

## Electronic Supplementary Information

### A Biocatalytic Approach to Capuramycin Analogues by Exploiting a Substrate Permissive N-Transacylase CapW

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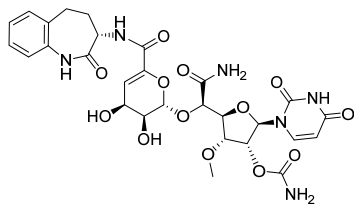
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<sup>d</sup>Natural Products Research Group, Discovery Science and Technology Group, Daiichi Sankyo RD Novare Co., Ltd., Tokyo 370-0503 (Japan).

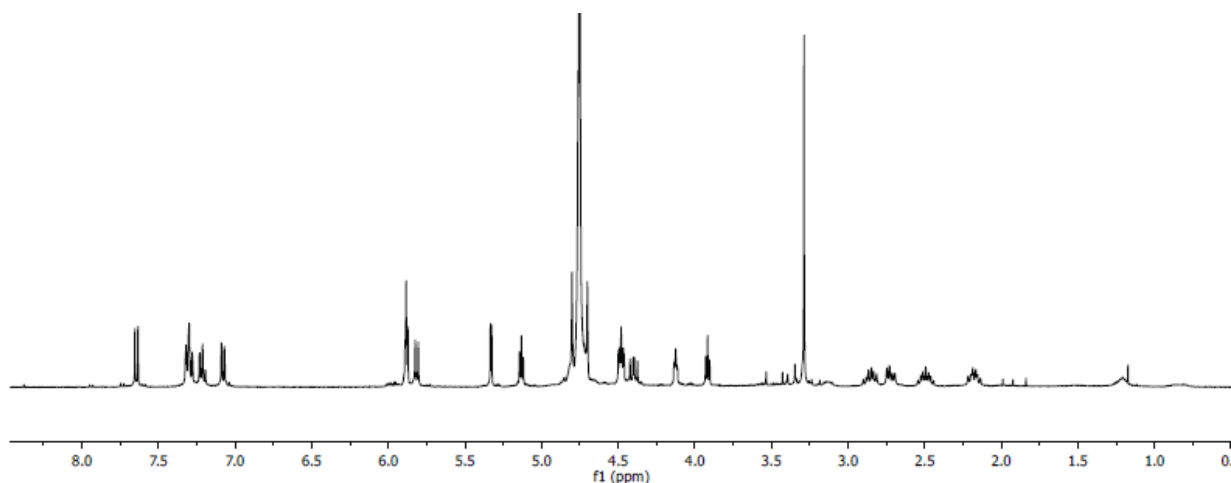
## 1. Supporting Results

### HPLC chromatograms and spectral assignments for compounds 10-53.

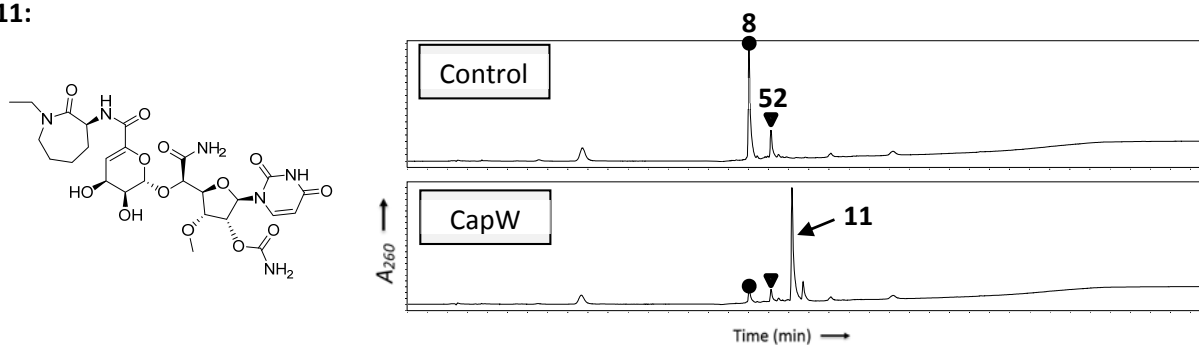
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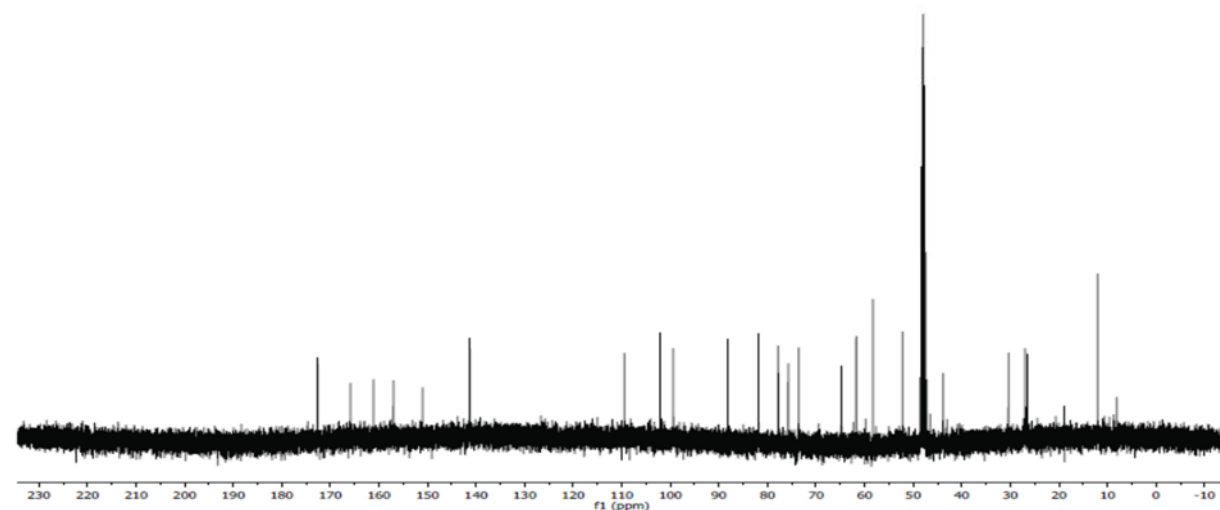
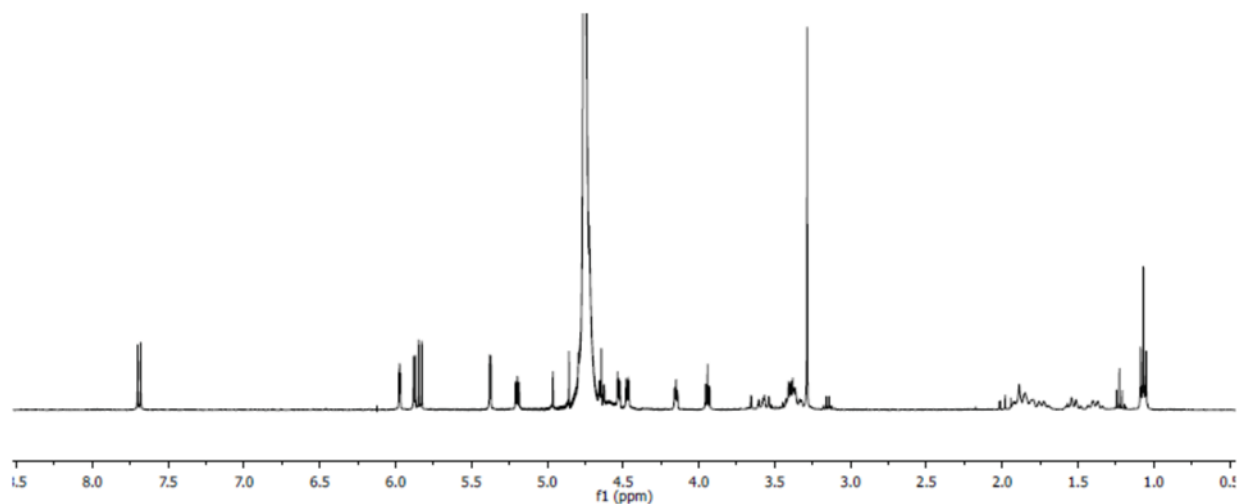
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.64 (d,  $J=8.0$  Hz, 1H), 7.30 (t,  $J=8.0$  Hz, 2H), 7.21 (t,  $J=6.9$  Hz, 1H), 7.08 (d,  $J=7.8$  Hz, 1H), 5.88 (t,  $J=3.6$  Hz, 2H), 5.81 (d,  $J=8.1$  Hz, 1H), 5.33 (d,  $J=3.3$  Hz, 1H), 5.13 (t,  $J=5.0$  Hz, 1H), 4.51-4.45 (m, 2H), 4.40 (dd,  $J=11.5, 8.1$  Hz, 1H), 4.14-4.10 (m, 1H), 3.91 (t,  $J=5.3$  Hz, 1H), 3.29 (s, 3H), 2.86 (td,  $J=13.3, 7.7$  Hz, 1H), 2.72 (dd,  $J=14.2, 6.7$  Hz, 1H), 2.49 (ddd,  $J=20.8, 13.0, 7.8$  Hz, 1H), 2.22-2.12 (m, 1H), 1.25-1.17 (m, 1H). HRMS (ESI):  $\text{C}_{28}\text{H}_{32}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 661.2100, Found: 661.2105.



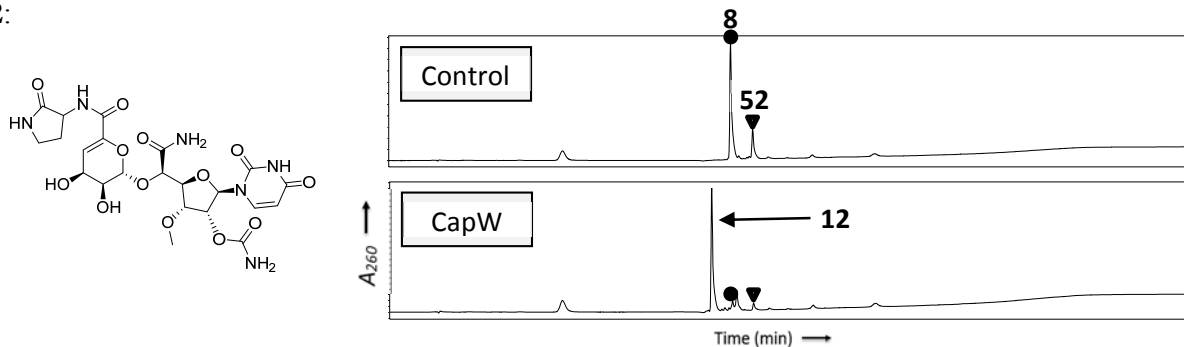
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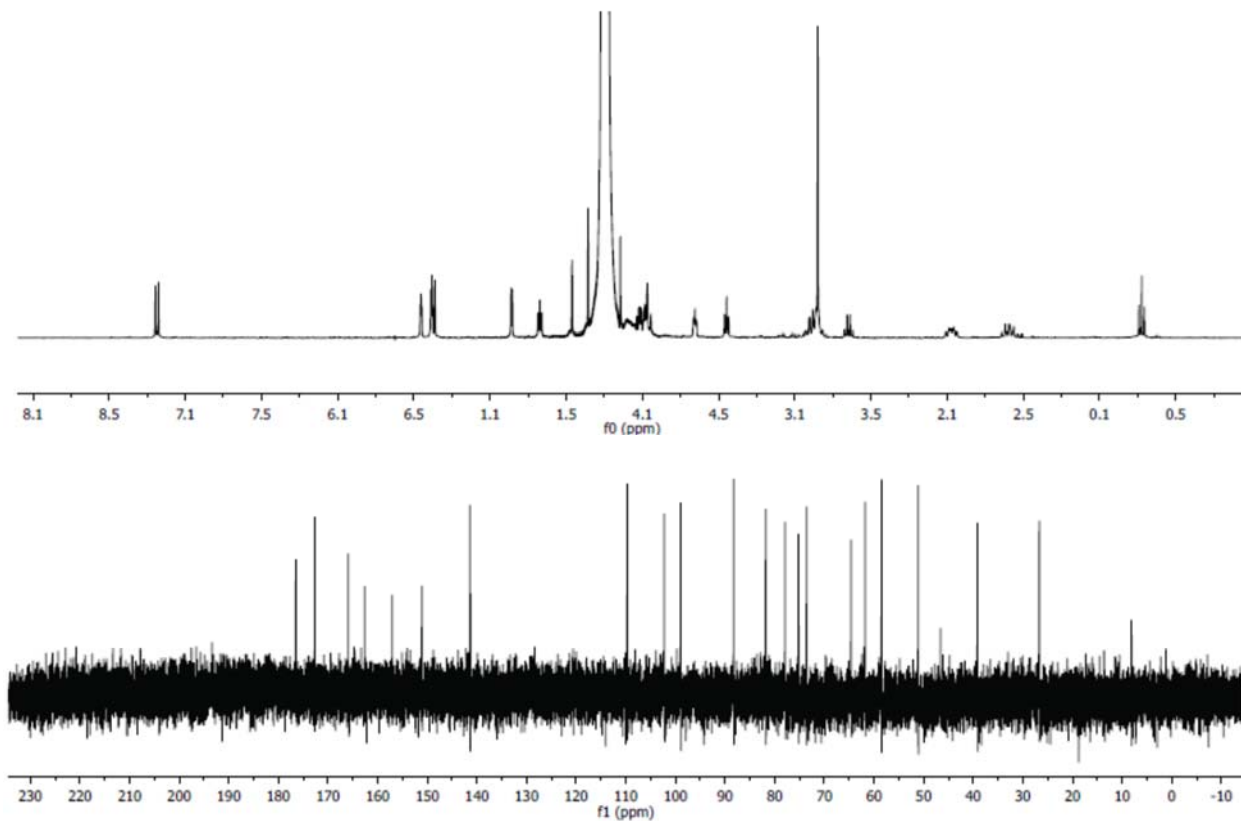
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.69 (d,  $J=8.1$  Hz, 1H), 5.97 (dd,  $J=2.7, 1.5$  Hz, 1H), 5.88 (d,  $J=4.3$  Hz, 1H), 5.84 (d,  $J=8.1$  Hz, 1H), 5.38 (d,  $J=3.5$  Hz, 1H), 5.22-5.17 (m, 1H), 4.53 (dd,  $J=4.6, 2.7$  Hz, 1H), 4.47 (dd,  $J=5.6, 2.4$  Hz, 1H), 4.17-4.12 (m, 1H), 3.94 (t,  $J=5.5$  Hz, 1H), 3.62-3.53 (m, 1H), 3.43-3.34 (m, 4H), 3.29 (s, 3H), 1.95-1.68 (m, 4H), 1.58-1.32 (m, 2H), 1.07 (dd,  $J=9.2, 5.2$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$  with methanol as standard)  $\delta$  172.62, 165.88, 161.09, 157.03, 151.01, 141.35, 141.29, 109.45, 102.10, 99.40, 88.17, 81.84, 77.79, 75.72, 73.54, 64.77, 61.73, 59.81, 58.29, 52.19, 43.83, 30.39, 26.95, 26.52, 12.03. HRMS (ESI):  $\text{C}_{26}\text{H}_{36}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 641.2413, Found: 641.2415.



