Molecular basis of fatty acid selectivity in the zDHHC family of S-acyltransferases revealed by click chemistry

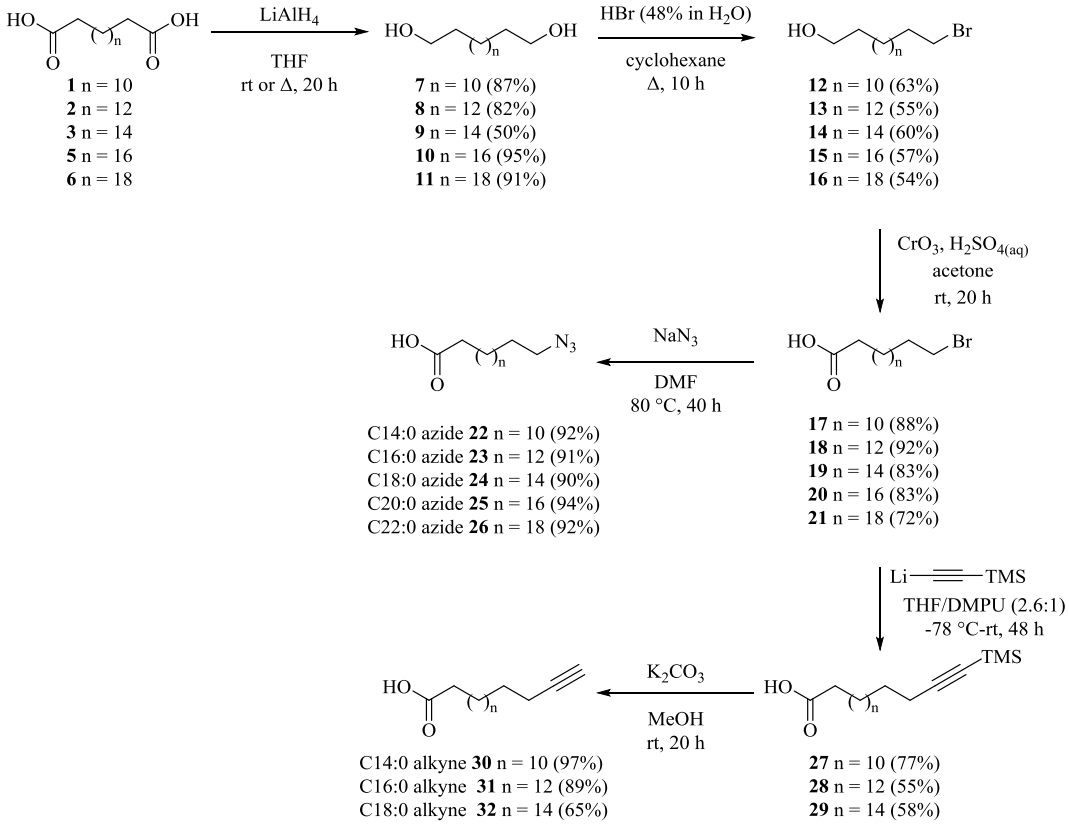
Greaves et al

SUPPLEMENTARY INFORMATION APPENDIX

General Experimental Details

Unless otherwise stated, all commercially available reagents were used as supplied without any further purification. ^{n}BuLi was purchased as a 2.5 M solution, and the solution was titrated with diphenylacetic acid prior to use. Dry THF was used directly from a PureSolv MD 5 Solvent Purification System by Innovative Technology Inc., and handled under inert atmosphere. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone) (DMPU) was dried by heating to reflux over calcium hydride and distilling under vacuum before being purged with, and stored under N_2 over 4 Å molecular sieves. Flash chromatography was carried out using Merck Kieselgel 60 H silica. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254} that were visualised using *p*-anisaldehyde. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz Ultrashield Magnet, Prodigy liquid nitrogen cryoprobe, AVIII console and a Z420 HP workstation running TopSpin 3.X running at 400 MHz (^{1}H NMR) and 101 MHz (^{13}C NMR); an 500 MHz Ascend magnet, BBO multi nuc' Smart probe, AVIIIHD500 console and Z420 HP workstation running TopSpin 3.X running at 500 MHz (^{1}H NMR) and 126 MHz (^{13}C NMR) or a 600 MHz Ultrashield magnet, BBO multi nuc' probe, AVII+ console and a Z420 HP workstation running TopSpin 3.X running at 600 MHz (^{1}H NMR) or 151 MHz (^{13}C NMR). Chemical shifts are reported in parts per million (ppm) in the scale relative to $CDCl_3$, 7.26 ppm for ^{1}H NMR and 77.16 for ^{13}C NMR; DMSO-d_6, 2.50 ppm for ^{1}H NMR and 39.52 for ^{13}C NMR. Coupling constants are measured in Hertz (Hz). Low-resolution mass spectra (LRMS) were recorded on an Agilent 6130 single quadrupole with APCI/ESI dual source, on a ThermoQuest Finnigan LCQ DUO electrospray, or on an Agilent 7890A GC system, equipped with a 30 m DB5MS column connected to a 5975C inert XL CI MSD with Triple-Axis Detector. MALDI were performed on an Axima-CFR from Kratos-Shimadzu. High-resolution mass spectra (HRMS) were obtained courtesy of the EPSRC National Mass Spectrometry Facility at Swansea University, UK. Infrared spectra were recorded on an Agilent 5500a FTIR equipped with ATR (Attenuated Total Reflectance) and were reported in cm^{-1}. *In vacuo* refers to evaporation under reduced pressure using a rotary evaporator connected to a diaphragm pump, followed by the removal of trace volatiles using a high vacuum (oil) pump. Melting points were determined with a Gallenkamp SG92 melting point apparatus and are uncorrected.

Preparation of fatty acid azide and alkyne chemical probes



1 n = 10
2 n = 12
3 n = 14
5 n = 16
6 n = 18

7 n = 10 (87%)
8 n = 12 (82%)
9 n = 14 (50%)
10 n = 16 (95%)
11 n = 18 (91%)

12 n = 10 (63%)
13 n = 12 (55%)
14 n = 14 (60%)
15 n = 16 (57%)
16 n = 18 (54%)

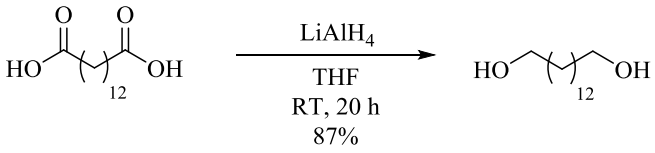
17 n = 10 (88%)
18 n = 12 (92%)
19 n = 14 (83%)
20 n = 16 (83%)
21 n = 18 (72%)

C14:0 azide **22** n = 10 (92%)
C16:0 azide **23** n = 12 (91%)
C18:0 azide **24** n = 14 (90%)
C20:0 azide **25** n = 16 (94%)
C22:0 azide **26** n = 18 (92%)

27 n = 10 (77%)
28 n = 12 (55%)
29 n = 14 (58%)

C14:0 alkyne **30** n = 10 (97%)
C16:0 alkyne **31** n = 12 (89%)
C18:0 alkyne **32** n = 14 (65%)

Preparation of 1,14-tetradecanediol 7(1)



Reaction performed under a N_2 atmosphere.

Solid $LiAlH_4$ (1.47 g, 38.7 mmol) was added to a solution of 1,14-tetradecanedioic acid (5.00 g, 19.35 mmol) in THF (194 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred at room temperature for 20 h. Upon completion, wet $NaSO_4$ was added portion-wise until the grey suspension turned white. The suspension was stirred at room temperature until the white solid was free-flowing, and solid $MgSO_4$ was added. The reaction was filtered and the filter cake washed with Et_2O (5 × 50 mL). The solvent was evaporated *in vacuo* to afford the product (3.88 g, 87%) as a white solid.

δ_H (600 MHz, DMSO-d_6) 4.29 (t, 2H, *J* 5.0 Hz, 2 × O*H*), 3.35–3.38 (m, 4H, 2 × C*H*$_2$OH), 1.36–1.42 (m, 4H, 2 × C*H*$_2$CH$_2$OH), 1.22–1.29 (m, 20H, 10 × C*H*$_2$).

δ_C (151 MHz, DMSO-d_6) 60.7, 32.5, 29.1, 29.0, 29.0, 28.9, 25.5.

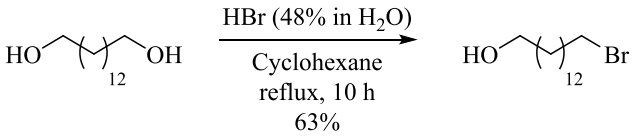
LR-MS (MALDI-TOF) 253.2 ($[M+Na]^+$)

HR-MS calcd for $C_{14}H_{31}O_2^+$ ($[M+H]^+$) 231.2318, found 231.2318.

υ_{max} (thin film, cm^{-1}) 3410, 3351, 2921, 2891, 2850.

Mp 88–90 °C (lit. 87–89 °C)(1)

Preparation of 14-bromotetradecan-1-ol 12(2, 3)



HBr (48% in H_2O, 41 mL) was added to a suspension of diol **7** (3.57 g, 15.5 mmol) in cyclohexane (41 mL). The biphasic mixture was heated to reflux for 10 h before being cooled to room temperature. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 30 mL). The combined organics were washed with $NaHCO_3$ (4 × 20 mL of a saturated aqueous solution), brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 95:5 petrol/EtOAc then 70:30 petrol/EtOAc, to afford the product (2.85 g, 63%) as a pale yellow solid.

δ_H (400 MHz, $CDCl_3$) 3.64 (t, 2H, *J* 6.6 Hz, C*H*$_2$OH), 3.40 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 1.80–1.90 (m, 2H, C*H*$_2$CH$_2$Br), 1.53–1.60 (m, 2H, C*H*$_2$CH$_2$OH), 1.25–1.46 (m, 20H, 10 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 63.2, 34.2, 33.0, 33.0, 29.7, 29.7, 29.6, 28.3, 28.9.

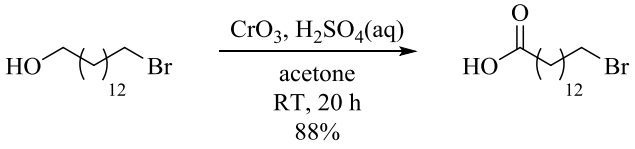
LR-MS (EI+) 294.9 ([M(^{81}Br)+H]$^+$), 292.9 ([M(^{79}Br)+H]$^+$), 276.9 ([M(^{81}Br)-H_2O+H]$^+$), 292.9 ([M(^{79}Br)-H_2O+H]$^+$), 213.9 ([M-Br+H]$^+$).

HR-MS calcd for $C_{14}H_{33}ON^{79}Br^+$ ([M+NH_4]$^+$) 310.1740, found 310.1744.

υ_{max} (thin film, cm^{-1}) 3274, 2919, 2850.

Mp 46–48 °C (lit. 42–43 °C)(3)

Preparation of 14-bromotetradecanoic acid 17(4, 5)



CrO_3 (3.89 g, 38.92 mmol) was dissolved in concentrated H_2SO_4 (7.2 mL). Cold H_2O (16.2 mL) was added slowly and the solution stirred at room temperature for 10 min. The resulting solution was added drop-wise to a solution of alcohol **12** (2.85 g, 9.73 mmol) in acetone (243 mL). The reaction was stirred at room temperature for 20 h before being H_2O (100 mL) and CH_2Cl_2 (40 mL) were added. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 30 mL). The combined organics were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (2.63 g, 88%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.41 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 2.35 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.81–1.89 (m, 2H, C*H*$_2$CH$_2$Br), 1.59–1.68 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.24–1.45 (m, 18H, 9 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 179.0, 34.2, 34.0, 33.0, 29.7, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8.

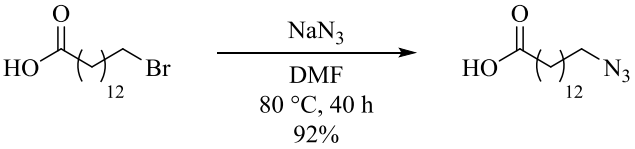
LR-MS (EI+) 309.0 ([M(^{81}Br)+H]$^+$), 307.0 ([M(^{79}Br)+H]$^+$), 291.0 ([M(^{81}Br)-H_2O+H]$^+$), 289.0 ([M(^{79}Br)-H_2O+H]$^+$), 227.1 ([M-Br+H]$^+$).

HR-MS calcd for $C_{14}H_{26}O^{79}Br^-$ ([M-H]$^-$) 305.1122, found 305.1123.

