Refactoring the formicamycin biosynthetic gene cluster to make high-level producing strains and new molecules

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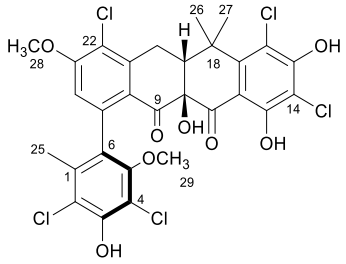
Structure determination of new fasamycin and formicamycin congeners

Structures were assigned based on our published data [4]. The substituent variations including chlorination and *O*-methylations were determined by 2D HSQC and NOESY NMR.

Formicamycin R



Molecular formula: $C_{29}H_{23}O_8Cl_5$

Isolated yield: 16.5 mg

UV (PDA): λ_{max} = 245 and 290 nm

Specific rotation: $[\alpha]_D^{20}$ = 305.1

HRMS (ESI) *m/z*: calculated $[M - H]^-$ = 672.9763, observed $[M - H]^-$ = 672.9759, Δ = -0.59 ppm

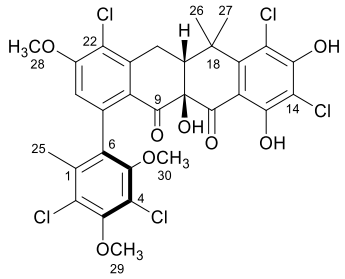
NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 1: Structural determination of Formicamycin R

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	134.5		25	
2	119.4		25	
3	153.3		29	
4	115.8			
5	150.8			
6	130.0		24, 25	
7	140.6		24	
8	124.5		20, 24	
9	195.3		19	
10	80.4		19, 20	
11	199.9		19	
12	115.0			
13	160.8			
14	108.9			
15	161.5			
16	109.9			

17	147.1		19, 26, 27	
18	41.3		19, 20, 26, 27	
19	51.3	2.5 (1H, dd, 10.16, 6.09)	20, 26, 27	20, 26, 27
20	30.3	2.8 (1H, dd, 18.86, 10.16)		19
		3.7 (1H, dd, 18.86, 6.09)		
21	143.4		20	
22	122.7		20, 24	
23	160.4		24, 28	
24	115.6	6.8 (1H, s)		25, 28
25	18.3	2.0 (3H, s)		24
26	27.3	1.7 (3H, s)	27	19, 20, 27
27	29.3	1.9 (3H, s)	19, 26	19, 26
28	57.5	4.0 (3H, s)		24
29	61.3	3.5 (3H, s)		

Formicamycin S



Molecular formula: $C_{30}H_{25}O_8Cl_5$

Isolated yield: 7.0 mg

UV (PDA): λ_{max} = 240 and 292 nm

Specific rotation: $[\alpha]_D^{20}$ = 278.0

HRMS (ESI) *m/z*: calculated $[M - H]^-$ = 686.9919, observed $[M - H]^-$ = 686.9918, Δ = -0.15 ppm

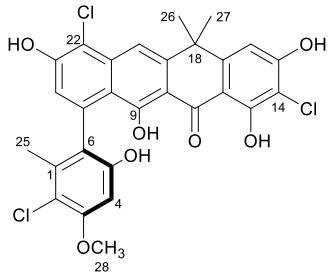
NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 2: Structural determination of Formicamycin S

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	135.5		25	
2	121.8		25	
3	153.7		29	
4	116.2			
5	153.7		30	
6	135.1		24, 25	
7	140.1		24	
8	124.3		20, 24	
9	195.4		19	
10	80.4		19, 20	
11	199.5		19	
12	126.2			
13	161.6			
14	108.9			
15	161.7			
16	109.5			
17	146.9		19, 26, 27	
18	41.3		19, 20, 26, 27	
19	51.3	2.5 (1H, dd, 9.81, 5.54)	20, 26, 27	20, 26, 27
20	30.3	2.8 (1H, dd, 18.91, 9.81)		19

		3.7 (1H, dd, 18.08, 5.54)		
21	143.5		20	
22	122.9		20, 24	
23	160.5		24, 28	
24	115.1	6.9 (1H, s)		25, 28
25	18.2	2.0 (3H, s)		24
26	27.3	1.7 (3H, s)	27	19, 20, 27
27	29.3	1.9 (3H, s)	19, 26	19, 26
28	57.5	3.92 (3H, s)		24
29	61.4	3.5 (3H, s)		
30	61.2	3.90 (3H, s)		

Fasamycin L



Molecular formula: $C_{28}H_{21}O_7Cl_3$

Isolated yield: 45.8 mg

UV (PDA): λ_{max} = 249, 290, 350 and 419 nm

Specific rotation: $[\alpha]_D^{20} = 6.8$

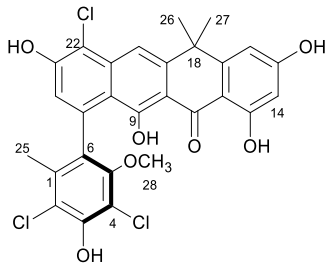
HRMS (ESI) *m/z*: calculated $[M + Na]^+$ = 597.0245, observed $[M + Na]^+$ = 597.0254, Δ = 1.51 ppm

NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 3: Structural determination of Fasamycin L

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	135.6		25	
2	113.7		4, 25	
3	157.1		4, 28	
4	98.6	6.5 (1H, s)		28
5	153.9		4	
6	126.6		4, 24, 25	
7	138.8			
8	118.8		20, 24	
9	166.7			
10	108.1		20	
11	191.6			
12	107.5		16	
13	162.0			
14	109.1		16	
15	162.5		14, 16	
16	107.2	6.8 (1H, s)		26, 27
17	153.2		16, 26, 27	
18	40.2		16, 20, 26, 27	
19	148.0		20, 26, 27	
20	112.5	7.9 (1H, s)		26, 27
21	139.0		20	
22	114.3		20, 24	
23	156.0		24	
24	122.1	6.8 (1H, s)		25
25	18.4	2.0 (3H, s)		24
26	35.0	1.7 (3H, s)	27	
27	35.0	1.7 (3H, s)	26	
28	56.3	3.6 (3H, s)		4

Fasamycin M



Molecular formula: $C_{28}H_{21}O_7Cl_3$

Isolated yield: 2.3 mg

UV (PDA): λ_{max} = 249, 289, 352 and 413 nm

Specific rotation: $[\alpha]_D^{20}$ = 29.1

HRMS (ESI) *m/z*: calculated $[M + Na]^+$ = 597.0245, observed $[M + Na]^+$ = 597.0246, Δ = 0.17 ppm

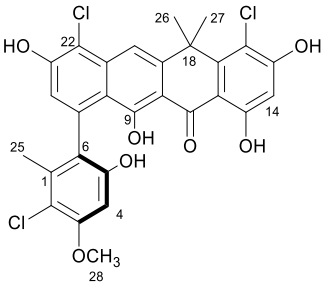
NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 4: Structural determination of Fasamycin M

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	134.7		25	
2	119.1		25	
3	150.6			
4	114.9			
5	153.0			
6	132.1		24, 25	
7	137.2			
8	118.2		20, 24	
9	165.7			
10	108.4		20	
11	191.7			
12	108.6		14, 16	
13	167.2			
14	102.4	6.2 (d, 2.22)	16	
15	167.4		14, 16	
16	107.7	6.7 (d, 2.22)	14	26, 27
17	156.0		26, 27	
18	40.4		16, 20, 26, 27	
19	148.7		20, 26, 27	
20	112.5	7.9 (1H, s)		26, 27
21	138.7			
22	115.0		20, 24	

23	156.4			
24	122.4	6.8 (1H, s)		25, 28
25	18.3	2.0 (3H, s)		24
26	35.0	1.8 (3H, s)	27	16, 20
27	35.1	1.8 (3H, s)	26	16, 20
28	60.9	3.5 (3H, s)		24

Fasamycin N



Molecular formula: $C_{28}H_{21}O_7Cl_3$

Isolated yield: 10.7 mg

UV (PDA): λ_{max} = 253, 293, 365 and 424 nm

Specific rotation: $[\alpha]_D^{20}$ = 3.9

HRMS (ESI) *m/z*: calculated $[M + Na]^+$ = 597.0245, observed $[M + Na]^+$ = 597.0262, Δ = 2.85 ppm

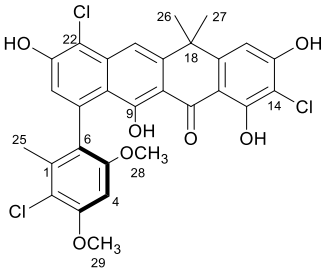
NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 5: Structural determination of Fasamycin N

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	135.6		25	
2	113.7		4, 25	
3	157.1		4, 28	
4	98.6	6.5 (1H, s)		28
5	153.9		4	
6	126.6		4, 24, 25	
7	139.1			
8	118.4		20, 24	
9	166.0			
10	106.9		20	
11	191.8			
12	109.4		14	
13	164.0		14	
14	103.9	6.5 (1H, s)		
15	166.0		14	
16	114.0			
17	151.1		26, 27	
18	41.4		20, 26, 27	
19	150.1		26, 27	
20	113.3	7.9 (1H, s)		26, 27
21	138.9			
22	114.0		20, 24	

23	155.9		24	
24	122.1	6.8 (1H, s)		25
25	18.4	2.0 (3H, s)		24
26	30.7	2.1 (3H, s)	27	20
27	30.8	2.1 (3H, s)	26	20
28	56.3	3.6 (3H, s)		4

Fasamycin O



Molecular formula: $C_{29}H_{23}O_7Cl_3$

Isolated yield: 3.2 mg

UV (PDA): λ_{max} = 252, 292, 336 and 420 nm

Specific rotation: $[\alpha]_D^{20}$ = 3.0

HRMS (ESI) *m/z*: calculated $[M - H]^-$ = 587.0437, observed $[M - H]^-$ = 587.0463, Δ = 4.43 ppm

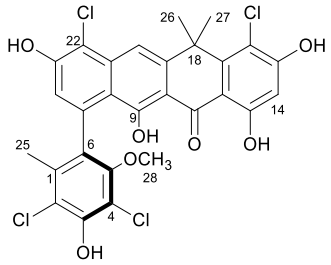
NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 6: Structural determination of Fasamycin O

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	135.7		25	
2	113.8		4, 25	
3	157.1		4, 28	
4	98.6	6.5 (1H, s)		29
5	157.1		4, 29	
6	126.8		4, 25	
7	138.1			
8	119.5		20	
9	170.2			
10	108.4		20	
11	191.1			
12	107.9		16	
13	166.6			
14	108.2			
15	164.9			
16	108.4	6.8 (1H, s)		26, 27
17	153.0		26, 27	
18	40.2		16, 20, 26, 27	
19	148.3		26, 27	
20	112.5	8.0 (1H, s)		26, 27
21	139.5			
22	116.7		20	

23	not detected			
24	117.4	7.0 (1H, s)		25, 28
25	18.5	2.0 (3H, s)		24
26	35.0	1.7 (3H, s)	27	16, 20
27	35.0	1.7 (3H, s)	26	16, 20
28	57.4	4.0 (3H, s)		24
29	56.3	3.6 (3H, s)		4

Fasamycin P



Molecular formula: $C_{28}H_{20}O_7Cl_4$

Isolated yield: 2.3 mg

UV (PDA): λ_{max} = 253, 294, 360 and 419 nm

Specific rotation: $[\alpha]_D^{20}$ = 41.7

HRMS (ESI) *m/z*: calculated $[M - H]^-$ = 606.9890, observed $[M - H]^-$ = 606.9903, Δ = 2.14 ppm

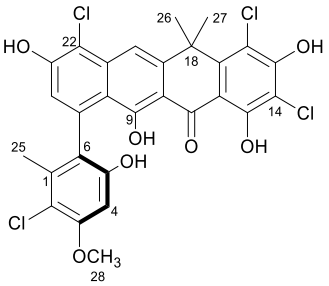
NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 7: Structural determination of Fasamycin P

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	134.8		25	
2	119.1		25	
3	not detected			
4	114.9			
5	153.0		28	
6	132.1		24, 25	
7	138.9			
8	117.9		20, 24	
9	165.0			
10	107.3		20	
11	191.4			
12	115.2			
13	not detected			
14	104.2	6.4 (1H, s)		
15	166.2		14	
16	108.5		14	
17	151.6		26, 27	
18	41.4		20, 26,27	
19	149.7		26, 27	
20	113.4	7.9 (1H, s)		26, 27
21	137.2			
22	114.7		20, 24	

23	155.9		24	
24	122.2	6.8 (1H, s)		25, 28
25	18.3	2.0 (3H, s)		24
26	30.7	2.1 (3H, s)	27	20
27	30.7	2.1 (3H, s)	26	20
28	60.9	3.5 (3H, s)		24

Fasamycin Q



Molecular formula: $C_{28}H_{20}O_7Cl_4$

Isolated yield: 5.0 mg

UV (PDA): λ_{max} = 254, 293, 361 and 429 nm

Specific rotation: $[\alpha]_D^{20}$ = 21.3

HRMS (ESI) *m/z*: calculated $[M - H]^-$ = 606.9890, observed $[M - H]^-$ = 606.9897, Δ = 1.15 ppm

