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Syntheses of sphingomyelin methylene, aza, and sulfur analogues by the versatile olefin cross-metathesis method

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ABSTRACT

The syntheses of optically homogeneous sphingomyelin analogues, which possess CH_2, NH, and S instead of the phosphate oxygen connecting the phosphocholine head group to the sphingosine backbone, were successfully achieved by employing the olefin cross-metathesis protocol between 1-pentadecene and the amino alcohol parts possessing the suitable building block or functional group for construction of the phosphocholine moiety. In addition, fluorescence-labeled sphingomyelin methylene analogue was also synthesized.

1. Introduction

Sphingolipids are regarded as lipid secondary messengers in mammalian cells and cell membranes, and are now accepted to play an important role in signal transduction and molecular recognition processes in cell membranes.[1] Among them, sphingomyelin (SM) is known as a major component that forms a raft domain, which is the lipid microdomain consisting of SM and cholesterol as the major components.[2] Generally, this particular domain is considered to play a key role in certain processes, such as membrane trafficking and signal transduction.[2c,3] However, the distinct role of SM in constructing the raft domain is still not clearly understood. It has been suggested that there exists an intramolecular hydrogen bond between the hydroxy group in the sphingosine backbone and the phosphate oxygen, which is the one connecting the phosphocholine head group to the sphingosine backbone.[4a,b] Meanwhile, the amide hydrogen has also been suggested to act as a donor of the intermolecular hydrogen bond.[4b,c] These two kinds of hydrogen bonds have been suggested to affect the phase transition temperature of bilayer membranes consisting of SM and cholesterol. The size of the phosphocholine head group in sphingolipids, which is connected by phosphate oxygen, has also been found important in the interactions with cholesterol.[5]

We then planned to synthesize three types of SM analogues, in which the phosphate oxygen was replaced with CH_2, NH, and S groups to examine their physical properties in the microdomain. Since the replacement of the phosphate oxygen is likely to affect the properties of the SM analogues, we intended to examine how these modifications affect the SM intermolecular property in addition to the SM/cholesterol interaction in monolayers and multilamellar vesicles. Meanwhile, we previously synthesized the corresponding saturated short chain analogues, which were originally designed as sphingomyelinase inhibitors.[6] However, both the double bond and the same number of the carbon chain to that of natural SM are required for understanding the property of the raft domain. The olefin cross-metathesis protocol is very attractive for providing these molecules,[7] and a number of papers on the functionalized sphingolipid synthesis were reported.[8] We also developed the olefin cross-metathesis protocol as the efficient synthesis of SM.[9] Furthermore, very recently, we have established a stereocontrolled versatile method for the synthesis of various kinds of natural sphingolipids, such as sphingosine, ceramide, sphingosine 1-phosphate, SM, and functionalized sphingosine derivatives by two types of combinations of the olefin cross-metathesis reaction. One was between the same olefin part and appropriate amino alcohols, which were prepared starting from L-serine, and the other was between the fluorescence and photoaffinity labeled olefin parts and the same amino alcohol part as shown in Figure 2.[10]

In this paper, we describe the highly efficient syntheses of the SM methylene **1**, aza **2**, and sulfur **3** analogues in addition to the synthesis of the fluorescence-labeled SM methylene analogue **4** using the versatile olefin cross-metathesis protocol as a key step (Fig. 1).

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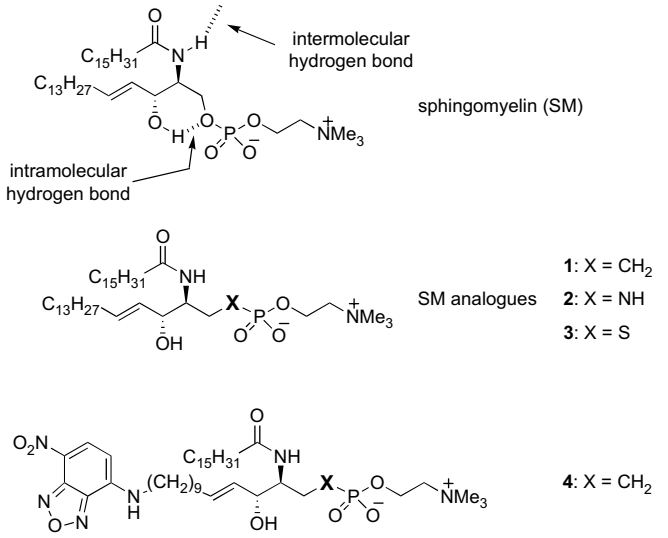


Figure 1. Sphingomyelin (SM) and SM analogues.

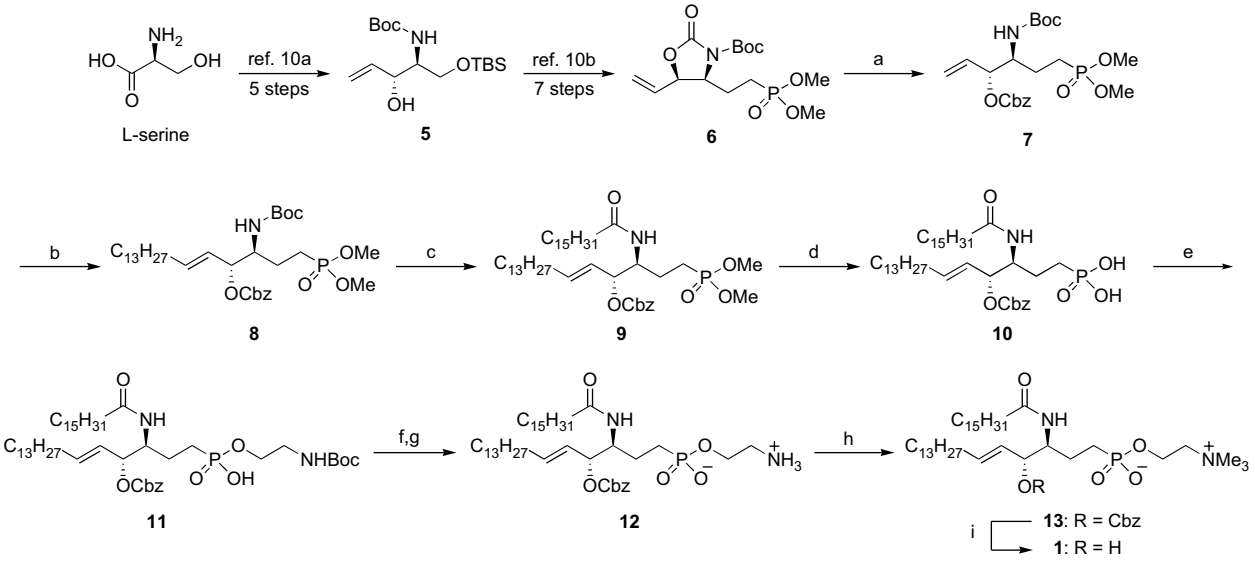
2. Results and discussion

2.1. Synthesis of sphingomyelin methylene analogue 1

We first planned to synthesize **1** in order to investigate the effect of the intramolecular hydrogen bond in the SM molecule in constructing the raft domain. The synthesis of **1** is shown in Scheme 1.

Dimethyl phosphonate **6**, a key synthetic intermediate of the SM methylene analogue **1**, was prepared by the method used for our previous synthesis of the fluorescence-labeled shingosine 1-phosphate methylene analogue.[10b] Thus, the intermediary **5**, which could be easily furnished from L-serine over five steps, was converted to **6** through the oxazolidinone formation and the replacement of the primary hydroxy group into the dimethyl phosphonylmethyl group over seven steps. The oxazolidinone ring opening of **6** was performed with benzyl alcohol in the presence of cesium carbonate[11] in THF to obtain the desired Cbz-protected amino alcohol derivative **7** in 78% yield. The olefin cross-metathesis reaction between **7** and 1-pentadecene required a rather longer reaction time to complete the reaction than the results of Figure 2, and the coupling product **8** was obtained in 68% yield after 7 h. In this case, the cis-isomer was not admitted. The Cbz protecting group at the secondary hydroxy group seemed to decrease the reactivity compared with that of the free hydroxy derivative in the olefin cross-metathesis reaction. The treatment of **8** with TFA followed by an acylation with palmitoyl chloride and K_2CO_3 produced **9**, which was transformed into the desired phosphonic acid derivative **10** by the TMSBr treatment in good yield.[12] The final part of the synthesis of **1** was the introduction of a choline group. The treatment of **10** with 3.0 equiv of *N*-Boc aminoethanol and 5.0 equiv of trichloroacetonitrile in pyridine at 65 °C successfully produced **11** in 89% yield.[6d,13] Meanwhile, the use of a large amount of the reagents afforded the undesired product, which possessed two choline moieties. The treatment of **11** with TFA to remove the Boc group gave the undesired product, which might be the benzyloxycarbonyl (Cbz) ester of the phosphonic acid resulting from the rearrangement of the Cbz group at the secondary hydroxy group. On the other hand, the Boc group was successfully removed after methylation of the phosphonic acid moiety. Thus, the successive treatment of **11** with Dowex 50W in chloroform to thoroughly acidify followed by a diazomethane treatment quantitatively produced the corresponding methyl ester, which was treated with bromotrimethylsilane and then methanol to produce the desired **12** resulting from the selective removal of both the Boc and the methyl groups rather than the 2-aminoethyl group.[14] Methylation of the primary amine in **12** with MeI and K_2CO_3 produced the desired compound **13** in 54% yield from **10**. Finally, the Cbz group of **13** was removed by hydrolysis with 2 N KOH in methanol at room temperature. The desired **1** was successfully obtained in 76% yield by neutralization of the reaction mixture with 2 N HCl, concentration without extraction, and then purification by silica gel.

Thus, we synthesized the SM methylene analogue **1**; however, very careful reaction conditions and purification procedures were required. In order to synthesize **1** more efficiently, we then tried to improve the construction method of the phosphonocholine moiety as shown in Scheme 2. The selective removal of one methyl group of the dimethyl phosphonate in **14**, which was prepared from **5** by the similar synthetic procedure to that of **6**, was successful by the



Scheme 1. (a) BnOH, Cs_2CO_3, THF, rt, 1 h, 78%; (b) 1-pentadecene, second generation Grubbs catalyst, CH_2Cl_2, reflux, 7 h, 68%; (c) TFA, CH_2Cl_2, 0 °C, 3 h, then $C_{15}H_{31}COCl$, K_2CO_3 aq, $CHCl_3$, 0 °C, 15 min, 76%; (d) TMSBr, CH_2Cl_2, rt, 2 h, then MeOH, rt, 1 h, 86%; (e) HOC_2H_4NHBoc, CCl_3CN, pyridine, 65 °C, 2 h, 89%; (f) Dowex 50W, $CHCl_3$, rt, 5 min, then CH_2N_2, $CHCl_3$, Et_2O, 0 °C, 99%; (g) TMSBr, CH_2Cl_2, 0 °C, 1 h, then MeOH, rt, 1 h; (h) MeI, K_2CO_3, $CHCl_3$, rt, 2 days, 61% for two steps; (i) 2 N KOH, MeOH, rt, 2 h, 76%.

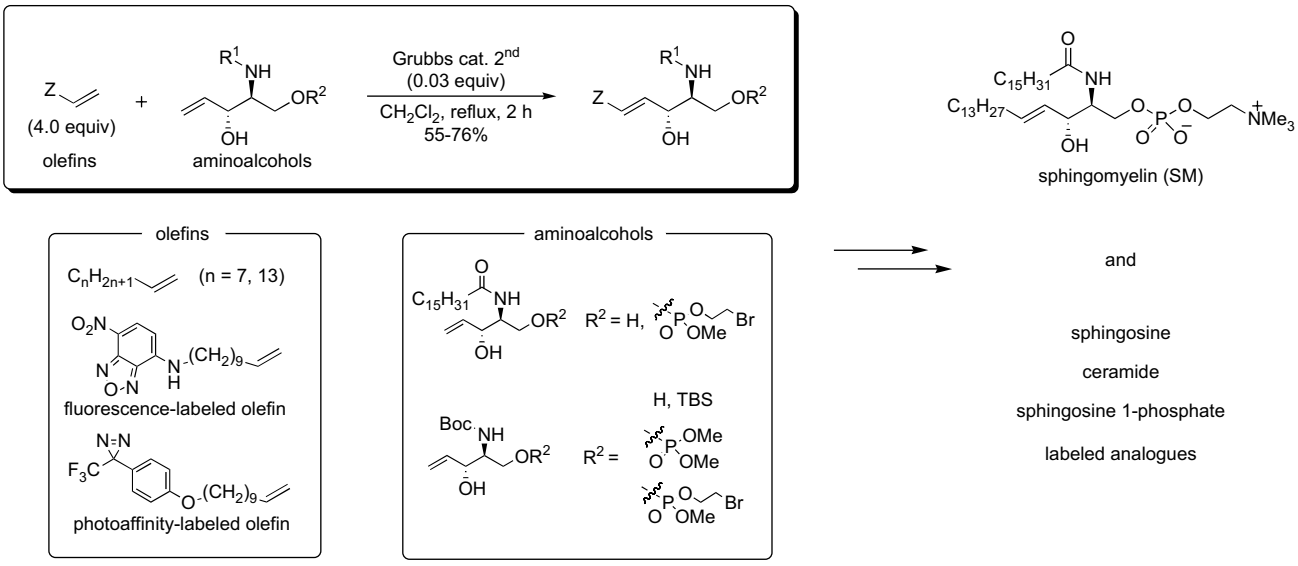
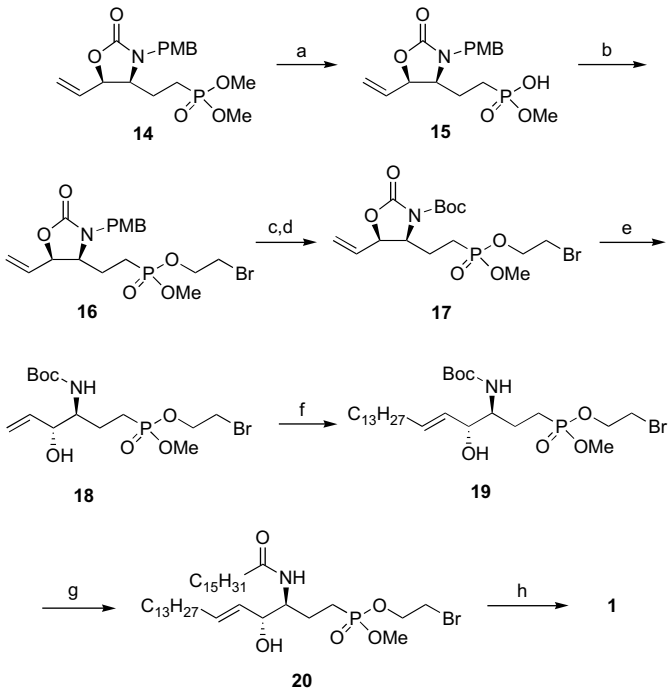


Figure 2. The established synthetic method of sphingolipids.

treatment with excess aqueous trimethylamine in methanol to afford **15** in 87% yield. Treatment of **15** with 5.0 equiv of 1,2-dibromoethane in the presence of potassium carbonate resulted in the introduction of the 2-bromoethyl group into **15** to afford **16** in 61% yield.[15,16] After the PMB group of **16** was exchanged with a Boc group, the resulting **17** was treated with methanol in the presence of cesium carbonate to give the amino alcohol **18** in 51% yield. The intermediary corresponding methylcarbonate of the secondary hydroxy group, which was detected by mass spectra, was gradually changed to **18**. In the olefin cross-metathesis between **18** and 1-pentadecene, the coupling smoothly proceeded again to produce **19** in 60% yield along with a very small amount of the corresponding cis-isomer. The treatment of the obtained **19** with TFA followed by the reaction with palmitoyl chloride produced **20** in 56% yield. The desired methylene analogue **1** was obtained in 73% yield by the treatment of **20** with trimethylamine in methanol for 2 days. Thus, the synthesis of **1** was efficiently achieved by modifying the introduction step of the phosphonocholine moiety.



