

Synthesis and Anti-bacterial Activity Study of Anti-Biofilm Agent Based on American Oyster Defensin Analog A4

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Supporting Information

Figures S1-S2: The structure and oxidation reaction curve of A4

Figures S3-S12: The ESI-MS spectrum of A4 and A4 and A4-derived peptides

Figures S13-S15: The HPLC of trypsin stability of A4, D-A4, A4-T3

S.1. Structure and reaction curve of A4

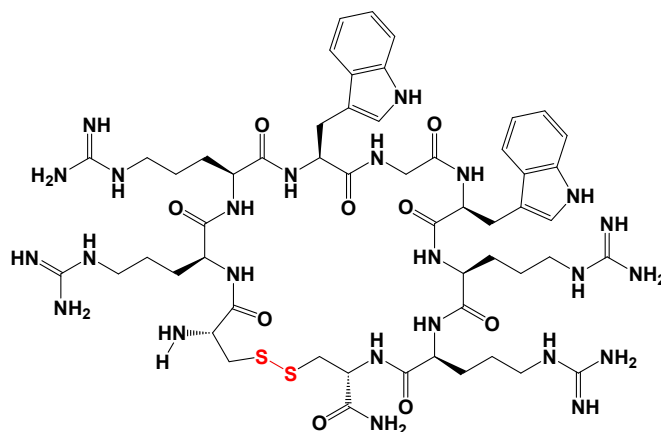


Figure S1. The chemical structures of A4

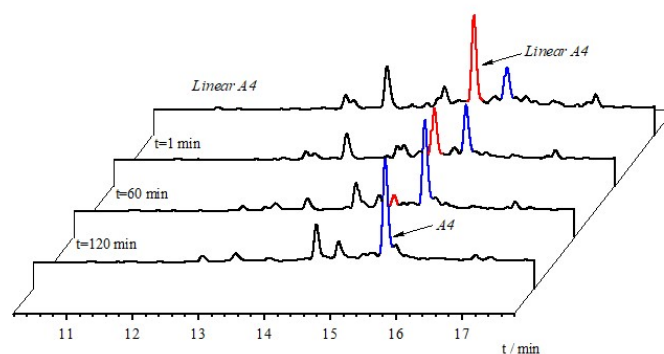


Figure S2. Linear A4 Oxidation Reaction

RP-HPLC conditions: a linear gradient of 20%~80% buffer B (0.1% TFA in acetonitrile) in buffer A (0.1% TFA in water) over 30 min (20% for 2 min, then 20%~80% for 30 min) on C18 column, $\lambda = 214$ nm.

S.2. Mass spectrum information of A4 and derived peptides

S.2.1 Mass spectrum information of A4

For Linear A4, the calculated ESI-MS m/z values are: 639.765 ($[M+2H]^{2+}$), 426.843 ($[M+3H]^{3+}$), and

320.3825 ($[M+4H]^{4+}$). The observed values are: 639.4916 ($[M+2H]^{2+}$), 426.6697 ($[M+3H]^{3+}$), and 319.8932 ($[M+4H]^{4+}$).

