Supporting Information

Caged Ceramide 1-Phosphate Analogues: Synthesis and Activity

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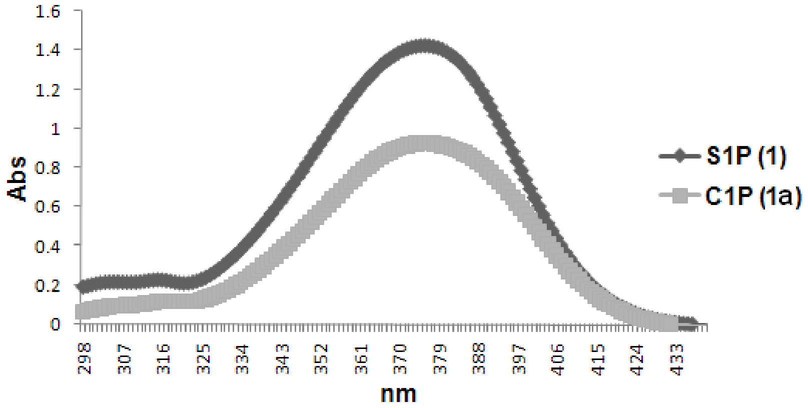
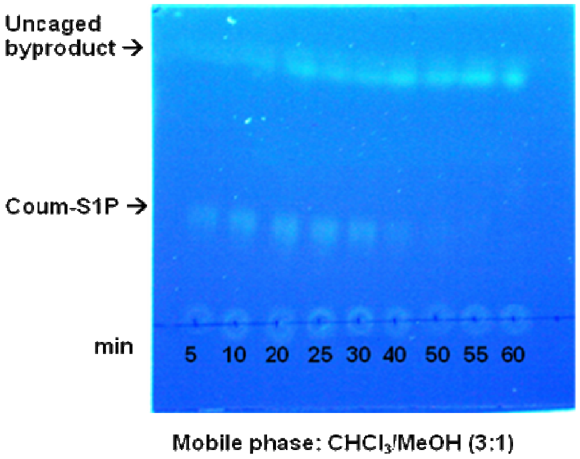
Absorption Spectra of DECM-caged S1P and C1P Analogues. Figure 1 shows the UV-visible spectra of compounds **1** and **1a**. DECM-caged S1P **1** shows a λ_{max} at 376 nm (ϵ = 15,792 $M^{-1}cm^{-1}$) in EtOH. DECM-caged C1P **1a** shows a λ_{max} at 376 nm (ϵ = 15,466 $M^{-1}cm^{-1}$) in CH_2Cl_2.

Figure 1. Absorption spectra of DECM-caged S1P (**1**) in EtOH and C1P (**1a**) in CH_2Cl_2.



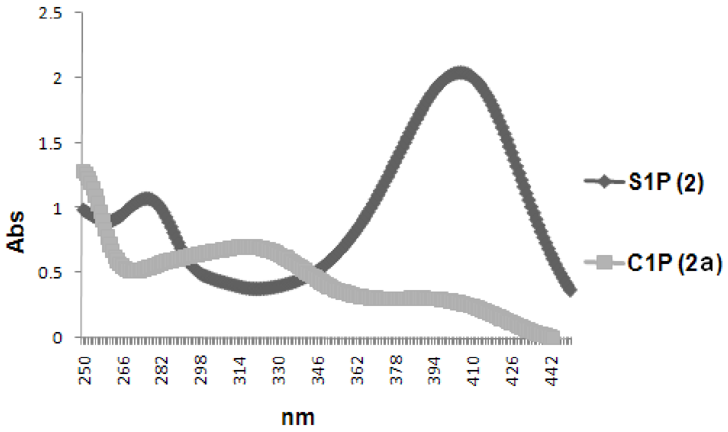
Qualitative Measurement of Uncaging Efficiency. A 100 µM solution of S1P **1** in 50% aqueous EtOH and 50% 10 mM Tris, pH 7.4, was placed in a quartz cuvette and placed at a distance of 1 cm from a conventional UV lamp (Entela UVGL-25 UV lamp). The compound was irradiated using long wavelength (365 nm) and, after a certain time, as indicated in the TLC (Figure 2), an aliquot was applied to a TLC plate, which was developed with $CHCl_3$/MeOH 3:1. Photodecomposition of 100% DECM-caged S1P **1** took place around 60 min.

Figure 2. Qualitative measurement of uncaging efficiency of DECM-caged S1P (**1**) in 50% aqueous EtOH, pH 7.4.



Absorption Spectra of BHNB-caged S1P and C1P Analogues. Figure 3 (page S4) shows the UV-visible spectra of compounds **2** and **2a**. BHNB-caged S1P **2** absorbs at 406 nm (ϵ = 12,000 $M^{-1}cm^{-1}$) and 278 nm (ϵ = 6,294 $M^{-1}cm^{-1}$) in 50% aqueous EtOH, 50% 10 mM Tris, pH 7.4. BHNB-caged C1P **2a** absorbs at 397 nm (ϵ = 1,747 $M^{-1}cm^{-1}$) and 320 nm (ϵ = 4,111 $M^{-1}cm^{-1}$) in EtOH.

Figure 3. Absorption spectra of BHNB-caged S1P (**2**) in 50% aqueous EtOH, pH 7.4, and BHNB-caged C1P (**2a**) in EtOH.

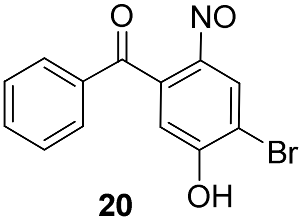


Stability of BHNPB-caged S1P (2) in Buffer in the Dark. The stability of BHNB-caged S1P (**2**) in 50% aqueous EtOH, 50% 10 mM Tris (170 µM), pH 7.4, was tested by withdrawing aliquots from the solution over a period of seven days. The analysis showed no change in the R_f value (0.35, $CHCl_3$/MeOH 3:1) of the product, and no new spot was observed.

Qualitative Measurement of Uncaging Efficiency. A 100 µM solution of BHNB-caged S1P (**2**) in 50% aqueous EtOH and 50% 10 mM Tris, pH 7.4, was placed in a quartz cuvette and irradiated at 365 nm at a distance of 1 cm with a conventional UV lamp (Entela UVGL-25 UV lamp). Aliquots were withdrawn at various times and analyzed by TLC (developed using $CHCl_3$/MeOH 2:1). The mechanism of photolysis of 2-nitrobenzyl caged compounds has been thoroughly studied and is known to generate 2-nitrosobenzaldehyde as the photo by-product of the reaction.[S1] Similarly, our BHNB uncaging experiment is expected to generate a 2-nitrosobenzophenone derivative as the byproduct (Figure 4). After being photoirradiated for 65 min, the sample in the cuvette was concentrated and analysis by ESI-HRMS showed a strong peak at *m/z* 327.9596

(M+Na)$^+$, which is the expected mass of the photo by-product. Figure 5 shows that 100% photodecomposition of BHNB-S1P (**2**) took place after 65 min.

Figure 4. Uncaged photo-byproduct, 4-bromo-5-hydroxy-2-nitroso-benzophenone.



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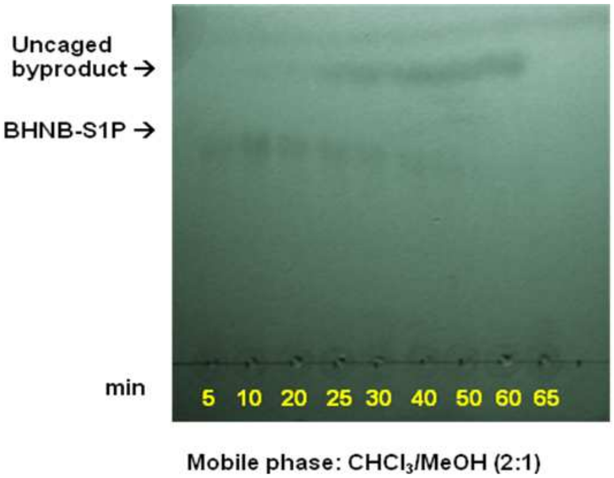
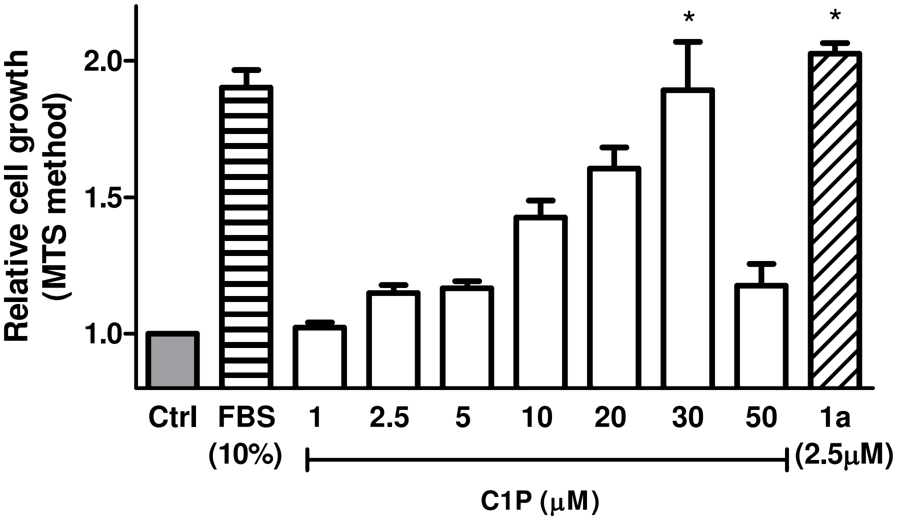
ESI-HRMS [M+Na]$^+$ $C_{13}H_8{}^{79}BrNO_3Na$ calcd for *m/z* 327.9585, found 327.9596

Figure 5. Qualitative measurement of uncaging efficiency of BHNB-caged S1P (**2**) in 50% aqueous EtOH, pH 7.4.



Cell Growth and Proliferation Assays. RAW 264.7 cells were obtained from the American Type Culture Collection (Rockville, MD). BMDMs were isolated from femurs of 6- to 8-week old female CD-1 mice from Charles River Laboratory as described previously.[S2] The cells were cultured as described previously. Cell growth was estimated by measuring the rate of reduction of the tetrazolium dye MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenol)-2-(4-sulfophenyl)-2H-tetrazolium). Aqueous dispersions of commercial N-palmitoyl-C1P were prepared by sonication and added to the cultured macrophages as described previously.[S3,S4] Figure 6 shows that the optimum concentration of exogenous N-palmitoyl-C1P for stimulation of RAW 264.7 macrophage cell growth was 30 μM; higher concentrations than 30 μM are growth inhibitory. Photolysis of 2.5 μM of compound **1a** (delivered to RAW 264.7 cells in EtOH) was equipotent as exogenous 30 μM C1P.

Figure 6. Stimulation of growth of RAW 264.7 cells on addition of FBS, exogenous N-palmitoyl-C1P, and compound **1a**. The cells were exposed to 400-500 nm light in a transilluminator equipped with a 9W lamp for 60 min at a distance of 1.5 cm at 37 °C.



General Experimental Methods. The solvents were dried as follows. THF and Et_2O were heated at reflux over sodium benzophenone ketyl. Acetonitrile, diisopropylethylamine, and CH_2Cl_2 were distilled over calcium hydride, benzene was distilled over sodium metal, and methanol was heated at reflux over magnesium. Silica gel 60 F254 aluminum TLC plates of 0.2-mm thickness were used to monitor the reactions, with short wavelength ultraviolet light to visualize the spots and by charring the TLC plate after spraying with 15% sulfuric acid. Phosphorus-containing compounds were detected with a molybdic acid spray. Flash chromatography was carried out with silica gel 60 (230-400 ASTM mesh). ^1H NMR spectra were recorded at 400 and 500 MHz, and chemical shifts are given in parts per million. ^{13}C NMR and ^{31}P NMR spectra were recorded at 100 MHz and 162 MHz, respectively.

Syntheses

(*S*)-*tert*-Butyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (3). A solution of *N*-Boc-(*S*)-serine methyl ester (12.0 g, 46.3 mmol) in toluene (100 mL) was cooled to -78 °C under nitrogen. To the cooled solution was slowly added DIBAL-H (83 mL, a 1.5 M solution in toluene). The reaction mixture was stirred for 2 h at -78 °C, and the reaction was quenched by slowly adding 100 mL of cold MeOH. The resulting white emulsion was slowly poured into 200 mL of ice-cold 1 M HCl with swirling over 20 min, and the aqueous mixture was extracted with EtOAc (3 x 120 mL). The combined organic layers were washed with brine (150 mL), dried (Na_2SO_4), and concentrated to give the crude product as a colorless oil, which was vacuum distilled to provide aldehyde **3** (7.5 g, 71%) as a colorless liquid, bp 115-125 °C (9.0 mm Hg).

(*S*)-*tert*-Butyl-4-((*R*)-1-hydroxyallyl)-2,2-dimethyloxazolidine-3-carboxylate (4). To a -78 °C solution of (*S*)-Garner aldehyde (**3**, 7.0 g, 30.5 mmol) in dry THF (50 mL) under N_2 was slowly added vinylmagnesium bromide (92 mL, 92 mmol, a 1 M solution in THF) via cannula. After the cloudy yellow solution was stirred for 2 h at -78 °C, it was warmed to 0 °C, and the reaction was quenched with saturated aqueous NH_4Cl solution (60 mL). The product was extracted with Et_2O (2 x 60 mL). The combined organic layers were washed with brine (60 mL), dried (Na_2SO_4), and concentrated. The mixture of erythro and threo diastereomers was separated by column chromatography by gravity (hexane/EtOAc 5:1) to afford **4** (4.8 g, 62%) and its C3-epimer **4a** (0.8 g, 10.2%). Compound **4**: $[\alpha]^{25}_D$ -37.7° (*c* 1.8, $CHCl_3$); R_f 0.26 (EtOAc/hexane 1:3); ^1H NMR ($CDCl_3$) δ 1.48 (s, 3H), 1.50 (s, 9H), 1.58 (s, 3H), 3.91 (m, 2H), 3.98 (m, 1H), 4.25 (m, 1H), 4.39 (m, 1H), 5.24 (m, 1H), 5.38 (m, 1H), 5.85 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.3, 28.3, 61.8, 64.4, 73.3, 81.4, 94.4, 116.1, 117.8, 137.6, 155.0; ESI-HRMS $[M+Na]^+$ $C_{13}H_{23}NO_4Na$ calcd for *m/z* 280.1525, found 280.1519.

***tert*-Butyl-(2*S*,3*R*)-1-hydroxy-3-(methoxymethoxy)pent-4-en-2-yl-carbamate (6).** To a solution of allylic alcohol **4** (900 mg, 3.5 mmol) in CH_2Cl_2 (25 mL) were added DIPEA (1.5 mL, 8.7 mmol) and MOMCl (0.56 mL, 7.0 mmol) at 0 °C. After 10 min, the cooling bath was removed, and the reaction mixture was stirred overnight at rt. After TLC indicated the complete protection of the alcohol, the reaction mixture was diluted with saturated aqueous NH_4Cl solution (40 mL) and the product was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to afford the MOM-protected alcohol **5** (960 mg, 91%). Without further purification, compound **5** (960 mg, 3.2 mmol) was dissolved in THF (25 mL), and 1 M HCl (5 mL) was added. The reaction mixture was stirred at rt until the starting material disappeared (overnight). The acid was neutralized by washing with saturated aqueous $NaHCO_3$ (30 mL) and the product was extracted with CH_2Cl_2 (2 x 25 mL). The organic layer was purified by chromatography (hexane/EtOAc 3:1) to afford alcohol **6** (705 mg, 85%): R_f 0.20 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.21 (br s, 1H), 3.39 (s, 3H), 3.68 (m, 2H), 3.88 (dd, 1H, *J* = 4.2, 11.6 Hz), 4.27 (t, 1H, *J* = 5.0 Hz), 4.57 (d, 1H, *J* = 6.6 Hz), 4.65 (d, 1H, *J* = 6.6 Hz), 5.32 (m, 3H), 5.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.9, 54.6, 55.4, 62.0, 76.0, 78.3, 79.4, 94.0, 119.0, 134.7, 156.3; ESI-HRMS $[M+Na]^+$ $C_{12}H_{23}NO_5Na$ calcd for *m/z* 284.1474, found 284.1468.

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-hydroxy-3-(methoxymethoxy)octadec-4-en-2-yl-carbamate (7).** To a solution of alcohol **6** (230 mg, 0.88 mmol) in CH_2Cl_2 (8 mL) was added 1-pentadecene (741 mg, 3.52 mmol). The solution was degassed twice using N_2 (2 x 10 min). To the reaction mixture was added benzylidene[1,3-bis(2,4,6-trimethyl-phenyl)-2-imidazolidinylidene]dichloro-(tricyclohexylphosphine)ruthenium catalyst (22.4 mg, 0.026 mmol) at rt under N_2. After the reaction mixture was stirred for 4 h under reflux, the solvent was removed and the product was purified by chromatography (hexane/EtOAc 4:1) to afford **7** (280 mg, 72%): R_f 0.45 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 7.0 Hz), 1.25 (m, 24H), 1.44 (m, 9H), 2.06 (q, 2H, *J* = 7.7 Hz), 2.89 (d, 1H, *J* = 5.2 Hz), 3.38 (s, 3H), 3.67 (m, 2H), 3.94 (dt, 1H, *J* = 3.2, 11.1 Hz), 4.22 (dd, 1H, *J* = 5.0, 7.6 Hz), 4.53 (d, 1H, *J* = 6.6 Hz), 4.67 (d, 1H, *J* = 6.6 Hz), 5.26 (d, 1H, *J* = 7.6 Hz), 5.36 (dd, 1H, *J* = 8.0, 15.4 Hz), 5.75 (dt, 1H, *J* = 6.6, 14.6 Hz); ^{13}C NMR

($CDCl_3$) δ 14.1, 22.7, 28.4, 29.0, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 54.9, 55.6, 62.3, 76.1, 78.3, 79.4, 93.8, 125.9, 136.9, 155.9; ESI-HRMS $[M+Na]^+$ $C_{25}H_{49}NO_5Na$ calcd for *m/z* 466.3508, found 466.3514.

