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Supplementary Information

Substrate scope for trimethyllysine hydroxylase catalysis

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Contents

1. TMLH production and purification

An MBP-TMLHa fusion construct (TMLHa residues 40-417) was expressed and purified according to a procedure based on the protocol described by Kazaks et al..[1] Briefly; competent *E.coli* BL21-AI cells, containing the pGro7 plasmid encoding for groEL-groES (Clontech #3340), were transformed with the plasmid encoding for the MBP-TMLHa fusion construct. A positive clone was selected and the fusion construct was expressed in TB medium supplemented with trace metals (50 μM $FeCl_3$, 20 μM $CaCl_2$, 10 μM $MnCl_2$ and $ZnSO_4$, 2 μM $CoCl_2$, $CuCl_2$, $NiCl_2$, $NaMoO_4$, Na_2SeO_3 and H_3BO_3) at 37°C, 200rpm. At OD_{600}~0.6 the cells were induced with 0.1mM IPTG and 1% L-Arabinose and cultured for 4 hours at 37°C, after which the cells were harvested and lysed. MBP-TMLHa was then purified by Ni-NTA affinity followed by size exclusion chromatography on a superdex 200 increase 10/300 GL at 1 mL min^{-1}, using 20 mM K_2HPO_4 (pH 7.0), 20 mM KCl, 2 mM DTT as mobile phase.

2. LC-MS experiments

LC-MS enzymatic experiments were conducted at 310 K in phosphate buffer (20 mM) and KCl (20 mM) and DTT (2 mM) at pH 7.0. To a premixed solution of TMLH (3 μM), $FeSO_4$ (500 μM), 2OG (2.5 mM) and ascorbate (5 mM), was added trimethyllysine (500 μM). After shaking for 30 minutes at 37 °C in Eppendorf, the reaction mixutre (100 μL) was quenched with methanol (100 μL). Subsequently, the sample was analyzed by LC-MS and was performed on a Thermo Finnigan LCQ-Fleet ESI-ion trap (Thermofischer, Breda, the Netherlands) equipped with a Phenomenex Gemini-NX C18 column, 50 x 2.0 mm, particle size 3 μM (Phenomenex, Utrecht, The Netherlands). An acetonitrile/water gradient containing 0.1 % formic acid was used for elution (5-100 %, 1-50 min, flow 0.2 mL min^{-1}).

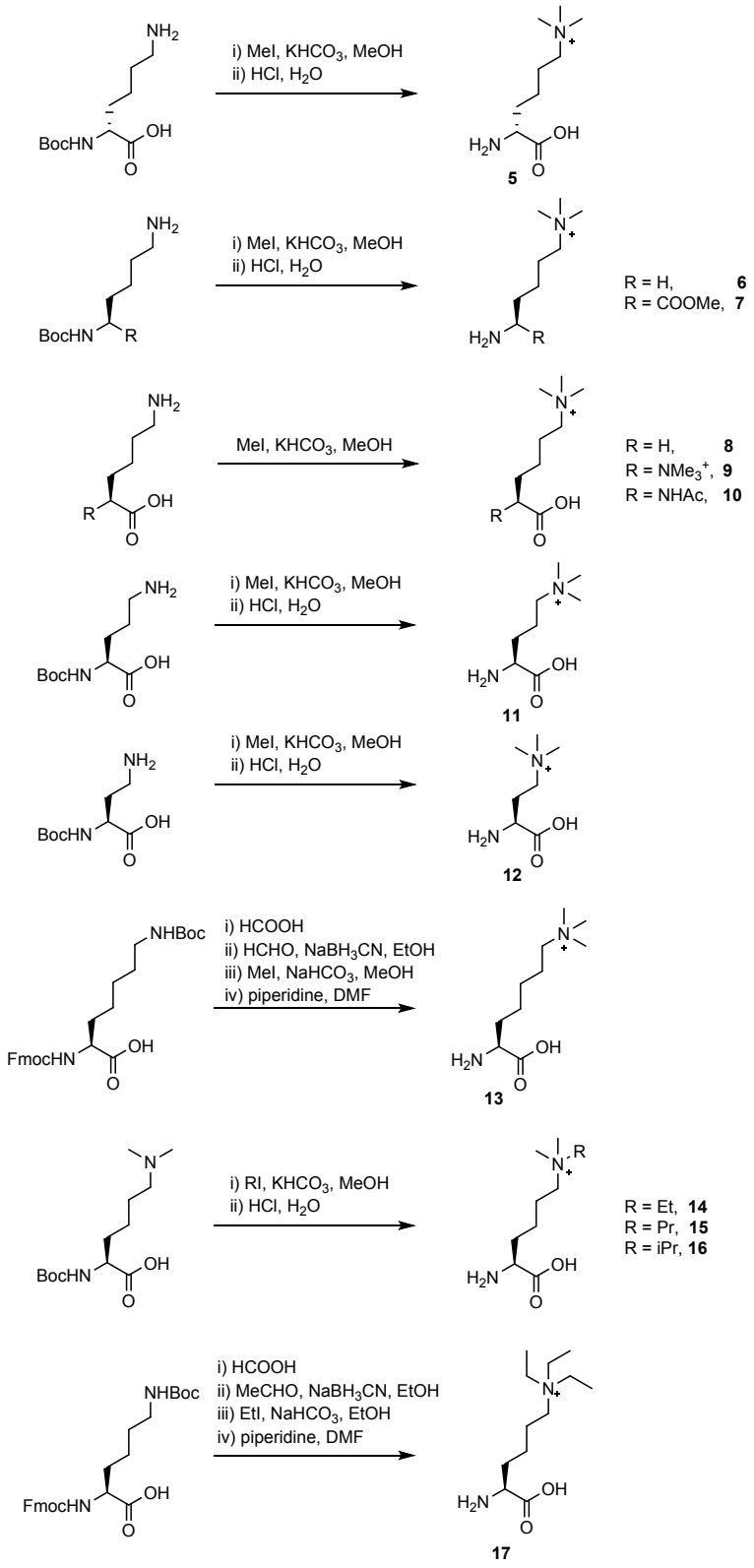
3. NMR experiments

NMR enzymatic experiments were conducted at 310 K in 20 mM Tris-D_{11}.HCl (pH 7.5). To a premixed solution of TMLH (3 μM), FeSO4 (100 μM), 2OG (2 mM) and ascorbate (500 μM), was added trimethyllysine (500 μM). After shaking for 30 minutes at 37 °C in Eppendorf, the reaction mixutre (500 μL) was transferred into the NMR tube and recorded by ^{1}H NMR. NMR spectra were recorded on a Bruker Avance III spectrometer paired with a 500 MHz magnet equipped with the Prodigy cryoprobe. ^{1}H 1D spectra were acquired using presaturation to suppress the water signal with 64 or 128 transients and a relaxation delay of 12 s. 2D COSY spectra were acquired with presaturation of the water resonance using 2k points per transient, 16 transients per increment with a relaxation delay of 2 s and 256 increments with a sweep width of 10 ppm in each dimension. 2D TOCSY spectra were acquired with presaturation of the water resonance using 1k points per transient, 16 transients per increment with a relaxation delay of 2 s

and 256 increments with a sweep width of 10 ppm in each dimension. 2D ^{1}H-^{13}C multiplicity-edited HSQC spectra were acquired using 1k points per transient, 16 transients per increment, a relaxation delay of 2 s, and 256 increments. The ^{13}C sweep width spanned from -10 ppm to 130 ppm. ^{1}H NMR characterization of substrates prior to enzymatic catalysis was performed using a 30° excitation pulse, 16-128 transients per compound, and a relaxation delay of 8 s. ^{13}C-^{1}H spectra of the substrates were recorded using a 30° excitation pulse, 512-4096 transients per compound and a relaxation delay of 2 s.

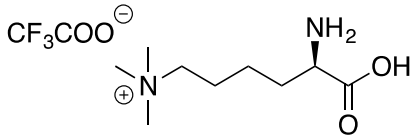
4. Synthetic procedures

Substrates used in this study were either commercially available (trimethyllysine **1**, dimethyllysine **2**, methyllysine **3**, lysine **4**, symmetric dimethylarginine **18**, asymmetric dimethylarginine **19**, mildronate **20** and γ-butyrobetaine **21**) or synthesised (**5**-**17**). Scheme S1 illustrates the reaction pathways for the preparation of compounds **5**-**17**. Detailed procedures are described for each compound. In general, trimethyllysine analogues were synthesised by the modification of the protocols for related compounds.[2-4] All final crude products were purified by preparative HPLC using gradient elution at constant flow rate of 10 mL min^{-1} and the temperature of 30 °C. A typical run was performed as follows: (C-18 reverse phase column; gradient: 10% B in 15 min, 100% B in 18 min, 100% B in 20 min, afterwards maintaining a constant polarity for 5 min (30 min total runtime)). Buffer A: 0.1% (v/v) TFA in water; buffer B: CH_3CN containing 0.1% (v/v) TFA. The fractions containing product were combined and freeze-dried to yield the product as a hygroscopic solid.

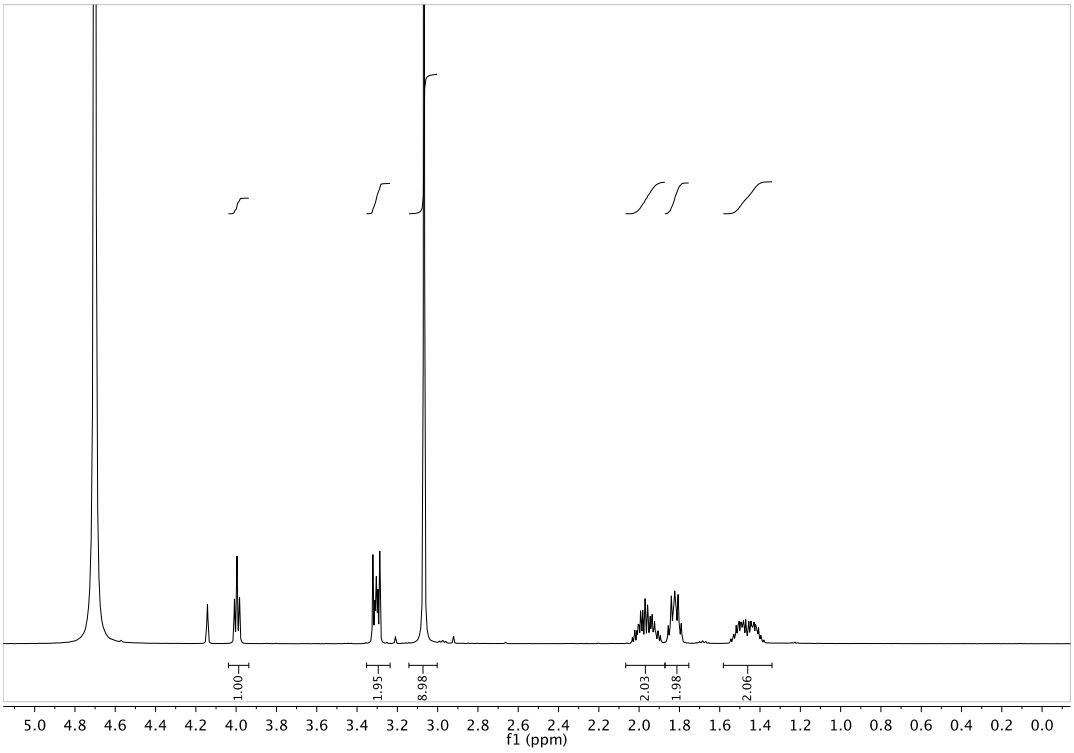


Scheme S1. Syntheses of trimethyllysine analogues **5**-**17**.

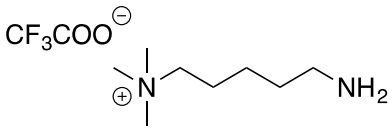
(*R*)-5-amino-5-carboxy-*N*,*N*,*N*-trimethylpentan-1-aminium 2,2,2-trifluoroacetate (5)



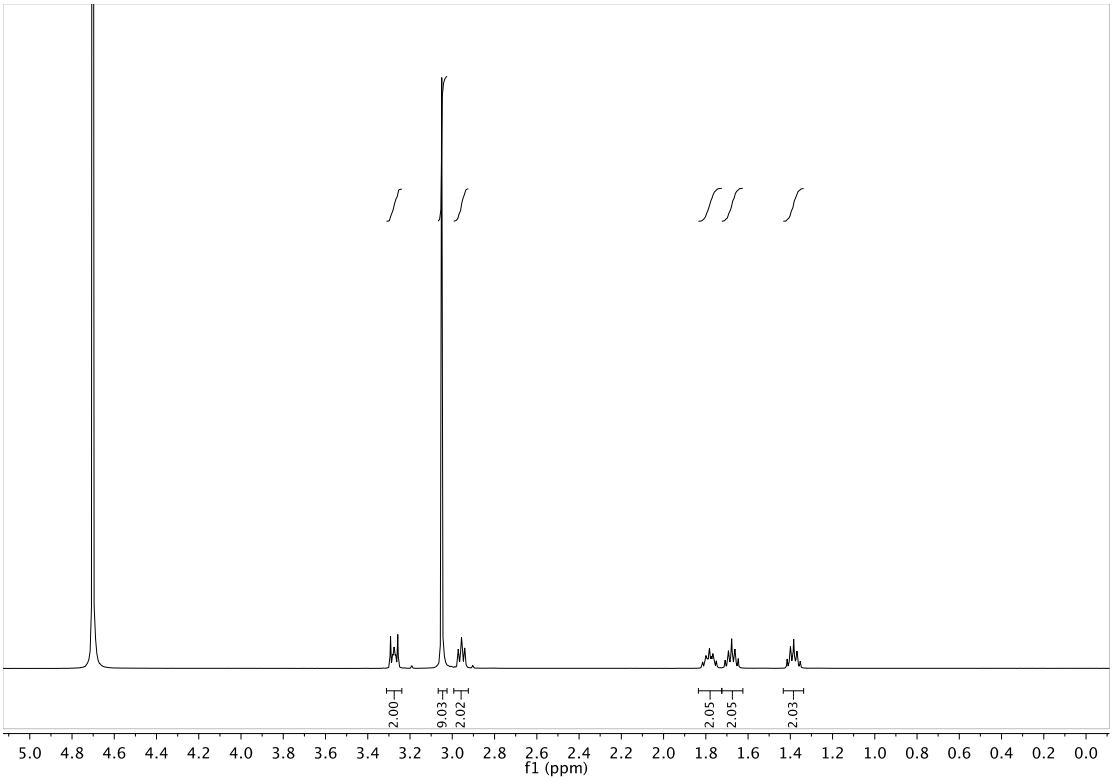
To a solution of Boc-D-Lys-OH (246 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 2 days. Methanol was then evaporated, and the remaining product dissolved in water (2 mL) and acidified with concentrated HCl (2 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC.^{1}H NMR (500 MHz, D_2O) δ 4.00 (t, J = 6.3 Hz, 1H), 3.32-3.21 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.07 (s, 9H), 2.08-1.88 (m, 2H), 1.87-1.78 (m, 2H), 1.57-1.32 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 172.4, 66.0 (1:1:1 t, $^{2}J_{NC}$ = 3.0 Hz, ε-CH_2), 53.0, 52.9 (1:1:1 t, $^{2}J_{NC}$ = 4.0 Hz, N-Me), 29.4, 22.0, 21.3 (br). HRMS (ESI) calc. for $C_9H_{21}N_2O_2$ $[M+H]^+$ 189.1603, found 189.1627.



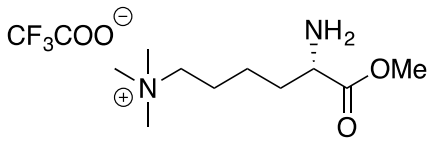
5-amino-*N*,*N*,*N*-trimethylpentan-1-aminium 2,2,2-trifluoroacetate (6)



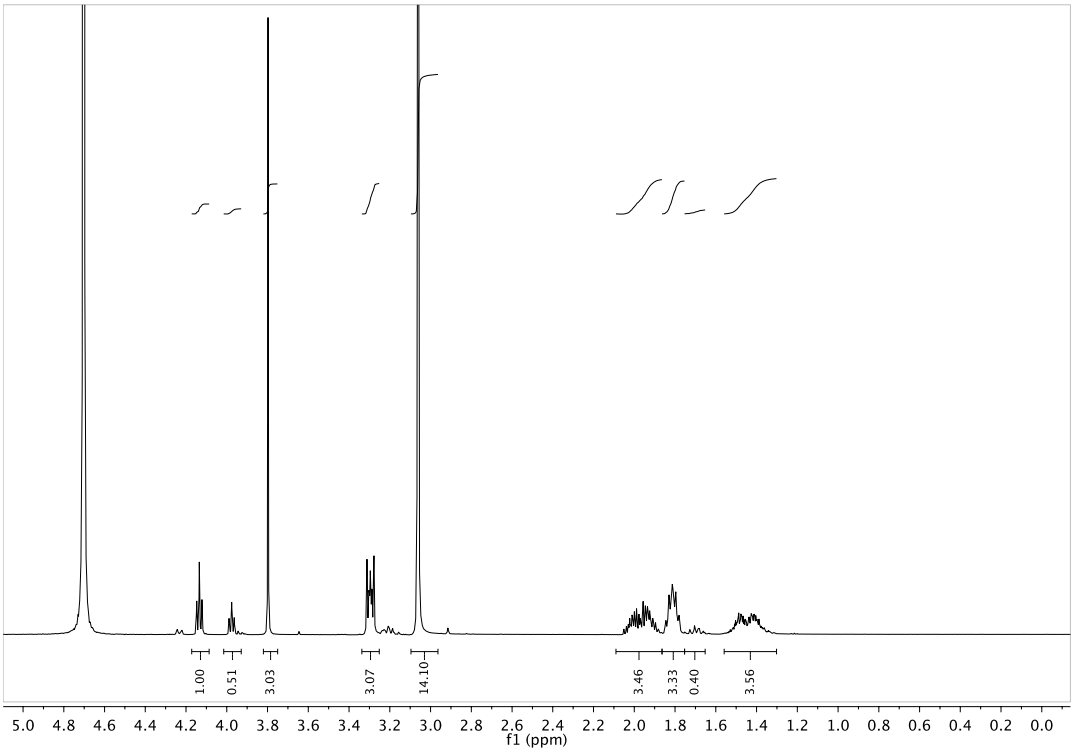
To a solution of Boc-N-5-aminopentylamine (202 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 1 day. Methanol was then evaporated, and the remaining product dissolved in water (2 mL) and acidified with concentrated HCl (2 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 3.39–3.26 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.05 (s, 9H), 2.96 (t, J = 7.7 Hz, 2H), 1.82-1.75 (m, 2H), 1.73-1.62 (m, 2H), 1.38 (quint, J = 7.7 Hz, 2H); ^{13}C NMR (126 MHz, D_2O) δ 66.2 (1:1:1 t, $^{2}J_{NC}$ = 3.0 Hz, CH_2), 52.8 (1:1:1 t, $^{2}J_{NC}$ = 4.0 Hz, N-Me), 39.1, 26.3, 22.5 (1:1:1 t, $^{2}J_{NC}$ = 1.7 Hz, CH_2), 21.9. HRMS (ESI) calc. for $C_8H_{21}N_2$ $[M+H]^+$ 145.1705, found 145.1727.



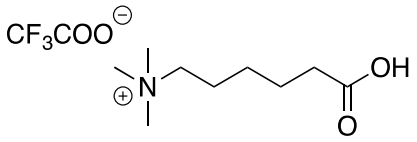
(*S*)-5-amino-6-methoxy-*N*,*N*,*N*-trimethyl-6-oxohexan-1-aminium 2,2,2-trifluoroacetate (7)



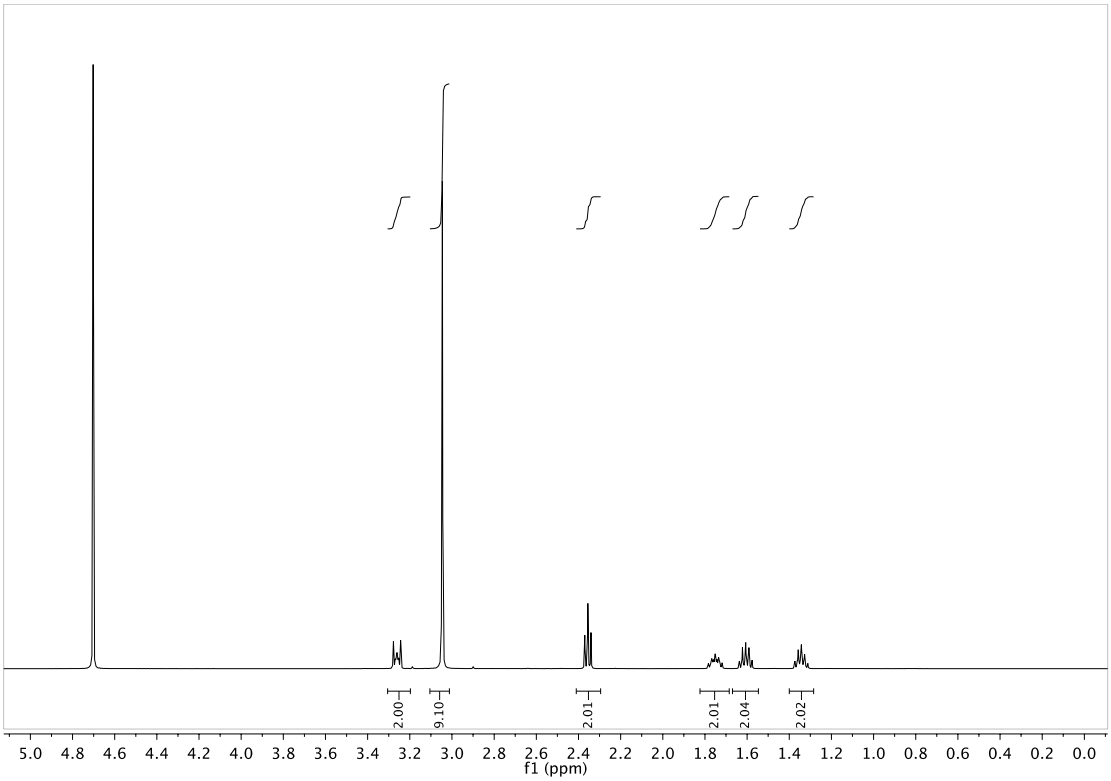
To a solution of Boc-Lys-OMe·HCl (296 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 2 days. Methanol was then evaporated, and the remaining crude product was purified by preparative HPLC. Methyl ester partially hydrolyzed in D_2O during the NMR acquisition. ^{1}H NMR (500 MHz, D_2O, 2:1 ratio of ester and acid) δ 4.13 (t, J = 6.5 Hz, 1H), 3.98 (t, J = 6.3 Hz, 1H, acid α-CH), 3.80 (s, 3H, OCH_3), 3.37-3.21 (m, 4H), 3.06 (d, J = 1.0 Hz, 18H), 2.18-1.87 (m, 4H), 1.85-1.76 (m, 4H), 1.55-1.29 (m, 4H); ^{13}C NMR (126 MHz, D_2O, 2:1 ratio of ester and acid) δ 172.4, 170.4, 65.9 (br), 53.7, 53.0 (1:1:1 t, $^2J_{NC}$ = 4.1 Hz, N-Me), 52.6, 29.4, 29.2, 22.0 (br), 21.3. HRMS (ESI) calc. for $C_{10}H_{23}N_2O_2$ $[M+H]^+$ 203.1760, found 203.1776.



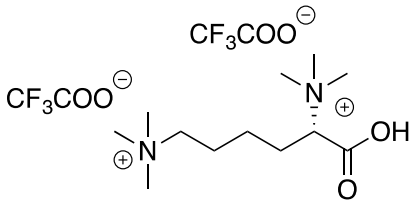
5-carboxy-*N*,*N*,*N*-trimethylpentan-1-aminium 2,2,2-trifluoroacetate (8)



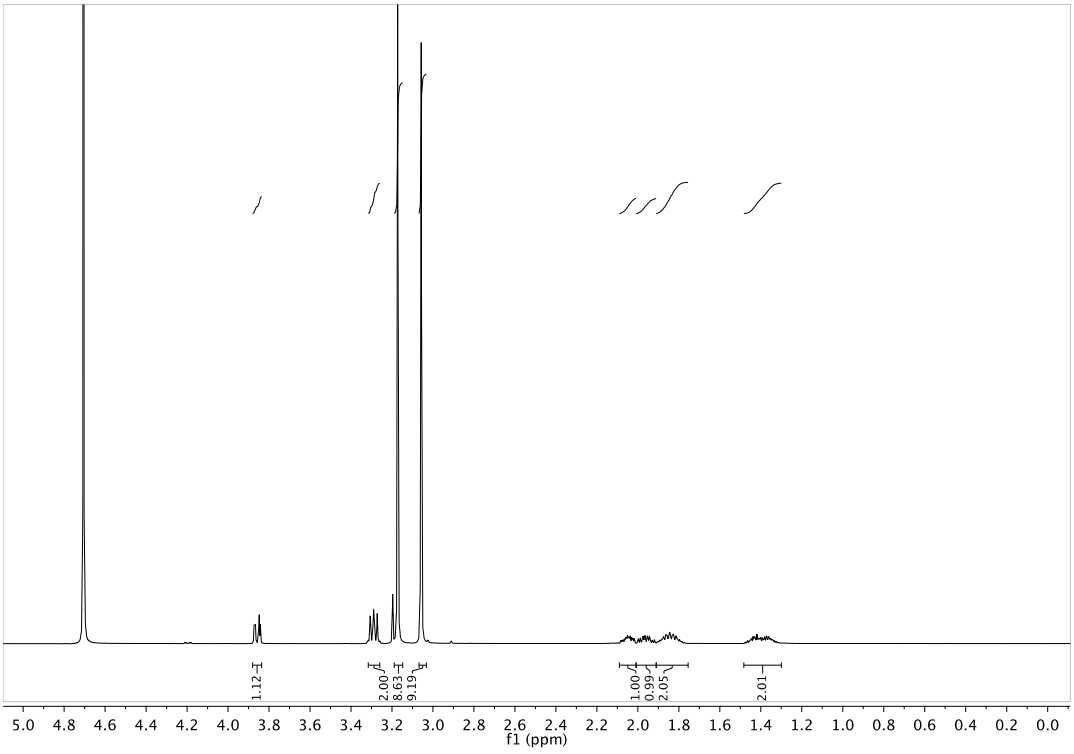
To a solution of 6-aminohexanoic acid (131 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 2 days. Methanol was then evaporated, and the remaining product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 3.38-3.17 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.05 (s, 9H), 2.36 (t, J = 7.4 Hz, 2H), 1.78-1.72 (m, 2H), 1.61 (quint, J = 7.4 Hz, 2H), 1.34 (quint, J = 7.5 Hz, 2H); ^{13}C NMR (126 MHz, D_2O) δ 178.7, 66.4 (1:1:1 t, $^{2}J_{NC}$ = 2.8 Hz, N-CH_2), 52.9 (1:1:1 t, $^{2}J_{NC}$ = 4.1 Hz, N-Me), 33.5, 24.9 (1:1:1 t, $^{2}J_{NC}$ = 1.6 Hz, CH_2), 23.7, 22.0. HRMS (ESI) calc. for $C_9H_{20}NO_2$ $[M+H]^+$ 174.1494, found 174.1524.



