

Table of contents

Figure 1-18. RP HPLC and MALDI-TOF MS characterization of analogs **1-18**.....S2-19

Figure 19. RP HPLC spectra obtained in the serum stability study of **6**.....S20

Figure 20. MALDI-TOF spectra of fractions obtained in the serum stability study of **6**S21

Figure 21. RP HPLC spectra obtained in stability study of **6** in EMEM mediaS22

Figure 22. MALDI-TOF spectra of fractions obtained in the stability study of **6** in EMEM.....S23

Figure 23. Stability of **6** in EMEM media.....S23

Table 1. Hemolytic activityS24

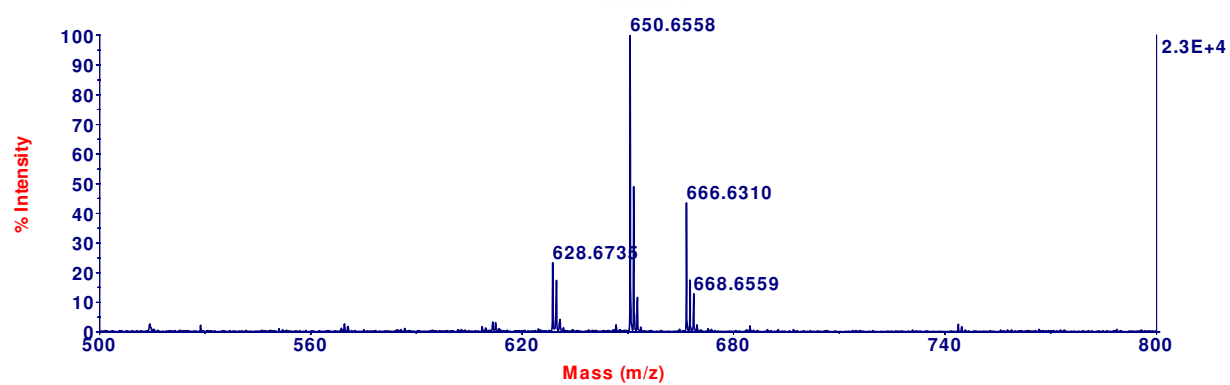
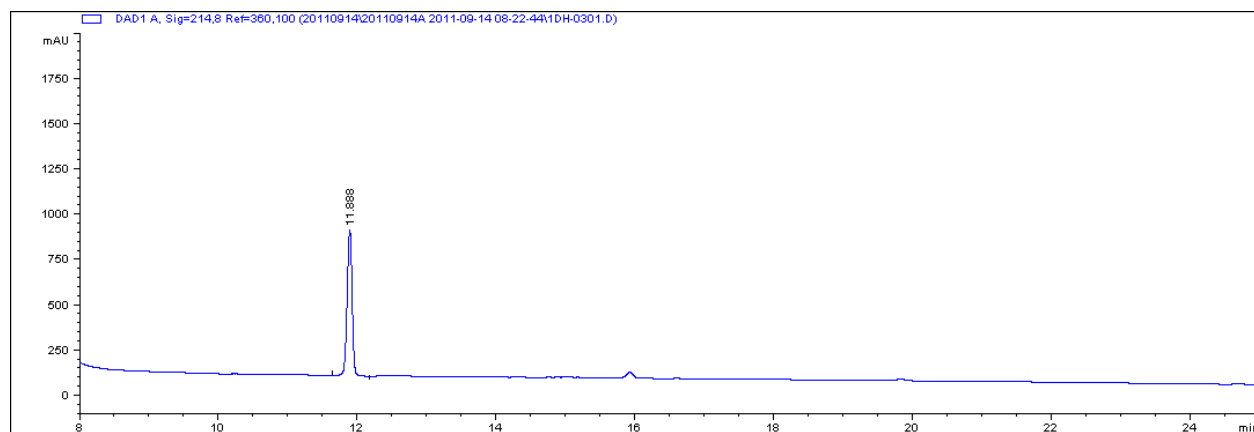


Figure 1. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 1.

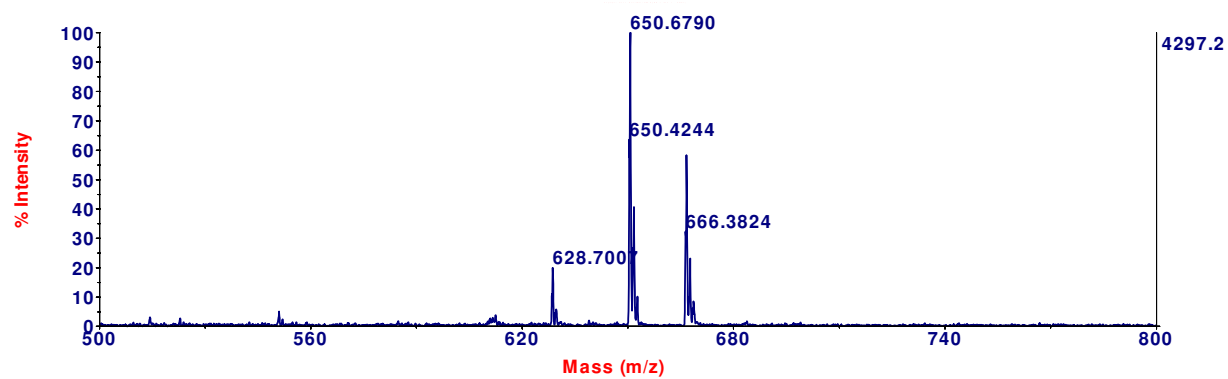
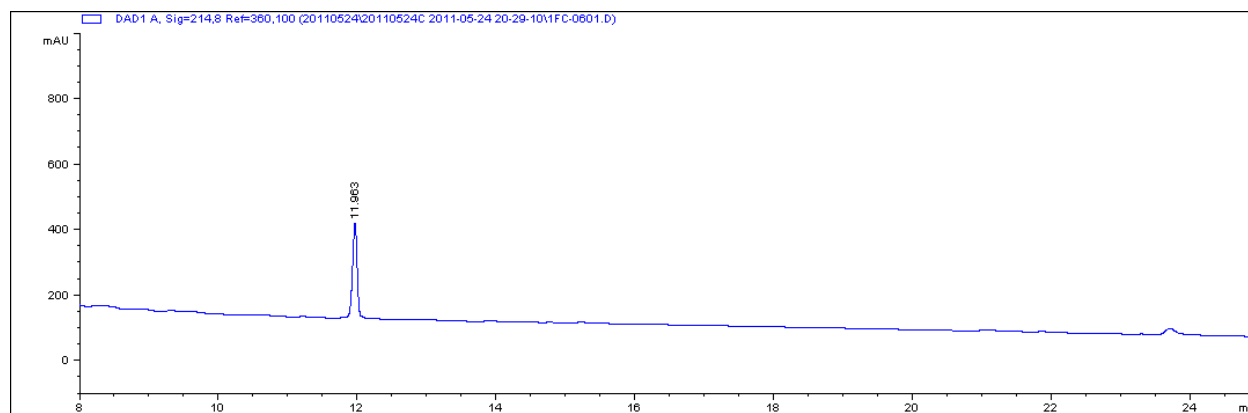


Figure 2. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 2.

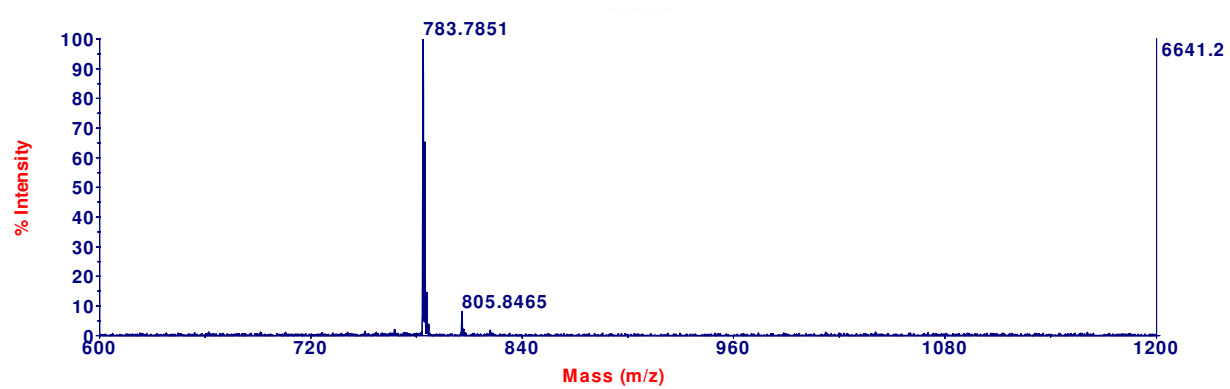
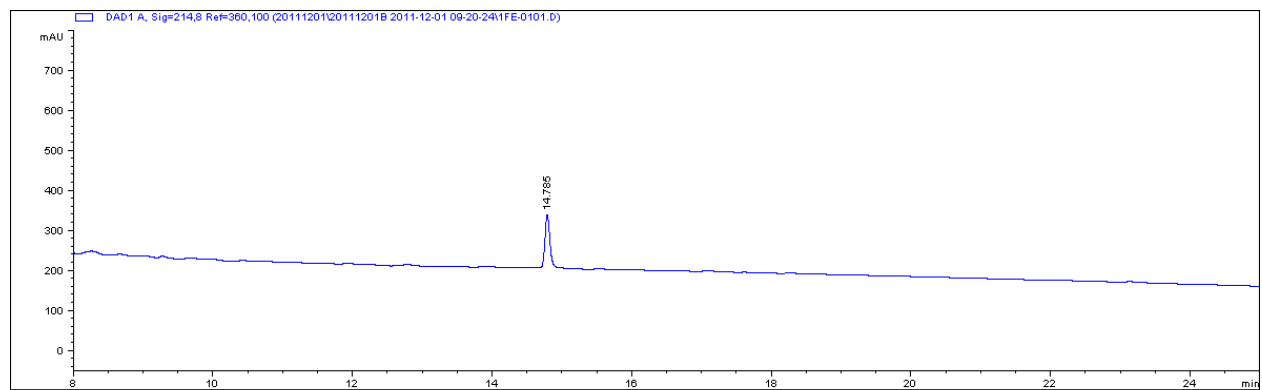


Figure 4. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog **3**.

