

## Supplementary Information

# Genomics- and Metabolomics-Based Investigation of the Deep-Sea Sediment-Derived Yeast, *Rhodotorula mucilaginosa* 50-3-19/20B

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**Table S1.** Bioactivity screening of extracts derived from six deep-sea *Rhodotorula* spp.

| Sample             | Cell culture |      |       | ESKAPE panel |        |        |        |    |    |     |    | Phytopatogenic bacteria |    |    | Yeasts |    | Phytopathogenic fungi |    |    | Dermatophytes |     |
|--------------------|--------------|------|-------|--------------|--------|--------|--------|----|----|-----|----|-------------------------|----|----|--------|----|-----------------------|----|----|---------------|-----|
|                    | MB231        | A375 | HaCaT | Efm          |        | MRSA   |        | Kp | Ab | Psa | Ec | Pss                     | Ea | Rs | Ca     | Cn | Pi                    | Po | Bc | Tr            | Tm  |
|                    |              |      |       | exp. 1       | exp. 2 | exp. 1 | exp. 2 |    |    |     |    |                         |    |    |        |    |                       |    |    |               |     |
| 50-3-19/20B_W      | -            | -    | -     | 32           | 59     | 99     | 96     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| 50-3-19/20B_P      | 74           | 73   | 20    | -            | 37     | -      | -      | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| 52-1-0/1B_W        | -            | -    | -     | 24           | 100    | 99     | 99     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| 52-1-0/1B_P        | -            | -    | -     | 78           | 100    | 99     | 100    | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| 54-4-0-/1B_W       | -            | -    | -     | 91           | 100    | 99     | 99     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| 54-4-0-/1B_P       | -            | -    | -     | -            | 100    | -      | 100    | -  | 22 | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| LR 28-14-1-1-1-1_W | -            | -    | -     | 62           | 86     | 99     | 96     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| LR 28-14-1-1-1-1_P | -            | -    | -     | 100          | 71     | 94     | 93     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| LR 28-17-4-1_W     | -            | -    | -     | 61           | 90     | 100    | 99     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| LR 28-17-4-1_P     | -            | -    | -     | 85           | 100    | 94     | 97     | -  | -  | -   | -  | -                       | -  | 29 | -      | -  | -                     | -  | -  | -             | -   |
| LR 5-2-4/4-1_W     | -            | -    | -     | 79           | 79     | 98     | 96     | -  | -  | -   | -  | -                       | -  | 23 | -      | -  | -                     | -  | -  | -             | -   |
| LR 5-2-4/4-1_P     | -            | -    | -     | 83           | 53     | 88     | 92     | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| WSP-30 blank       | -            | -    | -     | -            | -      | -      | -      | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |
| PDA blank          | 40           | 28   | 44    | 24           | 47     | 40     | -      | -  | -  | -   | -  | -                       | -  | 24 | -      | -  | -                     | -  | -  | -             | 28  |
| positive control   | 83           | 97   | 66    | 97           | 91     | 97     | 96     | 99 | 42 | 99  | 96 | 64                      | 92 | 94 | 98     | 99 | 98                    | 98 | 99 | 96            | 100 |
| solvent control    | -            | -    | -     | -            | -      | -      | -      | -  | -  | -   | -  | -                       | -  | -  | -      | -  | -                     | -  | -  | -             | -   |

MB231: breast cancer; A375: lung carcinoma; HaCaT: non-cancerous keratinocyte; Efm: *Enterococcus faecium*; MRSA: methicillin-resistant *Staphylococcus aureus*; Kp: *Klebsiella pneumoniae*; Ab: *Acinetobacter baumannii*; Psa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Pss: *Pseudomonas syringae*; Ea: *Erwinia amylovora*; Rs: *Ralstonia solanacearum*; Ca: *Candida albicans*; Cn: *Cryptococcus neoformans*; Pi: *Phytophthora infestans*; Po: *Pycularia oryzae*; Bc: *Botrytis cinerea*; Tr: *Trichophyton rubrum*; Tm: *T. mentagrophytes*

**Table S2.** Overview of the genomic locus for exo-inulinase enzyme in marine *R. mucilaginosa* 50-3-19/20B, as deduced via Omicsbox and Pfam annotations.

| Name     | Description  | Protein Length | Pfam annotation  | Pfam Domain     | Pfam ID    | E-value | Start | End  |
|----------|--|----------------|--|-----------------|------------|---------|-------|------|
| g1614.t1 | Endonuclease/exonuclease/phosphatase domain-containing protein | 1604           | Endonuclease/Exonuclease/phosphatase family                | Exo_endo_phos   | PF03372.22 | 9.0E-6  | 99    | 472  |
| g1615.t1 | hypothetical protein RHOSPDRAFT_19222                          | 463            | Translation initiation factor SUI1                         | SUI1            | PF01253.21 | 3.6E-27 | 353   | 429  |
| g1616.t1 | ---NA---   | 663            | Permease for cytosine/purines, uracil, thiamine, allantoin | Transp_cyt_pur  | PF02133.14 | 3.7E-42 | 326   | 579  |
|          |  |                | Permease for cytosine/purines, uracil, thiamine, allantoin | Transp_cyt_pur  | PF02133.14 | 2.3E-25 | 100   | 275  |
| g1617.t1 | hypothetical protein BMF94_3082                                | 66             |  |                 |            |         |       |      |
| g1618.t1 | FAD/NAD(P)-binding domain-containing protein                   | 592            | GDP dissociation inhibitor                                 | GDI             | PF00996.17 | 2.2E-32 | 5     | 284  |
| g1619.t1 | ---NA---   | 101            | Vacuolar protein sorting 55                                | Vps55           | PF04133.13 | 1.2E-24 | 6     | 97   |
| g1620.t1 | hypothetical protein RHOSPDRAFT_34835                          | 812            |  |                 |            |         |       |      |
| g1621.t1 | actin-like ATPase domain-containing protein                    | 1956           |  |                 |            |         |       |      |
| g1622.t1 | NAD-P-binding protein  | 1910           | short chain dehydrogenase                                  | adh_short       | PF00106.24 | 5.1E-24 | 1641  | 1861 |
| g1623.t1 | ---NA---   | 1041           |  |                 |            |         |       |      |
| g1624.t1 | ARM repeat-containing protein                                  | 1943           |  |                 |            |         |       |      |
| g1625.t1 | hypothetical protein RHOSPDRAFT_35524                          | 1198           |  |                 |            |         |       |      |
| g1626.t1 | L-iditol 2-dehydrogenase                                       | 617            | Alcohol dehydrogenase GroES-like domain                    | ADH_N           | PF08240.11 | 3.5E-24 | 59    | 171  |
|          |  |                | Zinc-binding dehydrogenase                                 | ADH_zinc_N      | PF00107.25 | 1.5E-13 | 211   | 343  |
| g1627.t1 | ferric reductase transmembrane component                       | 778            | Ferric reductase like transmembrane component              | Ferric_reduct   | PF01794.18 | 6.5E-18 | 186   | 302  |
|          |  |                | Ferric reductase NAD binding domain                        | NAD_binding_6   | PF08030.11 | 1.5E-09 | 504   | 659  |
| g1628.t1 | protein of ctr copper transporter family                       | 216            | Ctr copper transporter family                              | Ctr             | PF04145.14 | 6.2E-33 | 22    | 193  |
| g1629.t1 | beta-fructofuranosidase  | 679            | Glycosyl hydrolases family 32 N-terminal domain            | Glyco_hydro_32N | PF00251.19 | 6.5E-76 | 178   | 485  |
|          |  |                | Glycosyl hydrolases family 32 C terminal                   | Glyco_hydro_32C | PF08244.11 | 1.3E-5  | 506   | 673  |
| g1630.t1 | Dynactin, subunit p25  | 696            | RING-variant domain  | RINGv           | PF12906.6  | 9.5E-13 | 341   | 391  |

|          |                                       |      |  |              |            |         |      |      |
|----------|---------------------------------------|------|--|--------------|------------|---------|------|------|
| g1631.t1 | gpi anchored protein                  | 275  |  |              |            |         |      |      |
| g1632.t1 | lipoyl(octanoyl) transferase          | 356  |  |              |            |         |      |      |
| g1633.t1 | hypothetical protein RHOSPDRAFT_32393 | 744  | Glycine-rich domain-containing protein-like      | GRDP-like    | PF07173.11 | 6.8E-09 | 403  | 516  |
| g1634.t1 | voltage-gated chloride channel        | 1809 | Voltage gated chloride channel                   | Voltage_CLC  | PF00654.19 | 1.2E-85 | 1176 | 1538 |
|          |                                       |      | Rab-GTPase-TBC domain                            | RabGAP-TBC   | PF00566.17 | 4.9E-52 | 481  | 683  |
|          |                                       |      | CBS domain                                       | CBS          | PF00571.27 | 8.1E-4  | 1680 | 1735 |
| g1635.t1 | hypothetical protein RHOSPDRAFT_24861 | 600  |  |              |            |         |      |      |
| g1636.t1 | E3 ubiquitin-protein ligase UBR1      | 2049 | Putative zinc finger in N-recognin (UBR box)     | zf-UBR       | PF02207.19 | 1.2E-15 | 115  | 189  |
|          |                                       |      | ATP-dependent Clp protease adaptor protein ClpS  | ClpS         | PF02617.16 | 1.5E-13 | 429  | 500  |
| g1637.t1 | carbon-nitrogen hydrolase             | 332  | Carbon-nitrogen hydrolase                        | CN_hydrolase | PF00795.21 | 1.4E-26 | 34   | 319  |
| g1638.t1 | hypothetical protein RHOSPDRAFT_24864 | 805  |  |              |            |         |      |      |
| g1639.t1 | hypothetical protein RHOSPDRAFT_24865 | 920  |  |              |            |         |      |      |
| g1640.t1 | hypothetical protein RHOSPDRAFT_24865 | 564  |  |              |            |         |      |      |
| g1641.t1 | NAD(P)-binding protein                | 303  | Enoyl-(Acyl carrier protein) reductase           | adh_short_C2 | PF13561.5  | 8.6E-47 | 65   | 300  |
| g1642.t1 | Other/IRE protein kinase              | 1382 | Ribonuclease 2-5A                                | Ribonuc_2-5A | PF06479.11 | 1.8E-45 | 1249 | 1375 |
|          |                                       |      | Protein kinase domain                            | Pkinase      | PF00069.24 | 1.6E-31 | 941  | 1130 |
|          |                                       |      | Protein kinase domain                            | Pkinase      | PF00069.24 | 1.3E-7  | 1156 | 1243 |
| g1643.t1 | C6 transcription factor               | 1042 |  |              |            |         |      |      |
| g1644.t1 | taurine catabolism dioxygenase        | 377  | Taurine catabolism dioxygenase TauD, TfdA family | TauD         | PF02668.15 | 6E-40   | 54   | 352  |

**Table S3.** Annotation summary of putative exo-inulinase enzyme from *Rhodotorula mucilaginosa* 50-3-19/20B and its homologs in different fungi

| Sequence ID | Species                                  | Description                                    | Protein Length | e-Value | sim mean | #GO | GO IDs   | GO Names  | Enzyme Codes                                | Enzyme Names   | InterPro IDs   | InterPro GO IDs               | InterPro GO Names  |
|-------------|--|--|----------------|---------|----------|-----|--|---|---|--|--|-------------------------------|--|
| g1629.t1    | R.<br><i>mucilaginosa</i><br>50-3-19/20B | glycoside<br>hydrolase<br>family 32<br>protein | 679            | 0       | 74.14    | 3   | P:GO:0005987;<br>F:GO:0004575;<br>C:GO:0000324 | P:sucrose catabolic<br>process; F:sucrose<br>alpha-glucosidase<br>activity; C:fungal-<br>type vacuole | EC:3.2.1.20;<br>EC:3.2.1.26;<br>EC:3.2.1.48 | Alpha-glucosidase; Beta-<br>fructofuranosidase;<br>Sucrose alpha-<br>glucosidase | IPR013189 (PFAM);<br>G3DSA:2.60.120.560 (GENE3D);<br>IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>IPR013148 (PFAM); mobidb-lite<br>(MOBIDB_LITE);<br>PTHR42800:SF2 (PANTHER);<br>PTHR42800 (PANTHER);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY) | no GO terms                   | no GO terms  |
| AZR37516.1  | <i>Rhodotorula</i><br><i>paludigena</i>  | beta-<br>fructofurano<br>sidase                | 617            | 0       | 68.7     | 3   | P:GO:0005987;<br>F:GO:0004575;<br>C:GO:0000324 | P:sucrose catabolic<br>process; F:sucrose<br>alpha-glucosidase<br>activity; C:fungal-<br>type vacuole | EC:3.2.1.20;<br>EC:3.2.1.26;<br>EC:3.2.1.48 | Alpha-glucosidase; Beta-<br>fructofuranosidase;<br>Sucrose alpha-<br>glucosidase | IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>IPR013148 (PFAM);<br>G3DSA:2.60.120.560 (GENE3D);<br>mobidb-lite (MOBIDB_LITE);<br>mobidb-lite (MOBIDB_LITE);<br>PTHR42800:SF2 (PANTHER);<br>PTHR42800 (PANTHER);<br>IPR018053<br>(PROSITE_PATTERNS);                             | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |

|            |                            |                                       |     |   |       |   |                            |  |  |   |                            |  |
|------------|----------------------------|---------------------------------------|-----|---|-------|---|----------------------------|--|--|---|----------------------------|--|
|            |                            |                                       |     |   |       |   |                            |  |  | cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)   |                            |  |
| TKA53735.1 | Rhodotorula sp. CCFEE 5036 | glycoside hydrolase family 32 protein | 676 | 0 | 74.85 | 2 | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |  | IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); G3DSA:2.60.120.560 (GENE3D); IPR013148 (PFAM); mobidb-lite (MOBIDB_LITE); mobidb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY) | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KWU45911.1 | Rhodotorula sp. JG-1b      | glycoside hydrolase family 32 protein | 559 | 0 | 74.92 | 2 | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |  | IPR013189 (PFAM); IPR013148 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D); mobidb-lite (MOBIDB_LITE); mobidb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053   | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |

|            |                               |                                 |     |   |       |   |   |  |   |   |  |  |  |
|------------|-------------------------------|---------------------------------|-----|---|-------|---|---|--|---|---|--|--|--|
|            |                               |                                 |     |   |       |   |   |  |   | (PROSITE_PATTERNS);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY)  |  |  |  |
| POY72393.1 | Rhodotorula<br>taiwanensis    | beta-<br>fructofurano<br>sidase | 614 | 0 | 73.96 | 2 | P:GO:0005975;<br>F:GO:0004553                                   | P:carbohydrate<br>metabolic process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds                                      |   | G3DSA:2.60.120.560 (GENE3D);<br>IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>IPR013189 (PFAM); IPR013148<br>(PFAM); mobidb-lite<br>(MOBIDB_LITE); PTHR42800<br>(PANTHER); PTHR42800:SF2<br>(PANTHER); IPR018053<br>(PROSITE_PATTERNS);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553  | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |  |
| GFZ51952.1 | Saitozyma<br>sp. JCM<br>24511 | beta-<br>fructofurano<br>sidase | 523 | 0 | 77.08 | 4 | P:GO:0005987;<br>F:GO:0004575;<br>C:GO:0000324;<br>C:GO:0016021 | P:sucrose catabolic<br>process; F:sucrose<br>alpha-glucosidase<br>activity; C:fungal-<br>type vacuole;<br>C:integral<br>component of<br>membrane | EC:3.2.1.20;<br>EC:3.2.1.26;<br>EC:3.2.1.48 | Alpha-glucosidase; Beta-<br>fructofuranosidase;<br>Sucrose alpha-<br>glucosidase  | IPR013189 (PFAM); IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>IPR013148 (PFAM);<br>G3DSA:2.60.120.560 (GENE3D);<br>mobidb-lite (MOBIDB_LITE);<br>PTHR42800:SF2 (PANTHER);<br>PTHR42800 (PANTHER);<br>IPR018053<br>(PROSITE_PATTERNS);<br>cd18622 (CDD); IPR023296 | P:GO:0005975;<br>F:GO:0004553  | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |



|            |                     |  |     |   |       |   |                               |   |  |   |                               |  |
|------------|---------------------|--|-----|---|-------|---|-------------------------------|---|--|---|-------------------------------|--|
|            |                     |  |     |   |       |   |                               |   |  | (SUPERFAMILY); IPR013320<br>(SUPERFAMILY)   |                               |  |
| KKY24889.1 | Diplodia<br>seriata | glycoside<br>hydrolase<br>family 32<br>protein | 563 | 0 | 85.94 | 2 | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |  | IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>IPR013148 (PFAM); IPR013189<br>(PFAM); G3DSA:2.60.120.560<br>(GENE3D); PTHR42800:SF2<br>(PANTHER); PTHR42800<br>(PANTHER); IPR018053<br>(PROSITE_PATTERNS);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |
| OMP85659.1 | Diplodia<br>seriata | glycoside<br>hydrolase<br>family 32<br>protein | 563 | 0 | 86.07 | 2 | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |  | IPR013148 (PFAM); IPR013189<br>(PFAM); IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>G3DSA:2.60.120.560 (GENE3D);<br>PTHR42800:SF2 (PANTHER);<br>PTHR42800 (PANTHER);<br>IPR018053<br>(PROSITE_PATTERNS);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-<br>glycosyl<br>compounds |

|                |                          |                                       |     |   |       |   |  |   |  |   |                               |   |
|----------------|--------------------------|---------------------------------------|-----|---|-------|---|--|---|--|---|-------------------------------|---|
| XP_035362863.1 | Lasiodiplodia theobromae | glycoside hydrolase family 32 protein | 562 | 0 | 86.11 | 2 | P:GO:0005975;<br>F:GO:0004553                  | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |  | IPR013148 (PFAM); IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| XP_020129257.1 | Diplodia corticola       | glycoside hydrolase family 32 protein | 563 | 0 | 85.81 | 2 | P:GO:0005975;<br>F:GO:0004553                  | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |  | G3DSA:2.60.120.560 (GENE3D); IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KAF4312946.1   | Botryosphaeria dothidea  | glycoside hydrolase family 32 protein | 562 | 0 | 85.72 | 3 | P:GO:0005975;<br>F:GO:0004553;<br>C:GO:0016021 | P:carbohydrate metabolic process;<br>F:hydrolase activity,                                  |  | IPR013148 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); G3DSA:2.60.120.560 (GENE3D);   | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate metabolic process;<br>F:hydrolase  |

|            |                                |                                       |     |   |       |   |  |  |  |  |   |                               |  |
|------------|--------------------------------|---------------------------------------|-----|---|-------|---|--|--|--|--|---|-------------------------------|--|
|            |                                |                                       |     |   |       |   |  | hydrolyzing O-glycosyl compounds;<br>C:integral component of membrane  |  |  | PTHR42800 (PANTHER);<br>PTHR42800:SF2 (PANTHER);<br>IPR018053<br>(PROSITE_PATTERNS);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY)   |                               | activity,<br>hydrolyzing O-glycosyl<br>compounds   |
| KIJ52043.1 | Sphaerobolus stellatus SS14    | glycoside hydrolase family 32 protein | 562 | 0 | 84.23 | 2 | P:GO:0005975;<br>F:GO:0004553                  | P:carbohydrate metabolic process;<br>F:hydrolase activity,<br>hydrolyzing O-glycosyl<br>compounds                |  |  | IPR013148 (PFAM); IPR013189<br>(PFAM); IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>G3DSA:2.60.120.560 (GENE3D);<br>PTHR42800 (PANTHER);<br>PTHR42800:SF2 (PANTHER);<br>IPR018053<br>(PROSITE_PATTERNS);<br>cd18622 (CDD); IPR013320<br>(SUPERFAMILY); IPR023296<br>(SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-glycosyl<br>compounds |
| THU96730.1 | Dendrothele bispora CBS 962.96 | glycoside hydrolase family 32 protein | 523 | 0 | 84.95 | 3 | P:GO:0005975;<br>F:GO:0004553;<br>C:GO:0016021 | P:carbohydrate metabolic process;<br>F:hydrolase activity,<br>hydrolyzing O-glycosyl<br>compounds;<br>C:integral |  |  | G3DSA:2.60.120.560 (GENE3D);<br>IPR023296<br>(G3DSA:2.115.10.GENE3D);<br>IPR013148 (PFAM); IPR013189<br>(PFAM); PTHR42800<br>(PANTHER); PTHR42800:SF2<br>(PANTHER); IPR018053<br>(PROSITE_PATTERNS);  | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate<br>metabolic<br>process;<br>F:hydrolase<br>activity,<br>hydrolyzing O-glycosyl<br>compounds |

