties of single CER species only distinguished by a slightly different head group or chain length, pure synthetic CER as synthesized here are necessary. Only using very basic mixtures with these pure single CER or very basic mixtures of only a few CER, the complexity of the model system is low enough to contribute sometimes very minor differences to a specific component. Specifically, deuterated CER as synthesized here furthermore allow determination of the position of this label within the lamellar arrangement, due to the difference in the neutron scattering cross-section of H and D. This allows to determine the position and chain arrangement of the CER within the system. At the moment, this is the most efficient way to get an insight into the details of the nanostructure of a specific model system.

For the CER[NS]/CER[EOS]-br–based system investigated in this study, only a single phase was observed for all 4 investigated mixtures. This phase had a spacing of 3.94 ± 0.1 nm, thus, no long periodicity phase (LPP) with a spacing of 12 to 13 nm was observed.[34] The observed spacing however is also shorter than what would be expected for an short periodicity phase (SPP) with 2 opposing CER[NS] C18/18, which would be estimated as 4.42 nm.[35] The difference of 0.48 nm cannot be explained by interdigitating chains, temperature, or other minor influences. This difference is thus to be assumed, to be the result of a slightly tilted CER chain arrangement. A chain tilt that would result in a corresponding distance difference would have a tilt angle α of about 26° (solely considering geometrical factors). The position of the specific deuteration could be determined for all 3 deuterated CER. A distinct difference between the CER[EOS]-br-d_3 with its terminal end deuterated and the CER[EOS]-d_3-br with its branching side chain deuterated could be shown. For the terminally deuterated CER[EOS]-br-d_3, the difference is smaller than for the other CER but is observed over the whole range of measurements for the contrast variation and thus can be confirmed. The positions of the different deuterations also strongly support the assumption of a tilted chain arrangement. Furthermore, to fit the SPP arrangement, the CER[EOS] not including its ω-acyl group was determined to most likely span 2 whole lamellar leaflets. Its ω-acyl head group would then be located in the

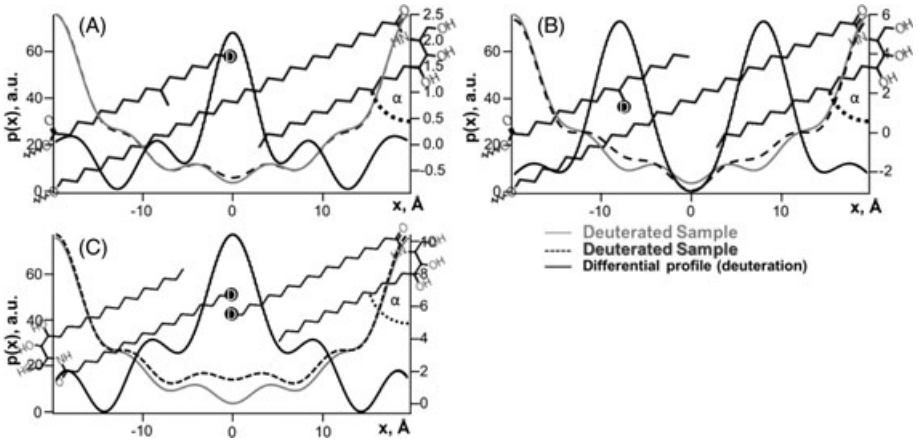


FIGURE 2 Neutron scattering length density profiles for the CER([NS]/[EOS])/SA/CHOL system with a molar ratio of (0.8:0.2)/1/0.7 in comparison to mixtures with deuterated CER. A, CER[EOS]-br-d_3. B, CER[EOS]-d_3-br. C, CER[NS]-d_3. The very long CER[EOS]-d_3-br spanning 2 lamellae is shown in 2 parts to fit a single profile

head group region of the next neighboring lamellar leaflet and its ω-hydroxy chain arranged parallel to the CER[NS] in a third leaflet. A possible arrangement is shown in Figure 2 for both the short CER[NS] and the very long CER[EOS]-br, which is split into 2 parts to fit a single profile.

3 | EXPERIMENTAL

3.1 | General procedures and materials

Lithium aluminum deuteride was purchased from ARMAR (Europe), GmbH (Leipzig, Germany), and PyBOP from Carbolution Chemicals GmbH (Saarbrücken, Germany). The aminoalcohols sphingosine and phytosphingosine were obtained from Evonik Goldschmidt GmbH (Essen, Germany). From Sigma-Aldrich (St Louis, Missouri) were received all other chemicals. Before use, all solvents were dried. Silica gel 60 (0.063-0.200 mm) were used from Merck (Darmstadt, Germany) for column chromatography. Thin-layer chromatography plates were obtained from Macherey-Nagel (Düren, Germany). For detection of the thin-layer chromatography plates, bromothymol blue solution was used. Mass spectrometry data were recorded at a Finnigan MAT 710C (Thermoseparation Products, San Jose, CA) for ESI-MS and a LTQ-Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) for HR-MS (high-resolution mass spectrometry). Nuclear magnetic resonance (NMR) data were received on Varian Gemini 2000 and Varian Inova 500 instruments. $CDCl_3$, CD_3OD, THF-d_8, and mixtures were used. An HP 1100 Agilent (Agilent Technologies, Waldbronn, Germany) with ELSD 2000 Alltech (Grace Davison, Columbia, Maryland) detector and a Nucleodur 100-5 125 × 2 column from Macherey-Nagel (Düren, Germany) were used for high-performance liquid chromatography (HPLC). Melting points were determined on a Boetius apparatus and were not corrected.

3.2 | Synthesis of the ceramides

3.2.1 | Synthesis of 1-bromo-6,6,6-d_3-hexane (2b)

To a stirred suspension of $LiAlD_4$ (2.77 g, 66 mmol) in 100 mL abs. ether (100 mL), a solution of methyl 6-bromohexanoate (**1**) (25.1 g, 120 mmol) in abs. ether (150 mL) was dropwise added under argon atmosphere at a temperature of −10°C. The mixture was stirred for further 4 hours at −10°C. For working up, D_2O (10 mL) was added dropwise under ice cooling and the reaction was stirred for 2 hours. To remove the formed precipitate, the mixture was filtered by suction and the frit was washed 3 times with ether (20 mL). The combined ether phases were washed with brine, dried over Na_2SO_4, concentrated to dryness under reduced pressure, and dried over P_2O_5 to give the deuterated alcohol as colorless liquid.

To a solution of the liquid residue of the alcohol and TEA (18.62 mL, 134 mmol) in $CHCl_3$ (250 mL), a solution of mesyl chloride (10.40 mL, 134 mmol) in $CHCl_3$ (100 mL) was added under ice cooling. Afterwards, the reaction was stirred for further 12 hours at room temperature. For working up, additional TEA (4.65 mL, 33 mmol) was given to the mixture and ice (100 g) was added. The $CHCl_3$ phase was separated, and the water layer was extracted 3 times with $CHCl_3$ (100 mL). The combined organic layers were evaporated to dryness under reduced pressure. The residue was taken up in a mixture of heptane (400 mL) and ether (200 mL). The organic layer was washed with water (150 mL). The water layer was extracted with ether (100 mL). The combined organic layers were evaporated to dryness under reduced pressure and were dried over P_2O_5 to give the mesylate.

A solution of the mesylate in abs. ether (80 mL) was added dropwise under argon atmosphere at a temperature of −10°C to a stirred suspension of $LiAlD_4$ (2.5 g, 60 mmol) in abs. ether (100 mL). The reaction was stirred for further 5 hours at a temperature of −10°C. H_2O (25 mL) was added dropwise under cooling, and the mixture was stirred for further 60 minutes. To remove the formed precipitate, the mixture was filtered by suction and the frit was washed 3 times with ether (20 mL). The combined ether phases were washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. For purification, the obtained liquid was distilled under reduced pressure to give the 1-bromo-6,6,6-d_3-hexane (**2b**) as colorless oil (15.58 g, 77%). The deuterated alkyl bromide was used without further characterization.

3.2.2 | Monoalkylation of diethyl malonate

Diethyl hexylmalonate (4a)

To a solution of sodium ethanolate (2.30 g, 100 mmol sodium in 50 mL abs. ethanol) was added under moderate boiling diethyl malonate (**3**) (16.02 g, 100 mmol) and then hexyl bromide (**2a**) (17.33 g, 105 mmol). The mixture was heated under reflux for 12 hours; afterwards, ethanol (prepared from 2,3 g sodium (100mmol) in 50 ml abs. ethanol) was removed under reduced pressure. For working up, ice water was added to the mixture and the organic layer was separated. The water layer was extracted 3 times with ether (50 mL). The combined organic layers were dried over Na_2SO_4 and filtered and evaporated to dryness under reduced pressure. For purification, the crude residue was distilled under reduced pressure to give **4a** as colorless liquid (18.75 g, 77%). Diethyl hexylmalonate (**4a**) was used without further characterization.

