Supplementary Information

Arginine N-glycosylation of melittin enhances its bacteriostatic activity and antiproliferative therapeutic index

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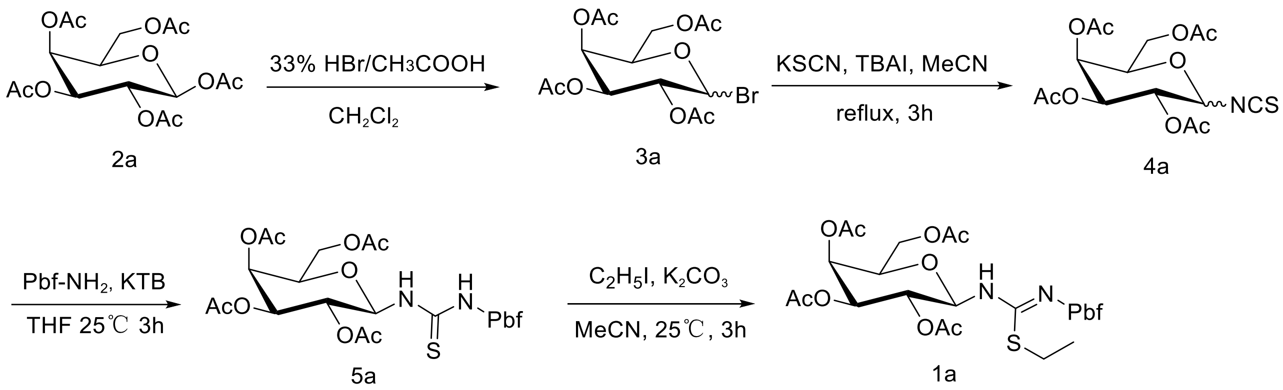


Scheme S1. Synthesis route of glycoligand (galactose ligands **1a** as example).

Compound **2a** (10.00 g) was dissolved in 40 mL anhydrous DCM, slowly add 33% HBr/CH_3COOH solution dropwise in ice bath, react at room temperature for 3 h. Concentrate under reduced pressure to obtain yellow oily substance, after dissolving by DCM, the crude product was extracted with saturated sodium bicarbonate solution, wash by saturated brine, dryed over anhydrous sodium sulfate, and purified by silica gel column chromatography to obtain white solid **3a**. TBAI (6.73 g), KSCN (3.54 g) and molecular sieve (6.0 g) were dissolved by anhydrous CH_3CN under nitrogen protection, and stirred for 2 h at room temperature. Anhydrous CH_3CN solution of compound **3a** was added to the reaction solution, then reflux at 75°C for 2 h. Concentrate under reduced pressure to remove the solvent, then extract with DCM and water, washed with saturated brine, dryed over anhydrous sodium sulfate, and purified by column chromatography to obtain white solid **4a**. Pbf-NH_2 (1.40 g) and KTB (0.60 g) was dissolved in anhydrous THF under N_2 conditions, stirred at room temperature for 2 h. Anhydrous THF solution of **4a** was added and continue to react for 1 h. After adjusting the pH to neutral with a cation exchange resin, filterred to remove the resin, and concentrated under reduced pressure to obtain yellow oily substance **5a**. Under N_2 conditions, the obtained compound **5a** was dissolved in anhydrous CH_3CN, K_2CO_3 (3.40 g) and ethyl iodide (1.56 g) were added, and reacted at room temperature for 16 h. Concentrated under reduced pressure, extracted with ethyl acetate and water, dried over anhydrous sodium sulfate, purified by column chromatography to obtain white solid **1a**.

Table S1. HPLC elution program of melittin analogs

Name	Gradient
MLT-1a	0~5 min，40%B：5~80 min，40%~70%B
MLT-2a	0~5 min，40%B：5~80 min，40%~70%B
MLT-3a	0~5 min，38%B：5~80 min，38%~68%B
MLT-1b	0~5 min，40%B：5~80 min，40%~70%B
MLT-2b	0~5 min，40%B：5~80 min，40%~70%B
MLT-3b	0~5 min，38%B：5~80 min，38%~68%B
MLT-1c	0~5 min，40%B：5~80 min，40%~60%B
MLT-2c	0~5 min，38%B：5~80 min，38%~55%B
MLT-3c	0~5 min，35%B：5~80 min，35%~70%B
MLT-1d	0~5 min，40%B：5~80 min，40%~70%B
MLT-2d	0~5 min，40%B：5~80 min，40%~70%B
MLT-3d	0~5 min，38%B：5~80 min，38%~68%B
MLT-1e	0~5 min，38%B：5~80 min，38%~65%B
MLT-2e	0~5 min，38%B：5~80 min，38%~55%B
MLT-3e	0~5 min，35%B：5~80 min，35%~50%B
MLT-1f	0~5 min，40%B：5~80 min，40%~70%B
MLT-2f	0~5 min，40%B：5~80 min，40%~65%B
MLT-3f	0~5 min，35%B：5~80 min，35%~50%B
MLT-1g	0~5 min，38%B：5~80 min，38%~50%B
MLT-2g	0~5 min，38%B：5~80 min，38%~55%B
MLT-3g	0~5 min，38%B：5~80 min，38%~55%B
MLT-1h	0~5 min，38%B：5~80 min，38%~65%B
MLT-2h	0~5 min，38%B：5~80 min，38%~60%B
MLT-3h	0~5 min，35%B：5~80 min，35%~55%B

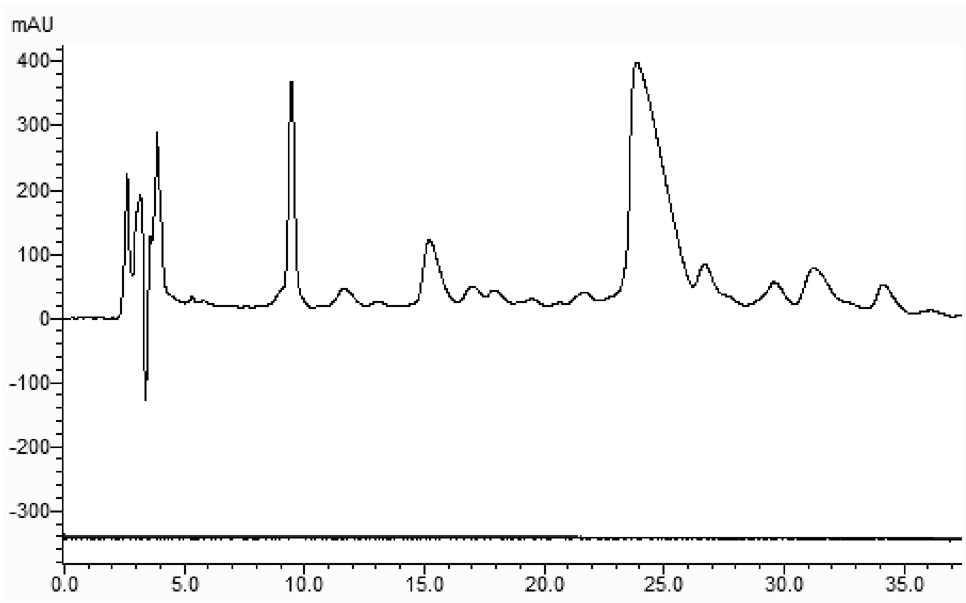


Figure S1. Preparative HPLC spectrum of melittin and its analogs.
(**MLT** as example, t_R: 23.84min, seperation yield > 15%).

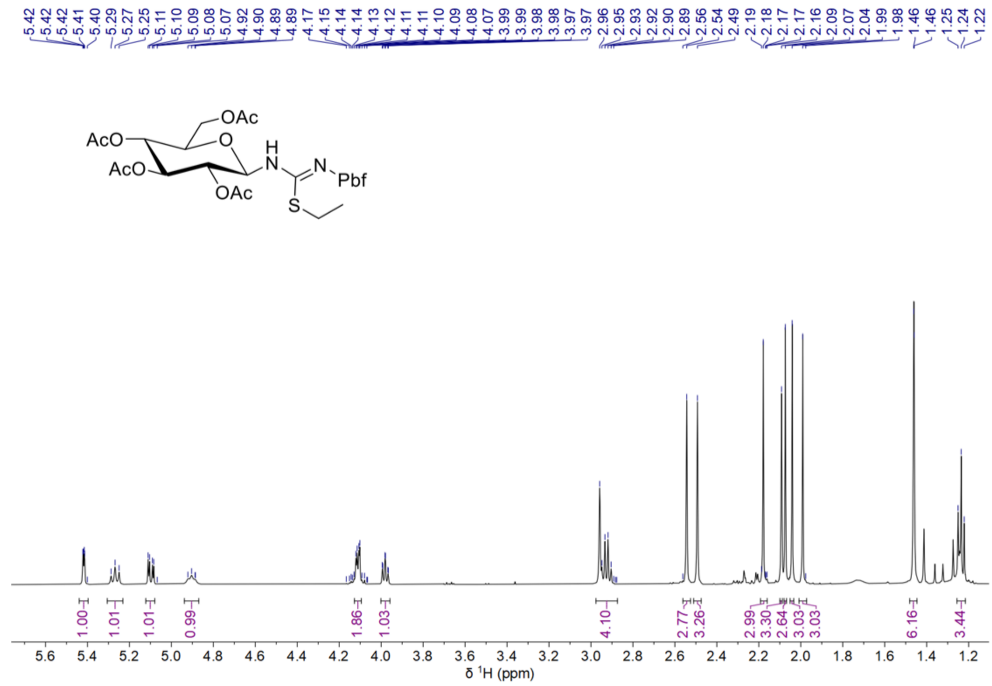


Figure S2. [1]H-NMR of compound **1a**. ^{1}H NMR (500 MHz, Chloroform-d) δ 5.42 (d, *J* = 2.2 Hz, 1H), 5.27 (t, *J* = 9.6 Hz, 1H), 5.10 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.99 - 4.86 (m, 1H), 4.11 (dd, *J* = 6.7, 2.6 Hz, 2H), 3.98 (t, *J* = 6.6 Hz, 1H), 2.98 - 2.89 (m, 4H), 2.54 (s, 3H), 2.49 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.46 (s, 6H), 1.26 - 1.23 (m, 3H).

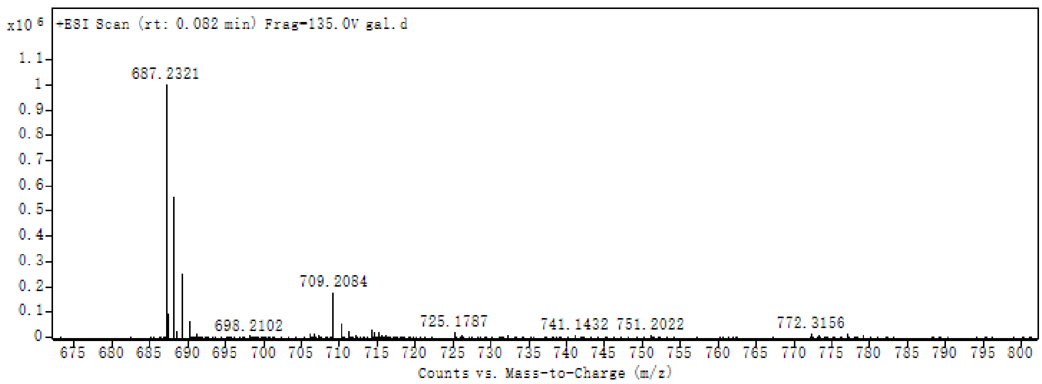


Figure S3. HR-Q-TOF-MS of compound **1a**. HR-Q-TOF-MS m/z calcd for $C_{30}H_{42}N_2O_{12}S_2$ 686.2179; found $[M+H]^+$ 687.2321; $[M+Na]^+$ 709.2084.

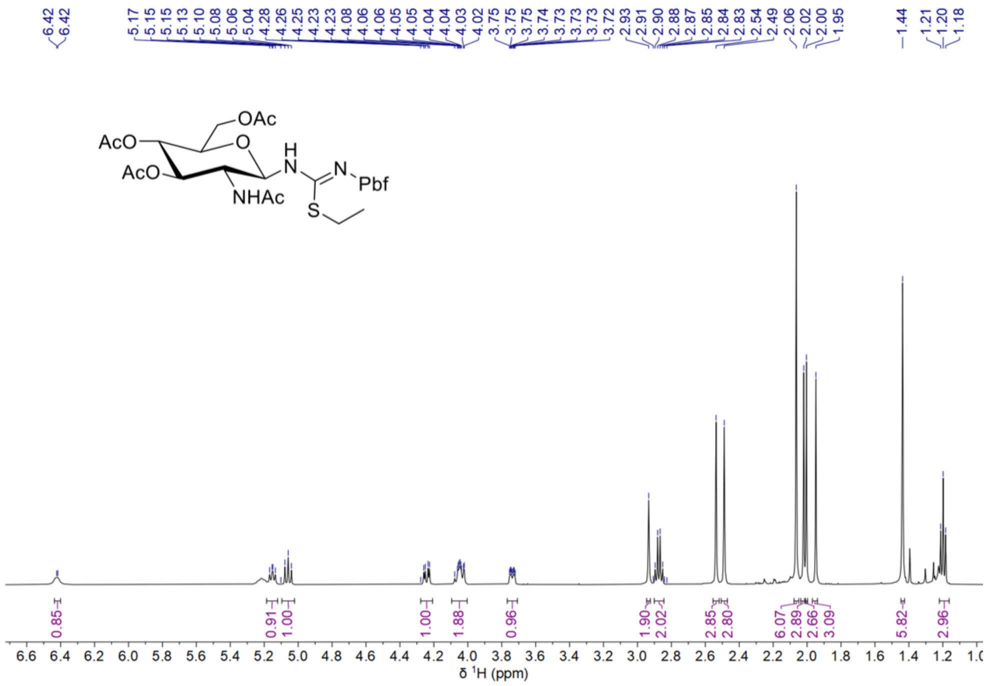


Figure S4. ^{1}H-NMR of compound **1b**. ^{1}H NMR (500 MHz, Chloroform-d) δ 5.42 (d, *J* = 2.2 Hz, 1H), 5.27 (t, *J* = 9.6 Hz, 1H), 5.10 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.99 - 4.86 (m, 1H), 4.11 (dd, *J* = 6.7, 2.6 Hz, 2H), 3.98 (t, *J* = 6.6 Hz, 1H), 2.98 - 2.89 (m, 4H), 2.54 (s, 3H), 2.49 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.46 (s, 6H), 1.26 - 1.23 (m, 3H).

