Electronic Supplementary Information

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

Xiaodong Liu,[a] Yuanyuan Jin,[b] Wenlong Cai,[a] Keith D. Green,[a] Anwesha Goswami,[a] Sylvie Garneau-Tsodikova,[a] Koichi Nonaka,[c] Satoshi Baba,[c] Masanori Funabashi,[d] Zhaoyong Yang,[b] Steven G. Van Lanen[a]*

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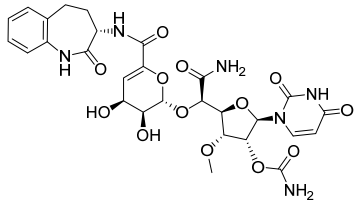
[a] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536 (USA). Email, svanlanen@uky.edu.*
[b] *Key Laboratory of Biotechnology of Antibiotics, Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing (China).*
[c] *Biologics Technology Research Laboratories, R&D Division, Daiichi Sankyo Co., Ltd., Gunma 370-0503 (Japan).*
[d] *Natural Products Research Group, Discovery Science and Technology Group, Daiichi Sankyo RD Novare Co., Ltd., Tokyo 370-0503 (Japan).*

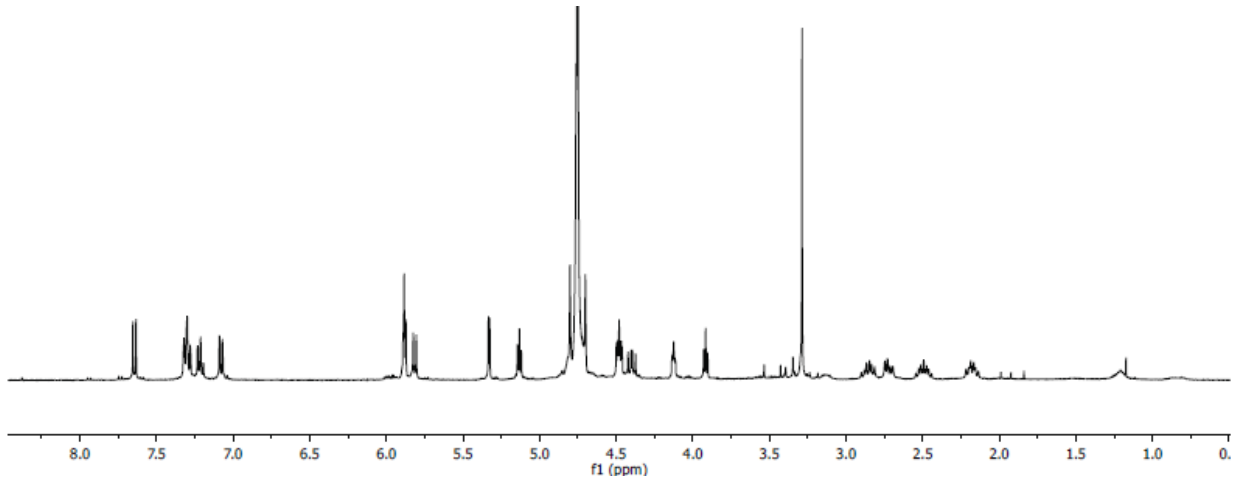
1. Supporting Results

HPLC chromatograms and spectral assignments for compounds 10-53.

10:



^{1}H NMR (400 MHz, D_2O) δ 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): $C_{28}H_{32}N_6O_{13}$+H^+, Calc: 661.2100, Found: 661.2105.



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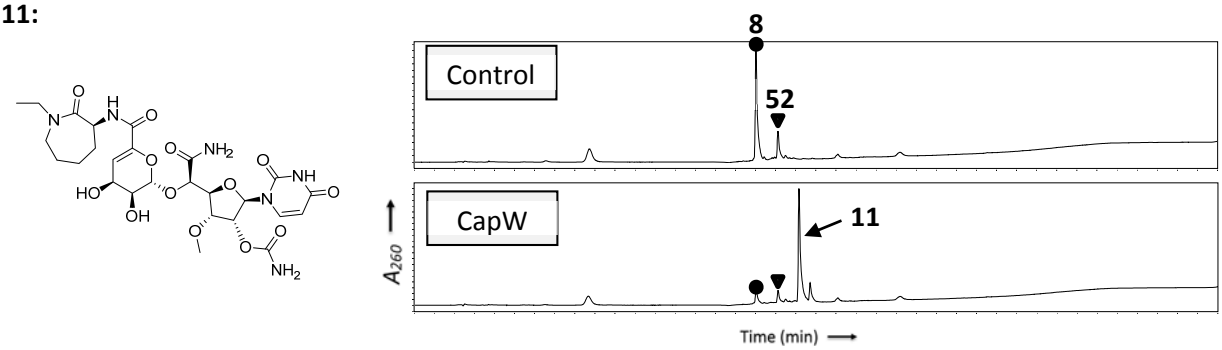
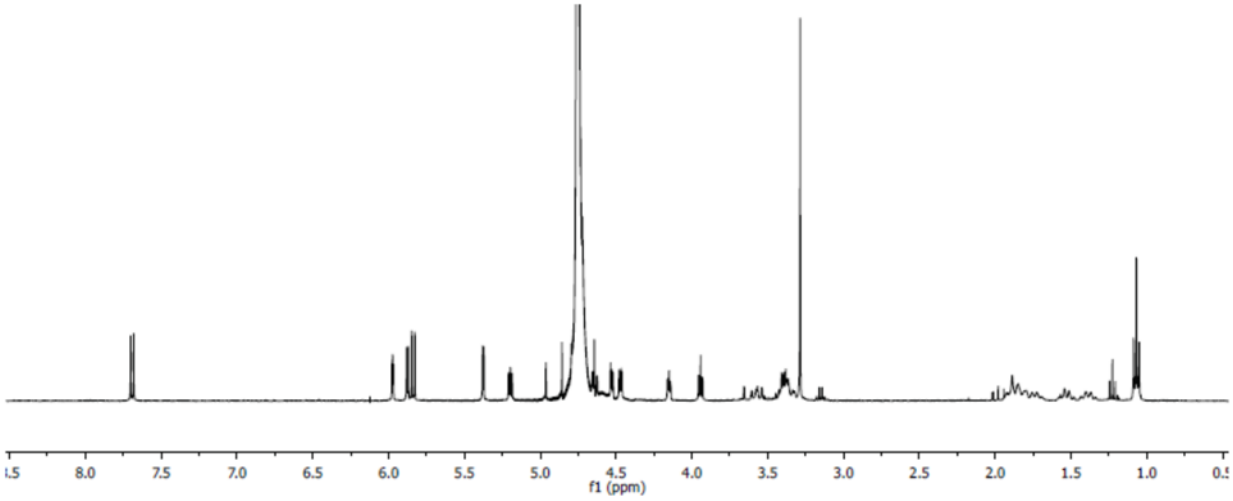
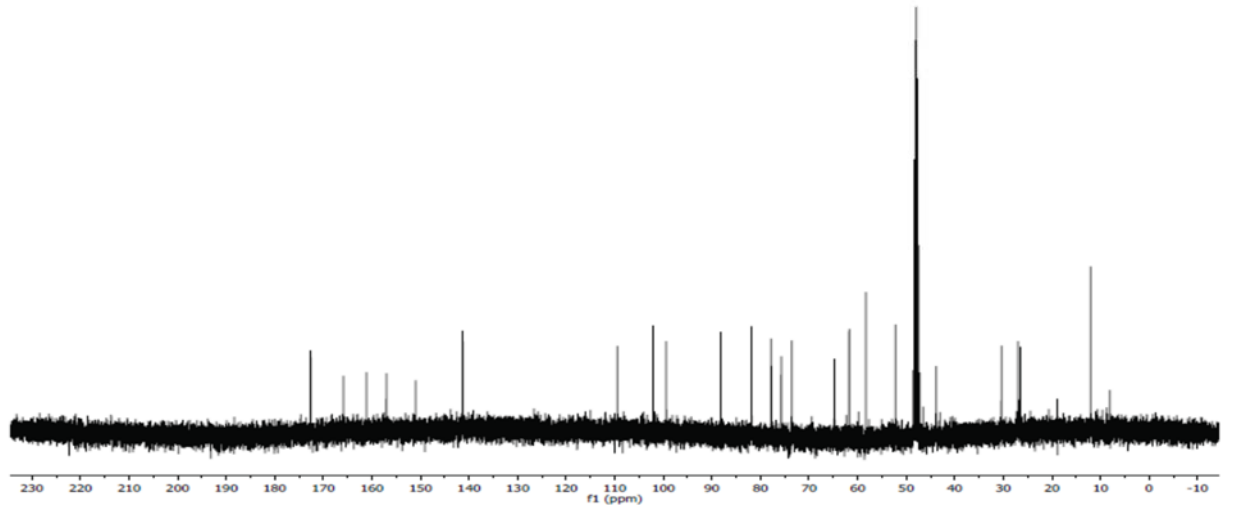


^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, *J*=8.1 Hz, 1H), 5.97 (dd, *J*=2.7, 1.5 Hz, 1H), 5.88 (d, *J*=4.3 Hz, 1H), 5.84 (d, *J*=8.1 Hz, 1H), 5.38 (d, *J*=3.5 Hz, 1H), 5.22-5.17 (m, 1H), 4.53 (dd, *J*=4.6, 2.7 Hz, 1H), 4.47 (dd, *J*=5.6, 2.4 Hz, 1H), 4.17-4.12 (m, 1H), 3.94 (t, *J*=5.5 Hz, 1H), 3.62-3.53 (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}C NMR (100 MHz, D_2O with methanol as standard) δ 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): $C_{26}H_{36}N_6O_{13}+H^+$, Calc: 641.2413, Found: 641.2415.





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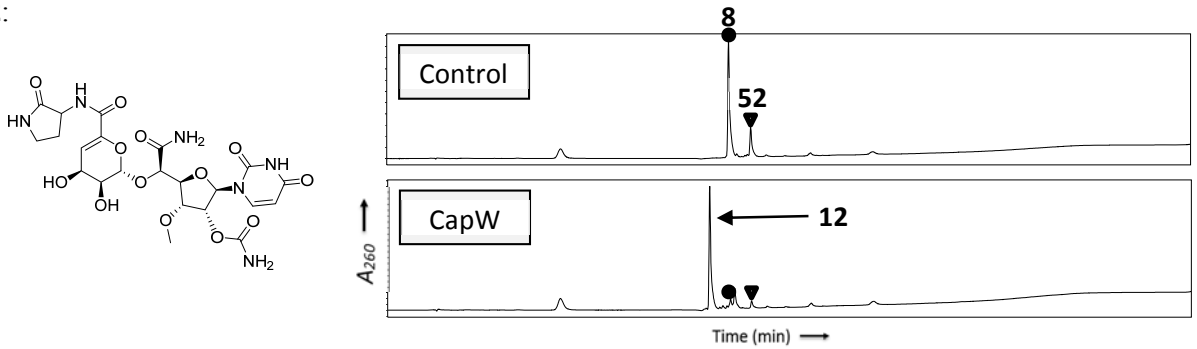
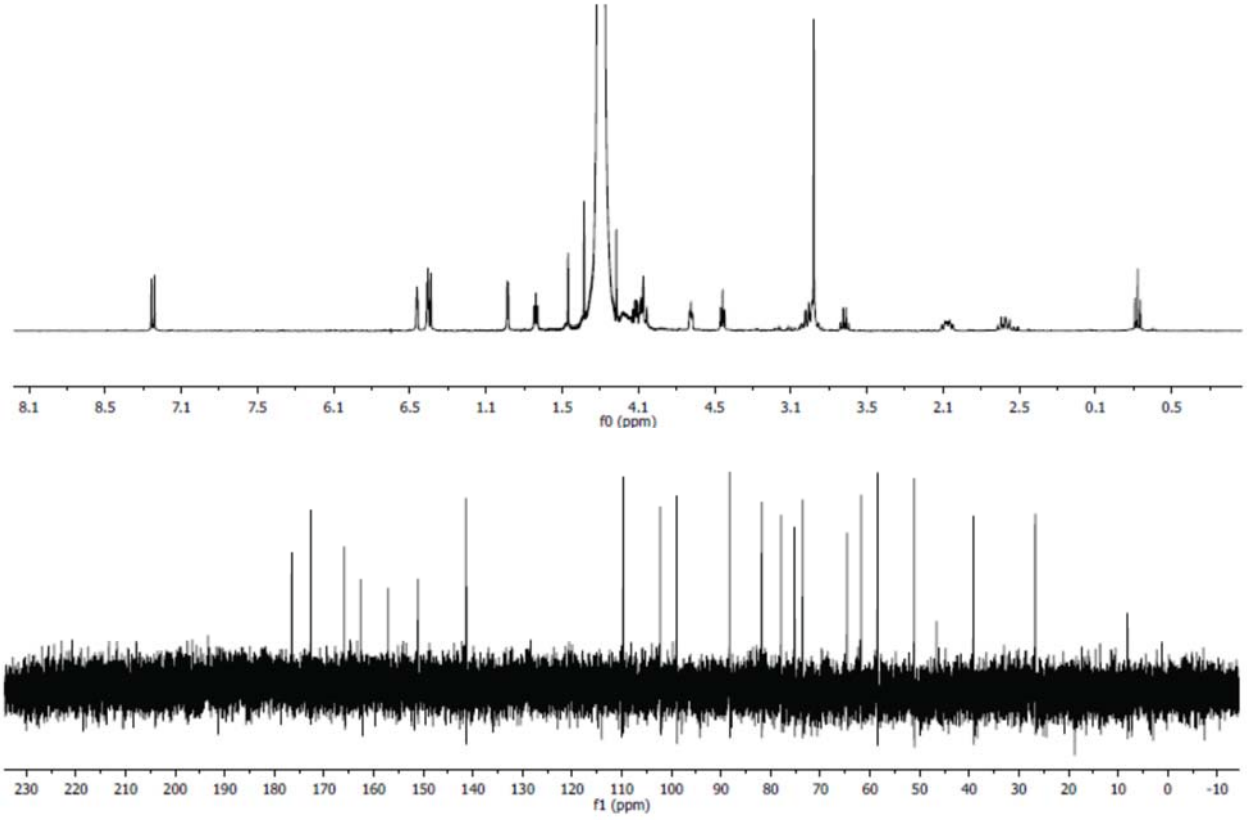


^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, *J*=8.1 Hz, 1H), 5.95 (dd, *J*=2.5, 1.6 Hz, 1H), 5.88 (dd, *J*=6.3, 4.5 Hz, 1H), 5.36 (d, *J*=3.2 Hz, 1H), 5.20-5.14 (m, 1H), 4.73-4.71 (m, 1H), 4.54-4.43 (m, 3H), 4.17-4.13 (m, 1H), 3.97-3.92 (m, 1H), 3.44-3.36 (m, 1H), 3.35 (s, 3H), 3.15 (dd, *J*=14.6, 7.3 Hz, 1H), 2.53-2.42 (m, 1H), 2.15-2.04 (m, 1H), 1.22 (t, *J*=7.3 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 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): $C_{22}H_{28}N_6O_{13}+H^+$, Calc: 585.1787, Found: 585.1788.



