

Improved Solid Phase Synthesis of Hydrogen Bond Surrogate Derived α -Helices: Resolving the Case of a Difficult Amide Coupling

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Supplementary Information

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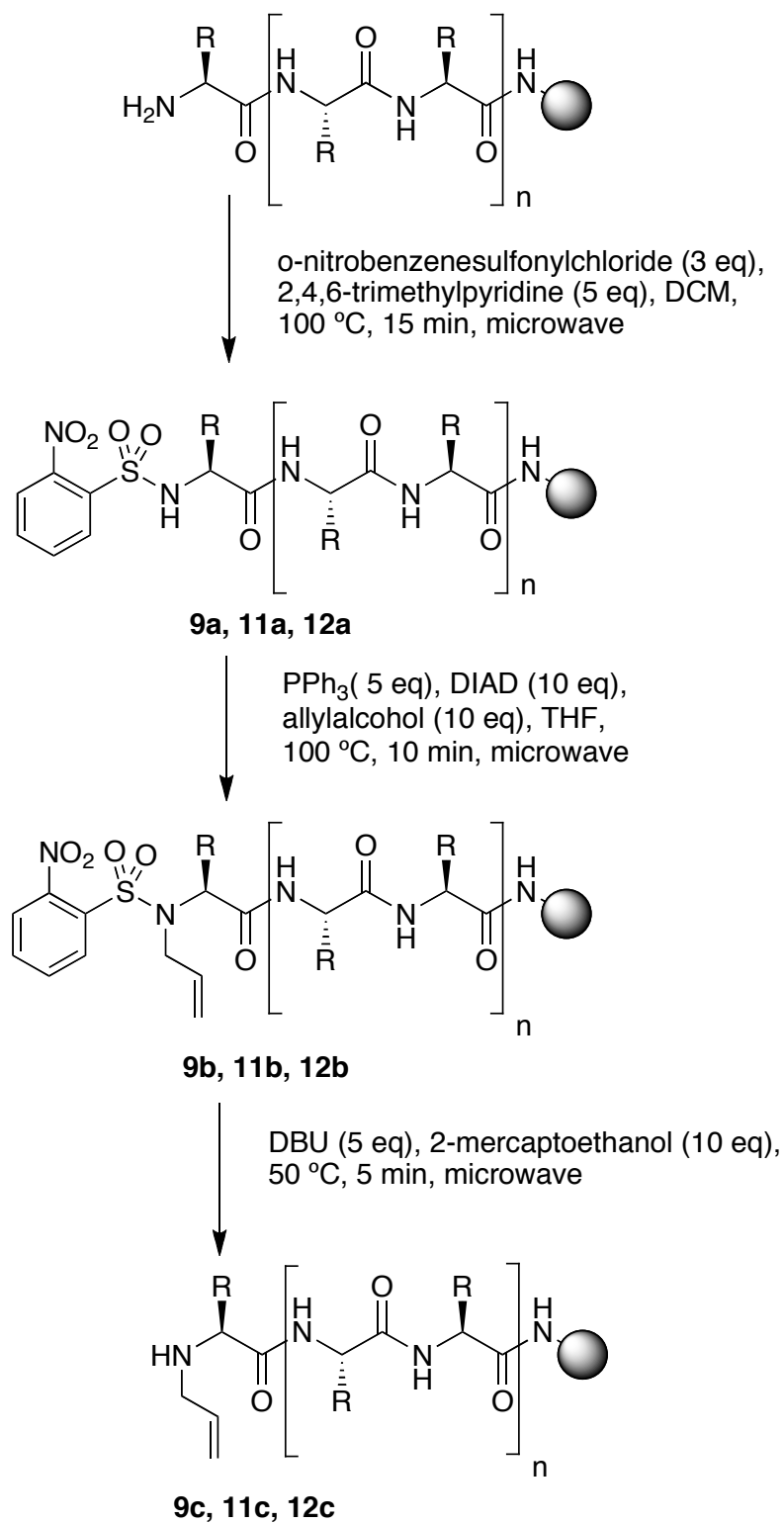
General. Commercial-grade reagents and solvents were used without further purification except as indicated. CH₂Cl₂, THF, and DMF were dried prior to use on an Innovative Technology PureSolve solvent drying system. Dichloroethane was distilled before use in the metathesis reaction. All reactions were stirred magnetically or mechanically shaken; moisture-sensitive reactions were performed under nitrogen in flame-dried glassware. Reverse-phase HPLC experiments were conducted with 4.6 x 150 mm Waters C18 reverse phase columns using a Beckman Coulter HPLC equipped with a System Gold 168 Diode array detector. The typical flow rate for analytical HPLC was 1 mL/min. In all cases, 0.1% aqueous trifluoroacetic acid and 0.1% trifluoroacetic acid in acetonitrile buffers were used. ESIMS data was obtained on an Agilent 1100 series LC/MSD (XCT) electrospray trap. The microwave reactions were performed in the CEM Discover single-mode reactor with controlled power, temperature, and time settings. The parent peptides were synthesized on a CEM Liberty series microwave peptide synthesizer.

