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Synthesis of 6-Hydroxysphingosine and α-Hydroxy Ceramide Using a Cross-Metathesis Strategy

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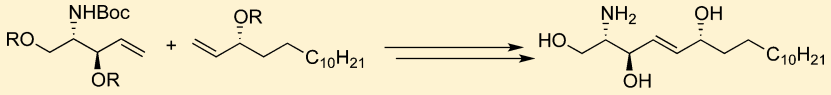
Supporting Information



ABSTRACT: In this paper, a new synthetic route toward 6-hydroxysphingosine and α-hydroxy ceramide is described. The synthesis employs a cross-metathesis to unite a sphingosine head allylic alcohol with a long-chain fatty acid alkene that also bears an allylic alcohol group. To allow for a productive CM coupling, the sphingosine head allylic alcohol was protected with a cyclic carbonate moiety and a reactive CM catalyst system, consisting of Grubbs II catalyst and CuI, was employed.

(Glyco)sphingolipids are a family of complex lipids found in all mammalian cells. Besides playing important structural roles as membrane components, they are involved in a multitude of intra- and intercellular signaling events and play a role in many (patho)physiological processes.[1] This structurally diverse class of lipids is composed of a sphingosine base (a 2-amino-1,3-dihydroxy long chain alkyl lipid) which can be acylated at the nitrogen with a variety of acyl chains.[2] Further structural variation comes from differences in substitution at the primary alcohol group, at which position a large variety of glycans and phosphate groups can be attached. Structural modifications in the sphingosine base are also found. The most recently reported members of the human sphingolipid family, the 6-hydroxyceramides (e.g., **2**, Figure 1), were discovered in 1989,[3] and their structure, based on a 6-hydroxysphingosine base (**1**, See Figure 1), was fully established in 1994.[4] These sphingolipids are important constituents of the human skin, especially the stratum corneum (SC), where they play a role in skin barrier pathologies.[5] Authentic samples of these sphingolipids are valuable to study their role in skin physiology processes. Because they are not commercially available and cannot be obtained from natural sources in pure form and sufficient quantity, the development of synthetic routes to access these molecules is important.[6] To date, three reports describing the synthesis of the 6-hydroxysphingosine base have appeared.[7−9] These three syntheses all hinge on the diastereoselective nucleophilic attack of an appropriately protected alkyne lipid to (*S*)-Garner aldehyde[10] (**3**, See Figure 1) using strongly basic conditions and necessitating a subsequent reduction step. We and others have previously described the assembly of (glyco)sphingolipids using a cross-metathesis (CM) strategy.[11−14] In this approach, an allylic sphingosine head is coupled with a long-chain alkene (varying in length and carrying different functionalities[12] or ^{13}C labels[13]) through the formation of the *E*-double bond. The mild conditions required for this transformation and the broad functional group tolerance make this an attractive strategy, and we therefore reasoned that it could be an effective approach to access 6-hydroxysphingosines. To make this strategy successful, we realized that difficulties associated with the cross-metathesis of two similar allylic alcohols (type II or III CM coupling partners)[15] had to be overcome. We here describe a strategy for the CM of two similar allylic alcohols and present the synthesis of 6-hydroxysphingosine **1** (see Figure 1) as well as the synthesis of ceramide **2.**

The synthesis of the required cross-metathesis partners **6** and **7** bearing different protecting group patterns is depicted in Scheme 1. The sphingosine head **6a** was accessed in six steps from L-serine as previously described by Yamamoto et al.[14] The long-chain allylic alcohol **7** was assembled using a "tellurium transposition" strategy, in which a primary allylic alcohol (**12**) is transformed into the regioisomeric secondary allylic alcohol (**7a**) following a Sharpless asymmetric epoxidation (SAE)−tellurium-mediated reductive elimination sequence.[16] To this end, we first generated the required primary allylic alcohol **12** from tridecanal **10**. Because we found that commercial grade tridecanal did not perform well in the ensuing olefination reaction, we generated this aldehyde from tridecanoic acid. Thus, this acid was first transformed via the acid chloride into the Weinreb amide (99% over two steps), which was reduced to give tridecanal **10**. The aldehyde was immediately used in the ensuing olefination event. We found that the best *E*/*Z*-selectivity for *trans*-olefin **11** (≥98% *E*) was achieved using a Horner−Wadsworth−Emmons (HWE) reaction with diisopropyl (ethoxycarbonylmethyl)phosphonate.[7] The Horner−Wittig reaction of tridecanal with (ethoxycarbonylmethyl)-triphenylphosphonium bromide, as well as the HWE reaction with diethyl (ethoxycarbonylmethyl)phosphonate, proceeded

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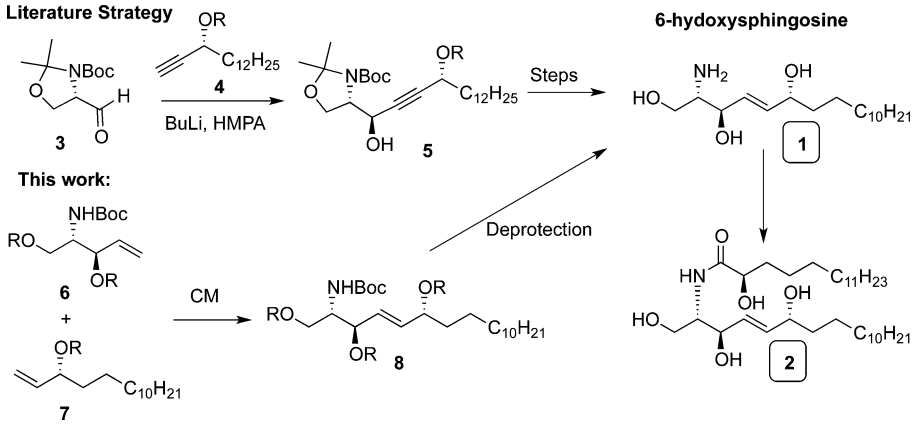
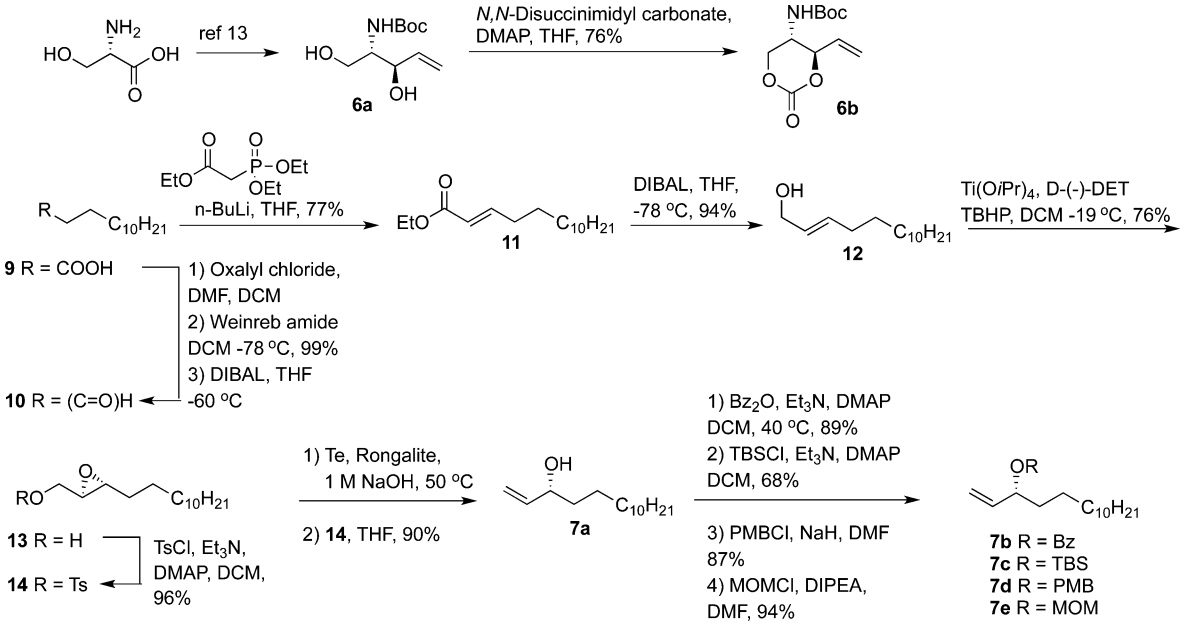


Figure 1. Literature strategy and this work.

