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Title: Cytotoxic C_{20} Diterpenoid Alkaloids from the Australian Endemic Rainforest Plant *Anopterus macleayanus*

Authors: Claire Levrier,[†,‡] Martin C. Sadowski,[‡] Colleen C. Nelson,[‡] and Rohan A. Davis[*,†]

Address:
[†]Eskitis Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia

[‡] Australian Prostate Cancer Research Centre−Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Princess Alexandra Hospital, Translational Research Institute, Brisbane, QLD 4102, Australia

Corresponding author contact details:
Tel.: +61 7 3735 6043; Fax: +61 7 3735 6001; E-mail: r.davis@griffith.edu.au

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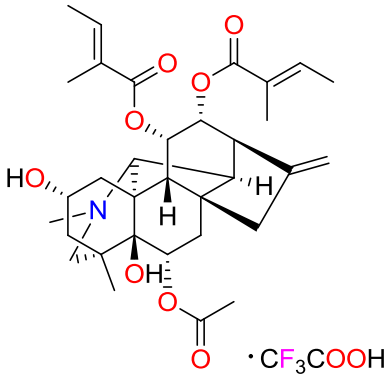

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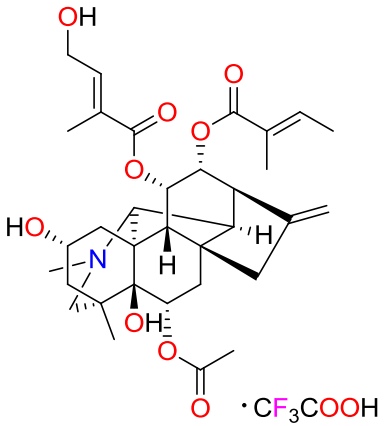

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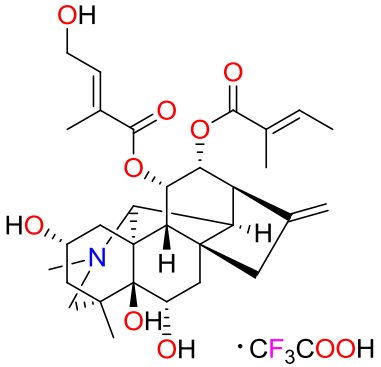

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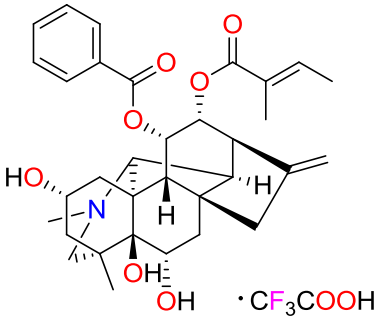

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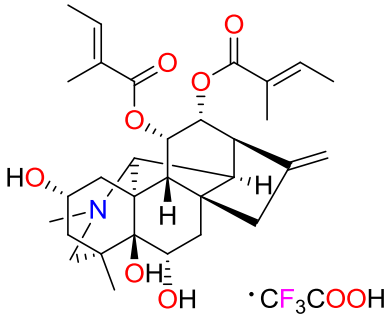

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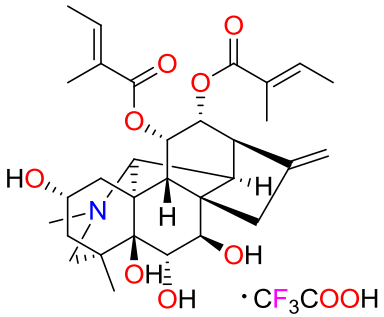

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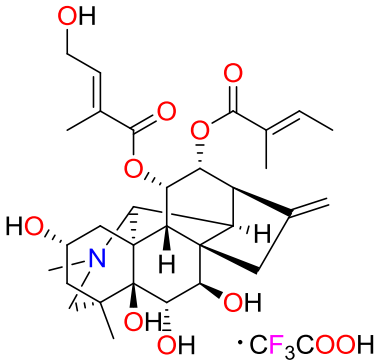

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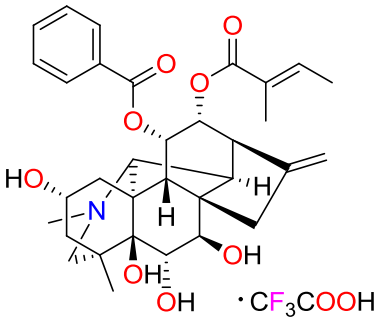

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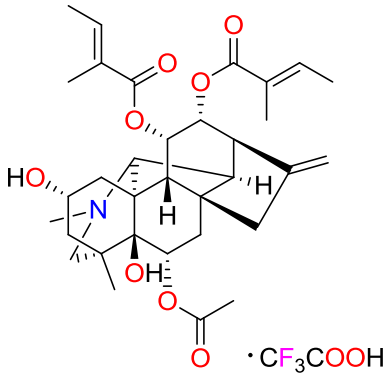
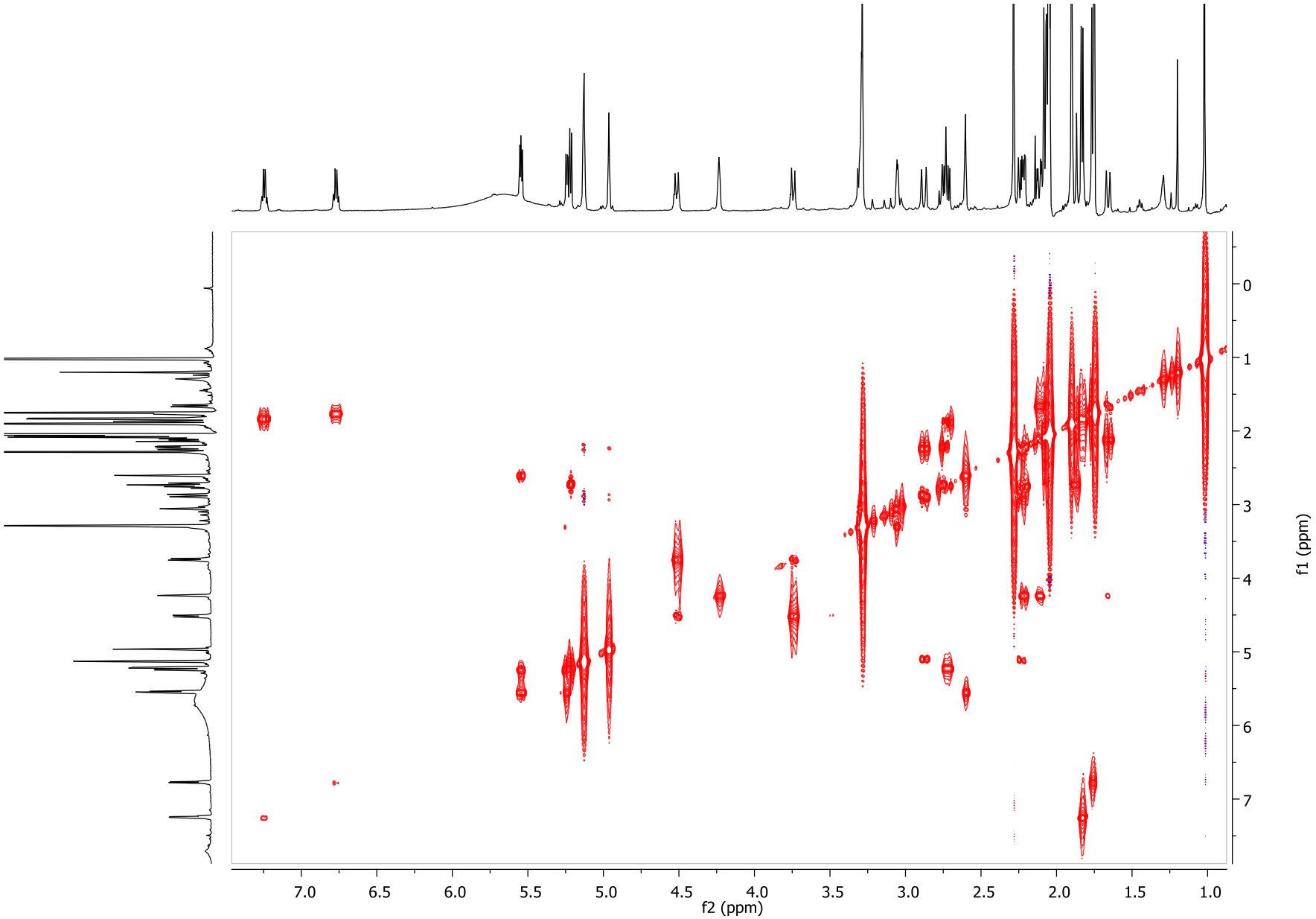
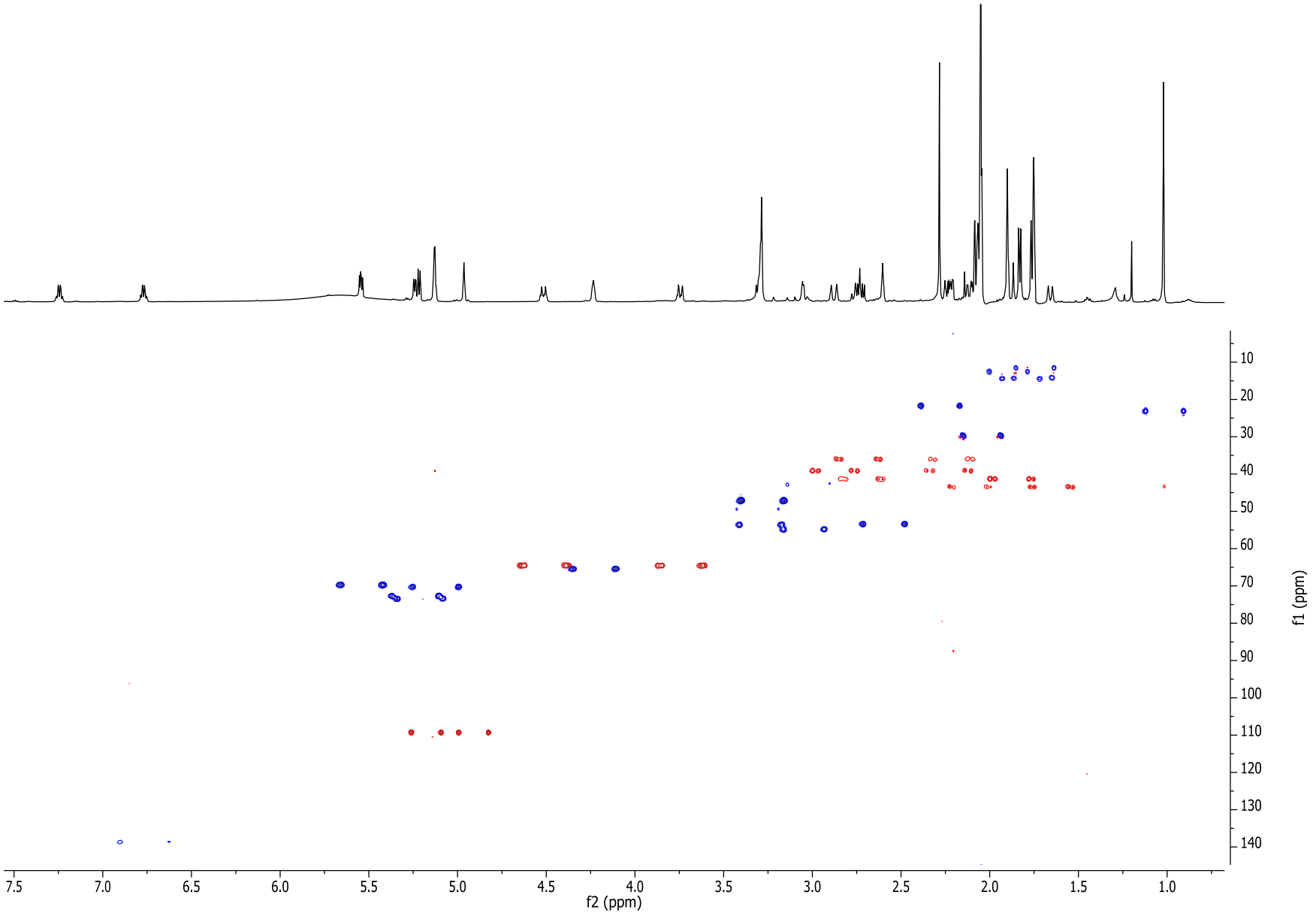
S1. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)



S2. COSY (Acetone-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)

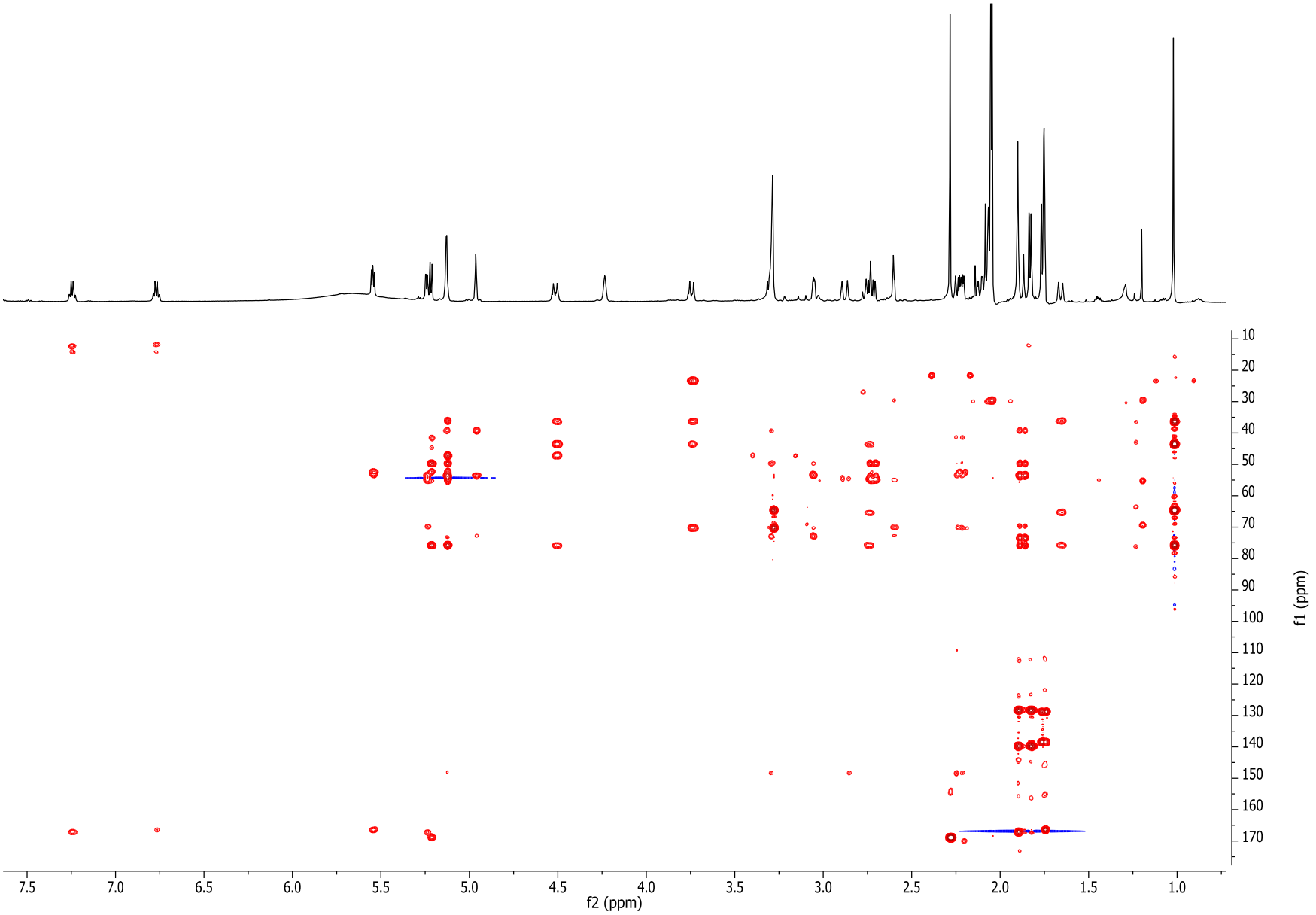


S3. HSQC* (Acetone-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)

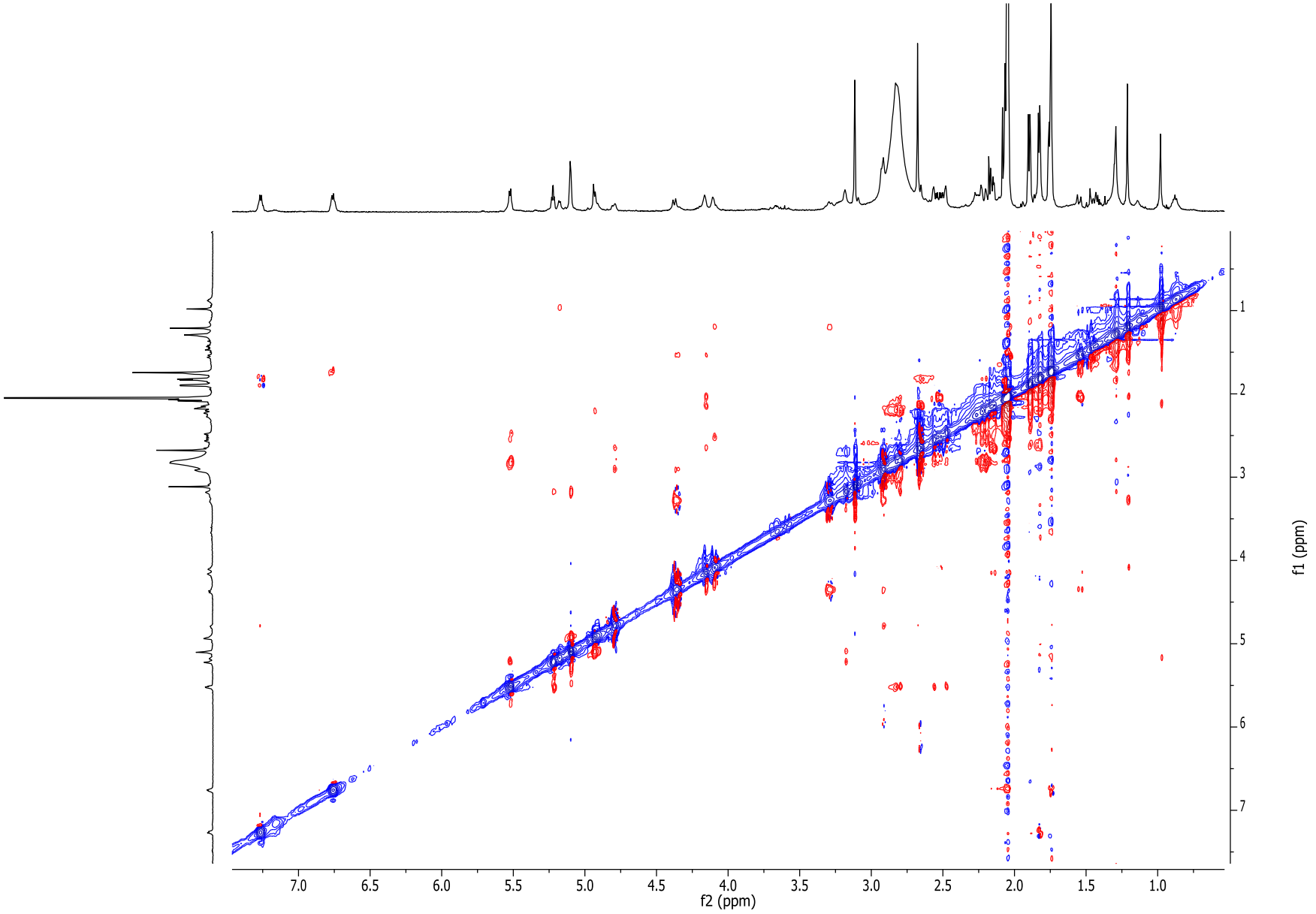


*^{13}C decoupler out of order at time of acquisition, resulting in signals being split by $^1J_{CH}$

