Electronic Supplementary Information (ESI)

Bioorthogonal labelling of living bacteria using unnatural amino acids containing nitrones and a nitrone derivative of vancomycin

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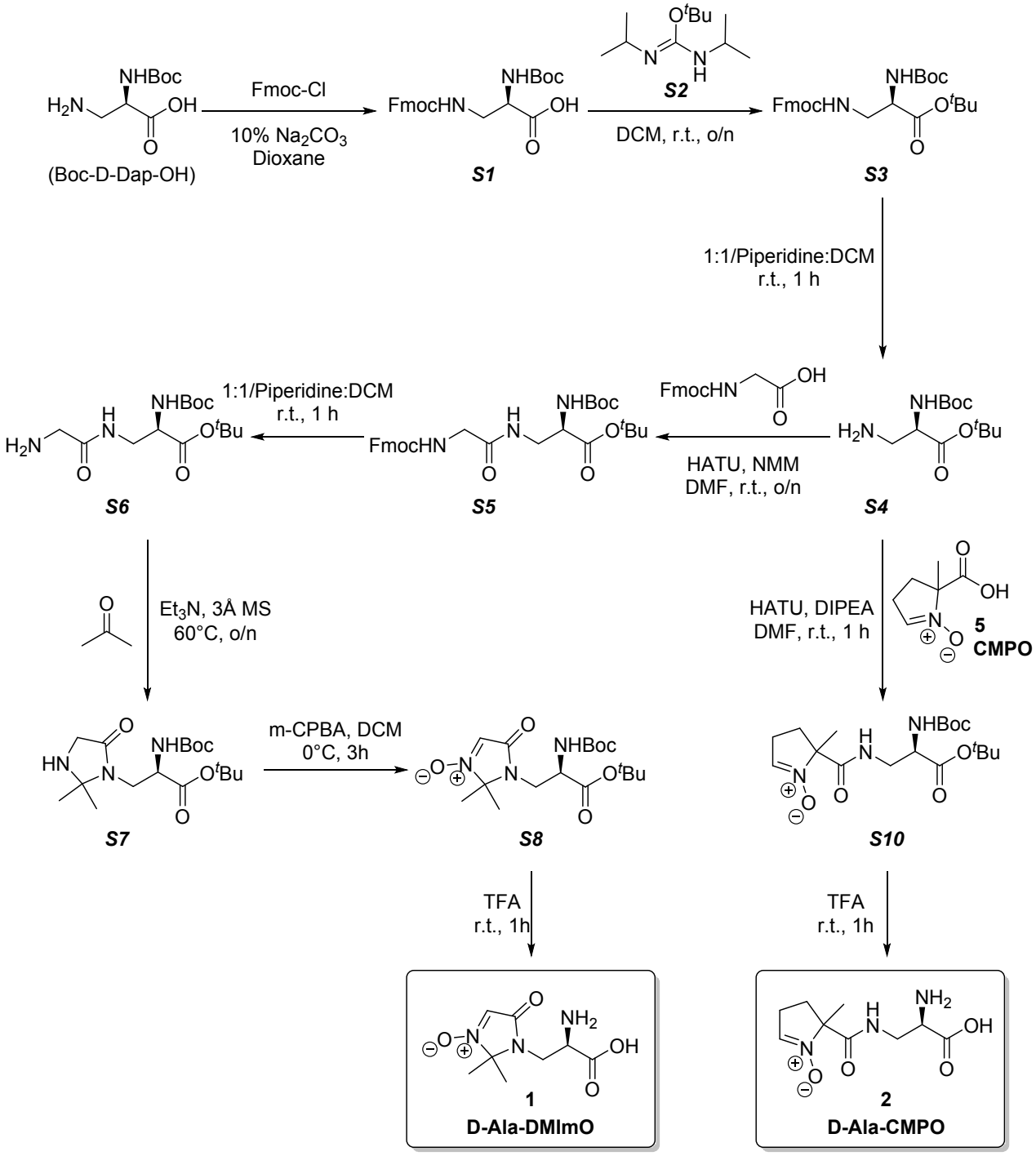
Table of Contents

Materials and Synthetic Methods

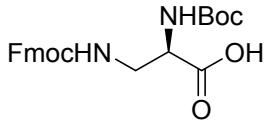
All reagents and solvents were purchased from Sigma-Aldrich, unless otherwise stated, and used without further purification. Deuterated solvents were purchased from Cambridge Isotope laboratories. Thin layer chromatography was performed on Analtech Uniplate® silica gel plates (60 Å F254, layer thickness 250μm). Flash chromatography was performed using silica gel (60 Å, particle size 40–63 μm). LC-MS/MS spectra were obtained using Waters Alliance 2795 liquid chromatograph quipped with Waters 996 PDA diode array detector and connected to Micromass ZQ2000 mass spectrometer equipped with pneumatically assisted electrospray ionization source, operating in both positive and negative mode. Samples were run with gradient elution of acetonitrile/water/0.1% formic acid on the Waters SunFire C18 (2.1 x 100 mm, 3.5 μm) column with flow rate of 0.2 mL/min. Preparatory HPLC was performed on a Waters Delta Prep 4000 equipped with Waters 996 PDA diode array detector and column Waters SunFire C18 (19 x 100 mm, 5 μm) and Waters fraction collector. Column effluents were monitored at wavelength specified in respective procedures. Mobile phase was acetonitrile/water/0.1% formic acid, degassed with helium sparge at flow rate of 17 mL/min. Aliquots were injected onto column via a Pheodyne 7125 injector fitted with a 5 mL loop. All ^{1}H and ^{13}C NMR spectra were obtained on a Bruker-DRX-400 spectrometer using a frequency of 400 MHz for ^{1}H and 100 MHz for ^{13}C and processed using Bruker TOPSPIN 2.1 software. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet or unresolved, br = broad signal and *J* = coupling constants in Hz.

Syntheses of DMImO-amino acid analogues were based on the literature procedures.[1]

Synthesis of D-Alanine analogues



(2R)-2-*tert*-Butoxycarbonylamino-3-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid

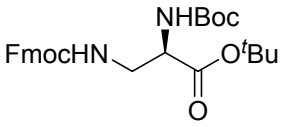


S1

Boc-D-Dap-OH (2.08g, 10.2 mmol, 1 eq) was partially dissolved in a 10% aqueous sodium carbonate and cooled to 0 ℃. A solution of 9-fluorenylmethyl chloroformate (3.18 g, 12.3 mmol, 1.2 eq) in 32 mL of 1,4-dioxane was added slowly. The reaction was stirred at 0 ℃ for 1 hour, then diluted with distilled water and ether and layers were separated. The aqueous phase was acidified to pH~1 with concentrated HCl and extracted with ethyl acetate. The organic extracts were dried over magnesium sulfate, filtered and concentrated to afford **S1** as fluffy white solid (~4.34 g, 10.2 mmol, quantitative yield). R_f = 0.05 in 5% MeOH in DCM. **MS (ESI+)** *m/z* calcd for **S1** ($C_{23}H_{26}N_2O_6$): 449.17 $[M+Na]^+$, found 449.1; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 9.76 (br s, 1H), 7.74 (d, *J*=7.5 Hz, 2H), 7.55 (d, *J*=7.4 Hz, 2H), 7.37 (t, *J*=7.3 Hz, 2H), 7.28 (t, *J*=7.2 Hz, 2H), 4.36 (m, 3H), 4.17 (t, *J*=6.7 Hz, 1H), 3.67-3.48 (br m, 1H), 1.43 (s, 9H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 173.1, 156.2, 143.7, 141.3, 141.3, 127.7, 127.1, 125.1, 120.0, 80.7, 67.2, 47.1, 42.8, 28.3.

2-tert-butyl-1,3-diisopropylurea (**S2**)[2] as synthesized according to literature procedure.

(2R)-2-*tert*-Butoxycarbonylamino-3-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid *tert*-butyl ester

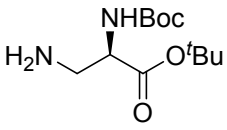


S3

S1 (2.29 mg, 5.38 mmol, 1 eq) was dissolved in 30 mL of dry DCM (dried over 3Å molecular sieves) and placed under Argon at room temperature. 2-tert-butyl-1,3-diisopropylurea (**S2**) (3.43 g, 17.1 mmol, 3.2 eq) was added dropwise and the mixture was stirred overnight at room temperature. Hexanes were added to precipitate the urea by-product, which was then filtered off. The filtrate was concentrated under reduced pressure. The crude was purified by column chromatography (8:2/Hx:EtOAc, R_f = 0.3) to afford **S3** as pale yellow oil (1.69 g, 3.5 mmol, 65%). **MS (ESI+)** *m/z* calcd for **S3** ($C_{27}H_{34}N_2O_6$): 483.24 $[M+H]^+$, found 483.3; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J*=7.4 Hz, 2H), 7.40 (t, *J*=7.4 Hz, 2H), 7.31 (t, *J*=7.4 Hz, 2H), 5.42 (d, *J*=6.2 Hz, 1H), 5.27 (br s, 1H), 4.37 (d, *J*=6.9 Hz, 2H), 4.29 (br s, 1H), 4.20 (t, *J*=7.0 Hz, 1H), 3.59 (t, *J*=5.3 Hz, 2H), 1.47 (s, 9H), 1.45 (s, 9H); **^{13}C-NMR**

(100 MHz, $CDCl_3$): δ 169.6, 156.5, 155.7, 143.9, 143.8, 141.3, 127.7, 127.1, 125.1, 120.0, 82.9, 80.2, 67.0, 54.4, 47.2, 43.4, 28.3, 27.9.

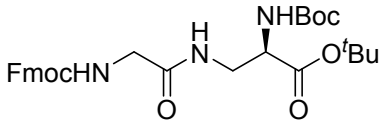
3-Amino-(2R)-2-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester



S4

S3 (1.69 g, 3.5 mmol) was dissolved in 10 mL of 50% (v/v) piperidine in DCM and stirred at room temperature for 1 hour. Upon reaction completion, the mixture was concentrated under reduced pressure and purified by column chromatography eluting 5% MeOH in DCM, followed by 5% MeOH in DCM+1% Et_3N (R_f = 0.3, stains well with ninhydrin stain) to afford **S4** as pale yellow solid (~0.9 g, 3.5 mmol, quantitative yield). **MS (ESI+)** *m/z* calcd for **S4** ($C_{12}H_{24}N_2O_4$): 261.17 $[M+H]^+$, found 261.3; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 5.37 (d, *J*=5.6 Hz, 1H), 4.18 (s, 1 H), 3.03 (dq, *J*=4.7, 13.3, 13.3, 13.3 Hz, 2H), 1.80 (d, *J*=6.8 Hz, 2H), 1.47 (s, 9H), 1.44 (s, 9H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 170.5, 155.6, 82.2, 79.8, 56.3, 44.2, 28.3, 28.0.

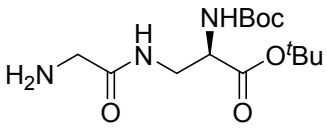
(2R)-2-*tert*-Butoxycarbonylamino-3-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-acetylamino]-propionic acid *tert*-butyl ester



S5

Fmoc-Gly-OH (458 mg, 1.54 mmol, 1 eq) was dissolved in DMF (8 mL), placed under Argon and HATU (586 mg, 1.54 mmol, 1 eq) and 4-methylmorpholine (NMM) (198 mg, 1.7 mmol, 1.1 eq) were added at room temperature. **S4** (400 mg, 1.54 mmol, 1 eq) was dissolved in DMF (8 mL), added dropwise to the stirring solution and the reaction mixture was stirred overnight. The reaction was diluted with distilled water and extracted with ethyl acetate. The organic phase was separated and washed with 1M HCl, water and saturated sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (1:1/Hx:EtOAc, R_f = 0.3) to afford **S5** as white foam (685 mg, 1.27 mmol, 82%). **MS (ESI+)** *m/z* calcd for **S5** ($C_{29}H_{37}N_3O_7$): 540.26 $[M+H]^+$, found 540.3; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.72 (d, *J*=7.5 Hz, 2 H), 7.62 (d, *J*=7.3 Hz, 1 H), 7.34 (t, *J*=7.3 Hz, 2 H), 7.26 (t, *J*=7.3 Hz, 2 H), 4.32 (d, *J*=7.0 Hz, 2 H), 4.17 (td, *J*=7.0, 7.0, 20.1 Hz, 2 H), 3.80 (m, 2 H), 3.57 (dq, *J*=6.2, 13.5, 13.3, 13.3 Hz, 2 H), 1.44 (s, 9 H), 1.42 (s, 9 H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 172.5, 172.4, 171.2, 158.7, 157.6, 145.0, 142.3, 128.6, 128.0, 126.1, 120.8, 83.0, 80.5, 68.0, 55.7, 48.1, 44.8, 41.6, 41.4, 28.6, 28.2.

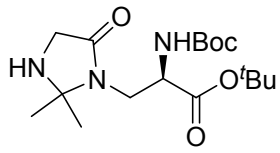
3-[(2R)-2-Amino-acetylamino]-2-tert-butoxycarbonylamino-propionic acid *tert*-butyl ester



S6

S5 (685 mg, 1.27 mmol) was dissolved in 50% (v/v) piperidine in DCM and stirred at room temperature for 1 hour. Upon reaction completion, the mixture was concentrated under reduced pressure and purified by column chromatography eluting 7:3/Hx:EtOAc, followed by 8:2/DCM:MeOH+1%Et_3N (R_f = 0.5, stains well with ninhydrin stain) to afford **S6** as white foam (~0.5 g, quantitative yield). **MS (ESI+)** *m/z* calcd for **S6** ($C_{14}H_{27}N_3O_5$): 318.20 $[M+H]^+$, found 318.3; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.64 (br s, 1H), 5.61 (d, *J*=6.9 Hz, 1H), 4.20 (d, *J*=5.1 Hz, 1H), 3.48-3.63 (m, 2H), 3.30 (s, 2H), 2.70 (br s, 2H), 1.39 (s, 9H), 1.36 (s, 9H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 173.1, 169.7, 155.7, 82.5, 79.8, 54.5, 44.4, 41.2, 28.2, 27.9.

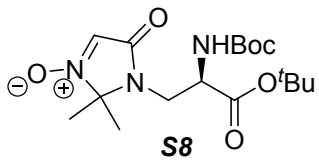
(2R)-2-*tert*-Butoxycarbonylamino-3-(2,2-dimethyl-5-oxo-imidazolidin-1-yl)-propionic acid *tert*-butyl ester



S7

S6 (158 mg, 0.50 mmol, 1 eq) was dissolved in $CHCl_3$ and transferred to a flame-dried round-bottom flask and concentrated. 3Å molecular sieves (0.1 g) were added followed by acetone (0.37 mL, 5.0 mmol, 10 eq) and Et_3N (0.347 mL, 2.5 mmol, 5 eq). The reaction was stirred at 60 ℃ overnight and monitored by TLC. Upon completion, the mixture was cooled to room temperature and dissolved in methanol, filtered through Celite, concentrated and purified by column chromatography (0-5% MeOH in DCM, R_f = 0.4) to afford **S7** as beige semi-solid (117 g, 0.33 mmol, 66%). **MS (ESI+)** *m/z* calcd for **S7** ($C_{17}H_{31}N_3O_5$): 358.23 $[M+H]^+$, found 358.3; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 5.57 (d, *J*=7.7 Hz, 1H), 4.24 (dt, *J*=5.5, 8.5, 8.4 Hz, 1H), 3.52 (dd, *J*=9.2, 14.2 Hz, 1H), 3.41 (m, 2H), 3.32 (dd, *J*=5.3, 14.3 Hz, 1H), 1.43 (s, 9H), 1.41, (s, 3H), 1.38, (s, 9H), 1.34, (s, 3H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 174.9, 169.4, 155.5, 82.4, 79.7, 78.5, 54.8, 48.0, 41.4, 28.3, 27.9, 26.3, 25.6.

(2R)-2-*tert*-Butoxycarbonylamino-3-(2,2-dimethyl-5-oxo-3-oxy-2,5-dihydro-imidazol-1-yl)-propionic acid *tert*-butyl ester

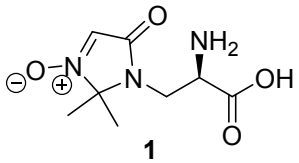


S8

S7 (58 mg, 0.16 mmol, 1 eq) was dissolved in dry DCM (purchased from Sigma-Aldrich) and cooled to 0 C, to which *m*-CPBA (92 mg at ≤77%, 0.41 mmol, 2.6 eq) was added. The mixture was stirred for 3

hours, concentrated, and purified by column chromatography eluting 40-50% EtOAc in Hx (R_f = 0.3 in 5% MeOH in DCM) to afford **S8** as translucent white semi-solid (49.8 mg, 0.13 mmol, 81%). **MS (ESI+)** *m/z* calcd for **S8** ($C_{17}H_{29}N_3O_6$): 372.21 $[M+H]^+$, found 372.2; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.32 (s, 1H), 4.46 (m, 1H), 3.91 (dd, *J*=5.4, 14.6 Hz, 1H), 3.64 (dd, *J*=9.2, 14.5 Hz, 1H), 1.68 (s, 3 H), 1.66 (s, 3 H), 1.50 (s, 9 H), 1.45 (s, 9 H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 169.7, 164.6, 157.6, 125.6, 92.2, 83.4, 80.8, 54.4, 42.6, 28.7, 28.2, 24.8, 24.6.