NMR (Methanol-d4; 600 MHz & 125 MHz): ^1H, ^{13}C, HSQC, HMBC, NOESY

Table 8: Structural determination of Fasamycin Q

Position	**δ_C ppm**	**δ_H ppm (no. of protons, multiplicity, J in Hz)**	**HMBC**	**NOESY**
1	135.6			
2	113.7		4	
3	157.1		4	
4	98.6	6.5 (1H, s)		28
5	153.9		4	
6	126.7		4, 24	
7	138.8		24	
8	118.5		20, 24	
9	165.6			
10	107.0		20	
11	190.5			
12	116.5			
13	161.5			
14	109.9			
15	not detected			
16	107.1			
17	151.1			
18	41.2		20, 26, 27	
19	147.2			
20	113.2	7.8 (1H, s)		26, 27
21	138.9			
22	114.0		20, 24	

23	155.6		24	
24	121.9	6.7 (1H, s)		25
25	18.5	2.0 (3H, s)		24
26	30.8	2.1 (3H, s)		20
27	30.9	2.1 (3H, s)		20
28	56.3	3.6 (3H, s)		4

^{1}H NMR Spectra

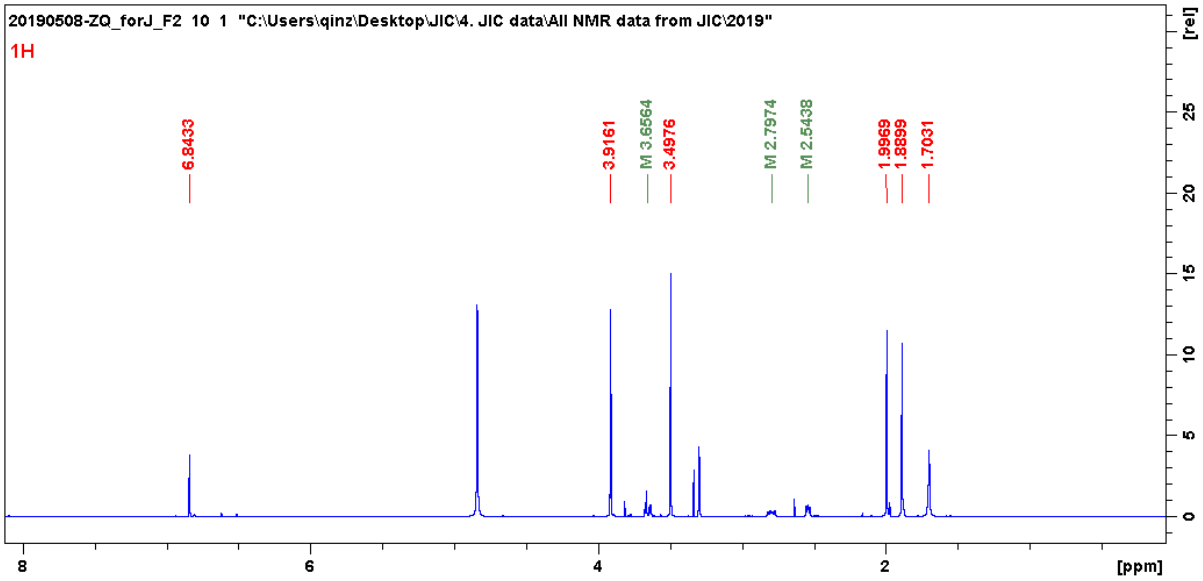


Figure 1. ^{1}H NMR spectrum for formicamycin R. CD_3OD, 600 MHz.

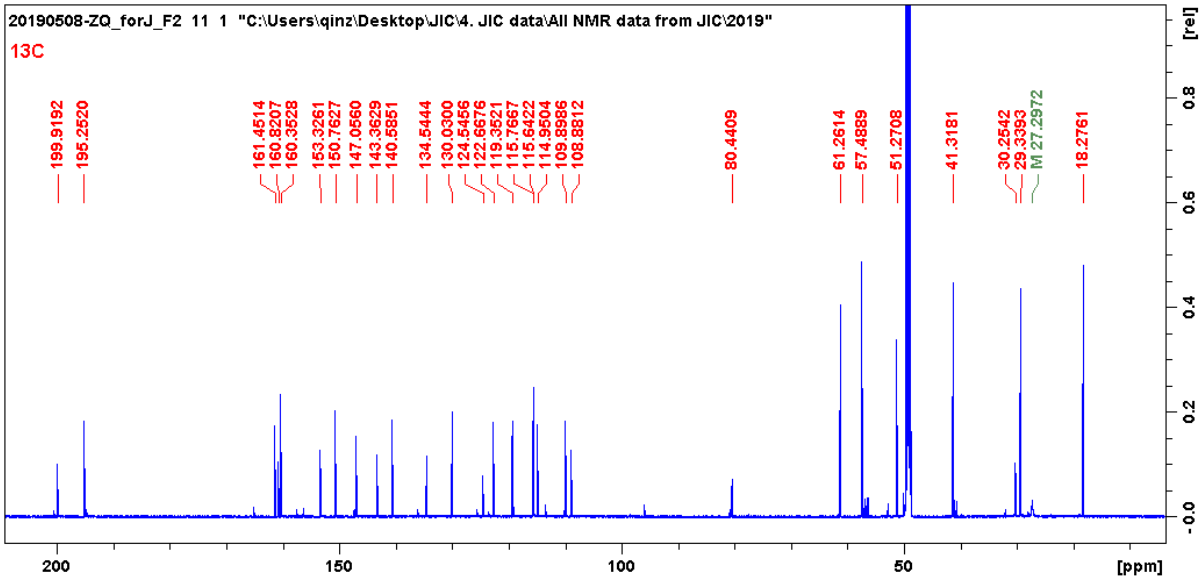


Figure 2. ^{13}C NMR spectrum for formicamycin R. CD_3OD, 150 MHz.

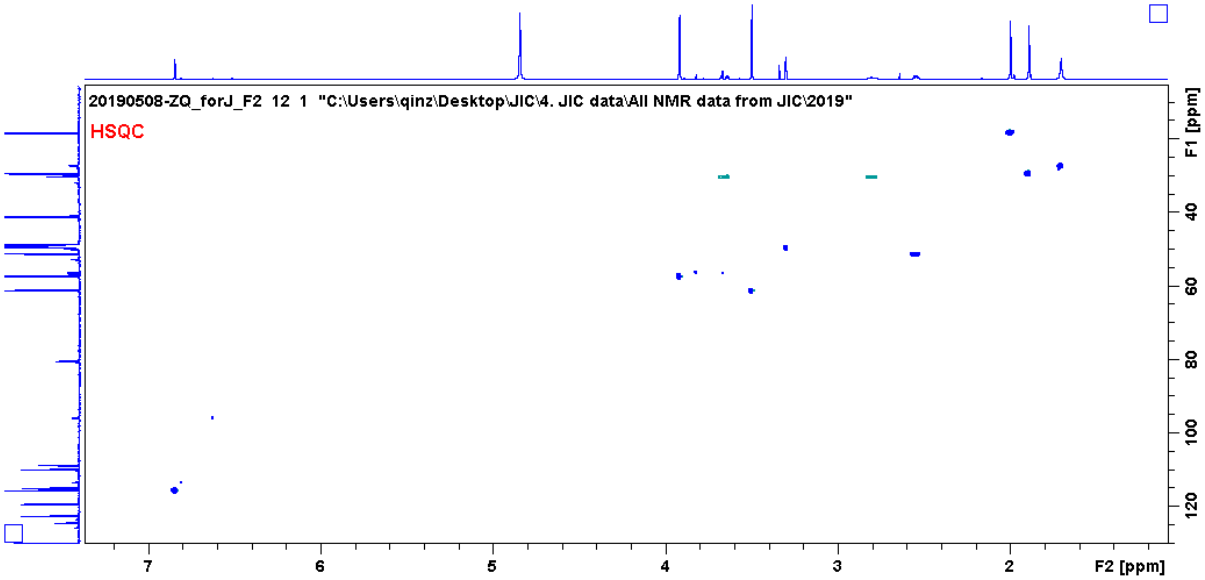


Figure 3. HSQC spectrum for formicamycin R. CD_3OD.

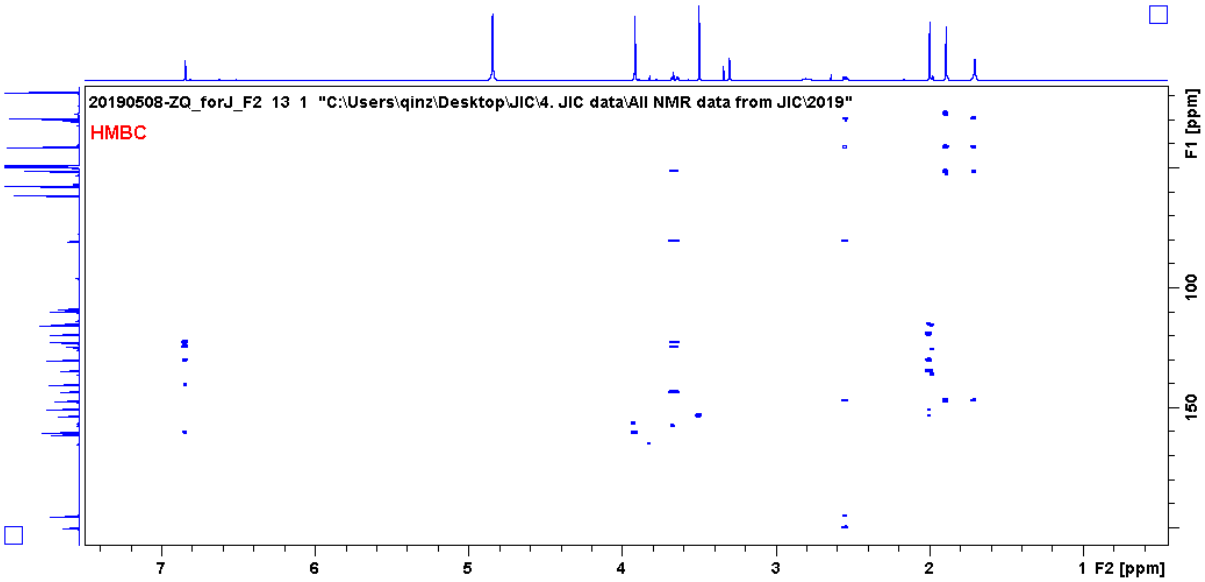


Figure 4. HMBC spectrum for formicamycin R. CD_3OD.

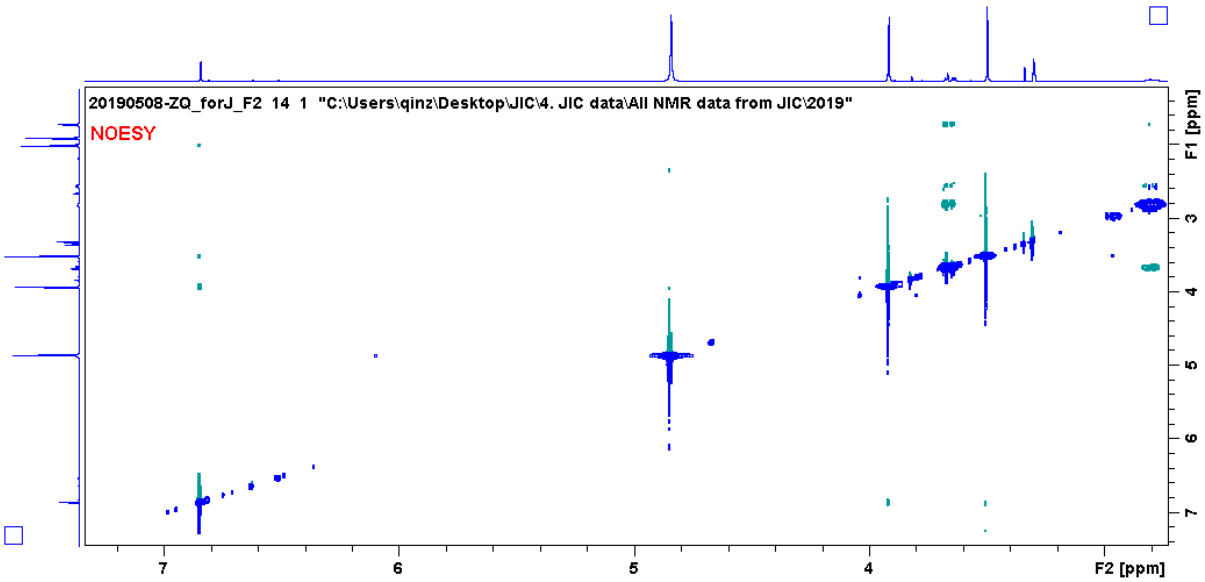


Figure 5. NOESY spectrum for formicamycin R. CD_3OD.