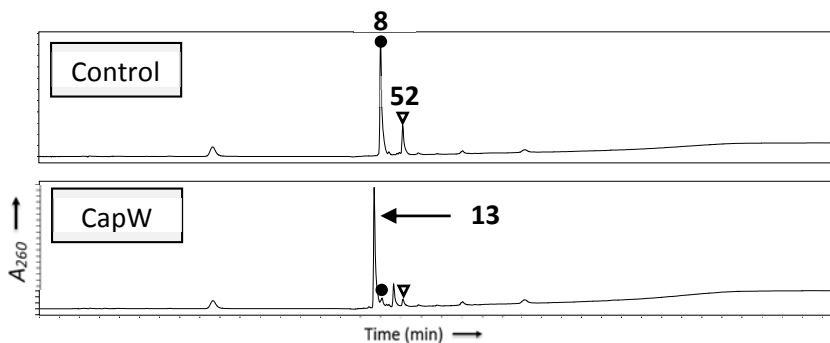
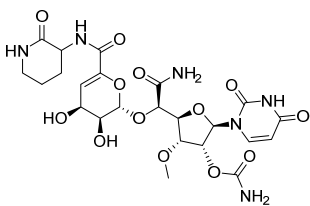
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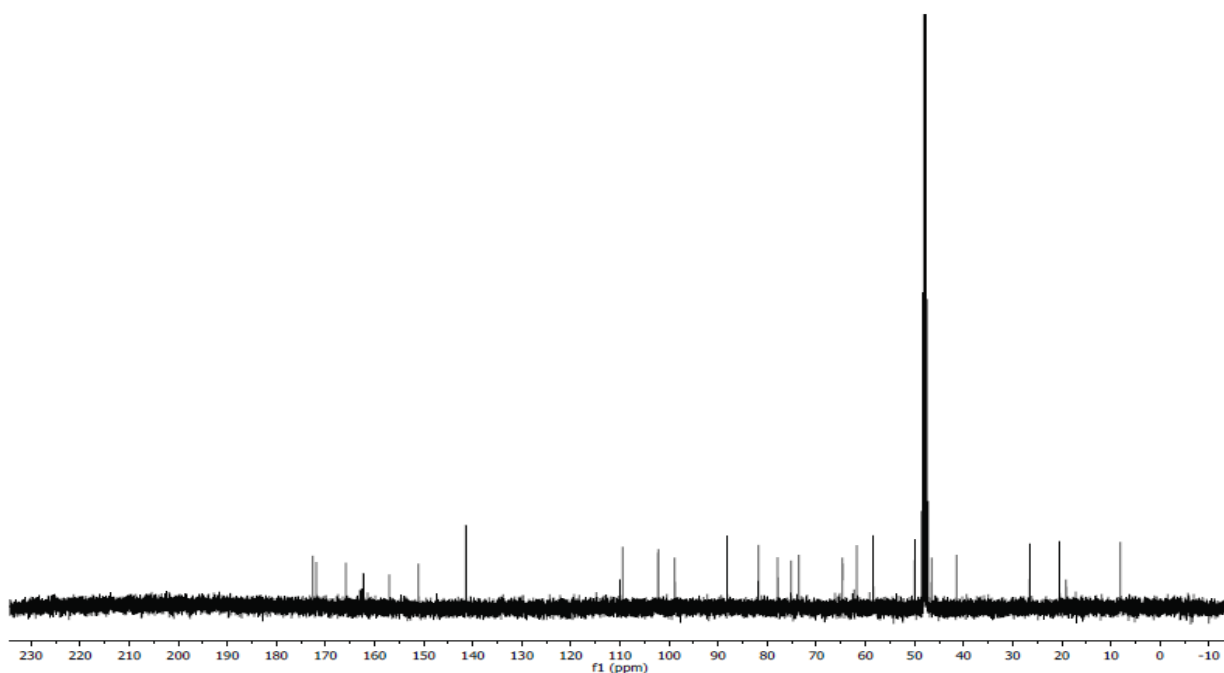
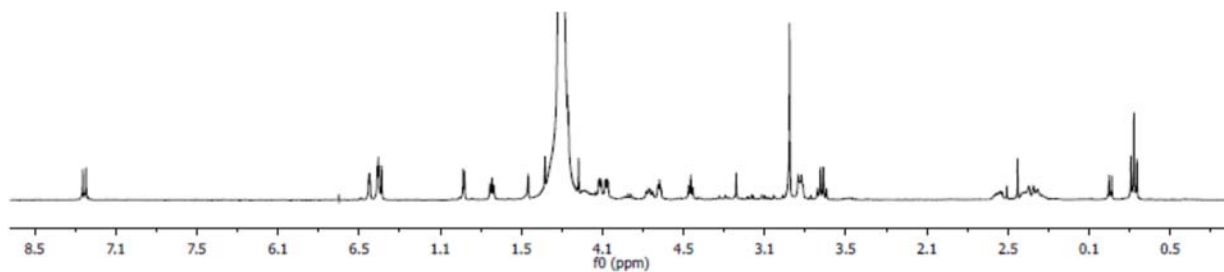
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.69 (d,  $J=8.1$  Hz, 1H), 5.95 (dd,  $J=2.5, 1.6$  Hz, 1H), 5.88 (dd,  $J=6.3, 4.5$  Hz, 1H), 5.36 (d,  $J=3.2$  Hz, 1H), 5.20-5.14 (m, 1H), 4.73-4.71 (m, 1H), 4.54-4.43 (m, 3H), 4.17-4.13 (m, 1H), 3.97-3.92 (m, 1H), 3.44-3.36 (m, 1H), 3.35 (s, 3H), 3.15 (dd,  $J=14.6, 7.3$  Hz, 1H), 2.53-2.42 (m, 1H), 2.15-2.04 (m, 1H), 1.22 (t,  $J=7.3$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  176.49, 172.64, 165.94, 162.62, 157.06, 151.09, 141.39, 141.24, 109.72, 102.28, 98.93, 88.24, 81.81, 77.89, 75.19, 73.57, 64.62, 61.75, 58.47, 51.10, 39.16, 26.69. HRMS (ESI):  $\text{C}_{22}\text{H}_{28}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 585.1787, Found: 585.1788.



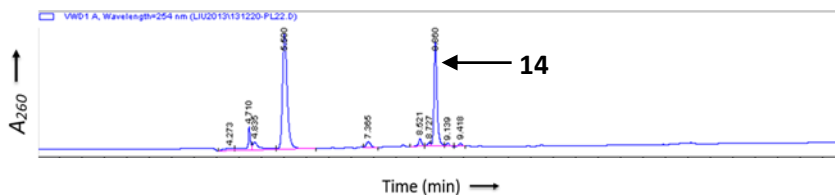
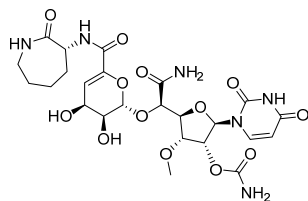
13:



$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.69 (d,  $J=8.1$  Hz, 1H), 5.94 (dd,  $J=2.5, 1.6$  Hz, 1H), 5.88 (dd,  $J=6.2, 4.3$  Hz, 2H), 5.35 (d,  $J=3.3$  Hz, 1H), 5.20-5.16 (m, 1H), 4.51 (dd,  $J=4.5, 2.6$  Hz, 1H), 4.47 (dd,  $J=5.2, 2.3$  Hz, 1H), 4.21 (dd,  $J=10.1, 6.3$  Hz, 1H), 4.17-4.12 (m, 1H), 3.96 (t,  $J=5.4$  Hz, 1H), 3.35 (s, 3H), 3.28 (d,  $J=8.1$  Hz, 2H), 2.04 (d,  $J=5.2$  Hz, 1H), 1.92-1.76 (m, 3H), 1.37 (d,  $J=7.0$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$  with methanol as standard)  $\delta$  172.58, 171.84, 165.88, 162.25, 157.01, 151.04, 141.37, 109.47, 102.22, 98.84, 88.17, 81.80, 77.88, 75.15, 73.55, 64.69, 61.76, 58.43, 49.94, 46.53, 41.46, 26.52, 20.47. HRMS (ESI):  $\text{C}_{23}\text{H}_{30}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 599.1944, Found: 599.1942.

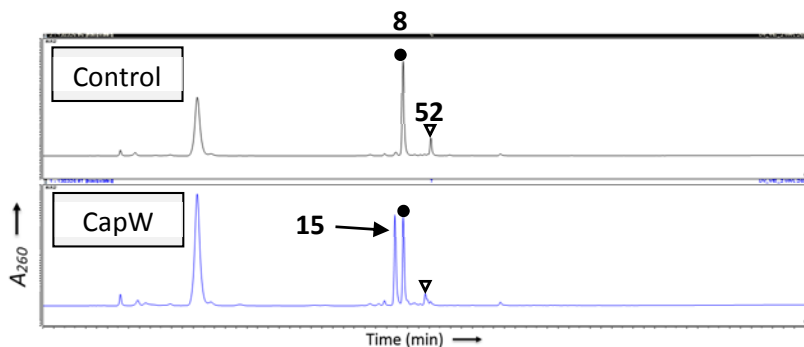
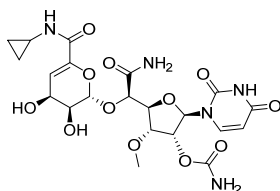


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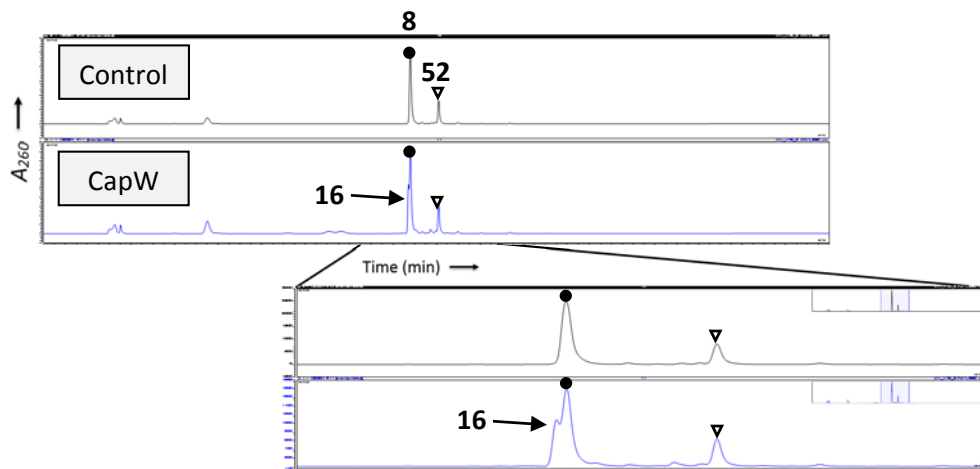
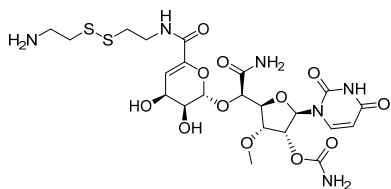
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.72 (d,  $J=8.2$  Hz, 1H), 6.02-5.98 (m, 1H), 5.93-5.86 (m, 2H), 5.39 (d,  $J=3.2$  Hz, 1H), 5.22 (t,  $J=4.7$  Hz, 1H), 4.79-4.78 (m, 1H), 4.62 (d,  $J=9.8$  Hz, 1H), 4.58-4.54 (m, 1H), 4.49 (dd,  $J=5.3, 2.2$  Hz, 1H), 4.18 (s, 1H), 3.98 (t,  $J=5.3$  Hz, 1H), 3.33 (s, 3H), 3.29 (d,  $J=9.4$  Hz, 2H), 1.96 (dd,  $J=32.2, 13.0$  Hz, 2H), 1.83 (d,  $J=11.4$  Hz, 2H), 1.76-1.60 (m, 2H). HRMS (ESI):  $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 613.2100, Found: 613.2101.

15:



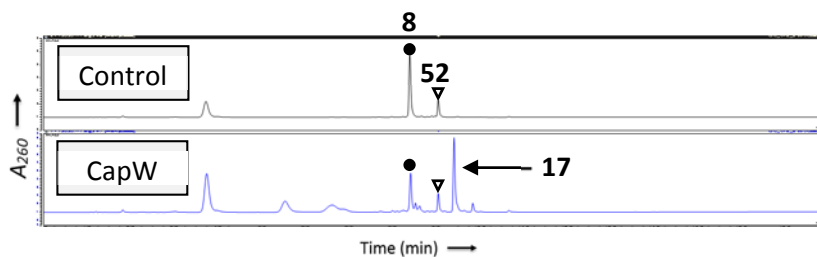
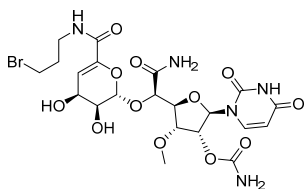
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.72 (d,  $J=8.2$  Hz, 1H), 6.02-5.98 (m, 1H), 5.93-5.86 (m, 2H), 5.39 (d,  $J=3.2$  Hz, 1H), 5.22 (t,  $J=4.7$  Hz, 1H), 4.79-4.78 (m, 1H), 4.62 (d,  $J=9.8$  Hz, 1H), 4.58-4.54 (m, 1H), 4.49 (dd,  $J=5.3, 2.2$  Hz, 1H), 4.18 (s, 1H), 3.98 (t,  $J=5.3$  Hz, 1H), 3.33 (s, 3H), 3.29 (d,  $J=9.4$  Hz, 2H), 1.96 (dd,  $J=32.2, 13.0$  Hz, 2H), 1.83 (d,  $J=11.4$  Hz, 2H), 1.76-1.60 (m, 2H). HRMS (ESI):  $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 613.2100, Found: 613.2101.

16:



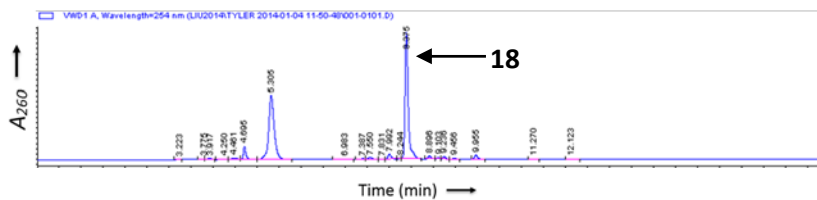
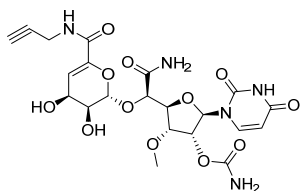
MS (ESI):  $C_{22}H_{32}N_6O_{12}S_2+H^+$ , Calc: 637.2, Found: 636.6.