Scheme 2. (a) NMe_3 aq, MeOH, rt, 1 day; (b) 1,2-dibromoethane, K_2CO_3, DMF, 80 °C, 4 h, 61%; (c) CAN, CH_3CN, H_2O, 0 °C, 15 min, 80%; (d) Boc_2O, NEt_3, DMAP, CH_2Cl_2, 0 °C, 15 min, 92%; (e) MeOH, Cs_2CO_3, THF, rt, 20 min, 51%; (f) 1-pentadecene, second generation Grubbs catalyst, CH_2Cl_2, reflux, 2 h, 60%; (g) TFA, CH_2Cl_2, 0 °C, 3 h, then $C_{15}H_{31}COCl$, K_2CO_3 aq, 0 °C, 15 min, 56%; (h) NMe_3, MeOH, rt, 2 days, 73%.

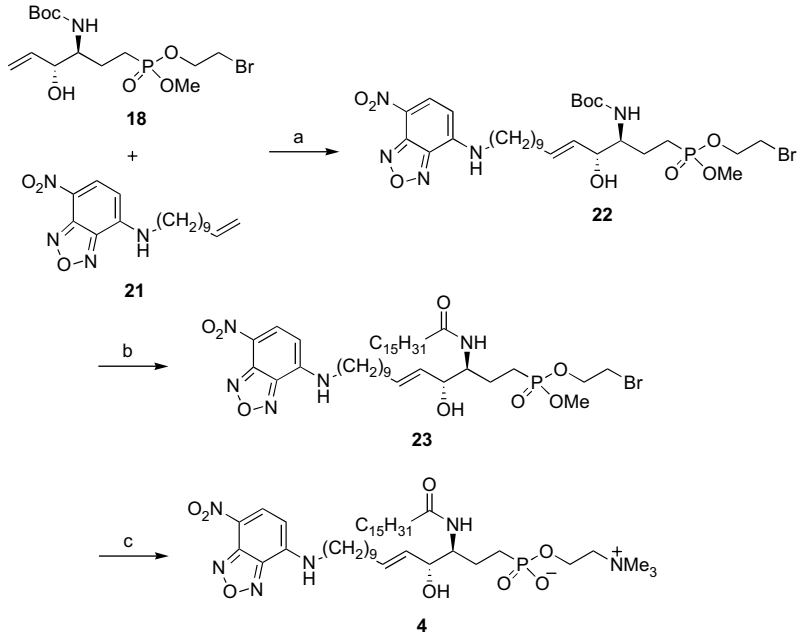
2.2. Synthesis of fluorescence-labeled analogue 4

As further demonstration, the synthetic route of **1** was applied for the synthesis of the fluorescence-labeled SM methylene analogue **4** as shown in Scheme 3. This analogue might be regarded as a second generation fluorescence-labeled one of SM, because the phosphate oxygen connecting the phosphocholine part with the backbone skeleton was replaced by a CH_2 group and hence compound **4** was not hydrolyzed at this position.[6a,d] Thus, the same amino alcohol **18** was coupled to the olefin part **21**, which possessed a fluorescent NBD (nitrobenzo-2-oxa-1,3-diazole) group at the terminus of the backbone skeleton, under the same reaction conditions used for the methylene analogue synthesis. As a result, the desired coupling product **22** was obtained in 78% yield as a mixture of 15:1 of trans- and cis-isomer. The treatment of the resulting **22** with TFA followed by acylation, and then a trimethylamine treatment easily produced the desired fluorescence-labeled methylene analogue **4** in a manner similar to that for the methylene analogue synthesis. Thus, we demonstrated the versatility of the olefin cross-metathesis strategy for the synthesis of various sphingolipids.

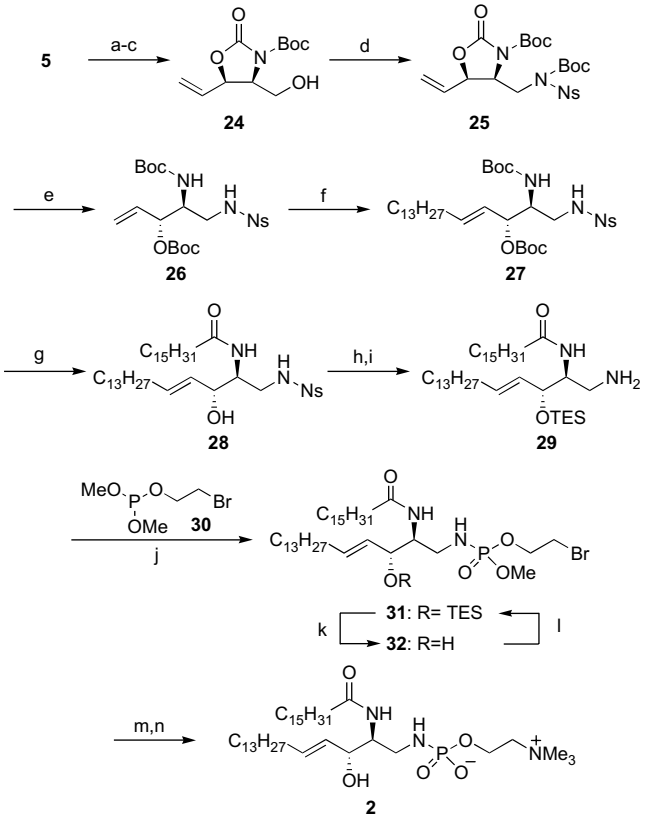
2.3. Synthesis of sphingomyelin aza analogue 2

We next planned to synthesize the aza analogue **2**, which has an NH group instead of the phosphate oxygen, from the same intermediate **5** for the SM synthesis (Scheme 4).

After several trials to introduce a nitrogen function at the terminal position of **5**, we found that the Mitsunobu reaction conditions gave the best results (Scheme 4). Thus, compound **5** was transformed into the *N*-Boc-oxazolidinone derivative **24** in three steps in 73% yield, which was then reacted with *N*-Boc-protected nosylamide in the presence of DIAD and triphenylphosphine[17] to successfully produce the nitrogen-substituted derivative **25**, although a similar Mitsunobu-type substitution reaction of the corresponding linear compound was not successful. The isolation of **25**



Scheme 3. (a) Second generation Grubbs catalyst, CH_2Cl_2, reflux, 2 h, 78%; (b) TFA, CH_2Cl_2, rt, 75 min, then $C_{15}H_{31}COCl$, K_2CO_3 aq, 0 °C, 82%; (c) NMe_3 aq, MeOH, rt, 2 days, 61%.



Scheme 4. (a) NaH, THF, 50 °C, 1 h, 93%; (b) Boc_2O, DMAP, NEt_3, CH_2Cl_2, 0 °C, 1 h; (c) 2 N HCl, MeOH, rt, 16 h, 78% for two steps; (d) BocNHNs, DIAD, PPh_3, toluene, rt, 16 h; (e) Cs_2CO_3, MeOH, rt, 1 h, 87% for two steps; (f) 1-pentadecene, second generation Grubbs catalyst, CH_2Cl_2, reflux, 7 h, 72%; (g) TFA, CH_2Cl_2, 0 °C, 3.5 h, then $C_{15}H_{31}COCl$, K_2CO_3, 0 °C, 30 min, 84%; (h) TESCl, imidazole, DMF, 0 °C, 5 min; (i) $CH_3(CH_2)_{11}SH$, NaH, DMF, 0 °C, 1 h, 89% for two steps; (j) CBr_4, 2,6-lutidine, 4 Å MS, CH_2Cl_2, 0 °C, 1.5 h; (k) Dowex 50W, MeOH, rt, 5 min, 54% for two steps; (l) TESCl, imidazole, DMF, 0 °C, 5 min, 95%; (m) NMe_3, toluene, 60 °C, h, 2 days; (n) Dowex 50W, MeOH, rt, 5 min, 30% for two steps.

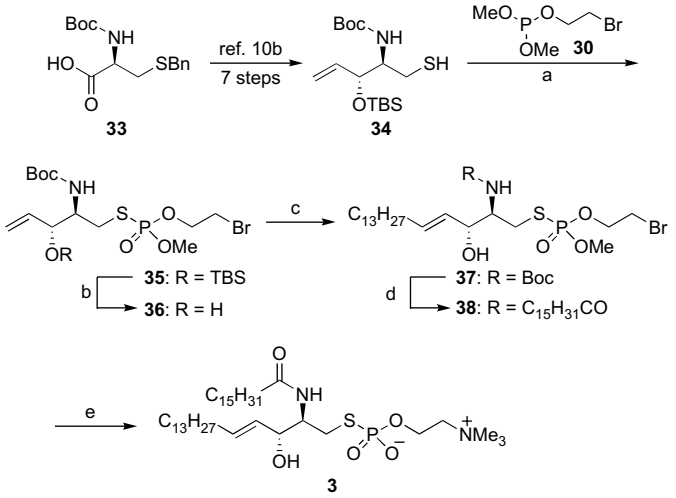
from the residue, however, was very difficult. The resulting mixture was semi-purified by column chromatography, and then treated with cesium carbonate in methanol. The 3-hydroxy-1,2-diamine derivative **26** could be isolated by careful column chromatography (2–3% of methanol in chloroform) in 87% yield for two steps.

The Boc group at the nosylamide of **25** transferred to the allylic oxygen resulting from the solvolysis of the oxazolidinone ring. Next was the key olefin cross-metathesis reaction. Compound **26** and 4.0 equiv of 1-pentadecene in the presence of 0.03 equiv of the second generation Grubbs catalyst in CH_2Cl_2 for 7 h under reflux produced the desired coupling product **27** in 72% yield. In this case, the catalyst was not deactivated until the reaction was completed, although the secondary hydroxy group was protected by a Boc group. The desired trans-isomer was exclusively obtained in usual cases of this reaction, but the cis-isomer was sometimes admitted less than 10% portion in the repeated same reactions. The activity of the used catalyst may be responsible. The treatment of **27** with TFA and then acylation produced the *N*-acyl derivative **28** in 82% yield for two steps. The hydroxy group of **28** was again protected with a TES group followed by removal of the nosyl group with an odorless dodecylmercaptane treatment[18] to afford the 3-alkoxy-1,2-diamine derivative **29** in 89% yield from **28**. The next step was the construction of the phosphocholine moiety. We applied 2-bromoethyl dimethyl phosphite (**30**),[19] which was previously used for the rapid synthesis of the natural SM.[9] Thus, compound **29** was stirred with **30** in the presence of carbon tetrabromide and catalytic amount of 2,6-lutidine to produce the desired phosphoramidate **31** along with a small amount of the residue produced from the reagent **30** in nearly 80% yield. The isolation of **31** was not successful even after very careful column chromatography. When the obtained compound **31** was reacted with trimethylamine in a sealed tube followed by Dowex 50W treatment, unfortunately the desired choline compound **2** was obtained in very low yield and the reaction was not reproducible under the reaction conditions applied for the syntheses of natural SM and methylene analogue **1**. In order to obtain **31** as a pure form, we attempted the purification of alcohol **32**, which was easily derived from **31**. The treatment of **31** with Dowex 50W in methanol gave the corresponding alcohol **32**, which was isolated by careful column chromatography. The

hydroxy group of the isolated **32** was again protected with a TES group to avoid the rearrangement of the phosphate moiety from the amino group to the secondary hydroxy group of **32** under basic condition such as the trimethylamine treatment. Thus, the pure **31** was obtained. The desired SM aza analogue **2** was finally synthesized in 30% yield by the trimethylamine treatment of **31** at 60 °C in a sealed tube for 2 days followed by the Dowex 50W treatment. This reaction condition was fortunately reproducible. Thus, we achieved the synthesis of the relatively unstable aza analogue **2** of SM.

2.4. Synthesis of sphingomyelin sulfur analogue 3

Finally, we synthesized SM sulfur analogue **3** starting from L-cystein as shown in Scheme 5. Thiol **34** corresponding to the intermediary amino alcohol **5** in the syntheses of methylene and aza analogues was derived from the commercially available *N*-Boc-*S*-Bn-L-cystein **33** over seven steps according to the previously reported procedure including monoalkylation of the corresponding Weinreb amide with a vinyl Grignard reagent, stereoselective reduction of the produced ketone, and selective protection of the resulting secondary hydroxy group.[10b] The phosphorylation of **34** smoothly proceeded to give thiophosphate **35** in 61% yield with phosphite **30** under the similar conditions to those of the SM and aza analogue **2** synthesis. The removal of the TBS group in **35** with 2 N HCl in MeOH afforded allyl alcohol **36** in 68% yield. The olefin cross-metathesis reaction between **36** and 1-pentadecene produced **37** in 74% yield as a mixture of approximately 15:1 of trans- and cis-isomer. The treatment of the obtained **37** with TFA followed by the reaction with palmitoyl chloride produced **38** in 92% yield. The synthesis of sulfur analogue **3** was achieved by the reaction of **38** with trimethylamine in methanol for 1 day in 50% yield.



Scheme 5. (a) CBr_4, 2,6-lutidine, 4 Å MS, CH_2Cl_2, 0 °C, 2 h, 61%; (b) 2 N HCl, MeOH, rt, 1 h, 68%; (c) 1-pentadecene, second generation Grubbs catalyst, CH_2Cl_2, reflux, 2 h, 74% (d) TFA, CH_2Cl_2, 0 °C, 2.5 h, then $C_{15}H_{31}COCl$, K_2CO_3, 0 °C, 15 min, 92%; (e) NMe_3, MeOH, rt, 1 day, 50%.

Thus, we accomplished the syntheses of four analogues, SM methylene **1**, aza **2**, and sulfur **3** analogues, and fluorescence-labeled SM methylene one **4** by employing the olefin cross-metathesis protocol.

2.5. The molecular properties and membrane behavior

The molecular properties and membrane behavior of the synthesized methylene (CH_2-SM) **1**, aza (NH-SM) **2**, and sulfur (S-SM) **3** analogues of SM were examined comparing with natural palmitoyl SM, by Slotte's group at åbo Academi University.[20] The results showed that the S-linkage increased and the NH- and CH_2-linkage decreased the stability of the SM-analogue bilayer membranes as compared to SM. The obtained data also showed that all compounds formed SM/cholesterol-rich domains, and the S-SM/cholesterol and SM/cholesterol domains displayed a similar thermostability, whereas the NH-SM/cholesterol and CH_2-SM/cholesterol domains were less thermostable. It was suggested from the obtained data that the properties of the bond linking the phosphocholine head group to the 1-hydroxy group on the ceramide molecule is important for the stability of the SM/SM and SM/cholesterol interactions.

3. Experimental

3.1. General

All commercially available reagents were used without further purification. All solvents were used after distillation. Tetrahydrofuran, diethyl ether, and toluene were refluxed over and distilled from sodium. Dichloromethane was refluxed over and distilled from P_2O_5. Dimethylformamide (DMF) was distilled from CaH_2. Preparative separation was usually performed by column chromatography on silica gel. ^1H and ^{13}C NMR spectra were recorded on JEOL α-400 or a JMM-ECX 400KA spectrometer and chemical shifts were represented as δ values relative to the internal standard TMS. IR spectra were recorded on a JASCO FT/IR-5300 and FT/IR-8100A Fourier Transform Infrared Spectrometer. High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-T100LC spectrometer for electrospray ionization. Optical rotations were determined using a JASCO DIP-370 polarimeter with a 100 mm cell.

