Supplementary Data

Hydropropidine: A novel, cell-impermeant fluorogenic probe for detecting extracellular superoxide

Radoslaw Michalski, Jacek Zielonka, Micael Hardy, Joy Joseph,
and Balaraman Kalyanaraman

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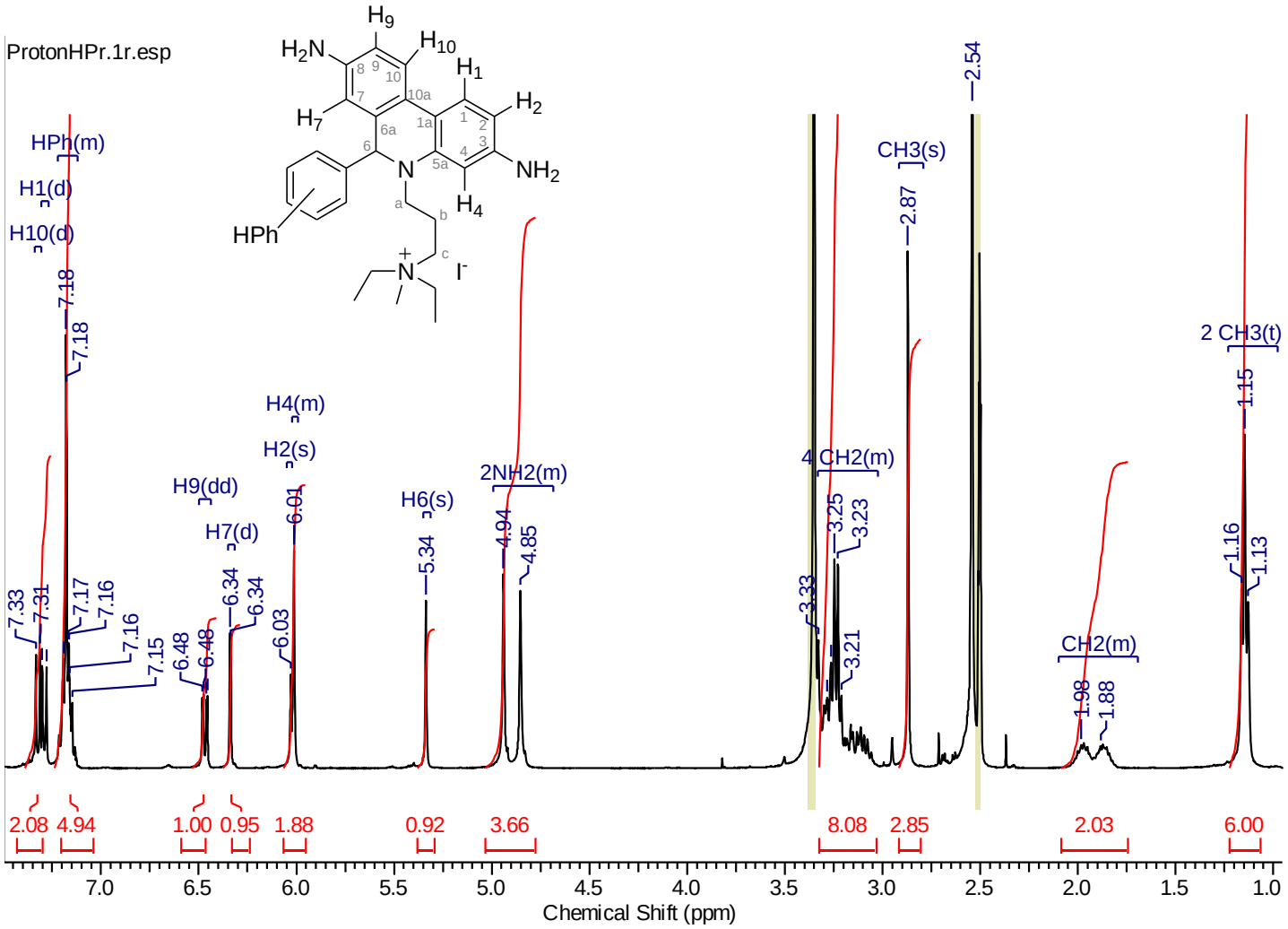
Supplementary Figures legends.

Supplementary Figure 1: NMR spectra of hydropropidine (HPr^+) in DMSO-*d6*. (a) ^{1}H NMR, (b) ^{13}C APT, (c) HSQC, (d) HMBC. **^{1}H NMR** (400.13 MHz): δ 7.32 (H_{10}, d, *J* = 8.3), 7.29 (H_1, d, *J* = 8.8), 7.22-7.12 (5H, m), 6.47 (H_9, d, *J* = 8.3, 2.3), 6.34 (H_7, d, *J* = 2.3), 6.03 (H_2, m, , *J* = 8.5), 6.01 (H_4, br.s), 5.34 (H_6, s), 4.94 (2H (NH_2), s), 4.85 (2H (NH_2), s), 3.32-3.04 (8H, m), 2.87 (3H, s), 2.07-1.81 (2H, m), 1.15, (6H, t, *J* = 6.9). **^{13}C APT** (75.47 MHz): δ 148.6 (C, s) 146.7, (C, s), 143.9 (C, s), 143.4 (C_{5a}, s), 135.0 (C_{6a}, s), 128.6 ($2C_{Ph}$, s), 127.4 (C_{Ph}, s), 126.8 ($2C_{Ph}$, s), 123.0 (C_1, s), 122.2 (C_{10}, s), 120.0 (C_{10a}, s), 114.3 (C_9, s), 112.7 (C_{1a}, s), 111.9 (C_7, s), 104.7 (C_2, s), 98.5 (C_4, s), 65.5 (C_6, s), 57.8 (C, CH_2, s), 56.2 (2C, CH_2, s), 47.0 (C, CH_3), 46.2 (C, CH_2), 20.2, (CH_2, s), 7.8 ($2CH_3$, s). HRMS calculated for $C_{27}H_{35}N_4I_2$ $[C_{27}H_{35}N_4]^+$ 415.2856, found 415.2858.

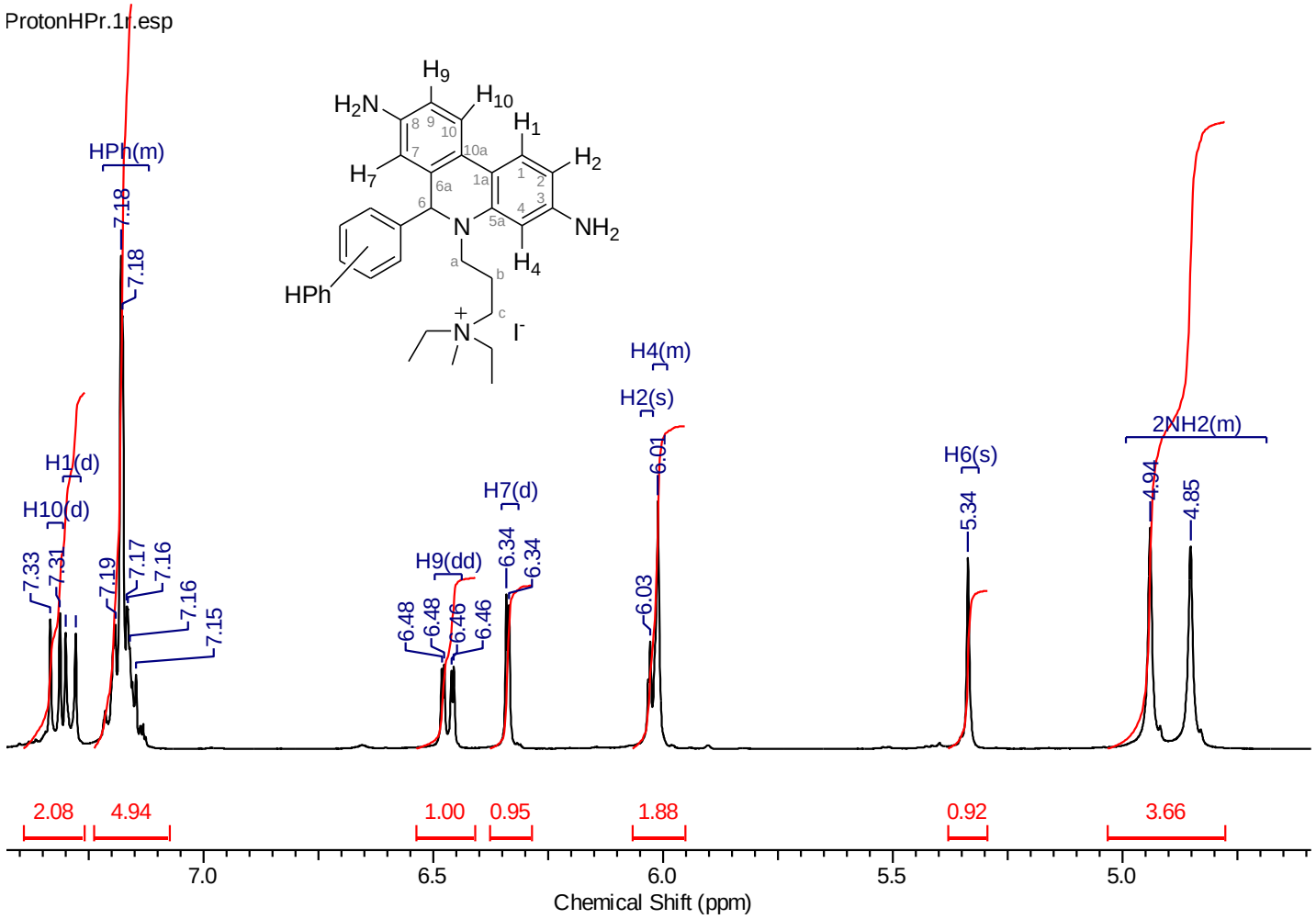
Supplementary Figure 2: NMR spectra of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6*. (a) ^{1}H NMR, (b) ^{13}C APT, (c) HSQC, (d) HMBC. **^{1}H NMR** (400.13 MHz): δ 8.35 (H_{10}, d, *J* = 9.3), 8.01 (H_1, s), 7.82-7.69 (5H, m), 7.54 (H_4, s), 7.52 (H_9, d, *J* = 9.0, 2.5), 6.25 (H_7, d, *J* = 2.3), 4.44 (2H, br.t), 3.21 (6H, ($3CH_2$) br.q), 2.84 (3H, s), 2.20 (2H, bquint.), 1.13, (6H, t, *J* = 7.2). **^{13}C APT** (75.47 MHz): δ 153.9 (C^{IV}, s), 148.3 (C^{IV}, s), 148.0 (C^{IV}, s), 142.8, (C^{IV}, s), 132.2 (C^{IV}, s), 131.9 (C^{IV}, s), 130.8 (C_{Ph}, s), 129.5 ($2C_{Ph}$, s), 128.7 ($2C_{Ph}$, s), 128.5 (C, s), 127.5 (C_9, s), 125.1 (C_{10a}, s), 122.5 (C_{10}, s), 119.2 (C_{1a}, s), 107.4 (C_7, s), 103.8 (C_1, s), 98.6 (C_4, s), 56.0 (2C, CH_2, s), 55.4 (C, CH_2), 50.2, (CH_{2a}, s), 46.6 (C, CH_3), 21.4 (CH_{2b}, s), 7.4 ($2CH_3$, s). HRMS calculated for $C_{27}H_{34}N_4I_2O[C_{27}H_{34}N_4O]^{++}$ 215.1360, found 215.1360.

Supplementary Figure 3: NMR spectra of propidium (Pr^{++}) in DMSO-*d6*. (a) ^{1}H NMR, (b) ^{13}C APT, (c) HSQC, (d) HMBC. **^{1}H NMR** (400.13 MHz): δ 8.70 (H_1, d, *J* = 9.3), 8.64 (H_{10}, d, *J* = 9.3), 7.82-7.73 (5H, m), 7.56 (H_4, d, *J* = 1.5), 7.54 (H_9, d, *J* = 9.3, 2.3), 7.36 (H_2, dd, *J* = 9.3, 1.5), 6.38 (2H (NH_2), s), 6.26 (H_7, d, *J* = 2.5), 5.99 (2H, (NH_2), s), 4.44 (2H, br.t), 3.23 (6H, ($3CH_2$) br.q), 2.89 (3H, s), 2.22 (2H, br.quint.), 1.14, (6H, t, *J* = 7.0). **^{13}C APT** (75.47 MHz): δ 158.7 (C_6, s), 151.2 (C_3, s), 148.0 (C_8, s), 134.2 (C_{5a}, s), 131.9 ($1C^{IV}$, s), 130.9 (1C, s), 129.5 (2C, s), 128.3 (2C, s), 128.2 (C_9, s), 127.6 ($1C^{IV}$, s), 125.0 ($1C^{IV}$, s), 124.8 (C_1, s), 122.7 (C_{10}, s), 120.0 (C_2, s), 117.5 (C_{1a}, s), 107.8 (C_7, s), 98.1 (C_4, s), 56.0 (2C, CH_2, s), 55.5 (C, CH_2), 50.2, (CH_{2a}, s), 46.7 (C, CH_3), 21.2 (CH_{2b}, s), 7.6 ($2CH_3$, s). HRMS calculated for $C_{27}H_{34}N_4I_2$ $[C_{27}H_{34}N_4]^{++}$ 207.1386, found 207.1385.

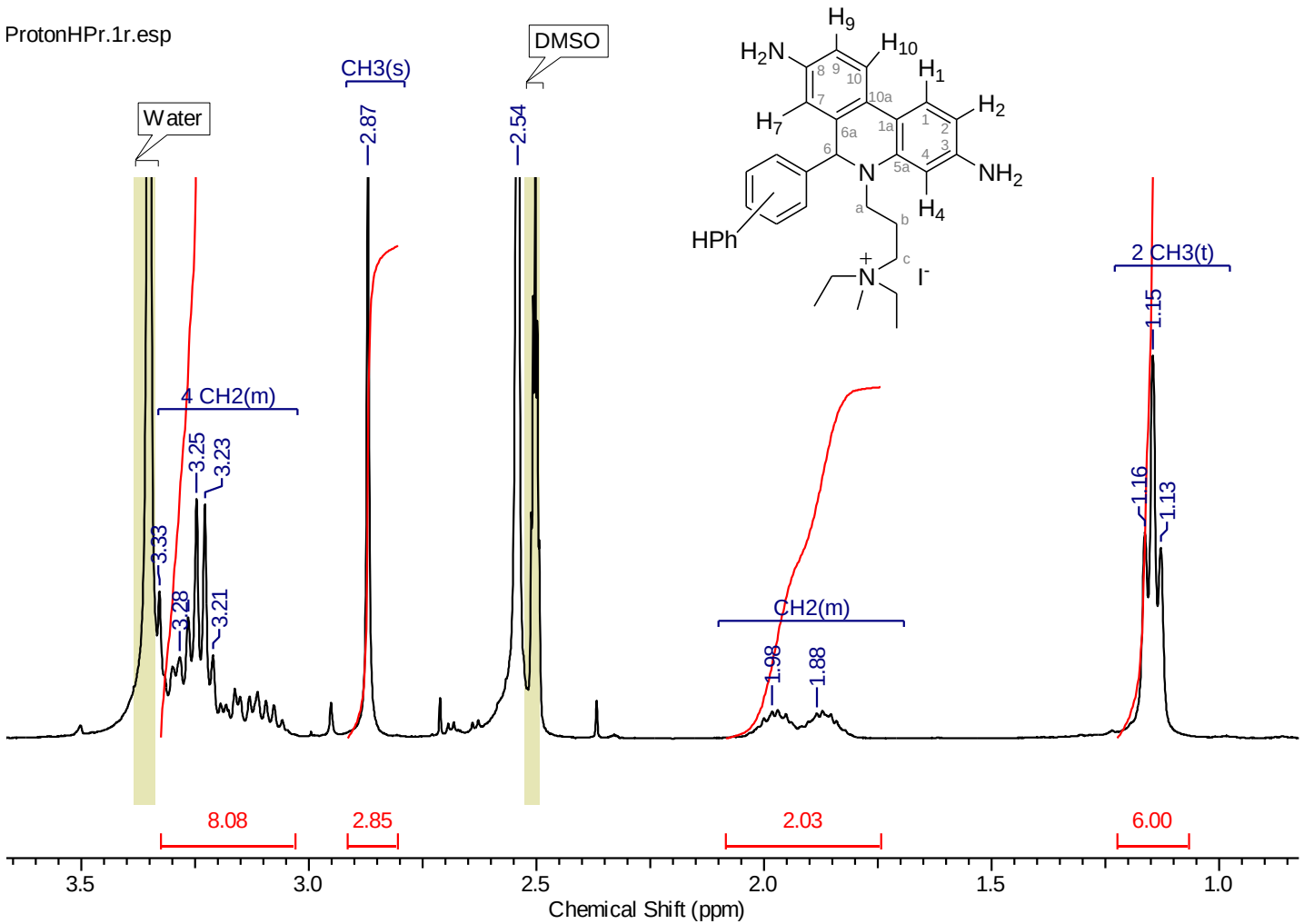
Supplementary Figure 4: NMR spectra of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6*. (a) ^{1}H NMR, (b) ^{13}C APT, (c) HSQC, (d) HMBC. **^{1}H NMR** (400.13 MHz): δ 8.70 (H_{10}, d, *J* = 9.0), 8.69 (H_1, s), 7.87-7.78 (5H, m), 7.73 (H_4, d, *J* = 6.5), 7.54 (H_9, d, *J* = 9.0, 2.1), 6.34 (H_7, d, *J* = 2.1), 4.53 (2H, br.t), 3.25 (6H, ($3CH_2$) br.q), 2.88 (3H, s), 2.28 (2H, br.quint.), 1.15, (6H, t, *J* = 7.1). **^{13}C APT** (75.47 MHz): δ 159.4 (C, s), 158.1 (C, s), 157.8 (C, s), 148.9 (C, s), 148.5 (C, s), 134.0 (C, s), 131.7 (C, s), 131.2 (C_{Ph}, s), 129.6 (C_{Ph}, s), 128.3 (C_4, s), 128.2 (C_9, s), 127.6 (C, s), 127.3 (C, s), 126.2 (C_1, s), 125.2 (C, s), 123.2 (C_{10}, s), 117.9 (C, s), 108.1 (C_7, s), 100.1 (C, s), 56.0 (2C, CH_2, s), 55.4 (C, CH_2), 50.4 (CH_2, s), 46.7 (CH_3), 21.3 (CH_2, s), 7.4 ($2CH_3$, s).



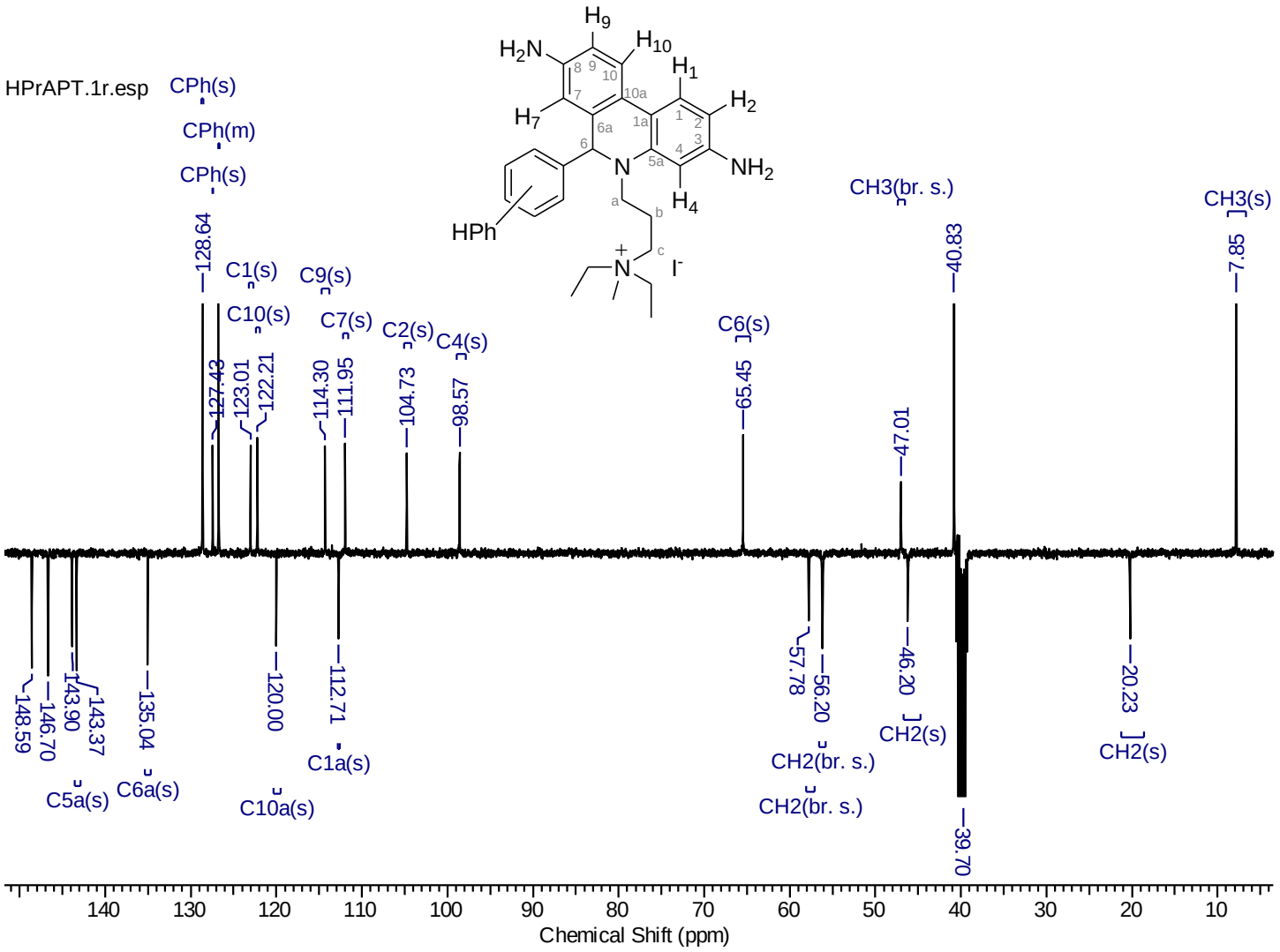
Supplementary Figure 1a. ^{1}H NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6*



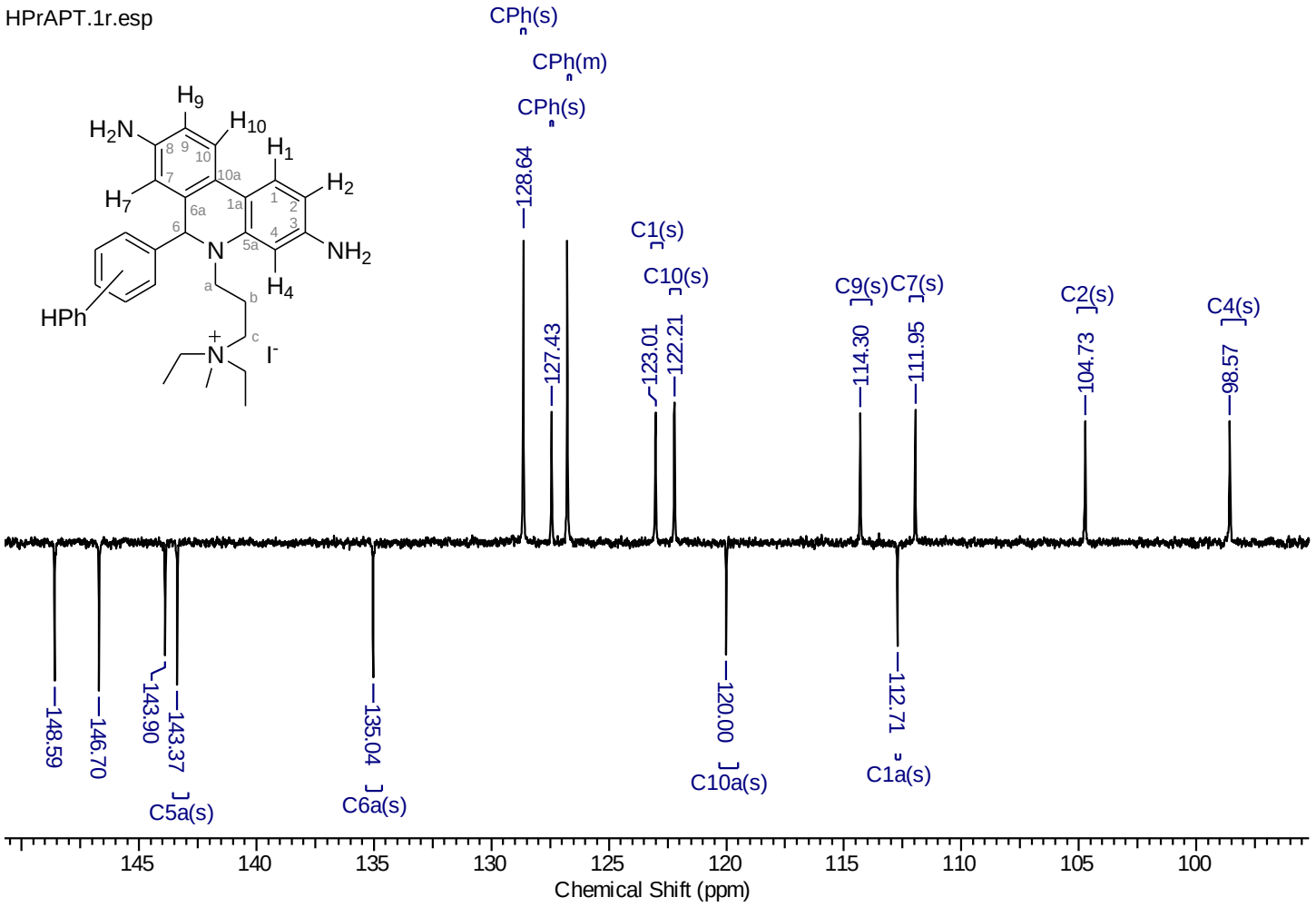
Supplementary Figure 1a. ^{1}H NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed region 4.5 – 7.5 ppm).