(2R)-2-Amino-3-(2,2-dimethyl-5-oxo-3-oxy-2,5-dihydro-imidazol-1-yl)-propionic acid (D-Ala-DMImO)

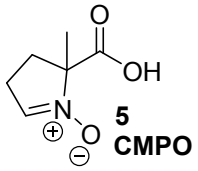


1

D-Ala-DMImO

Trifluoroacetic acid (neat, 1 mL, 13 mmol, large excess 26 eq) was added to **S8** (0.18 g, 0.5 mmol, 1 eq) and stirred for 1.5 hours. Upon completion, the reaction was diluted with methanol and evaporated to dryness under reduced pressure. The final product was azeotroped with 10 mL methanol three times to dryness each time and further dried on high vacuum pump to afford **1** as light yellow solid (108 mg, 0.49, quantitative yield). **MS (ESI+)** *m/z* calcd for **1** ($C_8H_{13}N_3O_4$): 216.09 $[M+H]^+$, found 216.3; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.37 (s, 1H), 4.39 (dd, *J*=4.7, 6.6 Hz, 1H), 4.03 (m, 2H), 1.70 (s, 3H), 1.68 (s, 3H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 169.4, 166.3, 125.3, 92.4, 54.4, 42.1, 24.4, 24.3.

5-Carboxyl-5-methyl pyrroline-*N*-oxide (CMPO)

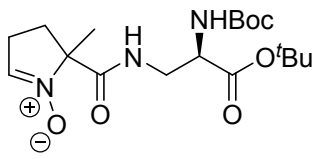


5

CMPO

5-Ethoxycarbonyl-5-methyl-1-pyrroline *N*-oxide (EMPO) was synthesized according to literature procedure.[3] EMPO (1.93 g, 11.3 mmol) was dissolved in aqueous sodium hydroxide (2.5% by weight, 13 mL) in a round bottom flask. The flask was equipped with a reflux condenser and the reaction was heated to 100°C for 2 hour. The reaction was cooled to room temperature and passed through a pre-flushed (rinsed and packed with distilled water) column containing Biorad AG 50W-X8 resin, 100-200 mesh, hydrogen form and eluted with water until all product was collected. The fractions were combined and lyophilized to afford **5** as pale yellow solid (1.02 g, 7.1 mmol, 63%). **MS (ESI+)** *m/z* calcd for **5** ($C_6H_9NO_3$): 144.06 $[M+H]^+$, found 144.2; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.30 (s, 1H), 2.91-2.71 (br s, 2H), 2.63 (ddd, *J*=4.2, 8.3, 12.7 Hz, 1H), 2.27 (td, *J*=8.6, 8.6, 13.6 Hz, 1H), 1.67 (s, 3 H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 172.6, 143.3, 80.3, 33.3, 27.3, 21.2.

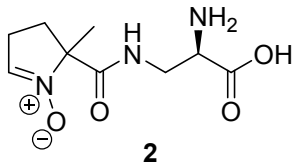
(2R)-2-*tert*-Butoxycarbonylamino-3-[(2-methyl-1-oxy-3,4-dihydro-2H-pyrrole-2-carbonyl)-amino]-propionic acid *tert*-butyl ester



S10

S4 (455 mg, 1.75 mmol, 1 eq), HATU (665 mg, 1.75 mmol, 1 eq) and **5** (300 mg, 2.1 mmol, 1.2 eq) were placed in a screw-cap vial and DMF (1.8 mL) was added, followed by *N*,*N*-diisopropylethylamine (532 uL, 3.06 mmol, 1.75 eq). The reaction mixture was stirred for 45 minutes. Upon confirmation of completion via HPLC-MS, the reaction was diluted with methanol, filtered and purified by preparatory HPLC using isocratic method with 30% MeCN over 6 minutes. **S10** eluted at ~6 minutes, its presence was confirmed by MS and the fractions were pooled and concentrated under reduced pressure to afford **S10** as light brown foam (184 mg, 0.48 mmol, 27%). **MS (ESI+)** *m/z* calcd for **S10** ($C_{18}H_{31}N_3O_6$): 386.22 $[M+H]^+$, found 386.3; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.27 (m, 1H), 4.21 (ddd, *J*=5.5, 7.8, 9.3 Hz, 1H), 3.66 (dt, *J*=4.8, 13.3, 13.3 Hz, 1H), 3.48 (ddd, *J*=7.7, 13.8, 18.1 Hz, 1H), 2.74 (m, 1 H), 2.22 (m, 1H), 1.68 (s, 3H), 1.49 (s, 9H), 1.47 (s, 9H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 172.7, 171.2, 164.3, 157.7, 143.2, 83.2, 83.2, 80.6, 80.3, 55.4, 55.3, 41.6, 32.1, 28.7, 28.2, 26.3, 23.1.

(2R)-2-Amino-3-[(2-methyl-1-oxy-3,4-dihydro-2H-pyrrole-2-carbonyl)-amino]-propionic acid (D-Ala-CMPO)

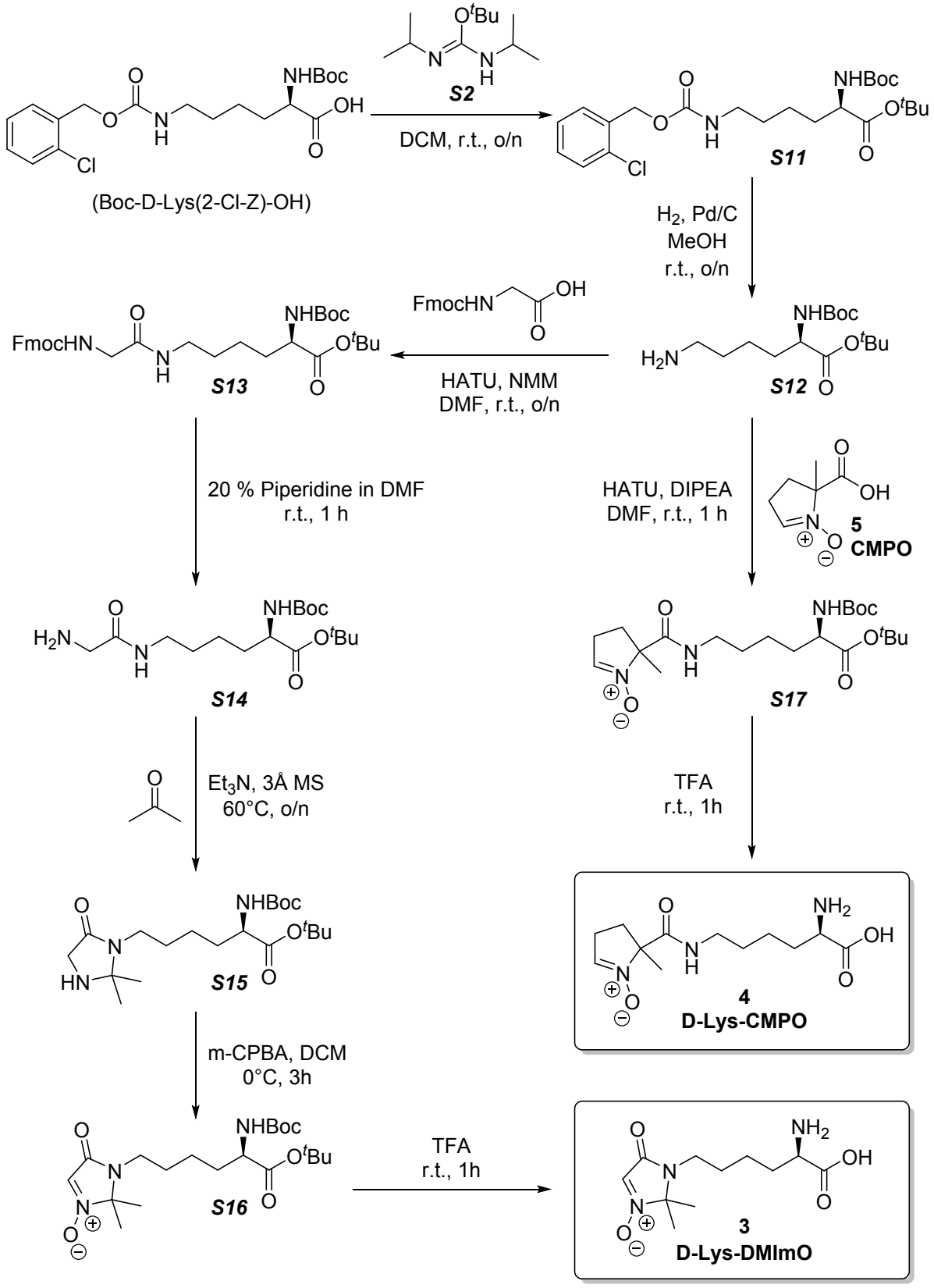


2

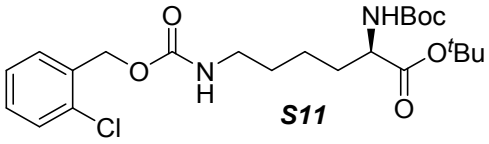
D-Ala-CMPO

Trifluoroacetic acid (neat, 1.8 mL, 24 mmol, large excess 50 eq) was added to **S8** (184 g, 0.48 mmol, 1 eq) and stirred for 1.5 hours. Upon completion, the reaction was diluted with methanol and evaporated to dryness under reduced pressure. The final product was azeotroped with 10 mL methanol three times to dryness each time and further dried on high vacuum pump to afford **2** as light yellow solid (109 mg, 0.48, quantitative yield). **MS (ESI+)** *m/z* calcd for **2** ($C_9H_{15}N_3O_4$): 230.11 $[M+H]^+$, found 230.3; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.29 (s, 1H), 4.17 (td, *J*=4.5, 4.5, 6.7 Hz, 1H), 3.91-3.67 (m, 2H), 2.78-2.64 (m, 3H), 2.27-2.17 (m, 1H), 1.69 (s, 3H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 173.6, 169.9, 143.9, 80.7, 54.3, 40.7, 32.5, 26.8, 22.3.

Synthesis of D-Lysine analogues



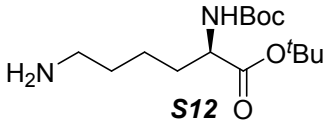
N_α-Boc-N_ε-(2-chloro-carboxybenzyl)-D-lysine *tert*-butyl ester



S11

Boc-D-Lys(2-Cl-Z)-OH (5 g, 12 mmol, 1 eq) was dissolved in 50 mL of DCM, 2-tert-butyl-1,3-diisopropylurea (**S2**) (~5.4 g, 27 mmol, 2.25 eq) was added and the mixture was stirred for 48 hours at room temperature. The crude reaction mixture was concentrated under reduced pressure and purified by column chromatography (6:4/Hx:EtOAc, R_f = 0.65) to afford **S11** as colourless oil (3.65 g, 7.8 mmol, 65%). **MS (ESI+)** *m/z* calcd for **S11** ($C_{23}H_{35}ClN_2O_6$) $[M+H]^+$: 470.22, found 471.2; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.40 (m, 1H), 7.36 (m, 1H), 7.24 (m, 2H), 5.19 (s, 2H), 5.07 (d, *J*=7.5 Hz, 1H), 4.94 (br s, 1H), 4.15 (dd, *J*=7.6, 12.6 Hz, 1H), 3.19 (dd, *J*=6.5, 12.8 Hz, 2H), 1.77 (ddd, *J*=5.6, 10.6, 14.6 Hz, 1H), 1.66-1.49 (m, 4H), 1.44 (s, 9H), 1.42 (s, 9H), 1.40-1.29 (m, 2H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 171.9, 156.2, 155.5, 134.4, 133.5, 129.7, 129.5, 129.3, 126.8, 81.9, 79.7, 63.8, 53.7, 40.8, 32.7, 29.3, 28.3, 28.0, 22.3.

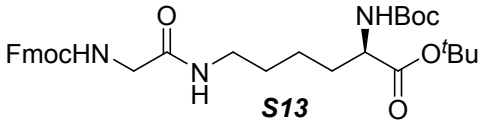
N_α-Boc-N_ε-(2-chloro-carboxybenzyl)-D-lysine *tert*-butyl ester



S12

S11 (3.65 g, 7.8 mmol, 1 eq) was dissolved in 58 mL of methanol. Pd/C (0.36 g) was added very cautiously in small portions to the stirring solution. The flask air was replaced with hydrogen gas. The flask was fitted with septa and a hydrogen gas balloon, and stirred overnight. The reaction was filtered through a short plug of Celite. The filter cake was **not** allowed to dry out. The filtrate was concentrated under reduced pressure to afford **S12** as viscous yellow oil (~2.4 g, 7.8 mmol, quantitative yield). **S12** was used without further purification. **MS (ESI+)** *m/z* calcd for **S12** ($C_{15}H_{30}N_2O_4$) $[M+H]^+$: 303.22, found 303.2.

(2R)-2-*tert*-Butoxycarbonylamino-6-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-acetylamino]-hexanoic acid *tert*-butyl ester

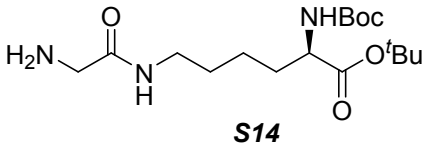


S13

Fmoc-Gly-OH (0.589 g, 1.98 mmol, 1 eq) and HATU (0.754 g, 1.98 mmol, 1 eq) were dissolved in DMF (10 mL) at room temperature and stirred for 5 minutes under a nitrogen atmosphere. **S12** (0.600 g, 1.98 mmol, 1 eq) was dissolved in DMF (10 mL) and added dropwise to the reaction mixture, followed by 4-methylmorpholine (NMM) (0.240 mL, 2.18 mmol, 1.1 eq). The reaction was stirred overnight at room temperature, concentrated, and

purified by column chromatography eluting 20-50% EtOAc in Hx (R_f = 0.15 in 1:1 EtOAc:Hx). **S13** is colourless oil (0.3 g, 0.52 mmol, 26%). **MS (ESI+)** *m/z* calcd for **S13** ($C_{32}H_{43}N_3O_7$): 582.31 $[M+H]^+$, found 582.5; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.75 (d, *J*=7.5 Hz, 2H), 7.58 (d, *J*=7.3 Hz, 2H), 7.39 (t, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 6.30 (br s, 1H), 5.73 (br s, 1H), 5.13 (d, *J*=8.0 Hz, 1H), 4.41 (d, *J*=7.0 Hz, 2H), 4.21 (t, *J*=6.9 Hz, 1H), 4.13 (dd, *J*=7.4, 12.3 Hz, 1H), 3.84 (br d, *J*=3.6 Hz, 2H), 3.33-3.16 (m, 2H), 1.75 (ddd, *J*=5.7, 10.2, 13.5 Hz, 1H), 1.65-1.48 (m, 3H), 1.45 (s, 9H), 1.43 (s, 9H), 1.40-1.30 (m, 2H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 171.9, 169.0, 156.7, 155.6, 143.7, 141.3, 127.8, 127.1, 125.1, 120.0, 81.9, 79.7, 67.2, 53.7, 47.1, 44.5, 39.3, 32.7, 28.9, 28.4, 28.0, 22.5.

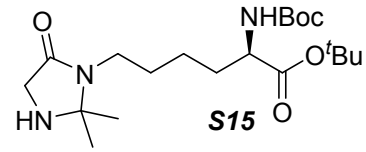
6-(2-Amino-acetylamino)-(2R)-2-tert-butoxycarbonylamino-hexanoic acid *tert*-butyl ester



S14

S13 (0.300 g, 0.52 mmol) was dissolved in 20% Piperidine in DMF (20 mL) and stirred at room temperature under nitrogen atmosphere overnight. The reaction was concentrated under reduced pressure, pre-adsorbed onto silica and purified by column chromatography eluting 1:1/EtOAc:Hx followed by 8:2/$CHCl_3$:EtOH+1% Et_3N (R_f = 0.05 in 5% MeOH in DCM) to afford **S14** as clear oil (156 mg, 0.43 mmol, 83%). **MS (ESI+)** *m/z* calcd for **S14** ($C_{17}H_{33}N_3O_5$): 360.24 $[M+H]^+$, found 360.3; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.31 (s, 1H), 5.10 (d, *J*=7.8 Hz, 1H), 4.08 (dd, *J*=8.2, 13.5 Hz, 1H), 3.31 (br s, 1H), 3.22 (dd, *J*=6.6, 13.0 Hz, 3H), 1.79-1.66 (m, 1H), 1.46-1.65 (m, 4H), 1.41 (s, 9H), 1.39 (s, 9H), 1.36-1.26 (m, 3H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 171.9, 155.5, 81.8, 79.6, 53.8, 45.8, 38.7, 32.6, 29.1, 28.3, 28.0, 22.6, 8.8.

(2R)-2-*tert*-Butoxycarbonylamino-6-(2,2-dimethyl-5-oxo-imidazolidin-1-yl)-hexanoic acid *tert*-butyl ester

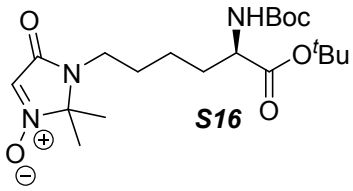


S15

S14 (156 mg, 0.43 mmol, 1 eq) was dissolved in acetone and transferred to a flame-dried round bottom flask, followed by removal of the solvent. 3Å molecular sieves (0.2 g) were added, followed by acetone (0.500 mL, 6.78 mmol, 15.8 eq) and triethylamine (0.307 mL, 2.2 mmol, 5.1). The flask was fitted with a reflux condenser and heated to 70°C overnight. The residue was diluted with methanol, filtered through Celite, concentrated and purified by column chromatography (5% MeOH in DCM, R_f = 0.3) to afford **S15** as obtained as brown oil (56 mg, 0.14 mmol, 33%). **MS (ESI+)** *m/z* calcd for **S15** ($C_{20}H_{37}N_3O_5$): 400.27 $[M+H]^+$, found 400.3;

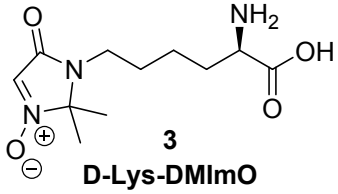
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.05 (d, *J*=8.2 Hz, 1H), 4.12 (td, *J*=4.8, 7.8 Hz, 1H), 3.41 (s, 2H), 3.10 (t, *J*=7.8 Hz, 2H), 1.82-1.69 (m, 1H), 1.65-1.52 (m, 3H), 1.43 (s, 9H), 1.41 (s, 9H), 1.39-1.25 (m, 8H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 173.5, 171.9, 155.4, 81.8, 79.6, 78.1, 53.7, 48.2, 40.1, 32.6, 29.7, 29.0, 28.3, 28.0, 26.5, 22.9.

