Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Fluorinated trimethyllysine as a ^{19}F NMR probe for trimethyllysine hydroxylase catalysis

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Contents

1. TMLH Production

The expression and purification of human TMLH were done following the reported protocol.[1]

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), FeSO4 (500 μM), 2OG (2.5 mM) and ascorbate (5 mM), was added *N*ε-trimethyllysine **1** or *N*ε-(fluoromethyl)dimethyllysine **3** (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 (pD 7.5). To a premixed solution of TMLH (3 μM), $FeSO_4$ (100 μM), 2OG (2 mM) and ascorbate (500 μM), was added *N*ε-trimethyllysine **1** or *N*ε-(fluoromethyl)dimethyllysine **3** (500 μM). After shaking for 1 hour at 37 °C in Eppendorf, the reaction mixutre (500 μL) was transferred into the NMR tube and recorded by ^{1}H NMR at 298 K. 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 4 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. Supplementary Figures

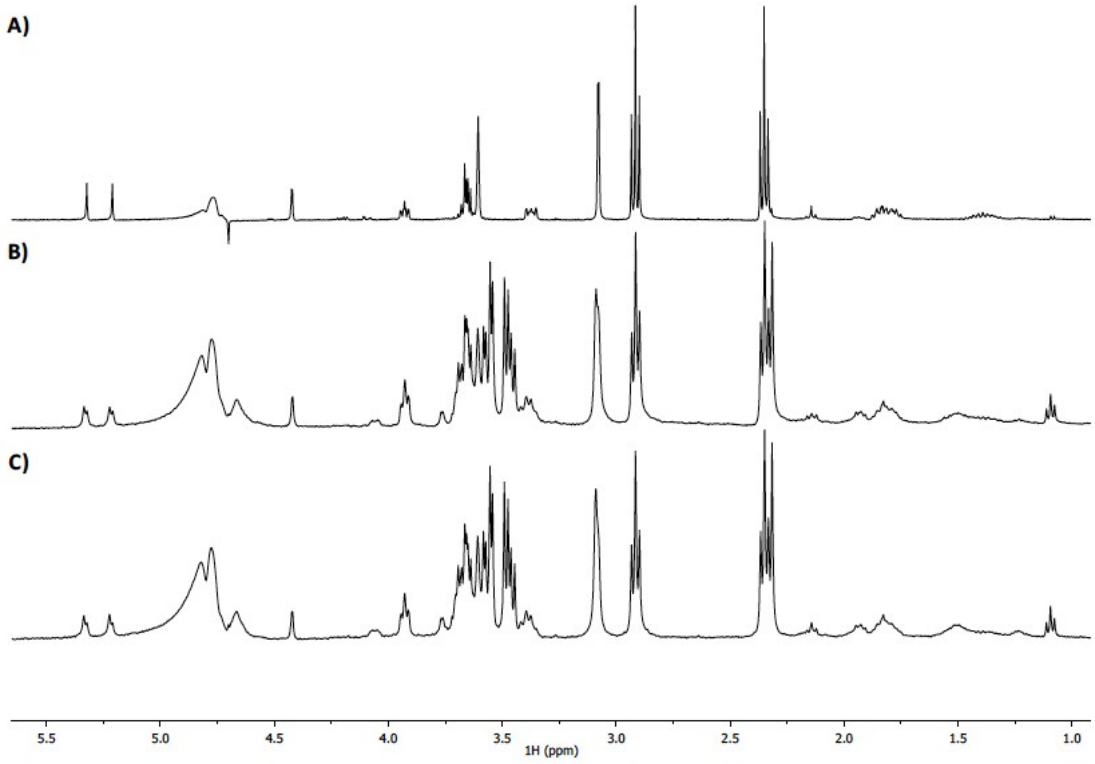


Fig. S1 ^{1}H NMR analyses of the time-course experiment of TMLH-catalysed hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**). A) ^{1}H NMR spectrum of the control experiment (i.e. in the absence of TMLH). B) ^{1}H NMR spectrum of TMLH-catalysed hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**) after 1 h incubation at 37 °C. C) ^{1}H NMR spectrum of TMLH-catalysed hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**) after 2 h incubation at 37 °C.

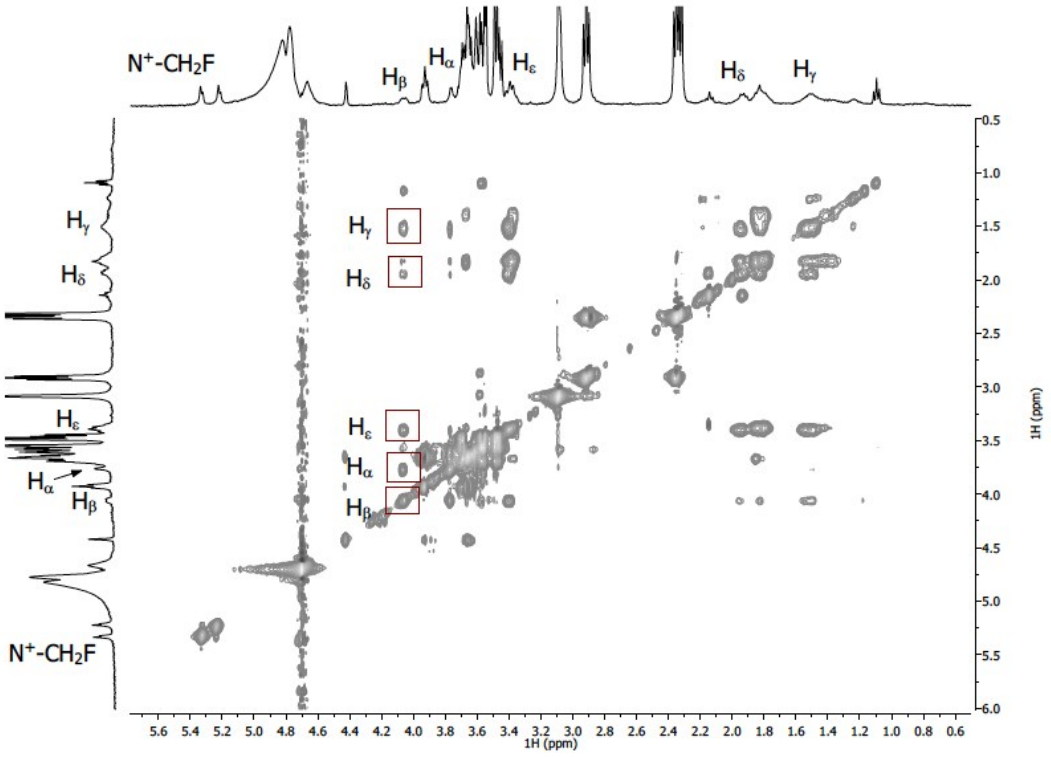


Fig. S2 Full TOCSY data of TMLH-catalysed C-3 hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**).

