Supporting Information for

Sortase A-Catalyzed Peptide Cyclization for the Synthesis of Macrocyclic Peptides and Glycopeptides

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1.1. Solid-phase Synthesis of Peptides 1, 6, 7, and 8

Automatic solid-phase peptide synthesis (SPPS) was carried out with a peptide synthesizer on a 0.1 mmol scale, starting from the commercial Wang resin loaded with a serine residue employing standard protocols and Fmoc chemistry. The completed peptides were released from the resin with simultaneous side-chain deprotection using 95% (v/v) trifluoroacetic acid (TFA) in water containing 2.5% (v/v) of triethylsilane (Et_3SiH). All of the products were purified by HPLC and characterized by MALDI-TOF MS (Figures 1-4). **1**, calcd for $C_{60}H_{101}N_{17}O_{24}$: 1443.7, observed 1445.1 $[M+H]^+$; **6**, calcd for $C_{62}H_{104}N_{18}O_{25}$: 1500.7, observed 1501.3 $[M+H]^+$; **7**, calcd for $C_{64}H_{107}N_{19}O_{26}$: 1557.8, observed 1558.3 $[M+H]^+$; **8**, calcd for $C_{66}H_{110}N_{20}O_{27}$: 1614.8, observed 1615.5 $[M+H]^+$

