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Synthesis of a Photoactivatable (2*S*,3*R*)-Sphingosylphosphorylcholine Analogue

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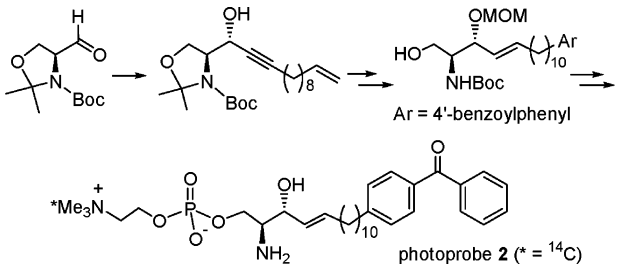

The receptor for the lipid mediator sphingosylphosphorylcholine (SPC) has not yet been identified. We describe here the synthesis of the first photoaffinity analogue of SPC. This probe, which contains a ^{14}C-isotopic label in the choline methyl groups and a photoreactive benzophenone in the long-chain base, may be a useful tool in the identification of the G protein coupled receptors that have been postulated to interact directly and specifically with SPC and in the definition of the ligand-binding sites. The key steps in the synthesis are selective reduction of the triple bond in enyne **6** to install the 4*E* double bond, Suzuki coupling to incorporate the benzophenone photophore at the end of the sphingoid chain, and reduction of the 2-azidoethyl phosphate headgroup of **13** followed by *N*,*N*,*N*-trimethylation to introduce the radiolabel into the choline moiety. The synthesis was completed by the release of the amino group at C2 of the sphingoid base of SPC analogue **2**.

Introduction

Sphingosylphosphorylcholine (SPC or lysosphingomyelin, **1**) is formed by *N*-deacylation of sphingomyelin (SPM).[1] SPC is a natural component of blood plasma[2] and high-density lipoproteins.[3] It accumulates in the brain of patients with Niemann−Pick type A disease[4] and in the malignant ascites of patients with ovarian cancer.[5] SPC participates in the regulation of many cellular functions, including proliferation, cell migration, smooth muscle contraction, and wound healing.[6] SPC also has potential pathophysiological roles in angiogenesis (the growth of new capillary blood vessels)[7] and in enhancement of the elasticity of human epithelial tumor cells.[8] Several putative receptors for SPC have been suggested.[6,9] Several years ago, it was concluded that the multiple cellular functions of SPC were directly transduced via a G protein-coupled receptor called GPR4.[10] However, this conclusion was retracted recently.[11] Nev-

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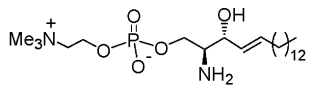
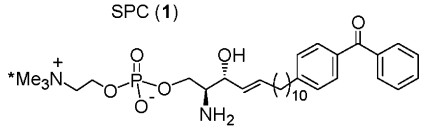
ertheless, very recently it was proposed that SPC-induced angiogenesis in endothelial cells is mediated by GPR4.[7] Thus, the molecular identity of the SPC receptor(s) is unclear at present. The availability of a photoreactive SPC analogue would provide the means for identifying the SPC receptor and help elucidate the molecular mechanisms underlying the normal and pathophysiological functions of SPC.

Photolabeling techniques employ molecules that are targeted to a biological system and, upon photolysis with UV light, form short-lived, highly reactive intermediates that form covalent bonds with adjacent molecules. Photoactivatable analogues of phospholipids have been used to identify lipid-binding membrane proteins.[12] In a previous study of photoactivatable analogues of the lipid mediator sphingosine 1-phosphate (S1P),[13] we found that a probe containing benzophenone was bound more tightly to a S1P receptor than a similar S1P analogue bearing a 3-trifluoromethyl-3-aryldiazirine probe, perhaps because of an electrostatic effect.[14] Therefore, the benzophenone photophore was selected for the present study. Among its many useful features are (a) a high degree of hydrophobicity, thus inserting spontaneously into membrane bilayers; (b) chemical stability with respect to many solvents and reaction conditions, and stability in the absence of light; (c) photoactivatability to a triplet state with long-wavelength UV light (λ > 350 nm), thus minimizing damage to proteins; and (d) the ability to undergo photochemical reactions by random insertion into accessible Cα−H bonds of amino acid residues. A radiolabel is generally incorporated into photoprobes to allow sensitive detection and identification of proteins covalently coupled to the probe. In the present study, we describe an efficient synthesis of a photoactivatable analogue **2** that bears a benzophenone moiety in the sphingoid chain and *N*-[^{14}C]-methyl groups in the polar headgroup.