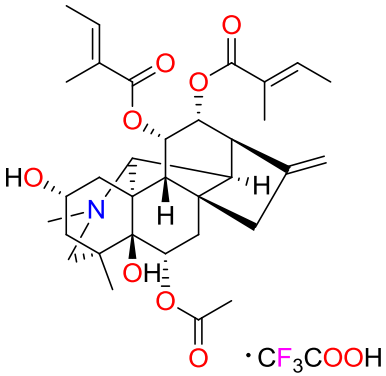
S4. HMBC (Acetone-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)



S5. ROESY (Acetone-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)



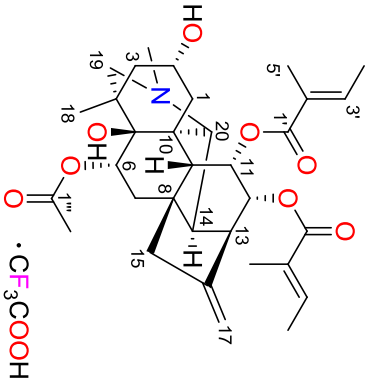
S6. ^{1}H NMR (600 MHz, DMSO-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)



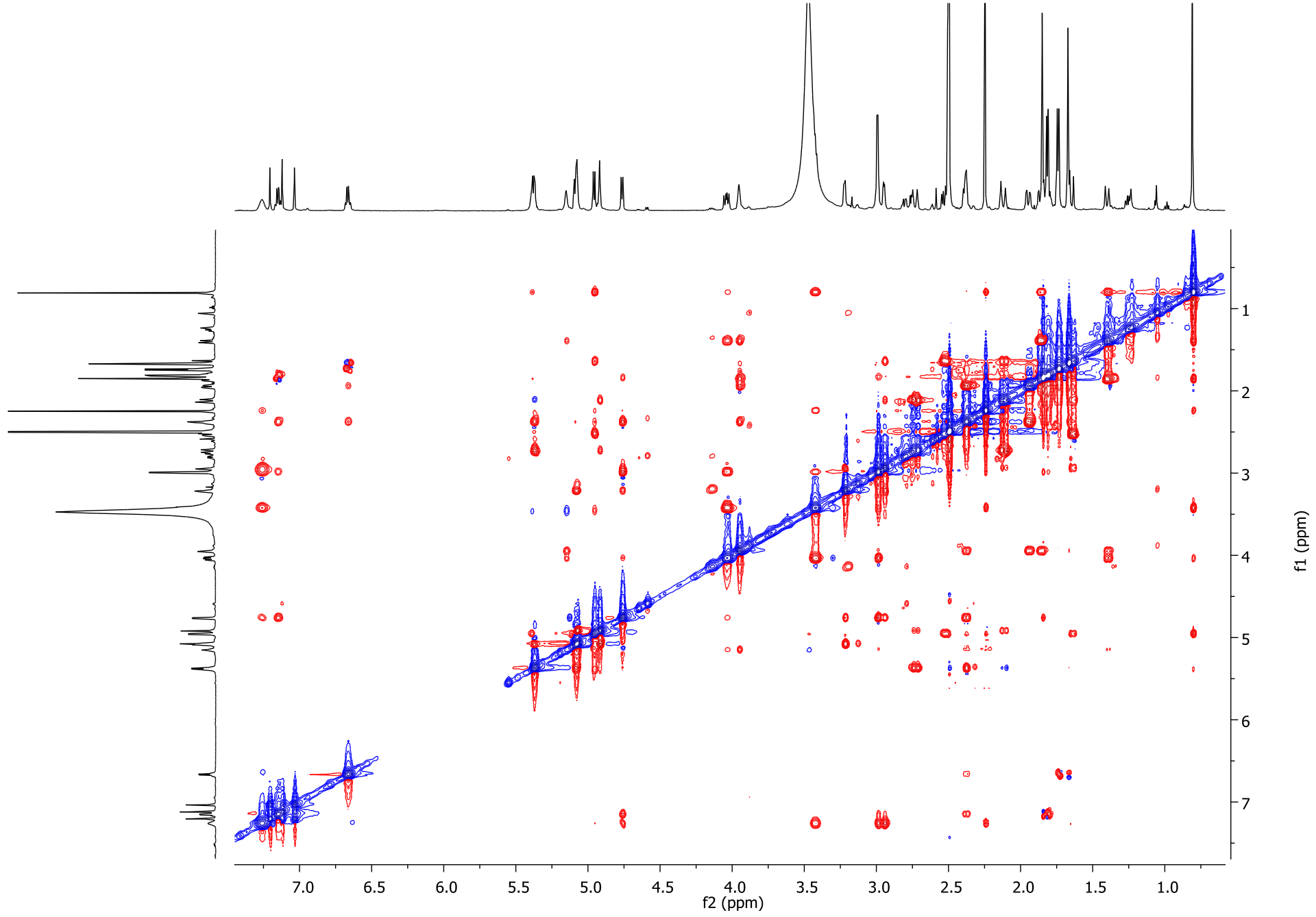
S7. Comparison of NMR Data of 6α-Acetoxyanopterine (**1**) in DMSO-d_6 and Acetone-d_6

Position	**1** [a]	**1** [b]
	δ_H (mult. *J* in Hz)	δ_H (mult. *J* in Hz)
1	2.22, dd (14.6, 4.7)	1.95, dd (14.5, 4.4)
	2.75, br d (14.6)	2.40, m
2	4.24, m	3.95, br s
OH-2		5.15, br s
3	1.66, br d (14.3)	1.40, br d (14.3)
	2.12, dd (14.3, 3.8)	1.88, m
OH-5		5.38, br s
6	5.22, br d (6.7)	4.96, d (6.9)
7	1.89, br d (16.2)	1.66, d (15.9)
	2.72, dd (16.2, 6.7)	2.53, dd (15.9, 6.9)
9	2.60, m	2.39, m
11	5.54, dd (6.1, 4.4)	5.38, dd (6.0, 5.0)
12	5.24, dd (6.1, 2.4)	5.09, dd (6.0, 2.3)
13	3.30, m	3.22, m
14	3.05, m	2.95, m
15	2.23, ddd (18.6, 2.1, 2.1)	2.12, brd (18.5)
	2.88, br d (18.6)	2.75, br d (18.5)
17	4.96, m	4.92, m
	5.13, m	5.08, m
18	1.02, s	0.81, s
19	3.74, m	3.44, m
	4.52, m	4.04, m
20	5.12, m	4.76, d (6.7)
N-CH_3	3.29, s	2.99, d (4.6)
H-N-CH_3		7.26, br s
3'	6.77, br q (6.7)	6.66, br q (6.8)
4'	1.76, d (6.7)	1.74, br d (6.8)
5'	1.75, s	1.67, s
3"	7.26, br q (7.0)	7.15, br q (7.0)
4"	1.83, d (7.0)	1.82, br d (7.0)
5"	1.90, s	1.85, s
2"'	2.29, s	2.25, s

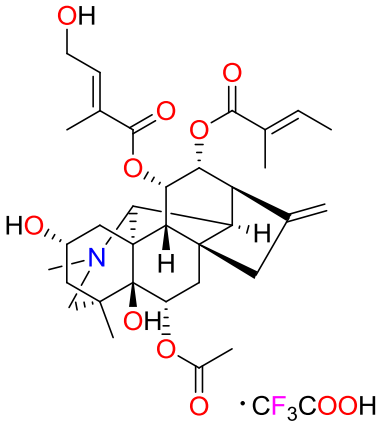
[a] Spectrum recorded at 600 MHz in acetone-d_6 at 30 °C.
[b] Spectrum recorded at 600 MHz in DMSO-d_6 at 30 °C.



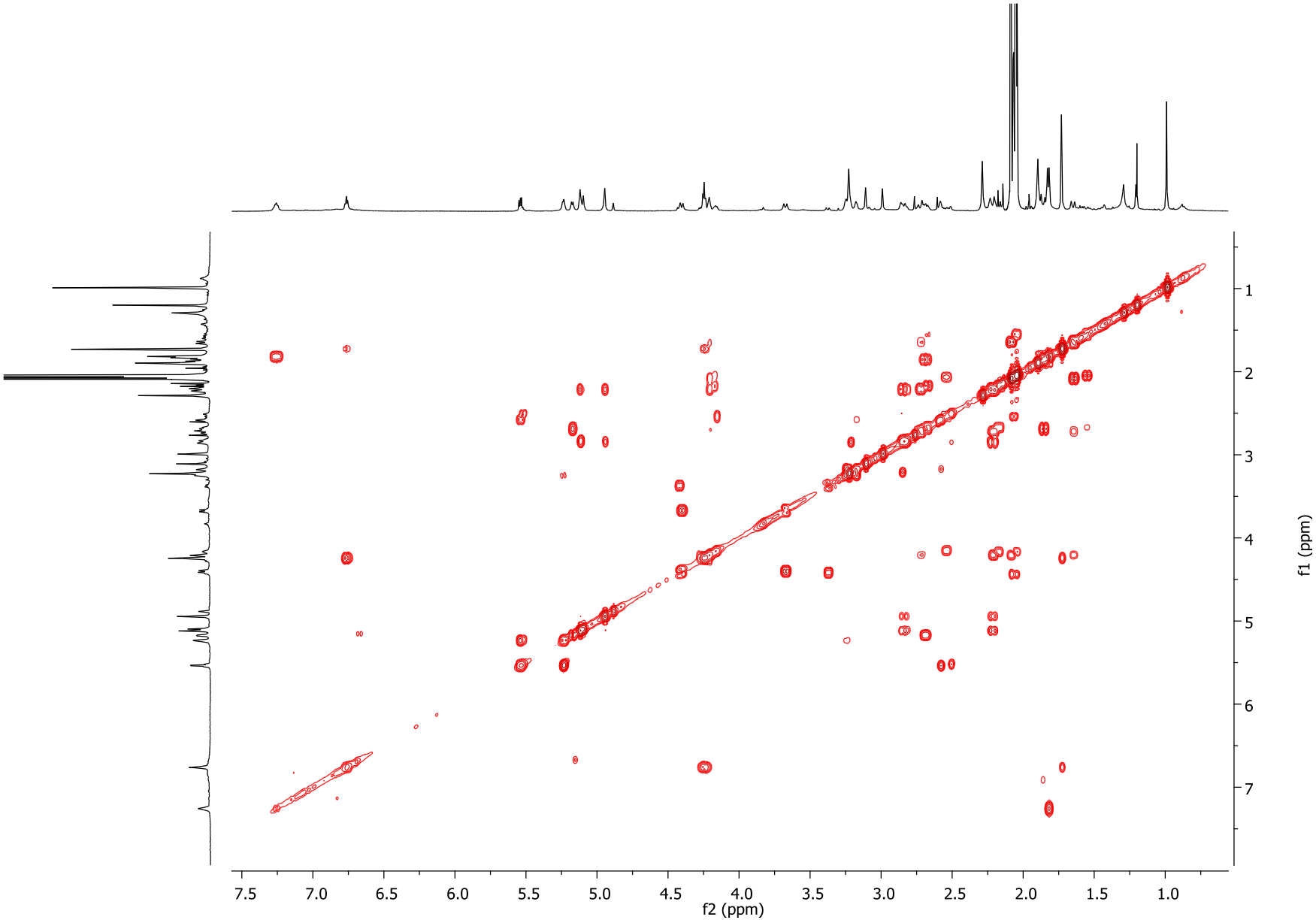
S8. ROESY (DMSO-d_6) Spectrum of 6α-Acetoxyanopterine (**1**)



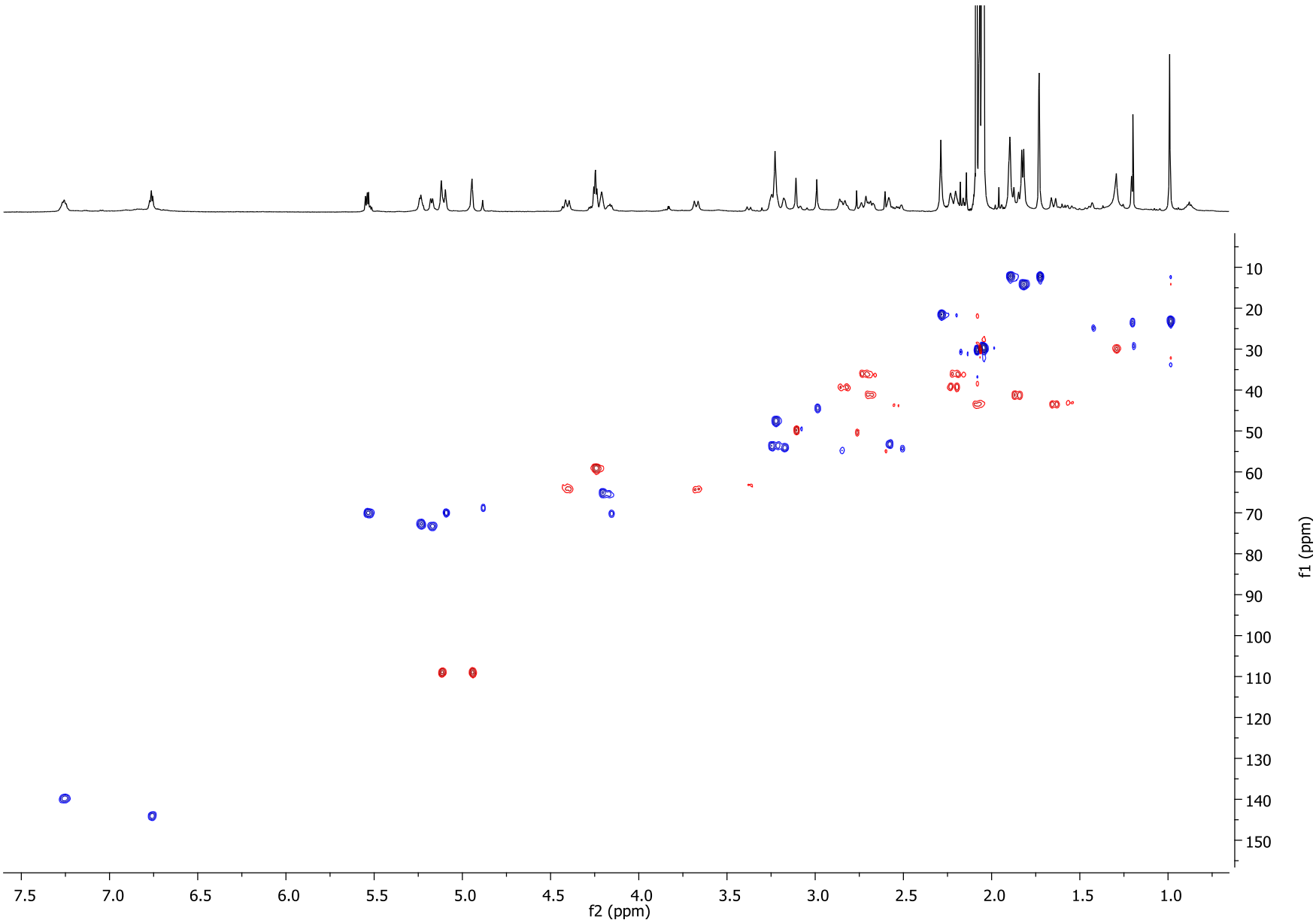
S9. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 4'-Hydroxy-6α-acetoxyanopterine (**2**)



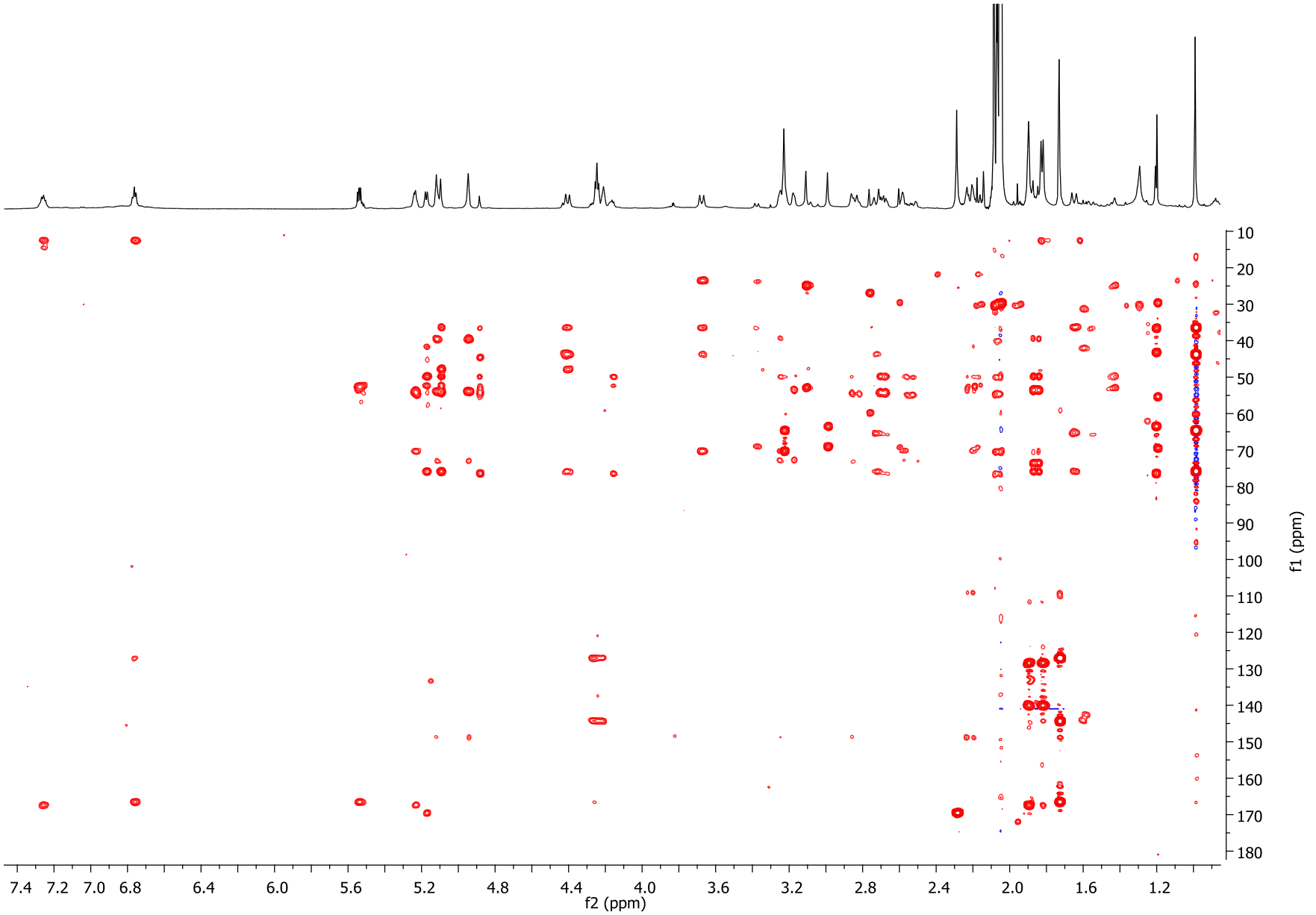
S10. COSY (Acetone-d_6) Spectrum of 4'-Hydroxy-6α-acetoxyanopterine (**2**)