|                |                                   |                                       |     |   |       |   |  |  |  |  |   |                            |  |
|----------------|-----------------------------------|---------------------------------------|-----|---|-------|---|--|--|--|--|---|----------------------------|--|
|                |                                   |                                       |     |   |       |   |  | component of membrane  |  |  | cd18622 (CDD); IPR013320 (SUPERFAMILY); IPR023296 (SUPERFAMILY)   |                            |  |
| XP_008034329.1 | Trametes versicolor FP-101664 SS1 | glycoside hydrolase family 32 protein | 540 | 0 | 83.59 | 3 | P:GO:0005975; F:GO:0004553; C:GO:0016021 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; C:integral component of membrane |  |  | IPR013189 (PFAM); G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KAF5355425.1   | Tetrapyrgos nigripes              | glycoside hydrolase family 32 protein | 521 | 0 | 83.08 | 3 | P:GO:0005975; F:GO:0004553; C:GO:0016021 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; C:integral component of membrane |  |  | IPR013148 (PFAM); G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR013320  | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |

|              |                           |                                       |     |   |       |   |  |  |   |   |   |  |   |
|--------------|---------------------------|---------------------------------------|-----|---|-------|---|--|--|---|---|---|--|---|
|              |                           |                                       |     |   |       |   |  |  |   | (SUPERFAMILY); IPR023296<br>(SUPERFAMILY)   |   |  |   |
| KAE8543285.1 | Cryptococcus cf. gattii   | beta-fructofuranosidase               | 519 | 0 | 97.55 | 3 | P:GO:0005987;<br>F:GO:0004575;<br>C:GO:0000324 | P:sucrose catabolic process; F:sucrose alpha-glucosidase activity; C:fungal-type vacuole                                   | EC:3.2.1.20;<br>EC:3.2.1.26;<br>EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; Sucrose alpha-glucosidase   | G3DSA:2.60.120.560 (GENE3D);<br>IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D);<br>IPR013148 (PFAM); mobidb-lite (MOBIDB_LITE); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS);<br>cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553  | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| OSD00128.1   | Trametes coccinea BREM310 | glycoside hydrolase family 32 protein | 524 | 0 | 83.56 | 3 | P:GO:0005975;<br>F:GO:0004553;<br>C:GO:0016021 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; C:integral component of membrane |   | IPR013148 (PFAM);<br>G3DSA:2.60.120.560 (GENE3D);<br>IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D);<br>PTHR42800 (PANTHER);<br>PTHR42800:SF2 (PANTHER);<br>IPR018053 (PROSITE_PATTERNS);<br>cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553   | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |   |

|              |                               |   |     |   |       |   |   |   |   |   |  |                               |   |
|--------------|-------------------------------|---|-----|---|-------|---|---|---|---|---|--|-------------------------------|---|
| EOD51241.1   | Neofusicoccum parvum UCRNP2   | glycoside hydrolase family 32 protein         | 563 | 0 | 84.03 | 2 | P:GO:0005975;<br>F:GO:0004553                                   | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds   |   |   | IPR013148 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D); mobidb-lite (MOBIDB_LITE); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KAF2091127.1 | Saccharata proteae CBS 121410 | SCF E3 ubiquitin ligase complex F-box protein | 537 | 0 | 79.88 | 4 | P:GO:0005975;<br>P:GO:0006629;<br>F:GO:0004553;<br>F:GO:0008081 | P:carbohydrate metabolic process;<br>P:lipid metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds;<br>F:phosphoric diester hydrolase activity |   |   | G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)                            | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate metabolic process;<br>F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KIR51126.1   | Cryptococcus gattii Ru294     | beta-fructofuranosidase                       | 519 | 0 | 97.81 | 3 | P:GO:0005987;<br>F:GO:0004575;<br>C:GO:0000324                  | P:sucrose catabolic process; F:sucrose alpha-glucosidase  | EC:3.2.1.20;<br>EC:3.2.1.26;<br>EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; | IPR023296 (G3DSA:2.115.10.GENE3D); G3DSA:2.60.120.560 (GENE3D);  | P:GO:0005975;<br>F:GO:0004553 | P:carbohydrate metabolic process;   |

|             |   |                         |     |   |       |   |  |  |                                       |   |   |                            |  |
|-------------|---|-------------------------|-----|---|-------|---|--|--|---------------------------------------|---|---|----------------------------|--|
|             |   |                         |     |   |       |   |  | activity; C:fungal-type vacuole  |                                       | Sucrose alpha-glucosidase   | IPR013189 (PFAM); IPR013148 (PFAM); PTHR42800 (PANTHER); PTHR42800:SF2 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY)   |                            | F:hydrolase activity, hydrolyzing O-glycosyl compounds                                   |
| XP_567775.1 | Cryptococcus neoformans var. neoformans JEC21 | beta-fructofuranosidase | 519 | 0 | 96.37 | 3 | P:GO:0005987; F:GO:0004575; C:GO:0000324 | P:sucrose catabolic process; F:sucrose alpha-glucosidase activity; C:fungal-type vacuole | EC:3.2.1.20; EC:3.2.1.26; EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; Sucrose alpha-glucosidase | G3DSA:2.60.120.560 (GENE3D); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013189 (PFAM); IPR013148 (PFAM); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053 (PROSITE_PATTERNS); cd18622 (CDD); IPR023296 (SUPERFAMILY); IPR013320 (SUPERFAMILY) | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds |
| KIR83202.1  | Cryptococcus gattii VGIV IND107               | beta-fructofuranosidase | 519 | 0 | 97.96 | 3 | P:GO:0005987; F:GO:0004575; C:GO:0000324 | P:sucrose catabolic process; F:sucrose alpha-glucosidase activity; C:fungal-type vacuole | EC:3.2.1.20; EC:3.2.1.26; EC:3.2.1.48 | Alpha-glucosidase; Beta-fructofuranosidase; Sucrose alpha-glucosidase | IPR013189 (PFAM); IPR023296 (G3DSA:2.115.10.GENE3D); IPR013148 (PFAM); G3DSA:2.60.120.560 (GENE3D); PTHR42800:SF2 (PANTHER); PTHR42800 (PANTHER); IPR018053   | P:GO:0005975; F:GO:0004553 | P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-                   |

|  |  |  |  |  |  |  |  |  |  |  |                       |
|--|--|--|--|--|--|--|--|--|--|--|-----------------------|
|  |  |  |  |  |  |  |  |  |  | (PROSITE_PATTERNS);<br>cd18622 (CDD); IPR023296<br>(SUPERFAMILY); IPR013320<br>(SUPERFAMILY) | glycosyl<br>compounds |
|--|--|--|--|--|--|--|--|--|--|--|-----------------------|



**Table S4.** Cytotoxic activity (%-inhibition at 100  $\mu\text{g}/\text{mL}$ ) of the fractionated PDA-K-DCM subextract of *R. mucilaginosa* 50-3-19/20B against the breast cancer cell line MDA-MB-231

| Fraction   | MDA-MB-231 |
|------------|------------|
| F1         | -          |
| F2         | -          |
| F3         | -          |
| F4         | 27         |
| F5-8       | 22         |
| F9-12      | -          |
| F13-16     | -          |
| F17        | -          |
| F18        | -          |
| F19        | 44         |
| F20        | 51         |
| <b>F21</b> | <b>86</b>  |
| <b>F22</b> | <b>94</b>  |
| <b>F23</b> | <b>88</b>  |
| <b>F24</b> | <b>97</b>  |
| F25        | 52         |
| F26        | -          |
| F27        | -          |
| F28        | -          |
| F29        | -          |
| F30        | -          |
| F31        | -          |

**Table S5.** Putative annotations of compounds detected in deep-sea *R. mucilaginosa* 50-3-19/20B extracts. Annotations were based on GNPS and manual dereplication of  $m/z$  ( $[M+H]^+$  or  $[M+Na]^+$ ), retention time ( $t_R$ ), fragmentation pattern and predicted molecular formula against Dictionary of Natural Products, DEREPIP and other literature data. Confidence levels of putative identification according to Sumner et al. [1] and Blaženovic et al. [2].

| I.D  | $m/z$    | $t_R$<br>(min) | MS/MS<br>( $m/z$ )*   | Ion type       | Molecular formula<br>(M) | Identified compound   | Compound class   | Confidence | Reference |
|--|----------|----------------|---|----------------|--------------------------|---|------------------|------------|-----------|
|  | 261.1304 | 2.15           | 233.17; 216.14; 188.14; 120.08  | $[M+H]^+$      | $C_{15}H_{20}N_2O_2$     | Cyclo-(Leu-Phe)   | Diketopiperazine | 2          | [3]       |
|  | 188.0713 | 3.83           | 170.06; 146.06; 118.07  | $[M-H_2O+H]^+$ | $C_{11}H_{11}NO_3$       | DL-Indole-3-lactic acid   | Indole alkaloid  | 2          | [4]       |
|  | 160.0764 | 3.84           | 132.08; 118.06  | $[M+H]^+$      | $C_{10}H_{11}NO_4S$      | 1-Hydroxy-2-(1H-indol-3-yl)ethanesulfonic acid                  | Indole alkaloid  | 3          |           |
|  | 206.08   | 3.94           | 188.07; 170.06; 160.08; 146.06;<br>130.07118.07   | $[M+H]^+$      | $C_{11}H_{11}NO_3$       | DL-Indole-3-lactic acid   | Indole alkaloid  | 2          | [4]       |
|  | 603.5312 | 9.54           | 273.10; 235.21; 231.09; 217.20; 189.08;<br>171.07; 161.13; 153.06; 135.12; 121.10;<br>111.04; 95.09; 81.07                            | $[M+H]^+$      | $C_{40}H_{58}O_4$        | Tetrahydroxydihydroycopene                                      | Carotenoid       | 3          | [5]       |
|  | 571.6362 | 10.37          | 263.24; 245.23; 231.09; 189.08; 175.15;<br>161.13; 153.06; 147.12; 133.10; 121.10;<br>111.05; 93.03; 81.07                            | $[M+H]^+$      | $C_{40}H_{60}O_2$        | Dihydroxyycopene  | Carotenoid       | 3          | [5]       |
|  | 553.5593 | 15.17          | 451.34; 391.32; 293.28; 275.27; 233.23;<br>219.21; 201.08; 177.16; 163.15; 149.13;<br>141.06; 135.12; 121.10; 109.10; 97.10;<br>81.03 | $[M+H]^+$      | $C_{40}H_{56}O$          | Cryptoxanthin   | Carotenoid       | 3          | [5]       |
| 5  | 220.0983 | 5.10           | 202.09; 170.06; 160.08; 132.08; 118.07  | $[M+H]^+$      | $C_{12}H_{14}NO_3$       | Methyl 2-hydroxy-3-(1H-indol-2-yl)propanoate                    | Indole           | 1          | [6]       |
|  | 411.3263 | 14.01          | 216535.14; 191.11; 173.10; 145.10   | $[M-H_2O+H]^+$ | $C_{28}H_{44}O_3$        | 5 $\alpha$ ,8 $\alpha$ -Epidioxyergosta-6,22-dien-3 $\beta$ -ol | Sterol           | 2          | [7]       |
|  | 288.2906 | 7.76           | 270.28; 106.09; 88.08   | $[M+H]^+$      | $C_{17}H_{37}NO_2$       | C17-Sphinganine   | Aminolipid       | 2          | [8]       |
| <i>PEFA (Polyol ester of fatty acid) glycolipid annotations (in molecular clusters as per Figure 4 in the main text)</i> |          |                |   |                |                          |   |                  |            |           |
|  | 767.4197 | 15.59          | 707.40; 679.37; 647.38; 619.34; 587.36;<br>517.31; 425.14; 365.12; 337.09; 265.25;<br>181.09; 153.05; 111.04                          | $[M+Na]^+$     | $C_{38}H_{64}O_{14}$     | Mannitol-pentaacetate-3-acetyloxy-C20                           | PEFA             | 2          |           |

|   |          |       |   |                     |   |                                       |      |   |         |
|---|----------|-------|---|---------------------|---|---------------------------------------|------|---|---------|
|   | 739.4256 | 15.48 | <b>679.44; 619.42; 559.37;</b> 535.33; <b>397.18;</b><br><b>369.11;</b> 365.27; 337.16; 309.09; 265.25;<br>253.06; 237.15; 199.14; 111.04   | [M+Na] <sup>+</sup> | C <sub>36</sub> H <sub>60</sub> O <sub>14</sub> | Mannitol-pentaacetate-3-acetyloxy-C18 | PEFA | 3 | [9,10]  |
|   | 695.3984 | 15.40 | <b>635.38; 575.36;</b> 553.37; 533.34; <b>515.34;</b><br>493.35; 451.35; 393.30; <b>325.09;</b> 293.28;<br>275.27; 265.07; 201.08; 141.04   | [M+Na] <sup>+</sup> | C <sub>35</sub> H <sub>60</sub> O <sub>12</sub> | Arabitol-tetraacetate-3-acetyloxy-C20 | PEFA | 3 |         |
|   | 613.2845 | 11.44 | <b>553.26; 493.24; 433.22; 411.24;</b> 373.20;<br><b>355.10; 295.08;</b> 281.17; <b>235.06.</b> 181.16;<br>153.06; 111.05   | [M+Na] <sup>+</sup> | C <sub>28</sub> H <sub>46</sub> O <sub>13</sub> | Mannitol-tetraacetate-3-acetyloxy-C12 | PEFA | 2 |         |
|   | 641.3152 | 12.62 | <b>581.29; 521.27; 461.25;</b> 439.27; <b>401.23;</b><br><b>355.19;</b> 309.20; <b>295.08;</b> 273.10; <b>235.06;</b><br>209.19; 153.06; 111.04   | [M+Na] <sup>+</sup> | C <sub>30</sub> H <sub>50</sub> O <sub>13</sub> | Mannitol-tetraacetate-3-acetyloxy-C14 | PEFA | 2 | [10,11] |
|   | 669.4626 | 13.61 | <b>609.33; 549.30; 489.28;</b> 467.30; <b>429.26;</b><br>407.28; <b>355.10;</b> 337.24; <b>295.08;</b> 273.10;<br>237.22; <b>235.06;</b> 231.09; 219.21; 171.07;<br>153.06; 135.12; 121.10; 115.04; 111.05        | [M+Na] <sup>+</sup> | C <sub>32</sub> H <sub>54</sub> O <sub>13</sub> | Mannitol-tetraacetate-3-acetyloxy-C16 | PEFA | 3 | [9-11]  |
| 3 | 697.3560 | 14.51 | <b>637.36; 577.34;</b> 535.33; <b>517.32;</b> 495.33;<br><b>457.29;</b> 435.31; 365.27; <b>355.11;</b> 337.27;<br><b>295.08;</b> 273.10; 265.25; <b>235.06;</b> 231.09;<br>171.07; 153.06; 135.12; 121.10; 111.05 | [M+Na] <sup>+</sup> | C <sub>34</sub> H <sub>58</sub> O <sub>13</sub> | Mannitol-tetraacetate-3-acetyloxy-C18 | PEFA | 3 | [9-11]  |
|   | 725.4203 | 15.22 | <b>665.39; 605.37; 545.35;</b> 523.36; <b>485.32;</b><br>393.30; <b>355.10; 295.08;</b> 273.10; <b>235.06;</b><br>231.09; 171.07; 153.06; 135.12; 111.05  | [M+Na] <sup>+</sup> | C <sub>36</sub> H <sub>62</sub> O <sub>13</sub> | Mannitol-tetraacetate-3-acetyloxy-C20 | PEFA | 3 | [10]    |
|   | 555.3145 | 12.70 | <b>495.29; 435.27;</b> 379.25; <b>375.25;</b> 353.27;<br>337.24; 289.27; <b>241.07;</b> 237.22; 219.21;<br>213.09; 195.09; 181.05; 159.07; 135.12;<br>121.10; 99.04   | [M+Na] <sup>+</sup> | C <sub>27</sub> H <sub>48</sub> O <sub>10</sub> | Arabitol-diacetate-3-acetyloxy-C16    | PEFA | 2 | [10]    |

|   |          |       |   |                     |   |                                      |      |   |         |
|---|----------|-------|---|---------------------|---|--------------------------------------|------|---|---------|
|   | 583.5362 | 13.83 | <b>523.33; 463.31;</b> 407.28; <b>403.28;</b> 381.30;<br>365.27; 265.25; <b>241.07;</b> 181.05; 159.07;<br>135.12; 99.05  | [M+Na] <sup>+</sup> | C <sub>29</sub> H <sub>52</sub> O <sub>10</sub> | Arabitol-diacetate-3-acetyloxy-C18   | PEFA | 3 |         |
|   | 529.3364 | 9.33  | <b>469.24;</b> 451.23; <b>409.22;</b> 392.21; 383.16;<br><b>349.20;</b> 323.18; 281.17; <b>271.08;</b> 153.06;<br>111.05  | [M+Na] <sup>+</sup> | C <sub>24</sub> H <sub>42</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C12   | PEFA | 3 |         |
|   | 557.2955 | 10.97 | <b>497.27;</b> 479.26; <b>437.25;</b> 419.24; 395.24;<br><b>377.23;</b> 351.21; 313.09; 309.20; <b>271.08;</b><br>253.07; 211.06  | [M+Na] <sup>+</sup> | C <sub>26</sub> H <sub>46</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C14   | PEFA | 2 | [11]    |
|   | 585.4353 | 12.32 | <b>525.30;</b> 508.30; <b>465.28;</b> 447.27; <b>405.26;</b><br>381.25; 337.24; 313.09; <b>271.08;</b> 253.07;<br>211.06; 129.06; 111.08  | [M+Na] <sup>+</sup> | C <sub>28</sub> H <sub>50</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C16   | PEFA | 3 | [10]    |
|   | 613.3557 | 13.51 | <b>553.34;</b> 535.33; <b>493.31;</b> 475.30; <b>433.29;</b><br>407.27; 365.27; <b>271.08,</b> 211.06   | [M+Na] <sup>+</sup> | C <sub>30</sub> H <sub>54</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C18   | PEFA | 2 | [10,11] |
|   | 417.2099 | 6.63  | <b>357.19;</b> 339.18; 253.14; <b>187.06</b>  | [M+Na] <sup>+</sup> | C <sub>18</sub> H <sub>34</sub> O <sub>9</sub>  | Mannitol- 3-acetyloxy C10            | PEFA | 2 |         |
|   | 445.2415 | 8.02  | <b>385.23;</b> 281.17; <b>187.06</b>  | [M+Na] <sup>+</sup> | C <sub>20</sub> H <sub>38</sub> O <sub>9</sub>  | Mannitol- 3-acetyloxy C12            | PEFA | 2 |         |
|   | 473.4443 | 9.44  | <b>413.25;</b> 309.20; <b>187.06</b>  | [M+Na] <sup>+</sup> | C <sub>22</sub> H <sub>42</sub> O <sub>9</sub>  | Mannitol- 3-acetyloxy C14            | PEFA | 3 |         |
|   | 501.2922 | 10.73 | <b>441.28;</b> 415.19; 397.17; 379.16; 337.23;<br><b>187.06</b>   | [M+Na] <sup>+</sup> | C <sub>24</sub> H <sub>46</sub> O <sub>9</sub>  | Mannitol- 3-acetyloxy C16            | PEFA | 2 | [11]    |
|   | 515.2836 | 10.11 | <b>455.26;</b> 437.25; <b>495.24;</b> 309.20; <b>229.07</b>   | [M+Na] <sup>+</sup> | C <sub>24</sub> H <sub>44</sub> O <sub>10</sub> | Mannitol-monocetate-3-acetyloxy-C18  | PEFA | 2 | [11]    |
| 2 | 543.3145 | 11.54 | <b>483.29;</b> <b>423.27;</b> 337.24; <b>229.07</b>   | [M+Na] <sup>+</sup> | C <sub>26</sub> H <sub>48</sub> O <sub>10</sub> | Mannitol-monocetate-3-acetyloxy-C16  | PEFA | 2 |         |
|   | 571.3468 | 12.57 | <b>511.32;</b> 493.31; <b>451.30;</b> 365.27; <b>229.07</b>   | [M+Na] <sup>+</sup> | C <sub>28</sub> H <sub>52</sub> O <sub>10</sub> | Mannitol-monocetate-3-acetyloxy-C18  | PEFA | 2 |         |
|   | 655.2946 | 12.32 | <b>595.27;</b> <b>535.25;</b> 513.27; 494.25; <b>475.23;</b><br>453.25; 433.22; 411.24; <b>397.11;</b> <b>337.09;</b><br>281.17; 273.10; 231.09; 213.08; 181.16;<br>153.06; 111.05    | [M+Na] <sup>+</sup> | C <sub>30</sub> H <sub>48</sub> O <sub>14</sub> | Mannitol-pentacetate-3-acetyloxy-C12 | PEFA | 2 |         |
|   | 739.3934 | 14.86 | <b>679.37;</b> <b>619.35;</b> <b>597.36;</b> 577.34; <b>559.33;</b><br><b>537.34;</b> 517.31; <b>499.30;</b> 495.33; 457.29;<br>435.31; <b>397.11;</b> 365.27; <b>337.09;</b> 315.11; | [M+Na] <sup>+</sup> | C <sub>36</sub> H <sub>60</sub> O <sub>14</sub> | Mannitol-pentacetate-3-acetyloxy-C18 | PEFA | 2 | [9,10]  |

