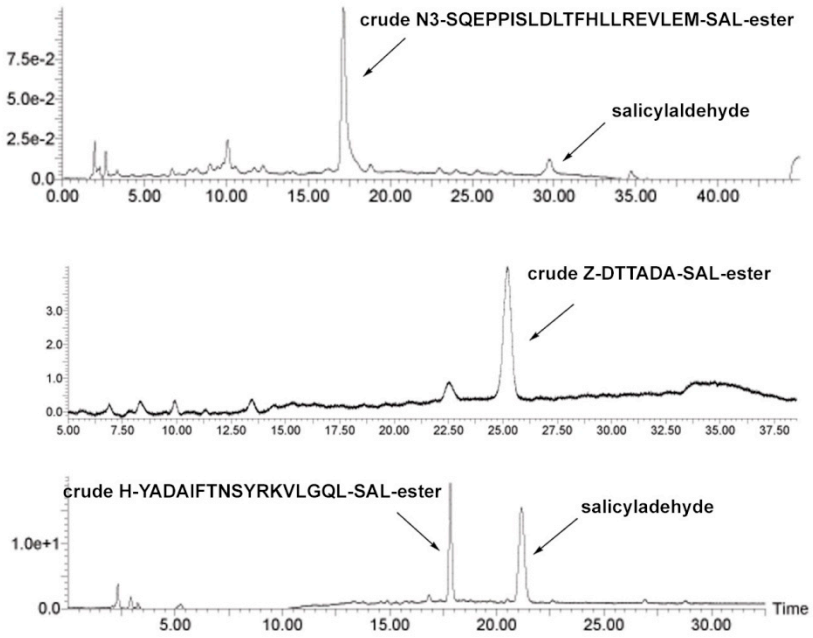
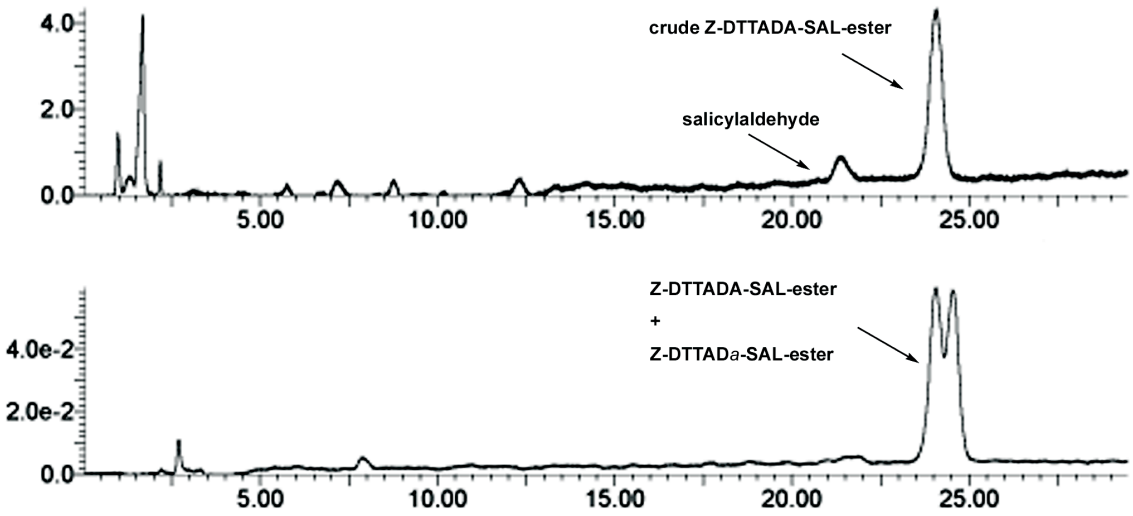
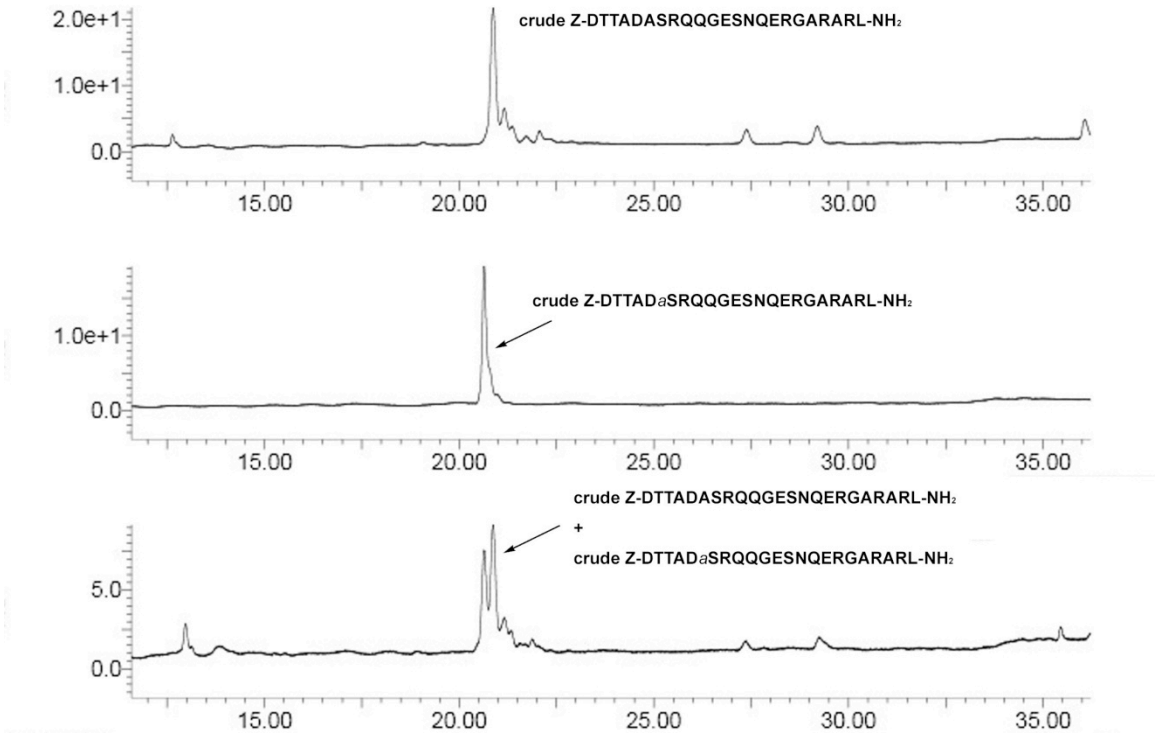


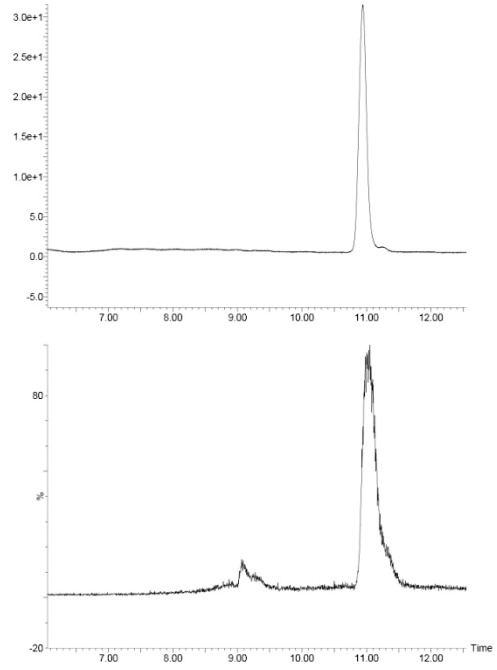
Spectrum 1. Representative analytical HPLC traces of the crude reaction mixtures revealing the formation of the peptide SAL-ester: gradient 5-50% CH_3CN/H_2O over 20 min followed by 50% isocratic CH_3CN/H_2O over another 10 min at a flow rate of 0.6 mL/min.



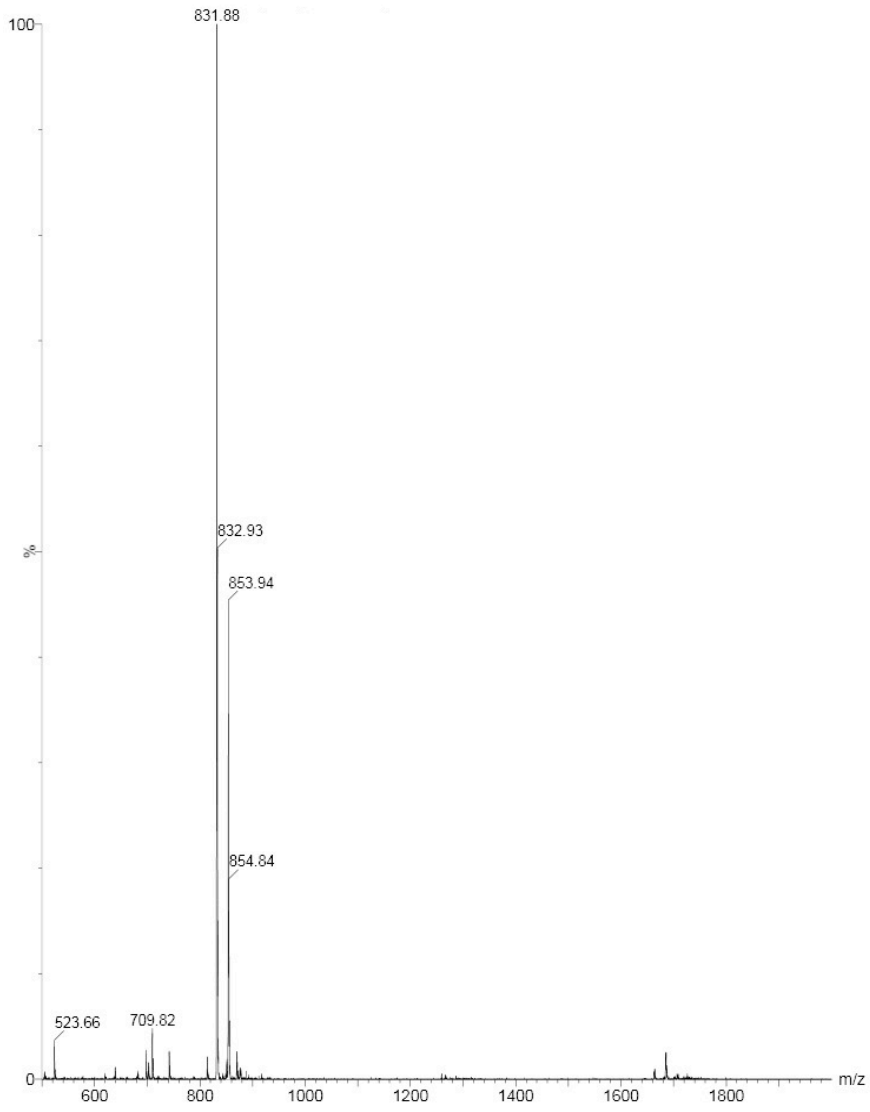
Spectrum 2. UV traces from LC-MS analysis of the epimerization study: gradient 25-35% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min. (top) HPLC trace of the preparation of Z-DTTADA SAL-ester from the crude reaction mixture; (bottom) HPLC trace of the co-injected Z-DTTADA SAL-ester and Z-DTTAD*a* SAL-ester.



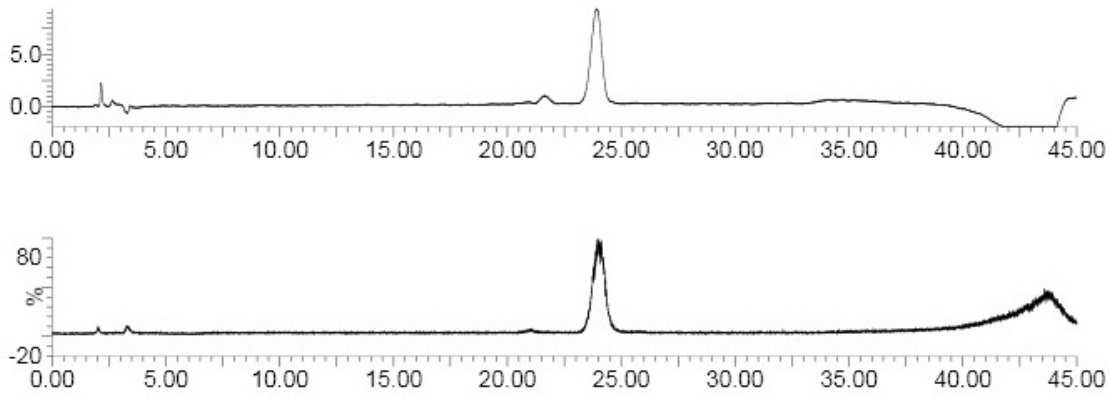
Spectrum 3. UV traces from LC-MS analysis of the epimerization study during ligation: gradient 5-30% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min.



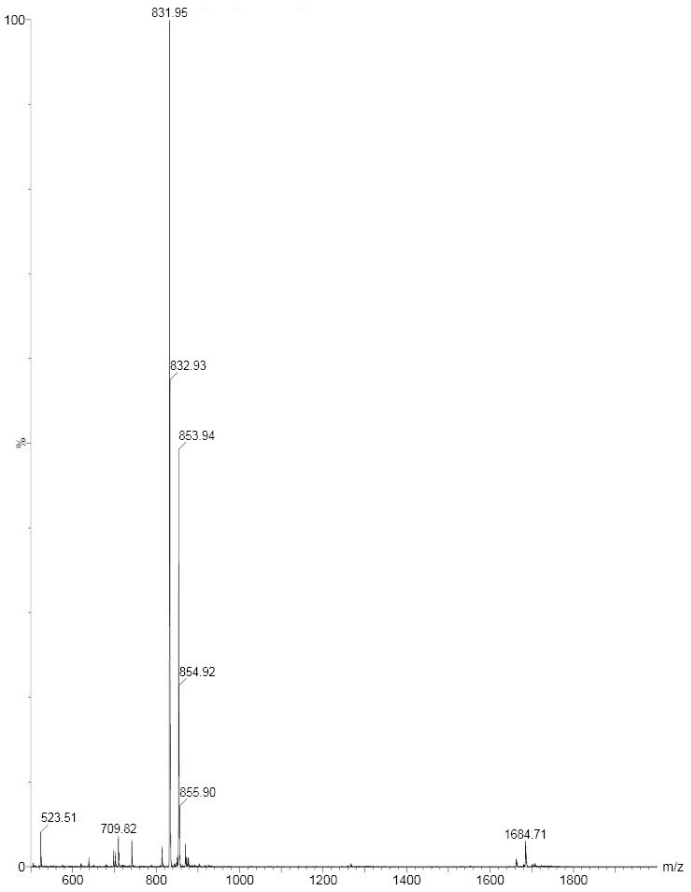
Spectrum 4. UV and MS traces from LC-MS analysis of compound **1**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