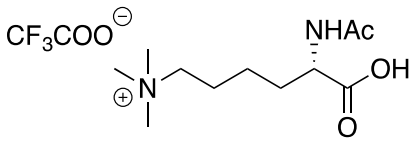
(*S*)-1-carboxy-N^1,N^1,N^1,N^5,N^5,N^5-hexamethylpentane-1,5-diaminium 2,2,2-trifluoroacetate (9)



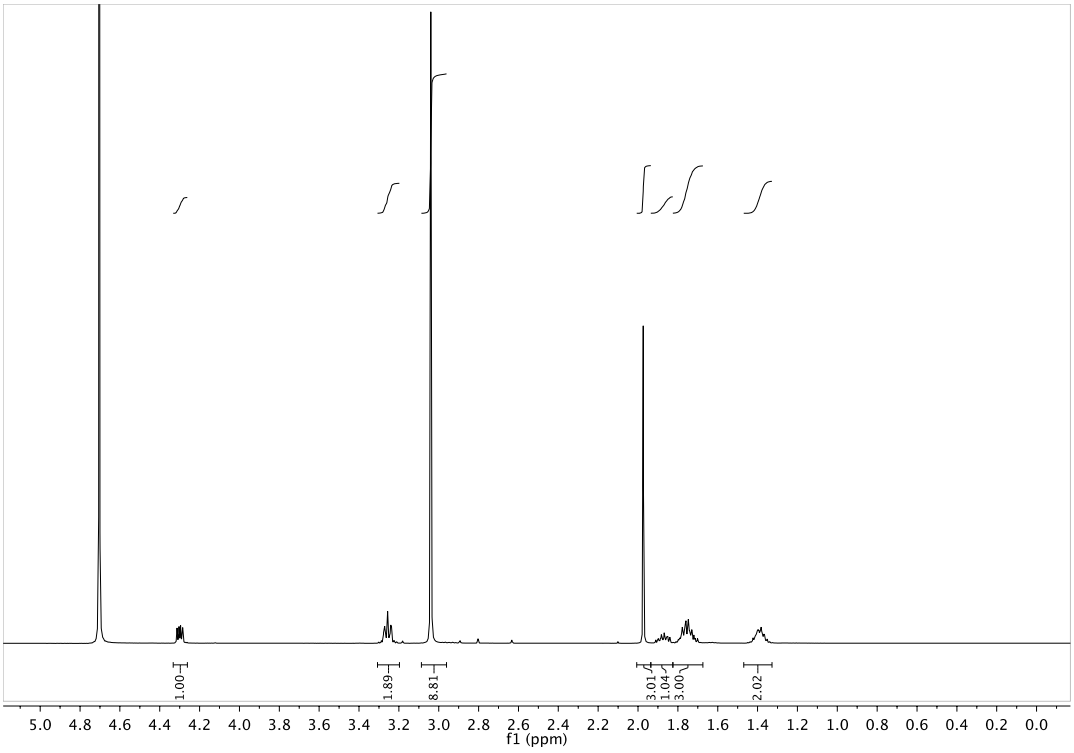
To a solution of lysine (146 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (800 mg, 8 mmol, 8 equiv.) and iodomethane (622 µL, 10 mmol, 10 equiv.). The reaction mixture was stirred at room temperature for 3 days. Methanol was then evaporated, and the remaining product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 3.86 (dd, J = 12.0, 3.5 Hz, 1H), 3.39-3.23 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.17 (s, 9H), 3.06 (s, 9H), 2.08-2.02 (m, 1H), 2.00-1.92 (m, 1H), 1.91-1.75 (m, 2H), 1.52-1.29 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 170.2, 76.2, 65.9 (1:1:1 t, $^2J_{NC}$ = 3.0 Hz, ε-CH_2), 53.0 (1:1:1 t, $^2J_{NC}$ = 4.0 Hz, N-Me), 52.9, 25.8, 22.1. HRMS (ESI) calc. for $C_{12}H_{28}N_2O_2$ $[M+H]^+$ 231.2072, found 231.2087.



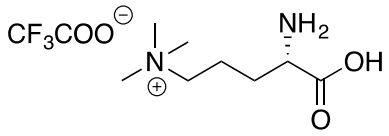
(*S*)-5-acetamido-5-carboxy-*N*,*N*,*N*-trimethylpentan-1-aminium 2,2,2-trifluoroacetate (10)



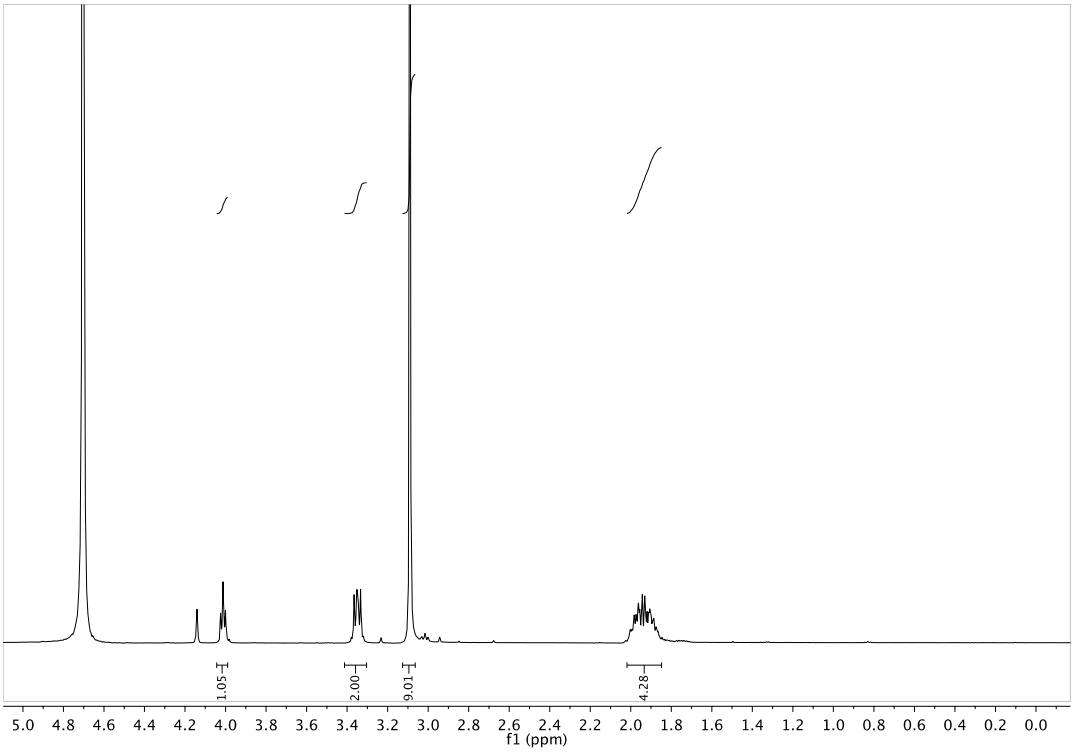
To a solution of Ac-Lys-OH (188 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 3 days. Methanol was then evaporated, and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 4.30 (dd, J = 9.1, 5.1 Hz, 1H), 3.30-3.21 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.04 (s, 9H), 1.97 (s, 3H), 1.91-1.84 (m, 1H), 1.81-1.66 (m, 3H), 1.49-1.28 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 175.7, 174.3, 66.2 (1:1:1 t, $^{2}J_{NC}$ = 2.9 Hz, ε-CH_2), 52.8 (1:1:1 t, $^{2}J_{NC}$ = 4.0 Hz, N-Me), 52.4, 29.9, 21.9, 21.8, 21.6. HRMS (ESI) calc. for $C_{11}H_{23}N_2O_3$ $[M+H]^+$ 231.1709, found 231.1720.



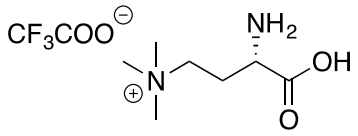
(*S*)-4-amino-4-carboxy-*N*,*N*,*N*-trimethylbutan-1-aminium 2,2,2-trifluoroacetate (11)



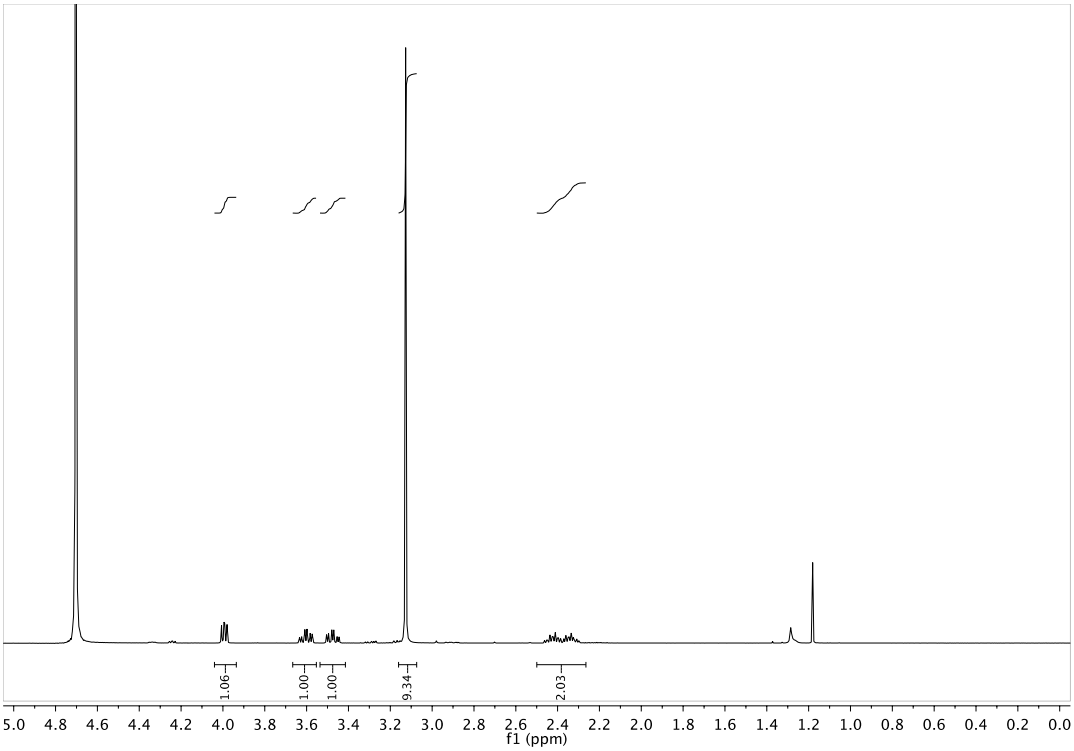
To a solution of Boc-Orn-OH (232 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 3 days. Methanol was then evaporated, and the remaining product dissolved in water (2 mL) and acidified with concentrated HCl (2 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 4.01 (t, J = 6.1 Hz, 1H), 3.38-3.32 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.09 (s, 9H), 2.03-1.84 (m, 4H); ^{13}C NMR (126 MHz, D_2O) δ 172.0, 65.4 (1:1:1 t, $^{2}J_{NC}$ = 3.1 Hz, δ-CH_2), 53.0 (1:1:1 t, $^{2}J_{NC}$ = 4.0 Hz, N-Me), 52.7, 26.6 (1:1:1 t, $^{2}J_{NC}$ = 1.9 Hz, γ-CH_2), 18.8. HRMS (ESI) calc. for $C_8H_{19}N_2O_2$ $[M+H]^+$ 175.1446, found 175.1464.



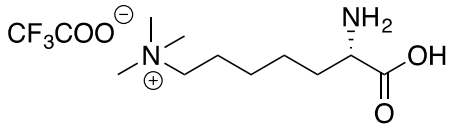
(*S*)-3-amino-3-carboxy-*N*,*N*,*N*-trimethylpropan-1-aminium 2,2,2-trifluoroacetate (12)



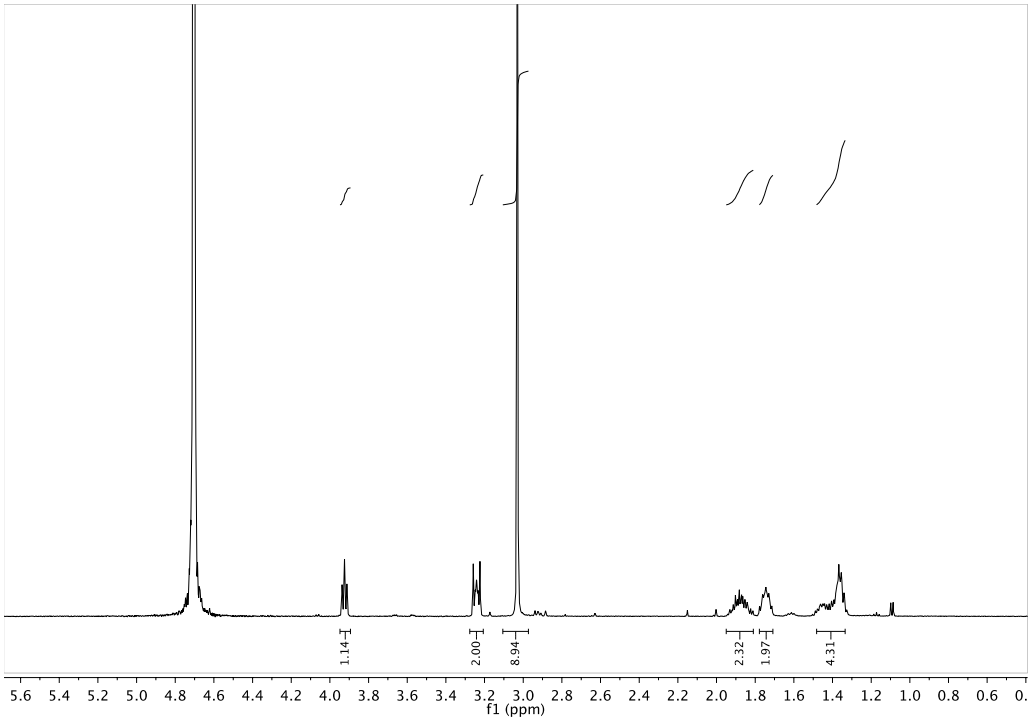
To a solution of Boc-Dab-OH (218 mg, 1 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (400 mg, 4 mmol, 4 equiv.) and iodomethane (311 µL, 5 mmol, 5 equiv.). The reaction mixture was stirred at room temperature for 3 days. Methanol was then evaporated, and the remaining product dissolved in water (2 mL) and acidified with concentrated HCl (2 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 3.99 (dd, J = 7.6, 5.5 Hz, 1H), 3.60 (td, J = 12.7, 5.0 Hz, 1H), 3.47 (td, J = 12.7, 4.7 Hz, 1H), 3.13 (s, 9H), 2.46-2.30 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 171.2, 62.4 (1:1:1 t, $^2J_{NC}$ = 3.4 Hz, γ-CH_2), 53.1 (1:1:1 t, $^2J_{NC}$ = 3.9 Hz, N-Me), 50.8, 23.9. HRMS (ESI) calc. for $C_7H_{17}N_2O_2$ $[M+H]^+$ 161.1290, found 161.1306.



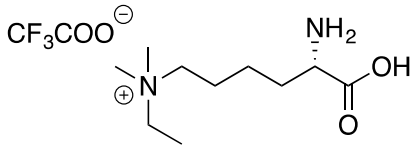
(*S*)-6-amino-6-carboxy-*N*,*N*,*N*-trimethylhexan-1-aminium 2,2,2-trifluoroacetate (13)



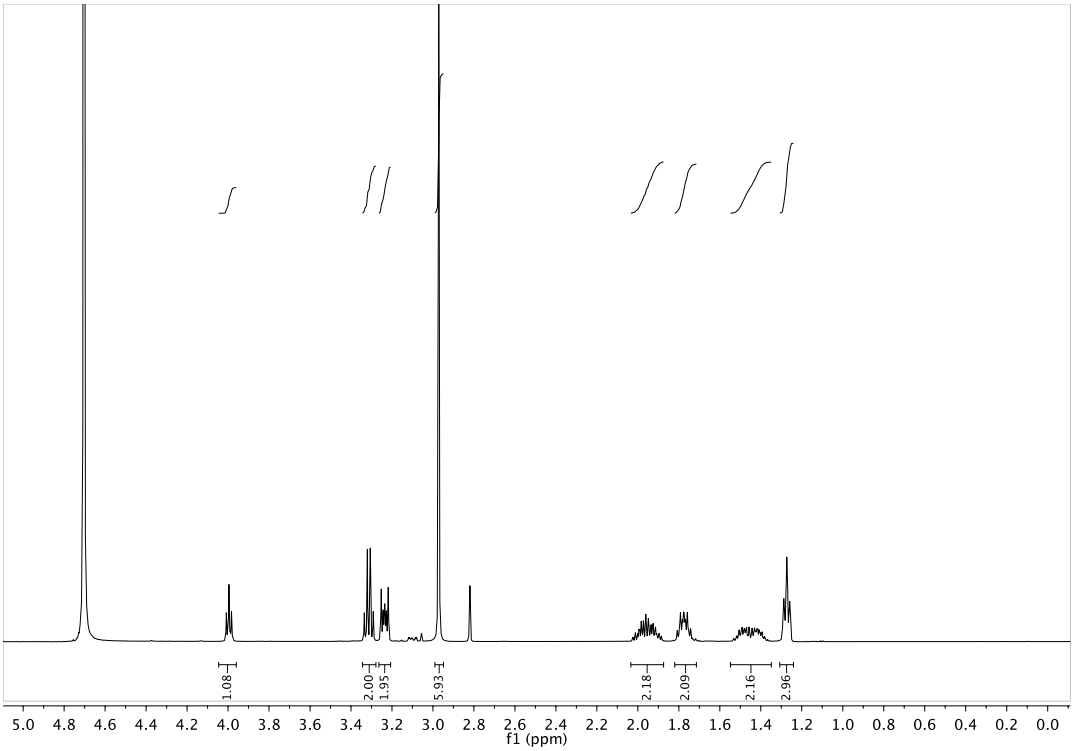
Fmoc-homoLys(Boc)-OH (241 mg, 0.5 mmol, 1 equiv.) was dissolved in formic acid (3mL) and the reaction mixture was stirred for 1 hour and then the solvent evaporated. To a stirred solution of Fmoc-protected homolysine in ethanol (5 mL) were slowly added $NaBH_3CN$ (63 mg, 1 mmol, 2 equiv.) and formaldehyde (90 mg, 3 mmol, 6 equiv.). The reaction mixture was stirred for 2 hours, until ESI-MS indicated reaction completion, and then quenched with concentrated HCl. The mixture was filtered and the residue was washed with ethanol (2 mL). The crude material was obtained after removal of solvents *in vacuo* and was used without further purification. To a stirred solution of crude Fmoc-protected dimethylhomolysine in methanol (5 mL) were added $NaHCO_3$ (126 mg, 1.5 mmol, 3 equiv.) and iodomethane (156 µL, 2.5 mmol, 5 equiv.), and the progress of the reaction was monitored by ESI-MS. After 2 days, the solvent was removed in vacuo, and the reaction mixture was dissolved in water (10 mL), basified with 20% piperidine (in DMF) for 30 minutes, and then washed with diethylether (3 × 10 mL). The aqueous phase was purified by preparative HPLC to yield pure trimethylhomolysine (**13**).^{1}H NMR (500 MHz, D_2O) δ 3.92 (t, J = 6.2 Hz, 1H), 3.28–3.20 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.03 (s, 9H), 1.91-1.80 (m, 2H), 1.78-1.71 (m, 2H), 1.59-1.29 (m, 4H); ^{13}C NMR (126 MHz, D_2O) δ 174.8, 66.4 (1:1:1 t, $^2J_{NC}$ = 2.8 Hz, ζ-CH_2), 54.6, 52.8 (1:1:1 t, $^2J_{NC}$ = 4.0 Hz, N-Me), 30.0, 25.1, 23.8, 22.0. HRMS (ESI) calc. for $C_{10}H_{23}N_2O_2$ $[M+H]^+$ 203.1760, found 203.1777.



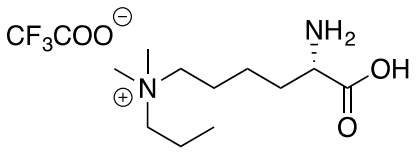
(*S*)-5-amino-5-carboxy-*N*-ethyl-*N*,*N*-dimethylpentan-1-aminium 2,2,2-trifluoroacetate (14)



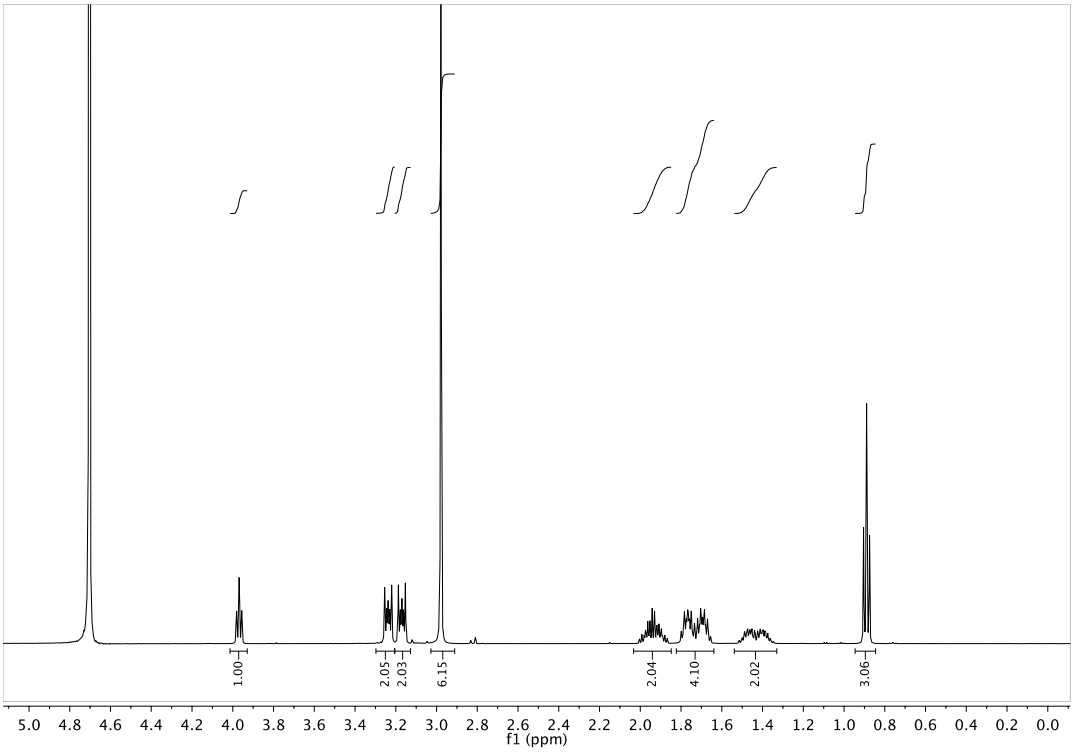
To a solution of Boc-Lys(Me2)-OH (55 mg, 0.2 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (40 mg, 0.4 mmol, 2 equiv.) and iodoethane (161 µL, 2 mmol, 10 equiv.). The reaction mixture was stirred at room temperature for 4 days. Methanol was then evaporated, and the remaining product dissolved in water (1 mL) and acidified with concentrated HCl (1 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 4.00 (t, J = 6.3 Hz, 1H), 3.31 (q, J = 7.3 Hz, 2H), 3.27-3.20 (m, 2H, multiplet due to ^{1}H-^{14}N), 2.97 (s, 6H), 2.01-1.86 (m, 2H), 1.81-1.74 (m, 2H), 1.52-1.38 (m, 2H), 1.27 (tt, J = 7.3, 2.0 Hz, 3H); ^{13}C NMR (126 MHz, D_2O) δ 172.2, 62.8 (1:1:1 t, $^{2}J_{NC}$ = 3.0 Hz, ε-CH_2), 59.7 (1:1:1 t, $^{2}J_{NC}$ = 3.1 Hz, N-$CH_2$$CH_3$), 52.8, 49.9 (1:1:1 t, $^{2}J_{NC}$ = 4.1 Hz, N-Me), 29.3, 21.5, 21.4, 7.4. HRMS (ESI) calc. for $C_{10}H_{23}N_2O_2$ $[M+H]^+$ 203.1760, found 203.1776.



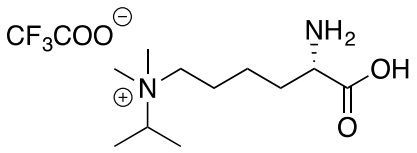
(*S*)-5-amino-5-carboxy-*N*,*N*-dimethyl-*N*-propylpentan-1-aminium 2,2,2-trifluoroacetate (15)



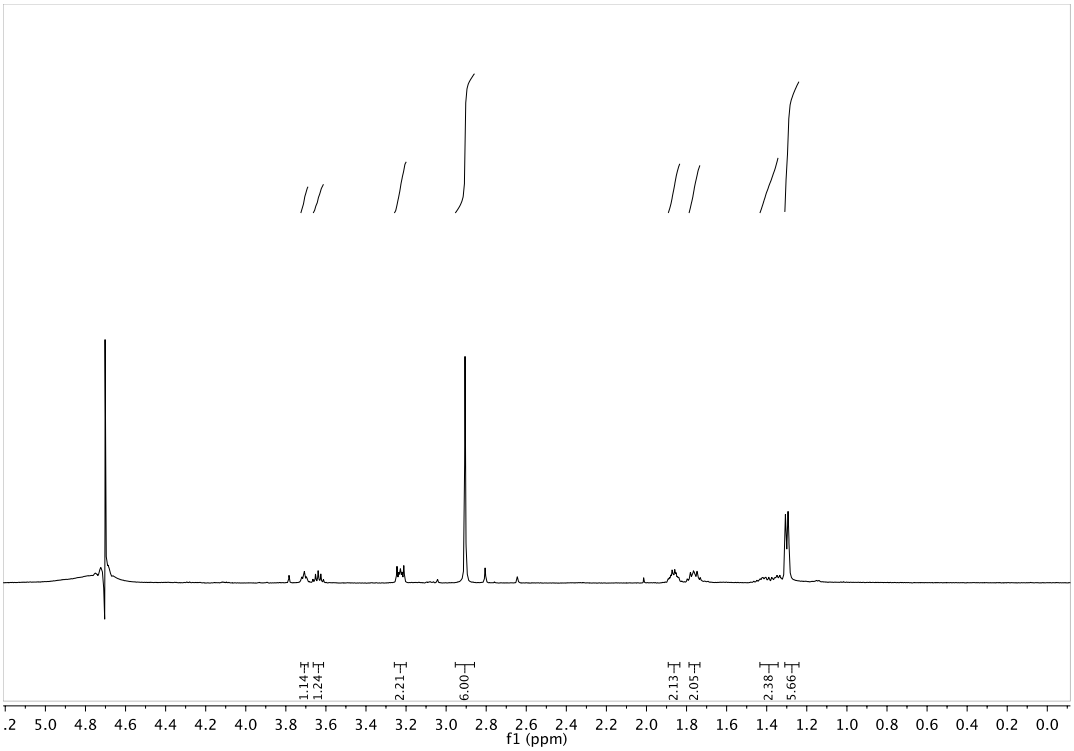
To a solution of Boc-Lys(Me2)-OH (55 mg, 0.2 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (40 mg, 0.4 mmol, 2 equiv.) and 1-iodopropane (195 µL, 2 mmol, 10 equiv.). The reaction mixture was stirred at room temperature for 5 days. Methanol was then evaporated, and the remaining product dissolved in water (1 mL) and acidified with concentrated HCl (1 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 3.97 (t, J = 6.3 Hz, 1H), 3.26-3.22 (m, 2H, multiplet due to ^{1}H-^{14}N), 3.19-3.15 (m, 2H, multiplet due to ^{1}H-^{14}N), 2.98 (s, 6H), 2.00-1.87 (m, 2H), 1.80–1.66 (m, 4H), 1.51-1.35 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, D_2O) δ 172.3, 65.6 (1:1:1 t, $^{2}J_{NC}$ = 2.8 Hz, CH_2), 63.4 (1:1:1 t, $^{2}J_{NC}$ = 3.0 Hz, CH_2), 52.9, 50.5 (1:1:1 t, $^{2}J_{NC}$ = 4.0 Hz, N-Me), 29.3, 21.6, 21.3, 15.6, 9.8. HRMS (ESI) calc. for $C_{11}H_{25}N_2O_2$ $[M+H]^+$ 217.1916, found 217.1931.



