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Synthesis and characterization of bichromophoric 1-deoxyceramides as FRET probes

Content

Table S1. Spectral overlap and Förster distances

Calculated spectral overlap integrals and Förster critical distances for the two possible donor-acceptor pairs in DMSO, EtOH and PBS buffer.

Fluorescent partners	Solvent	Spectral overlap integral ($J(\lambda)$) ($nm^4 \cdot M^{-1} \cdot cm^{-1}$)	R_0 (Å)
Donor: **RBM5-142** (MCC) Acceptor: **RBM5-154** (NBD)	DMSO	2.79E+14	23.5
	EtOH	2.34E+14	35.1
	PBS	1.61E+14	22.0
Donor: **RBM5-154** (NBD) Acceptor: **RBM5-143** (NR)	DMSO	2.77E+15	36.0
	EtOH	2.60E+15	46.8
	PBS	2.23E+15	39.1

Due to solubility issues, compound **RBM5-154** was used as a surrogate of the doxdhCer **RBM5-159**.

Table S2: Photophysical properties of the monochromophoric compounds

[a] Wavelength of maximum absorption (nm). [b] Molar extinction coefficients (ε) were calculated at the λ_{max}^{Abs} following Lambert-Beer's law. [c]Wavelength of the emission maximum upon excitation at the λ_{ex} indicated in parentheses. [d]Dulbecco's phosphate saline (PBS) buffer solution containing 1 % DMSO and 0.1 % Triton X-100. Fluorescence quantum yields (Φ) were determined according to the comparative method[3] using either [e]quinine sulfate (λ_{ex} = 340 nm, Φ = 0.546 in 0.5 M aq. H_2SO_4), [f]fluorescein (λ_{ex} = 470 nm, Φ_F = 0.91 in 0.01 M aq. NaOH) or [g]rhodamine B (λ_{ex} = 510 nm, Φ = 0.7 in EtOH) as a standard.

Compound	Solvent	λ_{max}^{Abs} [a]	ε ($M^{-1}\cdot cm^{-1}$) [b]	λ_{max}^{Em} (λ_{ex}) [c]	Φ
RBM5-142	DMSO	346	21,296	404 (340)	0.07 [e]
	EtOH	348	45,370	399 (340)	0.70 [e]
	PBS[d]	350	20,558	403 (340)	0.06 [e]
RBM5-143	DMSO	550	41,242	626 (510)	0.43 [g]
	EtOH	548	40,269	629 (510)	0.28 [g]
	PBS[d]	550	30,244	631 (510)	0.21 [g]
RBM5-154	DMSO	476	21,543	539 (470)	0.10 [f]
	EtOH	465	25,882	529 (470)	0.35 [f]
	PBS[d]	475	16,121	535 (470)	0.13 [f]
RBM5-159	EtOH	466	24,959	529 (470)	0.34 [f]

Table S3. Photophysical properties of the bichromophoric compounds

Compounds RBM5-160 and RBM5-161: [a]Wavelength of the absorption maximum (nm). [b] Molar extinction coefficients (ε) at the λ_{max}^{Abs}. [c]Wavelength of the emission maximum upon excitation at the λ_{ex} indicated in parentheses. [d]The ε of the compounds **RBM5-160** and **RBM5-161** could not be determined in PBS due to a lack of linearity between Abs and concentration, allegedly due to solubility issues.

Compound	Solvent	λ_{max}^{Abs} [a]	ε ($M^{-1}\cdot cm^{-1}$) [b]	λ_{max}^{Em} (λ_{ex}) [c]
RBM5-160	DMSO	348	25,594	403/538 (340)
		469	17,466	
	EtOH	345	26,928	399/529 (340)
		466	19,244	
	PBS	349	- [d]	399/544 (340)
		484	- [d]	
RBM5-161	DMSO	495	29,482	543/625 (470)
		551	35,577	
	EtOH	479	23,736	528/629 (470)
		549	30,389	
	PBS	492	- [d]	630 (470)
		548	- [d]	

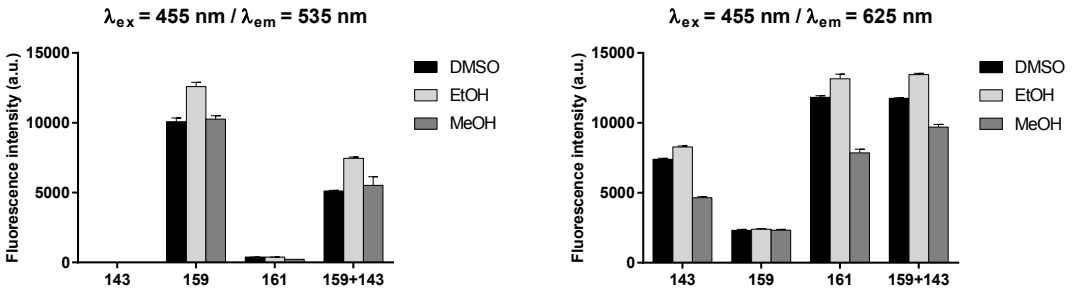


Figure S1.

Bar diagram representing the fluorescence emission at 535 nm (corresponding to NBD, left) and 625 nm (corresponding to NR, right), resulting from the excitation at 455 nm (NBD), of a reaction mixture containing the compounds **RBM5-159** (25 μM) and **RBM5-143** (50 μM) after 1 h at 37 ºC in DMSO (black), EtOH (light grey) and MeOH (dark grey). The corresponding starting materials (**RBM5-159** and **RBM5-143** at 25 μM) were used as the negative controls (0 % conversion), whereas the cycloadduct **RBM5-161** (25 μM) was used as the positive control (100 % conversion). The results correspond to the mean ± standard deviation of at least two independent experiments with triplicates.

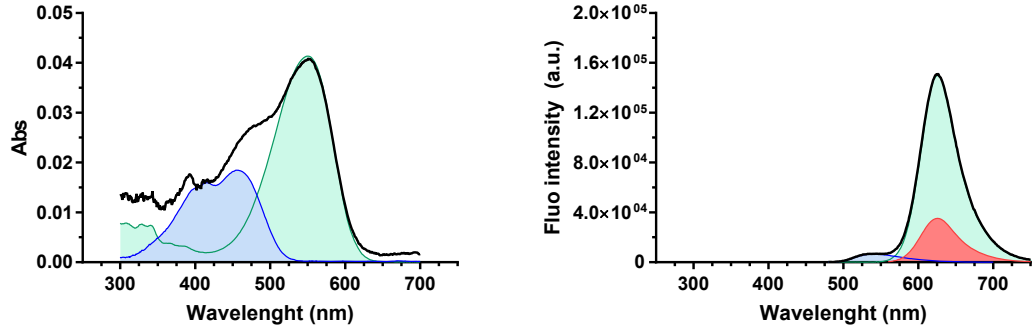


Figure S2

Deconvoluted absorption spectrum (left) and emission spectrum (right) upon excitation at 470 nm of compound **RBM5-161** at 1 µM in DMSO upon spectral deconvolution. Legend: total absorption (or emission) spectrum (black), absorption (or emission) of the donor component (blue), absorption (or emission) of the acceptor component (green), AEB ≡ emission of the acceptor component as a result of its direct excitation at 470 nm (red). Note in the left panel that there is an evident overlap between the absorption bands of the donor and the acceptor between 450-500 nm which is responsible for the appearance of AEB (see also Table S4).

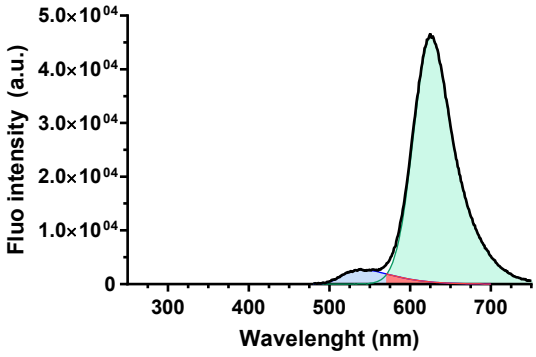


Figure S3

Emission spectrum (excitation at 470 nm) of compound **RBM5-161** at 0.5 µM in DMSO upon spectral deconvolution. Legend: total emission spectrum (black), emission of the donor component (blue), DEB ≡ emission of the donor component within the acceptor-specific wavelength interval [570,700 nm] (red), emission of the acceptor component (green) (see also Table S4).

Table S4. Study of the intramolecular FRET process of the bichromophoric compounds RBM5-160 and RBM5-161

Compound	Solvent	λ_{ex} (nm)	DEB[a] (%)	AEB[b] (%)
RBM5-161	DMSO	470	1.74	46.14
		455	1.92	39.01
	EtOH	470	1.73	36.28
		455	1.93	24.44

[a] Donor emission bleed-through (emission cross-talk) is expressed as a percentage over the total integrated fluorescence intensity observed within the acceptor-specific emission wavelength interval. [b] Acceptor emission bleed-through is expressed as a percentage over the total integrated fluorescence intensity observed within the acceptor-specific emission wavelength interval (see also Figure S2 and S3)

References

(1) Briggs, M. S. J.; Bruce, I.; Miller, J. N.; Moody, C. J.; Simmonds, A. C.; Swann, E. *J. Chem. Soc. Perkin Trans. 1* **1997**, No. 7, 1051–1058.

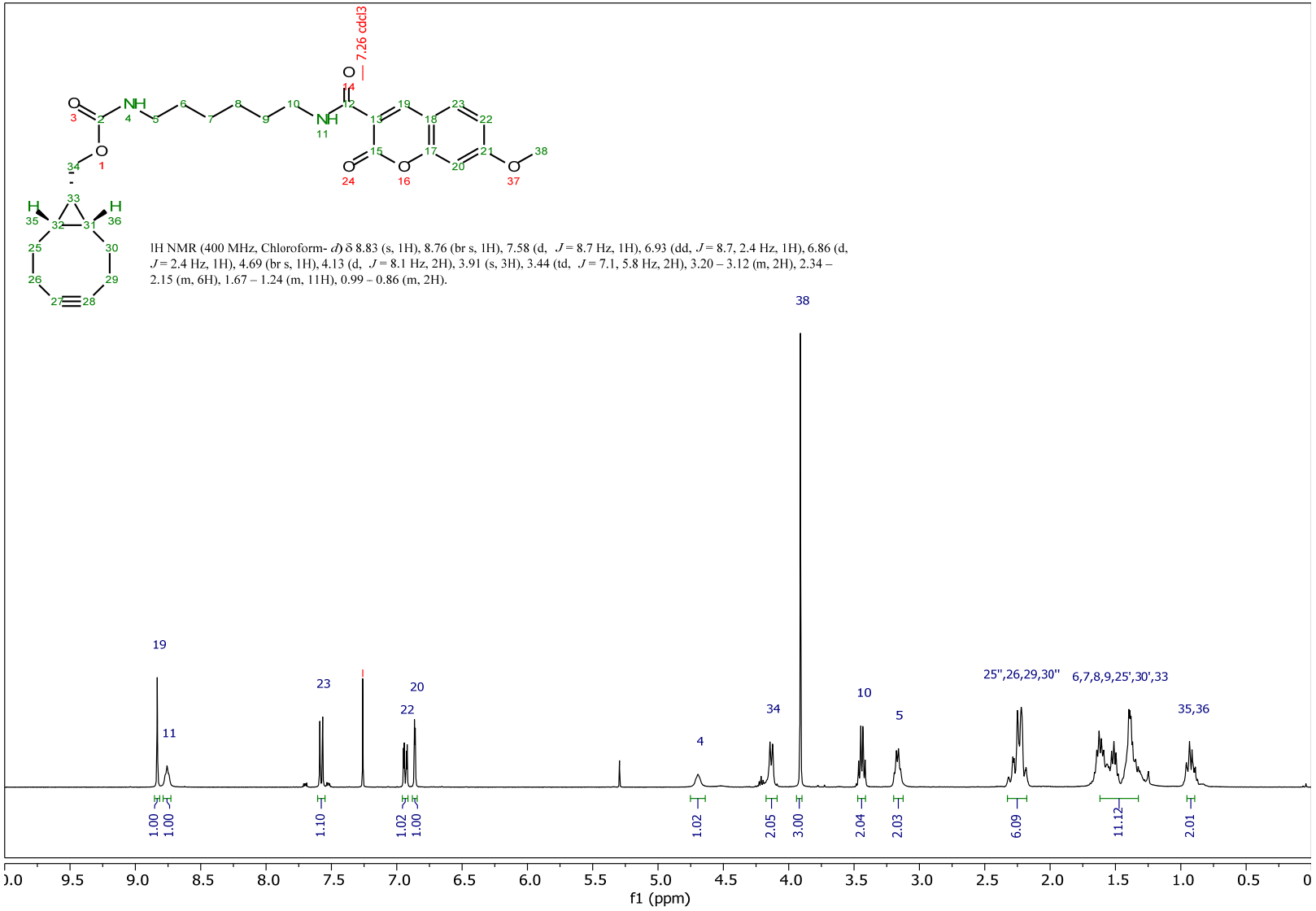
(2) Pinkert, T.; Furkert, D.; Korte, T.; Herrmann, A.; Arenz, C. *Angew. Chem. Int. Ed.* **2017**, *56* (10), 2790–2794.

(3) Resch-Genger, U.; Rurack, K. *Pure Appl. Chem.* **2013**, *85* (10), 2005–2013.

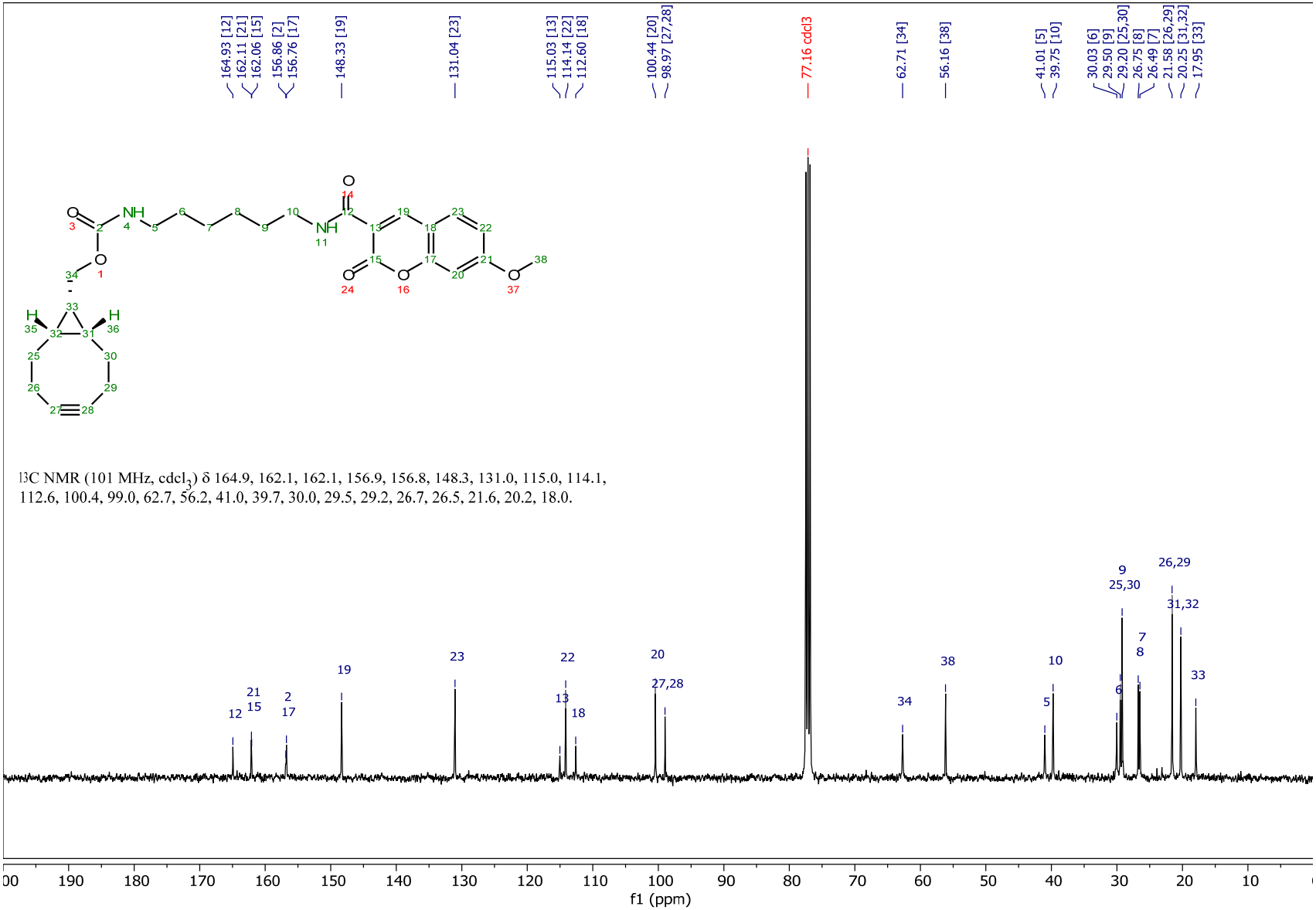
NMR of the synthesized compounds:

Compound RBM5-142

^{1}H NMR

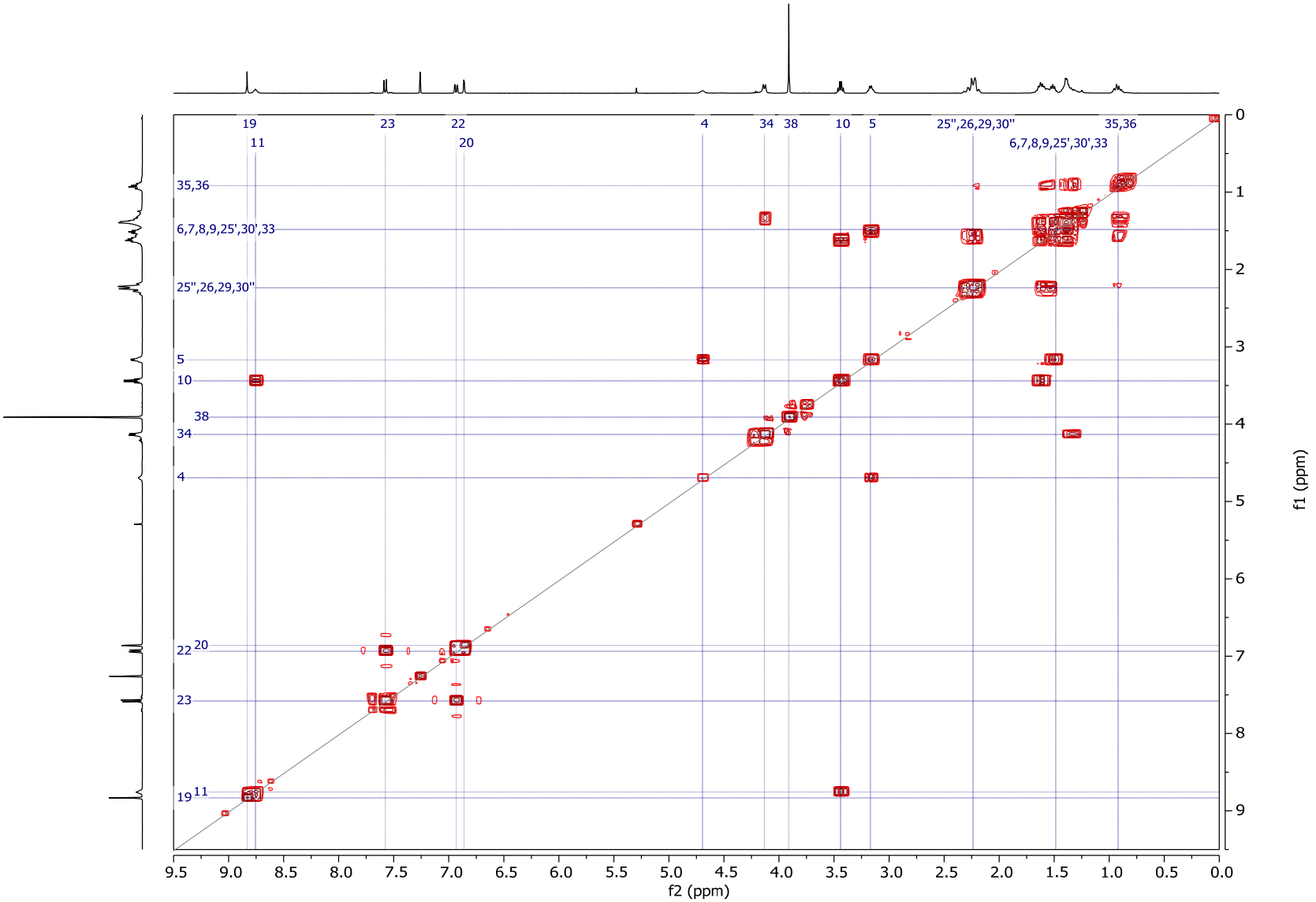


^{13}C NMR; RBM5-142

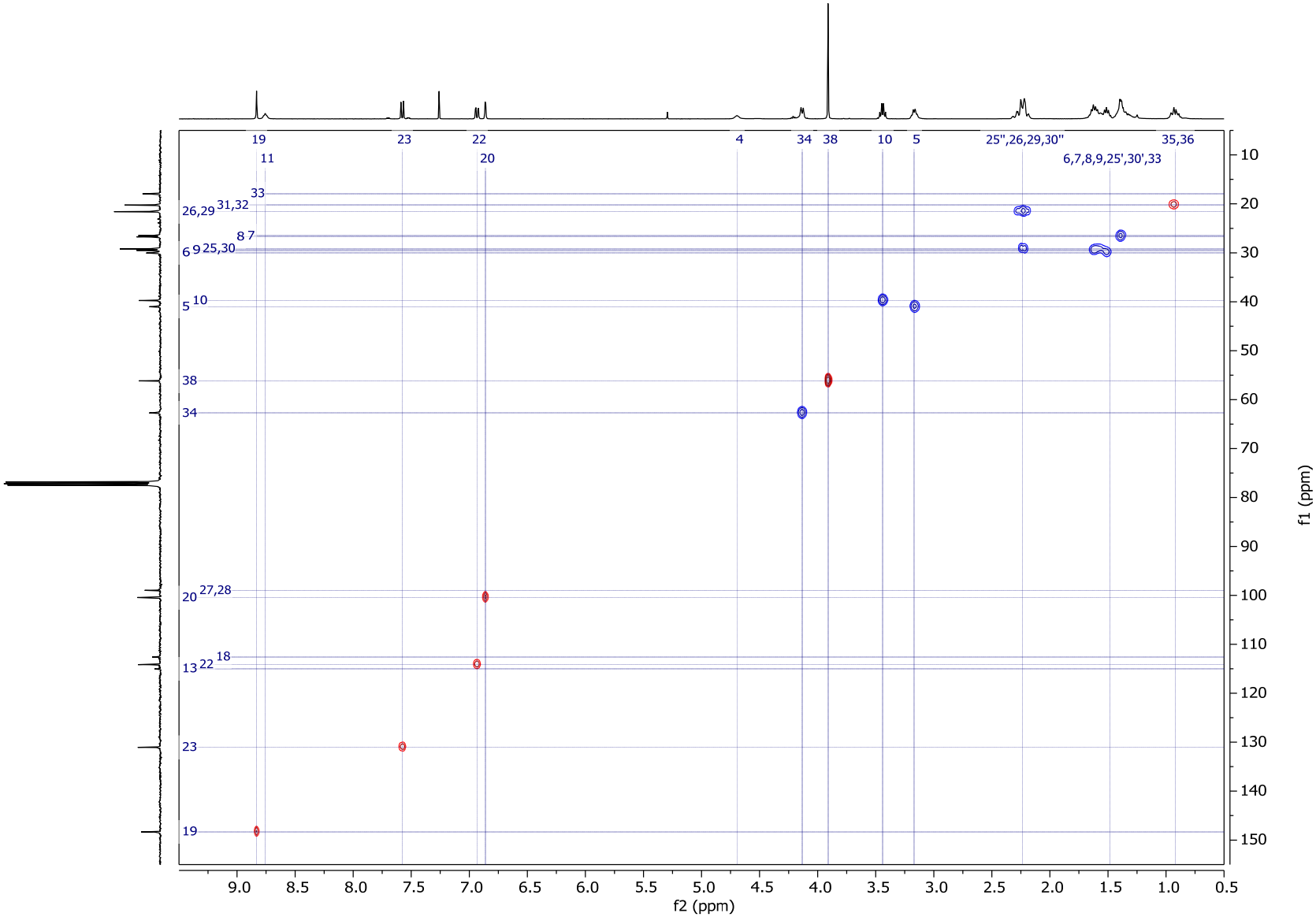


^{13}C NMR (101 MHz, cdcl$_3$) δ 164.9, 162.1, 162.1, 156.9, 156.8, 148.3, 131.0, 115.0, 114.1, 112.6, 100.4, 99.0, 62.7, 56.2, 41.0, 39.7, 30.0, 29.5, 29.2, 26.7, 26.5, 21.6, 20.2, 18.0.