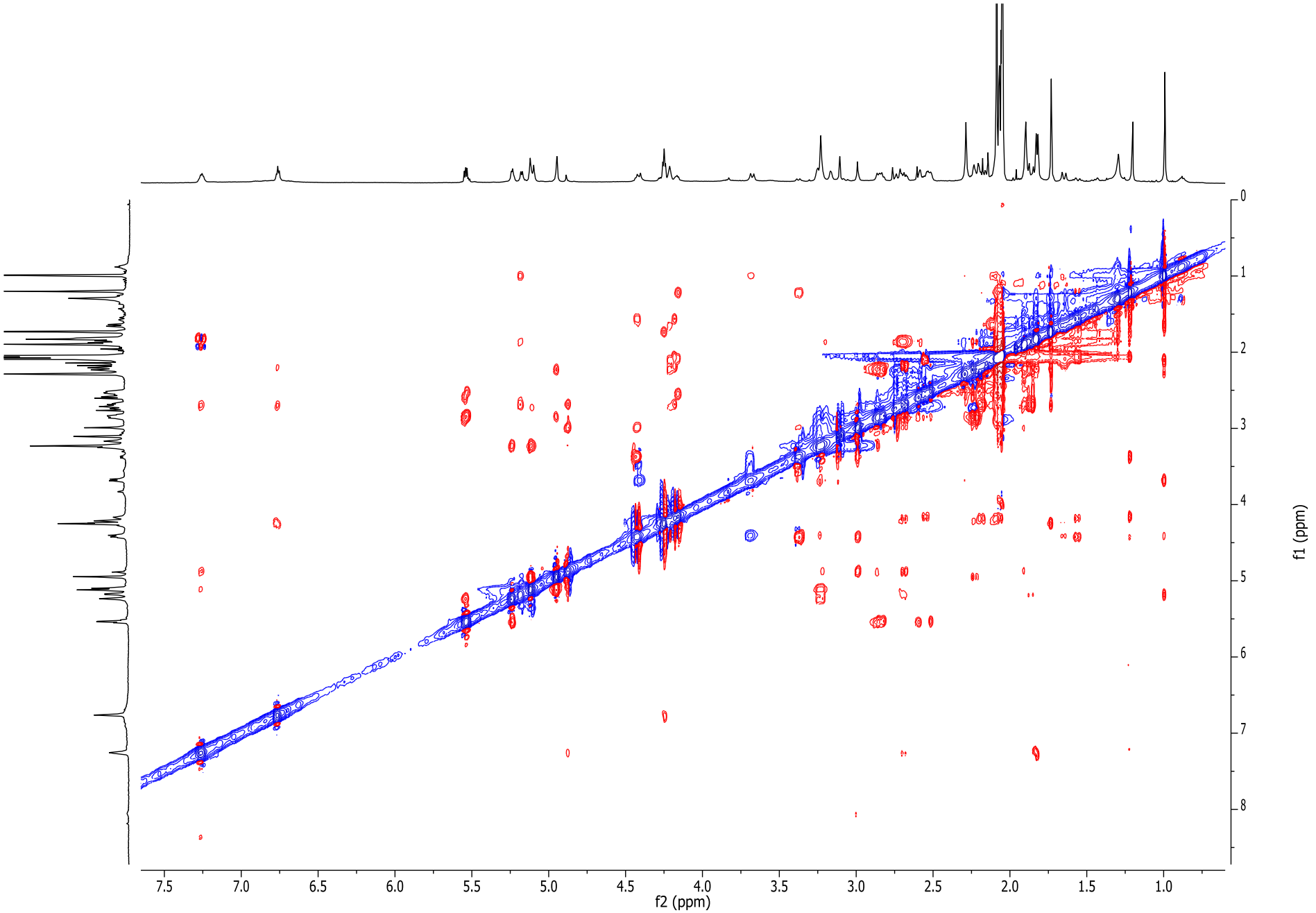
S11. HSQC (Acetone-d_6) Spectrum of 4'-Hydroxy-6α-acetoxyanopterine (**2**)



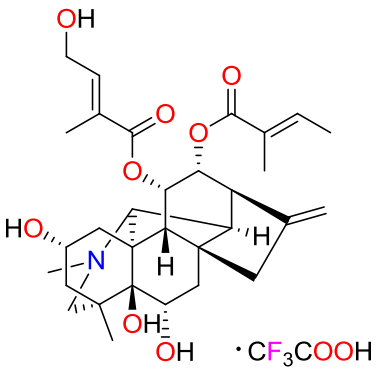
S12. HMBC (Acetone-d_6) Spectrum of 4'-Hydroxy-6α-acetoxyanopterine (**2**)



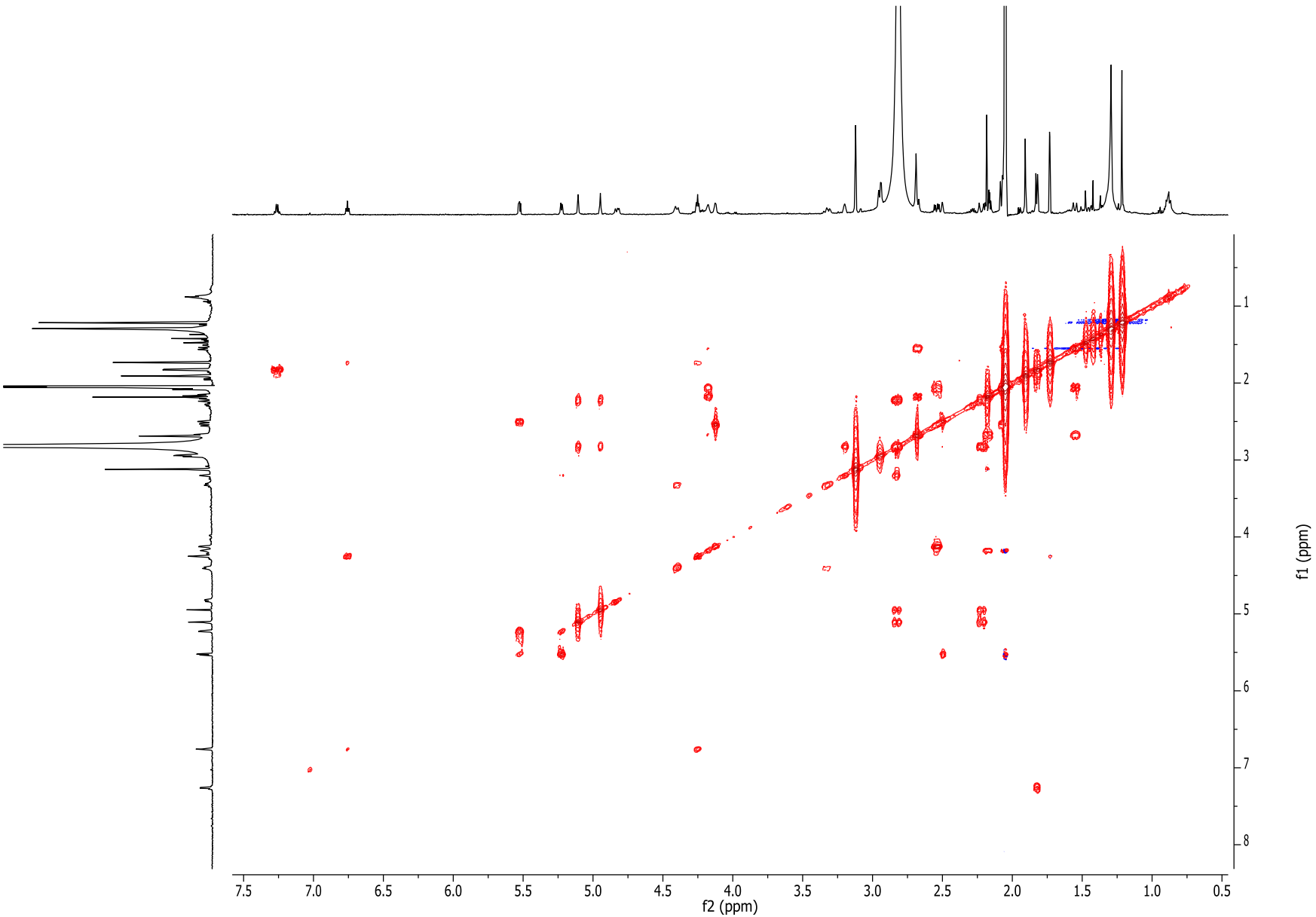
S13. ROESY (Acetone-d_6) Spectrum of 4'-Hydroxy-6α-acetoxyanopterine (**2**)



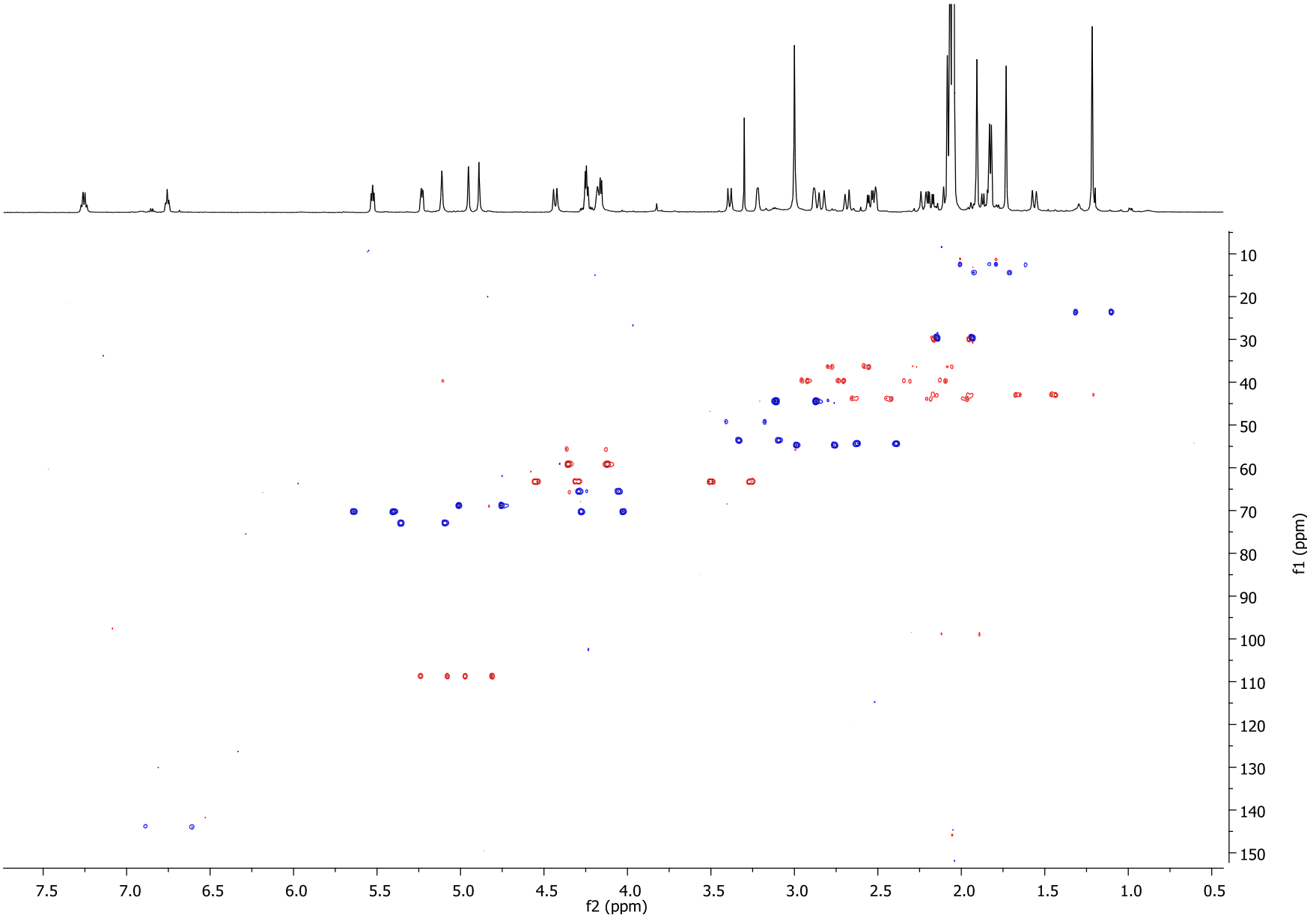
S14. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 4'-Hydroxyanopterine (**3**)



S15. COSY (Acetone-d_6) Spectrum of 4'-Hydroxyanopterine (**3**)

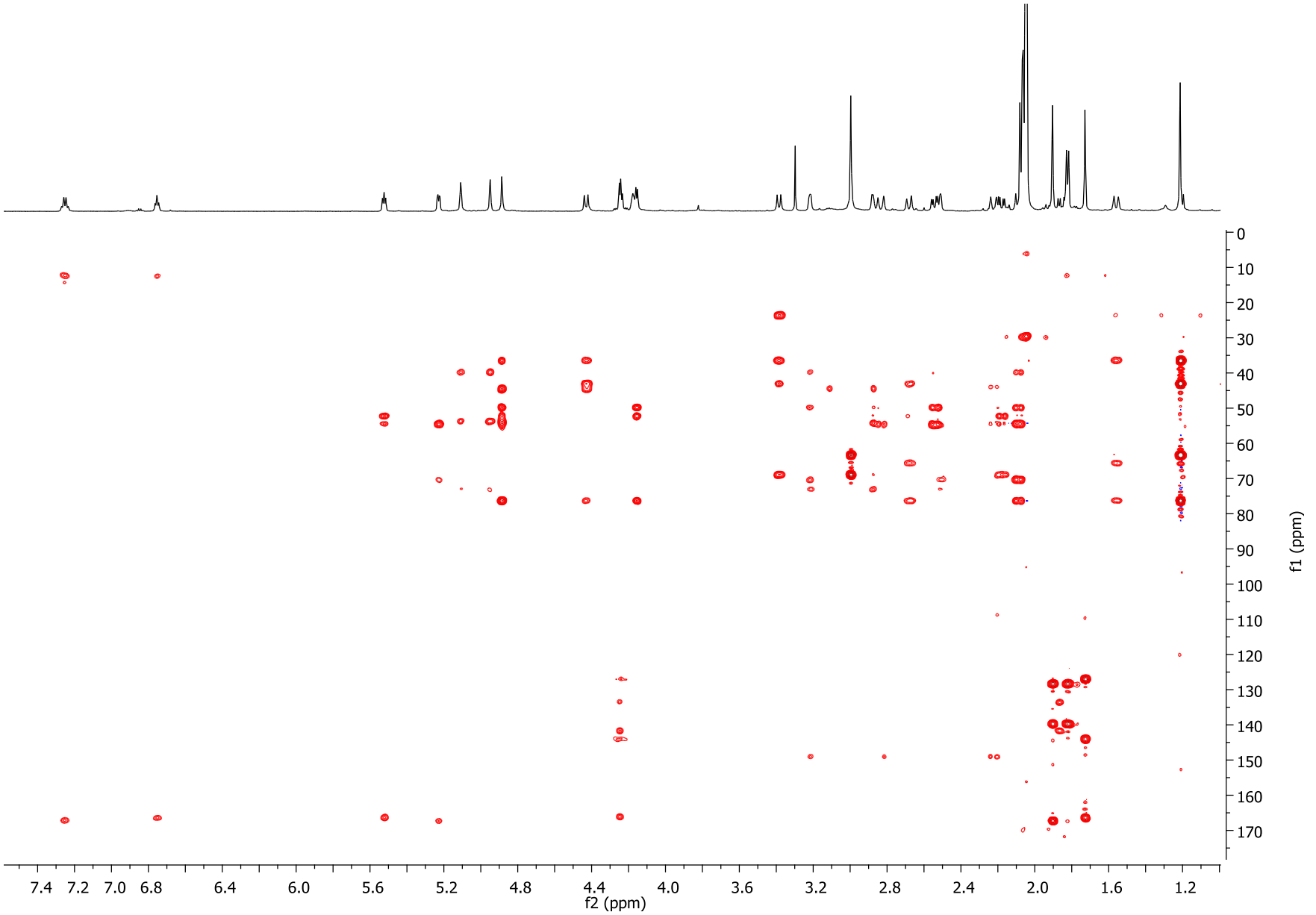


S16. HSQC* (Acetone-d_6) Spectrum of 4'-Hydroxyanopterine (**3**)

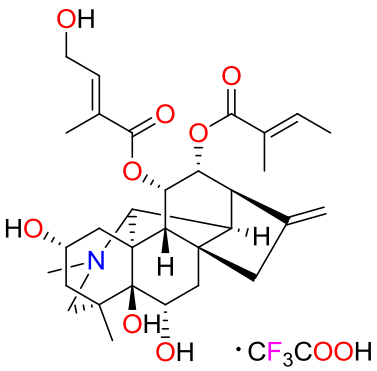


*^{13}C decoupler out of order at time of acquisition, resulting in signals being split by $^{1}J_{CH}$

S17. HMBC (Acetone-d_6) Spectrum of 4'-Hydroxyanopterine (**3**)

