

## Supplemental Material For:

**Title:** Tailoring Enzyme-Rich Environmental DNA Clones: A Source of Enzymes for Generating Libraries of Unnatural Natural Products

**Authors:** Jacob J. Banik, Jeffrey W. Craig, Paula Y. Calle, and Sean F. Brady\*

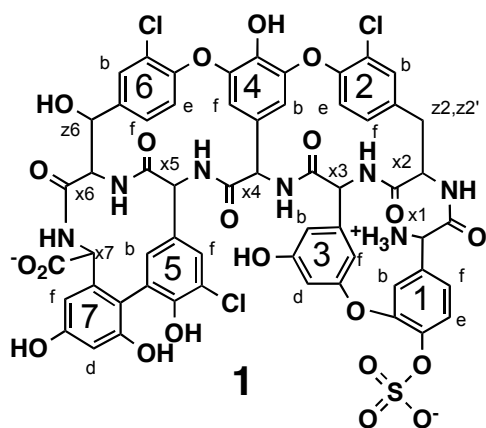
**Author affiliation:** Howard Hughes Medical Institute, Laboratory of Genetically Encoded Small Molecules, The Rockefeller University, 1230 York Avenue, New York, NY 10065.

**Corresponding Author:** Sean F. Brady

**Address:** Laboratory of Genetically Encoded Small Molecules  
The Rockefeller University  
1230 York Avenue  
New York, NY 10065  
Phone: 212-327-8280  
Fax: 212-327-8281  
Email: [sbrady@rockefeller.edu](mailto:sbrady@rockefeller.edu)

## Supplemental Material Table of Contents

|   |     |
|---|-----|
| <b>Table S1:</b> Numbering Scheme and $^1\text{H}$ Assignments for Compounds 1-5 Core                                   | S3  |
| <b>Table S2:</b> Compound specific $^1\text{H}$ and $^{13}\text{C}$ Assignments for Compounds 2-5                       | S4  |
| <b>Table S3:</b> Numbering scheme and $^1\text{H}$ and $^{13}\text{C}$ Assignments for Compound 4 – glucose             | S5  |
| <b>Figure S1:</b> $^1\text{H}$ of Compound 1, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$                     | S6  |
| <b>Figure S2:</b> $^1\text{H}-^{13}\text{C}$ HMQC of Compound 1, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$  | S7  |
| <b>Figure S3:</b> $^1\text{H}-^{13}\text{C}$ HMBC of Compound 1, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$  | S8  |
| <b>Figure S4:</b> $^1\text{H}$ of Compound 2, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$                     | S9  |
| <b>Figure S5:</b> $^1\text{H}-^{13}\text{C}$ HMQC of Compound 2, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$  | S10 |
| <b>Figure S6:</b> $^1\text{H}-^{13}\text{C}$ HMBC of Compound 2, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$  | S11 |
| <b>Figure S7:</b> $^1\text{H}$ of Compound 3, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$                     | S12 |
| <b>Figure S8:</b> $^1\text{H}-^{13}\text{C}$ HMQC of Compound 3, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$  | S13 |
| <b>Figure S9:</b> $^1\text{H}-^{13}\text{C}$ HMBC of Compound 3, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$  | S14 |
| <b>Figure S10:</b> $^1\text{H}$ of Compound 4, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$                    | S15 |
| <b>Figure S11:</b> $^1\text{H}-^{13}\text{C}$ HMQC of Compound 4, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$ | S16 |
| <b>Figure S12:</b> $^1\text{H}-^{13}\text{C}$ HMBC of Compound 4, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$ | S17 |
| <b>Figure S13:</b> $^1\text{H}$ of Compound 4, 313K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$                    | S18 |
| <b>Figure S14:</b> $^1\text{H}$ of Compound 5, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$                    | S19 |
| <b>Figure S15:</b> $^1\text{H}-^{13}\text{C}$ HMQC of Compound 5, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$ | S20 |
| <b>Figure S16:</b> $^1\text{H}-^{13}\text{C}$ HMBC of Compound 5, 298K in 3:1 $\text{D}_2\text{O}:\text{CD}_3\text{CN}$ | S21 |
| <b>Table S4:</b> Pathway CA37 ORFs  | S22 |
| <b>Table S5:</b> Pathway CA878 ORFs   | S23 |
| <b>Table S6:</b> Pathway CA915 ORFs   | S24 |

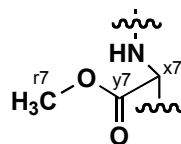
**Table S1:** Numbering Scheme and <sup>1</sup>H Assignments for Compounds 1-5 Core

|     | $\delta$ (ppm)<br>Compound 1 | $\delta$ (ppm)<br>Compound 2 | $\delta$ (ppm)<br>Compound 3 | $\delta$ (ppm)<br>Compound 4 | $\delta$ (ppm)<br>Compound 5 |
|-----|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| 1b  | 7.01                         | 7.02                         | 7.00                         | 7.02                         | 6.97                         |
| 1e  | 7.61                         | 7.60                         | 7.65                         | 7.61                         | 7.61                         |
| 1f  | 7.38                         | 7.35                         | 7.38                         | 7.38                         | 7.33                         |
| 2b  | 7.32                         | 7.31                         | 7.32                         | 7.33                         | 7.31                         |
| 2e  | 7.21                         | 7.19                         | 7.35                         | 7.22                         | 7.20                         |
| 2f  | 7.58                         | 7.57                         | 7.58                         | 7.63                         | 7.57                         |
| 3b  | 6.49                         | 6.49                         | 6.49                         | 6.50                         | 6.46                         |
| 3d  | 6.62                         | 6.61                         | 6.63                         | 6.62                         | 6.61                         |
| 3f  | 6.24                         | 6.27                         | 6.22                         | 6.26                         | 6.21                         |
| 4b  | 5.27                         | 5.26                         | 5.27                         | 5.30                         | 5.27                         |
| 4f  | 5.68                         | 5.68                         | 5.82                         | 5.70                         | 5.68                         |
| 5b  | 6.96                         | 6.97                         | 6.98                         | 6.97                         | 6.95                         |
| 5f  | 7.01                         | 6.98                         | 7.02                         | 7.01                         | 7.01                         |
| 6b  | 7.60                         | 7.59                         | 7.61                         | 7.60                         | 7.59                         |
| 6e  | 7.21                         | 7.18                         | 7.21                         | 7.21                         | 7.20                         |
| 6f  | 7.47                         | 7.47                         | 7.51                         | 7.47                         | 7.49                         |
| 7d  | 6.52                         | 6.58                         | 6.53                         | 6.52                         | 6.51                         |
| 7f  | 6.44                         | 6.23                         | 6.45                         | 6.44                         | 6.43                         |
| x1  | 5.40                         | 5.40                         | 5.40                         | 5.40                         | 4.94                         |
| x2  | 4.94                         | 4.98                         | 4.94                         | 4.94                         | 4.87                         |
| x3  | 5.49                         | 5.49                         | 5.49                         | 5.49                         | 5.43                         |
| x4  | 5.49                         | 5.52                         | 5.49                         | 5.49                         | 5.49                         |
| x5  | 4.35                         | 4.35                         | 4.36                         | 4.36                         | 4.35                         |
| x6  | 4.00                         | 4.03                         | 4.02                         | 4.02                         | 4.00                         |
| x7  | 4.53                         | 4.72                         | 4.53                         | 4.53                         | 4.53                         |
| z2  | 3.35                         | 3.35                         | 3.36                         | 3.34                         | 3.35                         |
| z2' | 3.10                         | 3.09                         | 3.11                         | 3.11                         | 3.10                         |
| z6  | 5.47                         | 5.43                         | 5.48                         | 5.47                         | 5.47                         |

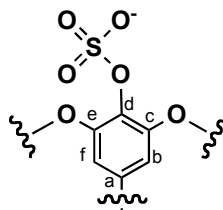
**Table S2:** Compound specific  $^1\text{H}$  and  $^{13}\text{C}$  Assignments for Compounds 2-5

The  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts reported in table S2 detail the specific chemical shifts used to make the structural assignments of compounds 2-5. For compound 2, these are the chemical shifts for both the parent compound (Compound 1) and the new compound at the C-terminus of the glycopeptide. For compounds 3 and 4, the chemical shifts reported are the key shifts vital to the assigning the position of the compound specific modification (sulfation and glycosylation, respectively) of the hydroxyl at the top of ring 4. For compound 5, the shifts reported are those new signals and the observed changes in the  $^{13}\text{C}$  at the C- $\alpha$ , x1, at the N-terminus.

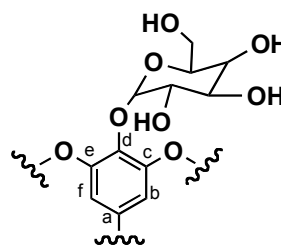
|          | Compound 1 | Compound 2 |
|----------|------------|------------|
| r7 (1H)  | -          | 3.85       |
| r7 (13C) | -          | 53.21      |
| y7 (13C) | 176.79     | 172.32     |
| x7 (13C) | 59.09      | 57.11      |



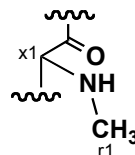
|          | Compound 1 | Compound 3 |
|----------|------------|------------|
| 4a (13C) | 135.27     | 138.22     |
| 4b (13C) | 107.5      | 108.93     |
| 4c (13C) | 146.53     | 151.65     |
| 4d (13C) | 132.98     | 128.41     |
| 4e (13C) | 147.08     | 151.84     |
| 4f (13C) | 104.16     | 104.22     |



|          | Compound 1 | Compound 4 |
|----------|------------|------------|
| 4a (13C) | 135.27     | 134.29     |
| 4b (13C) | 107.5      | 107.56     |
| 4c (13C) | 146.53     | 151.38     |
| 4d (13C) | 132.98     | 130.95     |
| 4e (13C) | 147.08     | 151.78     |
| 4f (13C) | 104.16     | 104.11     |

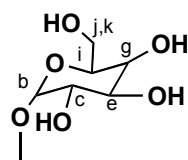


|          | Compound 1 | Compound 5 |
|----------|------------|------------|
| r1 (1H)  | -          | 2.48       |
| r1 (13C) | -          | 31.34      |
| x1 (13C) | 54.16      | 64.25      |