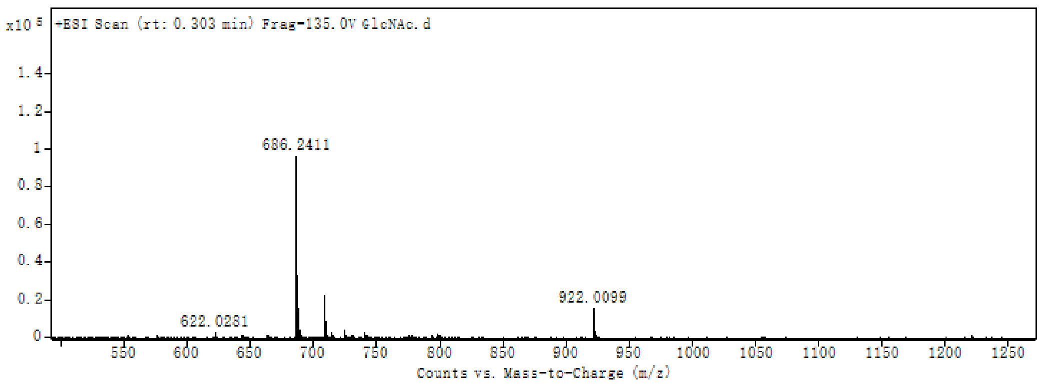


Figure S5. HR-Q-TOF-MS of compound **1b**. HR-Q-TOF-MS m/z calcd for $C_{30}H_{42}N_2O_{12}S_2$ 686.2179; found $[M+H]^+$ 687.2321; $[M+Na]^+$ 709.2084.

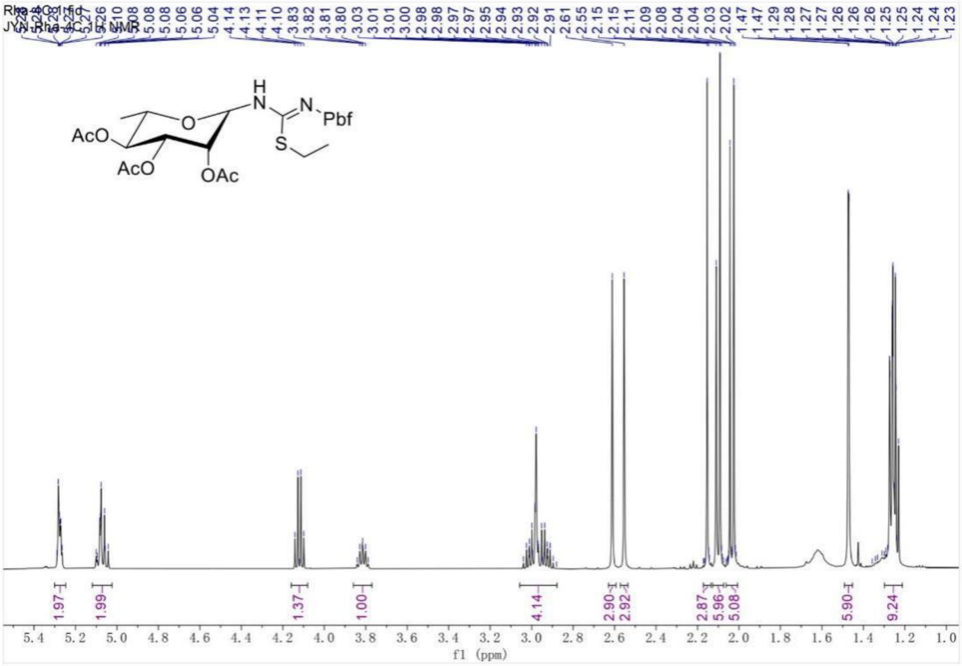


Figure S6. [1]H-NMR of compound **1c**. ^{1}H NMR (500 MHz, Chloroform-d) δ 5.30 - 5.25 (m, 2H), 5.12 - 5.02 (m, 2H), 4.12 (q, *J* = 7.1 Hz, 1H), 3.81 (p, *J* = 6.4 Hz, 1H), 3.06 - 2.88 (m, 4H), 2.61 (s, 3H), 2.55 (s, 3H), 2.15 (s, 3H), 2.10 (d, *J* = 8.9 Hz, 6H), 2.03 (d, *J* = 9.4 Hz, 5H), 1.47 (d, *J* = 1.6 Hz, 6H), 1.30 - 1.21 (m, 9H).

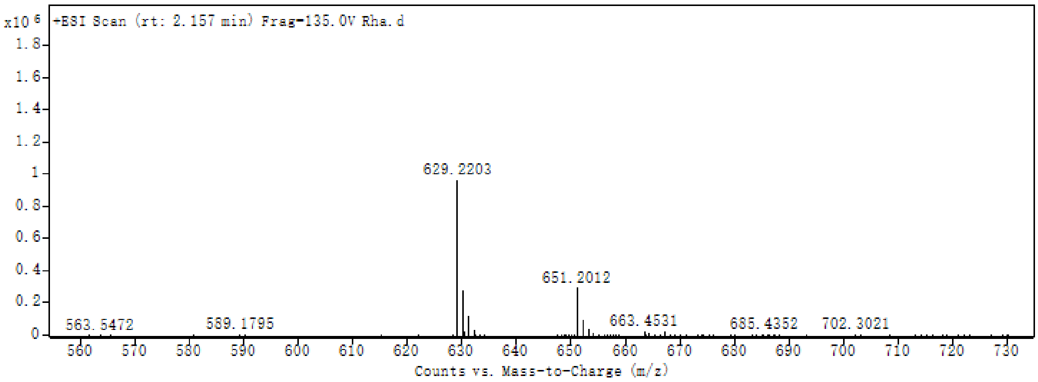


Figure S7. HR-Q-TOF-MS of compound **1c**. HR-Q-TOF-MS m/z calcd for $C_{28}H_{40}N_2O_{10}S_2$ 628.2124; found $[M+H]^+$ 629.2203; $[M+Na]^+$ 651.2015.

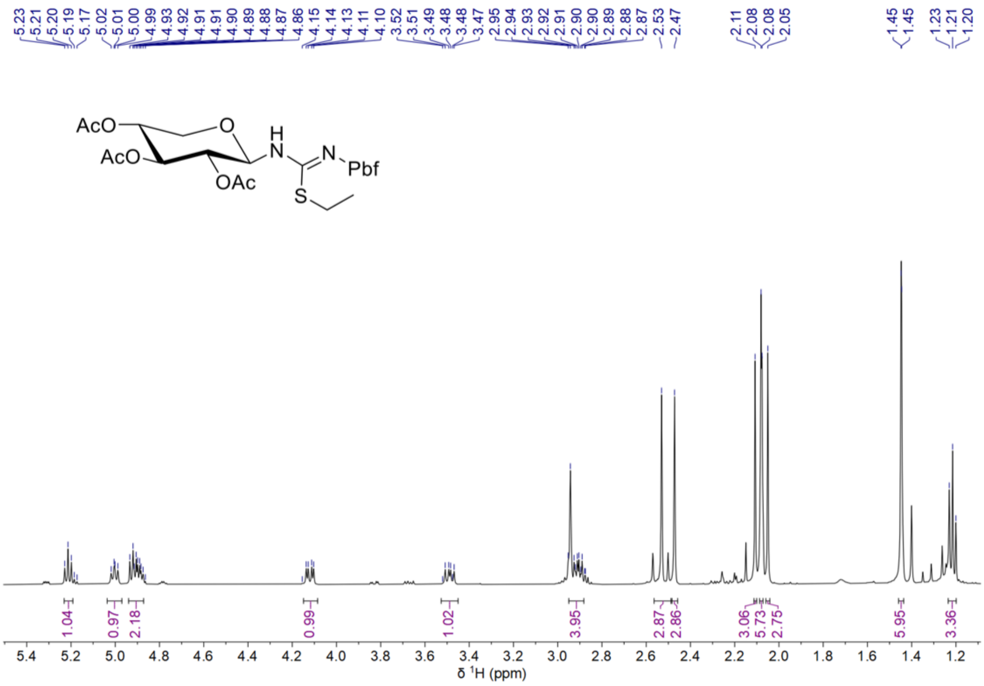


Figure S8. ^{1}H-NMR of compound **1d**. ^{1}H NMR (500 MHz, Chloroform-d) δ 5.26 - 5.16 (m, 1H), 5.03 - 4.98 (m, 1H), 4.89 (ddd, J = 12.8, 12.1, 6.2 Hz, 2H), 4.12 (dd, J = 12.2, 4.6 Hz, 1H), 3.48 (dd, J = 12.2, 7.9 Hz, 1H), 2.96 - 2.85 (m, 4H), 2.53 (d, J = 9.4 Hz, 3H), 2.47 (s, 3H), 2.10 (s, 3H), 2.07 (d, J = 2.0 Hz, 6H), 2.05 (s, 3H), 1.44 (s, 6H), 1.22 (q, J = 7.5 Hz, 3H).

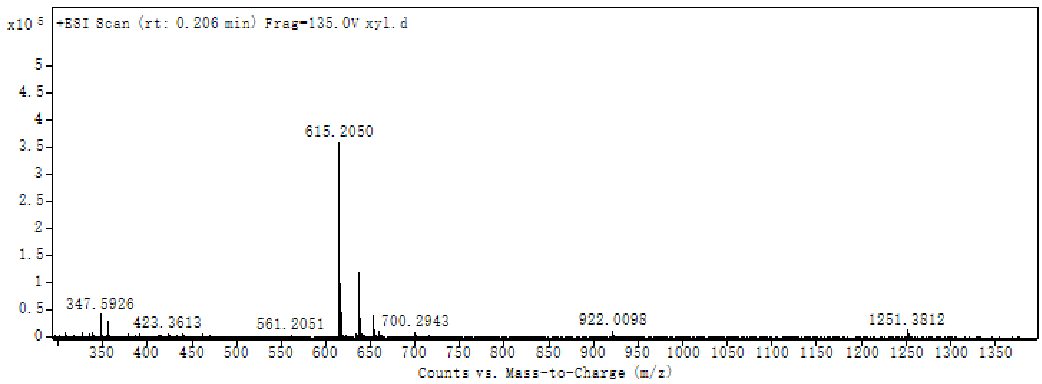


Figure S9. HR-Q-TOF-MS of compound **1d**. HR-Q-TOF-MS m/z calcd for $C_{27}H_{28}N_{2}O_{10}S_{2}$ 614.7250; found $[M+H]^{+}$ 615.2044; $[M+Na]^{+}$ 637.1859.

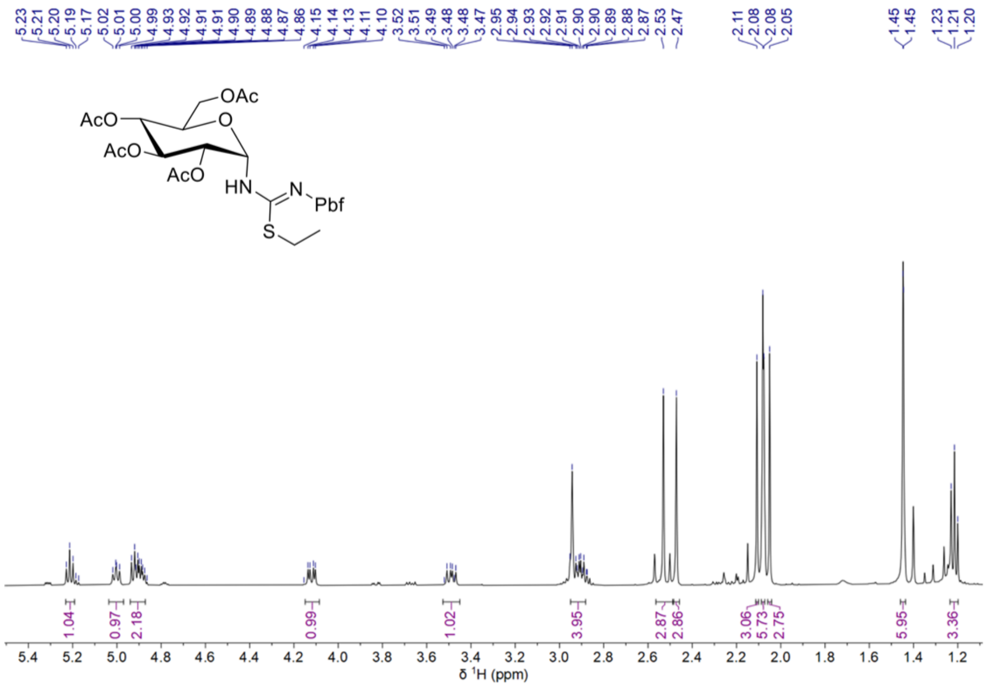


Figure S10. [1]H-NMR of compound **1e**. ^{1}H NMR (500 MHz, Chloroform-d) δ 5.29 (t, J = 9.4 Hz, 1H), 5.07 (td, J = 9.5, 6.6 Hz, 2H), 4.94 (s, 1H), 4.25 (dd, J = 12.4, 4.7 Hz, 1H), 4.12 (dd, J = 12.4, 2.3 Hz, 1H), 3.77 (ddd, J = 10.2, 4.7, 2.3 Hz, 1H), 2.98 - 2.88 (m, 4H), 2.54 (s, 3H), 2.49 (s, 3H), 2.09 (d, J = 2.0 Hz, 6H), 2.07 (s, 3H), 2.03 (d, J = 4.9 Hz, 5H), 1.46 (d, J = 1.2 Hz, 6H), 1.24 (t, J = 7.4 Hz, 3H).

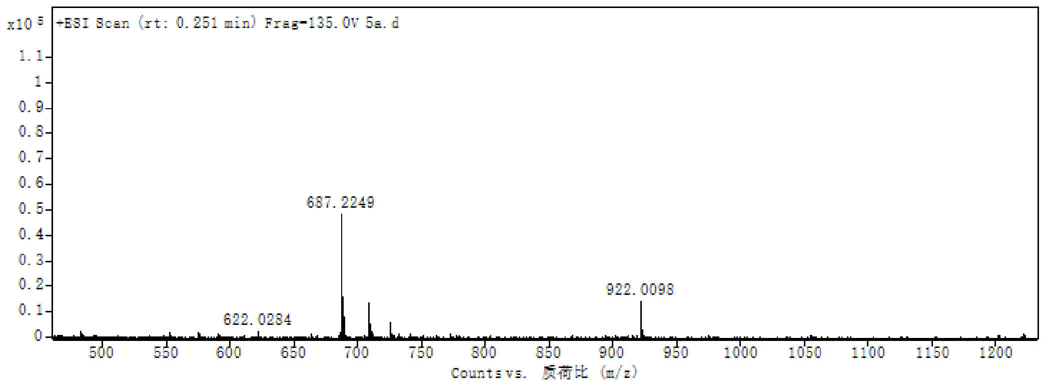


Figure S11. HR-Q-TOF-MS of compound **1e**. HR-Q-TOF-MS m/z calcd for $C_{30}H_{42}N_2O_{12}S_2$ 686.2179; found $[M+H]^+$ 687.2247; $[M+Na]^+$ 709.2078.