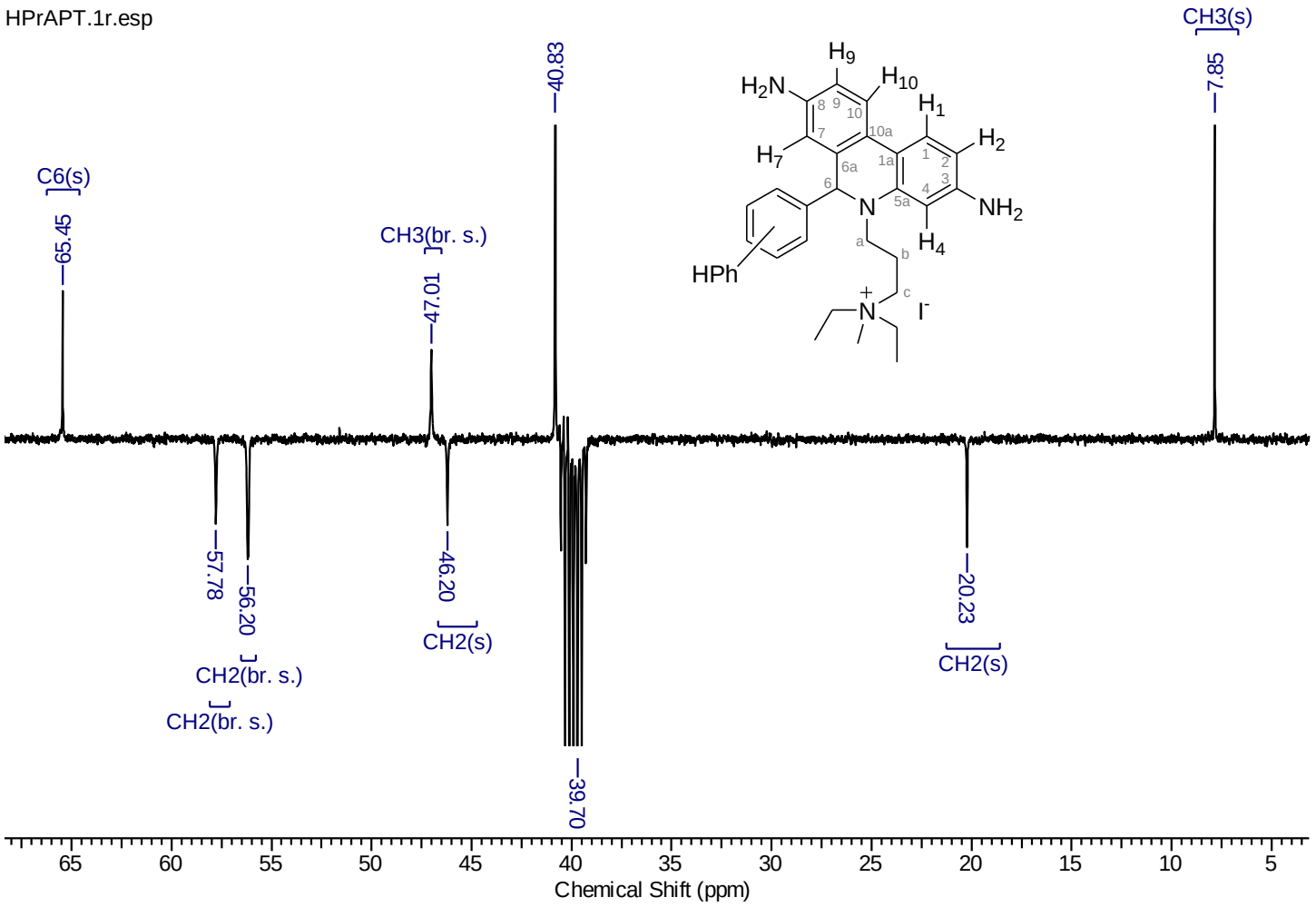
Supplementary Figure 1a. ^{1}H NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed region 0.8 – 3.2 ppm).



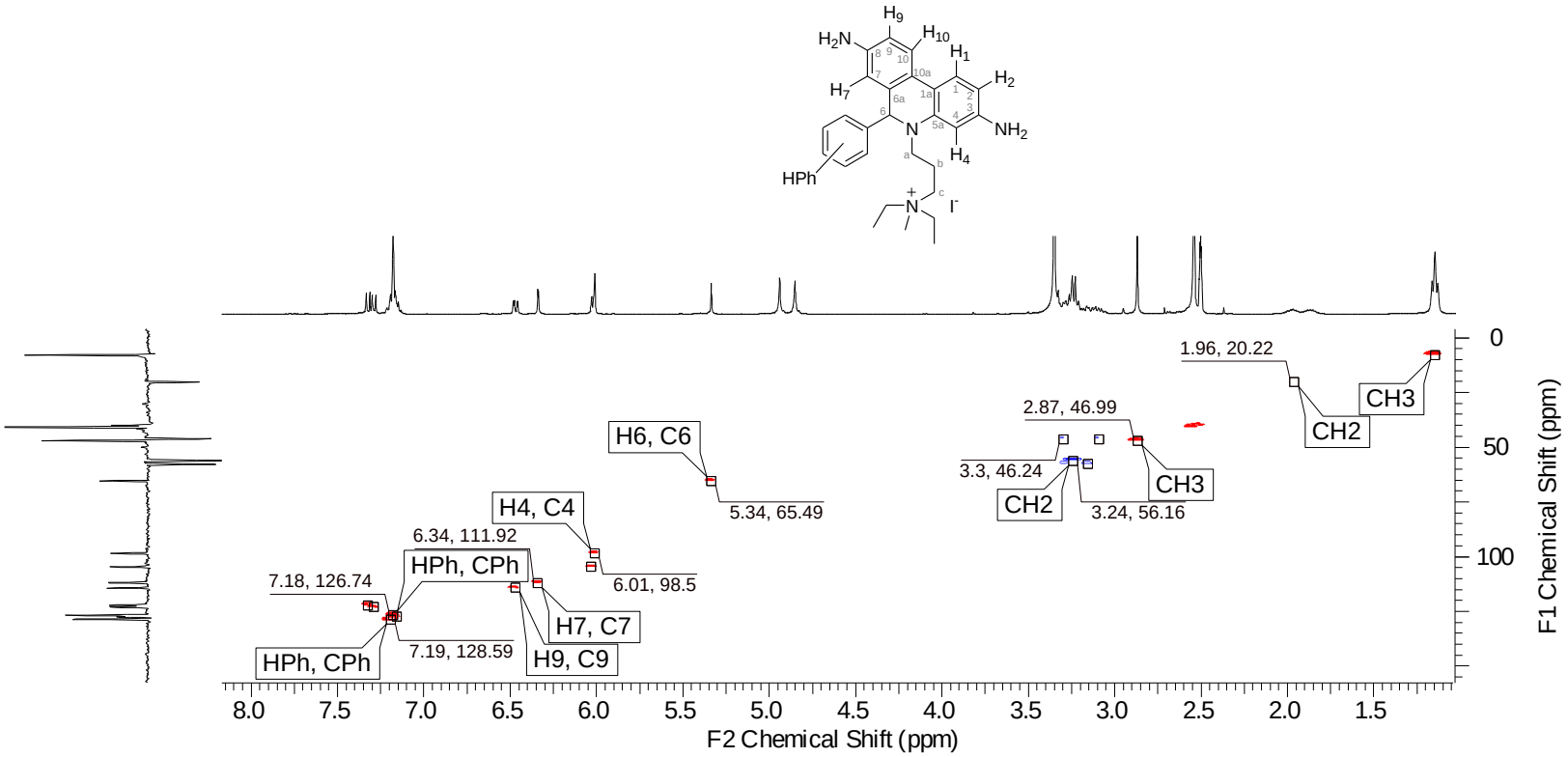
Supplementary Figure 1b. ^{13}C APT NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6*.



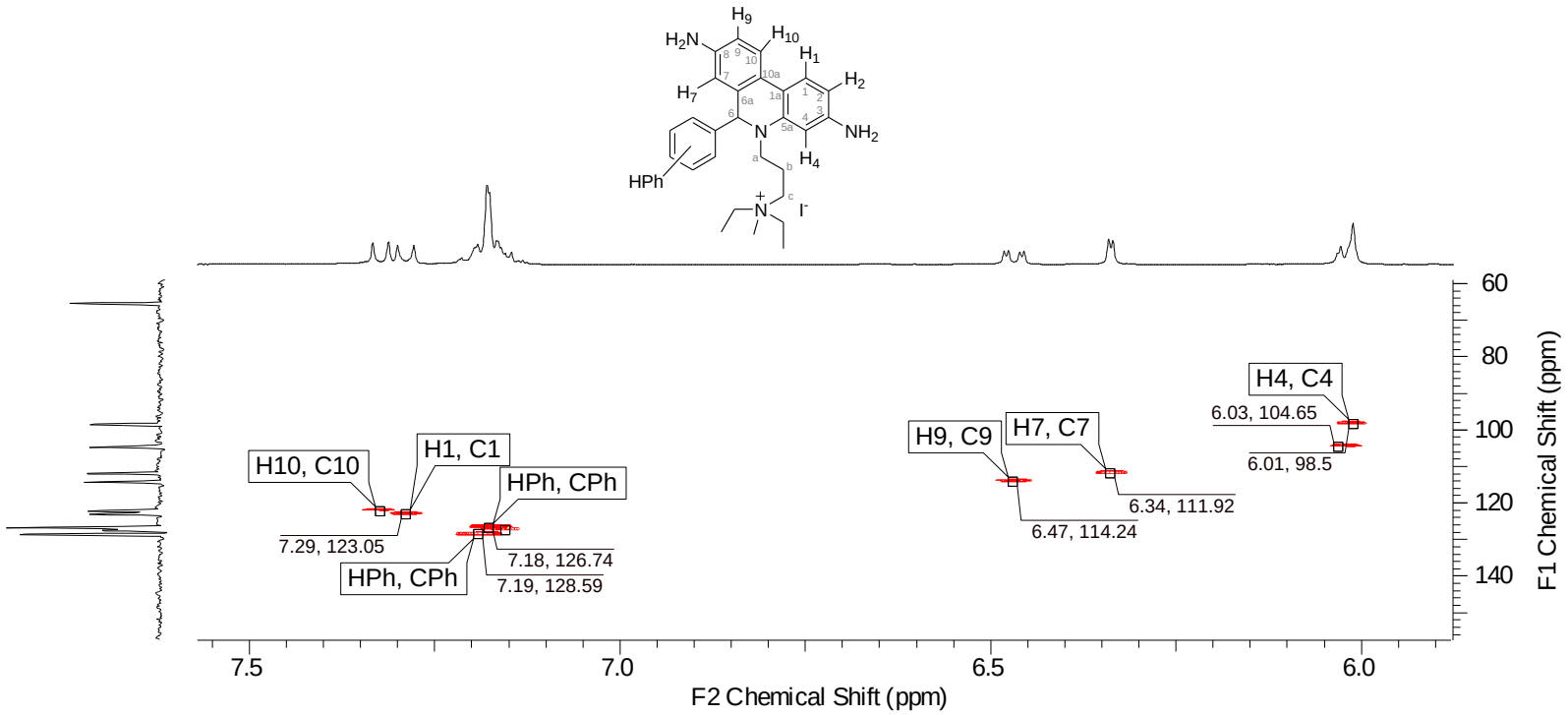
Supplementary Figure 1b. ^{13}C APT NMR spectrum of hydropropidine (HPr^+) in DMSO-*d6* (zoomed region 95 – 150 ppm).



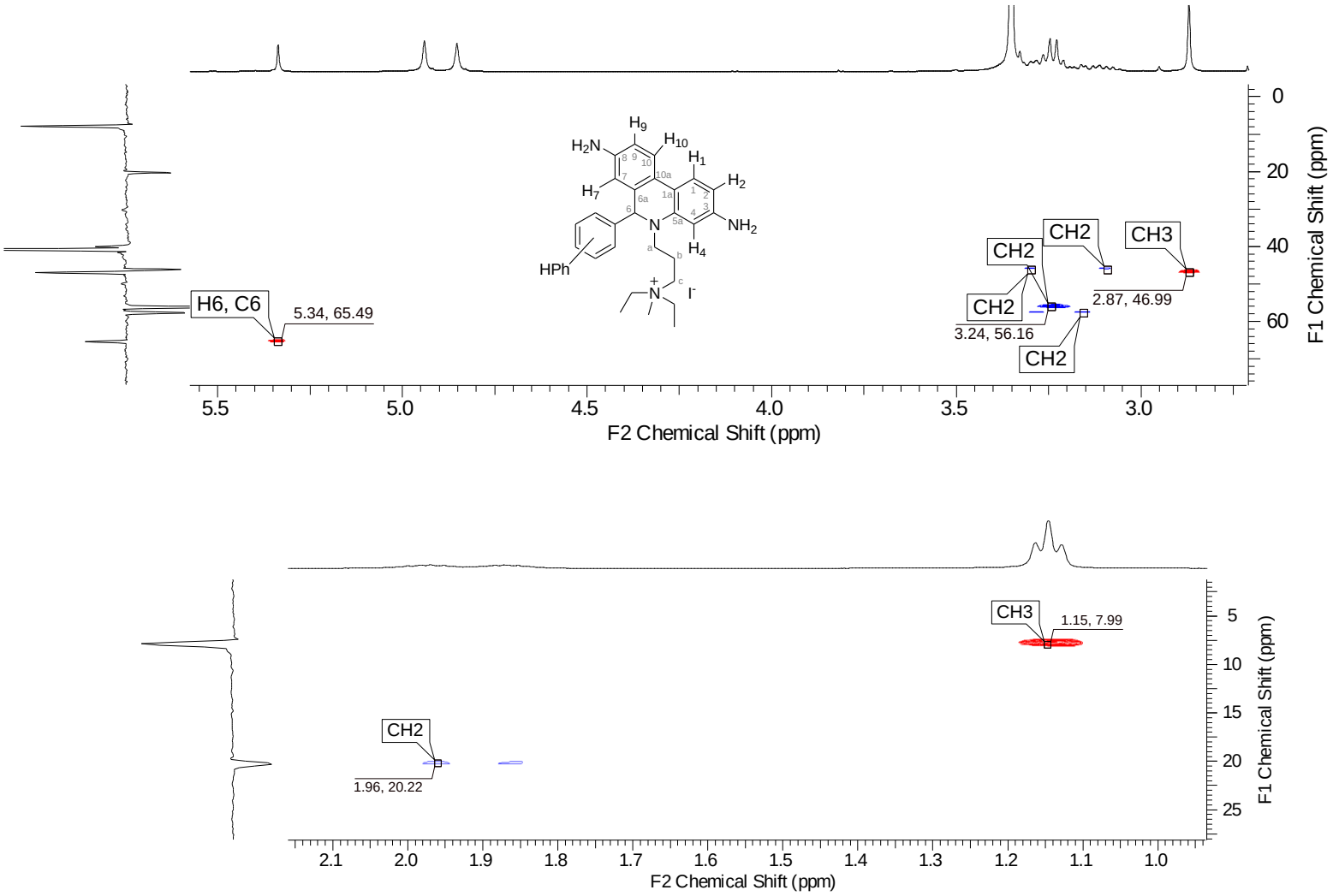
Supplementary Figure 1b. ^{13}C APT NMR spectrum of hydropropidine (HPr^+) in DMSO-*d6* (zoomed region 9 – 68 ppm).



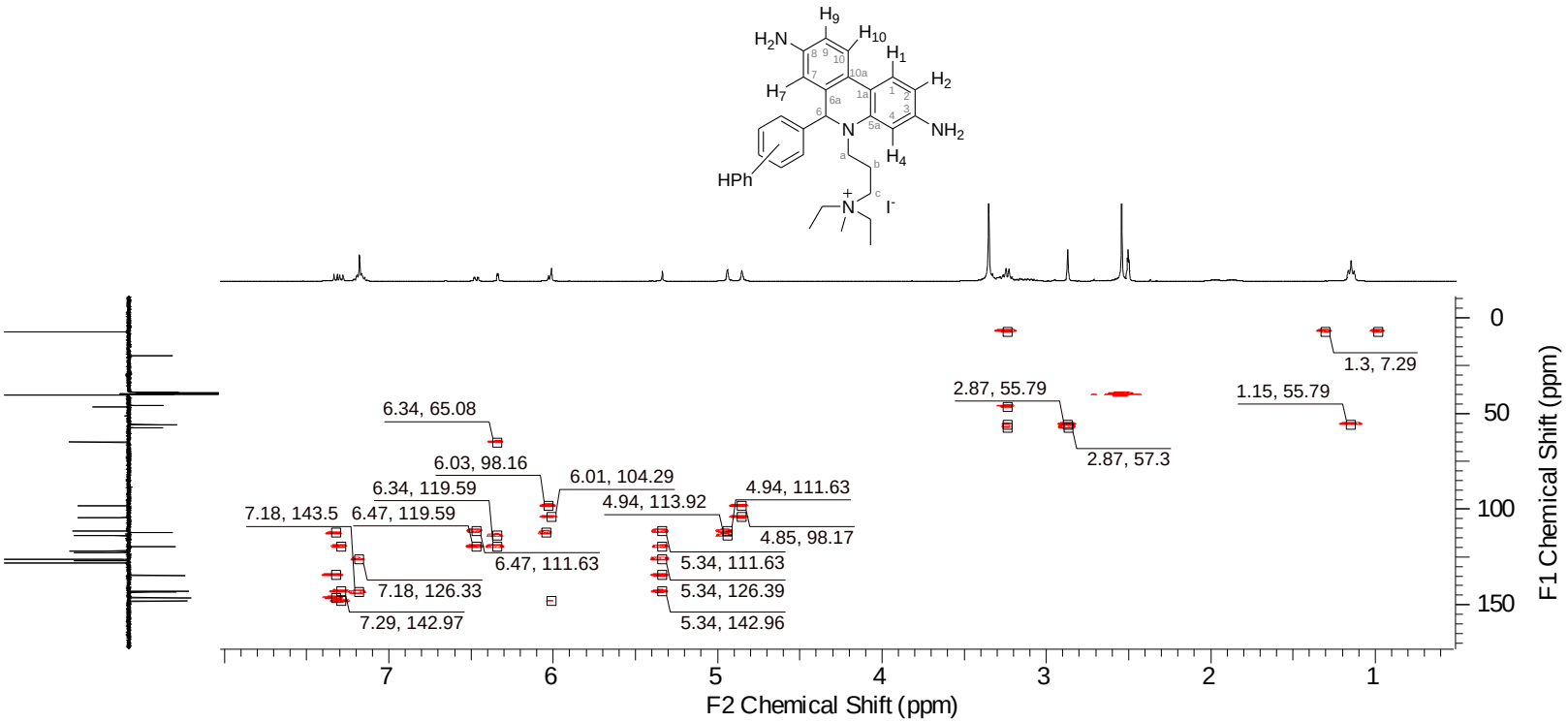
Supplementary Figure 1c. ^{1}H-^{13}C HSQC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6*.



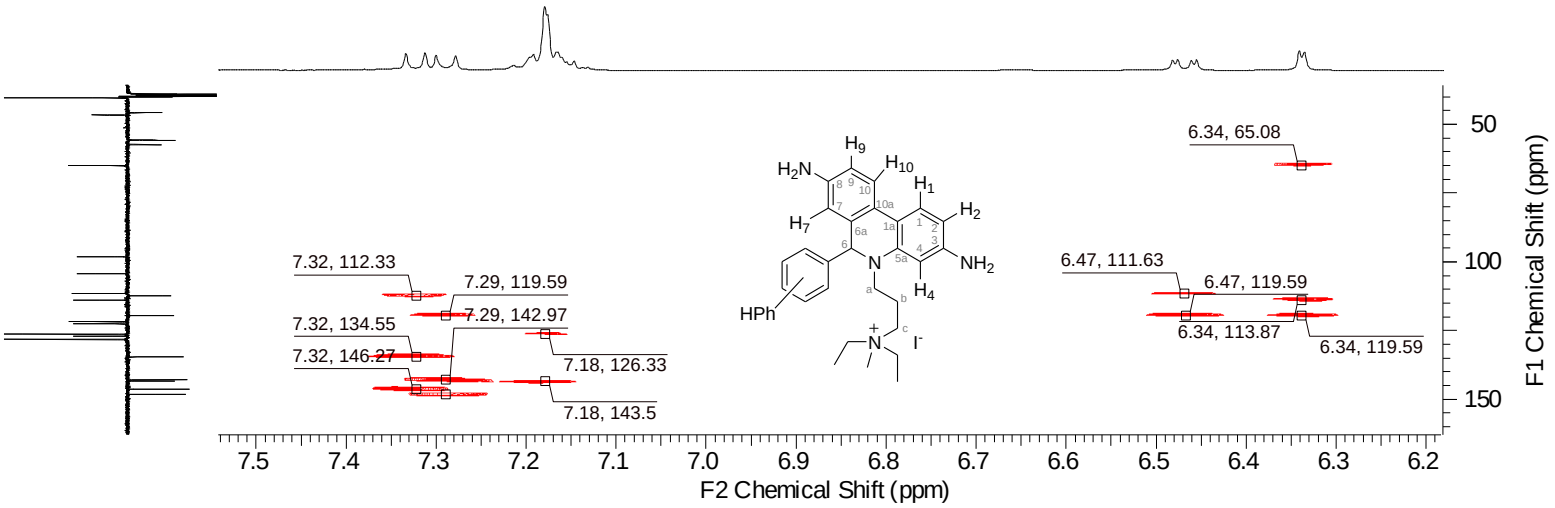
Supplementary Figure 1c. ^{1}H-^{13}C HSQC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed).