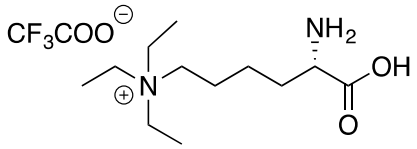
(*S*)-5-amino-5-carboxy-*N*-isopropyl-*N*,*N*-dimethylpentan-1-aminium 2,2,2-trifluoroacetate (16)



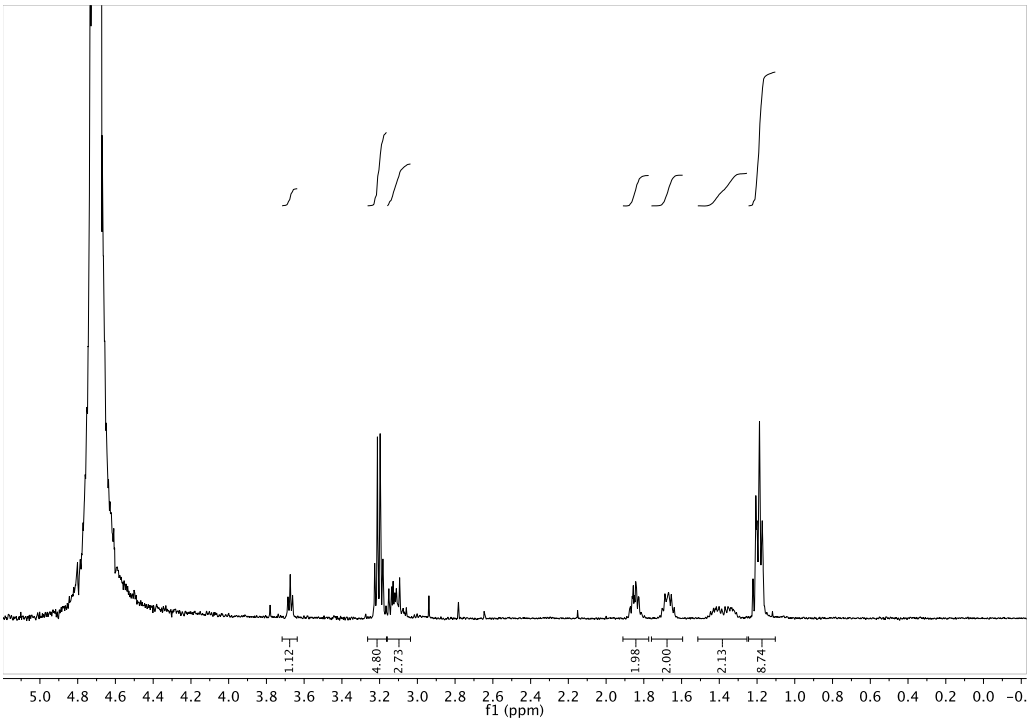
To a solution of Boc-Lys(Me2)-OH (55 mg, 0.2 mmol, 1 equiv.) in methanol (5 mL) were added $KHCO_3$ (40 mg, 0.4 mmol, 2 equiv.) and 2-iodopropane (300 µL, 3 mmol, 15 equiv.). The reaction mixture was stirred at room temperature for 5 days. Methanol was then evaporated, and the remaining product dissolved in water (1 mL) and acidified with concentrated HCl (1 mL). The mixture was stirred for 2 hours at room temperature and then washed with diethyl ether. The aqueous phase was evaporated *in vacuo* and the remaining crude product was purified by preparative HPLC. ^{1}H NMR (500 MHz, D_2O) δ 3.71 (dt, *J* = 6.9, 3.4 Hz, 1H), 3.64 (quint, *J* = 6.6 Hz, 1H), 3.26-3.20 (m, 2H, multiplet due to ^{1}H-^{14}N), 2.91 (s, 6H), 1.87 (tdd, *J* = 9.4, 4.5, 2.8 Hz, 2H), 1.76 (quint, *J* = 7.9 Hz, 2H), 1.51-1.32 (m, 2H), 1.30 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (126 MHz, D_2O) δ 173.75, 65.06, 61.95, 54.22, 47.26, 29.78, 21.44, 21.42, 15.44. HRMS (ESI) calc. for $C_{11}H_{25}N_2O_2$ $[M+H]^+$ 217.1916, found 217.1935.



(*S*)-5-amino-5-carboxy-*N*,*N*,*N*-triethylpentan-1-aminium 2,2,2-trifluoroacetate (17)



Fmoc-Lys(Boc)-OH (1.00 g, 2.13 mmol, 1 equiv.) was dissolved in formic acid (5 mL) and the reaction mixture was stirred for 1 hour and then the solvent evaporated. To a stirred solution of Fmoc-Lys-OH in ethanol (5 mL) were slowly added $NaBH_3CN$ (268 mg, 4.26 mmol, 2 equiv.) and acetaldehyde (564 mg, 12.8 mmol, 6 equiv.). The reaction mixture was stirred for 4 hours, and then quenched with concentrated HCl. The mixture was filtered and the residue was washed with ethanol (2 mL). The crude material was obtained after removal of solvents *in vacuo* and was used without further purification. To a stirred solution of crude Fmoc-Lys(Et2)-OH in ethanol (5 mL) were added $NaHCO_3$ (538 mg, 6.4 mmol, 3 equiv.) and iodoethane (860 µL, 10.7 mmol, 5 equiv.), and the progress of the reaction was monitored by ESI-MS. After 5 days, the solvent was removed *in vacuo*, and the reaction mixture was dissolved in water (10 mL), basified with 20% piperidine (in DMF) for 30 minutes, and then washed with diethylether (3 × 10 mL). The aqueous phase was purified by preparative HPLC to yield pure triethyllysine (**17**).^{1}H NMR (500 MHz, D_2O) δ 3.67 (t, *J* = 6.1 Hz, 1H), 3.20 (q, *J* = 7.3 Hz, 6H), 3.16-3.08 (m, 2H, multiplet due to ^{1}H-^{14}N), 1.94-1.77 (m, 2H), 1.67 (quint, *J* = 7.5 Hz, 2H), 1.45-1.31 (m, 2H), 1.19 (tt, *J* = 7.2, 2.1 Hz, 9H). HRMS (ESI) calc. for $C_{12}H_{27}N_2O_2$ $[M+H]^+$ 231.2072, found 231.2090.



5. Supplementary figures

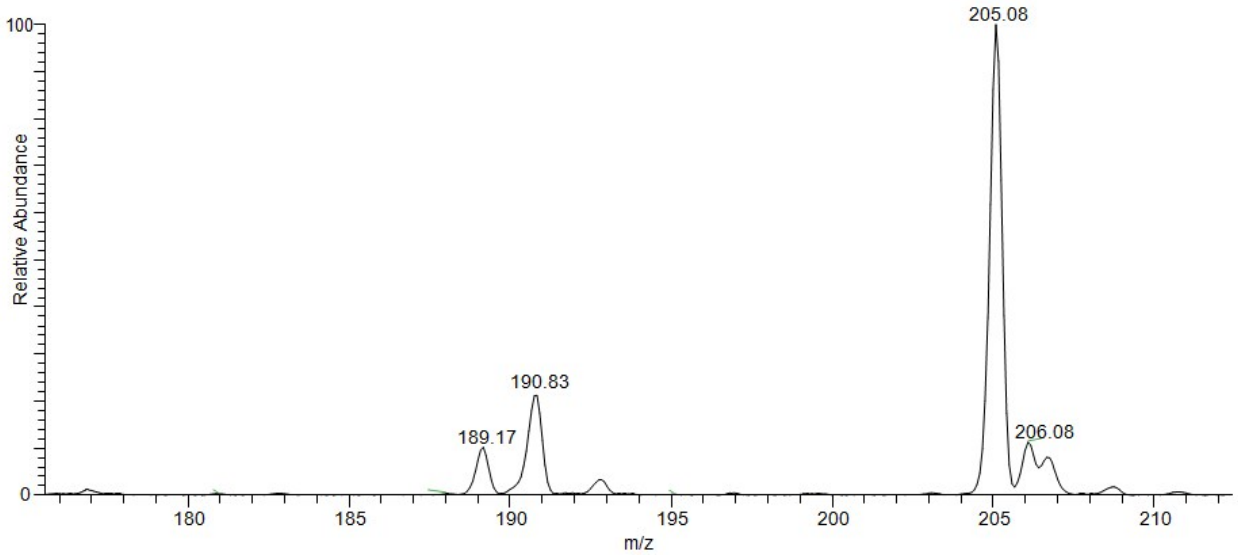


Fig. S1 Hydroxylation of trimethyllysine (**1**) in the presence of TMLH, Fe(II), 2OG and ascorbate as analysed by LC-MS.

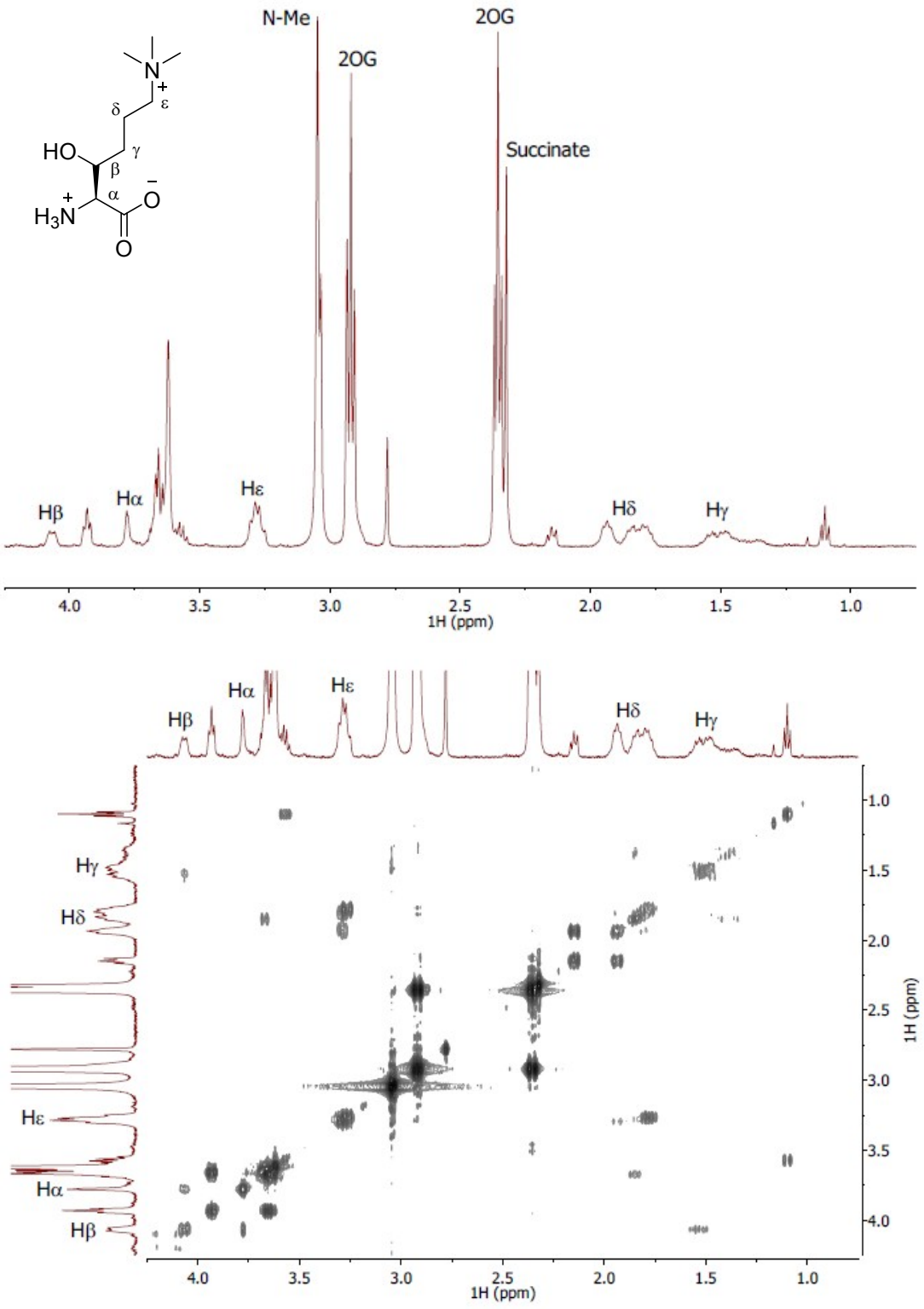


Fig. S2 NMR analyses of TMLH-catalysed hydroxylation of trimethyllysine (**1**). ^{1}H NMR data of TMLH-catalysed C-3 hydroxylation of trimethyllysine (top). COSY data of TMLH-catalysed C-3 hydroxylation of trimethyllysine (bottom).

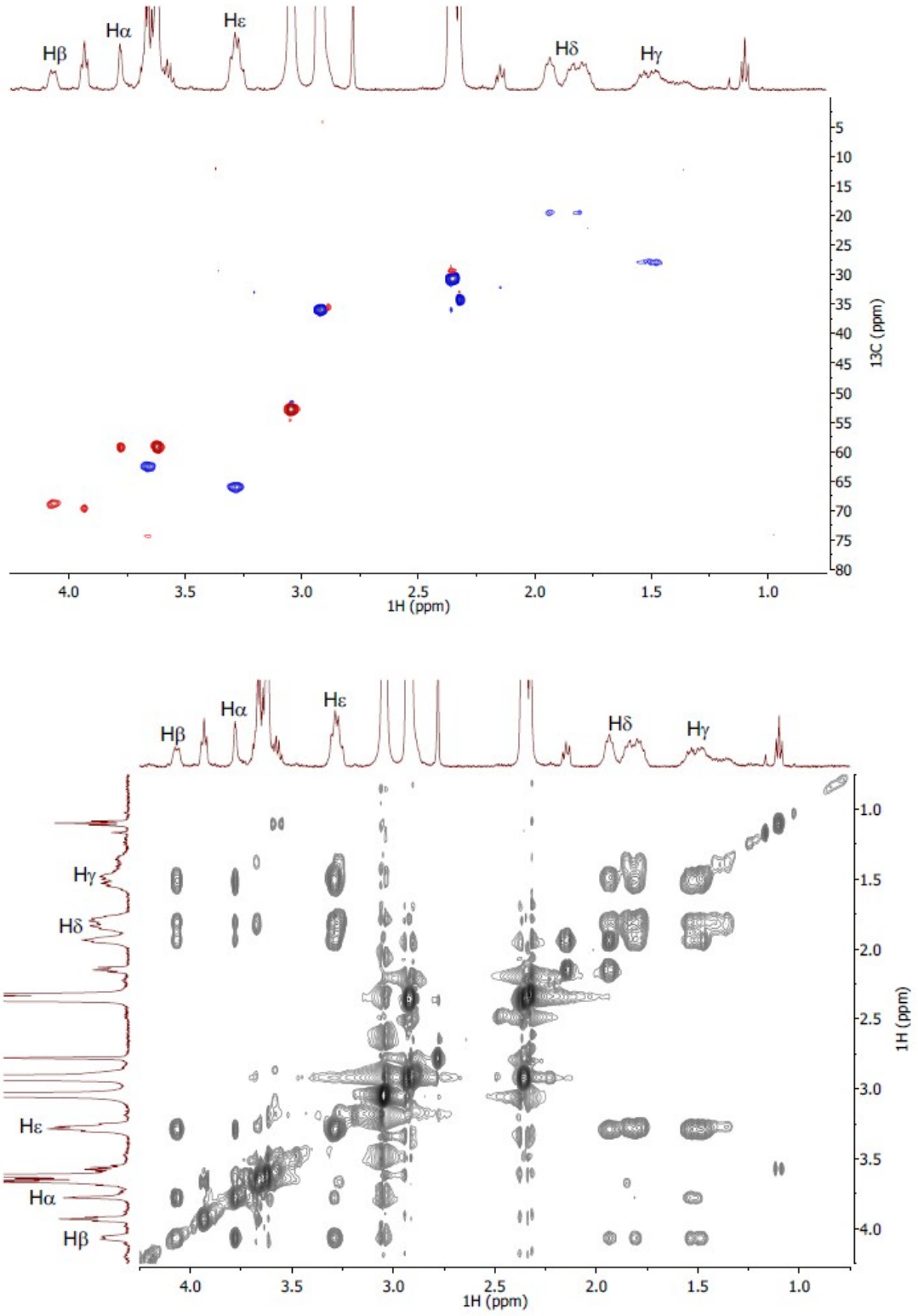


Fig. S3 NMR analyses of TMLH-catalysed hydroxylation of trimethyllysine (**1**). Multiplicity-edited HSQC data of TMLH-catalysed C-3 hydroxylation of trimethyllysine (top; red = positive, CH/CH_3, blue = negative, CH_2). TOCSY data of TMLH-catalysed C-3 hydroxylation of trimethyllysine (bottom).

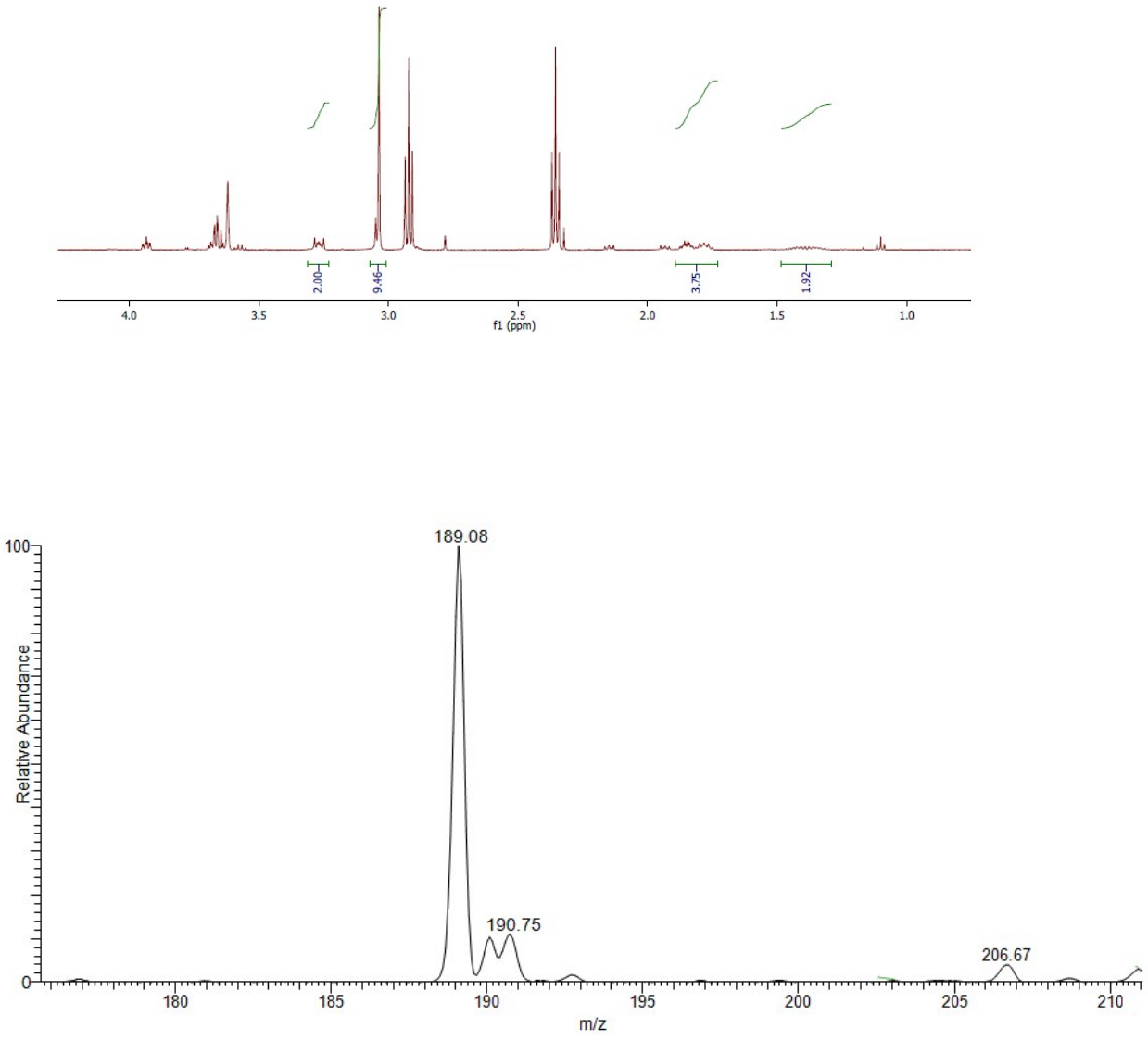


Fig. S4 Hydroxylation of trimethyllysine (**1**) in the absence of TMLH as analysed by ^{1}H NMR (top) and LC-MS (bottom).

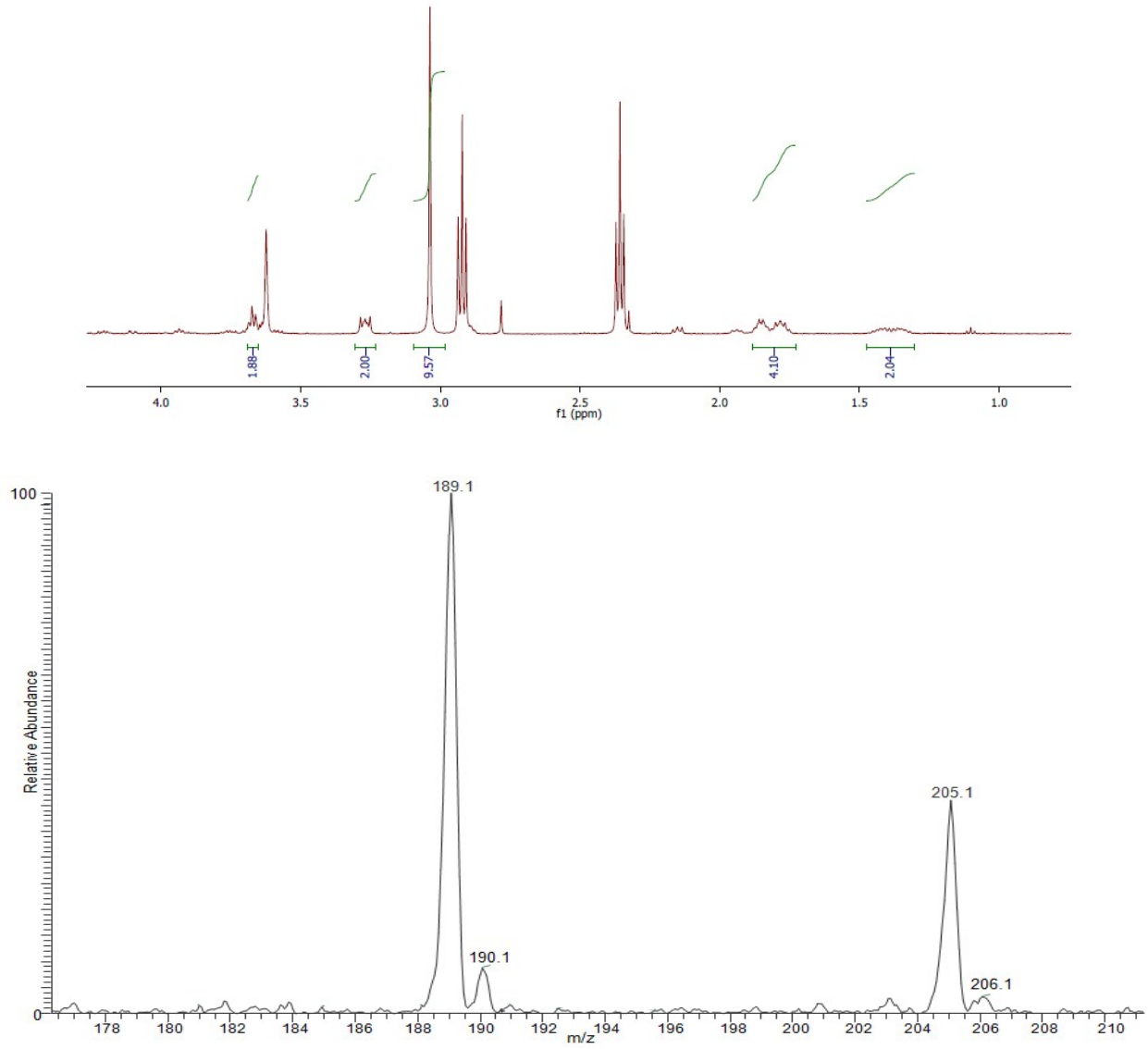


Fig. S5 Hydroxylation of trimethyllysine (**1**) in the absence of $FeSO_4$ as analysed by ^1H NMR (top) and LC-MS (bottom).