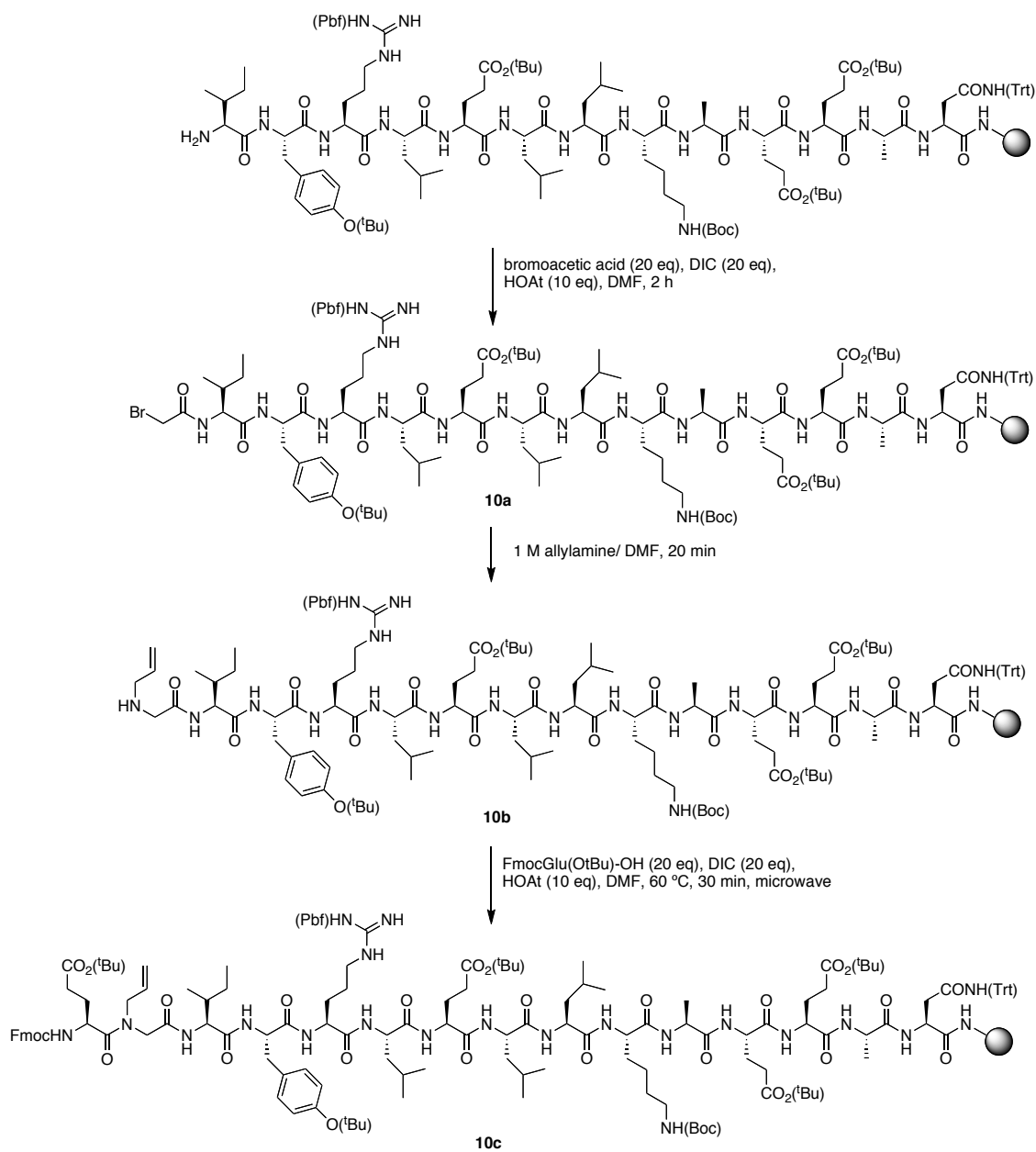
Synthesis and Characterization of HBS α -Helices

a) Solid phase peptide synthesis. Resin bound free amine peptides were synthesized by conventional Fmoc solid-phase chemistry on Rink Amide or Knorr resin (loading = 0.4mmole/g) on a CEM Liberty Microwave Peptide Synthesizer. Standard Fmoc amino acids (and 4-petenoic acid) (5 equiv) were activated with HBTU (4.9 equiv) in 6 % DIPEA/NMP solution for 15 min and added to the resin bound free amine. The coupling efficiency was monitored by the ninhydrin test. Fmoc groups were deprotected by treatment with 20% piperidine in NMP (2 x 20 min). The bis-olefin peptide containing resin was thoroughly washed with DMF and DCM respectively, and dried under vacuum overnight.

b) General method for microwave-based RCM of HBS helices. The resin bound bis-olefin peptide (0.1 mmole) was placed in a thick wall glass tube (CEM) with 20 mol% of Hoveyda Grubbs II catalyst and sealed. To this tube was added 900 μ l of freshly distilled dichloroethane, the tube was purged with nitrogen and the resin allowed to swell for 20 min. All reactions were carried out in the CEM discover microwave reactor with a ramp time of 2 minutes, hold time of 10 minutes and temperature of 120 °C as previously reported.¹ After the indicated time had elapsed, the solution was cooled rapidly by compressed air, and the resin washed with DMF (3x), Methanol (3x) and DCM (3x), then dried for 3 h under vacuum. The peptide was cleaved from the resin with 95% TFA: 2.5% triisopropylsilane: 2.5% water for 2 h, filtered, concentrated on a rotary evaporator at 60 °C, and precipitated from cold ether. The precipitate was centrifuged, and the pellet was washed with cold ether and lyophilized. Dry peptide was dissolved in 7:3 0.1% aqueous TFA:acetonitrile for analytical HPLC analysis.

(1) Chapman, R. N.; Arora, P. S. *Org. Lett.* **2006**, *8*, 5825-5828.

Scheme S1. Microwave-assisted Fukuyama-Mitsunobu reaction.

