Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

Succinct synthesis of saturated hydroxy fatty acids and *in vitro* evaluation of all hydroxylauric acids at FFA1, FFA4 and GPR84

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Contents

Synthetic procedures

2-Hydroxylauric acid (2-HLA).[1] Lauric acid (200 mg, 1.0 mmol) was dissolved in $SOCl_2$ (0.36 mL) and the reaction was heated to 50 °C for 15 min. Bromine (0.25 mL, 5.0 mmol) was added and the reaction was stirred at 50 °C for 22 h. The reaction mixture was cooled to 0°C and concentrated, aqueous Na_2SO_3 was added until the disappearance of the colour, and the solution was acidified to pH 1 with 2M HCl. The aqueous phase was extracted with dichloromethane (3×10 mL). The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was dissolved in 2M NaOH (4.75 mL) and heated to 85 °C for 17 h. The reaction mixture was cooled to 0 °C and acidified with concentrated HCl to pH 1. The reaction mixture was extracted with ethyl acetate (3×30 mL). The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo*. The crude was purified by column chromatography (1:1:100 MeOH:AcOH:DCM) yielding 110 mg (53%) of the product as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 4.02 (dd, J = 7.7, 4.4 Hz, 1H), 1.73 – 1.62 (m, 1H), 1.61 – 1.50 (m, 1H), 1.39 – 1.31 (m, 2H), 1.24 (d, J = 18.5 Hz, 14H), 0.82 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 178.1, 71.5, 35.4, 33.1, 30.7, 30.7, 30.6, 30.5, 30.5, 26.1, 23.7, 14.4; ESI-HRMS: ($M+Na^+$) calcd for $C_{12}H_{24}NaO_3$: 239.1618, found: 239.1617

Ethyl 3-oxolaurate. NaH (40 mg, 60 % w/w, 1.0 mmol) was suspended in dry THF (4.5 mL). The suspension was cooled to 0 °C and ethyl acetoacetate (110 µL, 0.87 mmol) was added. When bubbling had seized, *n*-butyl lithium (2.5 M in hexanes, 0.37 mL, 0.93 mmol) was added. After half an hour 1-bromooctane was added (135 µL, 0.78 mmol), the reaction was then allowed to reach room temperature and stirred for 16 h. The reaction mixture was concentrated, NH_4Cl (sat. aq., 3 mL) was added, the pH was adjusted to 7 with aqueous HCl (1 M), and the aqueous phase was extracted with ethyl acetate (3×15 mL). The combined organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The crude was purified by column chromatography (1:20 ethyl acetate:petroleum ether) yielding 180 mg (96%) of the product as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (q, J = 7.1 Hz, 2H), 3.43 (s, 2H), 2.53 (t, J = 7.4 Hz, 2H), 1.64 – 1.53 (m, 2H), 1.34 – 1.24 (m, 15H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.0, 167.3, 61.3, 49.3, 43.1,

31.9, 29.4, 29.3, 29.2, 29.0, 23.5, 22.6, 14.1, 14.1. NMR is consistent with previously reported data.[2] Approximately 15 % of the corresponding enol was observed in the spectra.

3-Hydroxylauric acid (3-HLA).[2] Ethyl 3-oxolaurate (147 mg, 0.61 mmol) was dissolved in THF (1 mL) and absolute ethanol (0.5 mL). The solution was cooled to 0 °C, $NaBH_4$ (29 mg, 0.79 mmol) was added and the reaction was stirred for 2 h before addition of aqueous HCl (0.5 M, 6 mL) and brine (9 mL). The aqueous phase was extracted with ethyl acetate (3×15 mL). The combined organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The crude was dissolved in THF (1.5 mL) and added $LiOH•H_2O$ (55 mg, 1.31 mmol) dissolved in H_2O (0.75 mL). The reaction was stirred 21 h and then acidified to pH 1 with HCl (2 M). Brine (9 mL) was added and the aqueous phase was extracted with ethyl acetate (3×15 mL). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude was purified by column chromatography (1:1:100 AcOH:MeOH:DCM) yielding the desired product as a white solid (44 mg, 36% over two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 4.02 – 3.93 (m, 1H), 3.73 – 3.66 (m, 1H), 2.40 (qd, J = 15.2, 6.5 Hz, 2H), 1.48 (d, J = 6.3 Hz, 2H), 1.30 (d, J = 1.8 Hz, 14H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.7, 69.4, 49.7, 49.5, 49.2, 49.0, 48.8, 48.6, 48.4, 43.3, 38.1, 33.1, 30.7, 30.7, 30.5, 26.7, 23.7, 14.4; ESI-HRMS: calcd for $C_{12}H_{24}NaO_3$ ($M+Na^+$) 239.1618, found 239.1610.

Sodium 4-hydroxylaurate (4-HLA).[3] 4-Dodecanolide (105 µL, 0.50 mmol) and NaOH (21 mg, 0.53 mmol) was dissolved in methanol (1 mL) and stirred for 24 h at room temperature. The solvent was blown away under a stream of nitrogen yielding the desired compound as a white, waxy solid (112 mg, 93 %): ^{1}H NMR (400 MHz, CD_3OD) δ 3.58 – 3.50 (m, 1H), 3.35 (s, 1H), 2.35 – 2.21 (m, 2H), 1.81 – 1.72 (m, 1H), 1.70 – 1.60 (m, 1H), 1.46 – 1.28 (m, 14H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 183.0, 72.9, 38.5, 35.8, 34.9, 33.1, 30.9, 30.8, 30.4, 26.9, 23.7, 14.4; ESI-HRMS: calcd for $C_{12}H_{24}NaO_3$ ($M+Na^+$) 239.1618, found 239.1618.

Sodium 5-hydroxylaurate (5-HLA).[4] 5-Dodecanolide (105 µL, 0.50 mmol) and NaOH (21 mg, 0.50 mmol) was dissolved in methanol (1 mL). It was stirred for 24 h at room temperature. The solvent was blown away under a stream of nitrogen yielding the desired compound as a white solid (105 mg, 88%): ^{1}H NMR (400 MHz, CD_3OD) δ 3.57 – 3.49 (m, 1H), 2.17 (t, J = 7.5 Hz, 2H), 1.79 – 1.57 (m,

2H), 1.52 – 1.27 (m, 14H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 182.9, 72.2, 39.1, 38.5, 38.4, 33.0, 30.9, 30.5, 26.9, 23.8, 23.7, 14.4; ESI-HRMS: ($M+Na^+$) calcd for $C_{12}H_{24}NaO_3$ 239.1618, found 239.1623.

Concentration-response curves

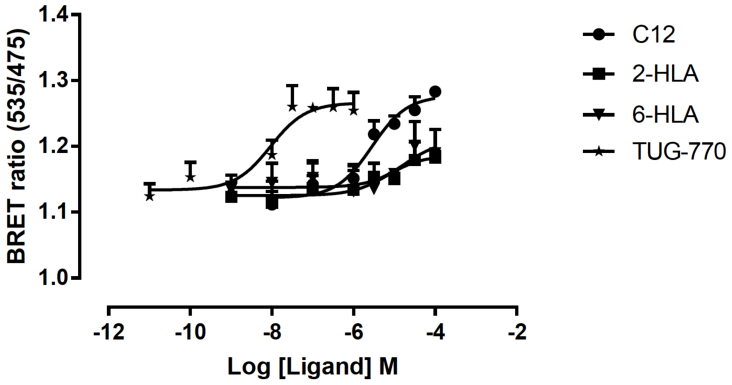

Figure S1. Concentration-response curves for lauric acid (C12), 2-HLA, 6-HLA and TUG-770 on FFA1.[5, 6]

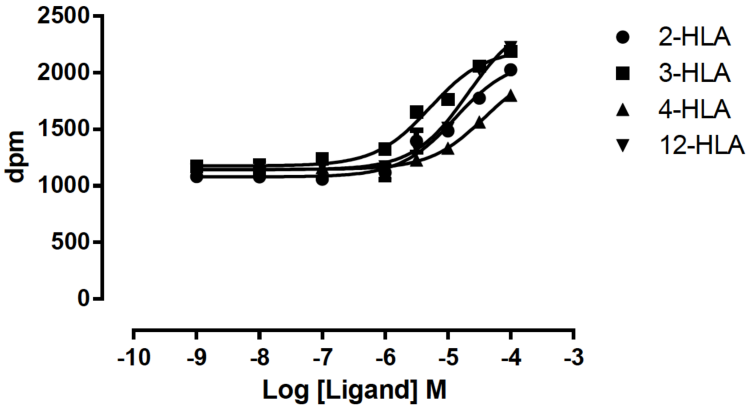

Figure S2. Concentration-response curves for 2-HLA, 3-HLA, 4-HLA and 12-HLA on GPR84.[7]

References

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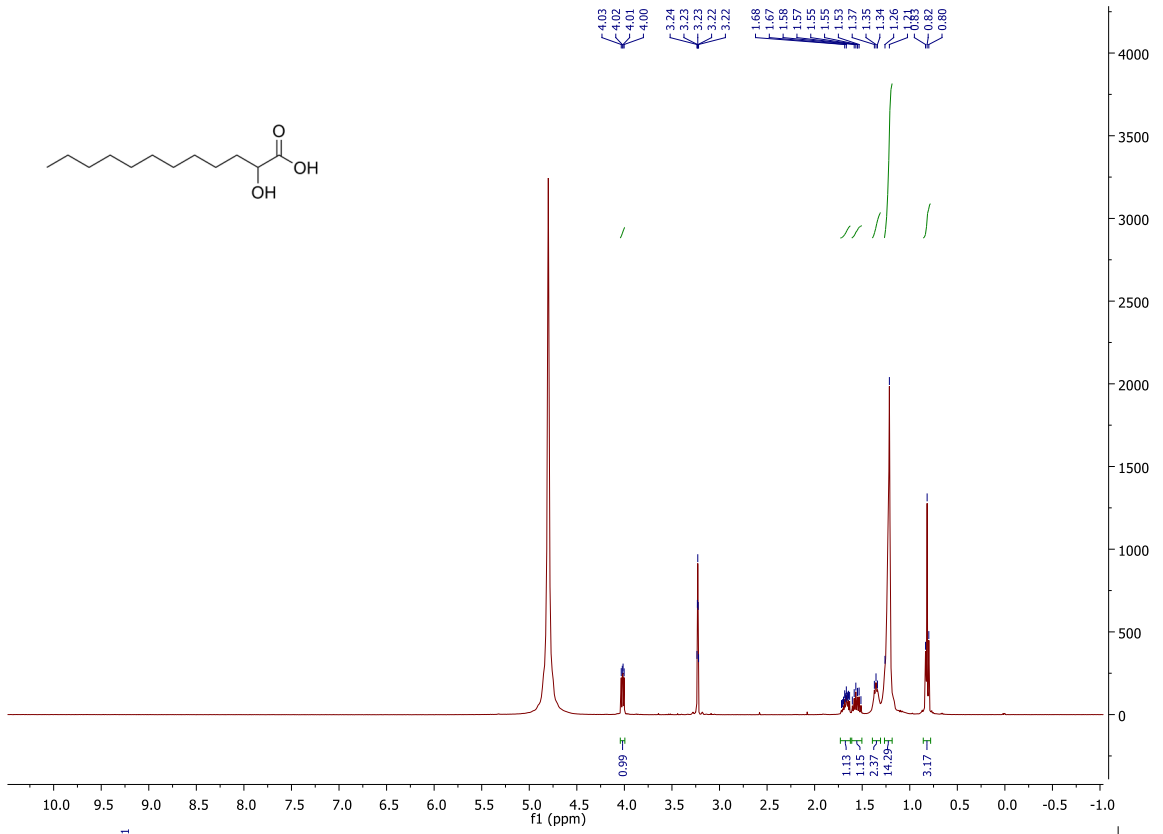
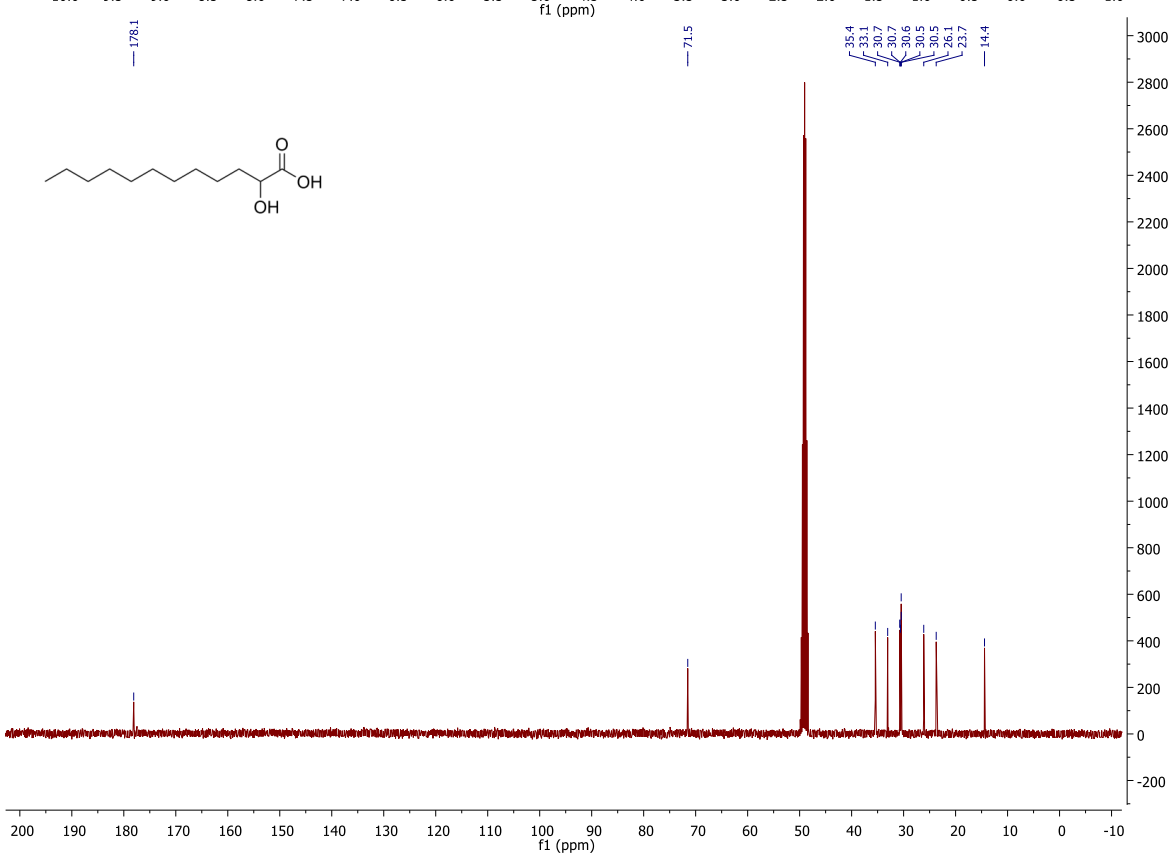
NMR spectra