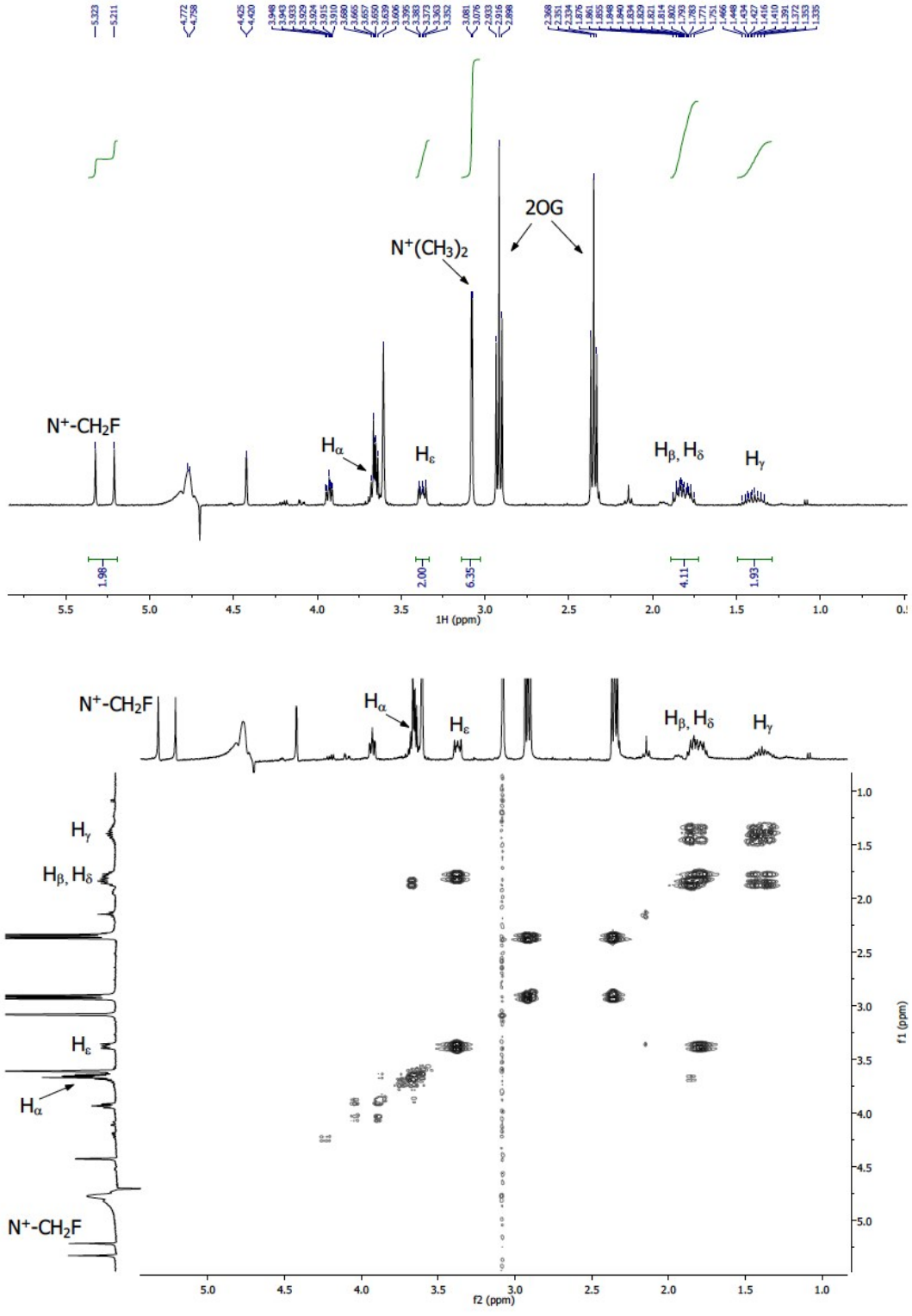


Fig. S3 NMR analyses of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) (in the absence of TMLH). ^{1}H NMR data of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) (top). ^{1}H-^{1}H COSY data of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) (bottom).

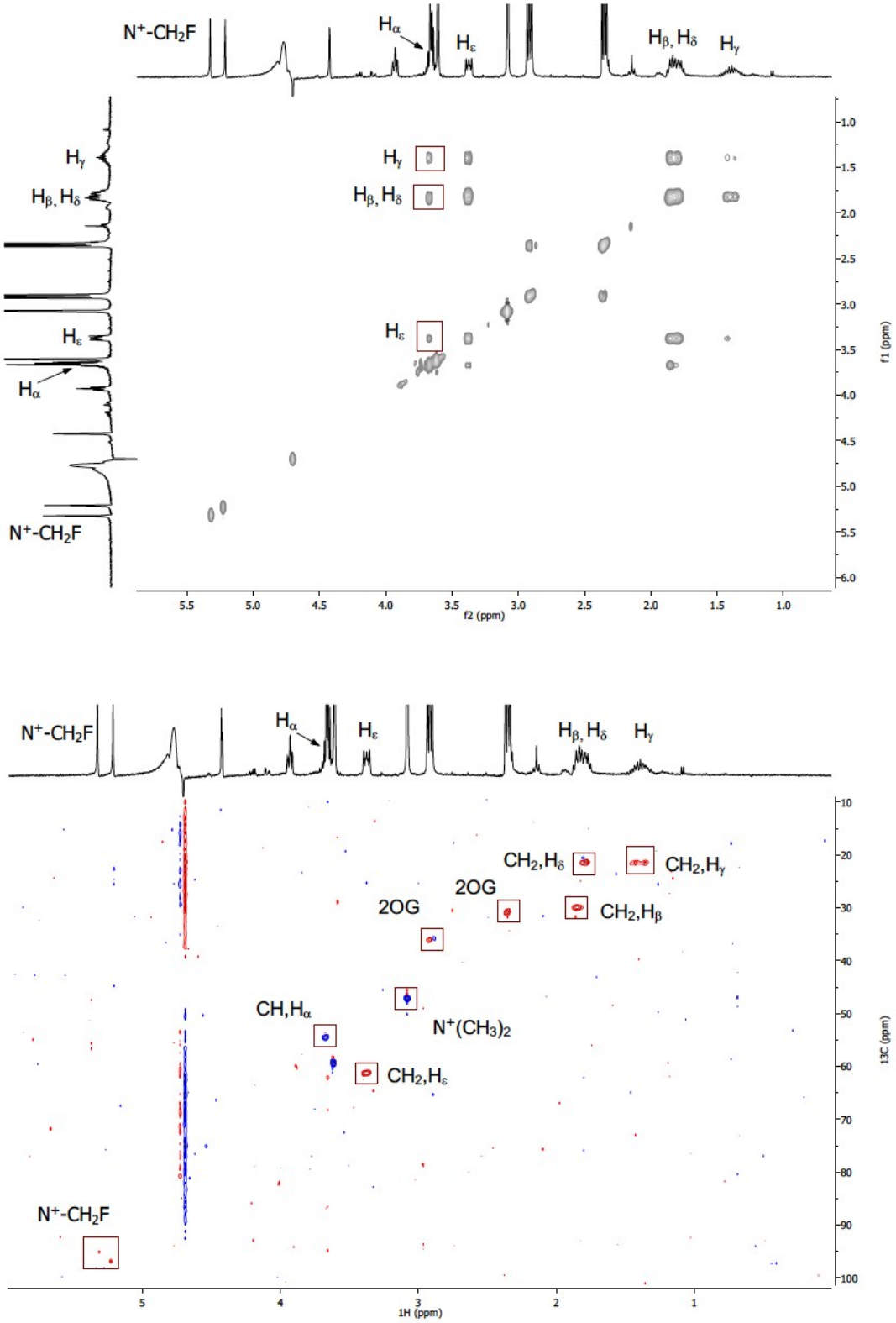


Fig. S4 NMR analyses of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) (in the absence of TMLH). TOCSY data of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) (top). Multiplicity-edited HSQC data of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) (bottom; blue = positive, CH/CH_3, red = negative, CH_2).

