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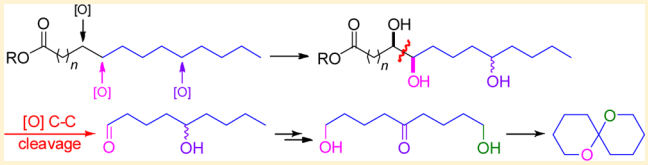
Oxidative Carbon–Carbon Bond Cleavage Is a Key Step in Spiroacetal Biosynthesis in the Fruit Fly *Bactrocera cacuminata*

Arti A. Singh, Jessica A. Rowley, Brett D. Schwartz, William Kitching, and James J. De Voss*

School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane 4072, Australia

Supporting Information

ABSTRACT: The early steps of spiroacetal biosynthesis in the fruit fly *Bactrocera cacuminata* (Solanum fly) have been investigated using a series of deuterium-labeled, oxygenated fatty acid like compounds. These potential spiroacetal precursors were administered to male flies, and their volatile emissions were analyzed for specific deuterium incorporation by GC/MS. This has allowed the order of early oxidative events in the biosynthetic pathway to be determined. Together with the already well-established later steps, the results of these in vivo investigations have allowed essentially the complete delineation of the spiroacetal biosynthetic pathway, beginning from products of primary metabolism. A fatty acid equivalent undergoes a series of enzyme-mediated oxidations leading to a trioxygenated fatty acid like species that includes a vicinal diol. This moiety then undergoes enzyme-mediated oxidative carbon–carbon bond cleavage as the key step to generate the C_9 unit of the final spiroacetal. This is the first time such an oxidative transformation has been reported in insects. A final hydroxylation step is followed by spontaneous spiro-cyclization. This distinct pathway adds further to the complexity and diversity of biosynthetic pathways to spiroacetals.



INTRODUCTION

The spiroacetal motif is found within many natural products isolated from terrestrial and marine, prokaryotic and eukaryotic sources. Simple spiroacetals are common in insects of the orders Diptera (true flies), Hymenoptera (wasps, ants, and bees) and Coleoptera (beetles) and are usually found as components of volatile semiochemical secretions.[1] 1,7-Dioxaspiro[5.5]undecane (**1**) was the first spiroacetal to be identified as an insect sex pheromone when it was isolated as the major component of the blend emitted by female *Bactrocera oleae* (Olive fly).[2] Over 30 structurally different spiroacetals, including hydroxylated and alkylated ones, have since been discovered and characterized,[1] with disubstituted **2** being most commonly observed in nature. While these deceptively simple structures have received a great deal of synthetic attention,[1−3] relatively little is known about their biosynthesis. Understanding sex pheromone biogenesis may form the basis for species-specific methods of pest control, and thus, there is interest in the biochemical origin of spiroacetals. Their biosynthesis in *Bactrocera* sp. has been investigated because of the highly pestiferous nature and economic importance of *B. oleae* and *B. tryoni* (Queensland fruit fly). Studies to date with these and other insects have indicated a surprising degree of complexity and diversity in the construction of such simple molecules.

The major spiroacetal produced by female *B. tryoni*,[4] male *B. cucumis* (Cucumber fly),[5] and female *Megarhyssa nortoni nortoni* (Giant Ichneumon Wasp)[6] is (*E*,*E*)-**2**. Isotopic labeling studies with these species have led to the delineation of three distinct, yet related, biosynthetic pathways to **2** from primary metabolites.[1,4−7] These pathways (Scheme 1) all involve a penultimate carbon hydroxylation step but display species-specific differences in their early steps and in the origin of the oxygen atoms of **2**. ^{18}O- and deuterium-labeling studies indicated that while all three pathways involve the oxidation of long-chain fatty acid equivalents (e.g., fatty acids or thioesters) to produce a common intermediate, 2,6-undecanedione (**3**), the sequence of steps that leads to **3** is distinctly different in each species.[1,4−7] Despite this, the steps from **3** to spiroacetal **2** are identical in all three species.[4,6,7] The derivation of at least one oxygen atom of **2** from O_2 suggested the involvement of the oxidative cytochrome P450 enzymes (P450s) in these biosynthetic pathways.

Only the later stages of the biosynthesis of the unsubstituted C_9 spiroacetal **1** in female *B. oleae* and male *B. cacuminata* (Solanum fly) are well established and bear a strong resemblance to those in the biosynthesis of **2** (Scheme 1). The biogenesis of **1** (Scheme 2) has so far proven to be identical in both species.[8] 5-Hydroxynonanal (**4**) undergoes reduction to form 1,5-nonanediol (**5**), which is then oxidized at C-9 (ω oxidation)[9] to give 1,5,9-nonanetriol (**6**). Further oxidation of triol **6** affords *meso* ketodiol **7**, which cyclizes to afford spiroacetal **1** as a racemate. Hydroxyspiroacetals formed by oxidation of **1** have been detected in both species.[5,10] These results have led to the proposal of a general paradigm for spiroacetal biosynthesis that appears to be applicable across insect genera: generation of a dioxygenated precursor via the oxidation/modification of a long chain fatty acid equivalent is

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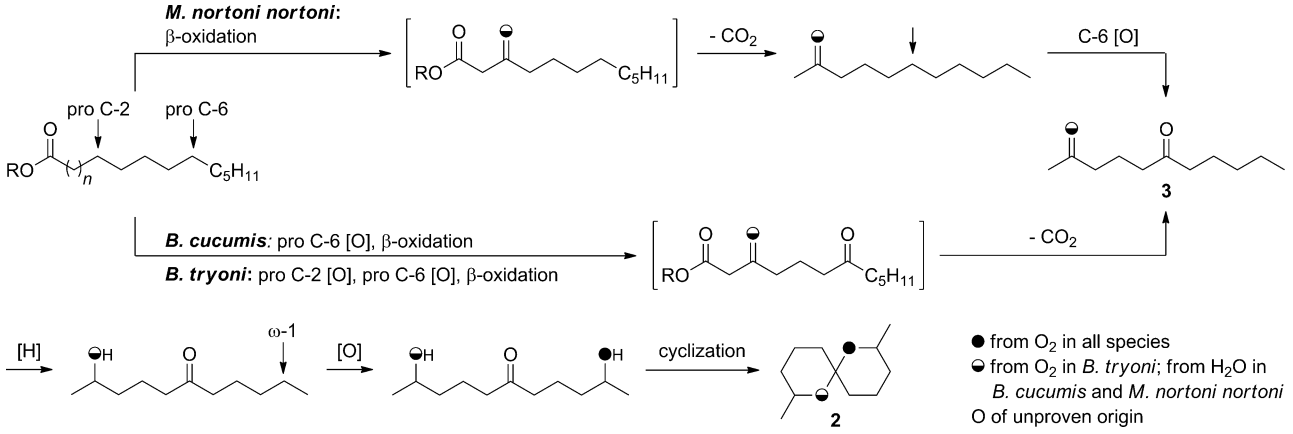
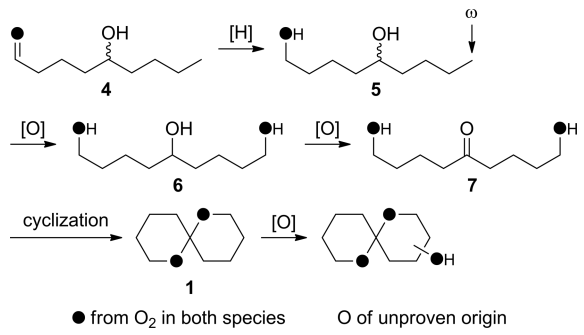
Scheme 1. Biosynthesis of Spiroacetal 2 in *M. nortoni nortoni*, *B. cucumis*, and *B. tryoni*, Showing the Origin of the Oxygen Atoms[1,4−7a]



[a]"Pro C-2" refers to the carbon atom of the fatty acid equivalent that becomes C-2 of 2,6-undecanedione (**3**) and subsequently C-2 (or C-10) of spiroacetal **2**, and "pro C-6" refers to the carbon atom that becomes C-6 of **3** and subsequently the spirocenter of **2**.

Scheme 2. Established Later Steps in the Biosynthesis of Spiroacetal 1 in *B. cacuminata* and *B. oleae*, Showing the Origin of the Oxygen Atoms[5−8,10]



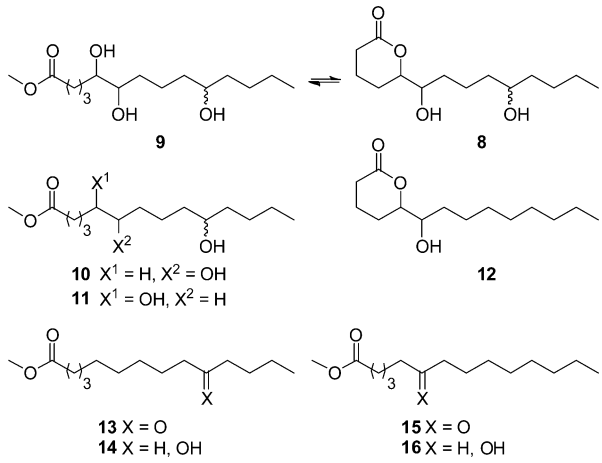


Figure 1. Synthetic targets in unlabeled form.

followed by carbon hydroxylation and cyclization of a resultant ketodiol as the final step.[6,7]

Both oxygen atoms in **1** are derived from O_2 in *B. oleae*[10] and *B. cacuminata*[5] (cf. spiroacetal **2** in *B. tryoni*), indicating that the C_9 unit of **1** does not come directly from a fatty acid or polyketide biosynthetic pathway. This strongly suggests the involvement of P450s in the generation of **4** from a long-chain fatty acid equivalent in these species. It was thus hypothesized[8] that **4** arose within these flies via P450-mediated oxidative carbon−carbon bond cleavage of a saturated chain. The majority of research into insect P450s to date has focused on their roles in xenobiotic metabolism (especially insecticide metabolism), although it has been suggested that approximately 35−50% of P450s found within an insect species are likely to be involved in hormone and pheromone production.[11]

Herein, we describe our investigations into the early steps of the biosynthetic pathway of spiroacetal **1** in male *B. cacuminata*, specifically focused on the involvement and stereoselectivity of the proposed oxidative C−C bond cleavage step.

RESULTS AND DISCUSSION

Several oxygenated fatty acid like compounds (Figure 1) were synthesized in deuterium-labeled form to investigate the biosynthetic steps that lead to the formation of 5-hydroxynonanal (**4**) and, thus, spiroacetal **1**, in male *B. cacuminata*. Carboxylic acids exist in their ionized form (i.e., are negatively charged) at biological pH and are unable to cross biological membranes easily. Thus, methyl esters or lactones were synthesized and administered in order to promote successful passage of the compounds across cell membranes and to render them accessible to enzymes in vivo. While fatty acid metabolizing P450s often have preferred substrate chain lengths,[12,13] the chain length specificity of the spiroacetal biosynthetic enzymes of *B. cacuminata* and *B. oleae* has not yet been investigated. The C_{14} chain length employed here was chosen because it was thought that longer chain lengths, closer to dietary fatty acids, might be more susceptible to catabolism by processes such as β-oxidation.

A series of stereoisomeric dihydroxylactones [$^2H_4$]-**8**/trihydroxyesters [$^2H_4$]-**9** (biosynthetically equivalent to trihydroxy-fatty acids) with defined vicinal diol stereochemistry was used to probe the involvement and stereoselectivity of an oxidative C−C bond cleavage step. Based on the results of feeding experiments with these compounds (vide infra), several compounds with dioxygenated ([$^2H_4$]-**10**, [$^2H_4$]-**11**, and [$^2H_2$]-**12**) and monooxygenated chains ([$^2H_4$]-**13**, [$^2H_4$]-**14**, [$^2H_4$]-**15**, and [$^2H_4$]-**16**) were also synthesized and employed to investigate the order of oxidative events that lead to the formation of a trioxygenated chain species.

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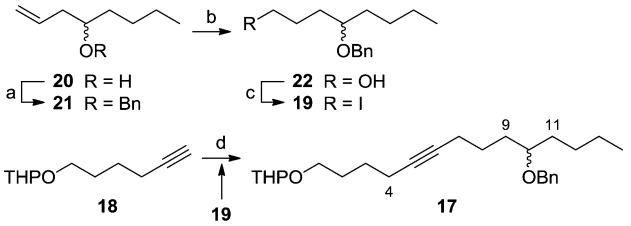
Synthesis of Compounds with Trioxygenated Chains. Compound **17** was the key intermediate in the construction of the isomeric 6,10-dihydroxylactones **8**/5,6,10-trihydroxyesters **9**, both in deuterium-labeled and unlabeled form. The alkyne moiety of **17** allowed formation of both (*E*)- and (*Z*)-alkenes, which were required for the stereoselective introduction of the 5,6-*threo* or *erythro* vicinal diol moieties; the protected alcohols at C-1 and C-10 were subsequently transformed into the desired ester and ketone functionalities, respectively. The ketone at C-10 allowed for regiospecific deuterium-labeling via base-catalyzed exchange and its chemoselective reduction provided the desired C-10 hydroxyl group; the stereochemistry of this is currently believed to be unimportant and thus was not controlled. Introduction of the *threo* or *erythro* vicinal diol moieties via osmium-mediated oxidation was initially envisioned as the final synthetic step.

Alkyne **17** was obtained by coupling of the anion of THP-protected 5-hexyn-1-ol (**18**)[7] and benzyl-protected 1-iodooctan-4-ol (**19**). The synthesis of iodide **19** commenced with racemic 1-octen-4-ol (**20**),[14] the hydroxyl group of which was easily protected as the benzyl ether to give **21** in 84% yield (Scheme 3). Hydroboration−oxidation of **21** afforded the primary alcohol **22** (87% yield), which was subsequently converted to the desired iodide **19** (I_2, PPh_3, imidazole, 98% yield). The terminal alkyne **18** was then deprotonated (*n*-BuLi, −40 °C), and the resulting anion was reacted with iodide **19** in the presence of HMPA to afford the key intermediate **17** in 83% yield.

Scheme 3. Synthesis of 17 from 1-Octen-4-ol (20) and Alkyne 18[a]



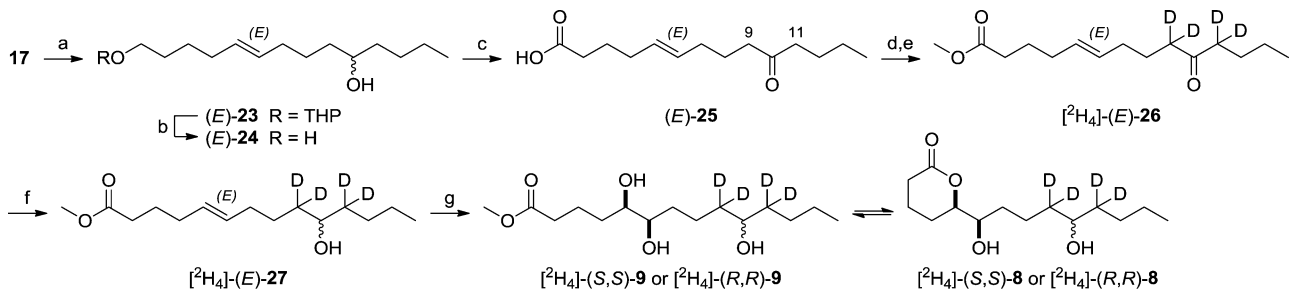
[a]Reagents and conditions: (a) (i) NaH, THF, 0 °C, (ii) BnBr, tetra-*n*-butylammonium iodide, rt, 84%; (b) (i) BH_3·DMS, CH_2Cl_2, 0 °C, (ii) NaOH, H_2O_2, rt, 87%; (c) I_2, PPh_3, imidazole, CH_3CN/Et_2O (1:3), 0 °C to rt, 98%; (d) (i) *n*-BuLi, THF, −40 °C, (ii) HMPA, iodide **19**, −40 °C to rt, 83%.

***Threo* Isomers.** Synthesis of *threo* [$^2H_4$]-(*S*,*S*)-**8**/**9** and [$^2H_4$]-(*R*,*R*)-**8**/**9** then commenced with lithium/liquid ammonia-mediated reduction of **17** (Scheme 4). This converted the alkyne to the desired (*E*)-alkene and simultaneously removed the benzyl ether to afford (*E*)-**23** in 93% yield. Elaboration of (*E*)-**23** via a series of standard transformations including base-catalyzed deuterium exchange then afforded the *δ*,*ε*-(*E*)-unsaturated hydroxyester [$^2H_4$]-(*E*)-**27** in good yield over five steps. Sharpless' asymmetric dihydroxylation (SAD)[15] of [$^2H_4$]-(*E*)-**27** then gave the *threo* diols [$^2H_4$]-(*S*,*S*)-**8**/**9** and [$^2H_4$]-(*R*,*R*)-**8**/**9**. Dihydroxylactones [$^2H_4$]-(*S*,*S*)-**8** and [$^2H_4$]-(*R*,*R*)-**8** presumably arose from the in situ lactonization of the initially formed trihydroxyesters [$^2H_4$]-(*S*,*S*)-**9** and [$^2H_4$]-(*R*,*R*)-**9**. However, difficulties were encountered in the purification and isolation of **8**/**9**, attributed to ring-opening or hydrolysis of **8** during work-up and/or chromatographic purification. An alternative synthetic route that circumvented these difficulties was thus developed.

Enantioselective dihydroxylation was again the key step, but the vicinal diol was introduced early during the synthesis and protected as the corresponding isopropylidene ketal. It was envisioned that acid-catalyzed cleavage of the ketal as the final synthetic step would produce only a volatile byproduct and would circumvent the requirement for chromatographic purification. Alkene (*E*)-**23** was thus reacted with either AD-mix-*α* or AD-mix-*β* (Scheme 5) under standard SAD conditions to form (5*S*,6*S*)- and (5*R*,6*R*)-diol moieties, respectively, based upon the Sharpless predictive model.[15] Both the desired 5,6,10-triols (*S*,*S*)-**28** and (*R*,*R*)-**28** were obtained in 95% yield, thus providing the 1,5,6,10-tetraoxygenated C_{14} skeleton required for the subsequent formation of the 5,6-*threo* isomers of **8**/**9**.[16]

Synthesis of the desired (*S*,*S*)- and (*R*,*R*) compounds, *threo*-**8**/**9**, was then carried out in parallel using identical methodology. Acid-catalyzed THP-deprotection of *threo*-**28** cleanly afforded the tetraol *threo*-**29** in excellent yield (Scheme 5), and the vicinal diol moiety was selectively protected as an isopropylidene ketal, *threo*-**30**. Oxidation (PDC, DMF) of *threo*-**30** to the ketoacid *threo*-**31** and subsequent esterification (CH_2N_2, $MeOH/Et_2O$) provided the ketoester *threo*-**32** in high yield. Enantioselective HPLC analysis (Chiralpak OD column) revealed an enantiomeric excess (ee) of 84% for (*S*,*S*)-**32** and 81% for (*R*,*R*)-**32**, in accord with ee values reported for similar systems in the literature.[17,18] Chemoselective ketone reduction of *threo*-**32** then afforded the hydroxyester *threo*-**33** with

Scheme 4. Initial Synthetic Route to *Threo* Compounds [$^2H_4$]-(*S*,*S*)-8/9 and [$^2H_4$]-(*R*,*R*)-8/9 from 17[a]



[a]Reagents and conditions: (a) Li (s)/NH_3 (l), *t*-BuOH/THF (3:5), −78 °C, 93%; (b) *p*-TsOH, MeOH, 94%; (c) Jones' reagent, acetone, 0 °C to rt, 95%, ≥95% *E* isomer; (d) LiOD, D_2O/THF; (e) CH_2N_2, $MeOH/Et_2O$, 0 °C to rt, 62% over two steps, ≥95% *E* isomer, ≤1% [$^2H_0$]; (f) (i) $NaBH_4$, MeOH, 0 °C to rt, (ii) chromatography with $AgNO_3$-impregnated silica gel, 87%, 100% *E* isomer, ≤1% [$^2H_0$]; (g) [$^2H_4$]-(*S*,*S*)-**8**/**9**: AD-mix-*α*, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0−4 °C, 2%; [$^2H_4$]-(*R*,*R*)-**8**/**9**: AD-mix-*β*, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0−4 °C, 6%.

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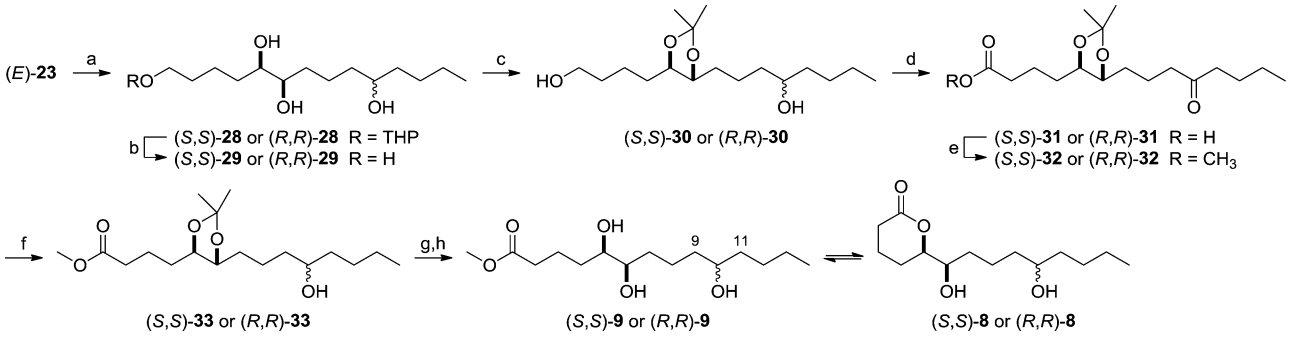
Scheme 5. Optimized Synthetic Route to Unlabeled *Threo* Compounds (*S,S*)-8/9 and (*R,R*)-8/9 from Compound (*E*)-23[a]



[a]Reagents and conditions: (a) (*S,S*)-**28**: AD-mix-α, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0−4 °C, 95%; (*R,R*)-**28**: AD-mix-β, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0−4 °C, 95%; (b) HCl, MeOH, (*S,S*)-**29** 96%; (*R,R*)-**29** 95%; (c) *p*-TsOH, acetone, 4 Å molecular sieves, (*S,S*)-**30** 97%; (*R,R*)-**30** 94%; (d) PDC, DMF, (*S,S*)-**31** 91%; (*R,R*)-**31** 95%; (e) CH_2N_2, MeOH/Et_2O, 0 °C to rt, (*S,S*)-**32** 94%, 84% ee; (*R,R*)-**32** 95%, 81% ee (Chiralpak OD column, 2% 2-propanol in *n*-hexane, flow rate 0.8 mL min^{-1}, PDA-UV detector 215 nm, retention times: (*R,R*)-**32** 10.8 min, (*S,S*)-**32** 11.2 min); (f) $NaBH_4$, MeOH/Et_2O, 0 °C to rt, (*S,S*)-**33** 93%; (*R,R*)-**33** 93%; (g) Amberlyst 15 acidic resin, MeOH/THF, 70 °C; (h) CH_2N_2, MeOH/Et_2O, (*S,S*)-**8**/**9** ∼95%; (*R,R*)-**8**/**9** ∼91%.

uncontrolled stereochemistry at C-10. Methanolysis of the ketals catalyzed by acidic Amberlyst 15 ion-exchange resin at 70 °C[19−21] and treatment of the crude products with diazomethane afforded mixtures of the desired dihydroxylactones (*S,S*)-**8** or (*R,R*)-**8** and their corresponding methyl trihydroxyesters (*S,S*)-**9** or (*R,R*)-**9** in excellent yields.

The final deprotection step worked well and no further purification was required, eliminating the earlier problems experienced with chromatography. The presence of both dihydroxylactone **8** and trihydroxyester **9** in each product mixture was confirmed by NMR spectroscopy and mass spectrometry, where $[M + Na]^{+\bullet}$ ions for both **8** (*m/z* 281) and **9** (*m/z* 313) were detected. The ^1H and ^{13}C NMR spectra of the products were complicated due to the presence of the diastereomeric dihydroxylactones **8**, trihydroxyesters **9**, and what appears to be the corresponding trihydroxy acids **34** (Figure 2) generated over the course of spectral acquisition.

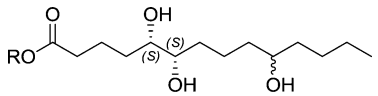
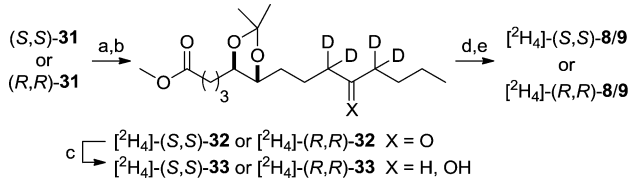


Figure 2. (5*S*,6*S*)-5,6,10-Trihydroxytetradecanoic acid [(*S,S*)-**34**] and its corresponding sodium carboxylate (*S,S*)-**35**.

However, only dihydroxylactones **8** were observed by GC/MS analysis in both cases, presumably due to intramolecular cyclization of **9** promoted by the high temperature within the GC injection port. Compounds **8** and **9** are equivalent in terms of being potential precursors to spiroacetal **1** in *B. cacuminata* and should cross cell membranes to the biosynthetic enzymes in vivo. A small amount of the (*S,S*) product mixture was treated with NaOD in D_2O in order to convert all of the diastereomeric tetraoxygenated compounds present into the single, diastereomeric sodium carboxylate (*S,S*)-**35** (Figure 2). The ^1H and ^{13}C NMR spectra of (*S,S*)-**35** were much simpler, with only two sets of resonances now observed in the ^{13}C NMR spectrum for C-5, C-6 and C-10, and a single signal present for C-1 (carboxylate carbon). This confirmed that the original product mixture was comprised only of pairs of equivalent tetraoxygenated compounds diastereomeric at C-10.

Scheme 6. Synthesis of Deuterium-Labeled *Threo* Compounds $[^2H_4]$-(*S,S*)-8/9 and $[^2H_4]$-(*R,R*)-8/9 from Ketoacids (*S,S*)-31 and (*R,R*)-31[a]



[a]Reagents and conditions: (a) NaOD, D_2O/THF; (b) CH_2N_2, MeOH/Et_2O, 0 °C to rt, $[^2H_4]$-(*S,S*)-**32** 91% over two steps, ≤1% $[^2H_0]$; $[^2H_4]$-(*R,R*)-**32** 93% over two steps, ≤1% $[^2H_0]$; (c) $NaBH_4$, MeOH/Et_2O, 0 °C to rt, $[^2H_4]$-(*S,S*)-**33** 97%, ≤1% $[^2H_0]$; $[^2H_4]$-(*R,R*)-**33** 98%, ≤1% $[^2H_0]$; (d) Amberlyst 15 acidic resin, MeOH/THF, 70 °C; (e) CH_2N_2, MeOH/Et_2O, $[^2H_4]$-(*S,S*)-**8**/**9** ∼96%, ≤1% $[^2H_0]$; $[^2H_4]$-(*R,R*)-**8**/**9** ∼92%, ≤1% $[^2H_0]$.

Synthesis of the deuterium-labeled analogues of *threo*-**8**/**9** was then undertaken via an analogous route (Scheme 6). Ketoacids (*S,S*)-**31** and (*R,R*)-**31** were thus treated with NaOD/D_2O, which resulted in H−D exchange adjacent to the C-10 ketone moiety. Esterification afforded $[^2H_4]$-(*S,S*)-**32** and $[^2H_4]$-(*R,R*)-**32** in 91% and 93% yields over two steps, respectively. NMR and GC/MS analysis confirmed the almost complete, regiospecific deuteration at C-9 and C-11 (≤1% $[^2H_0]$) and established the tetradeuterated species as the predominant products. Subsequent ketone reduction and acid-catalyzed ketal methanolysis afforded the desired dihydroxylactone/trihydroxyester mixtures $[^2H_4]$-(*S,S*)-**8**/**9** and $[^2H_4]$-(*R,R*)-**8**/**9** in high yields. GC/MS and NMR spectroscopy confirmed the retention of deuterium throughout this series of transformations. ESI mass spectrometry gave $[M + Na]^{+\bullet}$ ions for both the dihydroxylactones $[^2H_4]$-*threo*-**8** (*m/z* 285) and trihydroxyesters $[^2H_4]$-*threo*-**9** (*m/z* 317).

***Erythro* Isomers.** The synthesis of *erythro* dihydroxylactones **8**/trihydroxyesters **9** commenced with catalytic hydrogenation of **17** over Lindlar's catalyst (Scheme 7), which gave the desired alkene (*Z*)-**36** in 84% yield without affecting the benzyl ether at C-10. On occasion, GC/MS and NMR analysis of (*Z*)-**36** revealed that a small amount (∼10%) of the corresponding chromatographically inseparable (*E*)-alkene (*E*)-**36** had also been formed during the catalytic hydrogenation of

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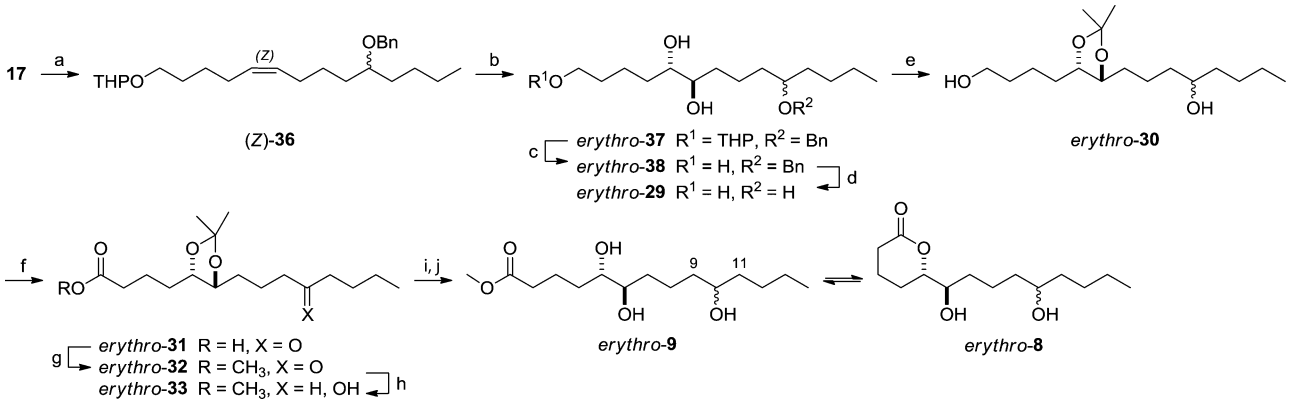
Scheme 7. Synthesis of Unlabeled *erythro*-8/9 from Compound 17 via (*Z*)-36[a]



[a]Reagents and conditions: (a) H_2, Lindlar's catalyst (5% $Pd/CaCO_3$ poisoned with lead), EtOAc, 84%; (b) OsO_4, NMO, THF/*t*-BuOH/H_2O (10:3:1), 90%; (c) *p*-TsOH, MeOH, quantitative; (d) H_2, 10% Pd/C, MeOH, 97%; (e) *p*-TsOH, acetone, 4 Å molecular sieves, 97%; (f) PDC, DMF, 89%; (g) CH_2N_2, MeOH/Et_2O, 0 °C to rt, quantitative; (h) $NaBH_4$, MeOH/Et_2O, 0 °C to rt, 94%; (i) Amberlyst 15 acidic resin, MeOH/THF, 70 °C; (j) CH_2N_2, MeOH/Et_2O, ∼94%.