2-Hydroxydodecanoic acid

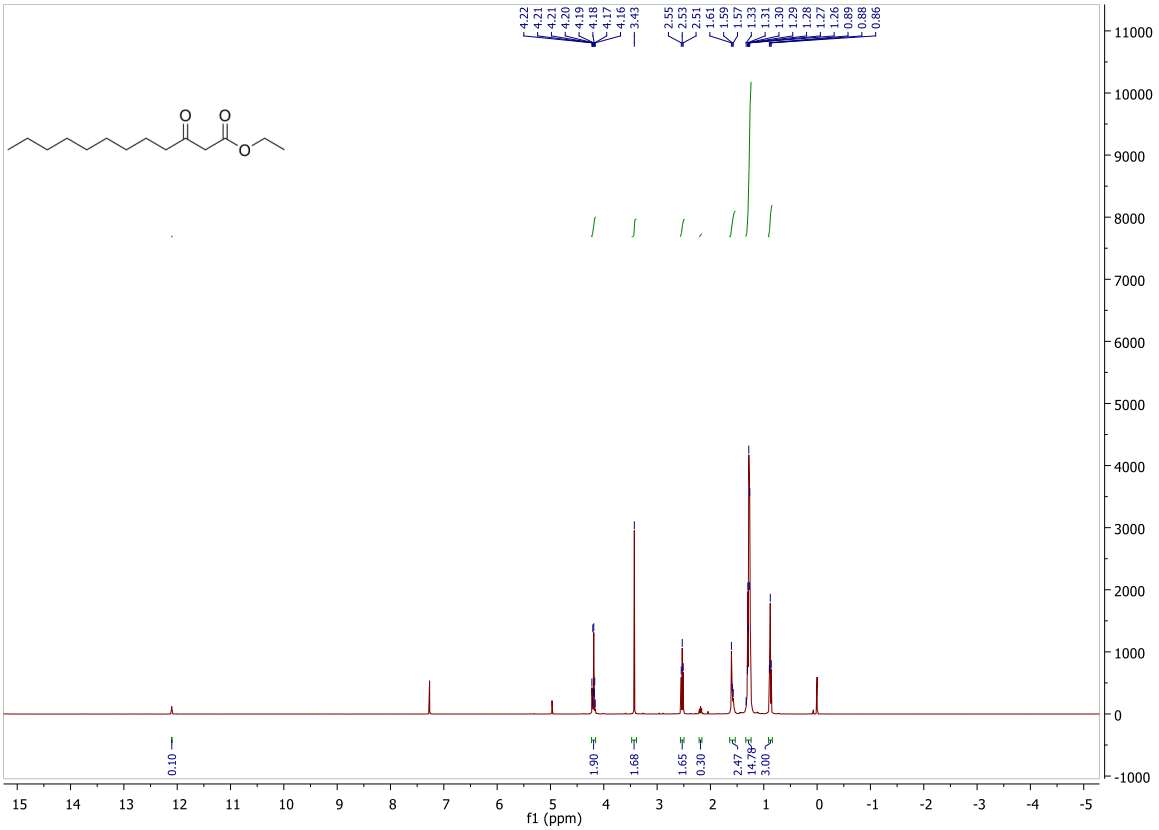
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

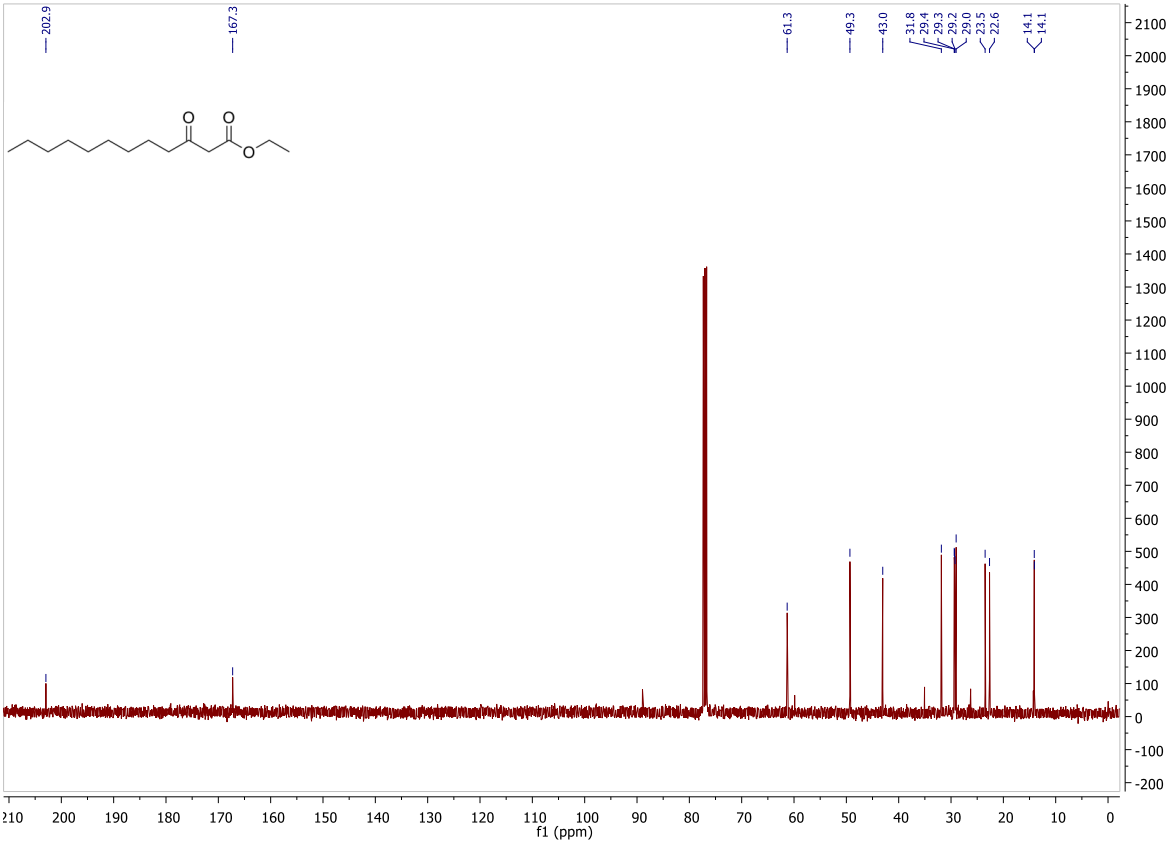




Ethyl 3-oxolaurate

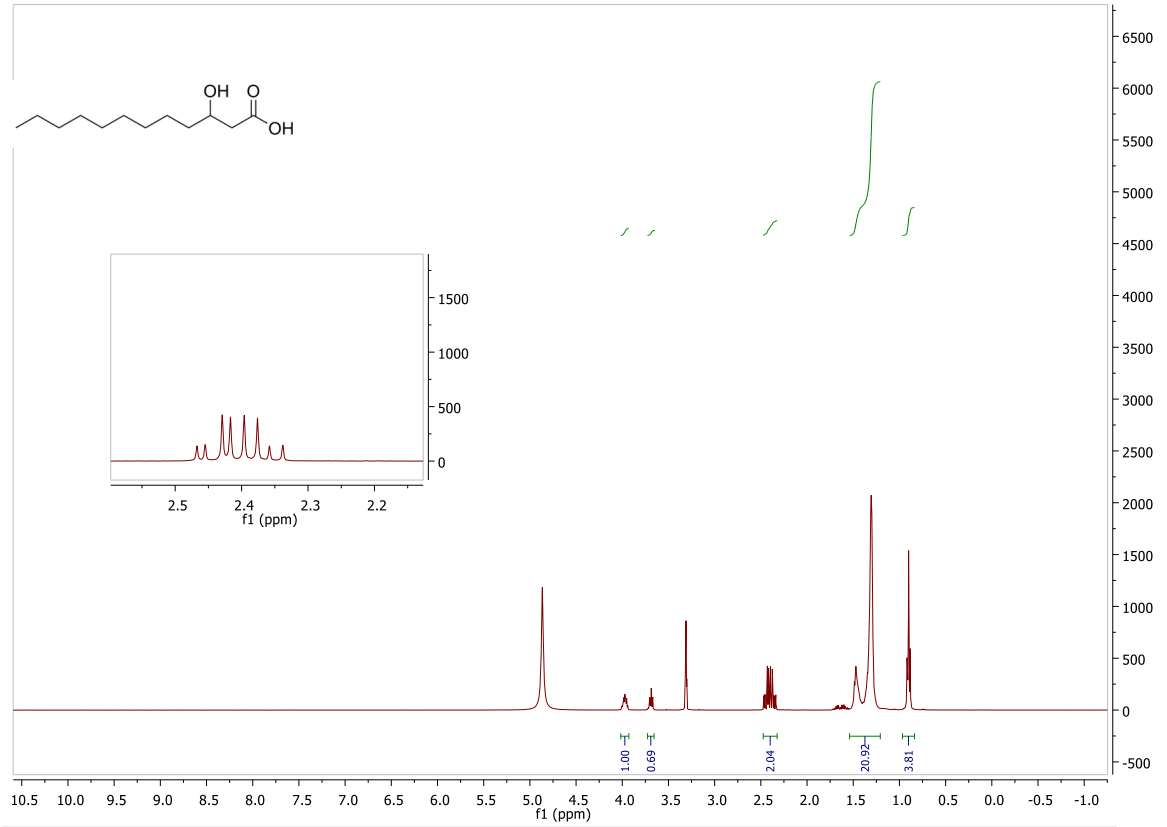
^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$)