υ_{max} (thin film, cm^{-1}) 3036, 2917, 2852, 1696

Mp 63–66 °C (lit. 64–65 °C)(5)

Preparation of 14-azidotetradecanoic acid 22(4, 6)



Reaction performed under a N_2 atmosphere

NaN_3 (636 mg, 9.78 mmol) was added to a solution of bromide **17** (500 mg, 1.63 mmol) in DMF (6.5 mL). The reaction was stirred at 80 °C for 40 h before being cooled to room temperature. A 1:1 mixture of EtOAc/H_2O (20 mL) was added, the layers separated and the aqueous phase extracted with EtOAc (3 × 10 mL). The combined organics were washed with brine (10 mL), dried

over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (403 mg, 92%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.25 (t, 2H, *J* 7.0 Hz, CH_2N_3), 2.34 (t, 2H, *J* 7.5 Hz, CH_2CO_2H), 1.55–1.68 (m, 4H, $CH_2CH_2N_3$, $CH_2CH_2CO_2H$), 1.23–1.38 (m, 18H, 9 × CH_2).

δ_C (101 MHz, $CDCl_3$) 180.1, 51.6, 34.2, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 26.9, 24.8.

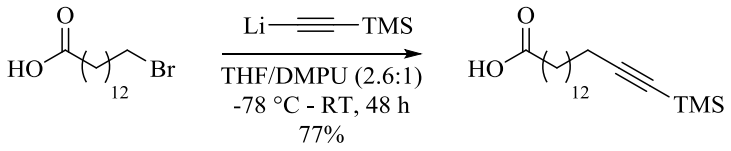
LR-MS (ES-) 268.1 ($[M-H]^-$), 240.1 ($[M-N_2-H]^-$).

HR-MS calcd for $C_{14}H_{26}N_3O_2^-$ ($[M-H]^-$) 268.2031, found 268.2028.

υ_{max} (thin film, cm^{-1}) 3016, 2915, 2848, 2101, 1701.

Mp 38–40 °C.

Preparation of 16-(trimethylsilyl)hexadec-15-ynoic acid 27



Reaction performed under a N_2 atmosphere.

^{n}BuLi (2.19 M, 7.46 mL, 16.3 mmol) was added drop-wise to a solution of (trimethylsilyl)acetylene (1.6 g, 2.3 mL, 16.3 mmol) in THF (10 mL) at -78 °C. The reaction was stirred at -78 °C for 1 h before DMPU (18 mL) was added and the reaction stirred for a further 30 min. A solution of bromide **17** (500 mg, 1.63 mmol) in THF (37 mL) was added drop-wise and the reaction stirred at room temperature for 48 h. Upon completion, the reaction was quenched with HCl (20 mL of a 1 M solution). The layers were separated and the aqueous phase extracted with Et_2O (4 × 20 mL). The combined organics were washed with brine (3 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (407 mg, 77%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 2.34 (t, 2H, *J* 7.5 Hz, CH_2CO_2H), 2.20 (t, 2H, *J* 7.2 Hz, $CH_2C{\equiv}CSi$), 1.58–1.67 (m, 2H, $CH_2CH_2CO_2H$), 1.46–1.55 (m, 2H, $CH_2CH_2C{\equiv}CSi$), 1.25–1.39 (m, 18H, 9 × CH_2), 0.14 (s, 9H, $Si(CH_3)_3$).

δ_C (101 MHz, $CDCl_3$) 180.3, 107.9, 84.4, 34.2, 29.7, 29.6, 29.6, 29.4, 29.2, 28.9, 28.8, 24.8, 20.0, 0.3.

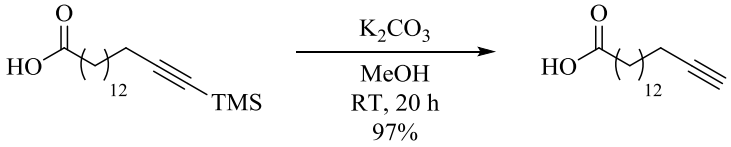
LR-MS (ES-) 323.2 ($[M-H]^-$).

HR-MS calcd for $C_{19}H_{35}O_2Si^-$ ($[M-H]^-$) 323.2412, found 323.2408.

υ_{max} (thin film, cm^{-1}) 2939, 2921, 2852, 2178, 1714, 1699.

Mp 37–40 °C

Preparation of hexadec-15-ynoic acid 30(7, 8)



K_2CO_3 (285 mg, 2.06 mmol) was added to a solution of TMS-alkyne **27** (268 mg, 0.82 mmol) in methanol (8.2 mL) at room temperature. The reaction was stirred at room temperature for 20 h before the solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc (15 mL) and HCl (15 mL of a 1 M aqueous solution) added. The layers were separated and the aqueous phase extracted with EtOAc (3 × 20 mL). The combined organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (200 mg, 97%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 2.18 (td, 2H, *J* 7.1 Hz, ^4J 2.7 Hz, C*H*$_2$C≡CH), 1.93 (t, 1H, ^4J 2.7 Hz, C≡C*H*), 1.58–1.67 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.47–1.55 (m, 2H, C*H*$_2$CH$_2$C≡CH), 1.25–1.43 (m, 18H, 9 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 180.3, 85.0, 68.2, 34.2, 29.7, 29.6, 29.6, 29.4, 29.2, 29.2, 28.9, 28.6, 24.8, 18.5.

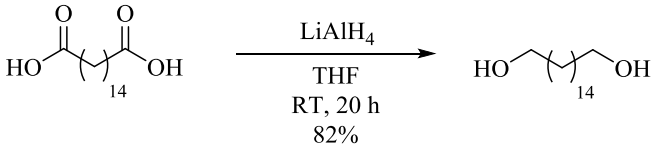
LR-MS (ES-) 251.2 ([M-H]$^-$).

HR-MS calcd for $C_{16}H_{27}O_2^-$ ([M-H]$^-$) 251.2017, found 251.2017.

υ_{max} (thin film, cm^{-1}) 3285, 3042, 2917, 2852, 2115, 1694.

Mp 59–61 °C.

Preparation of 1,16-hexadecanediol 8(9)



Reaction performed under a N_2 atmosphere.

Solid $LiAlH_4$ (1.33 g, 34.9 mmol) was added to a solution of 1,16-hexadecanedioic acid (5.00 g, 17.46 mmol) in THF (175 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred at room temperature for 20 h. Upon completion, wet $NaSO_4$ was added portion-wise until the grey suspension turned white. The suspension was stirred at room temperature until the white solid was free-flowing, and solid $MgSO_4$ was added. The reaction was filtered and the filter cake washed with Et_2O (5 × 50 mL). The solvent was evaporated *in vacuo* to afford the product (3.68 g, 82%) as a white solid.

δ_H (600 MHz, DMSO-d_6) 4.29 (t, 2H, *J* 4.9 Hz, 2 × O*H*), 3.34–3.39 (m, 4H, 2 × C*H*$_2$OH), 1.36–1.42 (m, 4H, 2 × C*H*$_2$CH$_2$OH), 1.22–1.29 (m, 24H, 12 × C*H*$_2$).

δ_C (151 MHz, DMSO-d_6) 60.7, 32.5, 29.1, 29.0, 29.0, 29.0, 28.9, 25.5.

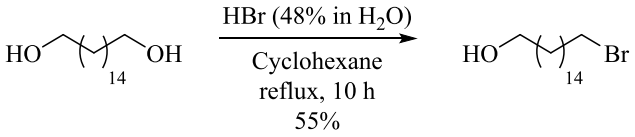
LR-MS (MALDI-TOF) 281.3 ([M+Na]$^+$), 297.3 ([M+K]$^+$).

HR-MS calcd for $C_{16}H_{35}O_2^+$ ([M+H]$^+$) 259.2632, found 259.2632.

υ_{max} (thin film, cm^{-1}) 3414, 3353, 2919, 2891, 2848.

Mp 91-94 °C (lit. 93–94 °C)(9)

Preparation of 16-bromohexadecan-1-ol 13(10, 11)



HBr (48% in H_2O, 36 mL) was added to a suspension of diol **8** (3.48 g, 13.5 mmol) in cyclohexane (36 mL). The biphasic mixture was heated to reflux for 10 h before being cooled to room temperature. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 30 mL). The combined organics were washed with $NaHCO_3$ (4 × 20 mL of a saturated aqueous solution), brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 95:5 petrol/EtOAc then 70:30 petrol/EtOAc, to afford the product (2.40 g, 55%) as a pale yellow solid.

δ_H (400 MHz, $CDCl_3$) 3.64 (t, 2H, *J* 6.6 Hz, C*H*$_2$OH), 3.41 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 1.80–1.91 (m, 2H, C*H*$_2$CH$_2$Br), 1.52–1.62 (m, 2H, C*H*$_2$CH$_2$OH), 1.25–1.46 (m, 24H, 12 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 63.3, 34.2, 33.0, 33.0, 29.8, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9.

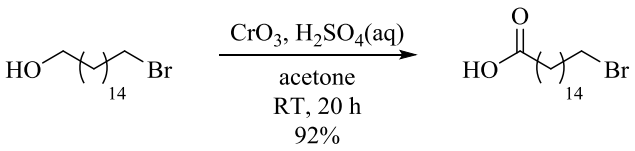
LR-MS (EI+) 320.9 ([M(^{81}Br)+H]$^+$), 318.9 ([M(^{79}Br)+H]$^+$), 304.9 ([M(^{81}Br)-H$_2$O+H]$^+$), 302.9 ([M(^{79}Br)-H$_2$O+H]$^+$), 241.8 ([M-Br+H]$^+$).

HR-MS calcd for $C_{16}H_{37}{}^{79}BrON^+$ ([M+NH$_4$]$^+$) 338.2053, found 338.2056.

υ_{max} (thin film, cm^{-1}) 3274, 2917, 2850.

Mp 54-56 °C (lit. 54–56 °C)(12)

Preparation of 16-bromohexadecanoic acid 18(13)



CrO_3 (3.00 g, 30.0 mmol) was dissolved in concentrated H_2SO_4 (5.5 mL). Cold H_2O (12.5 mL) was added slowly and the solution stirred at room temperature for 10 min. The resulting solution was added drop-wise to a solution of alcohol **13** (2.40 g, 7.50 mmol) in acetone (188 mL). The reaction was stirred at room temperature for 20 h before being H_2O (100 mL) and CH_2Cl_2 (40 mL) were added. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 30 mL). The combined organics were washed with brine (30 mL), dried over $MgSO_4$, filtered and

concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (2.30 g, 92%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.41 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 2.35 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.80–1.90 (m, 2H, C*H*$_2$CH$_2$Br), 1.59–1.68 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.48 (m, 22H, 11 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 178.6, 34.2, 33.9, 33.0, 29.8, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8.

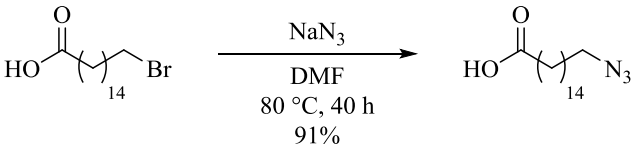
LR-MS (EI+) 337.0 ([M(^{81}Br)+H]$^+$), 335.0 ([M(^{79}Br)+H]$^+$), 317.0 ([M(^{81}Br)-H_2O+H]$^+$), 315.0 ([M(^{79}Br)-H_2O+H]$^+$), 257.1 ([M-Br+H]$^+$), 237.1 ([M-Br-H_2O+H]$^+$).

HR-MS calcd for $C_{16}H_{30}{}^{79}BrO^-$ ([M-H]$^-$) 333.1435, found 333.1430.

υ_{max} (thin film, cm^{-1}) 3034, 2917, 2850, 1696

Mp 72-74 °C (lit. 71 °C)(11)

Preparation of 16-azidohexadecanoic acid 23(6)



Reaction performed under a N_2 atmosphere

NaN_3 (582 mg, 8.96 mmol) was added to a solution of bromide **18** (500 mg, 1.49 mmol) in DMF (6 mL). The reaction was stirred at 80 °C for 40 h before being cooled to room temperature. A 1:1 mixture of EtOAc/H_2O (20 mL) was added, the layers separated and the aqueous phase extracted with EtOAc (3 × 10 mL). The combined organics were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (402 mg, 91%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.25 (t, 2H, *J* 7.0 Hz, C*H*$_2$N$_3$), 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.55–1.67 (m, 4H, C*H*$_2$CH$_2$N$_3$, C*H*$_2$CH$_2$CO$_2$H), 1.24–1.40 (m, 22H, 11 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 180.0, 51.7, 34.2, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 29.2, 29.2, 29.0, 26.9, 24.8.

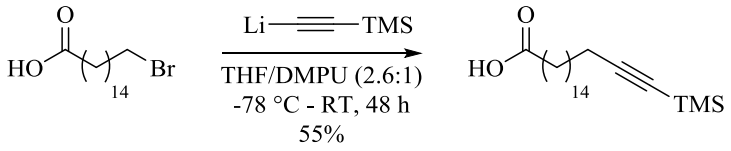
LR-MS (ES-) 268.2 ([M-N_2-H]$^-$), 296.2 ([M-H]$^-$).

HR-MS calcd for $C_{16}H_{32}NO_2^+$ ([M-N_2+H]$^+$) 270.2440, found 270.2433.

υ_{max} (thin film, cm^{-1}) 3014, 2915, 2848, 2103, 1701.

Mp 48–50 °C

Preparation of 18-(trimethylsilyl)octadec-17-ynoic acid 28



Reaction performed under a N_2 atmosphere.