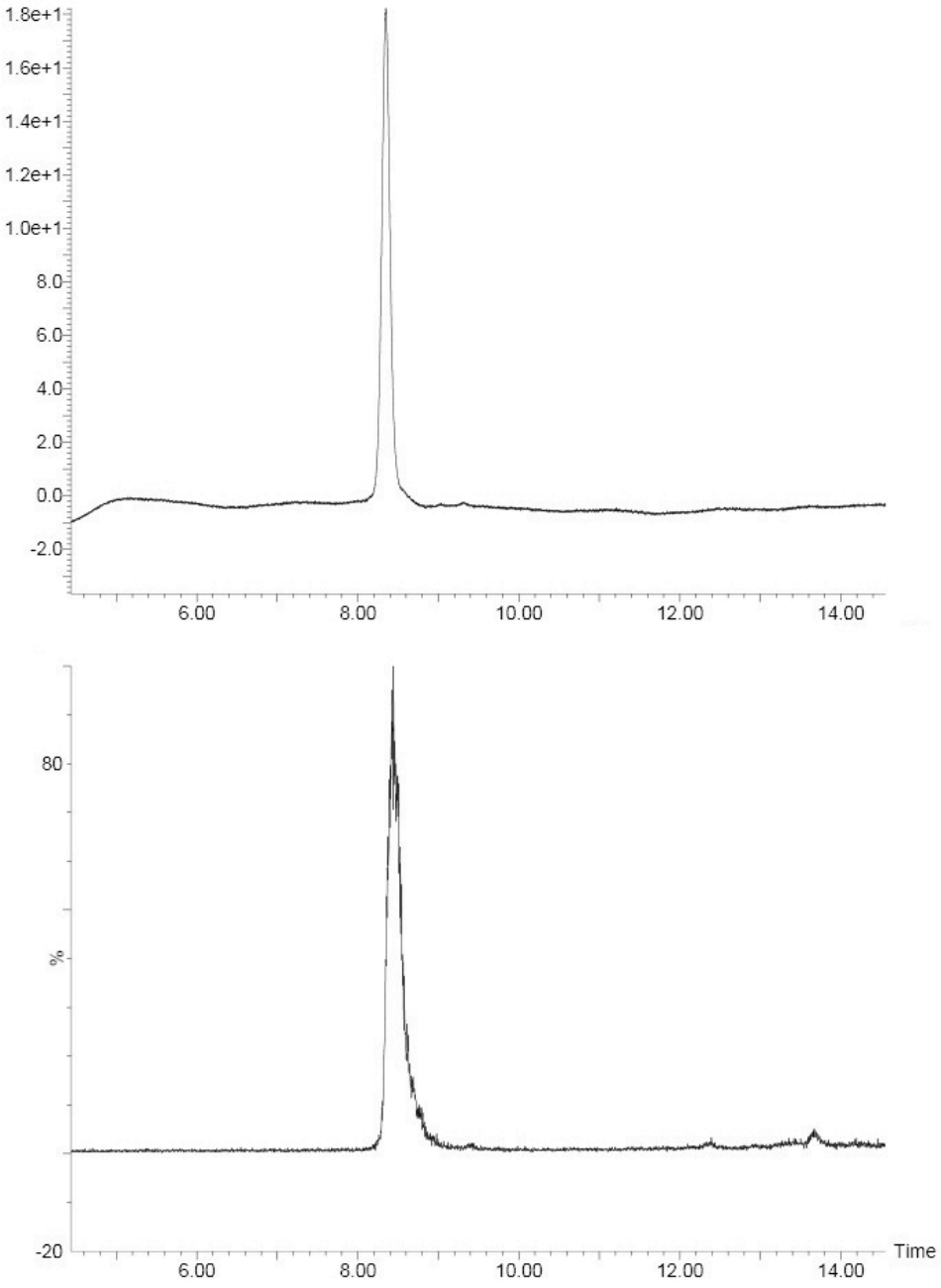
Spectrum 5. ESI calcd for $C_{37}H_{46}N_6O_{16}$ $[M+H]^+$ *m/z* = 831.79, found: 831.88.



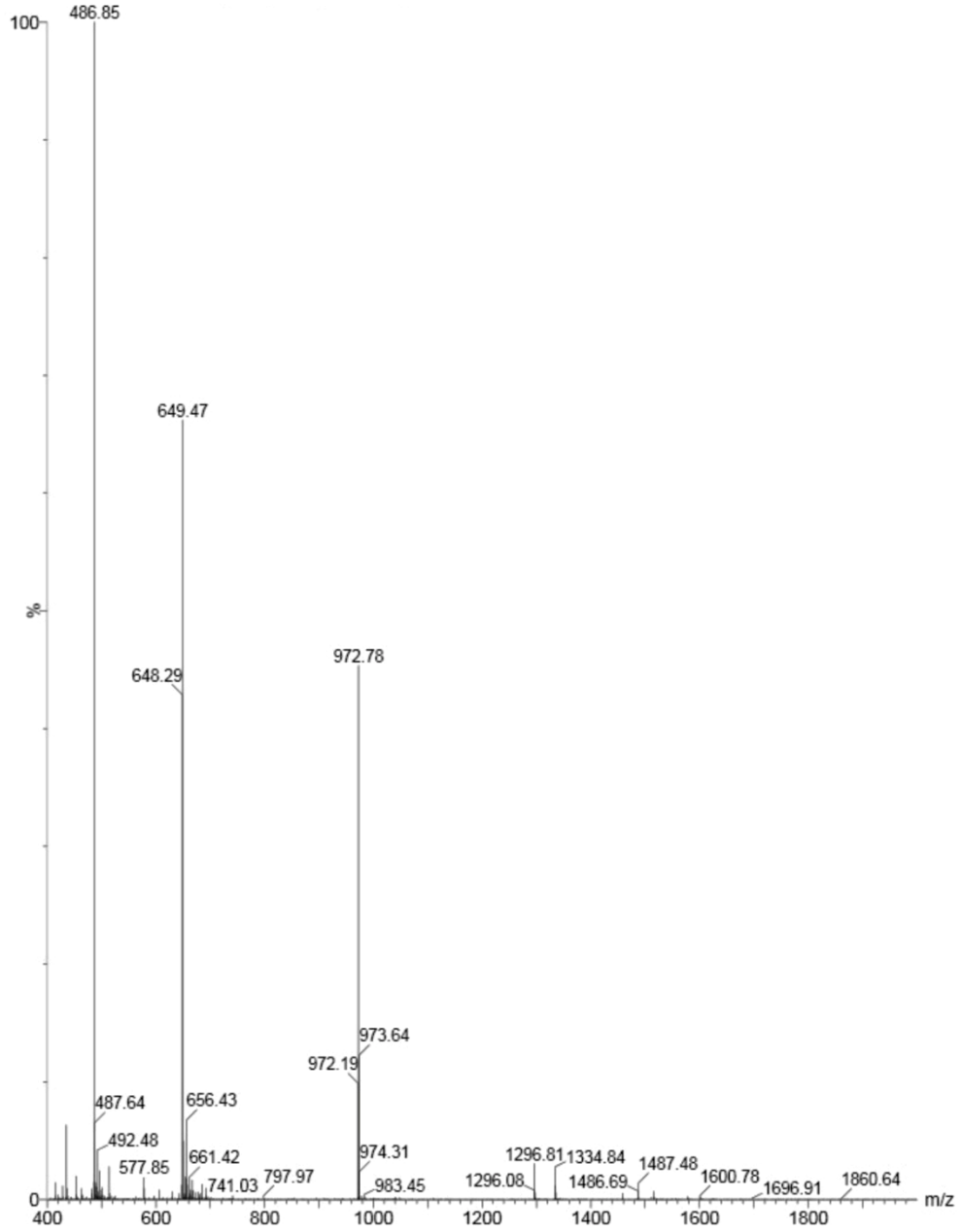
Spectrum 6. UV and MS traces from LC-MS analysis of compound **2**: gradient 25-35% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min.



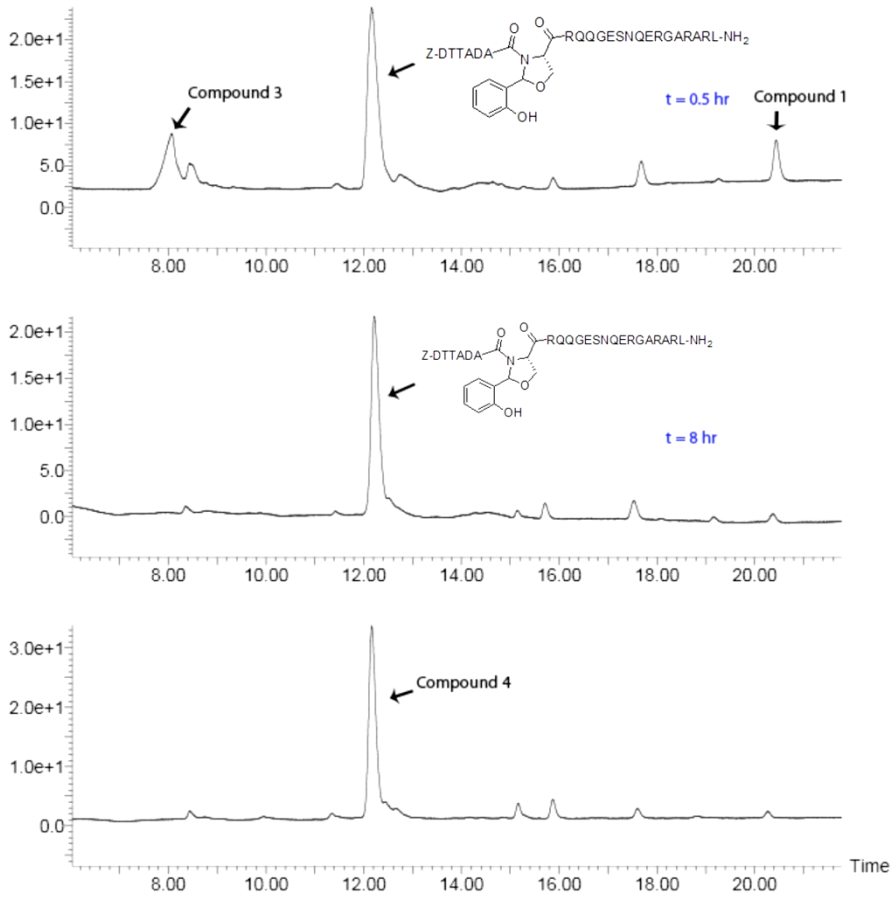
Spectrum 7. ESI calcd for $C_{37}H_{46}N_6O_{16}$ $[M+H]^+$ *m/z* = 831.79, found: 831.95.



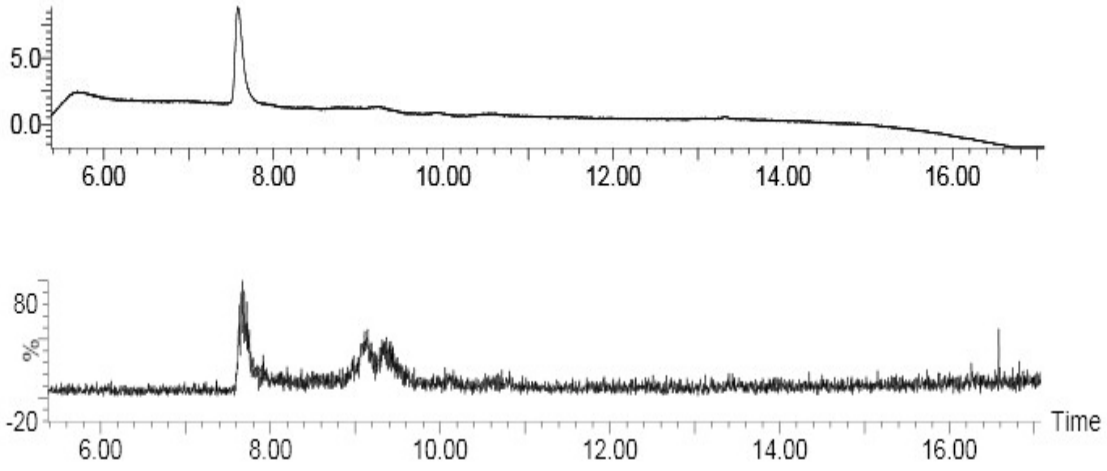
Spectrum 8. UV and MS traces from LC-MS analysis of compound **3**: gradient 5-50% CH_3CN/H_2O over 20 min followed by 50% isocratic CH_3CN/H_2O over another 10 min at a flow rate of 0.6 mL/min.



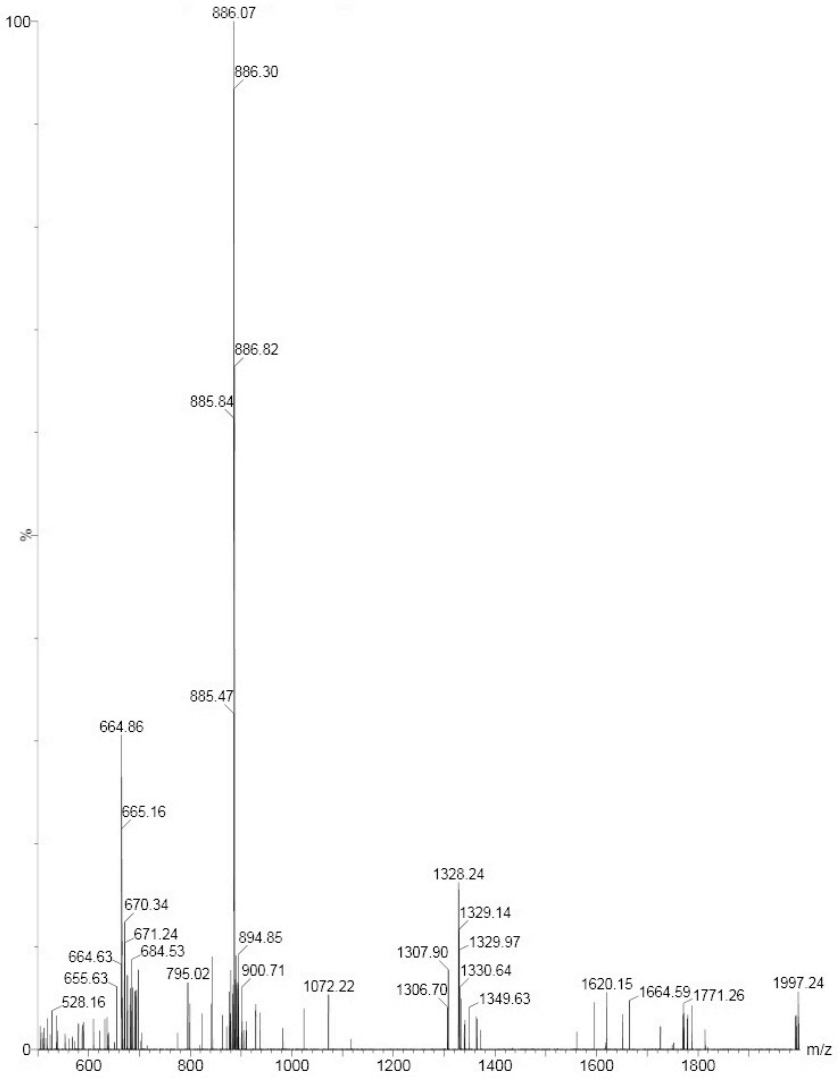
Spectrum 9. ESI calcd for $C_{75}H_{132}N_{34}O_{27}$ $[M+H]^{+}$ *m/z* = 1943.06 $[M+2H]^{2+}$ *m/z* = 972.03 $[M+3H]^{3+}$ *m/z* = 648.35 $[M+4H]^{4+}$ *m/z* = 486.52, found: 972.78, 649.47, 486.85.