17. Dihydroxylation of (*E*)-alkenes under standard SAD conditions usually occurs more quickly than that of the corresponding (*Z*)-alkenes. Thus, oxidation of the isomeric mixture **36** at this stage with 0.15 equiv of AD-mix-α (26 h) enabled complete dihydroxylation of the (*E*)-alkene impurity while leaving the majority of (*Z*)-**36** unreacted. Chromatographic separation of the diol from the unreacted alkene thus provided geometrically pure (*Z*)-**36**. The synthesis of racemic *erythro*-**8**/**9** was undertaken because there was some evidence that *erythro* diols were unlikely to be P450 substrates.[22] Dihydroxylation of alkene (*Z*)-**36** was thus performed using catalytic OsO_4 with *N*-methylmorpholine *N*-oxide (NMO) as the stoichiometric co-oxidant (Upjohn conditions) to provide a racemic mixture of *erythro*-**37** in 90% yield (Scheme 7). Reductive cleavage of the benzyl ether of *erythro*-**37** (by catalytic hydrogenation or with lithium/liquid ammonia) and then acid-catalyzed methanolysis of the THP moiety to afford the triol *erythro*-**28** proved to be inefficient. However, acid-catalyzed THP deprotection of *erythro*-**37** followed by hydrogenolysis of the benzyl ether of the resulting triol *erythro*-**38** (Pd/C, MeOH) proceeded cleanly to afford the desired tetraol *erythro*-**29** in near-quantitative yield. Having obtained the tetraol *erythro*-**29**, synthesis of the unlabeled and deuterium-labeled dihydroxylactone/trihydroxyester mixtures, *erythro*-**8**/**9** (Scheme 7) and [$^2H_4$]-*erythro*-**8**/**9** (Figure 3, cf. Scheme 6), proceeded in a manner analogous to that described for the corresponding *threo* compounds above.

As observed for the *threo* analogues, the ^1H and ^{13}C NMR spectra of both the final unlabeled and deuterium-labeled *erythro* product mixtures (*erythro*-**8**/**9**/**34** and [$^2H_4$]-*erythro*-**8**/**9**/**34**) were complicated. A small amount of the unlabeled *erythro* product mixture was treated with NaOD in D_2O, and both the ^1H and ^{13}C NMR spectra of the sodium carboxylate *erythro*-**35** were much simpler than the corresponding spectra of the *erythro*-**8**/**9**/**34** mixture, thus confirming that the original product mixture was composed only of pairs of equivalent, diastereomeric tetraoxygenated compounds. The difference in chemical shifts observed for the resonances of C-1, C-5, C-6, and C-10 of *erythro*-**35** and (*S*,*S*)-**35** also confirmed the diastereomeric relationship of these sodium carboxylates and, thus, of *erythro*-**8**/**9** and (*S*,*S*)-**8**/**9**. The presence of dihydroxylactones and trihydroxyesters in both the unlabeled and deuterium-labeled *erythro* product mixtures was confirmed by ESI mass spectrometry, but again only the dihydroxylactones *erythro*-**8** and [$^2H_4$]-*erythro*-**8** were observed by GC/MS analysis. Furthermore, comparison of the MS fragmentation patterns for *erythro*-**8** and [$^2H_4$]-*erythro*-**8** indicated that the tetradeuterated compound was the only clearly detectable labeled species present (≤1% [$^2H_0$]).

Synthesis of Compounds with Dioxygenated Chains. Several compounds with dioxygenated chains were then synthesized on the basis of the results of feeding experiments with the various stereoisomers of deuterium-labeled **8**/**9** (vide infra). The dihydroxyesters [$^2H_4$]-**10** (28% yield) and [$^2H_4$]-**11** (19%) were obtained in a 3:2 ratio from the standard hydroboration−oxidation of unsaturated hydroxyester [$^2H_4$]-(*E*)-**27**. Surprisingly, the 5,10-dihydroxyester [$^2H_4$]-**11** did not cyclize to form a 10-hydroxylactone (cf. compounds **8** and **12**), which led to difficulties in its chromatographic separation from [$^2H_4$]-**10**. Two ∼9:1 isomerically enriched mixtures (**10**:**11** and **11**:**10**) were thus obtained, and this level of enrichment was believed to be sufficient to allow differentiation between [$^2H_4$]-**10** and [$^2H_4$]-**11** (Figure 4) in terms of deuterium incorporation into spiroacetal **1** in feeding experiments. While the position of the newly introduced hydroxyl group (C-6 in [$^2H_4$]-**10** and C-5 in [$^2H_4$]-**11**) could not be determined from

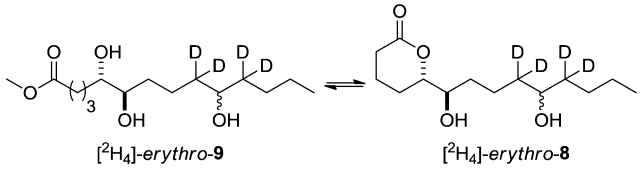


Figure 3. Deuterium-labeled trihydroxyester [$^2H_4$]-*erythro*-**9** and dihydroxylactone [$^2H_4$]-*erythro*-**8**.

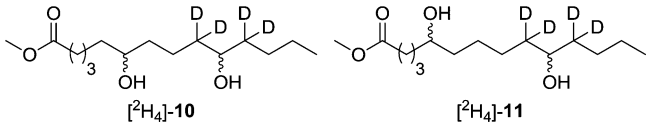


Figure 4. Deuterium-labeled methyl 6,10-dihydroxytetradecanoate [$^2H_4$]-**10** and methyl 5,10-dihydroxytetradecanoate [$^2H_4$]-**11**.

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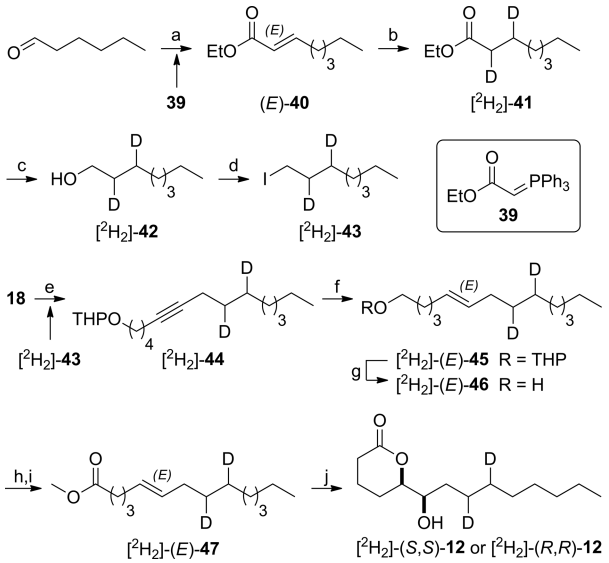
their NMR spectra, the mass spectra of these compounds (available from GC/MS) were clearly distinguishable and were thus used to assign their structures. Key mass fragments used to distinguish these compounds include m/z 113 for [$^2H_4$]-**10** and m/z 99 for [$^2H_4$]-**11** (thought to arise from lactonization and accompanied loss of methanol, with subsequent scission of the alkyl chain from the lactone ring), and m/z 145 for [$^2H_4$]-**10** (from scission of the bond between C-6 and C-7, adjacent to the newly introduced hydroxyl group).

Scheme 8. Synthesis of [$^2H_2$]-(*S*,*S*)-12 and [$^2H_2$]-(*R*,*R*)-12 from Hexanal and Alkyne 18[a]

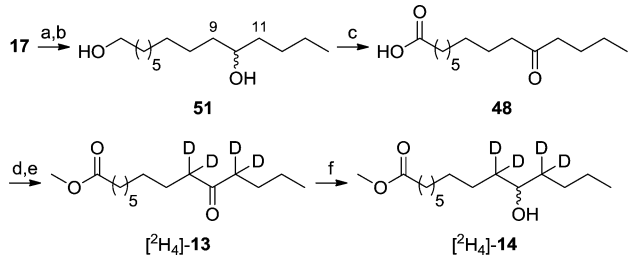


[a]Reagents and conditions: (a) stabilized ylide **39**, CH_2Cl_2, reflux, 92%, ≥95% *E* isomer; (b) $^2H_2$, 5% Pd/C, EtOH, quantitative, ∼20% [$^2H_0$]; (c) $LiAlH_4$, Et_2O, 0 °C to rt, 99%; (d) I_2, PPh_3, imidazole, CH_3CN/Et_2O (1:3), 0 °C to rt, 66%, ∼20% [$^2H_0$]; (e) (i) *n*-BuLi, THF, −40 °C, (ii) HMPA, iodide [$^2H_2$]-**43**, −40 °C to rt, 70%, ∼20% [$^2H_0$]; (f) Li (s)/NH_3 (l), *t*-BuOH/THF (3:5), −78 °C, 79%, ∼20% [$^2H_0$]; (g) *p*-TsOH, MeOH, quantitative, ∼20% [$^2H_0$]; (h) Jones' reagent, acetone, 0 °C to rt; (i) CH_2N_2, MeOH/Et_2O, 0 °C to rt, 44% over two steps, ∼20% [$^2H_0$]; (j) [$^2H_2$]-(*S*,*S*)-**12**: AD-mix-*α*, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0−4 °C, 9%, ∼20% [$^2H_0$]; [$^2H_2$]-(*R*,*R*)-**12**: AD-mix-*β*, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0−4 °C, 10%, ∼20% [$^2H_0$].

The synthesis of the enantiomeric, deuterium-labeled 6-hydroxylactones [$^2H_2$]-(*S*,*S*)-**12** and [$^2H_2$]-(*R*,*R*)-**12** (Scheme 8), with *threo* vicinal diols, commenced with Wittig addition of the stabilized ylide ethyl 2-(triphenylphosphoranylidene)-acetate (**39**)[23] to hexanal. This afforded ethyl 2-octenoate [(*E*)-**40**] in 92% yield as a 19:1 mixture of *E* and *Z* isomers, with the coupling constant for H-3 (δ_H 6.94 ppm) and H-2 (5.79 ppm) revealing the *E* stereochemistry of the major isomer (^3J = 15.7 Hz). Catalytic reduction of (*E*)-**40** with deuterium gas over Pd/C gave a mixture of labeled compounds (due to H−D exchange during the reaction)[3,4] with [$^2H_2$]-**41** as the predominant deuterated product (∼20% [$^2H_0$]), and subsequent $LiAlH_4$-mediated ester reduction yielded [$^2H_2$]-**42**. The alcohol [$^2H_2$]-**42** was converted to the iodide [$^2H_2$]-**43** (66% yield), which was subsequently reacted with the anion of protected 5-hexyn-1-ol **18**[7] to provide the target alkyne [$^2H_2$]-**44** in 70% yield. Lithium/liquid ammonia-mediated reduction and acid-catalyzed deprotection to [$^2H_2$]-**46**, and Jones' oxidation and subsequent esterification afforded the *δ*,*ε*-(*E*)-unsaturated ester [$^2H_2$]-(*E*)-**47** in good yield. Sharpless' asymmetric dihydroxylation of [$^2H_2$]-(*E*)-**47** under standard conditions then provided the desired hydroxylactones [$^2H_2$]-(*S*,*S*)-**12** (9% yield; from AD-mix-*α*) and [$^2H_2$]-(*R*,*R*)-**12** (10% yield; from AD-mix-*β*). Similar difficulties in yield and stability were experienced with [$^2H_2$]-*threo*-**12** as initially with [$^2H_4$]-*threo*-**8** (Scheme 3) but overall [$^2H_2$]-*threo*-**12** appeared to be slightly more stable and were available in usable quantities for feeding experiments from this synthesis.

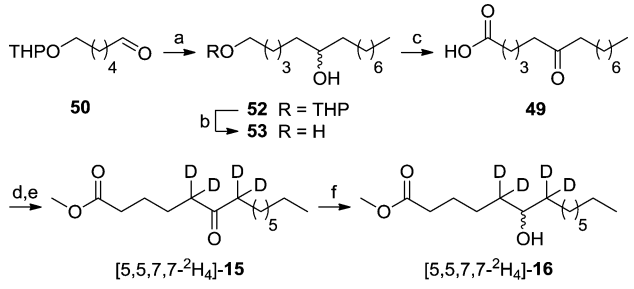
Synthesis of Monooxygenated Esters. Several monooxygenated esters were also synthesized, based on the results of feeding experiments with the various dioxygenated chain compounds (vide infra). The key intermediate in the synthesis of [$^2H_4$]-**13** and [$^2H_4$]-**14** (oxygenated at C-10) was 10-oxotetradecanoic acid (**48**), which was available in near-quantitative yield in three steps from alkyne **17** via a series of standard transformations (Scheme 9). Similarly, 6-oxotetradecanoic acid (**49**) was the key intermediate in the initial synthesis of [$^2H_4$]-**15** and [$^2H_4$]-**16** (oxygenated at C-6) and was obtained from THP-protected 6-hydroxyhexanal (**50**)[24] and commercially available 1-bromooctane in three steps (Scheme 10). Ketoacids [$^2H_4$]-**48** and [$^2H_4$]-**49** were produced by base-catalyzed deuterium exchange (≤1% [$^2H_0$]) and esterified to afford the ketoesters [$^2H_4$]-**13** and [5,5,7,7-$^2H_4$]-

Scheme 9. Synthesis of [$^2H_4$]-13 and [$^2H_4$]-14 from Compound 17[a]



[a]Reagents and conditions: (a) H_2, 10% Pd/C, MeOH; (b) *p*-TsOH, MeOH, 98% over two steps; (c) Jones' reagent, acetone, 0 °C to rt, 97%; (d) LiOD, D_2O/THF; (e) CH_2N_2, MeOH/Et_2O, 0 °C to rt, 95% over two steps, ≤1% [$^2H_0$]; (f) $NaBH_4$, MeOH, 0 °C to rt, 94%, ≤1% [$^2H_0$].

Scheme 10. Synthesis of [5,5,7,7-$^2H_4$]-15 and [5,5,7,7-$^2H_4$]-16 from Aldehyde 50[a]



[a]Reagents and conditions: (a) 1-octylmagnesium bromide, Et_2O, 0 °C, 70%; (b) *p*-TsOH, MeOH, 96%; (c) Jones' reagent, acetone, 0 °C to rt, 96%; (d) LiOD, D_2O/THF; (e) CH_2N_2, Et_2O, 0 °C to rt, 63% over two steps, ≤1% [$^2H_0$]; (f) $NaBH_4$, MeOH, 0 °C to rt, 88%, ≤1% [$^2H_0$].

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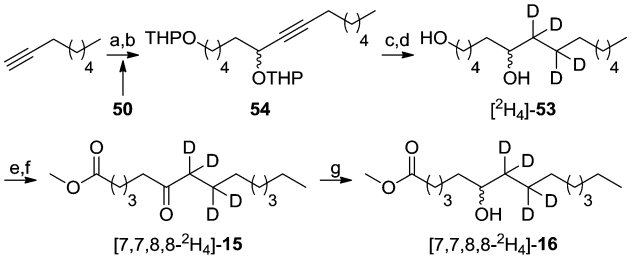
15, respectively; ketone reduction gave the desired hydroxyesters [$^2H_4$]-**14** and [5,5,7,7-$^2H_4$]-**16**.

Both [5,5,7,7-$^2H_4$]-**15** and [5,5,7,7-$^2H_4$]-**16** have deuterium at one of the sites of potential in vivo hydroxylation (C-5); this could hinder their incorporation into spiroacetal **1** as P450-mediated hydroxylation is known to exhibit significant primary kinetic isotope effects.[25] Thus, the synthesis of isotopomers [7,7,8,8-$^2H_4$]-**15** and [7,7,8,8-$^2H_4$]-**16**, which are labeled solely at sites not expected to undergo hydroxylation, was undertaken. Instead of base-catalyzed exchange, this synthetic route (Scheme 11) employed catalytic reduction of alkyne **54** with

Scheme 11. Synthesis of [7,7,8,8-$^2H_4$]-15 and [7,7,8,8-$^2H_4$]-16 from 1-Octyne and Aldehyde 50[a]



[a]Reagents and conditions: (a) (i) *n*-BuLi, THF, −40 °C, (ii) aldehyde **50**, −40 °C to rt; (b) DHP, *p*-TsOH, CH_2Cl_2, 0 °C to rt, 74% over two steps; (c) $^2H_2$, Wilkinson's catalyst, benzene; (d) *p*-TsOH, MeOH, 80% over two steps, ≤1% [$^2H_0$]; (e) PDC, DMF; (f) CH_2N_2, MeOH/Et_2O, 0 °C to rt, 71% over two steps, ≤1% [$^2H_0$]; (g) $NaBH_4$, MeOH/Et_2O, 0 °C to rt, 87%, ≤1% [$^2H_0$].

deuterium gas over Wilkinson's catalyst for the regiospecific introduction of deuterium atoms at C-7 and C-8. Obtained in two steps from aldehyde **50**[24] and commercially available 1-octyne, alkyne **54**, with both hydroxyl groups protected, was chosen as the substrate for deuteration because catalytic reduction of alkyne moieties adjacent to hydroxyl or ketone groups has been reported to co-occur with varying degrees of isomerization and hydrogenolysis.[26−29] Furthermore, comparison of the catalytic reduction of alkynols and their THP-derivatives has indicated that deuteration over Wilkinson's catalyst[30−32] proceeds to a greater extent and with greater regiospecificity when no exchangeable hydrogen atoms are present in the substrate.[32] Following the reduction of **54** using deuterium gas, standard THP-deprotection cleanly provided regiospecifically labeled [7,7,8,8-$^2H_4$]-1,6-tetradecanediol ([$^2H_4$]-**53**), which was further elaborated to afford the desired ketoester [7,7,8,8-$^2H_4$]-**15** and hydroxyester [7,7,8,8-$^2H_4$]-**16** (≤1% [$^2H_0$]). The use of a homogeneous catalytic system ensured the specific incorporation of four deuterium atoms at only positions C-7 and C-8, and the isotopomeric relationship of [7,7,8,8-$^2H_4$]-**15** and [7,7,8,8-$^2H_4$]-**16** with [5,5,7,7-$^2H_4$]-**15** and [5,5,7,7-$^2H_4$]-**16** was confirmed by NMR. No H−D scrambling or overincorporation of deuterium was detectable by NMR and GC/MS analysis, as is common when heterogeneous Pd/C catalysts are used[3,4] and was observed for [$^2H_2$]-**41**.

Administration of Potential Spiroacetal Precursors to Flies and Deuterium Incorporation Analysis. No permission from national or local authorities was required to perform in vivo studies with fruit flies. The deuterium-labeled compounds were administered to sexually mature male *B. cacuminata* (at least 10 days postemergence)[33] through their diet. Analysis of the volatile emissions of the flies was conducted by solid-phase microextraction (SPME) and GC/MS without disruption of either the flies or the incorporation experiments, as described previously.[34] SPME sampling was carried out once a day following administration of the deuterated substrates for up to 5 days (the average duration of an experiment was 2−3 days). An average of three separate experiments were conducted for each substrate. In all experiments, spiroacetal **1** was the only compound observed during SPME-GC/MS analysis. As previously summarized by Booth et al.,[1] spiroacetals undergo characteristic mass spectrometric fragmentations. This greatly facilitates the detection of specific in vivo deuterium incorporation into spiroacetals, as different mass fragment ions are produced by spiroacetals containing different numbers of deuterium atoms and by isotopomers with different sites of labeling (Figure 5).

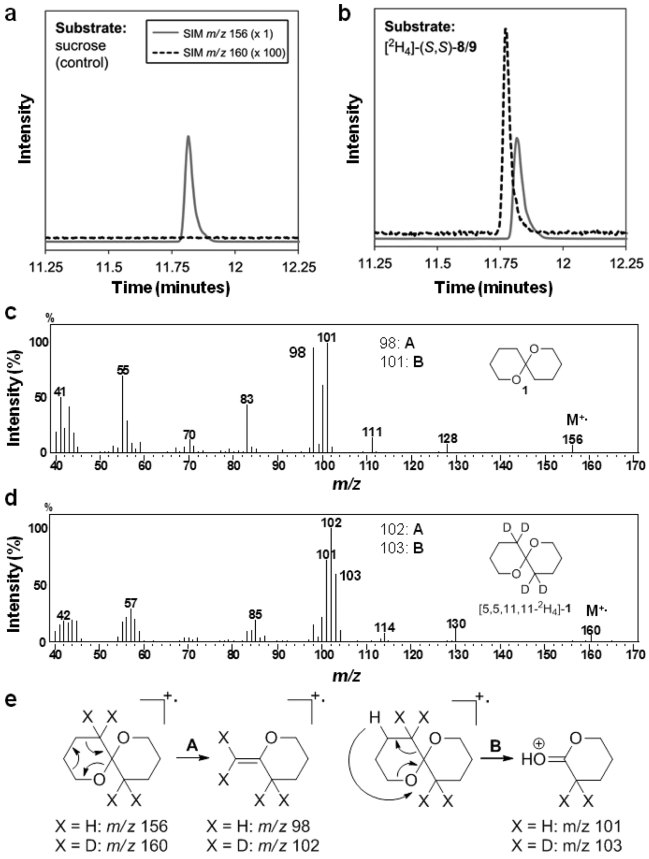


Figure 5. Example of feeding experiment results from SPME-GC/MS analysis. Selected GC/MS SIM traces for feeding experiments with (a) sucrose (control) and (b) [$^2H_4$]-(*S*,*S*)-**8**/**9**, 46 h following dietary administration (solid gray line: *m*/*z* 156, $M^{+\bullet}$ for **1**; dotted black line: *m*/*z* 160, $M^{+\bullet}$ for [$^2H_4$]-**1**). Mass spectra of spiroacetals (c) **1** and (d) [5,5,11,11-$^2H_4$]-**1** formed by specific in vivo deuterium incorporation from [$^2H_4$]-(*S*,*S*)-**8**/**9**. (e) Selected characteristic fragmentation patterns for **1** and [5,5,11,11-$^2H_4$]-**1**. Labels A and B in (c) and (d) correspond to the fragments depicted in (e).

In addition to monitoring the total ion current (TIC) of the GC/MS, selected ion monitoring (SIM) was also used to improve the sensitivity of the analyses. Several factors can influence deuterium incorporation observed in experiments using live insects; these relate to the health and age of the insects, the deuteration-levels of substrates, the method of analysis, and external conditions such as temperature and light

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exposure. Qualitative comparisons of deuterium incorporation are thus more meaningful than quantitative determinations. Analysis of GC/MS data allows qualitative estimates to be made, based upon which administered substrates are categorized into qualitative incorporation levels:[7] in this case, "some incorporation", "low incorporation", and "no detectable incorporation" (Supporting Information).

Dihydroxylactones/Trihydroxyesters. As diastereoselectivity between similar *threo* and *erythro* diol substrates has been observed previously in P450-mediated oxidative C−C bond cleavage reactions in vitro,[22] it was initially hypothesized that [$^2H_4$]-*erythro*-**8**/**9** may not be incorporated into spiroacetal **1** in vivo to as great an extent as the *threo* isomers, [$^2H_4$]-(*S*,*S*)-**8**/**9** or [$^2H_4$]-(*R*,*R*)-**8**/**9**. However, it was unclear whether any selectivity between the *threo* isomers would be observed, as only low enantioselectivity in vitro has been observed for such a reaction previously.[22] The specific incorporation of deuterium from the various stereoisomers of [$^2H_4$]-**8**/**9** would result in the formation of labeled spiroacetal [5,5,11,11-$^2H_4$]-**1**; thus, SIM monitoring of mass fragment ions identified for **1** and [5,5,11,11-$^2H_4$]-**1** was used to aid the detection of deuterium incorporation. Not unexpectedly, both the *threo* compound mixtures, [$^2H_4$]-(*S*,*S*)-**8**/**9** and [$^2H_4$]-(*R*,*R*)-**8**/**9** were found to be incorporated into spiroacetal **1** in vivo ("some incorporation") in several independent experiments, supporting the inclusion of an oxidative C−C bond cleavage step in the biosynthetic pathway. Incorporation was monitored by comparison of the magnitudes of the molecular ion currents (MIC) for *m*/*z* 156 ($M^{+\bullet}$ of natural **1**) and *m*/*z* 160 ($M^{+\bullet}$ of [$^2H_4$]-**1**) in GC/MS chromatograms. However, no consistent, appreciable difference in selectivity between the *threo* isomers ([$^2H_4$]-(*S*,*S*)-**8**/**9** and [$^2H_4$]-(*R*,*R*)-**8**/**9**) was detected across several experiments by SIM. Comparison of the SIM traces for mass ions that would result from the fragmentation of [5,5,11,11-$^2H_4$]-**1** against those due to the fragmentation of **1** (Figure 5) indicated that [5,5,11,11-$^2H_4$]-**1** was formed by the specific incorporation of the administered substrates, and was observed to increase over the duration of the experiments.

Administration of substrate mixtures containing ∼50% deuterated substrate with 50% unlabeled substrate of the opposite stereochemistry (i.e., [$^2H_4$]-(*S*,*S*)-**8**/**9** with (*R*,*R*)-**8**/**9** and (*S*,*S*)-**8**/**9** with [$^2H_4$]-(*R*,*R*)-**8**/**9**) was then conducted in order to investigate further any selectivity of incorporation between the *threo* isomers. These experiments also indicated no appreciable enantioselectivity. In contrast to the *threo* isomers, a very low level of deuterium incorporation was observed from [$^2H_4$]-*erythro*-**8**/**9** ("low incorporation"). This incorporation was not evident by examination of the mass spectrum of emitted spiroacetal **1** and required careful inspection of GC/MS SIM traces to be detected. The coadministration of the β-oxidation inhibitor 2-fluorostearic acid was not found to visibly increase deuterium incorporation from any of the *threo* or *erythro* substrates.

Interestingly, deuterium incorporation into **1** was observed following the administration of mixed substrates consisting of ∼50% [$^2H_4$]-*erythro*-**8**/**9** and 50% unlabeled (*S*,*S*)-**8**/**9** or (*R*,*R*)-**8**/**9**, which was comparable to that observed from [$^2H_4$]-*erythro*-**8**/**9** alone. Furthermore, lower levels of deuterium incorporation than those resulting from the administration of either [$^2H_4$]-(*S*,*S*)-**8**/**9** or [$^2H_4$]-(*R*,*R*)-**8**/**9** alone were observed following the administration of mixtures of unlabeled *erythro*-**8**/**9** with either [$^2H_4$]-(*S*,*S*)-**8**/**9** or [$^2H_4$]-(*R*,*R*)-**8**/**9**. The results of these experiments were surprising, as the incorporation levels from the experiments with single substrates suggested that the *threo* diols were the preferred substrates for oxidative C−C bond cleavage rather than *erythro* diols. Thus, the *threo* diols were expected to inhibit the incorporation of the *erythro* diols and the *erythro* diols were not expected to affect *threo* processing. The results of these mixed substrate experiments may reflect factors such as greater bioavailability of the *erythro* isomers in vivo or greater susceptibility of the *threo* compounds to processing by other enzymes. In addition to being processed at a low level by biosynthetic enzyme(s), it is possible that the *erythro* diols have an inhibitory effect on them and thus decrease *threo* diol incorporation into **1**.

Nevertheless, the diastereoselectivity observed in this biosynthetic C−C bond cleavage reaction, with the *threo* deuterium-labeled substrates [$^2H_4$]-(*S*,*S*)-**8**/**9** and [$^2H_4$]-(*R*,*R*)-**8**/**9** both being specifically processed into spiroacetal **1** at a higher level than [$^2H_4$]-*erythro*-**8**/**9**, strongly supports the hypothesis that this step is enzyme-mediated rather than an adventitious chemical process. This finding, and the lack of preference for either the (*S*,*S*) or (*R*,*R*) substrate, are reminiscent of the high degree of diastereoselectivity but low level of enantioselectivity between *threo* substrates observed in the C−C bond cleavage reaction mediated by $P450_{BioI}$.[22] This was thought to arise because, although dihydroxy-fatty acids themselves are cleaved by $P450_{BioI}$, acyl carrier protein-bound fatty acids are now known to be the true enzyme substrates; this may also be the situation for the enzyme(s) involved in the biosynthesis of spiroacetal **1**. While it is clear that both the *threo* vicinal diols are processed to **1** in *B. cacuminata* more efficiently than the *erythro* diols, this is not necessarily indicative of the natural substrate stereochemistry as both *threo* isomers may be enzyme substrates but only one may occur naturally.

