# *Supporting Information*

## Rifamorpholines A-E, Potential Antibiotics from a Locust-Associated *Amycolatopsis* sp. Hca4

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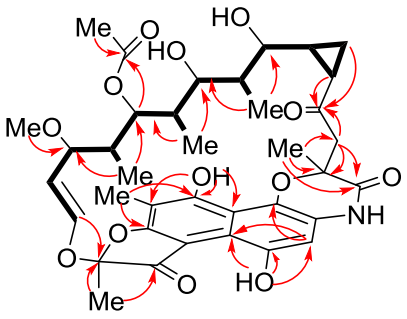
**Table S1.** Annotation of the Rifamorpholines biosynthetic gene cluster

| Name | No. of amino acids | Name of Rif | Proposed function | identity /similarity | accession no. |
|---|---|---|---|---|---|
| Orf-2 | 486 | | hypothetical protein | 76/83 | SEB34960 |
| Orf-1 | 74 | | hypothetical protein | 65/70 | EOD68504 |
| RMPC | 1303 | RpoC | DNA-dependent RNA polymerase β'-subunit | 99/99 | AAS07761 |
| RMPB | 1167 | RpoB | DNA-dependent RNA polymerase β -subunit | 99/99 | AAS07760 |
| RMP36 | 419 | Orf36 | regulatory protein | 94/95 | AAS07758 |
| RMPJ | 151 | RifJ | aminodehydroquinate dehydratase | 95/97 | AAS07762 |
| RMP16 | 420 | Orf16c | cytochrome P450 monooxygenase | 94/96 | AAC01740 |
| RMP15a | 303 | Orf15B | putative transketolase B subunit | 93/96 | AAC01739 |
| RMP15b | 230 | Orf15A | putative transketolase A subunit | 95/97 | AAS07757 |
| RMP14 | 272 | Orf14 | C-27 O-methyltransferase | 96/97 | AAC01738 |
| RMP13 | 424 | Orf13c | putative cytochrome P450 monooxygenase | 97/97 | AAC01737 |
| RMPR | 256 | RifR | thioesterase | 91/94 | AAG52991 |
| RMP20 | 403 | Orf20c | 25-O-acetyltransferase | 86/92 | AAG52990 |
| RMP19 | 500 | Orf19c | 3-(3-hydroxylphenyl) propionate hydroxylase | 91/95 | AAG52989 |
| RMP18 | 473 | Orf18 | putative 2,3-dehydratase | 96/98 | AAG52988 |
| RMP17 | 356 | Orf17 | putative alpha-chain alkanal monooxygenase | 91/96 | AAG52987 |
| RMP11 | 294 | Orf11 | putative flavin-dependent oxidoreductase | 89/94 | AAC01735 |
| RMP10 | 330 | Orf10c | putative dNTP-hexose 3-ketoreductase | 88/93 | AAC01734 |
| RMP9 | 290 | Orf9c | dNTP-hexose aminotransferase | 93/97 | AAC01733 |
| RMP8 | 215 | Orf8 | putative dNTP-hexose 3,5-epimerase | 93/96 | AAC01732 |
| RMP7 | 403 | Orf7 | putative dNTP-hexose glycosyltransferase | 95/97 | AAC01731 |
| RMP6 | 435 | Orf6c | putative dNTP-hexose dehydratase | 98/99 | AAC01730 |
| RMP5 | 421 | Orf5c | putative cytochrome P450 monooxygenase | 97/98 | AAC01729 |
| RMP4 | 403 | Orf4c | putative cytochrome P450 oxidoreductase | 95/98 | AAC01728 |
| RMP3 | 165 | Orf3c | Pyridoxamine 5'-phosphate oxidase | 93/97 | SEB30227 |
| RMPQ | 241 | RifQ | putative tetR-like transcription regulatory protein | 95/97 | AAC01726 |
| RMPP | 523 | RifP | efflux transporter protein | 87/92 | AAC01725 |
| RMP2 | 310 | Orf2 | putative esterase | 89/91 | AAC01724 |
| RMPO | 254 | RifO | putative regulatory protein | 93/96 | AAC01723 |
| RMPN | 300 | RifN | kanosamine kinase | 90/93 | AAC01722 |
| RMPM | 232 | RifM | phosphatase | 96/96 | AAC01721 |
| RMPL | 356 | RifL | oxidoreductase | 94/97 | AAS07754 |
| RMPK | 388 | RifK | AHBA synthase | 96/98 | AAC01720 |

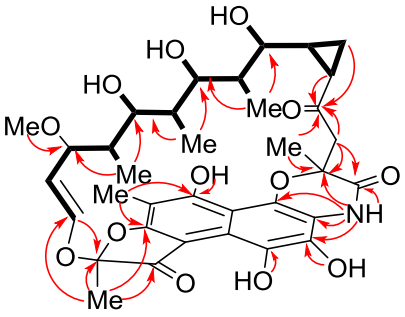
| | | | | | |
|---|---|---|---|---|---|
| RMPL | 263 | RifL | aminoquinate dehydrogenase | 87/92 | AAC01719 |
| RMPH | 443 | RifH | aminoDAHP sythase | 89/91 | AAC01718 |
| RMPG | 351 | RifG | aminodehydroquinate synthase | 92/93 | AAC01717 |
| RMP1 | 70 | Orf1 | hypothetical protein | 77/91 | AAC01716 |
| RMPF | 258 | RifF | amide synthase (*N*-acyl transferase) | 93/97 | AAC01715 |
| RMPE2 | 2363 | RifE | rifamycin polyketide synthase protein | 88/91 | AAC01714 |
| RMPE1 | 1033 | RifE | rifamycin polyketide synthase protein | 91/94 | AAC01714 |
| RMPD2 | 1163 | RifD | rifamycin polyketide synthase protein | 85/89 | AAC01713 |
| RMPD1 | 547 | RifD | rifamycin polyketide synthase protein | 96/98 | AAC01713 |
| RMPC | 1746 | RifC | rifamycin polyketide synthase protein | 90/93 | AAC01712 |
| RMPB | 5023 | RifB | rifamycin polyketide synthase protein | 91/94 | AAC01711 |
| RMPA2 | 1571 | RifA | rifamycin polyketide synthase protein | 91/94 | AAC01710 |
| RMPA1 | 3132 | RifA | rifamycin polyketide synthase protein | 89/93 | AAC01710 |
| RMP0 | 396 | Orf0 | cytochrome-P450-like protein | 96/97 | AAC01709 |
| RMP35 | 71 | Orf35 | hypothetical protein | 94/95 | AAS07753 |
| RMPT | 259 | RifT | putative NADH-dependent dehydrogenase | 84/90 | AAC01707 |
| RMPS | 330 | RifS | putative NADH-dependent dehydrogenase | 94/96 | AAS07752 |
| RMP31 | 328 | Orf31 | putative integral membrane protein | 83/86 | AAS07750 |
| RMP30 | 191 | Orf30 | putative membrane protein | 91/95 | AAS07749 |
| RMP29 | 421 | Orf29 | putative secreted protein | 96/98 | AAS07748 |
| RMP28 | 391 | Orf28 | putative secreted protein | 95/97 | AAS07747 |
| RMP27 | 394 | Orf27 | putative secreted protein | 96/98 | AAS07746 |
| RMP26 | 328 | Orf26 | putative lipoprotein | 97/98 | AAS07745 |
| RMP25 | 342 | Orf25 | putative lipoprotein | 97/98 | AAS07744 |
| RMP24 | 441 | Orf24 | putative secreted protein | 96/99 | AAS07743 |
| RMP23 | 278 | Orf23 | putative ABC-transporter permease | 99/99 | AAS07742 |
| RMP22 | 250 | Orf22 | putative ABC-transporter integral membrane protein | 99/100 | AAS07741 |
| RMP21 | 391 | Orf21 | putative ABC transporter ATP-binding protein | 98/98 | AAS07740 |
| RplI | 128 | RplI | ribosomal protein L7/L12 | 93/94 | AAS07739 |
| RplJ | 184 | RplJ | ribosomal protein L10 | 98/99 | AAS07738 |
| Orf+1 | 315 | | hypothetical protein | 83/86 | WP_013222528 |
| Orf+2 | 257 | | alpha/beta hydrolase | 95/97 | WP_003079255 |

a)



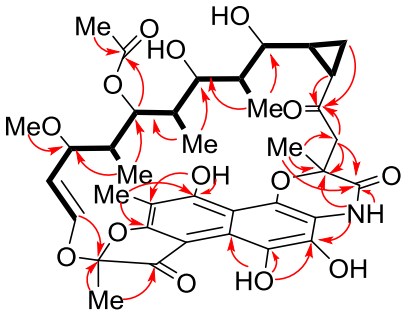

**3**

b)




**4**

c)

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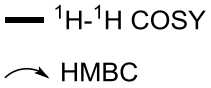


**Figure S1.** Key 2D NMR correlations of **3-5**.

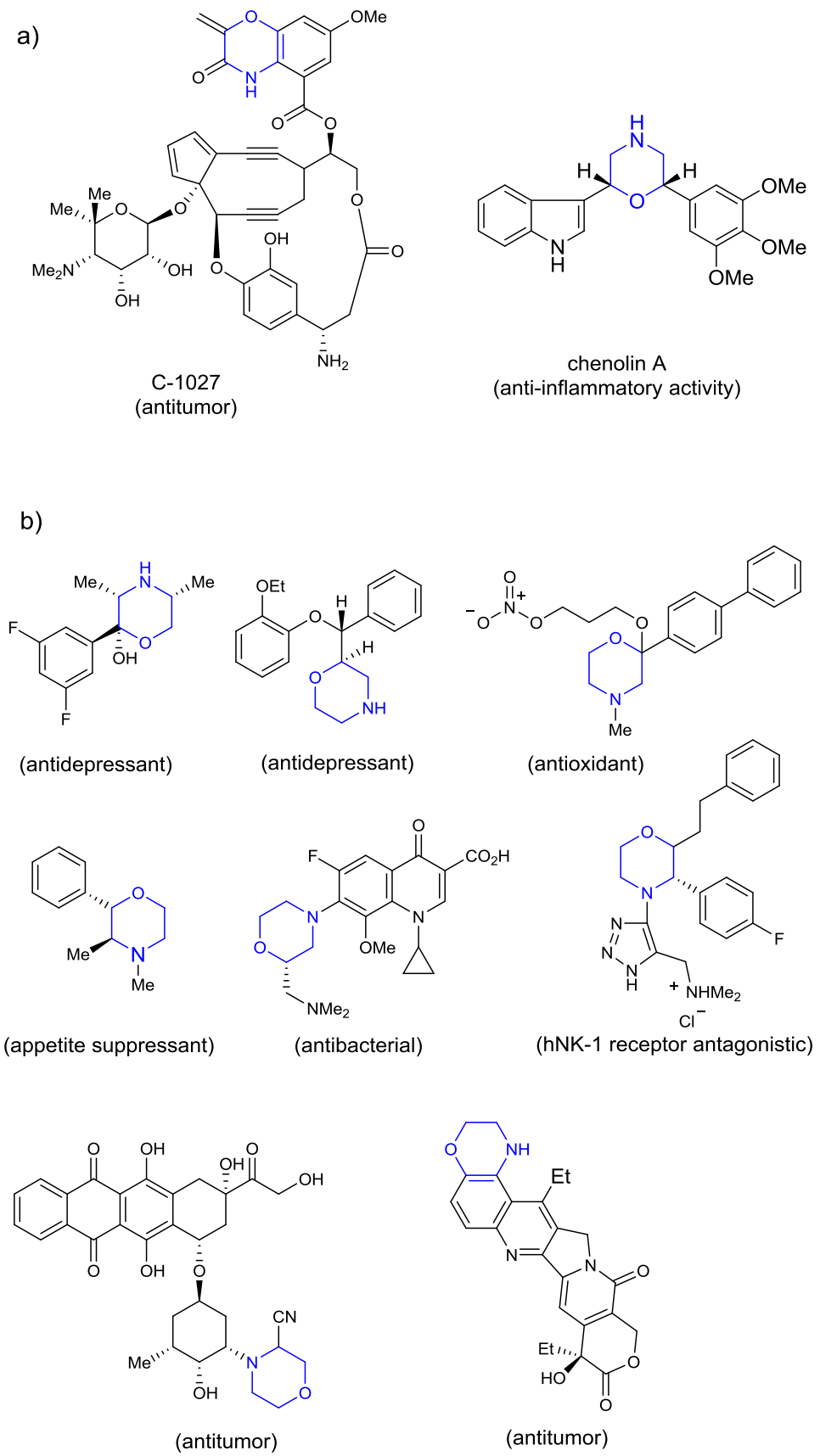


**Figure S2.** Morpholine-containing compounds (marked in blue). a) Natural products, b) synthetic or semi-synthetic compounds.[3]