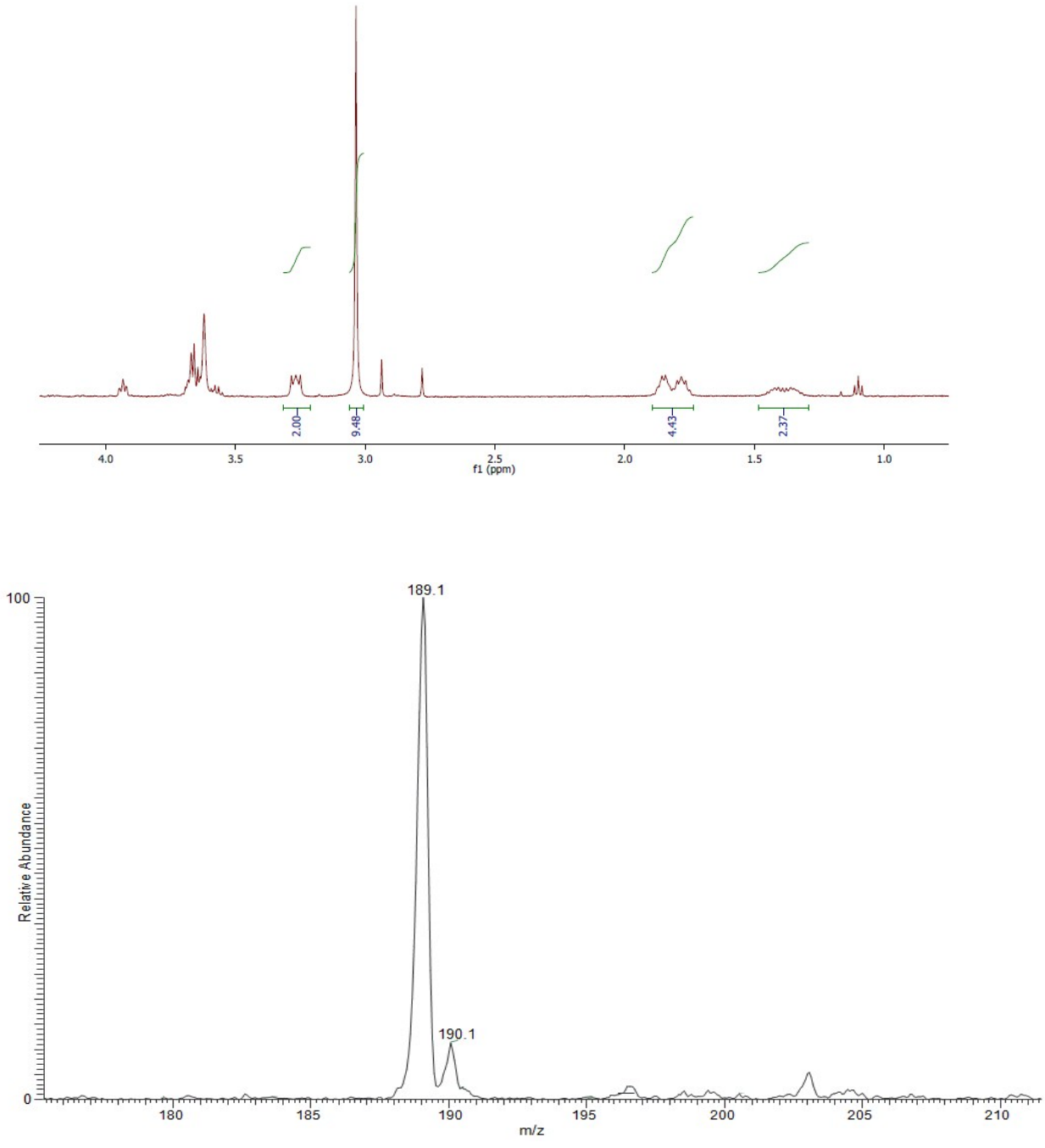


Fig. S6 Hydroxylation of trimethyllysine (**1**) in the absence of 2OG as analysed by ^{1}H NMR (top) and LC-MS (bottom).

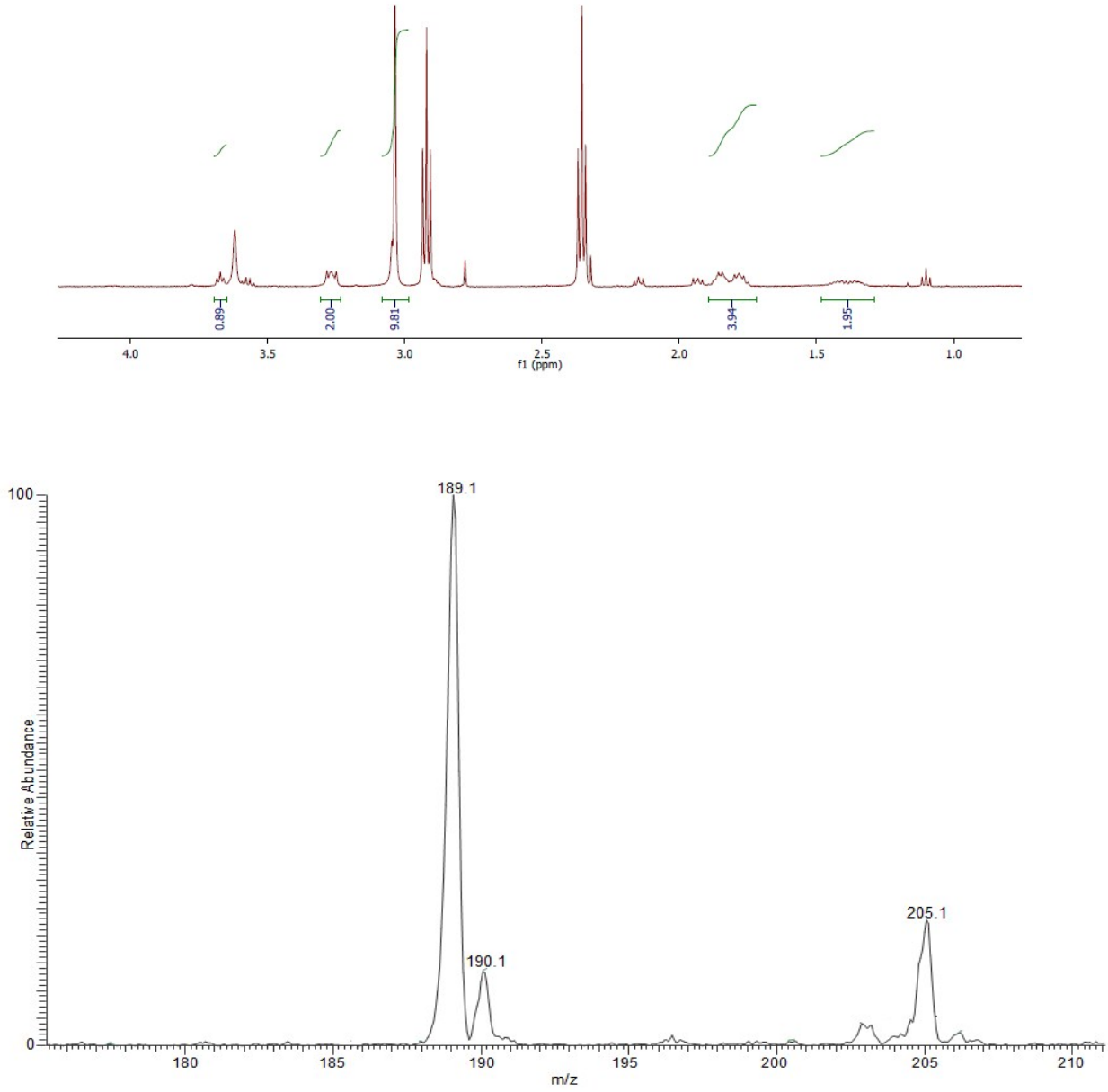


Fig. S7 Hydroxylation of trimethyllysine (**1**) in the absence of ascorbate as analysed by ^{1}H NMR (top) and LC-MS (bottom).

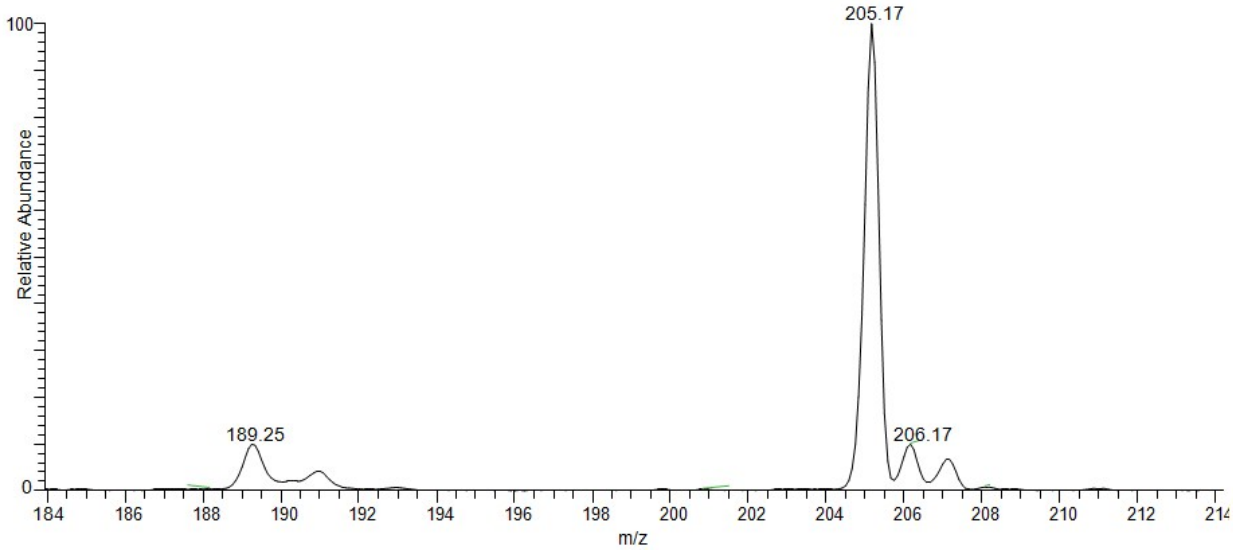


Fig. S8 Hydroxylation of trimethyllysine (**1**) in $H_2{}^{18}O$ under standard conditions as analysed by LC-MS.

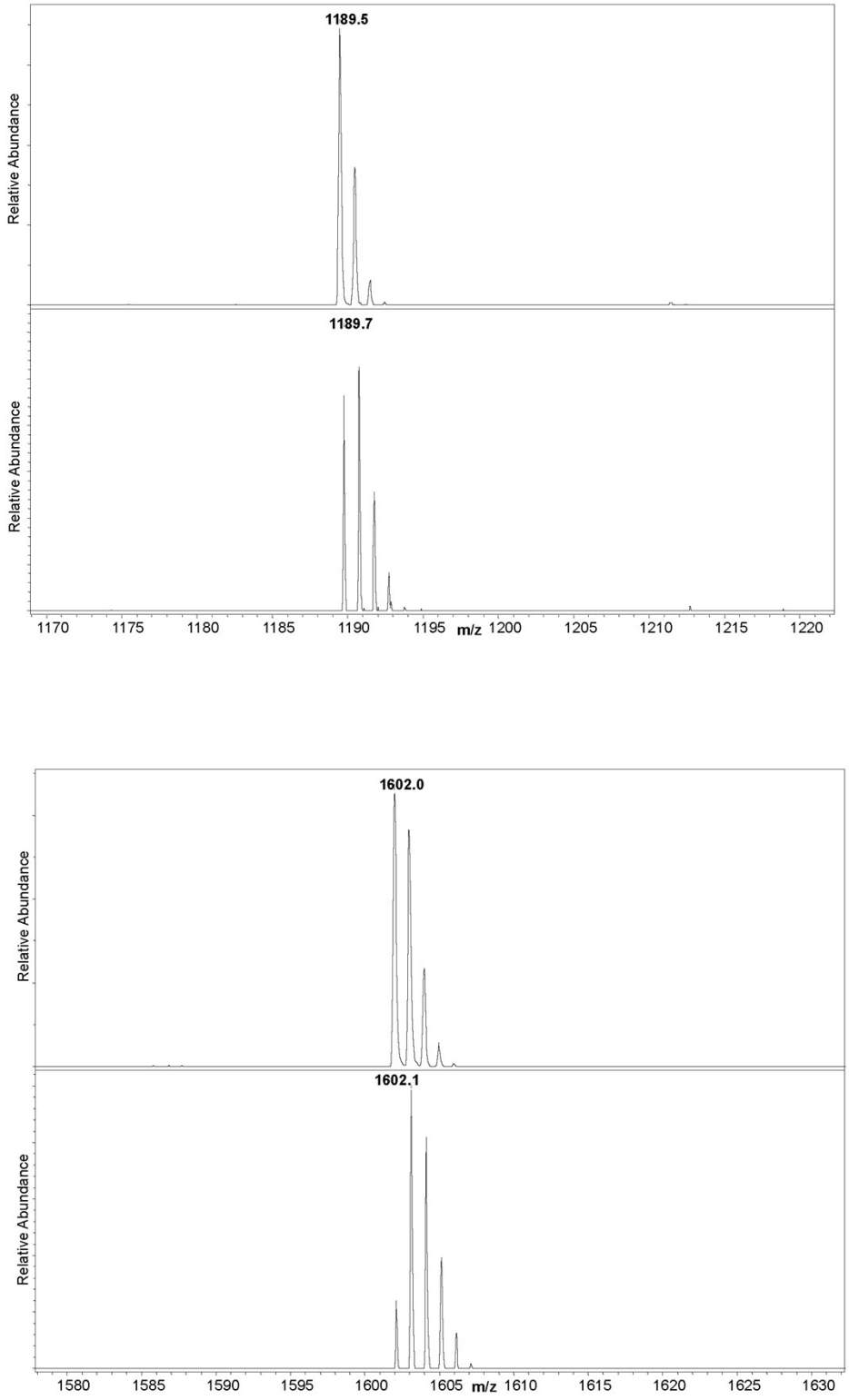


Fig. S9 Hydroxylation of H3K4me3 (residues 1-10, top) and H3K9me3 (residues 1-15, bottom) under standard conditions as analysed by MALDI-TOF MS. (top panel = starting peptide, bottom panel = TMLH-catalysed reaction)

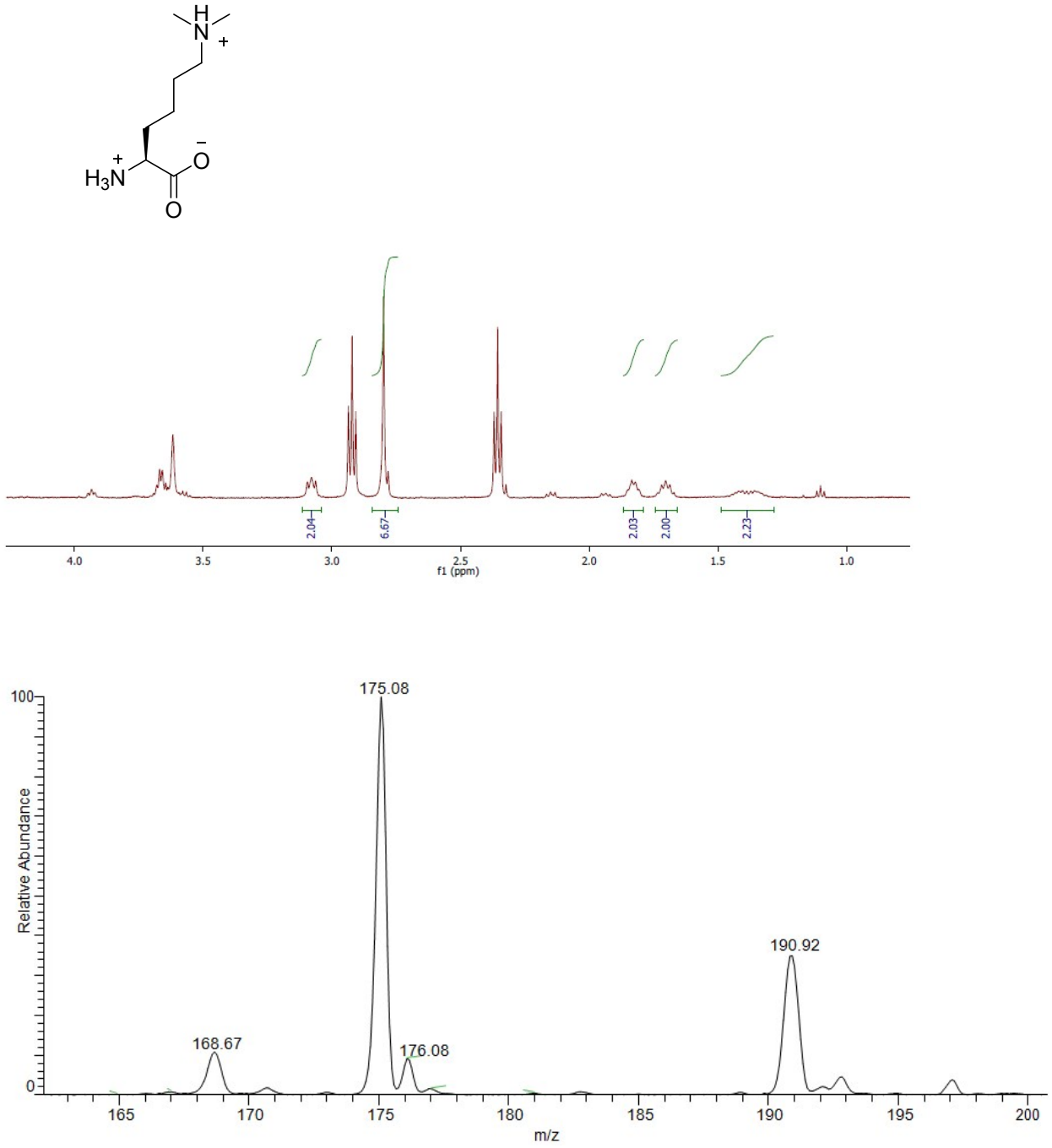


Fig. S10 Hydroxylation of dimethyllysine (**2**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

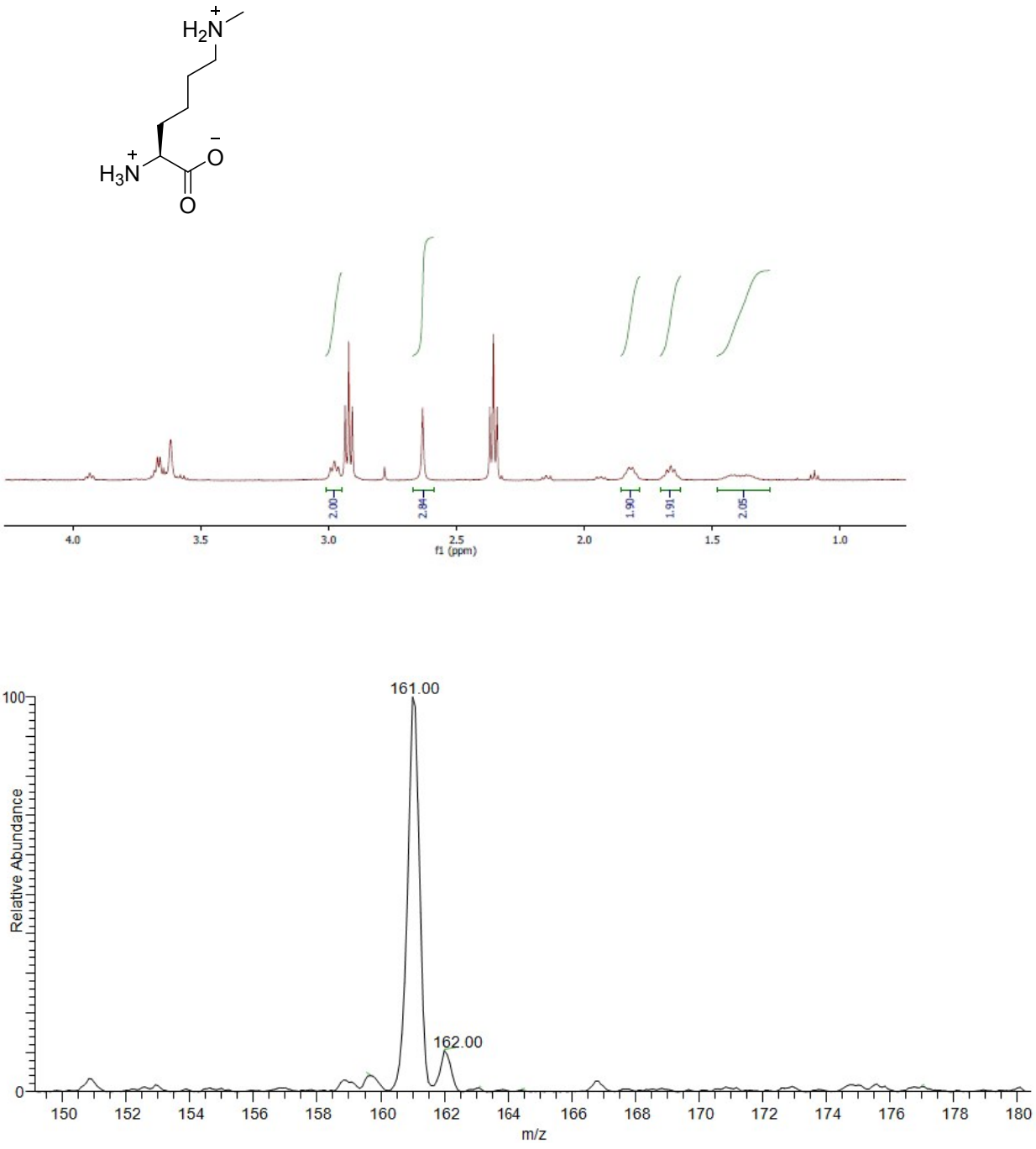


Fig. S11 Hydroxylation of methyllysine (**3**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

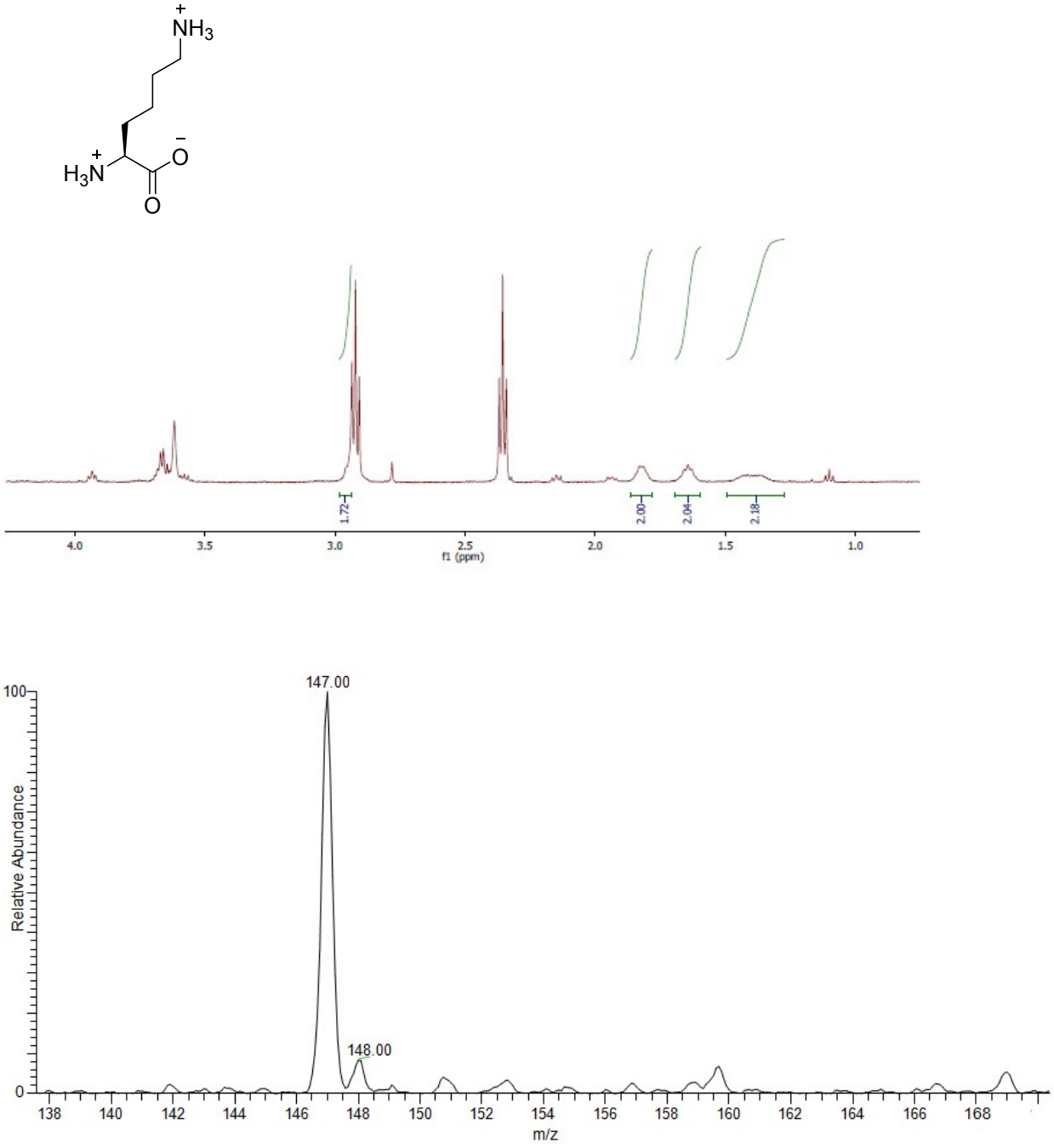


Fig. S12 Hydroxylation of lysine (**4**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

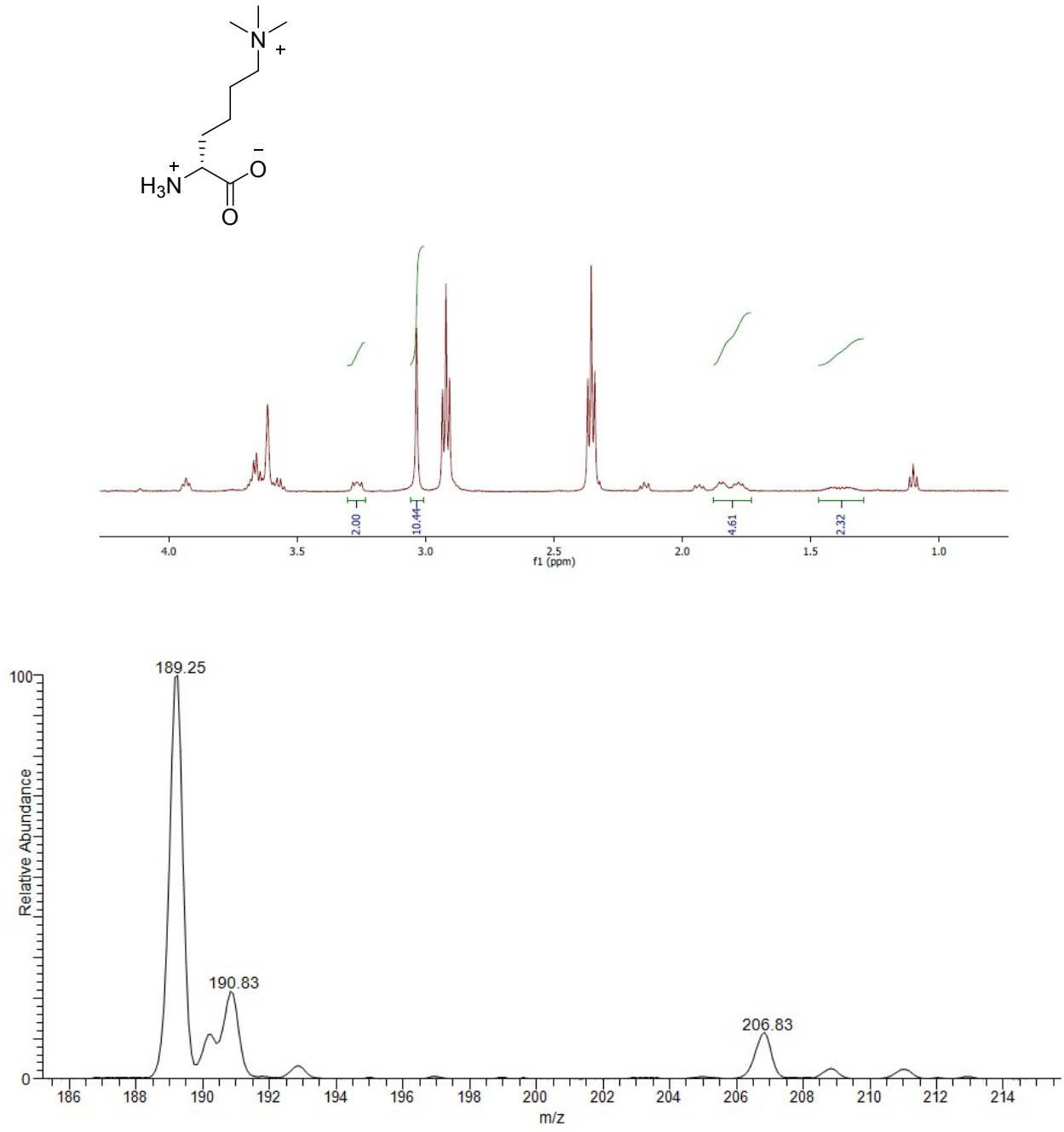


Fig. S13 Hydroxylation of D-trimethyllysine (**5**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

