Supplementary Material (ESI) for MedChemComm

Electronic Supporting Information

Novel sphingosine-containing analogues selectively inhibit sphingosine kinase (SK) isozymes, induce SK1 proteasomal degradation and reduce DNA synthesis in human pulmonary arterial smooth muscle cells

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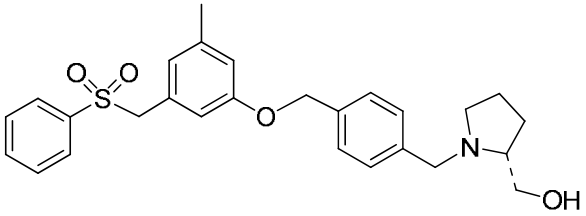
Table of Contents

A. Experimental Methods

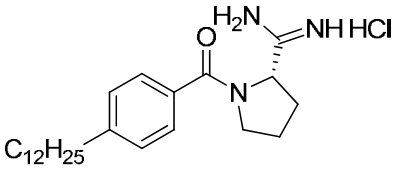
(a) Biological methods

Materials--All general biochemicals and the anti-actin antibody were from Sigma (Poole, UK). High glucose Dulbecco's modified Eagle's Medium (DMEM), European fetal calf serum, and penicillin-streptomycin (10000 U/mL penicillin and 10 mg/mL streptomycin) were from Invitrogen (Paisley, UK). Sphingosine (Sph) was from Avanti Polar Lipids (Alabaster, AL, USA). Purified SK2 was from Enzo Life Sciences (Exeter, UK). BML-258 was from Tocris (Bristol, UK). PF-543 was from Calbiochem (EMD Millipore). VPC96091 was synthesized by following the procedure of Kennedy et al.[S1] Human pulmonary aortic smooth muscle cells (PAMSC), human smooth muscle cell growth medium, and passaging solutions were from TCS Cellworks (Buckingham, UK). [γ-^{32}P]ATP (specific activity, 4.4×10^4 cpm/nmol) was from Perkin-Elmer (Buckingham, UK).

Figure S1. Structures of **PF-543** and **VPC96091**



PF-543



VPC96091

Cell Culture. PASMC were grown in human smooth muscle cell growth medium. HEK 293 cells stably over-expressing GFP-SK1 (30-fold increase in SK1 activity vs. vector-transfected cells) were cultured in DMEM supplemented with 10% European fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1% non-essential amino acids, and 0.8% geneticin at 37 °C in 5% CO_2.

Preparation of Whole Cell Extracts. PASMC cell extracts for SDS-PAGE and Western blot analysis were prepared according to ref. S2.

[^{3}H]-Thymidine Incorporation Assay. PASMC cells were plated at a density of 2.0×10^4 cells/well in 24-well plates and grown to 60% confluency in complete medium before being quiesced in human smooth muscle cell basal growth medium containing 0.1% eFCS for 24 h. Subsequently, cells were treated with varying concentrations of inhibitor dissolved in DMSO or vehicle control (DMSO, 0.1%) for 48 h in complete medium. During the final 24 h, the cells were incubated with [^{3}H]-thymidine (0.25 μCi/mL) before termination by washing 3 times with 1 mL of ice-cold 10% (w/v) trichloroacetic acid. [^{3}H]-Thymidine incorporated into DNA was harvested with 0.25 mL of 0.1% (w/v) SDS and 0.3 M NaOH and was quantified by liquid-scintillation counting with 2 mL of scintillation cocktail. Each concentration of the inhibitor was tested in triplicate.

Sphingosine Kinase Activity Assays. In order to measure SK2 activity, Sph was complexed with fatty acid free bovine serum albumin (final concentration, 0.2 mg/mL) in buffer A containing 20 mM Tris (pH 7.4), 1 mM EDTA, 1 mM Na_3VO_4, 40 mM β-

glycerophosphate, 1 mM NaF, 0.007% (v/v) β-mercaptoethanol, 20% (v/v) glycerol, 10 μg/mL aprotinin, 10 μg/mL soybean trypsin inhibitor, 1 mM PMSF, 0.5 mM 4-deoxypyridoxine, and 400 mM KCl. SK2 assays were performed using 37 ng of purified SK2 and incubating the assay for 30 min at 30 °C in the presence of 10 μM Sph, 250 μM [γ-^{32}P]ATP in 10 mM $MgCl_2$, and varying concentrations of the inhibitors dissolved in DMSO or control (5% v/v DMSO). To measure SK1 activity, Sph was solubilized in Triton X-100 (final concentration, 0.063% w/v) and combined with buffer A without KCl. Thirty micrograms of recombinant SK1 was incubated for 30 min at 30 °C, in the presence of 3 μM Sph, 250 μM [γ-^{32}P]ATP in 10 mM $MgCl_2$ with or without inhibitor dissolved in DMSO or control (5% v/v DMSO). Both assay reactions were terminated by the addition of 500 μL of 1-butanol. After 1 mL of 2 M KCl was added, with mixing, two phases were formed. The lower (aqueous) phase, which contains unreacted [γ-^{32}P]ATP, was removed and discarded. The organic phase containing [^{32}P]-S1P was extracted by washing twice with 2 M KCl (1 mL each time) before quantification by Cerenkov counting. To evaluate the test compounds as putative substrates of SK1 and SK2, the assay was conducted in the presence of 50 μM of the test compound (but in the absence of Sph) and radioactivity in the 1-butanol phase was quantified.

We designed the SK1 and SK2 assays to enable us to evaluate selectivity of compounds for either of these lipid kinases. These assays were performed using Sph at concentrations of 3 and 10 μM, which correspond to the K_m values of SK1 and SK2, respectively.[S3,S4]

Therefore, the assays are performed under conditions where both enzymes are saturated to 50% with Sph, which enables estimation of selectivity by comparing the % inhibition of SK1 with the % inhibition of SK2 using a fixed concentration of inhibitor.

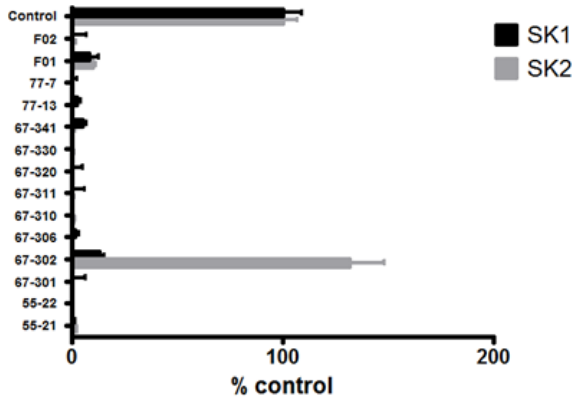


Figure S2. Evaluation of compounds as putative substrates of SK1 and SK2. SK1 and SK2 activity was measured using 50 µM compound and 250 µM ATP in the absence of Sph (n = 3 for each compound); results are expressed as mean ± S.D. Control = activity using Sph alone (3 µM for SK1 and 10 µM for SK2) and is represented as 100% against which each compound alone is compared.

Fig. S2 shows that **67-302** (*cis*-Sph; see structure on page S18) is an effective substrate of SK2; on the other hand, **F-01** is a very weak SK2 substrate. None of the analogues is an effective substrate at 50 µM, but **F-01**, **77-13**, **67-341**, and **67-302** are weak substrates of SK1.