17:



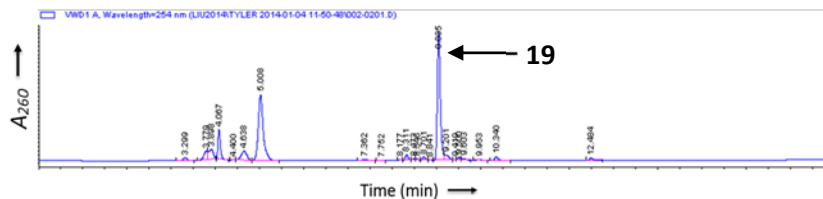
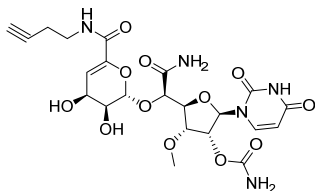
MS (ESI):  $C_{21}H_{28}BrN_5O_{12}+H^+$ , Calc: 622.1, Found: 622.5.

18:



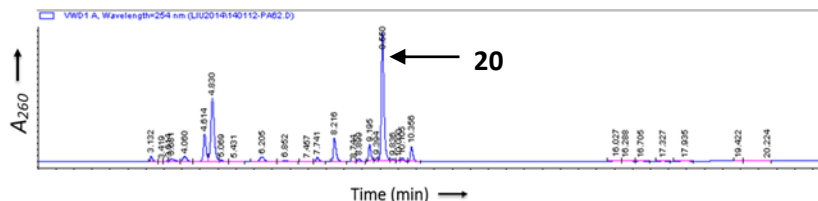
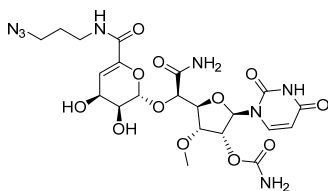
MS (ESI):  $C_{21}H_{25}N_5O_{12}-H^-$ , Calc: 538.2, Found: 538.6.

19:



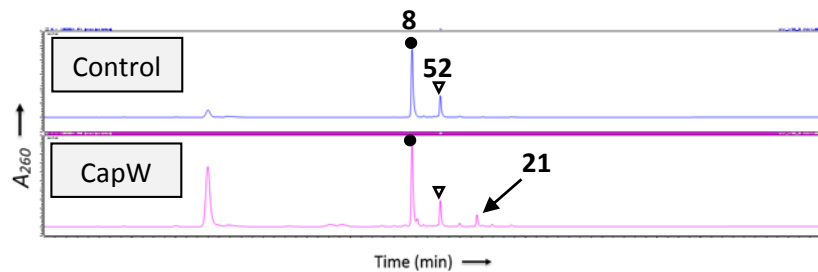
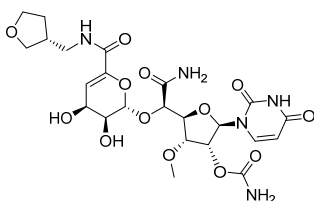
<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 7.71 (d, *J*=8.1 Hz, 1H), 5.98 (dd, *J*=2.9, 1.1 Hz, 1H), 5.90 (dd, *J*=6.2, 4.3 Hz, 2H), 5.39 (d, *J*=3.1 Hz, 1H), 5.24-5.20 (m, 1H), 4.71 (d, *J*=2.3 Hz, 1H), 4.56 (dd, *J*=4.5, 2.5 Hz, 1H), 4.49 (dd, *J*=5.5, 2.3 Hz, 1H), 4.23-4.17 (m, 1H), 3.95 (t, *J*=5.5 Hz, 1H), 3.47-3.42 (m, 2H), 3.36 (d, *J*=0.6 Hz, 3H), 2.46 (td, *J*=6.6, 2.6 Hz, 2H), 2.36 (td, *J*=2.6, 0.8 Hz, 1H). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O) δ 172.71, 165.96, 162.81, 157.08, 151.08, 141.48, 141.32, 109.52, 102.28, 98.94, 88.46, 82.08, 81.70, 77.72, 75.07, 73.47, 70.47, 64.51, 61.77, 58.45, 37.91, 18.31. HRMS (ESI): C<sub>22</sub>H<sub>27</sub>N<sub>5</sub>O<sub>12</sub>+H<sup>+</sup>, Calc: 554.1729, Found: 554.1731.

20:



MS (ESI): C<sub>21</sub>H<sub>28</sub>N<sub>8</sub>O<sub>12</sub>+H<sup>+</sup>, Calc: 585.2, Found: 584.7.

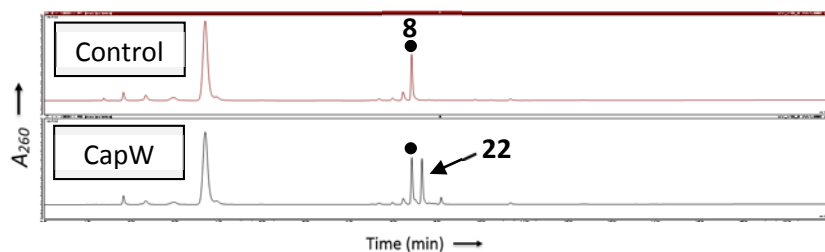
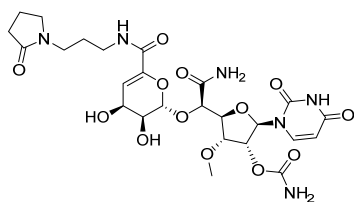
21:



MS (ESI): C<sub>23</sub>H<sub>31</sub>N<sub>5</sub>O<sub>13</sub>+H<sup>+</sup>, Calc: 586.2, Found: 586.6.

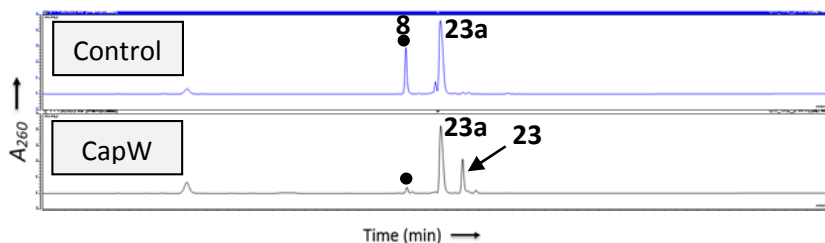
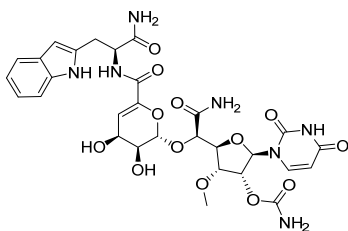


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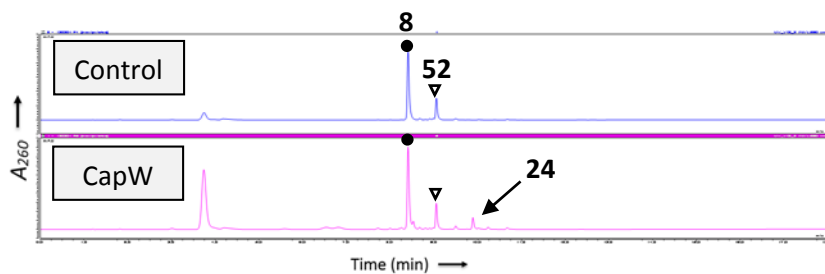
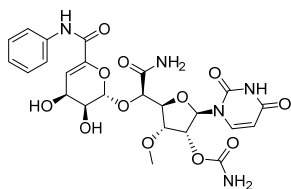
MS (ESI):  $C_{25}H_{34}N_6O_{13}+H^+$ , Calc: 627.2, Found: 626.6.

23:



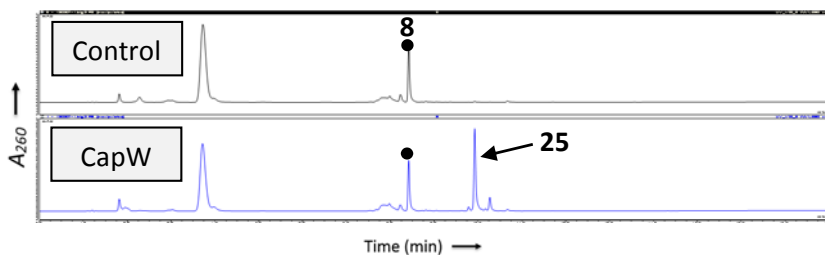
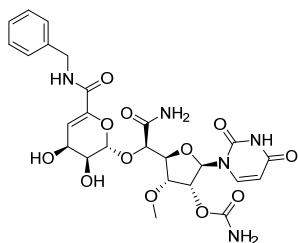
MS (ESI):  $C_{29}H_{33}N_7O_{13}+H^+$ , Calc: 688.2, Found: 687.6.

24:



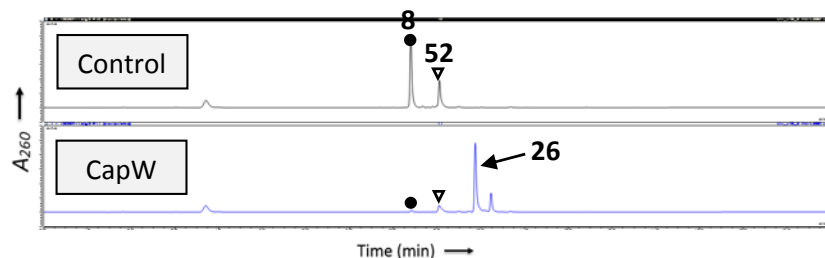
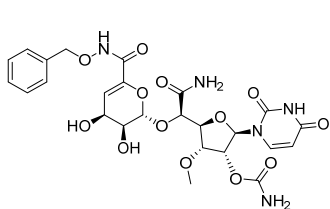
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25:

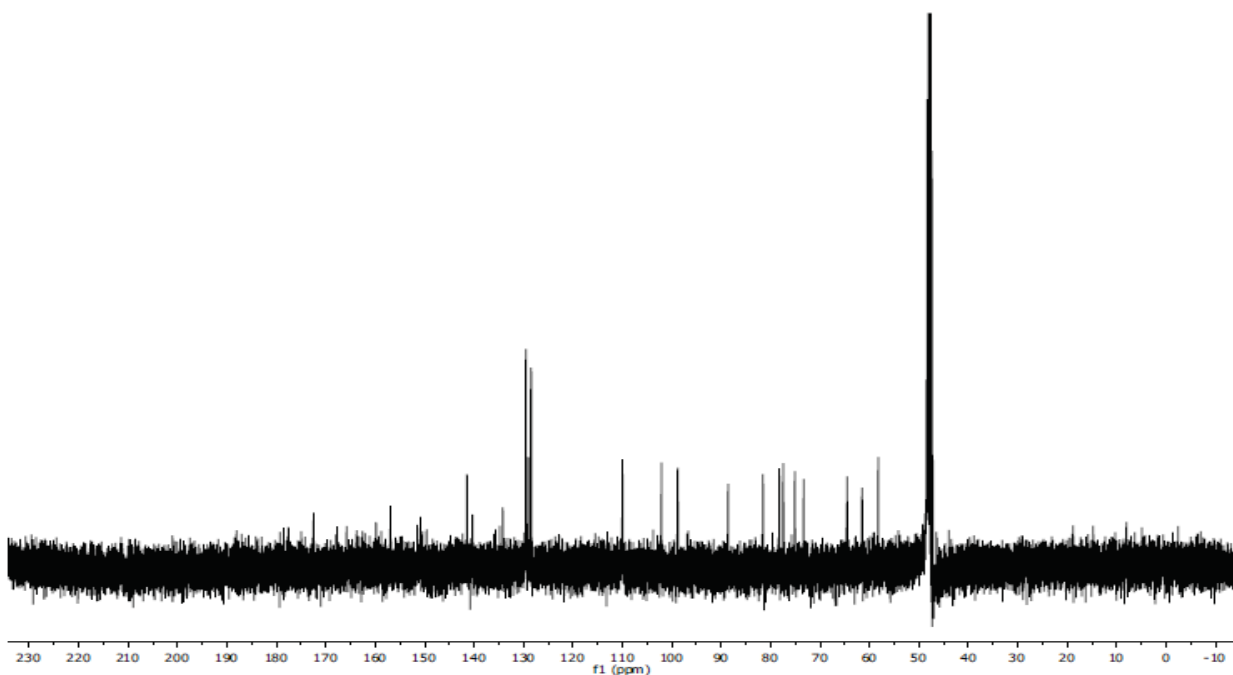
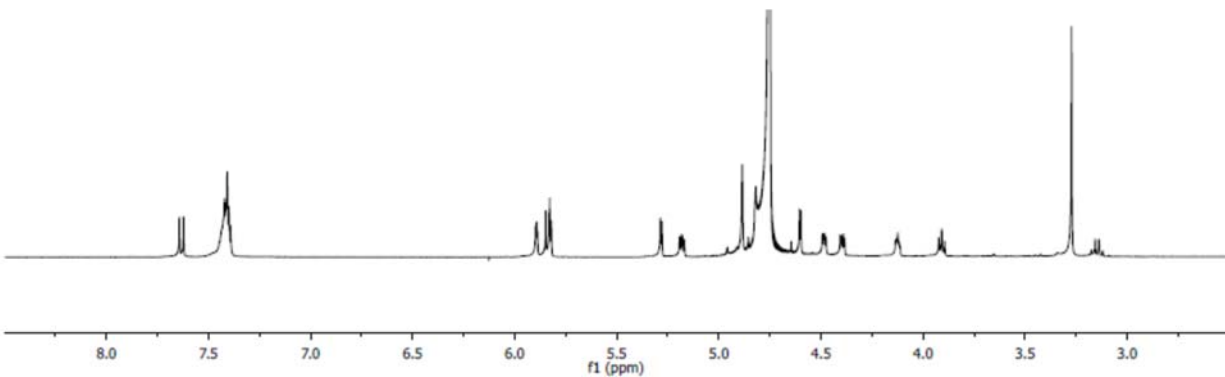


MS (ESI):  $C_{25}H_{29}N_5O_{12}+H^+$ , Calc: 592.2, Found: 591.7.