|          |       |  |   |   |                                     |      |   |        |  |
|----------|-------|--|---|---|-------------------------------------|------|---|--------|--|
|          |       |  | 295.08; 273.10; 265.25; 247.24; 231.09;<br>213.08; 171.07; 153.06; 111.05 |   |                                     |      |   |        |  |
| 683.3955 | 14.87 | <b>623.38; 563.36; 503.33; 443.30;</b> 435.31<br>393.30; <b>313.09;</b> 293.28; 253.07; 231.09;<br>211.06; 193.05; 189.08; 171.07; 153.06;<br>111.05 | [M+Na] <sup>+</sup>   | C <sub>34</sub> H <sub>60</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C20 | PEFA | 2 | [10]   |  |
| 655.3449 | 13.92 | <b>595.35; 535.33; 475.30;</b> 453.32; <b>415.28;</b><br>365.27; <b>313.09;</b> 265.25; 253.07; 231.09;<br>189.08; 153.06; 111.05                    | [M+Na] <sup>+</sup>   | C <sub>32</sub> H <sub>56</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C18 | PEFA | 3 | [9-11] |  |
| 653.3890 | 15.13 | <b>593.37; 533.35; 473.33;</b> 451.34; 413.30;<br>393.30; 293.28; <b>283.08;</b> 223.06; 201.08  | [M+Na] <sup>+</sup>   | C <sub>31</sub> H <sub>54</sub> O <sub>11</sub> | Arabitol-triacetate-3-acetyloxy-C20 | PEFA | 3 |        |  |
| 627.6426 | 12.82 | <b>567.32; 507.29; 447.27; 387.25;</b> 379.25;<br>337.24; <b>313.09;</b> 253.07; 237.22; 231.09;<br>189.08; 111.05                                   | [M+Na] <sup>+</sup>   | C <sub>30</sub> H <sub>52</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C16 | PEFA | 3 | [10]   |  |
| 625.3367 | 14.40 | <b>565.34; 505.31; 445.29;</b> 423.31; <b>385.25;</b><br>365.27; <b>283.08;</b> 265.25; 223.06; 202.08;  | [M+Na] <sup>+</sup>   | C <sub>31</sub> H <sub>54</sub> O <sub>11</sub> | Arabitol-triacetate-3-acetyloxy-C18 | PEFA | 3 | [9,10] |  |
| 599.4146 | 11.65 | <b>539.28; 479.26; 419.24;</b> 397.26; <b>359.22;</b><br>351.21; <b>313.09;</b> 253.07; 231.09; 209.19;<br>189.08; 153.06; 111.05                    | [M+Na] <sup>+</sup>   | C <sub>28</sub> H <sub>48</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C14 | PEFA | 3 | [11]   |  |
| 597.3263 | 13.46 | <b>537.30; 477.28; 417.26;</b> 395.28; <b>357.24;</b><br>337.24; <b>283.08;</b> 255.11; 237.22;<br>223.06201.08                                      | [M+Na] <sup>+</sup>   | C <sub>29</sub> H <sub>50</sub> O <sub>11</sub> | Arabitol-triacetate-3-acetyloxy-C16 | PEFA | 3 | [9,10] |  |
| 571.4611 | 10.55 | <b>511.25; 451.23; 391.21;</b> 396.23; <b>331.19;</b><br><b>313.08;</b> 281.17; 253.07; 231.09; 189.08;<br>181.16; 153.06; 111.05                    | [M+Na] <sup>+</sup>   | C <sub>26</sub> H <sub>44</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C12 | PEFA | 3 |        |  |
| 569.3000 | 12.30 | <b>509.27; 449.25; 389.23;</b> 367.25; <b>329.21;</b><br>309.20; <b>283.08;</b> 255.11; 223.06; 209.19   | [M+Na] <sup>+</sup>   | C <sub>27</sub> H <sub>46</sub> O <sub>11</sub> | Arabitol-triacetate-3-acetyloxy-C14 | PEFA | 3 |        |  |
| 541.2764 | 10.93 | <b>481.24; 421.22; 361.20;</b> 339.22; <b>301.18;</b><br><b>283.08;</b> 223.06; 201.08; 181.16; 141.06   | [M+Na] <sup>+</sup>   | C <sub>25</sub> H <sub>42</sub> O <sub>11</sub> | Arabitol-triacetate-3-acetyloxy-C12 | PEFA | 3 |        |  |

|          |          |       |   |                     |   |                                       |      |   |        |
|----------|----------|-------|---|---------------------|---|---------------------------------------|------|---|--------|
|          | 655.3052 | 14.16 | <b>595.35; 535.33; 475.30;</b> 453.32; <b>415.28;</b><br>365.27; <b>313.09;</b> 265.25; 253.07; 231.09;<br>189.08; 153.06; 111.05   | [M+Na] <sup>+</sup> | C <sub>32</sub> H <sub>56</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C18   | PEFA | 2 |        |
| <b>1</b> | 627.3359 | 12.84 | <b>567.32; 507.29; 447.27;</b> 425.29; <b>387.25;</b><br>337.24; <b>313.09;</b> 253.07; 231.09; 189.08;<br>111.05   | [M+Na] <sup>+</sup> | C <sub>30</sub> H <sub>52</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C16   | PEFA | 2 |        |
|          | 599.3064 | 11.65 | <b>539.28; 479.26; 419.24;</b> 397.26; <b>359.22;</b><br>355.21; <b>313.09;</b> 309.20; 253.07; 231.09;<br>209.19; 189.08; 153.06; 111.05                                   | [M+Na] <sup>+</sup> | C <sub>28</sub> H <sub>48</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C14   | PEFA | 2 |        |
|          | 571.2739 | 10.59 | <b>511.25; 451.23;</b> 395.20; <b>391.21;</b> 369.23;<br><b>331.13; 313.09;</b> 281.17; 253.07; 231.09;<br>197.04; 189.08; 171.01; 153.06; 111.05                           | [M+Na] <sup>+</sup> | C <sub>26</sub> H <sub>44</sub> O <sub>12</sub> | Mannitol-triacetate-3-acetyloxy-C12   | PEFA | 2 |        |
|          |          |       |   |                     |   |                                       |      |   |        |
|          | 639.3936 | 14.00 | <b>579.32; 519.29;</b> 497.31; 477.28; <b>459.27;</b><br>437.29; <b>399.31;</b> 395.28; 349.29; 337.24;<br><b>325.09;</b> 297.12; 283.26; 265.07; 237.22;<br>210.08; 141.06 | [M+Na] <sup>+</sup> | C <sub>33</sub> H <sub>56</sub> O <sub>12</sub> | Arabitol-tetraacetate-3-acetyloxy-C16 | PEFA | 3 | [9,10] |
| <b>4</b> | 667.4842 | 14.95 | <b>607.35; 547.33; 525.34; 505.31; 487.30;</b><br><b>465.31; 445.29;</b> 427.28; 423.31; 365.27;<br><b>325.09;</b> 303.11; 265.25; 247.24; 210.08;<br>141.06; 81.03         | [M+Na] <sup>+</sup> | C <sub>31</sub> H <sub>52</sub> O <sub>12</sub> | Arabitol-tetraacetate-3-acetyloxy-C18 | PEFA | 3 | [9,10] |
|          |          |       |   |                     |   |                                       |      |   |        |
|          | 459.2210 | 7.46  | <b>399.20; 339.18;</b> 253.14; <b>229.07;</b> 189.08;<br>169.05; 154.13; 129.06   | [M+Na] <sup>+</sup> | C <sub>20</sub> H <sub>36</sub> O <sub>10</sub> | Mannitol-monoacetate-3-acetyloxy-C10  | PEFA | 2 |        |
|          | 487.2520 | 8.85  | <b>427.23; 367.21;</b> 281.17; <b>229.07;</b> 189.08;<br>181.16; 169.05; 129.06; 111.05   | [M+Na] <sup>+</sup> | C <sub>22</sub> H <sub>40</sub> O <sub>10</sub> | Mannitol-monoacetate-3-acetyloxy-C12  | PEFA | 2 |        |
|          | 501.2312 | 8.39  | <b>441.21; 381.19; 321.17; 271.08;</b> 253.14;<br>211.06; 189.08; 153.13; 129.06; 111.05  | [M+Na] <sup>+</sup> | C <sub>22</sub> H <sub>38</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C10    | PEFA | 2 |        |
|          | 529.2628 | 9.56  | <b>469.24; 409.22;</b> 353.19; <b>349.20;</b> 323.16;<br>281.17; <b>271.08;</b> 211.06; 189.08; 181.16;<br>111.05   | [M+Na] <sup>+</sup> | C <sub>24</sub> H <sub>42</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C12    | PEFA | 2 |        |

|          |       |   |                     |   |                                       |      |   |      |
|----------|-------|---|---------------------|---|---------------------------------------|------|---|------|
| 585.3253 | 12.33 | <b>525.30; 465.28;</b> 409.26; <b>405.26;</b> 379.25;<br>337.24; <b>271.08;</b> 236.22; 211.06; 189.08;<br>111.05   | [M+Na] <sup>+</sup> | C <sub>28</sub> H <sub>50</sub> O <sub>11</sub> | Mannitol-diacetate-3-acetyloxy-C16    | PEFA | 2 | [11] |
| 583.2775 | 12.28 | <b>523.25; 463.23;</b> 441.25; 421; <b>403.21;</b><br>381.23; <b>343.19;</b> 339.22; <b>325.09;</b> 281.17;<br>256.07; 201.08; 181.16; 141.06                       | [M+Na] <sup>+</sup> | C <sub>28</sub> H <sub>48</sub> O <sub>11</sub> | Arabitol-tetraacetate-3-methoxy-C14   | PEFA | 3 |      |
| 655.2927 | 11.14 | <b>595.31; 535.29; 513.30; 475.27; 453.29;</b><br>433.26; <b>415.26; 393.27;</b> 353.23; 325.09;<br>293.21; 265.07; 235.21; 201.08; 141.06                          | [M+Na] <sup>+</sup> | C <sub>32</sub> H <sub>52</sub> O <sub>14</sub> | Mannitol-pentaacetate-3-acetyloxy-C12 | PEFA | 3 |      |
| 683.3435 | 12.51 | <b>623.34; 563.32; 541.34;</b> 521.31; <b>503.30;</b><br><b>443.27; 481.31;</b> 461.29; <b>421.28;</b> 381.26;<br>325.09; 321.24; 303.11; 265.07; 201.08;<br>141.06 | [M+Na] <sup>+</sup> | C <sub>30</sub> H <sub>48</sub> O <sub>14</sub> | Mannitol-pentaacetate-3-acetyloxy-C14 | PEFA | 3 | [10] |
| 611.3049 | 10.68 | <b>551.28; 491.26; 431.24;</b> 369.12; 351.21;<br>309.09; 291.19; <b>283.08;</b> 223.06; 210.08;<br>141.05  | [M+Na] <sup>+</sup> | C <sub>31</sub> H <sub>56</sub> O <sub>10</sub> | Arabitol-triacetate-3-hydroxy-C20     | PEFA | 3 |      |
| 653.3145 | 12.27 | <b>593.29; 533.27;</b> 511.29; 491.26; <b>473.25;</b><br>432.24; 351.21; <b>325.09;</b> 291.19; 265.07;<br>223.21; 201.08; 155.14; 141.06                           | [M+Na] <sup>+</sup> | C <sub>33</sub> H <sub>58</sub> O <sub>11</sub> | Arabitolpentaacetate-3-hydroxy-C20    | PEFA | 3 |      |

\* For PEFA annotations, fragments most indicative of loss of acetyl groups and the residual polyol moiety are highlighted in bold

**Table S6.** Theoretical masses of acetylated PEFAs

|     | Mannitol 0 acetyl hydroxyl | Mannitol 1 acetyl hydroxyl | Mannitol 2 acetyl hydroxyl | Mannitol 3 acetyl hydroxyl | Mannitol 4 acetyl hydroxyl | Mannitol 5 acetyl hydroxyl |
|-----|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| C14 | 431                        | 473                        | 515                        | 557                        | 599                        | 641                        |
| C16 | 459                        | 501                        | 543                        | 585                        | 627                        | 669                        |
| C18 | 487                        | 529                        | 571                        | 613                        | 655                        | 697                        |
| C20 | 515                        | 557                        | 599                        | 641                        | 683                        | 725                        |
|     |                            |                            |                            |                            |                            |                            |
|     | Mannitol 0 acetyl methoxy  | Mannitol 1 acetyl methoxy  | Mannitol 2 acetyl methoxy  | Mannitol 3 acetyl methoxy  | Mannitol 4 acetyl methoxy  | Mannitol 5 acetyl methoxy  |
| C14 | 445                        | 487                        | 529                        | 571                        | 613                        | 655                        |
| C16 | 473                        | 515                        | 557                        | 599                        | 641                        | 683                        |
| C18 | 501                        | 543                        | 585                        | 627                        | 669                        | 711                        |
| C20 | 529                        | 571                        | 613                        | 655                        | 697                        | 739                        |
|     |                            |                            |                            |                            |                            |                            |
|     | Mannitol 0 acetyl acetoxo  | Mannitol 1 acetyl acetoxo  | Mannitol 2 acetyl acetoxo  | Mannitol 3 acetyl acetoxo  | Mannitol 4 acetyl acetoxo  | Mannitol 5 acetyl acetoxo  |
| C14 | 473                        | 515                        | 557                        | 599                        | 641                        | 683                        |
| C16 | 501                        | 543                        | 585                        | 627                        | 669                        | 711                        |
| C18 | 529                        | 571                        | 613                        | 655                        | 697                        | 739                        |
| C20 | 557                        | 599                        | 641                        | 683                        | 725                        | 767                        |
|     |                            |                            |                            |                            |                            |                            |
|     | Arabitol 0 acetyl hydroxyl | Arabitol 1 acetyl hydroxyl | Arabitol 2 acetyl hydroxyl | Arabitol 3 acetyl hydroxyl | Arabitol 4 acetyl hydroxyl |                            |
| C14 | 401                        | 443                        | 485                        | 527                        | 569                        |                            |
| C16 | 429                        | 471                        | 513                        | 555                        | 597                        |                            |
| C18 | 457                        | 499                        | 541                        | 583                        | 625                        |                            |
| C20 | 485                        | 527                        | 569                        | 611                        | 653                        |                            |
|     |                            |                            |                            |                            |                            |                            |
|     | Arabitol 0 acetyl methoxy  | Arabitol 1 acetyl methoxy  | Arabitol 2 acetyl methoxy  | Arabitol 3 acetyl methoxy  | Arabitol 4 acetyl methoxy  |                            |
| C14 | 415                        | 457                        | 499                        | 541                        | 583                        |                            |
| C16 | 443                        | 485                        | 527                        | 569                        | 611                        |                            |
| C18 | 471                        | 513                        | 555                        | 597                        | 639                        |                            |
| C20 | 499                        | 541                        | 583                        | 625                        | 667                        |                            |
|     |                            |                            |                            |                            |                            |                            |
|     | Arabitol 0 acetyl acetoxo  | Arabitol 1 acetyl acetoxo  | Arabitol 2 acetyl acetoxo  | Arabitol 3 acetyl acetoxo  | Arabitol 4 acetyl acetoxo  |                            |
| C14 | 443                        | 485                        | 527                        | 569                        | 611                        |                            |
| C16 | 471                        | 513                        | 555                        | 597                        | 639                        |                            |
| C18 | 499                        | 541                        | 583                        | 625                        | 667                        |                            |
| C20 | 527                        | 569                        | 611                        | 653                        | 695                        |                            |



**Table S7.** MS/MS fragment ions indicative of the type of polyol group in PEFAs

|                | $m/z$ [M+H] <sup>+</sup> | $m/z$ [M+NH <sub>4</sub> ] <sup>+</sup> | $m/z$ [M+Na] <sup>+</sup> |
|----------------|--------------------------|---|---------------------------|
| Mannitol, 0 Ac | 165.1                    | 182.1                                   | 187.1                     |
| Mannitol, 1 Ac | 207.1                    | 224.1                                   | 229.1                     |
| Mannitol, 2 Ac | 249.1                    | 266.1                                   | 271.1                     |
| Mannitol, 3 Ac | 291.1                    | 308.1                                   | 313.1                     |
| Mannitol, 4 Ac | 333.1                    | 350.1                                   | 355.1                     |
| Mannitol, 5 Ac | 375.1                    | 392.1                                   | 397.1                     |
|                |                          |   |                           |
| Arabitol, 0 Ac | 135.1                    | 152.1                                   | 157.1                     |
| Arabitol, 1 Ac | 177.1                    | 194.1                                   | 199.1                     |
| Arabitol, 2 Ac | 219.1                    | 236.1                                   | 241.1                     |
| Arabitol, 3 Ac | 261.1                    | 278.1                                   | 283.1                     |
| Arabitol, 4 Ac | 303.1                    | 320.1                                   | 325.1                     |

**Table S8.** NMR data for compound 1 (MeOD, 600/150 MHz)

| Position | $\delta_{\text{H}}$ Multiplicity<br>(J in Hz) | $\delta_{\text{C}}$ | COSY             | HMBC<br>H $\rightarrow$ C        | TOCSY                         | NOE                 |
|----------|---|---------------------|------------------|----------------------------------|-------------------------------|---------------------|
| 1        | 3.63 (m), 3.80 (m)                            | 64.8                | H-2              | C-3                              | H-6                           |                     |
| 2        | 3.79 (m)                                      | 70.3                | H-1, H-3         | C-1, C-3, C-4,<br>C-11           | H-1, H-3,<br>H-4, H-5,<br>H-6 | H-5                 |
| 3        | 3.69 (m)                                      | 72.7                | H-2, H-4         | C-9                              | H-2                           |                     |
| 4        | 3.48 (m)                                      | 70.4                | H-3, H-5         | C-5                              | H-2, H-5,<br>H-6              | H-5                 |
| 5        | 3.87 (m)                                      | 70.0                | H-4, H-6         | C-4                              | H-2, H-4,<br>H-6              | H-2, H-4,<br>H-6    |
| 6        | 4.16 (m), 4.37 (m)                            | 67.7                | H-5              | C-4, C-7                         | H-2, H-4,<br>H-5, H-6         | H-5                 |
| 7        | -   | 172.1               |                  |                                  |                               |                     |
| 8        | 2.05 (s)                                      | 20.3 - 20.8         |                  | C-7                              |                               |                     |
| 9        | -   | 173.1               |                  |                                  |                               |                     |
| 10       | 2.03 (s)                                      | 20.3 - 20.8         |                  | C-9                              |                               |                     |
| 11       | -   | 172.9               |                  |                                  |                               |                     |
| 12       | 2.08 (s)                                      | 20.6                |                  | C-11                             |                               |                     |
| 1'       | -   | 172.3               |                  |                                  |                               |                     |
| 2'       | 2.61 (m), 2.65 (m)                            | 39.8                | H-3'             | C-1', C-3', C-4'                 | H-3', H-4',<br>H-5'           | H-4'                |
| 3'       | 5.22 (m)                                      | 71.8                | H-2', H-4'       | C-1', C-2', C-4',<br>C-5', C-17' | H-2', H-4',<br>H-5'           | H-2', H-4',<br>H-5' |
| 4'       | 1.61 (m), 1.61 (m)                            | 34.7                | H-3', H-5'       |                                  |                               | H-2', H-3',<br>H-5' |
| 5'       | 1.33 (m)                                      | 25.9                | H-4'             |                                  |                               |                     |
| 6' - 13' | 1.29 - 1.33 (m)                               | 30.2 - 31.0         |                  |                                  |                               |                     |
| 14'      | 1.29 (m)                                      | 32.9                | H-13', H-<br>15' |                                  |                               |                     |
| 15'      | 1.31 (m)                                      | 23.5                | H-14', H-<br>16' | C-14', C-16'                     |                               |                     |
| 16'      | 0.90 (t, 6.9)                                 | 14.1                | H-15'            | C-14', C-15'                     |                               |                     |
| 17'      | -   | 172.3               |                  |                                  |                               |                     |
| 18'      | 2.02 (s)                                      | 20.8                |                  | C-17'                            |                               |                     |

