Supporting Information

Efficient Chemical Synthesis for the Analogue of Ubiquitin-Based Probe Ub-AMC with Native Bioactivity

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Experimental Section

A. HPLC of peptide segments

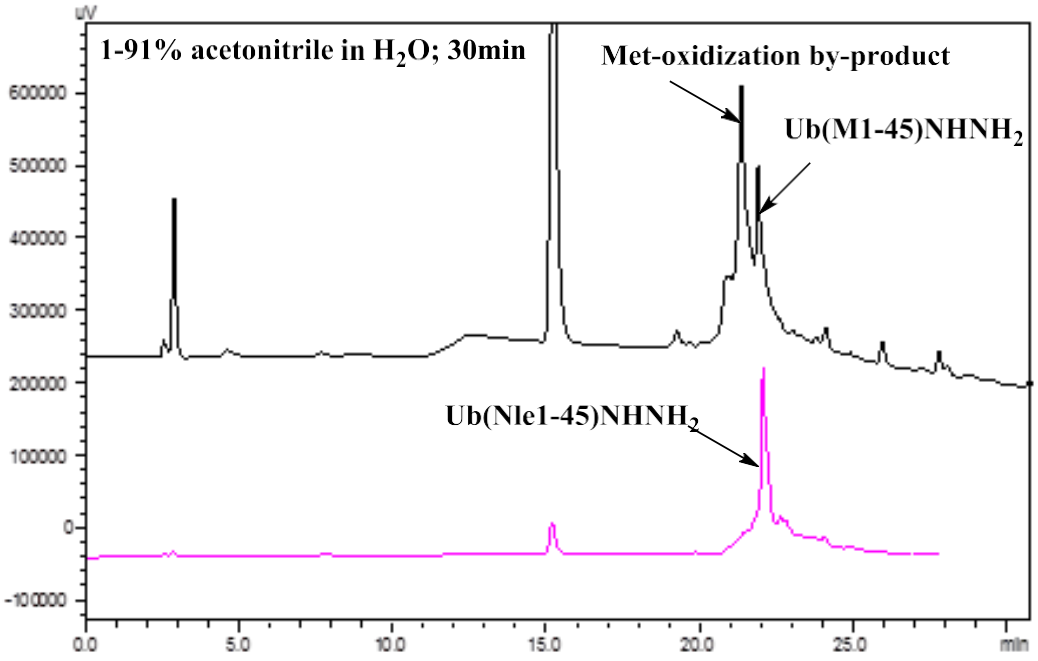


Figure S1 HPLC of Ub(M1-45)-$NHNH_2$ and Ub(M1Nle-45)-$NHNH_2$ segment