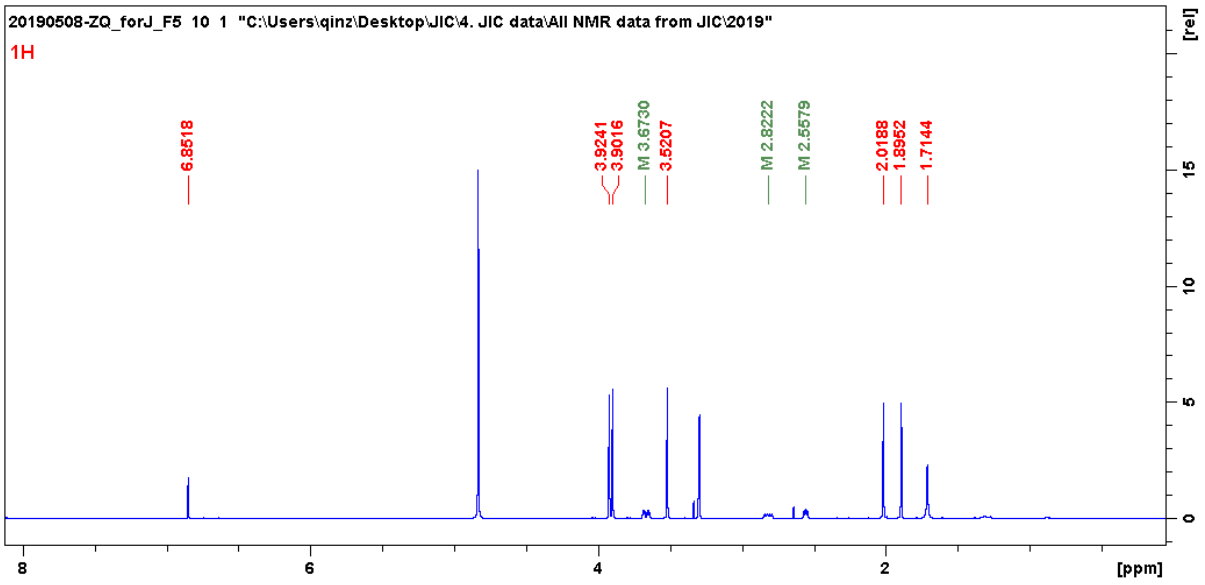


Figure 6. ^1H NMR spectrum for formicamycin S. CD_3OD, 600 MHz.

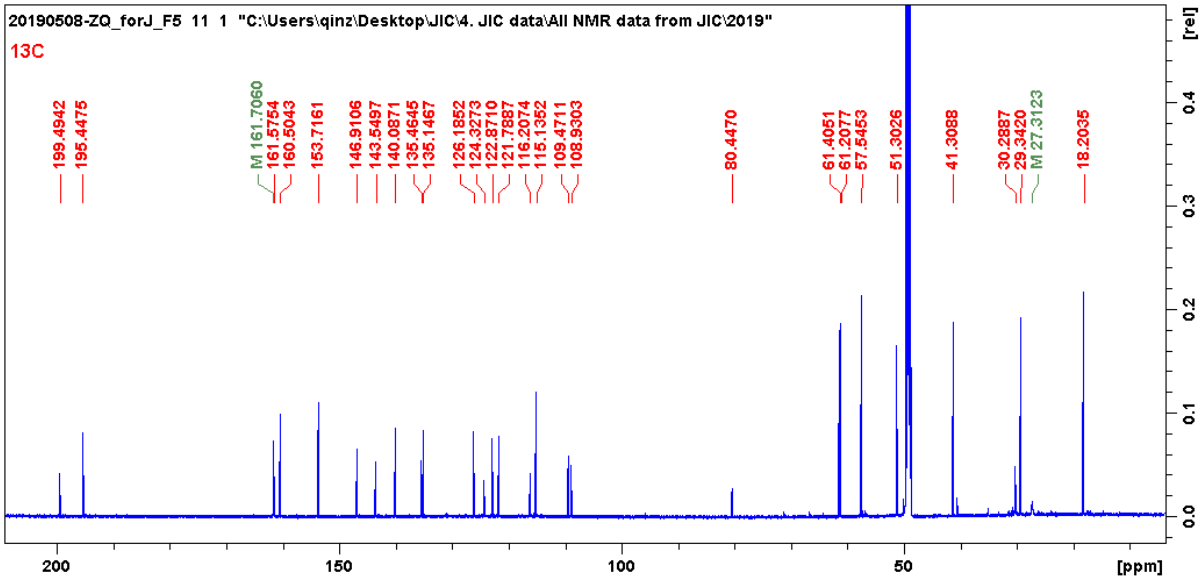


Figure 7. ^{13}C NMR spectrum for formicamycin S. CD_3OD, 150 MHz.

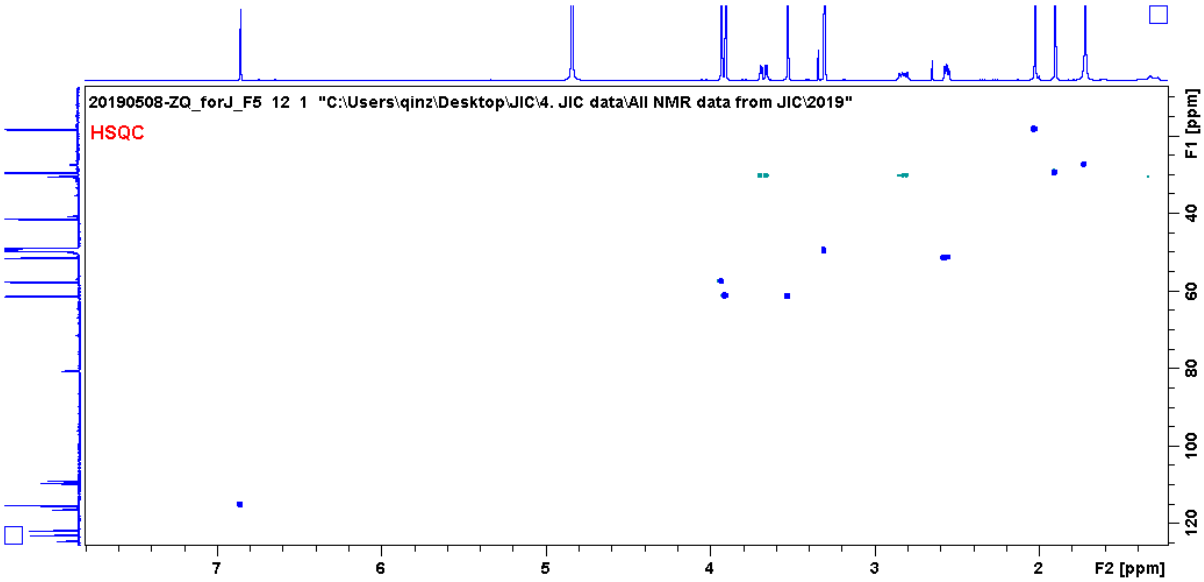


Figure 8. HSQC spectrum for formicamycin S. CD_3OD.

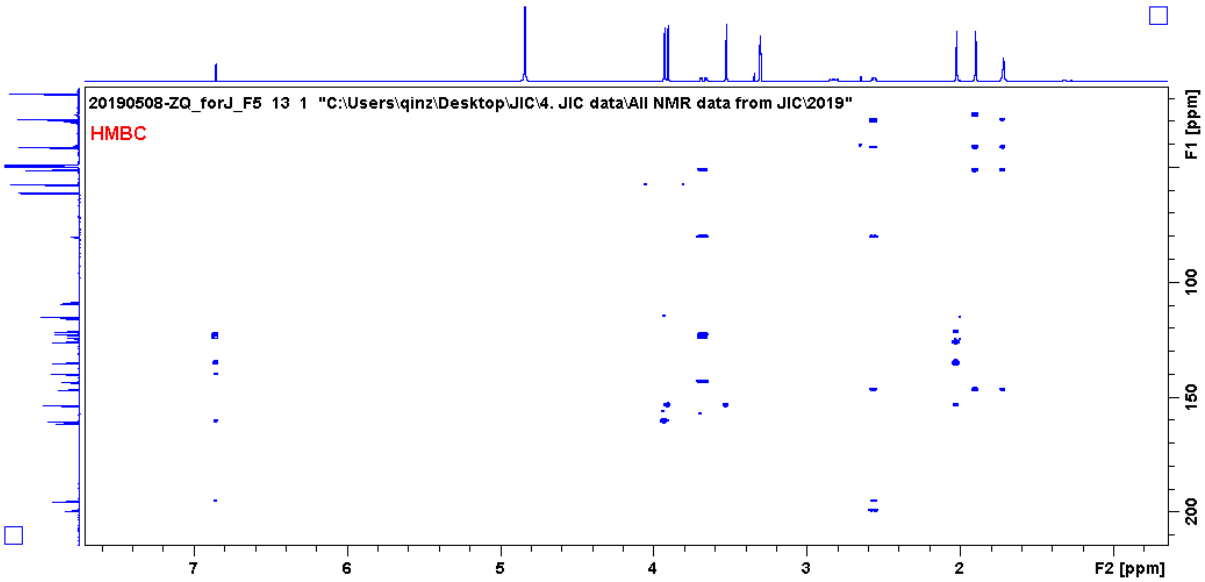


Figure 9. HMBC spectrum for formicamycin S. CD_3OD.

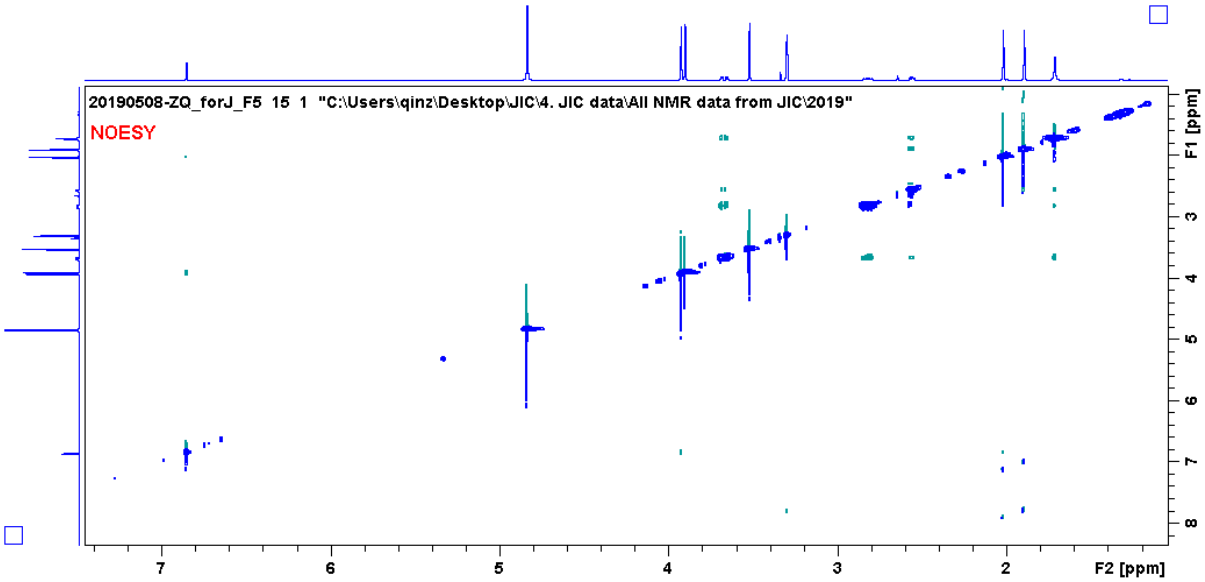


Figure 10. NOESY spectrum for formicamycin S. CD_3OD.

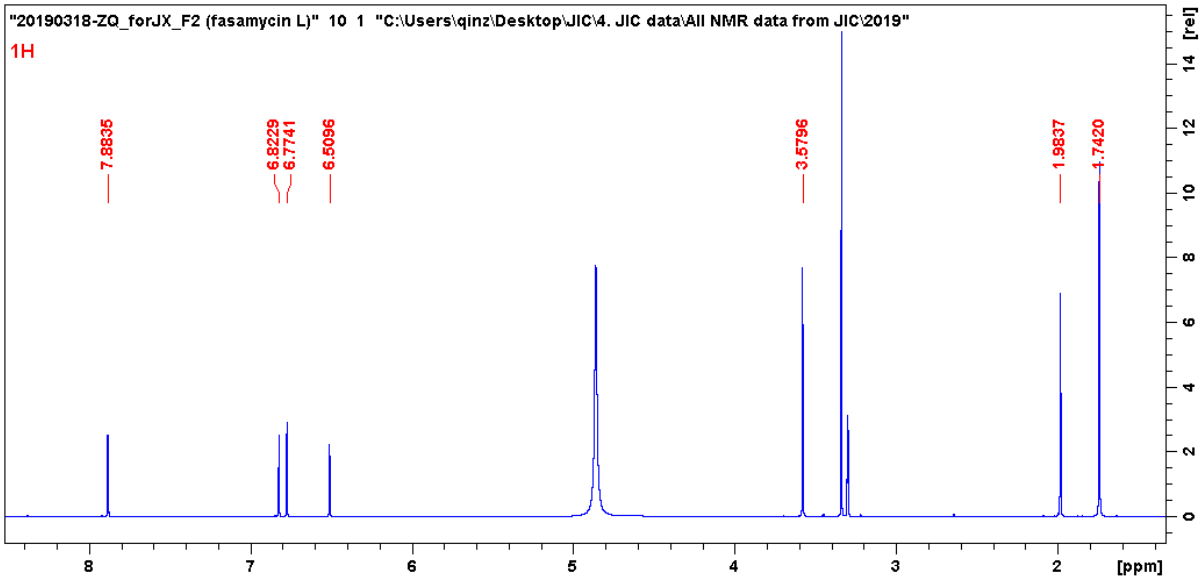


Figure 11. ^{1}H NMR spectrum for fasamycin L. CD_3OD, 600 MHz.