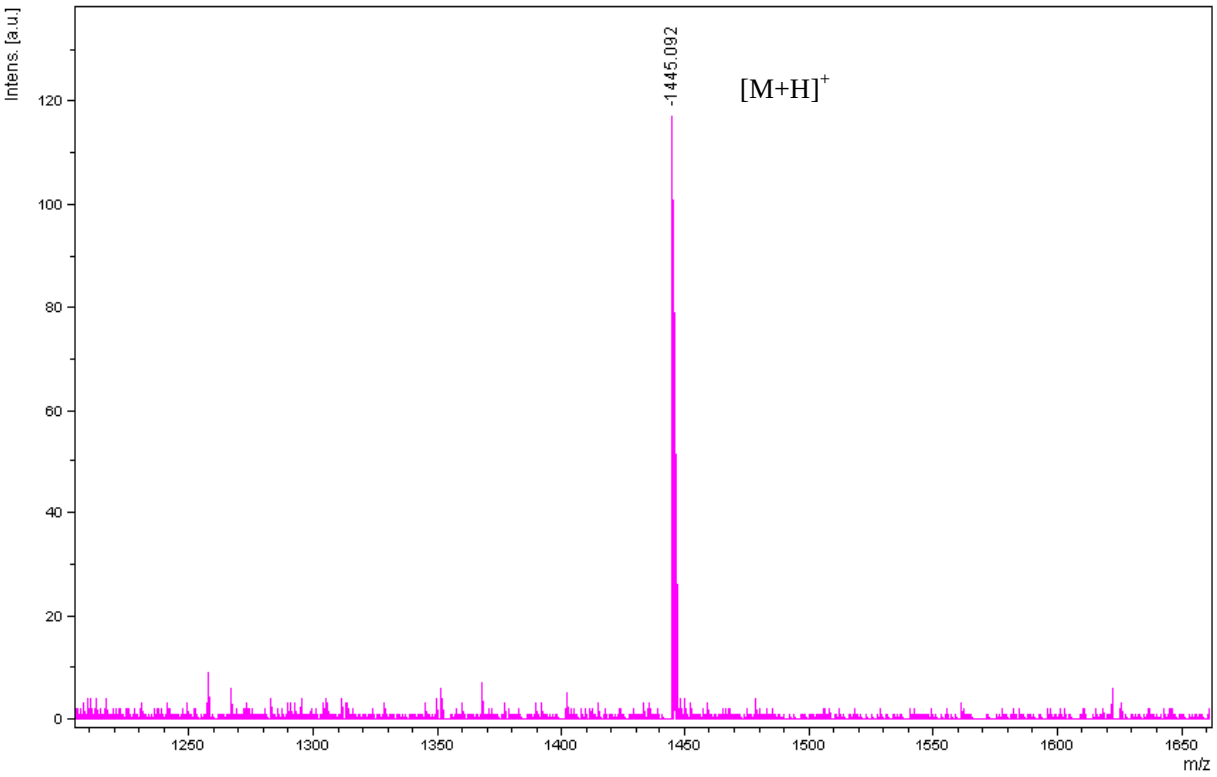


Figure 1. MALDI-TOF MS of **1**

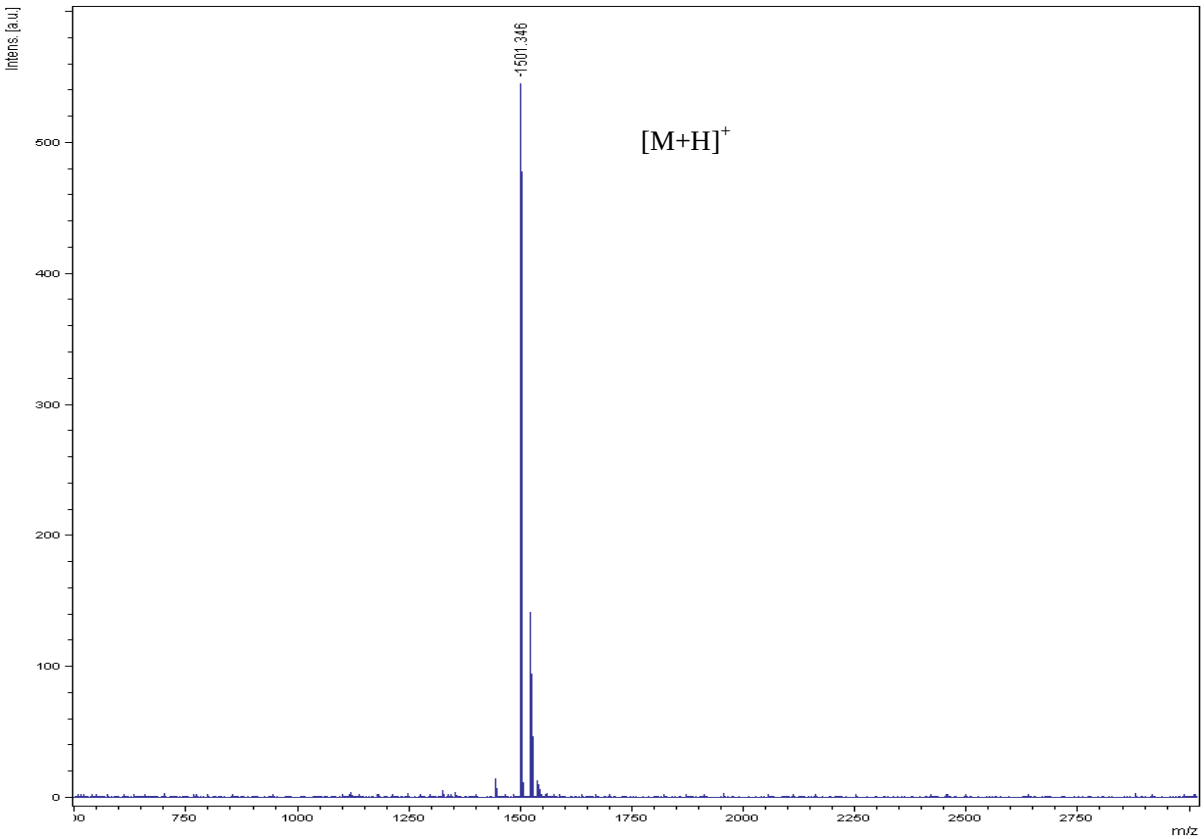


Figure 2. MALDI-TOF MS of **6**

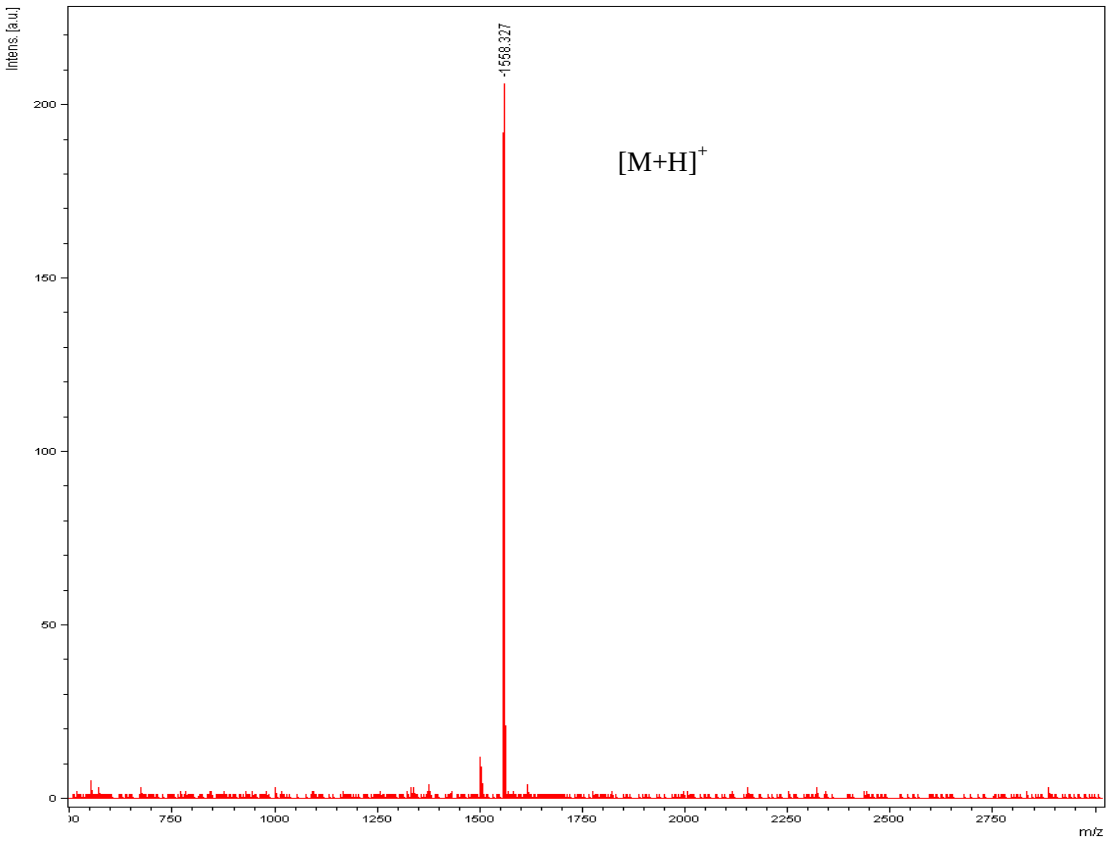


Figure 3. MALDI-TOF MS of **7**

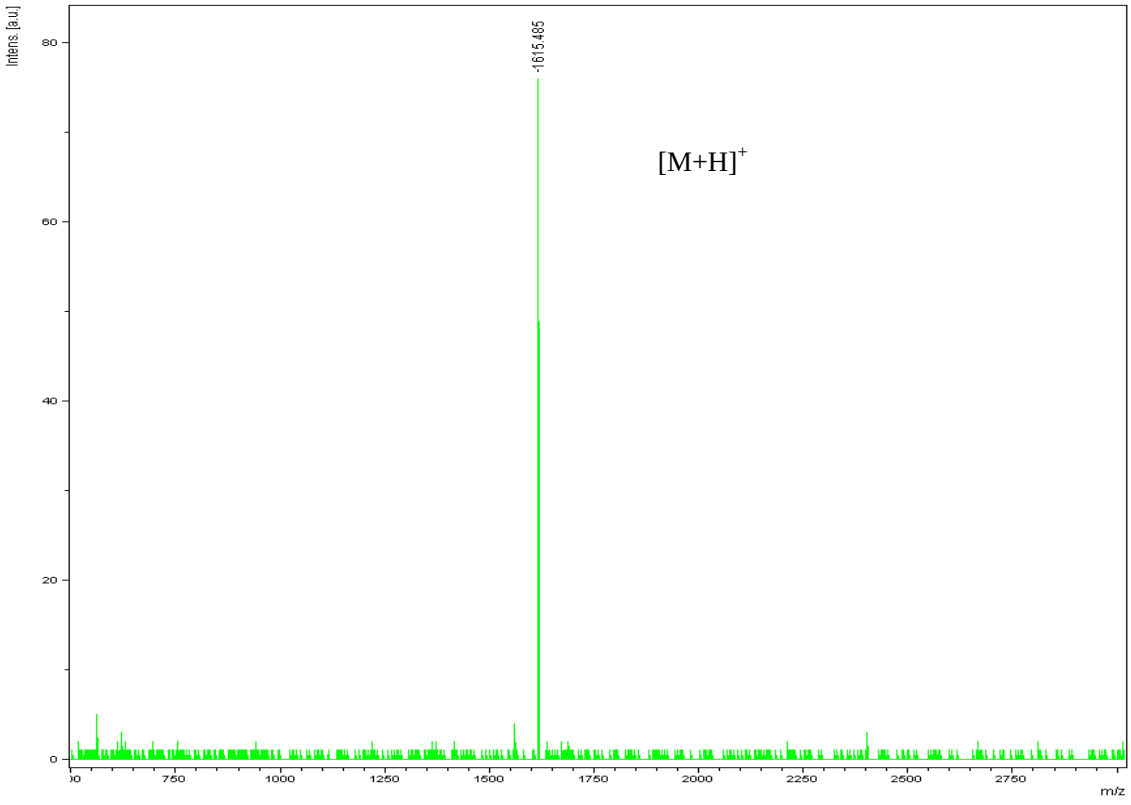


Figure 4. MALDI-TOF MS of **8**

1.2. Solid-Phase Synthesis of Glycopeptide 9

Glycopeptide **9** was prepared according to our previously reported method (Wu, Z. M.; Guo, X. Q.; Guo, Z. W., *Chem Commun* **2010,** *46*, 5773-5774). After the glycopeptide assembly on the resin was finished, the *N*-terminal Fmoc was removed by 10% of piperidine. Then, the resin was treated with 95% aq. TFA containing 2.5% Et_3SiH at rt for 2 h to release acetylated glycopeptide **13**, which was characterized with MALDI–TOF MS [Figure 5, calcd for $C_{102}H_{158}N_{20}O_{48}$ (m/z) 2431.1, observed 2432.3 $(M + H)^+$]. Glycopeptide **13** was dissolved in MeOH, to which was added 0.1 M solution of NaOMe in MeOH to adjust the pH to *ca*. 8.0. The reaction progress was monitored by MALDI-TOF. After the reaction was finished, it was neutralized by addition of acetic acid. The solvent was removed in *vacuum*, and the residue was dissolved in water and purified by preparative RP-HPLC (20% to 50% MeCN in H_2O, both containing 0.1% TFA) to afford **9** as a white solid, which was characterized with MALDI-TOF MS [Figure 6, calcd for $C_{84}H_{140}N_{20}O_{39}$ (m/z) 2052.9, observed 2054.0 $(M + H)^+$].

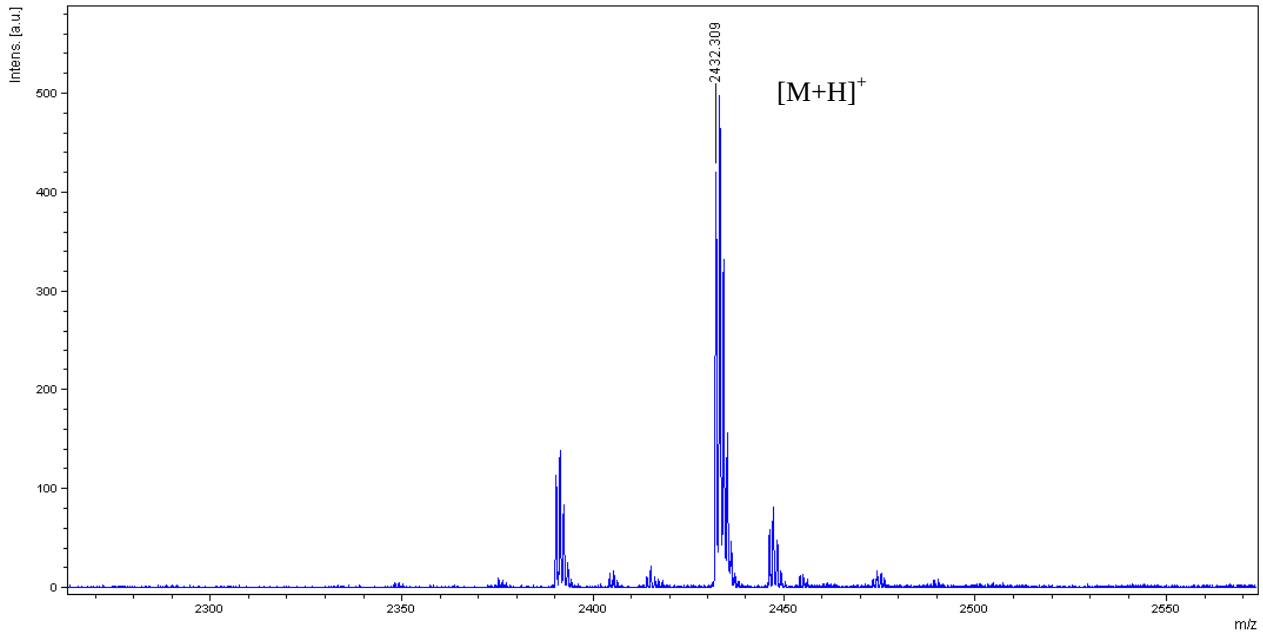


Figure 5. MALDI-TOF MS of **13**

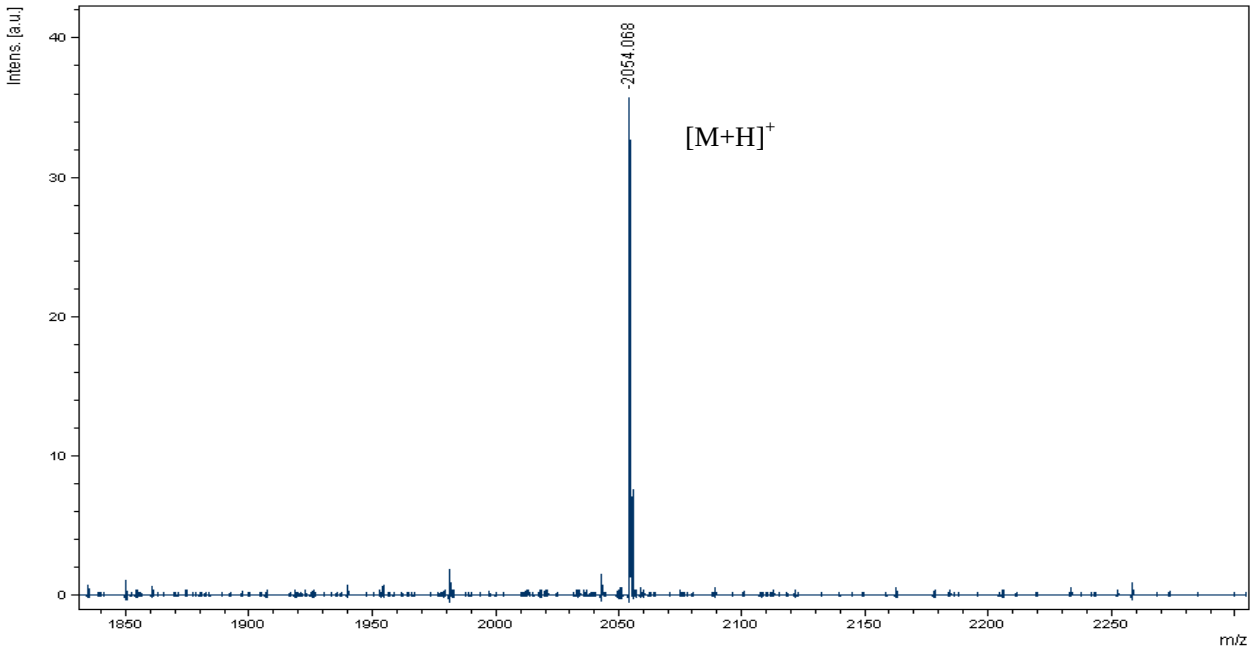
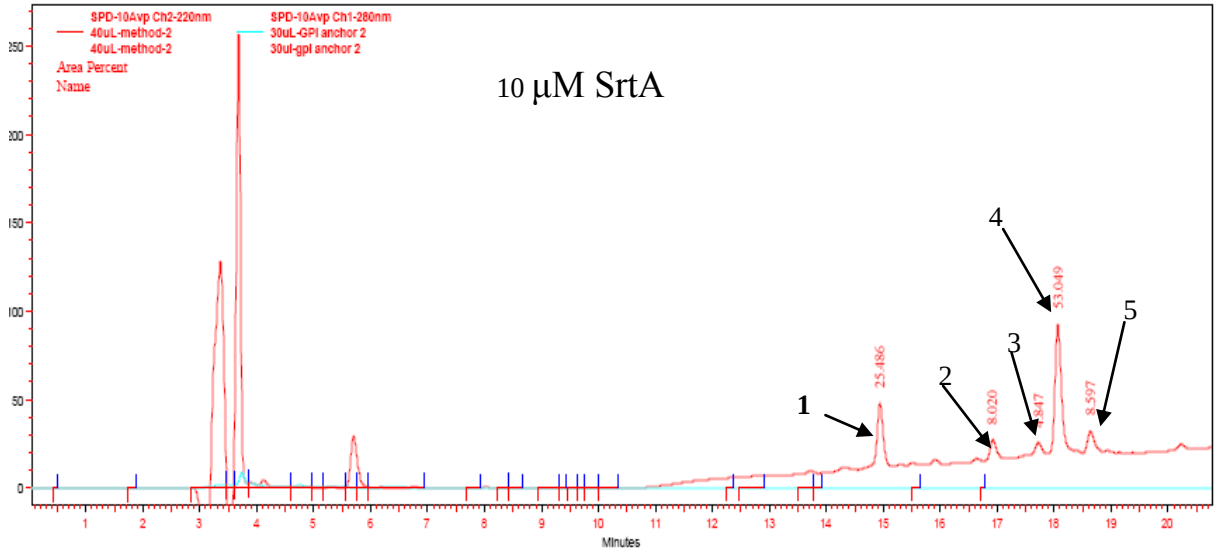


Figure 6. MALDI-TOF MS of **9**

2. SrtA-Catalyzed Peptide Oligomerization and Cyclization Reactions

2.1 SrtA-catalyzed reactions of 1 in the presence of different concentrations of SrtA

The reaction was performed in 100 μL solution with fixed concentration of peptide **1** (0.5 mM) and different concentrations of SrtA (10, 20, 40, and 60 μM) in 0.3 M Tris-HCl buffer (pH = 7.5) containing 0.15 M NaCl, 5 mM $CaCl_2$, and 2 mM 2-mercaptoethanol. After the reaction was kept at 37 ^oC for 20 h, 20 μL of the reaction mixture was withdrawn and quenched with the same volume of 0.1% of TFA. The aliquots were then analyzed by HPLC using a RP C18 column (Figure 7) and the fractions were analyzed by MALDI-TOF MS (Figures 11-14).