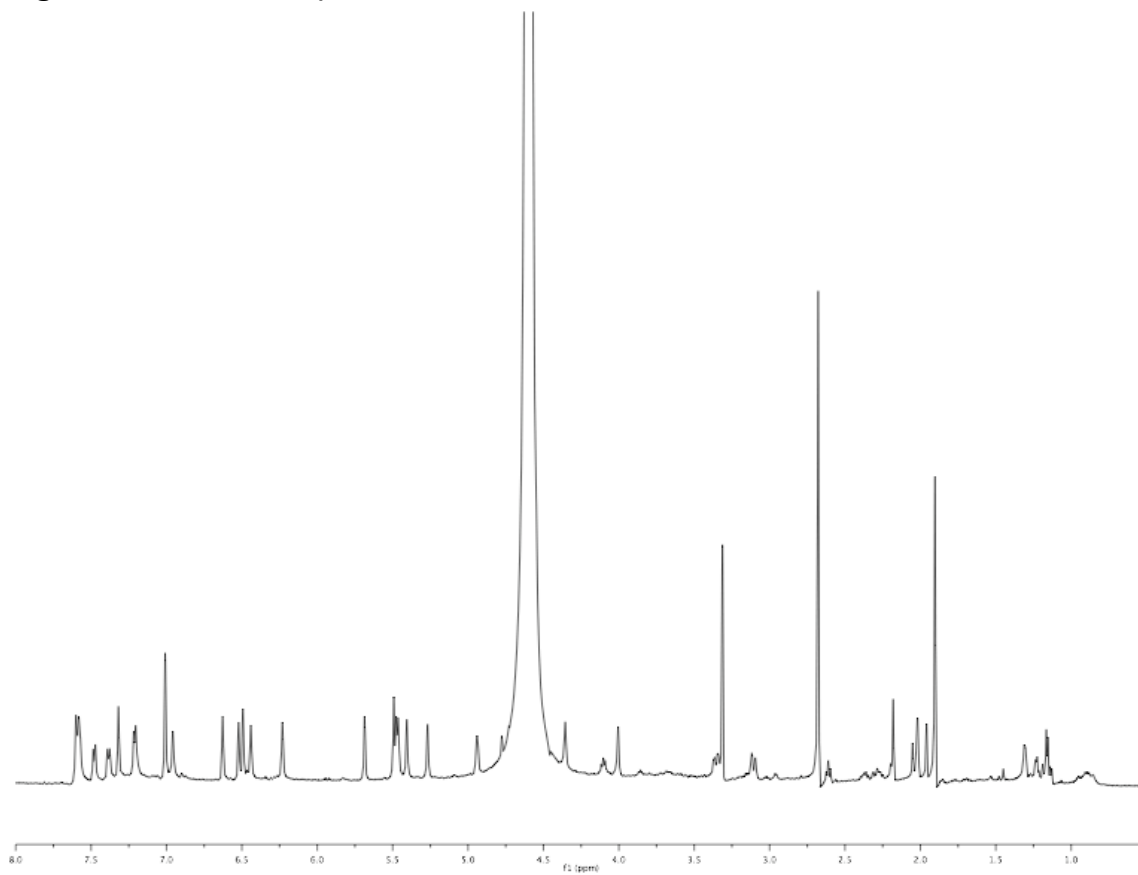


**Table S3:** Numbering scheme and  $^1\text{H}$  and  $^{13}\text{C}$  Assignments for Compound 4 - glucose

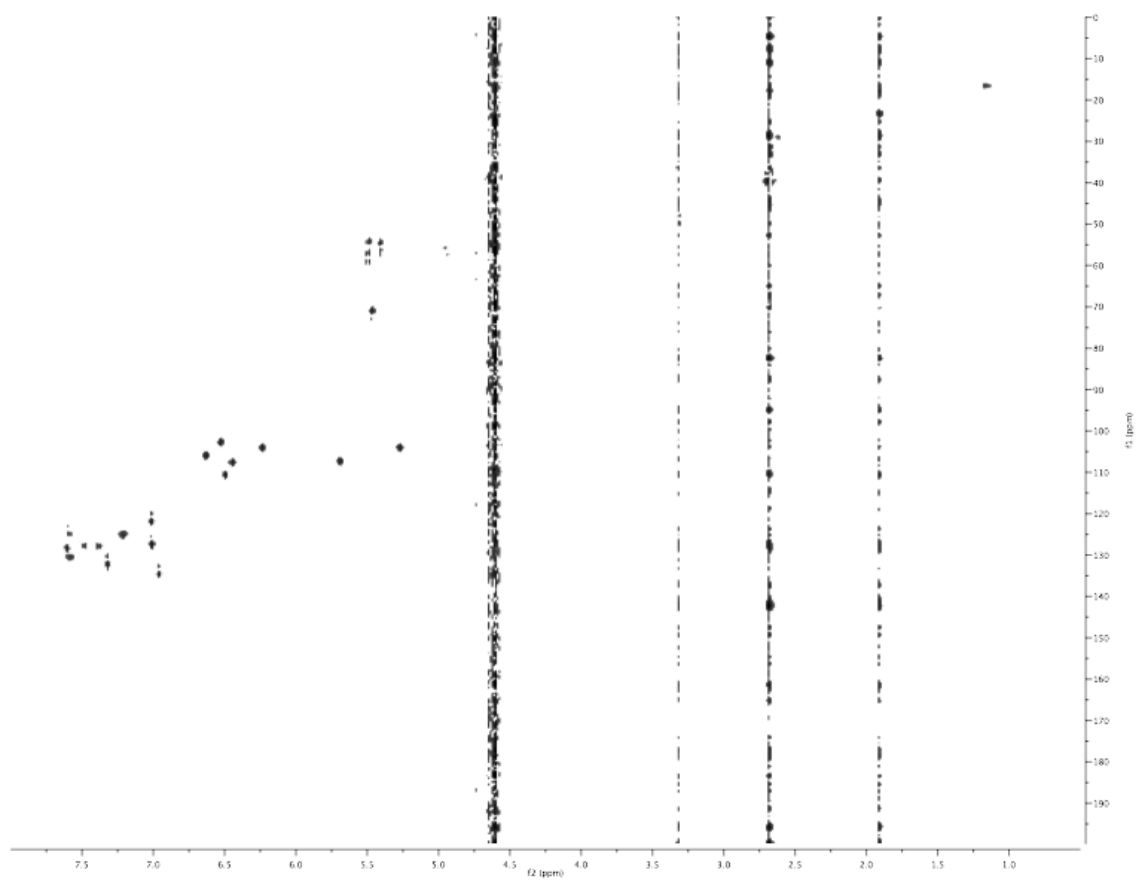
|   | $^1\text{H}$ (ppm) | $^{13}\text{C}$ (ppm) | Observed 3J (Hz) |
|---|--------------------|-----------------------|------------------|
| b | 5.33               | 104.49                | 7.80             |
| c | 3.75               | 73.20                 | 8.60             |
| e | 3.68               | 75.28                 | 9.50             |
| g | 3.63               | 68.69                 | 9.50             |
| I | 3.56               | 76.06                 | -                |
| j | 3.88               | 59.98                 | 5.18             |
| k | 3.80               | 59.98                 | -                |



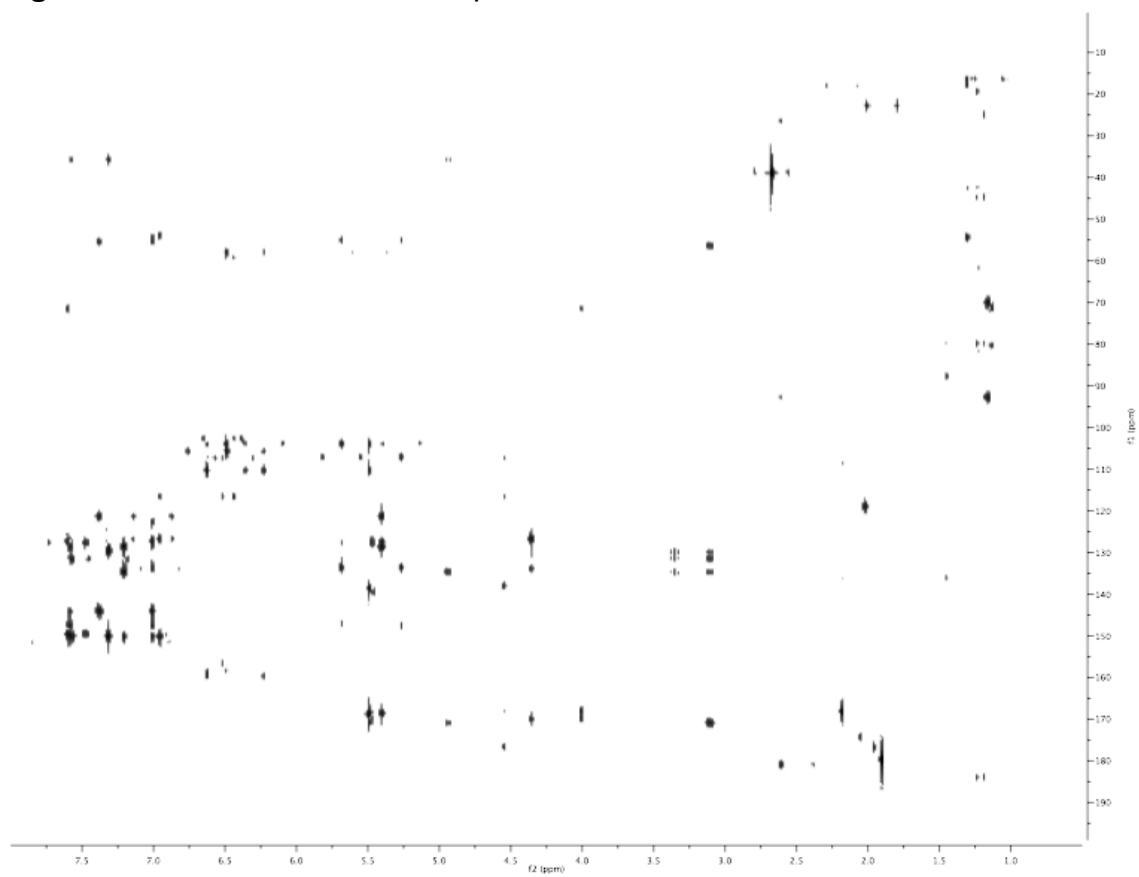
**Figure S1:**  $^1\text{H}$  of Compound 1, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



**Figure S2:**  $^1\text{H}$ - $^{13}\text{C}$  HMQC of Compound 1, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$