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-[7-diethylaminocoumarin-4-yl-methoxy(methoxy)phosphor-yloxy]-3-(methoxymethoxy)octadec-4-en-2-yl-carbamate (10).** To a solution of compound **7** (55 mg, 0.124 mmol) in THF (8 mL) was added DIPEA (86.4 μL, 0.49 mmol). Methyl diisopropylphosphoramidochloridite (48 μL, 0.24 mmol) was added dropwise to the reaction mixture at 0 °C under N_2. After the reaction mixture was stirred at the same temperature for 30 min, H_2O (10 mL) was added, and the product was extracted with EtOAc (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated, and the residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford phosphoramidite **8**: R_f 0.68 (hexane/EtOAc 3:1); ^{31}P NMR ($CDCl_3$) δ 140.8. 7-(Diethylamino)-4-hydroxymethylcoumarin (**9**) was prepared from 4-methyl-7-(diethylamino)coumarin by a known procedure.[S5,S6] A mixture of coumarin **9** (71 mg, 0.28 mmol) and phosphoramidite **8** (58 mg, 0.096 mmol) was dried by lyophilization from benzene, and the residue was dissolved in CH_3CN (8 mL). The resulting solution was transferred by cannula to a solution of 1*H*-tetrazole (34 mg, 0.48 mmol) in CH_3CN (3 mL) at rt. After 3 h, the consumption of the phosphoramidite was complete as observed by TLC (hexane/EtOAc 3:1). To the intermediate phosphite, *t*-BuOOH (0.14 mL, a 1 M solution in toluene, 0.14 mmol) was added at 0 °C, and the reaction mixture was warmed to rt. After 4 h, TLC indicated the complete conversion of the phosphite to the corresponding phosphate. The reaction mixture was diluted with

CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ solution (15 mL). The organic layer was dried (Na_2SO_4), concentrated, and the residue was purified by chromatography (hexane/EtOAc 2:1) to afford phosphate **10** (52 mg, 55%): $[\alpha]^{25}_{D}$ -26.5° (*c* 0.6, $CHCl_3$); R_f 0.22 (EtOAc/hexane/Et_3N 48:50:2); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20 (t, 6H, *J* = 7.1 Hz), 1.25 (m, 23H), 1.41 (s, 9H), 2.03 (q, 2H, *J* = 6.5 Hz), 3.35 (s, 3H), 3.42 (q, 4H, *J* = 7.1 Hz), 3.84 (d, 3H, *J* = 10.5 Hz), 3.91 (br s, 1H), 4.09 (t, 1H, *J* = 7.2 Hz), 4.25 (m, 1H), 4.31 (m, 1H), 4.50 (d, 1H, *J* = 6.6 Hz), 4.69 (d, 1H, *J* = 6.6 Hz), 4.95 (t, 1H, *J* = 6.4 Hz), 5.20 (m, 2H), 5.29 (dd, 1H, *J* = 8.4, 15.0 Hz), 5.75 (dt, 1H, *J* = 5.9, 14.1 Hz), 6.21 (s, 1H), 6.50 (d, 1H, *J* = 2.4 Hz), 6.58 (dd, 1H, *J* = 2.5, 9.0 Hz), 7.29 (d, 1H, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 12.4, 14.1, 22.7, 28.3, 29.0, 29.2, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 44.7, 54.7, 54.8, 55.7, 64.6, 67.0, 76.2, 79.6, 93.5, 97.8, 105.5, 106.3, 108.7, 124.3, 125.6, 138.1, 149.1, 150.7, 155.4, 156.2, 161.7; ^{31}P NMR ($CDCl_3$) δ 0.43; ESI-HRMS $[M+Na]^+$ $C_{40}H_{67}N_2O_{10}PNa$ calcd for *m/z* 789.4431, found 789.4417.

(2*S*,3*R*,4*E*)-2-Amino-3-hydroxyoctadec-4-enyl (7-(Diethylamino)coumarin-4-yl)-methyl Phosphate (1). To a solution of phosphate **10** (25 mg, 0.032 mmol) in THF (1 mL) was added 6 M HCl (2 mL). The reaction mixture was stirred at 60 °C for 6 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and brine (10 mL). The product was extracted with CH_2Cl_2 (3 x 15 mL), dried (K_2CO_3), and purified by chromatography ($CHCl_3$/MeOH 6:1). After removal of suspended silica gel by filtration of a solution of **1** in $CHCl_3$ through a 0.45-μm Cameo 30F syringe filter, compound **1** was obtained (15.5 mg, 78%): $[\alpha]^{25}_{D}$ +2.22° (*c* 0.6, $CHCl_3$/MeOH 1:1); R_f 0.70 ($CHCl_3$/MeOH/H_2O

65:35:8); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 5.5 Hz), 1.23 (m, 26H), 1.48 (m, 2H), 1.98 (q, 2H, *J* = 5.9 Hz), 3.20-3.79 (m, 9H), 4.15 (m, 2H), 4.41 (m, 1H), 5.07 (m, 2H), 5.40 (dd, 1H, *J* = 5.4, 15.2 Hz), 5.81 (dt, 1H, *J* = 5.6, 13.4 Hz), 6.26 (s, 1H), 6.41 (s, 1H), 6.51 (d, 1H, *J* = 8.0 Hz), 7.29 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 12.3, 14.0, 22.6, 28.2, 29.0, 29.3, 29.4, 29.6, 31.8, 32.3, 44.6, 55.8, 62.4, 63.1, 69.6, 97.3, 104.7, 108.8, 124.4, 126.2, 135.5, 150.6, 155.8, 163.0; ^{31}P NMR ($CDCl_3$) δ -0.38; ESI-HRMS $[M+H]^+$ $C_{32}H_{54}N_2O_7P$ calcd for *m/z* 609.3669, found 609.3670; UV: λ_{max} 376 nm (ϵ = 15792 $M^{-1}cm^{-1}$) in EtOH.

(2*S*,3*R*,4*E*)-2-Palmitamido-3-hydroxyoctadec-4-enyl (7-Diethylaminocoumarin-4-yl)methyl Phosphate (1a). A solution of compound **1** (7.0 mg, 0.010 mmol), *p*-nitrophenyl palmitate (9.4 mg, 0.023 mmol), and anhydrous potassium carbonate (3.6 mg, 0.024 mmol) was suspended in a solution of anhydrous DMF (2.5 mL) and CH_2Cl_2 (1 mL). After the reaction mixture had stirred for two days, the mixture was concentrated under high vacuum. The product was purified by chromatography ($CHCl_3$/MeOH 9:1), followed by removal of suspended silica gel by filtration of a solution of **1a** in $CHCl_3$ through a 0.45-μm Cameo 30F syringe filter, affording DECM-caged ceramide 1-phosphate **1a** (7.0 mg, 82%) as a yellow solid: R_f 0.60 ($CHCl_3$/MeOH 2:1); $[\alpha]^{25}_D$ +4.73° (*c* 0.27, $CHCl_3$:MeOH 1:1); R_f 0.62 ($CHCl_3$/MeOH 2:1); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, *J* = 6.5 Hz), 1.16-1.53 (m, 46H), 1.96 (q, 2H, *J* = 6.9 Hz), 2.13 (t, 1H, *J* = 7.3 Hz), 3.38 (m, 4H), 3.88 (m, 1H), 4.01 (m, 1H), 4.12 (t, 1H, *J* = 7.2 Hz), 4.26 (m, 1H), 5.04 (m, 2H), 5.44 (dd, 1H, *J* = 7.2, 14.8 Hz), 5.70 (dt, 1H, *J* = 6.3, 14.1 Hz), 6.28 (s, 1H), 6.43 (s, 1H), 6.54 (d, 1H, *J* = 8.0 Hz), 7.27 (d, 1H, *J* = 8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 22.9, 23.6, 25.8, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 30.2, 31.9, 32.4, 36.4, 38.6, 44.6, 68.2, 97.4, 105.8, 108.8, 128.7, 130.9, 132.2, 134.7, 150.6, 155.8, 163.3, 167.9, 174.2; ^{31}P NMR ($CDCl_3$) δ -0.83; ESI-HRMS $(M+H)^+$ $C_{48}H_{84}N_2O_8P$ calcd for *m/z* 847.5965, found 847.5973; UV: λ_{max} 376 nm (ϵ = 15466 $M^{-1}cm^{-1}$) in EtOH.

1-Bromo-2-methoxy-4-methyl-5-nitrobenzene (12). To a solution of nitrobenzene **11** (2.2 g, 13.1 mmol) in CH_3CN (8 mL) in a pressure tube was added *N*-bromosuccinimide (8.2 g, 45.8 mmol). The tube was sealed and the contents were heated to 140 °C overnight on an oil bath. The reaction mixture turned brownish-red overnight. After the reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (150 mL), the product was extracted with Et_2O (2 x 150 mL), dried (Na_2SO_4), and purified by chromatography (hexane/EtOAc 3:1) to afford brominated product **12** along with other byproducts that were not separable by chromatography (hexane/EtOAc 3:1). The product mixture (2.8 g, 87%) was used directly in the next step: R_f 0.29 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 2.66 (s, 3H), 3.98 (s, 3H), 6.76 (s, 1H), 8.31 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.7, 56.8, 108.9, 114.3, 125.2, 130.4, 136.3, 141.9, 159.3; ESI-HRMS $[M-Br-CH_3-H]^-$ $C_7H_6NO_3$ calcd for *m/z* 152.0348, found 152.0349.

4-Bromo-5-methoxy-2-nitrobenzaldehyde (13). To a stirred solution of compound **12** (2.3 g, 9.4 mmol) in dry DMF (15 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (DMF·DMA) (6.0 g, 24.5 mmol). After the reaction mixture was heated at 140 °C for 16 h, the dark red solution was cooled to 0 °C and added rapidly to a stirred solution of $NaIO_4$ (10.5 g, 49 mmol) in H_2O/DMF (4:1, 25 mL) at 0 °C. After 8 h of stirring, the brown solution was filtered, and the reaction flask was rinsed with toluene/EtOAc (1:1, 30 mL) and again filtered. The filtrate was washed with saturated aqueous NaCl solution (45 mL) and extracted with EtOAc (2 x 45 mL). The organic phase was dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford aldehyde **13** (1.3 g, 54%): R_f 0.22 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 4.09 (s, 3H), 7.38 (s, 1H), 8.43 (s, 1H), 10.50 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 57.4, 110.5, 116.8, 130.3, 132.6, 142.3, 160.5, 187.7; ESI-HRMS $[M-H]^-$ $C_8H_5{}^{79}BrNO_4$ calcd for *m/z* 257.9402, found 257.9407.

(4-Bromo-5-methoxy-2-nitrophenyl)(phenyl)methanol (14). To a solution of aldehyde **13** (200 mg, 0.77 mmol) in THF (10 mL) at -78 °C was added slowly a solution of PhMgBr (2.7 mL, a 1.0 M solution in THF, 2.7 mmol). After the reaction mixture was stirred at this temperature for 30 min, the reaction was quenched with 1 M HCl (15 mL). The product was extracted with Et_2O (2 x 20 mL). The organic layer was dried (Na_2SO_4), and concentrated, and the residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford **14** (240 mg, 92%): R_f 0.32 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 2.96 (br s, 1H), 3.96 (s, 3H), 6.52 (s, 1H), 7.27 (m, 5H), 7.37 (s, 1H), 8.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 57.0, 71.7, 110.5, 110.9, 127.1, 128.3, 128.7, 130.6, 140.6, 141.2, 160.0: ESI-HRMS $[M+HCO_2]^-$ $C_{15}H_{13}{}^{79}BrNO_6$ calcd for *m/z* 381.9926, found 381.9931.

(4*S*,5*R*,1'*E*)-*tert*-Butyl-4-(hydroxymethyl)-2,2-dimethyl-5-(pentadec-1'-enyl)-oxazolidine-3-carboxylate (16).[S7] To a solution of D-*erythro*-sphingosine (400 mg, 1.3 mmol) in EtOH/H_2O (2:1, 9 mL) was added aqueous 1 N NaOH (3.9 mL, 3.9 mmol) and

$(Boc)_2O$ (851 mg, 3.9 mmol). The reaction mixture was stirred at rt until the consumption of the starting material was observed by TLC (hexane/EtOAc 3:1): R_f 0.12. The reaction was quenched with saturated aqueous NH_4Cl solution (30 mL), and the product was extracted with EtOAc (2 x 25 mL), dried (Na_2SO_4), and concentrated. The resulting residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford the *N*-Boc-sphingosine derivative (495 mg, 95%): R_f 0.22. The Boc derivative (180 mg, 0.45 mmol) was thoroughly dried in vacuum and then was dissolved in CH_2Cl_2 (10 mL). After imidazole (122 mg, 1.8 mmol) and TBDPSCl (236 μL, 0.90 mmol) were added, the reaction mixture was stirred for 3 h at rt, and then was concentrated. The residue was purified by flash chromatography (hexane/EtOAc 3:1): R_f 0.65. To a solution of the silylated primary alcohol in benzene (30 mL) were added 2,2-dimethoxypropane (220 μL, 1.8 mmol) and catalytic *p*-$TsOH{\cdot}H_2O$ (1 mg, 0.010 mmol). The reaction mixture was heated at reflux in a Dean-Stark trap for 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (15 mL), and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford the isopropylidene derivative: R_f 0.75. The isopropylidene intermediate was treated with TBAF (11.25 mL, 1 M solution in THF, 11.25 mmol) for 6 h at rt. The reaction mixture was diluted with H_2O (10 mL), and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford protected sphingosine derivative **16** (92 mg, 47%): R_f 0.51 (hexane/EtOAc 3:1); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.26 (m, 22H), 1.49 (s, 9H), 1.54 (s, 3H), 1.60 (s, 3H), 2.07 (q, 2H, J = 5.6 Hz), 3.50 (br s, 1H), 3.65 (m, 1H), 3.80 (m, 1H), 4.09 (m, 1H), 4.57 (m, 1H), 5.46 (dd, 1H, J = 7.5, 15.2

Hz), 5.88 (dt, 1H, *J* = 6.5, 13.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.7, 27.8, 28.4, 28.8, 29.2, 29.3, 29.4, 29.5, 29.7, 31.9, 62.0, 63.8, 81.2, 92.9, 123.2, 137.5, 154.4; ESI-HRMS [M+Na]$^+$ $C_{26}H_{49}NNaO_4$ calcd for *m/z* 462.3559, found 462.3554.

(4*S*,5*R*,1”*E*)-*tert*-Butyl-4-((((4’-bromo-5’-methoxy-2’-nitrophenyl)(phenyl)methoxy)(methoxy)phosphoryloxy)methyl)-2,2-dimethyl-5-(pentadec-1”-enyl)oxazolidine-3-carboxylate (18). To a solution of compound **14** (44 mg, 0.13 mmol) in THF (8 mL) was added DIPEA (54 μL, 0.31 mmol). Methyl diisopropyl phosphoramidochloridite (51 μL, 0.26 mmol) was then added slowly to the reaction mixture at rt under N_2. After the reaction mixture was stirred at the same temperature for 3 h, H_2O (8 mL) was added and the product was extracted with EtOAc (2 x 8 mL). The organic layer was dried (Na_2SO_4), concentrated, and the product was purified by flash chromatography (hexane/EtOAc 3:1) to afford phosphoramidite **15**: R$_f$ 0.65 (hexane/EtOAc 3:1). A mixture of phosphoramidite **15** (52 mg, 0.10 mmol) and protected sphingosine **16** (88 mg, 0.20 mmol) was dried by lyophilization from benzene, and the residue was dissolved in CH_3CN (5 mL). The resulting solution was transferred by cannula to a solution of 1*H*-tetrazole (21 mg, 0.30 mmol) in CH_3CN (3 mL) at rt. After 3 h, complete consumption of the phosphoramidite took place as observed by TLC (hexane/EtOAc 3:1). The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford phosphite **17**: R_f 0.55. To the intermediate phosphite **17** was added *t*-BuOOH (0.3 mL, a 1 M solution in toluene) at rt. After 1 h, TLC indicated the complete conversion of the phosphite to the corresponding phosphate. The reaction mixture was concentrated and purified by chromatography (hexane/EtOAc 3:1) to afford phosphate **18** (60 mg, 70%): R_f 0.15 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.7 Hz), 1.26 (m, 22H), 1.46 (s, 9H), 1.53 (s, 3H), 1.64 (s, 3H), 2.07 (m, 2H), 3.64 (m, 3H), 3.86 (m, 1H), 3.99 (m, 1H), 4.06 (m, 4H), 4.50 (m, 1H), 5.48 (m, 1H), 5.88 (m, 1H), 7.25 (m, 1H), 7.32 (m, 6H), 7.51 (m, 1H), 8.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.3, 29.0, 29.4, 29.5, 29.6, 29.7, 31.9, 32.5, 54.4, 57.1, 59.2, 80.3, 93.1, 110.0, 111.3, 122.8, 127.9, 128.0, 128.6, 129.0, 130.8, 137.9, 139.8, 160.1; ^{31}P NMR ($CDCl_3$) δ -1.60, -1.46, -1.31, -1.13; ESI-HRMS $[M+Na]^+$ $C_{41}H_{62}BrN_2O_{10}PNa$ calcd for *m/z*, 875.3223 found 875.3218.

(4’-Bromo-5’-hydroxy-2’-nitrophenyl)(phenyl)methyl-(2*S*,3*R*,4*E*)-3-hydroxy-2-palmitamidooctadec-4-enyl Hydrogen Phosphate (2a). A solution of compound **2** (1.0 mg, 1.5 μmol), *p*-nitrophenyl palmitate (1.7 mg, 4.5 μmol), and anhydrous potassium carbonate (1 mg, 4.5 μmol) was suspended in a solution of anhydrous DMF (1.25 mL) and CH_2Cl_2 (0.5 mL). After the reaction mixture had stirred for 2 days, the mixture was concentrated under high vacuum. The compound was purified by chromatography ($CHCl_3$/MeOH 6:1), followed by removal of suspended silica gel by filtration of a solution of compound **2a** in $CHCl_3$ through a 0.45-μm 30F syringe filter, affording BHNB caged C1P **2a** (1 mg, 77%): R_f 0.33 ($CHCl_3$/MeOH 6:1); ^{1}H NMR ($CDCl_3$/CD_3OD 30:1) δ 0.83 (t, 6H, *J* = 6.8 Hz), 1.25 (m, 46H), 1.92 (m, 2H), 2.10 (m, 2H), 3.58 (m, 1H), 3.83 (m, 1H), 3.92 (m, 2H), 4.0 (m, 1H), 5.34 (m, 1H), 5.62 (m, 1H), 7.02 (m, 1H), 7.23 (m, 5H), 7.50 (m, 1H), 8.29 (m, 1H); ^{13}C DEPT-45 NMR ($CDCl_3$/CD_3OD 30:1) δ 14.1, 19.8, 22.7, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 30.2, 31.9, 32.4, 36.5, 53.5, 57.0, 71.4, 74.4, 77.4, 110.8, 127.5, 127.7, 128.2, 128.4, 130.3, 135.0; ^{31}P NMR ($CDCl_3/CD_3OD$ 30:1) δ -1.23, -1.11; ESI-HRMS $[M+Na]^+$ $C_{47}H_{76}BrN_2O_9PNa$ calcd for *m/z* 945.4369, found 945.4363; UV: λ_{max} 397 nm (ϵ = 1,747 $M^{-1}cm^{-1}$), 320 nm (ϵ = 4,111 $M^{-1}cm^{-1}$) in EtOH.