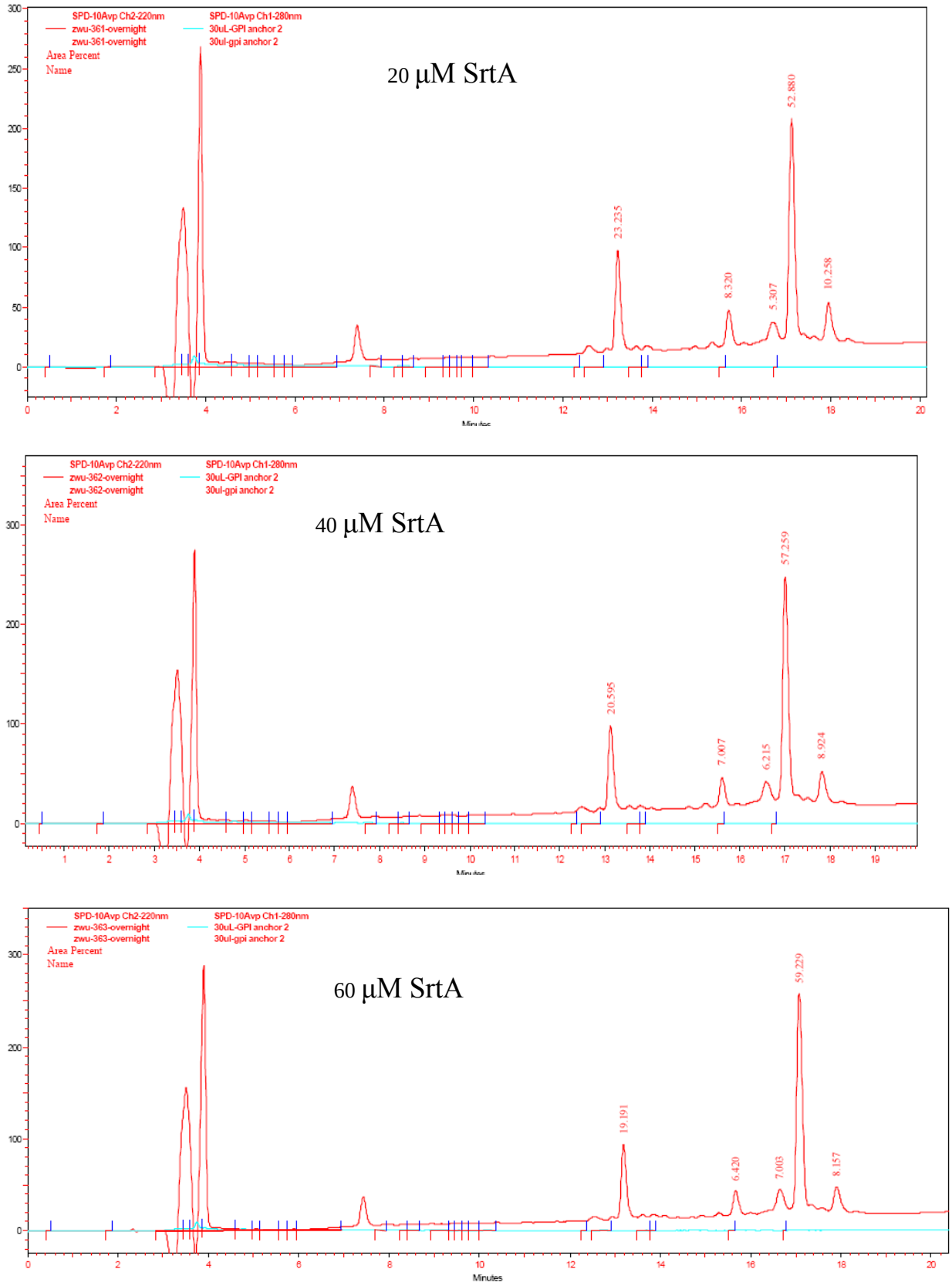
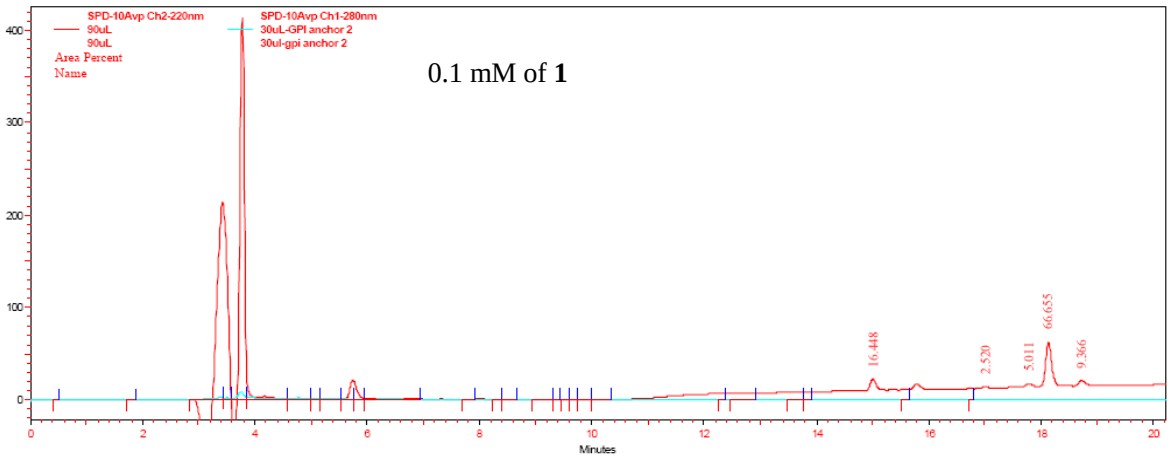
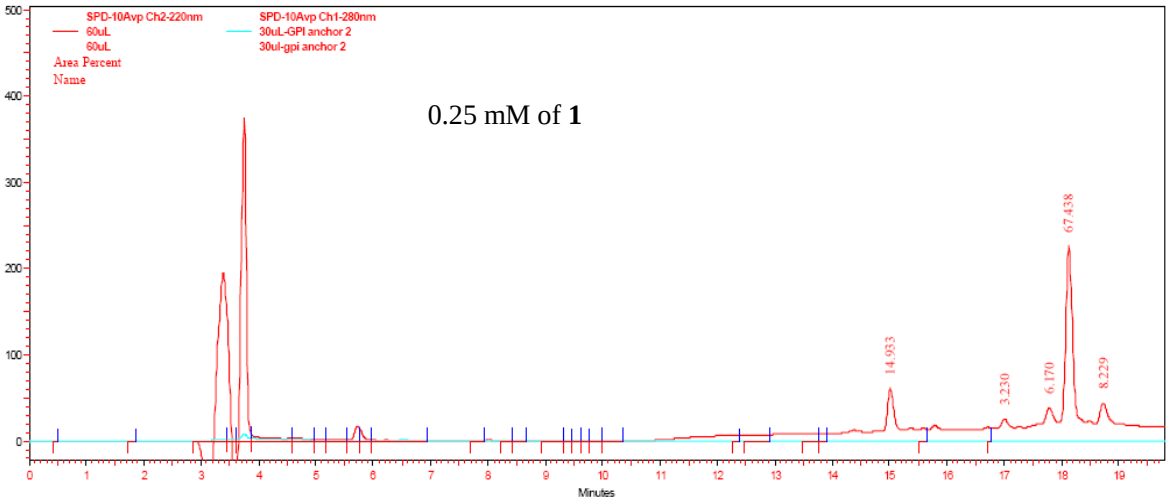


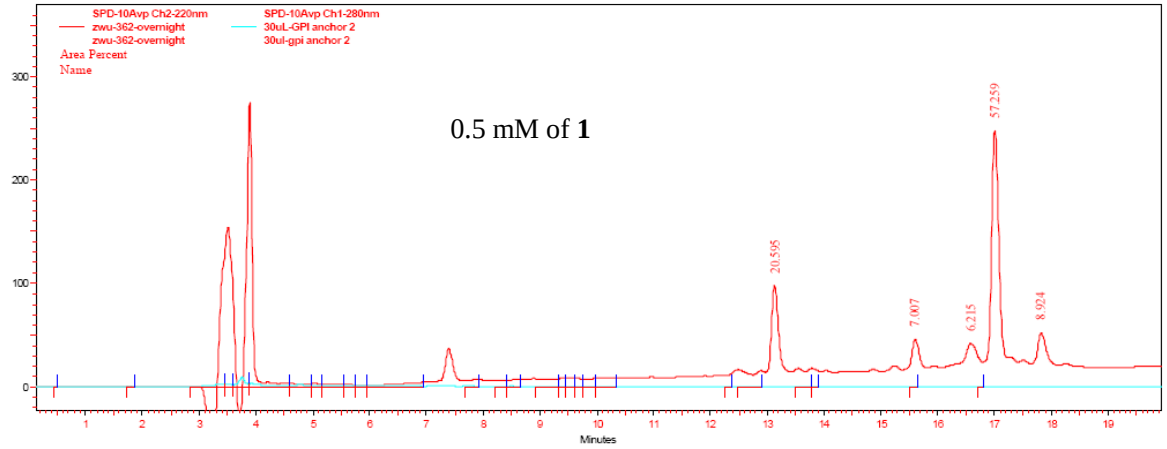
Figure 7. HPLC diagrams of the reactions of **1** in the presence of different concentrations of SrtA. Monitored with UV at 220 nm. Eluent: 10% to 40% MeCN in H_2O (both containing 0.1% TFA) in 15 min; flow rate: 1 mL/min; column: C-18 (250 x 4.6 mm).

2.2 SrtA-catalyzed reactions of different concentrations of 1

The reaction was performed in 100 μL solution with a fixed concentration of SrtA (40 μM) and different concentrations of **1** (0.1, 0.25, 0.5, 1, 2.5, 5, and 10 mM). Reactions were carried out in 0.3 M Tris-HCl buffer (pH = 7.5) containing 0.15 M NaCl, 5 mM $CaCl_2$, and 2 mM 2-mercaptoethanol. After the reaction was incubated at 37 ^{o}C for 20 h, 20 μL of the reaction mixture was taken and then quenched with the same volume of 0.1% of TFA. The aliquots were analyzed by HPLC using a RP C18 column (Figure 8), and the fractions were analyzed by MALDI-TOF MS (Figures 11-14).







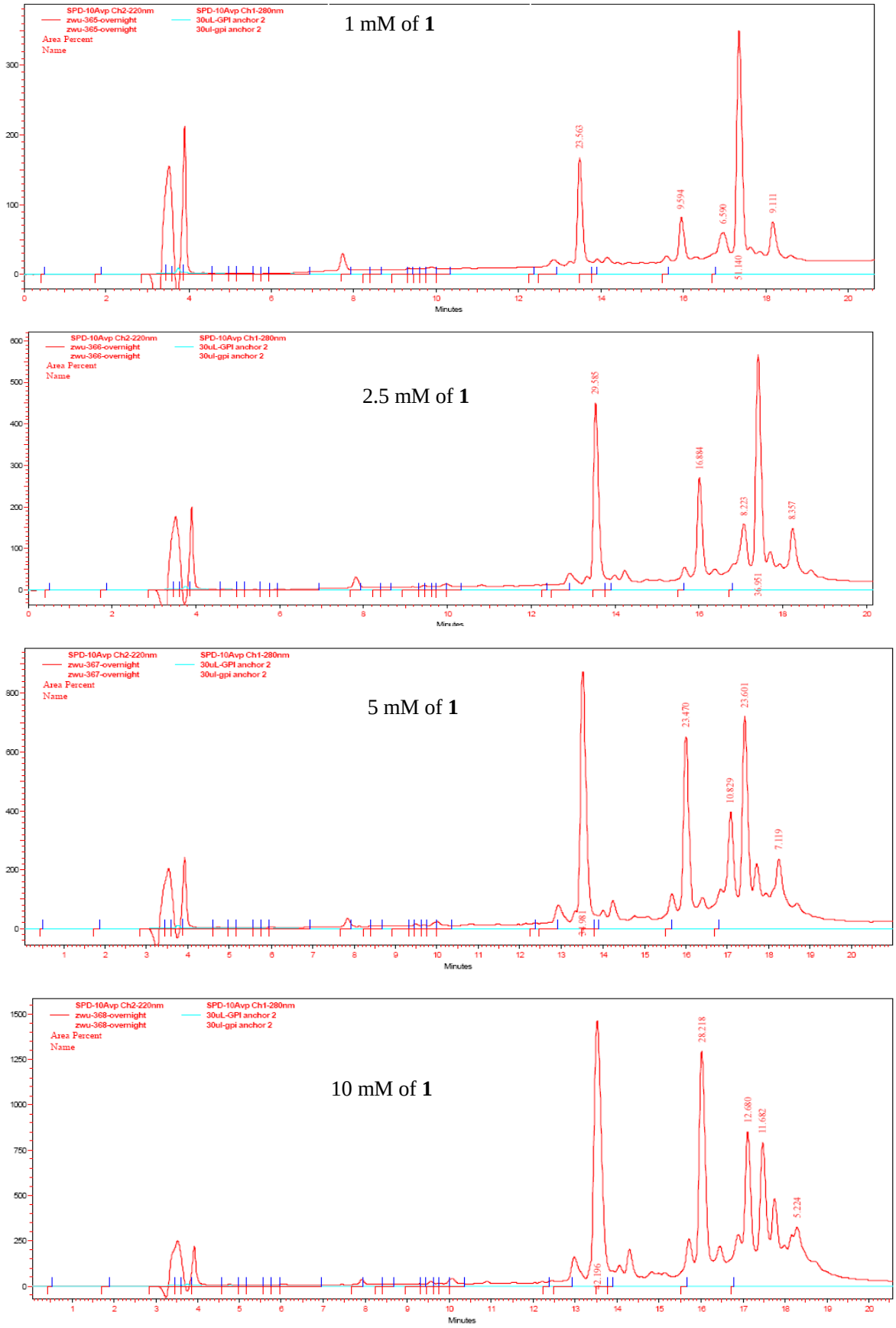
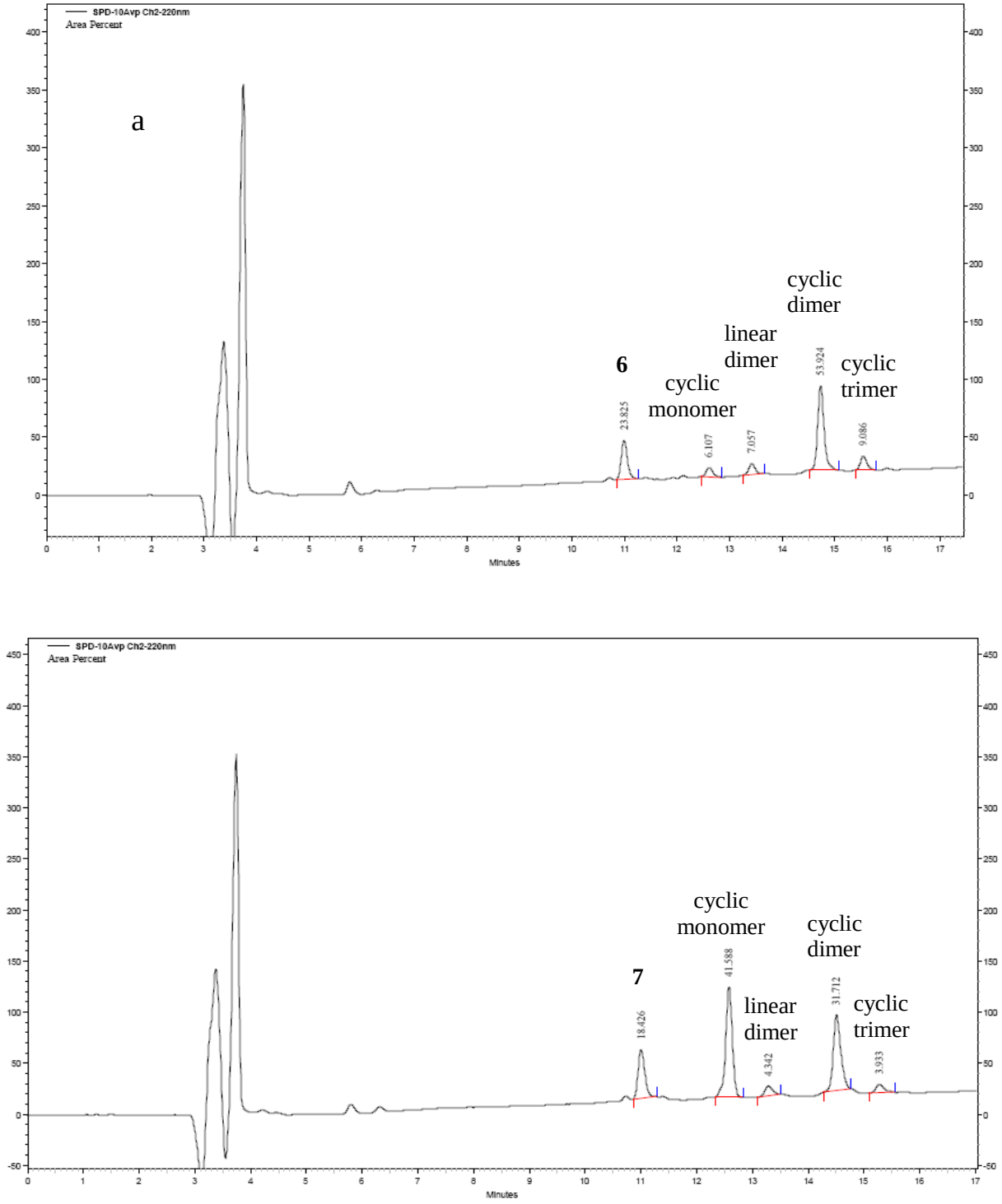


