

## Supplementary materials

### Discovery of novel highly bioactive pamamycins through transcriptional engineering of the biosynthetic gene cluster

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**Table S1.** Bacterial strains and plasmids

| Bacterial strains                                           | Description                                                                                                                                     | Source     |
|-------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>E. coli</i> ET12567 (pUB307)                             | Conjugative transfer of DNA                                                                                                                     | [1]        |
| <i>E. coli</i> GB05-redCC                                   | Derivative of GB2005 containing Red/ET mediating plasmid pSC101-BAD-gbaA                                                                        | [2]        |
| <i>E. coli</i> GB2005                                       | <i>E. coli</i> strain used in basic cloning procedures                                                                                          | [3]        |
| <i>S. albus</i> J1074                                       | Parental strain                                                                                                                                 | [4]        |
| <i>S. albus</i> J1074 R2                                    | <i>S. albus</i> J1074 containing the R2 cosmid with the native <i>pam</i> BGC, AmR                                                              | This study |
| <i>S. albus</i> J1074 P21pamW                               | <i>S. albus</i> J1074 with overexpression of pamamycin transporter gene <i>pamW</i> , markerless                                                | This study |
| <i>S. albus</i> J1074 P21pamW/R2                            | <i>S. albus</i> J1074 pTOS-P21pamW containing the R2 cosmid, AmR                                                                                | This study |
| <i>S. albus</i> J1074 P21pamW/R2 library                    | <i>S. albus</i> J1074 pTOS-P21pamW with derivatives of the R2 cluster, AmR                                                                      | This study |
| <i>S. albus</i> J1074 Del14                                 | <i>S. albus</i> J1074 derived heterologous expression host                                                                                      | [5]        |
| <i>S. albus</i> J1074 pGUS                                  | <i>S. albus</i> J1074 strain with the pGUS plasmid                                                                                              | [6]        |
| <i>S. albus</i> J1074 pGUS-p67up/p73up/p100up/pR2up         | <i>S. albus</i> J1074 strain with the pGUS plasmid with different upstream promoters of R2 constructs cloned in front of the reporter gene, AmR | This study |
| <i>S. albus</i> J1074 pGUS-p67down/p73down/p100down/pR2down | <i>S. albus</i> J1074 strain with the pGUS plasmid with different downstream promoters of R2 constructs cloned in front of the reporter gene    | This study |
| <i>S. albus</i> J1074 R2-p73up                              | <i>S. albus</i> J1074 strain with the R2 cluster and the semisynthetic promoter p73up                                                           | This study |
| <i>S. albus</i> J1074 R2-p73down                            | <i>S. albus</i> J1074 strain with the R2 cluster and the semisynthetic promoter p73down                                                         | This study |
| Plasmids/Cosmids                                            | Description                                                                                                                                     | Source     |
| pTOS                                                        | Apramycin resistance (Am <sup>R</sup> ), VWB-based integrative vector                                                                           | [7]        |
| pTOS-P21pamW                                                | pTOS derivative containing <i>pamW</i> gene under P21 promoter                                                                                  | This work  |
| pUWLH                                                       | pIJ101 replicon based replicative vector, HygR                                                                                                  | [8]        |
| pUWLHpamS                                                   | pUWLH with cloned <i>pamS</i> gene                                                                                                              | This work  |
| pUWLHpamW                                                   | pUWLH with cloned <i>pamW</i> gene                                                                                                              | This work  |
| R2                                                          | Derivative of pOJ436 containing the cluster for pamamycin biosynthesis                                                                          | [9]        |
| R2 mutant library                                           | R2 cosmids, containing a Hyg <sup>R</sup> cassette flanked by 2 random promoters between the <i>pamF</i> and <i>pamA</i> genes                  | This work  |
| R2- pamAp73                                                 | R2 cosmid containing a Hyg <sup>R</sup> cassette with the downstream oriented promoter of the R2 mutant R2-73                                   | This work  |
| R2- pamFp73                                                 | R2 cosmid containing a Hyg <sup>R</sup> cassette with the upstream oriented promoter of the R2 mutant R2-73                                     | This work  |
| pGUS                                                        | pSET152 derivative with promoterless <i>gusA</i> gene                                                                                           | [10]       |
| pGUS-pR2Up/p67Up/p73Up/p100Up                               | Derivative of pGUS containing the upstream promoter of the R2/R2-67/R2-73 or R2-100 cosmids cloned in front of the <i>gusA</i> reporter gene    | This work  |
| pGUS-pR2Down/p67Down/p73Down/p100Down                       | Derivative of pGUS containing the downstream promoter of the R2/R2-67/R2-73 or R2-100 cosmids cloned in front of the <i>gusA</i> reporter gene  | This work  |

**Table S2.** Oligonucleotides used in this study

| Pair N. | Name                                                           | Sequence (5'→3')                                                                                                                                                                                                                                                | Purpose                                                                                                                                                                     |
|---------|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1       | pamWFEcoRV<br>pamWRPstI                                        | GATATCAATCCCGAAGGAGGCCAGTC<br>CTGCAGCGGCTGCTTCTGTGGTCA                                                                                                                                                                                                          | Cloning of <i>pamW</i> gene into pUWL-Hyg vector                                                                                                                            |
| 2       | pamSFEcoRV<br>pamSRPstI                                        | GATATCATTAGGGTGGGAGCAAGCA<br>CTGCAGCCCCGTCCGTGCACGTAT                                                                                                                                                                                                           | Cloning of <i>pamS</i> gene into pUWL-Hyg vector                                                                                                                            |
| 3       | PamWIntro-F<br>PamWIntro-R                                     | ACGATCTGTTACGGCTCAGCGGCTGCCTCGCCGCCGCTGCTTC<br>GGTGCTTCTGTGTGTCATGTGCGGGCTCTAACACGTCTAGTAT                                                                                                                                                                      | Amplification of <i>pamW</i>                                                                                                                                                |
| 4       | pTOS-F<br>pTOS-R                                               | AAAATCCTGTATATCGTGCG<br>ACTCAGACTCACTGAGGCTC                                                                                                                                                                                                                    | Primer pair for checking for introduction of <i>pamW</i>                                                                                                                    |
| 5       | PamPrandomHyg <sup>R</sup> -F<br>PamPrandomHyg <sup>R</sup> -R | GATCAGTGCCTCGCGACCGGGATCCTGCTGCTGATGCGCCATGCGAC<br>TTCCTCCTTCANNNNNNNATCCTANNNNNNNNNNNNNNNNNNAG<br>CCNNNNNNCGCGGATGTATCAGGCGCC<br>CCCCGCGGTTTTCCGTGCAGCGGATTCAGCCTGTGCAGCCATAACGA<br>TTCCTCCGATANNNNNNNATCCTANNNNNNNNNNNNNNNNNNAGC<br>CNNNNAAATACTTGACATATCACTG | Primer pair for homologous recombination and introduction of degenerated promoter sequences                                                                                 |
| 6       | Hyg <sup>R</sup> -Seq-F<br>Hyg <sup>R</sup> -Seq-R             | AGGCTCGCTAGGAATCATCC<br>AACTGCATCTCAACGCCTTCC                                                                                                                                                                                                                   | Primer pair for partially sequencing of the introduced promoters attached to the Hyg <sup>R</sup> cassette                                                                  |
| 7       | R2-pamAp73-F<br>R2-pamAp73-R                                   | GGATTCTCCCGGTACCGGTGGGCGCATGTCCTCGAATTCCTCGGCTC<br>AGGCGCCGGGGCGGTGT<br>CCCCGCGGTTTTCCGTGCAGCGGATTCAGCCTGTGCAGCCATAACGA<br>TTCTCCGATACCTCGTATCTATGAATATCACGGGGATGAGCCGG<br>GATAAATACTTGACATATCACTG                                                              | Primer for amplification of the downstream p73Down promoter and introduction with an Hyg <sup>R</sup> cassette into R2                                                      |
| 8       | R2-pamFp73-F<br>R2-pamFp73-R                                   | TGCGTCGCGACCGGGATCCTGCTGCTGATGCGCCATGCGACTTCCCTC<br>CTTCACAACCTATCCTAACTCATCGCTAGCCGTGGAGCCGGTACTCGC<br>GGGATGTATCAGGCGCC<br>CGACCCGACCGCTCTAGCGTCGCGCGCTGTGACAGCGGCACGCA<br>AATACTTGACATATCACTG                                                                | Primer for amplification of the upstream p73Up promoter and introduction with an Hyg <sup>R</sup> cassette into R2                                                          |
| 9       | R2-pamFp67-GU-R<br>R2-GU-F-all                                 | AAAAGGTACCGCGACTTCCCTCCTTCAGCTGGTATCTAATTCTGGGT<br>TTTTCTCGAGCCCGGTGAAAATACTTGACATATCACTGT<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                     | Primer for the amplification of the P67-Up promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>            |
| 10      | R2-pamAp67-GU-R<br>R2-GU-F-all                                 | AAAAGGTACCAACGATTCTCCGATAGCGA<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                                                                                  | Primer for the amplification of the P67Down promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>           |
| 11      | R2-pamFp73-GU-R<br>R2-GU-F-all                                 | AAAAGGTACCGCGACTTCCCTCCTTCACAACCTATCCTAACTCATCGCT<br>AGCCGTTGGAGCCGGTACTAAATACTTGACATATCACTGT<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                  | Primer for the amplification of the p73Up promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>             |
| 12      | R2-pamAp73-GU-R<br>R2-GU-F-all                                 | AAAAGGTACCAACGATTCTCCGATACCTC<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                                                                                  | Primer for the amplification of the p73Down promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>           |
| 13      | R2-pamFp100-GU-R<br>R2-GU-F-all                                | AAAAGGTACCGCGACTTCCCTCCTTCATCTGCGATCTAGCACGAAAC<br>AGGGTATGACAGCTGTGTTAAATACTTGACATATCACTGT<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                    | Primer for the amplification of the P100Up promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>            |
| 14      | R2-pamAp100-GU-R<br>R2-GU-F-all                                | AAAAGGTACCAACGATTCTCCGATAGAGG<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                                                                                  | Primer for the amplification of the P100Down promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>          |
| 15      | R2-pamA-GU-R<br>R2-GU-F-all                                    | AAAAGGTACCGGAAAGCTATCCGCCGG<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                                                                                    | Primer for the amplification of the native downstream promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i> |
| 16      | R2-PL-Nat-R<br>R2-PL-Nat-F                                     | AAAAGGTACCGAGACTCCCTGTGTGCGTG<br>TATGTGAATCACAGTGATATGTCAAGTATTTCCGGGAATTCGAGGGAC                                                                                                                                                                               | Primer for the amplification of the native upstream promoter for the cloning into pGUS with restriction site <i>SpeI</i>                                                    |
| 17      | R2-PL-Nat-HygR-R<br>R2-GU-F-all                                | GGTGGGCGATGTCCCTCGAATTCGGGCAATACTTGACATATCACTGT<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                                                                | Primer for the amplification of a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction site <i>XbaI</i>                                                     |
| 18      | R2-pamF-GU-R<br>R2-GU-F-all                                    | AAAAGGTACCGAGACTCCCTGTGTGCGTG<br>AAAATCTAGATCAGGCGCCGGGGCGGTGT                                                                                                                                                                                                  | Primer for the amplification of the native upstream promoter and a Hyg <sup>R</sup> cassette for the cloning into pGUS with restriction sites <i>SpeI</i> and <i>XbaI</i>   |

**Table S3.** Schematic overview of pamamycin production by engineered R2 constructs expressed in *S. albus* J1074 pTOS-P21pamW.

| Name  | Production | Production | Name  |
|-------|------------|------------|-------|
| R2-1  |            |            | R2-28 |
| R2-2  |            |            | R2-29 |
| R2-3  |            |            | R2-30 |
| R2-4  |            |            | R2-31 |
| R2-5  |            |            | R2-32 |
| R2-6  |            |            | R2-33 |
| R2-7  |            |            | R2-34 |
| R2-8  |            |            | R2-35 |
| R2-9  |            |            | R2-36 |
| R2-10 |            |            | R2-37 |
| R2-11 |            |            | R2-38 |
| R2-12 |            |            | R2-39 |
| R2-13 |            |            | R2-40 |
| R2-14 |            |            | R2-41 |
| R2-15 |            |            | R2-42 |
| R2-16 |            |            | R2-43 |
| R2-17 |            |            | R2-44 |
| R2-18 |            |            | R2-45 |
| R2-19 |            |            | R2-46 |
| R2-20 |            |            | R2-47 |
| R2-21 |            |            | R2-48 |
| R2-22 |            |            | R2-49 |
| R2-23 |            |            | R2-50 |
| R2-24 |            |            | R2-51 |
| R2-25 |            |            | R2-52 |
| R2-26 |            |            | R2-53 |
| R2-27 |            |            |       |

| Name  | Production | Production | Name   |
|-------|------------|------------|--------|
| R2-54 |            |            | R2-81  |
| R2-55 |            |            | R2-82  |
| R2-56 |            |            | R2-83  |
| R2-57 |            |            | R2-84  |
| R2-58 |            |            | R2-85  |
| R2-59 |            |            | R2-86  |
| R2-60 |            |            | R2-87  |
| R2-61 |            |            | R2-88  |
| R2-62 |            |            | R2-89  |
| R2-63 |            |            | R2-90  |
| R2-64 |            |            | R2-91  |
| R2-65 |            |            | R2-92  |
| R2-66 |            |            | R2-93  |
| R2-67 |            |            | R2-94  |
| R2-68 |            |            | R2-95  |
| R2-69 |            |            | R2-96  |
| R2-70 |            |            | R2-97  |
| R2-71 |            |            | R2-98  |
| R2-72 |            |            | R2-99  |
| R2-73 |            |            | R2-100 |
| R2-74 |            |            | R2-101 |
| R2-75 |            |            | R2-102 |
| R2-76 |            |            | R2-103 |
| R2-77 |            |            | R2-104 |
| R2-78 |            |            | R2-105 |
| R2-79 |            |            | R2-106 |
| R2-80 |            |            |        |

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12 Legend: red – strains producing no pamamycin or with the yield significantly lower than in the

13 control R2 carrying strain; green – strains with the pamamycin production on par or higher than

14 that of *S. albus* J1074 P21pamW/R2.

15

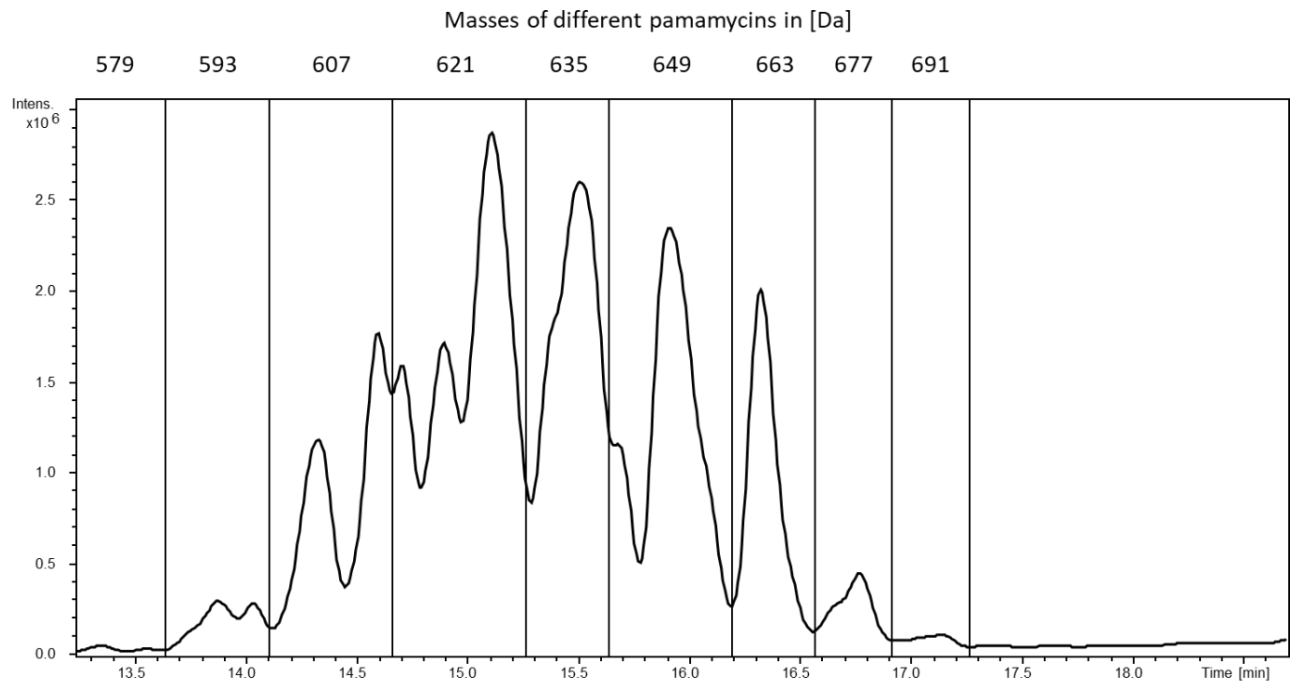


**Fig. S1.** High resolution MS-chromatograms of *S. albus* J1074 R2-73. Pamamycin derivatives are indicated by their molecular weight.