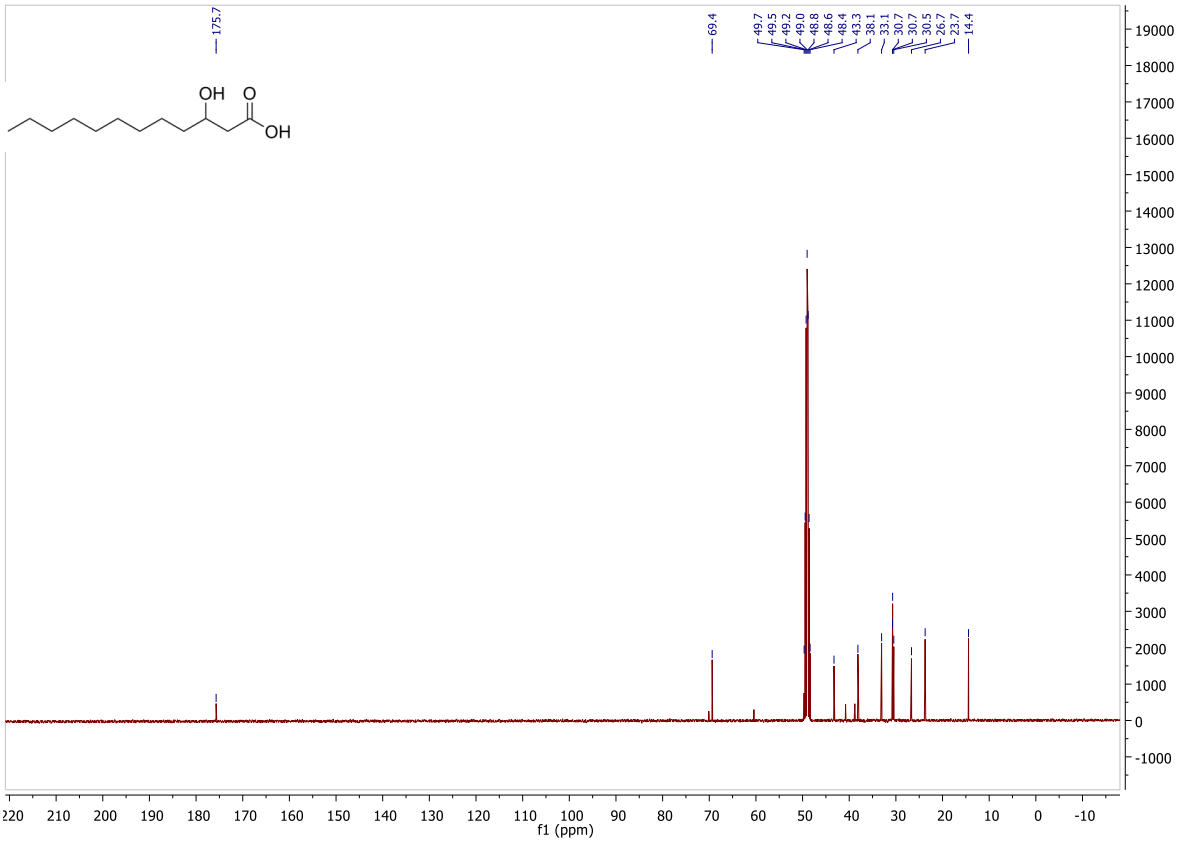




3-Hydroxylauric acid

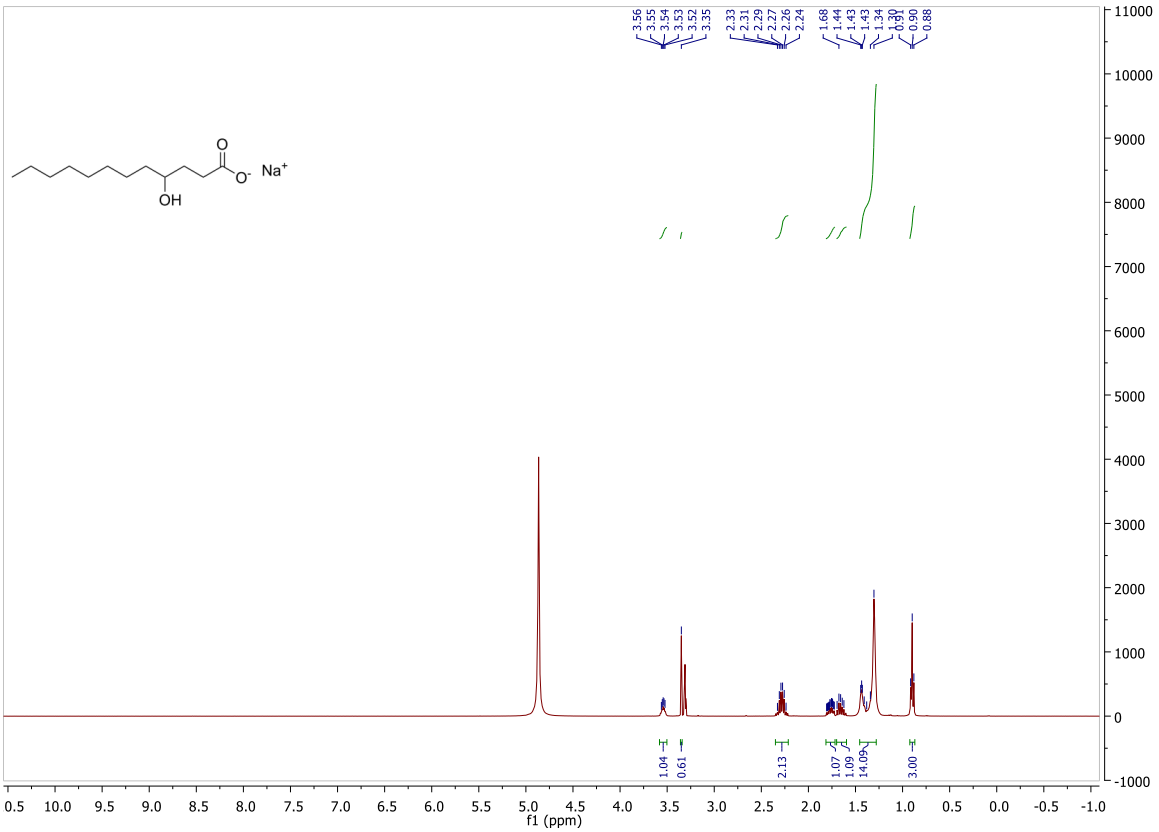
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

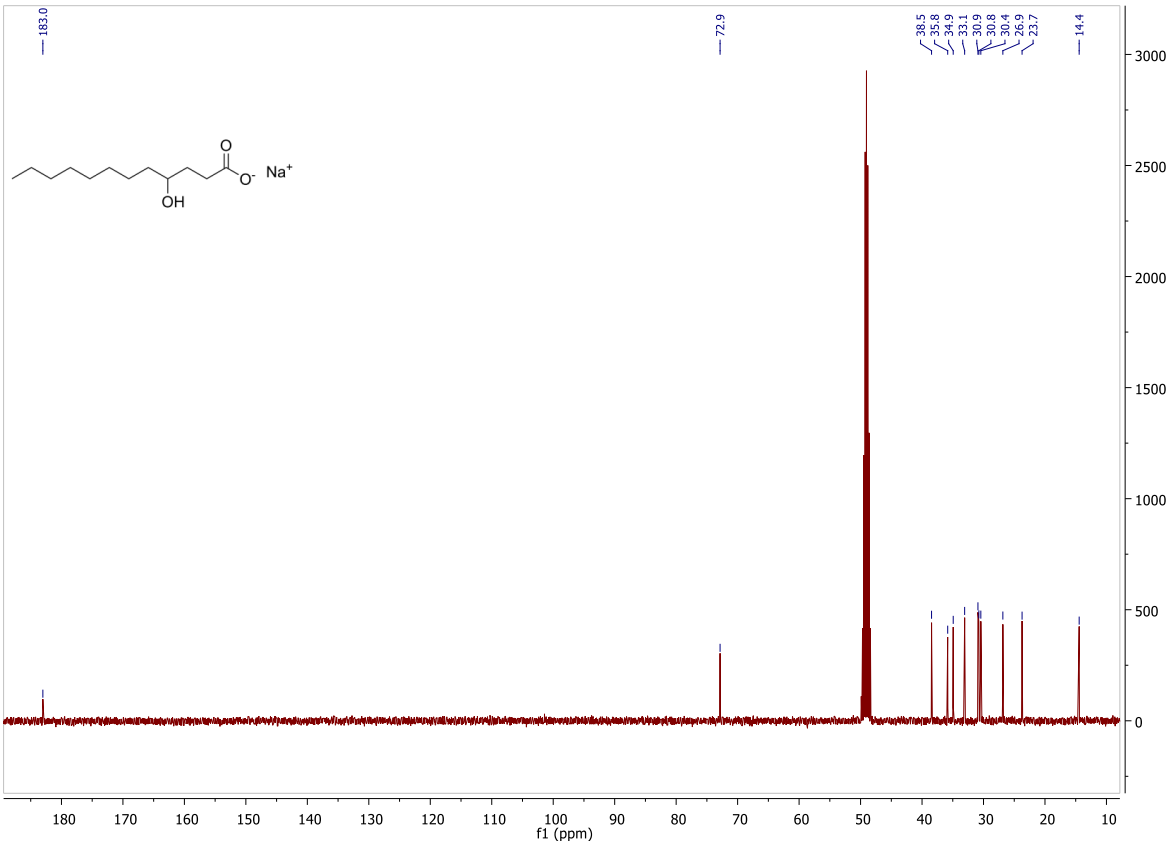




Sodium 4-hydroxylaurate

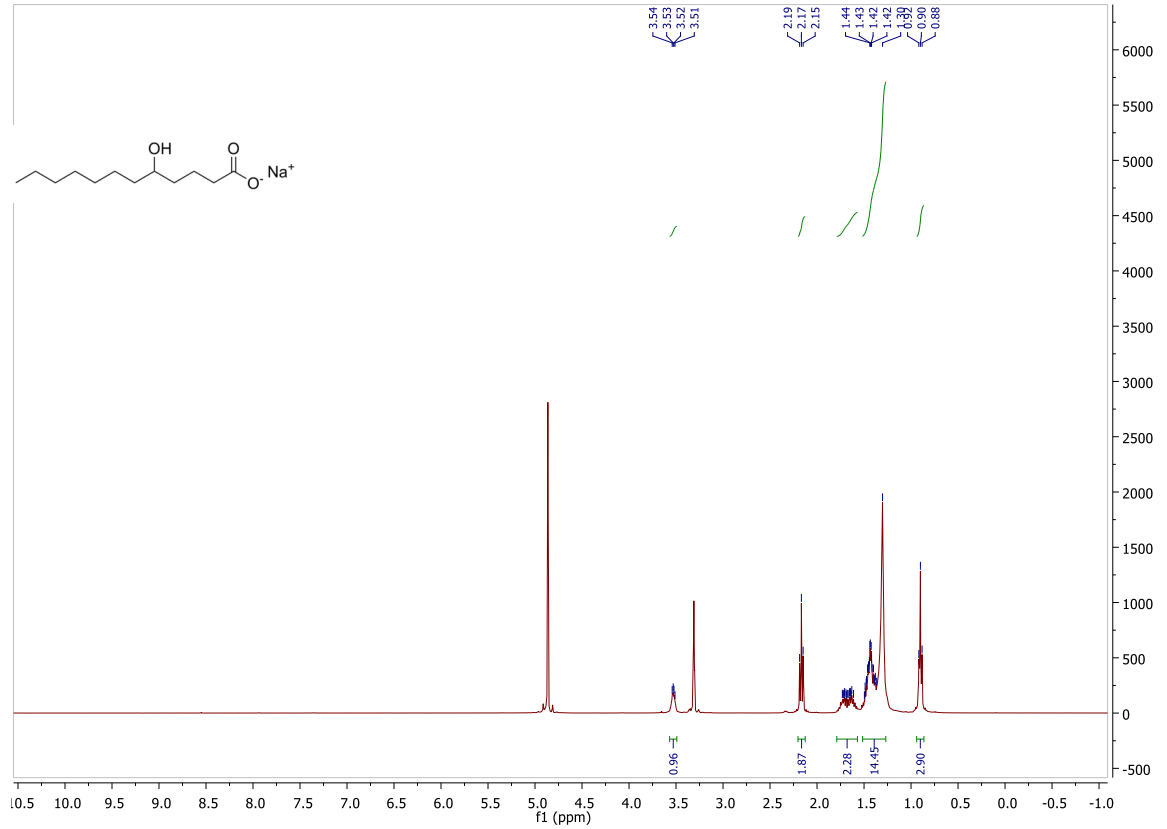
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