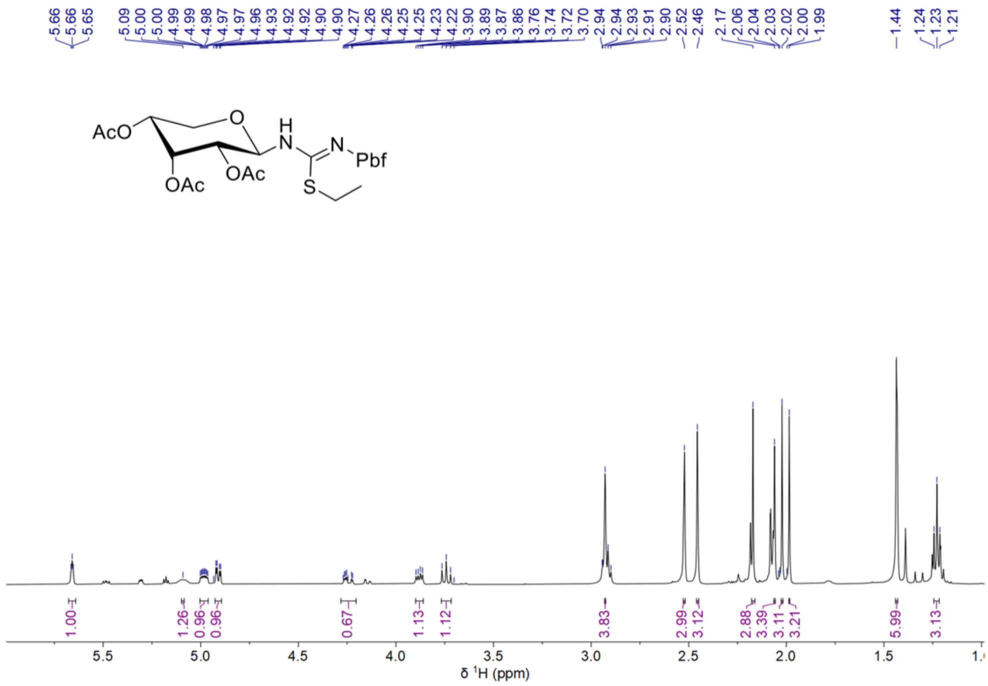


Figure S12. [1]H-NMR of compound **1f**. ^{1}H NMR (500 MHz, CDCl3) δ 5.66 (d, *J* = 9.5 Hz, 1H), 5.11 (s, 1H), 5.00 (ddd, *J* = 10.6, 5.4, 2.8 Hz, 1H), 4.92 (dt, *J* = 9.1, 4.6 Hz, 1H), 3.90 (dd, *J* = 11.0, 5.4 Hz, 1H), 3.76 (t, *J* = 11.0 Hz, 1H), 2.98 - 2.91 (m, 4H), 2.54 (s, 3H), 2.47 (s, 3H), 2.23 - 2.17 (m, 3H), 2.08 (d, *J* = 3.6 Hz, 3H), 2.04 (s, 3H), 2.01 (d, *J* = 5.4 Hz, 3H), 1.45 (d, *J* = 1.8 Hz, 6H), 1.24 (d, *J* = 7.4 Hz, 3H).

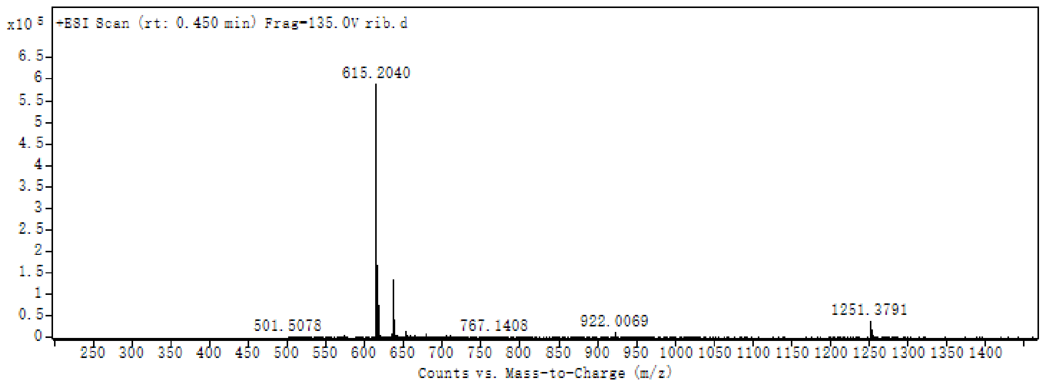


Figure S13. HR-Q-TOF-MS of compound **1f**. HR-Q-TOF-MS m/z calcd for $C_{27}H_{28}N_2O_{10}S_2$ 614.7250; found $[M+H]^+$ 615.2044; $[M+Na]^+$ 637.1842.

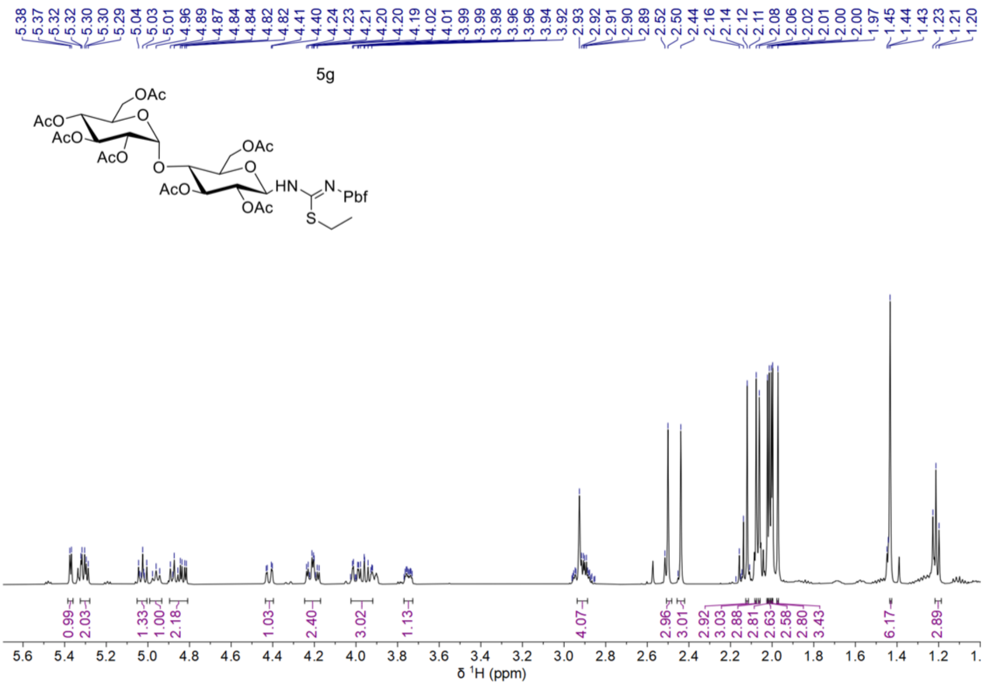


Figure S14. 1H-NMR of compound **1g**. 1H NMR (500 MHz, CDCl3) δ 5.38 (t, *J* = 5.6 Hz, 1H), 5.36 - 5.28 (m, 2H), 5.09 - 5.01 (m, 1H), 4.97 (t, *J* = 8.5 Hz, 1H), 4.93 - 4.88 (m, 1H), 4.88 - 4.82 (m, 1H), 4.43 (dd, *J* = 12.2, 2.2 Hz, 1H), 4.28 - 4.17 (m, 2H), 4.04 - 3.90 (m, 3H), 3.80 - 3.73 (m, 1H), 2.98 - 2.89 (m, 4H), 2.55 - 2.50 (m, 3H), 2.46 (d, *J* = 6.5 Hz, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.07 (d, *J* = 2.8 Hz, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.45 (s, 6H), 1.22 (t, *J* = 5.8 Hz, 3H).

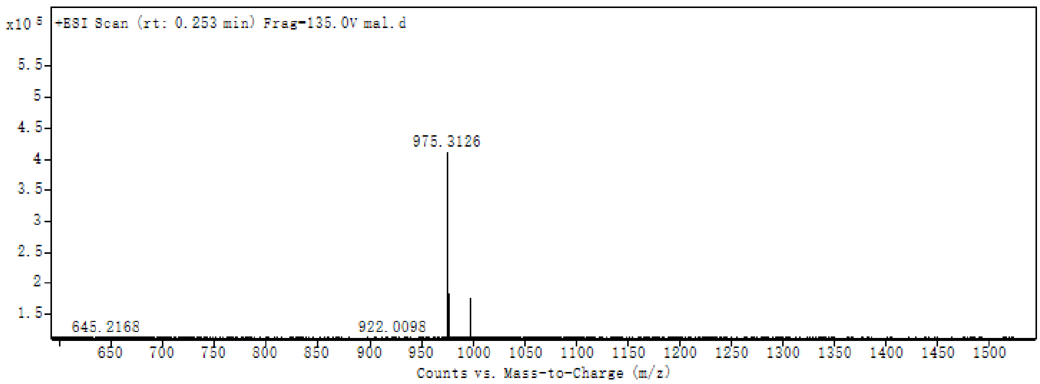


Figure S15. HR-Q-TOF-MS of compound **1g**. HR-Q-TOF-MS m/z calcd for $C_{42}H_{58}N_2O_{20}S_2$ 975.0400; found $[M+H]^+$ 975.3093; $[M+Na]^+$ 997.2908.

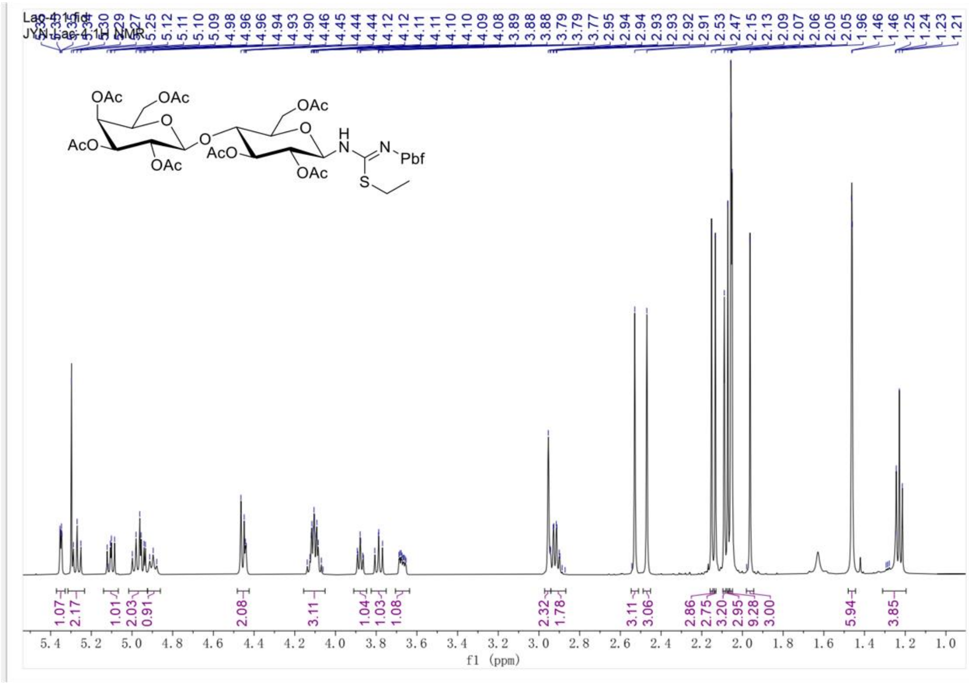


Figure S16. ^{1}H-NMR of compound **1h**. ^{1}H NMR (500 MHz, Chloroform-d) δ 5.35 (dd, J = 3.5, 1.1 Hz, 1H), 5.32 - 5.23 (m, 2H), 5.10 (dd, J = 10.4, 7.9 Hz, 1H), 5.02 - 4.92 (m, 2H), 4.90 (t, J = 8.6 Hz, 1H), 4.48 - 4.42 (m, 2H), 4.16 - 4.05 (m, 3H), 3.88 (td, J = 6.7, 1.2 Hz, 1H), 3.82 - 3.75 (m, 1H), 3.67 (ddd, J = 9.9, 5.1, 2.1 Hz, 1H), 2.95 (s, 2H), 2.91 (td, J = 7.4, 1.6 Hz, 2H), 2.53 (s, 3H), 2.47 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 - 2.05 (m, 9H), 1.96 (s, 3H), 1.46 (d, J = 1.4 Hz, 6H), 1.23 (t, J = 7.4 Hz, 4H).

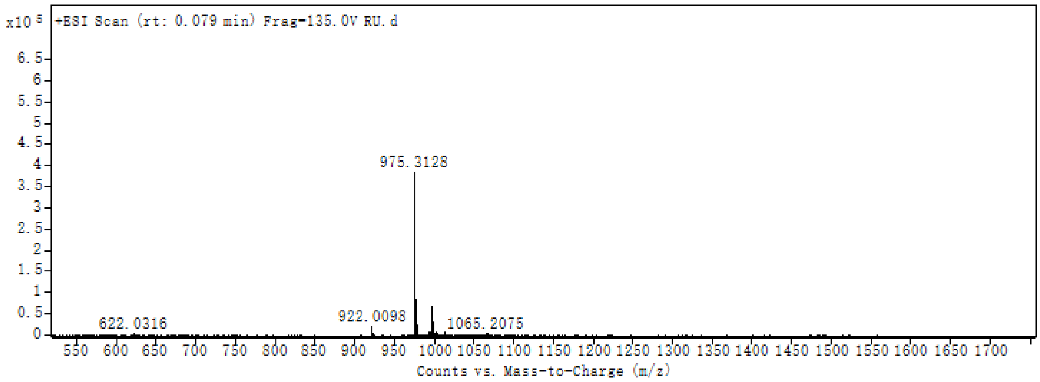


Figure S17. HR-Q-TOF-MS of compound **1h**. HR-Q-TOF-MS m/z calcd for $C_{42}H_{58}N_2O_{20}S_2$ 975.0400; found $[M+H]^+$ 975.3105.