Diethyl 6,6,6-d$_3$-hexylmalonate (4b)

To a stirred suspension of NaH (60% in paraffin, 5.45 g, 136 mmol) in abs. toluene (280 mL), a solution of **3** (21.83 g, 136 mmol) in abs. toluene (120 mL) was added under argon atmosphere. The mixture was warmed to 70°C for 2 hours, followed by dropwise adding of a solution of **2b** (15.0 g, 0.09 mol) in abs. toluene (60 mL). The reaction was heated under reflux for 24 hours. For working up, water (50 mL) was added. The organic layer was separated, and the water layer was extracted 3 times with ether (50 mL). The combined organic layers were dried over Na_2SO_4 and filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using gradient technique with heptane/Et_2O eluent to give diethyl 6,6,6-d$_3$-hexylmalonate (**4b**) as colorless liquid (12.36 g, 56%): R_f 0.40 ($CHCl_3$/heptane 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (*t*, *J* = 7.1 Hz, 6H, 2× -OCH$_2$C***H***$_3$), 1.29-1.32 (*m*, 8H, D$_3$C(C***H***$_2$)$_4$-), 1.85-1.91 (*m*, 2H, D$_3$C(CH$_2$)$_4$C***H***$_2$-), 3.30 (*t*, *J* = 7.5 Hz, 1H, -C***H***(COO-)$_2$), 4.19 (q, *J* = 7.1 Hz, 4H, 2× -OC***H***$_2$CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.2, 22.4, 27.4, 28.9, 29.0, 31.6, 52.3, 61.4, 169.8; ESI-MS *m/z*: 248.1 (M + H)$^+$, 270.0 (M + Na)$^+$, 516.8 (2M + Na)$^+$.

Synthesis of 2-(9-bromononyloxy)tetrahydro-2*H*-pyran (6)

The preparation was following the procedure described in the literature[27] starting from nonan-1,9-diol (**5**) to give **6** as colorless liquid: R_f 0.47 ($CHCl_3$/heptane 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30-1.43 (*m*, 10H, Br(CH$_2$)$_2$(C***H***$_2$)$_5$-), 1.50-1.61 (*m*, 6H, -C***H***$_2$CH$_2$OCH-CH$_2$(C***H***$_2$)$_2$-), 1.68-1.74 (*m*, 1H, -OCH-C***H***H′-), 1.78-1.88 (*m*, 3H, BrCH$_2$C***H***$_2$-, -OCH-CH***H***′-), 3.35-3.41 (*m*, 3H, BrC***H***$_2$(CH$_2$)$_7$C***H***H′-), 3.47-3.52 (*m*, 1H, -OCH-(CH$_2$)$_3$C***H***H′-), 3.72 (d*t*, *J* = 6.9, 9.5 Hz, 1H, Br(CH$_2$)$_8$CH***H***′-), 3.84-3.89 (*m*, 1H, -OCH-(CH$_2$)$_3$CH***H***′-), 4.56-4.57 (*m*, 1H, -OC***H***-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 19.9, 25.7, 26.3, 28.3, 29.5, 29.9, 30.9, 33.0, 34.1, 62.5, 67.8, 99.0.

3.2.3 | Second alkylation to diethyl dialkylmalonate

A solution of **4a,b** (10.0 g, 0.04 mol) in abs. toluene (40 mL) was added dropwise under argon atmosphere to a stirred suspension of KH (30% in paraffin, 6.03 g, 45 mmol) in abs. toluene (50 mL). The mixture was stirred for further 12 hours at room temperature. Afterwards, the reaction was warmed to 50°C before dropwise adding a solution of **6** (15.75 g, 51.25 mmol) in abs. toluene (60 mL). The mixture was heated under reflux for 24 hours. For working up, ice water (50 mL) was added, and the mixture was stirred for further 2 hours. Sat. NH4Cl (50 mL) was added, and the organic layer was separated. The water phase was extracted 2 times with ether (50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using gradient technique with heptane/Et_2O and TEA (0.5%) eluent to give diethyl dialkylmalonate **7a,b**.

Diethyl hexyl[9-(tetrahydro-2*H*-pyran-2-yloxy)nonyl] malonate (7a)

Colorless liquid (16.37 g, 85%): R_f 0.34 ($CHCl_3$/heptane 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (*t*, *J* = 6.6 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.09-1.38 (*m*, 26H, H$_3$C(C***H***$_2$)$_4$CH$_2$C(COOCH$_2$C***H***$_3$)$_2$CH$_2$(C***H***$_2$)$_6$-), 1.49-1.62 (*m*, 6H, -C***H***$_2$CH$_2$OCH-CH$_2$(C***H***$_2$)$_2$-), 1.67-1.74 (*m*, 1H, -OCH-C***H***H′-), 1.78-1.86 (*m*, 5H, -C***H***$_2$CC***H***$_2$-, -OCH-CH***H***′-), 3.37 (d*t*, *J* = 6.7, 9.5 Hz, 1H, -(CH$_2$)$_8$C***H***H′O-), 3.46-3.51 (*m*, 1H, -OCH-(CH$_2$)$_3$C***H***H′-), 3.71 (d*t*, *J* = 6.9, 9.4 Hz, 1H, -(CH$_2$)$_8$CH***H***′O-), 3.82-3.89 (*m*, 1H, -OCH-(CH$_2$)$_3$CH***H***′-), 4.16 (q, *J* = 7.1 Hz, 4H, 2× -OC***H***$_2$CH$_3$), 4.55-4.57 (*m*, 1H, -OC***H***-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.3, 19.8, 22.7, 24.0, 24.1, 25.7, 26.4, 29.4, 29.6, 29.7, 29.9, 30.0, 30.9, 31.7, 32.3, 57.7, 61.0, 62.5, 67.8, 99.0, 172.2; ESI-MS *m/z*: 493.3 (M + Na)$^+$.

Diethyl 6,6,6-d$_3$-hexyl[9-(tetrahydro-2*H*-pyran-2-yloxy) nonyl]malonate (7b)

Colorless liquid (15.90 g, 83%): R_f 0.40 ($CHCl_3$/heptane 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09-1.16 (*m*, 4H, 2× (-OOC)$_2$C(CH$_2$C***H***$_2$-)$_2$), 1.21 (*m*, 22H, D$_3$C(C***H***$_2$)$_3$(CH$_2$)$_2$C(COOCH$_2$C***H***$_3$)$_2$(CH$_2$)$_2$(C***H***$_2$)$_5$-), 1.48-1.62 (*m*, 6H, -C***H***$_2$CH$_2$OCH-CH$_2$(C***H***$_2$)$_2$-), 1.68-1.74 (*m*, 1H, -OCH-C***H***H′-), 1.79-1.87 (*m*, 5H, -C***H***$_2$CC***H***$_2$-, -OCH-CH***H***′-), 3.37 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -(CH$_2$)$_8$C***H***H′O-), 3.46-3.52 (*m*, 1H, -OCH-(CH$_2$)$_3$C***H***H′-), 3.71 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -(CH$_2$)$_8$CH***H***′O-), 3.83-3.89 (*m*, 1H, -OCH-(CH$_2$)$_3$CH***H***′-), 4.16 (q, *J* = 7.1 Hz, 4H, 2× -OC***H***$_2$CH$_3$), 4.55-4.57 (*m*, 1H, -OC***H***-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.8, 22.4, 24.0, 24.1, 25.7, 26.4, 29.4, 29.6, 29.7, 29.9, 30.0, 30.9, 31.6, 32.3, 57.7, 61.0, 62.5, 67.8, 99.0, 172.2; ESI-MS *m/z*: 496.3 (M + Na)$^+$.

Monosaponification and decarboxylation of diethyl dialkylmalonate

A mixture of **7a,b** (15.0 g, 32 mmol) and KOH (2.53 g, 38.4 mmol) in 96% ethanol (80 mL) was stirred at a temperature of 65°C for 24 hours. For working up, sat. NH_4Cl were added (80 mL) and the mixture was extracted 3 times with ether (80 mL). The combined ether phases were washed with brine, dried over Na_2SO_4, and filtered and evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography using $CHCl_3$ and TEA (1%) eluent to remove the byproducts followed by $CHCl_3$/MeOH (9:1) to wash the acid from the column. To the crude acid residue was given TEA (4.44 mL, 32 mmol), cumene (160 mL), and 1 drop pyridine. The mixture was heated for 2 hours

under reflux. Afterwards, the solvent was evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography using gradient technique with heptane/Et_2O and TEA (0.5%) eluent to give ethyl ester **8a,b**.