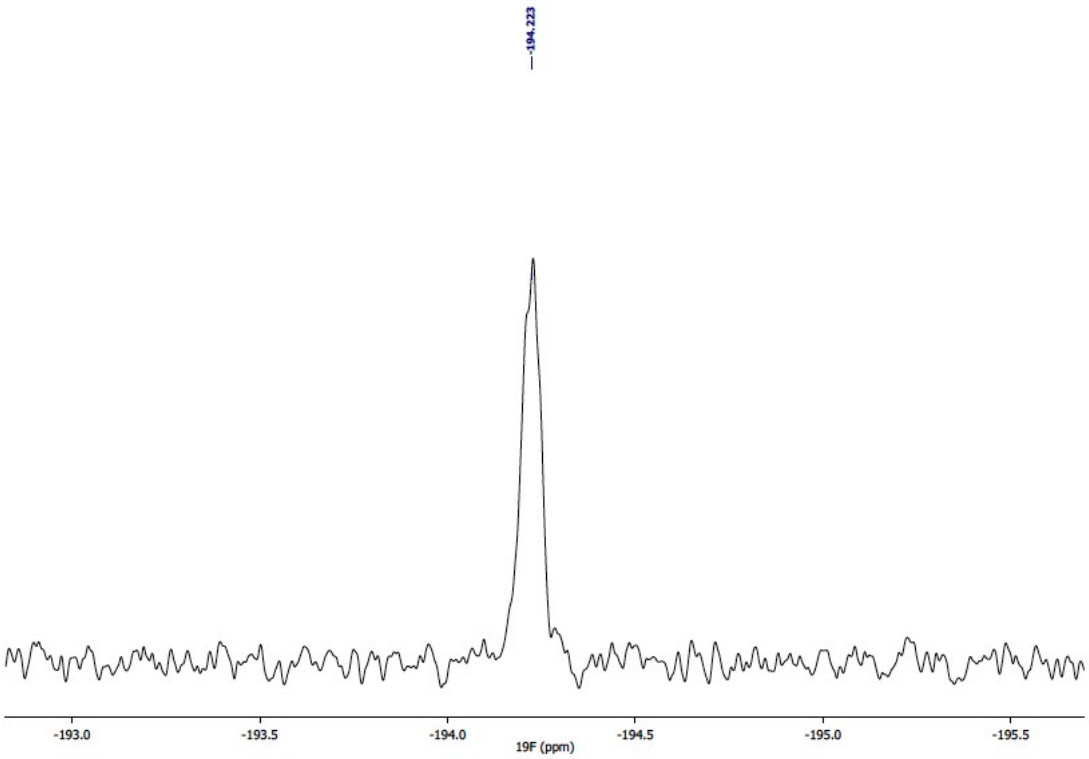


Fig. S5 ^{19}F NMR data of TMLH-catalysed C-3 hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**). Reaction conditions: Reaction performed under standard conditions with TMLH (3 μM) for 1 h at 37 °C, and then additional TMLH was added (6 μM final concentration). The enzymatic mixture was then incubated for another 1 h at 37 °C and the ^{19}F NMR spectrum was recorded.

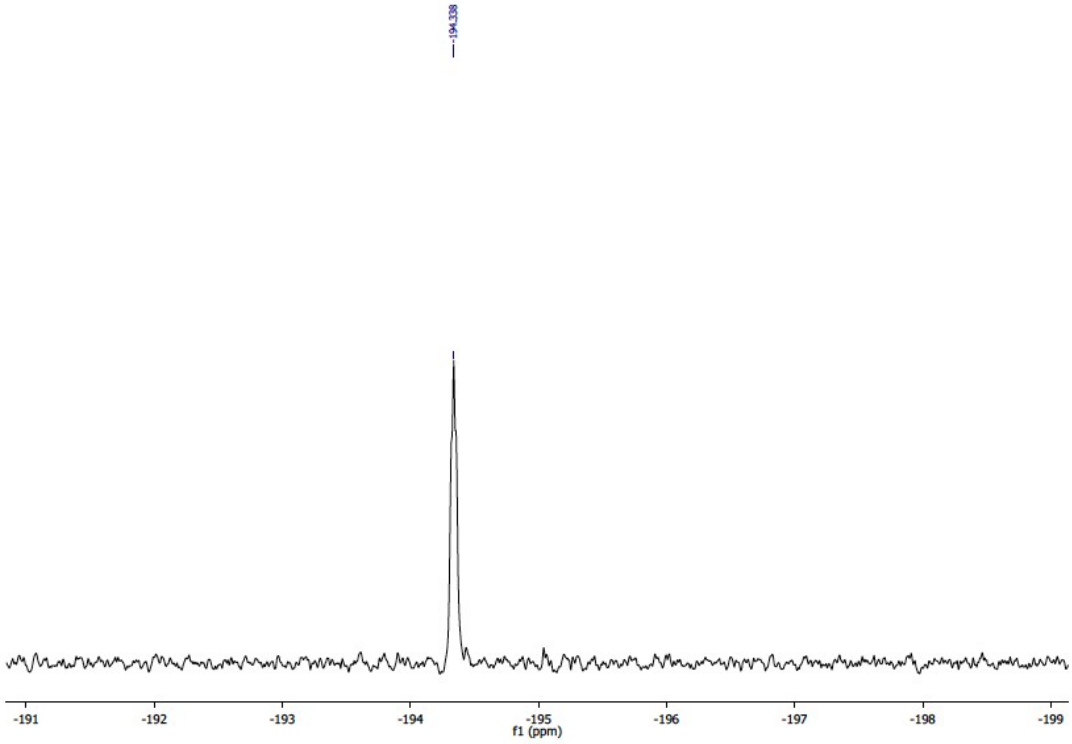


Fig. S6 ^{19}F NMR data of the control experiment of N^{ε}-(fluoromethyl)dimethyllysine (**3**) in the presence of $FeSO_4$, 2OG and ascorbate (but in the absence of TMLH) after incubation at 37 °C for 1 h.

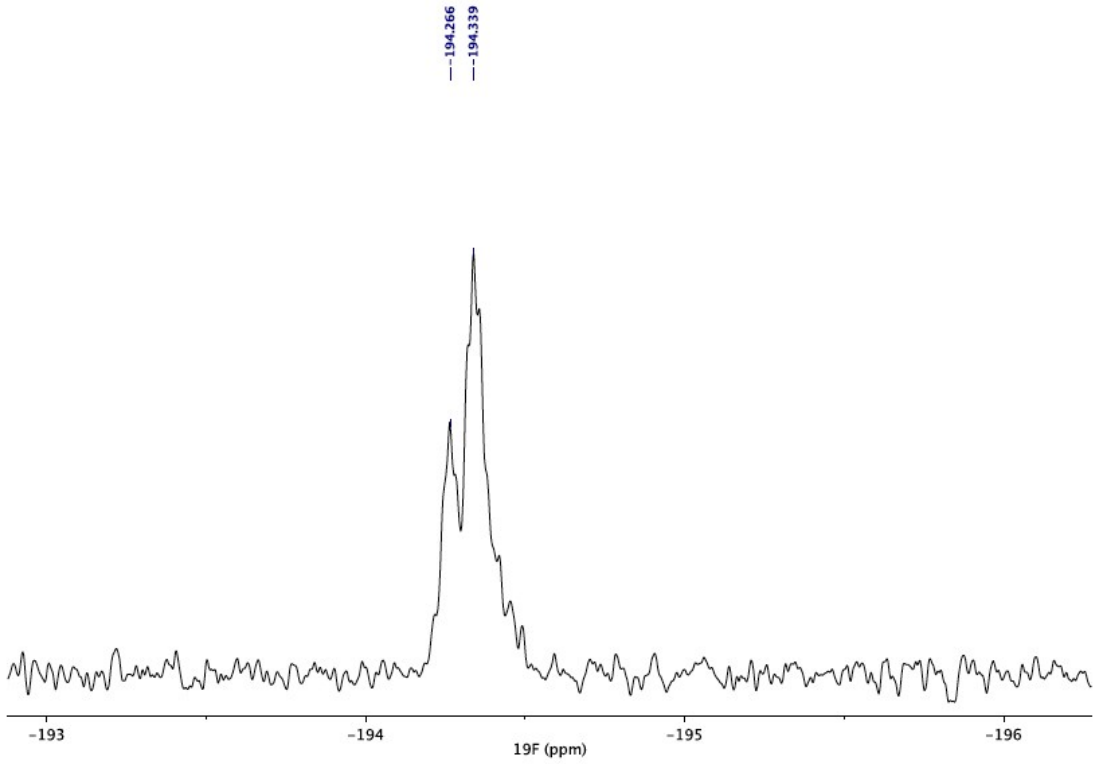


Fig. S7 ^{19}F NMR analysis of the mixture of N^{ε}-(fluoromethyl)dimethyllysine (**3**), $FeSO_4$, 2OG, ascorbate and cell lysates that contain TMLH after incubation at 37 °C for 2 h.