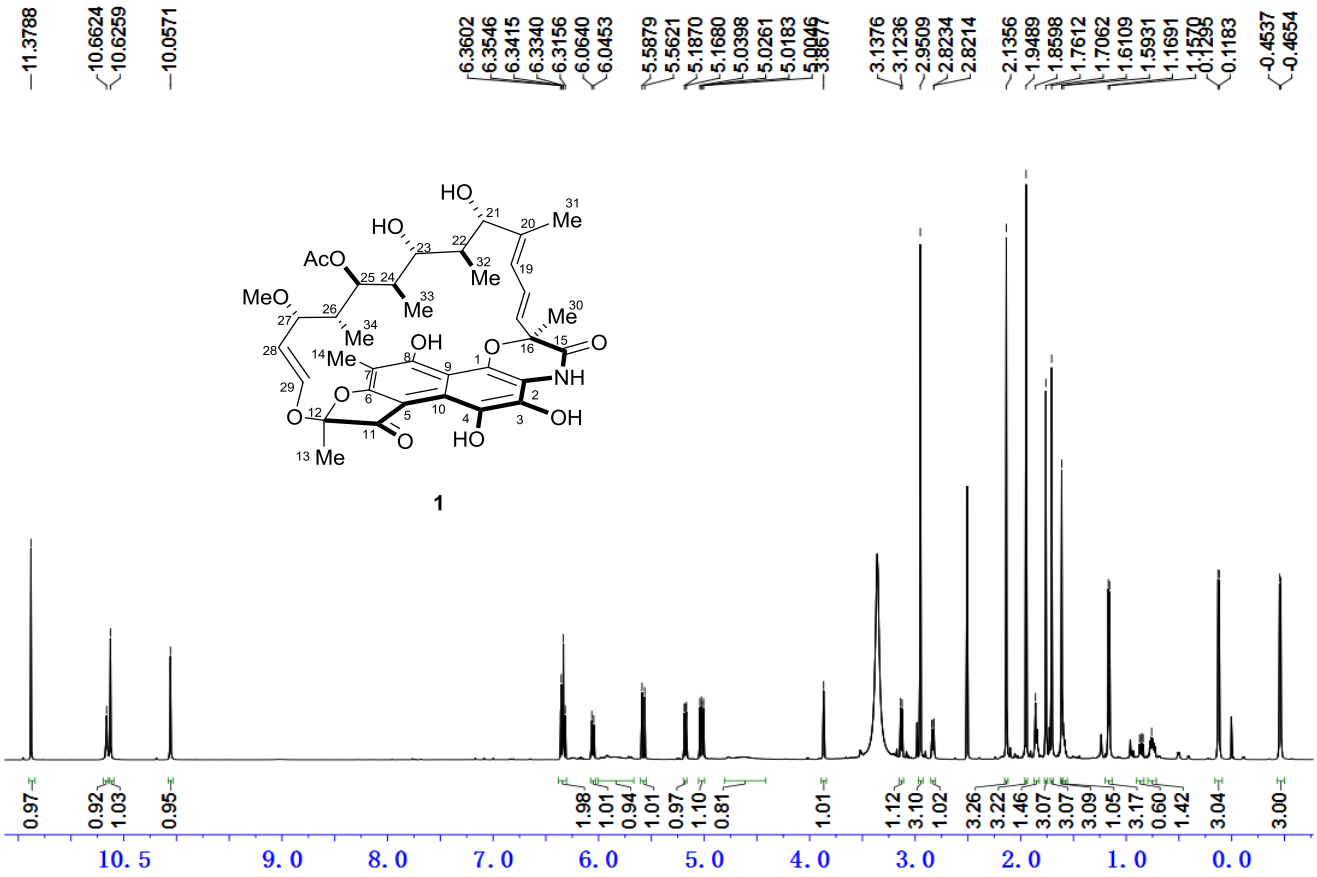


**Figure S3.** $^{1}$H NMR spectrum of **1** in DMSO-$d_6$ (600M Hz)

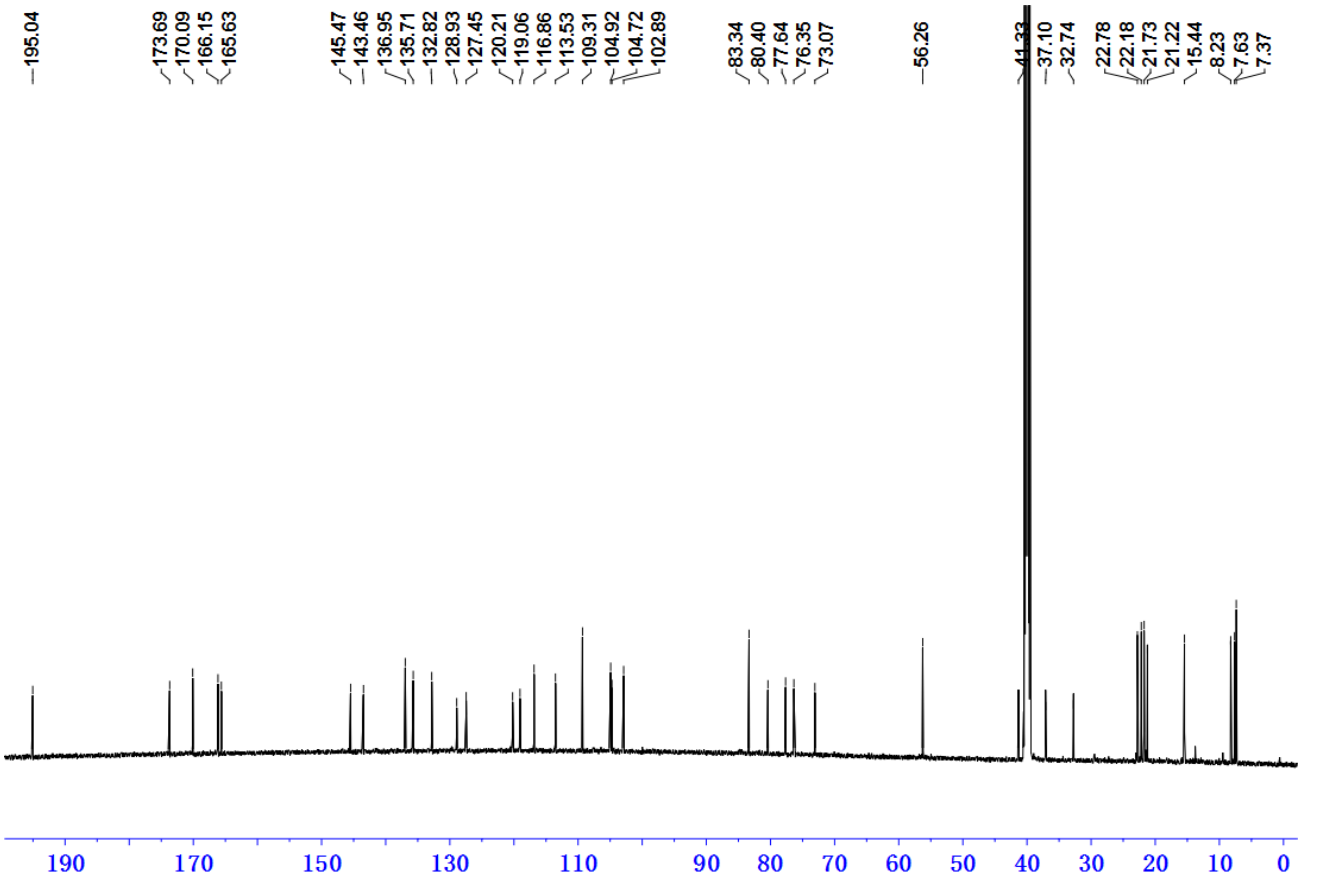


**Figure S4.** $^{13}$C NMR spectrum of **1** in DMSO-$d_6$ (150M Hz)

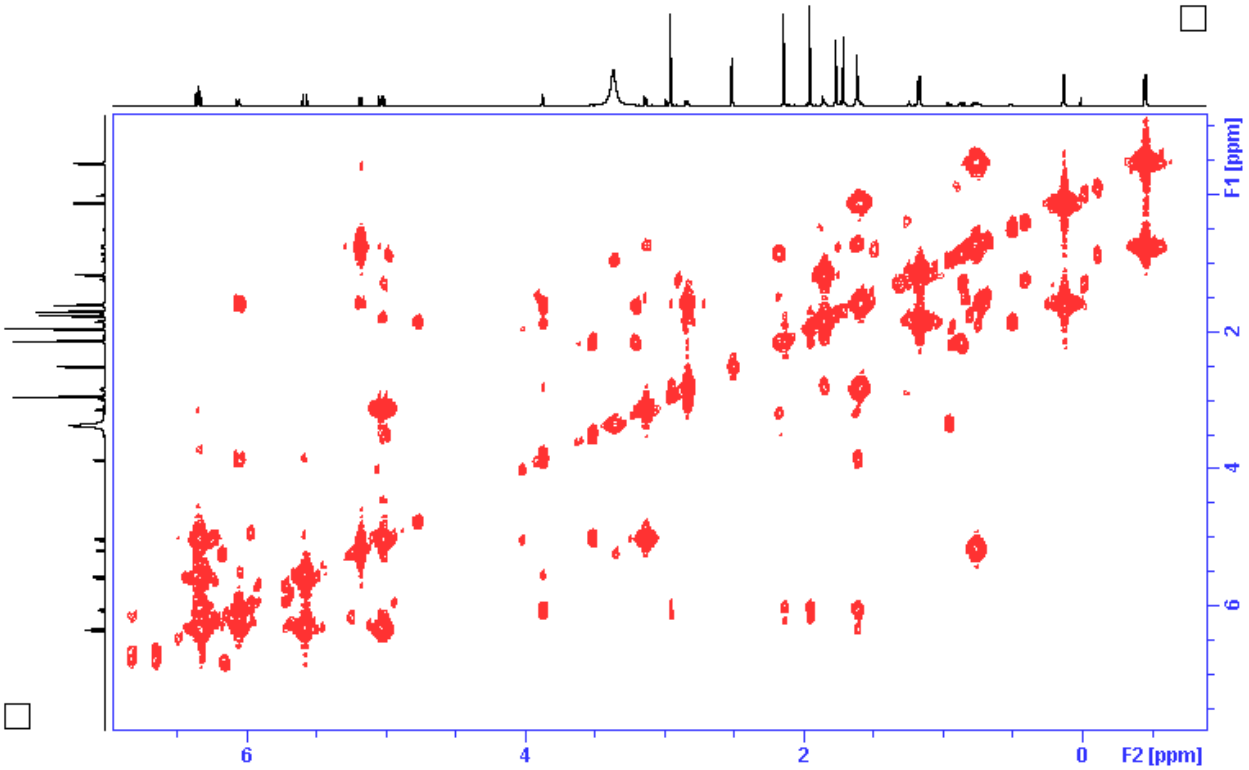


**Figure S5.** $^{1}$H -$^{1}$H COSY NMR spectrum of **1** in DMSO-$d_6$ (600M Hz)

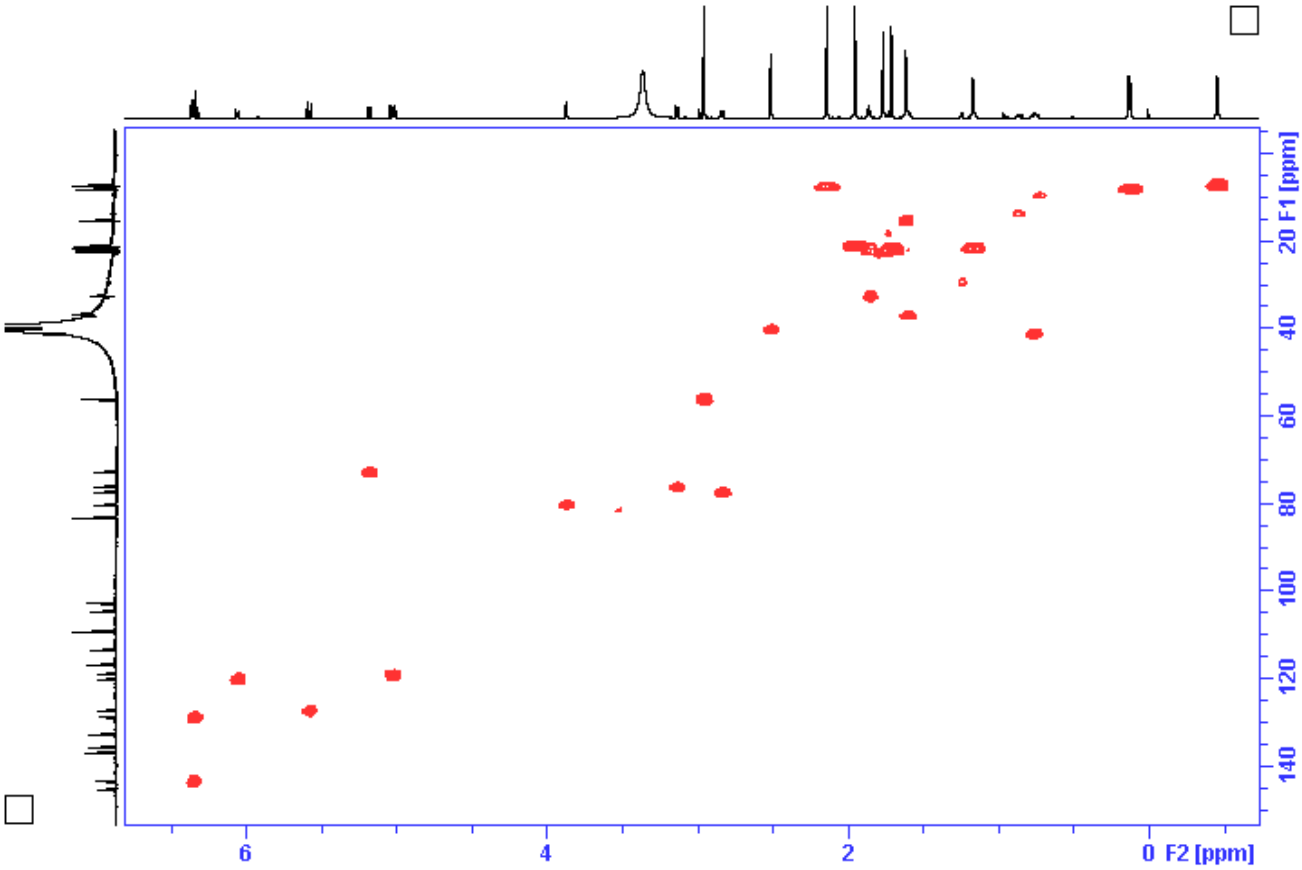


**Figure S6.** HSQC NMR spectrum of **1** in DMSO-$d_6$ (600M Hz)

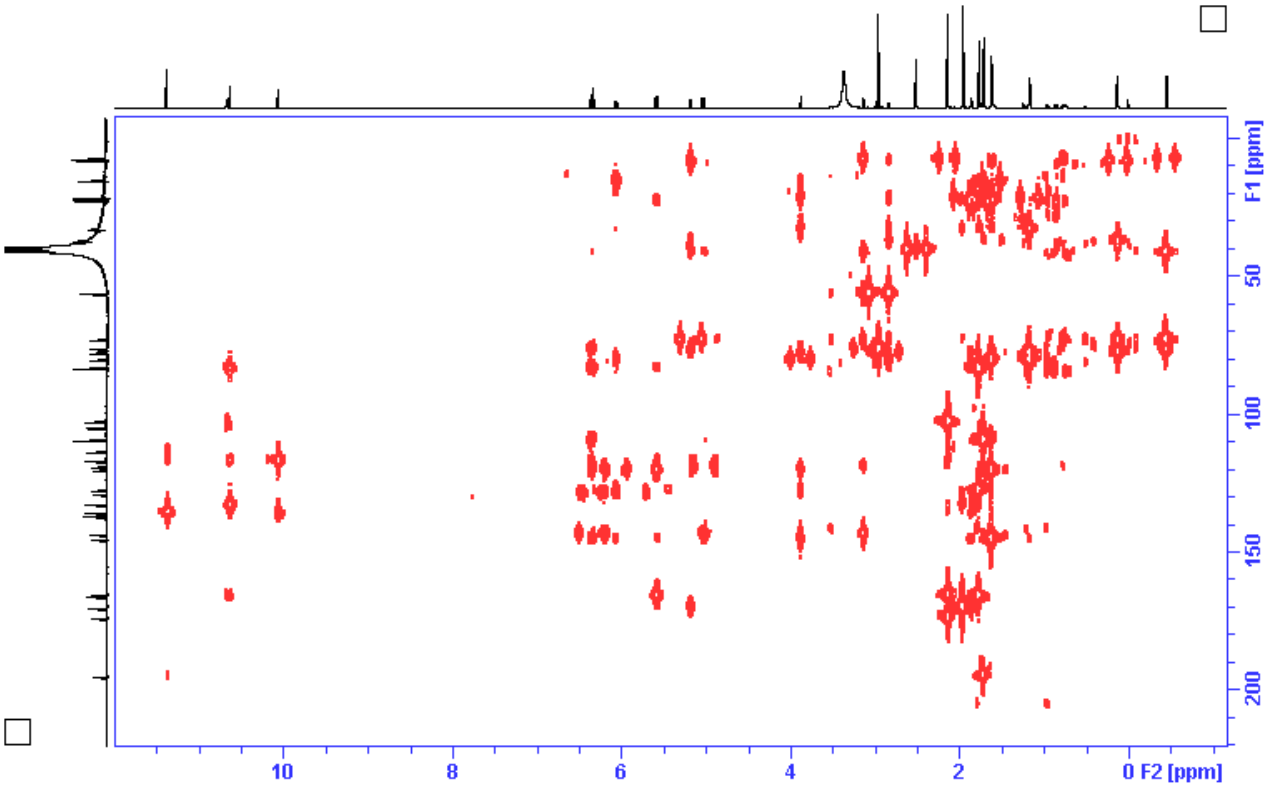


**Figure S7.** HMBC NMR spectrum of **1** in DMSO-$d_6$ (600M Hz)

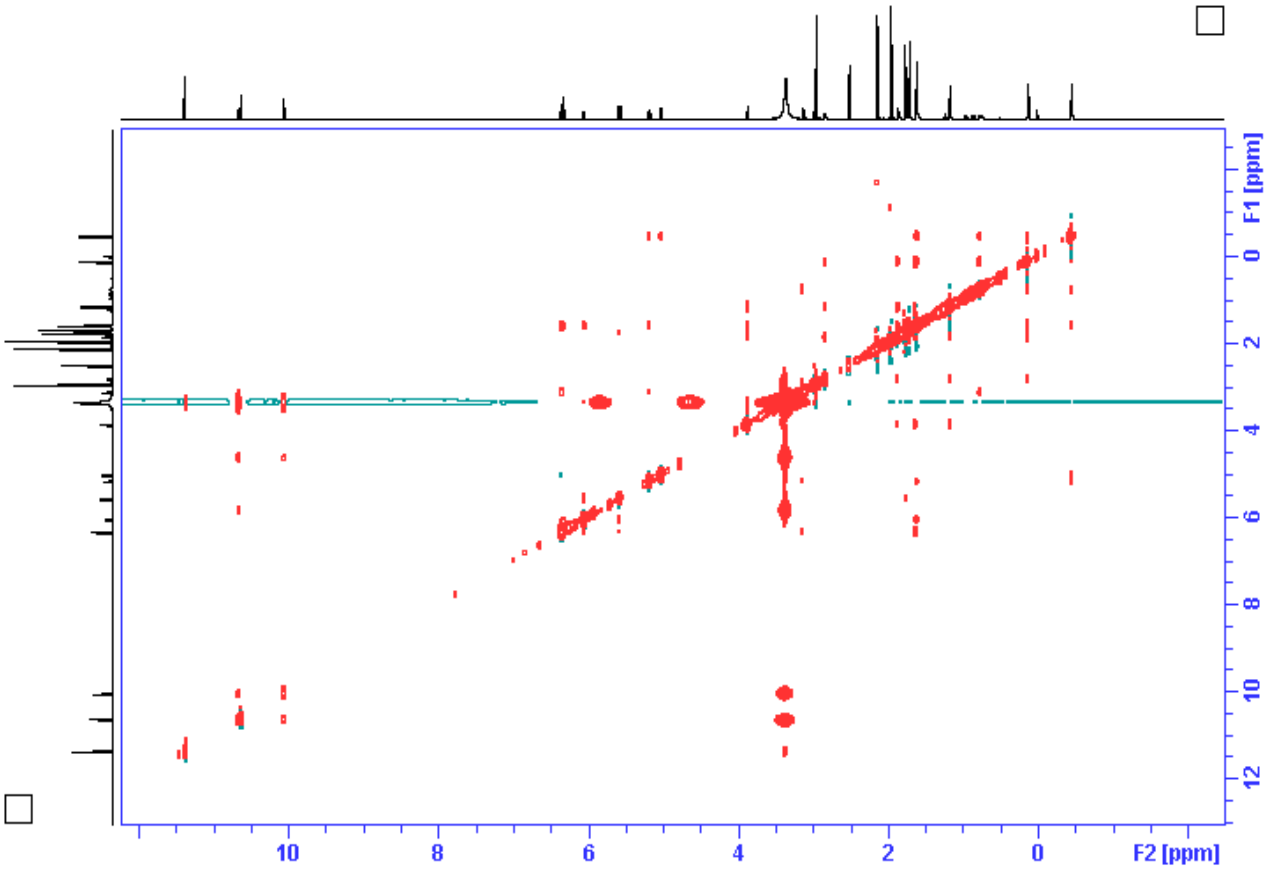


**Figure S8.** NOESY NMR spectrum of **1** in DMSO-$d_6$ (600M Hz)

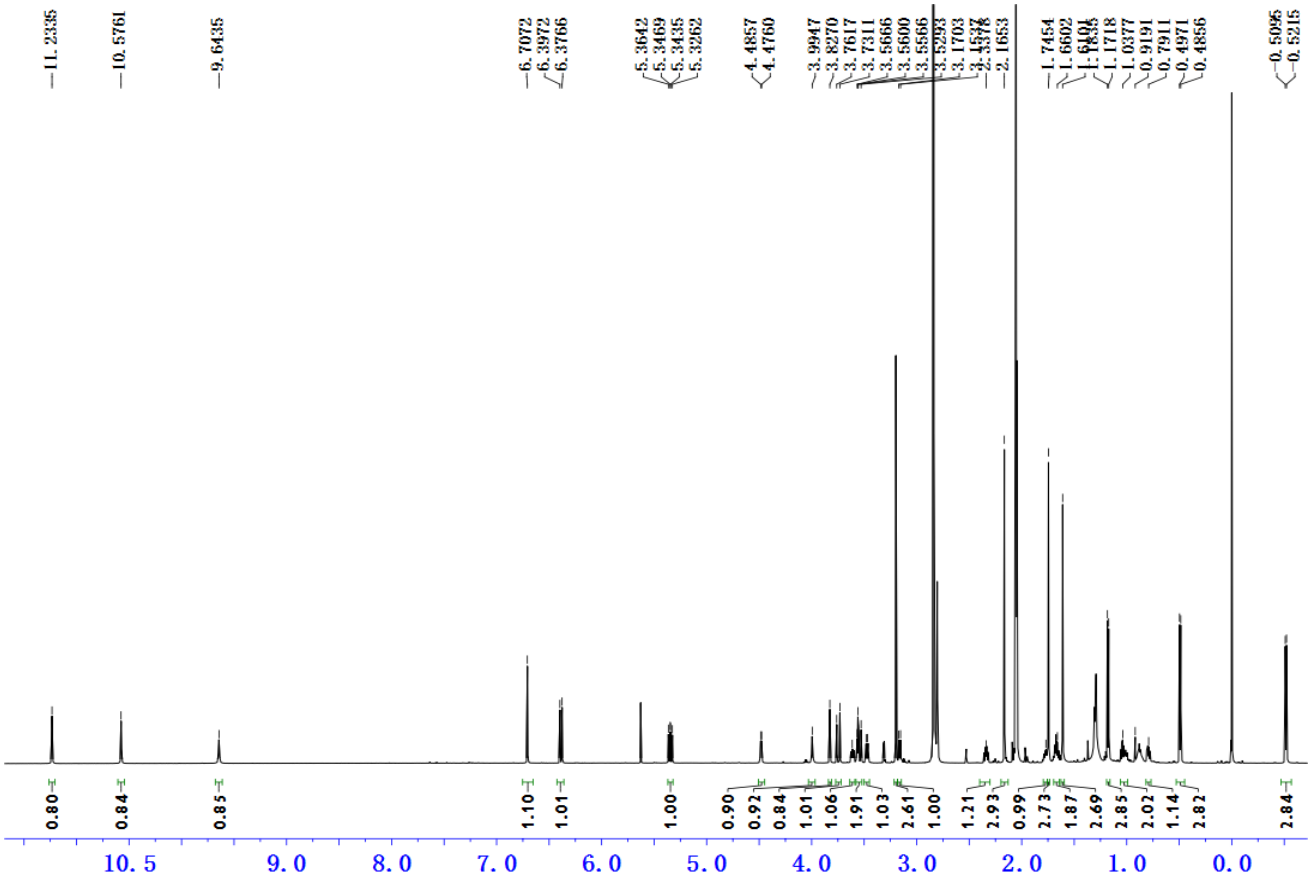