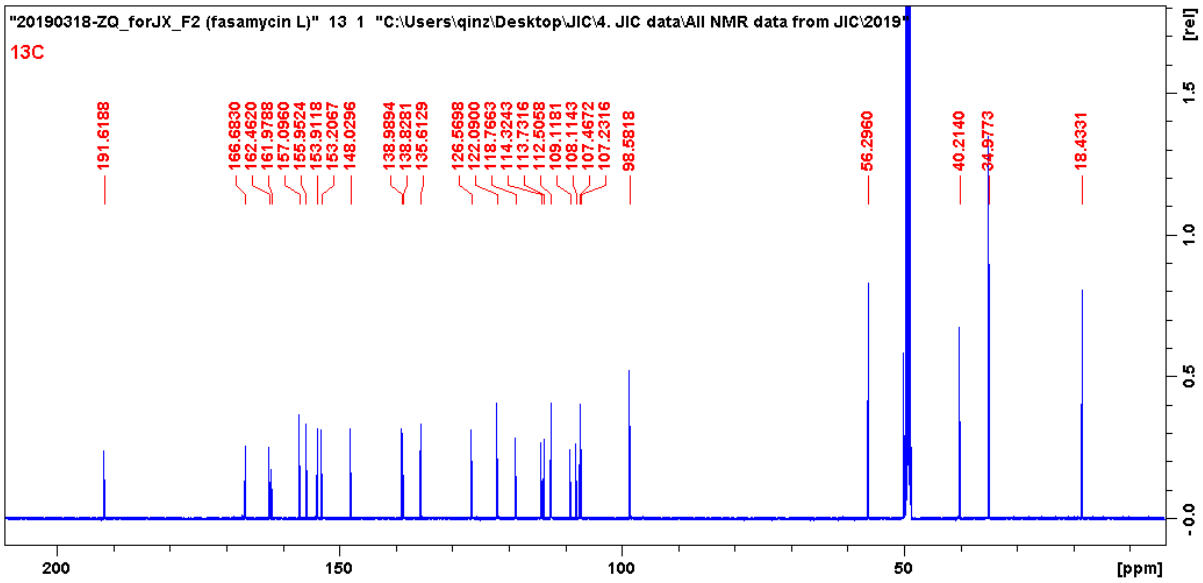


Figure 12. ^{13}C NMR spectrum for fasamycin L. CD_3OD, 150 MHz.

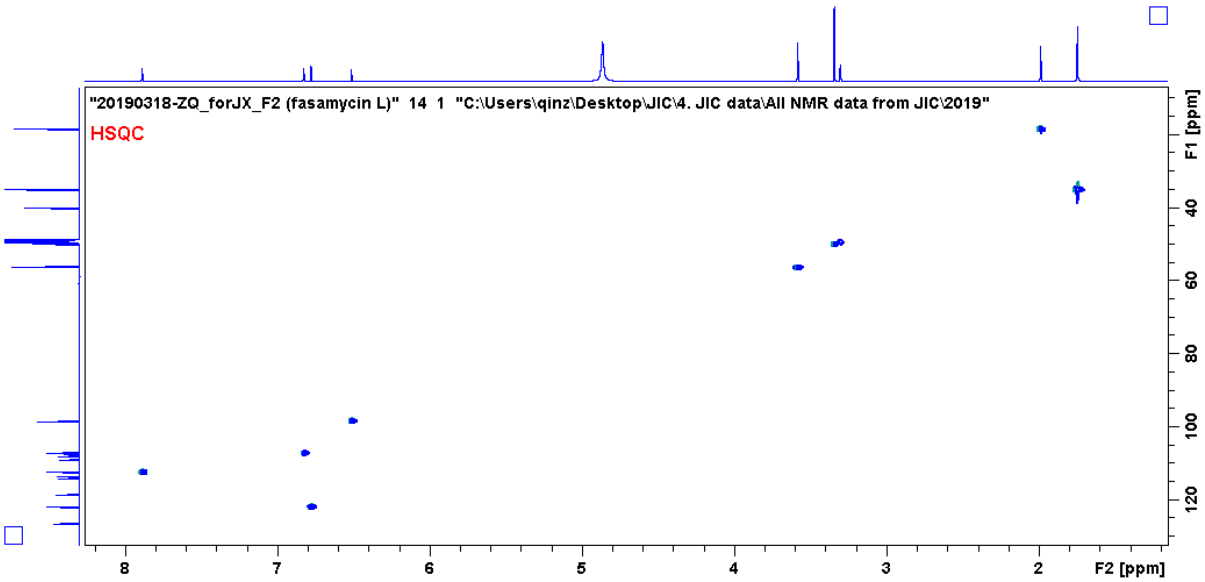


Figure 13. HSQC spectrum for fasamycin L. CD_3OD.

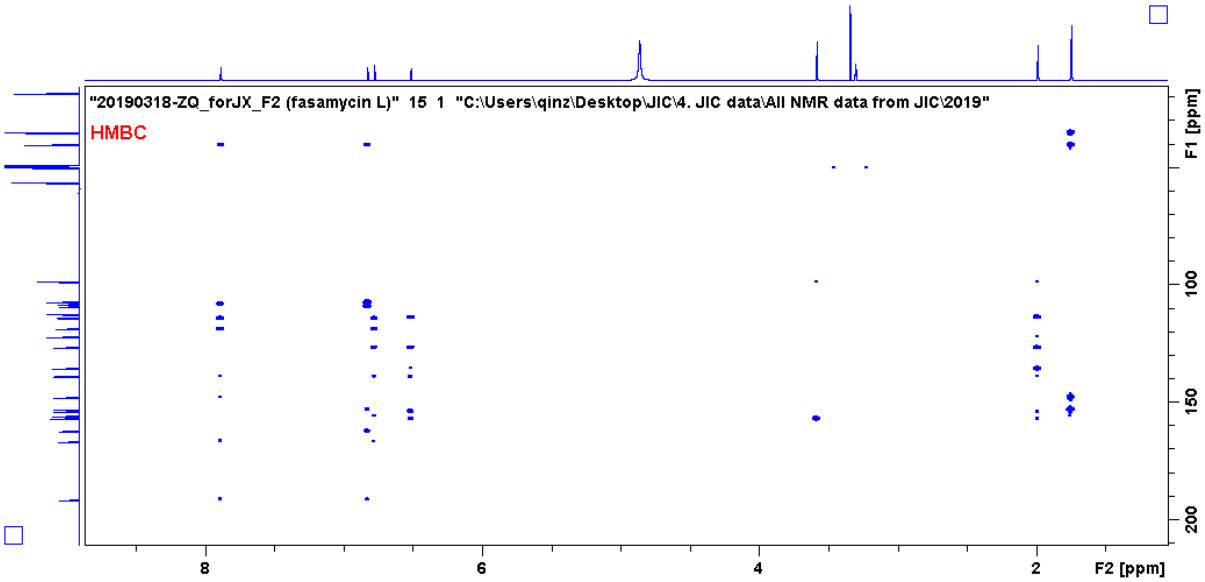


Figure 14. HMBC spectrum for fasamycin L. CD_3OD.

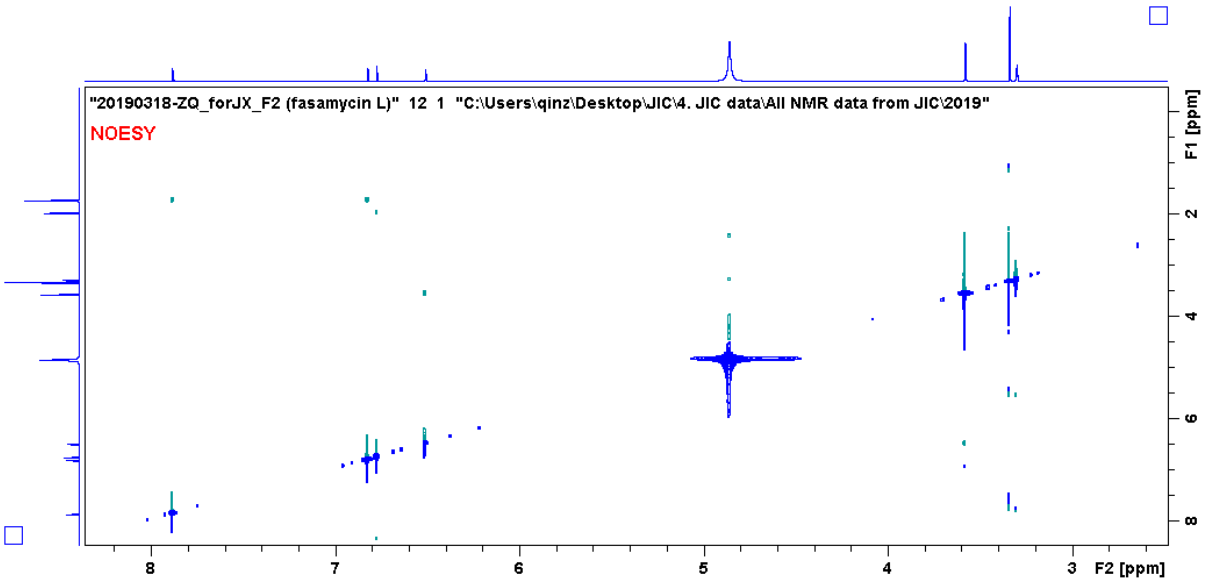


Figure 15. NOESY spectrum for fasamycin L. CD_3OD.

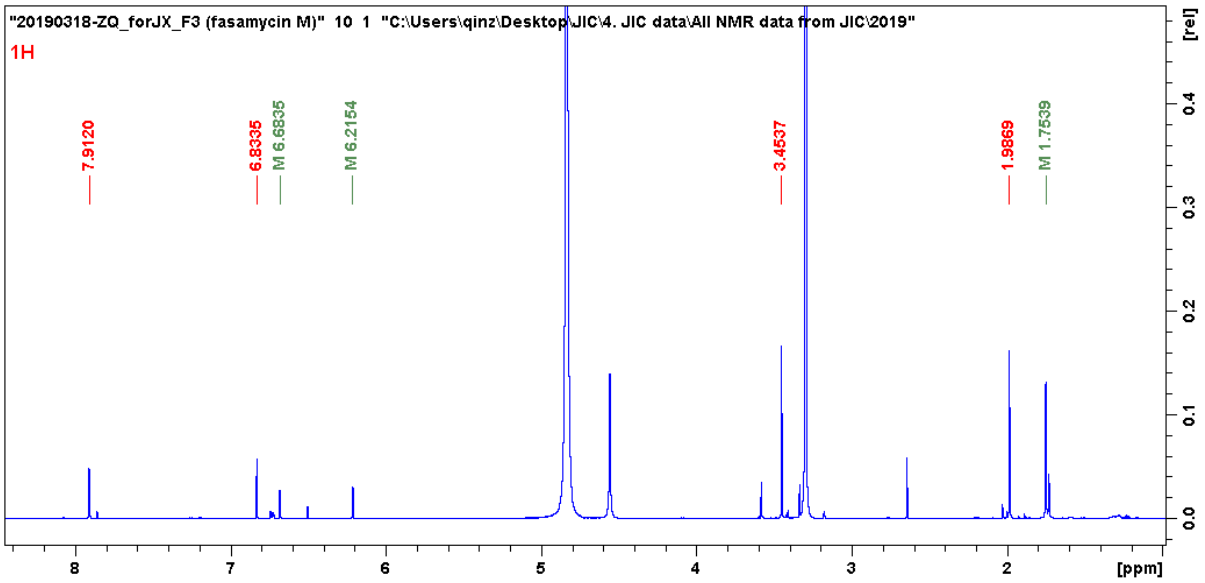


Figure 16. ^1H NMR spectrum for fasamycin M. CD_3OD, 600 MHz.

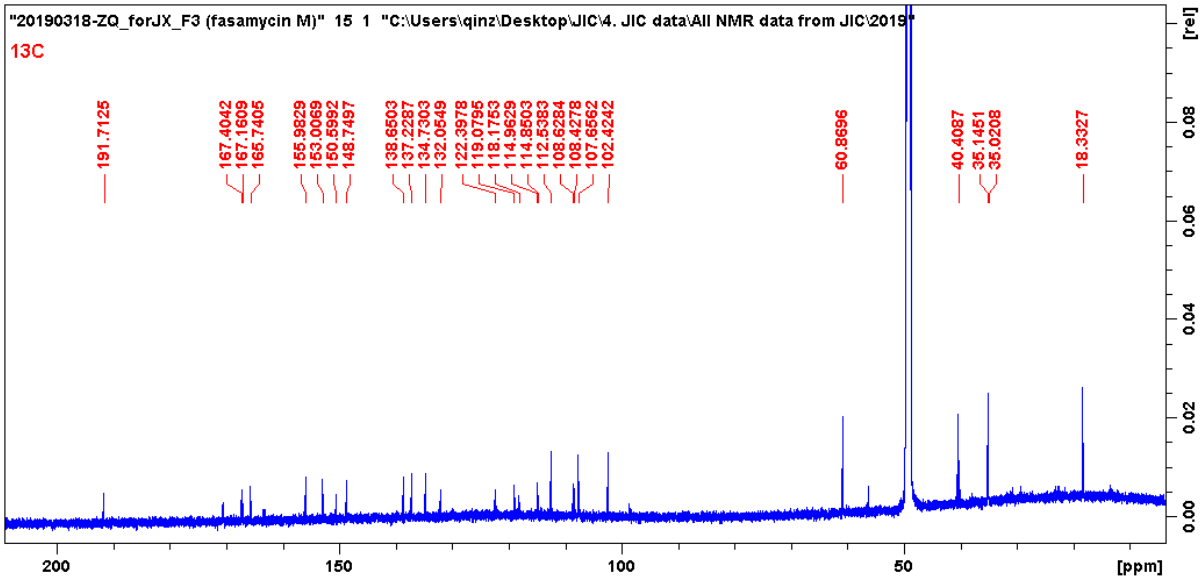


Figure 17. ^{13}C NMR spectrum for fasamycin M. CD_3OD, 150 MHz.