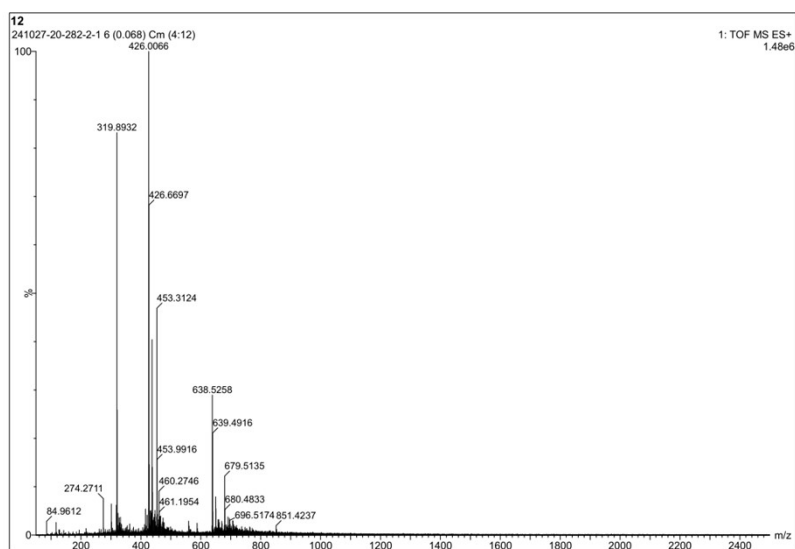


Figure S3. The ESI-MS spectrum of Linear A4

For A4, the calculated ESI-MS m/z values are: 638.765 ($[M+2H]^{2+}$), 426.1767 ($[M+3H]^{3+}$), and 319.8825 ($[M+4H]^{4+}$). The observed values are: 638.5186 ($[M+2H]^{2+}$), 426.0170 ($[M+3H]^{3+}$), and 319.8365 ($[M+4H]^{4+}$).

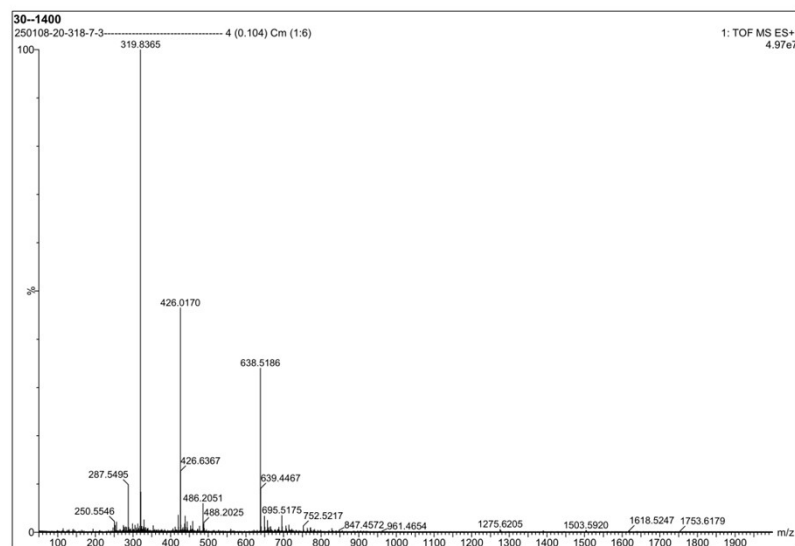


Figure S4. The ESI-MS spectrum of A4

S.2.2 Mass spectrum information of D-A4

For Linear D-A4, the calculated ESI-MS m/z values are: 639.765 ($[M+2H]^{2+}$), 426.843 ($[M+3H]^{3+}$), and 320.3825 ($[M+4H]^{4+}$). The observed values are: 639.4467 ($[M+2H]^{2+}$), 426.6367 ($[M+3H]^{3+}$), and 319.8365 ($[M+4H]^{4+}$).