**Figure S9.** $^{1}$H NMR spectrum of **2** in acetone-$d_6$ (600M Hz)

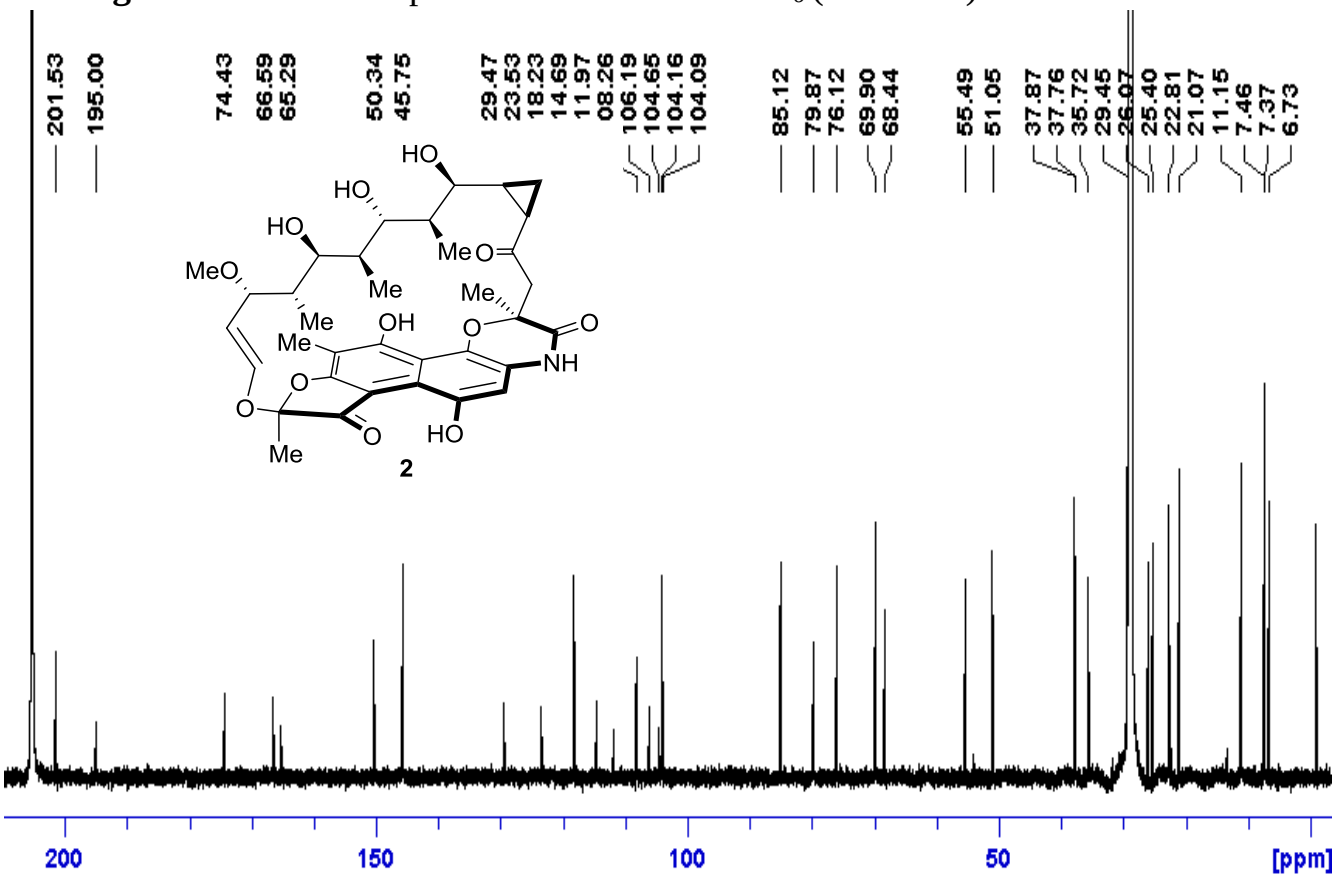

**Figure S10.** $^{13}$C NMR spectrum of **2** in acetone-$d_6$ (150M Hz)

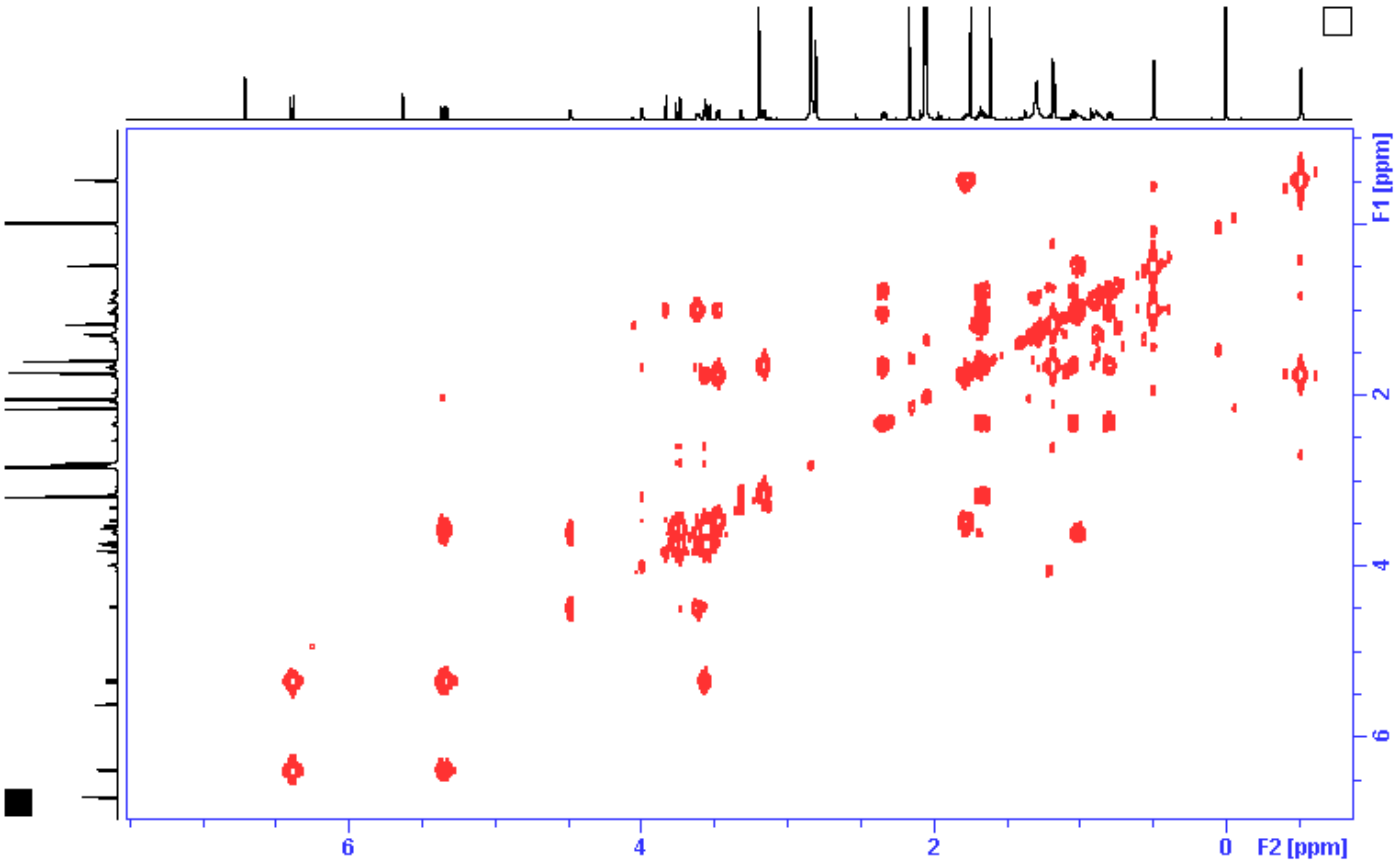


**Figure S11**. $^{1}$H-$^{1}$H COSY NMR spectrum of **2** in acetone-$d_6$ (600M Hz)

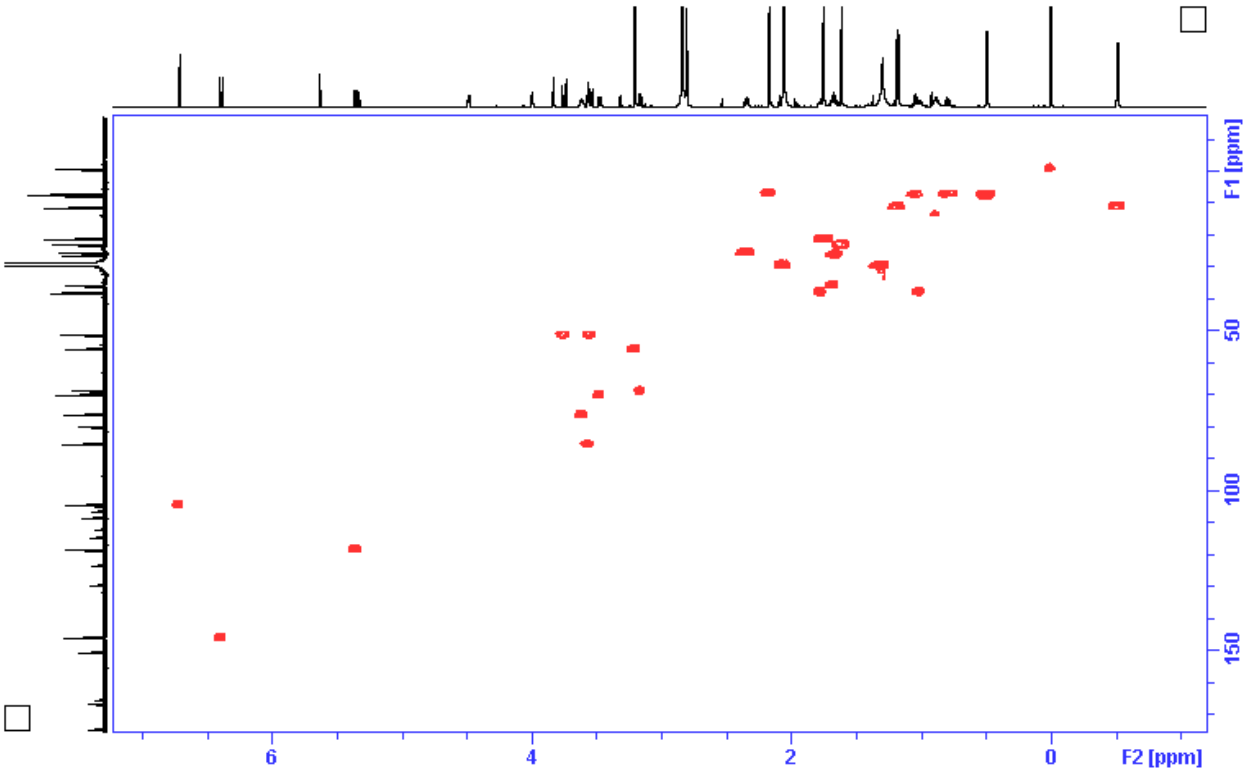


**Figure S12.** HSQC NMR spectrum of **2** in acetone-$d_6$ (600M Hz)

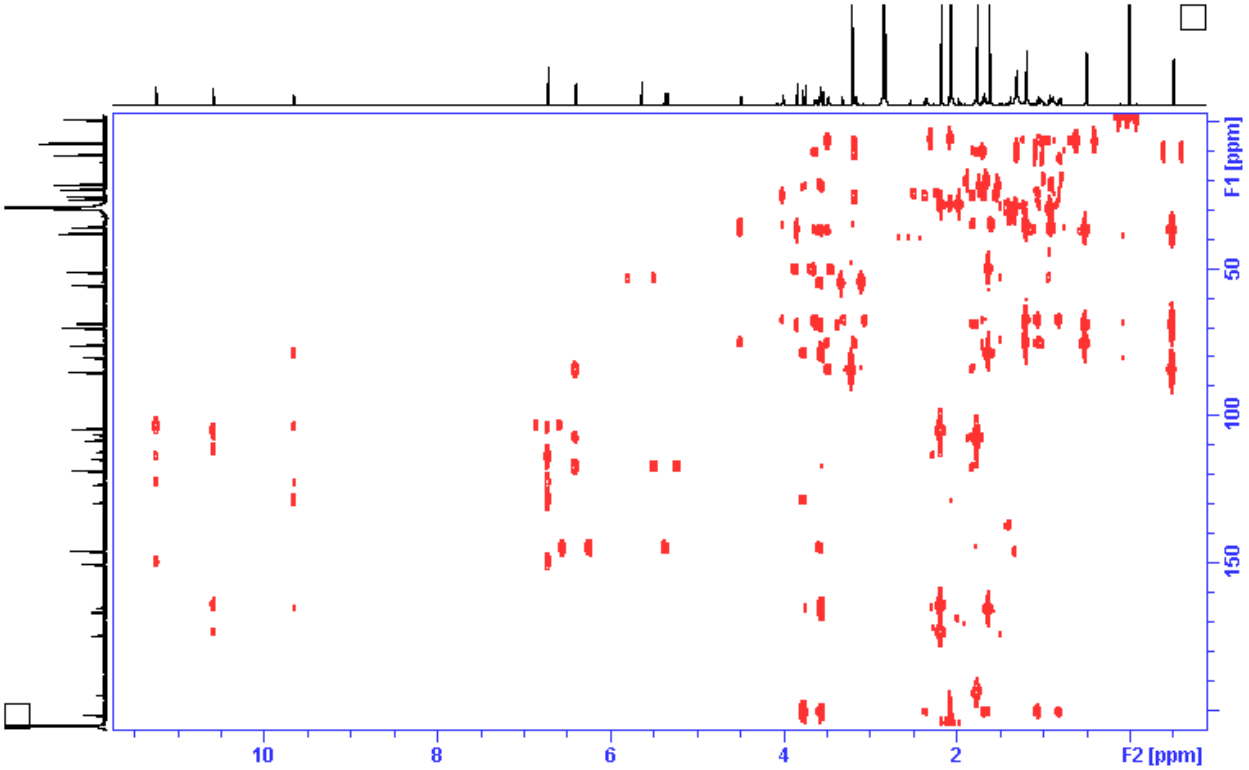


**Figure S13**. HMBC NMR spectrum of **2** in acetone-$d_6$ (600M Hz)

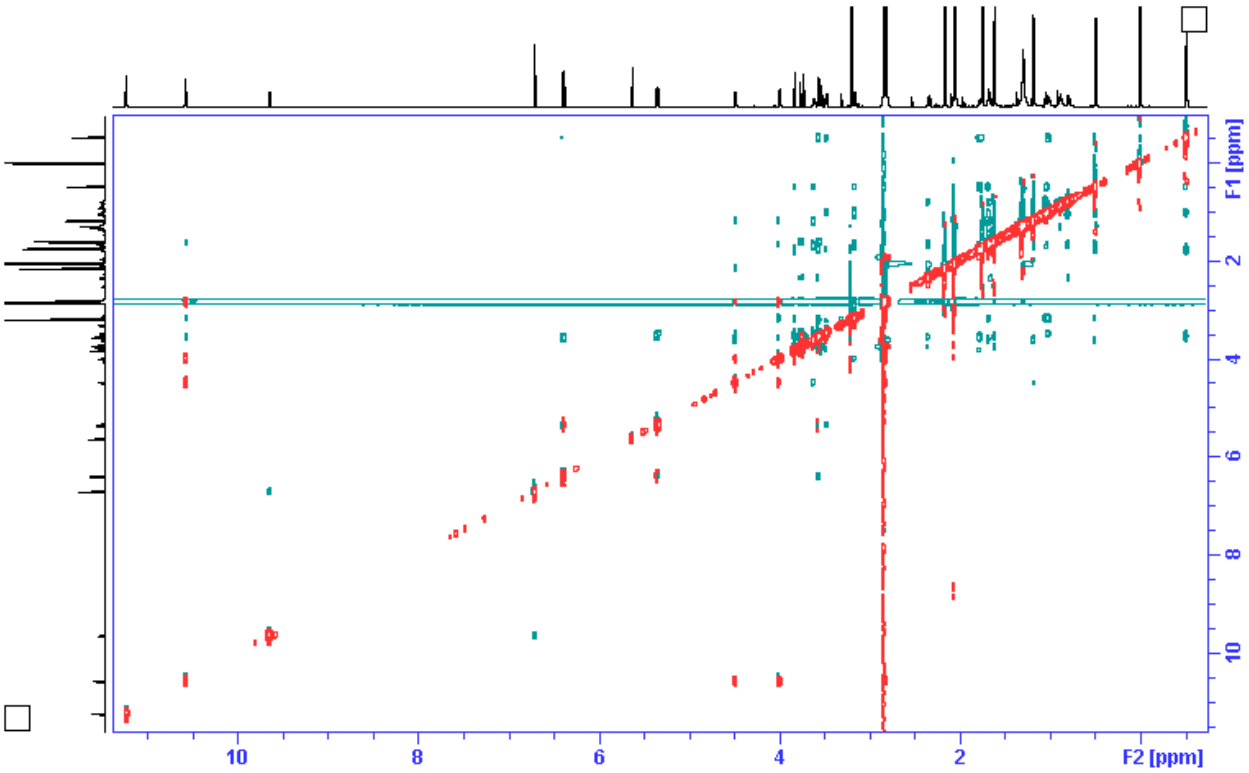


**Figure S14.** NOESY NMR spectrum of **2** in acetone-$d_6$ (600M Hz)

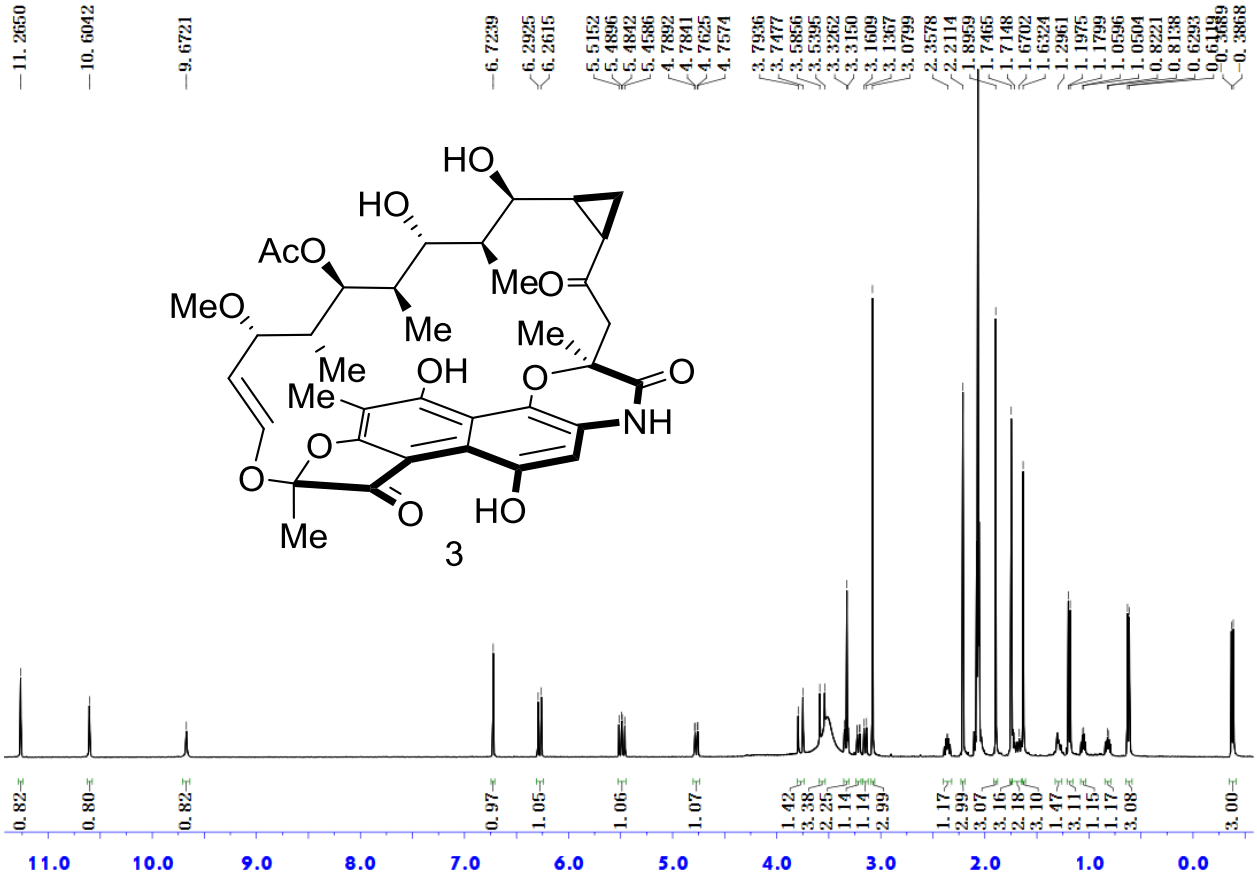