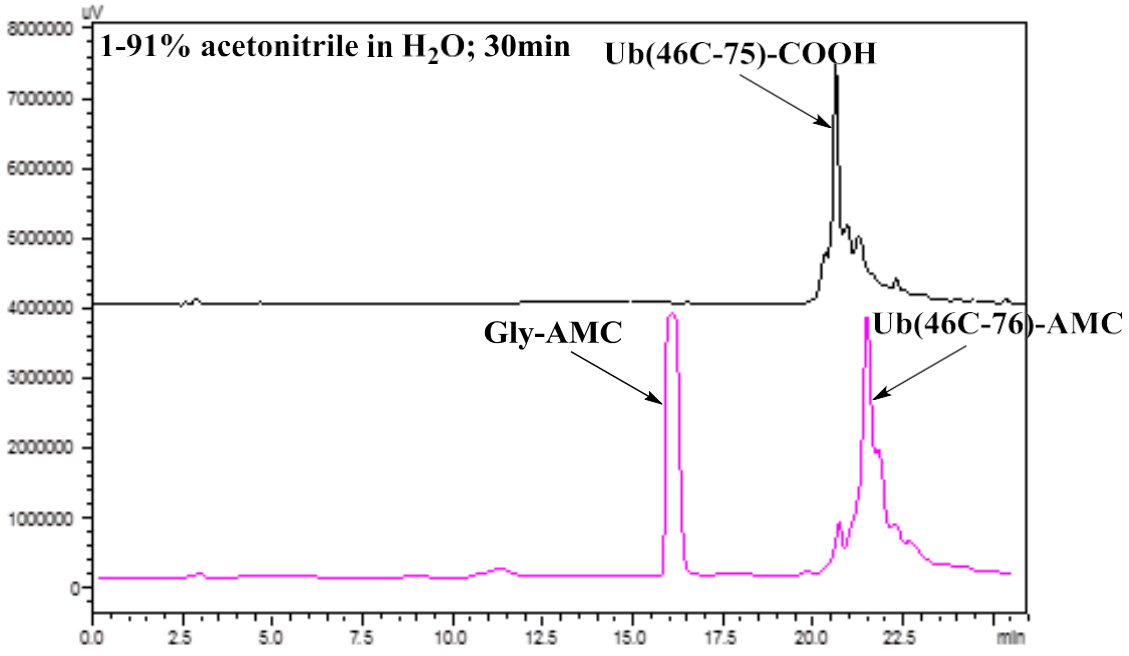


Figure S2 HPLC of Ub(46C-75)-COOH and Ub(46C-76)-AMC segment

B. One-pot ligation-desulfurization of Ub(Nle1-45)$NHNH_2$ and Ub(46C-76)AMC

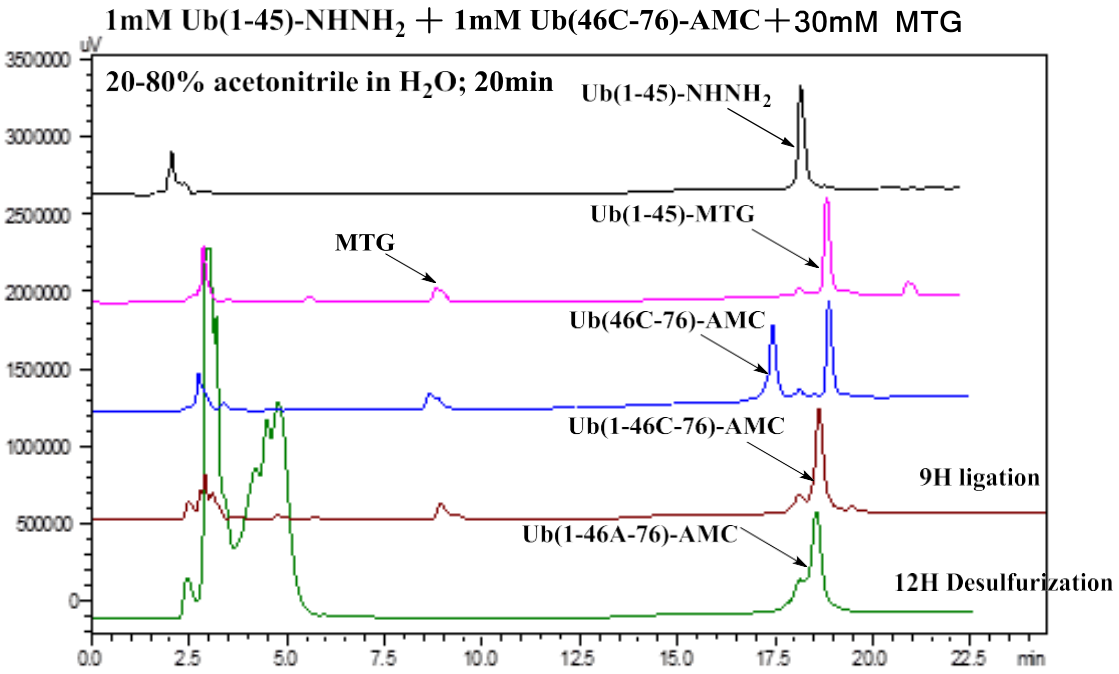
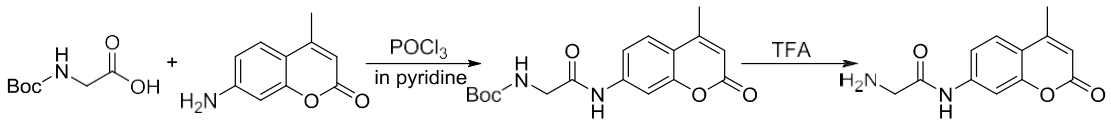


Figure S3 Ligation of two segments.