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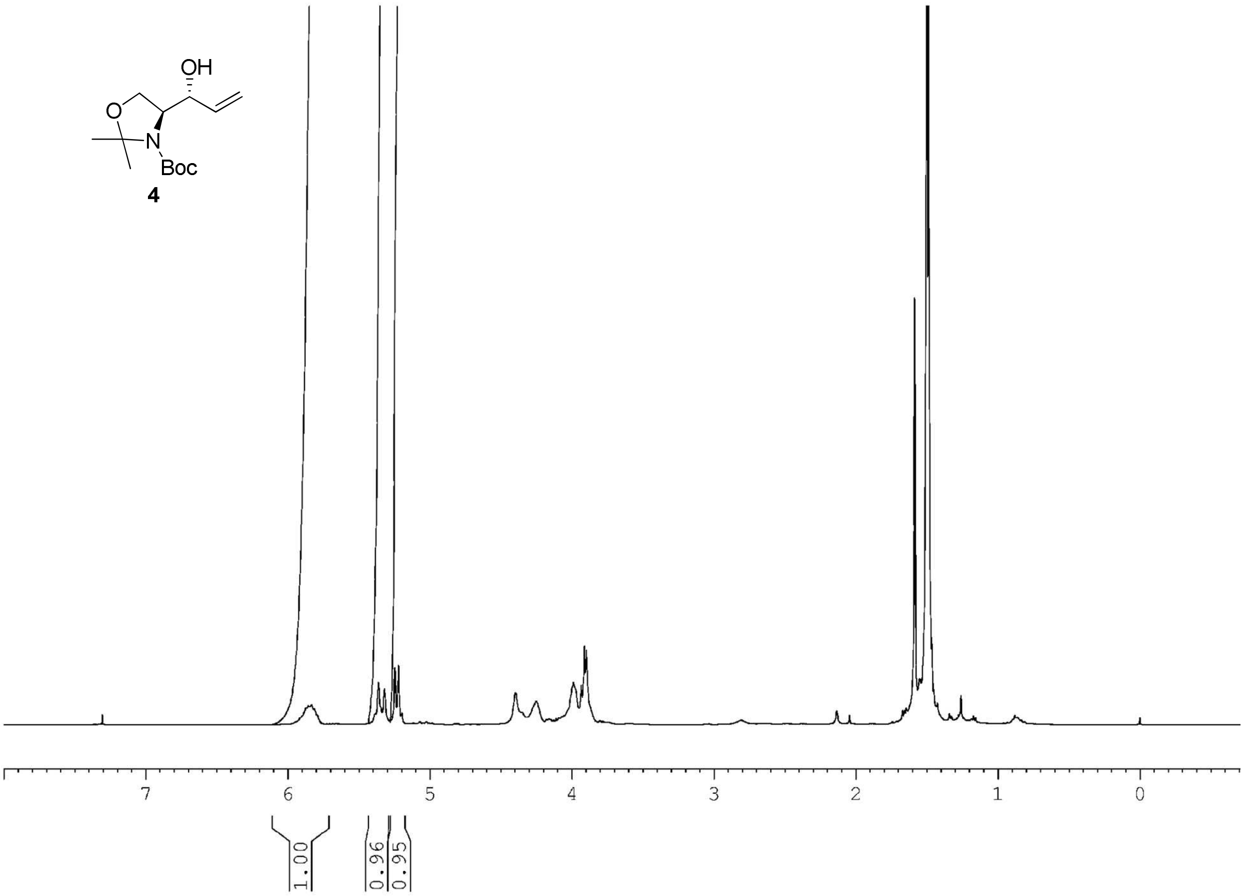
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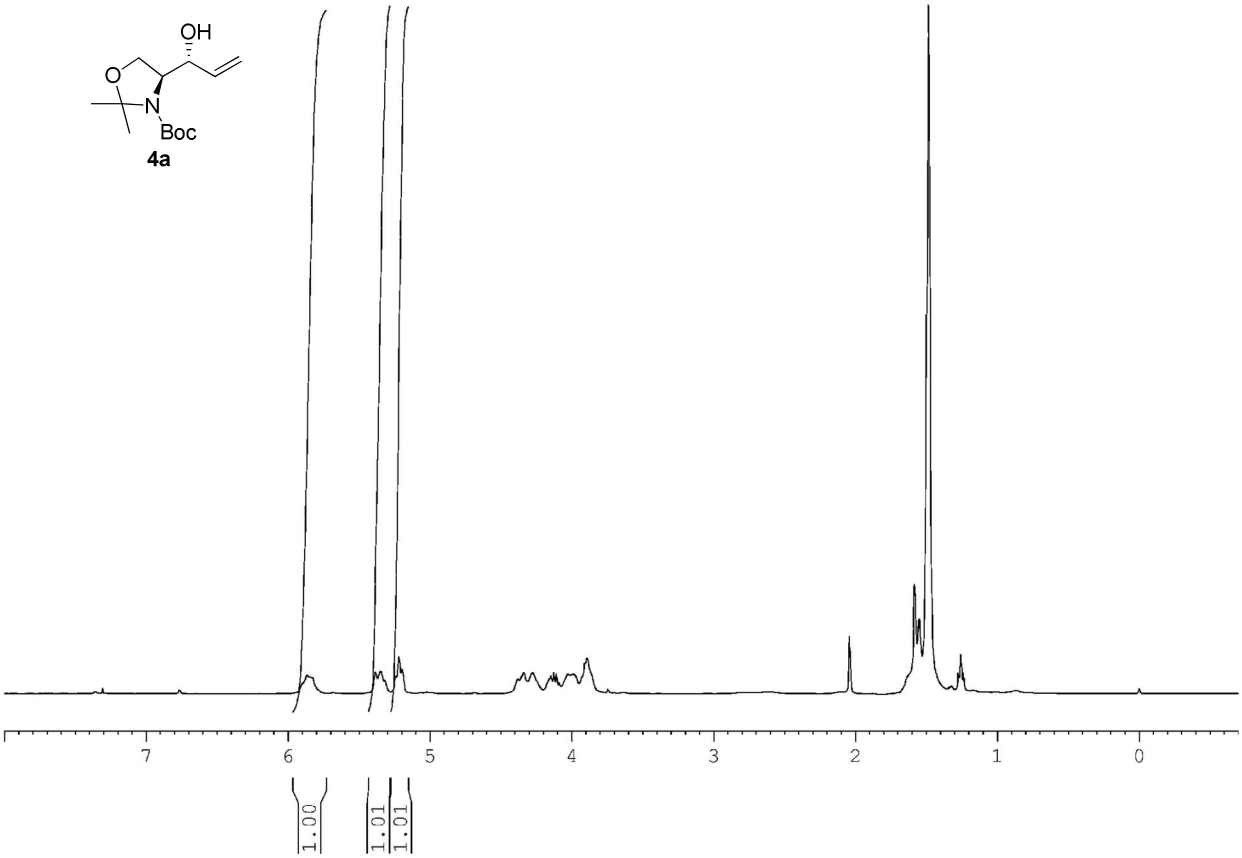

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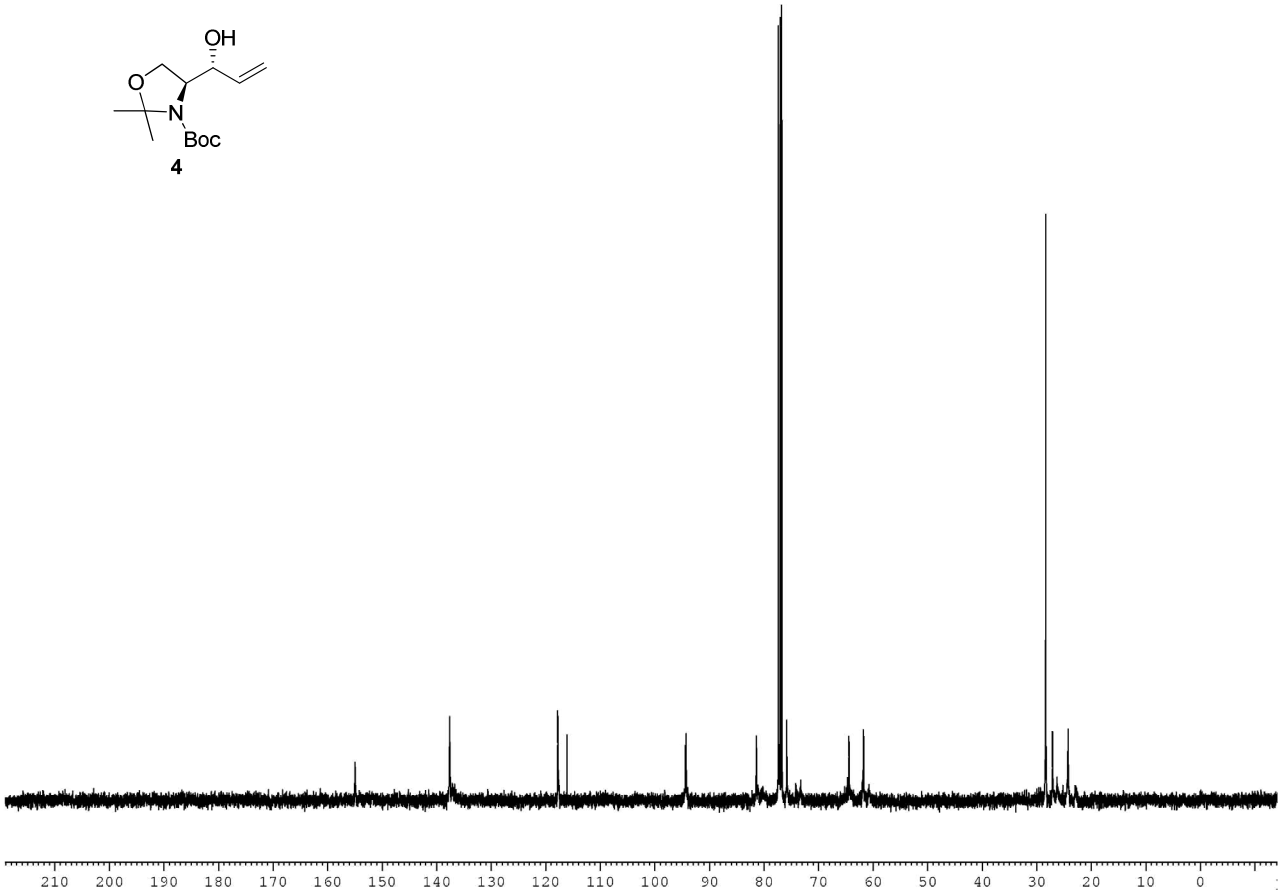
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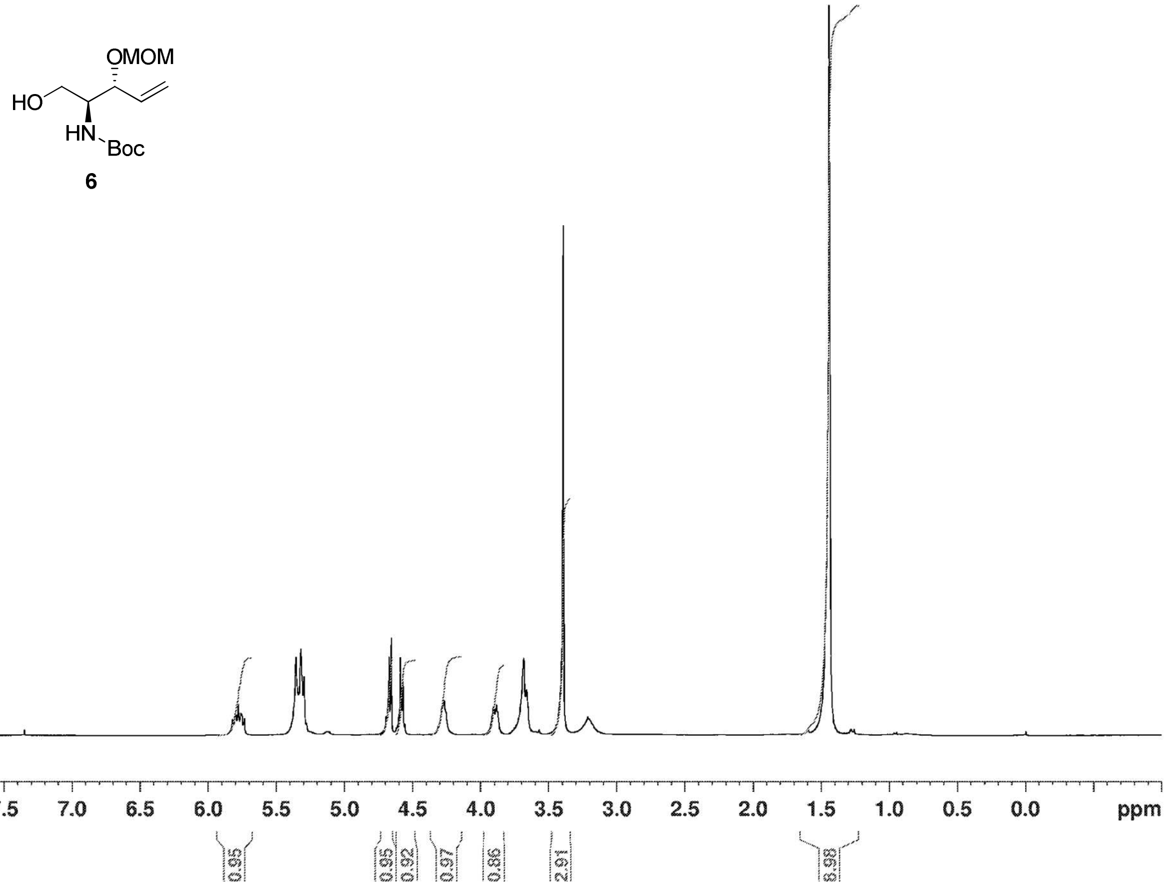
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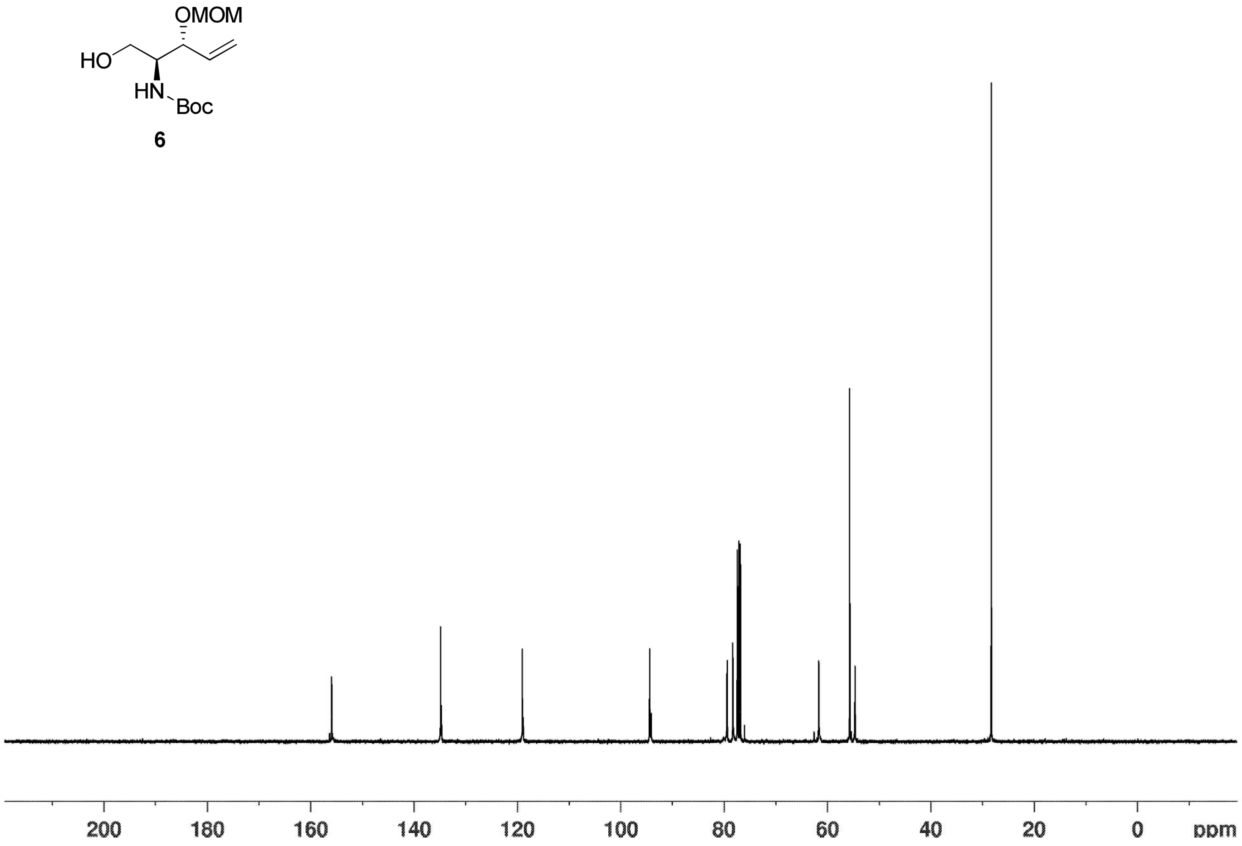
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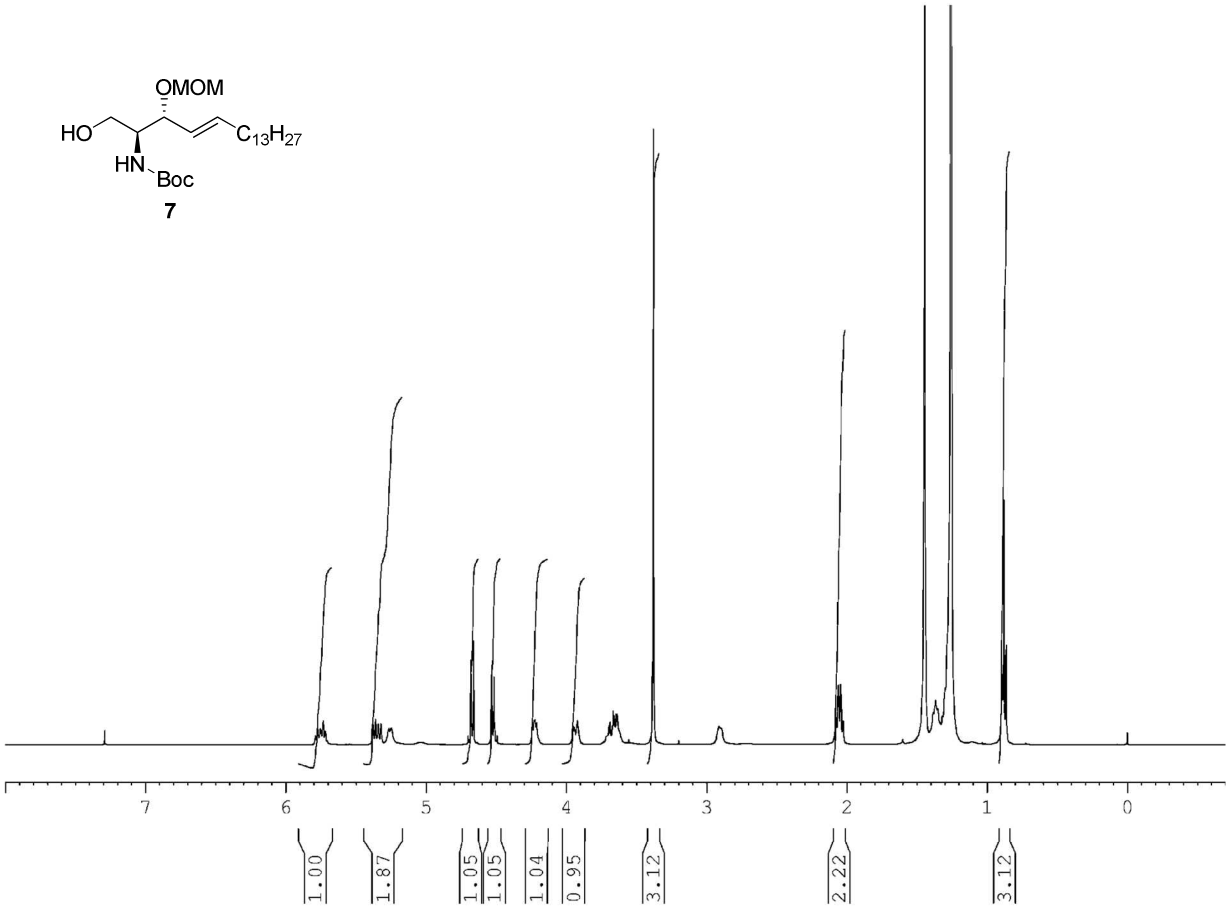
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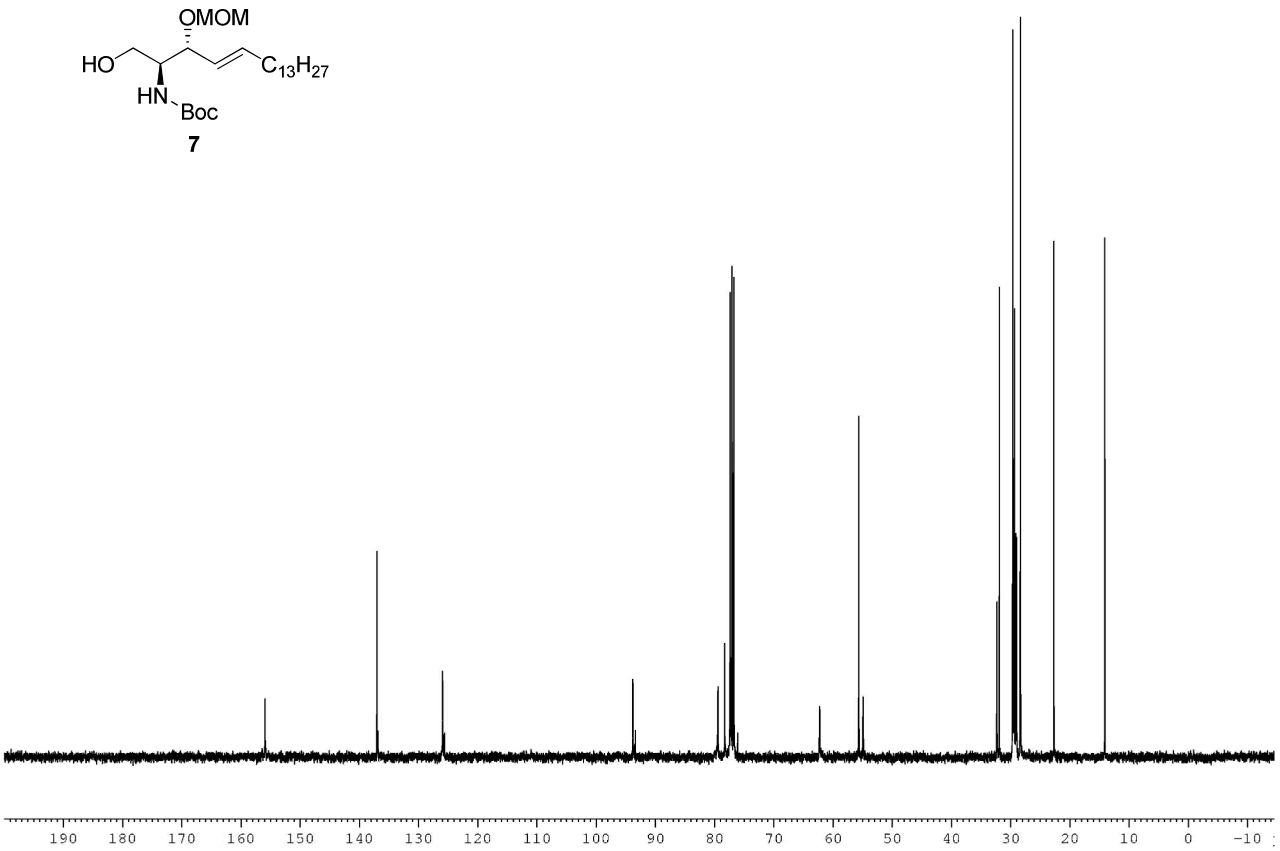