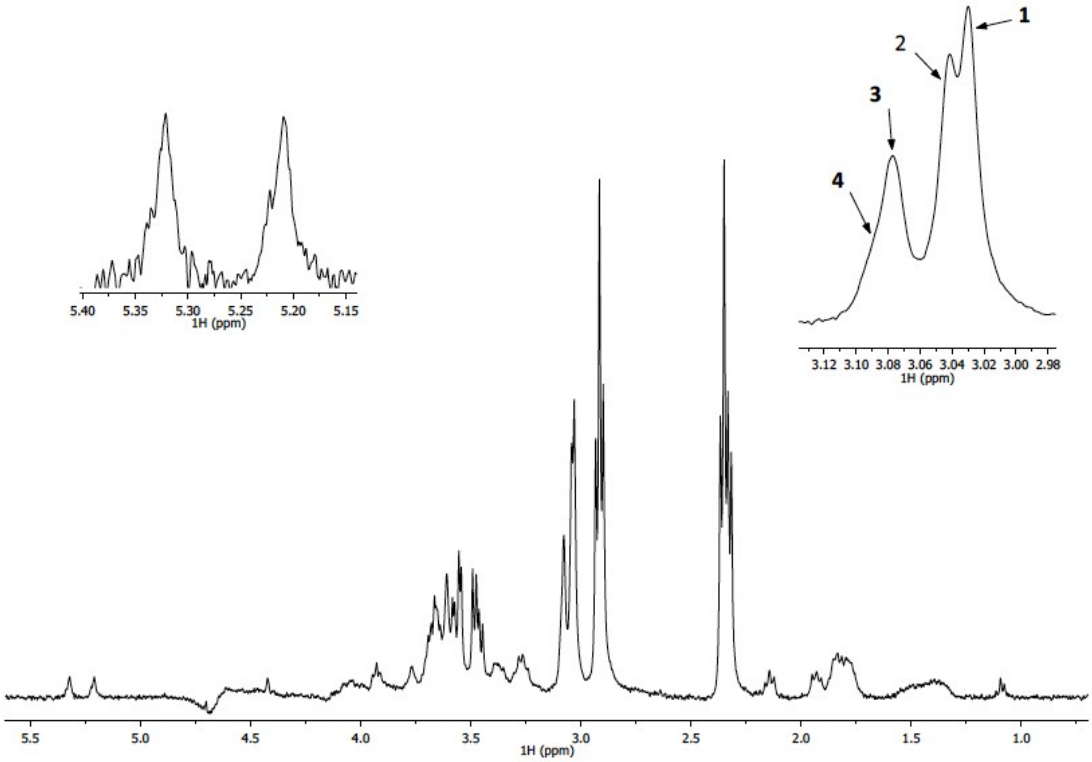


Fig. S8 ^{1}H NMR analysis of TMLH-catalysed hydroxylation of 1:1 mixture of N^{ε}-(fluoromethyl)dimethyllysine (**3**) and N^{ε}-trimethyllysine (**1**) after 1 h incubation at 37 °C. Zoomed views are also shown.

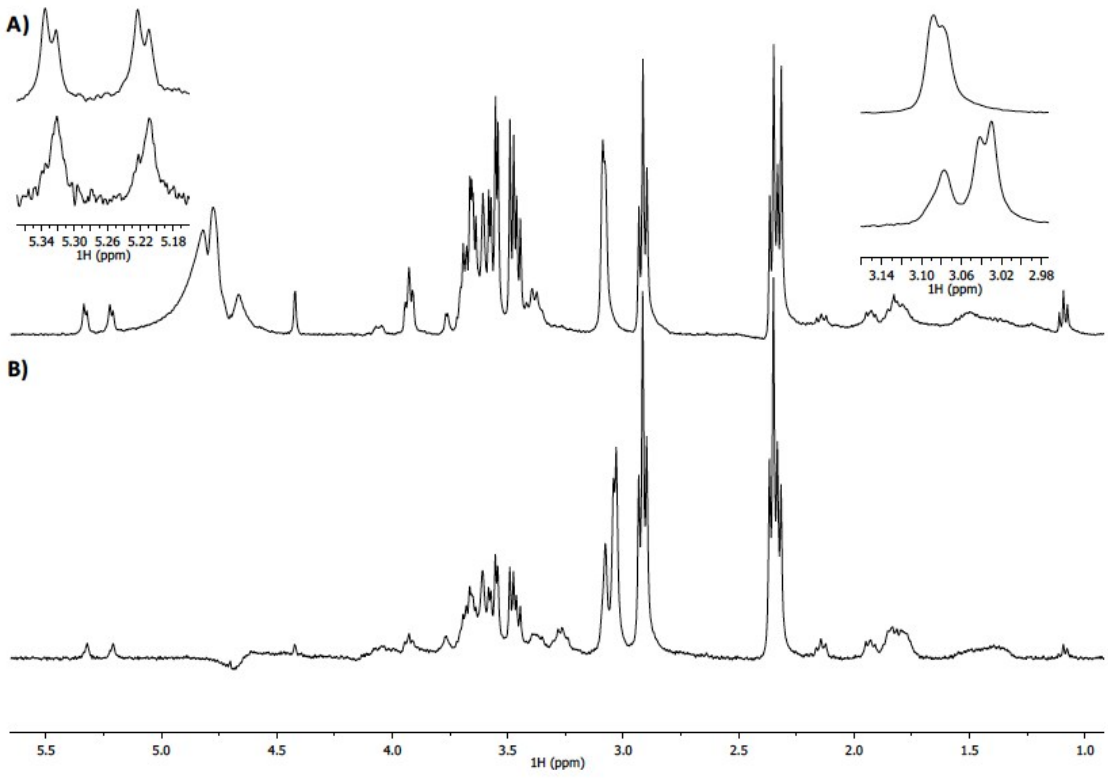


Fig. S9 Comparative ^{1}H NMR analyses of TMLH-catalysed hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**). A) ^{1}H NMR spectrum of TMLH-catalysed hydroxylation of N^{ε}-(fluoromethyl)dimethyllysine (**3**) for 1 h incubation at 37 °C (top). B) ^{1}H NMR spectrum of TMLH-catalysed hydroxylation of 1:1 mixture of N^{ε}-(fluoromethyl)dimethyllysine (**3**) and N^{ε}-trimethyllysine (**1**) after 1 h incubation at 37 °C (bottom).