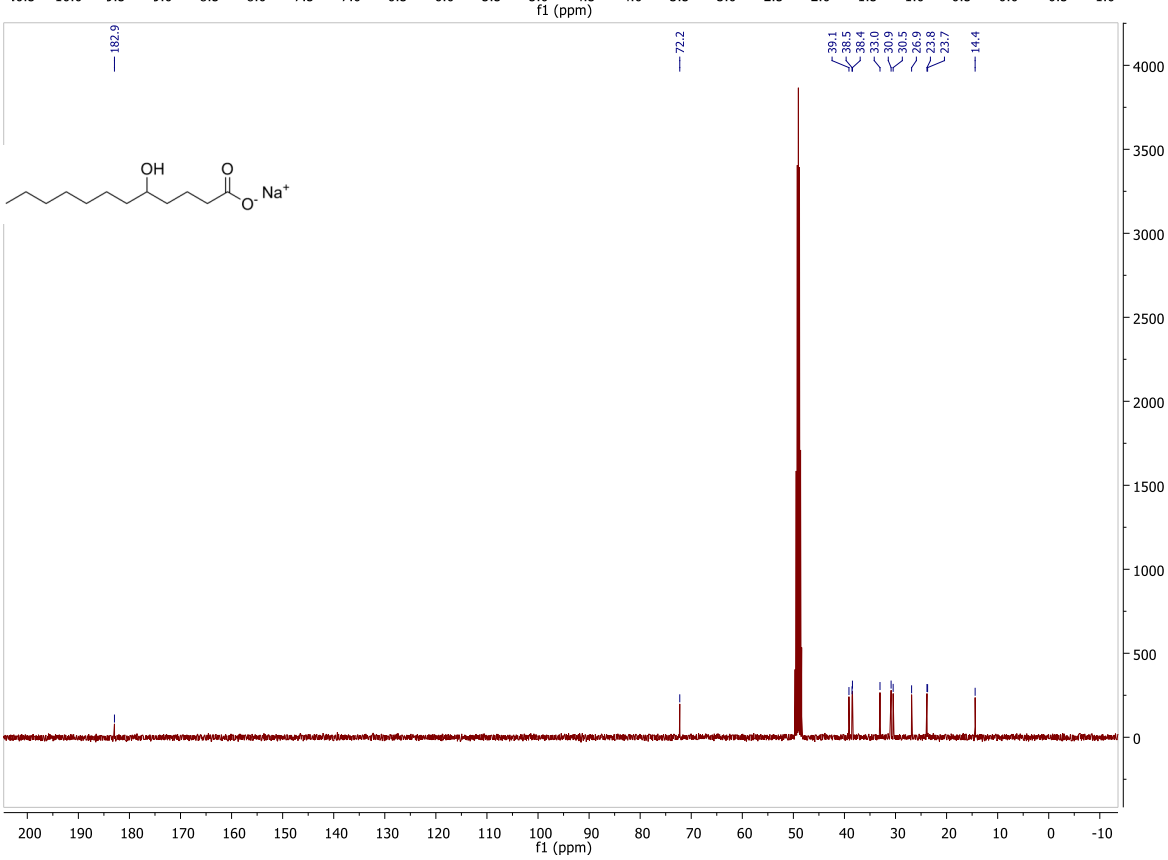




Sodium 5-hydroxylaurate

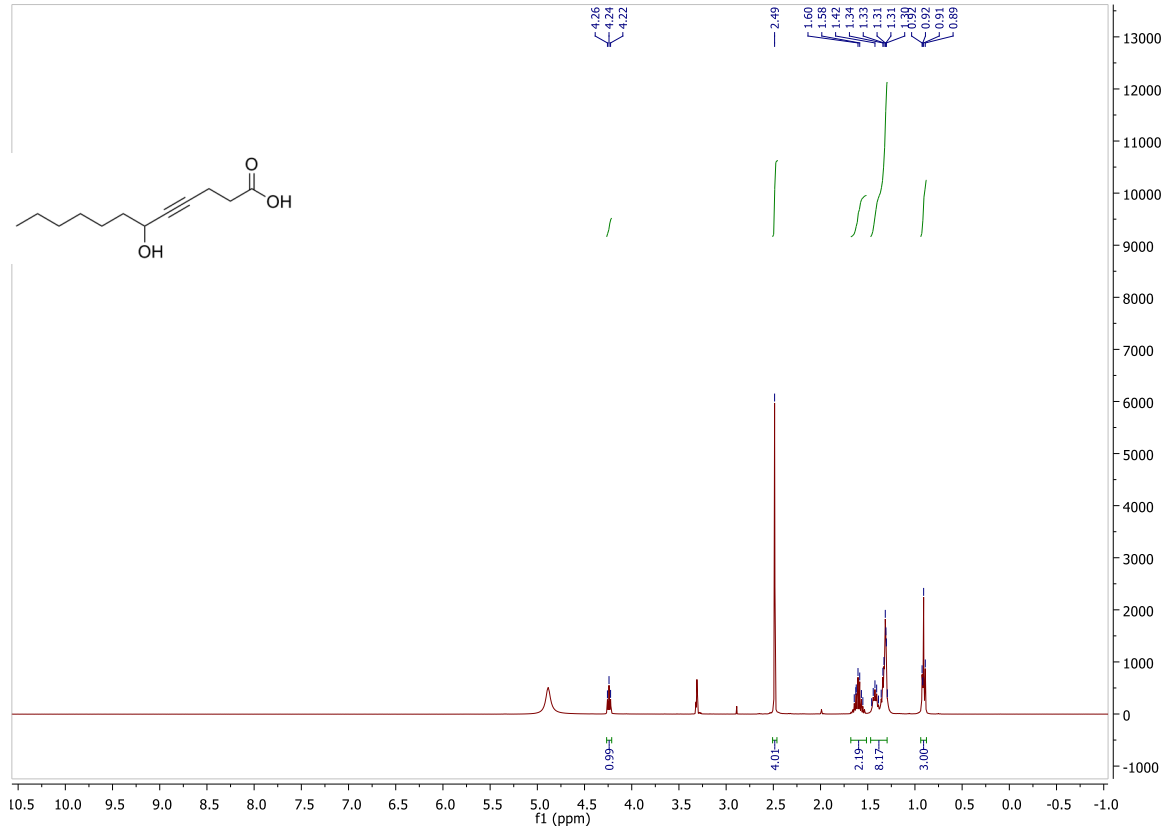
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

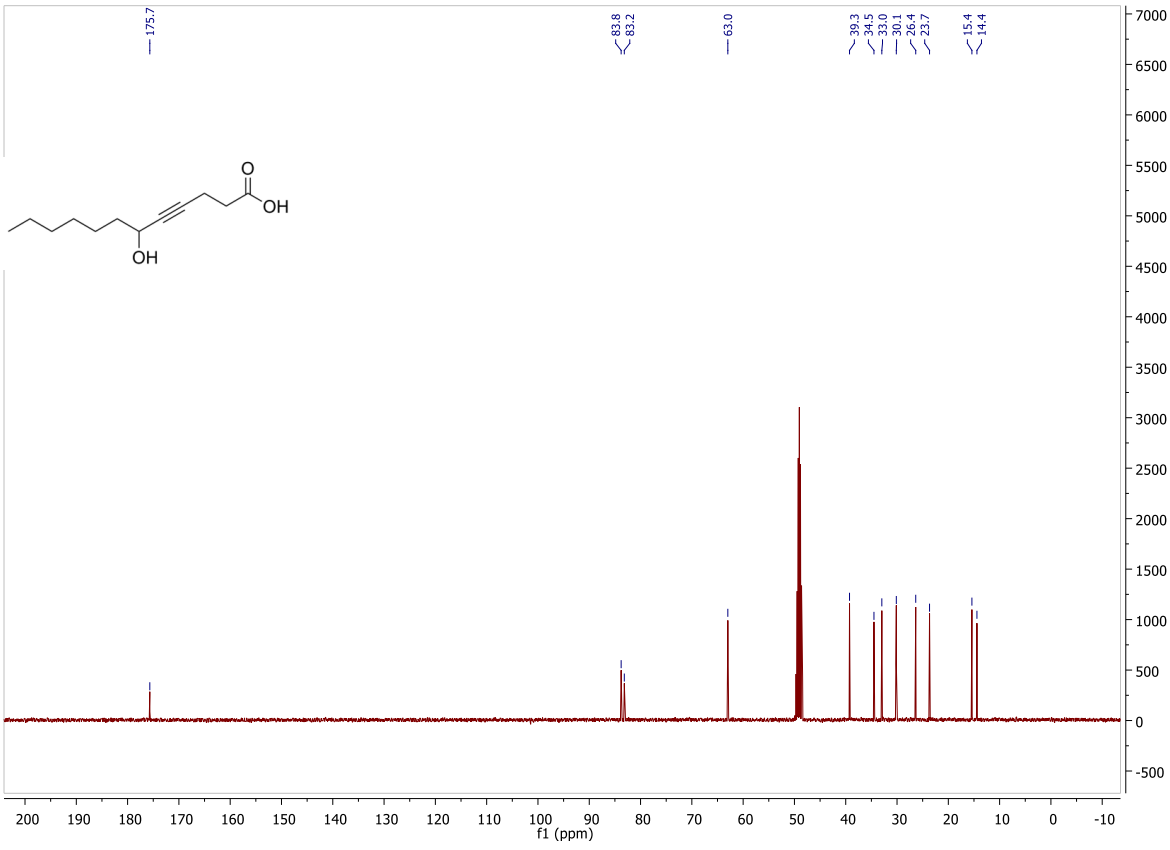




6-Hydroxy-4-dodecynoic acid

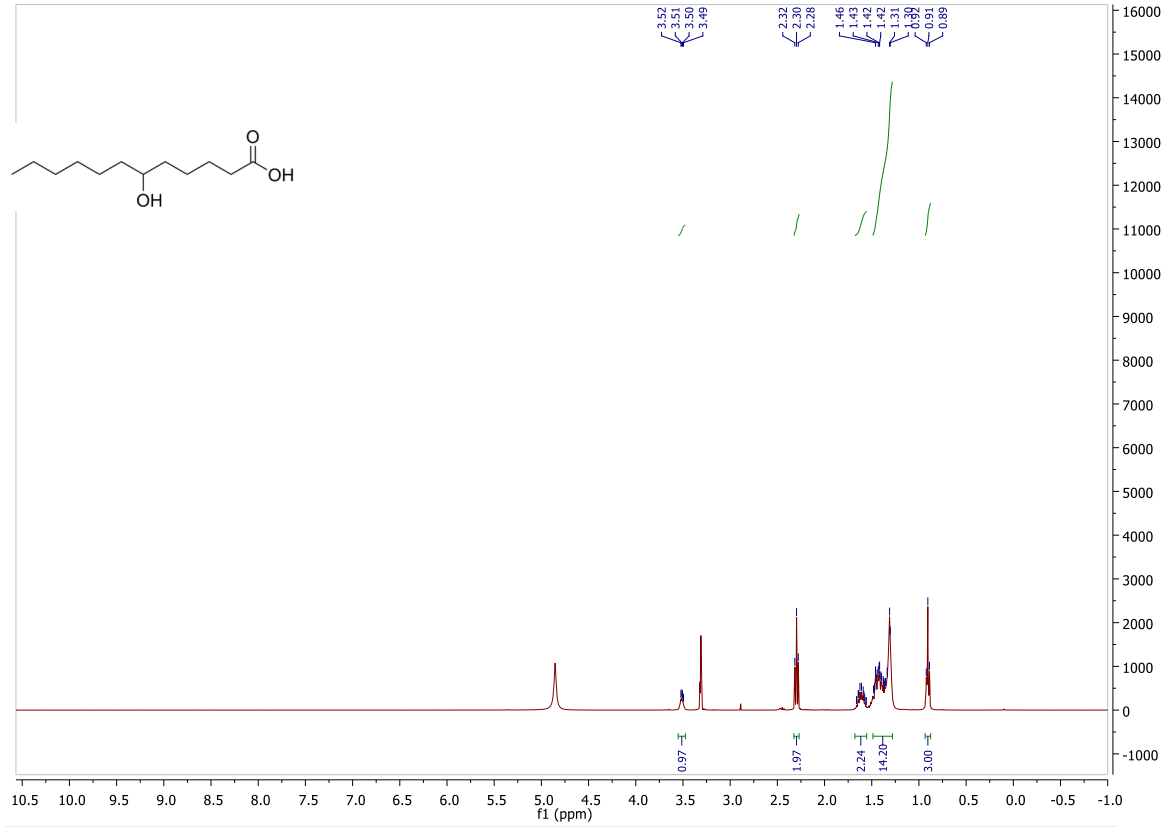
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