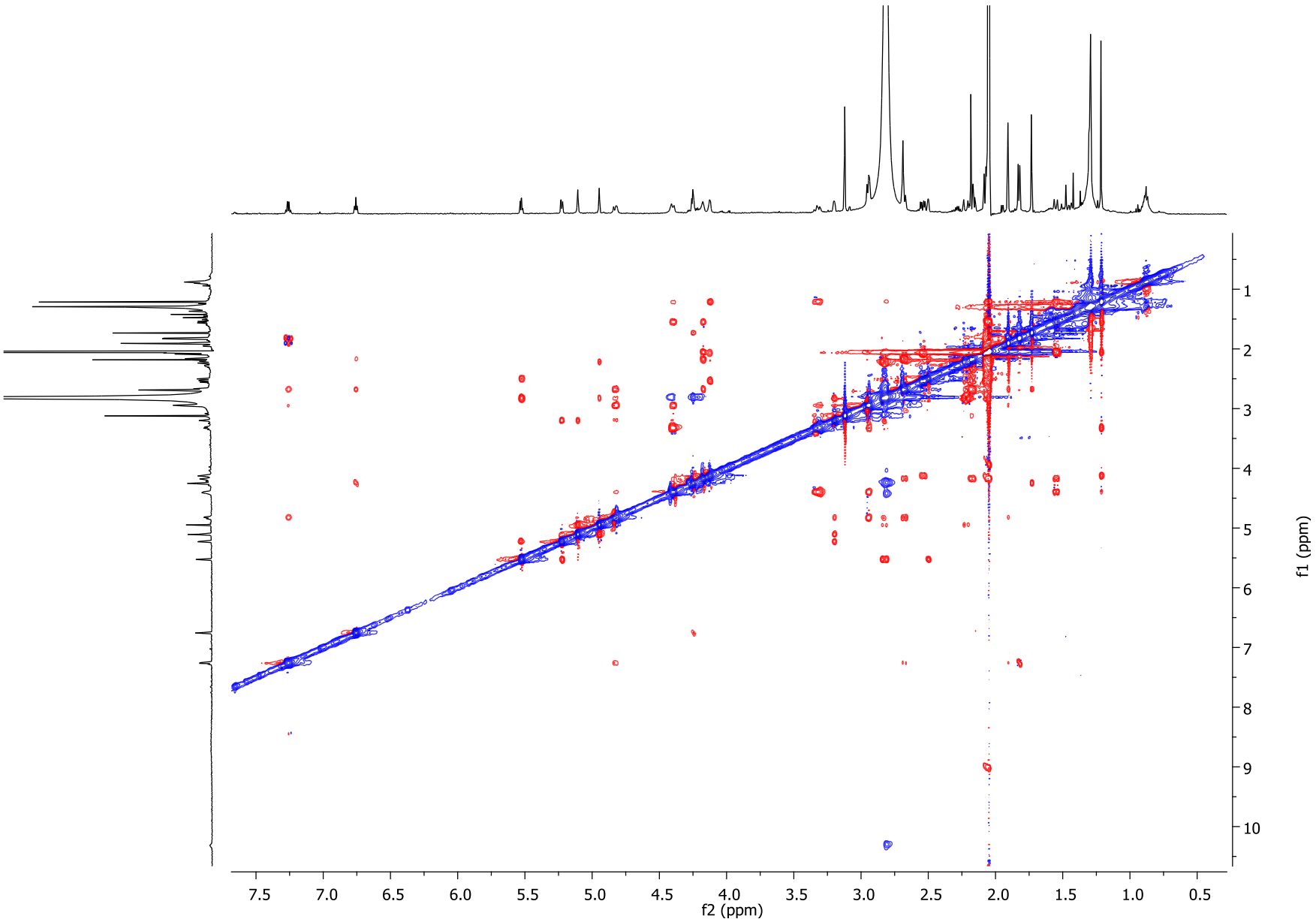


S18. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 4'-Hydroxyanopterine (**3**)[a]

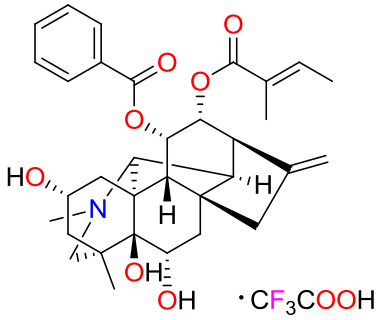


[a]Sample 2

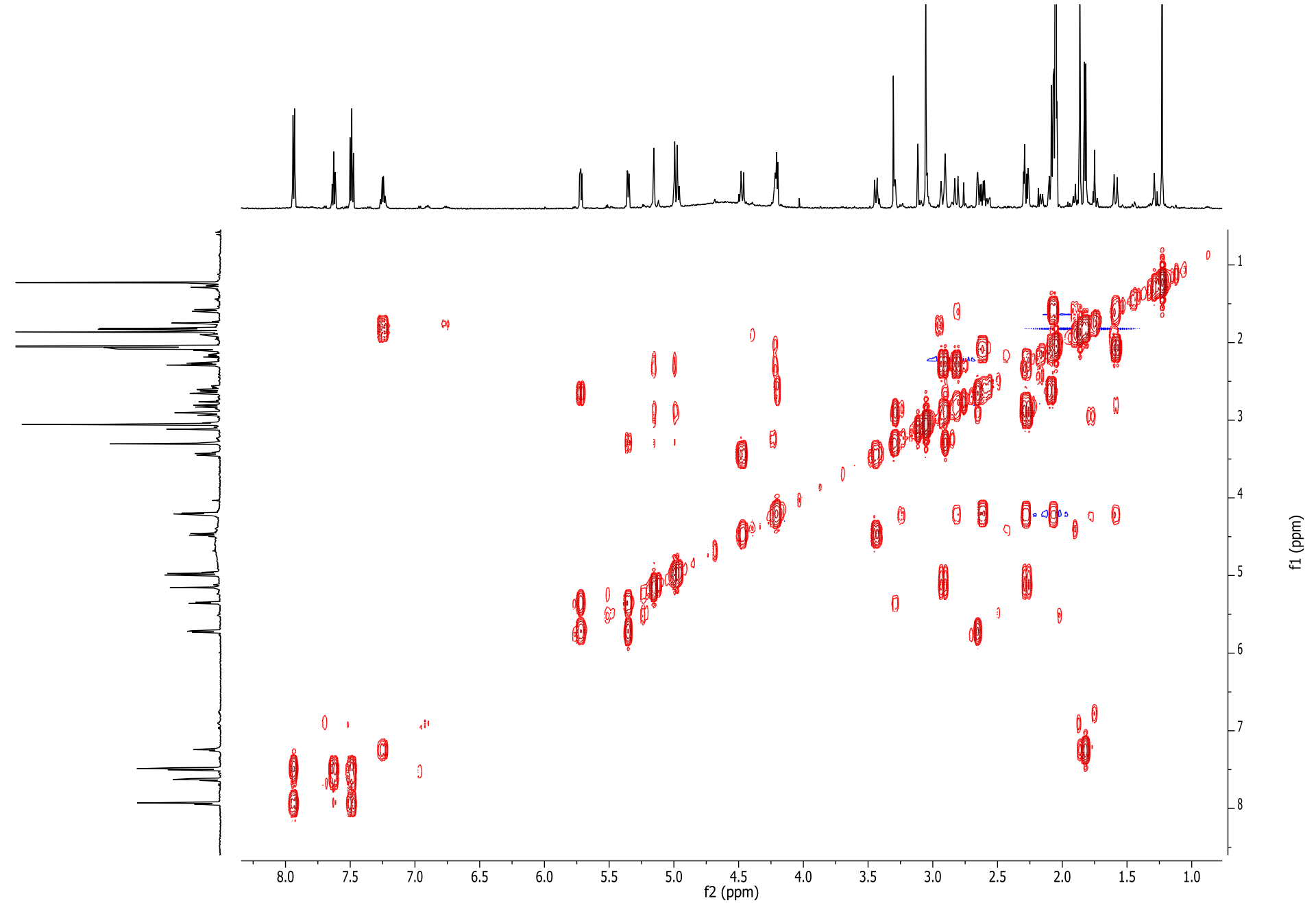
S19. ROESY (Acetone-d_6) Spectrum of 4'-Hydroxyanopterine (**3**)



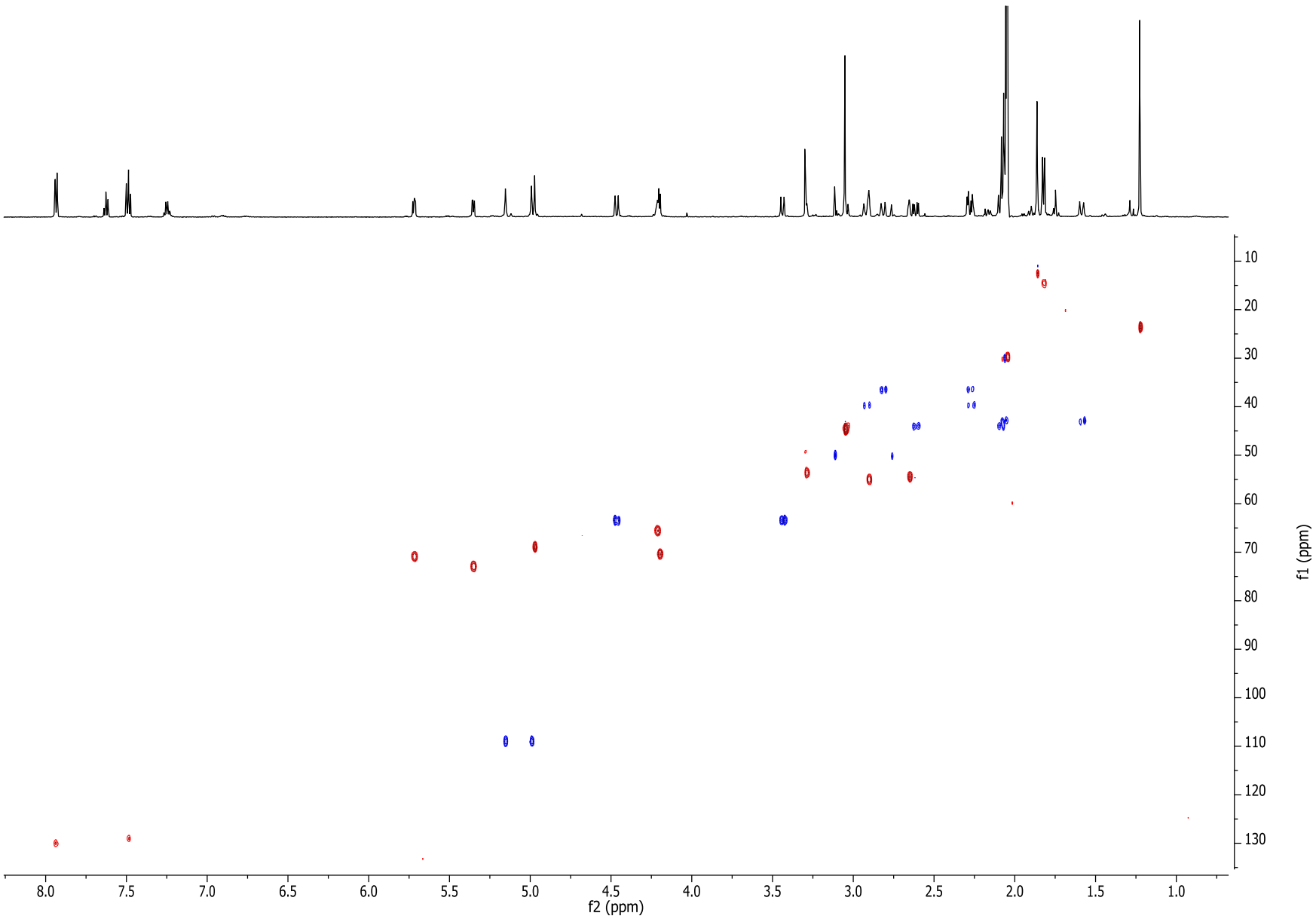
S20. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 11α-Benzoylanopterine (**4**)



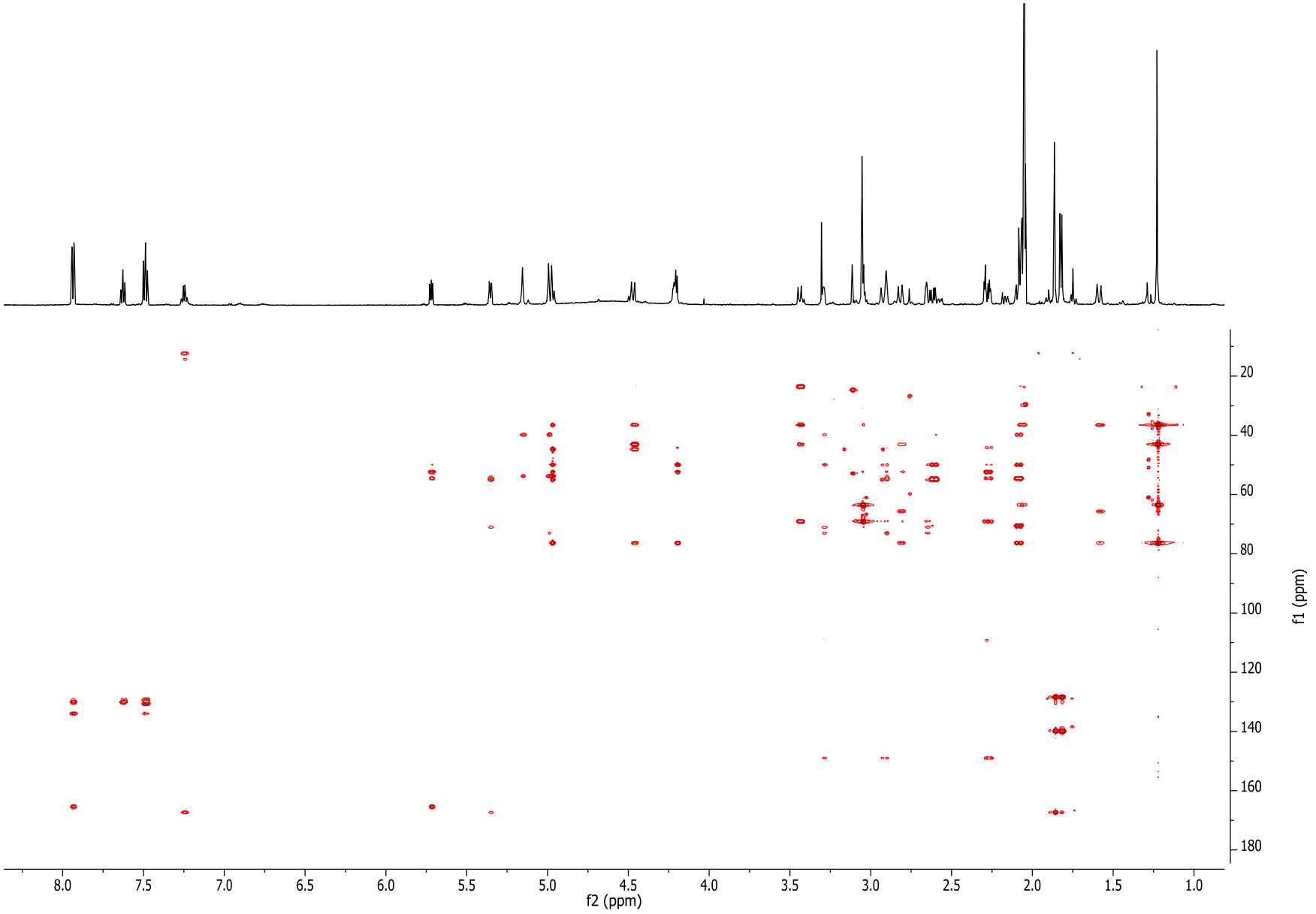
S21. COSY (Acetone-d_6) Spectrum of 11α-Benzoylanopterine (**4**)



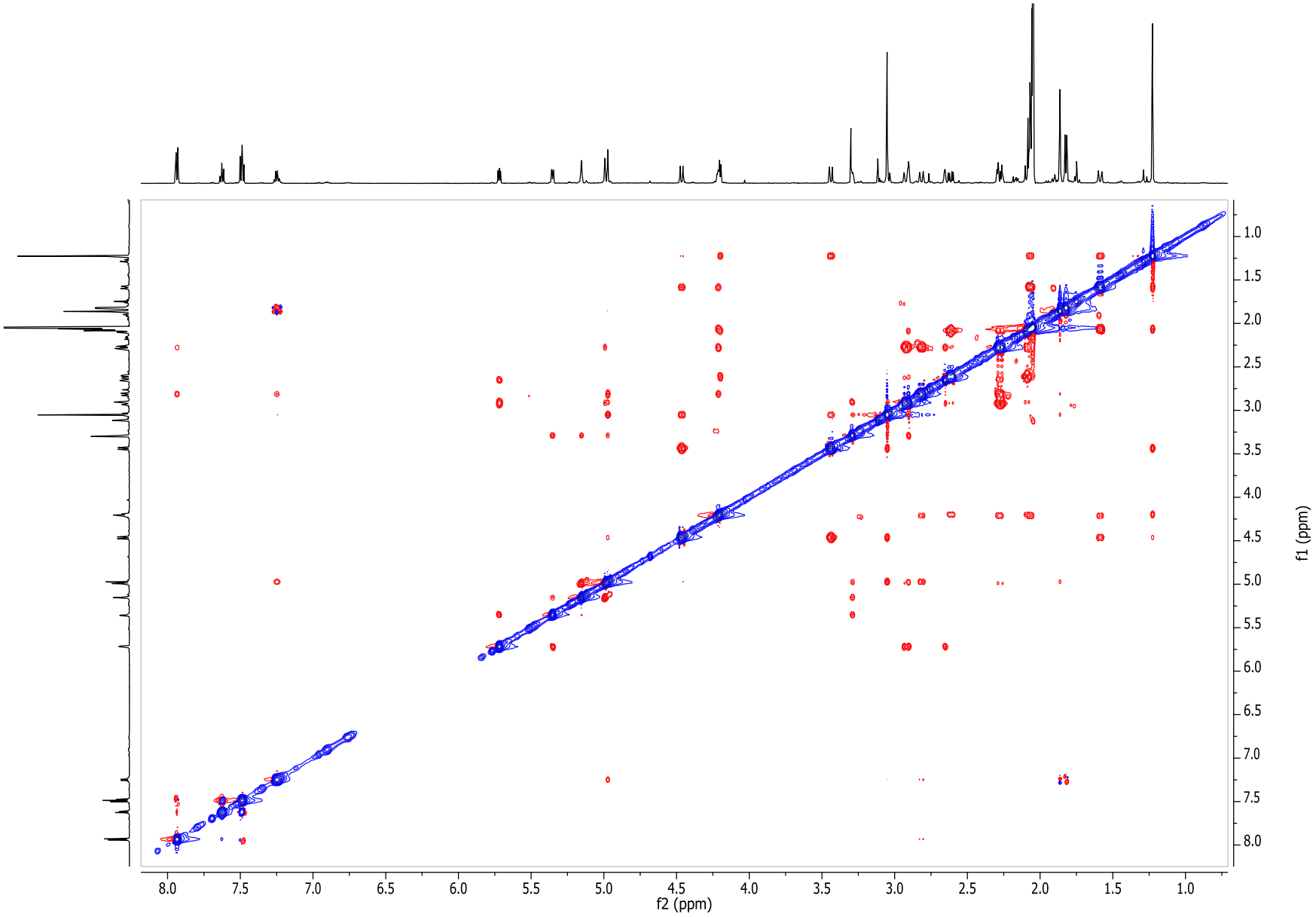
S22. HSQC (Acetone-d_6) Spectrum of 11α-Benzoylanopterine (**4**)



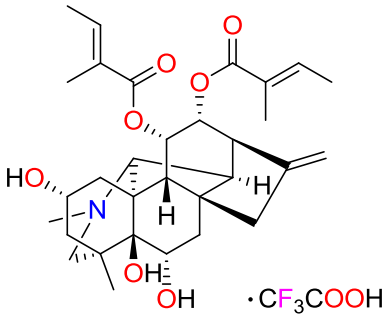
S23. HMBC (Acetone-d_6) Spectrum of 11α-Benzoylanopterine (**4**)