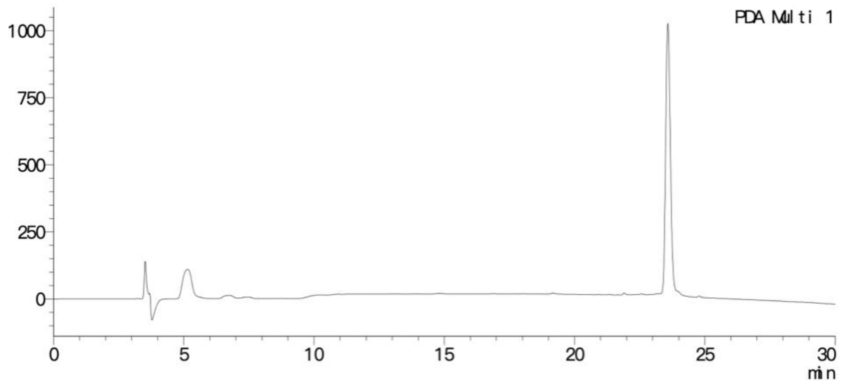


Figure S18. HPLC of compound **MTL-1a** (purity>95%)

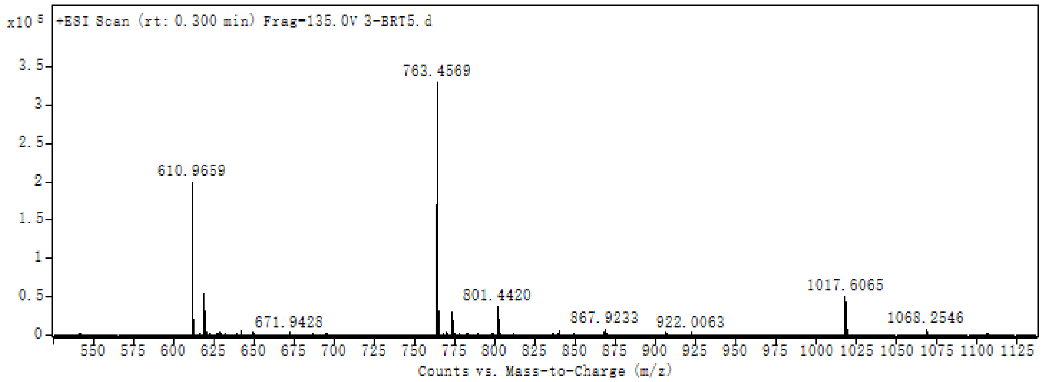


Figure S19. HR-Q-TOF-MS of compound **MTL-1a**

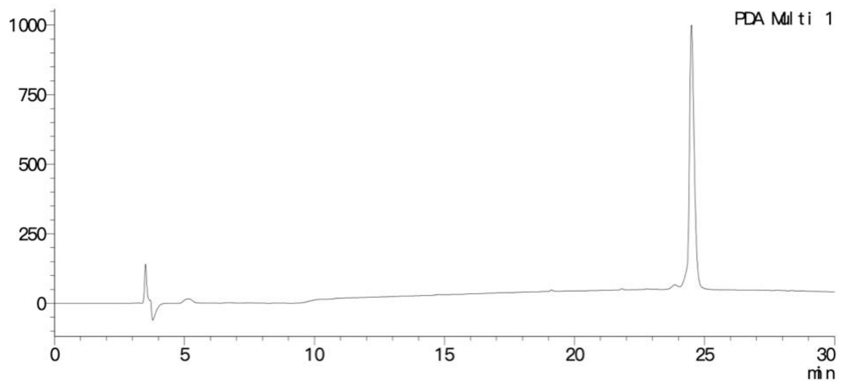


Figure S20. HPLC of compound **MTL-2a** (purity>95%)

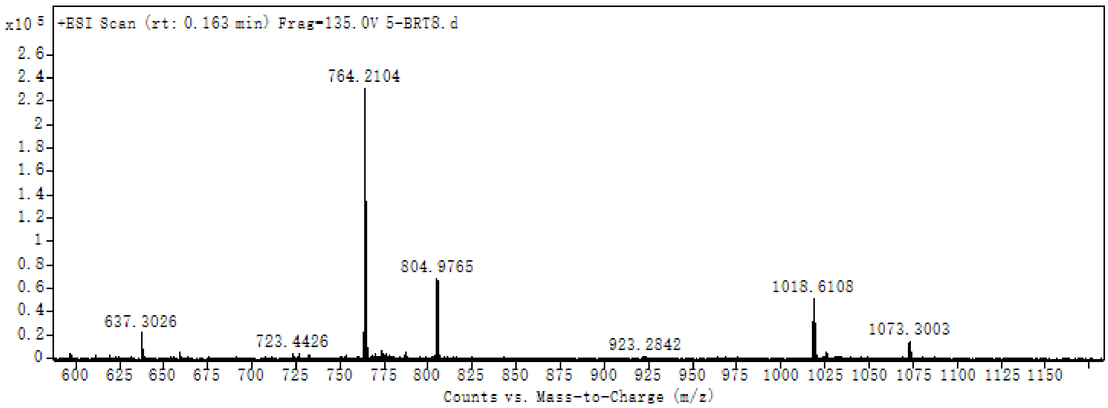


Figure S21. HR-Q-TOF-MS of compound **MTL-2a**

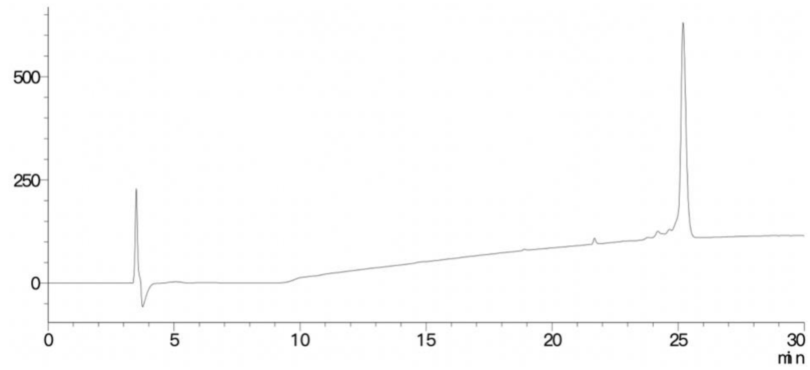


Figure S22. HPLC of compound **MTL-3a** (purity>95%)

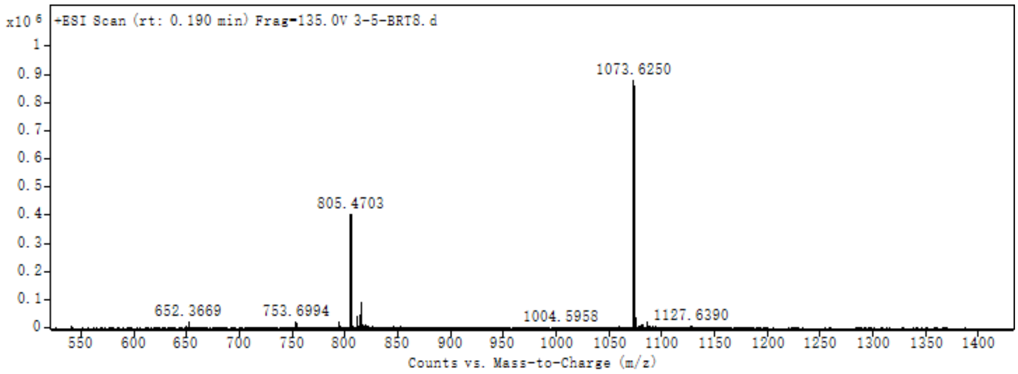


Figure S23. HR-Q-TOF-MS of compound **MTL-3a**

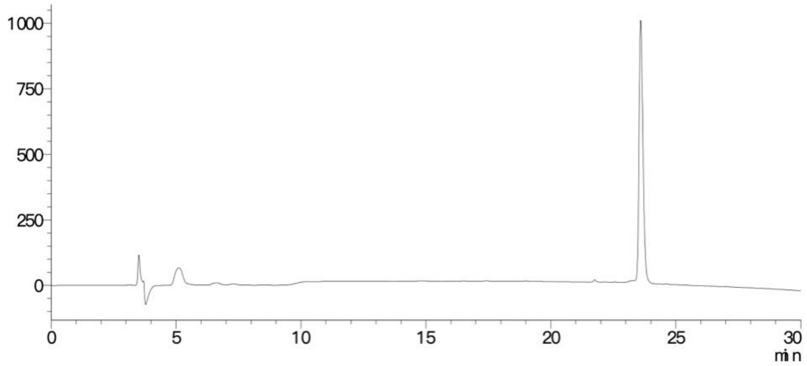


Figure S24. HPLC of compound **MTL-1b** (purity>95%)

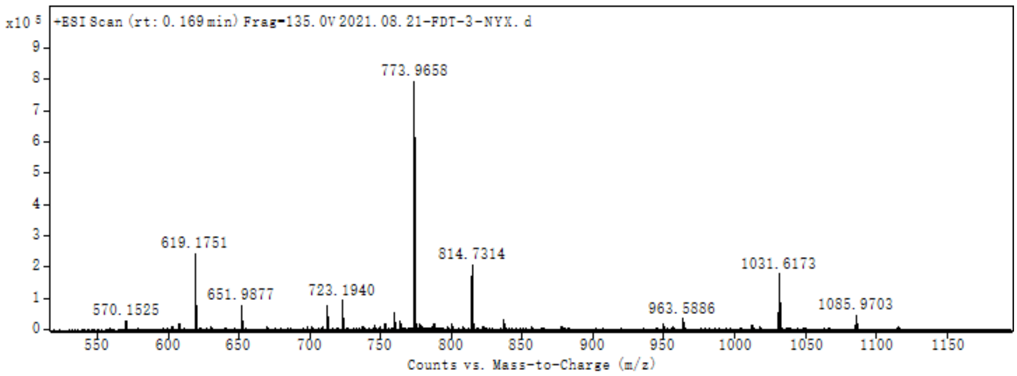


Figure S25. HR-Q-TOF-MS of compound **MTL-1b**

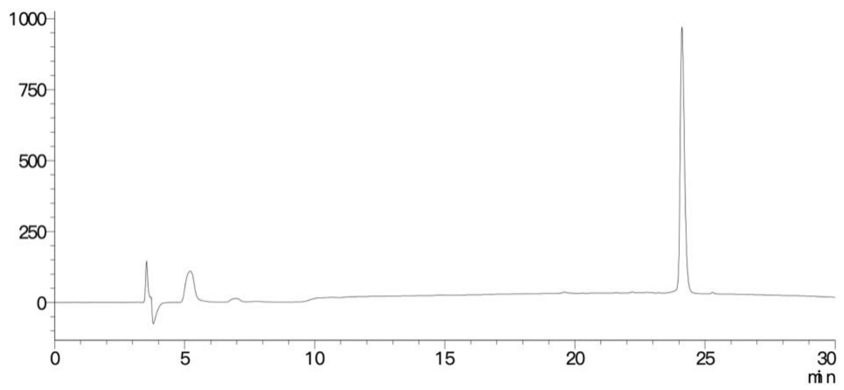


Figure S26. HPLC of compound **MTL-2b** (purity>95%)

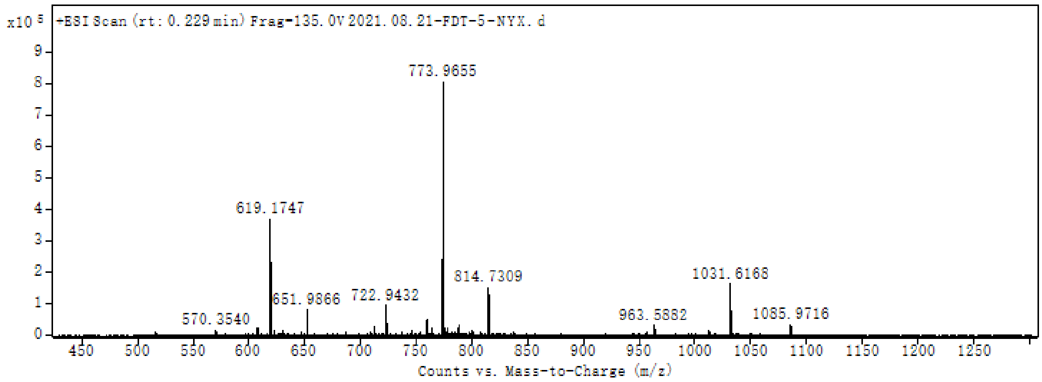


Figure S27. HR-Q-TOF-MS of compound **MTL-2b**

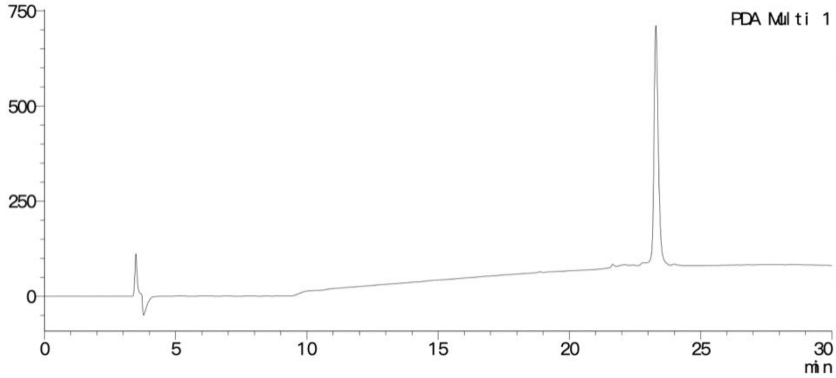


Figure S28. HPLC of compound **MTL-3b** (purity>95%)

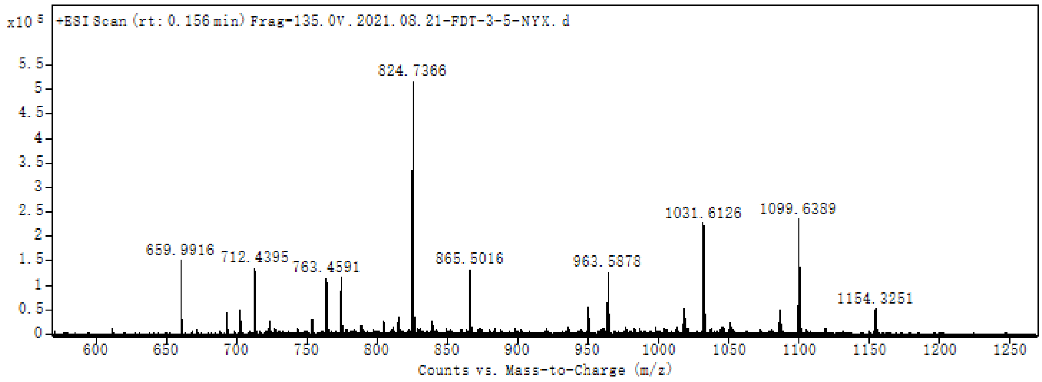


Figure S29. HR-Q-TOF-MS of compound **MTL-3b**

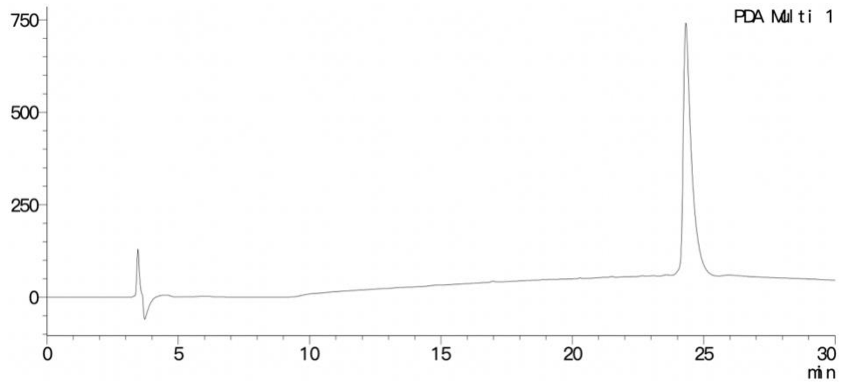


Figure S30. HPLC of compound **MTL-1c** (purity>95%)

