**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: C29H23O8Cl5

Isolated yield: 16.5 mg

UV (PDA): λmax = 245 and 290 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 1:** Structural determination of Formicamycin R

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C30H25O8Cl5

Isolated yield: 7.0 mg

UV (PDA): λmax = 240 and 292 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 2:** Structural determination of Formicamycin S

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C28H21O7Cl3

Isolated yield: 45.8 mg

UV (PDA): λmax = 249, 290, 350 and 419 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 3:** Structural determination of Fasamycin L

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C28H21O7Cl3

Isolated yield: 2.3 mg

UV (PDA): λmax = 249, 289, 352 and 413 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 4:** Structural determination of Fasamycin M

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C28H21O7Cl3

Isolated yield: 10.7 mg

UV (PDA): λmax = 253, 293, 365 and 424 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 5:** Structural determination of Fasamycin N

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C29H23O7Cl3

Isolated yield: 3.2 mg

UV (PDA): λmax = 252, 292, 336 and 420 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 6:** Structural determination of Fasamycin O

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C28H20O7Cl4

Isolated yield: 2.3 mg

UV (PDA): λmax = 253, 294, 360 and 419 nm

Specific rotation: [α]D2 0 = 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, 13C, HSQC, HMBC, NOESY

**Table 7:** Structural determination of Fasamycin P

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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: C28H20O7Cl4

Isolated yield: 5.0 mg

UV (PDA): λmax = 254, 293, 361 and 429 nm

Specific rotation: [α]D2 0 = 21.3

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

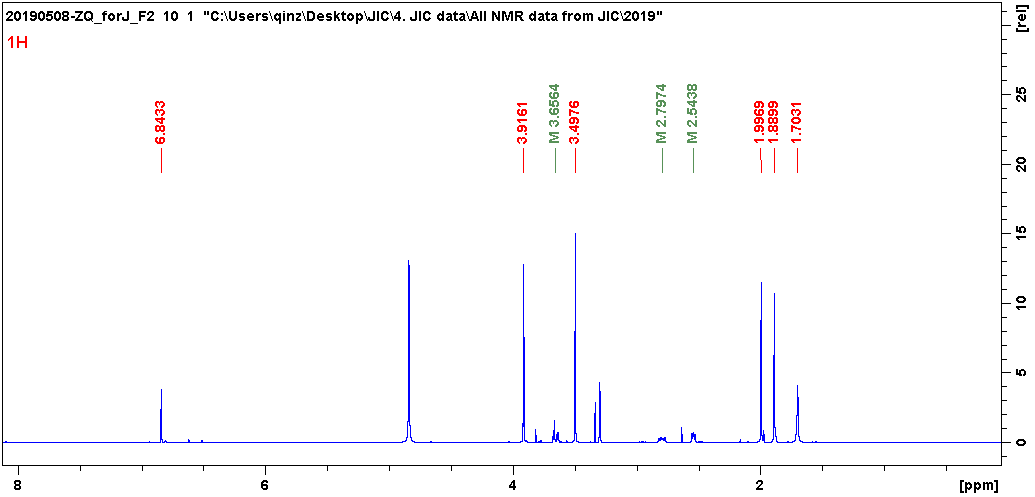
= 1.15 ppm

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

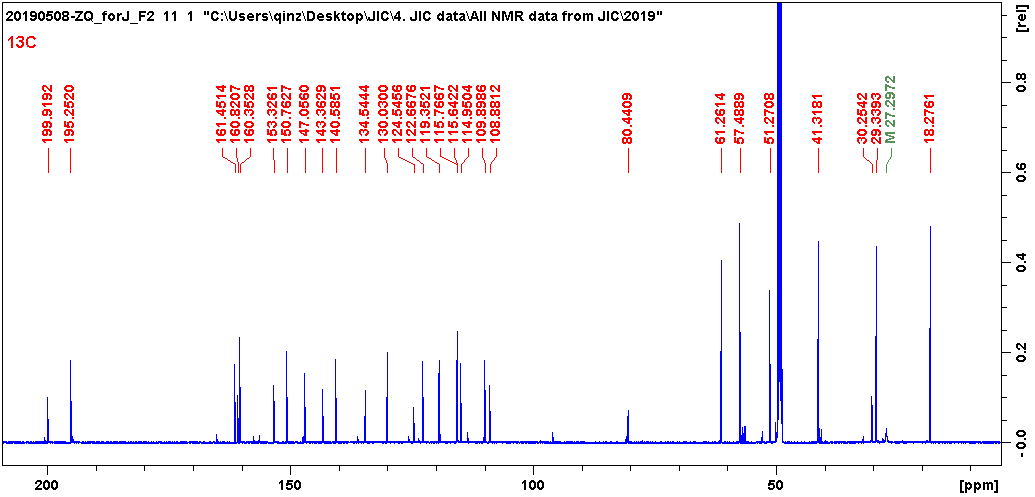
**Table 8:** Structural determination of Fasamycin Q

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position** | **δC ppm** | **δH 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 |

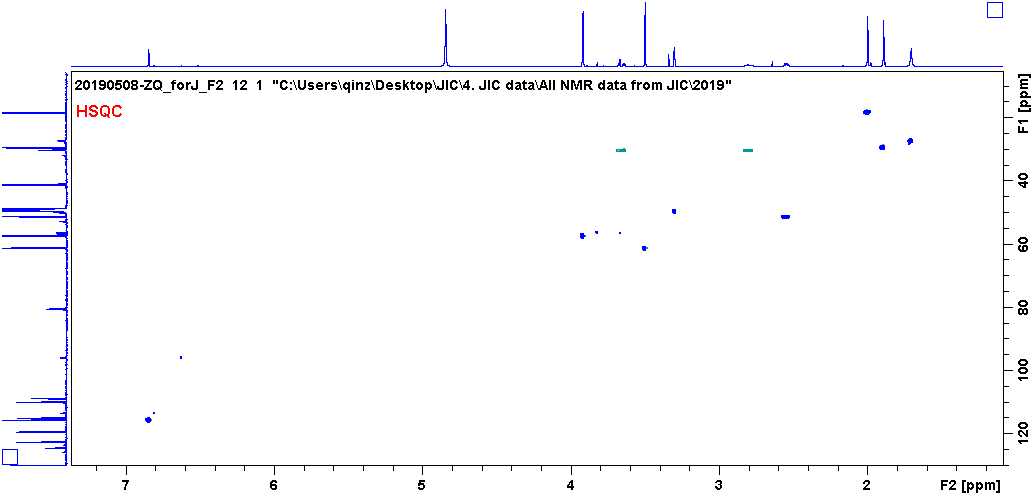
**1H NMR Spectra**



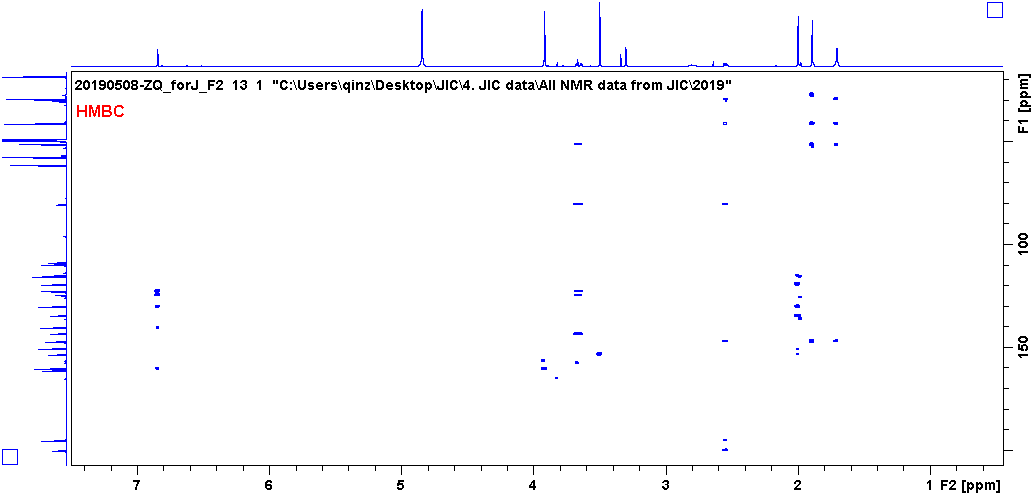
**Figure 1.** 1H NMR spectrum for formicamycin R. CD3OD, 600 MHz.