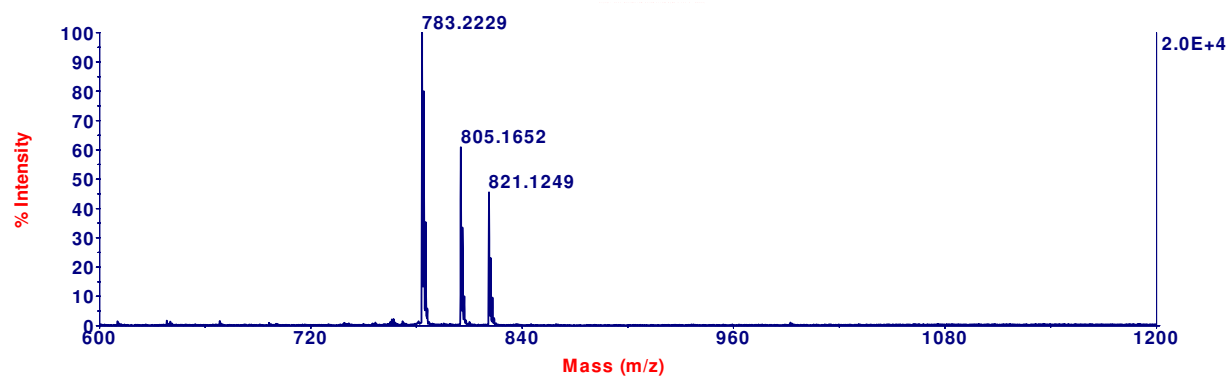
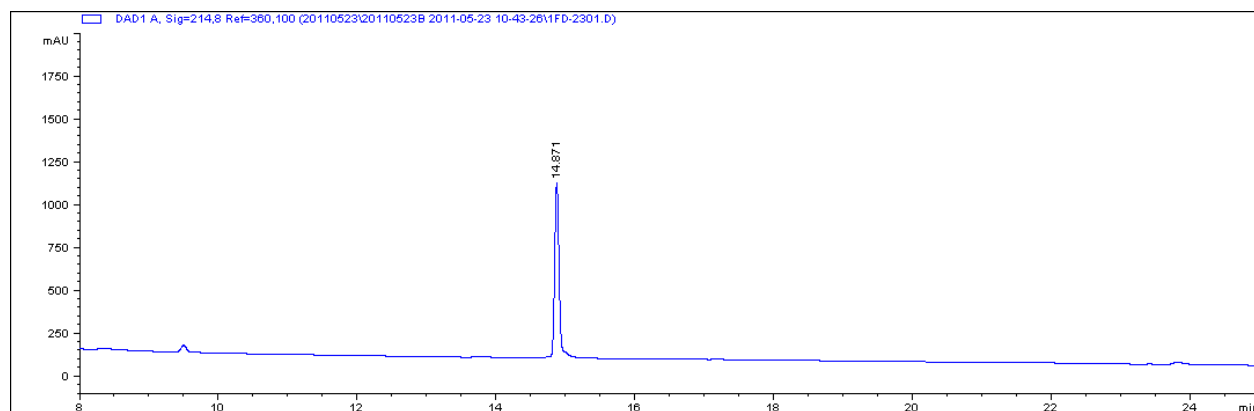


Figure 4. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 4.

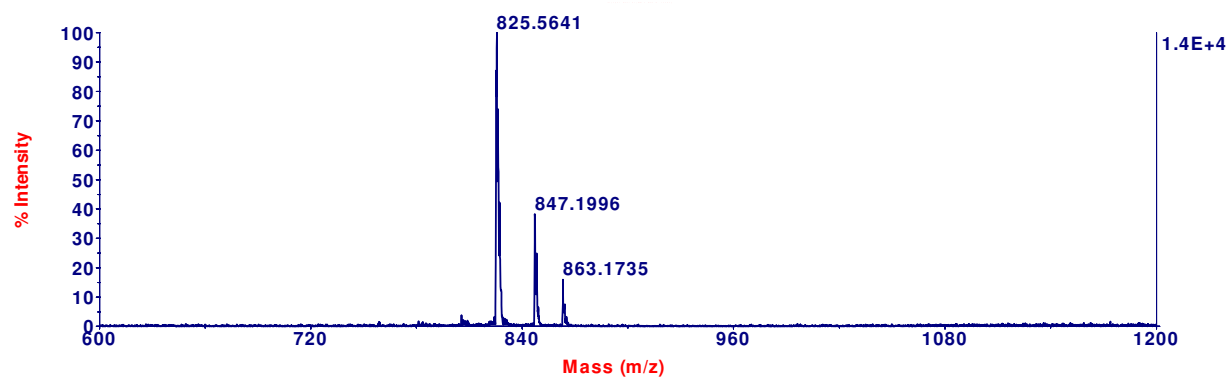
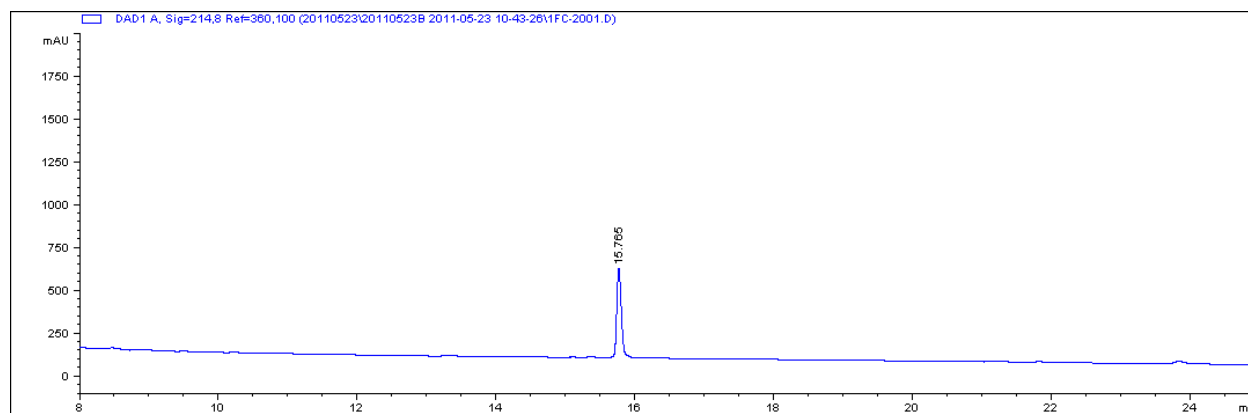


Figure 5. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 5.

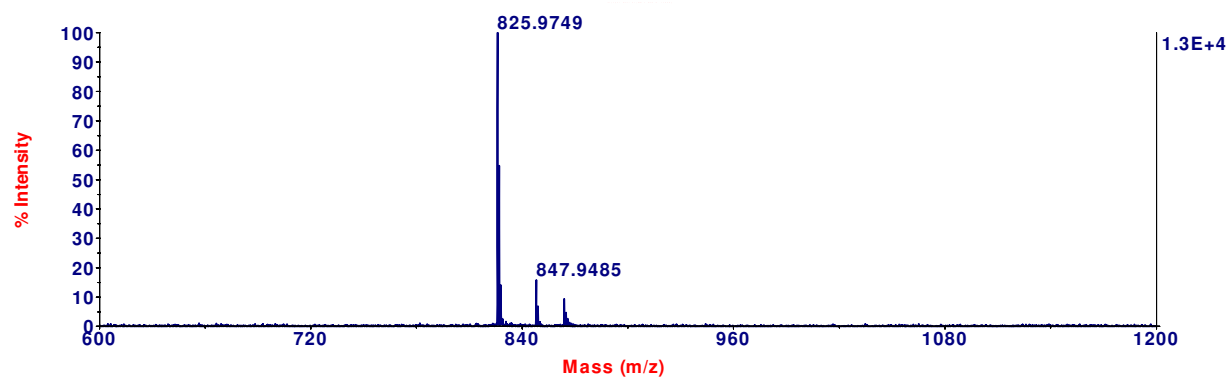
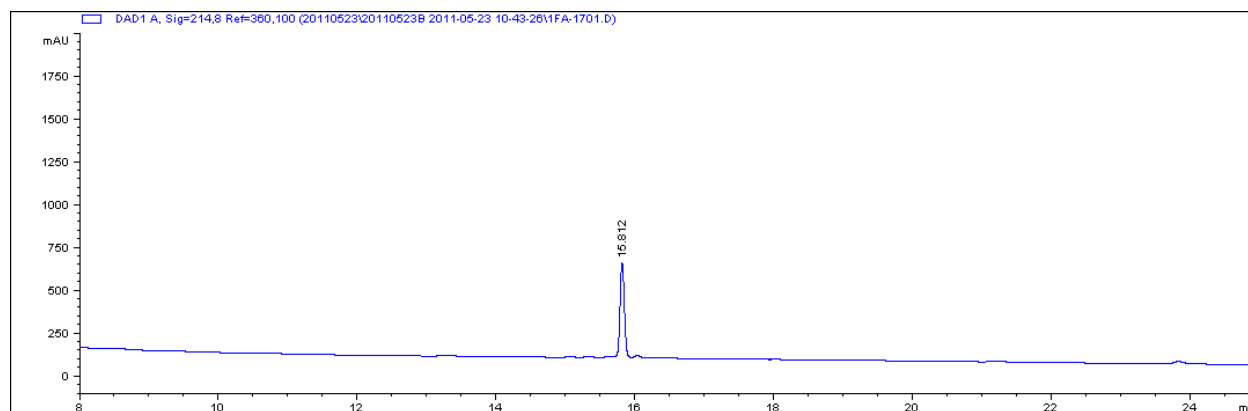


Figure 6. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 6.