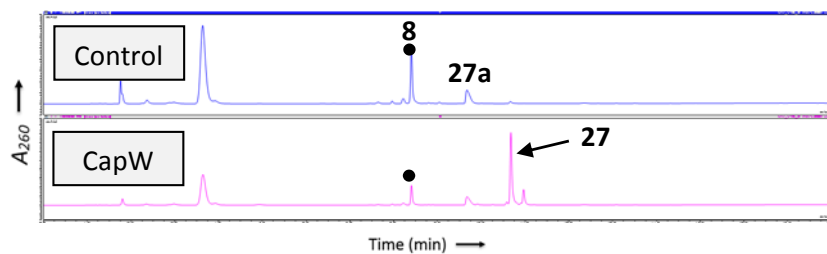
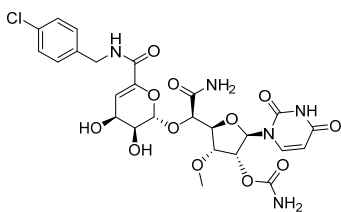
26:



$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.63 (d,  $J=8.1$  Hz, 1H), 7.45-7.38 (m, 5H), 5.89 (dd,  $J=2.5, 1.7$  Hz, 1H), 5.86-5.81 (m, 2H), 5.28 (d,  $J=3.2$  Hz, 1H), 5.18 (dd,  $J=5.3, 4.1$  Hz, 1H), 4.89 (s, 2H), 4.60 (d,  $J=2.7$  Hz, 1H), 4.48 (dd,  $J=4.5, 2.5$  Hz, 1H), 4.39 (dd,  $J=6.1, 2.6$  Hz, 1H), 4.14-4.10 (m, 1H), 3.91 (t,  $J=5.7$  Hz, 1H), 3.27 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$  with methanol as standard)  $\delta$  172.49, 156.93, 150.90, 141.47, 140.37, 134.25, 129.58, 129.48, 129.08, 128.45, 109.99, 102.07, 98.83, 88.58, 81.55, 78.26, 77.48, 75.07, 73.32, 64.54, 61.54, 58.18. HRMS (ESI):  $\text{C}_{25}\text{H}_{29}\text{N}_5\text{O}_{13}+\text{H}^+$ , Calc: 608.1835, Found: 608.1840.

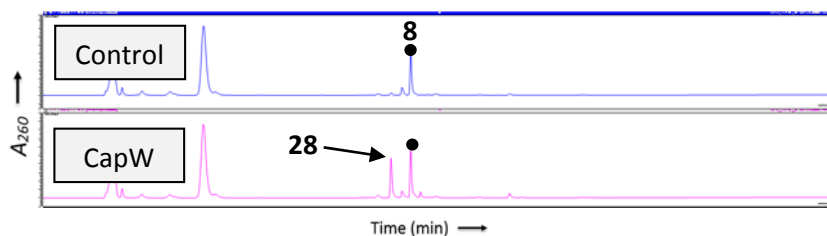
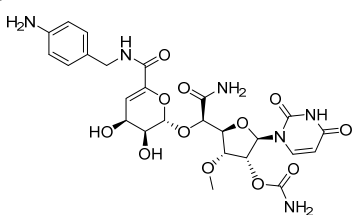


27:



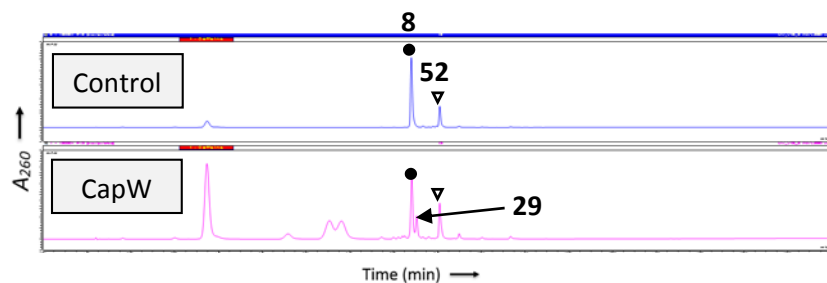
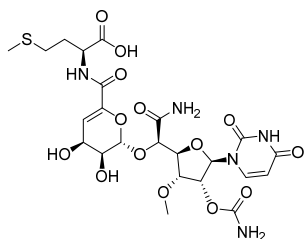
MS (ESI):  $C_{25}H_{28}ClN_5O_{12}-H^-$ , Calc: 624.1, Found: 623.5.

28:



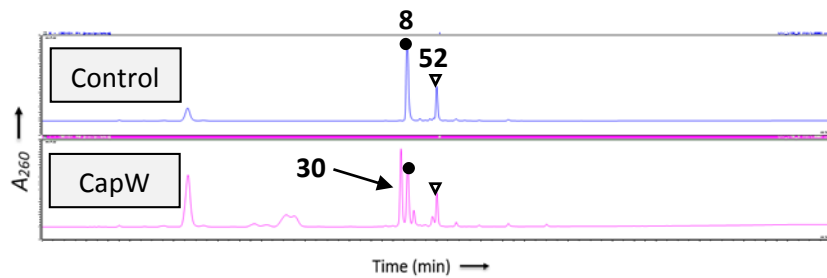
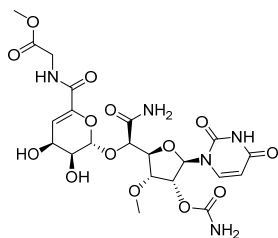
MS (ESI):  $C_{25}H_{30}N_6O_{12}+H^+$ , Calc: 607.2, Found: 606.6.

29:



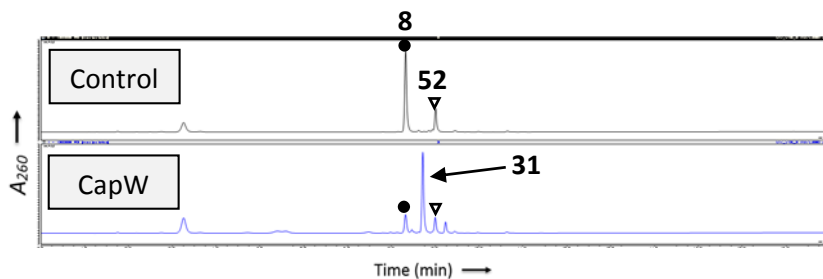
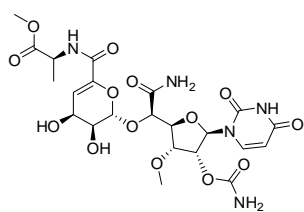
MS (ESI):  $C_{23}H_{31}N_5O_{14}+H^+$ , Calc: 634.2, Found: 633.5.

30:

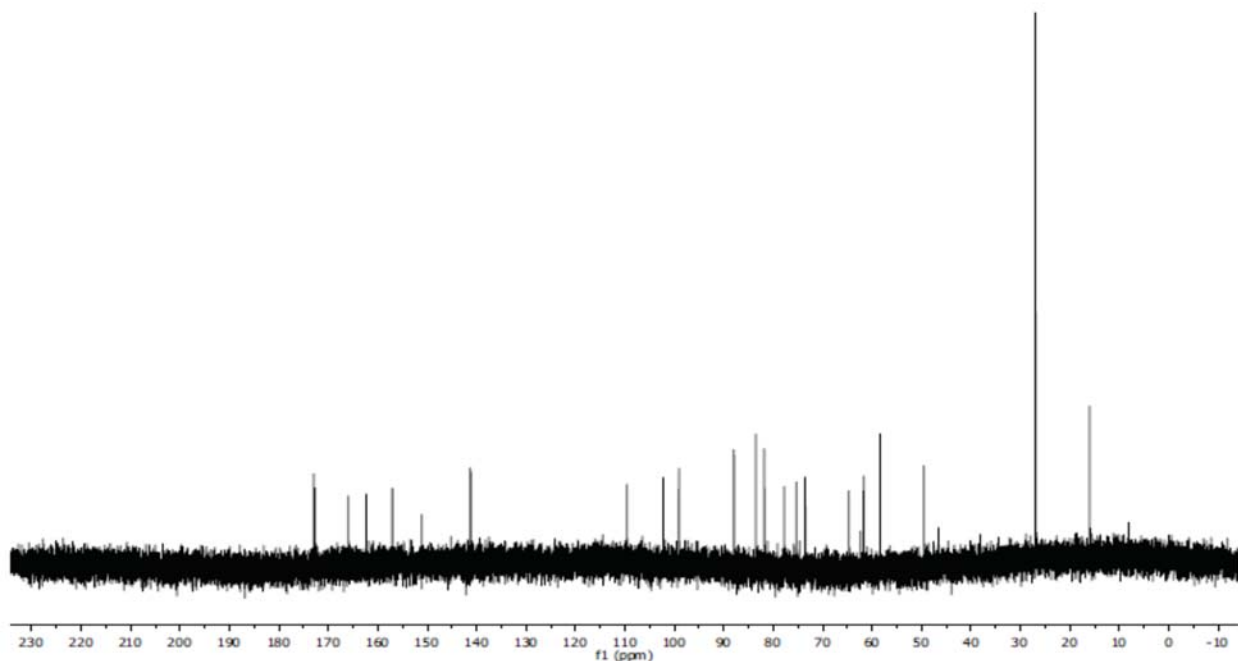
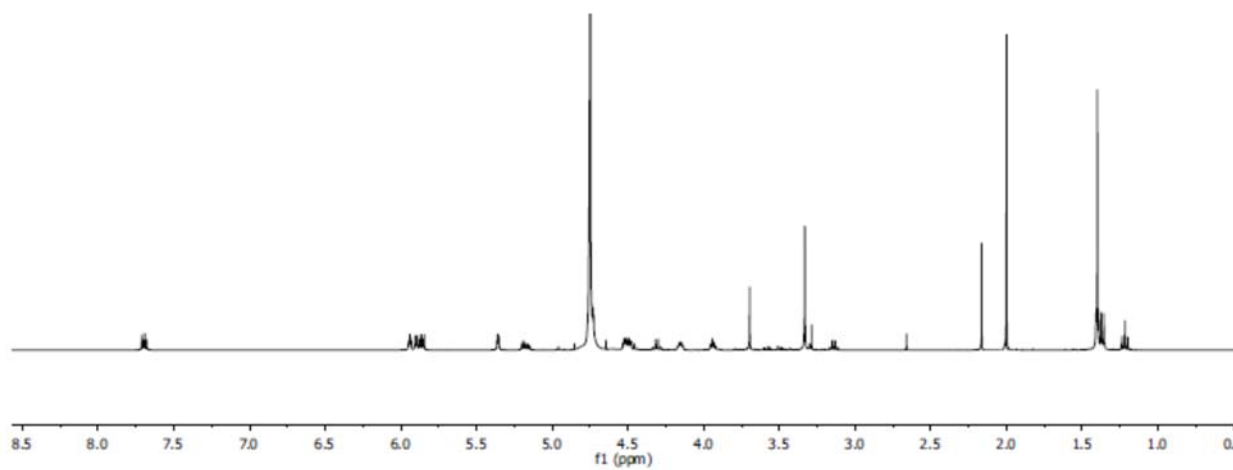


MS (ESI):  $C_{21}H_{27}N_5O_{14}+H^+$ , Calc: 574.2, Found: 573.7.

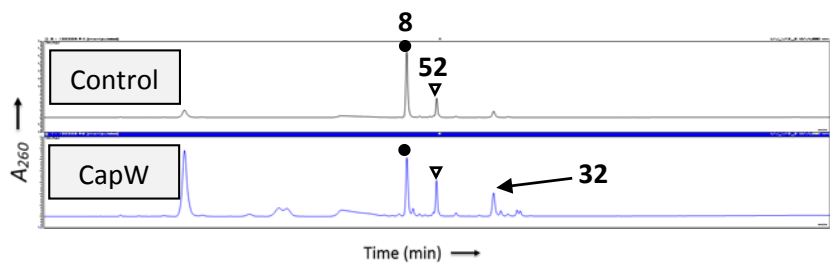
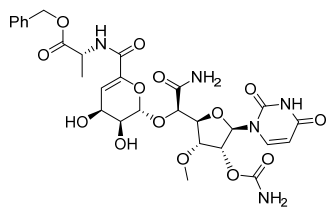
31:



$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.70 (dd,  $J=8.1, 4.7$  Hz, 1H), 5.94 (ddd,  $J=4.2, 2.5, 1.6$  Hz, 1H), 5.92-5.83 (m, 2H), 5.39-5.34 (m, 1H), 5.21-5.14 (m, 1H), 4.54-4.47 (m, 2H), 4.23-4.09 (m, 1H), 3.97-3.90 (m, 1H), 3.33 (s, 3H), 2.01-1.99 (m, 3H), 1.41-1.39 (m, 3H), 1.37 (d,  $J=7.3$  Hz, 2H), 1.22 (t,  $J=7.3$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  172.92, 172.69, 165.92, 162.30, 157.05, 141.29, 141.18, 109.57, 102.26, 99.08, 87.95, 83.52, 81.80, 77.79, 75.36, 73.53, 64.69, 61.71, 58.40, 49.50, 26.96, 16.01. HRMS (ESI):  $\text{C}_{22}\text{H}_{29}\text{N}_5\text{O}_{14}+\text{H}^+$ , Calc: 588.1784, Found: 588.1786.