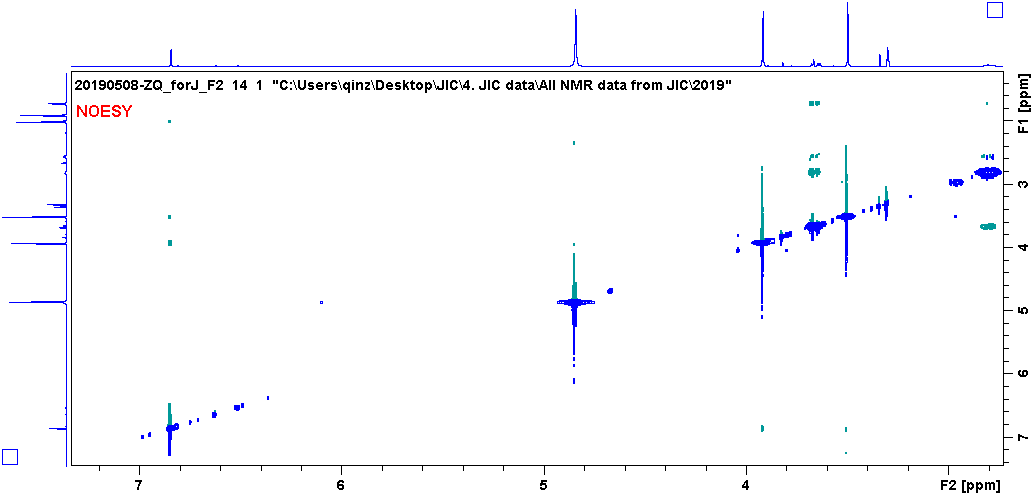
**Figure 2**. 13C NMR spectrum for formicamycin R. CD3OD, 150 MHz.



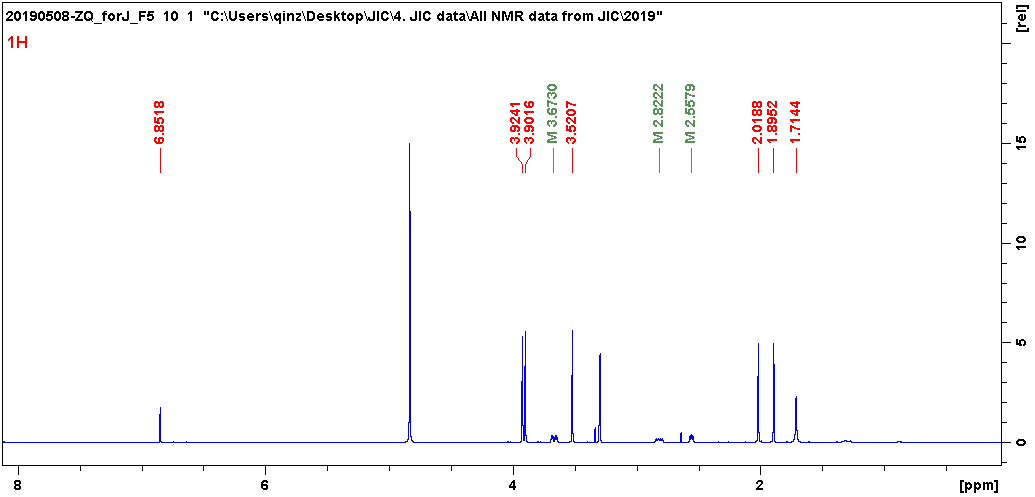
**Figure 3**. HSQC spectrum for formicamycin R. CD3OD.



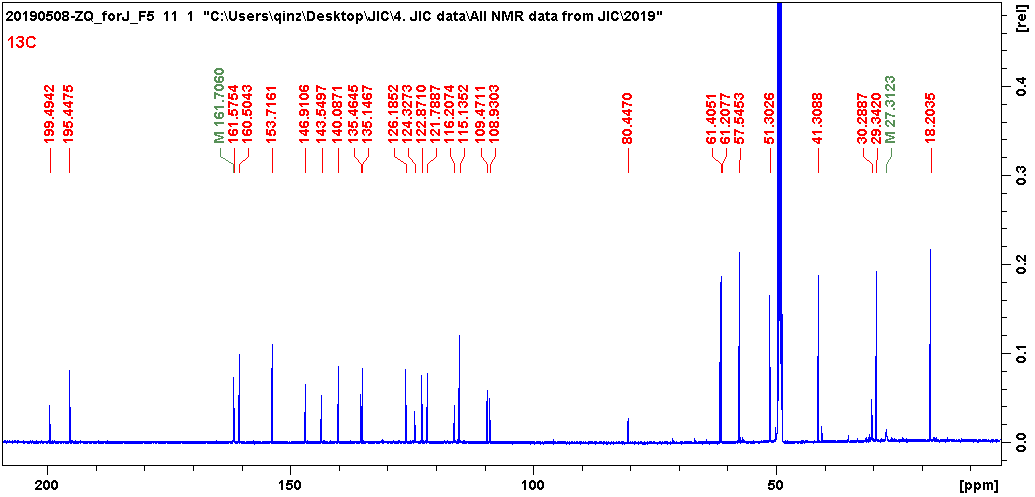
**Figure 4**. HMBC spectrum for formicamycin R. CD3OD.



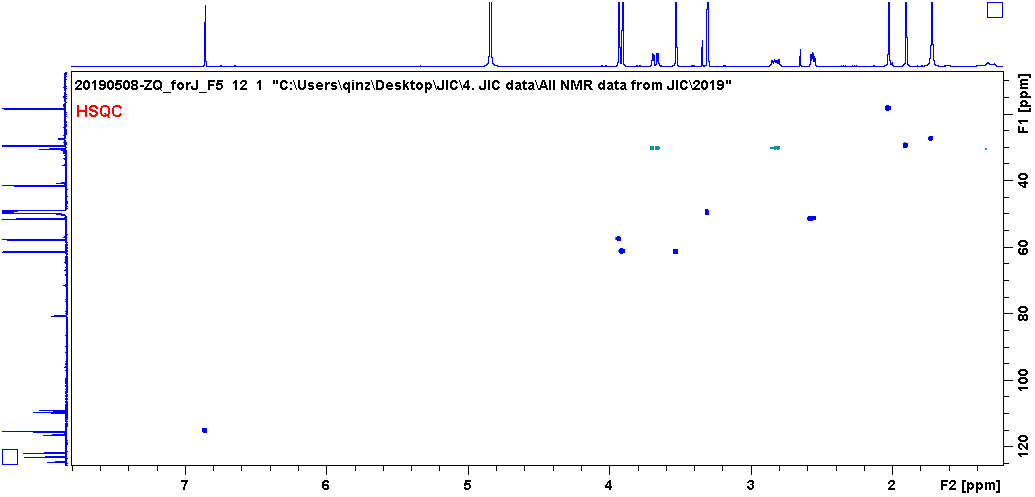
**Figure 5**. NOESY spectrum for formicamycin R. CD3OD.



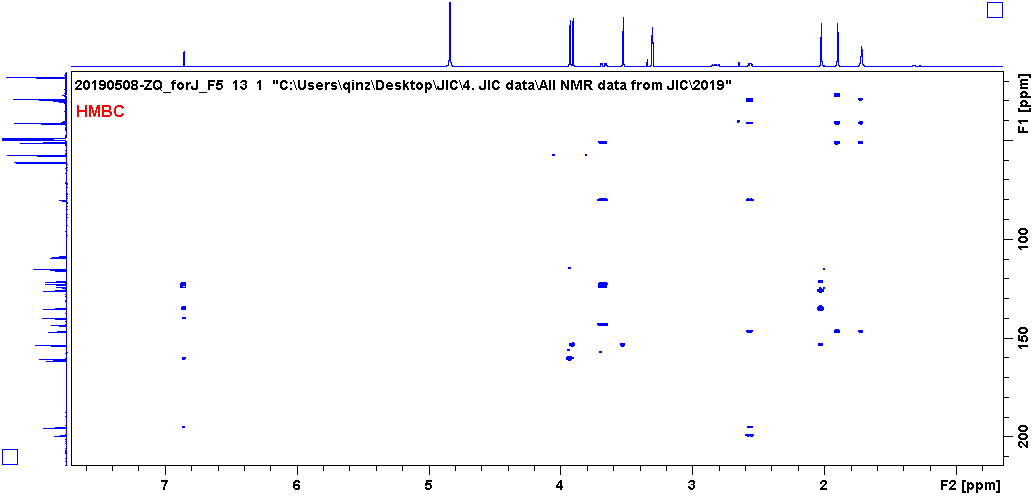
**Figure 6**. 1H NMR spectrum for formicamycin S. CD3OD, 600 MHz.