(b) Synthetic procedures and compound characterization

General experimental methods. All chemicals were reagent grade and were used as purchased. Reactions were carried out under a dry nitrogen atmosphere using oven-dried glassware and magnetic stirring. The solvents were dried as follows: THF was heated at reflux over sodium benzophenone ketyl; toluene was heated at reflux over sodium; CH_2Cl_2 were dried over CaH_2. The progress of the reactions was monitored by TLC analysis using aluminum-backed silica gel 60 F254 plates of 0.2-mm thickness. The spots were visualized with short wavelength ultraviolet light or by charring after spraying with 15% H_2SO_4. Flash chromatography was performed on silica gel grade 60 (230–400 ASTM mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in δ units relative to deuterated solvents ($CDCl_3$ δ = 7.26 ppm for ^{1}H NMR and 77.00 ppm for ^{13}C NMR); CD_3OD δ = 4.78, 3.31 ppm for ^{1}H NMR and 49.1 ppm for ^{13}C NMR), which served as an internal reference, at 400 or 500 (for ^{1}H NMR) and 100 MHz (for ^{13}C NMR), respectively. The purity of the products was >95% based on proton NMR spectra. High-resolution mass spectra were recorded on an Agilent Technologies G6520A Q-TOF mass spectrometer using electrospray ionization. Optical rotations were recorded on a digital polarimeter at the sodium-D line at rt.

Detailed synthetic procedures.

(2*S*,3*R*)-2-Azidooctadecane-1,3-diol (2). This compound was prepared by reduction of azidoester **1**[S5] with an excess of $NaBH_4$ in THF/MeOH (100:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H), 1.26 (m, 26H), 1.5-1.58 (m, 2H), 3.42 (q, *J* = 5.2 Hz,

1H), 3.90 (d, J = 1.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.1,22.7, 25.6, 29.3, 29.48, 29.52, 29.56, 29.62, 29.68, 31.9, 33.7, 62.5, 66.8, 72.1.

(2*S*,3*R*)-1-Deoxy-2-amino-3-octadecanol (55-21). To a solution of azide **2**[S4] (1.50 g, 4.58 mmol) in 50 mL of CH_2Cl_2 was added 430 μL (5.92 mmol) of $SOCl_2$, followed by 950 μL (11.7 mol) of pyridine at -78 °C. The reaction mixture was stirred at -78 °C for 2 h, then at rt for 2 h, and was filtered through a pad of silica gel, which was washed with hexane/EtOAc (10:1). The filtrate was concentrated to give a cyclic sulfite intermediate. To a solution of the cyclic sulfite in 25 mL of MeCN were added 1.30 g (6.07 mmol) of crystalline $NaIO_4$ and 30 mg (0.14 mmol) of $RuCl_3 \cdot 3H_2O$ in 5 mL of H_2O. The mixture was stirred at rt for 2 h, and then was diluted with 250 mL of Et_2O and washed with H_2O. The ether layer was dried over Na_2SO_4 and concentrated to give 2-azido-1,3-cyclic sulfate **3**. To a solution of **3** in 25 mL of DMF were added 380 mg (10.0 mmol) of $NaBH_4$ and 760 mg (5.07 mmol) of NaI at 0 °C. The mixture was stirred for 48 h at rt, and then was diluted with 200 mL of Et_2O, treated with 100 mL of 1 M of aqueous HCl solution for 4 h, and neutralized with 5 M of aqueous NaOH solution. The organic layer was separated, dried over Na_2SO_4, and concentrated. The product was purified by column chromatography on silica gel (eluting with $CHCl_3$/MeOH/concd NH_4OH 130:25:4) to afford 1.04 g (79%) of **55-21**. The product was dissolved in a minimum volume of $CHCl_3$ and passed through a Cameo filter to remove dissolved silica gel: $[\alpha]_D$ +5.0 (*c* 0.40, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.4 Hz), 1.01 (d, 2H, J = 6.4 Hz), 1.26 (m,

26H), 1.35 (m, 1H), 1.73 (br s, 3H), 2.98 (m, 1H), 3.44 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.5, 29.34, 29.60, 29.61, 29.64, 29.69, 29.8, 31.9, 33.8, 63.4, 70.8.

(2*S*,3*R*)-2-*N*,*N*-Dimethylamino-3-octadecanol (55-22). To a mixture of **55-21** (457 mg, 1.60 mmol) and paraformaldehyde (500 mg, 16.6 mmol) in 50 mL of MeOH was added $NaBH_3CN$ (1.10 g, 17.5 mmol) at 0 °C. After the mixture was stirred at rt for 48 h, it was diluted with 200 mL of EtOAc and was washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The product was purified by column chromatography on silica gel (eluting with hexane/ EtOAc 1:1) to afford 412 mg (82%) of **55-22**: $[\alpha]_D$ +4.9 (*c* 0.35, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 0.98 (d, 3H, *J* = 6.8 Hz), 1.26 (m, 26H), 1.53 (m, 1H), 2.21 (m, 2H), 2.30 (s, 6H), 3.72 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 14.1, 22.7, 26.5, 29.35, 29.61, 29.62, 29.65, 29.68, 29.8, 31.9, 33.8, 42.8, 63.6, 70.8.

(2*S*,3*R*)-*N*,*N*,*N*-Trimethyl-3-hydroxy-2-octadecanaminium *p*-toluenesulfonate (77-13). A mixture of **55-22** (81 mg, 0.26 mmol) and methyl *p*-toluenesulfonate (63 mg, 0.34 mmol) in 5 mL of THF was stirred overnight at rt. After the *N*-methylation reaction was completed, the mixture was diluted with 5 mL of hexane. Filtration provided 125 mg (100%) of **77-13**: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 0.98 (d, 3H, *J* = 6.8 Hz), 1.26 (m, 26H), 1.53 (m, 1H), 2.21 (m, 2H), 2.30 (s, 9H), 3.72 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 14.1, 22.7, 26.5, 29.35, 29.61, 29.62, 29.65, 29.68, 29.8, 31.9, 33.8, 42.8, 63.6, 70.8; HRMS (M^+) calcd for *m*/*z* $C_{21}H_{48}NO^+$ 328.3574, found 328.3579.