(2R)-2-*tert*-Butoxycarbonylamino-6-(2,2-dimethyl-5-oxo-3-oxy-2,5-dihydro-imidazol-1-yl)-hexanoic acid *tert*-butyl ester



S15 (56 mg, 0.14 mmol, 1 eq) was dissolved in DCM (5 mL, dried over 3Å molecular sieves) and cooled to 0°C. *m*-CPBA (83.6 mg at ≤77%, 0.37 mmol, 2.6 eq) was added in one portion and stirred at 0°C for 3 hours. Upon completion, excess *m*-CPBA was quenched with 1M aqueous sodium thiosulfate; saturated aqueous sodium bicarbonate was added and the layers were separated. The aqueous layer was extracted with DCM (3x10 mL), the organic phases were combined and washed with saturated sodium bicarbonate (10.0 mL) and brine (10.0 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude was purified by column chromatography (0-5% MeOH in DCM, R_f = 0.4 in 5% MeOH in DCM) to afford **S16** as pale yellow oil (42.2 mg, 0.102 mmol, 73%). **MS (ESI+)** *m/z* calcd for **S16** ($C_{20}H_{35}N_3O_6$): 414.25 $[M+H]^+$, found 414.3; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.00 (s, 1H), 5.06 (d, *J*=8.0 Hz, 1H), 4.15 (dd, *J*=7.4, 12.8 Hz, 1H), 3.43-3.28 (m, 2H), 1.84-1.74 (m, 1H), 1.74-1.66 (m, 2H), 1.66-1.54 (m, 7H), 1.44 (s, 9H), 1.42 (s, 9H), 1.41-1.35 (m, 2H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 171.8, 162.0, 155.4, 123.9, 90.2, 82.0, 79.7, 53.5, 40.5, 32.6, 28.7, 28.3, 28.0, 24.8, 24.7, 22.7.

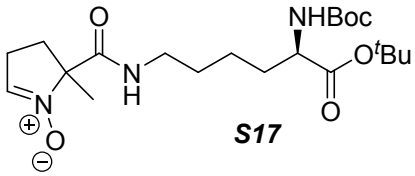
(2R)-2-Amino-6-(2,2-dimethyl-5-oxo-3-oxy-2,5-dihydro-imidazol-1-yl)-hexanoic acid (D-Lys-DMImO)



Trifluoracetic acid (0.300 mL, 3.91 mmol) was added to **S16** (10 mg, 0.024 mmol) and stirred at room temperature for 4 hours. Reaction progress was monitored by HPLC-MS. Upon completion, the reaction was diluted with methanol and evaporated to dryness under reduced pressure. The final product was azeotroped with 10 mL methanol three times to dryness each time and further dried on high vacuum pump to afford **3** as dark brown oil (~6 mg, quantitative yield). R_f = 0.09 (20% MeOH in DCM). **MS (ESI+)** *m/z* calcd for **3** ($C_{11}H_{19}N_3O_4$):

258.14 $[M+H]^+$, found 258.3; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.29 (s, 1H), 3.98 (t, J=6.4 Hz, 1H), 3.49 (t, J=7.4 Hz, 2H), 2.08-1.85 (m, 2H), 1.79-1.70 (m, 2H), 1.65 (s, 6H), 1.60-1.44 (m, 3H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 171.8, 164.2, 125.9, 92.0, 53.7, 41.0, 31.0, 29.7, 24.7, 24.7, 23.3.

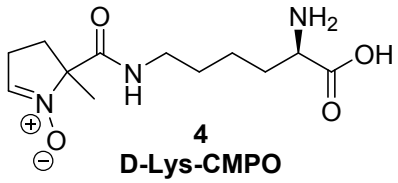
(2R)-2-*tert*-Butoxycarbonylamino-6-[(2-methyl-1-oxy-3,4-dihydro-2H-pyrrole-2-carbonyl)-amino]-hexanoic acid *tert*-butyl ester



S17

S12 (265 mg, 0.88 mmol, 1 eq), HATU (334 mg, 0.88 mmol, 1 eq) and **5** (150 mg, 1.05 mmol, 1.2 eq) were placed in a screw-cap vial and DMF (1.05 mL) was added, followed by *N*,*N*-diisopropylethylamine (270 uL, 1.55 mmol, 1.76 eq). The reaction mixture was stirred for 45 minutes. Upon confirmation of completion via HPLC-MS, the reaction was concentrated under reduced pressure and purified by preparatory HPLC using isocratic method with 35% MeCN over 6 minutes. **S17** eluted at ~6-7 minutes, its presence was confirmed by MS and the fractions were pooled and concentrated under reduced pressure to afford **S17** as clear yellow oil (45.8 mg, 0.11 mmol, 12%). **MS (ESI+)** *m/z* calcd for **S17** ($C_{21}H_{37}N_3O_6$): 428.27 $[M+H]^+$, found 428.3; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.23 (s, 1H), 3.94 (dd, J=5.0, 8.6 Hz, 1H), 3.25 (tt, J=5.2, 10.4 Hz, 2H), 2.82-2.62 (m, 3H), 2.27-2.16 (m, 1H), 1.82-1.72 (m, 1H), 1.69 (s, 3H), 1.66-1.53 (m, 3H), 1.48 (s, 9H), 1.46 (s, 9H), 1.44-1.33 (m, 2H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 173.8, 172.4, 158.2, 142.8, 82.5, 80.4, 55.8, 40.3, 32.4, 32.2, 29.8, 28.7, 28.3, 28.1, 26.4, 24.2, 23.0.

(2R)-2-Amino-6-[(2-methyl-1-oxy-3,4-dihydro-2H-pyrrole-2-carbonyl)-amino]-hexanoic acid (D-Lys-CMPO)

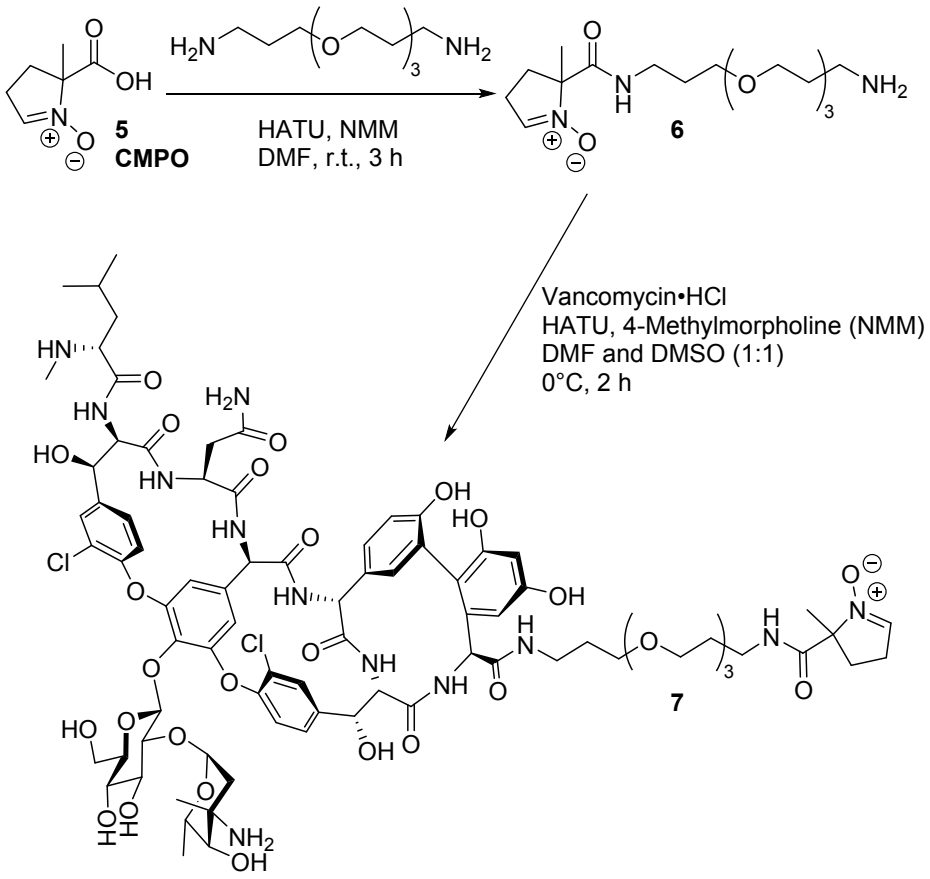


4
D-Lys-CMPO

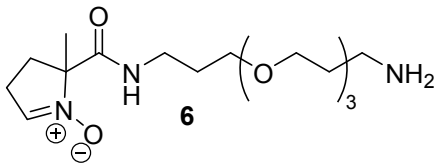
Trifluoracetic acid (0.45 mL, 5.9 mmol) was added to **S17** (45.8 mg, 0.11 mmol) and stirred at room temperature for 1 hour. Upon MS confirmation of completion, the reaction was diluted with methanol and evaporated to dryness under reduced pressure. The final product was azeotroped with 10 mL methanol three times to dryness each time and further dried on high vacuum pump to afford **4** as red oil (~29 mg, quantitative yield). **MS (ESI+)** *m/z* calcd for **4** ($C_{12}H_{21}N_3O_4$): 272.15 $[M+H]^+$, found 272.2; **^{1}H-NMR** (400 MHz, CD_3OD): δ 7.25 (s, 1H), 3.98 (t, J=5.7 Hz, 1H), 3.31-3.21 (m, 2H), 2.81-2.64 (m, 3H), 2.29-2.15

(m, 1H), 2.07-1.83 (m, 2H), 1.69 (s, 3H), 1.66-1.56 (m, 2H), 1.53-1.38 (m, 2H); **^{13}C-NMR** (100 MHz, CD_3OD): δ 172.3, 171.8, 143.0, 53.8, 39.9, 32.4, 31.0, 29.7, 26.5, 23.1, 23.0, 22.7.

Synthesis of Vancomycin-PEG3-CMPO



2-Methyl-1-oxy-3,4-dihydro-2H-pyrrole-2-carboxylic acid (3-(2-[2-(3-amino-propoxy)-ethoxy]-ethoxy)-propyl)-amide



5 (63 mg, 0.450 mmol, 1 eq) was dissolved in DMF to which HATU (0.171 g, 0.450 mmol, 1 eq) was added, followed by 4,7,10-trioxa-1,13-tridecane diamine (0.6 g, 2.72 mmol, 6 eq) and 4-methylmorpholine (NMM) (0.138 g, 1.36 mmol, 3 eq) were added. The reaction was stirred at room temperature for 3 hours, concentrated under reduced pressure and purified by column chromatography (20-60% EtOH in

$CHCl_3$+1% Et_3N, followed by pure MeOH+1% Et_3N) to afford 6 as yellow oil (62 mg, 0.18 mmol, 40%). **MS (ESI+)** *m/z* calcd for **6** ($C_{16}H_{31}N_3O_5$): 346.23$[M+H]^+$, found 346.4.

Vancomycin-PEG3-CMPO (7)

6 (0.06 g, 0.17 mmol, 2.8 eq) was dissolved in DMF and DMSO (1:1) to which Vancomycin hydrocholoride (0.089 g, 0.06 mmol, 1 eq) was added, followed by HATU (0.023 g, 0.06 mmol, 1 eq) and 4-methylmorpholine (NMM) (0.017 g, 0.17 mmol, 2.8 eq). The reaction was stirred at room temperature for 3 hours and then purified by preparatory HPLC using gradient of 10-25% MeCN over 10 minutes. **7** eluted at ~1.5 minutes, its presence was confirmed by MS and the fractions were pooled and concentrated under reduced pressure to remove acetonitrile, followed by lyophilization to afford **7** as white fluffy solid (22 mg, 0.012 mmol, 20%). Due to the complexity of the molecule, the compound identity was confirmed by mass spectrometry: *m/z* calcd for **7** ($C_{82}H_{104}N_{12}O_{28}Cl_2$): 1774.5228, found **MS (ESI+)** 598.6 $[M+3H/3]^+$, **HRMS** 1813.6211 $[M+K]^+$

Biological Experimental Methods

Fluorescence microscopy. All microscopy images herein were acquired using an Olympus 1 x 81 spinning-disk confocal microscope equipped with a Photometrics (Coolsnap ES) camera and FITC filter (Semrock, Excitation: 465-499 nm, Emission: 516-556 nm). Images were acquired with 100X magnification using both bright field and FITC channel (1-2s exposure). Images were processed using ImageJ software to apply pseudocolouring to the FITC channel, and to apply the same pixel-intensity ranges for samples and paired controls.

Metabolic labelling of bacterial cells via SPANC. Bacteria were inoculated into their appropriate growth medium (LB for *E. coli* and BHI for *L. lactis* and *L. innocua*) and cultured until mid-log phase growth at which point 5 mM of UAA (from a 500mM stock in DMSO) or equivalent volume of DMSO was introduced into the media for 1 hour. *L. innocua* were cultured at 30°C without agitation, while *E. coli* and *L. lactis* were cultured at 37°C with agitation. Bacteria were washed 3 times in PBS, reacted by SPANC with 25 μM DIBO-Alexa488 in PBS (10 minutes at 37°C), and then washed in PBS 3 more times prior to imaging live cells by fluorescence microscopy.

Supporting Figures. Metabolic Labelling

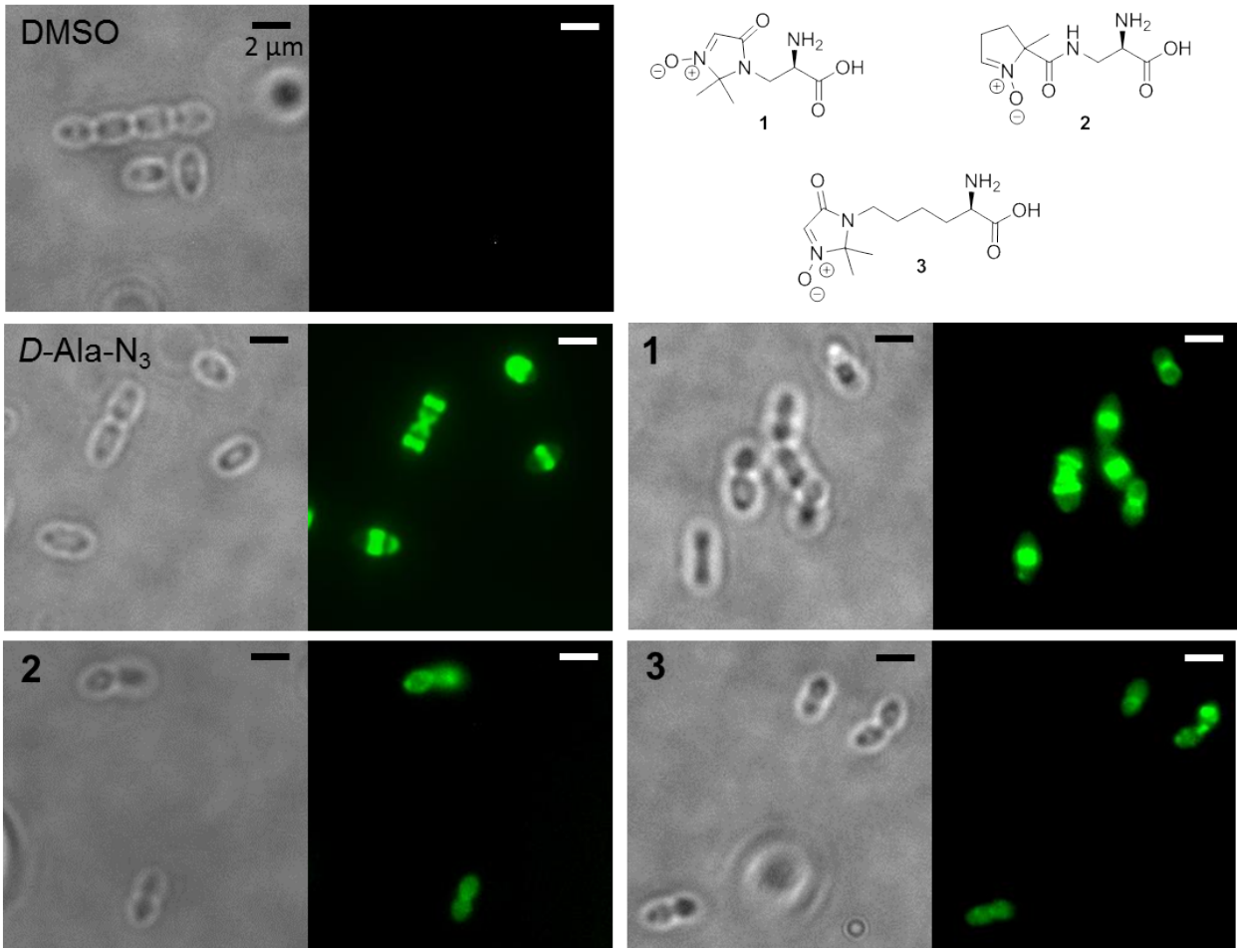


Figure S1. Metabolic labelling of *L. lactis* by UAA probes. *L. lactis* were cultured in BHI medium until mid-log phase growth, and then treated with DMSO, or 5 mM of the indicated UAA for 1 hour at 37°C. Cells were washed in PBS before and after SPANC reaction with DIBO-Alexa488 (10 minutes at 37°C), followed by fluorescence microscopy with bright field images on the left and fluorescence on the right. Fluorescence intensity is displayed above background labelling of DMSO control, with the same brightness for all images except D-Alanine-N_3, which is shown approximately 20 times lower due to signal saturation. Scale bars = 2 µm.