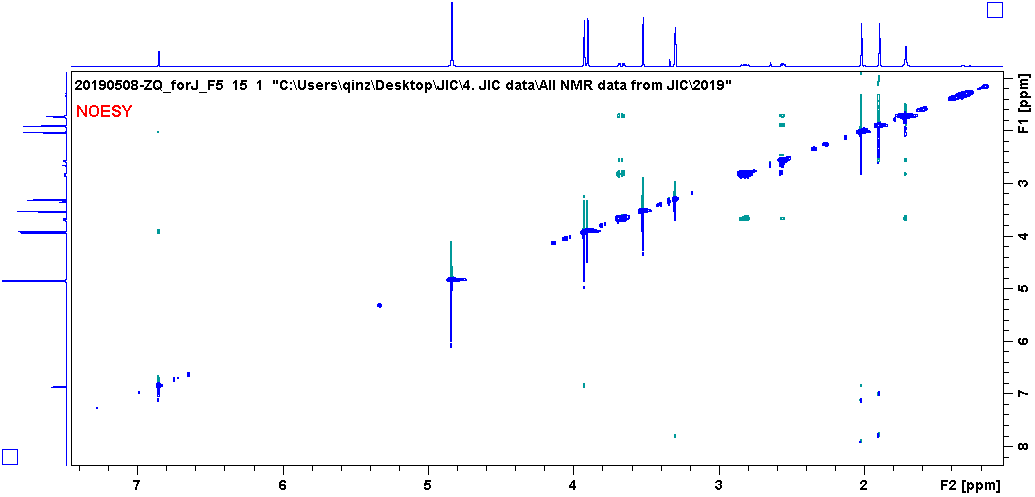
**Figure 7**. 13C NMR spectrum for formicamycin S. CD3OD, 150 MHz.



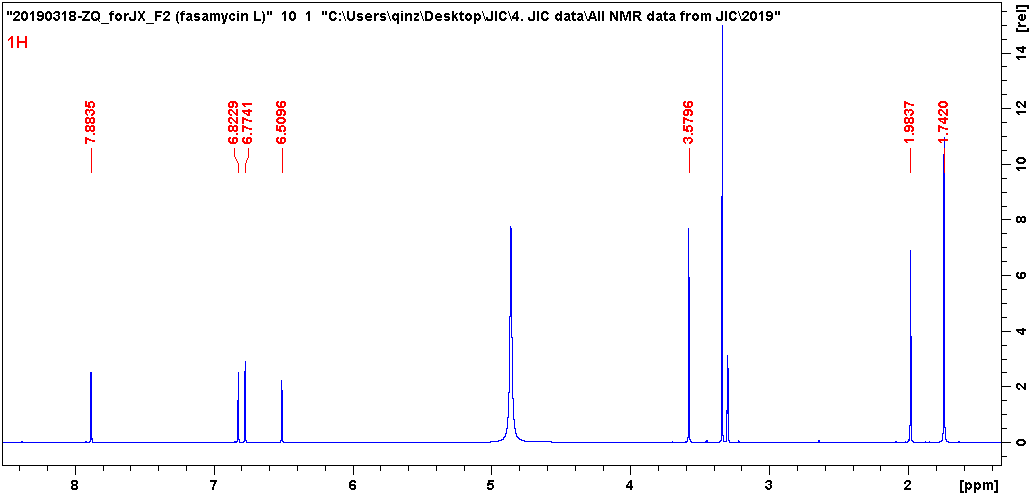
**Figure 8**. HSQC spectrum for formicamycin S. CD3OD.



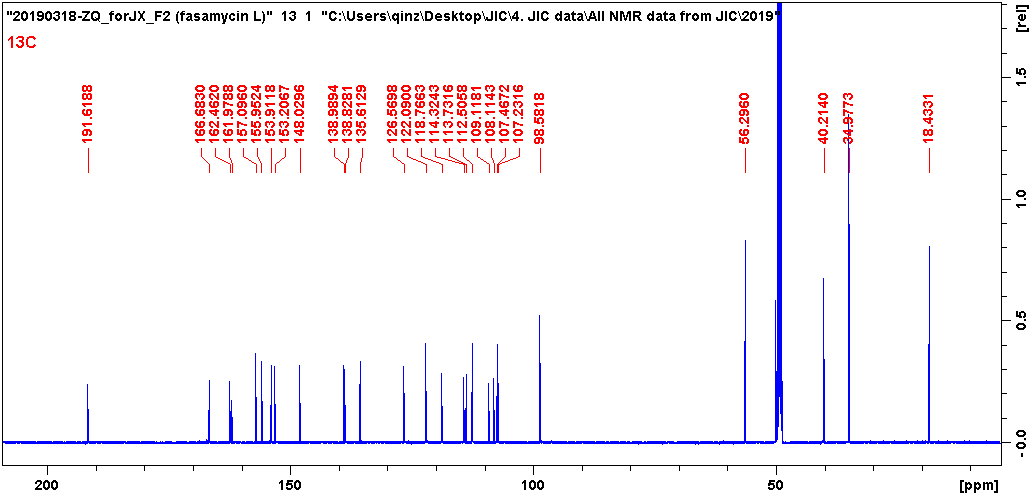
**Figure 9**. HMBC spectrum for formicamycin S. CD3OD.



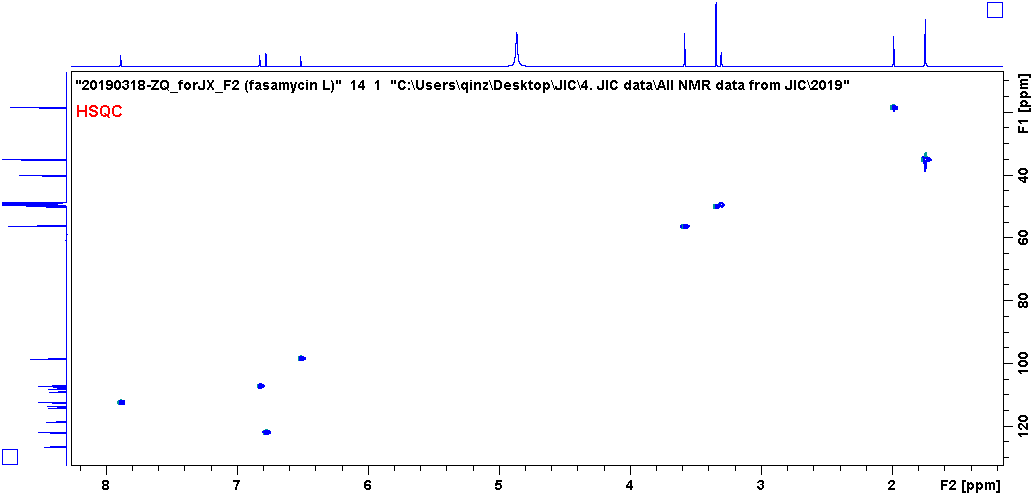
**Figure 10.** NOESY spectrum for formicamycin S. CD3OD.



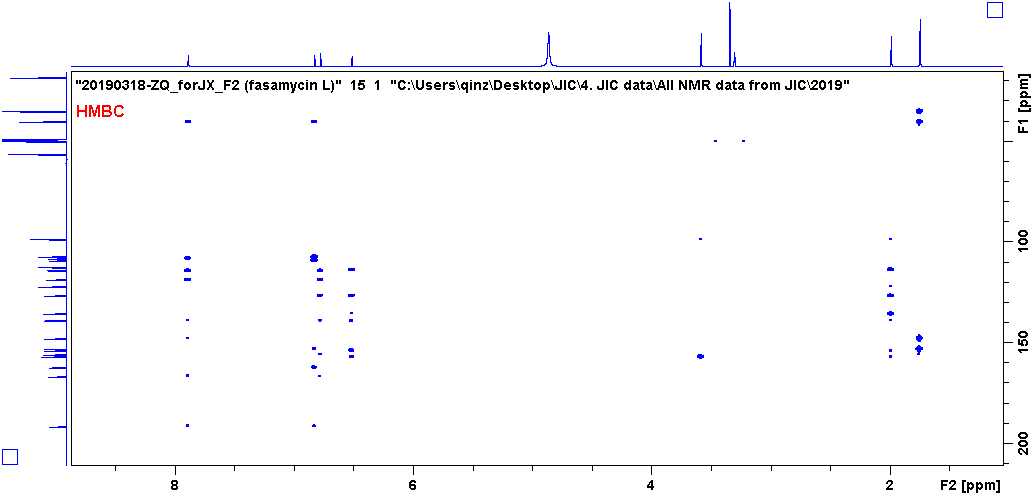
**Figure 11**. 1H NMR spectrum for fasamycin L. CD3OD, 600 MHz.