**Figure S15**. $^{1}$H NMR spectrum of **3** in acetone-$d_6$ (400M Hz)

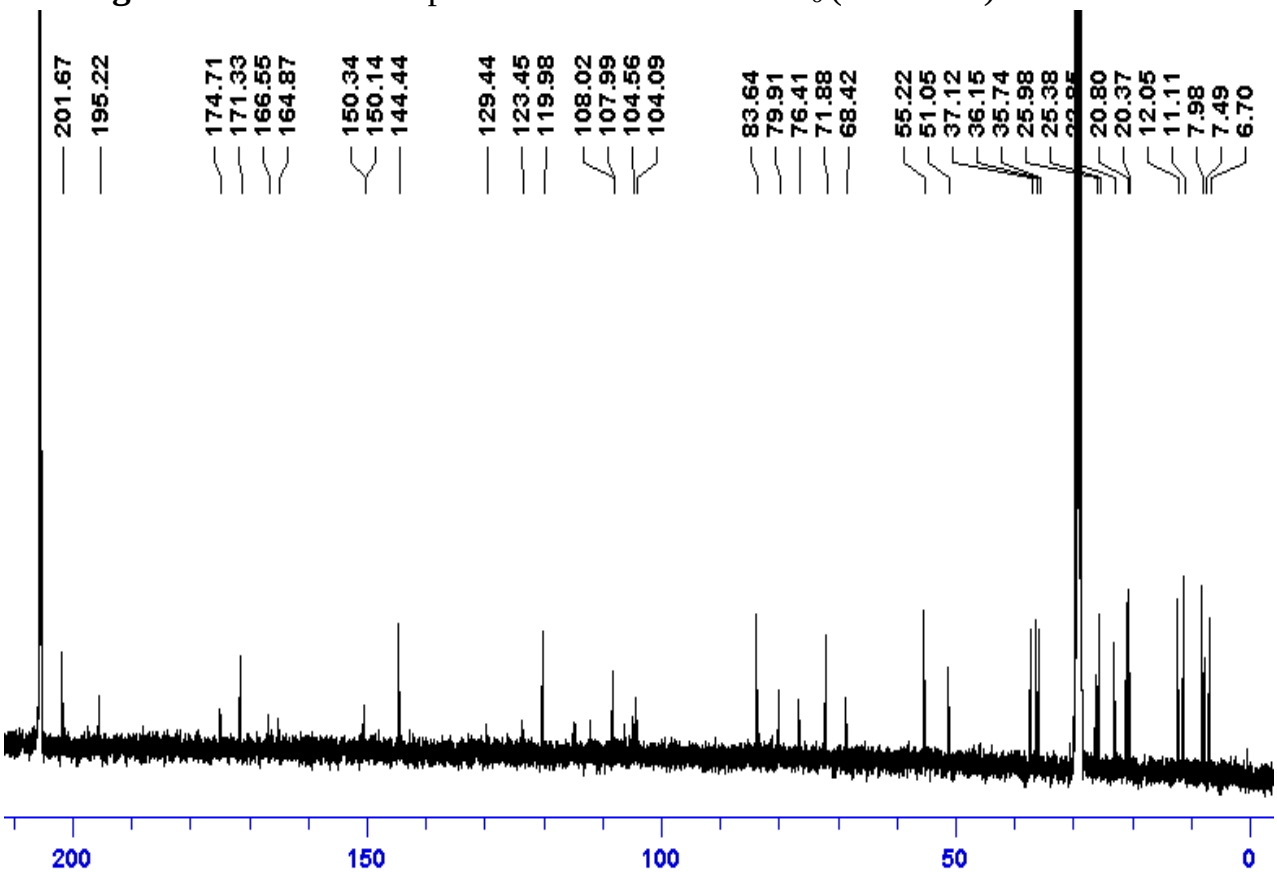

**Figure S16.** $^{13}$C NMR spectrum of **3** in acetone-$d_6$ (100M Hz)

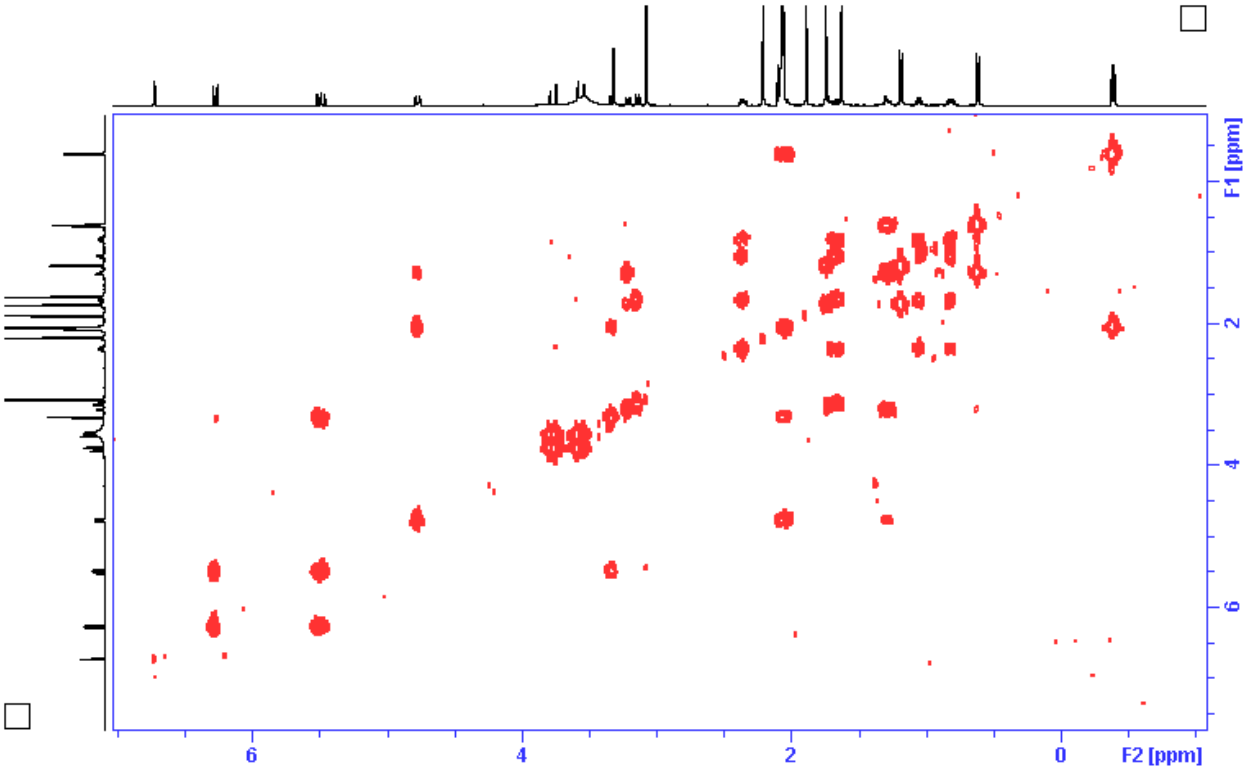


**Figure S17**. $^{1}$H-$^{1}$H COSY NMR spectrum of **3** in acetone-$d_6$ (400M Hz)

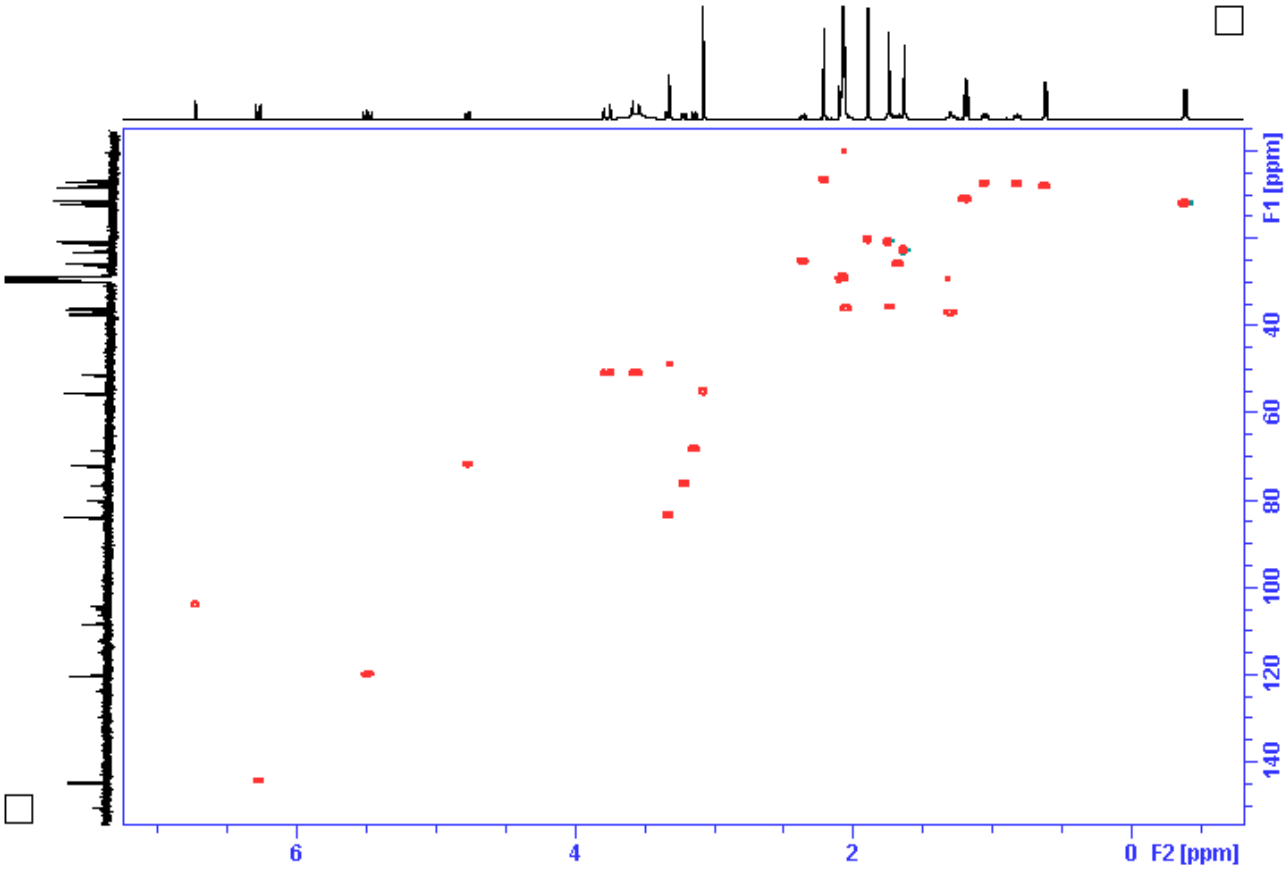


**Figure S18.** HSQC NMR spectrum of **3** in acetone-$d_6$ (400M Hz)

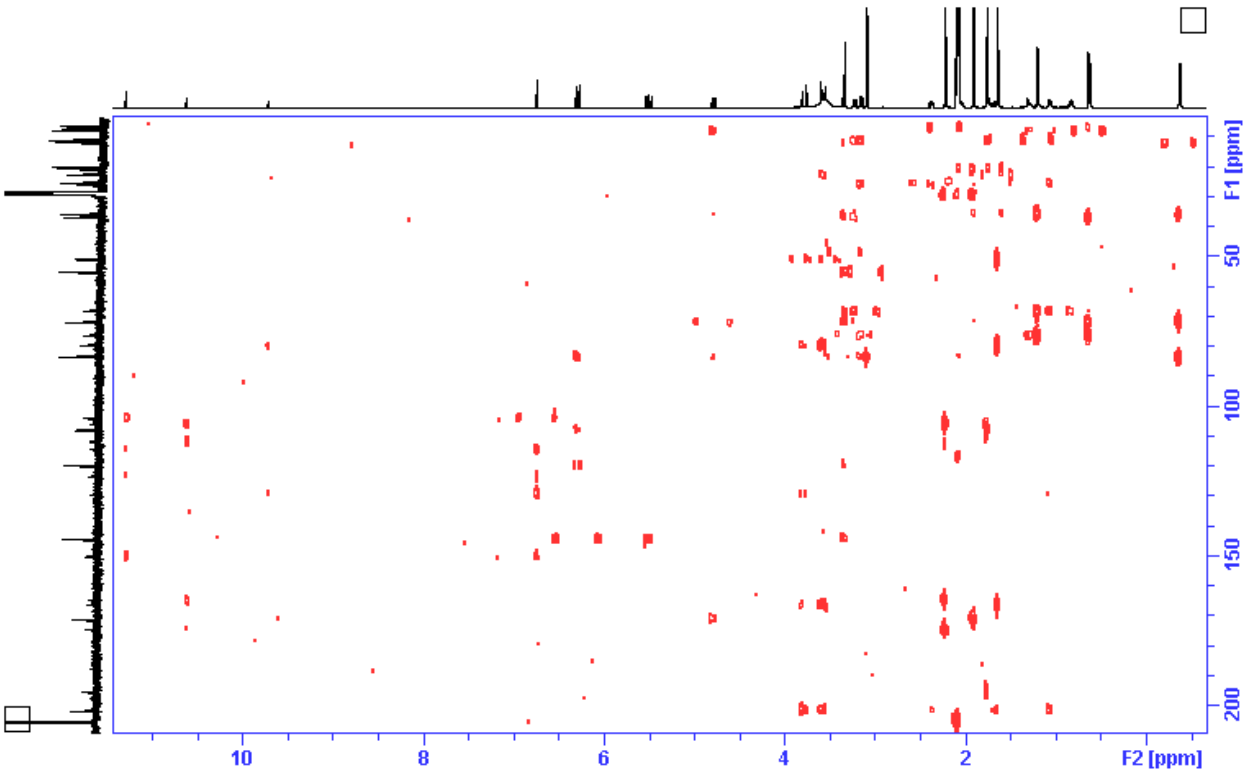


**Figure S19**. HMBC NMR spectrum of **3** in acetone-$d_6$ (400M Hz)

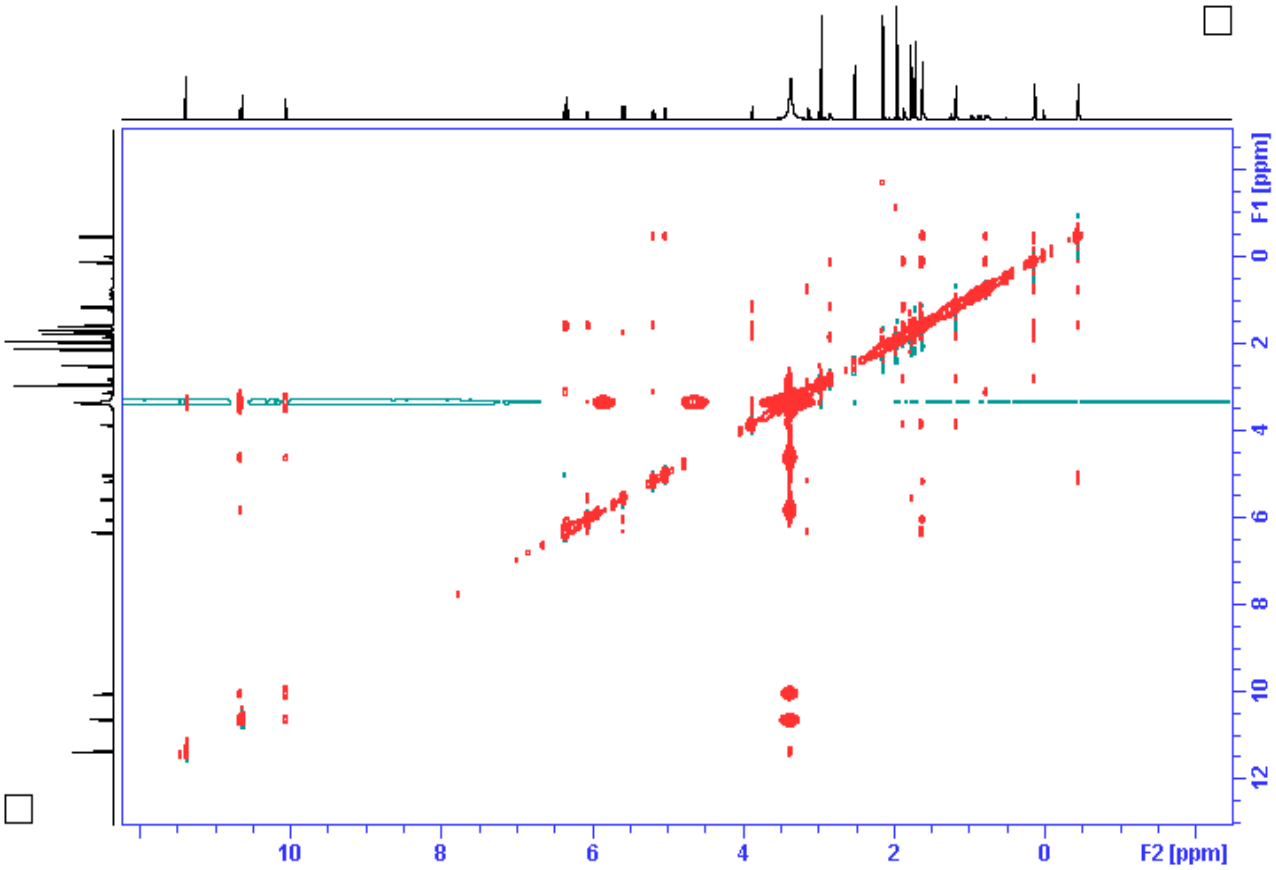


**Figure S20.** NOESY NMR spectrum of **3** in acetone-$d_6$ (400M Hz)

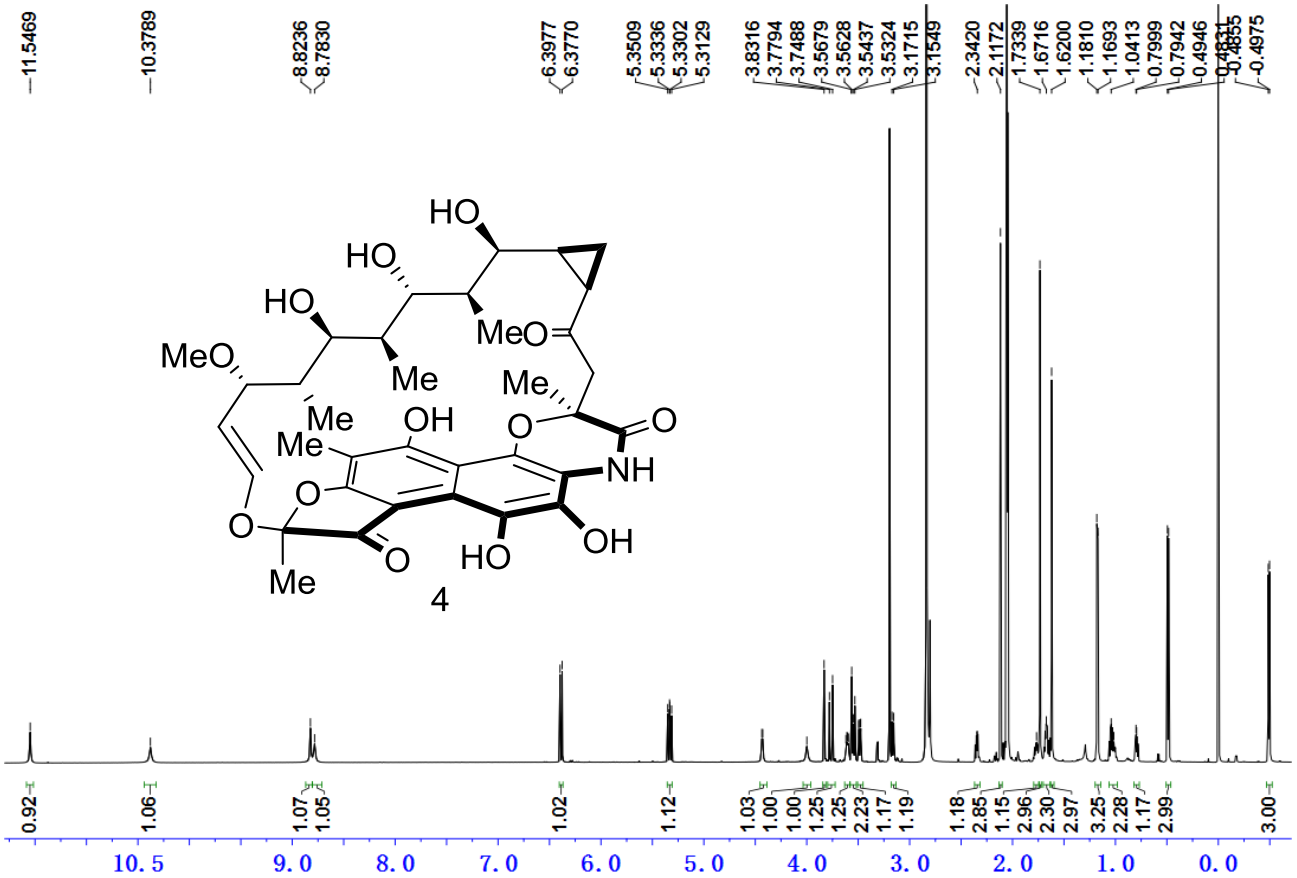