Scheme S2. Synthesis of *N*-allylglycine peptide **10c**

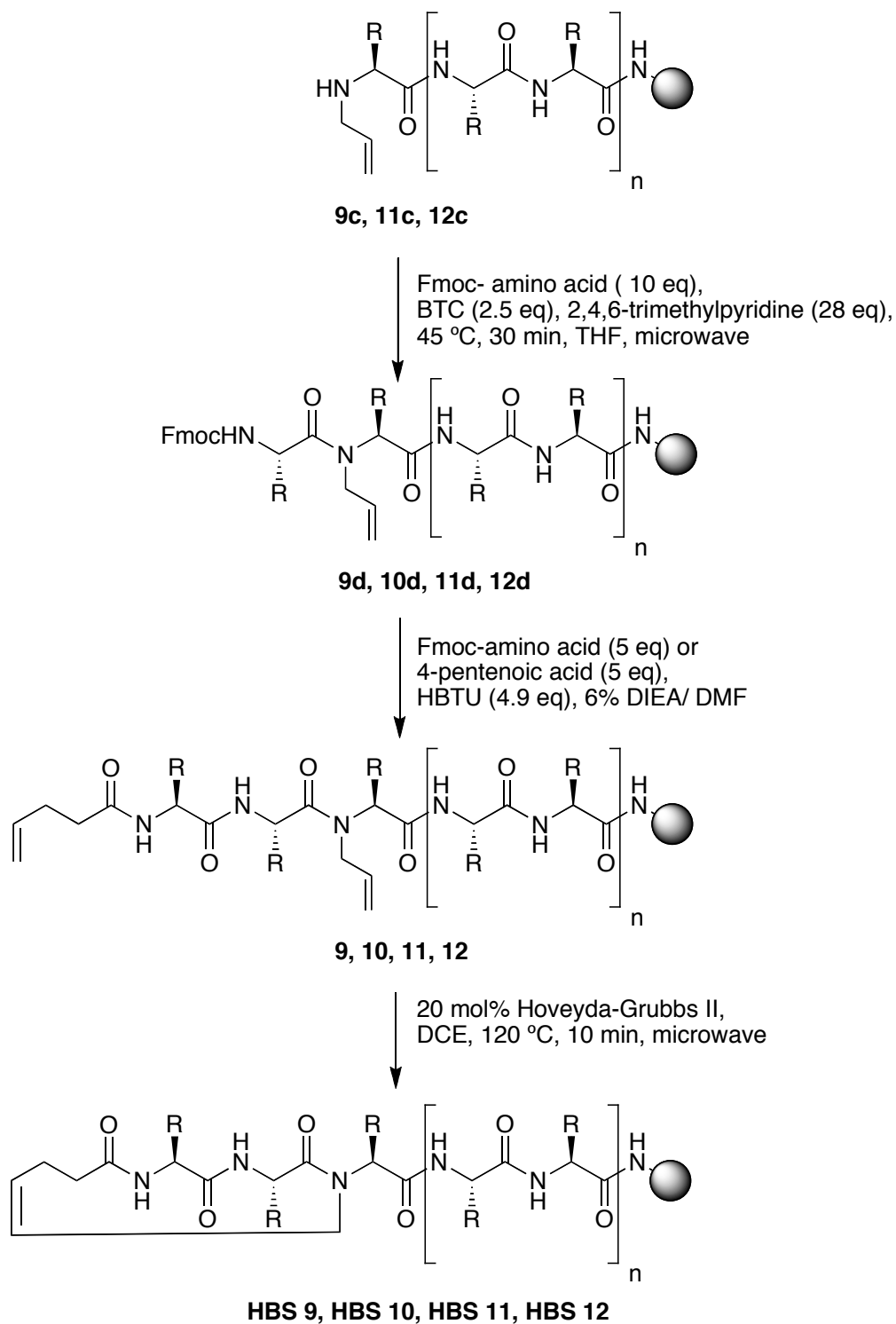