3.2. Synthesis of SM methylene analogue 1

*3.2.1. (4S)-(tert-Butyldimethylsilyloxymethyl)-(5R)-vinyloxazolidin-2-one (**5a**)*

To a solution of **5** (7.304 g, 22.03 mmol) in THF (110 ml) was added sodium hydride (793 mg, 33.0 mmol) at 0 °C. After the reaction mixture was stirred at 50 °C for 1 h, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 13% to 33% ethyl acetate in hexane) gave **5a** (5.264 g, 93%) as a colorless solid. $[\alpha]_D^{20.5}$ −51.1 (*c* 1.04, $CHCl_3$); IR (KBr disk, cm^{-1}) 3252, 2932, 1775, 1391, 1258, 1103; ^1H NMR ($CDCl_3$, 400 MHz), δ 5.90 (ddd, *J*=17.2, 10.6, 6.8 Hz, 1H), 5.49 (ddd, *J*=17.2, 2.1, 1.0 Hz, 1H), 5.37 (ddd, *J*=10.6, 2.0, 1.2 Hz, 1H), 5.09 (m, 1H), 3.89 (ddd, *J*=8.1, 7.5, 4.3 Hz, 1H), 3.59 (dd, *J*=10.3, 4.3 Hz, 1H), 3.54 (dd, *J*=10.3, 7.4 Hz, 1H), 0.89 (s, 9H), 0.061 (s, 3H), 0.057 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.7, 130.3, 120.2, 78.8, 62.4, 56.9, 25.7, 18.1, −5.5; ESI-HRMS *m*/*z* calcd for $C_{12}H_{23}NO_3SiNa$ $(M+Na)^+$ 280.1345, found 280.1349.

*3.2.2. (4S)-(Hydroxymethyl)-3-(p-methoxybenzyl)-(5R)-vinyloxazolidin-2-one (**5b**)*

To a solution of **5a** (7.795 g, 30.3 mmol) in THF (150 ml) was added sodium hydride (1.820 g, 45.49 mmol) at 0 °C. After the reaction mixture was stirred at the same temperature for 30 min, *p*-methoxybenzyl chloride (6.17 ml, 45.5 mmol) and tetrabutylammonium iodide (22.40 g, 60.52 mmol) were added at the same temperature. After the reaction mixture was stirred at room temperature for 16 h, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 3.3% to 20% ethyl acetate in

hexane) gave the PMB protected oxazolidinone (10.73 g, 94%) as a colorless solid. $[\alpha]_D^{20.5}$ −4.2 (*c* 0.62, $CHCl_3$); IR (KBr disk, cm^{-1}) 2928, 1748, 1514, 1418, 1253, 839; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.23 (md, *J*=8.7 Hz, 2H), 6.87 (md, *J*=8.7 Hz, 2H), 5.99 (ddd, *J*=17.4, 10.6, 7.1 Hz, 1H), 5.42 (ddd, *J*=17.4, 1.2, 1.1 Hz, 1H), 5.35 (ddd, *J*=10.5, 1.1, 0.9 Hz, 1H), 4.90 (dddd, *J*=8.2, 7.3, 0.9, 0.9 Hz, 1H), 4.86 (d, *J*=15.1 Hz, 1H), 3.99 (d, *J*=15.1 Hz, 1H), 3.81 (s, 3H), 3.68 (m, 1H), 3.55–3.62 (m, 2H), 0.90 (s, 9H), 0.070 (s, 3H), 0.053 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 158.1, 131.5, 129.4, 128.3, 120.3, 114.1, 77.6, 59.2, 58.5, 55.3, 45.9, 25.7, 18.0, −5.7; ESI-HRMS *m*/*z* calcd for $C_{20}H_{31}NO_4SiNa$ $(M+Na)^+$ 400.1920, found 400.1908.

To a solution of the compound obtained above (10.73 g, 28.43 mmol) in methanol (150 ml) was added 2 N HCl (57 ml) at room temperature. After the reaction mixture was stirred at the same temperature for 16 h, saturated aqueous $NaHCO_3$ solution was added and the solvent was removed in vacuo. The resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 66% ethyl acetate in hexane) gave **5b** (7.477 g, quant.) as a colorless solid. $[\alpha]_D^{20.5}$ −17.8 (*c* 1.06, $CHCl_3$); IR (KBr disk, cm^{-1}) 3370, 2928, 1721, 1514, 1447, 1244, 1038; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.26 (md, *J*=8.7 Hz, 2H), 6.88 (md, *J*=8.7 Hz, 2H), 6.05 (ddd, *J*=17.2, 10.5, 6.9 Hz, 1H), 5.50 (md, *J*=17.2 Hz, 1H), 5.40 (md, *J*=10.6 Hz, 1H), 4.94 (mdd, *J*=8.0, 7.1 Hz, 1H), 4.72 (d, *J*=15.0 Hz, 1H), 4.21 (d, *J*=15.0 Hz, 1H), 3.80 (s, 3H), 3.62–3.74 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.4, 158.1, 131.4, 129.4, 128.3, 120.5, 114.3, 77.3, 59.2, 58.8, 55.3, 46.2; ESI-HRMS *m*/*z* calcd for $C_{14}H_{17}NO_4Na$ $(M+Na)^+$ 286.1055, found 286.1066.

3.2.3. Dimethyl 2-[3-(p-methoxybenzyl)-2-oxo-(5R)-vinyloxazolidin-(4S)-yl]ethylphosphonate (**14**)

To a solution of **5b** (1.80 g, 6.84 mmol) in dichloromethane (68 ml) were added 2,6-lutidine (0.95 ml, 8.17 mmol) and trifluoromethanesulfonic anhydride (1.38 ml, 8.18 mmol) at −78 °C. After the reaction mixture was stirred at the same temperature for 20 min, it was poured into saturated aqueous $NaHCO_3$ solution and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 20% to 33% ethyl acetate in hexane) gave the roughly purified corresponding triflate as a colorless solid. $[\alpha]_D^{21.5}$ +2.1 (*c* 0.52, $CHCl_3$); IR (KBr disk, cm^{-1}) 2938, 1748, 1514, 1416, 1248, 1179; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.23 (md, *J*=8.7 Hz, 2H), 6.90 (md, *J*=8.7 Hz, 2H), 5.85 (ddd, *J*=17.1, 10.8, 6.4 Hz, 1H), 5.57 (ddd, *J*=17.1, 1.2, 1.2 Hz, 1H), 5.50 (ddd, *J*=10.8, 1.2, 1.2 Hz, 1H), 5.00 (dddd, *J*=8.5, 6.4, 1.2, 1.1 Hz, 1H), 4.85 (d, *J*=15.1 Hz, 1H), 4.48 (dd, *J*=10.8, 4.8 Hz, 1H), 4.39 (dd, *J*=10.8, 4.8 Hz, 1H), 4.10 (d, *J*=15.1 Hz, 1H), 3.89 (dt, *J*=8.2, 4.6 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.7, 156.9, 129.6, 129.0, 126.9, 122.0, 118.4 (q, J_{C-P}=320.1 Hz), 71.7, 55.8, 55.3, 46.5; ESI-HRMS *m*/*z* calcd for $C_{15}H_{16}F_3NO_6SNa$ $(M+Na)^+$ 418.0548, found 418.0539.

To a solution of dimethyl methyl phosphonate (2.47 ml, 22.8 mmol) in THF (38 ml) was added 1.6 N n-butyllithium in hexane (14.24 ml, 22.8 mmol) at −78 °C, and the reaction mixture was stirred at the same temperature for 30 min to prepare the corresponding lithium anion, which was already added to a solution of the triflate obtained above in THF (30 ml) at −78 °C until the triflate was consumed. After the reaction mixture was stirred at the same temperature for 10 min, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 4% methanol in chloroform) gave **14** (1.75 g, 70%, two steps) as a colorless solid. $[\alpha]_D^{20.5}$ +25.0 (*c* 1.02, $CHCl_3$); IR (KBr disk, cm^{-1}) 2955, 1744, 1514, 1252, 1038; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.23 (md, *J*=8.7 Hz, 2H), 6.88 (md, *J*=8.7 Hz, 2H), 5.90 (ddd, *J*=17.2, 10.5, 6.8 Hz, 1H), 5.51 (ddd, *J*=17.2, 1.2, 1.1 Hz, 1H), 5.44 (mdd, *J*=10.5, 1.1 Hz, 1H), 4.88 (mdd, *J*=7.8, 7.1 Hz, 1H), 4.80 (d, *J*=15.1 Hz, 1H), 3.97 (d, *J*=15.1 Hz, 1H), 3.80 (s, 3H), 3.71 (d, *J*=11.0 Hz, 3H), 3.70 (d, *J*=10.7 Hz, 3H), 3.66 (td, *J*=7.8, 3.2 Hz, 1H), 1.75–1.95 (m, 2H), 1.59–1.68 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 157.5, 130.2, 129.4, 127.5, 121.0, 114.2, 77.7, 56.6 (d, J_{C-P}=17.7 Hz), 52.45 (d, J_{C-P}=6.8 Hz), 52.40 (d, J_{C-P}=6.8 Hz), 45.6, 20.6, 19.9 (d, J_{C-P}=140 Hz). ESI-HRMS *m*/*z* calcd for $C_{17}H_{24}NO_6PNa$ $(M+Na)^+$ 392.1239, found 392.1248.

3.2.4. Dimethyl 2-[2-oxo-(5R)-vinyloxazolidin-(4S)-yl]-ethylphosphonate (**14a**)

To a solution of **14** (2.733 g, 7.399 mmol) in acetonitrile (22 ml) and water (7.5 ml) was added ceric ammonium nitrate (12.17 g, 22.19 mmol) at 0 °C. After the reaction mixture was stirred at the same temperature for 15 min, brine was added and the resulting mixture was extracted with ethyl acetate for more than three times. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave **14a** (1.482 g, 80%) as a colorless oil. $[\alpha]_D^{20.5}$ −11.1 (*c* 1.12, $CHCl_3$); IR (neat, cm^{-1}) 3245, 2957, 1759, 1393, 1236, 1035; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41 (br m, 1H), 5.90 (ddd, *J*=17.2, 10.5, 6.9 Hz, 1H), 5.48 (mdd, *J*=17.2, 1.2 Hz, 1H), 5.41 (mdd, *J*=10.5, 0.9 Hz, 1H), 5.07 (dd, *J*=7.8, 7.4 Hz, 1H), 3.94 (m, 1H), 3.75 (d, *J*=10.8 Hz, 3H), 3.74 (d, *J*=10.7 Hz, 3H), 1.65–2.13 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.9, 130.4, 120.6, 80.2, 55.8 (d, J_{C-P}=13.4 Hz), 52.7 (d, J_{C-P}=6.2 Hz), 52.7 (d, J_{C-P}=6.3 Hz), 24.4 (d, J_{C-P}=4.8 Hz), 21.2 (d, J_{C-P}=142.8 Hz); ESI-HRMS *m*/*z* calcd for $C_9H_{16}NO_5PNa$ $(M+Na)^+$ 272.0664, found 272.0674.

3.2.5. Dimethyl 2-[3-(tert-butyloxycarbonyl)-2-oxo-(5R)-vinyloxazolidin-(4S)-yl]ethylphosphonate (**6**)

To a solution of **14a** (320 mg, 1.28 mmol) in dichloromethane (13 ml) were added triethylamine (0.36 ml, 2.57 mmol), 4-dimethylaminopyridine (78 mg, 0.64 mmol), and di-*tert*-butyldicarbonate (420 mg, 1.93 mmol) at 0 °C. After the reaction mixture was stirred at the same temperature for 20 min, saturated aqueous NH_4Cl solution and brine were added, and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 1% ethyl methanol in chloroform) gave **6** (420 mg, 94%) as a colorless oil. $[\alpha]_D^{20.5}$ +18.2 (*c* 0.73, $CHCl_3$); IR (KBr disk, cm^{-1}) 2984, 1823, 1385, 1368, 1078, 1042; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.90 (ddd, *J*=17.2, 10.8, 6.6 Hz, 1H), 5.58 (md, *J*=17.2 Hz, 1H), 5.51 (md, *J*=10.8 Hz, 1H), 5.00 (m, 1H), 4.35 (td, *J*=6.6, 4.8 Hz, 1H), 3.741 (d, *J*=10.8 Hz, 3H), 3.737 (d, *J*=10.8 Hz, 3H), 2.02–2.14 (m, 1H), 1.84–1.97 (m, 1H), 1.70–1.82 (m, 2H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.3, 149.2, 129.0, 121.9, 84.3, 77.8, 58.1 (d, J_{C-P}= 20.1 Hz), 52.5 (d, J_{C-P}=6.7 Hz), 52.46 (d, J_{C-P}=6.7 Hz), 27.9, 22.5 (d, J_{C-P}=3.8 Hz), 20.6 (d, J_{C-P}=142.8 Hz); ESI-HRMS *m*/*z* calcd for $C_{14}H_{24}NO_7PNa$ $(M+Na)^+$ 372.1188, found 372.1176.

3.2.6. Dimethyl (4R)-(benzyloxycarbonyloxy)-(3S)-(tert-butyloxycarbonylamino)hex-5-enylphosphonate (7)

To a solution of **6** (200 mg, 0.573 mmol) in THF (3 ml) were added benzyl alcohol (0.30 ml, 2.86 mmol) and cesium carbonate (933 mg, 2.86 mmol) at room temperature. After the reaction mixture was stirred at the same temperature for 1 h, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 83% ethyl acetate in hexane) gave **7** (205 mg, 78%) as a colorless

oil. $[\alpha]_D^{20.5}$ +0.755 (*c* 1.02, $CHCl_3$); IR (neat, cm^{-1}) 3409, 2976, 1750, 1711, 1368, 1262, 1036, 820; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.39 (m, 5H), 5.79 (ddd, *J*=17.2, 10.8, 6.2 Hz, 1H), 5.34 (d, *J*=17.4 Hz, 1H), 5.30 (d, *J*=11.0 Hz, 1H), 5.16 (s, 2H), 5.11–5.15 (m, 1H), 4.68 (br d, *J*=9.2 Hz, 1H), 3.74–3.86 (m, 1H), 3.72 (d, *J*=10.9 Hz, 6H), 1.56–1.96 (m, 4H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 154.3, 134.9, 132.1, 128.54, 128.47, 128.3, 128.2, 119.3, 80.2, 79.8, 69.8, 53.3 (d, J_{C-P}=16.3 Hz), 52.41 (d, J_{C-P}=6.7 Hz), 52.40 (d, J_{C-P}=6.7 Hz), 28.3, 22.7 (d, J_{C-P}=2.9 Hz), 21.4 (d, J_{C-P}=142.8 Hz); ESI-HRMS *m*/*z* calcd for $C_{21}H_{32}NNaO_8P$ $(M+Na)^+$ 480.1749, found 480.1763.

3.2.7. Dimethyl (4R)-(benzyloxycarbonyloxy)-(3S)-(tert-butyloxycarbonylamino)nonadec-(5E)-enylphosphonate (**8**)

To a solution of **7** (200 mg, 0.437 mmol) in dichloromethane (2 ml) were added 1-pentadecene (368 mg, 1.750 mmol) and second generation Grubbs catalyst (11 mg, 0.013 mmol) at room temperature. After the reaction mixture was stirred for 7 h under reflux, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 75% ethyl acetate in hexane) gave **8** (190 mg, 68%) as a colorless oil. $[\alpha]_D^{20.5}$ −8.5 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3281, 2926, 2854, 1748, 1713, 1365, 1246, 1034, 822, 754; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.33–7.38 (m, 5H), 5.81 (dt, *J*=15.6, 6.9 Hz, 1H), 5.38 (dd, *J*=15.3, 7.1 Hz, 1H), 5.15 (s, 2H), 5.06 (m, 1H), 4.61 (br d, *J*=9.8 Hz, 1H), 3.73–3.77 (m, 1H), 3.74 (d, *J*=10.8 Hz, 6H), 2.03 (dt, *J*=7.3, 6.9 Hz, 2H), 1.58–1.90 (m, 4H), 1.42 (s, 9H), 1.25 (s, 22H), 0.88 (t, *J*=6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 154.3, 137.6, 135.1, 128.55, 128.50, 128.3, 123.4, 80.6, 79.7, 69.7, 53.5 (d, J_{C-P}=18.2 Hz), 52.7 (m), 32.4, 31.9, 29.68, 29.59, 29.46, 29.36, 29.18, 28.79, 28.38, 28.31, 28.23, 23.0 (m), 22.7, 21.4 (d, J_{C-P}=142.8 Hz), 14.1; ESI-HRMS *m*/*z* calcd for $C_{34}H_{58}NNaO_8P$ $(M+Na)^+$ 662.3770, found 662.3798.