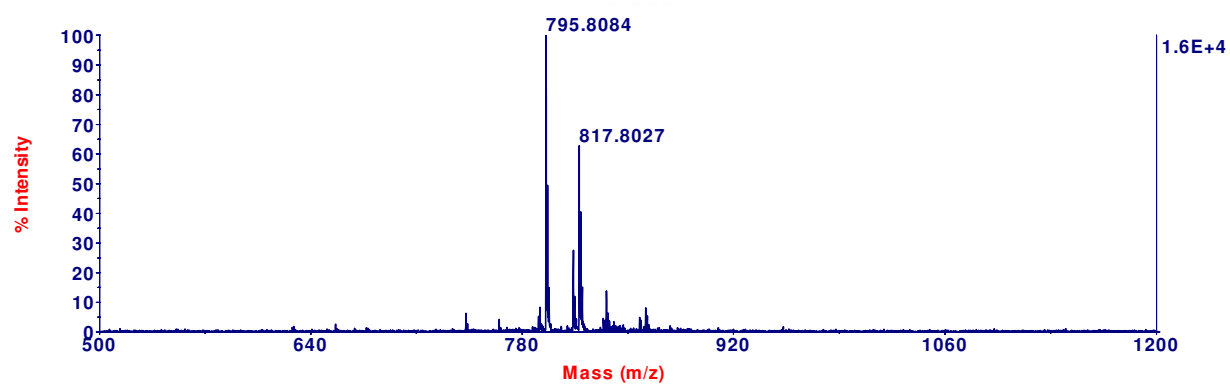
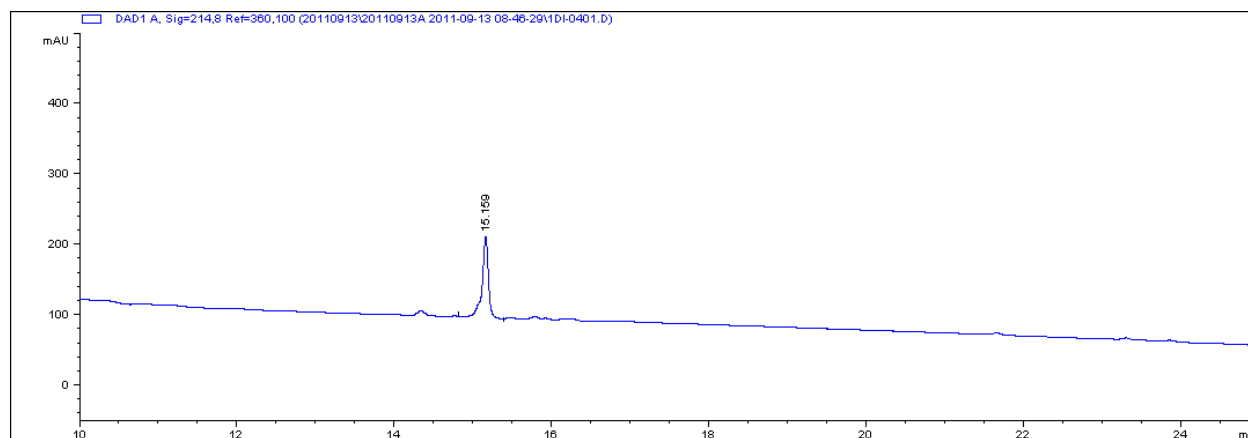


Figure 7. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 7.

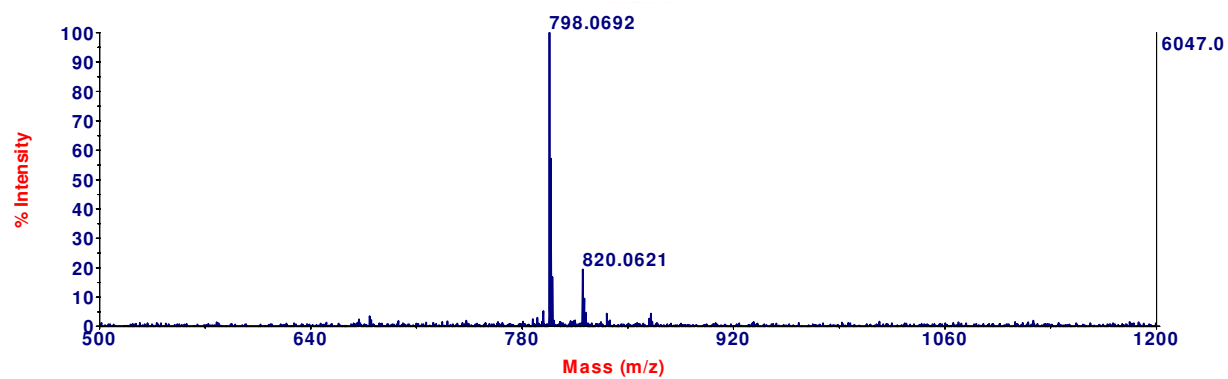
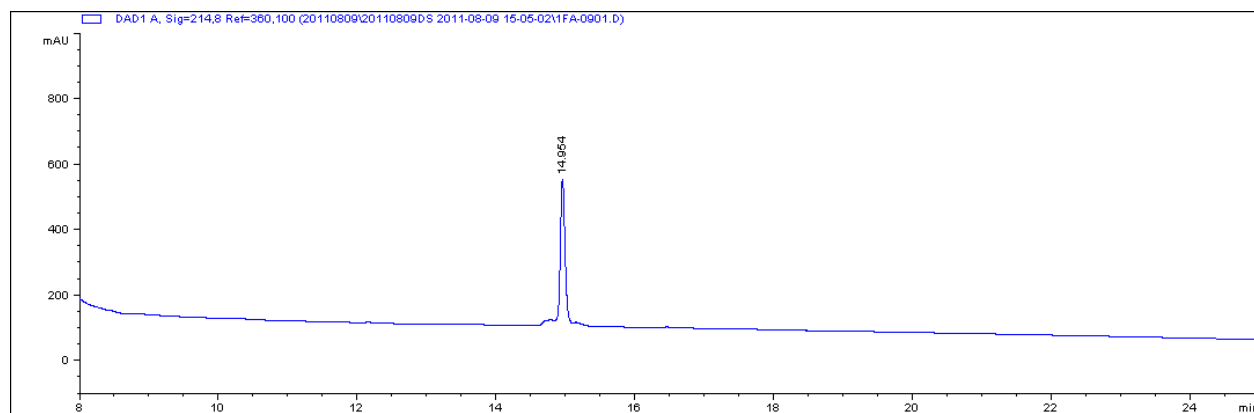


Figure 8. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog **8**.