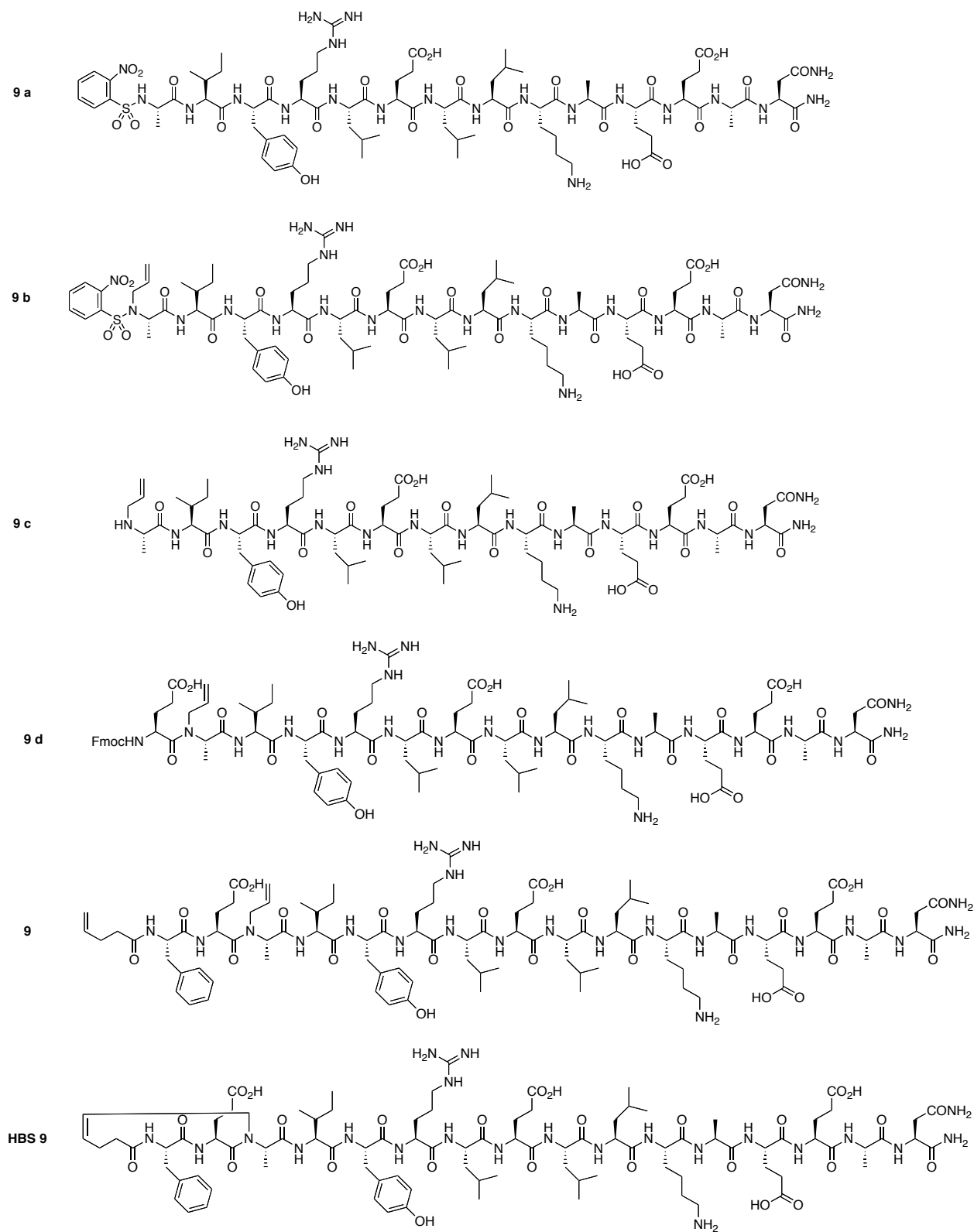
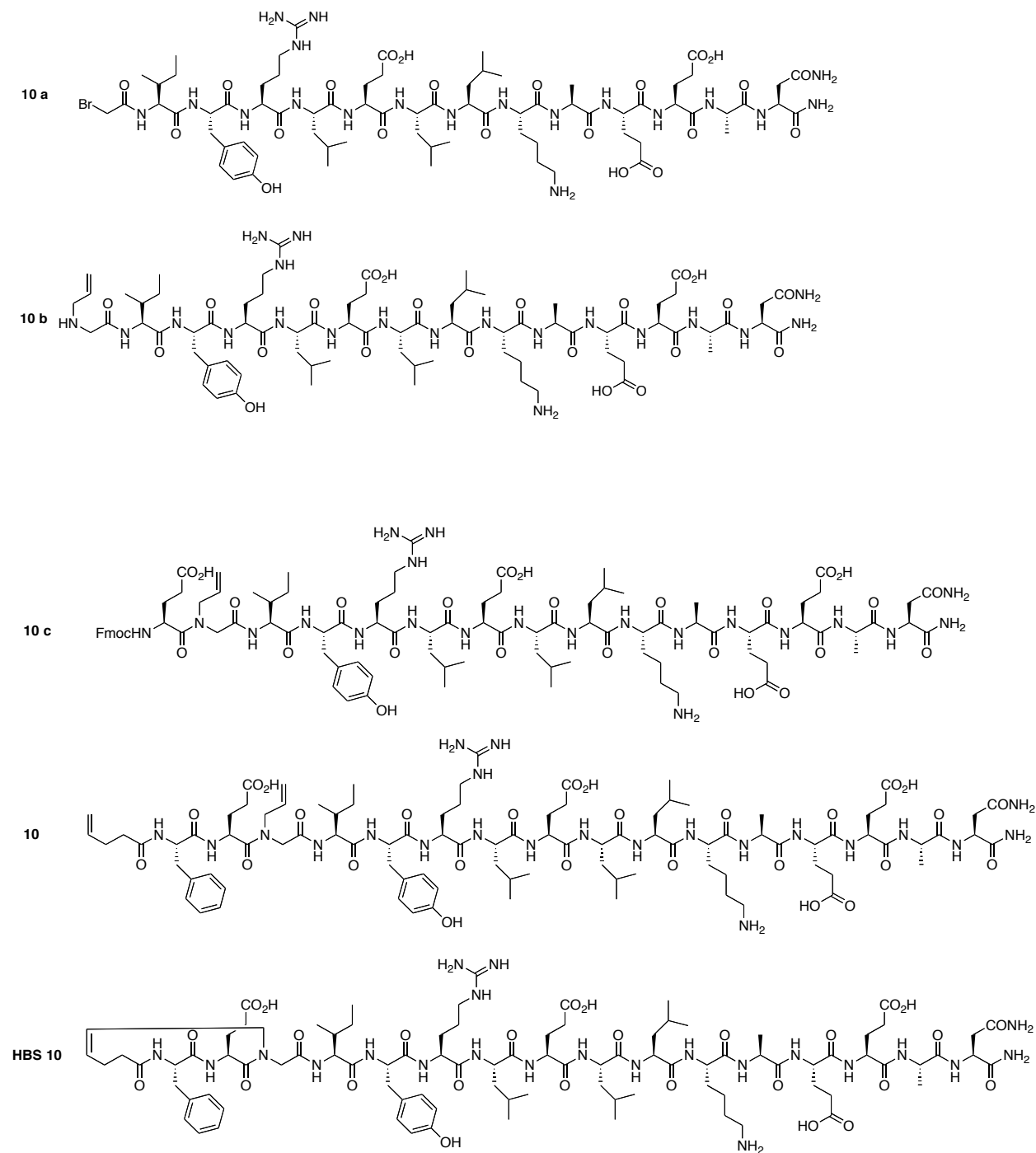