Ethyl (2*RS*)-2-hexyl-11-[(tetrahydro-2*H*-pyran-2-yl)oxy] undecanoate (8a)

Colorless liquid (9.40 g, 74%): R_f 0.44 ($CHCl_3$/heptane 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (*t*, *J* = 6.9 Hz, 3H, $\boldsymbol{H}_3C(CH_2)_5$-), 1.23-1.45 (*m*, 25H, $H_3C(C\boldsymbol{H}_2)_4C\boldsymbol{H}H'CH$-$C\boldsymbol{H}H'(C\boldsymbol{H}_2)_6$-, -$OCH_2C\boldsymbol{H}_3$), 1.50-1.62 (*m*, 8H, -$CH\boldsymbol{H}'CH$-$CH\boldsymbol{H}'(CH_2)_6C\boldsymbol{H}_2CH_2OCH$-$CH_2(C\boldsymbol{H}_2)_2$-), 1.68-1.73 (*m*, 1H, -OCH-$C\boldsymbol{H}H'$-), 1.79-1.86 (*m*, 1H, -OCH-$CH\boldsymbol{H}'$-), 2.26-2.32 (*m*, 1H, -$CH_2C\boldsymbol{H}$-CH_2-), 3.37 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -$(CH_2)_8C\boldsymbol{H}H'$O-), 3.47-3.51 (*m*, 1H, -OCH-$(CH_2)_3C\boldsymbol{H}H'$-), 3.72 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -$(CH_2)_8CH\boldsymbol{H}'$O-), 3.84-3.89 (*m*, 1H, -OCH-$(CH_2)_3CH\boldsymbol{H}'$-), 4.13 (q, *J* = 7.1 Hz, 2H, -$OC\boldsymbol{H}_2CH_3$), 4.56-4.57 (*m*, 1H, -$OC\boldsymbol{H}$-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.5, 19.9, 22.7, 25.7, 26.4, 27.5, 27.6, 29.4, 29.6, 29.7, 29.9, 31.0, 31.8, 32.7, 45.9, 60.1, 62.5, 67.8, 99.0, 176.7; ESI-MS *m/z*: 421.3 $(M + Na)^+$.

Ethyl (2*RS*)-2-(6,6,6-d_3-hexyl-11-[(tetrahydro-2*H*-pyran-2-yl)oxy]undecanoate (8b)

Colorless liquid (10.56 g, 83%): R_f 0.34 ($CHCl_3$/heptane 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23-1.45 (*m*, 25H, $D_3C(C\boldsymbol{H}_2)_4C\boldsymbol{H}H'CH$-$C\boldsymbol{H}H'(C\boldsymbol{H}_2)_6$-, -$OCH_2C\boldsymbol{H}_3$), 1.48-1.61 (*m*, 8H, -$CH\boldsymbol{H}'CH$-$CH\boldsymbol{H}'(CH_2)_6C\boldsymbol{H}_2CH_2OCH$-$CH_2(C\boldsymbol{H}_2)_2$-), 1.68-1.74 (*m*, 1H, -OCH-$C\boldsymbol{H}H'$-), 1.78-1.86 (*m*, 1H, -OCH-$CH\boldsymbol{H}'$-), 2.25-2.32 (*m*, 1H, -$CH_2C\boldsymbol{H}$-CH_2-), 3.37 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -$(CH_2)_8C\boldsymbol{H}H'$O-), 3.46-3.52 (*m*, 1H, -OCH-$(CH_2)_3C\boldsymbol{H}H'$-), 3.71 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -$(CH_2)_8CH\boldsymbol{H}'$O-), 3.83-3.89 (*m*, 1H, -OCH-$(CH_2)_3CH\boldsymbol{H}'$-), 4.12 (q, *J* = 7.1 Hz, 2H, -$OC\boldsymbol{H}_2CH_3$), 4.55-4.57 (*m*, 1H, -$OC\boldsymbol{H}$-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 19.9, 22.5, 25.7, 26.4, 27.5, 27.6, 29.4, 29.6, 29.7, 29.9, 30.9, 31.8, 32.7, 45.9, 60.0, 62.5, 67.8, 99.0, 176.7; ESI-MS *m/z*: 424.3 $(M + Na)^+$.

3.2.4 | Synthesis of (2*RS*)-2-hexyl-11-[(tetrahydro-2*H*-pyran-2-yl)oxy]undecanoic acid (10)

To a stirred suspension of NaH (60% in paraffin, 0.90 g, 22.5 mmol) and DIPA (3.16 mL, 22.5 mL) in abs. THF (22 mL), a solution of octanoic acid (**9**) (3.24 g, 22.5 mmol) in abs. THF (8 mL) was added dropwise under argon atmosphere at a temperature of 0°C. The mixture was warmed to 60°C until the gas development stopped. Afterwards, the mixture was cooled to −10°C and n-butyllithium (2.5 m in hexane, 9 mL, 22.5 mmol) was added. The reaction was warmed to 28°C within 45 minutes, followed by the warming to 30°C for 3 minutes. Then the mixture was stirred at 28°C. After 30 minutes, a solution of **6** (4.61 g, 15 mmol) in abs. THF (8 mL) was added and the mixture was stirred for further 12 hours at 28°C. For working up, the reaction was cooled to 0°C and cold sat. NH_4Cl (40 mL) was added before the mixture was extracted 3 times with ether (45 mL). The combined ether phases were washed with water and brine. The crude residue was purified by column chromatography using $CHCl_3$ and TEA (1%) eluent to remove the by-products followed by $CHCl_3$/MeOH (9:1) to wash the acid **10** from the column.

Liquid (2,82 g, 51%): R_f 0.55 ($CHCl_3$/Et_2O 8:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (*t*, *J* = 6.9 Hz, 3H, $\boldsymbol{H}_3C(CH_2)_5$-), 1.27-1.35 (*m*, 20H, $H_3C(C\boldsymbol{H}_2)_4CH_2CH$-$CH_2(C\boldsymbol{H}_2)_6$-), 1.40-1.45 (*m*, 2H, -$C\boldsymbol{H}H'CH$-$C\boldsymbol{H}H'$-), 1.49- 1.62 (*m*, 8H, -$CH\boldsymbol{H}'CH$-$CH\boldsymbol{H}'(CH_2)_6C\boldsymbol{H}_2CH_2OCH$-$CH_2(C\boldsymbol{H}_2)_2$-), 1.68-1.74 (*m*, 1H, -OCH-$C\boldsymbol{H}H'$-), 1.79-1.86 (*m*, 1H, -OCH-$CH\boldsymbol{H}'$-), 2.27-2.32 (*m*, 1H, -$CHH'C\boldsymbol{H}$-CHH'-), 3.38 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -$(CH_2)_8C\boldsymbol{H}H'$O-), 3.48-3.52 (*m*, 1H, -OCH-$(CH_2)_3C\boldsymbol{H}H'$-), 3.72 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -$(CH_2)_8CH\boldsymbol{H}'$O-), 3.85-3.89 (*m*, 1H, -OCH-$(CH_2)_3CH\boldsymbol{H}'$-), 4.57-4.59 (*m*, 1H, -$OC\boldsymbol{H}$-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 19.8, 22.7, 25.6, 26.4, 27.6, 29.4, 29.6, 29.7, 29.9, 30.9, 31.9, 32.6, 44.8, 62.4, 67.8, 98.8, 181.9; ESI-MS *m/z*: 369.5 $(M - Na)^-$, 739.3 $(2M - Na)^-$.

3.2.5 | Introduction of the branched methyl group

The methyl-branched THP-protected alcohols **11a** and **11b** were prepared from ethyl (2*RS*)-2-hexyl-11-[(tetrahydro-2*H*-pyran-2-yl)oxy]undecanoate (**8a**) (method A) and from (2*RS*)-2-hexyl-11-[(tetrahydro-2*H*-pyran-2-yl)oxy]undecanoic acid (**10**) (method B) and the methyl-branched THP-protected alcohols **11c** and **11d** from ethyl (2*RS*)-2-(6,6,6-d_3-hexyl-11-[(tetrahydro-2*H*-pyran-2-yl)oxy]undecanoate (**8b**). The methyl-branched THP-protected alcohols **12a-d** were purified by column chromatography using gradient technique with heptane/Et_2O and TEA (0.5%) eluent.

Method A: The ester **8a,b** (4.50 g, 11 mmol) were reduced using the procedure for **2b**. For the preparation of the compounds **11a** and **11c**, the reduction agent $LiAlH_4$ was used and for compounds **11b** and **11d** $LiAlD_4$. The reduction reactions were heated under reflux until complete conversion.

Method B: The acid **10** (1.11 g, 3 mmol) were reduced using the procedure for **2b**, however, for the first reduction, 0.9 equivalents were used of the reducing agent. For the preparation of the compound **11a**, the reduction agent $LiAlH_4$ was used and for compound **11b** $LiAlD_4$. The reduction reactions were heated under reflux until complete conversion.