C. The synthesis of Gly-AMC



To a 100 mL round-bottom flask fitted with a stir bar were added 7-Amino-4-methylcoumarin (1.75 g, 10 mmol), Boc-Gly-OH (1.75 g, 10 mmol) and dried pyridine (35 mL). Then the $POCl_3$ (1 mL, 11 mmol) was added to the mixture dropwise in a −15°C ice/salt bath. After 3 h, the reaction mixture was allowed to warm to room temperature and stirred for an additional 16 h. Then the reaction was quenched with water. The mixture was washed with 4 M HCl (remove pyridine) and extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography with a yield of 45% (1.49 g, 4.5 mmol).

To a 50 mL round-bottom flask fitted with a stir bar were added Boc-Gly-AMC (1.75 g, 10 mmol) and TFA (25 mL). The mixture was stirred for 3 h. The TFA was removed by rotary evaporation. The product H-Gly-AMC was sufficiently pure with a yield of 96% (1 g, 4.3 mmol). ^{1}H NMR (400 MHz, D_2O) δ 7.16 (d, J = 8.7 Hz, 1H), 7.09 (s, 1H), 6.91 (d, J = 8.6 Hz, 1H), 5.84 (s, 1H), 3.85 (s, 2H), 2.03 (s, 3H).

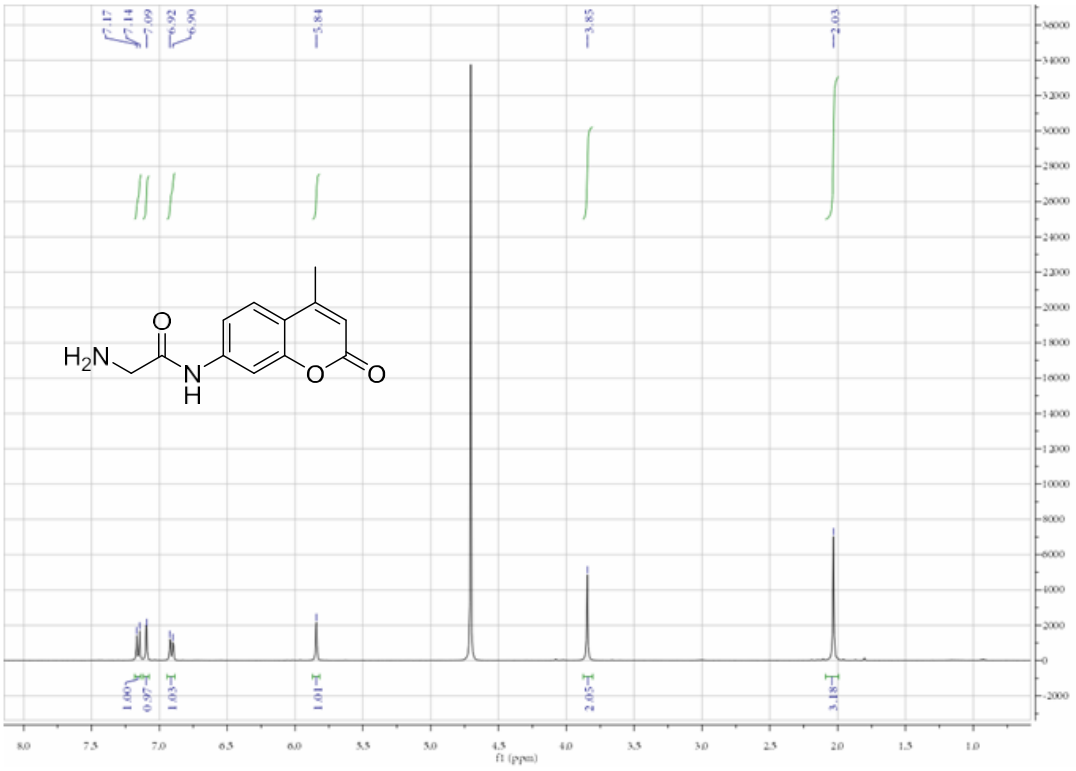


Figure S4 ^{1}H NMR of Gly-AMC

D. ESI-MS of peptide segments

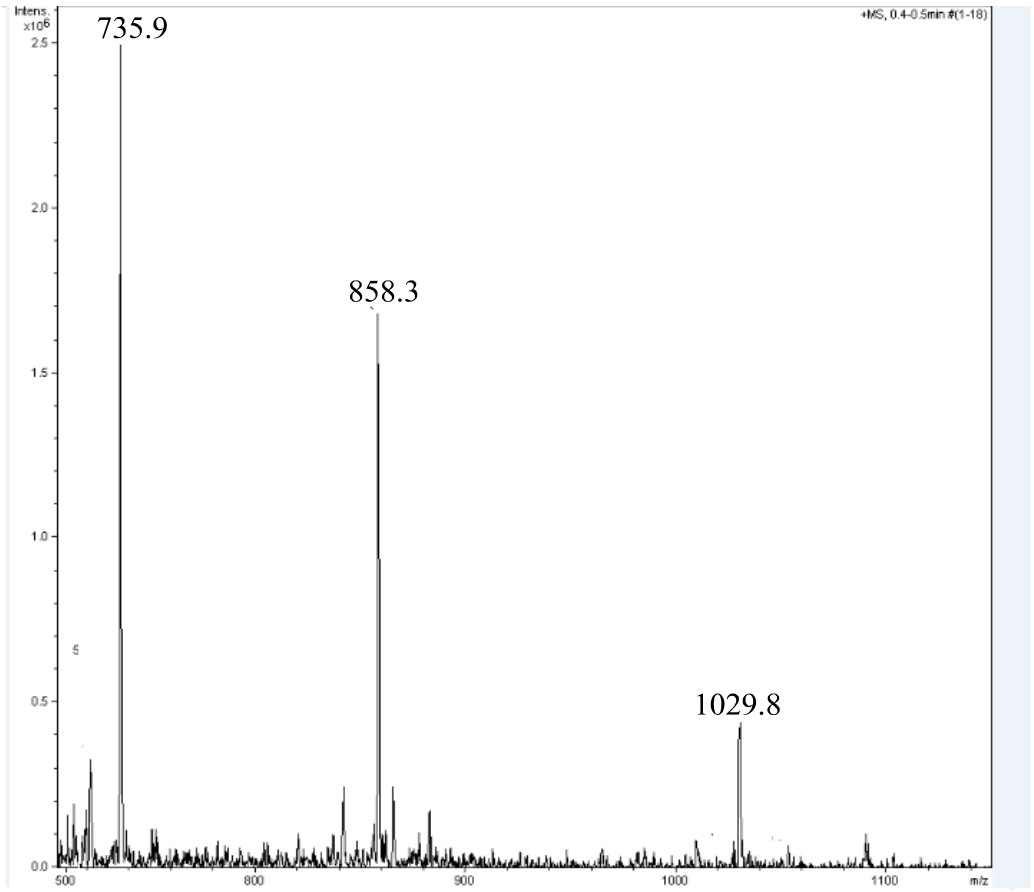


Figure S5 ESI-MS of Met-oxidization Ub (Met1-45) $NHNH_2$ (observed: 5144.0± 0.3 Da; calculated: 5143.9 Da).