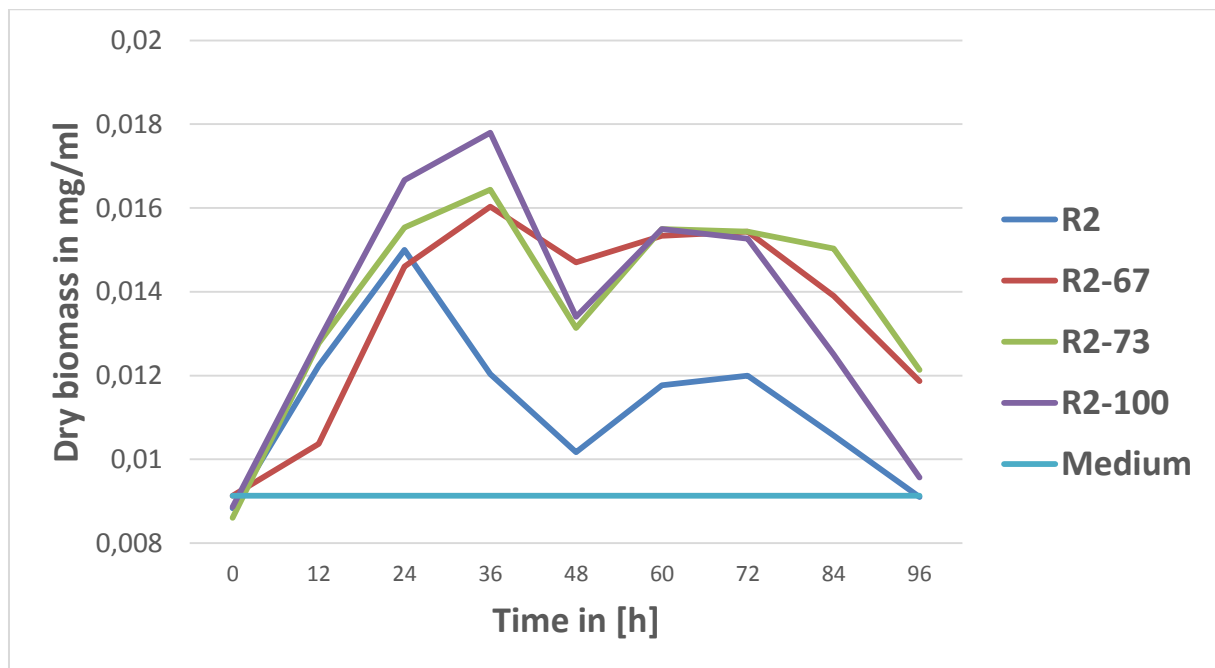


**Fig. S2.** Biomass of *S. albus* J1074 P21pamW strains containing different R2 cosmids over time: Blue: R2; Red: R2-67; green R2-73; R2-100; light blue: dry biomass of the medium.

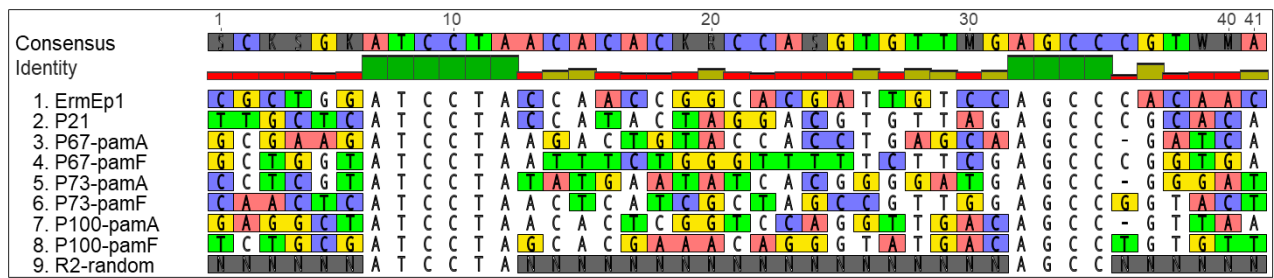


**Fig. S3.** Multiple sequence alignment of ErmEp1 to different synthetic promoters based on the -10 and -35 region of ErmEp1. Despite P21 all promoters were obtained in this research and P21 serves as an example for a very a promoter with strong activity.

**Table S4.** Sequences of the semisynthetic promoters (Sequence (5'→3')).

| Promoter Name | RBS   | Conserved | Random 5' UTR | -10 Region | Random spacer        | -35 Region | Random |
|---------------|-------|-----------|---------------|------------|----------------------|------------|--------|
| Promoter      | CCTCC | TTCA      | NNNNNN        | ATCCTA     | NNNNNNNNNNNNNNNNNNNN | AGCC       | NNNNNN |
| ErmE          |       |           | CGCTGG        | ATCCTA     | CCAACCGGCACGATTGTCC  | AGCC       | CACAAC |
| pamF67        | CCTCC | TTCA      | GCTGGT        | ATCCTA     | ATTTCTGGGTTTTCTTCG   | AGCC       | CGGTGA |
| pamF73        | CCTCC | TTCA      | CAACTC        | ATCCTA     | ACTCATCGCTAGCCGTTGG  | AGCC       | GGTACT |
| pamF 100      | CCTCC | TTCA      | TCTGCG        | ATCCTA     | GCACGAAACAGGGTATGAC  | AGCC       | TGTGTT |
| pamA67        | CCTCC | GATA      | GCGAAG        | ATCCTA     | AGACTGTACCACCTGAGCA  | AGCC       | GATCA  |
| pamA73        | CCTCC | GATA      | CCTCGT        | ATCCTA     | TATGAATATCACGGGGATG  | AGCC       | GGGAT  |
| pamA100       | CCTCC | GATA      | GAGGCT        | ATCCTA     | CACTCGGTCCAGGTTGAC   | AGCC       | GTTAA  |

**Table S5.** Results of antibacterial tests of different pamamycins.

| Indicator strain                         | MIC [ $\mu$ M] |          |          |
|------------------------------------------|----------------|----------|----------|
|                                          | Pam-607        | Pam-649A | Pam-663A |
| <i>E. coli</i> BW25113                   | > 128          | 32       | 32       |
| <i>E. coli</i> $\Delta$ acrB             | 32             | 32       | 32       |
| <i>P. aeruginosa</i> PA14                | > 128          | 128      | 128      |
| <i>P. aeruginosa</i> PA14 $\Delta$ mexAB | > 128          | 64       | 128      |
| <i>B. subtilis</i> DSM-10                | 8              | 2        | 2        |
| <i>E. faecium</i> DSM-20477              | 16             | 8        | 4        |
| <i>M. smegmatis</i> mc <sup>2</sup> 155  | 16             | 8        | 8        |
| <i>S. aureus</i> Newman                  | 8              | 2        | 1        |
| <i>S. aureus</i> N315 (MRSA)             | 16             | 2        | 1        |
| <i>S. pneumoniae</i> DSM-20566           | 4-8            | 1        | 1        |

**Table S6.** Results of activity tests of pamamycins with different molecular weight against cell lines

| Cell line | IC <sub>50</sub> [μM] |         |         |             |
|-----------|-----------------------|---------|---------|-------------|
|           | Pam-607               | Pam-649 | Pam-663 | Doxorubicin |
| KB-3.1    | 0.48                  | 0.06    | 0.02    | 0.00034     |
| HepG2     | 0.62                  | 0.01    | 0.002   | 0.00029     |

**Table S7.** Results of activity tests of pamamycins with different molecular weight against zebra fish embryos

| <i>Danio rerio</i> line and age | MTC [μM] |         |         |
|---------------------------------|----------|---------|---------|
|                                 | Pam-607  | Pam-649 | Pam-663 |
| TL; 1 dpf (embryos)             | < 10     | < 10    | < 10    |
| TL; 3 dpf (larvae)              | < 10     | 10      | < 10    |

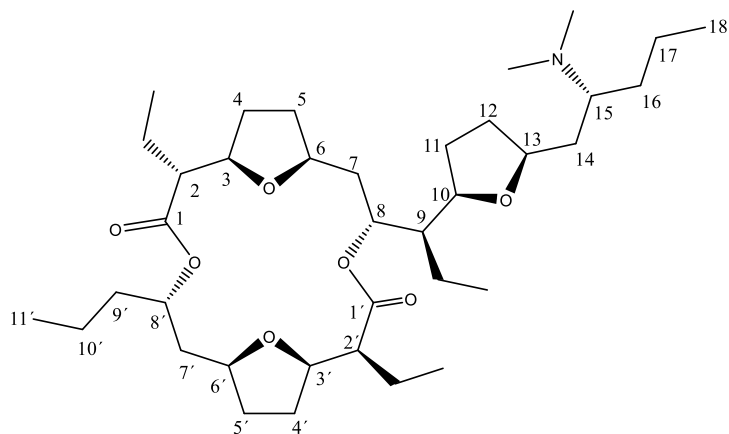
**Table S8.** Results of activity tests of pamamycins with different molecular weight against *Agrostis stolonifera*

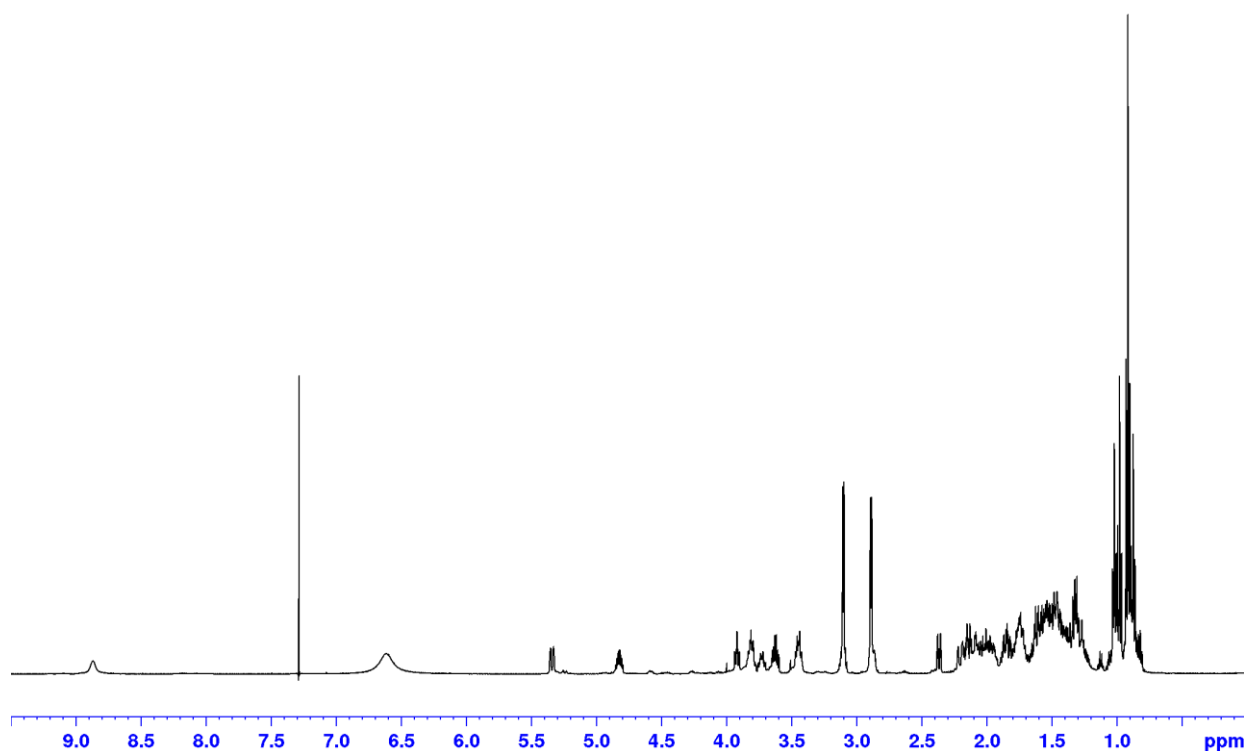
| <i>Agrostis stolonifera</i> | Different pamamycins and their Concentration [μM] |               |               |
|-----------------------------|---------------------------------------------------|---------------|---------------|
|                             | Number of grown plants                            |               |               |
| Dilution                    | Pam-607                                           | Pam-649A      | Pam-663A      |
| 0                           | 41.2<br>0                                         | 38.5<br>0     | 37.7<br>0     |
| 1                           | 20.6<br>2                                         | 19.25<br>3    | 18.85<br>4    |
| 2                           | 10.3<br>5                                         | 9.625<br>>5   | 9.425<br>5    |
| 3                           | 5.15<br>>5                                        | 4.8125<br>>5  | 4.7125<br>>5  |
| 4                           | 2.575<br>>5                                       | 2.40625<br>>5 | 2.35625<br>>5 |