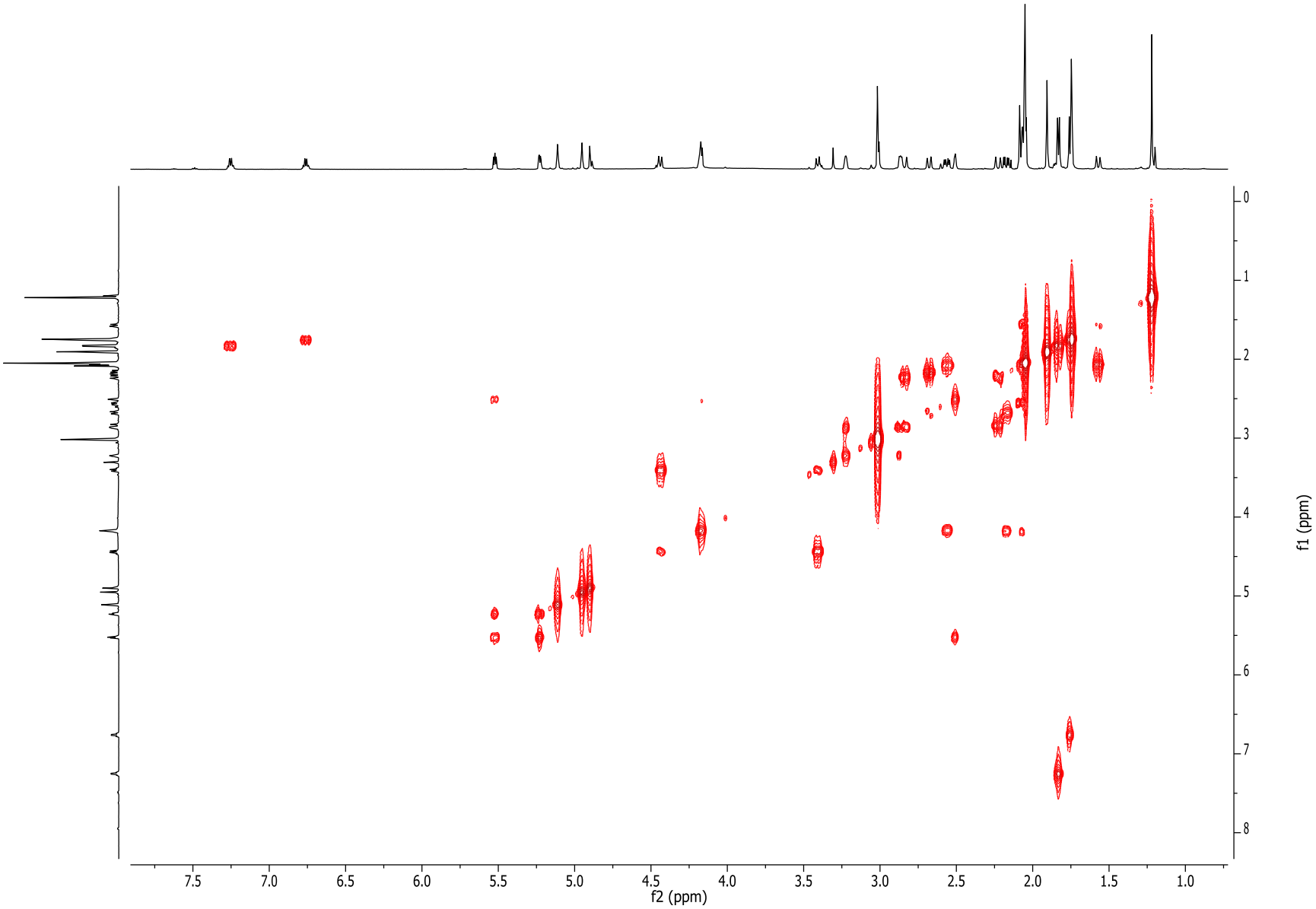
S24. ROESY Spectrum of 11α-Benzoylanopterine (**4**)



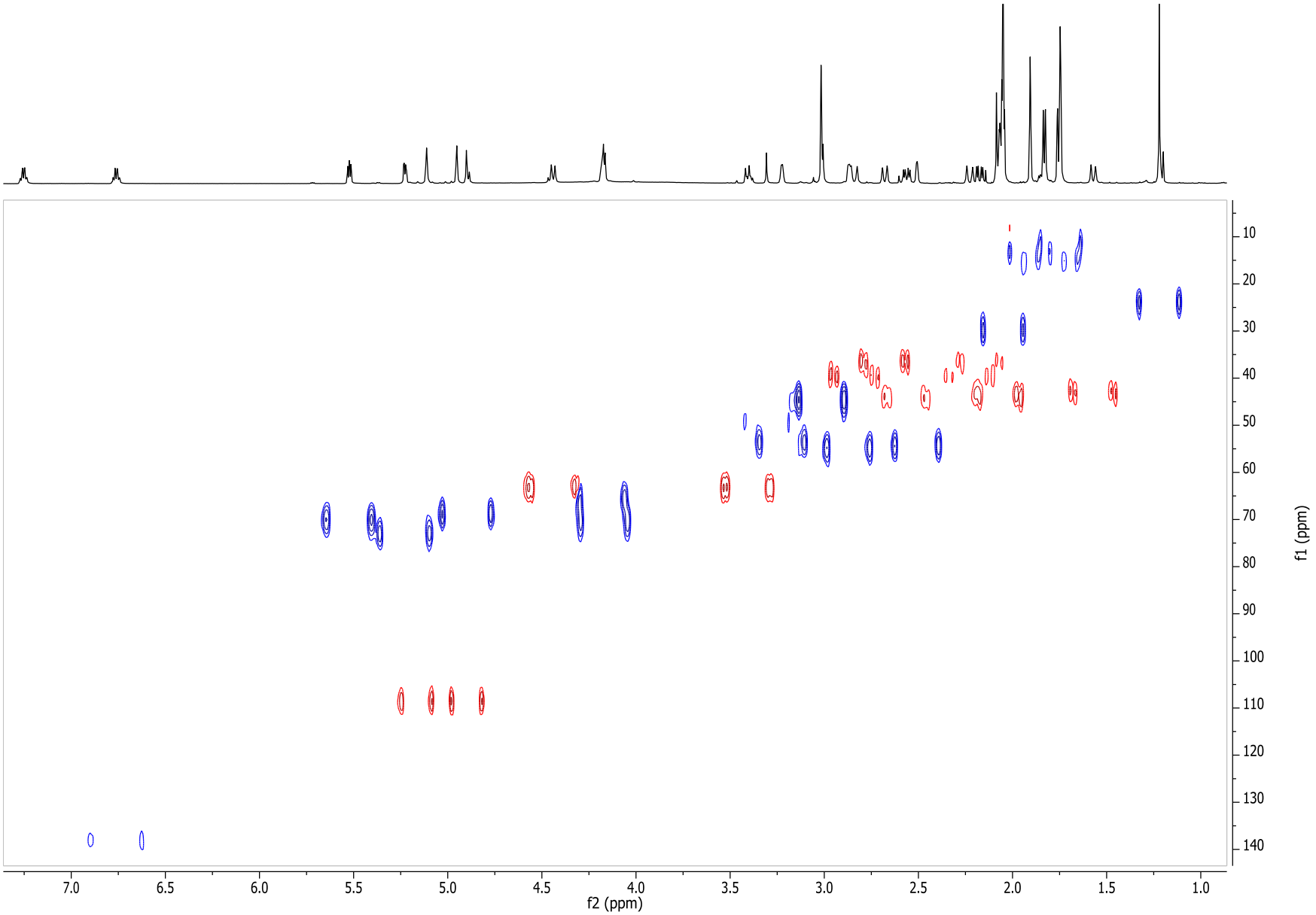
S25. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of Anopterine (**5**)



S26. COSY (Acetone-d_6) Spectrum of Anopterine (**5**)

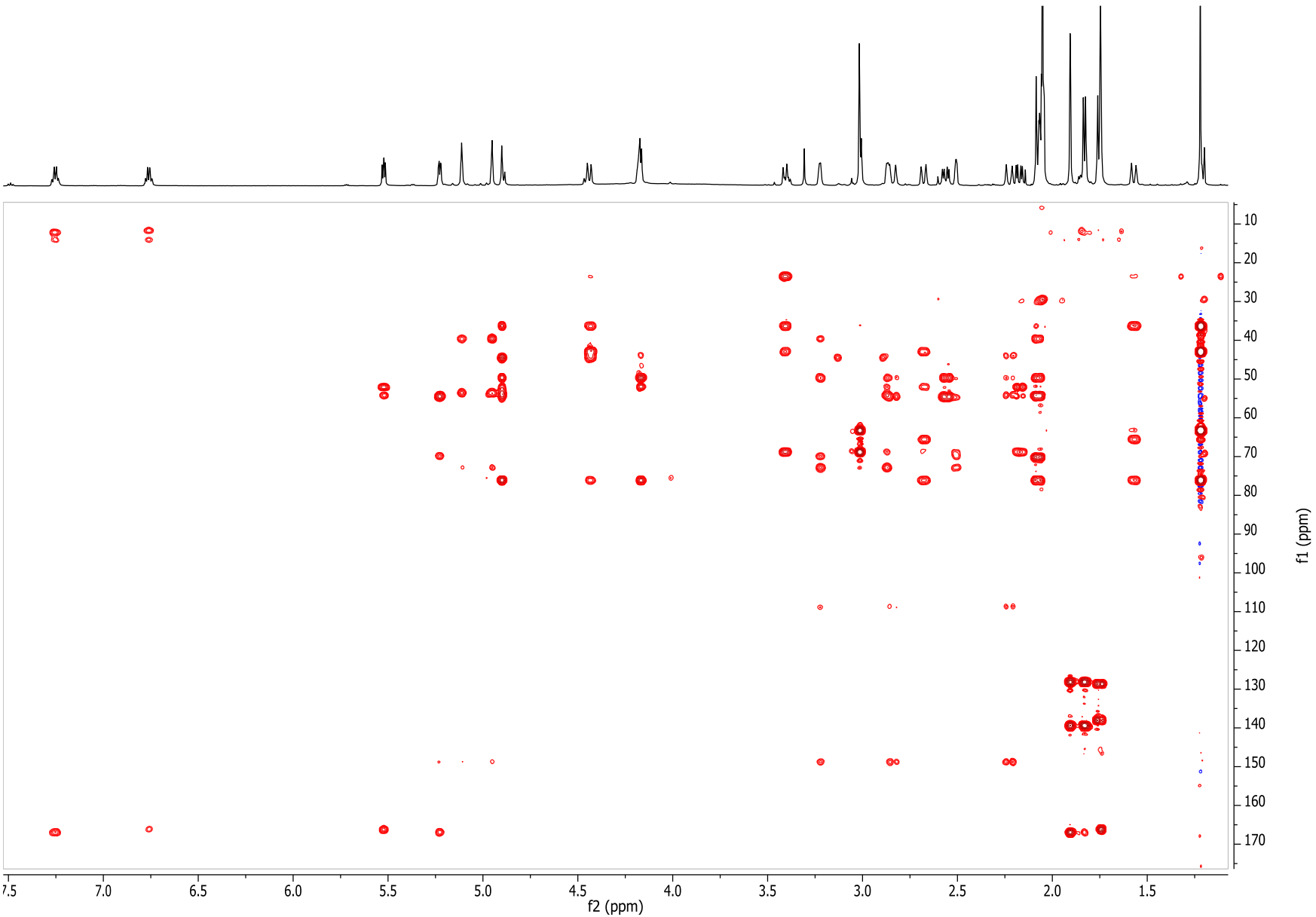


S27. HSQC* (Acetone-d_6) Spectrum of Anopterine (**5**)

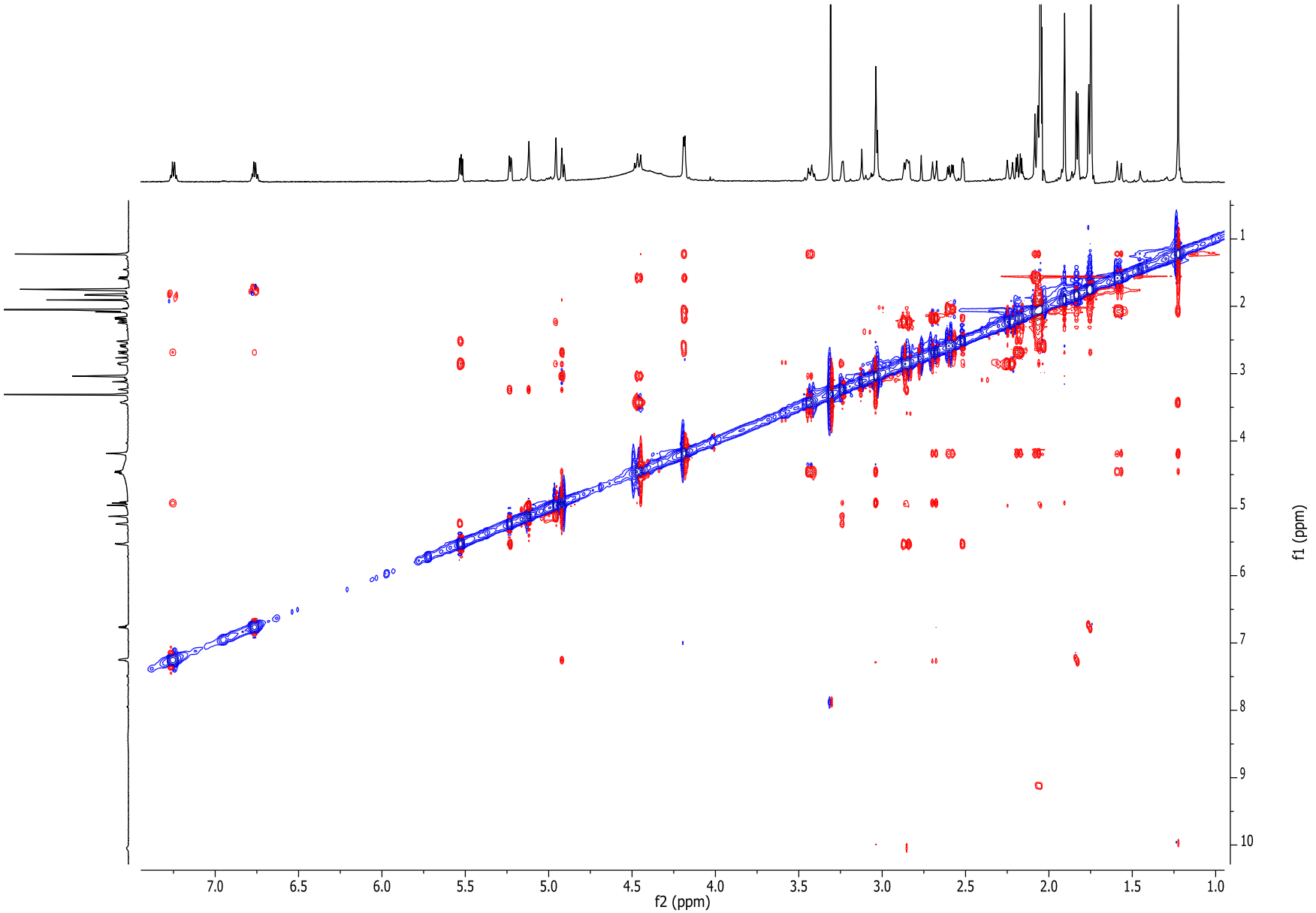


*^{13}C decoupler out of order at time of acquisition, resulting in signals being split by $^{1}J_{CH}$

S28. HMBC (Acetone-d_6) Spectrum of Anopterine (**5**)



S29. ROESY (Acetone-d_6) Spectrum of Anopterine (**5**)



S30. Comparison of NMR Data of TFA Salt and Free Base of Anopterine

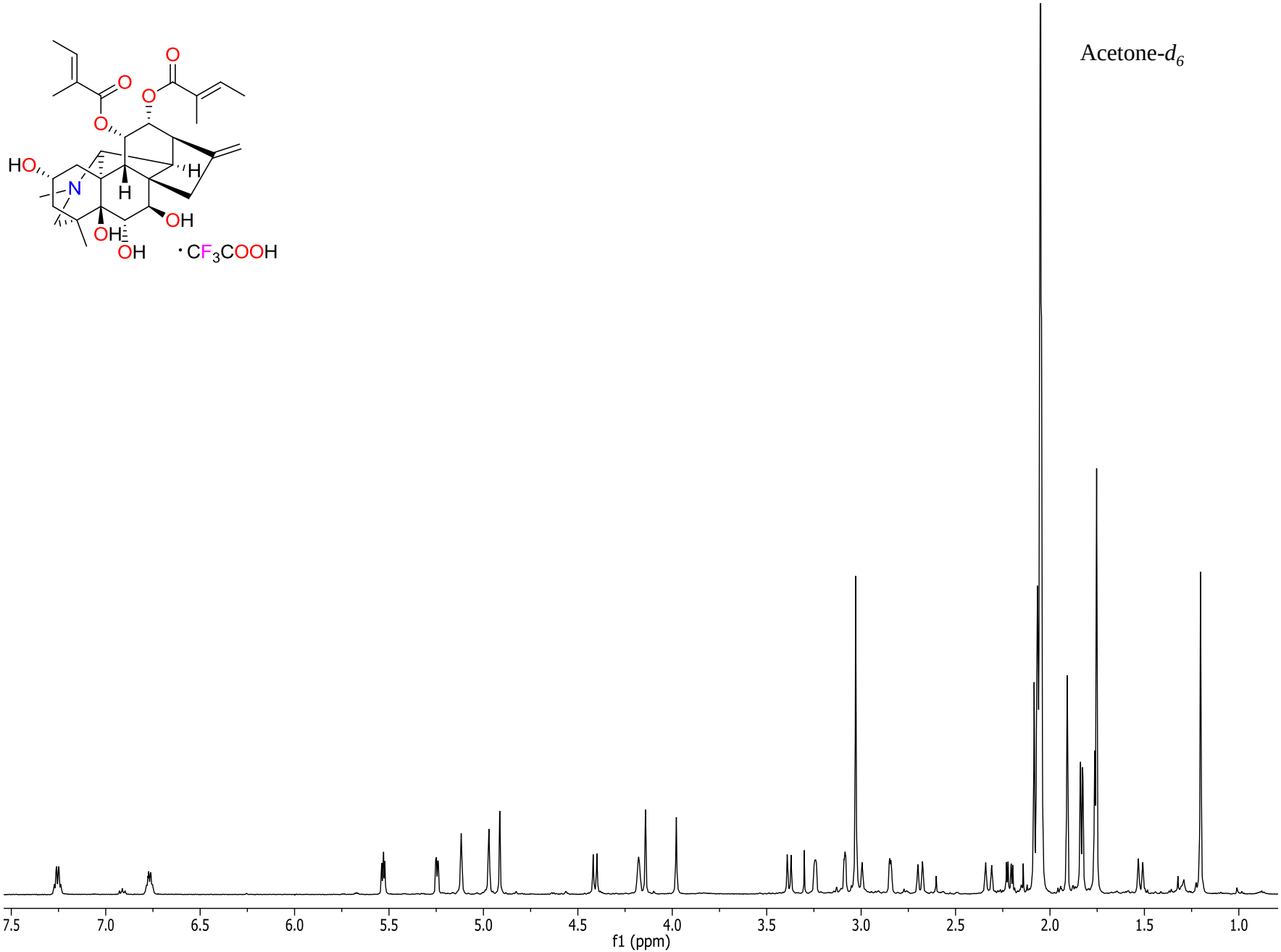
Position	TFA Salt of Anopterine [a]		Free Base of Anopterine [b]	
	δ_H (mult. *J* in Hz)	δ_C	δ_H (mult. *J* in Hz)	δ_C
1	2.18, dd (14.9, 5.0)	36.3	2.04 (15.5, 5.0)	36.8
	2.68, br d (14.9)		2.49 (15.5, 2.5, 2.0)	
2	4.18, m	66.4	4.14 (5.0, 5.0, 2.0, 2.0)	66.5
3	1.57, br d (14.3)	42.9	1.46 (14.5, 2.5, 2.0)	42.7
	2.09, m		1.92 (14.5, 5.0)	
4		36.4		36.4
5		76.1		78.8
6	4.16, d (5.0)	70.3	3.57 (5.5)	71.9
7	2.08, m	44.2	1.88 (14.5)	46.2
	2.56, dd (14.9, 5.4)		2.47 (14.5, 5.5)	
8		49.6		49.1
9	2.51, m	54.6	2.40 (4.0)	54.4
10		52.1		51.5
11	5.52, dd (6.0, 4.4)	70.0	5.52 (6.0, 4.0)	70.3
12	5.23, dd (6.0, 2.3)	73.2	5.16 (6.0, 2.5)	73.1
13	3.22, m	53.6	2.96 (4.5, 2.5)	53.3
14	2.87, m	55.0	2.35 (4.5)	57.0
15	2.23, br d (18.7)	39.5	2.17 (18.5, 2.5, 2.5)	39.8
	2.84, br d (18.7)		2.75 (18.5, 2.5, 2.5)	
16		148.9		148.7
17	4.95, m	108.8	4.90 (2.5, 2.5)	108.5
	5.11, m		5.06 (2.5, 2.5)	
18	1.22, s	24.0	1.21	24.2
19	3.41, m	63.3	2.72 (11.0)	61.9
	4.44, m		3.71 (11.0)	
20	4.90, s	68.9	4.04	65.8
N-CH_3	3.01, s	44.7	2.34	43.1
1'		166.2		166.7[#]
2'		128.7		128.6[#]
3'	6.77, br q (6.5)	138.1	6.75 (7.0)	137.8[#]
4'	1.76, d (6.5)	13.9	1.74 (7.0)	14.5[#]
5'	1.75, s	11.5	1.75	12.0[#]
1"		167.0		167.4[#]
2"		128.1		128.4[#]
3"	7.25, br q (7.0)	139.5	7.08 (7.0)	138.4[#]
4"	1.83, d (7.0)	14.1	1.84 (7.0)	14.5[#]
5"	1.90, s	12.0	1.90	12.5[#]

[a] ^{1}H and ^{13}C spectra recorded at 600 MHz and 150 MHz respectively in acetone-d_6 at 30 °C.