SPC (**1**)

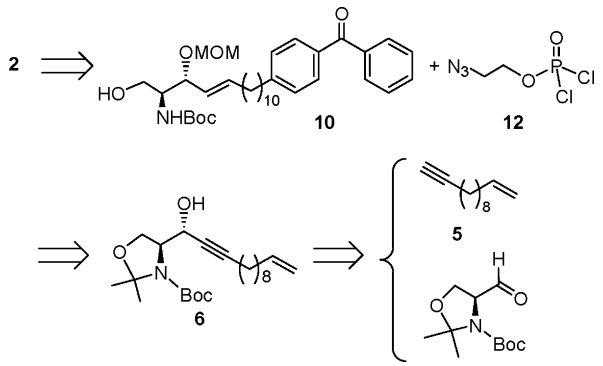


[^{14}C]-labeled photoprobe **2** (* = ^{14}C)

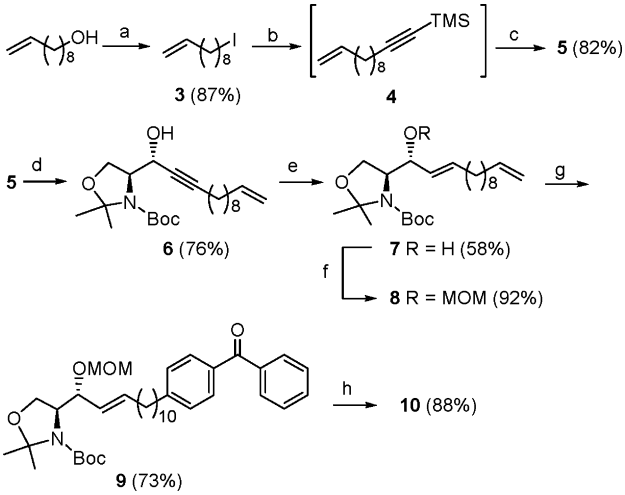
Results and Discussion

Synthetic Plan. As illustrated in the retrosynthetic analysis (Scheme 1), our synthesis of radiolabeled photoactivatable analogue **2** started with the addition of the acetylide ion derived from enyne **5** to *N*-Boc-*N*,*O*-isopropylidene-L-serinal ((*S*)-Garner aldehyde).[15] Use of nonchelation-controlled conditions afforded the requisite 2*S*,3*R* stereochemistry of the sphingoid backbone of **6**.

SCHEME 1. Retrosynthetic Plan



SCHEME 2. Synthesis of Alcohol 10 via ω-Alkyn-1-ene 5[a]



[a] Reagents and conditions: (a) PPh_3, I_2, imidazole, Et_2O/CH_3CN (3:1), 0−5 °C to rt, overnight; (b) TMS-acetylene, *n*-BuLi, HMPA, THF, −78 °C; (c) 1 N NaOH, Et_2O, rt, 1 h; (d) (*S*)-Garner aldehyde, *n*-BuLi, HMPA, THF, −78 °C, 2.5 h; (e) Red-Al, Et_2O, rt, 4 h; (f) MOMCl, EtN(Pr-*i*)$_2$, CH_2Cl_2, 0 °C to rt; (g) (i) 9-BBN, THF, 0 °C to rt, (ii) $Pd(PPh_3)_4$, K_3PO_4, 4-bromobenzophenone, H_2O, dioxane, 85 °C, overnight; (h) 80% HOAc, 80 °C, 5 h.

After Red-Al reduction of progarylic alcohol **6** and protection of the secondary alcohol, the benzophenone group was installed by Suzuki coupling. The oxazolidine ring was opened with retention of the *N*-Boc group to provide alcohol **10**. Reaction with 2-azidoethyl phosphorochloridate **12**[16] and introduction of radioactivity into the choline moiety, followed by deprotection of the *O*-MOM and *N*-Boc groups in a one-pot reaction, completed the synthesis of **2**.