55 **Table S'1.** NMR data (500 MHz, CDCl<sub>3</sub>) for Pamamycin-635 G

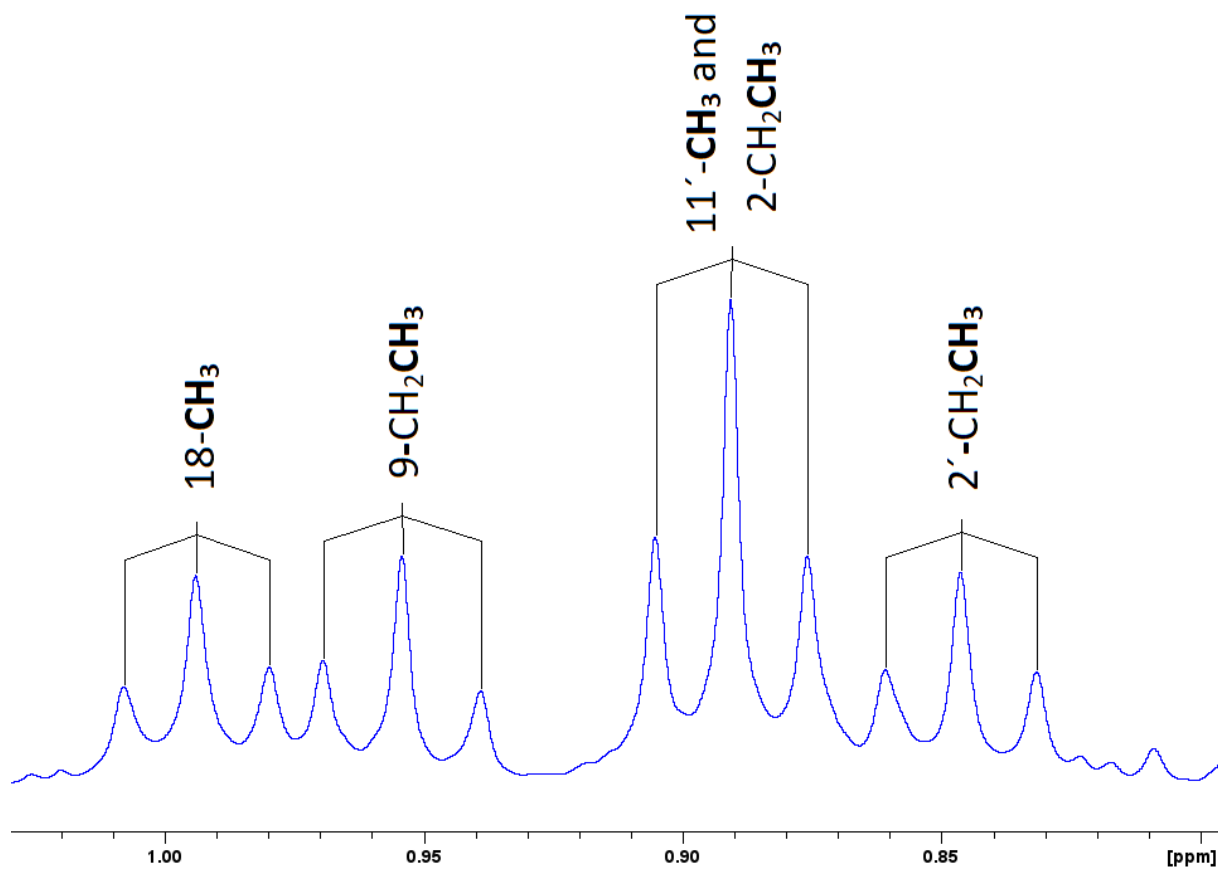
| 56 |                             | $\delta_c$ m           | $\delta_{CH}$ (J in Hz)   |
|----|-----------------------------|------------------------|---------------------------|
| 57 | <b>1</b>                    | 172.80 C               | -----                     |
| 58 | <b>2</b>                    | 54.86 CH               | 2.13 m                    |
| 59 | <b>3</b>                    | 81.77 CH               | 3.61 td (10.5, 5.0)       |
| 60 | <b>4</b>                    | 29.55 CH <sub>2</sub>  | 1.29 m and 1.93 m         |
| 61 | <b>5</b>                    | 30.59 CH <sub>2</sub>  | 1.47 m and 2.03 m         |
| 62 | <b>6</b>                    | 74.47 CH               | 3.79 m                    |
| 63 | <b>7</b>                    | 38.90 CH <sub>2</sub>  | 1.44 m and 2.13 m         |
| 64 | <b>8</b>                    | 70.06 CH               | 5.32 ddd (12.5, 3.0, 1.5) |
| 65 | <b>9</b>                    | 49.33 CH               | 1.30 m                    |
| 66 | <b>10</b>                   | 79.65 CH               | 3.42 dt (10.5, 7.0)       |
| 67 | <b>11</b>                   | 29.03 CH <sub>2</sub>  | 1.58 m and 2.05 m         |
| 68 | <b>12</b>                   | 30.89 CH <sub>2</sub>  | 1.52 m and 2.06 m         |
| 69 | <b>13</b>                   | 78.65 CH               | 3.80 br m                 |
| 70 | <b>14</b>                   | 33.73 CH <sub>2</sub>  | 1.73 m and 2.18 m         |
| 71 | <b>15</b>                   | 67.91 CH               | 3.40 m                    |
| 72 | <b>16</b>                   | 28.72 CH <sub>2</sub>  | 1.46 m and 1.72 m         |
| 73 | <b>17</b>                   | 19.56 CH <sub>2</sub>  | 1.40 m and 1.50 m         |
| 74 | <b>18</b>                   | 13.86 CH <sub>3</sub>  | 0.99 t (7.0, 3H)          |
| 75 | <b>1'</b>                   | 173.44 C               | -----                     |
| 76 | <b>2'</b>                   | 49.89 CH <sub>2</sub>  | 2.34 dt (11.3, 2.5)       |
| 77 | <b>3'</b>                   | 79.15 CH               | 3.89 ddd (9.3, 7.5, 2.5)  |
| 78 | <b>4'</b>                   | 27.35 CH <sub>2</sub>  | 1.70 m and 1.83 m         |
| 79 | <b>5'</b>                   | 31.44 CH <sub>2</sub>  | 1.36 m and 1.97 m         |
| 80 | <b>6'</b>                   | 74.38 CH               | 3.72 m                    |
| 81 | <b>7'</b>                   | 38.26 CH <sub>2</sub>  | 1.83 m and 1.58 m         |
| 82 | <b>8'</b>                   | 71.38 CH               | 4.80 m                    |
| 83 | <b>9'</b>                   | 36.62 CH <sub>2</sub>  | 1.47 m and 1.62 m         |
| 84 | <b>10'</b>                  | 18.02 CH <sub>2</sub>  | 1.30 m (2H)               |
| 85 | <b>11'</b>                  | 13.96 CH <sub>3</sub>  | 0.89 t (7.5, 3H)          |
| 86 | <b>2-Et</b>                 | 22.04 CH <sub>2</sub>  | 1.43 m and 1.54 m         |
| 87 |                             | 11.74 CH <sub>3</sub>  | 0.89 t (7.5, 3H)          |
| 88 | <b>9-Et</b>                 | 18.92 CH <sub>2</sub>  | 1.21 m and 1.52 m         |
| 89 |                             | 13.81 CH <sub>3</sub>  | 0.95 (3H)                 |
| 90 | <b>15-N(Me)<sub>2</sub></b> | 36.31 CH <sub>3a</sub> | 2.86 d (5.0, 3H)          |
| 91 |                             | 43.33 CH <sub>3b</sub> | 3.08 d (5.0, 3H)          |
| 92 | <b>2'-Et</b>                | 16.02 CH <sub>2</sub>  | 1.38 m and 1.75 m         |
| 93 |                             | 12.69 CH <sub>3</sub>  | 0.85 t (7.5, 3H)          |
| 94 | <b>NH</b>                   |                        | 8.87 br s                 |

| Position                      | HMBC correlations                       |     |
|-------------------------------|-----------------------------------------|-----|
| H-2                           | C-1, C-3, C-2-Et                        | 91  |
| H-3                           | C-1, C-2, C-4, C-5, C-2-Et              | 92  |
| H-6                           | C-3, C-8                                | 93  |
| H-8                           | C-6, C-7, C-9, C-10, C-1', C-9-Et       | 94  |
| H-10                          | C-8, C-9, C-9- C-2-Et                   | 95  |
| H-13                          | C-11, C-15                              | 96  |
| H-15                          | C-12, C-14, C-17, 15-N-CH <sub>3a</sub> | 97  |
| H-18                          | C-16, C-17                              | 98  |
| H-2'                          | C-1', C-3', C-2'-Et                     | 99  |
| H-3'                          | C-1', C-2', C-4', C-5', C-2'-Et         | 100 |
| H-6'                          | C-3', C-8'                              | 101 |
| H-8'                          | C-1, C-6', C-7', C-10'                  | 102 |
| H-11'                         | C-9', C-10'                             | 103 |
| CH <sub>3</sub> -2 (from Et)  | C-2                                     | 104 |
| CH <sub>3</sub> -9 (from Et)  | C-9                                     | 105 |
| CH <sub>3</sub> -2' (from Et) | C-2'                                    | 106 |
| CH <sub>3a</sub> -N           | C-15, 15-N-CH <sub>3b</sub>             | 107 |
| CH <sub>3b</sub> -N           | C-15, 15-N-CH <sub>3a</sub>             |     |

**Fig. S'1.** Structure of Pamamycin-635 G



120

121 **Fig. S'3.**  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{CDCl}_3$ ) of Pamamycin-635 G

122

123 **Fig. S'3.**  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{CDCl}_3$ ) of Pamamycin-635 G, methyl region