Figure 8. HPLC diagrams of the reactions of different concentrations of **1** in the presence of 40 μM SrtA. Monitored with UV at 220 nm. Eluent: 10% to 40% MeCN in H_2O (both containing 0.1% TFA) in 15 min; flow rate: 1 mL/min; column: C-18 (250 x 4.6 mm).

2.3 SrtA-catalyzed oligomerization and clyclization reactions of 6, 7, and 8

The reactions were performed in 100 μL solutions with a fixed concentration of SrtA (40 μM) and 0.5 mM of peptide **6, 7** or **8** in 0.3 M Tris-HCl buffer (pH = 7.5) containing 0.15 M NaCl, 5 mM $CaCl_2$, and 2 mM 2-mercaptoethanol. The reaction conditions and workup procedures were identical to that used in the reactions of **1**. The reaction aliquots were analyzed by HPLC with a RP C18 column (Figure 9), and fractions were analyzed by MALDI-TOF MS (Figures 15-24).



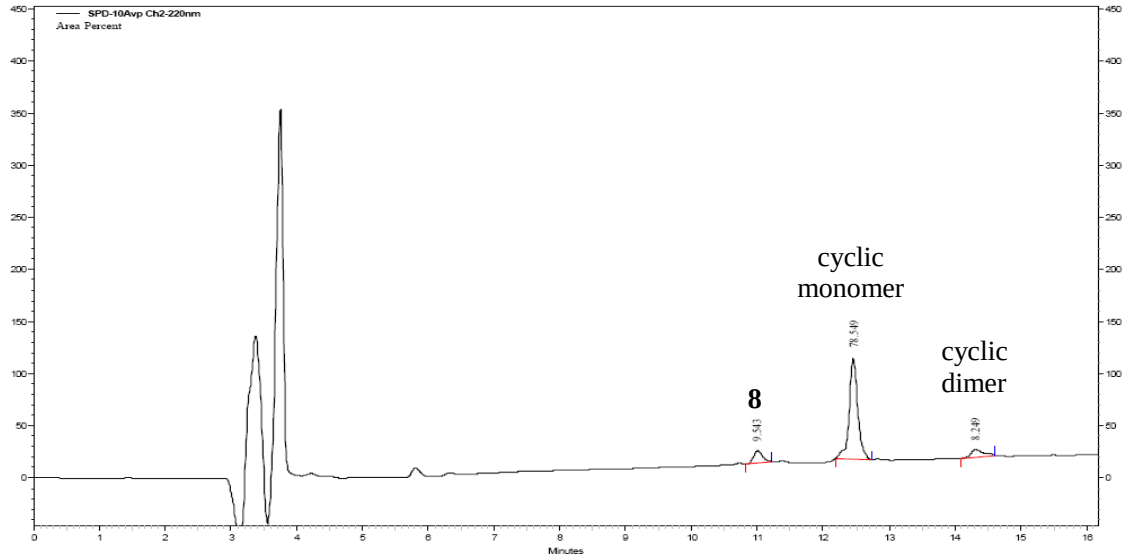


Figure 9. HPLC diagrams of reactions of **6**, **7**, and **8** in the presence of 40 μM of SrtA. Monitored with UV at 220 nm. Eluent: 10% to 40% MeCN in H_2O (both containing 0.1% TFA) in 15 min; flow rate: 1 mL/min; column: C-18 (250 x 4.6 mm).

2.4 SrtA-catalyzed oligomerization and clyclization reactions of glycopeptide 9

The reaction was performed at the optimized conditions with the concentrations of **9** at 0.25 mM and SrtA at 20 μM. After incubation at 37 ^{o}C for 20 h, 20 μL of the reaction mixture was taken and quenched with the same volume of 0.1% of TFA. The aliquots were analyzed by HPLC with a C18 column (Figure 10), and the fractions were analyzed by MALDI-TOF MS (Figures 25-27).

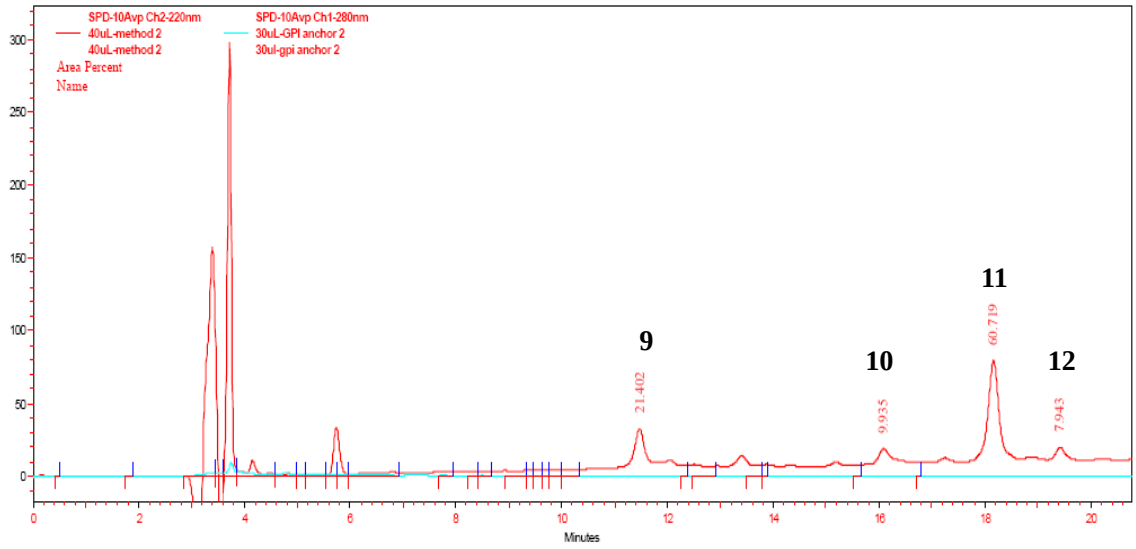


Figure 10. HPLC diagram of the reaction of **9** in the presence of 40 μM of SrtA. Monitored with UV at 220 nm. Eluent: 10% to 40% MeCN in H_2O (both containing 0.1% TFA) in 15 min; flow rate: 1 mL/min; column: C-18 (250 x 4.6 mm).