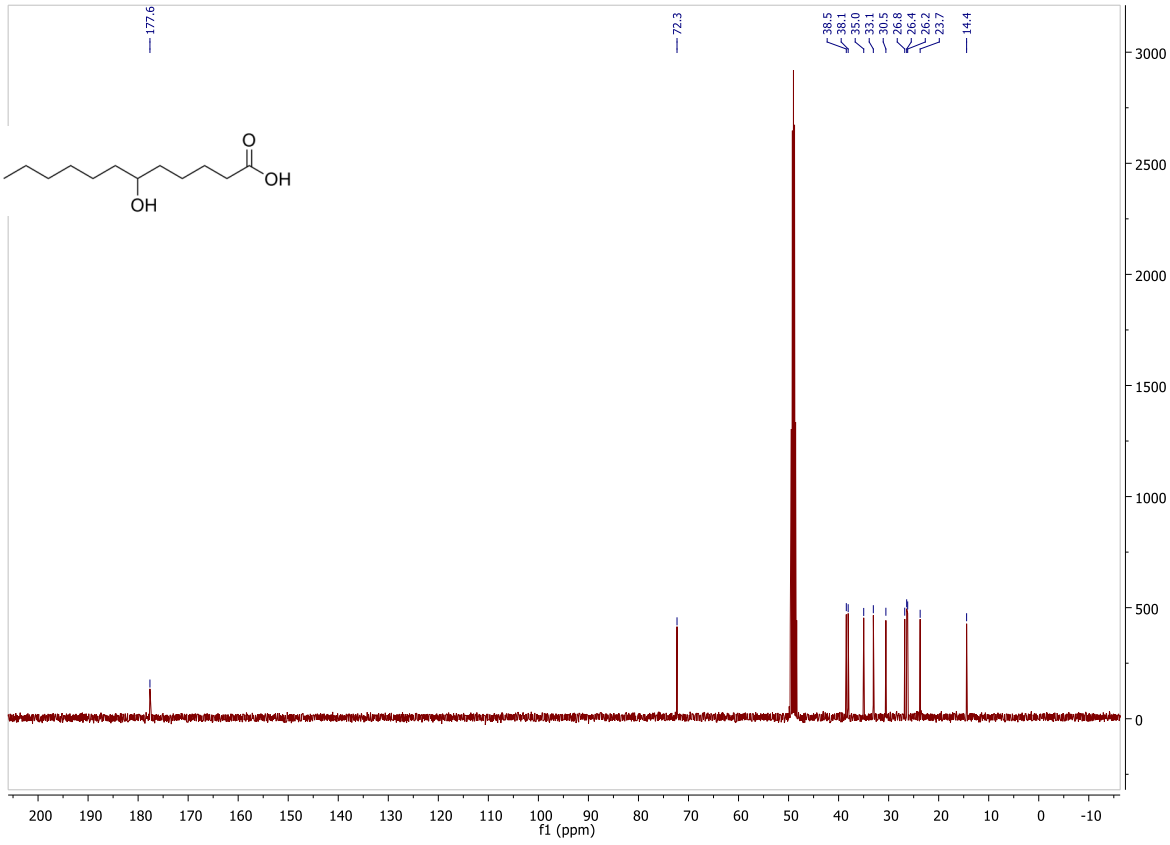




6-Hydroxylauric acid

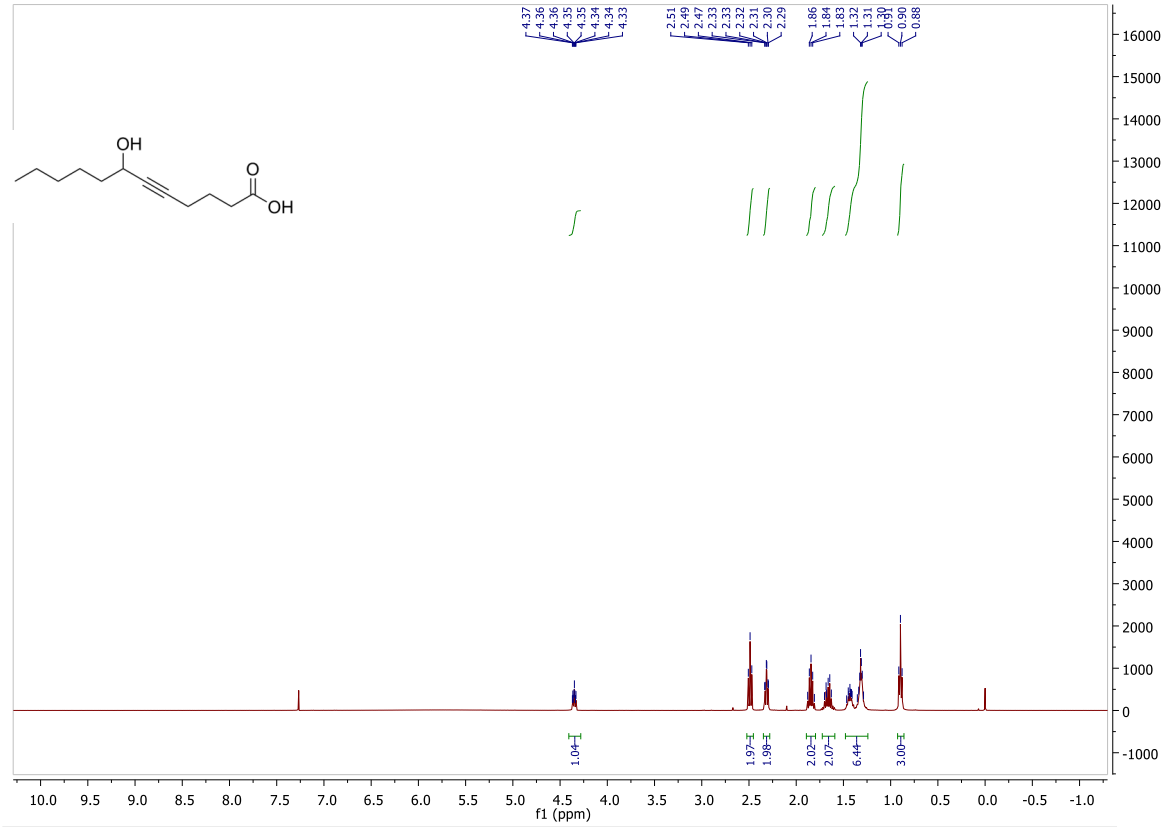
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

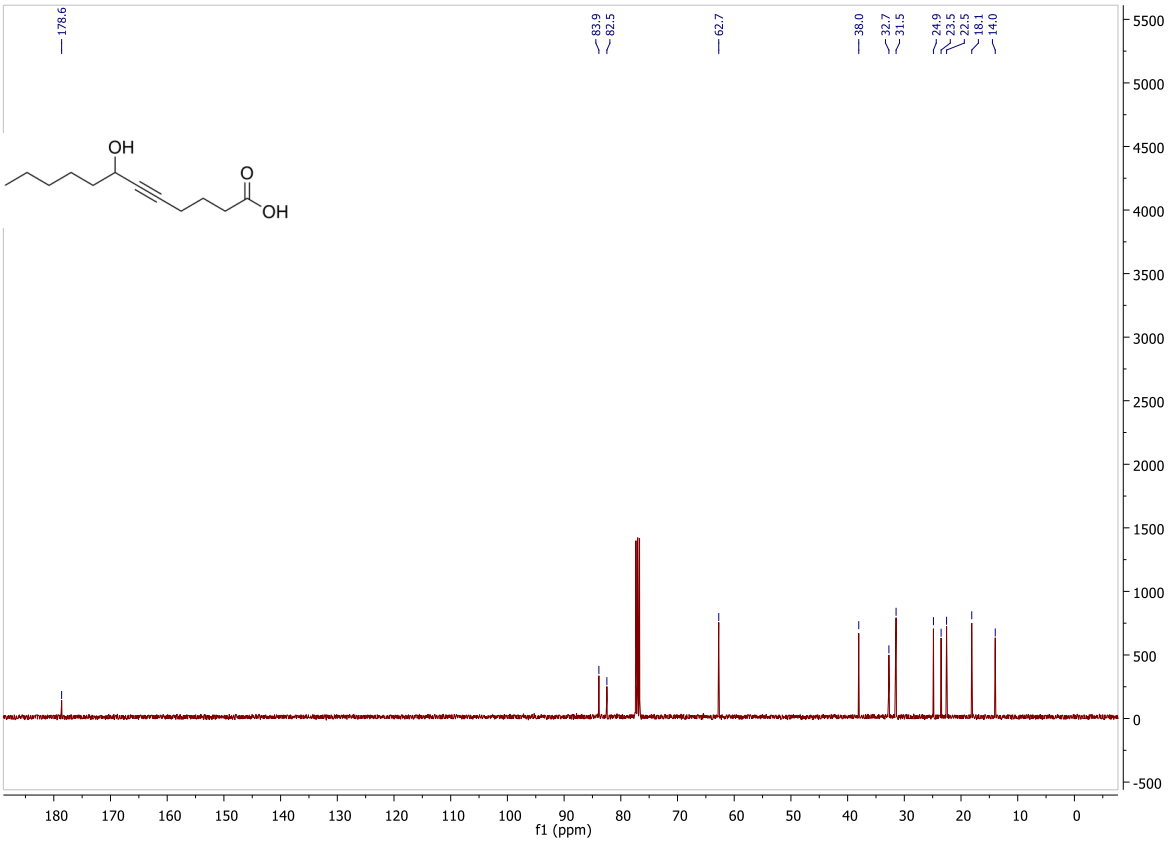




7-Hydroxy-5-dodecynoic acid

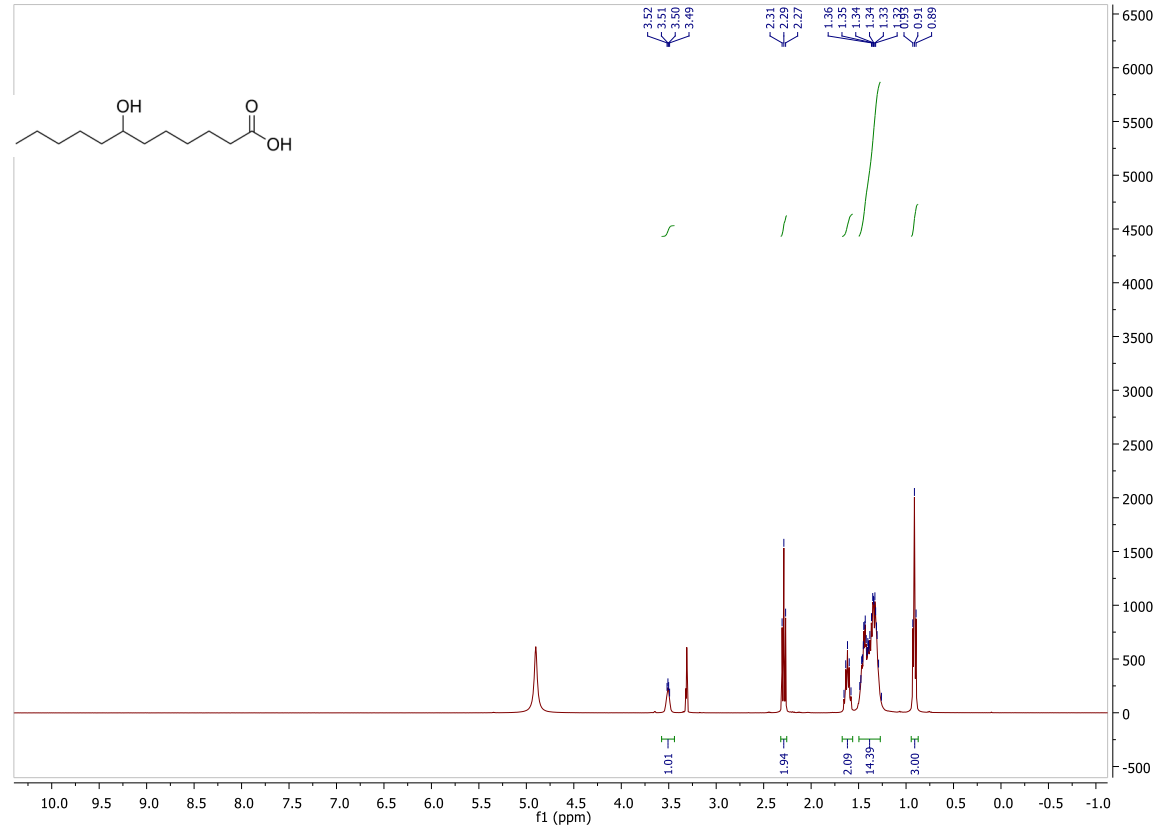
^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$)

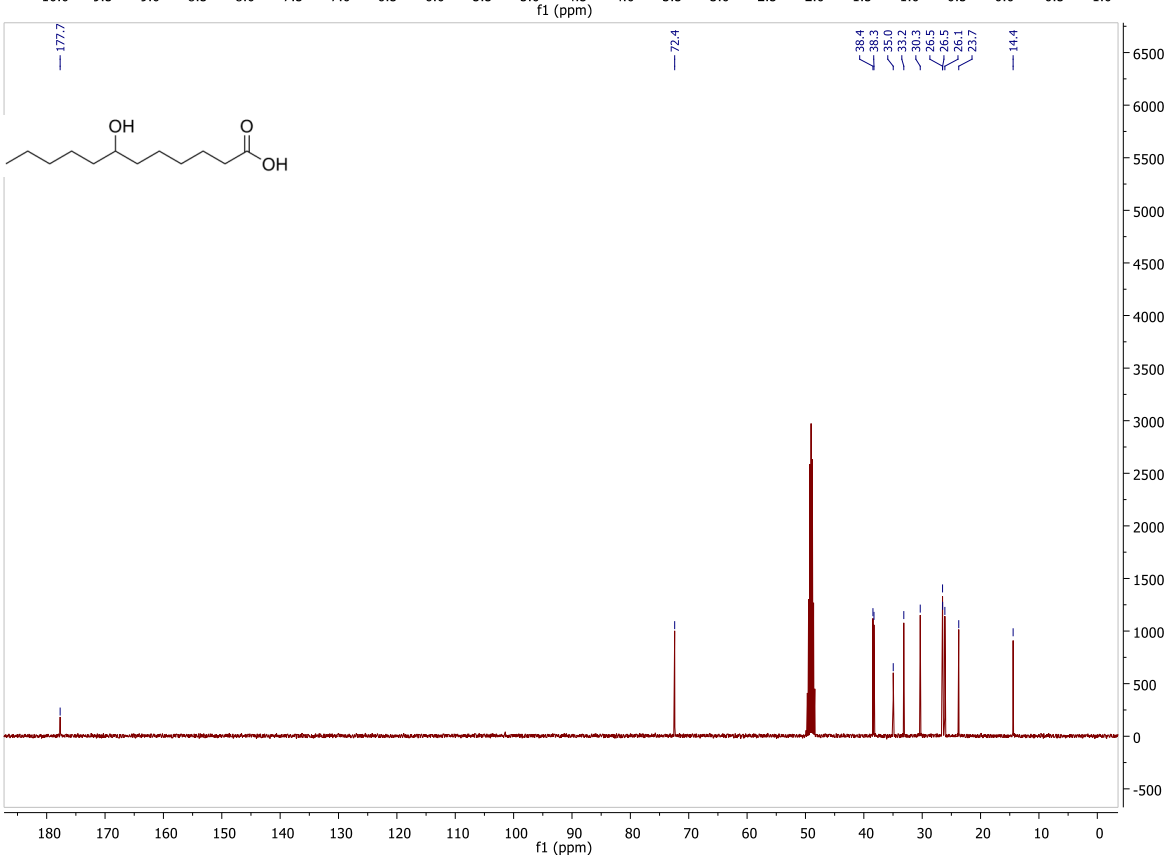




7-Hydroxylauric acid

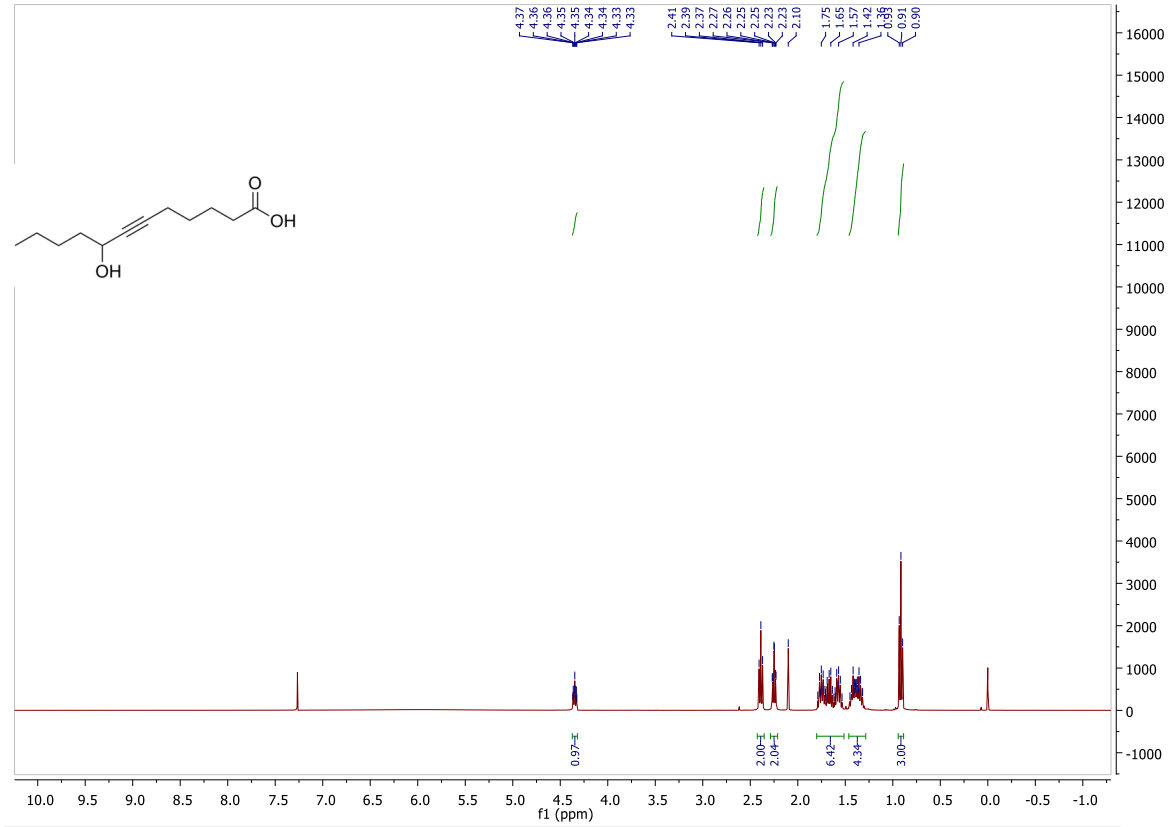
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