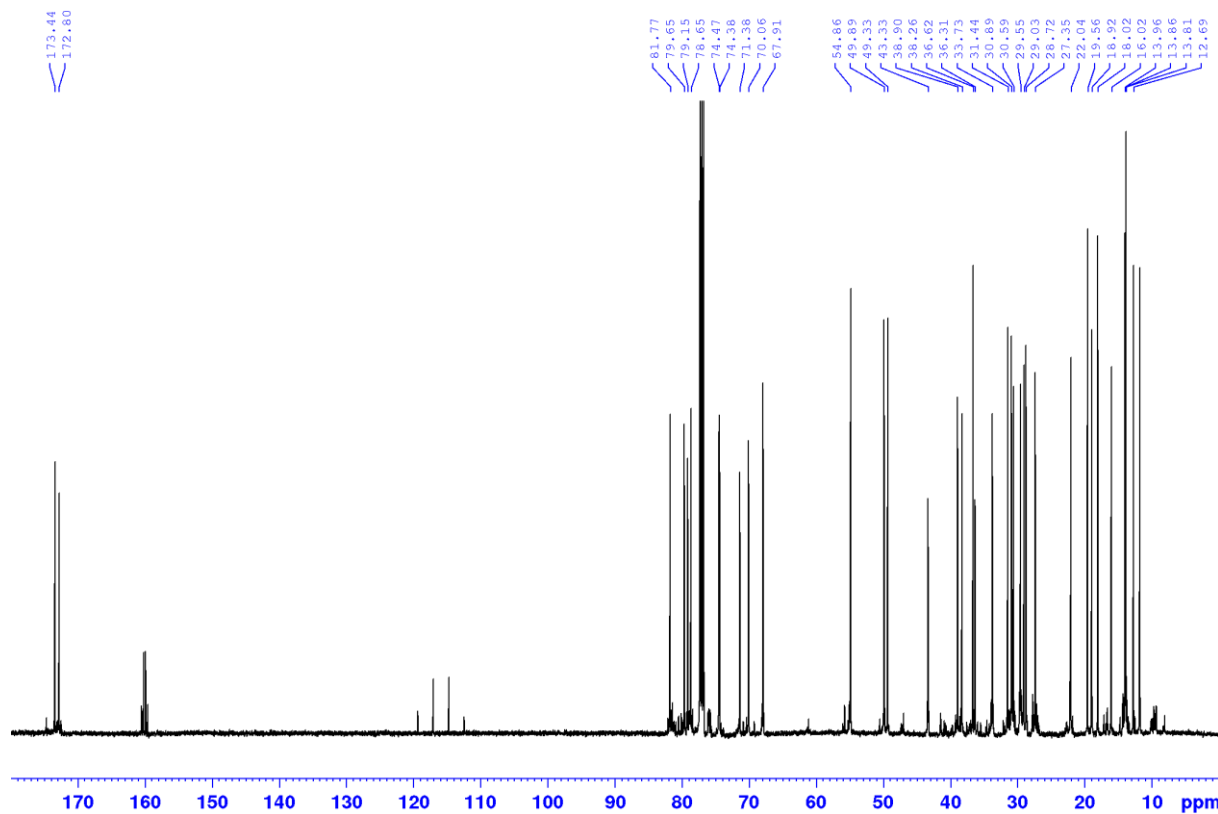


**Fig. S'4.**  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{CDCl}_3$ ) of Pamamycin-635G

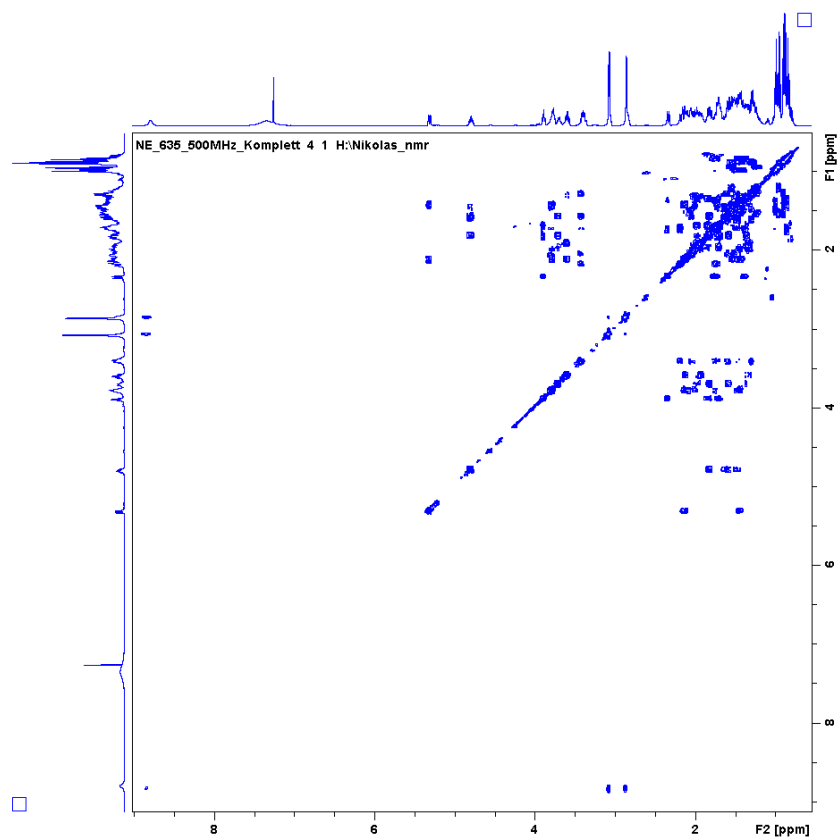


**Fig. S'4.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum ( $\text{CDCl}_3$ ) of Pamamycin-635 G

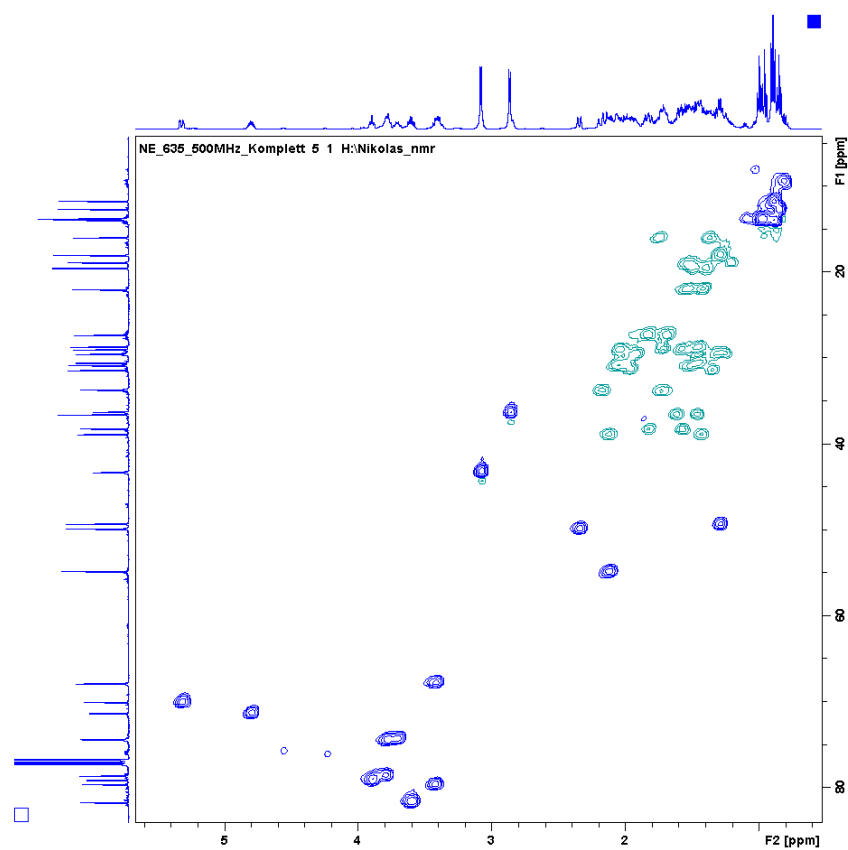


**Fig. S'5.** Edited HSQC spectrum (CDCl<sub>3</sub>) of Pamamycin-635 G

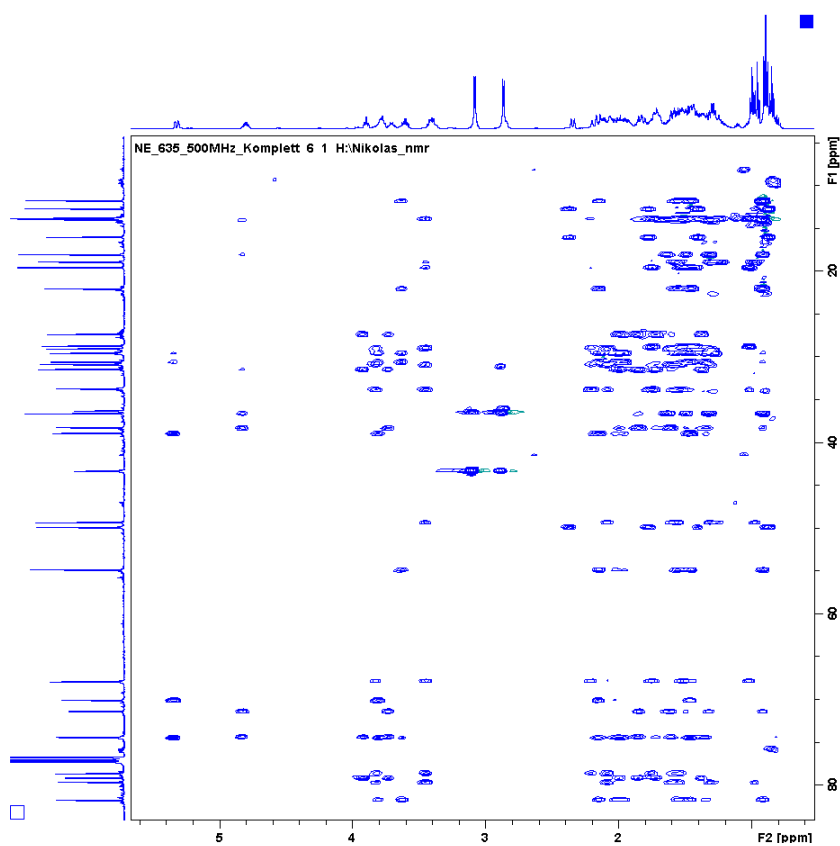


**Fig. S'6.** HSQC-TOCSY spectrum (CDCl<sub>3</sub>) of Pamamycin-635 G

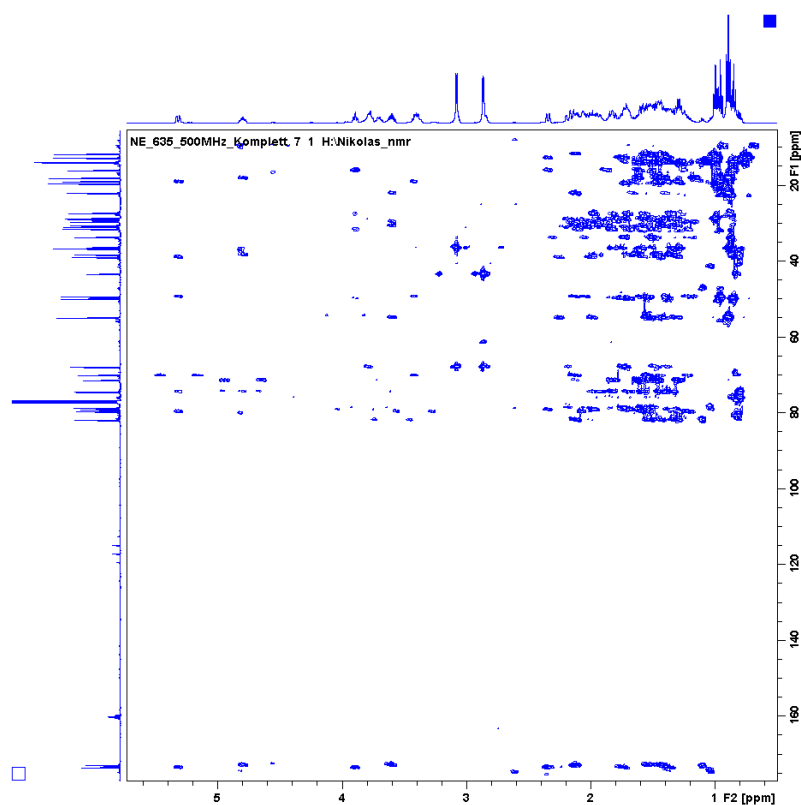


**Fig. S'7.** HMBC spectrum (CDCl<sub>3</sub>) of Pamamycin-635 G

155 **Table S'3.** NMR data (700 MHz, CDCl<sub>3</sub>) for Pamamycin-663 A

|                             | $\delta_c$ m           | $\delta_{CH}$ m (J in Hz)  |
|-----------------------------|------------------------|----------------------------|
| <b>1</b>                    | 172.31 C               | -----                      |
| <b>2</b>                    | 55.31 CH               | 2.11 ddd (14.0, 10.0, 4.0) |
| <b>3</b>                    | 81.34 CH               | 3.66 ddd (11.0, 10.0, 4.2) |
| <b>4</b>                    | 30.90 CH <sub>2</sub>  | 1.20 m and 1.94 m          |
| <b>5</b>                    | 27.51 CH <sub>2</sub>  | 1.71 m and 1.94 m          |
| <b>6</b>                    | 76.19 CH               | 4.21 ddd (9.0, 5.3, 2.2)   |
| <b>7</b>                    | 37.51 CH               | 1.93 m                     |
| <b>8</b>                    | 75.20 CH               | 4.91 d (11.0)              |
| <b>9</b>                    | 46.41 CH               | 1.53 m                     |
| <b>10</b>                   | 79.72 CH               | 3.36 dt (10.0, 7.0)        |
| <b>11</b>                   | 29.18 CH <sub>2</sub>  | 1.61 m and 2.06 m          |
| <b>12</b>                   | 30.99 CH <sub>2</sub>  | 1.53 m and 2.09 m          |
| <b>13</b>                   | 78.64 CH               | 3.75 br s                  |
| <b>14</b>                   | 33.81 CH <sub>2</sub>  | 1.71 m and 2.21 m          |
| <b>15</b>                   | 68.13 CH               | 3.39 br t (11.0)           |
| <b>16</b>                   | 28.72 CH <sub>2</sub>  | 1.46 m and 1.72 m          |
| <b>17</b>                   | 19.61 CH <sub>2</sub>  | 1.38 m and 1.52 m          |
| <b>18</b>                   | 13.81 CH <sub>3</sub>  | 1.00 t (7.0, 3H)           |
| <b>1'</b>                   | 173.07 C               | -----                      |
| <b>2'</b>                   | 50.12 CH <sub>2</sub>  | 2.36 dt (11.5, 2.5)        |
| <b>3'</b>                   | 78.13 CH               | 3.83 ddd (9.2, 6.0, 2.5)   |
| <b>4'</b>                   | 27.85 CH <sub>2</sub>  | 1.62 m and 1.81 m          |
| <b>5'</b>                   | 26.70 CH <sub>2</sub>  | 1.64 m and 1.77 m          |
| <b>6'</b>                   | 76.25 CH               | 3.94 td (7.6, 2.8)         |
| <b>7'</b>                   | 36.85 CH               | 1.87 m                     |
| <b>8'</b>                   | 75.77 CH               | 4.57 dt (11.0, 4.0)        |
| <b>9'</b>                   | 34.24 CH <sub>2</sub>  | 1.55 m and 1.68 m          |
| <b>10'</b>                  | 16.79 CH <sub>2</sub>  | 1.25 m and 1.34 m          |
| <b>11'</b>                  | 14.21 CH <sub>3</sub>  | 0.88 t (7.5, 3H)           |
| <b>2-Et</b>                 | 22.22 CH <sub>2</sub>  | 1.42 m and 1.56 m          |
|                             | 11.75 CH <sub>3</sub>  | 0.90 t (7.5, 3H)           |
| <b>7-Me</b>                 | 10.06 CH <sub>3</sub>  | 0.82 d (7.0, 3H)           |
| <b>9-Et</b>                 | 18.98 CH <sub>2</sub>  | 1.18 m and 1.54 m          |
|                             | 13.86 CH <sub>3</sub>  | 0.95 (3H)                  |
| <b>15-N(Me)<sub>2</sub></b> | 35.97 CH <sub>3a</sub> | 2.86 d (5.0, 3H)           |
|                             | 43.59 CH <sub>3b</sub> | 3.10 d (5.0, 3H)           |
| <b>2'-Et</b>                | 15.99 CH <sub>2</sub>  | 1.37 m and 1.77 m          |
|                             | 12.70 CH <sub>3</sub>  | 0.83 t (7.5, 3H)           |
| <b>7'-Me</b>                | 9.34 CH <sub>3</sub>   | 0.82 d (7.0, 3H)           |
| <b>NH</b>                   | -----                  | 8.74 br s                  |

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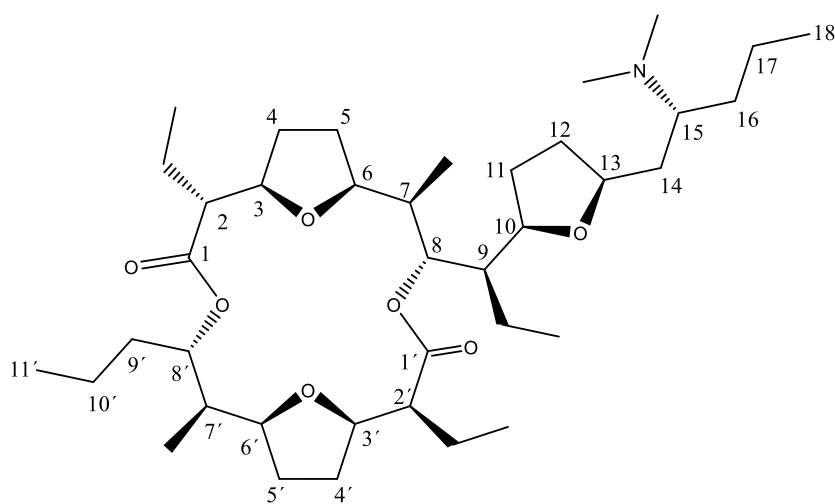
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161 **Table S'4.** HMBC Key correlations for Pamamycin-663 A

| Position                      | HMBC correlations                                       |
|-------------------------------|---------------------------------------------------------|
| H-2                           | C-1, C-3, C-4, C-2-Et                                   |
| H-3                           | C-1, C-2, C-5, C-2-Et                                   |
| H-6                           | C-7, C-8, -7-Me                                         |
| H-7                           | C-5, C-6, C-8, C-9, C-7-Me                              |
| H-8                           | C-6, C-7, C-9, C-10, C-1', C-7-CH <sub>3</sub> , C-9-Et |
| H-10                          | C-8, C-9, C-9- C-2-Et                                   |
| H-13                          | C-15                                                    |
| H-18                          | C-16, C-17                                              |
| H-2'                          | C-1', C-3', C-2'-Et                                     |
| H-3'                          | C-1', C-5', C-2'-Et                                     |
| H-6'                          | C-7', C-8', 7'-Me                                       |
| H-7'                          | C-8', C-9', 7'-Me                                       |
| H-8'                          | C-1, C-6', C-7', C-9', C-10', 7'-Me                     |
| H-11'                         | C-9', C-10'                                             |
| CH <sub>3</sub> -2 (from Et)  | C-2                                                     |
| CH <sub>3</sub> -7            | C-7                                                     |
| CH <sub>3</sub> -9 (from Et)  | C-9                                                     |
| CH <sub>3</sub> -2' (from Et) | C-2'                                                    |
| CH <sub>3</sub> -7'           | C-7'                                                    |
| CH <sub>3a</sub> -N           | C-15, 15-N-CH <sub>3b</sub>                             |
| CH <sub>3b</sub> -N           | C-15, 15-N-CH <sub>3a</sub>                             |