COSY NMR; RBM5-142

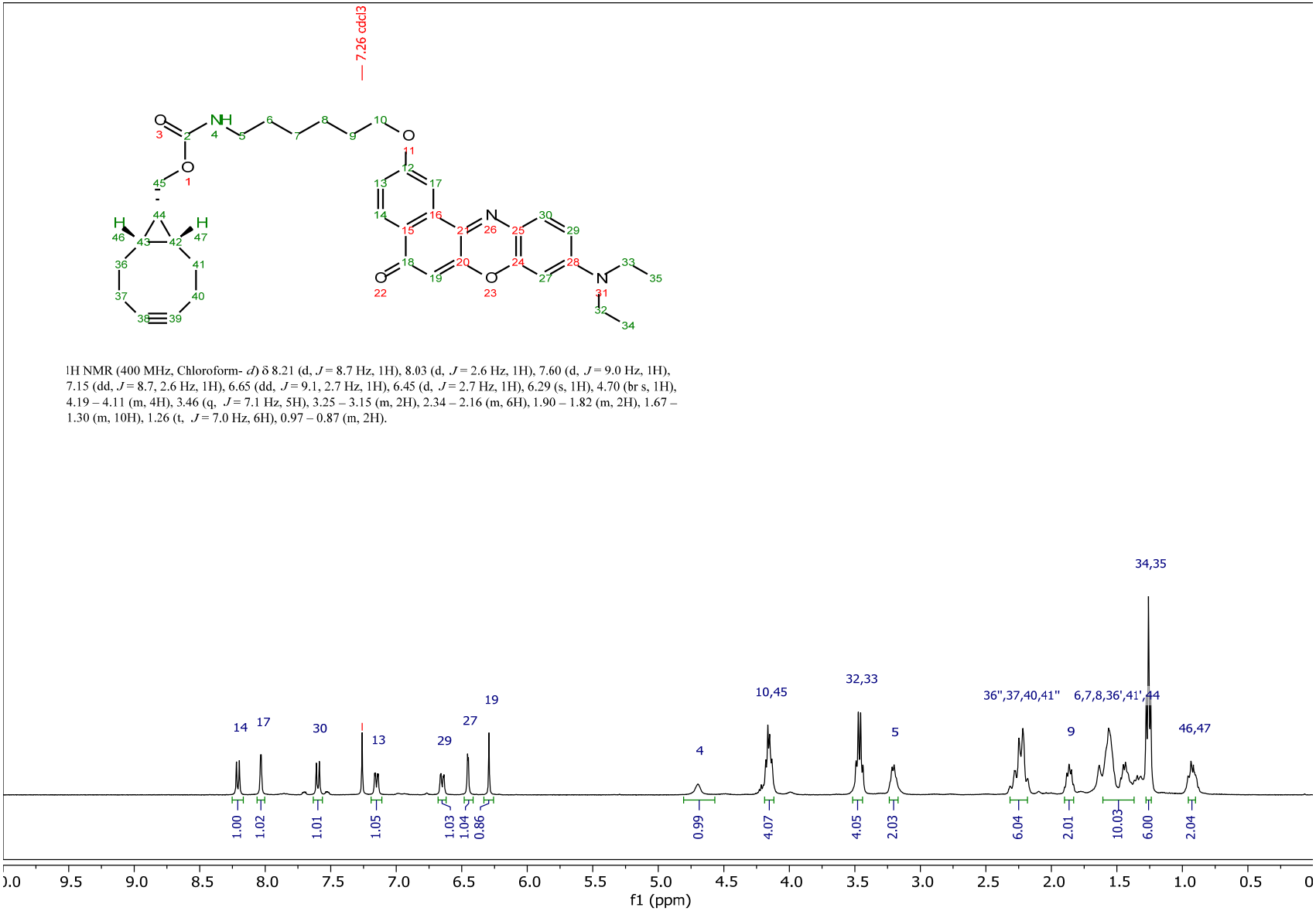


HSQC NMR; RBM5-142

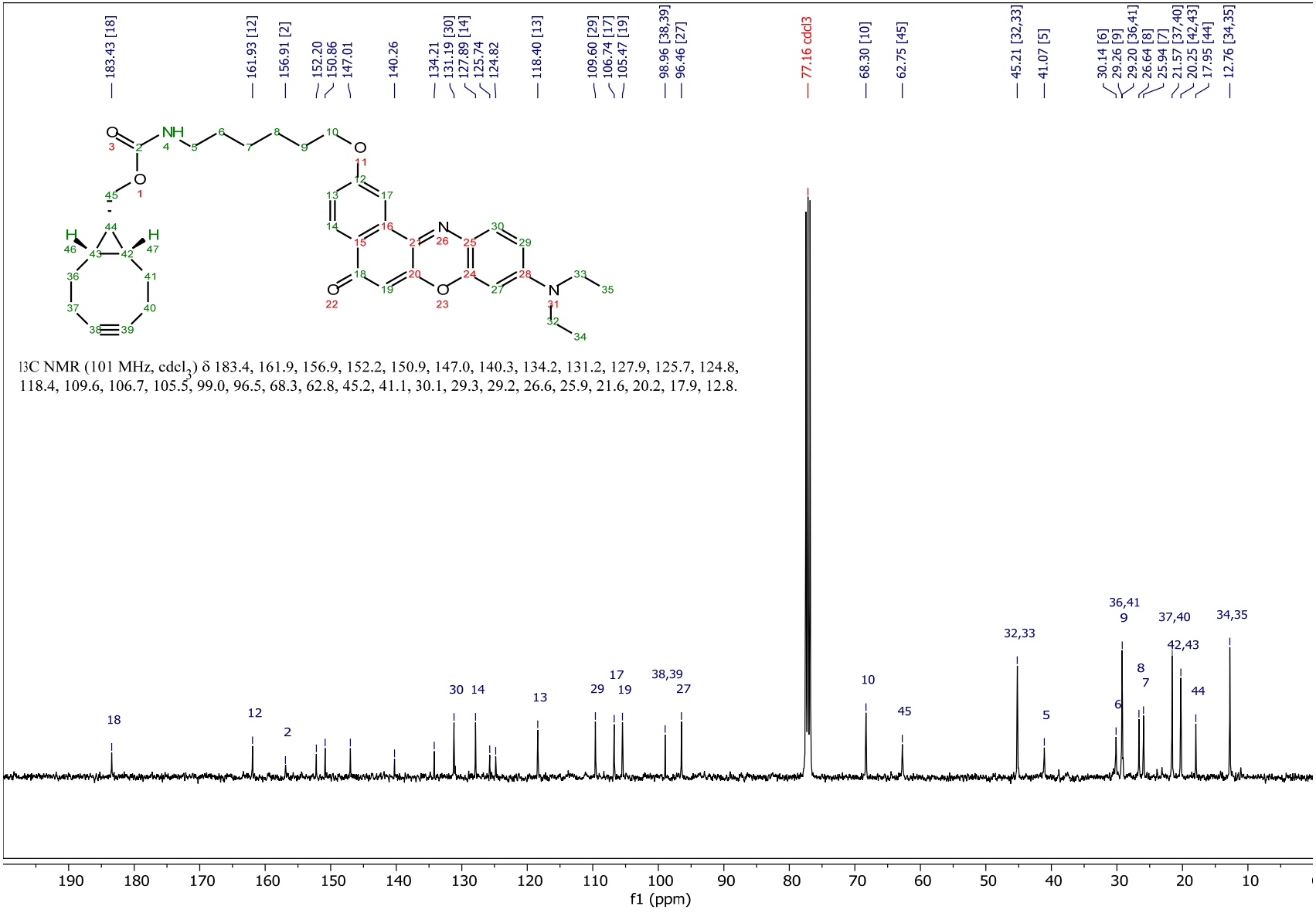


Compound RBM5-143

^{1}H NMR

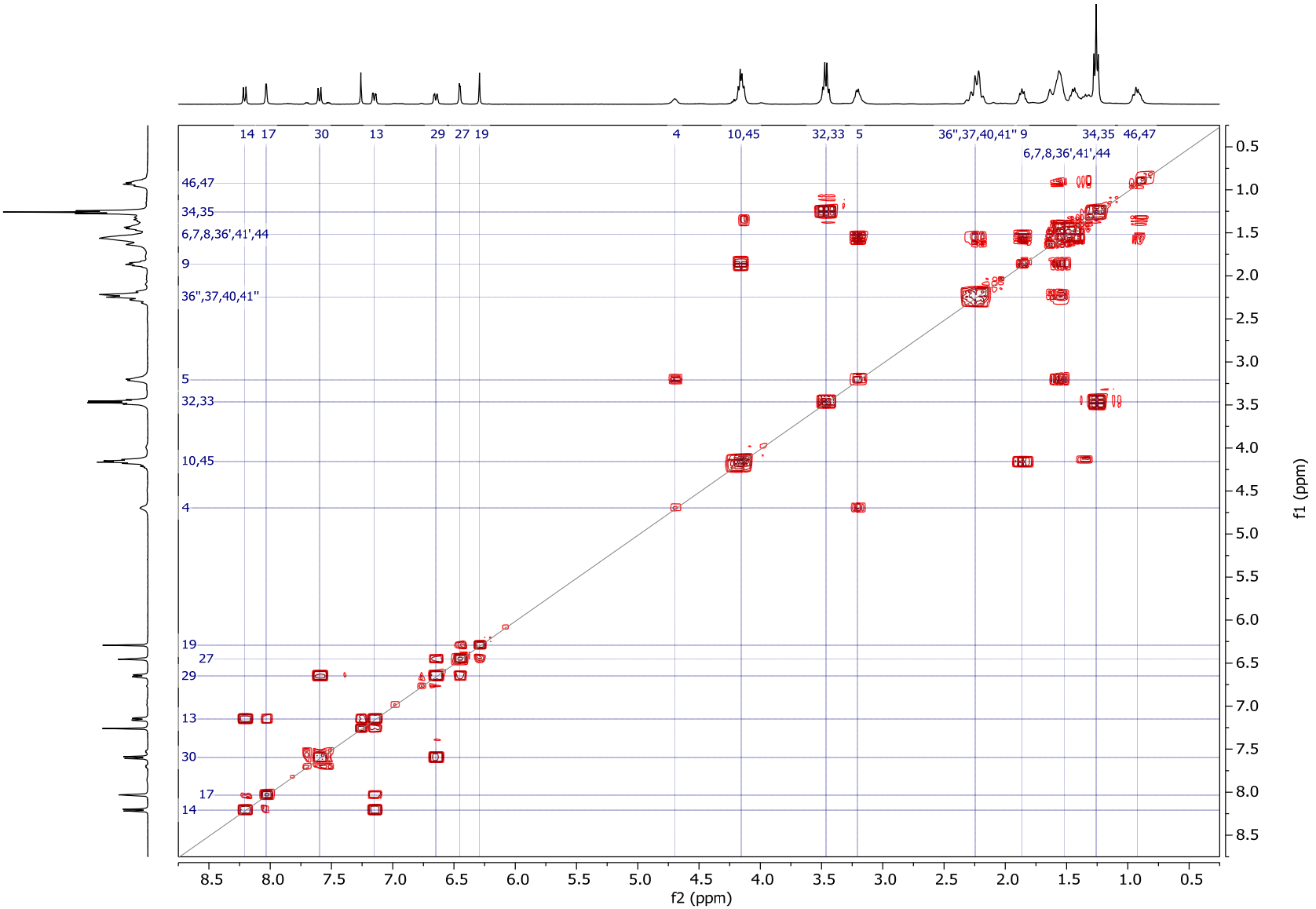


^{13}C NMR; RBM5-143

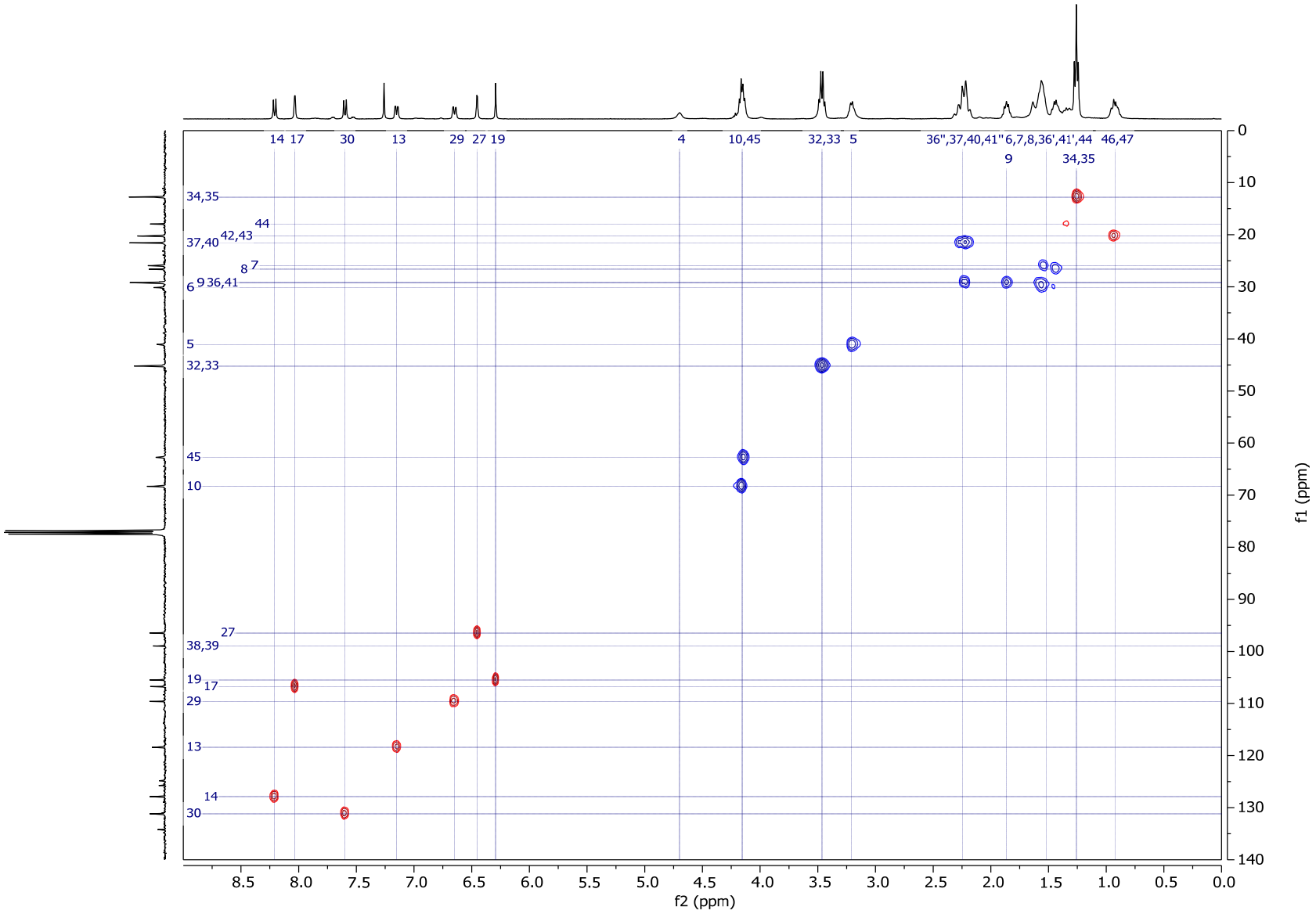


^{13}C NMR (101 MHz, cdcl$_3$) δ 183.4, 161.9, 156.9, 152.2, 150.9, 147.0, 140.3, 134.2, 131.2, 127.9, 125.7, 124.8, 118.4, 109.6, 106.7, 105.5, 99.0, 96.5, 68.3, 62.8, 45.2, 41.1, 30.1, 29.3, 29.2, 26.6, 25.9, 21.6, 20.2, 17.9, 12.8.