3. MALDI-TOF MS of Cyclic Peptide and Glycopeptide Products

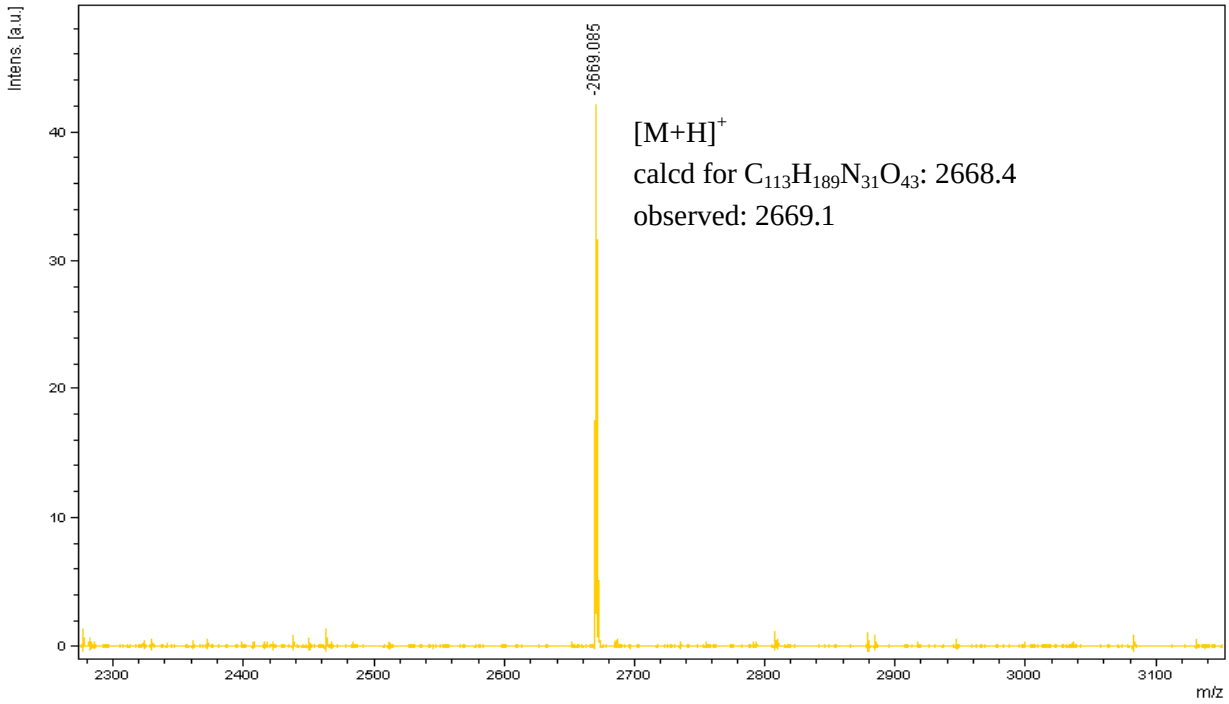


Figure 11. MALDI-TOF MS of purified **2**

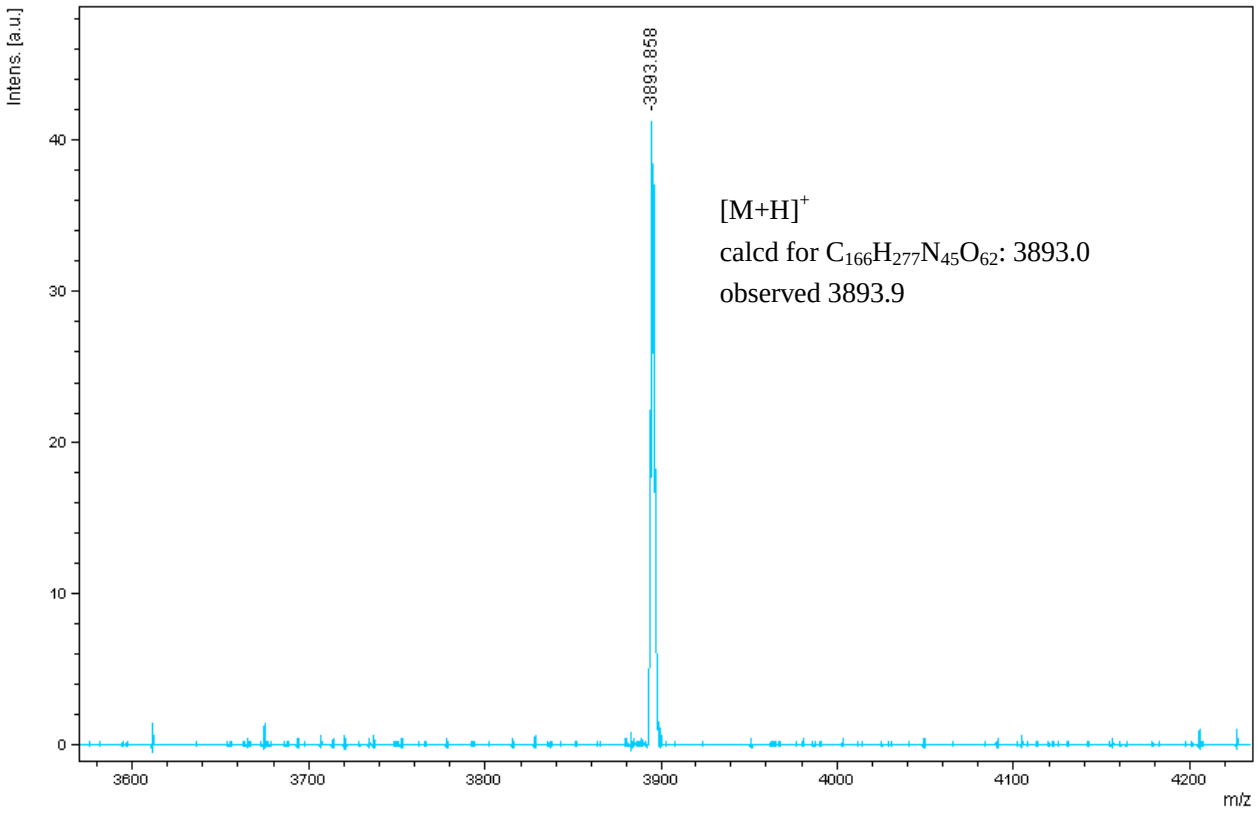


Figure 12. MALDI-TOF MS of purified **3**

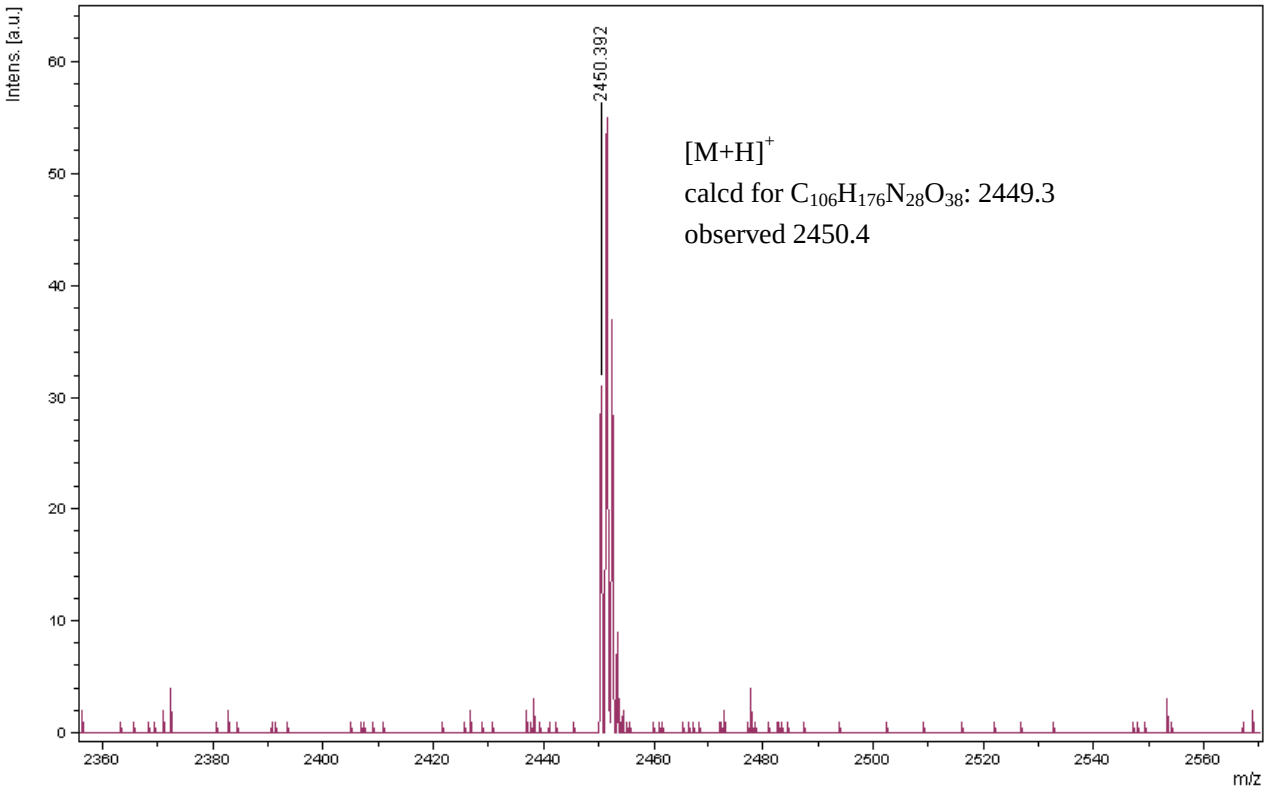


Figure 13. MALDI-TOF MS of purified **4**

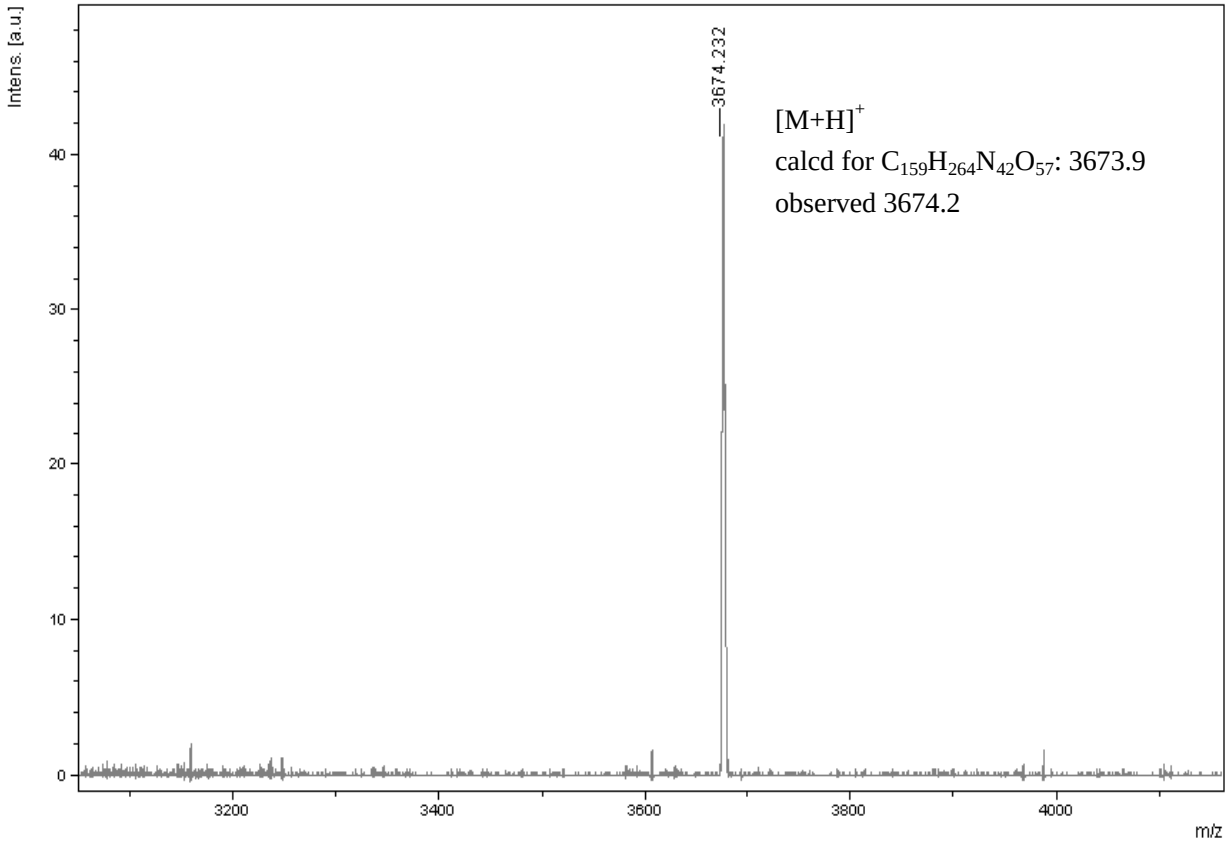


Figure 14. MALDI-TOF MS of purified **5**