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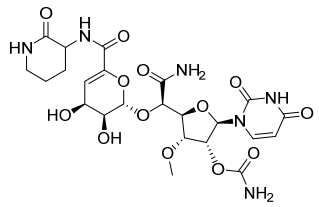
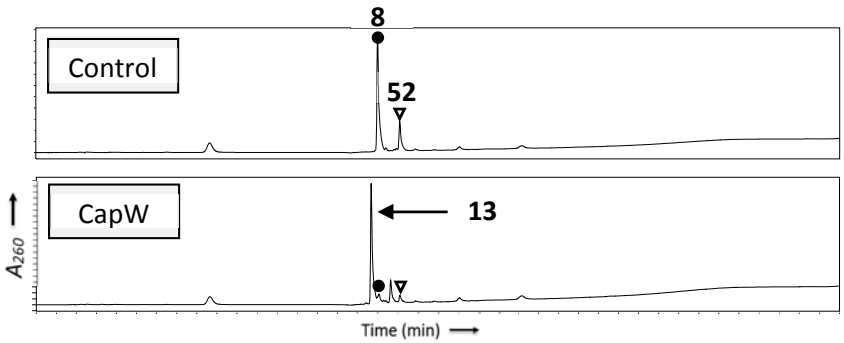
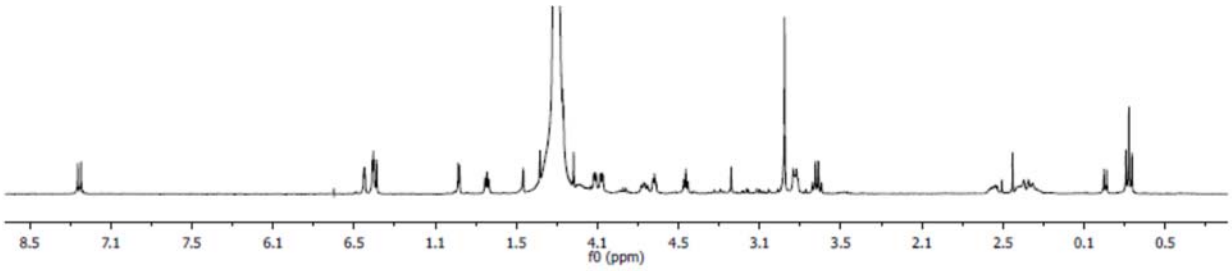
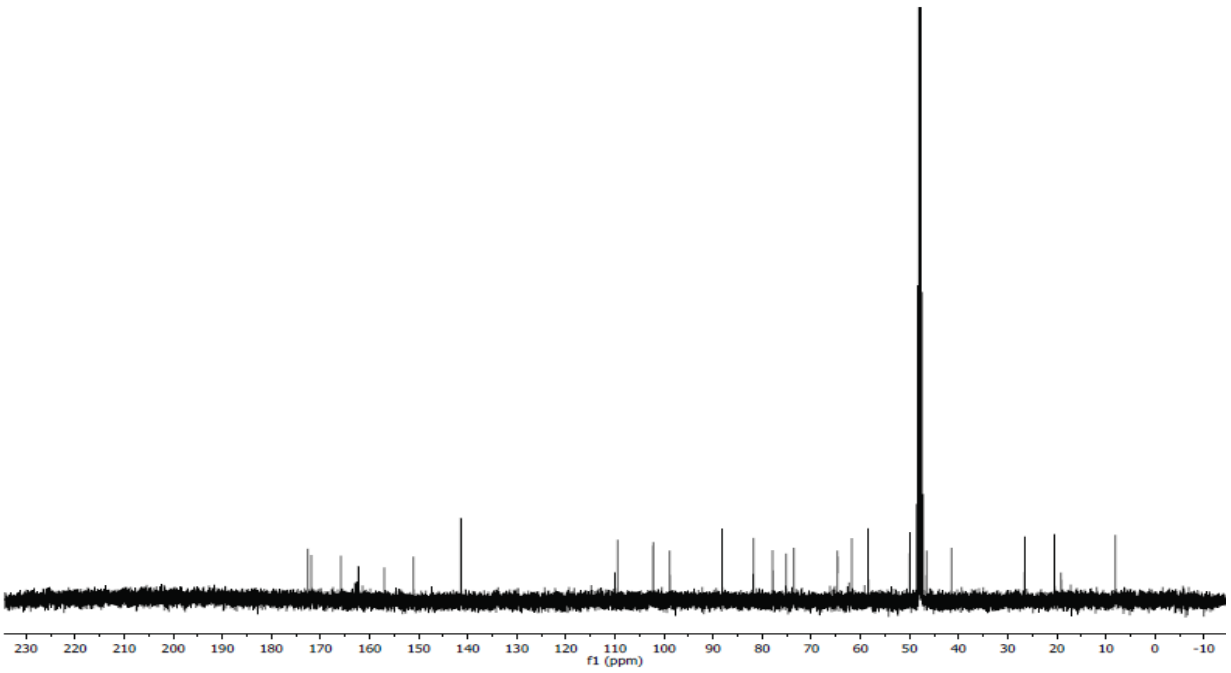




^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, *J*=8.1 Hz, 1H), 5.94 (dd, *J*=2.5, 1.6 Hz, 1H), 5.88 (dd, *J*=6.2, 4.3 Hz, 2H), 5.35 (d, *J*=3.3 Hz, 1H), 5.20-5.16 (m, 1H), 4.51 (dd, *J*= 4.5, 2.6 Hz, 1H), 4.47 (dd, *J*=5.2, 2.3 Hz, 1H), 4.21 (dd, *J*=10.1, 6.3 Hz, 1H), 4.17-4.12 (m, 1H), 3.96 (t, *J*=5.4 Hz, 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}C NMR (100 MHz, D_2O with methanol as standard) δ 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): $C_{23}H_{30}N_6O_{13}+H^+$, Calc: 599.1944, Found: 599.1942.





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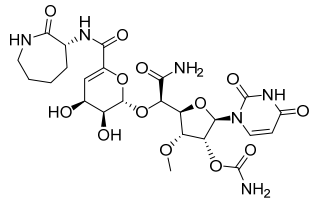
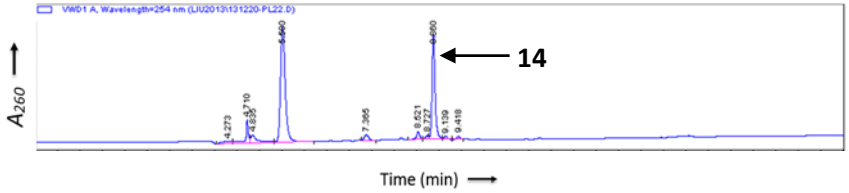
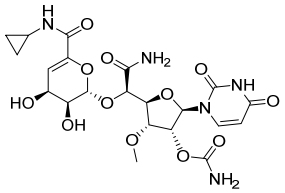
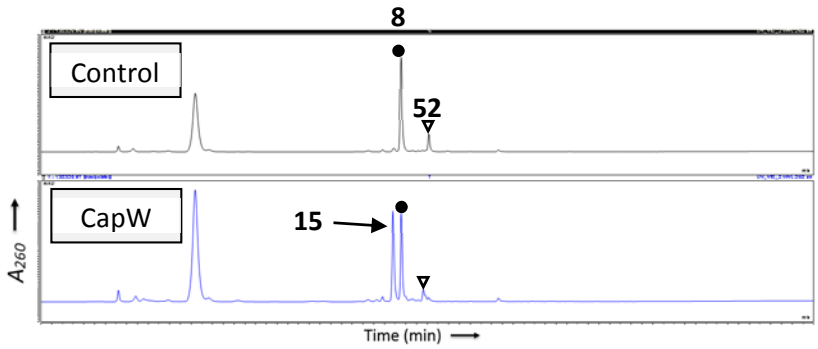




^{1}H NMR (400 MHz, D_2O) δ 7.72 (d, *J*=8.2 Hz, 1H), 6.02-5.98 (m, 1H), 5.93-5.86 (m, 2H), 5.39 (d, *J*=3.2 Hz, 1H), 5.22 (t, *J*=4.7 Hz, 1H), 4.79-4.78 (m, 1H), 4.62 (d, *J*=9.8 Hz, 1H), 4.58-4.54 (m, 1H), 4.49 (dd, *J*=5.3, 2.2 Hz, 1H), 4.18 (s, 1H), 3.98 (t, *J*=5.3 Hz, 1H), 3.33 (s, 3H), 3.29 (d, *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): $C_{24}H_{32}N_6O_{13}+H^+$, Calc: 613.2100, Found: 613.2101.

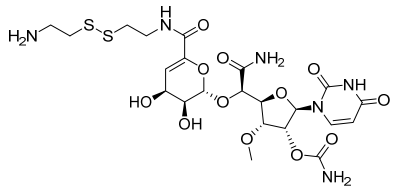
15:

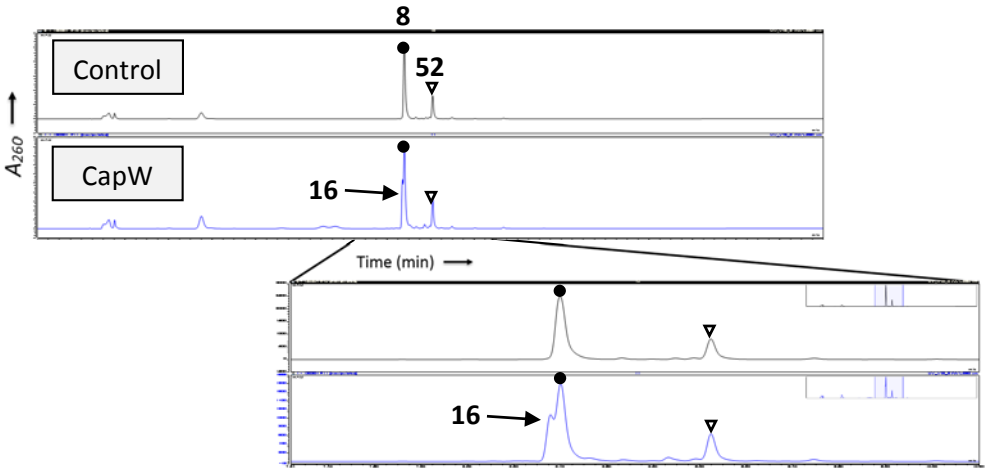




^{1}H NMR (400 MHz, D_2O) δ 7.72 (d, *J*=8.2 Hz, 1H), 6.02-5.98 (m, 1H), 5.93-5.86 (m, 2H), 5.39 (d, *J*=3.2 Hz, 1H), 5.22 (t, *J*=4.7 Hz, 1H), 4.79-4.78 (m, 1H), 4.62 (d, *J*=9.8 Hz, 1H), 4.58-4.54 (m, 1H), 4.49 (dd, *J*=5.3, 2.2 Hz, 1H), 4.18 (s, 1H), 3.98 (t, *J*=5.3 Hz, 1H), 3.33 (s, 3H), 3.29 (d, *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): $C_{24}H_{32}N_6O_{13}+H^+$, Calc: 613.2100, Found: 613.2101.

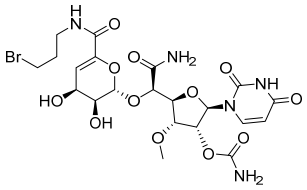
16:

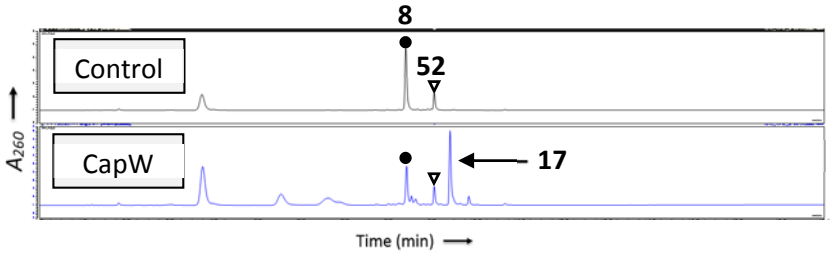




MS (ESI): $C_{22}H_{32}N_6O_{12}S_2+H^+$, Calc: 637.2, Found: 636.6.