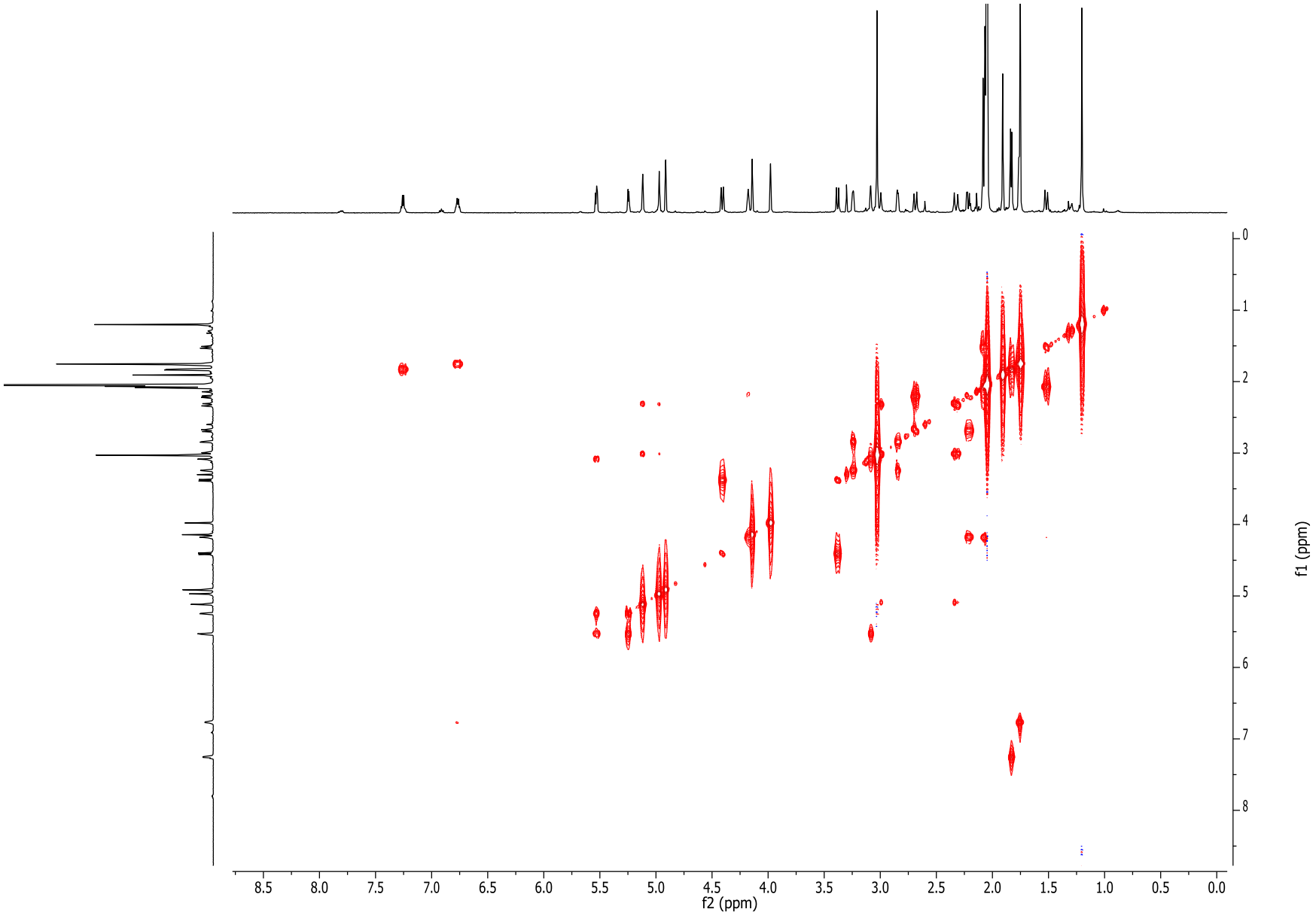
[b] ^{1}H and ^{13}C spectra recorded at 250 MHz and 62.9 MHz respectively in $CDCl_3$ from Johns, S. R.; Lamberton, J. A.; Suares, H.; Willing, R. I. *Aust. J. Chem.* **1985**, *38*, 1091-1106.

[#] ^{13}C spectra recorded at 22.6 MHz in $CDCl_3$, From Hart, N. K.; Johns, S. R.; Lamberton, J. A.; Suares, H.; Willing, R. I. *Aust. J. Chem.* **1976**, *29*, 1295-1318.

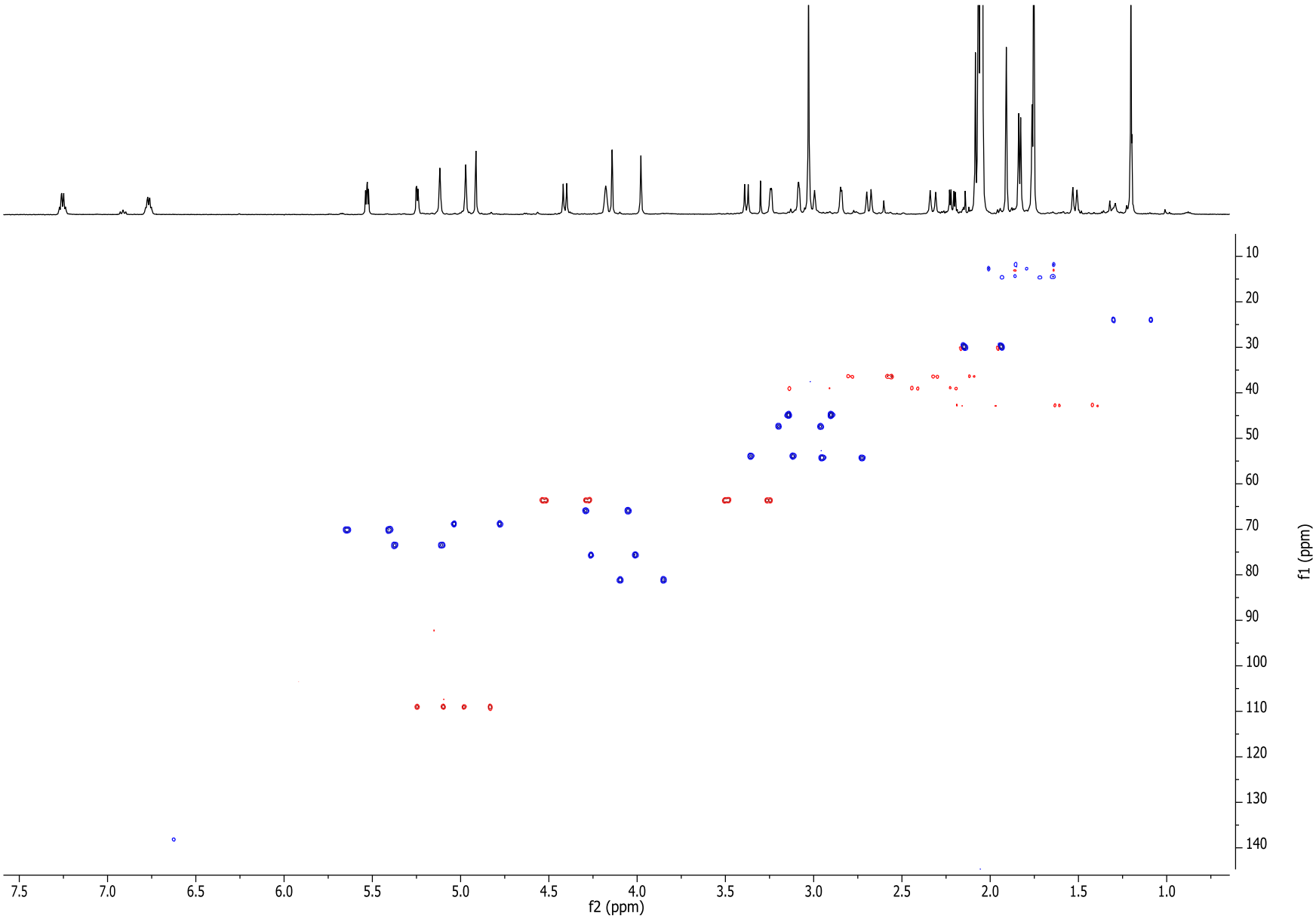
S31. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 7β-Hydroxyanopterine (**6**)



S32. COSY (Acetone-d_6) Spectrum of 7β-Hydroxyanopterine (**6**)

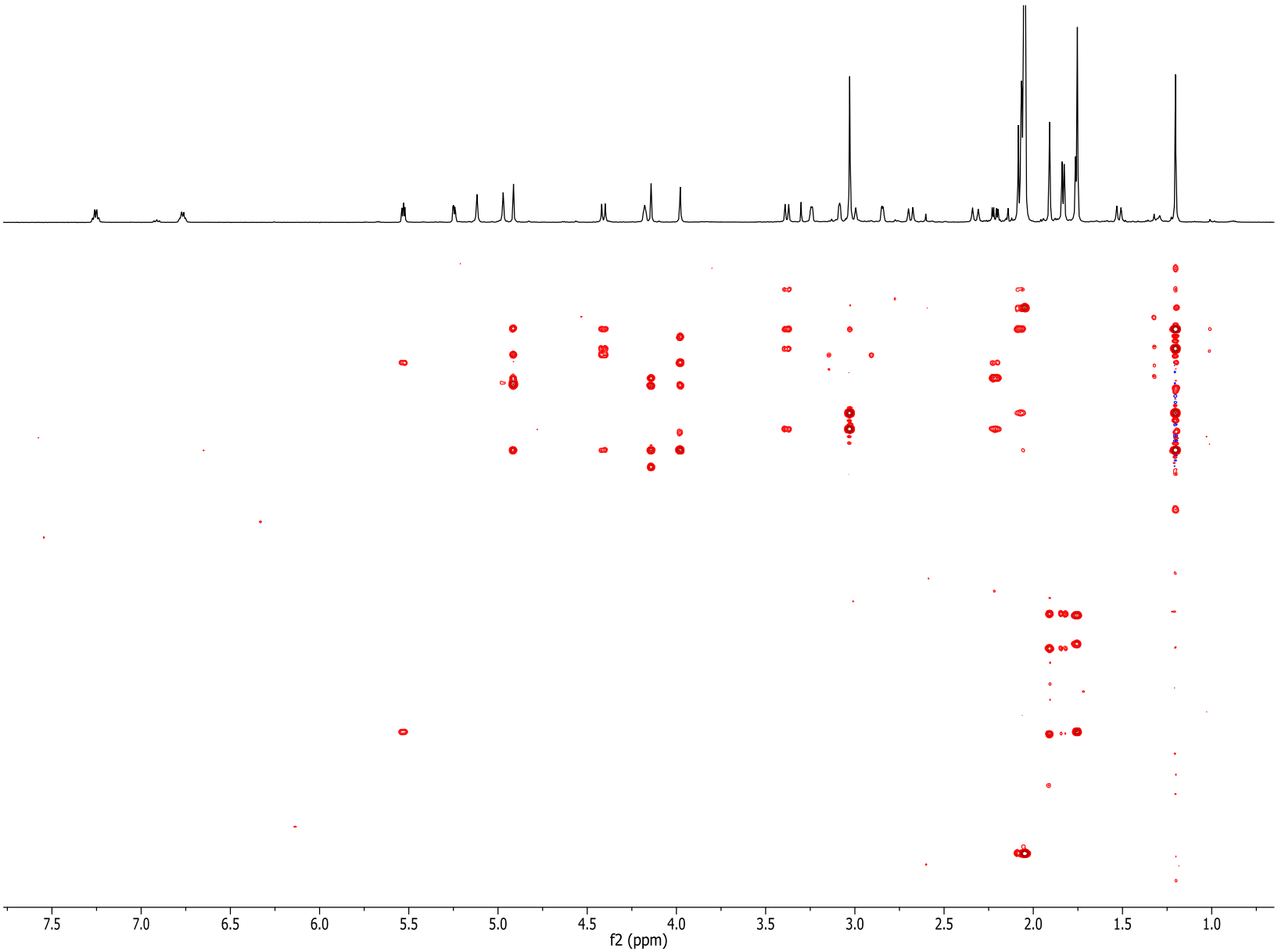


S33. HSQC* (Acetone-d_6) Spectrum of 7β-Hydroxyanopterine (**6**)

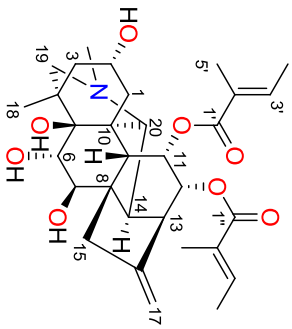


*^{13}C decoupler out of order at time of acquisition, resulting in signals being split by $^1J_{CH}$

S34. HMBC (Acetone-d_6) Spectrum of 7β-Hydroxyanopterine (**6**)



S35. Comparison of NMR Data of TFA Salt and Free Base of 7*β*-Hydroxyanopterine



Position	TFA Salt of 7*β*-Hydroxy-anopterine [a]		Free Base of 7*β*-Hydroxy-anopterine [b]	
	δ_H (mult. *J* in Hz)	δ_C	δ_H (mult. *J* in Hz)	δ_C
1	2.21, dd (14.7, 4.9)	36.4	2.13	36.2
	2.69, br d (14.7)		2.47	
2	4.18, m	65.8	4.15	66.5
3	1.52, br d (14.0)	42.7	1.42	41.8
	2.08, m		1.97	
4		36.7		36.2
5		75.5		77.9
6	4.14, s	75.6	3.62*	82.6*
7	3.98, br s	81.1	3.92*	76.0*
8		52.2		52.3
9	3.09, m	47.4	2.78	54.2*
10		54.7		47.0*
11	5.53, dd (6.0, 4.4)	70.1	5.49	70.3
12	5.23, dd (6.0, 2.4)	73.5	5.20	73.4
13	3.24, m	53.9	2.98	53.2
14	2.84, m	54.3	2.43	56.1
15	2.33, br d (18.7)	39.1	2.29	38.8
	3.02, br d (18.7)		3.03	
16		148.8		148.2
17	4.97, m	109.0	4.91	108.7
	5.12, m		5.09	
18	1.20, s	23.9	1.21	24.5
19	3.38, d (12.0)	63.6	2.66	61.6
	4.41, d (12.0)		3.75	
20	4.91, s	68.8	4.11	64.9
N-CH_3	3.00, s	44.8	2.31	42.8
1'		166.4		N.R
2'		128.8		N.R
3'	6.77, br q (6.5)	138.1	6.76	N.R
4'	1.76, d (6.5)	14.4	1.76	N.R
5'	1.75, s	11.6	1.75	N.R
1"		167.1		N.R
2"		128.4		N.R
3"	7.26, br q (7.0)	139.6	7.09	N.R
4"	1.83, d (7.0)	14.7	1.84	N.R
5"	1.91, s	12.8	1.92	N.R

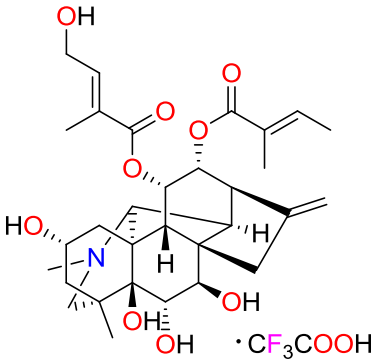
[a] ^1H and ^{13}C spectra recorded at 600 MHz and 150 MHz respectively in acetone-d_6 at 30 °C.

[b] ^1H and ^{13}C spectra recorded at 250 MHz and 62.9 MHz respectively in $CDCl_3$ from Johns, S. R.; Lamberton, J. A.; Suares, H.; Willing, R. I. *Aust. J. Chem.* **1985**, *38*, 1091-1106.