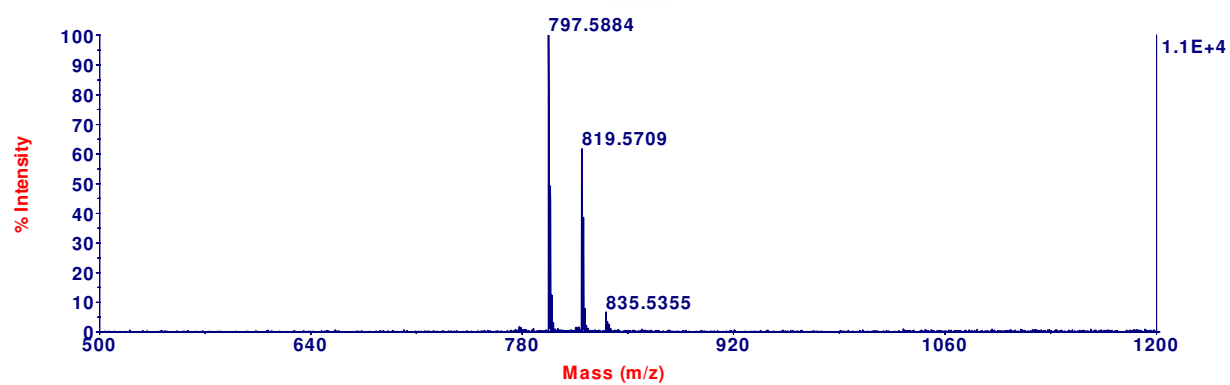
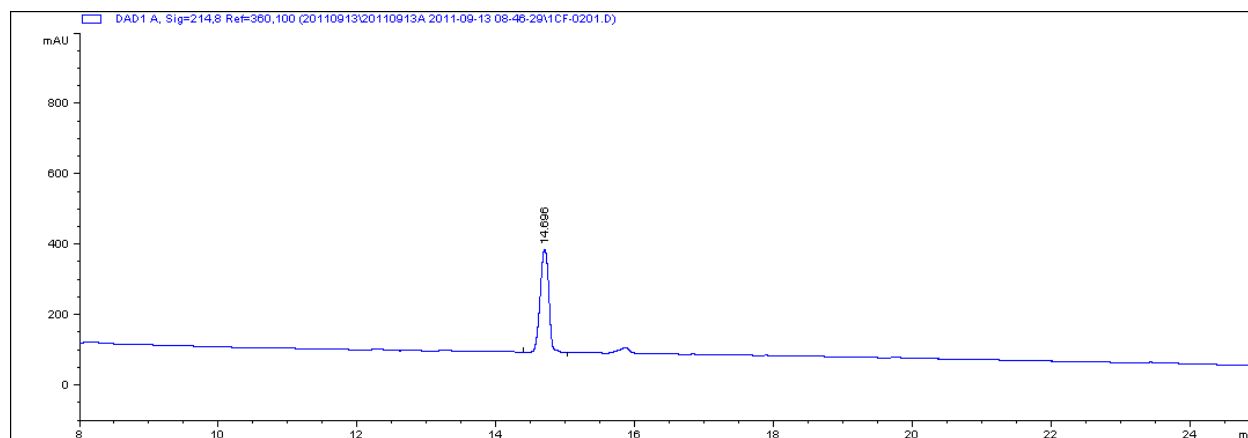


Figure 9. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog **9**.

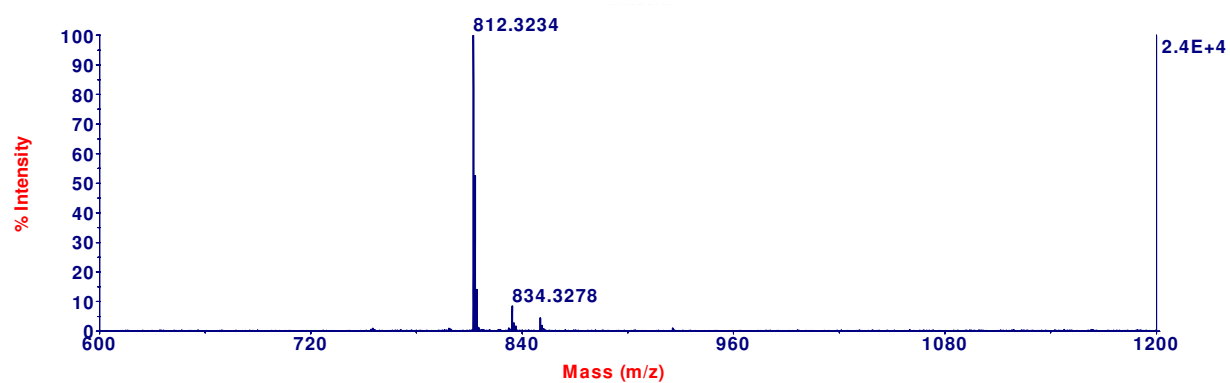
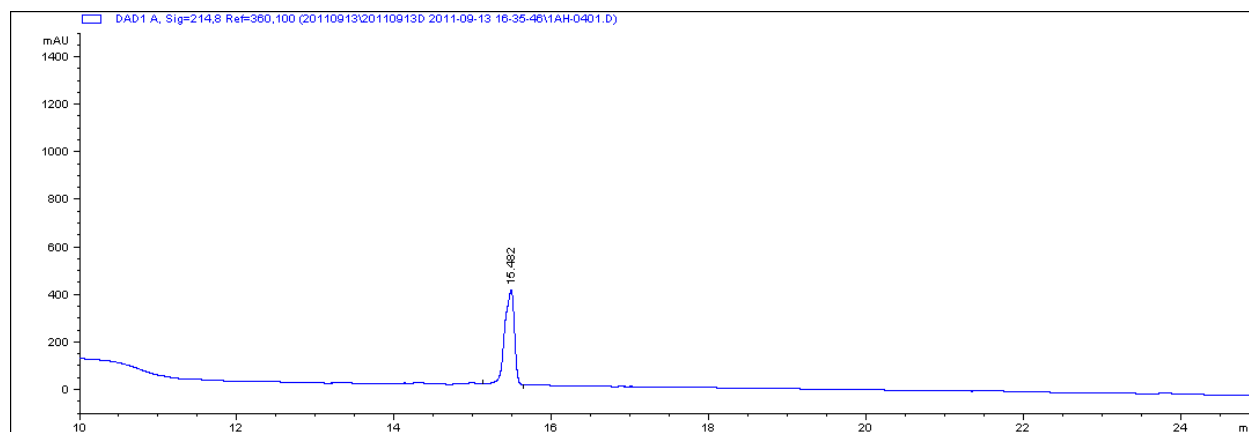


Figure 10. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 10.

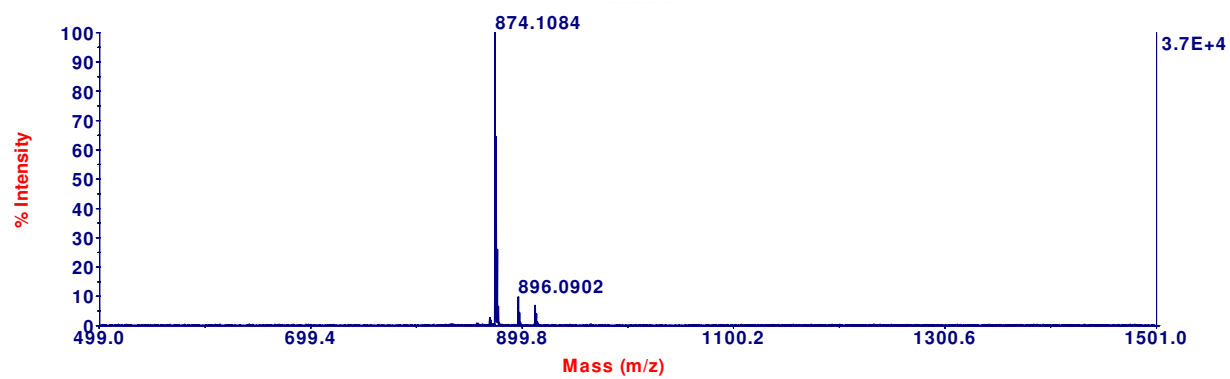
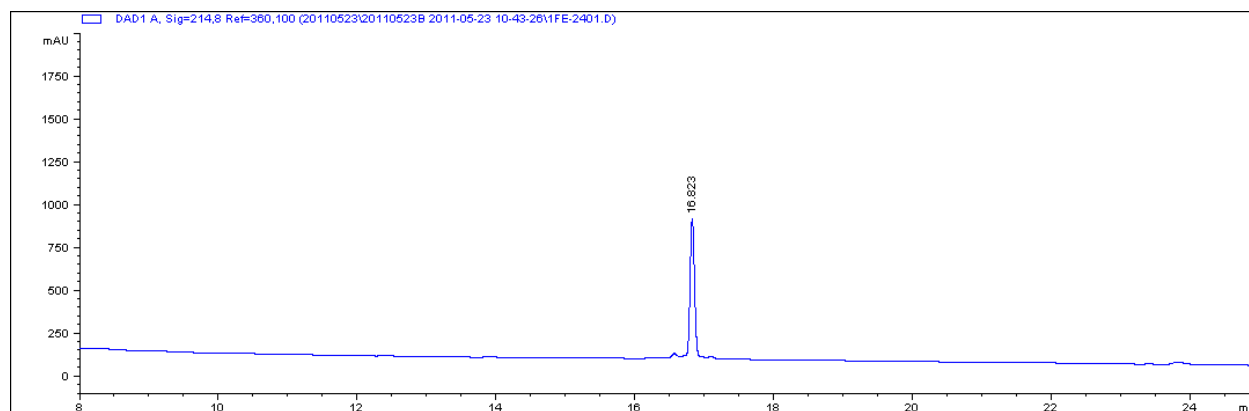


Figure 11. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 11.