^{n}BuLi (2.09 M, 3.6 mL, 7.46 mmol) was added drop-wise to a solution of (trimethylsilyl)acetylene (773 mg, 1.05 mL, 7.46 mmol) in THF (4.6 mL) at -78 °C. The reaction was stirred at -78 °C for 1 h before DMPU (8.3 mL) was added and the reaction stirred for a further 30 min. A solution of bromide **18** (500 mg, 1.49 mmol) in THF (16.7 mL) was added drop-wise and the reaction stirred at room temperature for 48 h. Upon completion, the reaction was quenched with HCl (20 mL of a 1 M solution). The layers were separated and the aqueous phase extracted with Et_2O (4 × 20 mL). The combined organics were washed with brine (3 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (288 mg, 55%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 2.20 (t, 2H, *J* 7.2 Hz, C*H*$_2$C≡CSi), 1.58–1.69 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.46–1.56 (m, 2H, C*H*$_2$CH$_2$C≡CSi), 1.24–1.40 (m, 22H, 11 × C*H*$_2$), 0.14 (s, 9H, Si(C*H*$_3$)$_3$).

δ_C (101 MHz, $CDCl_3$) 179.8, 108.0, 84.4, 34.1, 29.8, 29.7, 29.6, 29.6, 29.4, 29.2, 29.0, 28.8, 24.8, 20.0, 0.3.

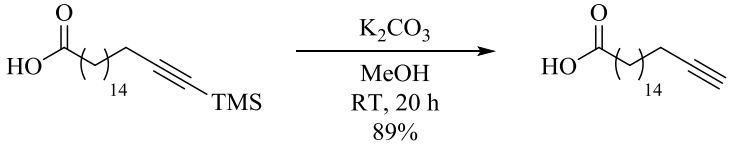
LR-MS (ES-) 351.3 ([M-H]$^-$).

HR-MS calcd for $C_{21}H_{39}O_2Si^-$ ([M-H]$^-$) 251.2725, found 351.2718.

υ_{max} (thin film, cm^{-1}) 2937, 2919, 2850, 2178, 1714, 1699.

Mp 50–53 °C

Preparation of octadec-17-ynoic acid 31(14)



K_2CO_3 (223 mg, 1.61 mmol) was added to a solution of TMS-alkyne **28** (228 mg, 0.65 mmol) in methanol (6.5 mL) at room temperature. The reaction was stirred at room temperature for 20 h before the solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc (15 mL) and HCl (15 mL of a 1 M aqueous solution) added. The layers were separated and the aqueous phase extracted with EtOAc (3 × 20 mL). The combined organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (161 mg, 89%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 2.18 (td, 2H, *J* 7.1 Hz, ^{4}*J* 2.6 Hz, C*H*$_2$C≡CH), 1.93 (t, 1H, ^{4}*J* 2.6 Hz, C≡C*H*), 1.59–1.67 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.48–1.56 (m, 2H, C*H*$_2$CH$_2$C≡CH), 1.24–1.44 (m, 22H, 11 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 180.1, 85.0, 68.2, 34.2, 29.8, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 28.9, 28.7, 28.4, 18.5.

LR-MS (ES-) 279.2 ($[M-H]^-$).

HR-MS calcd for $C_{18}H_{31}O_2^-$ ($[M-H]^-$) 279.2330, found 279.2331.

υ_{max} (thin film, cm^{-1}) 3284, 3042, 2917, 2850, 2115, 1694.

Mp 70–72 °C

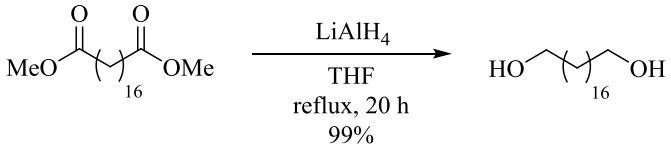
Preparation of 1,18-octadecanediol 9(15-17)

Method A

Reaction performed under a N_2 atmosphere.

Solid $LiAlH_4$ (1.21 g, 31.8 mmol) was added to a solution of 1,18-octadecanedioic acid (5.00 g, 15.9 mmol) in THF (160 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred at room temperature for 20 h. Upon completion, wet $NaSO_4$ was added portion-wise until the grey suspension turned white. The suspension was stirred at room temperature until the white solid was free-flowing, and solid $MgSO_4$ was added. The reaction was filtered and the filter cake washed with Et_2O (5 × 50 mL). The solvent was evaporated *in vacuo* to afford the product (2.26 g, 50%) as a white solid.

Method B



Reaction performed under a N_2 atmosphere.

Solid $LiAlH_4$ (1.44 g, 37.96 mmol) was added to a vigorously stirred solution of dimethyl octadecanedioate (5.00 g, 14.9 mmol) at room temperature. The reaction was heated to reflux and stirred at reflux for 20 h. Upon completion, the reaction was cooled to room temperature and wet $NaSO_4$ was added portion-wise until the grey suspension turned white. The suspension was stirred at room temperature until the white solid was free-flowing, and solid $MgSO_4$ was added. The reaction was filtered and the filter cake washed with Et_2O (5 × 50 mL). The solvent was evaporated *in vacuo* to afford the product (4.15 g, 99%) as a white solid.

δ_H (600 MHz, DMSO-d_6) 4.30 (t, 2H, *J* 5.1 Hz, 2 × O*H*), 3.34–3.39 (m, 4H, 2 × CH_2OH), 1.35–1.43 (m, 4H, 2 × CH_2CH$_2$OH), 1.23 (app. br. s., 28H, 14 × CH_2).

δ_C (151 MHz, DMSO-d_6) 60.6, 32.3, 28.8, 28.8, 28.7, 25.3.

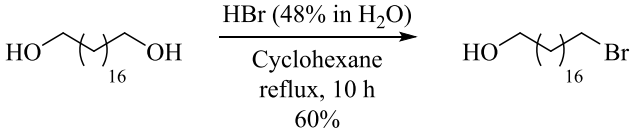
LR-MS (MALDI-TOF) 293.3 ([M+Li]$^+$), 309.3 ([M+Na]$^+$).

HR-MS calcd for $C_{18}H_{39}O_2^+$ ([M+H]$^+$) 287.2945, found 287.2946.

υ_{max} (thin film, cm^{-1}) 3416, 3353, 2919, 2891.

Mp 103–106 °C (lit. 98–99 °C)(16, 17)

Preparation of 18-bromooctadecan-1-ol 14(12)



HBr (48% in H_2O, 21 mL) was added to a suspension of diol **9** (2.26 g, 7.9 mmol) in cyclohexane (21 mL). The biphasic mixture was heated to reflux for 10 h before being cooled to room temperature. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 20 mL). The combined organics were washed with $NaHCO_3$ (4 × 20 mL of a saturated aqueous solution), brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 95:5 petrol/EtOAc then 70:30 petrol/EtOAc, to afford the product (1.65 g, 60%) as a pale yellow solid.

δ_H (400 MHz, $CDCl_3$) 3.64 (t, 2H, *J* 6.6 Hz, CH_2OH), 3.40 (t, 2H, *J* 6.9 Hz, CH_2Br), 1.81–1.89 (m, 2H, CH_2CH$_2$Br), 1.53–1.60 (m, 2H, CH_2CH$_2$OH), 1.25–1.46 (m, 28H, 14 × CH_2).

δ_C (101 MHz, $CDCl_3$) 63.3, 34.2, 33.0, 33.0, 29.8, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9.

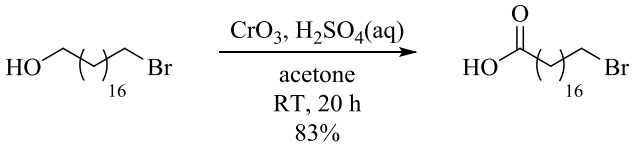
LR-MS (ES+) 332.5 ([M(^{81}Br)-H_2O+H]$^+$), 330.5 ([M(^{79}Br)-H_2O+H]$^+$).

HR-MS calcd for $C_{18}H_{41}{}^{79}BrON^+$ ([M+NH_4]$^+$) 366.2366, found 336.2368.

υ_{max} (thin film, cm^{-1}) 3274, 2917, 2850.

Mp 59–61 °C (lit. 60–62 °C)(12)

Preparation of 18-bromooctadecanoic acid 19(12, 18)



CrO_3 (1.42 g, 14.2 mmol) was dissolved in concentrated H_2SO_4 (3.5 mL). Cold H_2O (7.9 mL) was added slowly and the solution stirred at room temperature for 10 min. The resulting solution was added drop-wise to a solution of alcohol **14** (1.65 g, 4.73 mmol) in acetone (120 mL). The reaction was stirred at room temperature for 20 h before being H_2O (60 mL) and CH_2Cl_2 (40 mL) were added. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 20 mL). The combined

organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (1.43 g, 83%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.41 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 2.35 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.81–1.90 (m, 2H, C*H*$_2$CH$_2$Br), 1.59–1.68 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.46 (m, 26H, 13 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 179.6, 34.2, 34.1, 33.0, 29.8, 29.7, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8.

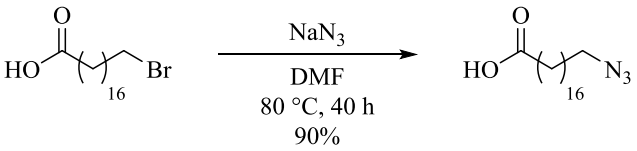
LR-MS (EI+) 365.0 ([M(^{81}Br)+H]$^+$), 363.0 ([M(^{79}Br)+H]$^+$), 347.0 ([M(^{81}Br)-H$_2$O+H]$^+$), 345.0 ([M(^{79}Br)-H$_2$O+H]$^+$), 284.0 ([M-Br+H]$^+$), 265.1 ([M-Br-H$_2$O+H]$^+$).

HR-MS calcd for $C_{18}H_{34}{}^{79}BrO_2^-$ ([M-H]$^-$) 361.1748, found 361.1741.

υ_{max} (thin film, cm^{-1}) 3034, 2915, 2850, 1696

Mp 77–80 °C (lit. 80 °C)(18)

Preparation of 18-azidooctadecanoic acid 24



Reaction performed under a N_2 atmosphere

NaN_3 (537 mg, 8.3 mmol) was added to a solution of bromide **19** (500 mg, 1.38 mmol) in DMF (5.5 mL). The reaction was stirred at 80 °C for 40 h before being cooled to room temperature. A 1:1 mixture of EtOAc/H_2O (30 mL) was added, the layers separated and the aqueous phase extracted with EtOAc (3 × 10 mL). The combined organics were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (403 mg, 90%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.25 (t, 2H, *J* 7.0 Hz, C*H*$_2$N$_3$), 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.55–1.67 (m, 4H, C*H*$_2$CH$_2$N$_3$, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.39 (m, 26H, 13 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 180.1, 51.6, 34.2, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 29.0, 26.7, 24.8.

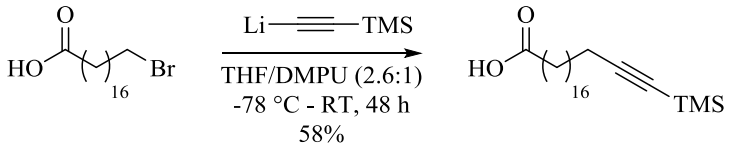
LR-MS (ES-) 296.3 ([M-N$_2$-H]$^-$), 324.1 ([M-H]$^-$).

HR-MS calcd for $C_{18}H_{34}N_3O_2^-$ ([M-H]$^-$) 324.2657, found 324.2649.

υ_{max} (thin film, cm^{-1}) 3040, 2915, 2850, 2098, 1696.

Mp 56–58 °C

Preparation of 20-(trimethylsilyl)icos-19-ynoic acid 29



Reaction performed under a N_2 atmosphere.

^{n}BuLi (2.00 M, 6.9 mL, 13.8 mmol) was added drop-wise to a solution of (trimethylsilyl)acetylene (1.36 g, 1.95 mL, 13.8 mmol) in THF (20 mL) at -78 °C. The reaction was stirred at -78 °C for 1 h before DMPU (15.3 mL) was added and the reaction stirred for a further 30 min. A solution of bromide **19** (500 mg, 1.38 mmol) in THF (14.5 mL) was added drop-wise and the reaction stirred at room temperature for 48 h. Upon completion, the reaction was quenched with HCl (20 mL of a 1 M solution). The layers were separated and the aqueous phase extracted with Et_2O (4 × 20 mL). The combined organics were washed with brine (3 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (305 mg, 58%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 2.21 (t, 2H, *J* 7.2 Hz, C*H*$_2$C≡CSi), 1.59–1.67 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.47–1.55 (m, 2H, C*H*$_2$CH$_2$C≡CSi), 1.25–1.39 (m, 26H, 13 × C*H*$_2$), 0.14 (s, 9H, Si(C*H*$_3$)$_3$).

δ_C (101 MHz, $CDCl_3$) 179.8, 108.0, 84.4, 34.1, 29.8, 29.7, 29.6, 29.6, 29.4, 29.2, 29.0, 28.8, 24.8, 20.0, 0.3.