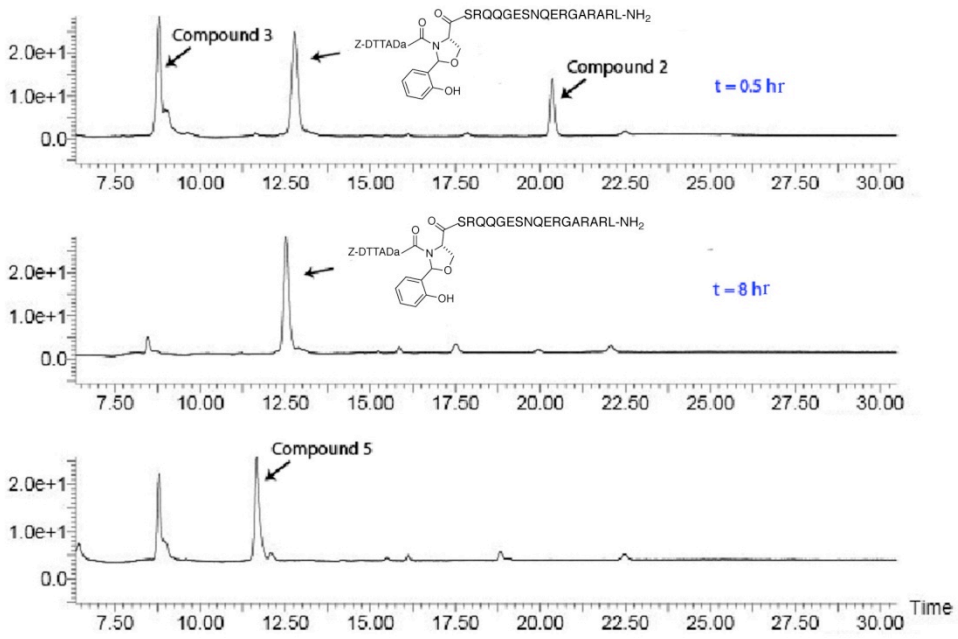
Spectrum 10. UV traces from LC-MS analysis of **serine ligation**: gradient 5-50% CH_3CN/H_2O over 20 min and 50-50% CH_3CN/H_2O over another 10 min at a flow rate of 0.6 mL/min.



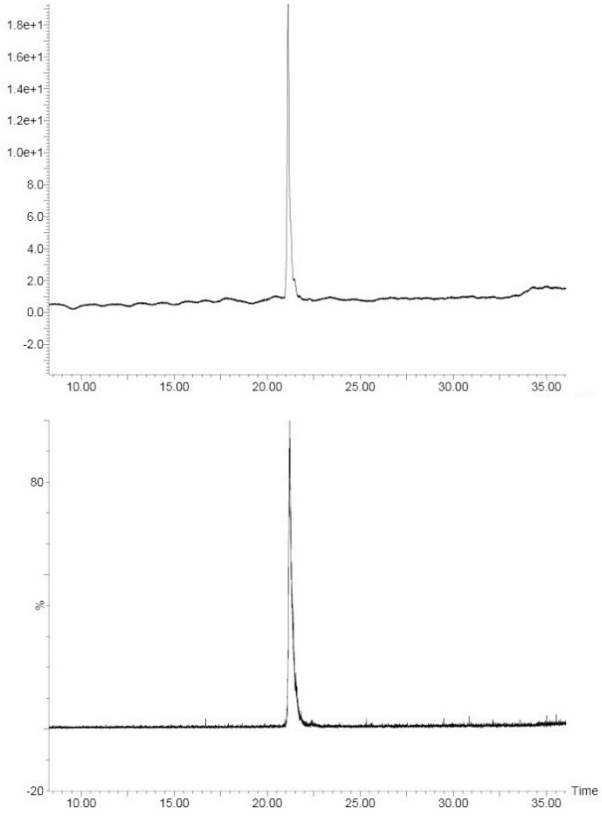
Spectrum 11. UV and MS traces from LC-MS analysis of purified compound **4**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



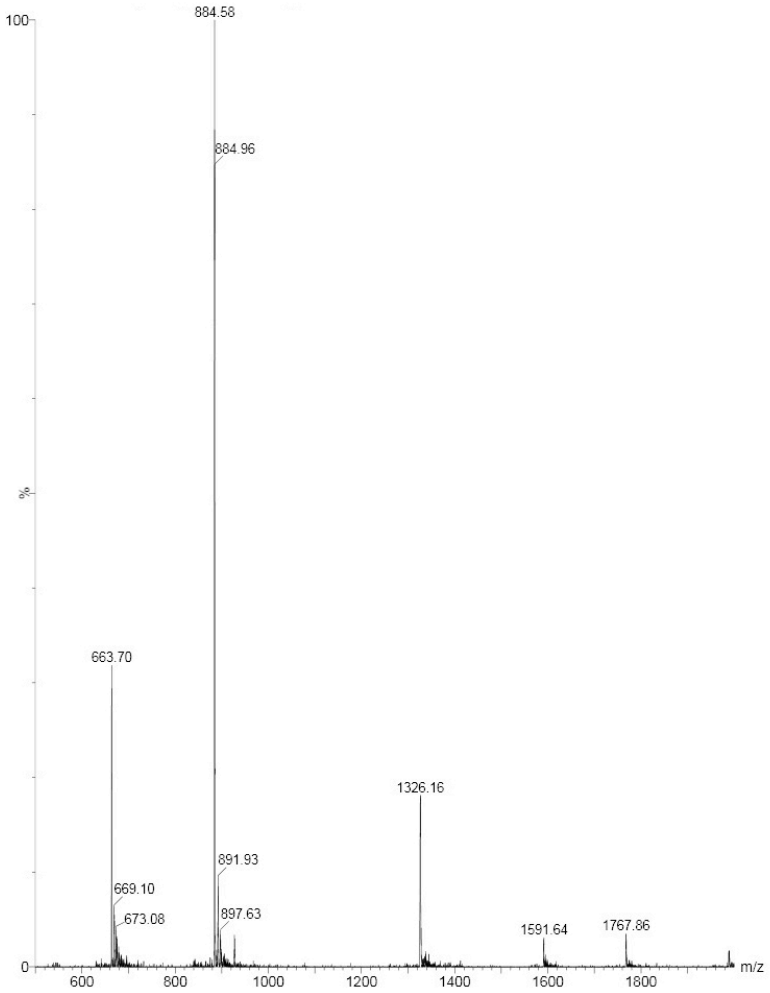
Spectrum 12. ESI calcd for $C_{105}H_{172}N_{40}O_{41}$ $[M+H]^+$ *m/z* = 2651.73 $[M+2H]^{2+}$ *m/z* = 1326.37 $[M+3H]^{3+}$ *m/z* = 884.58 $[M+4H]^{4+}$ *m/z* = 663.68, found: 1328.24, 886.07, 664.86.



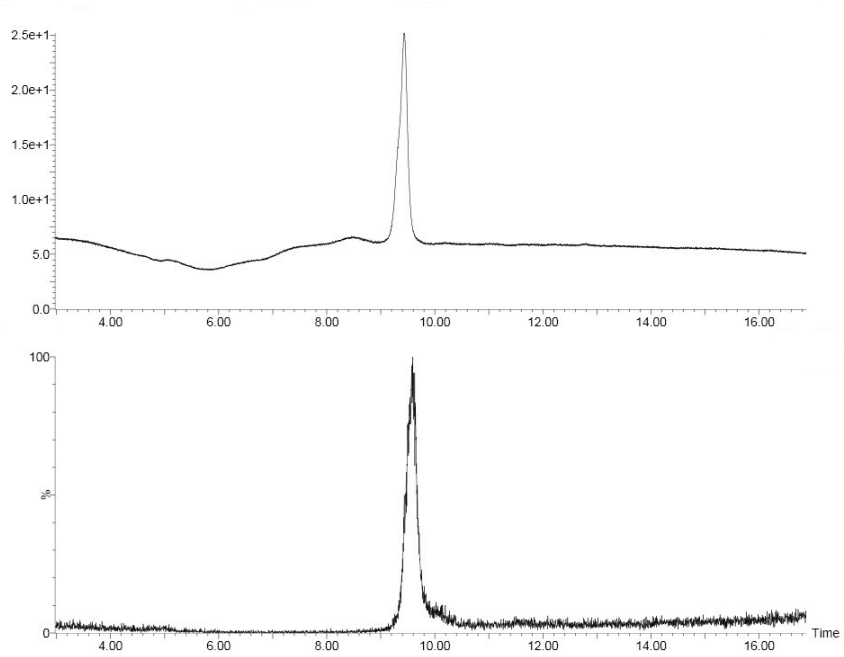
Spectrum 13. UV traces from LC-MS analysis of **serine ligation**: gradient 5-50% CH_3CN/H_2O over 20 min followed by 50% isocratic CH_3CN/H_2O over another 10 min at a flow rate of 0.6 mL/min.



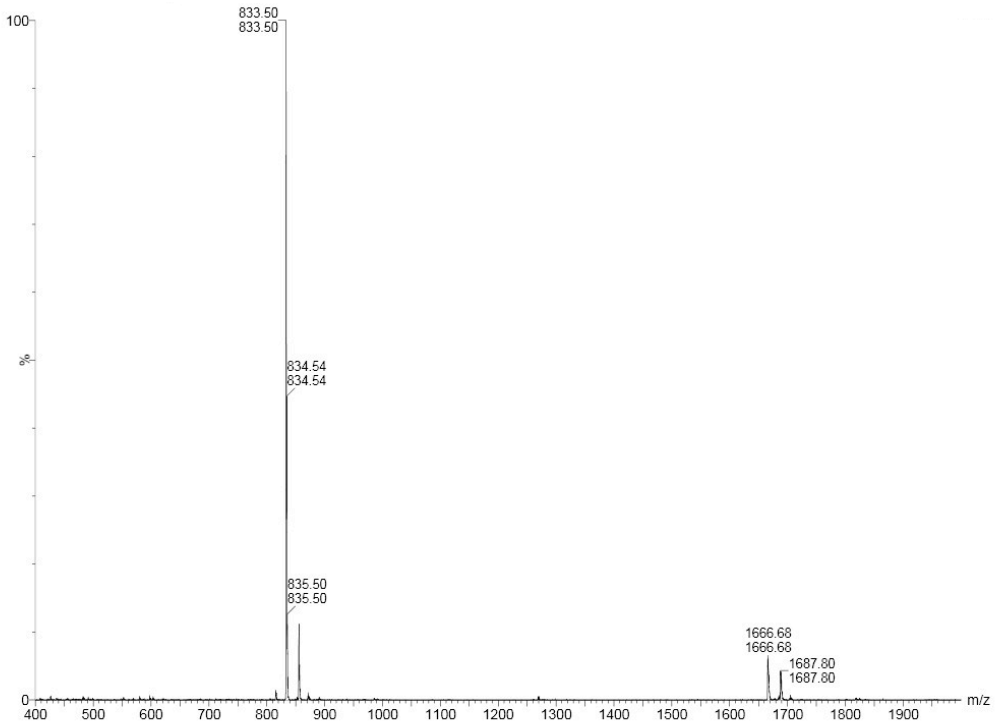
Spectrum 14. UV and MS traces from LC-MS analysis of purified compound **5**: gradient 5-30% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min.



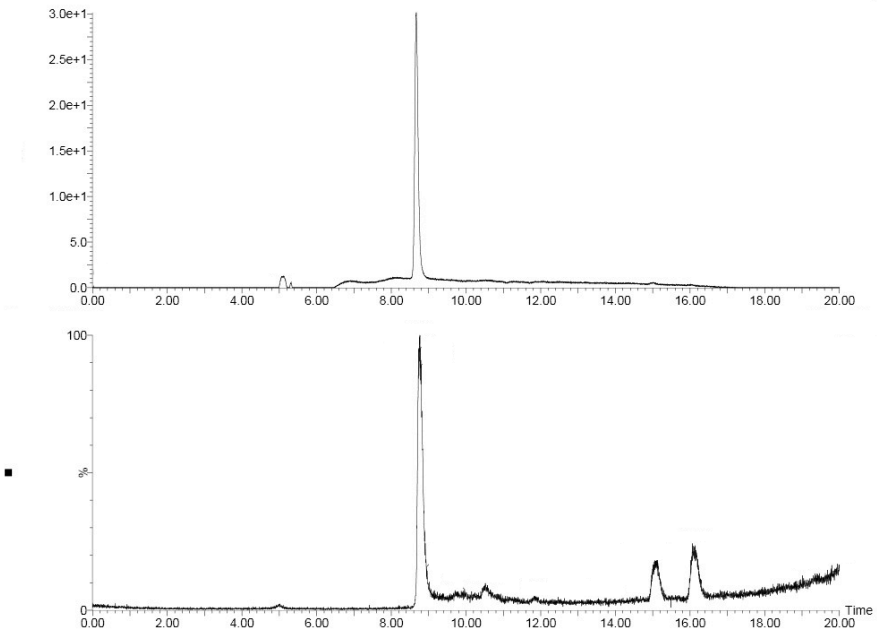
Spectrum 15. ESI calcd for $C_{105}H_{172}N_{40}O_{41}$ $[M+H]^+$ *m/z* = 2651.73 $[M+2H]^{2+}$ *m/z* = 1326.37 $[M+3H]^{3+}$ *m/z* = 884.58 $[M+4H]^{4+}$ *m/z* = 663.68, found: 1326.16, 884.58, 663.70.



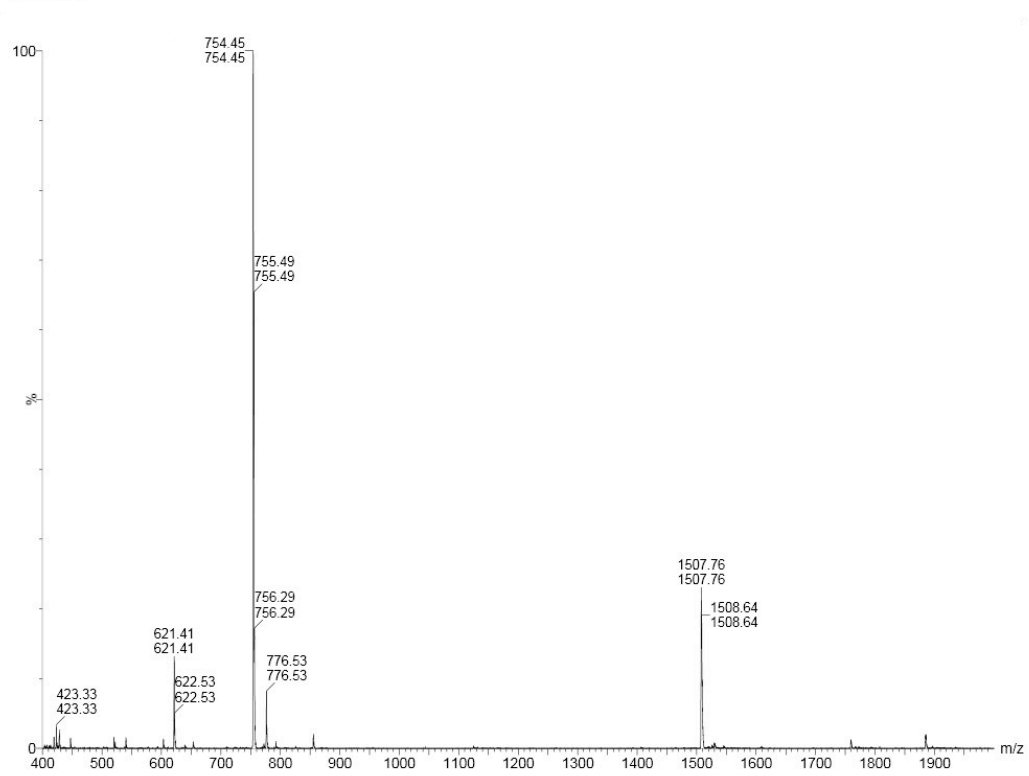
Spectrum 16. UV and MS traces from LC-MS analysis of purified compound **6**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



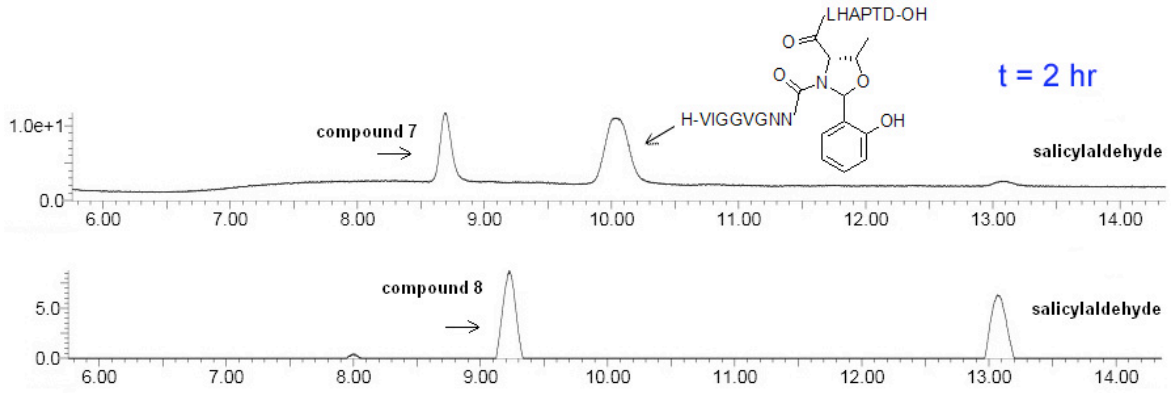
Spectrum 17. ESI calcd for $C_{37}H_{56}N_{10}O_{12}$ $[M+H]^+$ *m/z* =833.90, found: 833.50.