COSY NMR; RBM5-143

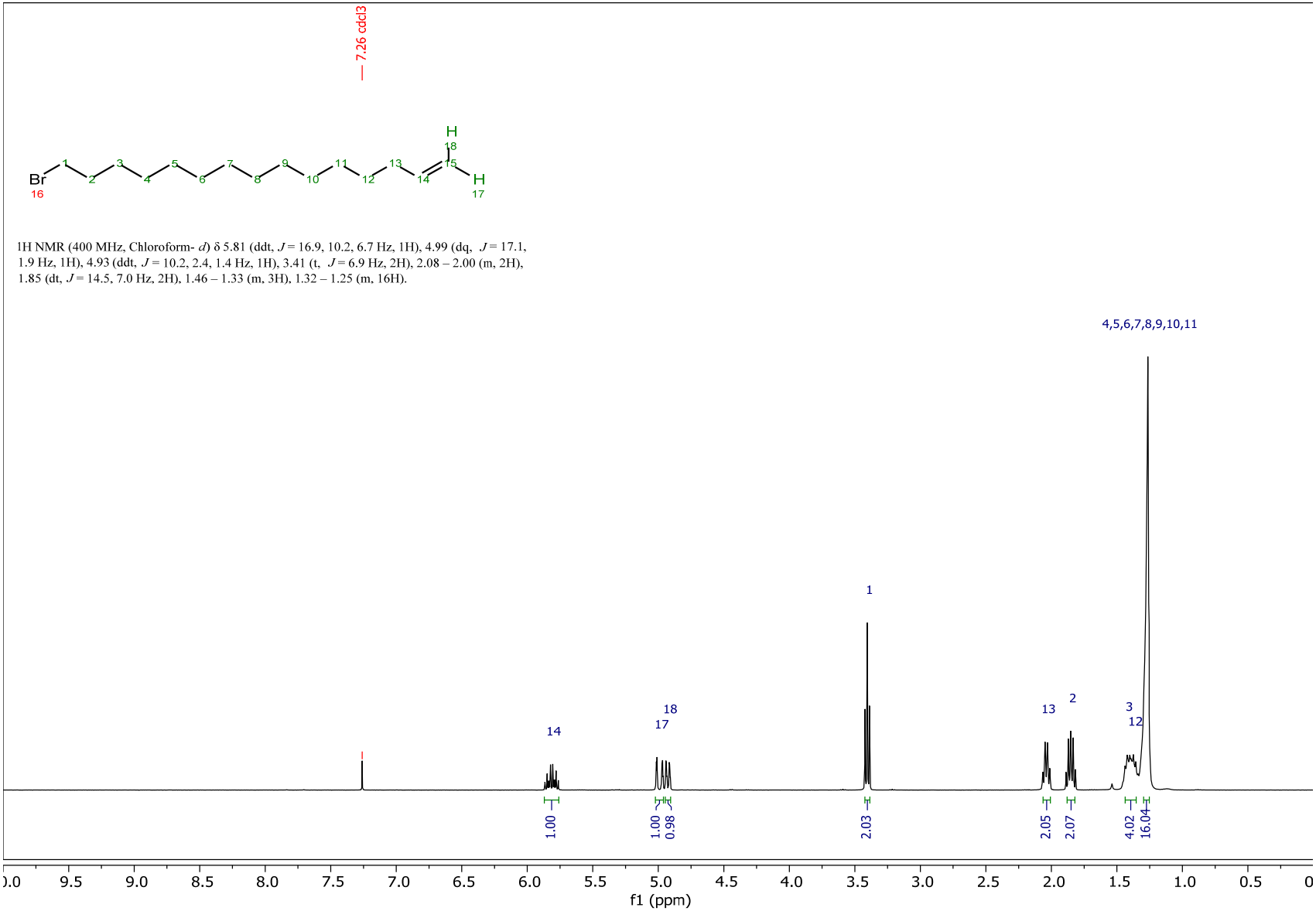


HSQC NMR; RBM5-143

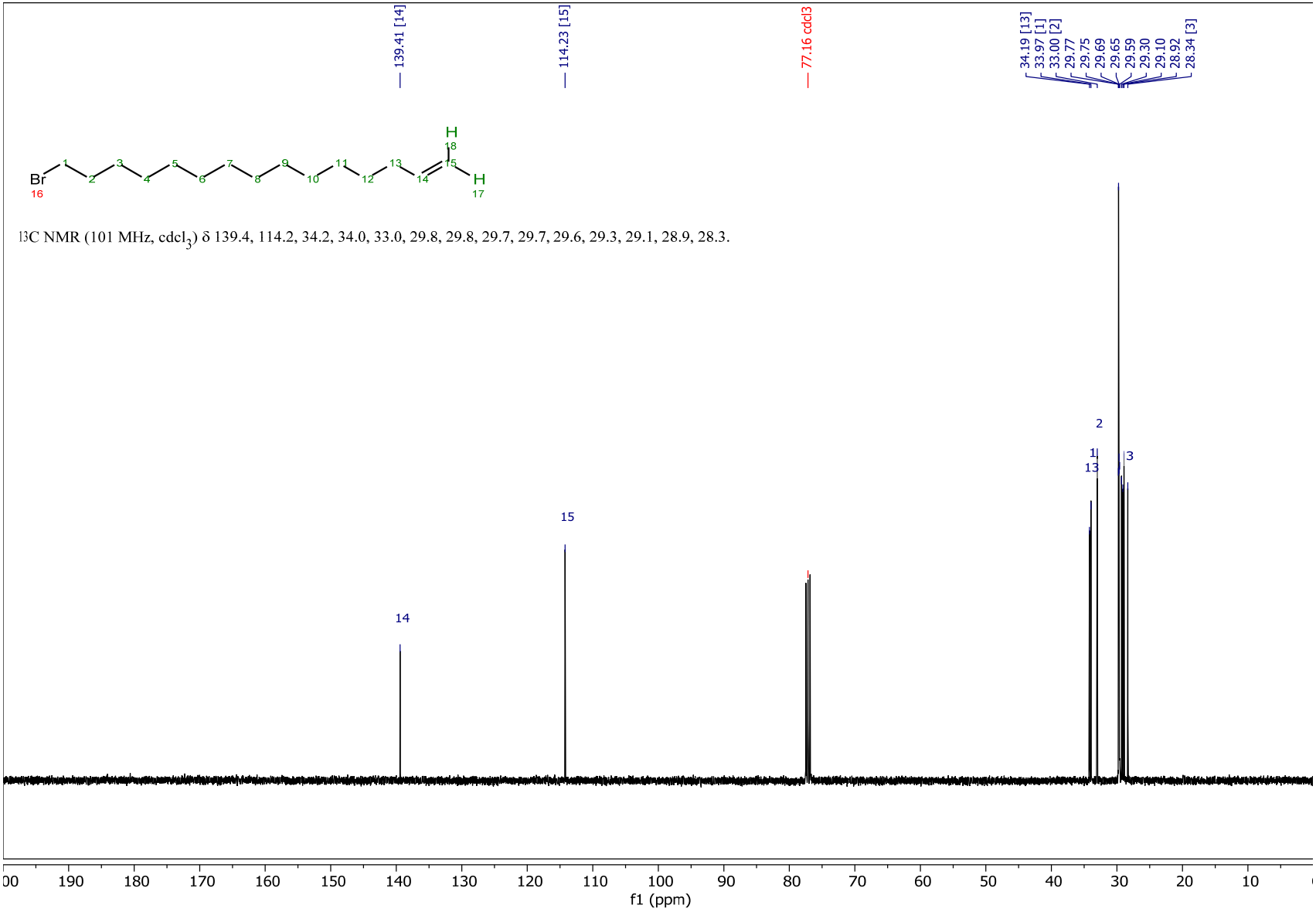


Compound RBM5-149

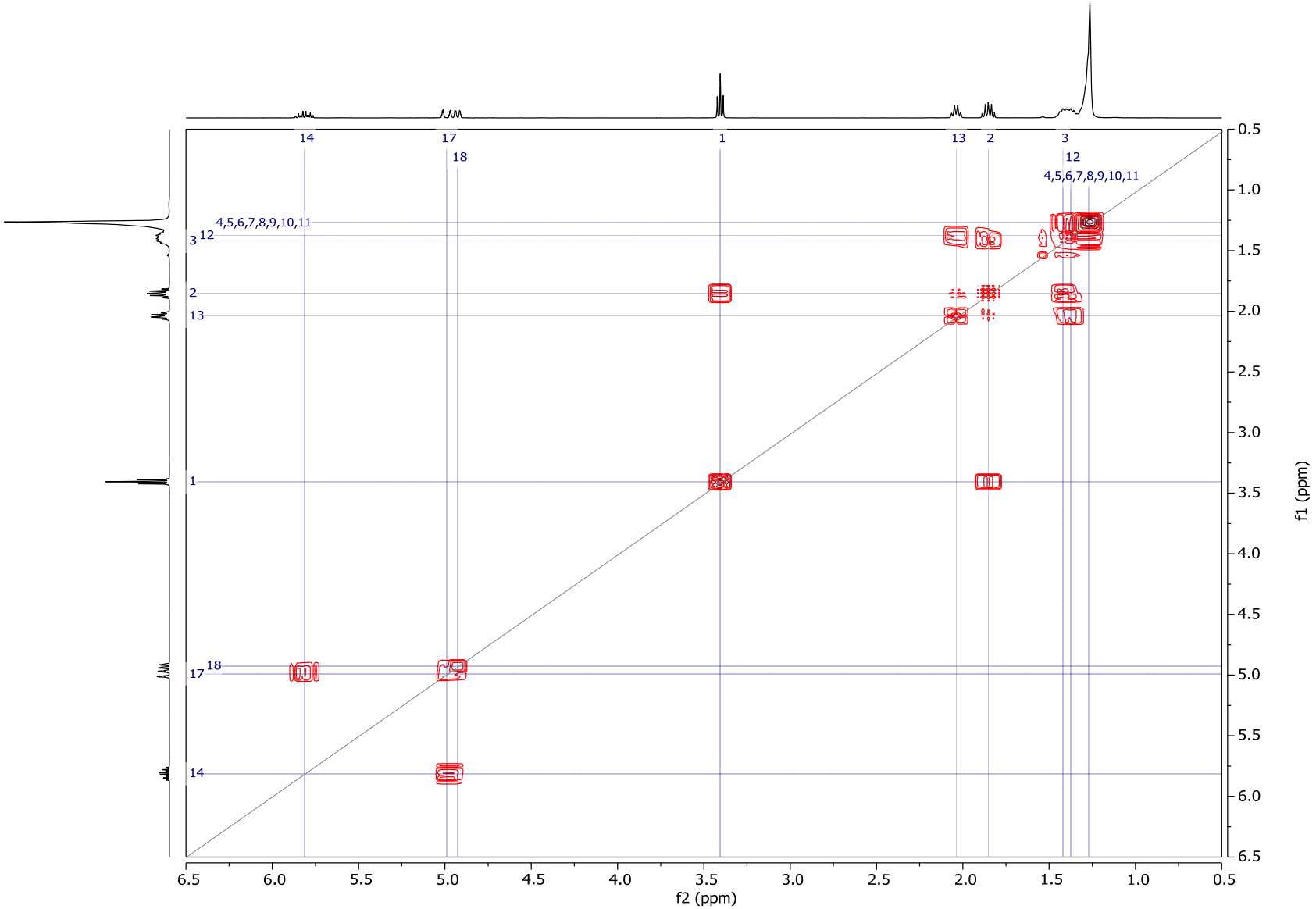
^{1}H NMR



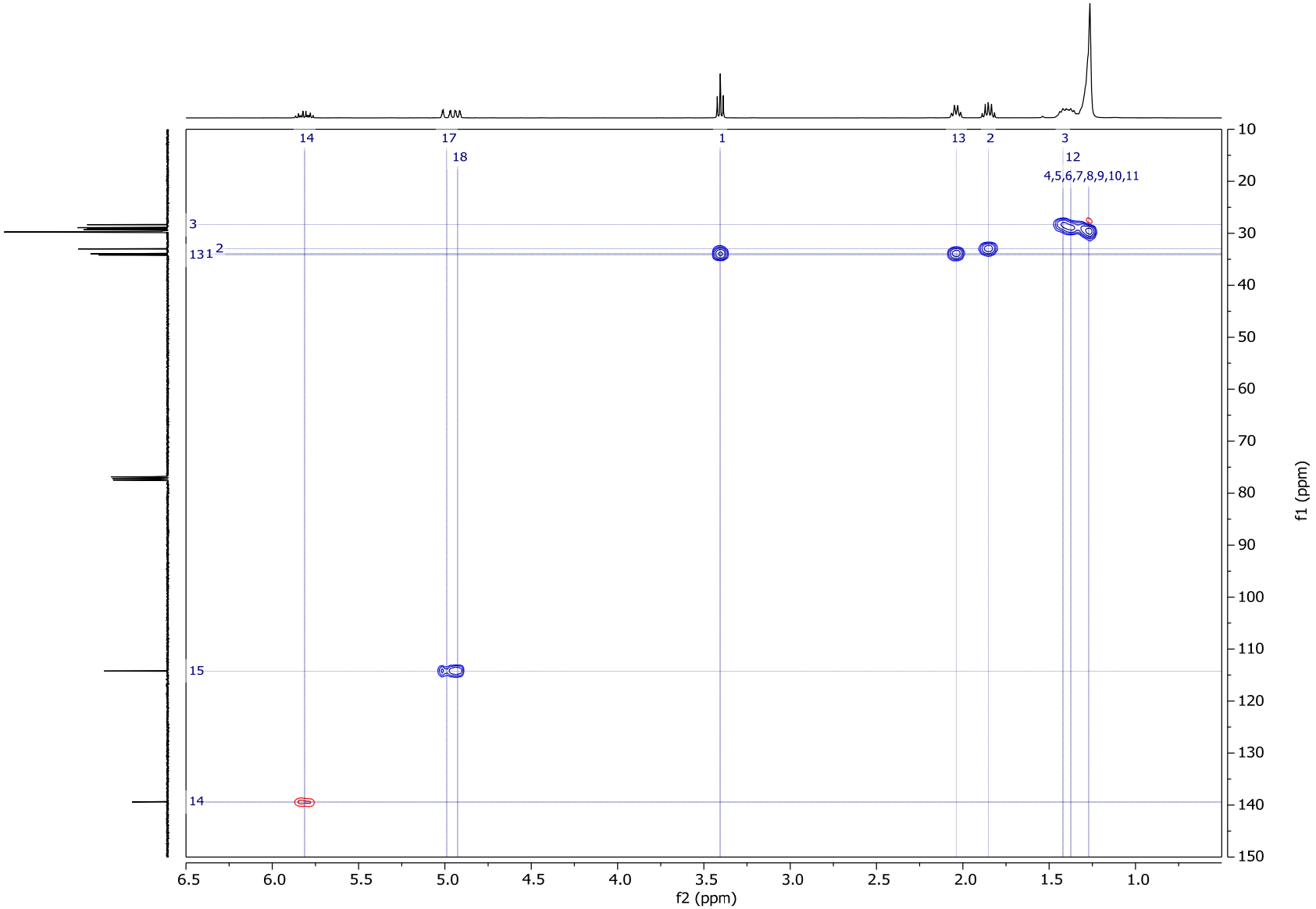
^{13}C NMR; RBM5-149



COSY NMR; RBM5-149

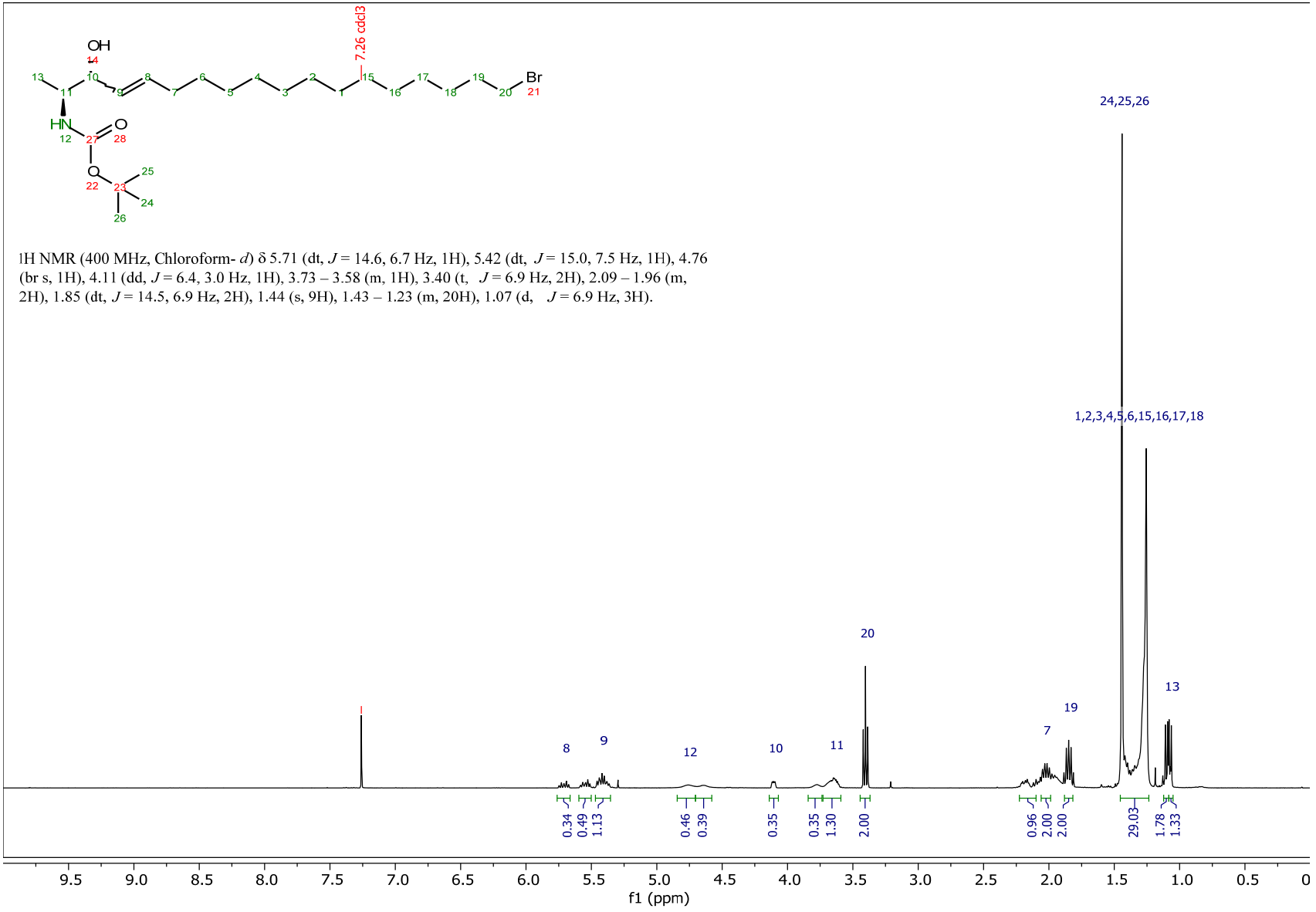


HSQC NMR; RBM5-149



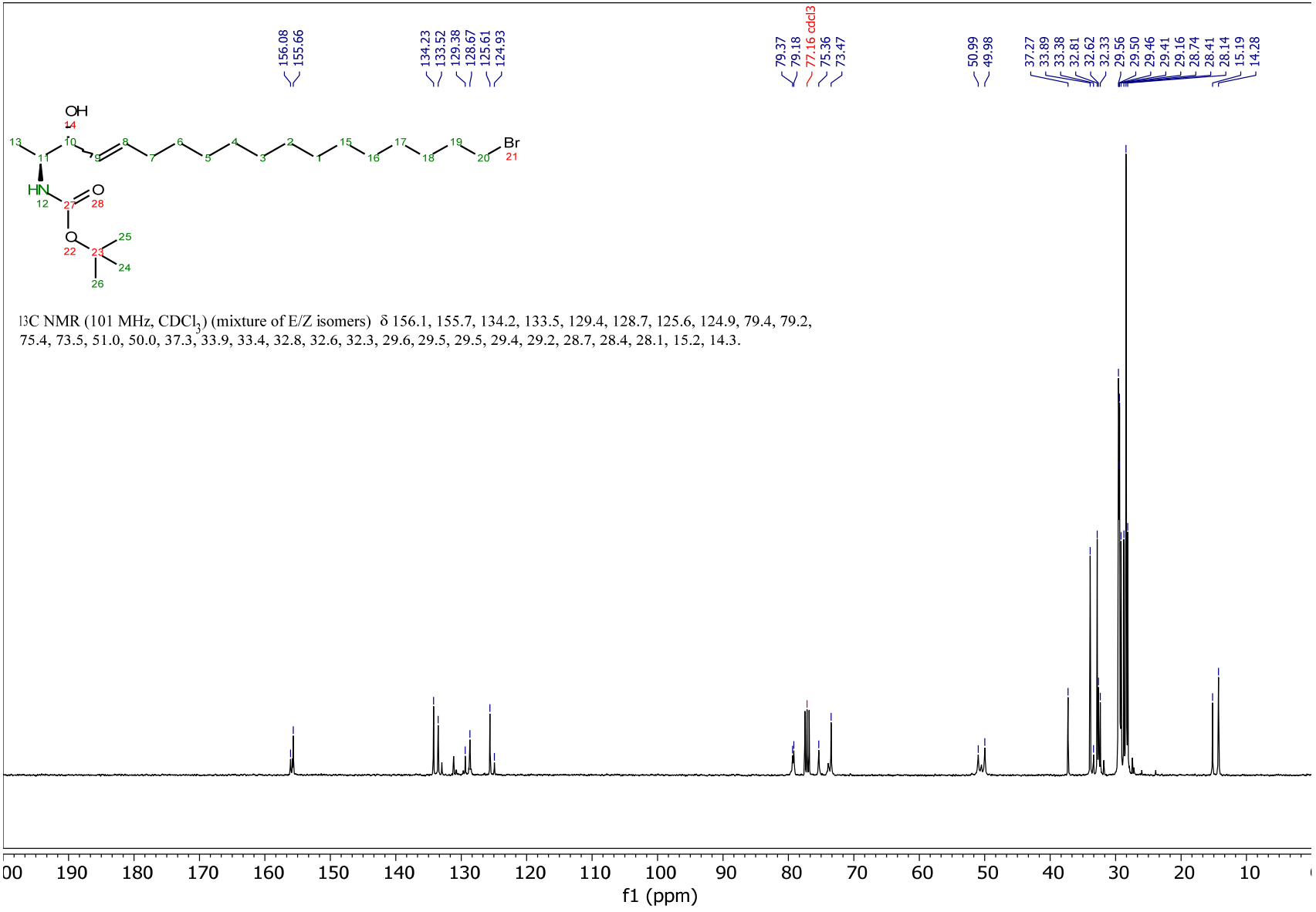
Compound RBM5-150

^{1}H NMR



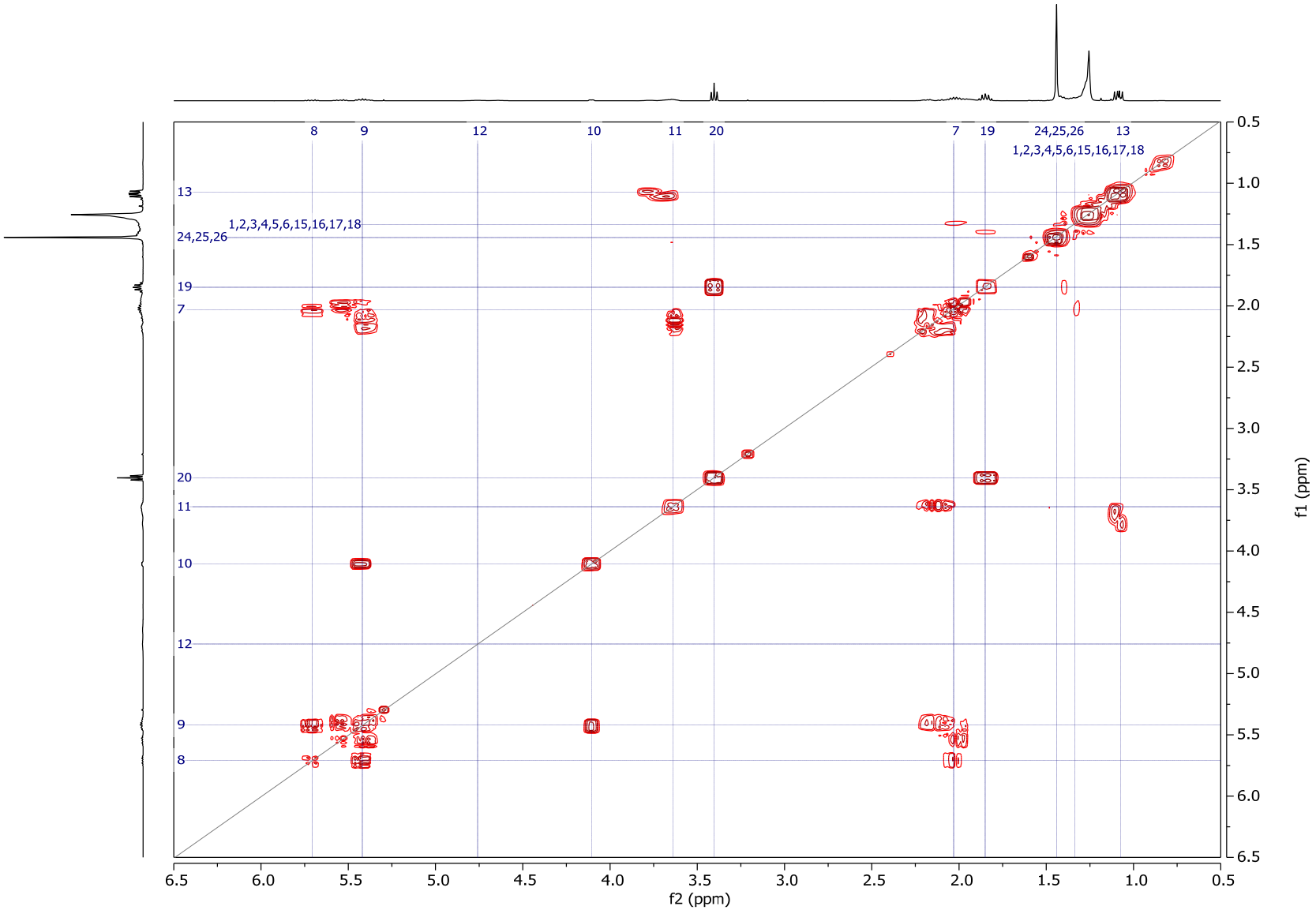
1H NMR (400 MHz, Chloroform- *d*) δ 5.71 (dt, *J* = 14.6, 6.7 Hz, 1H), 5.42 (dt, *J* = 15.0, 7.5 Hz, 1H), 4.76 (br s, 1H), 4.11 (dd, *J* = 6.4, 3.0 Hz, 1H), 3.73 – 3.58 (m, 1H), 3.40 (t, *J* = 6.9 Hz, 2H), 2.09 – 1.96 (m, 2H), 1.85 (dt, *J* = 14.5, 6.9 Hz, 2H), 1.44 (s, 9H), 1.43 – 1.23 (m, 20H), 1.07 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR; RBM5-150