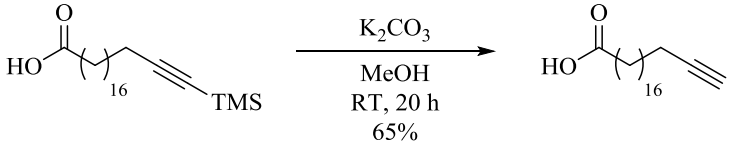
LR-MS (ES-) 379.3 ([M-H]$^-$).

HR-MS calcd for $C_{23}H_{43}O_2^-$ ([M-H]$^-$) 379.3038, found 379.3030.

υ_{max} (thin film, cm^{-1}) 2937, 2917, 2850, 2178, 1714, 1699.

Mp 58–60 °C

Preparation of icos-19-ynoic acid 32



K_2CO_3 (233 mg, 1.69 mmol) was added to a solution of TMS-alkyne **29** (257 mg, 0.67 mmol) in methanol (6.7 mL) at room temperature. The reaction was stirred at room temperature for 20 h before the solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc (15 mL) and HCl (15 mL of a 1 M aqueous solution) added. The layers were separated and the aqueous phase extracted with EtOAc (3 × 20 mL). The combined organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (134 mg, 65%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 2.34 (t, 2H, *J* 7.5 Hz, C$H_2$$CO_2H$), 2.18 (td, 2H, *J* 7.1 Hz, ^4J 2.6 Hz, CH_2C≡CH), 1.93 (t, 1H, ^4J 2.6 Hz, C≡C*H*), 1.58–1.68 (m, 2H, C$H_2$$CH_2CO_2H$), 1.48–1.56 (m, 2H, C$H_2$$CH_2$C≡CH), 1.24–1.43 (m, 26H, 13 × CH_2).

δ_C (101 MHz, $CDCl_3$) 179.9, 85.0, 68.2, 34.1, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 28.7, 24.8, 18.5.

LR-MS (ES-) 307.2 ($[M-H]^-$).

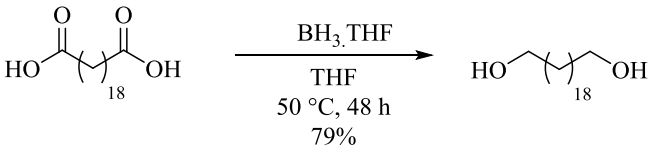
HR-MS calcd for $C_{20}H_{35}O_2^-$ ($[M-H]^-$) 307.2643, found 307.2636.

υ_{max} (thin film, cm^{-1}) 3282, 2915, 2850, 2115, 1694.

Mp 76–78 °C

Preparation of 1,20-icosanediol 10(19-22)

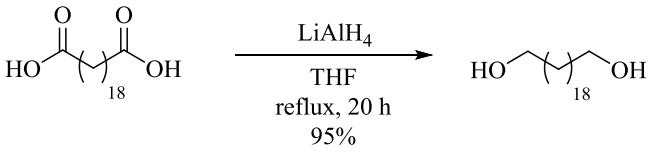
Method A



Reaction performed under a N_2 atmosphere.

BH_3.THF (1 M, 8.8 mL, 8.8 mmol) was added to a solution of icosanedioic acid (1.00 g, 2.92 mmol) in THF (58 mL). The reaction was heated to 50 °C for 24 h before additional BH_3.THF (1 M, 2.9 mL, 2.9 mmol) was added. The reaction was heated for a further 24 h before being cooled to 0 °C, quenched with MeOH (20 mL) and stirred for 30 min. HCl (20 mL of a 2 M aqueous solution) was added and the mixture stirred for 1 h. The solvent was evaporated *in vacuo* and the remaining aqueous phase extracted with $CHCl_3$ (3 × 30 mL). The combined organic phase was washed with H_2O (30 mL), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the product (724 mg, 79%) as white solid.

Method B



Reaction performed under a N_2 atmosphere.

Solid $LiAlH_4$ (1.44 g, 37.96 mmol) was added to a vigorously stirred solution of icosanedioic acid (5.00 g, 14.6 mmol) at room temperature. The reaction was heated to reflux and stirred at reflux for 20 h. Upon completion, the reaction was cooled to room temperature and wet $NaSO_4$ was added portion-wise until the grey suspension turned white. The suspension was stirred at room temperature until the white solid was free-flowing, and solid $MgSO_4$ was added. The reaction was filtered and the

filter cake washed with Et_2O (5 × 50 mL). The solvent was evaporated *in vacuo* to afford the product (4.35 g, 95%) as a white solid.

δ_H (600 MHz, DMSO-d_6) 3.38 (t, 4H, *J* 6.5 Hz, 2 × CH_2OH), 1.37–1.45 (m, 4H, 2 × CH_2CH_2OH), 1.23 (app. br. s., 32H, 16 × CH_2).

δ_C (151 MHz, DMSO-d_6) 60.6, 32.3, 28.8, 28.7, 28.7, 25.3.

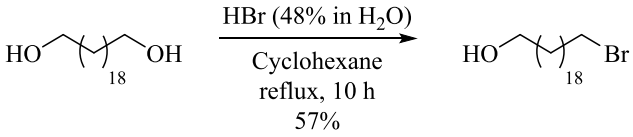
LR-MS (MALDI-TOF) 337.3 ($[M+Na]^+$).

HR-MS calcd for $C_{20}H_{43}O_2^+$ ($[M+H]^+$) 315.3258, found 315.3259.

υ_{max} (thin film, cm^{-1}) 3252, 2915, 2848.

Mp 103–105 °C (lit. 102–105 °C)(20)

Preparation of 20-bromoicosan-1-ol 15



HBr (48% in H_2O, 36 mL) was added to a suspension of diol **10** (4.35 g, 13.6 mmol) in cyclohexane (86 mL). The biphasic mixture was heated to reflux for 10 h before being cooled to room temperature. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 50 mL). The combined organics were washed with $NaHCO_3$ (4 × 30 mL of a saturated aqueous solution), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 95:5 petrol/EtOAc then 70:30 petrol/EtOAc, to afford the product (2.94 g, 57%) as a pale yellow solid.

δ_H (400 MHz, $CDCl_3$) 3.64 (t, 2H, *J* 6.6 Hz, CH_2OH), 3.41 (t, 2H, *J* 6.9 Hz, CH_2Br), 1.81–1.89 (m, 2H, CH_2CH_2Br), 1.53–1.59 (m, 2H, CH_2CH_2OH), 1.25–1.45 (m, 32H, 16 × CH_2).

δ_C (101 MHz, $CDCl_3$) 63.3, 34.2, 33.0, 33.0, 29.8, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9.

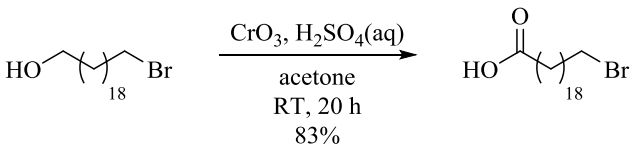
LR-MS (EI+) 376.4 ($[M(^{81}Br)+H]^+$), 374.4 ($[M(^{79}Br)+H]^+$), 361.1 ($[M(^{81}Br)-H_2O+H]^+$), 359.1 ($[M(^{79}Br)-H_2O+H]^+$,), 296.6 ($[M-Br+H]^+$).

HR-MS calcd for $C_{20}H_{40}{}^{79}Br^+$ ($[M-H_2O+H]^+$) 359.2313, found 359.2313.

υ_{max} (thin film, cm^{-1}) 3270, 2915, 2846.

Mp 66–68 °C

Preparation of 20-bromoicosanoic acid 20



CrO_3 (3.12 g, 31.2 mmol) was dissolved in concentrated H_2SO_4 (5.7 mL). Cold H_2O (13 mL) was added slowly and the solution stirred at room temperature for 10 min. The resulting solution was added drop-wise to a solution of alcohol **15** (2.94 g, 7.8 mmol) in acetone (195 mL). The reaction was stirred at room temperature for 20 h before being H_2O (100 mL) and CH_2Cl_2 (50 mL) were added. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 50 mL). The combined organics were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (2.53 g, 83%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.41 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 2.35 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.81–1.90 (m, 2H, C*H*$_2$CH$_2$Br), 1.59–1.68 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.46 (m, 30H, 15 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 178.9, 34.2, 34.0, 33.0, 29.8, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8.

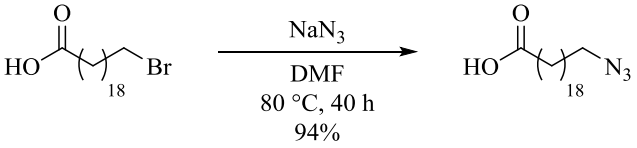
LR-MS (EI+) 393.0 ([M(^{81}Br)+H]$^+$), 391.0 ([M(^{79}Br)+H]$^+$), 375.0 ([M(^{81}Br)-H_2O+H]$^+$), 373.0 ([M(^{79}Br)-H_2O+H]$^+$), 312.0 ([M-Br+H]$^+$), 293.1 ([M-Br-H_2O+H]$^+$).

HR-MS calcd for $C_{20}H_{38}{}^{79}BrO^-$ ([M-H]$^-$) 389.2061, found 389.2057.

υ_{max} (thin film, cm^{-1}) 2914, 2848, 1693.

Mp 81–83 °C

Preparation of 20-azidoicosanoic acid 25



Reaction performed under a N_2 atmosphere

NaN_3 (499 mg, 7.7 mmol) was added to a solution of bromide **20** (500 mg, 1.28 mmol) in DMF (5 mL). The reaction was stirred at 80 °C for 40 h before being cooled to room temperature. A 1:1 mixture of EtOAc/H_2O (30 mL) was added, the layers separated and the aqueous phase extracted with EtOAc (3 × 15 mL). The combined organics were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (425 mg, 94%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.25 (t, 2H, *J* 7.0 Hz, C*H*$_2$N$_3$), 2.35 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.55–1.67 (m, 4H, C*H*$_2$CH$_2$N$_3$, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.39 (m, 30H, 15 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 179.6, 51.7, 34.1, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.0, 26.9, 24.8.

LR-MS (ES-) 324.3 ([M-N_2-H]$^-$), 352.3 ([M-H]$^-$).

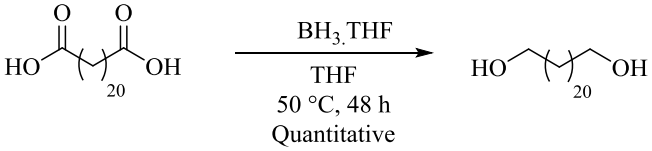
HR-MS calcd for $C_{20}H_{38}N_3O_2^-$ ([M-H]$^-$) 352.2970, found 352.2963.

υ_{max} (thin film, cm^{-1}) 3012, 2915, 2848, 2111, 1701.

Mp 63–65 °C

Preparation of 1,22-docosanediol 11(23)

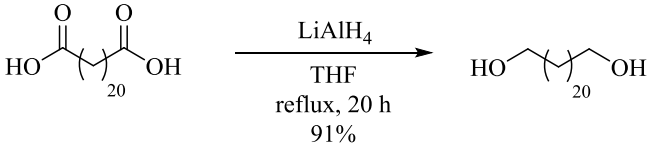
Method A



Reaction performed under a N_2 atmosphere.

BH_3.THF (1 M, 8.2 mL, 8.2 mmol) was added to a solution of docosanedioic acid (1.00 g, 2.70 mmol) in THF (51 mL). The reaction was heated to 50 °C for 24 h before additional BH_3.THF (1 M, 2.7 mL, 2.7 mmol) was added. The reaction was heated for a further 24 h before being cooled to 0 °C, quenched with MeOH (20 mL) and stirred for 30 min. HCl (20 mL of a 2 M aqueous solution) was added and the mixture stirred for 1 h. The solvent was evaporated *in vacuo* and the remaining aqueous phase extracted with $CHCl_3$ (3 × 30 mL). The combined organic phase was washed with H_2O (30 mL), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the product (974 mg, 100%) as white solid.

Method B



Reaction performed under a N_2 atmosphere.

Solid $LiAlH_4$ (1.36 g, 35.9 mmol) was added to a vigorously stirred solution of docosanedioic acid (5.12 g, 13.8 mmol) at room temperature. The reaction was heated to reflux and stirred at reflux for 20 h. Upon completion, the reaction was cooled to room temperature and wet $NaSO_4$ was added portion-wise until the grey suspension turned white. The suspension was stirred at room temperature until the white solid was free-flowing, and solid $MgSO_4$ was added. The reaction was filtered and the filter cake washed with Et_2O (5 × 50 mL). The solvent was evaporated *in vacuo* to afford the product (4.30 g, 91%) as a white solid.

δ_H (600 MHz, DMSO-d_6) 4.17 (t, 2H, *J* 5.1 Hz, 2 × O*H*), 3.36–3.40 (m, 4H, 2 × CH_2OH), 1.38–1.44 (m, 4H, 2 × CH_2CH_2OH), 1.25 (app. br. s., 36H, 18 × CH_2).