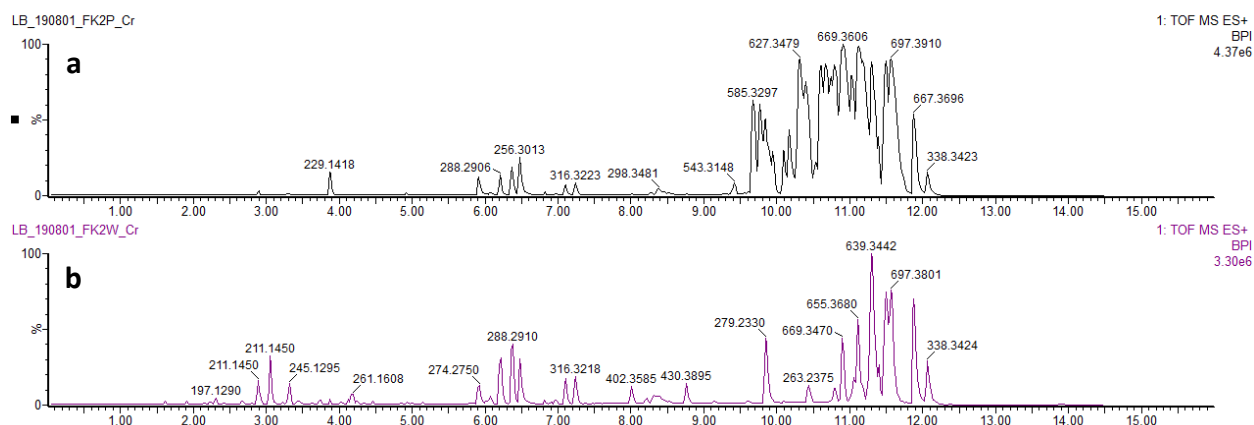
**Table S9.** NMR data for compound **2** (MeOD, 600/150 MHz)

| Position | $\delta_{\text{H}}$ Multiplicity<br>(J in Hz) | $\delta_{\text{C}}$ | COSY         | HMBC<br>H $\rightarrow$ C | NOE              |
|----------|---|---------------------|--------------|---------------------------|------------------|
| 1        | 3.63 (m), 3.80 (m)                            | 64.7                | H-2          | C-2                       | H-2              |
| 2        | 3.79 (m)                                      | 70.3                | H-1, H-3     | C-3                       | H-1, H-3, H-5    |
| 3        | 3.69 (m)                                      | 72.5                | H-2, H-4     |                           | H-2, H-4         |
| 4        | 3.47 (m)                                      | 70.7                | H-3, H-5     | C-7                       | H-3, H-5         |
| 5        | 3.87 (m)                                      | 70.0                | H-4, H-6     | C-4                       | H-2, H-4, H-6    |
| 6        | 4.18 (m), 4.39 (m)                            | 67.8                | H-5          |                           | H-5              |
| 7        |   | 172.9               |              |                           |                  |
| 8        | 2.08 (s)                                      | 20.5                |              | C-7                       |                  |
| 1'       |   | 172.3               |              |                           |                  |
| 2'       | 2.65 (m), 2.65 (m)                            | 39.8                | H-3'         | C-1', C-3', C-4'          | H-4'             |
| 3'       | 5.22 (m)                                      | 71.7                | H-2', H-4'   | C-1', C-17'               | H-2', H-4', H-5' |
| 4'       | 1.62 (m), 1.62 (m)                            | 34.8                | H-3', H-5'   | C-5'                      | H-2', H-3', H-5' |
| 5'       | 1.32 (m)                                      | 25.9                | H-4'         |                           |                  |
| 6' - 13' | 1.29 – 1.33 (m)                               | 30.2 – 31.0         |              |                           |                  |
| 14'      | 1.29 (m)                                      | 32.8                | H-13', H-15' |                           |                  |
| 15'      | 1.31 (m)                                      | 23.5                | H-14', H-16' | C-14', C-16'              |                  |
| 16'      | 0.90 (t, 6.9)                                 | 14.2                | H-15'        | C-15'                     |                  |
| 17'      |   | 172.3               |              |                           |                  |
| 18'      | 2.02 (s)                                      | 20.8                |              | C-17'                     |                  |

**Table S10.** NMR data of compound **5** (MeOD, 600/150 MHz)

| Position | $\delta_{\text{H}}$ m (J in ppm)                         | $\delta_{\text{C}}$ | HMBC<br>C $\rightarrow$ H          | NOE                               |
|----------|--|---------------------|------------------------------------|-----------------------------------|
| 1        |  | 175.7               | H-1, H <sub>2</sub> -3, OMe        |                                   |
| 2        | 4.43 (dd, 6.9, 5.4)                                      | 72.6                | H <sub>2</sub> -3                  | H <sub>2</sub> -3                 |
| 3        | 3.11 (ddd, 14.5, 6.5, 0.7)<br>3.21 (ddd, 14.5, 5.4, 0.7) | 31.3                | H-2<br>H-2                         | H-2, H-2', H-4'                   |
| 1'       |  | 137.7               | H-4', H-6'                         |                                   |
| 2'       | 7.09 (s)   | 124.5               | H <sub>2</sub> -3                  | H-2, H <sub>2</sub> -3, OMe       |
| 3'       |  | 110.8               | H <sub>2</sub> -3, H-2, H-4', H-2' |                                   |
| 3a'      |  | 127.3               | H <sub>2</sub> -3, H-2', H-7'      |                                   |
| 4'       | 7.53 (d, 7.8)  | 119.1               | H-6'                               | H-2, H <sub>2</sub> -3, H-5', OMe |
| 5'       | 6.99 (t, 7.8)  | 119.4               | H-7'                               | H-4', H-6'                        |
| 6'       | 7.07 (t, 7.8)  | 122.0               | H-4'                               | H-5', H-7'                        |
| 7'       | 7.31 (d)   | 111.9               | H-5'                               | H-6'                              |
| 7a'      |  | 110.1               | H-4, H-2, H-6'                     |                                   |
| OMe      | 3.64 (s)   | 52.0                |                                    | H-2', H-4'                        |

**Figure S1.** Differential metabolomes of *R. mucilaginosa* 50-3-19/20B (a) on PDA medium; (b) on WSP30 medium

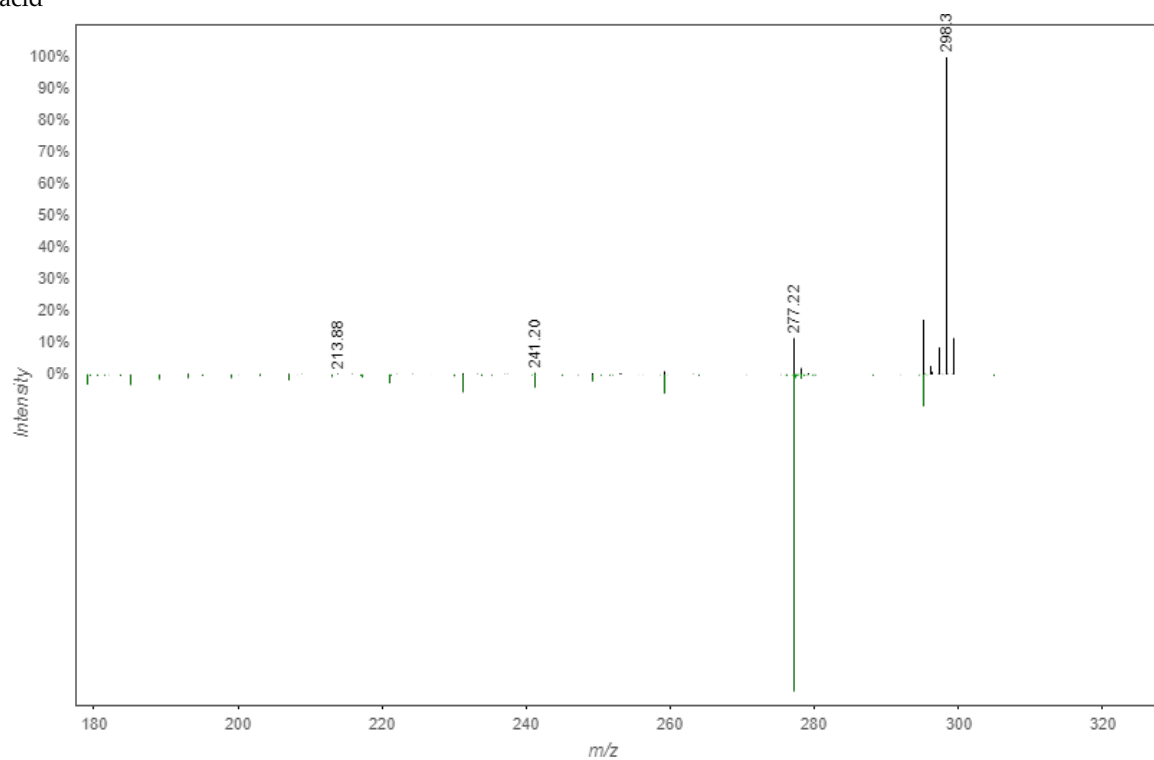


**Figure S2.** Overview of genomic statistics based on *de novo* genome assembly. (A) Genome Assembly statistics (B) Total repetitive elements

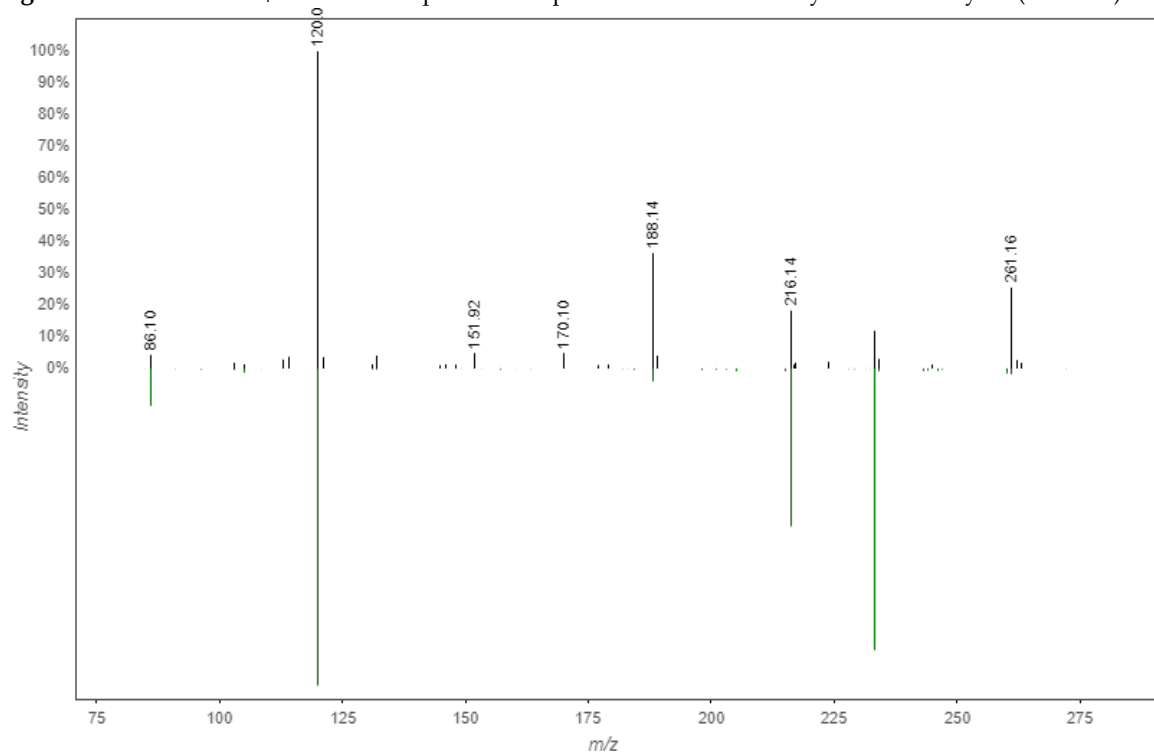
| <b>A</b>                   | <b>denovo Assembly</b> | <b>Definition</b>   |
|----------------------------|------------------------|---|
| <b>Assembled Size (Mb)</b> | 20.02                  | Total denovo assembled size   |
| <b>Total scaffolds</b>     | 265                    | Total number of scaffolds in assembled genome   |
| <b>N25 (Kb)</b>            | 498.8                  | Length of the scaffold until which sum of lengths of scaffolds are reached to 25% of 20.02 Mb |
| <b>L25</b>                 | 8                      | Number of scaffolds in the assembled genome that constitute N25                               |
| <b>N50 (Kb)</b>            | 295.8                  | Length of the scaffold until which sum of lengths of scaffolds are reached to 50% of 20.02 Mb |
| <b>L50</b>                 | 21                     | Number of scaffolds in the assembled genome that constitute N50                               |
| <b>N75 (Kb)</b>            | 163.5                  | Length of the scaffold until which sum of lengths of scaffolds are reached to 75% of 20.02 Mb |
| <b>L75</b>                 | 44                     | Number of scaffolds in the assembled genome that constitute N75                               |
| <b>%GC</b>                 | 60.47                  | Percentage of total G+C content of assembled genome (20.02 Mb)                                |

| <b>B</b> | <b>Types of repeats</b>      | <b>No. of Repeats*</b> | <b>Total length (bp)</b> | <b>Percentage of genomic sequence</b> |
|----------|------------------------------|------------------------|--------------------------|---------------------------------------|
|          | <b>Retroelements</b>         | <b>81</b>              | <b>55667</b>             | <b>0.28</b>                           |
|          | LINEs                        | 18                     | 3607                     | 0.02                                  |
|          | LTR elements                 | <b>63</b>              | <b>52060</b>             | <b>0.26</b>                           |
|          | Ty1/Copia                    | 28                     | 30103                    | 0.15                                  |
|          | Gypsy/DIRS1                  | 33                     | 21758                    | 0.11                                  |
|          | <b>DNA transposons</b>       | <b>9</b>               | <b>581</b>               | <b>0.00</b>                           |
|          | Tc1-IS630-Pogo               | 1                      | 15                       | 0.00                                  |
|          | Unclassified                 | 2                      | 887                      | 0.00                                  |
|          | <b>Small RNA</b>             | <b>10</b>              | <b>7904</b>              | <b>0.04</b>                           |
|          | <b>Simple repeats</b>        | <b>8318</b>            | <b>326295</b>            | <b>1.63</b>                           |
|          | <b>Low complexity</b>        | <b>759</b>             | <b>37594</b>             | <b>0.19</b>                           |
|          | Total interspersed repeats   |                        | 57135                    | 0.29                                  |
|          | <b>Total repeat contents</b> |                        | <b>428912</b>            | <b>2.14</b>                           |

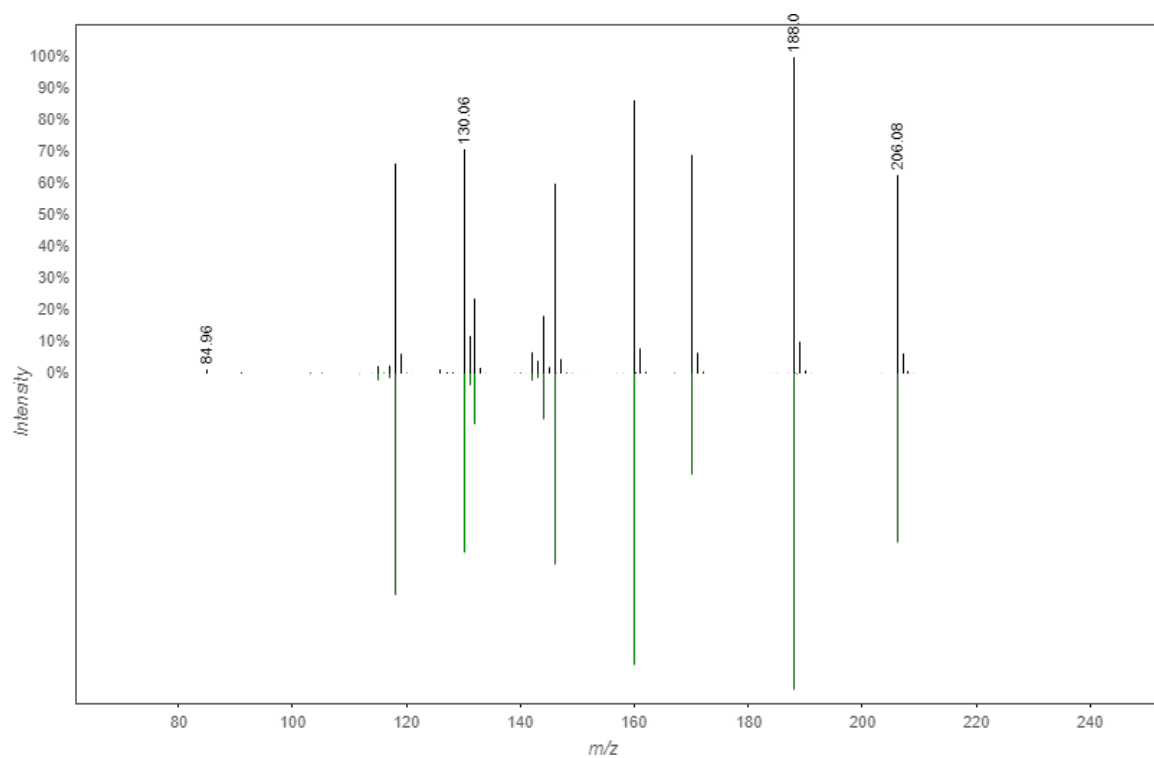
**Figure S3.** GNPS MS/MS mirror plot of experimental and library data of 9-oxo-10E,12Z-octadecadienoic acid



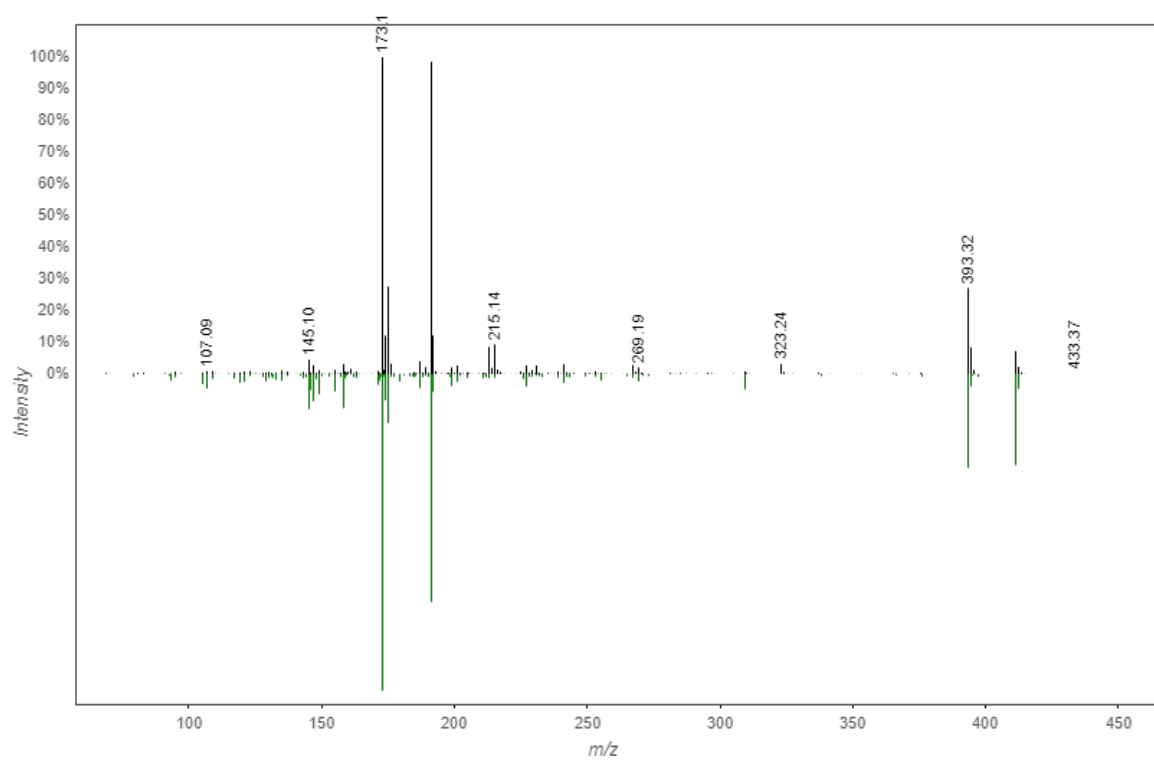
**Figure S4.** GNPS MS/MS mirror plot of experimental and library data of cyclo-(Leu-Phe)



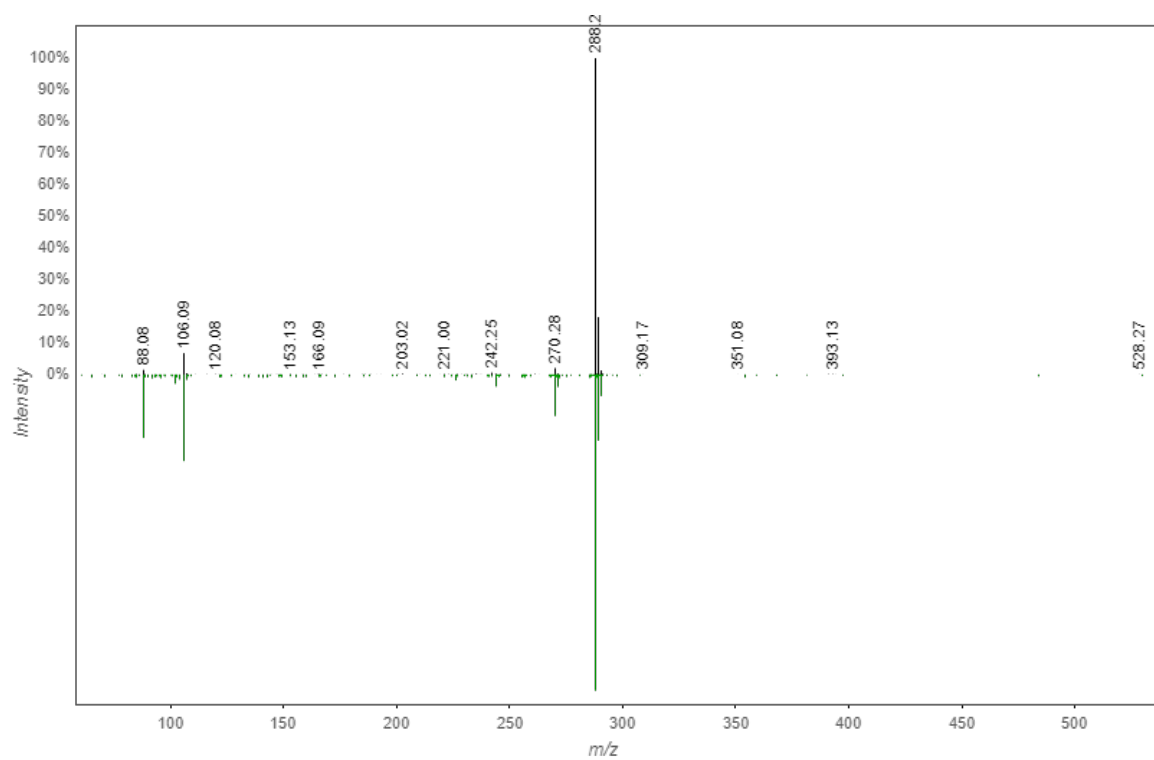
**Figure S5.** GNPS MS/MS mirror plot of experimental and library data of DL-Indole-3-lactic acid