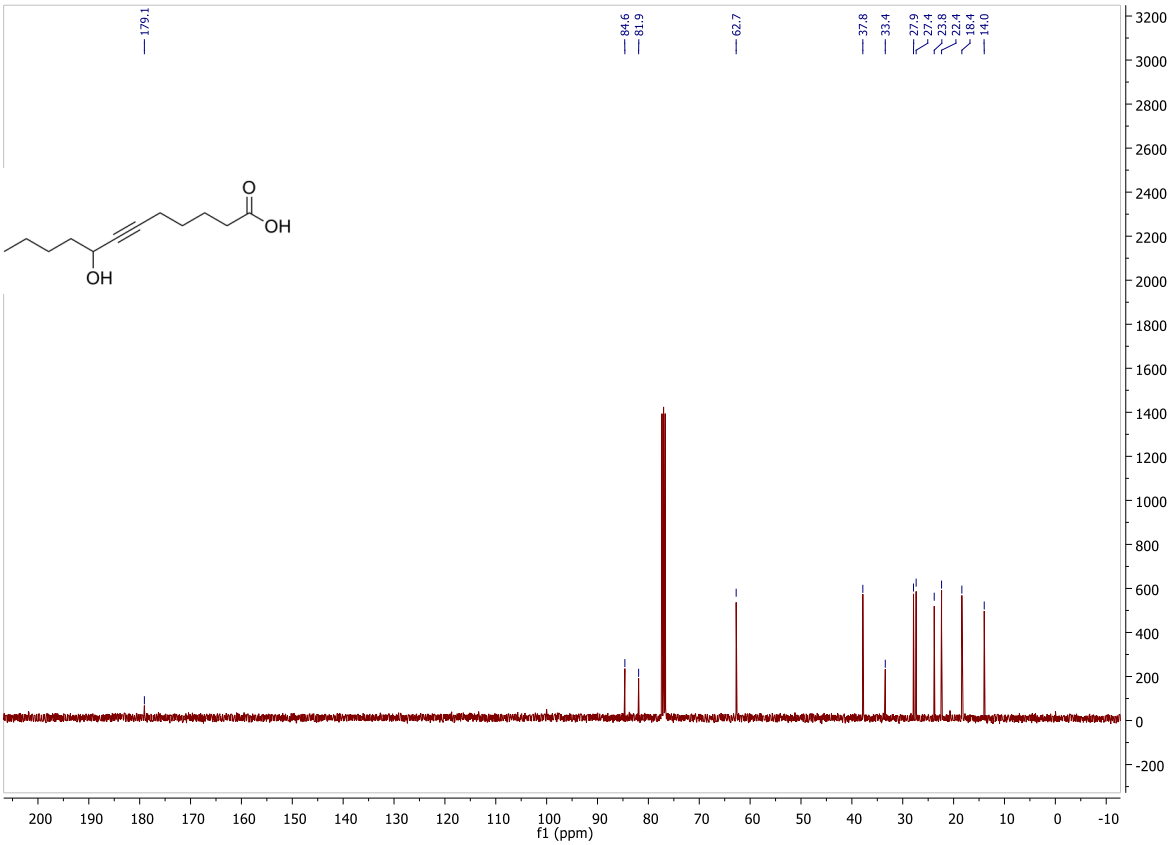




8-Hydroxy-6-dodecynoic acid

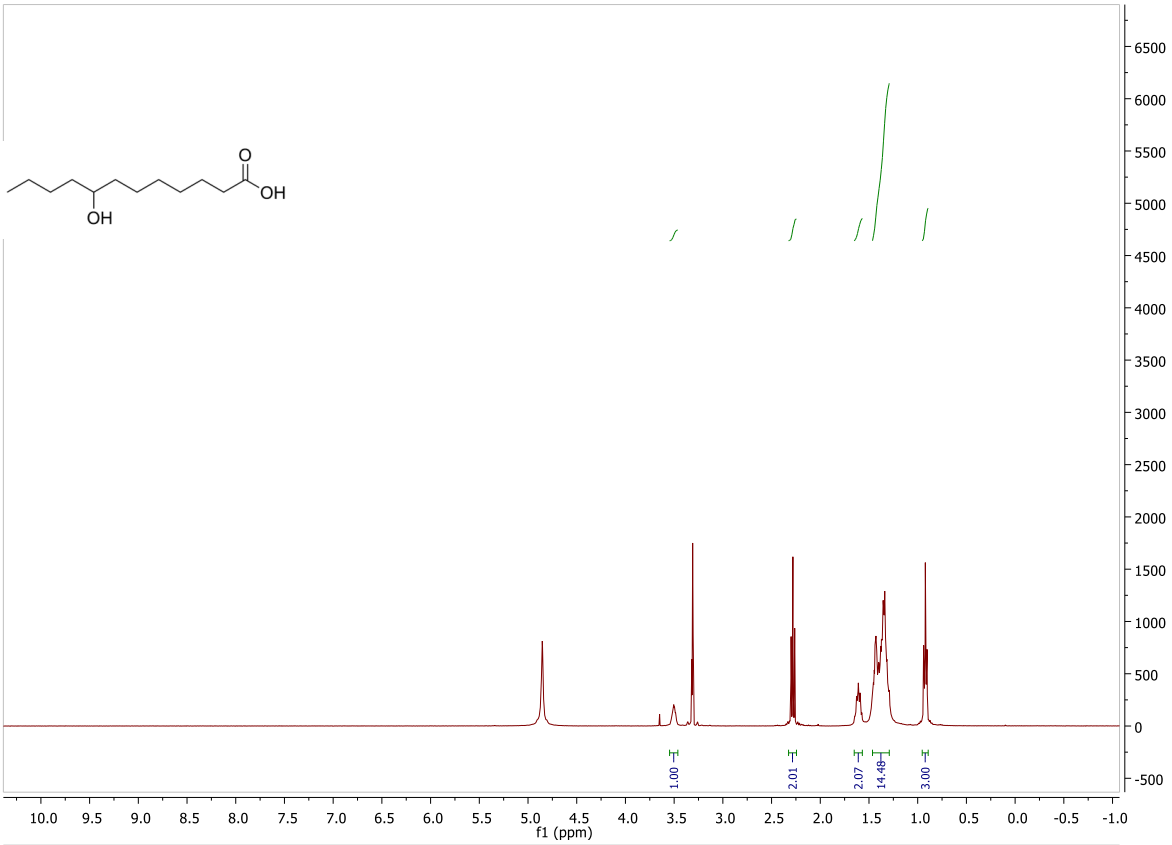
^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$)

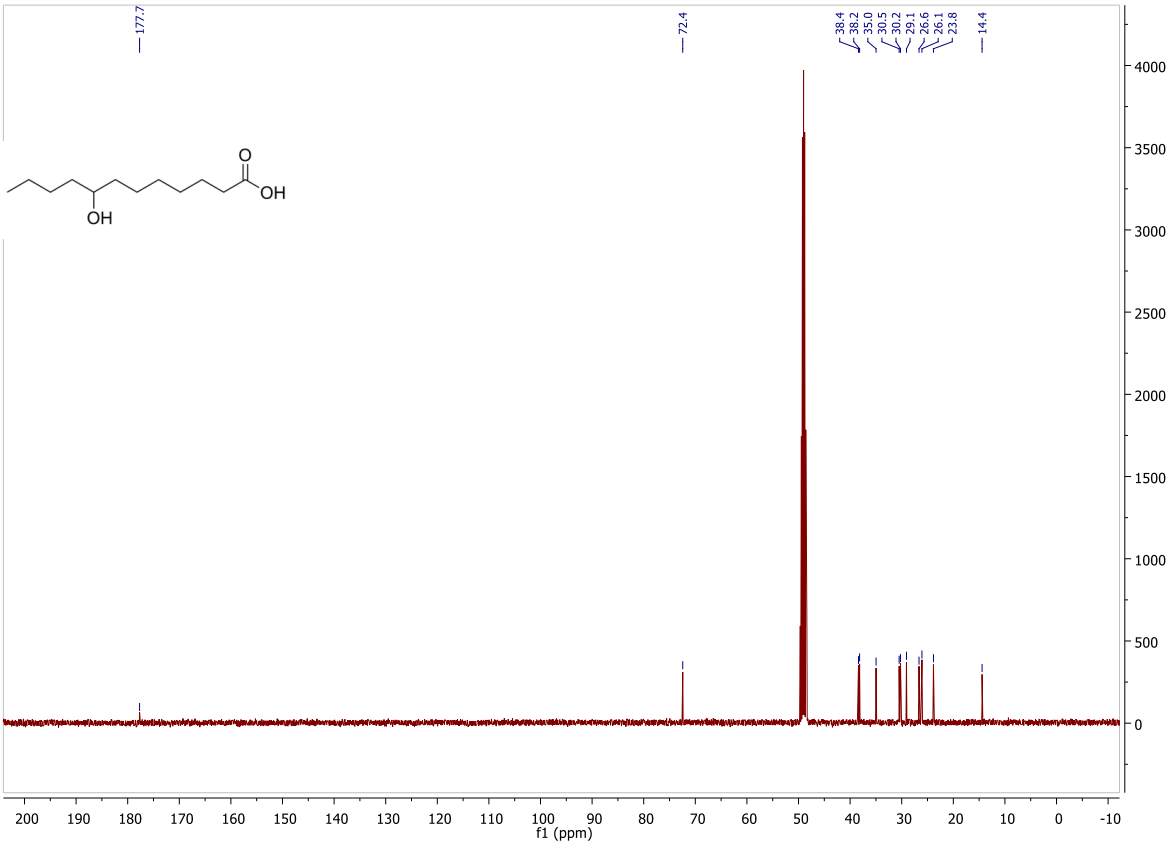




8-Hydroxylauric acid

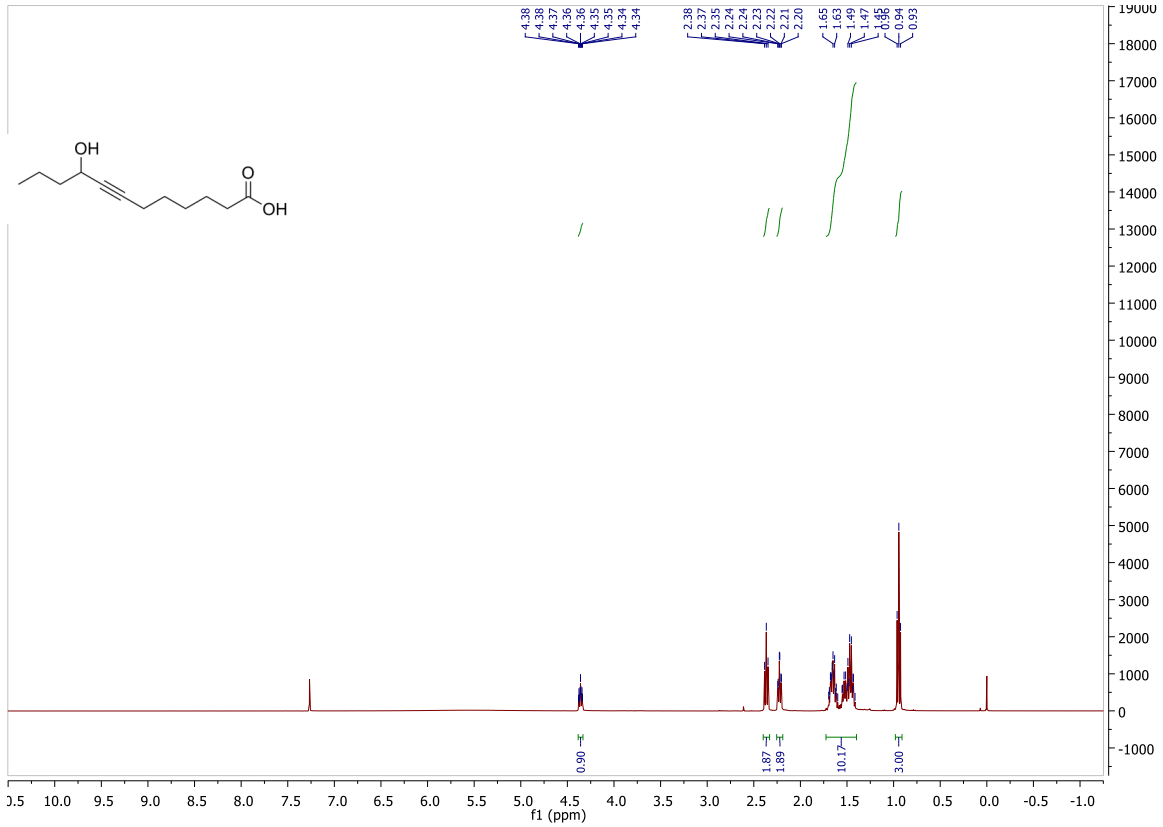
^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)