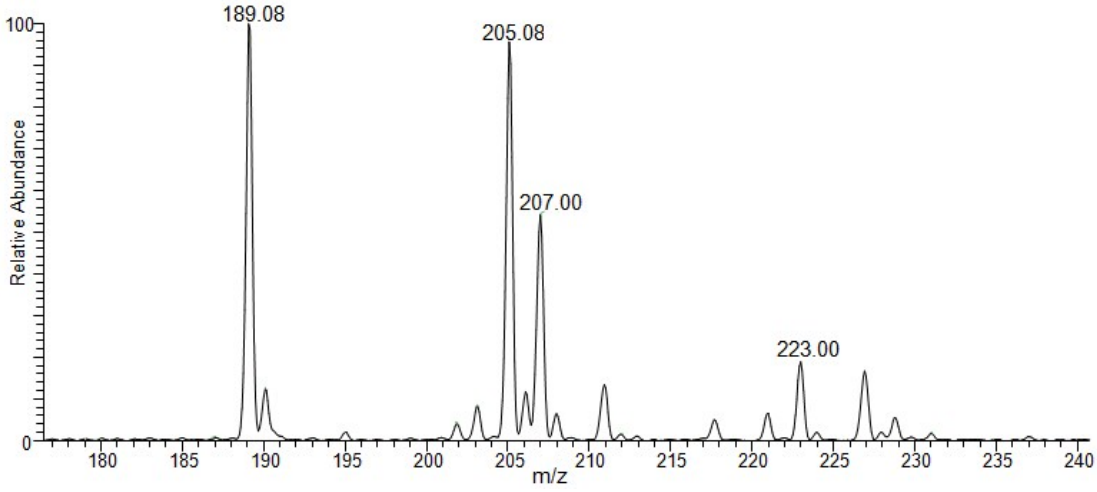
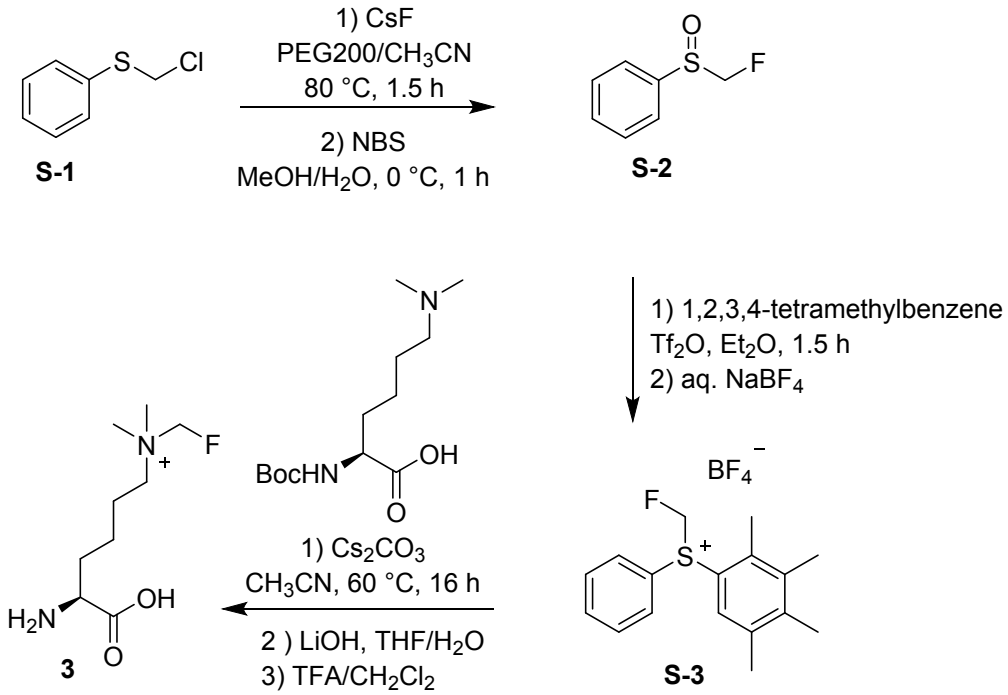


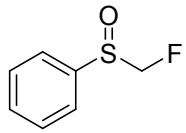
Fig. S10 LC-MS analysis of the competition experiment between N^{ε}-trimethyllysine (**1**) (500 μM) and N^{ε}-(fluoromethyl)dimethyllysine (**3**) (500 μM) in the presence of TMLH (3 μM), $FeSO_4$, 2OG and ascorbate after 1 hour at 37 °C. (**1** = 189.08 Da, **2** = 205.08 Da, **3** = 207.00 Da, **4** = 223.00 Da)

5. Syntheses and Characterisations



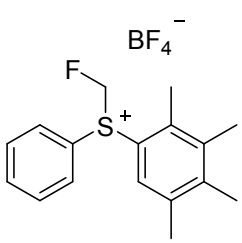
Scheme S1: Synthesis of N^{ε}-(fluoromethyl)dimethyllysine (**3**) [2]

((Fluoromethyl)sulfinyl)benzene (S-2)[2]



To a stirred suspension of chloromethyl phenyl sulphide (2.7 mL, 20.0 mmol) in PEG200/CH3CN (20 mL, 1:2 v/v) was added CsF (6.1 g, 40.0 mmol) and heated to 80 °C for 1.5 h until the disappearance of the chloride as monitored by LCQ-MS analysis. The reaction mixture was then cooled to room temperature and partitioned between water and CH_2Cl_2. The aqueous layer was then extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. Then the crude yellow oil was dissolved in a mixture of MeOH (30 mL) and water (3 mL) and cooled to 0 °C in an ice-water bath. To the stirring solution *N*-Bromosuccinimide (7.3 g, 41.0 mmol) was added slowly to maintain the internal temperature below 10 °C, and stirring continued for 1 h at 0 °C. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with 10% $Na_2S_2O_3$, 5% H_2SO_4, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by flash column chromatography to furnish the compound (1.65 g, 52%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (dd, J = 6.7, 3.0 Hz, 2H), 7.58 – 7.49 (m, 3H), 5.10 (d, J = 47.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2 (d, J = 6.4 Hz), 132.2, 129.6, 124.8, 98.0 (d, J = 221.1 Hz); ^{19}F NMR (471 MHz, $CDCl_3$) δ -212.27 (t, J = 47.7 Hz). MS: calcd for $C_7H_8FOS^+$ 159.0, found 159.0. The spectroscopic data were consistent with literature values.[2]

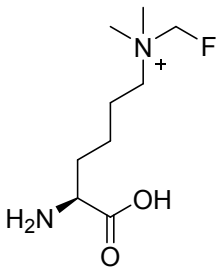
(Fluoromethyl)(phenyl)(2,3,4,5-tetramethylphenyl)sulfonium tetrafluoroborate (S-3)[2]



To a stirred solution of fluoromethylsulphinyl benzene (1.26 g, 8.0 mmol) and 1,2,3,4-tetramethylbenzene (1.20 mL, 8.0 mmol) in dry Et_2O (15 mL) was added triflic anhydride (1.35 mL, 8.0 mmol) slowly for 30 min at 0 °C. After stirring for 1 h, the precipitated solid was filtered, washed with ice cold Et_2O three times, dissolved in 60 mL DCM and washed with an aqueous $NaBF_4$ solution (1M). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure and concentrated under reduced pressure to furnish the compound (1.36 g, 47%) as an off-white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.79 (m, 2H), 7.76 (t, J = 7.4 Hz, 1H), 7.69 (t, J = 7.7 Hz, 2H), 7.45 (s, 1H), 6.59 (dd, J = 37.7, 9.5 Hz, 1H), 6.49 (dd, J = 36.6, 9.5 Hz, 1H), 2.52 (s, 3H), 2.40 (s, 3H), 2.33 (s, 3H), 2.31 (s, 3H); ^{13}C