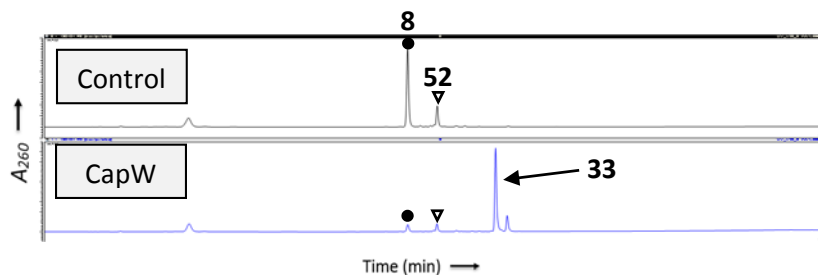
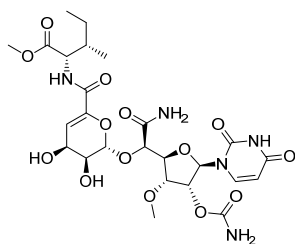


32:

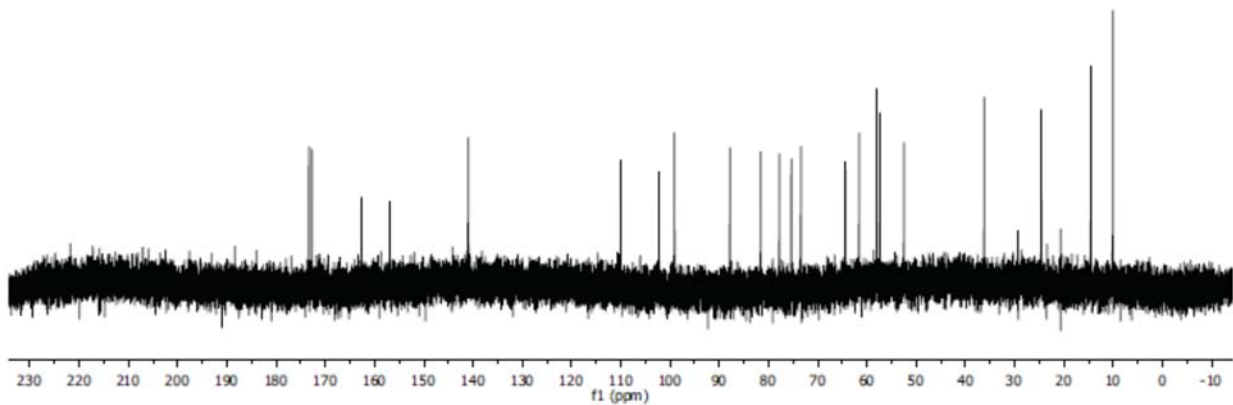
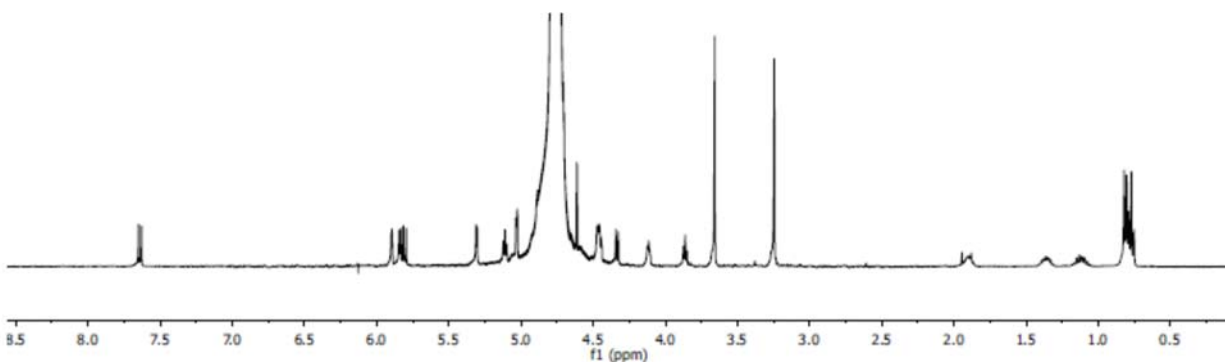


MS (ESI):  $C_{28}H_{33}N_5O_{14}+H^+$ , Calc: 664.2, Found: 663.6.

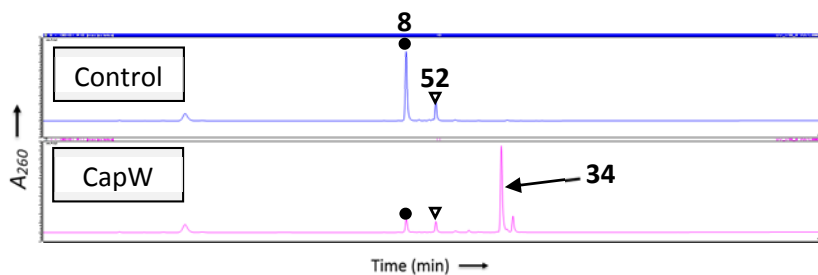
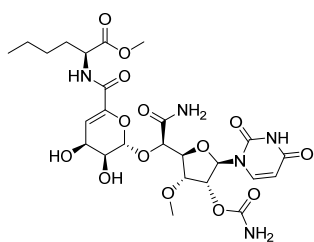
33:



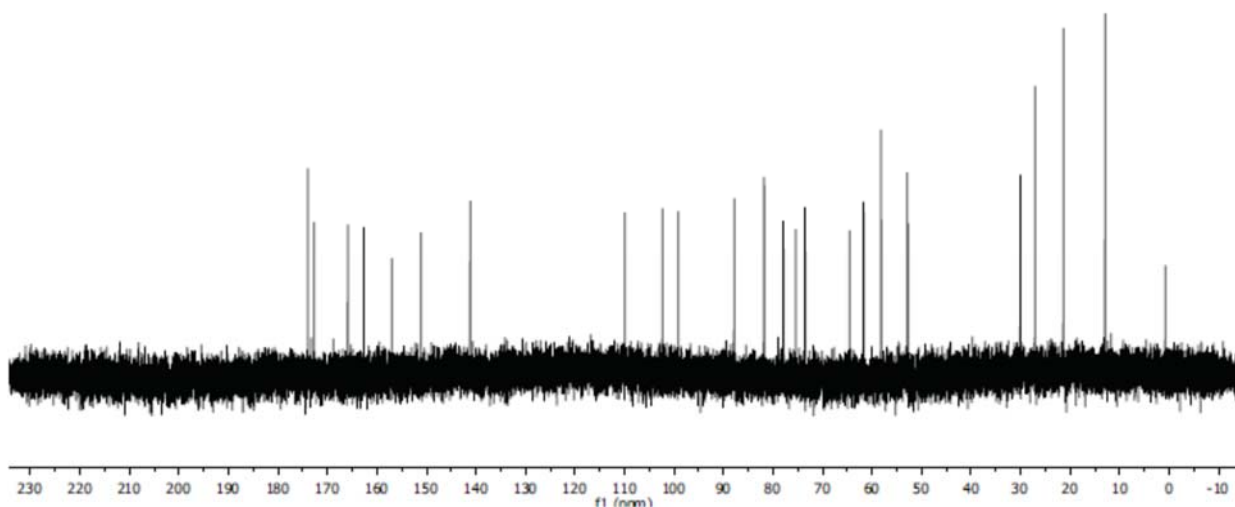
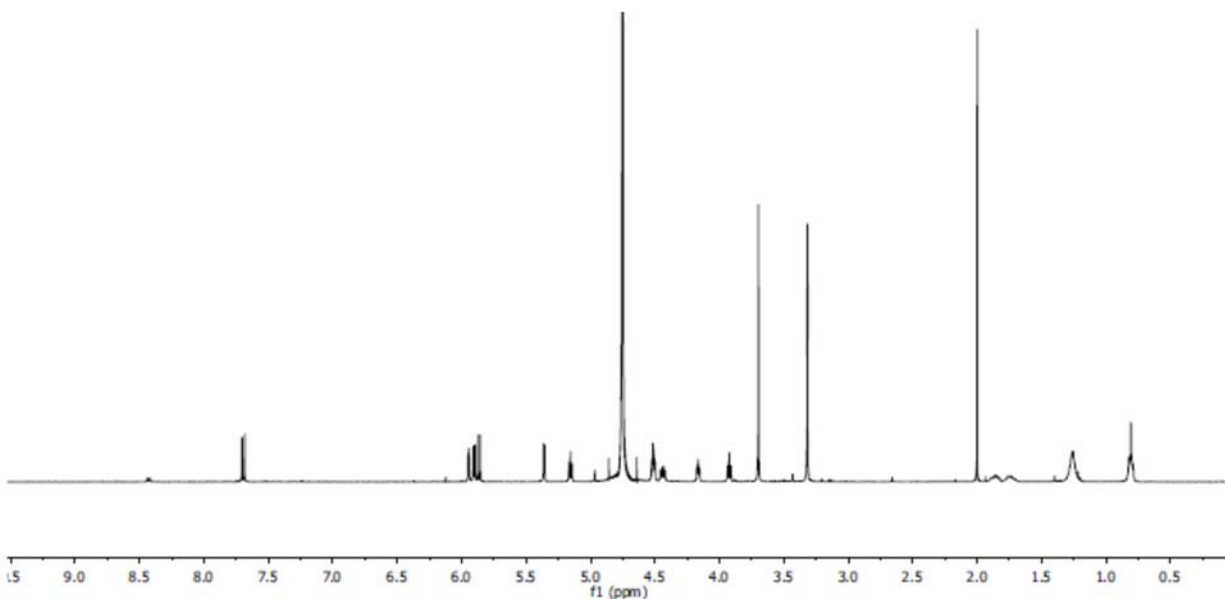
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.64 (d,  $J=8.1$  Hz, 1H), 5.92-5.88 (m, 1H), 5.84 (d,  $J=4.8$  Hz, 1H), 5.81 (d,  $J=8.1$  Hz, 1H), 5.31 (d,  $J=3.2$  Hz, 1H), 5.14-5.09 (m, 1H), 5.03 (ddd,  $J=5.5, 3.4, 2.7$  Hz, 1H), 4.49-4.43 (m, 1H), 4.33 (d,  $J=7.0$  Hz, 1H), 4.15-4.09 (m, 1H), 3.86 (t,  $J=5.2$  Hz, 1H), 3.66 (s, 3H), 3.25 (s, 3H), 1.95-1.84 (m, 1H), 1.41-1.30 (m, 1H), 1.18-1.04 (m, 1H), 0.85-0.73 (m, 5H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  173.33, 172.69, 162.64, 156.93, 140.99, 110.00, 102.23, 99.10, 87.84, 81.59, 77.80, 75.37, 73.43, 64.41, 61.61, 58.01, 57.35, 52.47, 36.19, 24.60, 14.52, 10.04. HRMS (ESI):  $\text{C}_{25}\text{H}_{35}\text{N}_5\text{O}_{14}+\text{H}^+$ , Calc: 630.2253, Found: 630.2260.



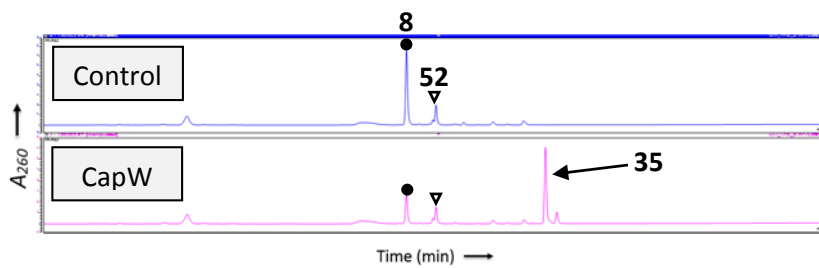
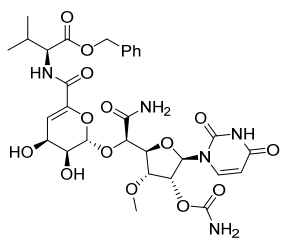
34:



$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.69 (d,  $J$  = 8.2 Hz, 1H), 5.94 (dd,  $J$  = 2.5, 1.7 Hz, 1H), 5.90 (d,  $J$  = 4.8 Hz, 1H), 5.86 (d,  $J$  = 8.1 Hz, 1H), 5.36 (d,  $J$  = 3.2 Hz, 1H), 5.16 (t,  $J$  = 5.1 Hz, 1H), 4.51 (td,  $J$  = 4.3, 2.3 Hz, 2H), 4.44 (dd,  $J$  = 9.2, 5.3 Hz, 1H), 4.16 (ddd,  $J$  = 4.7, 3.2, 1.7 Hz, 1H), 3.92 (t,  $J$  = 5.2 Hz, 1H), 3.70 (s, 3H), 3.32 (s, 3H), 2.00 (d,  $J$  = 2.1 Hz, 3H), 1.92-1.81 (m, 1H), 1.76-1.67 (m, 1H), 1.24 (d,  $J$  = 16.6 Hz, 4H), 0.81 (t,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  173.96, 172.70, 165.91, 162.68, 157.00, 151.11, 141.18, 109.92, 102.32, 99.12, 87.83, 81.79, 77.92, 75.42, 73.53, 64.53, 61.71, 58.17, 52.89, 52.70, 30.04, 27.08, 21.35, 12.93. HRMS (ESI):  $\text{C}_{25}\text{H}_{35}\text{N}_5\text{O}_{14} + \text{H}^+$ , Calc: 630.2253, Found: 630.2260.



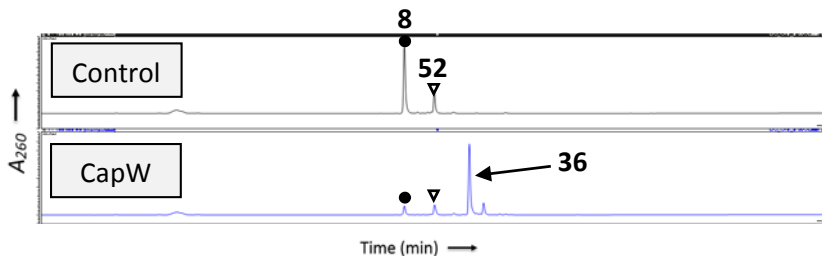
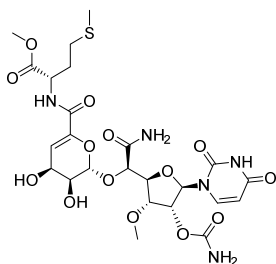
35:



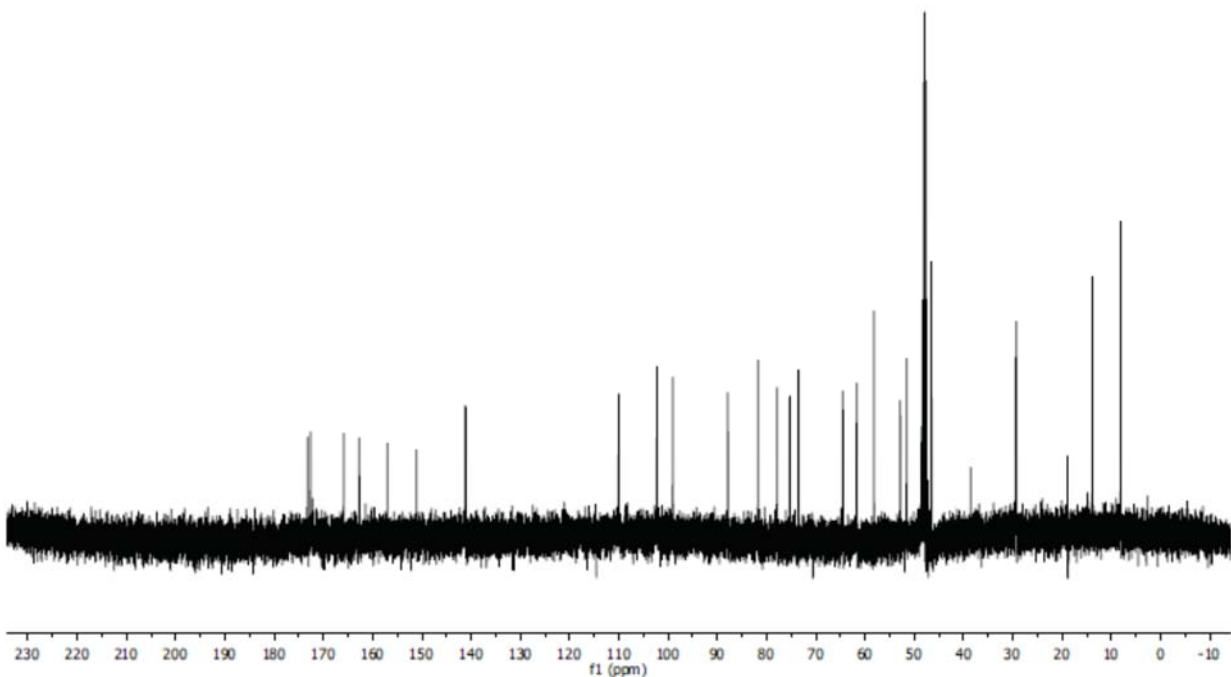
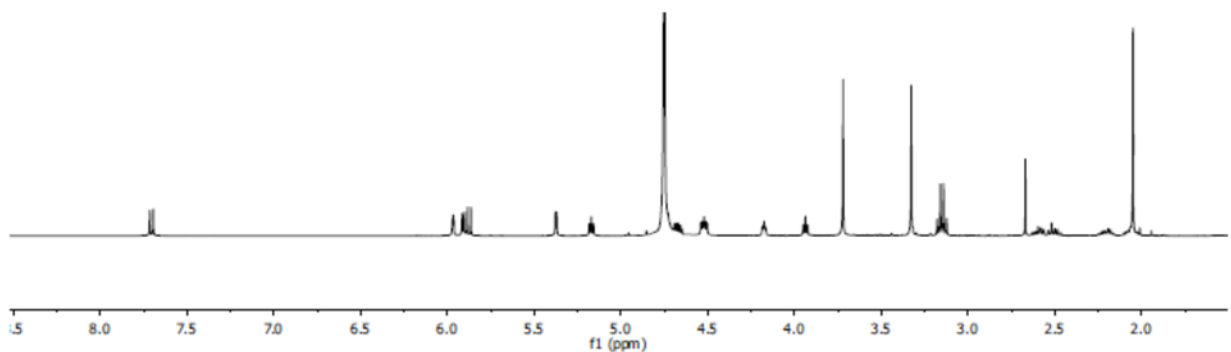
MS (ESI): C<sub>30</sub>H<sub>37</sub>N<sub>5</sub>O<sub>14</sub>+H<sup>+</sup>, Calc: 692.2, Found: 691.6.