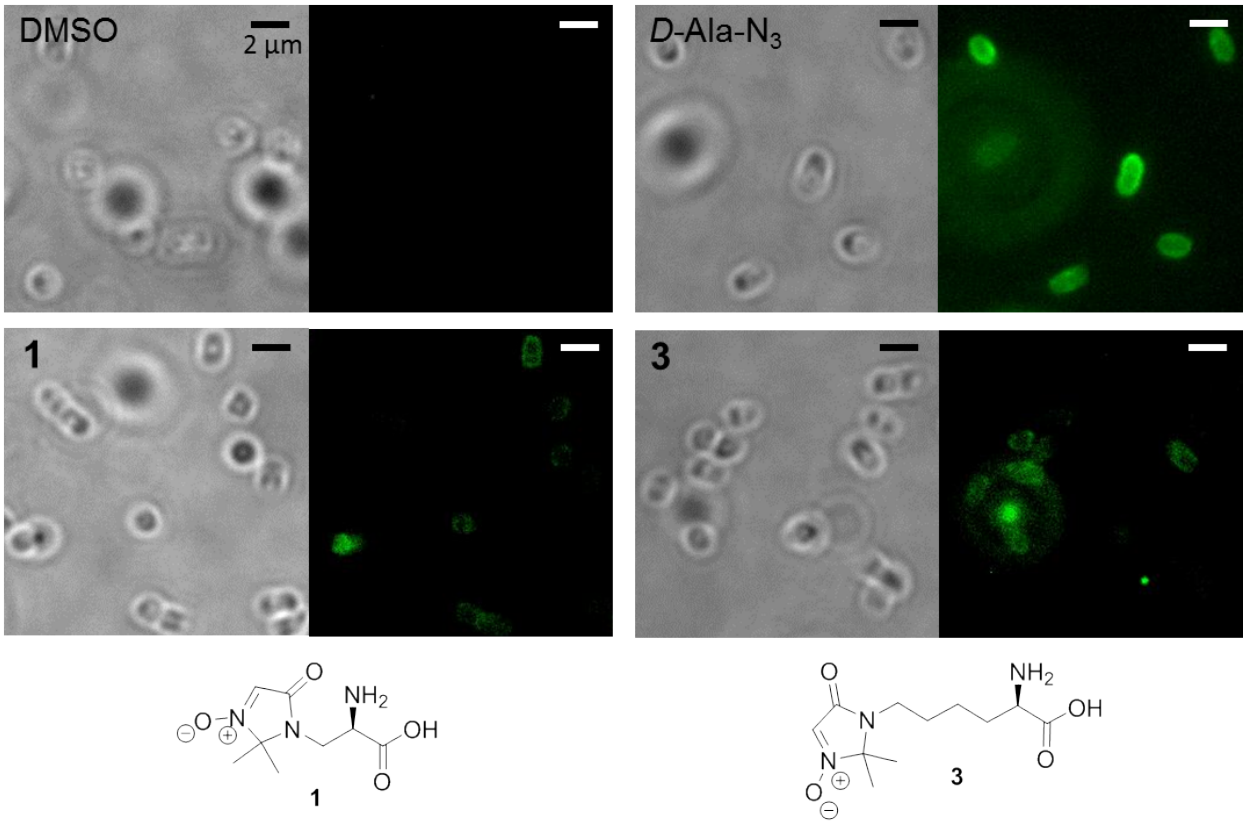
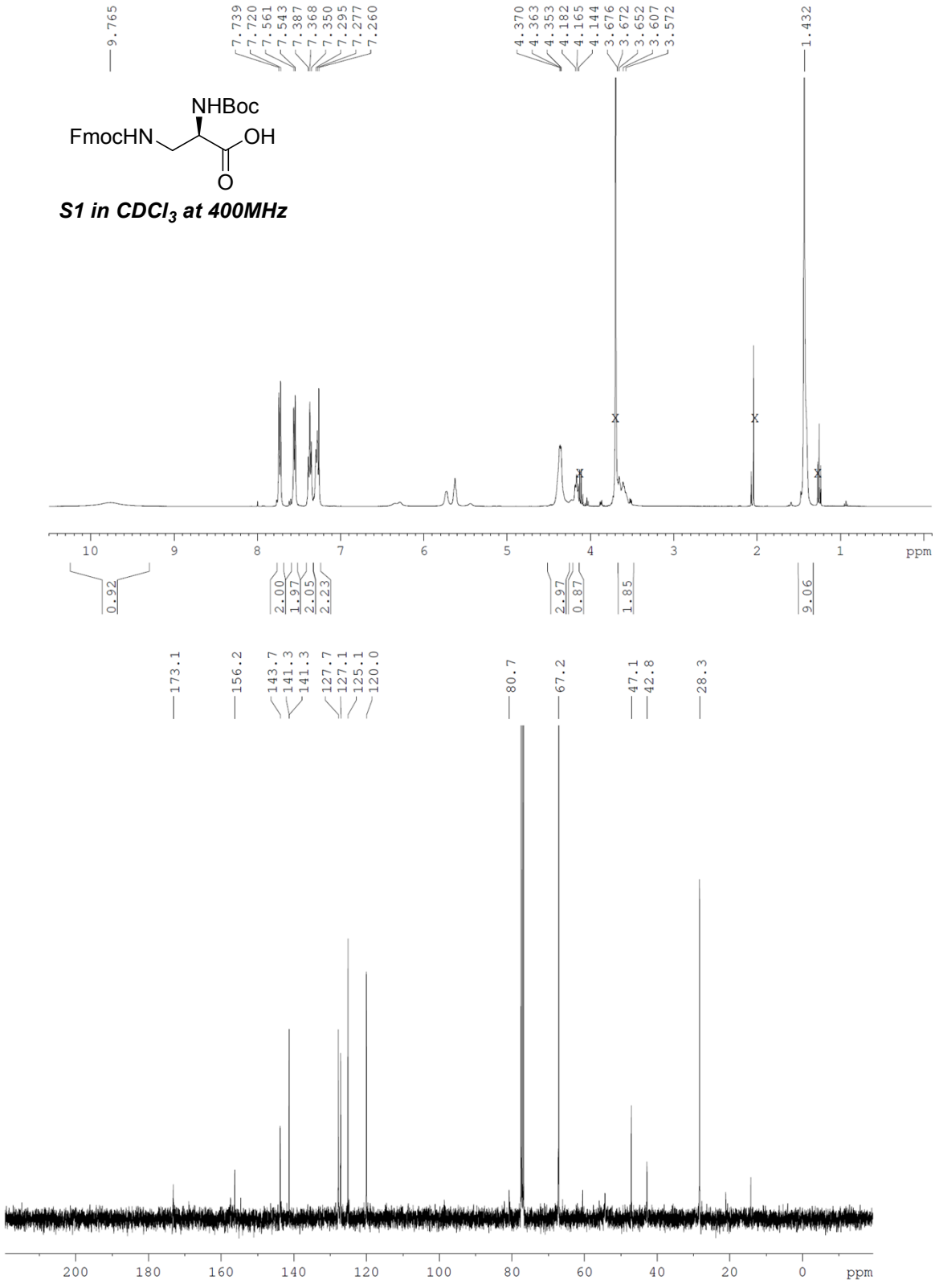
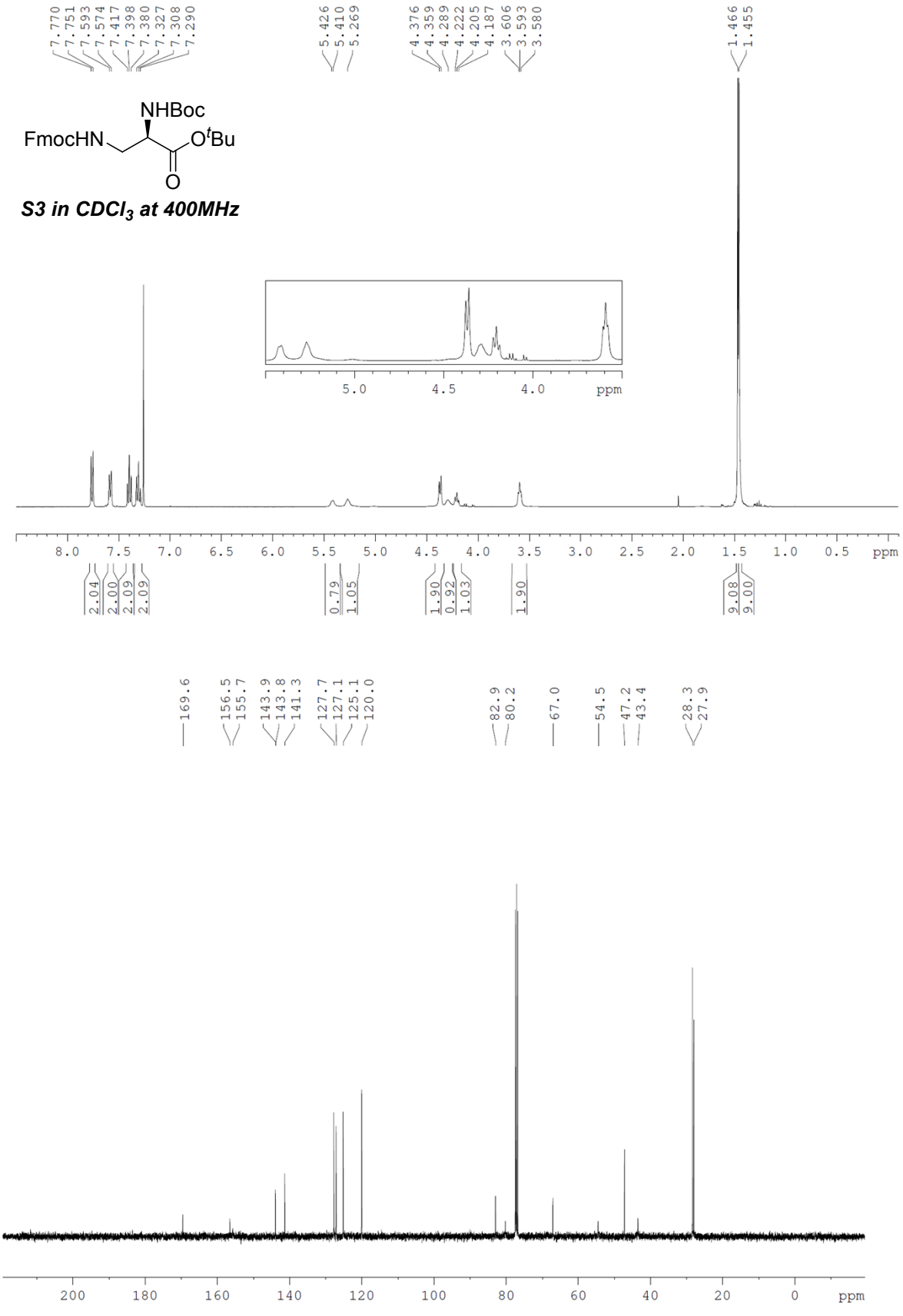


Figure S2. Metabolic labelling of K12 *E. coli* by UAA probes. *E. coli* were cultured in LB medium until mid-log phase growth, and then treated with DMSO, or 5 mM of the indicated UAA for 1 hour at 37°C. Cells were washed in PBS before and after SPANC reaction with DIBO-Alexa488 (10 minutes at 37°C), followed by fluorescence microscopy with bright field images on the left and fluorescence on the right. Fluorescence intensity is displayed above background labelling of DMSO control, with the same brightness for all images except D-Alanine-N_3, which is shown approximately 3 times lower due to signal saturation. Scale bars = 2 µm.