Scheme 1. Synthesis of Cross-Metathesis Partners 8 and 9



with less selectivity (E/Z = 9:1 to 95:5). The α,β-unsaturated ester **11** was then reduced to the allylic alcohol **12** with diisobutylaluminum hydride (DIBAL-H) to set the stage for Sharpless asymmetric epoxidation reaction, which was used to introduce the chirality in the molecule. After substantial optimization of this reaction, optimal conditions were found in the use of 13.5 mol % of Ti(O-*i*-Pr)$_4$, 17.5 mol % of D-(−)-diethyl tartrate (D-(−)-DET), 2.2 equiv of *tert*-butyl hydroperoxide (TBHP), and molecular sieves 4 Å (1 g/mol) in dichloromethane at −19 °C. This delivered the chiral epoxide **13** in 79% yield and 89% ee (determined from tosylate **14**). Installation of a tosylate function at the primary alcohol allowed for the tellurium-mediated reductive elimination reaction. To this end, an aqueous solution of Na_2Te was generated from tellurium and Rongalite ($HOCH_2SO_2Na$) in NaOH (aq).[16] Addition of **14** to this solution led to the nucleophilic displacement of the primary tosylate by tellurium ion, after which the ensuing epoxide ring opening generates an epitelluride ring that collapses upon exposure to air to give the "transposed" secondary allylic alcohol **7a**. With both allylic alcohols in hand, the stage was set for the crucial CM reaction. Generally, for a productive CM event, two reaction partners of differing reactivity are required. Grubbs and co-workers[14] have divided alkenes in four categories based on their ability to form homodimers in CM reactions. Type I alkenes undergo rapid homodimerization, and these can participate in an ensuing CM event. Type II alkenes undergo slow homodimerization leading to homodimers that can participate in CM to a limited extent. Type III alkenes do not form homodimers, but they are able to react with type I and II homodimers in a CM. Type IV alkenes are essentially "spectators to cross-metathesis" as they do not display any activity toward the catalyst. For a selective CM, the reaction partners are preferably from different types. The two allylic alcohols at hand are classified as type II CM partners, making the desired CM reaction a challenging process. To discriminate between the reactivity of the alkenes, we first decided to protect the long chain alcohol **7a** (generating a type III CM partner), and we masked the hydroxyl group as a benzoyl ester (**7b**), a *tert*-butyldimethylsilyl ether (**7c**), a *p*-methoxybenzyl (PMB) ether (**7d**), or methoxymethyl (MOM) ether (**7e**). The results of the CM reactions are summarized in Table 1. The first attempt, involving diol **6a** and alcohol **7a**, provided the desired CM **8a** product in 23% yield (entry 1). Raising the temperature of this reaction did not lead to an

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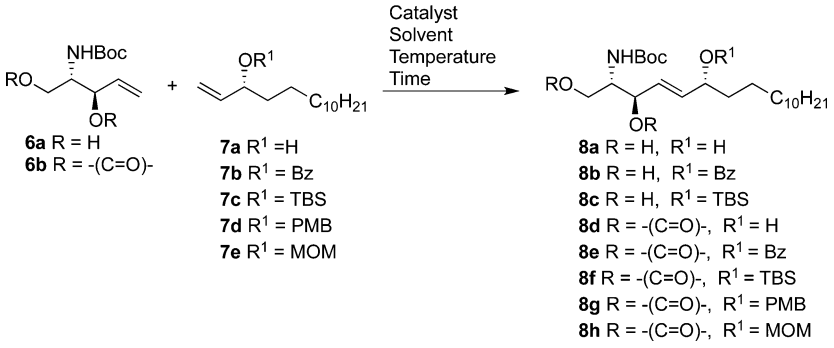
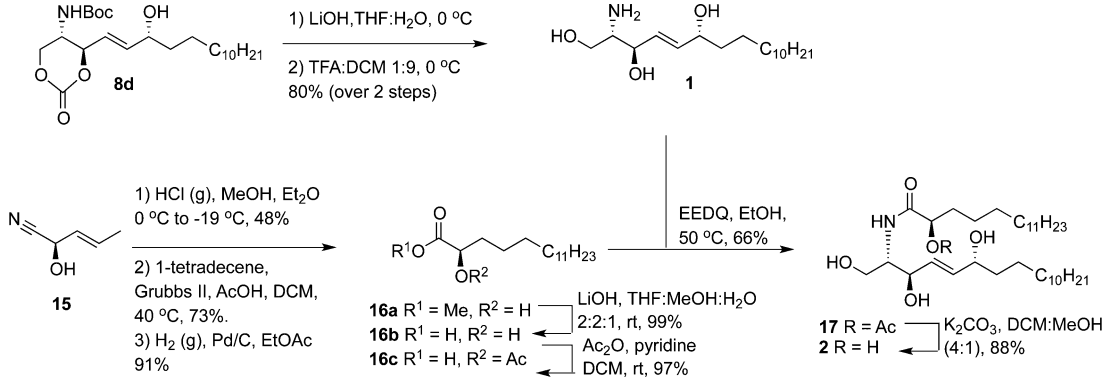
Table 1. Optimization of Cross-Metathesis



entry	6/7	catalyst[a]	solvent	temp (°C)	time (h)	product (yield, %)[b]
1	**6a**/**7a**	Grubbs II	DCM	40	48	**8a** (23%)
2	**6a**/**7a**	Grubbs II	DCE	80	48	**8a** (−)
3	**6a**/**7b**	Grubbs II	DCM	40	48	**8b** (−)
4	**6a**/**7c**	Grubbs II	DCM	40	48	**8c** (−)
5	**6b**/**7a**	Grubbs II	DCM	40	48	**8d** (48%)
6	**6b**/**7b**	Grubbs II	DCM	40	48	**8e** (−)[c]
7	**6b**/**7c**	Grubbs II	DCM	40	48	**8f** (−)[c]
8	**6b**/**7d**	Grubbs II	DCM	40	48	**8g** (37%)[c]
9	**6b**/**7e**	Grubbs II	DCM	40	66	**8h** (43%)[c]
10	**6b**/**7a**	Grubbs II	DCE	80	48	**8d** (−)
11	**6b**/**7a**	Hoveyda-Grubbs	DCM	40	70	**8d** (48%)
12	**6b**/**7a**	Grubbs II + CuI	tol	40	48	**8d** (78%)
13	**6b**/**7a**	Grubbs II + CuI	Et_2O	r.t.	48	**8d** (83%)

[a]Reaction conditions: molar ratio **6**:**7** (3:1), 20 mol % catalyst, 30 mol % (CuI). [b]Yields denote isolated yields after column chromatography. [c]Unreacted cyclic carbonate **6b** as well the protected allylic olefin (**7b**−**e**) could be recovered.