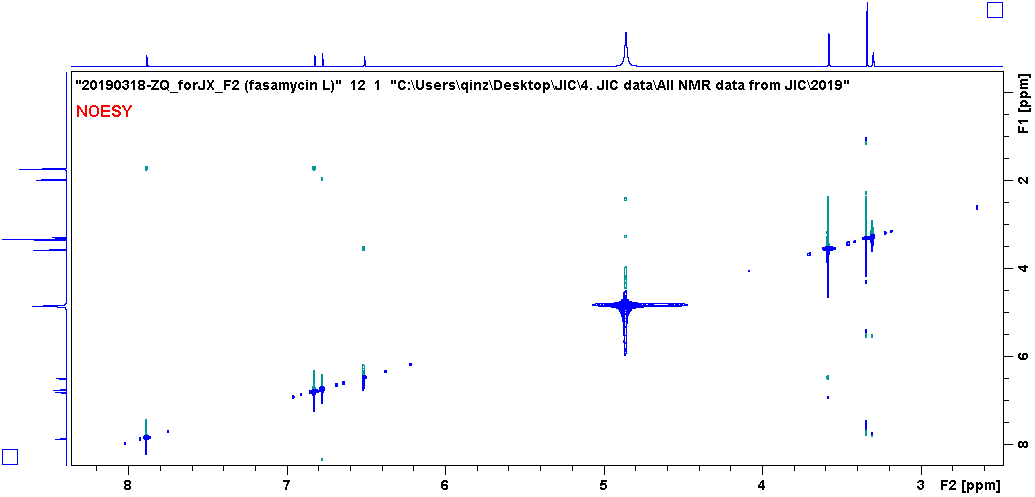
**Figure 12**. 13C NMR spectrum for fasamycin L. CD3OD, 150 MHz.



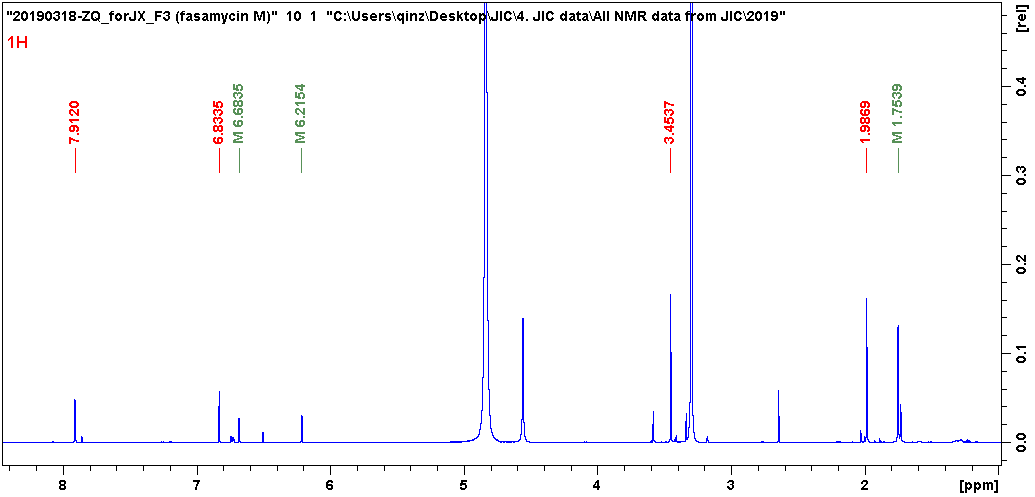
**Figure 13**. HSQC spectrum for fasamycin L. CD3OD.



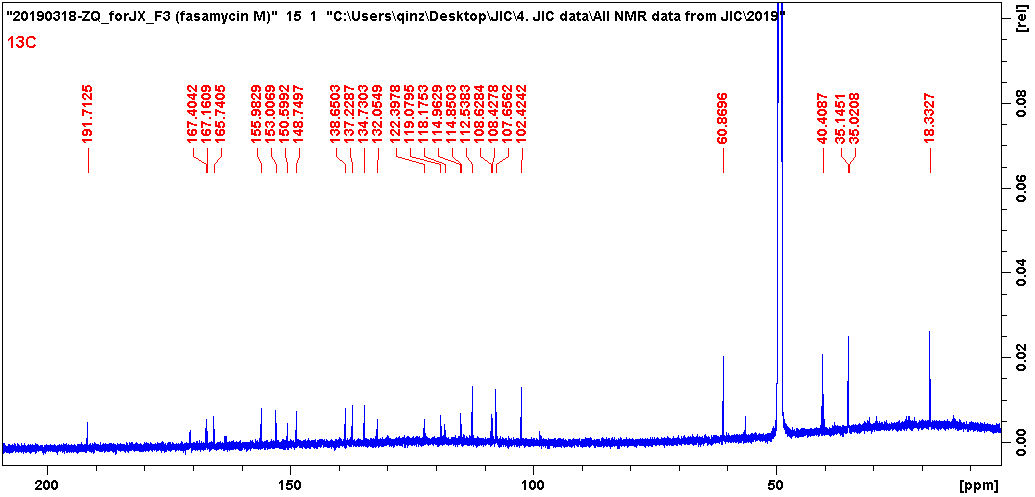
**Figure 14**. HMBC spectrum for fasamycin L. CD3OD.



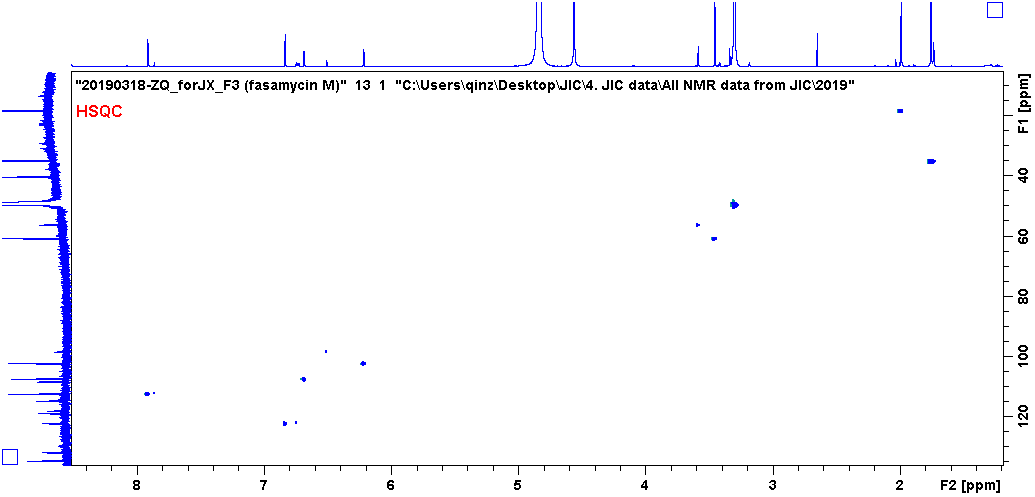
**Figure 15**. NOESY spectrum for fasamycin L. CD3OD.



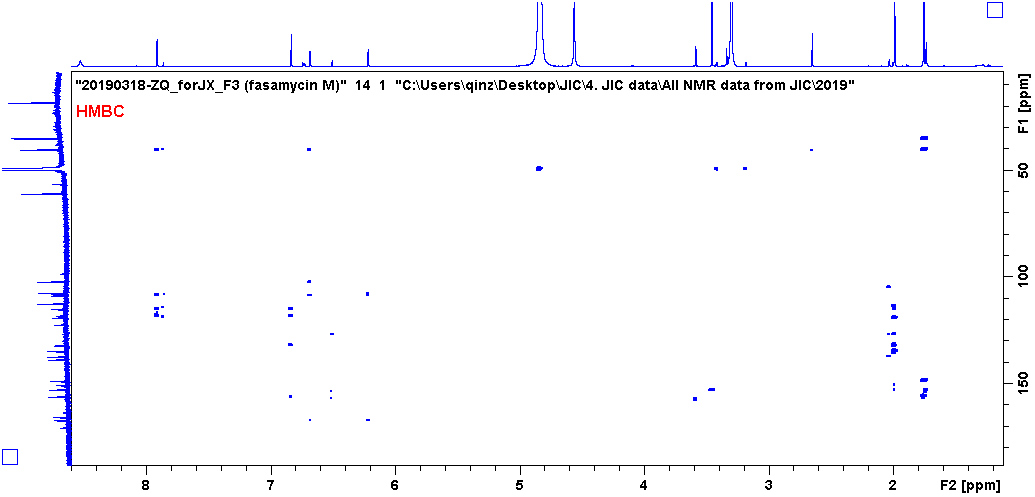
**Figure 16**. 1H NMR spectrum for fasamycin M. CD3OD, 600 MHz.