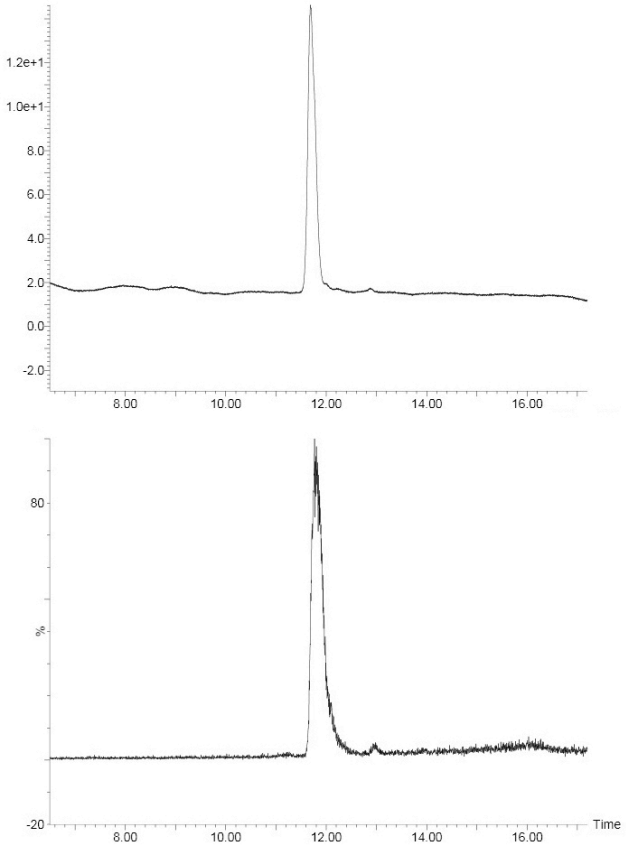
Spectrum 18. UV and MS traces from LC-MS analysis of purified compound **7**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



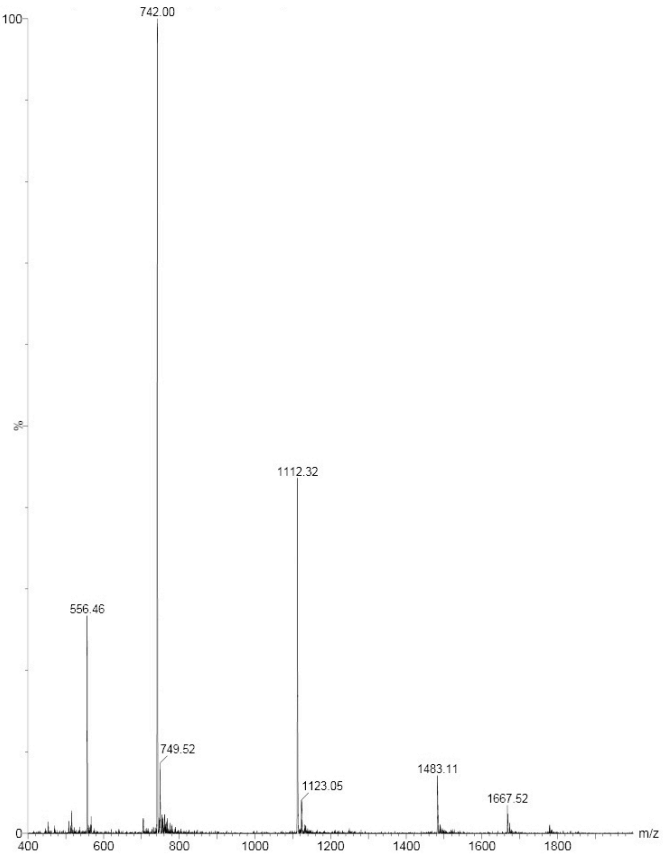
Spectrum 19. ESI calcd for $C_{32}H_{51}N_9O_{12}$ $[M+H]^+$ *m/z* =754.80, found: 754.45.



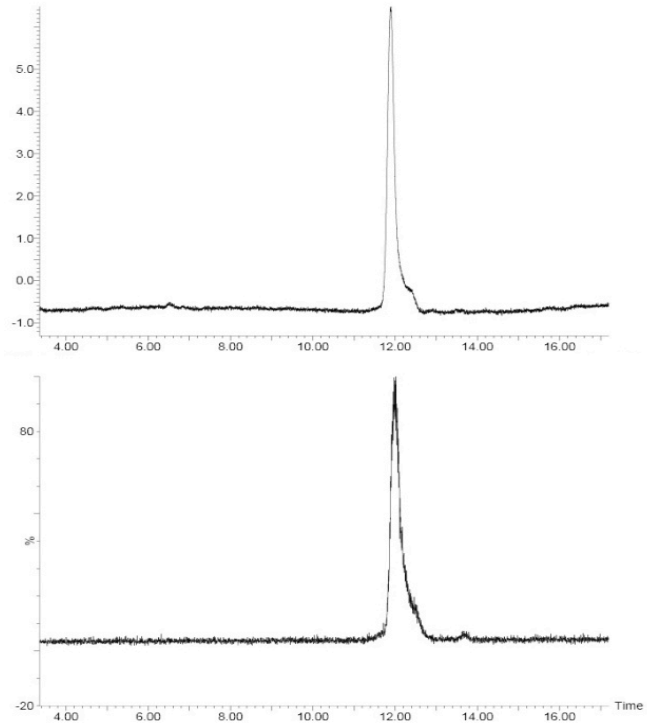
Spectrum 20. UV traces from LC-MS analysis of t**hreonine ligation**: gradient 5-95% CH_3CN/H_2O over 15min at a flow rate of 0.6 mL/min.



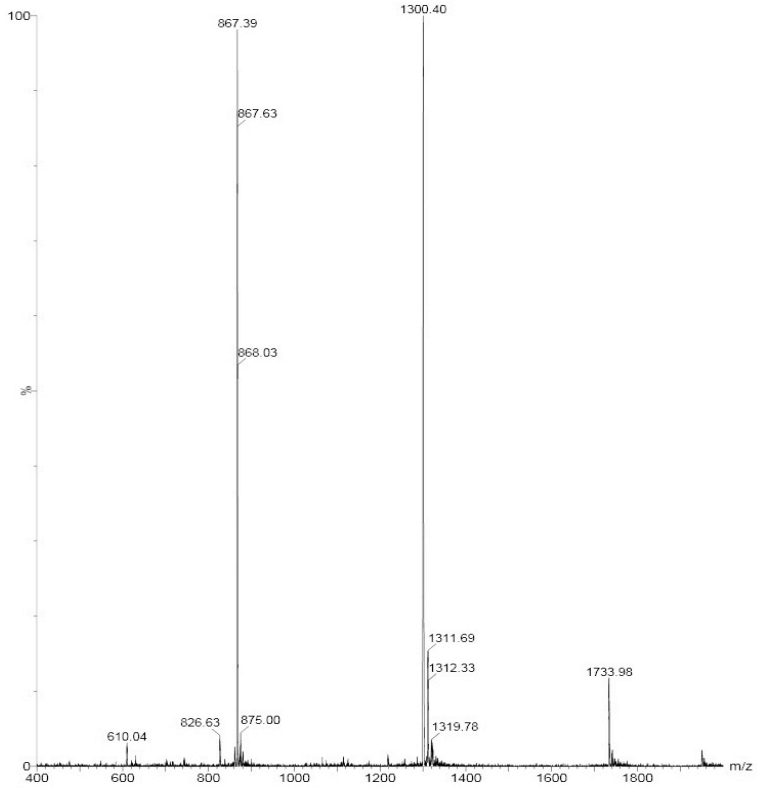
Spectrum 21. UV and MS traces from LC-MS analysis of purified compound **9**: gradient 5-50% CH_3CN/H_2O over 20 min and 50-50% CH_3CN/H_2O over another 10 min at a flow rate of 0.6 mL/min.



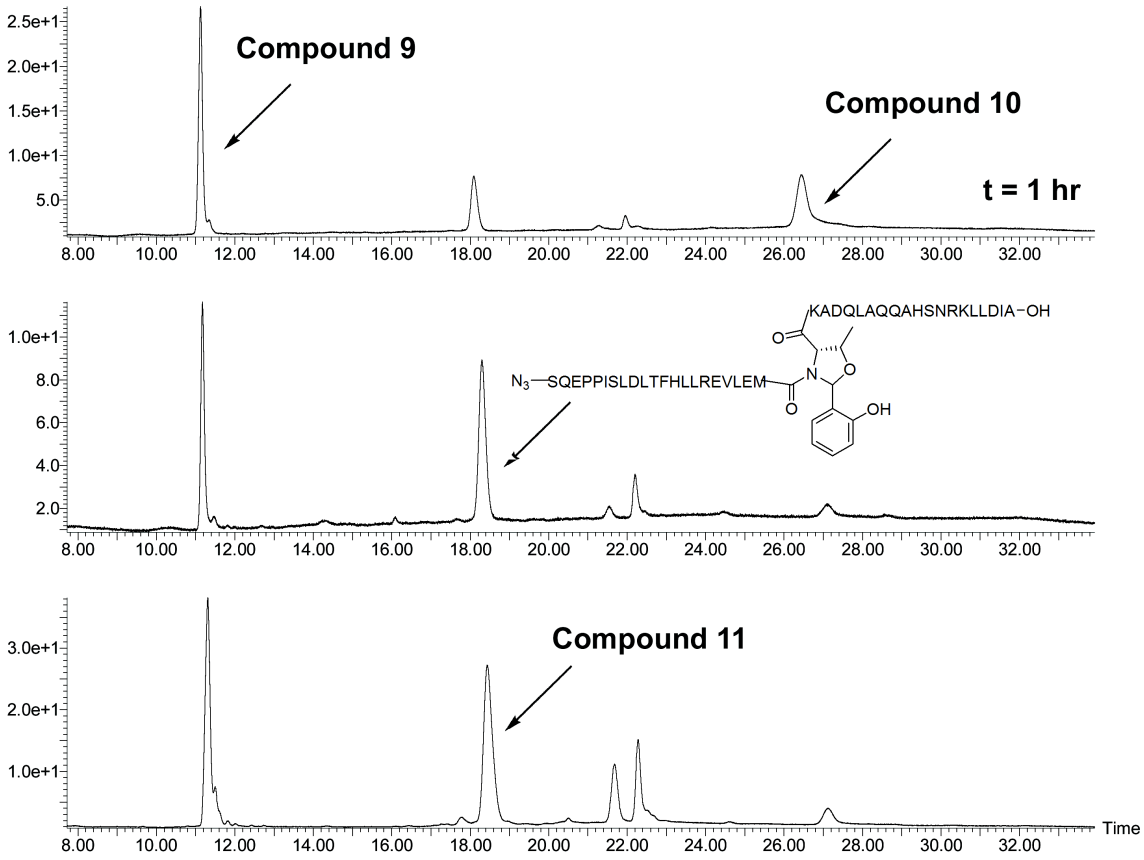
Spectrum 22. ESI calcd for $C_{94}H_{161}N_{31}O_{31}$ $[M+H]^+$ *m/z* = 2222.47 $[M+2H]^{2+}$ *m/z* = 1111.74 $[M+3H]^{3+}$ *m/z* = 741.49 $[M+4H]^{4+}$ *m/z* = 556.37, found: 1112.32, 742.00, 556.46.



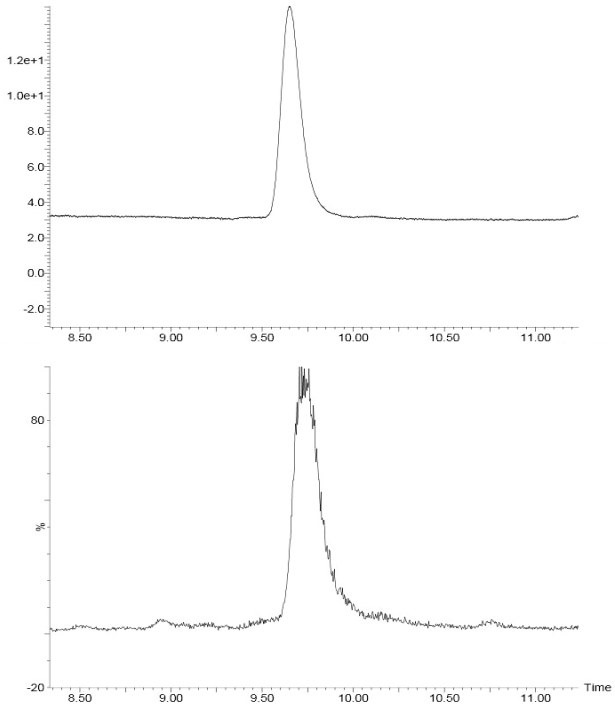
Spectrum 23. UV and MS traces from LC-MS analysis of purified compound **10**: gradient 40-60% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



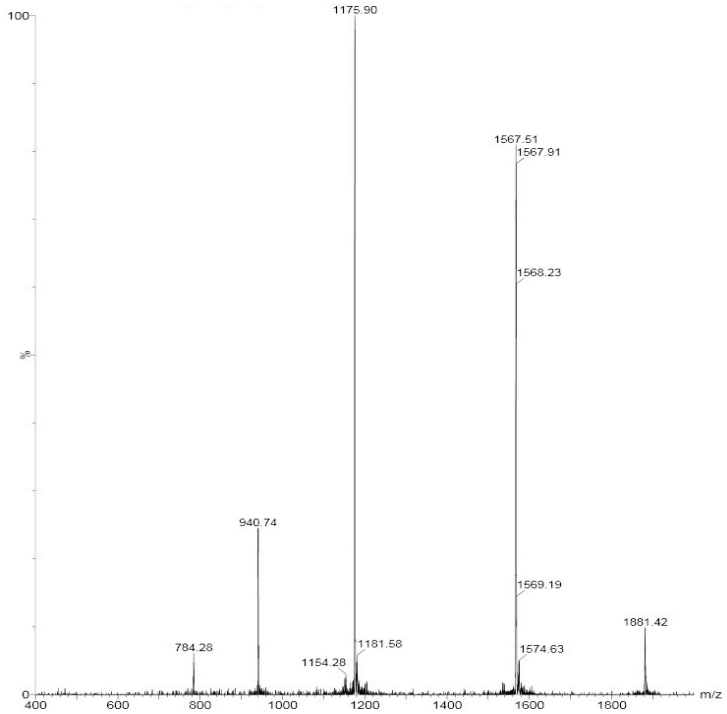
Spectrum 24. ESI calcd for $C_{118}H_{181}N_{29}O_{35}S$ $[M+H]^+$ *m/z* = 2598.94 $[M+2H]^{2+}$ *m/z* = 1299.97 $[M+3H]^{3+}$ *m/z* = 866.98, found: 1300.40, 867.39.