Scheme 2. Synthesis of 6-Hydroxysphingosine 1 and α-Hydroxy Ceramide 2



improved reaction (entry 2). Next, a CM was attempted with the more electron-poor benzoylated allylic alcohol **7b** in combination with diol **6a**, but this CM was unproductive (entry 3). Similarly, the use of silylated CM partner **7c** was to no avail (entry 4). Because modulation of the reactivity of the long-chain allylic alcohol proved unsuccessful, we decided to protect diol **6** and generated the cyclic carbonate **6b**. The cycic carbonate group is strongly electron withdrawing, changing the electronic properties of the alkene, and ties back the functionalities of the groups attached to the alkene, making it more accessible. When the allylic carbonate **6b** and alcohol **7a** were combined in a CM event, the desired *E*-alkene **8d** was obtained in an increased yield (48%, entry 5). The use of benzoylated and silylated CM partners **7b** and **7c** again led to unproductive CM reactions (entries 6 and 7). The use of PMB- and MOM-protected allylic alcohols **7d** and **7e** did deliver the desired CM products **8g** and **8h**, respectively, but in a lower yield than obtained for **8d** (entries 8 and 9). Having established that the most productive CM reaction occurred between carbonate **6b** and alcohol **7a**, this reaction was further optimized. Raising the temperature led to decomposition of the cyclic carbonate, and therefore, no product was obtained (entry 10). We then explored different catalyst systems. The use of the Grubbs−Hoveyda catalyst led to an identical result as compared to the Grubbs II catalyzed CM reaction (entry 11), and we therefore moved to the use of additives to improve the reactivity of the catalyst. As described by Voigtritter et al.,[17] copper iodide (CuI) can be used to generate a more stable catalyst (due to the iodide) while making the system more reactive because the copper sequesters the phosphine ligands. As shown in entries 12 and 13, the use of this additive proved very successful, and the cyclic carbonate protected alkene diol **6b** and allylic alcohol **7a** could be fused to provide the desired *E*-alkene **8d** in good yield. Having successfully constructed the

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6-hydroxysphingosine backbone, the base was globally deprotected by saponification of the carbonate group and the ensuing debocylation using dilute acid at low temperature (0 °C) to give 6-hydroxysphingosine **1**, as depicted in Scheme 2. The use of more forceful acidic conditions led to complex reaction mixtures, presumably as a result of acid-catalyzed allylic substitution reactions. The synthesis of the 6-hydroxysphingosine-based ceramide **2** featuring an α-hydroxyl side chain was finally accomplished. First, α-hydroxy fatty acid **16a** was generated from optically pure cyanohydrin **15**, obtained from crotonaldehyde by the action of almond hydroxynitrilase.[18] The cyanohydrin was transformed into the corresponding methyl ester using a Pinner reaction.[19] Next, a CM reaction of the alkene with 1-tetradecene gave the unsaturated long-chain lipid that was reduced and saponified to give the fatty acid **16b**. To condense the α-hydroxy fatty acid with the 6-hydroxysphingosine, the α-hydroxy group was first masked with an acetyl group. Activation of acid **16c** first with 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ)[20] and the ensuing condensation then gave acetylated ceramide **17**. Final saponification of the acetyl ester completed the synthesis of the target 6-hydroxyceramide **2**.

In conclusion, we have described a new synthetic route for 6-hydroxysphingosine and its α-hydroxyceramide counterpart, employing a cross-metathesis (CM) strategy. The crucial CM reaction on which the synthesis hinges unites two similar allylic alcohol alkenes. We have used a cyclic carbonate group to protect the diol system, present in the sphingosine head CM partner, with a cyclic carbonate functionality to serve two purposes. First, it makes the double bond less electron rich, discriminating it from its designated CM partner, the long-chain allylic alcohol. Second, it ties back the functional groups on the olefin, making the alkene sterically most accessible for the catalyst and the CM event. In combination with an activated catalyst system (the Grubbs II−CuI reagent pair), this led to an efficient CM connection of the sphingosine head and tail alkenes. The mild conditions required for this connection in conjunction with the straightforward deprotection scheme (mild base followed by mild acid) make the approach versatile and amenable to the use of a variety of CM coupling partners to generate labeled and tagged 6-hydroxysphingosine derived probes for future biochemical studies.[13,21]

EXPERIMENTAL SECTION

General Methods. Commercially available reagents and solvents were used as received. DCM and THF were dried and distilled by standard procedures. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out with silica gel 60 (40−63 μm mesh). IR spectra are reported in cm^{-1}. Optical rotations were measured with an automatic polarimeter (sodium D line, λ = 589 nm). The enantiomeric purity was determined by HPLC analysis using an OD column (hexane/isopropyl alchohol (98:2), 1 mL/min, UV 254 nm). NMR spectra were recorded on a 400 or 850 MHz spectrometer. Chemical shifts are reported as δ values (ppm) and were referenced to tetramethylsilane (δ = 0.00 ppm) directly in $CDCl_3$ or using the residual solvent peak (D_2O). High-resolution mass spectra were recorded on an LTQ-Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode with a 355 nm laser. Samples (1 μL, 100 mM in $CHCl_3$) were spotted on the MALDI plate, followed by addition of aqueous silver benzoate (0.5 μL, 10 mM), drying, and application of the matrix (2,5-dihydroxybenzoic acid, 0.5 μL, 0.5 M in methanol). A laser frequency of 1000 Hz (power set at 60%) was used.

***tert*-Butyl ((4*R*,5*S*)-2-Oxo-4-vinyl-1,3-dioxan-5-yl)carbamate (6b).** Allylic alcohol **6a** (3.03 g, 13.95 mmol, 1 equiv) was dissolved in anhydrous THF (400 mL) under an argon atmosphere. *N*,*N*′-Disuccinimidyl carbonate (8.93 g, 34.88 mmol, 2.5 equiv) and DMAP (4.26 g, 34.88 mmol, 2.5 equiv) were added, and the mixture was allowed to stir for 20 h at room temperature. The reaction mixture was concentrated in vacuo and purified by silica gel chromatography (30% EtOAc in pentane) to give cyclic carbonate **6b** (2.57 g, 10.56 mmol, 76%) as a thick, colorless oil: R_f = 0.6 (50% EtOAc in pentane); $[\alpha]_D$ = +46.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.92 (ddd, 1 H, J = 16.8, 10.8, 4.4 Hz), 5.58 (d, 1 H, J = 7.2 Hz), 5.50 (dd, 1 H, J = 16.8, 1.6 Hz), 5.47 (dd, 1 H, J = 10.8, 1.6 Hz), 5.02 (br s, 1 H), 4.54 (dd, 1 H, J = 11.2, 2.4 Hz), 4.31 (d, 1 H, J = 11.2 Hz), 3.97 (m, 1 H), 1.46 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.3, 147.7, 132.5, 119.7, 81.9, 80.9, 68.2, 45.8, 28.3; IR (neat) 3329, 2978, 2932, 1751,1701, 1165 cm^{-1}; HRMS (ESI) [M + H^+] calcd for $C_{11}H_{18}NO_5$ 244.1180, found 244.1184

Tridecanal (10). Tridecanoic acid **9** (7.50 g, 35.0 mmol, 1 equiv) was dissolved in anhydrous DCM (110 mL) under an argon atmosphere. The solution was cooled to 0 °C before addition of oxalyl chloride (2 M in DCM, 35 mL, 70 mmol, 2 equiv) and DMF (3 drops, cat.). The mixture was stirred for 4−5 h at room temperature. Once gas formation subsided, the reaction mixture was concentrated in vacuo, and the crude acyl chloride was immediately dissolved in anhydrous DCM (110 mL) under an argon atmosphere. The solution was cooled to −78 °C before slow addition of distilled *N*,*O*-dimethylhydroxylamine (6.42 mL, 87.5 mmol, 2.5 equiv). After 30 min, the reaction mixture was allowed to reach room temperature and stirred for 20 h. The reaction mixture was filtered, concentrated in vacuo, and purified by silica gel chromatography (5% → 10% EtOAc in pentane) to give *N*-methoxy-*N*-methyltridecanamide (8.94 g, 34.74 mmol, 99%) as an oil: R_f = 0.3 (10% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3 H), 3.18 (s, 3 H), 2.41 (t, 2 H, J = 7.6 Hz), 1.67−1.571 (m 2 H), 1.31−1.26 (m, 18 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 61.3, 32.3, 32.0 (×2), 29.8, 29.76 (×2), 29.64, 29.59, 29.56, 29.5, 24.8, 22.8, 14.3; IR (neat) 2922, 2853, 1668 cm^{-1}; HRMS (ESI) [M + H^+] calcd for $C_{15}H_{32}NO_2$ 258.2428, found 258.2426.