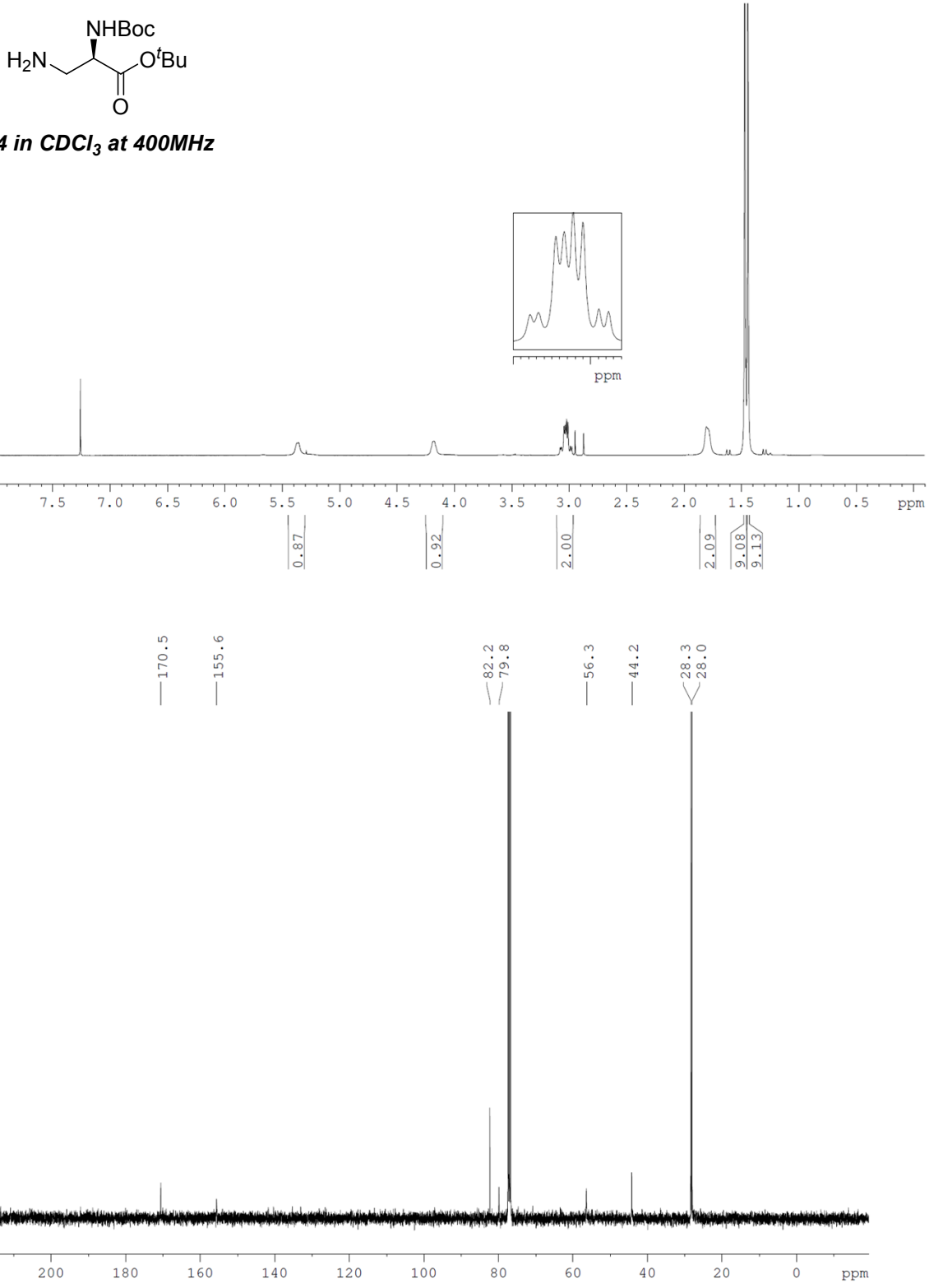
NMR Spectra



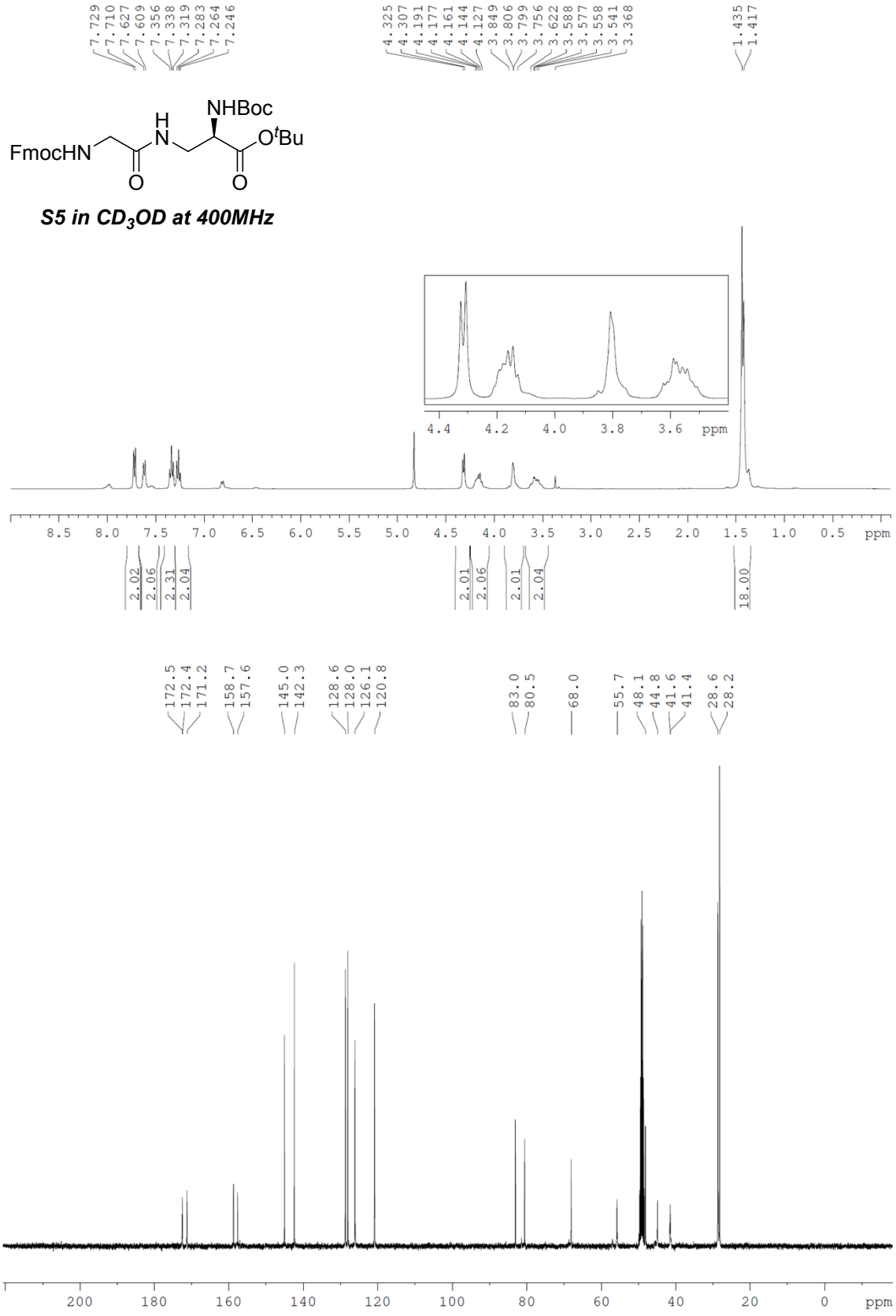
S1 in $CDCl_3$ at 400MHz



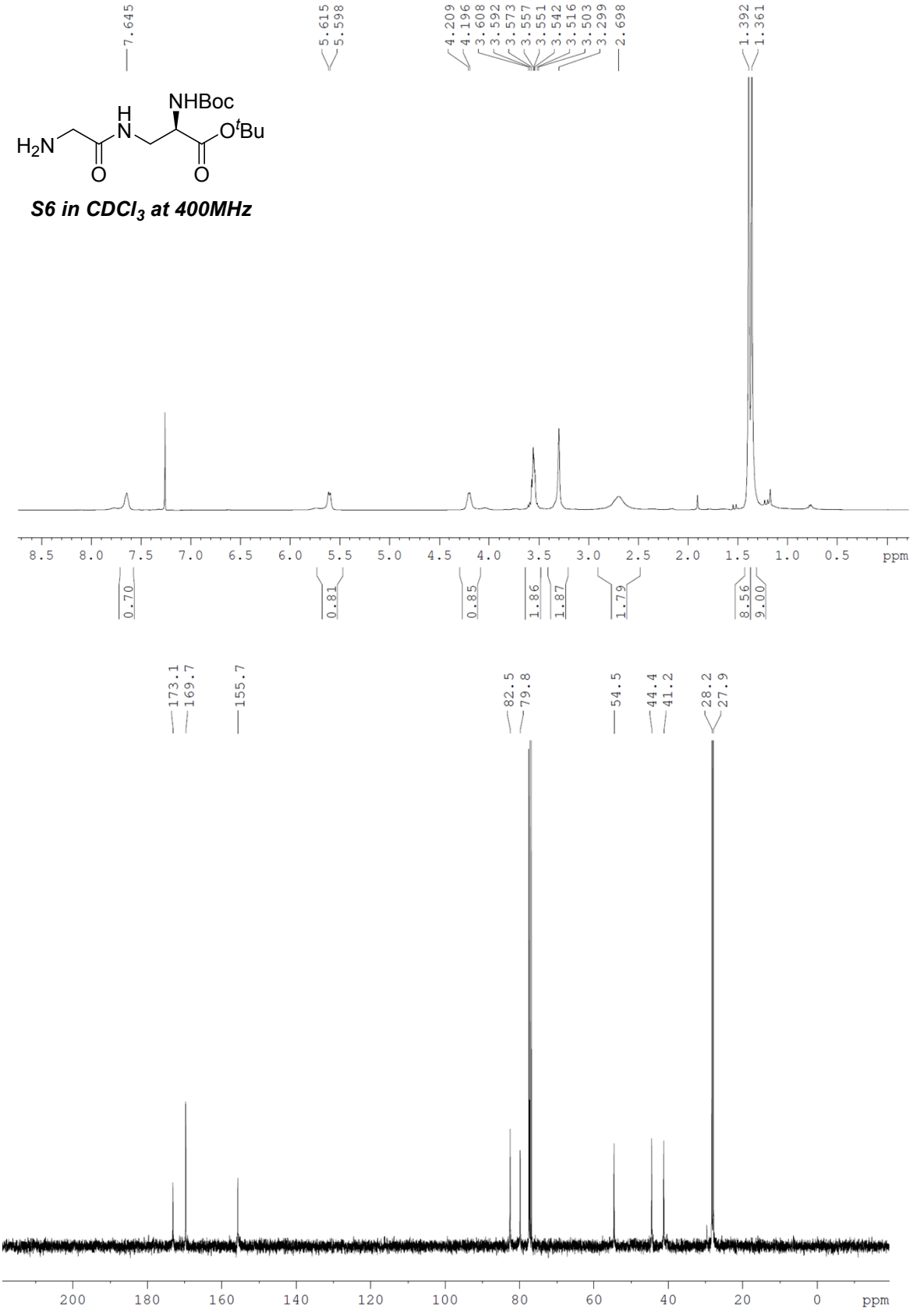
S3 in $CDCl_3$ at 400MHz



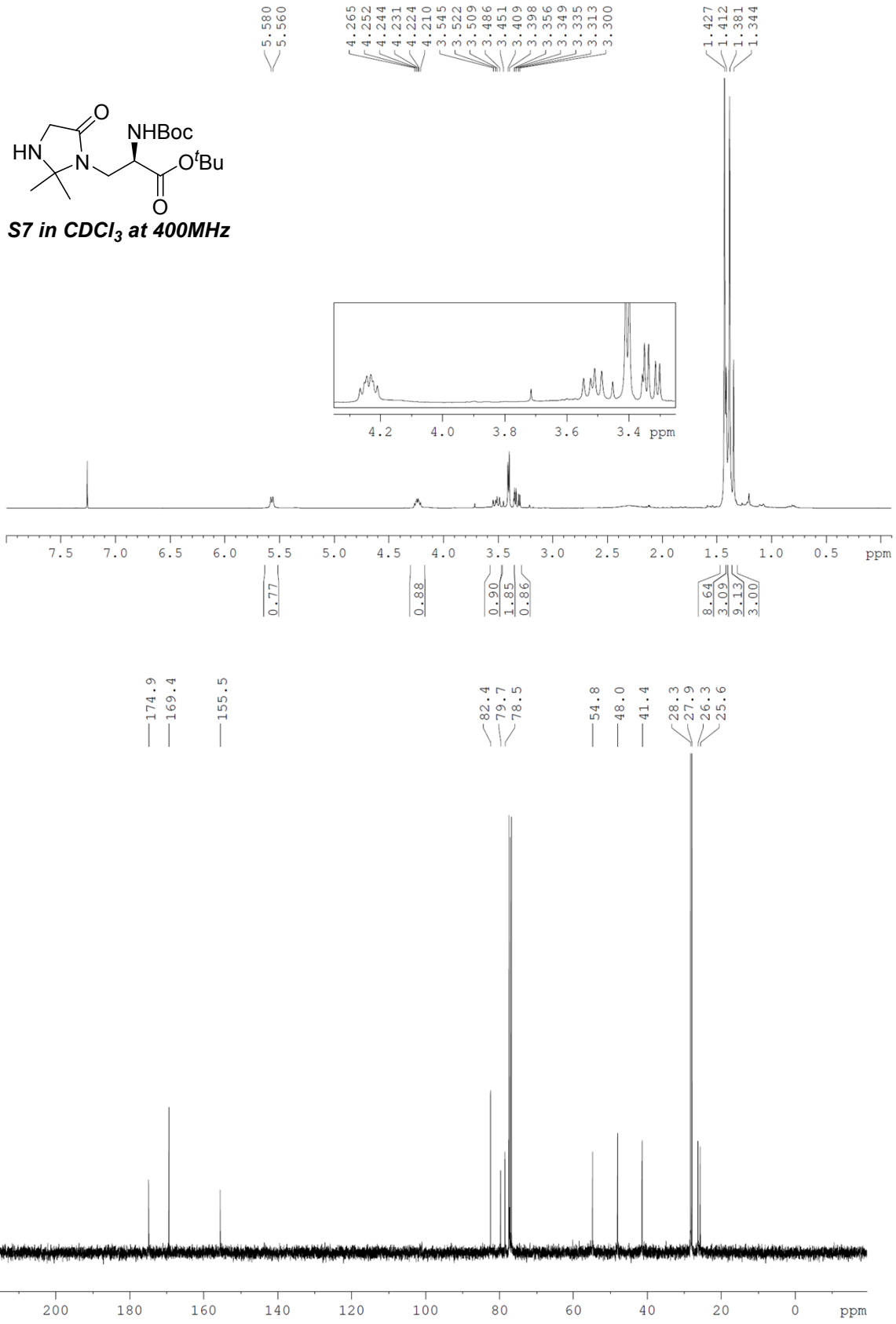
S4 in $CDCl_3$ at 400MHz



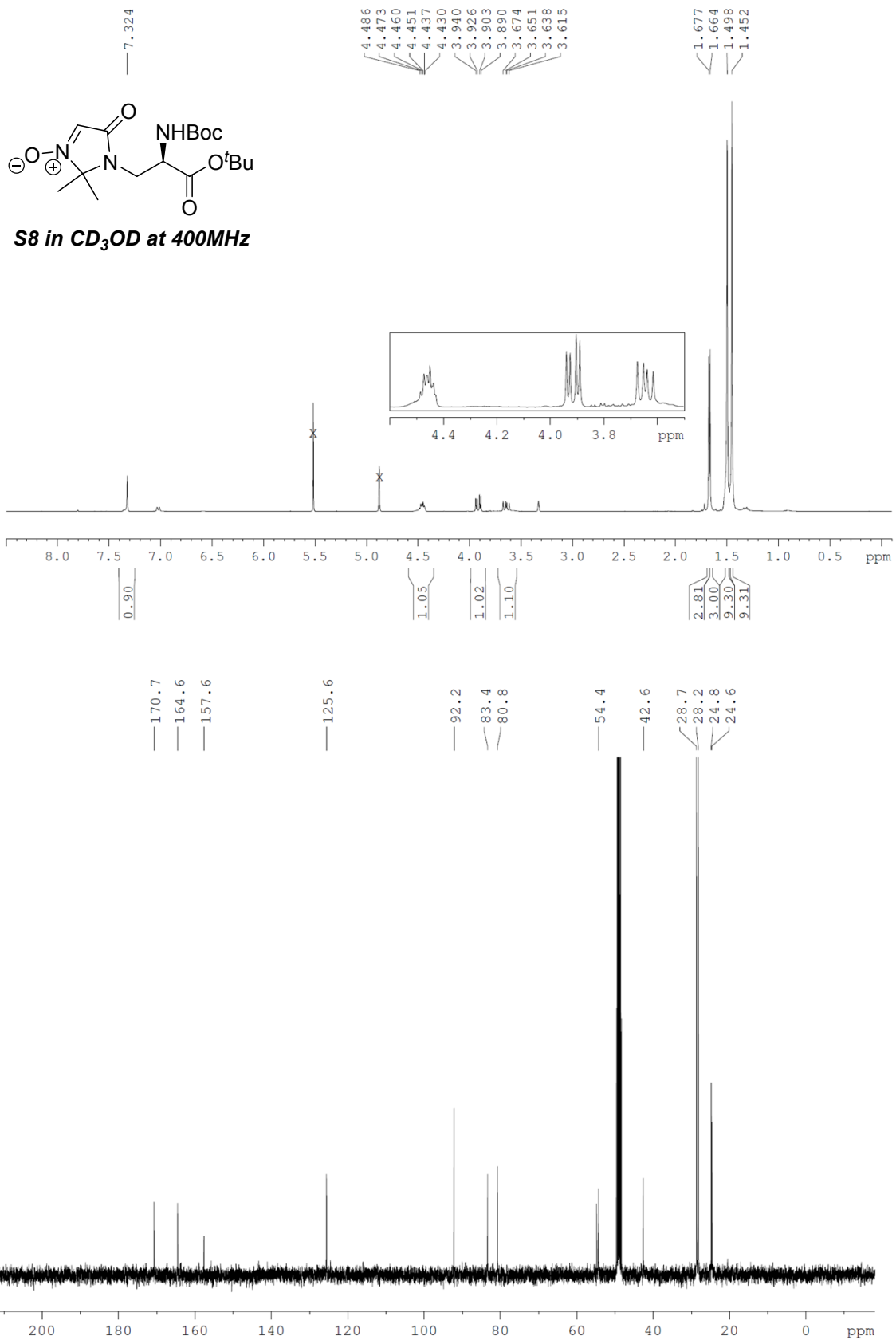
S5 in CD_3OD at 400MHz



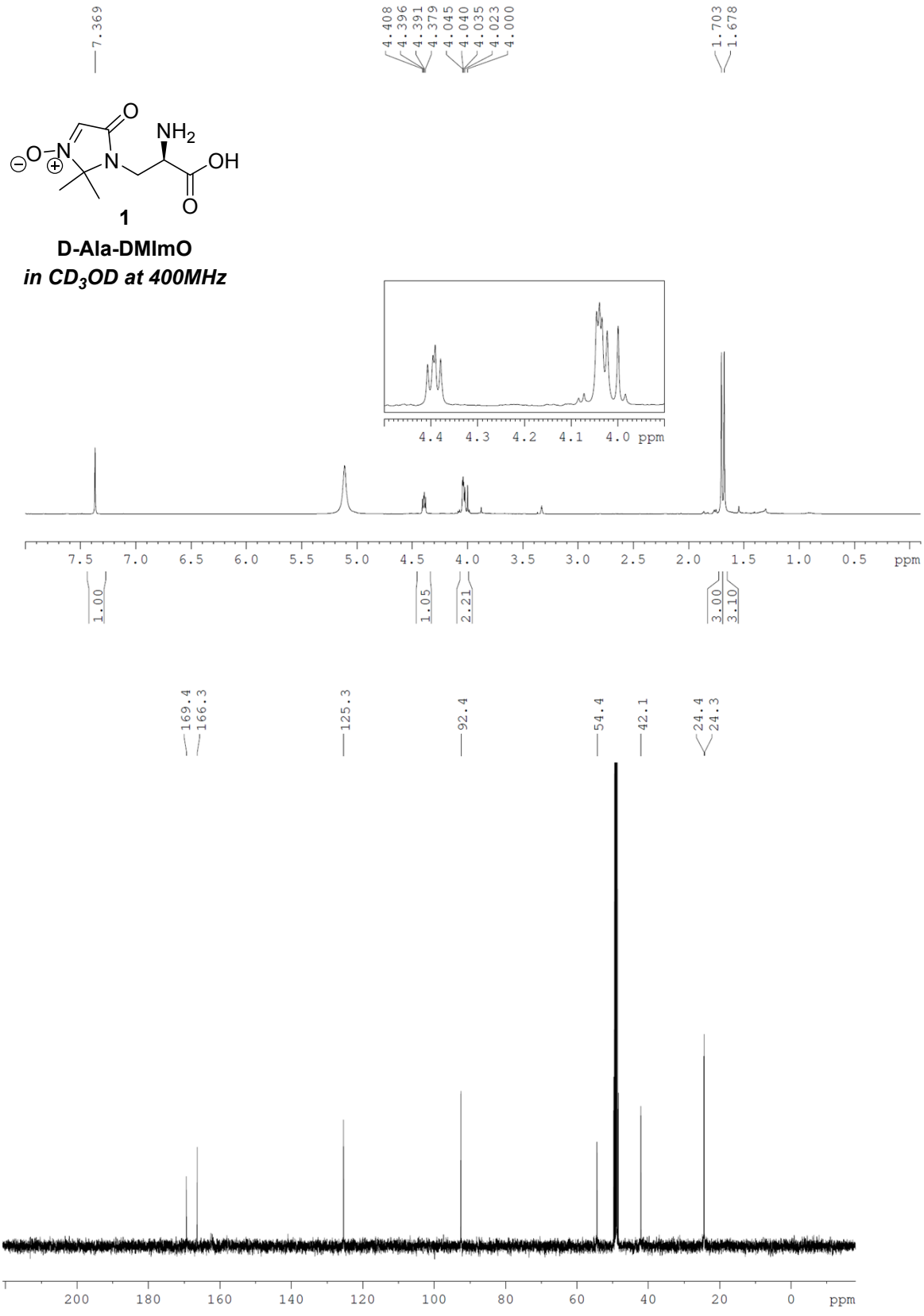
S6 in $CDCl_3$ at 400MHz

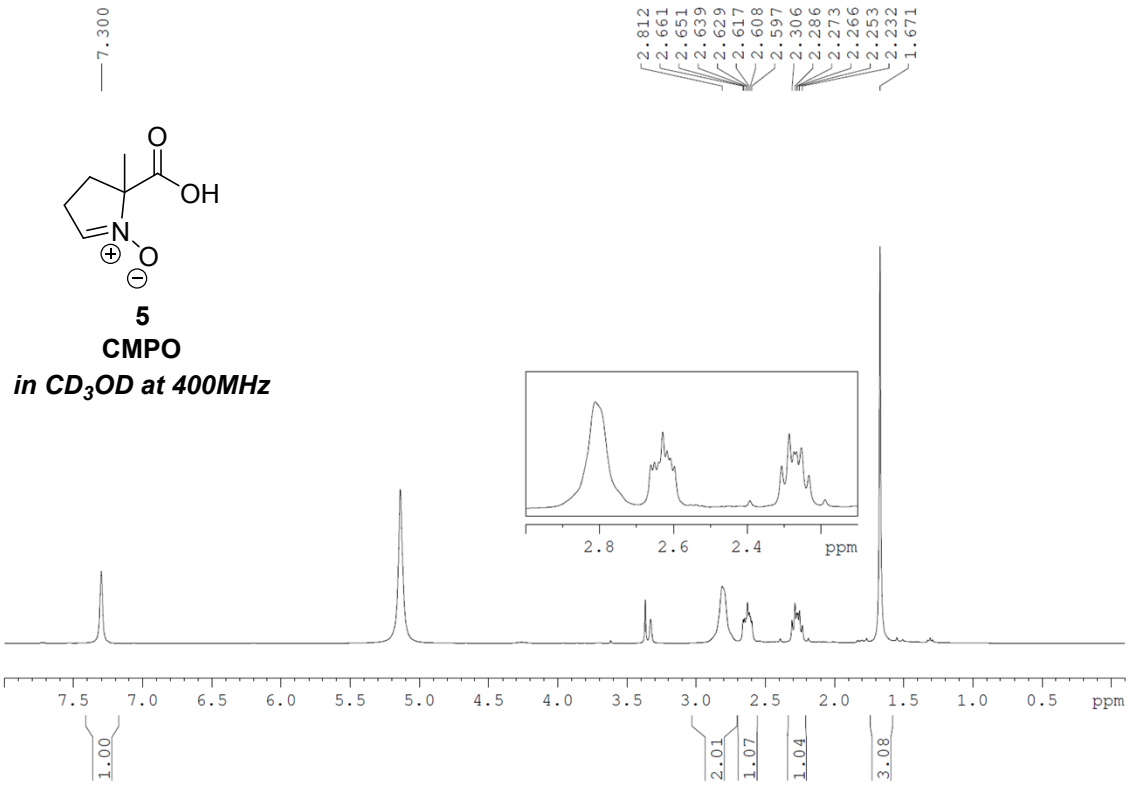


S7 in $CDCl_3$ at 400MHz



S8 in CD_3OD at 400MHz

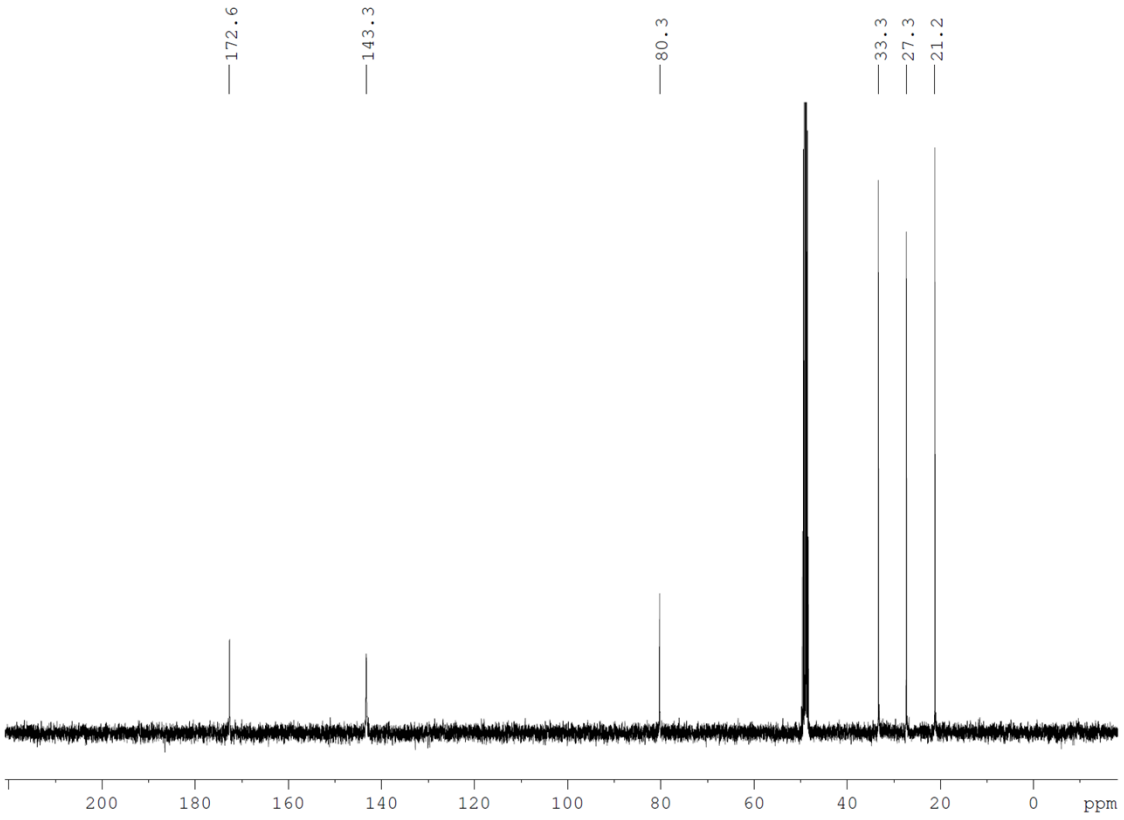

7.369
4.408
4.396
4.391
4.379
4.045
4.040
4.035
4.023
4.000
1.703
1.678
O
NH2
OH
N
O
O
1
D-Ala-DMImO
in CD3OD at 400MHz
4.4 4.3 4.2 4.1 4.0 ppm
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
1.05
2.21
3.00
3.10
169.4
166.3
125.3
92.4
54.4
42.1
24.4
24.3
200 180 160 140 120 100 80 60 40 20 0 ppm