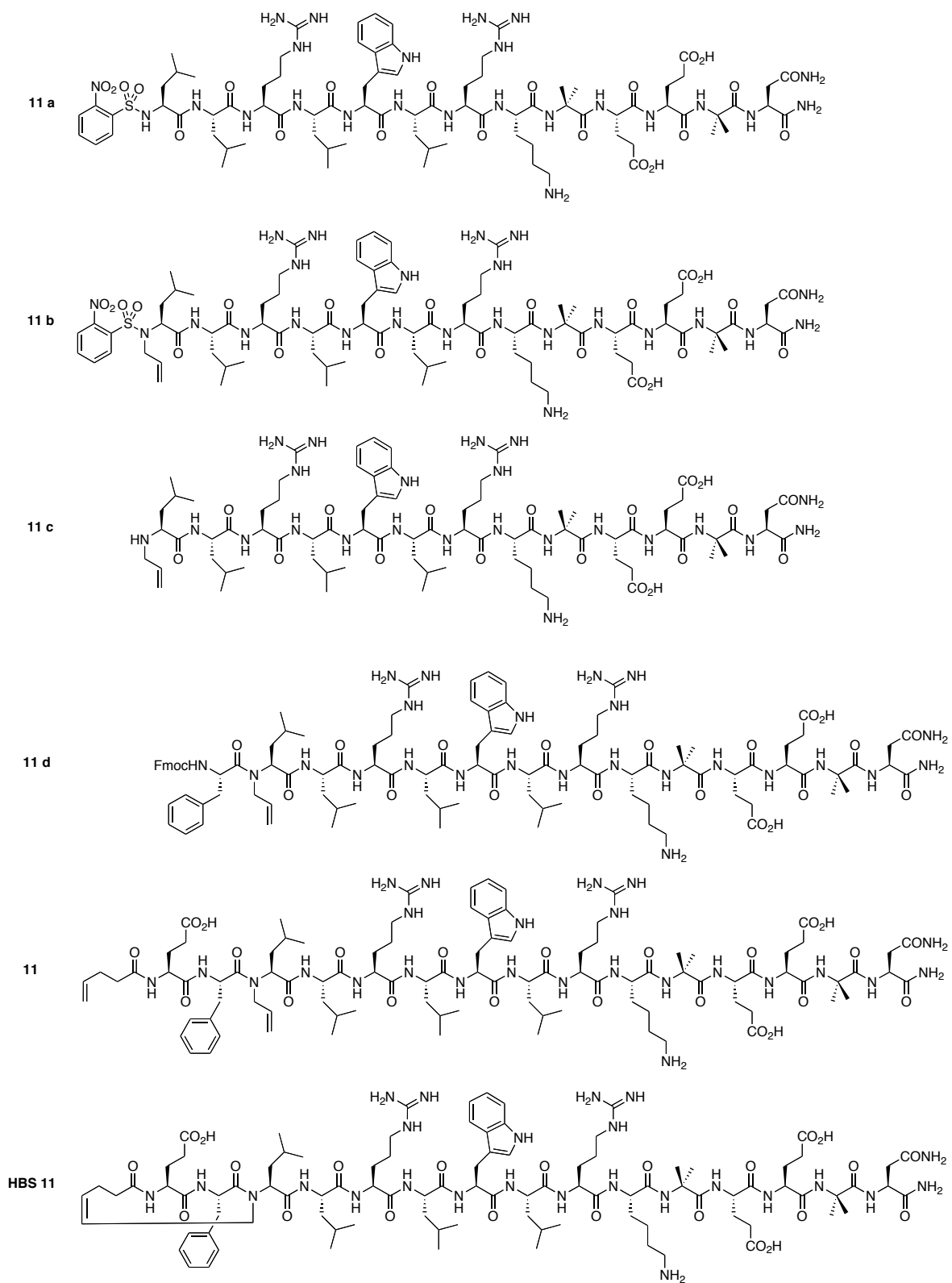
Scheme S3. Synthesis of HBS α - Helices **9-12**