3.2.8. Dimethyl (4R)-(benzyloxycarbonyloxy)-(3S)-(1-oxohexadecylamino)nonadec-(5E)-enylphosphonate (**9**)

To a solution of **8** (180 mg, 0.282 mmol) in dichloromethane (3.0 ml) was added trifluoroacetic acid (0.56 ml) at 0 °C. After the mixture was stirred for 3 h at the same temperature, saturated aqueous K_2CO_3 solution was added and the resulting mixture was stirred for further 5 min at the same temperature. To the mixture was added palmitoyl chloride (1.00 ml, 3.27 mmol) at 0 °C. After the reaction mixture was stirred for 15 min, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 80% ethyl acetate in hexane) gave **9** (167 mg, 76%) as a colorless solid. $[\alpha]_D^{23.0}$ −6.9 (*c* 0.99, $CHCl_3$); IR (KBr disk, cm^{-1}) 3306, 2919, 2851, 1742, 1649, 1260, 1032; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.38 (m, 5H), 5.81 (dt, *J*=15.1, 6.9 Hz, 1H), 5.67 (br d, *J*=9.6 Hz, 1H), 5.38 (dd, *J*=15.6, 7.1 Hz, 1H), 5.15 (s, 2H), 5.08 (dd, *J*=4.4, 6.9 Hz, 1H), 4.18 (m, 1H), 3.72 (d, *J*=10.8 Hz, 3H), 3.71 (d, *J*=10.8 Hz, 3H), 2.14 (t, *J*=7.6 Hz, 2H), 2.03 (td, *J*=6.9, 6.9 Hz, 2H), 1.69–1.98 (m, 6H), 1.25 (s, 46H), 0.88 (t, *J*=6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 154.3, 137.4, 135.1, 128.57, 128.54, 128.30, 123.5, 80.3, 69.7, 52.4 (d, J_{C-P}=6.7 Hz), 51.7 (d, J_{C-P}=16.3 Hz), 36.8, 32.3, 31.9, 29.70, 29.66, 29.61, 29.52, 29.47, 29.39, 29.36, 29.29, 29.20, 28.83, 25.7, 22.7, 22.5 (d, J_{C-P}=4.8 Hz), 21.3 (d, J_{C-P}=142.8 Hz), 14.1; ESI-HRMS *m*/*z* calcd for $C_{45}H_{80}NNaO_7P$ $(M+Na)^+$ 800.5552, found 800.5570.

3.2.9. (4R)-(Benzyloxycarbonyloxy)-(3S)-(1-oxohexadecylamino)-nonadec-(5E)-enylphosphonic acid (**10**)

To a solution of **9** (210 mg, 0.270 mmol) in dichloromethane (2 ml) was added bromotrimethylsilane (0.36 ml, 2.70 mmol) at room temperature. After the reaction mixture was stirred for 2 h at the same temperature, the solvent was removed in vacuo. The resulting crude silylester was dissolved in methanol (2 ml) and the solution was stirred for additional 1 h at room temperature. The solvent was removed in vacuo to give the crude product. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave **10** (173 mg, 86%) as a colorless solid. $[\alpha]_D^{23.0}$ −8.8 (*c* 1.12, $CHCl_3$); IR (KBr disk, cm^{-1}) 3281, 2922, 2853, 1748, 1647, 1545, 1466, 1254, 970; ^1H NMR (CD_3OD, 400 MHz) δ 7.31–7.36 (m, 5H), 5.81 (dt, *J*=15.6, 6.6 Hz, 1H), 5.43 (dd, *J*=15.3, 7.8 Hz, 1H), 5.13 (s, 2H), 5.04 (m, 1H), 4.09 (m, 1H), 2.18 (t, *J*=7.3 Hz, 2H), 2.05 (dt, *J*=6.9, 6.6 Hz, 2H), 1.53–1.79 (m, 6H), 1.28 (s, 46H), 0.88 (t, *J*=6.9 Hz, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.6, 155.8, 138.6, 137.1, 129.6, 129.4, 129.1, 125.6, 81.5, 70.5, 58.3, 53.3 (d, J_{C-P}=18.2 Hz), 33.4, 33.1, 30.9, 30.70, 30.65, 30.60. 30.52, 30.40, 30.3, 30.0, 27.2, 25.0(d, J_{C-P}=139.9 Hz), 24.5 (d, J_{C-P}=1.9 Hz), 23.8, 14.5; ESI-HRMS *m*/*z* calcd for $C_{43}H_{75}NNaO_7P$ $(M+Na)^+$ 794.5096, found 794.5077.

3.2.10. 2-(tert-Butyloxycarbonyl)ethyl hydrogen (4R)-(benzyloxycarbonyloxy)-(3S)-1-oxohexadecylaminononadec-(5E)-enylphosphonate (**11**)

To a solution of **10** (240 mg, 0.320 mmol) in pyridine (3 ml) were added 2-(*N*-*tert*-butyloxycarbonylamino)ethanol (155 mg, 0.960 mmol) and trichloroacetonitrile (0.16 ml, 1.60 mmol) at room temperature. After the reaction mixture was stirred for 2 h at 65 °C, 2 N HCl was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave **11** (255 mg, 89%) as a colorless solid. $[\alpha]_D^{23.0}$ −19.1 (*c* 0.97, $CHCl_3$); IR (KBr disk, cm^{-1}) 3416, 2920, 2851, 1736, 1684, 1638, 1468, 1262, 1071; ^1H NMR (CD_3OD, 400 MHz) δ 7.30–7.35 (m, 5H), 5.80 (dt, *J*=15.3, 6.6 Hz, 1H), 5.44 (dd, *J*=15.6, 7.8 Hz, 1H), 5.12 (s, 2H), 5.00 (m 1H), 4.22 (m, 1H), 3.84 (m, 2H), 3.23 (m, 2H), 2.20 (t, *J*=7.3 Hz, 2H), 2.04 (dt, *J*=6.9, 6.6 Hz, 2H), 1.53–1.68 (m, 6H), 1.42 (s, 9H) 1.28 (s, 46H), 0.89 (t, *J*=6.9 Hz, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.7, 158.4, 155.8, 138.7, 137.0, 129.5, 129.4, 129.1, 125.6, 81.8, 80.2, 71.3, 70.5, 63.85, 63.8, 42.5 (d, J_{C-P}=15.7 Hz), 40.2, 37.3, 33.4, 33.1, 31.6, 30.9, 30.8, 30.74, 30.68, 30.63, 30.5, 30.3, 30.2, 30.1, 28.8, 27, 25.2, 24.5 (d, J_{C-P}=141.1 Hz), 23.8, 18.4, 14.5, 14.4; ESI-HRMS *m*/*z* calcd for $C_{50}H_{89}N_2NaO_9P$ $(M+Na)^+$ 891.6238, found 891.6277.

3.2.11. (4R)-(Benzyloxycarbonyloxy)-(3S)-(1-oxohexadecylamino)-nonadec-(5E)-enylphosphonocholine (**13**)

To a solution of **11** (170 mg, 0.190 mmol) in chloroform (2 ml) was added a small amount of Dowex 50W-X4 at room temperature. After the reaction mixture was stirred for 5 min at the same temperature, it was filtered and a diazomethane diethyl ether solution was added dropwise to the filtrate at 0 °C until the reaction mixture became yellow. A saturated aqueous NH_4Cl solution was added to the reaction mixture and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave the corresponding methyl phosphonate (170 mg, 99%) as a colorless solid. $[\alpha]_D^{23.0}$ −6.8 (*c* 1.01, $CHCl_3$); IR (KBr disk, cm^{-1}) 3360, 2919, 2851, 1738, 1688, 1649, 1260, 1032; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.33–7.38 (m, 5H), 5.81 (dt, *J*=15.3, 6.9 Hz, 1H), 5.73 (br d, *J*=9.6 Hz, 1H), 5.39 (dd, *J*=15.6, 7.1 Hz, 1H), 5.14 (s, 2H), 5.08 (m, 1H), 4.21 (m, 1H), 4.05 (m, 2H), 3.71 (dd, *J*=10.8, 3.0 Hz, 3H), 3.37 (m, 2H), 2.15 (t, *J*=7.3 Hz, 2H), 2.03 (dt, *J*=6.9, 6.6 Hz, 2H), 1.72–1.82 (m, 2H), 1.57–1.61 (m, 4H), 1.44 (s, 9H), 1.25 (s, 46H), 0.88 (t, *J*=6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 155.8, 154.3, 137.4, 135.0, 128.56, 128.3, 123.5, 80.3, 79.5, 65.1, 65.0, 53.4, 52.5, 51.7, 51.5, 32.3, 31.9, 29.70, 29.66, 29.6, 29.52, 29.46, 29.4, 29.3, 29.2, 28.9, 28.4, 22.7, 22.6 (m), 21.7 (d, J_{C-P}= 142.8 Hz), 14.1; ESI-HRMS *m*/*z* calcd for $C_{51}H_{91}N_2NaO_9P$ $(M+Na)^+$ 929.6371, found 929.6360.

To a solution of the methyl phosphonate thus obtained (44 mg, 0.049 mmol) in dichloromethane (1 ml) was added bromotrimethylsilane (0.065 ml, 0.49 mmol) at 0 °C. After the reaction mixture was stirred for 1 h at the same temperature, the solvent was removed in vacuo. The resulting crude silylester was dissolved in methanol and the solution was stirred for additional 1 h at room temperature. The solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave the roughly purified corresponding amine **12**. To a solution of the obtained **12** in chloroform (1 ml) were added methyl iodide (0.03 ml, 0.485 mmol) and potassium carbonate (67 mg, 0.485 mmol) at room temperature. After the reaction mixture was stirred for 48 h at the same temperature, precipitates were removed by filtration and the filtrate was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% methanol in chloroform to 4.3% water and 26.6% methanol in chloroform) gave **13** (41 mg, 61% for two steps) as colorless solid. IR (KBr disk, cm^{-1}) 3424, 2920, 2851, 1638, 1468, 1256, 1188, 1053, 968 cm; ^1H NMR (CD_3OD, 400 MHz) δ 7.31–7.36 (m, 5H), 5.81 (dt, J=15.6, 6.9 Hz, 1H), 5.44 (dd, J=15.6, 7.8 Hz, 1H), 5.13 (s, 2H), 5.02 (m, 1H), 4.23 (m, 2H), 4.15 (m, 1H), 3.58 (m, 2H), 3.19 (s, 9H), 2.19 (t, J=7.6 Hz, 2H), 2.05 (dt, J=6.9, 6.9 Hz, 2H), 1.51–1.68 (m, 6H), 1.28 (s, 46H), 0.89 (t, J=6.9 Hz, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.6, 155.8, 138.6, 137.1 129.6, 129.4, 129.2, 125.5, 81.8, 70.5, 67.8, 58.6 (d, J_{C-P}=4.8 Hz), 54.72, 54.68, 54.72, 37.3 (d, J_{C-P}=5.8 Hz), 33.4, 33.1, 30.8, 30.69, 30.66, 30.59, 30.52, 30.3, 30.1, 27.3, 25.1 (d, J_{C-P}=3.8 Hz), 24.6 (d, J_{C-P}=137.0 Hz), 14.5; ESI-HRMS m/z calcd for $C_{48}H_{87}N_2NaO_7P$ $(M+Na)^+$ 857.6188, found 857.6149.

*3.2.12. SM methylene analogue **1***

To a solution of **13** (20 mg, 0.0239 mmol) in methanol (1 ml) was added 2 N KOH (1 ml) at room temperature. After the reaction mixture was stirred for 2 h at the same temperature, 2 N HCl was added and the resulting mixture was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% methanol in chloroform to 14% water and 43% methanol in chloroform) gave **1** (13 mg, 76%) as a colorless form. $[\alpha]_D^{23.0}$ −12.3 (c 0.13, $CHCl_3$); IR (KBr disk, cm^{-1}) 3441, 2920, 2851, 1638, 1468, 1192, 1053, 968; ^1H NMR (CD_3OD, 400 MHz) δ 5.57 (dt, J=15.3, 6.6 Hz, 1H), 5.34 (dd, J=15.3, 7.3 Hz, 1H), 4.15 (m, 2H), 3.77 (t, J=7.0 Hz, 1H), 3.67 (m, 1H), 3.51 (m, 2H), 3.12 (s, 9H), 2.08 (t, J=7.6 Hz, 2H), 1.93 (dt, J=7.1, 6.9 Hz, 2H), 1.43–1.57 (m, 6H), 1.19 (s, 46H), 0.80 (t, J=6.9 Hz, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.3, 134.6, 131.3, 76.2, 67.9, 58.7 (d, J_{C-P}=5.8 Hz), 54.73, 54.70, 54.66, 37.4, 33.5, 33.1, 30.84, 30.77, 30.7, 30.6, 30.51, 30.47, 27.3, 25.7 (d, J_{C-P}=3.9 Hz), 24.7 (d, J_{C-P}=137.0 Hz), 23.8, 14.5; ESI-HRMS m/z calcd for $C_{40}H_{81}N_2NaO_5P$ $(M+Na)^+$ 723.8655, found 723.5781.

3.3. Improved synthesis of SM methylene analogue 1

*3.3.1. Methyl hydrogen 2-[3-(p-methoxybenzyl)-2-oxo-(5R)-vinyloxazolidin-(4S)-yl]ethylphosphate (**15**)*

To a solution of **14** (1.078 g, 2.919 mmol) in methanol (8.1 ml) was added 30% trimethylamine aqueous solution (8.1 ml) at room temperature. After the reaction mixture was stirred for 24 h at the same temperature, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave **15** (1.031 g, 87%). $[\alpha]_D^{21.5}$ +17.0 (c 1.01, $CHCl_3$); IR (KBr disk, cm^{-1}) 3409, 2951, 1744, 1514, 1441, 1246, 1181, 1069, 949, 826; ^1H NMR (CD_3OD, 400 MHz) δ 7.25 (d, J=8.5 Hz, 2H), 6.90 (d, J=8.7 Hz, 2H), 6.03 (ddd, J=17.4, 10.5, 7.4 Hz, 1H), 5.49 (d, J=17.2 Hz, 1H), 5.41 (d, J=10.6 Hz, 1H), 4.99 (dd, J=7.5, 7.5 Hz, 1H), 4.66 (d, J=15.1 Hz, 1H), 4.11 (d, J=15.1 Hz, 1H), 3.77 (s, 3H), 3.75 (m, 1H), 3.51 (s, J=10.3 Hz, 3H), 1.90 (m, 1H), 1.78 (m, 1H), 1.45 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 160.8, 160.3, 132.4, 130.3, 129.3, 121.1, 115.2, 79.9, 59.4 (d, J_{C-P}=18.2 Hz), 55.7, 51.6 (d, J_{C-P}=4.8 Hz), 46.2, 23.1 (d, J_{C-P}=2.9 Hz), 22.7 (d, J_{C-P}=138.9 Hz); ESI-HRMS m/z calcd for $C_{16}H_{22}NO_6P$ $(M-H)^-$ 354.1106, found 354.1102.