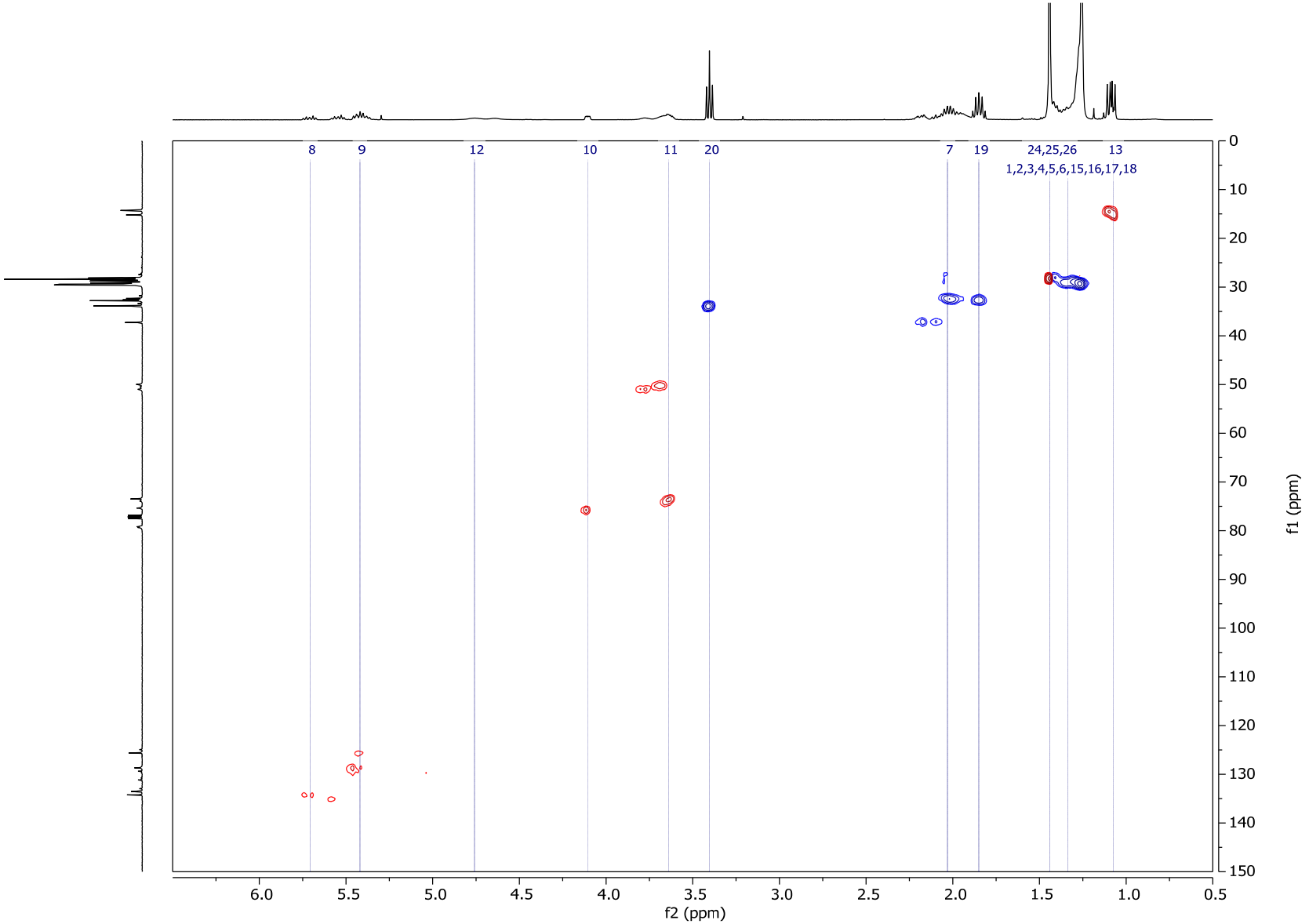


^{13}C NMR (101 MHz, $CDCl_3$) (mixture of E/Z isomers) δ 156.1, 155.7, 134.2, 133.5, 129.4, 128.7, 125.6, 124.9, 79.4, 79.2, 75.4, 73.5, 51.0, 50.0, 37.3, 33.9, 33.4, 32.8, 32.6, 32.3, 29.6, 29.5, 29.5, 29.4, 29.2, 28.7, 28.4, 28.1, 15.2, 14.3.

COSY NMR; RBM5-150

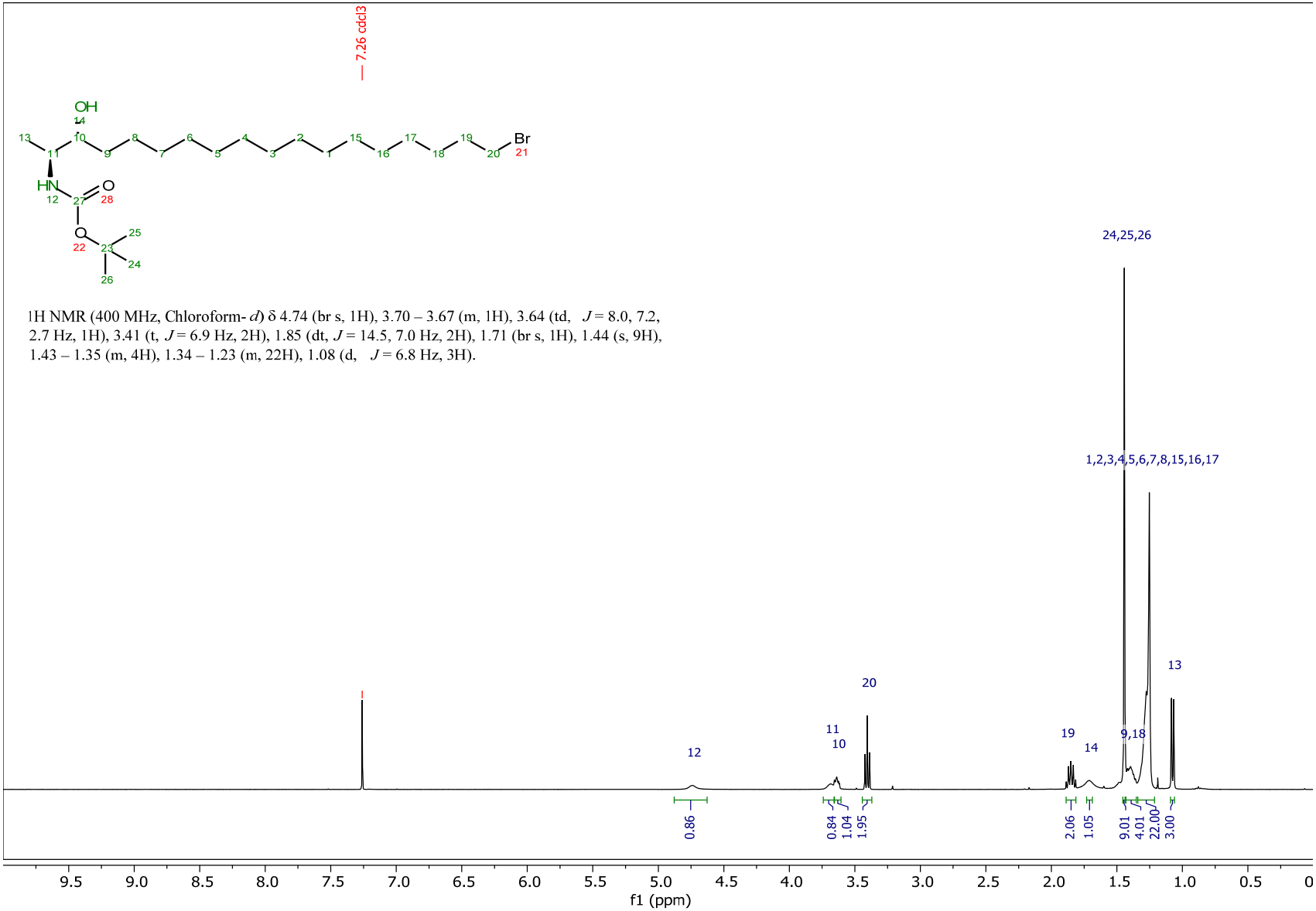


HSQC NMR; RBM5-150



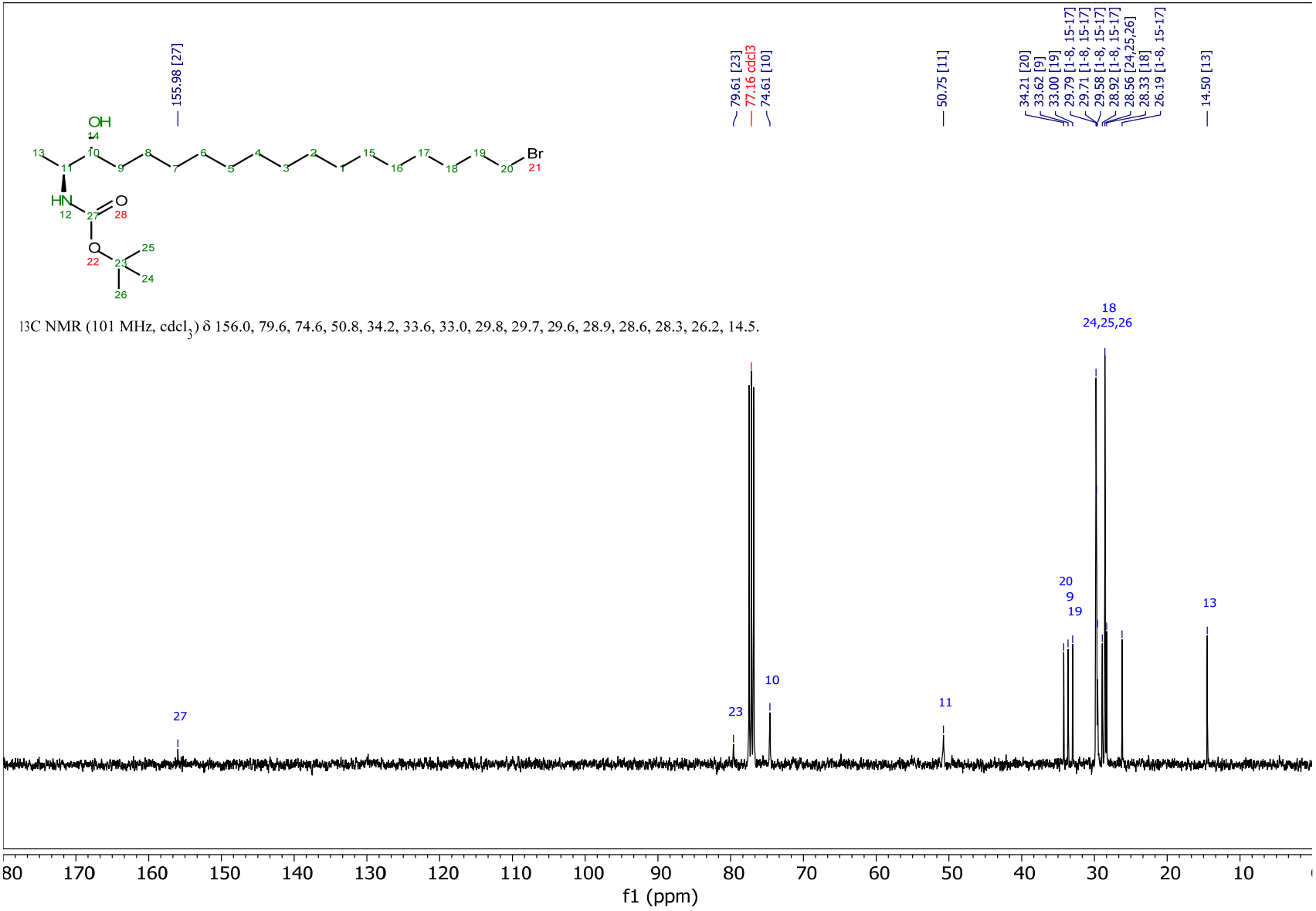
Compound RBM5-151

^{1}H NMR

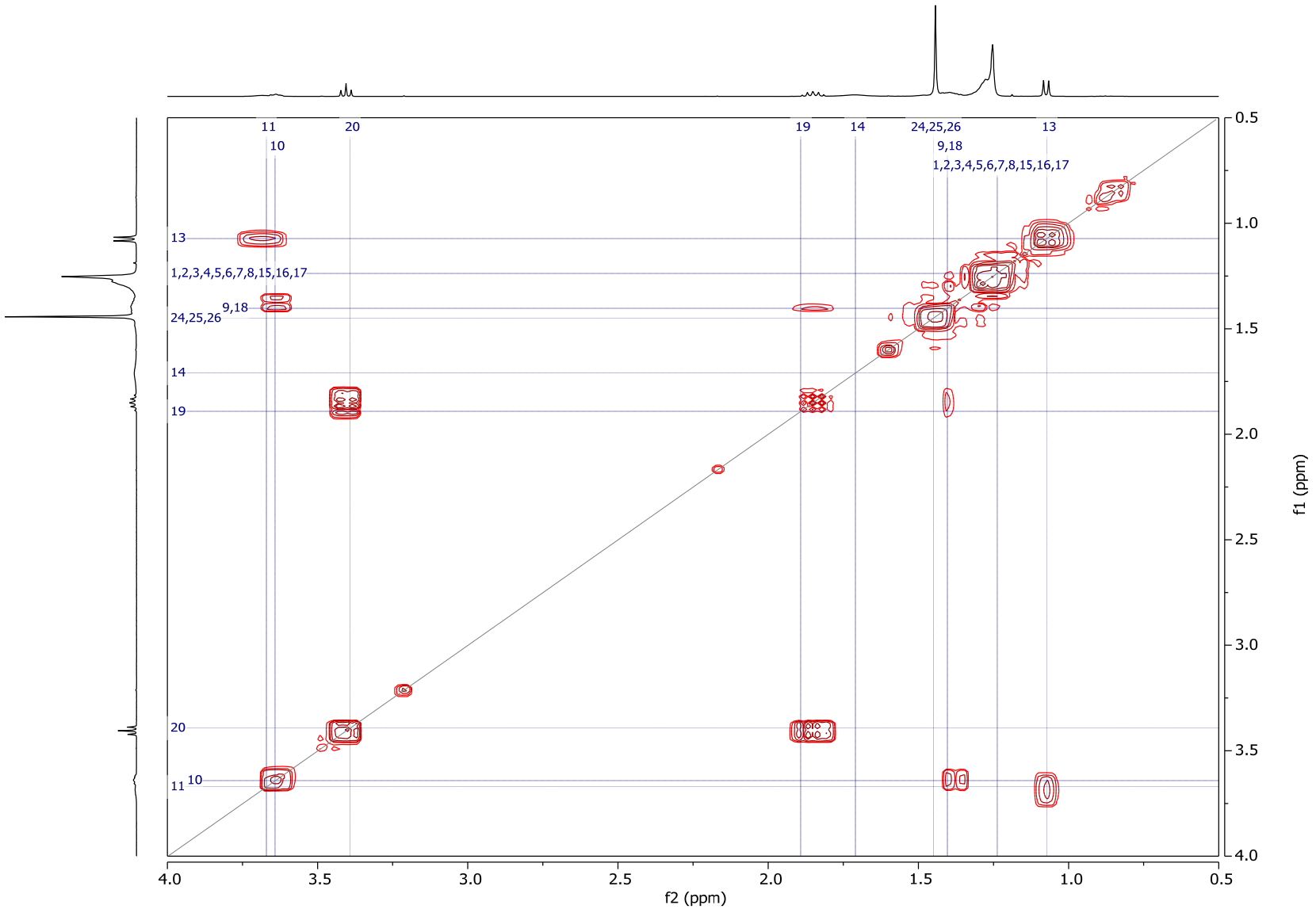


1H NMR (400 MHz, Chloroform-*d*) δ 4.74 (br s, 1H), 3.70 – 3.67 (m, 1H), 3.64 (td, *J* = 8.0, 7.2, 2.7 Hz, 1H), 3.41 (t, *J* = 6.9 Hz, 2H), 1.85 (dt, *J* = 14.5, 7.0 Hz, 2H), 1.71 (br s, 1H), 1.44 (s, 9H), 1.43 – 1.35 (m, 4H), 1.34 – 1.23 (m, 22H), 1.08 (d, *J* = 6.8 Hz, 3H).