Figure S1. Sequences of peptides **9**, **9a-d**, **HBS 9**, **10**, **10a-c**, **HBS 10**, **11**, **11a-d**, **HBS 11**, **12**, **12a-d** and **HBS 12**.







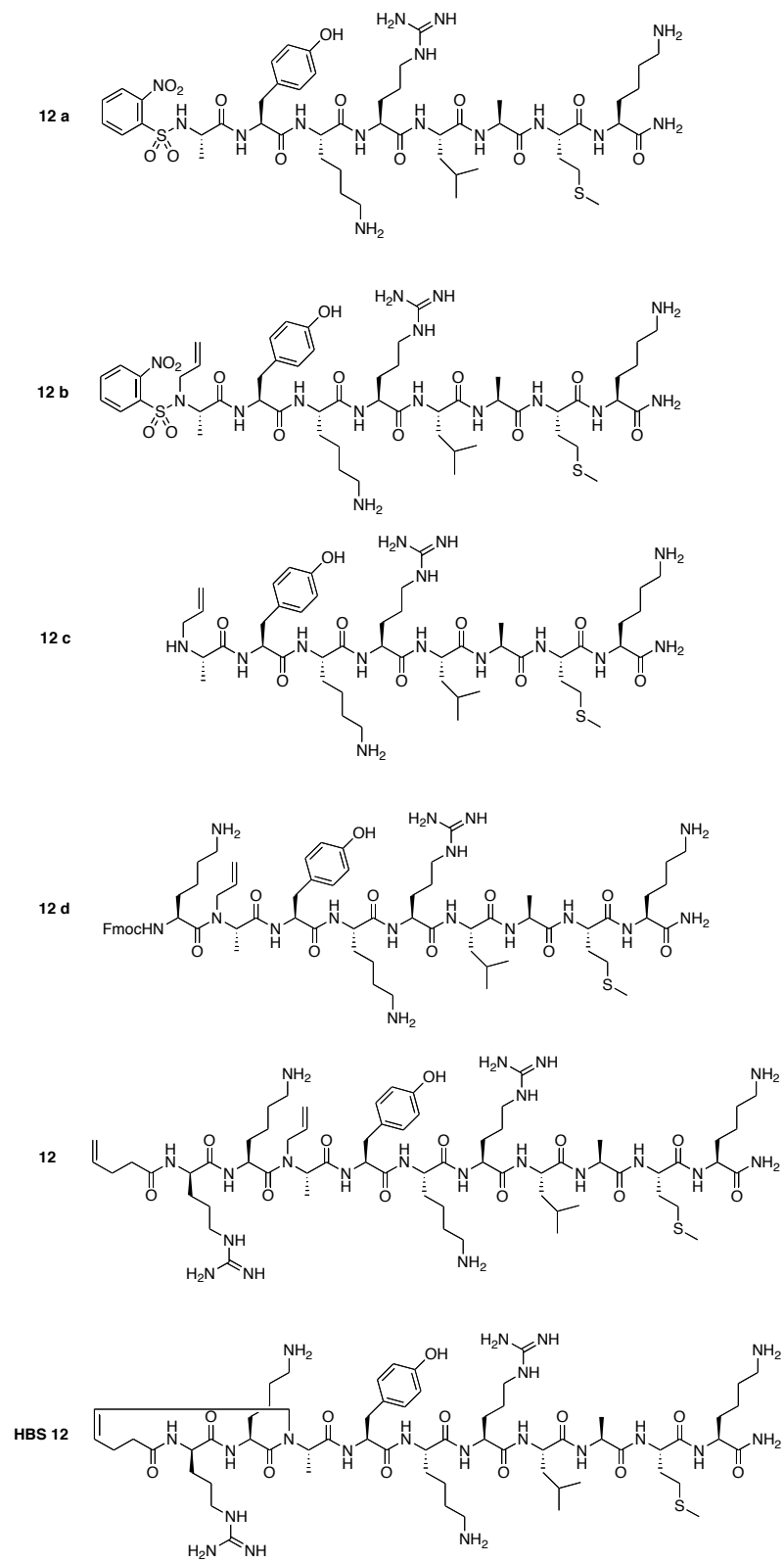


Table S1. Mass spectrometric characterization. ESIMS data was obtained on an Agilent 1100 Series LC/MSD (XCT) electrospray trap.

Compound	Calculated Mass [M+H]⁺	Observed Mass [M+H]⁺
9a	1818.03	1817.9
9b	1858.09	1857.9
9c	1672.93	1672.0
9d	2024.29	2024.1
9	2031.32	2031.2
HBS 9	2003.27	2003.0
10a	1682.73	1681.73
10b	1658.91	1658.0
10c	1788.02	1787.1
10	2017.30	2017.2
HBS 10	1989.24	1988.9
11a	1825.11	1824.9
11b	1864.18	1864.9
11 c	1680.02	1679.9
11d	2049.43	2049.0
11	2038.41	2038.1
HBS 11	2010.35	2010.1
12a	1165.40	1164.7
12b	1205.46	1204.7
12c	1020.31	1019.7
12d	1370.72	1369.9
12	1386.77	1385.9
HBS 12	1358.71	1358.0