*3.3.2. 2-Bromoethyl methyl 2-[3-(p-methoxybenzyl)-2-oxo-(5R)-vinyloxazolidin-(4S)-yl]ethylphosphonate (**16**)*

To a solution of potassium carbonate (1.167 mg, 8.444 mmol) and 1,2-dibromoethane (0.36 ml, 4.222 mmol) in acetonitrile (4.2 ml) was added **15** (300 mg, 0.844 mmol) in acetonitrile (4.2 ml) dropwise at 80 °C. After the reaction mixture was stirred for 4 h at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **16** (238 mg, 61%) as a colorless oil. $[\alpha]_D^{22.0}$ +19.0 (c 0.90, $CHCl_3$); IR (neat, cm^{-1}) 3459, 2928, 1748, 1512, 1418, 1250, 1038; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.23 (d, J=8.7 Hz, 2H), 6.87 (d, J=8.4 Hz, 2H), 5.90 (ddd, J=17.2, 10.5, 7.1 Hz, 1H), 5.51 (dd, J=17.2, 0.9 Hz, 1H), 5.44 (dd, J=15.0, 0.9 Hz, 1H), 4.88 (dd, J=7.5, 7.3 Hz, 1H), 4.80 (d, J=15.1 Hz, 1H), 4.28 (m, 2H), 3.98 (dd, J=15.1, 2.3 Hz, 1H), 3.80 (s, 3H), 3.719 (d, J=11.0 Hz, 3/2H), 3.716 (d, J=10.8 Hz, 3/2H), 3.67 (dd, J=7.8, 7.7 Hz, 1/2H), 3.66 (dd, J=8.1, 7.8 Hz, 1/2H), 3.51 (dd, J=6.0, 5.7 Hz, 1H), 3.50 (dd, J=5.9, 5.7 Hz, 1H), 1.80–1.97 (m, 2H), 1.64–1.73 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.4, 157.5, 130.2, 129.4, 127.5, 121.1, 114.2, 77.7, 65.10 (d, J_{C-P}=5.8 Hz, 1/2C), 65.05 (d, J_{C-P}=5.8 Hz, 1/2C), 56.62 (d, J=18.2 Hz, 1/2C), 56.58 (d, J=18.2 Hz, 1/2C), 55.3, 52.5 (d, J=4.8 Hz, 1/2C), 52.4 (d, J_{C-P}=5.8 Hz, 1/2C), 45.6, 30.4 (d, J_{C-P}=5.8 Hz, 1/2C), 30.3 (d, J_{C-P}=5.7 Hz, 1/2C), 20.6 (d, J_{C-P}=3.8 Hz), 20.5 (d, J_{C-P}=143.7 Hz); ESI-HRMS m/z calcd for $C_{18}H_{25}BrNO_6PNa$ $(M+Na)^+$ 484.0497, found 484.0501.

*3.3.3. 2-Bromoethyl methyl 2-[3-(tert-butyloxycarbonyl)-2-oxo-(5R)-vinyloxazolidin-(4S)-yl]ethylphosphonate (**17**)*

To a solution of **16** (235 mg, 0.508 mmol) in acetonitrile (1.5 ml) was added ceric ammonium nitrate (836 mg, 1.525 mmol) at 0 °C. After the reaction mixture was stirred at the same temperature for 15 min, brine was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave the PMB-deprotected oxazolidinone (185 mg, 80%) as a colorless oil. $[\alpha]_D^{22.0}$ −21.9 (c 0.32, $CHCl_3$); IR (neat, cm^{-1}) 3254, 2957, 1753, 1392, 1233, 1017, 957, 826, 770; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.71 (br d, J=8.3 Hz, 1H), 5.88 (ddd, J=10.5, 7.4, 7.1 Hz, 1H), 5.49 (ddd, J=17.2, 1.2, 1.1 Hz, 1H), 5.41 (ddd, J=10.5, 1.1, 1.0 Hz, 1H), 5.07 (dd, J=7.6, 7.6 Hz, 1H), 4.33 (m, 2H), 3.95 (dd, J=8.5, 8.4 Hz, 1/2H), 3.94 (dd, J=8.7, 8.4 Hz, 1/2H), 3.77 (d, J=11.0 Hz, 3/2H), 3.76 (d, J=10.9 Hz, 3/2H), 3.544 (dd, J=6.0, 5.7 Hz, 1H), 3.537 (dd, J=6.0, 5.7 Hz, 1H), 1.70–1.95 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.8, 130.4, 120.7, 80.1, 65.32 (d, J_{C-P}=5.7 Hz, 1/2C), 65.26 (d, J_{C-P}=5.8 Hz, 1/2C), 55.7 (d, J_{C-P}=12.4 Hz), 52.65 (d, J_{C-P}=6.7 Hz, 1/2C), 52.62 (d, J_{C-P}=6.7 Hz, 1/2C), 30.4 (d, J_{C-P}=6.7 Hz, 1/2C), 30.3 (d, J_{C-P}=7.7 Hz, 1/2C), 24.5 (d, J_{C-P}=4.8 Hz), 21.89 (d, J_{C-P}=143.6 Hz, 1/2C), 21.87 (d, J_{C-P}=143.6 Hz, 1/2C); ESI-HRMS m/z calcd for $C_{10}H_{17}BrNO_5PNa$ $(M+Na)^+$ 363.9925, found 363.9917.

To a solution of the oxazolidinone compound thus obtained (148 mg, 0.433 mmol) in dichloromethane (2 ml) were added triethylamine (0.12 ml, 0.865 mmol), DMAP (26 mg, 0.216 mmol), and Boc_2O (113 mg, 0.519 mmol) at 0 °C. After the reaction mixture was stirred at the same temperature for 15 min, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with dichloromethane. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to

give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **17** (176 mg, 92%) as a colorless oil. $[\alpha]_D^{22.0}$ +14.1 (*c* 0.33, $CHCl_3$); IR (neat, cm^{-1}) 3459, 2975, 1813, 1721, 1368, 1252, 1074; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.894 (ddd, *J*=17.2, 10.5, 6.7 Hz, 1/2H), 5.891 (ddd, *J*=17.2, 10.5, 6.4 Hz, 1/2H), 5.57 (dt, *J*=17.2, 1.2 Hz, 1H), 5.50 (dt, *J*=10.8, 1.2 Hz, 1H), 5.00 (m, 1H), 4.35 (m, 1H), 4.31 (m, 2H), 3.75 (d, *J*=11.0 Hz, 3/2H), 3.74 (d, *J*=11.0 Hz, 3/2H), 2.01–2.14 (m, 1H), 1.88–1.99 (m, 1H), 1.76–1.86 (m, 1H), 1.54 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.2, 149.2, 128.9, 122.0, 84.4, 77.8, 65.14 (d, J_{C-P}=5.8 Hz, 1/2C), 65.11 (d, J_{C-P}=5.7 Hz, 1/2C), 58.00 (d, J_{C-P}=10.2 Hz, 1/2C), 57.97 (d, J_{C-P}=9.1 Hz, 1/2C), 52.50 (d, J_{C-P}=6.7 Hz, 1/2C), 52.47 (d, J_{C-P}=6.7 Hz, 1/2C), 30.2 (d, J_{C-P}=6.7 Hz), 27.9, 22.5 (d, J_{C-P}=3.8 Hz), 21.2 (d, J_{C-P}=142.8 Hz); ESI-HRMS *m/z* calcd for $C_{15}H_{25}BrNO_7PNa$ $(M+Na)^+$ 464.0450, found 464.0436.

3.3.4. 2-Bromoethyl methyl (3S)-(tert-butyloxycarbonylamino)-(4R)-hydroxyhex-5-enylphosphate (**18**)

To a solution of **17** (90 mg, 0.204 mmol) in methanol (1 ml) was added potassium carbonate (42 mg, 0.244 mmol) at room temperature. After the reaction mixture was stirred for 20 min at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **18** (43 mg, 51%) as a colorless oil. $[\alpha]_D^{21.5}$ −2.9 (*c* 0.44, $CHCl_3$); IR (neat, cm^{-1}) 3356, 2976, 1528, 1453, 1368, 1244, 1171, 1047; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.86 (ddd, *J*=17.2, 10.5, 5.5 Hz, 1H), 5.35 (dt, *J*=17.4, 1.4 Hz, 1H), 5.25 (dt, *J*=10.5, 1.4 Hz, 1H), 4.88 (br d, *J*=2.9 Hz, 1H), 4.31 (m, 1H), 4.23 (md, *J*=2.7 Hz, 1H), 3.75 (d, *J*=11.0 Hz, 3H), 3.69 (m, 1H), 3.53 (t, *J*=5.9 Hz, 2H), 2.66 (s, 1H), 1.77–2.00 (m, 3H), 1.58–1.67 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4, 136.9, 116.8, 79.8, 75.1, 65.0 (d, J_{C-P}=5.8 Hz), 55.3 (d, J_{C-P}=17.2 Hz), 52.4 (d, J_{C-P}=5.7 Hz), 30.22 (d, J_{C-P}=6.7 Hz, 1/2C), 30.20 (d, J_{C-P}=6.7 Hz, 1/2C), 28.3, 22.4 (m), 22.02 (d, J_{C-P}=141.9 Hz, 1/2C), 22.00 (d, J_{C-P}=141.9 Hz, 1/2C); ESI-HRMS *m/z* calcd for $C_{14}H_{27}BrNO_6PNa$ $(M+Na)^+$ 438.0657, found 438.0653.

3.3.5. 2-Bromoethyl methyl (4R)-hydroxy-(3S)-(tert-butyloxycarbonylamino)nonadec-(5E)-enylphosphonate (**19**)

To a solution of **18** (0.250 g, 0.601 mmol) in dichloromethane (3.0 ml) were added 1-pentadecene (0.65 ml, 2.40 mmol) and second generation Grubbs catalyst (15 mg, 0.018 mmol) at room temperature. After the reaction mixture was stirred for 2 h under reflux, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **19** (0.216 mg, 60%) as a brownish foam. $[\alpha]_D^{25.5}$ −0.31 (*c* 2.9, $CHCl_3$); IR (neat, cm^{-1}) 3387, 2926, 1716, 1508, 1242, 1174, 1045; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.73 (dt, *J*=15.1, 7.3 Hz, 1H), 5.45 (dd, *J*=15.6, 6.6 Hz, 1H), 5.01 (br d, *J*=8.9 Hz, 1H), 4.25–4.37 (m, 2H), 4.14 (br m, 1H), 3.75 (d, *J*=11.0 Hz, 3H), 3.57–3.65 (m, 2H), 3.53 (t, *J*=6.2 Hz, 2H), 2.98 (br m, 1H), 2.03 (td, *J*=6.9, 6.9 Hz, 2H), 1.60–1.98 (m, 6H), 1.44 (s, 9H), 1.26–1.38 (m, 22H), 0.88 (t, *J*=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.3, 134.0, 128.4, 79.5, 75.0, 65.0 (d, J_{C-P}=5.7 Hz), 55.6 (d, J_{C-P}=15.3 Hz), 52.3 (d, *J*=6.7 Hz), 32.3, 31.8, 30.2 (d, J_{C-P}=6.7 Hz), 29.6, 29.6, 29.6, 29.5, 29.3, 29.2, 29.1, 28.3, 28.2, 22.6, 22.5 (d, J_{C-P}=2.9 Hz), 22.0 (d, J_{C-P}=141.8 Hz, 1/2C), 21.9 (d, J_{C-P}=141.8 Hz, 1/2C), 14.0; ESI-HRMS *m/z* calcd for $C_{29}H_{47}BrN_5O_9PNa$ $(M+Na)^+$ 620.2692, found 620.2697.

3.3.6. 2-Bromoethyl methyl (4R)-hydroxy-(3S)-(1-oxohexadecylamino)nonadec-(5E)-enylphosphonate (**20**)

To a solution of **19** (0.324 g, 0.541 mmol) in dichloromethane (5.4 ml) was added trifluoroacetic acid (1.1 ml) at 0 °C. After the reaction mixture was stirred for 3 h at the room temperature, it was cooled to 0 °C. Saturated aqueous K_2CO_3 solution was added and the mixture was stirred for 10 min at the same temperature, and then palmitoyl chloride (0.20 ml, 0.65 mmol) was added at the same temperature. After the mixture was stirred for 15 min at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **20** (0.223 g, 56%) as a colorless solid. $[\alpha]_D^{25.5}$ −7.95 (*c* 0.4, $CHCl_3$); IR (KBr disk, cm^{-1}) 3287, 2930, 1649, 1468, 1215, 1051, 721; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.13 (dd, *J*=8.5, 7.3 Hz, 1H), 5.74 (dt, *J*=14.9, 6.8 Hz, 1H), 5.44 (dd, *J*=14.6, 6.4 Hz, 1H), 4.26–4.38 (m, 2H), 4.16–4.13 (m, 1H), 3.95–4.05 (m, 1H), 3.75 (d, *J*=11.0 Hz, 3/2H), 3.74 (d, *J*=11.0 Hz, 3/2H), 3.53 (td, *J*=6.0, 1.8 Hz, 2H), 2.20 (t, *J*=7.8 Hz, 2H), 2.04 (dt, *J*=7.1, 7.1 Hz, 2H), 1.74–1.92 (m, 6H), 1.56–1.67 (m, 2H), 1.18–1.30 (m, 48H), 0.88 (t, *J*=7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.3, 134.4, 128.1, 75.1, 65.1 (d, J_{C-P}=5.7 Hz), 54.4 (d, J_{C-P}=15.3 Hz), 52.5 (d, J_{C-P}=6.7 Hz), 36.8, 35.9, 32.4, 32.3, 31.9, 30.2 (d, J_{C-P}=3.8 Hz), 29.7, 29.7, 29.5, 29.5, 29.4, 25.8, 25.5, 22.7, 14.1; ESI-HRMS *m/z* calcd for $C_{38}H_{75}BrNO_5PNa$ $(M+Na)^+$ 758.4464, found 758.4448.

3.3.7. SM methylene analogue **1**

To a solution of **20** (0.258 g, 0.350 mmol) in methanol (1.8 ml) was added trimethylamine (1.1 ml) at room temperature. After the reaction mixture was stirred for 48 h at the same temperature, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 9% methanol in chloroform to 14% water and 43% methanol in chloroform) gave **1** (0.179 g, 73%) as a colorless foam.

3.4. Synthesis of fluorescence-labeled SM methylene analogue 4

3.4.1. 2-Bromoethyl methyl 15-(7-nitrobenzo-2-oxa-1,3-diazol-4-ylamino)-(3S)-(tert-butyloxycarbonylamino)-(4R)-hydroxypent-(5E)-enylphosphonate (**22**)

To a solution of **18** (40 mg, 0.096 mmol) in dichloromethane (2 ml) were added **21** (128 mg, 0.384 mmol) and second generation Grubbs catalyst (2.5 mg, 0.029 mmol) at room temperature. After the reaction mixture was stirred for 2 h at 40 °C, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **22** (54 mg, 78%) as a brownish foam. $[\alpha]_D^{25.0}$ −12.2 (*c* 0.30, $CHCl_3$); IR (KBr disk) 3322, 2928, 2855, 1692, 1588, 1300, 1252, 1047 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.49 (d, *J*=8.5 Hz, 1H), 6.51 (s, 1H), 6.17 (d, *J*=8.7 Hz, 1H), 5.73 (td, *J*=6.5, 16.0 Hz, 1H), 5.45 (dd, *J*=6.6, 15.6 Hz, 1H), 4.81 (br d, *J*=6.9 Hz, 1H), 4.32 (m, 2H), 4.16 (m, 1H), 3.75 (d, *J*=10.9 Hz, 3H), 3.64 (m, 1H), 3.53 (t, *J*=6.0 Hz, 2H), 3.49 (td, *J*=6.0, 7.3 Hz, 2H), 2.35 (s, 1H), 2.04 (dt, *J*=7.1, 7.1 Hz, 2H), 1.81 (tt, *J*=7.3, 7.6 Hz, 2H), 1.77–1.96 (m, 3H), 1.58–1.69 (m, 1H), 1.44 (s, 9H), 1.24–1.38 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.3, 156.4, 144.3, 144.0, 143.9, 136.5, 134.2, 128.4, 98.5, 79.8, 75.2, 65.1 (d, J_{C-P}=6.8 Hz), 55.6 (d, J_{C-P}=16.3 Hz), 52.4 (d, J_{C-P}=6.7 Hz), 44.0, 32.2, 30.3 (d, J_{C-P}=5.7 Hz), 29.23, 29.18, 29.1, 29.9 (d, J_{C-P}=2.9 Hz), 28.4, 28.3, 26.9, 22.6, 22.0 (d, J_{C-P}=141.8 Hz); ESI-HRMS *m/z* calcd for $C_{29}H_{47}BrN_5O_9PNa$ $(M+Na)^+$ 742.2208, found 742.2192.