173 **Fig. S'8.** Structure of Pamamycin-663 A

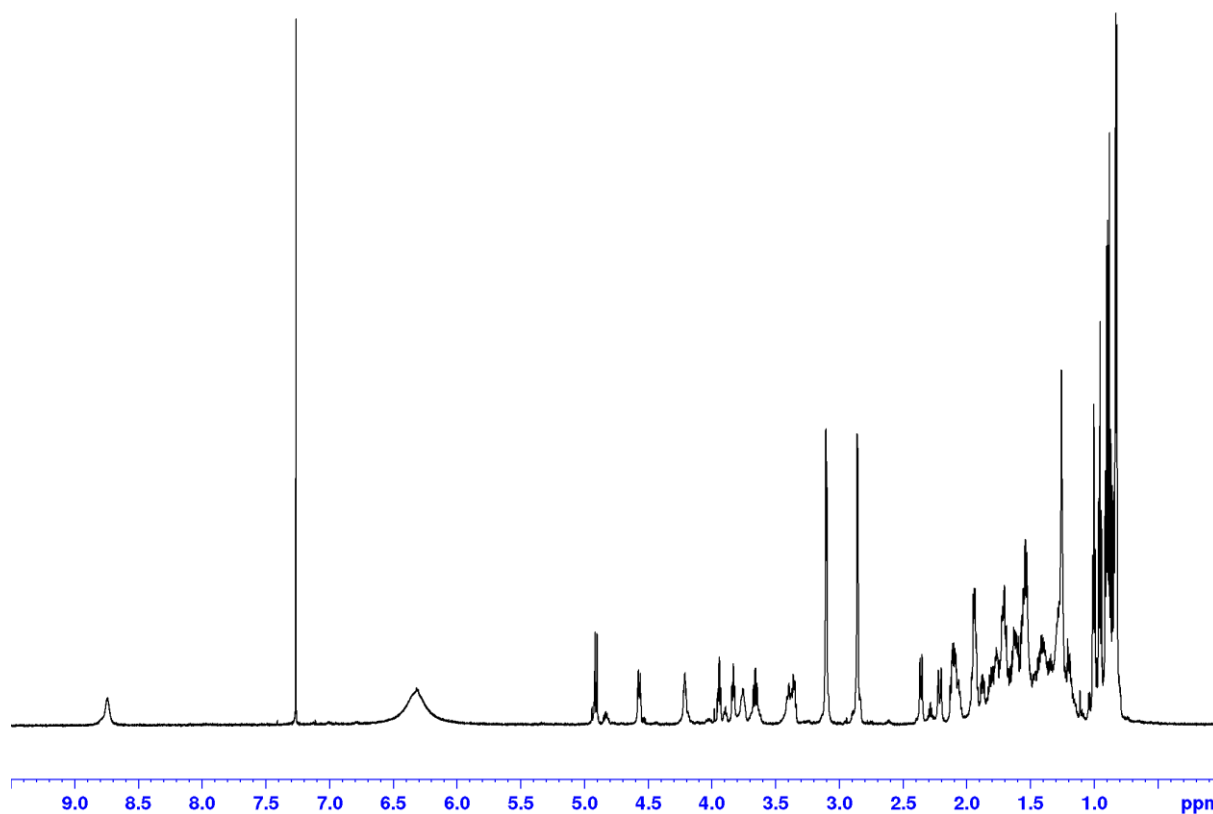


**Fig. S'9.**  $^1\text{H}$  NMR spectrum (700 MHz,  $\text{CDCl}_3$ ) of Pamamycin-663 A

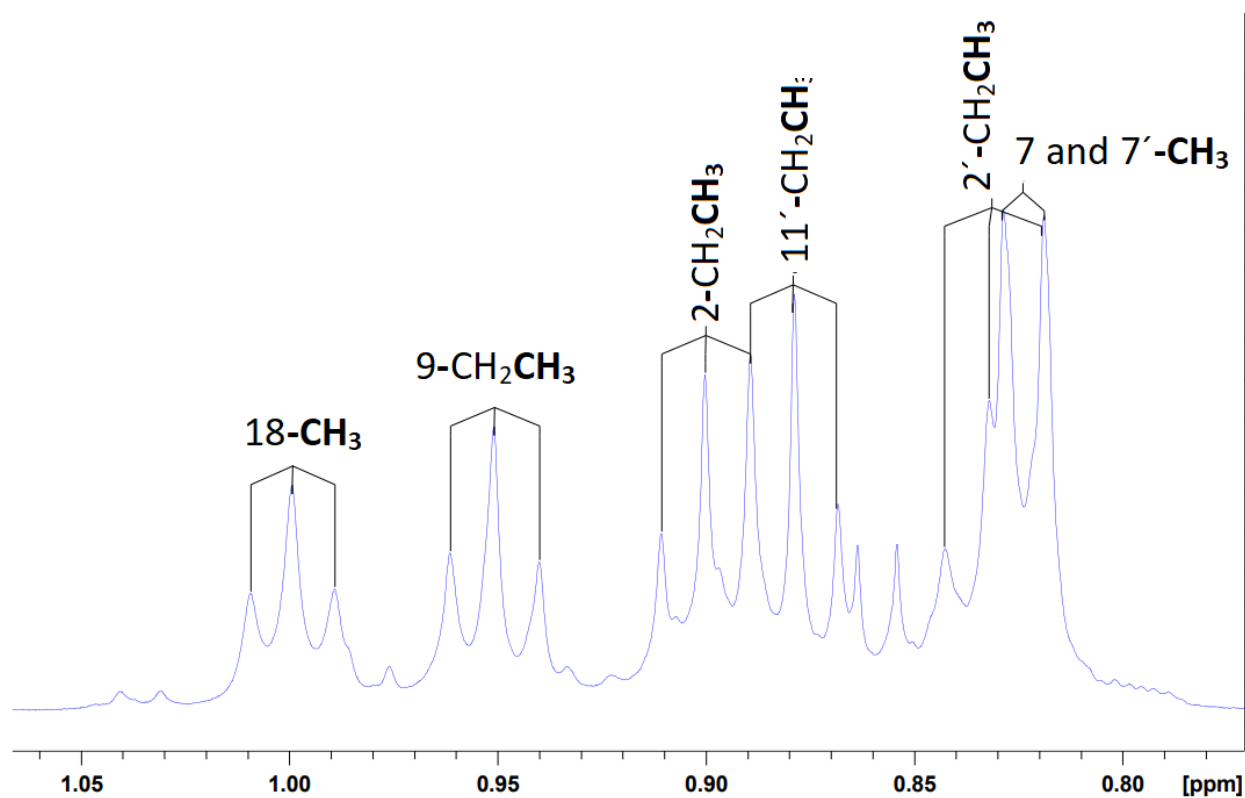


**Fig. S'10.**  $^1\text{H}$  NMR spectrum (700 MHz,  $\text{CDCl}_3$ ) of Pamamycin-663 A, Methyl region

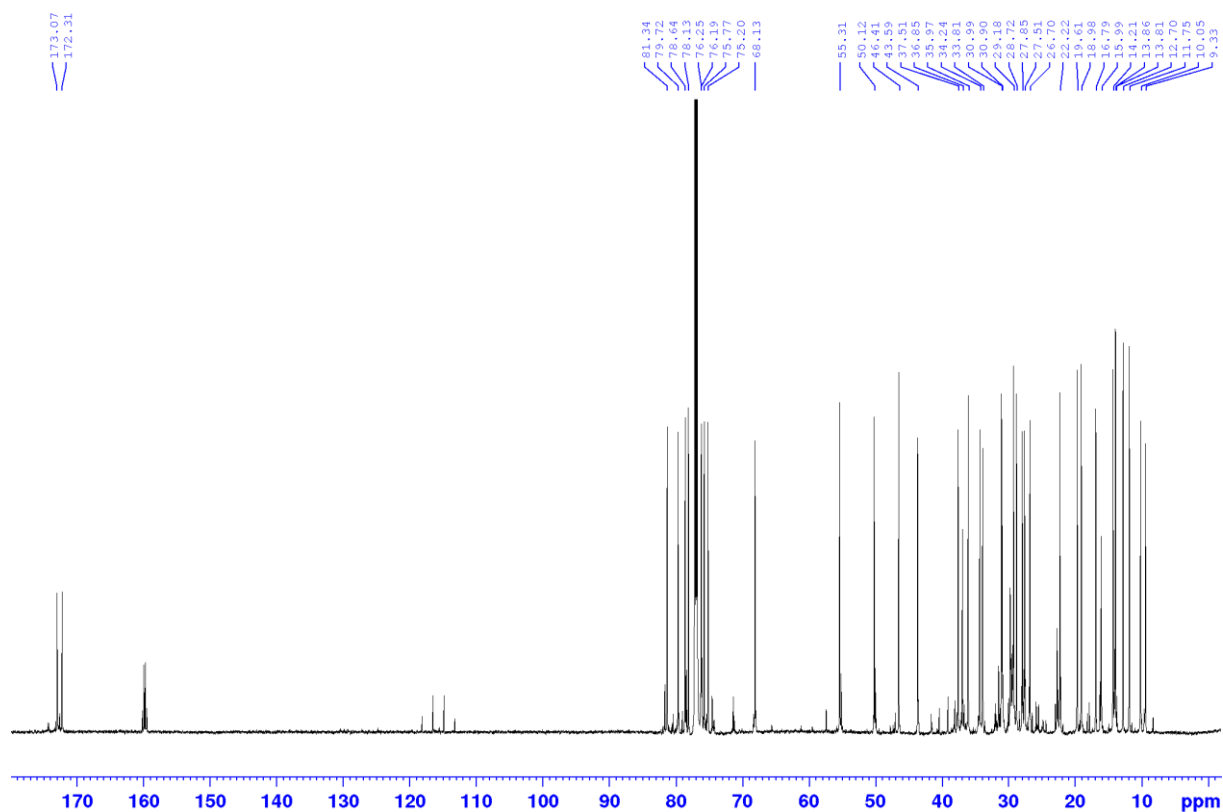


**Fig. S'11.**  $^{13}\text{C}$  NMR spectrum (175 MHz,  $\text{CDCl}_3$ ) of Pamamycin-663 A

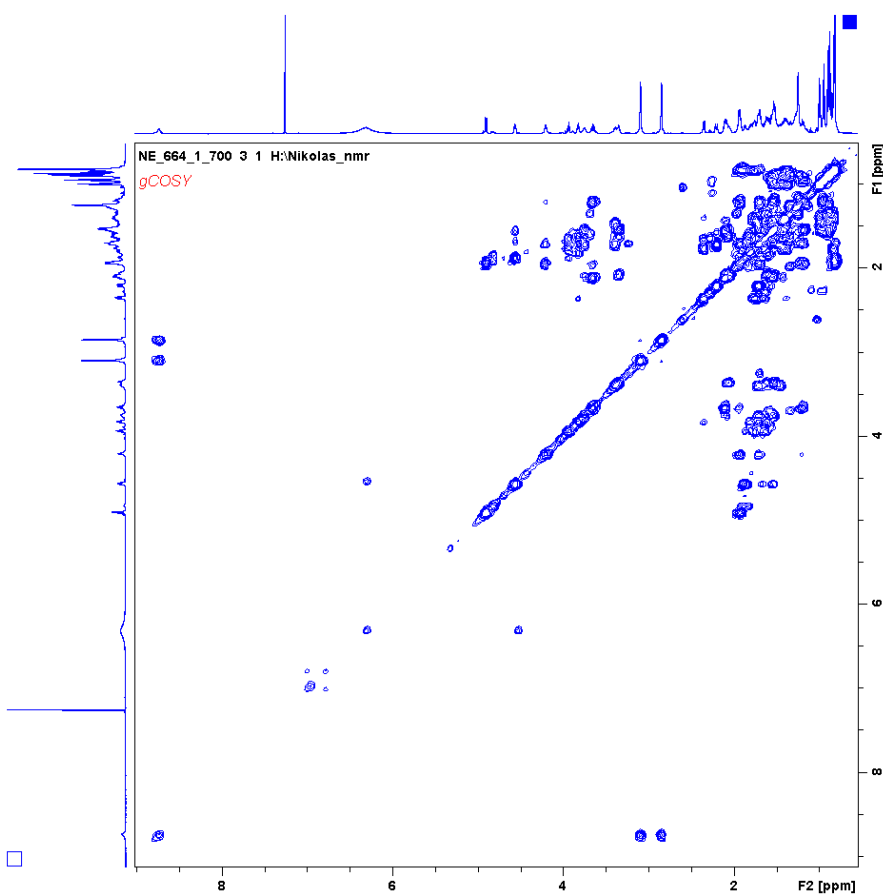


**Fig. S'12.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum ( $\text{CDCl}_3$ ) of Pamamycin-663 A

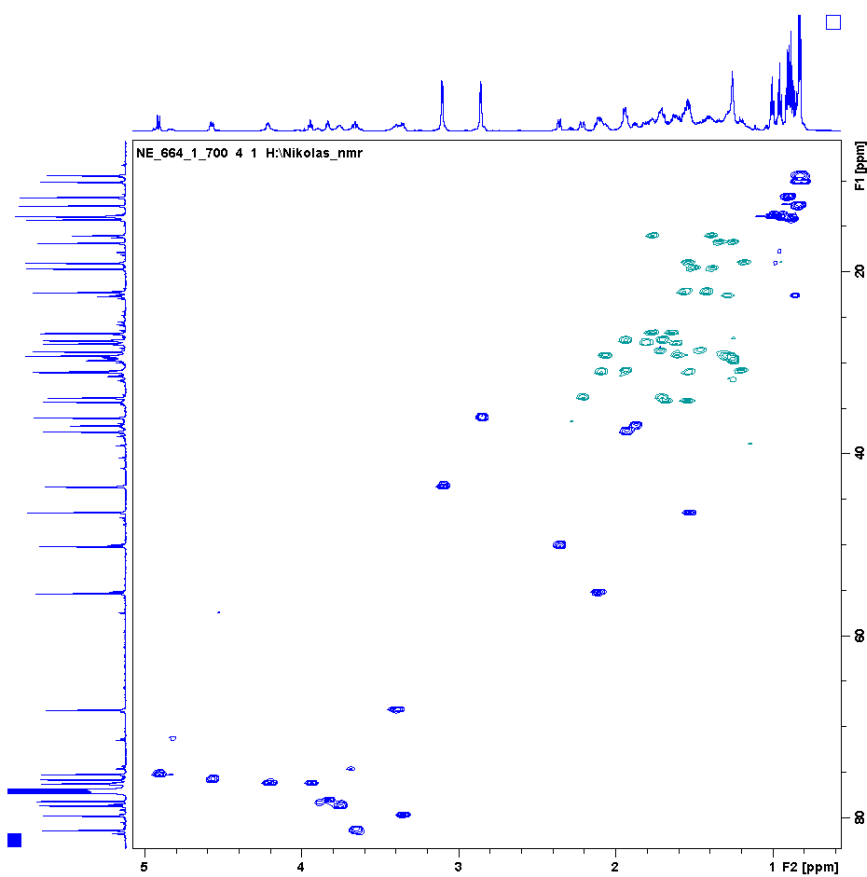


**Fig. S'13.** Edited HSQC spectrum (CDCl<sub>3</sub>) of Pamamycin-663 A

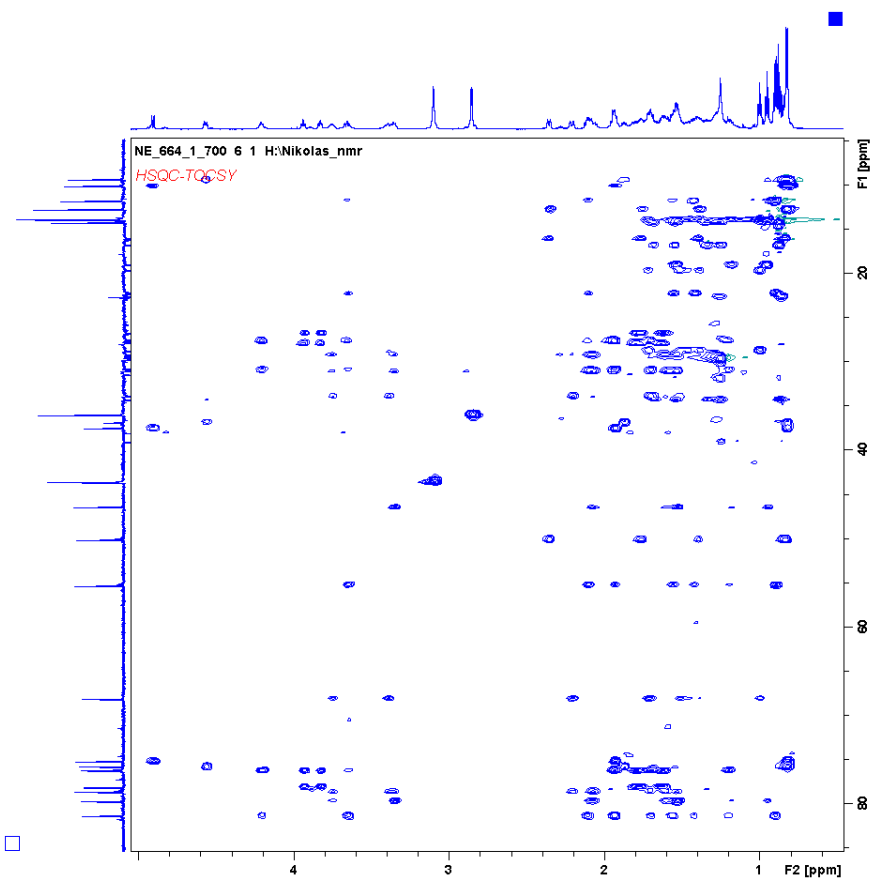


**Fig. S'14.** HSQC-TOCSY spectrum (CDCl<sub>3</sub>) of Pamamycin-663 A

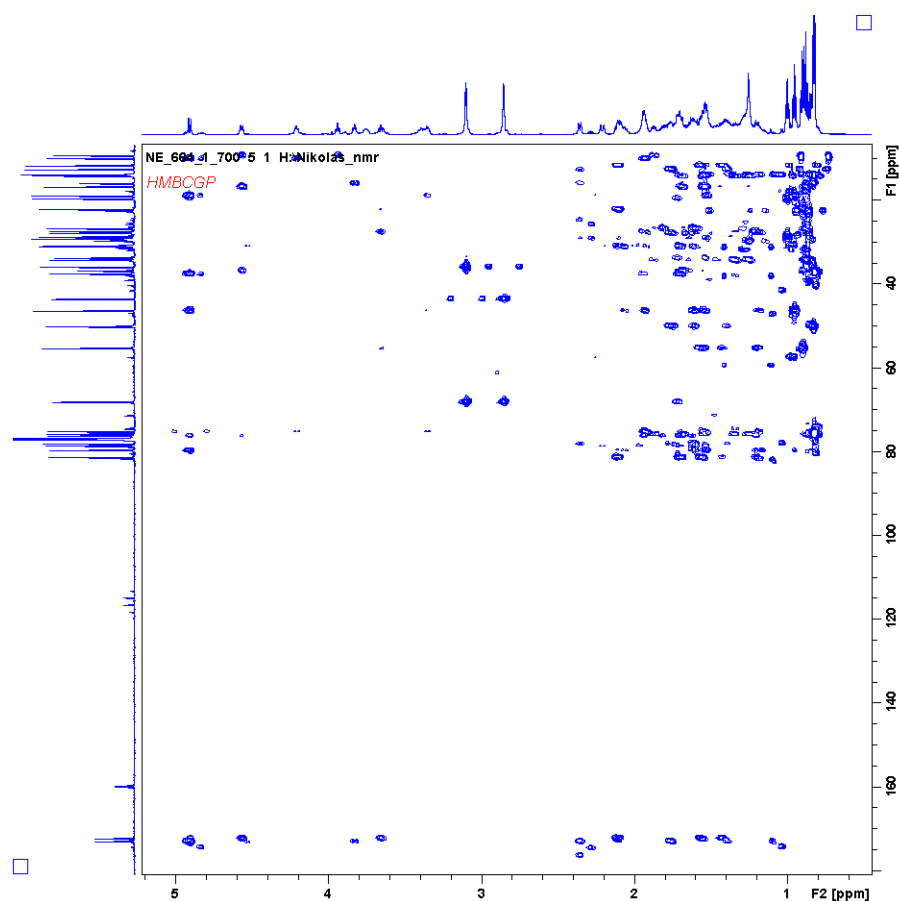


**Fig. S'15.** HMBC spectrum (CDCl<sub>3</sub>) of Pamamycin-663 A

206 **Table S'5.** NMR data (700 MHz, CDCl<sub>3</sub>) of Homopamamycin-677 A

|                             | $\delta_c$ m           | $\delta_{CH}$ m (J in Hz) |
|-----------------------------|------------------------|---------------------------|
| <b>1</b>                    | 172.30 C               | -----                     |
| <b>2</b>                    | 55.28 CH               | 2.11 m                    |
| <b>3</b>                    | 81.34 CH               | 3.65 td (11.0, 4.0)       |
| <b>4</b>                    | 30.99 CH <sub>2</sub>  | 1.20 m and 1.94 m         |
| <b>5</b>                    | 27.51 CH <sub>2</sub>  | 1.70 m and 1.94 m         |
| <b>6</b>                    | 76.15 CH               | 4.22 m                    |
| <b>7</b>                    | 37.46 CH               | 1.93 m                    |
| <b>8</b>                    | 75.15 CH               | 4.92 d (11.0)             |
| <b>9</b>                    | 46.34 CH               | 1.53 m                    |
| <b>10</b>                   | 79.65 CH               | 3.34 m                    |
| <b>11</b>                   | 29.14 CH <sub>2</sub>  | 1.61 m and 2.07 m         |
| <b>12</b>                   | 30.94 CH <sub>2</sub>  | 1.53 m and 2.09 m         |
| <b>13</b>                   | 78.59 CH               | 3.76 m                    |
| <b>14</b>                   | 33.71 CH <sub>2</sub>  | 1.71 m and 2.21 m         |
| <b>15</b>                   | 68.05 CH               | 3.38 br t (11.0)          |
| <b>16</b>                   | 28.67 CH <sub>2</sub>  | 1.46 m and 1.72 m         |
| <b>17</b>                   | 19.61 CH <sub>2</sub>  | 1.38 m and 1.50 m         |
| <b>18</b>                   | 13.81 CH <sub>3</sub>  | 0.99 t (7.0, 3H)          |
| <b>1'</b>                   | 173.04 C               | -----                     |
| <b>2'</b>                   | 50.07 CH <sub>2</sub>  | 2.35 dt (11.5, 2.5)       |
| <b>3'</b>                   | 78.12 CH               | 3.82 td (6.5, 2.5)        |
| <b>4'</b>                   | 27.83 CH <sub>2</sub>  | 1.61 m and 1.81 m         |
| <b>5'</b>                   | 26.66 CH <sub>2</sub>  | 1.65 m and 1.77 m         |
| <b>6'</b>                   | 76.22 CH               | 3.91 td (7.6, 2.8)        |
| <b>7'</b>                   | 36.54 CH               | 1.89 m                    |
| <b>8'</b>                   | 75.84 CH               | 4.59 dt (11.0, 4.0)       |
| <b>9'</b>                   | 34.12 CH <sub>2</sub>  | 1.50 m and 1.79 m         |
| <b>10'</b>                  | 25.34 CH <sub>2</sub>  | 1.25 m and 1.34 m         |
| <b>11'</b>                  | 22.89 CH <sub>2</sub>  | 1.28 m (2H)               |
| <b>12'</b>                  | 14.01 CH <sub>3</sub>  | 0.87 t (7.5, 3H)          |
| <b>2-Et</b>                 | 22.25 CH <sub>2</sub>  | 1.43 m and 1.57 m         |
|                             | 11.77 CH <sub>3</sub>  | 0.90 t (7.5, 3H)          |
| <b>7-Me</b>                 | 10.03 CH <sub>3</sub>  | 0.82 d (7.0, 3H)          |
| <b>9-Et</b>                 | 18.98 CH <sub>2</sub>  | 1.18 m and 1.54 m         |
|                             | 13.91 CH <sub>3</sub>  | 0.95 t (7.5, 3H)          |
| <b>15-N(Me)<sub>2</sub></b> | 35.87 CH <sub>3a</sub> | 2.84 m                    |
|                             | 43.50 CH <sub>3b</sub> | 3.09 m                    |
| <b>2'-Et</b>                | 15.99 CH <sub>2</sub>  | 1.39 m and 1.77 m         |
|                             | 12.65 CH <sub>3</sub>  | 0.83 t                    |
| <b>7'-Me</b>                | 9.27 CH <sub>3</sub>   | 0.82 m                    |
| <b>NH</b>                   | -----                  | 8.74 br s                 |