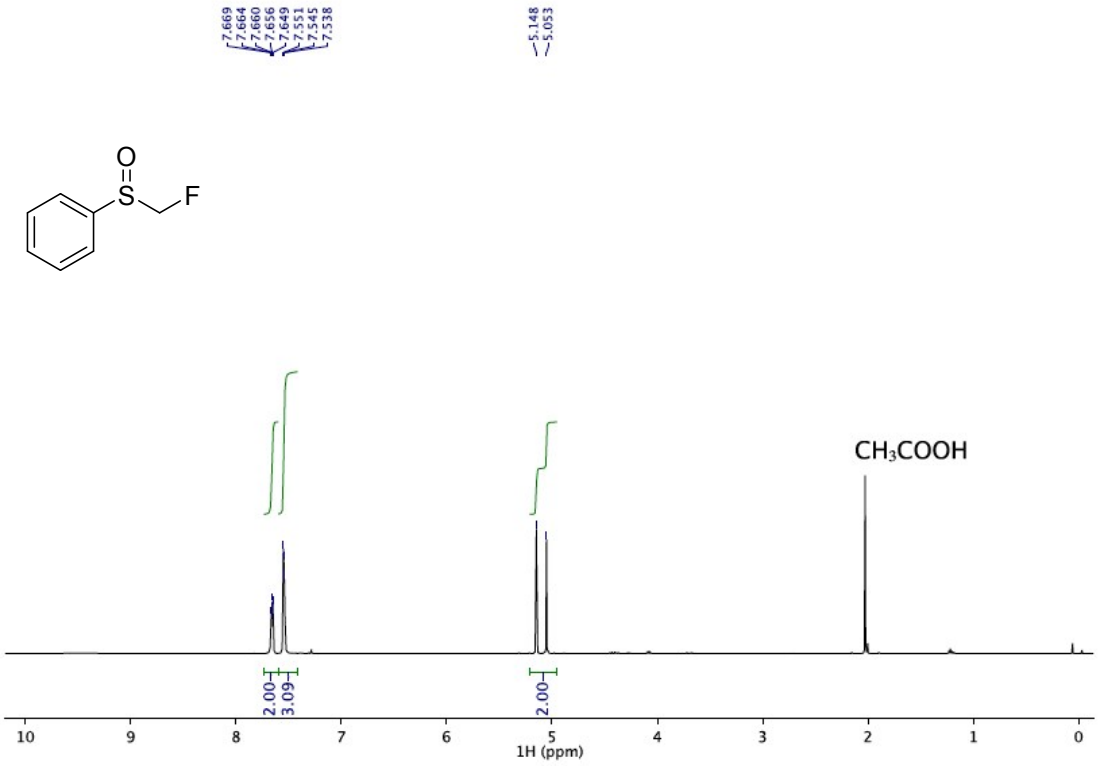
NMR (125 MHz, $CDCl_3$) δ 143.9, 139.3, 138.3, 137.2, 134.6, 131.5, 130.8, 128.2, 128.1, 121.0, 115.9, 90.0, 88.1, 20.8, 17.5, 16.8, 16.8, 16.8, 16.7; ^{19}F NMR (471 MHz, $CDCl_3$) δ -151.46, -151.52, -207.16 (t, J = 46.6 Hz). MS: calcd for $C_{17}H_{20}FS^+$ 275.1, found 275.0. The spectroscopic data were consistent with literature values.[2]

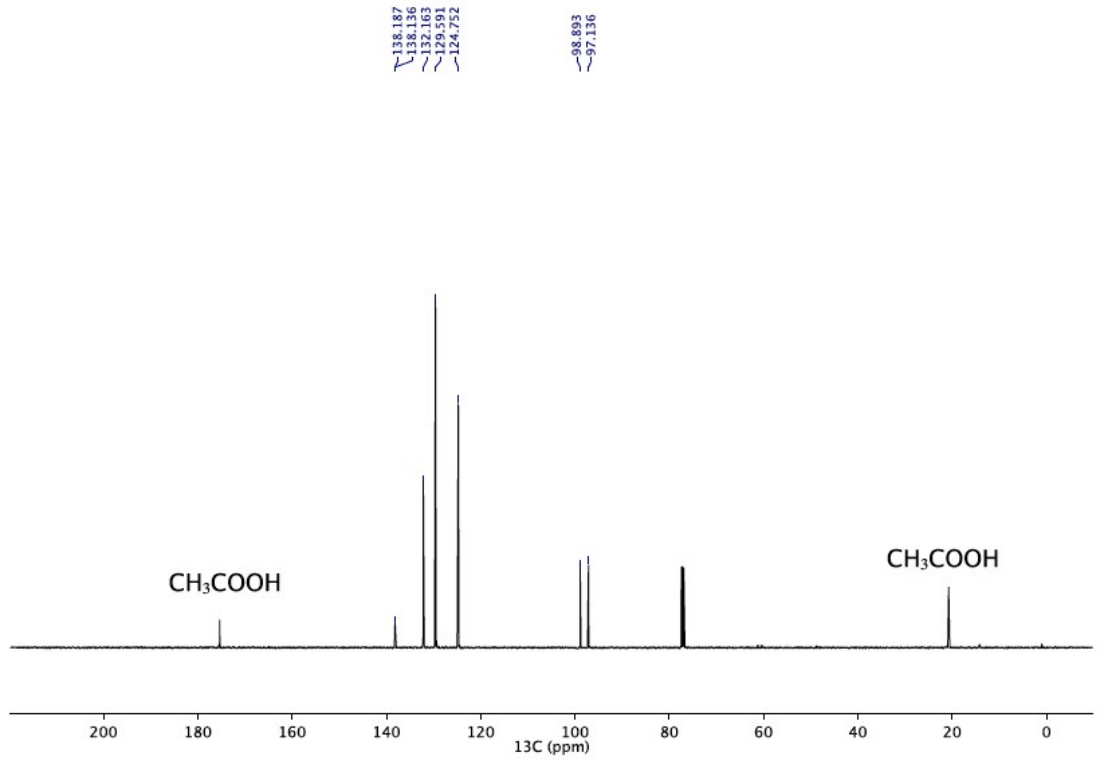
N^{ε}-(Fluoromethyl)dimethyllysine (3) [2]

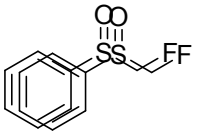


To a solution of *N*-Boc dimethyllysine (50 mg, 0.18 mmol) in CH_3CN (10 mL) was added Cs_2CO_3 (88 mg, 0.27 mmol) and the reaction mixture was stirred vigorously at room temperature, and then monofluoromethyl sulphonium salt **S-3** (196 mg, 0.54 mmol) was added in one portion. The resultant suspension was stirred at 60 °C overnight and filtered. The filtrate was extracted with cyclohexane for three times and co-evaporated with MeOH and CH_2Cl_2. The residue was dissolved in TFA/MeOH (1:1 v/v, 5 mL) and was added a solution of LiOH (20 mg) in water (5 mL). The reaction mixture was stirred at room temperature for 4 h, and then the volatiles were evaporated. The residue was suspended in MeOH, filtered and the filtrate was concentrated under reduced pressure. The resultant residue was re-dissolved in CH_2Cl_2 (5 mL) and TFA (200 μL) was added. The reaction mixture was stirred at room temperature for 2 h and then the volatiles were evaporated under reduced pressure, the crude was purified by reverse phase HPLC to furnish N^{ε}-(fluoromethyl)dimethyllysine (**3**) as a TFA salt: ^1H NMR (500 MHz, D_2O) δ 5.27 (d, J = 45.0 Hz, 2H), 3.76 (td, J = 6.2, 2.0 Hz, 1H), 3.47 – 3.30 (m, 2H), 3.08 (d, J = 2.0 Hz, 6H), 1.93 – 1.84 (m, 2H), 1.84 – 1.75 (m, 2H), 1.41 (dddd, J = 37.2, 13.6, 10.6, 6.3 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 173.0, 96.0 (d, J = 220.7 Hz), 61.1, 53.4, 47.1, 29.4, 21.3, 21.2; ^{19}F NMR (471 MHz, D_2O) δ -194.27 (t, J = 45.2 Hz). The spectroscopic data were consistent with literature values.[2]