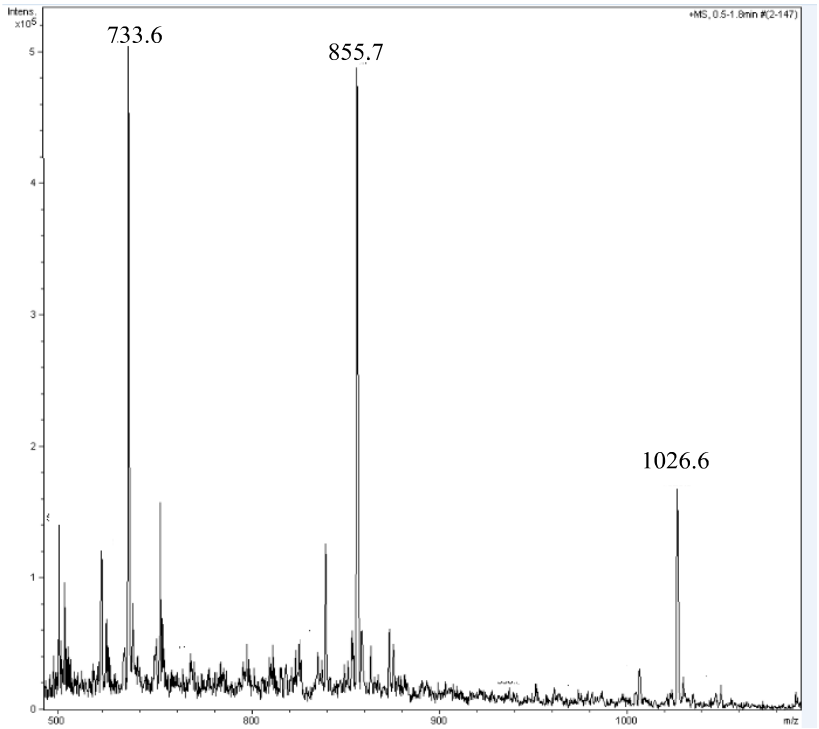


Figure S6 ESI-MS of Ub (Met1-45) $NHNH_2$ (observed: 5128.0± 0.3 Da; calculated: 5127.9 Da)

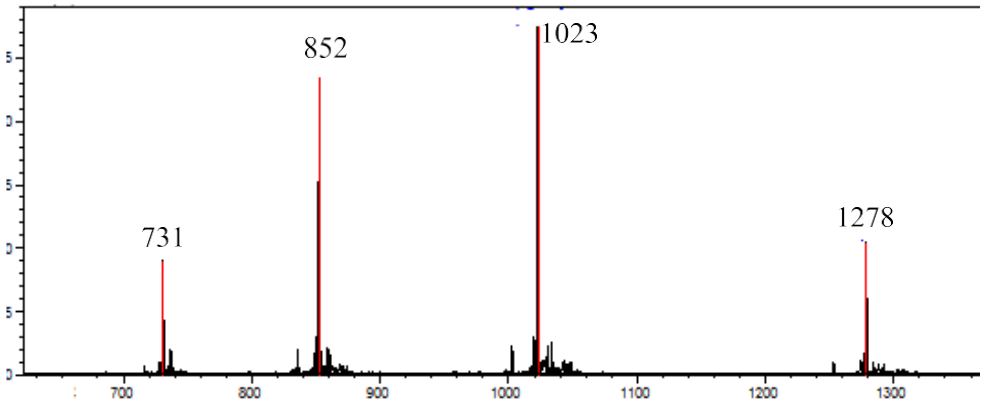


Figure S7 ESI-MS of Ub (Nle1-45) $NHNH_2$ (observed: 5108.5± 1.5 Da; calculated: 5109.9 Da)