3.2.6 | 2-[(10*RS*)-10-Methylhexadecyloxy] tetrahydro-2*H*-pyran (11a)

Colorless liquid (method A: 2.88 g, 75%; method B: 0.43 g, 42%): the analytical spectra corresponds with the data described in the literature.[20]

2-[(10*RS*)-10-(Methyl-d_3)hexadecyloxy]tetrahydro-2*H*-pyran (11b)

Colorless liquid (method A: 2.69 g, 70%; method B: 0.40 g, 39%): R_f 0.43 ($CHCl_3$/heptane 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (*t*, *J* = 6.9 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.04-1.38 (*m*, 25H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.48-1.62 (*m*, 6H, -(CH$_2$)$_7$C***H***$_2$CH$_2$OCH-CH$_2$(C***H***$_2$)$_2$-), 1.68-1.74 (*m*, 1H, -OCH-C***H***H′-), 1.80-1.86 (*m*, 1H, -OCH-CH***H***′-), 3.38 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -(CH$_2$)$_8$C***H***H′O-), 3.47-3.52 (*m*, 1H, -OCH-(CH$_2$)$_3$C***H***H′-), 3.73 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -(CH$_2$)$_8$CH***H***′O-), 3.85-3.89 (*m*, 1H, -OCH-(CH$_2$)$_3$CH***H***′-), 4.56-4.58 (*m*, 1H, -OC***H***-O-); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 19.9, 22.9, 25.7, 26.4, 27.2, 29.7, 29.8, 29.9, 30.2, 31.0, 32.1, 32.7, 37.2, 62.5, 67.9, 99.0; ESI-MS *m/z*: 366.2 $(M + Na)^+$.

2-[(10*RS*)-16,16,16-d_3-10-Methylhexadecyloxy]tetrahydro-2*H*-pyran (11c)

Colorless liquid (method A: 3.04 g, 79%): R_f 0.38 ($CHCl_3$/heptane 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 1.04-1.36 (*m*, 25H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.50-1.63 (*m*, 6H, -(CH$_2$)$_7$C***H***$_2$CH$_2$OCH-CH$_2$(C***H***$_2$)$_2$-), 1.68-1.75 (*m*, 1H, -OCH-C***H***H′-), 1.79-1.87 (*m*, 1H, -OCH-CH***H***′-), 3.38 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -(CH$_2$)$_8$C***H***H′O-), 3.47-3.52 (*m*, 1H, -OCH-(CH$_2$)$_3$C***H***H′-), 3.73 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -(CH$_2$)$_8$CH***H***′O-), 3.84-3.90 (*m*, 1H, -OCH-(CH$_2$)$_3$CH***H***′-), 4.56-4.57 (*m*, 1H, -OC***H***-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.9, 25.7, 26.4, 27.2, 29.7, 29.8, 29.9, 30.2, 31.0, 32.0, 32.9, 37.3, 62.5, 67.9, 99.0; ESI-MS *m/z*: 366.2 $(M + Na)^+$, 709.0 $(2M + Na)^+$.

2-[(10*RS*)-16,16,16-d_3-10-(Methyl-d_3)hexadecyloxy] tetrahydro-2*H*-pyran (11d)

Colorless liquid (method A: 2.80 g, 72%): R_f 0.35 ($CHCl_3$/heptane 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 1.04-1.36 (*m*, 25H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.49-1.63 (*m*, 6H, -(CH$_2$)$_7$C***H***$_2$CH$_2$OCH-CH$_2$(C***H***$_2$)$_2$-), 1.68-1.75 (*m*, 1H, -OCH-C***H***H′-), 1.79-1.87 (*m*, 1H, -OCH-CH***H***′-), 3.38 (d*t*, *J* = 6.7, 9.6 Hz, 1H, -(CH$_2$)$_8$C***H***H′O-), 3.47-3.52 (*m*, 1H, -OCH-(CH$_2$)$_3$C***H***H′-), 3.73 (d*t*, *J* = 6.9, 9.6 Hz, 1H, -(CH$_2$)$_8$CH***H***′O-), 3.84-3.90 (*m*, 1H, -OCH-(CH$_2$)$_3$CH***H***′-), 4.56-4.58 (*m*, 1H, -OC***H***-O-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 25.7, 26.4, 27.2, 29.7, 29.8, 29.9, 30.2, 31.0, 32.0, 32.7, 37.2, 62.5, 67.9, 99.0; ESI-MS *m/z*: 369.9 $(M + Na)^+$, 715.6 $(2M + Na)^+$.

3.2.7 | Deprotecting and oxidation to the methyl-branched fatty acid

A solution of the THP-protected alcohol **11a-d** (2.10 g, 6 mmol) and catalytic amounts of PPTS in methanol (120 mL) were heated under reflux for 4 hours. Afterwards, the solvent was removed under reduced pressure and the obtained residue was purified by column chromatography using gradient technique with heptane/$CHCl_3$ eluent to give the methyl-branched alcohol.

A solution of the methyl-branched alcohol (1.50 g, 5.8 mmol) in acetone (65 mL) was added dropwise over 90 minutes to ice-cooled stirred suspension of CrO_3 (1.45 g, 14.5 mmol) in 10 N H_2SO_4 (7.25 mL). The mixture was stirred for further 2 hours under ice cooling. For working up, the mixture was extracted 3 times with ether (50 mL). The combined ether phases were extracted 4 times with 5% aqueous KOH solution (25 mL). The combined aqueous layers were acidified with diluted H_2SO_4 before 3-time ether (50 mL) extraction. The combined ether phases were dried over Na_2SO_4 and filtered and evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography using gradient technique with heptane/$CHCl_3$/Et_2O eluent to give the methyl-branched acids **12a-d**.

(10*RS*)-10-Methylhexadecanoic acid (12a)

(10*RS*)-10-Methylhexadecanol: colorless liquid (1.53 g, 97%): R_f 0.27 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 0.87 (*t*, *J* = 6.8 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.03-1.35 (*m*, 25H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.52-1.59 (*m*, 2H, -C***H***$_2$CH$_2$OH), 3.62 (*t*, *J* = 6.7 Hz, 2H, -C***H***$_2$OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 19.8, 22.8, 25.9, 27.2, 29.6, 29.8, 30.2, 32.1, 32.9, 37.2, 37.3, 63.1.

(10*RS*)-10-Methylhexadecanoic acid (**12a**): colorless liquid (1.09 g, 69%): R_f 0.31 ($CHCl_3$/Et_2O 8:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 0.88 (*t*, *J* = 6.8 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.03-1.36 (*m*, 23H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-), 1.60-1.67 (*m*, 2H, -C***H***$_2$CH$_2$COOH), 2.35 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.9, 22.9, 24.9, 27.2, 29.2, 29.4, 29.6, 29.9, 30.1, 32.1, 32.9, 34.2, 37.2, 37.3, 179.9; ESI-MS *m/z*: 269.4 $(M - H)^-$, 539.0 $(2M - H)^-$.

(10*RS*)-10-(Methyl-d_3)hexadecanoic acid (12b)

(10*RS*)-10-(Methyl-d_3)hexadecanol: colorless liquid (1.52 g, 96%): R_f 0.28 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (*t*, *J* = 6.7 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.04-1.40 (*m*, 25H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.53-1.60 (*m*, 2H, -C***H***$_2$CH$_2$OH), 3.64 (*t*, *J* = 6.6 Hz, 2H, -C***H***$_2$OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.9, 25.9, 27.2, 29.6, 29.8, 29.9, 30.2, 32.1, 32.7, 33.0 37.2, 37.2, 63.3.

(10*RS*)-10-(Methyl-d_3)hexadecanoic acid (**12b**): colorless liquid (1.11 g, 70%); R_f 0.42 ($CHCl_3$/Et_2O 8:2); ^{1}H NMR

(400 MHz, $CDCl_3$) δ 0.88 (*t*, *J* = 6.8 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.04-1.36 (*m*, 23H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-), 1.60-1.67 (*m*, 2H, -C***H***$_2$CH$_2$COOH), 2.35 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.9, 24.9, 27.2, 29.2, 29.4, 29.6, 29.9, 30.1, 32.1, 32.7, 34.2, 37.2, 37.3, 180.2; ESI-MS *m/z*: 272.5 $(M - H)^-$, 545.1 $(2M - H)^-$.

(10*RS*)-16,16,16-d$_3$-10-Methylhexadecanoic acid (12c)

(10*RS*)-16,16,16-d$_3$-10-Methylhexadecanol: colorless liquid (1.53 g, 97%): R_f 0.33 ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.6 Hz, 3H, -CH-C***H***$_3$), 1.03-1.38 (*m*, 25H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.54-1.59 (*m*, 2H, -C***H***$_2$CH$_2$OH), 3.64 (*t*, *J* = 6.7 Hz, 2H, -C***H***$_2$OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 25.9, 27.2, 29.6, 29.8, 29.9, 30.2, 32.0, 32.9, 33.0, 37.3, 63.3.