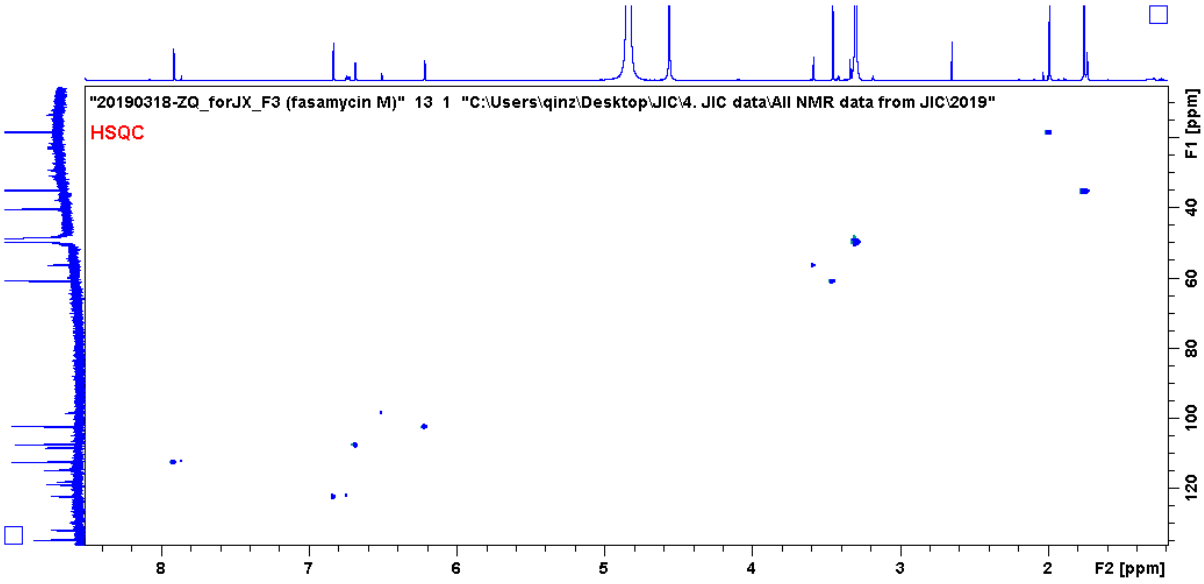


Figure 18. HSQC spectrum for fasamycin M. CD_3OD.

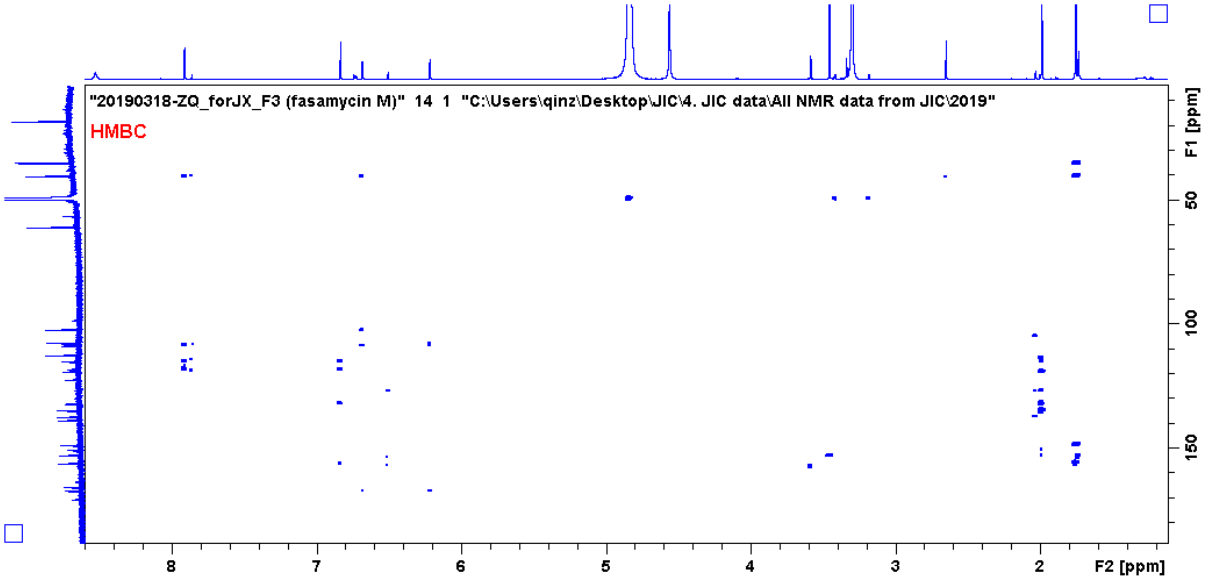


Figure 19. HMBC spectrum for fasamycin M. CD_3OD.

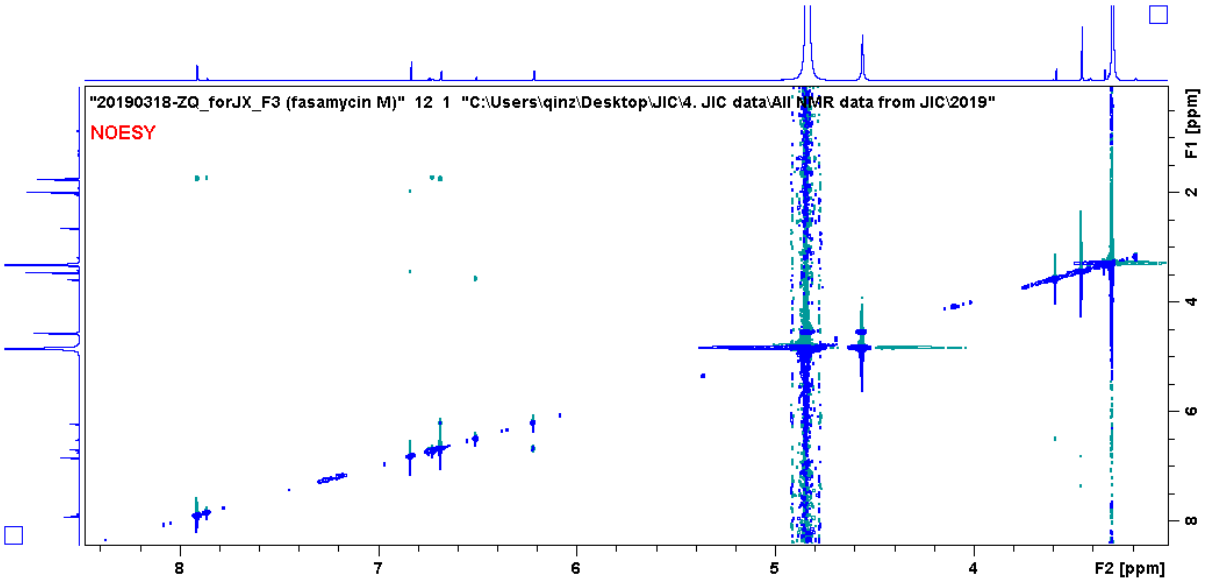


Figure 20. NOESY spectrum for fasamycin M. CD_3OD.

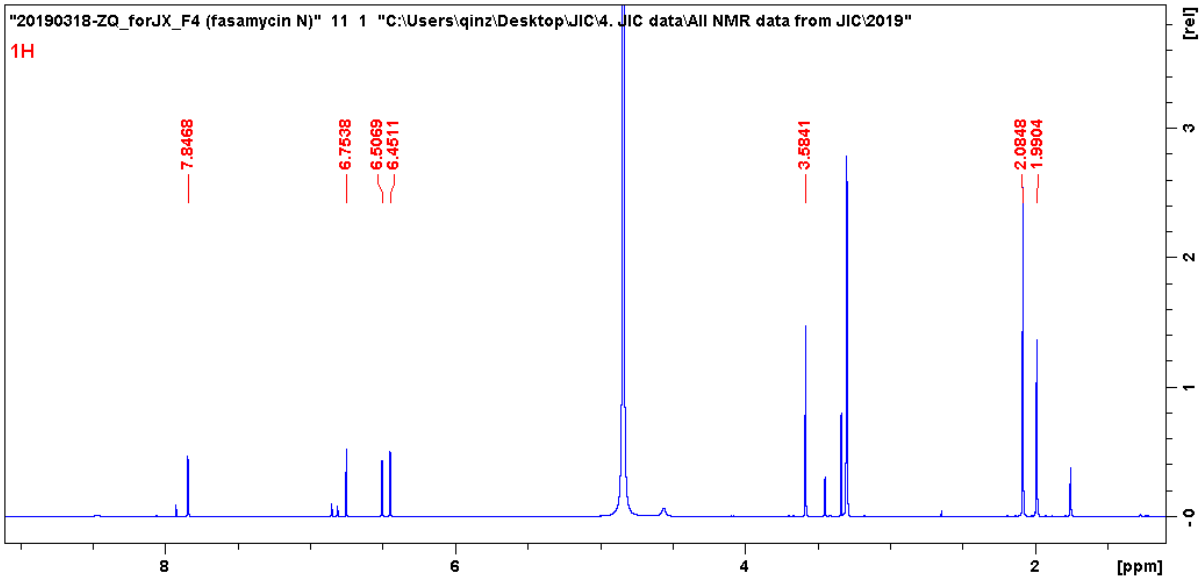


Figure 21. ^{1}H NMR spectrum for fasamycin N. CD_3OD, 600 MHz.

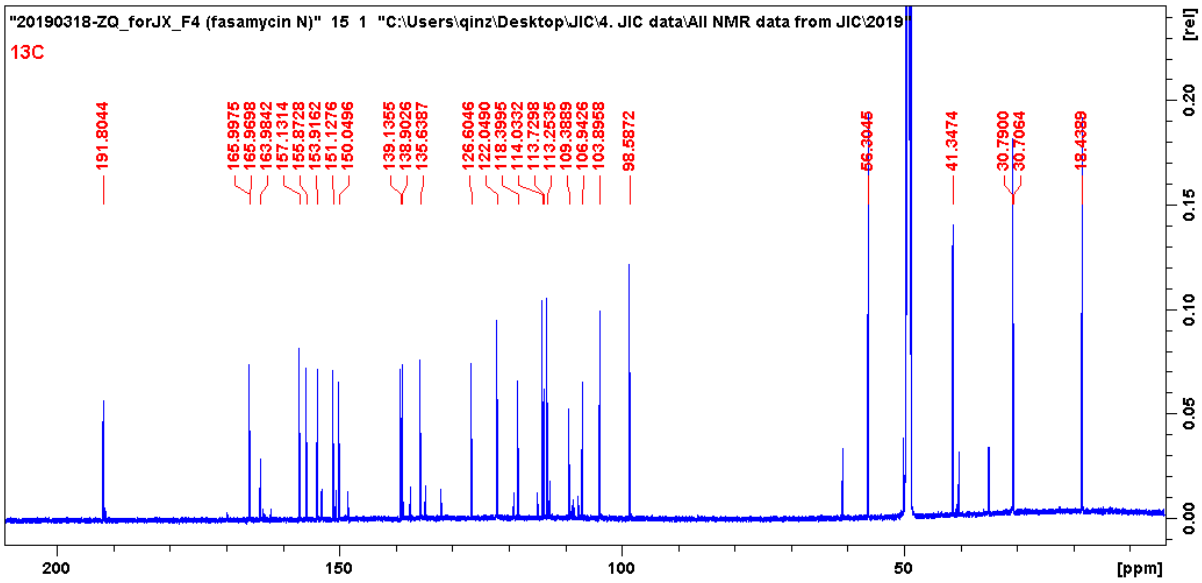


Figure 22. ^{13}C NMR spectrum for fasamycin N. CD_3OD, 150 MHz.