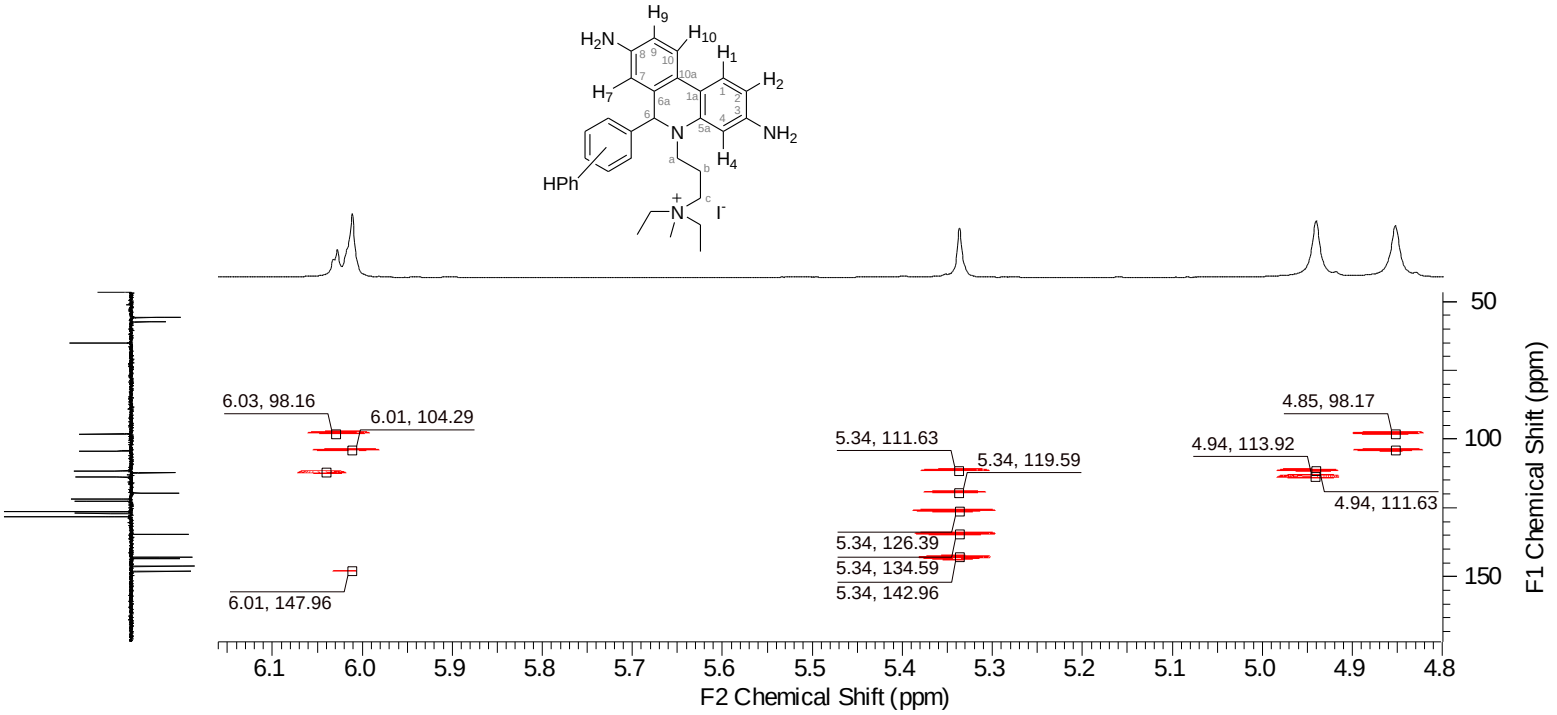
Supplementary Figure 1c. ^{1}H-^{13}C HSQC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed).



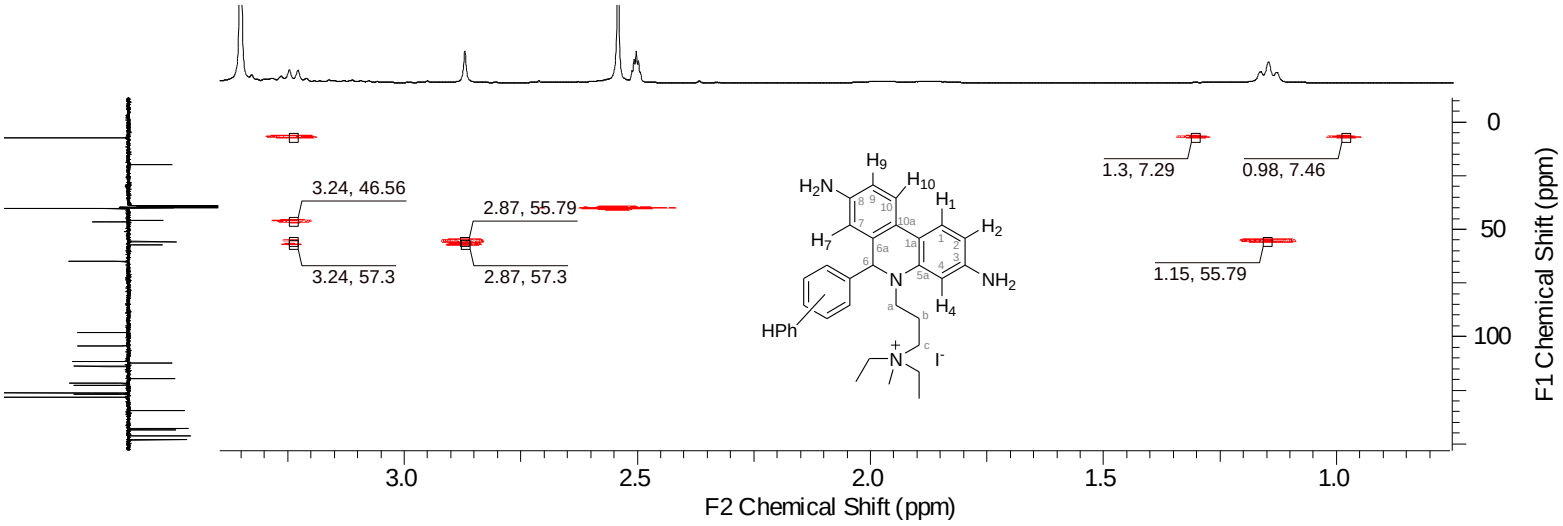
Supplementary Figure 1d. ^{1}H-^{13}C HMBC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6*.



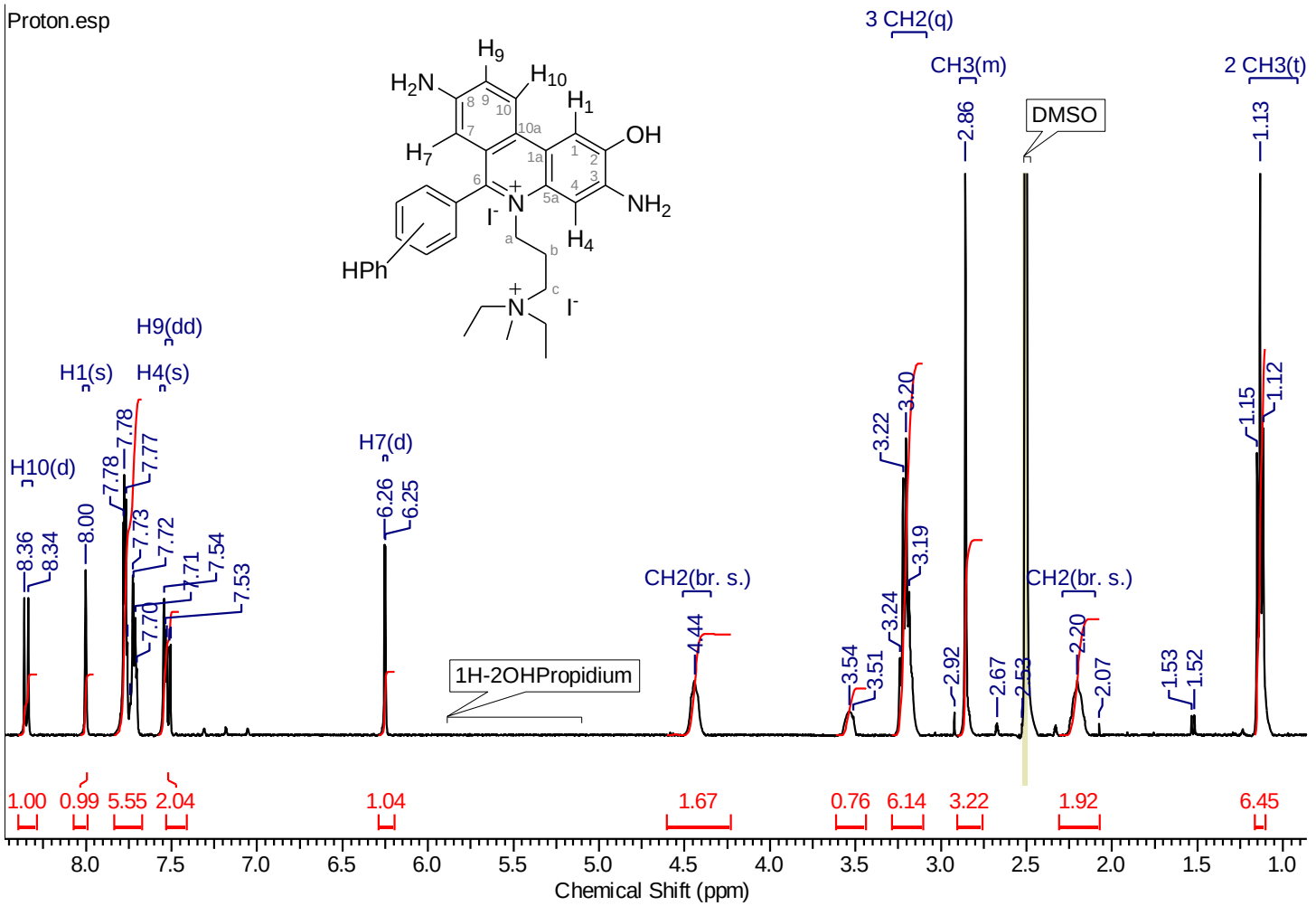
Supplementary Figure 1d. ^{1}H-^{13}C HMBC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed).



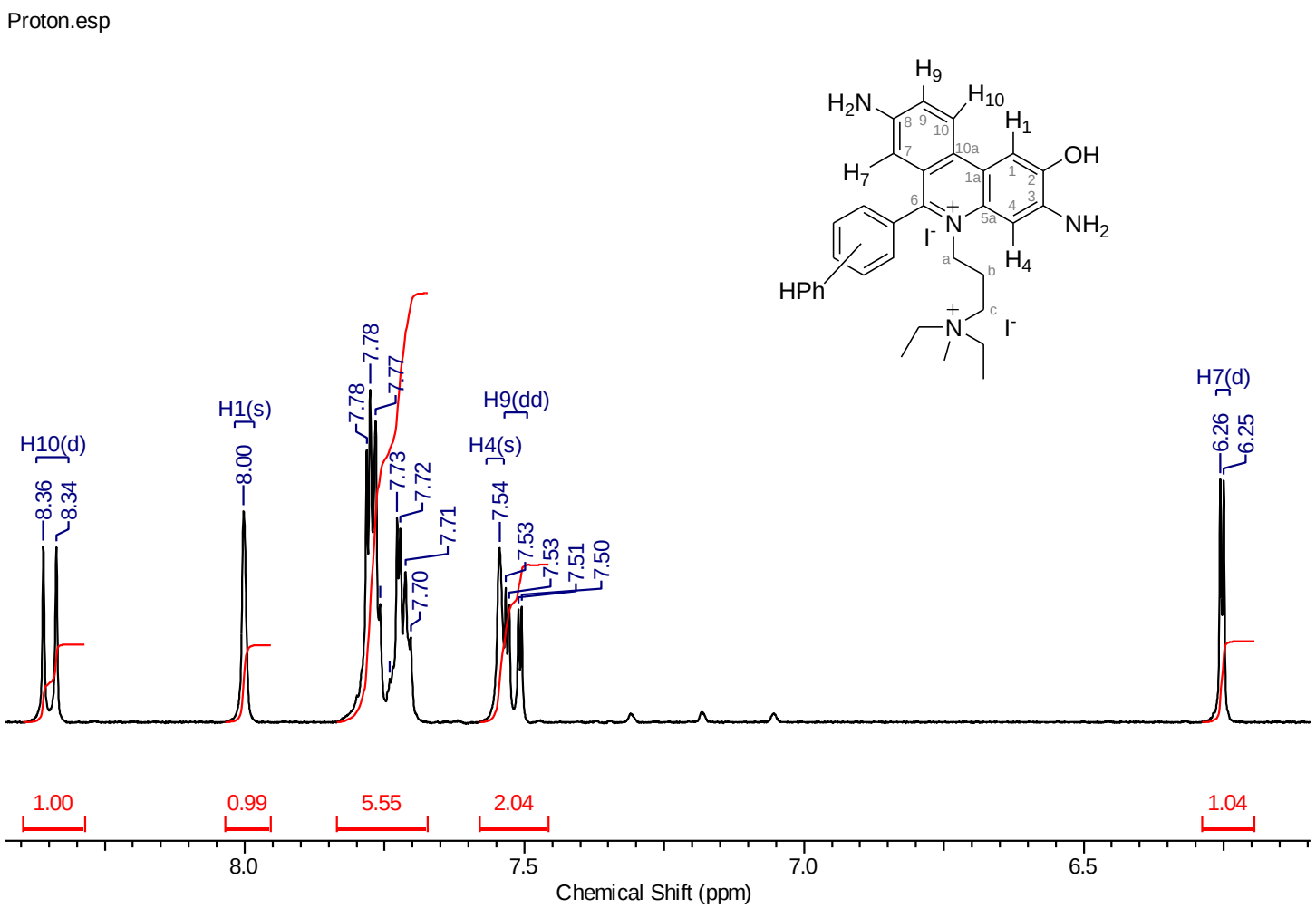
Supplementary Figure 1d. ^{1}H-^{13}C HMBC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed).



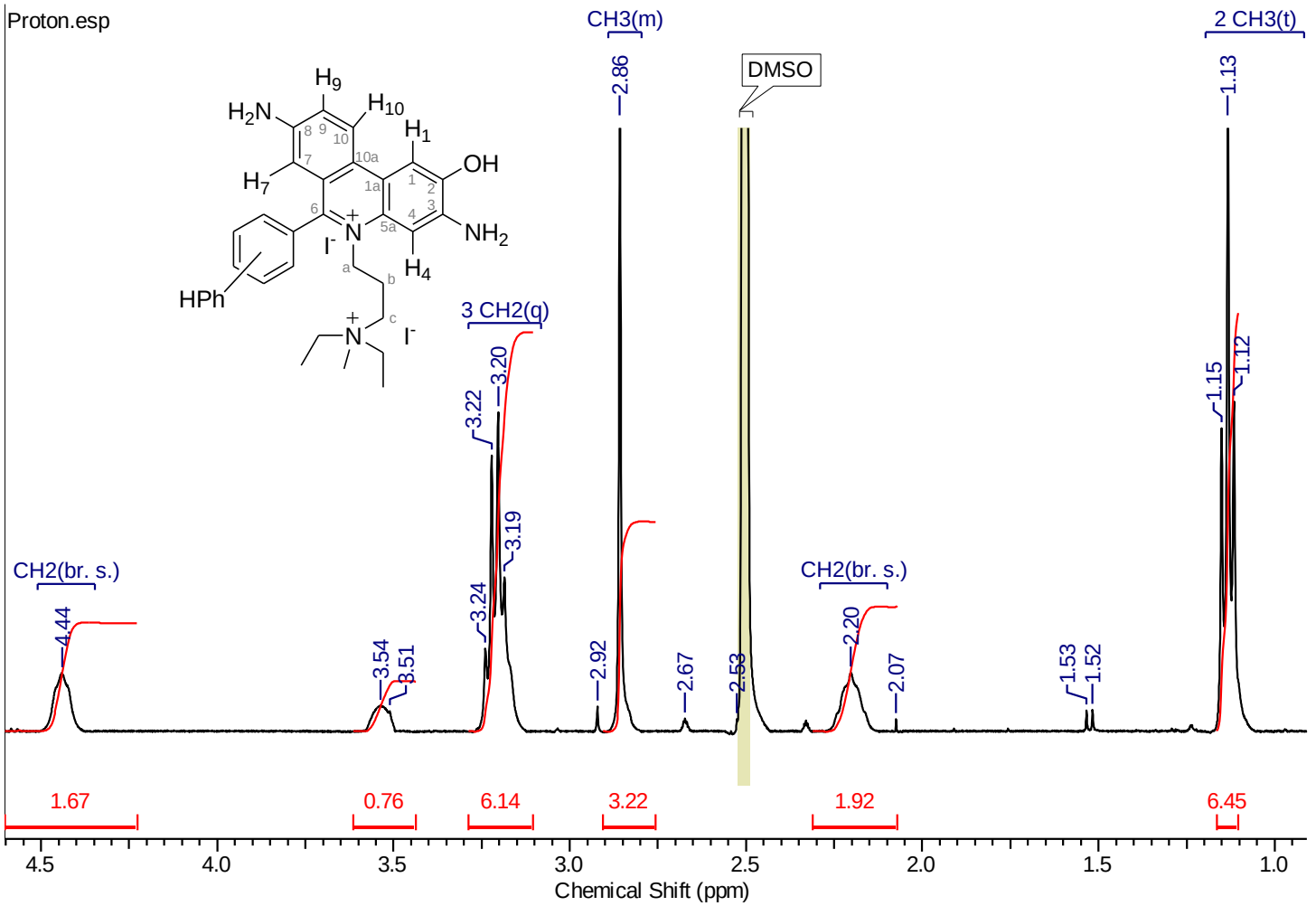
Supplementary Figure 1d. ^{1}H-^{13}C HMBC NMR spectrum of hydropropidine (HPr^{+}) in DMSO-*d6* (zoomed).



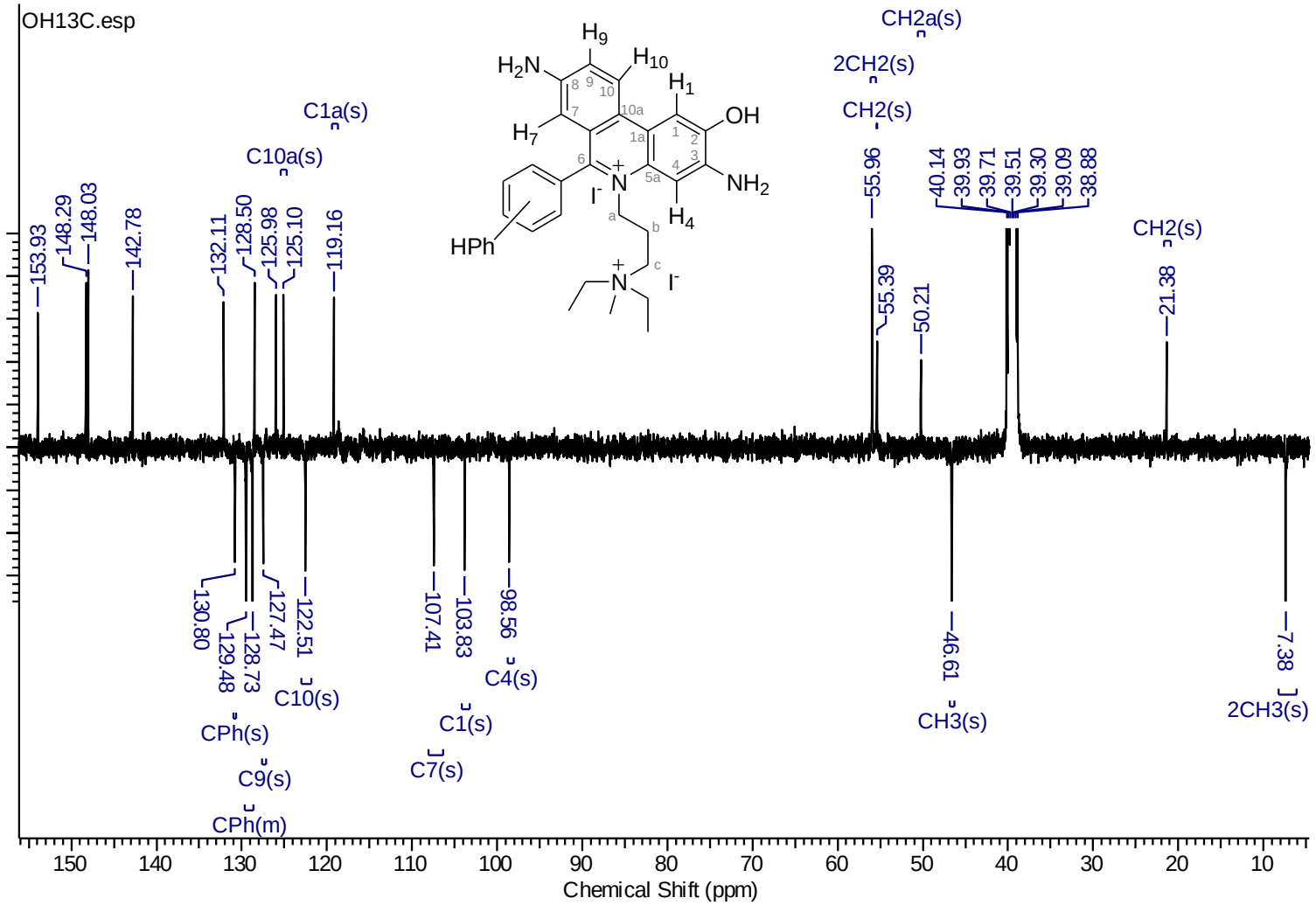
Supplementary Figure 2a. ^{1}H NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6*.



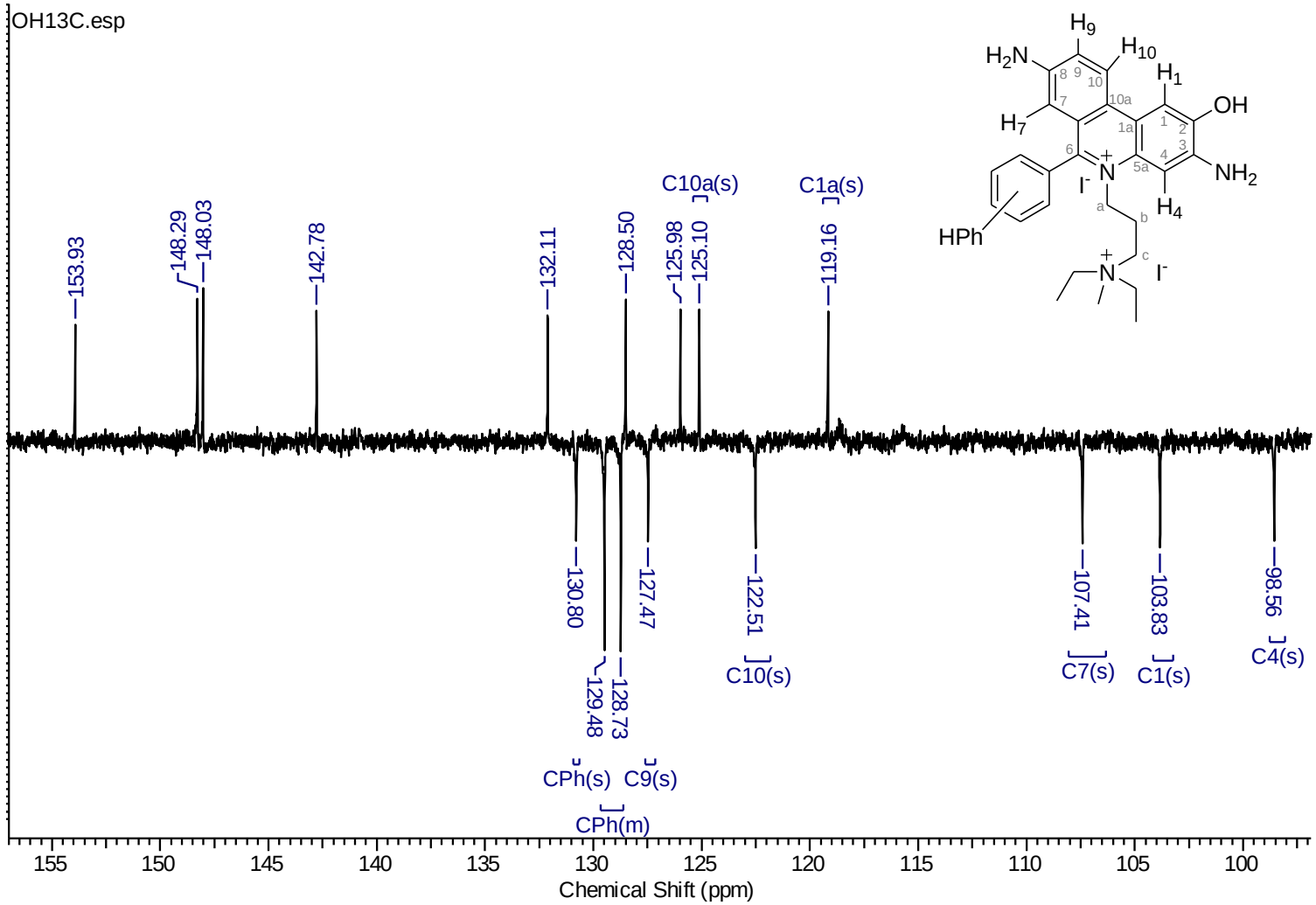
Supplementary Figure 2a. ^{1}H NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed region 5.3 – 8.4 ppm).