**Figure S6.** GNPS MS/MS mirror plot of experimental and library data of 5 $\alpha$ ,8 $\alpha$ -epidioxyergosta-6,22-dien-3 $\beta$ -ol



**Figure S7.** GNPS MS/MS mirror plot of experimental and library data of C17-sphinganine



**Figure S8.** GNPS MS/MS mirror plot of experimental and library data of 9,10-Epoxy-12-octadecenoic acid

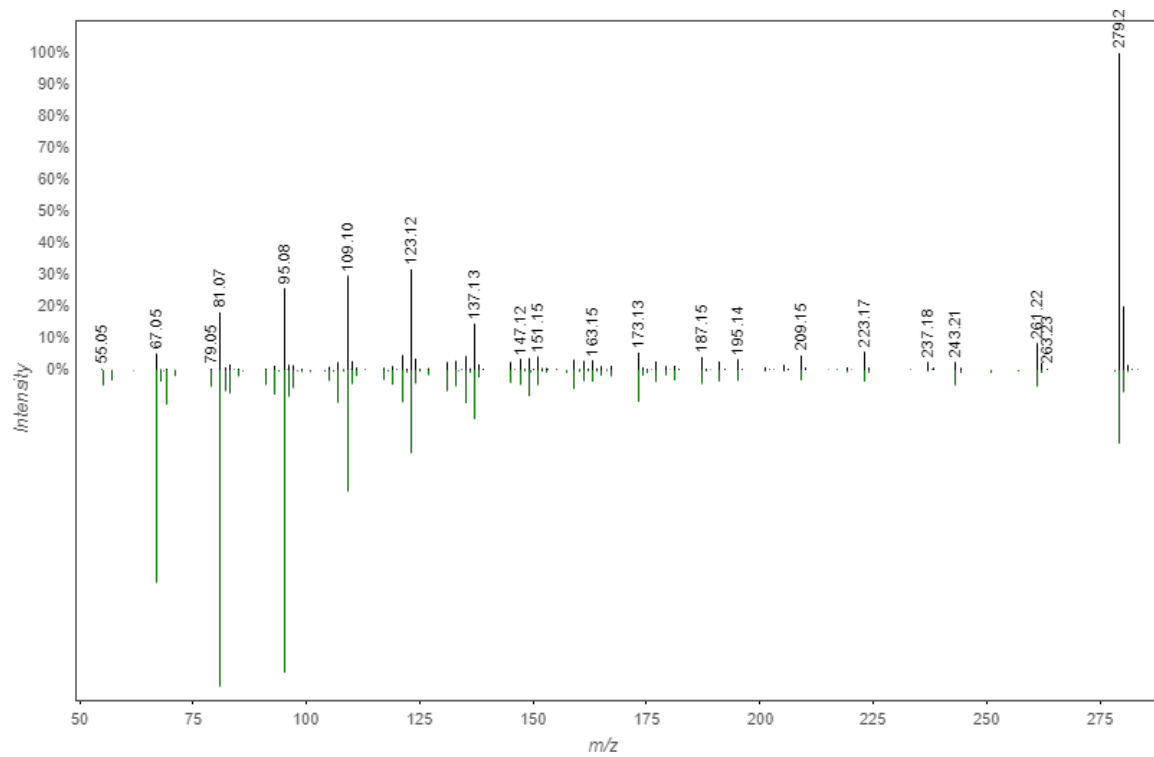
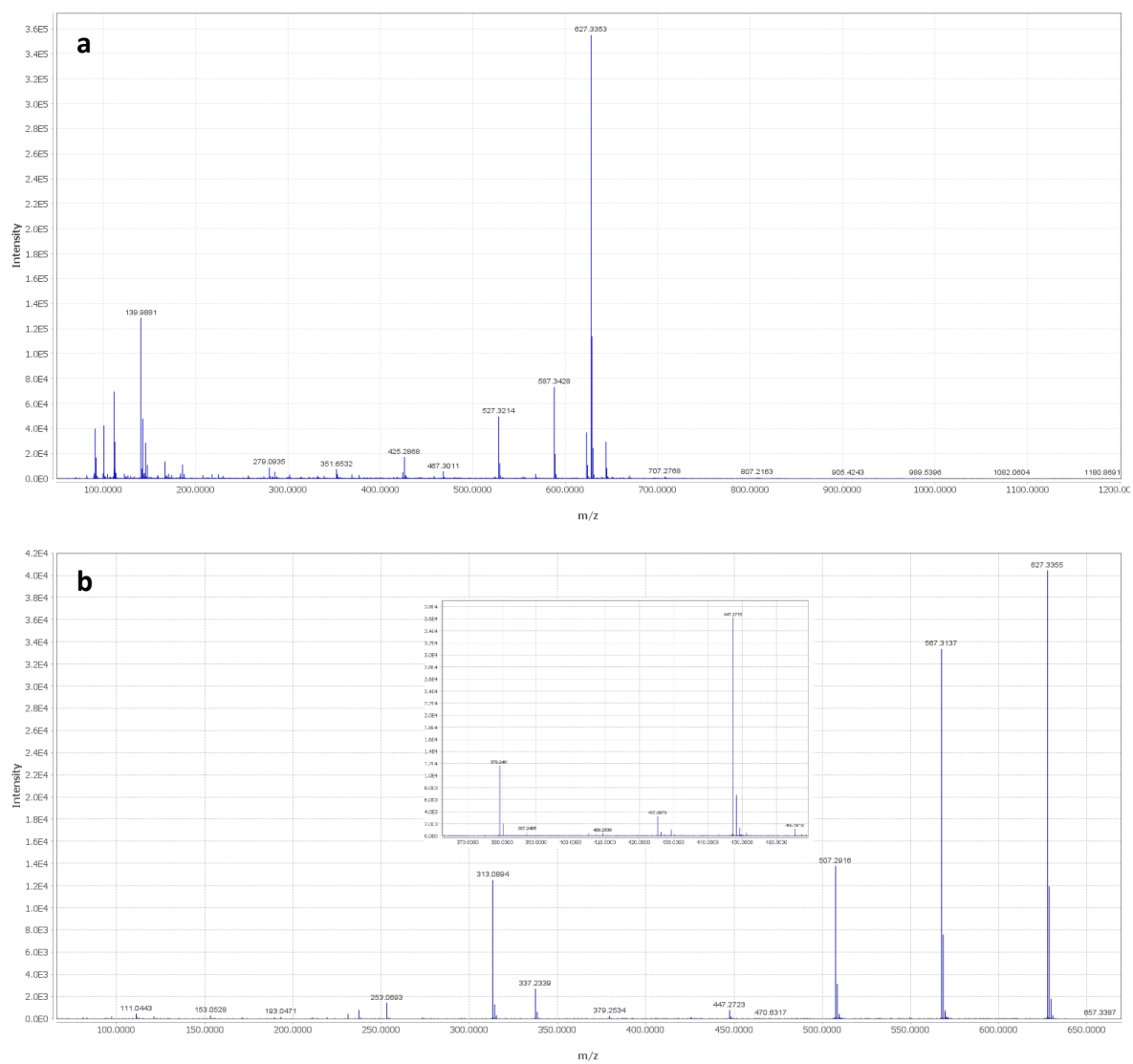
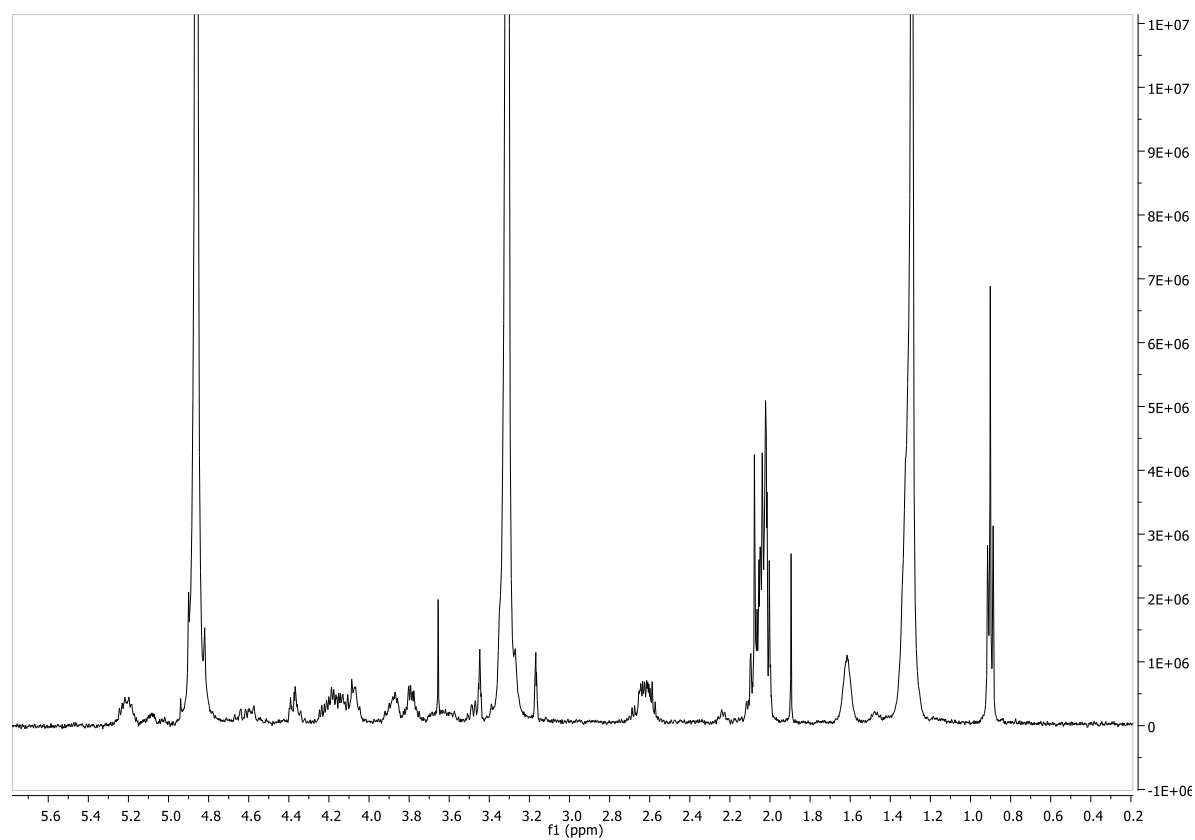




Figure S9. (a) HR-MS and (b) MS/MS spectra of 1



**Figure S10.**  $^1\text{H}$  NMR spectrum of compound **1** (MeOD, 600 MHz)



**Figure S11.** HSQC spectrum of compound **1** (MeOD, 600/150 MHz)

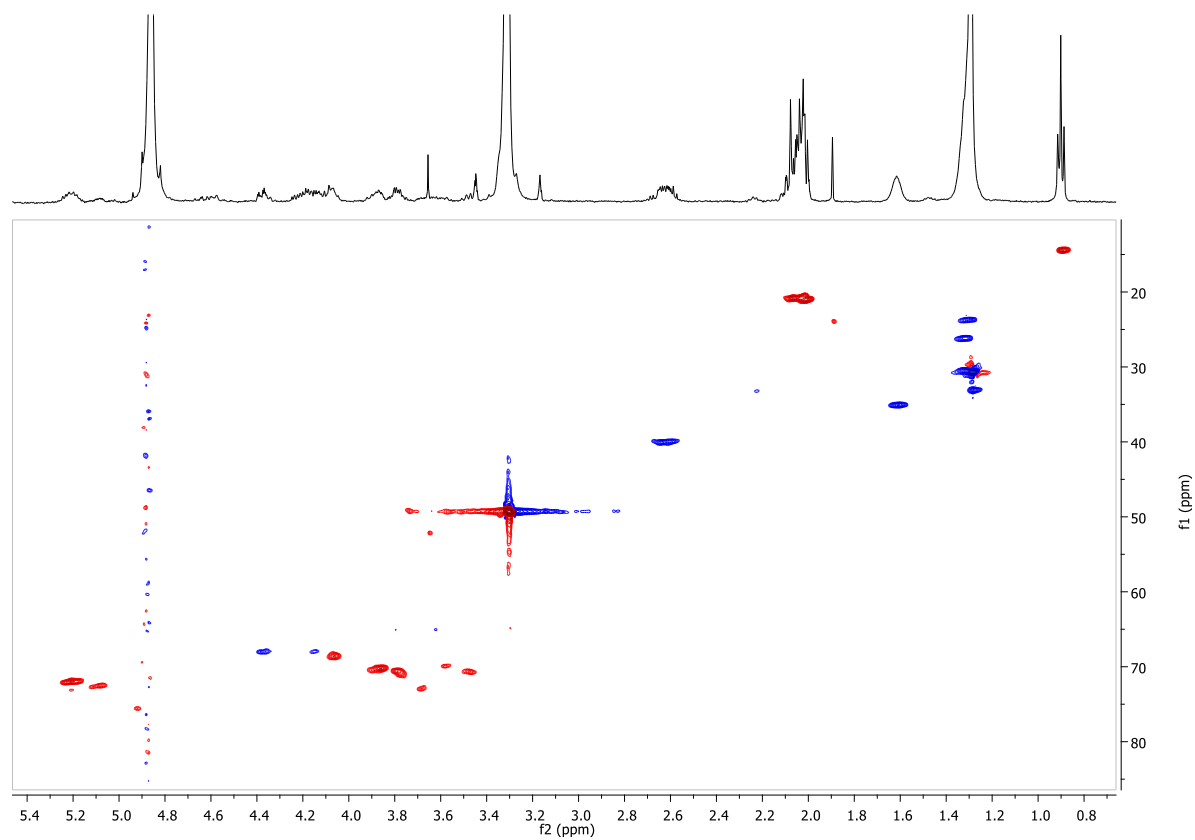


Figure S12. COSY spectrum of compound 1 (MeOD, 600 MHz)

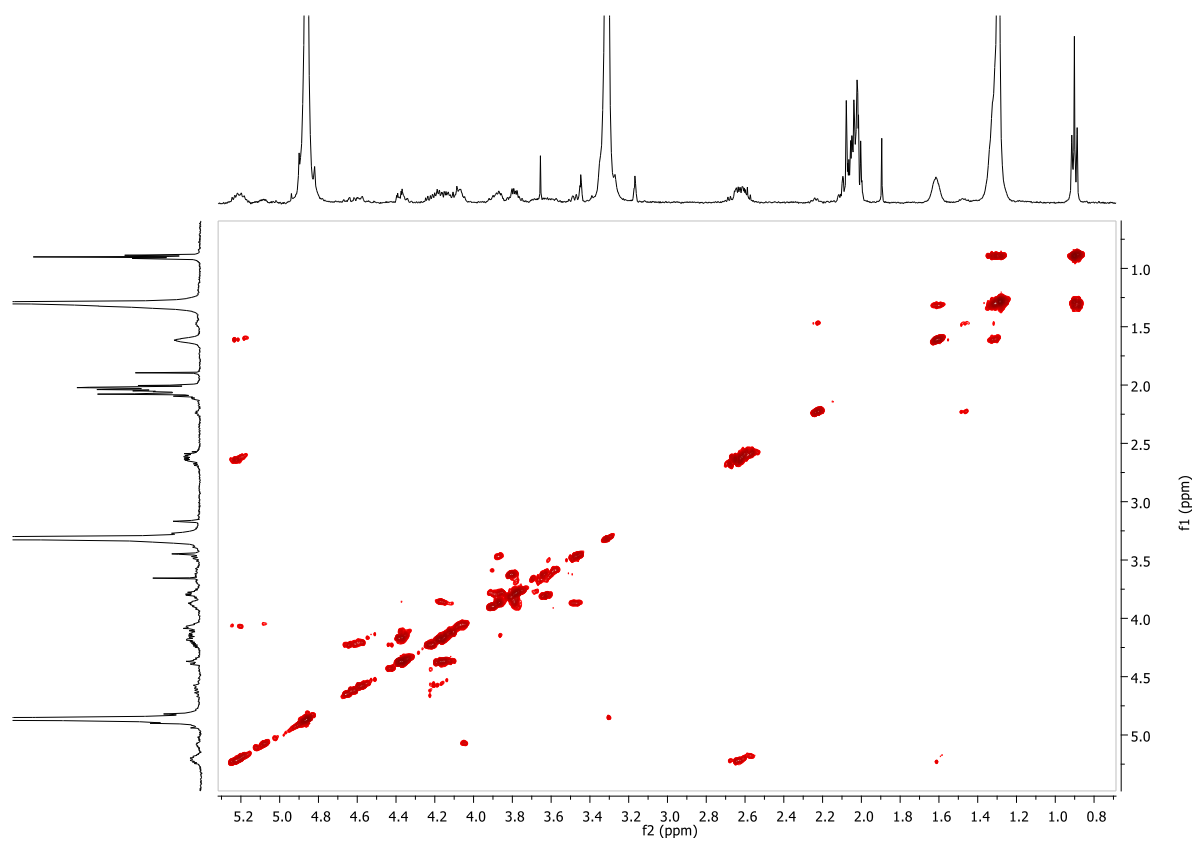


Figure S13. HMBC spectrum of compound 1 (MeOD, 600/150 MHz)

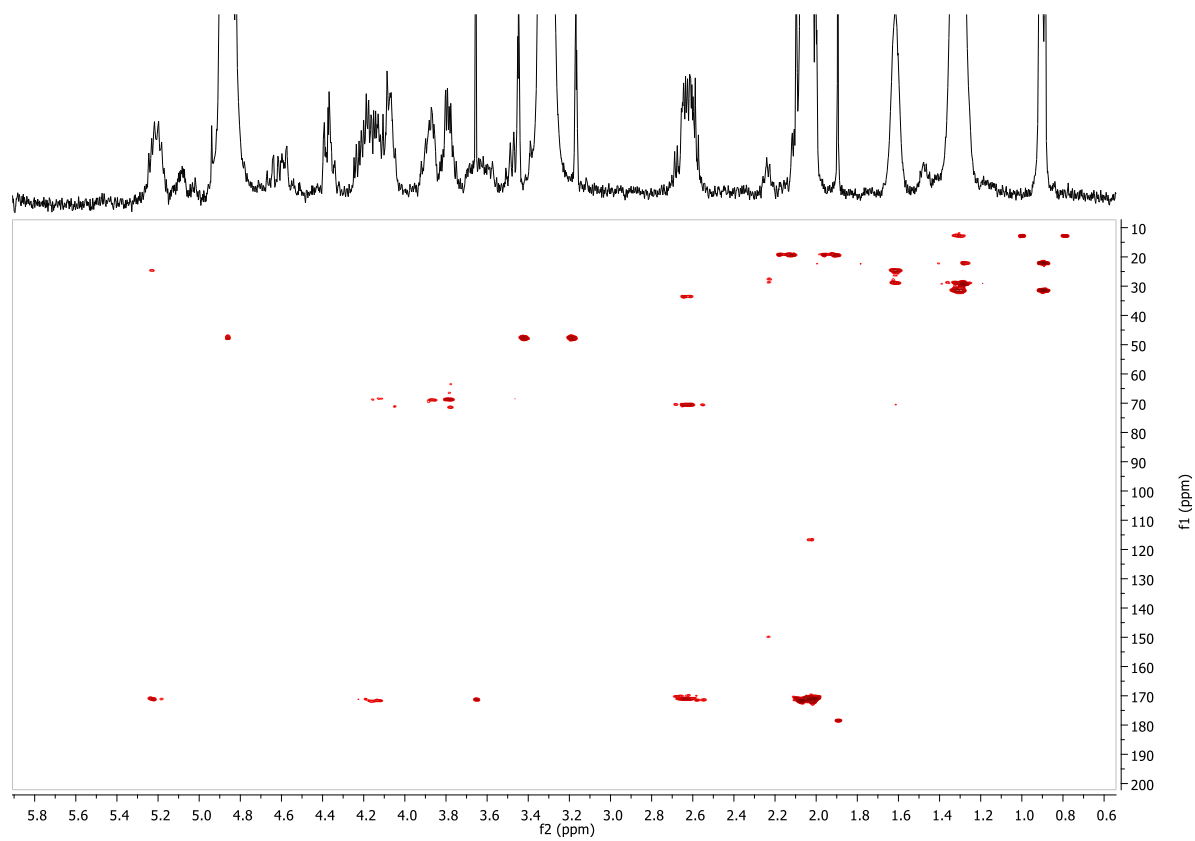


Figure S14. NOESY spectrum of compound 1 (MeOD, 600 MHz)