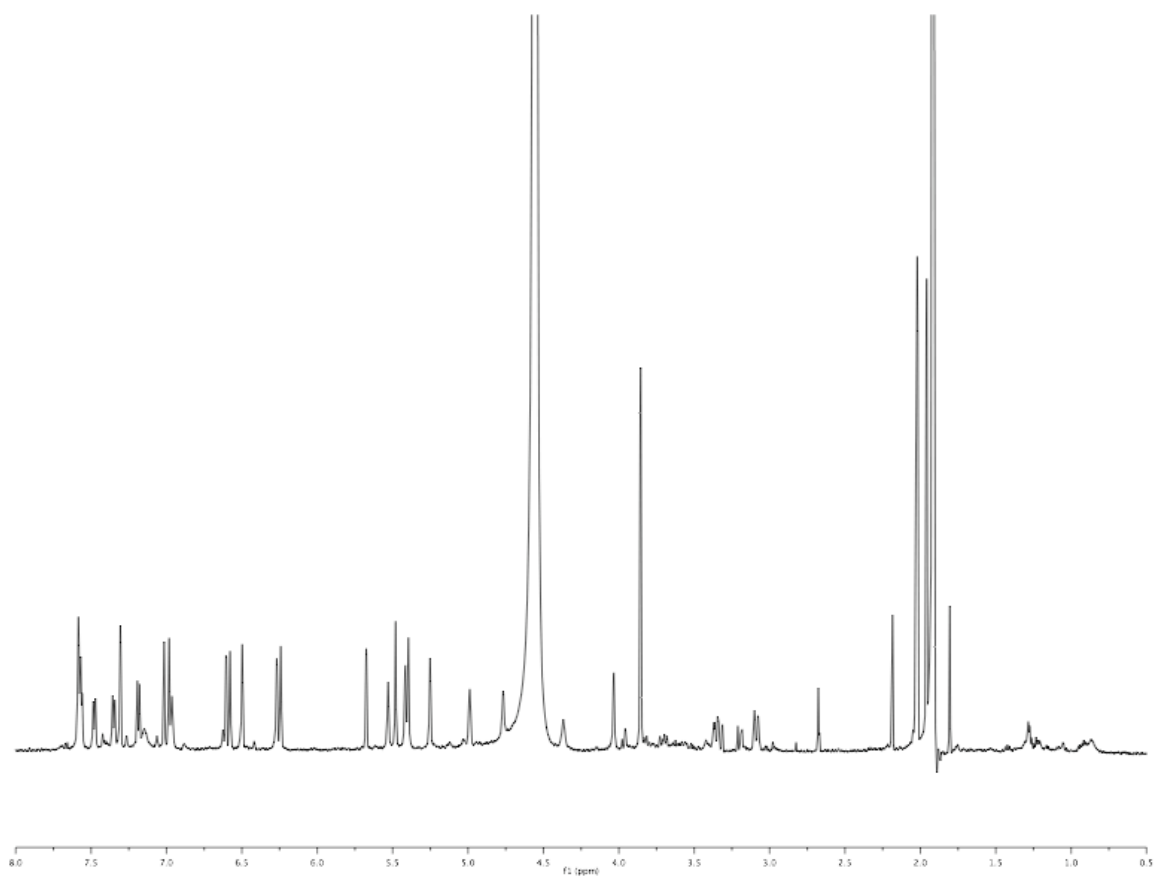


**Figure S3:**  $^1\text{H}$ - $^{13}\text{C}$  HMBC of Compound 1, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$

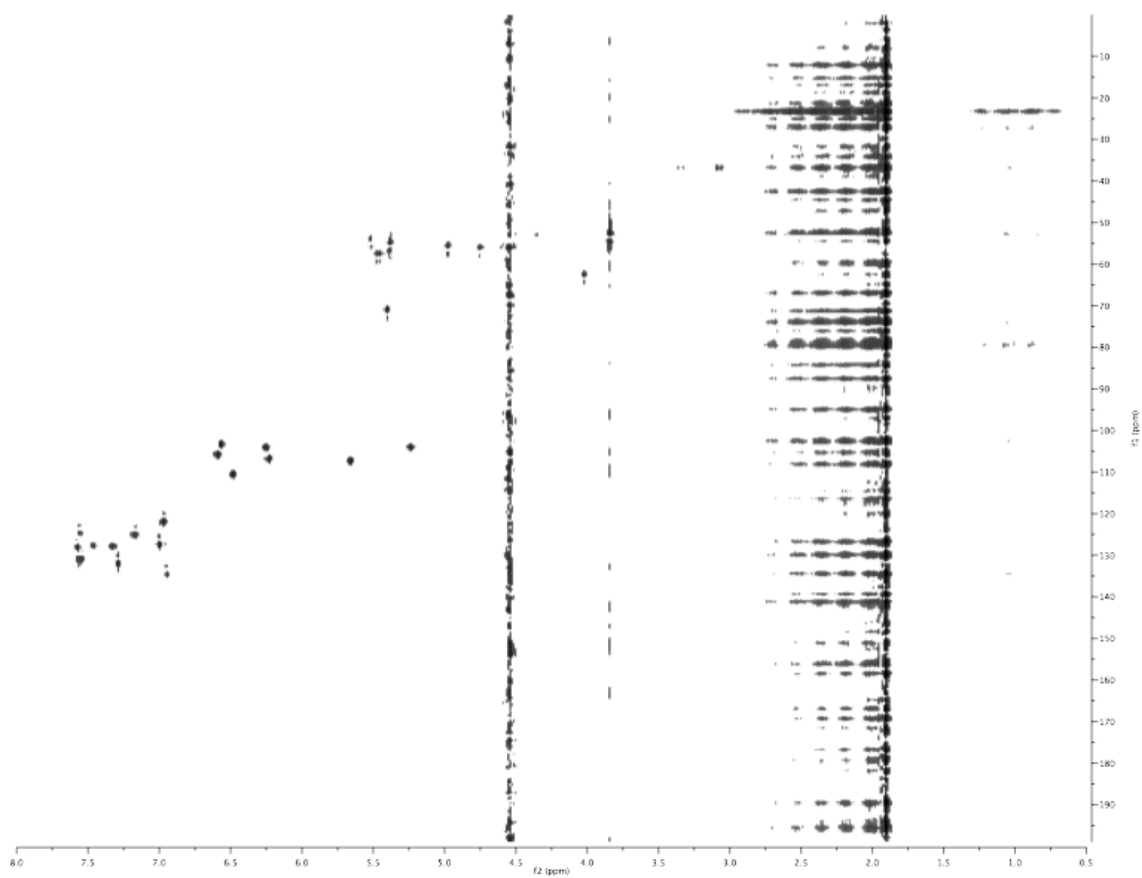




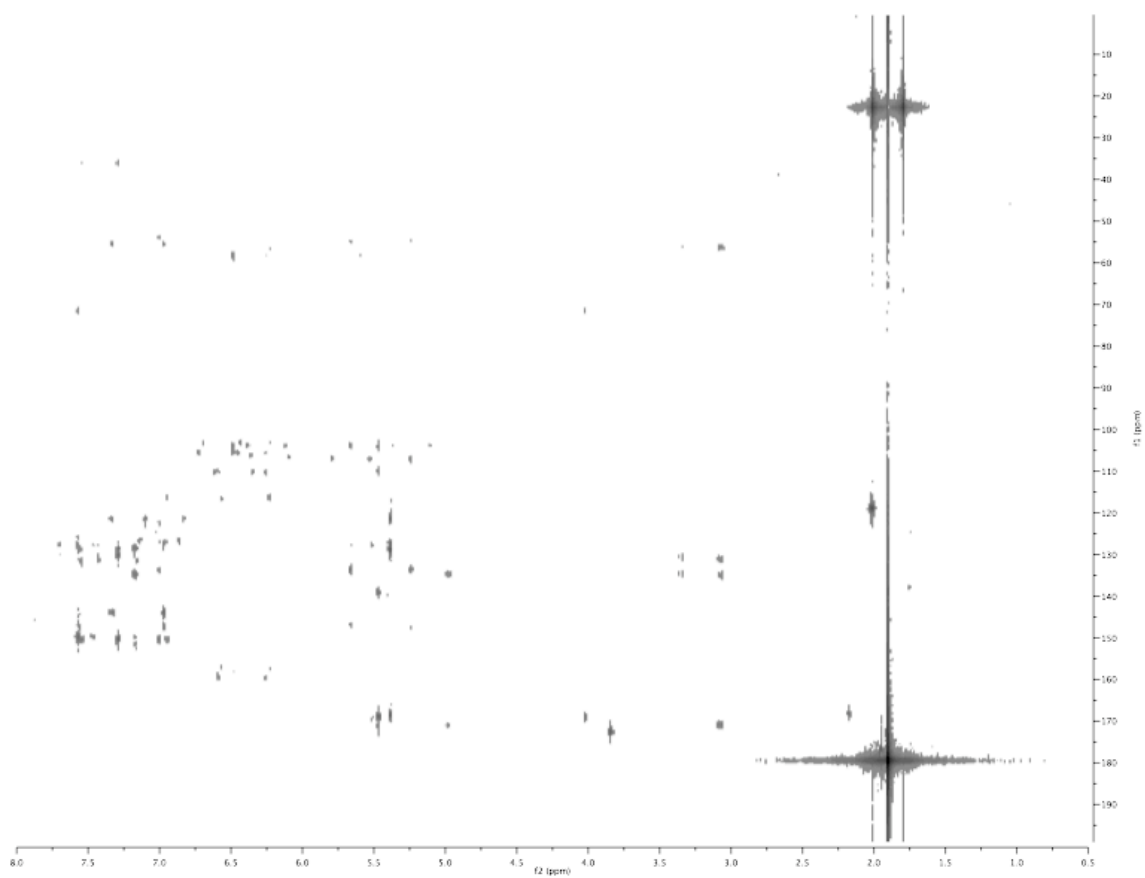
**Figure S4:**  $^1\text{H}$  of Compound 2, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



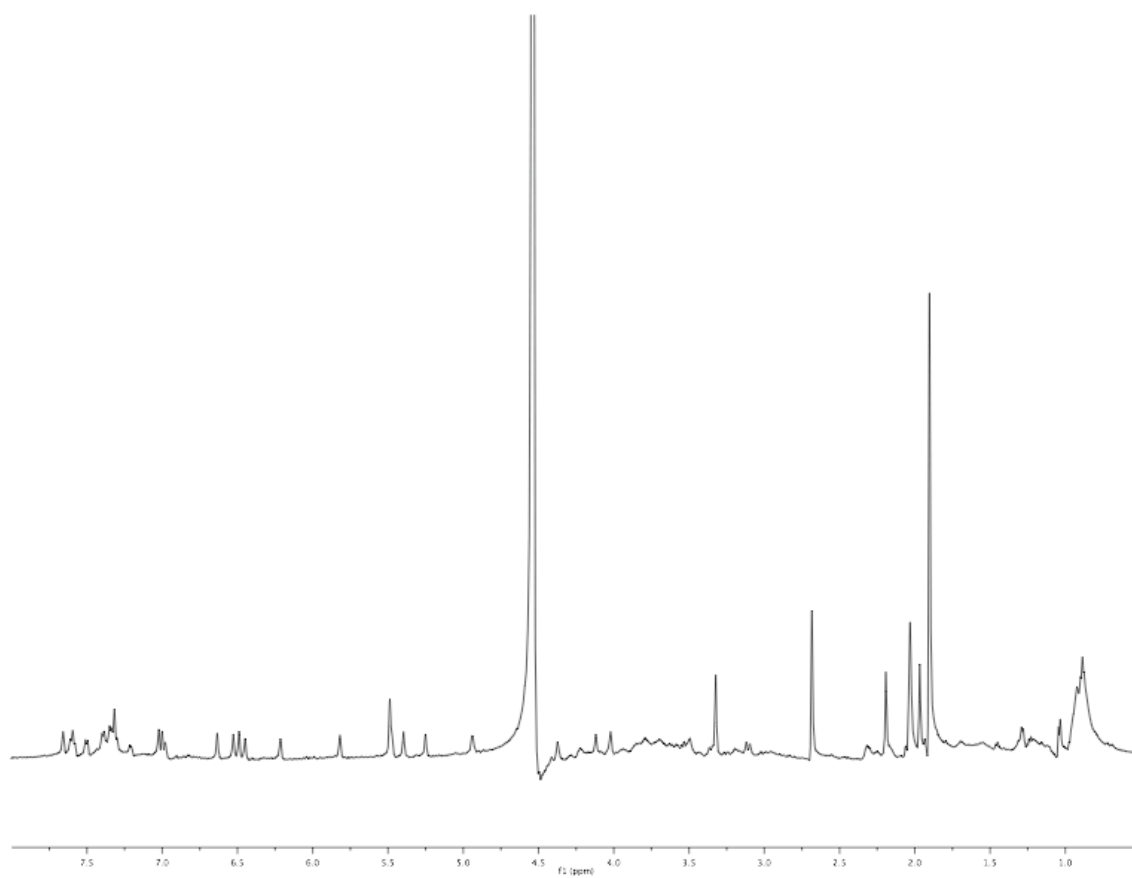
**Figure S5:**  $^1\text{H}$ - $^{13}\text{C}$  HMQC of Compound 2, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