Ethyl 4-Tetradecyloxy-2(*E*)-butenonate (6). To a solution of $NaIO_4$ (4.12 g, 19.2 mmol) in 25 mL of water was added a solution of 1-*O*-tetradecyl-*rac*-glycerol (**4**, 4.27 g, 14.8 mmol)[S6] in 25 mL of THF at 0 °C, followed by stirring at rt. After the oxidative glycol cleavage was completed (about 2 h at rt), the mixture was concentrated under reduced pressure in order to remove THF and the formaldehyde formed, providing aldehyde **5**. To the residue of crude **5** was added a solution of triethyl phosphonoacetate (4.68 g, 20.9 mmol) in 50 mL of 2-propanol, followed by dropwise addition of a solution of K_2CO_3 (26.0 g, 187 mmol) in 50 mL of water at 0 °C. The reaction mixture was gradually warmed to rt. After the mixture was stirred overnight at rt, the olefination product **6** was extracted with Et_2O (3 x 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography on silica gel (elution with hexane/EtOAc 25:1) to give 4.40 g (91%) of **6**: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H), 1.25 (s, 22H), 1.31(t, J = 7.2 Hz, 3H), 1.57-1.63 (m, 2H), 3.46 (t, J = 6.6 Hz, 2H), 4.13 (dd, J = 2.0, 4.3 Hz, 2H), 4.21 (q, J = 7.2 Hz, 2H), 6.08 (dt, J = 2.0, 15.7 Hz, 1H), 6.97 (dt, J = 4.3, 15.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.2, 22.7, 26.1, 29.3, 29.45, 29.56, 29.58, 29.63, 29.64, 29.66, 31.9, 60.3, 69.3, 71.2, 121.1, 144.7, 166.3; HRMS (MH^+) calcd for *m*/*z* $C_{20}H_{39}O_3^+$ 327.2894, found 327.2893.

Ethyl (2*R*,3*R*)-4-Tetradecyloxy-2,3-dihydroxybutanonate (7). After a solution of 14.0 g of AD-mix-β in 100 mL of *t*-BuOH/H_2O (1:1) was stirred vigorously at rt for 1 h, 950 mg (10.0 mmol) of $MeSO_2NH_2$ was added, and stirring was continued for an additional 10 min. After 3.27 g (10.0 mmol) of **6** was added, the mixture was allowed to warm to rt. After the α,β-unsaturated ester

was completely consumed (TLC), the reaction was quenched by the addition of sodium sulfite (1.5 g, 14.6 mmol). The product was extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated. Chromatography on silica gel (elution with hexane/EtOAc 2:1) gave 3.07 g (89%) of **7**: $[\alpha]_D$ +7.8 (*c* 1.61, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.6 Hz, 3H), 1.24 (s, *J* = 22H), 1.31 (t, *J* = 7.2 Hz, 3H), 1.54-1.57 (m, 2H), 2.61 (d, *J* = 7.4 Hz, 1H), 3.27 (d, *J* = 5.9 Hz, 1H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.51-3.62 (m, 2H), 4.06-4.13 (m, 1H), 4.23 (dd, *J* = 2.1, 5.8 Hz, 1H), 4.27 (q, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.08, 14.10, 22.7, 26.0, 29.3, 29.4, 29.53, 29.56, 29.58, 29.62, 29.63, 29.65, 31.9, 62.0, 70.8, 71.0, 71.2, 71.7, 173.1; HRMS (MH^+) calcd for *m*/*z* $C_{20}H_{31}O_5^+$ 361.2949, found 361.2952.

Ethyl (2*S*,3*R*)-4-Tetradecyloxy-2-azido-3-hydroxybutanonate (9). To a solution of 2.89 g (8.01 mmol) of **7** in 50 mL of CH_2Cl_2 was added 1.3 mL (16.1 mmol) of pyridine at rt. After the mixture was stirred and chilled to 0 °C, 760 μL (10.4 mmol) of $SOCl_2$ was added slowly. The reaction mixture was stirred for 30 min, and then was filtered through a pad of silica gel in a sintered glass funnel. The pad was washed with hexane/EtOAc 10:1, and the filtrate was concentrated and further dried under high vacuum (~1 torr) for 2 h. To a solution of the crude cyclic sulfite in 20 mL of MeCN was added 2.57 g (12.0 mmol) of $NaIO_4$. The heterogeneous mixture was stirred vigorously while a solution of 2.8 mg (0.080 mmol) of $RuCl_3 \cdot 3H_2O$ in 8 mL of H_2O was added. After the full consumption of the starting cyclic sulfite was observed (~1 h), the reaction mixture was diluted with 250 mL of Et_2O and washed with brine. The organic phase was dried (Na_2SO_4) and passed through a small pad of silica gel. Concentration of the filtrate gave crude

cyclic sulfate **8**, which was used without further purification. To a solution of **8** in 30 mL of acetone was added 1.56 g (24 mmol) of NaN_3, followed by 15 mL of H_2O. The reaction mixture was stirred at rt until **8** was fully consumed. After acetone and water were removed, the residue was dissolved in 100 mL of Et_2O, and the solution was treated with 50 mL of 20% aqueous H_2SO_4 in a fume hood with vigorous stirring of the heterogeneous mixture until the hydrolysis was completed. The layers were separated, and the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organic layers were treated with anhydrous K_2CO_3 (~100 mg) to remove the dissolved H_2SO_4 and then were dried (Na_2SO_4). After concentration, the product was purified by chromatography on silica gel (elution with hexane/EtOAc 10:1) to give 2.44 g (79%) of azide **9**: $[\alpha]_D$ +40.9 (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.26 (s, 22H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.54-1.57 (m, 2H), 2.77 (d, *J* = 7.4 Hz, 1H), 3.41-3.51 (m, 2H), 3.52-3.61 (m, 2H), 4.03-4.13 (m, 2H), 4.28 (q, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.08, 14.10, 22.7, 26.0, 29.3, 29.4, 29.56, 29.59, 29.63, 29.67, 31.9, 62.0, 63.2, 70.3, 70.8, 71.8, 168.7; HRMS (MNa^+) calcd for *m*/*z* $C_{20}H_{39}N_3NaO_4^+$ 408.2833, found 408.2837.

(2*S*,3*R*)-4-Tetradecyloxy-2-azido-1,3-butanediol (10). To a solution of 2.43 g (6.30 mmol) of **9** in 100 mL of THF was added 380 mg (10.0 mmol) of $NaBH_4$, followed by 1 mL of MeOH at 0 °C. The reaction mixture was stirred vigorously while the reaction mixture was allowed to warm to rt until the disappearance of **9** (monitored by TLC). After THF and water were removed, the residue was dissolved in 200 mL of EtOAc, and then was washed with brine and water. The organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel (elution with hexane/EtOAc 10:1, 8:1, and 6:1) to

give 1.11 g (51%) of **10**: $[\alpha]_D$ +3.5 (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.7 Hz, 3H), 1.26 (s, 22H), 1.53-1.63 (m, 2H), 2.51 (t, *J* = 5.7 Hz, 1H), 2.78 (d, *J* = 6.0 Hz, 1H), 3.40-3.65 (m, 5H), 3.77-3.87 (m, 2H), 3.88-3.97 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.0, 29.51, 29.56, 29.59, 29.63, 29.65, 29.67, 31.9, 62.7, 64.0, 70.7, 71.2, 71.8; HRMS (MH^+) calcd for *m*/*z* $C_{20}H_{39}O_3^+$ 327.2894, found 327.2893.