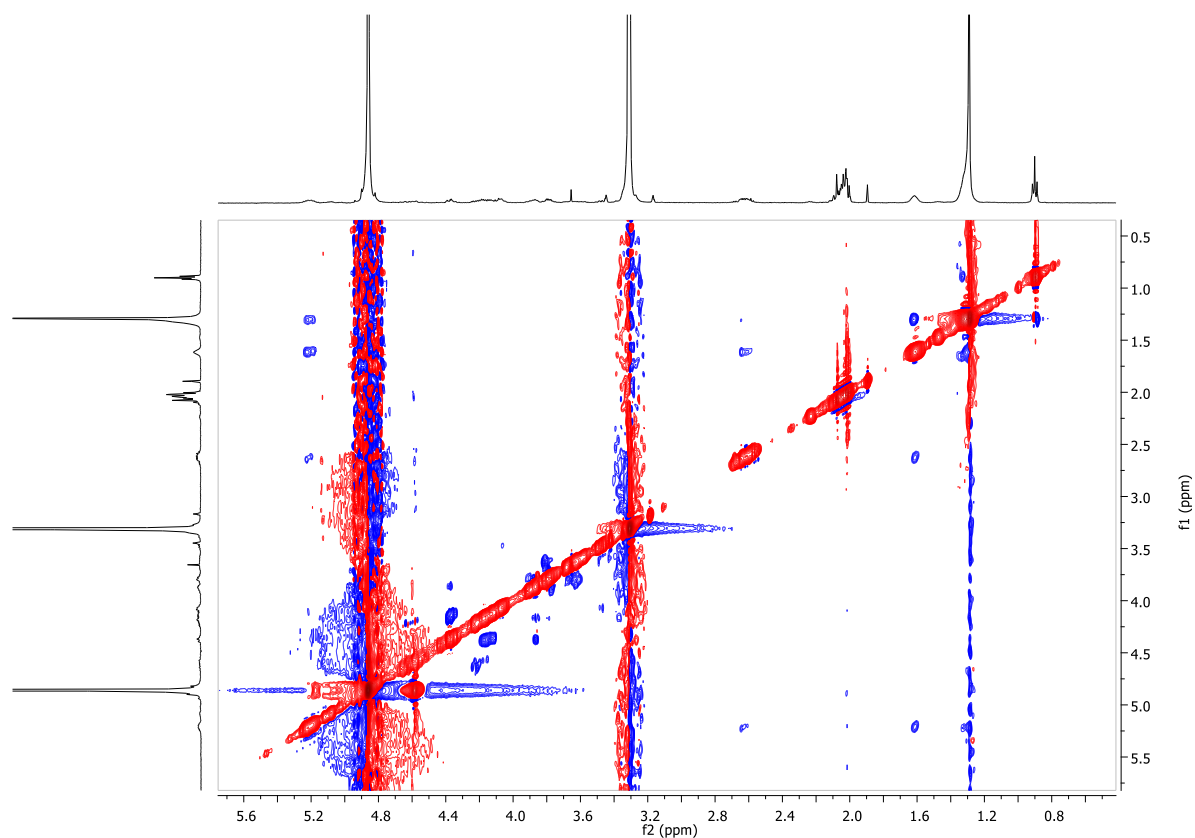


Figure S15. TOCSY spectrum of compound 1 (MeOD, 600 MHz)

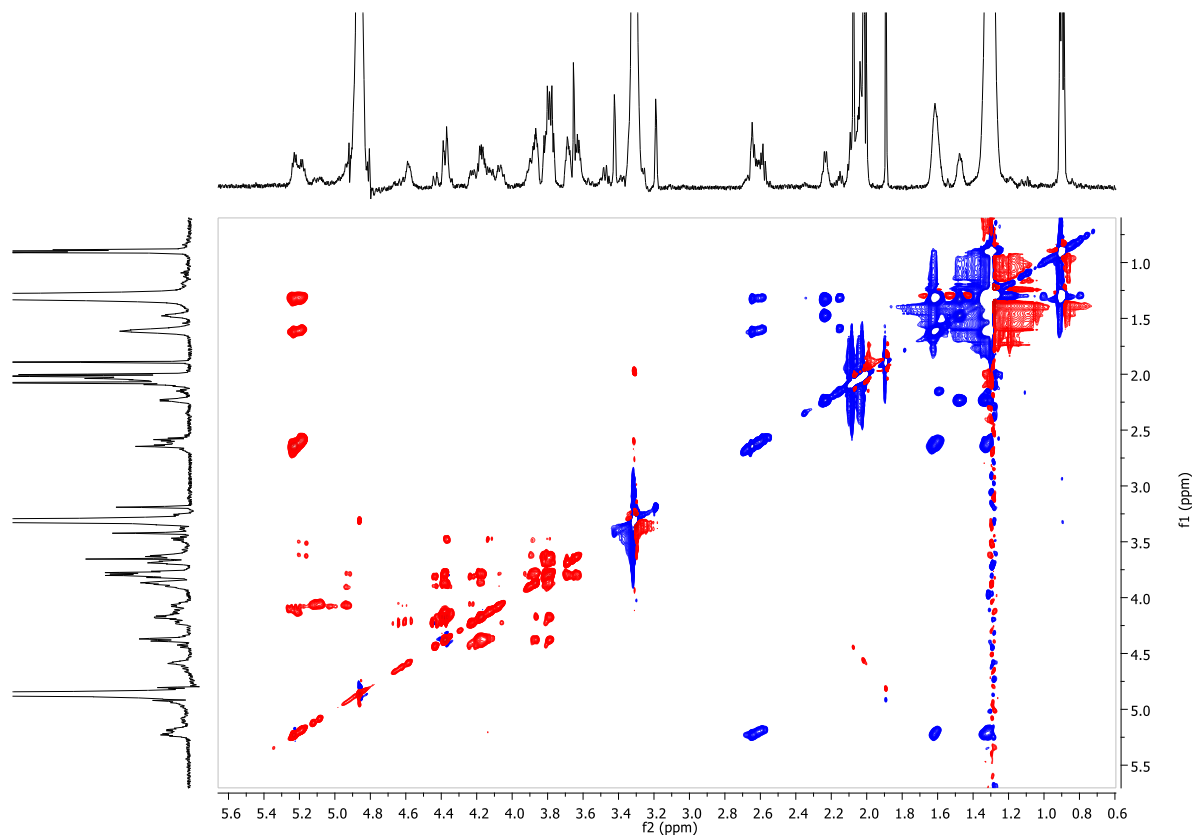


Figure S16. (a) HR-ESIMS and (b) MS/MS spectra of 2

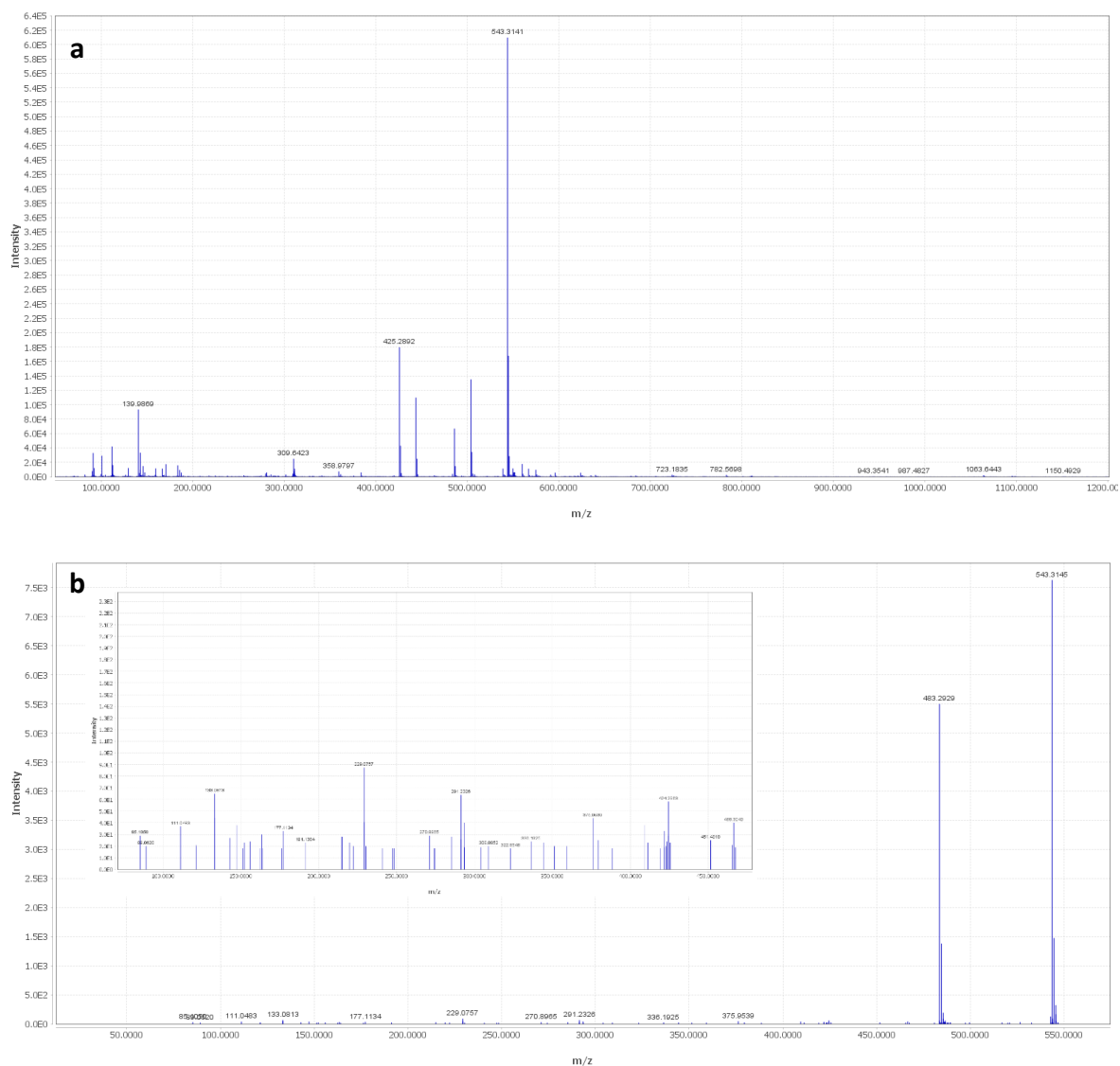


Figure S17. <sup>1</sup>H NMR spectrum of compound 2 (MeOD, 600 MHz)

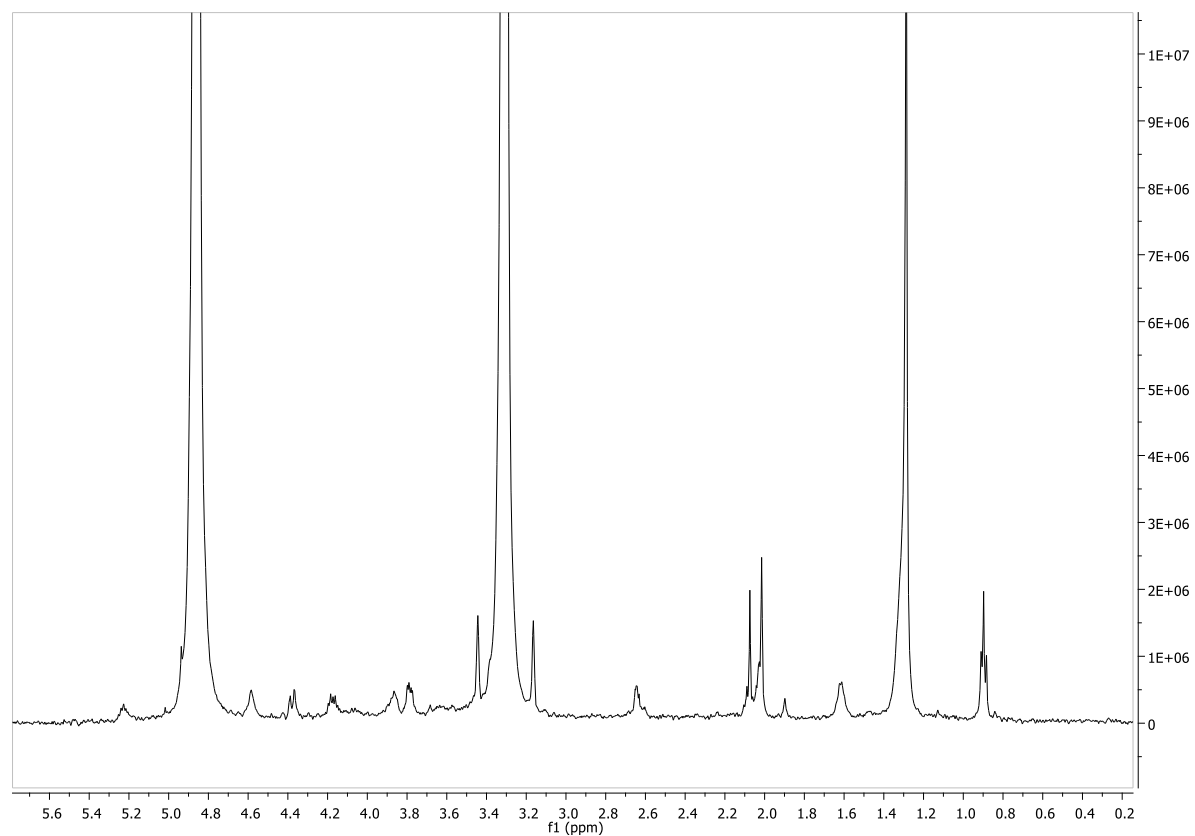


Figure S18. HSQC spectrum of compound 2 (MeOD, 600/150 MHz)

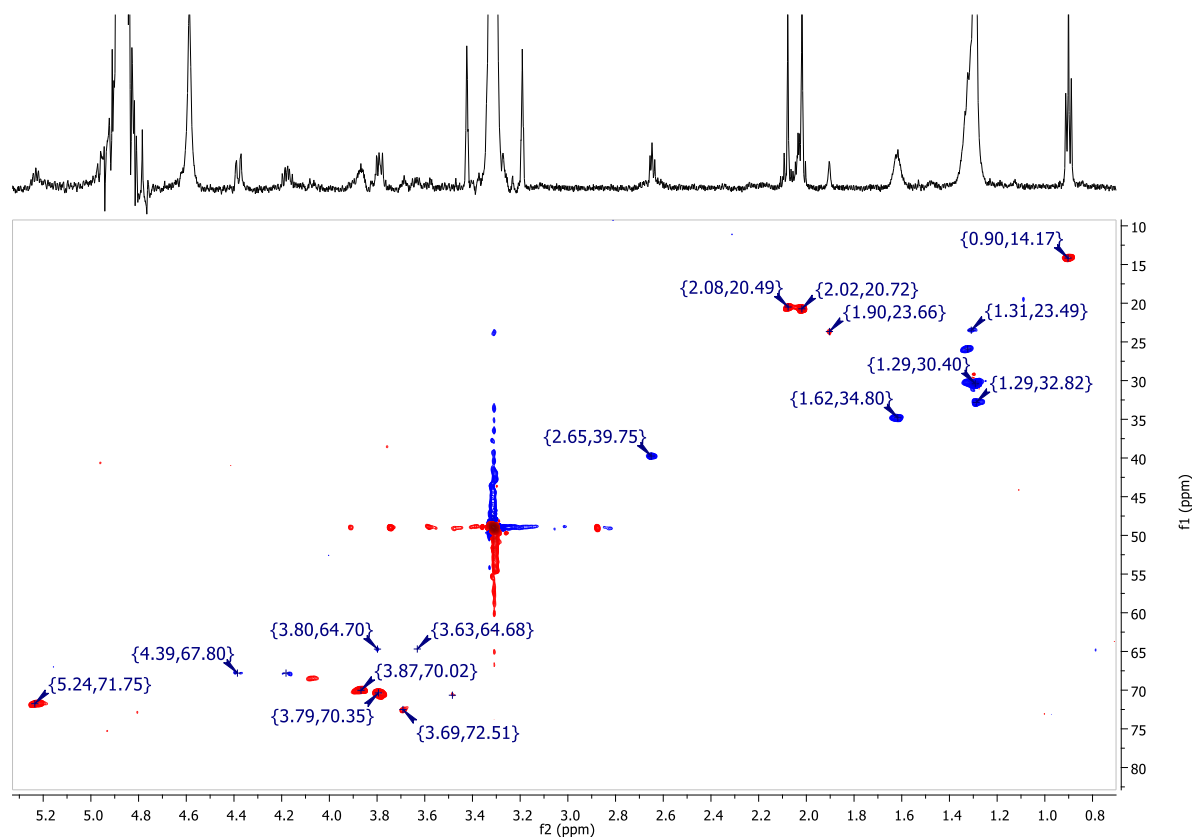


Figure S19. COSY spectrum of compound 2 (MeOD, 600 MHz)

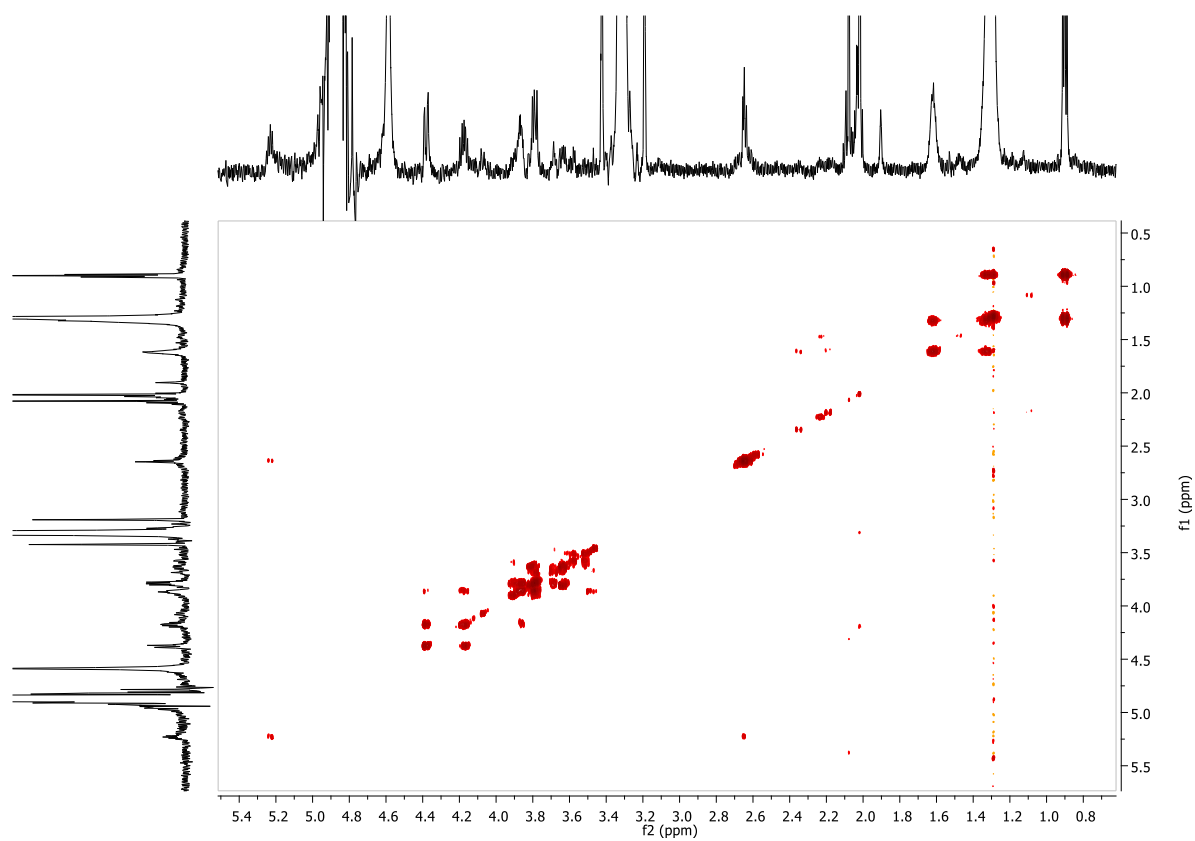


Figure S20. NOESY spectrum of compound 2 (MeOD, 600 MHz)