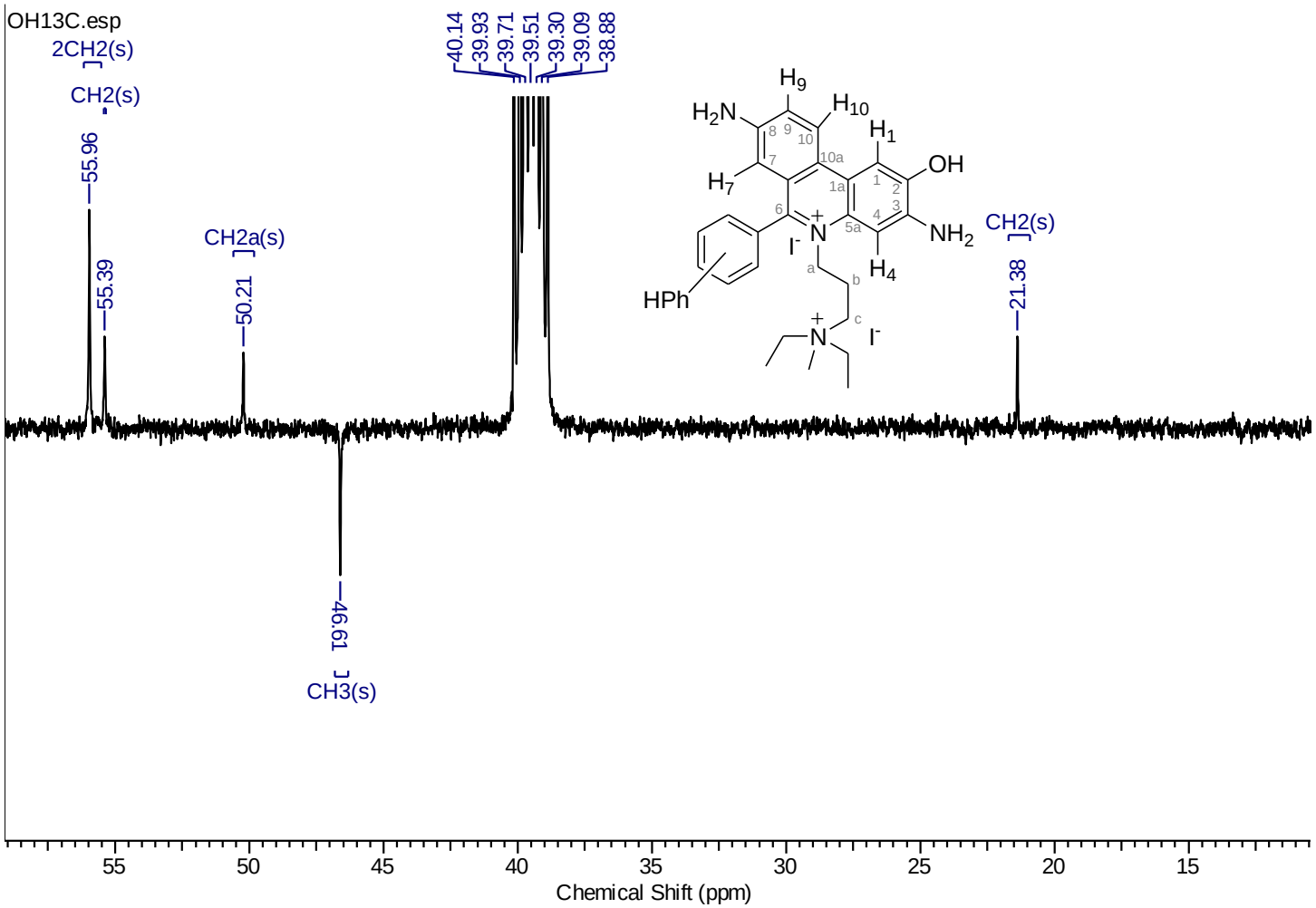
Supplementary Figure 2a. ^{1}H NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed region 0.9 – 4.6 ppm).



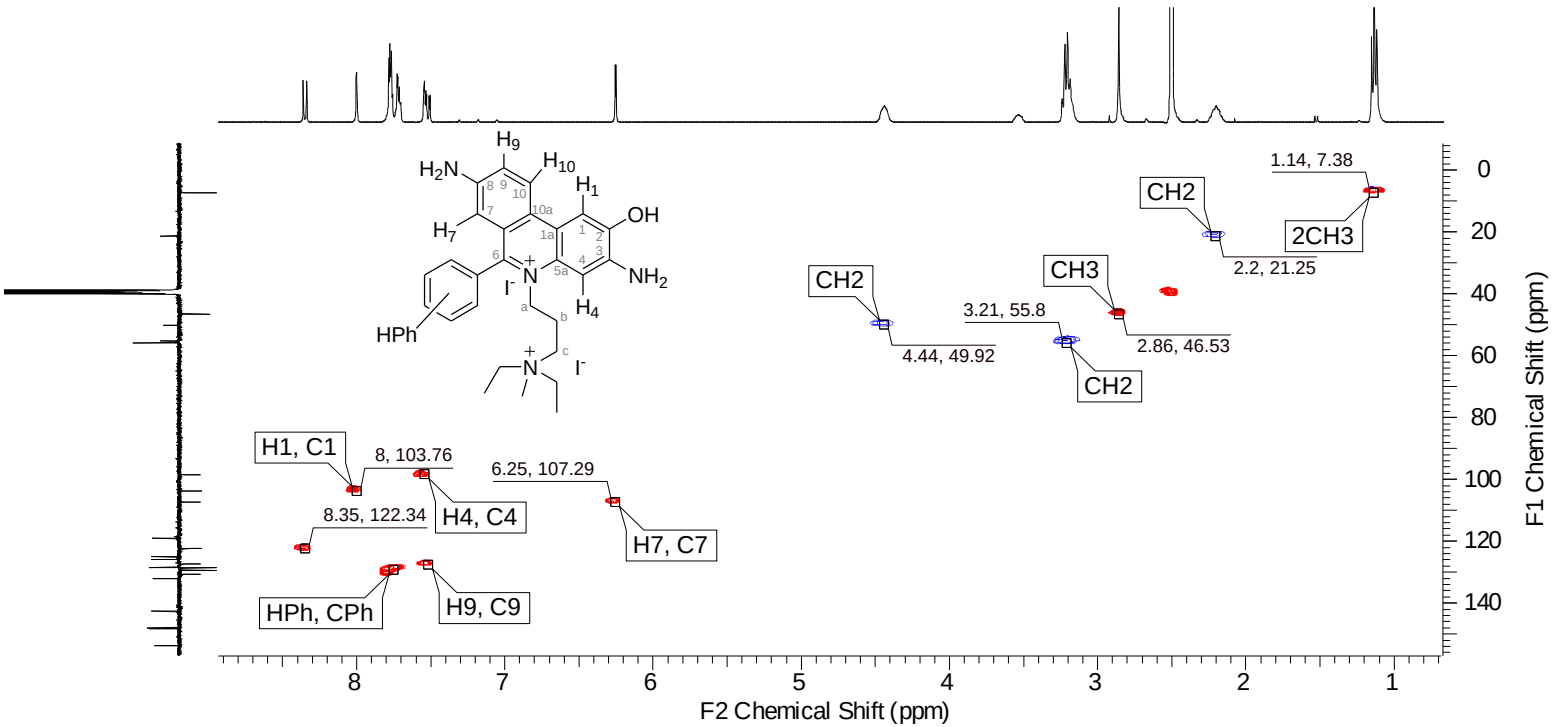
Supplementary Figure 2b. ^{13}C APT NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6*.



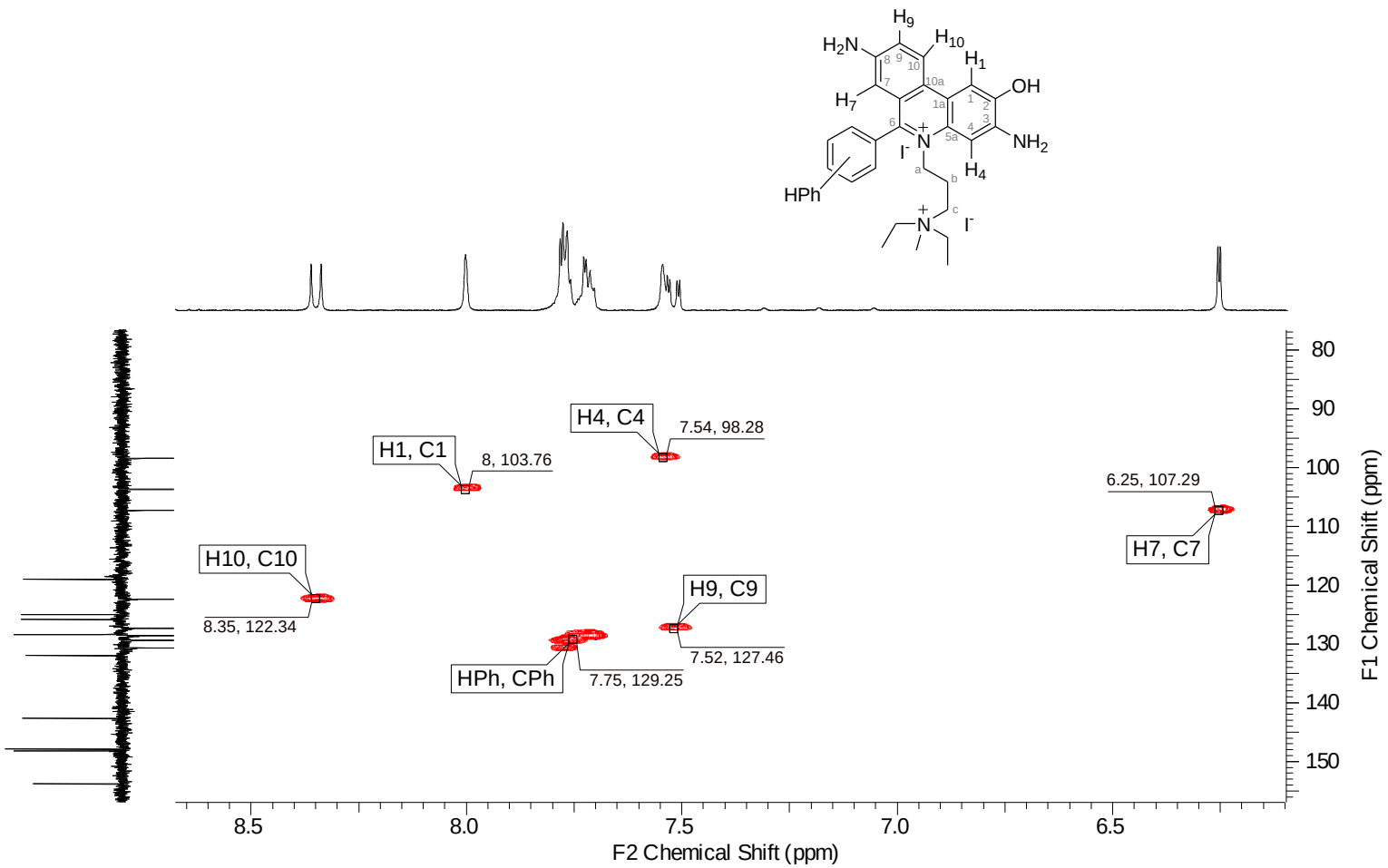
Supplementary Figure 2b. ^{13}C APT NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed region 95 – 156 ppm).



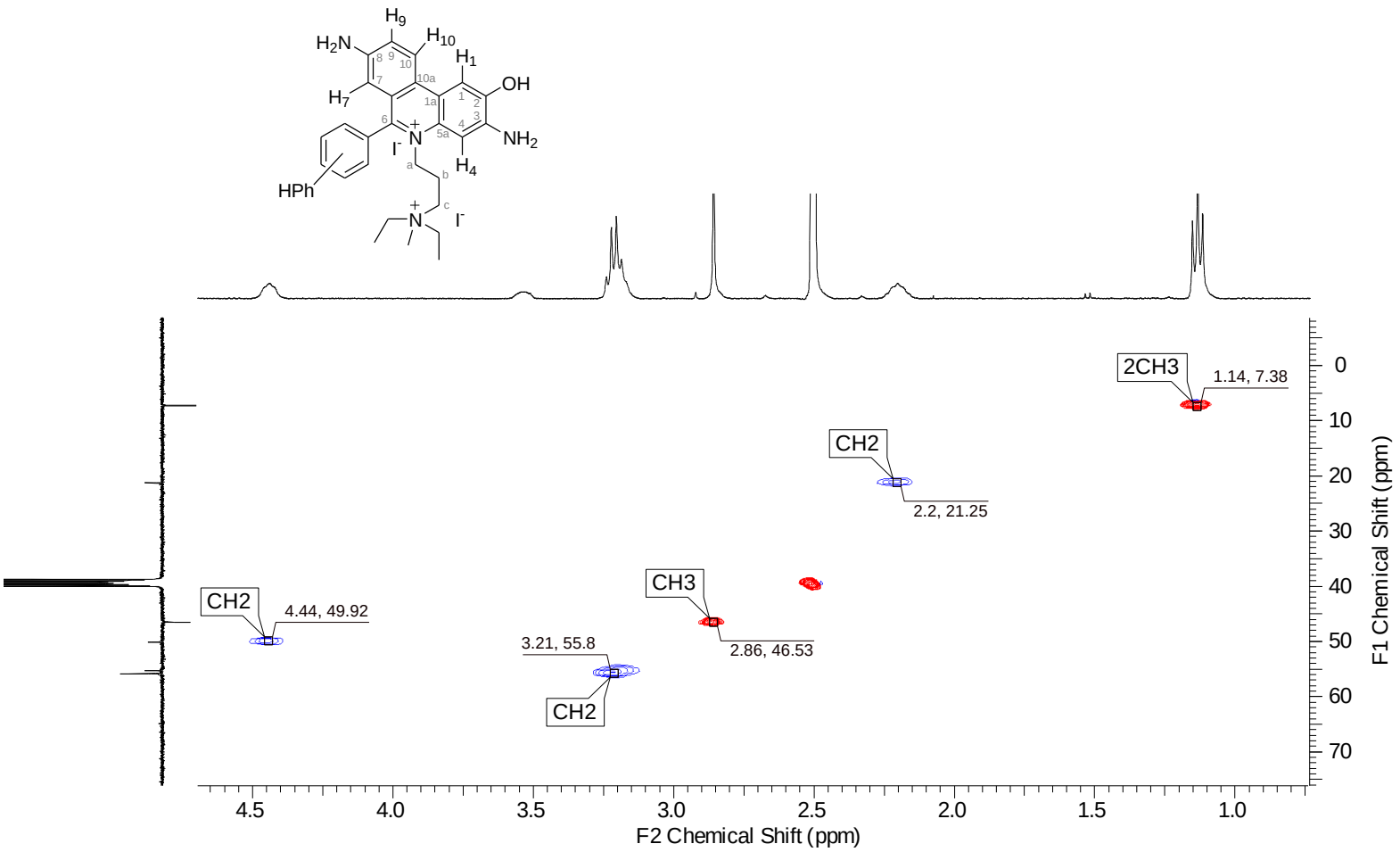
Supplementary Figure 2b. ^{13}C APT NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed region 11 – 58 ppm).



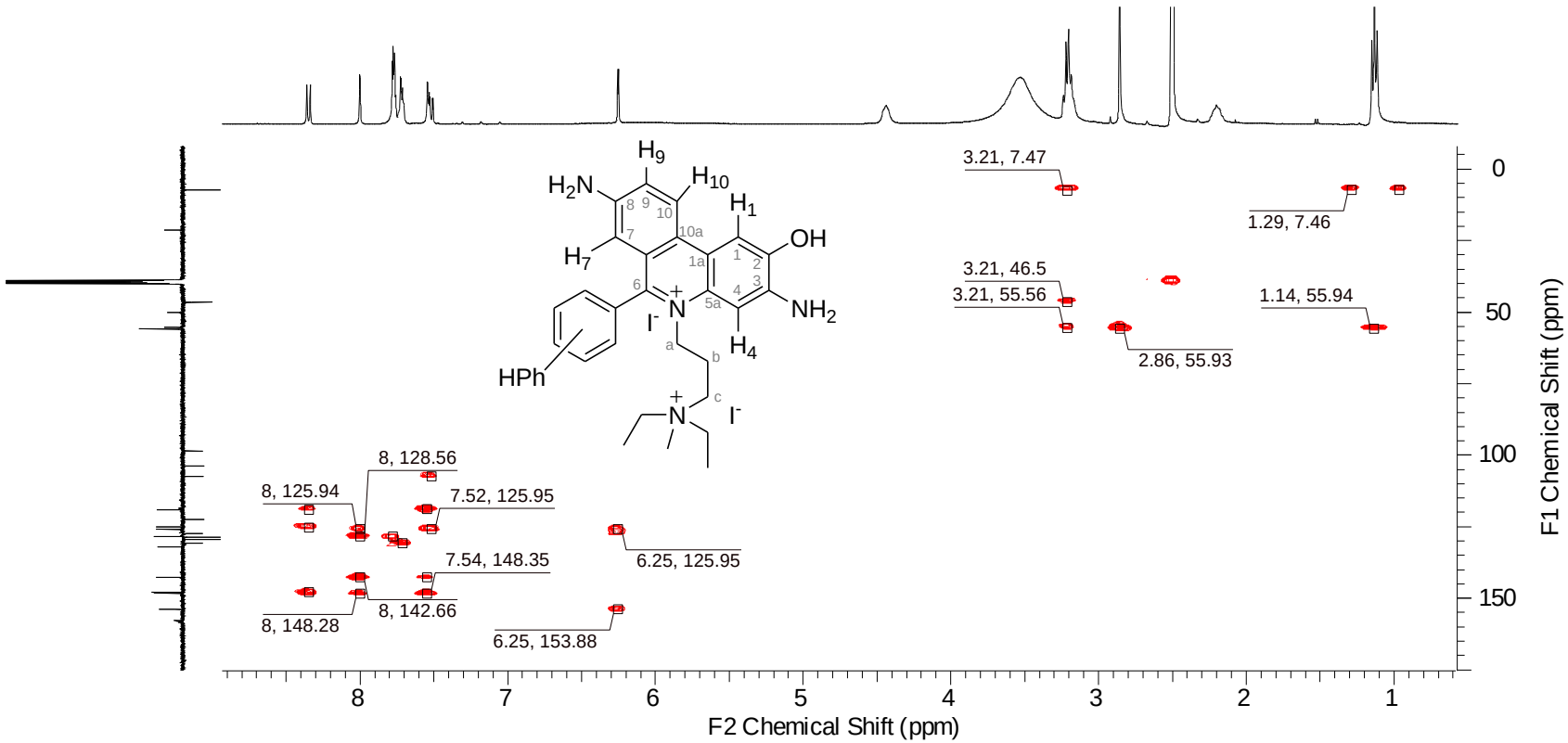
Supplementary Figure 2c. ^{1}H-^{13}C HSQC NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6*.



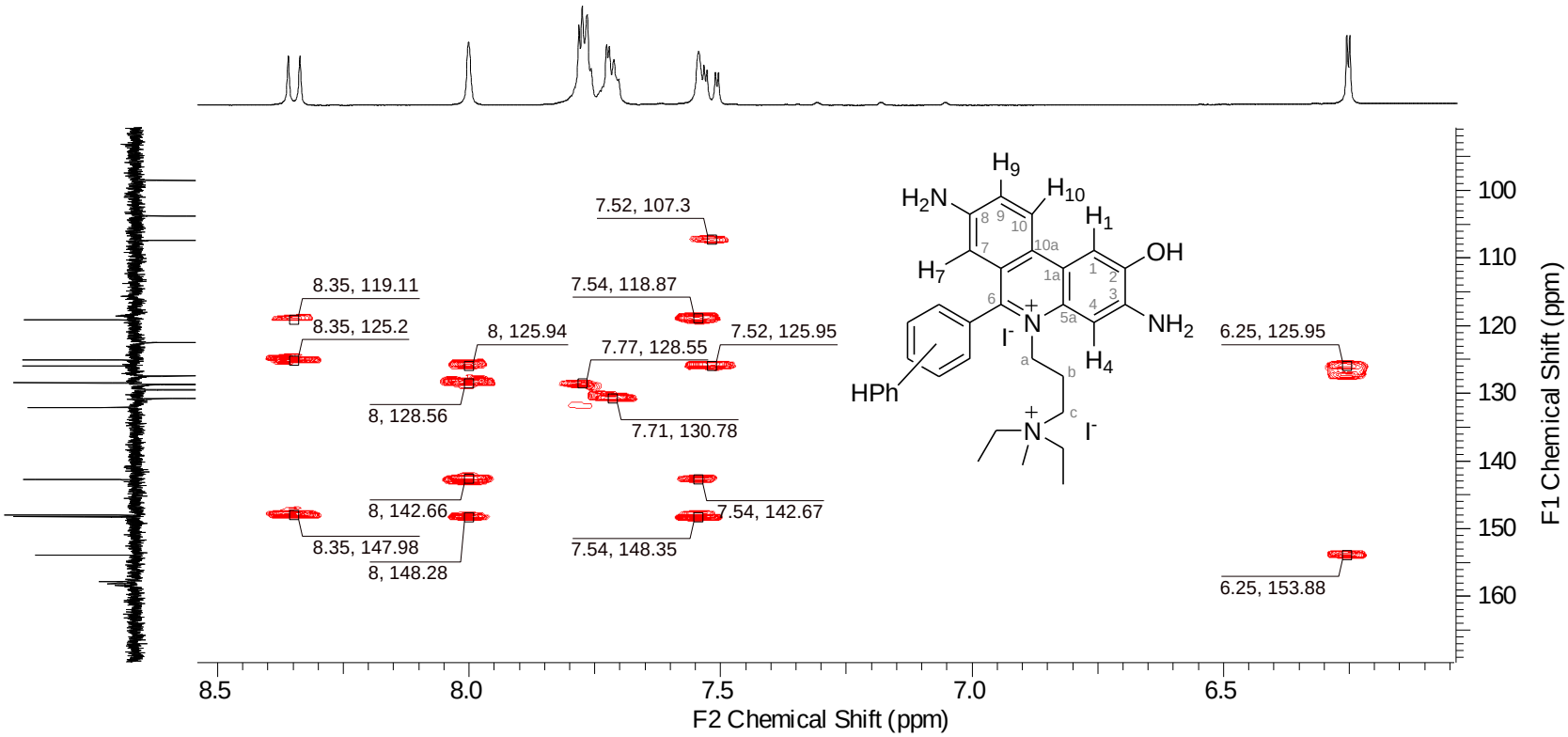
Supplementary Figure 2c. ^{1}H-^{13}C HSQC NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed).