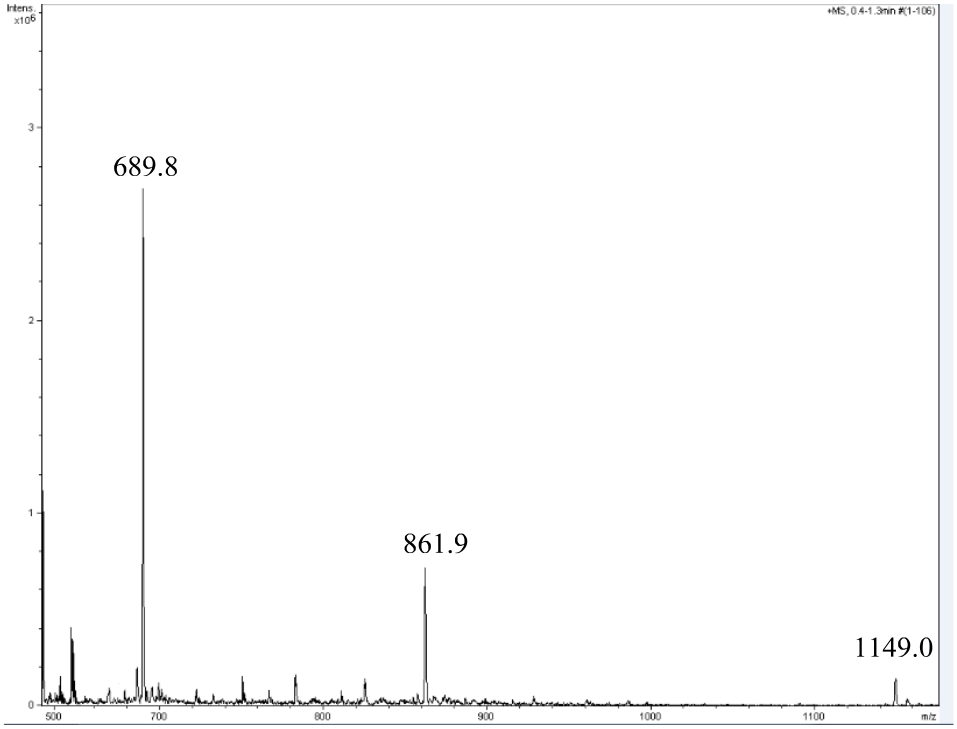


Figure S8 ESI-MS of Ub (46C-75) COOH (observed: 3443.9.0± 0.3 Da; calculated: 3443.9 Da)

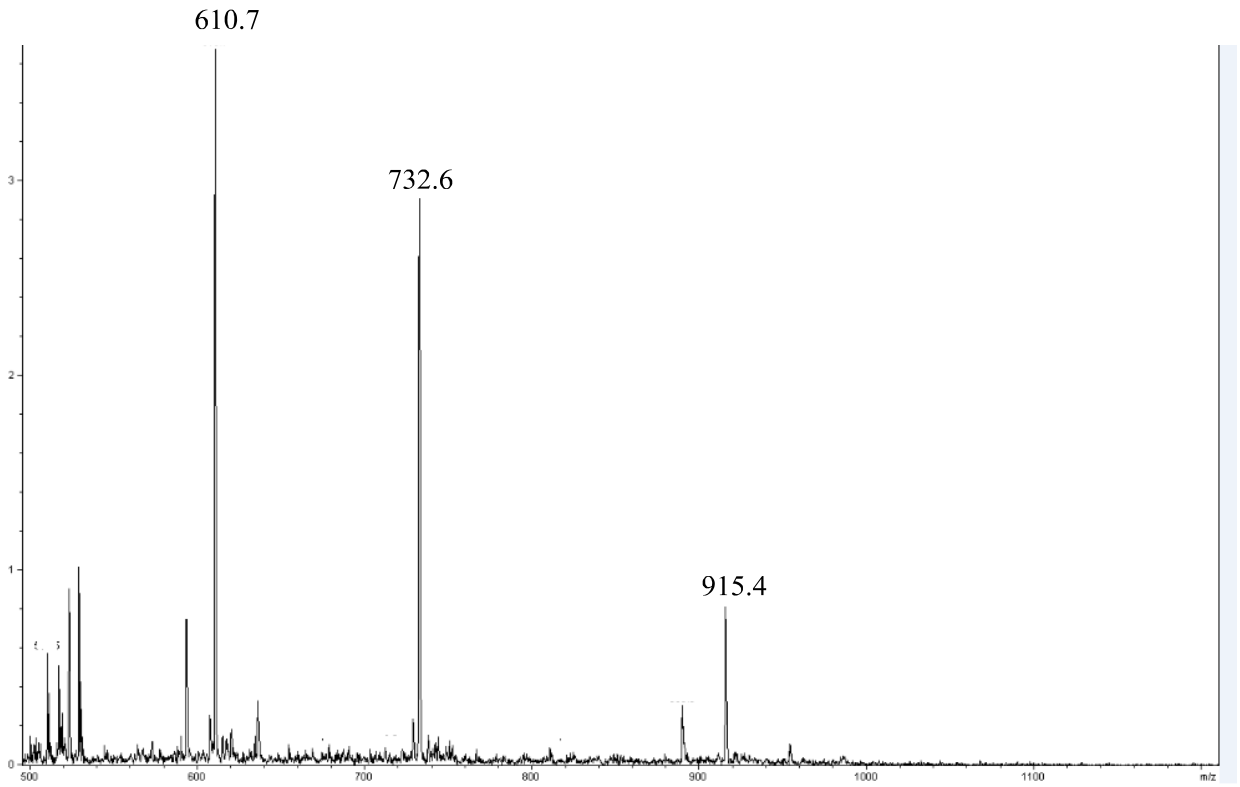


Figure S9 ESI-MS of Ub (46C-76) AMC (observed: 3657.9± 0.3 Da; calculated: 3658.2 Da)