3.4.2. 2-Bromoethyl methyl 15-(7-nitrobenzo-2-oxa-1,3-diazol-4-ylamino)-(4R)-hydroxy-(3S)-(1-oxohexadecylamino)pentadec-(5E)-enylphosphonate (**23**)

To a solution of **22** (33 mg, 0.046 mmol) in dichloromethane (0.5 ml) was added trifluoroacetic acid (0.09 ml) at 0 °C. After the reaction mixture was stirred for 2 h at the same temperature, it was warmed to room temperature and then stirred for another 1 h at

the same temperature. Saturated aqueous $NaHCO_3$ solution was added at 0 °C and the mixture was stirred for 5 min at the same temperature, and then palmitoyl chloride (0.017 ml, 0.055 mmol) was added at the same temperature. After the reaction mixture was stirred for 15 min, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with dichloromethane. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **23** (32 mg, 82%) as a brownish foam. $[\alpha]_D^{23.0}$ −8.7 (*c* 0.38, $CHCl_3$); IR (neat, cm^{-1}) 3285, 2924, 2853, 1588, 1300, 1046; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.50 (d, *J*=8.4 Hz, 1H), 6.63 (m, 1H), 6.17 (d, *J*=8.7 Hz, 1H), 6.08 (m, 1H), 5.72 (dt, *J*=15.3, 6.7 Hz, 1H), 5.45 (dd, *J*=15.6, 6.6 Hz, 1H), 4.32 (m, 2H), 4.16 (m, 1H), 4.01 (mdd, *J*=9.4, 9.3 Hz, 1H), 3.748 (d, *J*=11.0 Hz, 3/2H), 3.746 (d, *J*=11.0 Hz, 3/2H), 3.53 (t, *J*=6.2 Hz, 2H), 3.48 (dt, *J*=6.7, 6.2 Hz, 2H), 2.84 (br d, *J*=4.1 Hz, 1H), 2.21 (t, *J*=7.5 Hz, 2H), 2.03 (dt, *J*=6.9, 6.4 Hz, 2H), 1.81 (tt, *J*=7.8, 7.5 Hz, 2H), 1.69–1.96 (m, 4H), 1.62 (tt, *J*=6.6, 6.4 Hz, 2H), 1.46 (m, 2H), 1.24–1.37 (m, 34H), 0.87 (t, *J*=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 144.3, 144.0, 143.9, 136.6, 134.3, 128.4, 123.7, 98.4, 75.2, 65.14 (d, J_{C-P}=5.7 Hz, 1/2C), 65.11 (d, J_{C-P}=6.7 Hz, 1/2C), 54.5 (d, J_{C-P}=4.4 Hz), 52.5 (d, J_{C-P}=7.7 Hz), 44.0, 36.8, 32.2, 31.9, 30.3 (d, J_{C-P}=6.7 Hz), 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 28.4, 26.8, 25.8, 22.7, 22.0 (d, J_{C-P}=1.9 Hz), 21.9 (d, J_{C-P}=145.6 Hz), 14.1; ESI-HRMS *m*/*z* calcd for $C_{40}H_{69}BrN_5O_8PNa$ $(M+Na)^+$ 880.3965, found 880.3957.

3.4.3. *Fluorescence-labeled SM methylene analogue* ***4***

To a solution of **23** (0.186 g, 0.217 mmol) in methanol (2 ml) was added trimethylamine (0.6 ml) at room temperature. After the reaction mixture was stirred for 48 h at the same temperature, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 9% methanol in chloroform to 14% water and 43% methanol in chloroform) gave **4** (0.109 g, 61%) as a brownish foam. $[\alpha]_D^{25.5}$ −7.3 (*c* 0.4, MeOH); IR (KBr disk, cm^{-1}) 3289, 2916, 2849, 1489, 1327, 1184, 968; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.49 (d, *J*=8.7 Hz, 1H), 6.63 (d, *J*=8.9 Hz, 1H), 5.67 (dt, *J*=15.3, 7.1 Hz, 1H), 5.44 (dd, *J*=15.3, 7.1 Hz, 1H), 4.29 (br m, 2H), 3.89 (dd *J*=6.9, 7.1 Hz, 1H), 3.76–3.80 (m, 1H), 3.64–3.67 (m, 2H), 3.43–3.56 (m, 2H), 3.23 (s, 9H), 2.18 (t, *J*=7.6 Hz, 2H), 2.01 (dt, *J*=6.6, 6.7 Hz, 2H), 1.77 (tt, *J*=7.3, 7.3 Hz, 2H), 1.24–1.67 (m, 42H), 0.88 (t, *J*=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.2, 146.6, 145.8, 145.5, 138.6, 134.4, 131.3, 122.8, 99.6, 76.1, 67.8, 58.7 (d, J_{C-P}=4.8 Hz), 57.1, 56.1, 54.78, 54.75, 54.71, 44.8, 37.4, 33.4, 33.1, 30.8, 30.75, 30.66, 30.61, 30.57, 30.47, 30.43, 30.35, 29.3, 28.1, 27.3, 25.6 (d, J_{C-P}=2.9 Hz), 24.6 (d, J_{C-P}=137.0 Hz), 23.7, 14.5; ESI-HRMS *m*/*z* calcd for $C_{42}H_{75}N_6O_8PNa$ $(M+Na)^+$ 845.5282, found 845.5915.

3.5. Synthesis of SM aza analogue 2

3.5.1. *(4S)-(Hydroxymethyl)-3-(tert-butyloxycarbonyl)-(5R)-vinyloxazolidinone (****24****)*

To a solution of **5a** (2.734 g, 10.62 mmol) in dichloromethane (53.1 ml) were added DMAP (0.649 g, 5.31 mmol), triethylamine (2.96 ml, 231.2 mmol), and Boc_2O (2.92 ml, 15.9 mmol) at 0 °C. After the reaction mixture was stirred for 1 h at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 5% to 16% ethyl acetate in hexane) gave the Boc-protected oxazolidinone (3.536 g, 93%) as a colorless solid. $[\alpha]_D^{24.0}$ +27.9 (*c* 0.9, $CHCl_3$); IR (KBr disk, cm^{-1}) 2955, 1792, 1373, 1089, 835; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.12 (ddd, *J*=18.1, 10.3, 7.7 Hz, 1H), 5.48 (ddd, *J*=18.5, 1.1, 1.1 Hz, 1H), 5.44 (ddd, *J*=10.5, 0.9, 0.9 Hz, 1H), 4.94 (dd, *J*=7.8, 7.8 Hz, 1H), 4.19 (ddd, *J*=7.6, 3.2, 1.4 Hz, 1H), 3.99 (dd, *J*=11.0, 3.4 Hz, 1H), 3.71 (dd, *J*=11.0, 1.1 Hz, 1H), 1.55 (s, 9H), 0.88 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.6, 149.4, 130.8, 121.9, 83.6, 77.9, 59.5, 59.2, 28.0, 25.6, 17.9, −5.7; ESI-HRMS *m*/*z* calcd for $C_{17}H_{51}NO_5SiNa$ $(M+Na)^+$ 380.1869, found 380.1850.

To a solution of the obtained oxazolidinone derivative (3.000 g, 8.391 mmol) in methanol (52 ml) was added 1 N HCl (25.2 ml) at room temperature. After the reaction mixture was stirred for 16 h at the same temperature, saturated aqueous $NaHCO_3$ solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 83% ethyl acetate in hexane) gave **24** (1.718 g, 84%) as a colorless solid. $[\alpha]_D^{24.0}$ +28.8 (*c* 1.01, $CHCl_3$); IR (KBr disk, cm^{-1}) 3483, 2982, 1788, 1381, 1091, 779; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.08 (ddd, *J*=17.4, 10.5, 6.9 Hz, 1H), 5.57 (ddd, *J*=17.1, 1.1, 1.1 Hz, 1H), 5.48 (ddd, *J*=10.5, 0.9, 0.9 Hz, 1H), 5.03 (dddd, *J*=8.0, 6.9, 1.1, 1.1 Hz, 1H), 4.31 (ddd, *J*=7.6, 3.7, 3.7 Hz, 1H), 3.96 (ddd, *J*=12.1, 6.6, 4.1 Hz, 1H), 3.78 (ddd, *J*=12.1, 5.5, 3.4 Hz, 1H), 2.19 (dd, *J*=6.6, 5.5 Hz, 1H), 1.56 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.8, 149.7, 130.1, 121.6, 84.3, 78.8, 59.7, 59.6, 27.9; ESI-HRMS *m*/*z* calcd for $C_{11}H_{17}NO_5Na$ $(M+Na)^+$ 266.1004, found 266.1003.

3.5.2. *N-[(3R)-(tert-Butyloxycabonyloxy)-(2S)-(tert-butyloxycabonylamino)pent-4-enyl]-4-nitrobenzenesulfonamide (****26****)*

To a solution of **24** (0.500 g, 2.06 mmol) in toluene (10.3 ml) were added *N*-Boc nosylamide (0.934 g, 3.09 mmol), triphenylphosphine (0.810 g, 3.09 mmol), and DIAD (0.60 ml, 3.09 mmol) at room temperature. After the reaction mixture was stirred for 16 h at the same temperature, it was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 5% to 50% ethyl acetate in hexane) gave the roughly purified **25**. To a solution of **25** in methanol (4.1 ml) was added cesium carbonate (0.805 g, 2.47 mmol) at room temperature. After the reaction mixture was stirred for 1 h at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 2% to 3% methanol in chloroform) gave **26** (0.903 g, 87%, two steps) as a colorless solid. $[\alpha]_D^{24.0}$ −2.9 (*c* 1.04, $CHCl_3$); IR (KBr disk, cm^{-1}) 3371, 2980, 1695, 1348, 1159, 856; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.33–8.38 (m, 2H), 8.01–8.06 (m, 2H), 5.73 (ddd, *J*=16.9, 10.5, 6.0 Hz, 1H), 5.62 (m, 1H), 5.35 (ddd, *J*=12.6, 2.3, 1.1 Hz, 1H), 5.31 (ddd, *J*=6.2, 2.3, 1.1 Hz, 1H), 5.07 (dddd, *J*=6.6, 4.8, 1.4, 1.4 Hz, 1H), 4.87–4.89 (m, 1H), 3.80–3.86 (m, 1H), 3.30 (ddd, *J*=11.0, 7.3, 3.7 Hz, 1H), 3.01–3.07 (m, 1H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 152.6, 150.0, 145.8, 132.0, 128.42, 124.4, 119.6, 83.4, 80.7, 77.1, 52.3, 43.8, 28.3, 27.7; ESI-HRMS *m*/*z* calcd for $C_{21}H_{51}N_3O_9SNa$ $(M+Na)^+$ 524.1679, found 524.1674.

3.5.3. *N-[(3R)-(tert-Butyloxycabonyloxy)-(2S)-(tert-butyloxycabonylamino)octadec-(4E)-enyl]-4-nitrobenzenesulfonamide (****27****)*

To a solution of **26** (1.000 g, 1.990 mmol) in dichloromethane (20 ml) were added 1-pentadecene (2.15 ml, 7.980 mmol) and second generation Grubbs catalyst (0.051 g, 0.060 mmol). After the reaction mixture was stirred for 7 h under reflux, it was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 3% to 20% ethyl acetate in hexane) gave **27** (0.983 g, 72%) as a colorless solid. $[\alpha]_D^{23.5}$ −14.5 (*c* 1.12, $CHCl_3$); IR (KBr disk, cm^{-1}) 3373, 2924, 1693, 1342, 1159, 734; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.34–8.36 (m, 2H), 8.03–8.05 (m, 2H), 5.78 (td, *J*=14.4, 6.6 Hz, 1H), 5.66 (m, 1H), 5.32 (dd, *J*=15.3, 7.3 Hz, 1H), 4.97 (dd, *J*=6.4, 6.4 Hz, 1H), 4.83 (m, 1H), 3.77–3.79 (m, 1H), 3.29 (ddd, *J*=13.0, 7.6, 3.7 Hz, 1H), 2.98–3.04 (m, 1H), 2.01 (dt, *J*=6.6, 6.6 Hz,

2H), 1.44 (s, 9H), 1.44 (s, 9H), 1.21–1.36 (m, 2H), 1.26 (s, 20H), 0.88 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 152.7, 150.0, 145.9, 137.9, 128.2, 124.4, 123.4, 83.1, 80.5, 52.5, 44.0, 32.3, 31.9, 29.7, 29.6, 29.4, 29.3, 29.1, 28.7, 28.3, 27.7, 22.7, 14.1; ESI-HRMS m/z calcd for $C_{34}H_{57}N_3O_9SNa$ $(M+Na)^+$ 706.3713, found 706.3681.

3.5.4. *N-[(3R)-Hydroxy-(2S)-1-(oxohexadecylamino)octadec-(4E)-enyl]-4-nitrobenzenesulfonamide (**28**)*

To a solution of **27** (2.031 g, 2.970 mmol) in dichloromethane (15.0 ml) was added dropwise trifluoroacetic acid (6.00 ml) at 0 °C. After the reacting mixture was stirred for 3.5 h at the same temperature, it was poured into a mixture of saturated aqueous $NaHCO_3$ solution and chloroform at 0 °C, and the resulting mixture was stirred for another 5 min at the same temperature. To this mixture were added potassium carbonate (4.105 g, 29.70 mmol) and palmitoyl chloride (1.00 ml, 3.267 mmol) at 0 °C, and the resulting mixture was stirred for 30 min. Saturated aqueous NH_4Cl solution was added and the mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 2% methanol in chloroform) gave **28** (1.800 g, 84%) as a colorless solid. $[\alpha]_D^{24.0}$ −13.0 (c 0.97, $CHCl_3$); IR (KBr disk, cm^{-1}) 3294, 2920, 1649, 1350, 1161, 738; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33–8.37 (m, 2H), 8.01–8.05 (m, 2H), 6.06 (d, J=8.0 Hz, 1H), 5.98 (dd, J=6.6, 5.3 Hz, 1H), 5.73 (dtd, J=14.6, 6.6, 0.9 Hz, 1H), 5.38 (tdd, J=15.3, 6.4, 0.9 Hz, 1H), 4.23 (dd, J=5.5, 5.5 Hz, 1H), 3.91–3.96 (m, 1H), 3.27 (ddd, J=13.0, 7.6, 3.7 Hz, 1H), 3.16 (ddd, J=13.0, 6.4, 5.0 Hz, 1H), 2.18 (t, J=7.1 Hz, 2H), 2.00 (dt, J=7.1, 7.1 Hz, 2H), 1.62 (m, 2H), 1.20–1.36 (m, 48H), 0.88 (t, J=6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 150.0, 145.9, 135.2, 128.2, 127.9, 124.3, 77.2, 73.6, 52.6, 43.7, 36.8, 32.3, 31.9, 29.7, 29.7, 29.5, 29.5, 29.4, 29.3, 29.3, 29.1, 25.7, 22.7, 14.1; ESI-HRMS m/z calcd for $C_{40}H_{71}N_3O_6SNa$ $(M+Na)^+$ 744.4961, found 744.4931.