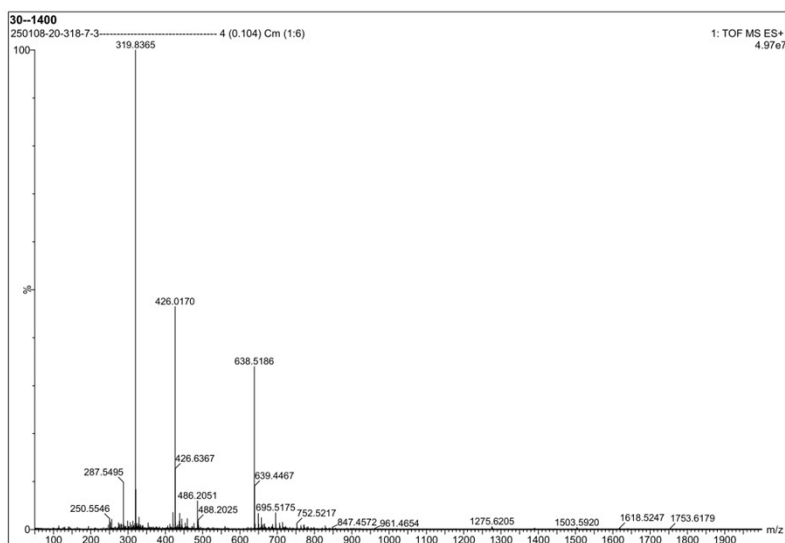


Figure S5. The ESI-MS spectrum of Linear D-A4

For D-A4, the calculated ESI-MS m/z values are: 638.765 ($[M+2H]^{2+}$), 426.1767 ($[M+3H]^{3+}$), and 319.8825 ($[M+4H]^{4+}$). The observed values are: 638.5248 ($[M+2H]^{2+}$), 426.0049 ($[M+3H]^{3+}$), and 319.8321 ($[M+4H]^{4+}$).

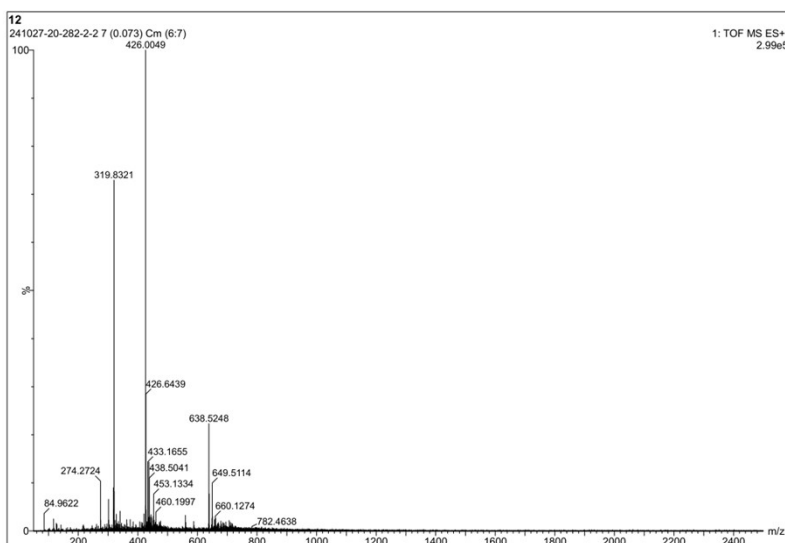


Figure S6. The ESI-MS spectrum of D-A4

S.2.3. Mass spectrum information of A4-T1

For Linear A4-T1, the calculated ESI-MS m/z values are: 647.245 ($[M+2H]^{2+}$), 431.83 ($[M+3H]^{3+}$), and 324.1225 ($[M+4H]^{4+}$). The observed values are: 647.0710 ($[M+2H]^{2+}$), 431.6965 ($[M+3H]^{3+}$), and 324.0839 ($[M+4H]^{4+}$).