Compounds with Dioxygenated, Monooxygenated, and Unsubstituted Chains. Feeding experiments were next carried out with deuterium-labeled, racemic dihydroxyesters [$^2H_4$]-**10** and [$^2H_4$]-**11** and hydroxylactones [$^2H_2$]-(*S*,*S*)-**12** and [$^2H_2$]-(*R*,*R*)-**12**, all potential precursors of **8**/**9**. The greatest degree of deuterium incorporation into spiroacetal **1** was observed from the 6,10-dihydroxyester [$^2H_4$]-**10** ("some incorporation"), while a much lower level of incorporation was observed from the 5,10-dihydroxyester [$^2H_4$]-**11**, and none from either of the 5,6-dioxygenated hydroxylactones [$^2H_2$]-(*S*,*S*)-**12** or [$^2H_2$]-(*R*,*R*)-**12**. Specifically labeled spiroacetal [5,5,11,11-$^2H_4$]-**1** was detected following administration of [$^2H_4$]-**10**, thus indicating that ω-4 hydroxylation of a fatty acid equivalent occurs prior to vicinal diol formation and that ω-9 hydroxylation occurs as the final step prior to oxidative C−C bond cleavage.

Feeding experiments were next carried out with a series of monooxygenated esters to investigate the order in which ω-4 and ω-8 oxidation occurs. No detectable deuterium incorporation into **1** was observed from either the 6-ketoester [5,5,7,7-$^2H_4$]-**15** or the 6-hydroxyester [5,5,7,7-$^2H_4$]-**16**, even following the coadministration of the β-oxidation inhibitor 2-fluorostearic acid. This lack of observable incorporation into **1** does not exclude such compounds as natural biosynthetic precursors in vivo and is likely to be due to their extensive diversion into other metabolic pathways because of their strong resemblance to endogenous fatty acids. Indeed, the incorporation of [$^2H_4$]-**10** into **1** was observed to increase following the coadministration of 2-fluorostearic acid, thus supporting the hypothesis that fatty acid like potential precursors are naturally degraded via the β-oxidation pathway in vivo. We also

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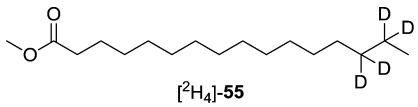
hypothesized that the lack of observed deuterium incorporation into **1** from [5,5,7,7-$^2H_4$]-**15** and [5,5,7,7-$^2H_4$]-**16** may have arisen because of the presence of deuterium atoms at C-5, a site of further hydroxylation, as P450-mediated oxygen insertion exhibits a significant primary isotope effect.[25] Administration of the isotopomers [7,7,8,8-$^2H_4$]-**15** and [7,7,8,8-$^2H_4$]-**16** was then carried out but deuterium incorporation from these compounds was again not observed and the coadministration of 2-fluorostearic acid did not change these results. This suggested that $\omega-4$ oxidation is likely to precede $\omega-8$ oxidation. In order to test this hypothesis, the administration of 10-ketoester [$^2H_4$]-**13** and 10-hydroxyester [$^2H_4$]-**14** was conducted. Deuterium incorporation was not observed from the hydroxyester [$^2H_4$]-**14**, with or without coadministration of 2-fluorostearic acid. A very low level of deuterium incorporation was, however, observed from ketoester [$^2H_4$]-**13** upon careful examination and comparison of SIM traces for fragment ions of [5,5,11,11-$^2H_4$]-**1** and **1**. This observation tentatively suggests that $\omega-4$ oxidation occurs prior to $\omega-8$ oxidation.

Deuterium incorporation from monooxygenated and unsubstituted esters has not previously been observed in *B. cacuminata* across several independent experiments, despite the coadministration of β-oxidation inhibitors. This is likely to be because of their diversion into other metabolic pathways, which also explains why a very low level of deuterium incorporation was observed from [$^2H_4$]-**13** (and thus, somewhat surprisingly, none from hydroxyester [$^2H_4$]-**14**). Such a result has in fact been observed previously during in vivo investigations of spiroacetal pheromone biosynthesis in *B. tryoni*, in which deuterium-labeled ketones were incorporated into spiroacetals efficiently while their corresponding alcohols were not detectably incorporated.[4] Deuterium-labeled methyl hexadecanoate [$^2H_4$]-**55** (Figure 6), available to us from other work, was then administered to flies. Surprisingly, a low level of incorporation was observed from [$^2H_4$]-**55**, which was comparable to that observed from [$^2H_4$]-*erythro*-**8/9** and greater than that observed from ketoester [$^2H_4$]-**13**. The presence of labeled spiroacetal [3,3,4,4-$^2H_4$]-**1** could only be detected by careful examination of SIM traces.



[$^2H_4$]-**55**

Figure 6. Deuterium-labeled methyl hexadecanoate [$^2H_4$]-**55**.

Despite the ketoester [$^2H_4$]-**13** being a more advanced precursor for spiroacetal [$^2H_4$]-**1** than the ester [$^2H_4$]-**55**, the lower level of deuterium incorporation observed from [$^2H_4$]-**13** may reflect greater metabolic susceptibility or its greater toxicity compared to [$^2H_4$]-**55**. Several experiments demonstrated that flies administered ketoester substrates died much sooner than those administered hydroxyesters or other compounds, even when sugar loadings of less than 2% w/w were used. Nevertheless, the very low level of incorporation observed from [$^2H_4$]-**13** and the lack of observable incorporation from [$^2H_4$]-**15** and [$^2H_4$]-**16** suggests that the biosynthesis of spiroacetal **1** in *B. cacuminata* commences with ω-4 oxidation of a fatty acid equivalent. The surprising level of deuterium incorporation from labeled methyl hexadecanoate [$^2H_4$]-**55** could also be an indication that fatty acid equivalents with C_{16} (or longer) chains may be better substrates for the responsible spiroacetal biosynthetic enzyme(s) than those with C_{14} chains (cf. compounds **8**–**16**). Although fatty acid metabolizing P450s often have preferred substrate chain lengths, the chain-length specificity of the spiroacetal biosynthetic enzymes of *B. cacuminata* (or *B. oleae*) has not yet been investigated.

The relative levels of in vivo deuterium incorporation into spiroacetal **1** observed from the various administered fatty ester-type compounds are shown in Table 1. These in vivo

Table 1. Incorporation Levels of Deuterium-Labeled Precursors into Spiroacetal [^2H_x]-1

oxygenation of fatty ester chain	some incorporation	low incorporation	no detectable incorporation
trioxygenated	[$^2H_4$]-(*R,R*)-**8/9**	[$^2H_4$]-*erythro*-**8/9**	
	[$^2H_4$]-(*S,S*)-**8/9**		
dioxygenated	[$^2H_4$]-**10**	[$^2H_4$]-**11**	[$^2H_2$]-(*R,R*)-**12**
			[$^2H_2$]-(*S,S*)-**12**
monooxygenated		[$^2H_4$]-**13**	[$^2H_4$]-**14**
			[5,5,7,7-$^2H_4$]-**15**
			[5,5,7,7-$^2H_4$]-**16**
			[7,7,8,8-$^2H_4$]-**15**
			[7,7,8,8-$^2H_4$]-**16**
unsubstituted		[$^2H_4$]-**55**	

investigations have established that an oxidative process culminating in a C–C bond cleavage step is integral to the generation of the nine-carbon dioxygenated unit that forms spiroacetal **1** in male *B. cacuminata*. The results are consistent with this being an enzyme-mediated process and indicate that it occurs stereoselectively in nature. The in vivo transformation of dihydroxylactones/trihydroxyesters [$^2H_4$]-**8/9**, trihydroxy-fatty acid equivalents containing stereochemically defined vicinal diol moieties, into spiroacetal **1** was observed to occur with clear *threo* diastereoselectivity, although no preference in incorporation was observed for either the (*R,R*) or (*S,S*) enantiomers. Incorporation of dihydroxyester [$^2H_4$]-**10** strongly suggested that the trioxygenated biosynthetic intermediate represented by **8/9** is formed by ω-9 oxidation of its dioxygenated precursor, and investigations into the earlier biosynthetic steps using keto- and hydroxyesters have tentatively indicated that the dioxygenated precursor itself is formed by ω-4 oxidation of a fatty acid equivalent, followed by ω-8 oxidation of the monooxygenated species.

The spiroacetal biosynthetic pathway in *B. cacuminata* (and presumably *B. oleae*) is now proposed to commence with the ω-4 oxidation of a fatty acid equivalent, followed by ω-8 oxidation to afford a dioxygenated precursor (Scheme 12). Subsequent ω-9 oxidation affords a trioxygenated fatty acid equivalent with a *threo* diol moiety that appears to undergo enzyme-mediated oxidative C–C bond cleavage to afford 5-hydroxynonanal (**4**). Elaboration of **4** to the spiroacetal **1** and hydroxyspiroacetal derivatives then occurs by a series of well established steps (Scheme 2).[8]

Oxidative C–C bond cleavage is one of the most impressive yet relatively uncommon transformations that P450s catalyze. Often occurring as the final step in sequential substrate oxidations, oxidative C–C bond scission can occur adjacent to hydroxyl groups, carbonyl groups, and amines. The majority of well-known and characterized examples are found in eukaryotic steroid biosynthesis, with a small number having roles in biodegradation and a sole example known from a prokaryotic

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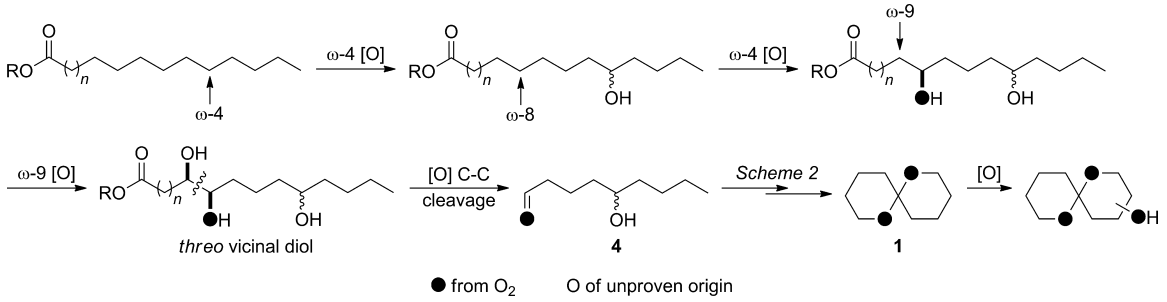
Scheme 12. Proposed Biosynthesis of Spiroacetal 1 and Hydroxyspiroacetal Derivatives in *B. cacuminata* from a Fatty Acid Equivalent, Showing the Origin of the Oxygen Atoms



biosynthetic pathway. Only two examples of oxidative diol cleavage are currently well characterized[22,35,36] and these reactions are reported to occur stereoselectively. It is possible that the actual species that undergoes C−C bond cleavage in vivo is not the diol but is rather a hydroxyketone (cf. reactions mediated by mammalian CYP17 during steroid biosynthesis[37−39] and by P450s involved in olanexidine biodegradation[40−42]) or a dione (cf. the anaerobic cleavage of cyclohexane-1,2-dione to form 6-oxohexanoate that is mediated by the thiamine pyrophosphate-dependent enzyme cyclohexane-1,2-dione hydrolase[43−45] from the bacterium *Azoarcus* sp. strain 22Lin). However, the diastereoselectivity with which the incorporation of dihydroxylactones/trihydroxyesters [$^2H_4$]-**8**/**9** into spiroacetal **1** was observed to occur is reminiscent of the diol cleavage reactions that are mediated by mammalian $P450_{scc}$ during steroid biosynthesis[35,36] and by the bacterial enzyme $P450_{BioI}$ during biotin biosynthesis.[22] Thus, the results obtained here are consistent with 5-hydroxynonanal (**4**) being produced via an enzyme-mediated oxidative diol cleavage reaction. Given the derivation of both the oxygen atoms in **1** from molecular oxygen, it is likely that the enzyme(s) involved are cytochromes P450.

■ CONCLUSION

Following the efficient synthesis of a series of deuterium-labeled potential spiroacetal precursors, the in vivo investigations described here have allowed the order of oxidative events in the biosynthetic pathway to spiroacetal **1** in *B. cacuminata* to be determined. Building upon previous work,[8] this has allowed (essentially) the complete delineation of the biosynthetic pathway from a fatty acid equivalent to **1** and its hydroxyspiroacetal derivatives (Scheme 12). In addition, it has justified the inclusion of an enzyme-mediated, multistep, oxidative C−C bond cleavage process as a key step. This is the first time such an oxidative transformation has been reported in insects. Future work will involve demonstrating that this pathway is also followed by the more pestiferous *B. oleae*, identifying the actual P450s involved in the biosynthesis, and defining their catalytic roles and substrate specificity.

■ EXPERIMENTAL SECTION

Deuterium-Labeled Compounds. For mixtures of deuterium-labeled products obtained using selective labeling methods (base-catalyzed exchange adjacent to ketones, or catalytic reduction of multiple bonds using deuterium gas), the predominant products present were determined from analysis of NMR and MS data, and the levels of unlabeled products present were determined by comparison of mass fragment peaks obtained from GC/MS. In experimental schemes and in the discussion of these compounds, the labeled mixtures are represented by the predominant labeled products using the appropriate prefix, [$^2H_4$] or [$^2H_2$], but without position descriptors except when reference is made to different isotopomers, in which case the position(s) of deuterium labeling are specified. However, nomenclature for selective labeling[46] has been used throughout the Experimental Section as only the complete mixture of products can account for the spectroscopic data observed.

Synthesis of Key Intermediate 17 from 1-Octen-4-ol (20). *((Oct-1-en-4-yloxy)methyl)benzene (**21**).* 1-Octen-4-ol (**20**)[14] (8.87 g, 69.2 mmol) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 5.08 g, 127.0 mmol) in anhydrous THF (60 mL) with stirring at 0 °C under a N_2 atmosphere. The mixture was allowed to warm to room temperature and stirred for 1 h. BnBr (98%, 9.0 mL, 74.2 mmol) was added dropwise, followed by tetra-*n*-butylammonium iodide (250 mg, cat.). The reaction mixture was stirred at room temperature for 92 h and then was recooled to 0 °C. Aqueous HCl solution (5 M, 50 mL) was added cautiously to quench the reaction, and the mixture was extracted with petroleum spirits 40−60 (6 × 40 mL). The combined organic extract was washed with saturated aqueous $NaHCO_3$ solution (40 mL) and brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography (silica gel, 100% petroleum spirits 40−60 to 2% Et_2O in petroleum spirits 40−60) to afford **21** (12.73 g, 58.3 mmol, 84%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.1 Hz, CH_3-8), 1.22−1.60 (m, 6H, CH_2-7, CH_2-6 and CH_2-5), 2.31 (m, 2H, CH_2-3), 3.42 (m, 1H, C*H*-4), 4.48 and 4.55 (AB q, 2H, J_{AB} = 11.6 Hz, ROCH_2Ph), 5.02−5.11 (m, 2H, CH_2-1), 5.84 (ddt, 1H, J = 17.2, 10.1, 7.1 Hz, C*H*-2), 7.22−7.36 ppm (m, 5H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-8), 22.8 (C-7), 27.6 (C-6), 33.5 (C-5), 38.3 (C-3), 70.9 (ROCH_2Ph), 78.6 (C-4), 116.8 (C-1), 127.4 (Ar CH), 127.7 (2 × Ar CH), 128.3 (2 × Ar CH), 135.1 (C-2), 139.0 ppm (Ar C). GC/MS (EI) m/z: 218 (0.03, $M^{+\bullet}$), 177 (2), 161 (2), 107 (1), 92 (9), 91 (100, $C_7H_7^{+\bullet}$), 77 (2), 65 (11), 57 (1), 51 (3), 43 (1), 41 (13). HRMS (EI) m/z: $M^{+\bullet}$ calcd for $C_{15}H_{22}O$ 218.1671, found 218.1601. Anal. Calcd for $C_{15}H_{22}O$: C, 82.52; H, 10.16. Found: C, 82.48; H, 10.17.

*4-(Benzyloxy)octan-1-ol (**22**).* Borane−dimethyl sulfide complex (2.80 mL, 29.5 mmol) was added dropwise to a solution of alkene **21** (4.39 g, 20.1 mmol) in anhydrous CH_2Cl_2 (50 mL) with stirring at 0 °C under a N_2 atmosphere. The solution was allowed to warm to room temperature, stirred for 2.5 h, and then recooled to 0 °C. EtOH (15 mL) was added dropwise, the mixture was stirred for a further 30 min, and then aqueous NaOH solution (20%, 25.0 mL, 125.0 mmol) was added dropwise, followed by H_2O_2 (30%, 25.0 mL, 220.5 mmol). The reaction mixture was allowed to warm to room temperature and stirred for a further 66 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (6 × 20 mL). The combined organic extract was washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 5% EtOAc in petroleum spirits 40−60 to 100% EtOAc) to afford alcohol **22** (4.13 g, 17.5 mmol, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.1 Hz, CH_3-8), 1.22−1.40 (m, 4H, CH_2-6 and CH_2-7), 1.44−1.73 (m, 6H, CH_2-2, CH_2-3 and CH_2-5), 3.41 (m, 1H, C*H*-4), 3.62 (m, 2H, CH_2-1), 4.48 and 4.53 (AB q, 2H, J_{AB} = 11.5 Hz, ROCH_2Ph), 7.22−7.34 ppm (m, 5H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0

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(C-8), 22.9 (C-7), 27.6 (C-6), 28.6, 30.3, 33.3 (C-5), 63.1 (C-1), 70.9 ($ROCH_2Ph$), 78.9 (C-4), 127.5 (Ar CH), 127.8 (2 × Ar CH), 128.4 (2 × Ar CH), 138.8 ppm (Ar C). GC/MS (EI) *m/z*: 236 (0.2, $M^{+\bullet}$), 218 (0.1, $M^{+\bullet} - H_2O$), 177 (0.3), 129 (2), 107 (15), 92 (10), 91 (100, $C_7H_7^{+\bullet}$), 77 (2), 65 (9), 57 (2), 55 (5), 45 (1), 43 (4). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{24}NaO_2$ 259.1674, found 259.1675. Anal. Calcd for $C_{15}H_{24}O_2$: C, 76.23; H, 10.24. Found: C, 76.54; H, 10.20.

*1-((1-Iodooctan-4-yloxy)methyl)benzene (**19**).* I_2 (99%, 2.46 g, 9.6 mmol) was added portionwise to a solution of alcohol **22** (1.40 g, 5.9 mmol), PPh_3 (99%, 2.56 g, 9.7 mmol), and imidazole (890 mg, 13.1 mmol) in a mixture of anhydrous Et_2O (12 mL) and CH_3CN (4 mL) with stirring at 0 °C under a N_2 atmosphere. The reaction mixture was allowed to warm to room temperature, stirred for a further 3 h, and then recooled to 0 °C. MeOH (5 mL) was added dropwise to quench the reaction, and the solvent was removed in vacuo. The residue was purified by flash column chromatography (silica gel, 100% petroleum spirits 40−60 to 5% EtOAc in petroleum spirits 40−60) to afford iodide **19** (2.01 g, 5.8 mmol, 98%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H, *J* = 7.1 Hz, CH_3-8), 1.25−1.40 (m, 4H, CH_2-6 and CH_2-7), 1.42−1.51 (m, 1H, CH-5), 1.53−1.69 (m, 3H, CH_2-3 and CH-5), 1.80−1.99 (m, 2H, CH_2-2), 3.16 (t, 2H, *J* = 7.0 Hz, CH_2-1), 3.38 (m, 1H, CH-4), 4.46 and 4.51 (AB q, 2H, J_{AB} = 11.6 Hz, $ROCH_2Ph$), 7.24−7.38 ppm (m, 5H, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 7.5 (C-1), 14.1 (C-8), 22.9 (C-7), 27.5 (C-6), 29.3 (C-2), 33.4 (C-5), 34.6 (C-3), 70.7 ($ROCH_2Ph$), 77.8 (C-4), 127.5 (Ar CH), 127.8 (2 × Ar CH), 128.3 (2 × Ar CH), 138.8 ppm (Ar C). GC/MS (EI) *m/z*: 289 (2), 255 (0.3, $M^{+\bullet} - C_7H_7$), 219 (0.3, $M^{+\bullet} - I$), 177 (2), 155 (1), 141 (0.2), 127 (4), 107 (1), 92 (10), 91 (100, $C_7H_7^{+\bullet}$), 77 (2), 65 (9), 55 (4), 43 (6). HRMS (EI) *m/z*: $M^{+\bullet}$ calcd for $C_{15}H_{23}IO$ 346.0794, found 346.0806.

*2-(10-(Benzyloxy)tetradec-5-yn-1-yloxy)tetrahydro-2H-pyran (**17**).* A solution of *n*-BuLi (1.78 M in hexanes, 6.0 mL, 10.7 mmol) was added dropwise to a solution of 2-(hex-5-yn-1-yloxy)tetrahydro-2*H*-pyran (**18**)[7] (2.02 g, 11.1 mmol) in anhydrous THF (10 mL) with stirring at −40 °C under a N_2 atmosphere. The solution was stirred at −40 °C for 3 h, then HMPA (99%, 2.5 mL, 14.2 mmol) was added, followed by the dropwise addition of a solution of iodide **19** (4.86 g, 14.0 mmol) in anhydrous THF (4 mL). The reaction mixture was allowed to warm to room temperature and stirred for 20 h. Saturated aqueous NH_4Cl solution (20 mL) was added to quench the reaction and the mixture was extracted with petroleum spirits 40−60 (6 × 20 mL). The combined organic extract was washed with aqueous LiCl solution (4 M, 6 × 20 mL) and brine (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 2% to 17% Et_2O in petroleum spirits 40−60) to afford **17** (3.69 g, 9.2 mmol, 83%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 7.1 Hz, CH_3-14), 1.23−1.87 (m, 20H), 2.16 (m, 4H, CH_2-4 and CH_2-7), 3.38 (m, 2H, CH-1 and CH-10), 3.47 (m, 1H, CH-6′), 3.73 (dt, 1H, *J* = 9.7, 6.6 Hz, CH-1), 3.84 (m, 1H, CH-6′), 4.48 (s, 2H, $ROCH_2Ph$), 4.55 (dd, 1H, *J* = 4.1, 2.9 Hz, CH-2′), 7.20−7.35 ppm (m, 5H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 18.6, 18.9, 19.6, 22.9, 24.9, 25.5, 26.0, 27.6, 29.0, 30.8, 32.9, 33.5, 62.3 (C-6′), 67.1 (C-1), 70.7 ($ROCH_2Ph$), 78.6 (C-10), 80.1 (RC≡CR), 80.2 (RC≡CR), 98.8 (C-2′), 127.4 (Ar CH), 127.7 (2 × Ar CH), 128.3 (2 × Ar CH), 139.1 ppm (Ar C). GC/MS (EI) *m/z*: 400 (0.03, $M^{+\bullet}$), 315 (1, $M^{+\bullet}$ − THP), 309 (0.3), 259 (1), 243 (2), 223 (1), 221 (1), 209 (2), 195 (1), 191 (2), 181 (1), 177 (1), 169 (1), 157 (1), 151 (5), 143 (1), 135 (3), 129 (1), 107 (5), 101 (2), 92 (9), 91 (100, $C_7H_7^{+\bullet}$), 85 (95, $THP^{+\bullet}$), 79 (12), 77 (6), 67 (17), 57 (11), 55 (20), 43 (10), 41 (17). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{26}H_{40}NaO_3$ 423.2875, found 423.2857. Anal. Calcd for $C_{26}H_{40}O_3$: C, 77.95; H, 10.06. Found: C, 77.78; H, 10.00.

Synthesis of Unsaturated Hydroxyester [$^2H_4$]-(*E*)-27 from Compound 17. *(E)-14-(Tetrahydro-2H-pyran-2-yloxy)tetradec-9-en-5-ol [(E)-**23**].* Lithium metal (350 mg, 50.4 mmol) was added portion-wise to liquid NH_3 (100 mL) with stirring at −78 °C until the solution remained blue in color. *t*-BuOH (9 mL) was added, followed by a solution of **17** (3.94 g, 9.8 mmol) in anhydrous THF (15 mL). The reaction mixture was stirred at −78 °C for 4 h and then was allowed to warm to room temperature while stirring overnight, during which time NH_3 evaporated. Saturated aqueous NH_4Cl solution (20 mL) was added to quench the reaction and the mixture was extracted with Et_2O (6 × 20 mL). The combined organic extract was washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 9% to 17% Et_2O in petroleum spirits 40−60) to afford (*E*)-**23** (2.85 g, 9.1 mmol, 93%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 7.0 Hz, CH_3-14), 1.20−1.87 (m, 20H), 1.98 (m, 4H, CH_2-4 and CH_2-7), 3.36 (dt, 1H, *J* = 9.6, 6.6 Hz, CH-1), 3.47 (m, 1H, CH-6′), 3.56 (m, 1H, CH-10), 3.70 (dt, 1H, *J* = 9.6, 6.8 Hz, CH-1), 3.84 (m, 1H, CH-6′), 4.55 (dd, 1H, *J* = 4.2, 2.7 Hz, CH-2′), 5.38 ppm (m, 2H, CH-5 and CH-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 19.6, 22.7, 25.5, 25.6, 26.2, 27.8, 29.2, 30.8, 32.3, 32.5, 36.9, 37.2, 62.3 (C-6′), 67.5 (C-1), 71.8 (C-10), 98.8 (C-2′), 130.3 (RHC=CHR), 130.4 ppm (RHC=CHR). GC/MS (EI) *m/z*: 312 (0.2, $M^{+\bullet}$), 155 (0.1), 115 (0.3), 101 (2), 85 (100, $THP^{+\bullet}$), 81 (6), 69 (8), 67 (22), 57 (17), 55 (26), 43 (26), 41 (49). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ Calcd for $C_{19}H_{36}NaO_3$ 335.2562, found 335.2572. Anal. Calcd for $C_{19}H_{36}O_3$: C, 73.03; H, 11.61. Found: C, 72.70; H, 11.26.

*(E)-5-Tetradecene-1,10-diol [(E)-**24**].* A solution of (*E*)-**23** (800 mg, 2.56 mmol) and *p*-TsOH.H_2O (25 mg, cat.) in MeOH (20 mL) was stirred at room temperature for 1 h. Saturated aqueous $NaHCO_3$ solution (30 mL) was added to quench the reaction and MeOH was evaporated in vacuo. The aqueous solution was extracted with EtOAc (2 × 50 mL) and the combined organic extract was washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 20% EtOAc in *n*-hexane) to afford diol (*E*)-**24** (550 mg, 2.41 mmol, 94%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H, *J* = 7.1 Hz, CH_3-14), 1.22−1.50 (m, 14H), 1.55 (m, 2H), 1.99 (m, 4H, CH_2-4 and CH_2-7), 3.57 (m, 1H, CH-10), 3.62 (t, 2H, *J* = 6.6 Hz, CH_2-1), 5.39 ppm (m, 2H, CH-5 and CH-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1 (C-14), 22.7, 25.55, 25.60, 27.8, 32.18, 32.22, 32.5, 36.9, 37.2, 62.9 (C-1), 71.9 (C-10), 130.3 (RHC=CHR), 130.5 ppm (RHC=CHR). GC/MS (EI) *m/z*: 228 (0.1, $M^{+\bullet}$), 210 (0.3, $M^{+\bullet} - H_2O$), 186 (1), 171 (1), 149 (0.4), 135 (2), 113 (6), 98 (7), 93 (11), 82 (17), 79 (23), 67 (36), 57 (26), 55 (38), 41 (100). HRMS (EI) *m/z*: $M^{+\bullet}$ calcd for $C_{14}H_{28}O_2$: 228.2089, found 228.2093.

*(E)-10-Oxo-5-tetradecenoic acid [(E)-**25**].* Jones' reagent (8 N) was added dropwise to a solution of diol (*E*)-**24** (300 mg, 1.31 mmol) in acetone (5 mL) with stirring at 0 °C until the orange color of the reaction mixture persisted. The reaction mixture was allowed to warm to room temperature with stirring over 15 min and then was quenched with water (5 mL). The mixture was extracted with Et_2O (3 × 20 mL) and the combined organic extract was washed with brine (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 10% EtOAc in *n*-hexane) to afford ketoacid (*E*)-**25** (300 mg, 1.25 mmol, 95%, ≥95% *E* isomer) as a colorless solid. Mp: 36−38 °C. ^{1}H NMR (500 MHz, $CDCl_3$): (mixture of *E* and *Z* isomers) δ 0.87 (t, 3H, *J* = 7.4 Hz, CH_3-14), 1.27 (sextet, 2H, *J* = 7.4 Hz), 1.52 (quintet, 2H, *J* = 7.5 Hz), 1.60 (quintet, 2H, *J* = 7.4 Hz), 1.67 (quintet, 2H, *J* = 7.4 Hz), 1.96 (m, 2H), 2.01 (m, 2H), 2.32 (t, 2H, *J* = 7.5 Hz), 2.36 (2 overlapping t, 4H, *J* = 7.4 Hz and 7.5 Hz), 5.36 ppm (m, 2H, CH-5 and CH-6). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of *E* and *Z* isomers) δ 13.8 (C-14), 22.3, 23.5, 23.6 (*Z* isomer), 24.3, 24.5 (*Z* isomer), 25.9, 26.4 (*Z* isomer), 26.6 (*Z* isomer), 31.7, 31.9, 33.2, 33.3 (*Z* isomer), 41.9, 42.0 (*Z* isomer), 42.55 (*Z* isomer), 42.58, 42.7 (*Z* isomer), 129.2 (RHC=CHR of *Z* isomer), 129.8 (RHC=CHR of *E* isomer), 130.1 (RHC=CHR of *Z* isomer), 130.7 (RHC=CHR of *E* isomer), 179.6 (C-1 of *E* isomer), 179.8 (C-1 of *Z* isomer), 211.5 (C-10 of *Z* isomer), 211.7 ppm (C-10 of *E* isomer). GC/MS (EI) *m/z*: (*E* isomer, as methyl ester) 254 (1, $M^{+\bullet}$), 236 (1), 223 (2, $M^{+\bullet} - OCH_3$), 222 (3, $M^{+\bullet}$ − MeOH), 212 (3), 194 (2), 180 (2), 165 (4), 154 (9), 147 (6), 137 (10), 123 (21), 122 (15), 95 (31), 94 (61), 85 (86, $C_5H_9O^{+\bullet}$), 80 (100), 74 (22), 67 (41), 59 (19, $C_2H_3O_2^{+\bullet}$), 57 (88, $C_4H_9^{+\bullet}$), 55 (58), 43 (40), 41 (94). GC/MS (EI) *m/z*: (*Z* isomer, as

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methyl ester) 254 (1, $M^{+\bullet}$), 246 (1), 223 (2, $M^{+\bullet} - OCH_3$), 222 (2, $M^{+\bullet}$ − MeOH), 212 (2), 194 (1), 180 (2), 165 (6), 154 (11), 147 (9), 137 (11), 123 (22), 122 (17), 95 (31), 94 (66), 85 (85, $C_5H_9O^{+\bullet}$), 80 (97), 74 (20), 67 (44), 59 (20, $C_2H_3O_2^{+\bullet}$), 57 (100, $C_4H_9^{+\bullet}$), 55 (62), 43 (44), 41 (100). Anal. Calcd for $C_{14}H_{24}O_3$: C, 69.96: H, 10.07. Found: C, 69.86; H, 10.43.

*Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-(E)-10-oxo-5-tetradecenoate ([$^2H_4$]-(E)-**26**).* Lithium metal (~100 mg, 14.41 mmol) was added cautiously to D_2O (99.9 atom % D, 15 mL) at 0 °C, and the resulting LiOD solution was added to a solution of ketoacid (*E*)-**25** (270 mg, 1.12 mmol) in anhydrous THF (1 mL). The pale yellow reaction mixture was stirred at room temperature for 24 h and then cold aqueous HCl (0.05 M, 10 mL) was added to acidify the basic reaction mixture. The acidic mixture was extracted with cold EtOAc (2 × 50 mL), and the combined organic extract was washed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in MeOH (5 mL) and the solution was cooled to 0 °C. Ethereal CH_2N_2 was added dropwise until the yellow color of the reaction mixture persisted. The yellow solution was stirred at 0 °C for 15 min and then was allowed to warm to room temperature with stirring over a further 15 min. Excess CH_2N_2 was evaporated under a stream of N_2 gas and the colorless reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, 10% EtOAc in *n*-hexane) to afford a viscous, colorless oil containing [$^2H_4$]-(*E*)-**26** (180 mg, 0.70 mmol, 62% over two steps, ≥95% *E* isomer, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^1H NMR (500 MHz, $CDCl_3$) (mixture of *E* and *Z* isomers): δ 0.86 (t, 3H, J = 7.4 Hz, CH_3-14), 1.26 (m, 2H), 1.49 (br t, 2H, J = 7.6 Hz), 1.57 (br t, 2H, J = 7.3 Hz), 1.64 (quintet, 2H, J = 7.4 Hz), 1.96 (m, 4H), 2.26 (t, 2H, J = 7.6 Hz, CH_2-2), 2.28−2.42 (m, 0.2H, residual hydrogen from CH_2 or CHD at C-9 and/or C-11 of [$^2H_0$]-[$^2H_3$]-analogues), 3.62 (s, 3H, RCO_2CH_3), 5.34 ppm (m, 2H, CH-5 and CH-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8 (C-14), 22.3, 23.3, 23.5 (*Z* isomer), 24.6, 24.8 (*Z* isomer), 25.8, 26.5 (*Z* isomer), 31.8, 31.9, 33.3, 33.4 (*Z* isomer), 40.8−42.2 (m, incl. 41.2 [quintet, $J_{C,D}$ = 19.2 Hz, CD_2], 41.8 [quintet, $J_{C,D}$ = 19.0 Hz, CD_2], C-9 and C-11), 51.4 (RCO_2CH_3), 129.3 (RHC=CHR of *Z* isomer), 129.87 (RHC=CHR of *E* isomer), 129.92 (RHC=CHR of *Z* isomer), 130.5 (RHC=CHR of *E* isomer), 174.1 (C-1), 211.7 ppm (C-10). GC/MS (EI) m/z: (*E* isomer) 258 (1, $M^{+\bullet}$), 240 (1), 227 (3, $M^{+\bullet} - OCH_3$), 226 (6, $M^{+\bullet}$ − MeOH), 216 (3), 198 (2), 184 (2), 167 (4), 154 (10), 149 (4), 139 (8), 123 (24), 122 (23), 95 (26), 94 (60), 87 (75, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 80 (100), 74 (25), 67 (34), 62 (47), 59 (96, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (27), 43 (41), 41 (48). GC/MS (EI) m/z: (*Z* isomer) 258 (1, $M^{+\bullet}$), 240 (1), 227 (2, $M^{+\bullet} - OCH_3$), 226 (6, $M^{+\bullet}$ − MeOH), 216 (3), 198 (2), 184 (2), 167 (4), 154 (11), 149 (4), 139 (8), 123 (20), 122 (21), 95 (28), 94 (60), 87 (80, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 80 (93), 74 (23), 67 (31), 62 (47), 59 (100, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (31), 43 (41), 41 (48). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{15}H_{22}D_4NaO_3$ 281.2031, found 281.2022.

*Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-(E)-10-hydroxy-5-tetradecenoate ([$^2H_4$]-(E)-**27**).* $NaBH_4$ (4 mg, 0.11 mmol) was added to a solution of ketoester [$^2H_4$]-(*E*)-**26** (82 mg, 0.32 mmol) in MeOH (5 mL) with stirring at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for a further 1 h and then was quenched with aqueous HCl (0.05 M, 10 mL). The reaction mixture was extracted with EtOAc (3 × 20 mL), and the combined organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel impregnated with 10% $AgNO_3$, 10% EtOAc in *n*-hexane) to afford a viscous, colorless oil containing [$^2H_4$]-(*E*)-**27** (72 mg, 0.28 mmol, 87%, 100% *E* isomer, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^1H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.0 Hz, CH_3-14), 1.22−1.51 (m, 7H), 1.67 (quintet, 2H, J = 7.4 Hz), 2.00 (m, 4H), 2.29 (t, 2H, J = 7.6 Hz, CH_2-2), 3.56 (m, 1H, CH-10), 3.65 (s, 3H, RCO_2CH_3), 5.38 ppm (m, 2H, CH-5 and CH-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0 (C-14), 22.6, 24.6, 25.2, 27.5, 31.8, 32.4, 33.3, 35.5−36.8 (m, incl. 35.9 [quintet, $J_{C,D}$ = 18.3 Hz, CD_2], 36.2 [quintet, $J_{C,D}$ = 18.3 Hz, CD_2], C-9 and C-11), 51.4 (RCO_2CH_3), 71.4 (C-10), 129.1 (RHC=CHR), 131.2 (RHC=CHR), 174.1 ppm (C-1). GC/MS (EI) m/z: 260 (0.2, $M^{+\bullet}$), 242 (1, $M^{+\bullet} - H_2O$), 218 (1), 201 (1), 183 (1), 168 (4), 154 (15), 140 (12), 126 (9), 122 (12), 98 (17), 94 (49), 87 (21), 81 (44), 80 (100), 74 (31), 71 (27), 67 (30), 59 (33), 55 (34), 43 (55), 41 (57). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{15}H_{24}D_4NaO_3$ 283.2187, found 283.2193.

Synthesis of Unlabeled *Threo* Dihydroxylactones/Trihydroxyesters (*S,S*)-8/9 and (*R,R*)-8/9 from (*E*)-23. *(5S,6S)-1-(Tetrahydro-2H-pyran-2-yloxy)tetradecane-5,6,10-triol [(S,S)-**28**].* Methanesulfonamide (97%, 385 mg, 3.93 mmol) and AD-mix-α (Aldrich, 5.166 g) were added to a solution of alkene (*E*)-**23** (924 mg, 2.96 mmol) in a mixture of *t*-BuOH and H_2O (1:1, 20 mL) with stirring at 0 °C. The yellow reaction mixture was stirred at 4 °C for 64 h, and then a solution of sodium sulfite (~1 g, 7.93 mmol) in water (10 mL) was added. The mixture was allowed to warm to room temperature and stirred for 1 h and then was extracted with Et_2O (6 × 30 mL). The combined organic extract was washed with aqueous NaOH solution (5%, 30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 33% Et_2O in petroleum spirits 40−60 to 100% Et_2O) to afford triol (*S,S*)-**28** (978 mg, 2.82 mmol, 95%) as a waxy, white solid. Mp: 40−46 °C. $[\alpha]_D^{24}$ −18.2 (*c* 0.61, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.9 Hz, CH_3-14), 1.10−1.87 (m, 24H), 2.52 (br s, 3H, 3 × ROH), 3.39 (m, 3H, CH-1, CH-5 and CH-6), 3.47 (m, 1H, CH-6′), 3.57 (m, 1H, CH-10), 3.73 (m, 1H, CH-1), 3.84 (m, 1H, CH-6′), 4.54 ppm (m, 1H, CH-2′). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.1 (C-14), 19.7, 19.8, 21.6, 21.8, 22.3, 22.5, 22.7, 25.4, 27.9, 29.5, 29.6, 30.75, 30.78, 33.2, 33.3, 33.5, 37.0, 37.20, 37.23, 37.3, 62.5 (C-6′), 62.6 (C-6′), 67.5 (C-1), 71.76 (C-10), 71.80 (C-10), 74.21, 74.23, 74.27, 74.28, 74.30, 74.36, 74.38, 74.40, 99.0 (C-2′), 99.1 ppm (C-2′). GC/MS (EI) m/z: 245 (0.1), 227 (2), 209 (1), 187 (2), 169 (3), 159 (2), 157 (1), 141 (8), 123 (4), 115 (1), 111 (2), 101 (2), 87 (2), 85 (100, $THP^{+\bullet}$), 81 (7), 67 (14), 57 (19), 55 (20), 43 (14), 41 (21). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{19}H_{38}NaO_5$ 369.2617, found 369.2626. Anal. Calcd for $C_{19}H_{38}O_5$: C, 65.86; H, 11.05. Found: C, 65.82; H, 11.05.

*(5R,6R)-1-(Tetrahydro-2H-pyran-2-yloxy)tetradecane-5,6,10-triol [(R,R)-**28**].* Alkene (*E*)-**23** (930 mg, 2.98 mmol) was dihydroxylated with AD-mix-β (Aldrich, 5.212 g) and methanesulfonamide (97%, 398 mg, 4.06 mmol) in a mixture of *t*-BuOH and H_2O (1:1, 20 mL) at 4 °C over 64 h, as described for the synthesis of (*S,S*)-**28**. Purification by flash column chromatography (silica gel, 33% Et_2O in petroleum spirits 40−60 to 100% Et_2O) afforded triol (*R,R*)-**28** (983 mg, 2.84 mmol, 95%) as a waxy, white solid. Mp: 40−46 °C. $[\alpha]_D^{24}$ +19.6 (*c* 0.75, MeOH). This compound was spectroscopically identical to (*S,S*)-**28**. HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{19}H_{38}NaO_5$ 369.2617, found 369.2611. Anal. Calcd for $C_{19}H_{38}O_5$: C, 65.86; H, 11.05. Found: C, 65.59; H, 10.97.

*(5S,6S)-1,5,6,10-Tetradecanetetraol [(S,S)-**29**].* A solution of (*S,S*)-**28** (797 mg, 2.30 mmol) and concentrated aqueous HCl (32%, 0.5 mL) in MeOH (10 mL) was stirred at room temperature for 22 h. Solid $NaHCO_3$ (420 mg) was added to quench the reaction, and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (silica gel, 5% to 9% MeOH in CH_2Cl_2) to afford (*S,S*)-**29** (580 mg, 2.21 mmol, 96%) as a white solid. Mp: 65−67 °C. $[\alpha]_D^{24}$ −20.3 (*c* 0.62, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 0.91 (t, 3H, J = 7.1 Hz, CH_3-14), 1.22−1.71 (m, 18H), 3.39 (m, 2H, CH-5 and CH-6), 3.52 (m, 1H, CH-10), 3.55 ppm (t, 2H, J = 6.4 Hz, CH_2-1). ^{13}C NMR (100 MHz, CD_3OD): (mixture of diastereomers) δ 14.4 (C-14), 23.21, 23.25, 23.4, 23.8, 29.1, 33.68, 33.71, 33.9, 34.0, 38.1, 38.2, 38.4, 38.5, 62.9 (C-1), 72.3, 72.4, 75.17, 75.23 ppm. HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{14}H_{30}NaO_4$ 285.2042, found 285.2039. Anal. Calcd for $C_{14}H_{30}O_4$: C, 64.08; H, 11.52. Found: C, 63.92; H, 11.52.

*(5R,6R)-1,5,6,10-Tetradecanetetraol [(R,R)-**29**].* The THP moiety of (*R,R*)-**28** (768 mg, 2.21 mmol) was cleaved with HCl (32%, 0.5 mL) in MeOH (10 mL) over 22 h, as described for the synthesis of (*S,S*)-**29**. Purification by flash column chromatography (silica gel, 5%

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to 9% MeOH in CH_2Cl_2) afforded (*R,R*)-**29** (552 mg, 2.10 mmol, 95%) as a white solid. Mp: 65−67 °C. $[\alpha]_D^{24}$ +21.4 (*c* 0.78, MeOH). This compound was spectroscopically identical to (*S,S*)-**29**. HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{30}NaO_4$ 285.2042, found 285.2028. Anal. Calcd for $C_{14}H_{30}O_4$: C, 64.08; H, 11.52. Found: C, 63.98; H, 11.54.

*1-((4S,5S)-5-(4-Hydroxybutyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-octan-4-ol [(S,S)-**30**].* Anhydrous *p*-TsOH (100 mg, cat.) was added to a suspension of tetraol (*S,S*)-**29** (492 mg, 1.88 mmol) and 4 Å molecular sieves in freshly distilled acetone (12 mL) with stirring at room temperature under a N_2 atmosphere. The reaction mixture was stirred for 19 h then solid $NaHCO_3$ (300 mg) was added to quench the reaction. The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography (silica gel, 17% Et_2O in petroleum spirits 40−60 to 100% Et_2O) to afford (*S,S*)-**30** (548 mg, 1.81 mmol, 97%) as a colorless, viscous oil. $[\alpha]_D^{24}$ −25.6 (*c* 0.77, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 7.1 Hz, CH_3-14), 1.15−1.65 (m, 26H, incl. 1.34 [s, 6H, 2 × ketal CH_3]), 3.58 (m, 3H, C*H*-5, C*H*-6 and C*H*-10), 3.61 ppm (t, 2H, *J* = 6.4 Hz, CH_2-1). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.0 (C-14), 22.1, 22.3, 22.7, 27.2 (2 × ketal CH_3), 27.80, 27.82, 32.42, 32.44, 32.6, 32.7, 37.1, 37.2, 37.27, 37.29, 62.5 (C-1), 71.6 (C-10), 80.78, 80.79, 80.81, 80.9, 107.84 (ketal $C(CH_3)_2$), 107.85 ppm (ketal $C(CH_3)_2$). GC/MS (EI) *m/z*: 287 (5, $M^{+\bullet} - CH_3$), 285 (1, $M^{+\bullet}$ − OH), 269 (2), 227 (26), 209 (15), 191 (7), 169 (10), 151 (5), 141 (7), 135 (12), 129 (2), 121 (18), 115 (7), 109 (24), 101 (5), 100 (15), 95 (25), 87 (5), 85 (87), 79 (23), 73 (4), 69 (48), 67 (40), 59 (69), 57 (58), 55 (56), 45 (8), 43 (100), 41 (62). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{17}H_{34}NaO_4$ 325.2355, found 325.2350. Anal. Calcd for $C_{17}H_{34}O_4$: C, 67.51; H, 11.33. Found: C, 67.58; H, 11.44.

*1-((4R,5R)-5-(4-Hydroxybutyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-octan-4-ol [(R,R)-**30**].* Tetraol (*R,R*)-**29** (461 mg, 1.76 mmol) was reacted with acetone in the presence of *p*-TsOH (100 mg, cat.) and 4 Å molecular sieves under a N_2 atmosphere over 19 h, as described for the synthesis of (*S,S*)-**30**. Purification by flash column chromatography (silica gel, 17% Et_2O in petroleum spirits 40−60 to 100% Et_2O) afforded (*R,R*)-**30** (501 mg, 1.66 mmol, 94%) as a colorless, viscous oil. $[\alpha]_D^{24}$ +27.5 (*c* 0.81, MeOH). This compound was spectroscopically identical to (*S,S*)-**30**. HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{17}H_{34}NaO_4$ 325.2355, found 325.2340. Anal. Calcd for $C_{17}H_{34}O_4$: C, 67.51; H, 11.33. Found: C, 67.57; H, 11.40.

*4-((4S,5S)-2,2-Dimethyl-5-(4-oxooctyl)-1,3-dioxolan-4-yl)-butanoic acid [(S,S)-**31**].* A solution of diol (*S,S*)-**30** (373 mg, 1.23 mmol) and PDC (98%, 2.42 g, 6.30 mmol) in DMF (20 mL) was stirred at room temperature under an Ar atmosphere for 18 h. The reaction mixture was diluted with Et_2O (50 mL) and filtered through a pad of Celite that was washed thoroughly with additional Et_2O. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography (silica gel, 17% EtOAc in petroleum spirits 40−60 to 100% EtOAc) to afford (*S,S*)-**31** (354 mg, 1.13 mmol, 91%) as a colorless, viscous oil. $[\alpha]_D^{25}$ −25.3 (*c* 0.59, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 7.3 Hz, CH_3-14), 1.26 (m, 2H), 1.33 (s, 6H, 2 × ketal CH_3), 1.42−1.90 (m, 10H), 2.33−2.46 (m, 6H, CH_2-2, CH_2-9 and CH_2-11), 3.56 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (C-14), 20.4, 21.3, 22.3, 25.9, 27.2 (2 × ketal CH_3), 31.9, 32.1, 33.8, 42.4, 42.5, 80.4, 80.6, 108.1 (ketal $C(CH_3)_2$), 178.8 (C-1), 211.2 ppm (C-10). GC/MS (EI) *m/z*: 314 (0.03, $M^{+\bullet}$), 299 (3, $M^{+\bullet} - CH_3$), 221 (8), 140 (5), 137 (11), 127 (1), 119 (4), 113 (2), 100 (10), 99 (1), 87 (3), 85 (44, $C_5H_9O^{+\bullet}$), 73 (2), 59 (18, $C_2H_3O_2^{+\bullet}$), 57 (43, $C_4H_9^{+\bullet}$), 45 (10), 43 (100). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{17}H_{30}NaO_5$ 337.1991, found 337.2000. Anal. Calcd for $C_{17}H_{30}O_5$: C, 64.94; H, 9.62. Found: C, 64.73; H, 9.62.

*4-((4R,5R)-2,2-Dimethyl-5-(4-oxooctyl)-1,3-dioxolan-4-yl)-butanoic acid [(R,R)-**31**].* Diol (*R,R*)-**30** (368 mg, 1.22 mmol) was oxidized with PDC (98%, 2.46 g, 6.41 mmol) in DMF (20 mL) under an Ar atmosphere over 18 h, as described for the synthesis of (*S,S*)-**31**. Purification by flash column chromatography (silica gel, 17% EtOAc in petroleum spirits 40−60 to 100% EtOAc) afforded (*R,R*)-**31** (362 mg, 1.15 mmol, 95%) as a colorless, viscous oil. $[\alpha]_D^{25}$ +27.2 (*c* 0.66, MeOH). This compound was spectroscopically identical to (*S,S*)-**31**. HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{17}H_{30}NaO_5$ 337.1991, found 337.1989. Anal. Calcd for $C_{17}H_{30}O_5$: C, 64.94; H, 9.62. Found: C, 64.82; H, 9.61.

*Methyl 4-((4S,5S)-2,2-dimethyl-5-(4-oxooctyl)-1,3-dioxolan-4-yl)-butanoate [(S,S)-**32**].* Ethereal CH_2N_2 was added dropwise to a solution of ketoacid (*S,S*)-**31** (140 mg, 0.45 mmol) in a mixture of MeOH (3 mL) and Et_2O (2 mL) with stirring at 0 °C until the yellow color persisted. The yellow reaction mixture was stirred at 0 °C for 15 min and then was allowed to warm to room temperature with stirring over a further 15 min. Excess CH_2N_2 was evaporated under a stream of N_2 gas. The colorless reaction mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography (silica gel, 5% to 17% EtOAc in petroleum spirits 40−60) to afford (*S,S*)-**32** (138 mg, 0.42 mmol, 94%) as a colorless oil. 84% ee (Chiralpak OD column, 2% 2-propanol in *n*-hexane, flow rate 0.8 mL min^{-1}, PDA-UV detector 215 nm, retention times: (*R,R*)-**32** 10.8 min, (*S,S*)-**32** 11.2 min). $[\alpha]_D^{25}$ −16.7 (*c* 0.55, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 7.3 Hz, CH_3-14), 1.28 (m, 2H), 1.33 (s, 6H, 2 × ketal CH_3), 1.39−1.93 (m, 10H), 2.28−2.46 (m, 6H, CH_2-2, CH_2-9 and CH_2-11), 3.56 (m, 2H, C*H*-5 and C*H*-6), 3.65 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (C-14), 20.4, 21.6, 22.3, 25.9, 27.3 (2 × ketal CH_3), 32.10, 32.13, 33.9, 42.4, 42.5, 51.5 (RCO_2CH_3), 80.4, 80.6, 108.1 (ketal $C(CH_3)_2$), 173.8 (C-1), 211.0 ppm (C-10). GC/MS (EI) *m/z*: 328 (0.2, $M^{+\bullet}$), 313 (17, $M^{+\bullet} - CH_3$), 271 (1), 254 (2), 253 (15), 239 (11), 221 (12), 210 (1), 203 (3), 193 (1), 185 (1), 181 (4), 175 (3), 169 (2), 155 (2), 140 (16), 137 (19), 129 (5), 127 (3), 115 (19), 113 (6), 101 (2), 99 (5), 98 (14), 93 (12), 87 (2, $C_4H_7O_2^{+\bullet}$), 85 (100, $C_5H_9O^{+\bullet}$), 74 (3), 73 (3), 71 (9), 59 (17, $C_2H_3O_2^{+\bullet}$), 57 (41, $C_4H_9^{+\bullet}$), 55 (31), 43 (49). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{32}NaO_5$ 351.2147, found 351.2138. Anal. Calcd for $C_{18}H_{32}O_5$: C 65.82, H 9.82; found: C 65.90, H 9.69.

*Methyl 4-((4R,5R)-2,2-dimethyl-5-(4-oxooctyl)-1,3-dioxolan-4-yl)-butanoate [(R,R)-**32**].* Ketoacid (*R,R*)-**31** (134 mg, 0.43 mmol) was esterified with CH_2N_2 in a mixture of MeOH (3 mL) and Et_2O (2 mL) at 0 °C, as described for the synthesis of (*S,S*)-**32**. Purification by flash column chromatography (silica gel, 5% to 17% EtOAc in petroleum spirits 40−60) afforded (*R,R*)-**32** (133 mg, 0.40 mmol, 95%) as a colorless oil. 81% ee (Chiralpak OD column, 2% 2-propanol in *n*-hexane, flow rate 0.8 mL min^{-1}, PDA-UV detector 215 nm, retention times: (*R,R*)-**32** 10.8 min, (*S,S*)-**32** 11.2 min). $[\alpha]_D^{25}$ +18.5 (*c* 0.68, MeOH). This compound was spectroscopically identical to (*S,S*)-**32**. HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{32}NaO_5$ 351.2147, found 351.2133. Anal. Calcd for $C_{18}H_{32}O_5$: C, 65.82; H, 9.82. Found: C, 65.81; H, 9.75.

*Methyl 4-((4S,5S)-5-(4-hydroxyoctyl)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate [(S,S)-**33**].* $NaBH_4$ (19 mg, 0.50 mmol) was added to a solution of ketoester (*S,S*)-**32** (66 mg, 0.20 mmol) in a mixture of MeOH (3 mL) and Et_2O (1 mL) with stirring at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for a further 2 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) to afford (*S,S*)-**33** (62 mg, 0.19 mmol, 93%) as a viscous, pale yellow oil. $[\alpha]_D^{25}$ −23.8 (*c* 0.67, MeOH). ^1H NMR (400 MHz, C_6D_6): δ 0.91 (t, 3H, *J* = 7.0 Hz, CH_3-14), 1.18−1.67 (m, 20H, incl. 1.42 [s, 3H, ketal CH_3], 1.43 [s, 3H, ketal CH_3]), 1.74 (m, 1H), 1.90 (m, 1H), 2.18 (ABX_2 system, 2H, J_{AB} = 16.1, J_{AX} = 7.5, J_{BX} = 7.1 Hz, CH_2-2), 3.37 ppm (s, 3H, RCO_2CH_3), 3.42 (m, 1H, C*H*-10), 3.57 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (100 MHz, C_6D_6): (mixture of diastereomers) δ 14.3 (C-14), 22.1, 22.8, 23.0, 23.1, 27.57 (ketal CH_3), 27.61 (ketal CH_3), 28.2, 32.39, 32.41, 33.16, 33.19, 33.8, 37.74, 37.78, 37.82, 37.9, 50.9 (RCO_2CH_3), 71.4 (C-10), 71.5 (C-10), 81.0 (2C), 81.2, 81.3, 108.06 (ketal $C(CH_3)_2$), 108.07 (ketal $C(CH_3)_2$), 173.19 (C-1), 173.21 ppm (C-1). GC/MS (EI) *m/z*: 315 (3, $M^{+\bullet} - CH_3$), 313 (2, $M^{+\bullet}$ − OH), 256 (4), 255 (26), 237 (5), 223 (23), 215 (2), 205 (18), 197 (7), 187 (12), 177 (14), 163 (12), 157 (6), 137 (11), 129 (4), 121 (22), 115 (32), 109 (18), 101 (5), 95 (25), 87 (6), 85 (56), 81 (24), 74 (7), 73 (7), 69 (30), 59 (41, $C_2H_3O_2^{+\bullet}$), 57 (35, $C_4H_9^{+\bullet}$), 55 (55), 43 (100). HRMS (ESI) *m/z*:

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$[M + Na]^{+\bullet}$ calcd for $C_{18}H_{34}NaO_5$: 353.2304, found 353.2295. Anal. Calcd for $C_{18}H_{34}O_5$: C, 65.42; H, 10.37. Found: C, 65.41; H, 10.34.

*Methyl 4-((4R,5R)-5-(4-hydroxyoctyl)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate [(R,R)-**33**].* Ketoester (*R*,*R*)-**32** (73 mg, 0.22 mmol) was reduced with $NaBH_4$ (21 mg, 0.56 mmol) in a mixture of MeOH (3 mL) and Et_2O (1 mL) over 2 h, as described for the synthesis of (*S*,*S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded hydroxyester (*R*,*R*)-**33** (68 mg, 0.21 mmol, 93%) as a viscous, pale yellow oil. $[\alpha]_D^{25}$ +21.7 (*c* 0.52, MeOH). This compound was spectroscopically identical to (*S*,*S*)-**33**. HRMS (ESI) *m*/*z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{34}NaO_5$: 353.2304, found 353.2299. Anal. Calcd for $C_{18}H_{34}O_5$: C, 65.42; H, 10.37. Found: C, 65.38; H, 10.38.

*(S)-6-((1S)-1,5-Dihydroxynonyl)-tetrahydropyran-2-one [(S,S)-**8**] and Methyl (5S,6S)-5,6,10-trihydroxytetradecanoate [(S,S)-**9**].* Amberlyst 15 ion-exchange resin (cat.) was added to a solution of (*S*,*S*)-**33** (27.6 mg, 83.5 μmol) in a mixture of anhydrous THF (1 mL) and MeOH (2 mL). The reaction mixture was stirred at 70 °C for 12 h and then allowed to cool to room temperature. The mixture was filtered through a pad of anhydrous Na_2SO_4 and concentrated in vacuo. The residue was dissolved in a mixture of MeOH (2 mL) and Et_2O (2 mL), and ethereal CH_2N_2 was added dropwise until the yellow color persisted. The yellow reaction mixture was stirred for 30 min and then excess CH_2N_2 was evaporated under a stream of N_2 gas. The colorless reaction mixture was concentrated in vacuo to afford a mixture of dihydroxylactone (*S*,*S*)-**8** and trihydroxyester (*S*,*S*)-**9** (23.1 mg, 79.5 μmol, 95% assuming all the product was (*S*,*S*)-**9**) as a viscous, pale yellow oil. $[\alpha]_D^{25}$ −14.5 (*c* 0.15, CH_3CN). ^1H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H, *J* = 7.0 Hz, C*H*$_3$-14), 1.20−2.66 (m, ∼18H, incl. 2.34 [apparent q, 1.2H, *J* = 7.0 Hz, C*H*$_2$-2]), 3.39 (m, 2.7H), 3.59 (m, 1.5H), 3.66 (s, 1.1H, RCO_2CH_3 of ester (*S*,*S*)-**9**), 4.08 ppm (apparent t, 0.5H, *J* = 6.4 Hz, C*H*-5 of lactone (*S*,*S*)-**8**). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers; also contains (5*S*,6*S*)-5,6,10-trihydroxytetradecanoic acid [(*S*,*S*)-**34**]) δ 14.0 (C-14), 18.4, 20.88, 20.91, 21.47, 21.54, 21.55, 21.65, 22.7, 24.1, 25.4, 26.1, 26.3, 26.7, 26.9, 27.2, 27.9 (br), 28.7, 29.6, 29.7, 30.1, 30.28, 30.32, 32.4, 32.8 (br), 33.1, 33.3, 33.7, 34.0, 36.8, 36.86, 36.90, 37.1 (br), 37.2, 37.3, 51.6 (RCO_2CH_3 of ester (*S*,*S*)-**9**), 58.5, 58.6, 62.66, 62.70, 64.2, 70.1, 70.6, 70.76, 70.82, 71.56, 71.64, 71.7 (br), 72.1, 72.5, 72.6, 72.7, 73.19, 73.21, 73.4, 73.5, 73.8 (br), 74.0 (br), 74.1 (br), 74.25, 74.29, 83.2, and 83.4 (C-5 of lactone (*S*,*S*)-**8**), 171.6 (C-1 of lactone (*S*,*S*)-**8**), 173.9 (C-1 of ester (*S*,*S*)-**9**), 174.3 ppm (C-1 of acid (*S*,*S*)-**34**). GC/MS (EI) *m*/*z*: (dihydroxylactone (*S*,*S*)-**8**) 240 (0.1, $M^{+\bullet} - H_2O$), 238 (2), 221 (1), 207 (1), 196 (1), 183 (23), 165 (16), 159 (3), 157 (3), 154 (7), 143 (1), 141 (46), 137 (19), 129 (14), 123 (57), 119 (32), 115 (1), 111 (14), 101 (11), 100 (79), 99 (36), 87 (5), 81 (68), 71 (60), 67 (45), 57 (59, $C_4H_9^{+\bullet}$), 55 (100), 43 (76), 41 (82). HRMS (ESI) *m*/*z*: (*S*,*S*)-**8**: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{26}NaO_4$: 281.1729, found 281.1717; (*S*,*S*)-**9**: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{30}NaO_5$: 313.1991. Found: 313.1976.