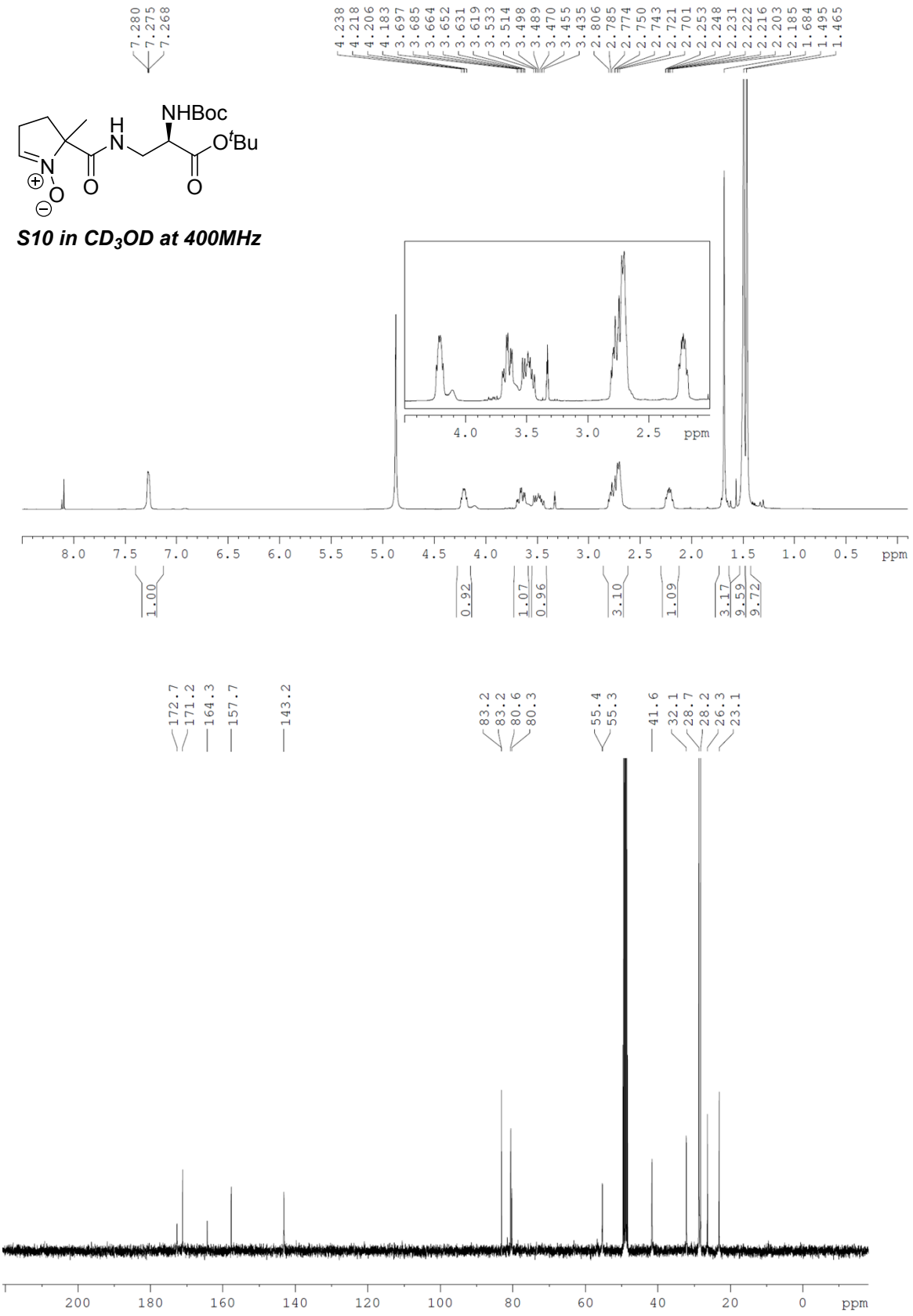


5

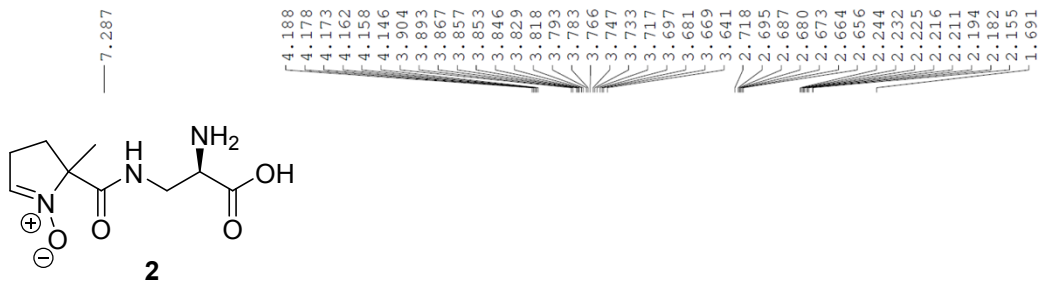
CMPO

in CD_3OD at 400MHz





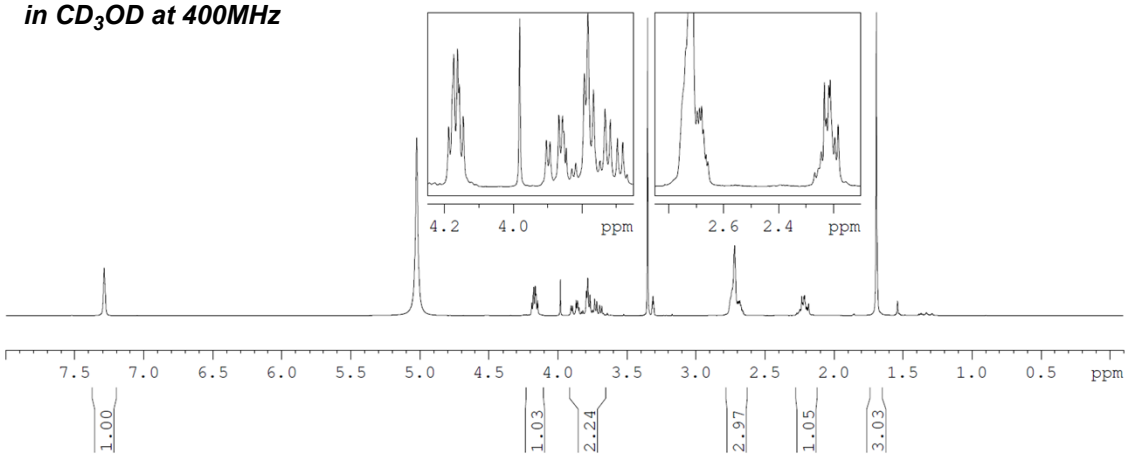
S10 in CD_3OD at 400MHz

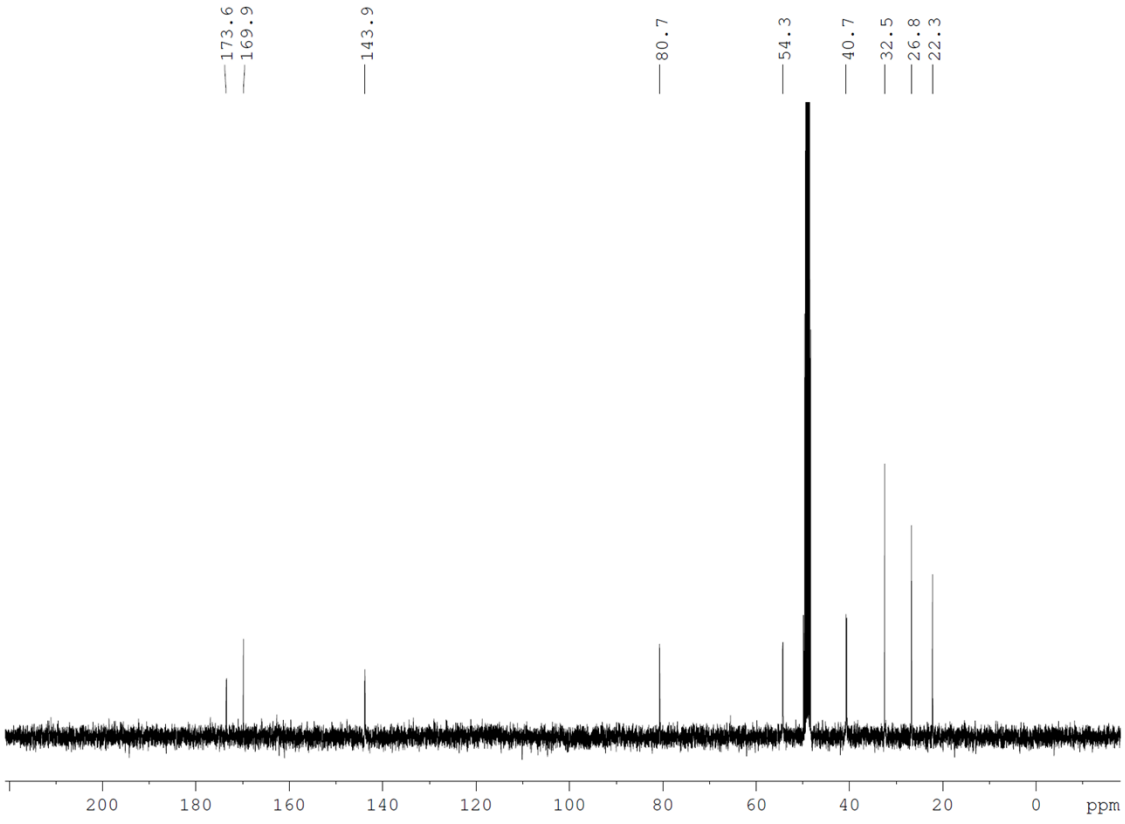


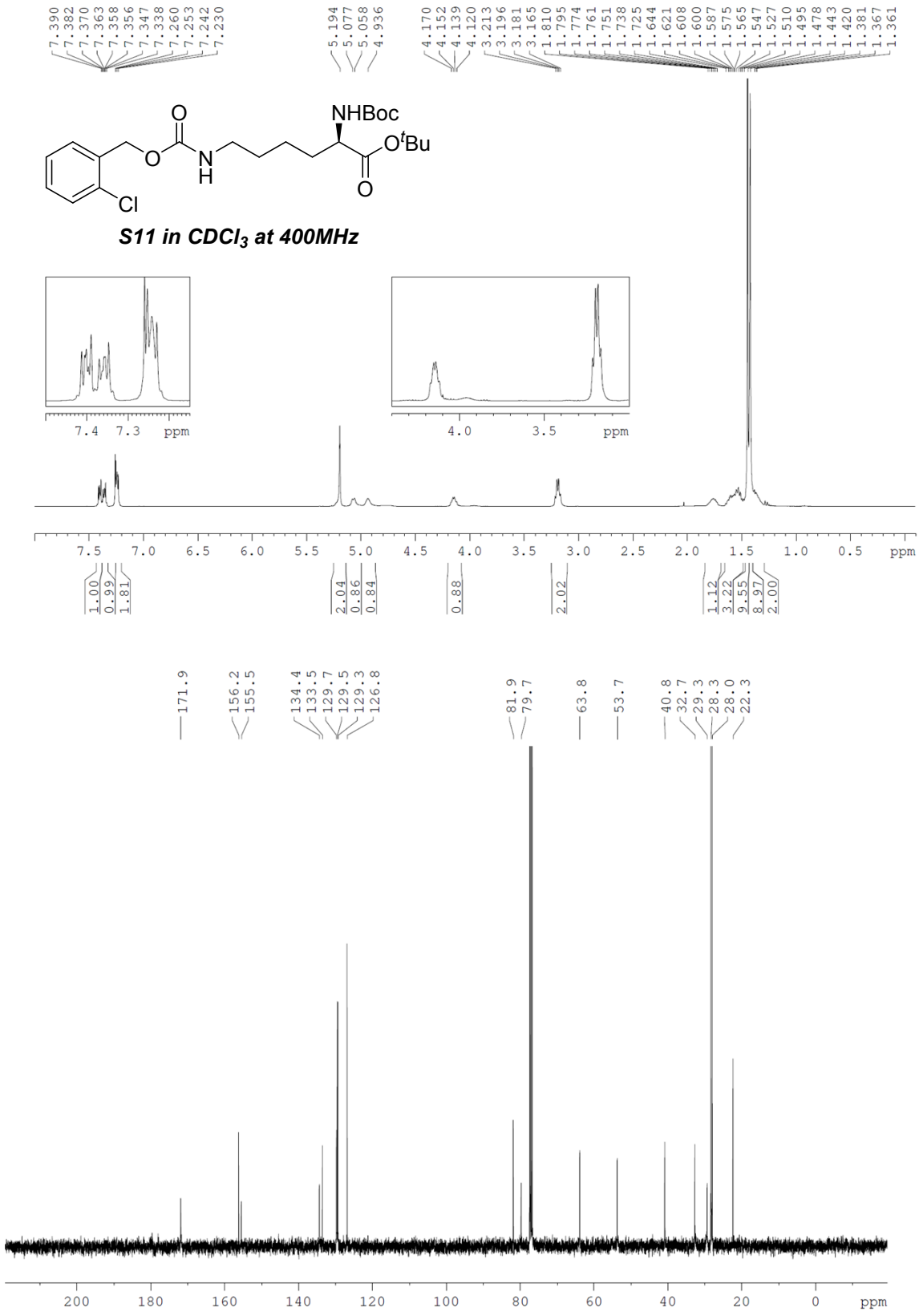
2

D-Ala-CMPO

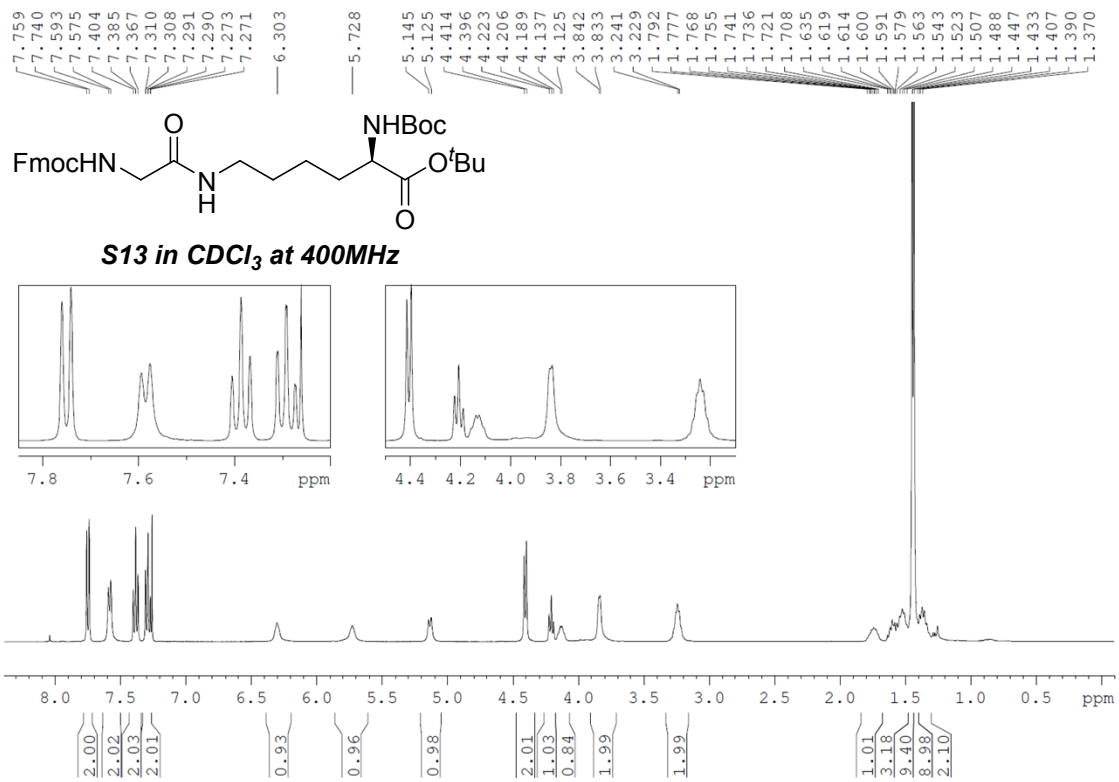
in CD_3OD at 400MHz



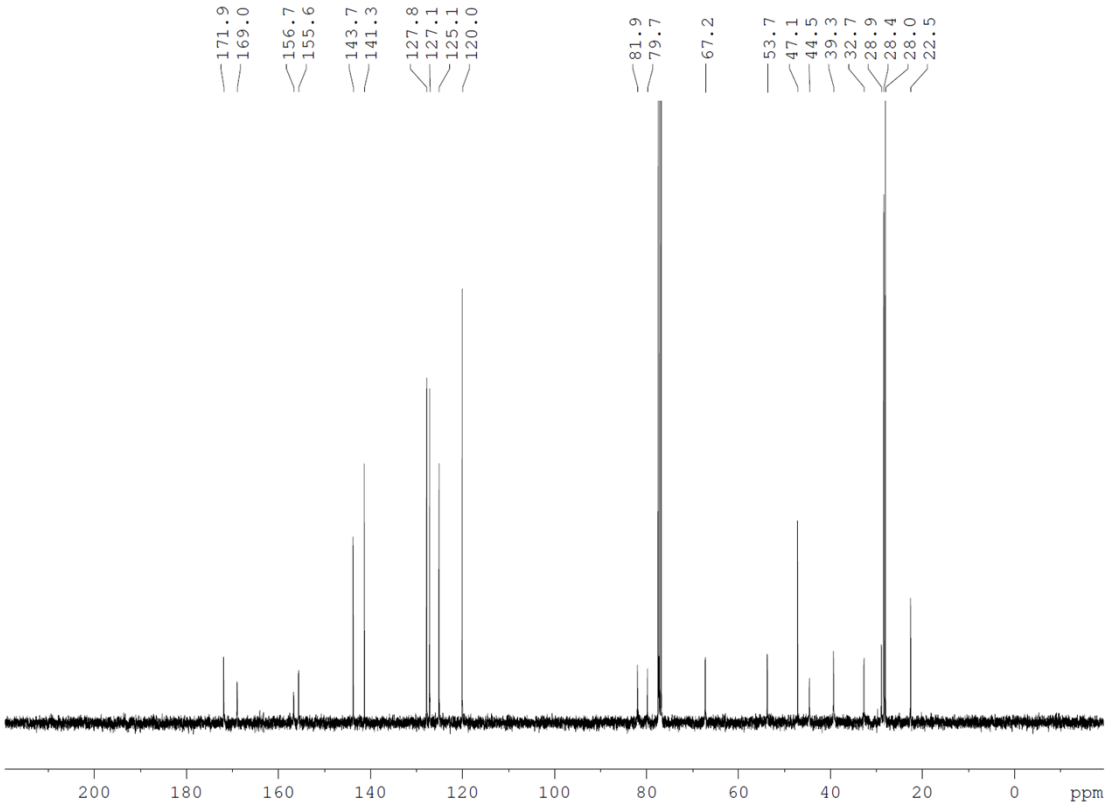


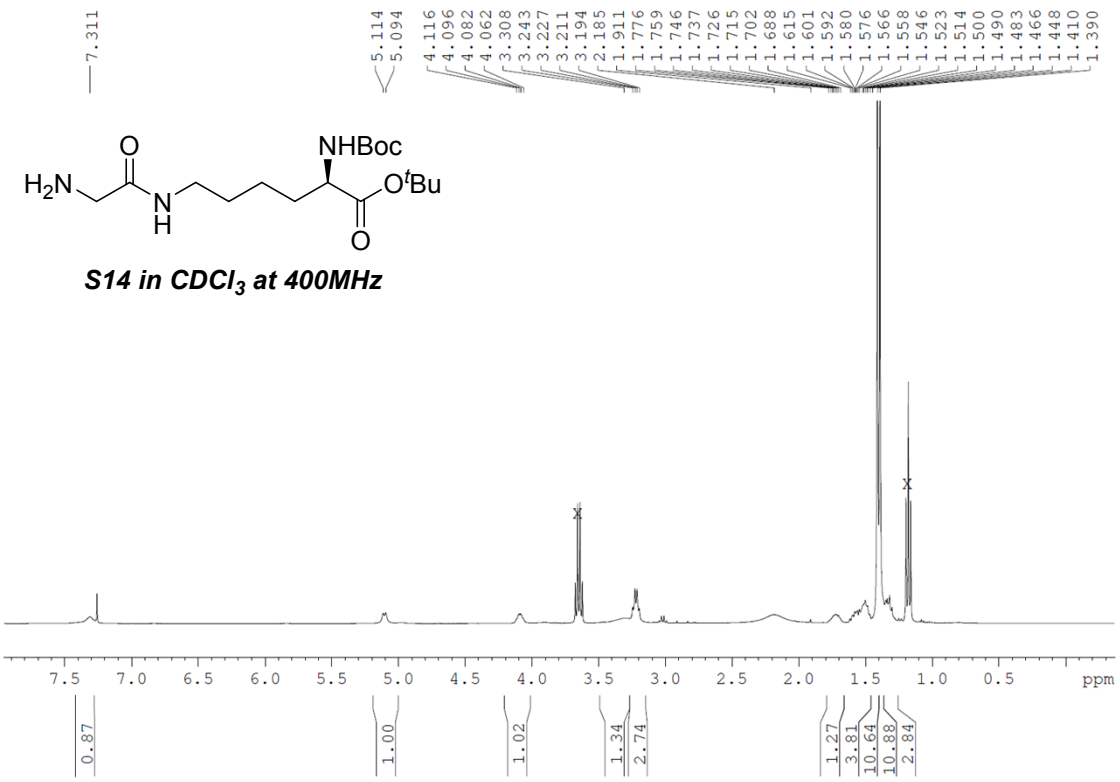


S11 in $CDCl_3$ at 400MHz

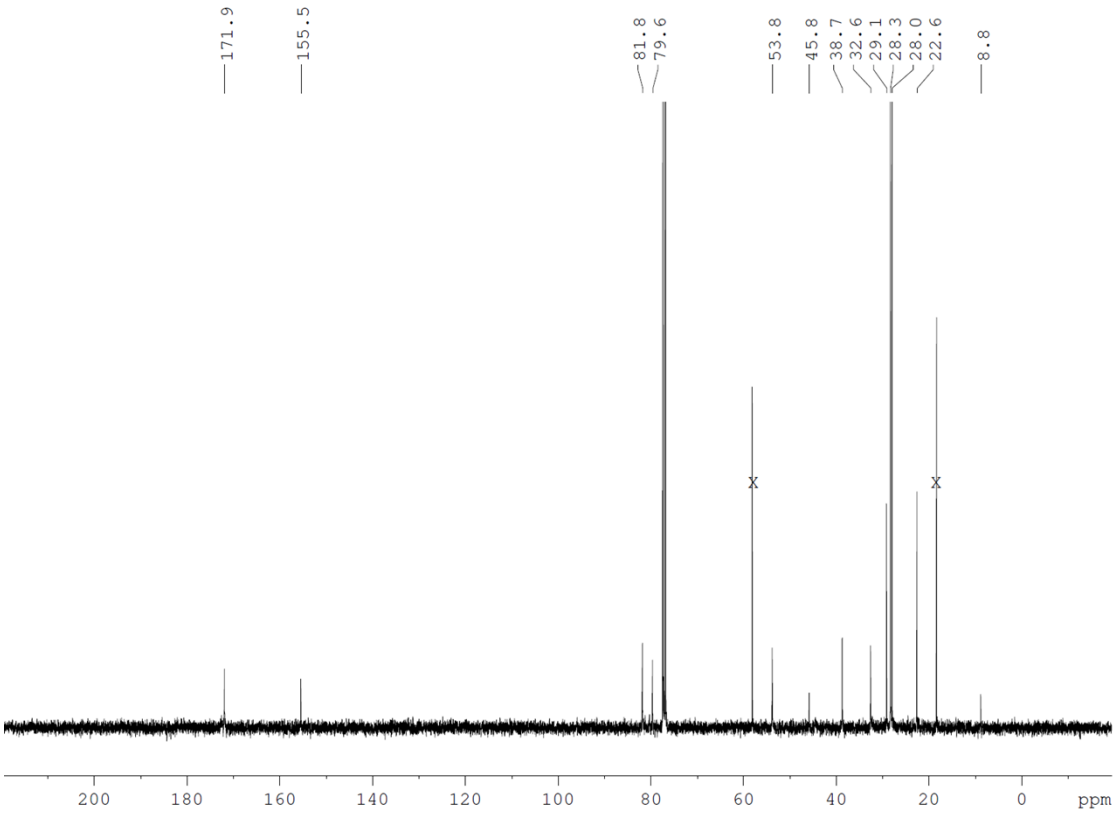


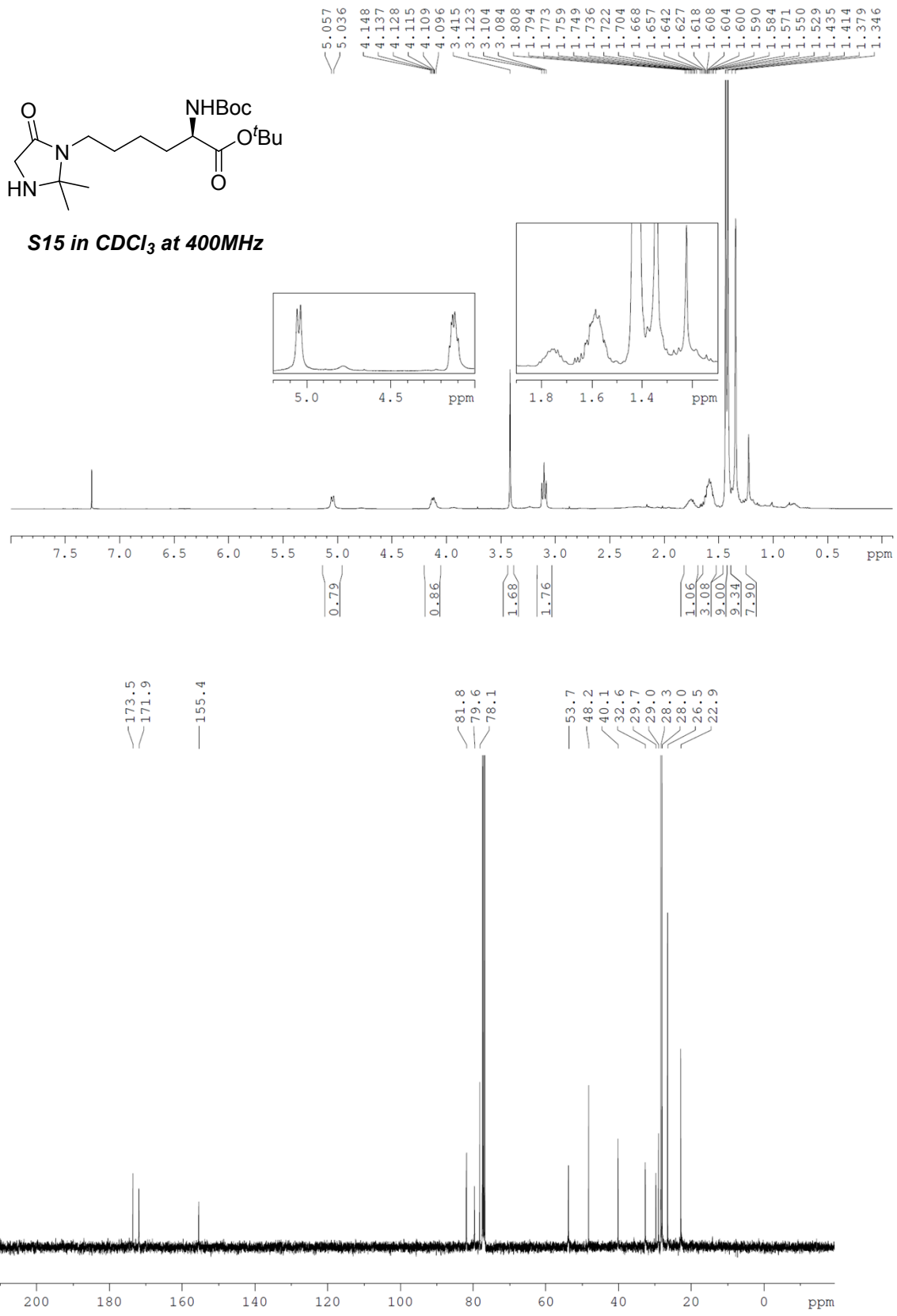
S13 in $CDCl_3$ at 400MHz



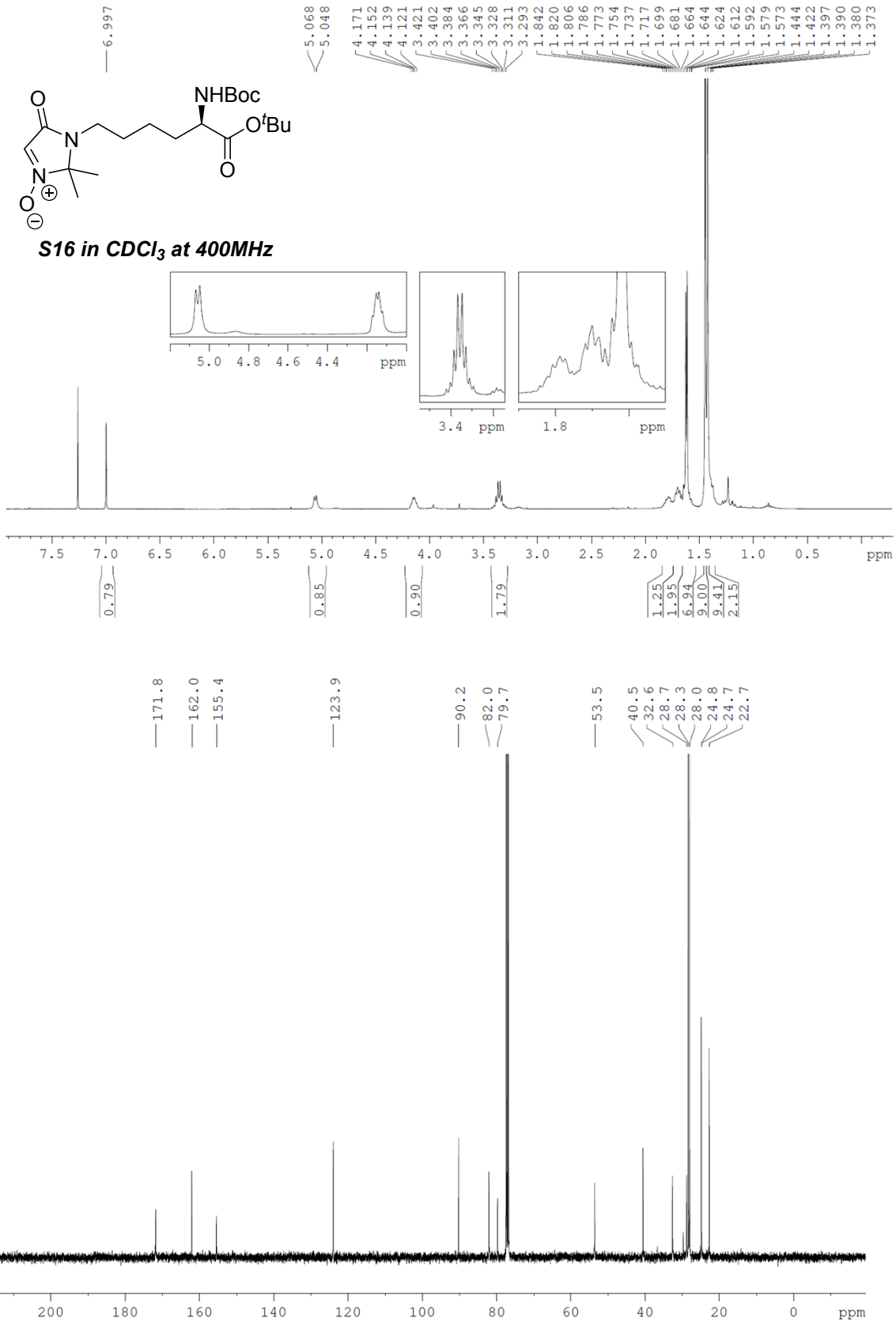


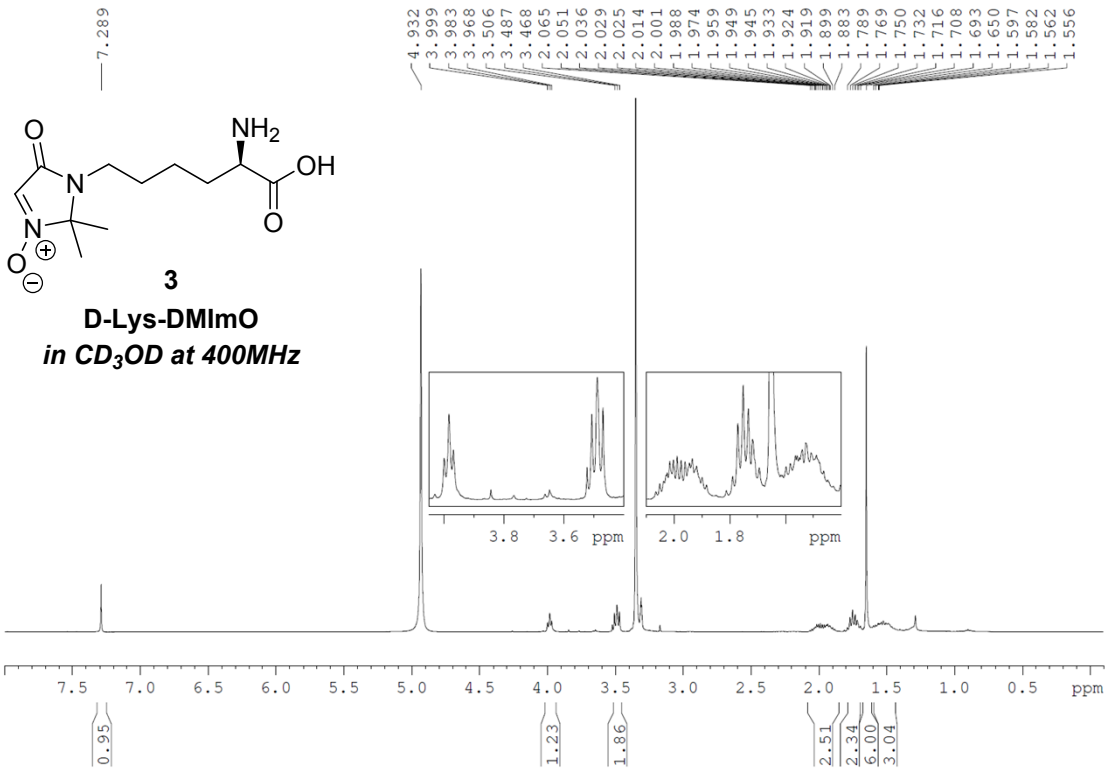
S14 in $CDCl_3$ at 400MHz

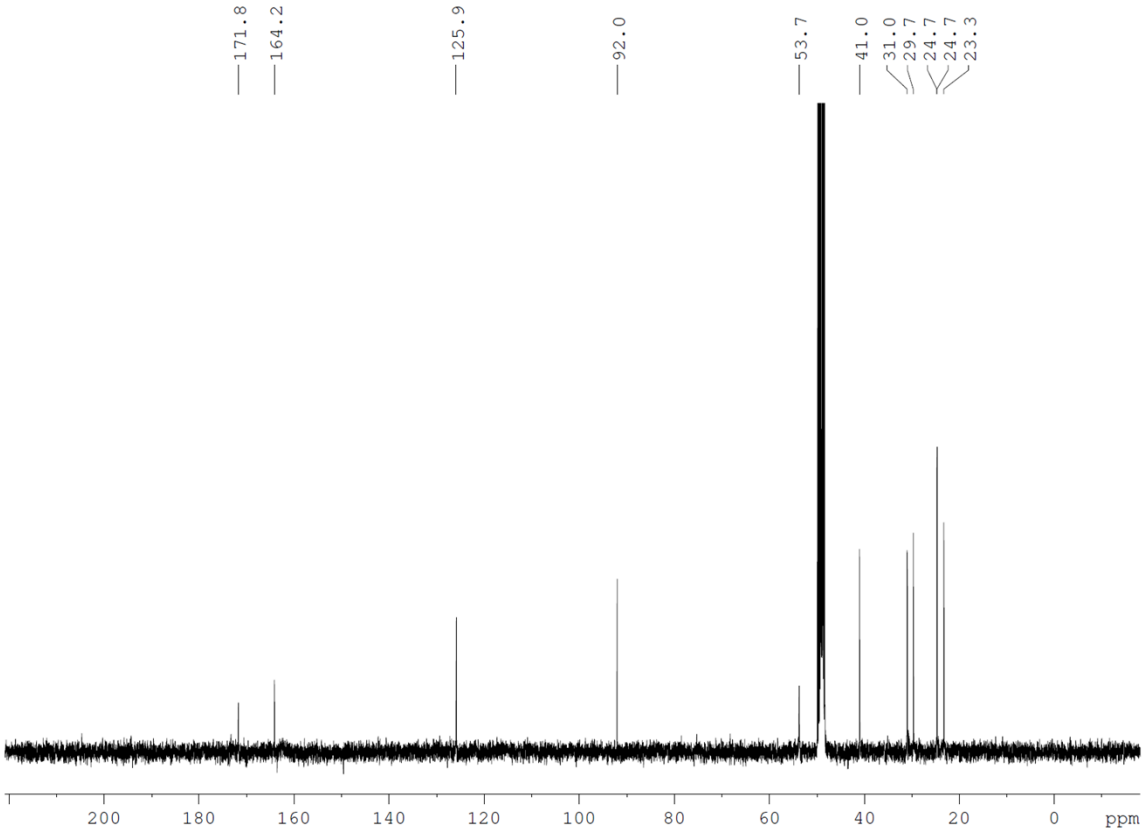


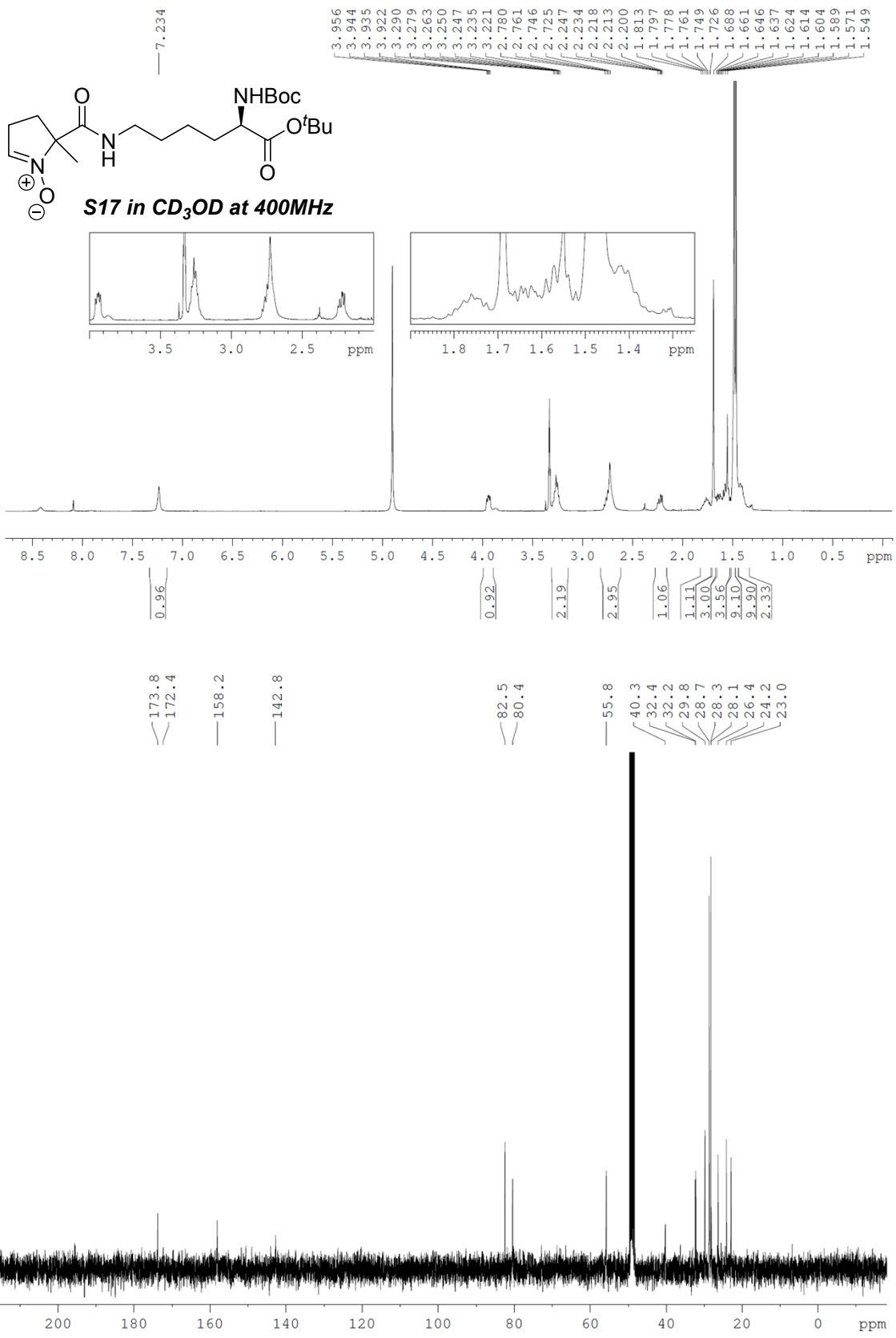


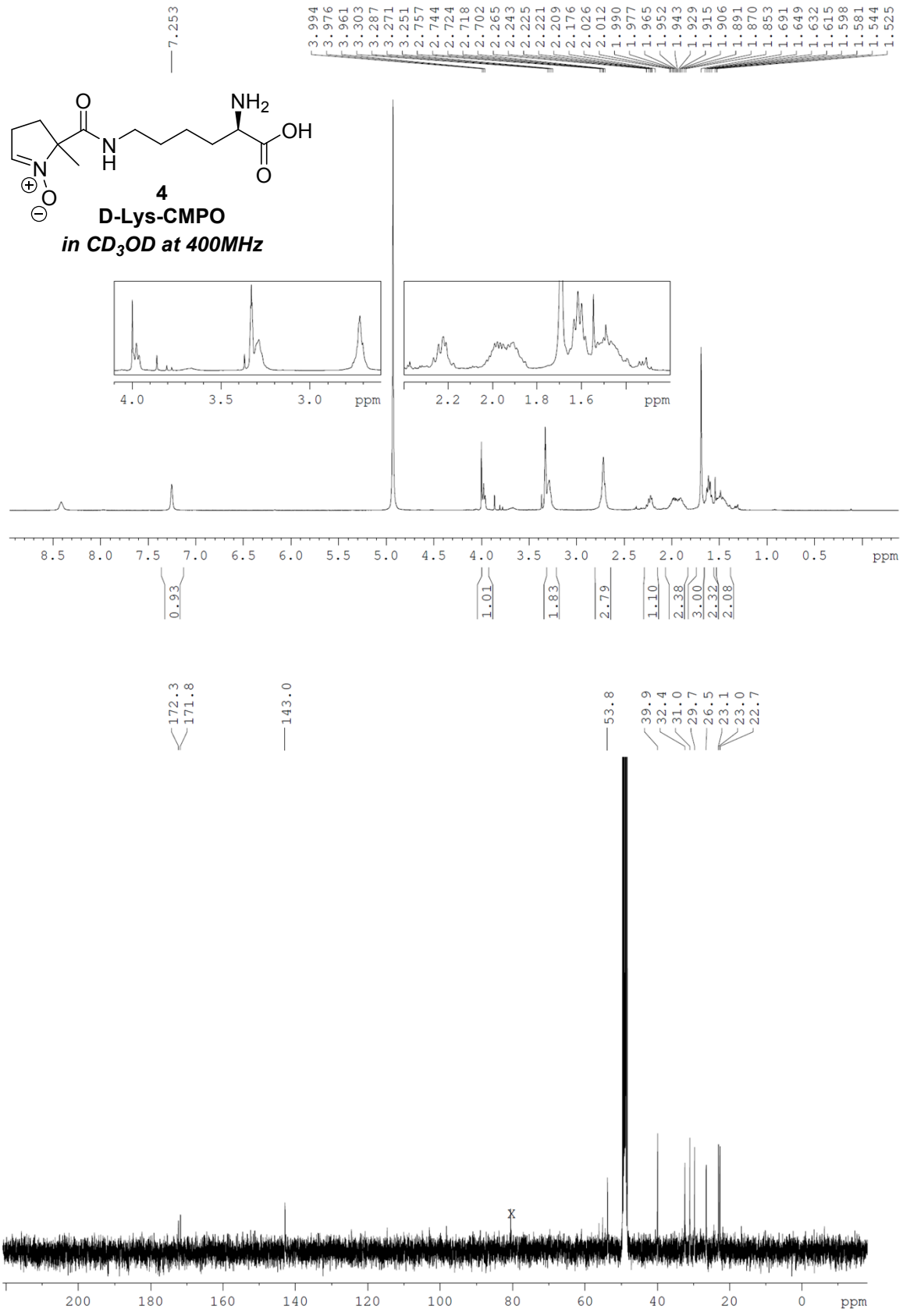
S15 in $CDCl_3$ at 400MHz


6.997
5.068
5.048
4.171
4.152
4.139
4.121
3.421
3.402
3.384
3.366
3.345
3.328
3.311
3.293
1.842
1.820
1.806
1.786
1.773
1.754
1.737
1.717
1.699
1.681
1.664
1.644
1.624
1.612
1.592
1.579
1.573
1.444
1.422
1.397
1.390
1.380
1.373
NHBoc
O^{t}Bu