N-Methoxy-*N*-methyltridecanamide (2.59 g, 10.05 mmol, 1 equiv) was dissolved in anhydrous THF (25 mL) under an argon atmosphere. The solution was cooled to −60 °C followed by addition of diisobutylaluminum hydride (1 M in THF, 12 mL, 12 mmol, 1.2 equiv). The reaction mixture was stirred for 3 h at −60 °C. The reaction was quenched by addition of Rochelle salt solution (satd, 20 mL) at −60 °C. The mixture was then allowed to reach room temperature before extraction with EtOAc (2 × 150 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude tridecanal **10** was collected as an oil, which was used for the next reaction without further purification: R_f = 0.7 (5% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, 1 H, J = 1.6 Hz), 2.42 (dt, 2 H, J = 7.2, 1.6 Hz), 1.67−1.57 (m, 2 H), 1.30−1.26 (m, 18 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.9, 44.0, 32.3, 29.76, 29.74, 29.70, 29.55, 29.48, 29.47, 29.28, 22.8, 22.2, 14.2; IR (neat) 3024, 2984, 2941, 1732, 1236 cm^{-1}.

Ethyl (*E*)-Pentadec-2-enoate (11). Diisopropyl (ethoxycarbonylmethyl)phosphonate (3.53 g, 14 mmol, 1.4 equiv) was dissolved in anhydrous THF (40 mL) under an argon atmosphere. *n*-Butyllithium (2.5 M in hexanes, 5 mL, 12.5 mmol, 1.25 equiv) was added and the mixture stirred for 15 min at room temperature followed by addition of a solution of crude tridecanal **10** in anhydrous THF (15 mL). The reaction mixture was stirred for 20 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (0% → 1% → 2% EtOAc in pentane). The α,β-unsaturated ester **11** (2.08 g, 7.73 mmol, 77% over two steps, ≥98% *E*) was collected as a light yellow oil: R_f = 0.5 (2% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (dt, 1 H, J = 15.6, 6.8 Hz), 5.81 (dt, 1 H, J = 15.6, 1.6 Hz), 4.18 (q, 2 H, J = 7.2 Hz), 2.23−

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2.15 (m, 2 H), 1.48−1.40 (m, 2 H), 1.32−1.26 (m, 21 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 149.6, 121.3, 60.2, 32.3, 32.4, 29.78, 29.76 (×2), 29.65, 29.52, 29.48, 29.28, 28.1, 22.8, 14.4, 14.2; IR (neat) 2922, 2853, 1722, 1655, 1179 cm^{-1}; ESI HRMS (ESI) [M + H$^+$] calcd for $C_{17}H_{33}O_2$ 269.2475, found 269.2475. Spectroscopic data was identical to literature values.[7]

(*E*)-Pentadec-2-en-1-ol (12). Ethyl (*E*)-pentadec-2-enoate (**11**) (2.39 g, 8.89 mmol, 1 equiv) was dissolved in anhydrous THF (45 mL) under an argon atmosphere. The solution was cooled to −78 °C followed by addition of diisobutylaluminum hydride (1 M in THF, 26.7 mL, 26.7 mmol, 3 equiv). The reaction mixture was stirred for 3 h, slowly warming to −60 °C. The reaction was quenched with NH_4Cl solution (satd) at −60 °C. The mixture was then allowed to warm to room temperature before addition of HCl (5%, 100 mL). The mixture was extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (2.5% → 5% EtOAc in pentane). Allylic alcohol **12** (1.89 g, 8.34 mmol, 94%) was collected as a colorless oil which slowly crystallized into a white solid: R_f = 0.3 (5% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73−5.59 (m, 2 H), 4.08 (d, 2 H, J = 6.0 Hz), 2.04 (q, 2 H, J = 7.4 Hz), 1.50 (br s, 1 H), 1.42−1.33 (m, 2 H), 1.32−1.26 (m, 18 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.8, 128.9, 64.0, 32.4, 32.1, 29.83, 29.81, 29.79, 29.76, 29.65, 29.5, 29.3, 29.28, 22.8, 14.3; IR (neat) 3292, 2955, 2918, 2849, 1672, 1462 cm^{-1}. Spectroscopic data was identical to literature values.[7]

((2*R*,3*R*)-3-Dodecyloxiran-2-yl)methanol (13). Activated, powdered molecular sieves (∼5 g, 4 Å) were added to a flame-dried flask with freshly distilled, anhydrous DCM (15 mL) under an argon atmosphere. The suspension was stirred for 10 min at room temperature, completely drying DCM in the process. (−)-Diethyl D-tartrate (0.15 mL, 0.86 mmol, 0.17 equiv) and titanium(IV) isopropoxide (0.2 mL, 0.69 mmol, 0.14 equiv) were added to this solution at −19 °C. Allylic alcohol **12** (1.13 g, 5.00 mmol, 1 equiv) was coevaporated with toluene (2×) and dissolved in freshly distilled, anhydrous DCM (10 mL). After the titanium tartrate solution was stirred for 30 min, the solution of allylic alcohol **12** was added followed by addition of *tert*-butyl hydroperoxide (∼5.5 M in decane, 2 mL, 11.0 mmol, 2.2 equiv). The reaction mixture was stirred for 5 h at −19 °C. The reaction was quenched by addition of H_2O (20 mL) at −19 °C. After the mixture was warmed to room temperature, NaOH (30% in brine, 2.5 mL) was added to aid in the hydrolysis of (−)-diethyl D-tartrate. The mixture was stirred for 15 min, followed by extraction with DCM (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtrated, and concentrated in vacuo. The remaining *tert*-butyl hydroperoxide was removed by coevaporation with toluene. The crude product was purified by silica gel chromatography (10 → 15% EtOAc in pentane). Epoxide **13** (0.92 g, 3.80 mmol, 76%, ee = 89% determined from tosylate **14**) was collected as a white solid. The analytical sample was recrystallized from petroleum ether (60%): R_f = 0.3 (20% EtOAc in pentane); $[\alpha]_D$ = +23.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (ddd, 1 H, J = 12.4, 5.2, 2.4 Hz), 3.63 (ddd, 1 H, J = 12.0, 7.2, 4.4 Hz), 2.96 (m, 1 H), 2.99−2.90 (m, 1 H), 1.69−1.63 (m, 1 H), 1.59−1.54 (m, 2 H), 1.49−1.39 (m, 2 H), 1.35−1.26 (m, 18 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 61.8, 58.5, 56.1, 32.1, 31.7, 29.81, 29.78 (×2), 29.70, 29.68, 29.55, 29.5, 26.1, 22.8, 14.3; IR (neat) 3252, 2953, 2916, 2870, 2847, 1458, 1248, 868 cm^{-1}; HRMS (ESI) [M + H$^+$] calcd for $C_{15}H_{31}O_2$ 243.2319, found 243.2321. Spectroscopic data was identical to literature values.[8,22]