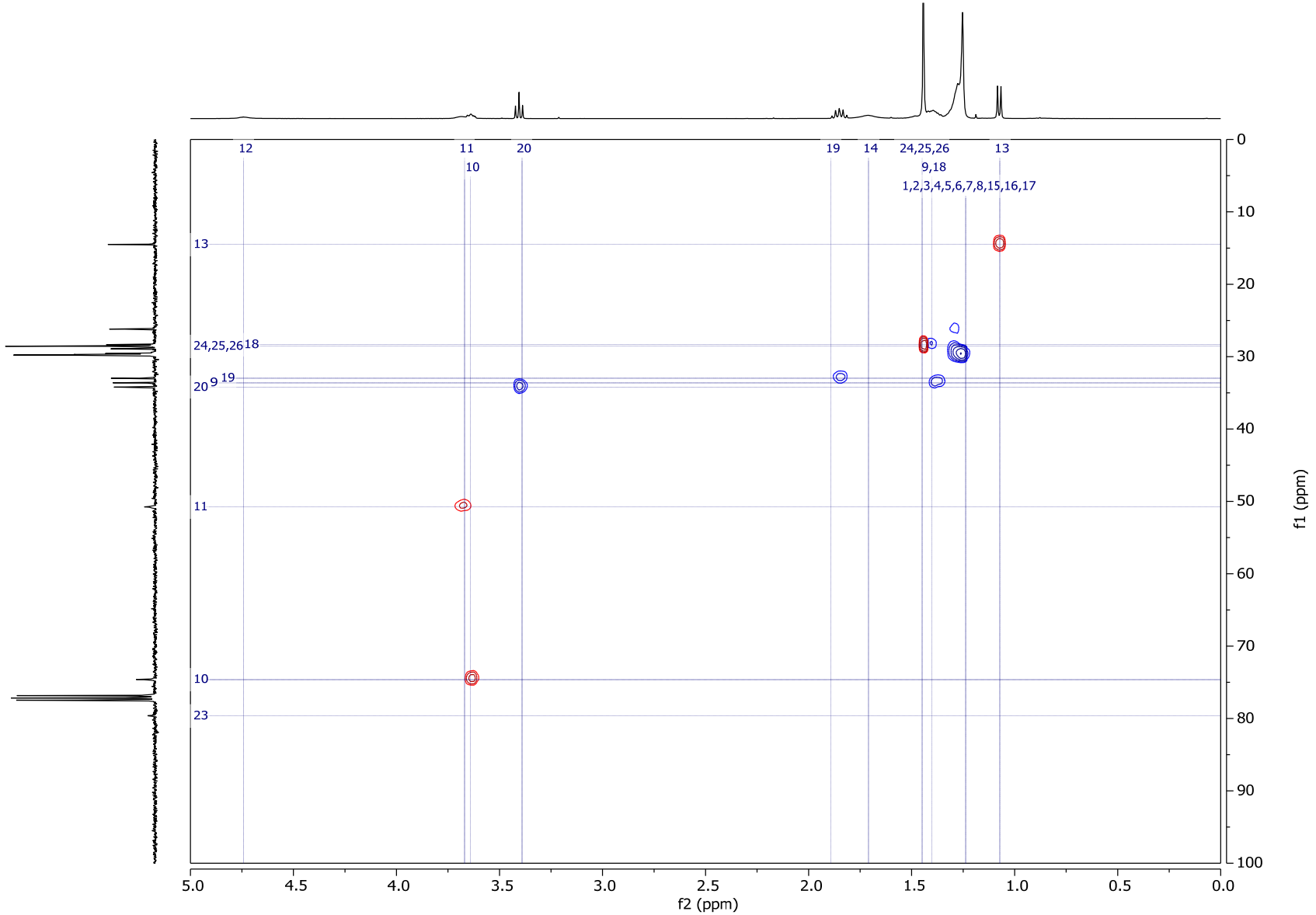
^{13}C NMR; RBM5-151



COSY NMR; RBM5-151

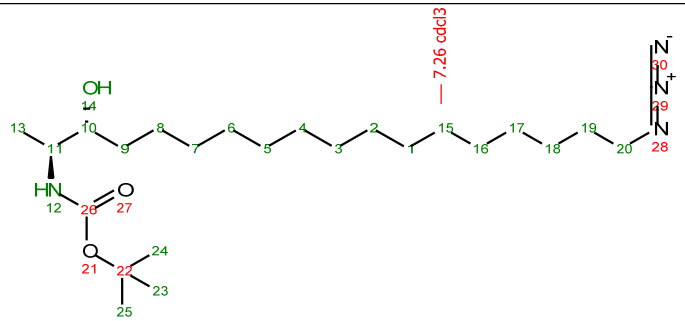


HSQC NMR; RBM5-151

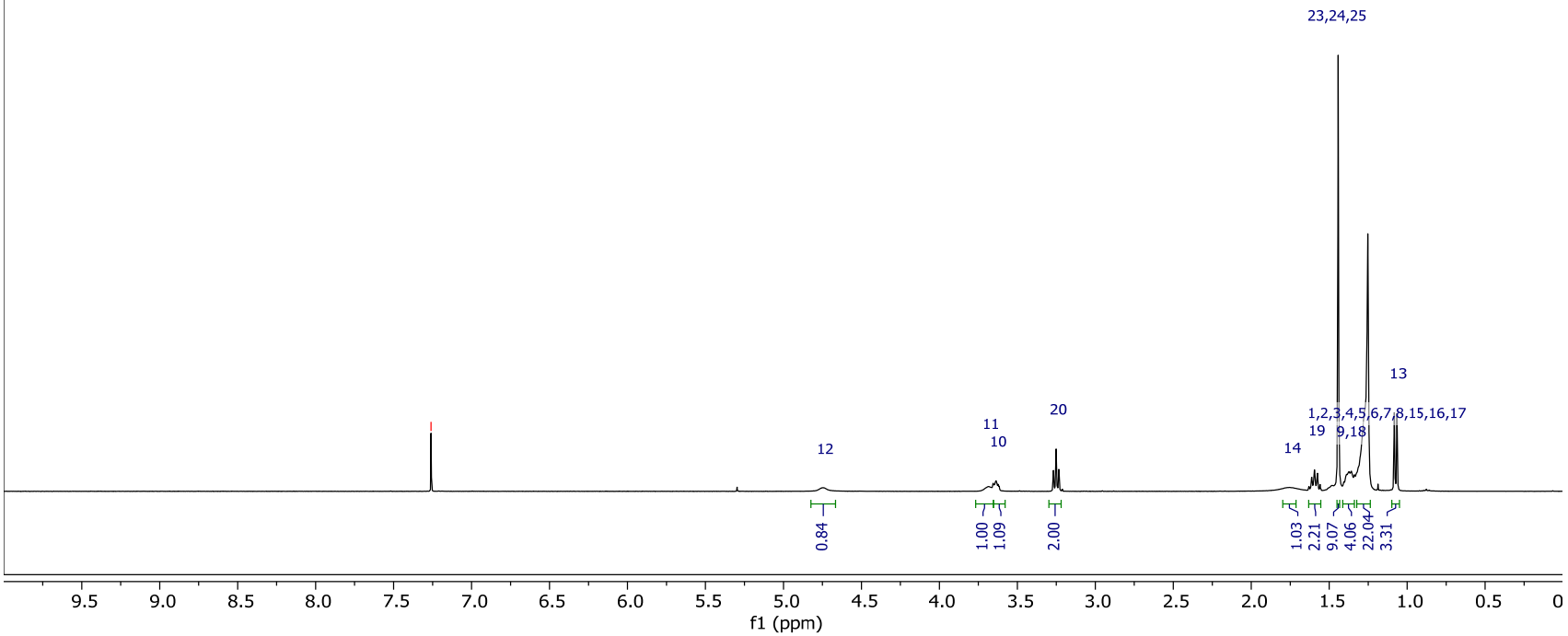


Compound RBM5-152

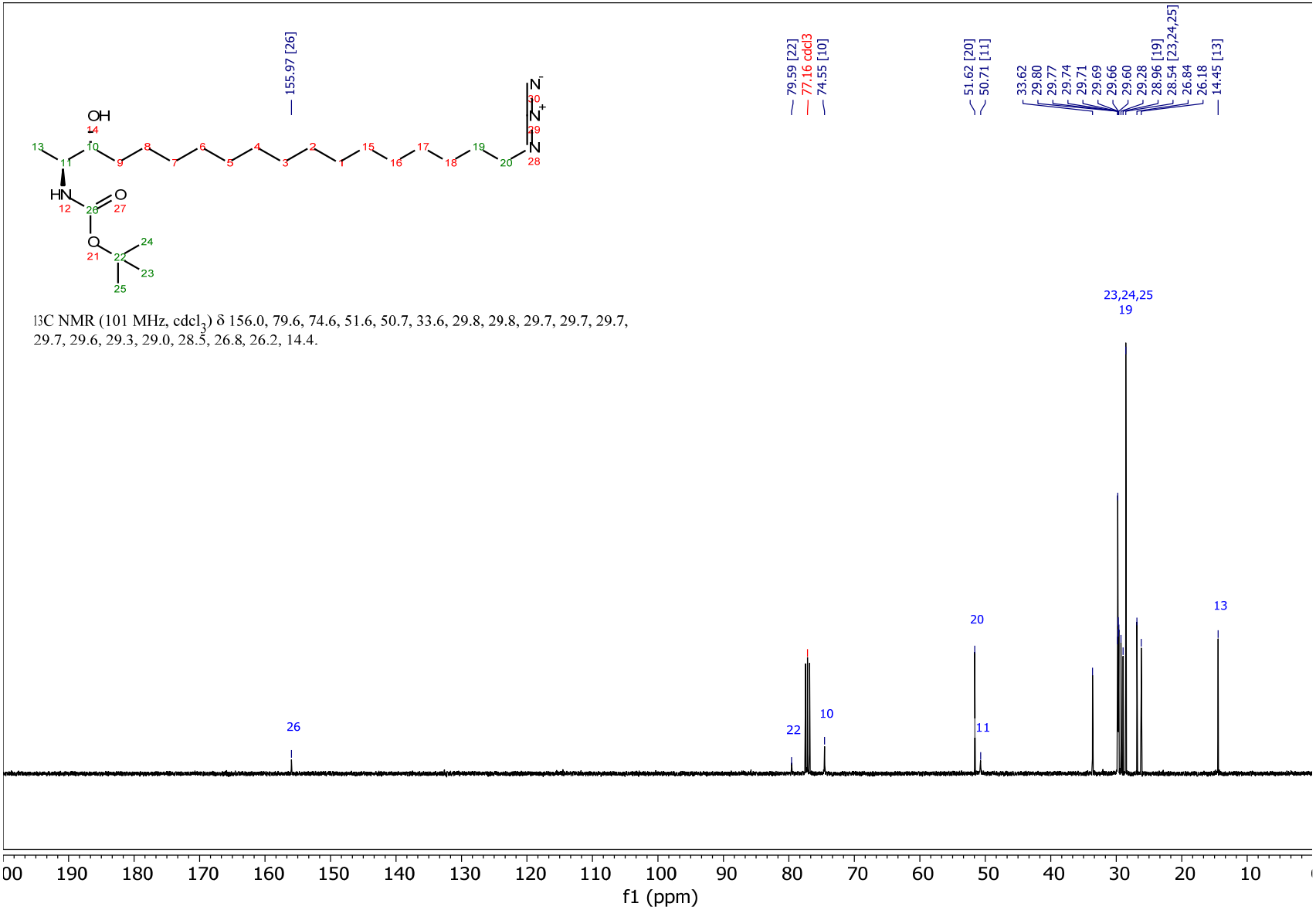
^{1}H NMR



1H NMR (400 MHz, Chloroform- *d*) δ 4.75 (s, 1H), 3.71 – 3.67 (m, 1H), 3.66 – 3.61 (m, 1H), 3.25 (t, *J* = 7.0 Hz, 2H), 1.75 (br s, 1H), 1.66 – 1.54 (m, 2H), 1.44 (s, 9H), 1.40 – 1.35 (m, 4H), 1.34 – 1.23 (m, 22H), 1.07 (d, *J* = 6.8 Hz, 3H).

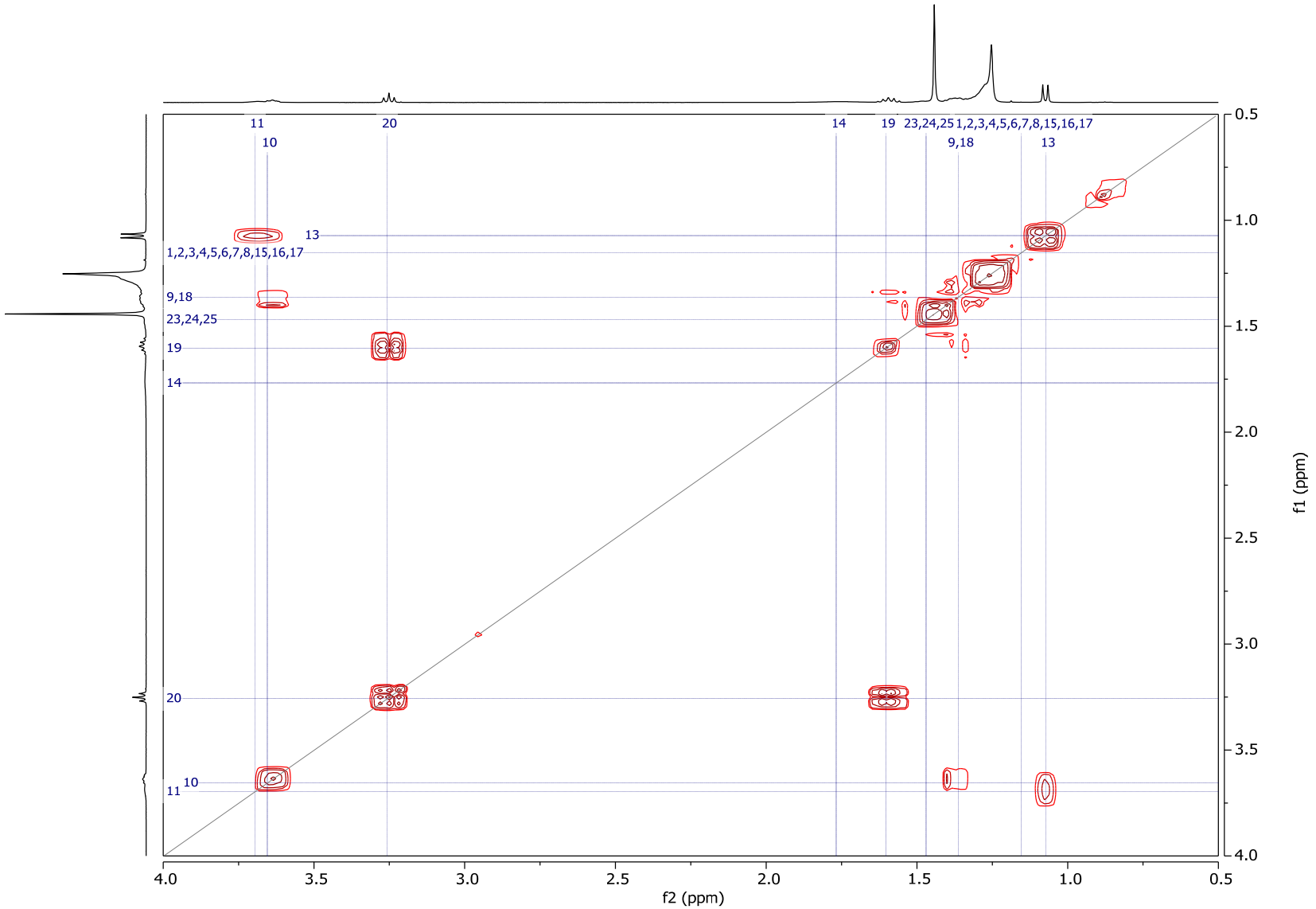


^{13}C NMR; RBM5-152

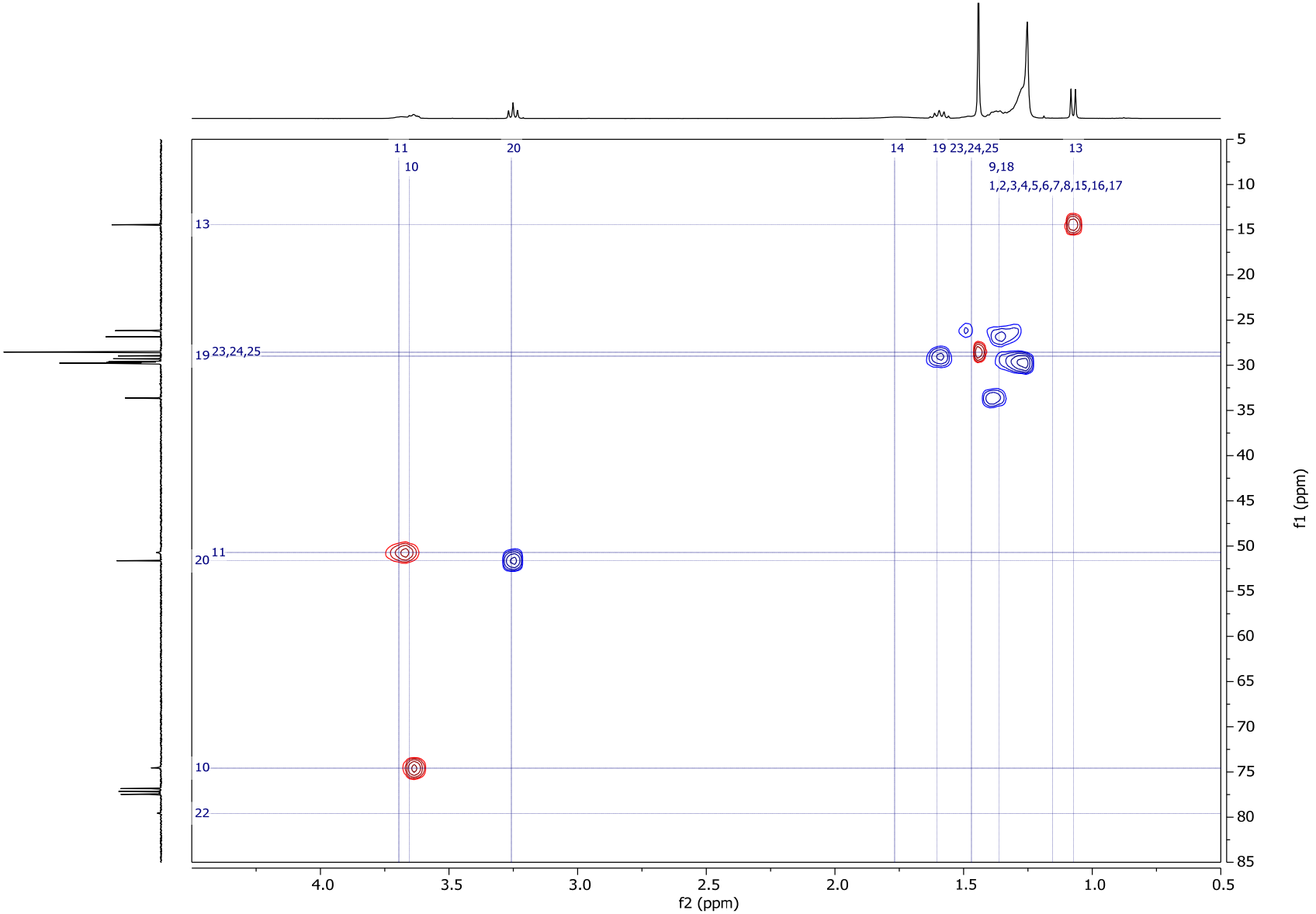


^{13}C NMR (101 MHz, cdcl$_3$) δ 156.0, 79.6, 74.6, 51.6, 50.7, 33.6, 29.8, 29.8, 29.7, 29.7, 29.7, 29.7, 29.6, 29.3, 29.0, 28.5, 26.8, 26.2, 14.4.

COSY NMR; RBM5-152

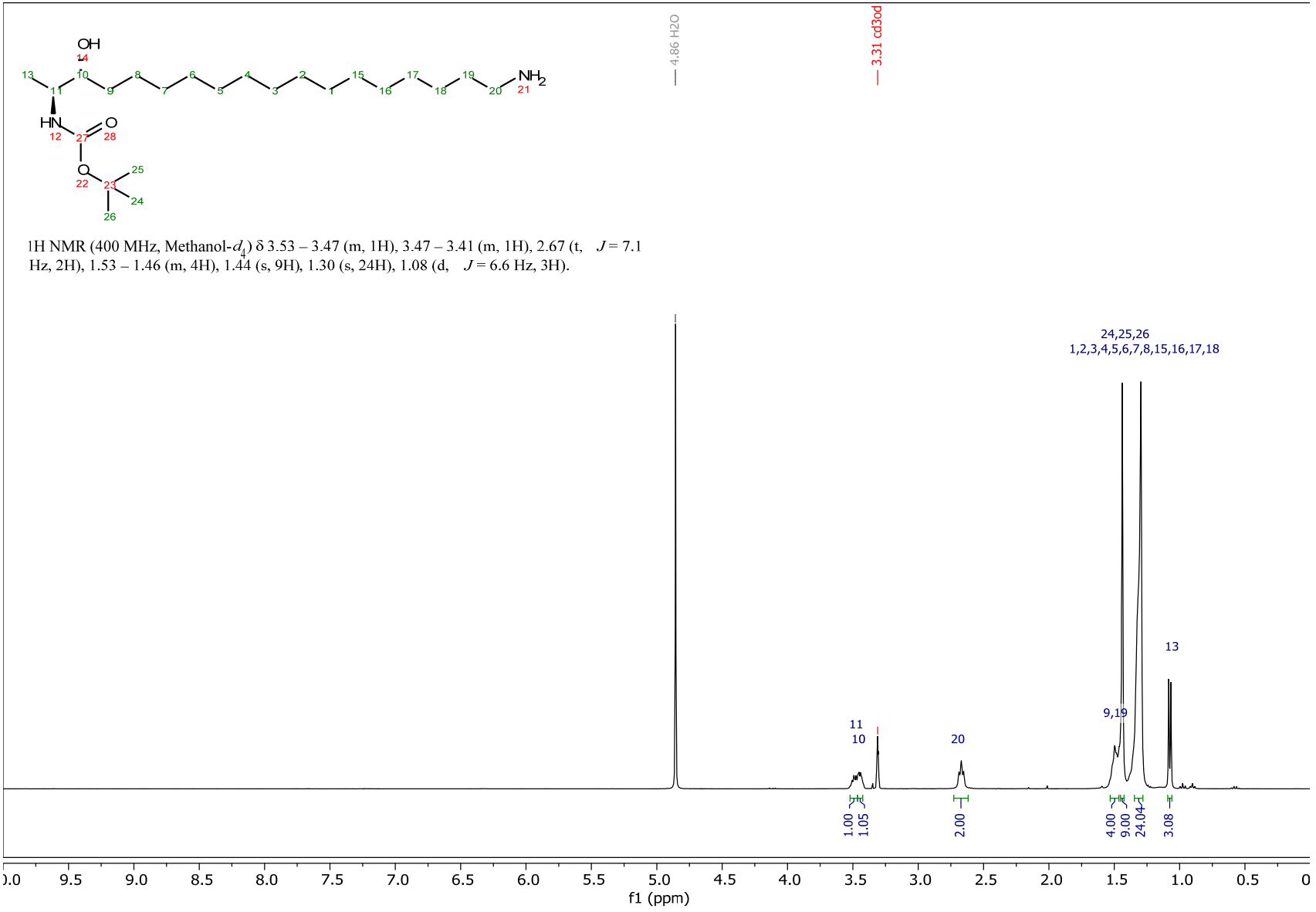


HSQC NMR; RBM5-152



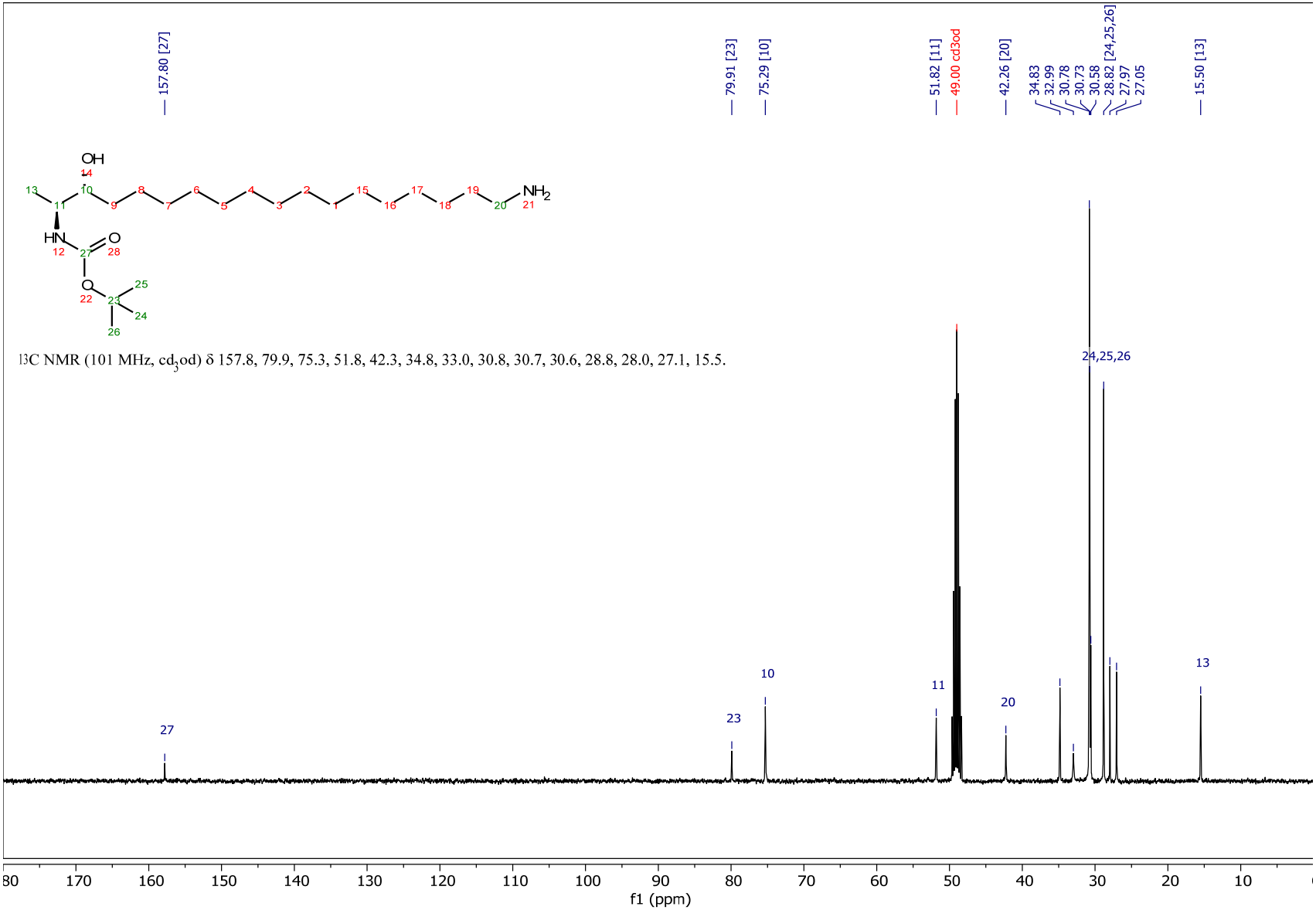
Compound RBM5-153

^{1}H NMR

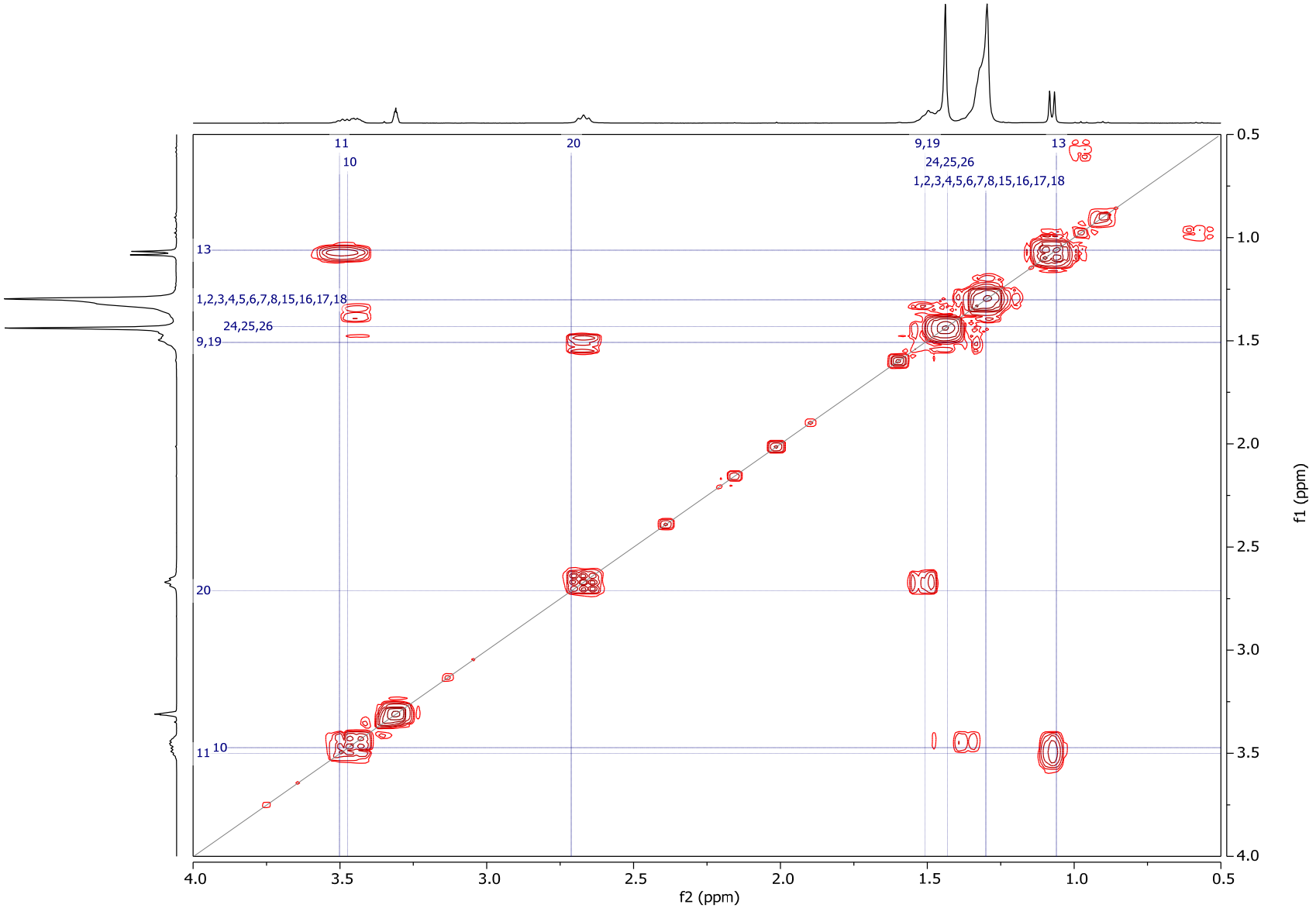


1H NMR (400 MHz, Methanol-d_4) δ 3.53 – 3.47 (m, 1H), 3.47 – 3.41 (m, 1H), 2.67 (t, J = 7.1 Hz, 2H), 1.53 – 1.46 (m, 4H), 1.44 (s, 9H), 1.30 (s, 24H), 1.08 (d, J = 6.6 Hz, 3H).