*Sodium (5S,6S)-5,6,10-trihydroxytetradecanoate [(S,S)-**35**].* A small amount of the (*S*,*S*)-**8**/**9**/**34** mixture was treated with NaOD in D_2O and analyzed by NMR. ^1H NMR (400 MHz, D_2O/1,4-dioxane): δ 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$-14), 1.22−1.76 (m, 18H), 2.20 (ABX_2 system, 2H, J_{AB} = 14.9, J_{AX} = 7.2, J_{BX} = 7.1 Hz, C*H*$_2$-2), 3.51 (m, 2H, C*H*-5 and C*H*-6), 3.67 ppm (m, 1H, C*H*-10). ^{13}C NMR (100 MHz, D_2O/1,4-dioxane): (mixture of diastereomers) δ 14.0 (C-14), 21.6, 21.7, 22.7, 22.8, 27.6, 32.5, 32.6, 36.2, 36.4, 36.5, 38.0, 72.1 (C-10), 72.2 (C-10), 74.2, 74.26, 74.30, 74.4, 184.2 ppm (C-1).

*(R)-6-((1R)-1,5-Dihydroxynonyl)-tetrahydropyran-2-one [(R,R)-**8**] and Methyl (5R,6R)-5,6,10-trihydroxytetradecanoate [(R,R)-**9**].* As described for the synthesis of (*S*,*S*)-**8**/**9** from (*S*,*S*)-**33**, acid-catalyzed ketal methanolysis of (*R*,*R*)-**33** (27.4 mg, 82.9 μmol) and treatment with CH_2N_2 afforded a mixture of dihydroxylactone (*R*,*R*)-**8** and trihydroxyester (*R*,*R*)-**9** (22.0 mg, 75.8 μmol, 91% assuming all the product was (*R*,*R*)-**9**) as a viscous, pale yellow oil. $[\alpha]_D^{25}$ +12.9 (*c* 0.13, CH_3CN). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 6.8 Hz, C*H*$_3$-14), 1.20−2.66 (m, ∼19H, incl. 2.33 [apparent t, 1.0H, *J* = 7.0 Hz, C*H*$_2$-2]), 3.40 (m, 4.5H), 3.59 (m, 1.7H), 3.64 (s, 0.4H, RCO_2CH_3 of ester (*R*,*R*)-**9**), 4.07 ppm (apparent t, 0.8H, *J* = 6.4 Hz, C*H*-5 of lactone (*R*,*R*)-**8**). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers; appears to primarily contain lactone (*R*,*R*)-**8**) δ 14.0 (C-14), 18.4, 21.4, 21.6, 22.7, 24.1, 25.4, 26.1, 26.3, 26.7, 26.9, 27.8 (br), 29.6, 29.7, 30.1, 30.28, 30.32, 32.3, 32.5, 32.8 (br), 34.0, 36.9, 37.1 (br), 37.2 (br), 37.27, 37.32, 58.48, 58.54, 62.66, 62.70, 64.2, 70.1, 70.75, 70.81, 71.57, 71.65, 71.7 (br), 72.1, 72.5, 72.6, 72.8, 73.2 (br), 83.2 and 83.3 (C-5 of lactone (*R*,*R*)-**8**), 171.5 (C-1 of lactone (*R*,*R*)-**8**), 173.9 ppm (C-1 of ester (*R*,*R*)-**9**). GC/MS (EI) *m*/*z*: (dihydroxylactone (*R*,*R*)-**8**) 240 (0.1, $M^{+\bullet} - H_2O$), 238 (2), 221 (1), 207 (1), 196 (1), 183 (19), 165 (15), 159 (3), 157 (3), 154 (6), 143 (1), 141 (41), 137 (18), 129 (13), 123 (53), 119 (31), 115 (1), 111 (13), 101 (12), 100 (75), 99 (34), 87 (5), 81 (64), 71 (59), 67 (44), 57 (58, $C_4H_9^{+\bullet}$), 55 (100), 43 (74), 41 (82). HRMS (ESI) *m*/*z*: (*R*,*R*)-**8**: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{26}NaO_4$: 281.1729, found 281.1720; (*R*,*R*)-**9**: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{30}NaO_5$: 313.1991, found 313.1996.

Synthesis of Deuterium-Labeled *Threo* Dihydroxylactones/Trihydroxyesters $[^2H_4]$-(*S*,*S*)-8/9 and $[^2H_4]$-(*R*,*R*)-8/9. *Methyl 4-((4S,5S)-2,2-dimethyl-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-oxooctyl)-1,3-dioxolan-4-yl)butanoate ($[^2H_4]$-(S,S)-**32**).* Sodium metal (∼200 mg, 8.70 mmol) was added to D_2O (99.9 atom % D, 5 mL) at 0 °C, and the resulting NaOD solution was added to a solution of ketoacid (*S*,*S*)-**31** (127 mg, 0.40 mmol) in anhydrous THF (1.5 mL). The pale yellow reaction mixture was stirred at room temperature for 28 days and then solid oxalic acid (∼2.3 g, 25.55 mmol) was added to acidify the basic reaction mixture. The acidic mixture was extracted with Et_2O (6 × 20 mL) and the combined organic extract was washed with brine (8 × 20 mL) until the aqueous wash obtained was neutral. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo, affording ketoacid $[^2H_4]$-(*S*,*S*)-**31** as the major deuterium-labeled product (≤1% $[^2H_0]$).

*4-((4S,5S)-2,2-Dimethyl-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-oxooctyl)-1,3-dioxolan-4-yl)butanoic acid ($[^2H_4]$-(S,S)-**31**).* GC/MS (EI) *m*/*z*: 318 (0.04, $M^{+\bullet}$), 303 (3, $M^{+\bullet} - CH_3$), 301 (1), 243 (2), 225 (2), 201 (1), 197 (1), 187 (1), 183 (2), 173 (1), 158 (5), 139 (16), 131 (2), 121 (12), 117 (1), 103 (1), 100 (28), 93 (9), 87 (42, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 79 (9), 73 (7), 71 (10), 59 (70, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (39), 45 (15), 43 (100).

The crude $[^2H_4]$-(*S*,*S*)-**31** was dissolved in a mixture of MeOH (3 mL) and Et_2O (2 mL) and the solution was cooled to 0 °C. Ethereal CH_2N_2 was added dropwise until the yellow color of the reaction mixture persisted. The yellow solution was stirred at 0 °C for 15 min and then allowed to warm to room temperature with stirring over a further 15 min. Excess CH_2N_2 was evaporated under a stream of N_2 gas. The colorless reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography (silica gel, 25% to 66% Et_2O in petroleum spirits 40−60) to afford a pale yellow oil containing $[^2H_4]$-(*S*,*S*)-**32** (122 mg, 0.37 mmol, 91% over 2 steps, ≤1% $[^2H_0]$) as the major deuterium-labeled product. $[\alpha]_D^{25}$ −21.7 (*c* 0.55, MeOH). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 7.4 Hz, C*H*$_3$-14), 1.28 (m, 2H), 1.33 (s, 6H, 2 × ketal C*H*$_3$), 1.38−1.94 (m, 10H), 2.33 (ABX_2 system, 2H, J_{AB} = 16.2, J_{AX} = 7.5, J_{BX} = 7.3 Hz, C*H*$_2$-2), 2.38−2.44 (m, 0.1H, residual hydrogen from C*H*$_2$ or C*H*D at C-9 and/or C-11 of $[^2H_0]$-$[^2H_3]$-analogues), 3.55 (m, 2H, C*H*-5 and C*H*-6), 3.64 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0 (C-14), 20.4, 21.7, 22.4, 25.9, 27.4 (2 × ketal C*H*$_3$), 32.2, 34.0, 41.4−42.3 (m, incl. 41.8 [quintet, $J_{C,D}$ = 18.4 Hz, C*D*$_2$], 41.9 [quintet, $J_{C,D}$ = 18.5 Hz, C*D*$_2$], C-9 and C-11), 51.6 (RCO_2CH_3), 80.6, 80.7, 108.2 (ketal $C(CH_3)_2$), 173.9 (C-1), 211.3 (minor singlet from β-shift, C-10 of $[^2H_3]$-analogues), 211.4 ppm (C-10 of $[^2H_4]$-(*S*,*S*)-**32**). GC/MS (EI) *m*/*z*: 332 (0.2, $M^{+\bullet}$), 317 (19, $M^{+\bullet} - CH_3$), 275 (1), 258 (3), 257 (16), 243 (11), 225 (9), 215 (1), 207 (2), 197 (2), 187 (1), 183 (5), 179 (2), 173 (2), 155 (2), 144 (19), 140 (19), 131 (3), 129 (9), 117 (1), 115 (25), 103 (1), 102 (6), 101 (9), 93 (10), 87 (100, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 74 (4), 73 (7), 71 (9), 59 (60, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (23), 43 (56). HRMS (ESI) *m*/*z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{28}D_4NaO_5$: 355.2399, found 355.2404.

*Methyl 4-((4R,5R)-2,2-dimethyl-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-oxooctyl)-1,3-dioxolan-4-yl)butan-oate ($[^2H_4]$-(R,R)-**32**).* As described for the synthesis of $[^2H_4]$-(*S*,*S*)-**32**, ketoacid (*R*,*R*)-**31** (123 mg, 0.39 mmol)

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was deuterated with NaOD/D_2O over 28 days to provide [$^2H_4$]-(*R,R*)-**31** as the major product (≤1% [$^2H_0$]). The crude [$^2H_4$]-(*R,R*)-**31** was then esterified with CH_2N_2. Purification by flash column chromatography (silica gel, 25% to 66% Et_2O in petroleum spirits 40−60) afforded a pale yellow oil that contained [$^2H_4$]-(*R,R*)-**32** (121 mg, 0.36 mmol, 93% over 2 steps, ≤1% [$^2H_0$]) as the major deuterium-labeled product. $[\alpha]_D^{25}$ +21.3 (*c* 0.48, MeOH). This compound was spectroscopically identical to [$^2H_4$]-(*S,S*)-**32**. HRMS (ESI) *m/z*: [M + Na]$^{+\bullet}$ calcd for $C_{18}H_{28}D_4NaO_5$: 355.2399, found 355.2404.

*Methyl 4-((4S,5S)-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-hydroxyoctyl)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate ([$^2H_4$]-(S,S)-**33**).* Ketoester [$^2H_4$]-(*S,S*)-**32** (76 mg, 0.23 mmol) was reduced with $NaBH_4$ (18 mg, 0.48 mmol) in a mixture of MeOH (3 mL) and Et_2O (1 mL) over 2 h, as described for the synthesis of (*S,S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded a viscous, pale yellow oil that contained [$^2H_4$]-(*S,S*)-**33** (74 mg, 0.22 mmol, 97%, ≤1% [$^2H_0$]) as the major deuterium-labeled product. $[\alpha]_D^{25}$ −30.2 (*c* 0.67, MeOH). ^1H NMR (500 MHz, C_6D_6): δ 0.91 (t, 3H, *J* = 7.0 Hz, C*H*$_3$-14), 1.19−1.65 (m, 16H, incl. 1.425 [s, 3H, ketal C*H*$_3$], 1.432 [s, 3H, ketal C*H*$_3$]), 1.73 (m, 1H), 1.90 (m, 1H), 2.18 (ABX_2 system, 2H, J_{AB} = 16.1, J_{AX} = 7.4, J_{BX} = 7.2 Hz, C*H*$_2$-2), 3.36 (s, 3H, RCO_2CH_3), 3.40 (m, 1H, C*H*-10), 3.57 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (125 MHz, C_6D_6): (mixture of diastereomers) δ 14.3 (C-14), 22.1, 22.6, 22.8, 23.1, 27.57 (ketal *C*H$_3$), 27.61 (ketal *C*H$_3$), 28.0, 32.38, 32.40, 33.11, 33.14, 33.8, 36.4−37.4 (br m, C-9 and C-11), 50.9 (RCO_2CH_3), 71.19 (C-10), 71.25 (C-10), 81.0 (2C), 81.2, 81.3, 108.1 (ketal $C(CH_3)_2$), 173.19 (C-1), 173.21 ppm (C-1). GC/MS (EI) *m/z*: 319 (3, M$^{+\bullet}$ − CH_3), 317 (2, M$^{+\bullet}$ − OH), 260 (4), 259 (29), 245 (2), 240 (4), 229 (1), 227 (17), 215 (1), 208 (9), 199 (5), 190 (7), 180 (9), 167 (16), 157 (6), 143 (12), 139 (11), 133 (2), 131 (12), 121 (13), 115 (42), 111 (13), 105 (2), 101 (8), 95 (13), 89 (4), 87 (47), 83 (22), 74 (9), 73 (12), 71 (33), 59 (59, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (37), 43 (100). HRMS (ESI) *m/z*: [M + Na]$^{+\bullet}$ calcd for $C_{18}H_{30}D_4O_5Na$: 357.2555, found 357.2558.

*Methyl 4-((4R,5R)-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-hydroxyoctyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-butanoate ([$^2H_4$]-(R,R)-**33**).* Ketoester [$^2H_4$]-(*R,R*)-**32** (85 mg, 0.26 mmol) was reduced with $NaBH_4$ (20 mg, 0.53 mmol) in a mixture of MeOH (3 mL) and Et_2O (1 mL) over 2 h, as described for the synthesis of (*S,S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded a viscous, pale yellow oil that contained [$^2H_4$]-(*R,R*)-**33** (84 mg, 0.25 mmol, 98%, ≤1% [$^2H_0$]) as the major deuterium-labeled product. $[\alpha]_D^{25}$ +29.7 (*c* 0.59 in MeOH). This compound was spectroscopically identical to [$^2H_4$]-(*S,S*)-**33**. HRMS (ESI) *m/z*: [M + Na]$^{+\bullet}$ calcd for $C_{18}H_{30}D_4O_5Na$: 357.2555, found 357.2543.

*(S)-6-([4-$^2H_{0;1;2}$,6-$^2H_{0;1;2}$]-(1S)-1,5-Dihydroxynonyl)-tetrahydropyran-2-one ([$^2H_4$]-(S,S)-**8**) and Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-(5S,6S)-5,6,10-trihydroxytetradecanoate ([$^2H_4$]-(S,S)-**9**).* As described for the synthesis of (*S,S*)-**8**/**9** from (*S,S*)-**33**, acid-catalyzed ketal methanolysis of [$^2H_4$]-(*S,S*)-**33** (30.1 mg, 90.0 μmol) and treatment with CH_2N_2 afforded a viscous, pale yellow oil that contained a mixture of dihydroxylactone [$^2H_4$]-(*S,S*)-**8** and trihydroxyester [$^2H_4$]-(*S,S*)-**9** (25.4 mg, 86.3 μmol, 96% assuming all the product was [$^2H_4$]-(*S,S*)-**9**, ≤1% [$^2H_0$]) as the major deuterium-labeled products. $[\alpha]_D^{23}$ −11.8 (*c* 0.10, CH_3CN). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 6.8 Hz, C*H*$_3$-14), 1.18−2.66 (m, ∼15H, incl. 2.33 [apparent t, 1.2H, *J* = 6.9 Hz, C*H*$_2$-2), 3.32−3.44 (m, 2.3H, incl. 3.37 [t, *J* = 6.2 Hz]), 3.56 (m, 1H), 3.64 (s, 0.8H, RCO_2CH_3 of ester [$^2H_4$]-(*S,S*)-**9**), 4.07 ppm (apparent t, 0.8H, *J* = 6.4 Hz, C*H*-5 of lactone [$^2H_4$]-(*S,S*)-**8**). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers; also contains [$^2H_4$]-(5*S*,6*S*)-5,6,10-trihydroxytetradecanoic acid [$^2H_4$]-(*S,S*)-**34**; * denotes major signals) δ 14.1* (C-14), 18.4, 20.87*, 20.89*, 21.2, 21.3, 21.5, 22.5, 22.7*, 24.07, 24.09, 24.13, 25.4*, 26.1*, 27.3, 27.6*, 28.7, 29.63, 29.67*, 30.3, 31.9, 32.1, 32.3, 32.5, 32.6, 32.7, 32.8* (br), 33.1*, 33.3*, 33.7*, 33.9, 34.0*, 35.7−37.0 (br m, C-9 and C-11), 51.5 (RCO_2CH_3 of ester [$^2H_4$]-(*S,S*)-**9**), 58.5, 58.6*, 64.2*, 71.30, 71.37, 71.44*, 71.49*, 71.53*, 72.14*, 73.2, 73.47, 73.49, 73.8* (br), 74.0* (br), 74.1*, 74.25*, 74.28, 80.48, 80.51, 80.7, 80.8, 82.9, 83.0, 83.2, 83.3, 171.5 (C-1 of lactone [$^2H_4$]-(*S,S*)-**8**), 173.9* (C-1 of ester [$^2H_4$]-(*S,S*)-**9**), 174.3* ppm (C-1 of acid [$^2H_4$]-(*S,S*)-**34**). GC/MS (EI) *m/z*: (dihydroxylactone [$^2H_4$]-(*S,S*)-**8**) 244 (0.1, M$^{+\bullet}$ − H_2O), 241 (3), 226 (0.4), 218 (1), 207 (1), 199 (1), 193 (1), 185 (26), 174 (1), 167 (10), 163 (3), 157 (2), 156 (7), 145 (50), 143 (3), 138 (20), 133 (1), 129 (16), 126 (55), 121 (26), 119 (4), 111 (17), 105 (2), 101 (42), 100 (87), 99 (45), 89 (5), 83 (55), 73 (27), 71 (85), 59 (37, $C_4H_7D_2^{+\bullet}$), 57 (66), 55 (86), 43 (100), 41 (68). HRMS (ESI) *m/z*: [$^2H_4$]-(*S,S*)-**8**: [M + Na]$^{+\bullet}$ calcd for $C_{14}H_{22}D_4NaO_4$: 285.1980, found 285.1945; [$^2H_4$]-(*S,S*)-**9**: [M + Na]$^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_5$: 317.2242; found: 317.2210.

*(R)-6-([4-$^2H_{0;1;2}$,6-$^2H_{0;1;2}$]-(1R)-1,5-Dihydroxynonyl)-tetrahydropyran-2-one ([$^2H_4$]-(R,R)-**8**) and Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-(5R,6R)-5,6,10-trihydroxytetradecanoate ([$^2H_4$]-(R,R)-**9**).* As described for the synthesis of (*S,S*)-**8**/**9** from (*S,S*)-**33**, acid-catalyzed ketal methanolysis of [$^2H_4$]-(*R,R*)-**33** (20.9 mg, 62.5 μmol) and treatment with CH_2N_2 afforded a viscous, pale yellow oil that contained a mixture of dihydroxylactone [$^2H_4$]-(*R,R*)-**8** and trihydroxyester [$^2H_4$]-(*R,R*)-**9** (16.9 mg, 57.4 μmol, 92% assuming all the product was [$^2H_4$]-(*R,R*)-**9**, ≤1% [$^2H_0$]) as the major deuterium-labeled products. $[\alpha]_D^{23}$ +14.1 (*c* 0.15, CH_3CN). ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$-14), 1.20−2.66 (m, ∼15H, incl. 2.34 [apparent q, 1.7H, *J* = 6.9 Hz, C*H*$_2$-2), 3.34−3.46 (m, 2.4H, incl. 3.37 [t, *J* = 6.2 Hz]), 3.56 (m, 1H), 3.65 (s, 1.5H, RCO_2CH_3 of ester [$^2H_4$]-(*R,R*)-**9**), 4.07 ppm (apparent t, 0.6H, *J* = 6.4 Hz, C*H*-5 of lactone [$^2H_4$]-(*R,R*)-**8**). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers; appears to contain mostly ester [$^2H_4$]-(*R,R*)-**9** and [$^2H_4$]-(5*R*,6*R*)-5,6,10-trihydroxytetradecanoic acid [$^2H_4$]-(*R,R*)-**34**; * denotes major signals) δ 14.1* (C-14), 20.85*, 20.88*, 21.4*, 21.5*, 21.6*, 22.7*, 24.07, 24.09*, 25.4*, 26.1*, 26.3, 26.9, 27.3, 27.6*, 28.7, 29.63, 29.67*, 30.3, 30.4, 31.9, 32.7, 32.9* (br), 33.1*, 33.3*, 33.7*, 33.9, 34.0*, 35.7−37.0 (br m, C-9 and C-11), 51.5 (RCO_2CH_3 of ester [$^2H_4$]-(*R,R*)-**9**), 58.5*, 58.6*, 62.7, 64.2*, 70.77, 70.84, 71.50*, 71.54*, 72.2*, 72.5, 73.5, 73.8* (br), 74.0* (br), 74.1*, 74.2*, 74.26*, 74.29*, 80.5, 80.7, 80.8, 82.9 and 83.0 (C-5 of lactone [$^2H_4$]-(*R,R*)-**8**), 173.9* (C-1 of ester [$^2H_4$]-(*R,R*)-**9**), 174.3* ppm (C-1 of acid [$^2H_4$]-(*R,R*)-**34**). GC/MS (EI) *m/z*: (dihydroxylactone [$^2H_4$]-(*R,R*)-**8**) 244 (0.1, M$^{+\bullet}$ − H_2O), 241 (3), 226 (0.4), 218 (1), 207 (1), 199 (1), 193 (1), 185 (26), 174 (1), 167 (10), 163 (3), 157 (2), 156 (8), 145 (50), 143 (3), 138 (22), 133 (1), 129 (16), 126 (58), 121 (26), 119 (4), 111 (18), 105 (2), 101 (43), 100 (90), 99 (48), 89 (5), 83 (56), 73 (26), 71 (85), 59 (38, $C_4H_7D_2^{+\bullet}$), 57 (66), 55 (88), 43 (100), 41 (66). HRMS (ESI) *m/z*: [$^2H_4$]-(*R,R*)-**8**: [M + Na]$^{+\bullet}$ calcd for $C_{14}H_{22}D_4NaO_4$: 285.1980, found 285.1939; [$^2H_4$]-(*R,R*)-**9**: [M + Na]$^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_5$: 317.2242, found 317.2205.

Synthesis of Dihydroxylactones/Trihydroxyesters *erythro*-8/9 and [$^2H_4$]-*erythro*-8/9. *(Z)-2-(10-(Benzyloxy)tetradec-5-en-1-yloxy)tetrahydro-2H-pyran [(Z)-**36**].* A mixture of alkyne **17** (737 mg, 1.84 mmol) and Lindlar's catalyst (5% Pd/$CaCO_3$ poisoned with Pb, 120 mg) in EtOAc (10 mL) was degassed-purged twice with N_2 (g) and then twice with H_2 (g). The reaction mixture was stirred under a H_2 atmosphere at room temperature for 4 h and then was filtered through a pad of Celite that was washed thoroughly with additional EtOAc. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (silica gel, 100% *n*-hexane to 2% EtOAc in *n*-hexane) to afford the alkene (*Z*)-**36** (625 mg, 1.55 mmol, 84%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 7.0 Hz, C*H*$_3$-14), 1.22−1.65 (m, 18H), 1.69 (m, 1H), 1.82 (m, 1H), 2.03 (m, 4H, C*H*$_2$-4 and C*H*$_2$-7), 3.36 (m, 2H, C*H*-1 and C*H*-10), 3.48 (m, 1H, C*H*-6′), 3.72 (dt, 1H, *J* = 9.6, 6.8 Hz, C*H*-1), 3.85 (m, 1H, C*H*-6′), 4.48 (s, 2H, ROC*H*$_2$Ph), 4.56 (dd, 1H, *J* = 4.2, 2.8 Hz, C*H*-2′), 5.35 (m, 2H, C*H*-5 and C*H*-6), 7.21−7.35 ppm (m, 5H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 19.7, 22.9, 25.45, 25.51, 26.4, 27.1, 27.3, 27.6, 29.4, 30.8, 33.5, 33.6, 62.3 (C-6′), 67.5 (C-1), 70.7 (ROCH_2Ph), 79.0 (C-10), 98.8 (C-2′), 127.3 (Ar CH), 127.7 (2 × Ar CH), 128.3 (2 × Ar CH), 129.8 (RHC═CHR), 129.9 (RHC═CHR), 139.2 ppm (Ar C). GC/MS (EI) *m/z*: 318 (1), 227 (1), 210 (2), 191 (1), 153 (1), 135 (2), 121 (1), 107 (3), 101 (2), 91 (64, $C_7H_7^{+\bullet}$), 85 (100, THP$^{+\bullet}$), 77 (1), 67 (16), 57 (9), 55 (15), 43 (14), 41 (21). HRMS (ESI) *m/z*: [M + Na]$^{+\bullet}$ calcd for

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$C_{26}H_{42}NaO_3$: 425.3032, found 425.3017. Anal. Calcd for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.20; H, 10.80.

*(5,6-erythro)-10-(Benzyloxy)-1-(tetrahydro-2H-pyran-2-yloxy)-tetradecane-5,6-diol (erythro-**37**).* OsO_4 (2.5% w/v in *t*-BuOH, 3.0 mL, 0.30 mmol) was added dropwise to a solution of NMO (97%, 457 mg, 3.78 mmol) and alkene (*Z*)-**36** (1.187 g, 2.95 mmol) in a mixture of THF (10 mL) and H_2O (1 mL). The orange reaction mixture was stirred at room temperature for 96 h. A solution of sodium sulfite (~1.0 g, 7.93 mmol) in H_2O (10 mL) was added to quench the reaction and the mixture was extracted with Et_2O (6 × 40 mL). The combined organic extract was washed with aqueous NaOH solution (5%, 2 × 40 mL) and brine (40 mL), dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 33% Et_2O in petroleum spirits 40−60 to 100% Et_2O) to afford racemic *erythro*-**37** (1.160 g, 2.66 mmol, 90%) as a viscous, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.0 Hz, C*H*$_3$-14), 1.22−2.10 (m, 26H), 3.38 (m, 2H, C*H*-1 and C*H*-10), 3.48 (m, 1H, C*H*-6′), 3.56 (m, 2H, C*H*-5 and C*H*-6), 3.74 (m, 1H, C*H*-1), 3.85 (m, 1H, C*H*-6′), 4.48 (m, 2H, ROC*H*$_2$Ph), 4.55 (dd, 1H, J = 4.4, 2.7 Hz, C*H*-2′), 7.21−7.36 ppm (m, 5H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.1 (C-14), 19.7, 21.7, 22.0, 22.8, 22.9, 25.49, 27.54, 27.5, 29.6, 30.8, 30.9, 31.0, 31.3, 31.4, 33.4, 33.5, 33.7, 33.8, 62.5 (C-6′), 67.45 (C-1), 67.51 (C-1), 70.77 (ROCH_2Ph), 70.85 (ROCH_2Ph), 74.50, 74.53 (br), 74.57, 74.59, 78.9 (C-10), 79.0 (C-10), 98.98 (C-2′), 99.00 (C-2′), 127.41 (Ar *C*H), 127.44 (Ar *C*H), 127.76 (2 × Ar *C*H), 127.80 (2 × Ar *C*H), 128.29 (2 × Ar *C*H), 128.30 (2 × Ar *C*H), 139.0 (Ar *C*), 139.1 ppm (Ar *C*). GC/MS (EI) m/z: 351 (0.1), 281 (0.1), 249 (2), 245 (5), 227 (11), 209 (4), 191 (2), 187 (4), 177 (1), 169 (3), 157 (1), 143 (1), 141 (12), 129 (1), 123 (5), 115 (1), 107 (4), 101 (3), 91 (97, $C_7H_7^{+\bullet}$), 85 (100, THP$^{+\bullet}$), 81 (7), 77 (5), 69 (7), 67 (14), 57 (17), 55 (23), 43 (13), 41 (18). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{26}H_{44}NaO_5$: 459.3086, found 459.3086. Anal. Calcd for $C_{26}H_{44}O_5$: C, 71.52; H, 10.16. Found: C, 71.20; H, 10.13.

*(5,6-erythro)-10-(Benzyloxy)tetradecane-1,5,6-triol (erythro-**38**).* A solution of *erythro*-**37** (805 mg, 1.84 mmol) and *p*-TsOH.H_2O (100 mg, cat.) in MeOH (15 mL) was stirred at room temperature for 20 h. Solid $NaHCO_3$ (400 mg) was added to quench the reaction and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (silica gel, 50% EtOAc in petroleum spirits 40−60 to 100% EtOAc) to afford the racemic triol *erythro*-**38** (650 mg, 1.84 mmol, quantitative) as a cream-colored solid. Mp: 56−58 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.9 Hz, C*H*$_3$-14), 1.21−1.67 (m, 18H), 1.80 (br, 2H, 2 × RO*H*), 2.29 (br, 1H, RO*H*), 3.37 (m, 1H, C*H*-10), 3.55 (m, 2H, C*H*-5 and C*H*-6), 3.62 (t, 2H, J = 5.6 Hz, C*H*$_2$-1), 4.48 (m, 2H, ROC*H*$_2$Ph), 7.21−7.37 ppm (m, 5H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.1 (C-14), 21.7, 21.9, 22.2, 22.9, 27.47, 27.52, 30.67, 30.70, 31.3, 31.4, 32.4, 33.4, 33.5, 33.7, 33.8, 62.6 (C-1), 70.75 (ROCH_2Ph), 70.84 (ROCH_2Ph), 74.5 (br), 74.6, 78.9 (C-10), 79.1 (C-10), 127.4 (Ar *C*H), 127.5 (Ar *C*H), 127.78 (2 × Ar *C*H), 127.84 (2 × Ar *C*H), 128.30 (2 × Ar *C*H), 128.31 (2 × Ar *C*H), 138.9 (Ar *C*), 139.0 ppm (Ar *C*). GC/MS (EI) m/z: 249 (0.4), 243 (0.3), 228 (1), 207 (1), 187 (1), 177 (1), 169 (2), 141 (8), 133 (1), 130 (4), 123 (5), 107 (4), 103 (2), 91 (100, $C_7H_7^{+\bullet}$), 85 (11), 81 (7), 77 (4), 73 (1), 67 (8), 59 (1), 57 (11), 55 (10), 45 (1), 43 (8), 41 (10). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{21}H_{36}NaO_4$: 375.2511, found 375.2511. Anal. Calcd for $C_{21}H_{36}O_4$: C, 71.55; H, 10.29. Found: C, 71.49; H, 10.21.