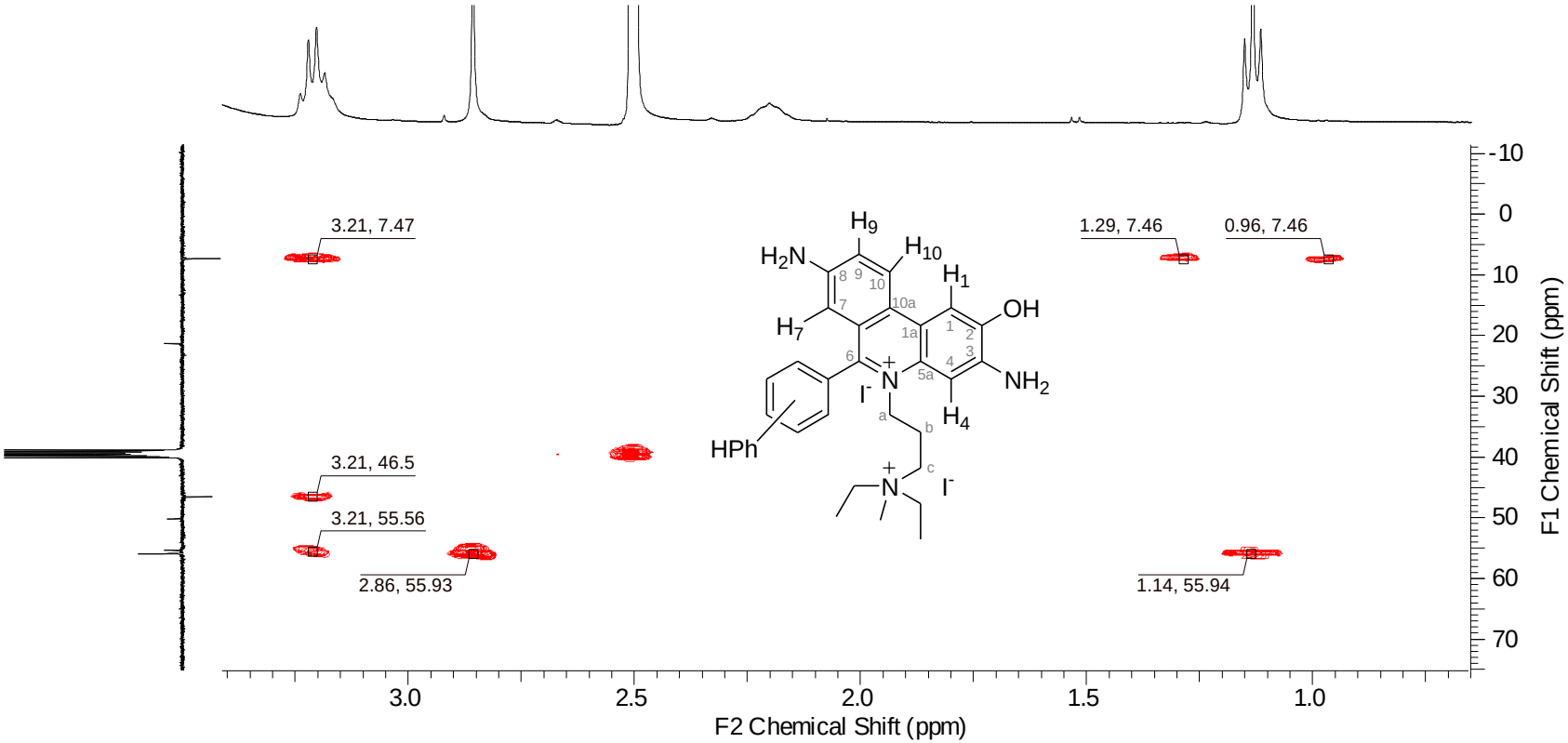
Supplementary Figure 2c. ^{1}H-^{13}C HSQC NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed).



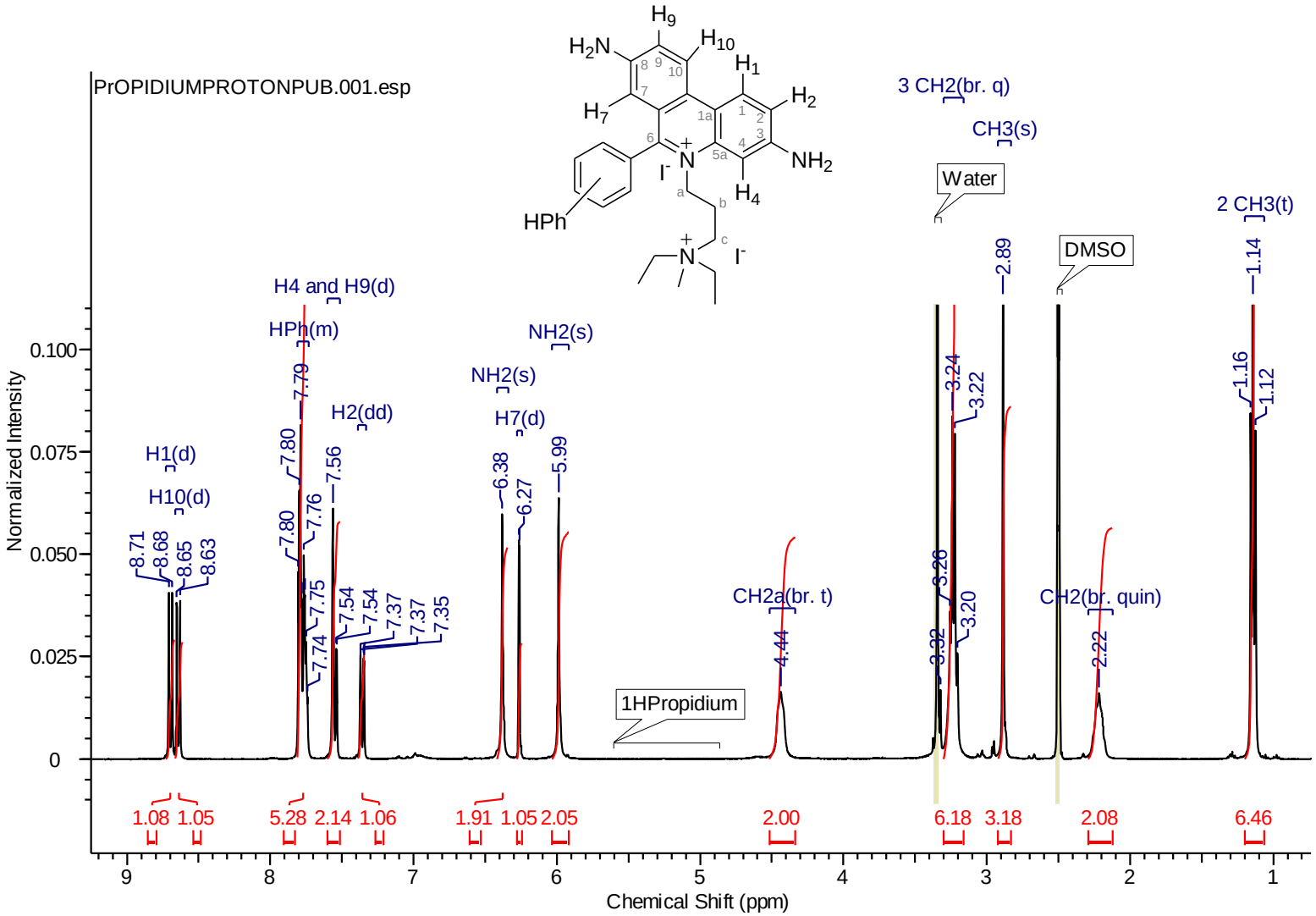
Supplementary Figure 2d. ^{1}H-^{13}C HMBC NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6*.



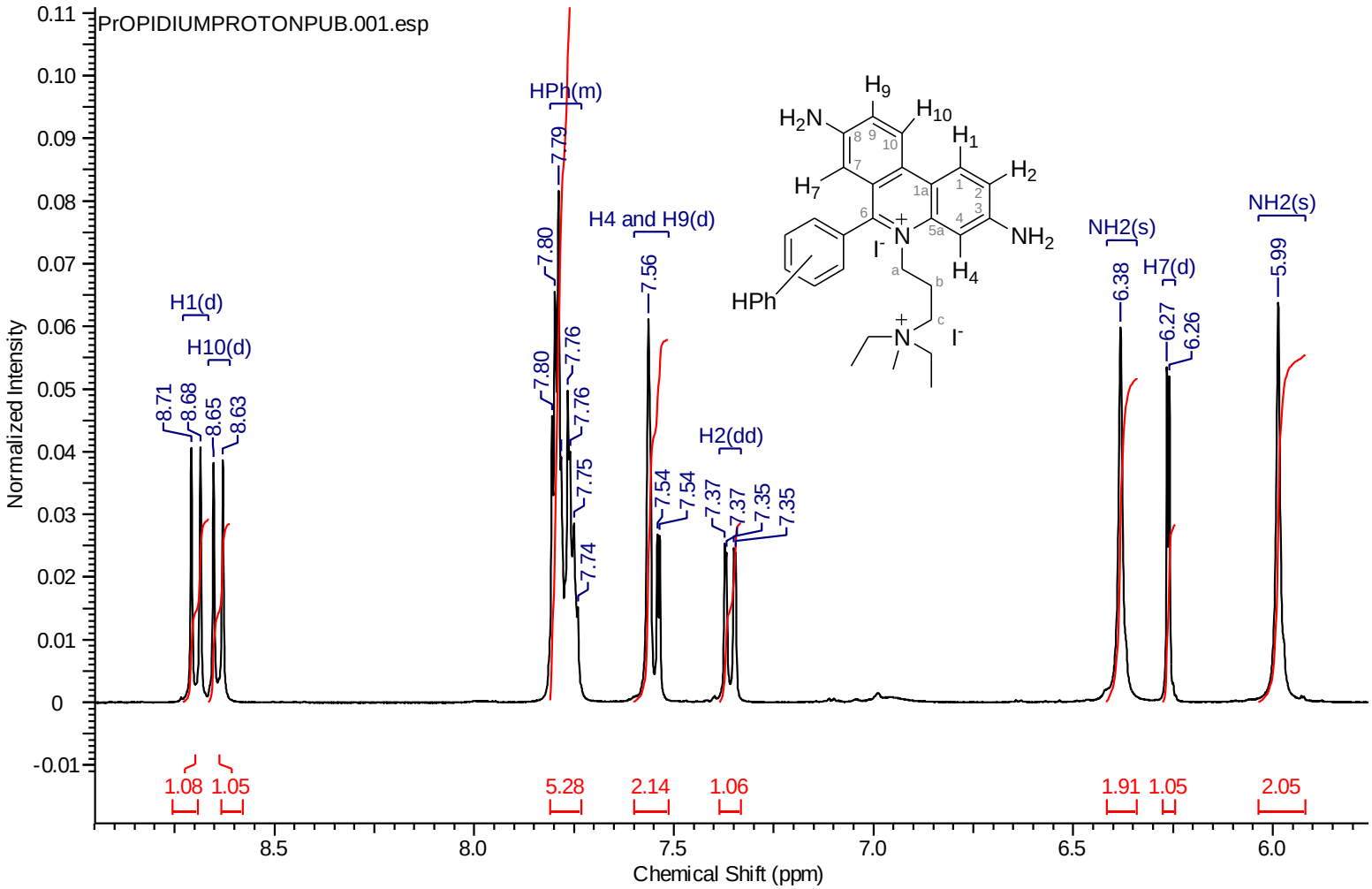
Supplementary Figure 2d. ^{1}H-^{13}C HMBC NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed).



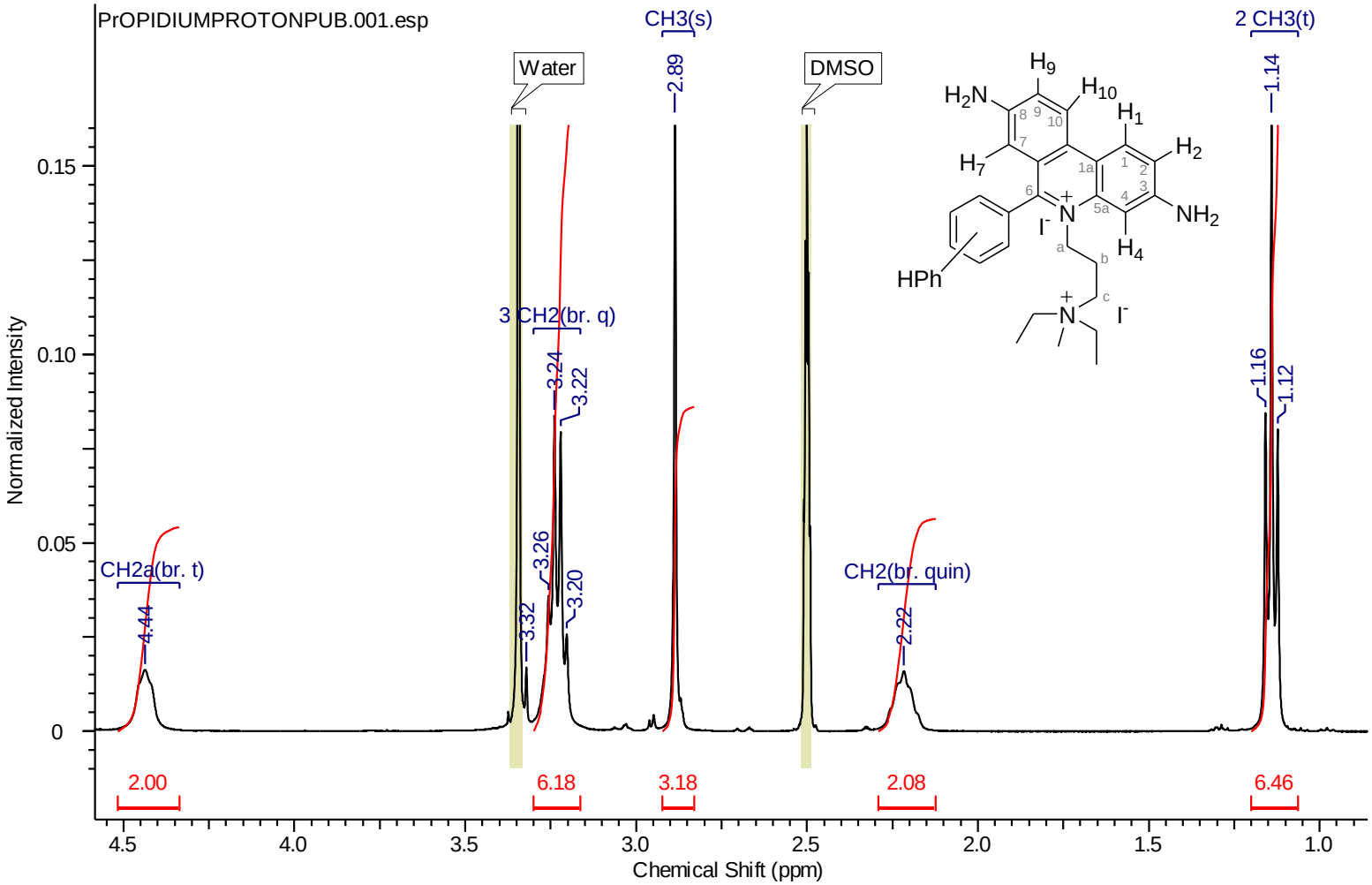
Supplementary Figure 2d. ^{1}H-^{13}C HMBC NMR spectrum of 2-hydroxypropidium (2-OH-Pr^{++}) in DMSO-*d6* (zoomed).



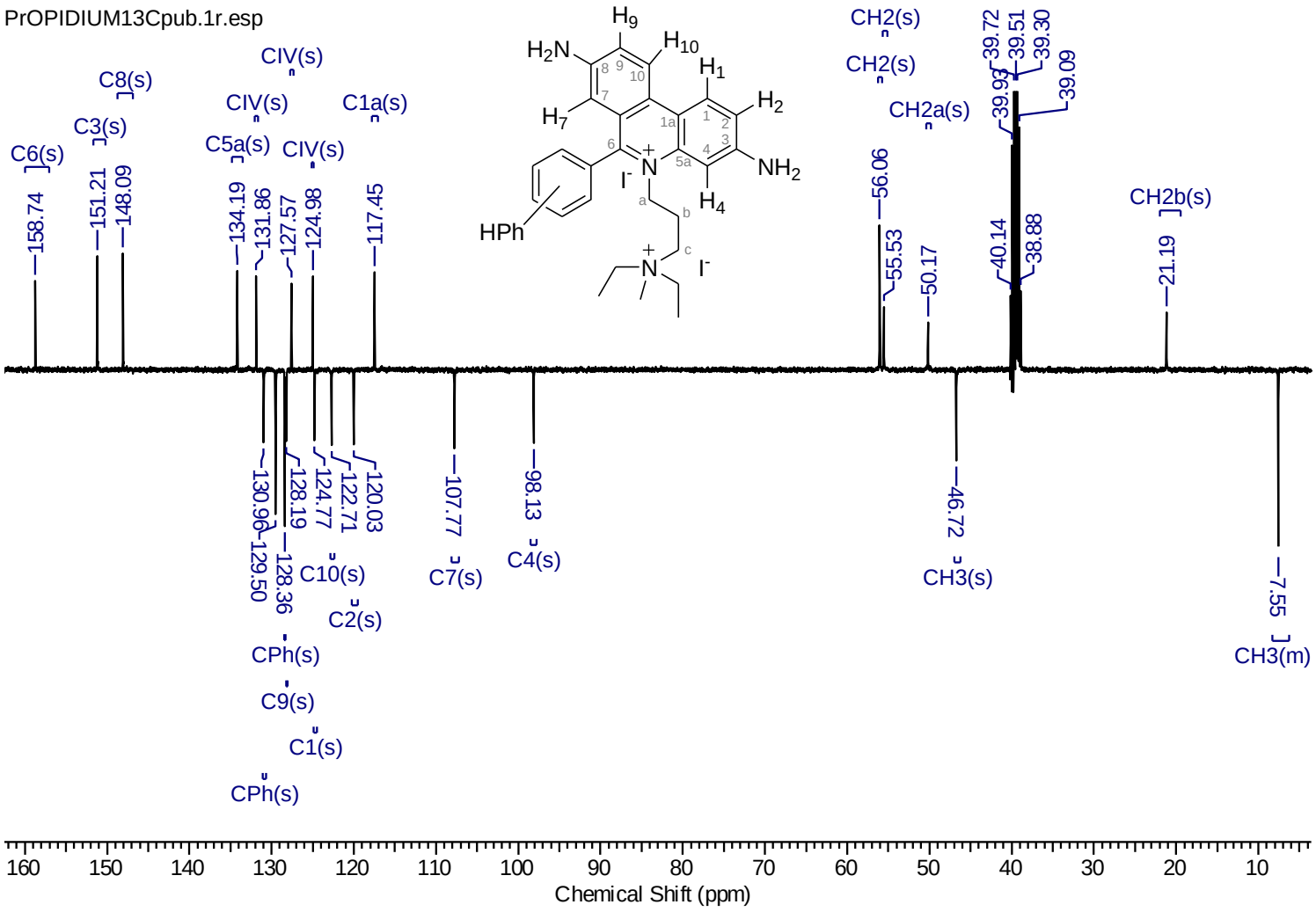
Supplementary Figure 3a. ^{1}H NMR spectrum of propidium (Pr^{++}) in DMSO-*d6*.



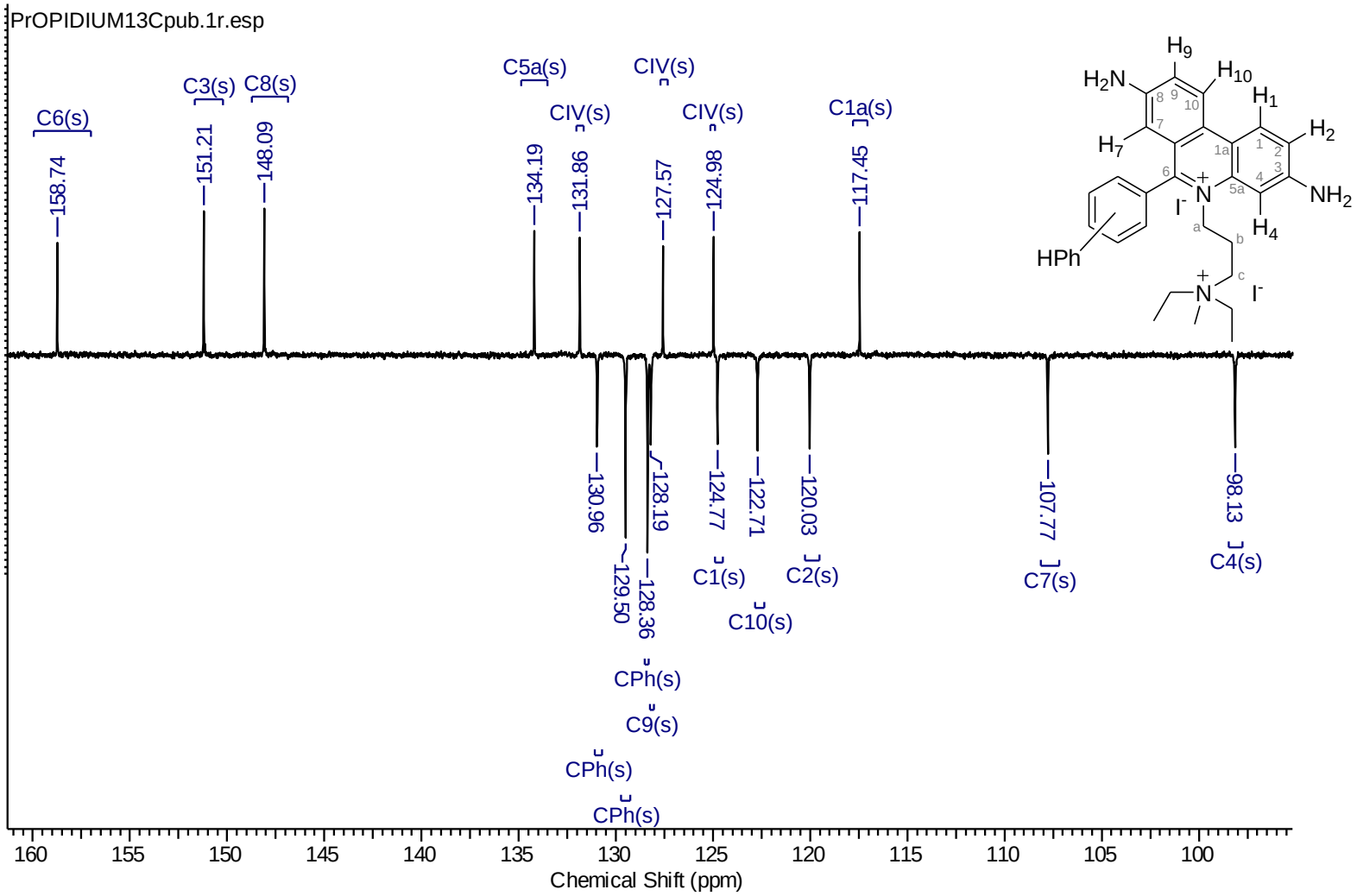
Supplementary Figure 3a. ^{1}H NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed region 5.8 – 8.8 ppm).