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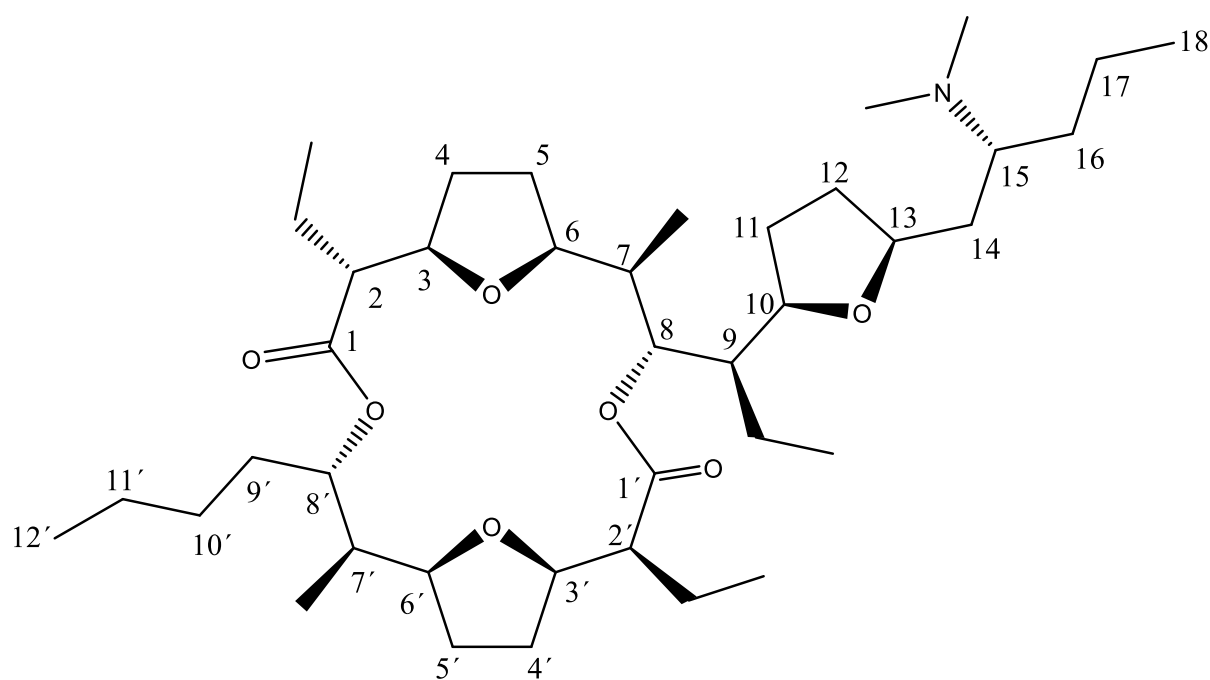
208

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210

211 **Table S'6.** HMBC Key correlations for Homopamamycin-677 A

| Position                      | HMBC correlations                    |
|-------------------------------|--------------------------------------|
| H-2                           | C-1, C-3, C-2-Et                     |
| H-3                           | C-1                                  |
| H-6                           | C-7-Me                               |
| H-8                           | C-7, C-9, C-10, C-1', C-7-Me, C-9-Et |
| H-10                          | C-8, C-9, C-9-Et                     |
| H-13                          | C-15                                 |
| H-18                          | C-16, C-17                           |
| H-3'                          | C-1', C-2'-Et                        |
| H-6'                          | C-7'-Me                              |
| H-8'                          | C-1, C-6', C-7', C-7'-Me             |
| CH <sub>3</sub> -2 (from Et)  | C-2                                  |
| CH <sub>3</sub> -7            | C-7                                  |
| CH <sub>3</sub> -9 (from Et)  | C-9                                  |
| CH <sub>3</sub> -2' (from Et) | C-2'                                 |
| CH <sub>3</sub> -7'           | C-7'                                 |
| CH <sub>3a</sub> -N           | C-15, 15-N-CH <sub>3b</sub>          |
| CH <sub>3b</sub> -N           | C-15, 15-N-CH <sub>3a</sub>          |