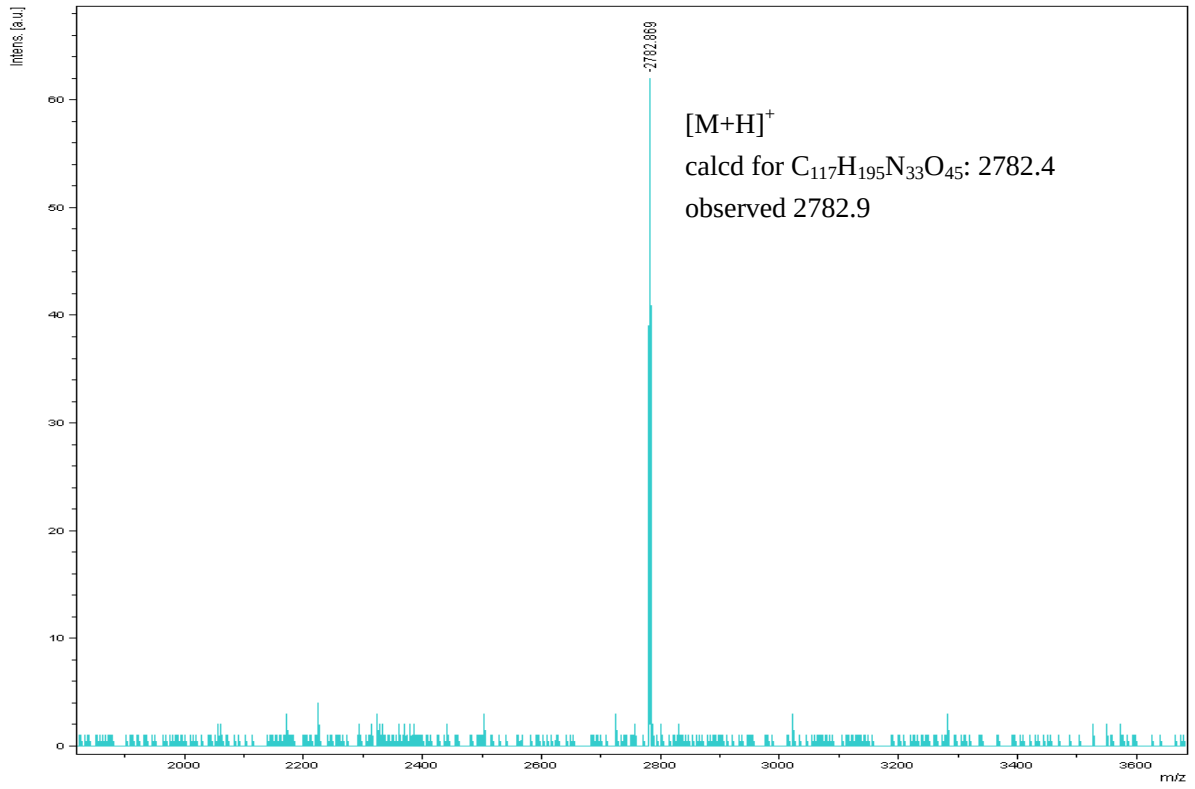


Figure 15. MALDI-TOF MS of purified linear dimer of **6**

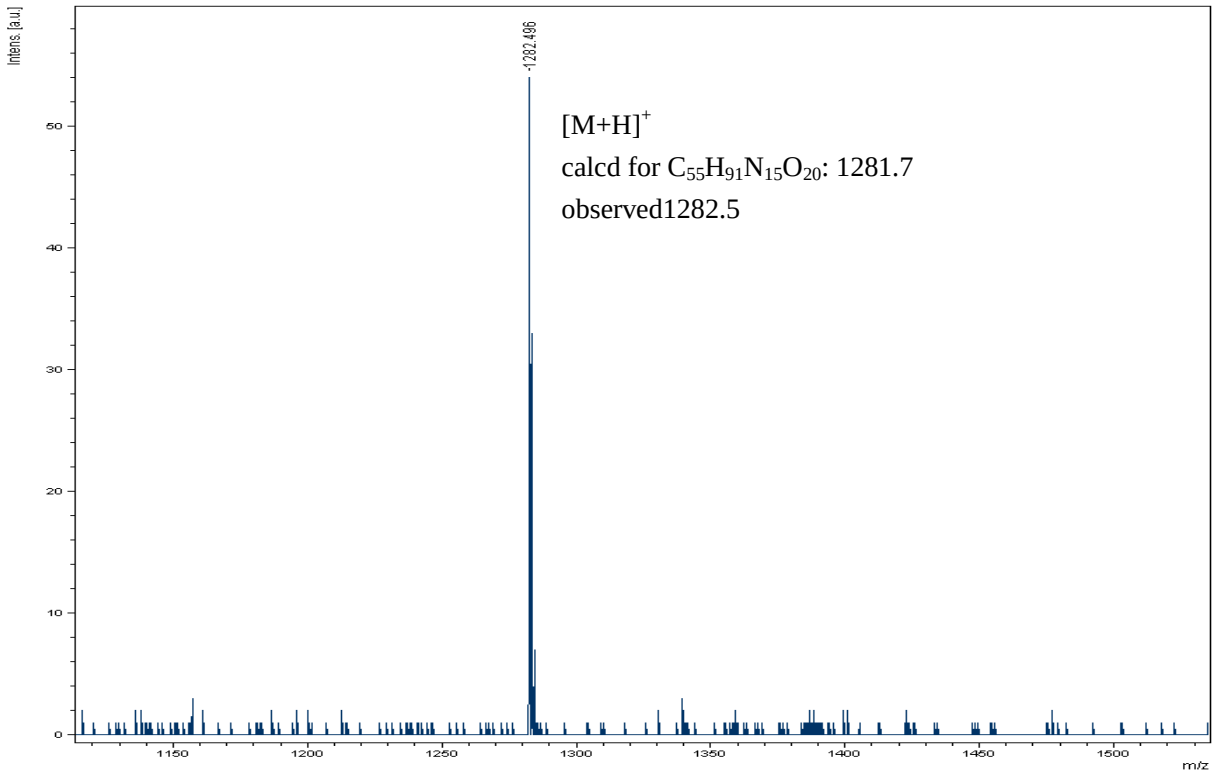


Figure 16. MALDI-TOF MS of purified cyclic monomer of **6**

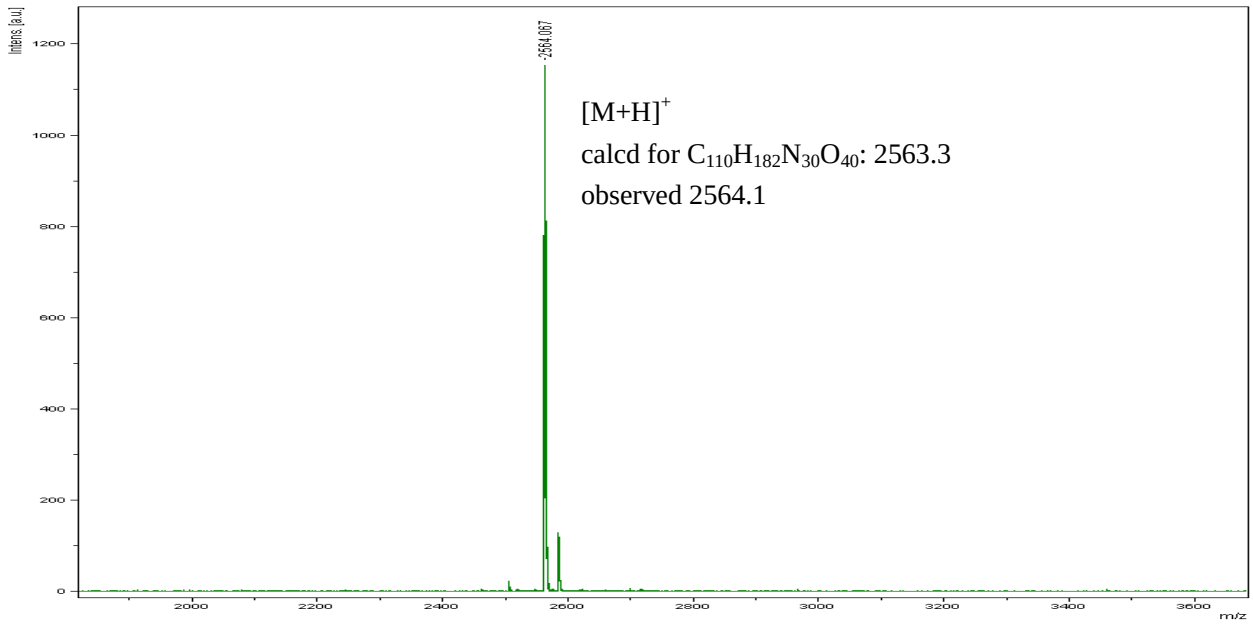


Figure 17. MALDI-TOF MS of purified cyclic dimer of **6**

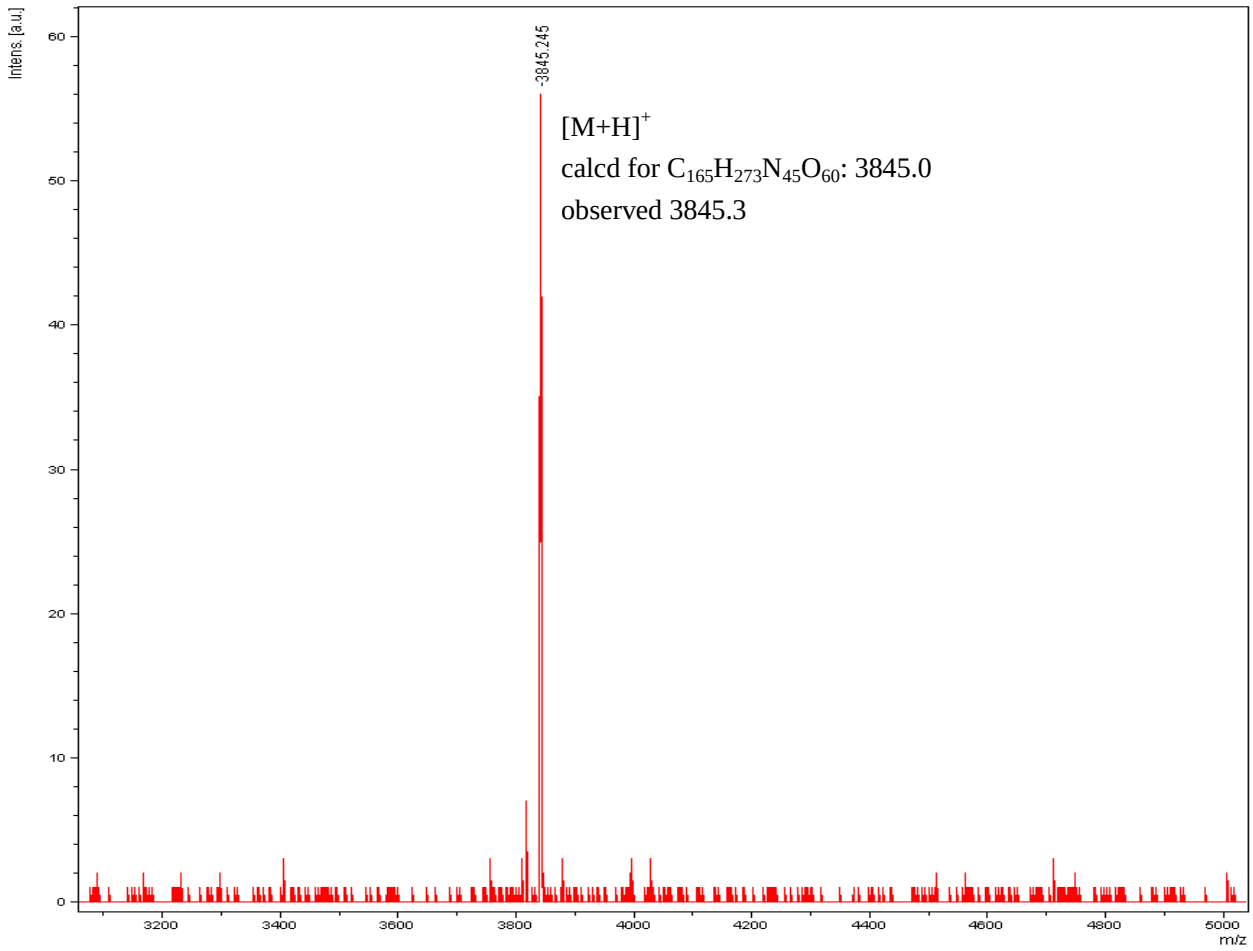


Figure 18. MALDI-TOF MS of purified cyclic trimer of **6**

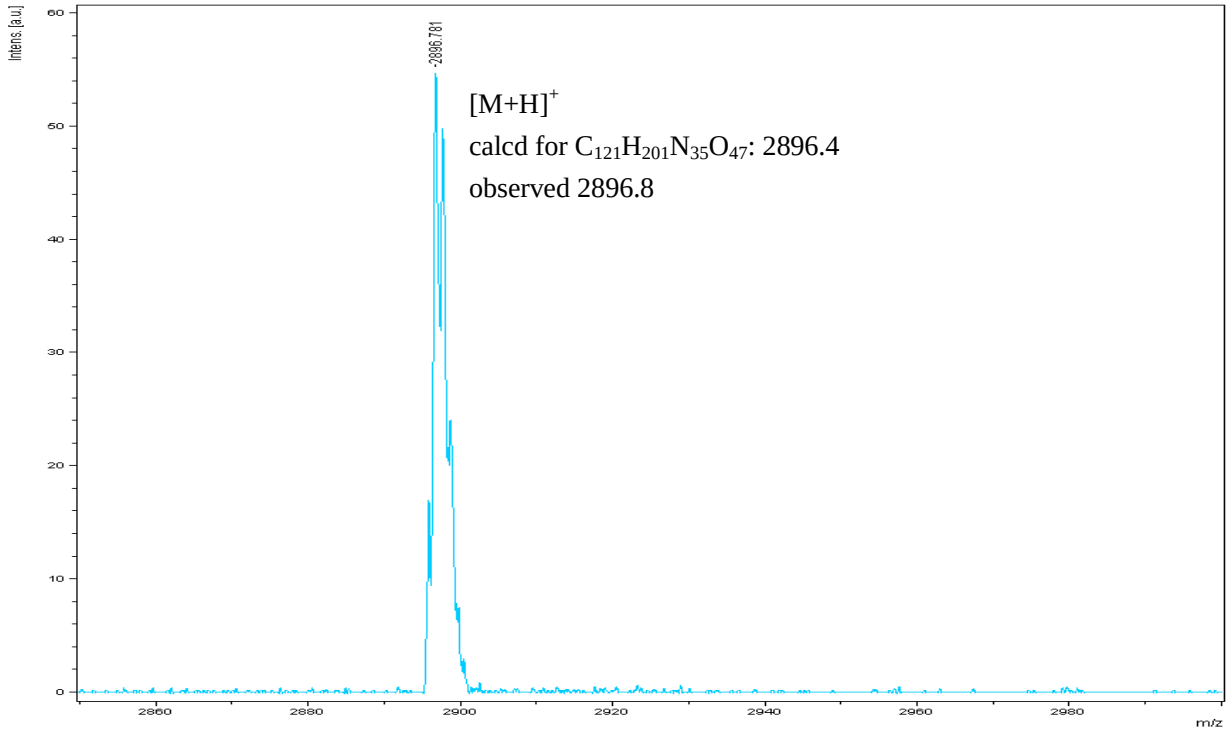