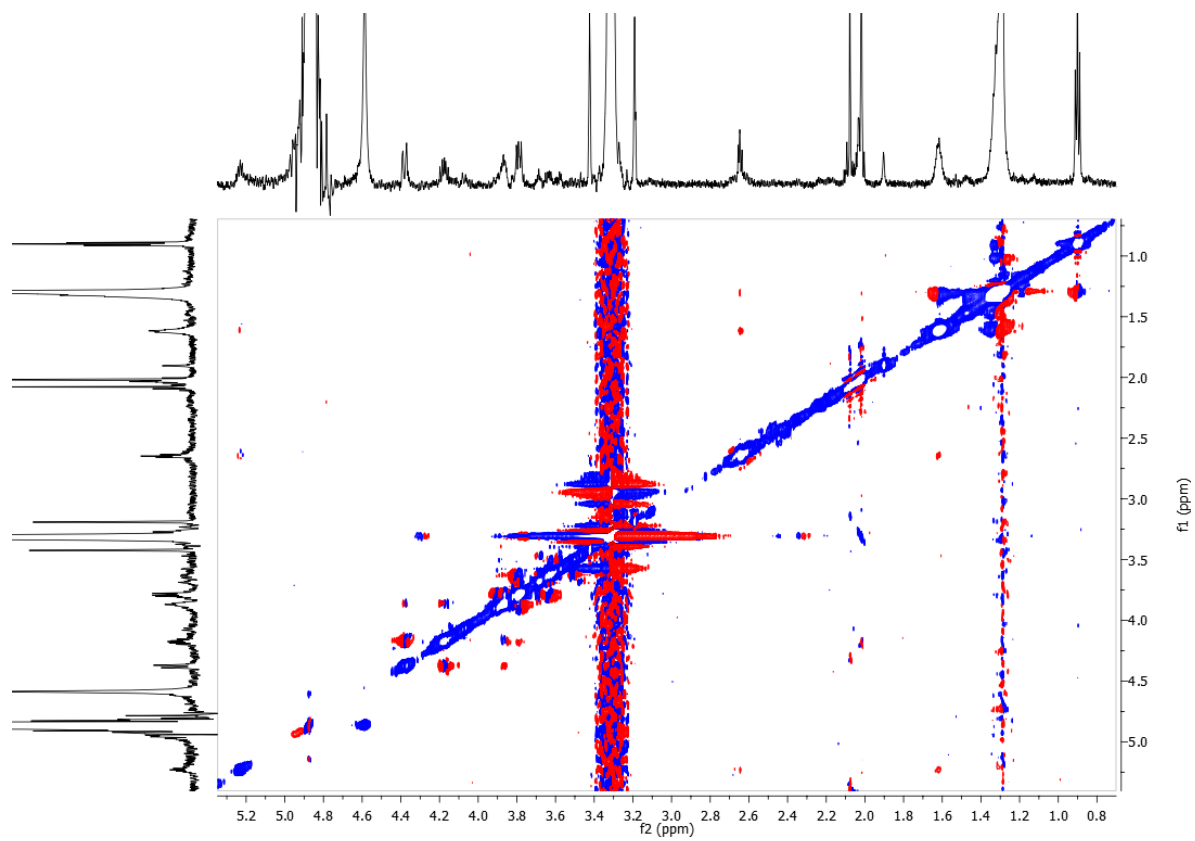


Figure S21. HMBC spectrum of compound 2 (MeOD, 600/150 MHz)

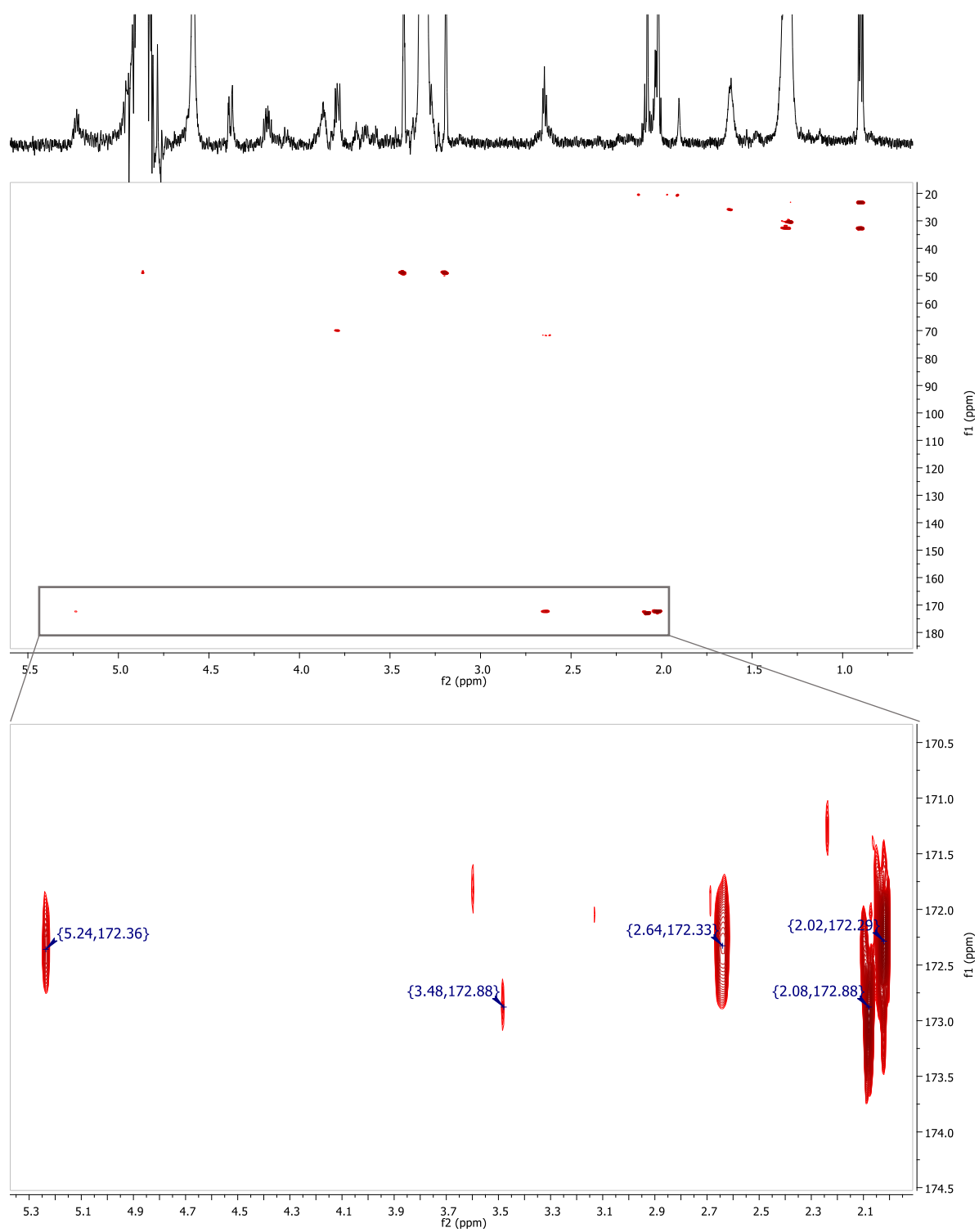




Figure S22. (a) HR-ESIMS and (b) MS/MS spectra of 3

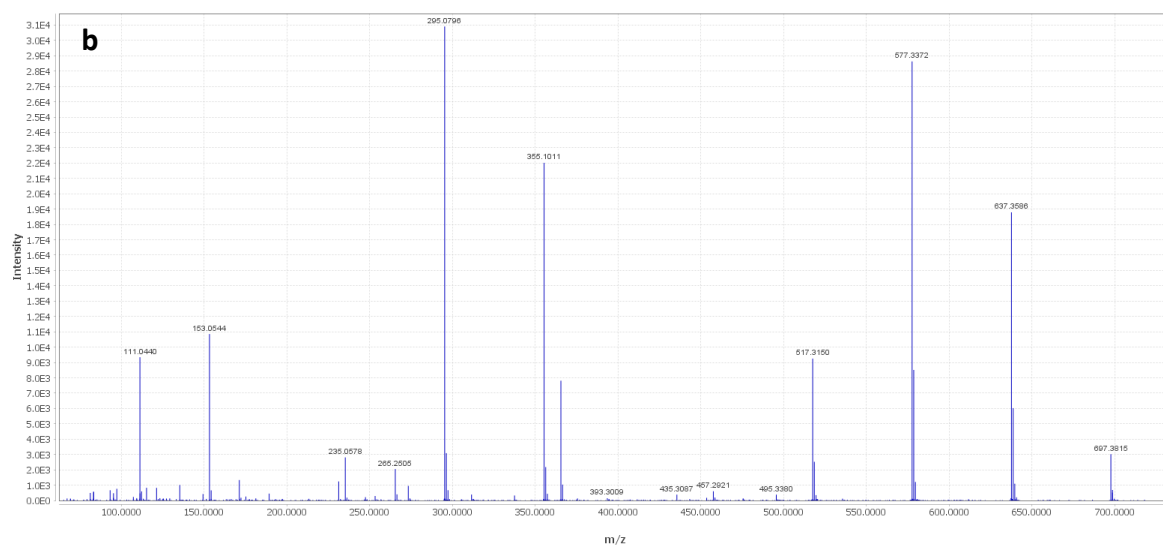
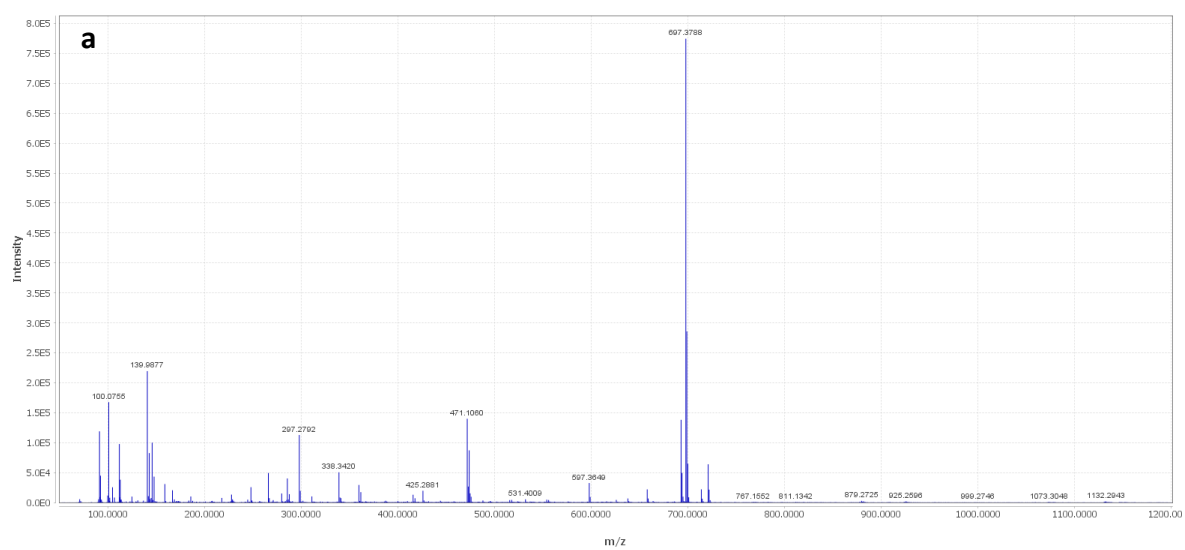


Figure S23. (a) HR-ESIMS and (b) MS/MS spectra of 4

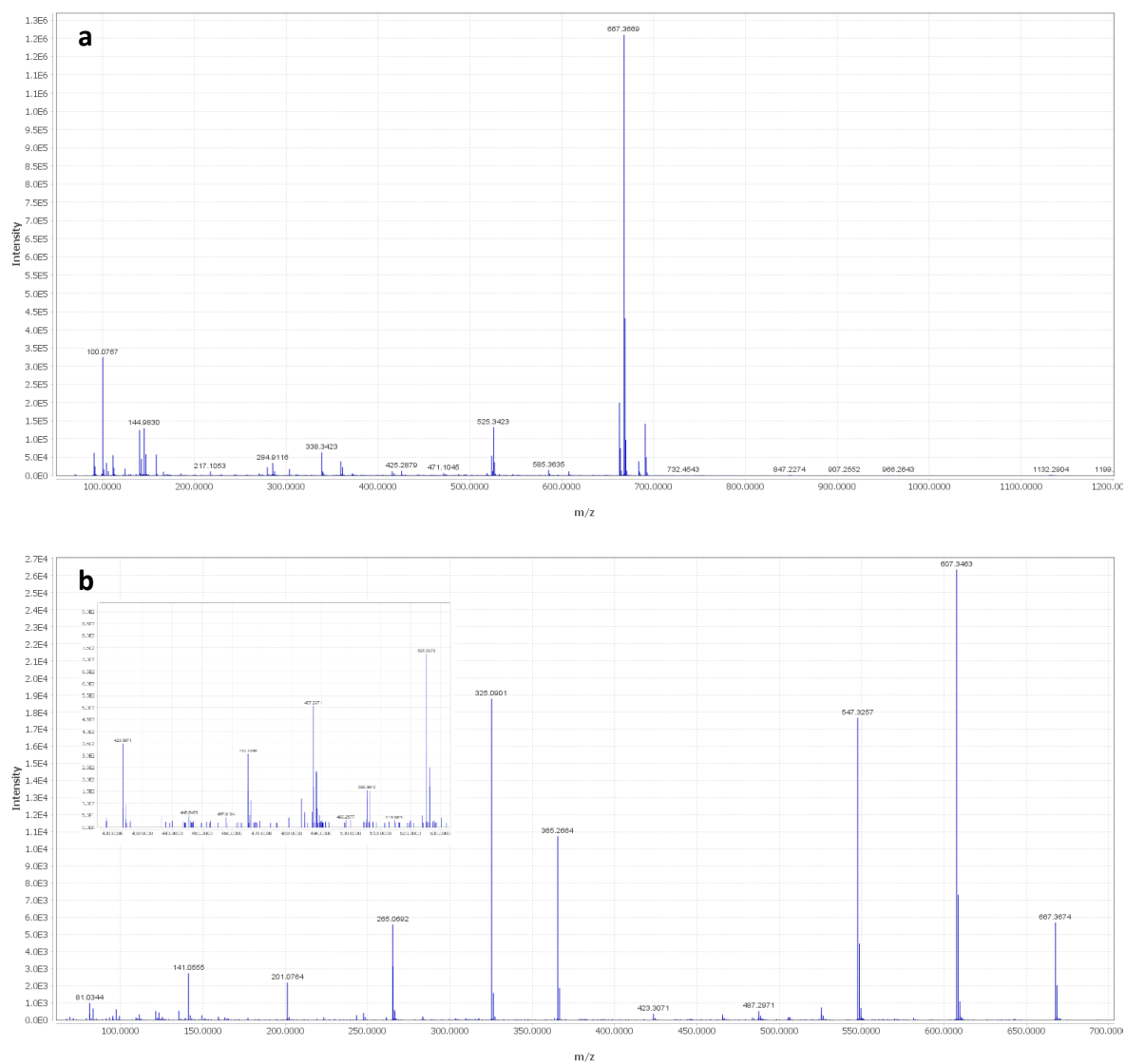
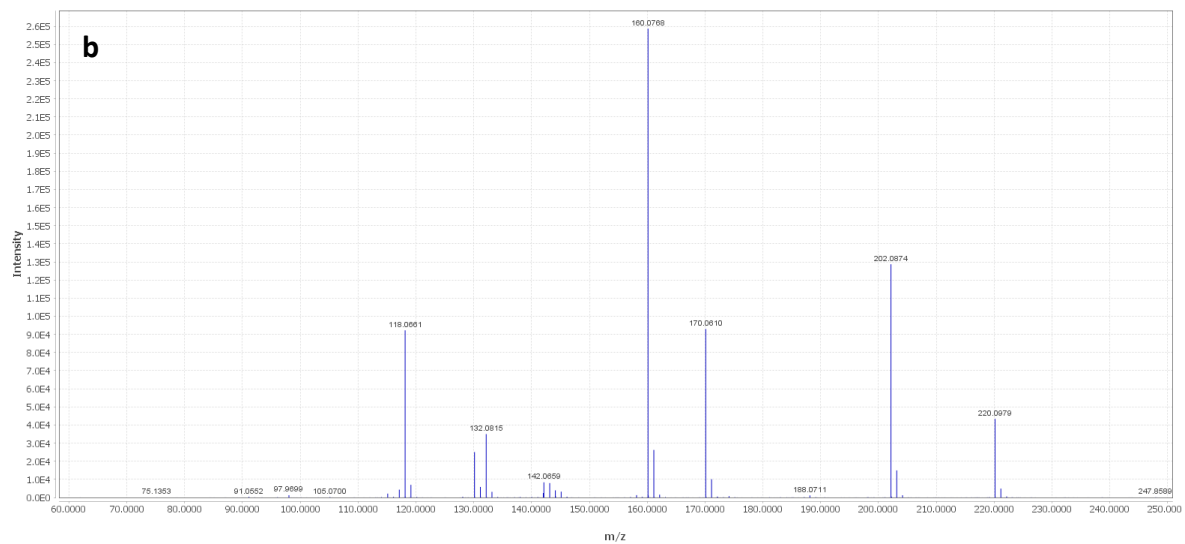
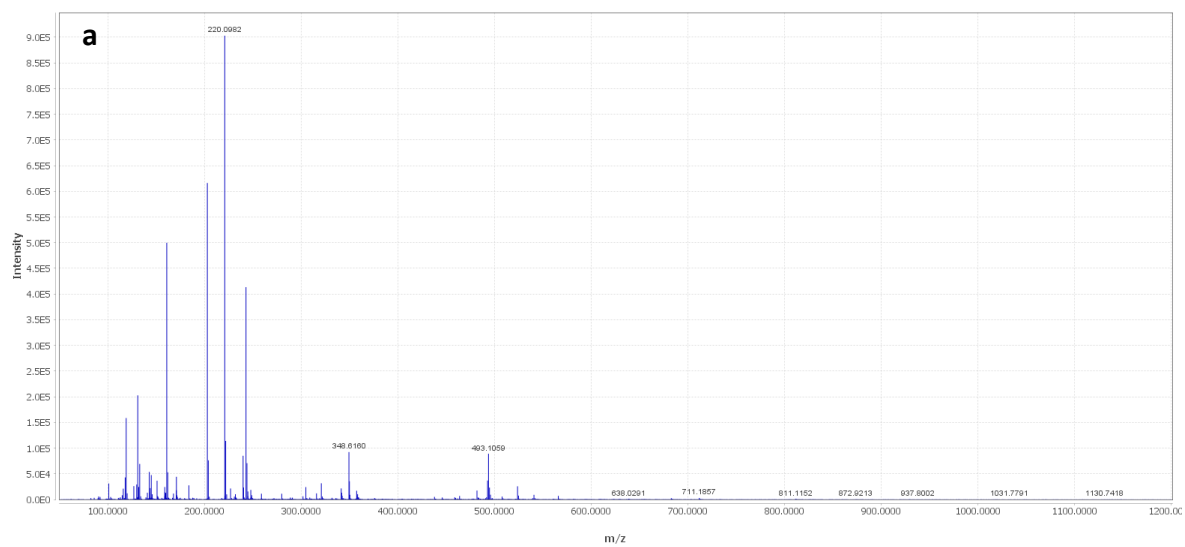
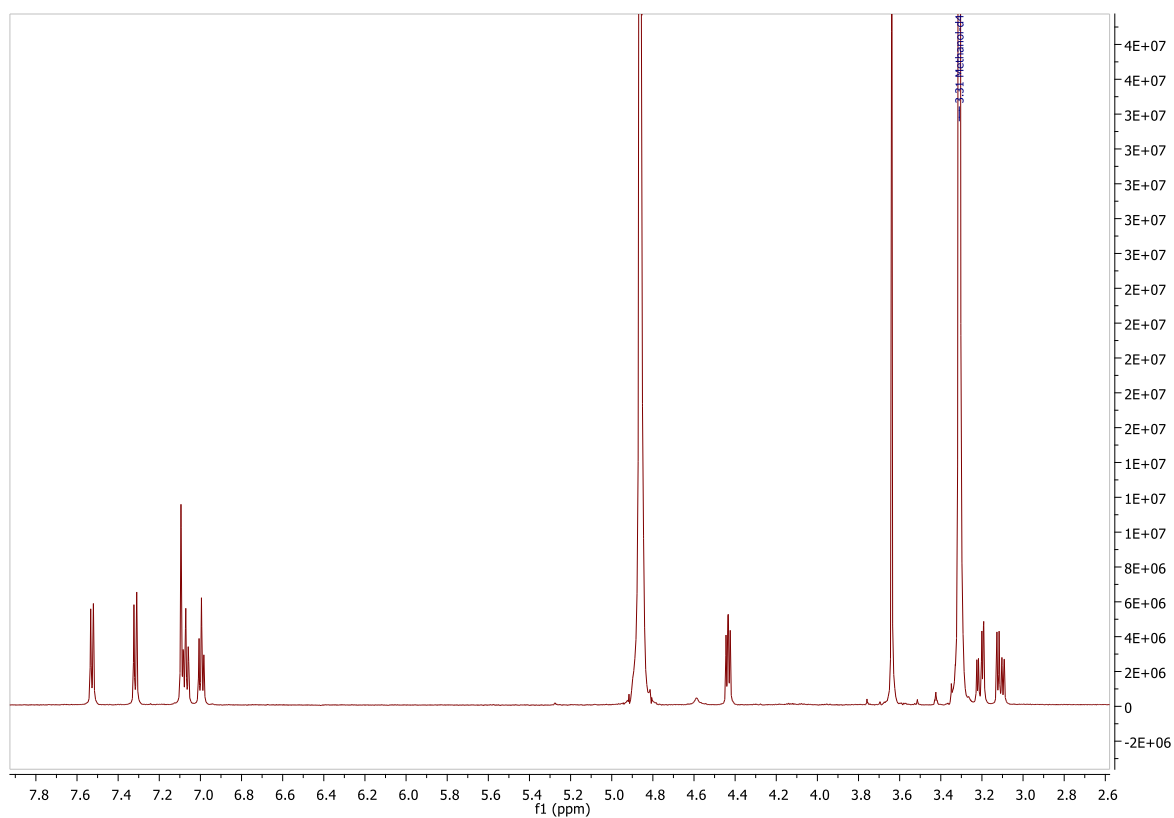