228 **Fig. S'16.** Structure of Homopamamycin-677 A

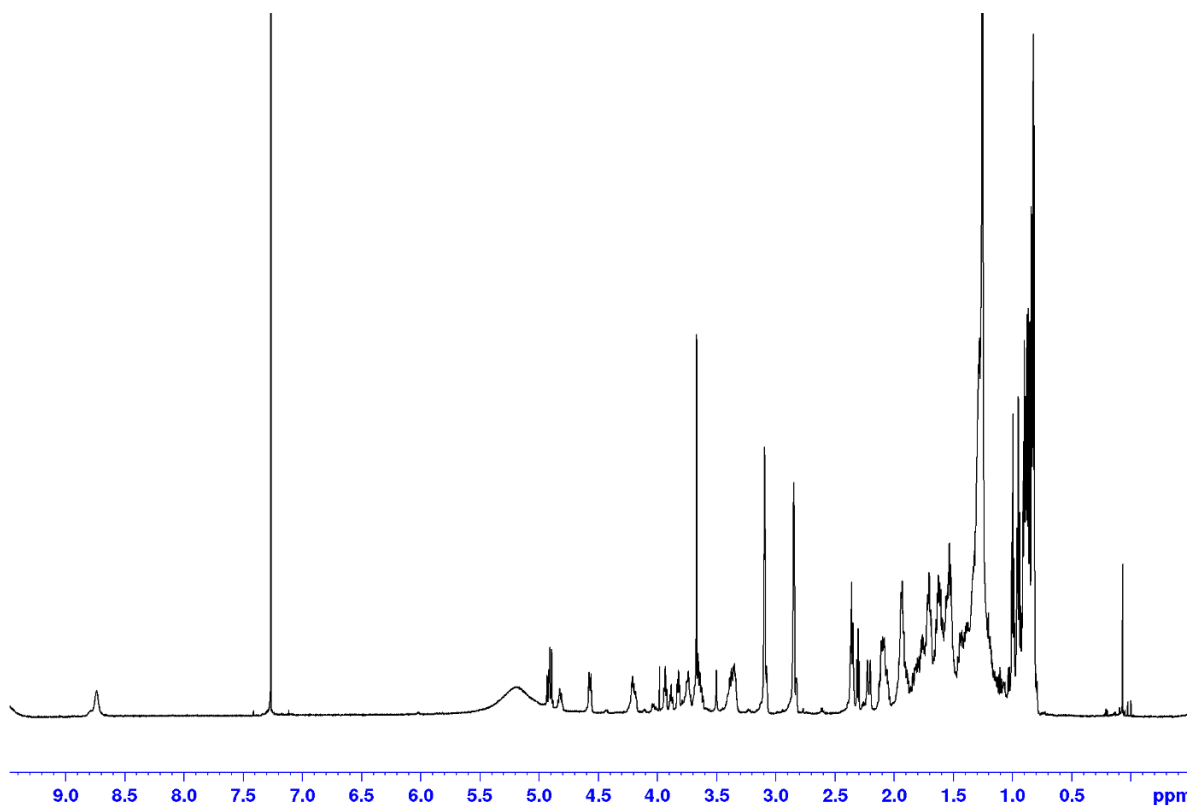


**Fig. S'17.**  $^1\text{H}$  NMR spectrum (700 MHz,  $\text{CDCl}_3$ ) of Homopamamycin-677 A

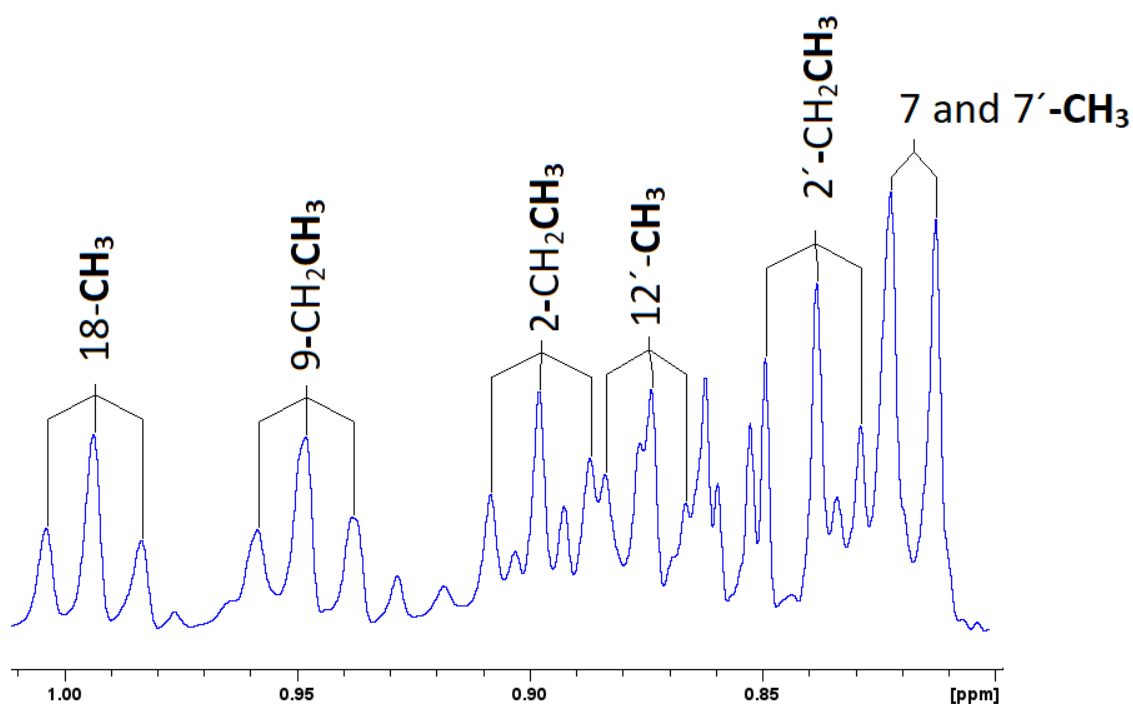


**Fig. S'18.**  $^1\text{H}$  NMR spectrum (700 MHz,  $\text{CDCl}_3$ ) of Homopamamycin-677 A, Methyl region

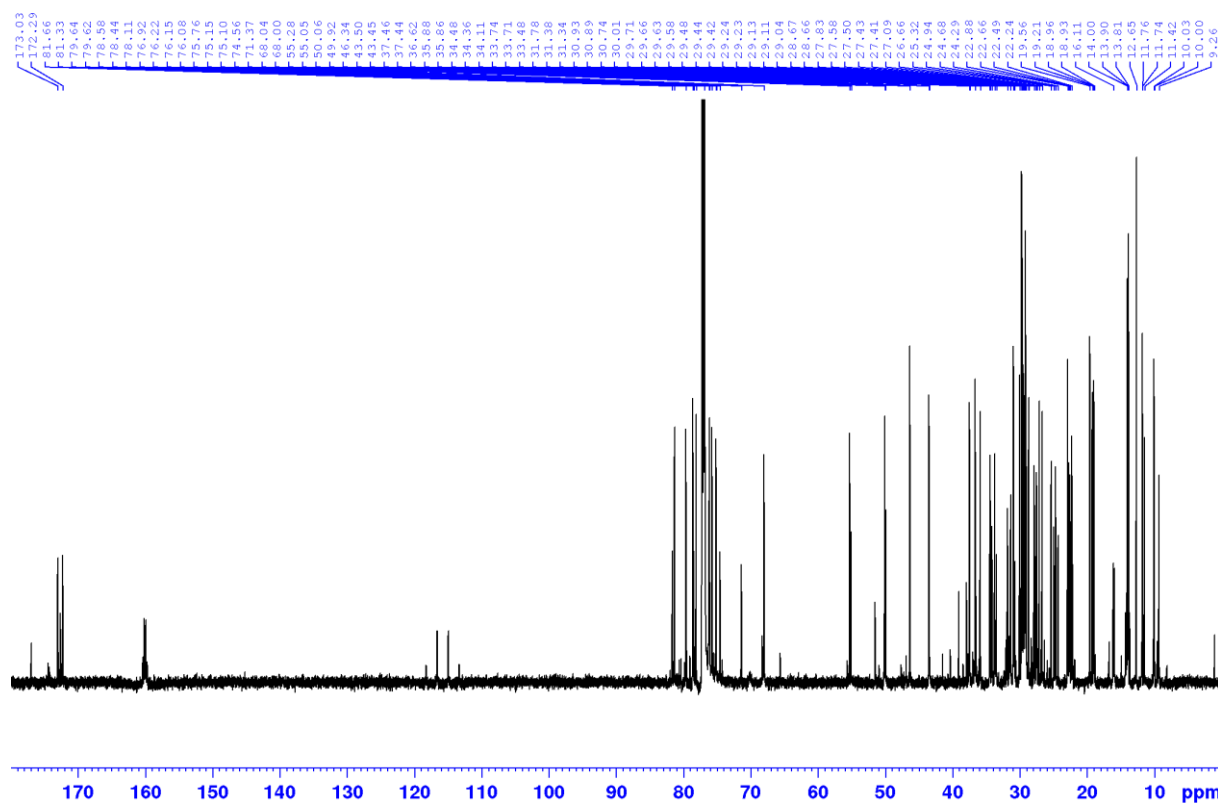


**Fig. S'19.**  $^{13}\text{C}$  NMR spectrum (175 MHz,  $\text{CDCl}_3$ ) of Homopamamycin-677 A

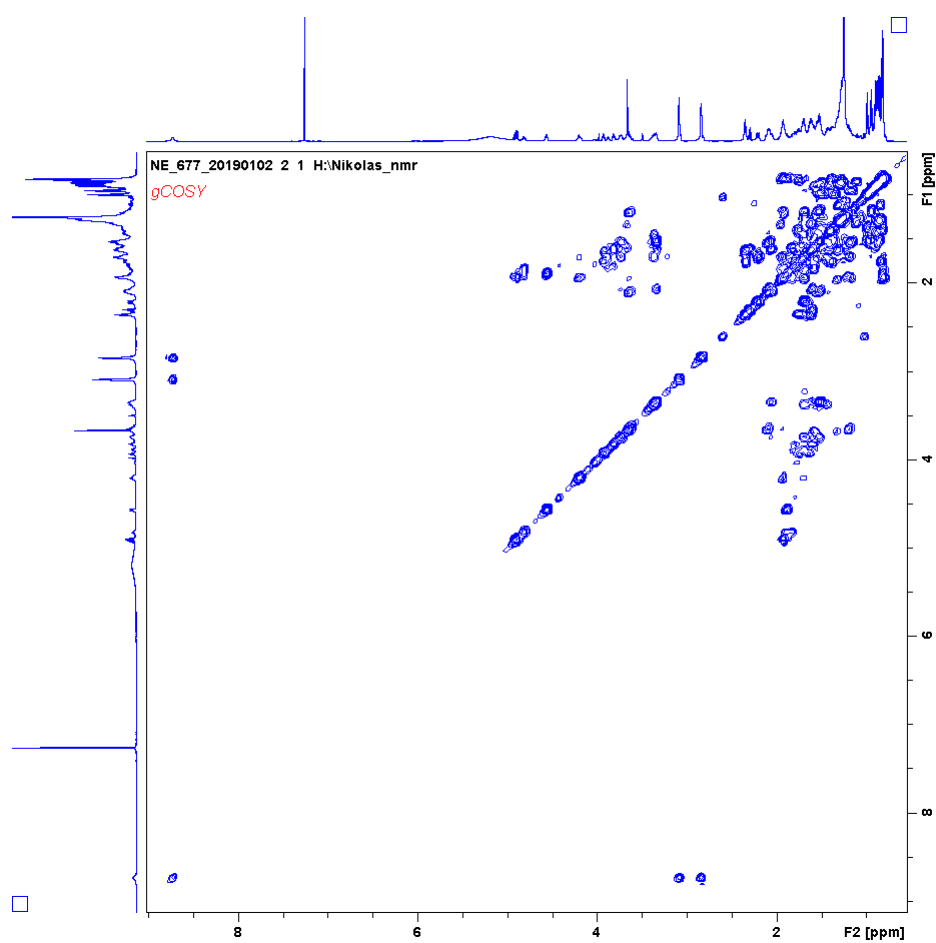


**Fig. S'20.**  $^1\text{H}$ - $^1\text{H}$ COSY spectrum ( $\text{CDCl}_3$ ) of Homopamamycin-677 A

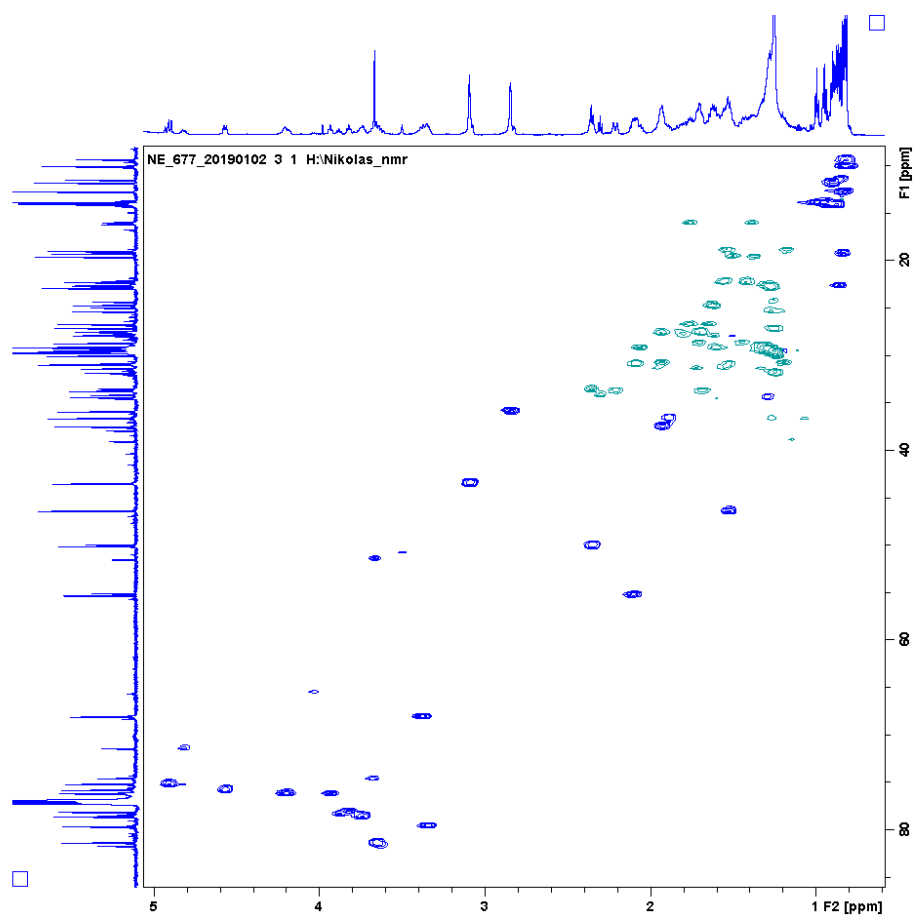


**Fig. S'21.** Edited HSQC spectrum (CDCl<sub>3</sub>) of Homopamamycin-677 A

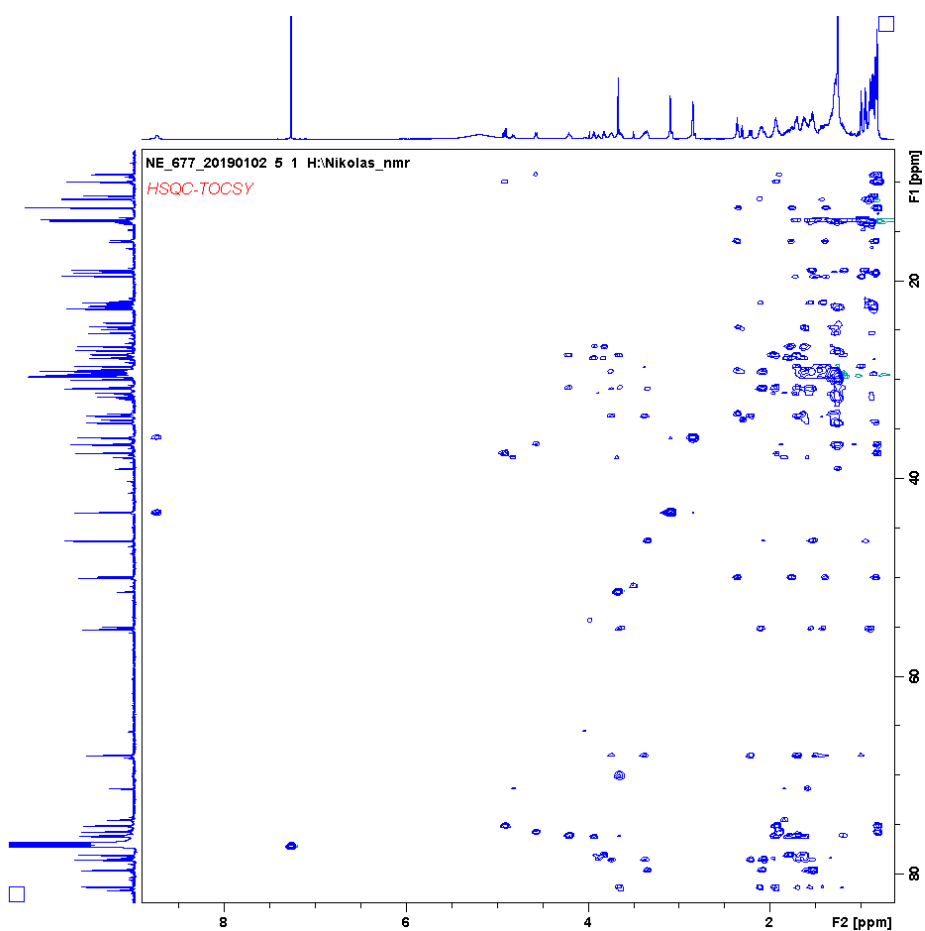


**Fig. S'22.** HSQC-TOCSY spectrum (CDCl<sub>3</sub>) of Homopamamycin-677 A

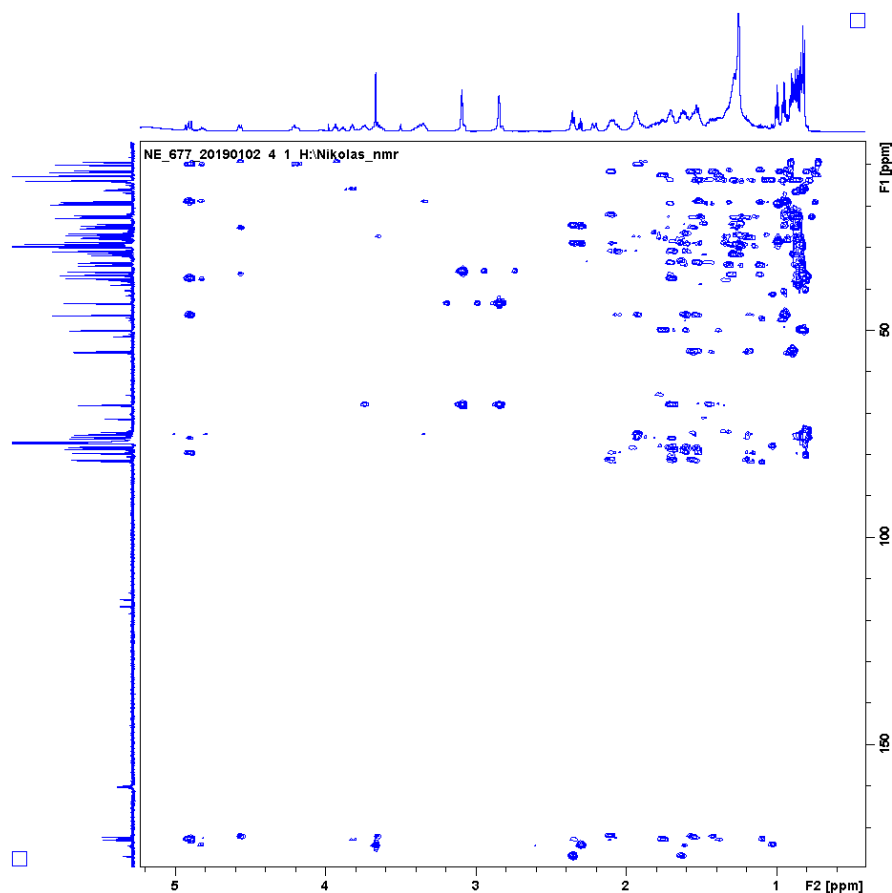
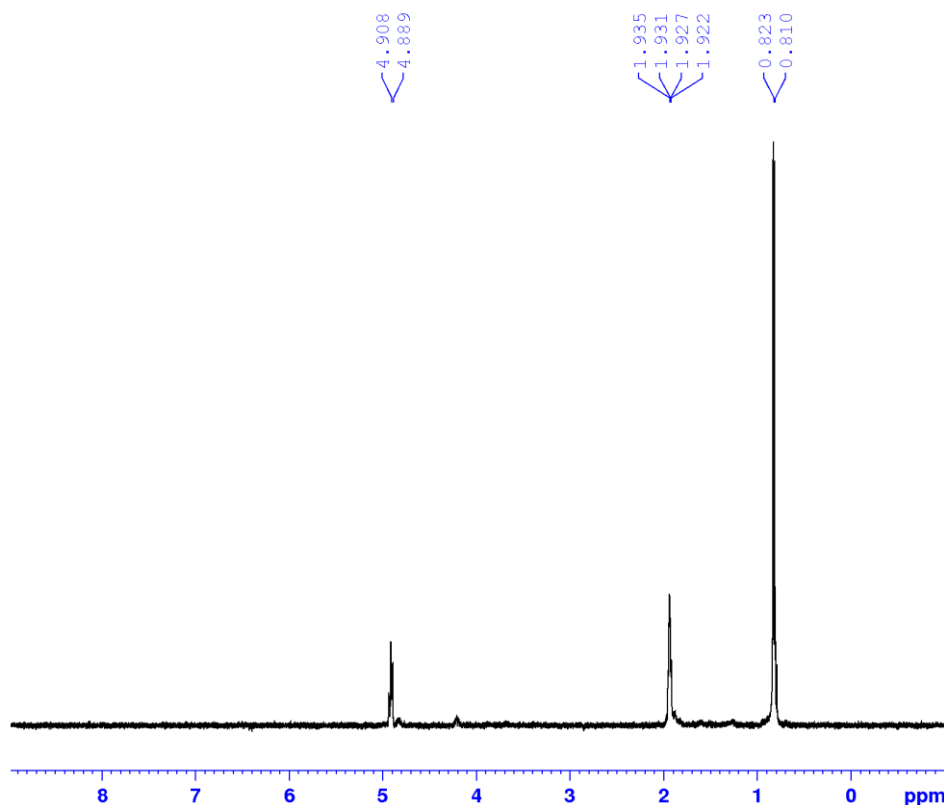


**Fig. S'23.** HMBC spectrum (CDCl<sub>3</sub>) of Homopamamycin-677 A

1D Selective Gradient TOCSY  
freq: 4.92 ppm



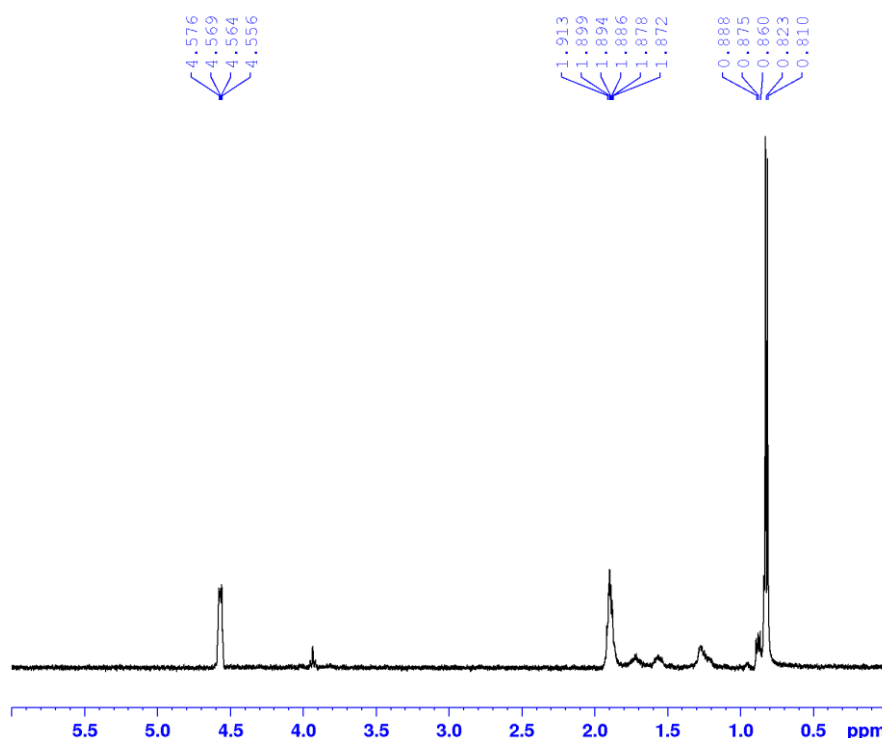
Current Data Parameters  
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EXPNO 14  
PROCNO 1

F2 - Acquisition Parameters  
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Time 10.10 h  
INSTRUM spect  
PROBHD Z44881\_0060 (C  
PULPROG selmlgp  
TD 65536  
SOLVENT CDCl<sub>3</sub>  
NS 32  
DS 4  
SWH 5000.000 Hz  
FIDRES 0.152588 Hz  
AQ 6.5535998 sec  
RG 32  
DW 100.000 usec  
DE 10.00 usec  
TE 290.8 K  
D1 2.00000000 sec  
D9 0.12500000 sec  
D16 0.00020000 sec  
LI 60  
TD0 1  
ZGPGTNS -DCALC\_SPOFFS  
SFO1 500.5320021 MHz  
NUC1 1H  
CNST21 4.9320211  
P1 7.10 usec  
P5 20.01 usec  
P6 30.00 usec  
P7 60.00 usec  
P12 25825.76 usec  
P17 2500.00 usec  
PLW0 0 W  
PLW1 7.07950020 W  
PLW10 0.39653000 W  
SPNAM[2] Gauss\_180r.1000  
SPOAL2 0.500  
spofffs2 466.50 Hz  
SPW2 0.00001263 W  
GPNAM[1] SMSQ10.100  
GPZ1 15.00 %  
P16 1000.00 usec

F2 - Processing parameters  
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SF 500.5300107 MHz  
WDW EM  
SSB 0  
LB 0.10 Hz  
GB 0  
PC 1.00

**Fig. S'24.** Sel. 1D TOCSY of Homopamamycin-677 A, excitation at 4.92 ppm

1D Selective Gradient TOCSY  
freq: 4.589ppm



Current Data Parameters  
NAME NE\_677\_1D-Tocsy\_cryo50(  
EXPNO 24  
PROCNO 1

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Time 10.15 h  
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PROBHD Z44881\_0060 (C  
PULPROG selmlgp  
TD 65536  
SOLVENT CDCl3  
NS 32  
DS 4  
SWH 5000.000 Hz  
FIDRES 0.152588 Hz  
AQ 6.5535998 sec  
RG 32  
DW 100.000 usec  
DE 10.00 usec  
TE 290.8 K  
D1 2.00000000 sec  
D9 0.12500000 sec  
D16 0.00020000 sec  
L1 60  
TD0 1  
ZGPTNS -DCALC\_SPOFFS  
SFO1 500.5320021 MHz  
NUC1 1H  
CNST21 4.5891032  
P1 7.10 usec  
P5 20.01 usec  
P6 30.00 usec  
P7 60.00 usec  
P12 29026.90 usec  
P17 2500.00 usec  
PLW0 0 W  
PLW1 7.07950020 W  
PLW10 0.39653000 W  
SPNAM[2] Gauss180r.1000  
SFOAL2 0.500  
spocfs2 294.86 Hz  
SPW2 0.00001000 W  
GPNAM[1] SMSQ10.100  
GP21 15.00 %  
P16 1000.00 usec

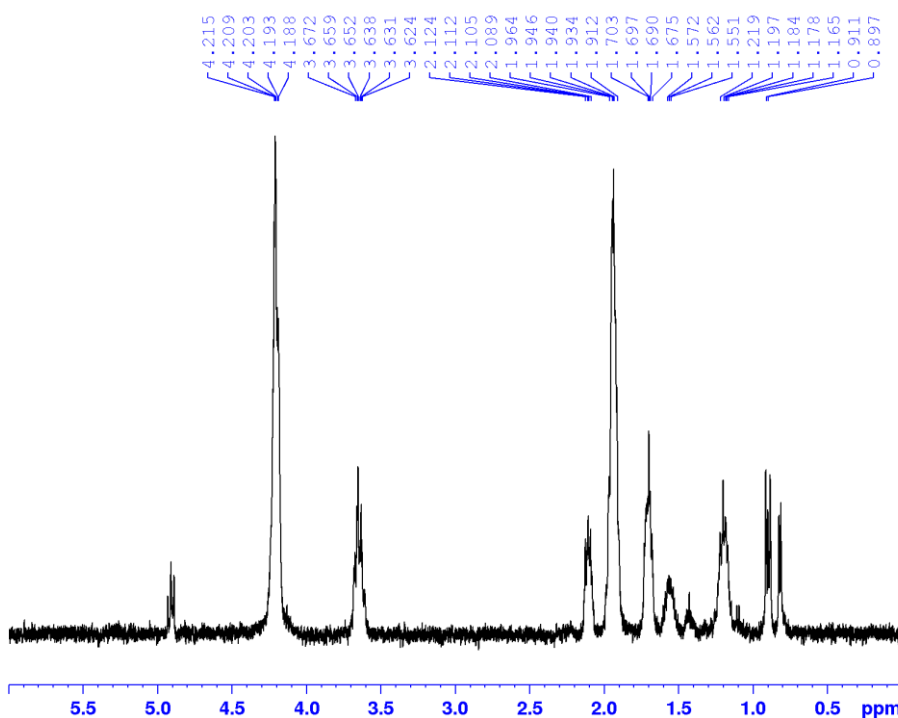
F2 - Processing parameters  
SI 32768  
SF 500.5300107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