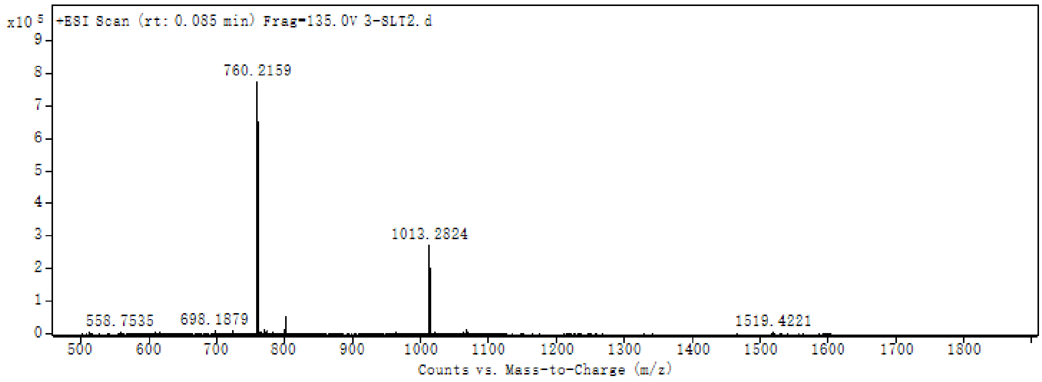


Figure S31. HR-Q-TOF-MS of compound **MTL-1c**

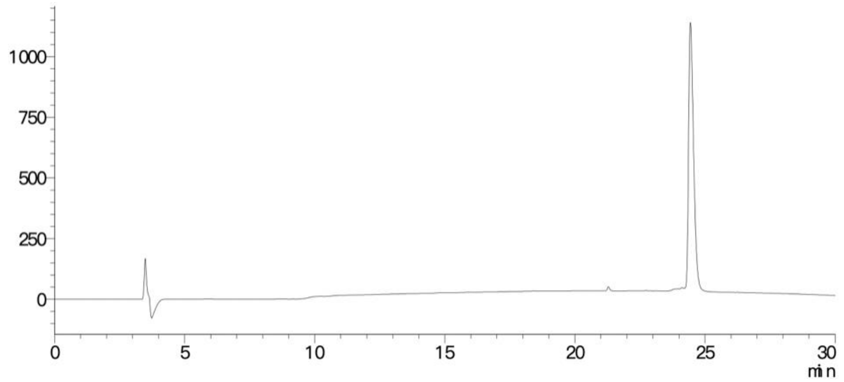


Figure S32. HPLC of compound **MTL-2c** (purity>95%)

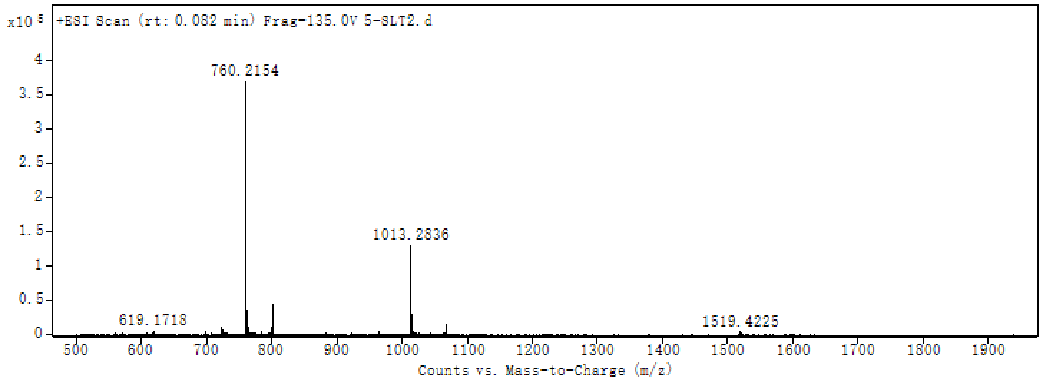


Figure S33. HR-Q-TOF-MS of compound **MTL-2c**

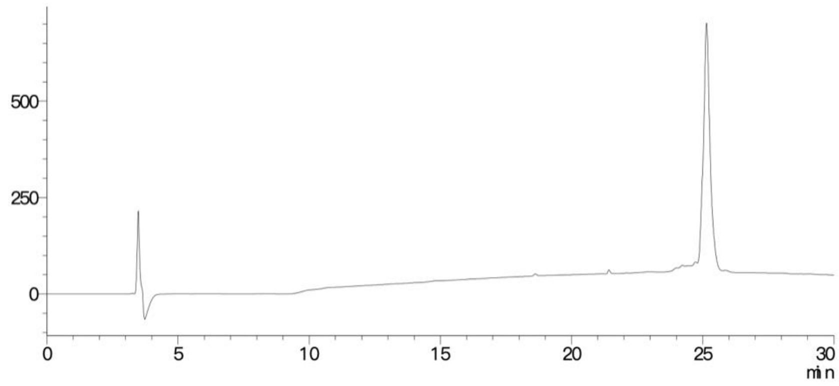


Figure S34. HPLC of compound **MTL-3c** (purity>95%)

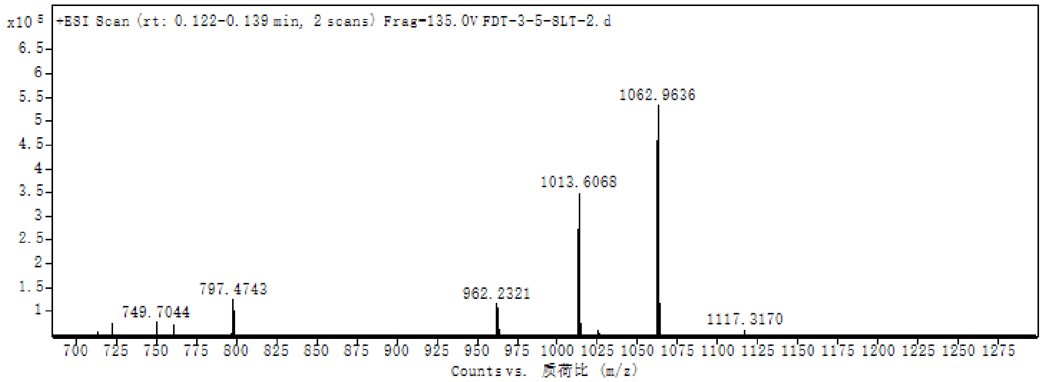


Figure S35. HR-Q-TOF-MS of compound **MTL-3c**

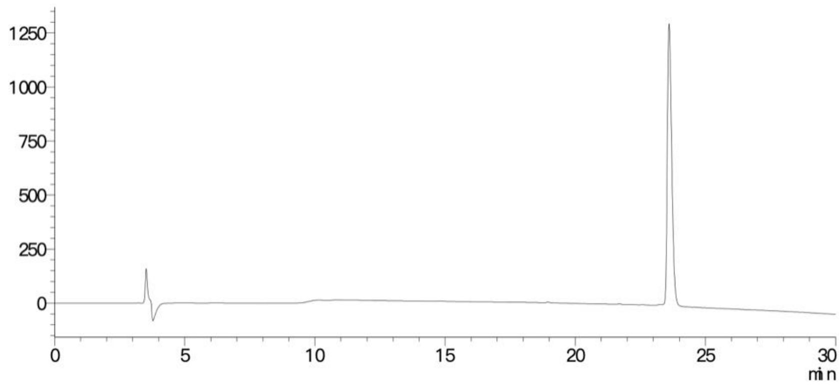


Figure S36. HPLC of compound **MTL-1d** (purity>95%)

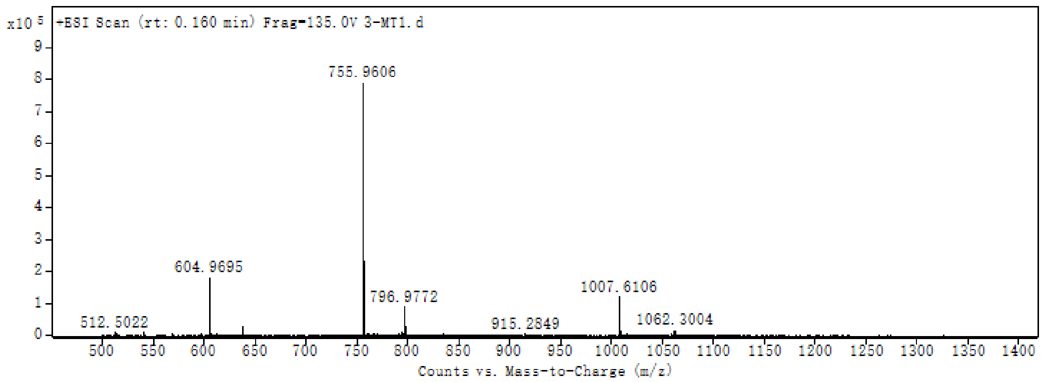


Figure S37. HR-Q-TOF-MS of compound **MTL-1d**

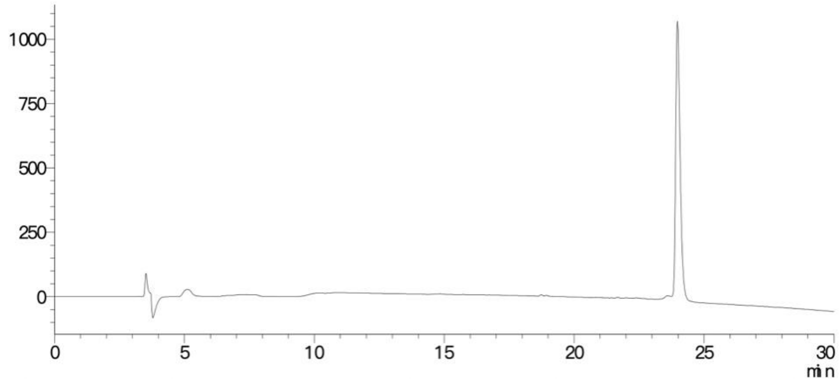


Figure S38. HPLC of compound **MTL-2d** (purity>95%)

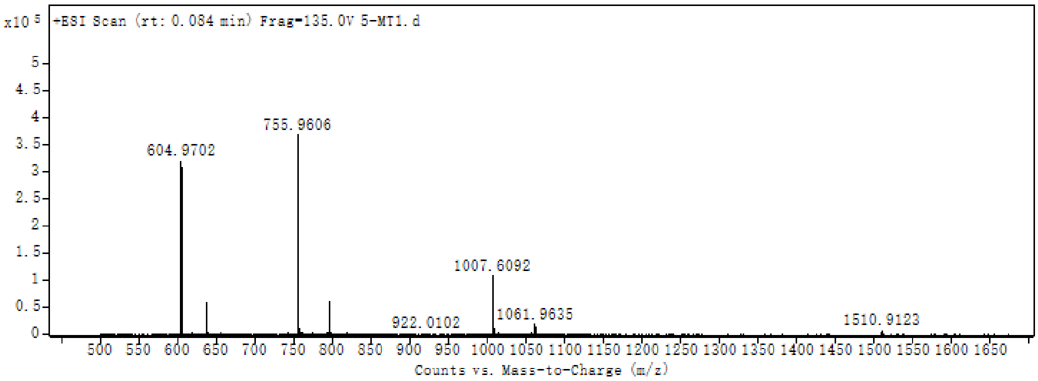


Figure S39. HR-Q-TOF-MS of compound **MTL-2d**

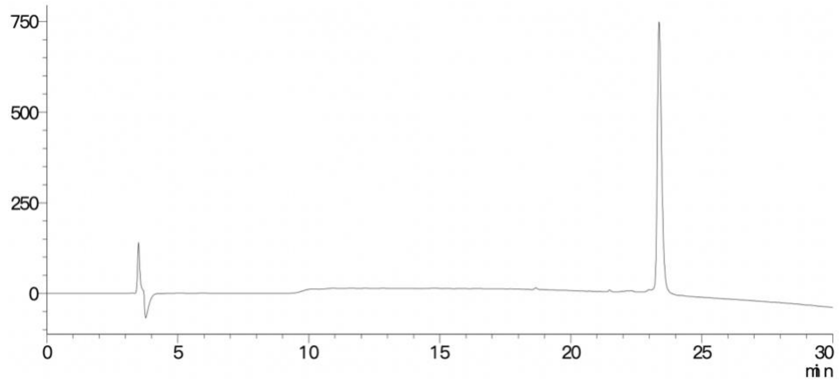


Figure S40. HPLC of compound **MTL-3d** (purity>95%)