Figure 19. MALDI-TOF MS of purified linear dimer of **7**

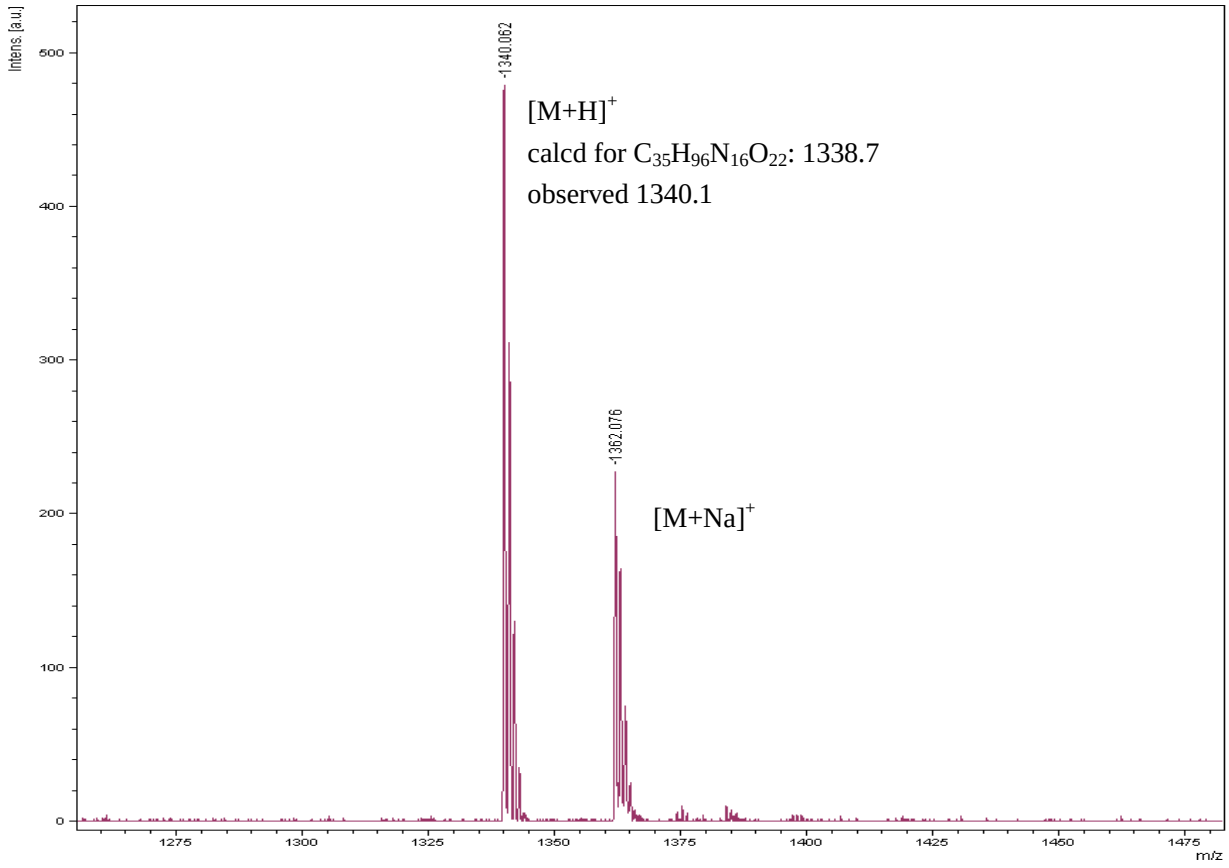


Figure 20. MALDI-TOF MS of purified cyclic monomer of **7**

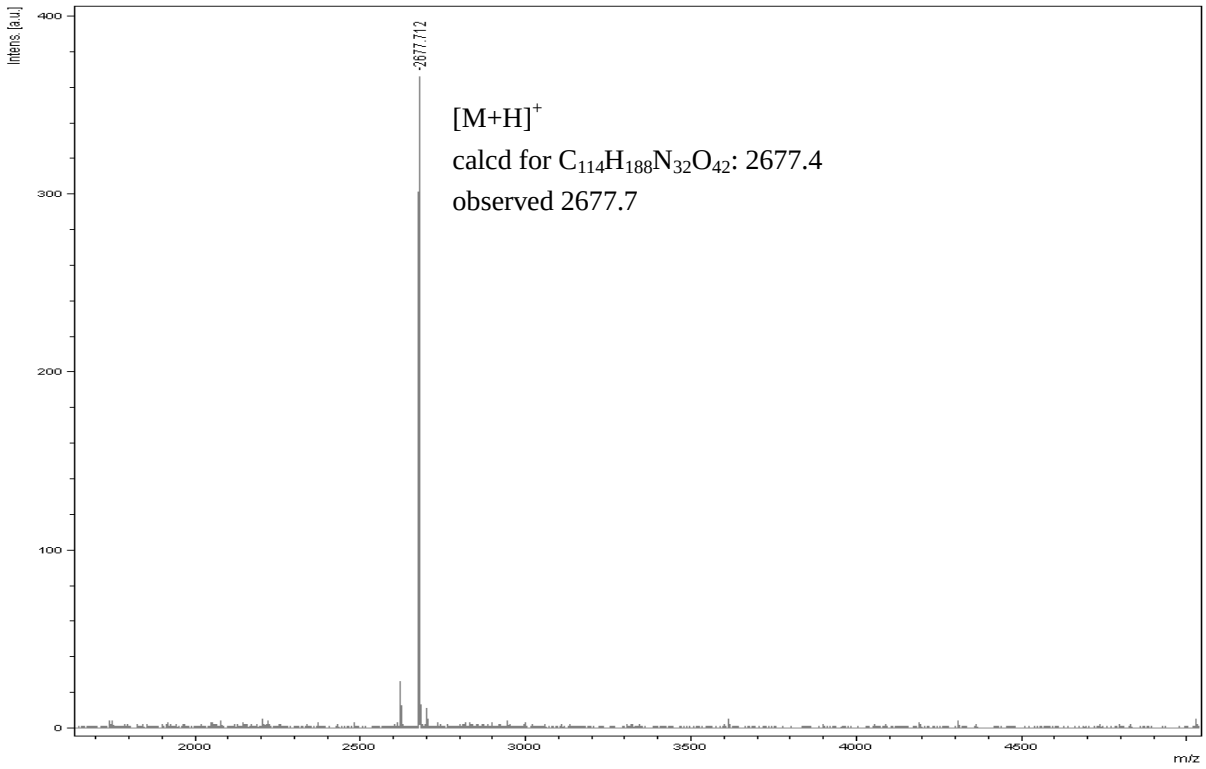


Figure 21. MALDI-TOF MS of purified cyclic dimer of **7**

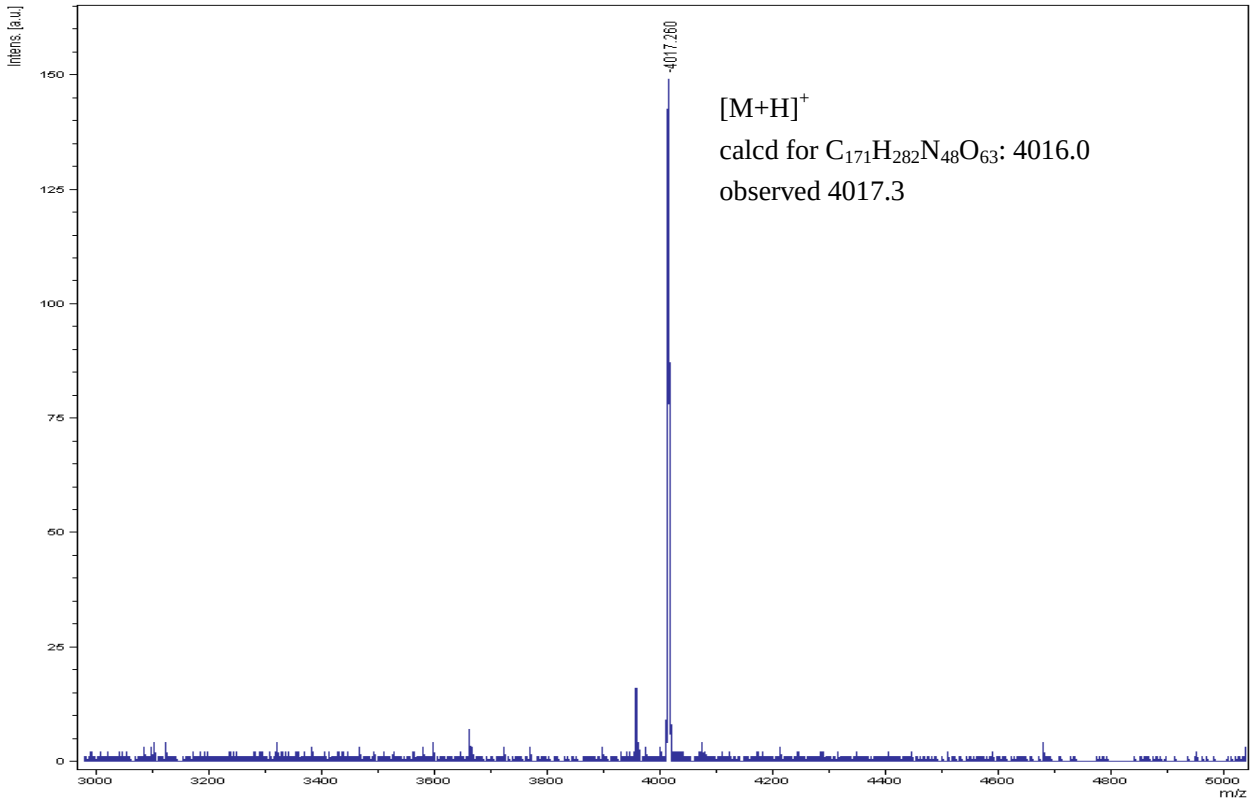


Figure 22. MALDI-TOF MS of purified cyclic trimer of **7**

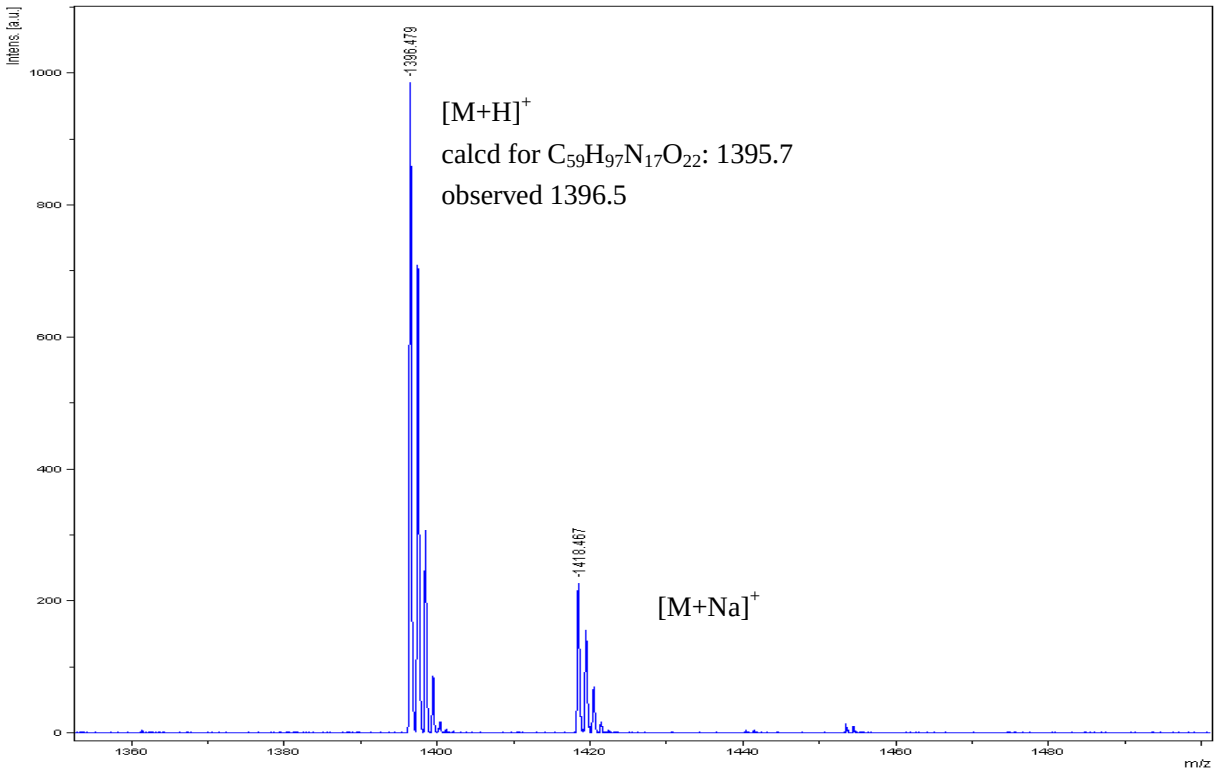