δ_C (151 MHz, DMSO-d_6) 60.6, 32.3, 28.8, 28.8, 28.7, 25.3.

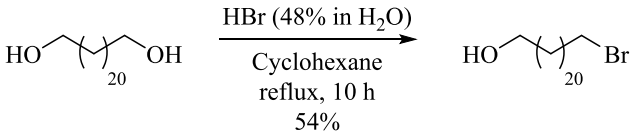
LR-MS (MALDI-TOF) 365.4 ($[M+Na]^+$).

HR-MS calcd for $C_{22}H_{47}O_2^+$ ($[M+H]^+$) 343.3571, found 343.3574.

υ_{max} (thin film, cm^{-1}) 3255, 2915, 2848.

Mp 98–100 °C (lit. 96–98 °C)(23)

Preparation of 22-bromodocosan-1-ol 16(24)



HBr (48% in H_2O, 32 mL) was added to a suspension of diol **11** (4.16 g, 12.2 mmol) in cyclohexane (64 mL). The biphasic mixture was heated to reflux for 10 h before being cooled to room temperature. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 50 mL). The combined organics were washed with $NaHCO_3$ (4 × 30 mL of a saturated aqueous solution), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 95:5 petrol/EtOAc then 70:30 petrol/EtOAc, to afford the product (2.65 g, 54%) as a pale yellow solid.

δ_H (400 MHz, $CDCl_3$) 3.64 (t, 2H, *J* 6.6 Hz, C*H*$_2$OH), 3.40 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 1.81–1.89 (m, 2H, C*H*$_2$CH$_2$Br), 1.53–1.60 (m, 2H, C*H*$_2$CH$_2$OH), 1.25–1.44 (m, 36H, 18 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 63.3, 34.2, 33.0, 33.0, 29.8, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9.

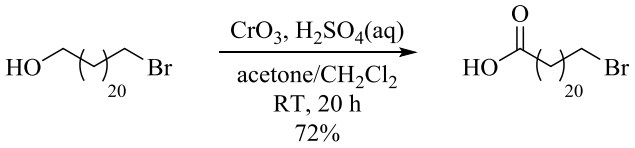
LR-MS (EI+) 405.5 ([M(^{81}Br)+H]$^+$), 403.5 ([M(^{79}Br)+H]$^+$), 388.5 ([M(^{81}Br)-H_2O+H]$^+$), 386.5 ([M(^{79}Br)-H_2O+H]$^+$), 324.4 ([M-Br+H]$^+$).

HR-MS calcd for $C_{22}H_{49}{}^{79}BrON$ ([M+NH_4]$^+$) 422.2992, found 422.2990.

υ_{max} (thin film, cm^{-1}) 3276, 2915, 2846.

Mp 66–68 °C (lit. 72 °C)(24)

Preparation of 22-bromodocosanoic acid 21



CrO_3 (696 mg, 6.96 mmol) was dissolved in concentrated H_2SO_4 (1.3 mL). Cold H_2O (2.9 mL) was added slowly and the solution stirred at room temperature for 10 min. The resulting solution was added drop-wise to a solution of alcohol **16** (703 mg, 1.74 mmol) in acetone/CH_2Cl_2 (3:1, 65 mL). The reaction was stirred at room temperature for 20 h before being H_2O (50 mL) and CH_2Cl_2 (30 mL) were added. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 30 mL). The combined organics were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (522 mg, 72%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.40 (t, 2H, *J* 6.9 Hz, C*H*$_2$Br), 2.35 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.81–1.90 (m, 2H, C*H*$_2$CH$_2$Br), 1.59–1.68 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.44 (m, 34H, 17 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 179.4, 34.2, 34.1, 33.0, 29.8, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8.

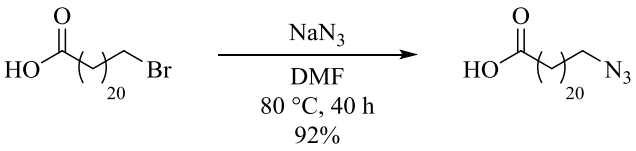
LR-MS (EI+) 421.0 ([M(^{81}Br)+H]$^+$), 419.0 ([M(^{79}Br)+H]$^+$), 393.0 ([M(^{81}Br)-H_2O+H]$^+$), 391.0 ([M(^{79}Br)-H_2O+H]$^+$), 341.1 ([M-Br+H]$^+$), 293.1 ([M-Br-H_2O+H]$^+$).

HR-MS calcd for $C_{22}H_{42}{}^{79}BrO_2^-$ ([M-H]$^-$) 417.2374, found 417.2368.

υ_{max} (thin film, cm^{-1}) 2913, 2846, 1693.

Mp 78–80 °C

Preparation of 22-azidodocosanoic acid 26



Reaction performed under a N_2 atmosphere

NaN_3 (195 mg, 3.0 mmol) was added to a solution of bromide **21** (208 mg, 0.5 mmol) in DMF (2 mL). The reaction was stirred at 80 °C for 40 h before being cooled to room temperature. A 1:1 mixture of EtOAc/H_2O (20 mL) was added, the layers separated and the aqueous phase extracted with EtOAc (3 × 10 mL) and CH_2Cl_2 (10 mL). The combined organics were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography, eluting with 90:10 petrol/EtOAc (+0.1% AcOH), to afford the product (176 mg, 92%) as a white solid.

δ_H (400 MHz, $CDCl_3$) 3.25 (t, 2H, *J* 7.0 Hz, C*H*$_2$N$_3$), 2.34 (t, 2H, *J* 7.5 Hz, C*H*$_2$CO$_2$H), 1.56–1.67 (m, 4H, C*H*$_2$CH$_2$N$_3$, C*H*$_2$CH$_2$CO$_2$H), 1.25–1.37 (m, 34H, 17 × C*H*$_2$).

δ_C (101 MHz, $CDCl_3$) 179.9, 51.7, 34.1, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 29.2, 29.0, 26.9, 24.8.

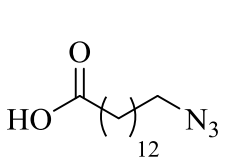
LR-MS (ES-) 352.3 ([M-N_2-H]$^-$), 380.3 ([M-H]$^-$).

HR-MS calcd for $C_{22}H_{42}N_3O_2^-$ ([M-H]$^-$) 380.3283, found 380.3275.

υ_{max} (thin film, cm^{-1}) 2913, 2846, 2095, 1693.

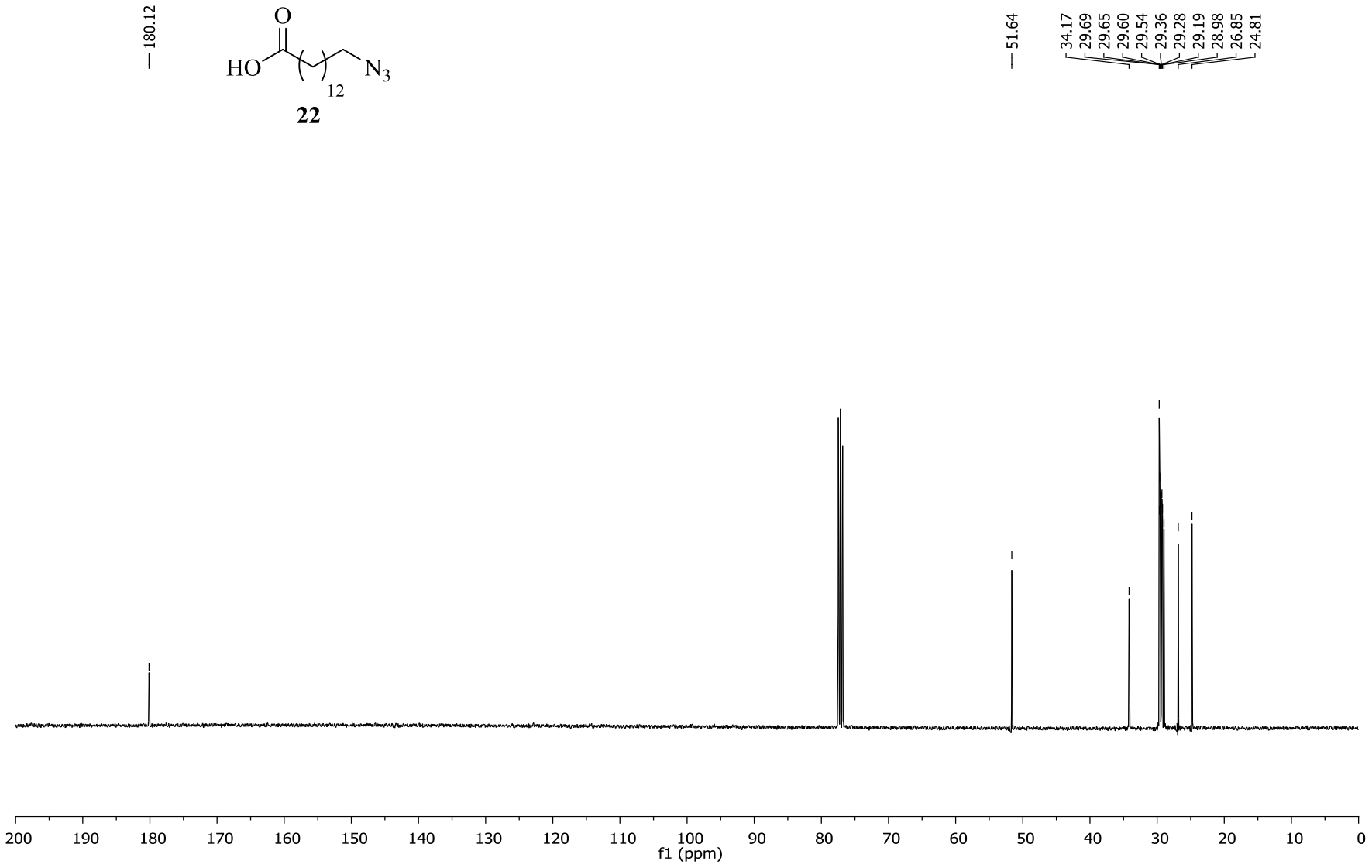
Mp 71–73 °C

14-Azidotetradecanoic acid 22 ^{1}H NMR (400 MHz, $CDCl_3$)

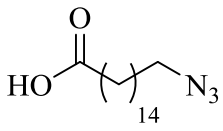


22

14-Azidotetradecanoic acid 22 ^{13}C NMR (101 MHz, $CDCl_3$)

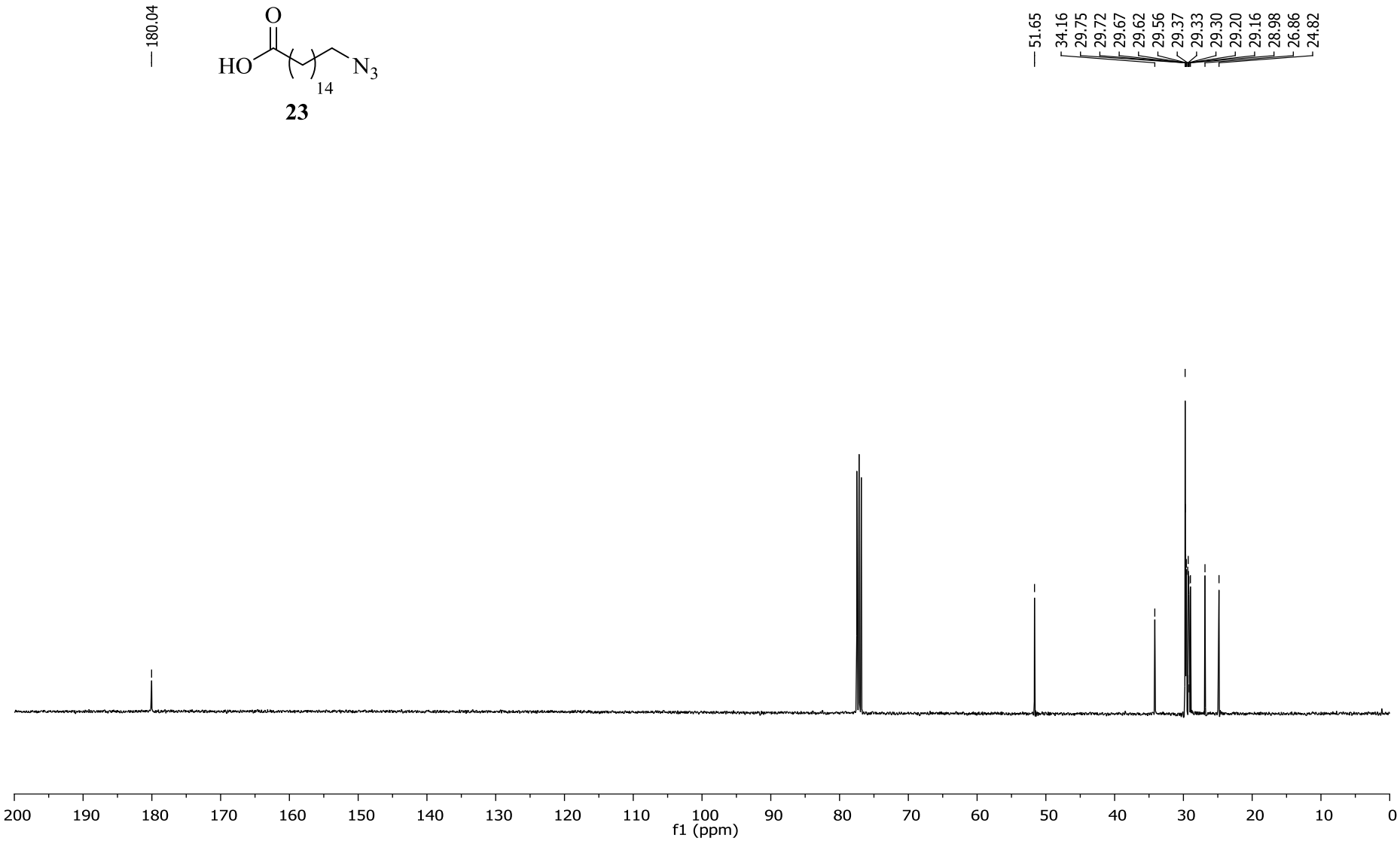


16-Azidohexadecanoic acid 23 ^{1}H NMR (400 MHz, $CDCl_3$)