(10*RS*)-16,16,16-d$_3$-10-Methylhexadecanoic acid (**12c**): colorless liquid (1.14 g, 72%): R_f 0.59 ($CHCl_3$/Et_2O 8:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 1.02-1.36 (*m*, 23H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-), 1.60-1.67 (*m*, 2H, -C***H***$_2$CH$_2$COOH), 2.35 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 24.8, 27.2, 29.2, 29.4, 29.6, 29.9, 30.1, 32.0, 32.9, 34.2, 37.2, 37.3, 180.0; ESI-MS *m/z*: 272.9 $(M - H)^-$, 545.5 $(2M - H)^-$.

(10*RS*)-16,16,16-d$_3$-10-(Methyl-d$_3$)hexadecanoic acid (12d)

(10*RS*)-16,16,16-d$_3$-10-(Methyl-d$_3$)hexadecanol: colorless liquid (1.54 g, 97%): R_f 0.30 ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04-1.36 (*m*, 25H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_7$-), 1.53-1.59 (*m*, 2H, -C***H***$_2$CH$_2$OH), 3.63 (*t*, *J* = 6.7 Hz, 2H, -C***H***$_2$OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.6, 25.9, 27.2, 29.6, 29.8, 29.9, 30.2, 32.0, 32.7, 32.9, 37.2, 63.2.

(10*RS*)-16,16,16-d$_3$-10-(Methyl-d$_3$)hexadecanoic acid (**12d**): colorless liquid (1.18 g, 74%): R_f 0.53 ($CHCl_3$/Et_2O 8:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04-1.34 (*m*, 23H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-), 1.60-1.66 (*m*, 2H, -C***H***$_2$CH$_2$COOH), 2.35 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COOH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.6, 24.9, 27.2, 29.2, 29.4, 29.6, 29.9, 30.1, 32.0, 32.7, 34.2, 37.2, 179.9; ESI-MS *m/z*: 275.7 $(M - H)^-$.

3.2.8 | Synthesis of the 1,30-triacontanediol (14)

The preparation was following the procedure described in the literature[30] starting from 15-pentadecanolide (**13**) to give **14.**

3.2.9 | Coupling of the methyl-branched acid with 1,30-triacontandiol

A mixture of **12a-d** (0.63 mg, 2.3 mmol) and fresh distilled thionyl chloride (1.67 mL, 23 mmol) was heated under reflux for 4 hours. Afterwards, the mixture was stirred under argon atmosphere for 12 hours. The thionyl chloride excess was distilled off, and the residue was dried over KOH. The obtained acid chloride was dissolved in $CHCl_3$ (20 mL), and the solution was added dropwise over 90 minutes to a boiling solution of **14** (3.14 g, 6.9 mmol) in $CHCl_3$ (140 mL). After adding, the mixture was heated under reflux for further 6 hours. For a full crystallization of the diol excess, the mixture was allowed to stand at room temperature for 3 hours. To remove the diol excess, the mixture was filtrated. The filter was washed 3 times with $CHCl_3$ (20 mL). The filtrate was evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography using gradient technique with heptane/$CHCl_3$ eluent to give the ω-esterified alcohols **15a-d**.

30-Hydroxytriacontyl (10*RS*)-10-methylhexadecanoate (15a)

White solid (1.34 g, 82%): *mp* 70.5-71°C; R_f 0.33 ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.6 Hz, 3H, -CH-C***H***$_3$), 0.88 (*t*, *J* = 6.9 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.04-1.44 (*m*, 75H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{26}$(CH$_2$)$_2$OH), 1.54-1.64 (*m*, 6H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$OH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.64 (*t*, *J* = 6.6 Hz, 2H, -C***H***$_2$OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.9, 22.9, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.6, 29.7, 29.8, 29.9, 30.1, 32.1, 32.9, 33.0, 34.6, 37.3, 63.3, 64.6, 174.2; ESI-MS *m/z*: 707.4 $(M + H)^+$, 729.6 $(M + H)^+$, 1435.6 $(2M + Na)^+$.

30-Hydroxytriacontyl (10*RS*)-10-(methyl-d$_3$)hexadecanoate (15b)

White solid (1.36 g, 83%): *mp* 70-70.5°C; R_f 0.37 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (*t*, *J* = 6.7 Hz, 3H, ***H***$_3$C(CH$_2$)$_5$-), 1.04-1.43 (*m*, 75H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{26}$(CH$_2$)$_2$OH), 1.53-1.64 (*m*, 6H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$OH), 2.28 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.64 (*t*, *J* = 6.6 Hz, 2H, -C***H***$_2$OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.9, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.6, 29.7, 29.8, 29.9, 30.1, 32.1, 32.7, 33.0, 34.6, 37.2, 63.2, 64.6, 174.2; ESI-MS *m/z*: 732.8 $(M + H)^+$.

30-Hydroxytriacontyl (10*RS*)-16,16,16-d$_3$-10-methylhexadecanoate (15c)

White solid (1.38 g, 84%): *mp* 70-70.5°C; R_f 0.35 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 1.04-1.44 (*m*, 75H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{26}$(CH$_2$)$_2$OH), 1.53-1.65 (*m*, 6H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$OH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.64 (*t*, *J* = 6.7 Hz, 2H, -C***H***$_2$OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.1, 32.0, 32.9, 33.0, 34.6, 37.3, 63.3, 64.6, 174.2; ESI-MS *m/z*: 711.0 $(M + H)^+$, 732.8 $(M + H)^+$.

30-Hydroxytriacontyl (10*RS*)-16,16,16-d$_3$-10-(methyl-d$_3$) hexadecanoate (15d)

White solid (1.34 g, 82%): *mp* 69-70.5°C; R_f 0.33 ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04-1.43 (*m*, 75H, $D_3C(CH_2)_5CH$-$(CH_2)_6$-, -$(CH_2)_{26}(CH_2)_2OH$), 1.54-1.64 (*m*, 6H, -$CH_2CH_2COOCH_2CH_2$-, -CH_2CH_2OH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -CH_2COO-), 3.64 (*t*, *J* = 6.7 Hz, 2H, -CH_2OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -$COOCH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.6, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.1, 32.0, 32.7, 33.0, 34.6, 37.2, 63.3, 64.6, 174.2; ESI-MS *m/z*: 713.7 $(M + H)^+$, 735.8 $(M + H)^+$, 1447.4 $(2M + Na)^+$.

3.2.10 | Oxidation to the ω-esterified acid

To an ice-cooled mixture of CrO_3 (0.37 g, 3.7 mmol) in 10 N H_2SO_4 (1.90 mL), a solution of **15a-d** (1.20 g, 1.7 mmol) in acetone (20 mL) and $CHCl_3$ (35 mL) was added dropwise over a period of 90 minutes. The mixture was stirred for further 60 minutes at a temperature of 0°C. For working up, brine (25 mL) and aqua (25 mL) were added. The mixture was extracted 3 times with $CHCl_3$ (75 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered and evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography using gradient technique with heptane/$CHCl_3$/Et_2O eluent to give the ω-esterified acids **16a-d**.

30-{[(10*RS*)-10-Methylhexadcanoyl]oxy}triacontanoic acid (16a)

White solid (0.86 g, 70%): *mp* 77-78°C; R_f 0.10 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.5 Hz, 3H, -CH-CH_3), 0.88 (*t*, *J* = 6.8 Hz, 3H, $H_3C(CH_2)_5$-), 1.04-1.35 (*m*, 73H, $H_3C(CH_2)_5CH$-$(CH_2)_6$-, -$(CH_2)_{25}(CH_2)_2COOH$), 1.58-1.67 (*m*, 6H, -$CH_2CH_2COOCH_2CH_2$-, -CH_2CH_2COOH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -CH_2COO-), 2.35 (*t*, *J* = 7.5 Hz, 2H, -CH_2COOH), 4.06 (*t*, *J* = 6.7 Hz, 2H, -$COOCH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.9, 22.9, 24.9, 25.2, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.1, 32.1, 32.9, 33.9, 34.6, 37.3, 64.6, 174.2, 178.6; ESI-MS *m/z*: 719.7 $(M - H)^-$.