((2*R*,3*R*)-3-Dodecyloxiran-2-yl)methyl 4-Methylbenzenesulfonate (14). Epoxide **13** (716 mg, 2.95 mmol, 1 equiv) was dissolved in anhydrous DCM (30 mL) under an argon atmosphere. The solution was cooled to 0 °C followed by addition of *p*-toluenesulfonyl chloride (1.13 g, 5.91 mmol, 2 equiv), DMAP (25 mg, 0.2 mmol, cat.), and triethylamine (1.24 mL, 8.86 mmol, 3 equiv). The reaction mixture was stirred for 20 h at room temperature. The reaction was quenched with H_2O (30 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were washed with H_2O (2×), dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (2 → 3 → 5% EtOAc in pentane). Tosylate **14** (1.12 g, 2.82 mmol, 96%) was collected as a colorless oil that slowly crystallized into a white solid: R_f = 0.3 (5% EtOAc in pentane); $[\alpha]_D$ = +24.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, 2 H, J = 8.4 Hz), 7.35 (d, 2 H, J = 8.0 Hz), 4.18 (dd, 1 H, J = 11.2, 4.0 Hz), 3.97 (dd, 1 H, J = 11.2, 6.0 Hz), 2.95 (ddd, 1 H, J = 6.0, 4.0, 2.0 Hz), 2.78 (m, 1 H), 2.45 (s, 3 H), 1.55−1.47 (m, 2 H), 1.43−1.33 (m, 2 H), 1.32−1.25 (m, 18 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.2, 132.9, 130.0, 128.1, 70.3, 57.0, 54.7, 32.0, 31.4, 29.79, 29.77, 29.76, 29.65, 29.61, 29.5, 29.4, 25.9, 22.8, 21.8, 14.3; IR (neat) 2953, 2916, 2870, 2849, 1599, 1364, 1177, 829, 808 cm^{-1}; HRMS (ESI) [M + H$^+$] calcd for $C_{22}H_{37}O_4S$ 397.2407, found 397.2404.

(*R*)-Pentadec-1-en-3-ol (7a). Sodium hydroxymethanesulfinate hydrate (6.50 g, 55.0 mmol, 7.3 equiv) was dissolved in NaOH (1 M, 150 mL). Argon was purged through the solution before addition of tellurium (powder, −200 mesh, 1.92 g, 15.1 mmol, 2 equiv). The reaction mixture was stirred for 2 h at 50 °C. The purple solution of tellurides was cooled to 0 °C followed by slow addition of a solution of tosylate **14** (2.99 g, 7.53 mmol, 1 equiv) in THF (75 mL). The reaction was stirred for 20 h at room temperature. The reaction was quenched by bubbling air through the solution. The crude reaction mixture was then filtrated over a pad of Celite and concentrated in vacuo. The aqueous layer was extracted with Et_2O (2 × 150 mL). The combined organic layers were washed with H_2O_2 (3%, 75 mL), sodium thiosulfate (10%), and brine before being dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (5% → 10% Et_2O in pentane). Allylic alcohol **7a** (1.54 g, 6.78 mmol, 90%) was collected as a white solid: R_f = 0.4 (20% Et_2O in pentane); $[\alpha]_D$ = −7.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (ddd, 1 H, J = 16.8, 10.4, 6.4 Hz), 5.22 (dt, 1 H, J = 17.2, 1.2 Hz), 5.10 (dt, 1 H, J = 10.4, 1.2 Hz), 4.13−4.08 (m, 1 H), 1.69 (br s, 1 H), 1.59−1.48 (m, 2 H), 1.43−1.26 (m, 20 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.5, 114.7, 73.5, 37.2, 32.1, 29.82, 29.80 (×2), 29.74 (×2), 29.70, 29.51, 25.48, 22.8, 14.3; IR (neat) 3348, 2918, 2853, 1643, 1466 cm^{-1}. Spectroscopic data was identical to literature values.[22]

(*R*)-Pentadec-1-en-3-yl Benzoate (7b). Allylic alcohol **7a** (325 mg, 1.44 mmol, 1 equiv) was dissolved in anhydrous DCM (17 mL) under an argon atmosphere. Benzoic anhydride (974 mg, 4.31 mmol, 3 equiv), triethylamine (0.80 mL, 5.74 mmol, 4 equiv), and DMAP (17 mg, 0.14 mmol, cat.) were added, and the reaction mixture was stirred under reflux for 20 h. The reaction was quenched by addition of NH_4Cl (satd, 25 mL). The aqueous layer was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (0 → 2% EtOAc in pentane). The benzoylated product **7b** (424 mg, 1.28 mmol, 89%) was collected as an oil: R_f = 0.8 (10% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (m, 2 H), 7.55 (m, 1 H), 7.44 (m, 2 H), 5.89 (ddd, 1 H, J = 17.2, 10.4, 6.4 Hz), 5.49 (m, 1 H), 5.32 (dt, 1 H, J = 17.2, 1.2 Hz), 5.20 (dt, 1 H, J = 10.4, 1.2 Hz), 1.84−1.68 (m, 2 H), 1.46−1.25 (m, 20 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 136.8, 133.0, 130.7, 129.7, 128.4, 166.7, 75.5, 34.5, 32.1, 29.80, 29.77 (×2), 29.70, 29.64, 29.54, 29.49, 25.2, 22.8, 14.3; IR (neat) 3088, 3030, 2922, 2853, 1719, 1267 cm^{-1}. HRMS (MALDI-TOF) [M + Ag]$^+$ calcd for $C_{22}H_{34}O_2Ag$ [M + Ag]$^+$ 437.1610, found 437.1602.

(*R*)-*tert*-Butyldimethyl(pentadec-1-en-3-yloxy)silane (7c). Allylic alcohol **7a** (259 mg, 1.14 mmol, 1 equiv) was dissolved in anhydrous DMF (5 mL) under an argon atmosphere. DMAP (6 mg, 0.05 mmol, cat.) was added, and the solution was cooled to 0 °C followed by addition of *tert*-butyldimethylsilyl chloride (175 mg, 1.16 mmol, 1.02 equiv) and triethylamine (0.18 mL, 1.29 mmol, 1.1 equiv). The reaction mixture was stirred for 20 h at room temperature. The reaction was quenched with H_2O (>50 mL) and extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (0% → 2% EtOAc in pentane). The silylated product **7c** was collected as a colorless oil

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(265 mg, 0.78 mmol, 68%): R_f = 0.4 (pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddd, 1 H, J = 16.8, 10.4, 6.0 Hz), 5.12 (dt, 1 H, J = 17.2, 1.6 Hz), 5.01 (dt, 1 H, J = 10.4, 1.6 Hz), 4.07 (q, 1 H, J = 6.2 Hz), 1.58−1.40 (m, 2 H), 1.37−1.26 (m, 20 H), 0.90 (s, 9 H), 0.88 (t, 3 H, J = 6.8 Hz), 0.05 (s, 3 H), 0.03 (s, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.1, 113.5, 74.1, 38.3, 32.1, 29.85, 29.83, 29.82, 29.80, 29.79 (×2), 29.5, 26.1, 25.4, 22.9, 18.4, 14.3, −4.2, −4.7; IR (neat) 2955, 2924, 2853, 1252, 1080, 814 cm^{-1}; HRMS (MALDI-TOF) [M + Ag]$^+$ calcd for $C_{21}H_{44}OSiAg$ 447.2212, found 447.2229.