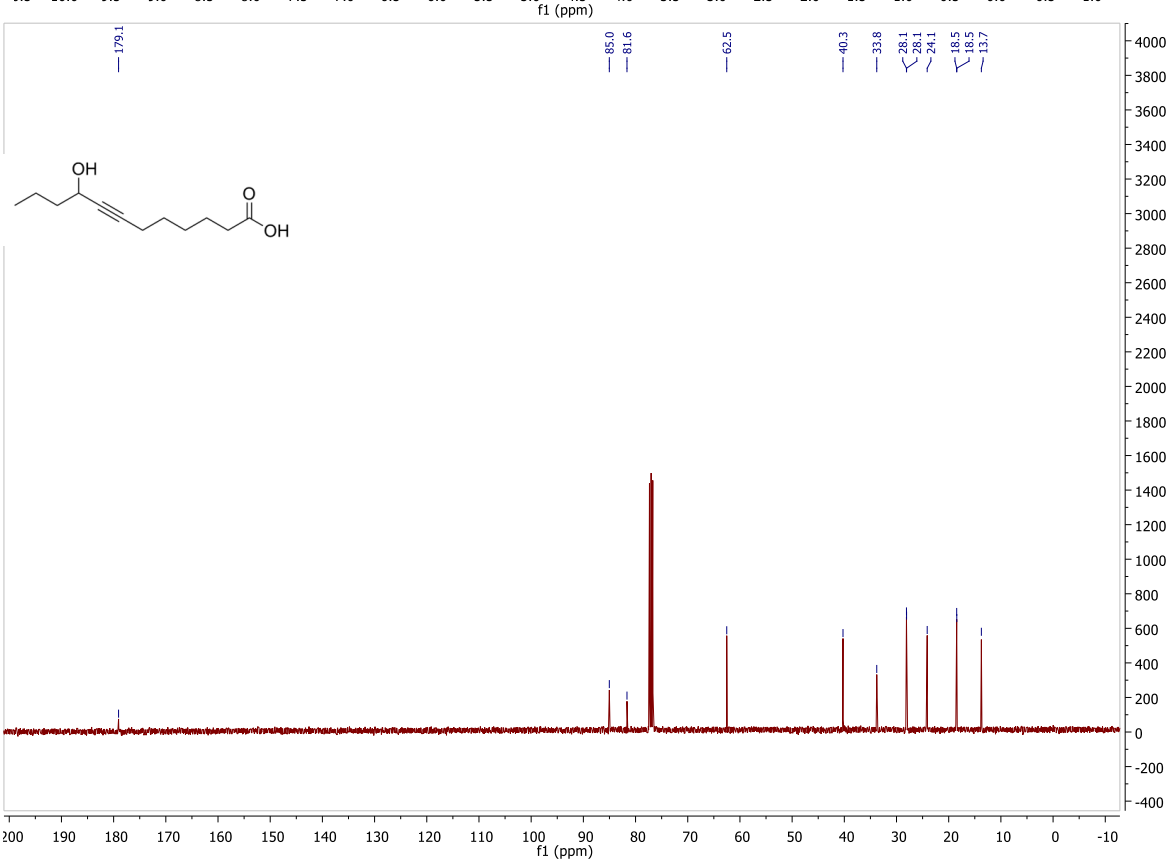




9-Hydroxy-7-dodecynoic acid

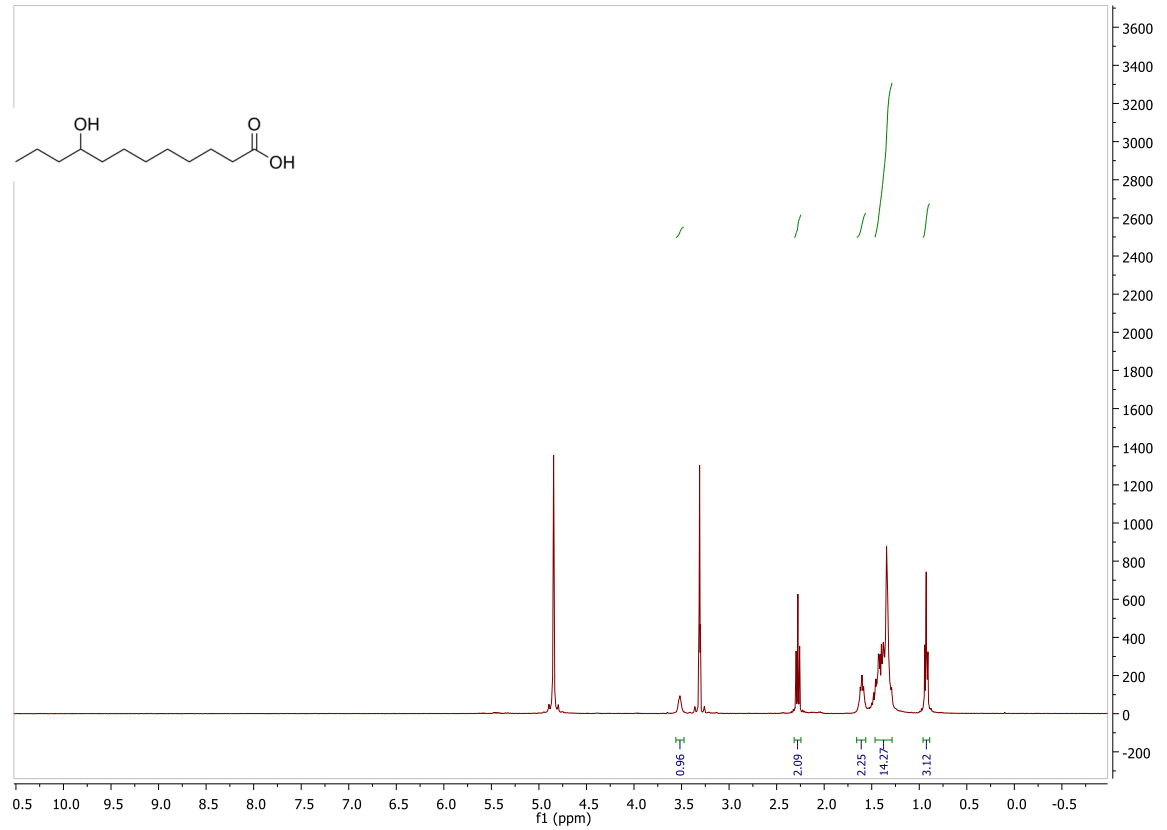
^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$)

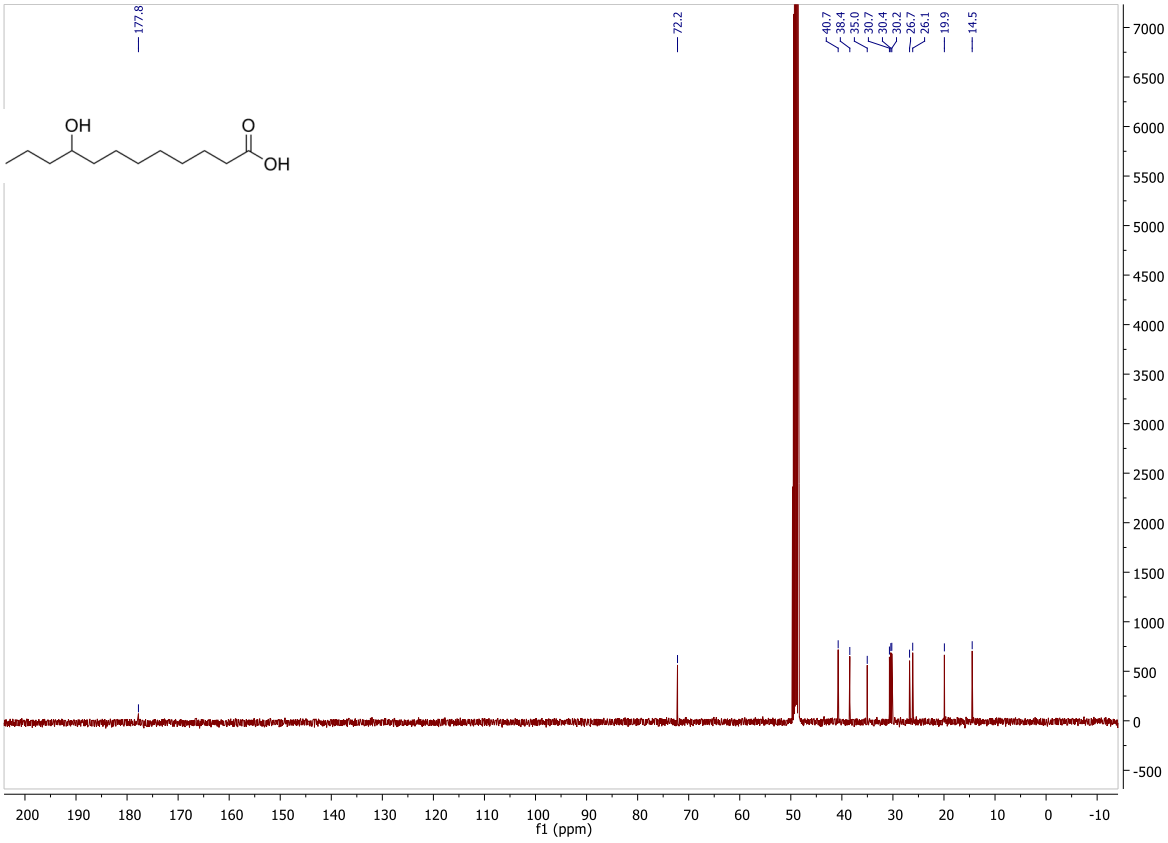




9-Hydroxylauric acid

^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)