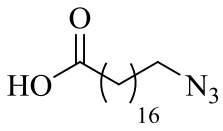


23

16-Azidohexadecanoic acid 23 ^{13}C NMR (101 MHz, $CDCl_3$)

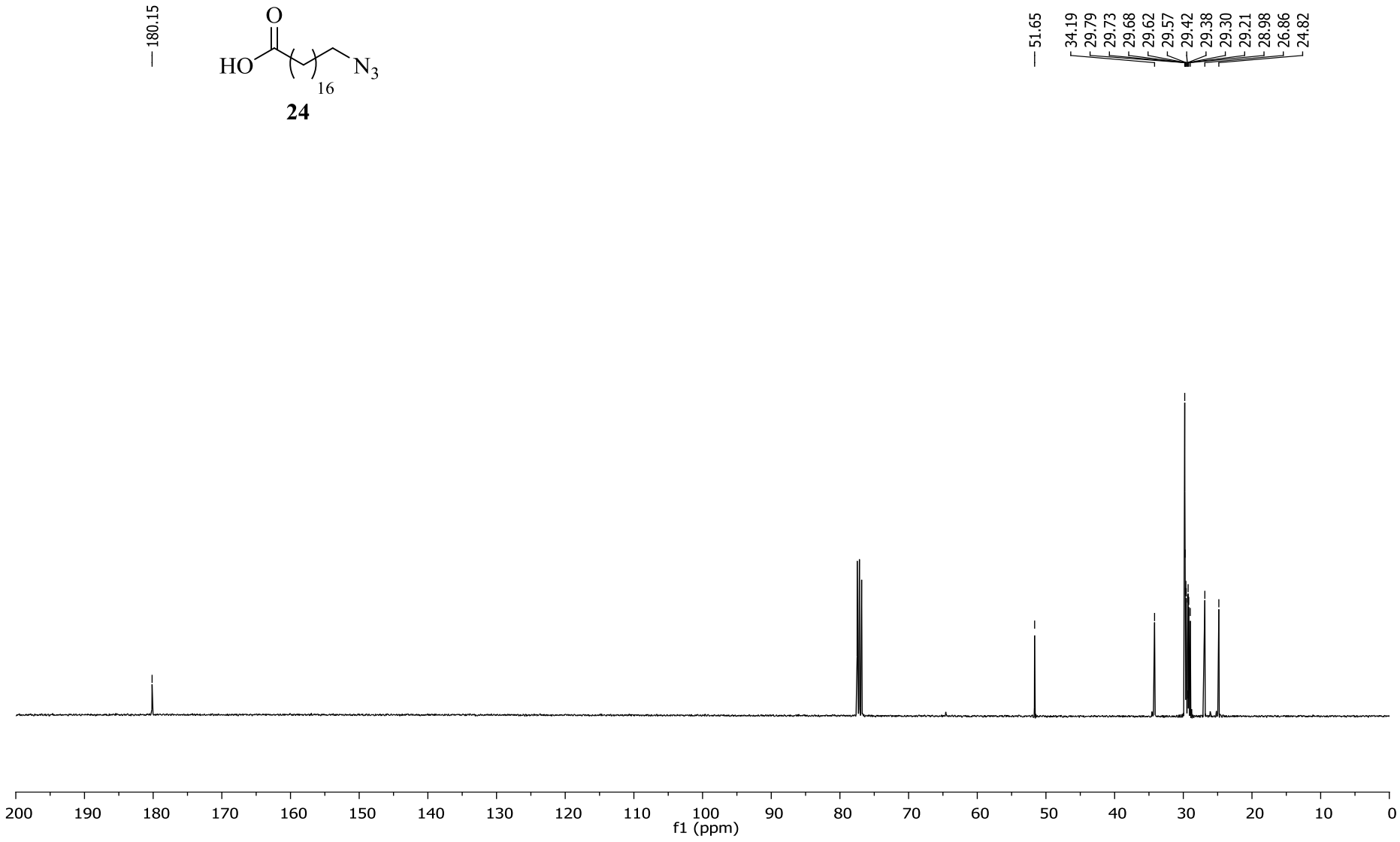


18-Azidooctadecanoic acid 24 ^{1}H NMR (400 MHz, $CDCl_3$)

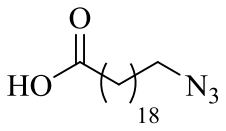


24

18-Azidooctadecanoic acid 24 ^{13}C NMR (101 MHz, $CDCl_3$)

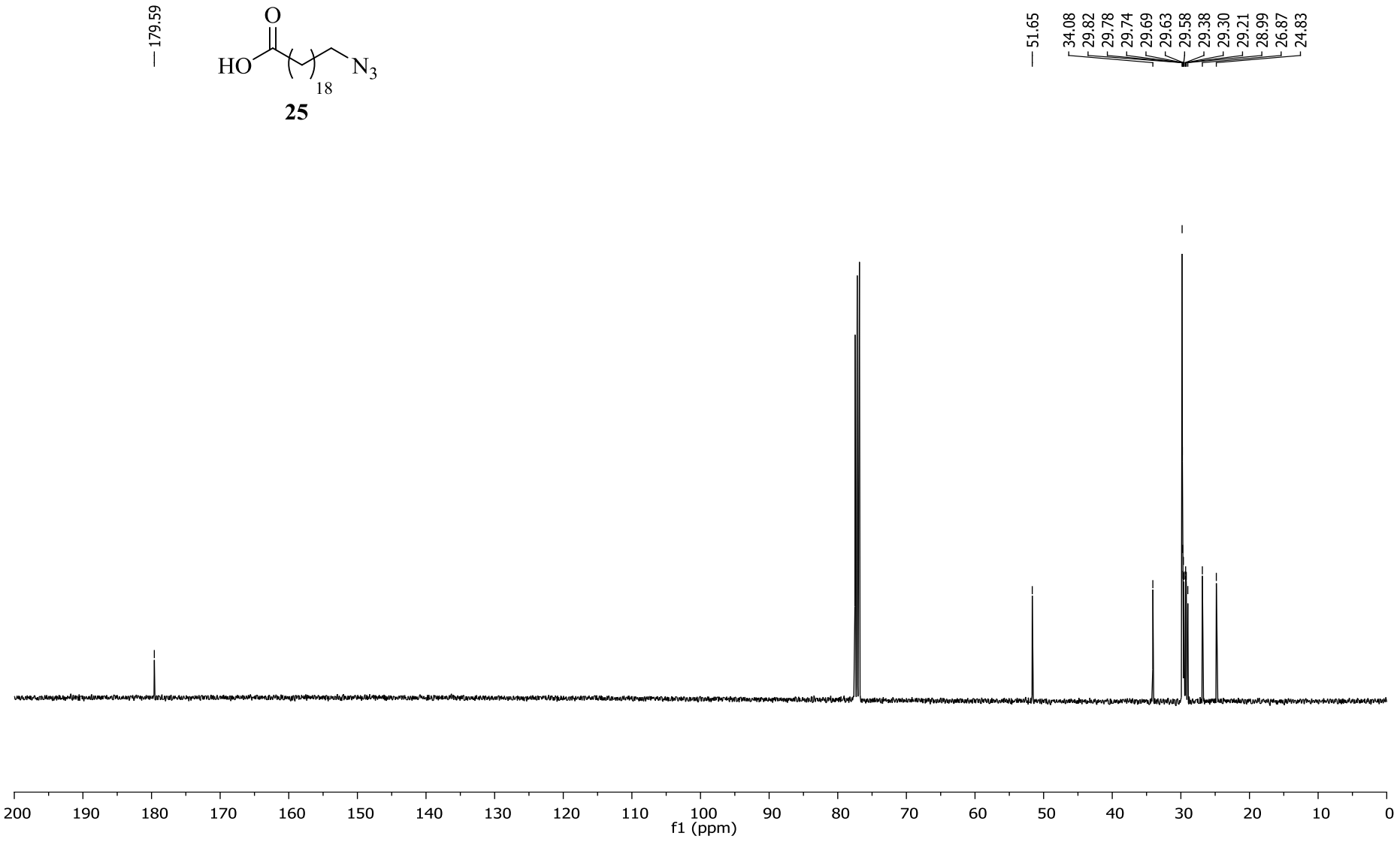


20-Azidoicoscanoic acid 25 ^{1}H NMR (400 MHz, $CDCl_3$)

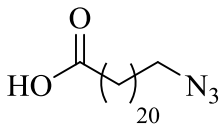


25

20-Azidoicoscanoic acid 25 ^{13}C NMR (101 MHz, $CDCl_3$)

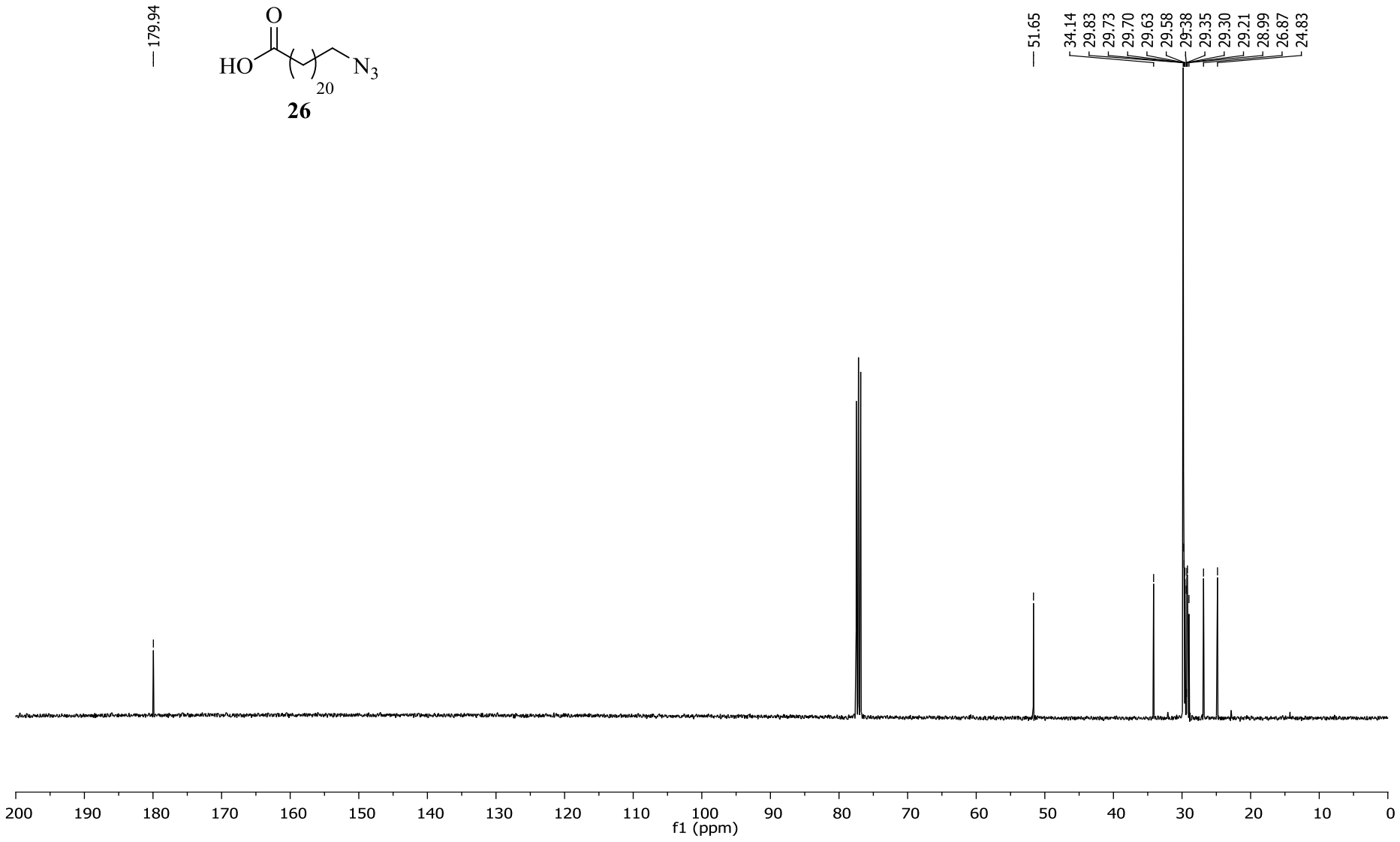


22-Azidodocoscanoic acid 26 ^{1}H NMR (400 MHz, $CDCl_3$)