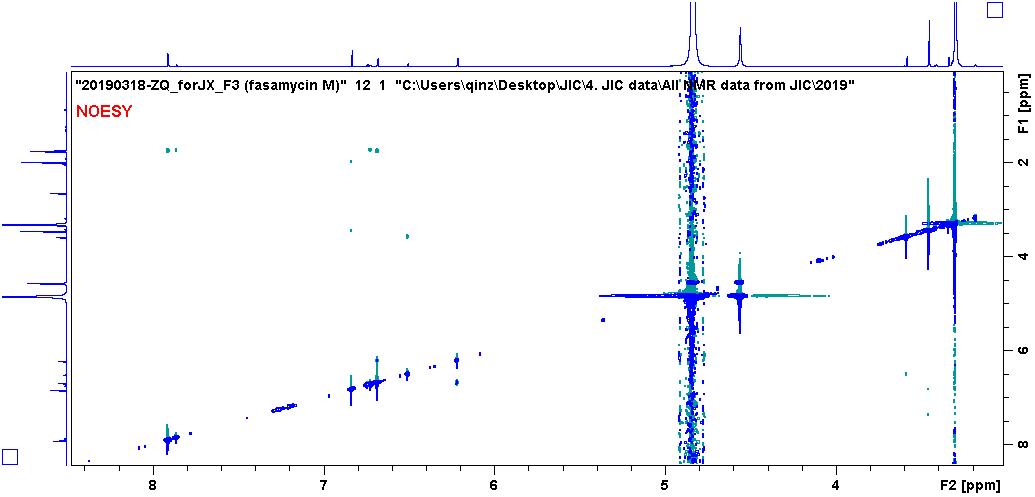
**Figure 17**. 13C NMR spectrum for fasamycin M. CD3OD, 150 MHz.



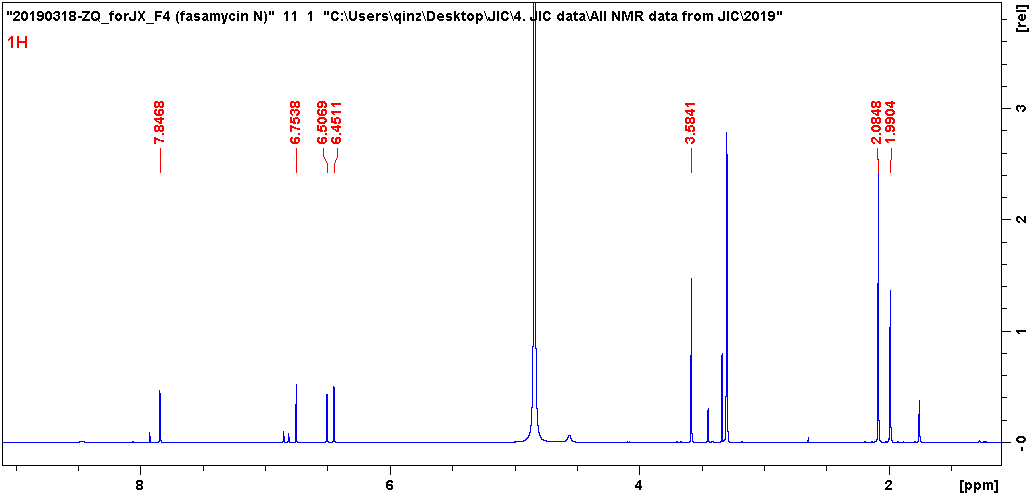
**Figure 18**. HSQC spectrum for fasamycin M. CD3OD.



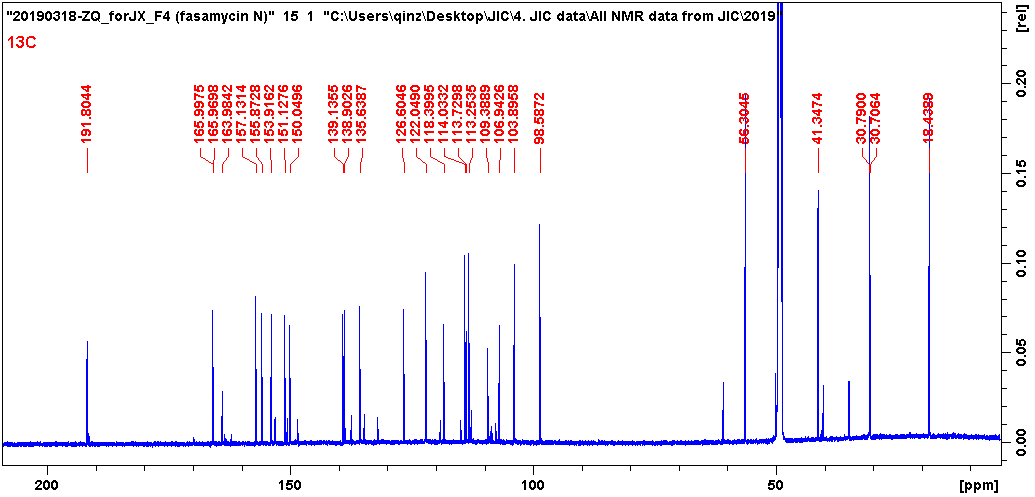
**Figure 19**. HMBC spectrum for fasamycin M. CD3OD.



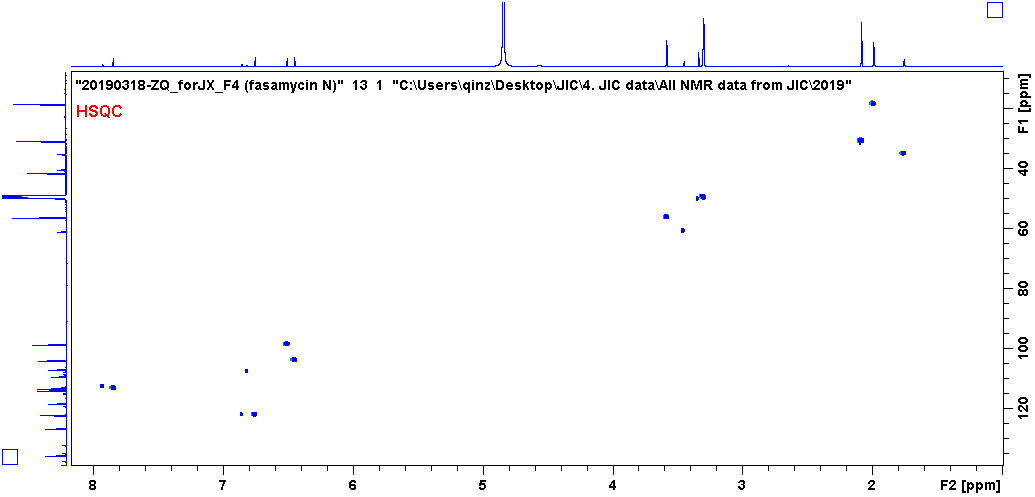
**Figure 20**. NOESY spectrum for fasamycin M. CD3OD.



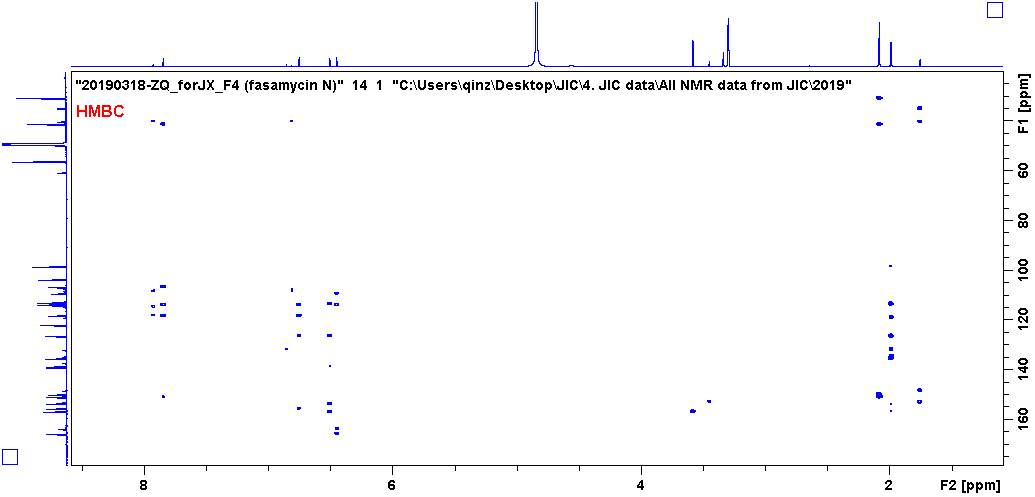
**Figure 21**. 1H NMR spectrum for fasamycin N. CD3OD, 600 MHz.