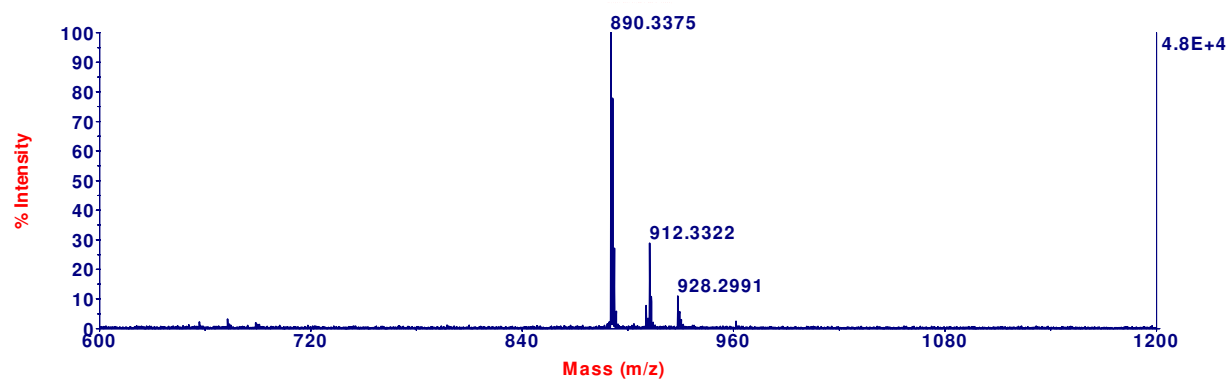
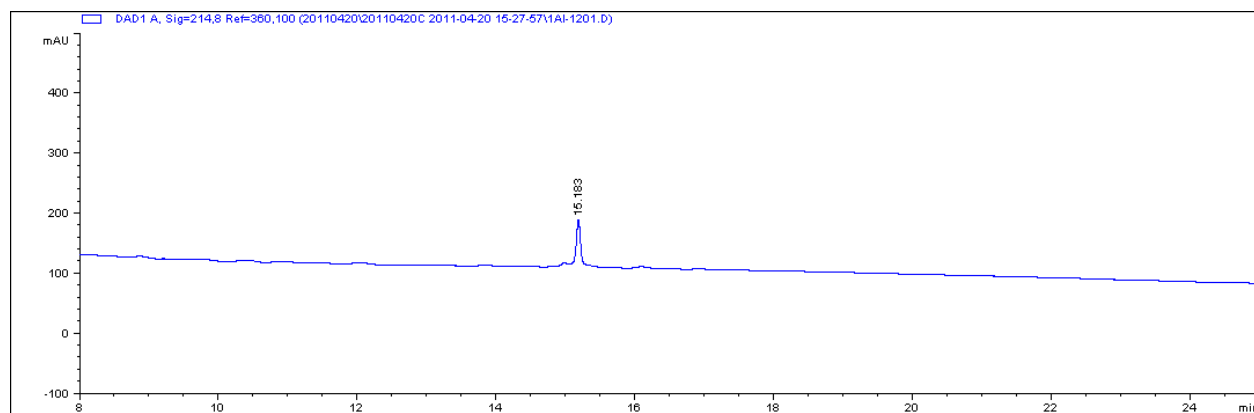


Figure 12. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog **12**.

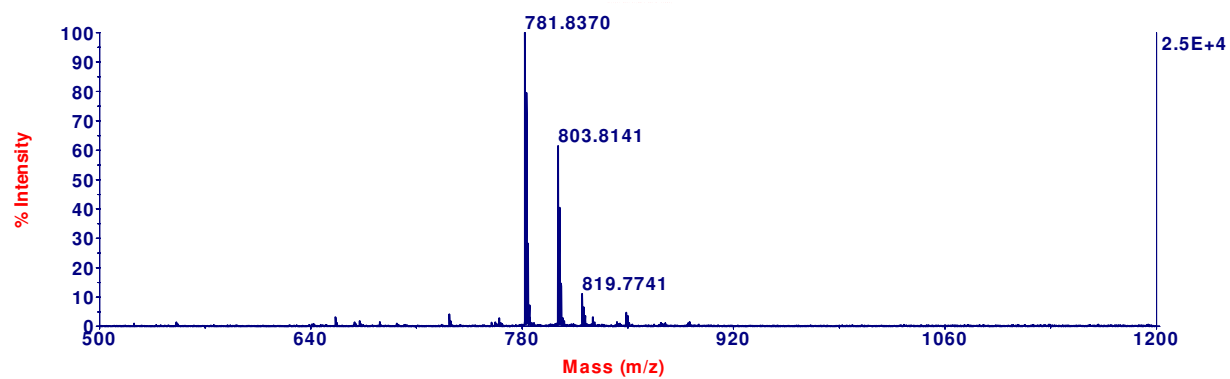
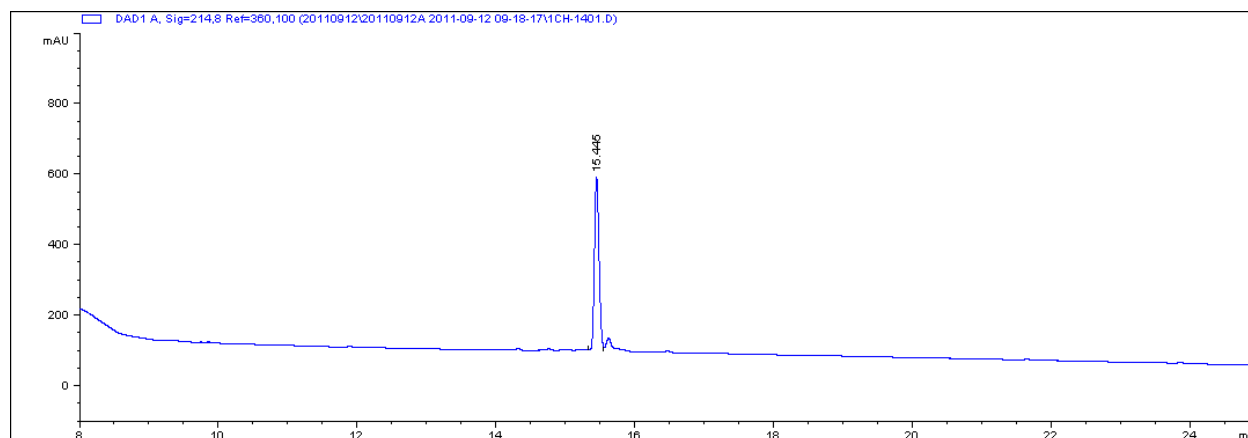


Figure 13. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 13.

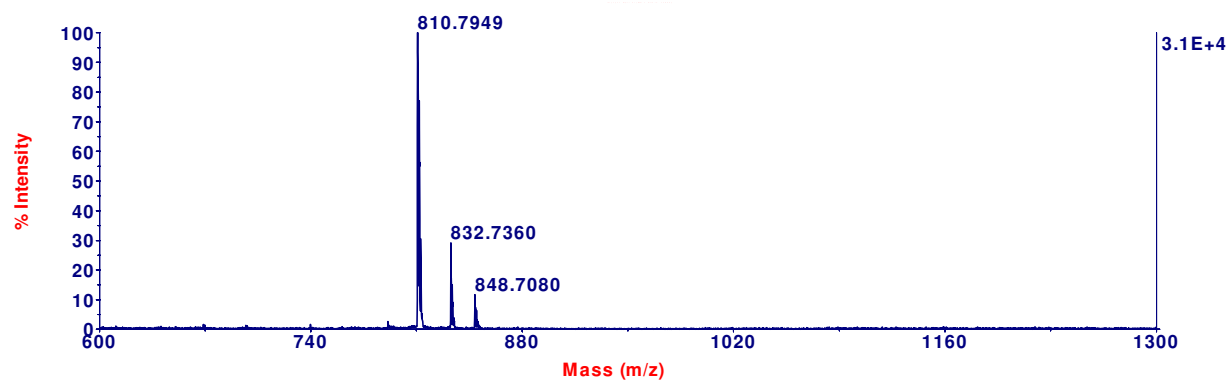
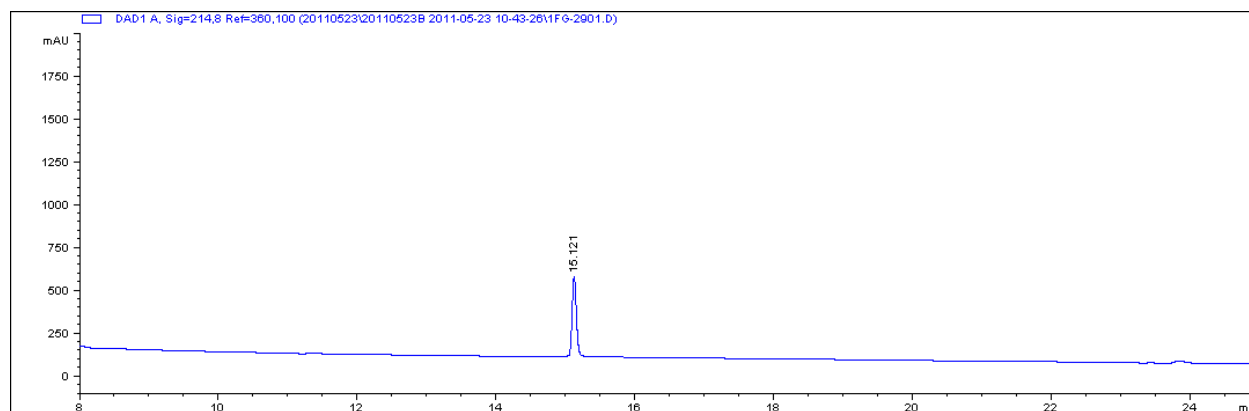


Figure 14. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 14.