Synthesis of Alcohol 10. 9-Decen-1-ol was converted to iodide **3** as described previously[17] (Scheme 2). Alkynylation with lithium trimethylsilylacetylene afforded intermediate **4**, and removal of the TMS group provided ω-alkyn-1-ene **5** (82% yield for two steps). The acetylide anion derived from **5** was coupled diastereoselectively with (*S*)-Garner aldehyde in the presence of HMPA, giving *erythro* isomer **6** in 76% yield.[15] Reduction of propargylic alcohol **6** with 2 equiv of Red-Al afforded (4*E*)-diene **7** in moderate yield; 30% of starting material **6** was

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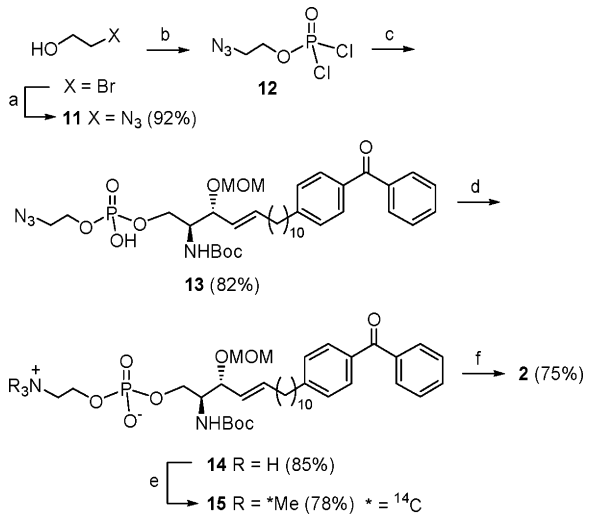
SCHEME 3. Synthesis of SPC Photoprobe 2[a]



[a] Reagents and conditions: (a) NaN_3, *n*-Bu_4NBr, 15 h, 110 °C; (b) $POCl_3$, 0 °C; (c) (i) **10**, pyridine, Et_2O, rt (30 min), reflux (3 h), (ii) H_2O; (d) $HS(CH_2)_3SH$, Et_3N, MeOH, rt; (e) (i) [^{14}C]MeI, $NaHCO_3$, MeOH, 50 °C, pressure tube, 3 h, (ii) MeI (excess), 50 °C, pressure tube, 3 h; (f) 3 M HCl/THF (1:1), 70 °C, 3 h.

recovered. Use of longer reaction times or additional Red-Al resulted in oxazolidine ring opening. Protection of the hydroxy group of **7** as a MOM ether furnished **8**, which was subjected to hydroboration with 9-BBN (0 °C, overnight). After unreacted 9-BBN was destroyed, Suzuki coupling[18] with 4-bromobenzophenone afforded benzophenone-labeled sphingosine analogue **9** in 73% yield. Selective deprotection of **9** with 80% HOAc at 80 °C yielded primary alcohol **10** with retention of the *N*-Boc group.[19]

Synthesis of SPC Analogue 2. To prepare 2-azidoethyl chlorophosphate (**12**),[16] 2-bromoethanol was reacted with sodium azide, and the resulting 2-azidoethanol (**11**)[16] was added to phosphorus oxychloride (Scheme 3). Phosphorylation was carried out by adding a solution of alcohol **10** in Et_2O to **12** in the presence of pyridine, providing 2-azidoethyl phosphate ester **13** in good yield. Reduction of the azido group to an amino group with PPh_3 or polymer-supported PPh_3 failed but reduction with 1,3-propanedithiol[20] in the presence of dry triethylamine afforded amine **14** in 85% yield. The radiolabel was introduced into the polar headgroup of **2** by *N*,*N*-dimethylation of 2-aminoethyl phosphate ester **14** with ~2.5 equiv of [^{14}C]methyl iodide in the presence of $NaHCO_3$ and MeOH at 50 °C in a pressure tube for 3 h. After complete *N*-methylation with a large excess (20 equiv) of unlabeled methyl iodide, the solvent and excess methyl iodide were evaporated, and the residue was purified on a short silica gel column to provide [^{14}C]-phosphocholine **15** in 78% yield. Deprotection of the *N*-Boc and *O*-MOM groups at the same time with 3 N HCl at 70 °C gave final product **2** in 75% yield.