**Figure S21**. $^{1}$H NMR spectrum of **4** in acetone-$d_6$ (600M Hz)

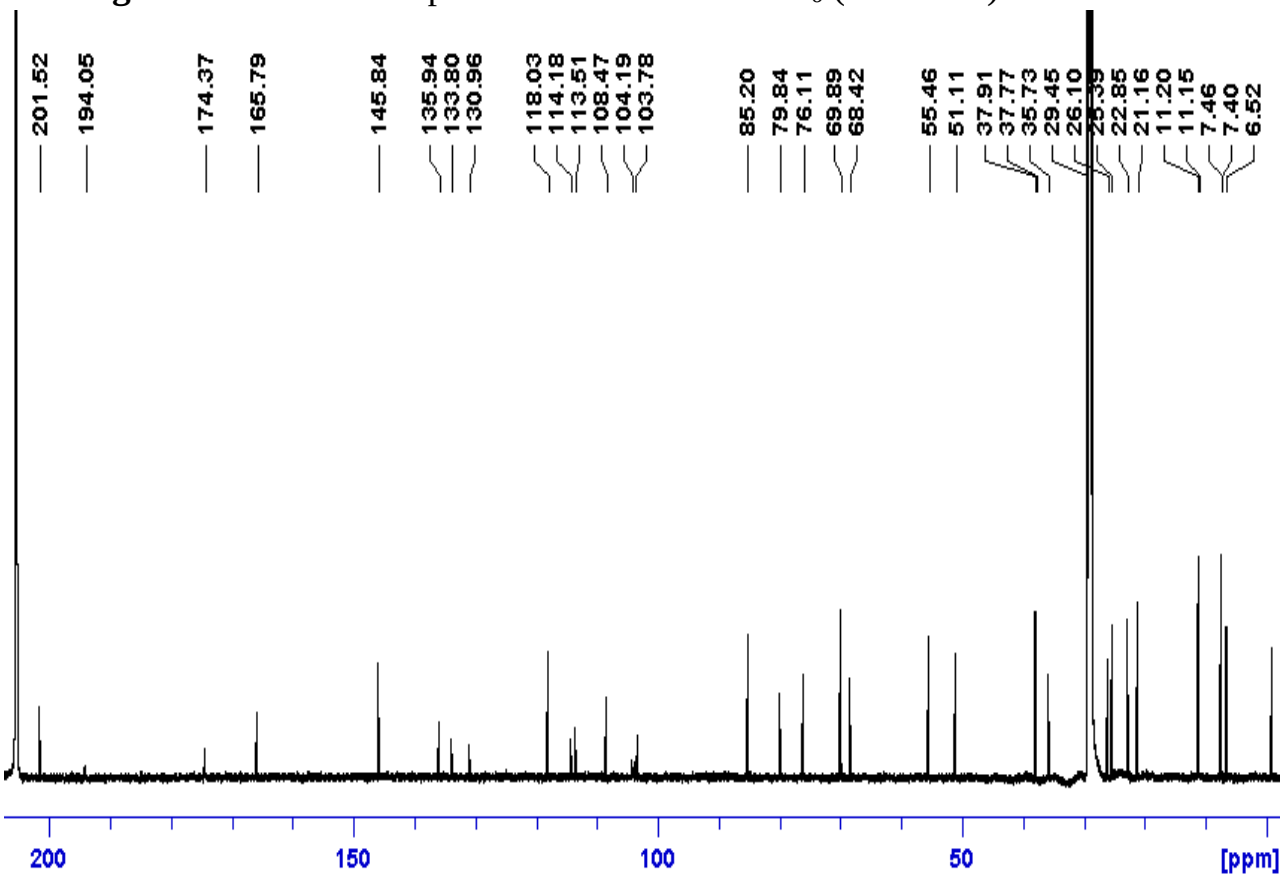


**Figure S22.** $^{13}$C NMR spectrum of **4** in acetone-$d_6$ (150M Hz)

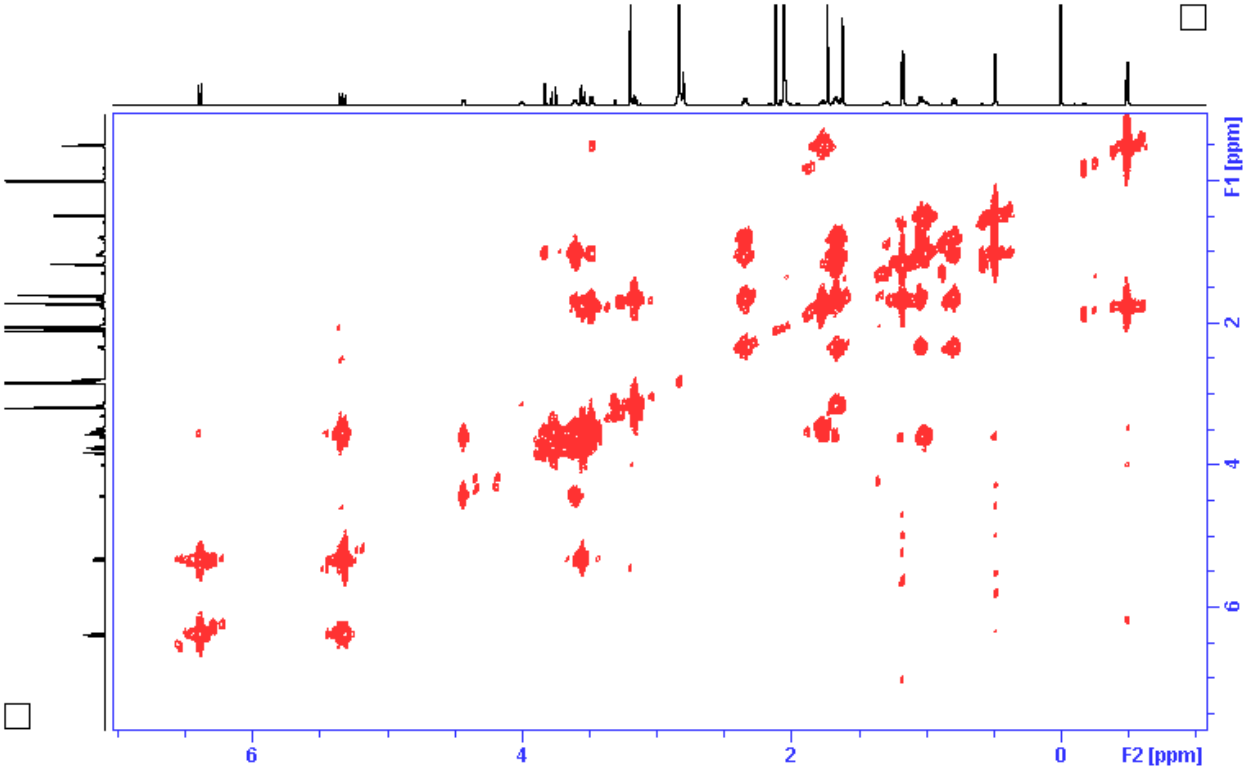


**Figure S23.** $^{1}$H-$^{1}$H COSY NMR spectrum of **4** in acetone-$d_6$ (600M Hz)

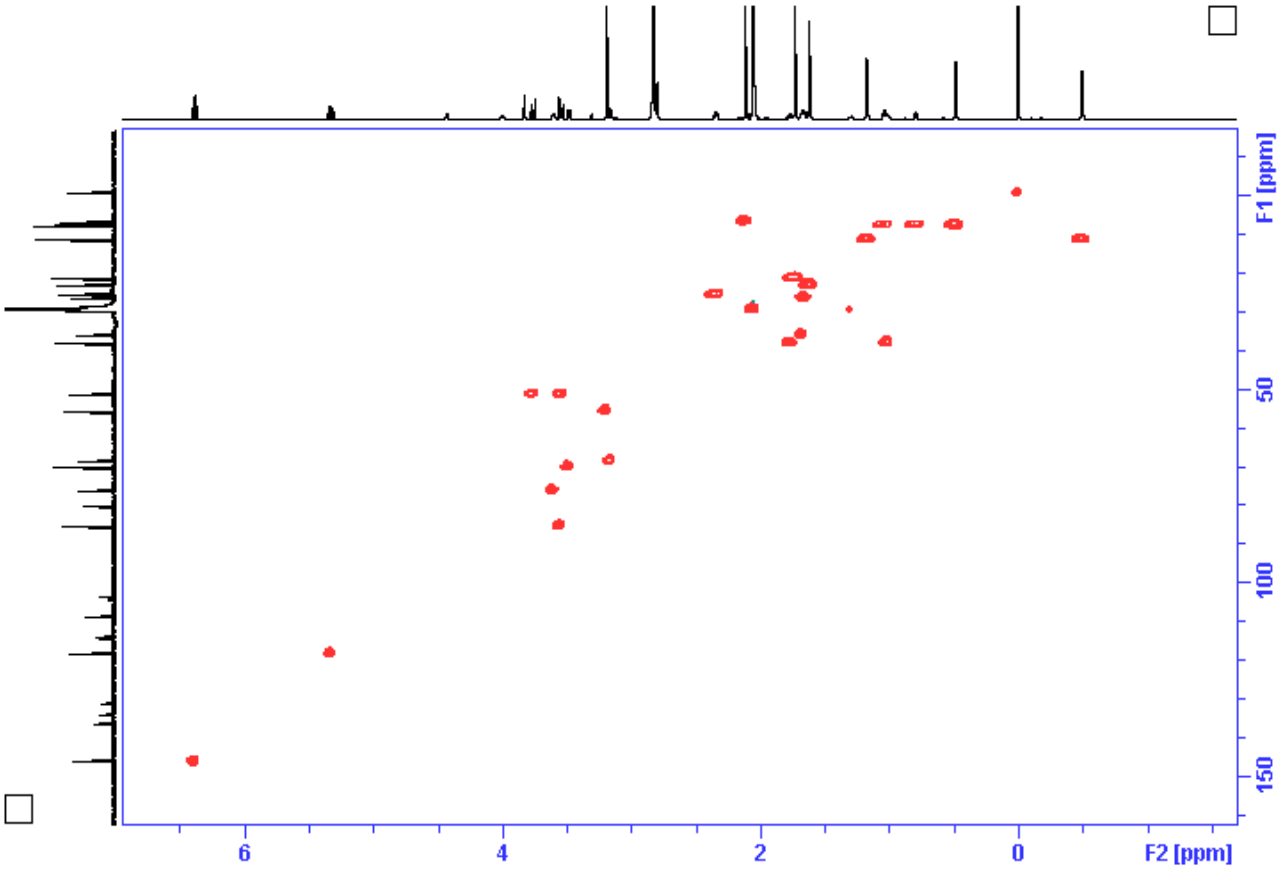


**Figure S24.** HSQC NMR spectrum of **4** in acetone-$d_6$ (600M Hz)

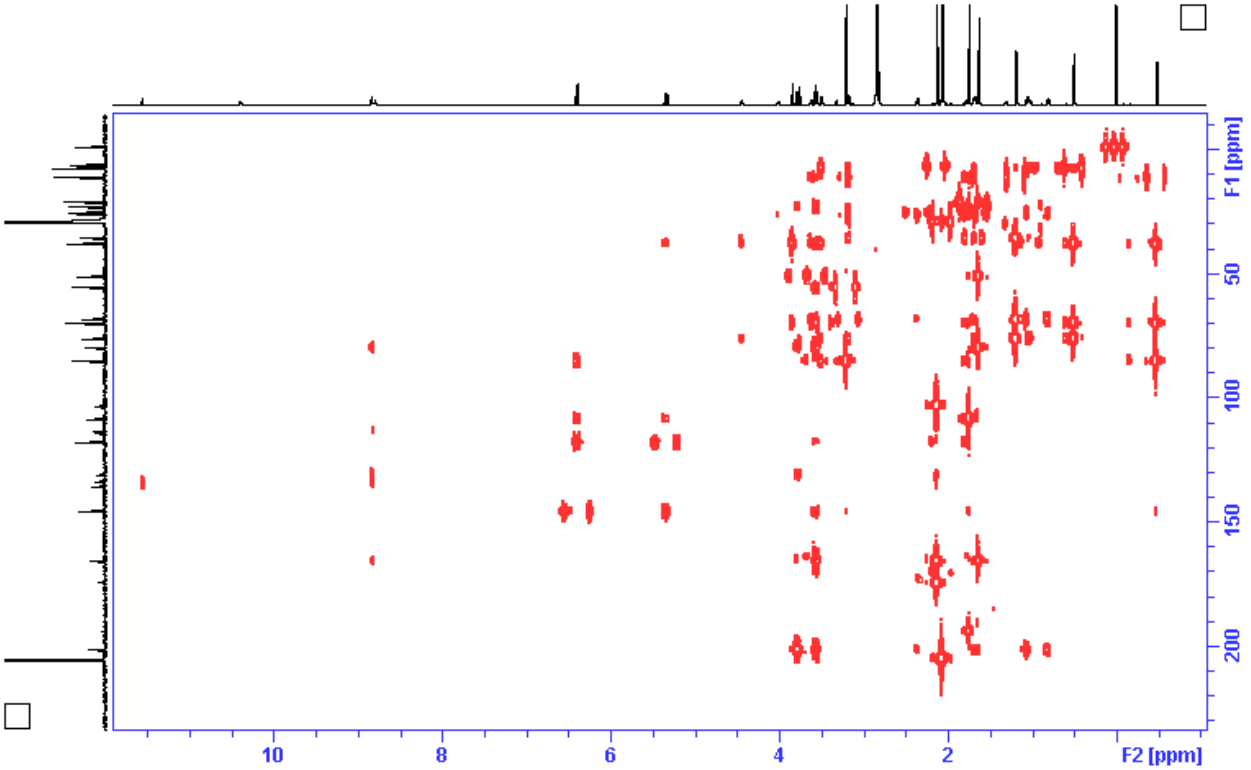


**Figure S25**. HMBC NMR spectrum of **4** in acetone-$d_6$ (600M Hz)

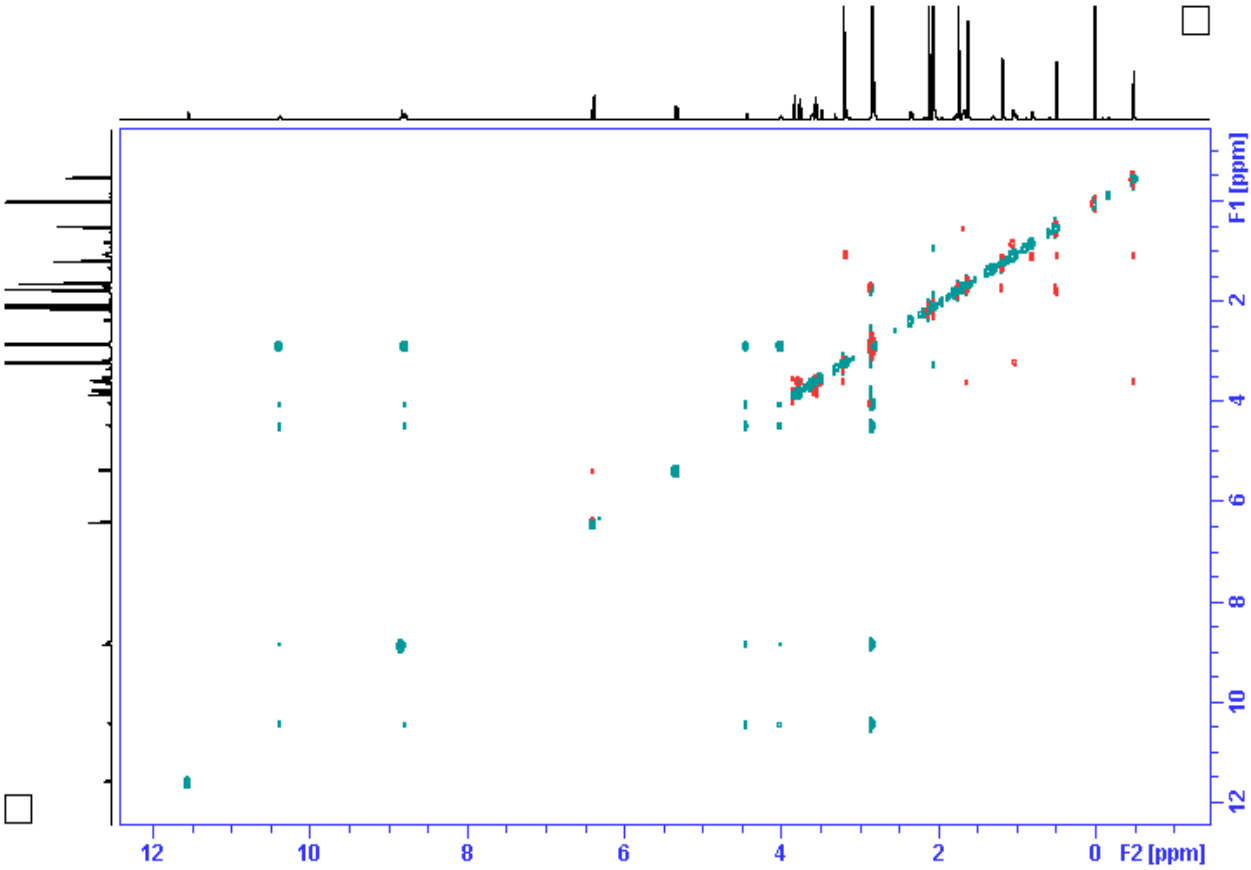


**Figure S26.** NOESY NMR spectrum of **4** in acetone-$d_6$ (600M Hz)

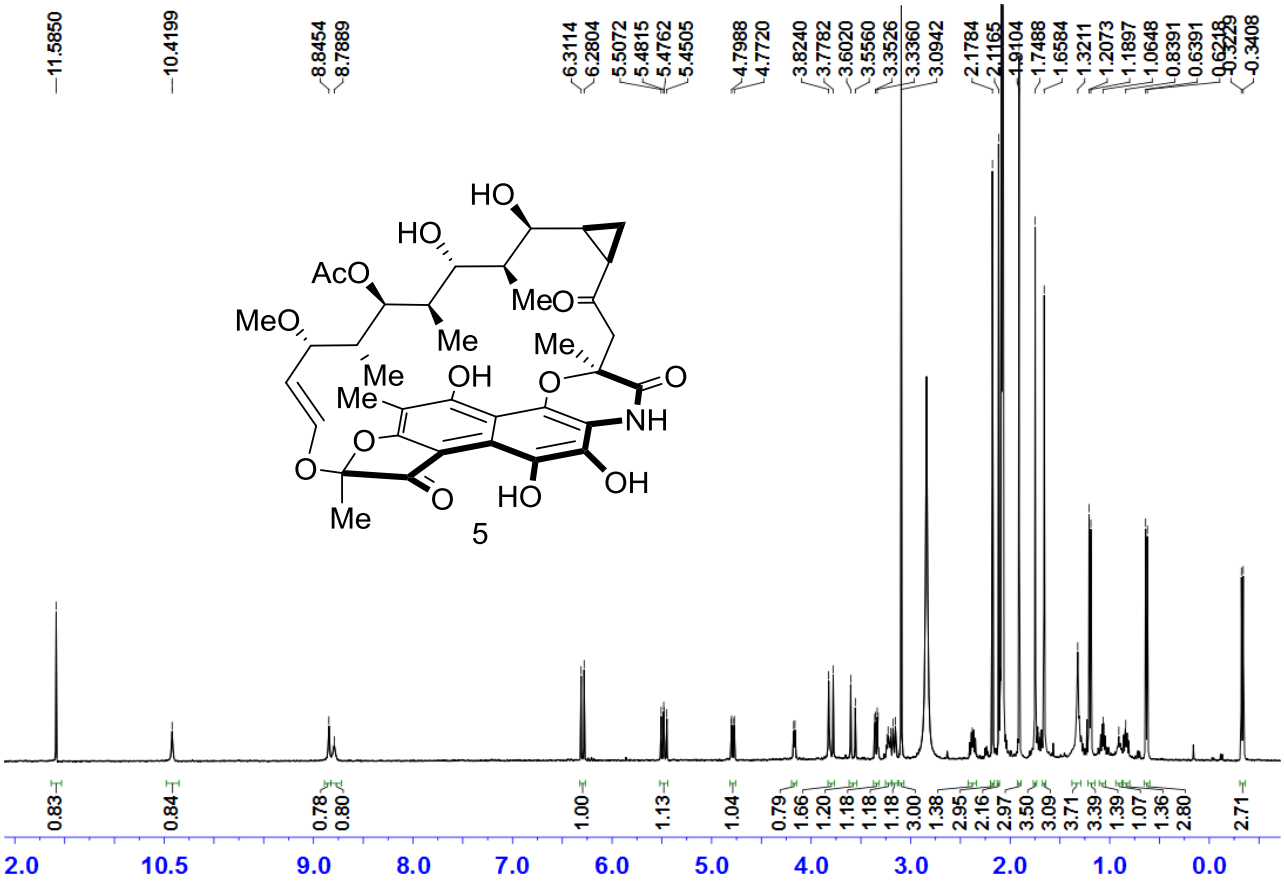