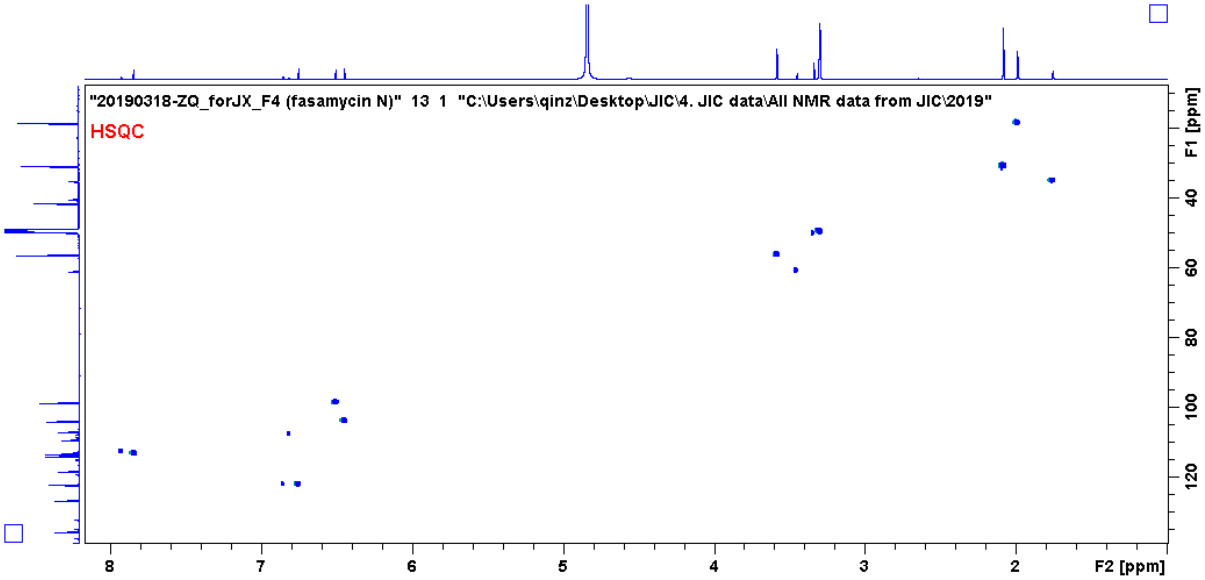


Figure 23. HSQC spectrum for fasamycin N. CD_3OD.

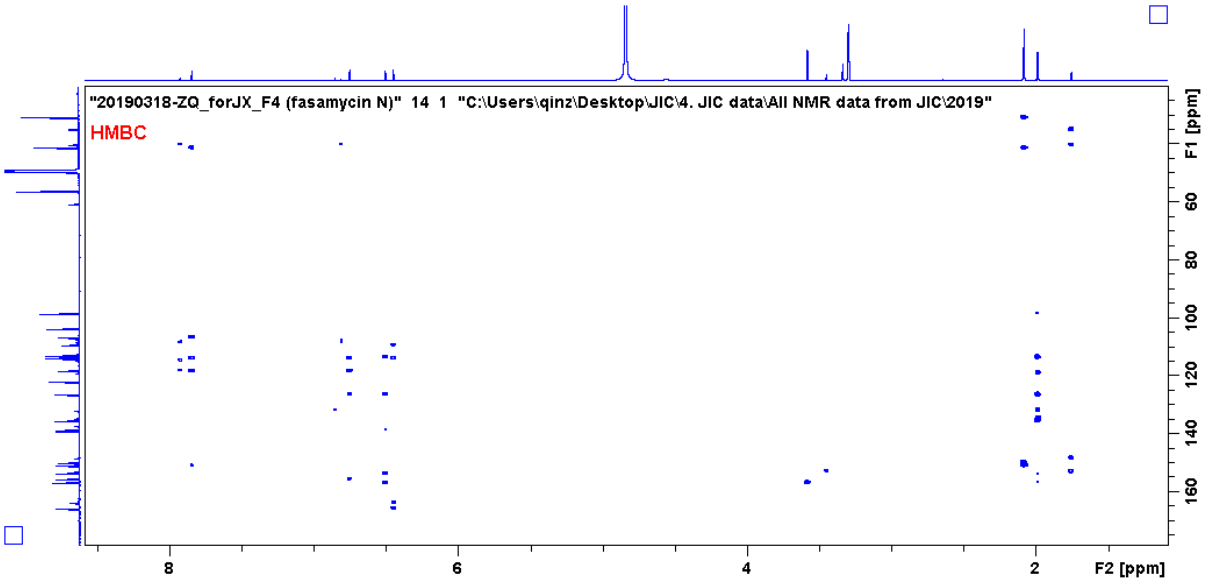


Figure 24. HMBC spectrum for fasamycin N. CD_3OD.

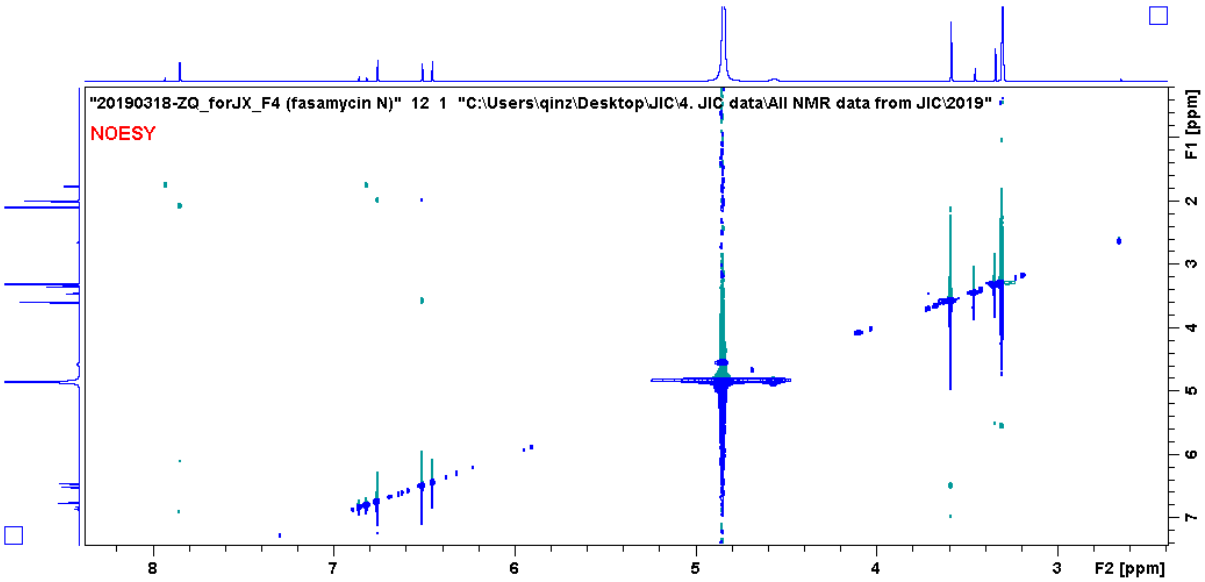


Figure 25. NOESY spectrum for fasamycin N. CD_3OD.

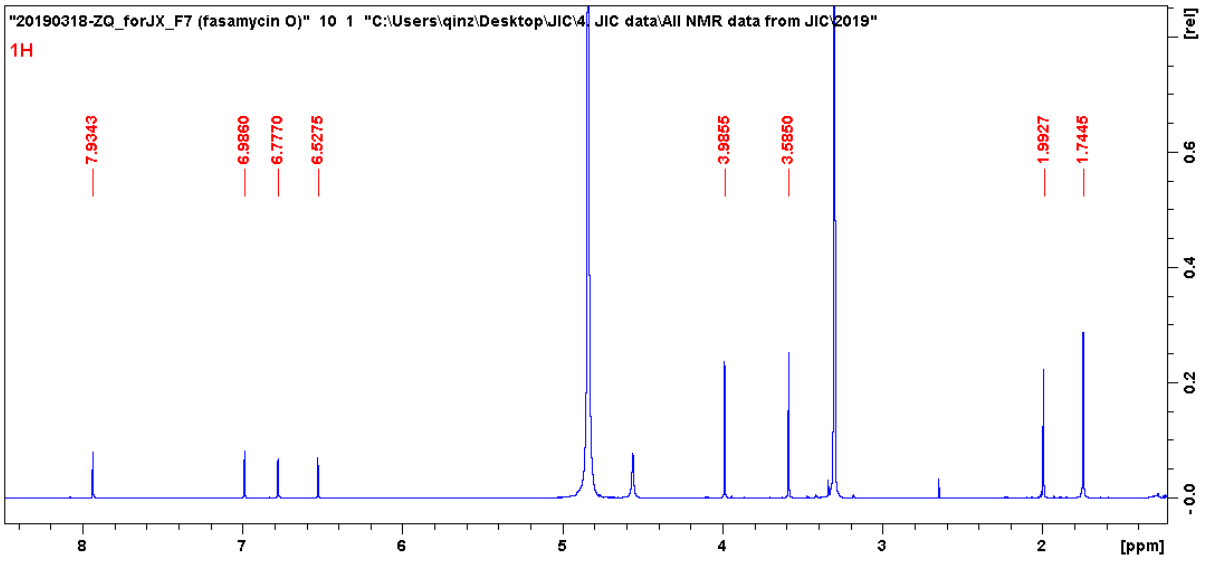


Figure 26. ^1H NMR spectrum for fasamycin O. CD_3OD, 600 MHz.

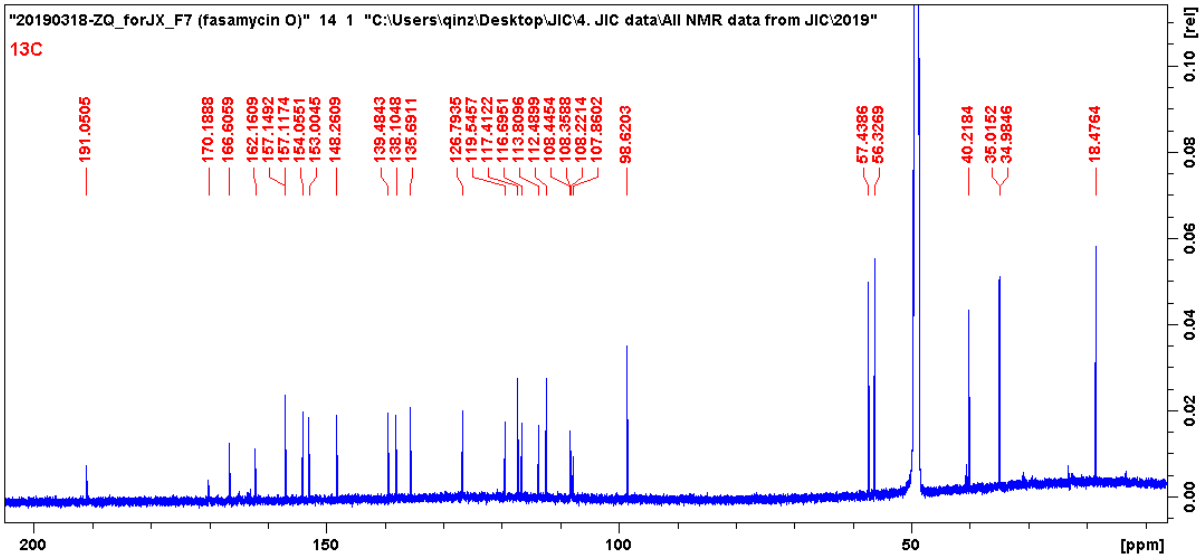


Figure 27. ^{13}C NMR spectrum for fasamycin O. CD_3OD, 150 MHz.

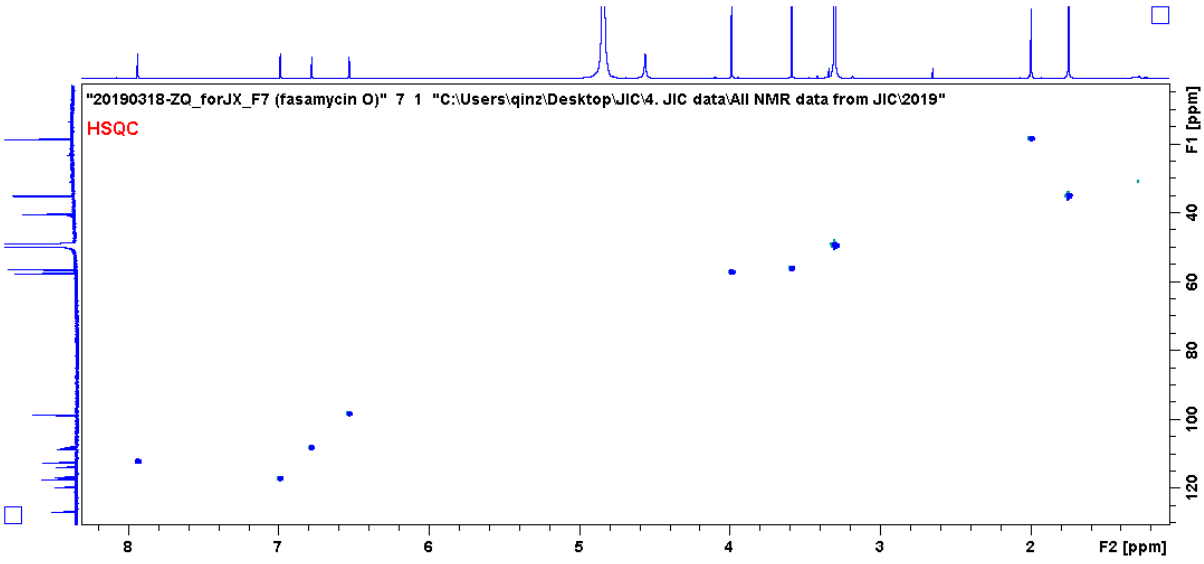


Figure 28. HSQC spectrum for fasamycin O. CD_3OD.