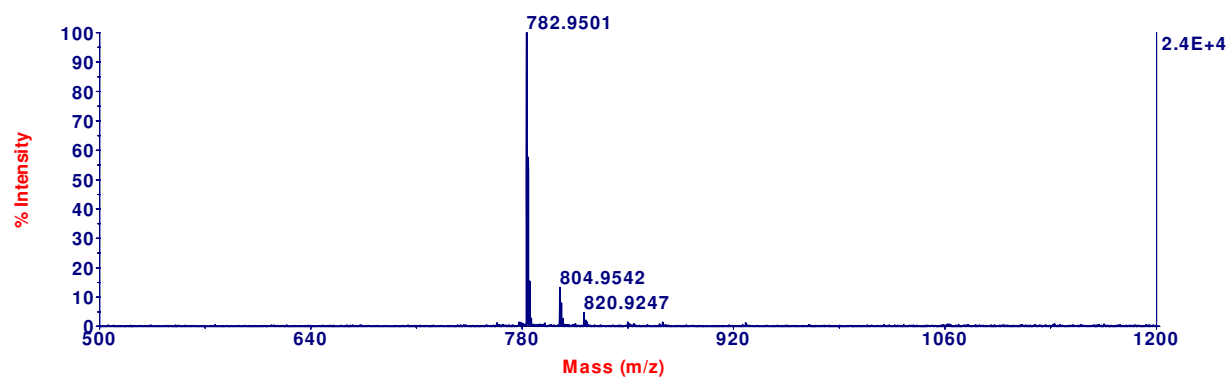
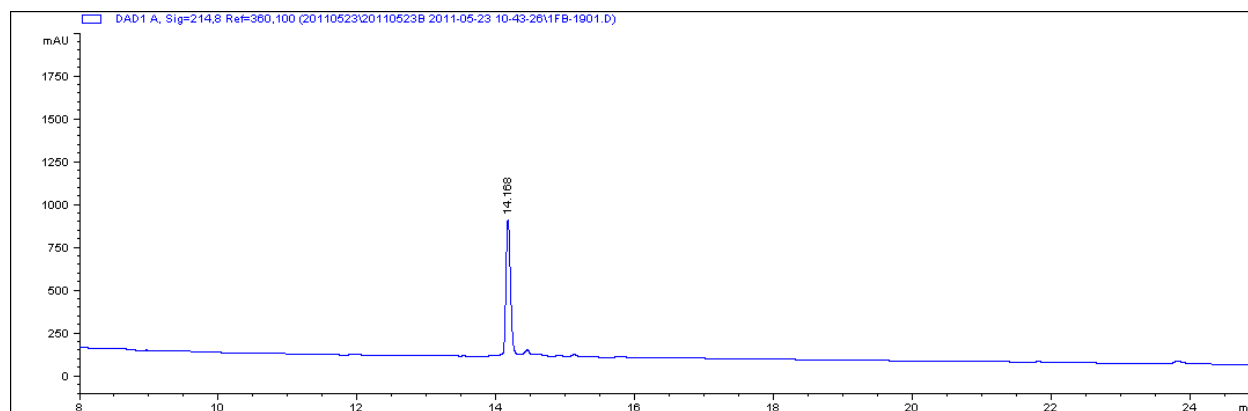


Figure 15. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 15.

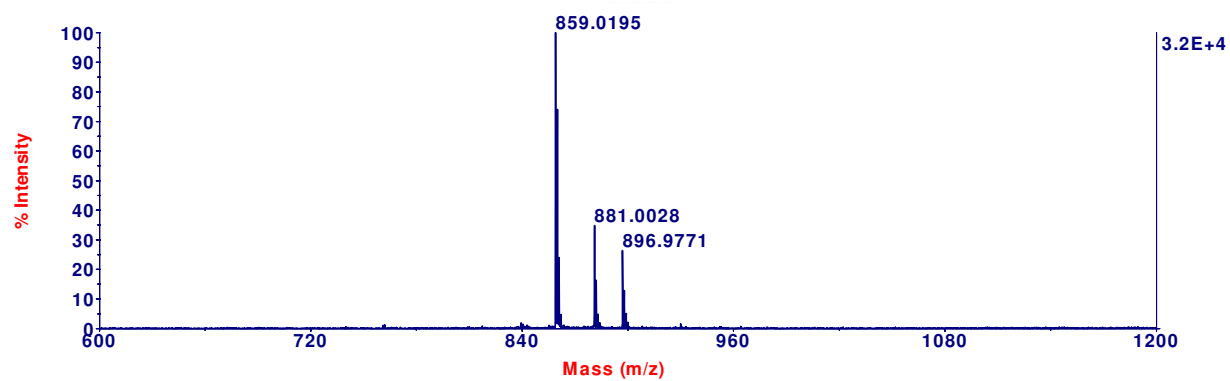
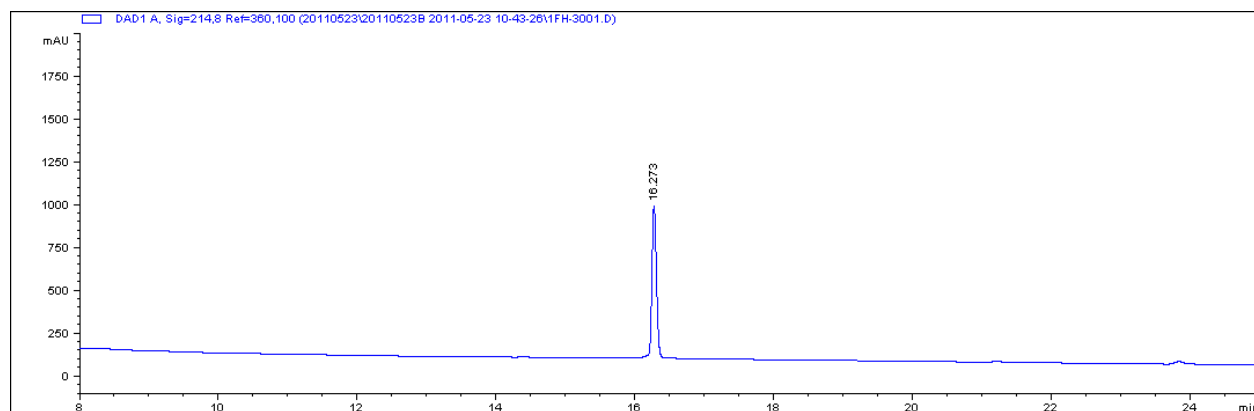


Figure 16. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog **16**.

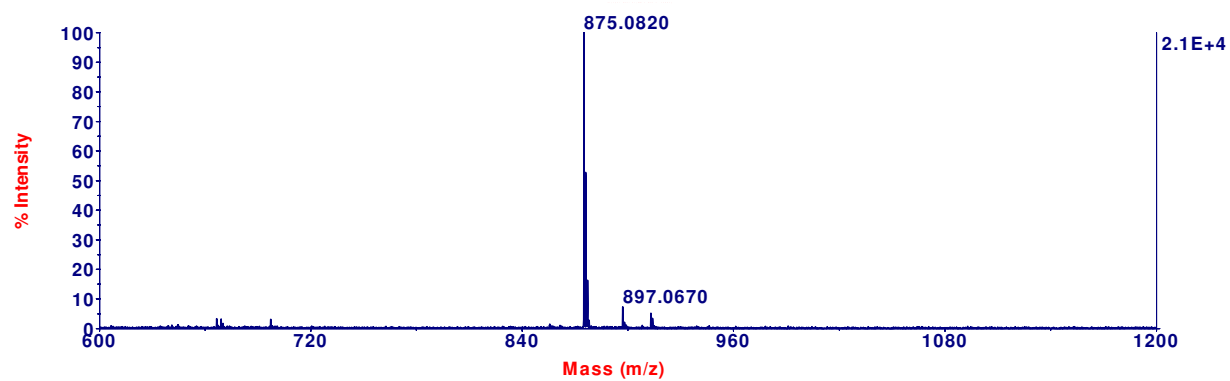
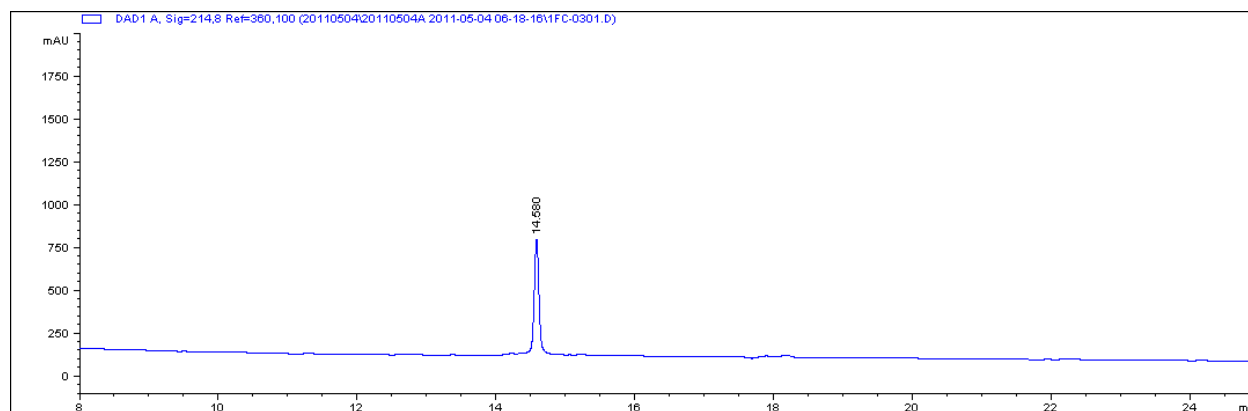


Figure 17. RP HPLC trace (up) and MALDI-TOF spectrum (down) of analog 17.