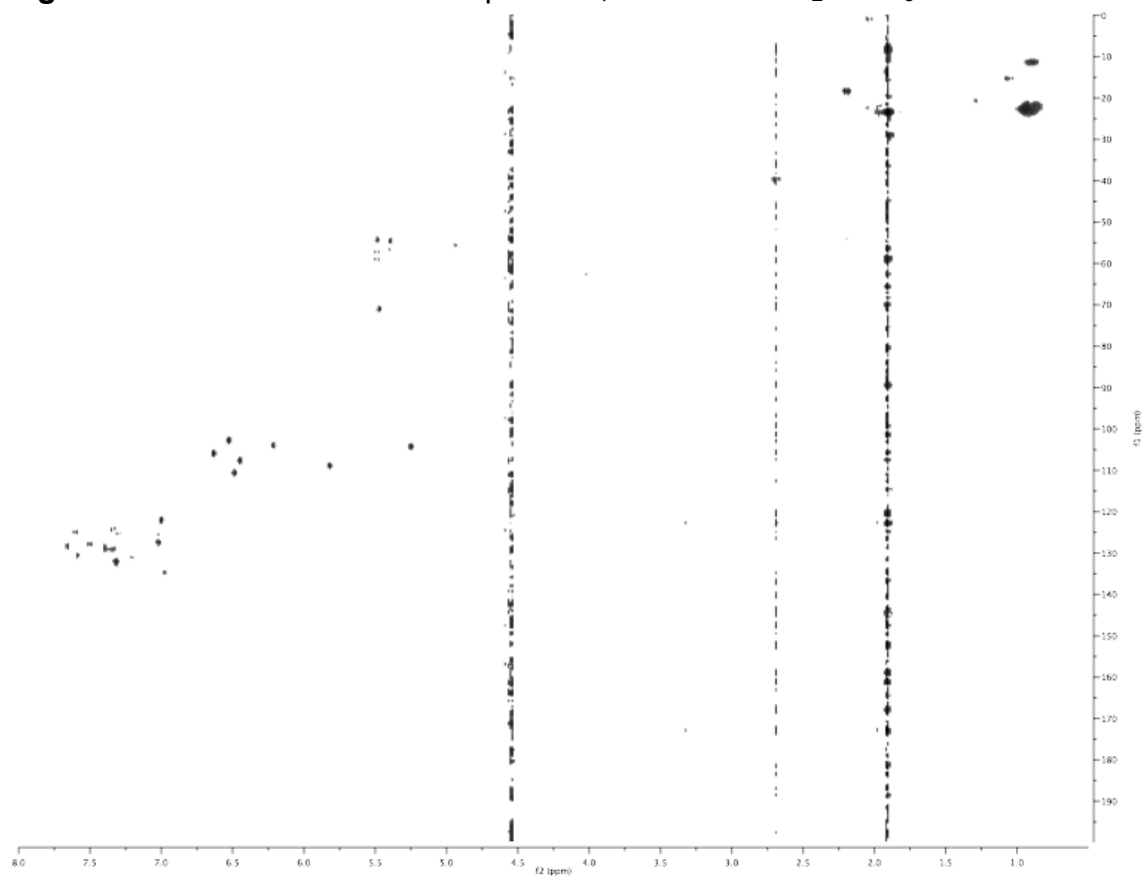
**Figure S6:**  $^1\text{H}$ - $^{13}\text{C}$  HMBC of Compound 2, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



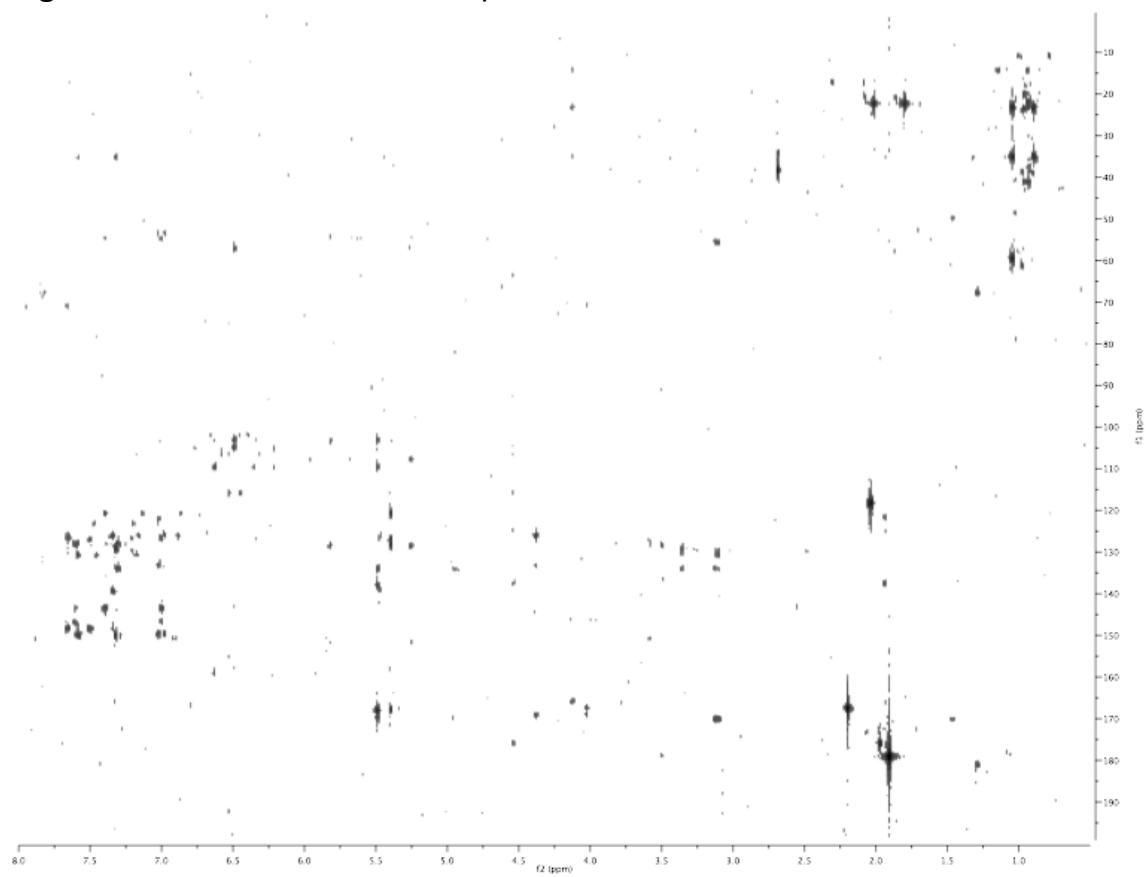
**Figure S7:**  $^1\text{H}$  of Compound 3, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



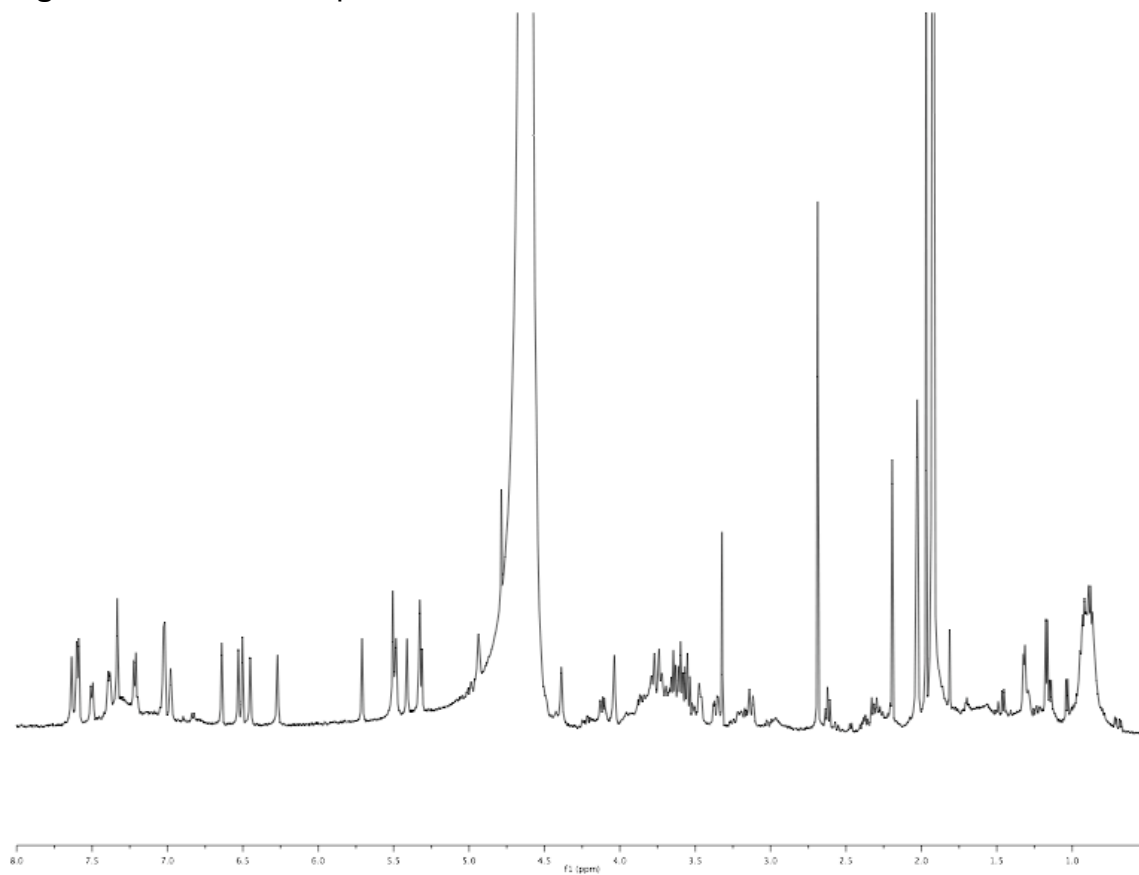
**Figure S8:**  $^1\text{H}$ - $^{13}\text{C}$  HMQC of Compound 3, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