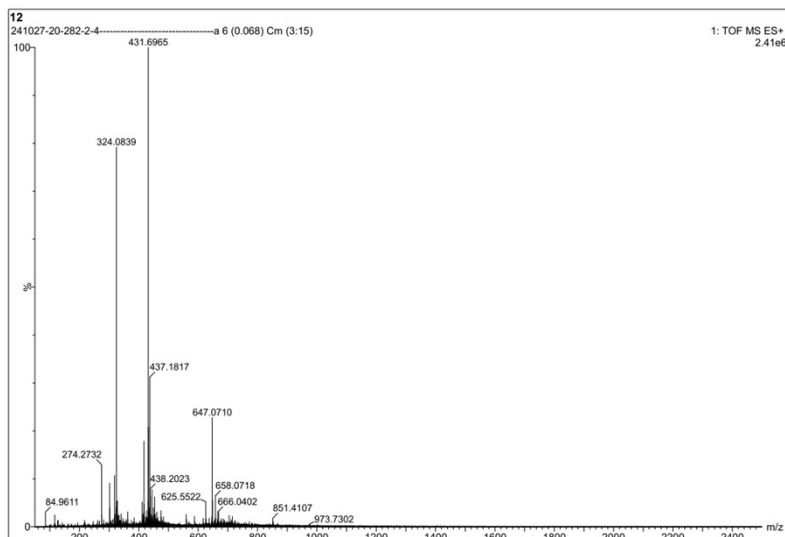


Figure S7. The ESI-MS spectrum of Linear A4-T1

For A4-T1, the calculated ESI-MS m/z values are: 647.245 ($[M+2H]^{2+}$), 431.83 ($[M+3H]^{3+}$), and 324.1225 ($[M+4H]^{4+}$). The observed values are: 647.0701 ($[M+2H]^{2+}$), 431.7180 ($[M+3H]^{3+}$), and 324.0912 ($[M+4H]^{4+}$).

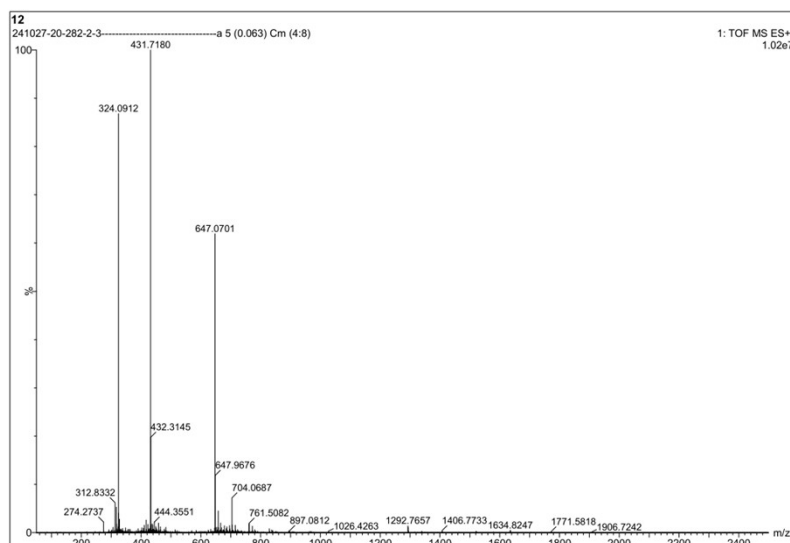


Figure S8. The ESI-MS spectrum of A4-T1

S.2.4. Mass spectrum information of A4-T2

For Linear A4-T2, the calculated ESI-MS m/z values are: 654.255 ($[M+2H]^{2+}$), 436.5033 ($[M+3H]^{3+}$), and 327.6275 ($[M+4H]^{4+}$). The observed values are: 654.0773 ($[M+2H]^{2+}$), 436.3814 ($[M+3H]^{3+}$), and 327.5899 ($[M+4H]^{4+}$).