6. NMR Spectra



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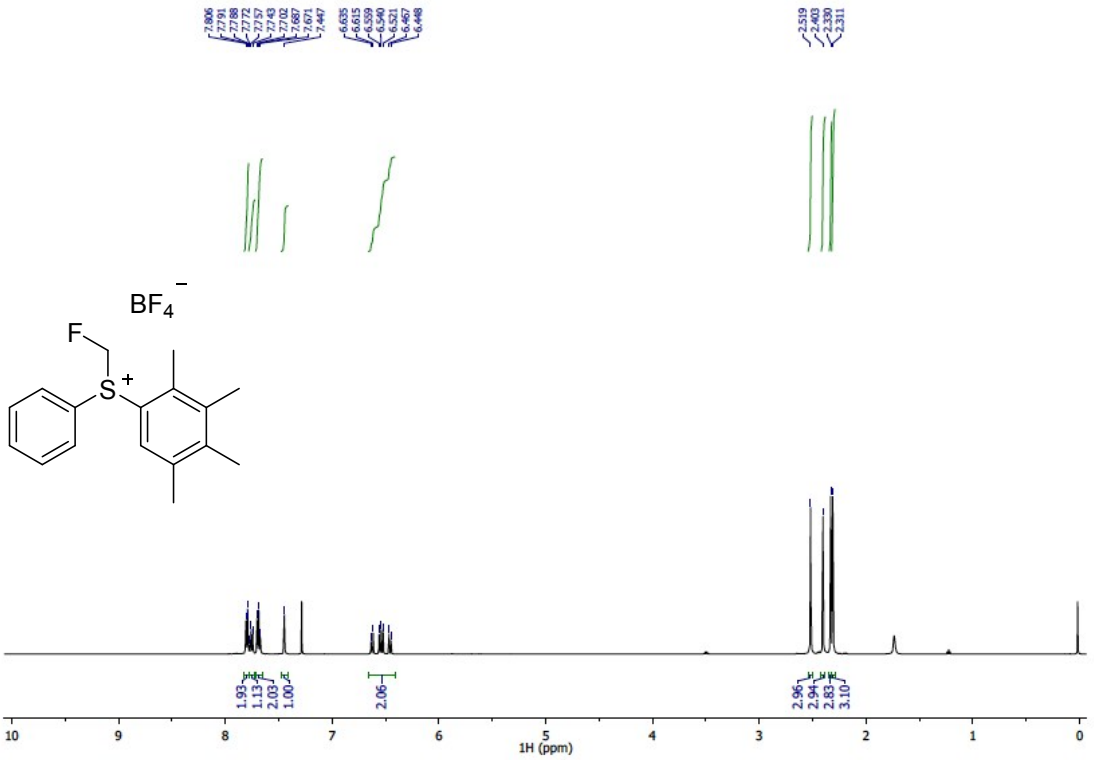
19F (ppm)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1.13
2.03
1.00
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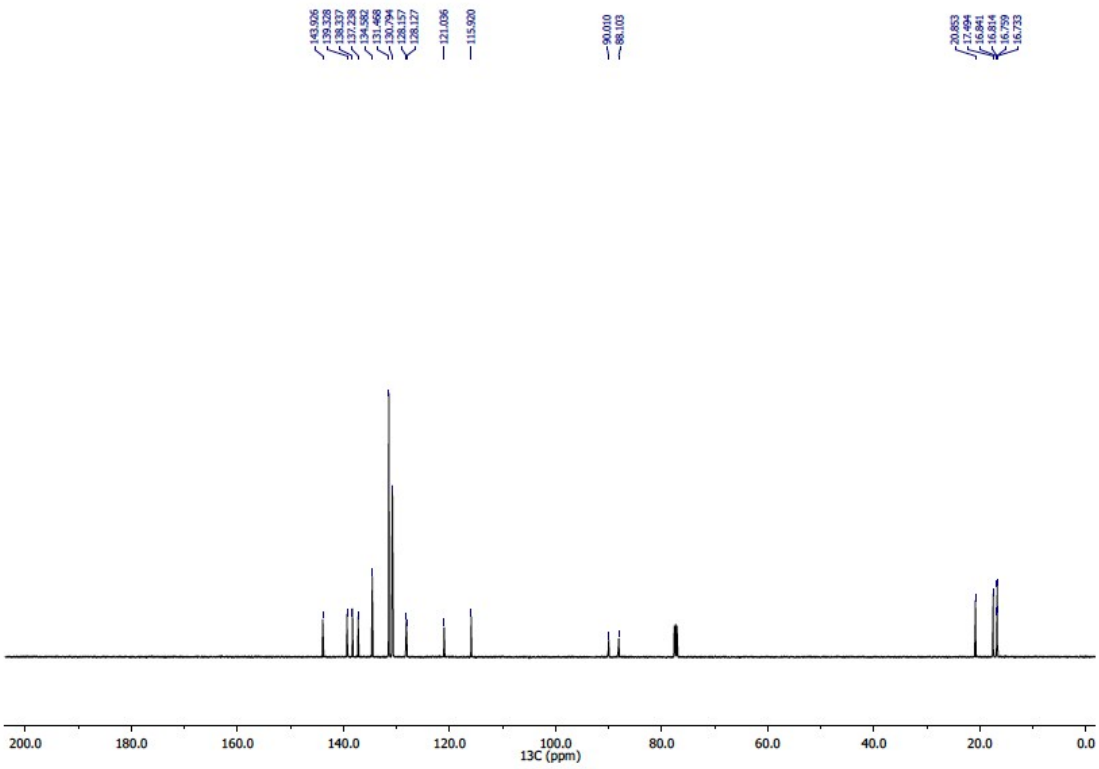
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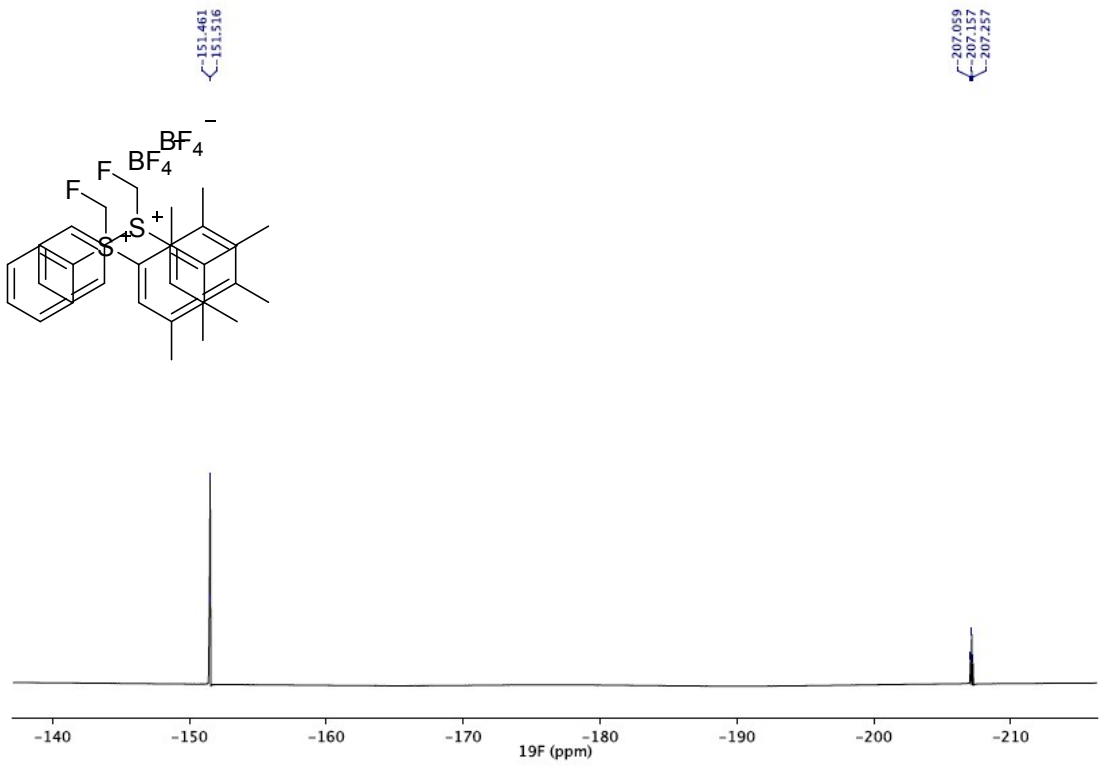
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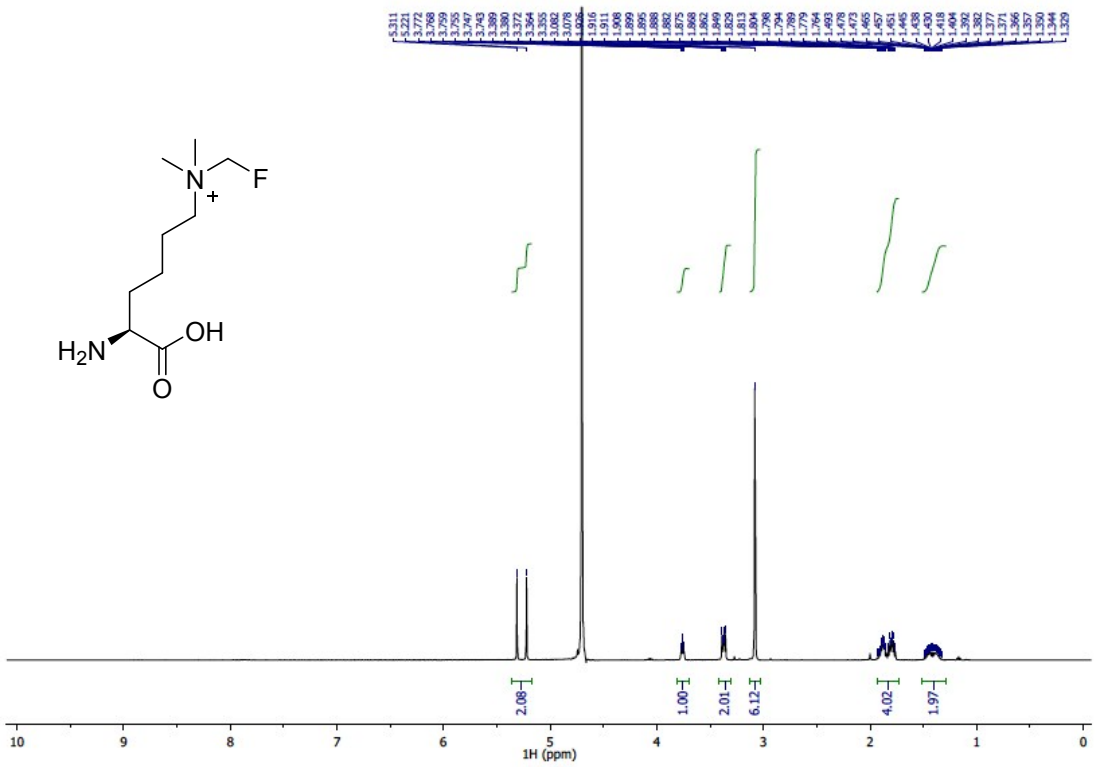