In summary, a photoactivatable analogue of SPC bearing a benzophenone in the sphingoid base and radioactivity in the polar headgroup was prepared in 12 steps and 7.6% overall yield starting from Garner aldehyde. Suzuki coupling was used to install the photophore, and [^{14}C]-*N*-methyl groups were introduced in the penultimate step. The specific activity of **2** was 3.2 mCi/mmol. It is anticipated that the availability of [^{14}C]-**2** will help unravel the identity of the SPC receptor(s), the nature of which has been elusive until now.[11]

Experimental Section

The general methods have been described previously.[21]

1-Dodecen-11-yne (5). To a solution of trimethylsilylacetylene (0.75 g, 7.5 mmol) in dry THF (20 mL) at −78 °C was added *n*-BuLi (2.89 M in hexanes, 2.6 mL, 7.5 mmol) over 5 min. After 30 min, HMPA (5 mL) was added. A solution of iodide **3** (1.33 g, 5.0 mmol) in dry THF (5 mL) was added dropwise, the cold bath was removed, and stirring was continued overnight at room temperature. The reaction was quenched with saturated aqueous NH_4Cl solution (20 mL), the layers were separated, the aqueous layer was extracted with EtOAc (3 × 10 mL), and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to give crude TMS-enyne **4**. To a solution of crude **4** in 15 mL of Et_2O was added 15 mL of aqueous 1 N NaOH solution. The mixture was stirred at room temperature for 1 h and then neutralized with 15 mL of 1 M HCl. The organic layer was isolated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (elution with hexane) gave ω-alkyn-1-ene **5** (0.67 g, 82%) as a colorless oil: R_f 0.80 (hexane); ^{1}H NMR ($CDCl_3$) δ 1.24−1.56 (m, 12H), 1.93 (t, 1H, J = 2.8 Hz), 2.04 (m, 2H), 2.17 (m, 2H), 4.94 (m, 2H), 5.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 18.4, 28.5, 28.7, 28.9, 29.1, 29.3, 29.7, 33.8, 68.4, 84.8, 114.1, 139.2.

***N*-*tert*-Butoxycarbonyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-hydroxy-2′-dodecyn-11′-enyl)oxazolidine (6).** To a solution of alkyne **5** (0.51 g, 3.1 mmol) in dry THF (50 mL) was added *n*-BuLi (2.5 M in hexane, 1.24 mL, 3.1 mmol) at −78 °C under N_2. The mixture was stirred for 2 h before HMPA (45 mg, 44 mL, 0.25 mmol) was added. After the mixture was stirred for 30 min, a solution of (*S*)-Garner aldehyde (0.57 g, 2.5 mmol) in 5 mL of dry THF was added slowly. The solution was stirred at −78 °C for 2.5 h and then quenched with aqueous saturated NH_4Cl solution (40 mL). The mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with brine (100 mL) and dried ($MgSO_4$). The crude oil was purified by flash chromatography (EtOAc/hexane 1:3) to afford **6** (0.77 g, 76%) as a colorless oil: R_f 0.59 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ −34.1 (*c* 1.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24−1.65 (m, 27H), 2.04 (m, 2H), 2.20 (m, 2H), 3.91 (m, 1H), 4.11 (m, 2H), 4.52 (m, 1H), 4.94 (m, 2H), 5.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 18.8, 25.8, 28.4, 28.6, 29.1, 29.4, 33.8, 60.4, 62.8, 64.2, 77.9, 81.2, 86.6, 94.9, 114.2, 139.2, 154.1; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{23}H_{39}NO_4Na^+$ 416.2771, found 416.2777.

***N*-*tert*-Butoxycarbonyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-hydroxy-2′,11′-dodecadienyl)oxazolidine (7).** To a solution of **6** (0.68 g, 1.67 mmol) in anhydrous Et_2O (20 mL) at 0 °C was added a solution of Red-Al (0.96 mL, 70% in toluene, 3.34 mmol) dropwise. After 10 min, the cooling bath was removed, and the reaction mixture was stirred at room temperature for 4 h. An aqueous saturated solution of NH_4Cl (2 mL) was slowly added (*Caution! very exothermic*). The resulting white slurry was diluted with Et_2O (10 mL), 1 N NaOH (5 mL), and water (5 mL), and the layers were separated. The aqueous phase was re-extracted with Et_2O (3 × 5 mL), and the combined organic

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phase was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc/hexane 1:3) to afford 396 mg (58%) of diene **7** as a colorless oil: R_f 0.54 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ −22.3 (*c* 1.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24−1.57 (m, 27H), 2.04 (m, 4H), 3.91 (m, 1H), 4.02 (m, 1H), 4.11 (m, 1H), 4.20 (m, 1H), 4.94 (m, 2H), 5.48 (m, 1H), 5.80 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.7, 26.2, 28.6, 28.9, 29.1, 29.2, 29.4, 29.7, 32.4, 33.8, 60.4, 62.3, 64.9, 74.0, 81.0, 94.4, 114.1, 128.2, 133.3, 139.2, 154.1; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{23}H_{41}NO_4Na^+$ 418.2928, found 418.2926.