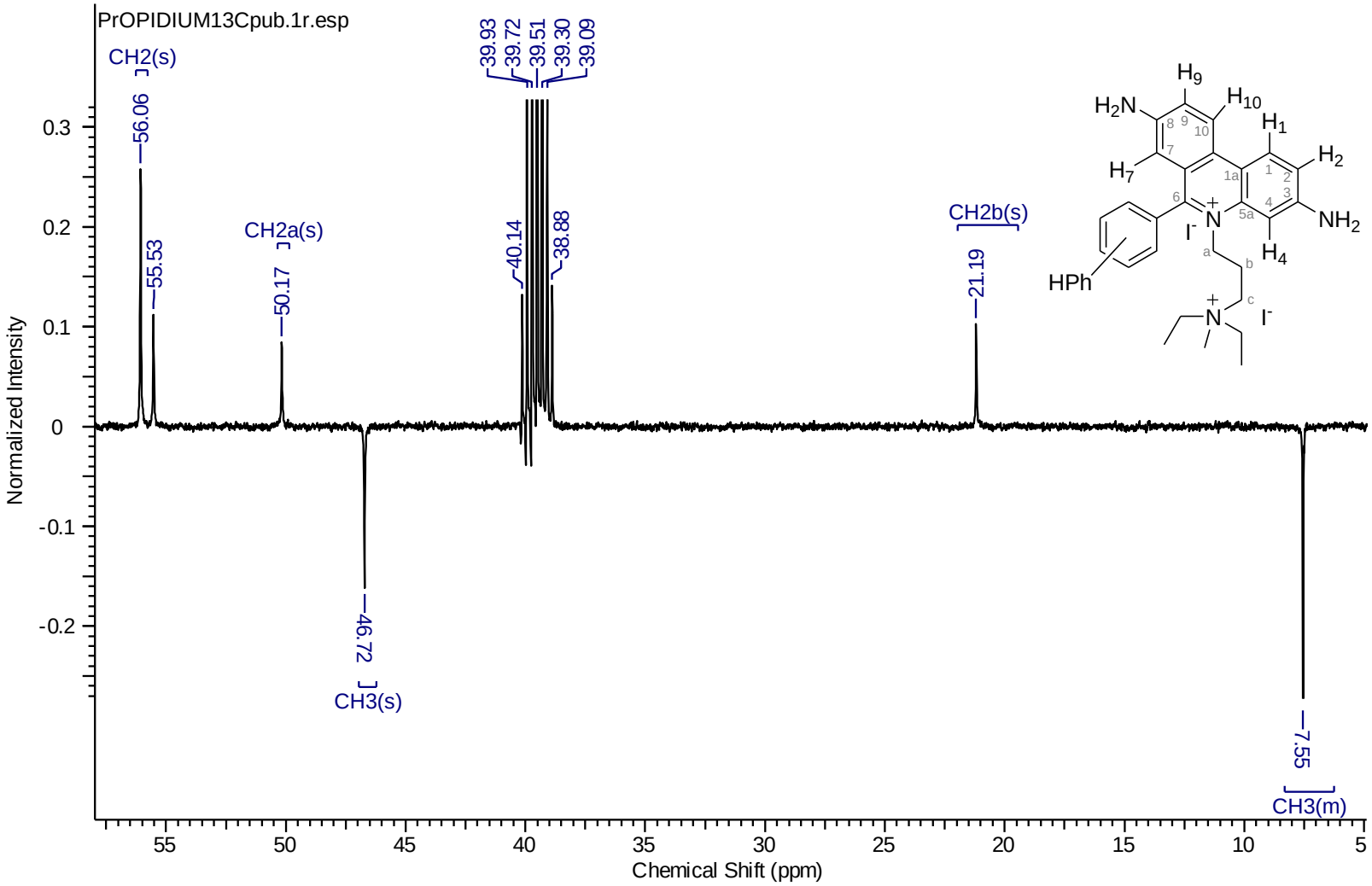
Supplementary Figure 3a. ^{1}H NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed region 0.9 – 4.5 ppm).



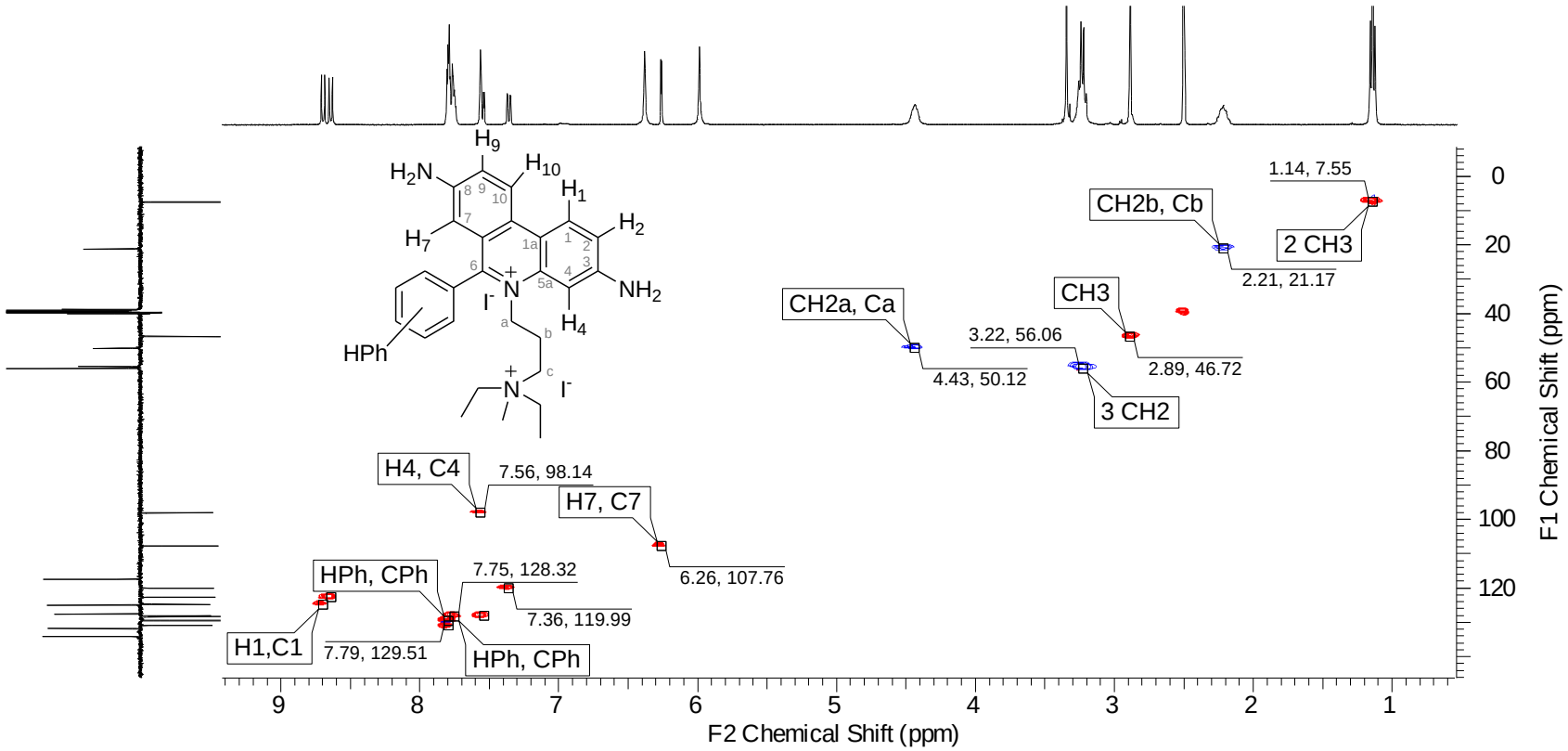
Supplementary Figure 3b. ^{13}C APT NMR spectrum of propidium (Pr^{++}) in DMSO-*d6*.



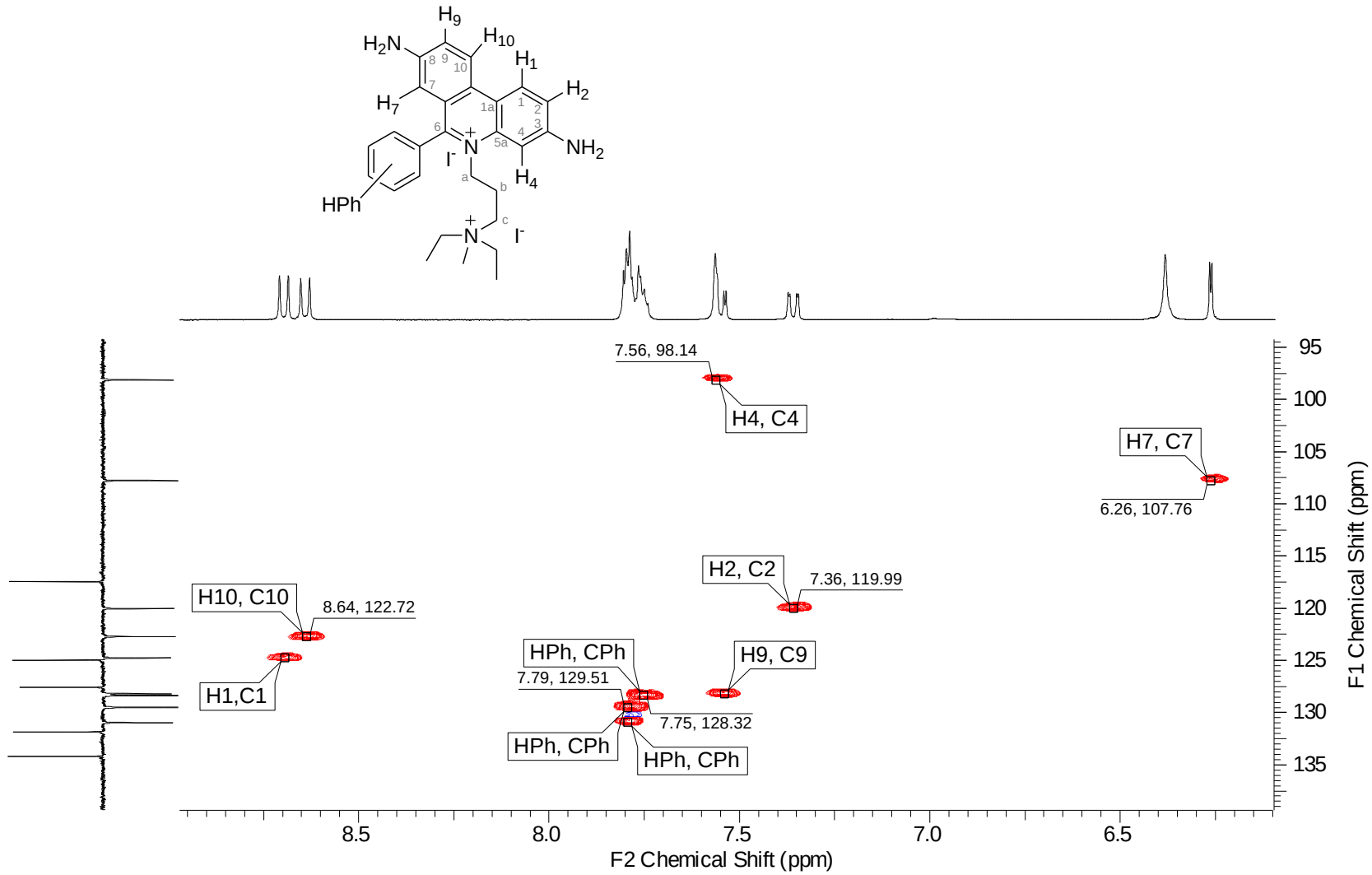
Supplementary Figure 3b. ^{13}C APT NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed region 96 – 161 ppm).



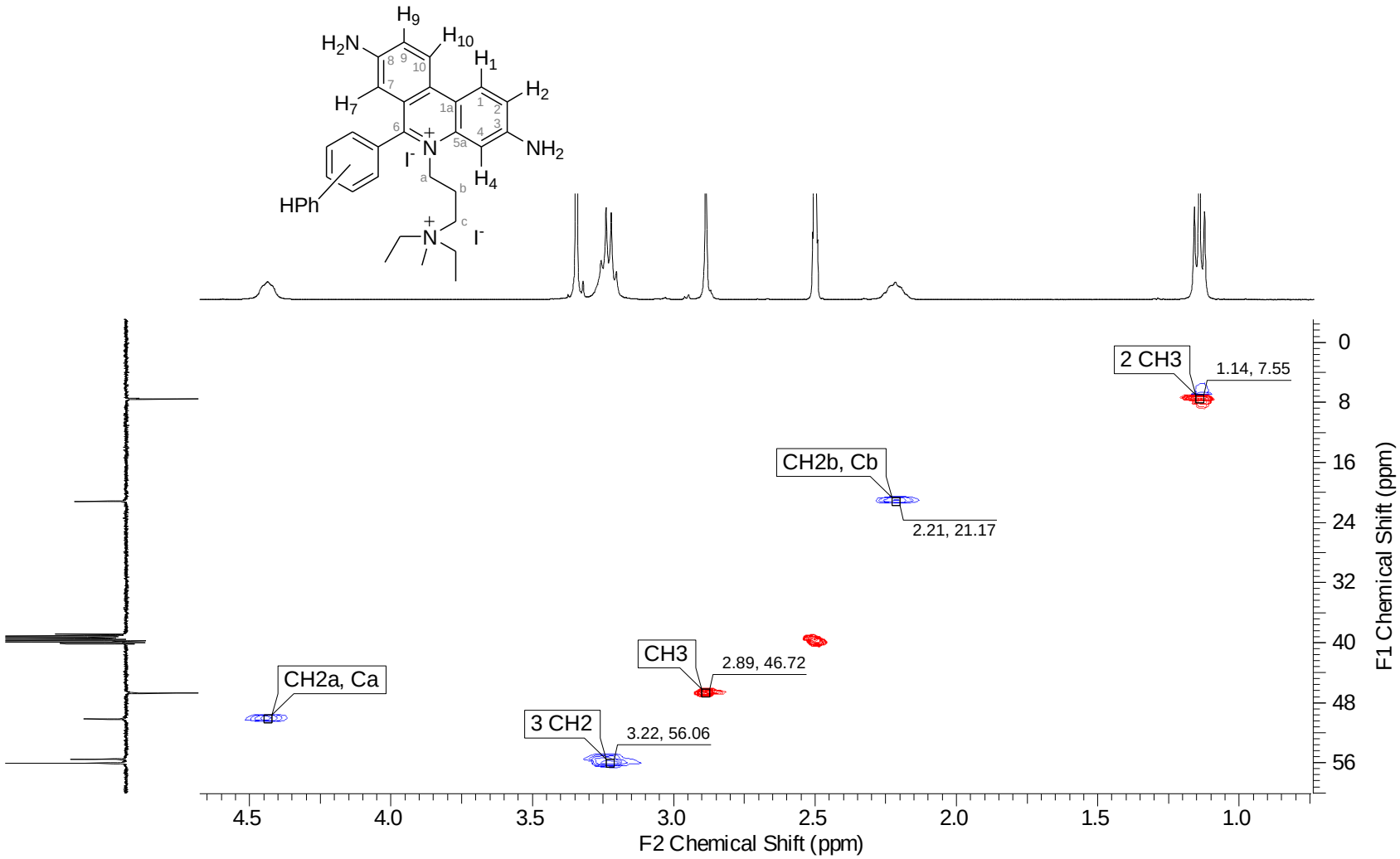
Supplementary Figure 3b. ^{13}C APT NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed region 5 – 57 ppm).



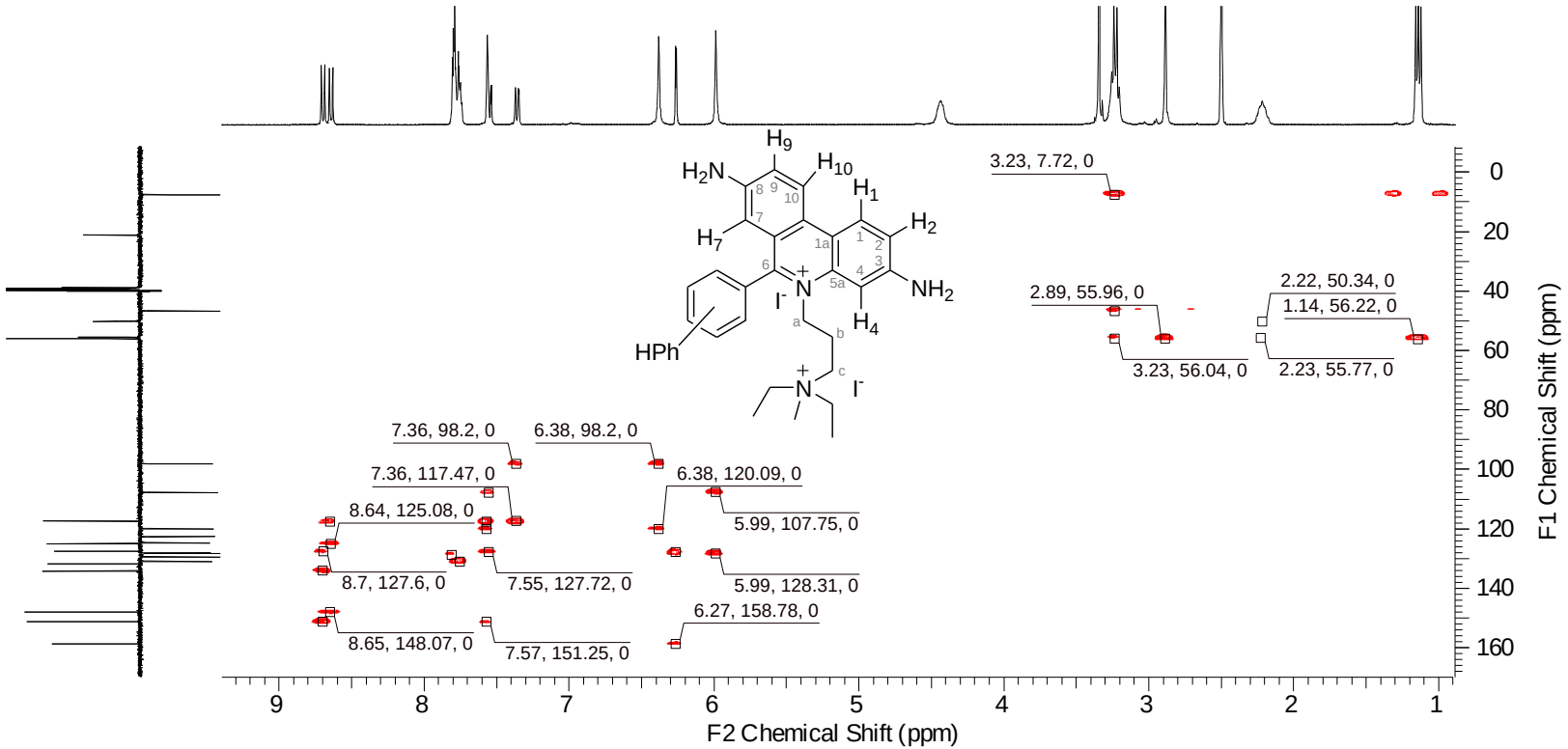
Supplementary Figure 3c. ^{1}H-^{13}C HSQC NMR spectrum of propidium (Pr^{++}) in DMSO-*d6*.



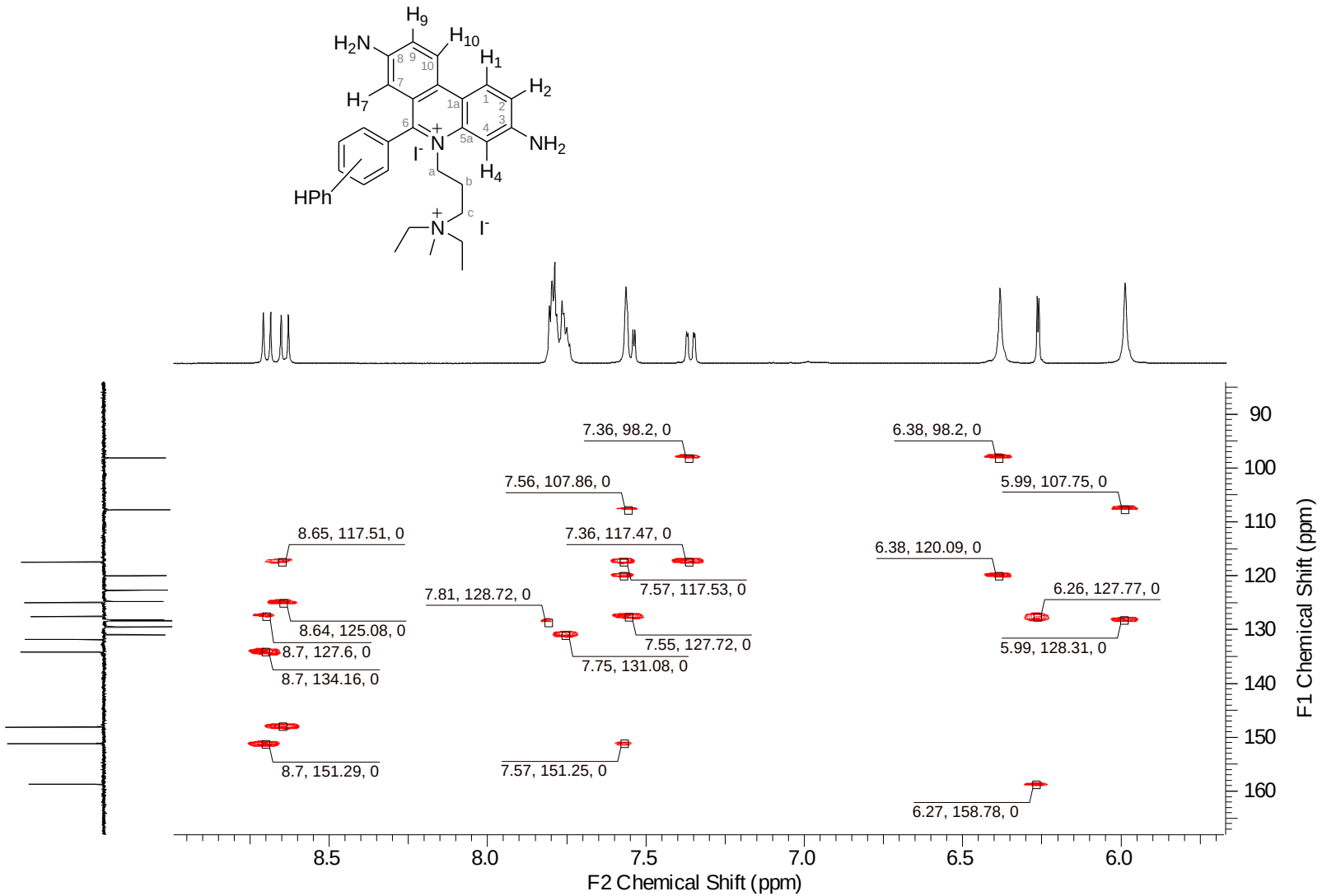
Supplementary Figure 3c. ^{1}H-^{13}C HSQC NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed).



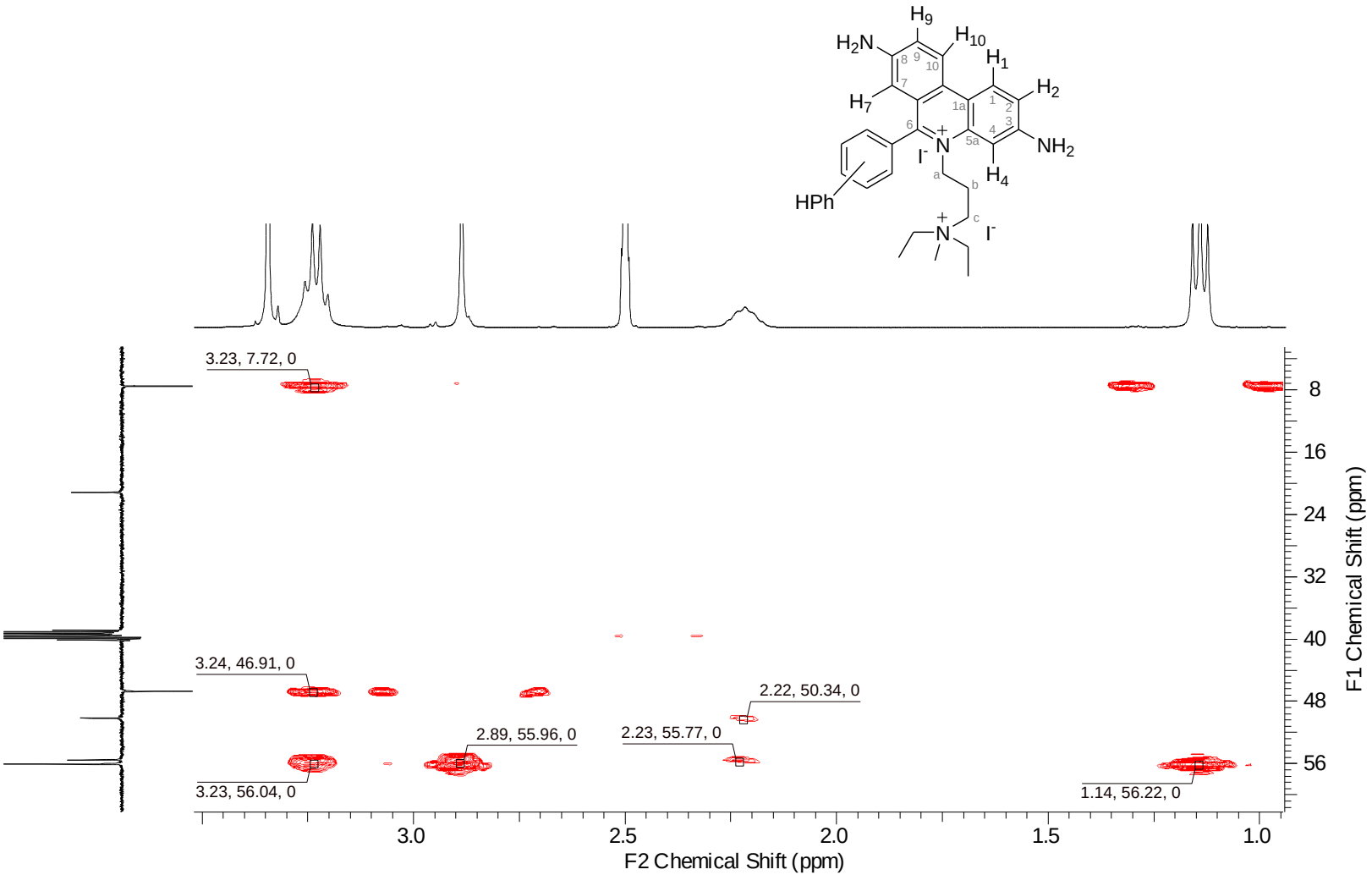
Supplementary Figure 3c. ^{1}H-^{13}C HSQC NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed).