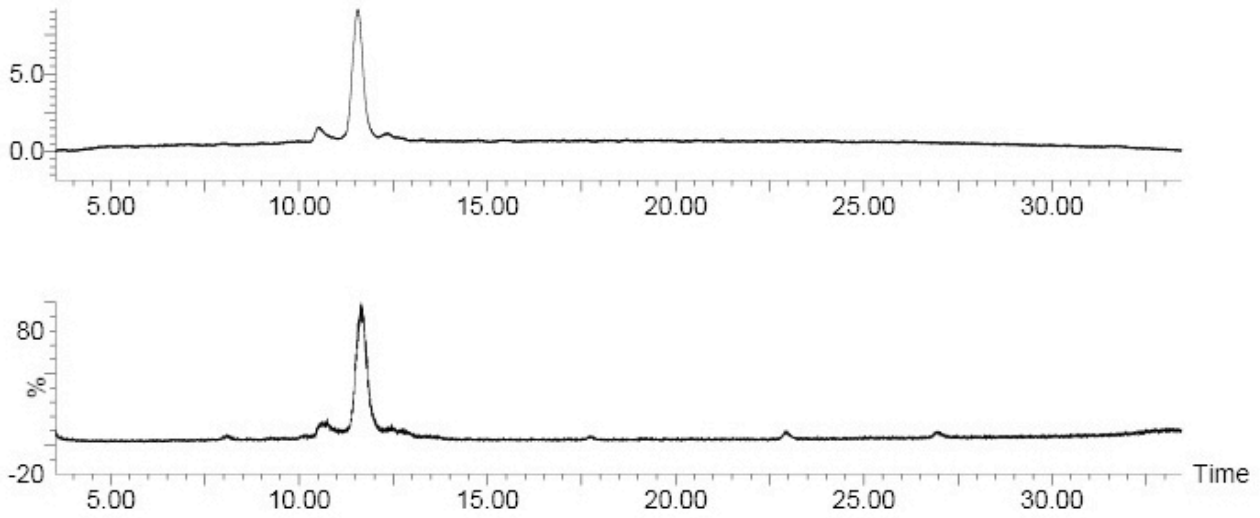
Spectrum 25. UV traces from LC-MS analysis of **threonine ligation**: gradient 5-50% CH_3CN/H_2O over 20 min followed by 50% isocratic CH_3CN/H_2O over another 10 min at a flow rate of 0.6 mL/min.



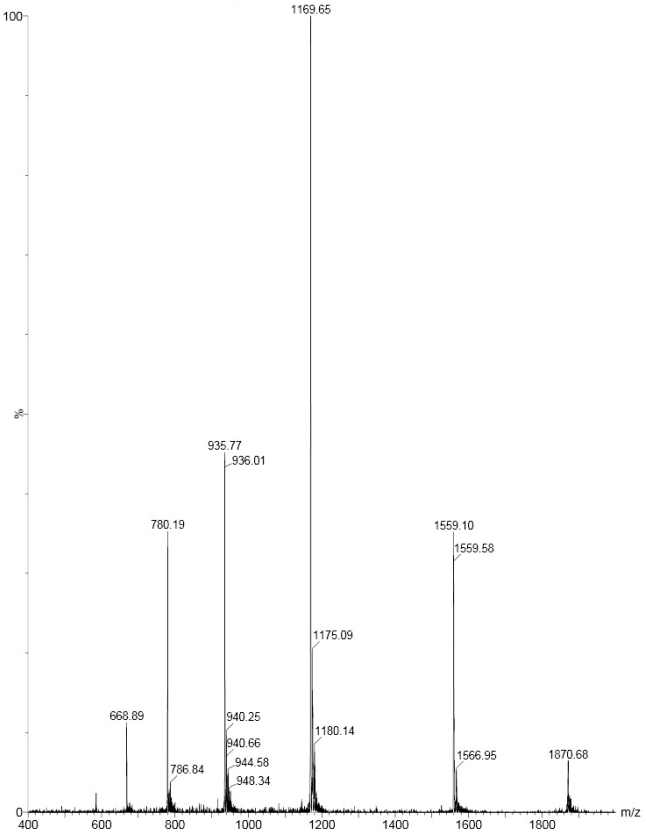
Spectrum 26. UV and MS traces from LC-MS analysis of purified compound **11**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



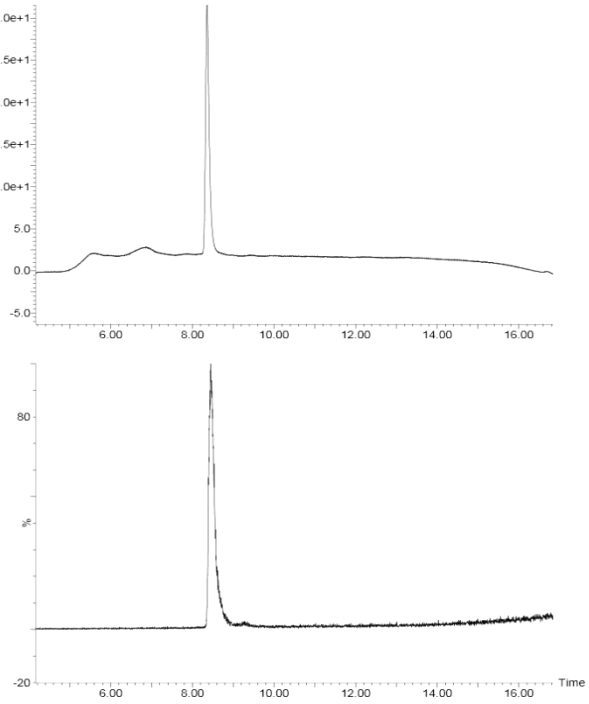
Spectrum 27. ESI calcd for $C_{205}H_{336}N_{60}O_{64}S$ $[M+H]^+$ *m/z* = 4698.29 $[M+3H]^{3+}$ *m/z* = 1566.76 $[M+4H]^{4+}$ *m/z* = 1175.32 $[M+5H]^{5+}$ *m/z* = 940.46 $[M+6H]^{6+}$ *m/z* = 783.88, found: 1567.51, 1175.90, 940.74, 784.28.



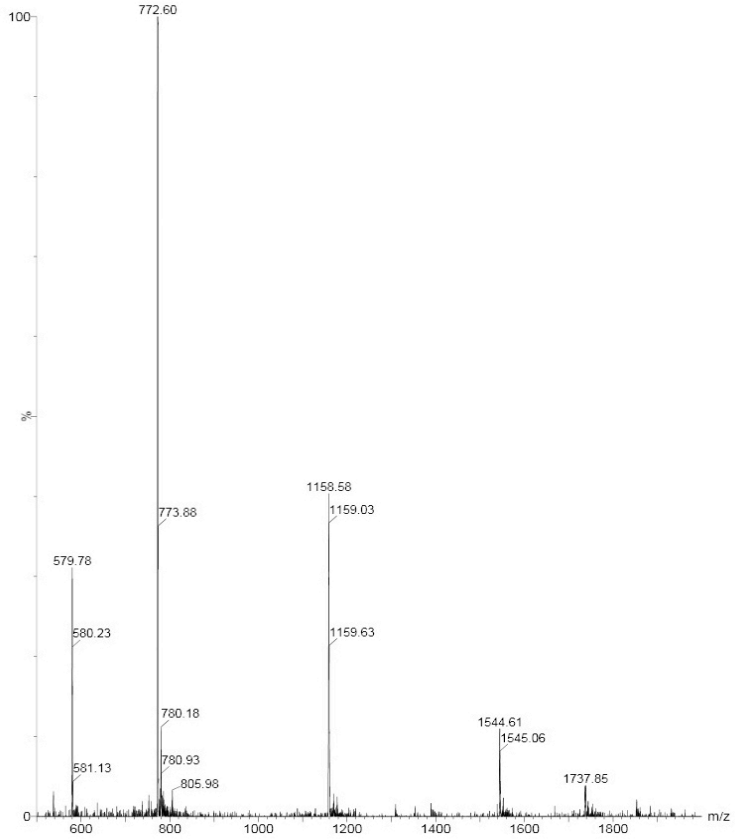
Spectrum 28. UV and MS traces from LC-MS analysis of crude compound **12**: gradient 25-70% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min.



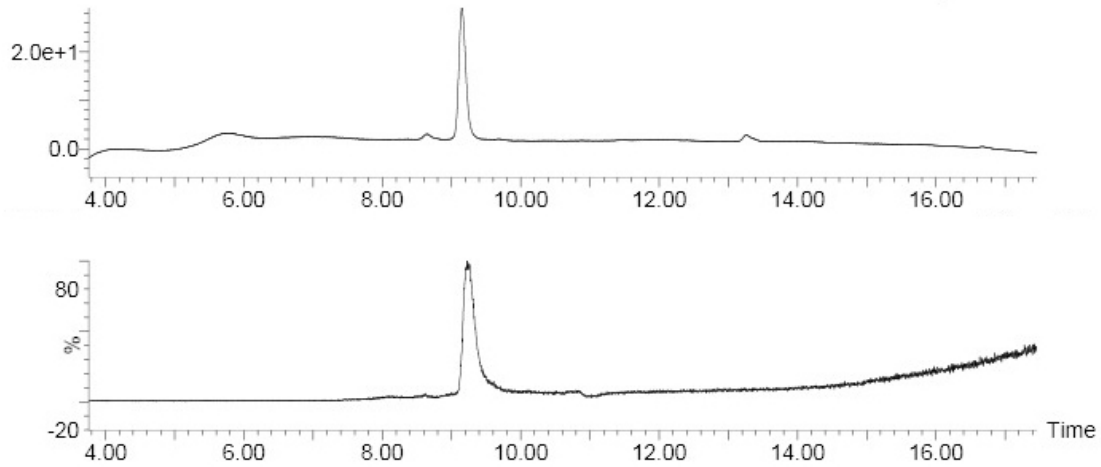
Spectrum 29. ESI calcd for $C_{205}H_{338}N_{58}O_{64}S$ $[M+H]^+$ *m/z* = 4672.29 $[M+3H]^{3+}$ *m/z* = 1558.10 $[M+4H]^{4+}$ *m/z* = 1168.82 $[M+5H]^{5+}$ *m/z* = 935.26 $[M+6H]^{6+}$ *m/z* = 779.55 $[M+7H]^{7+}$ *m/z* = 668.33, found: 1559.10, 1169.65, 935.77, 780.19, 668.89.



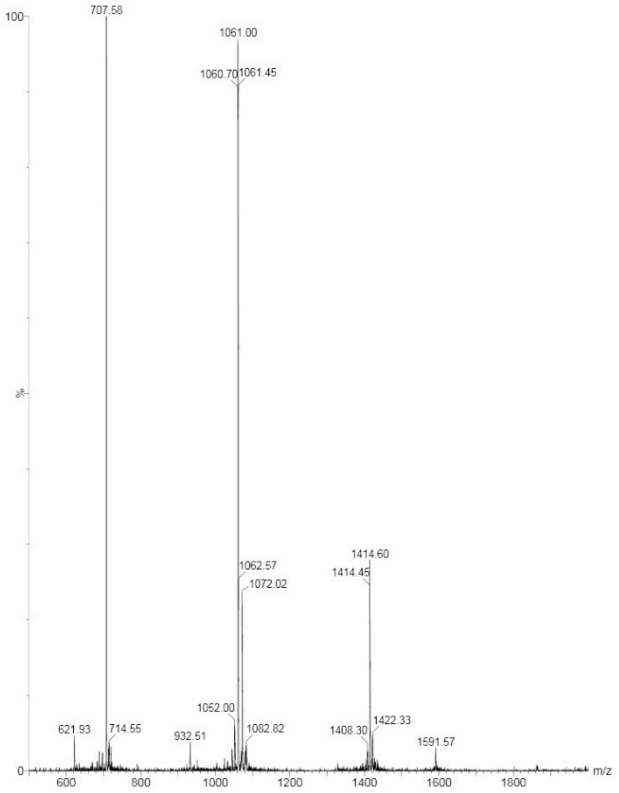
Spectrum 30. UV and MS traces from LC-MS analysis of purified compound **13**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



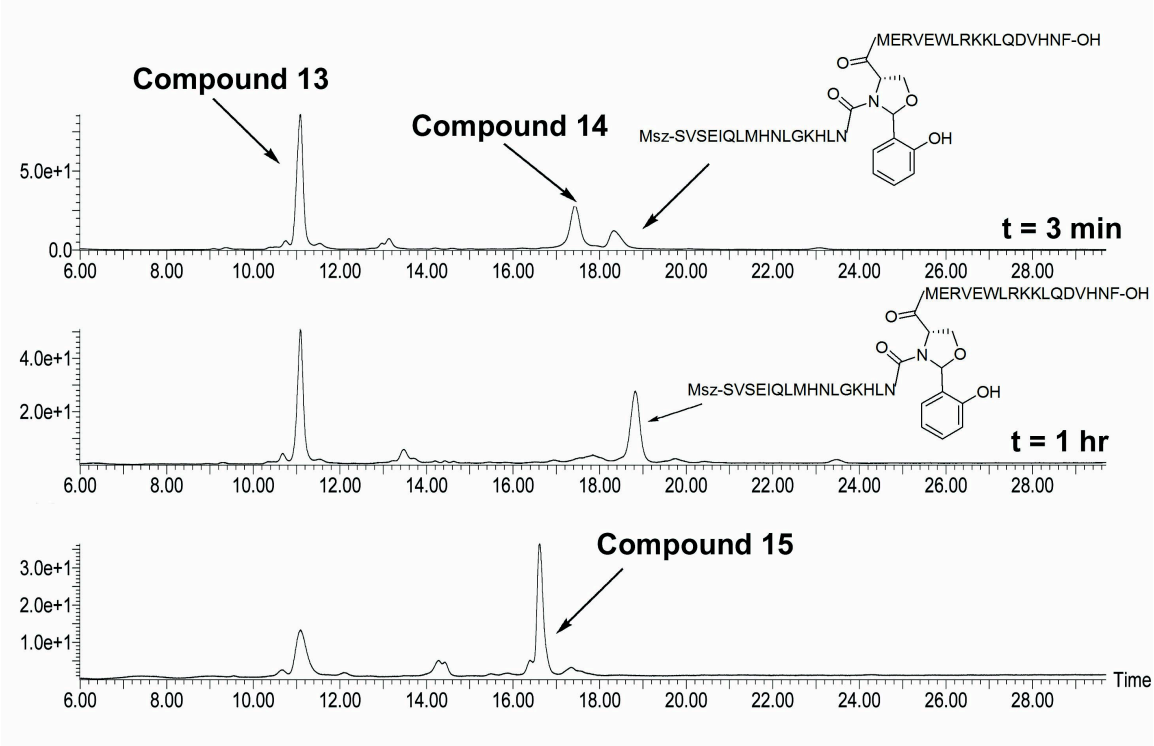
Spectrum 31. ESI calcd for $C_{103}H_{163}N_{31}O_{28}S$ $[M+H]^+$ *m/z* = 2316.65 $[M+2H]^{2+}$ *m/z* = 1158.83 $[M+3H]^{3+}$ *m/z* = 772.88 $[M+4H]^{4+}$ *m/z* = 579.91, found: 1158.58, 772.60, 579.78.