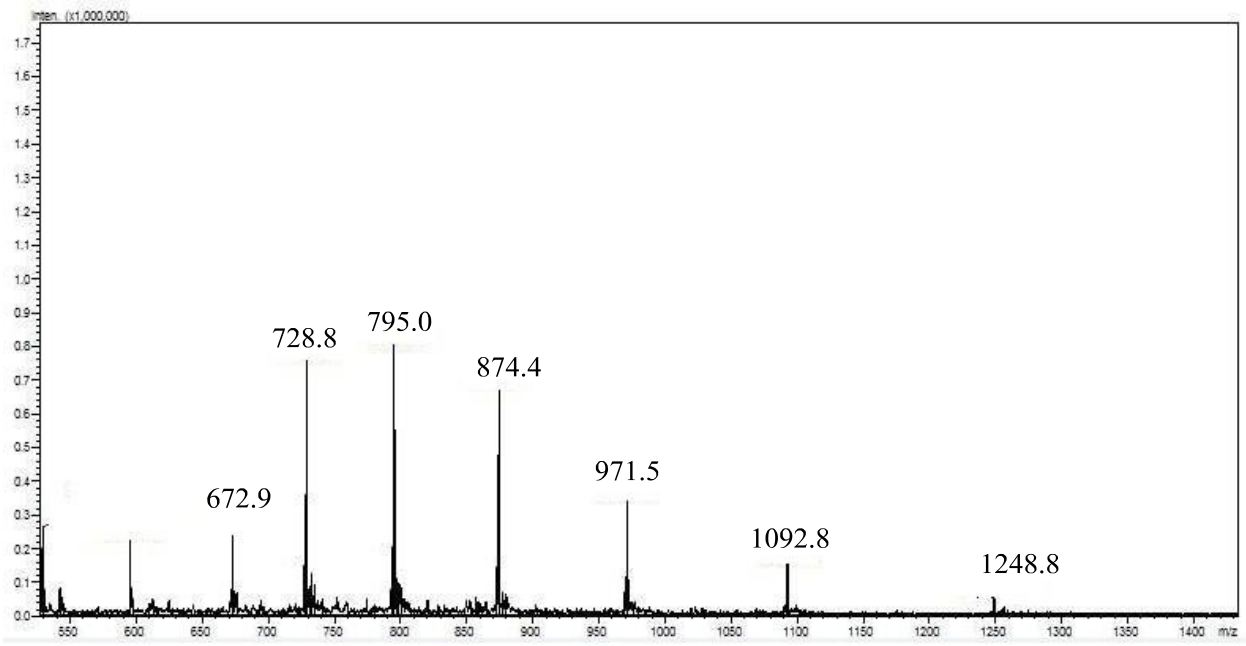


Figure S10 ESI-MS of Ub (Nle1-46C-76)-AMC (observed: 8734.2± 0.6 Da; calculated: 8736.1 Da)