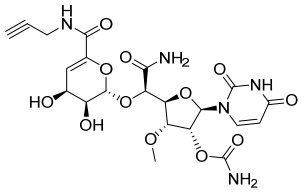
17:

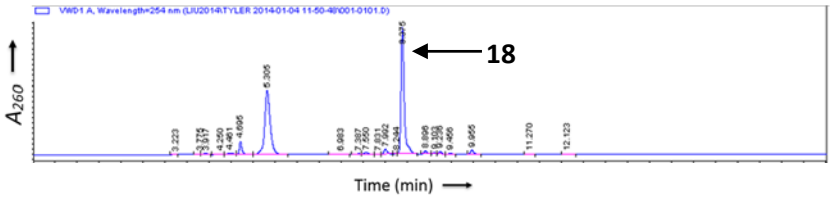




MS (ESI): $C_{21}H_{28}BrN_5O_{12}+H^+$, Calc: 622.1, Found: 622.5.

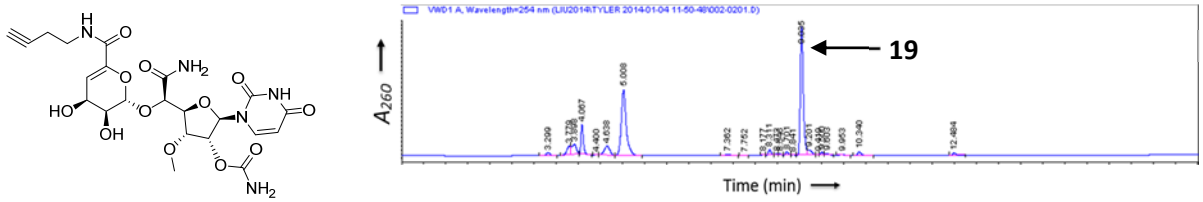
18:





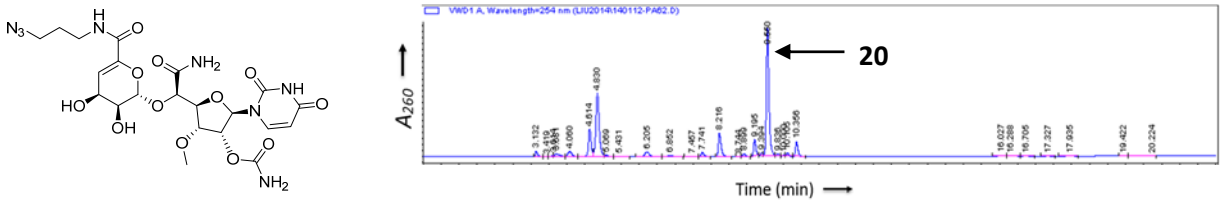
MS (ESI): $C_{21}H_{25}N_5O_{12}-H^-$, Calc: 538.2, Found: 538.6.

19:



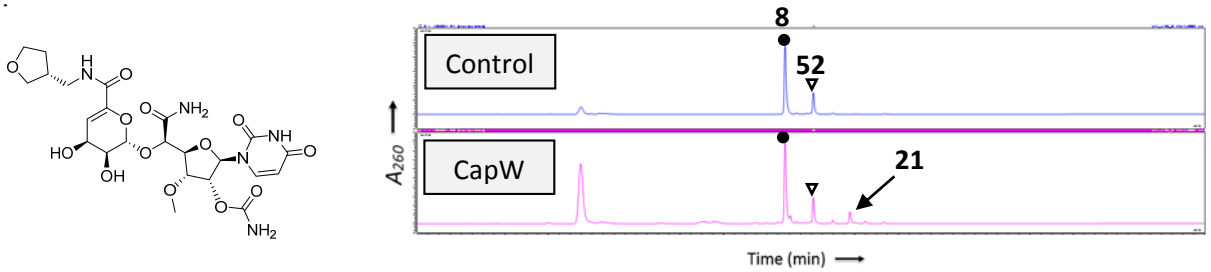
^{1}H NMR (400 MHz, D_2O) δ 7.71 (d, *J*=8.1 Hz, 1H), 5.98 (dd, *J*=2.9, 1.1 Hz, 1H), 5.90 (dd, *J*=6.2, 4.3 Hz, 2H), 5.39 (d, *J*=3.1 Hz, 1H), 5.24-5.20 (m, 1H), 4.71 (d, *J*=2.3 Hz, 1H), 4.56 (dd, *J*=4.5, 2.5 Hz, 1H), 4.49 (dd, *J*=5.5, 2.3 Hz, 1H), 4.23-4.17 (m, 1H), 3.95 (t, *J*=5.5 Hz, 1H), 3.47-3.42 (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). ^{13}C NMR (100 MHz, D_2O) 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): $C_{22}H_{27}N_5O_{12}$+H^+, Calc: 554.1729, Found: 554.1731.

20:



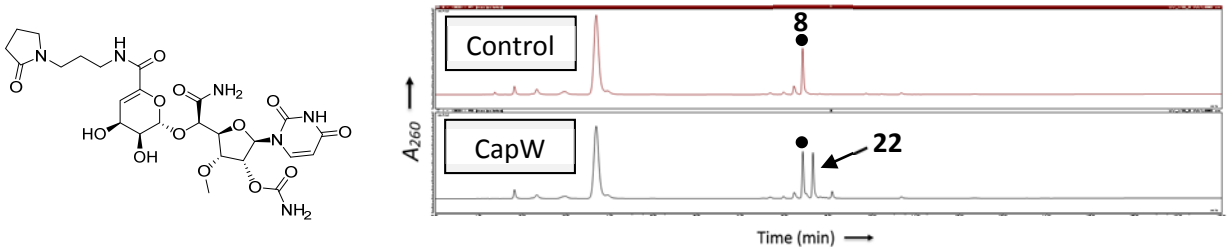
MS (ESI): $C_{21}H_{28}N_8O_{12}$+H^+, Calc: 585.2, Found: 584.7.

21:



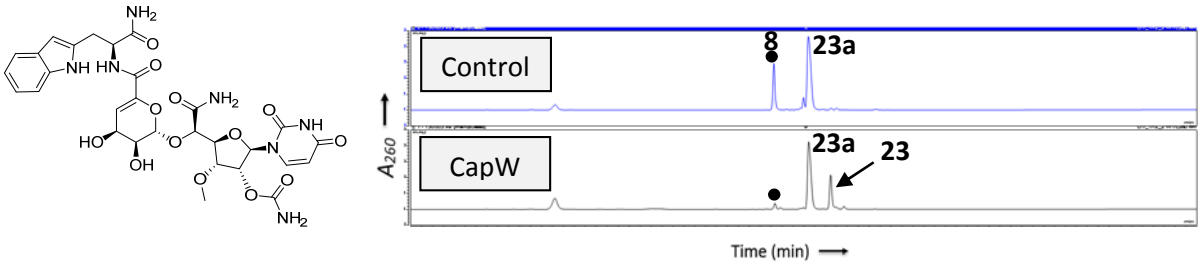
MS (ESI): $C_{23}H_{31}N_5O_{13}$+H^+, Calc: 586.2, Found: 586.6.

22:



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

23:



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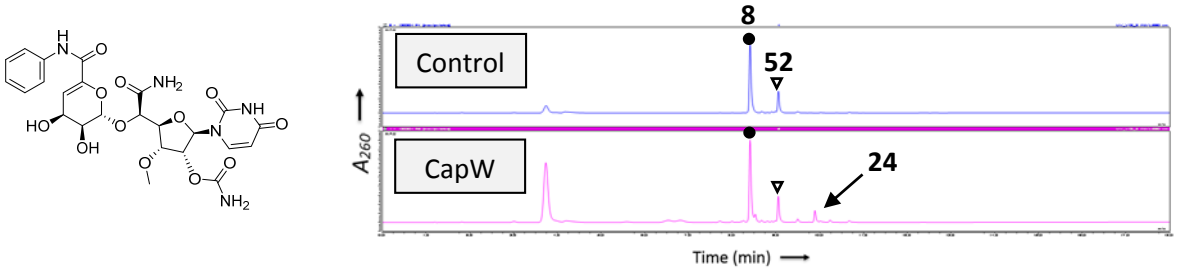
24:



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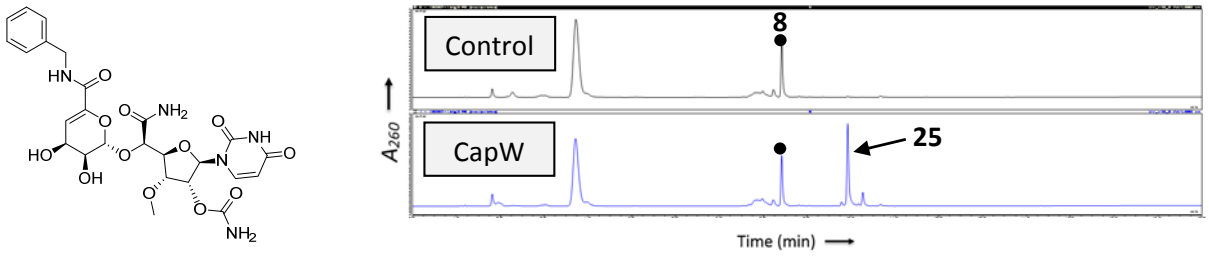
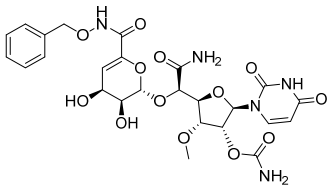
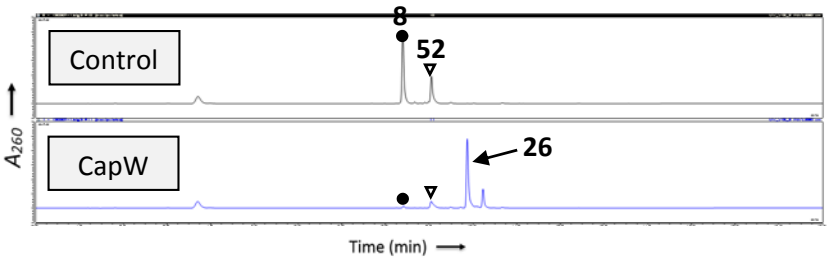
25:



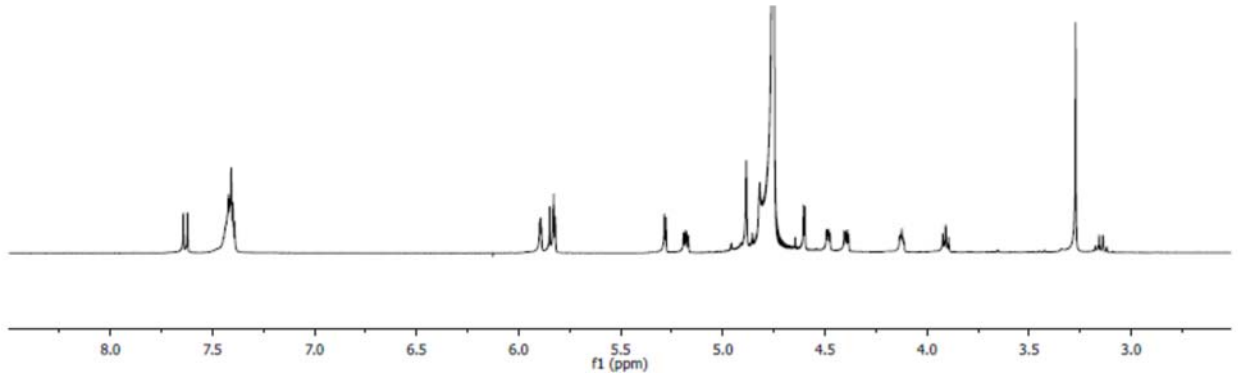
MS (ESI): $C_{25}H_{29}N_5O_{12}+H^+$, Calc: 592.2, Found: 591.7.

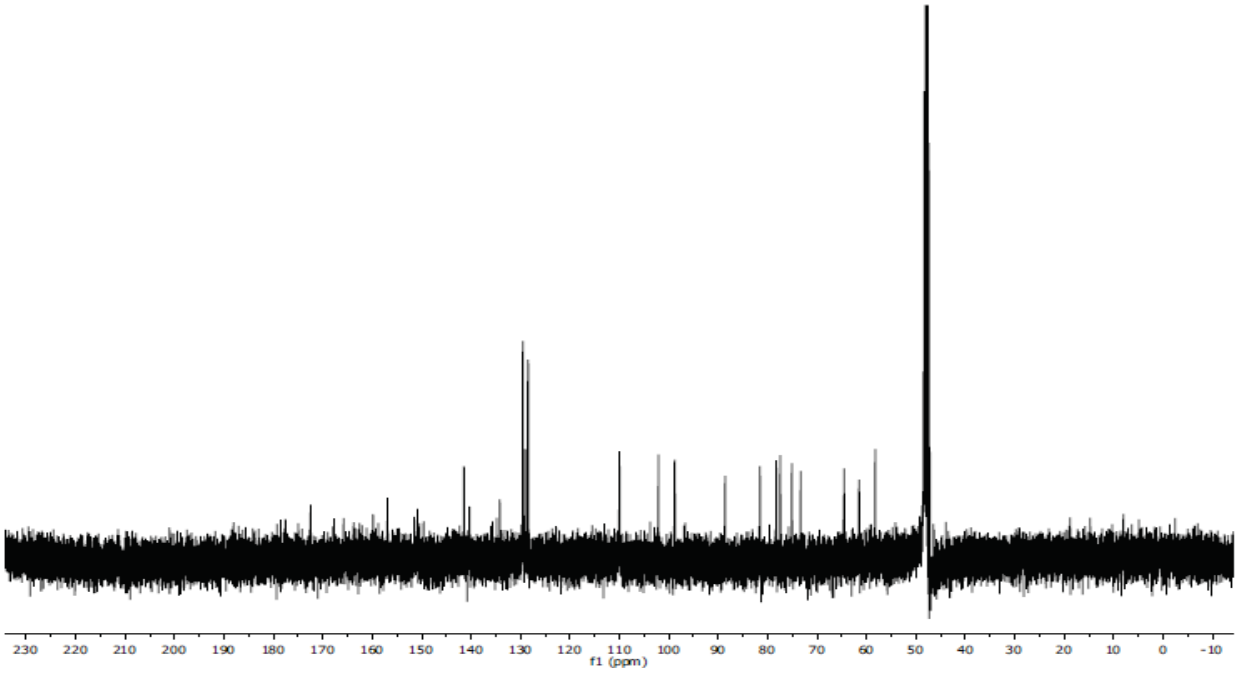
26:





^{1}H NMR (400 MHz, D_2O) δ 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}C NMR (100 MHz, D_2O with methanol as standard) δ 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): $C_{25}H_{29}N_5O_{13}$+H^+, Calc: 608.1835, Found: 608.1840.