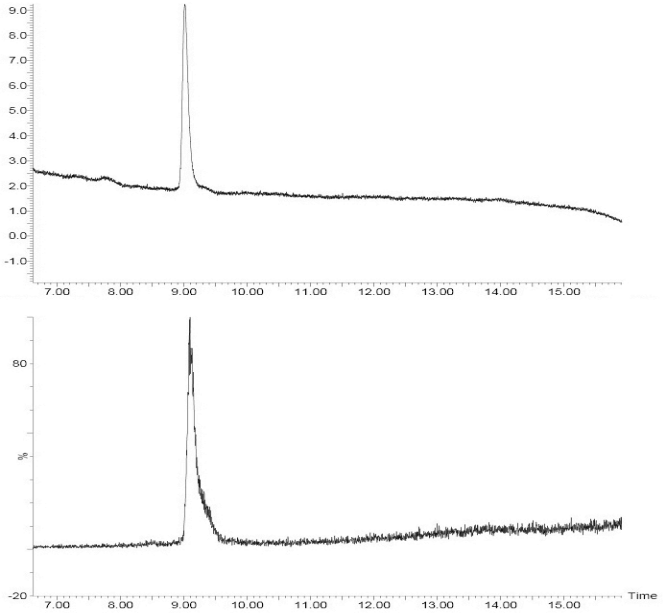
Spectrum 32. UV and MS traces from LC-MS analysis of purified compound **14**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



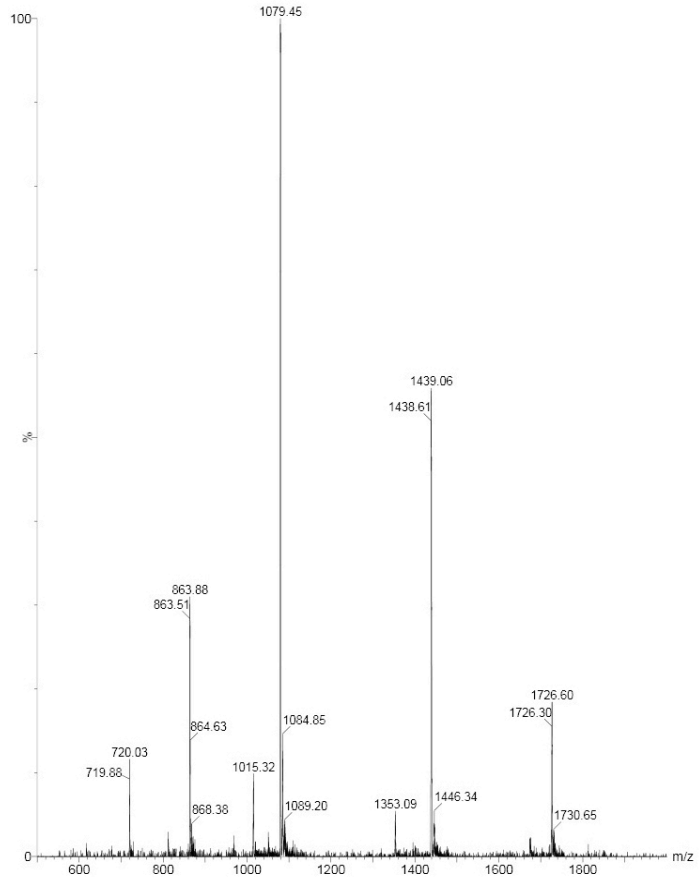
Spectrum 33. ESI calcd for $C_{94}H_{142}N_{24}O_{28}S_2$ $[M+H]^+$ *m/z* = 2121.41 $[M+2H]^{2+}$ *m/z* = 1061.20 $[M+3H]^{3+}$ *m/z* = 707.80, found: 1061.00, 707.58.



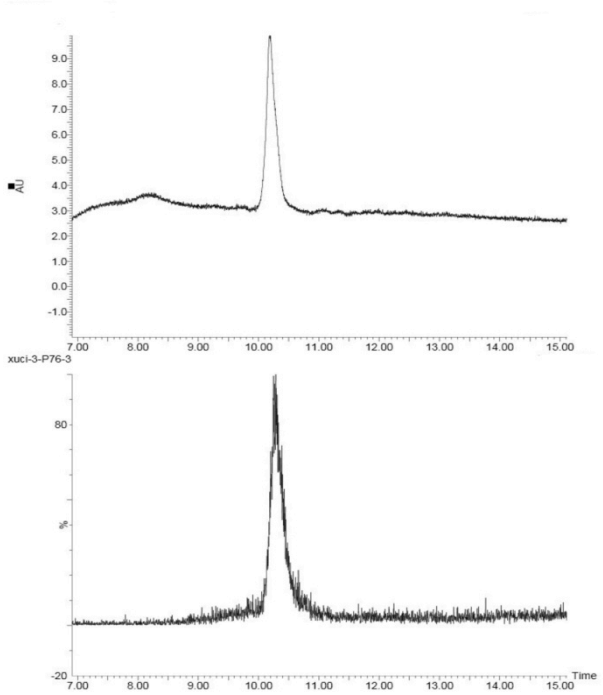
Spectrum 34. UV traces from LC-MS analysis of **serine ligation**: gradient 20-45% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min.



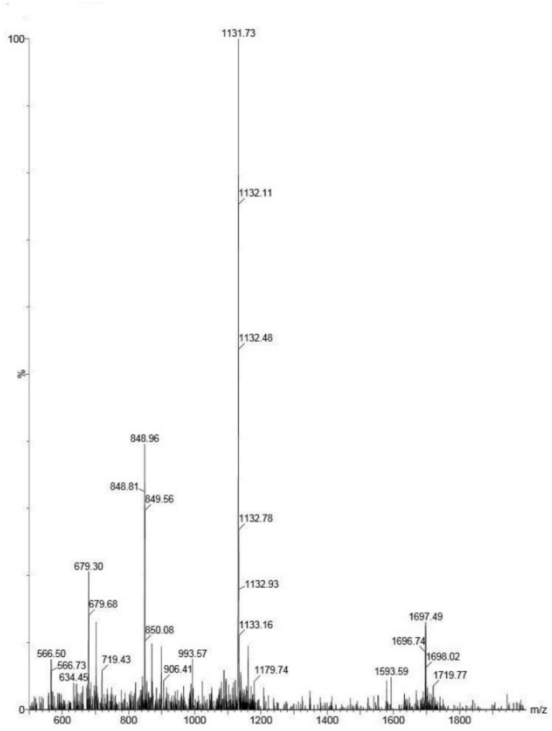
Spectrum 35. UV and MS traces from LC-MS analysis of purified compound **15**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



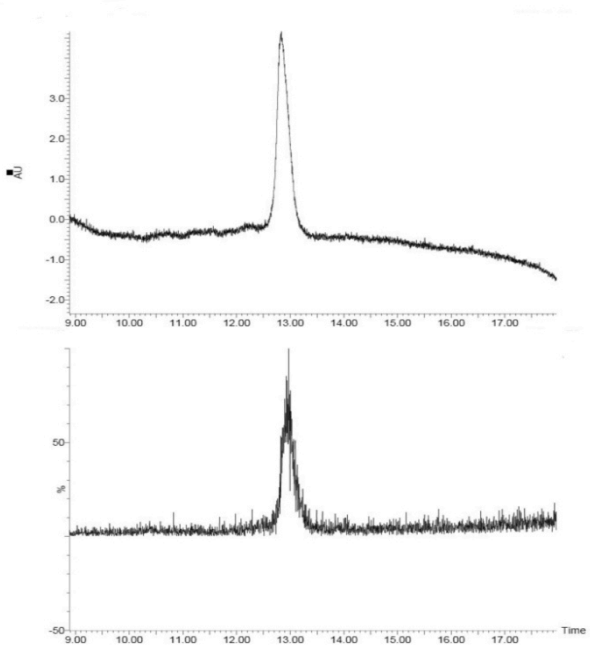
Spectrum 36. ESI calcd for $C_{190}H_{299}N_{55}O_{54}S_3$ $[M+H]^+$ *m/z* = 4314.94 $[M+3H]^{3+}$ *m/z* = 1438.98 $[M+4H]^{4+}$ *m/z* = 1079.48 $[M+5H]^{5+}$ *m/z* = 863.79 $[M+6H]^{6+}$ *m/z* = 719.99, found: 1439.06, 1079.45, 863.88, 720.03.



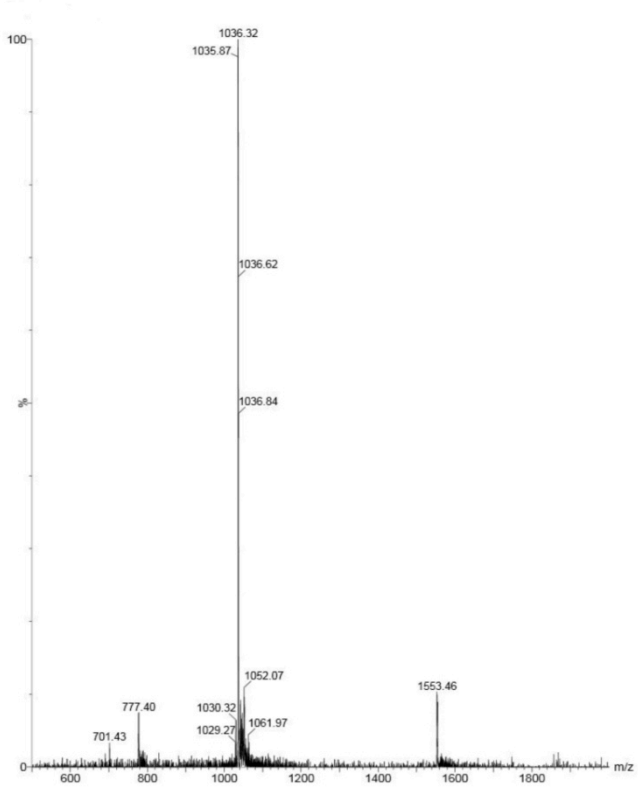
Spectrum 37. UV and MS traces from LC-MS analysis of purified compound **16**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



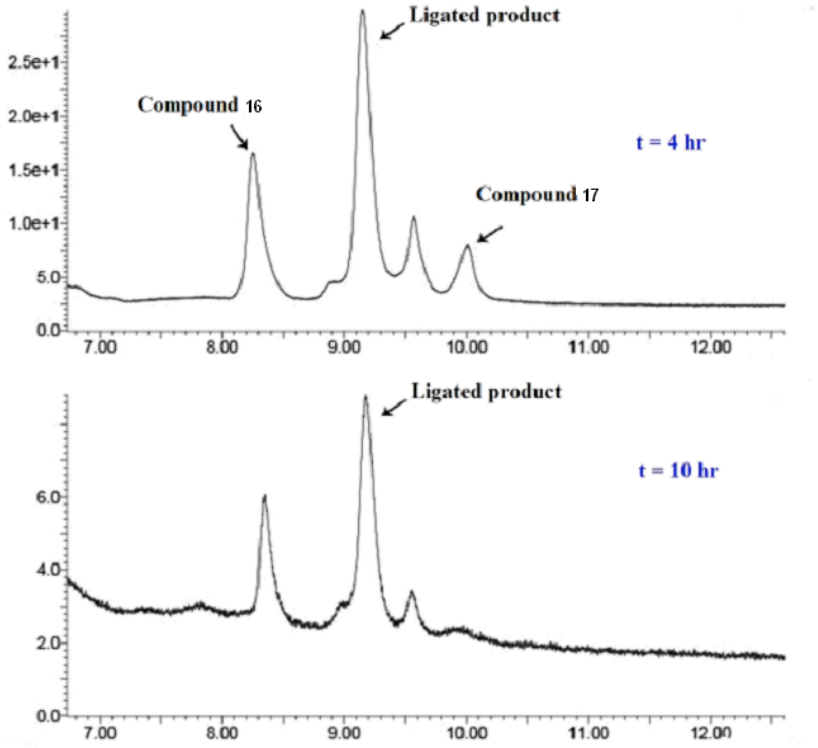
Spectrum 38. ESI calcd for $C_{154}H_{244}N_{40}O_{46}$ $[M+2H]^{2+}$ *m/z* = 1696.92 $[M+3H]^{3+}$ *m/z* = 1131.61 $[M+4H]^{4+}$ *m/z* = 848.96 $[M+5H]^{5+}$ *m/z* = 679.37, $[M+6H]^{6+}$ *m/z* = 566.30, found: 1697.49, 1131.73, 848.96, 679.30, 566.50.



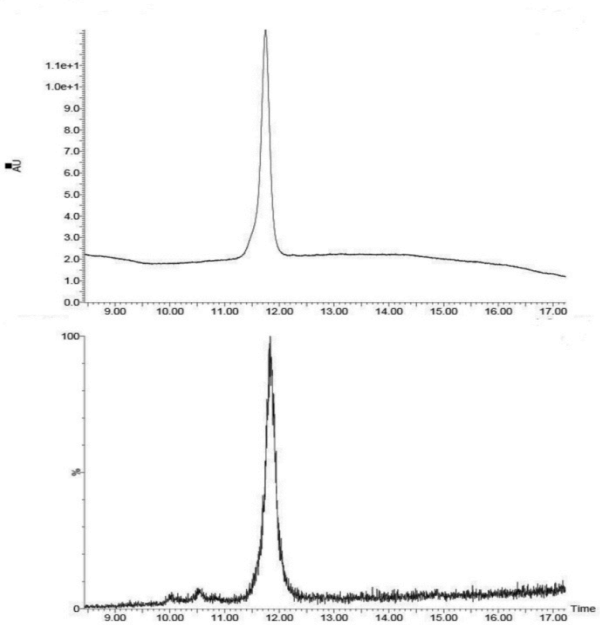
Spectrum 39. UV and MS traces from LC-MS analysis of compound **17**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