*(5,6-erythro)-1,5,6,10-Tetradecanetetraol (erythro-**29**).* A mixture of *erythro*-**38** (580 mg, 1.65 mmol) and Pd/C (10%, 61 mg) in MeOH (20 mL) was degassed-purged twice with N_2 (g) and then twice with H_2 (g). The reaction mixture was stirred under a H_2 atmosphere at room temperature for 20 h and then was filtered through a pad of Celite that was washed thoroughly with additional MeOH. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography (silica gel, 5% to 9% MeOH in CH_2Cl_2) to afford racemic *erythro*-**29** (420 mg, 1.60 mmol, 97%) as a white solid. Mp: 110−112 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.91 (t, 3H, J = 7.1 Hz, C*H*$_3$-14), 1.24−1.77 (m, 18H), 3.36 (m, 2H, C*H*-5 and C*H*-6), 3.52 (m, 1H, C*H*-10), 3.56 ppm (t, 2H, J = 6.4 Hz, C*H*$_2$-1). ^{13}C NMR (100 MHz, CD_3OD): (mixture of diastereomers) δ 14.4 (C-14), 23.2, 23.3, 23.8, 29.1, 33.4, 33.6, 33.7, 38.1, 38.2, 38.4, 38.5, 63.0 (C-1), 72.37, 72.43, 75.88, 75.93 ppm. HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{30}NaO_4$: 285.2042, found 285.2027. Anal. Calcd for $C_{14}H_{30}O_4$: C, 64.08; H, 11.52. Found: C, 64.04; H, 11.33.

*1-((4,5-erythro)-5-(4-Hydroxybutyl)-2,2-dimethyl-1,3-dioxolan-4-yl)octan-4-ol (erythro-**30**).* Tetraol *erythro*-**29** (375 mg, 1.43 mmol) was reacted with acetone in the presence of *p*-TsOH (100 mg, cat.) and 4 Å molecular sieves under a N_2 atmosphere over 18 h, as described for the synthesis of (*S*,*S*)-**30**. Purification by flash column chromatography (silica gel, 33% Et_2O in petroleum spirits 40−60 to 100% Et_2O) afforded *erythro*-**30** (420 mg, 1.39 mmol, 97%) as a colorless, viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.0 Hz, C*H*$_3$-14), 1.21−1.70 (m, 26H, incl. 1.31 [s, 3H, ketal C*H*$_3$], 1.41 [s, 3H, ketal C*H*$_3$]), 3.59 (m, 1H, C*H*-10), 3.64 (t, 2H, J = 6.1 Hz, C*H*$_2$-1), 4.02 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.1 (C-14), 22.3, 22.49, 22.51, 22.53, 22.7, 25.95 (ketal *C*H$_3$), 25.96 (ketal *C*H$_3$), 27.8, 28.6 (ketal *C*H$_3$), 29.39, 29.44, 29.7, 32.6, 37.1, 37.25, 37.31, 37.4, 62.8 (C-1), 71.7 (C-10), 71.8 (C-10), 77.90, 77.93, 77.95, 107.4 ppm (ketal *C*(CH$_3$)$_2$). GC/MS (EI) m/z: 287 (4, $M^{+\bullet} - CH_3$), 285 (1, $M^{+\bullet} -$ OH), 269 (2), 227 (33), 209 (16), 191 (8), 169 (8), 151 (5), 141 (5), 135 (13), 129 (1), 121 (17), 115 (12), 109 (23), 101 (5), 100 (10), 95 (25), 87 (4), 85 (73), 79 (21), 73 (6), 69 (50), 67 (39), 59 (58), 57 (52), 55 (54), 45 (7), 43 (100), 41 (58). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{17}H_{34}NaO_4$: 325.2355, found 325.2352. Anal. Calcd for $C_{17}H_{34}O_4$: C, 67.51; H, 11.33. Found: C, 67.25; H, 11.23.

*4-((4,5-erythro)-2,2-Dimethyl-5-(4-oxooctyl)-1,3-dioxolan-4-yl)-butanoic acid (erythro-**31**).* Diol *erythro*-**30** (305 mg, 1.01 mmol) was oxidized with PDC (98%, 2.18 g, 5.68 mmol) in DMF (12 mL) under an Ar atmosphere over 20 h, as described for the synthesis of (*S*,*S*)-**31**. Purification by flash column chromatography (silica gel, 17% EtOAc in petroleum spirits 40−60 to 100% EtOAc) afforded *erythro*-**31** (283 mg, 0.90 mmol, 89%) as a pale yellow, viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.3 Hz, C*H*$_3$-14), 1.22−1.91 (m, 18H, incl. 1.30 [s, 3H, ketal C*H*$_3$], 1.40 [s, 3H, ketal C*H*$_3$]), 2.33−2.46 (m, 6H, C*H*$_2$-2, C*H*$_2$-9 and C*H*$_2$-11), 4.01 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (C-14), 20.6, 21.5, 22.4, 25.91 (ketal *C*H$_3$), 25.95, 28.5 (ketal *C*H$_3$), 29.0, 29.1, 33.6, 42.4, 42.5, 77.5, 77.7, 107.7 (ketal *C*(CH$_3$)$_2$), 178.6 (br, C-1), 211.1 ppm (C-10). GC/MS (EI) m/z: 314 (0.02, $M^{+\bullet}$), 299 (0.1, $M^{+\bullet} - CH_3$), 221 (1), 140 (1), 137 (3), 127 (1), 119 (1), 113 (1), 100 (2), 99 (1), 87 (3), 85 (13, $C_5H_9O^{+\bullet}$), 73 (1), 59 (13, $C_2H_3O_2^{+\bullet}$), 57 (33, $C_4H_9^{+\bullet}$), 45 (14), 43 (100). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{17}H_{30}NaO_5$: 337.1991, found 337.1997. Anal. Calcd for $C_{17}H_{30}O_5$: C, 64.94; H, 9.62. Found: C, 64.97; H, 9.55.

*Methyl 4-((4,5-erythro)-2,2-dimethyl-5-(4-oxooctyl)-1,3-dioxolan-4-yl)butanoate (erythro-**32**).* Ketoacid *erythro*-**31** (45 mg, 0.14 mmol) was esterified with ethereal CH_2N_2 in a mixture of MeOH (2 mL) and Et_2O (1 mL) at 0 °C, as described for the synthesis of (*S*,*S*)-**32**. Purification by flash column chromatography (silica gel, 25% to 33% EtOAc in petroleum spirits 40−60 to 100% EtOAc) afforded *erythro*-**32** (47 mg, 0.14 mmol, quantitative) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.3 Hz, C*H*$_3$-14), 1.20−1.90 (m, 18H, incl. 1.30 [s, 3H, ketal C*H*$_3$], 1.39 [s, 3H, ketal C*H*$_3$]), 2.28−2.46 (m, 6H, C*H*$_2$-2, C*H*$_2$-9 and C*H*$_2$-11), 3.65 (s, 3H, RCO$_2$C*H*$_3$), 4.00 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (C-14), 20.6, 21.7, 22.4, 25.9 (2C, incl. one × ketal *C*H$_3$), 28.6 (ketal *C*H$_3$), 29.1 (2C), 33.8, 42.4, 42.5, 51.5 (RCO$_2$*C*H$_3$), 77.5, 77.7, 107.6 (ketal *C*(CH$_3$)$_2$), 173.9 (C-1), 211.0 ppm (C-10). GC/MS (EI) m/z: 328 (0.2, $M^{+\bullet}$), 313 (17, $M^{+\bullet} - CH_3$), 271 (1), 254 (3), 253 (18), 239 (16), 221 (18), 210 (1), 203 (3), 193 (2), 185 (1), 181 (4), 175 (5), 169 (5), 155 (3), 140 (11), 137 (19), 129 (10), 127 (4), 115 (17), 113 (7), 101 (3), 99 (6), 98 (13), 93 (14), 87 (2, $C_4H_7O_2^{+\bullet}$), 85 (100, $C_5H_9O^{+\bullet}$), 74 (3), 73 (4), 71 (10), 59 (18, $C_2H_3O_2^{+\bullet}$), 57 (47, $C_4H_9^{+\bullet}$), 55 (35), 43 (56). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{32}NaO_5$: 351.2147, found 351.2145. Anal. Calcd for $C_{18}H_{32}O_5$: C, 65.82; H, 9.82. Found: C, 65.71; H, 9.64.

*Methyl 4-((4,5-erythro)-2,2-dimethyl-5-(4-hydroxyoctyl)-1,3-dioxolan-4-yl)butanoate (erythro-**33**).* Ketoester *erythro*-**32** (40 mg, 0.12

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mmol) was reduced with $NaBH_4$ (10 mg, 0.26 mmol) in a mixture of MeOH (3 mL) and Et_2O (1 mL) over 2 h, as described for the synthesis of (*S,S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded *erythro*-**33** (38 mg, 0.11 mmol, 94%) as a viscous, pale yellow oil. ^{1}H NMR (500 MHz, C_6D_6): δ 0.92 (t, 3H, J = 7.0 Hz, CH_3-14), 1.16−1.82 (m, 21H, incl. 1.35 [s, 3H, ketal CH_3], 1.489 and 1.492 [2 × overlapping s, 3H, ketal CH_3]), 1.92 (m, 1H), 2.20 (ABX_2 system, 2H, J_{AB} = 16.0, J_{AX} = 7.2, J_{BX} = 7.2 Hz, CH_2-2), 3.369 and 3.370 (2 × overlapping s, 3H, RCO_2CH_3), 3.44 (m, 1H, C*H*-10), 3.90 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (125 MHz, C_6D_6): (mixture of diastereomers) δ 14.3 (C-14), 22.2, 22.9, 23.1, 23.2, 26.0 (ketal CH_3), 26.1 (ketal CH_3), 28.2, 28.81 (ketal CH_3), 28.82 (ketal CH_3), 29.56, 29.58, 30.08, 30.15, 33.8, 37.74, 37.76, 37.81, 37.9, 50.9 (RCO_2CH_3), 71.4 (C-10), 71.5 (C-10), 77.8, 78.08, 78.11, 107.5 (ketal $C(CH_3)_2$), 173.26 (C-1), 173.29 ppm (C-1). GC/MS (EI) *m/z*: 315 (4, $M^{+\bullet}$ − CH_3), 313 (4, $M^{+\bullet}$ − OH), 256 (9), 255 (53), 237 (11), 223 (37), 215 (4), 205 (22), 197 (7), 187 (18), 177 (23), 163 (19), 157 (5), 137 (20), 129 (9), 121 (34), 115 (36), 109 (28), 101 (8), 95 (35), 87 (7), 85 (66), 81 (33), 74 (8), 73 (11), 69 (37), 59 (45, $C_2H_3O_2^{+\bullet}$), 57 (38, $C_4H_9^{+\bullet}$), 55 (64), 43 (100). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{34}NaO_5$: 353.2304, found 353.2294. Anal. Calcd for $C_{18}H_{34}O_5$: C, 65.42; H, 10.37. Found: C, 65.49; H, 10.50.

*(5,6-erythro)-6-(1,5-Dihydroxynonyl)-tetrahydropyran-2-one (erythro-**8**) and Methyl (5,6-erythro)-5,6,10-trihydroxytetradecanoate (erythro-**9**).* As described for the synthesis of (*S,S*)-**8**/**9** from (*S,S*)-**33**, acid-catalyzed ketal methanolysis of *erythro*-**33** (27.7 mg, 83.8 μmol) and treatment with CH_2N_2 afforded a mixture of dihydroxylactone *erythro*-**8** and trihydroxyester *erythro*-**9** (23.0 mg, 79.2 μmol, 94% assuming all the product was *erythro*-**9**) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, J = 7.0 Hz, CH_3-14), 1.20−2.66 (m, ∼19H, incl. 2.34 [apparent t, 1.6H, J = 7.2 Hz, CH_2-2]), 3.33−3.44 (m, 3.3H, incl. 3.39 [t, J = 5.8 Hz]), 3.52−3.62 (m, 5H, incl. 3.39 [t, J = 6.0 Hz]), 3.64 ppm (s, 2.6H, RCO_2CH_3 of ester *erythro*-**9**). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers; appears to contain mostly (5,6-*erythro*)-5,6,10-trihydroxytetradecanoic acid (*erythro*-**34**); * denotes major signals) δ 14.0* (C-14), 18.2, 21.1, 21.2*, 21.3*, 21.88*, 21.95, 22.7, 26.17, 26.22*, 26.4, 26.6*, 26.8, 27.8* (br), 28.6, 29.3, 29.6*, 29.7, 30.0*, 30.19, 30.24*, 30.58*, 30.62*, 30.79*, 31.3*, 31.5, 31.7*, 31.9, 33.7* (br), 34.2, 36.7, 36.8*, 36.9, 37.0, 37.1*, 37.2*, 37.3*, 51.4 (RCO_2CH_3 of ester *erythro*-**9**), 58.4, 58.5*, 60.0, 62.5*, 62.6, 65.5, 67.6, 67.7, 70.5 (br), 70.7, 70.8, 71.1, 71.3, 71.40*, 71.43, 71.5, 71.6, 71.7*, 72.1, 72.2, 72.4, 72.45, 72.53, 72.7*, 74.08*, 74.14*, 74.3*, 74.5*, 82.9, 83.0, 83.5, 83.6, 172.1 (C-1 of lactone *erythro*-**8**), 173.9 (C-1 of ester *erythro*-**9**), 174.32* and 174.33* ppm (C-1 of acid *erythro*-**34**). GC/MS (EI) *m/z*: (dihydroxylactone *erythro*-**8**) 241 (0.4, $M^{+\bullet}$ − OH), 183 (32), 165 (23), 159 (3), 157 (4), 154 (1), 143 (1), 141 (67), 137 (22), 129 (12), 123 (80), 119 (36), 115 (3), 111 (12), 101 (12), 100 (100), 99 (42), 87 (7), 81 (81), 71 (53), 67 (50), 57 (50, $C_4H_9^{+\bullet}$), 55 (82), 43 (57), 41 (53). HRMS (ESI) *m/z*: *erythro*-**8**: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{26}NaO_4$: 281.1729, found 281.1729; *erythro*-**9**: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{30}NaO_5$: 313.1991, found 313.1986.

*Sodium (5,6-erythro)-5,6,10-trihydroxytetradecanoate (erythro-**35**).* A small amount of the *erythro*-**8**/**9**/**34** mixture was treated with NaOD in D_2O and analyzed by NMR. ^{1}H NMR (400 MHz, D_2O/1,4-dioxane) δ 0.87 (t, 3H, J = 7.0 Hz, CH_3-14), 1.23−1.78 (m, 18H), 2.20 (ABX_2 system, 2H, J_{AB} = 14.7, J_{AX} = 7.2, J_{BX} = 7.2 Hz, CH_2-2), 3.56 (m, 2H, C*H*-5 and C*H*-6), 3.67 ppm (m, 1H, C*H*-10). ^{13}C NMR (100 MHz, D_2O/1,4-dioxane): (mixture of diastereomers) δ 13.3 (C-14), 21.1, 21.2, 22.0, 22.3, 26.9, 27.0, 30.85, 30.88, 30.91, 31.1, 35.5, 35.6, 35.7, 35.9, 37.4, 71.4 (C-10), 71.5 (C-10), 74.08 (br), 74.14, 74.2, 183.6 ppm (C-1).

*Methyl 4-((4,5-erythro)-2,2-dimethyl-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-oxooctyl)-1,3-dioxolan-4-yl)-butanoate ([$^2H_4$]-erythro-**32**).* As described for the synthesis of [$^2H_4$]-(*S,S*)-**32**, ketoacid *erythro*-**31** (120 mg, 0.38 mmol) was deuterated with NaOD/D_2O over 30 days to provide [$^2H_4$]-*erythro*-**31** (≤1% [$^2H_0$]) as the major product.

*4-((4,5-erythro)-2,2-Dimethyl-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-oxooctyl)-1,3-dioxolan-4-yl)butanoic acid ([$^2H_4$]-erythro-**31**).* GC/MS (EI) *m/z*: 318 (0.1, $M^{+\bullet}$), 303 (19, $M^{+\bullet}$ − CH_3), 301 (1), 243 (8), 225 (9), 201 (1), 197 (1), 187 (1), 183 (6), 173 (4), 158 (5), 139 (16), 131 (2), 121 (10), 117 (2), 103 (1), 100 (17), 93 (9), 87 (51, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 79 (7), 73 (5), 71 (6), 59 (55, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (27), 45 (16), 43 (100).

The crude [$^2H_4$]-*erythro*-**31** was then esterified with CH_2N_2 in a mixture of MeOH (2 mL) and Et_2O (1 mL). Purification by flash column chromatography (silica gel, 25% to 66% Et_2O in petroleum spirits 40−60) afforded a colorless oil that contained [$^2H_4$]-*erythro*-**32** (116 mg, 0.35 mmol, 91% over 2 steps, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, J = 7.3 Hz, CH_3-14), 1.19−1.87 (m, 18H, incl. 1.29 [s, 3H, ketal CH_3], 1.39 [s, 3H, ketal CH_3]), 2.28−2.43 (m, 2.1H, incl. 2.34 [ABX_2 system, 2H, J_{AB} = 16.0, J_{AX} = 7.4, J_{BX} = 7.3 Hz, CH_2-2] + residual hydrogen from CH_2 or C*H*D at C-9 and/or C-11 of [$^2H_0$]-[$^2H_3$]-analogues), 3.64 (s, 3H, RCO_2CH_3), 4.00 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8 (C-14), 20.4, 21.7, 22.3, 25.8, 25.9 (ketal CH_3), 28.5 (ketal CH_3), 29.0, 29.1, 33.8, 41.2−42.2 (m, incl. 42.6 [quintet, $J_{C,D}$ = 19.0 Hz, CD_2], 42.8 [quintet, $J_{C,D}$ = 18.9 Hz, CD_2], C-9 and C-11), 51.5 (RCO_2CH_3), 77.5, 77.7, 107.6 (ketal $C(CH_3)_2$), 173.9 (C-1), 211.3 ppm (C-10). GC/MS (EI) *m/z*: 332 (0.1, $M^{+\bullet}$), 317 (17, $M^{+\bullet}$ − CH_3), 275 (1), 258 (3), 257 (17), 243 (16), 225 (13), 215 (1), 207 (2), 197 (3), 187 (2), 183 (6), 179 (4), 173 (5), 155 (3), 144 (13), 140 (17), 131 (4), 129 (15), 117 (2), 115 (21), 103 (1), 102 (5), 101 (10), 93 (12), 87 (100, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 74 (5), 73 (8), 71 (10), 59 (67, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (27), 43 (72). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{28}D_4NaO_5$: 355.2399, found 355.2398.

*Methyl 4-((4,5-erythro)-2,2-dimethyl-5-([3-$^2H_{0;1;2}$,5-$^2H_{0;1;2}$]-4-hydroxyoctyl)-1,3-dioxolan-4-yl)butanoate ([$^2H_4$]-erythro-**33**).* Ketoester [$^2H_4$]-*erythro*-**32** (76 mg, 0.23 mmol) was reduced with $NaBH_4$ (18 mg, 0.48 mmol) in a mixture of MeOH (3 mL) and Et_2O (1 mL) over 2 h, as described for the synthesis of (*S,S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded a viscous, pale yellow oil that contained [$^2H_4$]-*erythro*-**33** (74 mg, 0.22 mmol, 97%, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^{1}H NMR (500 MHz, C_6D_6): δ 0.92 (t, 3H, J = 7.0 Hz, CH_3-14), 1.15−1.81 (m, 17H, incl. 1.35 [s, 3H, ketal CH_3], 1.492 and 1.494 [2 × overlapping s, 3H, ketal CH_3]), 1.93 (m, 1H), 2.20 (ABX_2 system, 2H, J_{AB} = 16.1, J_{AX} = 7.5, J_{BX} = 7.2 Hz, CH_2-2), 3.367 and 3.368 (2 × overlapping s, 3H, RCO_2CH_3), 3.41 (m, 1H, C*H*-10), 3.90 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (125 MHz, C_6D_6): (mixture of diastereomers) δ 14.3 (C-14), 22.2, 22.7, 22.9, 23.1, 26.04 (ketal CH_3), 26.06 (ketal CH_3), 28.0, 28.8 (br, ketal CH_3), 29.56, 29.58, 30.0, 30.1, 33.8, 36.4−37.5 (m, C-9 and C-11), 50.9 (RCO_2CH_3), 71.1 (C-10), 71.3 (C-10), 77.8, 78.09, 78.11, 107.5 (ketal $C(CH_3)_2$), 173.25 (C-1), 173.28 ppm (C-1). GC/MS (EI) *m/z*: 319 (3, $M^{+\bullet}$ − CH_3), 317 (5, $M^{+\bullet}$ − OH), 260 (5), 259 (33), 245 (2), 240 (5), 229 (1), 227 (18), 215 (1), 208 (10), 199 (4), 190 (7), 180 (11), 167 (13), 157 (5), 143 (22), 139 (12), 133 (2), 131 (13), 121 (13), 115 (34), 111 (17), 105 (2), 101 (10), 95 (13), 89 (3), 87 (53), 83 (24), 74 (9), 73 (13), 71 (34), 59 (65, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (41), 43 (100). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{18}H_{30}D_4NaO_5$: 357.2555, found 357.2549.

*(5,6-erythro)-6-([4-$^2H_{0;1;2}$,6-$^2H_{0;1;2}$]-1,5-Dihydroxynonyl)-tetrahydropyran-2-one ([$^2H_4$]-erythro-**8**) and Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-(5,6-erythro)-5,6,10-trihydroxytetradecanoate ([$^2H_4$]-erythro-**9**).* Following the procedure described for the synthesis of (*S,S*)-**8**/**9** from (*S,S*)-**33**, acid-catalyzed ketal methanolysis of [$^2H_4$]-*erythro*-**33** (31.7 mg, 94.8 μmol) and treatment with ethereal CH_2N_2 afforded a white solid that contained a mixture of dihydroxylactone [$^2H_4$]-*erythro*-**8** and trihydroxyester [$^2H_4$]-*erythro*-**9** (25.3 mg, 85.9 μmol, 91% assuming all the product was [$^2H_4$]-*erythro*-**9**, ≤1% [$^2H_0$]) as the major deuterium-labeled products. ^{1}H NMR (750 MHz, $CDCl_3$): δ 0.85 (2 overlapping t, 3H, J = 7.0 Hz, CH_3-14), 1.18−2.60 (m, ∼15H, incl. 2.31 [m, 1.4H, CH_2-2]), 2.89 (br m, 3H), 3.32−3.42 (m, 2H, incl. 3.35 [t, J = 6.3 Hz]), 3.49−3.60 (m, 2.8H), 3.62 (s, 0.6H, RCO_2CH_3 of ester [$^2H_4$]-*erythro*-**9**), 4.04 ppm (t, 0.9H, J = 6.5 Hz, C*H*-5 of lactone [$^2H_4$]-*erythro*-**8**). ^{13}C NMR (188 MHz, $CDCl_3$): (mixture of diastereomers; appears to contain mostly lactone [$^2H_4$]-*erythro*-**8**

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with some ester [$^2H_4$]-*erythro*-**9** and [$^2H_4$]-(5,6-*erythro*)-5,6,10-trihydroxytetradecanoic acid ([$^2H_4$]-*erythro*-**34**); * denotes major signals) δ 13.9 (C-14), 14.0* (C-14), 18.2*, 21.27, 21.28, 21.33, 21.37, 21.40, 21.45, 21.48*, 21.56*, 21.61*, 21.67*, 21.74, 22.4, 22.60*, 22.61*, 25.35*, 25.44, 26.0*, 26.16, 26.20*, 26.21*, 26.23, 26.25, 26.26, 26.28, 26.32, 26.34, 26.5*, 26.7*, 27.3*, 27.58* (br), 27.61* (br), 28.6 (br), 29.3, 29.6*, 29.7*, 29.9*, 30.1*, 30.2*, 30.6 (br), 31.3 (br), 31.5*, 31.7*, 31.8, 32.0, 33.74*, 33.75*, 33.98*, 34.00*, 34.1*, 34.1*, 34.4 (br), 35.5−36.7 (br m, C-9 and C-11), 50.4*, 51.4*, 58.40*, 58.44*, 58.5*, 62.47*, 62.52*, 64.1 (br), 70.1, 70.41, 70.42, 70.45, 70.54, 70.6*, 70.7*, 71.11, 71.15, 71.19*, 71.22, 71.3, 71.4*, 71.5 (br), 72.0, 72.1* (br), 72.2*, 72.4*, 72.5 (br), 72.7*, 73.7−73.8 (br), 74.05, 74.11, 74.3, 74.5, 82.9, 83.0, 83.49* and 83.53* (C-5 of lactone [$^2H_4$]-*erythro*-**8**), 83.6, 83.7, 172.0* (C-1 of lactone [$^2H_4$]-*erythro*-**8**), 173.88 and 173.90 (C-1 of ester [$^2H_4$]-*erythro*-**9**), 174.26 and 174.28 ppm (C-1 of acid [$^2H_4$]-*erythro*-**34**). GC/MS (EI) *m/z*: (dihydroxylactone [$^2H_4$]-*erythro*-**8**) 244 (0.1, $M^{+\bullet} - H_2O$), 241 (2), 226 (0.4), 218 (1), 207 (1), 199 (1), 193 (1), 185 (25), 174 (1), 167 (11), 163 (3), 157 (2), 156 (8), 145 (59), 143 (3), 138 (21), 133 (1), 129 (11), 126 (57), 121 (25), 119 (4), 111 (15), 105 (2), 101 (43), 100 (95), 99 (52), 89 (6), 83 (57), 73 (24), 71 (92), 59 (37, $C_4H_7D_2^{+\bullet}$), 57 (68), 55 (83), 43 (100), 41 (65). HRMS (ESI) *m/z*: [$^2H_4$]-*erythro*-**8**: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{22}D_4NaO_4$: 285.1980, found 285.1984; [$^2H_4$]-*erythro*-**9**: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_5$: 317.2242, found 317.2255.

Synthesis of Compounds with Dioxygenated Chains. *Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-6,10-dihydroxytetradecanoate ([$^2H_4$]-**10**) and methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-5,10-dihydroxytetradecanoate ([$^2H_4$]-**11**).* Following the procedure described for the synthesis of alcohol **22**, alkene [$^2H_4$]-(*E*)-**27** (60 mg, 0.23 mmol) was hydroborated using borane-dimethylsulfide complex (2.0 M in THF, 0.12 mL, 0.24 mmol) in anhydrous CH_2Cl_2 (5 mL) under a N_2 atmosphere over 2 h, and then was oxidized using aqueous NaOH solution (20%, 2.0 mL, 10.00 mmol) and H_2O_2 (30%, 2.0 mL, 17.64 mmol). Purification by flash column chromatography (silica gel, 20% EtOAc in *n*-hexane) afforded an isomeric mixture enriched in the 6,10-dihydroxyester [$^2H_4$]-**10** (18 mg, 64.7 μmol, 28%, ∼9:1 [$^2H_4$]-**10**:[$^2H_4$]-**11**, ≤1% [$^2H_0$]) and a mixture enriched in the 5,10-dihydroxyester [$^2H_4$]-**11** (12 mg, 43.1 μmol, 19%, ∼9:1 [$^2H_4$]-**11**:[$^2H_4$]-**10**, ≤1% [$^2H_0$]) as colorless oils.

*Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-6,10-dihydroxytetradecanoate ([$^2H_4$]-**10**).* ^1H NMR (400 MHz, C_6D_6): δ 0.93 (t, 3H, *J* = 7.0 Hz, CH_3-14), 1.20−1.67 (m, 14H), 2.15 (t, 2H, *J* = 7.3 Hz, CH_2-2), 3.36−3.46 ppm (m, 5H, incl. 3.38 [s, 3H, RCO_2CH_3]). ^{13}C NMR (100 MHz, C_6D_6): (Mixture of diastereomers; signals for C-9 and C-11 not observed) δ 14.3 (C-14), 21.8, 21.9, 23.1, 25.2, 25.49, 25.51, 28.1, 34.0, 37.5, 37.58, 37.60, 37.8, 50.9 (RCO_2CH_3), 71.1, 71.21, 71.24, 71.3, 173.5 ppm (C-1). GC/MS (EI) *m/z*: 278 (0.2, $M^{+\bullet}$), 261 (0.3), 228 (0.2), 210 (2), 201 (3), 183 (8), 169 (13), 151 (17), 145 (76), 126 (30), 113 (71), 87 (78), 71 (59), 67 (61), 59 (72), 57 (99), 55 (100), 43 (92), 41 (80). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_4$: 301.2293, found 301.2295.

*Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-5,10-dihydroxytetradecanoate ([$^2H_4$]-**11**).* ^1H NMR (400 MHz, C_6D_6): δ 0.93 (t, 3H, *J* = 7.0 Hz, CH_3-14), 1.20−1.81 (m, 14H), 2.15 (t, 2H, *J* = 7.3 Hz, CH_2-2), 3.32−3.44 ppm (m, 5H, incl. 3.37 [s, 3H, RCO_2CH_3]). ^{13}C NMR (100 MHz, C_6D_6): (Mixture of diastereomers; signals for C-9 and C-11 not observed) δ 14.3 (C-14), 21.4, 23.1, 25.8, 25.9, 26.0, 28.0, 33.9, 37.2, 37.86, 37.88, 51.0 (RCO_2CH_3), 70.9, 71.0, 71.2, 71.3, 173.6 ppm (C-1). GC/MS (EI) *m/z*: 228 (0.1), 187 (6), 169 (22), 151 (13), 132 (5), 123 (26), 122 (33), 100 (7), 99 (46), 95 (15), 71 (100), 55 (72), 42 (74). HRMS (ESI): $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_4$: 301.2293, found 301.2296.

Synthesis of Hydroxylactones [$^2H_2$]-(*S,S*)-12 and [$^2H_2$]-(*R,R*)-12. *Ethyl (E)-2-octenoate [(E)-**40**].* A solution of hexanal (98%, 0.26 mL, 2.06 mmol) and ethyl 2-(triphenylphosphoranylidene)acetate (**39**)[23] (1.02 g, 2.93 mmol) in anhydrous CH_2Cl_2 (20 mL) was heated at reflux under a N_2 atmosphere for 20 h. The reaction mixture was cooled to room temperature and concentrated in vacuo, and the residue was purified by flash column chromatography (silica gel, 2% to 5% EtOAc in *n*-hexane) to afford (*E*)-**40** (322 mg, 1.89 mmol, 92%, ≥95% *E* isomer) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 6.7 Hz, CH_3-8), 1.22−1.34 (m, 7H, incl. 1.26 [t, 3H, *J* = 7.1 Hz, $RCO_2CH_2CH_3$]), 1.44 (m, 2H), 2.17 (qd, 1.90H, *J* = 7.3, 1.4 Hz, CH_2-4 of *E* isomer), 2.62 (qd, 0.10H, *J* = 7.5, 1.6 Hz, CH_2-4 of *Z* isomer), 4.16 (2 overlapping q, 2H, *J* = 7.1 Hz, $RCO_2CH_2CH_3$), 5.73 (dt, 0.05H, *J* = 11.5, 1.7 Hz, C*H*-2 of *Z* isomer), 5.79 (dt, 0.95H, *J* = 15.7, 1.5 Hz, C*H*-2 of *E* isomer), 6.19 (dt, 0.05H, *J* = 11.5, 7.5 Hz, C*H*-3 of *Z* isomer), 6.94 ppm (dt, 0.95H, *J* = 15.6, 7.0 Hz, C*H*-3 of *E* isomer). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9 (CH_3), 14.3 (CH_3), 22.4, 27.7, 28.7 (*Z* isomer), 28.9 (*Z* isomer), 31.3, 31.5 (*Z* isomer), 32.1, 59.7 ($RCO_2CH_2CH_3$ of *Z* isomer), 60.1 ($RCO_2CH_2CH_3$ of *E* isomer), 119.6 (C-2 of *Z* isomer), 121.2 (C-2 of *E* isomer), 149.5 (C-3 of *E* isomer), 150.6 (C-3 of *Z* isomer), 166.8 ppm (C-1). GC/MS (EI) *m/z*: (*E* isomer) 170 (1, $M^{+\bullet}$), 155 (1, $M^{+\bullet} - CH_3$), 142 (1), 141 (3, $M^{+\bullet} - Et$), 127 (10, $M^{+\bullet} - Pr$), 125 (32, $M^{+\bullet} - OEt$), 124 (15, $M^{+\bullet} - EtOH$), 101 (26), 99 (27, $M^{+\bullet} - C_5H_{11}$), 96 (22), 88 (15), 86 (14), 82 (20), 81 (14), 73 (38), 71 (5), 68 (25), 57 (14), 55 (100), 43 (28), 41 (69). GC/MS (EI) *m/z*: (*Z* isomer) 170 (15, $M^{+\bullet}$), 155 (1, $M^{+\bullet} - CH_3$), 142 (3), 141 (3, $M^{+\bullet} - Et$), 127 (67, $M^{+\bullet} - Pr$), 125 (30, $M^{+\bullet} - OEt$), 124 (4, $M^{+\bullet} - EtOH$), 101 (9), 99 (100, $M^{+\bullet} - C_5H_{11}$), 96 (12), 88 (12), 86 (20), 82 (24), 81 (29), 73 (24), 71 (13), 68 (34), 57 (13), 55 (92), 43 (56), 41 (94). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{10}H_{18}NaO_2$: 193.1204, found 193.1207.

*Ethyl [2-$^2H_{0;1;2}$,3-$^2H_{0;1;2}$]octanoate ([$^2H_2$]-**41**).* A mixture of (*E*)-**40** (5.00 g, 29.4 mmol) and Pd/C (5%, 25 mg) in EtOH (200 mL) was degassed-purged twice with N_2 (g) and then twice with $^2H_2$ (g). The reaction mixture was stirred under a $^2H_2$ (D_2, 99.7 atom % D) atmosphere at room temperature for 4 h and then was filtered through a pad of Celite that was washed thoroughly with Et_2O (3 × 50 mL). The solvent was removed in vacuo to afford a colorless oil that contained [$^2H_2$]-**41** (5.12 g, 29.4 mmol, quantitative, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 6.8 Hz, CH_3-8), 1.18−1.45 (m, 11H, incl. 1.23 [t, 3H, *J* = 7.1 Hz, $RO_2CH_2CH_3$]), 1.59 (m, 1.5H, C*H*D-3 of [2,3-$^2H_2$]-**41** and [3-$^2H_1$] or [2,2,3-$^2H_3$]-analogues + CH_2-3 of [$^2H_0$], [2-$^2H_1$], or [2,2-$^2H_2$]-analogues), 2.26 (br t, 1.7H, *J* = 7.5 Hz, C*H*D-2 of [2,3-$^2H_2$]-**41** and [2-$^2H_1$] or [2,3,3-$^2H_3$]-analogues + CH_2-2 of [$^2H_0$], [3-$^2H_1$], or [3,3-$^2H_2$]-analogues), 4.10 ppm (q, 2H, *J* = 7.1 Hz, $RO_2CH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0 (CH_3), 14.2 (CH_3), 22.6, 24.9, 25.0 (minor singlet from isotope shift), 28.7−29.0 (m), 29.1, 31.6 (br), 34.2−34.4 (m), 60.1 ($RO_2CH_2CH_3$), 173.9 ppm (C-1). GC/MS (EI) *m/z*: 174 (1, $M^{+\bullet}$), 143 (2), 129 (27, $M^{+\bullet} - OEt$), 115 (5), 101 (22), 88 (100), 70 (40), 61 (63), 43 (53). HRMS (ESI) *m/z*: $[M + Na]^{+\bullet}$ calcd for $C_{10}H_{18}D_2NaO_2$: 197.1487, found 197.1497.

*[2-$^2H_{0;1;2}$,3-$^2H_{0;1;2}$]-1-Octanol ([$^2H_2$]-**42**).* A solution of ester [$^2H_2$]-**41** (4.00 g, 23.0 mmol) in anhydrous Et_2O (20 mL) was added dropwise to a solution of $LiAlH_4$ (95%, 1.74 g, 43.6 mmol) in anhydrous Et_2O (100 mL) with stirring under a N_2 atmosphere at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Sodium sulfate decahydrate (20 g) was added to quench the reaction. The mixture was stirred until it was white in color and then filtered through a pad of Celite that was washed thoroughly with Et_2O (3 × 50 mL). The filtrate was concentrated in vacuo to afford a colorless oil that contained the alcohol [$^2H_2$]-**42** (3.00 g, 22.7 mmol, 99%, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 6.7 Hz, CH_3-8), 1.18−1.36 (m, 9.6H, incl. ∼1.6H for C*H*D-3 of [2,3-$^2H_2$]-**42** and [3-$^2H_1$] or [2,2,3-$^2H_3$]-analogues + CH_2-3 of [$^2H_0$], [2-$^2H_1$], or [2,2-$^2H_2$]-analogues), 1.54 (m, 1.6H, C*H*D-2 of [2,3-$^2H_2$]-**42** and [2-$^2H_1$] or [2,3,3-$^2H_3$]-analogues + CH_2-2 of [$^2H_0$], [3-$^2H_1$], or [3,3-$^2H_2$]-analogues), 1.78 (br s, 1H, RO*H*), 3.61 ppm (br t, 2H, *J* = 6.6 Hz, CH_2-1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (C-8), 22.6, 25.5 (br m, C-3), 29.2−29.4 (br m), 31.8, 32.6−32.9 (m, C-2), 63.1 ppm (br, C-1). GC/MS (EI) *m/z*: 115 (0.2, $M^{+\bullet} - OH$), 114 (0.4, $M^{+\bullet} - H_2O$), 98 (1), 84 (32), 70 (77), 56 (100), 43 (87), 42 (88), 41 (90).

*[2-$^2H_{0;1;2}$,3-$^2H_{0;1;2}$]-1-Iodooctane ([$^2H_2$]-**43**).* Following the procedure described for the synthesis of iodide **19**, alcohol [$^2H_2$]-**42** (380 mg, 2.87 mmol) was iodinated using I_2 (99%, 950 mg, 3.71 mmol), PPh_3 (99%, 980 mg, 3.70 mmol), and imidazole (390 mg, 5.73 mmol)

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in a mixture of anhydrous Et_2O (7.5 mL) and CH_3CN (2.5 mL) at 0 °C under a N_2 atmosphere over 2 h. Purification by flash column chromatography (silica gel, 20% EtOAc in *n*-hexane) afforded a pale pink oil that contained iodide [$^2H_2$]-**43** (460 mg, 1.90 mmol, 66%, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.9 Hz, CH_3-8), 1.18−1.40 (m, 9.5H, incl. ∼1.5H for C*H*D-3 of [2,3-$^2H_2$]-**43** and [3-$^2H_1$] or [2,2,3-$^2H_3$]-analogues + CH_2-3 of [$^2H_0$], [2-$^2H_1$], or [2,2-$^2H_2$]-analogues), 1.79 (br q, 1.6H, J = 7.1 Hz, C*H*D-2 of [2,3-$^2H_2$]-**43** and [2-$^2H_1$] or [2,3,3-$^2H_3$]-analogues + CH_2-2 of [$^2H_0$], [3-$^2H_1$] or [3,3-$^2H_2$]-analogues), 3.16 ppm (t, 2H, J = 6.8 Hz, CH_2-1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.2 (minor singlet from isotope shift) and 7.3 (both C-1), 14.1 (C-8), 22.6, 28.3−28.6 (m), 28.7−29.1 (m), 29.6−30.6 (m), 30.9, 31.8 (br), 32.8−33.6 ppm (m). GC/MS (EI) m/z: 242 (1, $M^{+\bullet}$), 184 (0.2), 155 (3), 141 (1), 127 (2), 115 (2), 86 (0.3), 72 (57), 71 (47), 58 (71), 57 (73), 43 (100), 41 (70).

*[8-$^2H_{0;1;2}$,9-$^2H_{0;1;2}$]-2-(Tetradec-5-yn-1-yloxy)tetrahydro-2H-pyran ([$^2H_2$]-**44**).* Following the procedure described for the synthesis of **17**, alkyne **18** (700 mg, 3.84 mmol) was deprotonated with *n*-BuLi (1.50 M in hexanes, 3.07 mL, 4.61 mmol) in THF (38 mL) at −40 °C under an Ar atmosphere over 2 h and then was reacted with iodide [$^2H_2$]-**43** (1.10 g, 4.54 mmol) in the presence of HMPA (99%, 1.90 mL, 10.81 mmol) over 12 h. Purification by flash column chromatography (silica gel, 10% EtOAc in *n*-hexane) afforded a colorless oil that contained alkyne [$^2H_2$]-**44** (800 mg, 2.70 mmol, 70%, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.8 Hz, CH_3-14), 1.22−1.87 (m, ∼21H), 2.08−2.21 (m, 4H, CH_2-4 and CH_2-7), 3.39 (dt, 1H, J = 9.7, 6.3 Hz, C*H*-1), 3.48 (m, 1H, C*H*-6′), 3.73 (dt, 1H, J = 9.7, 6.5 Hz, C*H*-1), 3.85 (m, 1H, C*H*-6′), 4.56 ppm (dd, 1H, J = 4.3, 2.9 Hz, C*H*-2′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (C-14), 18.55, 18.63, 18.8, 19.6, 22.7, 25.4, 25.5, 26.0, 28.9, 29.0, 29.1 (br), 30.8, 30.9, 31.9, 62.3 (C-6′), 67.1 (C-1), 79.8 (RC≡CR), 80.6 (RC≡CR), 98.8 ppm (C-2′). GC/MS (EI) m/z: 296 (0.1, $M^{+\bullet}$), 251 (0.1), 223 (2), 195 (1), 181 (4), 167 (1), 153 (1), 137 (1), 111 (5), 101 (6), 95 (15), 85 (100, $THP^{+\bullet}$), 67 (23), 55 (19). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{19}H_{32}D_2NaO_2$ 319.2582, found 319.2563.

*[8-$^2H_{0;1;2}$,9-$^2H_{0;1;2}$]-(E)-2-(Tetradec-5-en-1-yloxy)tetrahydro-2H-pyran ([$^2H_2$]-(E)-**45**).* Following the procedure described for the synthesis of (*E*)-**23**, alkyne [$^2H_2$]-**44** (400 mg, 1.35 mmol) was reduced with lithium metal (50 mg, 7.20 mmol) in a mixture of *t*-BuOH (3 mL), THF (5 mL), and liquid NH_3 (50 mL) at −78 °C over 2 h. Purification by flash column chromatography (silica gel, 1% EtOAc in *n*-hexane) afforded a colorless oil that contained [$^2H_2$]-(*E*)-**45** (320 mg, 1.07 mmol, 79%, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.6 Hz, CH_3-14), 1.18−1.87 (m, ∼21H), 1.90−2.00 (m, 4H, CH_2-4 and CH_2-7), 3.36 (dt, 1H, J = 9.6, 6.6 Hz, C*H*-1), 3.47 (m, 1H, C*H*-6′), 3.71 (dt, 1H, J = 9.3, 6.8 Hz, C*H*-1), 3.84 (m, 1H, C*H*-6′), 4.55 (dd, 1H, J = 4.4, 2.8 Hz, C*H*-2′); 5.37 ppm (m, 2H; C*H*-5 and C*H*-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (C-14), 19.7, 22.7, 25.5, 26.3, 29.0−29.7 (obscured m), 29.2, 30.8, 30.9, 31.9, 32.4, 32.5 (obscured m), 62.3 (C-6′), 67.5 (C-1), 98.8 (C-2′), 129.9 (RHC═CHR), 130.8 ppm (RHC═CHR). GC/MS (EI): m/z: 298 (0.1, $M^{+\bullet}$), 254 (0.1), 225 (1), 195 (1), 183 (0.2), 167 (0.3), 156 (0.1), 125 (1), 111 (1), 101 (2), 97 (2), 85 (100, $THP^{+\bullet}$), 67 (17), 55 (15). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{19}H_{34}D_2NaO_2$ 321.2739, found 321.2724.

*[8-$^2H_{0;1;2}$,9-$^2H_{0;1;2}$]-(E)-5-Tetradecen-1-ol ([$^2H_2$]-(E)-**46**).* As described for the synthesis of (*E*)-**24**, the THP moiety of [$^2H_2$]-(*E*)-**45** (320 mg, 1.07 mmol) was cleaved with *p*-TsOH.H_2O (5 mg, cat.) in MeOH (10 mL) over 2 h to afford a colorless oil that contained [$^2H_2$]-(*E*)-**46** (230 mg, 1.07 mmol, quantitative, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.8 Hz, CH_3-14), 1.18−1.46 (m, ∼15H), 1.90−2.04 (m, 4H, CH_2-4 and CH_2-7), 3.62 (t, 2H, J = 6.5 Hz, CH_2-1), 5.38 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (C-14), 22.7, 25.7, 28.9−29.7 (m), 31.9 (br), 32.3, 32.4−32.6 (m), 62.9 (C-1), 129.7 (RHC═CHR), 130.9 ppm (RHC═CHR). GC/MS (EI) m/z: 198 (2), 155 (1), 138 (2), 125 (4), 110 (8), 96 (33), 82 (82), 67 (100), 55 (77).

*Methyl [8-$^2H_{0;1;2}$,9-$^2H_{0;1;2}$]-(E)-5-tetradecenoate ([$^2H_2$]-(E)-**47**).* Alcohol [$^2H_2$]-(*E*)-**46** (200 mg, 0.93 mmol) was oxidized with Jones' reagent (8 N) in acetone (4 mL) at 0 °C following the procedure described for the synthesis of (*E*)-**25**. The crude acid obtained was then esterified with ethereal CH_2N_2 in MeOH (5 mL) at 0 °C, as described for the synthesis of (*S*,*S*)-**32**. Purification by flash column chromatography (silica gel, 10% EtOAc in *n*-hexane) afforded a colorless oil that contained [$^2H_2$]-(*E*)-**47** (100 mg, 0.41 mmol, 44% over 2 steps, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.8 Hz, CH_3-14), 1.16−1.37 (m, ∼11H), 1.67 (quintet, 2H, J = 7.2 Hz, CH_2-3), 1.90−2.04 (m, 4H, CH_2-4 and CH_2-7), 2.28 (t, 2H, J = 7.5 Hz, CH_2-2), 3.64 (s, 3H, RCO_2CH_3), 5.36 ppm (m, 2H, C*H*-5 and C*H*-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (C-14), 22.7, 24.8, 28.9−29.6 (m), 30.9, 31.89, 31.91, 32.6 (br), 33.4, 51.4 (RCO_2CH_3), 128.8 (RHC═CHR), 131.7 (RHC═CHR), 174.2 ppm (C-1). GC/MS (EI) m/z: 242 (1, $M^{+\bullet}$), 223 (0.1), 210 (6), 192 (3), 181 (1), 167 (11), 152 (2), 139 (5), 125 (11), 111 (16), 96 (47), 84 (43), 81 (39), 74 (100), 55 (61).

*(6S)-6-([3-$^2H_{0;1;2}$,4-$^2H_{0;1;2}$]-(1S)-1-Hydroxynonyl)tetrahydro-2H-pyran-2-one ([$^2H_2$]-(S,S)-**12**).* Following the procedure described for the synthesis of (*S*,*S*)-**28**, alkene [$^2H_2$]-(*E*)-**47** (50 mg, 0.21 mmol) was dihydroxylated with AD-mix-α (Aldrich, 290 mg) and methanesulfonamide (97%, 21 mg, 0.21 mmol) in a mixture of *t*-BuOH and H_2O (1:1, 6 mL) at 4 °C over 24 h. Purification by flash column chromatography (silica gel, 40% EtOAc in *n*-hexane) afforded a white solid that contained hydroxylactone [$^2H_2$]-(*S*,*S*)-**12** (4.40 mg, 18.0 μmol, 9%, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. Mp: 50−52 °C. ^1H NMR (400 MHz, C_6D_6): δ 0.83−1.58 (m, ∼21H, incl. 0.95 [t, 3H, J = 6.8 Hz, CH_3-14]), 1.90−2.20 (m, 2H, CH_2-2), 3.22 (m, 1H), 3.58 ppm (m, 1H). ^{13}C NMR (100 MHz, C_6D_6): δ 14.3 (C-14), 18.4, 23.1, 24.0, 25.8, 25.9, 29.0−30.3 (obscured m), 29.7, 32.26, 32.28, 32.8−33.2 (m), 73.3, 82.6, 169.8 ppm (C-1). GC/MS (EI) m/z: 243 (0.03, $M^{+\bullet}$ − H), 225 (0.1), 165 (0.1), 144 (0.3), 129 (1), 112 (1), 100 (100), 85 (3), 71 (14), 55 (28), 43 (27), 41 (26). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{24}D_2NaO_3$ 267.1905, found 267.1903.

*(6R)-6-([3-$^2H_{0;1;2}$,4-$^2H_{0;1;2}$]-(1R)-1-Hydroxynonyl)tetrahydro-2H-pyran-2-one ([$^2H_2$]-(R,R)-**12**).* Following the procedure described for the synthesis of (*S*,*S*)-**28**, alkene [$^2H_2$]-(*E*)-**47** (50 mg, 0.21 mmol) was dihydroxylated with AD-mix-β (Aldrich, 290 mg) and methanesulfonamide (97%, 21 mg, 0.21 mmol) in a mixture of *t*-BuOH and H_2O (1:1, 6 mL) at 4 °C over 24 h. Purification by flash column chromatography (silica gel, 40% EtOAc in *n*-hexane) afforded a white solid that contained hydroxylactone [$^2H_2$]-(*R*,*R*)-**12** (4.80 mg, 19.6 μmol, 10%, ∼20% [$^2H_0$]) as the predominant deuterium-labeled product. Mp: 53−54 °C. This compound was spectroscopically identical to [$^2H_2$]-(*S*,*S*)-**12**. HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{24}D_2NaO_3$ 267.1905, found 267.1900.

Synthesis of Deuterium-Labeled Monooxygenated Esters. *1,10-Tetradecanediol (**51**).* Reduction of **17** (580 mg, 1.45 mmol) was carried out with H_2 (g) and Pd/C (10%, 72 mg) in MeOH (10 mL) over 18 h following the procedure described for the synthesis of *erythro*-**29**. The THP moiety of the crude product was then cleaved with *p*-TsOH·H_2O (100 mg, cat.) in MeOH (10 mL) over 4 h, as described for the synthesis of *erythro*-**38**. Purification by flash column chromatography (silica gel, 9% to 67% Et_2O in petroleum spirits 40−60) afforded the diol **51** (328 mg, 1.42 mmol, 98% over 2 steps) as a white solid. Mp: 55−56 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.0 Hz, CH_3-14), 1.20−1.50 (m, 22H), 1.54 (m, 2H), 3.56 (m, 1H, C*H*-10), 3.62 ppm (t, 2H, J = 6.6 Hz, CH_2-1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 22.8, 25.6, 25.7, 27.8, 29.4, 29.5 (2C), 29.7, 32.8, 37.2, 37.5, 63.1 (C-1), 72.0 ppm (C-10). GC/MS (EI) m/z: 207 (0.4), 186 (0.3), 173 (12), 155 (5), 137 (26), 124 (2), 109 (4), 101 (1), 95 (68), 87 (37), 86 (38), 81 (70), 69 (100), 67 (32), 57 (32), 55 (45), 43 (22), 41 (39). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{30}NaO_2$ 253.2143, found 253.2141. Anal. Calcd for $C_{14}H_{30}O_2$: C, 72.99; H, 13.12. Found: C, 72.99; H, 13.20.

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*10-Oxotetradecanoic acid (**48**).* Jones' reagent (8 N) was added dropwise to a solution of diol **51** (280 mg, 1.22 mmol) in acetone (10 mL) with stirring at 0 °C until the orange color of the reaction mixture persisted. The reaction mixture was allowed to warm to room temperature with stirring over 30 min and then was quenched with water (20 mL). The mixture was extracted with Et_2O (6 × 20 mL), and the combined organic extract was washed with brine (2 × 20 mL) and then extracted with aqueous NaOH solution (5%, 6 × 20 mL). The combined basic aqueous extract was cooled to 0 °C, and concentrated aqueous HCl (32%, 30 mL) was added dropwise until the solution was strongly acidic. The acidic aqueous solution was extracted with Et_2O (6 × 20 mL), and the combined organic extract was then washed with brine (20 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed in vacuo to afford ketoacid **48** (285 mg, 1.18 mmol, 97%) as a white solid. Mp: 66−68 °C (lit.[47] mp: 67.6−68.6 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.3 Hz, CH_3-14), 1.18−1.36 (m, 10H), 1.47−1.65 (m, 6H), 2.29−2.39 ppm (m, 6H, CH_2-2, CH_2-9 and CH_2-11, incl. 2.32 [t, 2H, J = 7.5 Hz, CH_2-2]). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (C-14), 22.4, 23.8, 24.6, 26.0, 28.9, 29.0, 29.1 (2C), 34.0, 42.5, 42.7, 179.7 (C-1), 211.8 ppm (C-10). GC/MS (EI) m/z: (as methyl ester) 256 (1, $M^{+\bullet}$), 227 (3), 225 (15, $M^{+\bullet}$ − OCH_3), 214 (23), 199 (25), 182 (6), 171 (9), 169 (10), 157 (30), 155 (1), 143 (1), 141 (5), 139 (37), 125 (85), 121 (28), 113 (13), 101 (17), 97 (52), 87 (12, $C_4H_7O_2^{+\bullet}$), 85 (100, $C_5H_9O^{+\bullet}$), 74 (12), 69 (40), 59 (21, $C_2H_3O_2^{+\bullet}$), 58 (69), 57 (99, $C_4H_9^{+\bullet}$), 55 (67), 43 (30), 41 (50). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{14}H_{26}NaO_3$ 265.1780, found 265.1778. Anal. Calcd for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81. Found: C, 69.38; H, 10.83.

*Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-10-oxotetradecanoate ([$^2H_4$]-**13**).* Following the procedure described for the synthesis of [$^2H_4$]-(*S,S*)-**32**, ketoacid **48** (218 mg, 0.90 mmol) was deuterated with NaOD/D_2O over 7 days and esterified with ethereal CH_2N_2 in MeOH (5 mL) at 0 °C. Purification by flash column chromatography (silica gel, 5% to 66% Et_2O in petroleum spirits 40−60) afforded a pale yellow, low-melting solid that contained ketoester [$^2H_4$]-**13** (223 mg, 0.86 mmol, 95% over two steps, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.3 Hz, CH_3-14), 1.18−1.33 (m, 10H), 1.51 (m, 4H), 1.59 (m, 2H), 2.27 (t, 2H, J = 7.5 Hz, CH_2-2), 2.30−2.37 (m, 0.1H, residual hydrogen from CH_2 or C*H*D at C-9 and/or C-11 of [$^2H_0$]-[$^2H_3$]-analogues), 3.64 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (C-14), 22.3, 23.7, 24.9, 25.8, 29.0 (2C), 29.1, 29.2, 34.1, 41.3−42.7 (m, incl. 41.8 [quintet, $J_{C,D}$ = 19.0 Hz, CD_2], 42.2 [quintet, $J_{C,D}$ = 19.0 Hz, CD_2], C-9 and C-11), 51.4 (RCO_2CH_3), 174.3 (C-1), 211.9 ppm (C-10). GC/MS (EI) m/z: 260 (2, $M^{+\bullet}$), 231 (3), 229 (23, $M^{+\bullet}$ − OCH_3), 218 (31), 201 (27), 186 (8), 169 (10), 159 (9), 157 (42), 145 (7), 143 (7), 141 (41), 125 (92), 115 (9), 97 (50), 87 (91, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 74 (31), 62 (79), 59 (100, $C_4H_7D_2^{+\bullet}$ and/or $C_2H_3O_2^{+\bullet}$), 55 (65), 43 (48). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{15}H_{24}D_4NaO_3$ 283.2187, found 283.2184.

*Methyl [9-$^2H_{0;1;2}$,11-$^2H_{0;1;2}$]-10-hydroxytetradecanoate ([$^2H_4$]-**14**).* Ketoester [$^2H_4$]-**13** (122 mg, 0.47 mmol) was reduced with $NaBH_4$ (31 mg, 0.82 mmol) in a mixture of MeOH (2 mL) and Et_2O (2 mL) over 2 h, as described for the synthesis of (*S,S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded a white solid that contained hydroxyester [$^2H_4$]-**14** (116 mg, 0.44 mmol, 94%, ≤1% [$^2H_0$]) as the major deuterium-labeled product. Mp: 34−36 °C (lit.[48] mp: (unlabeled compound) 35.5−36 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.0 Hz, CH_3-14), 1.20−1.45 (m, 15H), 1.59 (m, 2H), 2.27 (t, 2H, J = 7.6 Hz, CH_2-2), 3.53 (m, 1H, C*H*-10), 3.63 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1 (C-14), 22.7, 24.9, 25.4, 27.6, 29.1, 29.2, 29.4, 29.5, 34.1, 35.9−36.9 (m, C-9 and C-11), 51.4 (RCO_2CH_3), 71.7 (C-10), 174.3 ppm (C-1). GC/MS (EI) m/z: 261 (0.1, $M^{+\bullet}$ − H), 245 (0.4, $M^{+\bullet}$ − OH), 244 (0.4, $M^{+\bullet}$ − H_2O), 231 (1, $M^{+\bullet}$ − OCH_3), 229 (2), 212 (4), 203 (28, $M^{+\bullet}$ − $C_4H_7D_2$ and/or $M^{+\bullet}$ − $C_2H_3O_2$), 193 (1), 174 (38), 171 (100), 157 (3), 153 (6), 143 (34), 131 (33), 129 (10), 125 (18), 115 (5), 105 (1), 101 (15), 89 (17), 87 (99, $C_5H_7D_2O^{+\bullet}$ and/or $C_4H_7O_2^{+\bullet}$), 74 (66), 73 (13), 71 (55), 59 (35), 43 (35). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_3$ 285.2344, found 285.2333.