S16 in CDCl$_3$ at 400MHz
5.0 4.8 4.6 4.4 ppm
3.4 ppm
1.8 ppm
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.79
0.85
0.90
1.79
1.25
1.95
6.94
9.00
9.41
2.15
171.8
162.0
155.4
123.9
90.2
82.0
79.7
53.5
40.5
32.6
28.7
28.3
28.0
24.8
24.7
22.7
200 180 160 140 120 100 80 60 40 20 0 ppm


7.289
4.932
3.999
3.983
3.968
3.506
3.487
3.468
2.065
2.051
2.036
2.029
2.025
2.014
2.001
1.988
1.974
1.959
1.949
1.945
1.933
1.924
1.919
1.899
1.883
1.789
1.769
1.750
1.732
1.716
1.708
1.693
1.650
1.597
1.582
1.562
1.556
O
NH2
OH
N
N
O
O
3
D-Lys-DMImO
in CD3OD at 400MHz
3.8
3.6
ppm
2.0
1.8
ppm
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.95
1.23
1.86
2.51
2.34
6.00
3.04


171.8
164.2
125.9
92.0
53.7
41.0
31.0
29.7
24.7
24.7
23.3
200
180
160
140
120
100
80
60
40
20
0
ppm


7.234
3.956
3.944
3.935
3.922
3.290
3.279
3.263
3.250
3.247
3.235
3.221
2.780
2.761
2.746
2.725
2.247
2.234
2.218
2.213
2.200
1.813
1.797
1.778
1.761
1.749
1.726
1.688
1.661
1.646
1.637
1.624
1.614
1.604
1.589
1.571
1.549
O
NHBoc
N
H
O^{t}Bu
O
N
O
S17 in CD3OD at 400MHz
3.5
3.0
2.5
ppm
1.8
1.7
1.6
1.5