30-{[(10*RS*)-10-(Methyl-d$_3$)hexadecanoyl]oxy}triacontanoic acid (16b)

White solid (0.83 g, 68%): *mp* 76.5-77°C; R_f 0.10 ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (*t*, *J* = 6.9 Hz, 3H, $H_3C(CH_2)_5$-), 1.04-1.35 (*m*, 73H, $H_3C(CH_2)_5CH$-$(CH_2)_6$-, -$(CH_2)_{25}(CH_2)_2COOH$), 1.59-1.66 (*m*, 6H, -$CH_2CH_2COOCH_2CH_2$-, -CH_2CH_2COOH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -CH_2COO-), 2.35 (*t*, *J* = 7.5 Hz, 2H, -CH_2COOH), 4.06 (*t*, *J* = 6.7 Hz, 2H, -$COOCH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 24.8, 25.2, 26.1, 27.2, 28.8, 29.3, 29.4, 29.6, 29.7, 29.8, 30.1, 32.1, 32.7, 34.1, 34.6, 37.2, 64.5, 174.2, 179.4; ESI-MS *m/z*: 722.7 $(M - H)^-$.

30-{[(10*RS*)-16,16,16-d$_3$-10-Methylhexadcanoyl]oxy} triacontanoic acid (16c)

White solid (0.78 g, 64%): *mp* 76-78°C; R_f 0.14 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.6 Hz, 3H, -CH-CH_3), 1.04-1.35 (*m*, 73H, $D_3C(CH_2)_5CH$-$(CH_2)_6$-, -$(CH_2)_{25}(CH_2)_2COOH$), 1.58-1.66 (*m*, 6H, -$CH_2CH_2COOCH_2CH_2$-, -CH_2CH_2COOH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -CH_2COO-), 2.35 (*t*, *J* = 7.5 Hz, 2H, -CH_2COOH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -$COOCH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 24.9, 25.2, 26.1, 27.2, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.1, 32.0, 32.9, 34.0, 34.6, 37.3, 64.6, 174.2, 179.1; ESI-MS *m/z*: 722.8 $(M - H)^-$.

30-{[(10*RS*)-16,16,16-d$_3$-10-(Methyl-d$_3$)hexadcanoyl]oxy} triacontanoic acid (16d)

White solid (0.87 g, 71%): *mp* 76-78.5°C; R_f 0.13 ($CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04-1.35 (*m*, 73H, $D_3C(CH_2)_5CH$-$(CH_2)_6$-, -$(CH_2)_{25}(CH_2)_2COOH$), 1.58-1.67 (*m*, 6H, -$CH_2CH_2COOCH_2CH_2$-, -CH_2CH_2COOH), 2.29 (*t*, *J* = 7.5 Hz, 2H, -CH_2COO-), 2.35 (*t*, *J* = 7.5 Hz, 2H, -CH_2COOH), 4.06 (*t*, *J* = 6.7 Hz, 2H, -$COOCH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.6, 24.9, 25.2, 26.1, 27.2, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.1, 32.0, 32.7, 34.0, 34.6, 37.2, 64.6, 174.2, 179.0; ESI-MS *m/z*: 727.2 $(M - H)^-$.

3.2.11 | Coupling to the methyl-branched ceramide [mEOS]*

DIPEA (94 μL, 0.55 mmol) was added to a stirred suspension of **16a-d** (200 mg, 0.28 mmol) and PyBOP (157 mg, 0.30 mmol) in dry CH_2Cl_2 (5 mL). After 15 minutes, sphingosine (91 mg, 0.30 mmol) was added to the clear solution and the mixture was stirred for further 16 hours. Afterwards, the solvent was evaporated and the residue was purified by column chromatography using gradient technique with $CHCl_3$/MeOH eluent to give the ceramides **Ia-d**.

***N*-[(2*S*,3*R*,4*E*)-1,3-Dihydroxyoctadec-4-en-2-yl]-30-{[(10*RS*)-10-methylhexadecanoyl]oxy}-triacontanamid (CER[mEOS] Ia)**

White waxy solid (245 mg, 88%): *mp* 84.5-86°C; R_f 0.42 ($CHCl_3$/MeOH 95:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.5 Hz, 3H, -CH-CH_3), 0.88 (*t*, *J* = 6.7 Hz, 6H, 2× H_3CCH_2-), 1.04-1.38 (*m*, 95H, $H_3C(CH_2)_5CH$-$(CH_2)_6$-, -$(CH_2)_{25}(CH_2)_2CONH$-, -$(CH_2)_{11}CH_2CH$=CH-), 1.57-1.64

*Differing to the IUPAC nomenclature, the name is based on the amid-bonding backbone of ceramides.

(*m*, 6H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-), 2.02-2.07 (*m*, 2H, -C***H***$_2$CH=CH-), 2.22 (*t*, *J* = 7.6 Hz, 2H, -C***H***$_2$CONH-), 2.28 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.69 (dd, *J* = 2.5, 10.6 Hz, 1H, -C***H***H′OH), 3.88-3.95 (*m*, 2H, -C***H***-CH***H′***OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.28-4.31 (*m*, 1H, -CH=CHC***H***OH-), 5.52 (dd, *J* = 6.4, 15.4 Hz, 1H, -CH$_2$CH=C***H***-), 5.74-5.81 (*m*, 1H, -CH$_2$C***H***=CH-), 6.28 (d, *J* = 7.2 Hz, 1 H, -CON***H***-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.9, 22.8, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.7, 29.8, 29.9, 30.1, 32.1, 32.4, 32.9, 34.6, 37.0, 37.2, 37.3, 54.7, 62.6, 64.6, 74.7, 129.0, 134.3, 174.1, 174.2; HRMS [M + Na]$^+$ *m/z* calcd for $C_{65}H_{127}NO_5Na$ 1024.9606, found 1024.9588; HPLC purity >99.5%.

***N*-[(2*S*,3*R*,4*E*)-1,3-Dihydroxyoctadec-4-en-2-yl]-30-{[(10*RS*)-10-(methyl-d$_3$)hexadecanoyl]-oxy}triacontanamid (CER[mEOS] *m*-d$_3$ Ib)**

White waxy solid (233 mg, 84%): *mp* 83-84°C; R_f 0.46 ($CHCl_3$/MeOH 95:5); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87-0.89 (*m*, 6H, 2× ***H***$_3$CCH$_2$-), 1.04-1.38 (*m*, 95H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{11}$CH$_2$CH=CH-), 1.58-1.67 (*m*, 6H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-), 2.04-2.08 (*m*, 2H, -C***H***$_2$CH=CH-), 2.23 (*t*, *J* = 7.6 Hz, 2H, -C***H***$_2$CONH-), 2.29 (*t*, *J* = 7.4 Hz, 2H, -C***H***$_2$COO-), 3.71 (dd, *J* = 3.3, 11.2 Hz, 1H, -C***H***H′OH), 3.91 (d*t*, 2H, *J* = 3.7, 11.2 Hz, 1H, -C***H***-CH$_2$OH), 3.96 (dd, *J* = 3.8, 11.2 Hz, 2H, CH***H′***OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.31-4.33 (*m*, 1H, -CH=CHC***H***OH-), 5.53 (dd, *J* = 6.4, 15.5 Hz, 1H, -CH$_2$CH=C***H***-), 5.76-5.81 (*m*, 1H, -CH$_2$C***H***=CH-), 6.23 (d, *J* = 7.3 Hz, 1 H, -CON***H***-); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.9, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.7, 29.8, 29.9, 30.1, 32.1, 32.4, 32.7, 34.6, 37.0, 37.2, 37.3, 54.7, 62.7, 64.6, 74.9, 129.0, 134.5, 174.0, 174.2; HRMS [M + Na]$^+$ *m/z* calcd for $C_{65}H_{124}D_3NO_5Na$ 1027.9795, found 1027.9777; HPLC purity 98.5%.

***N*-[(2*S*,3*R*,4*E*)-1,3-Dihydroxyoctadec-4-en-2-yl]-30-{[(10*RS*)-16,16,16-d$_3$-10-methyl-hexadecanoyl]oxy}triacontanamid (CER[mEOS] *t*-d$_3$ Ic)**

White waxy solid (235 mg, 84%): *mp* 82.5-84°C; R_f 0.53 ($CHCl_3$/MeOH 95:5); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.6 Hz, 3H, -CH-C***H***$_3$), 0.88 (*t*, *J* = 7.0 Hz, 3H, ***H***$_3$CCH$_2$-), 1.04-1.38 (*m*, 95H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{11}$CH$_2$CH=CH-), 1.58-1.67 (*m*, 6H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-), 2.03-2.07 (*m*, 2H, -C***H***$_2$CH=CH-), 2.23 (*t*, *J* = 7.6 Hz, 2H, -C***H***$_2$CONH-), 2.28 (*t*, *J* = 7.6 Hz, 2H, -C***H***$_2$COO-), 3.70 (dd, *J* = 3.3, 11.7 Hz, 1H, -C***H***H′OH), 3.90 (d*t*, 2H, *J* = 3.7, 11.3 Hz, 1H, -C***H***-CH$_2$OH), 3.96 (dd, 2H, *J* = 3.5, 11.2 Hz, 1H -CH***H′***OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.30-4.32 (*m*, 1H, -CH=CHC***H***OH-), 5.53 (dd, *J* = 6.3, 15.5 Hz, 1H, -CH$_2$CH=C***H***-), 5.75-5.81 (*m*, 1H, -CH$_2$C***H***=CH-), 6.33 (d, *J* = 4.5 Hz, 1H, -CON***H***-); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 19.9, 22.6, 22.9, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.7, 29.8, 29.9, 30.1, 32.1, 32.5, 32.9, 34.6, 37.0, 37.2, 37.3, 54.7, 62.7, 64.6, 74.8, 129.0, 134.3, 174.0, 174.2; HRMS [M + Na]$^+$ *m/z* calcd for $C_{65}H_{124}D_3NO_5Na$ 1027.9795, found 1027.9779; HPLC purity 99.5%.