10-Hydroxy-8-dodecynoic acid

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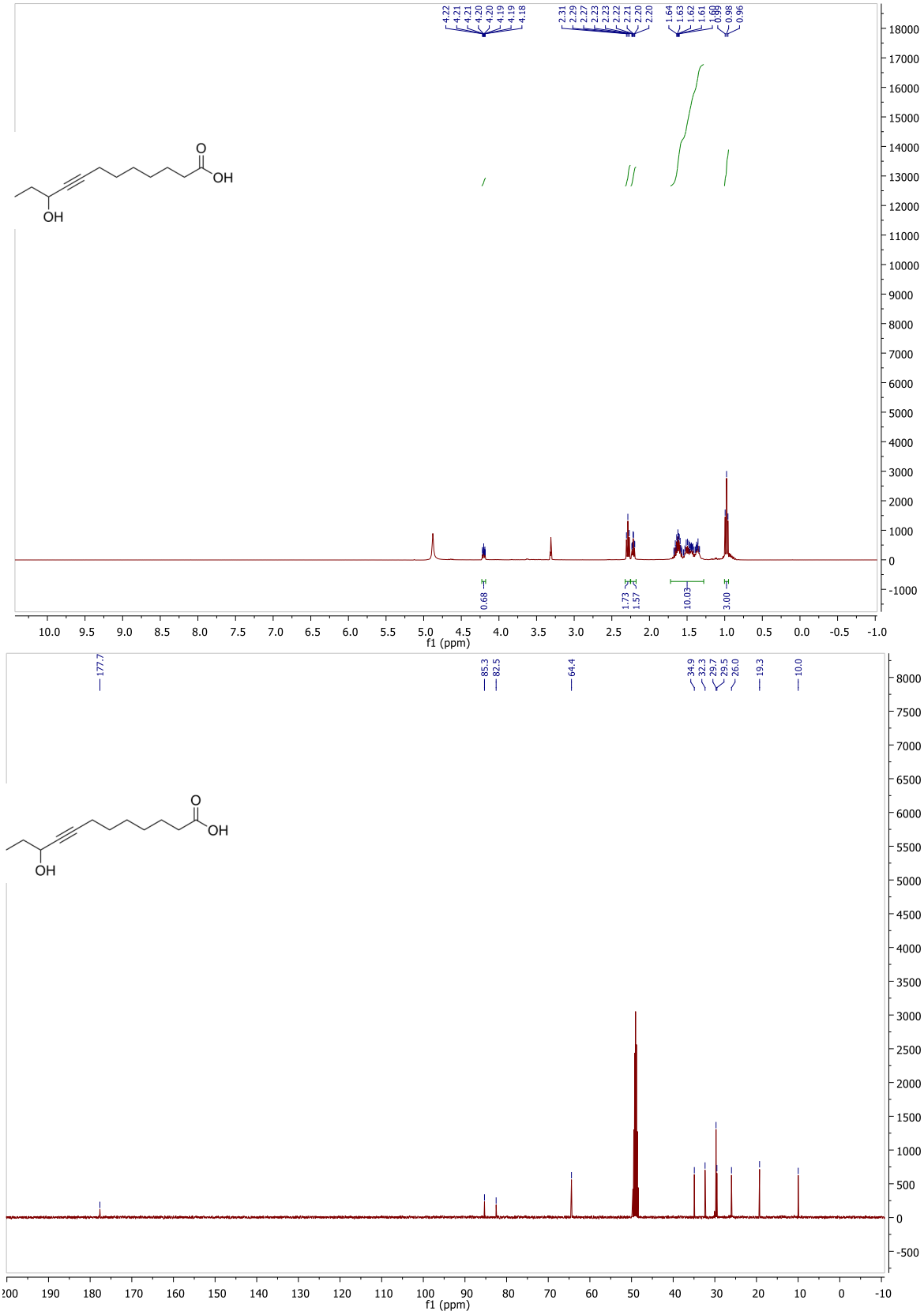
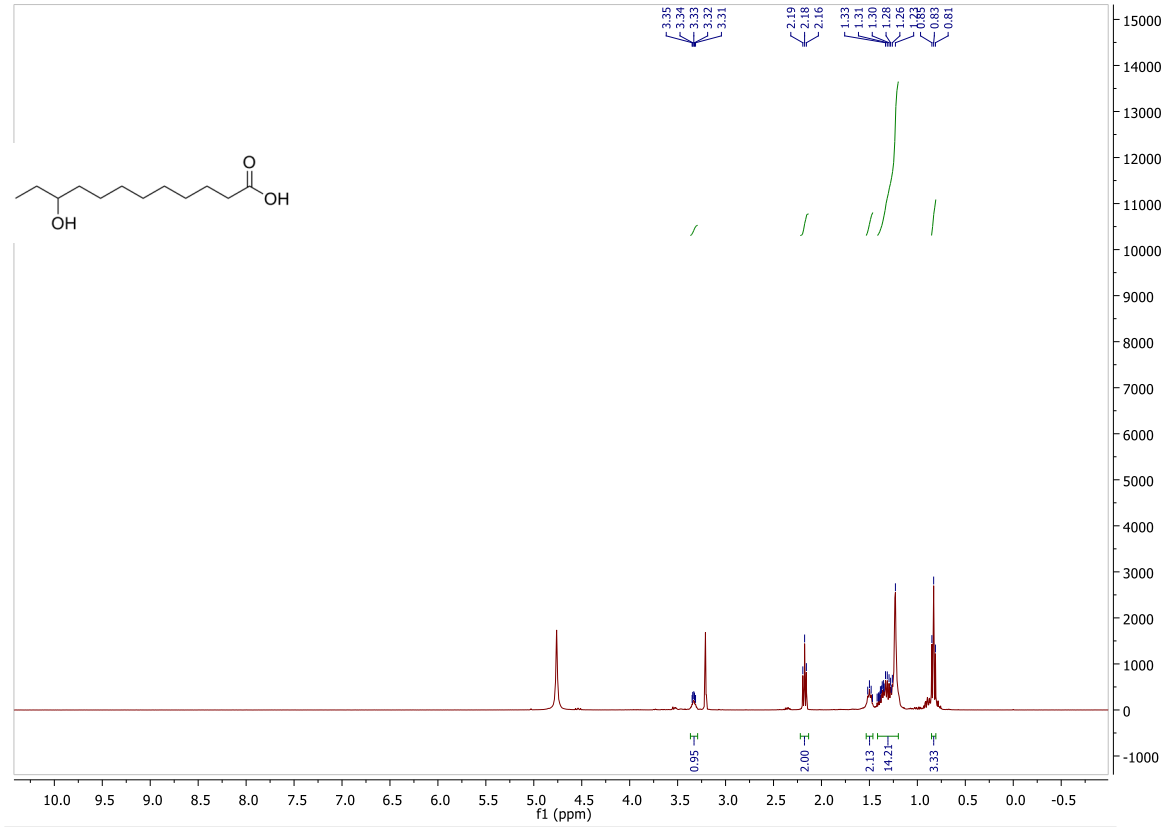
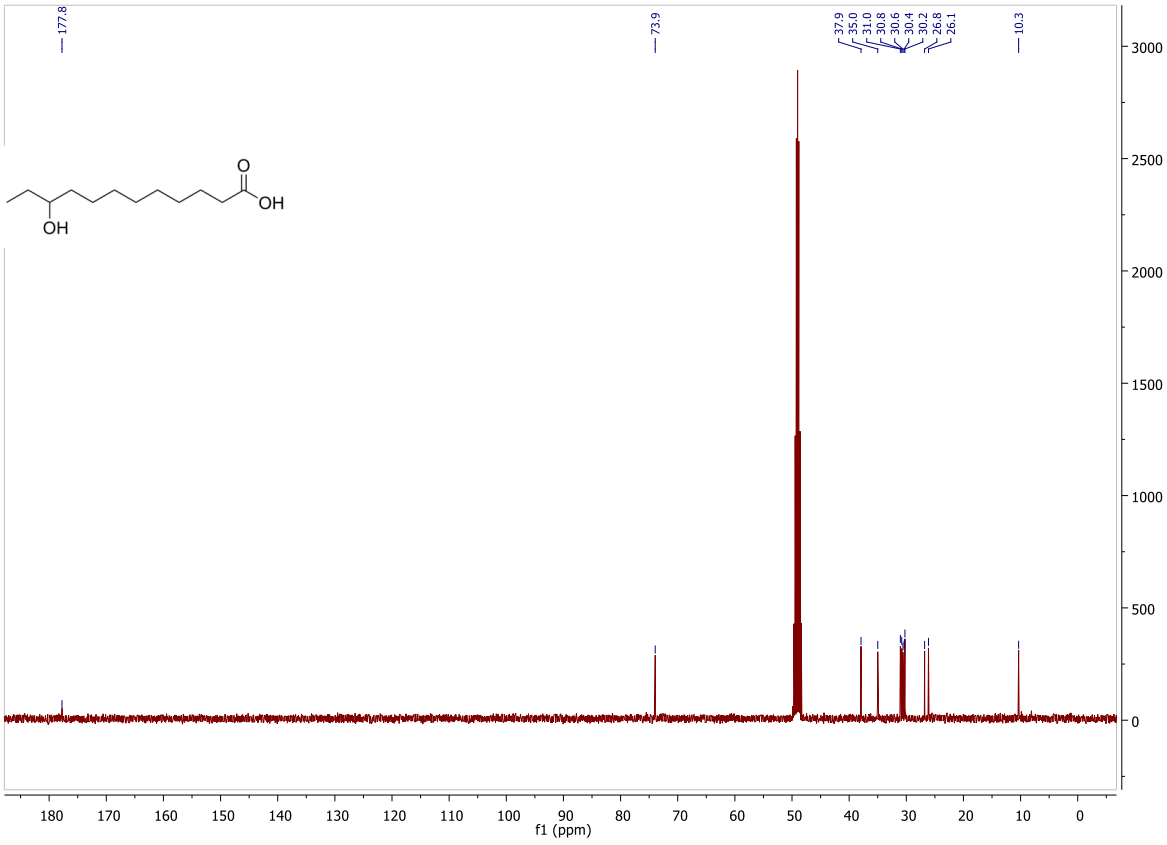


10-Hydroxylauric acid

^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)





11-Hydroxy-9-dodecynoic acid

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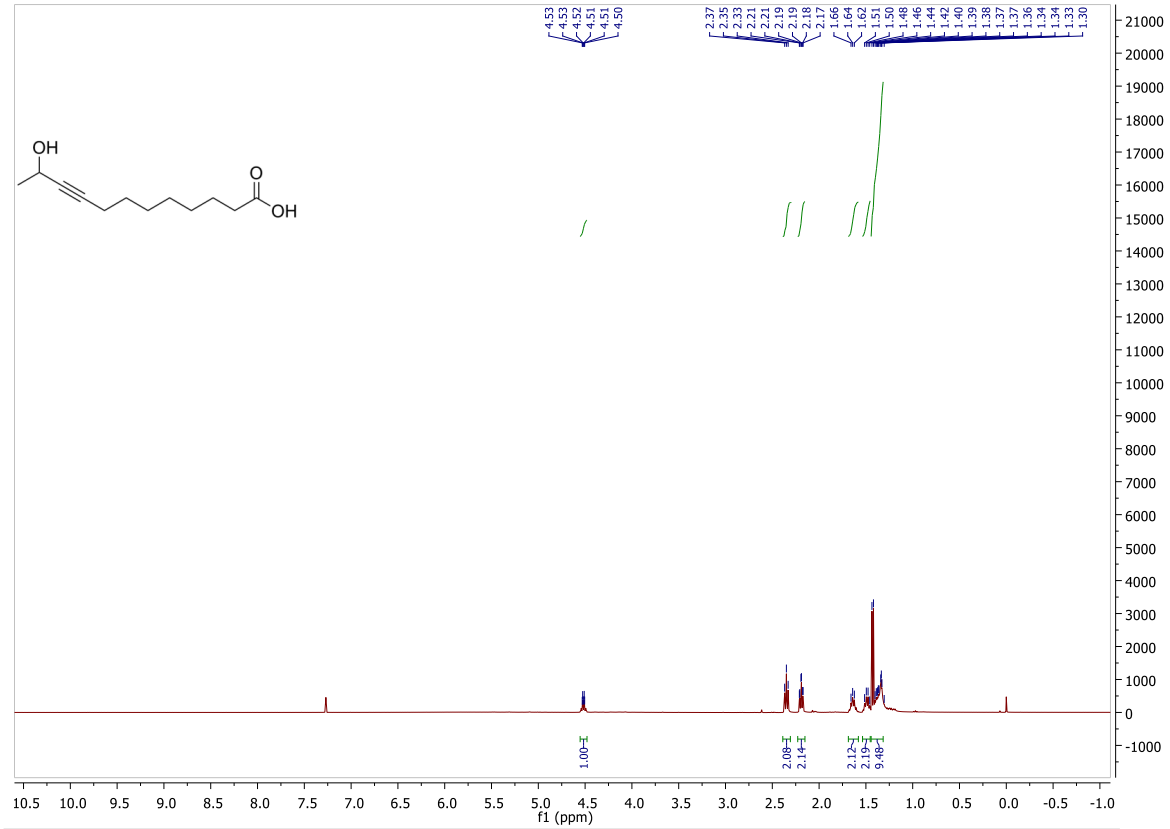

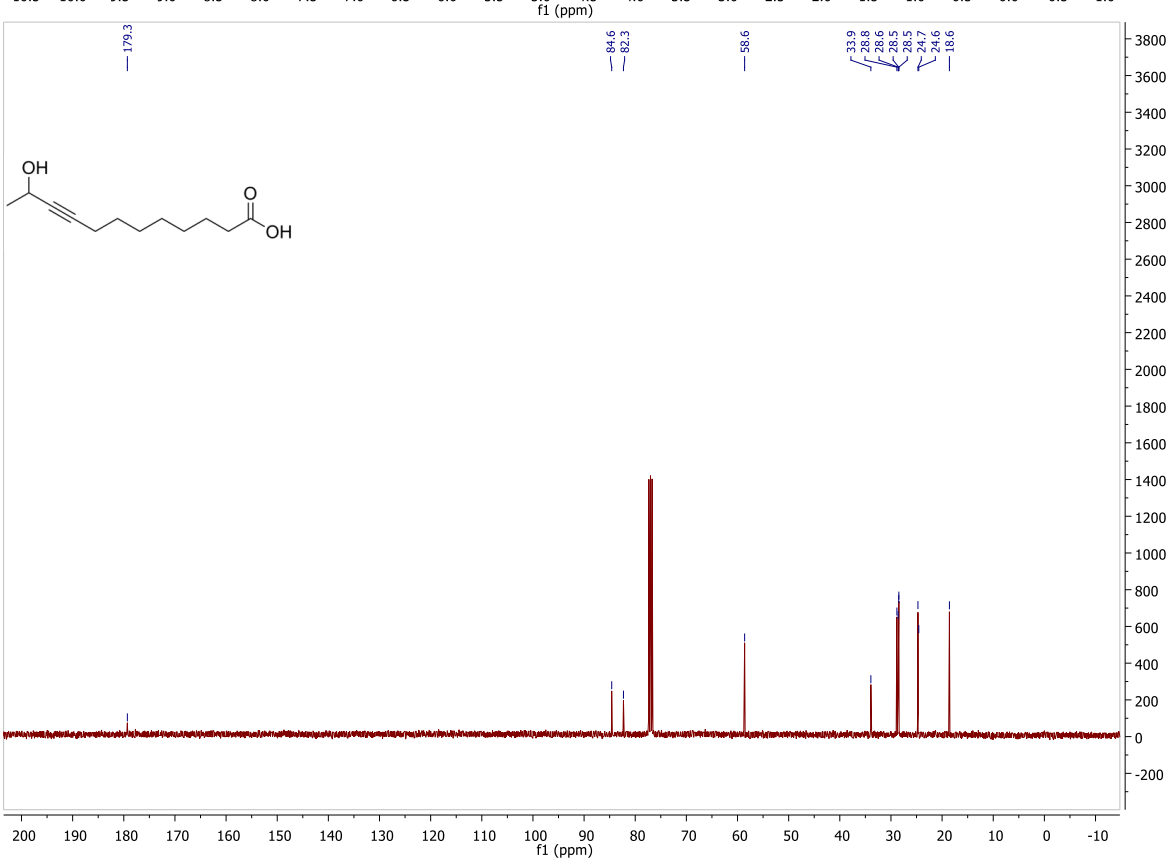

11-Hydroxylauric acid

^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (101 MHz, CD_3OD)