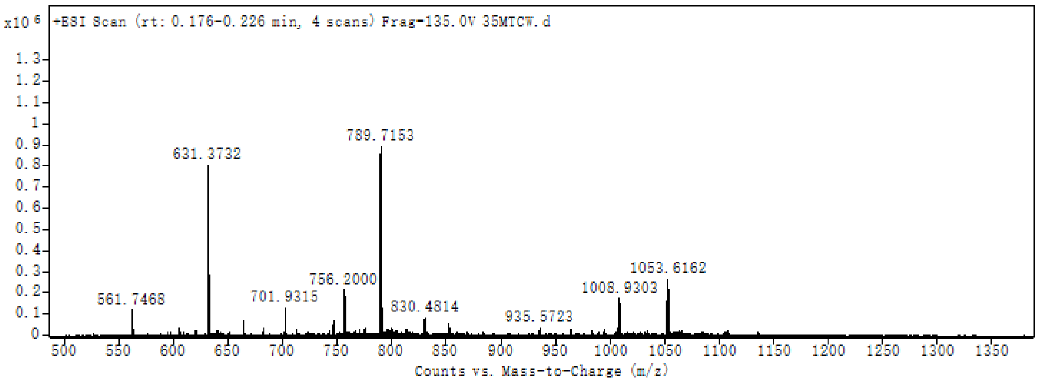


Figure S41. HR-Q-TOF-MS of compound **MTL-3d**

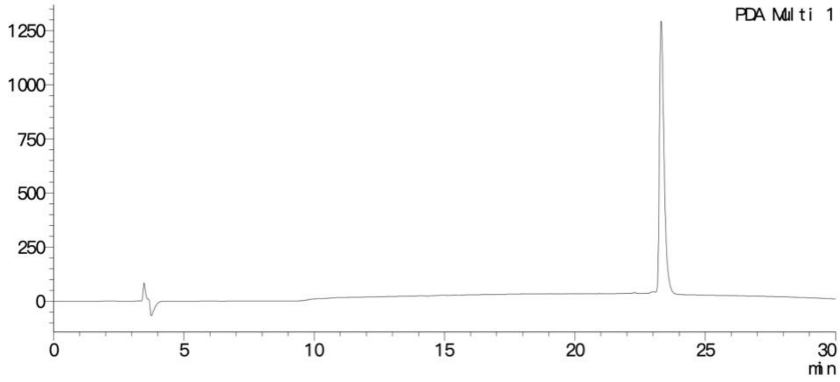


Figure S42. HPLC of compound **MTL-1e** (purity>95%)

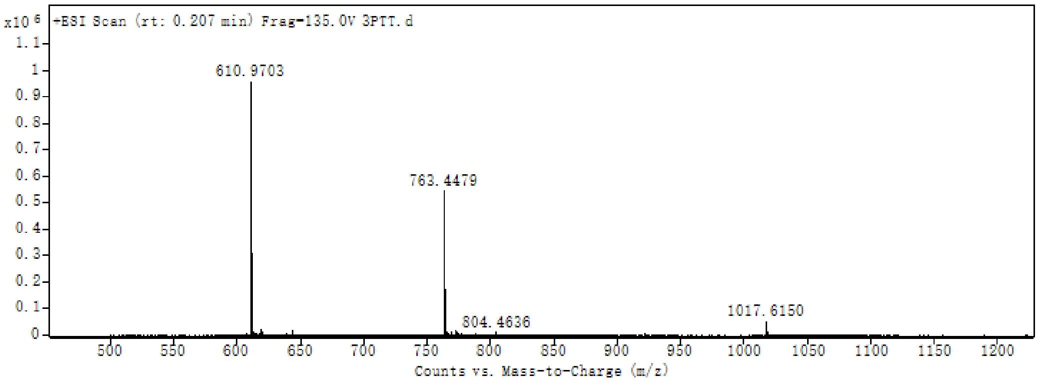


Figure S43. HR-Q-TOF-MS of compound **MTL-1e**

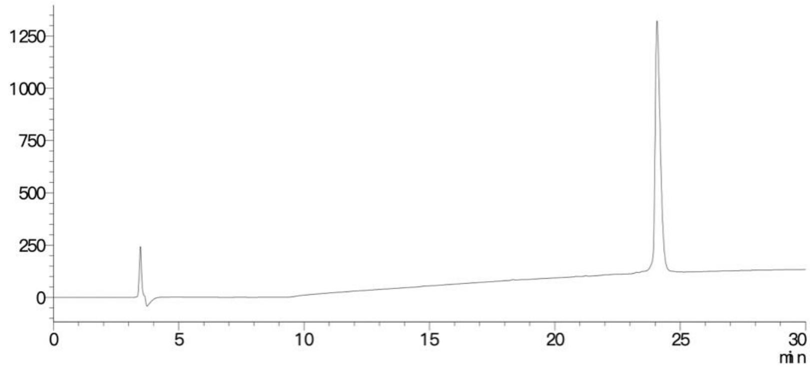


Figure S44. HPLC of compound **MTL-2e** (purity>95%)

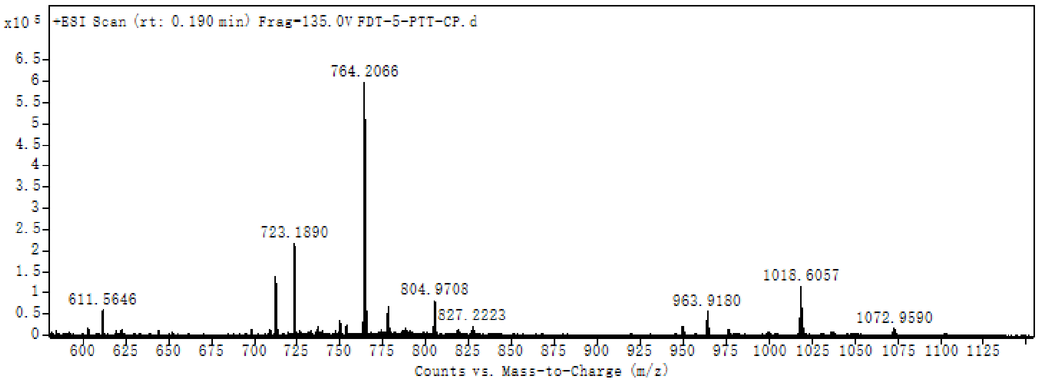


Figure S45. HR-Q-TOF-MS of compound **MTL-2e**

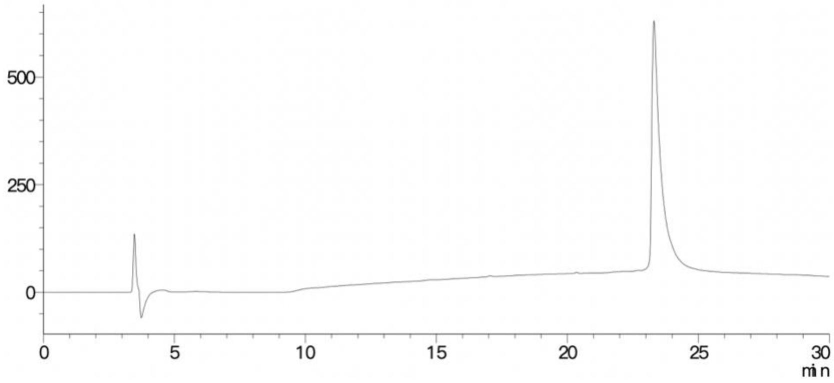


Figure S46. HPLC of compound **MTL-3e** (purity>95%)

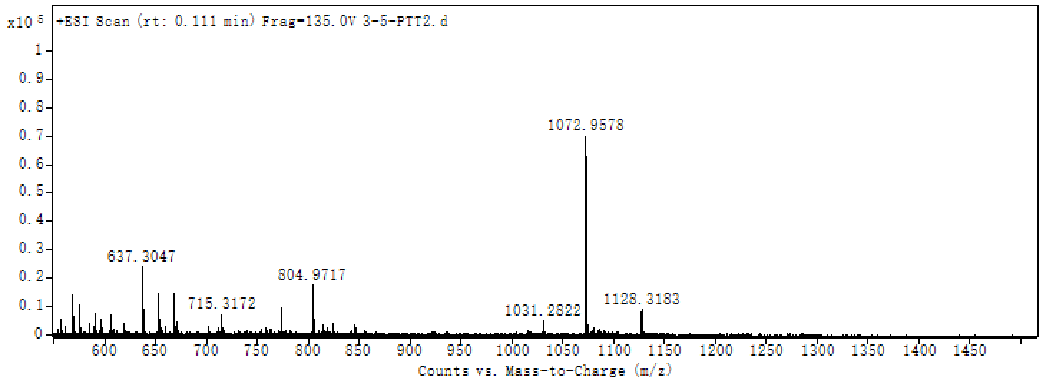


Figure S47. HR-Q-TOF-MS of compound **MTL-3e**

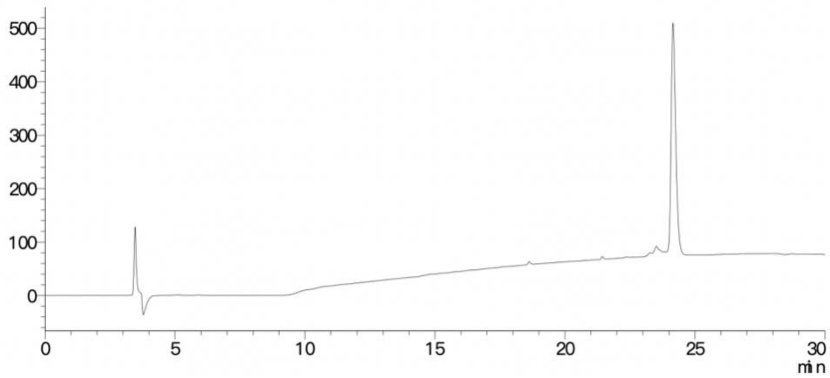


Figure S48. HPLC of compound **MTL-1f** (purity>95%)

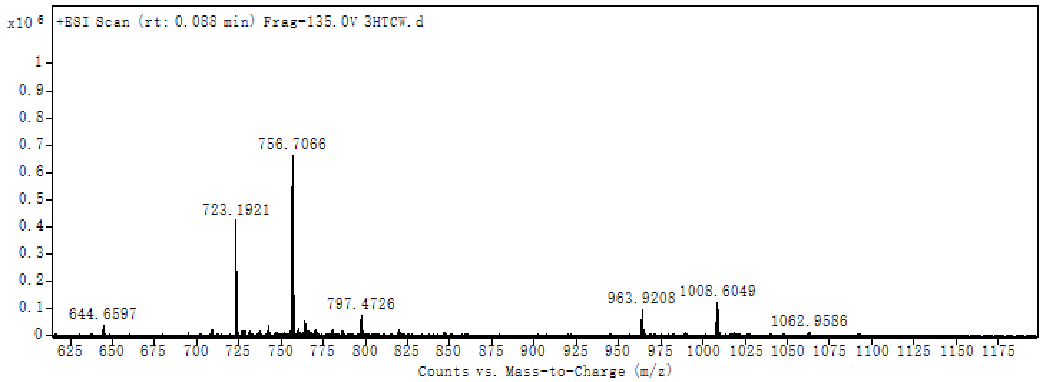


Figure S49. HR-Q-TOF-MS of compound **MTL-1f**

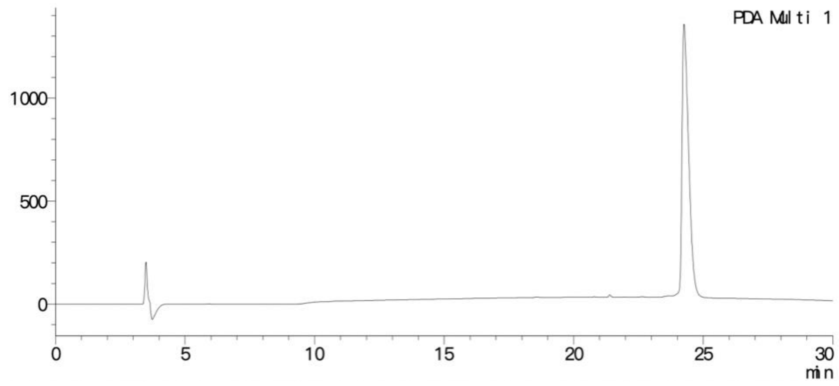


Figure S50. HPLC of compound **MTL-2f** (purity>95%)

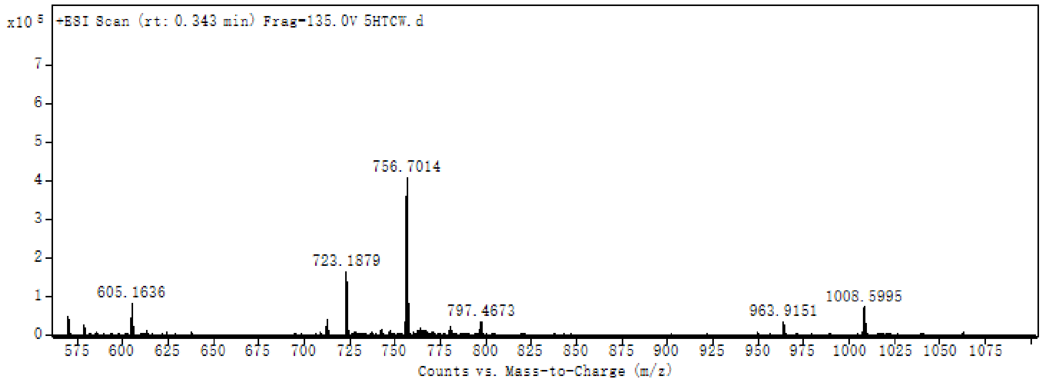


Figure S51. HR-Q-TOF-MS of compound **MTL-2f**

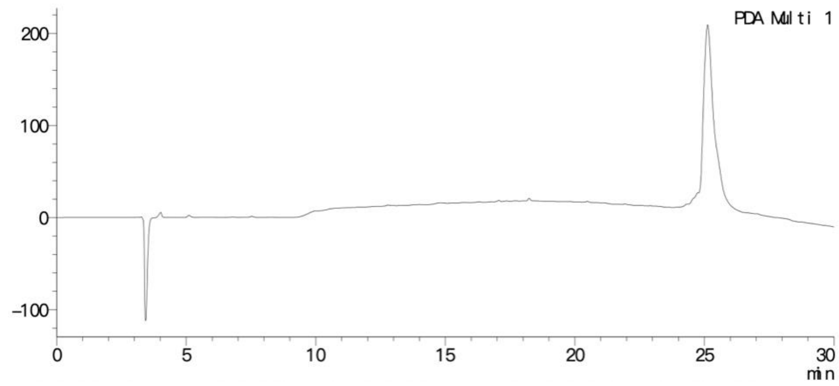


Figure S52. HPLC of compound **MTL-3f** (purity>95%)