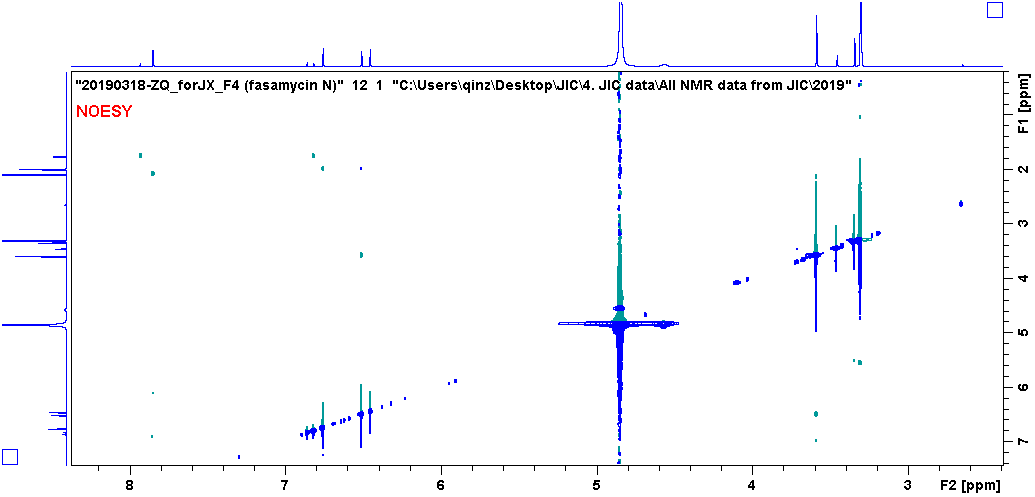
**Figure 22**. 13C NMR spectrum for fasamycin N. CD3OD, 150 MHz.



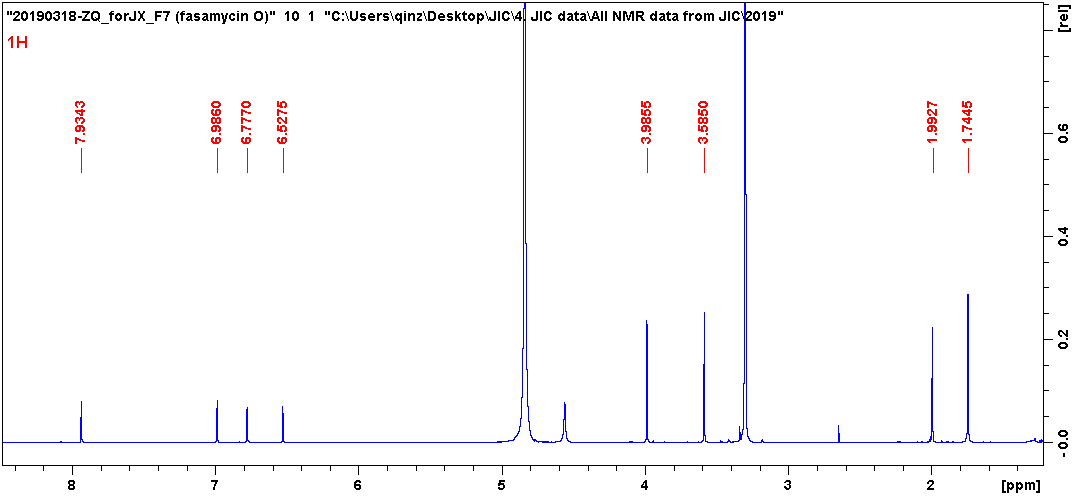
**Figure 23**. HSQC spectrum for fasamycin N. CD3OD.



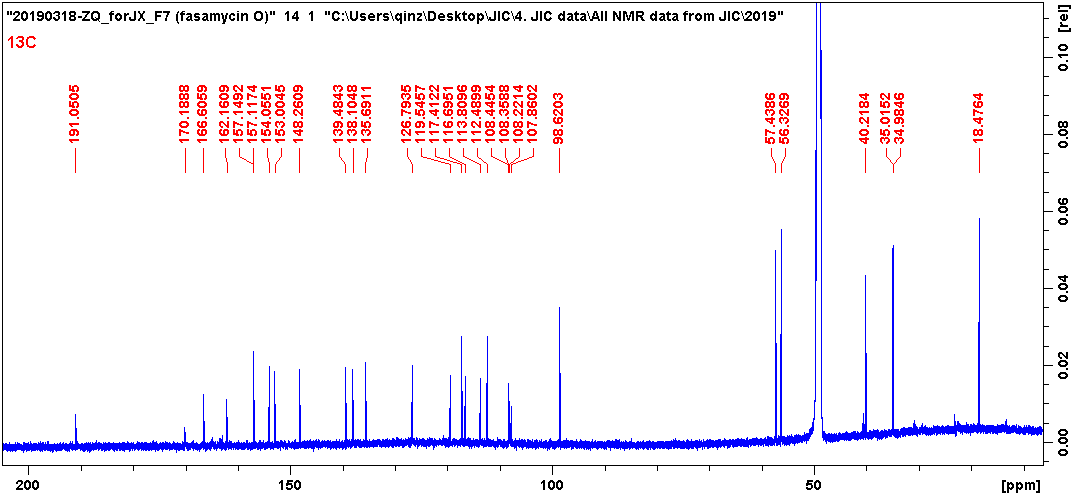
**Figure 24**. HMBC spectrum for fasamycin N. CD3OD.



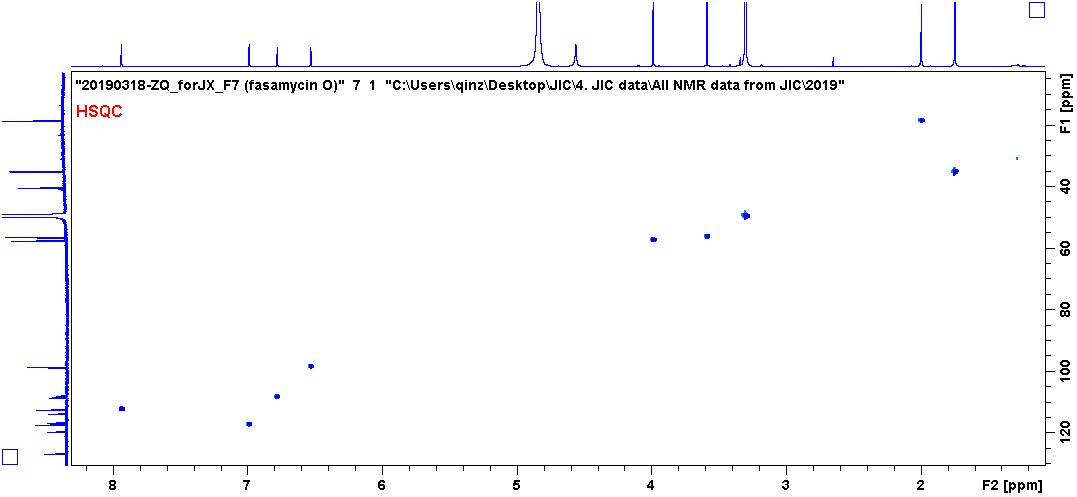
**Figure 25**. NOESY spectrum for fasamycin N. CD3OD.



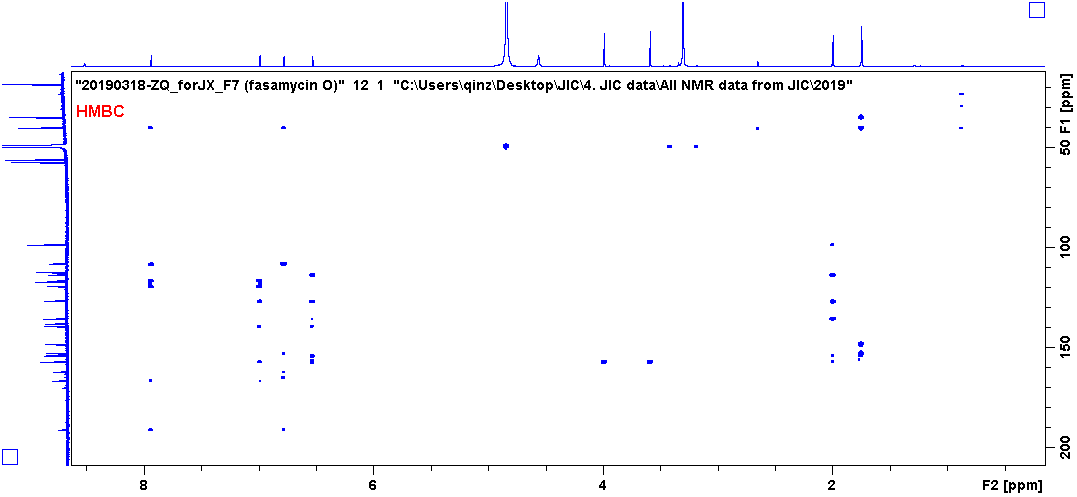
**Figure 26**. 1H NMR spectrum for fasamycin O. CD3OD, 600 MHz.