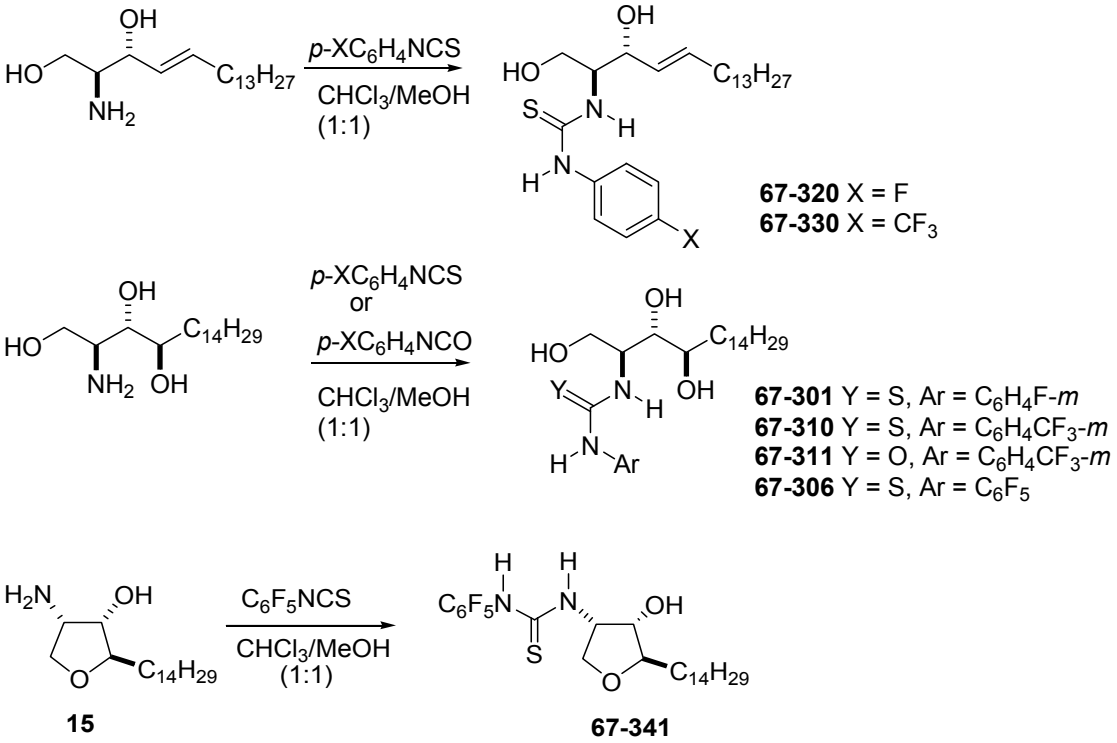
(2*S*,3*R*)-4-Tetradecyloxy-2-amino-3-butanol (77-6b). A mixture of **10** (382 mg, 1.11 mmol) and *n*-Bu_2SnO (280 mg, 1.12 mmol) in 25 mL of toluene was heated at reflux until a clear solution was formed. The solvent was removed under reduced pressure and the residue (**11**) was dried further under high vacuum for 2 h. After the dry residue was dissolved in 25 mL of CH_2Cl_2, 220 mg (1.15 mmol) of *p*-toluenesulfonyl chloride was added at 0 °C. After the mixture was stirred overnight at rt, the reaction was quenched by addition of H_2O (20 μL, 1.11 mmol). The mixture was filtered through a pad of Celite, which was washed with 100 mL of Et_2O. The filtrate was washed with brine, aqueous saturated $NaHCO_3$ solution, and water. The organic layer was dried (Na_2SO_4) and concentrated to give crude tosylate **12**. To a solution of **12** in 25 mL of THF was added 100 mg (2.64 mmol) of $NaBH_4$ at 0 °C. The mixture was stirred for 48 h at rt, diluted with 200 mL of Et_2O, and washed with brine. The ether layer was dried (Na_2SO_4) and concentrated. To the residue was added Pearlman's catalyst (50 mg) in 25 mL of MeOH, and the mixture was stirred overnight at rt under a hydrogen atmosphere. After the catalyst was removed by filtration, the product was purified by column chromatography on silica gel (eluting with $CHCl_3$/MeOH/conc. NH_4OH 130:25:4) to afford 241 mg (66%) of **77-6b**. The product was dissolved in a

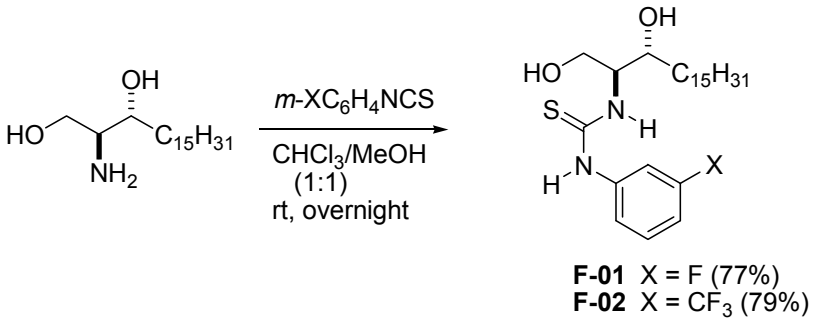
minimum volume of $CHCl_3$ and passed through a Cameo filter to remove dissolved silica gel: $[\alpha]_D$ +4.5 (*c* 0.40, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.4 Hz, 3H), 1.10 (d, *J* = 6.4 Hz, 2H), 1.26 (m, 26H), 1.57 (m, 2H), 2.45 (br s, 3H), 3.05 (m, 1H), 3.43-3.49 (m, 4H), 3.62 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.6, 22.7, 26.1, 29.33, 29.44, 29.56, 29.58, 31.9, 48.8, 71.7, 71.8, 73.6; HRMS (MH^+) calcd for *m*/*z* $C_{18}H_{40}NO_2^+$ 302.3054, found 302.3057.

(2*S*,3*R*)-4-Tetradecyloxy-2-(*N*,*N*-dimethylamino)-3-butanol (77-7). To a mixture of paraformaldehyde (50 mg, 1.6 mmol) and **77-6b** (43 mg, 0.14 mmol) in 10 mL of MeOH was added $NaBH_3CN$ (110 mg, 1.75 mmol) at 0 °C. The mixture was stirred at rt for 48 h, and then was diluted with 200 mL of EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The product was purified by column chromatography on silica gel (eluting with $CHCl_3$, $CHCl_3$/MeOH 25:1, and then $CHCl_3$/MeOH/conc. NH_4OH 130:25:4) to afford 38 mg (80%) of product: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.02 (d, 3H, *J* = 6.7 Hz), 1.26 (m, 26H), 1.58 (q, *J* = 6.7 Hz, 2H), 2.27 (s, 6H), 2.49 (q, *J* = 6.6 Hz, 1H), 3.37 (dd, *J* = 7.9, 9.5 Hz, 1H), 3.46 (t, *J* = 6.6 Hz, 2H), 3.54 (dd, *J* = 3.6, 9.5 Hz, 1H), 3.87-3.82 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 8.5, 14.1, 22.7, 26.1, 29.35, 29.46, 29.59, 29.64, 29.67, 31.9, 41.7, 61.2, 70.8, 71.5, 73.1; HRMS (MH^+) calcd for *m*/*z* $C_{20}H_{44}NO_2^+$ 330.3367, found 330.3365.

General Procedure for Preparation of *N*-Arylthiourea and *N*-Arylurea Derivatives. To a solution of the amino-sphingoid base (1.02 mmol) in 30 mL of $CHCl_3$/MeOH (1:1) was added the aryl isothiocyanate or aryl isocyanate (XC_6H_4NCS or XC_6H_4NCO,

1.00 mmol) at rt. The mixture was stirred overnight, and then was concentrated under reduced pressure. The product was purified by column chromatography on silica gel.