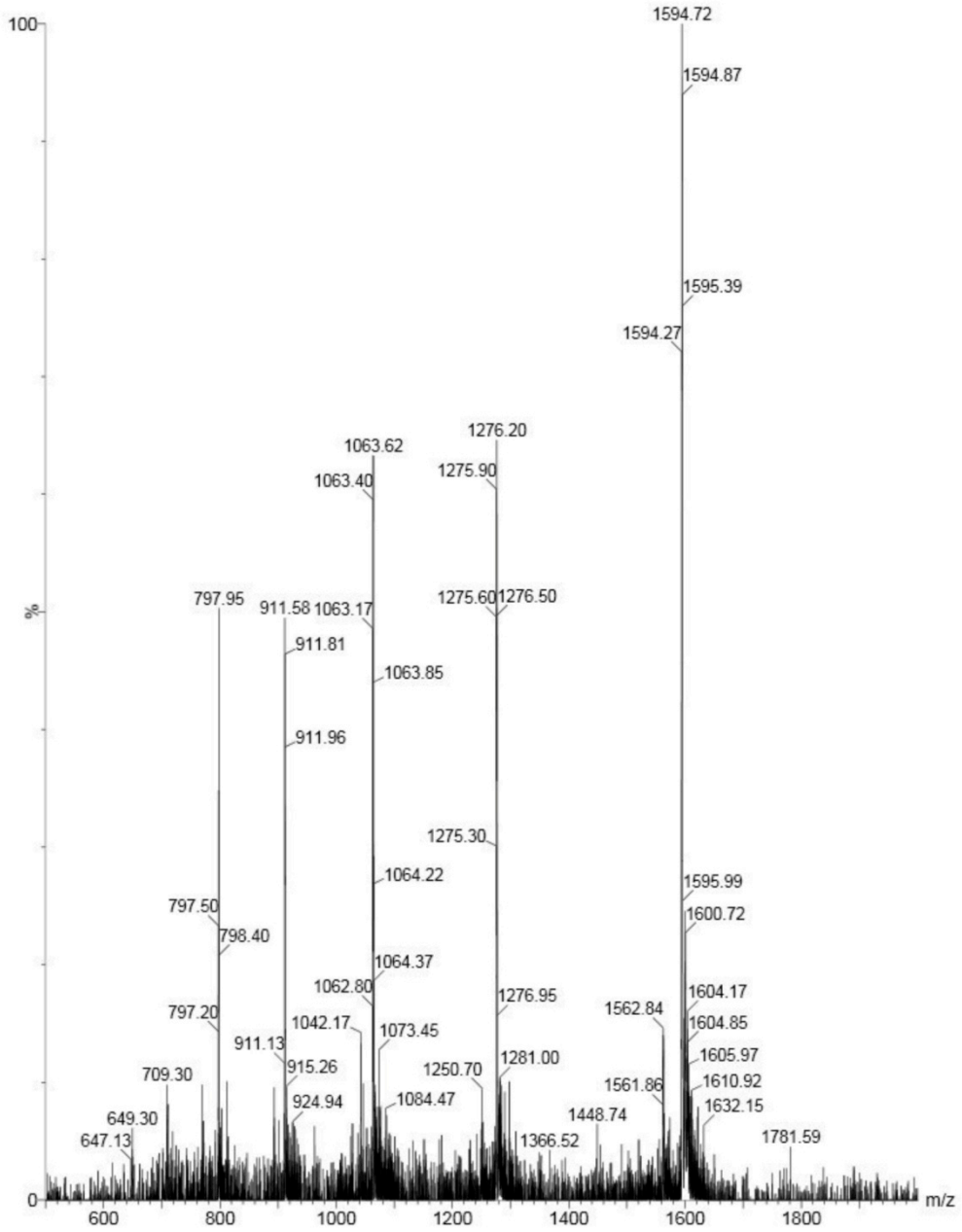
Spectrum 40. ESI calcd for $C_{142}H_{210}N_{38}O_{39}S$ $[M+2H]^{2+}$ m/z = 1553.74 $[M+3H]^{3+}$ m/z = 1036.16 $[M+4H]^{4+}$ m/z = 777.37, found: 1553.46, 1036.32, 777.40.



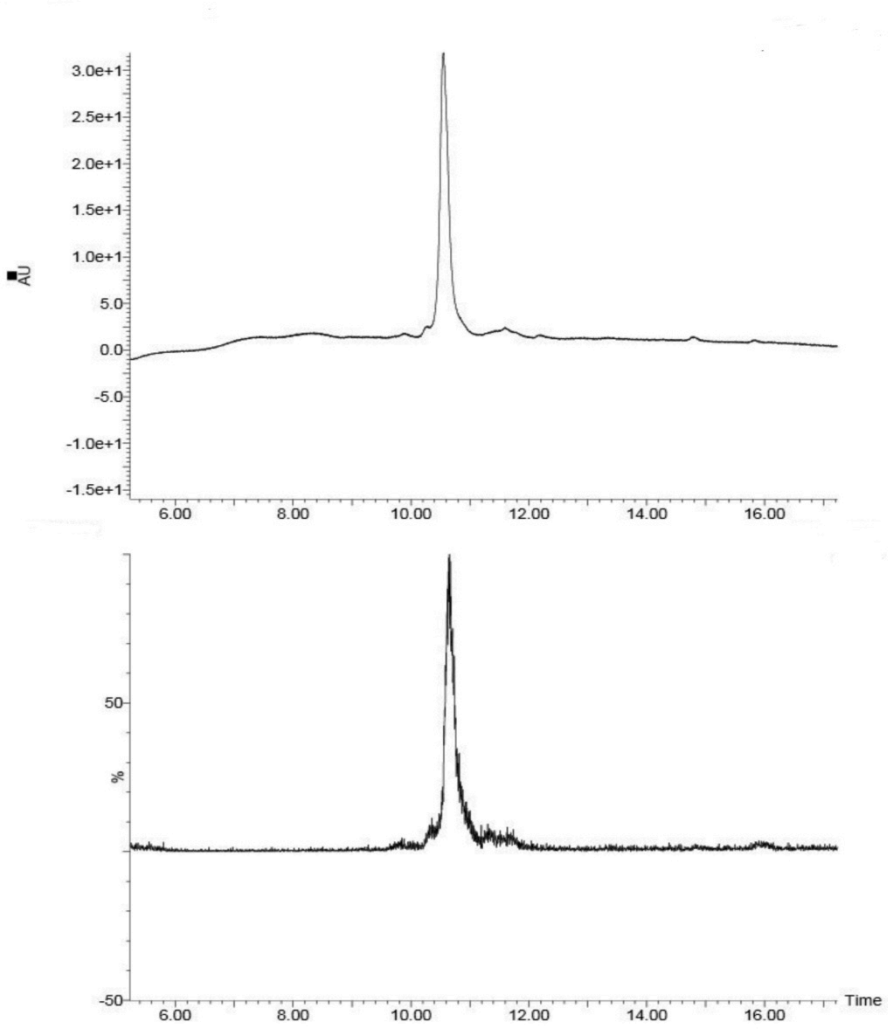
Spectrum 41. UV traces from LC-MS analysis of **serine ligation**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



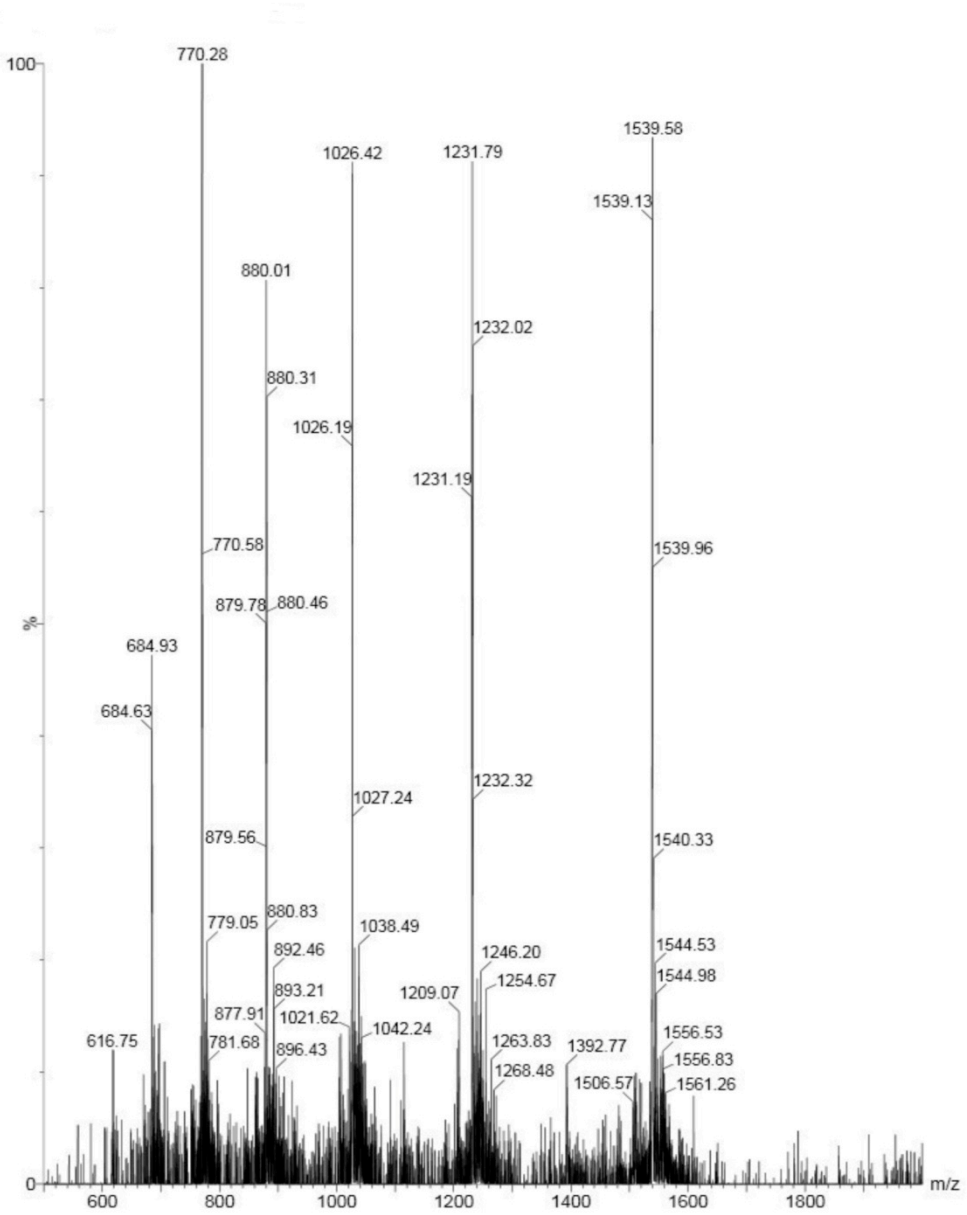
Spectrum 42. UV and MS traces from LC-MS analysis of purified compound **18**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



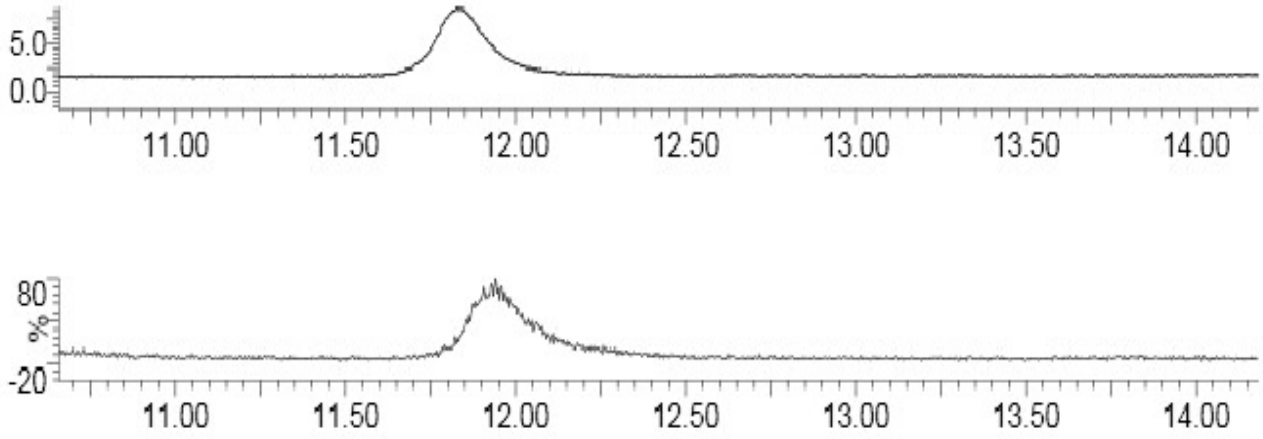
Spectrum 43. ESI calcd for $C_{289}H_{448}N_{78}O_{83}S$ $[M+4H]^{4+}$ m/z = 1594.80 $[M+5H]^{5+}$ m/z = 1276.04 $[M+6H]^{6+}$ m/z = 1063.53 $[M+7H]^{7+}$ m/z = 911.74 $[M+8H]^{8+}$ m/z = 797.90 $[M+9H]^{9+}$ m/z = 709.35 found: 1574.72, 1276.20, 1063.62, 911.58, 797.95, 709.35.



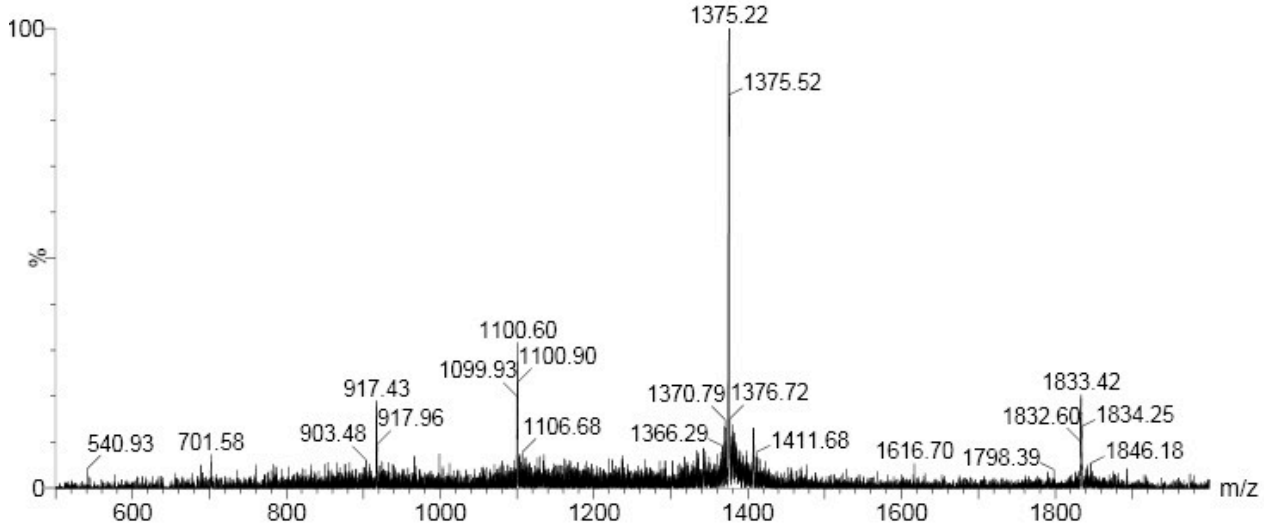
Spectrum 44. UV and MS traces from LC-MS analysis of compound **19**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



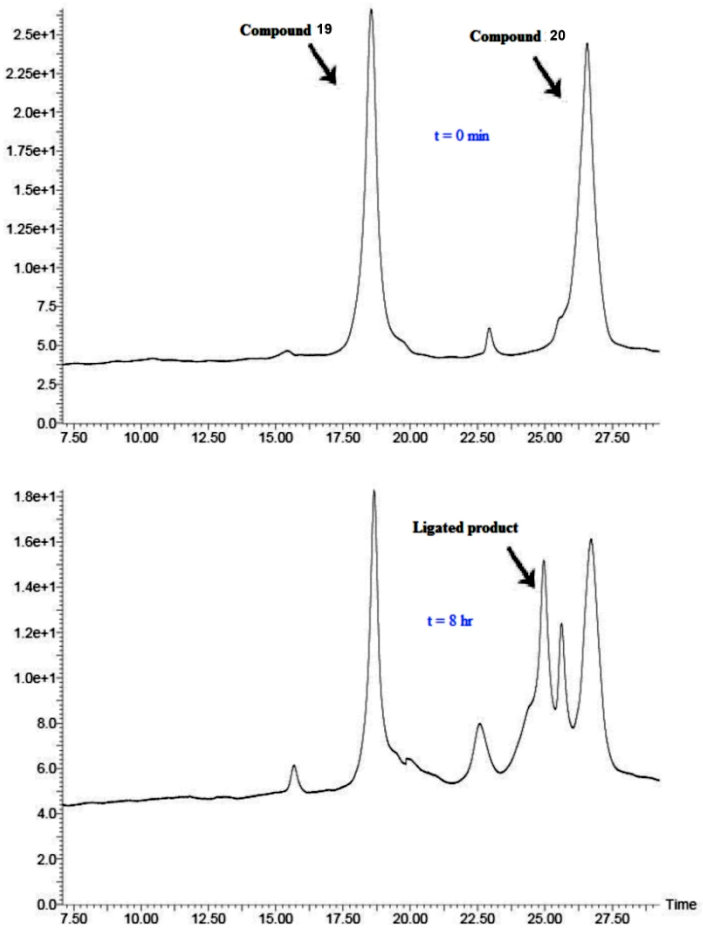
Spectrum 45. ESI calcd for $C_{274}H_{438}N_{78}O_{81}S$ $[M+4H]^{4+}$ m/z = 1539.24 $[M+5H]^{5+}$ m/z = 1231.59 $[M+6H]^{6+}$ m/z = 1026.49 $[M+7H]^{7+}$ m/z = 879.99 $[M+8H]^{8+}$ m/z = 770.12 $[M+9H]^{9+}$ m/z = 684.66 $[M+10H]^{10+}$ m/z = 616.30, found: 1539.58, 1231.79, 1026.42, 880.01, 770.28, 684.93, 616.75.