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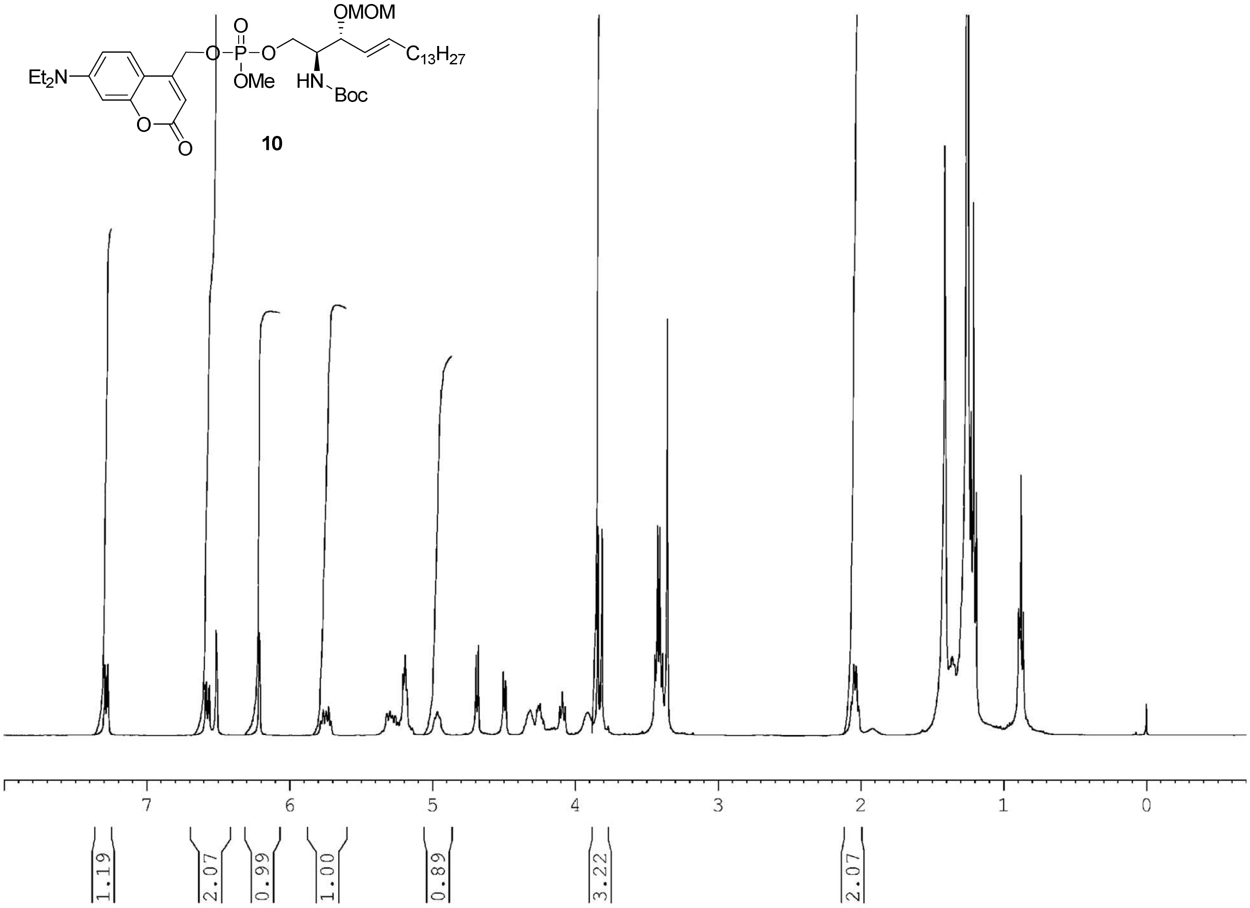

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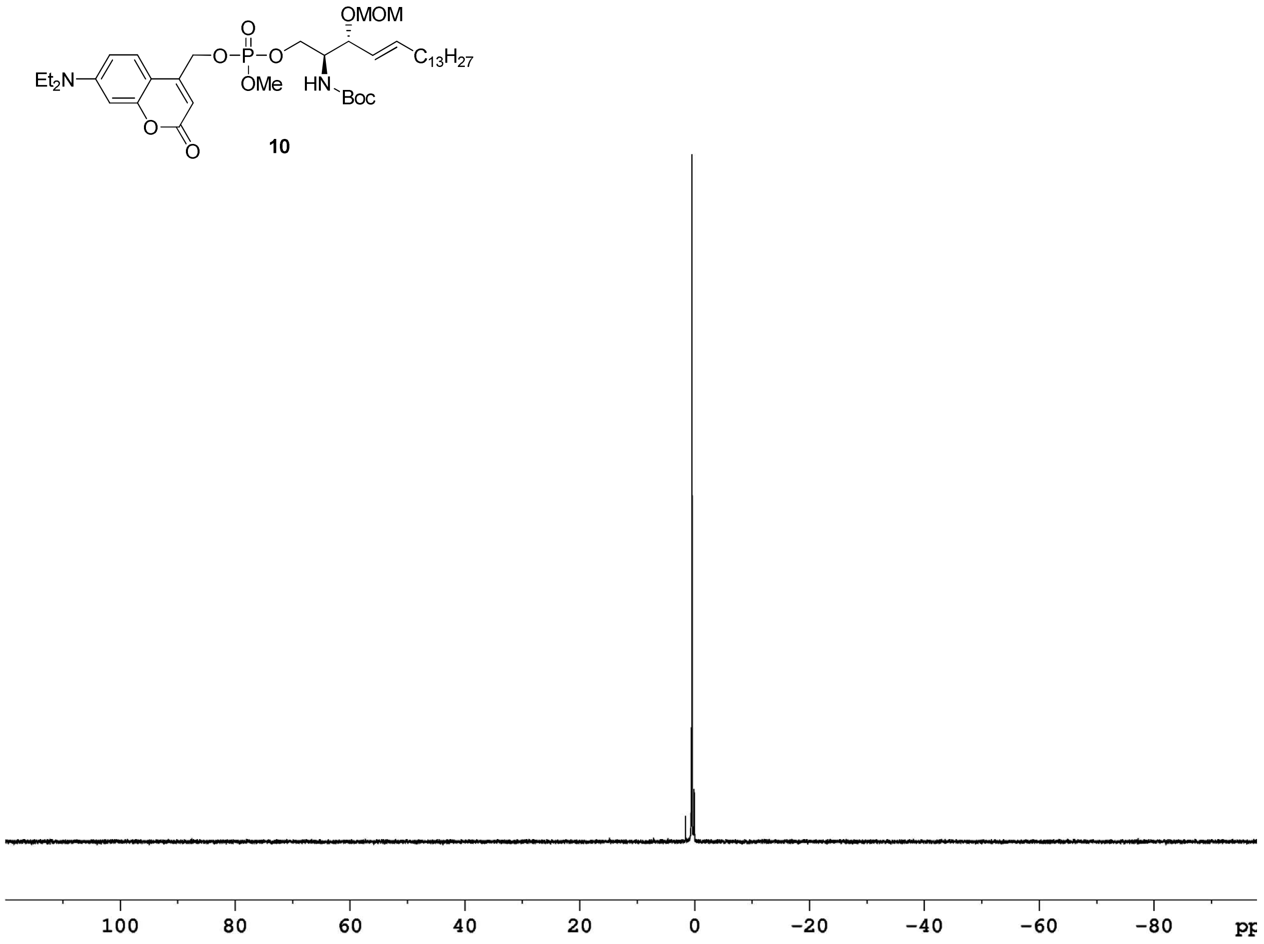

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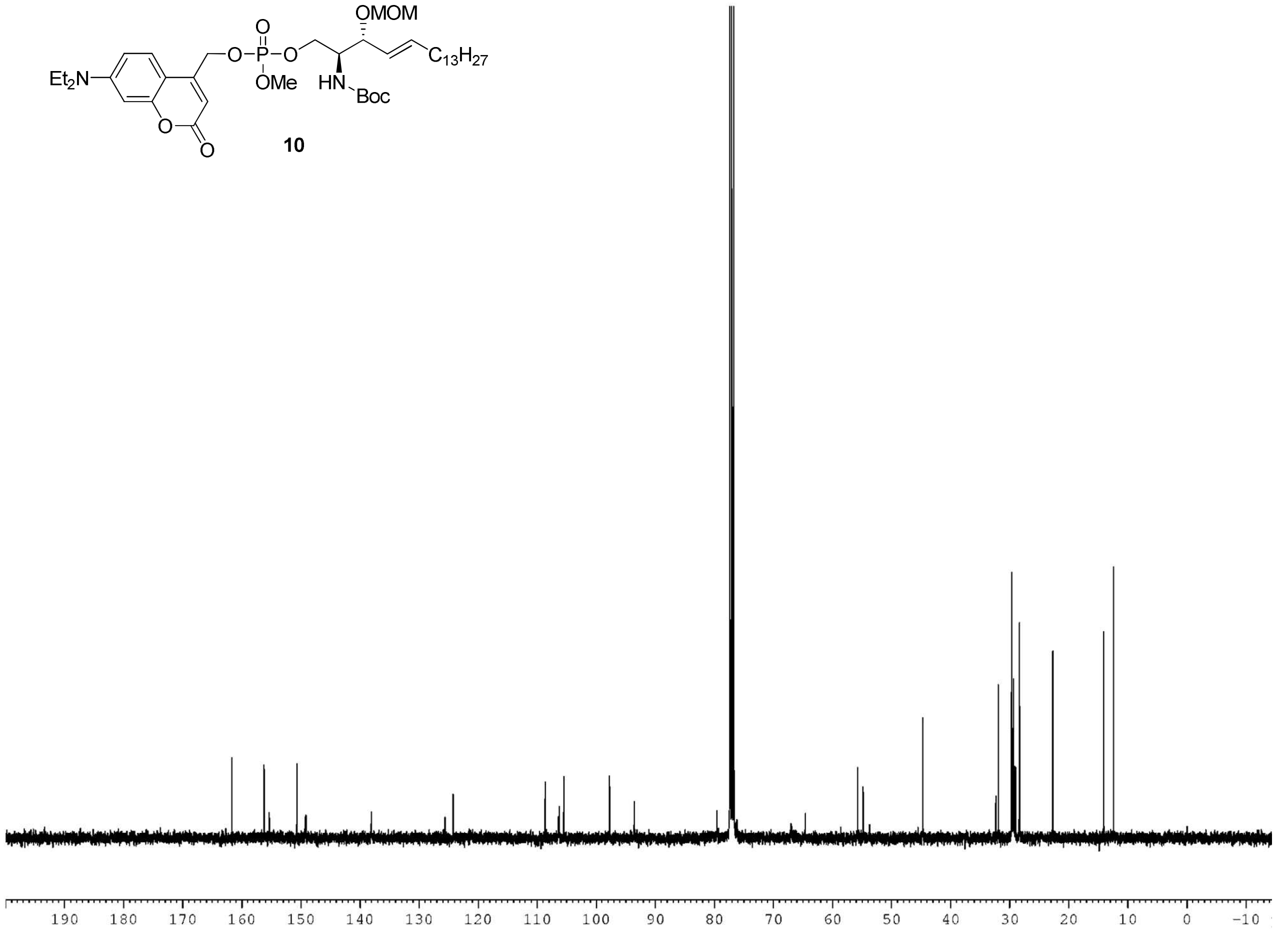
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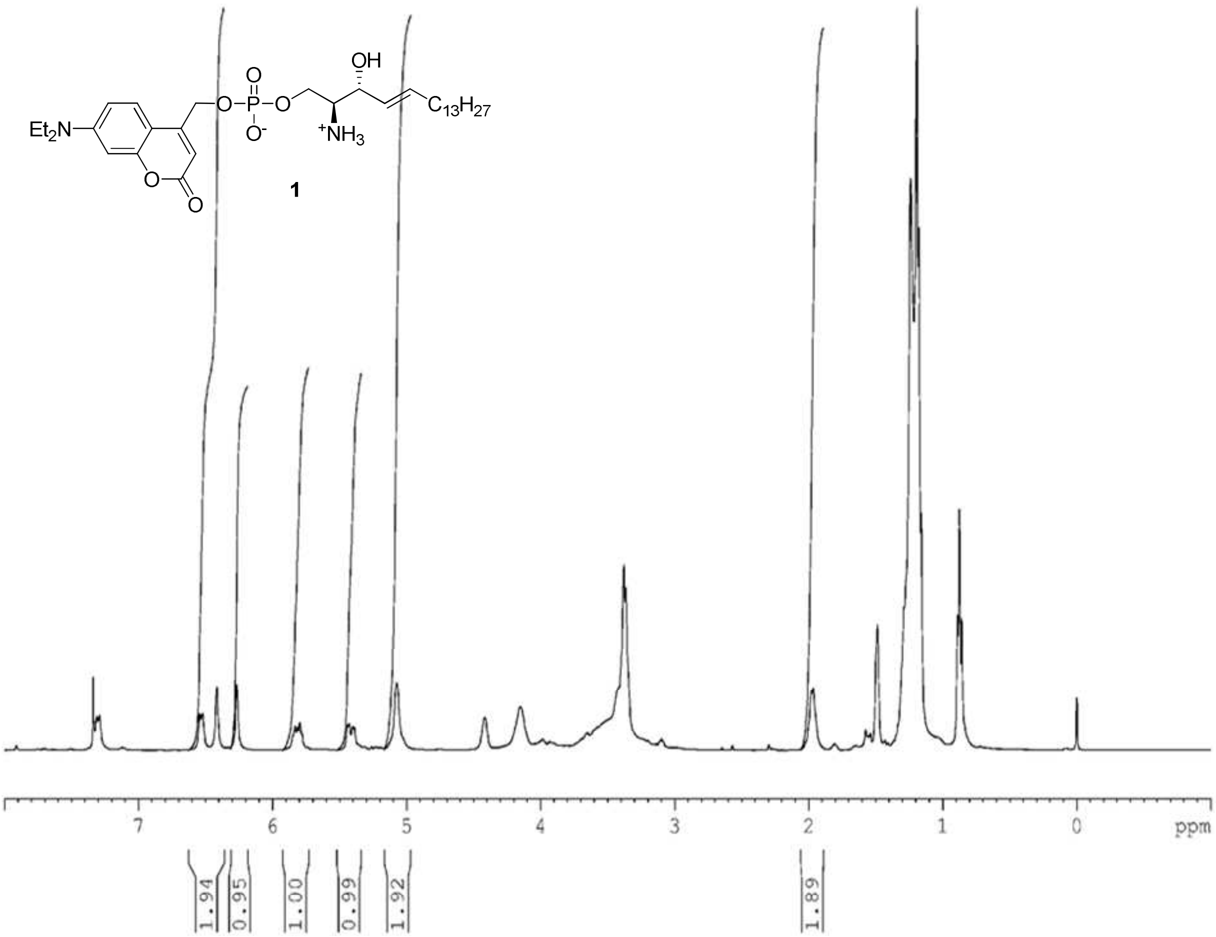
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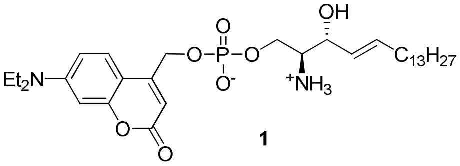