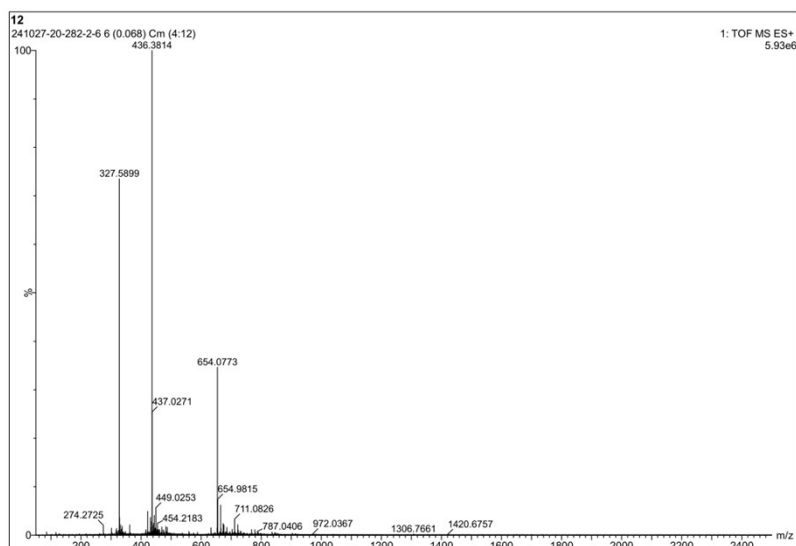


Figure S9. The ESI-MS spectrum of Linear A4-T2

For A4-T2, the calculated ESI-MS m/z values are: 654.255 ($[M+2H]^{2+}$), 436.5033 ($[M+3H]^{3+}$), and 327.6275 ($[M+4H]^{4+}$). The observed values are: 654.0862 ($[M+2H]^{2+}$), 436.3984 ($[M+3H]^{3+}$), and 327.6061 ($[M+4H]^{4+}$).

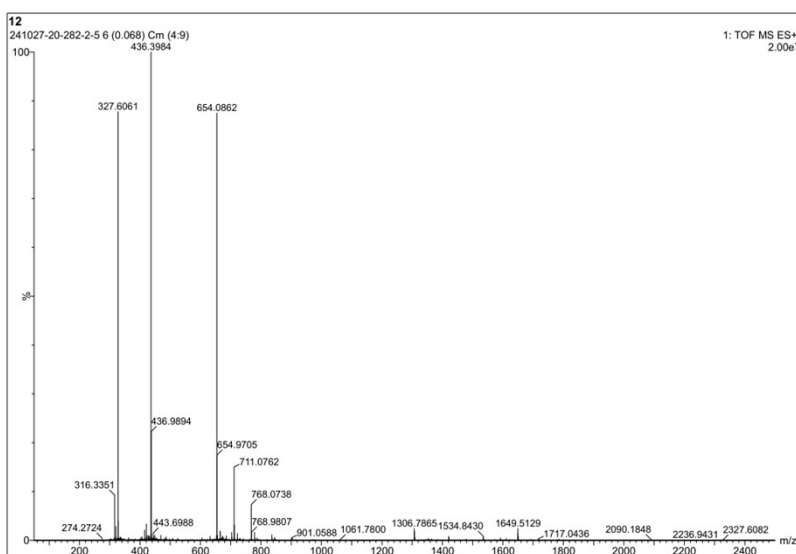


Figure S10. The ESI-MS spectrum of A4-T2

S.2.5. Mass spectrum information of A4-T3

For Linear A4-T3, the calculated ESI-MS m/z values are: 661.27 ($[M+2H]^{2+}$), 441.18 ($[M+3H]^{3+}$), and 331.135 ($[M+4H]^{4+}$). The observed values are: 661.0945 ($[M+2H]^{2+}$), 441.0436 ($[M+3H]^{3+}$), and 331.0896 ($[M+4H]^{4+}$).

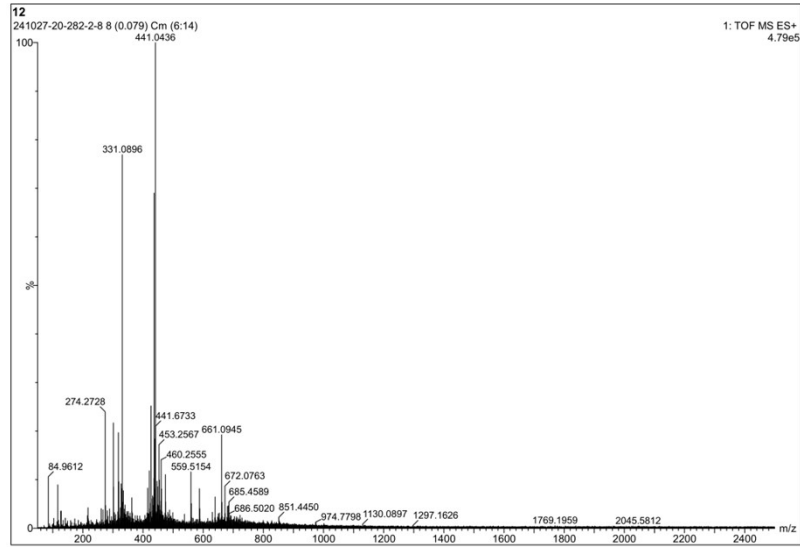


Figure S11. The ESI-MS spectrum of Linear A4-T3

For A4-T3, the calculated ESI-MS m/z values are: 661.27 ($[M+2H]^{2+}$), 441.18 ($[M+3H]^{3+}$), and 331.135 ($[M+4H]^{4+}$). The observed values are: 661.0874 ($[M+2H]^{2+}$), 441.0526 ($[M+3H]^{3+}$), and 331.0941 ($[M+4H]^{4+}$).