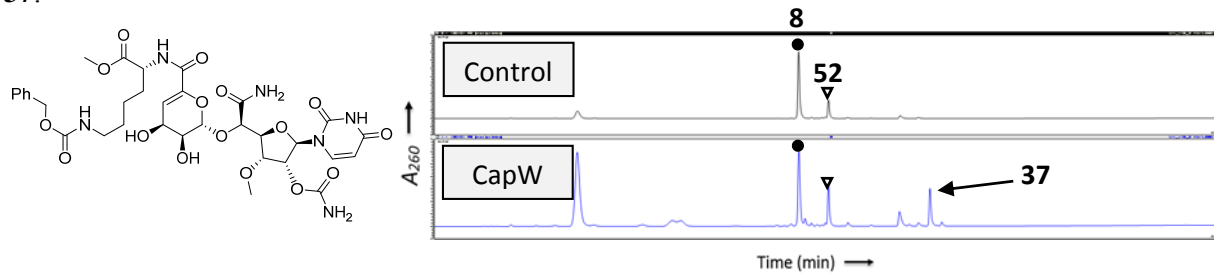
36:



$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.70 (d,  $J=8.1$  Hz, 1H), 5.97 (dd,  $J=2.4, 1.7$  Hz, 1H), 5.91 (d,  $J=4.7$  Hz, 1H), 5.87 (d,  $J=8.1$  Hz, 1H), 5.37 (d,  $J=3.2$  Hz, 1H), 5.17 (t,  $J=5.0$  Hz, 1H), 4.70-4.64 (m, 1H), 4.55-4.49 (m, 2H), 4.18 (ddd,  $J=4.6, 3.2, 1.7$  Hz, 1H), 3.94 (t,  $J=5.2$  Hz, 1H), 3.72 (s, 3H), 3.33 (s, 3H), 2.64-2.45 (m, 2H), 2.25-2.16 (m, 1H), 2.11-2.02 (m, 3H), 2.05 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$  with methanol as standard)  $\delta$  173.19, 172.65, 165.90, 162.71, 157.00, 151.10, 141.19, 141.07, 110.04, 102.28, 99.06, 87.88, 81.77, 77.87, 75.29, 73.52, 64.50, 61.70, 58.19, 52.86, 51.56, 46.53, 29.40, 13.84. HRMS (ESI):  $\text{C}_{24}\text{H}_{33}\text{N}_5\text{O}_{14}\text{S}+\text{H}^+$ , Calc: 648.1817, Found: 648.1829.

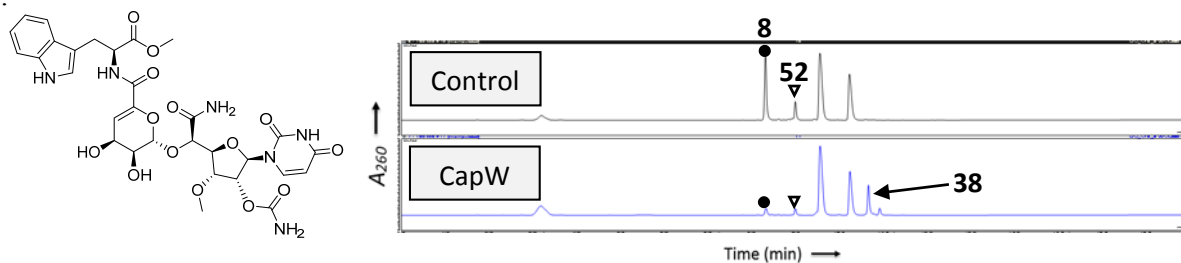


37:



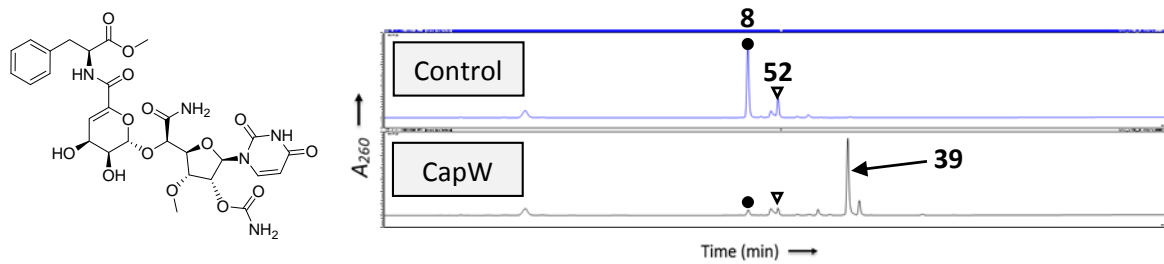
MS (ESI):  $C_{33}H_{42}N_6O_{16}+H^+$ , Calc: 778.3, Found: 778.6.

38:



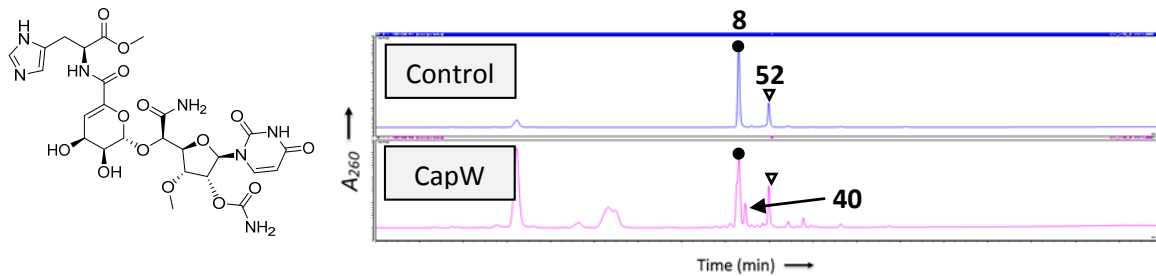
MS (ESI):  $C_{30}H_{34}N_6O_{14}+H^+$ , Calc: 703.2, Found: 702.6.

39:



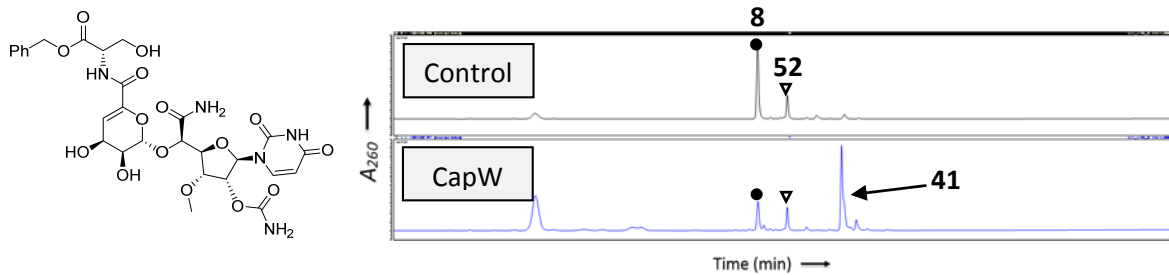
MS (ESI):  $C_{28}H_{33}N_5O_{14}+H^+$ , Calc: 664.2, Found: 663.6.

40:



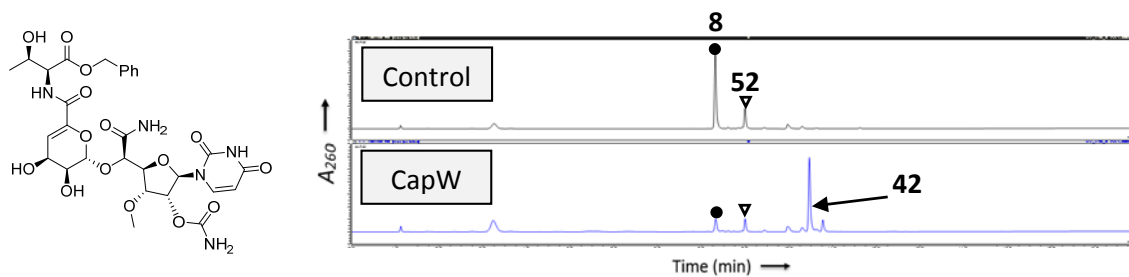
MS (ESI):  $C_{28}H_{33}N_5O_{14}+H^+$ , Calc: 654.2, Found: 653.7.

41:



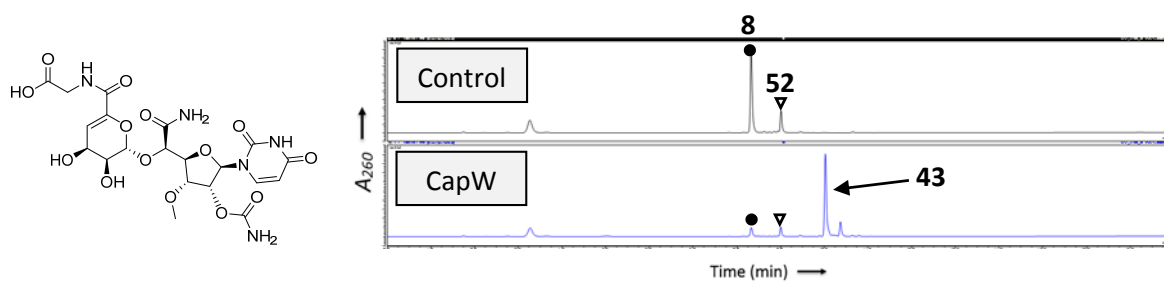
MS (ESI):  $C_{28}H_{33}N_5O_{15}+H^+$ , Calc: 680.2, Found: 679.6.

42:



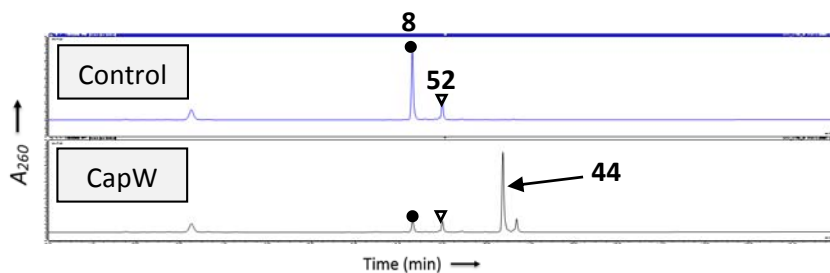
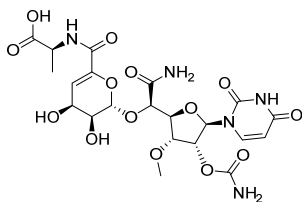
MS (ESI):  $C_{29}H_{35}N_5O_{15}+H^+$ , Calc: 694.2, Found: 693.6.

43:

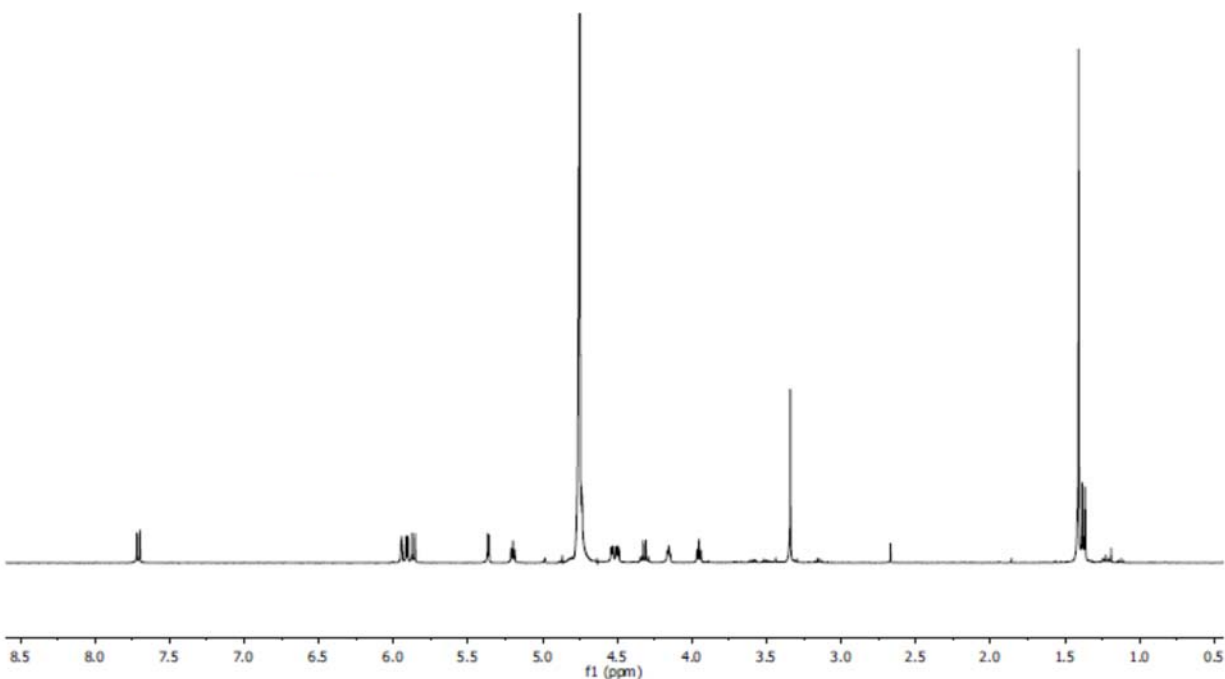


MS (ESI):  $C_{20}H_{25}N_5O_{14}+H^+$ , Calc: 560.1, Found: 559.6.