***N*-*tert*-Butoxycarbonyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-methoxymethoxy-2′,11′-dodecadienyl)oxazolidine (8).** To a solution of 409 mg (1.0 mmol) of alcohol **7** in 15 mL of anhydrous CH_2Cl_2 were added 269 mL (1.55 mmol) of $(i\text{-Pr})_2$NEt and 118 mL (1.55 mmol) of MOMCl at 0 °C. After 10 min, the cooling bath was removed, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into H_2O (70 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Purification by flash chromatography (hexane/EtOAc 6:1) gave 417 mg (92%) of ether **8** as a colorless oil: R_f 0.82 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ −73.8 (*c* 1.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24−1.60 (m, 27H), 2.04 (m, 4H), 3.36 (s, 3H), 3.92 (m, 2H), 4.07 (m, 1H), 4.28 (m, 1H), 4.51 (d, 1H, J = 6.4 Hz), 4.73 (d, 1H, J = 6.4 Hz), 4.94 (m, 2H), 5.32 (m, 1H), 5.69 (m, 1H), 5.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 22.7, 24.9, 26.2, 28.4, 29.0, 29.4, 32.4, 33.8, 55.8, 60.3, 64.6, 76.3, 79.9, 93.7, 94.3, 114.1, 126.7, 136.8, 139.2, 152.4; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{25}H_{45}NO_5Na^+$ 462.3190, found 462.3202.

***N*-*tert*-Butoxycarbonyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-methoxymethoxy-2′-dodecene-13′-benzoylphenyl)oxazolidine (9).** To a solution of 363 mg (0.80 mmol) of **8** in 5 mL of dry THF was added 1.8 mL (0.90 mmol) of a 0.5 M solution of 9-BBN in THF. The solution was stirred overnight until **8** had completely disappeared (TLC, EtOAc/hexane 1:6). Unreacted 9-BBN was destroyed by adding 2 drops of H_2O with stirring for 10 min. To this reaction mixture was added a solution of 209 mg (0.80 mmol) of 4-bromobenzophenone in 4 mL of dioxane, followed by $Pd(PPh_3)_4$ (28 mg, 0.024 mmol) and K_3PO_4 (0.92 g, 40 mmol). The reaction mixture was heated overnight at reflux (85 °C). After the solvents were removed, the residue was purified by chromatography (EtOAc/hexane 1:6), providing **9** (363 mg, 73%) as a colorless oil: R_f 0.51 (EtOAc/hexane 1:6); $[\alpha]^{25}_D$ −40.6 (*c* 0.83, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27−1.65 (m, 31H), 2.13 (m, 2H), 2.68 (t, 2H, J = 7.6 Hz), 3.36 (s, 3H), 3.92 (m, 2H), 4.07 (m, 1H), 4.28 (m, 1H), 4.51 (d, 1H, J = 6.4 Hz), 4.73 (d, 1H, J = 6.4 Hz), 5.30 (m, 1H), 5.69 (m, 1H), 7.29 (d, 2H, J = 6.4 Hz), 7.47 (t, 2H, J = 7.2 Hz), 7.56 (m, 1H), 7.73 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 25.6, 28.4, 29.1, 29.4, 29.5, 31.2, 36.0, 55.8, 60.3, 64.6, 76.3, 79.9, 93.7, 94.3, 126.7, 128.2, 128.3, 130.0, 132.1, 135.0, 136.8, 137.9, 148.2, 152.4, 196.4; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{38}H_{55}NO_6Na^+$ 644.3922, found 644.3951.