***N*-[(2*S*,3*R*,4*E*)-1,3-Dihydroxyoctadec-4-en-2-yl]-30-{[(10*RS*)-16,16,16-d$_3$-10-(methyl-d$_3$)-hexadecanoyl]oxy} triacontanamid (CER[mEOS] *m*,*t*-d$_3$ Id)**

White waxy solid (257 mg, 93%): *mp* 84-85.5°C; R_f 0.52 ($CHCl_3$/MeOH 95:5); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (*t*, *J* = 7.0 Hz, 3H, ***H***$_3$C-), 1.04-1.38 (*m*, 95H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{11}$CH$_2$CH=CH-), 1.58-1.66 (*m*, 6H, -C***H***$_2$CH$_2$COO CH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-), 2.03-2.07 (*m*, 2H, -C***H***$_2$CH=CH-), 2.22 (*t*, *J* = 7.7 Hz, 2H, -C***H***$_2$CONH-), 2.28 (*t*, *J* = 7.6 Hz, 2H, -C***H***$_2$COO-), 3.70 (dd, *J* = 3.3, 11.2 Hz, 1H, -C***H***H′OH), 3.90 (d*t*, 2H, *J* = 3.6, 11.2 Hz, 1H, -C***H***-CH$_2$OH), 3.95 (dd, 2H, *J* = 3.8, 11.2 Hz, 1H, -CH***H′***OH), 4.05 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.30-4.32 (*m*, 1H, -CH=CHC***H***OH-), 5.53 (dd, *J* = 6.4, 15.4 Hz, 1H, -CH$_2$CH=C***H***-), 5.75-5.81 (*m*, 1H, -CH$_2$C***H***=CH-), 6.33 (d, *J* = 7.1 Hz, 1H, -CON***H***-); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.6, 22.9, 25.2, 25.9, 26.1, 27.2, 28.8, 29.3, 29.4, 29.5, 29.7, 29.8, 29.9, 30.1, 32.0, 32.1, 32.4, 32.7, 34.6, 37.0, 37.2, 54.6, 62.7, 64.6, 74.8, 129.0, 134.4, 174.1, 174.2; HRMS [M + Na]$^+$ *m/z* calcd for $C_{65}H_{121}D_6NO_5Na$ 1030.9983, found 1030.9970; HPLC purity 99.5%.

3.2.12 | Coupling to the methyl-branched ceramide [mEOP]

DIPEA (94 µL, 0.55 mmol) was added to a stirred suspension of **16a-d** (200 mg, 0.28 mmol) and PyBOP (157 mg, 0.30 mmol) in dry CH_2Cl_2 (5 mL). After 15 minutes, phytosphingosine (96 mg, 0.30 mmol) was added to the clear solution and the mixture was stirred for further 16 hours. Afterwards, the formed precipitate was filtered and the filter was washed 2 times with CH_2Cl_2 (2 mL). For purification, the residue was adsorbed on silica gel before column chromatography using gradient technique with $CHCl_3$/MeOH and NH_3 (0.5%) eluent to give the ceramides **IIa-d**.

***N*-[(2*S*,3*S*,4*R*)-1,3,4-Trihydroxyoctadec-2-yl]-30-{[(10*RS*)-10-methylhexadecanoyl]oxy}-triacontanamid (CER[mEOP] IIa)**

White waxy solid (227 mg, 80%): *mp* 102-103°C; R_f 0.21 ($CHCl_3$/MeOH/NH_3 95:5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 40°C) δ 0.84 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 0.89 (*t*, *J* = 6.7 Hz, 6H, 2× ***H***$_3$CCH$_2$-), 1.05-1.36 (*m*, 97H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{12}$CH$_2$CHOH-), 1.48-1.78 (*m*, 8H,

-C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-, -C***H***$_2$CHOH-), 2.24 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$CONH-), 2.29 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.58-3.68 (*m*, 2H, -CH$_2$C***H***OHC***H***OH-), 3.76 (dd, *J* = 5.3, 11.6 Hz, 1H, -C***H***H′OH), 3.95 (dd, *J* = 2.7, 11.6 Hz, 1H, -CH***H***′OH), 4.06 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.12-4.16 (*m*, 1H, -C***H***-CH$_2$OH), 6.32 (d, *J* = 7.6 Hz, 1H, -CON***H***-); ^{13}C NMR (100 MHz, THF-d$_8$, 40°C) δ 14.6, 20.3, 23.7, 26.8, 26.9, 27.1, 28.1, 28.2, 29.9, 30.2, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 31.0, 31.1, 33.0, 34.0, 34.5, 34.9, 37.0, 38.2, 54.0, 62.7, 64.7, 73.4, 77.4, 173.4; HRMS [M + Na]$^+$ *m/z* calcd for $C_{65}H_{129}NO_6Na$ 1042.9712, found 1042.9694; HPLC purity >99.5%.

N-[(2*S*,3*S*,4*R*)-1,3,4-Trihydroxyoctadec-2-yl]-30-{[(10*RS*)-10-(methyl-d$_3$)-hexadecanoyl]-oxy}triacontanamid (CER[mEOP] *m*-d$_3$ IIb)

White waxy solid (223 mg, 79%): *mp* 101-103°C; R_f 0.25 ($CHCl_3$/MeOH/NH_3 95:5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 40°C) δ 0.89 (*t*, *J* = 6.7 Hz, 6H, 2× ***H***$_3$CCH$_2$-), 1.05-1.36 (*m*, 97H, H$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{12}$CH$_2$CHOH-), 1.45-1.82 (*m*, 8H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-, -C***H***$_2$CHOH-), 2.24 (*t*, *J* = 7.6 Hz, 2H, -C***H***$_2$CONH-), 2.29 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.59-3.67 (*m*, 2H, -CH$_2$C***H***OHC***H***OH-), 3.76 (dd, *J* = 5.43, 11.3 Hz, 1H, -C***H***H′OH), 3.95 (dd, *J* = 2.2, 11.4 Hz, 1H, -CH***H***′OH), 4.06 (*t*, *J* = 6.8 Hz, 2H, -COOC***H***$_2$-), 4.13-4.17 (*m*, 1H, -C***H***-CH$_2$OH), 6.32 (d, *J* = 7.8 Hz, 1H, -CON***H***-); ^{13}C NMR (100 MHz, THF-d$_8$, 40°C) δ 14.6, 23.7, 26.8, 27.0, 27.1, 28.1, 28.2, 29.9, 30.2, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 31.0, 31.1, 33.0, 33.7, 34.5, 34.9, 37.0, 38.2, 54.1, 62.7, 64.7, 73.4, 77.4, 173.4; HRMS [M + Na]$^+$ *m/z* calcd for $C_{65}H_{129}NO_6Na$ 1042.9712, found 1042.9694; HPLC purity >99.5%.

N-[(2*S*,3*S*,4*R*)-1,3,4-Trihydroxyoctadec-2-yl]-30-{[(10*RS*)-16,16,16-d$_3$-10-methylhexa-decanoyl]oxy}triacontanamid (CER[mEOP] *t*-d$_3$ IIc)

White waxy solid (215 mg, 76%): *mp* 103-105°C; R_f 0.22 ($CHCl_3$/MeOH/NH_3 95:5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 40°C) δ 0.84 (d, *J* = 6.5 Hz, 3H, -CH-C***H***$_3$), 0.89 (*t*, *J* = 6.8 Hz, 3H, ***H***$_3$CCH$_2$-), 1.05-1.37 (*m*, 97H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{12}$CH$_2$CHOH-), 1.44-1.81 (*m*, 8H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-, -C***H***$_2$CHOH-), 2.24 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$CONH-), 2.29 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.58-3.67 (*m*, 2H, -CH$_2$C***H***OHC***H***OH-), 3.77 (dd, *J* = 5.3, 11.2 Hz, 1H, -C***H***H′OH), 3.96 (dd, *J* = 1.9, 11.2 Hz, 1H, -CH***H***′OH), 4.06 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.12-4.16 (*m*, 1H, -C***H***-CH$_2$OH), 6.32 (d, *J* = 7.3 Hz, 1 H, -CON***H***-); ^{13}C NMR (100 MHz, THF-d$_8$, 40°C) δ 14.6, 20.3, 23.5, 23.7, 26.8, 27.0, 27.1, 28.2, 29.9, 30.3, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 31.0, 31.1, 33.0, 34.0, 34.5, 34.9, 37.0, 38.2, 54.0, 62.7, 64.7, 73.4, 77.4, 173.4; HRMS [M + H]$^+$ *m/z* calcd for $C_{65}H_{127}D_3NO_6$ 1024.0081, found 1024.0099; HPLC purity >99.5%.