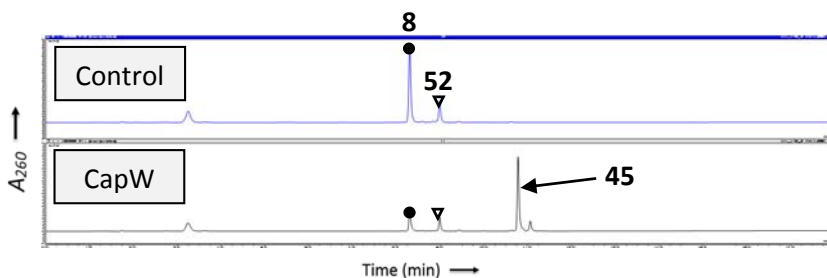
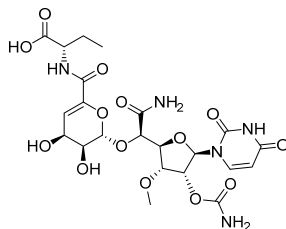
44:



$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.71 (d,  $J=8.1$  Hz, 1H), 5.94 (dd,  $J=2.6, 1.6$  Hz, 1H), 5.91 (d,  $J=4.6$  Hz, 1H), 5.86 (d,  $J=8.1$  Hz, 1H), 5.36 (d,  $J=3.3$  Hz, 1H), 5.21-5.18 (m, 1H), 4.74 (dd,  $J=1.9, 1.2$  Hz, 1H), 4.53 (dd,  $J=4.4, 2.7$  Hz, 1H), 4.50 (dd,  $J=5.3, 2.2$  Hz, 1H), 4.32 (q,  $J=7.2$  Hz, 1H), 4.17-4.14 (m, 1H), 3.95 (t,  $J=5.3$  Hz, 1H), 3.34 (s, 3H), 1.37 (d,  $J=7.3$  Hz, 3H). HRMS (ESI):  $\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_{14}+\text{H}^+$ , Calc: 574.1627, Found: 574.1632.

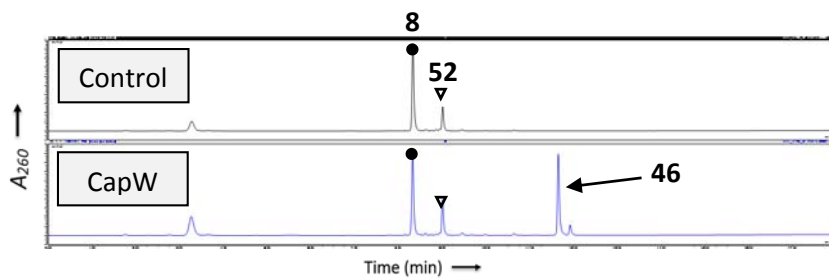
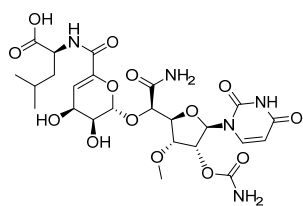


45:



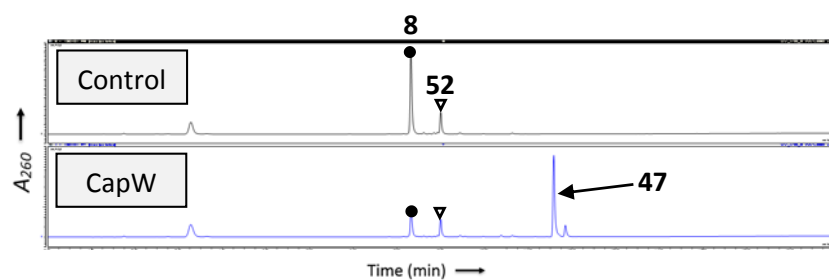
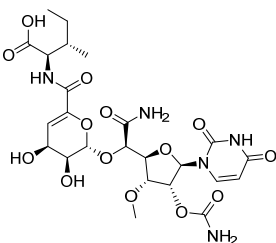
MS (ESI):  $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_{14}+\text{H}^+$ , Calc: 588.2, Found: 587.6.

46:



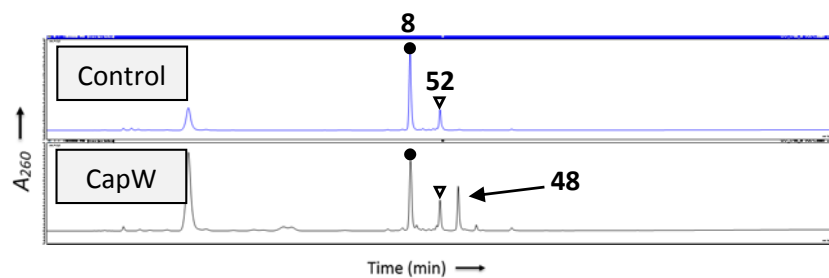
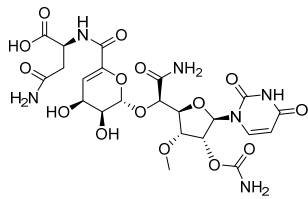
MS (ESI):  $C_{24}H_{33}N_5O_{14}+H^+$ , Calc: 616.2, Found: 615.7.

47:



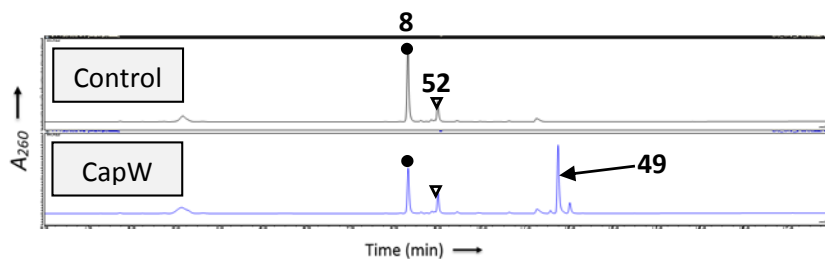
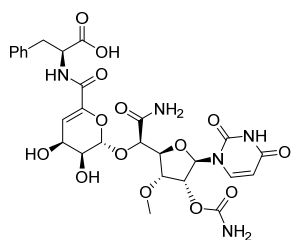
MS (ESI):  $C_{24}H_{33}N_5O_{14}+H^+$ , Calc: 616.2, Found: 615.6.

48:

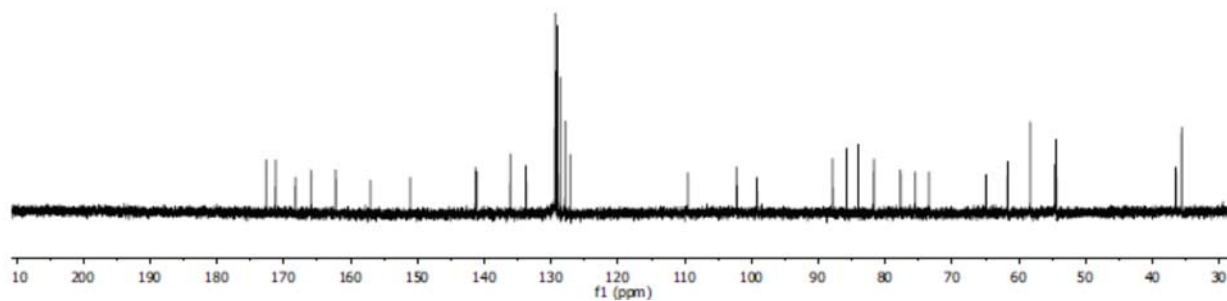
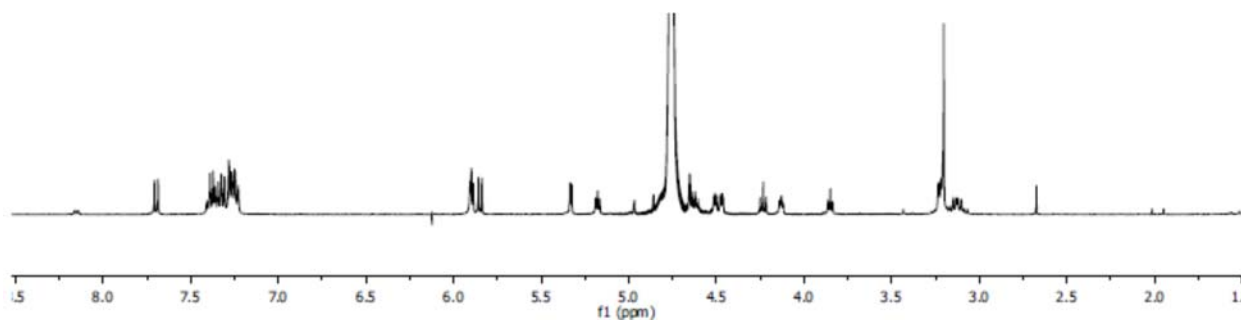


MS (ESI):  $C_{22}H_{28}N_6O_{15}+H^+$ , Calc: 617.2, Found: 616.6.

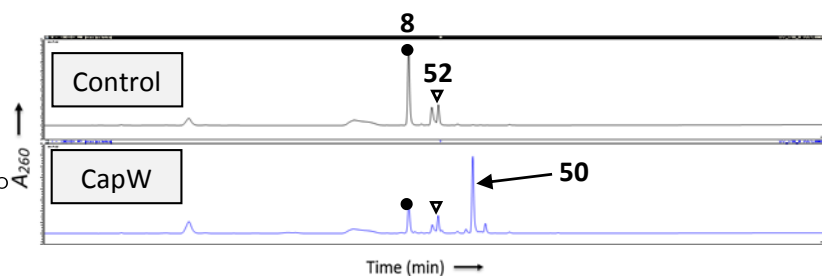
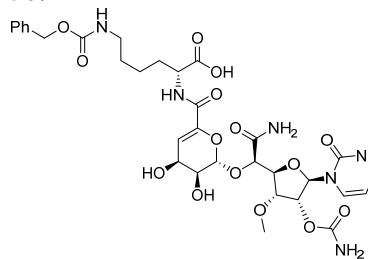
49:



$^1\text{H}$  NMR (400 MHz,  $d$   $\text{D}_2\text{O}$ )  $\delta$  7.70 (d,  $J=8.2$  Hz, 1H), 7.39-7.24 (m, 5H), 5.90 (dd,  $J=5.3, 2.9$  Hz, 2H), 5.85 (d,  $J=8.1$  Hz, 1H), 5.33 (d,  $J=3.7$  Hz, 1H), 5.20 – 5.16 (m, 1H), 4.64-4.60 (m, 1H), 4.51 (dd,  $J=4.3, 2.8$  Hz, 1H), 4.47 (dd,  $J=5.3, 2.2$  Hz, 1H), 4.23 (t,  $J=6.8$  Hz, 1H), 4.14-4.11 (m, 1H), 3.85 (t,  $J=5.3$  Hz, 1H), 3.23 (d,  $J=2.6$  Hz, 1H), 3.20 (s, 3H), 3.13 (dd,  $J=11.1, 7.2$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  172.65, 171.23, 168.25, 165.90, 162.23, 157.02, 151.05, 141.31, 141.12, 133.75, 127.89, 127.12, 109.54, 102.24, 99.21, 85.79, 84.05, 81.71, 77.74, 75.52, 73.47, 64.93, 61.69, 54.63, 36.52. HRMS (ESI):  $\text{C}_{27}\text{H}_{31}\text{N}_5\text{O}_{14}+\text{H}^+$ , Calc: 650.1940, Found: 650.1948.

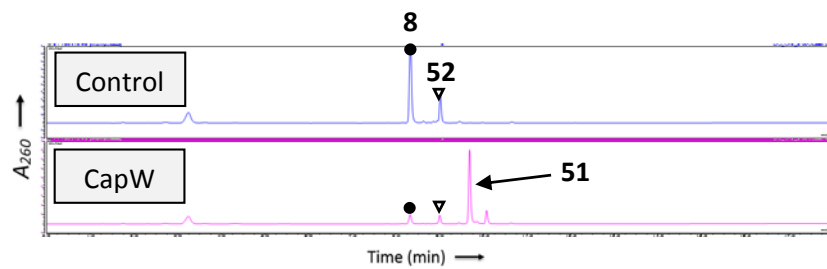
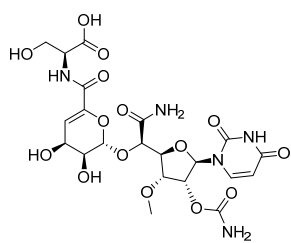


50:



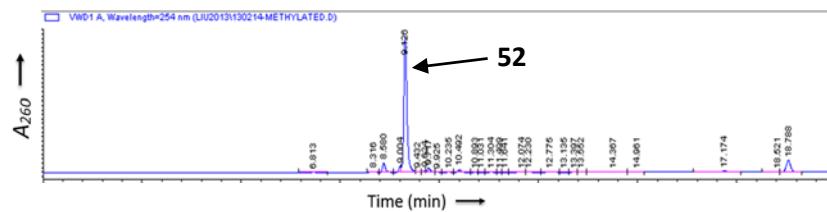
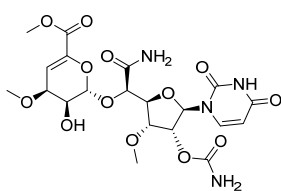
MS (ESI):  $\text{C}_{32}\text{H}_{40}\text{N}_6\text{O}_{16}+\text{H}^+$ , Calc: 765.3, Found: 764.8.