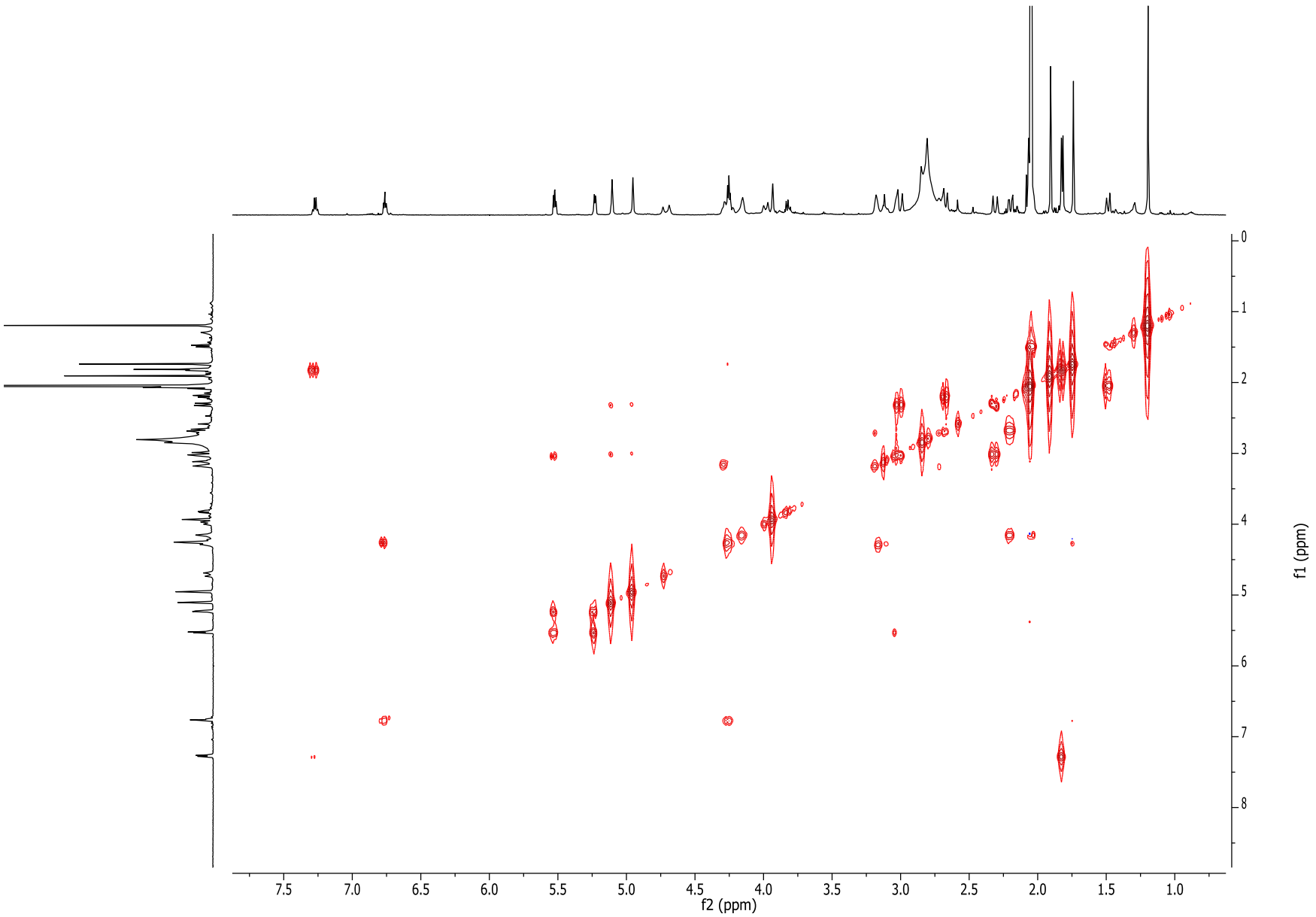
*Initially misassigned by Johns et al. C-6, C-7, C-9 and C-10 have been reassigned based on our data

N.R: Not reported

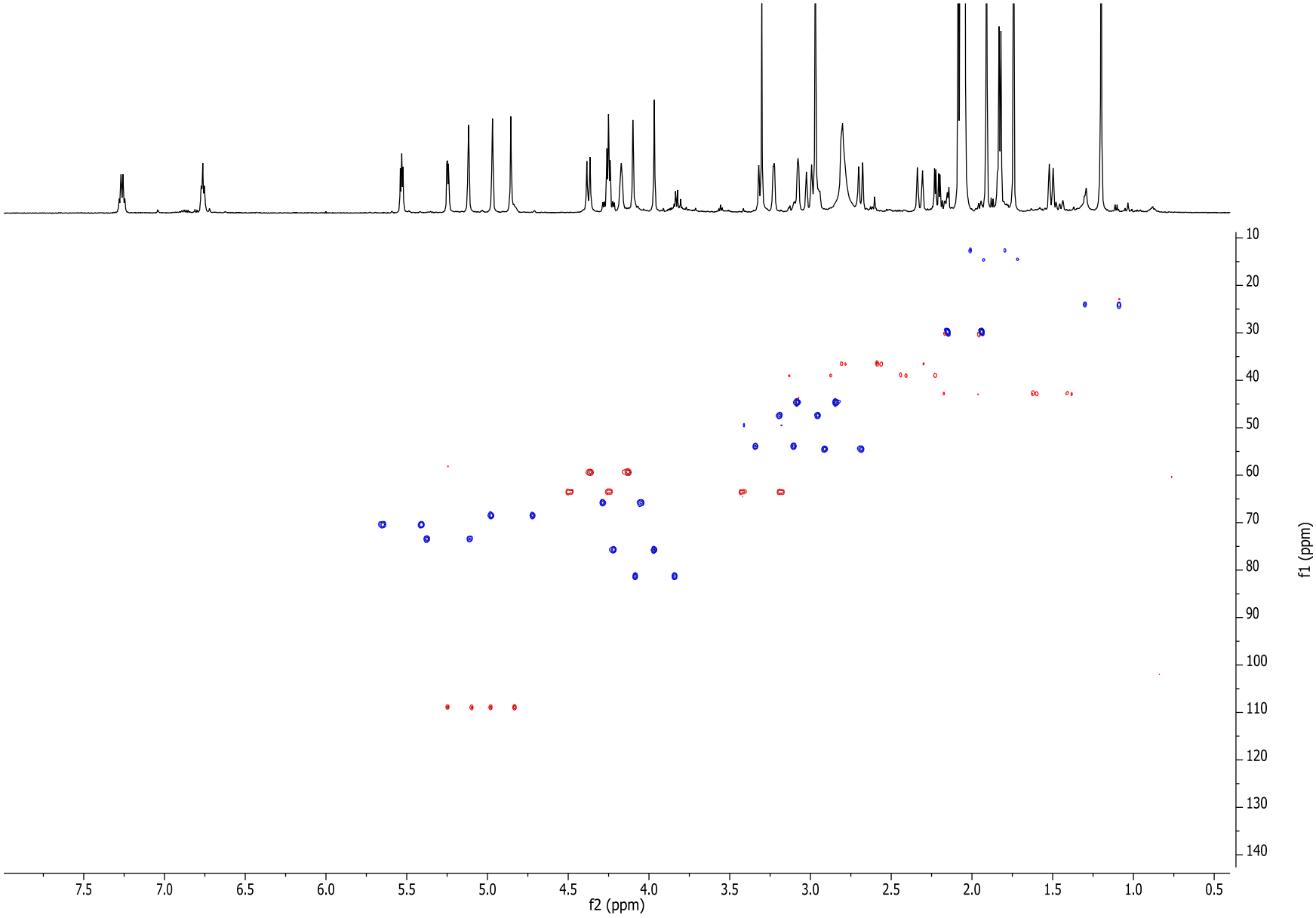
S36. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 7β,4'-Dihydroxyanopterine (**7**)



S37. COSY (Acetone-d_6) Spectrum of 7β,4'-Dihydroxyanopterine (**7**)

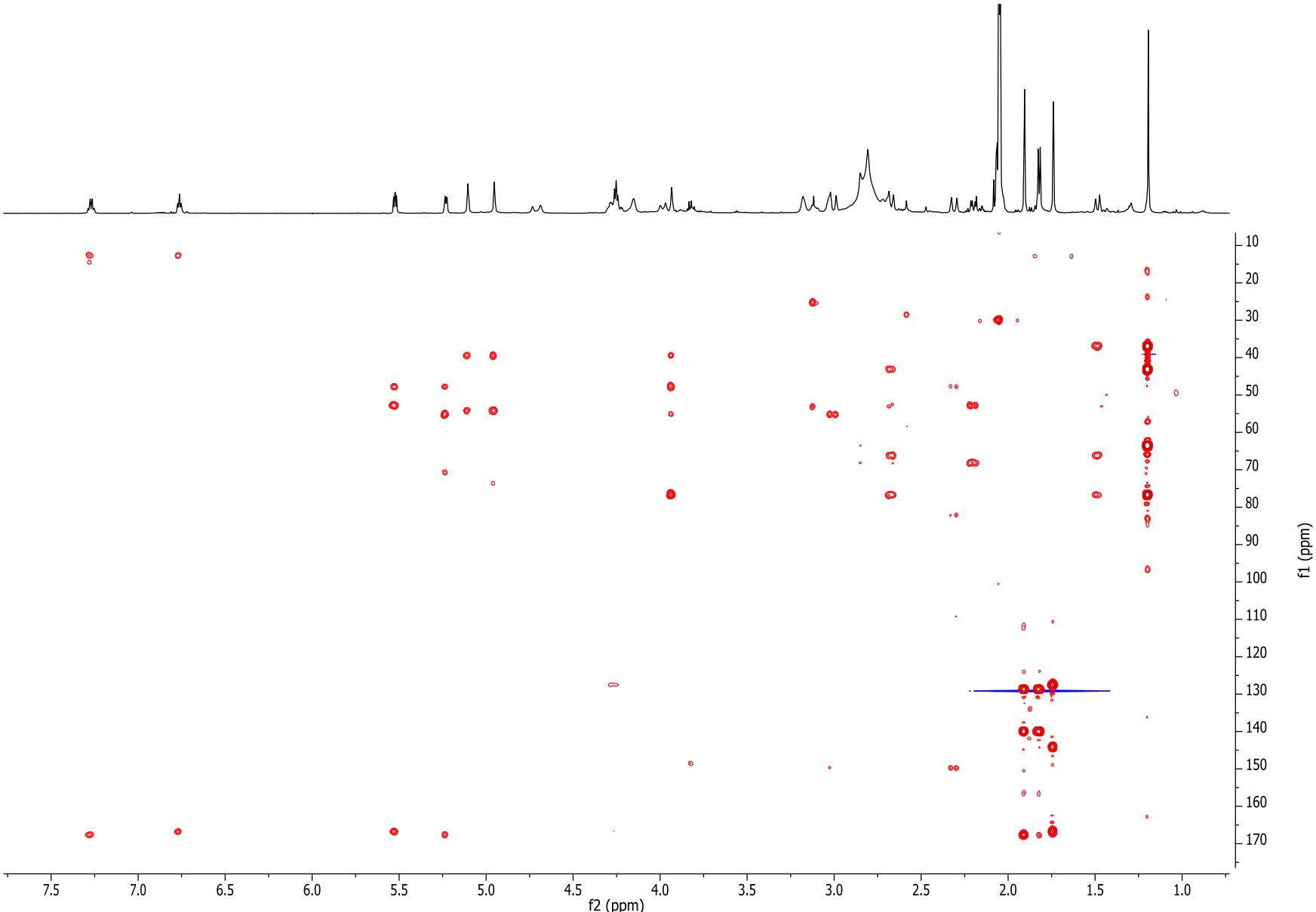


S38. HSQC* (Acetone-d_6) Spectrum of 7β,4'-Dihydroxyanopterine (**7**)



*^{13}C decoupler out of order at time of acquisition, resulting in signals being split by $^1J_{CH}$

S39. HMBC (Acetone-d_6) Spectrum of 7β,4'-Dihydroxyanopterine (**7**)



S40. Comparison of NMR Data of TFA Salt and Free Base of 7*β*,4'-Dihydroxyanopterine

Position	TFA Salt of 7*β*,4'-Dihydroxy-anopterine[a]		Free Base of 7*β*,4'-Dihydroxy-anopterine[b]	
	δ_H (mult. *J* in Hz)	δ_C	δ_H (mult. *J* in Hz)	δ_C
1	2.21, dd (14.8, 4.9)	36.4	2.15	37.4
	2.69, br d (14.8)		2.42	
2	4.17, m	65.8	4.10	65.4
3	1.51, br d (14.2)	42.7	1.42	41.7
	2.07, m		1.96	
4		37.0		36.6
5		76.2		78.2
6	4.10, s	75.6	3.54*	82.7*
7	3.97, s	81.4	3.75*	75.7*
8		52.3		52.6
9	3.08, m	47.5	2.78	54.3*
10		54.7		47.2*
11	5.53, dd (5.9, 4.3)	70.4	5.50	70.8
12	5.24, dd (5.9, 2.1)	73.5	5.20	73.3
13	3.23, m	53.9	2.98	53.5
14	2.80, m	54.6	2.39	55.8
15	2.32, br d (18.8)	38.9	2.28	38.9
	3.00, br d (18.8)		3.02	
16		148.5		148.5
17	4.97, m	108.9	4.93	108.9
	5.12, m		5.10	
18	1.20, s	23.9	1.19	24.4
19	3.31, br d (11.9)	63.5	2.56	61.5
	4.37, br d (11.9)		3.81	
20	4.86, s	68.7	4.10	65.4
N-CH_3	2.97, s	44.6	2.32	42.8
1'		166.7		N.R
2'		127.5		N.R
3'	6.76, ddq (5.6, 5.6, 1.5)	144.1	6.70	N.R
4'	4.23, dd (15.4, 5.6)	59.2	4.26	N.R
	4.27, dd (15.4, 5.6)			
5'	1.74, s	12.5	1.76	N.R
1''		167.6		N.R
2''		128.7		N.R
3''	7.26, br q (7.0)	140.1	7.16	N.R
4''	1.83, d (7.0)	14.8	1.85	N.R
5''	1.91, s	12.6	1.92	N.R

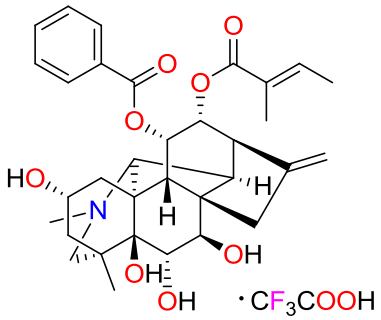
[a] ^1H and ^{13}C spectra recorded at 600 MHz and 150 MHz respectively in acetone-d_6 at 30 °C.

[b] ^1H and ^{13}C spectra recorded at 250 MHz and 62.9 MHz respectively in $CDCl_3$ from Johns, S. R.; Lamberton, J. A.; Suares, H.; Willing, R. I. *Aust. J. Chem.* **1985**, *38*, 1091-1106.

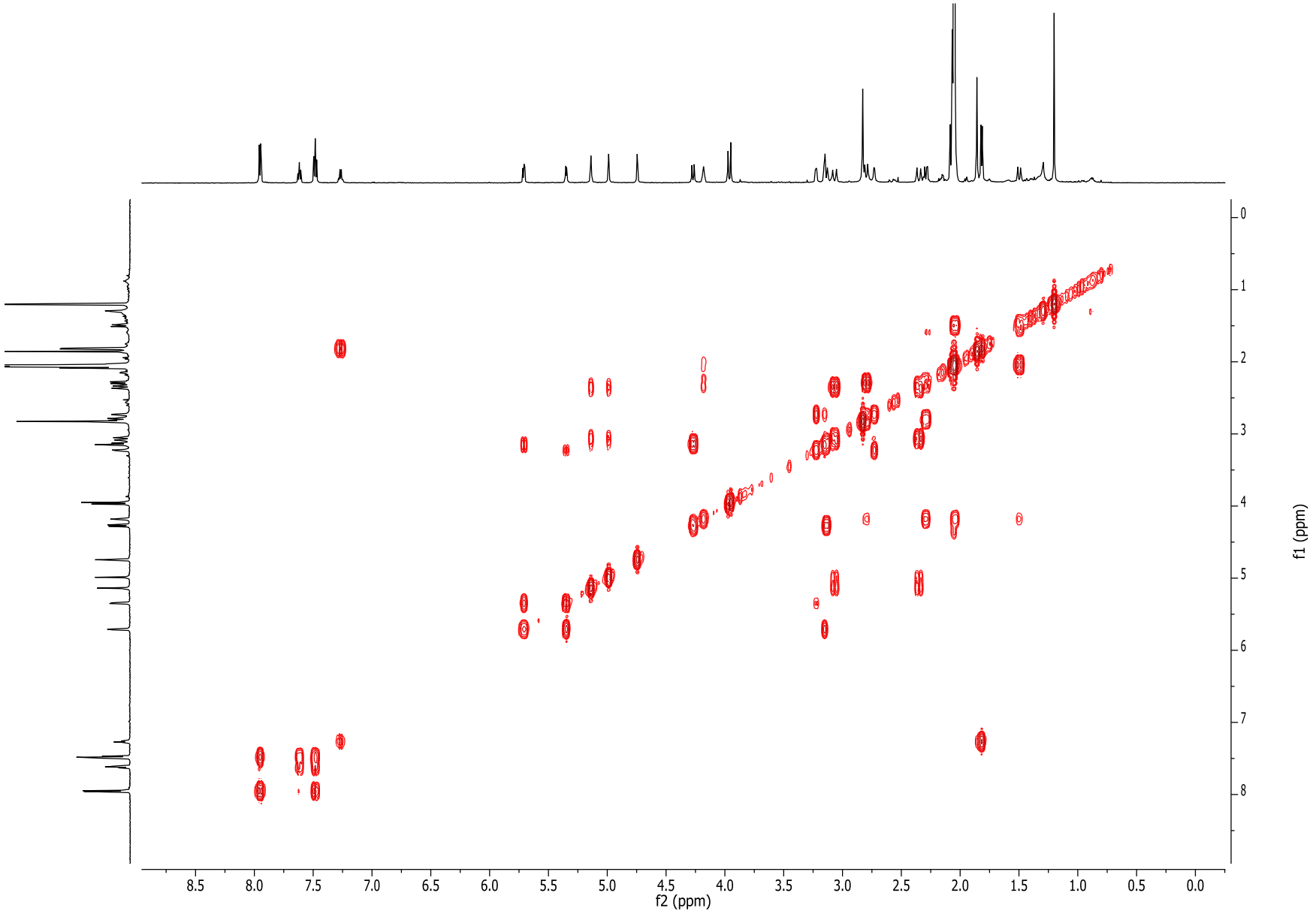
*Initially misassigned by Johns et al. C-6, C-7, C-9 and C-10 have been reassigned based on our data