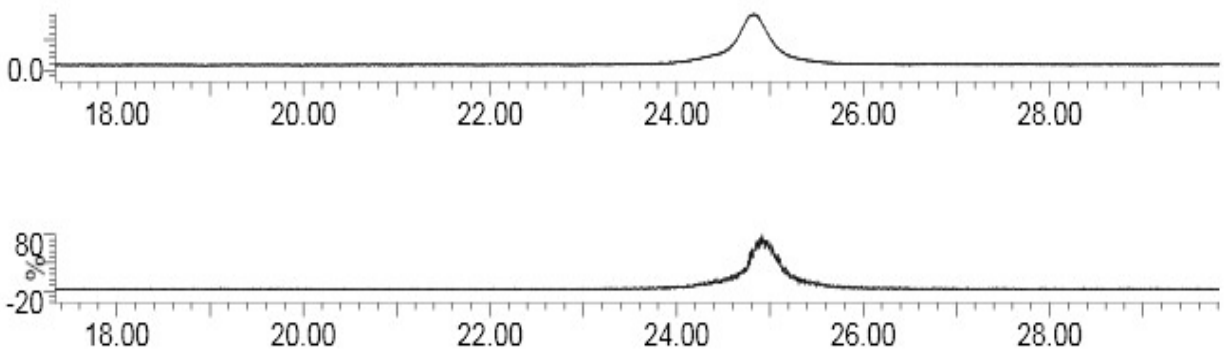
Spectrum 46. UV and MS traces from LC-MS analysis of purified compound **20**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



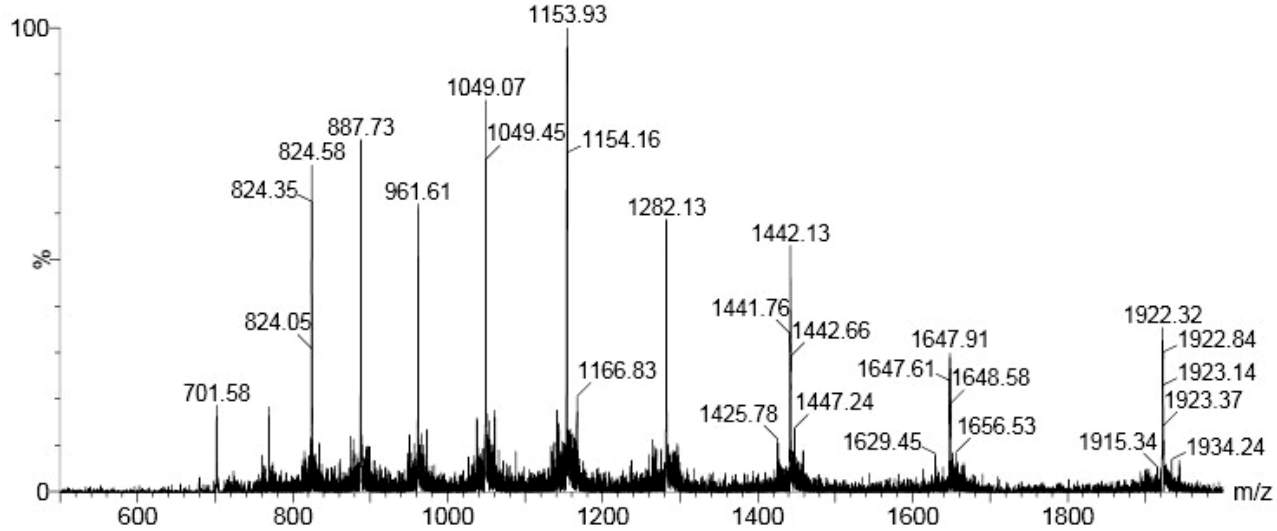
Spectrum 47. ESI calcd for $C_{253}H_{375}N_{63}O_{75}$ $[M+3H]^{3+}$ *m/z* = 1834.02 $[M+4H]^{4+}$ *m/z* = 1375.77 $[M+5H]^{5+}$ *m/z* = 1100.81 $[M+6H]^{6+}$ *m/z* = 917.51 , found: 1833.42, 1375.22, 1100.60, 917.43.



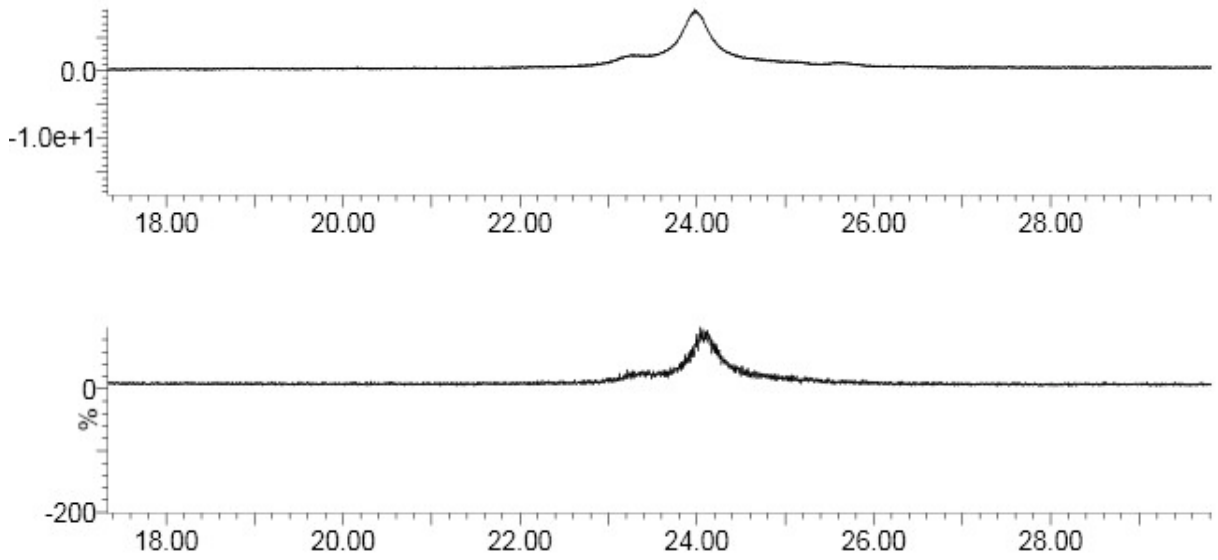
Spectrum 48. UV traces from LC-MS analysis of **threonine ligation**: gradient 20-45% CH_3CN/H_2O over 30min and at a flow rate of 0.6 mL/min.



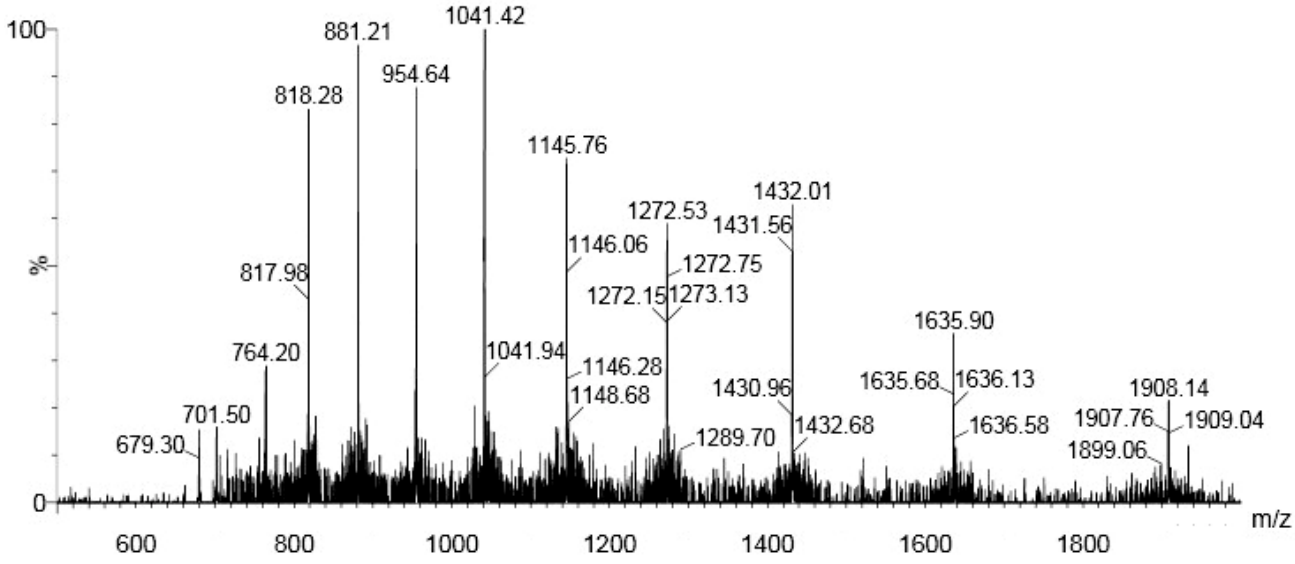
Spectrum 49. UV and MS traces from LC-MS analysis of purified compound **21**: gradient 20-45% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



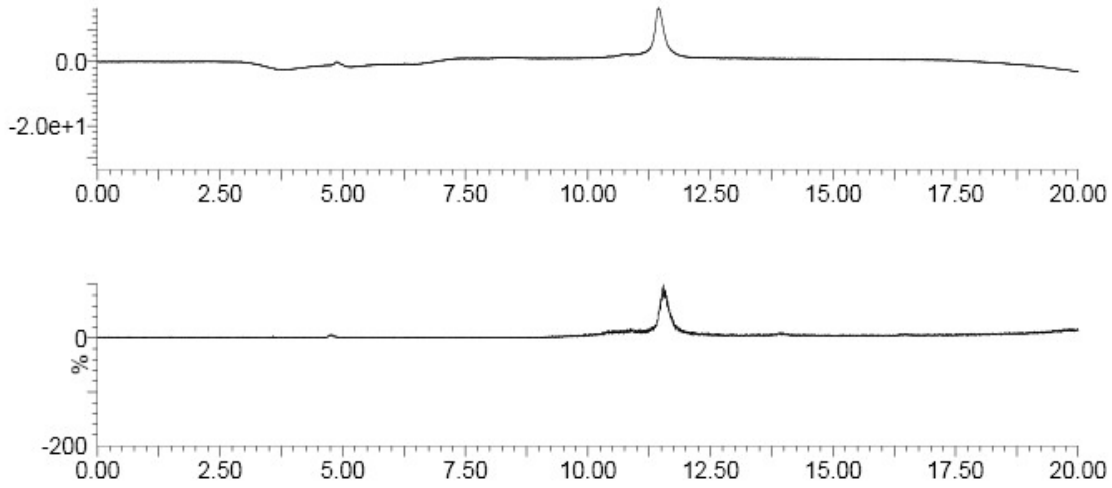
Spectrum 50. ESI calcd for $C_{520}H_{807}N_{141}O_{154}S$ $[M+6H]^{6+}$ *m/z* = 1922.65 $[M+7H]^{7+}$ *m/z* = 1648.13 $[M+8H]^{8+}$ *m/z* = 1442.24 $[M+9H]^{9+}$ *m/z* = 1282.10 $[M+10H]^{10+}$ *m/z* = 1153.99 $[M+11H]^{11+}$ *m/z* = 1049.17 $[M+12H]^{12+}$ *m/z* = 961.82 $[M+13H]^{13+}$ *m/z* = 887.91, $[M+14H]^{14+}$ *m/z* = 824.56, found: 1922.32, 1647.91, 1442.13, 1282.13, 1153.93, 1049.07, 961.61, 887.73, 824.58.



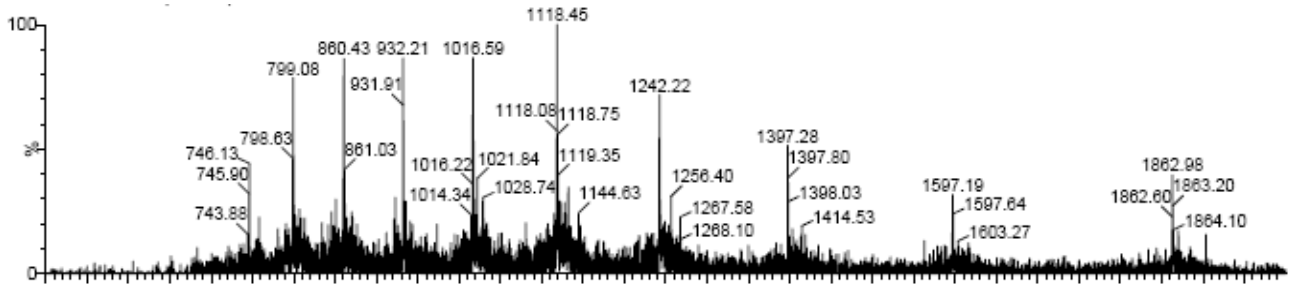
Spectrum 51. UV and MS traces from LC-MS analysis of crude compound **22** gradient 20 - 45% CH_3CN/H_2O over 30 min at a flow rate of 0.6 mL/min.



Spectrum 52. ESI calcd for $C_{516}H_{803}N_{141}O_{152}S$ $[M+6H]^{6+}$ *m/z* = 1908.64 $[M+7H]^{7+}$ *m/z* = 1636.12 $[M+8H]^{8+}$ *m/z* = 1431.73, $[M+9H]^{9+}$ *m/z* = 1272.76 $[M+10H]^{10+}$ *m/z* = 1145.58 $[M+11H]^{11+}$ *m/z* = 1041.53 $[M+12H]^{12+}$ *m/z* = 954.82 $[M+13H]^{13+}$ *m/z* = 881.45 $[M+14H]^{14+}$ *m/z* = 818.56 $[M+15H]^{15+}$ *m/z* = 764.058, found: 1908.14, 1635.90, 1432.01, 1272.53, 1145.76, 1041.42, 954.64, 881.21, 818.28, 764.20.



Spectrum 53. UV and MS traces from LC-MS analysis of compound **23**: gradient 5-95% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min.



Spectrum 54. ESI calcd for $C_{500}H_{787}N_{141}O_{148}S$ $[M+6H]^{6+}$ *m/z* = 1863.25 $[M+7H]^{7+}$ *m/z* = 1597.22 $[M+8H]^{8+}$ *m/z* = 1397.69, $[M+9H]^{9+}$ *m/z* = 1242.50 $[M+10H]^{10+}$ *m/z* = 1118.35 $[M+11H]^{11+}$ *m/z* = 1016.77 $[M+12H]^{12+}$ *m/z* = 932.13 $[M+13H]^{13+}$ *m/z* = 860.50 $[M+14H]^{14+}$ *m/z* = 799.11 $[M+15H]^{15+}$ *m/z* = 745.90, found: 1862.98, 1597.19, 1397.28, 1242.22, 1118.45, 1016.59, 932.21, 860.43, 799.08, 746.13.