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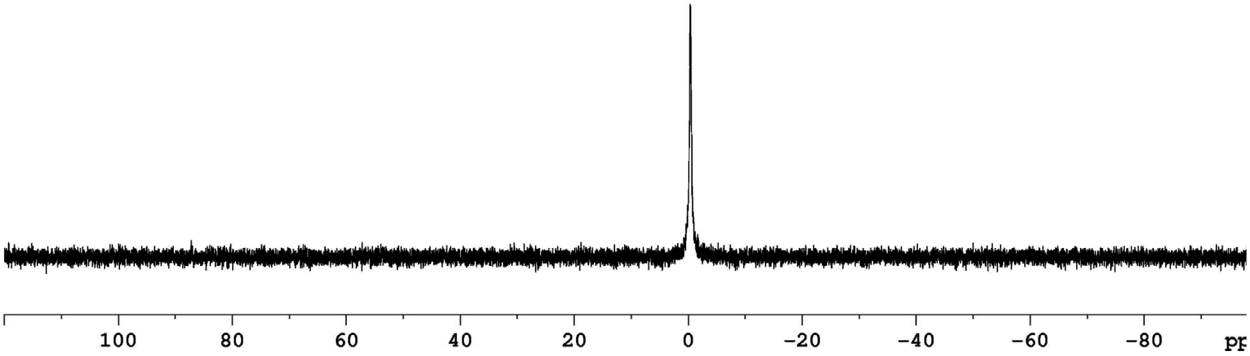

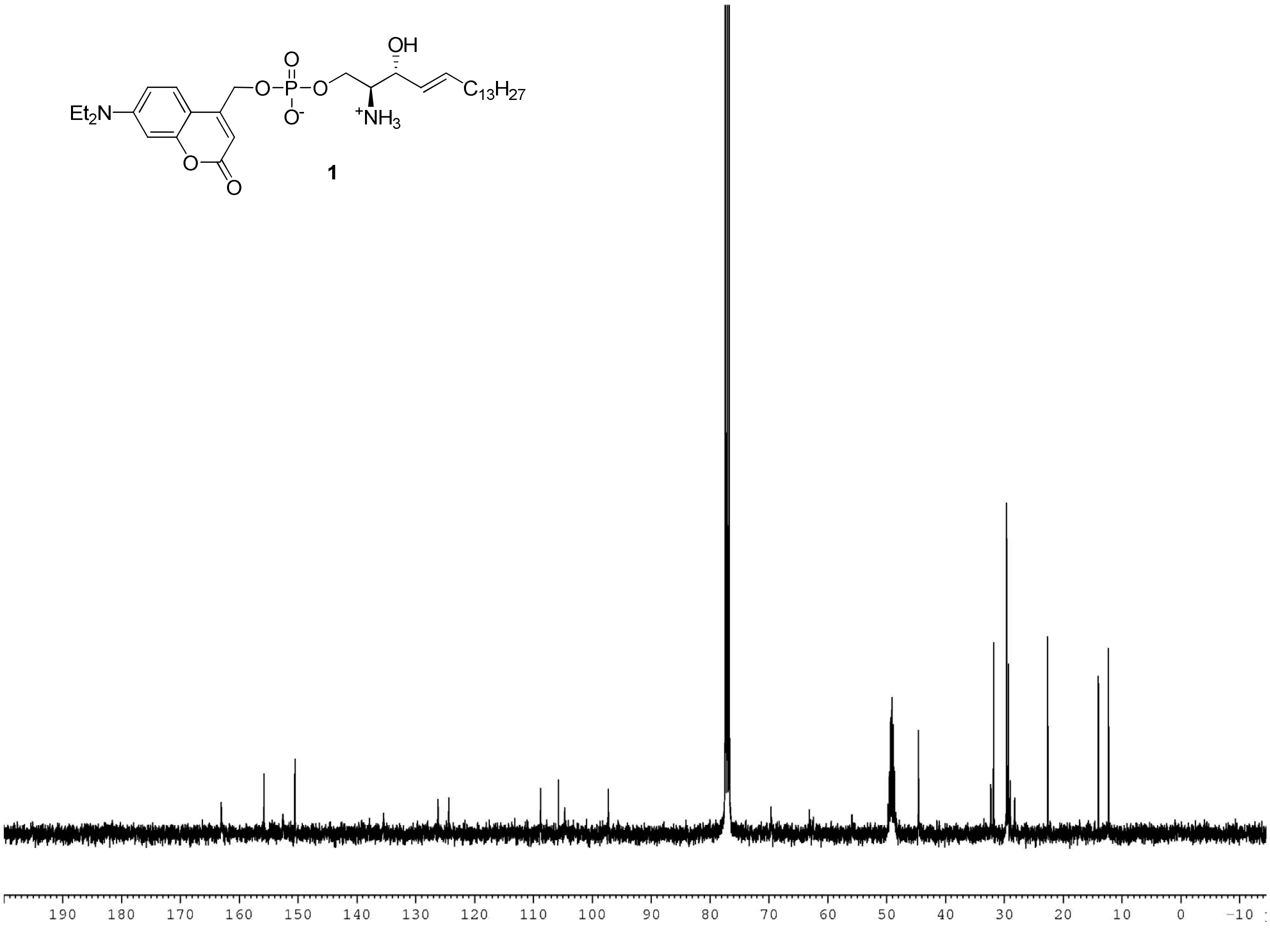
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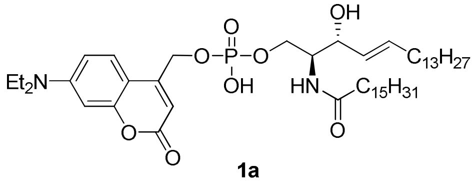
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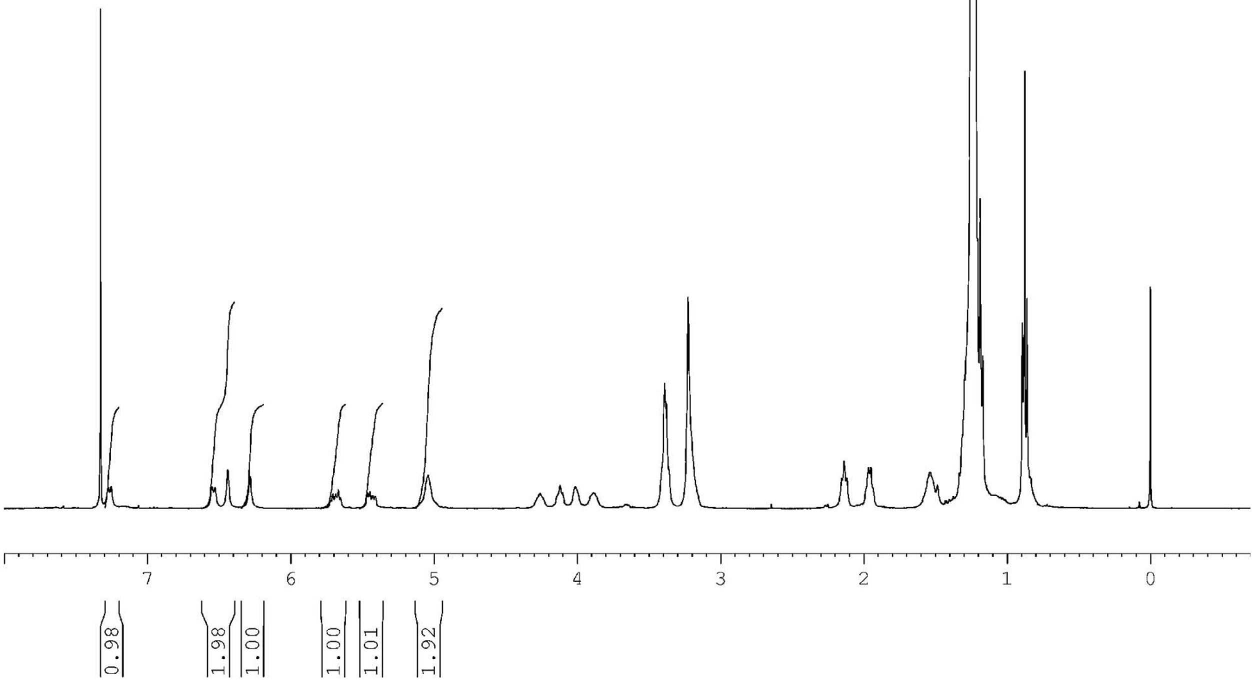
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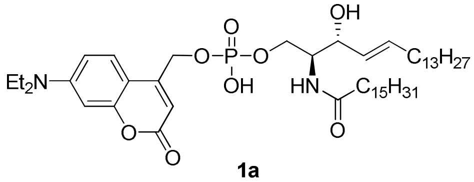

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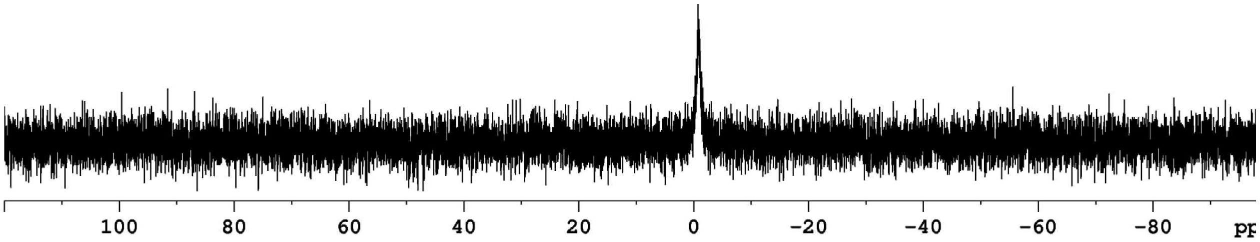

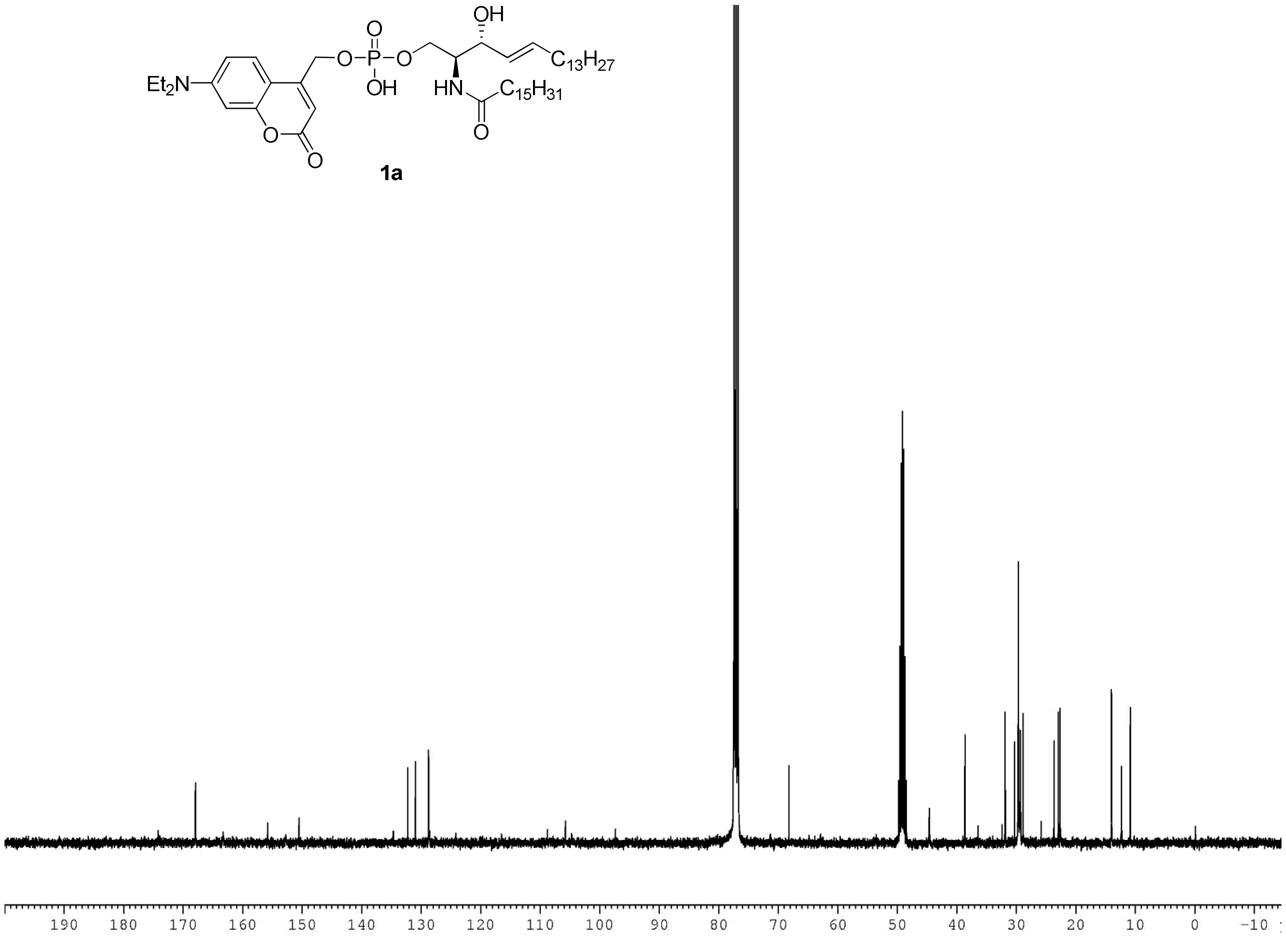
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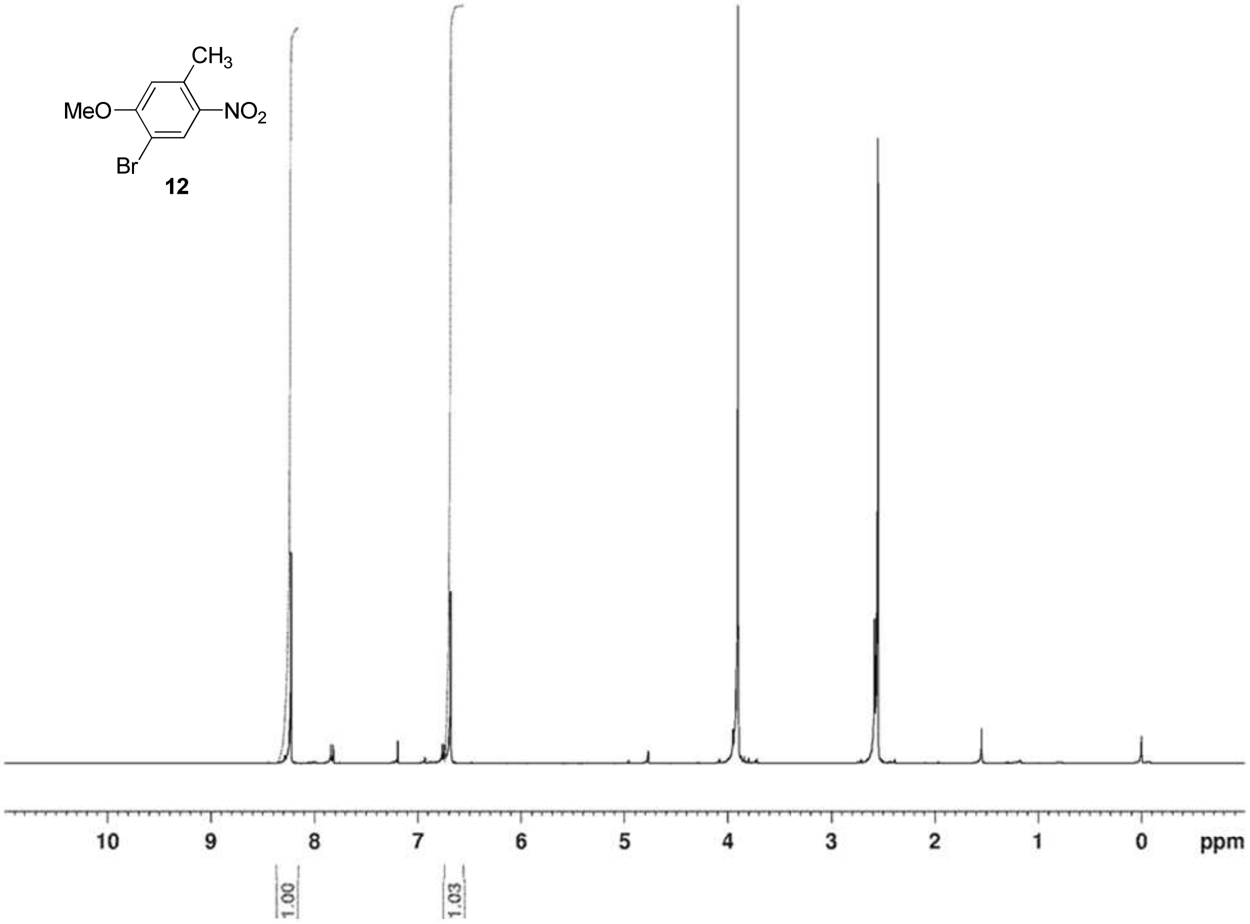
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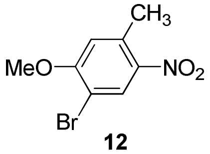