3.5.5. *N-[1-Amino-(3R)-triethylsilyloxyoctadec-(4E)-en-(2S)-yl]hexadecanamide (**29**)*

To a solution of **28** (0.500 g, 0.693 mmol) in DMF (4.0 ml) were added imidazole (0.283 g, 4.158 mmol) and chlorotriethylsilane (0.29 ml, 1.663 mmol) at room temperature. After the reaction mixture was stirred for 5 min at the same temperature, saturated aqueous $NaHCO_3$ solution was added and the resulting mixture was extracted with diethyl ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 5% to 16% ethyl acetate in hexane containing 3% triethylamine) gave the corresponding TES product (0.573 g, 99%) as a colorless oil. $[\alpha]_D^{24.0}$ −16.7 (c 1.08, $CHCl_3$); IR (neat, cm^{-1}) 3287, 2924, 1649, 1348, 1167, 736; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.34–8.37 (m, 2H), 8.00–8.04 (m, 2H), 6.11 (m, 1H), 5.96 (d, J=7.8 Hz, 1H), 5.62 (dtd, J=14.9, 6.9, 0.9 Hz, 1H), 5.23 (tdd, J=15.6, 6.6, 1.4 Hz, 1H), 4.27 (dd, J=6.0, 3.4 Hz, 1H), 3.82–3.88 (m, 1H), 3.25 (dd, J=12.6, 3.4 Hz, 1H), 3.11 (dd, J=12.6, 5.7 Hz, 1H), 2.15 (dt, J=7.3, 2.7 Hz, 2H), 2.00 (dt, J=7.1, 7.1 Hz, 2H), 1.61 (tt, J=7.3, 7.3 Hz, 2H), 1.20–1.33 (m, 48H), 0.94 (t, J=7.8 Hz, 9H), 0.88 (t, J=6.6 Hz, 6H), 0.59 (q, J=7.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 149.9, 145.9, 134.2, 128.3, 128.2, 124.2, 77.2, 75.0, 52.5, 46.1, 43.7, 36.7, 32.1, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.3, 29.2, 29.0, 25.5, 22.7, 14.1, 6.7, 4.8; ESI-HRMS m/z calcd for $C_{46}H_{85}N_3O_6SSiNa$ $(M+Na)^+$ 858.5826, found 858.5805.

To a solution of the TES product obtained above (0.452 g, 0.540 mmol) in DMF (10.8 ml) were added dodecylmercaptane (1.08 ml, 1.081 mmol) and 60% sodium hydride (0.058 g, 2.430 mmol) at 0 °C. After the reaction mixture was stirred for 1 h at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with diethyl ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 9% methanol in chloroform) gave **29** (0.317 g, 90%) as a colorless oil. $[\alpha]_D^{24.0}$ −13.0 (c 1.0, $CHCl_3$); IR (KBr disk, cm^{-1}) 3298, 2932, 1639, 1468, 1074, 744; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.21 (d, J=8.0 Hz, 1H), 5.67 (td, J=15.3, 6.9 Hz, 1H), 5.42 (dd, J=15.3, 6.6 Hz, 1H), 4.28 (dd, J=6.2, 3.9 Hz, 1H), 3.83–3.89 (m, 1H), 2.97 (dd, J=13.0, 5.7 Hz, 1H), 2.81 (dd, J=13.0, 4.3 Hz, 1H), 2.20 (dt, J=10.8, 2.7 Hz, 2H), 2.02 (dt, J=6.9, 6.9 Hz, 2H), 1.63 (tt, J=7.1, 7.1 Hz, 2H), 1.22–1.35 (m, 48H), 0.94 (t, J=7.8 Hz, 9H), 0.88 (t, J=6.6 Hz, 6H), 0.58 (q, J=7.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 133.2, 129.7, 75.2, 54.8, 40.9, 37.0, 32.2, 31.9, 29.7, 29.7, 29.5, 29.4, 29.4, 29.4, 29.2, 29.1, 25.8, 22.7, 14.1, 6.9, 4.9; ESI-HRMS m/z calcd for $C_{40}H_{82}N_2O_2Si$ $(M+H)^+$ 651.6224, found 651.6238.

3.5.6. *2-Bromoethyl dimethyl phosphite (**30**)*

To a solution of methyl dichlorophosphite (5.0 ml, 55.0 mmol) in THF (275 ml) were added diisopropylethylamine (28.7 ml, 165 mmol) and 2-bromoethanol (3.90 ml, 55.0 mmol) at −78 °C. After the reaction mixture was stirred for 1 h, methanol (2.23 ml, 55.0 mmol) was added to the reaction mixture at −78 °C. After the reaction mixture was stirred for 30 min at the same temperature, it was slowly warmed to room temperature. Diethyl ether was added and the precipitate was removed by filtration through Celite, and then the filtrate was concentrated in vacuo. The residue was distilled under reduced pressure (bp 65 °C at 7 mmHg) to give **30** (7.57 g, 64%) as a colorless liquid. IR (neat, cm^{-1}) 2948, 2838, 1456, 1287, 1181, 1005; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.10 (dt, J=7.6, 6.6 Hz, 2H), 3.55 (d, J=10.7 Hz, 6H), 3.49 (t, J=6.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 62.0 (J_{C-P}=11.6 Hz), 49.3 (J_{C-P}=10.8 Hz), 31.2 (J_{C-P}=4.1 Hz); ^{31}P NMR ($CDCl_3$, 300 MHz) δ 141.2.

3.5.7. *2-Bromoethyl methyl [(3R)-hydroxy-(2S)-(1-oxohexadecylamino)octadec-(4E)-enyl]phosphoramidate (**32**)*

To a mixture of 4 Å molecular sieves (0.300 g) and carbon tetrabromide (0.612 g, 1.844 mmol) in dichloromethane (3.0 ml) was added 2-bromoethyl dimethyl phosphite **30** (0.28 ml, 1.844 mmol) at 0 °C. After the reaction mixture was stirred for 20 min, a solution of **29** (0.300 g, 0.461 mmol) in dichloromethane and 2,6-lutidine (0.02 ml, 0.184 mmol) were added to the reaction mixture at the same temperature. After the reaction mixture was stirred for 1.5 h at the same temperature, chloroform was added and the precipitate was removed by filtration. Saturated aqueous $NaHCO_3$ solution was added and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% to 66% ethyl acetate in hexane containing 3% triethylamine) gave the roughly purified **31**. To a solution of **31** obtained above in methanol (5 ml) was added Dowex 50W (pH=5) at room temperature. After the reaction mixture was stirred for 5 min at the same temperature, methanol was added and the precipitate was removed by filtration. The filtrate was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 0% to 3% methanol in chloroform) gave **32** (0.185 g, 54%, two steps) as a colorless solid. $[\alpha]_D^{24.0}$ −9.3 (c 0.79, $CHCl_3$); IR (KBr disk, cm^{-1}) 3277, 2953, 1713, 1643, 1545, 1466, 1364; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.45 (br d, J=6.4 Hz, 1H), 5.75 (dt, J=15.3, 6.6 Hz, 1H), 5.47 (dd, J=15.1, 6.6 Hz, 1H), 4.27 (m, 2H), 4.20 (m, 1H), 3.96 (m, 1H), 3.74 (d, J=11.8 Hz, 3/2H), 3.73 (d, J=11.2 Hz, 3/2H), 3.54 (dt, J=6.2, 2.3 Hz, 2H), 3.08–3.26 (m, 3H), 2.21 (t, J=7.5 Hz, 2H), 2.04 (dt, J=7.1, 7.1 Hz, 2H), 1.63 (tt, J=7.3, 7.3 Hz, 2H), 1.26–1.36 (m, 48H), 0.88 (t, J=6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.1, 134.2, 128.6, 73.8, 65.9 (d, J_{C-P}=4.8 Hz, 1/2C), 65.8 (d, J_{C-P}=5.7 Hz, 1/2C), 54.3 (1/2C), 54.2 (1/2C), 53.55 (d, J_{C-P}=5.7 Hz, 1/2C), 53.52 (d, J_{C-P}=5.7 Hz, 1/2C), 41.3, 36.8, 32.4, 31.9, 29.70, 29.66, 29.56, 29.55, 29.45, 29.40, 29.36, 29.31, 29.2, 25.8, 22.7, 14.1; ESI-HRMS m/z calcd for $C_{37}H_{74}BrN_2O_5PNa$ $(M+Na)^+$ 759.4416, found 759.4415.

*3.5.8. 2-Bromoethyl methyl [(3R)-triethylsilyloxy-(2S)-(1-oxohexadecylamino)octadec-(4E)-enyl]phosphoramidate (**31**)*

To a solution of **32** (0.262 g, 0.356 mmol) in DMF (5.0 ml) were added imidazole (0.145 g, 0.288 mmol) and chlorotriethylsilane (0.14 ml, 0.854 mmol) at room temperature. After the reaction mixture was stirred for 5 min at the same temperature, saturated aqueous $NaHCO_3$ solution was added and the resulting mixture was extracted with diethyl ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% to 50% ethyl acetate in hexane containing 3% triethylamine) gave **31** (0.288 g, 95%) as a colorless oil. $[\alpha]_D^{24.0}$ −9.6 (*c* 0.8, $CHCl_3$); IR (KBr disk, cm^{-1}) 3278, 2924, 1655, 1460, 1240; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.22 (dd, *J*=8.2, 6.6 Hz, 1H), 5.67 (dtd, *J*=15.1, 6.6, 0.9 Hz, 1H), 5.42 (dd, *J*=15.3, 6.6 Hz, 1H), 4.30–4.34 (m, 1H), 4.22–4.29 (m, 2H), 3.84–3.91 (m, 1H), 3.74 (d, *J*=11.0 Hz, 3/2H), 3.72 (d, *J*=11.2 Hz, 3/2H), 3.54 (dt, *J*=6.2, 3.9 Hz, 2H), 3.21 (m, 1H), 3.00–3.14 (m, 1H), 2.17–2.21 (m, 2H), 2.03 (dt, *J*=6.9, 6.9 Hz, 2H), 1.63 (tt, *J*=7.3, 7.3 Hz, 2H), 1.22–1.36 (m, 48H), 0.95 (t, *J*=7.8 Hz, 9H), 0.88 (t, *J*=6.6 Hz, 6H), 0.58 (q, *J*=8.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1 (1/2C), 173.1 (1/2C), 132.8, 129.7 (1/2C), 129.6 (1/2C), 77.2, 74.4 (1/2C), 74.2 (1/2C), 65.4 (d, J_{C-P}=4.8 Hz, 1/2C), 65.3 (d, J_{C-P}=4.8 Hz, 1/2C), 53.2 (d, J_{C-P}=6.7 Hz, 1/2C), 53.0 (d, J_{C-P}=5.7 Hz, 1/2C), 52.3 (1/2C), 52.1 (1/2C), 47.8 (d, J_{C-P}=5.7 Hz, 1/2C), 47.8 (d, J_{C-P}=6.7 Hz, 1/2C), 36.9, 33.6 (d, J_{C-P}=3.8 Hz, 1/2C), 33.5 (d, J_{C-P}=3.8 Hz, 1/2C), 32.1, 31.9, 30.0, 29.9, 29.6, 29.5, 29.4, 29.3, 29.1, 29.1, 25.5, 22.7, 14.0, 6.8, 4.9; ESI-HRMS *m*/*z* calcd for $C_{44}H_{90}BrN_2O_5PSiNa$ $(M+Na)^+$ 887.5438, found 887.5431.

*3.5.9. SM aza analogue **2***

To a solution of **31** (0.147 g, 0.173 mmol) in toluene (3.0 ml) in a sealed tube was added trimethylamine (2.5 ml) at −78 °C. After the reaction mixture was stirred for 48 h at 60 °C, trimethylamine was removed in vacuo. To a solution of the residue in methanol (2.0 ml) was added Dowex 50W (pH=4) at room temperature. After the reaction mixture was stirred for 5 min at the same temperature, methanol was added to the reaction mixture, Dowex 50W was removed by filtration, and then the filtrate was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% methanol in chloroform to 3% water and 27% methanol in chloroform) gave **2** (0.037 g, 30%) as a colorless foam. $[\alpha]_D^{24.0}$ −1.5 (*c* 0.8, $CHCl_3$); IR (KBr disk, cm^{-1}) 3387, 2919, 1655, 1200, 1053, 968; ^1H NMR (CD_3OD, 400 MHz) δ 5.68 (td, *J*=15.1, 6.6 Hz, 1H), 5.44 (dd, *J*=15.3, 7.3 Hz, 1H), 4.17 (m, 2H), 4.02 (dd, *J*=7.6, 7.6 Hz, 1H), 3.74–3.79 (m, 2H), 3.60 (m, 2H), 3.22 (s, 9H), 3.08 (m, 2H), 2.19 (t, *J*=6.9 Hz, 2H), 2.02 (dt, *J*=6.9, 6.9 Hz, 2H), 1.58 (m, 2H), 1.29 (s, 48H), 0.90 (t, *J*=7.1 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.2, 134.5, 131.2, 73.8, 67.7 (m), 59.5 (d, J_{C-P}=3.8 Hz), 56.6 (d, J_{C-P}=4.8 Hz), 54.75, 54.71, 54.67, 43.1, 37.6, 33.5, 33.1, 30.9, 30.74, 30.66, 30.53, 30.49, 27.2, 23.8, 14.5; ESI-HRMS *m*/*z* calcd for $C_{39}H_{80}N_3O_5PNa$ $(M+Na)^+$ 724.5733, found 724.5732.

3.6. Synthesis of SM sulfur analogue 3

*3.6.1. Benzyl (2S)-(tert-butyloxycarbonylamino)-3-oxopent-4-eny sulfide (**33b**)*

To a solution of *N*-Boc-*S*-Bn-L-cystein **33** (10.00 g, 32.11) in dichloromethane (64 ml) were added *N*-methylmorpholine (3.88 ml, 35.3 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (3.759 g, 38.54 mmol), at −15 °C. To the mixture was then added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (7.387 g, 38.54 mmol) over 30 min at the same temperature. After the reaction mixture was stirred at the same temperature for 1 h, ice and 1 N HCl were added and the resulting mixture was extracted with chloroform. The organic layers were combined, washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude Weinreb amide **33a** as a colorless solid. To a solution of **33a** obtained above in THF (96 ml) was added a solution of vinylmagnesium bromide in THF, which was prepared with Mg (2.342 g, 96.34 mmol) and vinyl bromide (14.73 g, 144.5 mmol) in THF (96 ml) under reflux, at room temperature. After the reaction mixture was stirred for 10 min at the same temperature, it was poured into 2 N HCl and ice at 0 °C. The resulting mixture was then extracted with hexane. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (25% ethyl acetate in hexane) gave the roughly purified corresponding vinyl ketone **33b** (9.033 g, 87%, two steps) as a pale yellow oil. $[\alpha]_D^{24.0}$ +4.1 (*c* 1.19, $CHCl_3$); IR (neat, cm^{-1}) 3376, 2982, 1682, 1510, 1277, 1163; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28 (m, 5H), 6.44 (dd, *J*=17.3, 10.5 Hz, 1H), 6.33 (dd, *J*=17.3, 1.2 Hz, 1H), 5.84 (dd, *J*=10.2, 1.2 Hz, 1H), 5.37–5.46 (br m, 1H), 4.75 (m, 1H), 3.73 (s, 2H), 2.89 (dd, *J*=13.7, 5.6 Hz, 1H), 2.67 (dd, *J*=13.9, 6.1 Hz, 1H), 14.6 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.7, 155.0, 137.5, 132.7, 130.2, 128.8, 128.4, 127.0, 79.8, 56.3, 36.4, 32.6, 28.1; ESI-HRMS *m*/*z* calcd for $C_{17}H_{23}NO_3SNa$ $(M+Na)^+$ 344.1296, found 344.1295.

*3.6.2. 1-Benzylthio-(2S)-(tert-butyloxycarbonylamino)pent-4-en-(3R)-ol (**33c**)*

To a solution of **33b** (8.38 g, 26.08 mmol) in ethanol (130 ml) and THF (30.0 ml) was added lithium tri-*tert*-butoxyaluminohydride (14.58 g, 57.37 mmol) at −78 °C. After the reaction mixture was stirred at the same temperature for 15 min, 2 N HCl was added. The resulting mixture was then extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% to 33% ethyl acetate in hexane) gave **33c** (8.28 g, 98%) as a colorless solid. $[\alpha]_D^{24.0}$ −44.0 (*c* 1.20, $CHCl_3$); IR (KBr disk, cm^{-1}) 3351, 2982, 1686, 1528, 1292, 1173, 1013; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28 (m, 5H), 5.79 (ddd, *J*=17.3, 10.5, 5.6 Hz, 1H), 5.30 (ddd, *J*=17.3, 1.5, 1.5 Hz, 1H), 5.20 (ddd, *J*=10.5, 1.5, 1.5 Hz, 1H), 4.77–4.90 (br m, 1H), 4.27 (m, 1H), 3.82 (m, 1H), 3.73 (s, 2H), 2.75–2.83 (br m, 1H), 2.63 (dd, *J*=13.9, 4.9 Hz, 1H), 2.54 (dd, *J*=13.9, 7.8 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) 156.3, 137.9, 136.6, 128.9, 128.5, 127.1, 116.9, 79.9, 74.5, 54.0, 36.4, 31.6, 28.3; ESI-HRMS *m*/*z* calcd for $C_{17}H_{25}NO_3SNa$ $(M+Na)^+$ 346.1453, found 346.1450.