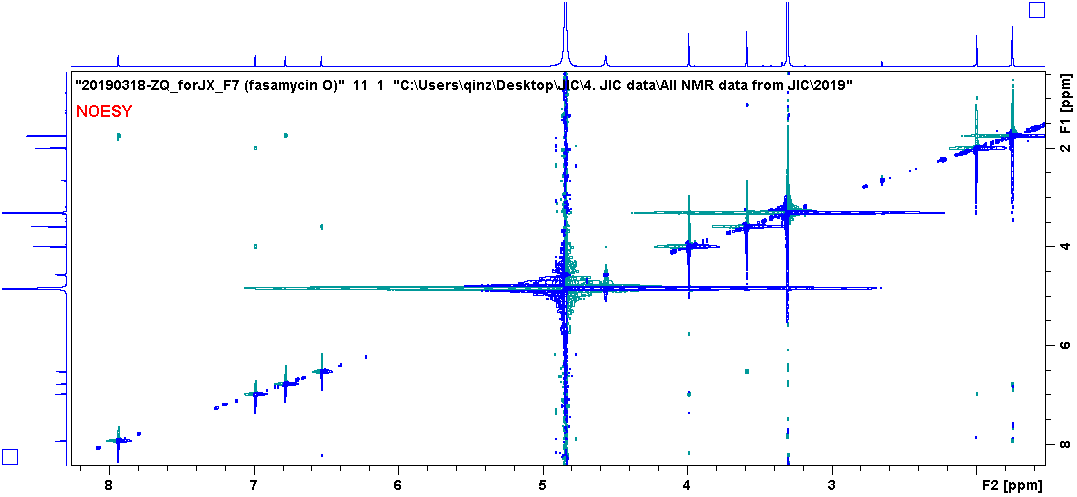
**Figure 27**. 13C NMR spectrum for fasamycin O. CD3OD, 150 MHz.



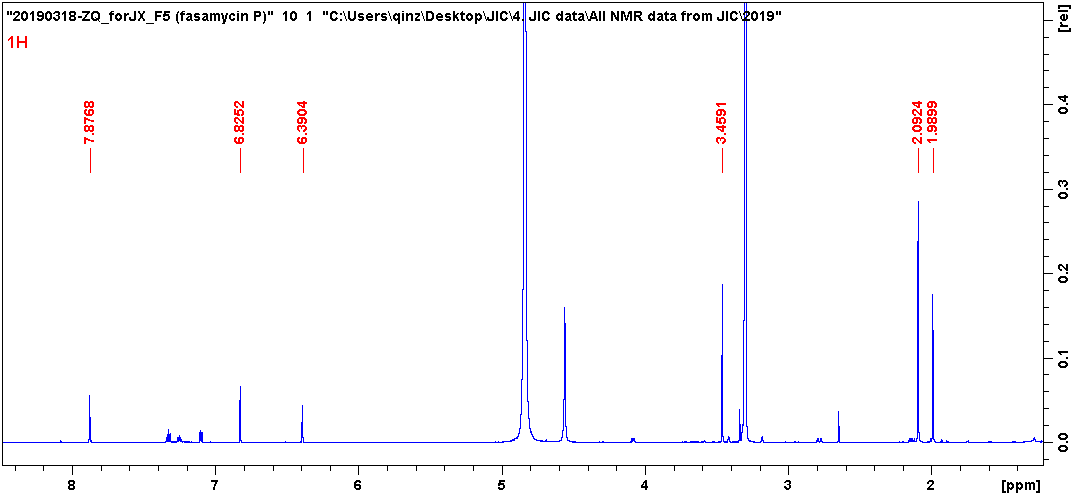
**Figure 28**. HSQC spectrum for fasamycin O. CD3OD.



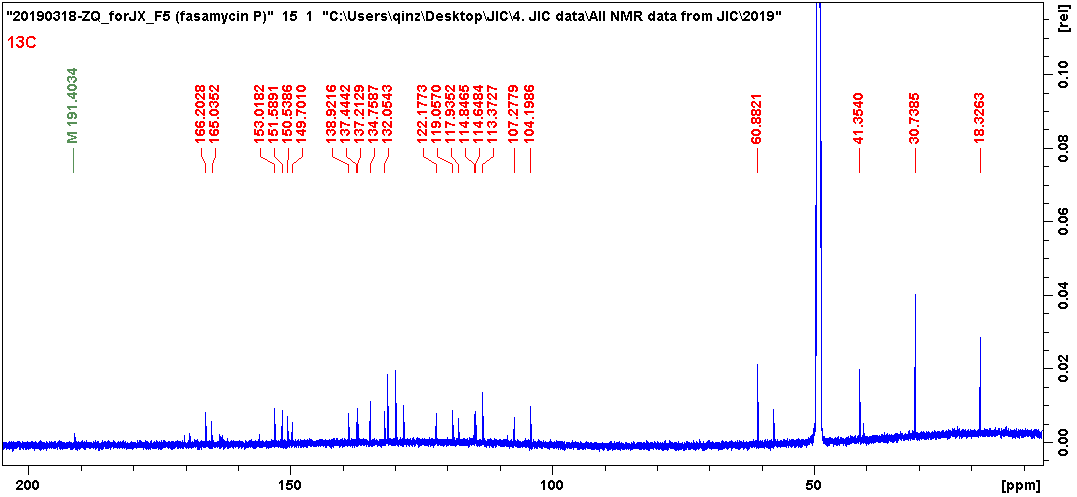
**Figure 29**. HMBC spectrum for compound **6**. CD3OD.



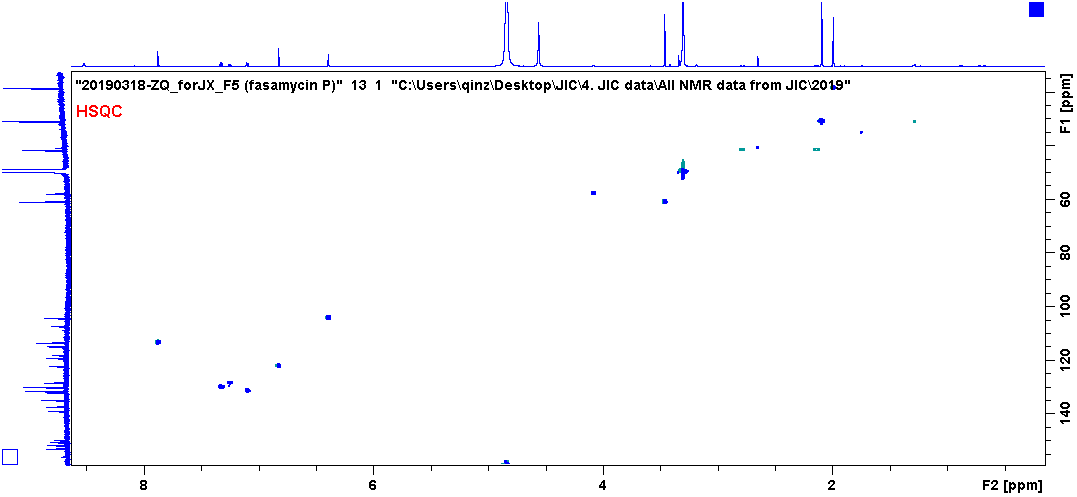
**Figure 30**. NOESY spectrum for fasamycin O. CD3OD.



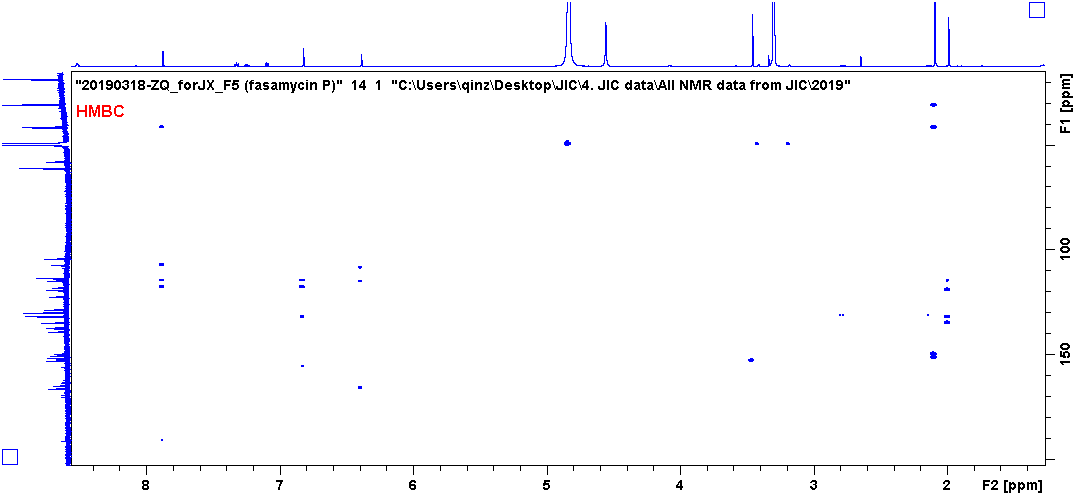
**Figure 31**. 1H NMR spectrum for fasamycin P. CD3OD, 600 MHz.