*1-(Tetrahydro-2H-pyran-2-yloxy)tetradecan-6-ol (**52**).* 1-Bromooctane (0.2 mL, 1.15 mmol) was added dropwise to a mixture of magnesium (217 mg, 8.93 mmol) and I_2 (cat.) in anhydrous Et_2O (2 mL) with stirring at 0 °C under a N_2 atmosphere, in order to initiate formation of the Grignard reagent. When the resulting yellow solution became colorless, it was diluted with anhydrous Et_2O (4 mL), and additional 1-bromooctane (0.4 mL, 2.29 mmol) was added dropwise. The Grignard reagent mixture was allowed to warm to room temperature with continuous stirring for 1 h and then was cooled to 0 °C. A solution of aldehyde **50**[24] (360 mg, 1.80 mmol) in anhydrous Et_2O (1 mL) was added dropwise, and the reaction mixture was allowed to warm to room temperature and stirred for a further 1.5 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (20 mL) and extracted with Et_2O (3 × 10 mL). The combined organic extract was washed with brine (3 × 10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 9% EtOAc in *n*-hexane) to afford **52** (398 mg, 1.27 mmol, 70%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.0 Hz, CH_3-14), 1.20−1.65 (m, 27H), 1.69 (m, 1H), 1.80 (m, 1H), 3.37 (dt, 1H, J = 9.6, 6.6 Hz, C*H*-1), 3.48 (m, 1H, C*H*-6′), 3.57 (m, 1H, C*H*-6), 3.72 (m, 1H, C*H*-1), 3.85 (m, 1H, C*H*-6′), 4.55 ppm (m, 1H, C*H*-2′). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.1 (C-14), 19.72, 19.74, 22.7, 25.4, 25.5, 25.7, 26.28, 26.34, 29.3, 29.6, 29.7, 30.8, 31.9, 37.4, 37.5, 62.39 (C-6′), 62.43 (C-6′), 67.5 (C-1), 67.6 (C-1), 71.89 (C-6), 71.93 (C-6), 98.89 (C-2′), 98.91 ppm (C-2′). GC/MS (EI) m/z: 229 (0.2), 213 (0.2), 171 (0.1), 157 (1), 143 (0.4), 129 (0.3), 115 (1), 101 (22, $THPO^{+\bullet}$), 99 (8), 85 (100, $THP^{+\bullet}$), 84 (11), 83 (13), 81 (11), 69 (21), 67 (12), 57 (34), 56 (15), 55 (52), 44 (9), 43 (44), 42 (14), 41 (57). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{19}H_{38}NaO_3$ 337.2719, found 337.2708. Anal. Calcd for $C_{19}H_{38}O_3$: C, 72.56; H, 12.18. Found: C, 72.43; H, 12.19.

*1,6-Tetradecanediol (**53**).* The THP moiety of **52** (202 mg, 0.64 mmol) was cleaved with *p*-TsOH.H_2O (5 mg, cat.) in MeOH (5 mL) over 3 h, as described for the synthesis of (*E*)-**24**. Purification by flash column chromatography (silica gel, 50% EtOAc in *n*-hexane) afforded diol **53** (142 mg, 0.62 mmol, 96%) as a pale yellow solid. Mp: 56−58 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.0 Hz, CH_3-14), 1.18−1.60 (m, 24H), 3.57 (m, 1H, C*H*-6), 3.63 ppm (t, 2H, J = 6.6 Hz, CH_2-1). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1 (C-14), 22.7, 25.4, 25.7, 25.8, 29.3, 29.6, 29.7, 31.9, 32.7, 37.4, 37.6, 62.9 (C-1), 71.9 ppm (C-6). GC/MS (EI) m/z: 157 (0.1), 145 (0.2), 143 (3), 117 (17, $C_6H_{13}O_2^{+\bullet}$), 99 (36), 85 (2), 83 (20), 82 (12), 81 (68), 71 (10), 70 (21), 69 (55), 67 (14), 57 (66), 56 (11), 55 (100), 44 (25), 43 (89), 42 (44), 41 (98). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{14}H_{30}NaO_2$ 253.2143, found 253.2135. Anal. Calcd for $C_{14}H_{30}O_2$: C, 72.99; H, 13.12. Found: C, 72.82; H, 13.32.

*6-Oxotetradecanoic acid (**49**).* Diol **53** (136 mg, 0.59 mmol) was oxidized with Jones' reagent (8 N) in acetone (5 mL) at 0 °C, as described for the synthesis of **48**. Purification by acid−base extraction afforded ketoacid **49** (138 mg, 0.57 mmol, 96%) as a white solid. Mp: 67−69 °C (lit.[49] mp: 68−69 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, 3H, J = 7.2 Hz, CH_3-14), 1.20−1.30 (m, 10H), 1.55 (m, 2H, CH_2-8), 1.60 (m, 4H, CH_2-3 and CH_2-4), 2.31−2.42 ppm (m, 6H, CH_2-2, CH_2-5 and CH_2-7). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (C-14), 22.6 (C-13), 23.1 (C-4), 23.9 (C-8), 24.2 (C-3), 29.1, 29.2, 29.3, 31.8 (C-12), 33.7 (C-2), 42.2 (C-5), 42.9 (C-7), 179.0 (C-1), 211.0 ppm (C-6). GC/MS (EI) m/z: (as methyl ester) 225 (0.5, $M^{+\bullet}$ − OCH_3), 171 (1), 158 (23), 143 (8), 141 (11), 126 (41), 115 (6), 111 (38), 101 (13), 98 (17), 84 (16), 83 (18), 73 (16), 71 (37), 59 (33), 58 (19), 57 (63), 55 (94), 43 (100), 41 (95). HRMS (ESI) m/z: [M + Na]$^{+\bullet}$ calcd for $C_{14}H_{26}NaO_3$ 265.1780, found 265.1775. Anal. Calcd for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81. Found: C, 69.31; H, 10.69.

*Methyl [5-$^2H_{0;1;2}$,7-$^2H_{0;1;2}$]-6-oxotetradecanoate ([5,5,7,7-$^2H_4$]-**15**).* As described for the synthesis of [$^2H_4$]-(*S,S*)-**32**, ketoacid **49** (116 mg, 0.48 mmol) was deuterated with NaOD/D_2O over 9 days, and the crude [$^2H_4$]-**49** was then esterified with CH_2N_2 in Et_2O (2 mL) at 0 °C. Purification by flash column chromatography (silica gel, 25% to

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66% Et_2O in petroleum spirits 40−60) afforded a yellow, low-melting solid that contained ketoester [5,5,7,7-$^2H_4$]-**15** (79 mg, 0.30 mmol, 63% over two steps, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, 3H, J = 7.2 Hz, CH_3-14), 1.21−1.29 (m, 10H), 1.50−1.57 (m, 2H, CH_2-8), 1.57−1.67 (m, 4H, CH_2-3 and CH_2-4), 2.32 (t, 2H, J = 6.6 Hz, CH_2-2), 3.66 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 22.6 (C-13), 23.1 (C-4), 23.7 (C-8), 24.4 (C-3), 29.1, 29.2, 29.4, 31.8, 33.8 (C-2), 41.1−42.6 (m, incl. 41.5 [quintet, $J_{C,D}$ = 19.0 Hz, CD_2], 42.1 [quintet, $J_{C,D}$ = 19.2 Hz, CD_2], C-5 and C-7), 51.5 (RCO_2CH_3), 173.9 (C-1), 211.2 ppm (C-6). GC/MS (EI) m/z: 260 (0.2, $M^{+\bullet}$), 229 (1, $M^{+\bullet} - OCH_3$), 173 (1), 162 (27), 145 (10), 143 (12), 130 (46), 117 (9), 113 (28), 112 (32), 102 (23), 101 (28), 99 (8), 87 (8), 85 (20), 75 (18), 74 (15), 73 (35), 71 (15), 62 (18), 59 (82), 57 (88), 56 (49), 55 (47), 45 (47), 44 (49), 43 (100), 41 (84). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{24}D_4NaO_3$ 283.2187, found 283.2182.

*Methyl [5-$^2H_{0;1;2}$,7-$^2H_{0;1;2}$]-6-hydroxytetradecanoate ([5,5,7,7-$^2H_4$]-**16**).* Ketoester [5,5,7,7-$^2H_4$]-**15** (44 mg, 0.17 mmol) was reduced with $NaBH_4$ (11 mg, 0.29 mmol) in MeOH (5 mL) over 25 min, as described for the synthesis of (*S*,*S*)-**33**. Purification by flash column chromatography (silica gel, 17% EtOAc in *n*-hexane) afforded a yellow, crystalline solid that contained [5,5,7,7-$^2H_4$]-**16** (39 mg, 0.15 mmol, 88%, ≤1% [$^2H_0$]) as the major deuterium-labeled product. Mp: 32−34 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.2 Hz, CH_3-14), 1.18−1.70 (m, 17H), 2.31 (t, 2H, J = 6.6 Hz, CH_2-2), 3.56 (m, 1H, C*H*-6), 3.65 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 22.6 (C-13), 24.8, 25.0, 25.4, 29.2, 29.57, 29.61, 31.9, 34.0 (C-2), 35.6−37.1 (m, incl. 36.1 [quintet, $J_{C,D}$ = 19.0 Hz, CD_2], 36.8 [quintet, $J_{C,D}$ = 19.2 Hz, CD_2], C-5 and C-7), 51.5 (RCO_2CH_3), 71.5 (C-6), 174.2 ppm (C-1). GC/MS (EI) m/z: 147 (21), 118 (26), 115 (57), 101 (3), 99 (3), 88 (40), 87 (100), 85 (7), 74 (11), 73 (5), 71 (10), 70 (11), 69 (26), 68 (15), 59 (27), 58 (19), 57 (35), 56 (24), 55 (34), 45 (20), 44 (22), 43 (59), 42 (28), 41 (51). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_3$ 285.2344, found 285.2336.

*2-(6-(Tetrahydro-2H-pyran-2-yloxy)tetradec-7-ynyloxy)tetrahydro-2H-pyran (**54**).* A solution of *n*-BuLi (1.10 M in hexanes, 4.0 mL, 4.40 mmol) was added dropwise to a solution of 1-octyne (96%, 1.0 mL, 6.51 mmol) in anhydrous THF (5 mL) with stirring under a N_2 atmosphere at −40 °C. The solution was stirred at −40 °C for 3 h, then a solution of aldehyde **50**[24] (257 mg, 1.28 mmol) in anhydrous THF (5 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for a further 42 h. Saturated aqueous NH_4Cl solution (20 mL) was added to quench the reaction, and the mixture was extracted with Et_2O (6 × 20 mL). The combined organic extract was washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude propargylic alcohol was dissolved in CH_2Cl_2 (30 mL), and *p*-TsOH·H_2O (200 mg, cat.) was added. The solution was cooled to 0 °C with stirring under a N_2 atmosphere, and DHP (97%, 0.4 mL, 4.25 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for a further 18 h. Aqueous NaOH solution (5%, 50 mL) was added to quench the reaction, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (6 × 20 mL), and the combined organic extract was washed with brine (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 1% to 5% Et_2O in petroleum spirits 40−60) to afford **54** (374 mg, 0.95 mmol, 74% over 2 steps) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, J = 7.1 Hz, CH_3-14), 1.20−1.65 (m, 22H), 1.65−1.75 (m, 4H), 1.75−1.88 (m, 2H), 2.17 (td, 2H, J = 7.1, 1.9 Hz, CH_2-9), 3.37 (m, 1H, C*H*-1), 3.48 (m, 2H, C*H*-6′ and C*H*-6″), 3.72 (m, 1H, C*H*-1), 3.78 (m, 1H), 3.85 (m, 1H), 4.36 (tt, 1H, J = 6.7, 1.9 Hz, C*H*-6), 4.55 (dd, 1H, J = 4.3, 2.8 Hz, C*H*-2′), 4.95 ppm (dd, 1H, J = 4.0, 2.9 Hz, C*H*-2″). ^{13}C NMR (125 MHz, $CDCl_3$): (mixture of diastereomers) δ 14.0 (C-14), 18.7, 19.5, 19.7, 22.6, 25.40, 25.41, 25.50, 25.53, 26.05, 26.06, 28.5, 28.7, 29.7, 30.6, 30.8, 31.3, 36.1 (C-5), 62.3 (2C, C-6′ and C-6″), 65.2 (C-6), 67.6 (C-1), 78.9 (C-8), 86.0 (C-7), 95.3 (C-2″), 98.8 ppm (C-2′). GC/MS (EI) m/z: 309 (0.2), 293 (1), 275 (0.3), 225 (1), 207 (1), 171 (1), 135 (1), 121 (1), 115 (1), 109 (2), 101 (4), 85 (100, $THP^{+\bullet}$), 67 (11), 57 (8), 55 (20), 43 (9), 41 (16). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{24}H_{42}NaO_4$ 417.2981, found 417.2975. Anal. Calcd for $C_{24}H_{42}O_4$: C, 73.05; H, 10.73. Found: C, 73.09; H, 10.61.

*[7-$^2H_{0;1;2}$,8-$^2H_{0;1;2}$]-1,6-Tetradecanediol ([$^2H_4$]-**53**).* A solution of **54** (276 mg, 0.70 mmol) in benzene (8 mL) was degassed−purged twice with N_2 (g) and then twice with $^2H_2$ (g). Wilkinson's catalyst (tris(triphenylphosphine)rhodium(I) chloride, 150 mg, 0.16 mmol) was added, and the reaction mixture was stirred under a $^2H_2$ (D_2, 99.98 atom % D) atmosphere at room temperature for 18 h. The mixture was diluted with *n*-hexane (50 mL) and filtered through a pad of Celite that was washed thoroughly with additional *n*-hexane. The filtrate was concentrated under reduced pressure, and the residue was dissolved in MeOH (10 mL). *p*-TsOH·H_2O (50 mg, cat.) was added, and the reaction mixture was stirred at room temperature for 16 h. Solid $NaHCO_3$ (200 mg) was added to quench the reaction, and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (silica gel, 10% to 66% Et_2O in petroleum spirits 40−60) to afford a white solid that contained [$^2H_4$]-**53** (131 mg, 0.56 mmol, 80% over 2 steps, ≤1% [$^2H_0$]) as the major deuterium-labeled product. Mp: 54−55 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.9 Hz, CH_3-14), 1.18−1.50 (m, 18H), 1.57 (m, 2H), 3.57 (m, 1H, C*H*-6), 3.62 ppm (t, 2H, J = 6.6 Hz, CH_2-1). ^{13}C NMR (100 MHz, $CDCl_3$): (signals for C-7 and C-8 not observed) δ 14.1 (C-14), 22.7, 25.4, 25.8, 29.3, 29.4, 29.5, 31.9, 32.7, 37.3, 62.9 (C-1), 71.8 ppm (C-6). GC/MS (EI) m/z: 215 (0.2), 207 (0.2), 198 (0.1), 186 (0.1), 173 (1), 160 (1), 147 (15), 129 (3), 117 (47, $C_8H_{13}D_4^{+\bullet}$ and/or $C_6H_{13}O_2^{+\bullet}$), 99 (90), 87 (11), 85 (25), 81 (100), 73 (14), 71 (45), 70 (62), 59 (17), 57 (45), 55 (58), 45 (12), 43 (48). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{14}H_{26}D_4NaO_2$ 257.2395, found 257.2395.

*Methyl [7-$^2H_{0;1;2}$,8-$^2H_{0;1;2}$]-6-oxotetradecanoate ([7,7,8,8-$^2H_4$]-**15**).* Diol [$^2H_4$]-**53** (118 mg, 0.50 mmol) was oxidized with PDC (98%, 924 mg, 2.41 mmol) in DMF (10 mL) under an Ar atmosphere over 18 h, following the procedure described for the synthesis of (*S*,*S*)-**31**. The crude acid obtained was then esterified with ethereal CH_2N_2 in MeOH (5 mL) at 0 °C, as described for the synthesis of (*S*,*S*)-**32**. Purification by flash column chromatography (silica gel, 5% to 66% Et_2O in petroleum spirits 40−60) afforded a pale yellow, low-melting solid that contained [7,7,8,8-$^2H_4$]-**15** (93 mg, 0.36 mmol, 71% over two steps, ≤1% [$^2H_0$]) as the major deuterium-labeled product. ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, 3H, J = 6.9 Hz, CH_3-14), 1.17−1.30 (m, 10H), 1.51−1.64 (m, 4H), 2.29 (t, 2H, J = 7.1 Hz, CH_2-2), 2.39 (t, 2H, J = 7.0 Hz, CH_2-5), 3.64 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-14), 22.6 (C-13), 23.0 (obscured quintet, $J_{C,D}$ = 19.3 Hz, CD_2 at C-8), 23.1, 24.5, 29.0, 29.1, 29.3, 31.8 (C-12), 33.8 (C-2), 42.0 (obscured quintet, $J_{C,D}$ = 19.1 Hz, CD_2 at C-7), 42.2 (C-5), 51.5 (RCO_2CH_3), 173.8 (C-1), 211.1 ppm (C-6). GC/MS (EI) m/z: 260 (1, $M^{+\bullet}$), 229 (3, $M^{+\bullet} - OCH_3$), 211 (5), 183 (2), 173 (4), 160 (69), 159 (10), 145 (30, $C_9H_{13}D_4O^{+\bullet}$), 143 (21, $M^{+\bullet} - C_8H_{13}D_4$), 128 (100), 117 (2), 115 (17, $M^{+\bullet} - C_9H_{13}D_4O$), 111 (71), 101 (34), 100 (35), 87 (14), 85 (21), 83 (39), 73 (45), 71 (11), 59 (49), 57 (23), 55 (54), 43 (40). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{24}D_4NaO_3$ 283.2187, found 283.2181.

*Methyl [7,7,8,8-$^2H_4$]-6-hydroxytetradecanoate ([7,7,8,8-$^2H_4$]-**16**).* Ketoester [7,7,8,8-$^2H_4$]-**15** (25 mg, 96.0 μmol) was reduced with $NaBH_4$ (7 mg, 185.0 μmol) in a mixture of MeOH (2 mL) and Et_2O (2 mL) at 0 °C over 4 h, as described for the synthesis of (*S*,*S*)-**33**. Purification by flash column chromatography (silica gel, 17% to 66% Et_2O in petroleum spirits 40−60) afforded a white solid that contained [7,7,8,8-$^2H_4$]-**16** (22 mg, 83.8 μmol, 87%, ≤1% [$^2H_0$]) as the major deuterium-labeled product. Mp: 34−36 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.9 Hz, CH_3-14), 1.19−1.55 (m, 15H), 1.63 (m, 2H), 2.31 (t, 2H, J = 7.5 Hz, CH_2-2), 3.56 (m, 1H, C*H*-6), 3.65 ppm (s, 3H, RCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): (Signals for C-7 and C-8 not observed) δ 14.1 (C-14), 22.7, 24.9, 25.2, 29.3, 29.4, 29.5, 31.9, 34.0, 37.0 (C-5), 51.5 (RCO_2CH_3), 71.6 (C-6), 174.2 ppm (C-1). GC/MS (EI) m/z: 244 (0.3), 231 (0.4, $M^{+\bullet} - OCH_3$), 229 (1), 212 (3), 194 (1), 170 (3), 161 (2), 160 (15), 145 (32, $M^{+\bullet} - C_8H_{13}D_4$), 128 (24), 116 (33), 113 (69), 101 (13), 100 (16), 87 (100), 85 (63), 74 (29), 73 (20), 71 (19), 67 (30), 59 (34), 57 (37),

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55 (66), 43 (55), 41 (52). HRMS (ESI) m/z: $[M + Na]^{+\bullet}$ calcd for $C_{15}H_{26}D_4NaO_3$ 285.2344, found 285.2340.

In Vivo Deuterium Incorporation Studies. *Fruit Flies.* No permission from national or local authorities was required to perform in vivo studies with fruit flies. *B. cacuminata* pupae were hatched out and housed in a cotton mesh cage at room temperature. They were fed a diet of sugar (sucrose), water, and a concentrated yeast extract and were subjected to a normal light−dark cycle. Feeding experiments were conducted at least 10 days after emergence because *B. cacuminata* are sexually mature at this time.[33]

Preparation of Precursors and Equipment. The potential precursors and β-oxidation inhibitor 2-fluorostearic acid were dissolved in Et_2O and enough sucrose was added to form ∼2% w/w compound-to-sucrose mixtures. The mixtures were thoroughly stirred, then Et_2O was evaporated and the mixtures were placed under high vacuum for ∼2 h. Feeding experiments were carried out at 28 °C in 100 mL conical flasks sealed with fresh rubber septa. The conical flasks were thoroughly rinsed with *n*-hexane, ethanol, concentrated aqueous HCl solution, water, and acetone and were then baked in an oven at 150 °C for several days prior to their use.

Administration of Potential Spiroacetal Precursors to Flies and Deuterium Incorporation Analysis. Five male flies were placed in a flask with three small pieces of sponge soaked in water and approximately 10 mg of the compound−sucrose mixture. Control groups were fed sucrose in a similar manner. In experiments involving the coadministration of 2-fluorostearic acid, the deuterated compound−sugar mixture was introduced to the flies ∼6−18 h following the administration of the 2-fluorostearic acid−sugar mixture. Sampling of the headspace volatile emissions was carried out using Supelco SPME units with 75 μm carboxen-PDMS fibers. The SPME fiber was inserted into the experiment flask through the rubber septum and sampling was conducted for 10−40 min. After each sampling period, the septum was removed from the flask for 15 s to allow air to enter and carbon dioxide to escape from the flask. The SPME fiber was then inserted into the GC/MS injection port, allowing direct analysis of the adsorbed volatiles. SPME sampling[34] was carried out once a day following administration of the deuterated compounds for 5 days or until the flies had died. GC/MS program: DB-5 column (30 m, J&W Scientific): splitless mode; column flow 1.6 mL min^{-1}; total flow 78.8 mL min^{-1}; injector 250 °C; detector 250 °C; oven 40 °C (1.0 min equilibration) held for 4.0 min, ramp 10 °C min^{-1} to 270 °C and held for 25.0 min (total program time 52.0 min). Masses monitored in SIM experiments: m/z 55, 57, 58, 73, 77, 83, 85, 86, 98, 99, 100, 101, 102, 103, 104, 105, 111, 113, 114, 115, 126, 128, 130, 156, 157, 158, 159, 160, 161, 162.

■ ASSOCIATED CONTENT

Supporting Information

Materials and general methods, copies of ^1H and ^{13}C NMR spectra of new compounds and final products, an explanation of the categories and criteria used for qualitative classification of in vivo deuterium incorporation from labeled substrates into spiroacetals, and characteristic spiroacetal mass fragmentation patterns. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: j.devoss@uq.edu.au.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Booth, Y. K.; Kitching, W.; De Voss, J. J. *Nat. Prod. Rep.* **2009**, *26*, 490−525.
(2) Baker, R.; Herbert, R.; Howse, P. E.; Jones, O. T.; Francke, W.; Reith, W. *J. Chem. Soc., Chem. Commun.* **1980**, 52−53.
(3) Schwartz, B. D.; Hayes, P. Y.; Kitching, W.; De Voss, J. J. *J. Org. Chem.* **2005**, *70*, 3054−3065.
(4) Booth, Y. K.; Kitching, W.; De Voss, J. J. *ChemBioChem* **2011**, *12*, 155−172.
(5) Fletcher, M. T.; Wood, B. J.; Brereton, I. M.; Stok, J. E.; De Voss, J. J.; Kitching, W. *J. Am. Chem. Soc.* **2002**, *124*, 7666−7667.
(6) Schwartz, B. D.; Moore, C. J.; Rahm, F.; Hayes, P. Y.; Kitching, W.; De Voss, J. J. *J. Am. Chem. Soc.* **2008**, *130*, 14853−14860.
(7) Schwartz, B. D.; Booth, Y. K.; Fletcher, M. T.; Kitching, W.; De Voss, J. J. *Chem. Commun.* **2010**, *46*, 1526−1528.
(8) Schwartz, B. D.; McErlean, C. S. P.; Fletcher, M. T.; Mazomenos, B. E.; Konstantopoulou, M. A.; Kitching, W.; De Voss, J. J. *Org. Lett.* **2005**, *7*, 1173−1176.
(9) When referring to an aliphatic chain, this notation describes the distance between the position of interest and the chain terminus (often a methyl group), referred to as the ω position. The position adjacent to the terminus is described as the ω-1 position, the one two bonds away from the terminus is the ω-2 position, then the ω-3 position, and so forth. Thus, ω-*n* describes the position *n* bonds away from the terminus.
(10) Fletcher, M. T.; Mazomenos, B. E.; Georgakopoulos, J. H.; Konstantopoulou, M. A.; Wood, B. J.; De Voss, J. J.; Kitching, W. *Chem. Commun.* **2002**, 1302−1303.
(11) Scott, J. G. In *Recent Advances in Insect Physiology, Toxicology and Molecular Biology*; Liu, N., Ed.; Research Signpost: Trivandrum, India, 2008; pp 117−124.
(12) For an example of P450 chain length specificity, see Helvig, C.; Tijet, N.; Feyereisen, R.; Walker, F. A.; Restifo, L. L. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 1495−1502.
(13) For an example of P450 chain length specificity, see: Narhi, L. O.; Fulco, A. J. *J. Biol. Chem.* **1986**, *261*, 7160−7169.
(14) Paddon-Jones, G. C.; McErlean, C. S. P.; Hayes, P.; Moore, C. J.; Konig, W. A.; Kitching, W. *J. Org. Chem.* **2001**, *66*, 7487−7495.
(15) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.
(16) All of the synthesized (*S*,*S*) compounds had negative optical rotations, while all the (*R*,*R*) isomers exhibited positive optical rotations, e.g., (*S*,*S*)-**28**: $[\alpha]_D^{24}$ −18.2 (*c* 0.61, MeOH); (*R*,*R*)-**28**: $[\alpha]_D^{24}$ +19.6 (*c* 0.75, MeOH). A similar result was observed previously with (7*S*,8*S*)- and (7*R*,8*R*)-methyl 7,8-dihydroxytetradecanoate synthesized via SAD (Singh, A. A.; Zulkifli, S. N. A.; Meyns, M.; Hayes, P. Y.; De Voss, J. J. *Tetrahedron: Asymmetry* **2011**, *22*, 1709−1719), where stereochemical assignments were confirmed by comparison with material derived from chiral, nonracemic tartrate substrates (Cryle, M. J.; De Voss, J. J. *Chem. Commun.* **2004**, 86−87). This result strongly supports our stereochemical assignments of (*S*,*S*)-**28** and (*R*,*R*)-**28**.
(17) Lohray, B. B.; Rao, B. S.; Baskaran, S.; Venkateswarlu, S.; Bhushan, V. *Indian J. Chem., Sect B* **1998**, *37*, 209−218.
(18) Plate, M.; Overs, M.; Schaefer, H. J. *Synthesis* **1998**, 1255−1258.
(19) Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999.
(20) Coutrot, P.; Grison, C.; Bomont, C. *J. Organomet. Chem.* **1999**, *586*, 208−217.
(21) Keck, G. E.; McHardy, S. F.; Murry, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 7289−7290.
(22) Cryle, M. J.; De Voss, J. J. *Chem. Commun.* **2004**, 86−87.
(23) Xiao, Y.; Liu, P. *Angew. Chem., Int. Ed.* **2008**, *47*, 9722−9725.
(24) Banfi, L.; Cabri, W.; Poli, G.; Potenza, D.; Scolastico, C. *J. Org. Chem.* **1987**, *52*, 5452−5457.
(25) de Visser, S. P. *Chem.—Eur. J.* **2006**, *12*, 8168−8177.

(26) Rylander, P. N. *Catalytic Hydrogenation in Organic Syntheses*; Academic Press: New York, 1979.
(27) Rylander, P. N. *Hydrogenation Methods*; Academic Press: London, 1985.
(28) Jakubowski, A. A.; Guziec, F. S.; Sugiura, M.; Tam, C. C.; Tishler, M. *J. Org. Chem.* **1982**, *47*, 1221−1228.
(29) Tedeschi, R. J. *J. Org. Chem.* **1962**, *27*, 2398−2402.
(30) Birch, A. J.; Walker, K. A. M. *J. Chem. Soc. C* **1966**, 1894−1896.
(31) Osborn, J. A.; Jardine, F. H.; Young, J. F.; Wilkinson, G. *J. Chem. Soc. A* **1966**, 1711−1732.
(32) Rakoff, H.; Rohwedder, W. K. *Lipids* **1992**, *27*, 567−569.
(33) Raghu, S.; Clarke, A. R. *Physiol. Entomol.* **2003**, *28*, 175−184.
(34) Fletcher, M. T.; Wood, B. J.; Schwartz, B. D.; Rahm, F.; Lambert, L. K.; Brereton, I. M.; Moore, C. J.; De Voss, J. J.; Kitching, W. *ARKIVOC* **2004**, 109−117.
(35) Burstein, S.; Middleditch, B. S.; Gut, M. *J. Biol. Chem.* **1975**, *250*, 9028−9037.
(36) Byon, C.-Y.; Gut, M. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 549−552.
(37) Shimizu, K. *J. Biol. Chem.* **1978**, *253*, 4237−4241.
(38) Nakajin, S.; Hall, P. F. *J. Biol. Chem.* **1981**, *256*, 3871−3876.
(39) Nakajin, S.; Takahashi, M.; Shinoda, M.; Hall, P. F. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 708−713.
(40) Umehara, K.; Kudo, S.; Hirao, Y.; Morita, S.; Uchida, M.; Odomi, M.; Miyamoto, G. *Drug Metab. Dispos.* **2000**, *28*, 887−894.
(41) Umehara, K.; Kudo, S.; Hirao, Y.; Morita, S.; Ohtani, T.; Uchida, M.; Miyamoto, G. *Drug Metab. Dispos.* **2000**, *28*, 1417−1424.
(42) Umehara, K.; Shimokawa, Y.; Koga, T.; Ohtani, T.; Miyamoto, G. *Xenobiotica* **2004**, *34*, 61−71.
(43) Fraas, S.; Steinbach, A. K.; Tabbert, A.; Harder, J.; Ermler, U.; Tittmann, K.; Meyer, A.; Kroneck, P. M. H. *J. Mol. Catal. B: Enzym.* **2009**, *61*, 47−49.
(44) Steinbach, A. K.; Fraas, S.; Harder, J.; Tabbert, A.; Brinkmann, H.; Meyer, A.; Ermler, U.; Kroneck, P. M. H. *J. Bacteriol.* **2011**, *193*, 6760−6769.
(45) Steinbach, A.; Fraas, S.; Harder, J.; Warkentin, E.; Kroneck, P. M. H.; Ermler, U. *FEBS J.* **2012**, *279*, 1209−1219.
(46) Rigaudy, J.; Klesney, S. P. *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F, and H*; Pergamon Press: Oxford, 1979.
(47) Breusch, F. L.; Ozeris, S. *Istanbul Univ. Fen Fak. Mecm., Seri C* **1964**, *29*, 34−39.
(48) Tulloch, A.; Hoffman, L. *Lipids* **1973**, *8*, 617−622.
(49) Christie, W. W.; Gunstone, F. D.; Prentice, H. G.; Sen Gupta, S. G. *J. Chem. Soc.* **1964**, *1*, 5833−5837.