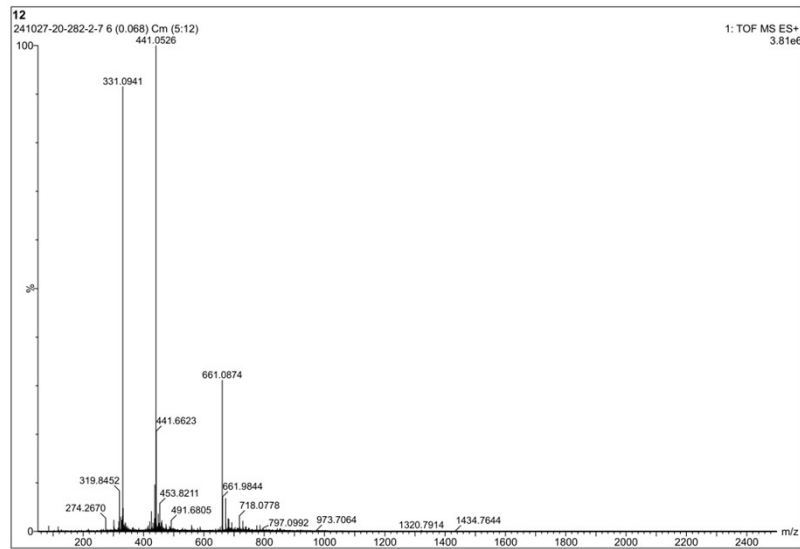


Figure S12. The ESI-MS spectrum of A4-T3

S.3. Stability of A4 and its Derived Peptides

S.3.1. The original HPLC spectra of A4 under trypsin conditions

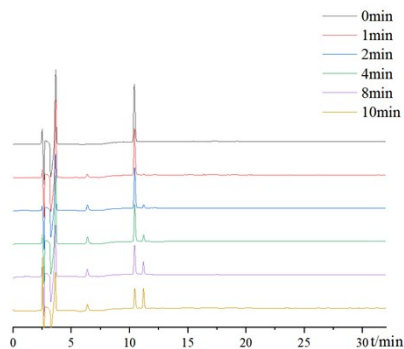


Figure S13. The HPLC spectra of A4 under trypsin conditions

S.3.2. The original HPLC spectra of D-A4 under trypsin conditions

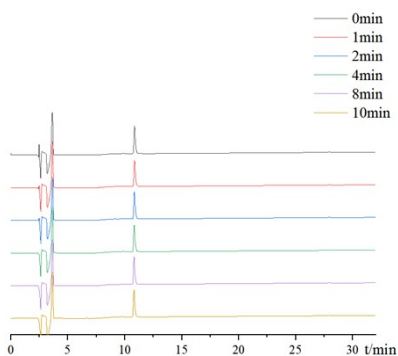


Figure S14. The HPLC spectra of D-A4 under trypsin conditions

S.3.3. The original HPLC spectra of A4-T3 under trypsin conditions

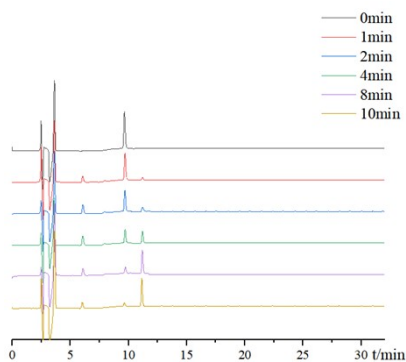


Figure S15. The HPLC spectra of A4-T3 under trypsin conditions