**Figure S27**. $^{1}$H NMR spectrum of **5** in acetone-$d_6$ (400M Hz)

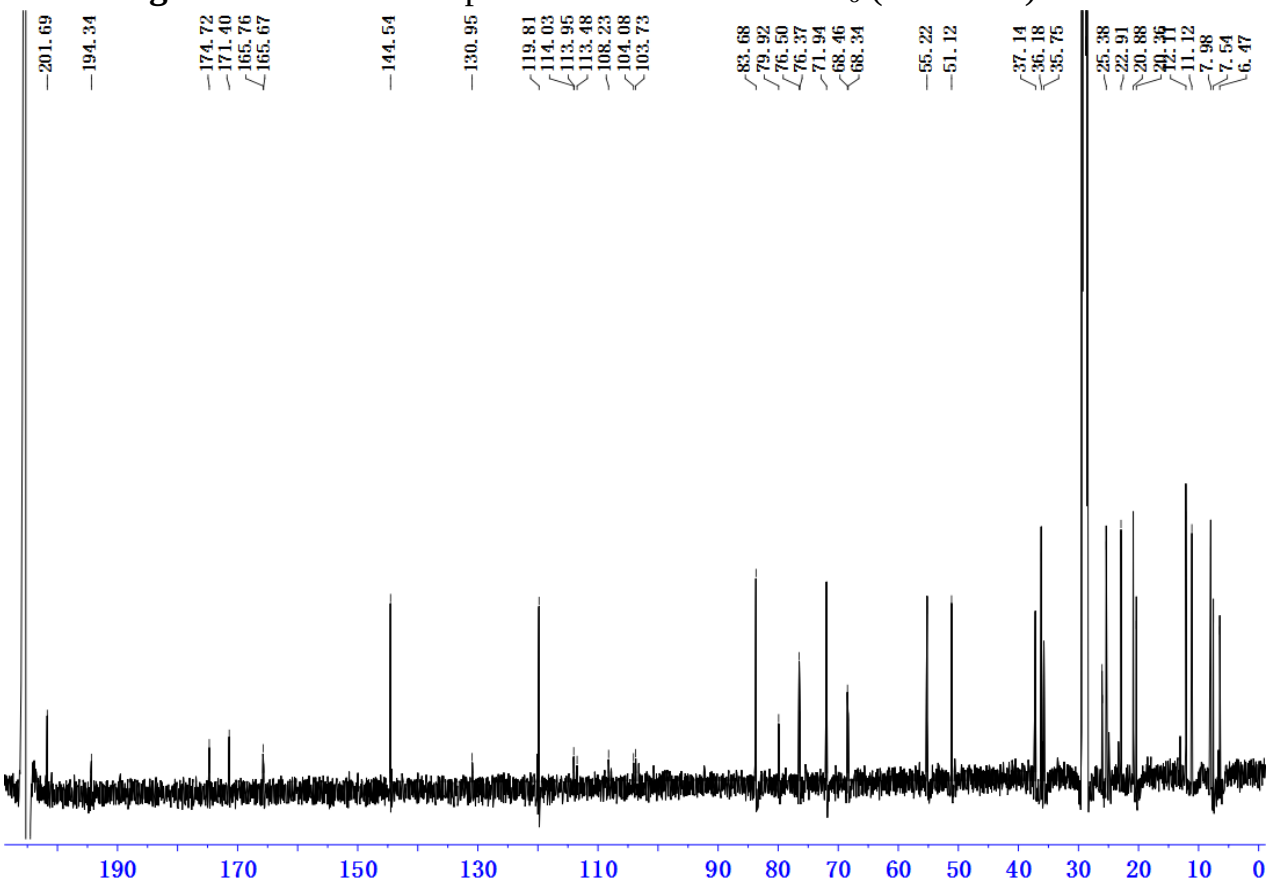

**Figure S28.** $^{13}$C NMR spectrum of **5** in acetone-$d_6$ (100M Hz)

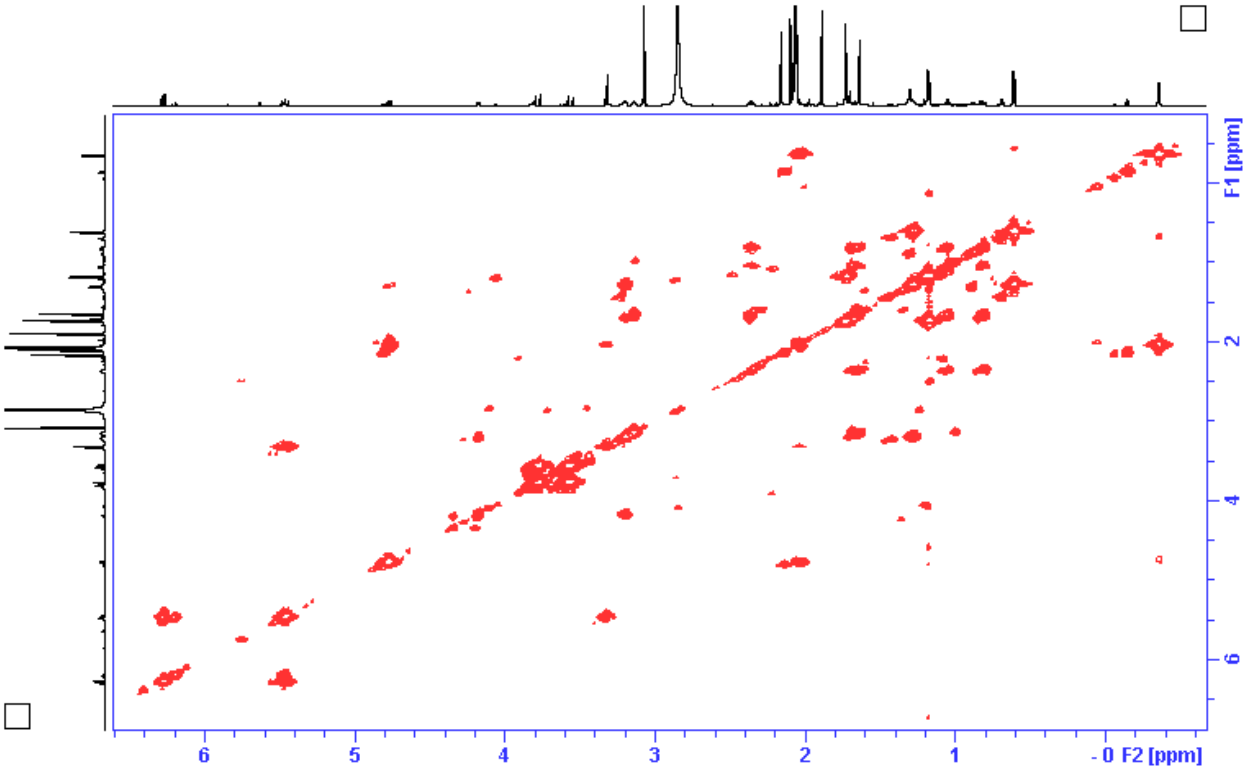


**Figure S29.** $^{1}$H-$^{1}$H COSY NMR spectrum of **5** in acetone-$d_6$ (400M Hz)

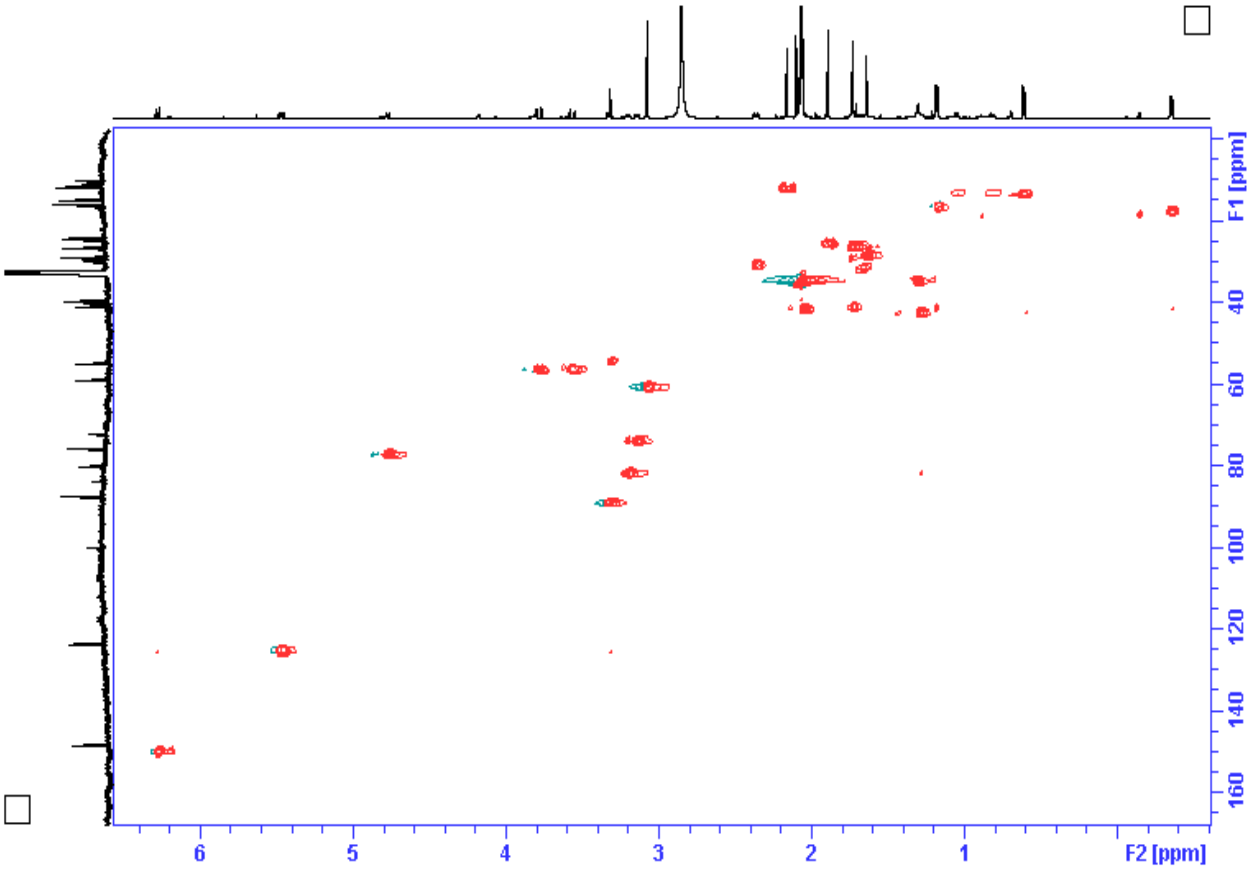


**Figure S30.** HSQC NMR spectrum of **5** in acetone-$d_6$ (400M Hz)

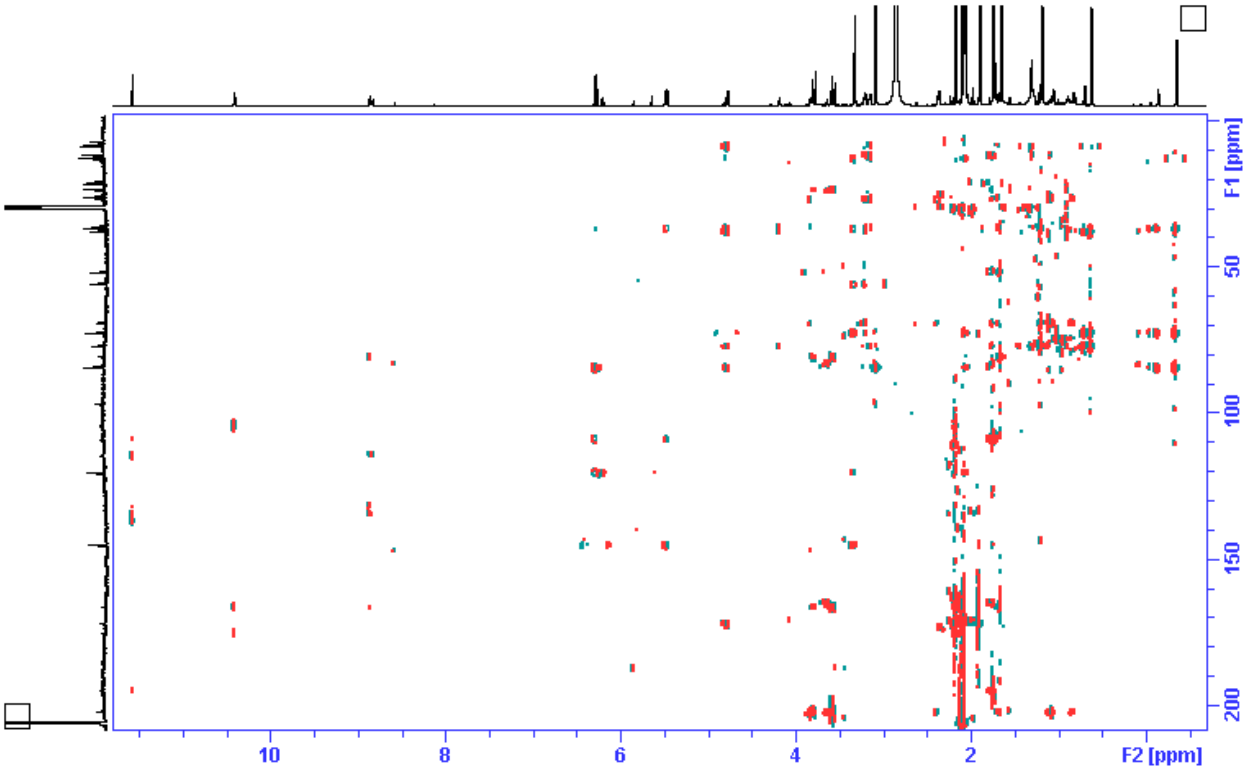


**Figure S31**. HMBC NMR spectrum of **5** in acetone-$d_6$ (400M Hz)

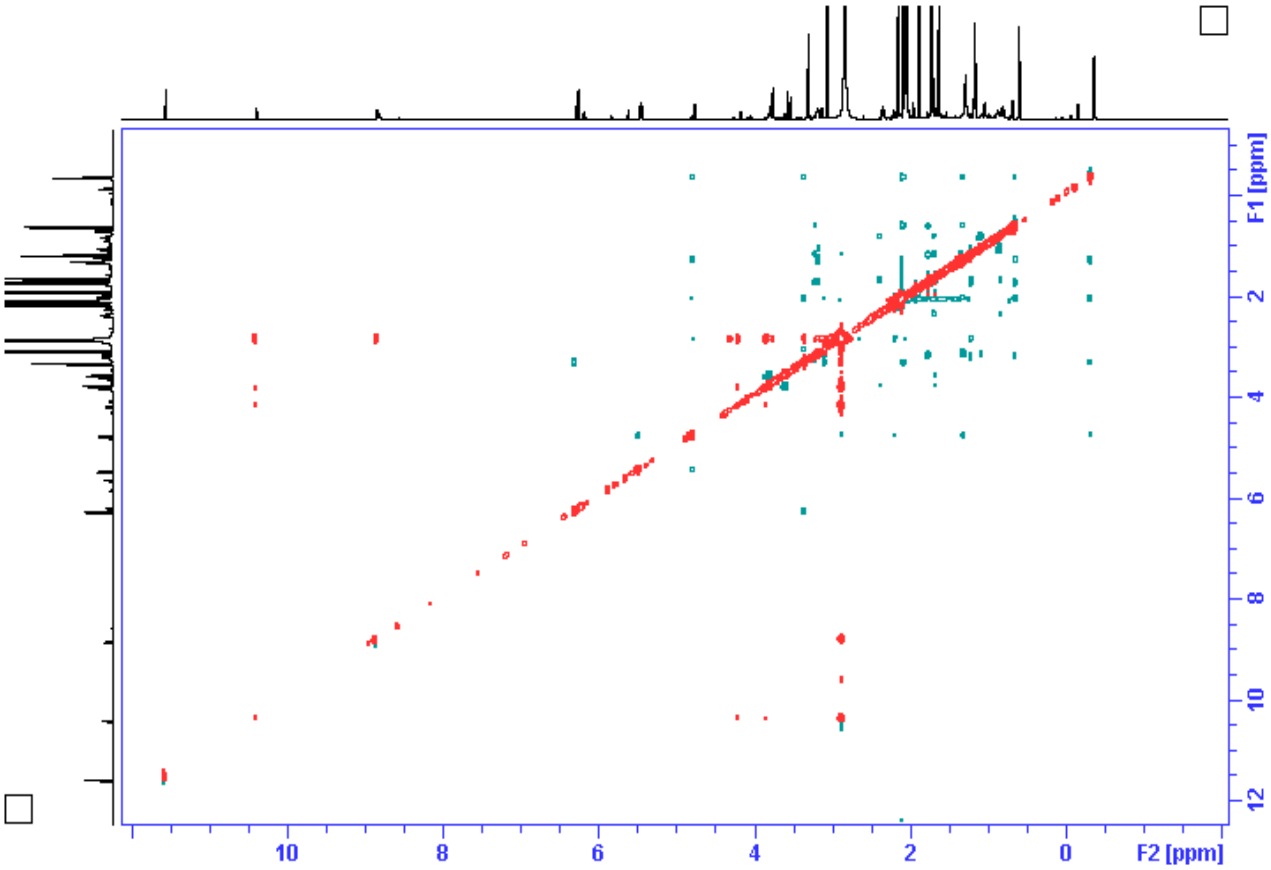