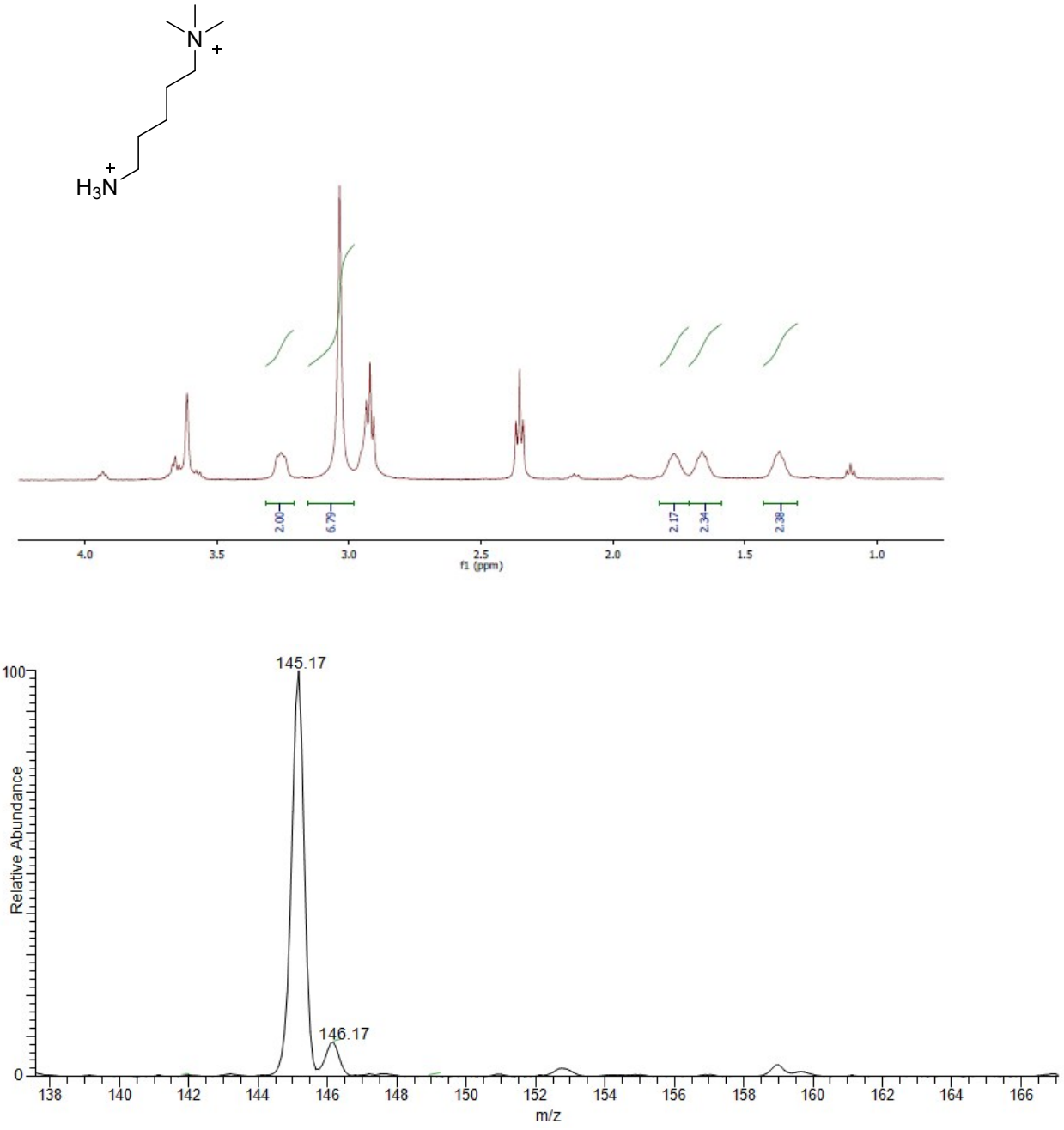


Fig. S14 Hydroxylation of 5-trimethylamino-1-aminopentane (**6**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

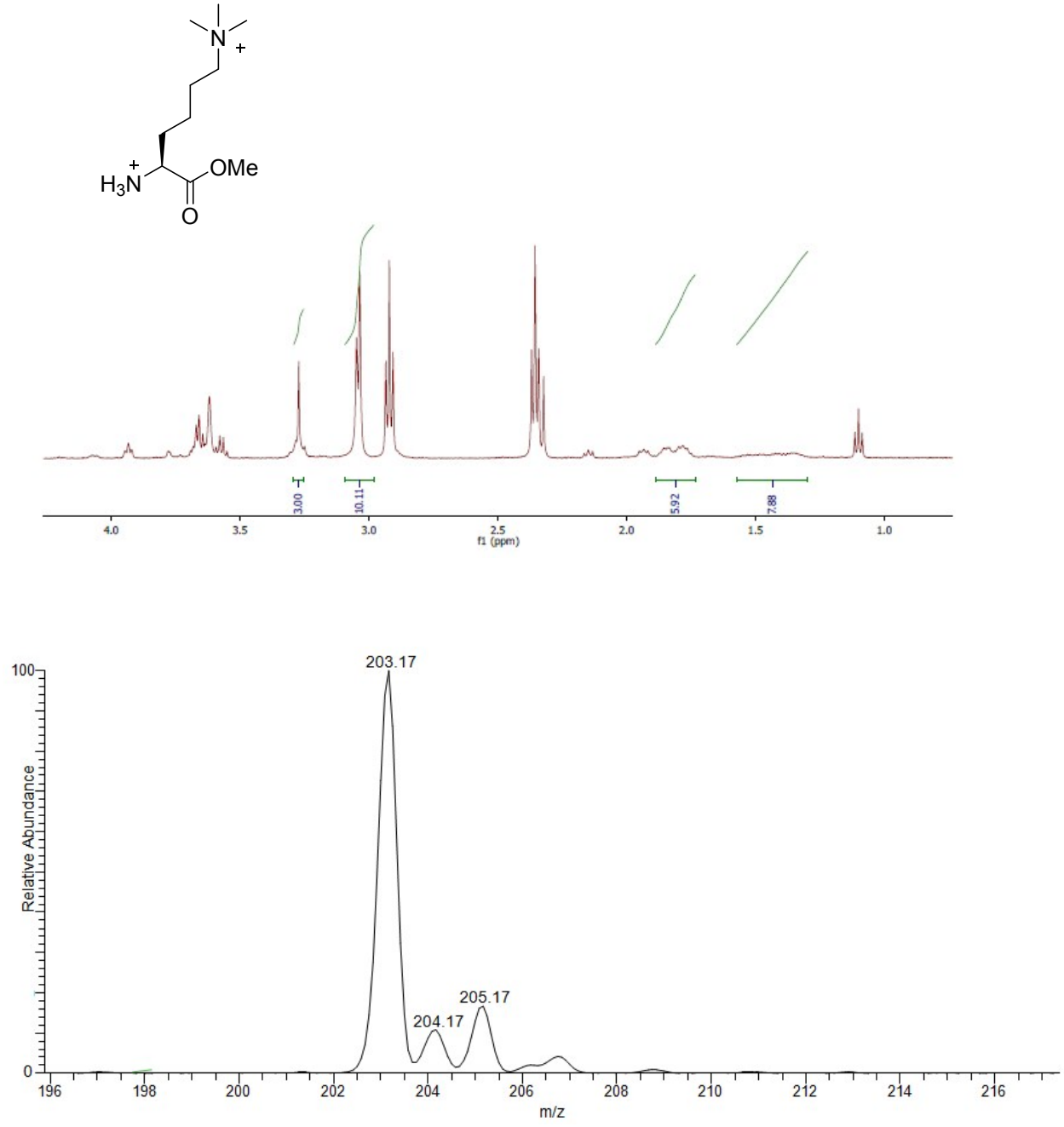


Fig. S15 Hydroxylation of the methyl ester of trimethyllysine (**7**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom). Small peaks of 3-hydroxytrimethyllysine derive from hydroxylation of trimethyllysine (a product of methyl ester hydrolysis under assay conditions).

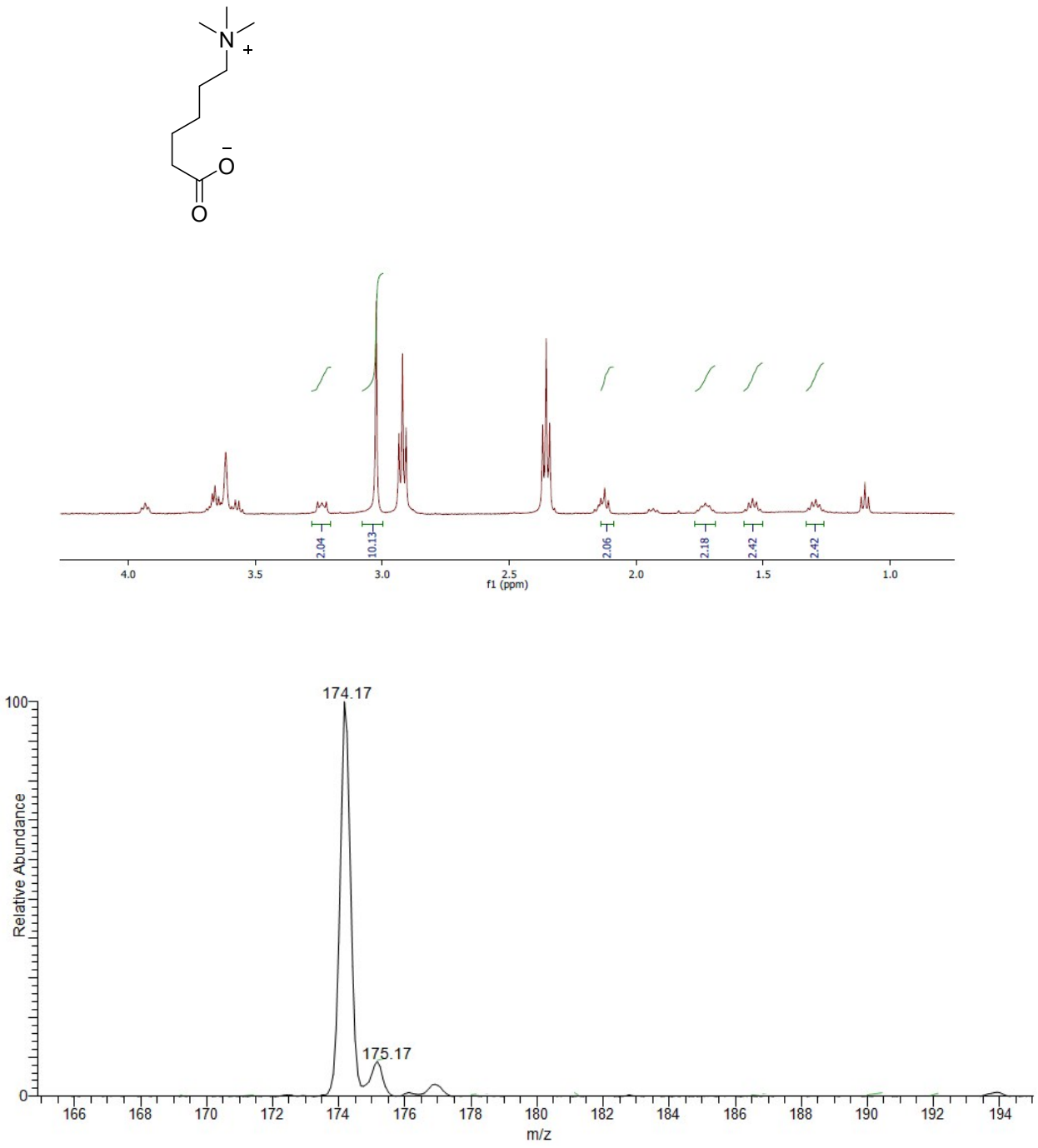


Fig. S16 Hydroxylation of 6-trimethylaminohexanoic acid (**8**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

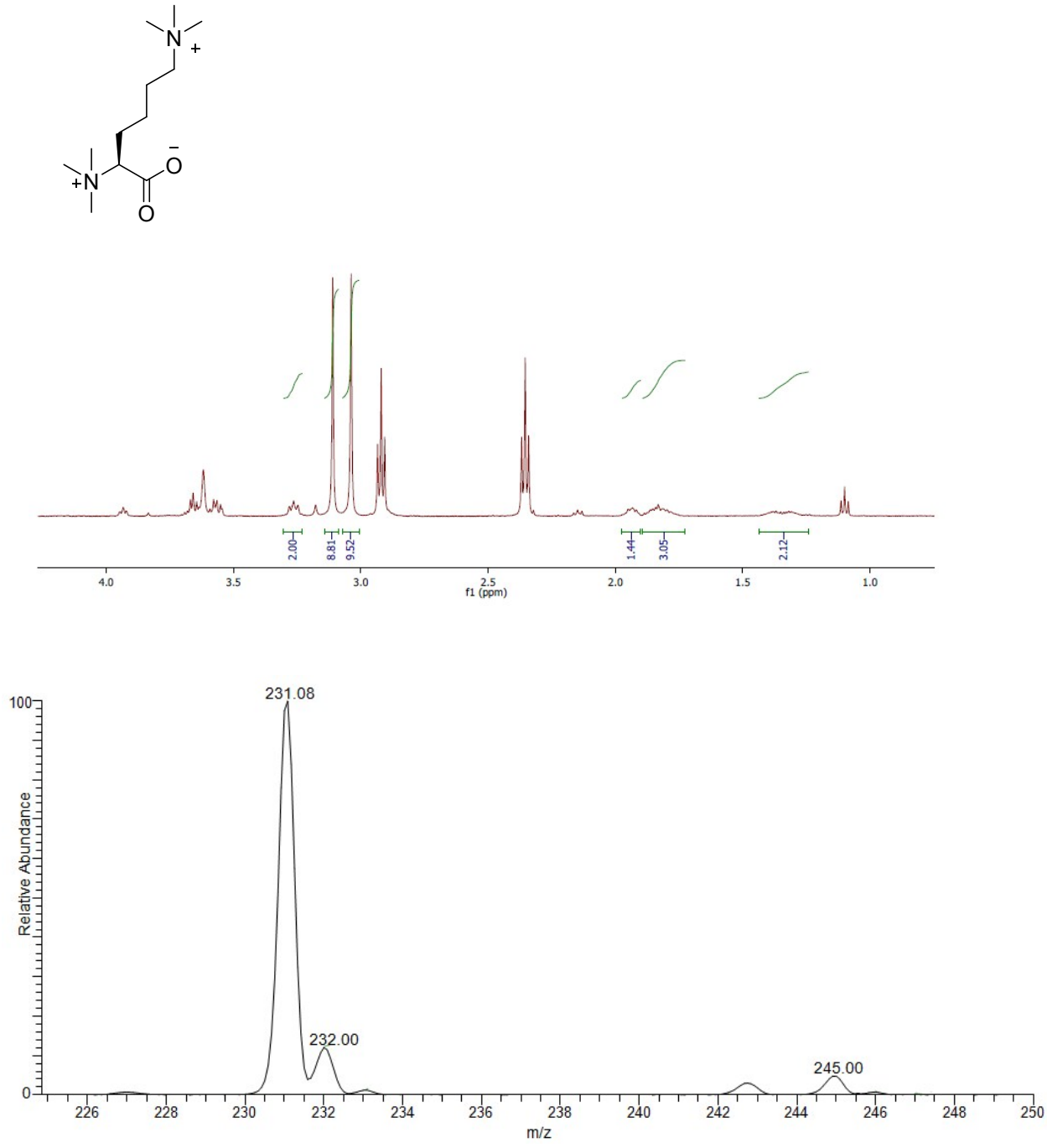


Fig. S17 Hydroxylation of hexamethyllysine (**9**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

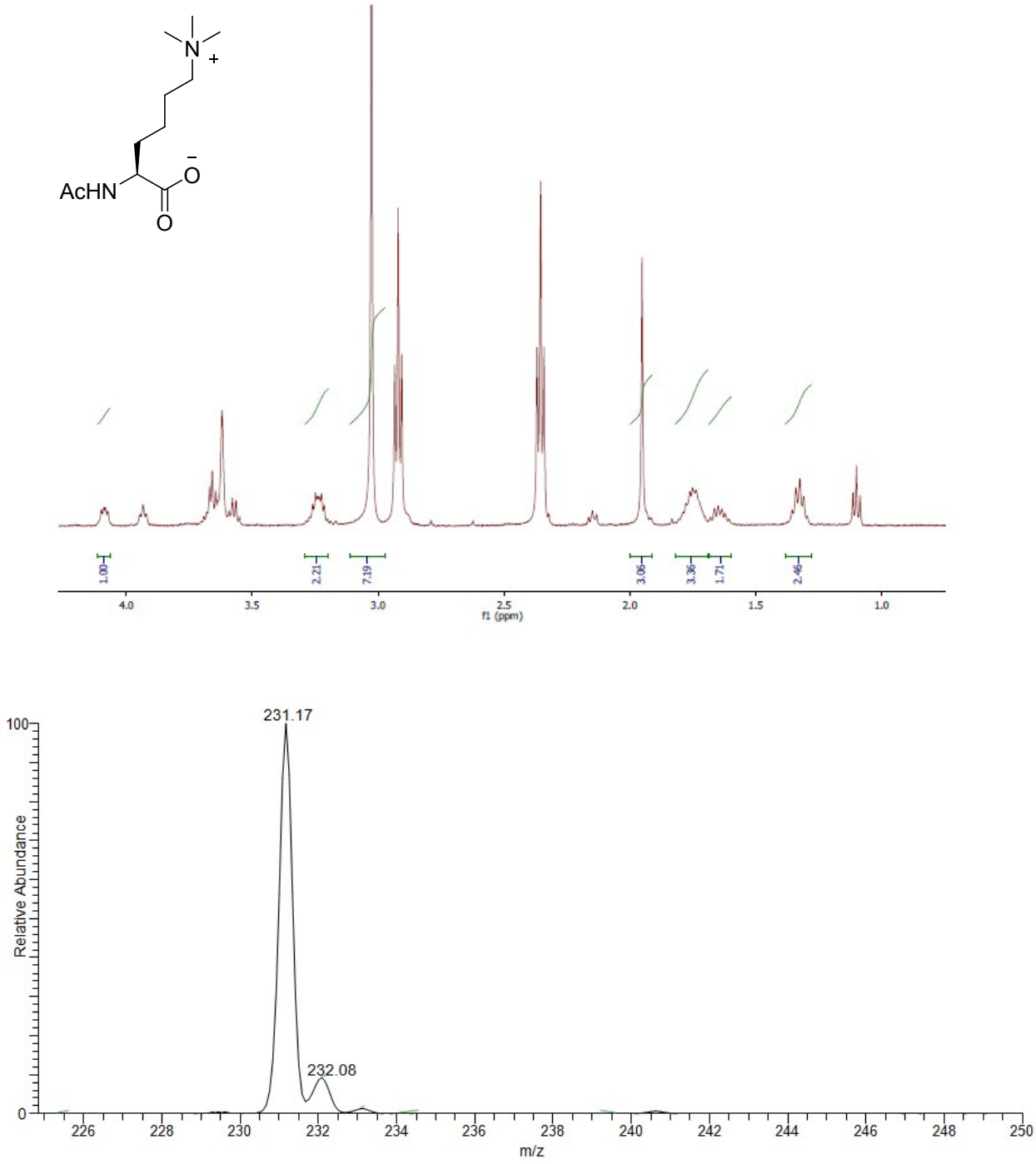


Fig. S18 Hydroxylation of N-acetyl trimethyllysine (**10**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

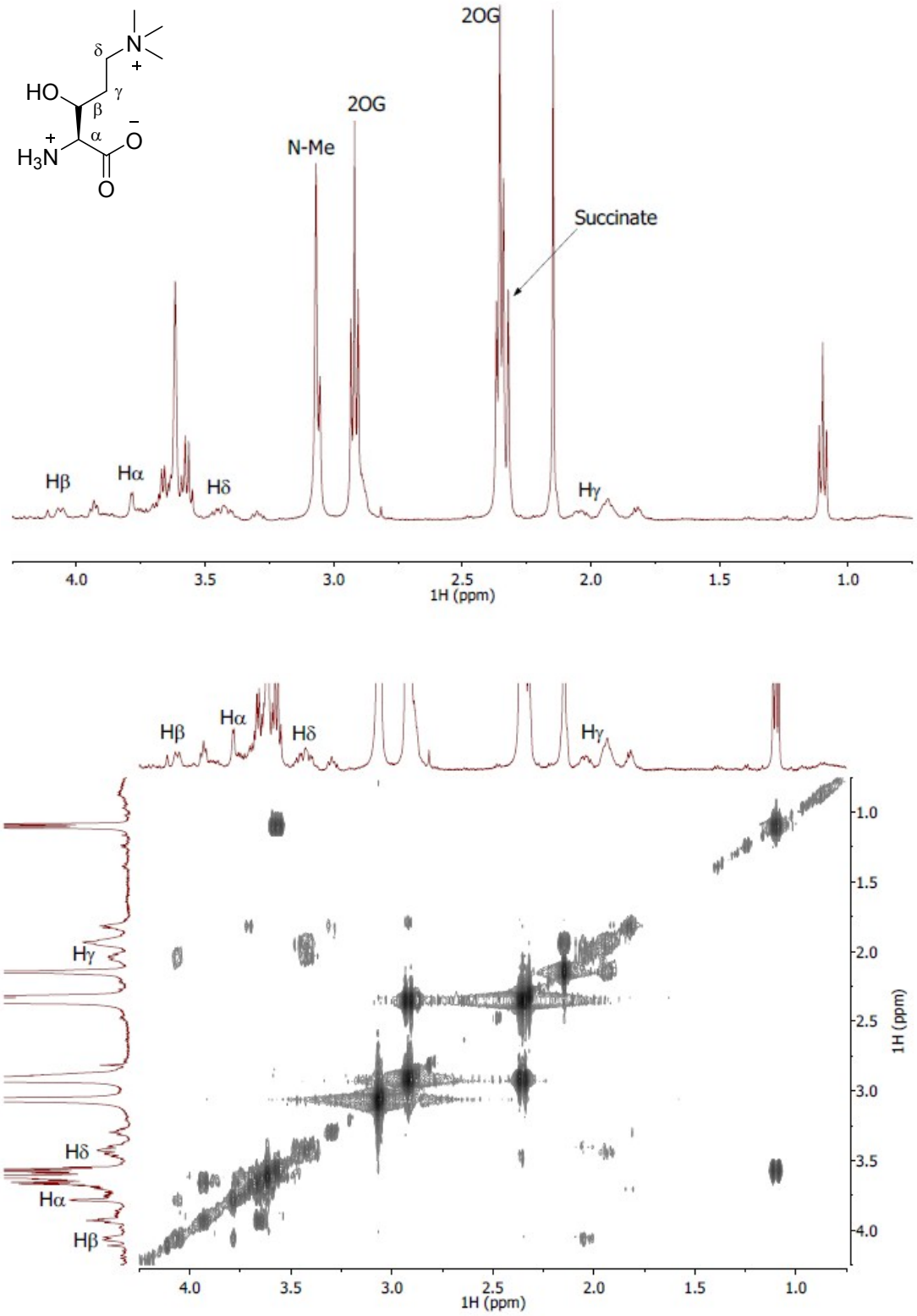


Fig. S19 NMR analyses of TMLH-catalysed hydroxylation of trimethylornithine (**11**). ^{1}H NMR data of TMLH-catalysed C-3 hydroxylation of trimethylornithine (top). COSY data of TMLH-catalysed C-3 hydroxylation of trimethylornithine (bottom).

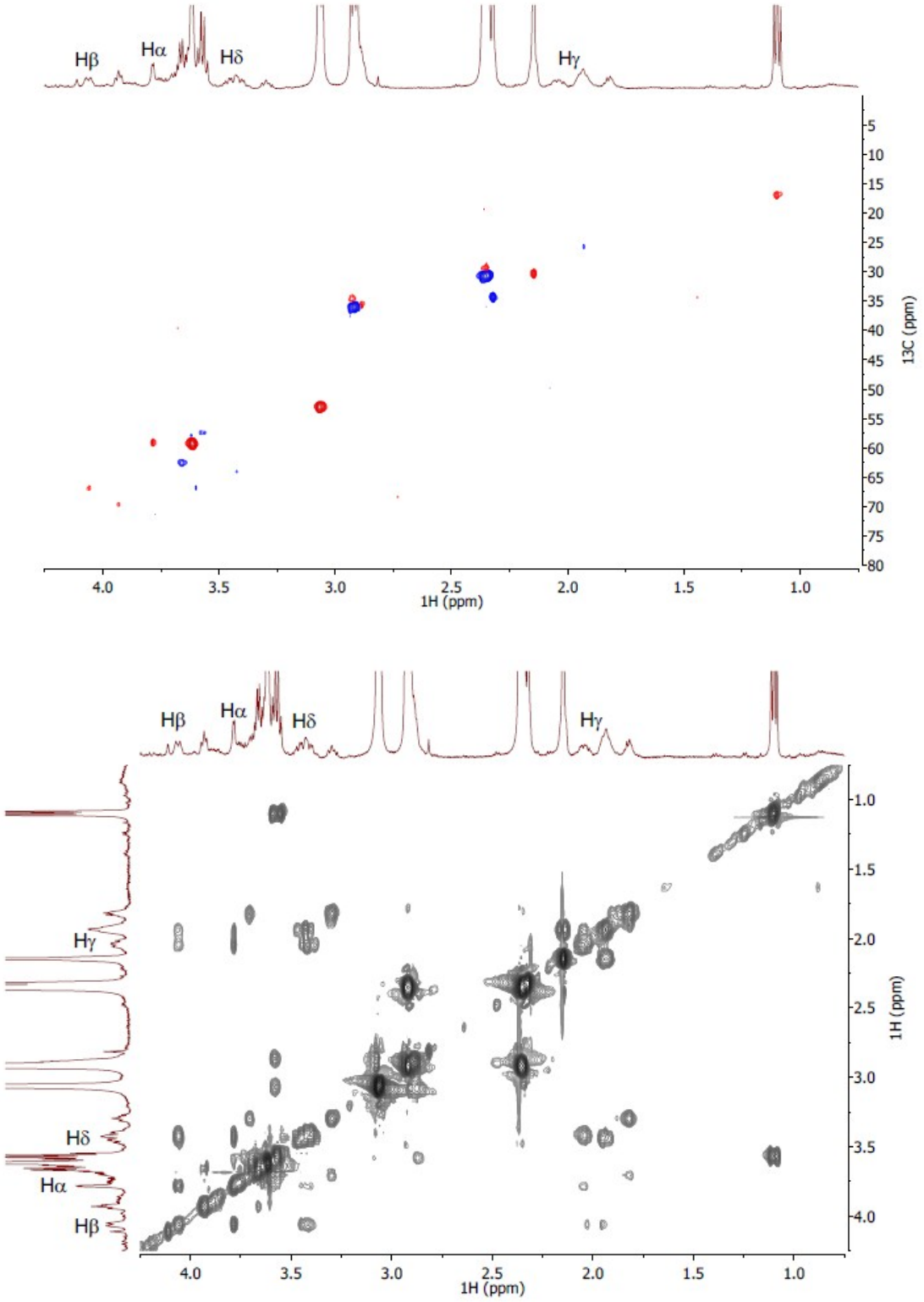


Fig. S20 NMR analyses of TMLH-catalysed hydroxylation of trimethylornithine (**11**). Multiplicity-edited HSQC data of TMLH-catalysed C-3 hydroxylation of ornithine (top; red = positive, CH/CH_3, blue = negative, CH_2). TOCSY data of TMLH-catalysed C-3 hydroxylation of trimethylornithine (bottom).