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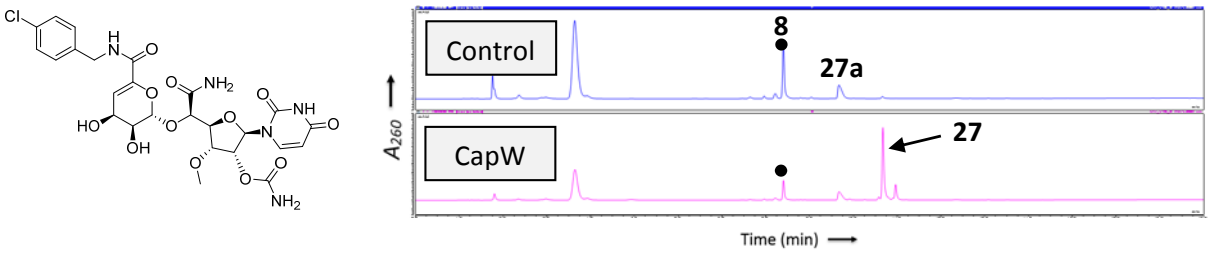
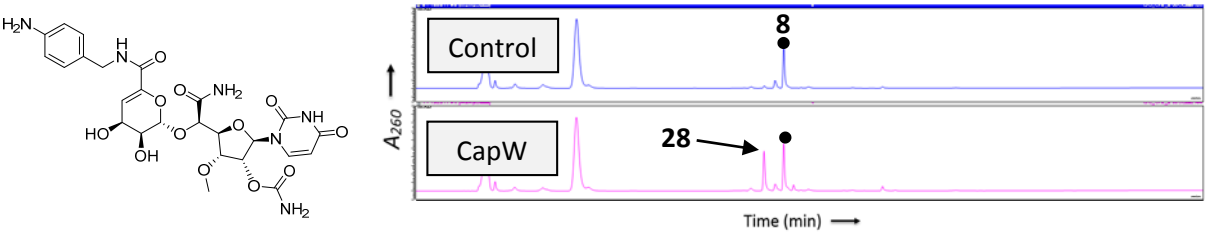


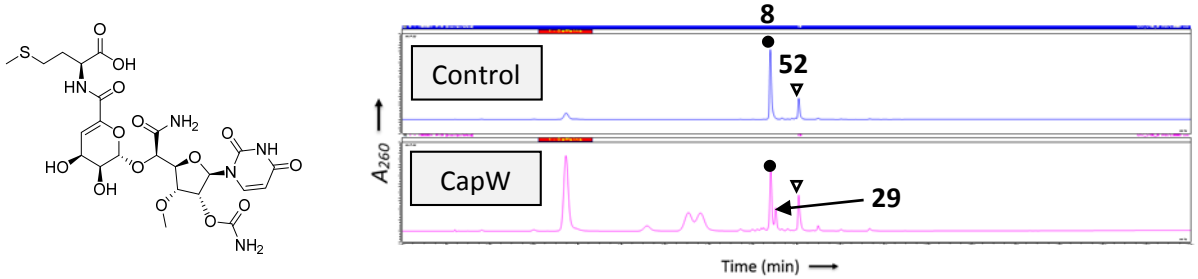
MS (ESI): $C_{25}H_{28}ClN_5O_{12}$-H^-, Calc: 624.1, Found: 623.5.

28:



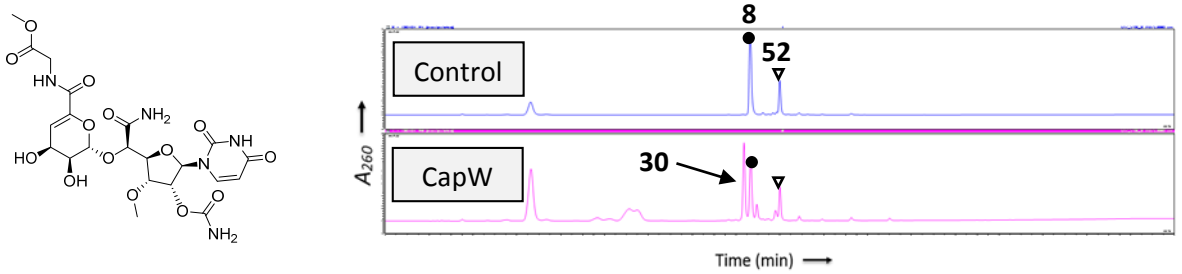
MS (ESI): $C_{25}H_{30}N_6O_{12}$+H^+, Calc: 607.2, Found: 606.6.

29:



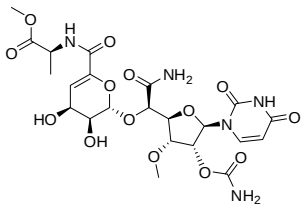
MS (ESI): $C_{23}H_{31}N_5O_{14}$+H^+, Calc: 634.2, Found: 633.5.

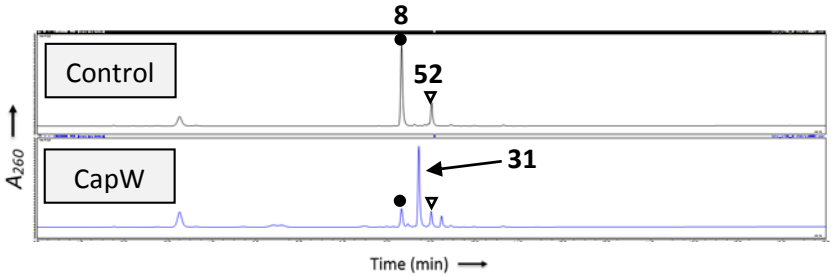
30:



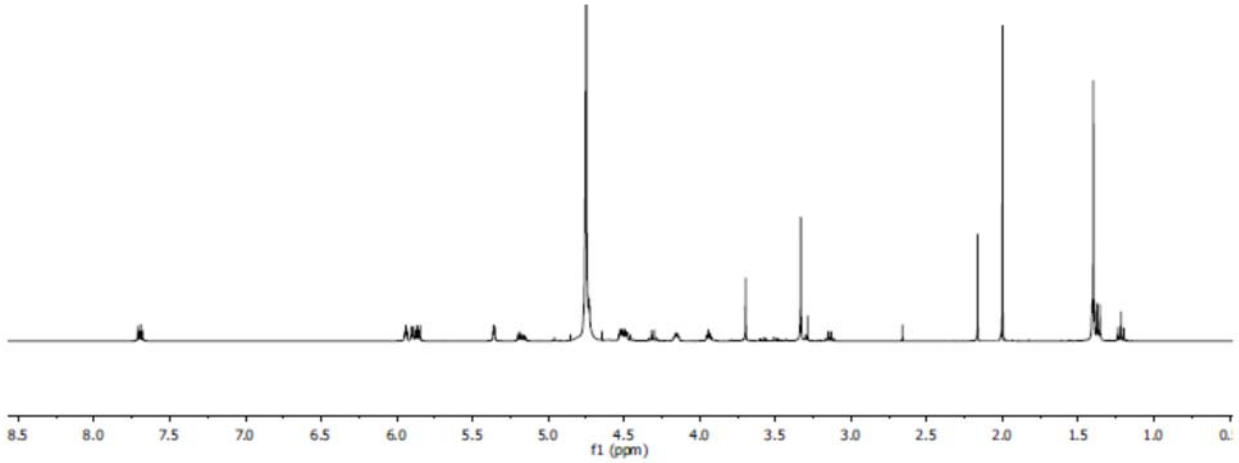
MS (ESI): $C_{21}H_{27}N_5O_{14}$+H^+, Calc: 574.2, Found: 573.7.

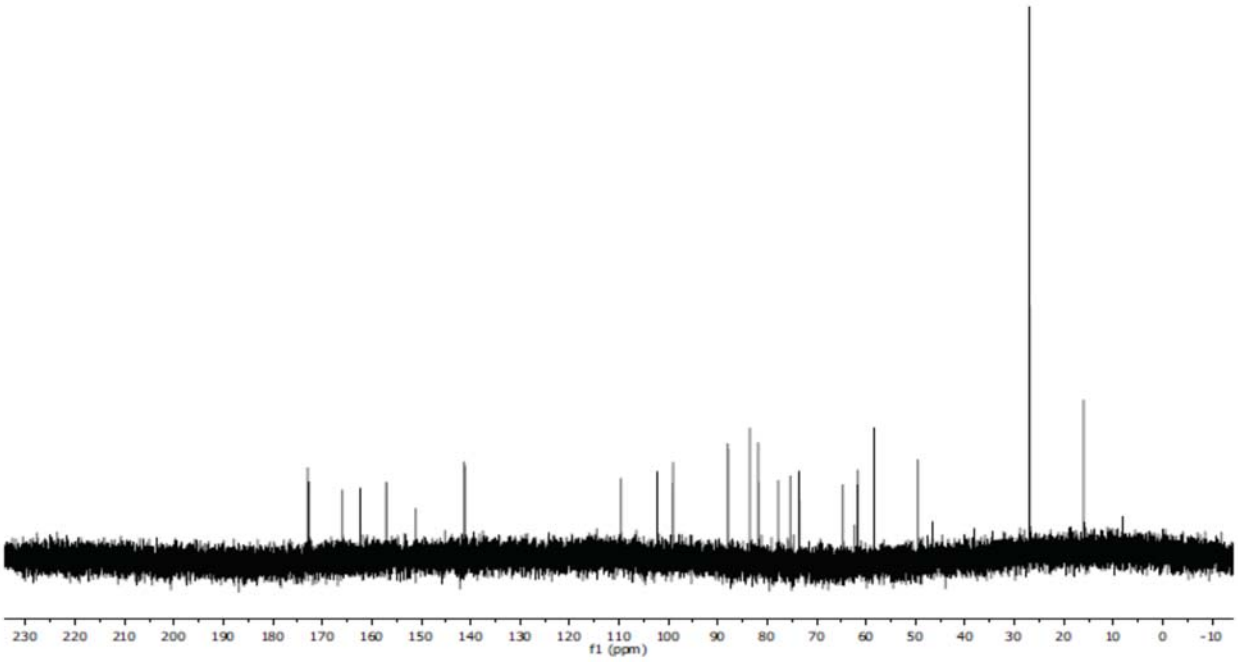
31:



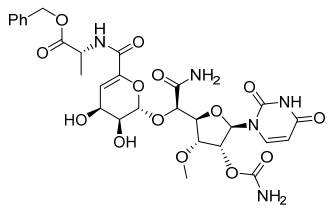


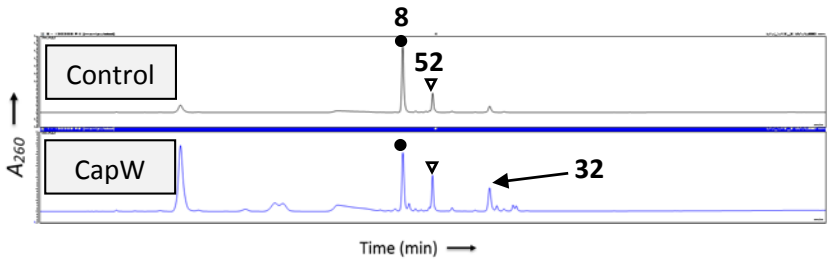
^{1}H NMR (400 MHz, D_2O) δ 7.70 (dd, *J*=8.1, 4.7 Hz, 1H), 5.94 (ddd, *J*=4.2, 2.5, 1.6 Hz, 1H), 5.92-5.83 (m, 2H), 5.39-5.34 (m, 1H), 5.21-5.14 (m, 1H), 4.54-4.47 (m, 2H), 4.23-4.09 (m, 1H), 3.97-3.90 (m, 1H), 3.33 (s, 3H), 2.01-1.99 (m, 3H), 1.41-1.39 (m, 3H), 1.37 (d, *J*=7.3 Hz, 2H), 1.22 (t, *J*=7.3 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 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): $C_{22}H_{29}N_5O_{14}+H^+$, Calc: 588.1784, Found: 588.1786.





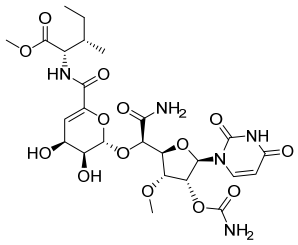
32:

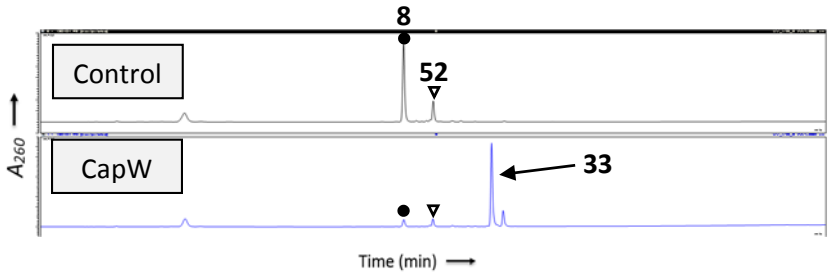




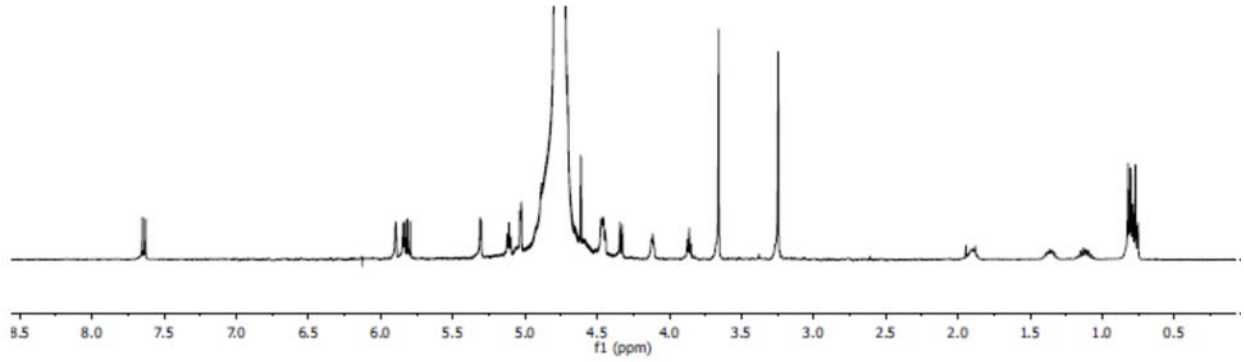
MS (ESI): $C_{28}H_{33}N_5O_{14}+H^+$, Calc: 664.2, Found: 663.6.