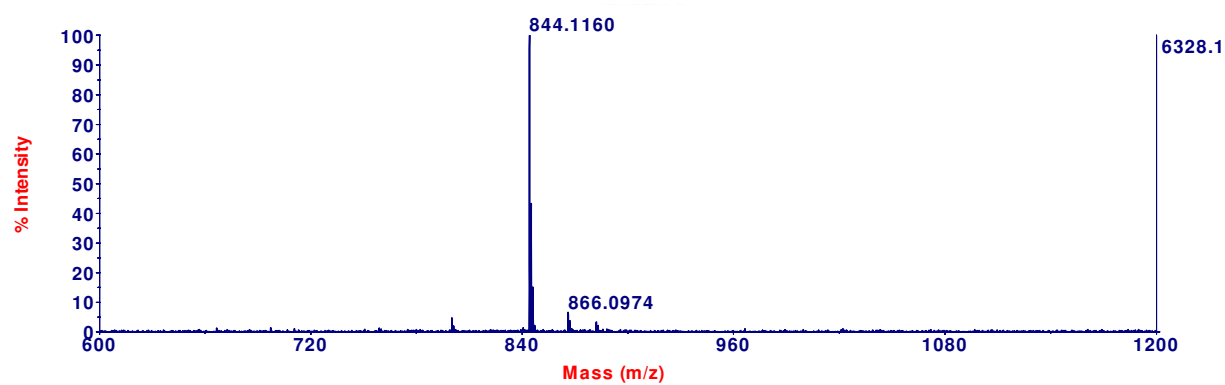
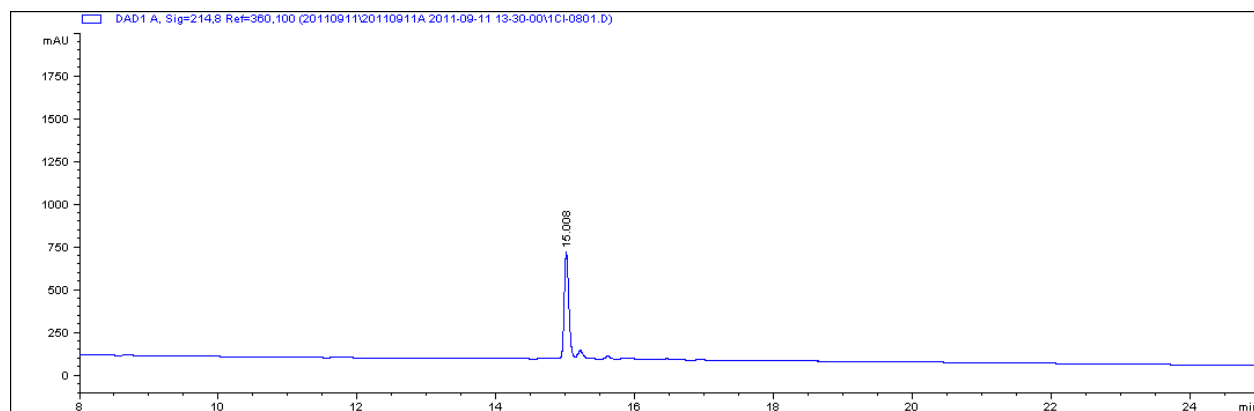


Figure 18. RP HPLC trace (up) and MALDI-TOF spectrum (down) of control lipopeptide **18**.

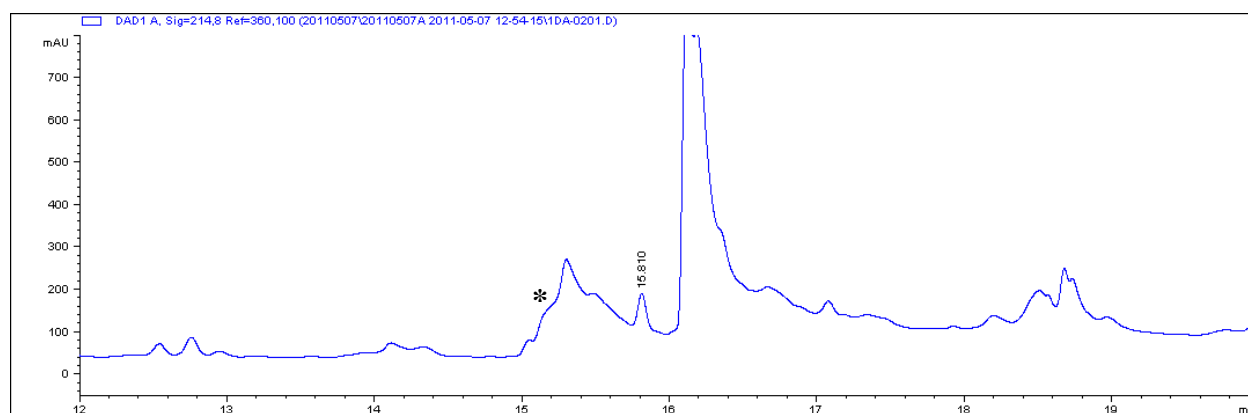
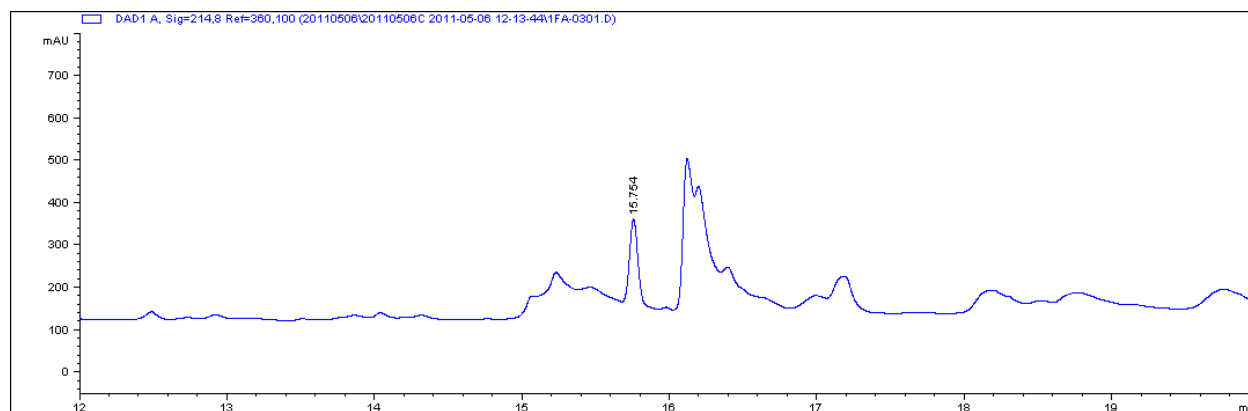


Figure 19. RP HPLC trace of analog **6** in 50% human serum at time 0 (up) and after 24 h incubation at 37°C (down). Peak at $R_t=15.8$ corresponds to unchanged **6**. (*) Marks approximate elution time ($R_t= 15-15.2$ min.) of the major degradation product.

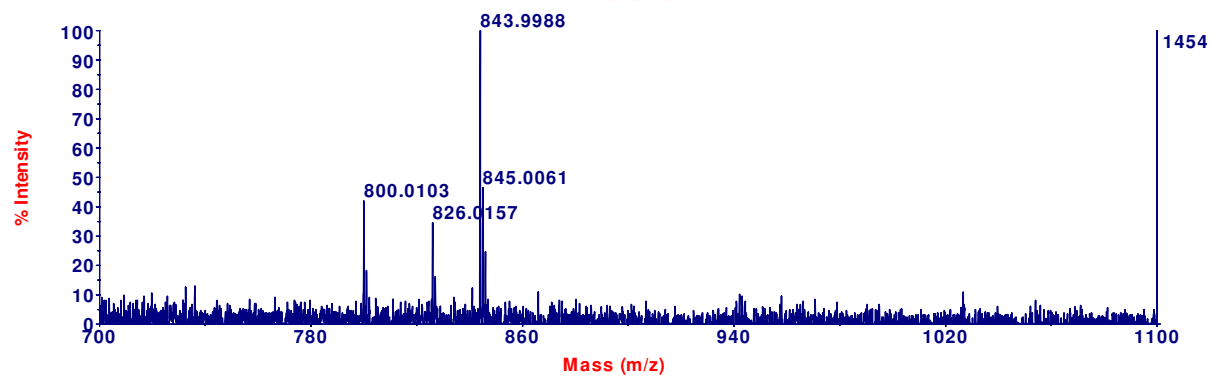
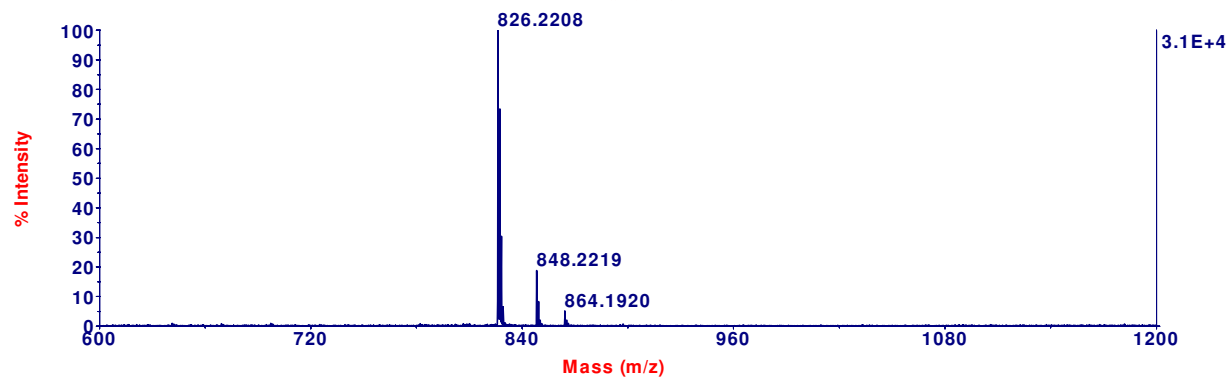


Figure 20. MALDI-TOF MS analysis of RP HPLC fractions obtained after 24h incubation of **6** in 50% human serum. Up: fraction collected at $R_t=15.7$ min., corresponding to unchanged **6**, down: fraction collected at $R_t=15-15.2$ min., indicating ester bond hydrolysis in **6** to generate the major degradation product (m/z and RP HPLC R_t are almost identical, within experimental error, to control linear lipopeptide **18**).