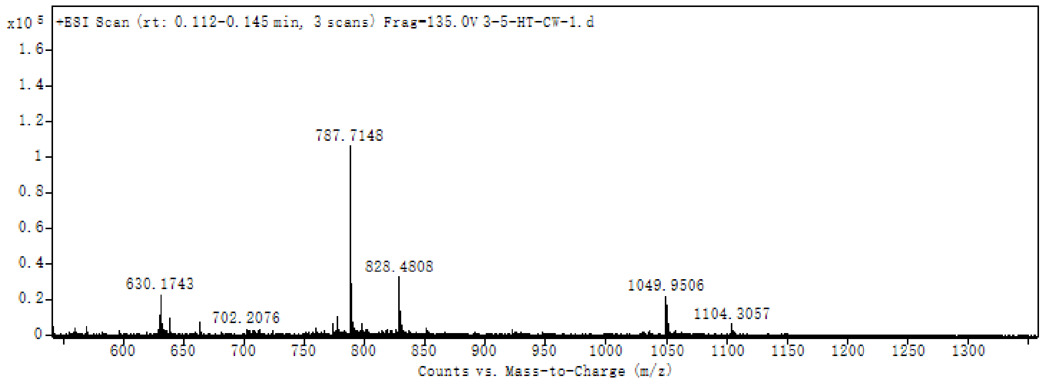


Figure S53. HR-Q-TOF-MS of compound **MTL-3f**

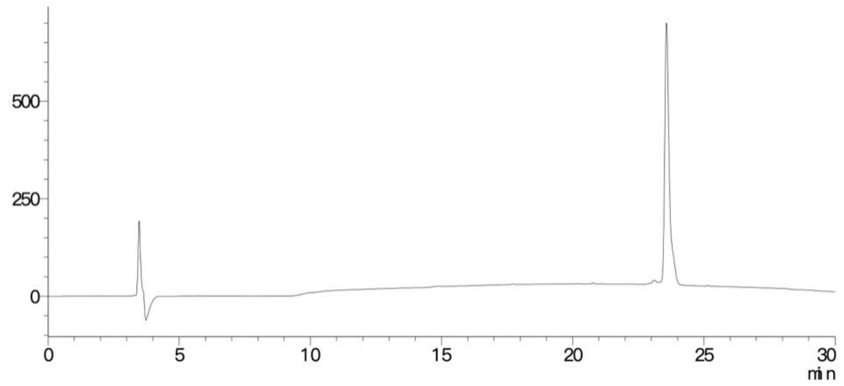


Figure S54. HPLC of compound **MTL-1g** (purity>95%)

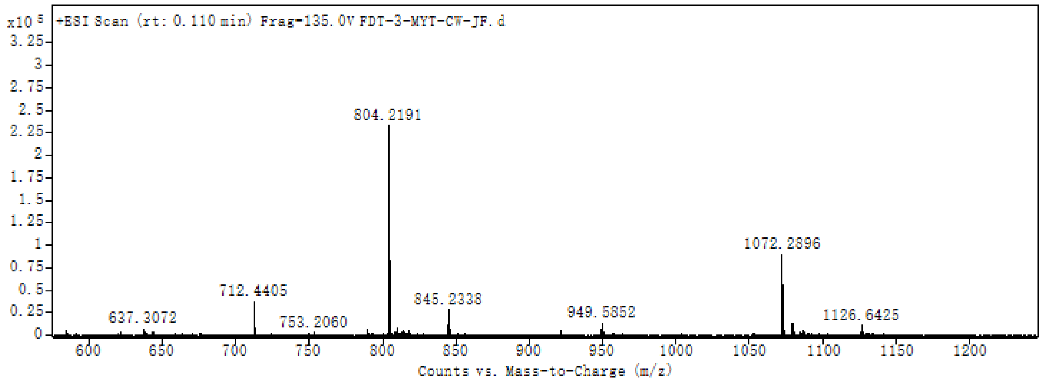


Figure S55. HR-Q-TOF-MS of compound **MTL-1g**

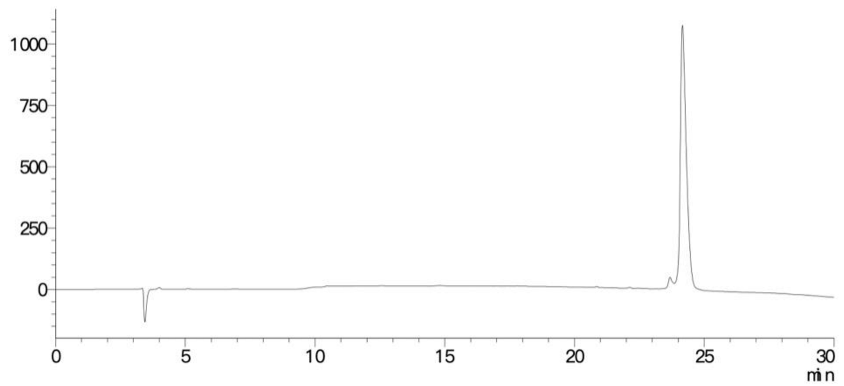


Figure S56. HPLC of compound **MTL-2g** (purity>95%)

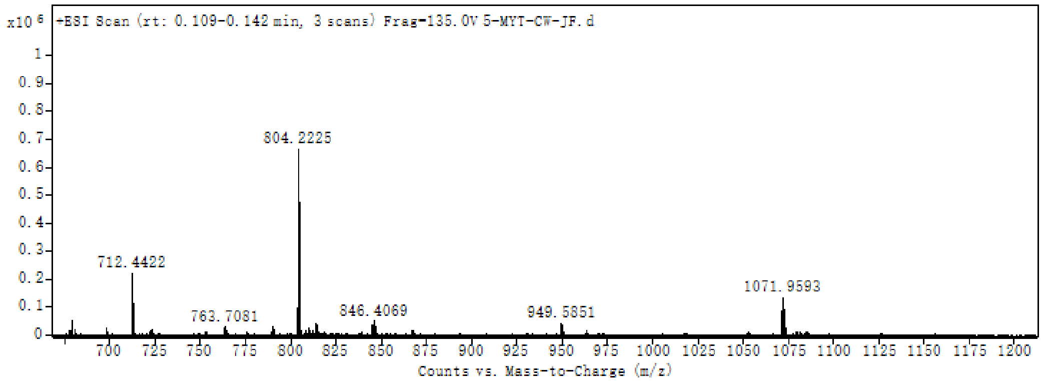


Figure S57. HR-Q-TOF-MS of compound **MTL-2g**

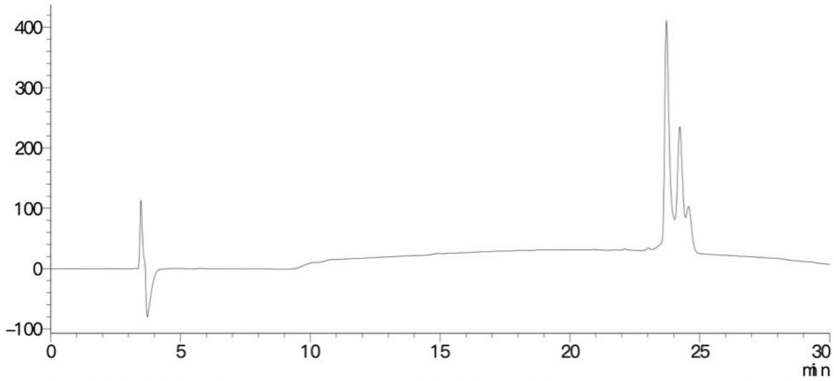


Figure S58. HPLC of compound **MTL-3g** (purity<95%)

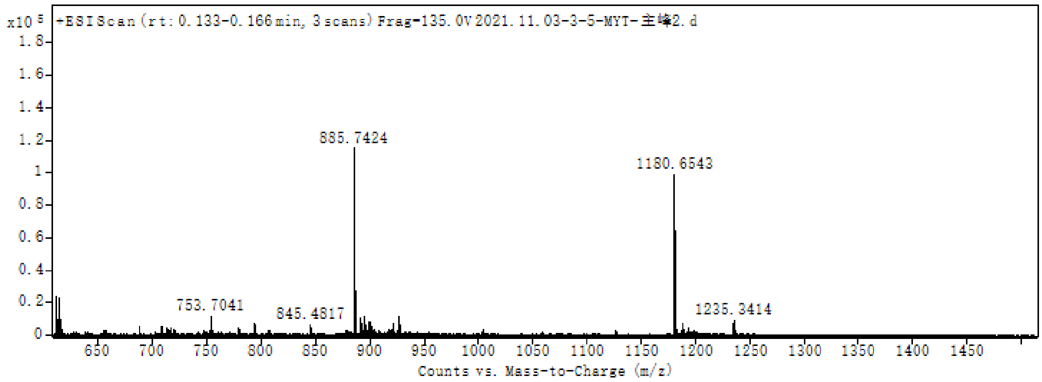


Figure S59. HR-Q-TOF-MS of compound **MTL-3g**

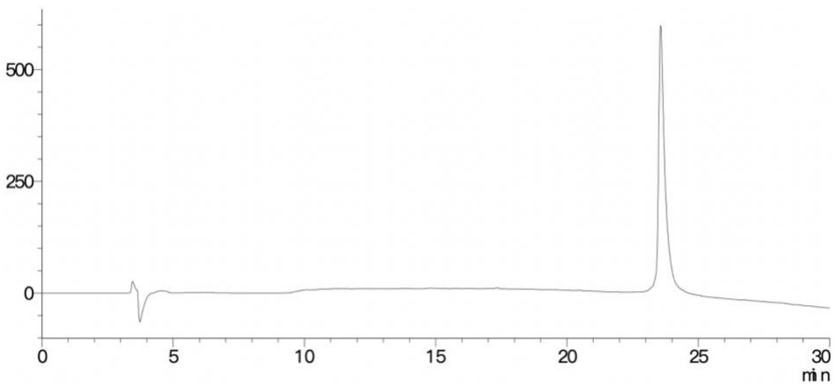


Figure S60. HPLC of compound **MTL-1h** (purity>95%)

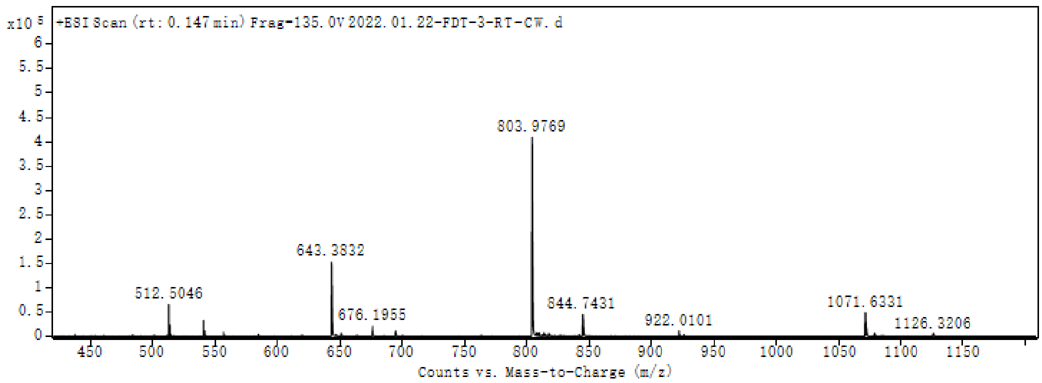


Figure S61. HR-Q-TOF-MS of compound **MTL-1h**

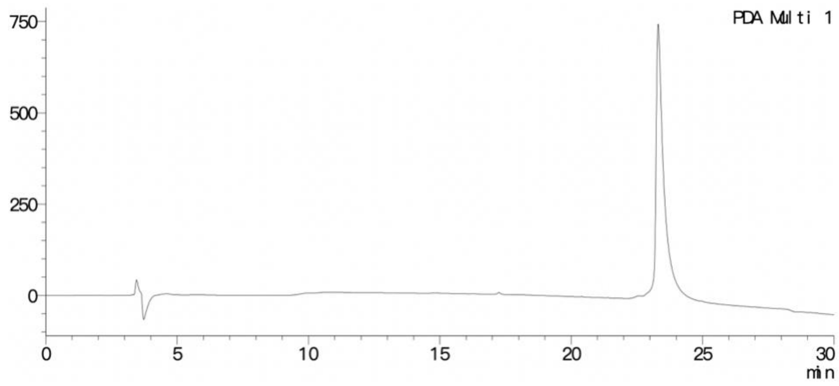


Figure S62. HPLC of compound **MTL-2h** (purity>95%)

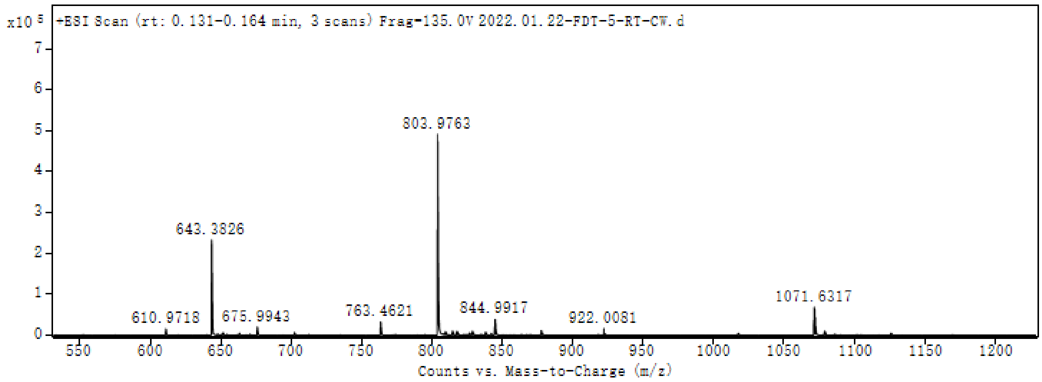


Figure S63. HR-Q-TOF-MS of compound **MTL-2h**

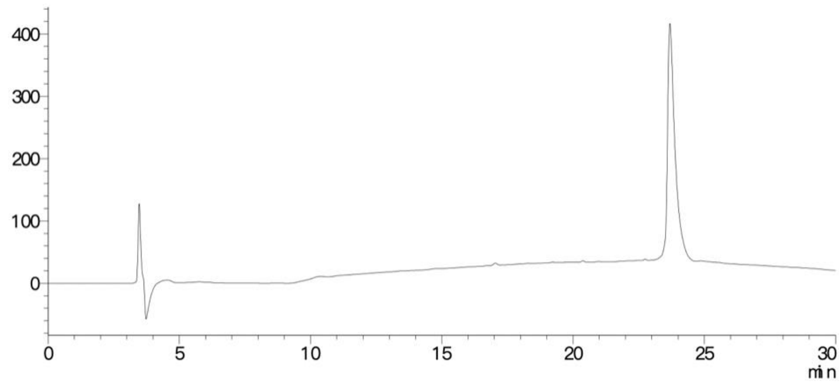


Figure S64. HPLC of compound **MTL-3h** (purity>95%)