51:

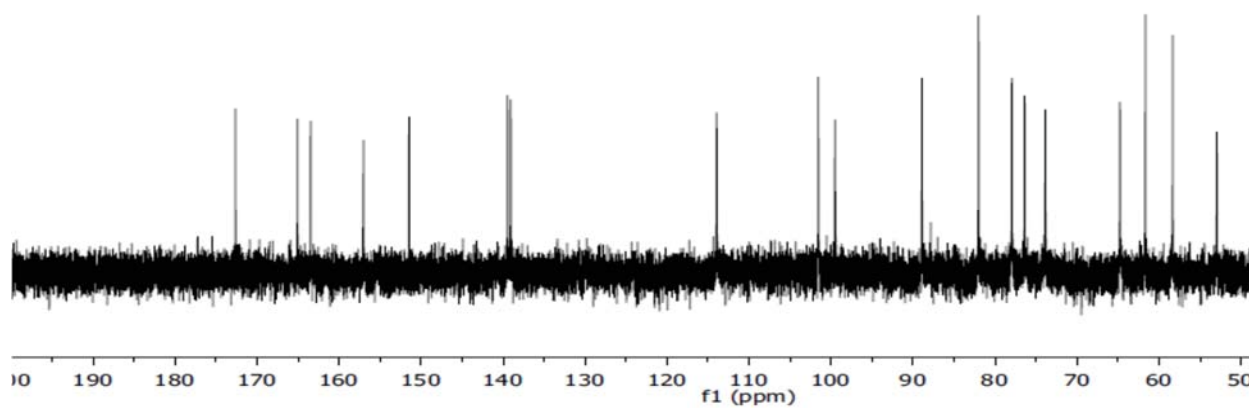
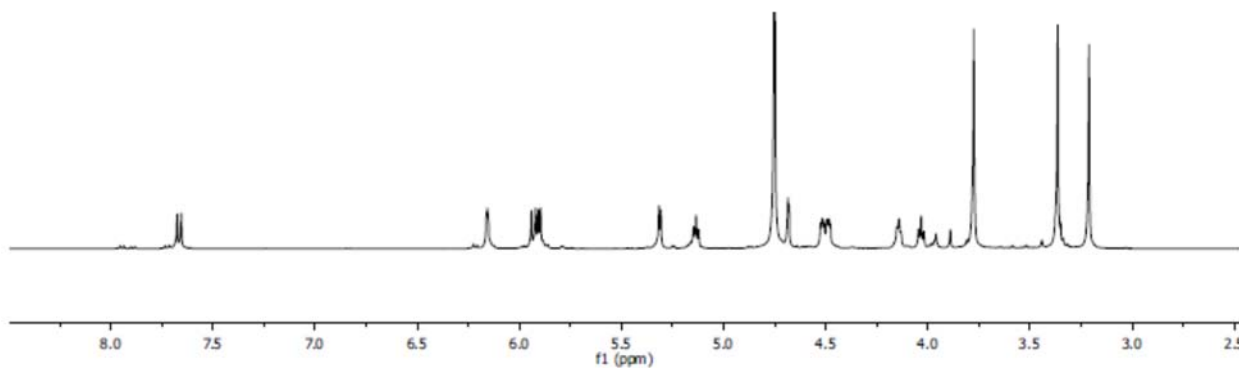


MS (ESI):  $C_{21}H_{27}N_5O_{15}+H^+$ , Calc: 590.2, Found: 589.6.

52:

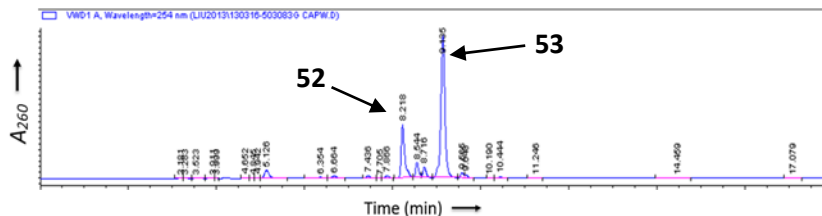
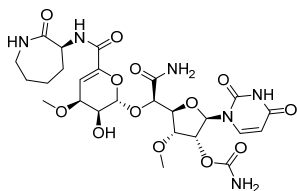


MS (ESI):  $C_{21}H_{27}N_5O_{15}+H^+$ , Calc: 531.1, Found: 530.7.

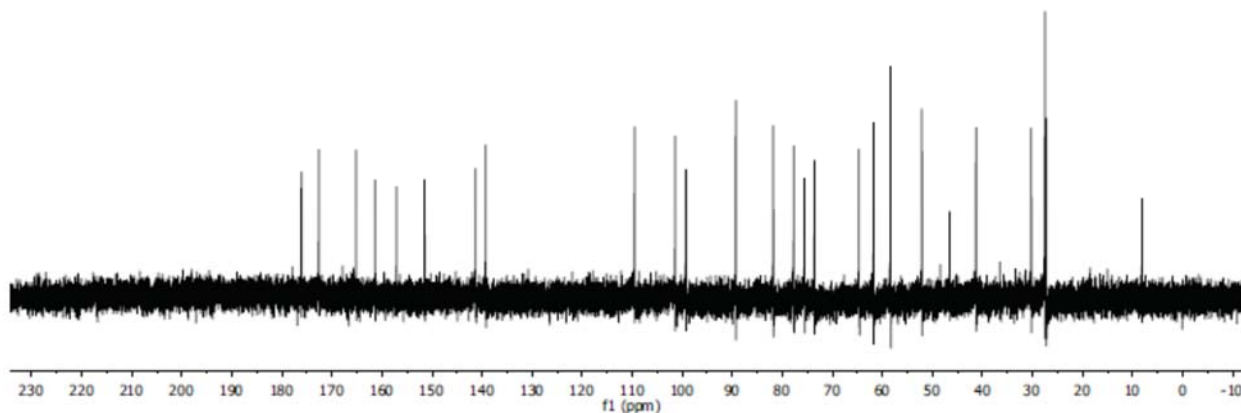
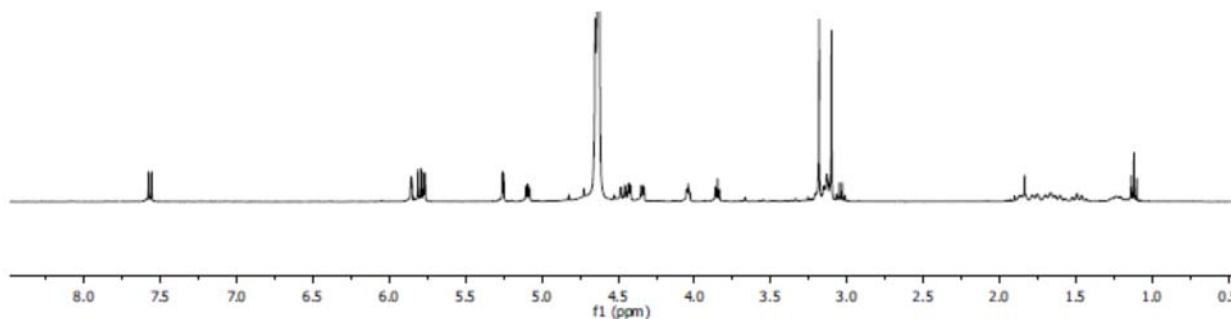




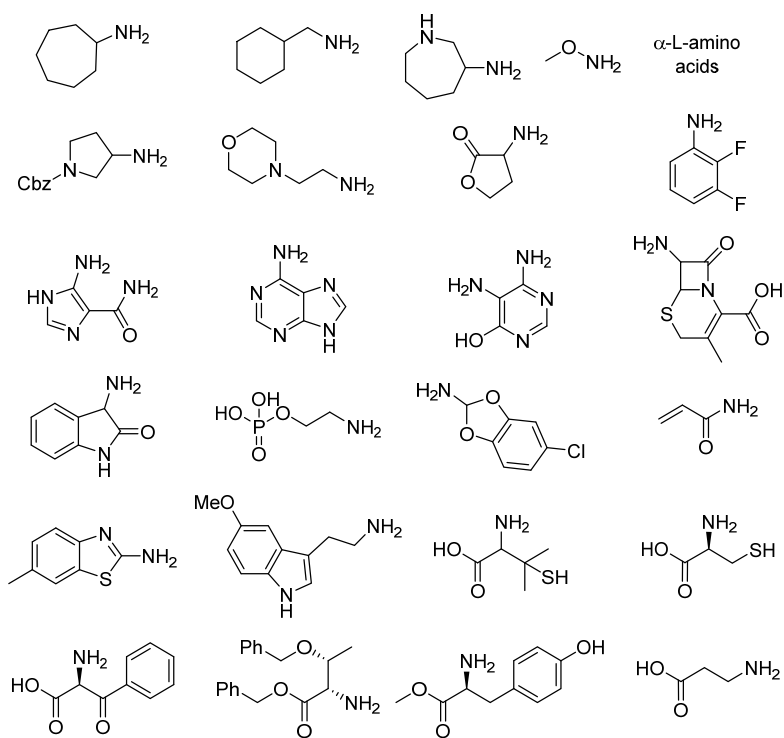
53:



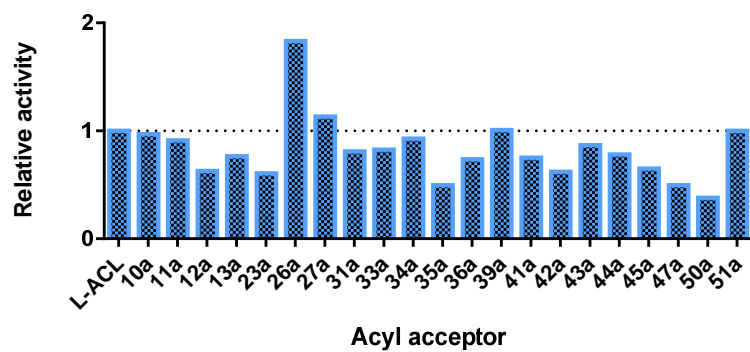
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.69 (d,  $J=8.1$  Hz, 1H), 5.98 (dd,  $J=2.5, 1.6$  Hz, 1H), 5.93 (d,  $J=8.1$  Hz, 1H), 5.89 (d,  $J=4.1$  Hz, 1H), 5.38 (d,  $J=3.3$  Hz, 1H), 5.24-5.19 (m, 1H), 4.62-4.57 (m, 1H), 4.55 (dd,  $J=4.4, 2.6$  Hz, 1H), 4.46 (dd,  $J=5.8, 2.5$  Hz, 1H), 4.19-4.14 (m, 1H), 3.97 (t,  $J=5.6$  Hz, 1H), 3.31 (s, 3H), 3.29-3.24 (m, 2H), 3.23 (s, 3H), 2.02-1.93 (m, 1H), 1.89 (d,  $J=12.3$  Hz, 1H), 1.84-1.67 (m, 2H), 1.65-1.57 (m, 1H), 1.40-1.30 (m, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  176.12, 172.64, 165.17, 161.37, 157.08, 151.49, 141.32, 139.28, 109.53, 101.42, 99.26, 89.29, 81.80, 77.71, 75.58, 73.58, 64.69, 61.76, 58.36, 52.10, 46.56, 41.28, 30.25, 27.53, 27.28. HRMS (ESI):  $\text{C}_{25}\text{H}_{34}\text{N}_6\text{O}_{13}+\text{H}^+$ , Calc: 627.2257, Found: 627.2264.



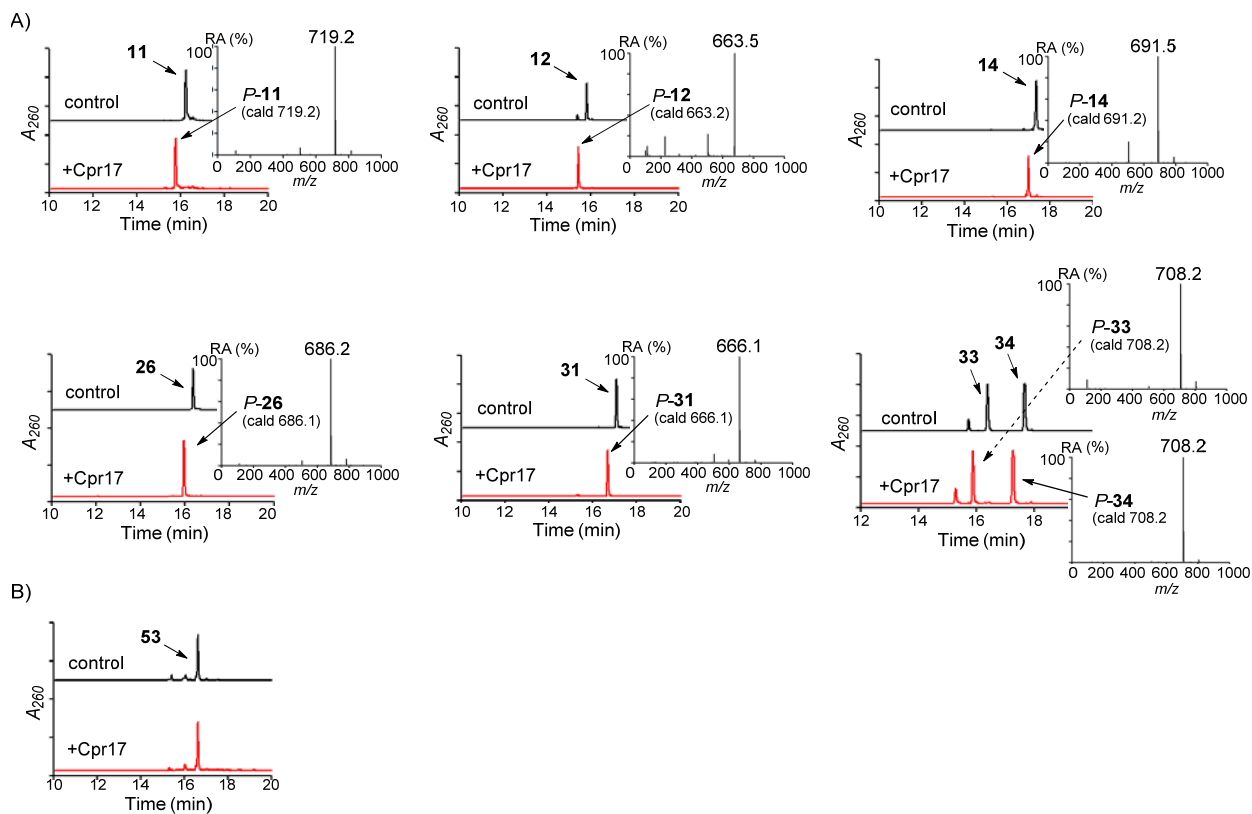
## 2. Supporting Figures



**Fig. S1** Tested acyl acceptors not incorporated by CapW. All compounds were provided as the HCl salt. A racemic mixture was used for compounds without any indicated stereochemistry.



**Fig. S2** Relative specific activity of CapW with different acyl acceptors.



**Fig. S3** Enzymatic phosphorylation of analogues by Cpr17. A) HPLC traces for representative analogues following the reaction with Cpr17 (+Cpr17) or control (denatured Cpr17). The inset shows the mass spectrum in negative ionisation mode for the monophosphorylated product. B) HPLC traces demonstrating Cpr17 cannot phosphorylate *N*-3-methyl-3.