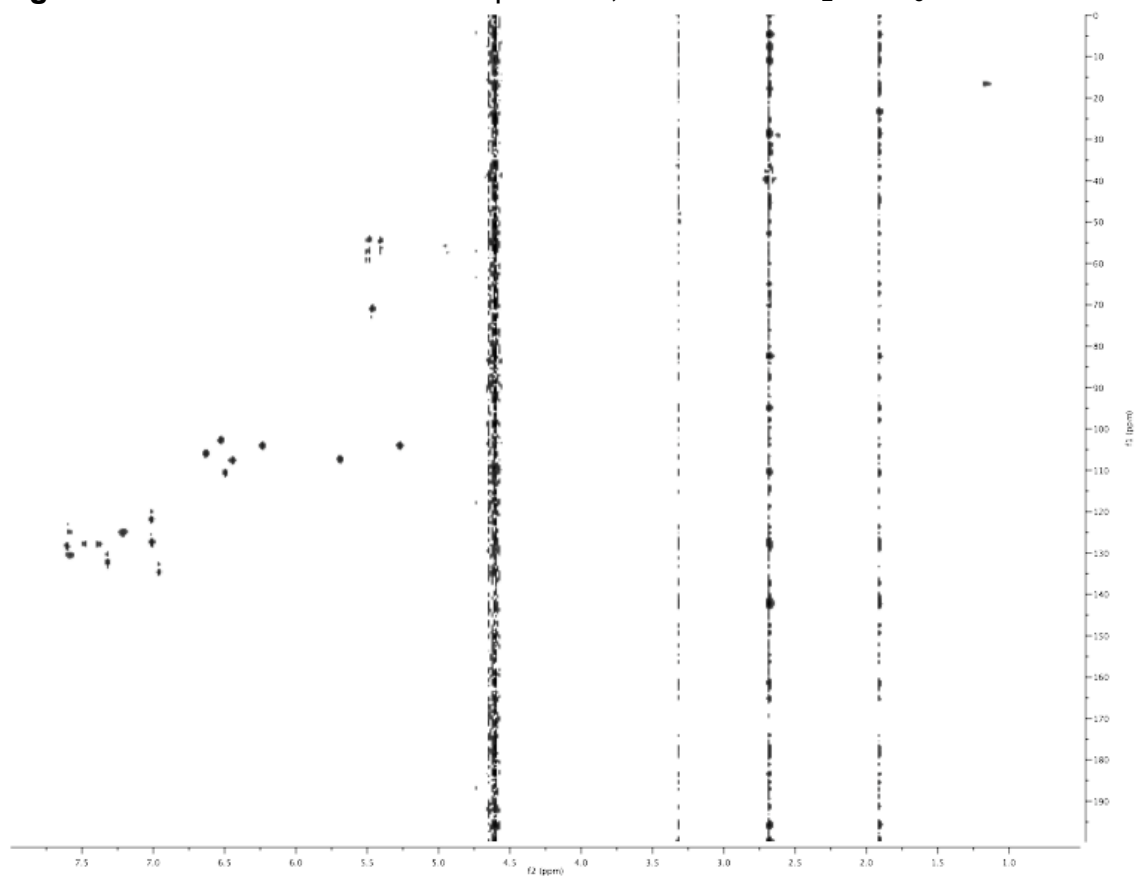
**Figure S9:**  $^1\text{H}$ - $^{13}\text{C}$  HMBC of Compound 3, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



**Figure S10:**  $^1\text{H}$  of Compound 4, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$

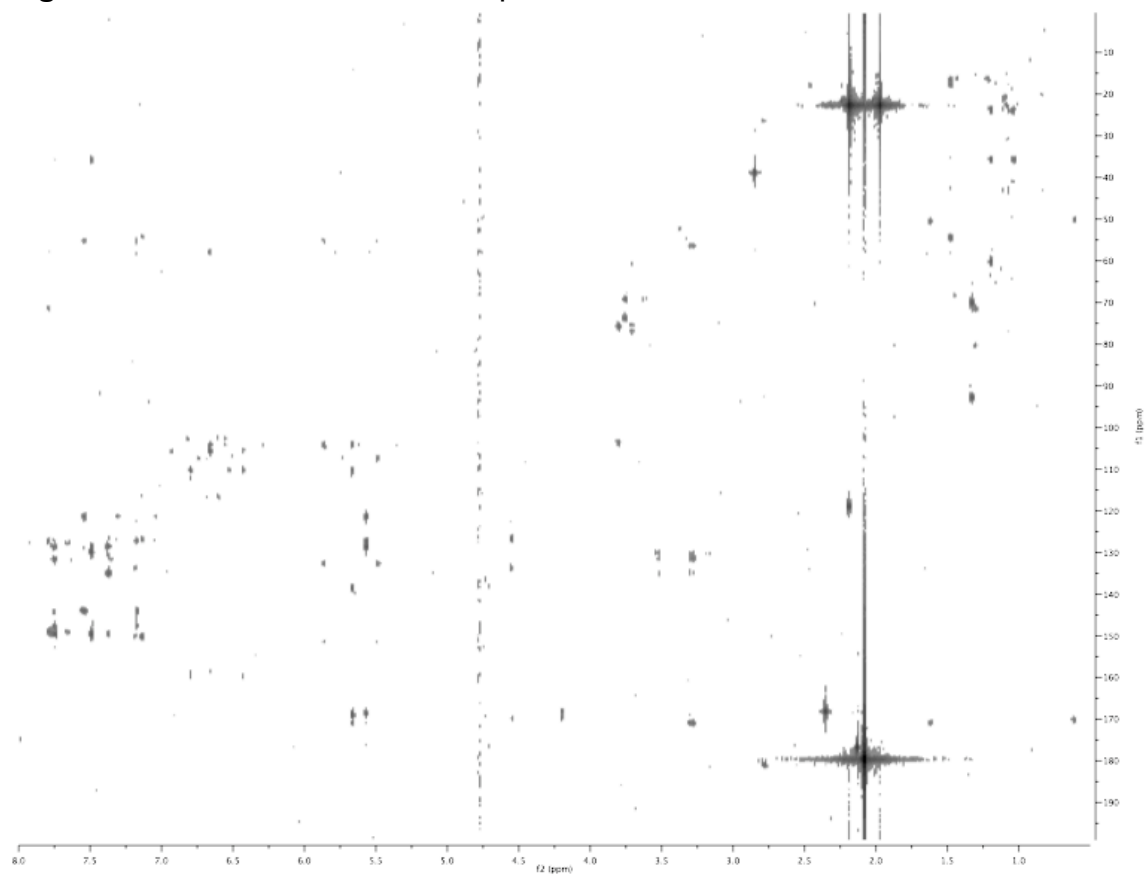


**Figure S11:**  $^1\text{H}$ - $^{13}\text{C}$  HMQC of Compound 4, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$