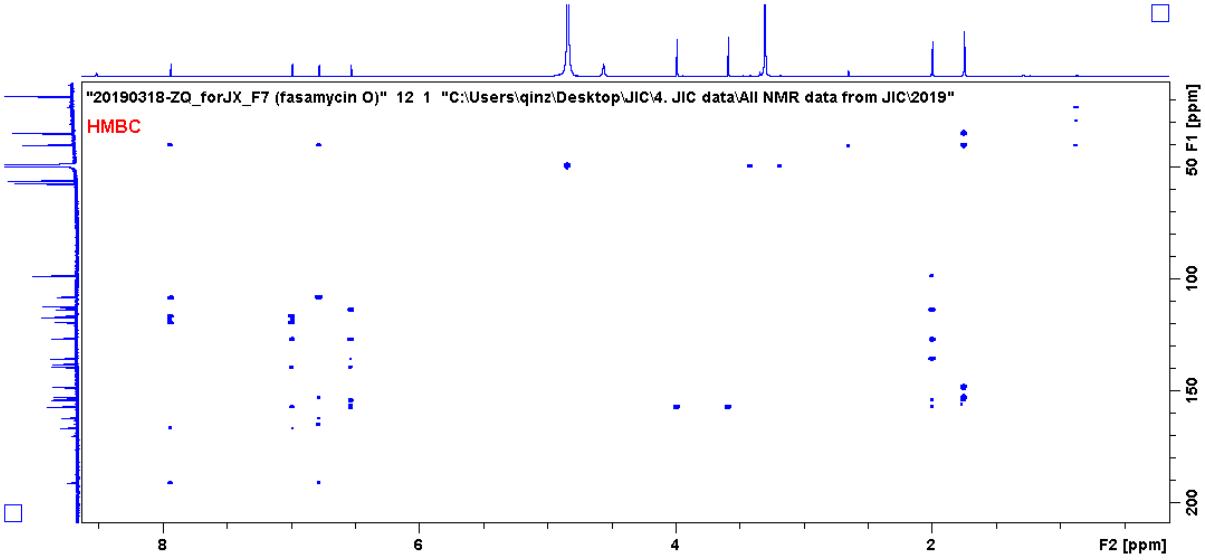


Figure 29. HMBC spectrum for compound **6**. CD_3OD.

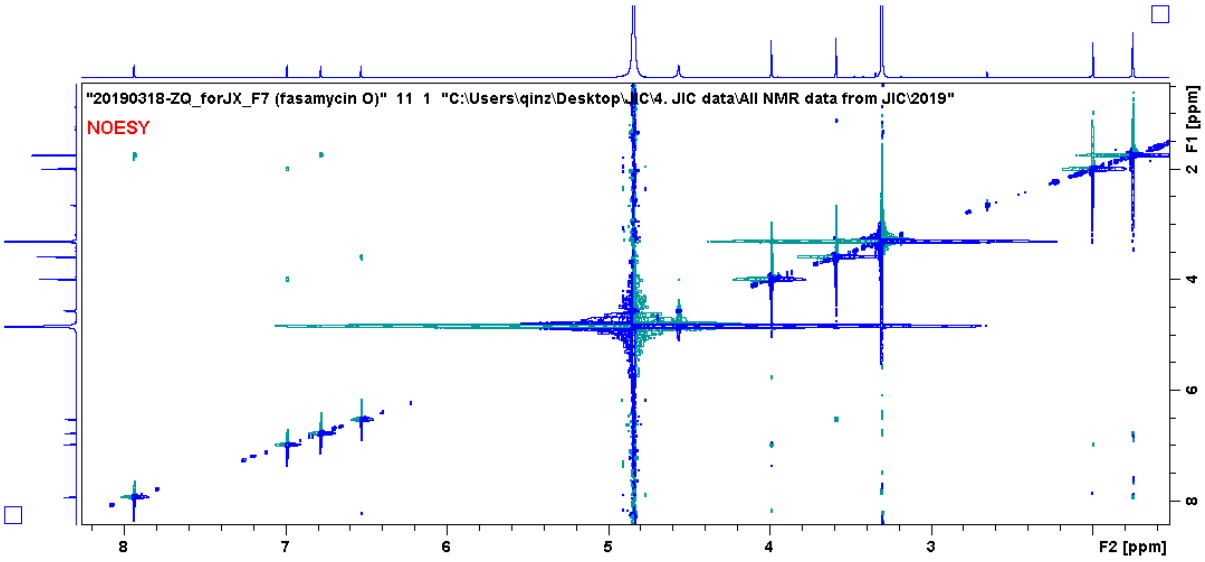


Figure 30. NOESY spectrum for fasamycin O. CD_3OD.

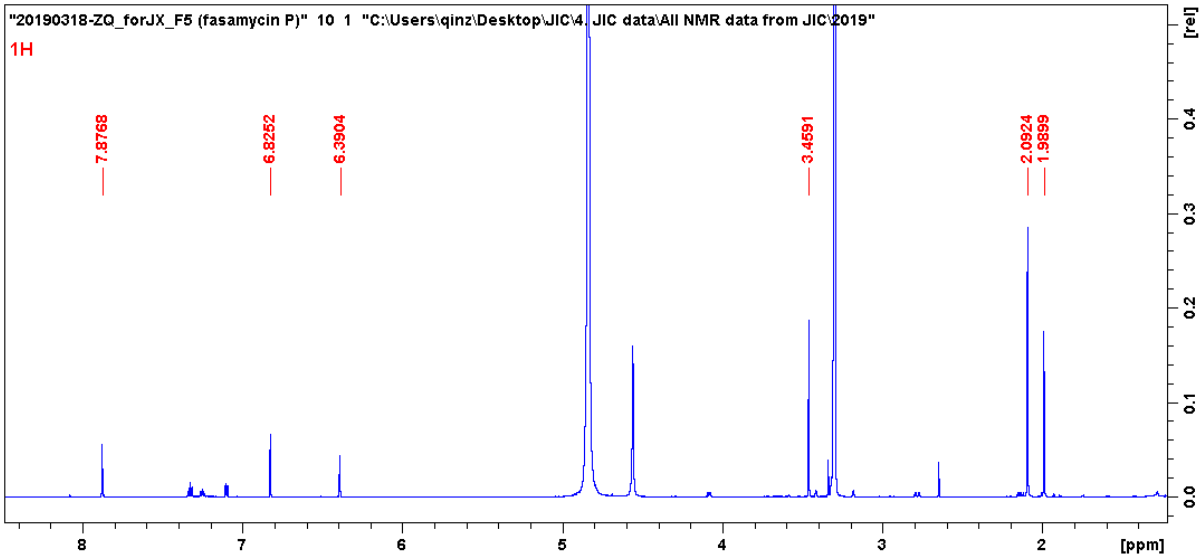


Figure 31. ^{1}H NMR spectrum for fasamycin P. CD_3OD, 600 MHz.

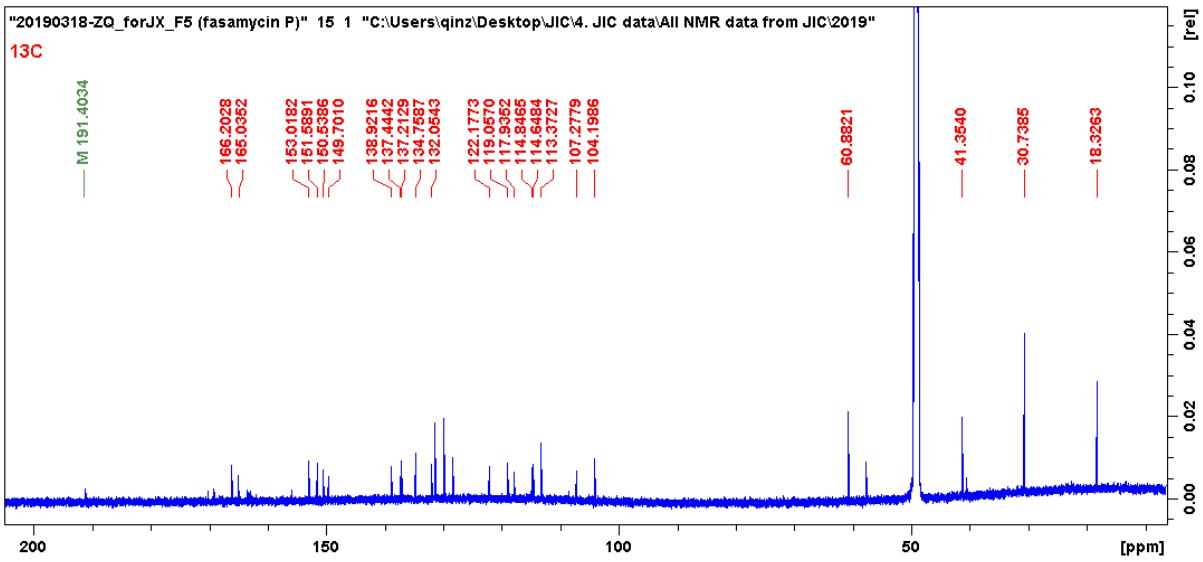


Figure 32. ^{13}C NMR spectrum for fasamycin P. CD_3OD, 150 MHz.

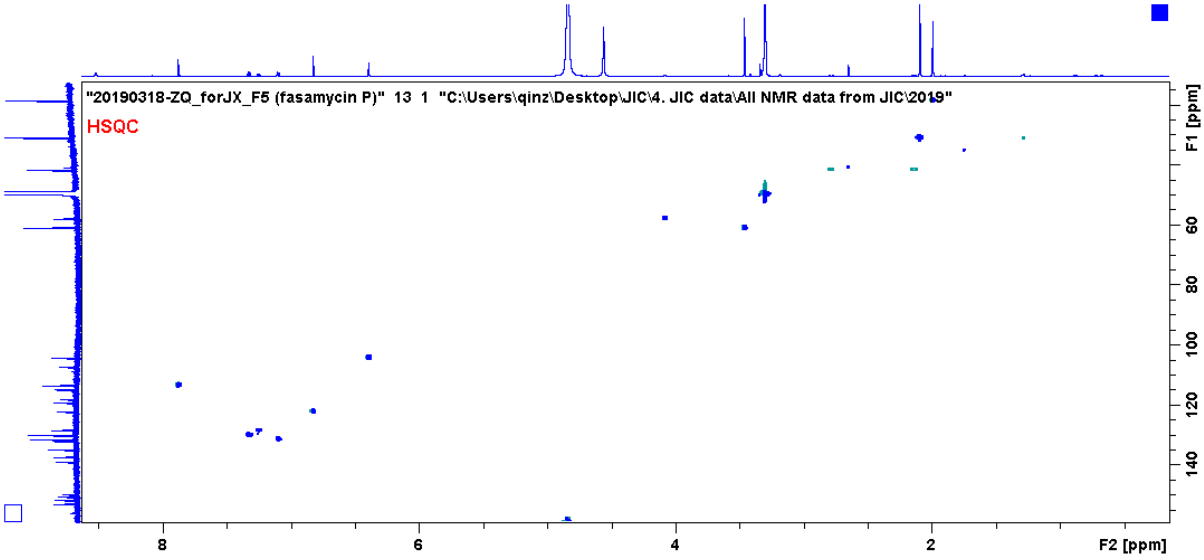


Figure 33. HSQC spectrum for fasamycin P. CD_3OD.

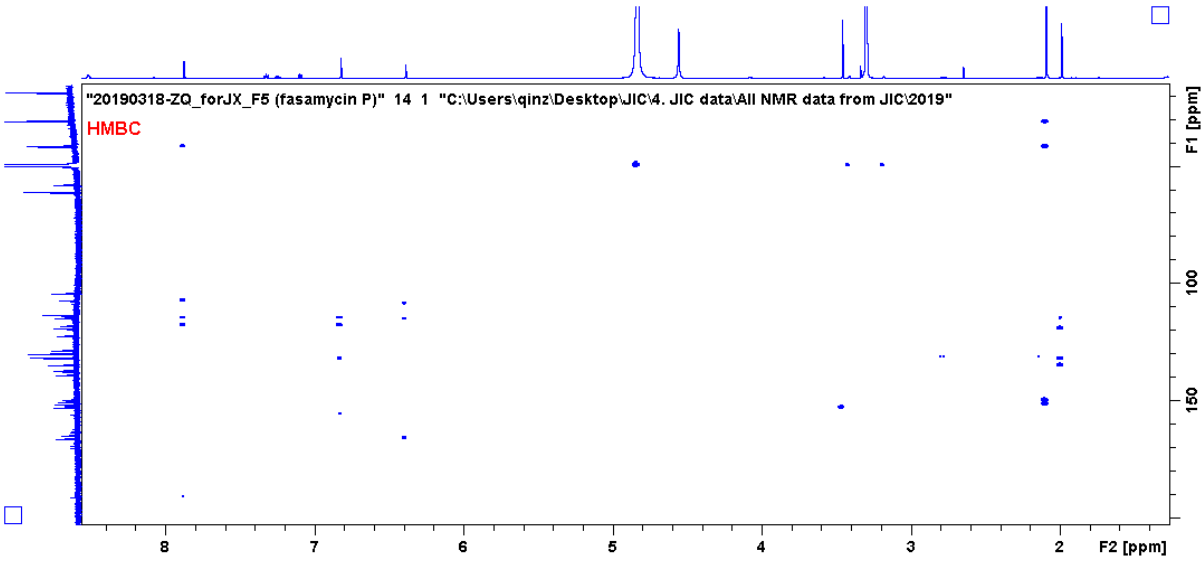


Figure 34. HMBC spectrum for fasamycin P. CD_3OD.