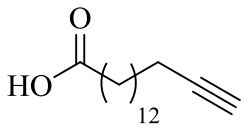


26

22-Azidodcoscanoic acid 26 ^{13}C NMR (101 MHz, $CDCl_3$)

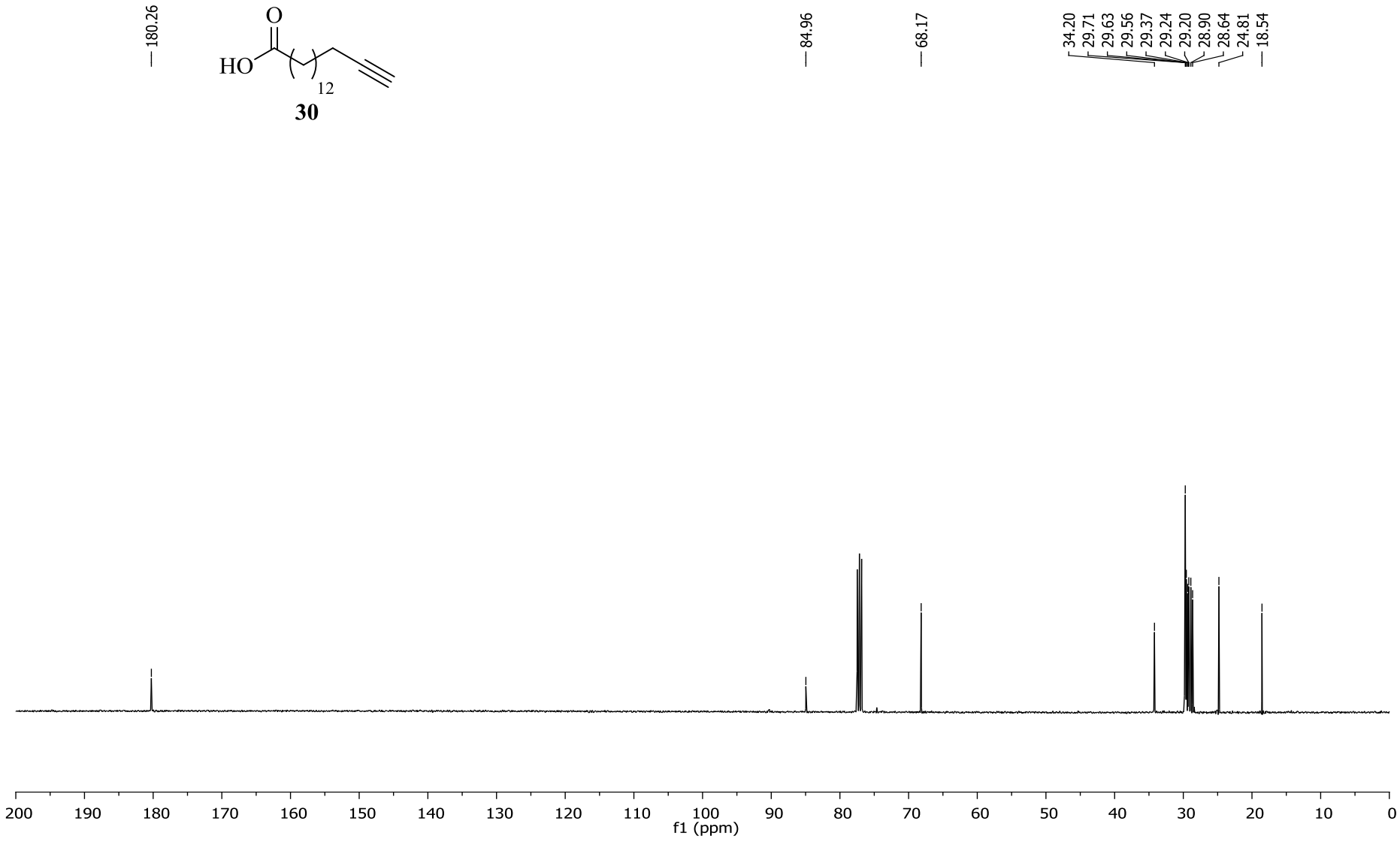


Hexadec-15-ynoic acid 30 ^{1}H NMR (400 MHz, $CDCl_3$)

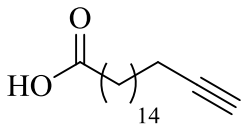


30

Hexadec-15-ynoic acid 30 ^{13}C NMR (101 MHz, $CDCl_3$)

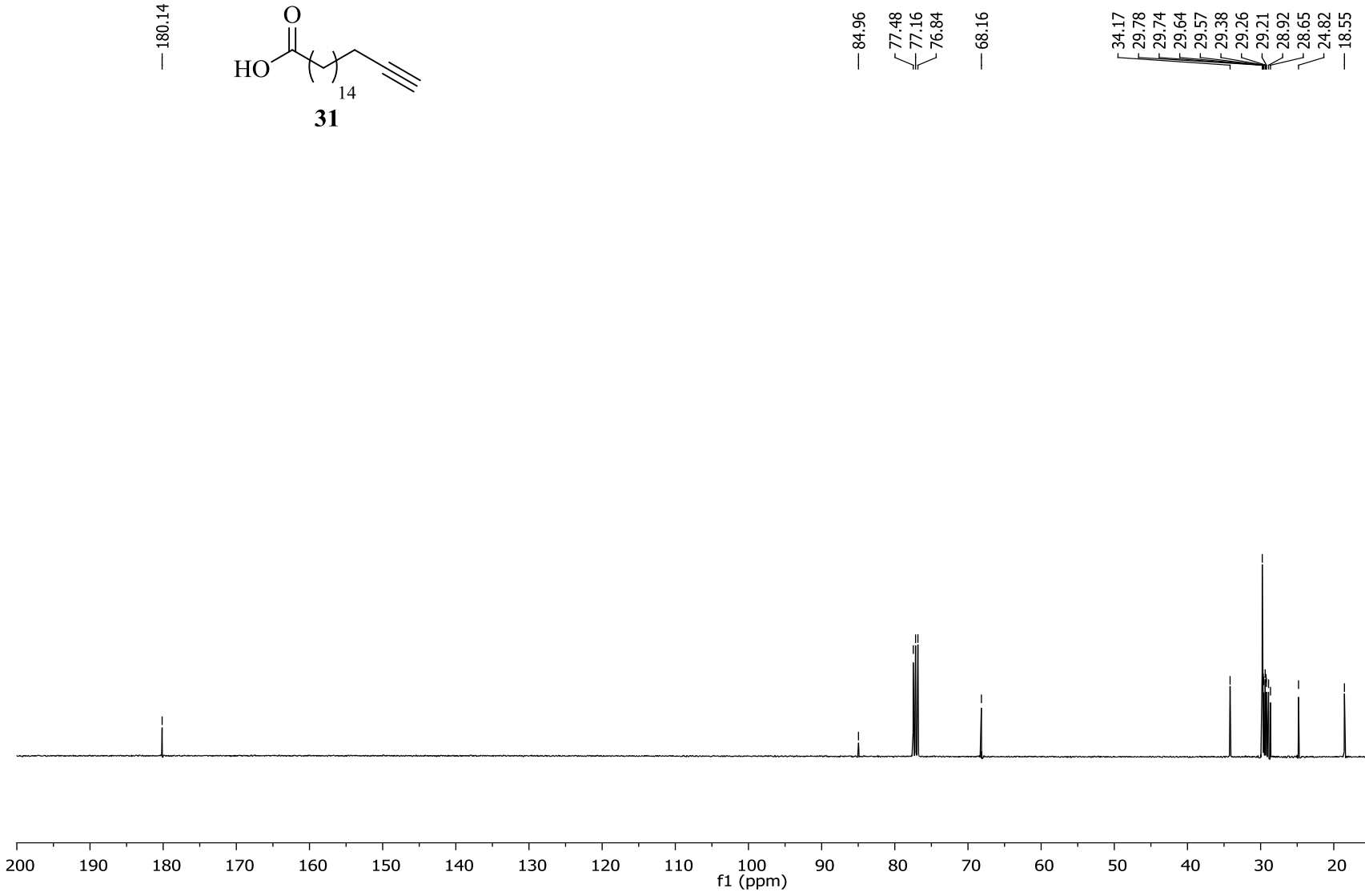


Octadec-17-ynoic acid 31 ^{1}H NMR (400 MHz, $CDCl_3$)

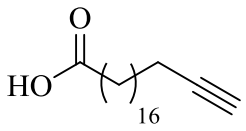


31

Octadec-17-ynoic acid 31 ^{13}C NMR (101 MHz, $CDCl_3$)

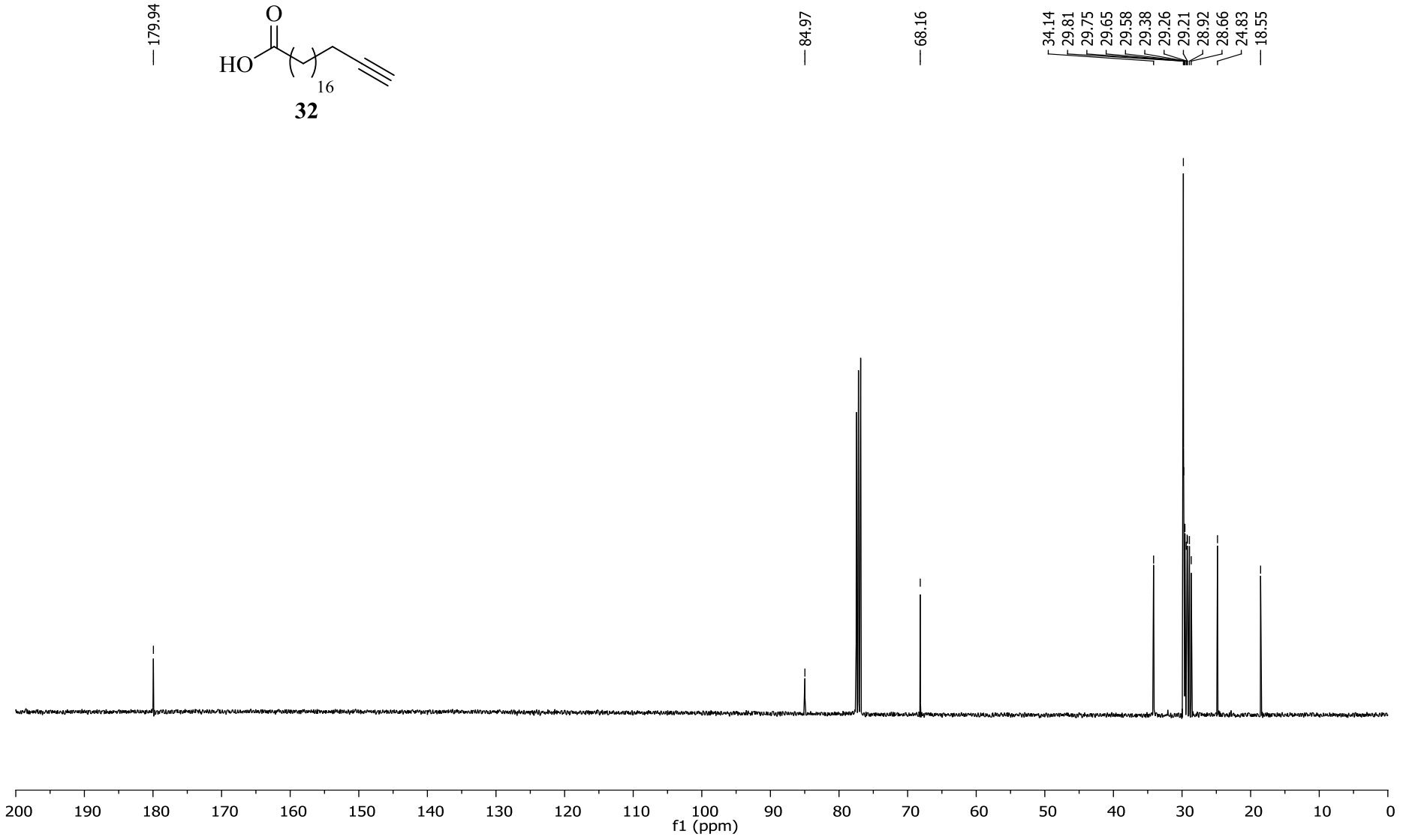


Icos-19-ynoic acid 32 ^{1}H NMR (400 MHz, $CDCl_3$)