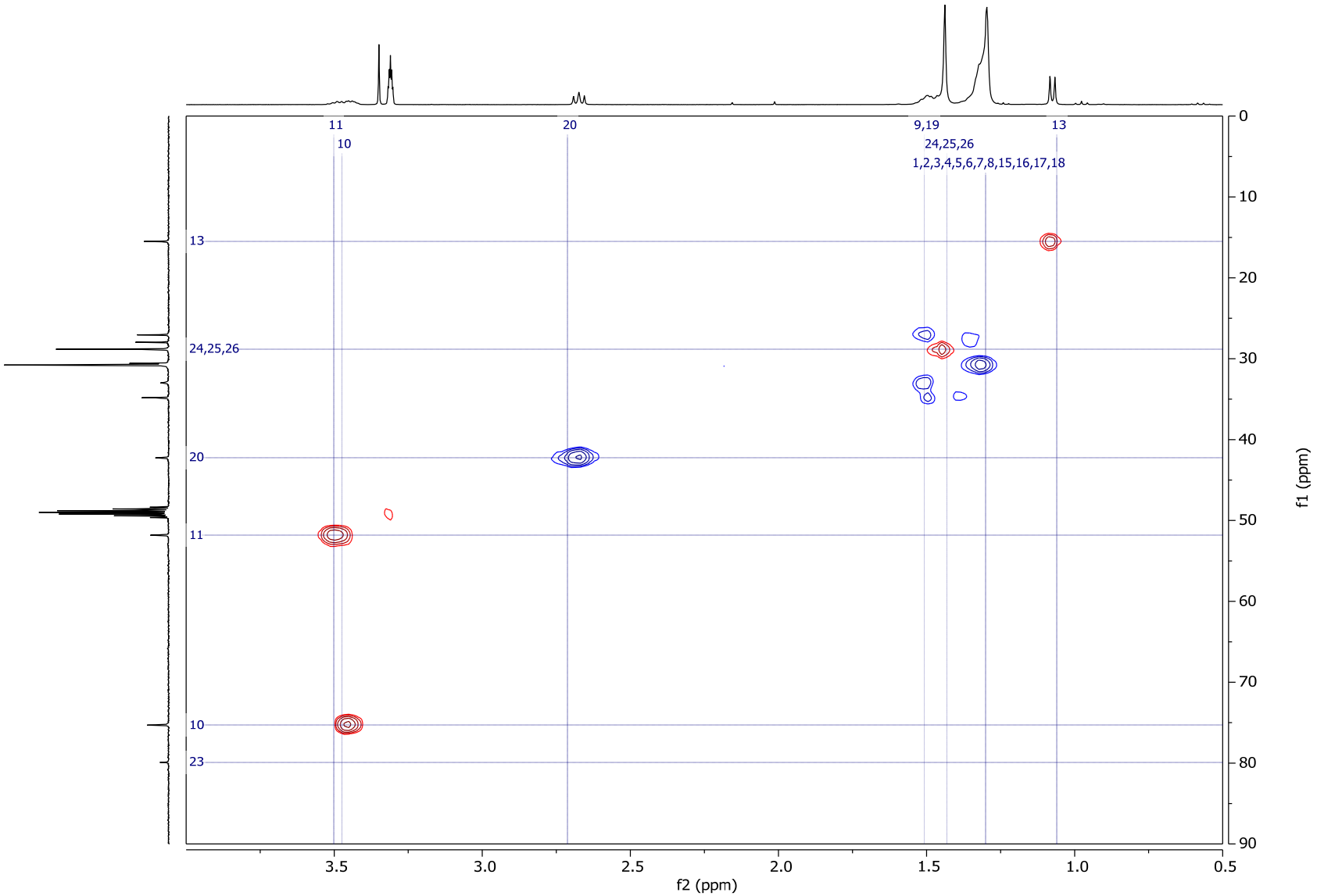
^{13}C NMR; RBM5-153



COSY NMR; RBM5-153

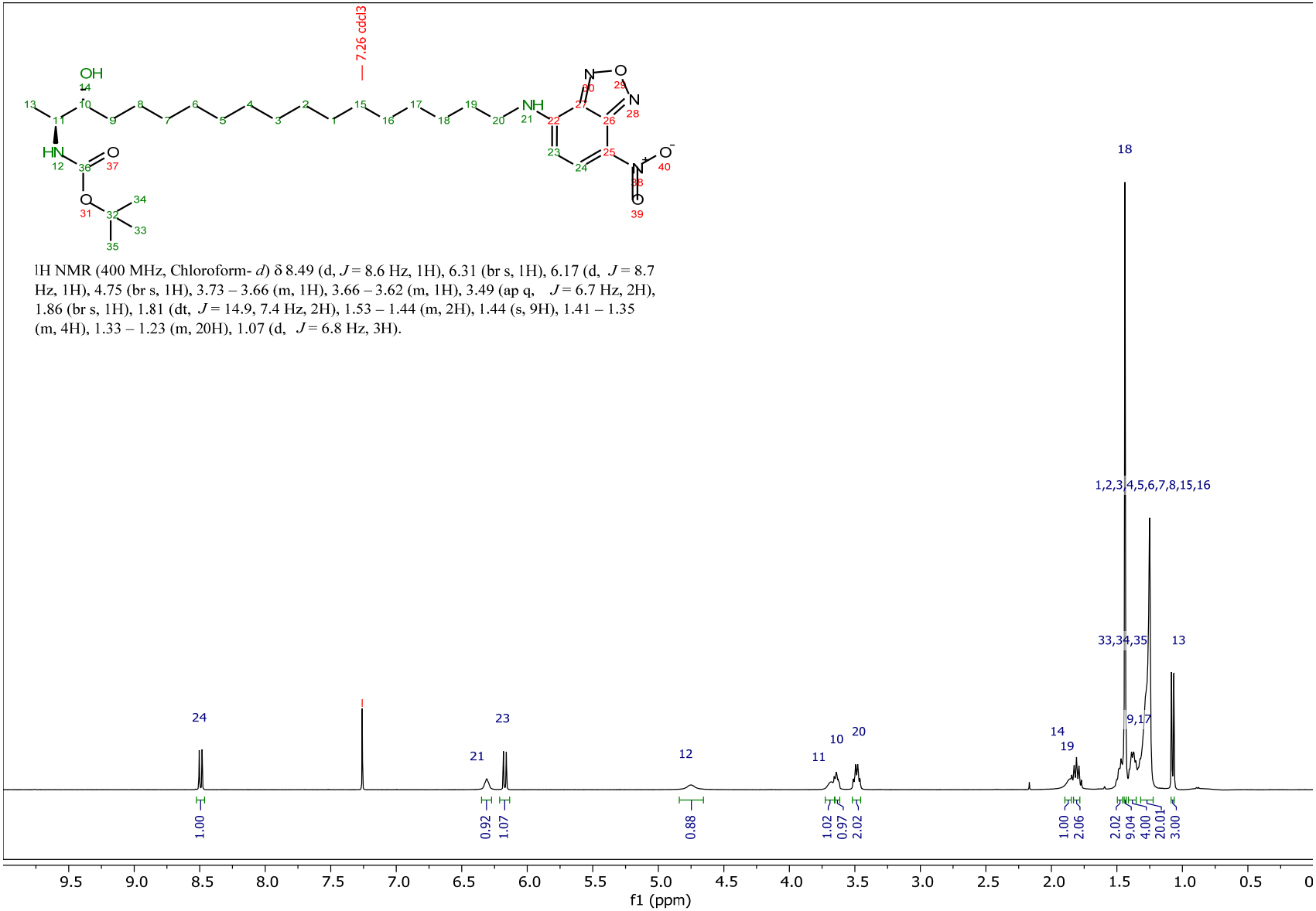


HSQC NMR; RBM5-153

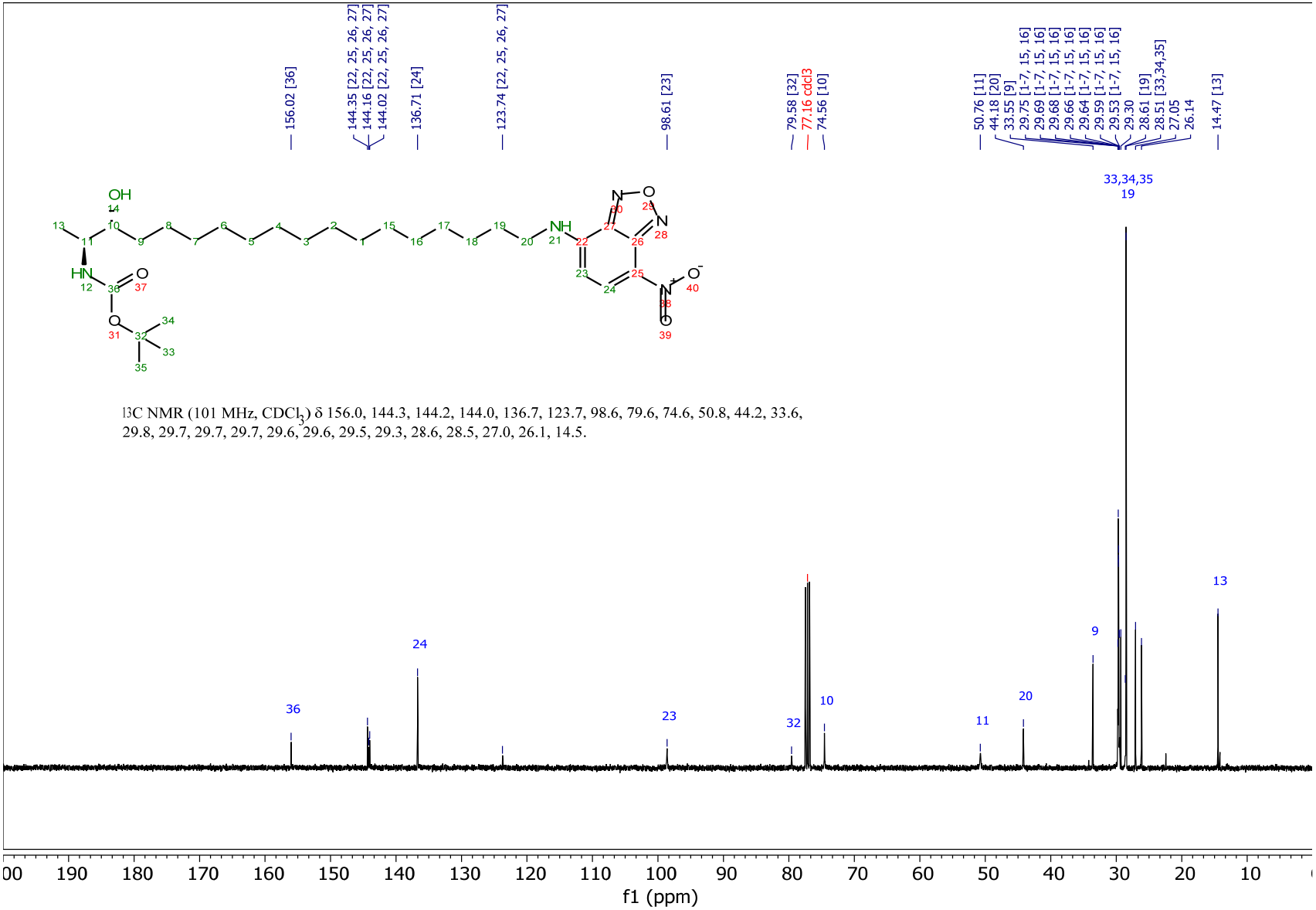


Compound RBM5-154

^{1}H NMR

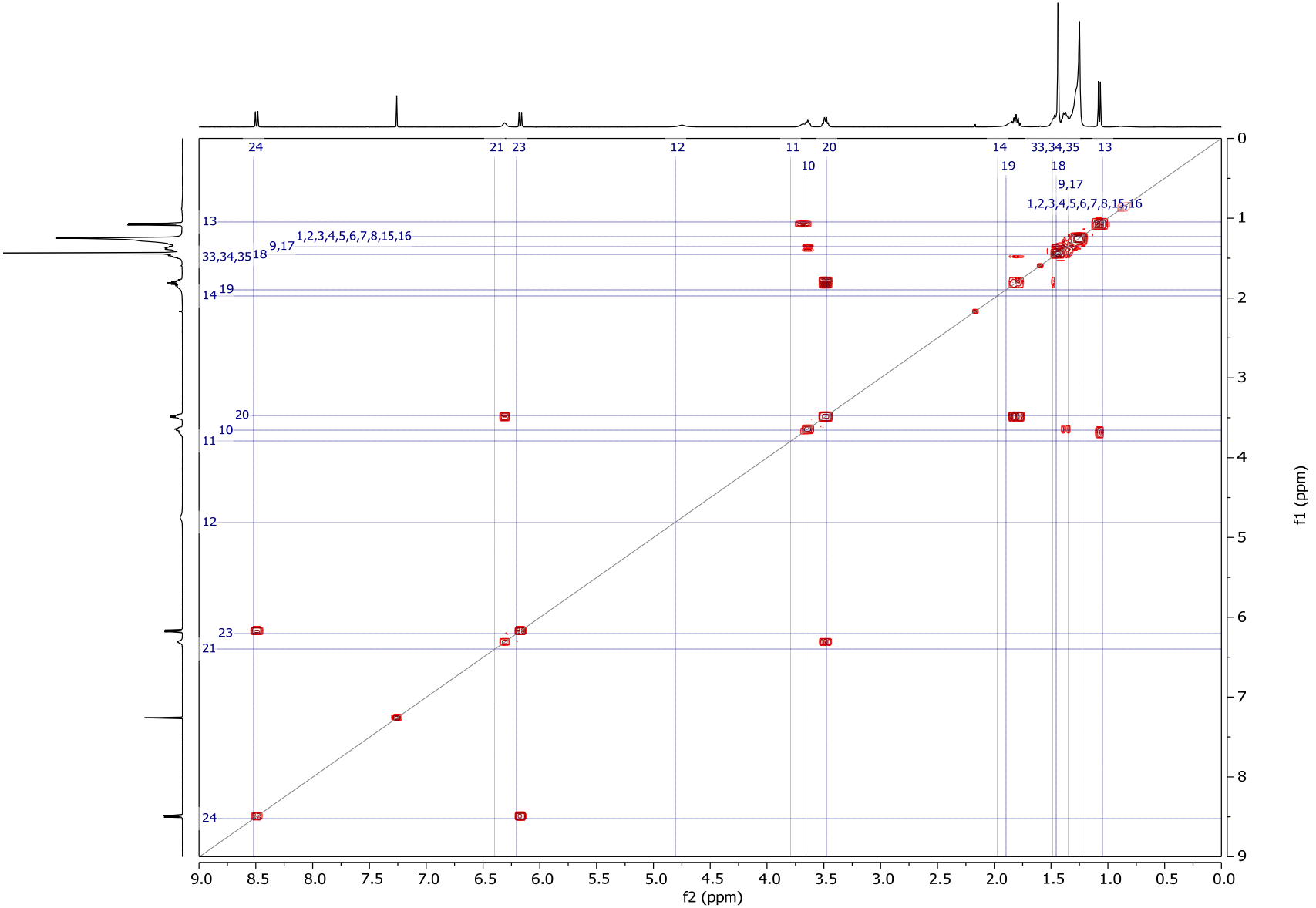


^{13}C NMR; RBM5-154

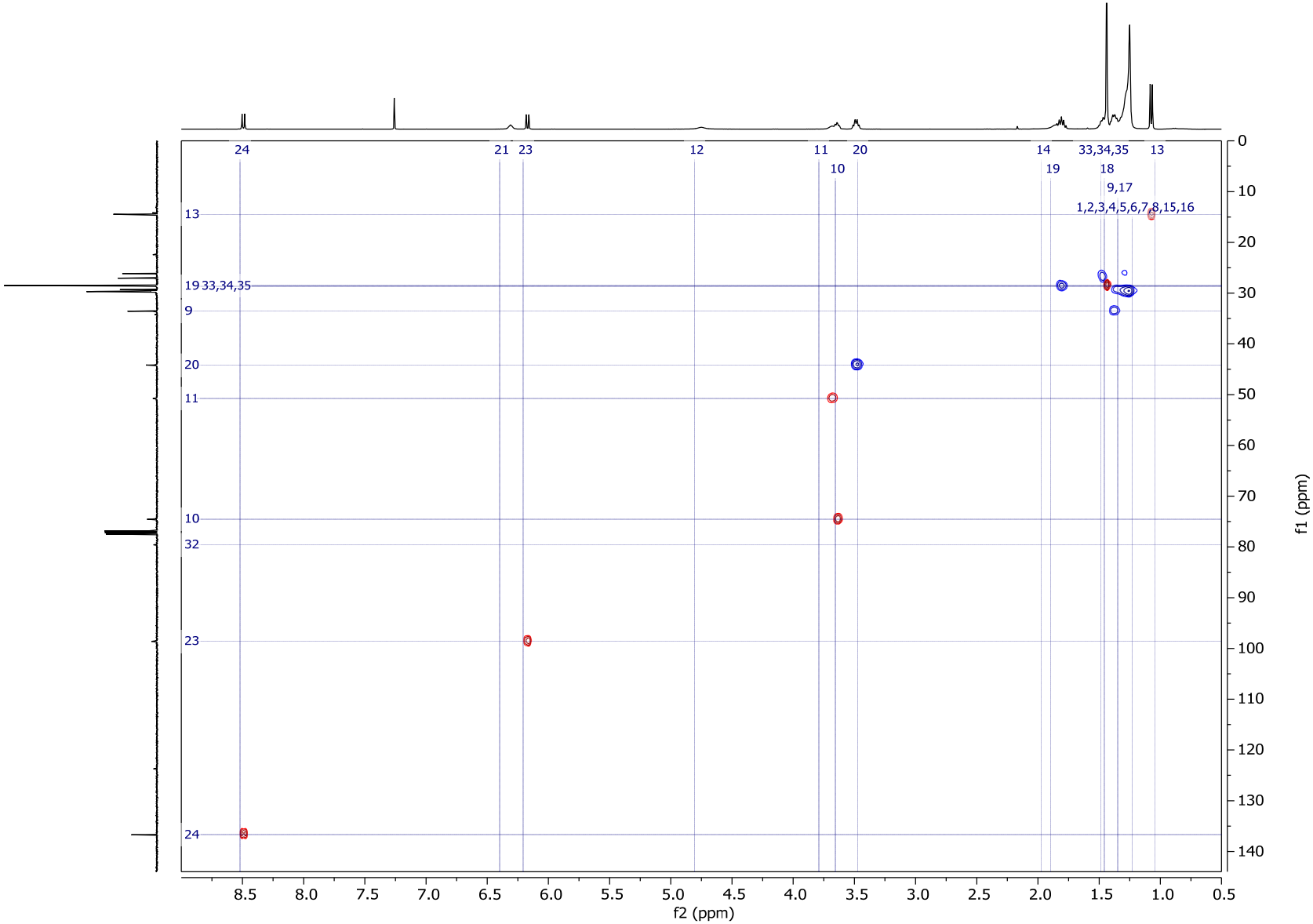


^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 144.3, 144.2, 144.0, 136.7, 123.7, 98.6, 79.6, 74.6, 50.8, 44.2, 33.6, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.3, 28.6, 28.5, 27.0, 26.1, 14.5.