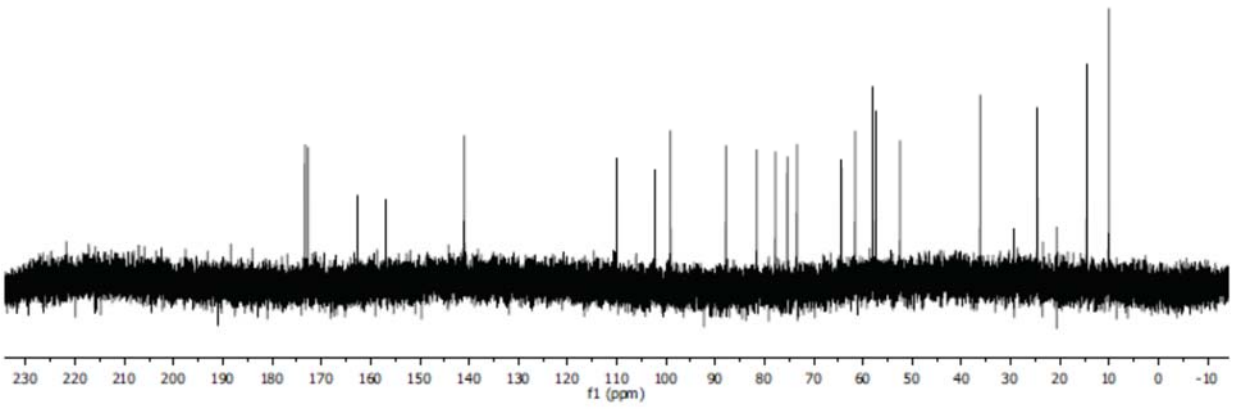
33:



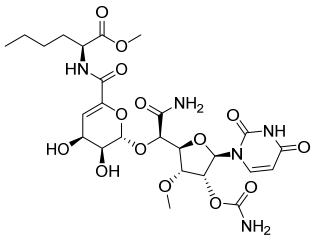


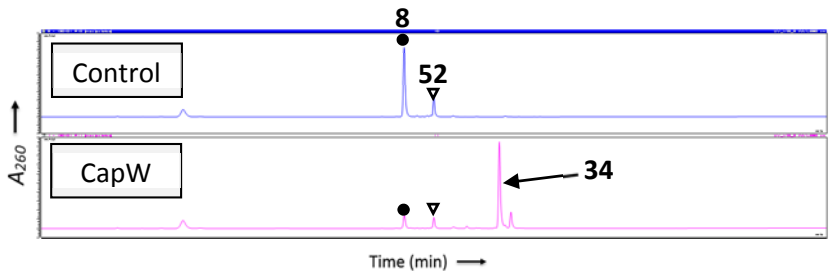
^{1}H NMR (400 MHz, D_2O) δ 7.64 (d, *J*=8.1 Hz, 1H), 5.92-5.88 (m, 1H), 5.84 (d, *J*=4.8 Hz, 1H), 5.81 (d, *J*=8.1 Hz, 1H), 5.31 (d, *J*=3.2 Hz, 1H), 5.14-5.09 (m, 1H), 5.03 (ddd, *J*=5.5, 3.4, 2.7 Hz, 1H), 4.49-4.43 (m, 1H), 4.33 (d, *J*=7.0 Hz, 1H), 4.15-4.09 (m, 1H), 3.86 (t, J=5.2 Hz, 1 H), 3.66 (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}C NMR (100 MHz, D_2O) δ 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): $C_{25}H_{35}N_5O_{14}$+H^+, Calc: 630.2253, Found: 630.2260.



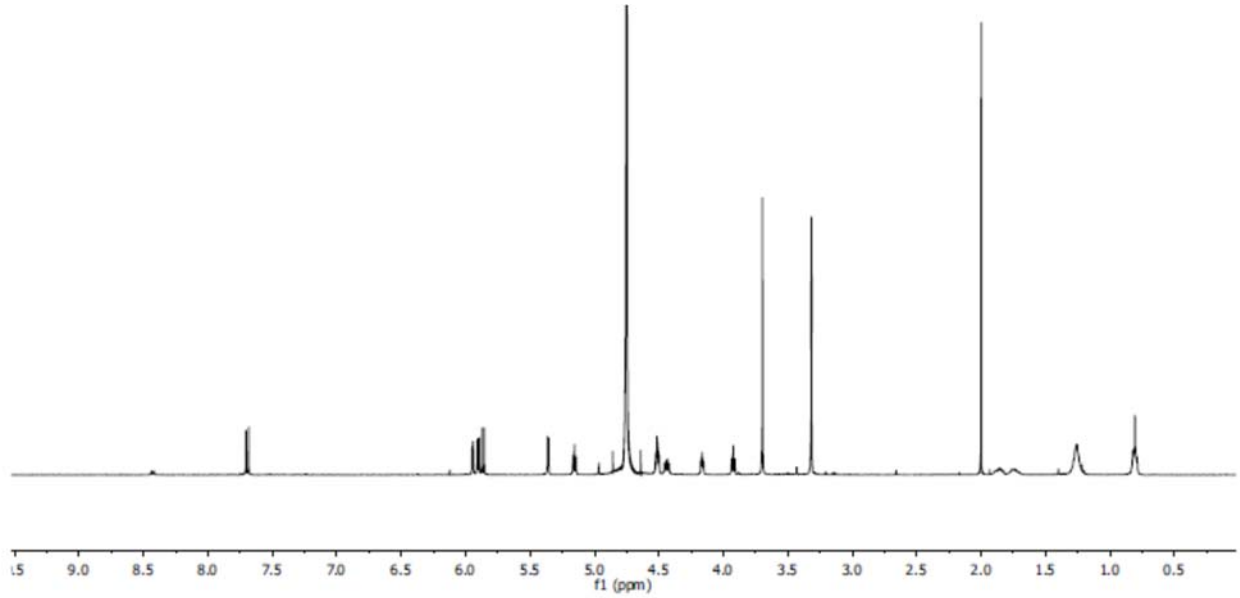


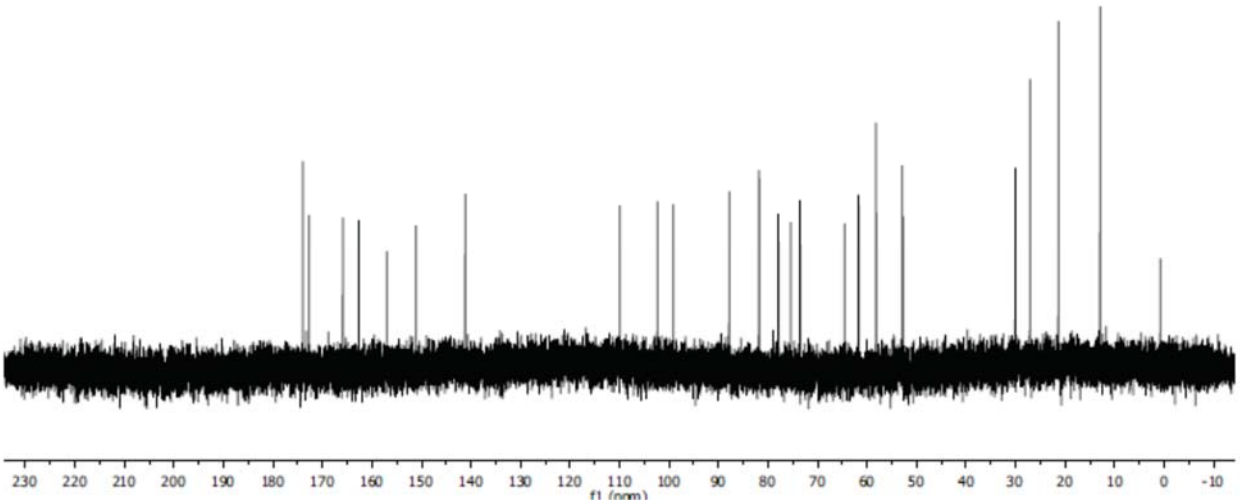
34:



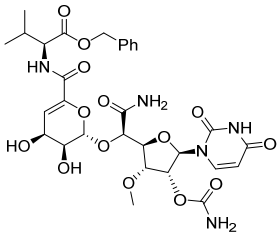


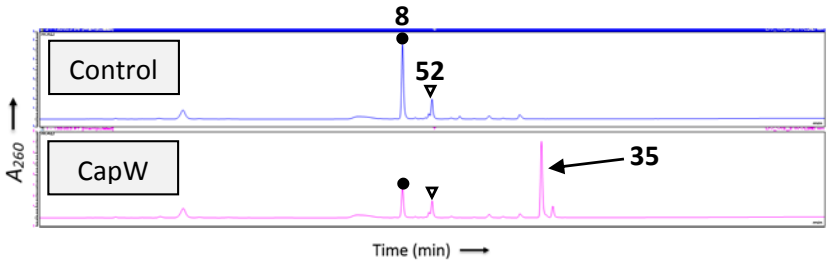
^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, *J* = 8.2 Hz, 1H), 5.94 (dd, *J*=2.5, 1.7 Hz, 1H), 5.90 (d, *J*=4.8 Hz, 1H), 5.86 (d, *J*=8.1 Hz, 1H), 5.36 (d, *J*=3.2 Hz, 1H), 5.16 (t, *J*=5.1 Hz, 1H), 4.51 (td, *J*=4.3, 2.3 Hz, 2H), 4.44 (dd, *J*=9.2, 5.3 Hz, 1H), 4.16 (ddd, *J*=4.7, 3.2, 1.7 Hz, 1H), 3.92 (t, *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}C NMR (100 MHz, D_2O) δ 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): $C_{25}H_{35}N_5O_{14}+H^+$, Calc: 630.2253, Found: 630.2260.





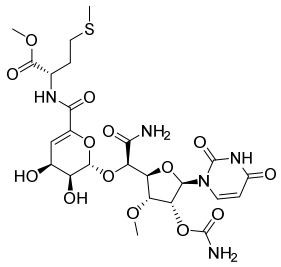
35:

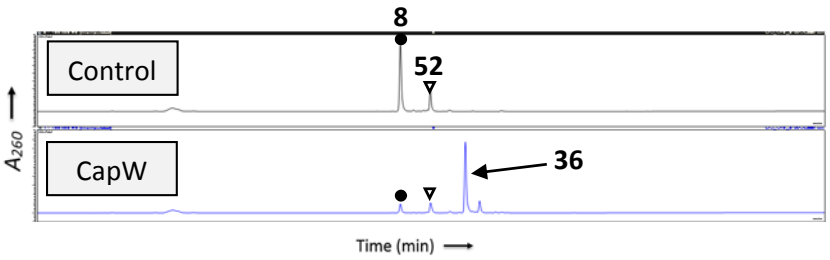




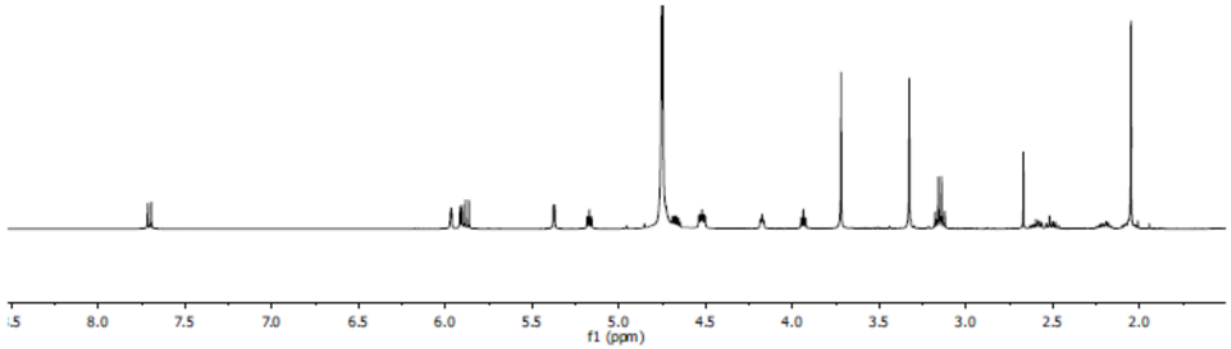
MS (ESI): $C_{30}H_{37}N_5O_{14}+H^+$, Calc: 692.2, Found: 691.6.