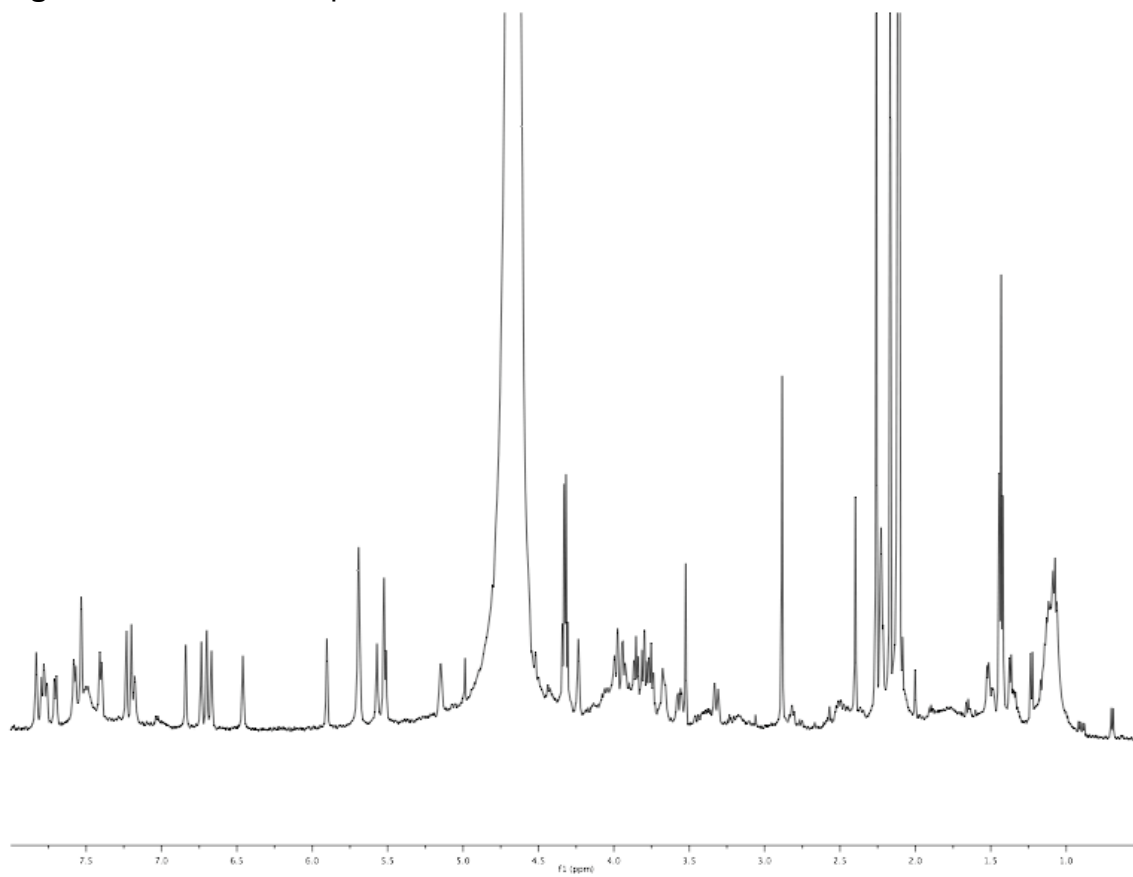




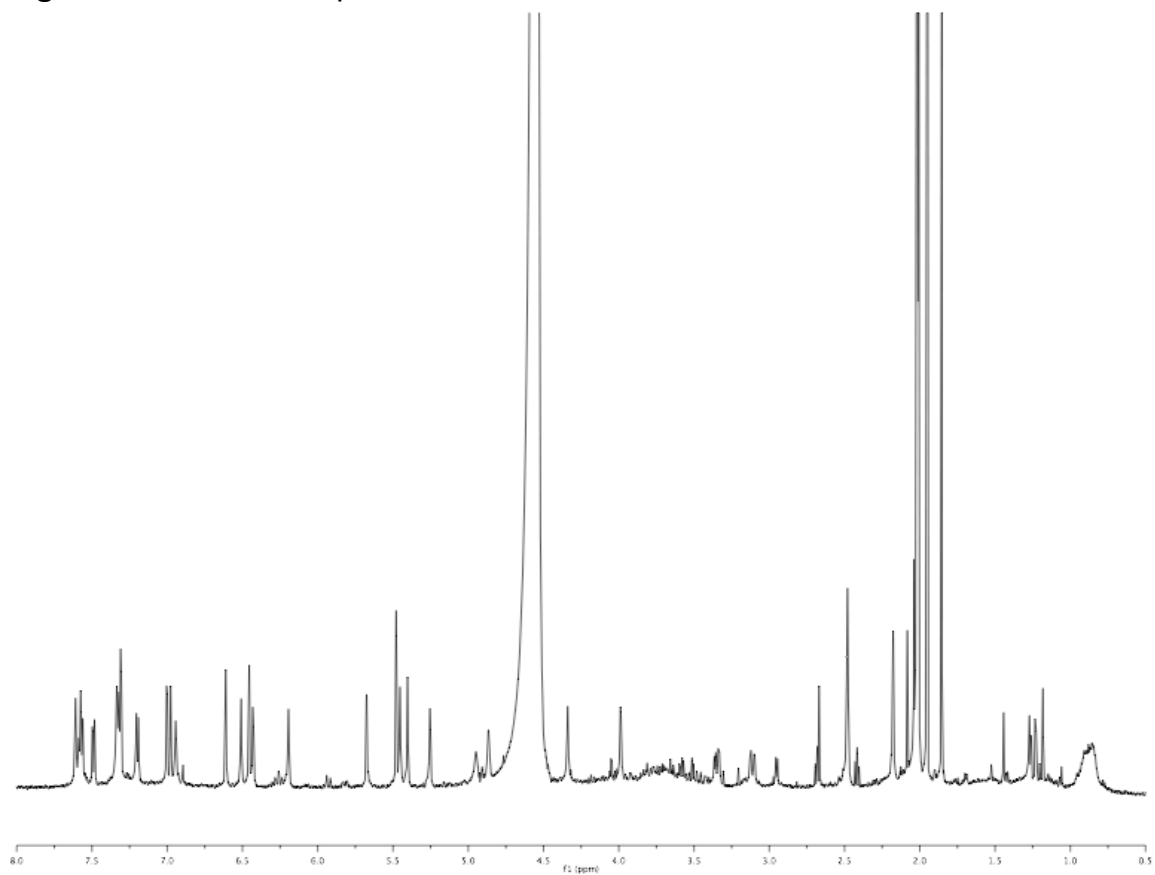
**Figure S12:**  $^1\text{H}$ - $^{13}\text{C}$  HMBC of Compound 4, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



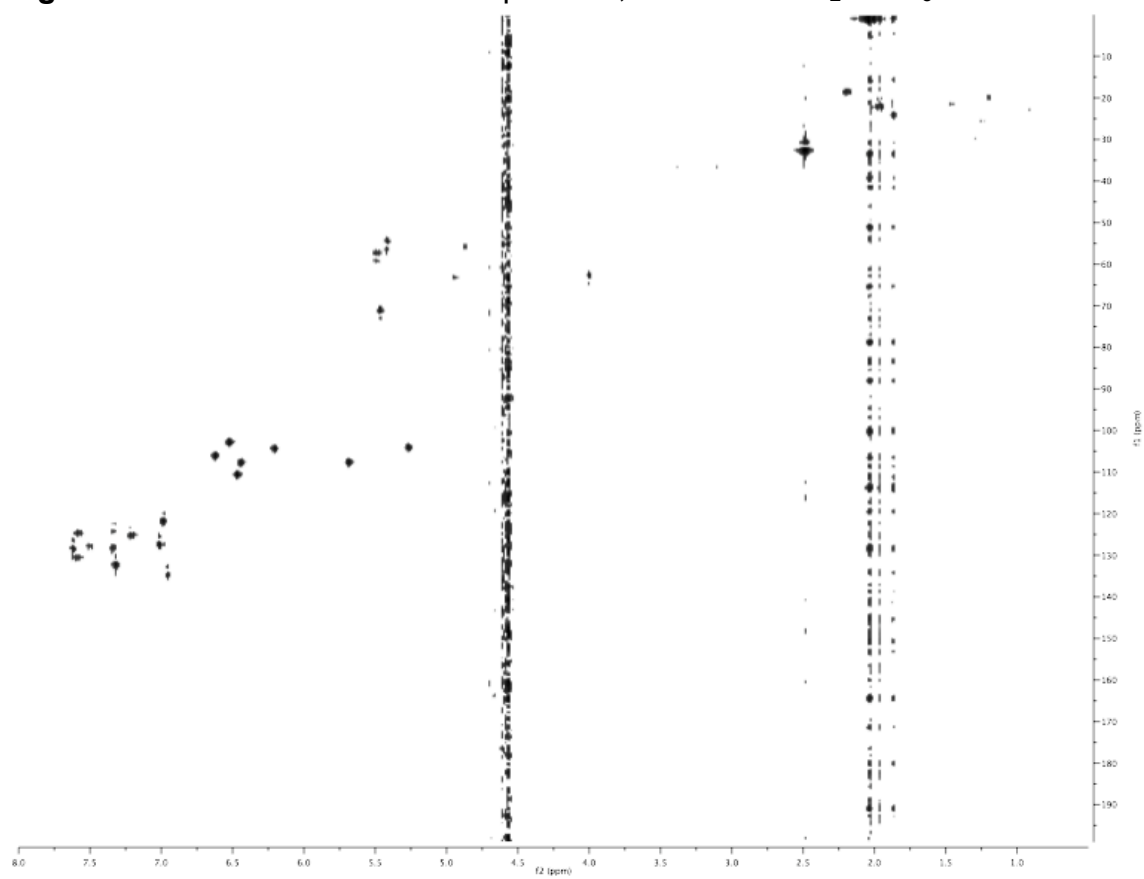
**Figure S13:**  $^1\text{H}$  of Compound 4, 313K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



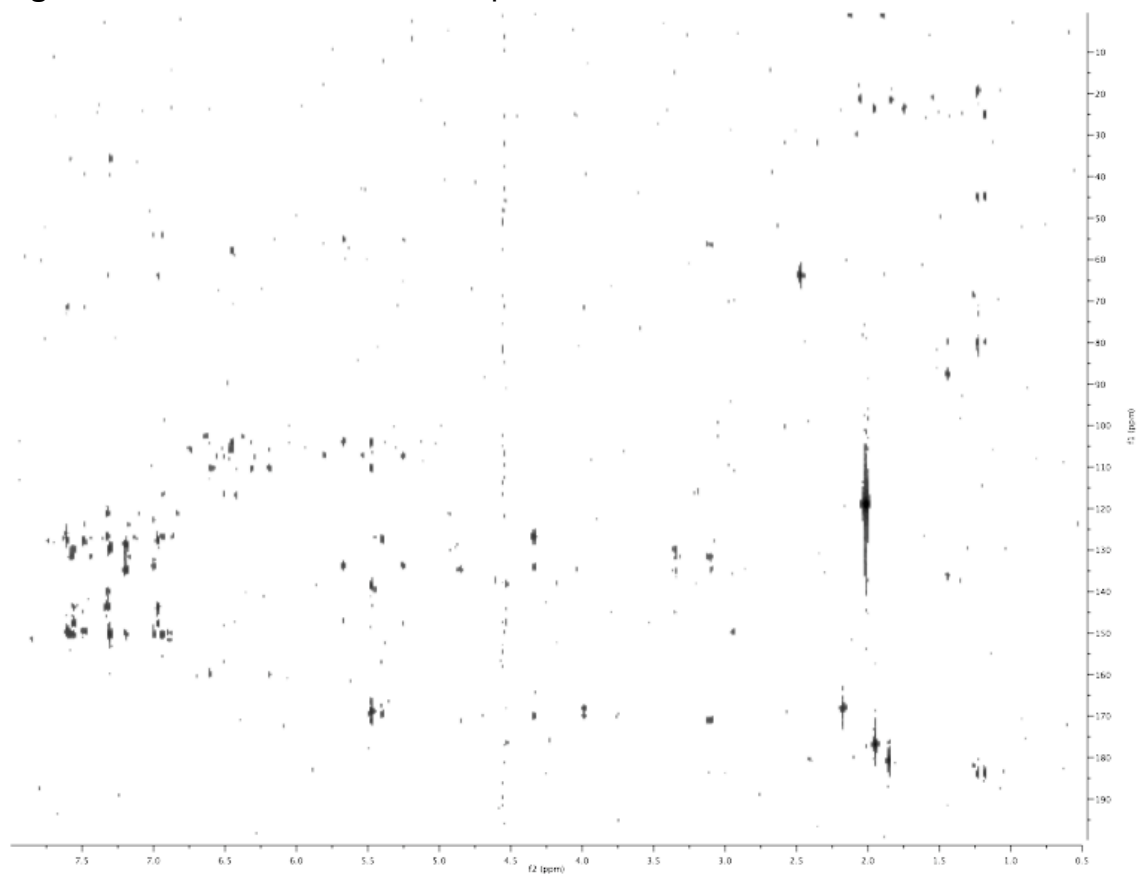
**Figure S14:**  $^1\text{H}$  of Compound 5, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



**Figure S15:**  $^1\text{H}$ - $^{13}\text{C}$  HMQC of Compound 5, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



**Figure S16:**  $^1\text{H}$ - $^{13}\text{C}$  HMBC of Compound 5, 298K in 3:1  $\text{D}_2\text{O}:\text{CD}_3\text{CN}$