F-01 X = F (77%)
F-02 X = CF_3 (79%)

Scheme S1. Synthesis of *N*-arylthiourea and *N*-arylurea derivatives by the reaction of Sph, phytosphingosine (PHS), 2-*epi*-pachastrissamine, or sphinganine with an aryl isothiocyanate (X = S) or an aryl isocyanate (X = O).

F-02. $[\alpha]^{30}_{D}$ = +22.6 (*c*, 9.65); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.26 (m, 26H), 1 58 (m, 2H), 2.76 (br s, 2H), 3.93 (d, *J* = 2.8 Hz, 1H), 3.96 (d, *J* = 2.0 Hz, 1H), 4.09 (d, *J* = 10.2 Hz, 1H), 4.44 (br s, 1H), 7.41-7.59 (m, 5H), 8.74 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.9, 29.3, 29.5, 29.56, 29.62, 29.65, 29.67, 31.9, 34.6, 57.9, 61.9, 74.6, 119.4, 120.61, 120.64, 120.86, 120.72, 122.1, 122.8, 124.8, 127.0, 130.3, 132.0132.3, 137.6, 179.8; HRMS (MH^+) calcd for *m/z* $C_{26}H_{44}F_3N_2O_2S^+$ 505.3070, found 505.3074; HRMS (MNa^+) calcd for *m/z* $C_{26}H_{43}F_3N_2O_2SNa^+$ 527.2890, found 527.2891.

67-301. Yield: 91%; ^{1}H NMR (pyridine-d_5) δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.25 (m, 24H), 1.57-1.74 (m, 1H), 1.73-1.81 (m, 2H), 2.16-2.31 (m, 1H), 4.20-4.29 (m, 1H), 4.34-4.43 (m, 1H), 4.50-4.66 (m, 2H), 5.74 (br s, 1H), 7.05 (t, *J* = 8.6, 2H), 7.53-7.69 (m, 2H),

8.07 (br s, 1H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 13.8, 22.5, 25.6, 29.1, 29.4, 29.5, 31.7, 33.1, 56.2, 60.7, 72.6, 75.4, 116.0, 116.2, 126.6, 126.7, 159.4, 161.9, 180.1; HRMS (MH^+) *m*/*z* calcd. for $C_{25}H_{44}FN_2O_3S^+$ 471.3051, found 471.3051.

67-310. Yield: 89%; ^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.88 (t, *J* = 6.3 Hz, 3H), 1.26 (m, 24H), 1.50-1.61 (m, 1H), 1.70-1.79 (m, 1H), 3.62-3.96 (m, 10H), 7.60 (s, 4H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 13.8, 22.5, 25.6, 29.1, 29.4, 29.5, 31.7, 33.1, 55.8, 60.7, 72.6, 75.4, 122.49, 122.67, 122.70, 122.76, 125.2, 125.93, 125.97, 126.02, 126.3, 126.7, 141.4, 179.9; HRMS (MH^+) *m*/*z* calcd. for $C_{26}H_{44}F_3N_2O_3S^+$, 521.3019, found 521.3019.

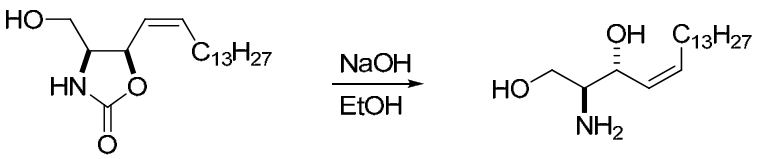
67-311. Yield: 76%; ^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.89 (t, *J* = 6.2 Hz, 3H), 1.26 (m, 24H), 1.51-1.62 (m, 1H), 1.69-1.80 (m, 1H), 3.35 (br s, 1H), 3.59 (m, 2H), 3.78 (dd, *J* = 4.7, 11.3 Hz, 1H), 3.85 (dd, *J* = 4.4, 11.3 Hz, 1H), 4.05 (m, 1H), 4.26 (br s, 1H), 7.48-7.57 (m, 4H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 13.2, 22.1, 25.2, 28.8, 29.08, 29.12, 31.4, 32.5, 51.5, 60.9, 71.9, 75.5, 114.1 136.4, 138.9, 141.7, 142.9, 145.3, 183.4; HRMS (MH^+) *m*/*z* calcd. for $C_{26}H_{44}F_3N_2O_4^+$, 505.3248, found 505.3246.

67-306. Yield: 85%;^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.89 (t, *J* = 6.5 Hz, 3H), 1.28 (m, 24H), 1.57 (m, 1H), 1.76 (m, 1H), 3.62 (m, 1H), 3.73 (m, 1H), 3.87 (m, 1H), 3.97 (m, 1H), 4.71 (br s, 1H), 7.46 (br s, 1H); ^{13}C NMR δ 13.6, 22.3, 25.6, 29.0, 29.3, 31.6, 32.7, 56.3, 60.4, 72.5, 75.0, 82.5, 85.1, 114.1 136.1, 138.7, 141.4, 142.8, 145.3, 182.8; HRMS (MH^+) *m*/*z* calcd. for $C_{25}H_{40}F_5N_2O_3S^+$, 543.2674, found 543.2676.

67-341. Yield: 84%; ^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.88 (t, *J* = 3.7 Hz, 3H), 1.26 (m, 24H), 1.41-1.49 (m, 2H), 3.62 (t, *J* = 9.4 Hz, 1H), 3.73-3.78 (m, 1H), 4.00- 4.05 (m, 1H), 4.22-4.34 (m, 1H), 4.77 (br s, 1H), 7.33 (s, 1H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 13.9, 22.5, 25.6, 29.2, 29.42, 29.46, 29.52, 29.55, 31.8, 33.3, 56.7, 70.0, 74.0, 82.5, 85.2, 114.1, 136.4, 138.9, 141.7, 142.9, 145.3, 183.4; HRMS (MH^+) *m/z* calcd. for $C_{25}H_{38}F_5N_2O_2S^+$ 525.2569, found 525.2570.

67-320 (H_2O). $[\alpha]^{30}_D$ = +21.8 (*c*, 4.35); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H), 1.26 (m, 20H), 1.32-1.40 (m, 2H), 2.05 (q, *J* = 6.9 Hz, 2H), 2.35 (br s, 1H), 2.48 (br s, 1H), 3.75-3.86 (m, 1H), 4.03 (d, *J* = 11.6 Hz, 1H), 4.15 (br s, 1H), 5.55 (dd, *J* = 6.0, 15.4 Hz, 1H), 5.80 (dt, *J* = 6.7, 15.4 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 7.00-7.16 (m, 2H), 7.22-7.28 (m, 2H), 7.74 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.0, 29.2, 29.3, 29.45, 29.59, 29.64, 29.66, 31.9, 32.3, 58.9, 61.9, 74.5, 116.1, 117.0, 126.96, 127.05, 128.3, 132.2, 134.2, 157.0, 162.4, 180.3; HRMS (MH^+) *m/z* calcd. for $C_{25}H_{42}FN_2O_2S^+$, 453.2946, found 453.2943.