COSY NMR; RBM5-154

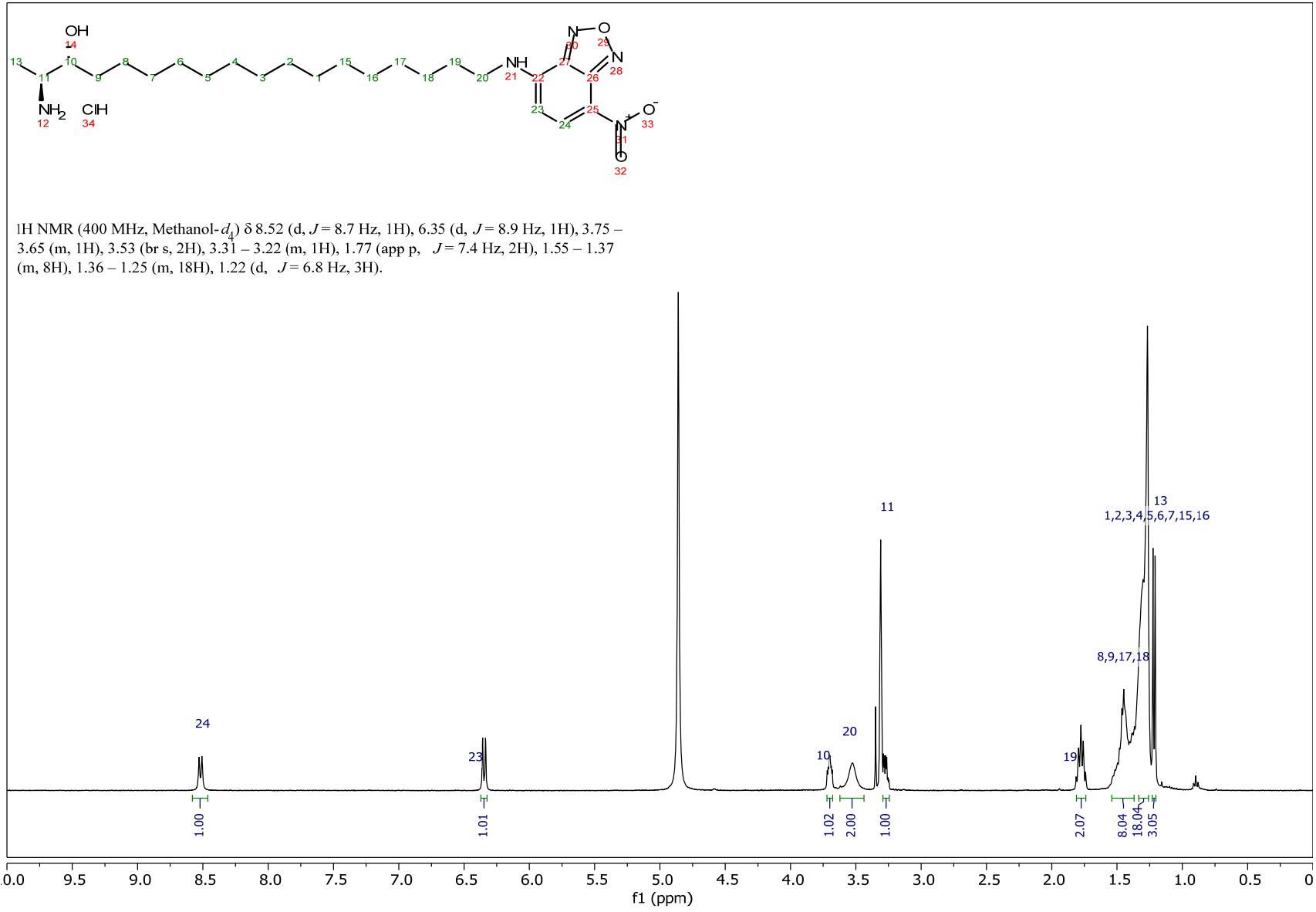


HSQC NMR; RBM5-154

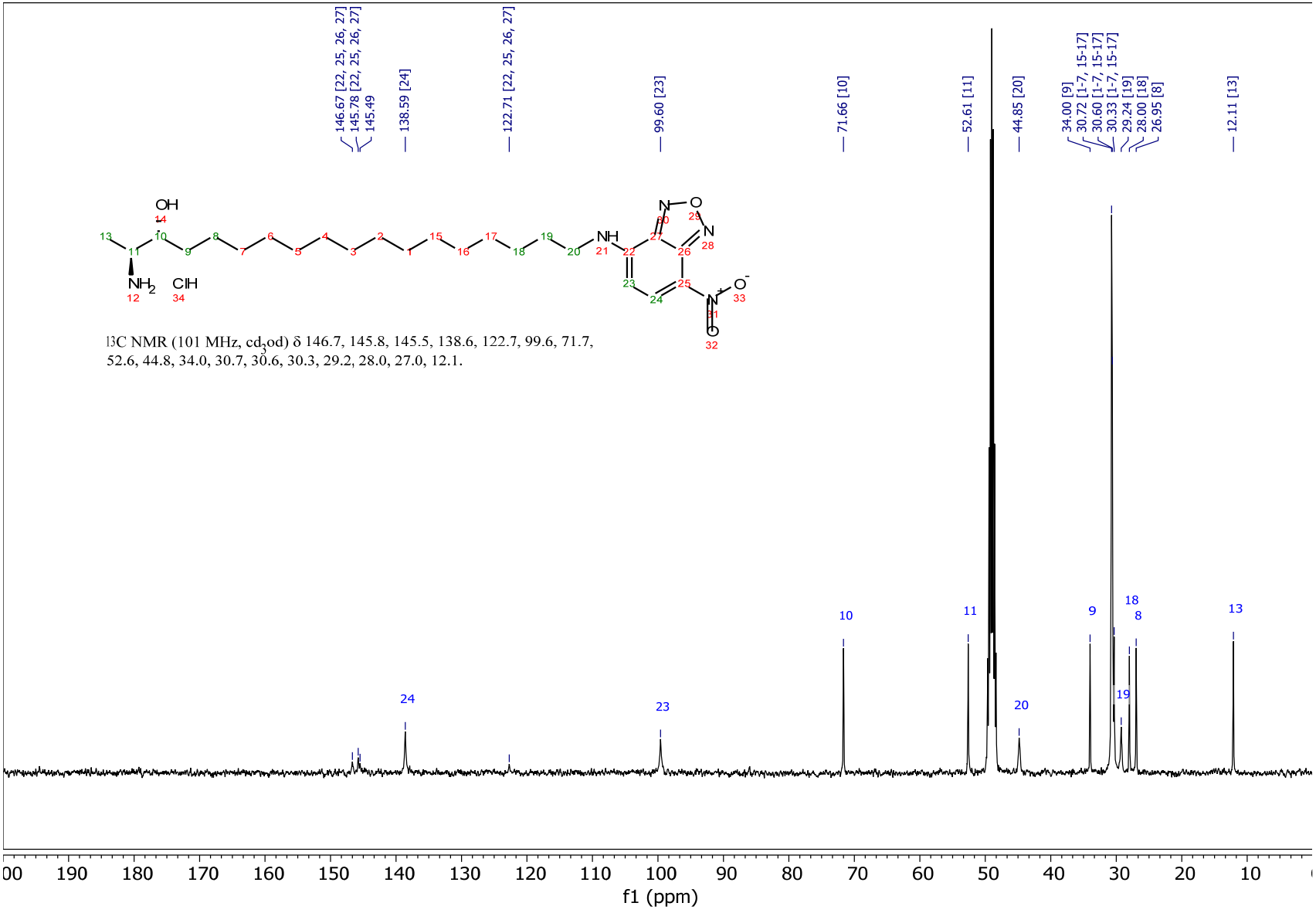


Compound RBM5-155

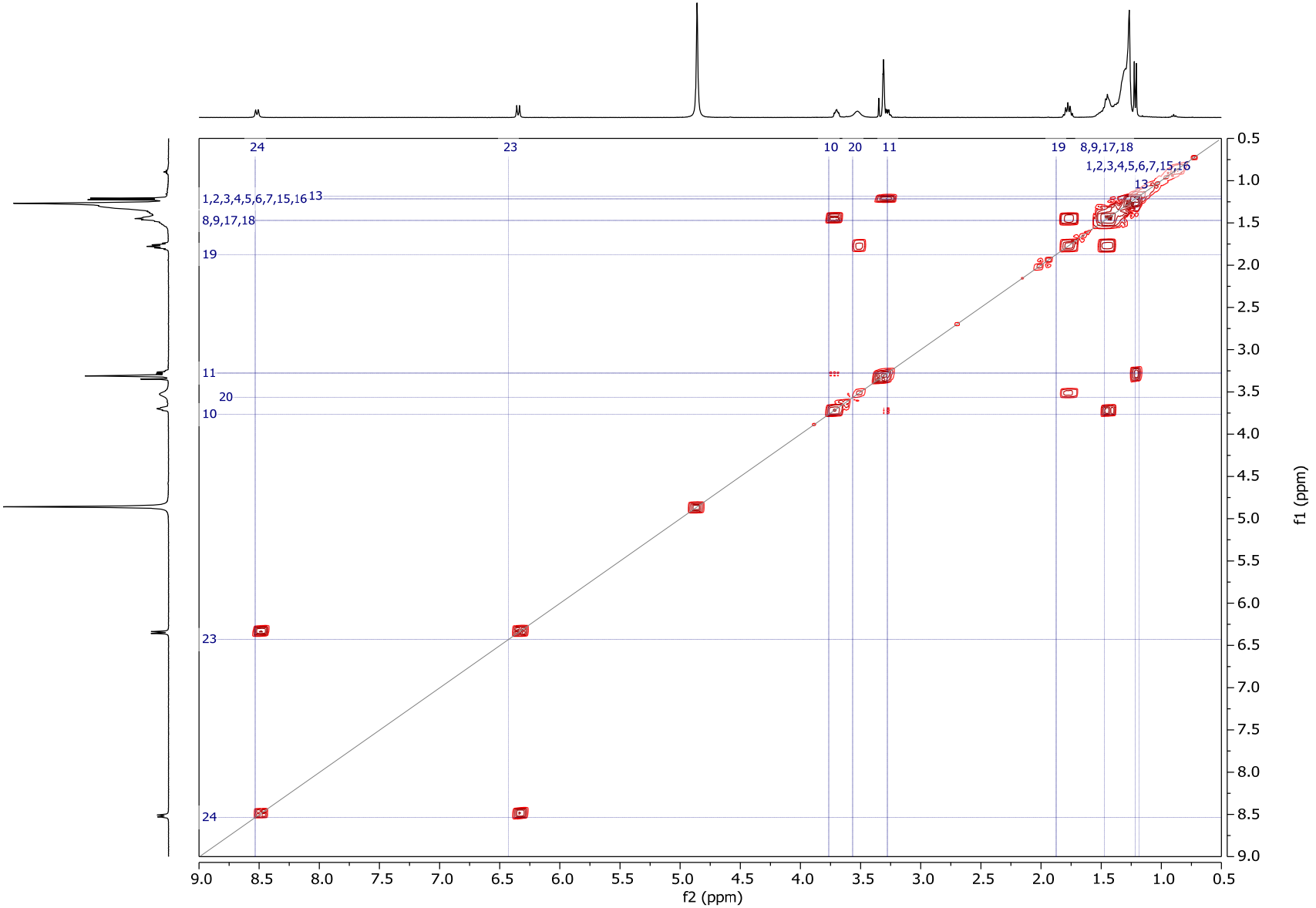
^{1}H NMR



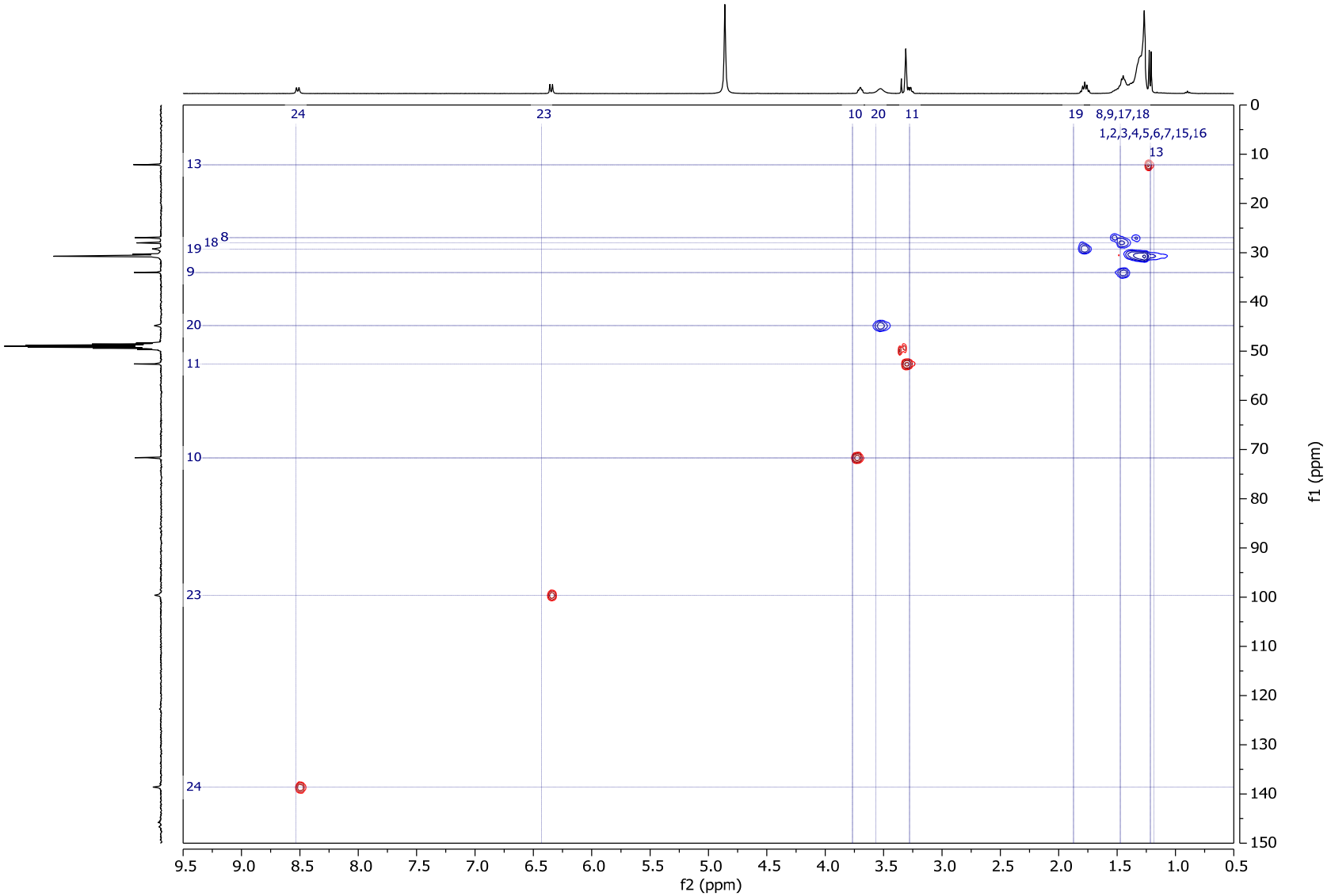
^{13}C NMR; RBM5-155



COSY NMR; RBM5-155

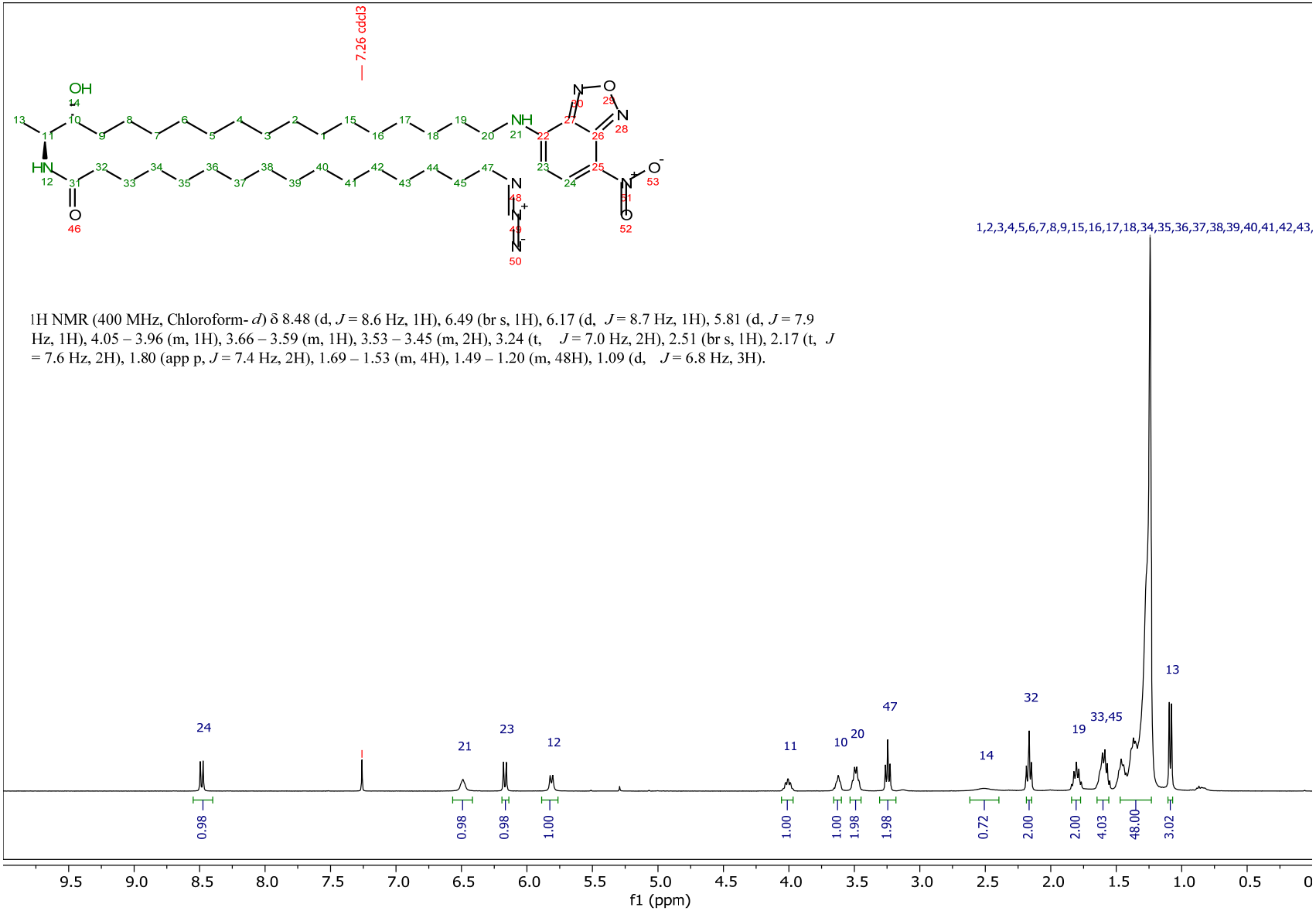


HSQC NMR; RBM5-155



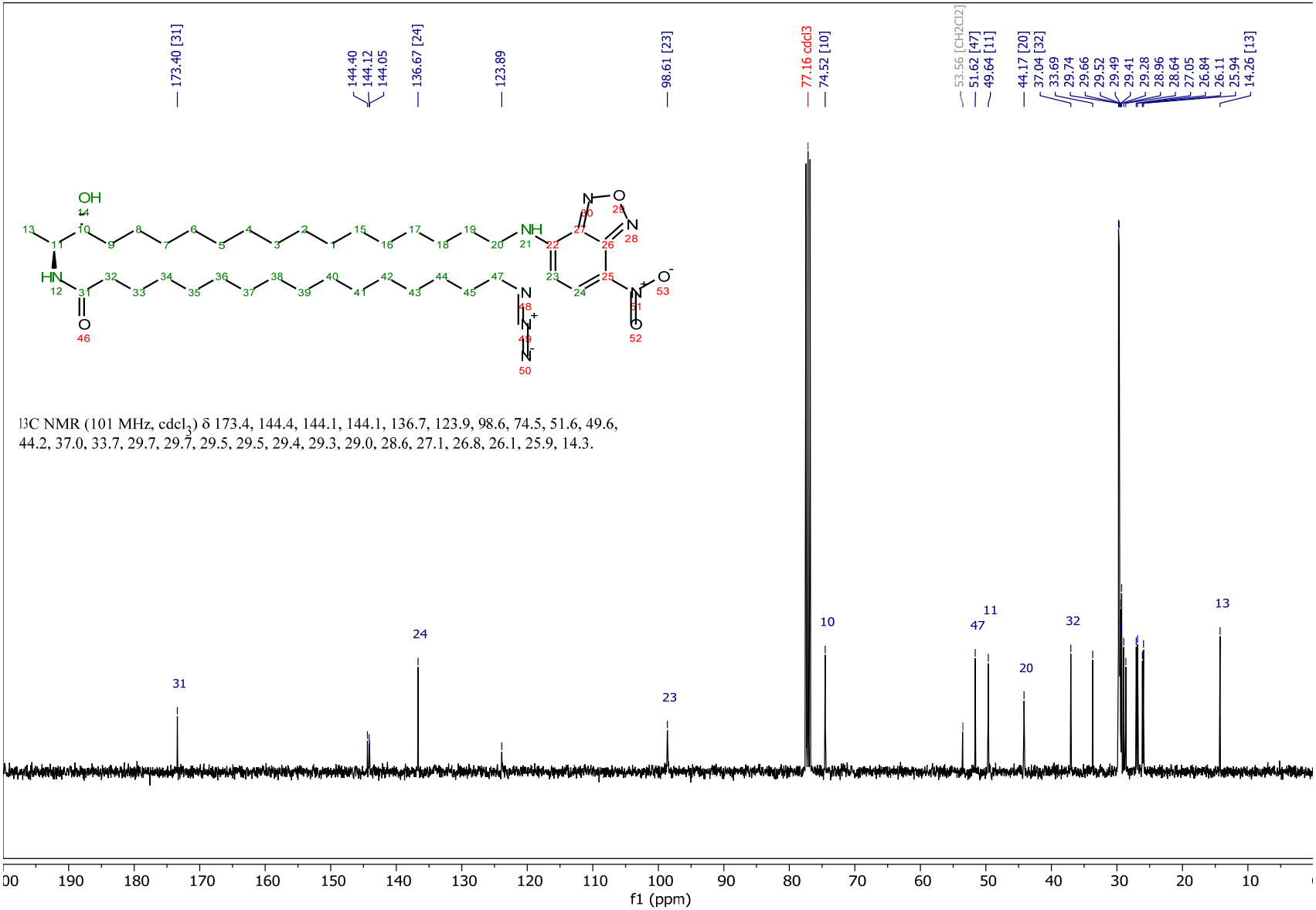
Compound RBM5-159

^{1}H NMR



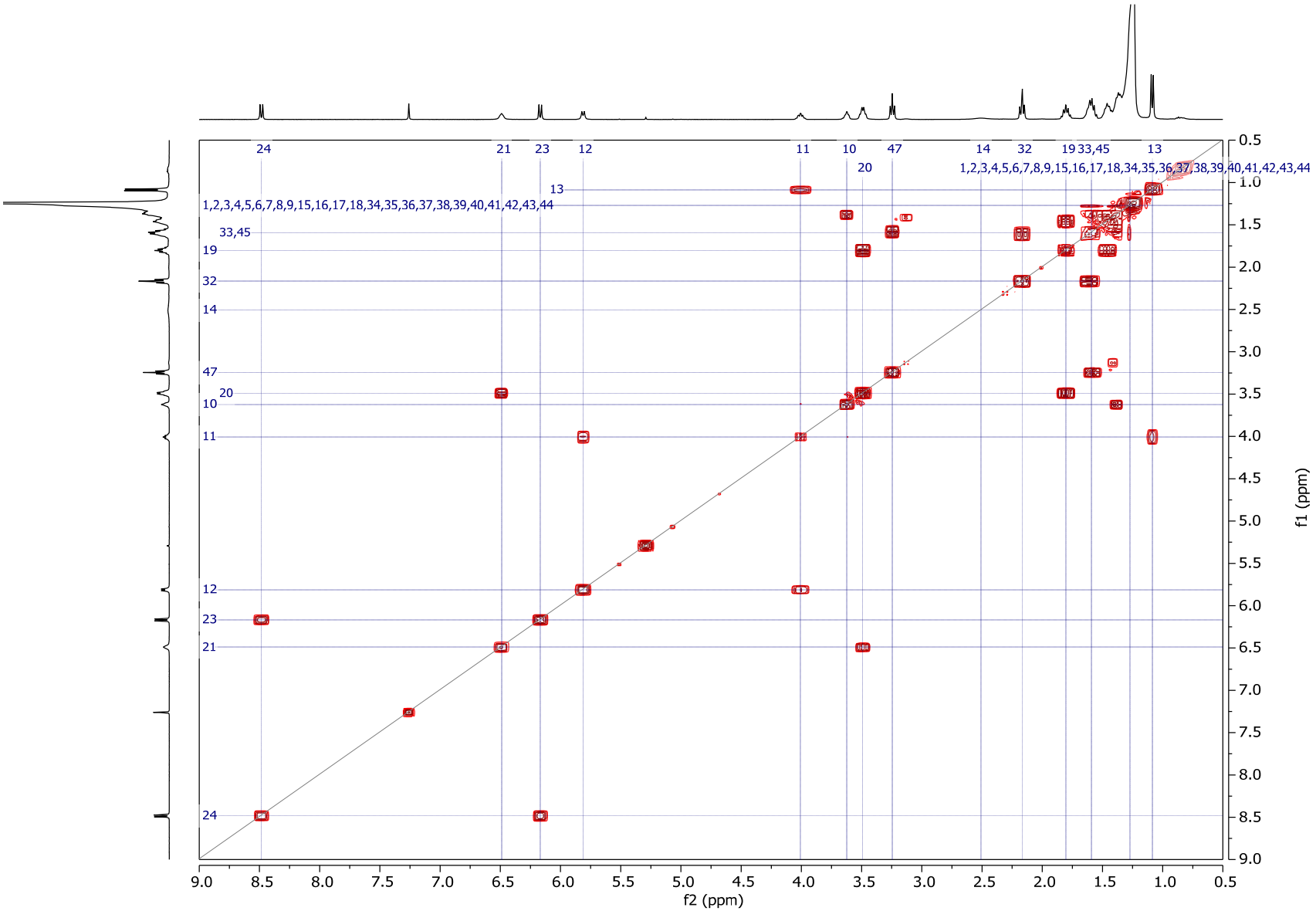
1H NMR (400 MHz, Chloroform-*d*) δ 8.48 (d, *J* = 8.6 Hz, 1H), 6.49 (br s, 1H), 6.17 (d, *J* = 8.7 Hz, 1H), 5.81 (d, *J* = 7.9 Hz, 1H), 4.05 – 3.96 (m, 1H), 3.66 – 3.59 (m, 1H), 3.53 – 3.45 (m, 2H), 3.24 (t, *J* = 7.0 Hz, 2H), 2.51 (br s, 1H), 2.17 (t, *J* = 7.6 Hz, 2H), 1.80 (app p, *J* = 7.4 Hz, 2H), 1.69 – 1.53 (m, 4H), 1.49 – 1.20 (m, 48H), 1.09 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR; RBM5-159

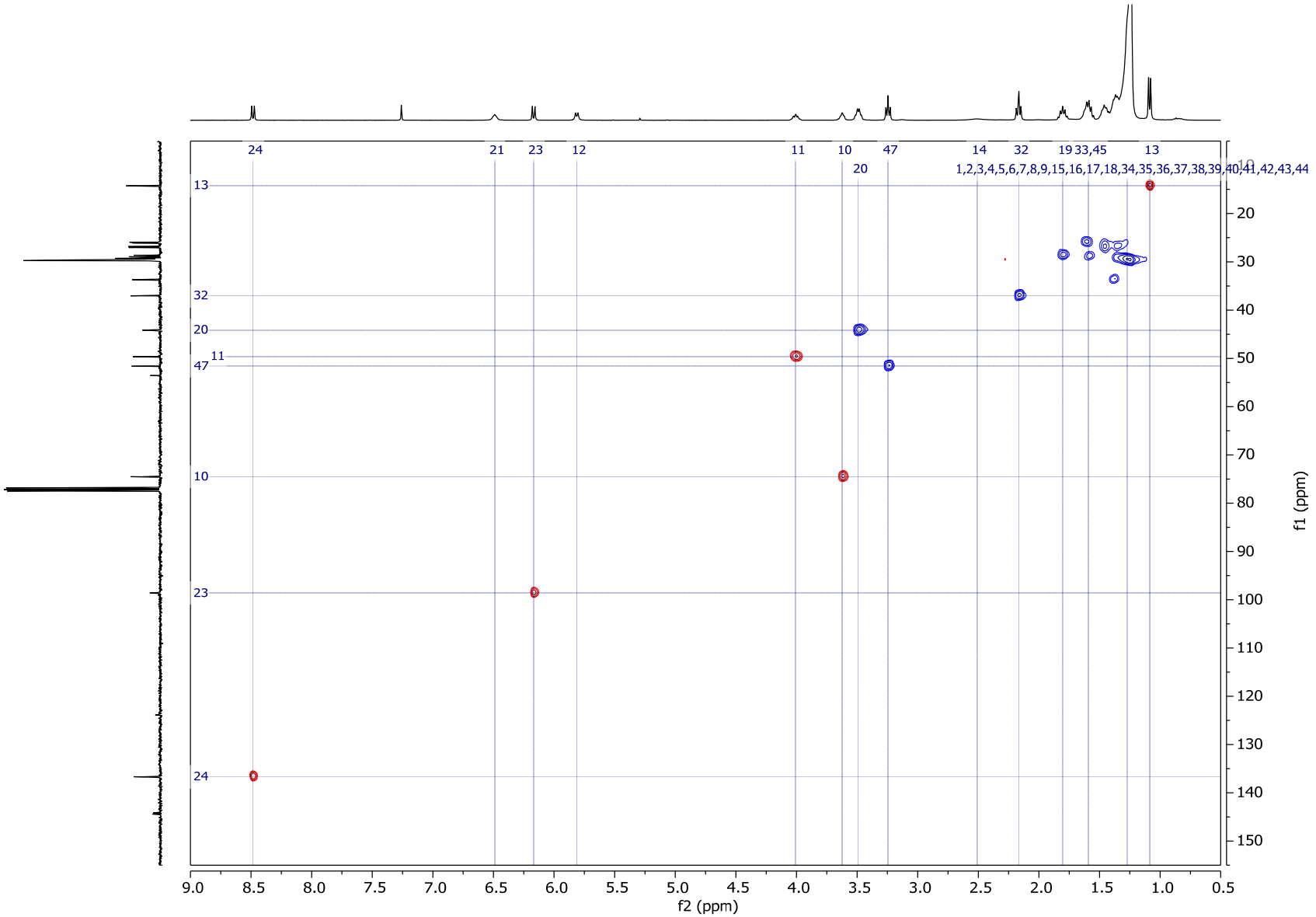


13C NMR (101 MHz, $cdcl_3$) δ 173.4, 144.4, 144.1, 144.1, 136.7, 123.9, 98.6, 74.5, 51.6, 49.6, 44.2, 37.0, 33.7, 29.7, 29.7, 29.5, 29.5, 29.4, 29.3, 29.0, 28.6, 27.1, 26.8, 26.1, 25.9, 14.3.

COSY NMR; RBM5-159

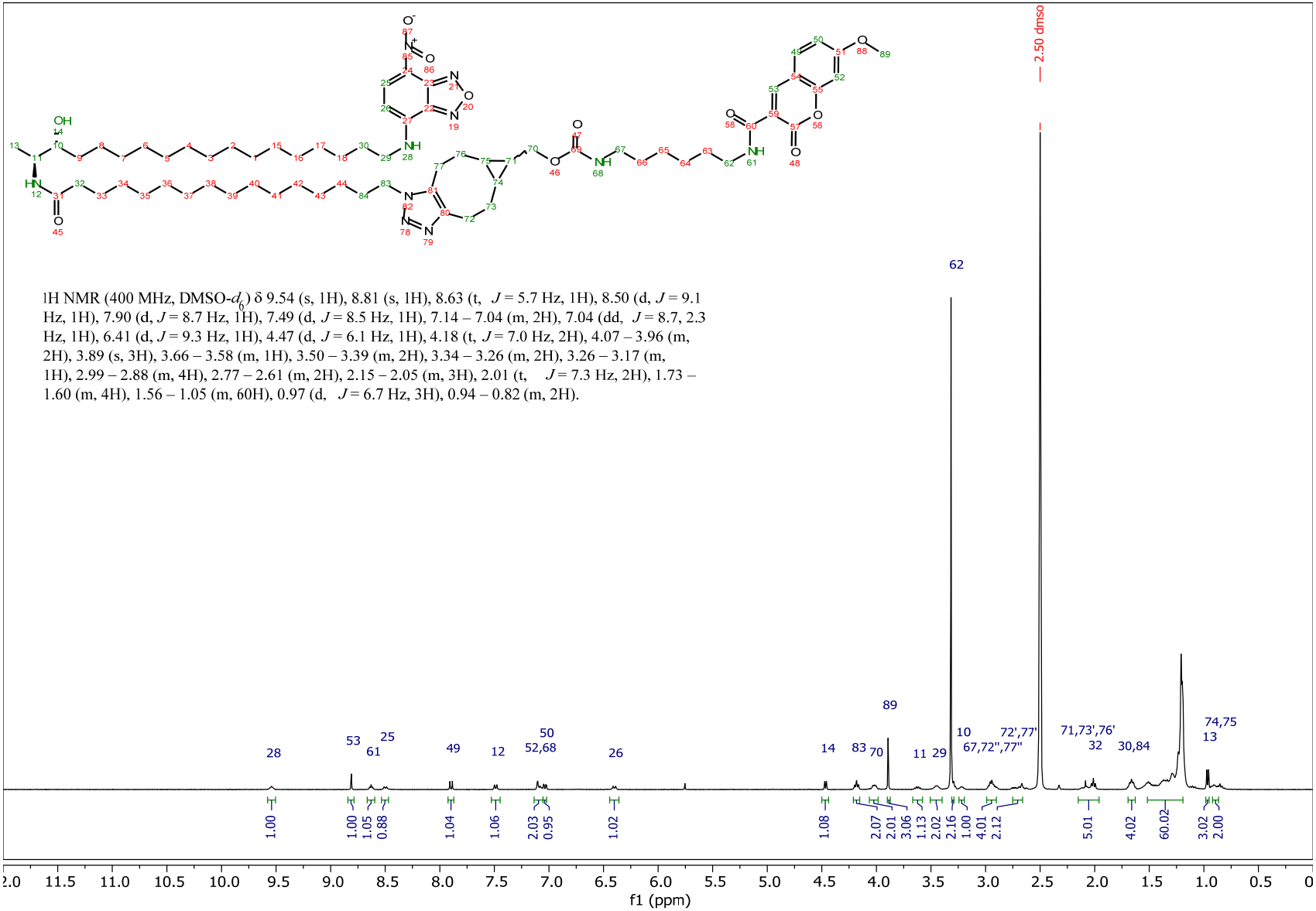


HSQC NMR; RBM5-159



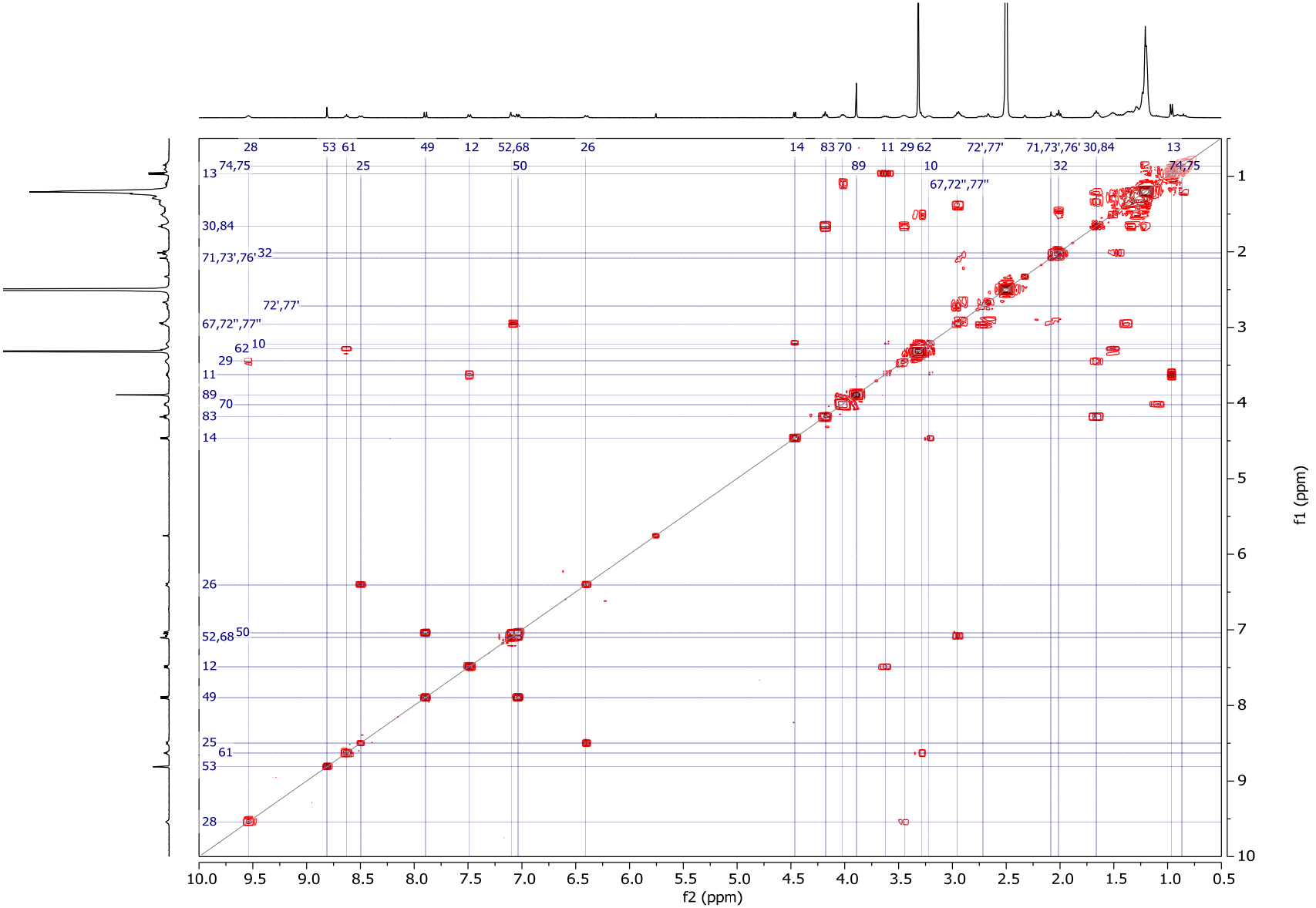
Compound RBM5-160

^{1}H NMR



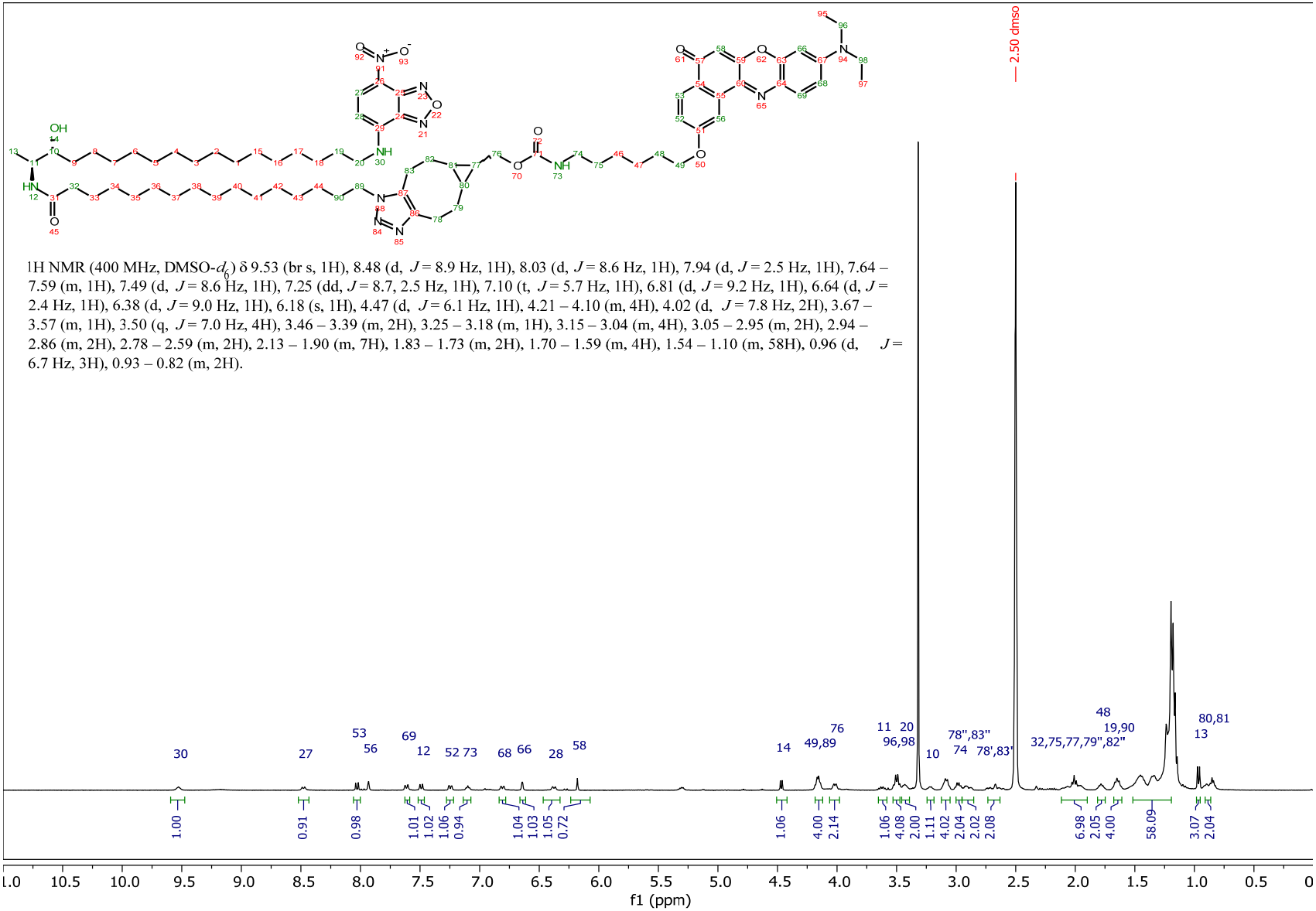