(*R*)-1-Methoxy-4-((pentadec-1-en-3-yloxy)methyl)benzene (7d). Allylic alcohol **7a** (232 mg, 1.02 mmol, 1 equiv) was dissolved in anhydrous DMF (5 mL) under an argon atmosphere. The solution was cooled to 0 °C, followed by addition of sodium hydride (60% in mineral oil, 48 mg, 2.0 mmol, 2 equiv). After the mixture was stirred for 15 min, 4-methoxybenzyl chloride (0.27 mL, 2.0 mmol, 2 equiv) was slowly added to the reaction mixture. The solution was stirred for 20 h at room temperature. The reaction was quenched by adding dropwise of NH_4Cl (satd, 20 mL). After quenching, H_2O (>50 mL) was added and extracted with Et_2O (2 × 100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (0% → 1% Et_2O in pentane). The PMB-protected product **7d** (309 mg, 0.89 mmol, 87%) was collected as a light yellow oil: R_f = 0.2 (1% Et_2O in pentane); $[\alpha]_D$ = +19.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, 2 H, J = 8.8 Hz), 6.87 (d, 2 H, J = 8.8 Hz), 5.72 (ddd, 1 H, J = 18.4, 10.4, 8.0 Hz), 5.22−5.18 (m, 1 H), 5.17−5.14 (m, 1 H), 4.52 (d, 1 H, J = 11.6 Hz), 4.28 (d, 1 H, J = 11.6 Hz), 3.80 (s, 3 H), 3.69 (q, 1 H, J = 6.8 Hz), 1.68−1.56 (m, 1 H), 1.53−1.45 (m, 1 H), 1.40−1.25 (m, 20 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.1, 139.5, 131.1, 129.5, 117.0, 113.9, 80.4, 69.8, 55.4, 35.7, 32.1, 29.83, 29.81 (×2), 29.78, 29.75, 29.70, 29.5, 25.5, 22.9, 14.3; IR (neat) 2922, 2853, 1612, 1246, 1038 cm^{-1}. HRMS (MALDI-TOF) [M + Ag]$^+$ calcd for $C_{17}H_{34}O_2Ag$ 377.1610, found 377.1598.

(*R*)-3-(Methoxymethoxy)pentadec-1-ene (7e). Allylic alcohol **7a** (229 mg, 1.01 mmol, 1 equiv) was dissolved in anhydrous DCM (6 mL) under an argon atmosphere. DMAP (16 mg, 0.13 mmol, cat.) was added, and the solution was cooled to 0 °C. DIPEA (0.87 mL, 5.0 mmol, 5 equiv) and chloromethyl methyl ether (0.34 mL, 4.5 mmol, 4.5 equiv) were added. The reaction was stirred for 20 h at room temperature. The reaction was quenched by addition of NH_4Cl (satd, 20 mL). The reaction mixture was diluted with H_2O and extracted with DCM (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (1% → 10% → 20% toluene in pentane). The MOM-protected product **7e** (256 mg, 0.95 mmol, 94%) was collected as a light yellow oil: R_f = 0.2 (20% Toluene in pentane); $[\alpha]_D$ = +50.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (ddd, 1 H, J = 17.6, 10.4, 7.6 Hz), 5.23−5.20 (m, 1 H), 5.19−5.15 (m, 1 H), 4.70 (d, 1 H, J = 6.8 Hz), 4.53 (d, 1 H, J = 6.8 Hz), 3.98 (q, 1 H, J = 6.8 Hz), 3.37 (s, 3 H), 1.68−1.58 (m, 1 H), 1.52−1.42 (m, 1 H), 1.42−1.26 (m, 20 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 117.1, 93.8, 77.5, 55.5, 35.6, 32.1, 29.82, 29.80 (×2), 29.76, 29.75, 29.70, 29.5, 25.5, 22.8, 14.3; IR (neat) 2924, 2853, 1036 cm^{-1}.HRMS (MALDI-TOF) [M + Ag]$^+$ calcd for $C_{22}H_{34}O_2Ag$ 437.1610, found 437.1602.

***tert*-Butyl ((4*R*,5*S*)-4-((*R*,*E*)-3-Hydroxypentadec-1-en-1-yl)-2-oxo-1,3-dioxan-5-yl)carbamate (8d).** Allylic alcohol **7a** (24 mg, 0.11 mmol, 1 equiv) and cyclic carbonate **6b** (73 mg, 0.30 mmol, 3 equiv) were combined and coevaporated with toluene in a 50 mL round-bottom flask. The mixture was dissolved in anhydrous Et_2O (0.5 mL) under an argon atmosphere. The solution was stirred briefly before addition of second-generation Grubbs catalyst (17 mg, 0.02 mmol, 0.2 equiv) and copper(I) iodide (6 mg, 0.03 mmol, 0.3 equiv). The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was concentrated in vacuo. The crude product was purified by silica gel chromatography (20% EtOAc → 25% EtOAc → 30% EtOAc in pentane). The metathesized product **8d** was collected as a brown oil that slowly crystallized (39 mg, 0.088 mmol, 83%): R_f = 0.4 (40% EtOAc in pentane); $[\alpha]_D$ = +24.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (dd, 1 H, J = 15.6, 4.8 Hz), 5.78 (dd, 1 H, J = 15.2, 4.8 Hz), 5.53 (d, 1 H, J = 7.2 Hz), 5.03−4.96 (m, 1 H), 4.55 (br d, 1 H, J = 9.6 Hz), 4.30 (br d, 1 H, J = 10.4 Hz), 4.22−4.15 (m, 1 H), 3.94 (br s, 1 H), 2.41 (br s, 1 H), 1.56−1.46 (m, 2 H), 1.46 (s, 9 H), 1.32−1.26 (m, 20 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.3, 147.8, 139.2, 123.8, 81.7, 80.9, 71.3, 68.4, 46.2, 37.2, 32.0, 29.76, 29.74 (×2), 29.71, 29.66, 29.61, 29.4, 28.4, 25.4, 22.8, 14.2; IR (neat) 3464, 3352, 2951, 2917, 2850, 1759, 1695, 1190, 1165 cm^{-1}; HRMS (ESI) [M − Boc + H$^+$] calcd for $C_{19}H_{36}NO_4$ 342.2639, found 342.2641.

6-Hydroxysphingosine (1). Protected 6-hydroxysphingosine **8d** (35 mg, 0.080 mmol) was dissolved in THF/H_2O (3:1, 1.5 mL) at room temperature. The solution was cooled to 0 °C before addition of lithium hydroxide monohydrate (9 mg, 0.21 mmol, 2.7 equiv). The reaction mixture was stirred for 3 h at 0 °C. The reaction mixture was acidified by addition of Amberlyst. The reaction mixture was filtrated, washed with MeOH/EtOAc, and concentrated in vacuo. The crude *tert*-butyl ((2*S*,3*R*,6*R*,*E*)-1,3,6-trihydroxyoctadec-4-en-2-yl)carbamate was collected as a solid which was used for the next reaction without further purification: R_f = 0.4 (80% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (dd, 1 H, J = 15.6, 5.2 Hz), 5.74 (dd, 1 H, J = 15.6, 5.2 Hz), 5.46 (d, 1 H, J = 8.4 Hz,), 4.33 (t, 1 H, J = 4.6 Hz), 4.11 (q, 1 H, J = 6.0 Hz), 3.88 (dd, 1 H, J = 11.2, 3.6 Hz), 3.67 (dd, 1 H, J = 11.2, 3.6 Hz), 3.63−3.56 (m, 1 H), 1.55−1.45 (m, 2 H), 1.44 (s, 9 H), 1.28−1.26 (m, 20 H), 0.88 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.6, 135.7, 129.6, 80.1, 73.6, 72.0, 62.3, 55.4, 37.4, 32.1, 29.83 (×3), 29.80 (×2), 29.76, 29.5, 28.6, 25.7, 22.8, 14.3; HRMS (ESI) [M + Na]$^+$ calcd for $C_{23}H_{45}NO_5Na$ 438.3190, found 438.3187.