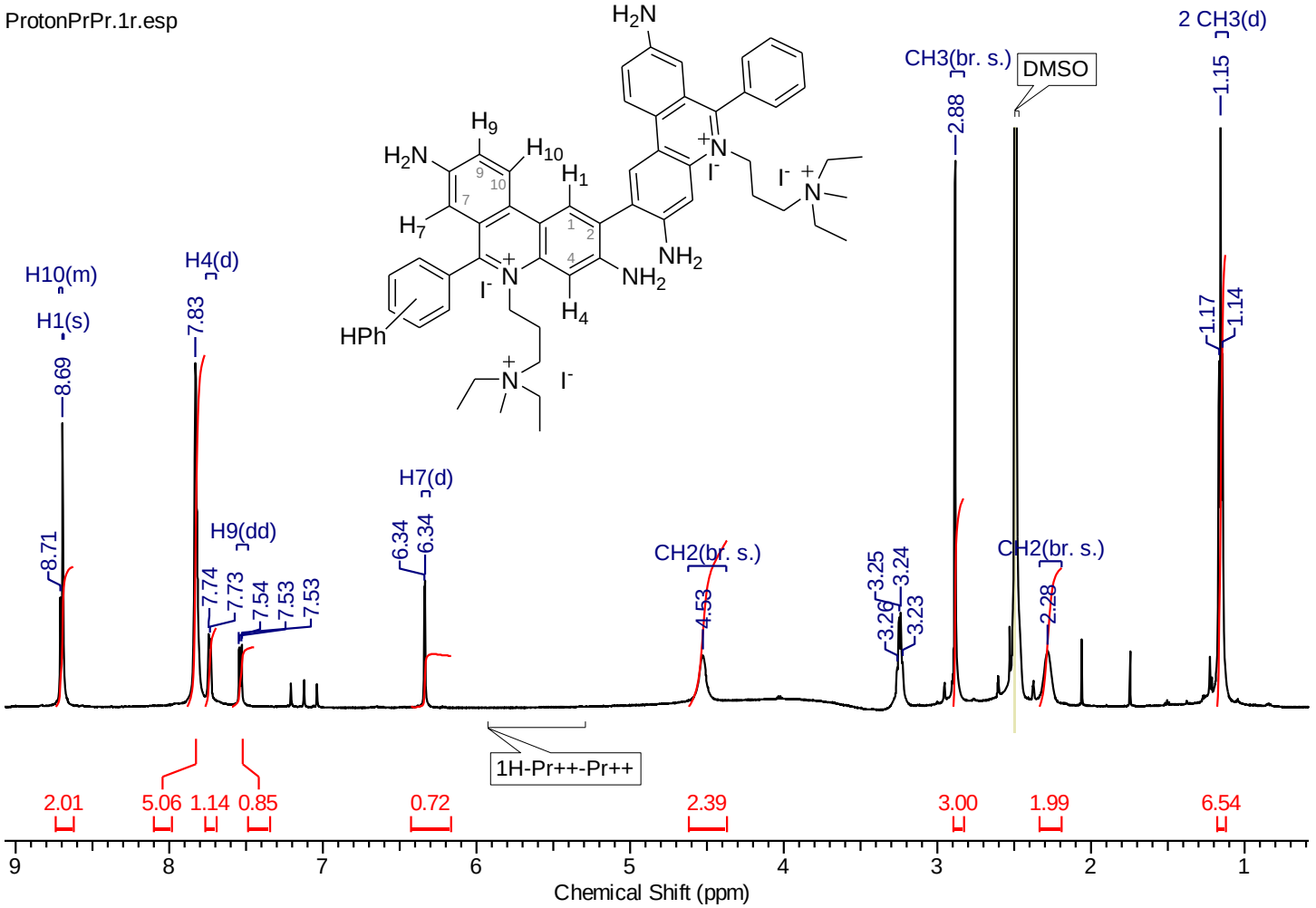
Supplementary Figure 3d. ^{1}H-^{13}C HMBC NMR spectrum of propidium (Pr^{++}) in DMSO-*d6*.



Supplementary Figure 3d. ^{1}H-^{13}C HMBC NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed).

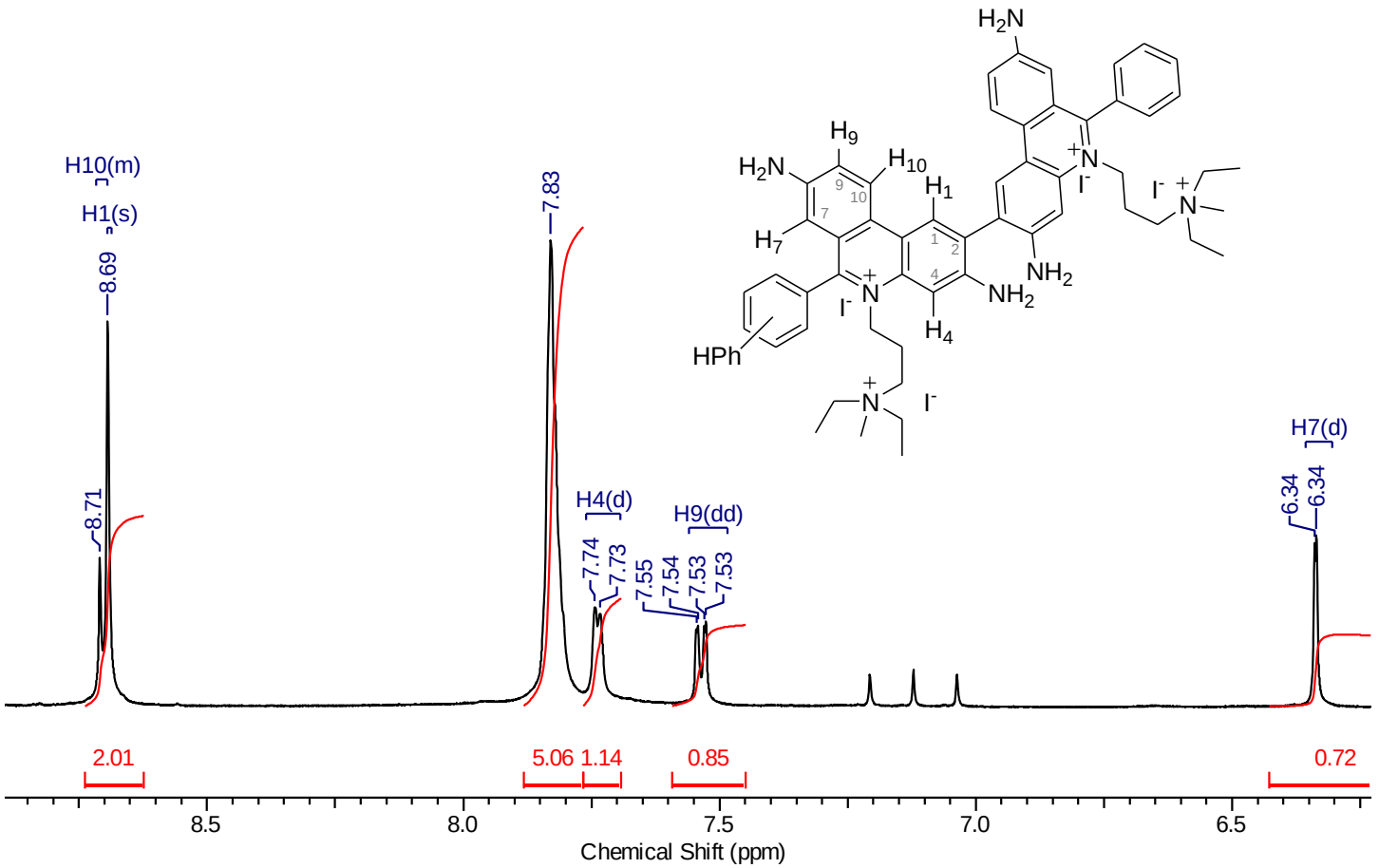


Supplementary Figure 3d. ^{1}H-^{13}C HMBC NMR spectrum of propidium (Pr^{++}) in DMSO-*d6* (zoomed).

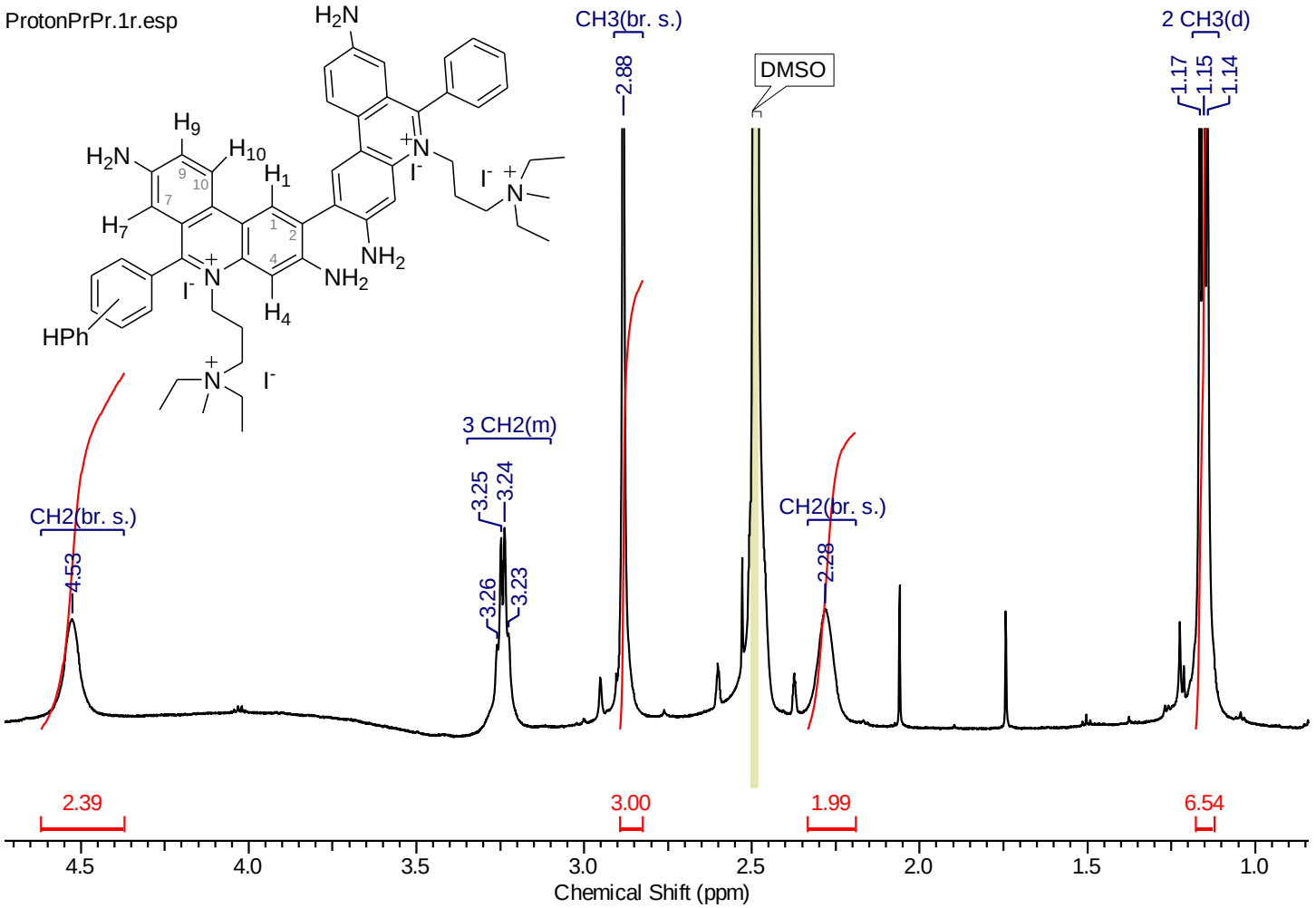


Supplementary Figure 4a. ^{1}H NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6*.

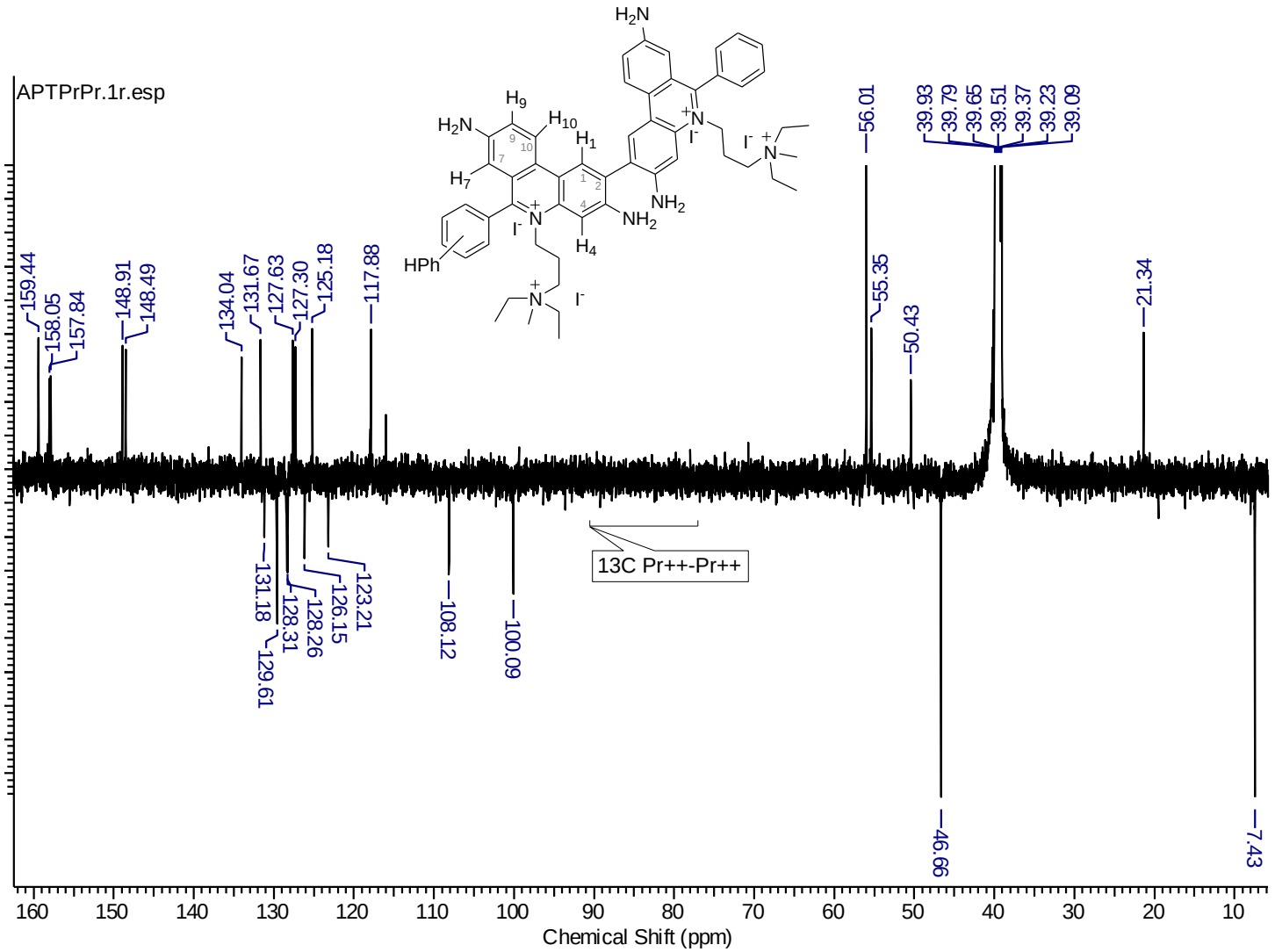
ProtonPrPr.1r.esp



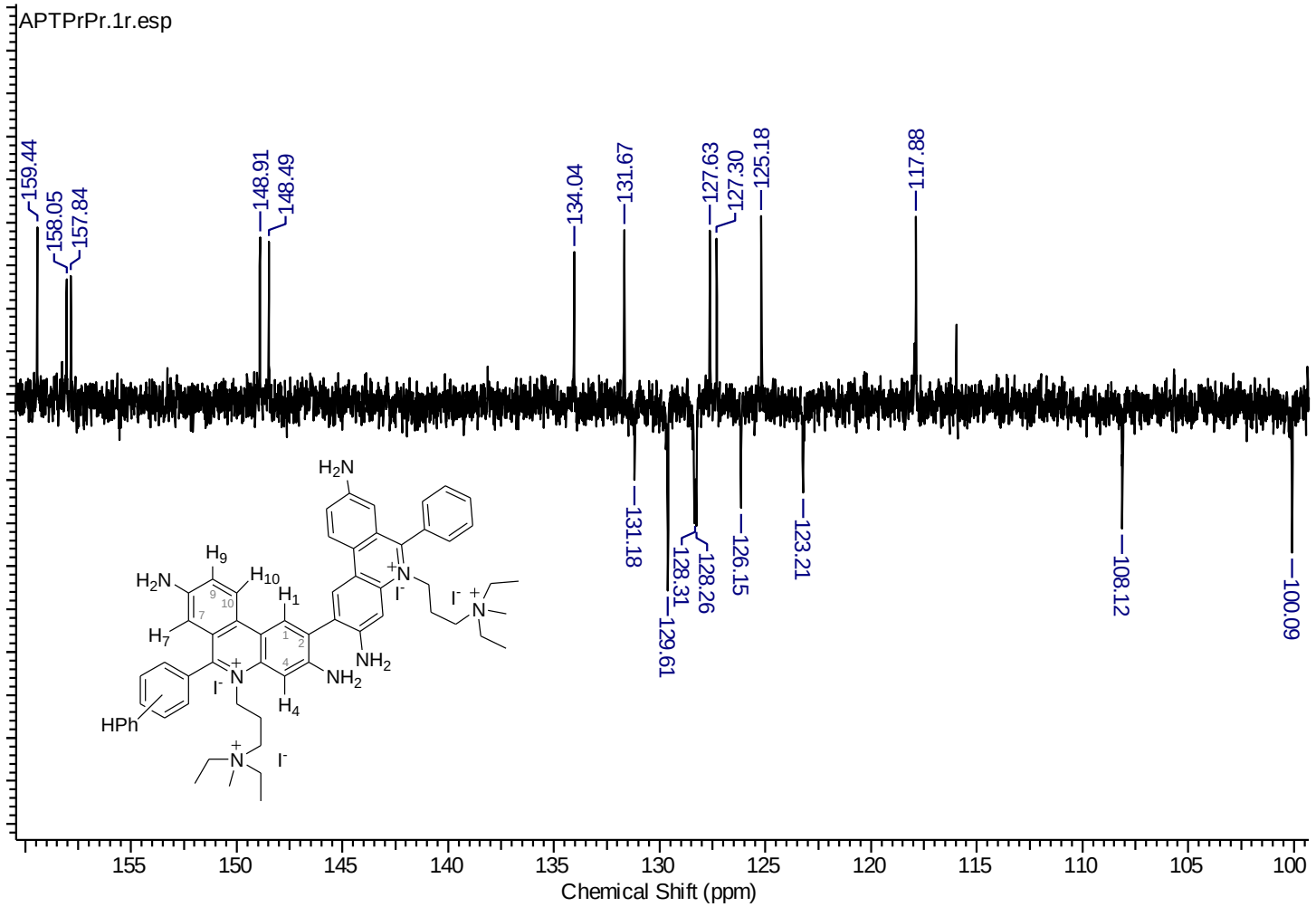
Supplementary Figure 4a. ^{1}H NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed region 6.3 – 8.8 ppm).



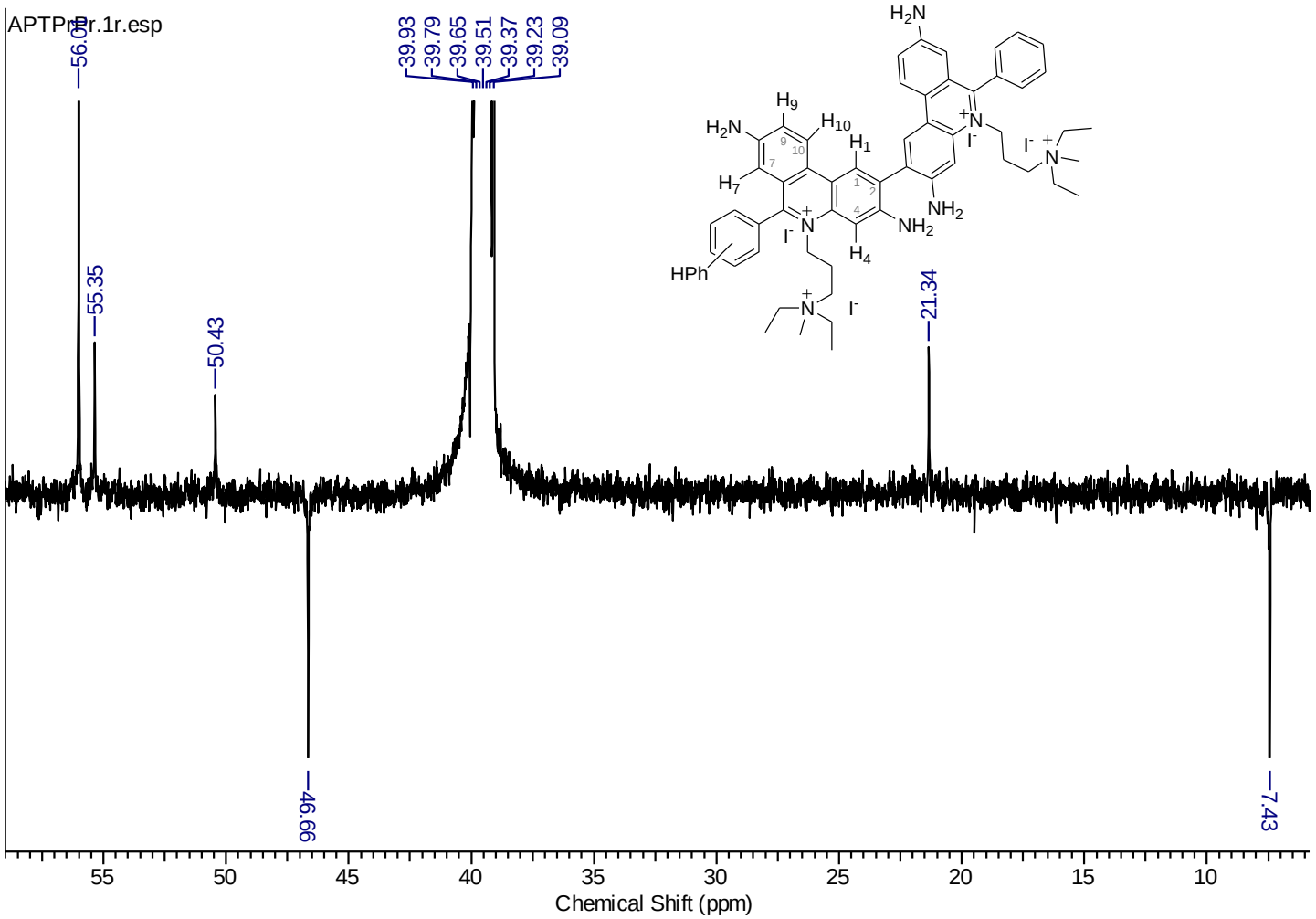
Supplementary Figure 4a. ^{1}H NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed region 0.9 – 4.6 ppm).