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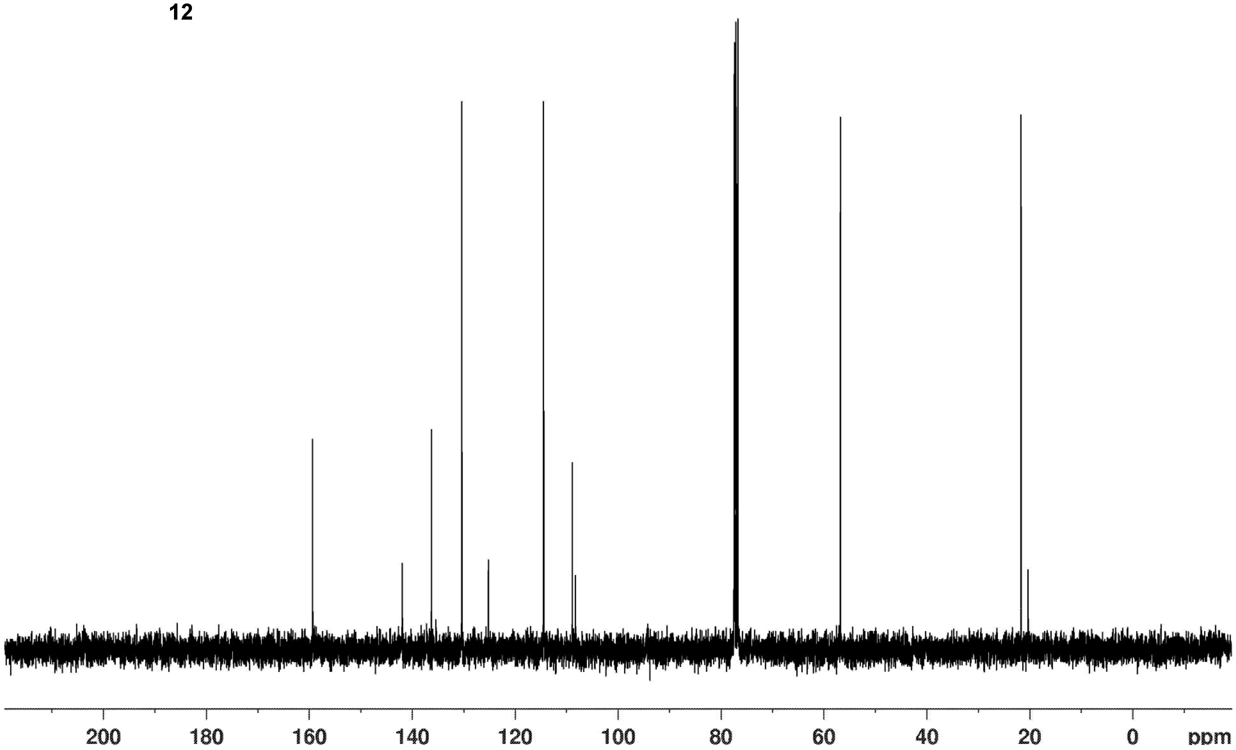

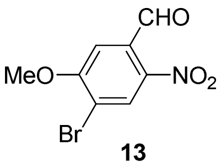

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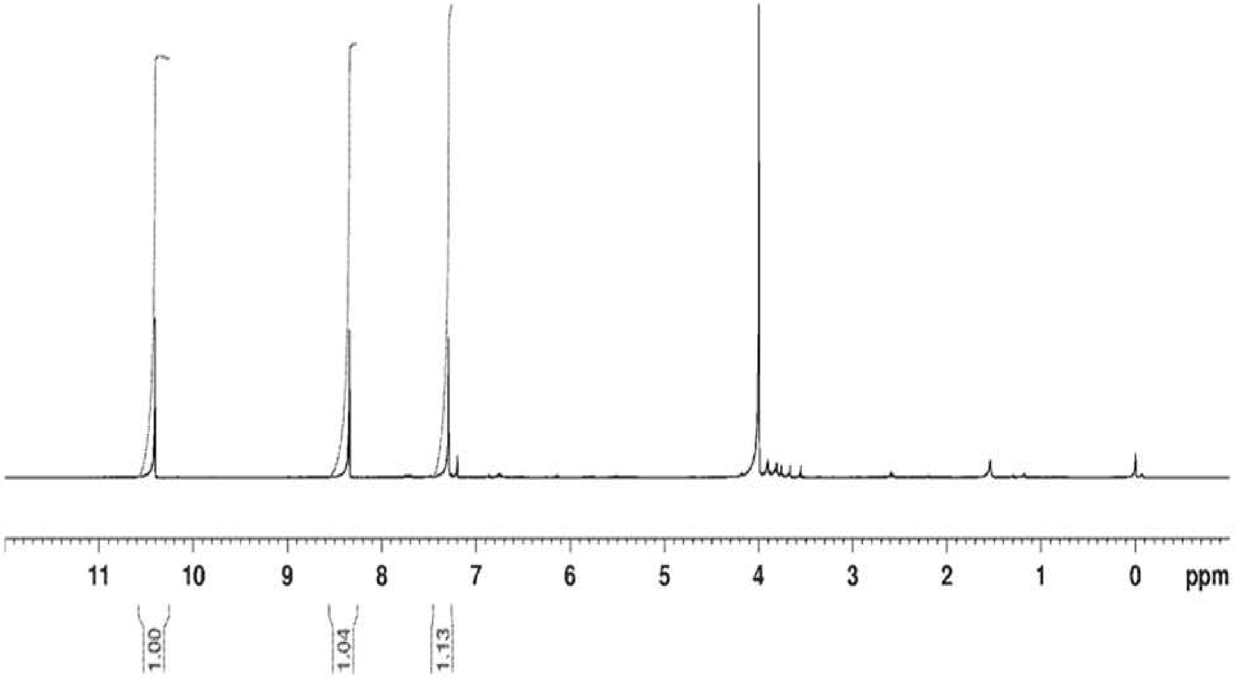

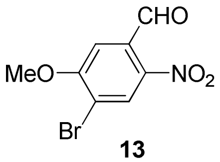

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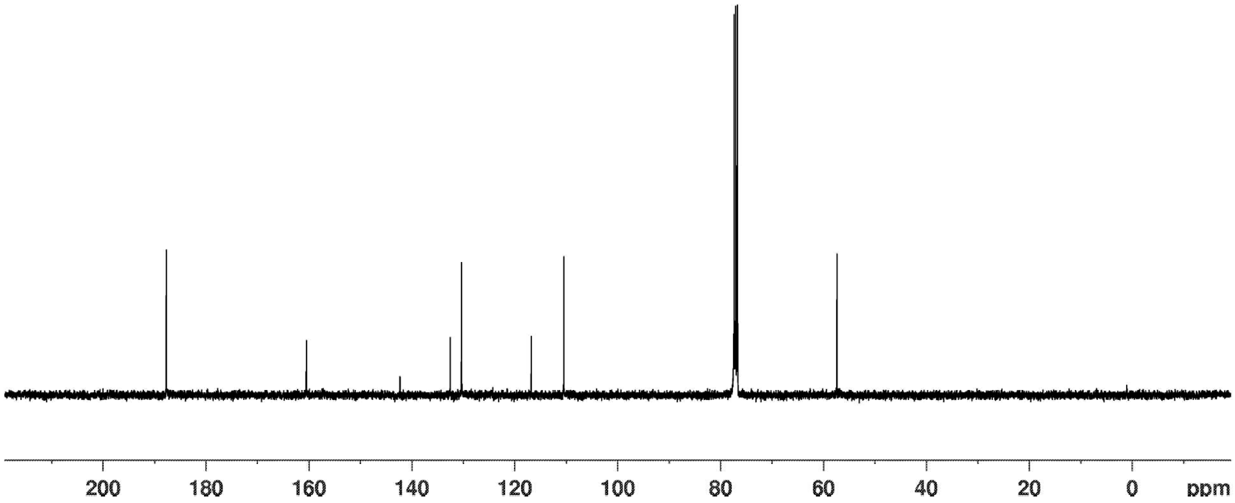

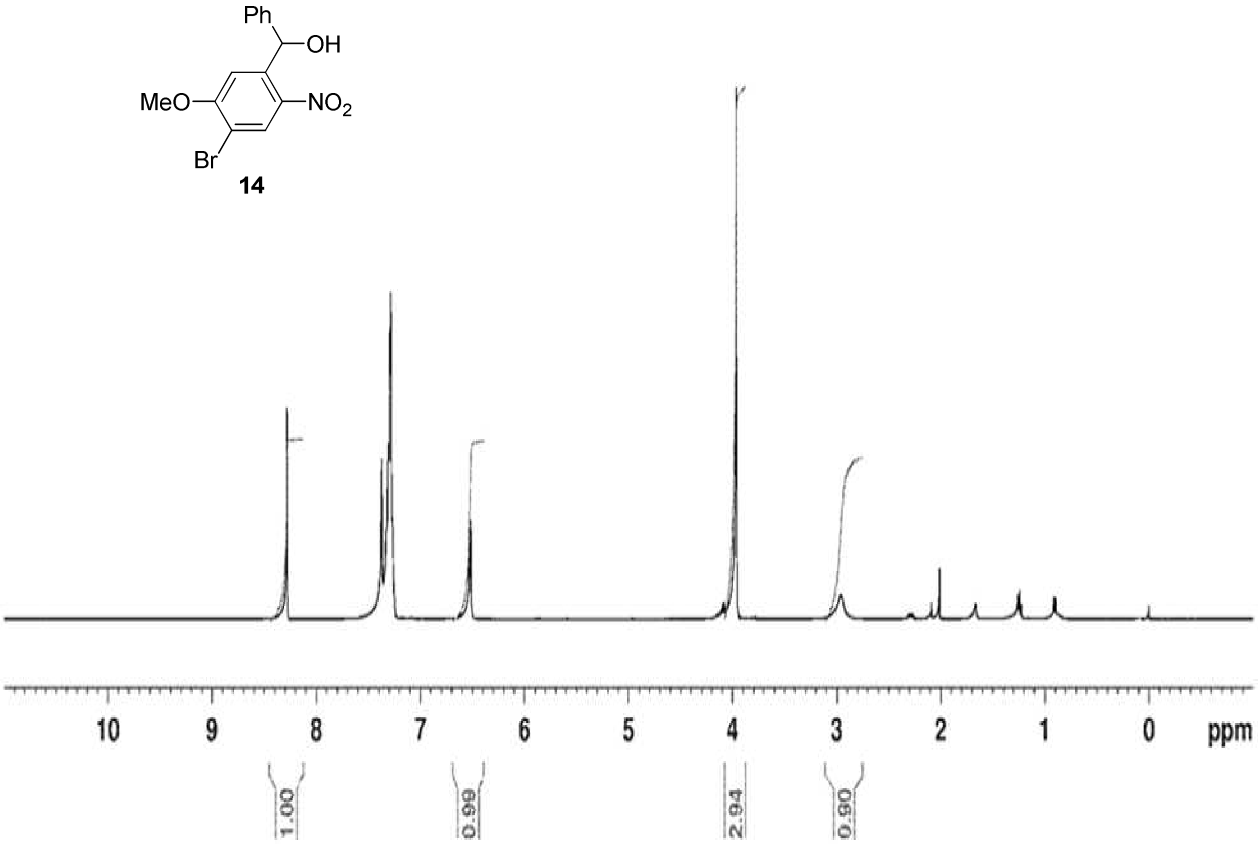
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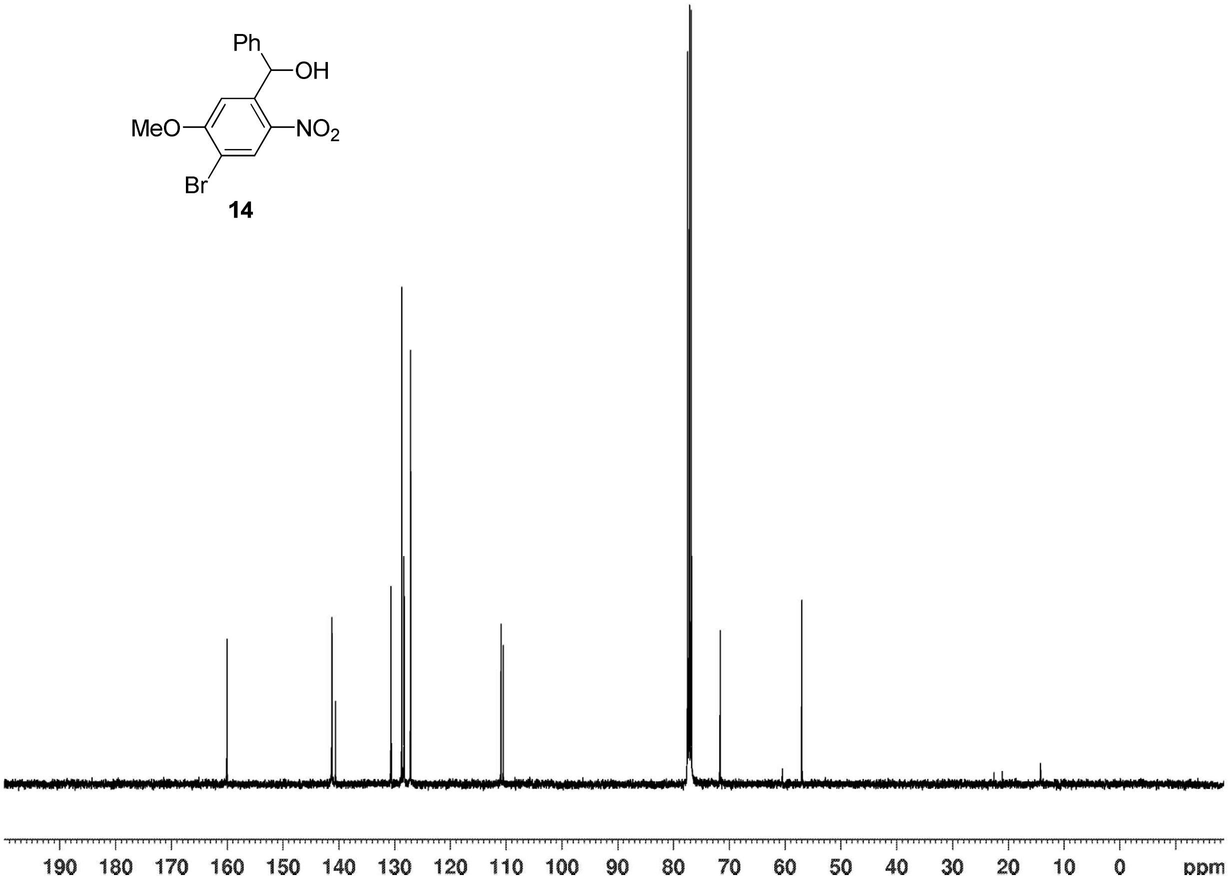
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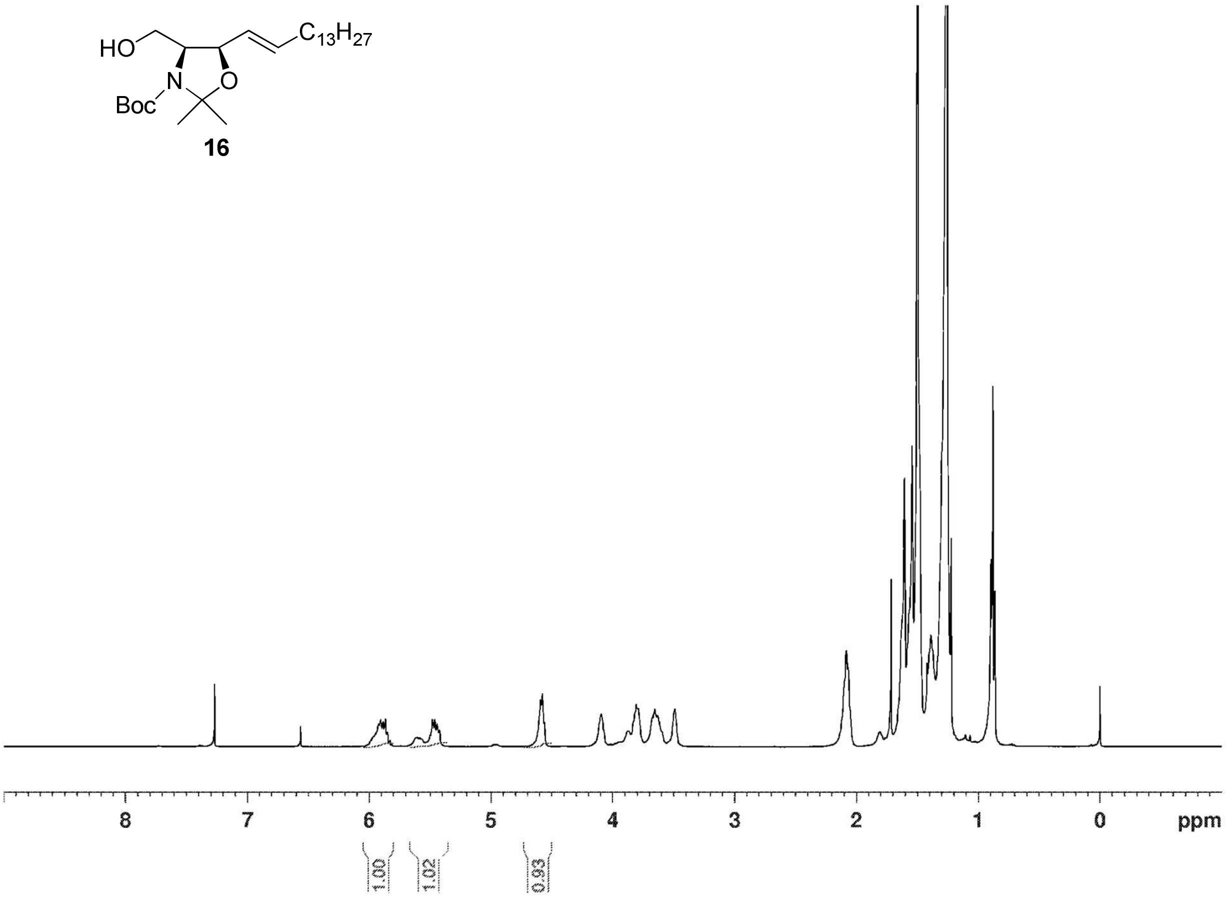
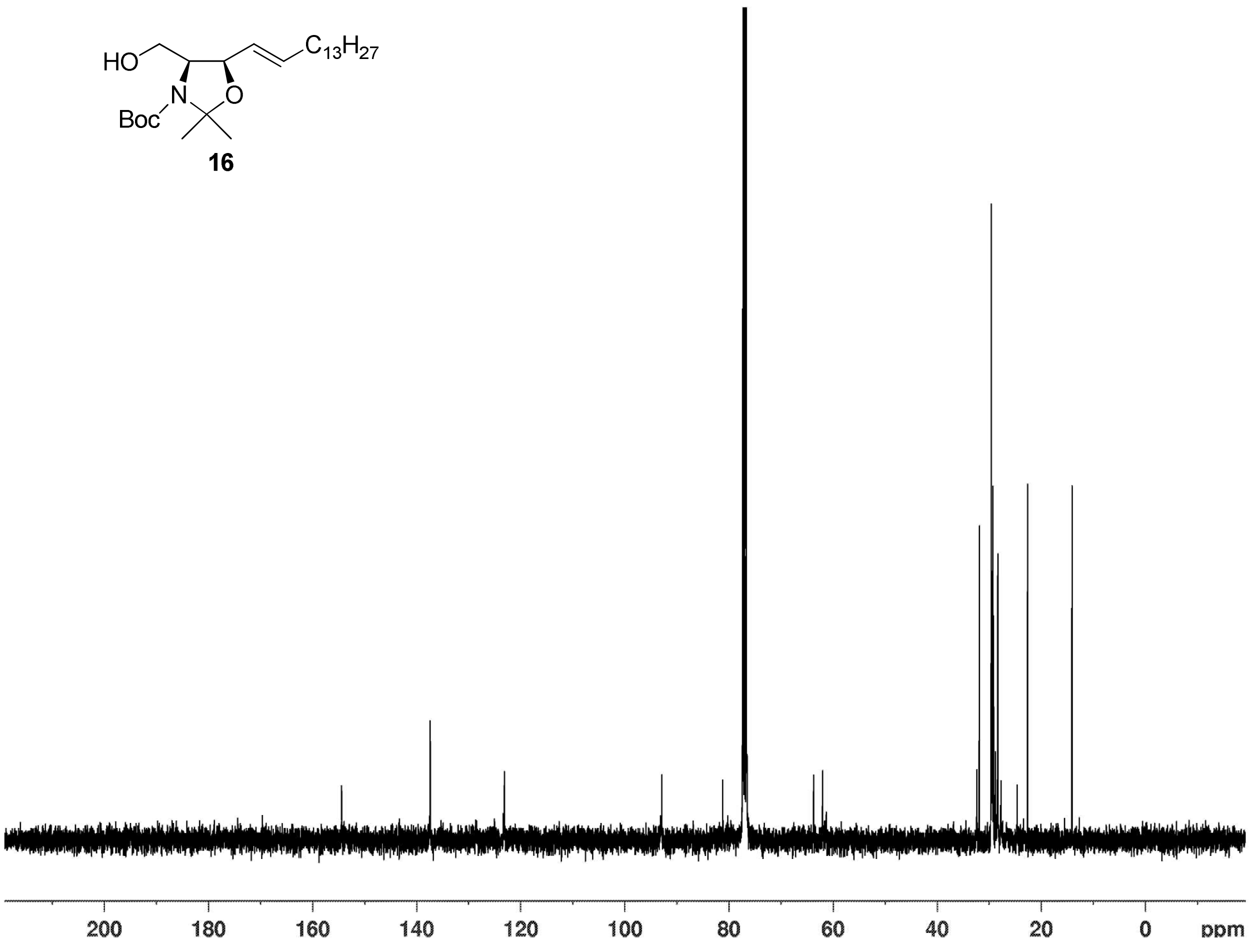
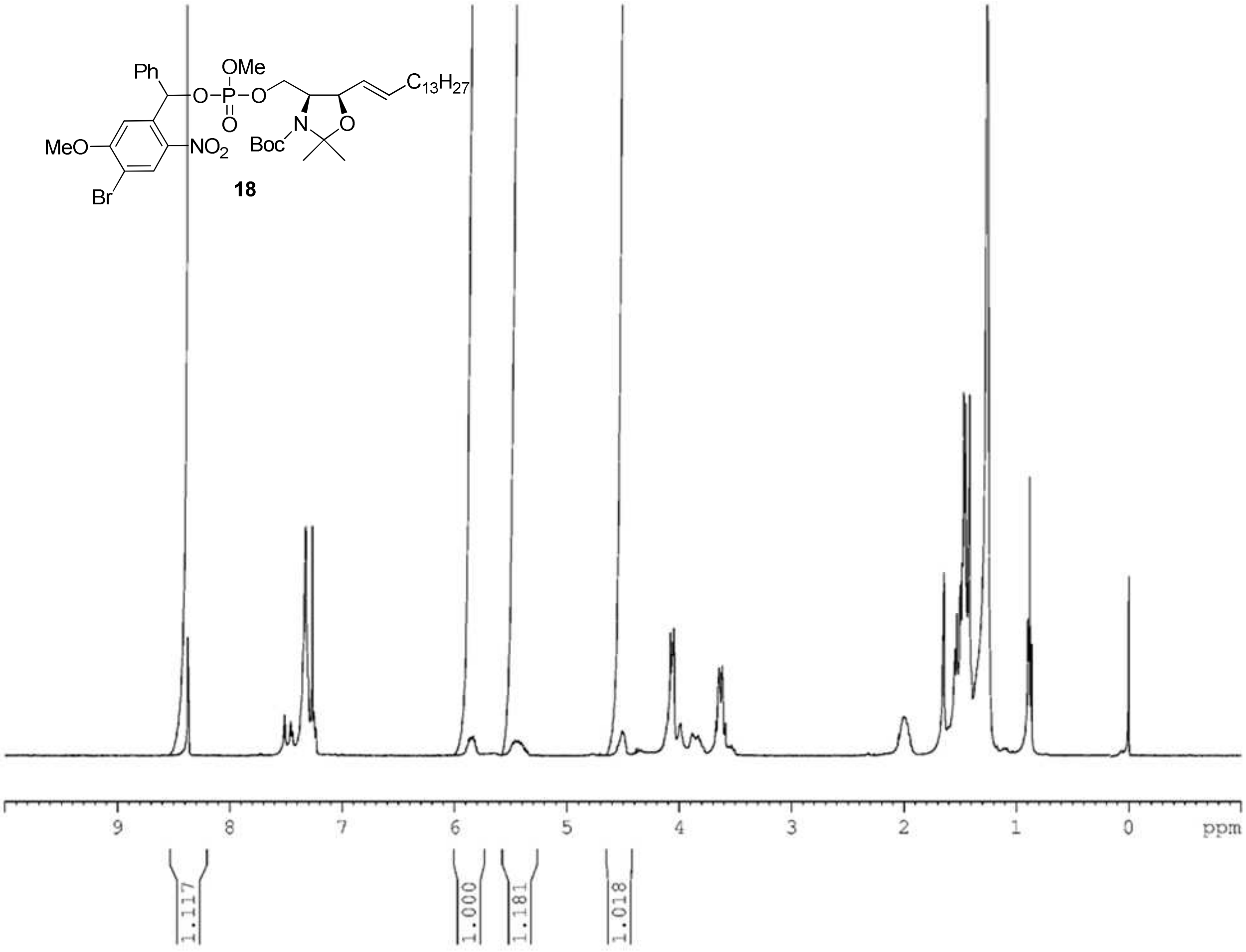
ppm