The crude *tert*-butyl ((2*S*,3*R*,6*R*,*E*)-1,3,6-trihydroxyoctadec-4-en-2-yl)carbamate previously described was dissolved in DCM (2.7 mL). The solution was cooled to 0 °C before slow addition of TFA (0.3 mL). The solution was stirred for 90 min at 0 °C. The reaction mixture was diluted with toluene (~20 mL) followed by concentration in vacuo. Before complete evaporation of all solvents, the reaction mixture was diluted with toluene 2 more times (2 × 20 mL). The crude product was purified by silica gel chromatography (neutralized silica, 5% → 7.5% MeOH in $CHCl_3$). 6-Hydroxysphingosine (**1**) was collected as a waxy solid (20 mg, 0.063 mmol, 80% over two steps): R_f = 0.3 (30% MeOH in $CHCl_3$); $[\alpha]_D$ = −9.6 (c = 0.5, MeOH); ^{1}H NMR (400 MHz, MeOD) δ 5.86 (dd, 1 H, J = 15.6, 6.0 Hz), 5.67 (dd, 1 H, J = 15.6, 6.4 Hz), 4.37 (t, 1 H, J = 5.2 Hz,), 4.09 (q, 1 H, J = 6.0 Hz), 3.80 (dd, 1 H, J = 11.6, 4.0 Hz), 3.70 (dd, 1 H, J = 11.6, 8.0 Hz), 3.28−3.21 (m, 1 H), 1.58−1.48 (m, 2 H), 1.46−1.29 (m, 20 H), 0.90 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, MeOD) δ 138.3, 128.7, 72.5, 70.4, 59.3, 58.3, 38.3, 33.1, 30.77 (×2), 30.74 (×4), 30.5, 26.5, 23.7, 14.4; IR (neat) 3329, 3096, 2953, 2922, 2853, 1668, 1464, 1456, 1435, 1200, 1186, 1136 cm^{-1}; HRMS (ESI) [M + H]$^+$ calcd for $C_{18}H_{37}NO_3$ 316.2846, found 316.2847. Spectroscopic data was identical to literature.[8]

Methyl (*R*)-2-Hydroxyhexadecanoate (16a). (*R*,*E*)-2-Hydroxypent-3-enenitrile **15** (1.95 g, 20.10 mmol, 1 eq, ee >99%) was dissolved in anhydrous Et_2O (25.0 mL) in a flame-dried, three-necked, round-bottom flask under an argon atmosphere. Anhydrous MeOH (1.66 mL, 20.98 mmol, 2.0 equiv) was added. This solution was purged with dry HCl gas (1.47 g, 40.19 mmol, 2 equiv). The acidified reaction mixture was stored at −20 °C for 20 h under an argon atmosphere. H_2O (10 mL) was added and the mixture stirred for 40 min. The aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with $NaHCO_3$ (satd, 40 mL) and brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude product was purified by silica gel chromatography (10% DCM, 10% Et_2O in pentane, isocratic) to give methyl (*R*,*E*)-2-hydroxypent-3-enoate as a yellow oil (1.26 g, 9.66 mmol, 48%): R_f = 0.2 (10% DCM, 10% Et_2O in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95−5.85 (m, 1 H), 5.58−5.50 (m, 1 H), 4.61 (d, 1 H, J = 6.4 Hz), 3.80 (s, 3 H), 3.20 (br s, 1 H), 1.74 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 129.9, 127.3, 71.5, 52.8, 17.7; IR (neat) 3439, 2922, 2855, 1732, 1445, 1198 cm^{-1}. Spectroscopic data was identical to literature values.[18]

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Methyl (*R*,*E*)-2-hydroxypent-3-enoate (1.42 g, 10.94 mmol, 1 equiv) was dissolved in anhydrous DCM (6 mL) under an argon atmosphere in a 250 mL round-bottom flask. 1-Tetradecene (5.55 mL, 21.88 mmol, 2 equiv), acetic acid (63 μL, 1.09 mmol, 0.5 equiv), and second-generation Grubbs catalyst (93 mg, 0.11 mmol, 0.05 equiv) were added to the reaction mixture. The reaction mixture was stirred for 60 h at 50 °C. Afterward, the reaction mixture was concentrated in vacuo. The crude product was purified by silica gel chromatography (5% Et_2O, 10% DCM → 7.5% Et_2O, 10% DCM → 10% Et_2O, 10% DCM in pentane). The metathesized product methyl (*R*,*E*)-2-hydroxyhexadec-3-enoate was collected as a brown oil (2.27 g, 7.99 mmol, 73%): R_f = 0.2 (10% Et_2O, 10% DCM in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (dt, 1 H, *J* = 14.4, 6.8 Hz), 5.50 (dd, 1 H, *J* = 15.2, 6.0 Hz), 4.61 (t, 1 H, *J* = 4.4 Hz), 3.80 (s, 3 H), 2.80 (br s, 1 H), 2.06 (q, 2 H, *J* = 6.8 Hz), 1.45−1.35 (m, 2 H), 1.35−1.26 (m, 18 H), 0.88 (t, 3 H, *J* = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 135.3, 126.0, 71.6, 52.9, 32.3, 32.1, 29.82, 29.80, 29.79, 29.75, 29.6, 29.5, 29.3, 29.0, 22.8, 14.3; IR (neat) 3458, 2922, 2853, 1713, 1466 cm^{-1}.

Methyl (*R*,*E*)-2-hydroxyhexadec-3-enoate (1.16 g, 4.08 mmol, 1 equiv) was dissolved in EtOAc (40 mL) under an argon atmosphere. The solution was purged with argon followed by addition of palladium on carbon (10% loading, 22 mg, 0.2 mmol, 0.05 equiv). The mixture was then stirred for 30 min under a flow of hydrogen gas and was then left for 60 h under a hydrogen atmosphere. The mixture was filtered over a pad of Celite and concentrated in vacuo to give the crude product **16a** as a solid (1.07 g, 3.72 mmol, 91%): R_f = 0.2 (10% Et_2O, 10% DCM in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (dd, 1 H, *J* = 7.2, 4.4 Hz), 3.79 (s, 3 H), 2.69 (br s, 1 H), 1.85−1.76 (m, 1 H), 1.68−1.58 (m, 1 H), 1.54−1.25 (m, 24 H), 0.88 (t, 3 H, *J* = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.0, 70.6, 52.6, 34.6, 32.1, 29.84, 29.83, 29.81 (×2), 29.78, 29.70, 29.6, 29.51, 29.45, 24.9, 22.9, 14.3; IR (neat) 3462, 2920, 2853, 1736, 1460, 1215 cm^{-1}; HRMS (MALDI-TOF) $[M + Ag]^+$ calcd for $C_{17}H_{34}O_3Ag$ 393.1559, found 393.1572.