N.R: Not reported

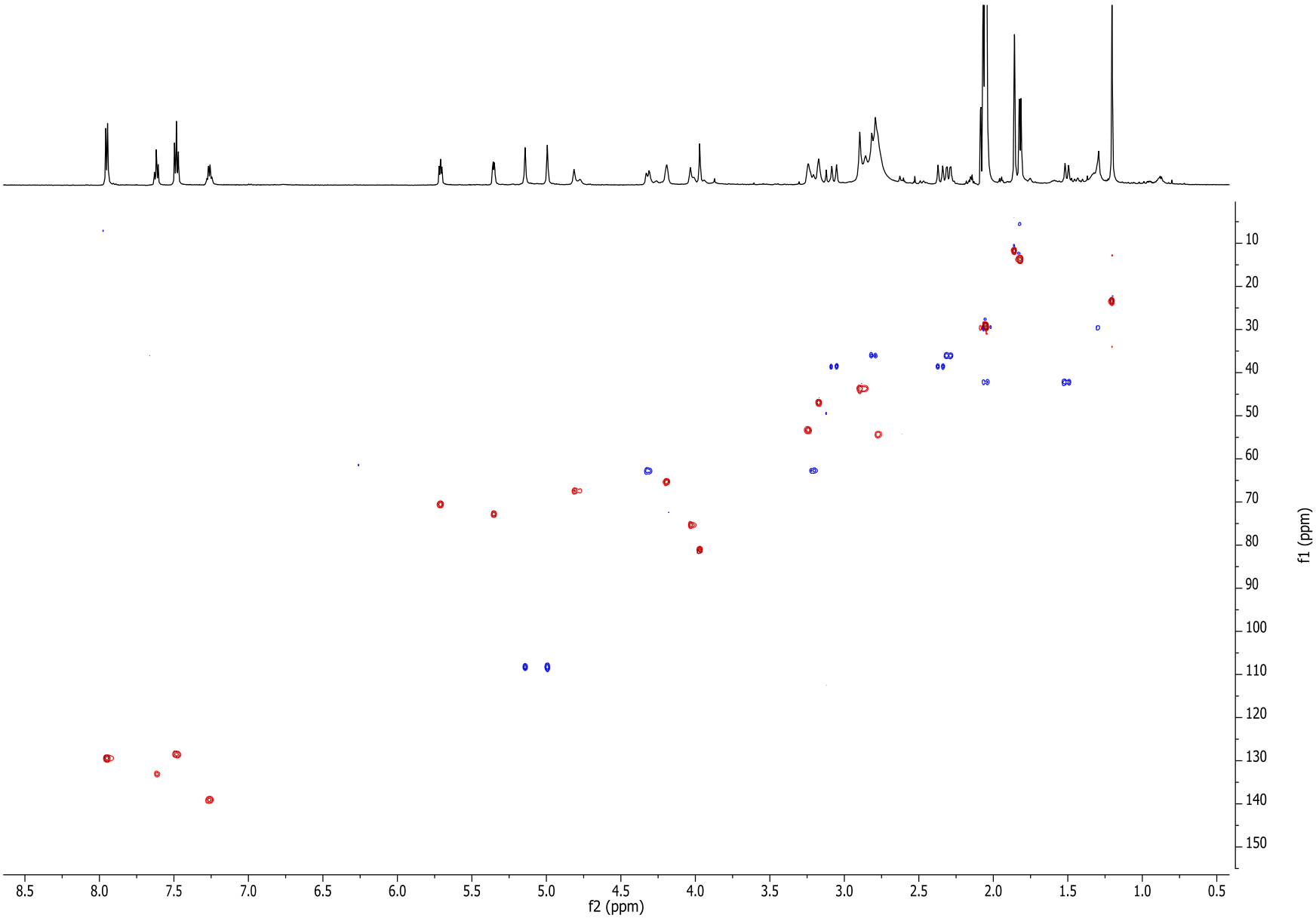
S41. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of 7β-Hydroxy-11α-benzoylanopterine (**8**)



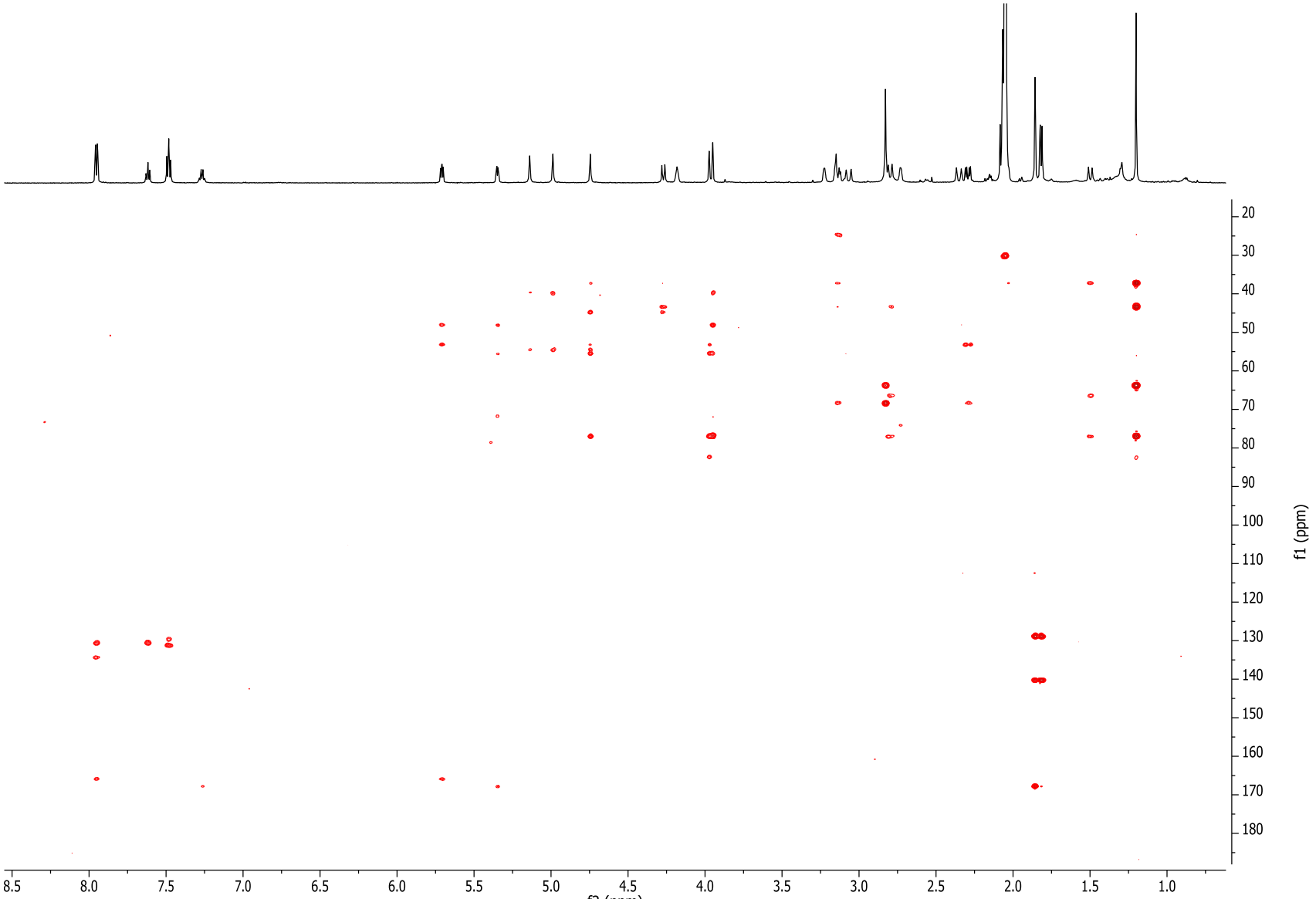
S42. COSY (Acetone-d_6) Spectrum of 7β-Hydroxy-11α-benzoylanopterine (**8**)



S43. HSQC (Acetone-d_6) Spectrum of 7β-Hydroxy-11α-benzoylanopterine (**8**)



S44. HMBC (Acetone-d_6) Spectrum of 7β-Hydroxy-11α-benzoylanopterine (**8**)



S45. Comparison of NMR Data of TFA Salt and Free Base of 7β-Hydroxy-11α-benzoylanopterine

Position	TFA Salt of 7β-Hydroxy-11α-benzoyl-anopterine[a]		Free Base of 7β-Hydroxy-11α-benzoyl-anopterine[b]	
	δ_H (mult. J in Hz)	δ_C	δ_H (mult. J in Hz)	δ_C
1	2.30, dd (14.6, 5.0)	36.7	2.22	N.R
	2.81, br d (14.6)		2.60	
2	4.19, m	65.9	4.17	N.R
3	1.56, br d (14.2)	42.8	1.42	N.R
	2.05, m		1.92	
4		36.6		N.R
5		76.4		N.R
6	3.98, s	75.9*	3.60*	N.R
7	3.95, s	81.7*	3.93*	N.R
8		52.6		N.R
9	3.15, m	47.5	2.90	N.R
10		54.8		N.R
11	5.71, dd (6.0, 5.0)	71.2	5.67	N.R
12	5.35, dd (6.0, 2.5)	73.4	5.31	N.R
13	3.23, m	53.9	3.03	N.R
14	2.73, m	55.0	2.46	N.R
15	2.36, ddd (18.8, 2.0, 2.0)	39.3	2.30	N.R
	3.07, br d (18.8)		3.03	
16		149.4		N.R
17	4.99, m	108.9	4.95	N.R
	5.14, m		5.13	
18	1.20, s	24.0	1.21	N.R
19	3.14, m	63.4	2.64	N.R
	4.27, br d (11.8)		3.75	
20	4.75, br s	68.0	4.13	N.R
N-CH_3	2.83, s	44.3	2.30	N.R
1'		165.4		N.R
2'		130.1		N.R
3'	7.95, m	130.1	7.92	N.R
4'	7.48, m	128.2	7.41	N.R
5'	7.61, m	133.8	7.55	N.R
6'	7.48, m	128.2	7.41	N.R
7'	7.95, m	130.1	7.92	N.R
1"		167.3		N.R
2"		128.4		N.R
3"	7.27, br q (7.1)	139.7	7.11	N.R
4"	1.82, d (7.1)	14.3	1.83	N.R
5"	1.86, s	12.3	1.89	N.R

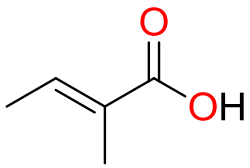
[a] ^{1}H and ^{13}C spectra recorded at 600 MHz and 150 MHz respectively in acetone-d_6 at 30 °C.

[b] ^{1}H spectrum recorded at 250 MHz in $CDCl_3$ from Johns, S. R.; Lamberton, J. A.; Suares, H.; Willing, R. I. *Aust. J. Chem.* **1985**, *38*, 1091-1106.

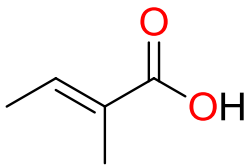
*Initially misassigned by Johns et al. H-6, H-7 have been reassigned based on our data

N.R: Not reported

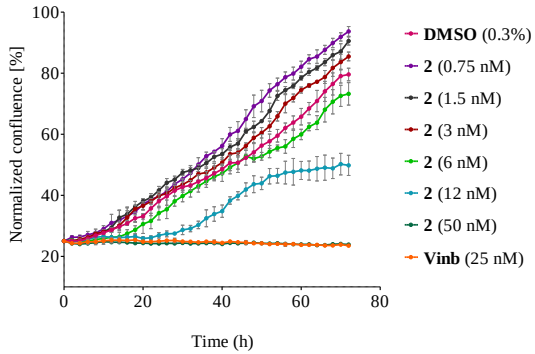
S46. ^{1}H NMR (600 MHz, Acetone-d_6) Spectrum of Tiglic Acid

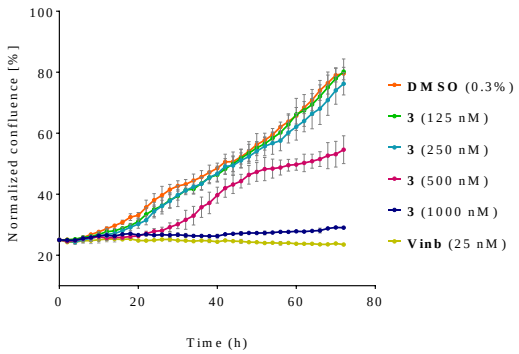


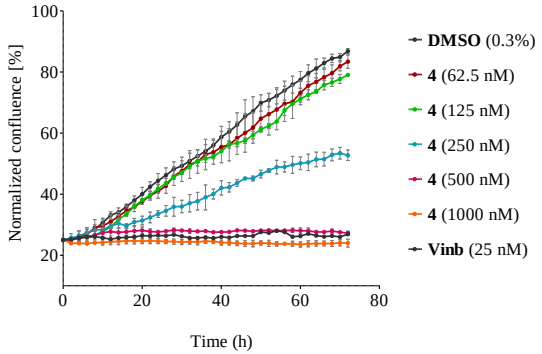
S47. ^{13}C NMR (150 MHz, Acetone-d_6) Spectrum of Tiglic Acid

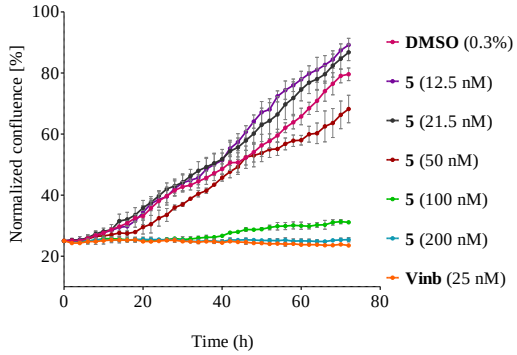


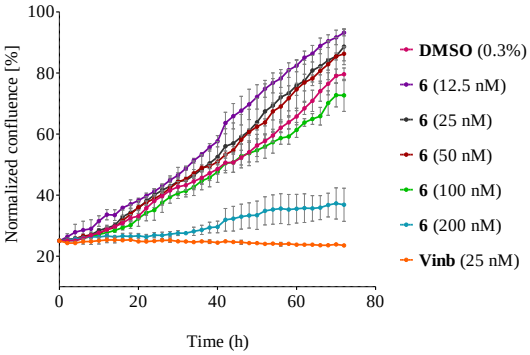
S48. Cytotoxicity Evaluation of Compounds **2**–**8** Using a Live-Cell Imaging Assay (Confluence)

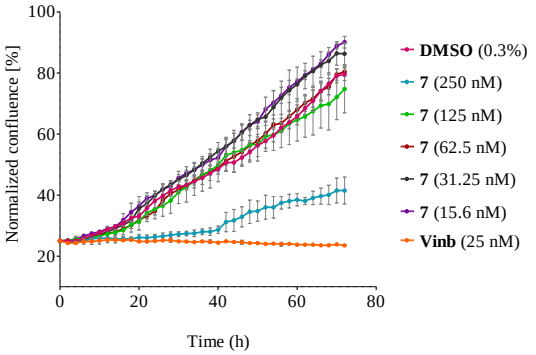


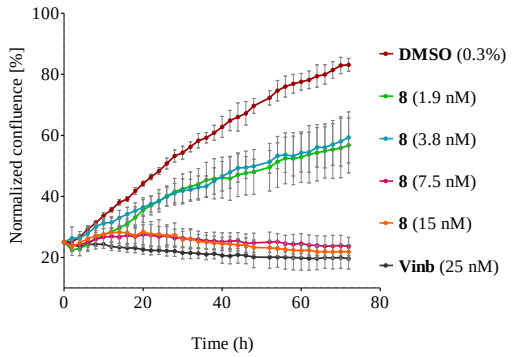


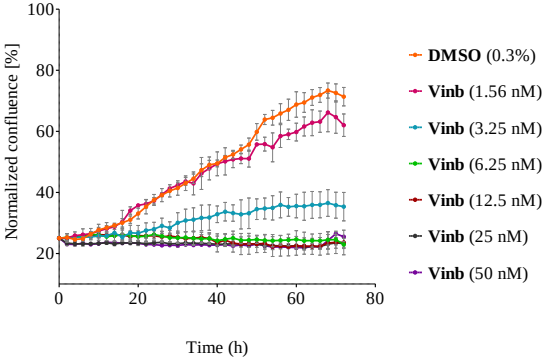






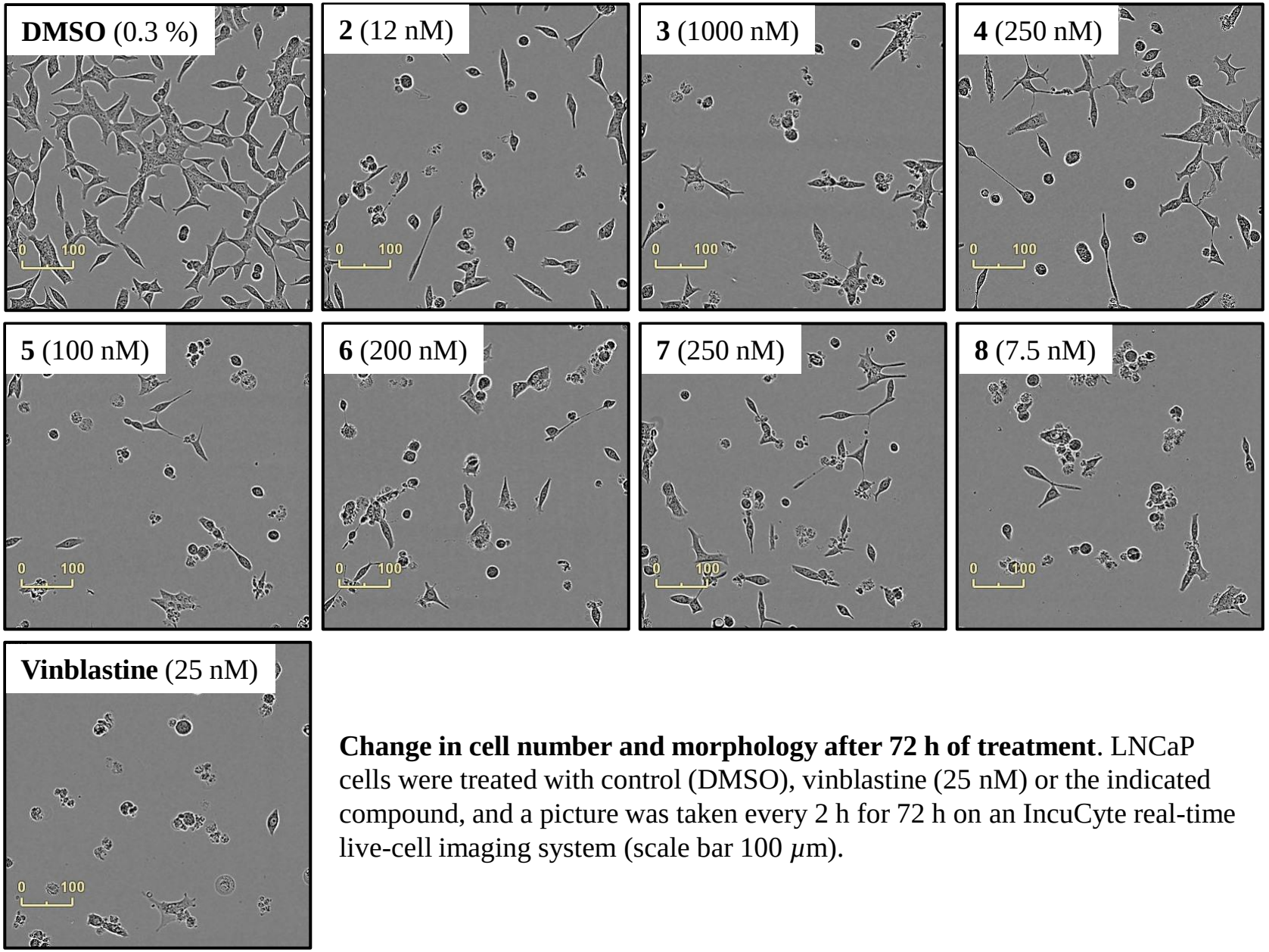






Proliferation as a function of cell confluence. LNCaP cells were treated with control (DMSO), vinblastine (25 nM) or the indicated compound, and confluence was measured every 2 h for 72 h on an IncuCyte real-time live-cell imaging system.

S49. Cytotoxicity Evaluation of Compounds **2**–**8** Using a Live-Cell Imaging Assay (Cell Morphology)



Change in cell number and morphology after 72 h of treatment. LNCaP cells were treated with control (DMSO), vinblastine (25 nM) or the indicated compound, and a picture was taken every 2 h for 72 h on an IncuCyte real-time live-cell imaging system (scale bar 100 μm).