1H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.81 (s, 1H), 8.63 (t, J = 5.7 Hz, 1H), 8.50 (d, J = 9.1 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.14 – 7.04 (m, 2H), 7.04 (dd, J = 8.7, 2.3 Hz, 1H), 6.41 (d, J = 9.3 Hz, 1H), 4.47 (d, J = 6.1 Hz, 1H), 4.18 (t, J = 7.0 Hz, 2H), 4.07 – 3.96 (m, 2H), 3.89 (s, 3H), 3.66 – 3.58 (m, 1H), 3.50 – 3.39 (m, 2H), 3.34 – 3.26 (m, 2H), 3.26 – 3.17 (m, 1H), 2.99 – 2.88 (m, 4H), 2.77 – 2.61 (m, 2H), 2.15 – 2.05 (m, 3H), 2.01 (t, J = 7.3 Hz, 2H), 1.73 – 1.60 (m, 4H), 1.56 – 1.05 (m, 60H), 0.97 (d, J = 6.7 Hz, 3H), 0.94 – 0.82 (m, 2H).

COSY NMR; RBM5-160



Compound RBM5-161

^{1}H NMR;



1H NMR (400 MHz, DMSO-d_6) δ 9.53 (br s, 1H), 8.48 (d, J = 8.9 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 7.94 (d, J = 2.5 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.49 (d, J = 8.6 Hz, 1H), 7.25 (dd, J = 8.7, 2.5 Hz, 1H), 7.10 (t, J = 5.7 Hz, 1H), 6.81 (d, J = 9.2 Hz, 1H), 6.64 (d, J = 2.4 Hz, 1H), 6.38 (d, J = 9.0 Hz, 1H), 6.18 (s, 1H), 4.47 (d, J = 6.1 Hz, 1H), 4.21 – 4.10 (m, 4H), 4.02 (d, J = 7.8 Hz, 2H), 3.67 – 3.57 (m, 1H), 3.50 (q, J = 7.0 Hz, 4H), 3.46 – 3.39 (m, 2H), 3.25 – 3.18 (m, 1H), 3.15 – 3.04 (m, 4H), 3.05 – 2.95 (m, 2H), 2.94 – 2.86 (m, 2H), 2.78 – 2.59 (m, 2H), 2.13 – 1.90 (m, 7H), 1.83 – 1.73 (m, 2H), 1.70 – 1.59 (m, 4H), 1.54 – 1.10 (m, 58H), 0.96 (d, J = 6.7 Hz, 3H), 0.93 – 0.82 (m, 2H).

COSY NMR; RBM5-161