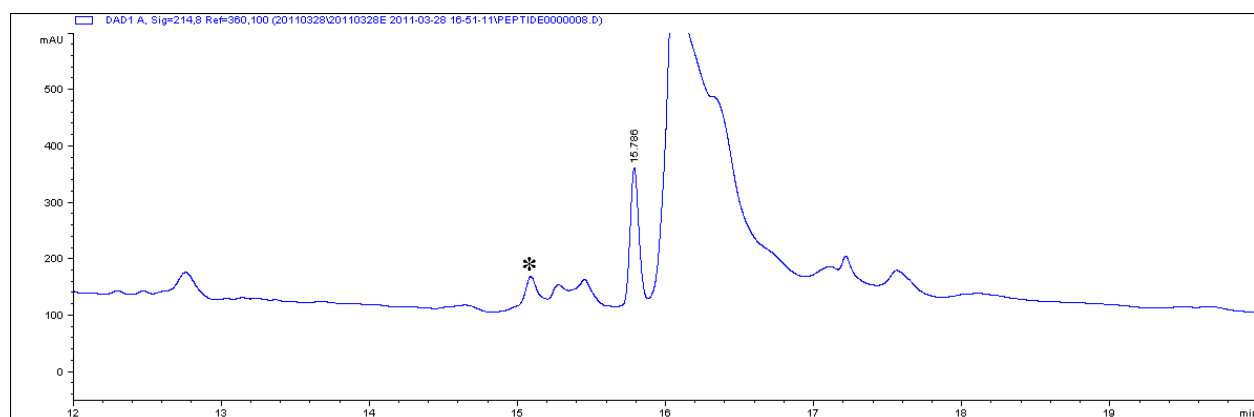
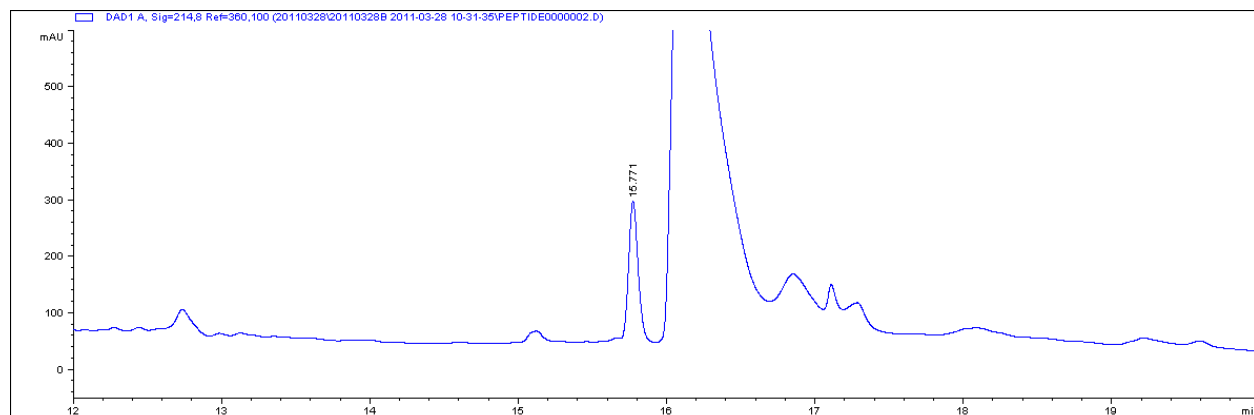


Figure 21. RP HPLC trace of analog **6** in EMEM medium at time 0 (up) and after 24 h incubation at 37°C (down). Peak at $R_t=15.7$ corresponds to unchanged **6**. (*) Marks elution time ($R_t=15.1$ min.) of the major degradation product.

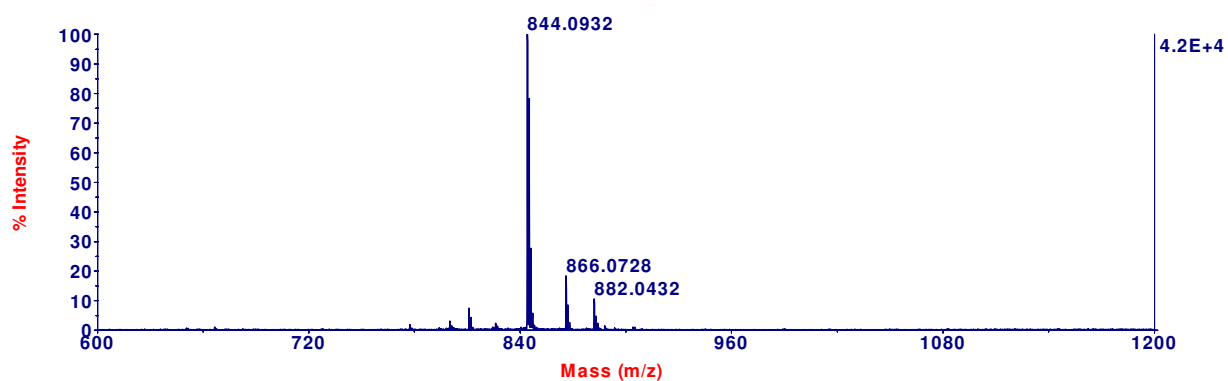
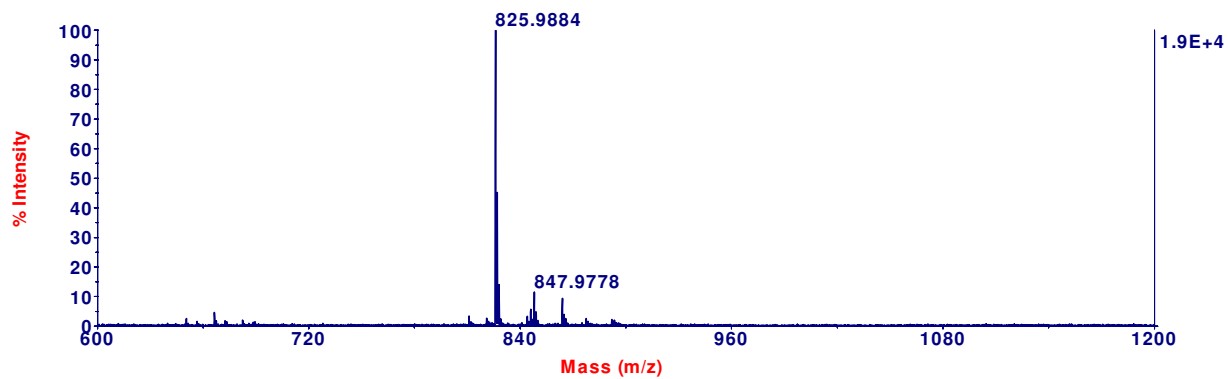


Figure 22. MALDI-TOF MS analysis of RP HPLC fractions obtained after 24h incubation of **6** in EMEM medium. Up: fraction at 15.7 min, down: fraction at 15.1 min.

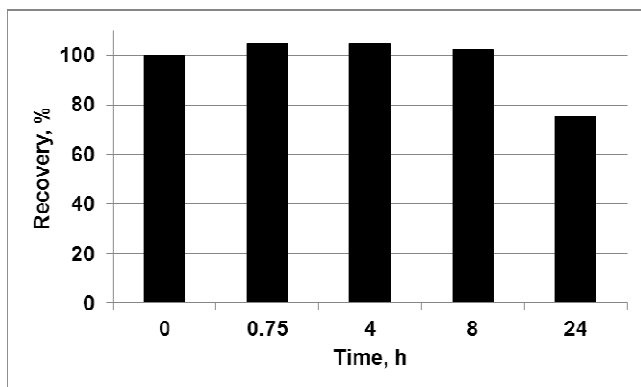


Figure 23. Serum stability of fusaricidin analog **6** in EMEM medium containing 10% FBS.

Table 1. Hemolytic activity of selected fusaricidin A /LI-F04a analogs^a.

Peptide concentration (µg/mL)	Analog									
	2	4	5	6	8	11	14	15	16	18
256	- ^b	-	-	109.2	-	96.8	14.9	-	52.5	-
128	-	-	-	88.7	-	95.2	6.3	-	25.3	-
64	nd ^c	nd	nd	43.5	29.2	63.6	3.9	1.1	1.6	8.1
32	nd	nd	nd	1.4	1.2	27.1	nd	nd	nd	8.3
16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

^a Expressed as % of hemolysis caused by Triton X-100; ^b not tested; ^c nd=no hemolysis detected.