(2*S*,3*R*)-2-*N*-(*tert*-Butoxycarbonylamido)-3-*O*-methoxymethyl-15-(4′-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (10). Oxazolidine **9** (311 mg, 0.50 mmol) was dissolved in acetic acid (0.8 mL) and water (0.2 mL), and the mixture was stirred at 80 °C for 5 h. The mixture was concentrated and coevaporated with heptane (2 × 1 mL) to provide a residue that was purified by chromatography (hexane/EtOAc 1:1), affording *N*-Boc alcohol **10** (256 mg, 88%) as a colorless oil: R_f 0.18 (EtOAc/hexane 1:3); $[\alpha]^{25}_D$ −38.4 (*c* 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27−1.65 (m, 25H), 2.04 (m, 2H), 2.68 (t, 2H, J = 7.6 Hz), 2.80 (br s, 1H), 3.36 (s, 3H), 3.68 (m, 2H), 3.93 (m, 1H), 4.24 (m, 1H), 4.51 (d, 1H, J = 6.4 Hz), 4.66 (d, 1H, J = 6.4 Hz), 5.26 (m, 1H), 5.36 (dd, 1H, J = 8.0, 15.6 Hz), 5.73 (m, 1H), 7.29 (d, 2H, J = 8.0 Hz), 7.47 (t, 2H, J = 7.2 Hz), 7.57 (m, 1H), 7.73 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 26.2, 26.4, 28.4, 29.0, 29.3, 29.6, 31.2, 32.3, 36.0, 55.7, 61.6, 62.4, 78.5, 79.5, 93.9, 126.0, 128.2, 128.3, 130.0, 132.1, 135.0, 137.0, 138.0, 148.2, 156.0, 196.5; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{35}H_{51}NO_6Na^+$ 604.3609, found 604.3586.

2-Azidoethyl Phosphorochloridate (12). To a 50-mL flask containing 8.86 g (58 mmol) of $POCl_3$ was added 2.5 g (28.7 mmol) of **11** (see the Supporting Information) dropwise at 0 °C. The mixture was heated at 70 °C for 20 h, and the remaining $POCl_3$ was evaporated at room temperature (1 Torr, 2 days) to give crude **12**, which was used without further purification.

(2*S*,3*R*)-1-*O*-[2′-Azidoethyl(hydroxy)phosphoryl]-2-*N*-(*tert*-butoxycarbonylamido)-3-*O*-methoxymethyl-15-(4′-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (13). To a well-dried 50-mL flask containing 202 mg (1.0 mmol) of crude **12** in 15 mL of anhydrous Et_2O was added 0.16 mL (2.0 mmol) of anhydrous pyridine. After 30 min of stirring, a solution of 200 mg (0.34 mmol) of alcohol **10** in 2 mL of Et_2O was added dropwise. The reaction mixture was stirred at room temperature for 30 min, and then was heated at reflux for about 3 h until the starting material (alcohol **10**) disappeared. Water (2 mL) was added at 0 °C, and stirring was continued at room temperature overnight. The solvent was removed, and the residue was purified by chromatography ($CHCl_3$/MeOH 9:1, then 9:2) to give 206 mg (82%) of **13** as a wax: R_f 0.48 ($CHCl_3$/MeOH 9:2); $[\alpha]^{25}_D$ −24.4 (*c* 6.40, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26−1.65 (m, 25H), 2.03 (m, 2H), 2.68 (t, 2H, J = 7.6 Hz), 3.36 (s, 3H), 3.48 (m, 2H), 3.85 (m, 1H), 4.09 (m, 5H), 4.53 (m, 1H), 4.67 (m, 1H), 5.33 (m, 1H), 5.71 (m, 1H), 7.28 (d, 2H, J = 7.2 Hz), 7.47 (t, 2H, J = 7.2 Hz), 7.56 (m, 1H), 7.74 (d, 2H, J = 7.6 Hz), 7.78 (d, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 28.4, 29.1, 29.3, 29.4, 29.5, 29.6, 31.2, 32.4, 36.0, 51.3, 54.0, 55.7, 64.8, 65.3, 79.4, 93.9, 125.9, 127.9, 128.2, 128.3, 130.0, 130.3, 132.1, 135.0, 137.4, 138.0, 148.2, 155.9, 196.5; ^{31}P NMR ($CDCl_3$) δ −0.28; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{37}H_{55}N_4O_9PNa^+$ 753.3599, found 753.3567.