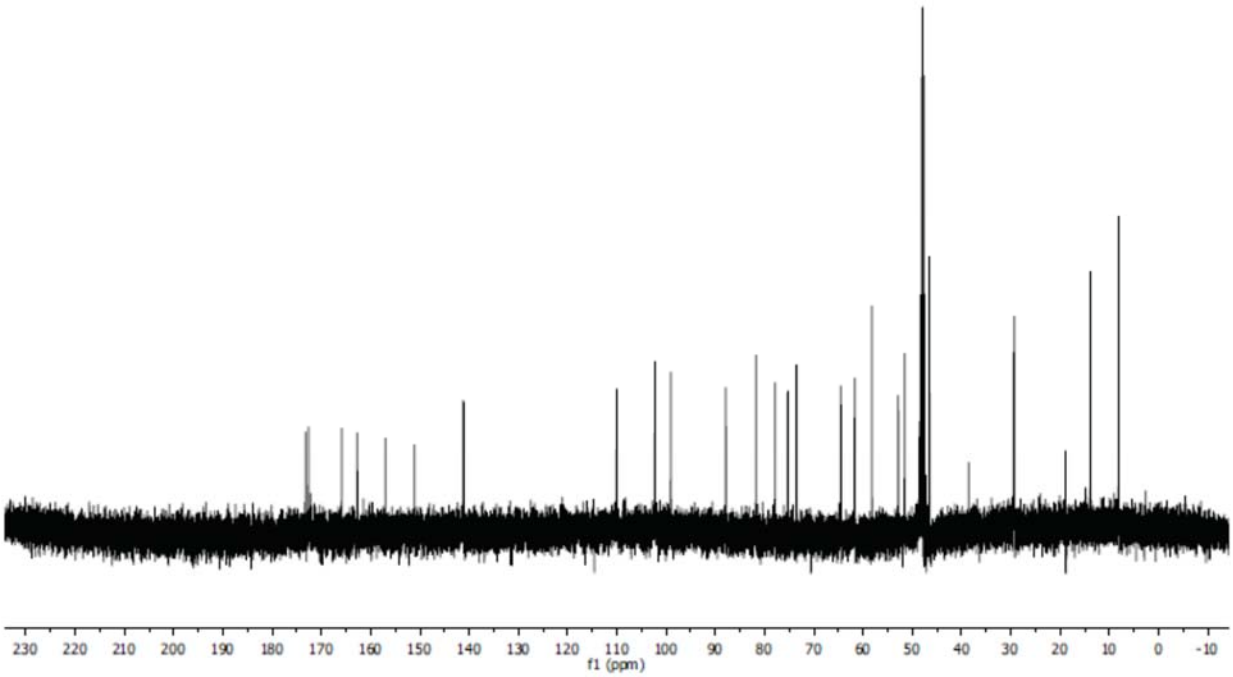
36:



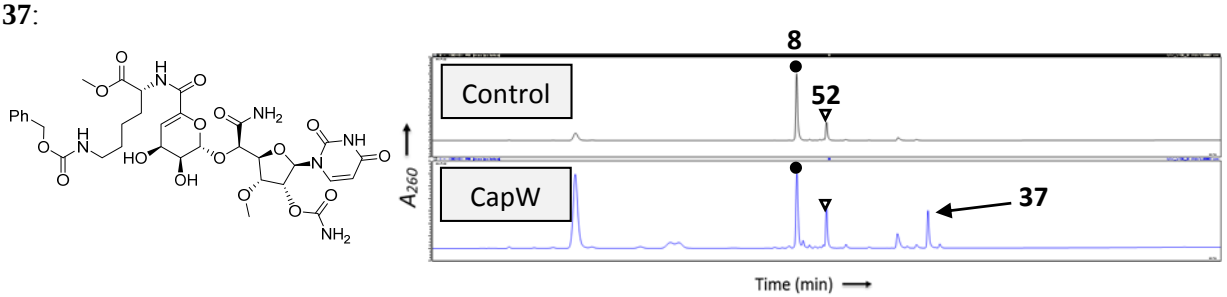


^{1}H NMR (400 MHz, D_2O) δ 7.70 (d, *J*=8.1 Hz, 1H), 5.97 (dd, *J*=2.4, 1.7 Hz, 1H), 5.91 (d, *J*=4.7 Hz, 1H), 5.87 (d, *J*=8.1 Hz, 1H), 5.37 (d, *J*=3.2 Hz, 1H), 5.17 (t, *J*=5.0 Hz, 1H), 4.70-4.64 (m, 1H), 4.55-4.49 (m, 2H), 4.18 (ddd, *J*=4.6, 3.2, 1.7 Hz, 1H), 3.94 (t, *J*=5.2 Hz, 1H), 3.72 (s, 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}C NMR (100 MHz, D_2O with methanol as standard) δ 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): $C_{24}H_{33}N_5O_{14}S+H^+$, Calc: 648.1817, Found: 648.1829.



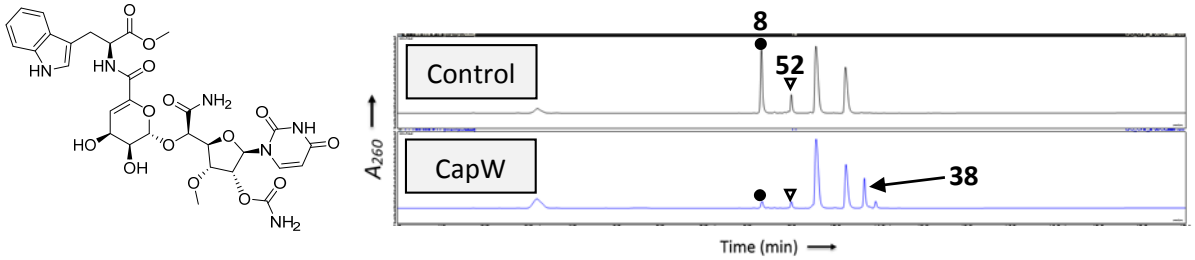


37:



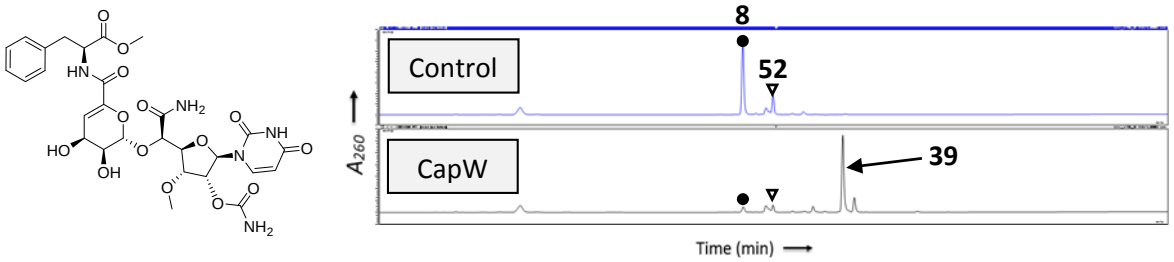
MS (ESI): $C_{33}H_{42}N_6O_{16}+H^+$, Calc: 778.3, Found: 778.6.

38:



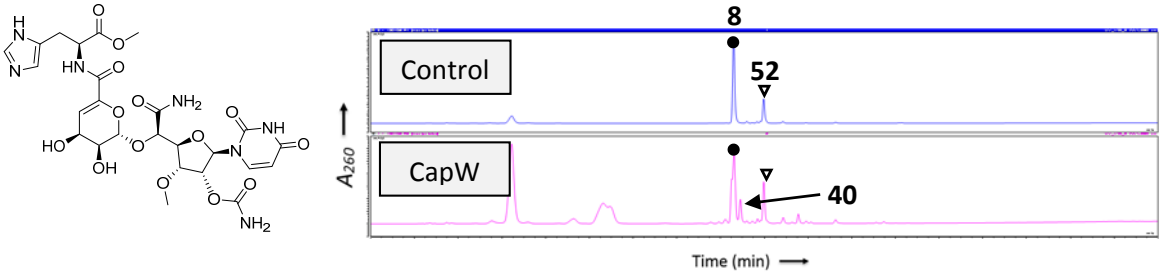
MS (ESI): $C_{30}H_{34}N_6O_{14}+H^+$, Calc: 703.2, Found: 702.6.

39:



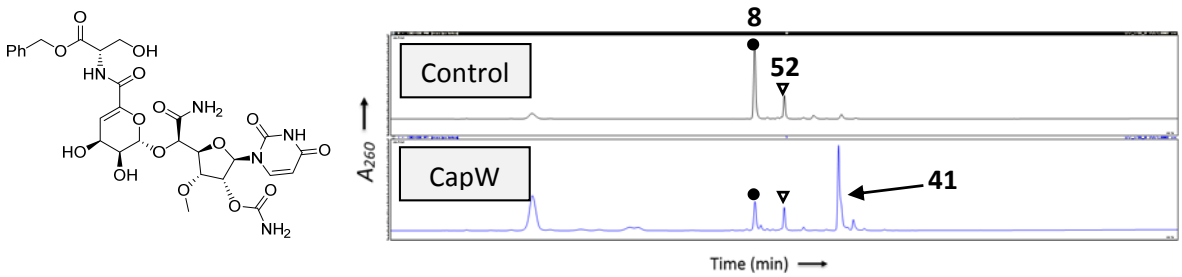
MS (ESI): $C_{28}H_{33}N_5O_{14}+H^+$, Calc: 664.2, Found: 663.6.

40:



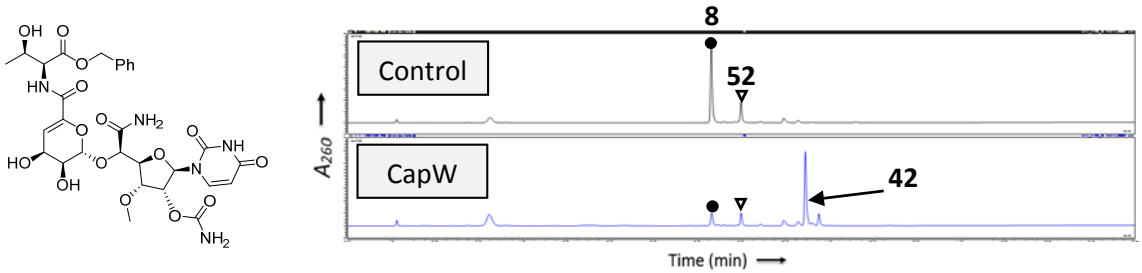
MS (ESI): $C_{28}H_{33}N_5O_{14}+H^+$, Calc: 654.2, Found: 653.7.

41:



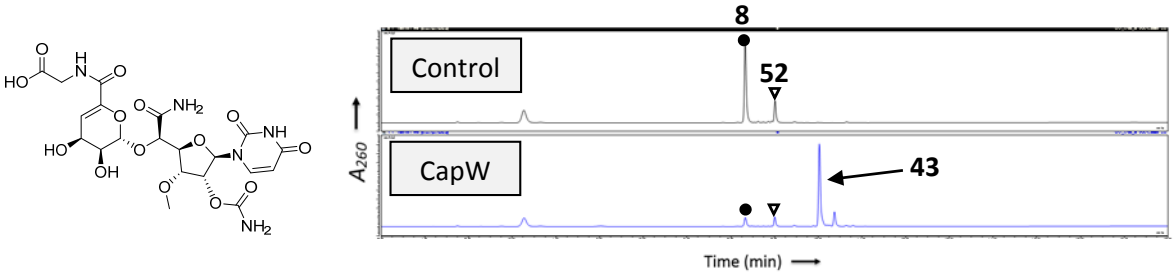
MS (ESI): $C_{28}H_{33}N_5O_{15}+H^+$, Calc: 680.2, Found: 679.6.

42:



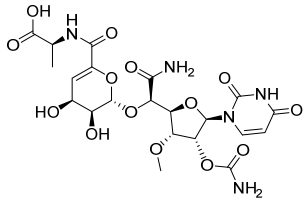
MS (ESI): $C_{29}H_{35}N_5O_{15}+H^+$, Calc: 694.2, Found: 693.6.

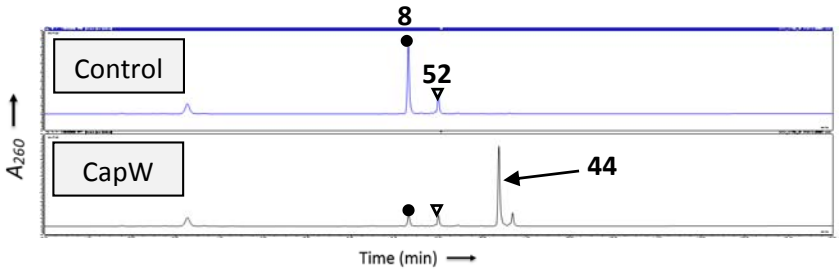
43:



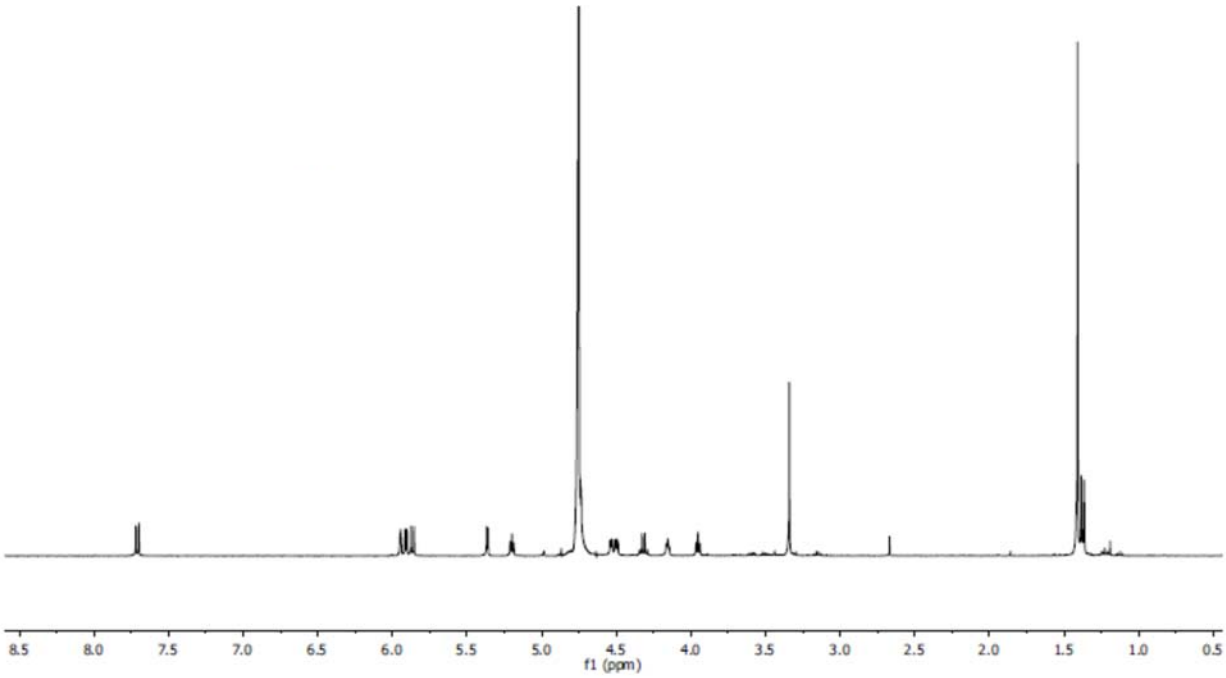
MS (ESI): $C_{20}H_{25}N_5O_{14}+H^+$, Calc: 560.1, Found: 559.6.