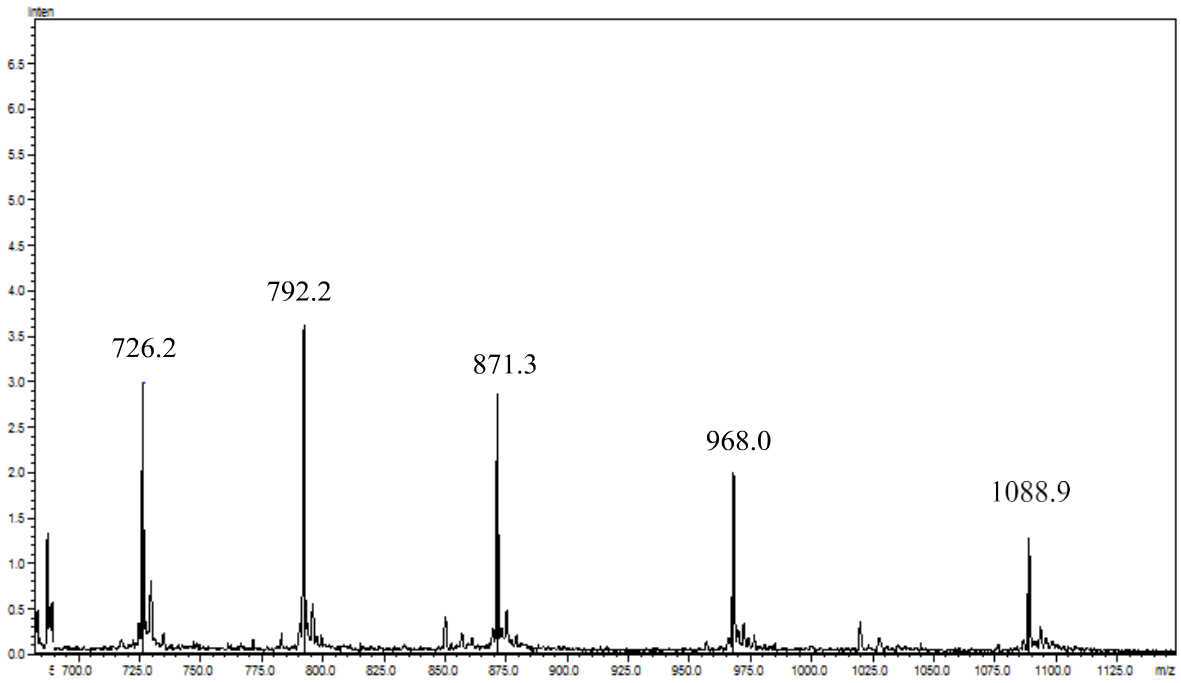


Figure S11 ESI-MS of Ub (Nle1-76)-AMC (observed: 8703.0± 0.6 Da; calculated: 8704.1 Da)

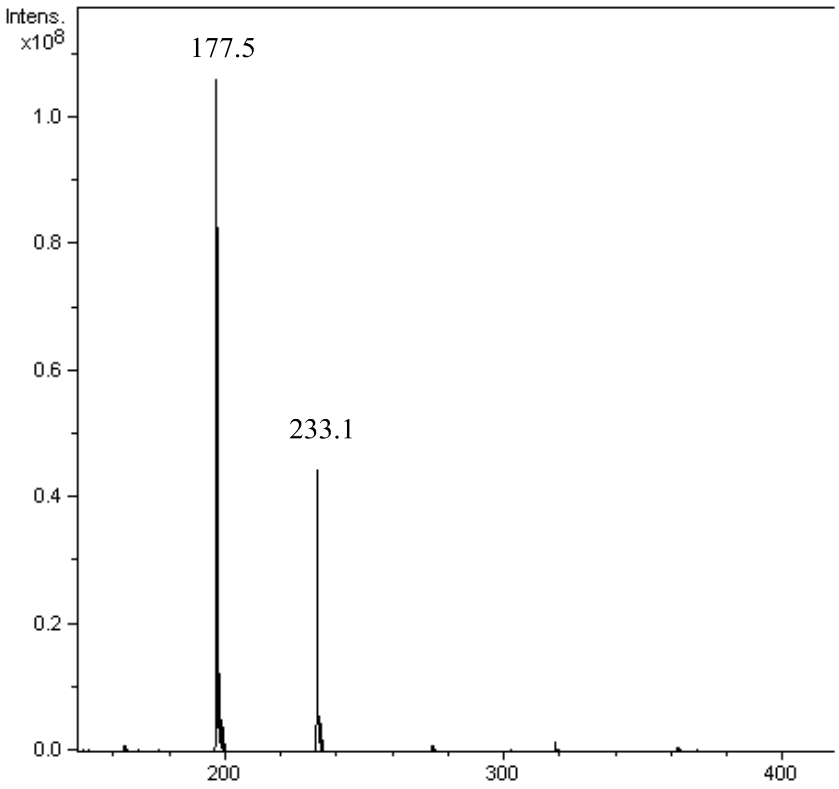


Figure S12 ESI-MS of Gly-AMC (observed: 233.1 Da; calculated: 233.2 Da)

Reference:

1 J. Liang, G. M. Fang, X. L. Huang, Z. Q. Mei, J. Li, C. L. Tian, L. Liu, *Sci. China. Chem.*, 2013, **56**, 1301-1306.