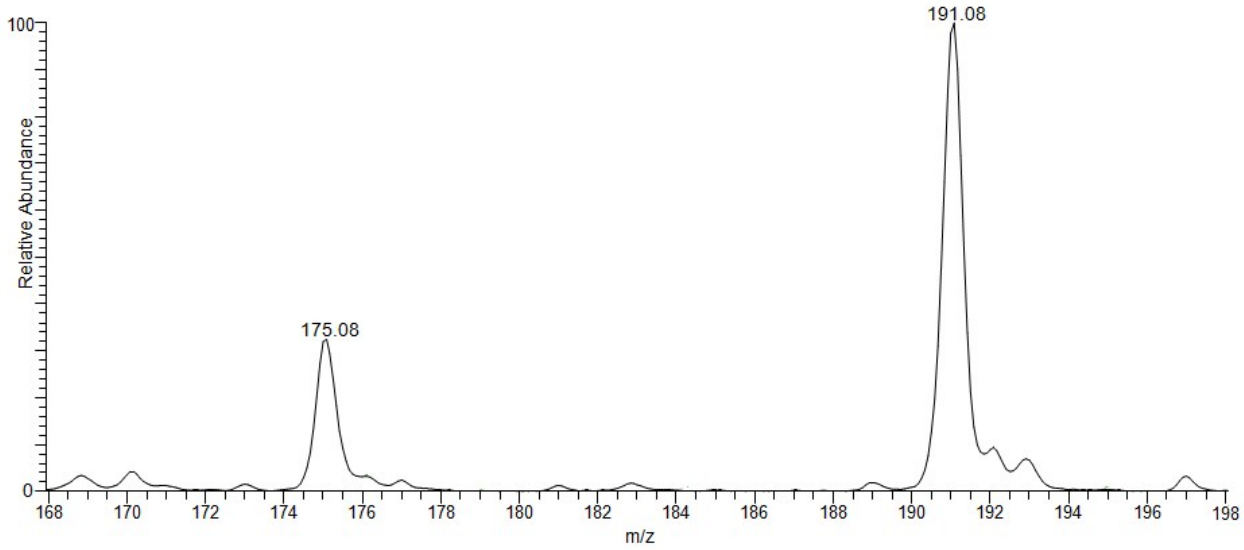


Fig. S21 Hydroxylation of trimethylornithine (**11**) under standard conditions as analysed by LC-MS.

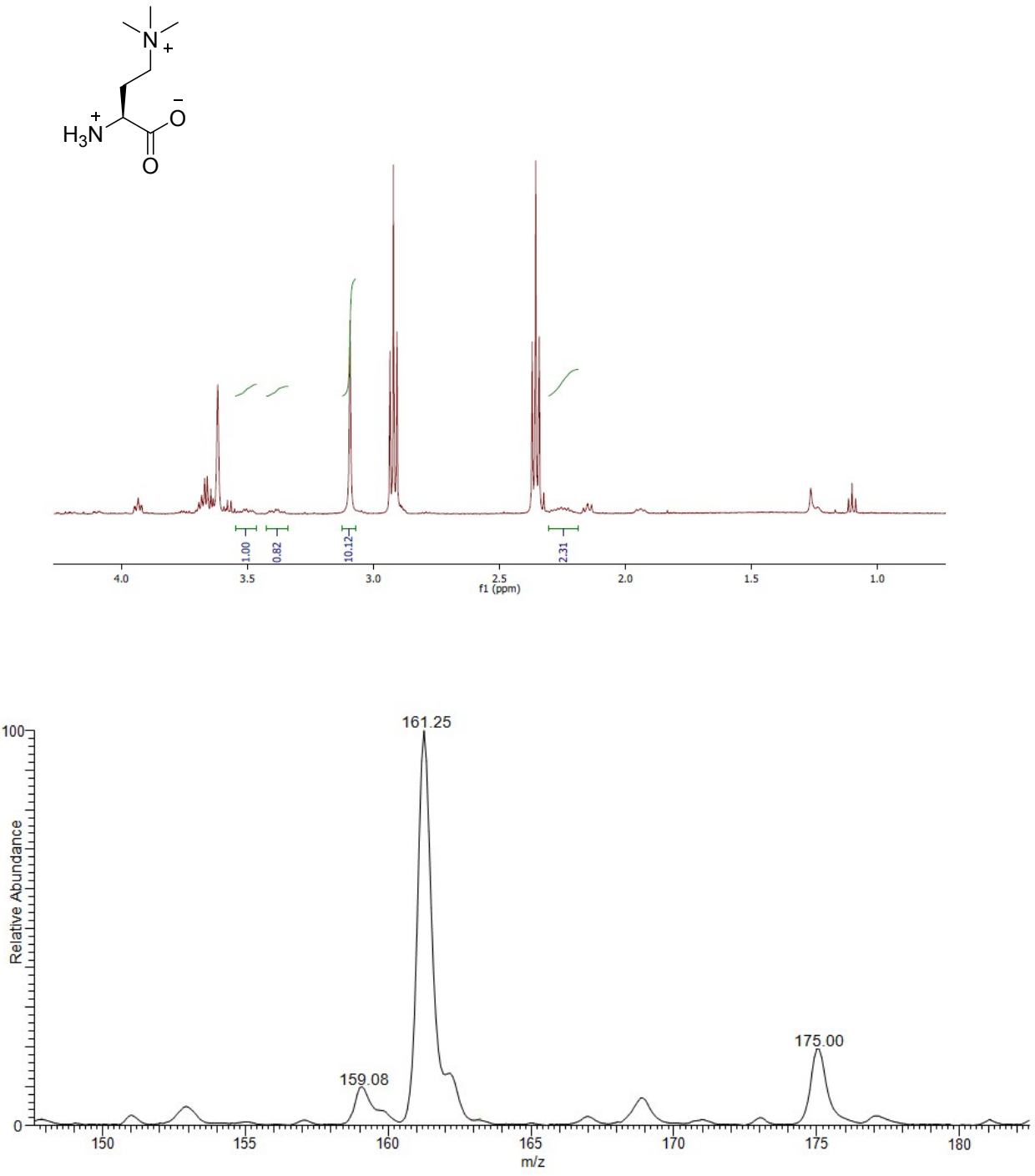


Fig. S22 Hydroxylation of trimethyldiaminobutyric acid (**12**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

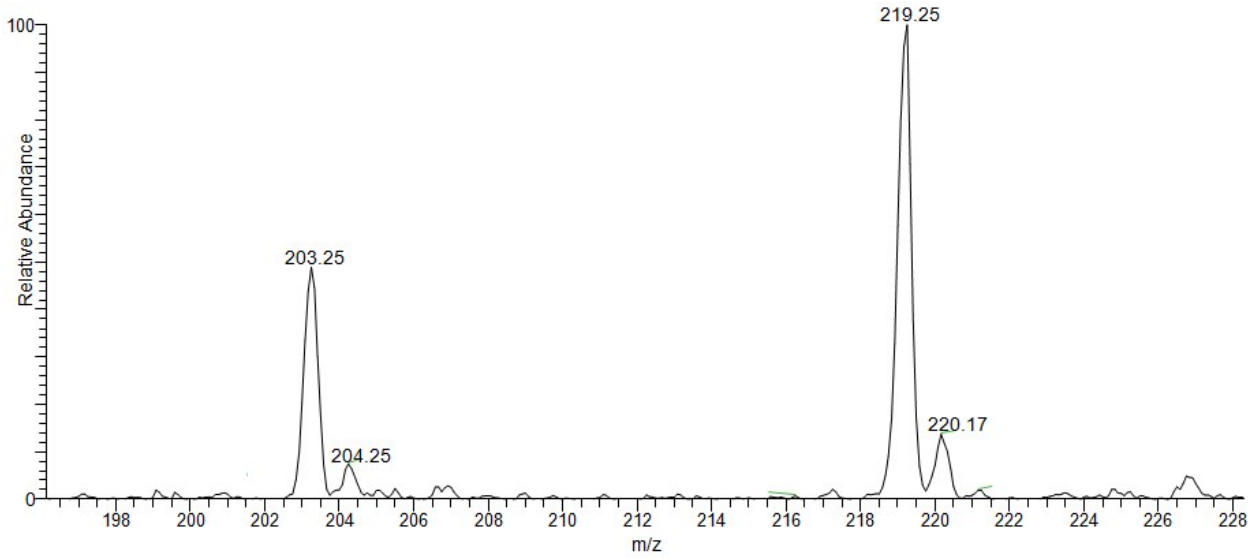


Fig. S23 Hydroxylation of trimethylhomolysine (**13**) under standard conditions as analysed by LC-MS.

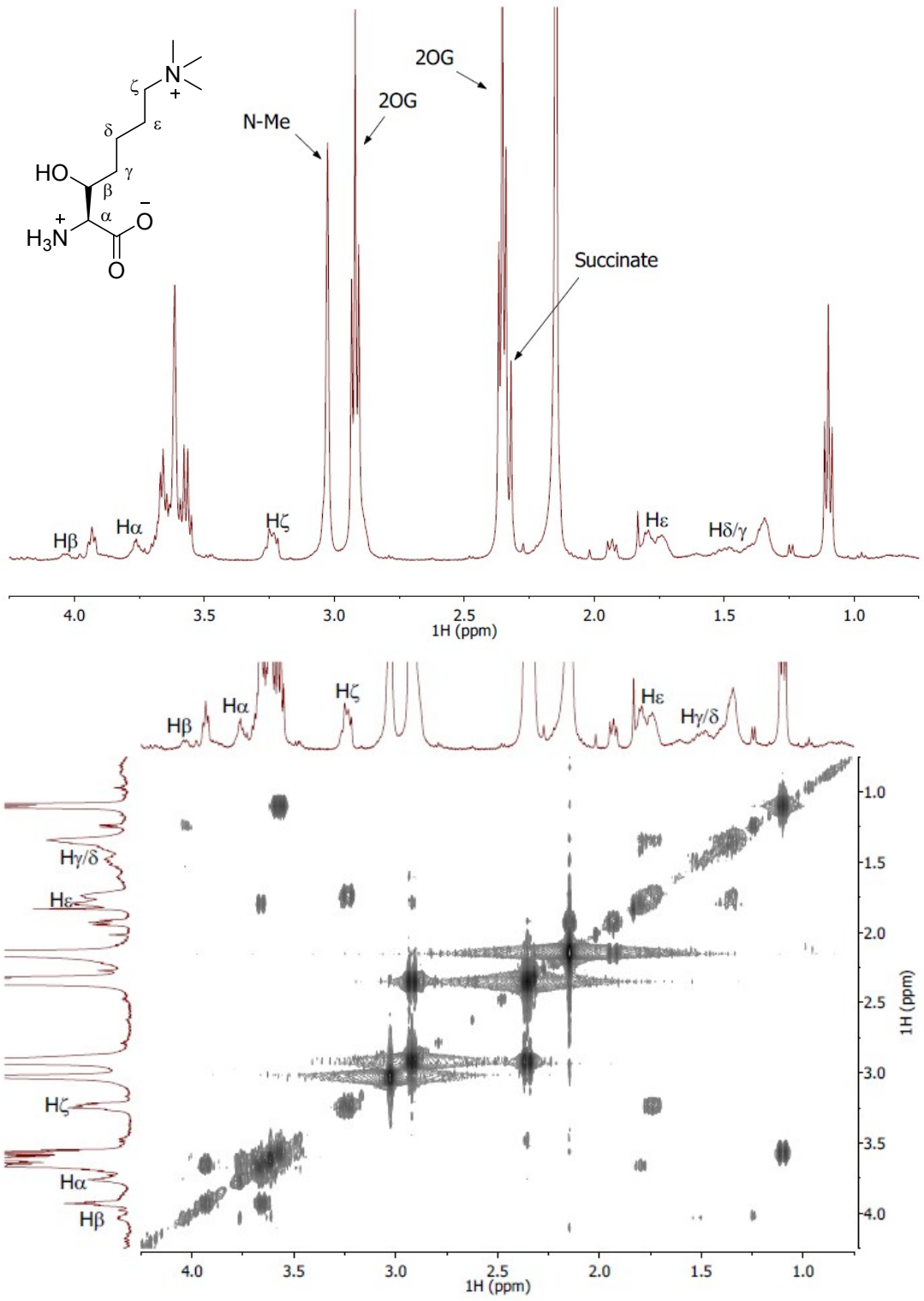


Fig. S24 NMR analyses of TMLH-catalysed hydroxylation of trimethylhomolysine (**13**). ^{1}H NMR data of TMLH-catalysed C-3 hydroxylation of trimethylhomolysine (top). COSY data of TMLH-catalysed C-3 hydroxylation of trimethylhomolysine (bottom).

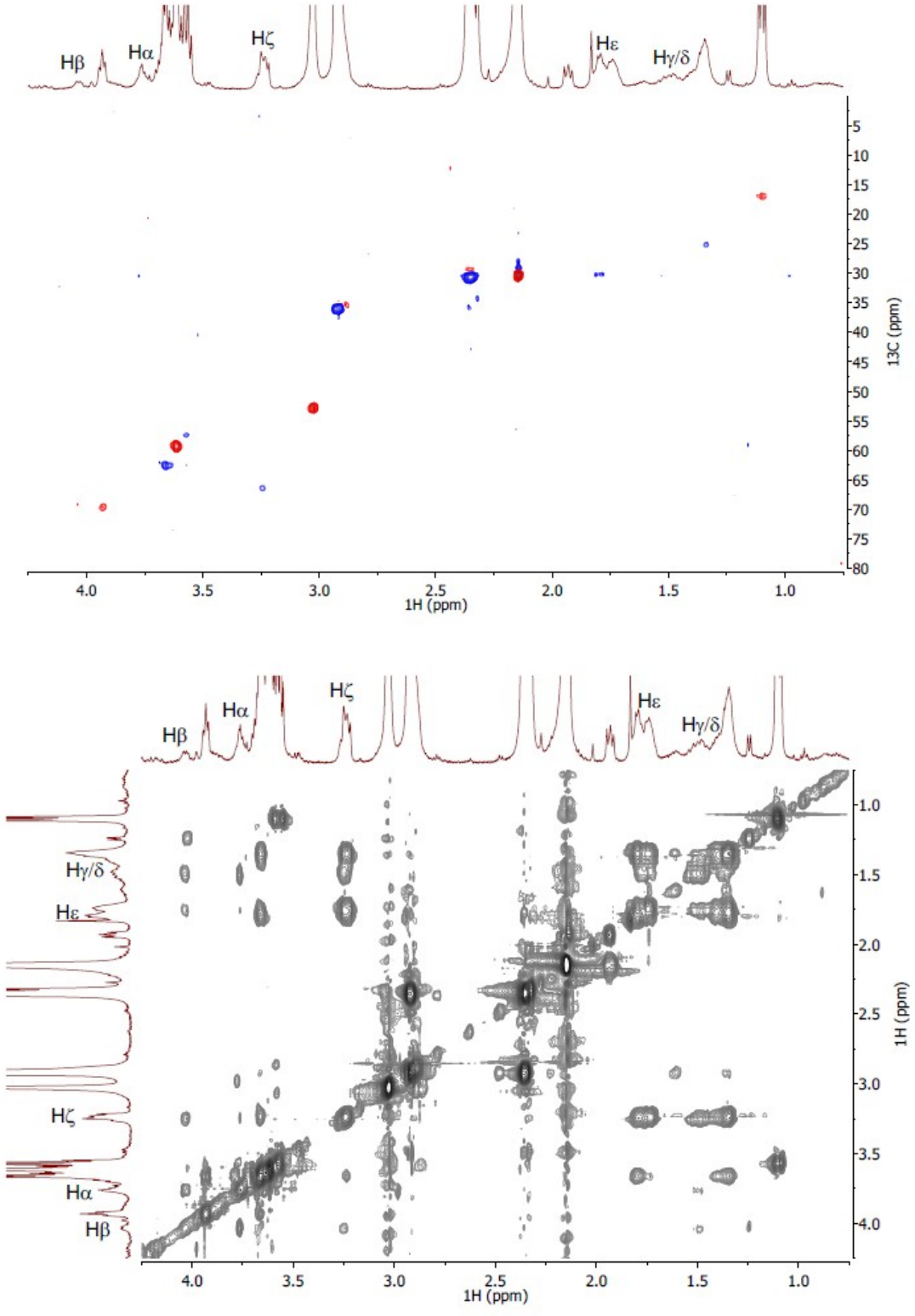


Fig. S25 NMR analyses of TMLH-catalysed hydroxylation of trimethylhomolysine (**13**). Multiplicity-edited HSQC data of TMLH-catalysed C-3 hydroxylation of trimethylhomolysine (top; red = positive, CH/CH_3,

blue = negative, CH_2). TOCSY data of TMLH-catalysed C-3 hydroxylation of trimethylhomolysine (bottom).

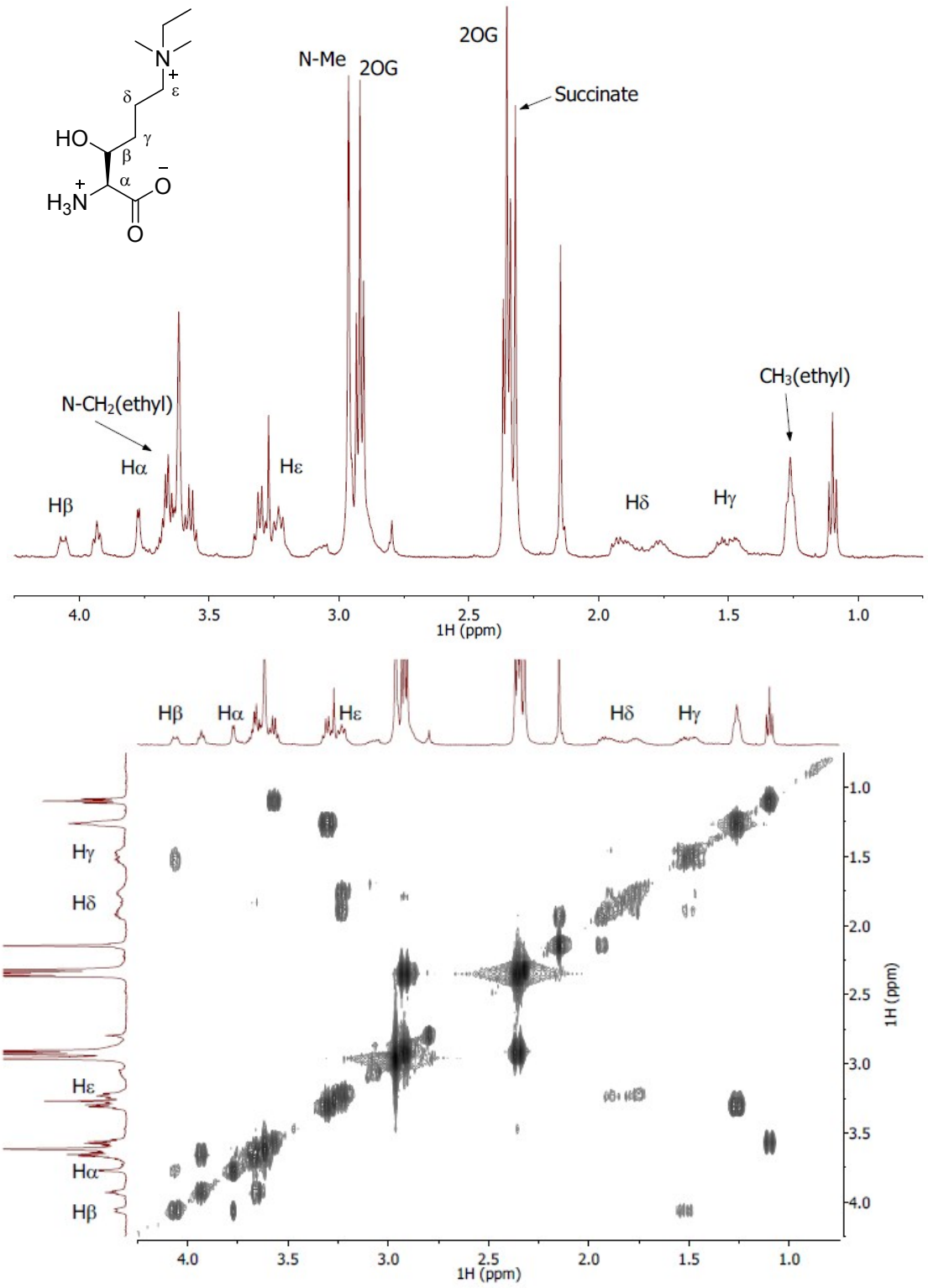
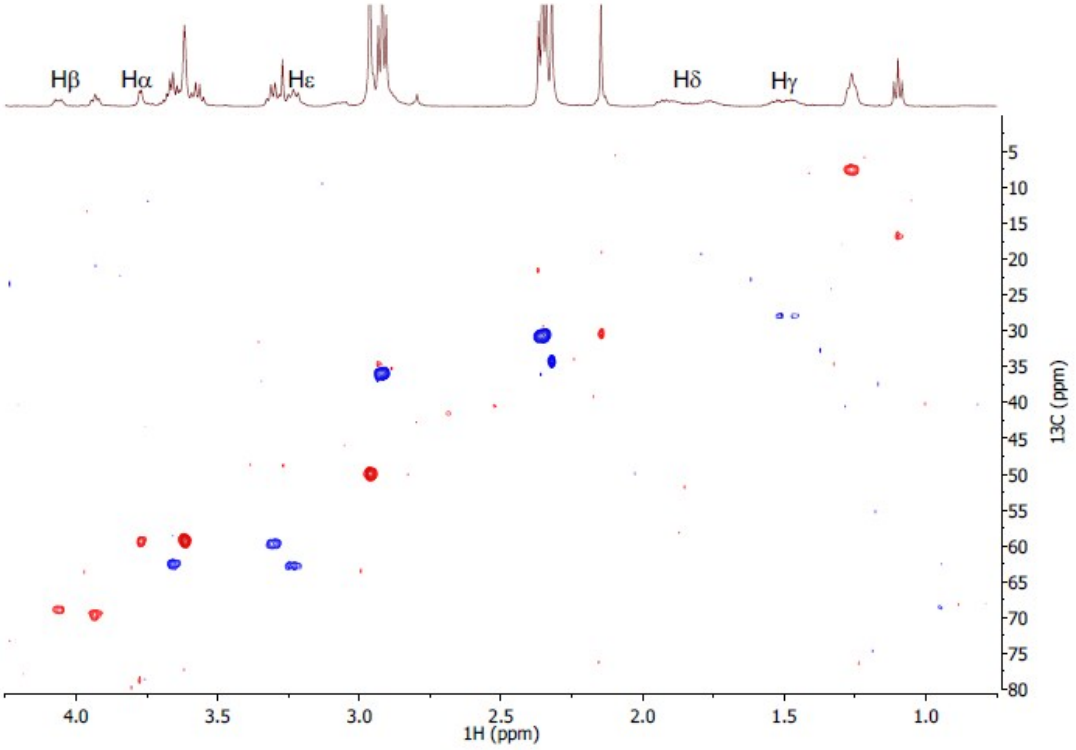


Fig. S26 NMR analyses of TMLH-catalysed hydroxylation of dimethylethyllysine (**14**). ^{1}H NMR data of TMLH-catalysed C-3 hydroxylation of dimethylethyllysine (top). COSY data of TMLH-catalysed C-3 hydroxylation of dimethylethyllysine (bottom).



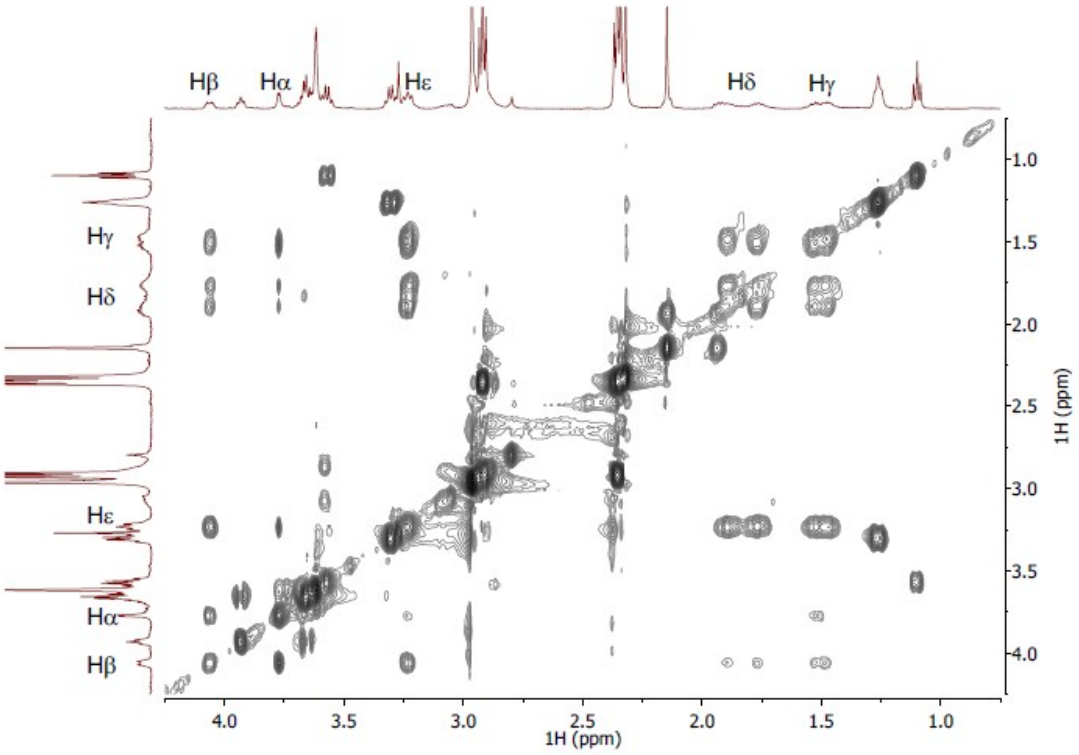


Fig. S27 NMR analyses of TMLH-catalysed hydroxylation of dimethylethyllysine (**14**). Multiplicity-edited HSQC data of TMLH-catalysed C-3 hydroxylation of dimethylethyllysine (top; red = positive, CH/CH_3, blue = negative, CH_2). TOCSY data of TMLH-catalysed C-3 hydroxylation of dimethylethyllysine (bottom).