44:

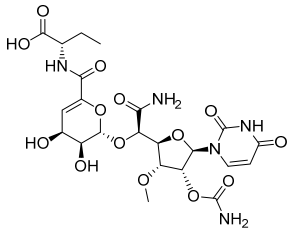


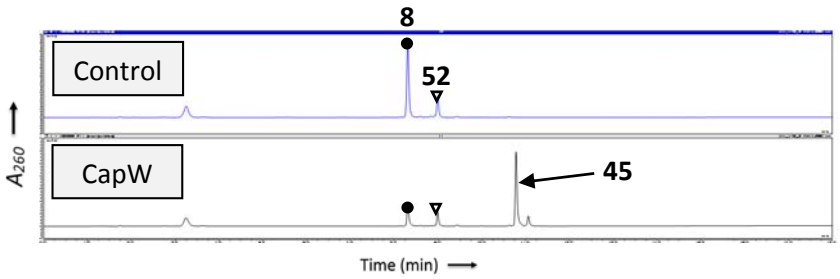


^{1}H NMR (400 MHz, D_2O) δ 7.71 (d, *J*=8.1 Hz, 1H), 5.94 (dd, *J*=2.6, 1.6 Hz, 1H), 5.91 (d, *J*=4.6 Hz, 1H), 5.86 (d, *J*=8.1 Hz, 1H), 5.36 (d, *J*=3.3 Hz, 1H), 5.21-5.18 (m, 1H), 4.74 (dd, *J*=1.9, 1.2 Hz, 1H), 4.53 (dd, *J*=4.4, 2.7 Hz, 1H), 4.50 (dd, *J*=5.3, 2.2 Hz, 1H), 4.32 (q, *J*=7.2 Hz, 1H), 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): $C_{21}H_{27}N_5O_{14}$+H^+, Calc: 574.1627, Found: 574.1632.



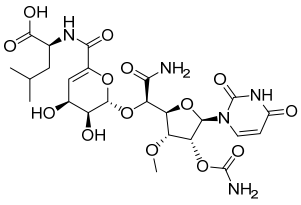
45:

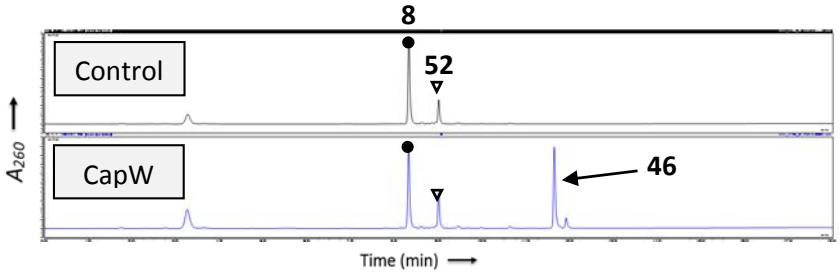




MS (ESI): $C_{20}H_{25}N_5O_{14}$+H^+, Calc: 588.2, Found: 587.6.

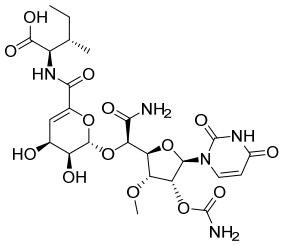
46:

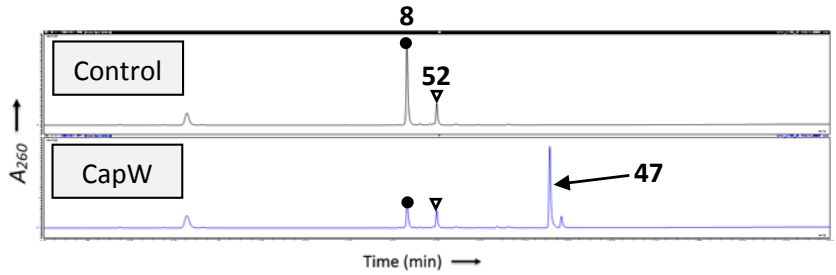




MS (ESI): $C_{24}H_{33}N_5O_{14}+H^+$, Calc: 616.2, Found: 615.7.

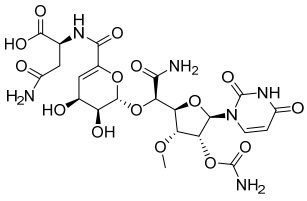
47:

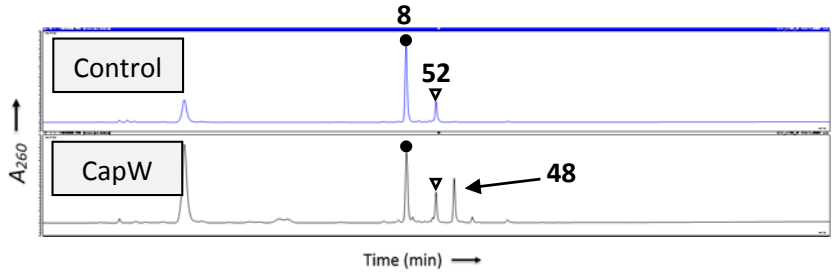




MS (ESI): $C_{24}H_{33}N_5O_{14}+H^+$, Calc: 616.2, Found: 615.6.

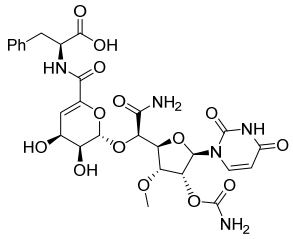
48:

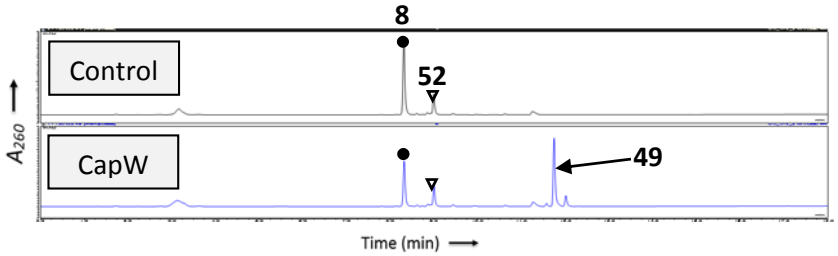




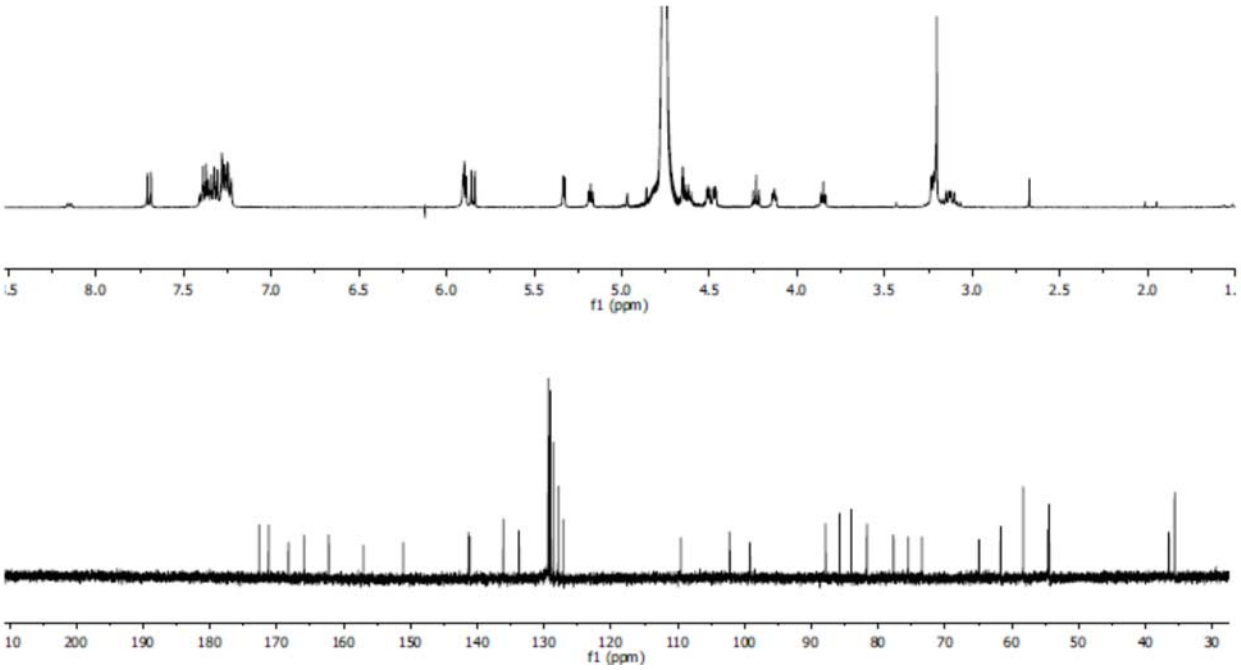
MS (ESI): $C_{22}H_{28}N_6O_{15}+H^+$, Calc: 617.2, Found: 616.6.

49:





^{1}H NMR (400 MHz, d D_2O) δ 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}C NMR (100 MHz, D_2O) δ 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): $C_{27}H_{31}N_5O_{14}$+H^+, Calc: 650.1940, Found: 650.1948.



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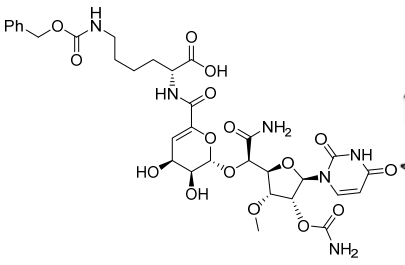
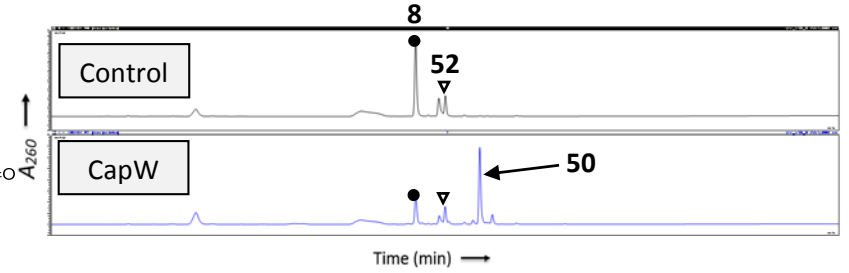
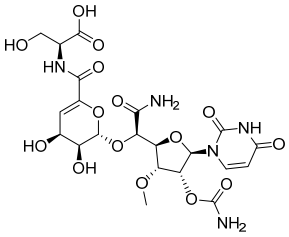
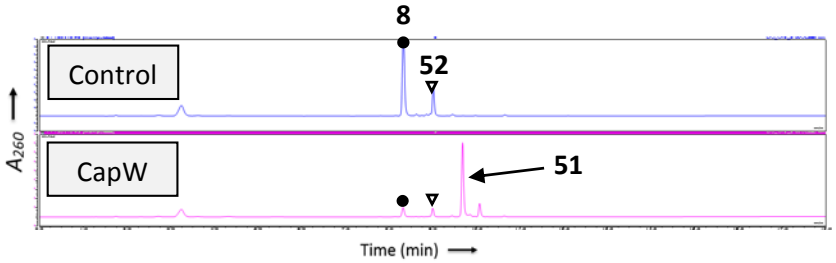




MS (ESI): $C_{32}H_{40}N_6O_{16}$+H^+, Calc: 765.3, Found: 764.8.

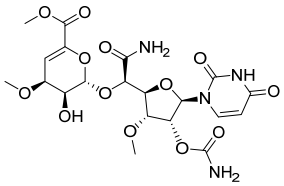
51:

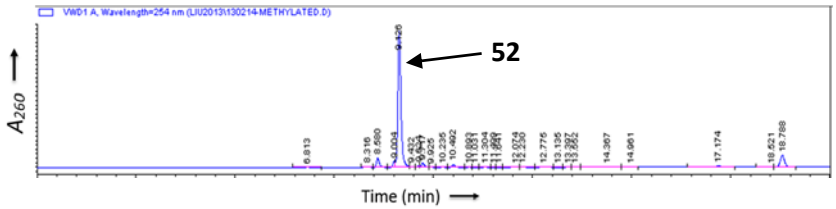




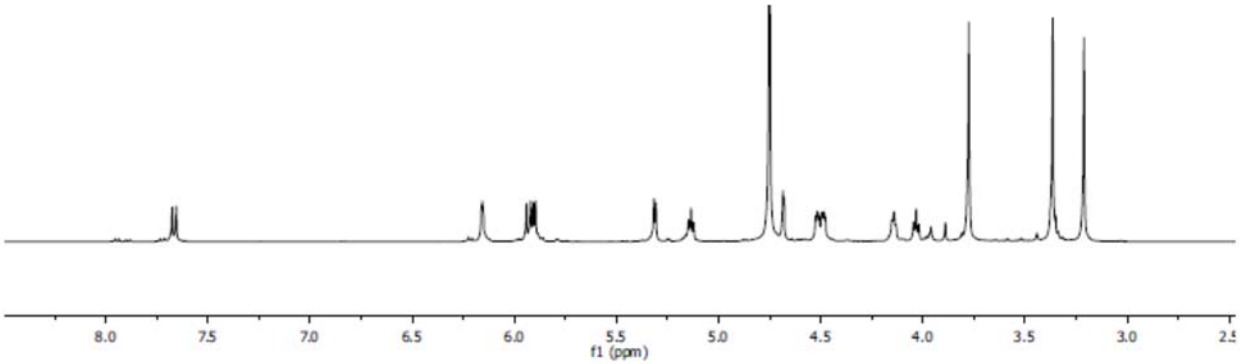
MS (ESI): $C_{21}H_{27}N_5O_{15}+H^+$, Calc: 590.2, Found: 589.6.