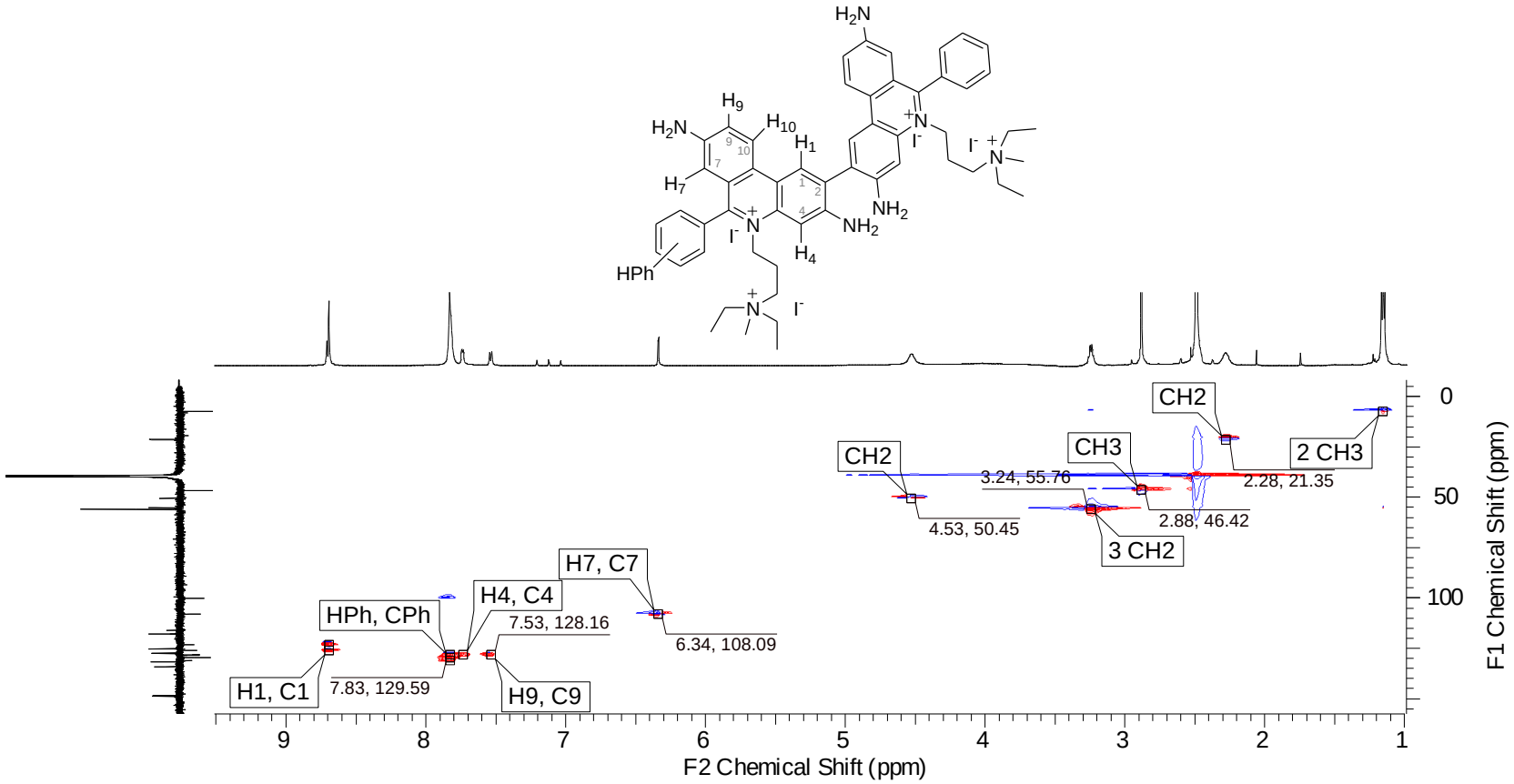
Supplementary Figure 4b. ^{13}C APT NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6*.



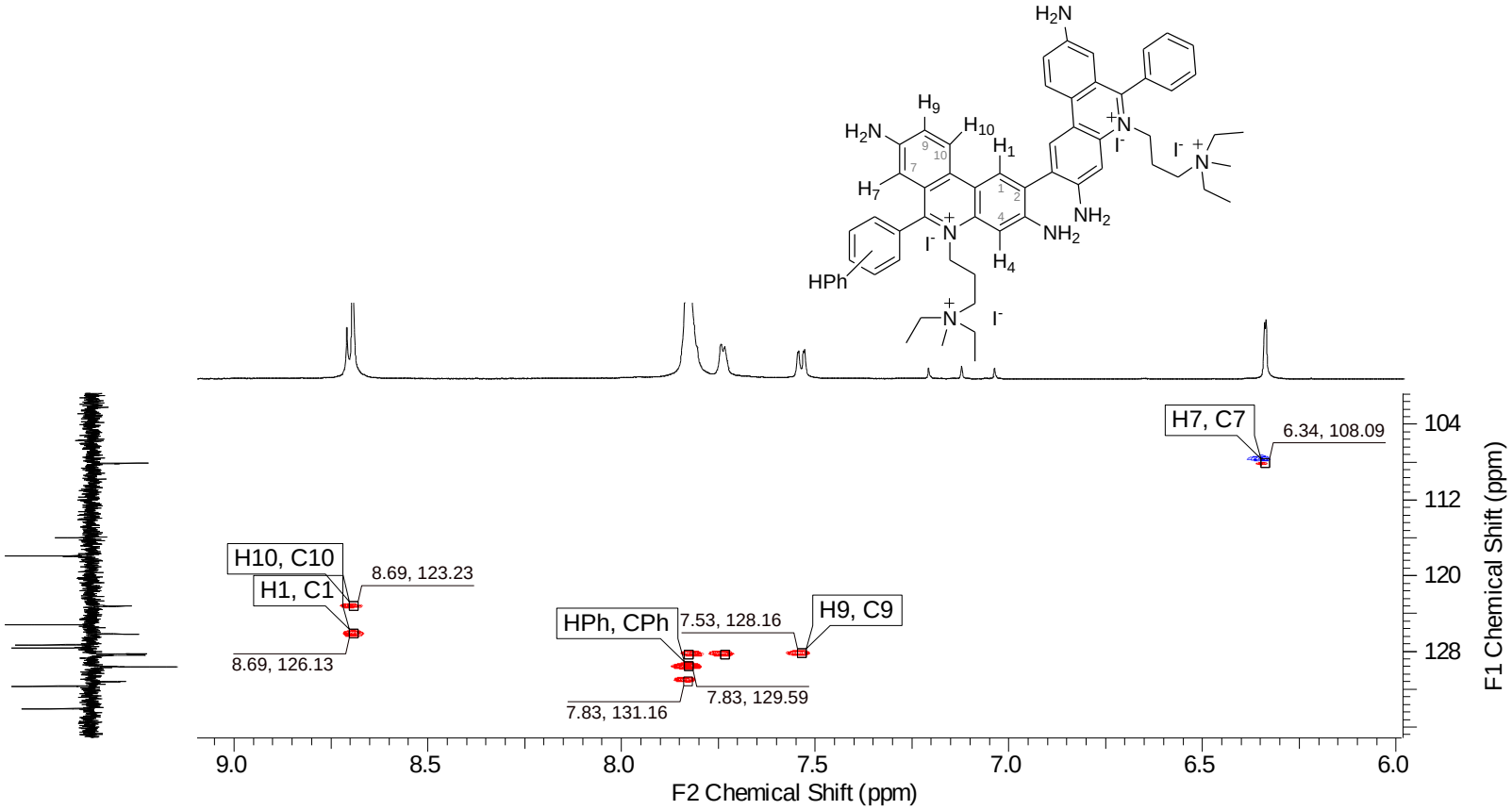
Supplementary Figure 4b. ^{13}C APT NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed region 99.5 – 160.5 ppm).



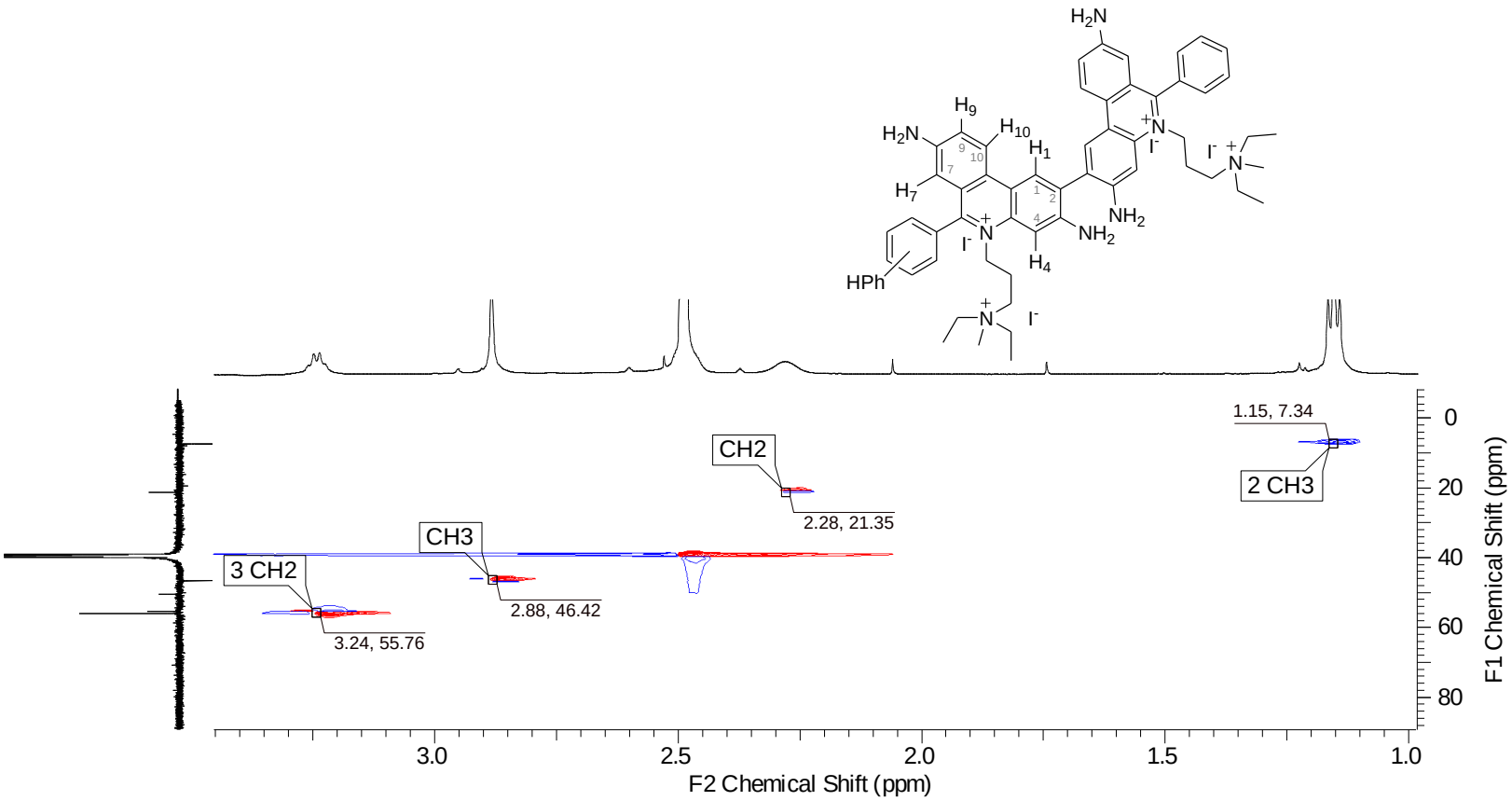
Supplementary Figure 4b. ^{13}C APT NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed region 6 – 58 ppm).



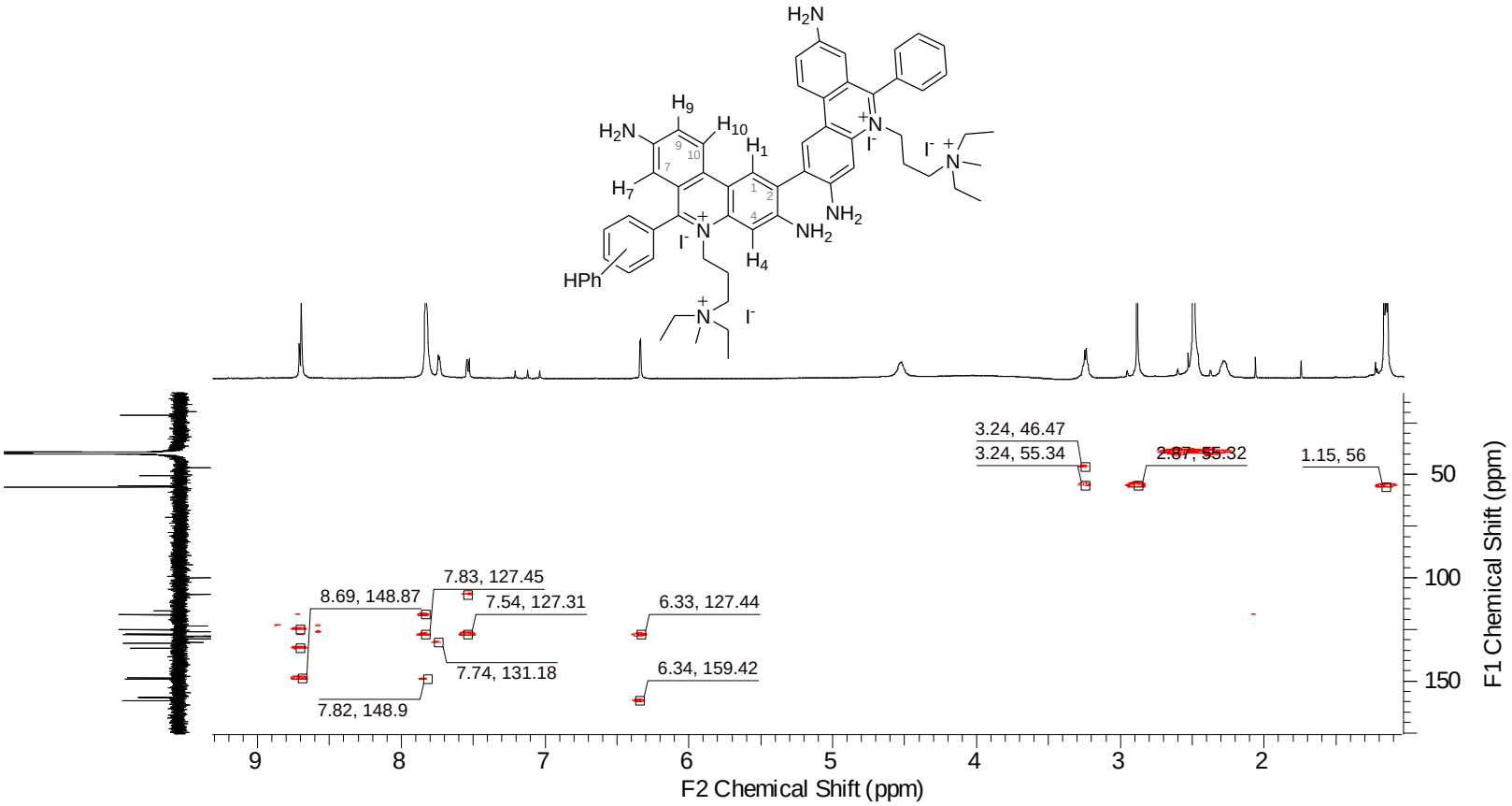
Supplementary Figure 4c. ^{1}H-^{13}C HSQC NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6*.



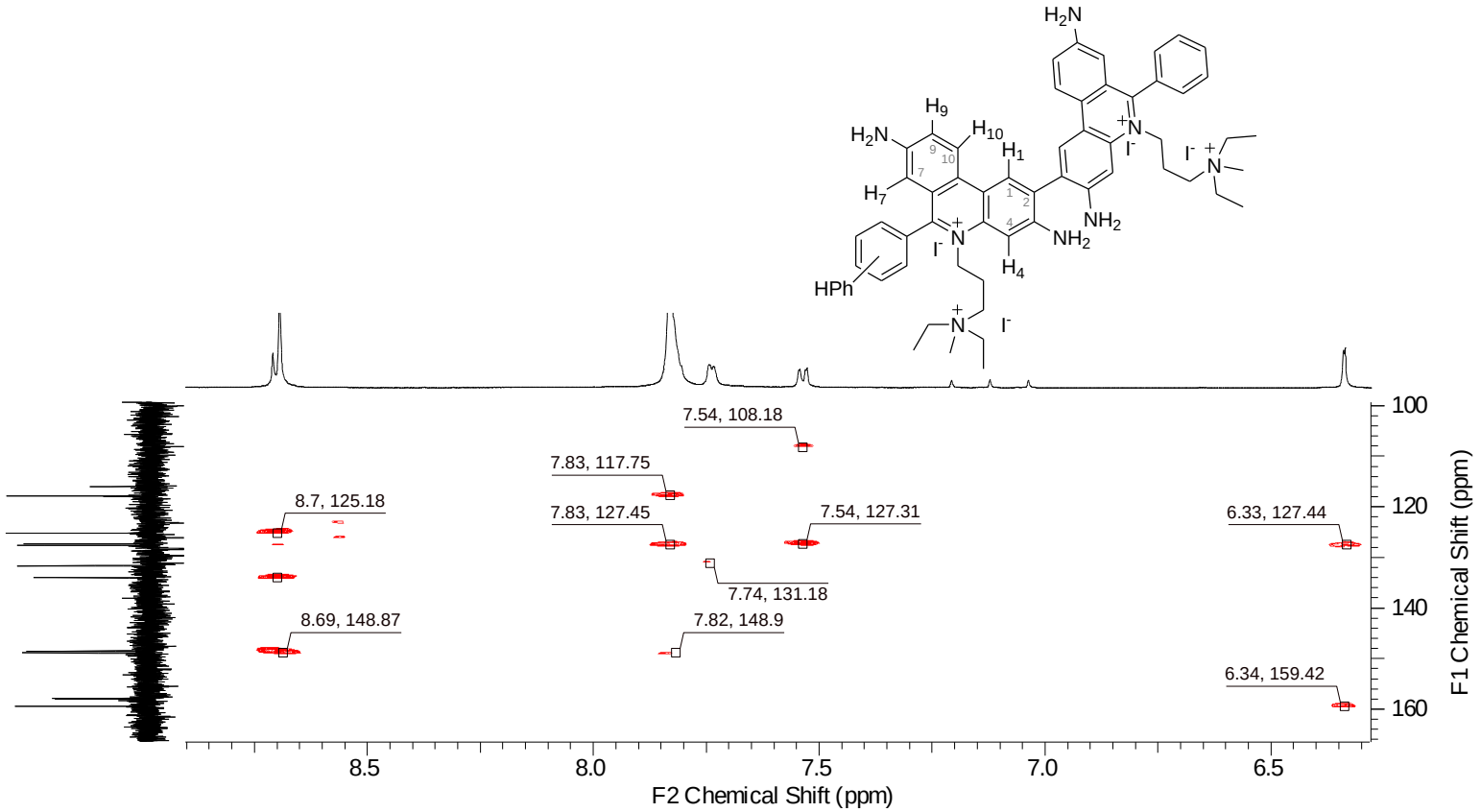
Supplementary Figure 4c. ^{1}H-^{13}C HSQC NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed).



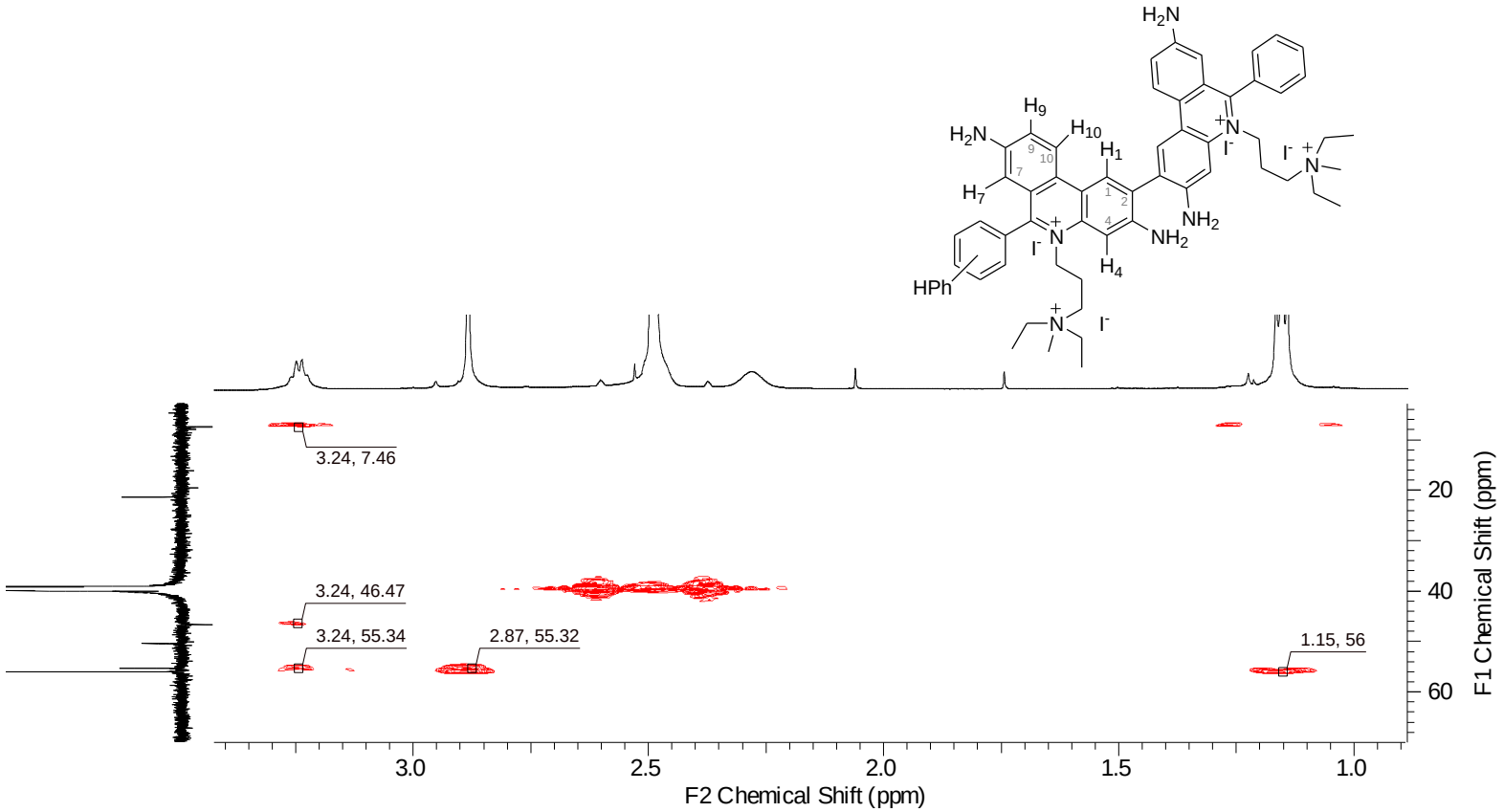
Supplementary Figure 4c. ^{1}H-^{13}C HSQC NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed).



Supplementary Figure 4d. ^{1}H-^{13}C HMBC NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6*.



Supplementary Figure 4d. ^{1}H-^{13}C HMBC NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed).



Supplementary Figure 4d. ^{1}H-^{13}C HMBC NMR spectrum of dipropidium (Pr^{++}-Pr^{++}) in DMSO-*d6* (zoomed).