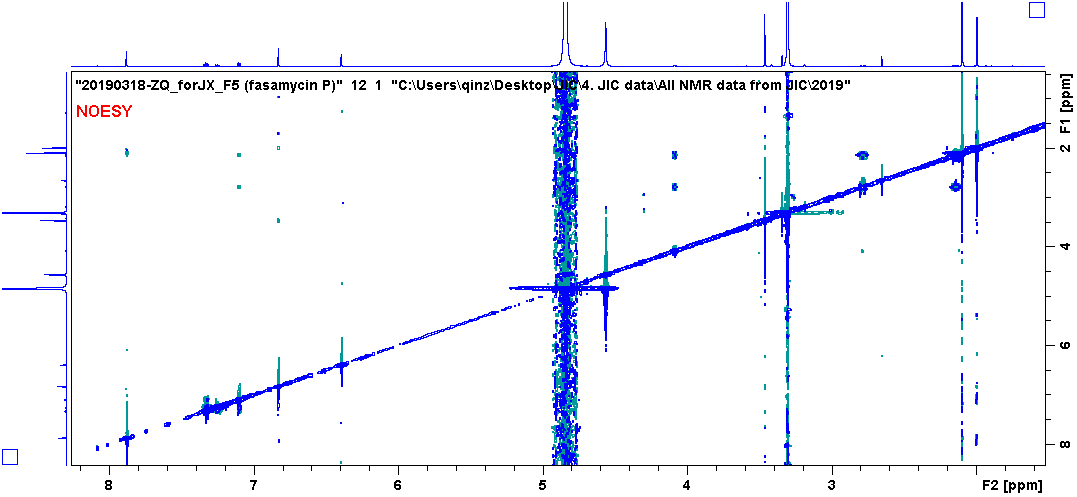
**Figure 32**. 13C NMR spectrum for fasamycin P. CD3OD, 150 MHz.



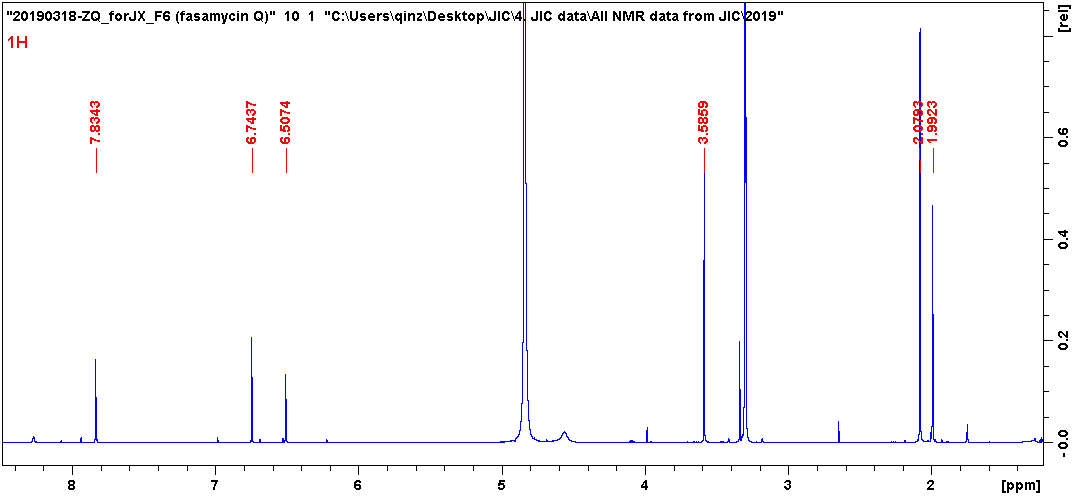
**Figure 33**. HSQC spectrum for fasamycin P. CD3OD.



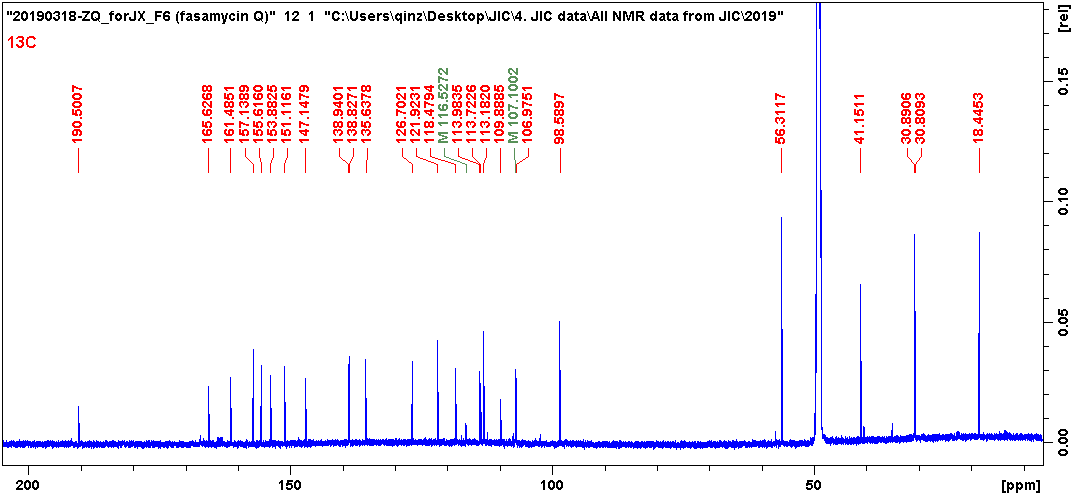
**Figure 34**. HMBC spectrum for fasamycin P. CD3OD.



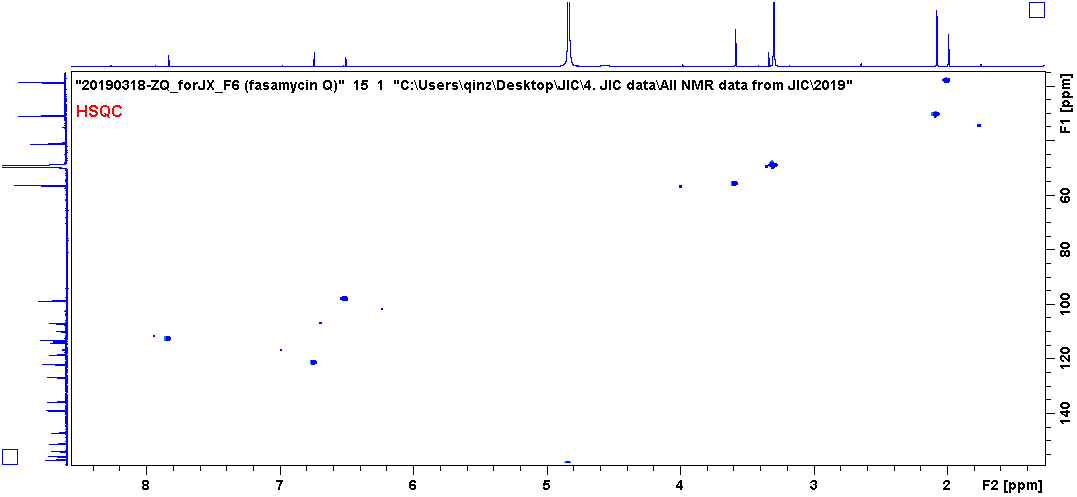
**Figure 35**. NOESY spectrum for fasamycin P. CD3OD.



**Figure 36**. 1H NMR spectrum for fasamycin Q. CD3OD, 600 MHz.



**Figure 37**. 13C NMR spectrum for fasamycin Q. CD3OD, 150 MHz.



**Figure 38**. HSQC spectrum for fasamycin Q. CD3OD.

A screenshot of a cell phone

Description automatically generated

**Figure 39**. HMBC spectrum for fasamycin Q. CD3OD.

A close up of a map

Description automatically generated

**Figure 40**. NOESY spectrum for fasamycin Q. CD3OD.