N-[(2*S*,3*S*,4*R*)-1,3,4-Trihydroxyoctadec-2-yl]-30-{[(10*RS*)-16,16,16-d$_3$-10-(methyl-d$_3$)-hexa-decanoyl]oxy} triacontanamid (CER[mEOP] *m*,*t*-d$_6$ IId)

White waxy solid (217 mg, 77%): *mp* 100-103°C; R_f 0.18 ($CHCl_3$/MeOH/NH_3 95:5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 40°C) δ 0.89 (*t*, *J* = 6.9 Hz, 3H, ***H***$_3$CCH$_2$-), 1.05-1.36 (*m*, 97H, D$_3$C(C***H***$_2$)$_5$C***H***-(C***H***$_2$)$_6$-, -(C***H***$_2$)$_{25}$(CH$_2$)$_2$CONH-, -(C***H***$_2$)$_{12}$CH$_2$CHOH-), 1.47-1.82 (*m*, 8H, -C***H***$_2$CH$_2$COOCH$_2$C***H***$_2$-, -C***H***$_2$CH$_2$CONH-, -C***H***$_2$CHOH-), 2.24 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$CONH-), 2.29 (*t*, *J* = 7.5 Hz, 2H, -C***H***$_2$COO-), 3.58-3.67 (*m*, 2H, -CH$_2$C***H***OHC***H***OH-), 3.76 (dd, *J* = 5.6, 11.5 Hz, 1H, -C***H***H′OH), 3.96 (dd, *J* = 2.4, 11.5 Hz, 1H, -CH***H***′OH), 4.06 (*t*, *J* = 6.7 Hz, 2H, -COOC***H***$_2$-), 4.12-4.16 (*m*, 1H, -C***H***-CH$_2$OH), 6.32 (d, *J* = 7.3 Hz, 1H, -CON***H***-); ^{13}C NMR (100 MHz, THF-d$_8$, 40°C) δ 14.6, 23.4, 23.7, 26.8, 26.9, 27.1, 28.1, 28.2, 29.9, 30.2, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 31.0, 31.1, 33.0, 33.7, 34.5, 34.9, 37.0, 38.2, 54.0, 62.7, 64.7, 73.4, 77.4, 173.4; HRMS [M + H]$^+$ *m/z* calcd for $C_{65}H_{124}D_3NO_6$ 1027.0269, found 1027.0261; HPLC purity >99.5%.

3.3 | Sample preparation

The SC model membranes were prepared on quartz glass slides. For the sample application, the pure substances were solved in a 2:1 mixture of chloroform and methanol (analytical grade, by Sigma-Aldrich) with a concentration of 10 mg/mL. These solutions were then combined, to generate CER[NS]/CER[EOS]-br/CHOL/FFA mixtures with a molar ratio of 0.8/0.2/0.7/1, see Table 1. For deposition, the mixtures were sprayed onto the surface, using an airbrush instrument (Harder & Steenbeck, Norderstedt, Germany).

3.4 | Neutron diffraction experiments

Diffraction data were collected at the membrane diffractometer V1 located at the Hahn-Meitner campus of the Helmholtz-Zentrum Berlin. Neutrons generated by the research reactor BER II were cooled to a thermal level, using an H_2 cold source. A gallium monochromator was used, to filter neutrons with a wavelength of λ = 4.567 Å. The distance between sample and detector for this device was 101.8 cm. The intensity of the scattered neutrons was recorded as a function of the detector angle 2Θ. For contrasting against the water background, the 3 different H_2O/D_2O ratios of 0:100, 50:50, and 92:8 (*w*/w) were applied. A higher D_2O content also generates a higher signal-to-background ratio, which allows for detection of peaks of higher diffraction orders with high precision. These higher order peaks are very

TABLE 1 Measurement conditions for the neutron scattering measurements

Mixture	x, mol/mol	ϑ, °C	R.H., %	D_2O, %
NS(18)/EOS-br/CHOL/SA	0.8/0.2/0.7/1	32	98	98/50/8
NS(18)-D3/EOS-br/CHOL/SA	0.8/0.2/0.7/1	32	98	98/50/8
NS(18)/EOS-br-D3/CHOL/SA	0.8/0.2/0.7/1	32	98	98/50/8
NS(18)/EOS-D3-br/CHOL/SA	0.8/0.2/0.7/1	32	98	98/50/8

important for calculation of a detailed scattering length density profile.

Conditions according to Table 1 were applied for the measurements. For the base system and the sample containing the CER[EOS]-d_3-br, an additional measurement at a hydration of 57%, which is about the natural moisture content of the skin, and 100% D_2O, which gives the best signal, was performed to investigate possible swelling under excess hydration. Temperature was kept at 32°C, which is about the average temperature of the human skin. Since it could be observed that no more changes in the diffraction signal occurred after about 6 hours of equilibration, samples were given a time of at least 7 hours after any change of temperature, humidity, or H_2O/D_2O ratio. To determine the membrane spacing, the scattering vector Q was calculated. Q is dependent of the scattering angle by $Q = \frac{4\pi \sin(\Theta)}{\lambda}$; it is a result of the incoming wave vector $\vec{k_i}$ and the scattering vector $\vec{k_s}$, with λ being the neutron wavelength. The repeat distance d of a series of equidistant peaks Q_n is then calculated as $d = \frac{2n\pi}{Qn}$, with n being the diffraction order. The neutron scattering length density profile as function $p_s(x)$ was calculated by Fourier synthesis of the structure factors F_h according to

$$p_s(x) = a + b\frac{2}{d}\sum_{n=1}^{n_{max}} F_n\left(\frac{2\pi n x}{d}\right).$$

The coefficients a and b are for relative normalisation of $p_s(x)$, d is the lamellar periodicity, and n the order of diffraction. The absolute values of the structure factors could be calculated as $|F_n| = \sqrt{hA_nI_n}$, with h as the Lorentz correction, A_n as absorption correction,[36] and I_n as intensity of the nth peak. For calculation of $p_s(x)$, at least 3 diffraction orders have to be determined. Contrasting with D_2O as mentioned above can be used, to determine diffractions of a higher order. Necessary Bragg peak determination and integration were performed using the IGOR Pro 6.1 software (WaveMetrics Inc., Portland, Oregon). For the Fourier transform, both the absolute value and the phase of each structure factor F_n are required. Assuming a Gaussian water distribution with a maximum position $x = \frac{d}{2}$ within the hydrophilic head group region allows determination of the F_n by application of different H_2O/D_2O contrast, representing an *isomorphous replacement*.[36,37] The centrosymmetric lipid arrangement that is to be expected of CER membranes leads to a phase of either + or – for each F_n. A 3-point measurement with 3 different H_2O/D_2O contrasts is thus sufficient for the calculation of the phase of each structure factor. A detailed description of this procedure (contrast variation) and of the neutron diffraction data evaluation can be assessed elsewhere.[36,38,39]

4 | CONCLUSION

Long chained ceramides [EOS] and [EOP] containing a methyl-branched fatty acid instead of linoleic acid were synthesized. Besides, the full protonated ceramides, a specifically deuteration in the branched methyl group and/or at the end of the fatty acid, were introduced. Two methods for the preparation of the methyl-branched fatty acid were presented, a classic method by dialkylation of diethyl malonate and a shorter method by α-alkylation of an acid dianion, which needs some higher demands to the equipment. For the ester formation of the branched fatty acid with a diol, a modification of the "normal acylation" procedure was necessary because of the insolubility of the diol at low reaction temperatures. Regarding the synthesis of natural CER[EOS] or CER[EOP] with linoleic acid, the preparation of the derivatives is possible in a shorter way without a complex blocking group strategy. For the amid formation of the methyl-branched ω-acyl ceramides, PyBOP was used as coupling reagent, which ensures high yield and a simple workup. First results for the neutron scattering of a CER[NS]-based system, containing the 2 variants of the synthesized deuterated CER[EOS]-br, were already obtained. These results clearly show that the CER[EOS]-br is incorporated into the SPP of CER-based model membranes. It can be used to obtain further information on the lamellar nanostructure. It was even possible to distinguish the 2 differently deuterated long-chain ceramides.

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