14

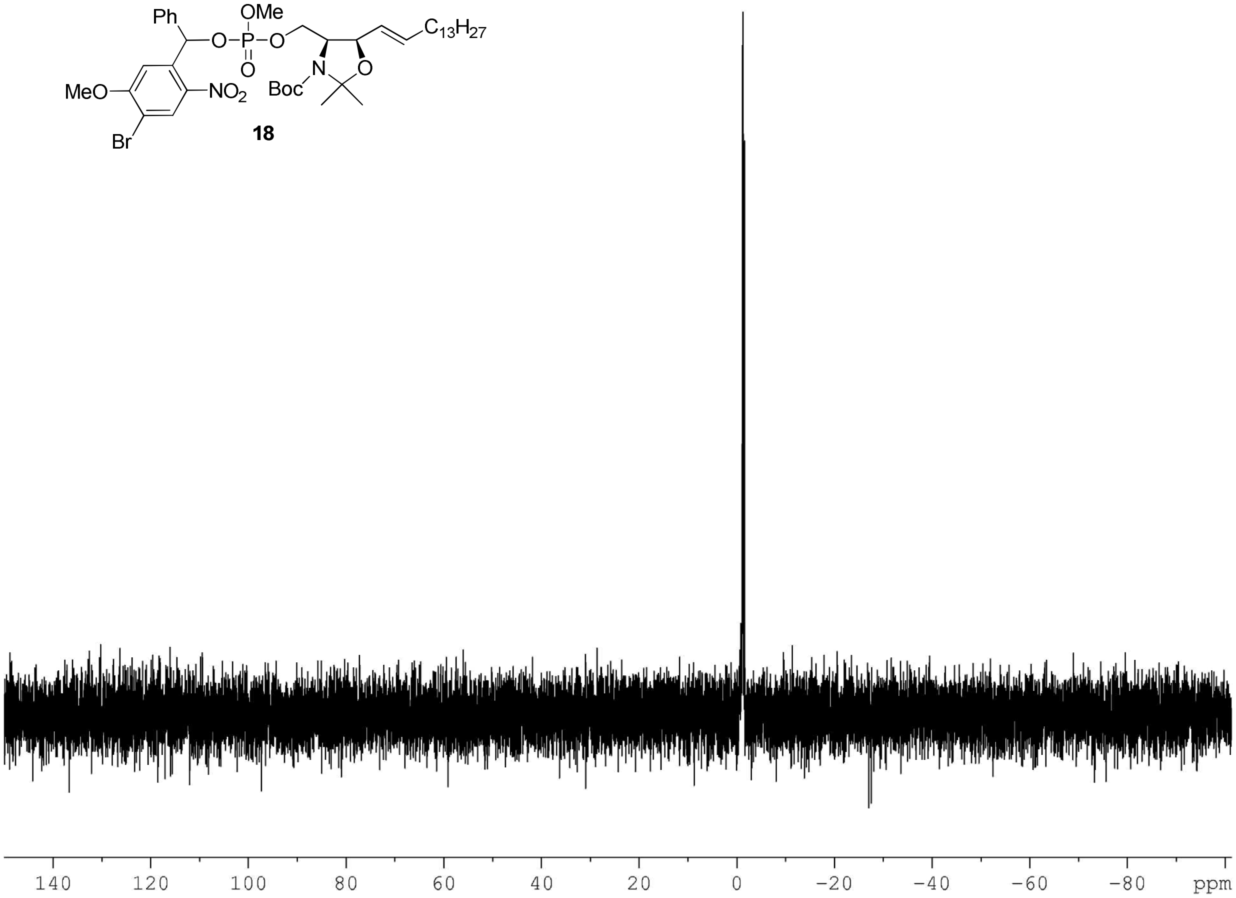

HO
N
O
Boc
$C_{13}H_{27}$
16
8
7
6
5
4
3
2
1
0
ppm
1.00
1.02
0.93



16

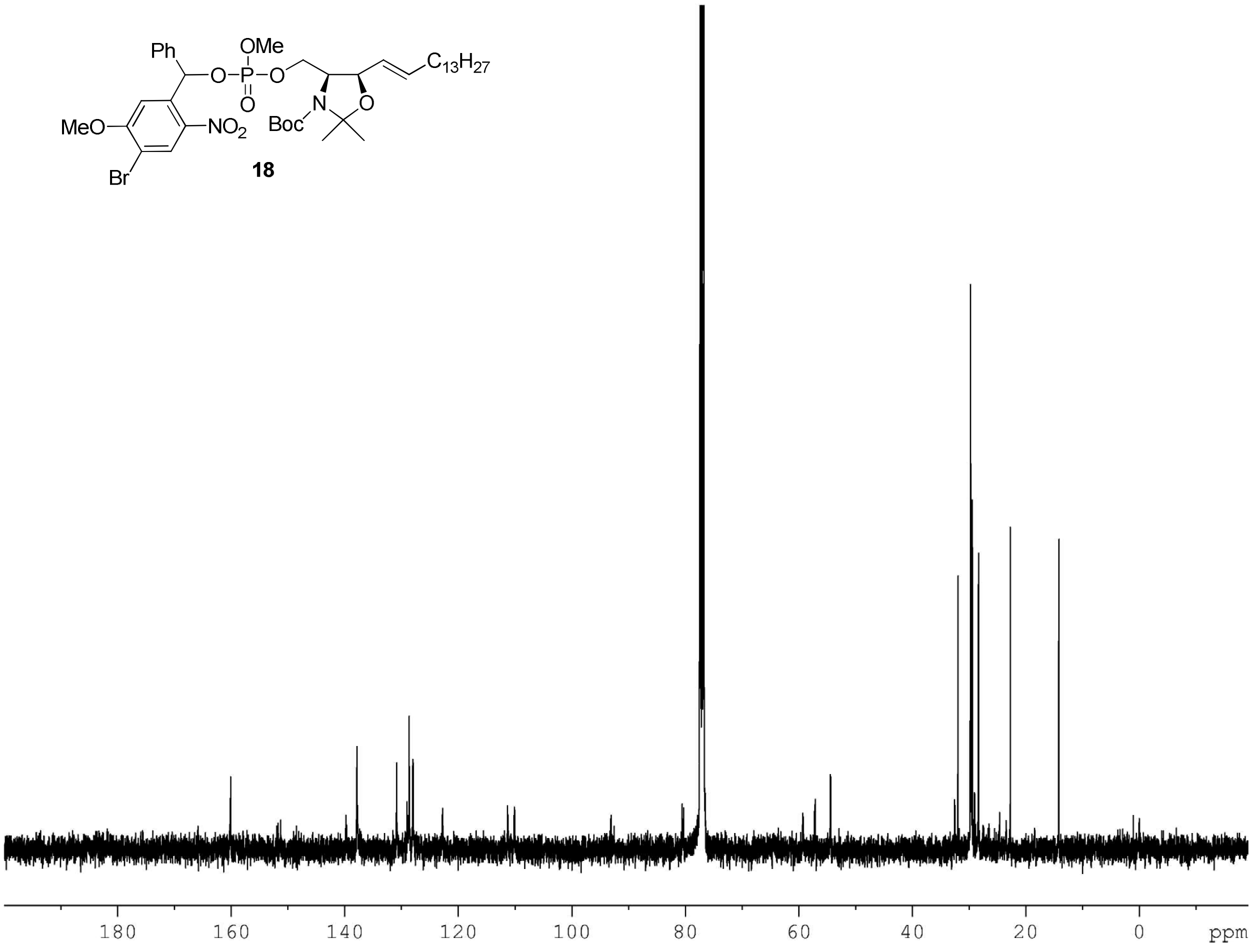

18

Ph
OMe
O
P
O
O
O
C$_{13}$H$_{27}$
MeO
NO$_2$
N
O
Boc
Br
9
8
7
6
5
4
3
2
1
0
ppm
1.117
1.000
1.181
1.018

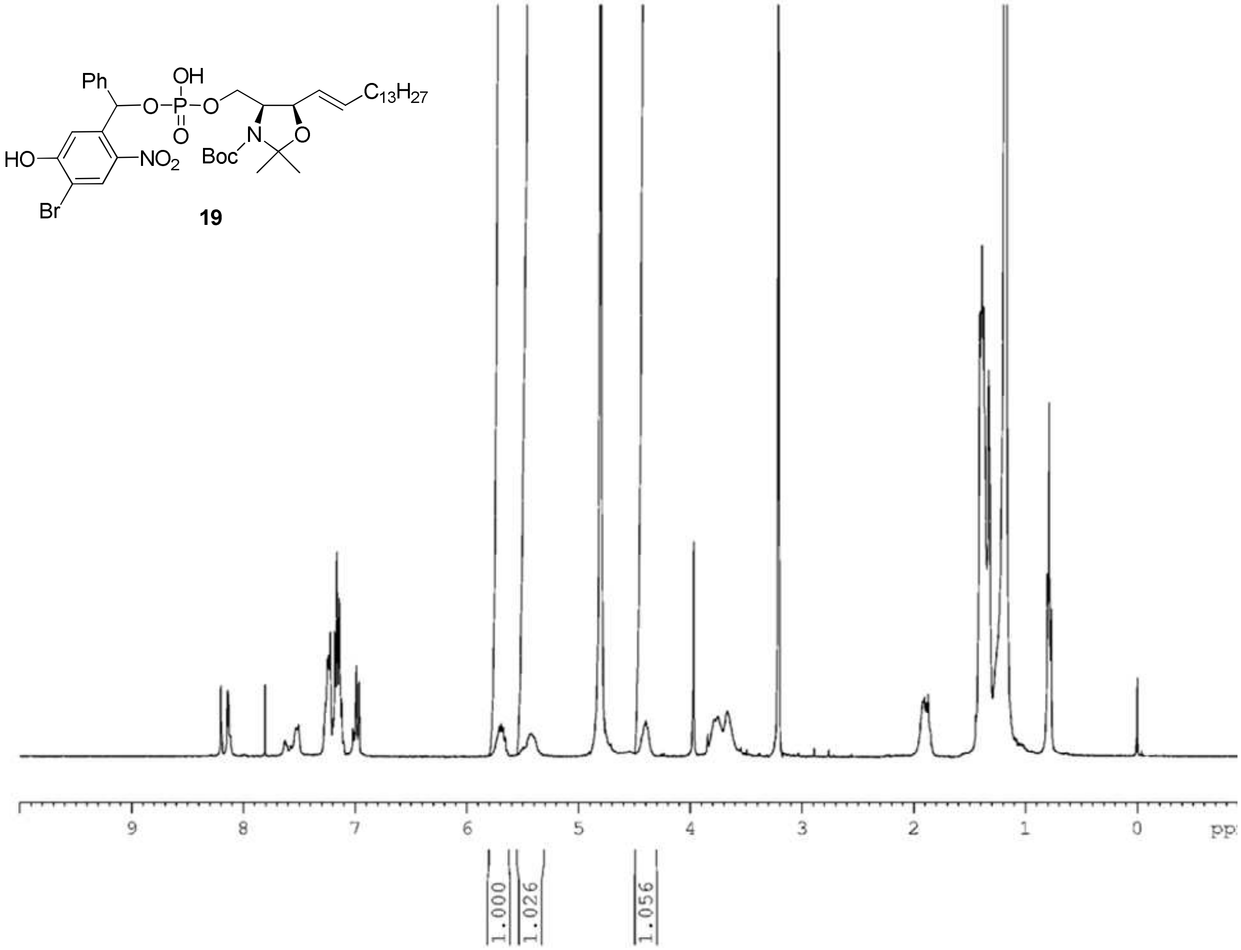


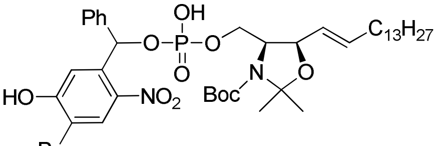
18

Ph
OMe
O
P
O
O
MeO
NO$_2$
Br
N
Boc
O
C$_{13}$H$_{27}$
140
120
100
80
60
40
20
0
-20
-40
-60
-80
ppm