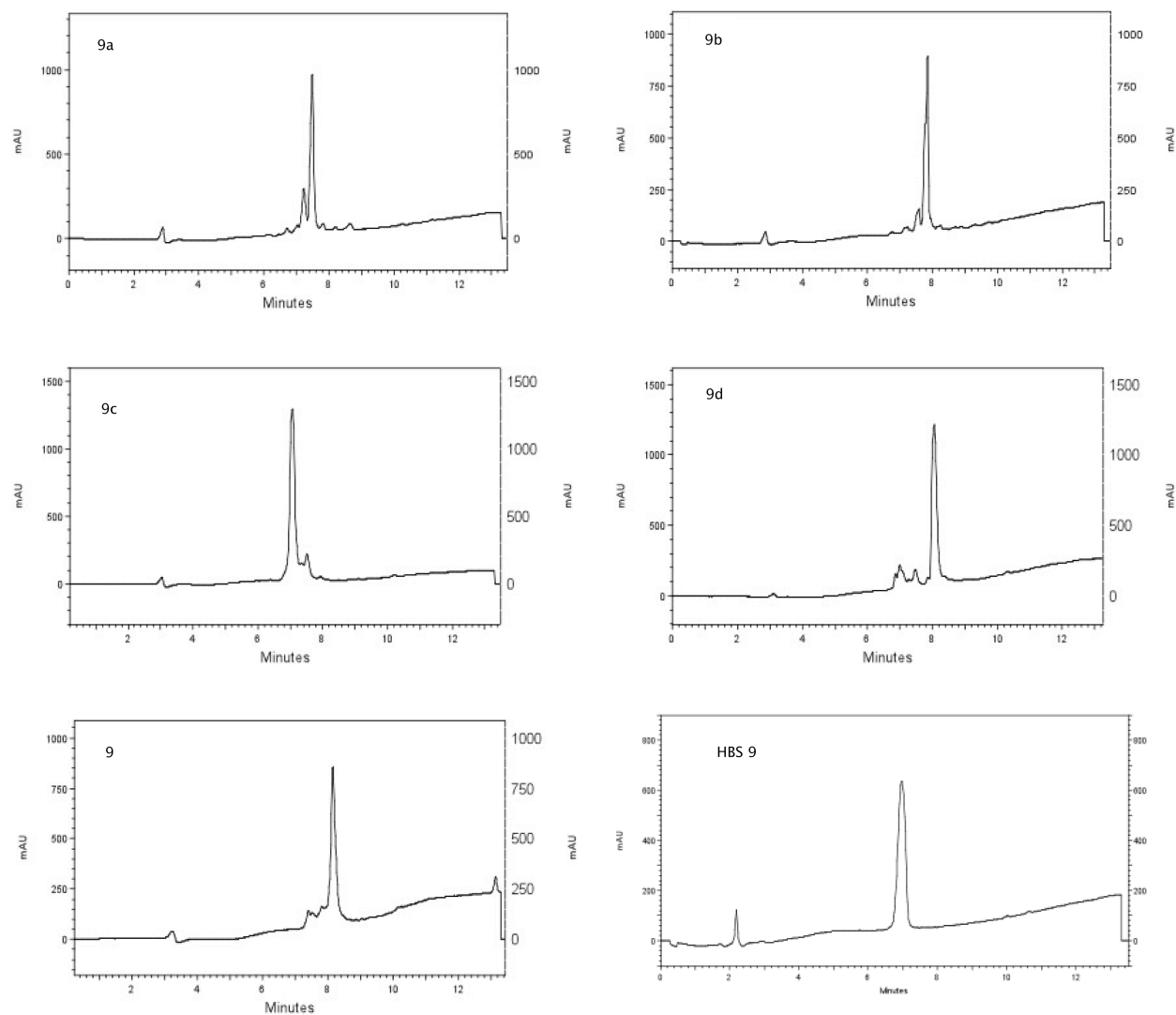


Figure S2. Analytical HPLC traces of crude peptides **9a**, **9b**, **9c**, **9d**, **9** and purified **HBS 9** (monitored at 220 nm).