67-330 (H_2O). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H), 1.26 (m, 20H), 1.32-1.42 (m, 2H), 2.07 (q, *J* = 6.9 Hz, 2H), 2.41 (br s, 1H), 2.54 (br s, 1H), 3.85 (d, *J* = 10.7 Hz, 1H), 4.08-4.11 (m, 2H), 4.50-4.51 (m, 1H), 4.61 (m, 1H), 5.58 (dd, *J* = 6.0, 15.4 Hz, 1H), 5.84 (dt, *J* = 6.7, 15.4 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 7.00-7.16 (m, 2H), 7.22-7.28 (m, 2H), 7.74 (br s, 1H); HRMS (MH^+) *m/z* calcd. for $C_{26}H_{42}F_3N_2O_2S^+$ 503.2914, found 503.2914.



67-304 **67-302**

67-304. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H), 1.26 (m, 20H), 1.31-1.40 (m, 2H), 1.98-2.14 (m, 2H), 3.64 (d, *J* = 4.7 Hz, 2H), 3.86 (dt, *J* = 4.7, 8.1 Hz, 1H), 5.46 (t, *J* = 8.6 Hz, 1H), 5.57-5.63 (m, 1H), 5.75-5.82 (m, 1H), 6.64 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.9, 29.2, 29.32, 29.34, 29.42, 29.5, 29.64, 29.67, 31.9, 57.3, 61.6, 75.2, 122.0, 137.7, 160.6; HRMS (MNa^+) *m*/*z* calcd. for $C_{19}H_{35}NNaO_3^+$ 348.2509, found 348.2509.

67-302 (*cis*-Sphingosine). ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.27 (m, 22H), 2.00-2.22 (m, 2H), 3.19-3.24 (m, 1H), 3.65-3.86 (m, 2H), 4.69-4.75 (m, 1H), 5.30-5.45 (m, 1H), 5.63-5.74 (m, 1H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 13.5, 22.2, 27.4, 28.9, 29.08, 29.13, 29.18, 29.21, 29.24, 31.5, 67.1, 57.8, 64.5, 125.7, 135.2; HRMS (MH^+) *m*/*z* calcd. for $C_{18}H_{38}NO_2^+$ 300.2897, found 300.2899.

B. References

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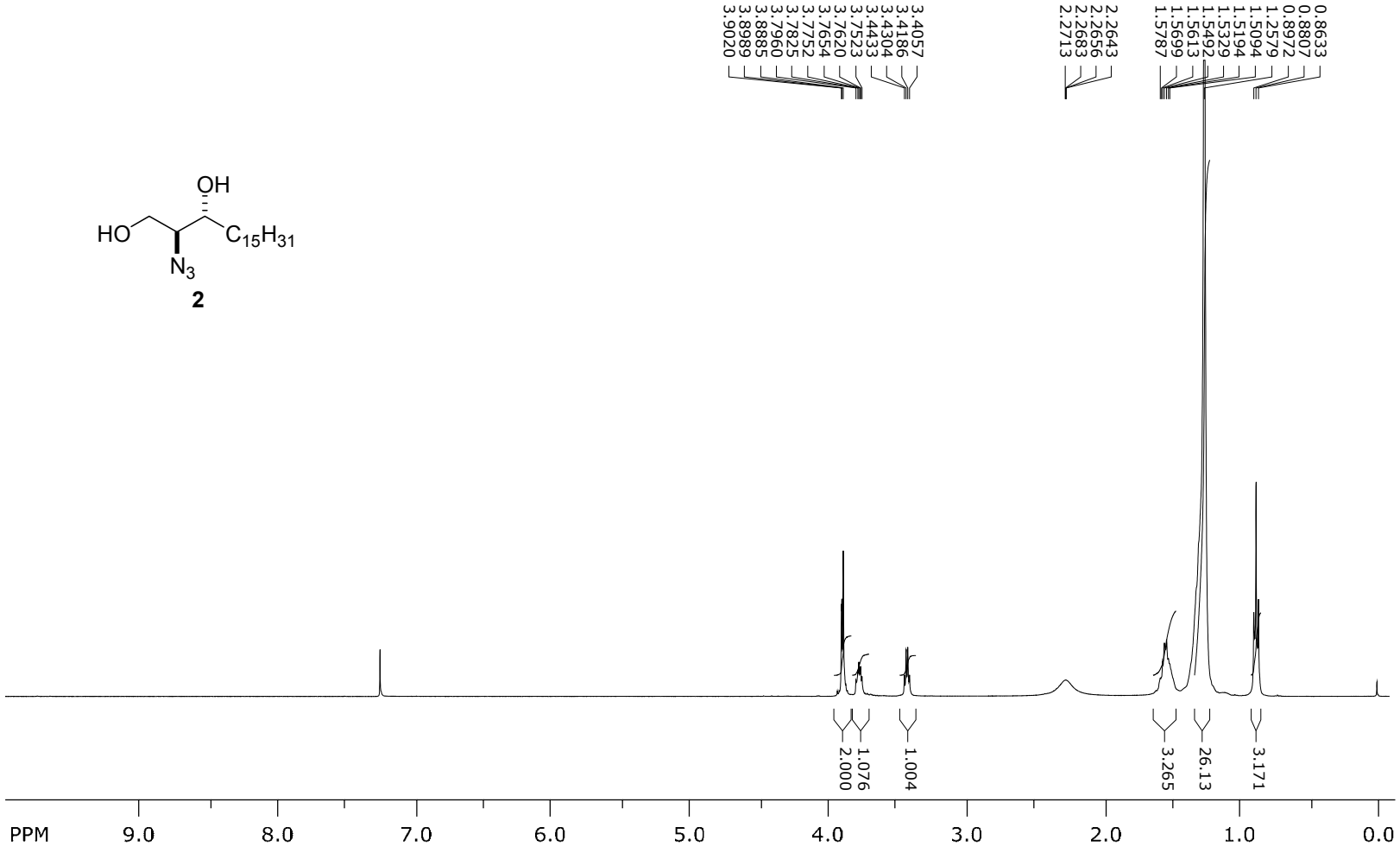
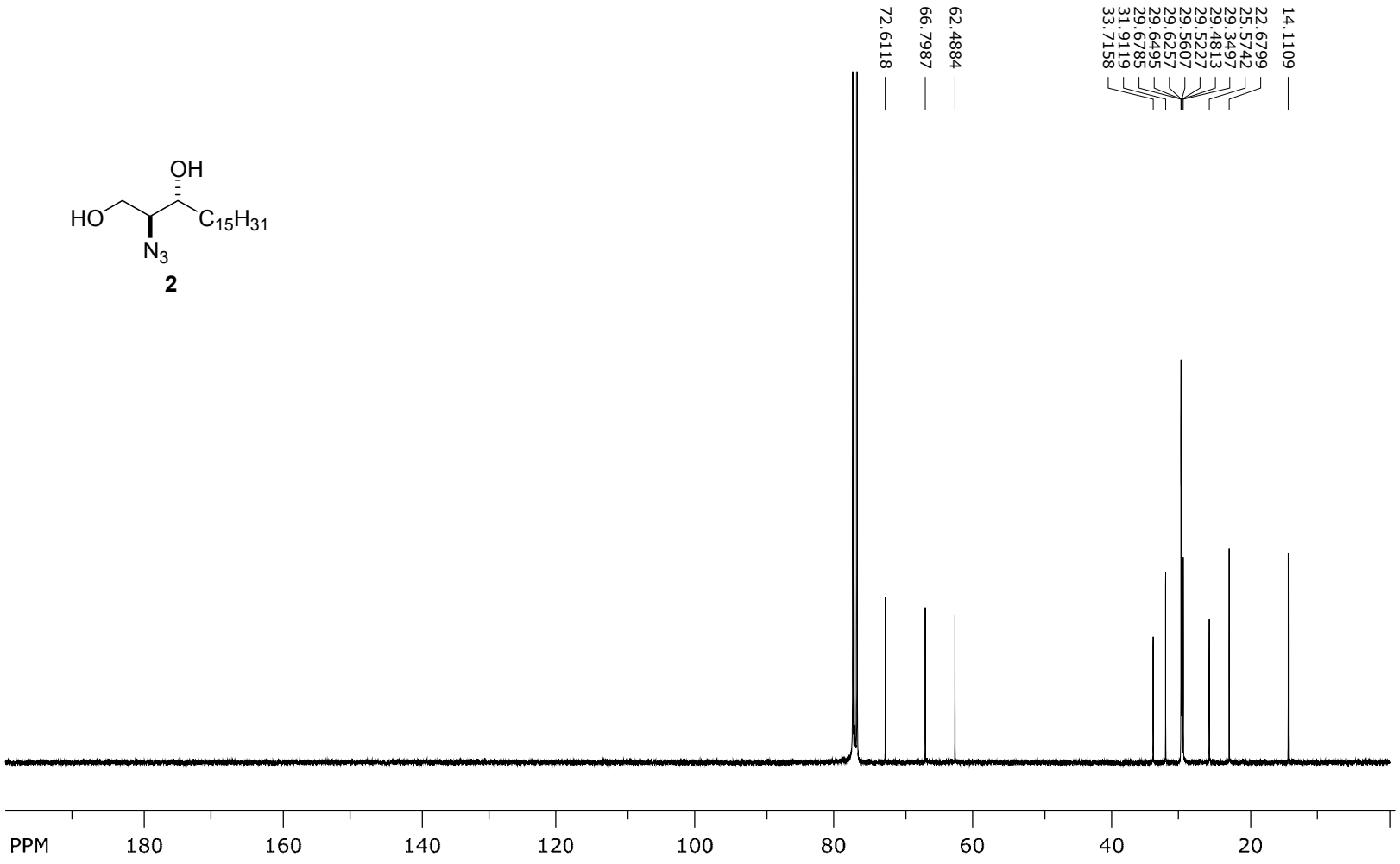