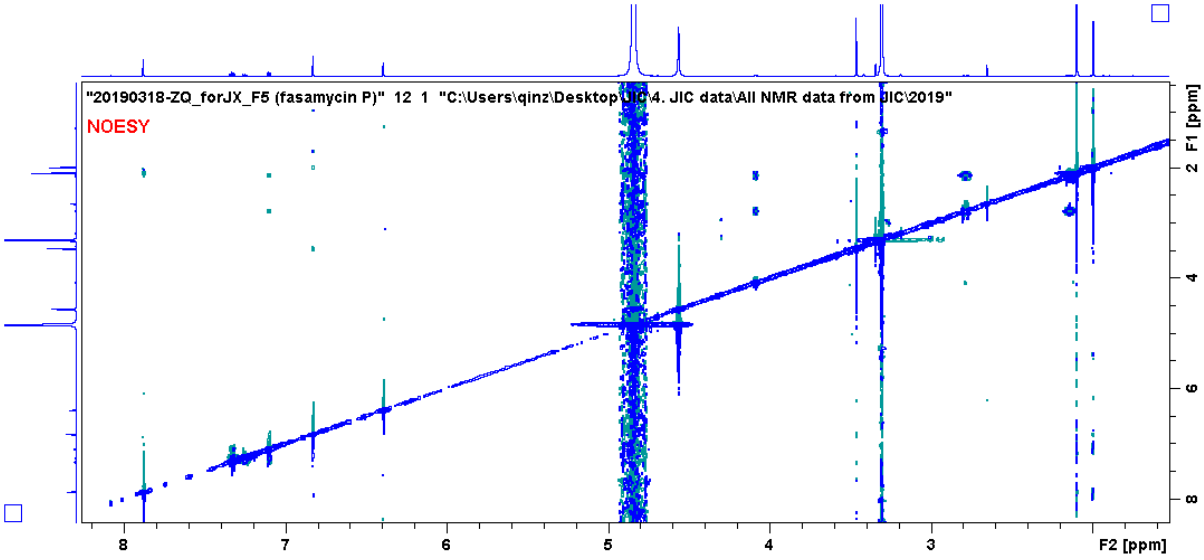


Figure 35. NOESY spectrum for fasamycin P. CD_3OD.

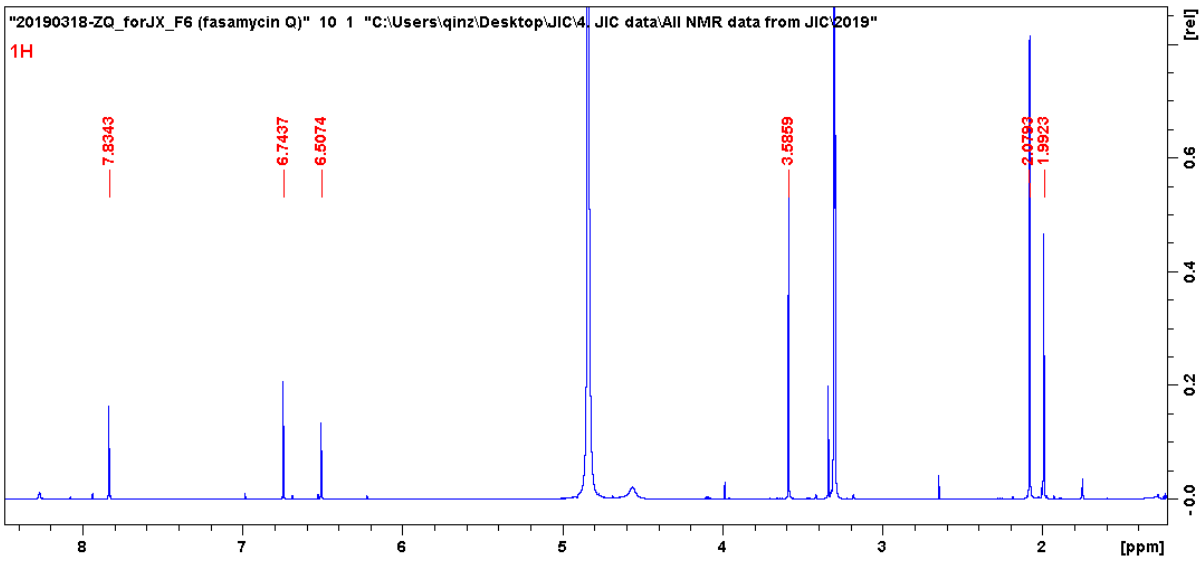


Figure 36. ^1H NMR spectrum for fasamycin Q. CD_3OD, 600 MHz.

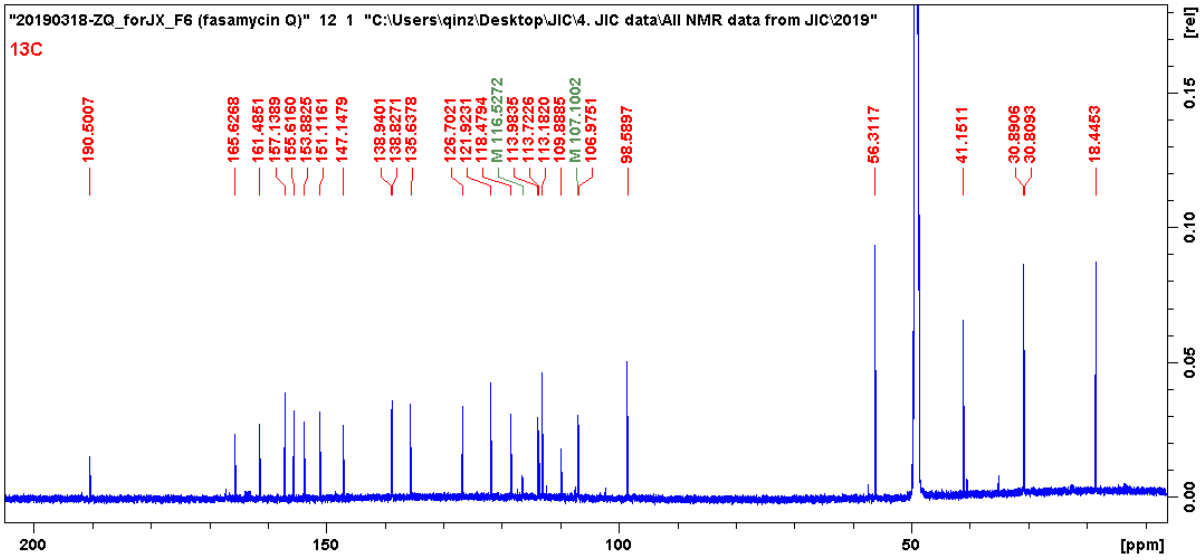


Figure 37. ^{13}C NMR spectrum for fasamycin Q. CD_3OD, 150 MHz.

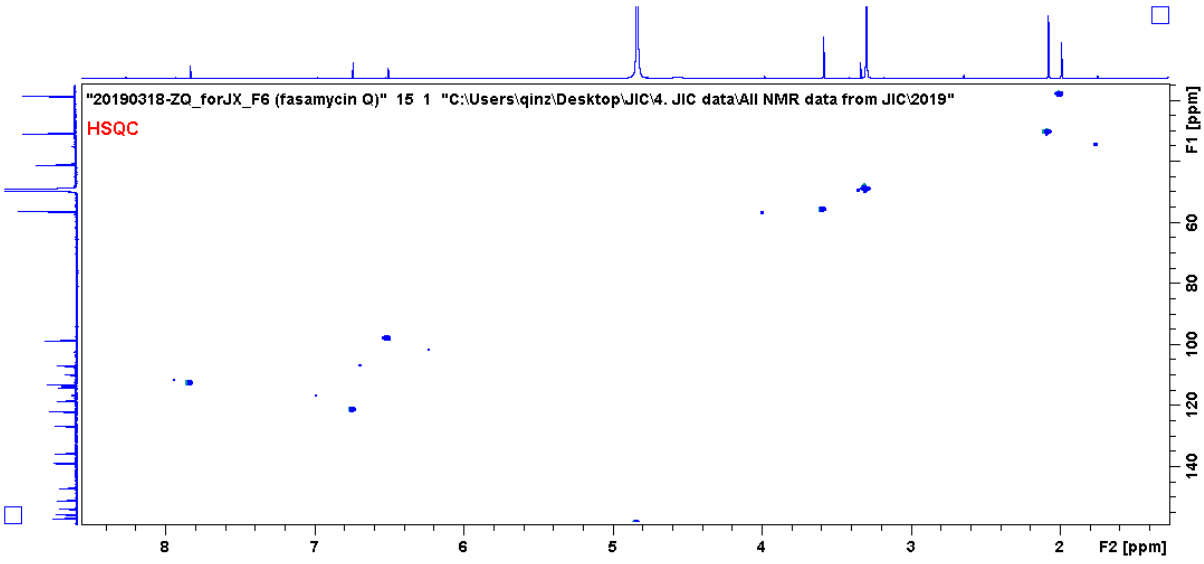


Figure 38. HSQC spectrum for fasamycin Q. CD_3OD.

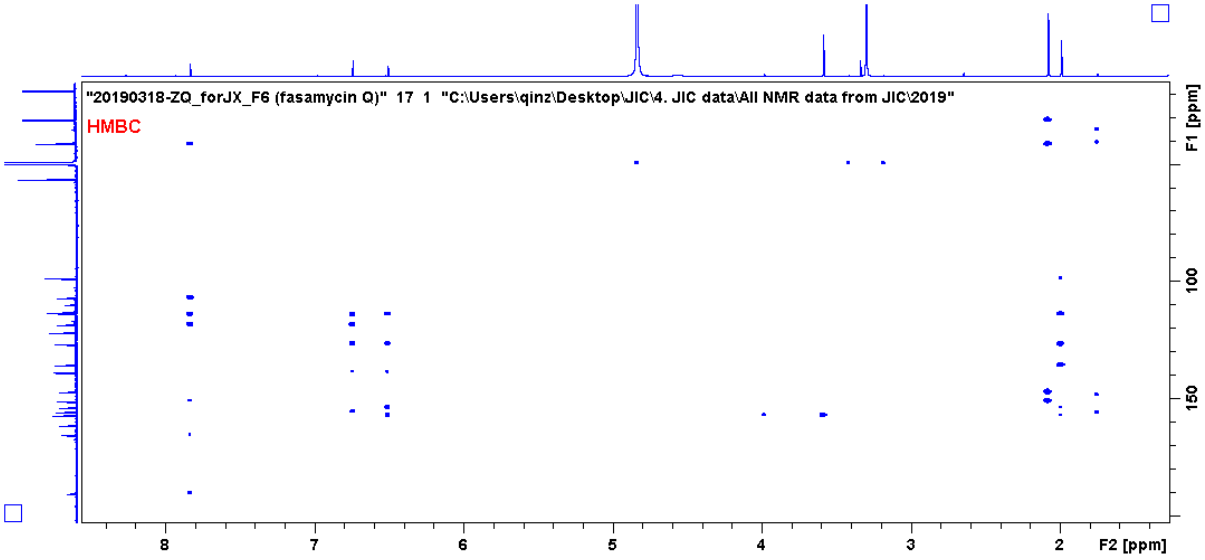


Figure 39. HMBC spectrum for fasamycin Q. CD_3OD.

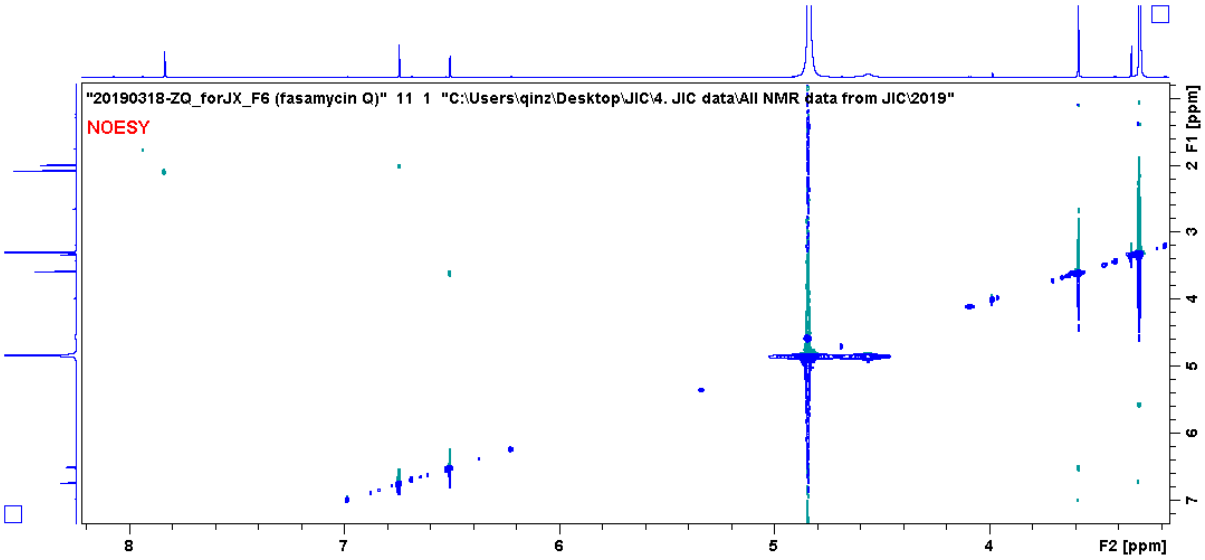


Figure 40. NOESY spectrum for fasamycin Q. CD_3OD.