1.4
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.96
0.92
2.19
2.95
1.06
1.11
3.00
3.56
9.10
9.90
2.33
173.8
172.4
158.2
142.8
82.5
80.4
55.8
40.3
32.4
32.2
29.8
28.7
28.3
28.1
26.4
24.2
23.0
200
180
160
140
120
100
80
60
40
20
0
ppm


7.253
3.994
3.976
3.961
3.303
3.287
3.271
3.251
2.757
2.744
2.724
2.718
2.702
2.265
2.243
2.225
2.221
2.209
2.176
2.026
2.012
1.990
1.977
1.965
1.952
1.943
1.929
1.915
1.906
1.891
1.870
1.853
1.691
1.649
1.632
1.615
1.598
1.581
1.544
1.525
O
NH2
OH
N
H
N
O
O
4
D-Lys-CMPO
in CD3OD at 400MHz
4.0
3.5
3.0
ppm
2.2
2.0
1.8
1.6
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.93
1.01
1.83
2.79
1.10
2.38
3.00
2.32
2.08
172.3
171.8
143.0
53.8
39.9
32.4
31.0
29.7
26.5
23.1
23.0
22.7
200
180
160
140
120
100
80
60
40
20
0
ppm

References

(1) Dai, X.; Miller, M. W.; Stamford, A. W. *Org. Lett.* **2010**, *12*, 2718.
(2) Hodges, J. C.; Wang, W.; Riley, F. *J. Org. Chem.* **2004**, *69*, 2504.
(3) Tsai, P.; Ichikawa, K.; Mailer, C.; Pou, S.; Halpern, H. J.; Robinson, B. H.; Nielsen, R.; Rosen, G. M. *J. Org. Chem.* **2003**, *68*, 7811.