**Table S4: Pathway CA37 ORFs**

| ORF | Length (AA) | Predicted Gene Product                           | Glycopeptide Homolog        | AA% ID ( <i>Organism</i> )       | Accession #  |
|-----|-------------|--|-----------------------------|----------------------------------|--------------|
| 1   | 202         | TetR Family Transcriptional Regulator            | None                        | 62%( <i>K. flavida</i> )         | ZP_03865120  |
| 2   | 229         | Short Chain Oxidoreductase                       | None                        | 82%( <i>K. flavida</i> )         | ZP_03863529  |
| 3   | 291         | Phytanoyl CoA Dioxygenase                        | None                        | 43%( <i>M. carbonacea</i> )      | ZP_04605278  |
| 4   | 217         | D-ala-D-ala Peptidase                            | VanX                        | 85%( <i>A. teichomyceticus</i> ) | CAE53345     |
| 5   | 346         | D-ala-D-lac Ligase                               | VanA                        | 86%( <i>A. teichomyceticus</i> ) | CAE53344     |
| 6   | 347         | D-lactate Dehydrogenase                          | VanH                        | 85%( <i>A. teichomyceticus</i> ) | CAE53343     |
| 7   | 430         | D-ala-D-ala Carboxypeptidase                     | VanY                        | 65%( <i>A. balhimycina</i> )     | CAG25753     |
| 8   | 74          | Two-Component Regulatory System                  | VanR                        | 63% ( <i>A. balhimycina</i> )    | CAG25752     |
| 9   | 387         | Two-Component Regulatory System                  | VanS                        | 72%( <i>A. teichomyceticus</i> ) | CAE53347     |
| 10  | 146         | N-acetyltransferase                              | None                        | 38%( <i>B. cepacia</i> )         | YP_368498    |
| 11  | 448         | UDP-N-acetylmuramoyltripectide D-ala-Dala Ligase | MurF                        | 78%( <i>A. teichomyceticus</i> ) | CAE53342     |
| 12  | 369         | DalaDala Ligase                                  | None                        | 62%( <i>T. fusca</i> YX)         | YP_289698    |
| 13  | 370         | UDP-muramoyl-pentapeptide transferase            | MurG                        | 88%( <i>K. flavida</i> )         | ZP_03862660  |
| 14  | 396         | Ferredoxin Reductase                             | None                        | 71%( <i>S. avermitilis</i> )     | NP_822785    |
| 15  | 348         | C/O-Methyltransferase                            | None                        | 48%( <i>S. ghanaensis</i> )      | ZP_04690473  |
| 16  | 2085        | Nonribosomal Peptide Synthetase, modules 1-2     | NRPS 1+ 2                   | 79%( <i>A. teichomyceticus</i> ) | CAE53350     |
| 17  | 1482        | Nonribosomal Peptide Synthetase, module 3        | NRPS3                       | 83%( <i>A. teichomyceticus</i> ) | CAE53351     |
| 18  | 4075        | Nonribosomal Peptide Synthetase, modules 4-6     | NRPS 4-6                    | 82%( <i>A. teichomyceticus</i> ) | CAE53352     |
| 19  | 1850        | Nonribosomal Peptide Synthetase, module 7        | NRPS 7                      | 86%( <i>A. teichomyceticus</i> ) | CAE53353     |
| 20  | 491         | NDP-Hexose-2,3-Dehydratase                       | EvaA                        | 56%( <i>A. balhimycina</i> )     | CAC48374     |
| 21  | 357         | Glucose Thymidyltransferase                      | Glucose Thymidyltransferase | 69%( <i>S. sviveus</i> )         | YP_002205669 |
| 22  | 378         | Glycosyltransferase                              | GtfA                        | 65%( <i>A. teichomyceticus</i> ) | CAE53349     |
| 23  | 407         | Glycosyltransferase                              | GtfB                        | 72%( <i>A. teichomyceticus</i> ) | CAE53364     |
| 24  | 572         | Mannosyltransferase                              | MtfA                        | 77%( <i>A. teichomyceticus</i> ) | CAE53356     |
| 25  | 629         | ABC Transporter                                  | ABC transporter             | 83%( <i>A. teichomyceticus</i> ) | CAE53357     |
| 26  | 392         | P450 Monooxygenase OxyA                          | OxyA                        | 88%( <i>A. teichomyceticus</i> ) | CAE53359     |
| 27  | 385         | P450 Monooxygenase OxyB                          | OxyB                        | 86%( <i>A. teichomyceticus</i> ) | CAE53360     |
| 28  | 399         | P450 Monooxygenase OxyD                          | OxyD                        | 86%( <i>A. teichomyceticus</i> ) | CAE53361     |
| 29  | 411         | P450 Monooxygenase OxyC                          | OxyC                        | 87%( <i>A. teichomyceticus</i> ) | CAE53363     |
| 30  | 441         | Halogenase                                       | Halogenase                  | 69%( <i>A. teichomyceticus</i> ) | CAE53362     |
| 31  | 507         | Non-heme Iron Dioxygenase                        | Non-Heme Iron dioxygenase   | 93%( <i>A. teichomyceticus</i> ) | CAE53366     |
| 32  | 356         | DAHPh Synthase                                   | DAHPh synthase              | 90%( <i>A. teichomyceticus</i> ) | CAE53368     |
| 33  | 331         | Str Family Transcriptional Regulator             | Transcriptional Regulator   | 83%( <i>A. teichomyceticus</i> ) | CAE53369     |
| 34  | 230         | Response Regulator                               | Res. Regulator (2 comp)     | 88%( <i>A. teichomyceticus</i> ) | CAE53348     |
| 35  | 836         | LuxR Regulator                                   | LuxR (2 comp)               | 78%( <i>A. teichomyceticus</i> ) | CAE53370     |
| 36  | 368         | Dihydroxyphenylacetic Acid Synthase              | DpgA                        | 93%( <i>A. teichomyceticus</i> ) | CAE53371     |
| 37  | 222         | Enoyl-CoA Hydratase/Isomerase                    | DpgB                        | 84%( <i>A. teichomyceticus</i> ) | CAE53372     |
| 38  | 434         | Dihydroxyphenylacetyl-CoA Oxygenase              | DpgC                        | 88%( <i>A. teichomyceticus</i> ) | CAE53373     |
| 39  | 239         | Enoyl-CoA Hydratase/Isomerase                    | DpgD                        | 91%( <i>A. teichomyceticus</i> ) | CAE53374     |
| 40  | 199         | GTP Cyclohydrolase                               | GTP Cyclohydrolase          | 91%( <i>A. teichomyceticus</i> ) | CAE53376     |
| 41  | 420         | Hydroxyphenylglycine Transaminase                | HpgT                        | 89%( <i>A. teichomyceticus</i> ) | CAE53377     |
| 42  | 374         | Prephenate Dehydrogenase                         | Prephenate Dehydrogenase    | 83%( <i>A. teichomyceticus</i> ) | CAG15036     |
| 43  | 301         | Transcriptional Regulator                        | None                        | 50%( <i>S. kasugaensis</i> )     | BAC53615     |
| 44  | 329         | Transcriptional Regulator                        | None                        | 50%( <i>S. griseus</i> )         | YP_001827443 |
| 45  | 522         | Non-heme Iron Dioxygenase                        | Non-heme Iron Dioxygenase   | 70%( <i>A. teichomyceticus</i> ) | CAG15037     |
| 46  | 352         | Hydroxymandelate Synthase                        | HmaS                        | 92%( <i>A. teichomyceticus</i> ) | CAE53378     |
| 47  | 365         | Hydroxymandelate Oxygenase                       | Hmo                         | 87%( <i>A. teichomyceticus</i> ) | CAE53379     |

**Table S5: Pathway CA878 ORFs**

| ORF | Length (AA) | Predicted Gene Product                       | Glycopeptide Homolog     | AA %ID ( <i>Orgnaism</i> )        | Accession # |
|-----|-------------|--|--------------------------|-----------------------------------|-------------|
| 1   | 350         | D-lactate Dehydrogenase                      | VanH                     | 74% ( <i>A. teichomyceticus</i> ) | CAG15002    |
| 2   | 346         | D-ala-D-lac Ligase                           | VanA                     | 77% ( <i>A. teichomyceticus</i> ) | CAE53344    |
| 3   | 203         | D-ala-D-ala Dipeptidase                      | VanX                     | 81% ( <i>A. teichomyceticus</i> ) | CAE53345    |
| 4   | 344         | Two-Component Regulatory System              | VanR                     | 69% ( <i>S. toyocaensis</i> )     | AAM80542    |
| 5   | 224         | Two-Component Regulatory System              | VanS                     | 90% ( <i>S. toyocaensis</i> )     | AAM80541    |
| 6   | 161         | D-ala-D-ala Carboxypeptidase                 | VanY                     | 73% ( <i>A. balhimycina</i> )     | CAG25753    |
| 7   | 322         | Str Family Transcriptional Regulator         | Trans Reg                | 85% ( <i>A. balhimycina</i> )     | CAG25754    |
| 8   | 358         | Prephenate Dehydrogenase                     | Prephenate Dehydrogenase | 83% ( <i>A. balhimycina</i> )     | CAG25755    |
| 9   | 709         | ABC Transporter                              | ABC                      | 81% ( <i>A. teichomyceticus</i> ) | CAE53357    |
| 10  | 1858        | Nonribosomal Peptide Synthetase, modules 1-2 | NRPS 1-2                 | 77% ( <i>A. orientalis</i> )      | CAA11794    |
| 11  | 1058        | Nonribosomal Peptide Synthetase, modules 3   | NRPS 3                   | 80% ( <i>A. teichomyceticus</i> ) | CAE53351    |
| 12  | 4078        | Nonribosomal Peptide Synthetase, modules 4-6 | NRPS 4-6                 | 84% ( <i>A. orientalis</i> )      | CAA11795    |
| 13  | 1835        | Nonribosomal Peptide Synthetase, modules 7   | NRPS 7                   | 87% ( <i>A. orientalis</i> )      | CAA11796    |
| 14  | 364         | P450 Monooxygenase OxyA                      | OxyA                     | 81% ( <i>A. teichomyceticus</i> ) | CAE53359    |
| 15  | 386         | P450 Monooxygenase OxyB                      | OxyB                     | 78% ( <i>A. teichomyceticus</i> ) | CAE53360    |
| 16  | 356         | P450 Monooxygenase OxyD                      | OxyD                     | 86% ( <i>A. orientalis</i> )      | CAA11798    |
| 17  | 346         | DAHP Synthase                                | DAHP Synthase            | 72% ( <i>A. teichomyceticus</i> ) | CAE53368    |
| 18  | 411         | P450 Monooxygenase OxyC                      | OxyC                     | 81% ( <i>A. teichomyceticus</i> ) | CAG15021    |
| 19  | 492         | Halogenase                                   | Halogenase               | 93% ( <i>A. orientalis</i> )      | CAA11780    |
| 20  | 388         | Glycosyltransferase                          | GtfA                     | 70% ( <i>A. orientalis</i> )      | AAB49292    |
| 21  | 409         | Glycosyltransferase                          | GtfB                     | 76% ( <i>A. orientalis</i> )      | AAB49293    |
| 22  | 407         | Glycosyltransferase                          | GtfC                     | 72% ( <i>A. orientalis</i> )      | AAB49294    |
| 23  | 278         | Methyltransferase                            | None                     | 54% ( <i>S. coelicolor</i> )      | NP_628799   |
| 24  | 385         | Glycosyltransferase                          | GtfA                     | 69% ( <i>A. teichomyceticus</i> ) | CAE53349    |
| 25  | 286         | Deacetylase                                  | Deacetylase              | 71% ( <i>A. teichomyceticus</i> ) | CAE53355    |
| 26  | 577         | Mannosyltransferase                          | Mannosyltransferase      | 79% ( <i>A. teichomyceticus</i> ) | CAE53356    |
| 27  | 347         | C/O-Methyltransferase                        | None                     | 51% ( <i>S. ghanaensis</i> )      | ZP_04690473 |
| 28  | 435         | Phenylglycine Aminotransferase               | HpgT                     | 89% ( <i>A. balhimycina</i> )     | CAC48367    |
| 29  | 277         | BHT Perhydrolase                             | Bhp                      | 92% ( <i>A. orientalis</i> )      | CAA11784    |
| 30  | 581         | BHT Peptide Synthetase                       | BpsD                     | 92% ( <i>A. orientalis</i> )      | CAA11773    |
| 31  | 397         | BHT Oxygenase                                | OxyD*                    | 89% ( <i>A. orientalis</i> )      | CAA11772    |
| 32  | 354         | Hydroxymandelate Synthase                    | HmaS                     | 82% ( <i>A. orientalis</i> )      | CAA11761    |
| 33  | 359         | Hydroxymandelate Oxidase                     | Hmo                      | 88% ( <i>A. orientalis</i> )      | CAA11762    |
| 34  | 456         | Na <sup>+</sup> /H <sup>+</sup> Antiporter   | Putative Antiporter      | 85% ( <i>A. balhimycina</i> )     | CAC48373    |
| 35  | 477         | NDP-Hexose-2,3-Dehydratase                   | EvaA                     | 90% ( <i>A. balhimycina</i> )     | CAC48374    |
| 36  | 326         | NDP-Hexose-4-Ketoreductase                   | EvaE                     | 86% ( <i>A. orientalis</i> )      | CAA11764    |
| 37  | 370         | NDP-Hexose-3-Aminotransferase                | EvaB                     | 91% ( <i>A. orientalis</i> )      | CAA11782    |
| 38  | 206         | NDP-Hexose-3,5-Epimerase                     | EvaD                     | 82% ( <i>A. orientalis</i> )      | CAA11781    |
| 39  | 608         | Mannosyltransferase                          | None                     | 64% ( <i>S. avermitilis</i> )     | NP_822291   |
| 40  | 373         | Dihydroxyphenylacetic Acid Synthase          | DpgA                     | 94% ( <i>A. orientalis</i> )      | CAA11765    |
| 41  | 219         | Enoyl-CoA Hydratase/Isomerase                | DpgB                     | 85% ( <i>A. balhimycina</i> )     | CAC48379    |
| 42  | 379         | Dihydroxyphenylacetyl-CoA Oxygenase          | DpgC                     | 87% ( <i>A. orientalis</i> )      | CAA11787    |
| 43  | 268         | Enoyl-CoA Hydratase/Isomerase                | DpgD                     | 89% ( <i>A. balhimycina</i> )     | CAC48381    |