α-Hydroxy Fatty Acid (16b). Methyl ester **16a** (926 mg, 3.23 mmol, 1 equiv) was dissolved in THF/MeOH/H_2O (2:2:1, 30 mL) at room temperature. Lithium hydroxide monohydrate (420 mg, 10.0 mmol, 3.1 equiv) was added, and the reaction mixture was stirred for 20 h at room temperature. The reaction mixture was quenched by HCl (1 M, 15 mL). The aqueous mixture was extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with H_2O (100 mL) and brine, dried over $MgSO_4$, filtrated, and concentrated in vacuo. The crude α-hydroxy fatty acid **16b** was collected as a solid (873 mg, 3.20 mmol, 99%) and was used for the next step without further purification: R_f = 0.1 (30% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (dd, 1 H, *J* = 7.6, 4.4 Hz), 1.91−1.81 (m, 1 H), 1.75−1.65 (m, 1 H), 1.52−1.42 (m, 2 H), 1.38−1.26 (m, 22 H), 0.88 (t, 3 H, *J* = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.4, 70.4, 34.4, 32.1, 29.84 (×2), 29.81 (×2), 29.79, 29.71, 29.6, 29.5, 29.4, 24.9, 22.9, 14.3; IR (neat) 3445, 2920, 2851, 1732, 1466 cm^{-1}. Spectroscopic data was identical to literature values.[23]

(*R*)-2-Acetoxyhexadecanoic Acid (16c). The crude α-hydroxy fatty acid **16b** (808 mg, 2.97 mmol, 1 equiv) was dissolved in anhydrous DCM (30 mL) under an argon atmosphere at room temperature. Acetic anhydride (4.49 mL, 47.45 mmol, 16 equiv) and pyridine (7.45 mL, 92.15 mmol, 31 equiv) were added, and the reaction mixture was stirred for 20 h. The reaction was quenched by addition of $NaHCO_3$ (satd, 50 mL). The aqueous layer was extracted with $CHCl_3$ (2 × 50 mL). The combined organic layers were washed with $KHSO_4$ (0.5 M, 75 mL), dried over $MgSO_4$, filtrated, and concentrated in vacuo. The acetylated product **16c** was collected as a yellow solid (905 mg, 2.88 mmol, 97%) which was used for the next step without further purification: R_f = 0.2 (30% EtOAc in pentane); $[\alpha]_D$ = +9.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.61 (br s, 1 H), 5.00 (t, 1 H, *J* = 6.4 Hz), 2.14 (s, 3 H), 1.91−1.82 (m, 2 H), 1.46−1.38 (m, 2 H), 1.37−1.26 (m, 22 H), 0.88 (t, 3 H, *J* = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.0, 170.8, 72.0, 32.1, 31.1, 29.83 (×2), 29.80 (×2), 29.76, 29.68, 29.51, 29.49, 29.3, 25.3, 22.9, 20.7, 14.3; IR (neat) 2955, 2916, 2849, 1742, 1722, 1228 cm^{-1}. HRMS (MALDI-TOF) $[M + Ag]^+$ calcd for $C_{18}H_{34}O_4Ag$ 421.1508, found 421.1516. Spectroscopic data was identical to literature values.[23]

(*R*)-1-Oxo-1-(((2*S*,3*R*,6*R*,*E*)-1,3,6-trihydroxyoctadec-4-en-2-yl)amino)hexadecan-2-yl Acetate (17). 6-Hydroxysphingosine (**1**) (11 mg, 0.035 mmol, 1 equiv) and carboxylic acid **16c** (14 mg, 0.045 mmol, 1.3 equiv) were dissolved in anhydrous EtOH (1.5 mL) under an argon atmosphere. 2-Ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (17 mg, 0.069 mmol, 2 equiv) was added, and the reaction mixture was stirred for 20 h at 50 °C. The reaction mixture was concentrated in vacuo and purified by silica gel chromatography (0% → 1% → 3% MeOH in $CHCl_3$). Acetylated ceramide **17** was collected as a waxy white solid (14 mg, 0.023 mmol, 66%): R_f = 0.3 (5% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (d, 1 H, *J* = 8.0 Hz,), 5.84 (dd, 1 H, *J* = 15.6, 6.4 Hz), 5.73 (dd, 1 H, *J* = 15.6, 5.6 Hz), 4.99 (t, 1 H, *J* = 6.4 Hz), 4.37 (t, 1 H, *J* = 4.8 Hz), 4.13 (q, 1 H, *J* = 6.4 Hz), 3.97 (dd, 1 H, *J* = 11.2, 3.2 Hz), 3.92−3.86 (m, 1 H), 3.69 (dd, 1 H, *J* = 11.2, 3.6 Hz), 3.24 (br s, 1 H), 2.86 (br s, 1 H), 2.16 (s, 3 H), 1.88−1.78 (m, 2 H), 1.56−1.47 (m, 2 H), 1.37−1.26 (m, 44 H), 0.88 (t, 6 H, *J* = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 170.8, 136.3, 129.5, 74.8, 73.6, 72.2, 61.9, 54.2, 37.3, 32.1, 32.0, 29.85−29.81 (m, ×12), 29.73 (×2), 29.6, 29.5 (×2), 29.4, 25.6, 25.2, 22.8, 21.1, 14.3; HRMS (ESI) $[M + Na]^+$ calcd for $C_{36}H_{69}NO_6Na$ 634.5017, found 634.5011.

Ceramide (2). Acetylated ceramide **17** (11 mg, 0.018 mmol, 1 equiv) was dissolved in DCM/MeOH (4:1, 0.2 mL) under an argon atmosphere. Potassium carbonate (catalytic amount) was added, and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was acidified by addition of Amberlyst IR120, followed by filtration and concentration in vacuo. The crude product was purified by silica gel chromatography (0% → 1% → 5% MeOH in $CHCl_3$) giving ceramide (**2**) as a white solid (9 mg, 0.016 mmol, 88%): R_f = 0.5 (10% MeOH in $CHCl_3$); ^{1}H NMR (850 MHz, $CDCl_3$/MeOD, 9:1) δ 7.42 (d, 1 H, *J* = 8.5 Hz), 5.77 (dd, 1 H, *J* = 15.3, 6.0 Hz), 5.66 (dd, 1 H, *J* = 15.3, 6.0 Hz), 4.23 (t, 1 H, *J* = 5.1 Hz), 4.08−4.05 (m, 1 H), 4.03 (dd, 1 H, *J* = 8.5, 3.4 Hz), 3.87−3.83 (m, 1 H), 3.81 (dd, 1 H, *J* = 12.0, 4.3 Hz), 3.69 (dd, 1 H, *J* = 11.9, 2.6 Hz), 2.35−2.28 (m, 1 H), 2.09−2.04 (m, 1H), 2.03−1.98 (m, 1 H), 1.80 (m, 1 H), 1.58−1.44 (m, 4 H), 1.41−1.26 (m, 44 H), 0.88 (t, 6 H, *J* = 6.8 Hz); ^{13}C NMR (214 MHz, $CDCl_3$/MeOD, 9:1) δ 176.2, 135.8, 129.2, 72.6, 72.2, 71.8, 61.5, 54.6, 37.1, 34.4, 32.0, 29.75, 29.73 (×2), 29.72 (×2), 29.71 (×2), 29.70 (×2), 29.68 (×2), 29.66, 29.63, 29.5, 29.4 (×2), 25.6, 22.7, 14.1; IR (neat) 3377, 3264, 2953, 2916, 2849, 1738, 1715, 1651, 1620, 1470, 1074, 1043 cm^{-1}; HRMS (ESI) $[M + H]^+$ calcd for $C_{34}H_{68}NO_5$ 570.5092, found 570.5087.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR and ^{13}C NMR spectra of all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b00823.

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Notes

The authors declare no competing financial interest.

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