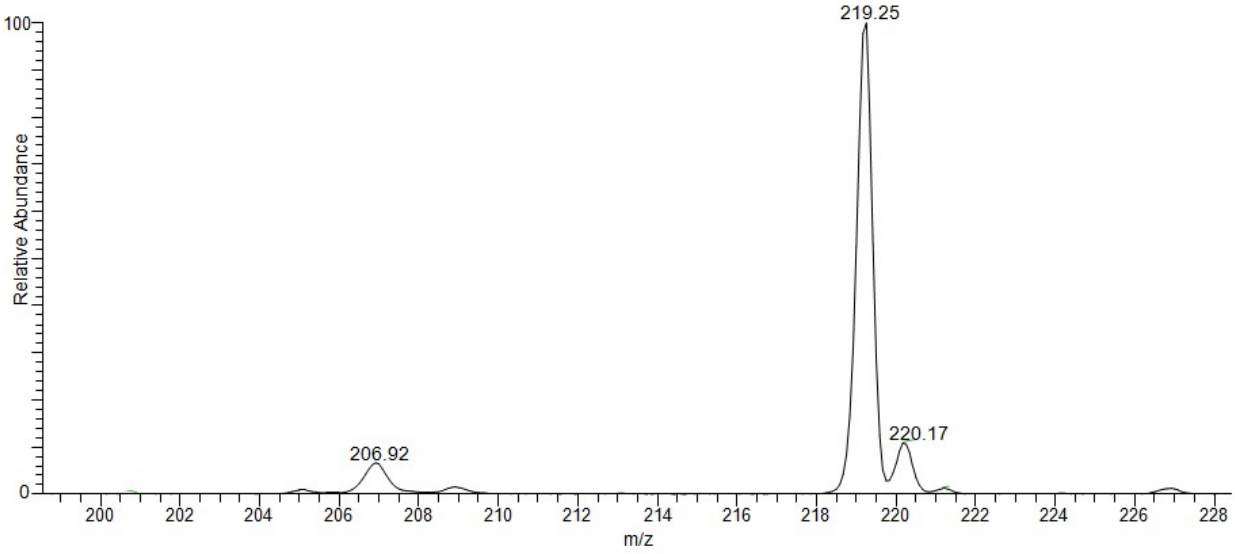
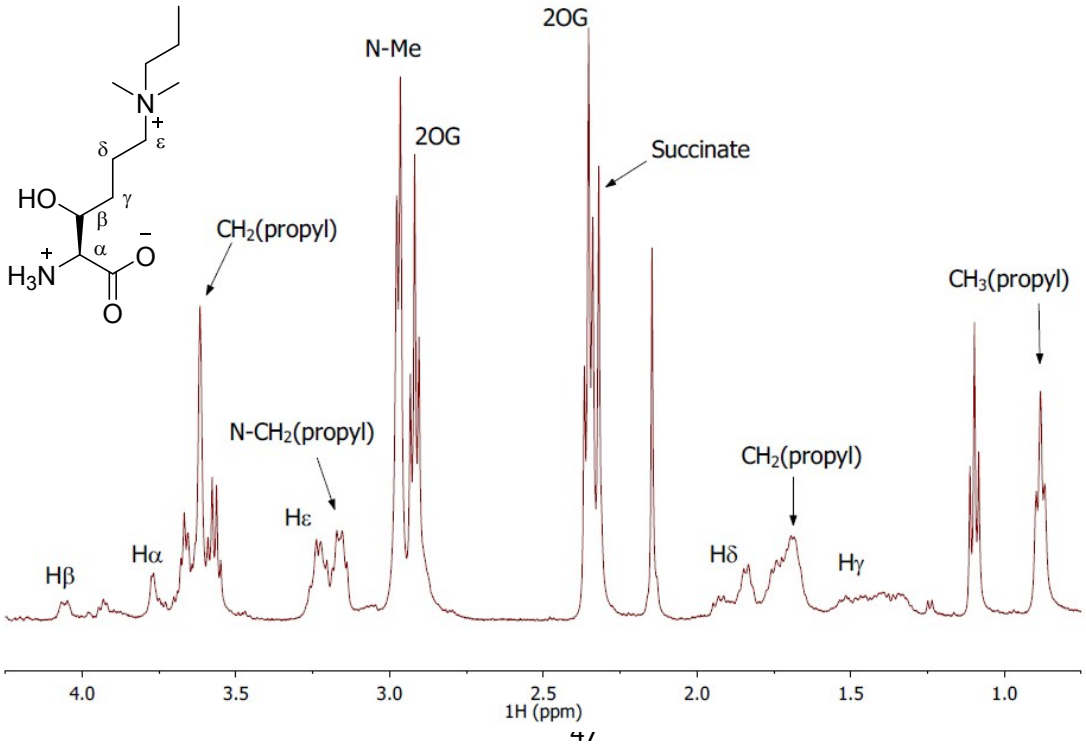


Fig. S28 Hydroxylation of dimethylethyllysine (**14**) under standard conditions as analysed by LC-MS.


HO
δ
ε
γ
β
α
O
O
2OG
N-Me
2OG
Succinate
CH2(propyl)
CH3(propyl)
N-CH2(propyl)
CH2(propyl)
Hε
Hβ
Hα
Hδ
Hγ
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1H (ppm)

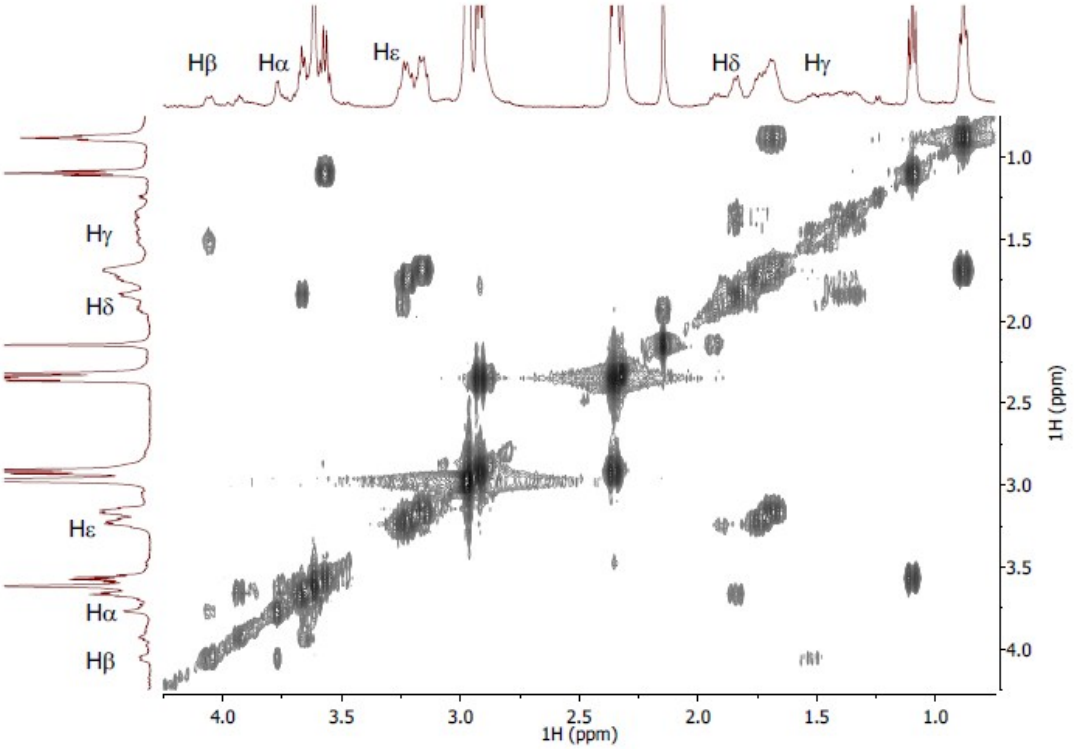
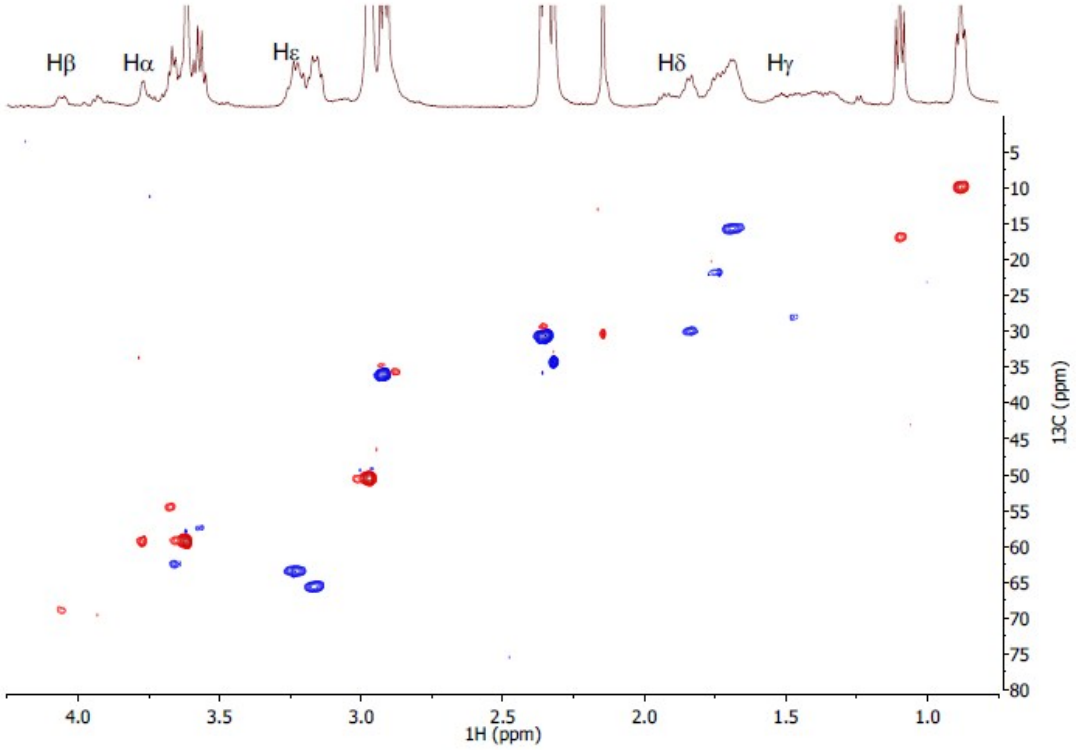


Fig. S29 NMR analyses of TMLH-catalysed hydroxylation of dimethylpropyllysine (**15**). ^{1}H NMR data of TMLH-catalysed C-3 hydroxylation of dimethylpropyllysine (top). COSY data of TMLH-catalysed C-3 hydroxylation of dimethylpropyllysine (bottom).



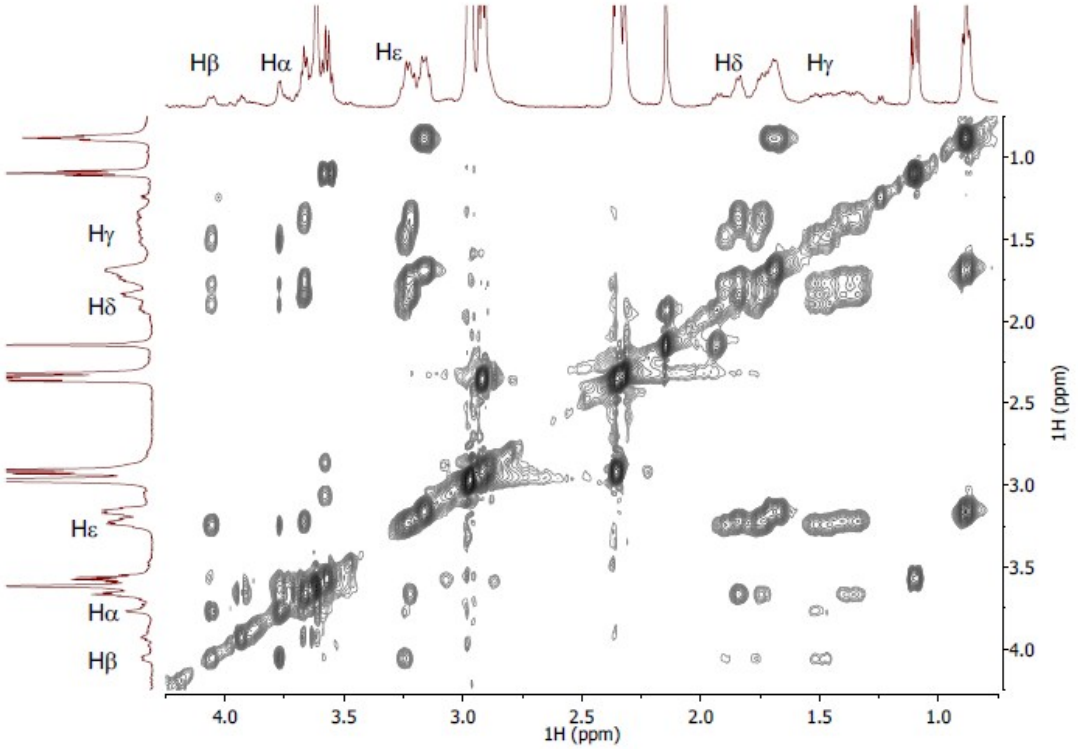


Fig. S30 NMR analyses of TMLH-catalysed hydroxylation of dimethylpropyllysine (**15**). Multiplicity-edited HSQC data of TMLH-catalysed C-3 hydroxylation of diimethylpropyllysine (top; red = positive, CH/CH_3, blue = negative, CH_2). TOCSY data of TMLH-catalysed C-3 hydroxylation of dimethylpropyllysine (bottom).

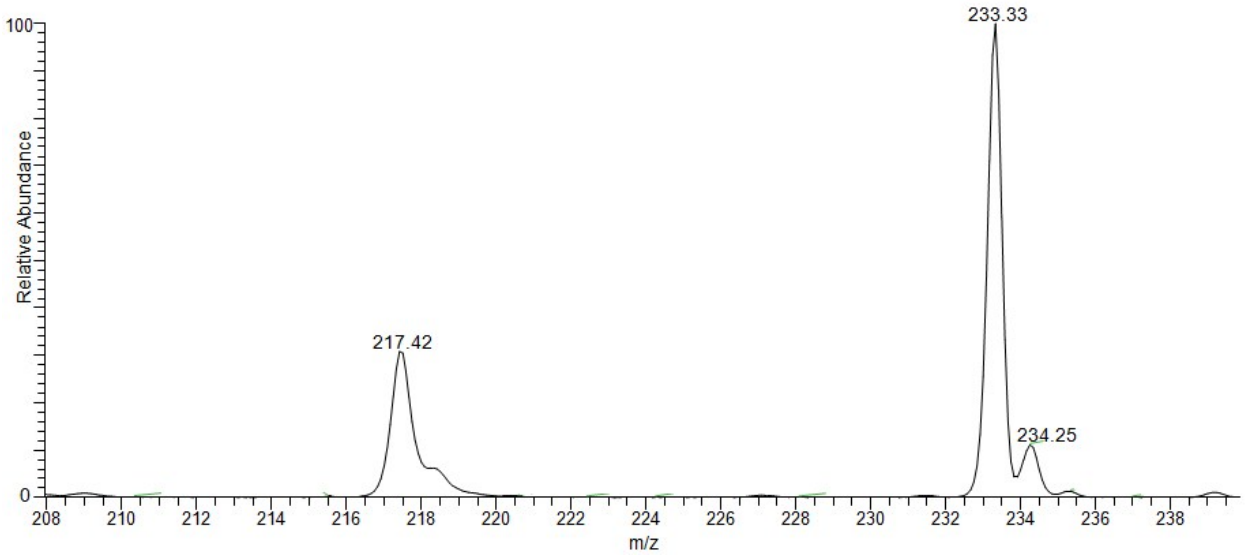
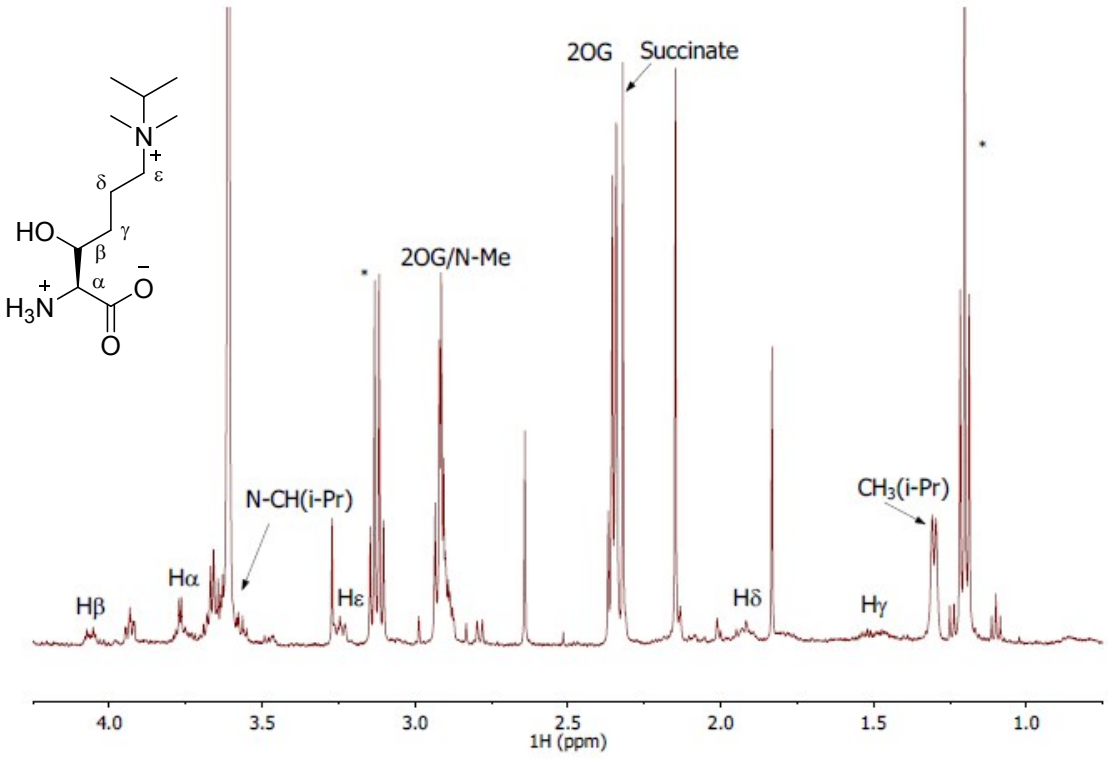


Fig. S31 Hydroxylation of dimethypropyllysine (**15**) under standard conditions as analysed by LC-MS.


2OG
Succinate
2OG/N-Me
N-CH(i-Pr)
CH3(i-Pr)
Hβ
Hα
Hε
Hδ
Hγ
HO
H3N
δ
ε
γ
β
α
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1H (ppm)

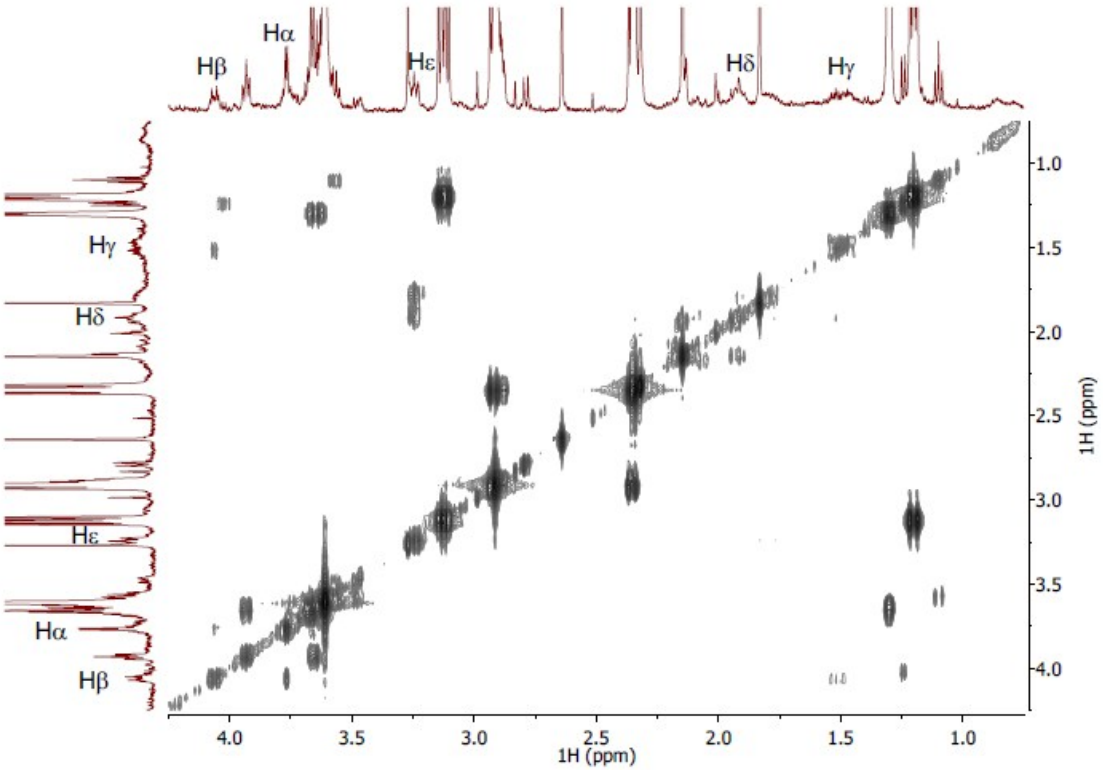
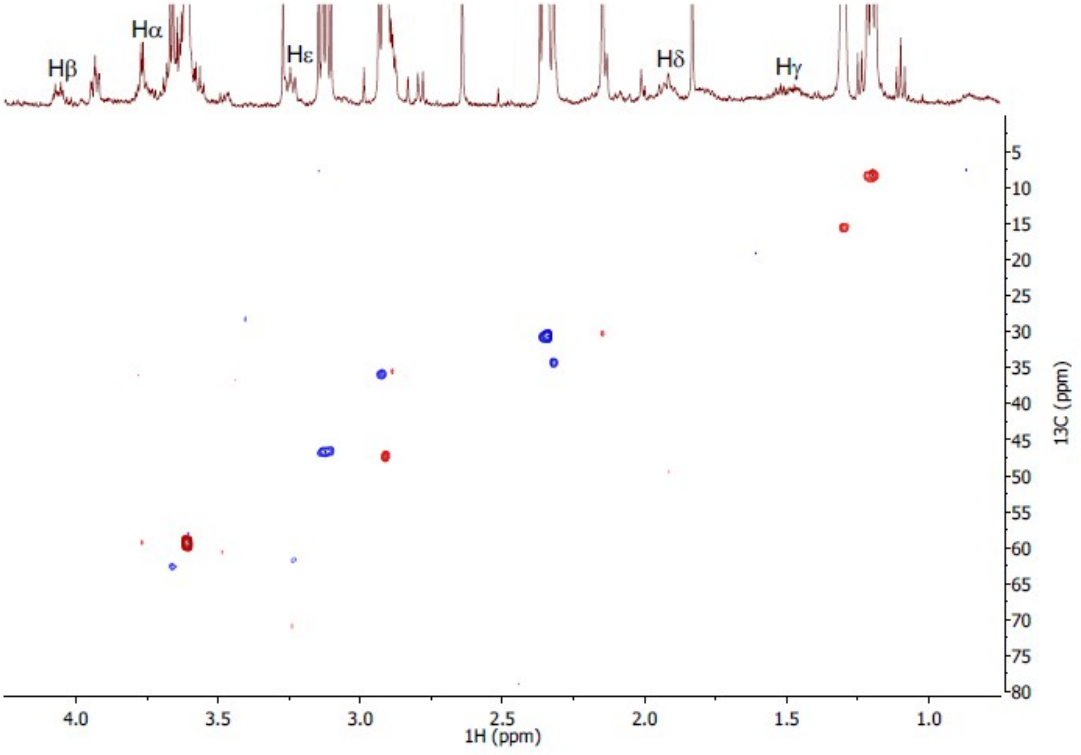


Fig. S32 NMR analyses of TMLH-catalysed hydroxylation of dimethylisopropyllysine (**16**). ^{1}H NMR data of TMLH-catalysed C-3 hydroxylation of dimethylisopropyllysine (top). COSY data of TMLH-catalysed C-3 hydroxylation of dimethylisopropyllysine (bottom).



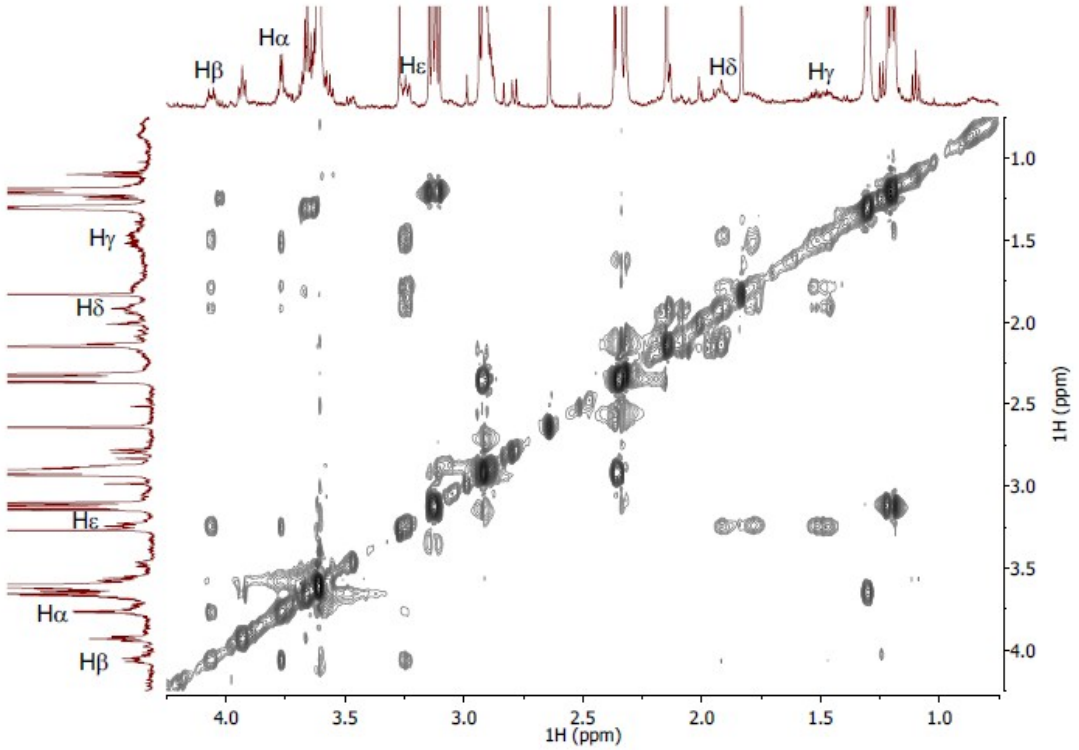


Fig. S33 NMR analyses of TMLH-catalysed hydroxylation of dimethylisopropyllysine (**16**). Multiplicity-edited HSQC data of TMLH-catalysed C-3 hydroxylation of diimethylisopropyllysine (top; red = positive, CH/CH_3, blue = negative, CH_2). TOCSY data of TMLH-catalysed C-3 hydroxylation of dimethylisopropyllysine (bottom).