**Table S6: Pathway CA915 ORFs**

| ORF | Length (AA) | Predicted Gene Product                             | Glycopeptide Homolog        |                           |              |
|-----|-------------|--|-----------------------------|---------------------------|--------------|
| 1   | 330         | D-ala-D-ala Ligase                                 | None                        | 61% (T. fusca YX)         | YP_289698    |
| 2   | 214         | D-ala-D-ala Dipeptidase                            | VanX                        | 81% (A. teichomyceticus)  | CAE53345     |
| 3   | 347         | D-ala-D-lac-Ligase                                 | VanA                        | 79% (A. teichomyceticus)  | CAE53344     |
| 4   | 419         | Betalactamase                                      | None                        | 51% (A. mirum)            | ZP_03818051  |
| 5   | 277         | Phytanoyl CoA Dioxygenase                          | None                        | 46%(M. carbonacea)        | ZP_04605278  |
| 6   | 448         | UDP-N-acetylmuramoyltri-peptide-D-ala-D-ala Ligase | MurF                        | 75% (A. teichomyceticus)  | CAE53342     |
| 7   | 333         | D-lactate Dehydrogenase                            | VanH                        | 70% (A. teichomyceticus)  | CAG15002     |
| 8   | 399         | Two-component Regulatory System                    | VanS                        | 67% (Frankia sp. EAN1pec) | YP_001511361 |
| 9   | 230         | Two-component Regulatory System                    | VanR                        | 87% (S. toyocaensis)      | AAM80541     |
| 10  | 206         | D-ala-D-ala Carboxypeptidase                       | VanY                        | 54% (S. nassauensis)      | ZP_04486505  |
| 11  | 368         | C/O-Methyltransferase                              | None                        | 50% (S. ghanaensis)       | ZP_04690473  |
| 12  | 1054        | Nonribosomal Peptide Synthetase Module 1           | NRPS1                       | 75% (S. toyocaensis)      | AAM80539     |
| 13  | 1106        | Nonribosomal Peptide Synthetase Module 2           | NRPS2                       | 76% (A. teichomyceticus)  | CAE53350     |
| 14  | 1060        | Nonribosomal Peptide Synthetase Module 3           | NRPS3                       | 77% (A. teichomyceticus)  | CAE53351     |
| 15  | 4068        | Nonribosomal Peptide Synthetase Modules 4-6        | NRPS4-6                     | 78% (A. teichomyceticus)  | CAE53352     |
| 16  | 1872        | Nonribosomal Peptide Synthetase Module 7           | NRPS7                       | 83% (A. teichomyceticus)  | CAE53353     |
| 17  | 501         | NDP-Hexose-2,3-Dehydratase                         | EvaA                        | 66% (S. cyanogenus)       | AAD13549     |
| 18  | 357         | Glucose Thymidyltransferase                        | Glucose Thymidyltransferase | 70% (S. svuceus)          | YP_002205669 |
| 19  | 375         | Glycosyltransferase                                | GtfA (glycosyltransferase)  | 61% (A. orientalis)       | AAB49292     |
| 20  | 408         | Glycosyltransferase                                | GtfB (glycosyltransferase)  | 70% (Nonomurea sp.)       | CAD91204     |
| 22  | 407         | Glycosyltransferase                                | GtfC (glycosyltransferase)  | 67% (A. orientalis)       | AAB49294     |
| 21  | 274         | Methyltransferase Type 11                          | None                        | 57% (Frankia sp. EAN1pec) | YP_001508433 |
| 23  | 381         | Glycosyltransferase                                | GtfA (glycosyltransferase)  | 63% (A. teichomyceticus)  | CAE53349     |
| 24  | 374         | NDP-Hexose-3-Aminotransferase                      | EvaB                        | 80% (S. arenicola)        | YP_001537186 |
| 25  | 201         | NDP-Hexose-3,5-Epimerase                           | EvaD                        | 68% (S. arenicola)        | YP_001537187 |
| 26  | 334         | NDP-Hexose-4-Ketoreductase                         | EvaE                        | 64% (S. venezuelae)       | AAL14256     |
| 27  | 238         | Methyltransferase Type 12                          | None                        | 74% (S. arenicola)        | YP_001539299 |
| 28  | 579         | Mannosyltransferase                                | Mannosyltransferase         | 71% (A. teichomyceticus)  | CAE53356     |
| 29  | 659         | ABC Transporter                                    | ABC Transporter             | 81% (A. teichomyceticus)  | CAE53357     |
| 30  | 231         | Str Family Transcriptional Regulator               | None                        | 42% (S. griseus)          | YP_001822146 |
| 31  | 392         | P450 Monooxygenase, OxyA                           | OxyA                        | 84% (A. teichomyceticus)  | CAE53359     |
| 32  | 385         | P450 Monooxygenase, OxyD                           | OxyD                        | 80% (A. teichomyceticus)  | CAE53360     |
| 33  | 399         | P450 Monooxygenase, OxyB                           | OxyB                        | 78% (A. teichomyceticus)  | CAE53361     |
| 34  | 411         | P450 Monooxygenase, OxyC                           | OxyC                        | 86% (A. teichomyceticus)  | CAE53363     |
| 35  | 412         | Halogenase   | Halogenase                  | 66% (A. teichomyceticus)  | CAE53362     |
| 36  | 531         | Non-Heme Iron Dioxygenase                          | Non-Heme Iron Dioxygenase   | 84% (A. teichomyceticus)  | CAE53366     |
| 37  | 352         | DAHPSynthase                                       | DAHPSynthase                | 87% (A. teichomyceticus)  | CAE53368     |
| 38  | 339         | Str Family Transcriptional Regulator               | Str Family Trans Reg        | 77% (A. teichomyceticus)  | CAE53369     |
| 39  | 513         | Non-Heme Iron Dioxygenase                          | Non-Heme Iron Dioxygenase   | 56% (S. toyocaensis)      | AAM80528     |
| 40  | 389         | Hydroxymandelate Oxygenase                         | Hmo                         | 70% (S. toyocaensis)      | AAM80552     |
| 41  | 806         | Transcriptional Regulator                          | Transcriptional Regulator   | 58% (A. teichomyceticus)  | CAE53370     |
| 42  | 199         | GTP Cyclohydrolase                                 | GTP Cyclohydrolase          | 92% (A. teichomyceticus)  | CAE53376     |
| 43  | 420         | Hydroxyphenylglycine Transaminase                  | HpgT                        | 88% (A. teichomyceticus)  | CAG15035     |
| 44  | 122         | Chorismate Mutase                                  | None                        | 68% (N. dassonvillei)     | ZP_04335576  |
| 45  | 354         | Hydroxymandelate Synthetase                        | HmaS                        | 64% (Frankia sp. Ccl3)    | YP_481550    |
| 46  | 365         | Prephenate Dehydrogenase                           | Prephenate Dehydrogenase    | 73% (A. teichomyceticus)  | CAG15036     |
| 47  | 369         | Dihydroxyphenylacetic Acid Synthase                | DpgA                        | 84% (A. teichomyceticus)  | CAE53371     |
| 48  | 221         | Enoyl-CoA Hydratase/Isomerase                      | DpgB                        | 67% (A. teichomyceticus)  | CAE53372     |
| 49  | 434         | Dihydroxyphenylacetyl-CoA Oxygenase                | DpgC                        | 78% (A. teichomyceticus)  | CAE53373     |
| 50  | 239         | Enoyl-CoA Hydratase/Isomerase                      | DpgD                        | 83% (A. teichomyceticus)  | CAE53374     |
| 51  | 395         | UDP muramoyl pentapeptide transferase              | MurG                        | 60% (S. arenicola)        | YP_001535200 |

\*This is the fourth oxidative enzyme found in balhimycin biosynthesis, OxyD, which is essential for the biosynthesis of  $\beta$ -HT. This differs from the OxyD which forms one of the four macrocycles in the biosynthesis of type III and type IV glycopeptides.