Figure 23. MALDI-TOF MS of purified cyclic monomer of **8**

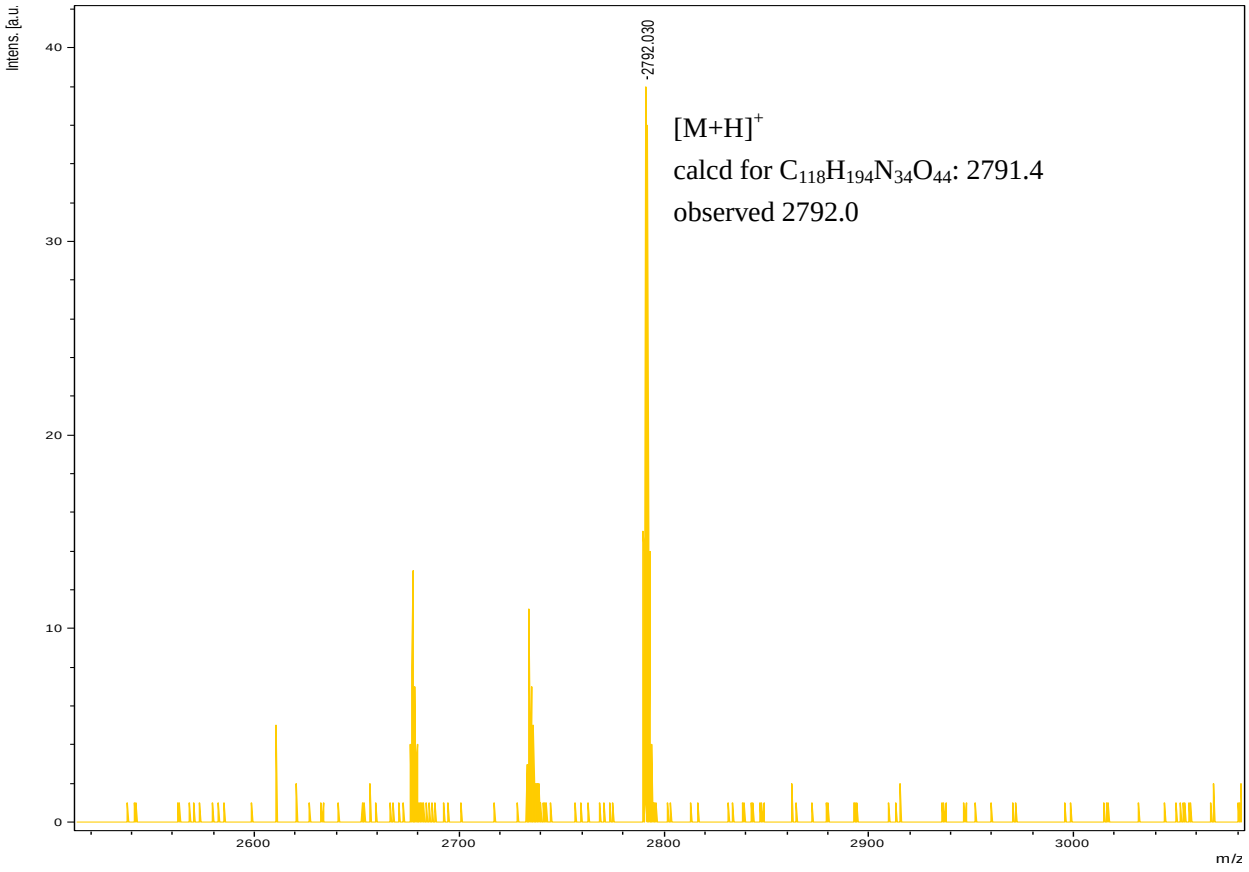


Figure 24. MALDI-TOF MS of purified cyclic dimer of **8**

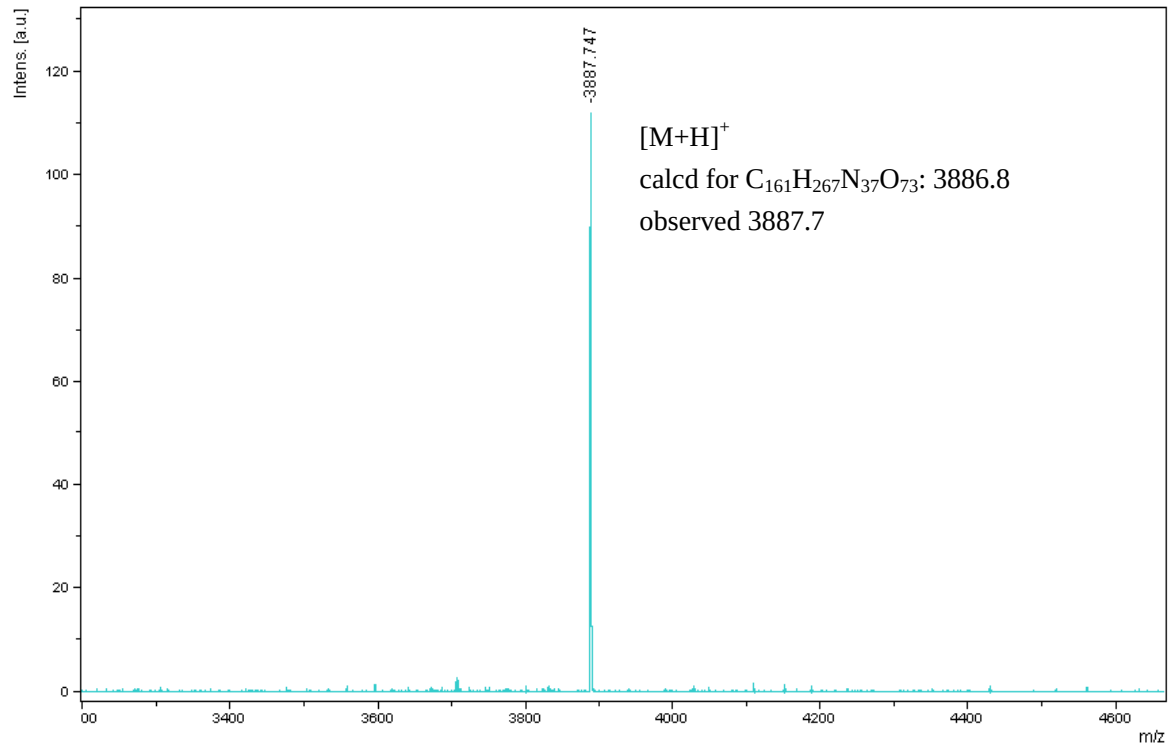


Figure 25. MALDI-TOF MS of purified **10**

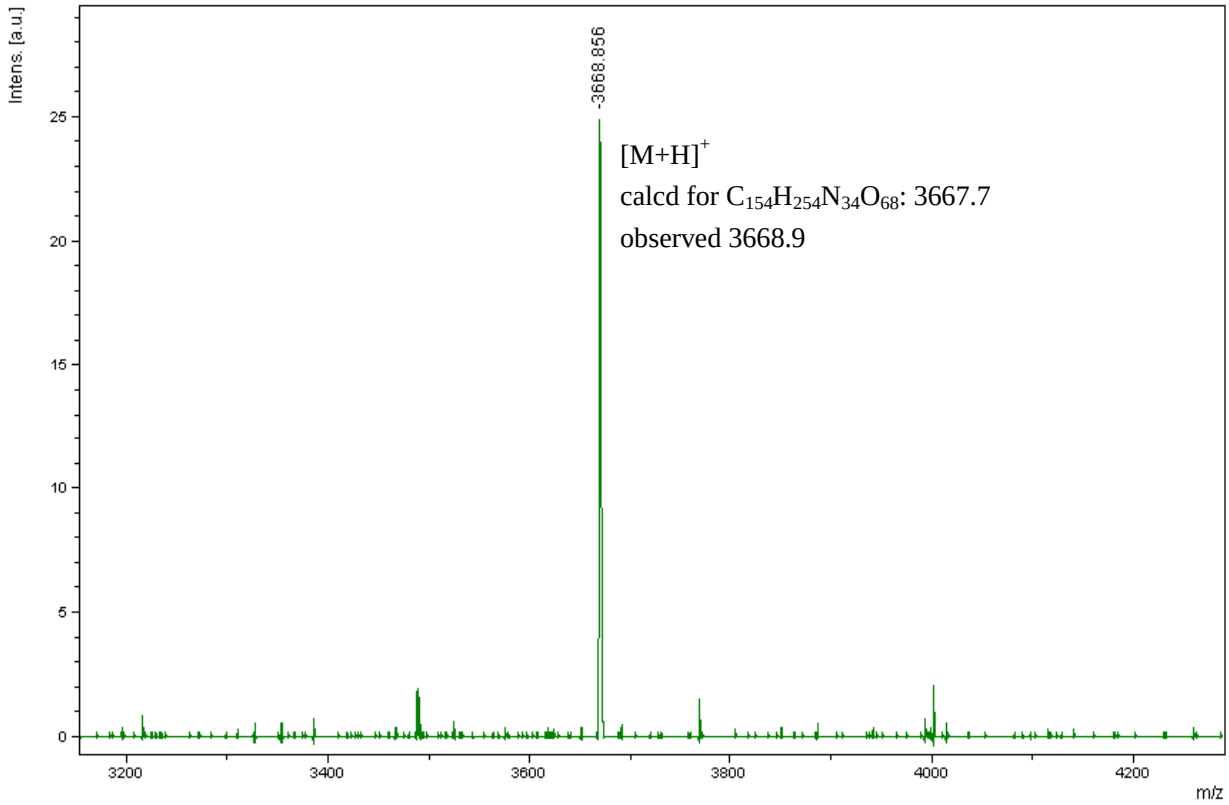


Figure 26. MALDI-TOF MS of purified **11**

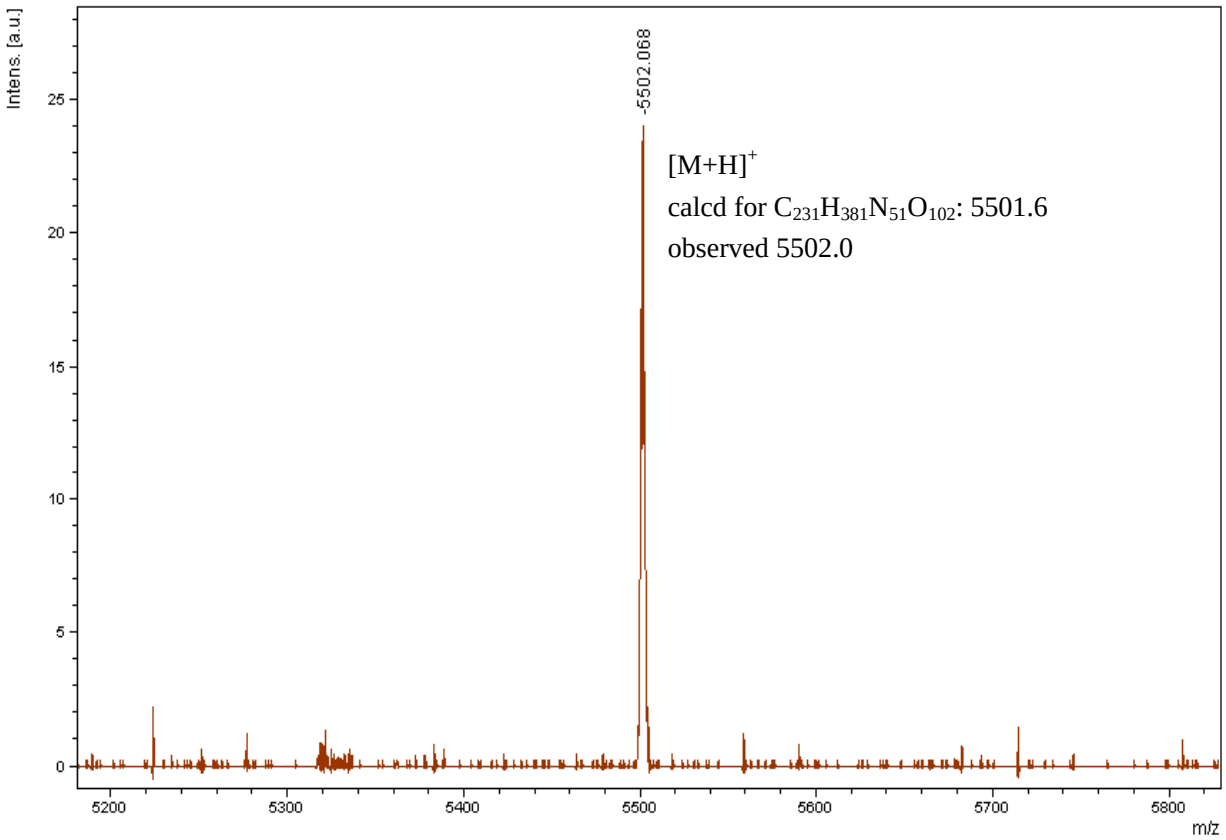


Figure 27. MALDI-TOF MS of purified **12**