*3.6.3. (3R)-Hydroxy-(2R)-(tert-butyloxycarbonylamino)pent-4-ene-1-thiol (**33d**)*

To a solution of lithium (0.63 g, 90.4 mmol) in liquid ammonia (338.9 ml) was added a solution of **33c** (7.31 g, 22.59 mmol) in THF (113 ml) at −78 °C. After the reaction mixture was stirred for 3 h at reflux, ammonium chloride was added at −78 °C. After ammonia was removed at room temperature, water was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% to 33% ethyl acetate in hexane) gave **33d** (5.22 g, 99%) as a colorless solid. $[\alpha]_D^{23.5}$ −3.2 (*c* 1.05, $CHCl_3$); IR (neat, cm^{-1}) 3358, 2980, 1688, 1530, 1171; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.90 (ddd, *J*=17.3, 10.5, 5.6 Hz, 1H), 5.37 (ddd, *J*=17.3, 1.5, 1.5 Hz, 1H), 5.27 (ddd, *J*=10.5, 1.5, 1.5 Hz, 1H), 4.88–4.99 (br m, 1H), 4.31 (m, 1H), 3.88 (m, 1H), 2.77 (dd, *J*=8.5, 6.6 Hz, 1H), 2.75 (dd, *J*=8.8, 4.7 Hz, 3/2H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 137.0, 117.1, 79.9, 73.9, 56.0, 28.3, 25.2; ESI-HRMS *m*/*z* calcd for $C_{10}H_{19}NO_3SNa$ $(M+Na)^+$ 256.0983, found 256.0972.

*3.6.4. (3R)-(tert-Butyldimethylsilyloxy)-(2R)-(tert-butyloxycarbonylamino)pent-4-ene-1-thiol (**34**)*

To a solution of **33d** (1.92 g, 8.23 mmol) in DMF (41 ml) were added triethylamine (2.87 ml, 20.57 mmol), DMAP (1.005 g,

8.229 mmol), and TBSCl (3.101 g, 20.57 mmol) at 0 °C. After the reaction mixture was stirred for 14 h at room temperature, ice was added and the resulting mixture was extracted with hexane. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude *O,S*-silylated product **33e**. To a solution of crude **33e** in THF (20.6 ml) was added 1.0 M TBAF in THF solution (7.8 ml) at −78 °C. After the reaction mixture was stirred for 10 min at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude **34** containing with small amount of disulfide **34**. To a solution of crude **34** in CH_3CN (41 ml) and H_2O (8.2 ml) was added tributylphosphine (2.06 ml, 8.23 mmol) at room temperature. After the reaction mixture was stirred for 1 h at the same temperature, saturated aqueous NH_4Cl solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude **34**. Column chromatography on silica gel (from 1% to 9% ethyl acetate in hexane) gave **34** (2.241 g, 78%, three steps) as a colorless oil. $[\alpha]_D^{23.5}$ −26.2 (*c* 0.92, $CHCl_3$); IR (neat, cm^{-1}) 3366, 2957, 1716, 1496, 1253, 1171; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.80 (ddd, *J*=17.2, 10.3, 5.7 Hz, 1H), 5.28 (ddd, *J*=17.2, 1.4, 1.4 Hz, 1H), 5.19 (ddd, *J*=10.3, 1.4, 1.4 Hz, 1H), 4.78 (br d, *J*=8.2 Hz, 1H), 4.32 (m, 1H), 3.69 (m, 1H), 2.75 (ddd, *J*=14.0, 7.1, 7.1 Hz, 1H), 2.67 (ddd, *J*=14.0, 9.4, 4.6 Hz, 1H), 1.45 (s, 9H), 0.90 (s, 9H), 0.07 (s, 3H), 0.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 137.9, 116.5, 79.5, 74.6, 56.3, 28.4, 25.8, 24.6, 18.1, −4.4, −5.0; ESI-HRMS *m*/*z* calcd for $C_{16}H_{33}NO_3SSiNa$ $(M+Na)^+$ 370.1848, found 370.1844.

3.6.5. *O-(2-Bromoethyl) S-[(3R)-(tert-butyldimethylsilyloxy)-(2R)-(tert-butyloxycarbonylamino)pent-4-enyl] O-methyl thiophosphate (**35**)*

To a mixture of 4 Å molecular sieves (0.300 g) and carbon tetrabromide (0.612 g, 1.844 mmol) in dichloromethane (3.0 ml) was added 2-bromoethyl dimethyl phosphite **30** (0.28 ml, 1.844 mmol) at 0 °C. After the reaction mixture was stirred for 20 min, a solution of **34** (0.103 g, 0.296 mmol) in dichloromethane (0.58 ml) and 2,6-lutidine (0.007 ml, 0.059 mmol) were added to the reaction mixture at the same temperature. After the reaction mixture was stirred for 2 h at the same temperature, the solvent was removed in vacuo to give the crude products. Column chromatography on silica gel (from 5% to 17% ethyl acetate in hexane) gave **35** (96 mg, 61%) as a colorless oil. $[\alpha]_D^{26.0}$ −9.7 (*c* 0.90, $CHCl_3$); IR (neat, cm^{-1}) 3327, 2957, 2857, 1711, 1522, 1254, 1017; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.79 (ddd, *J*=16.9, 10.6, 5.2 Hz, 1H), 5.30 (d, *J*=17.2 Hz, 1H), 5.20 (d, *J*=10.5 Hz, 1H), 5.17–5.09 (br m, 1H), 4.27–4.43 (m, 3H), 3.82 (d, *J*=12.6 Hz, 3/2H), 3.80 (d, *J*=12.6 Hz, 3/2H), 3.76 (m, 1H), 3.54 (t, *J*=6.5 Hz, 2/2H), 3.52 (d, *J*=5.5 Hz, 2/2H), 3.071 (ddd, *J*=17.2, 13.7, 10.1 Hz, 1/2H), 3.065 (m, 1/2H), 2.935 (ddd, *J*=16.2, 13.7, 10.1 Hz, 1/2H), 2.935 (ddd, *J*=17.2, 13.7, 10.1 Hz, 1/2H), 1.44 (s, 9/2H), 1.43 (s, 9/2H), 0.90 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 137.4, 116.6, 79.4, 74.7 (1/2C), 74.6 (1/2C), 66.2 (d, J_{C-P}=4.5 Hz), 54.0 (d, J_{C-P}=5.4 Hz), 44.8, 30.3 (1/2C), 30.1 (1/2C), 28.2, 25.7, 20.1 (m), 18.0, −4.6, −5.2; ESI-HRMS *m*/*z* calcd for $C_{19}H_{39}BrNO_6PSSiNa$ $(M+Na)^+$ 570.1086, found 570.1086.

3.6.6. *O-(2-Bromoethyl) S-[(3R)-hydroxy-(2R)-(tert-butyloxycarbonylamino)pent-4-enyl] O-methyl thiophosphate (**36**)*

To a solution of **35** (0.326 g, 0.594 mmol) in methanol (5.9 ml) was added 2 N HCl (1.2 ml) at room temperature. After the reaction mixture was stirred for 4 h at the same temperature, saturated aqueous $NaHCO_3$ solution was added and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 9% to 50% ethyl acetate in hexane) gave **36** (0.213 g, 83%) as a colorless oil. $[\alpha]_D^{26.0}$ +12.2 (*c* 0.93, $CHCl_3$); IR (neat, cm^{-1}) 3403, 2978, 1701, 1524, 1248, 1171, 1017; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.89 (ddd, *J*=16.9, 10.6, 5.7 Hz, 1H), 5.38 (d, *J*=17.2 Hz, 1H), 5.27 (d, *J*=10.6 Hz, 1H), 5.15–5.23 (br d, *J*=8.2 Hz, 1H), 4.31–4.44 (m, 2H), 4.13 (m, 1H), 3.87 (m, 1H), 3.85 (d, *J*=12.6 Hz, 3/2H), 3.83 (d, *J*=12.6 Hz, 3/2H), 3.563 (t, *J*=6.1 Hz, 2/2H), 3.559 (t, *J*=6.1 Hz, 2/2H),3.04–3.21 (m, 2H), 1.451 (s, 9/2H), 1.447 (s, 9/2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 136.6, 117.3, 80.00 (1/2C), 79.96 (1/2C), 74.0, 66.6 (m), 55.4, 54.4 (d, J_{C-P}=6.7 Hz), 31.2 (m), 29.2 (1/2C), 29.1 (1/2C), 28.4; ESI-HRMS *m*/*z* calcd for $C_{13}H_{25}BrNO_6PSNa$ $(M+Na)^+$ 456.0221, found 456.0213.

3.6.7. *O-(2-Bromoethyl) S-[(3R)-hydroxy-(2R)-(tert-butyloxycarbonylamino)octadec-(4E)-enyl] O-methyl thiophosphate (**37**)*

To a solution of **36** (0.423 g, 0.974 mmol) in dichloromethane (9.7 ml) were added 1-pentadecene (1.06 ml, 3.90 mmol) and second generation Grubbs catalyst (3 mg, 0.003 mmol). After the reaction mixture was stirred for 2 h under reflux, it was concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 17% to 33% ethyl acetate in hexane) gave **37** (0.450 g, 74%) as a colorless oil. $[\alpha]_D^{27.0}$ +2.0 (*c* 0.75, $CHCl_3$); IR (neat, cm^{-1}) 3289, 2919, 2851, 1647, 1541, 1252, 1013; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.76 (dt, *J*=15.3, 6.7 Hz, 1H), 5.47 (dd, *J*=15.3, 6.9 Hz, 1H), 5.13 (br m, *J*=1H), 4.31–4.44 (m, 2H), 4.16 (m, 1H), 3.85 (d, *J*=12.6 Hz, 3/2H), 3.83 (d, *J*=13.0 Hz, 3/2H), 3.561 (t, *J*=6.4 Hz, 2/2H), 3.557 (t, *J*=6.4 Hz, 2/2H), 2.98–3.21 (m, 3H), 2.04 (dt, *J*=6.9, 6.9 Hz, 2H), 1.447 (s, 9/2H), 1.442 (s, 9/2H), 1.37 (m, 2H), 1.26–1.32 (m, 20H), 0.88 (t, *J*=6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 135.1, 128.1, 79.9, 73.9 (m), 66.59 (d, J_{C-P}=4.8 Hz, 1/2C), 66.54 (d, J_{C-P}=5.7 Hz, 1/2C), 55.6, 54.4 (d, J_{C-P}=6.7 Hz), 32.4, 31.9, 31.5 (m), 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.4, 22.7, 14.1; ESI-HRMS *m*/*z* calcd for $C_{26}H_{51}BrNO_6PSNa$ $(M+Na)^+$ 638.2256, found 638.2253.

3.6.8. *O-(2-Bromoethyl) S-[(3R)-hydroxy-(2R)-(1-oxohexadecylamino)octadec-(4E)-enyl] O-methyl thiophosphate (**38**)*

To a solution of **37** (73 mg, 0.118 mmol) in dichloromethane (0.59 ml) was added TFA (0.24 ml) at 0 °C. After the reaction mixture was stirred for 2.5 h at the same temperature, chloroform and saturated K_2CO_3 solution were added until the mixture turned basic. To this mixture was added palmitoyl chloride (0.043 ml, 0.142 mmol) at 0 °C, and the resulting mixture was stirred for 25 min. Saturated aqueous NH_4Cl solution was added and the mixture was extracted with chloroform. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 25% to 50% ethyl acetate in hexane) gave **38** (82 mg, 92%) as a colorless solid. $[\alpha]_D^{27.0}$ +1.3 (*c* 0.76, $CHCl_3$); IR (KBr disk, cm^{-1}) 3405, 2926, 2855, 1711, 1524, 1250, 1013; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.46 (t, *J*=6.9 Hz, 1H), 5.75 (dt, *J*=15.3, 6.6 Hz, 1H), 5.46 (dd, *J*=15.3, 6.6 Hz, 1H), 4.29–4.42 (m, 2H), 4.12–4.18 (m, 2H), 3.83 (d, *J*=12.8 Hz, 3H), 3.553 (t, *J*=6.2 Hz, 2/2H), 3.545 (t, *J*=6.2 Hz, 2/2H), 3.46 (br d, *J*=15.6 Hz, 1H), 3.03–3.19 (m, 2H), 2.22 (t, *J*=7.3 Hz, 2H), 2.04 (dt, *J*=7.1, 7.1 Hz, 2H), 1.63 (m, 2H), 1.26–1.36 (m, 46H), 0.88 (t, *J*=6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4 (1/2C), 174.3 (1/2C), 135.0, 127.9, 74.3 (1/2C), 74.2 (1/2C), 66.8 (d, J_{C-P}=4.5 Hz), 55.1 (m), 54.6 (d, J_{C-P}=6.7 Hz), 36.69 (1/2C), 36.67 (1/2C), 32.4, 31.9, 30.7 (m), 29.71, 29.67, 29.5, 29.41, 29.36, 29.28, 29.17, 25.7, 22.7, 14.1; ESI-HRMS *m*/*z* calcd for $C_{37}H_{73}BrNO_5PSNa$ $(M+Na)^+$ 776.4028, found 776.4033.

3.6.9. *SM sulfur analogue **3***

To a solution of **38** (48 mg, 0.064 mmol) in methanol (1.3 ml) was added trimethylamine (0.64 ml) at room temperature. After

the reaction mixture was stirred for 1 day at the same temperature, the solvent was filtered and removed in vacuo to give the crude products. Column chromatography on silica gel (from 9% methanol in chloroform to 4% water and 27% methanol in chloroform) gave **3** (23 mg, 50%) as a colorless foam. $[\alpha]_D^{27.0}$ −8.3 (*c* 0.42, CH_3OH); IR (KBr disk, cm^{-1}) 3887, 2920, 1638, 1468, 1233, 1074; ^1H NMR (CD_3OD, 400 MHz) δ 5.69 (dt, *J*=15.3, 6.9 Hz, 1H), 5.44 (dd, *J*=15.3, 6.9 Hz, 1H), 4.28 (m, 2H), 3.98 (m, 2H), 3.67 (m, 2H), 3.23 (s, 9H), 3.15 (m, 1H), 2.83 (ddd, *J*=14.6, 13.7, 8.5 Hz, 1H), 2.19 (t, *J*=7.1 Hz, 2H), 2.02 (dt, *J*=7.1, 6.8 Hz, 2H), 1.59 (m, 2H), 1.28–1.36 (m, 46H), 0.90 (t, *J*=6.6 Hz, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.2, 134.9, 130.9, 75.2, 67.5 (m), 60.5 (d, J_{C-P}=5.7 Hz), 56.1 (m), 54.78, 54.74, 54.71, 37.4, 33.5, 33.1, 32.9, 30.9, 30.8, 30.7, 30.6, 30.52, 30.48, 27.3, 23.8, 14.5; ESI-HRMS *m*/*z* calcd for $C_{39}H_{79}N_2O_5PSNa$ $(M+Na)^+$ 741.5345, found 741.5333.

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Supplementary data

^1H and ^{13}C NMR spectra of compounds **1**–**4**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.10.018.

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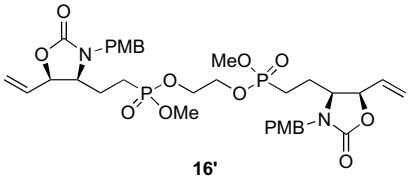
16. A small amount of the by-product **16′** was detected.



16'

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