(2*S*,3*R*)-1-*O*-[2′-Aminoethyl(hydroxy)phosphoryl]-2-*N*-(*tert*-butoxycarbonylamido)-3-*O*-methoxymethyl-15-(4′-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (14). To a solution of azide **13** (197 mg, 0.27 mmol) in dry MeOH (5 mL) were added dry Et_3N (0.14 mL, 1.0 mmol) and 1,3-propanedithiol (0.10 mL, 1.0 mmol). The solution was stirred overnight at room temperature. A white precipitate formed, which was removed by filtration, and the filtrate was concentrated. The residue was purified by chromatography ($CHCl_3$/MeOH 2:1) to give 162 mg (85%) of **14** as a wax: R_f 0.47 ($CHCl_3$/MeOH 2:1); $[\alpha]^{25}_D$ −36.4 (*c* 0.78, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26−1.65 (m, 25H), 2.03 (m, 2H), 2.68 (t, 2H, J = 7.6 Hz), 3.17 (m, 2H), 3.36 (s, 3H), 3.77 (m, 1H), 3.97−4.10 (m, 5H), 4.50 (m, 1H), 4.67 (m, 1H), 5.33 (m, 1H), 5.71 (m, 1H), 7.27 (d, 2H, J = 7.2 Hz), 7.47 (t, 2H, J = 7.2 Hz), 7.56 (m, 1H), 7.74 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 6.8 Hz), 8.51 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 28.5, 29.2, 29.4, 29.5, 29.6, 31.2, 32.4, 36.0, 40.3, 54.2, 55.6, 62.1, 64.7, 79.0, 93.7, 126.4, 127.9, 128.2, 128.3, 130.0, 130.1, 130.3, 132.1, 135.1, 137.2, 138.0, 148.2, 155.8, 196.5; ^{31}P NMR ($CDCl_3$) δ 0.61; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{37}H_{57}N_2O_9PNa^+$ 727.3694, found 727.3690.

(2*S*,3*R*)-1-*O*-[2′-[^{14}C]Trimethylaminoethyl(hydroxy)-phosphoryl]-2-*N*-(*tert*-butoxycarbonylamido)-3-*O*-methoxymethyl-15-(4′-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (15). To a solution of 12 mg (0.017 mmol) of **14** in 2 mL of dry MeOH in a pressure tube with a stirring bar were added 6 mg (0.043 mmol, ∼2.5 equiv) of [^{14}C]MeI (2.0 mCi, specific activity, 47.0 mCi/mmol) and 72 mg (0.85 mmol) of anhydrous $NaHCO_3$. After the tip of the tube containing [^{14}C]MeI was broken, the contents were transferred to the pressure tube and the vial was washed with MeOH (3 × 0.5 mL). The pressure tube was sealed, the contents were heated to 50 °C (no higher than 65 °C) in an oil bath for 3 h and then cooled to 0 °C in an ice bath, and 50 mg (0.35 mmol) of unlabeled MeI was added. The reaction mixture was again heated to 50 °C in an oil bath for 3 h. The reaction mixture was cooled to room temperature, and the contents of the tube were transferred to a 25-mL

round-bottom flask. The tube was washed with MeOH (3 × 1 mL), and the washings were transferred to the flask. The solvent and excess MeI were removed by evaporation. The residue was dissolved in 10 mL of CH_2Cl_2/ H_2O (1:1), and the solution was transferred to a separatory funnel. The organic layer was collected, and the aqueous layer was washed with CH_2Cl_2 (3 × 3 mL). The combined CH_2Cl_2 layers were washed with brine, water, dried (Na_2SO_4), and concentrated. The residue was purified on a short silica gel column (3 cm); elution was first with 20 mL of $CHCl_3$/MeOH 9:1 (to remove an impurity), and then with 50 mL of $CHCl_3$/MeOH/H_2O 65:25:4 (to collect the product), affording compound **15** (10 mg, 78%) as a white wax. To ensure that the product had been eluted completely, the fraction that was UV active and had R_f 0.23 (developed with $CHCl_3$/MeOH/H_2O 65:25:4) was monitored by TLC. For unlabeled **15**: R_f 0.23 ($CHCl_3$/MeOH/H_2O 65:25:4); $[\alpha]^{25}_D$ −23.6° (*c* 0.75, $CHCl_3$:MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26−1.39 (m, 23H), 1.63 (m, 2H), 2.03 (m, 2H), 2.68 (t, 2H, J = 7.6 Hz), 3.34 (s, 3H), 3.39 (s, 9H), 3.76 (m, 1H), 3.86 (m, 2H), 3.99 (m, 1H), 4.10 (m, 2H), 4.36 (m, 2H), 4.50 (d, 1H, J = 6.4 Hz), 4.67 (d, 1H, J = 6.4 Hz), 5.33 (dd, 1H, J = 8.0, 15.6 Hz), 5.56 (m, 1H), 5.70 (m, 1H), 7.27 (d, 2H, J = 7.2 Hz), 7.47 (t, 2H, J = 7.2 Hz), 7.56 (m, 1H), 7.74 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 28.5, 29.1, 29.3, 29.5, 29.6, 31.2, 32.4, 36.0, 54.5, 55.7, 59.4, 64.2, 66.4, 78.9, 93.8, 126.2, 128.2, 128.3, 130.0, 130.1, 130.3, 132.2, 135.1, 137.2, 138.0, 148.2, 155.8, 196.6; ^{31}P NMR ($CDCl_3$) δ 0.61; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{40}H_{63}N_2O_9PNa^+$ 769.4163, found 769.4159.