32

Icos-19-ynoic acid 32 ^{13}C NMR (101 MHz, $CDCl_3$)



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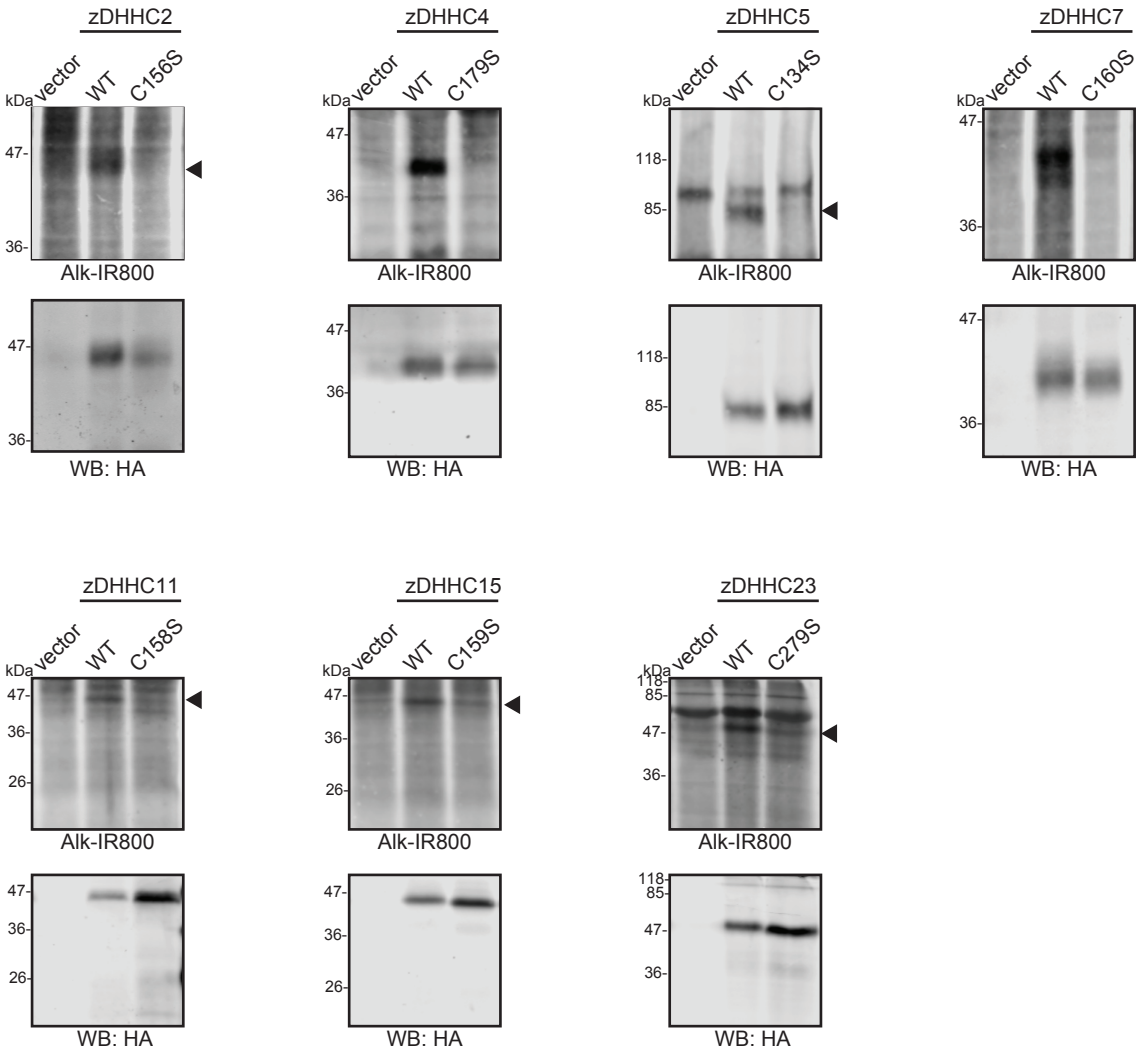
Acyl-CoA	MRM Transition	Collision Energy (eV)	Declustering Potential (eV)	Dwell Time (ms)	Response factor
14:0	976→471	40	40	500	2.25
16:0	1006→499	40	40	500	2.38
16:1	1004→497	40	40	500	2.3
17:0	1020→513	40	40	100	2.32
18:0	1034→527	45	40	250	2.62
18:1	1032→525	45	40	250	2.5
18:2	1030→523	50	45	500	2.4
20:0	1062→555	50	45	500	2.48
20:2	1058→551	50	45	500	2.3
20:4	1054→547	50	45	250	2.23
20:5	1052→545	55	45	500	2.2
22:0	1090→583	50	45	500	2.2
22:4	1082→575	55	45	500	2.1
22:5	1080→573	55	45	500	2.1
22:6	1078→571	50	45	500	2.1
C14:0-azide	1019→512	40	40	500	2.3
C16:0-azide	1047→540	40	40	500	2.3
C16:1-azide	1045→538	40	40	500	2.3
C18:0-azide	1075→568	45	40	500	2.3
C18:1-azide	1073→566	45	40	500	2.2
C18:2-azide	1071→564	45	40	500	2.1
C20:0-azide	1113→596	50	45	500	2.3
C20:1-azide	1111→594	50	45	500	2.3
C22:0-azide	1141→624	50	45	500	2.2

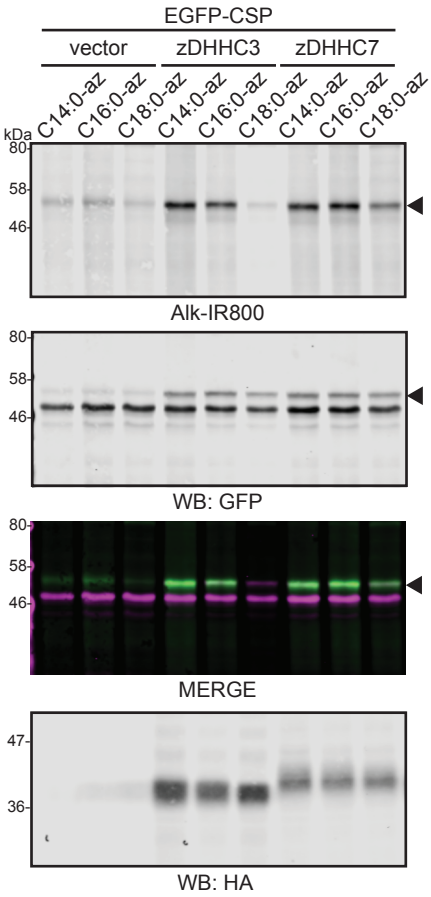
Table 1. MRM Transitions:

Multiple reaction monitoring transitions were based upon optimized fragmentation in positive ion mode. Interface temp 30 C, Nanomate: gas pressure 0.5 psi, Tip voltage 1.25-1.3kV, EP 8, CXP 12.

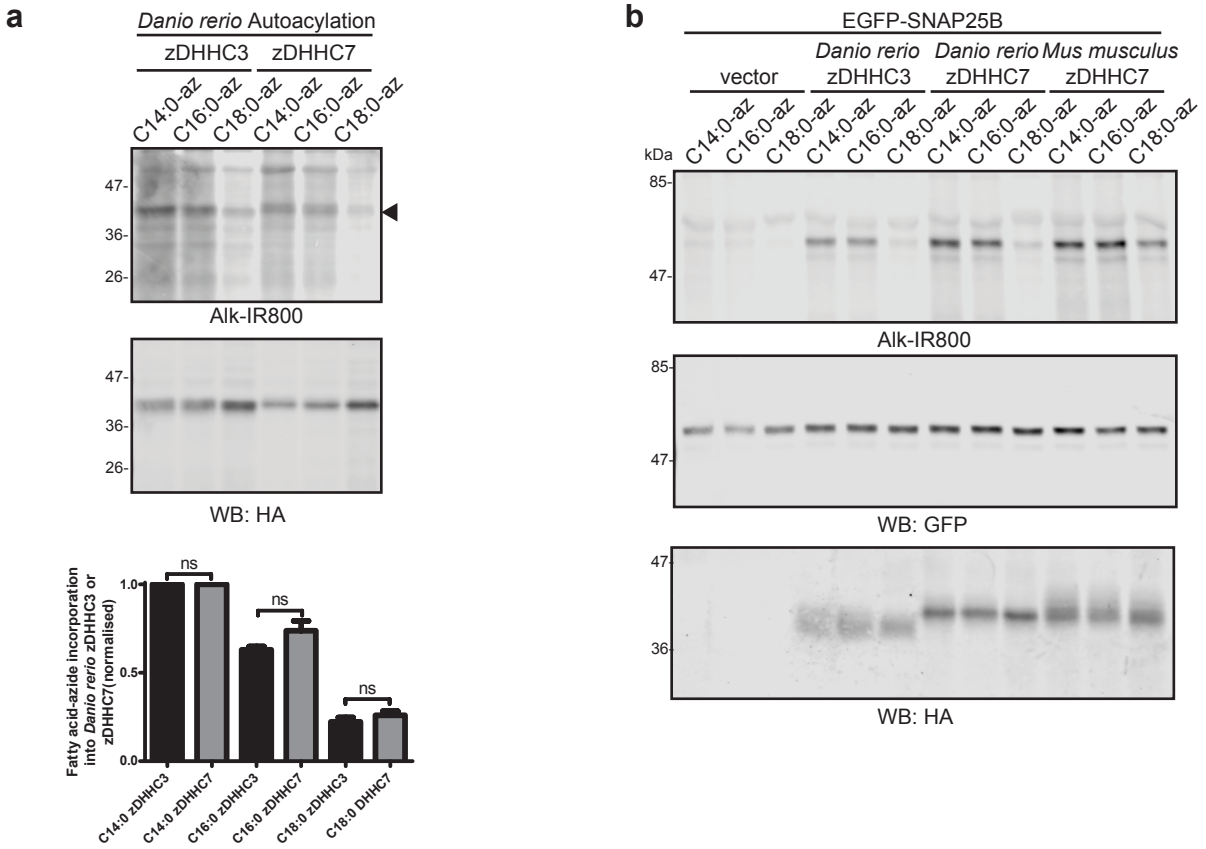
[1] Resp Factor[1] (10^6 cpm/pmole) are based upon standard curve point (1000, 500, 100, 10, 5, 2.5 and 1 pmole(s)) has its own response in terms of number of specific ions observed by the mass spectrometer under those specific conditions. Values in blue represent estimates in the absence of actual standards and similar related response factors observed previously (doi: 10.1194/jlr.D800001-JLR200))



Supplementary Figure 1: *S*-acylation of wild-type and inactive zDHHC enzymes by C16:0- azides.
HEK293T cells transfected with HA-tagged wild-type or mutant zDHHC enzyme constructs containing a cysteine-to-serine mutation of the catalytic DHHC cysteine residue. Cells were then incubated with C16:0 fatty acid azides for 4 h at 37 ºC. Fatty acid azides were then labelled by click chemistry using an alkyne-800 infrared dye. Isolated proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Representative click signals and western blots are shown. Position of molecular weight markers are shown on the left. Arrowheads indicate the zDHHC bands that incorporate label.



Supplementary Figure 2: *S*-acylation of EGFP-Cysteine-string protein by different zDHHC enzymes.
HEK293T cells were transfected with EGFP-CSP together with pEF-BOS-HA (vector), HA-zDHHC3 or HA-zDHHC7. Cells were then incubated with C14:0, C16:0 or C18:0 fatty acid azides as indicated for 4 h at 37 ºC. Fatty acid azides were labelled by click chemistry using an alkyne-800 infrared dye. Isolated proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Representative click signals and western blots are shown. Position of molecular weight markers are shown on the left. Note that CSP migrates as two bands, the upper band (*arrowhead*) is S-acylated, whereas the lower band is non-acylated.



Supplementary Figure 3: S-acylation profiles of *Danio rerio* zDHHC3 and zDHHC7 by fatty-acid azides.
(A) HEK293T cells transfected with HA-tagged zDHHC3 or zDHHC7 from *Danio rerio* were incubated with C14:0, C16:0 or C18:0 fatty acid azides for 4 h at 37 ºC. Fatty acid azides were then labelled by click chemistry using an alkyne-800 infrared dye. Isolated proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Representative click signals and western blots and quantified data (mean ± SEM) are shown. n = 5; ns = not significant. (B) HEK293T cells were transfected with EGFP-SNAP25B together with either pEF-BOS-HA (vector control), zDHHC3 or zDHHC7 from *Danio rerio*, or mouse zDHHC7. Cells were then incubated with C14:0, C16:0 or C18:0 fatty acid azides for 4 h at 37 ºC, and fatty acid azides were then labelled by click chemistry using an alkyne-800 infrared dye. Isolated proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Representative click signals and western blots are shown.