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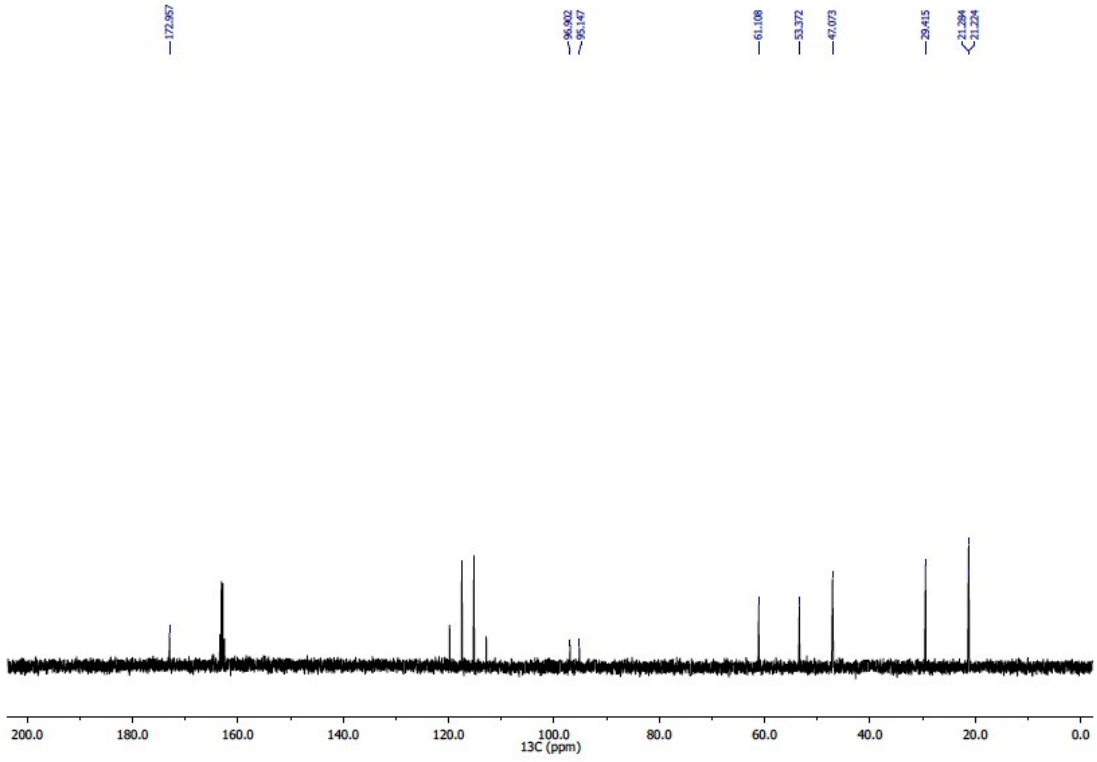
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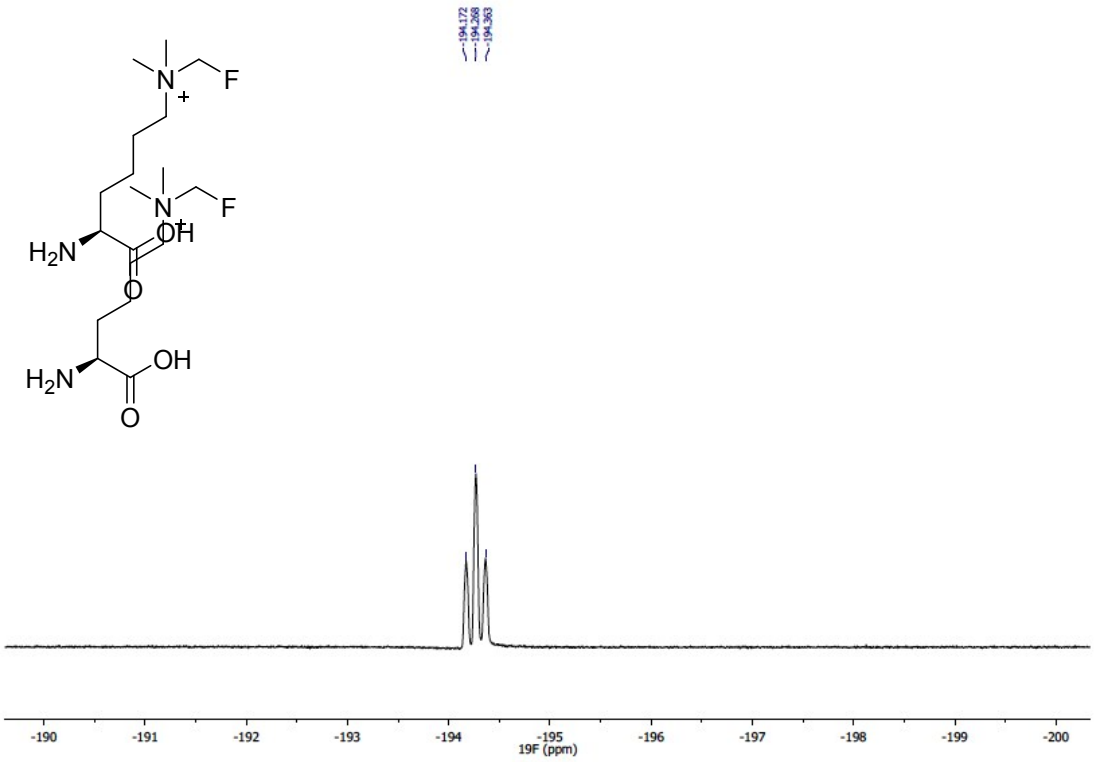

7. References

1. A. H. K.; Al Temimi, B. J. G. E. Pieters, Y. V. Reddy, P. B. White, J. Mecinović, *Chem. Commun.,* 2016**, 52**, 12849-12852.

2. A. M. Rydzik, I. K. H. Leung, A. Thalhammer, G. T. Kochan, T. D. W. Claridge and C. J. Schofield, *Chem. Commun.*, 2014, **50**, 1175-1177.