Figure S24. (a) HR-ESIMS and (b) MS/MS spectra of 5



**Figure S25.**  $^1\text{H}$  NMR spectrum of compound 5 (MeOD, 600 MHz)



**Figure S26.**  $^{13}\text{C}$  NMR spectrum of compound 5 (MeOD, 150 MHz)

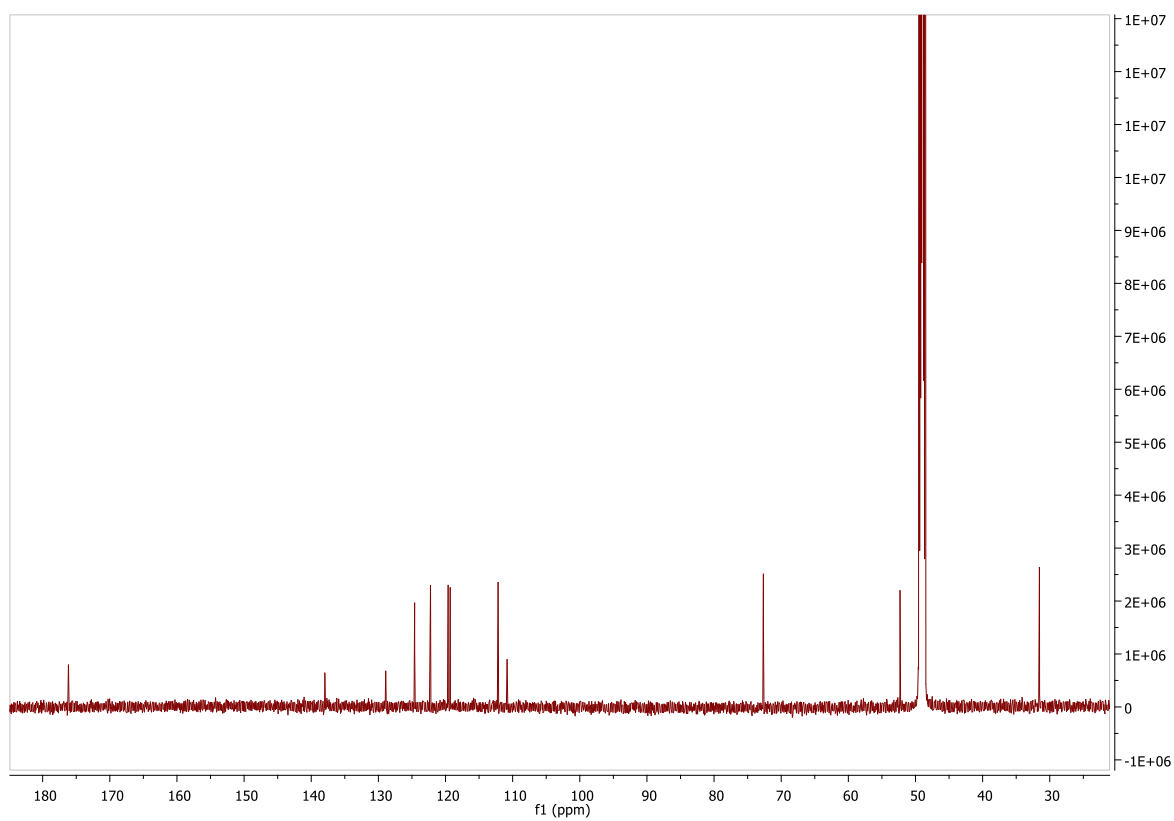


Figure S27. HSQC spectrum of compound 5 (MeOD, 600/150 MHz)

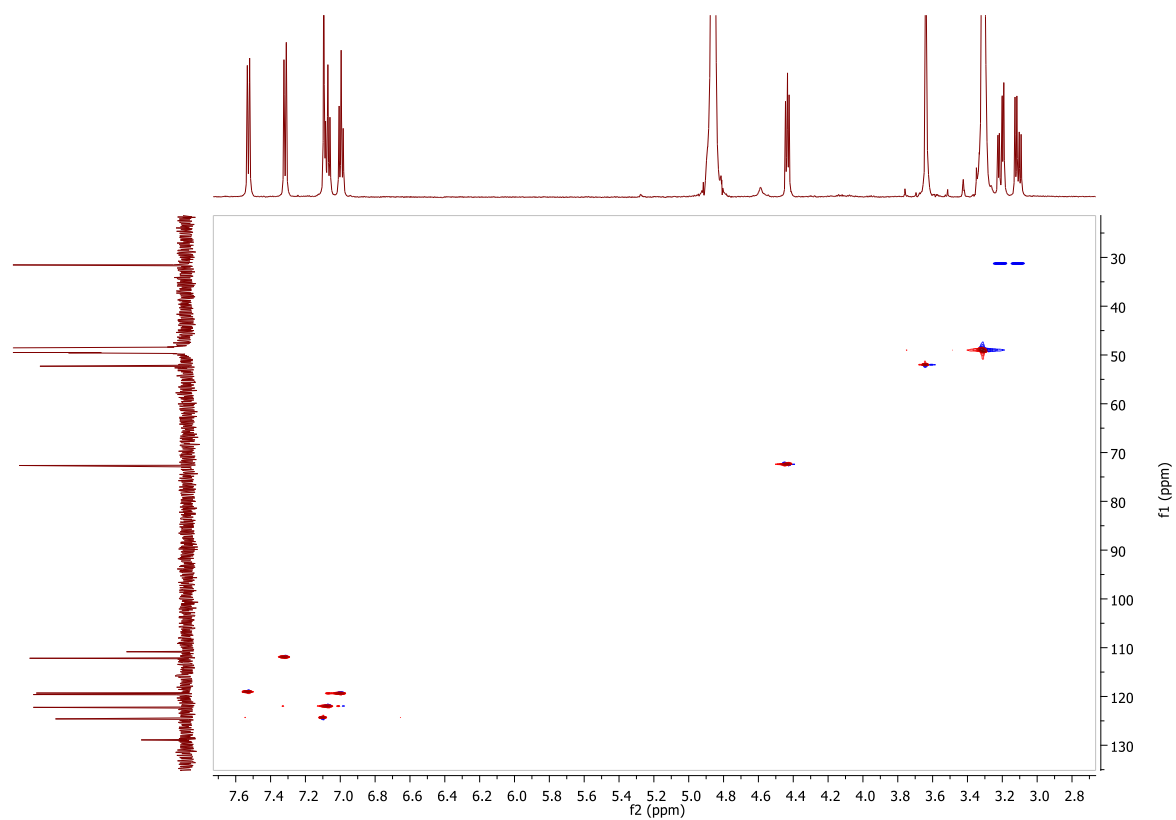


Figure S28. COSY spectrum of compound 5 (MeOD, 600 MHz)

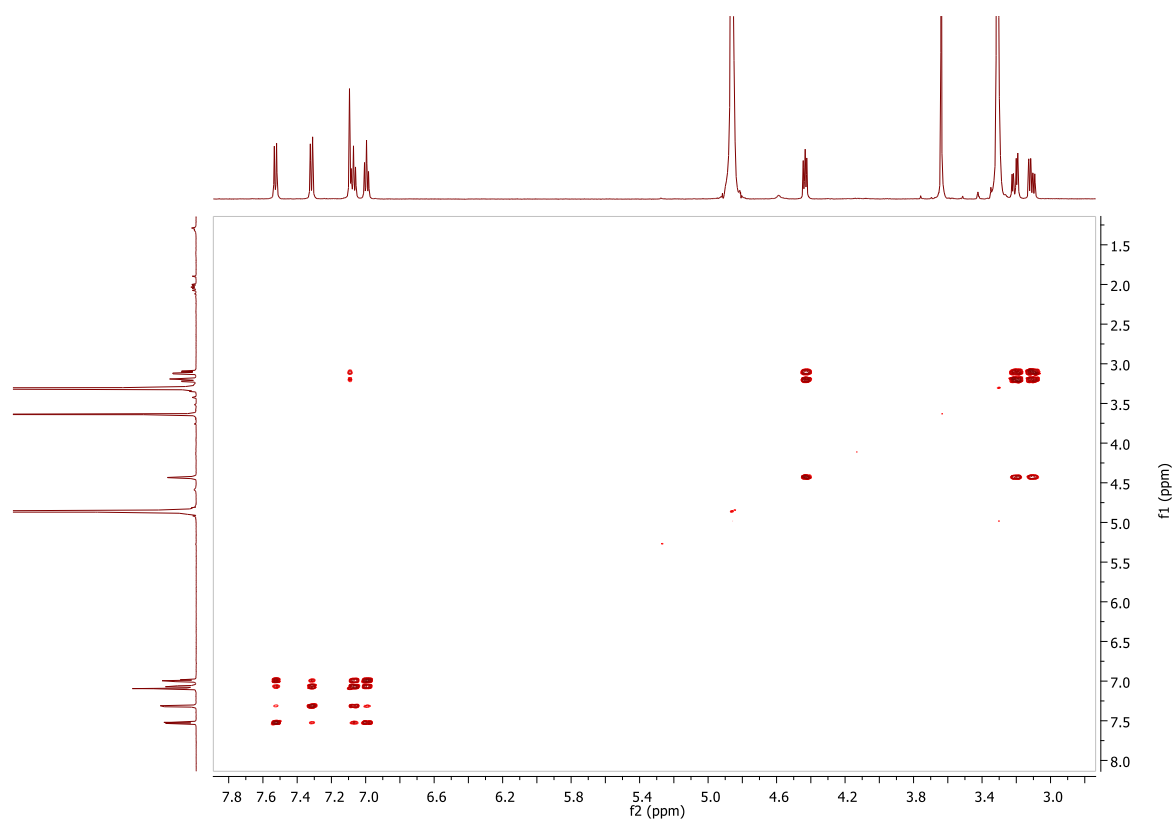


Figure S29. HMBC spectrum of compound 5 (MeOD, 600/150 MHz)

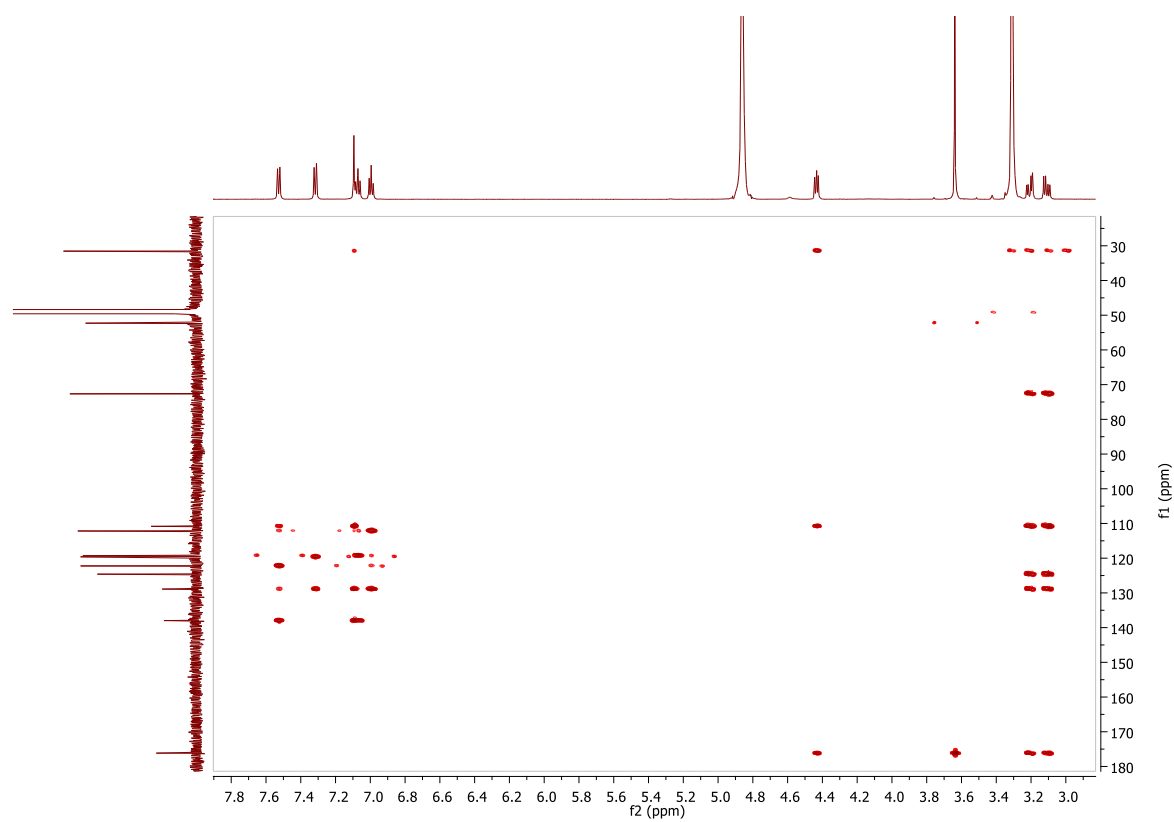
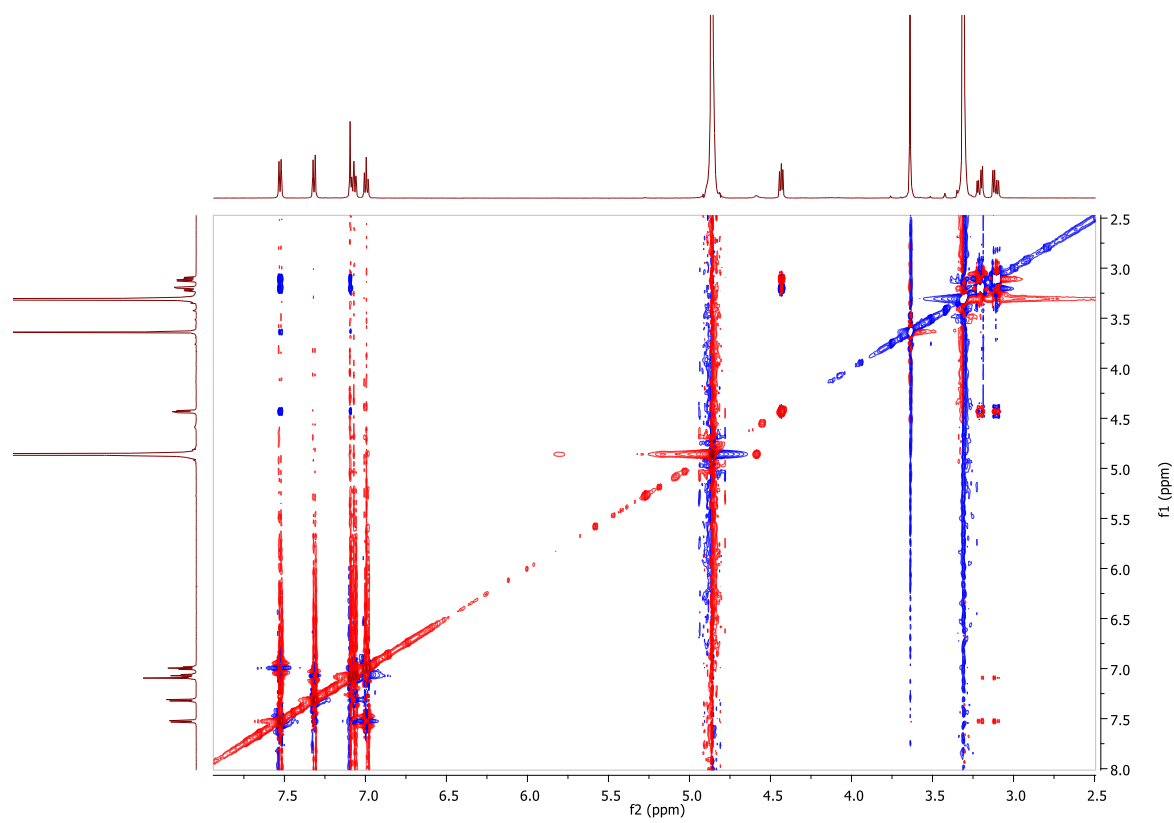
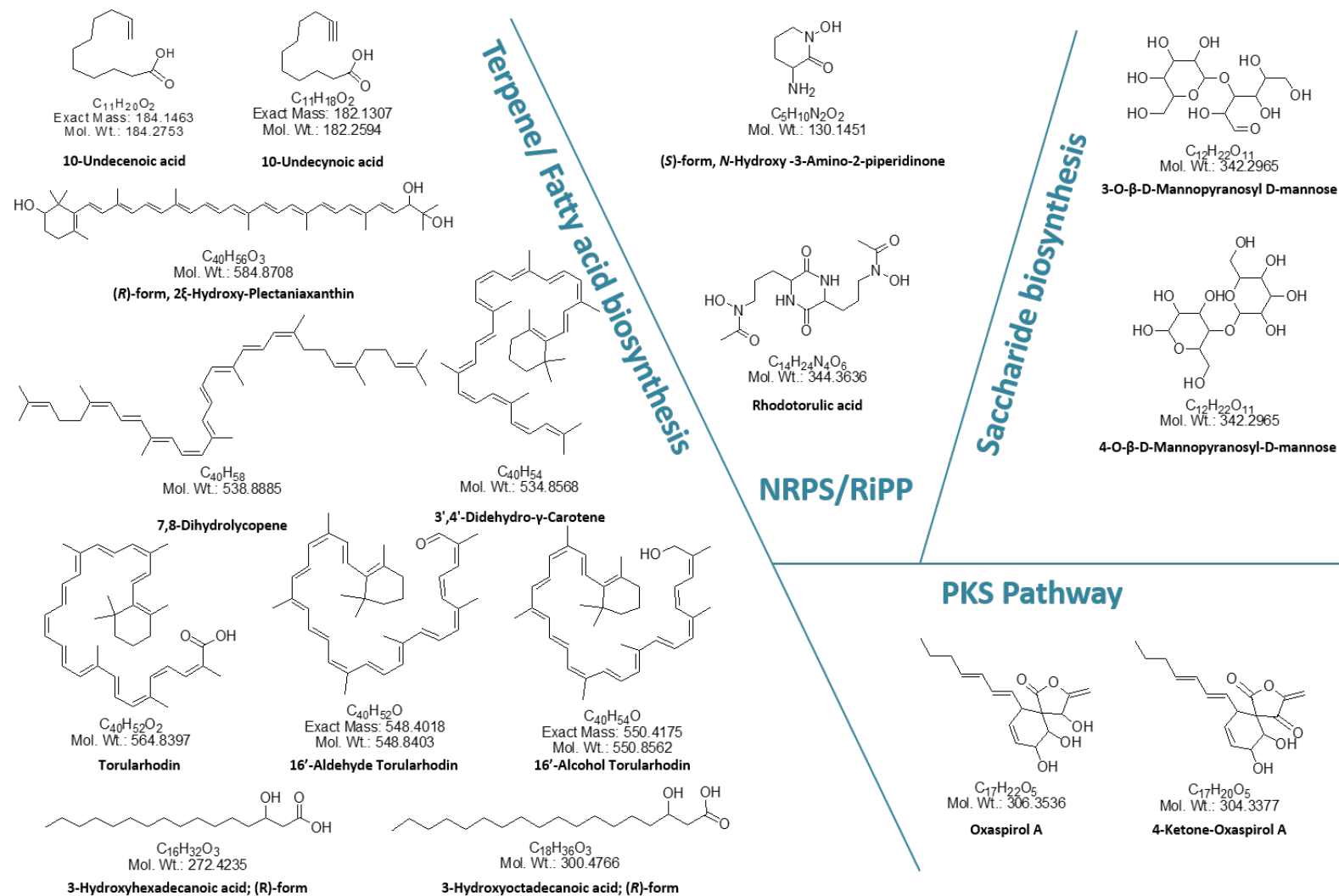


Figure S30. NOESY spectrum of compound 5 (MeOD, 600 MHz)



**Figure S31.** Metabolites reported in the Dictionary of Natural Products for the genus *Rhodotorula*



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