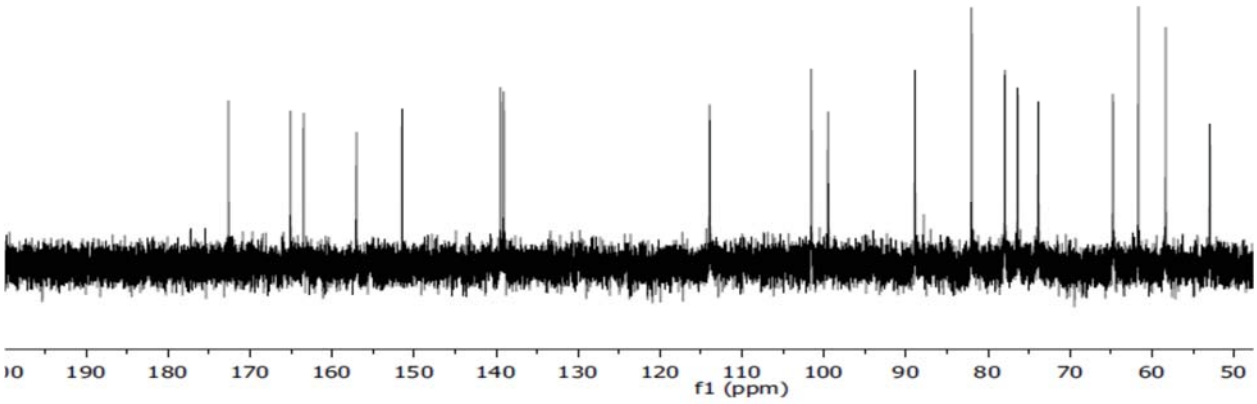
52:





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





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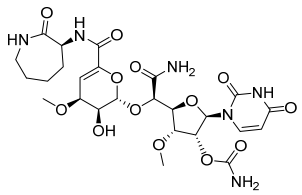
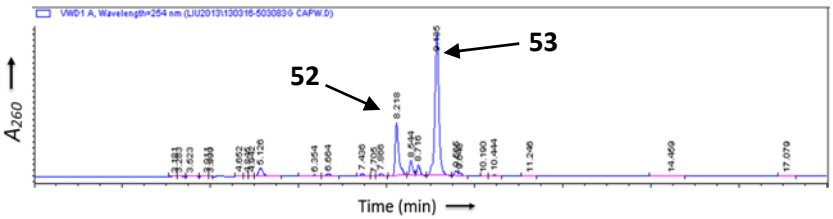
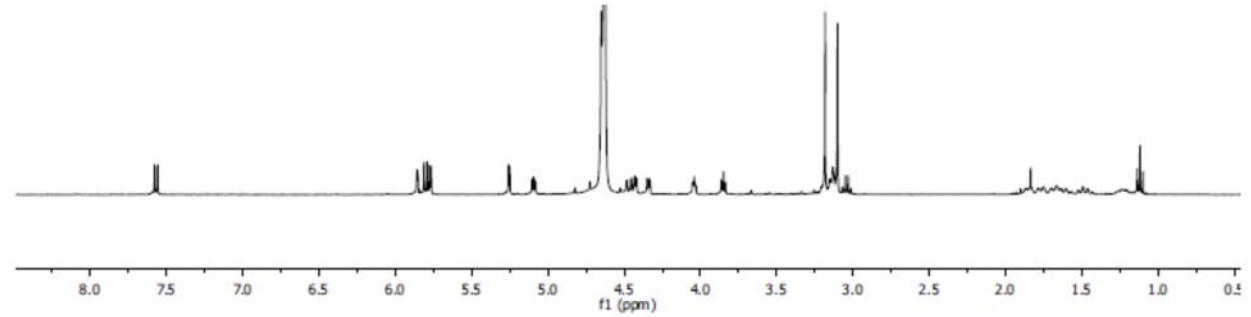
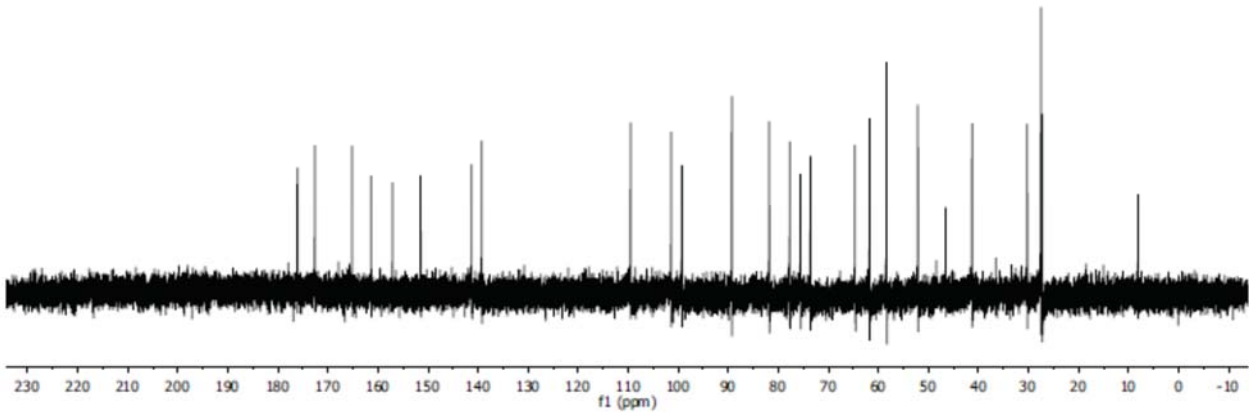




^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, *J*=8.1 Hz, 1H), 5.98 (dd, *J*=2.5, 1.6 Hz, 1H), 5.93 (d, *J*=8.1 Hz, 1H), 5.89 (d, *J*=4.1 Hz, 1H), 5.38 (d, *J*=3.3 Hz, 1H), 5.24-5.19 (m, 1H), 4.62-4.57 (m, 1H), 4.55 (dd, *J*=4.4, 2.6 Hz, 1H), 4.46 (dd, *J*=5.8, 2.5 Hz, 1H), 4.19-4.14 (m, 1H), 3.97 (t, *J*=5.6 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}C NMR (100 MHz, D_2O) δ 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): $C_{25}H_{34}N_6O_{13}$+H^+, Calc: 627.2257, Found: 627.2264.





2. Supporting Figures

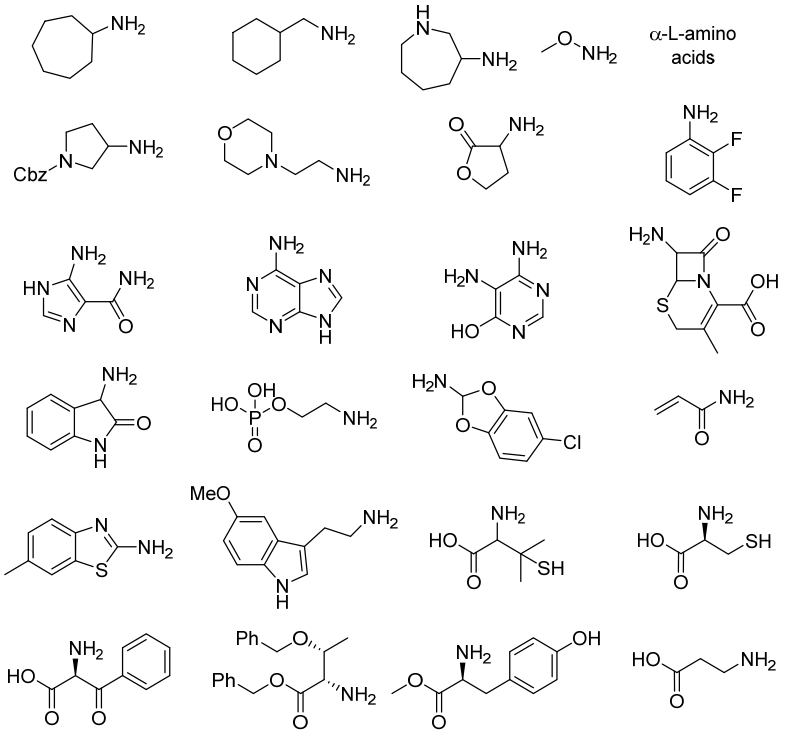


Fig. S1 Tested acyl acceptors not incorporated by CapW. All compounds were provided as the HCl salt. A racemic mixture was used for compounds without any indicated stereochemistry.

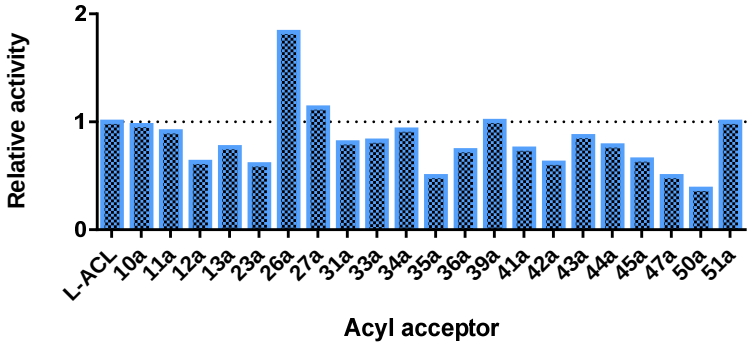


Fig. S2 Relative specific activity of CapW with different acyl acceptors.

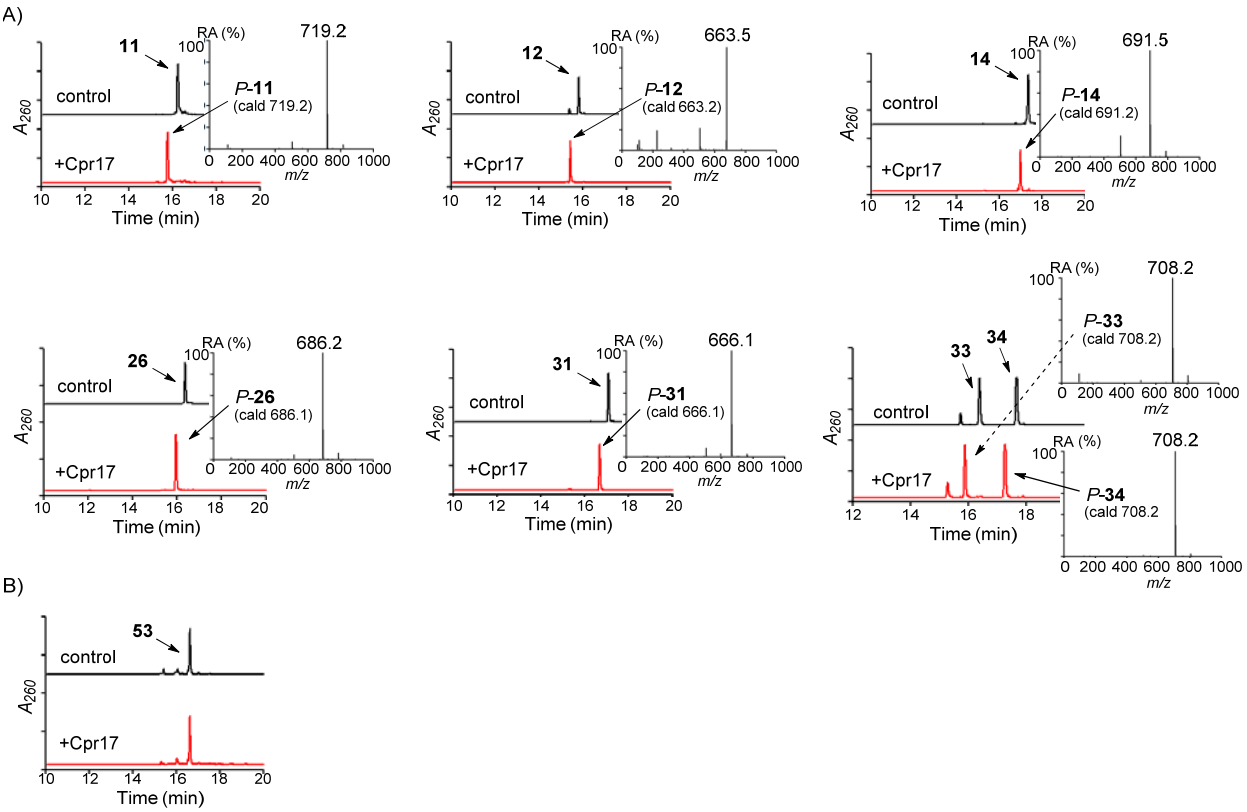


Fig. S3 Enzymatic phosporylation of analogues by Cpr17. A) HPLC traces for representative analogues following the reaction with Cpr17 (+Cpr17) or control (denatured Cpr17). The inset shows the mass spectrum in negative ionisation mode for the monophosphorylated product. B) HPLC traces demonstrating Cpr17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