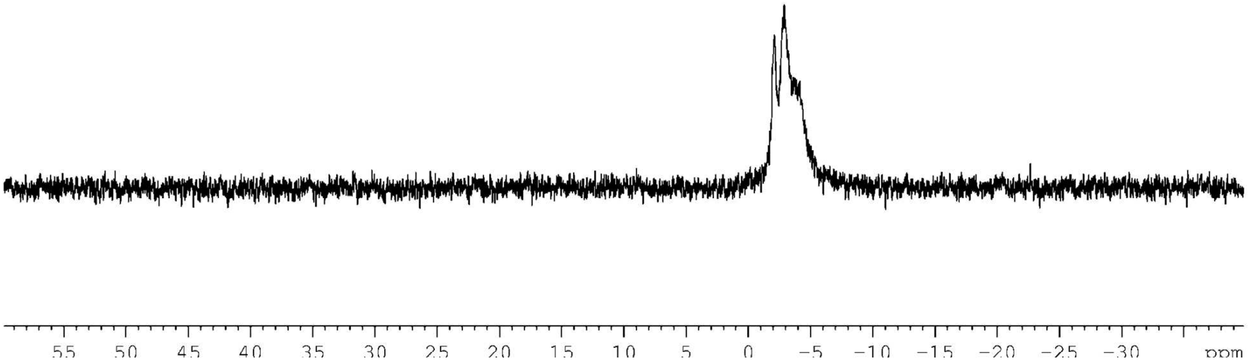

Ph
OMe
O
P
O
O
C13H27
MeO
NO2
Boc
N
O
Br
180
160
140
120
100
80
60
40
20
0
ppm

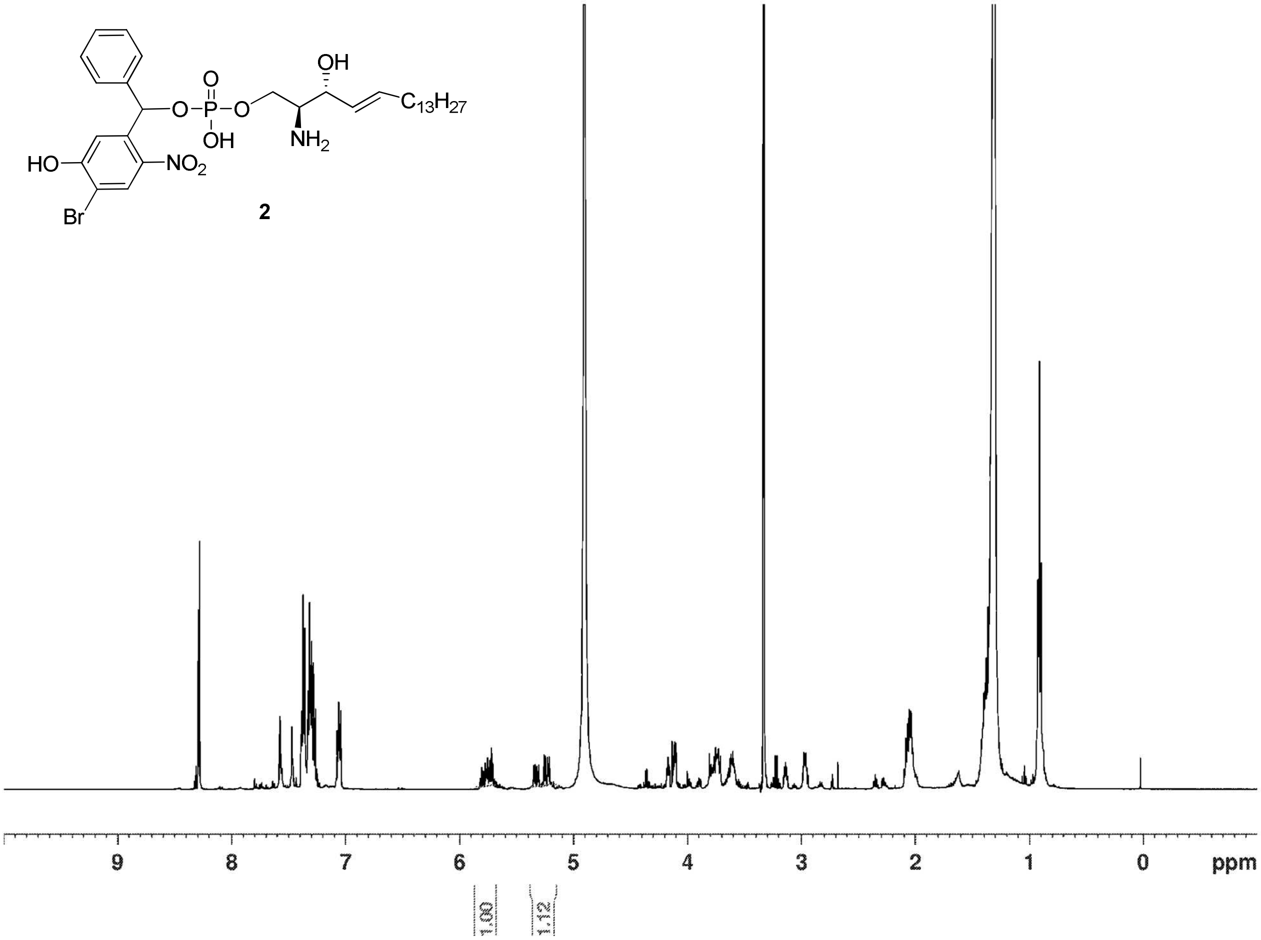
18


Ph
OH
O
P
O
O
$C_{13}H_{27}$
HO
NO_2
Boc
N
O
Br
19
9
8
7
6
5
4
3
2
1
0
pp
1.000
1.026
1.056

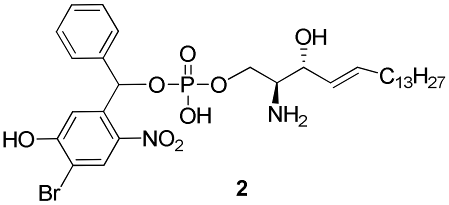

Ph
OH
O
P
O
O
HO
NO2
Br
Boc
N
O
C13H27

19

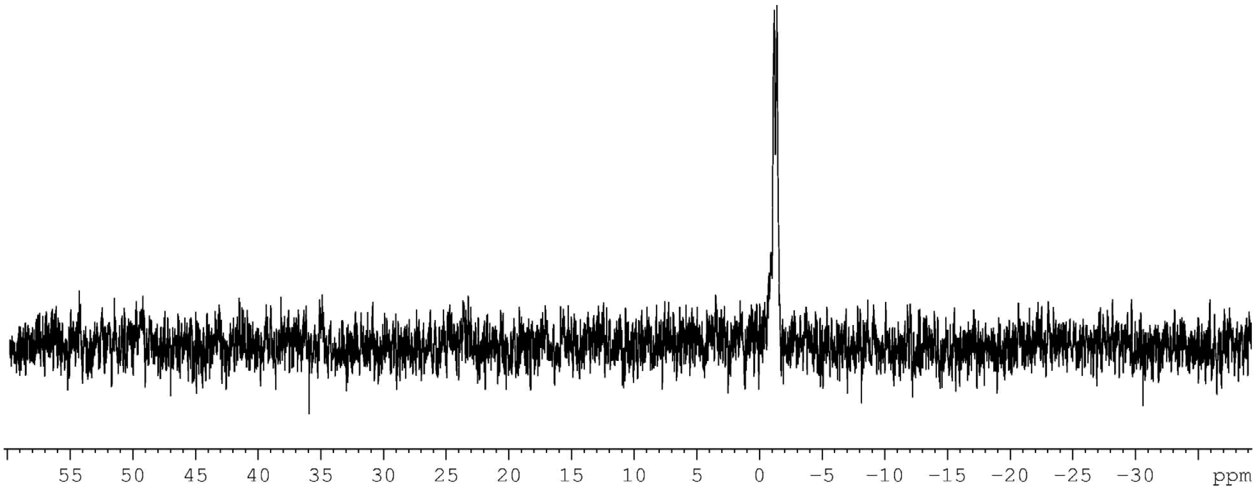

55
50
45
40
35
30
25
20
15
10
5
0
−5
−10
−15
−20
−25
−30
ppm

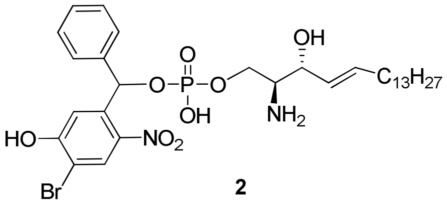


2



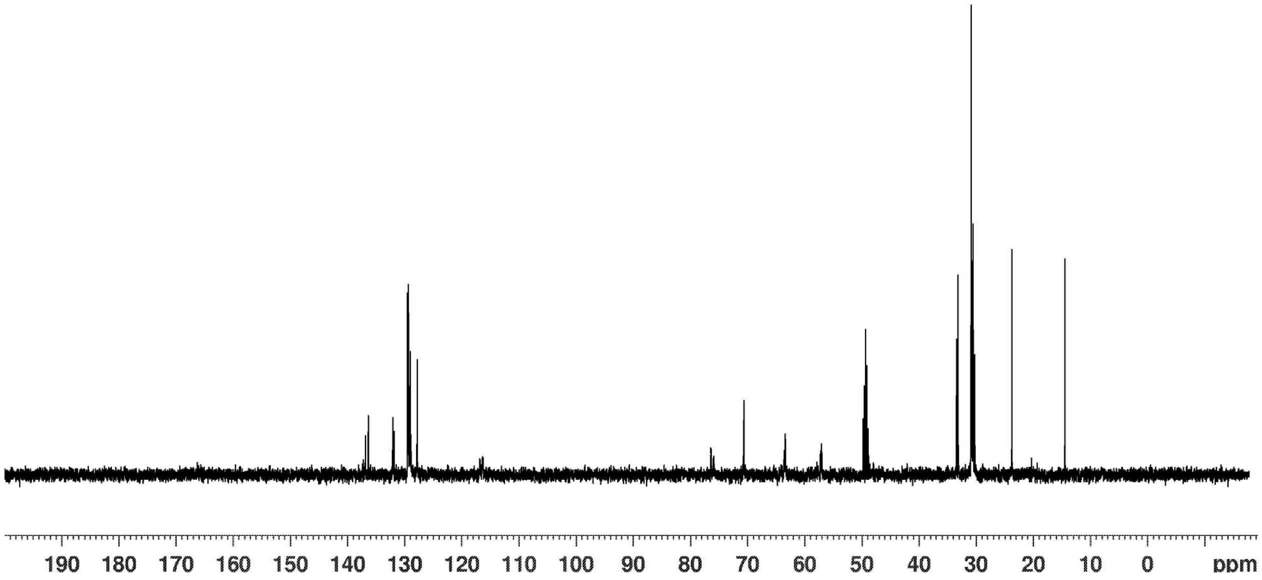
2



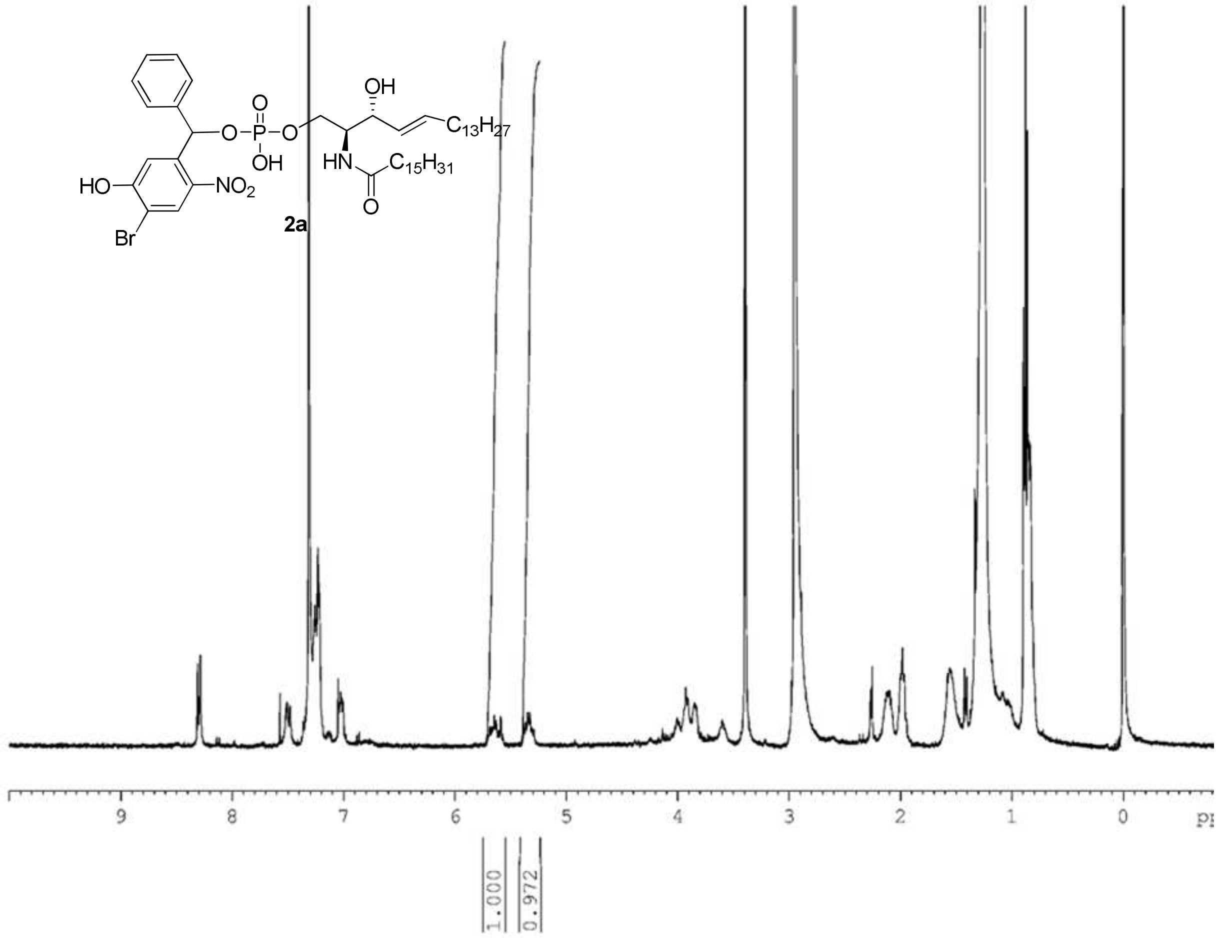


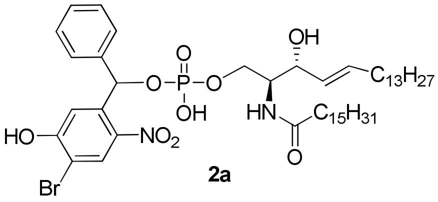
OH
O
O–P–O
OH
C13H27
NH2
HO
NO2
Br

2



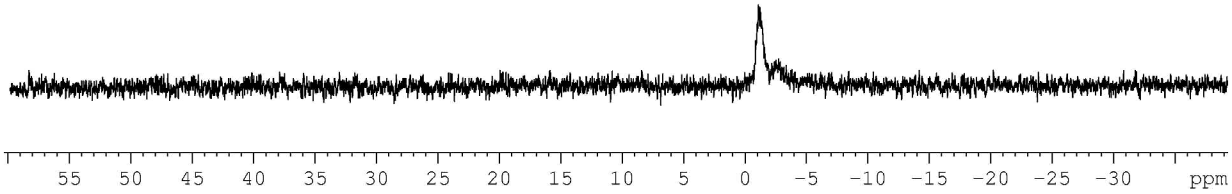
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


HO
Br
NO2
O
P
OH
OH
HN
C13H27
C15H31
O
2a
9
8
7
6
5
4
3
2
1
0
1.000
0.972

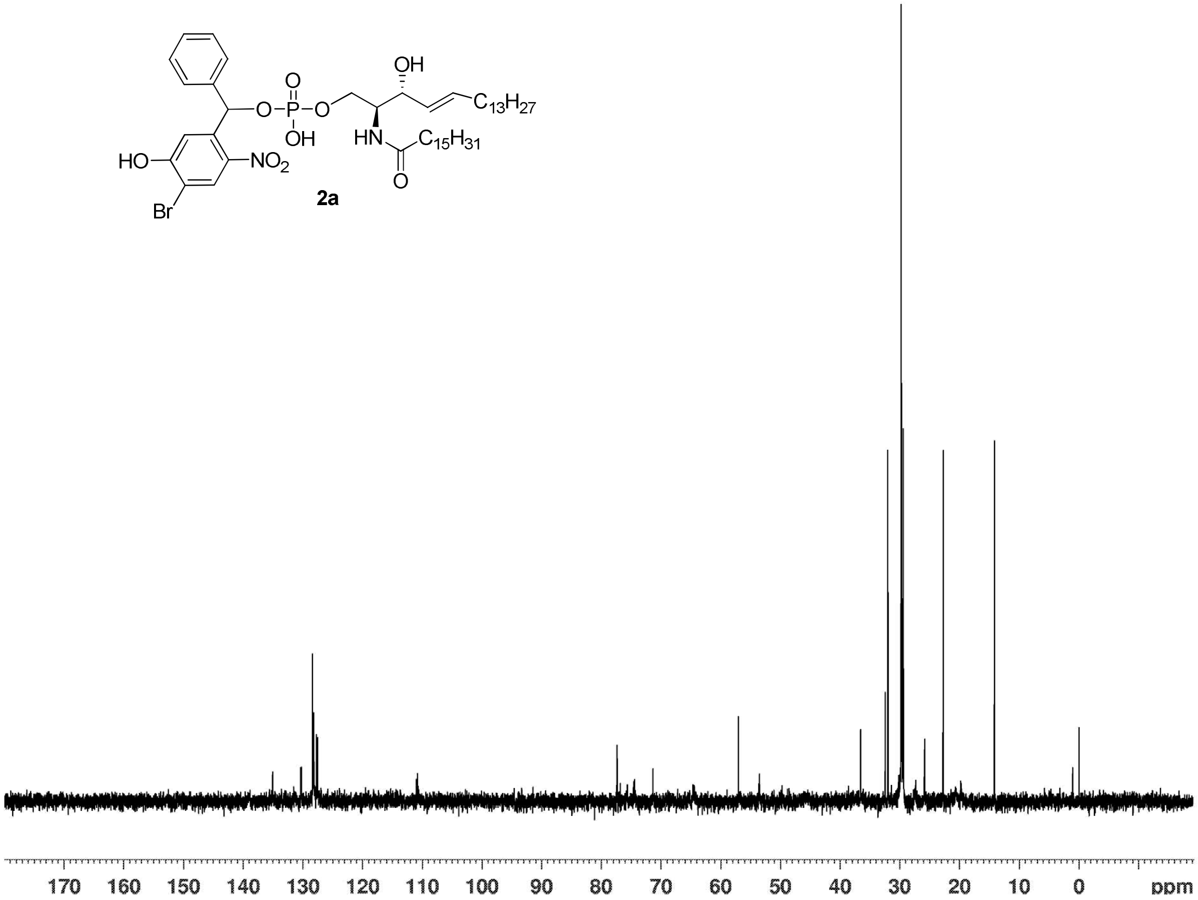


OH
O
O
P
O
OH
HN
C13H27
C15H31
O
HO
NO2
Br

2a



55
50
45
40
35
30
25
20
15
10
5
0
−5
−10
−15
−20
−25
−30
ppm


OH
O
O–P–O
OH
C13H27
HN
C15H31
O
HO
NO2
Br
2a
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm