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Exploring *Leishmania major* Inositol Phosphorylceramide Synthase (*Lmj*IPCS): Insights into the ceramide binding domain

ELECTRONIC SUPPLEMENTARY INFORMATION

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CHEMISTRY EXPERIMENTAL

General Considerations

Compound Numbering System

Numbered compounds in the manuscript have the same number in the ESI. All other compounds are numbered following the pattern S##.

Spectroscopic Data

Infrared spectra were recorded using a golden gate (ATR) on a Perkin-Elmer FT-IR 1600 spectrometer and reported in the following format ν_{max} frequency (s, strong; br, broad and w, weak) cm^{-1}. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ or CD_3OD unless otherwise stated on a Varian VXR-400 (^{1}H at 400 MHz, ^{13}C at 101 MHz) and reported as follows:

Chemical shift was reported in the following format; δ (ppm) (number of protons, multiplicity, coupling constant J (Hz), assignment).

The residual protic solvent was used as internal reference:
$CHCl_3$ δ_H = 7.26 ppm; $CDCl_3$ δ_C = 77.16 ppm
CHD_2OD δ_H= 3.31 ppm, 1.09 ppm; CD_3OD δ_C = 49.0 ppm

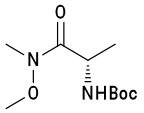
Assignment of stereochemistry was carried out using COSY, HSQC, HMBC and NOESY experiments.

Mass Spectrometry

Low Resolution Mass Spectra were obtained on Waters Micromass LCT Mass spectrometer. Gas-Chromatography Mass Spectra (GC-MS) were taken using a Thermo-Finnigan Trace. High-resolution mass spectra (HRMS) were performed on a Thermo-Finnigan LTQ FT Mass Spectrometer by Durham University Mass Spectroscopy service

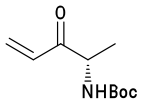
Experimental Procedures

Synthesis of Intermediate compounds



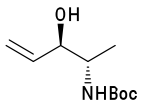
S01 (*S*)-tert-Butyl (1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate[1]

Procedure:[2] To a solution of *N*-(*tert*-butoxycarbonyl)-L-alanine (1.513 g, 8.0 mmol) in anhydrous CH_2Cl_2 (50 ml) was added *N*-methylmorpholine (0.967 ml, 8.8 mmol, 1.1 eq.) and *N*,*O*-dimethylhydroxylamine hydrochloride (0.859 g, 8.8 mmol, 1.1 eq.) at –15 °C. To the reaction mixture was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.53 g, 8.8 mmol, 1.1 eq.) over 30 min at the same temperature. The reaction mixture was stirred for 4 hrs then poured into ice and 1N HCl. The resulting mixture was extracted with CH_2Cl_2. The organic layers were combined, and washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude Weinreb amide as a white solid. Column chromatography on silica gel (from 25 % to 50 % EtOAc in pet. ether) gave 101 (1.69 g, 98 %) as a white solid. ν_{max} (ATR) 3293 (s), 2976, 1703, 1658, 1537, 1293, 1173, 1066, 981 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.24 (1H, m, N*H*), 4.68 (1H, m, 2-*H*), 3.77 (3H, s, OCH_3), 3.20 (3H, s, NCH_3), 1.44 (9H, s, C(CH_3)$_3$), 1.31 (3H, d, *J* 6.9, 3-H_3); δ_C (176 MHz, $CDCl_3$) 173.8 *C1*, 155.3 NH*C*OO, 79.7 *C*$(CH_3)_3$, 61.8 O*C*H_3, 46.7 *C2*, 32.3 N*C*H_3, 28.5 C(*C*H_3)$_3$, 18.9 *C3*; *m/z* (ES^+) 232.9 $[M]^+$ $C_{10}H_{20}O_4N_2^{23}$ (Expected: 232.1), 255.1 $[M+Na]^+$ $C_{10}H_{20}O_4N_2^{23}Na$ (Expected: 255.1).



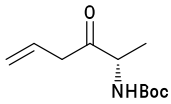
13 (*S*)-*tert*-Butyl (3-oxopent-4-en-2-yl)carbamate[3]

Procedure:[2] Vinyl magnesium bromide (12 ml of 0.6M solution in THF, 7.2 mmol, 4 eq.) was added dropwise at 0 °C to a solution of S01 (400 mg, 1.8 mmol) in anhydrous THF (10 ml). The reaction mixture was allowed to warm up to room temperature. After the reaction mixture was stirred for 1 hr at the same temperature, the reaction mixture was poured into ice cooled 2N HCl. The resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (from 10 % to 50 % EtOAc in pet. ether) gave 13 (335 mg, 98 %) as a white solid (R_f ≈ 0.37; EtOAc/pet. ether 25/75). ν_{max} (ATR) 3382 (s), 2975, 2931, 1710, 1694, 1612, 1518, 1282, 1246, 1162, 1003 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 6.45 (1H, dd, *J* 17.4, 10.5, 4-*H*), 6.37 (1H, dd, *J* 17.4, 1.3, 5-H_{trans}), 5.88 (1H, d, *J* 10.5, 1.3, 5-H_{cis}), 5.36 (1H, m, N*H*), 4.61 (1H, p, *J* 7.2, 2-*H*), 1.43 (9H, s, C(CH_3)$_3$), 1.33 (3H, d, *J* 7.2, , 1-H_3); δ_C (176 MHz, $CDCl_3$) 198.8 *C3*, 155.3 NH*C*OO, 132.9 *C4*, 130.3 *C5*, 79.8 *C*$(CH_3)_3$, 53.2 *C2*, 28.5 C(*C*H_3)$_3$, 18.6 *C1*; *m/*z (ES^+) 222.2 $[M+Na]^+$ $C_{10}H_{17}O_3N^{23}Na$ (Expected: 222.1).



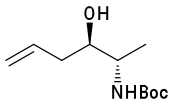
16 *tert*-Butyl ((2*S*,3*R*)-3-hydroxypent-4-en-2-yl)carbamate[4]

Procedure:[2] To a solution of 13 (500 mg, 2.5 mmol) in spectrophotometric grade ethanol (31 ml) was added lithium *tri-tert*-butoxy aluminium hydride (1.40 g, 5.5 mmol, 2.2 eq.) at –78 °C. After the reaction mixture was stirred at the same temperature for 2 hrs, 0.1N HCl (15 ml) was added followed by Celite and EtOAc (15 ml). The resulting slurry was filtered through Celite and the filtering bed was washed with EtOAc (15ml). The two phases were separated and the aqueous phase was re-extracted with EtOAc. The organic extracts were combined, washed with $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the product as a single diastereoisomer 16 as ascertained by crude ^{1}H NMR. Column chromatography on silica gel (from 25 % to 50 % ethyl acetate in pet. ether) gave 16 (450 mg, 89 %) as a white solid (R_f ≈ 0.3; EtOAc/pet. ether 25/75). ν_{max} (ATR) 3351 (br, s), 2986, 2937, 1679, 1529, 1280, 1161, 1021 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.85 (1H, ddd, *J* 17.2, 10.6, 5.5, 4-*H*), 5.33 (1H, dt, *J* 17.2, 1.5, 5-H_{trans}), 5.23 (1H, dd, *J* 10.6, 1.5, 5-H_{cis}), 4.65 (1H, m, N*H*), 4.22–4.16 (1H, m, 3-*H*), 3.84 (1H, m, 2-*H*), 1.44 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 6.9, 1-H_3); δ_C (176 MHz, $CDCl_3$) 156.5 NH*C*OO, 137.0 *C*4, 116.7 *C*5, 79.9 *C*(CH$_3$)$_3$, 75.9 *C*3, 50.9 *C*2, 28.5 C(*C*H$_3$)$_3$, 15.5 *C*1; *m/z* (ES$^+$) 224.3 [M+Na]$^+$ $C_{10}H_{19}O_3N^{23}Na$ (Expected: 224.1).



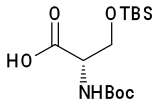
19 (*S*)-*tert*-Butyl (3-oxohex-5-en-2-yl)carbamate[5, 6]

Procedure:[6] A solution of *n*-BuLi (1.4 M solution in hexanes, 7.2 ml, 10 mmol, 1.0 eq.) was added dropwise at −10 °C to a solution of *N*-(*tert*-butoxycarbonyl)-L-alanine (1.89 g, 10 mmol) in anhydrous THF (100 ml). The resulting thick gelatinous suspension was stirred at −10 °C for 30 minutes, cooled to −78 °C and treated with a solution of allylmagnesium bromide (1.0 M solution in ether, 23.0 ml, 23 mmol, 2.3 eq.). The resultant light grey slurry was stirred for 1 hour at −78 °C, warmed to room temperature over 1 hour, stirred at this temperature for an additional 30 minutes and then poured into a mixture of sat. aq. NH_4Cl solution, ice and ether. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under *vacuo* to yield the desired product 19 as a crystalline white solid (2.03 g, 9.5 mmol, 95 %). The product was used without further purification (R_f ≈ 0.80; EtOAc/pet. ether 67/33). ν_{max} (ATR) 3386, 2975, 2932, 1726, 1694, 1642, 1516, 1166 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.91 (1 H, ddt, *J* 17.1, 10.2, 6.9, 5-*H*), 5.15 (3H, m, N*H* and 6-H_2), 4.41–4.31 (1H, m, 2-*H*), 3.34–3.21 (2H, m, 4-H_2), 1.43 (9H, s, C(CH_3)$_3$), 1.33 (3H, d, *J* 7.1, 1-H_3); δ_C (126 MHz, $CDCl_3$) 207.6 *C3*, 155.3 NH*C*OO, 129.9 *C5*, 119.5 *C4*, 79.9 *C*(CH$_3$)$_3$, 54.9 *C2*, 44.2 *C3*, 28.5 C(*C*H$_3$)$_3$, 17.8 *C1*; *m/z* (ES$^-$) 212.1 [M−H]$^-$; (ES$^+$) 236.2 [M+Na]$^+$; HRMS (ES$^+$) found [M+Na]$^+$ 236.1257, $C_{11}H_{19}O_3N^{23}Na$ requires M^+ 236.1257.



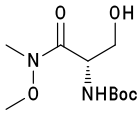
20 *tert*-Butyl (2*S*,3*R*)-3-hydroxyhex-5-en-2-ylcarbamate[6]

Procedure:[6] A solution of 19 (2 g, 9.4 mmol) in anhydrous methanol (60 ml) was treated with sodium borohydride (0.72 g, 18.5 mmol, 1.97 eq.) at −78 °C. The resulting mixture was stirred for 90 minutes, carefully quenched by addition of a sat. aq. NH_4Cl solution at −78 °C, warmed to room temperature, and diluted with 1M NaOH solution (24 ml) and ether (60 ml). The organic layer was separated and the aqueous layer was extracted with ether (2 × 20 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum to yield the crude product as a white solid consists of an 82:18 mixture of 20 and the other diastereoisomer respectively as determined by analysis of the ^1H NMR spectra of the crude product. The crude mixture was purified by chromatography (25 % ethyl acetate in pet. ether) to yield 0.73 g of a 70/30 mixture of the diastereoisomers followed by 1.14 g of pure 20 with total yield 92 % (1.87 g, 8.7 mmol) (R_f 20 ≈ 0.30, R_f 20' ≈ 0.32; EtOAc/pet. ether 25/75). 20; v_{max} (ATR) 3358 (br, s), 2979, 2940, 1681 (s), 1526 (s), 1174 (s), 1024 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.84 (1H, ddt, *J 7.3, 10.3, 14.4*, 5-*H*), 5.19–5.09 (2H, m, 6-*H*), 4.75 (1H, br s, N*H*), 3.71 (2H, m, 2-*H* and 3-*H*), 2.38–2.11 (3H, m, O*H*, 4-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.12 (3H, d, *J 6.8*, 1-H_3); δ_C (101 MHz, $CDCl_3$) 156.0 NH*C*OO, 134.8 C*5*, 118.2 C*6*, 79.6 *C*$(CH_3)_3$, 73.4 *C*3, 50.4 *C2*, 38.5 *C*4, 28.5 C(*C*H_3)$_3$, 14.7 *C*1; *m/z* (ES^+) 238.2 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 238.1414, $C_{11}H_{21}O_3N^{23}Na$ requires M^+ 238.1414.



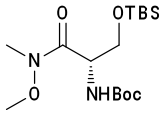
S02 (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)propanoic acid[2, 6]

Procedure:[6] To a solution of *N*-(*tert*-butoxycarbonyl)-L-serine (512 mg, 2.5 mmol) in anhydrous DMF (12 mL), imidazole (509 mg, 7.5 mmol, 3 eq.) was added and the reaction mixture was cooled to 0 °C followed by addition of TBSCl (490 mg, 3.25 mmol, 1.3 eq.). The resulting mixture was slowly warmed to room temperature and stirred overnight and followed by TLC. Upon total consumption of the starting material (monitored by TLC), the reaction mixture was poured into a mixture of ice cooled 1N HCl (5 mL) and ether (20 mL) to hydrolyze the silyl ester. The organic layer was separated and the aqueous layer was extracted with ether (2 × 20 ml). The combined organic extracts were washed with brine, dried over MgSO4, filtered and concentrated under vacuum to yield the desired product S02 as pale yellow sticky oil (630 mg, 1.97 mmol, 79 %). v_{max} (ATR) 3380-3492 (br), 3451 (s), 2952, 2569, 1724, 1691, 1505 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 8.49 (1H, br s, acidic O*H*), 5.35 (1H, br d, *J* 8.2, N*H*), 4.36 (1H, m, 2-*H*), 4.09 (1H, A of ABX syst., m, 3-H*H*), 3.83 (1H, B of ABX syst., dd, *J* 10.1, 3.5, 3-*H*H), 1.44 (9H, s, OC(CH_3)$_3$), 0.86 (9H, s, SiC(CH_3)$_3$), 0.03, 0.2 (2 × 3H, s, SiCH_3); δ_C (75 MHz, CDCl3) 175.3 *C*OOH, 155.5 NH*C*OO, 80.1 O*C*$(CH_3)_3$, 63.7 *C*3, 55.6 *C*2, 28.2 OC(*C*H_3)$_3$, 25.7 SiC(*C*H_3)$_3$, 18.4 Si*C*$(CH_3)_3$, -5.5 Si*C*H_3, -5.6 Si*C*H_3; *m/z* (ES^+) 320.3 $[M+H]^+$ $C_{14}H_{30}O_5N$ (Expected: 320.2).



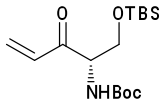
S03 (*S*)-*tert*-Butyl (3-hydroxy-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate[2, 7]

Procedure:[2] To a solution of *N*-(*tert*-butoxycarbonyl)-L-serine (3.075 g, 15 mmol) in anhydrous CH_2Cl_2 (100 ml) was added *N*-methylmorpholine (1.812 ml, 16.5 mmol, 1.1 eq.) and *N*,*O*-dimethylhydroxylamine hydrochloride (1.610 g, 16.5 mmol, 1.1 eq.) at –15 °C. To the reaction mixture was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.874 g, 16.5 mmol, 1.1 eq.) over 30 min at the same temperature. The reaction mixture was stirred for 4 hrs then poured into ice and 1N HCl. The resulting mixture was extracted with CH_2Cl_2. The organic layers were combined, and washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude Weinreb amide S03 (3.400 gm, 91%) as a colourless solid. The crude product was at high purity and was used directly without further purification. ν_{max} (ATR) 3329, 2935, 1715, 1665, 1498, 1173 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.57 (1H, br s, N*H*), 4.89–4.74 (1H, m, 2-*H*), 3.86–3.79 (2H, m, 3-H_2), 3.78 (3H, s, OCH_3), 3.24 (3H, s, NCH_3), 2.49 (1H, br s, O*H*), 1.45 (9H, s, C(CH_3)$_3$); δ_C (126 MHz, $CDCl_3$) 170.9 *C*1, 156.0 NH*C*OO, 80.3 *C*(CH$_3$)$_3$, 64.1 *C*3, 61.8 O*C*H$_3$, 52.5 *C*2, 31.1 N*C*H$_3$, 28.5 C(*C*H$_3$)$_3$; *m/z* (ES$^+$) 271.2 $[M+Na]^+$ $C_{10}H_{20}O_5N_2{}^{23}Na$ (Expected: 271.1).



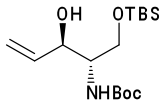
S04 (*S*)-*tert*-Butyl (3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxa-3-aza-8-siladecan-5-yl)carbamate[2]

Procedure:[7] To a solution of S03 (500 mg, 2.02 mmol) in anhydrous DMF (2.5 mL) were added imidazole (410 mg, 6.12 mmol, 3 eq.) and a catalytic amount of DMAP (25 mg, 0.2 mmol, 0.1 eq.) at 0 °C followed by addition of a solution of TBSCl (349 mg, 2.32 mmol, 1.15 eq.) in anhydrous DMF (2.5 ml). The resulting mixture was allowed to warm up to room temperature, stirred overnight, and poured into sat. aq. NH_4Cl solution (5 ml) and extracted with CH_2Cl_2 (3 × 20 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum to yield the crude product as yellowish viscous oil. Column chromatography on silica gel (from 25% to 50% EtOAc in pet. ether) gave S04 (650 mg, 1.80 mmol, 89%) as a sticky oil (R_f ≈ 0.25; EtOAc/pet. ether 25/75). ν_{max} (ATR) 2931, 2857, 2350, 1711, 1661, 1494, 1468, 1168, 1111, 837 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.35 (1H, br d, *J* 8.5, N*H*), 4.80–4.70 (1H, m, 5-*H*), 3.84 (1H, dd, *J* 9.4, 4.5, 6-*H*H), 3.78 (1H, dd, *J* 9.7, 4.5, 6-H*H*), 3.74 (3H, s, 1-H_3), 3.20 (3H, s, NCH_3), 1.43 (9H, s, C(CH_3)$_3$), 0.85 (9H, s, SiC(CH_3)$_3$), 0.02, 0.01 (2 × 3H, s, SiCH_3); δ_C (176 MHz, $CDCl_3$) 170.9, 155.5, 79.7, 63.7, 61.6, 52.6, 32.3, 28.5, 25.9, -5.4, -5.4; *m/z* (ES$^+$) 385.2 $[M+Na]^+$ $C_{16}H_{34}O_5N_2{}^{28}Si^{23}Na$ (Expected: 385.1).



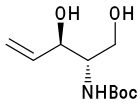
12 (*S*)-*tert*-Butyl (1-((*tert*-butyldimethylsilyl)oxy)-3-oxopent-4-en-2-yl)carbamate[2]

Procedure:[2] A (0.6 M) solution of vinyl magnesium bromide (*c.a.* 11 ml, 6.64 mmol, 4 eq.) in THF was added dropwise at 0 °C to a solution of S04 (600 mg, 1.66 mmol) in anhydrous THF (10 ml). The reaction mixture was allowed to warm up to room temperature. After the reaction mixture was stirred for 1 hour at the same temperature, the reaction mixture was poured into ice cooled 2N HCl. The resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 5% to 20% EtOAc in pet. ether) gave 12 (920 mg, 84%) as a viscous oil ($R_f \approx 0.78$; EtOAc/pet. ether 25/75). ν_{max} (ATR) 3460, 2865, 1695, 1489, 1359, 1173 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 6.55 (1H, dd, *J* 17.5, 10.7, 4-C*H*), 6.34 (1H, dd, *J* 17.5, 1.0, 5-CH_{trans}), 5.83 (1H, d, *J* 10.7, 1.0, 5-CH_{cis}), 5.52 (1H, br d, *J* 6.8, N*H*), 4.63–4.56 (1H, m, 2-C*H*), 4.00 (1H, dd, *J* 10.2, 3.1, 1-C*H*H), 3.85 (1H, dd, *J* 10.2, 4.4, 1-CH*H*), 2.04 (1H, s, O*H*), 1.44 (9H, s, C(CH_3)$_3$), 0.83 (9H, s, SiC(CH_3)$_3$), 0.02, 0.01 (2 × 3H, s, SiCH_3); δ_C (176 MHz, $CDCl_3$) 196.8, 155.3, 133.1, 129.3, 79.7, 63.4, 59.5, 28.3, 25.7, 18.2, -5.6; *m/z* (ES^+) 330.5 $[M+H]^+$ $C_{16}H_{32}O_4N_1{}^{28}Si$ (Expected: 330.2).



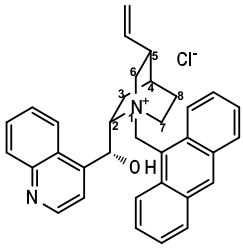
14 *tert*-Butyl ((2*S*,3*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypent-4-en-2-yl)carbamate[2]

Procedure:[2] To a solution of the enone 12 (910 mg, 2.77 mmol) in spectrophotometric grade ethanol (35 ml) was added lithium *tri-tert*-butoxy aluminium hydride (1.546 g, 6.1 mmol, 2.2 eq.) at –78 °C. After the reaction mixture was stirred at the same temperature for 2 hrs, 0.1N HCl (16.5 ml) was added followed by Celite and EtOAc (16.5 ml). The resulting slurry was filtered through Celite and the residue was washed with EtOAc (16.5ml). The two phases were separated and the aqueous phase re-extracted with EtOAc. The organic extracts were combined, washed with $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product as a single diastereomer 14 as indicated by the ^1H NMR analysis. Column chromatography on silica gel (from 20% to 50% ethyl acetate in pet. ether) gave 14 (870 mg, 95%) as a viscous oil ($R_f \approx 0.40$; EtOAc/pet. ether 25/75). ν_{max} (ATR) 3445, 2940, 1699, 1494, 1168, 840 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.91 (1H, ddd, *J* 17.1, 10.6, 4.9, 4-*H*), 5.37 (1H, dd, *J* 17.1, 1.6, 5-H_{trans}), 5.27 (1H, br d, *J* 7.9, N*H*), 5.23 (1H, dd, *J* 10.6, 1.6, 5-H_{cis}), 4.29–4.22 (1H, m, 3-*H*), 3.91 (1H, dd, *J* 10.4, 2.9, 1-*H*H), 3.74 (1H, dd, *J* 8.3, 2.9 1-H*H*), 3.65–3.58 (1H, br s, O*H*), 3.47 (1H, m, 2-*H*), 1.44 (9H, s, OC(CH_3)$_3$), 0.88 (9H, s, SiC(CH_3)$_3$), 0.05, 0.04 (2 × 3H, s, SiCH_3); δ_C (126 MHz, $CDCl_3$) 155.9, 138.0, 115.9, 79.7, 74.9, 63.6, 54.2, 28.5, 28.5, 25.9, 25.9, 18.2, -5.52, -5.54; *m/z* (ES^+) 332.4 $[M+H]^+$ $C_{16}H_{33}O_4N_1Si_1$ (Expected: 332.2), 354.4 $[M+Na]^+$ $C_{16}H_{33}O_4N_1{}^{28}Si^{23}Na$ (Expected: 354.2).



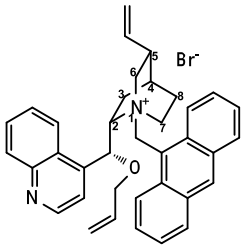
15 *tert*-Butyl ((2*S*,3*R*)-1,3-dihydroxypent-4-en-2-yl)carbamate[2]

Procedure: A solution of 14 (100 mg, 0.3 mmol) in methanol (1.5 ml) was treated with 2N HCl (150 µl) dropwise at −0 °C. The resulting mixture was stirred for 15 minutes at the same temperature and monitored by TLC. Upon complete consumption of the starting material, the reaction was quenched by addition of a brine solution (5 ml) and extracted with EtOAc (2 × 10 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum to yield the crude product. Column chromatography on silica gel (from 25% to 50% ethyl acetate in pet. ether and elution with MeOH) gave 15 (60 mg, 2.76 mmol, 92 %) as viscous oil ($R_f \approx 0.13$; EtOAc/pet. ether 50/50). v_{max} (ATR) 3456, 2931, 1705, 1510, 1177, 842 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.94 (1H, ddd, *J* 16.9, 10.6, 5.3, 4-*H*), 5.40 (1H, d, *J* 16.9, 5-H_{trans}), 5.35 (1H, br s, N*H*), 5.27 (1H, d, *J* 10.6, 5-H_{cis}), 4.39 (1H, br s, 3-*H*), 3.93 (1H, dd, *J* 11.2, 3.5, 1-H*H*), 3.74–3.68 (1H, m, 1-*H*H), 3.68–3.61 (1H, m, 2-*H*), 2.85 (1H, app d, *J* 4.5, 3-O*H*), 2.57 (1 H, br s, 1-O*H*), 1.45 (9H, s, C(CH_3)$_3$); δ_C (176 MHz, $CDCl_3$) 156.3, 137.6, 116.7, 80.1, 75.1, 62.7, 55.1, 28.5; *m/z* (ES^+) 240.4 $[M+Na]^+$ $C_{10}H_{19}O_4N^{23}Na$ (Expected: 240.1).



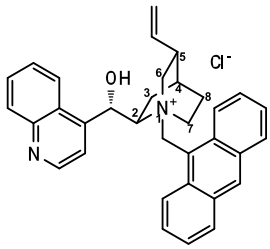
S05 (1*S*,2*S*,4*S*,5*R*)-1-(Anthracen-9-ylmethyl)-2-((*R*)-hydroxy(quinolin-4-yl)methyl)-5-vinyl-1-azoniabicyclo [2.2.2]octane chloride[8]

Procedure:[8] To a suspension of cinchonidine (294 mg, 1.0 mmol) in toluene (4 ml) was added 9-(chloromethyl) anthracene (237 mg, 1.05 mmol, 1.05 eq.), and the mixture was stirred at reflux 110 °C for 3 hrs. The mixture was cooled to room temperature, poured into 20 ml of ether. The solid residue was collected by centrifugation and decantation of the liquid phase. The residue was washed by ether (2 × 20 ml) and collected by centrifugation. The mother liquor was concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2/ether and kept at −20 °C to precipitate another fraction of the product. The combined solid residue was recrystallized from CH_2Cl_2/ether and kept at −20 °C to afford S05 (460 mg, 0.88 mmol, 88%) as light yellow solid ($R_f \approx 0.30$; CH_2Cl_2/MeOH 93/7). δ_H (400 MHz, $CDCl_3$, 20 mg/ml)[9] 8.97 (1H, d, *J* 8.4), 8.86 (1H, d, *J* 4.3), 8.79 (2H, d, *J* 8.9), 8.17 (1H, d, *J* 4.9), 8.06–8.01 (2H, m, *J* 4.7), 7.71 (1H, d, *J* 8.2), 7.68–7.64 (1H, m), 7.61 (1H, d, *J* 7.8), 7.47–7.40 (1H, m), 7.29–7.21 (4H, m), 7.19–7.09 (1H, m), 7.09–7.04 (1H, m), 6.74 (2H, s), 5.42 (1H, ddd, *J* 16.8, 10.5, 6.1), 5.21 (1H, d, *J* 16.8), 4.90 (1H, dd, *J* 10.5, 1.4), 4.85–4.73 (1H, m), 4.72–4.58 (1H, m), 3.97 (1H, d, *J* 13.0), 2.58 (1H, dd, *J* 12.9, 10.6), 2.47 (1H, app. t, *J* 12.6), 2.18–2.06 (1H, m), 1.95–1.77 (2H, m), 1.77–1.56 (1H, m), 1.22–1.11 (1 H, m), 1.11–0.95 (1H, m); δ_C (176 MHz, $CDCl_3$) 149.01, 136.52, 133.38, 132.76, 131.21, 130.45, 130.39, 128.98, 128.86, 128.38, 127.76, 127.46, 127.23, 125.98, 125.73, 124.93, 124.91, 124.38, 124.06, 120.26, 118.26, 117.86, 67.22, 67.18, 61.58, 54.98, 50.66, 38.64, 25.99, 25.81, 23.50; *m/z* (ES^+) 485.5 $[M]^+$ $C_{34}H_{33}ON_2$ (Expected: 485.3).



29 (1*S*,2*S*,4*S*,5*R*)-2-((*R*)-Allyloxy(quinolin-4-yl)methyl)-1-(anthracen-9-ylmethyl)-5-vinyl-1-azoniabicyclo [2.2.2]octane bromide[8]

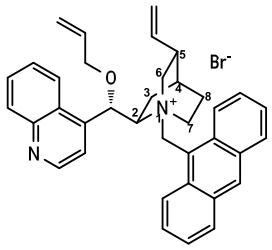
Procedure:[8] To a suspension of S05 (200 mg, 0.39 mmol) in CH_2Cl_2 (5 ml) was added allyl bromide (100 µl, 1.17 mmol, 3.05 eq.) and 50% aq. KOH solution (200 µl, 1.9 mmol, 5 eq.). The resulting mixture was stirred vigorously at room temperature for 4 hrs. The mixture was diluted with water (6.25 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic extracts were dried over MgSO4, filtered and concentrated *in vacuo*. Recrystallization of the residue from MeOH/ether at −20 °C afforded the desired product 29 (190 mg, 0.32 mmol, 84%) as an orange solid. δ_H (700 MHz, CD_3OD, 25 mg/ml)[9] 9.07 (1H, d, *J* 4.4), 8.93 (1H, s), 8.77 (1H, d, *J* 9.0), 8.63–8.56 (1H, m), 8.48 (1H, d, *J* 9.1), 8.31–8.26 (2H, m), 8.25 (1H, d, *J* 8.3), 8.01–7.93 (3H, m, *J* 15.0, 7.2), 7.83 (1H, dd), 7.79 (1H, dd, *J* 8.5, 7.2), 7.68 (2H, dd, *J* 14.4, 6.3), 6.98 (1H, s), 6.44 (2H, ddd, *J* 15.9, 11.0, 5.6), 5.92 (1H, d, *J* 13.9), 5.76–5.67 (2H, m), 5.58 (1H, d, *J* 10.4), 5.06–4.98 (2H, m, *J* 13.2), 4.59–4.52 (2H, m, *J* 13.0, 6.0), 4.51–4.42 (2H, m), 3.80 (1H, d, *J* 13.0), 3.30–3.24 (1H, m), 2.94 (1H, td, *J* 11.6, 5.1), 2.54–2.42 (2H, m, *J* 23.5, 13.6), 2.21 (1H, s), 2.00 (1H, s), 1.70–1.58 (2H, m); δ_C (176 MHz, CD_3OD) 151.05, 149.38, 142.94, 138.50, 134.80, 134.67, 134.61, 133.94, 133.10, 133.04, 131.52, 131.31, 131.18, 130.66, 129.41, 129.26, 127.08, 126.66, 126.57, 125.28, 124.88, 121.65, 119.12, 117.80, 71.44, 70.12, 63.55, 57.42, 53.69, 49.53, 39.48, 27.30, 26.26, 23.31; *m/z* (ES^+) 525.5 $[M]^+$ $C_{37}H_{37}ON_2$ (Expected: 525.3).



S06 (1*S*,2*R*,4*S*,5*R*)-1-(Anthracen-9-ylmethyl)-2-((*S*)-hydroxy(quinolin-4-yl)methyl)-5-vinyl-1-azoniabicyclo [2.2.2]octane chloride[9]

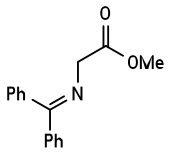
Procedure:[9] To a suspension of cinchonine (294 mg, 1.0 mmol) in toluene (4 ml) was added 9-(chloromethyl)anthracene (237 mg, 1.05 mmol, 1.05 eq.), and the mixture was stirred at reflux 110 °C for 4 days. The mixture was cooled to room temperature, poured into 20 ml of ether. The solid residue was collected by centrifugation and decantation of the liquid phase. The residue was washed by ether (2 × 20 ml) and collected by centrifugation. The mother liquor was concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2/ether and kept at −20 °C to precipitate another fraction of the product. The combined solid residue was recrystallized from CH_2Cl_2/ether and kept at −20 °C to afford S06 (250 mg, 0.48 mmol, 48%) as light yellow solid (R_f ≈ 0.30;

CH_2Cl_2/MeOH 93/7). δ_H (700 MHz, $CDCl_3$, 25 mg/ml)[9] 9.24 (1H, d, *J* 8.6), 8.92 (1H, d, *J* 6.7), 8.81 (1H, s), 8.41 (1H, d, *J* 8.7), 8.20 (1H, s), 8.02 (1H, d, *J* 3.7), 7.85 (1H, s), 7.55 (1H, d, *J* 8.1), 7.53 (1H, d, *J* 8.3), 7.45 (1H, d, *J* 8.2), 7.27–7.23 (1H, m), 7.21–7.16 (2H, m), 7.11–7.06 (1H, m, *J* 6.7), 7.06–7.02 (1H, m, *J* 7.2), 6.98–6.89 (3H, m), 6.46 (1H, d, *J* 13.7), 5.56 (1H, ddd, *J* 17.2, 10.5, 6.6), 5.00 (1H, d, *J* 10.5), 4.84 (1H, d, *J* 17.3), 4.75–4.68 (1H, m), 4.44–4.36 (1H, m), 4.24 (1H, t, *J* 11.5), 2.45 (2H, t, *J* 12.0), 2.30 (1H, dd, *J* 20.5, 10.7), 1.92 (1H, t, *J* 12.6), 1.73–1.64 (2H, m, *J* 23.5, 10.1), 1.49 (1H, s), 1.34 (1H, t, *J* 11.1), 0.65 – 0.57 (1H, m); δ_H (176 MHz, $CDCl_3$) 149.4, 147.0, 145.6, 135.6, 133.1, 132.8, 130.9, 130.4, 130.1, 129.0, 128.5, 128.2, 127.6, 127.3, 126.9, 124.9,124.9, 124.61, 120.1, 118.1, 117.5, 67.8, 66.8, 57.6, , 54.3, 54.0, 38.1, 26.4, 24.1, 22.7; *m/z* (ES^+) 485.8 $[M]^+$ $C_{34}H_{33}ON_2$ (Expected: 485.3).



28 (1*S*,2*R*,4*S*,5*R*)-2-((*S*)-Allyloxy(quinolin-4-yl)methyl)-1-(anthracen-9-ylmethyl)-5-vinyl-1-azoniabicyclo [2.2.2]octane bromide[10]

Procedure:[8] To a suspension of S06 (272 mg, 0.52 mmol) in CH_2Cl_2 (7 ml) was added allyl bromide (141 µl, 1.63 mmol, 3.05 eq.) and 50% aq. KOH solution (286 µl, 2.6 mmol, 5 eq.). The resulting mixture was stirred vigorously at room temperature for 4 hrs. The mixture was diluted with water (9 ml) and extracted with CH_2Cl_2 (3 × 7 ml). The combined organic extracts were dried over MgSO4, filtered and concentrated *in vacuo*. Recrystallization of the residue from MeOH/ether at −20 °C afforded the desired product 28 (275 mg, 0.45 mmol, 87%) as orange solid. δ_H (700 MHz, CD_3OD, 18 mg/ml)[9] 9.04 (1H, d, *J* 4.0), 9.01 (1H, d, *J* 9.0), 8.77 (2H, m), 8.27 (1H, d, *J* 8.7), 8.20–8.14 (3H, m), 8.00 (1H, s), 7.94–7.90 (2H, m), 7.84–7.79 (1H, m), 7.70–7.66 (1H, m), 7.62 – 7.57 (2H, m, *J* 10.4, 5.4), 6.90 (1H, s), 6.46–6.38 (1H, m), 6.13–5.99 (2H, m), 5.93 (1H, ddd, *J* 17.2, 10.6, 6.9), 5.72 (1H, d, *J* 17.2), 5.63 (1H, d, *J* 10.6), 5.19 (1H, d, *J* 10.5), 5.04 (1H, d, *J* 17.3), 4.58 (1H, m), 4.55 (1H, dd, *J* 12.8, 6.0), 4.41–4.34 (3H, m), 3.11 (1H, app. t, *J* 11.5), 2.77 (1H, m), 2.55–2.50 (1H, m), 2.25–2.14 (1H, m), 1.85–1.78 (2H, m), 1.64–1.55 (1H, m), 1.20–1.12 (1H, m), 0.70 (0.45H, t, *J* 7.4); *m/z* (ES^+) 525.7 $[M]^+$ $C_{37}H_{37}ON_2$ (Expected: 525.3).

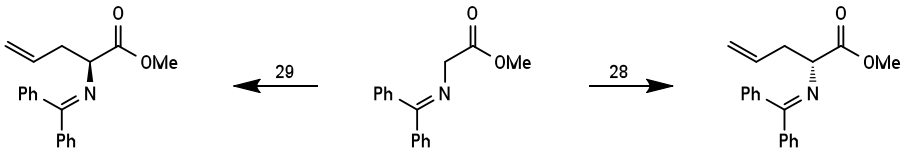


22 Methyl 2-((diphenylmethylene)amino)acetate[11]

Procedure:[11] To a suspension of methyl glycine hydrochloride (753 mg, 6 mmol) in anhydrous CH_2Cl_2 (20 ml), an equimolar amount of benzophenone imine (1 ml, 6 mmol) was added and the reaction mixture was stirred at room temperature for 24 hr. The reaction mixture was filtered to remove NH_4Cl and evaporated to dryness on a rotary evaporator. The residue was dissolved in 20 mL of ether, filtered, washed with 20 mL of water, and dried over MgSO4 to yield the crude product. Column chromatography on silica gel (from 10% to 50% ethyl acetate in pet. ether) gave 22 (1.221 g, 4.8 mmol, 80 %) as white waxy solid ($R_f \approx 0.33$; EtOAc/pet. ether 33/67). ν_{max} (ATR) 2948, 2890, 2361, 1970, 1752, 1622, 1573, 1384, 1195, 682 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.66 (2H, m, Ar*H*), 7.49–7.42 (3H, m, Ar*H*), 7.40 (1H, m, Ar'*H*), 7.33 (2H, m, Ar'*H*), 7.18 (2H, m, Ar'*H*), 4.22 (2H, s 2-H_2), 3.74 (3H, s, OCH_3); δ_C (176 MHz, $CDCl_3$) 172.0, 171.2, 139.3, 136.0, 130.6, 128.9, 128.9, 128.8, 128.2, 127.8, 55.7, 52.1; m/z (ES^+) 254.4 $[M+H]^+$ $C_{16}H_{16}O_2N$ (Expected: 254.1).

General Procedure for Phase Transfer Catalysis[8, 12]

To an ice cooled solution of the Schiff base 22 in Toluene/CH_2Cl_2 (7:3 v/v), the cinchona catalyst 29 or 28 was added (5 mol%) followed by dropwise addition of allyl bromide (5.0 eq.) at 0 °C. The reaction mixture was stirred for 5 min before the dropwise addition of 50% aq. KOH (5 eq.). The reaction was stirred vigorously overnight from 0°C to room temperature. The reaction was monitored by TLC and upon completion; the reaction mixture was diluted with water (10 ml) and extracted with EtOAc (3 × 5 ml). The organics were dried over $MgSO_4$, filtered, and evaporated in vacuo, affording the crude products.

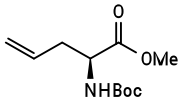


S07 22 S08

S07 (*S*)-Methyl 2-((diphenylmethylene)amino)pent-4-enoate[8]

S08 (*R*)-Methyl 2-((diphenylmethylene)amino)pent-4-enoate

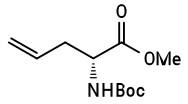
ν_{max} (ATR) 2954, 2385, 1704, 1491, 1168, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.65–7.61 (2H, m, Ar*H*), 7.47–7.42 (3H, m, Ar*H*), 7.40–7.36 (1H, m, Ar*H*), 7.35–7.30 (2H, m, Ar*H*), 7.19–7.14 (2H, m, Ar*H*), 5.67 (1H, ddt, *J* 17.1, 10.1, 7.2, 4-*H*), 5.07 (1H, dd, *J* 17.1, 2.0, 5-H*H*), 5.02 (1H, dd, *J* 10.1, 2.0, 5-*H*H), 4.16 (1H, dd, *J* 7.8, 5.3, 2-*H*), 3.72 (3H, s, OCH_3), 2.75–2.56 (2H, m, 3-H_2); δ_C (101 MHz, $CDCl_3$) 172.4 (*C*$OOCH_3$), 170.8 (Ph_2CN), 139.6 (Ar), 136.4 (Ar), 134.4 (Ar), 130.5 (*C*4), 128.94 (Ar), 128.78 (Ar), 128.61 (Ar), 128.14 (Ar), 128.03 (Ar), 117.8 (*C*5), 65.4 (*C*2), 52.1 (O*C*H_3), 38.3 (*C*3).



S09 (*S*)-Methyl 2-((*tert*-butoxycarbonyl)amino)pent-4-enoate[13, 14]

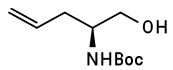
Procedure:[15] A mixture of the starting material S07 (735 mg, 2.51 mmol) and 4N HCl (3.1 ml, excess) in MeOH (10 ml) was refluxed for 1 hr. The reaction mixture was allowed to cool down to room temperature. The reaction mixture was extracted by ether (2 × 5 ml). The ether layer was discarded and the aqueous layer was basified by $NaHCO_3$ to pH 8 followed by addition of Boc2O (Boc anhydride) (824 mg, 3.77 mmol, 1.5 eq.) and the reaction was stirred for 2 hrs at room temperature and monitored by TLC. The reaction volume was reduced under vacuum and extracted with EtOAc (3 × 10 ml). The combined organic extracts were washed with brine, dried over MgSO4, filtered and evaporated in vacuo to afford the crude product. Column chromatography on silica gel (from 20% to

50% ethyl acetate in pet. ether) gave S09 (442 g, 1.9 mmol, 76%) as viscous liquid (Rf ≈ 0.42; EtOAc/pet. ether 33/67). v_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.68 (1H, ddt, *J* 17.0, 9.7, 7.2, 4-*H*), 5.17–5.08 (2H, m, 5-H_2), 5.03 (1H, br d, *J* 6.2, N*H*), 4.37 (1H, dd, *J* 13.3, 6.2, 2-*H*), 3.72 (3H, s, OCH_3), 2.61 – 2.39 (2H, m, 3-H_2), 1.42 (9H, s, C(CH_3)$_3$); δ_C (126 MHz, $CDCl_3$) 171.2, 155.3, 132.4, 119.1, 79.9, 52.3, 36.9, 28.4; *m/*z (ES^+) 253.0 $[M+Na]^+$ $C_{11}H_{19}O_2N_1{}^{23}Na_1$ (Expected: 253.1).



S10 (*R*)-Methyl 2-((*tert*-butoxycarbonyl)amino)pent-4-enoate[13, 14]

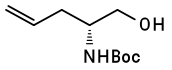
Procedure:[15] A mixture of the starting material S08 (690 mg, 2.35 mmol) and 4N HCl (3.0 ml, excess) in MeOH (10 ml) was refluxed for 1 hr. The reaction mixture was allowed to cool down to room temperature. The reaction mixture was extracted by ether (2 × 5 ml). The ether layer was discarded and the aqueous layer was basified by $NaHCO_3$ to pH 8 followed by addition of Boc_2O (Boc anhydride) (775 mg, 3.53 mmol, 1.5 eq.) and the reaction was stirred for 2 hrs at room temperature and monitored by TLC. The reaction volume was reduced under vacuum and extracted with EtOAc (3 × 10 ml). The combined organic extracts were washed with brine, dried over MgSO4, filtered and evaporated in vacuo to afford the crude product. Column chromatography on silica gel (from 20% to 50% ethyl acetate in pet. ether) gave S10 (408 g, 1.78 mmol, 75%) as viscous liquid (Rf ≈ 0.42; EtOAc/pet. ether 33/67). v_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.68 (1H, ddt, *J* 17.0, 9.7, 7.2, 4-*H*), 5.17–5.08 (2H, m, 5-H_2), 5.03 (1H, br d, *J* 6.2, N*H*), 4.37 (1H, dd, *J* 13.3, 6.2, 2-*H*), 3.72 (3H, s, OCH_3), 2.61–2.39 (2H, m, 3-H_2), 1.42 (9H, s, C(CH_3)$_3$); δ_C (126 MHz, $CDCl_3$) 171.2, 155.3, 132.4, 119.1, 79.9, 52.3, 36.9, 28.4; *m/*z (ES^+) 253.1 $[M+Na]^+$ $C_{11}H_{19}O_2N_1{}^{23}Na_1$ (Expected: 253.1).



25 (*S*)-*tert*-Butyl (1-hydroxypent-4-en-2-yl)carbamate[16]

Procedure: A solution of S09 (332 mg, 1.45 mmol) in anhydrous ether (5 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (100 mg, 3.19 mmol, 2.2 eq.) in anhydrous ether (5 ml) at −0 °C. The reaction mixture was allowed to warm up to room temperature, was stirred for additional 1 hr and followed by TLC. Upon completion, the reaction was carefully quenched with dropwise addition of water (1 ml) followed by addition of 15% aq. NaOH (1 ml), water (3 ml), Celite and EtOAc (10 ml). The resulting slurry was filtered through Celite and the filtering bed was washed with EtOAc (2 × 5 ml). The two phases were separated and the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo* to afford the crude product 25 (265 mg, 1.32 mmol, 91%) as a pale yellow oil (R_f ≈ 0.25; EtOAc/pet. ether 33/67). The product was pure enough and was used without further purification. v_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.78 (1H, ddt, *J* 17.2, 10.2, 7.1, 4-*H*), 5.11 (2H, m, 5-H_2), 4.72 (1H, br s, N*H*), 3.74–3.58 (3H, m, 1-CH_2 and 2-*H*), 2.67 (1H, br s, O*H*),

2.31 (1H, m, 3-*H*H), 2.23 (1H, m, 3-H*H*), 1.43 (9H, s, C(C*H*$_3$)$_3$); δ_C (101 MHz, $CDCl_3$) 156.56, 134.28, 118.23, 79.89, 65.54, 52.30, 36.10, 28.49; *m/z* (ES$^+$) 224.0 $[M+Na]^+$ $C_{10}H_{19}O_3N_1{}^{23}Na_1$ (Expected: 224.1).

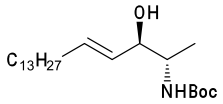


24 (*R*)-*tert*-Butyl (1-hydroxypent-4-en-2-yl)carbamate[16]

Procedure: A solution of S10 (388 mg, 1.69 mmol) in anhydrous ether (6 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (102 mg, 3.72 mmol, 2.2 eq.) in anhydrous ether (6 ml) at −0 °C. The reaction mixture was allowed to warm up to room temperature, was stirred for additional 1 hr and followed by TLC. Upon completion, the reaction was carefully quenched with dropwise addition of water (1 ml) followed by addition of 15% aq. NaOH (1 ml), water (3 ml), Celite and EtOAc (12 ml). The resulting slurry was filtered through Celite and the filtering bed was washed with EtOAc (2 × 5 ml). The two phases were separated and the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo* to afford the crude product 24 (270 mg, 1.34 mmol, 79%) as a pale yellow oil (R_f ≈ 0.25; EtOAc/pet. ether 33/67). The product was pure enough and was used without further purification. ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.78 (1H, ddt, *J* 17.2, 10.2, 7.1, 4-*H*), 5.11 (2H, m, 5-H_2), 4.72 (1H, br s, N*H*), 3.74–3.58 (3H, m, 1-H_2 and 2-*H*), 2.67 (1H, br s, O*H*), 2.31 (1H, m, 3-*H*H), 2.23 (1H, m, 3-H*H*), 1.43 (9H, s, C(C*H*$_3$)$_3$ δ_C (101 MHz, $CDCl_3$) 156.56 (NH*C*OO), 134.28, 118.23, 79.89, 65.54, 52.30, 36.10, 28.49; *m/z* (ES$^+$) 224.0 $[M+Na]^+$ $C_{10}H_{19}O_3N_1{}^{23}Na_1$ (Expected: 224.1).

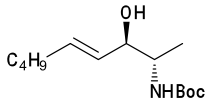
Olefin Cross Metathesis

To a solution of Grubbs II (0.3% mol) in CH_2Cl_2, the starting material was added as a solution in CH_2Cl_2 followed by the addition of the coupling olefin in excess (5 eq.). The reaction mixture was refluxed, typically for 4~8 hrs. The reaction was followed by TLC and quenched by addition of potassium 2-isocyanoacetate (1.32% mol, 4.4 eq. to Grubbs catalyst).[17] The reaction was stirred for 15 min at room temperature then evaporated *in vacuo* to yield the crude mixture which was purified by column chromatography on silica.[2]



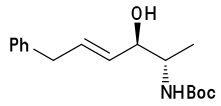
18a ***tert*-Butyl ((2*S*,3*R*,*E*)-3-hydroxyoctadec-4-en-2-yl)carbamate**

Yield (52%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.75–5.66 (1H, m, 5-*H*), 5.43 (1H, dd, *J* 15.5, 6.6, 4-*H*), 4.68–4.60 (1H, m, N*H*), 4.14–4.06 (1H, m, 3-*H*), 3.83–3.75 (1H, m, 2-*H*), 2.07–2.01 (2H, m, 6-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.39–1.22 (22H, m, 7-H_2 to 17-H_2), 1.07 (3H, d, *J* 6.4, 1-H_3), 0.88 (3H, t, *J* 6.9, 18-H_3); δ_C (101 MHz, $CDCl_3$) 156.3 (NH*C*OO), 134.2, 128.5, 79.8, 75.9, 32.5, 32.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 28.5, 22.8, 15.6, 14.3; *m/z* (ES^+) 406.5 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 384.3474, $C_{23}H_{46}O_3N_1$ requires M^+ 384.3472.



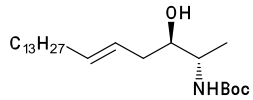
18b ***tert*-Butyl ((2*S*,3*R*,*E*)-3-hydroxynon-4-en-2-yl)carbamate**

Yield (67%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.76–5.65 (1H, m, 5-*H*), 5.42 (1H, ddt, *J* 15.4, 6.6, 1.4, 4-*H*), 4.74–4.61 (1H, m, N*H*), 4.13–4.05 (1H, m, 3-*H*), 3.84–3.72 (1H, m, 2-*H*), 2.01-2.07 (2H, m, 6-H_2), 1.43 (9H, s, C(CH_3)$_3$), 1.40–1.25 (4H, m, 7-H_2 and 8-H_2), 1.07 (3H, d, *J* 6.9, 1-H_3), 0.88 (3H, t, *J* 7.1, 9-H_3); δ_C (101 MHz, $CDCl_3$) 156.4, 134.1, 128.6, 79.7, 75.9, 51.2, 32.1, 31.5, 28.5, 22.3, 15.7, 14.0; *m/z* (ES^+) 280.0 $[M+Na]^+$; $C_{14}H_{27}O_3N_1Na_1$; HRMS (ES^+) found $[M+H]^+$ 258.2064, $C_{14}H_{28}O_3N_1$ requires M^+ 258.2064, $[M+Na]^+$ 280.1884, $C_{14}H_{27}O_3N_1{}^{23}Na_1$ requires M^+ 280.1883.



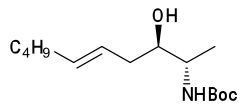
18c *tert*-Butyl ((2*S*,3*R*,*E*)-3-hydroxy-6-phenylhex-4-en-2-yl)carbamate

Yield (64%) as viscous oil; ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.32–7.24 (2H, m, Ar*H*), 7.23–7.14 (3H, m, Ar*H*), 5.93–5.83 (1H, m, 5-*H*), 5.53 (1H, ddt, *J* 15.3, 6.3, 1.4, 4-*H*), 4.74–4.66 (1H, m, N*H*), 4.19–4.13 (1H, m, 3-*H*), 3.86–3.73 (1H, m, 2-*H*), 3.40 (2H, d, *J* 6.8, 6-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.08 (3H, d, *J* 6.9, 1-H_3); δ_C (101 MHz, $CDCl_3$) 156.4, 140.1, 132.2, 130.2, 128.6, 128.9, 126.3, 79.8, 75.6, 51.2, 38.9, 28.5, 15.6; *m/z* (ES^+) 314.3 $[M+Na]^+$; $C_{17}H_{25}O_3N_1Na_1$; HRMS (ES^+) found $[M+H]^+$ 292.1908, $C_{17}H_{26}O_3N_1$ requires M^+ 292.1907.



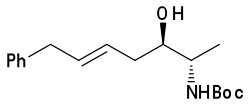
21a *tert*-Butyl ((2*S*,3*R*,*E*)-3-hydroxynonadec-5-en-2-yl)carbamate

Yield (53%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.55 (1H, dt, *J* 12.8, 6.7, 6-*H*), 5.43–5.36 (1H, m, 5-*H*), 4.83–4.68 (1H, m, N*H*), 3.73–3.58 (2H, m, 3-*H* and 2-*H*), 3.48 (1H, d, *J* 7.8, O*H*), 2.27–2.15 (4H, m, 4-H_2 and 7-H_2), 2.14–1.98 (2H, m, 7-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.38–1.22 (94 H, m, 8-H_2 to 18-H_2 and 1-H_3), 0.88 (3H, t, *J* 7.1, 19-H_3); δ_C (126 MHz, $CDCl_3$) 156.24, 135.03, 125.50, 79.56, 73.69, 50.35, 37.46, 32.88, 32.15, 29.90, 29.88, 29.85, 29.73, 29.66, 29.58, 29.45, 29.44, 28.63, 22.92, 14.35; *m/z* (ES^+) 398.5 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 398.3627, $C_{24}H_{48}O_3N_1$ requires M^+ 398.3629; $[M+Na]^+$ 420.3446, $C_{24}H_{47}O_3N_1{}^{23}Na_1$ requires M^+ 420.3448.



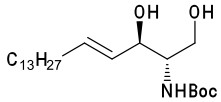
21b *tert*-Butyl ((2*S*,3*R*,*E*)-3-hydroxydec-5-en-2-yl)carbamate

Yield (56%);waxy solid; ν_{max} (ATR) 3449, 2928, 1715, 1497, 1173, 839 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.55 (1H, dt, *J* 13.2, 6.6, 6-C*H*), 5.43–5.34 (1H, m, 5-*H*), 4.83–4.70 (1H, m, N*H*), 3.74–3.58 (2H, m, 2-*H* and 3-*H*), 2.23–1.96 (4H, m, 4-H_2 and 7-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.39–1.23 (4H, m, 8-H_2 and 9-H_2), 1.15–1.02 (3H, d, *J* 6.8, 1-H_3), 0.89 (3H, t, *J* 7.1, 10-H_3); δ_C (101 MHz, $CDCl_3$) 156.0, 134.8, 125.6, 79.6, 73.5, 50.4, 37.2, 32.3, 31.6, 28.4, 22.2, 13.9; *m/z* (ES^+) 294.3 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 272.2220, $C_{15}H_{30}O_3N_1$ requires M^+ 272.2220; $[M+Na]^+$ 294.2040, $C_{15}H_{29}O_3N_1{}^{23}Na_1$ requires M^+ 294.2040.



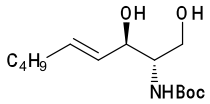
21c ***tert*-Butyl ((2*S*,3*R*,*E*)-3-hydroxy-7-phenylhept-5-en-2-yl)carbamate**

Yield (64%) as viscous oil; ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.30–7.27 (2H, m, Ar*H*), 7.21–7.15 (3H, m, Ar*H*), 5.71 (1H, dtd, *J* 15.2, 11.8, 6.6, 6-*H*), 5.53 (1H, dd, *J* 15.2, 6.3, 5-*H*), 4.80 (1H, br s, N*H*), 3.75–3.62 (2H, m, 3-*H* and 2-*H*), 3.36 (2H, d, *J* 6.6, 7-H_2), 2.24–2.05 (2H, m, 4-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 6.8, 1-H_3); δ_C (176 MHz, $CDCl_3$) 155.88, 140.56, 135.89, 133.24, 128.57, 127.42, 126.16, 79.58, 73.75, 50.37, 39.23, 39.22, 37.17, 28.55, 14.72; *m/z* (ES^+) 328.3 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 306.2063, $C_{18}H_{28}O_3N_1$ requires M^+ 306.2064; $[M+Na]^+$ 328.1883, $C_{18}H_{27}O_3N_1{}^{23}Na_1$ requires M^+ 328.1883.



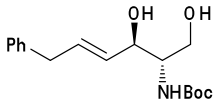
17a ***tert*-Butyl ((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-2-yl)carbamate[2]**

Yield (44%, 50%); ν_{max} (ATR) 3410, 2886, 2874, 1687, 1512, 1173 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.83–5.73 (1H, m, 5-*H*), 5.53 (1H, ddt, *J* 15.6, 6.5, 1.2, 4-H), 5.30 (1H, br d, *J* 6.4, N*H*), 4.38-4.28 (1H, m, 3-*H*), 3.94 (1H, app. d, *J* 10.0, 1-H*H*), 3.76–3.67 (1H, m, 1-*H*H), 3.63–3.54 (1H, m, 2-*H*), 2.50 (2H, br s, 2 × O*H*), 2.05(2H, q, *J* 7.3, 6-*H*), 1.45 (9H, s, C(CH_3)$_3$), 1.42–1.18 (22H, m, 7-H_2 to 17-H_2), 0.88 (3H, t, *J* 6.9, 18-H_3); δ_C (101 MHz, $CDCl_3$) 158.1, 134.2, 128.9, 79.7, 74.9, 62.9, 56.0, 32.3, 31.9, 29.8, 29.6, 29.5, 29.3, 29.1, 29.1, 28.4, 22.6, 14.1; *m/z* (ES^+) 400.9 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 400.3419, $C_{23}H_{46}O_4N_1$ requires M^+ 400.3421, $[M+Na]^+$ 422.3238, $C_{23}H_{45}O_4N_1{}^{23}Na_1$ requires M^+ 422.3241.



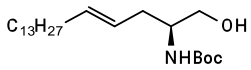
17b ***tert*-Butyl ((2*S*,3*R*,*E*)-1,3-dihydroxynon-4-en-2-yl)carbamate**

Yield (59%, 57%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.76 (1H, dtd, *J* 15.4, 6.7, 1.1, 5-*H*), 5.51 (1H, ddt, *J* 15.4, 6.5, 1.3, 4-*H*), 5.32 (1H, br d, N*H*), 4.34–4.21 (1H, m, 3-*H*), 3.90 (1H, d, *J* 11.4, 1-*H*H), 3.68 (1H, d, *J* 11.0, 1-H*H*), 3.63–3.51 (1H, m, 2-*H*), 2.97 (2H, br s, 2 × O*H*), 2.05 (2H, dd, *J* 13.8, 6.7, 6-H_2), 1.44 (9H, s, C(CH_3)$_3$), 1.39–1.23 (4H, m, 7-H_2 and 8-H_2), 0.88 (3H, t, *J* 7.1, 9-H_3); δ_C (101 MHz, $CDCl_3$) 156.4, 134.2, 129.1, 79.9, 74.8, 62.8, 55.6, 32.1, 31.4, 28.5, 22.3, 14.0; *m/z* (ES^+) 274.6 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 274.2012, $C_{14}H_{28}O_4N_1$ requires M^+ 274.2013, $[M+Na]^+$ 296.1831, $C_{14}H_{27}O_4N_1{}^{23}Na_1$ requires M^+ 296.1832.



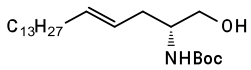
17c *tert*-Butyl ((2*S*,3*R*,*E*)-1,3-dihydroxy-6-phenylhex-4-en-2-yl)carbamate

Yield (62%, 43%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.32–7.26 (2H, m, Ar*H*), 7.23–7.19 (1H, m, Ar*H*), 7.18–7.14 (2H, m, Ar*H*), 6.00–5.90 (1H, m, 5-*H*), 5.61 (1H, dd, *J* 15.4, 6.2, 4-*H*), 5.30 (1H, br s, N*H*), 4.40–4.32 (1H, m, 3-*H*), 3.93 (1H, dd, *J* 11.2, 3.5, 1-H*H*), 3.71 (1H, dd, *J* 11.4, 3.5, 1-*H*H), 3.67–3.58 (1H, m, 2-*H*), 3.40 (2H, d, *J* 6.8, 6-*H*), 2.71–2.44 (2H, br m, 2 × O*H*), 1.45 (9H, s, C(C*H*$_3$)$_3$); δ_C (125 MHz, $CDCl_3$) 156.7, 139.3, 132.8, 130.7, 128.66, 128.62, 126.4, 80.7, 74.8, 62.8, 38.8, 28.5; *m/z* (ES^+) 329.9 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 308.1855, $C_{17}H_{26}O_4N_1$ requires M^+ 308.1856, $[M+Na]^+$ 330.1673, $C_{17}H_{25}O_4N_1{}^{23}Na_1$ requires M^+ 330.1676.



27a (*S*,*E*)-*tert*-Butyl (1-hydroxyoctadec-4-en-2-yl)carbamate[18]

Yield (53%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.52 (1H, dt, *J* 13.4, 6.7, 5-*H*), 5.41–5.27 (1H, m, 4-*H*), 4.66 (1H, br s, N*H*), 3.73–3.48 (3H, m, 1-*H*$_2$ and 2-*H*), 2.57 (1H, br s, O*H*), 2.33–2.10 (2H, m, 3-*H*$_2$), 2.00 (2H, dt, *J* 14.0, 6.7, 6-*H*$_2$), 1.44 (9H, s, C(C*H*$_3$)$_3$), 1.39–1.14 (22H, m), 0.88 (3H, t, *J* 6.8, 18-*H*$_3$); δ_C (101 MHz, $CDCl_3$) 155.98, 134.82, 125.20, 79.85, 65.99, 52.74, 34.89, 32.73, 32.07, 29.84, 29.81, 29.78, 29.66, 29.54, 29.51, 29.32, 28.51, 22.84, 14.28; *m/z* (ES^+) 384.9 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 384.3470, $C_{23}H_{46}O_3N_1$ requires M^+ 384.2472, $[M+Na]^+$ 406.3288, $C_{23}H_{45}O_3N_1{}^{23}Na_1$ requires M^+ 406.3292.

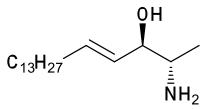


26a (*R*,*E*)-*tert*-Butyl (1-hydroxyoctadec-4-en-2-yl)carbamate[18]

Yield (62%); ν_{max} (ATR) 3375, 2971, 2879, 1716, 1491, 1173, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.52 (1H, dtt, *J* 15.4, 6.6, 1.0, 5-*H*), 5.35 (1H, dtt, *J* 15.4, 7.1, 1.3, 4-*H*), 4.66 (1H, br s, N*H*), 3.70–3.53 (3H, m, 1-*H*$_2$ and 2-*H*), 2.57 (1H, br s, O*H*), 2.30–2.11 (2H, m, 3-*H*$_2$), 1.99 (2H, dd, *J* 13.7, 6.7, 6-*H*$_2$), 1.44 (9H, s, OC(C*H*$_3$)$_3$), 1.36–1.22 (22H, m, 7-*H*$_2$ to 17-*H*$_2$), 0.88 (3H, t, *J* 6.9, 18-*H*$_3$); δ_C (101 MHz, $CDCl_3$) 155.98, 134.82, 125.20, 79.85, 65.99, 52.74, 34.89, 32.73, 32.07, 29.84, 29.81, 29.78, 29.66, 29.54, 29.51, 29.32, 28.51, 22.84, 14.28; *m/z* (ES^+) 384.9 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 384.3470, $C_{23}H_{46}O_3N_1$ requires M^+ 384.2472, $[M+Na]^+$ 406.3288, $C_{23}H_{45}O_3N_1{}^{23}Na_1$ requires M^+ 406.3292.

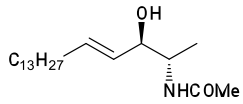
General procedure for BOC de-protection - Acylation Reactions

To a solution of the *N*-Boc protected starting material in CH_2Cl_2, TFA (excess) was added dropwise at room temperature and the reaction was monitored by TLC. Typically the reaction was complete in less than 1 hr. The reaction mixture was dried on the rotary evaporator to remove the excess TFA. The resulting residue (unprotected amine) was dissolved in CH_2Cl_2 (2 ml) and basified to pH 8 with aq. $NaHCO_3$ followed by the addition of the corresponding acid chloride (1.2 eq.). The reaction was monitored by TLC. Upon completion, the reaction was diluted with sat. aq. NH_4Cl. The phases were separated and the aqueous layer was extracted with twice CH_2Cl_2. The combined organic extracts were washed with brine, dried on anhydrous $MgSO_4$ and evaporated to yield the crude product. Assessment of the crude product purity was based on crude ^{1}H NMR. Compounds that were ≥80% pure were used as they soon as produced. Others were purified by standard column chromatography. All compounds used in the screening were characterised as an array (^{1}H NMR and Mass Spectrometry data).



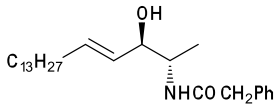
18an (2*S*,3*R*,*E*)-2-aminooctadec-4-en-3-ol

Yield (62%); δ_H (400 MHz, $CDCl_3$) 7.80 (2H, br s, N*H*$_2$), 5.77 (1H, dt, *J* 15.2, 6.4, 5-*H*), 5.37 (1H, dd, *J* 15.2, 6.1, 4-*H*), 4.41–4.24 (1H, m, 3-*H*), 3.40–3.28 (2H, m, 2-*H* and O*H*), 2.02 (2H, dd, *J* 13.7, 6.7, 6-*H*$_2$), 1.29–1.21 (25H, m, 7-*H*$_2$ to 17-*H*$_2$ and 1-*H*$_3$), 0.87 (3H, t, *J* 6.8, 18-*H*$_3$); *m/z* (ES$^+$) 284.3 $[M+H]^+$; HRMS (ES$^+$) found $[M+H]^+$ 284.2947, $C_{18}H_{38}O_3N_1$ requires M^+ 284.2948.



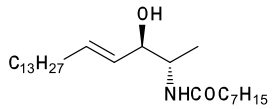
18az *N*-((2*S*,3*R*,*E*)-3-hydroxyoctadec-4-en-2-yl)acetamide

Yield (80%); δ_H (400 MHz, $CDCl_3$) 5.75–5.66 (1H, m, 5-*H*), 5.60 (1H, d, *J* 2.4, N*H*), 5.48–5.35 (1H, m, 4-*H*), 4.12 (1H, dd, *J* 6.7, 3.2, 3-*H*), 4.10–4.04 (1H, m, 2-*H*), 2.11–1.95 (5H, m, 6-*H*$_2$ and COC*H*$_3$), 1.40–1.11 (22H, m, 7-*H*$_2$ to 18-*H*$_2$), 1.08 (3H, d, *J* 6.9, 1-*H*$_3$), 0.87 (3H, t, *J* 6.8, 18-*H*$_3$); *m/z* (ES$^+$) 348.4 $[M+Na]^+$; HRMS (ES$^+$) found $[M+Na]^+$ 348.2871, $C_{20}H_{39}O_2N_1{}^{23}Na_1$ requires M^+ 348.2873.



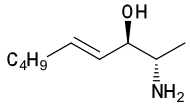
18ay N-((2S,3R,E)-3-hydroxyoctadec-4-en-2-yl)-2-phenylacetamide

Yield (22%); δ_H (400 MHz, $CDCl_3$) 7.39–7.23 (5H, m, Ar*H*), 5.64 (1H, dtd, *J* 15.0, 7.0, 0.9, 5-*H*), 5.51 (1H, br d, N*H*), 5.31 (1H, dd, *J* 15.0, 6.3, 4-*H*), 4.13–4.03 (2H, m, 3-*H* and 2-*H*), 3.58 (2H, s, PhC*H*$_2$), 1.98 (2H, dd, *J* 13.8, 7.0, 6-*H*$_2$), 1.39-1.18 (22H, m, 7-*H*$_2$ to 17-*H*$_2$), 1.01 (3H, d, *J* 6.8, 1-*H*$_3$), 0.88 (3H, t, *J* 6.9, 18-*H*$_3$); *m/z* (ES$^+$) 424.5 $[M+Na]^+$; HRMS (ES$^+$) found $[M+Na]^+$ 424.3182, $C_{26}H_{43}O_2N_1{}^{23}Na_1$ requires M^+ 424.3186.



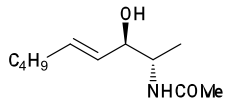
18ax *N*-((2*S*,3*R*,*E*)-3-hydroxyoctadec-4-en-2-yl)octanamide

Yield (41%); δ_H (400 MHz, $CDCl_3$) 5.71 (1H, dtd, *J* 14.9, 6.7, 1.1, 5-*H*), 5.43 (1H, ddt, *J* 15.4, 6.6, 1.4, 4-*H*), 4.64 (1H, br s, N*H*), 4.14–4.06 (1H, m, 3-*H*), 3.85–3.72 (1H, m, 2-*H*), 2.56 (1H, br s, O*H*), 2.04 (2H, dd, *J* 14.9, 7.0, 6-H_2), 1.40–1.20 (22 H, m, 7-H_2 to 17-H_2), 1.07 (3H, d, *J* 6.9, 1-H_3), 0.88 (3H, t, *J* 6.9, 18-H_3); *m/z* (ES^+) 432.6 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 432.3806, $C_{26}H_{51}O_2N_1{}^{23}Na_1$ requires M^+ 432.3812.



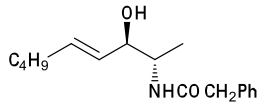
18bn (2*S*,3*R*,*E*)-2-aminonon-4-en-3-ol

Yield (73%); δ_H (400 MHz, $CDCl_3$) 5.79–5.69 (1H, m, 5-*H*), 5.39 (1H, dd, *J* 14.9, 5.3, 4-*H*), 5.30 (2H, br s, NH_2), 4.32–4.24 (1H, m, 3-*H*), 3.33–3.21 (1H, m, 2-*H*), 2.03 (2H, dd, *J* 12.8, 5.2, 6-H_2), 1.41–1.26 (4H, m, 7-H_2 and 8-H_2), 1.15 (1H, d, *J* 6.5, 1-H_3), 0.88 (1H, t, *J* 6.9, 9-H_3); *m/z* (ES^+) 158.1 $[M+H]^+$; HRMS (ES^+) found $[M+Na]^+$ 158.1539, $C_9H_{19}O_1N_1{}^{23}Na_1$ requires M^+ 158.1539.



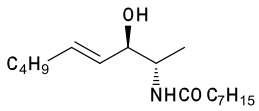
18bz *N*-((2*S*,3*R*,*E*)-3-hydroxynon-4-en-2-yl)acetamide

Yield (79%); δ_H (400 MHz, $CDCl_3$) 6.03 (1H, br s, N*H*), 5.74–5.62 (1H, m, 5-*H*), 5.41 (1H, dd, *J* 15.3, 6.4, 4-*H*), 4.16–4.00 (1H, m, 3-*H* and 2-*H*), 3.73 (1H, s, O*H*), 2.14–1.87 (5H, m, 6-H_2 and COCH_3), 1.44–1.25 (4H, m, 7-H_2 and 8-H_2), 1.06 (1H, d, *J* 6.8, 1-H_3), 0.88 (3H, t, *J* 6.8, 9-H_3); *m/z* (ES^+) 222.2 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 222.1463, $C_{11}H_{21}O_2N_1{}^{23}Na_1$ requires M^+ 222.1465.



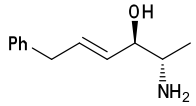
18by *N*-((2*S*,3*R*,*E*)-3-hydroxynon-4-en-2-yl)-2-phenylacetamide

Yield (50%); δ_H (400 MHz, $CDCl_3$) 7.35–7.22 (5H, m, Ar*H*), 5.69–5.59 (1H, m, 5-*H*), 5.32 (1H, dd, *J* 15.9, 6.3, 4-*H*), 4.19–3.98 (2H, m, 3-*H* and 2-*H*), 3.56 (2H, s, PhCH_2), 1.99 (2H, dd, *J* 13.3, 6.6, 6-H_2), 1.34–1.21 (2H, m, 7-H_2 and 8-H_2), 1.01 (1H, d, *J* 6.6, 1-H_3), 0.89 (1H, t, *J* 6.5, 9-H_3); *m/z* (ES^+) 298.3 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 298.1775, $C_{17}H_{25}O_2N_1{}^{23}Na_1$ requires M^+ 298.1778.



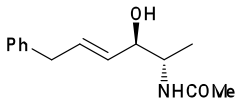
18bx *N*-((2*S*,3*R*,*E*)-3-hydroxynon-4-en-2-yl)octanamide

Yield (23%); δ_H (400 MHz, $CDCl_3$) 5.76–5.67 (1H, m, 5-*H*), 5.63 (1H, br d, *J* 7.7, N*H*), 5.41 (1H, dd, *J* 15.5, 6.3, 4-*H*), 4.11 (2H, m, 3-*H* and 2-*H*), 2.20–2.15 (2H, m, COCH_2), 2.10–2.02 (2H, m, 6-H_2), 1.66–1.58 (2H, m, $COCH_2CH_2$), 1.39–1.22 (12H, m, 7-H_2, 8-H_2 and 4 × octanoyl aliphatic CH_2), 1.09 (3H, d, *J* 6.8, 1-H_3), 0.92–0.85 (6H, m, 9-H_3 and octanoyl terminal CH_3); *m/z* (ES^+) 306.4 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 306.2402, $C_{17}H_{33}O_2N_1{}^{23}Na_1$ requires M^+ 306.2404.



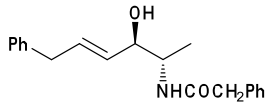
18cn (2*S*,3*R*,*E*)-2-amino-6-phenylhex-4-en-3-ol

Yield (77%); δ_H (400 MHz, $CDCl_3$) 7.85 (2H, br s, NH_2), 7.29–7.23 (2H, m, Ar*H*), 7.20–7.16 (1H, m, Ar*H*), 7.14–7.11 (2H, m, Ar*H*), 5.97–5.88 (1H, m, 5-*H*), 5.45 (1H, dd, *J* 15.5, 6.2, 4-*H*), 4.48–4.39 (1H, m, 3-*H*), 3.43–3.37 (1H, m, 2-*H*), 3.35 (2H, d, *J* 7.7, 6-H_2), 2.89 (1H, br s, O*H*), 1.19 (3H, d, *J* 6.5, 1-H_3); *m/z* (ES^+) 192.2 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 192.1383, $C_{12}H_{18}O_1N_1$ requires M^+ 192.1383.



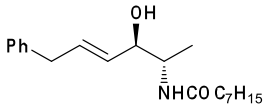
18cz *N*-((2*S*,3*R*,*E*)-3-hydroxy-6-phenylhex-4-en-2-yl)acetamide

Yield (67%); δ_H (400 MHz, $CDCl_3$) 7.31–7.27 (2H, m, Ar*H*), 7.22–7.14 (3H, m, Ar*H*), 5.98 (1H, br s, N*H*),5.93–5.82 (1H, m, 5-*H*), 5.50 (1H, dd, *J* 15.3, 6.2, 4-*H*), 4.21-4.13 (1H, m, 3-*H*), 4.06 (1H, dt, *J* 8.0, 4.0, 2-*H*), 3.38 (1H, d, *J* 6.7, 6-H_2), 2.85 (2H, br s, O*H*), 1.94 (3H, s, COCH_3), 1.08 (3H, d, *J* 6.9, 1-H_3); *m/z* (ES^+) 256.3 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 256.1307, $C_{14}H_{19}O_2N_1{}^{23}Na_1$ requires M^+ 256.1308.



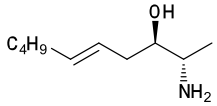
18cy *N*-((2*S*,3*R*,*E*)-3-hydroxy-6-phenylhex-4-en-2-yl)-2-phenylacetamide

Yield (87%); δ_H (400 MHz, $CDCl_3$) 7.35–7.10 (10H, m, Ar*H*), 5.91 (1H, br s, N*H*), 5.86–5.77 (1H, m, 5-*H*), 5.40 (1H, dd, *J* 15.4, 6.0, 4-*H*), 4.18–3.96 (1H, m, 3-*H*), 3.60–3.49 (1H, m, 2-*H*), 3.42 (2H, s, COCH_2), 3.32 (2H, d, *J* 6.7, 6-H_2), 1.01 (3H, d, *J* 6.8, 1-H_3); *m/z* (ES^+) 332.3 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 332.1618, $C_{20}H_{23}O_2N_1{}^{23}Na_1$ requires M^+ 332.1621.



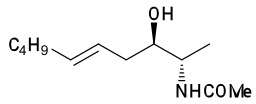
18cx ***N*-((2*S*,3*R*,*E*)-3-hydroxy-6-phenylhex-4-en-2-yl)octanamide**

Yield (90%); δ_H (400 MHz, $CDCl_3$) 7.31–7.24 (2H, m, Ar*H*), 7.22–7.12 (3H, m, Ar*H*), 5.95–5.82 (2H, m, 5-*H* and N*H*), 5.50 (1H, dd, *J* 15.4, 6.2, 4-*H*), 4.20–4.03 (2H, m, 3-*H* and 2-*H*), 3.38 (1H, d, *J* 6.7, 6-H_2), 2.17–2.10 (2H, m, COCH_2), 1.66–1.51 (2H, m, $COCH_2CH_2$), 1.32–1.20 (8H, m, 4 × CH_2), 1.08 (3H, d, *J* 6.8, 1-H_3), 0.87 (3H, t, *J* 6.8, octanoyl terminal CH_3); *m/z* (ES^+) 318.2 $[M+H]^+$, 340.4 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 318.2431, $C_{20}H_{32}O_2N_1$ requires M^+ 318.2428.



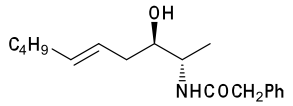
21bn **(2*S*,3*R*,*E*)-2-aminodec-5-en-3-ol**

Yield (79%); δ_H (400 MHz, $CDCl_3$) 5.62–5.45 (1H, m, 6-*H*), 5.41–5.26 (1H, m, 5-*H*), 5.14 (1H, br s, NH_2), 3.83–3.71 (1H, m, 2-*H*), 3.31–3.13 (1H, m, 3-*H*), 2.27–1.93 (4H, m, 4-H_2 and 7-H_2), 1.33–1.29 (4H, m, 8-H_2 and 9-H_2), 1.16 (3H, d, *J* 6.1, 1-H_3), 0.87 (3H, t, *J* 7.0, 10-H_3); *m/z* (ES^+) 172.3 $[M+H]^+$, 194.3 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 172.1696, $C_{10}H_{22}O_1N_1$ requires M^+ 172.1696.



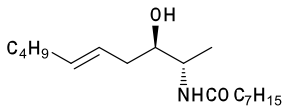
21bz ***N*-((2*S*,3*R*,*E*)-3-hydroxydec-5-en-2-yl)acetamide**

Yield (70%); δ_H (400 MHz, $CDCl_3$) 5.88 (1H, br s, N*H*), 5.55 (1H, dt, *J* 13.4, 6.6, 6-*H*), 5.44–5.34 (1H, m, 5-*H*), 4.01 (1H, ddd, *J* 8.5, 6.8, 3.0, 2-*H*), 3.64 (1H, ddt, *J* 8.5, 4.2, 3.1, 3-*H*), 2.11–1.99 (4H, m, 4-H_2 and 7-H_2), 1.98 (3H, s, COCH_3), 1.38–1.25 (4H, m, 8-H_2 and 9-H_2), 1.10 (3H, d, *J* 6.8, 1-H_3), 0.88 (3H, t, *J* 7.1, 10-H_3); *m/z* (ES^+) 236.3 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 236.1620, $C_{12}H_{23}O_2N_1{}^{23}Na_1$ requires M^+ 236.1621.



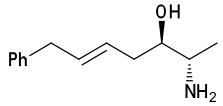
21by ***N*-((2*S*,3*R*,*E*)-3-hydroxydec-5-en-2-yl)-2-phenylacetamide**

Yield (85%); δ_H (400 MHz, $CDCl_3$) 7.40–7.22 (5H, m, Ar*H*), 5.73 (1H, br s, N*H*), 5.50 (1H, dt, *J* 15.2, 6.6, 6-*H*), 5.35 (1H, ddd, *J* 15.2, 7.7, 6.3, 5-*H*), 3.99 (1H, ddd, *J* 8.5, 6.9, 3.1, 2-*H*), 3.56 (1H, m, 3-*H* and PhCH_2), 2.22–2.08 (2H, m, 4-H_2), 2.06–1.92 (2H, m, 7-H_2), 1.34–1.28 (4H, m, 8-H_2 and 9-H_2), 1.04 (3H, d, *J* 6.9, 1-H_3), 0.88 (3H, t, *J* 7.1, 10-H_3); *m/z* (ES^+) 312.4 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 312.1935, $C_{18}H_{27}O_2N_1{}^{23}Na_1$ requires M^+ 312.1934.



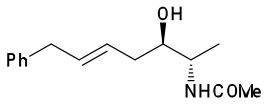
21bx *N*-((2*S*,3*R*,*E*)-3-hydroxydec-5-en-2-yl)octanamide

Yield (83%); δ_H (400 MHz, $CDCl_3$) 6.03-5.98 (1H, br s, N*H*), 5.54–5.43 (1H, m, 6-*H*), 5.35–5.25 (1H, m, 5-*H*), 4.23–4.11 (1H, m, 2-*H*), 4.05–3.98 (1H, m, 3-*H*), 2.35–2.27 (2H, m, COCH_2), 2.20–2.11 (2H, m, 4-H_2), 2.06–1.94 (2H, m, 7-H_2), 1.68–1.50 (2H, m, $COCH_2CH_2$), 1.39–1.19 (12H, m, 8-H_2, 9-H_2 and 4 × aliphatic CH_2), 1.14–1.05 (3H, d, *J* 6.9, 1-H_3), 0.86 (6H, 10-H_3 and octanoyl terminal CH_3); *m/z* (ES^+) 298.5 $[M+H]^+$, 320.5 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 320.2563, $C_{18}H_{35}O_2N_1{}^{23}Na_1$ requires M^+ 320.2560.



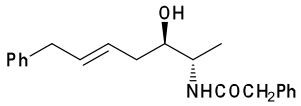
21cn (2*S*,3*R*,*E*)-2-amino-7-phenylhept-5-en-3-ol

Yield (46%); δ_H (700 MHz, $CDCl_3$) 7.88 (2H, br s, NH_2), 7.20–7.11 (5H, m, Ar*H*), 5.71–5.65 (1H, m, 6-*H*), 5.45–5.40 (1H, m, 5-*H*), 3.93–3.89 (1H, m, 3-*H*), 3.36 (1H, d, *J* 7.7, 2-*H*), 3.32 (2H, d, *J* 6.7, 7-H_2), 2.28–2.09 (2H, m, 4-H_2), 1.20 (3H, d, *J* 6.3, 1-H_3). *m/z* (ES^+) 206.3 $[M+H]^+$, 228.3 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 206.1540, $C_{13}H_{20}O_1N_1$ requires M^+ 206.1539.



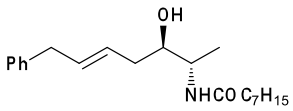
21cz *N*-((2*S*,3*R*,*E*)-3-hydroxy-7-phenylhept-5-en-2-yl)acetamide

Yield (86%); δ_H (400 MHz, $CDCl_3$) 7.34–7.24 (2H, m, Ar*H*), 7.24-7.15 (3H, m, Ar*H*), 5.79 (1H, br s, N*H*), 5.73 (1H, ddd, *J* 15.2, 8.4, 4.3, 6-*H*), 5.52 (1H, ddd, *J* 15.2, 7.7, 6.4, 5-*H*), 4.03 (1H, dqd, *J* 13.8, 6.9, 3.0, 2-*H*), 3.72–3.65 (1H, m, 3-*H*), 3.37 (1H, d, *J* 6.7, 7-H_2), 2.30–2.04 (2H, m, 4-H_2), 1.98 (3H, s. COCH_3), 1.11 (3H, d, *J* 6.9, 1-H_3); *m/z* (ES^+) 248.4 $[M+H]^+$, 270.3 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 270.1464, $C_{15}H_{21}O_2N_1{}^{23}Na_1$ requires M^+ 270.1465.



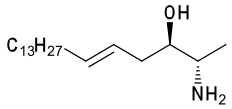
21cy *N*-((2*S*,3*R*,*E*)-3-hydroxy-7-phenylhept-5-en-2-yl)-2-phenylacetamide

Yield (27%); δ_H (400 MHz, $CDCl_3$) 7.43–7.08 (10H, m, Ar*H*), 5.78–5.59 (1H, m, 6-*H*), 5.57–5.37 (1H, m, 5-*H*), 4.07–3.93 (1H, m, 2-*H*), 3.64–3.50 (3H, m, 2-*H* and PhCH_2), 3.34 (1H, d, *J* 6.7, 7-H_2), 2.25–1.93 (2H, m, 4-H_2), 1.03 (3H, d, *J* 6.8, 1-H_3); *m/z* (ES^+) 346.4 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 346.1779, $C_{21}H_{25}O_2N_1{}^{23}Na_1$ requires M^+ 346.1778.



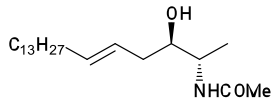
21cx *N*-((2*S*,3*R*,*E*)-3-hydroxy-7-phenylhept-5-en-2-yl)octanamide

Yield (70%); δ_H (400 MHz, $CDCl_3$) 7.31–7.27 (2H, m, Ar*H*), 7.23–7.15 (3H, m, Ar*H*), 5.87–5.65 (1H, m, N*H* and 6-*H*), 5.52 (1H, dt, *J* 14.2, 6.6, 5-*H*), 4.13-3.94 (1H, m, 2-*H*), 3.78–3.59 (1H, m, 3-*H*), 3.37(1H, d, *J* 6.6, 7-H_2), 2.32–2.04 (2H, m, COCH_2), 1.70–1.54 (2H, m, $COCH_2CH_2$), 1.37–1.21 (8H, m, 4 × aliphatic CH_2), 1.13 (3H, d, *J* 6.9, 1-H_3), 0.87 (3H, t, *J* 6.6, octanoyl terminal CH_3); *m/z* (ES^+) 332.5 $[M+H]^+$, 354.4 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 354.2403, $C_{21}H_{33}O_2N_1{}^{23}Na_1$ requires M^+ 354.2404.



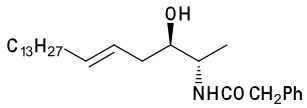
21an (2*S*,3*R*,*E*)-2-aminononadec-5-en-3-ol

Yield (70%); δ_H (700 MHz, $CDCl_3$) 8.12 (2H, br s, NH_2), 5.56–5.48 (1H, m, 6-*H*), 5.38 (1H, dd, *J* 14.0, 6.7, 5-*H*), 3.73–3.67 (1H, m, 3-*H*), 3.65–3.60 (1H, m, 2-*H*), 2.15–2.07 (1H, m, 4-*H*H), 2.04–1.95 (3H, m, 4-H*H* and 7-H_2), 1.38–1.09 (26H, m, 8-H_2 to 18-H_2 and 1-H_3), 0.86 (3H, t, *J* 7.0, 19-H_3). *m/z* (ES^+) 298.4 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 298.3099, $C_{19}H_{40}O_1N_1$ requires M^+ 298.3110.



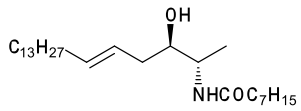
21az *N*-((2*S*,3*R*,*E*)-3-hydroxynonadec-5-en-2-yl)acetamide

Yield (82%); δ_H (400 MHz, $CDCl_3$) 5.96 (1H, br d, *J* 6.0, N*H*), 5.54 (1H, dt, *J* 15.0, 6.6, 6-*H*), 5.45–5.34 (1H, m, 5-*H*), 4.00 (1 H, ddd, *J* 8.5, 6.9, 3.0, 3-*H*), 3.64 (1H, ddd, *J* 8.8, 4.4, 3.0, 2-*H*), 2.23–1.99 (4 H, m, 7-H_2 and 4-H_2), 1.97 (3H, s, COCH_3), 1.38–1.21 (22H, m, 8-H_2 to 18-H_2), 1.10 (3H, d, *J* 6.8, 1-H_3), 0.87 (3H, t, *J* 6.9, 19-H_3). *m/z* (ES^-) 338.4 $[M-H]^-$; (ES^+) 362.4.3 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 340.3219, $C_{21}H_{42}O_2N_1$ requires M^+ 340.3216.



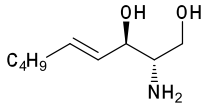
21ay *N*-((2*S*,3*R*,*E*)-3-hydroxynonadec-5-en-2-yl)-2-phenylacetamide

Yield (86%); δ_H (400 MHz, $CDCl_3$) 7.37–7.32 (2H, m, Ar*H*), 7.31–7.24 (3H, m, Ar*H*), 5.74 (1H, br d, *J* 8.3, N*H*), 5.50 (1H, dt, *J* 14.7, 6.6, 6-*H*), 5.34 (1H, ddd, *J* 15.2, 7.6, 6.4, 5-*H*), 3.99 (1H, ddd, *J* 8.4, 6.9, 3.1, 3-*H*), 3.73–3.64 (1H, m, 2-*H*), 3.55 (2H, s, PhCH_2), 2.15–2.08 (2H, m, 4-H_2), 2.06–1.94 (2H, m, 7-H_2), 1.36–1.22 (22H, m, 8-H_2 to 18-H_2), 1.04 (3H, d, *J* 6.8, 1-H_2), 0.88 (3H, t, *J* 6.9, 19-H_3). *m/z* (ES^-) 414.4 $[M-H]^-$; (ES^+) 438.4 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 416.3533, $C_{27}H_{46}O_2N_1$ requires M^+ 416.3529.



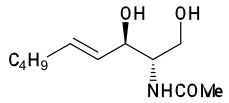
21ax *N*-((2*S*,3*R*,*E*)-3-hydroxynonadec-5-en-2-yl)octanamide

Yield (85%); δ_H (400 MHz, $CDCl_3$) 5.85 (1H, br d, *J* 8.4, N*H*), 5.55 (1H, dt, *J* 14.9, 6.6, 6-*H*), 5.39 (1H, ddd, *J* 15.0, 7.5, 6.4, 5-*H*), 4.03 (1H, ddd, *J* 8.7, 7.0, 3.0, 3-*H*), 3.64 (1H, ddd, *J* 8.8, 4.1, 3.0, 2-*H*), 2.33 (2H, t, *J* 7.5, COCH_2), 2.19–2.14 (2H, m, 4-H_2), 2.00 (2H, dd, *J* 13.7, 6.6, 7-H_2), 1.62 (2H, dd, *J* 13.3, 6.0, COCH2CH_2), 1.34–1.22 (26H, m, 8-H_2 to 18-H_2 and 4 × aliphatic CH_2), 1.10 (3H, d, *J* 6.8, 1-H_3), 0.87 (3H, t, *J* 6.7, 19-H_3). *m/z* (ES^-) 422. $[M-H]^-$; (ES^+) 446.4 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 424.4174, $C_{27}H_{54}O_2N_1$ requires M^+ 424.4155.



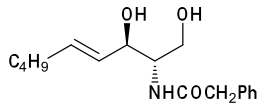
17bn (2*S*,3*R*,*E*)-2-aminonon-4-ene-1,3-diol

Yield (54%); δ_H (400 MHz, $CDCl_3$) 5.80–5.71 (1H, m, 5-*H*), 5.42 (1H, dd, *J* 15.2, 6.7, 4-*H*), 4.31 (4H, br s, NH_2 and 2 × O*H*), 4.25–4.19 (1H, m, 3-*H*), 3.78–3.63 (2H, m, 2-*H* and 1-*H*H), 3.06 (1H, dd, *J* 8.7, 4.2, 1-H*H*), 2.04 (2H, dd, *J* 13.1, 6.5, 6-H_2), 1.39–1.26 (4H, m, 7-H_2 and 8-H_2), 0.88 (3H, t, *J* 7.0, 9-H_3); *m/z* (ES^+) 174.3 $[M+H]^+$, 196.4 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 174.1490, $C_9H_{20}O_2N_1$ requires M^+ 174.1489.



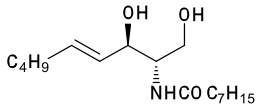
17bz *N*-((2*S*,3*R*,*E*)-1,3-dihydroxynon-4-en-2-yl)acetamide

Yield (73%); δ_H (700 MHz, $CDCl_3$) 6.44 (1H, br d, *J* 13.7, N*H*), 5.82–5.76 (1H, m, 5-*H*), 5.41 (1H, dd, *J* 15.1, 5.7, 4-*H*), 4.47–4.39 (1H, m, 3-*H*), 3.87–3.77 (2H, m, 2-*H* and 1-*H*H), 3.35–3.29 (1H, m, 1-H*H*), 2.09 –2.00 (5H, m, 6-H_2 and COCH_3), 1.41 – 1.23 (4H, m, 7-H_2 and 8-H_2), 0.88 (3H, t, *J* 7.3, 9-H_3). *m/z* (ES^+) 238.2 $[M+Na]^+$ $C_{11}H_{21}O_3N_1{}^{23}Na_1$; HRMS ($ES^+$) found $[M+Na]^+$ 238.1419, $C_{11}H_{21}O_3N_1{}^{23}Na_1$ requires M^+ 238.1419.



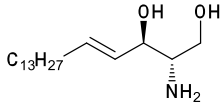
17by *N*-((2*S*,3*R*,*E*)-1,3-dihydroxynon-4-en-2-yl)-2-phenylacetamide

Yield (65%); δ_H (400 MHz, $CDCl_3$) 7.35–7.23 (5H, m, Ar*H*), 6.34 (1H, br d, *J* 6.6, N*H*), 5.66 (1H, dt, *J* 14.9, 6.7, 5-*H*), 5.40 (1H, dd, *J* 14.9, 6.5, 4-*H*), 4.23–4.16 (1H, m, 3-*H*), 3.88–3.79 (2H, m, 2-*H* and 1-*H*H), 3.61 (1H, dd, *J* 10.9, 2.7, 1-H*H*), 3.56 (2H, s, PhCH_2), 1.98 (1H, dd, *J* 13.5, 6.8, 6-H_2), 1.32–1.25 (4H, m, 7-H_2 and 8-H_2), 0.88 (3H, t, *J* 7.0, 9-H_3); *m/z* (ES^+) 292.2 $[M+H]^+$, 314.2 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 292.1907, $C_{17}H_{26}O_3N_1$ requires M^+ 292.1913.



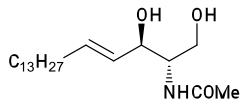
17bx *N*-((2*S*,3*R*,*E*)-1,3-dihydroxynon-4-en-2-yl)octanamide

Yield (68%); δ_H (400 MHz, $CDCl_3$) 6.44 (1H, br s, N*H*), 5.74 (1H, dt, *J* 15.9, 6.7, 5-*H*), 5.49 (1H, dd, *J* 15.9, 6.0, 4-*H*), 4.30–4.21 (1H, m, 3-*H*), 3.94–3.84 (2H, m, 2-*H* and 1-*H*H), 3.71–3.63 (1H, m, 1-H*H*), 2.24–2.16 (2H, m, COCH_2), 2.04 (2H, dd, *J* 13.0, 6.7, 6-H_2), 1.64–1.55 (2H, m, $COCH_2CH_2$), 1.34–1.21 (4H, m, 7-H_2 and 8-H_2), 0.87, 0.86 (2 × 3H, t, *J* 6.5, 9-H_3 and octanoyl terminal CH_3); *m/z* (ES^+) 300.3 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 300.2536, $C_{17}H_{34}O_3N_1$ requires M^+ 300.2539.



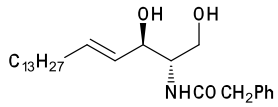
17an (2*S*,3*R*,*E*)-2-aminooctadec-4-ene-1,3-diol[2]

Yield (64%); δ_H (400 MHz, $CDCl_3$) 5.87–5.79 (1H, m, 5-*H*), 5.53 (1H, dd, *J* 15.4, 6.5, 4-*H*), 4.78 (2H, br s, NH_2), 4.41–4.36 (1H, m, 3-*H*), 4.10 (1H, dd, *J* 11.5, 2.7, 1-*H*H), 3.93 (1H, m, 2-*H*), 3.73 (1H, dd, *J* 11.5, 3.5, 1-H*H*), 2.77 (2H, br s, 2 × O*H*), 2.06 (2H, dd, *J* 14.0, 7.0, 6-H_2), 1.67 (1 H, dq, *J* 12.2, 6.0, 17-H_2), 1.48–1.22 (20H, m, 7-H_2 to 16-H_2), 0.89 (1H, t, *J* 7.5, 18-H_3); *m/z* (ES^+) 300.5 $[M+H]^+$, 322.5 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 300.2902, $C_{18}H_{38}O_2N_1$ requires M^+ 300.2897.



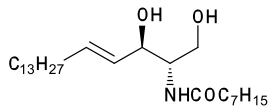
17az *N*-((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-2-yl)acetamide

Yield (71%); δ_H (400 MHz, $CDCl_3$) 5.81 (1H, dt, *J* 14.6, 7.1, 5-*H*), 5.51 (1H, dd, *J* 15.4, 6.4, 4-*H*), 5.39 (1H, br s, N*H*), 4.38–4.32 (1H, m, 3-*H*), 4.07(1 H, dd, *J* 11.6, 2.7, 2-*H*), 3.83–3.78 (1H, m, 1-H*H*), 3.73 (1H, dd, *J* 11.6, 2.9, 1-*H*H), 2.09–1.98 (5H, m, 6-H_2 and COCH_3), 1.39–1.20 (22H, m, 7-H_2 to 17-H_2), 0.87 (1H, t, *J* 6.7, 18-H_3); *m/z* (ES^+) 364.4 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 364.2829, $C_{20}H_{39}O_3N_1{}^{23}N_1$ requires M^+ 364.2822.



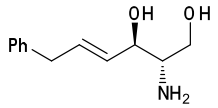
17ay *N*-((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-2-yl)-2-phenylacetamide

Yield (44%); δ_H (400 MHz, $CDCl_3$) 7.38–7.25 (5H, m, Ar*H*), 6.21 (1H, br d, *J* 7.0, N*H*), 5.73–5.63 (1H, m, 5-*H*), 5.42 (1H, dd, *J* 15.4, 6.5, 4-*H*), 4.27–4.20 (1H, m, 3-*H*), 3.90–3.83 (2H, m, 2-*H* and 1-*H*H), 3.65 (1H, dd, *J* 11.8, 3.9, 1-H*H*), 3.60 (2H, s, PhCH_2), 2.97–2.64 (2H, m, 2 × O*H*), 1.99 (2H, dd, *J* 13.8, 6.9, 6-H_2), 1.34–1.16 (22H, m, 7-H_2 to 17-H_2), 0.88 (1H, t, *J* 6.8, 18-H_3); *m/z* (ES^+) 440.5 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 418.3324, $C_{26}H_{44}O_3N_1$ requires M^+ 418.3316.



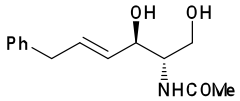
17ax *N*-((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-2-yl)octanamide

Yield (38%); δ_H (400 MHz, $CDCl_3$) 6.25 (1H, br d, *J* 7.8, N*H*), 5.84–5.74 (1H, m, 5-*H*), 5.53 (1H, dd, *J* 15.5, 6.5, 4-*H*), 4.36–4.30 (1H, m, 3-*H*), 3.96 (1H, dd, *J* 11.1, 3.7, 1-H*H*), 3.93–3.88 (1 H, m, 2-*H*), 3.70 (1H, dd, *J* 11.1, 2.9, 1-*H*H), 2.71 (2H, br s, 2 × O*H*), 2.27–2.20 (2H, m, COCH_2), 2.06 (2H, dd, *J* 14.6, 7.4, 6-H_2), 1.71–1.57 (2H, m, $COCH_2CH_2$), 1.43–1.18 (28H, m, 7-H_2 to 17-H_2 and 4'-H_2 to 7'-H_2), 0.92–0.83 (6H, m, 18-H_3 and octanoyl terminal CH_3); *m/*z (ES^+) 426.4 $[M+H]^+$; HRMS (ES^+) found $[M+Na]^+$ 448.3761, $C_{26}H_{44}O_3N_1$ requires M^+ 448.3767.



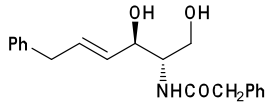
17cn (2*S*,3*R*,*E*)-2-amino-6-phenylhex-4-ene-1,3-diol

Yield (67%); δ_H (400 MHz, $CDCl_3$) 7.25–7.20 (2H, m, Ar*H*), 7.16–7.07 (3H, m, Ar*H*), 5.88 (1H, dt, *J* 15.0, 6.6, 5-*H*), 5.42 (1H, dd, *J* 15.0, 5.0, 4-*H*), 4.87 (2H, br s, NH_2), 4.40–4.29 (1H, m, 3-*H*), 3.76–3.67 (2H, m, 3-*H* and 1-*H*H), 3.41–3.33 (1H, m, 1-H*H*), 3.30 (2H, d, *J* 6.6, 6-H_2), 3.21–3.14 (2H, br s, 2 × O*H*); *m/*z (ES^+) 208.2 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 208.1331, $C_{12}H_{18}O_2N_1$ requires M^+ 208.1338.



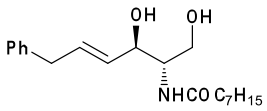
17cz *N*-((2*S*,3*R*,*E*)-1,3-dihydroxy-6-phenylhex-4-en-2-yl)acetamide

Yield (31%); δ_H (400 MHz, $CDCl_3$) 7.32–7.27 (2H, m, Ar*H*), 7.23–7.14 (3H, m, Ar*H*), 6.32 (1H, br d, N*H*), 5.96 (1H, dt, *J* 15.4, 6.7, 5-*H*), 5.61 (1H, dd, *J* 15.4, 6.0, 4-*H*), 4.38–4.32 (1H, m, 3-*H*), 3.99–3.88 (2H, m, 2-*H* and 1-*H*H), 3.70 (1H, dd, *J* 11.0, 2.8, 1-H*H*), 3.40 (1H, d, *J* 6.7, 6-H_2), 2.01 (3H, s, COCH_3); *m/*z (ES^+) 250.1 $[M+H]^+$, 272.1 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 272.1248, $C_{14}H_{19}O_3N_1{}^{23}Na_1$ requires M^+ 272.1263.



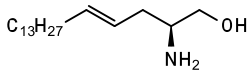
17cy *N*-((2*S*,3*R*,*E*)-1,3-dihydroxy-6-phenylhex-4-en-2-yl)-2-phenylacetamide

Yield (85%); δ_H (400 MHz, $CDCl_3$) 7.34–7.25 (6H, m, Ar*H*), 7.22–7.11 (4H, m, Ar*H*), 6.32 (1H, br d, *J* 7.5, N*H*), 5.82 (1H, dt, *J* 15.0, 6.8, 5-*H*), 5.47 (1H, dd, *J* 15.0, 5.8, 4-*H*), 4.24–4.18 (1H, m, 3-*H*), 3.89–3.84 (1H, m, 2-*H*), 3.81 (1H, dd, *J* 11.1, 4.0, 1-*H*H), 3.59 (1H, dd, *J* 11.1, 3.2, 1-H*H*), 3.51 (2H, s, COCH_2), 3.31 (2H, d, *J* 6.8, 6-H_2), 2.25 (2H, br s, 2 × O*H*); *m/*z (ES^+) 326.2 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 326.1767, $C_{20}H_{24}O_3N_1$ requires M^+ 326.1756.



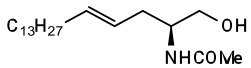
17cx ***N*-((2*S*,3*R*,*E*)-1,3-dihydroxy-6-phenylhex-4-en-2-yl)octanamide**

Yield (82%); δ_H (400 MHz, $CDCl_3$) 7.31–7.23 (3H, m, Ar*H*), 7.22–7.18 (1H, m, Ar*H*), 7.17–7.11 (2H, m, Ar*H*), 6.41 (1H, br d, *J* 6.6, N*H*), 5.92 (1H, dt, *J* 15.3, 6.8, 5-*H*), 5.58 (1H, dd, *J* 15.3, 6.1, 4-*H*), 4.33–4.27 (1H, m, 3-*H*), 3.89 (2H, m, 2-*H* and 1-*H*H), 3.66 (1H, dd, *J* 12.6, 4.7, 1-H*H*), 3.38 (2H, d, *J* 6.8, 6-H_2), 2.23–2.10 (2H, m, COCH_2), 1.63–1.53 (2H, m, $COCH_2CH_2$), 1.32–1.22 (8H, m, 4 × aliphatic CH_2), 0.87 (3H, t, *J* 6.8, octanoyl terminal CH_3); *m/z* (ES^+) 334.2 $[M+H]^+$; HRMS (ES^+) found $[M+Na]^+$ 334.2393, $C_{20}H_{32}O_3N_1$ requires M^+ 334.2382.



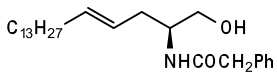
27an **(*S*,*E*)-2-aminooctadec-4-en-1-ol**

Yield (61%); δ_H (400 MHz, $CDCl_3$) 5.51 (1H, dt, *J* 13.9, 6.5, 5-*H*), 5.38–5.30 (1H, m, 4-*H*), 3.60 (1H, d, *J* 8.2, 1-H*H*), 3.33 (1H, dd, *J* 10.2, 8.2, 1-*H*H), 2.97-2.83 (1H, m, 2-*H*), 2.34 (3H, br s, NH_2 and O*H*), 2.20–2.10 (1H, m, 3-H_2), 1.99 (2H, dd, *J* 13.5, 6.6, 6-H_2), 1.37–1.20 (22H, m, 7-H_2 to 17-H_2), 0.87 (3H, t, *J* 6.5, 18-H_3); *m/z* (ES^+) 284.2 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 284.2947, $C_{18}H_{38}O_1N_1$ requires M^+ 284.2953.



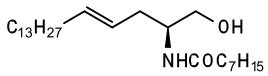
27az **(*S*,*E*)-*N*-(1-hydroxyoctadec-4-en-2-yl)acetamide**

Yield (68%); δ_H (700 MHz, $CDCl_3$) 5.82 (1H, br d, *J* 14.8, N*H*), 5.51 (1H, dt, *J* 15.0, 6.7, 5-*H*), 5.34 (1H, dt, *J* 15.1, 7.1, 4-*H*), 3.91 (1H, tdd, *J* 11.1, 7.2, 3.6, 2-*H*), 3.65 (1H, dd, *J* 11.0, 3.3, 1-H*H*), 3.58 (1H, dd, *J* 11.1, 5.7, 1-*H*H), 3.25 (1H, br s, O*H*), 2.27–2.22 (1H, m, 3-H*H*), 2.17 (1H, dt, *J* 14.1, 7.1, 3-*H*H), 1.98 (5H, m, 6-H_2 and COCH_3), 1.35–1.30 (2H, m, 7-H_2), 1.30 – 1.22 (20H, m, 8-H_2 to 17-H_2), 0.87 (3H, t, *J* 7.1, 18-H_3); *m/z* (ES^-) 324.4 $[M-H]^-$; (ES^+) 326.4 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 326.3046, $C_{20}H_{40}O_2N_1$ requires M^+ 326.3035.



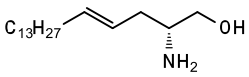
27ay **(*S*,*E*)-*N*-(1-hydroxyoctadec-4-en-2-yl)-2-phenylacetamide**

Yield (82%); δ_H (700 MHz, $CDCl_3$) 7.36–7.33 (1 H, m, Ar*H*), 7.30–7.28 (1 H, m, Ar*H*), 7.25–7.22 (1 H, m, Ar*H*), 5.58 (1H, br d, *J* 5.6, N*H*), 5.32 (1H, dt, *J* 14.7, 6.6, 5-*H*), 5.23–5.18 (1 H, m, 4-*H*), 3.88 (1H, dtd, *J* 13.2, 6.5, 3.8, 2-*H*), 3.61 (1H, dd, *J* 11.1, 3.5, 1-*H*H), 3.58 (2H, s, PhCH_2), 3.54 (1H, dd, *J* 11.1, 6.2, 1-H*H*), 2.19–2.14 (1H, m, 3-*H*H), 2.09–2.04 (1H, m, 3-H*H*), 1.88 (2H, dd, *J* 13.2, 6.5, 6-H_2), 1.31–1.23 (22H, m, 7-H_2 to 17-H_2), 0.88 (3H, t, *J* 7.0, 18-H_3); *m/z* (ES^-) 400.5 $[M-H]^-$; (ES^+) 402.4 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 402.3356, $C_{26}H_{44}O_2N_1$ requires M^+ 402.3348.



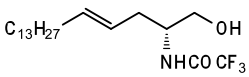
27ax (*S*,*E*)-*N*-(1-hydroxyoctadec-4-en-2-yl)octanamide

Yield (85%); δ_H (700 MHz, $CDCl_3$) 5.71 (1 H, br s, N*H*), 5.52 (1H, dt, *J* 14.8, 6.8, 5-*H*), 5.37–5.31 (1H, m, 4-*H*), 3.91 (1H, tdd, *J* 10.8, 6.9, 3.6, 2-*H*), 3.65 (1H, dd, *J* 10.9, 3.1, 1-*H*H), 3.58 (1H, dd, *J* 11.0, 6.0, 1-H*H*), 3.28 (1H, br s, O*H*), 2.26 (1H, dt, *J* 13.5, 6.6, 3-*H*H), 2.20–2.14 (3H, m, 3-H*H* and COCH_2), 1.99 (2H, dd, *J* 14.3, 7.1, 6-H_2), 1.60 (2H, dt, *J* 14.6, 7.4, $COCH_2CH_2$), 1.35–1.22 (30H, m, 7-H_2 to 17-H_2 and 4 × aliphatic CH_2), 0.87 (6H, t, *J* 7.1, 18-H_3 and terminal octanoyl CH_3); *m/z* (ES^-) 408.5 $[M-H]^-$; (ES^+) 410.5 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 410.3999, $C_{26}H_{52}O_2N_1$ requires M^+ 410.3998.



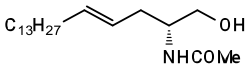
26an (*R*,*E*)-2-aminooctadec-4-en-1-ol

Yield (62%); δ_H (400 MHz, $CDCl_3$) 5.51 (1H, dt, *J* 13.9, 6.5, 5-*H*), 5.38–5.30 (1H, m, 4-*H*), 3.60 (1H, d, *J* 8.2, 1-H*H*), 3.33 (1H, dd, *J* 10.2, 8.2, 1-*H*H), 2.97-2.83 (1H, m, 2-*H*), 2.34 (3H, br s, NH_2 and O*H*), 2.20–2.10 (1H, m, 3-H_2), 1.99 (2H, dd, *J* 13.5, 6.6, 6-H_2), 1.37–1.20 (22H, m, 7-H_2 to 17-H_2), 0.87 (3H, t, *J* 6.5, 18-H_3); *m/z* (ES^+) 284.2 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 284.2947, $C_{18}H_{38}O_1N_1$ requires M^+ 284.2953.



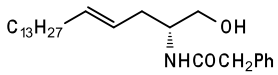
26af (*R*,*E*)-2,2,2-trifluoro-*N*-(1-hydroxyoctadec-4-en-2-yl)acetamide

Yield (38%); δ_H (400 MHz, $CDCl_3$) 6.56 (1H, br d, *J* 7.0, N*H*), 5.56 (1H, dt, *J* 15.0, 6.8, 5-*H*), 5.35 (1H, dt, *J* 15.0, 7.2, 4-*H*), 4.05–3.97 (1H, m, 2-*H*), 3.73 (2H, app. d, *J* 3.8, 1-H_2), 2.39–2.25 (2H, m, 3-H_2), 2.00 (2H, dd, *J* 13.8, 6.9, 6-H_2), 1.38–1.20 (22H, m, 7-H_2 to 17-H_2), 0.88 (3H, t, *J* 6.9, 18-H_3); δ_F (376 MHz, $CDCl_3$) −75.87 CF_3; *m/z* (ES^-) 378.4 $[M-H]^-$; (ES^+) 402.4 $[M+Na]^+$; HRMS (ES^+) found $[M+Na]^+$ 402.2603, $C_{20}H_{36}O_2N_1F_3{}^{23}Na_1$ requires M^+ 402.2608.



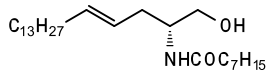
26az (*R*,*E*)-*N*-(1-hydroxyoctadec-4-en-2-yl)acetamide

Yield (88%); δ_H (700 MHz, $CDCl_3$) 5.82 (1H, br d, *J* 14.8, N*H*), 5.51 (1H, dt, *J* 15.0, 6.7, 5-*H*), 5.34 (1H, dt, *J* 15.1, 7.1, 4-*H*), 3.91 (1H, tdd, *J* 11.1, 7.2, 3.6, 2-*H*), 3.65 (1H, dd, *J* 11.0, 3.3, 1-H*H*), 3.58 (1H, dd, *J* 11.1, 5.7, 1-*H*H), 3.25 (1H, br s, O*H*), 2.27–2.22 (1H, m, 3-H*H*), 2.17 (1H, dt, *J* 14.1, 7.1, 3-*H*H), 1.98 (5H, m, 6-H_2 and COCH_3), 1.35–1.30 (2H, m, 7-H_2), 1.30–1.22 (20H, m, 8-H_2 to 17-H_2), 0.87 (3H, t, *J* 7.1, 18-H_3); *m/z* (ES^-) 324.4 $[M-H]^-$; (ES^+) 326.4 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 326.3046, $C_{20}H_{40}O_2N_1$ requires M^+ 326.3035.



26ay (*R*,*E*)-*N*-(1-hydroxyoctadec-4-en-2-yl)-2-phenylacetamide

Yield (47%); δ_H (700 MHz, $CDCl_3$) 7.36–7.33 (2H, m, Ar*H*), 7.30–7.28 (1H, m, Ar*H*), 7.25–7.22 (2H, m, Ar*H*), 5.58 (1H, br d, *J* 5.6, N*H*), 5.32 (1H, dt, *J* 14.7, 6.6, 5-*H*), 5.23–5.18 (1H, m, 4-*H*), 3.88 (1H, dtd, *J* 13.2, 6.5, 3.8, 2-*H*), 3.61 (1H, dd, *J* 11.1, 3.5, 1-*H*H), 3.58 (2H, s, PhCH_2), 3.54 (1H, dd, *J* 11.1, 6.2, 1-H*H*), 2.19–2.14 (1H, m, 3-*H*H), 2.09 – 2.04 (1H, m, 3-H*H*), 1.88 (2H, dd, *J* 13.2, 6.5, 6-H_2), 1.31–1.23 (22H, m, 7-H_2 to 17-H_2), 0.88 (3H, t, *J* 7.0, 18-H_3); *m/z* (ES^-) 400.5 $[M-H]^-$; (ES^+) 402.4 $[M+H]^+$; HRMS (ES^+) found $[M+H]^+$ 402.3356, $C_{26}H_{44}O_2N_1$ requires M^+ 402.3348.



26ax (*R*,*E*)-*N*-(1-hydroxyoctadec-4-en-2-yl)octanamide

Yield (76%); δ_H (700 MHz, $CDCl_3$) 5.71 (1 H, br s, N*H*), 5.52 (1 H, dt, *J* 14.8, 6.8, 5-*H*), 5.37–5.31 (1H, m, 4-*H*), 3.91 (1H, tdd, *J* 10.8, 6.9, 3.6, 2-*H*), 3.65 (1H, dd, *J* 10.9, 3.1, 1-*H*H), 3.58 (1H, dd, *J* 11.0, 6.0, 1-H*H*), 3.28 (1H, br s, O*H*), 2.26 (1H, dt, *J* 13.5, 6.6, 3-*H*H), 2.20–2.14 (3H, m, 3-H*H* and COCH_2), 1.99 (2H, dd, *J* 14.3, 7.1, 6-H_2), 1.60 (2H, dt, *J* 14.6, 7.4, $COCH_2CH_2$), 1.35–1.22 (30H, m, 7-H_2 to 17-H_2 and 4 × aliphatic CH_2), 0.87 (6H, t, *J* 7.1, 18-H_3 and terminal octanoyl CH_3); *m/z* (ES^-) 408.5 $[M-H]^-$; (ES^+) 410.5 $[M+Na]^+$; HRMS (ES^+) found $[M+H]^+$ 410.3999, $C_{26}H_{52}O_2N_1$ requires M^+ 410.3998.

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BIOLOGICAL METHODS

Preparation of the Screening Compounds

All compounds were first dissolved in MeOH and 1.0 mM stock solutions were prepared in DMSO/MeOH (9:1 v/v) and 0.20 mM working solutions in DMSO/MeOH (98:2). All prepared solutions were stored at -20 °C until use.

The Assay Protocol of the Inhibition Assays

The hydrophobic nature of most of the synthesised substrate analogues required special attention to ensure their solubility/dispersion in the reaction mixture. For each inhibition reaction, the donor substrate (PI) and test inhibitors were first dried into a reaction Eppendorf tube followed by addition of the reaction buffer, CHAPS and NBD-C_6-ceramide followed by sonication for 3 minutes and incubation at 30 °C for 15 minutes. The reaction was started by the addition of *Lmj*IPCS microsomes (0.6 u). Final concentrations of the test compound, PI and NBD-C_6-ceramide were 20 μM, 100 μM and 5 μM respectively. After 15 minutes the reaction was quenched by the addition of MeOH and the product sphingolipids separated from unreacted NBD-C6-ceramide by ion exchange chromatography.[19] The amount of fluorescent product was then quantified using a fluorescence plate reader. All reactions were done in triplicates with the inhibitory effect quantified by the change in the formation of the labelled product, NBD-C_6-IPC.

Mass Spectrometry Analyses

Analyses were performed on an LTQFT (ThermoFinnigan Corp); an FTICR MS instrument equipped with a 7.0 T magnet. A different assay protocol[20] was deployed to minimise CHAPS content using PI-depleted CHAPS-washed microsomal membranes.[21] The organic extracts were dried and re-suspended in chloroform and introduced into the electrospray ion source by direct infusion from a syringe at a flow rate 3 μl/min. Positive ion measurements were made with the source voltage at 4.0 kV and negative ion measurements were made with the source voltage at 3.5 kV. The tube lens was kept at 100 V and the source temperature at 275 °C for all experiments.

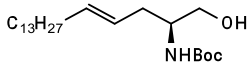
The spectra are presented below in the following format,

A: Full spectrum of the organic extract of the reaction mixture.

B: Expansion of the spectra showing the formation NBD-C_6-IPC.

C: Predicted response of the product that will hypothetically arise if the test compound functions as an alternative substrate.

D: Expansion of the spectra showing the region identified in C above.

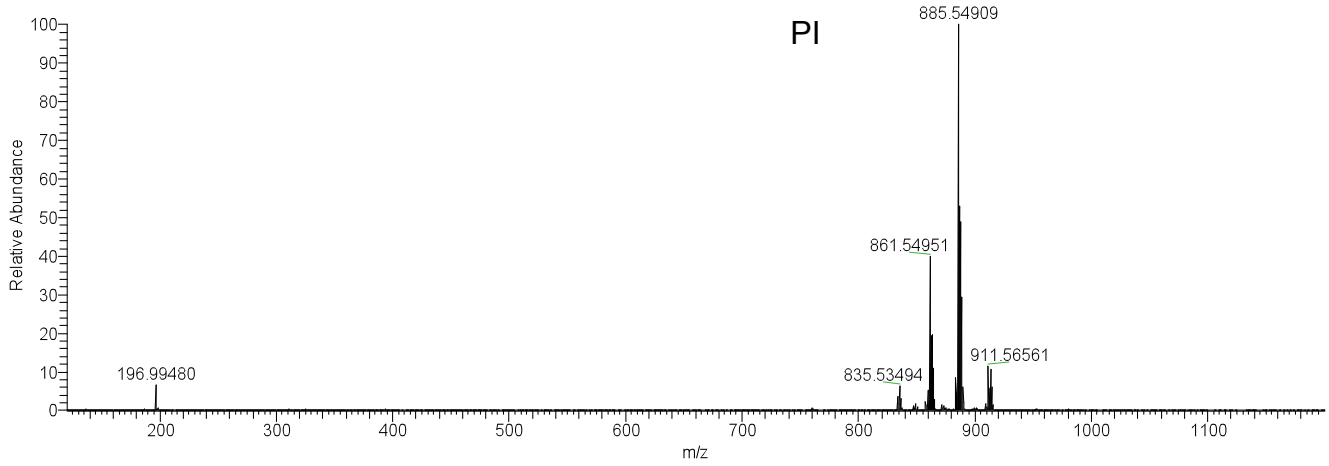


27a

Negative Ion Spectra

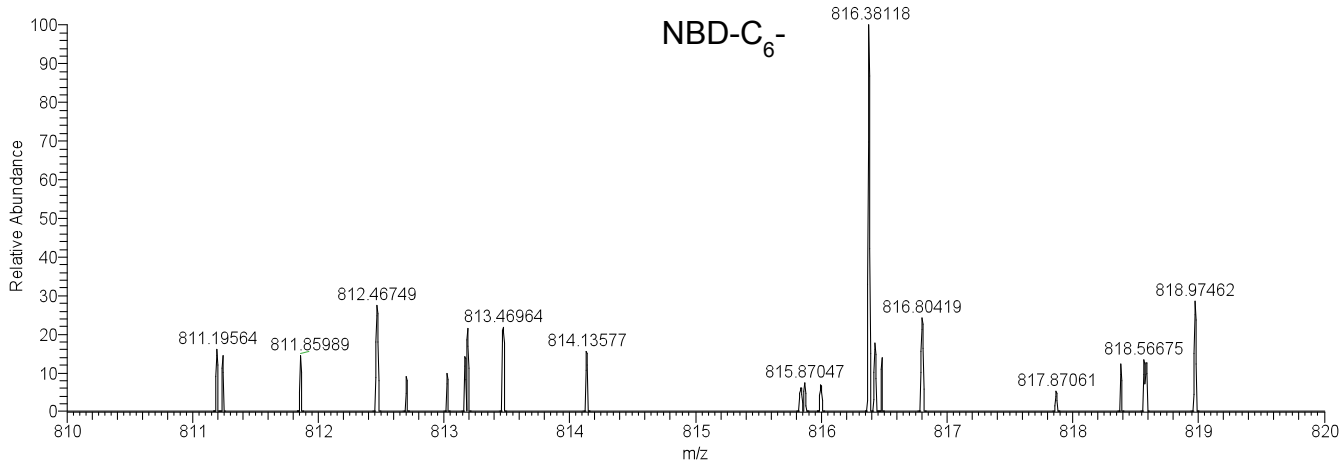
A

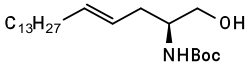
Full Spectrum



B

NBD-C_6-IPC: **Detected**



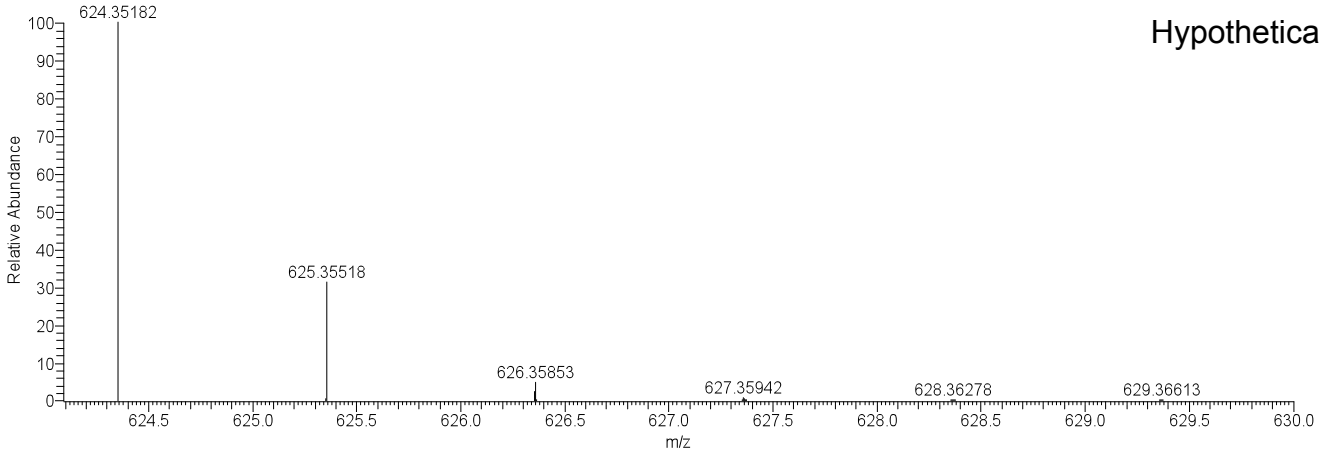


27a

Negative Ion Spectra

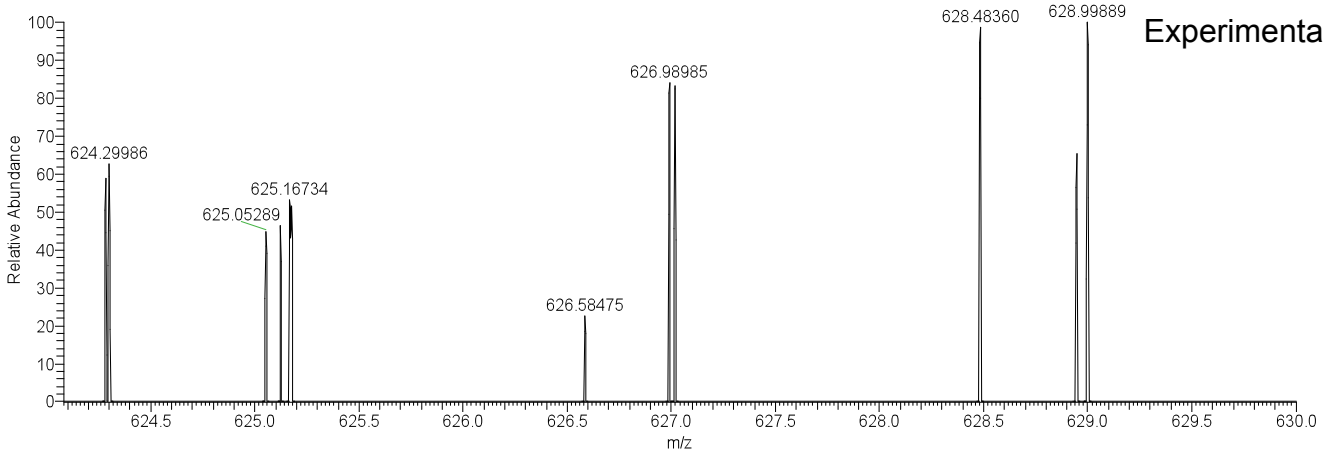
C

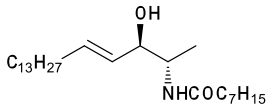
Peak $[M+241]^-$: 27a-Phosphoryl Inositol



D

27a-Phosphoryl Inositol: **Not Detected**



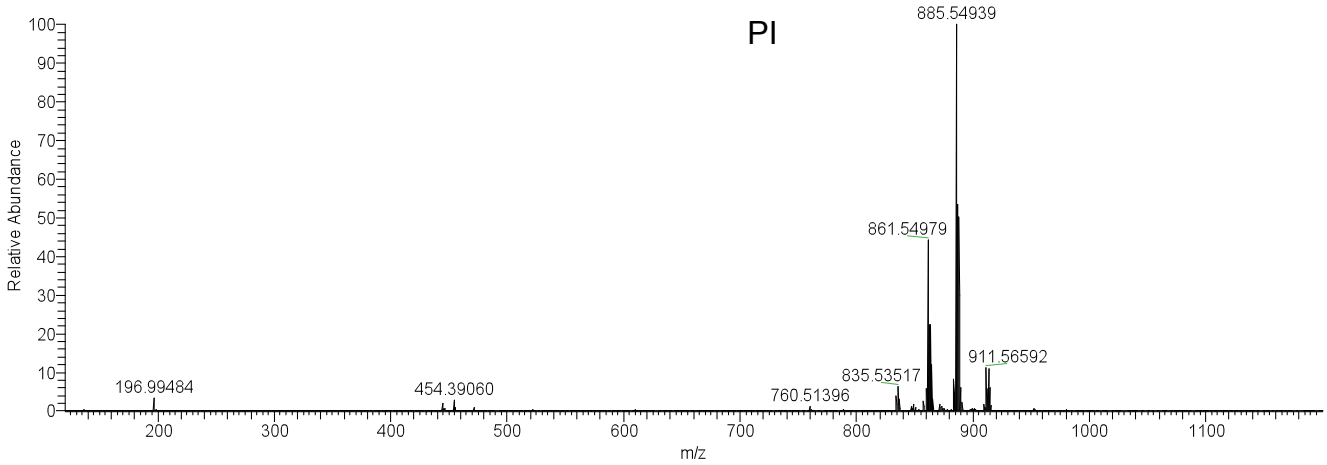


18ax

Negative Ion Spectra

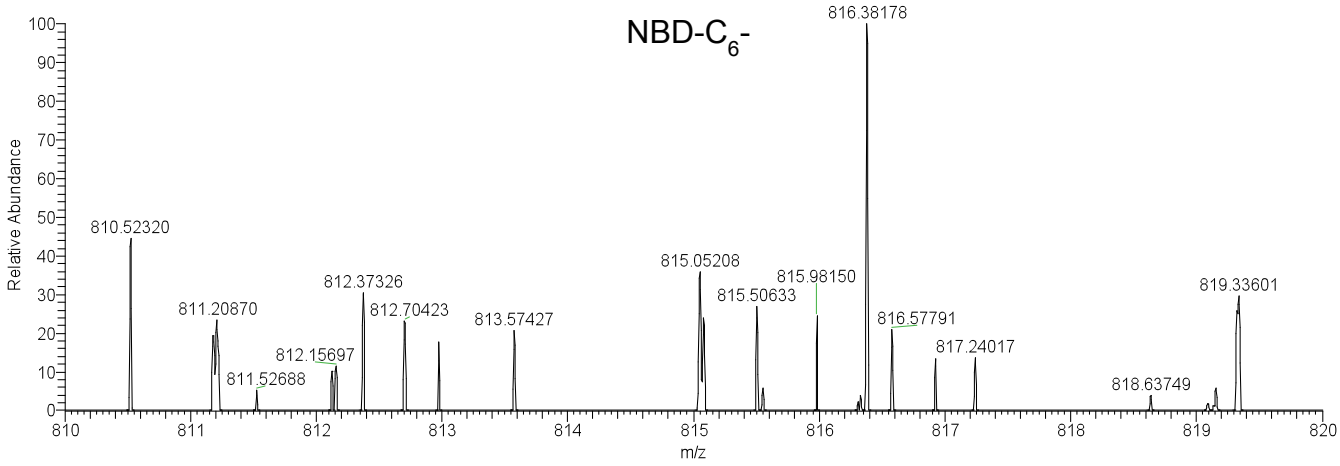
A

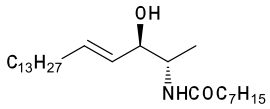
Full Spectrum



B

NBD-C_6-IPC: **Detected**



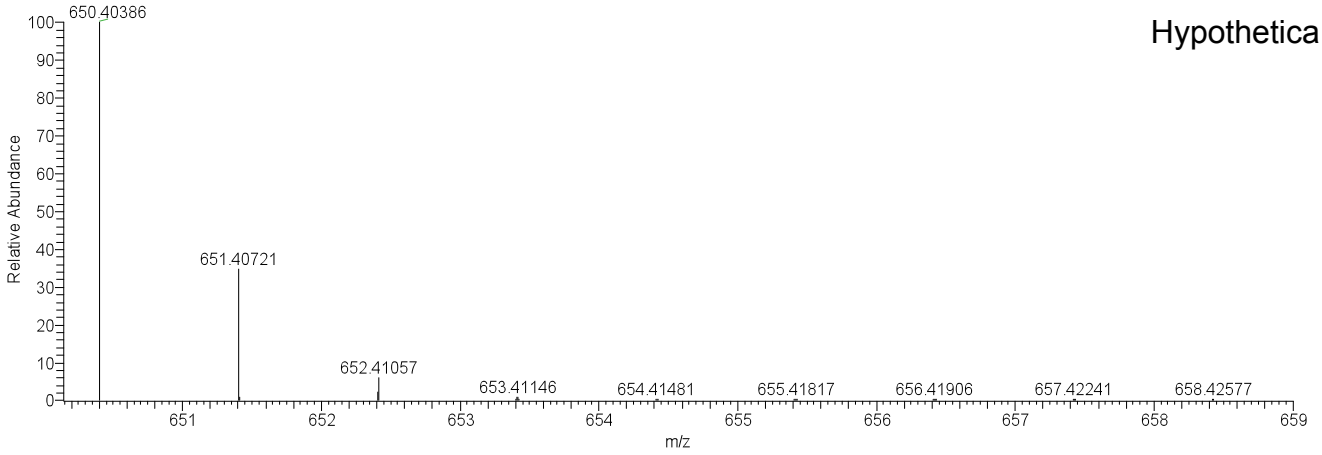


18ax

Negative Ion Spectra

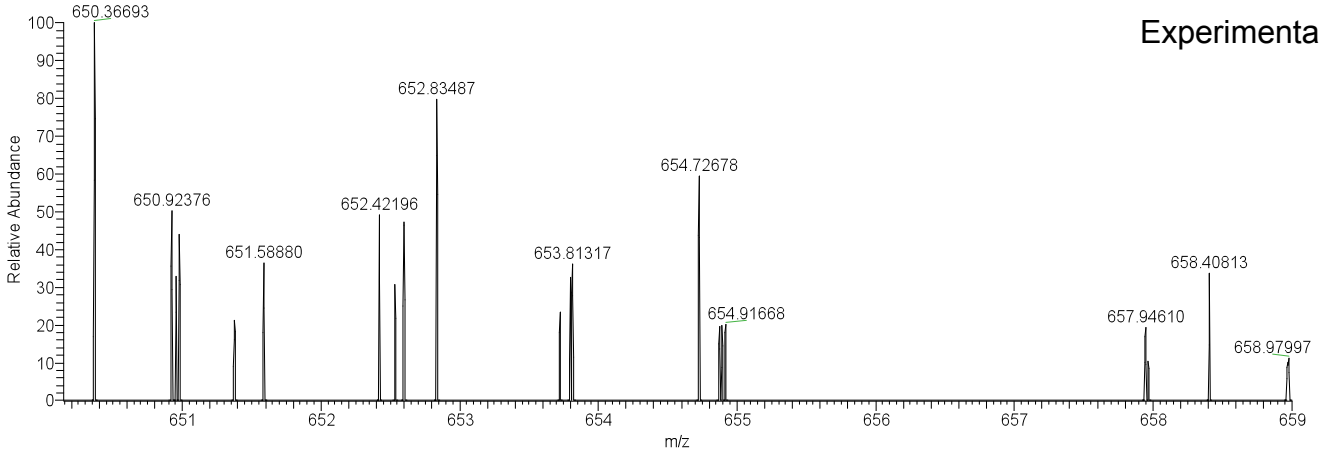
C

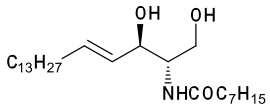
Peak $[M+241]^-$: 18ax-Phosphoryl Inositol



D

18ax-Phosphoryl Inositol: **Not Detected**



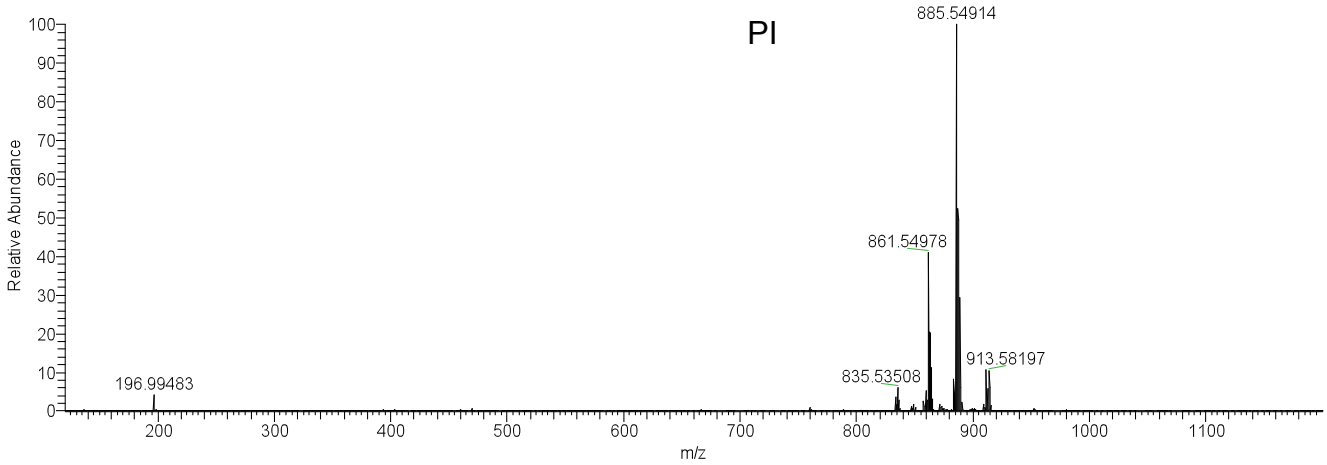


17ax

Negative Ion Spectra

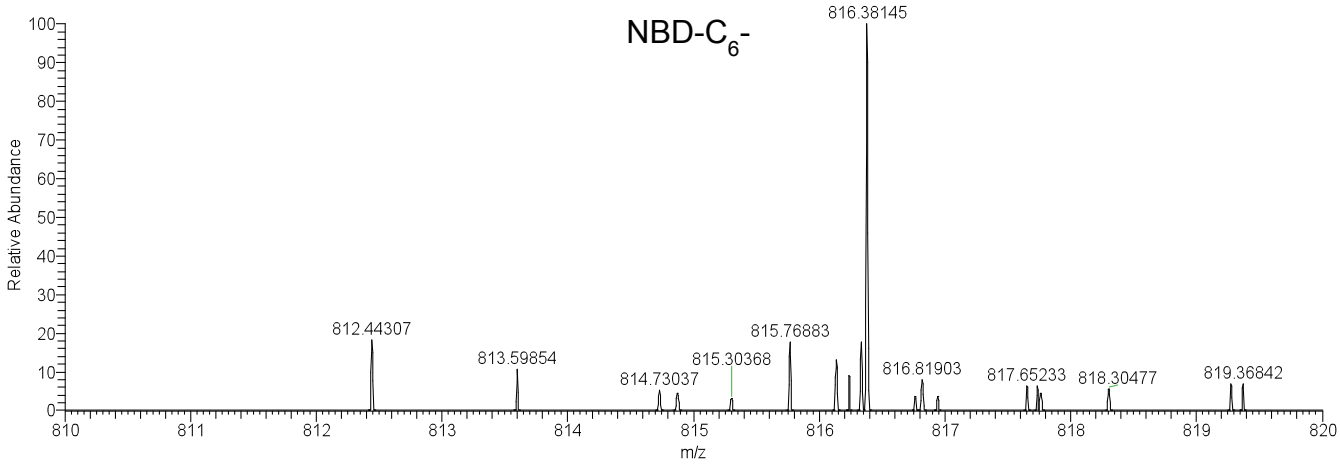
A

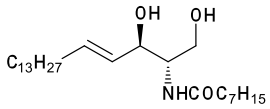
Full Spectrum



B

NBD-C_6-IPC: **Detected**





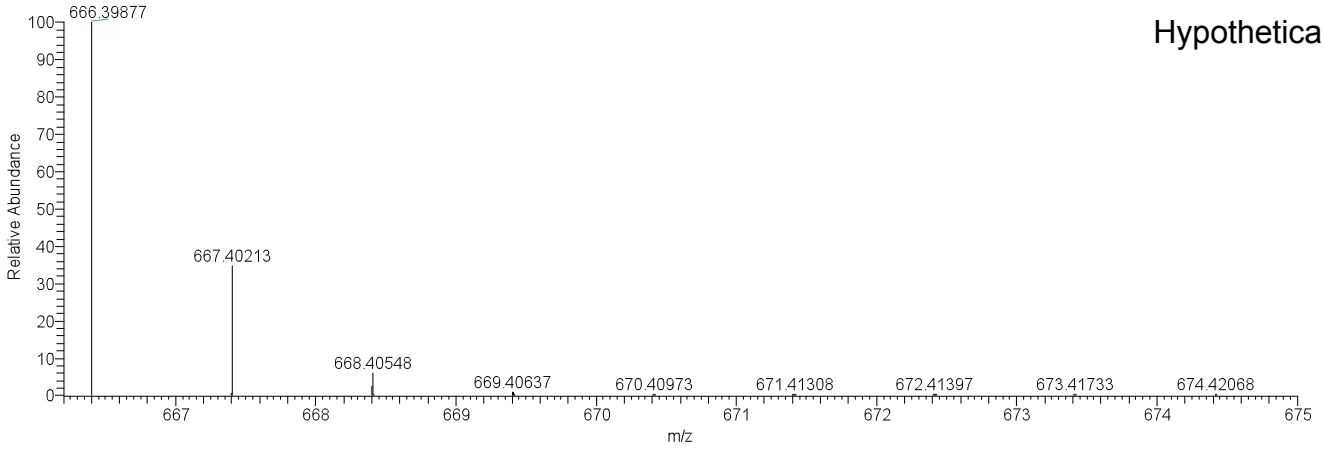
17ax

Negative Ion Spectra

C

Peak [M+241]$^-$: 17ax-Phosphoryl Inositol

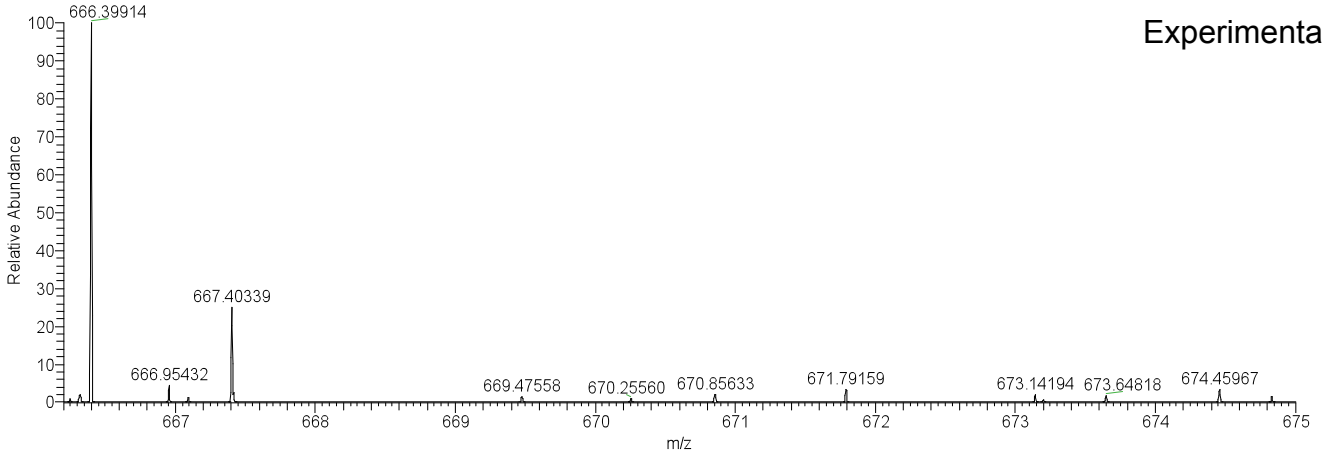
Hypothetical

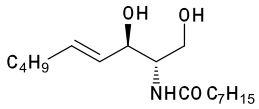


D

17ax-Phosphoryl Inositol: **Detected**

Experimenta



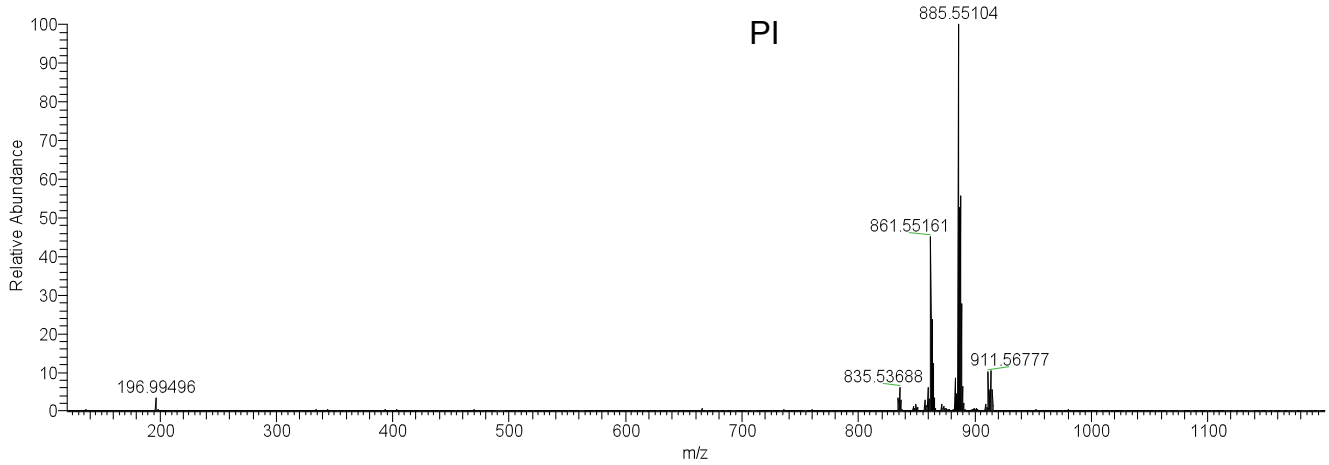


17bx

Negative Ion Spectra

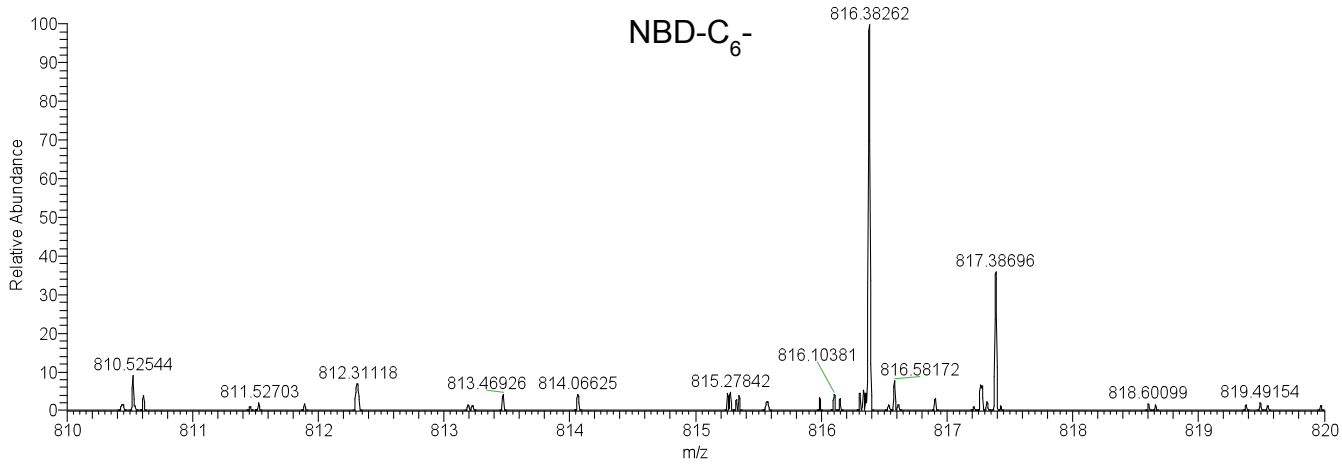
A

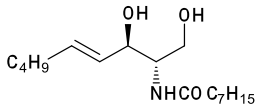
Full Spectrum



B

NBD-C_6-IPC: **Detected**



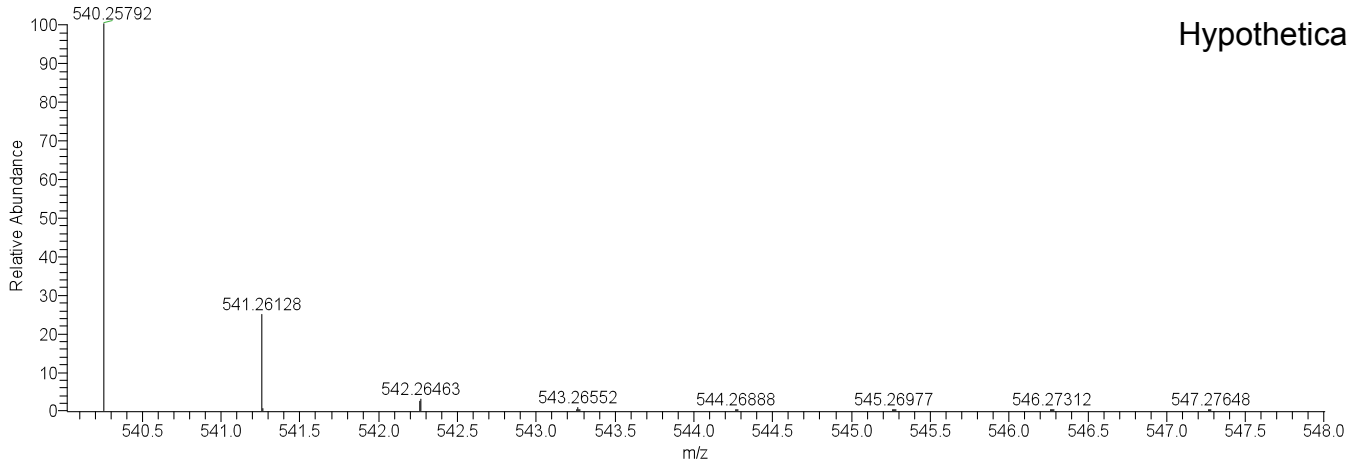


17bx

Negative Ion Spectra

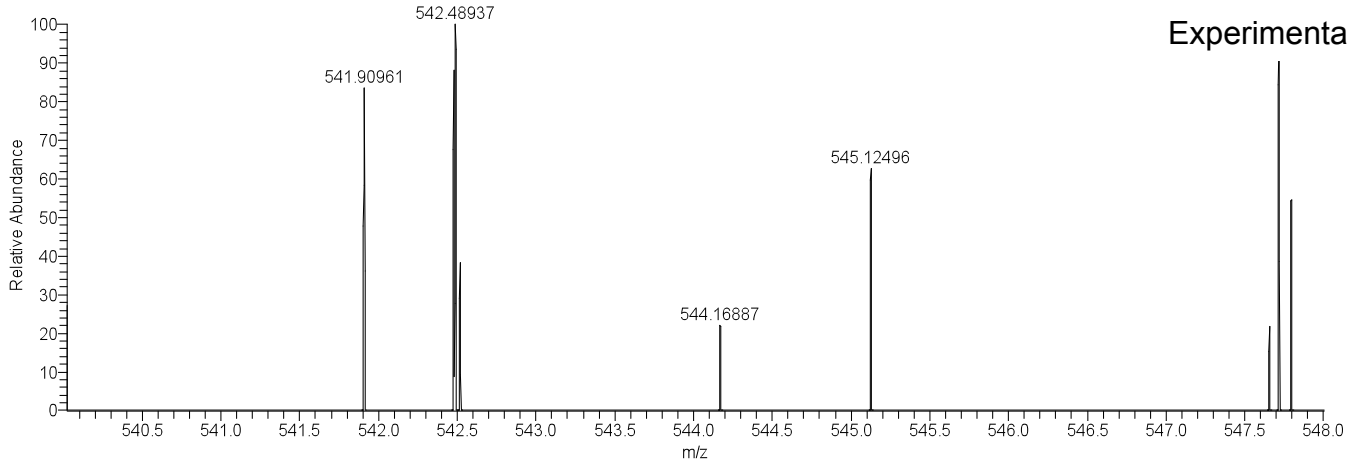
C

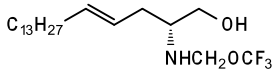
Peak [M+241]⁻ : 18bx-Phosphoryl Inositol



D

18bx-Phosphoryl Inositol: **Not Detected**



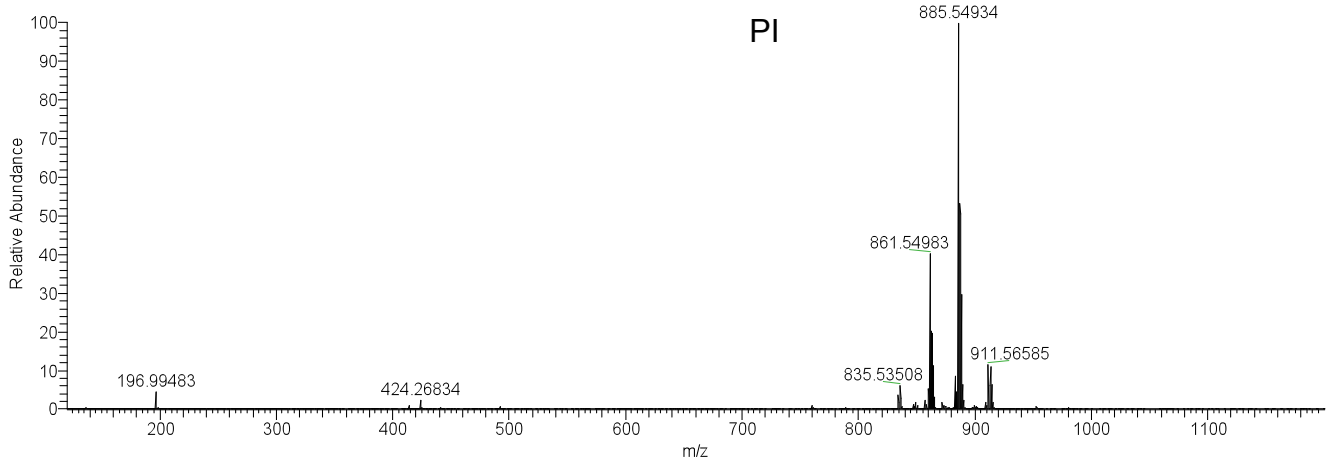


26af

Negative Ion Spectra

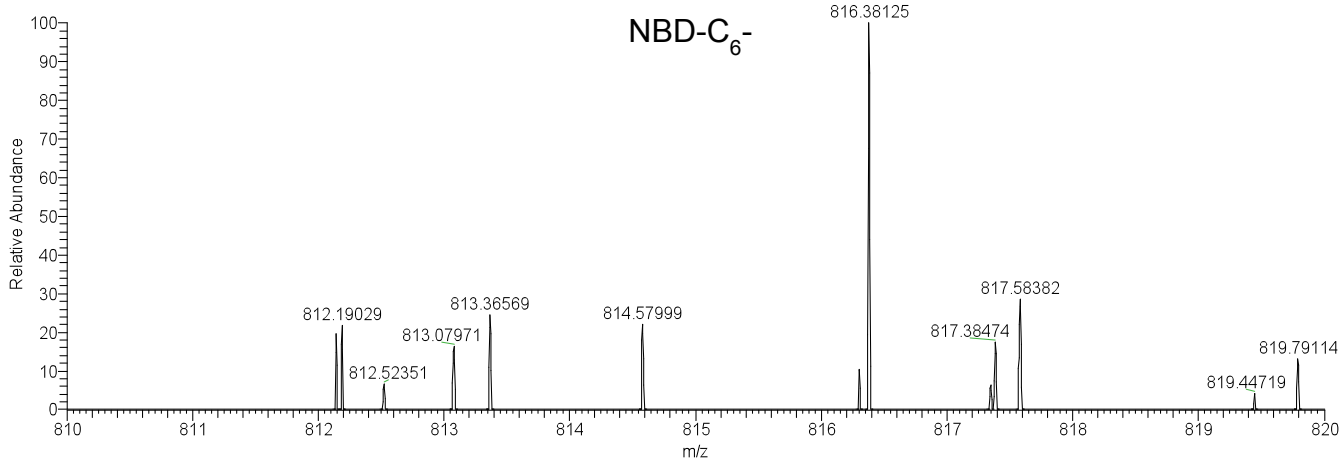
A

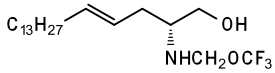
Full Spectrum



B

NBD-C_6-IPC: **Detected**



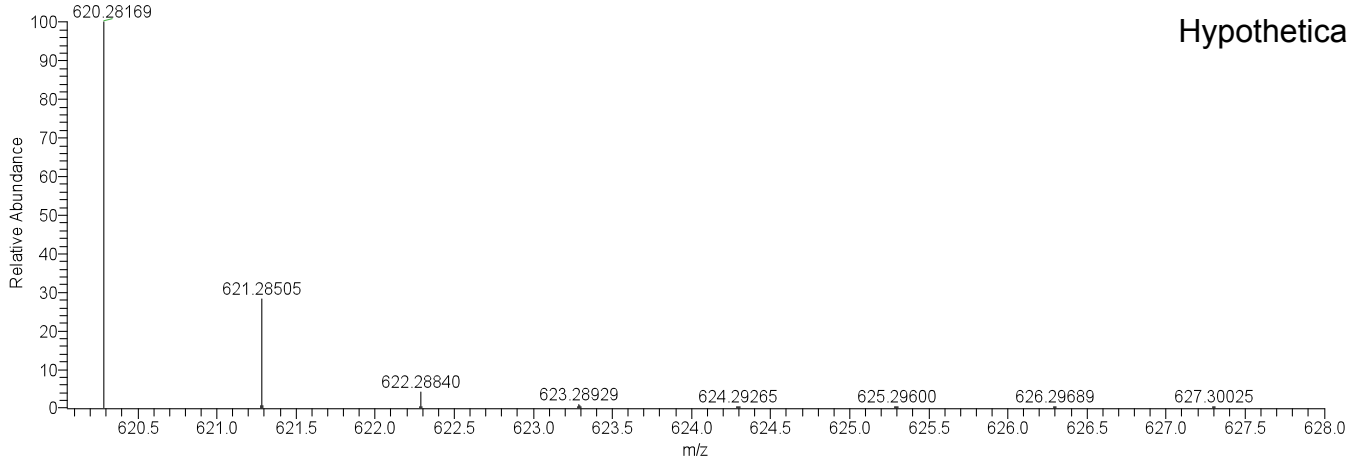


26af

Negative Ion Spectra

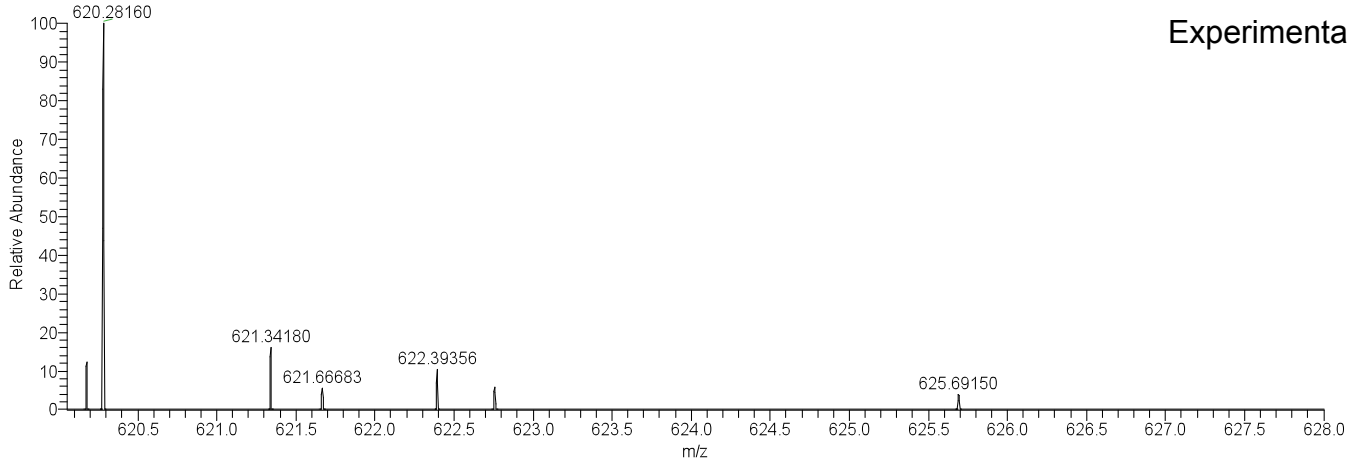
C

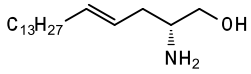
Peak [M+241]⁻ : 18ax-Phosphoryl Inositol



D

18ax-Phosphoryl Inositol: **Detected**



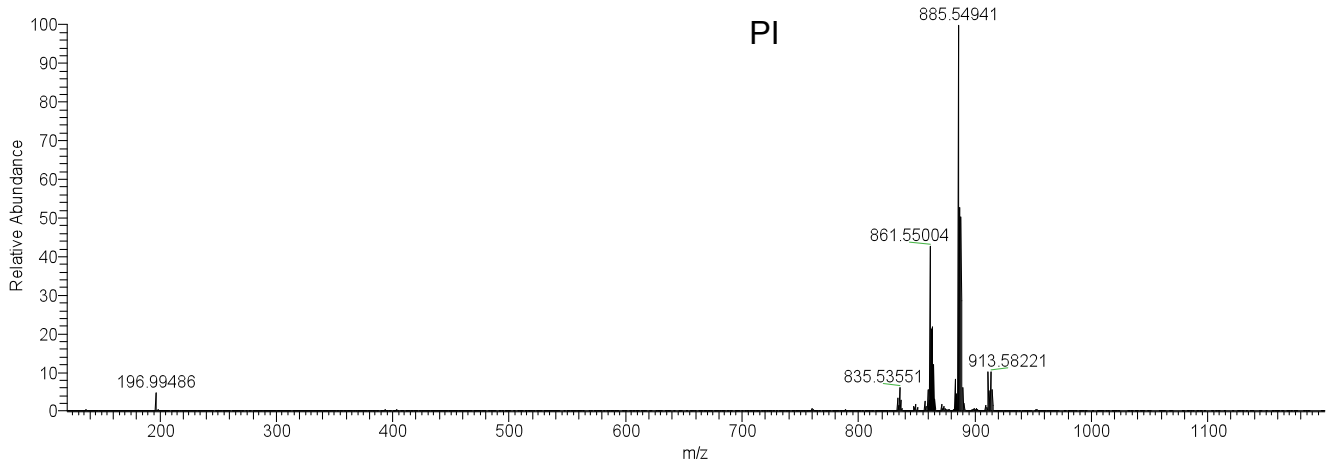


26an

Negative Ion Spectra

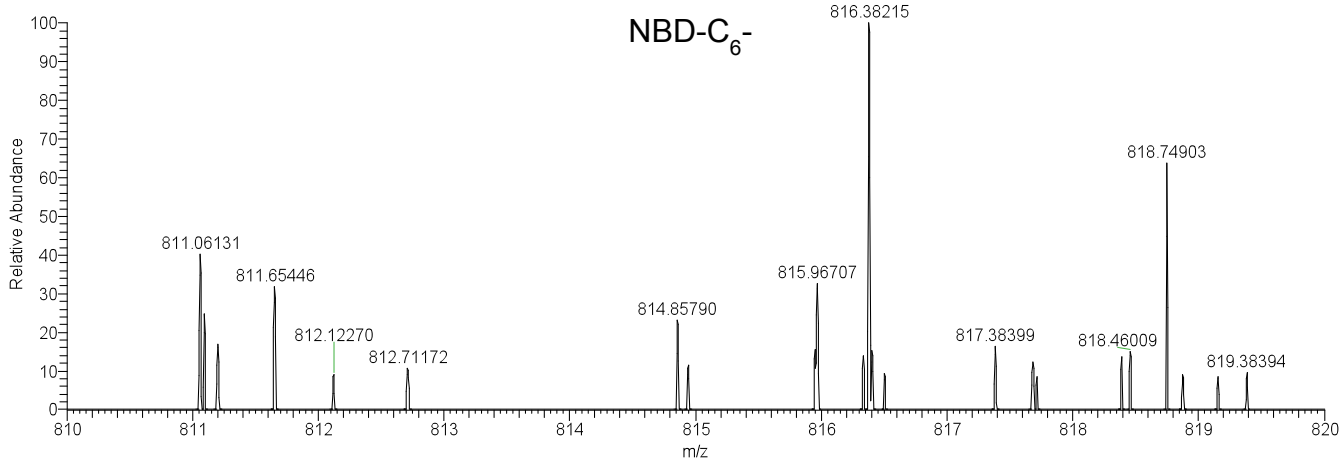
A

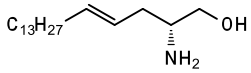
Full Spectrum



B

NBD-C_6-IPC: **Detected**



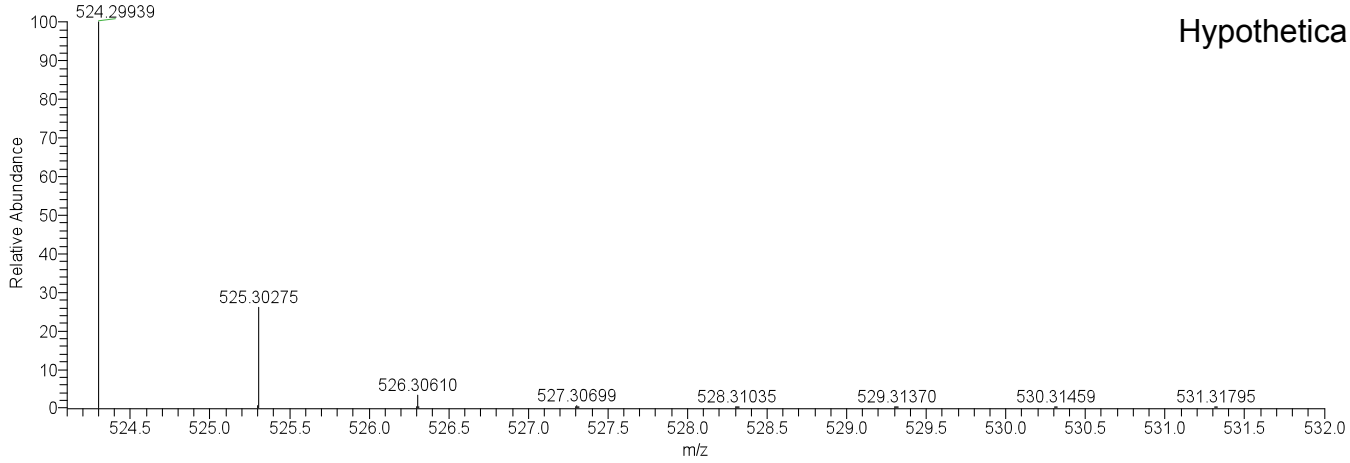


26an

Negative Ion Spectra

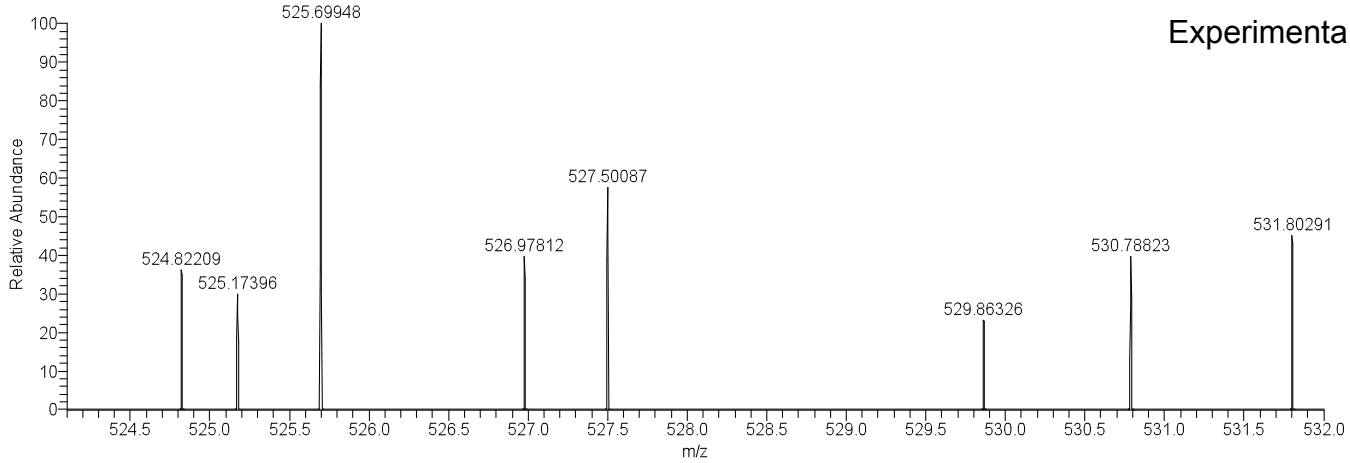
C

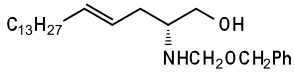
Peak $[M+241]^-$: 26an-Phosphoryl Inositol



D

26an-Phosphoryl Inositol: **Not Detected**



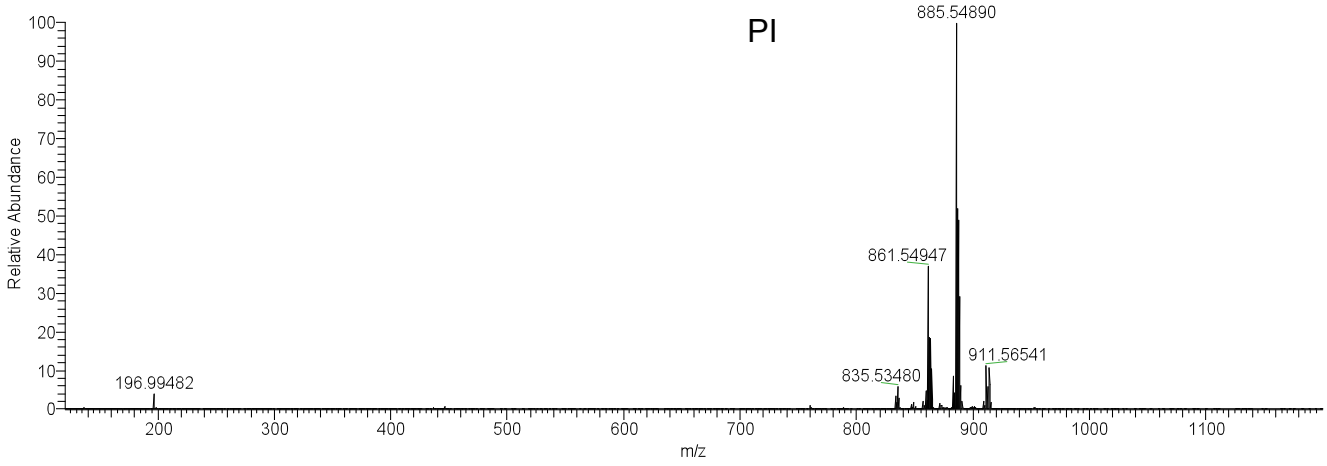


26ay

Negative Ion Spectra

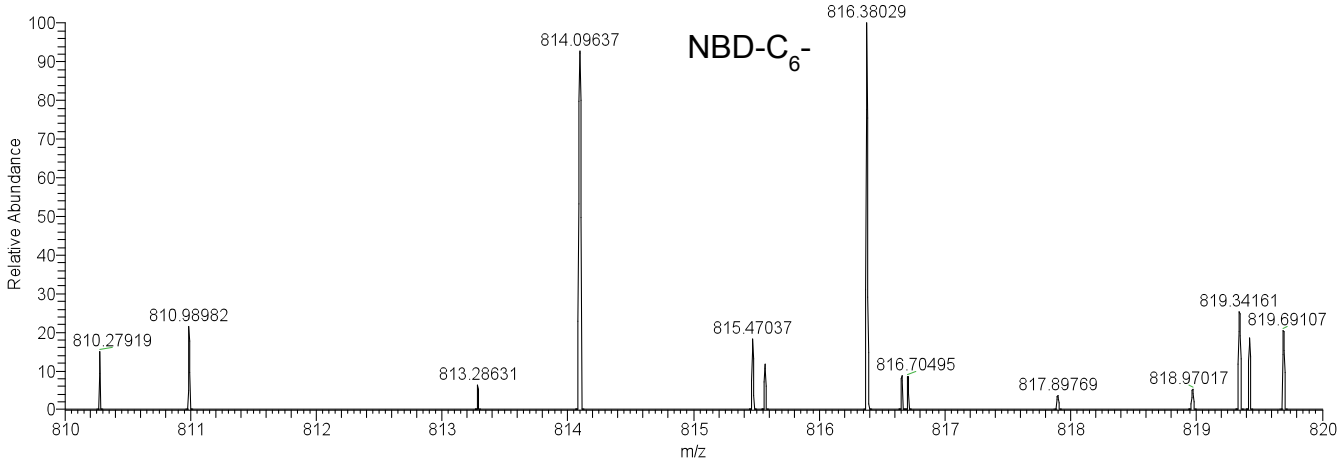
A

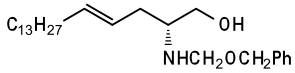
Full Spectrum



B

NBD-C_6-IPC: **Detected**



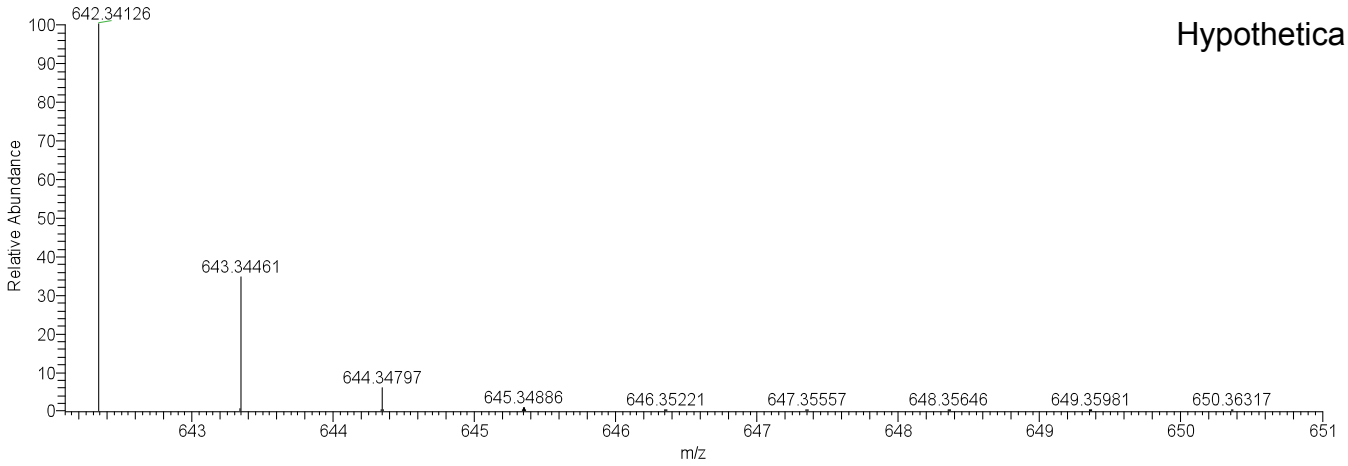


26ay

Negative Ion Spectra

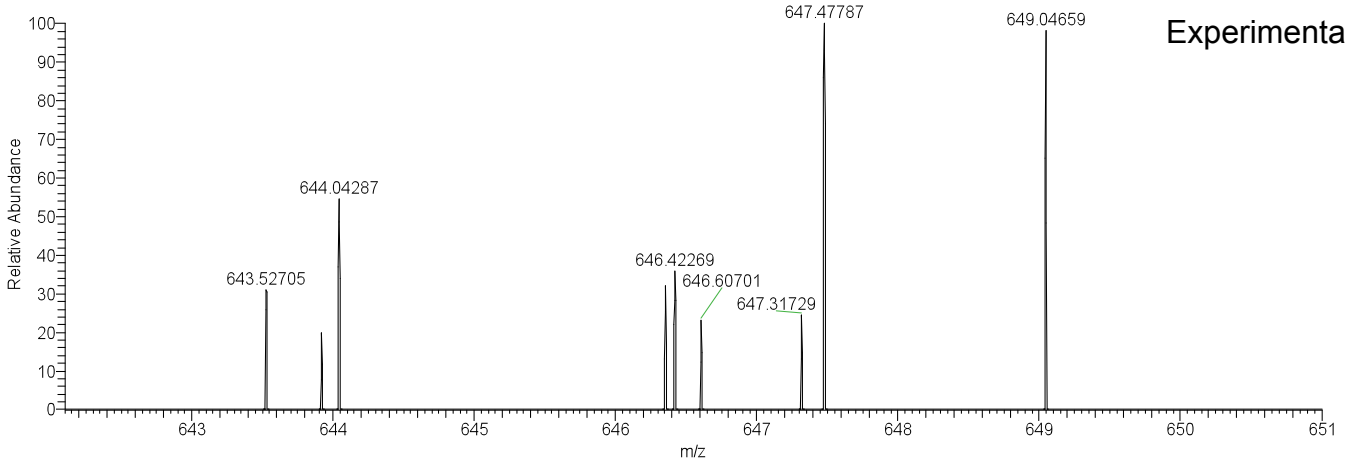
C

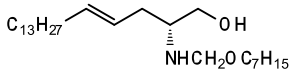
Peak [M+241]⁻ : 26ay-Phosphoryl Inositol



D

26ay-Phosphoryl Inositol: **Not Detected**



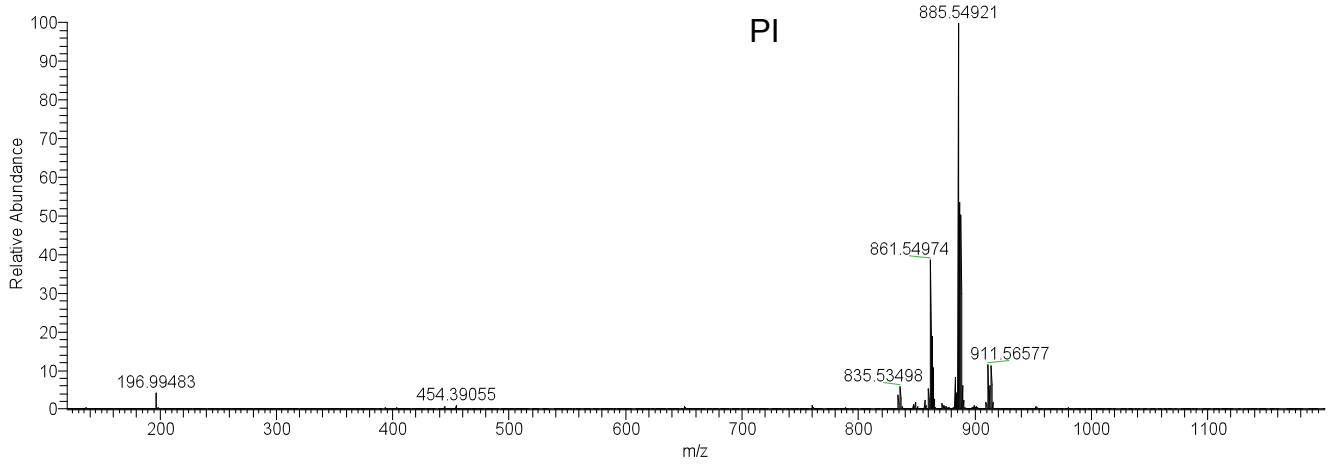


26ax

Negative Ion Spectra

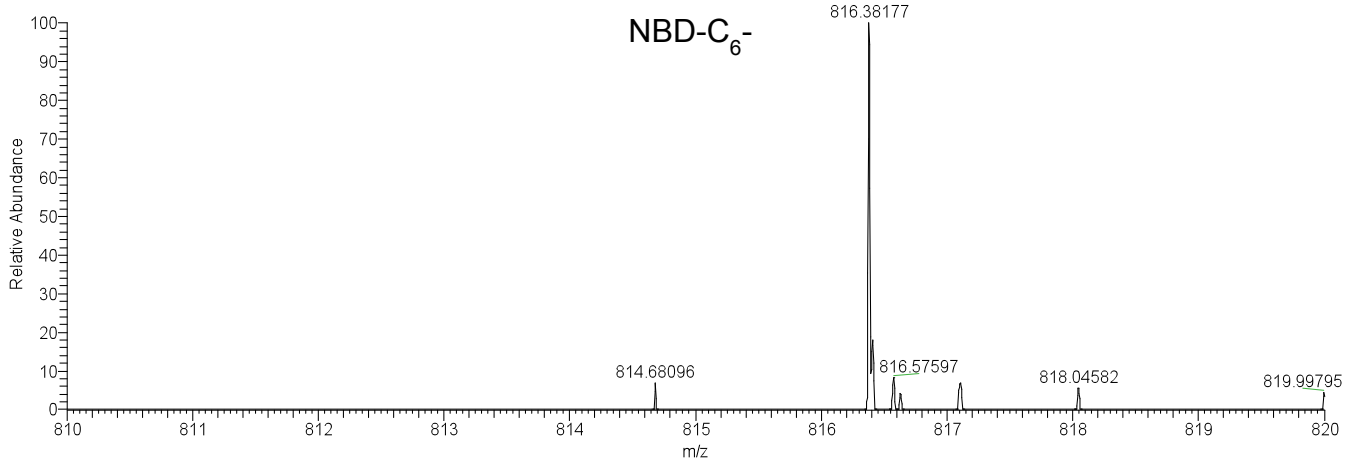
A

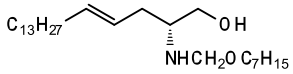
Full Spectrum



B

NBD-C_6-IPC: **Detected**



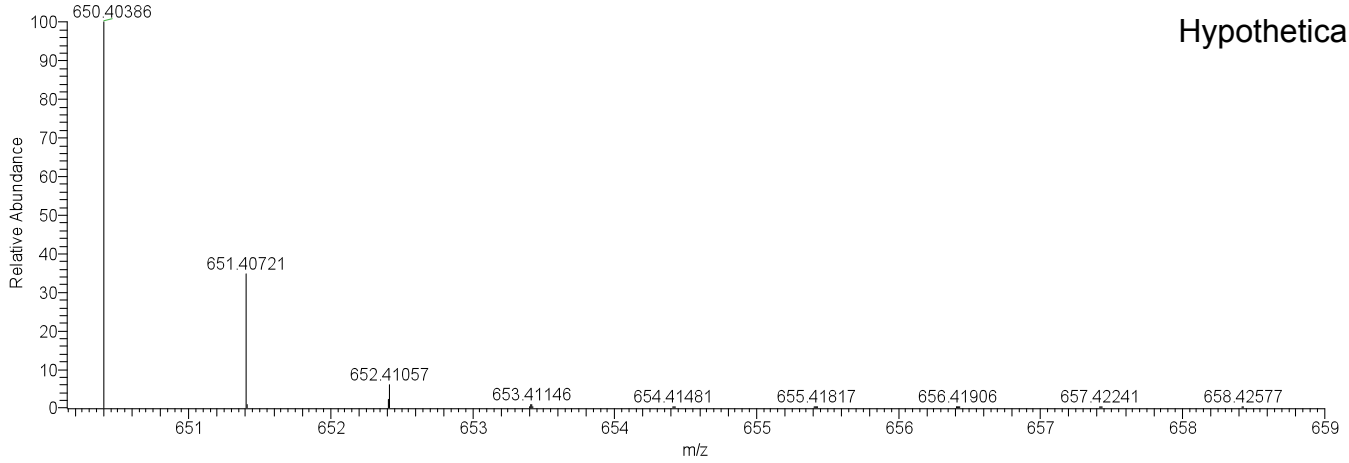


26ax

Negative Ion Spectra

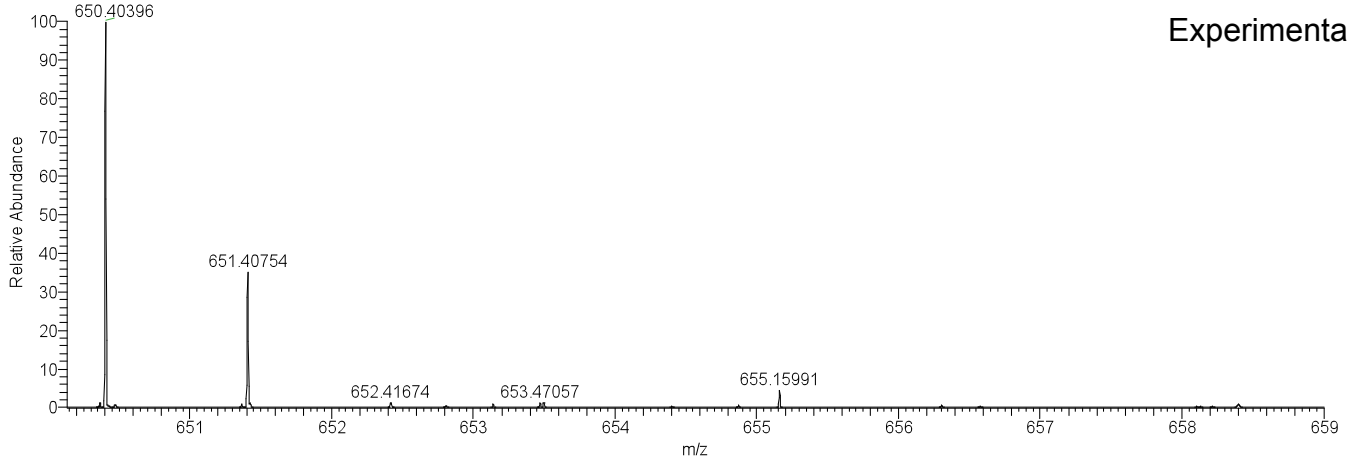
C

Peak [M+241]⁻ : 26ax-Phosphoryl Inositol



D

26ax-Phosphoryl Inositol: **Detected**



Cytotoxicity screening

L. major (MHOM/IL/81/Friedlin) promatigotes parasites were maintained at 26 ◦C in Schneider's Drosophila media (Sigma–Aldrich) supplemented with 15% heat inactivated foetal bovine sera (Biosera).

In 96-well plates (Nunc) parasites at $4x10^5$ ml^{-1} were incubated with compounds in triplicate (including miltefosine (Cayman Chemical) as a positive control, and untreated parasites and media as negative controls) for 24 h before incubation with Alamar Blue (Invitrogen) for 4 h prior to assessing cell viability using a fluorescent plate reader (Biotek; 560EX nm/600EMnm).

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