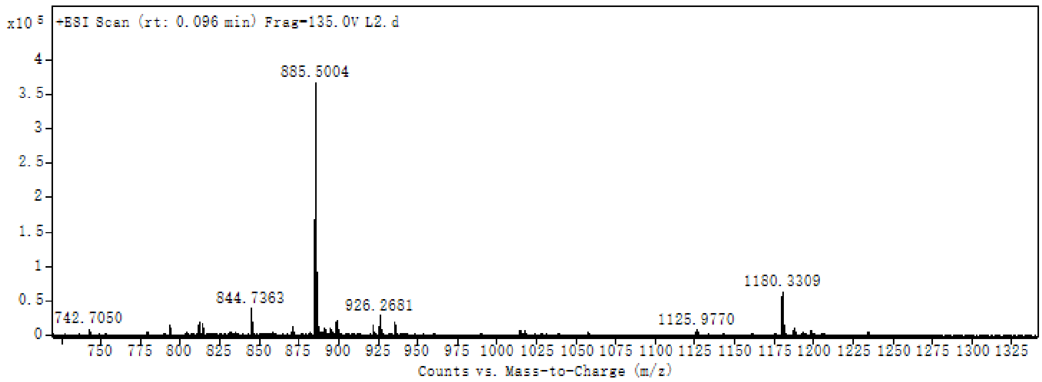


Figure S65. HR-Q-TOF-MS of compound **MTL-3h**

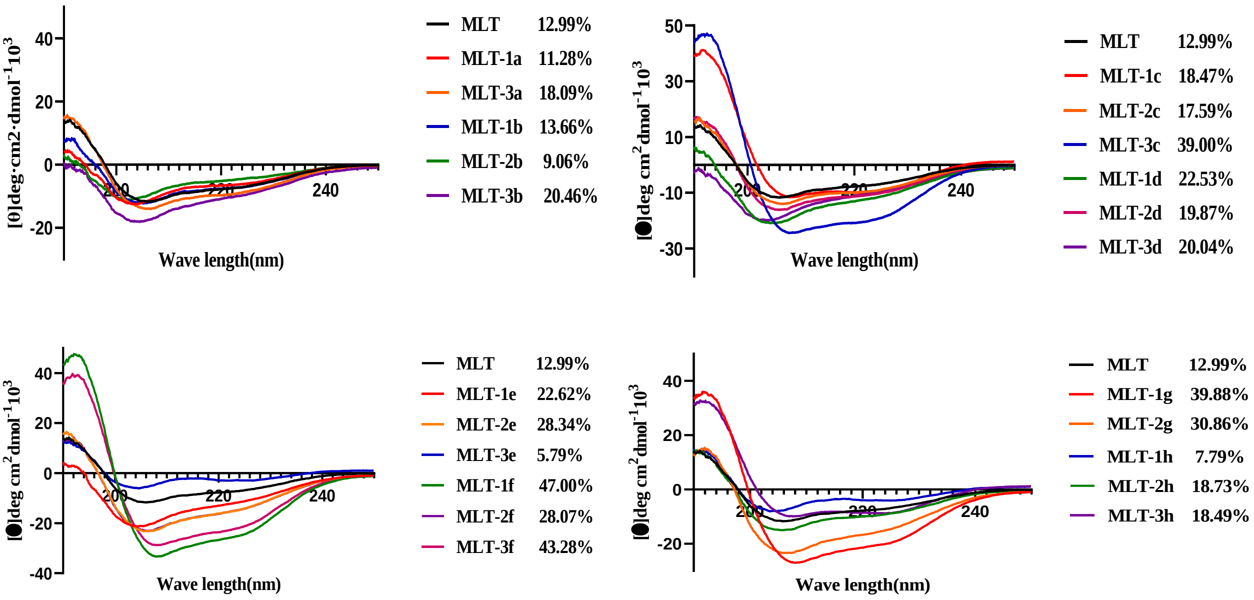
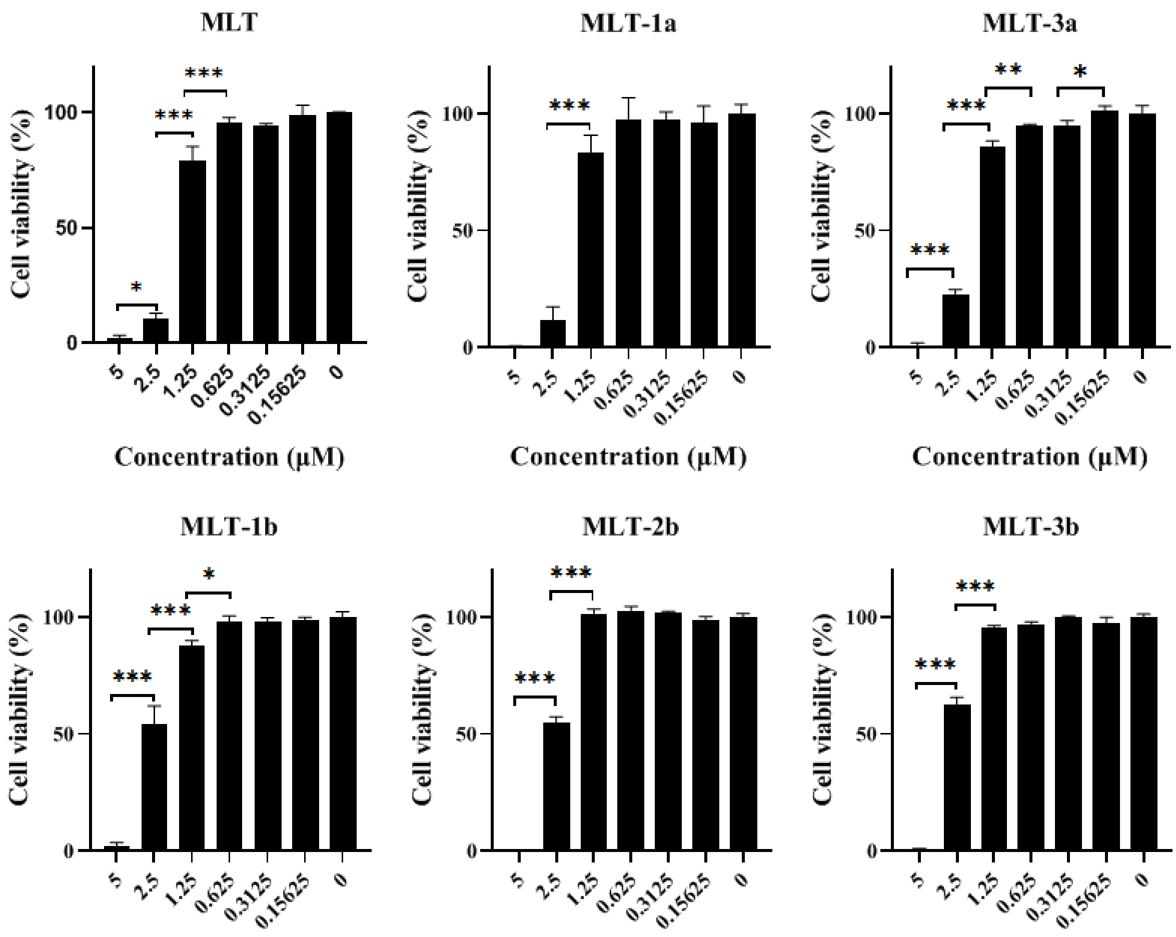
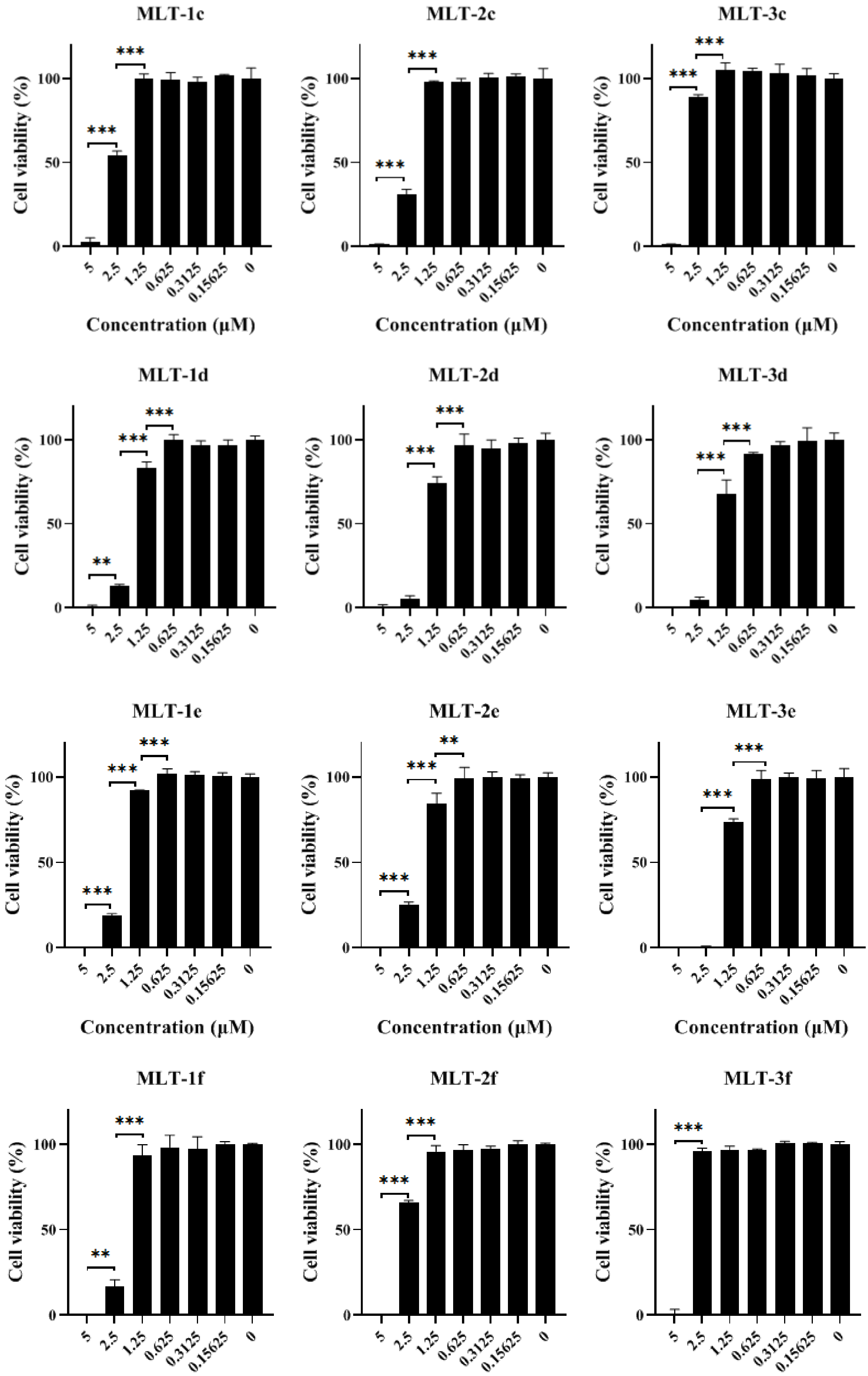


Figure S66. CD spectrum of natural and glycosylated melittin




MLT-1c
MLT-2c
MLT-3c
MLT-1d
MLT-2d
MLT-3d
MLT-1e
MLT-2e
MLT-3e
MLT-1f
MLT-2f
MLT-3f
Cell viability (%)
0
50
100
5
2.5
1.25
0.625
0.3125
0.15625
0
Concentration (μM)
***
**

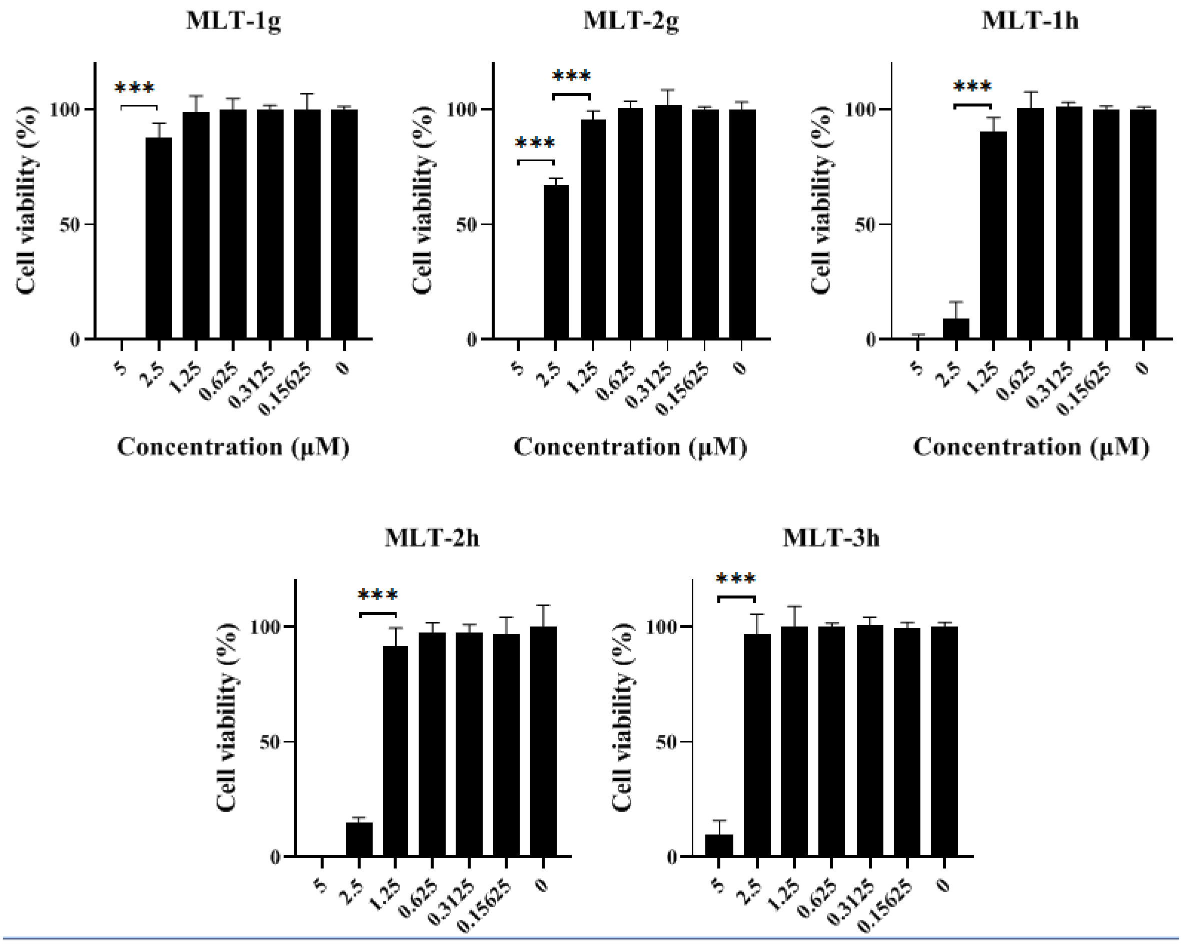


Figure S67. Cytotoxicity of melittin and its analogues against HCT116. Results were represented as mean ± standard deviation ($\bar{x}$ ± *SD*), *: *p <0.05, **: 0.01 <p <0.05, ***: 0.001 <p <0.01*.