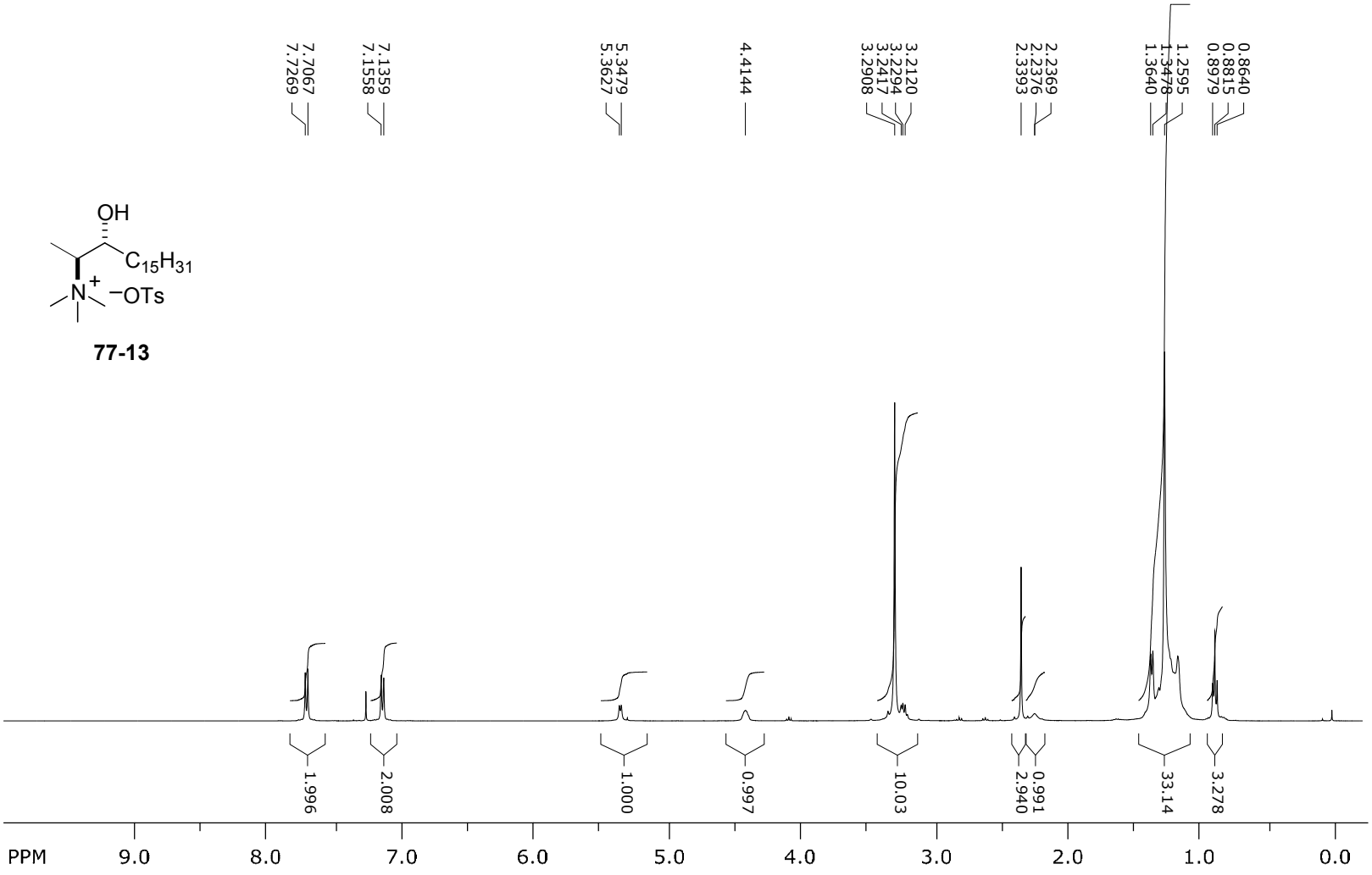
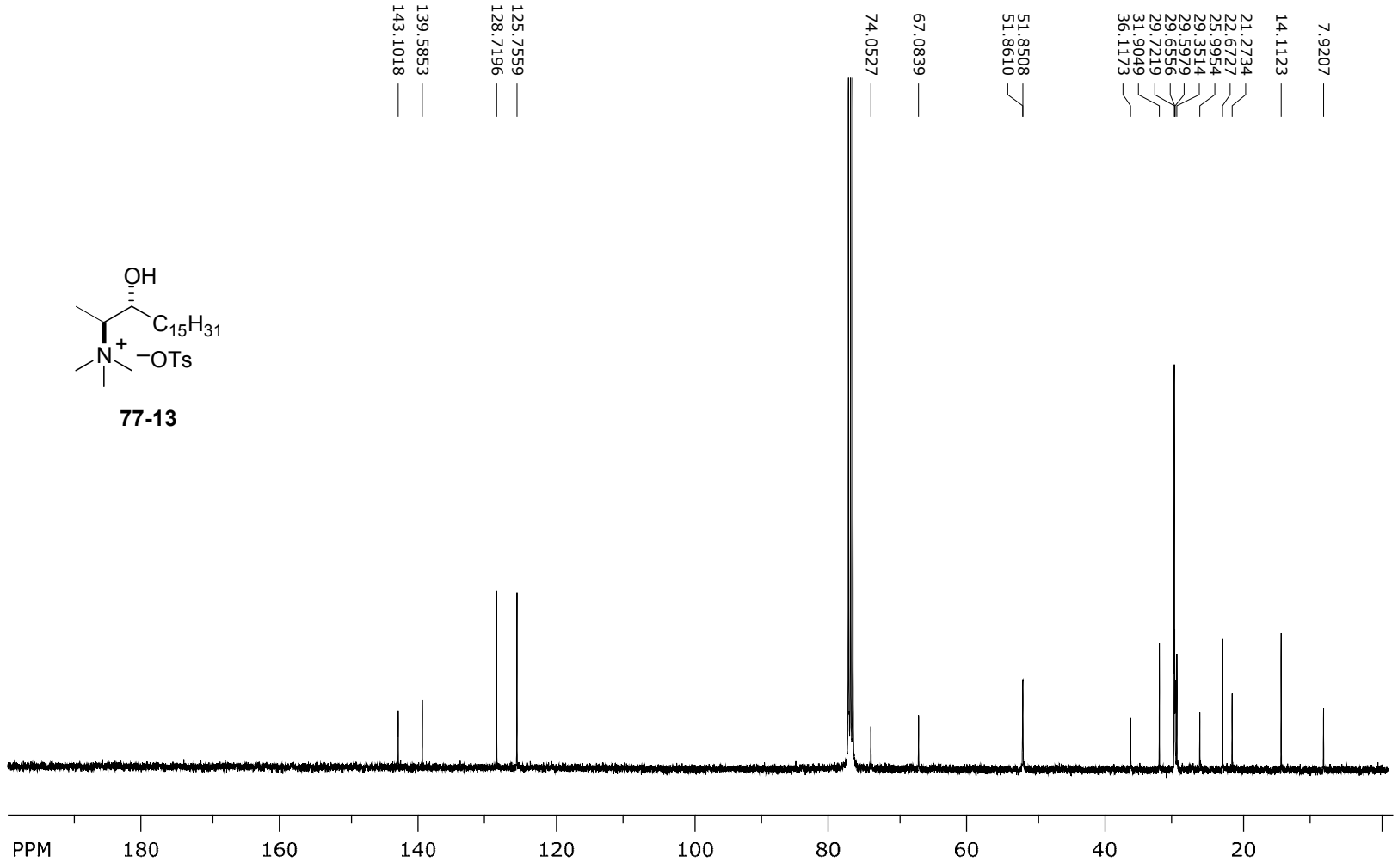
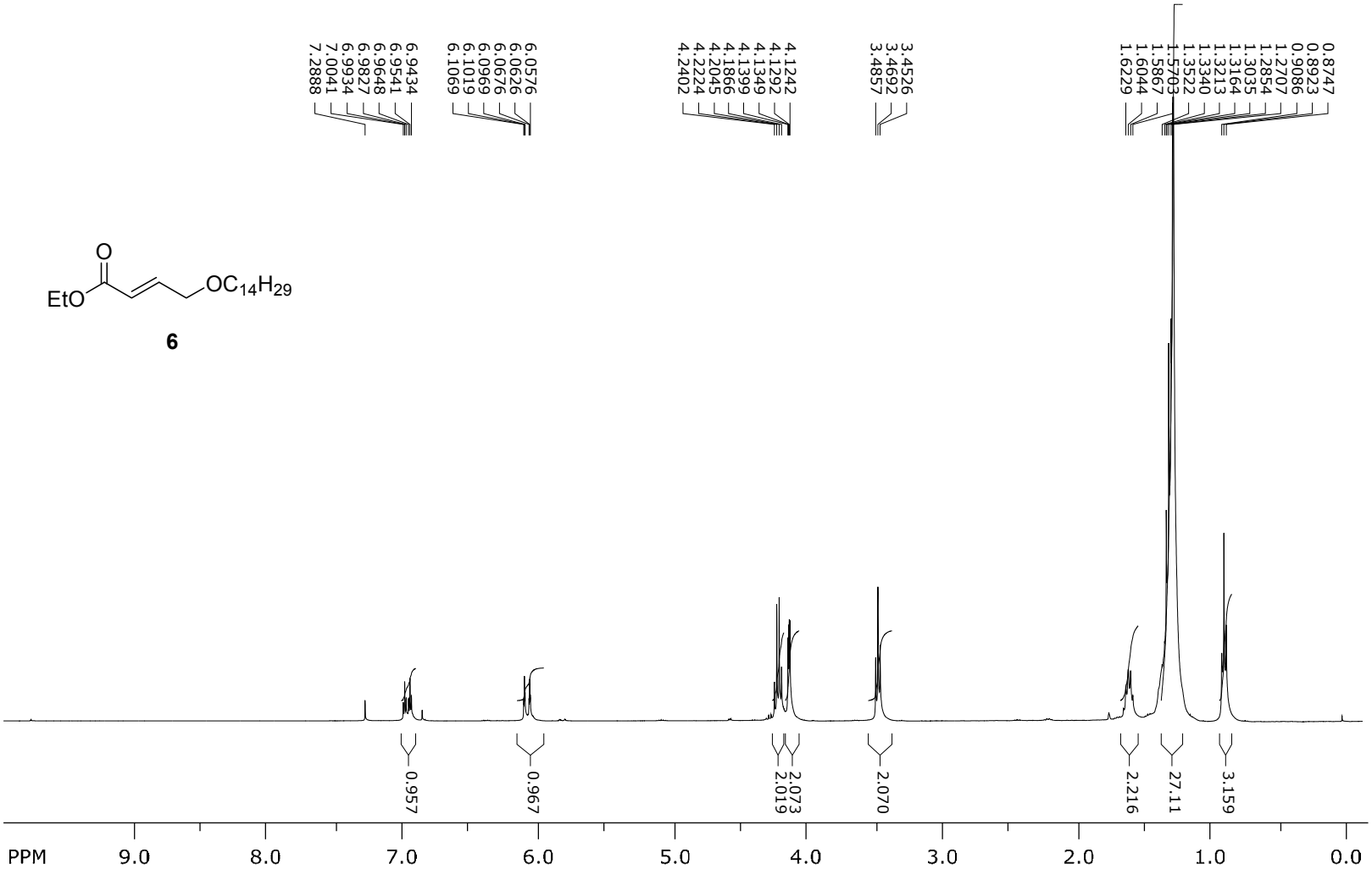
C. NMR Spectra











EtO, $OC_{14}H_{29}$

6