249

250 **Fig. S'25.** Sel. 1D TOCSY of Homopamamycin-677 A, excitation at 4.59 ppm

251

1D Selective Gradient TOCSY  
freq: 4.220ppm



Current Data Parameters  
NAME NE\_677\_1D-Tocsy\_cryo50(  
EXPNO 34  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190115  
Time 10.21 h  
INSTRUM spect  
PROBHD Z44881\_0060 (C  
PULPROG selmlgp  
TD 65536  
SOLVENT CDCl3  
NS 32  
DS 4  
SWH 5000.000 Hz  
FIDRES 0.152588 Hz  
AQ 6.5535998 sec  
RG 32  
DW 100.000 usec  
DE 10.00 usec  
TE 290.8 K  
D1 2.00000000 sec  
D9 0.12500000 sec  
D16 0.00020000 sec  
L1 60  
TD0 1  
ZGPTNS -DCALC\_SPOFFS  
SFO1 500.5320021 MHz  
NUC1 1H  
CNST21 4.2201071  
P1 7.10 usec  
P5 20.01 usec  
P6 30.00 usec  
P7 60.00 usec  
P12 26290.76 usec  
P17 2500.00 usec  
PLW0 0 W  
PLW1 7.07950020 W  
PLW10 0.39653000 W  
SPNAM[2] Gauss180r.1000  
SFOAL2 0.500  
spocfs2 110.17 Hz  
SPW2 0.00001219 W  
GPNAM[1] SMSQ10.100  
GP21 15.00 %  
P16 1000.00 usec

F2 - Processing parameters  
SI 32768  
SF 500.5300107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

252

253 **Fig. S'26.** Sel. 1D TOCSY of Homopamamycin-677 A, excitation at 4.22 ppm

254

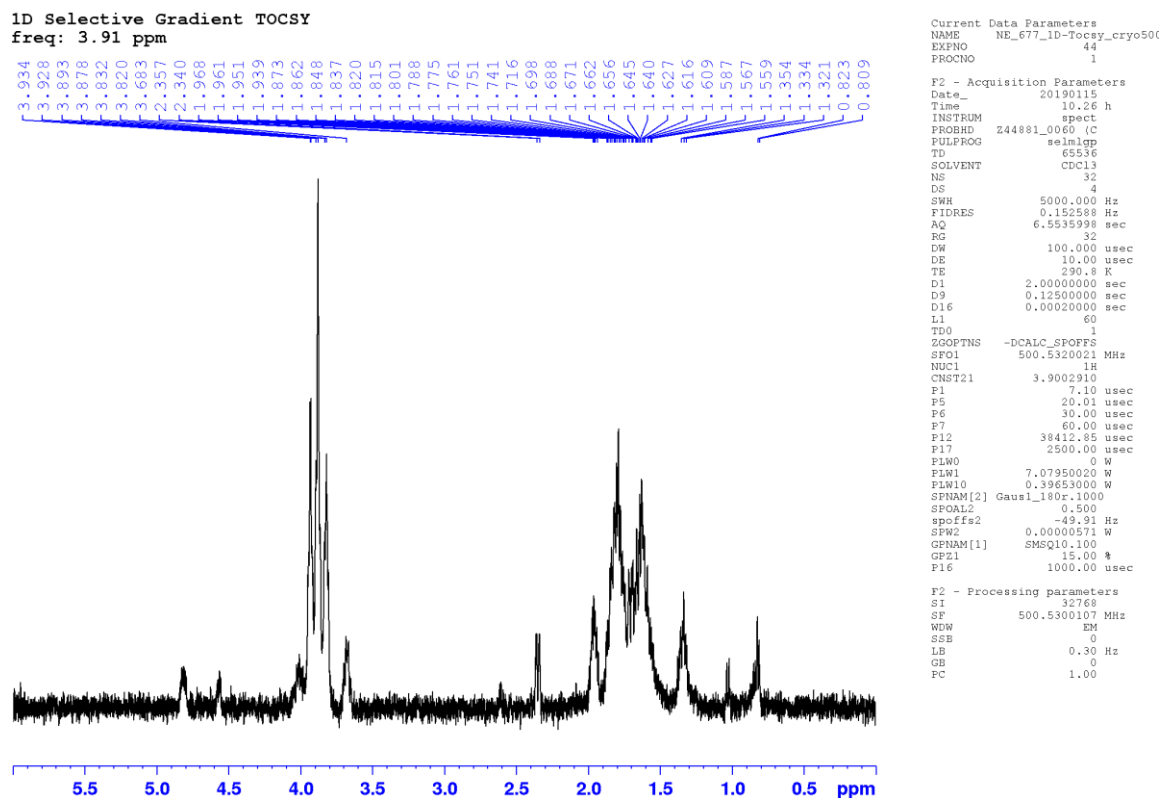


**Fig. S'27.** Sel. 1D TOCSY of Homopamamycin-677 A, excitation at 3.91 ppm

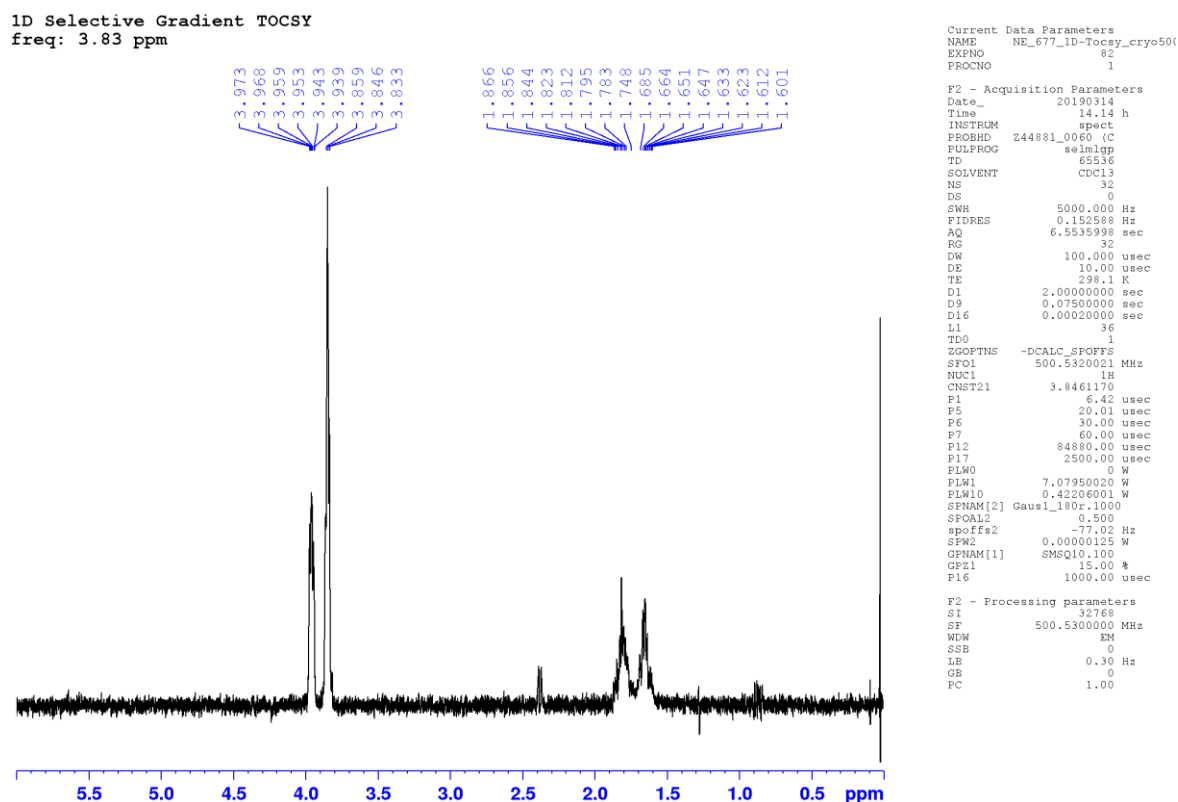
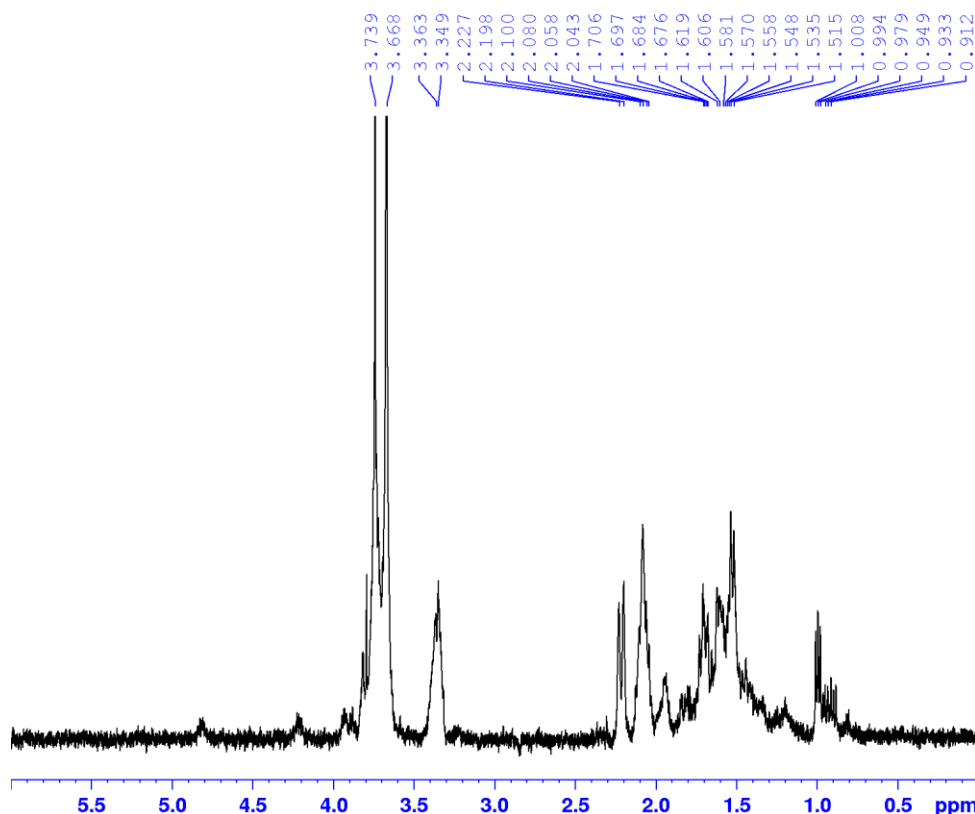


**Fig. S'28.** Sel. 1D TOCSY of Homopamamycin-677 A, excitation at 3.83 ppm

1D Selective Gradient TOCSY  
freq: 3.761 ppm

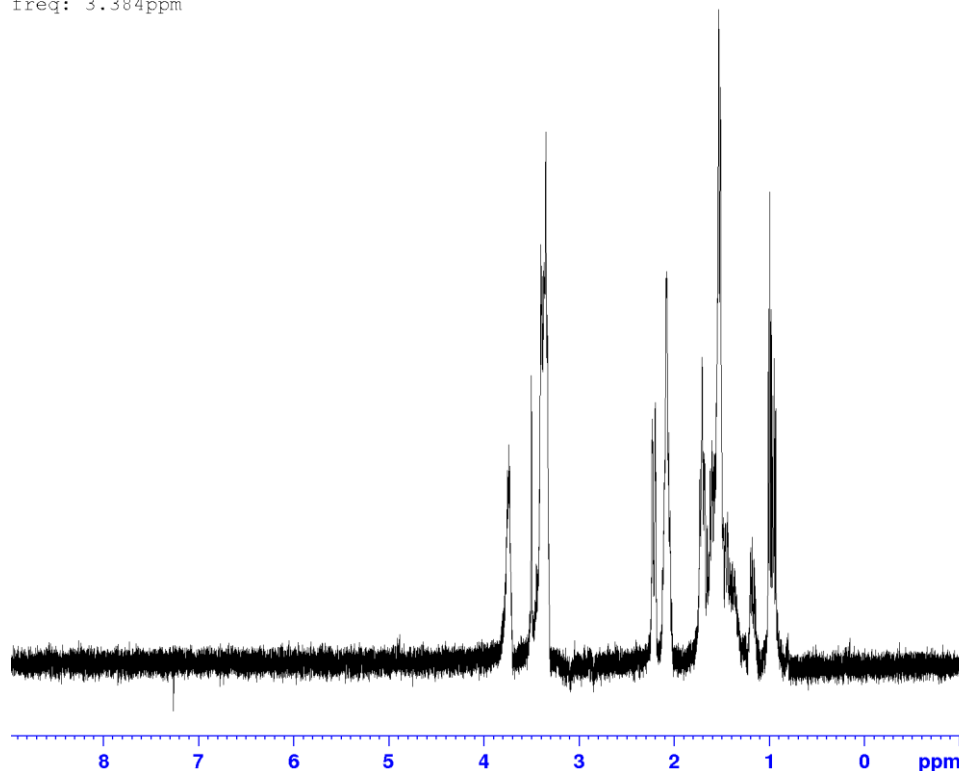


Current Data Parameters  
NAME NE\_677\_ID-Tocsy\_cryo500  
EXPNO 54  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190115  
Time 10.31 h  
INSTRUM spect  
PROBHD Z44881\_0060 (C  
PULPROG seimlpg  
TD 65536  
SOLVENT CDCl3  
NS 32  
DS 4  
SWH 5000.000 Hz  
FIDRES 0.152588 Hz  
AQ 6.5535998 sec  
RG 32  
DW 100.000 usec  
DE 10.00 usec  
TE 290.8 K  
D1 2.00000000 sec  
D9 0.12500000 sec  
D16 0.00020000 sec  
L1 60  
TD0 1  
ZGPTNS -DCALC\_SPOFFS  
SFO1 500.5320021 MHz  
NUC1 1H  
CNST21 3.7605090  
P1 7.10 usec  
P5 20.01 usec  
P6 30.00 usec  
P7 60.00 usec  
P12 31445.81 usec  
P17 2500.00 usec  
PLW0 0 W  
PLW1 7.07950020 W  
PLW10 0.39653000 W  
SPNAM[2] Gausl\_180r.1000  
SFOAL2 0.500  
spoffs2 -119.87 Hz  
SPW2 0.00000852 W  
GPNAM[1] SMSQ10.100  
GPZ1 15.00 %  
P16 1000.00 usec

F2 - Processing parameters  
SI 32768  
SF 500.5300107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

1D Selective Gradient TOCSY  
freq: 3.384ppm



Current Data Parameters  
NAME NE\_677\_ID-Tocsy\_cryo500  
EXPNO 64  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190115  
Time 10.37 h  
INSTRUM spect  
PROBHD Z44881\_0060 (C  
PULPROG seimlpg  
TD 65536  
SOLVENT CDCl3  
NS 32  
DS 4  
SWH 5000.000 Hz  
FIDRES 0.152588 Hz  
AQ 6.5535998 sec  
RG 32  
DW 100.000 usec  
DE 10.00 usec  
TE 290.9 K  
D1 2.00000000 sec  
D9 0.12500000 sec  
D16 0.00020000 sec  
L1 60  
TD0 1  
ZGPTNS -DCALC\_SPOFFS  
SFO1 500.5320021 MHz  
NUC1 1H  
CNST21 3.3843410  
P1 7.10 usec  
P5 20.01 usec  
P6 30.00 usec  
P7 60.00 usec  
P12 17337.69 usec  
P17 2500.00 usec  
PLW0 0 W  
PLW1 7.07950020 W  
PLW10 0.39653000 W  
SPNAM[2] Gausl\_180r.1000  
SFOAL2 0.500  
spoffs2 -308.16 Hz  
SPW2 0.00002803 W  
GPNAM[1] SMSQ10.100  
GPZ1 15.00 %  
P16 1000.00 usec

F2 - Processing parameters  
SI 32768  
SF 500.5300107 MHz  
WDW EM  
SSB 0  
LB 0.10 Hz  
GB 0  
PC 1.00

Fig. S'30. Sel. 1D TOCSY of Homopamamycin-677 A, excitation at 3.38 ppm

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