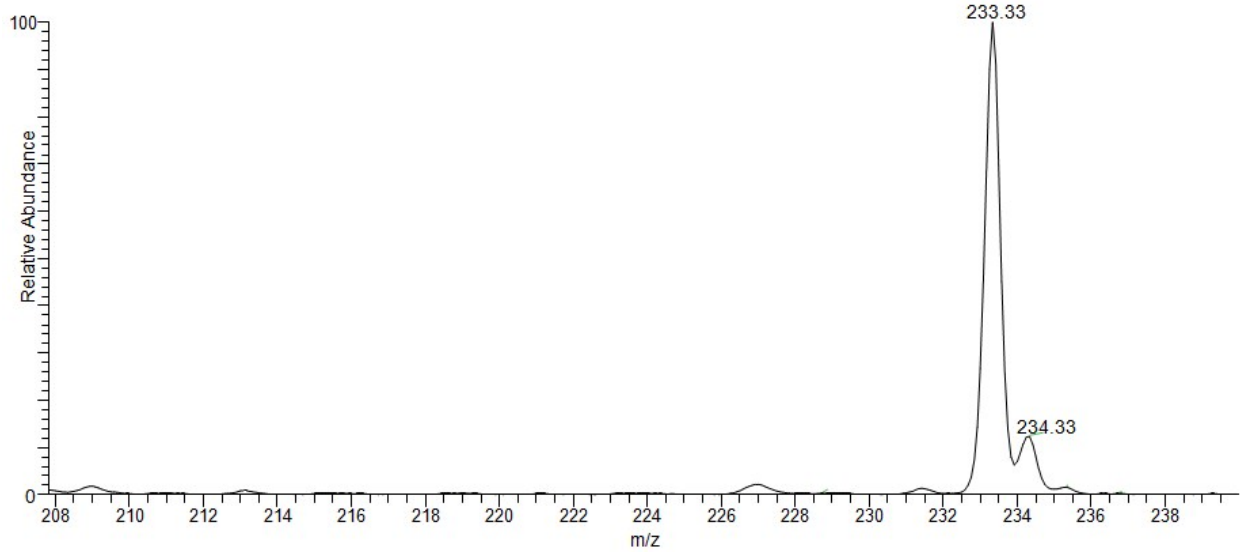
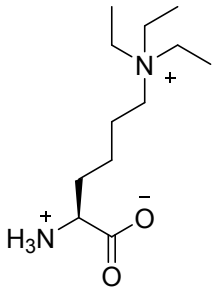
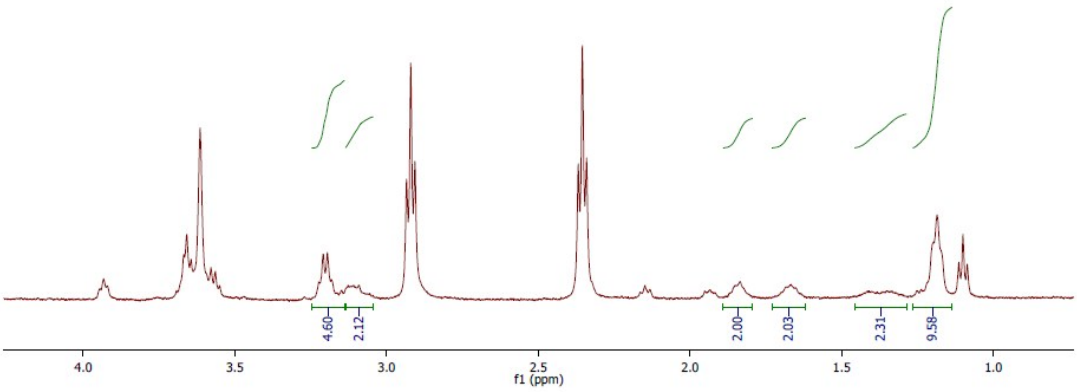


Fig. S34 Hydroxylation of dimethylisopropyllysine (**16**) under standard conditions as analysed by LC-MS.


N
+
H3N
+
O
−
O


4.60
2.12
2.00
2.03
2.31
9.58
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

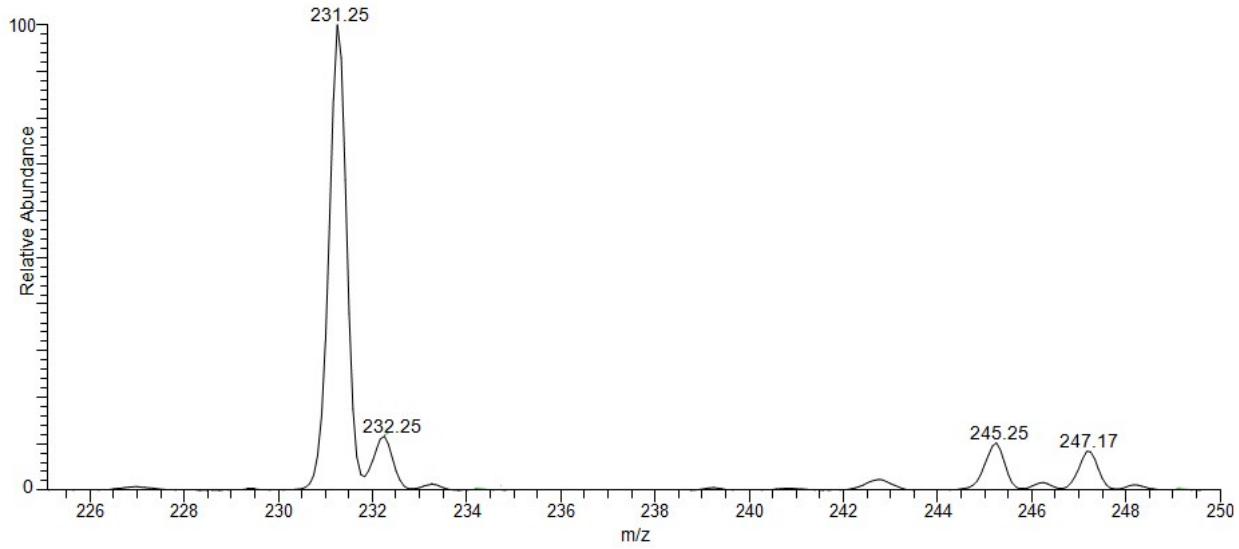
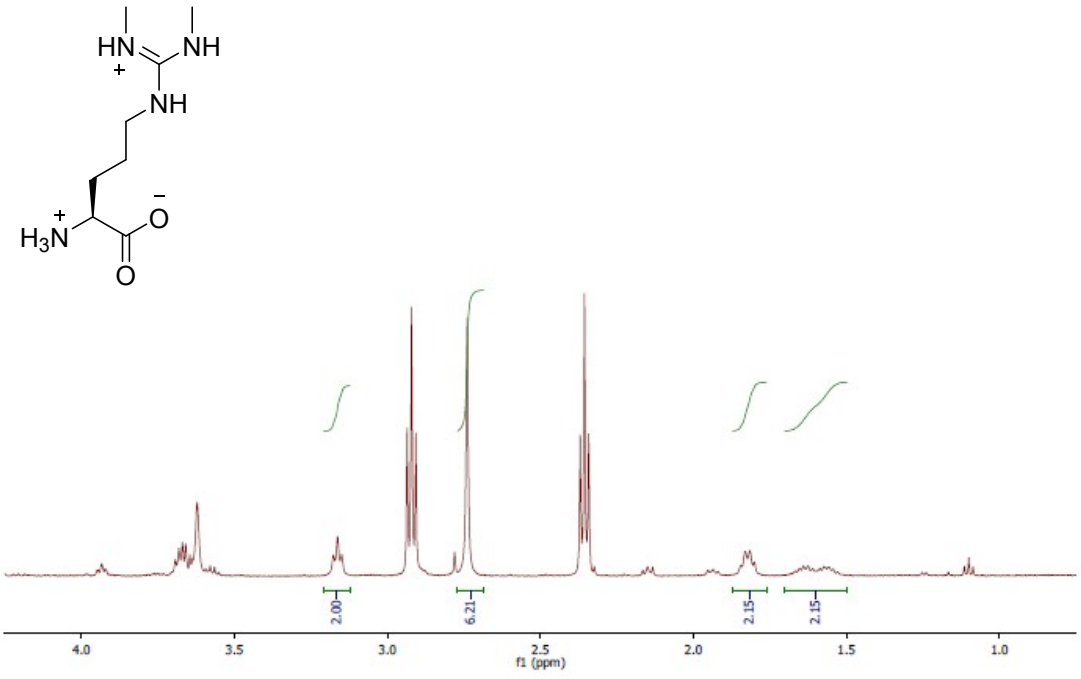


Fig. S35 Hydroxylation of triethyllysine (**17**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).



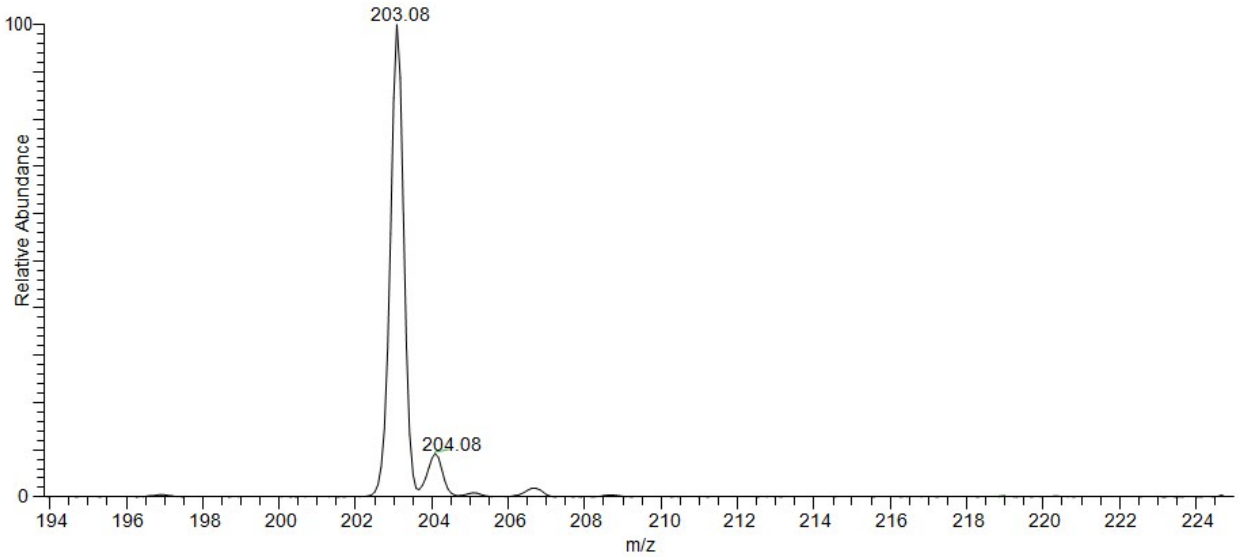
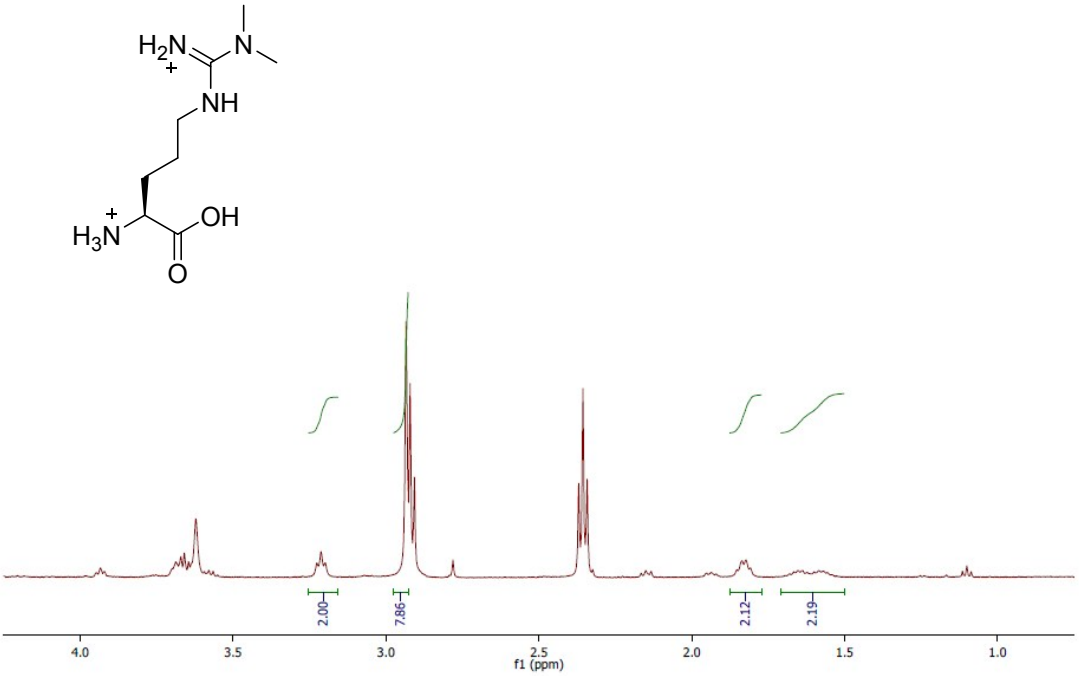


Fig. S36 Hydroxylation of symmetric dimethylarginine (**18**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).



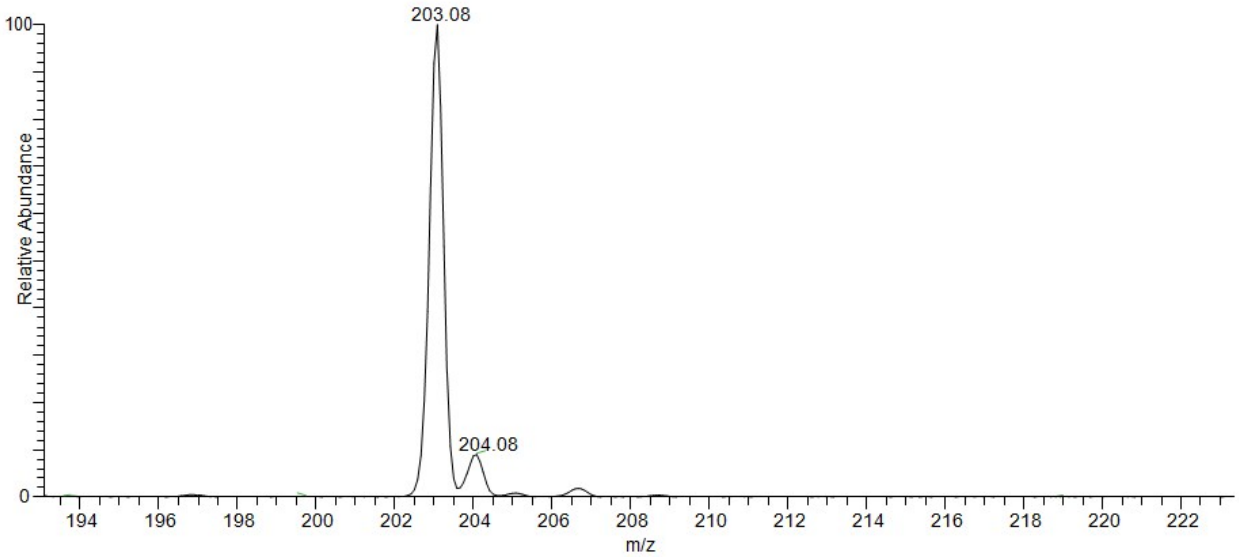
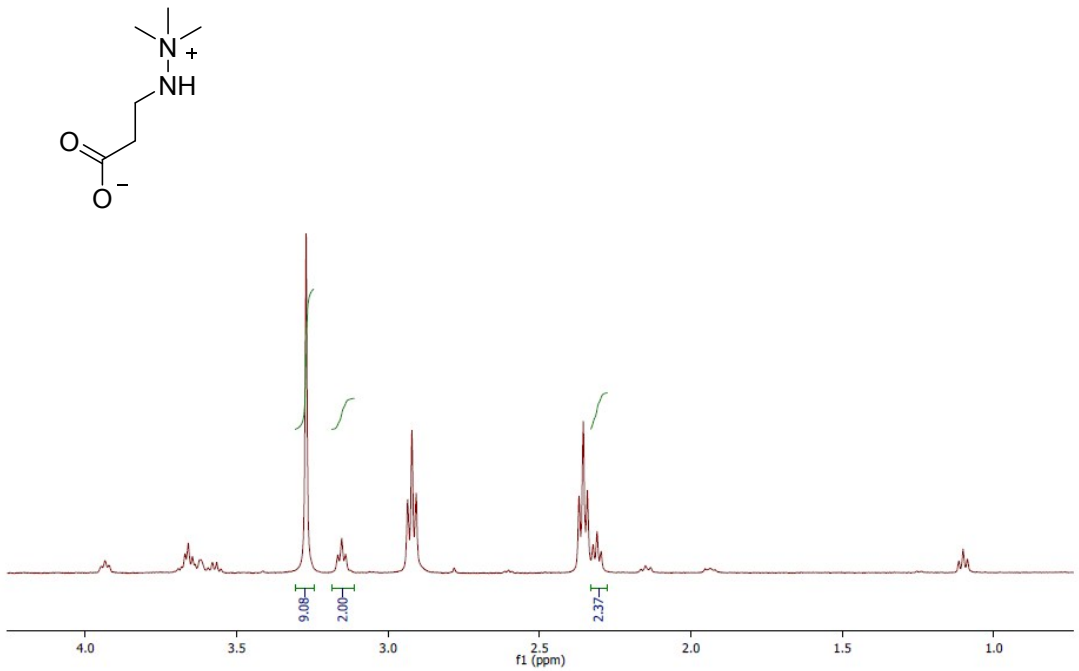


Fig. S37 Hydroxylation of asymmetric dimethylarginine (**19**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).



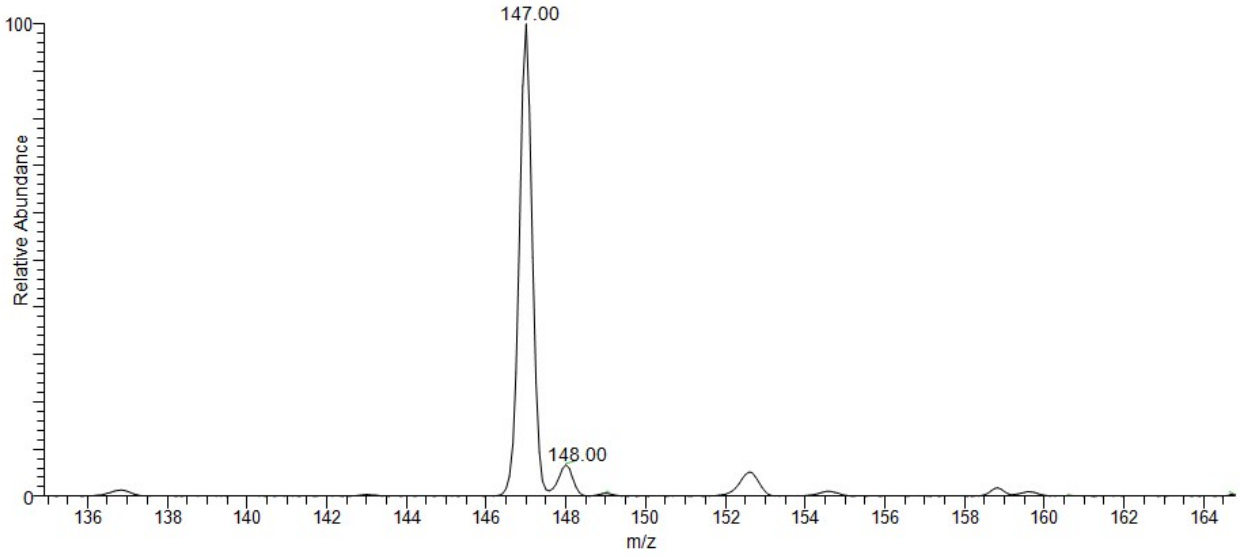


Fig. S38 Hydroxylation of mildronate (**20**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

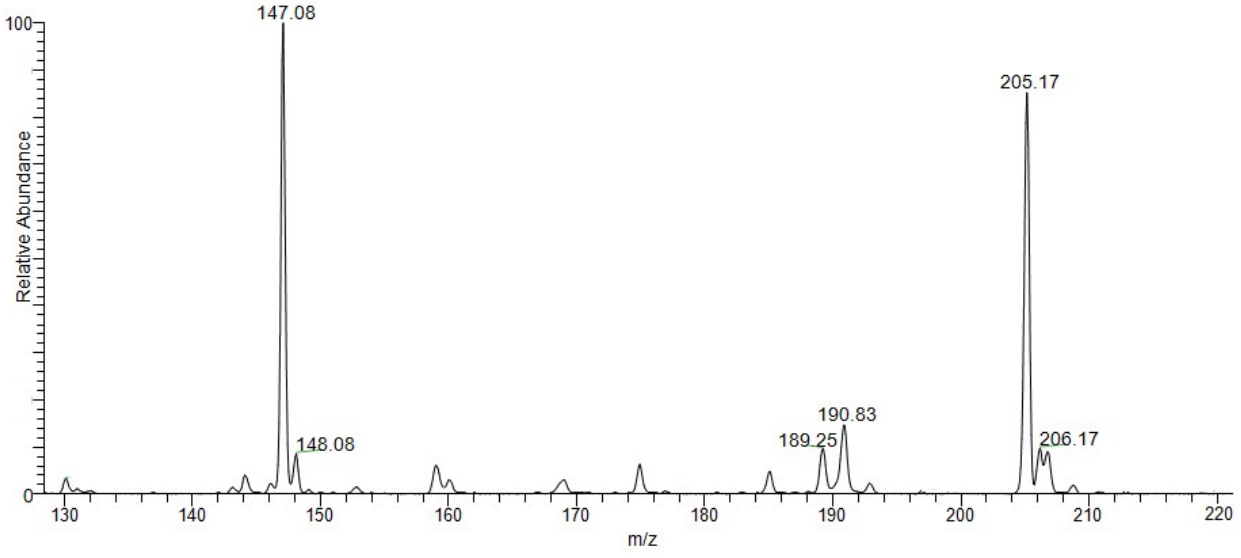
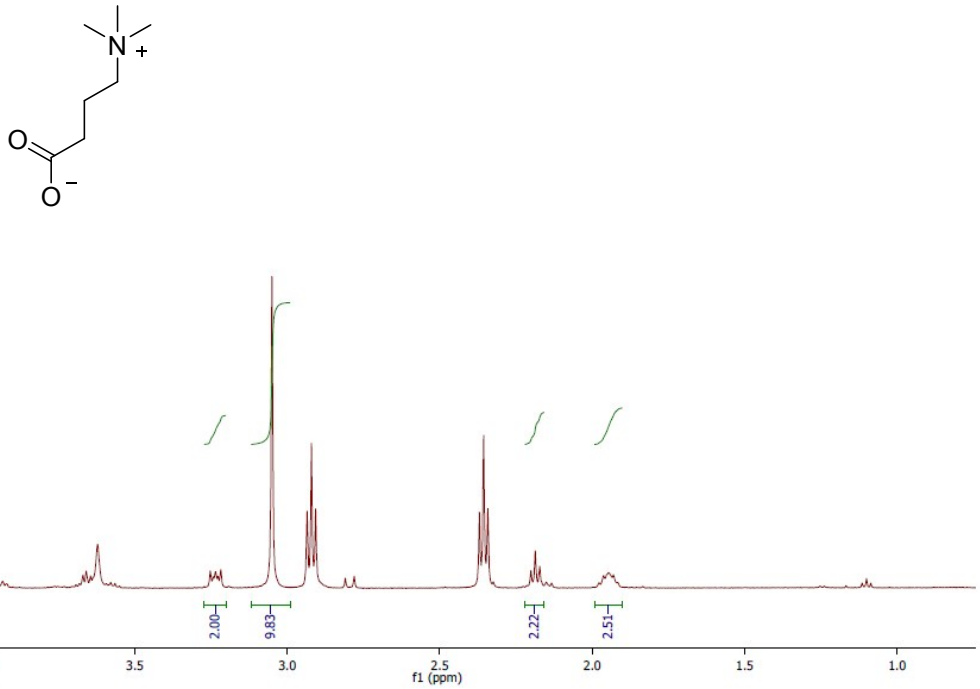


Fig. S39 Hydroxylation of trimethyllysine (**1**) in the presence of 1 mM of mildronate (**20**) under standard conditions as analysed by LC-MS.


N +
O
O –
2.00
9.83
2.22
2.51
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

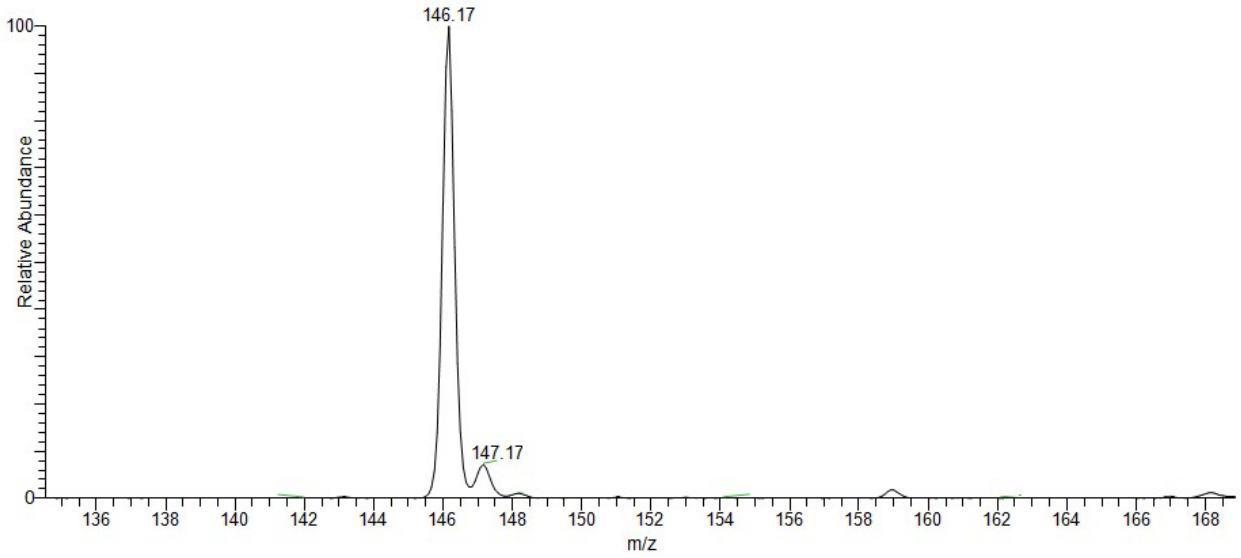


Fig. S40 Hydroxylation of γ-butyrobetaine (**21**) under standard conditions as analysed by ^{1}H NMR (top) and LC-MS (bottom).

6. References

1. A. Kazaks, M. Makrecka-Kuka, J. Kuka, T. Voronkova, I. Akopjana, S. Grinberga, O. Pugovics and K. Tars, *Protein Expr. Purif.*, 2014, **104**, 1-6.

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3. I. K. H. Leung, T. J. Krojer, G. T. Kochan, L. Henry, F. von Delft, T. D. W. Claridge, U. Oppermann, M. A. McDonough and C. J. Schofield, *Chem. Biol.*, 2010, **17**, 1316-1324.

4. R. J. Hopkinson, L. J. Walport, M. Muenzel, N. R. Rose, T. J. Smart, A. Kawamura, T. D. W. Claridge, C. J. Schofield, *Angew. Chem. Int. Ed.*, 2013, **52**, 7709-7713.