**Figure S32.** NOESY NMR spectrum of **5** in acetone-$d_6$ (400M Hz)

## References

1. (a) K. Yoshida, Y. Minami, R. Azuma, M. Saeki, T. Otani, *Tetrahedron Lett.* **1993**, *34*, 2637. (b) M. Somei, K. Aoki, Y. Nagaham, K. Nakagawa, *Heterocycles* **1995**, *41*, 5. (c) P. Melloni, A. Della Torre, E. Lazzari, G. Mazzini, M. Meroni, *Tetrahedron* **1985**, *41*, 1393. (d) J. L. Kelley, D. L. Musso, G. E. Boswell, F. E. Soroko, B. R. Cooper, *J. Med. Chem*. **1996**, *39*, 347. (e) R. B. Rothman, M. Katsnelson, N. Vu, J. S. Partilla, C. M. Dersch, B. E. Blough, M. H. Baumann, *Eur. J. Pharmacol*. **2002**, 51. (f) D. Kim, D. H. Ryu, J. Y. Lee, N. Lee, Y. Kim, J. Kim, K. Chang, G. Im, T. Kim, W. Choi, *J. Med. Chem*. **2001**, *44*, 1594. (g) E. M. Acton, G. L. Tong, C. W. Mosher, R. L. Wolgemuth, *J. Med. Chem.* **1984**, *27*, 638. (h) M. C. Chrysselis, E. A. Rekka, I. C. Siskou, P. N. Kourounakis, *J. Med. Chem.* **2002**, *45*, 5406. (i) K. Araki, T. Kuroda, S. Uemori, A. Moriguchi, Y. Ikeda, F. Hirayama, Y. Yokoyama, E. Iwao, T. Yakushiji, *J. Med. Chem*. **1993**, *36*, 1356. (j) N. Sakurai, M. Sano, F. Hirayama, T. Kuroda, S. Uemori, A. Moriguchi, K. Yamamoto, Y. Ikeda, T. Kawakita, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2185. (k) T. Harrison, A. P. Owens, B. J. Williams, C. J. Swain, A. Williams, E. J. Carlson, W. Rycroft, F. D. Tattersall, M. A. Cascieri, G. G. Chicchi, S. Sadowski, N. M. J. Rupniak, R. Hargreaves, *J. Med. Chem*. **2001**, *44*, 4296.