(2*S*,3*R*)-1-*O*-[2′-[^{14}C]Trimethylaminoethyl(hydroxy)-phosphoryl]-2-amino-15-(4′-benzoylphenyl)-(4*E*)-pentadecene-1,3-diol (2). (Unlabeled **2** is needed for competitive binding studies with putative membrane proteins.) For unlabeled probe **2**: A solution of **15** (10 mg, 0.013 mmol) in 5 mL of THF and 5 mL of 3 M HCl in a 25-mL round-bottom flask was heated at 70 °C in an oil bath for 5 h. After the solution was cooled to room temperature, the solvents were removed by vacuum evaporation. The residue was dried, and a drop of concentrated NH_4OH was added to neutralize the residue. After about 5 min, NH_4OH was removed, and the residue was dried under vacuum. Dry MeOH (5 mL) was added with stirring, which dissolved the product, leaving some NH_4Cl remaining as a solid. The mixture was filtered through filter paper, which was washed with 3 mL of dry MeOH. The combined MeOH solutions were collected, and the solvent was removed by vacuum evaporation. The residue was dissolved in $CHCl_3$/MeOH/H_2O (65:25:4) and loaded onto a TLC plate, which was developed with $CHCl_3$/MeOH/H_2O (65:25:4). The UV-active band (R_f 0.2) was scraped from the plate and extracted with $CHCl_3$/MeOH/H_2O (65:25:4). The solution was passed through a Cameo syringe filter (elution with $CHCl_3$/MeOH 65:25) to remove traces of suspended silica gel. The solvents were removed and the residue was dried under vacuum to give product **2** as a white solid (6.0 mg, 75%): R_f 0.20 ($CHCl_3$/MeOH/H_2O 65:25:4); mp 178.5 °C-182.3 °C; $[\alpha]^{25}_D$ −5.4 (*c* 0.36, $CHCl_3$/MeOH 1:1); ^{1}H NMR (CD_3OD) δ 1.26−1.39 (m, 14H), 1.63 (m, 2H), 2.13 (m, 2H), 2.74 (t, 2H, J = 7.6 Hz), 3.26 (s, 9H), 3.33 (m, 1H), 3.69 (m, 2H), 4.11 (m, 2H), 4.31 (m, 3H), 5.50 (dd, 1H, J = 8.0, 15.6 Hz), 5.88 (m, 1H), 7.37 (d, 2H, J = 8.0 Hz), 7.55 (t, 2H, J = 8.0 Hz), 7.66 (m, 1H), 7.74 (d, 2H, J = 8.0 Hz), 7.77 (d, 2H, J = 7.2 Hz); ^{13}C NMR (CD_3OD) δ 30.2, 30.3, 30.5, 30.6, 30.7, 32.4, 33.4, 36.9, 54.7, 57.5, 60.7, 62.8, 63.6, 67.7, 70.7, 128.3, 129.5, 129.6, 130.9, 131.4, 133.7, 136.3, 137.2, 139.2, 149.9, 198.5; ^{31}P NMR (CD_3OD) δ −0.38; HR-MS (FAB, MNa^+) *m/z* calcd for $C_{33}H_{51}N_2O_6PNa^+$ 625.3377, found 625.3403. Radioactivity was determined on a liquid-scintillation counter. The specific activity of [^{14}C]-labeled probe **2** was determined to be 3.2 mCi/mmol.

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Supporting Information Available: Preparation of compound **11**; ^{1}H and ^{13}C NMR spectra for compounds **2**, **5**−**10**, and **13**−**15**; and ^{31}P NMR spectra for compounds **2** and **13**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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