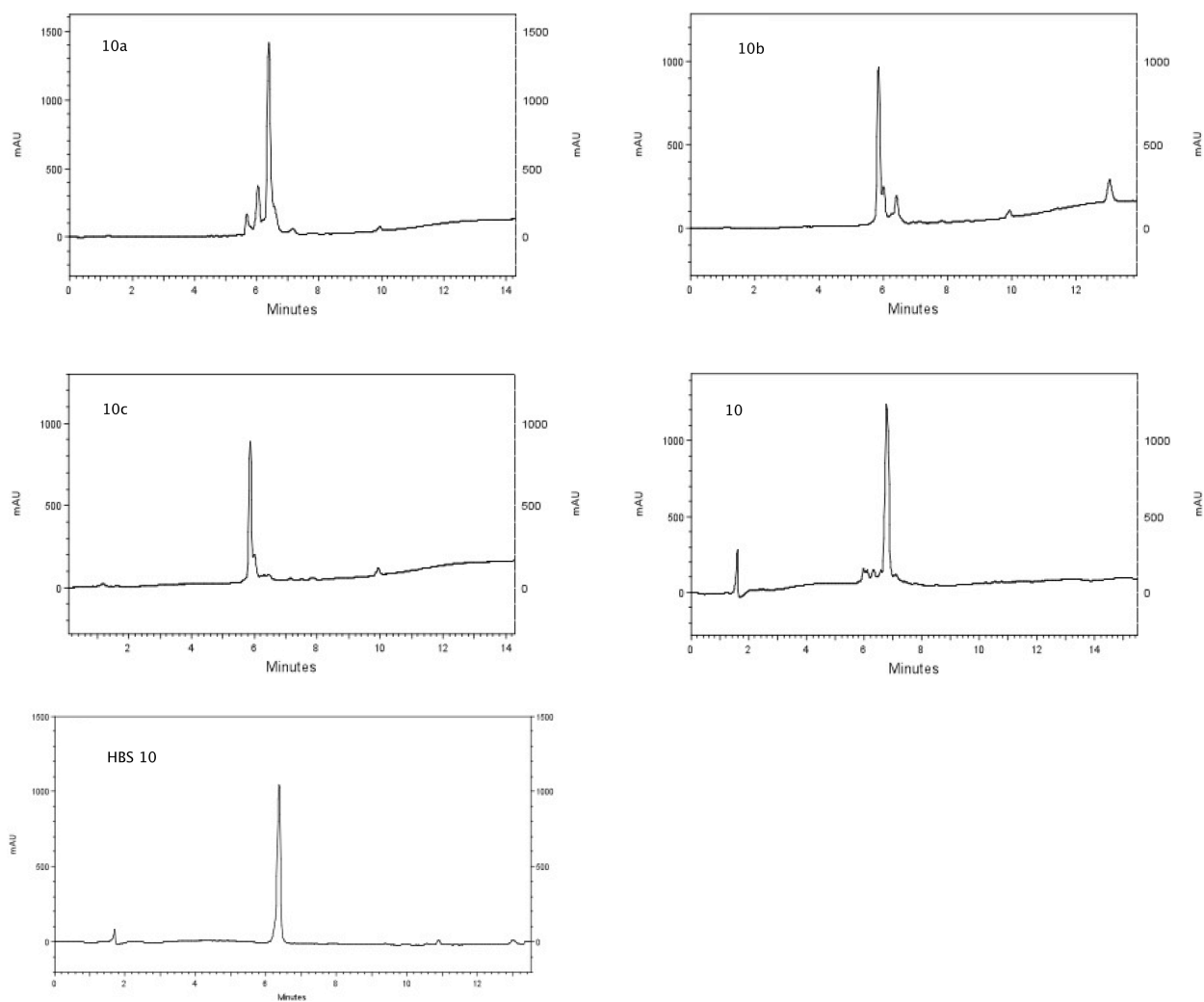


Figure S3. Analytical HPLC traces of crude peptides **10a**, **10b**, **10c**, **10** and purified **HBS 10** (monitored at 220 nm).

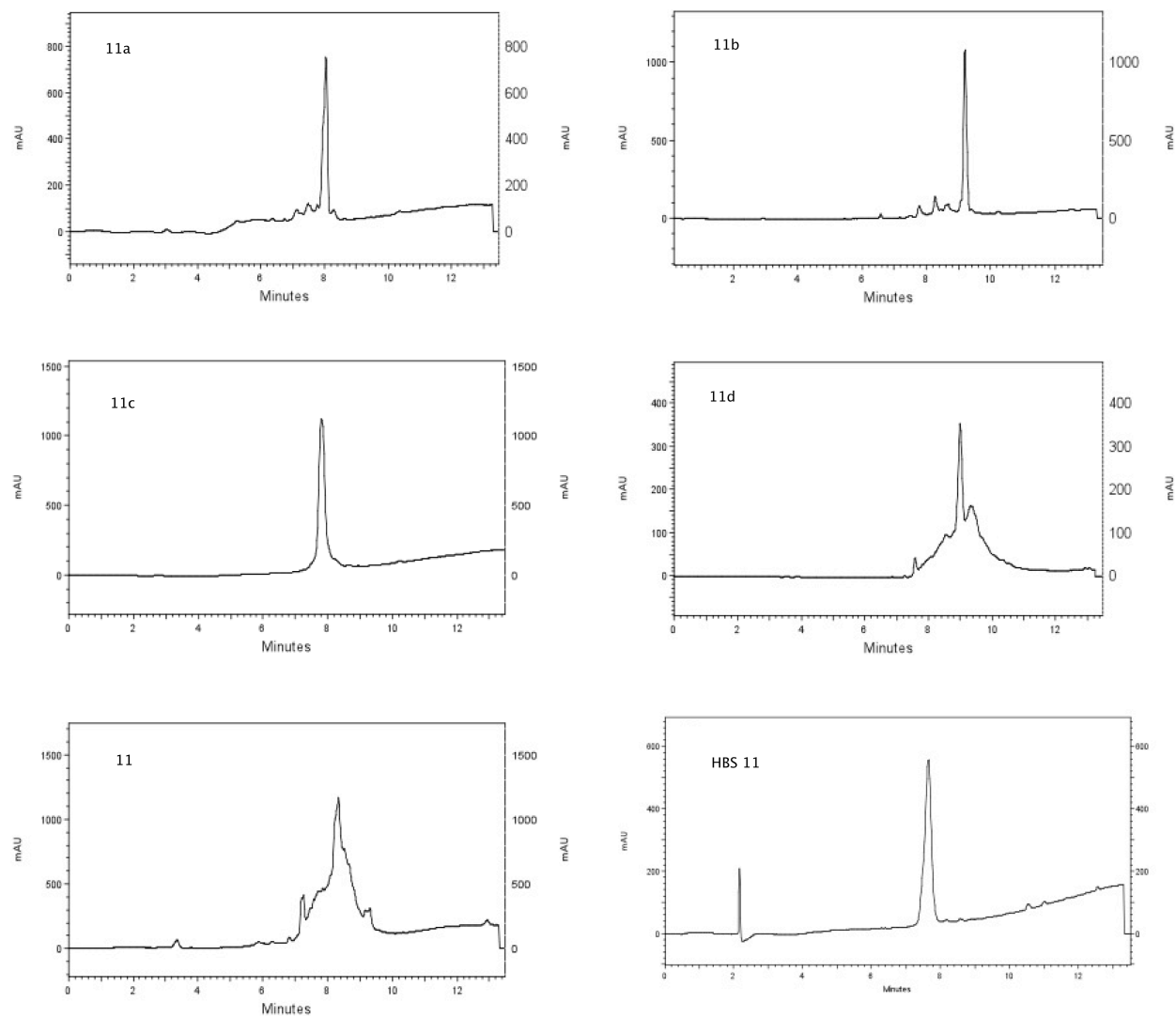


Figure S4. Analytical HPLC traces of crude peptides **11a**, **11b**, **11c**, **11d**, **11** and purified **HBS 11** (monitored at 220 nm).

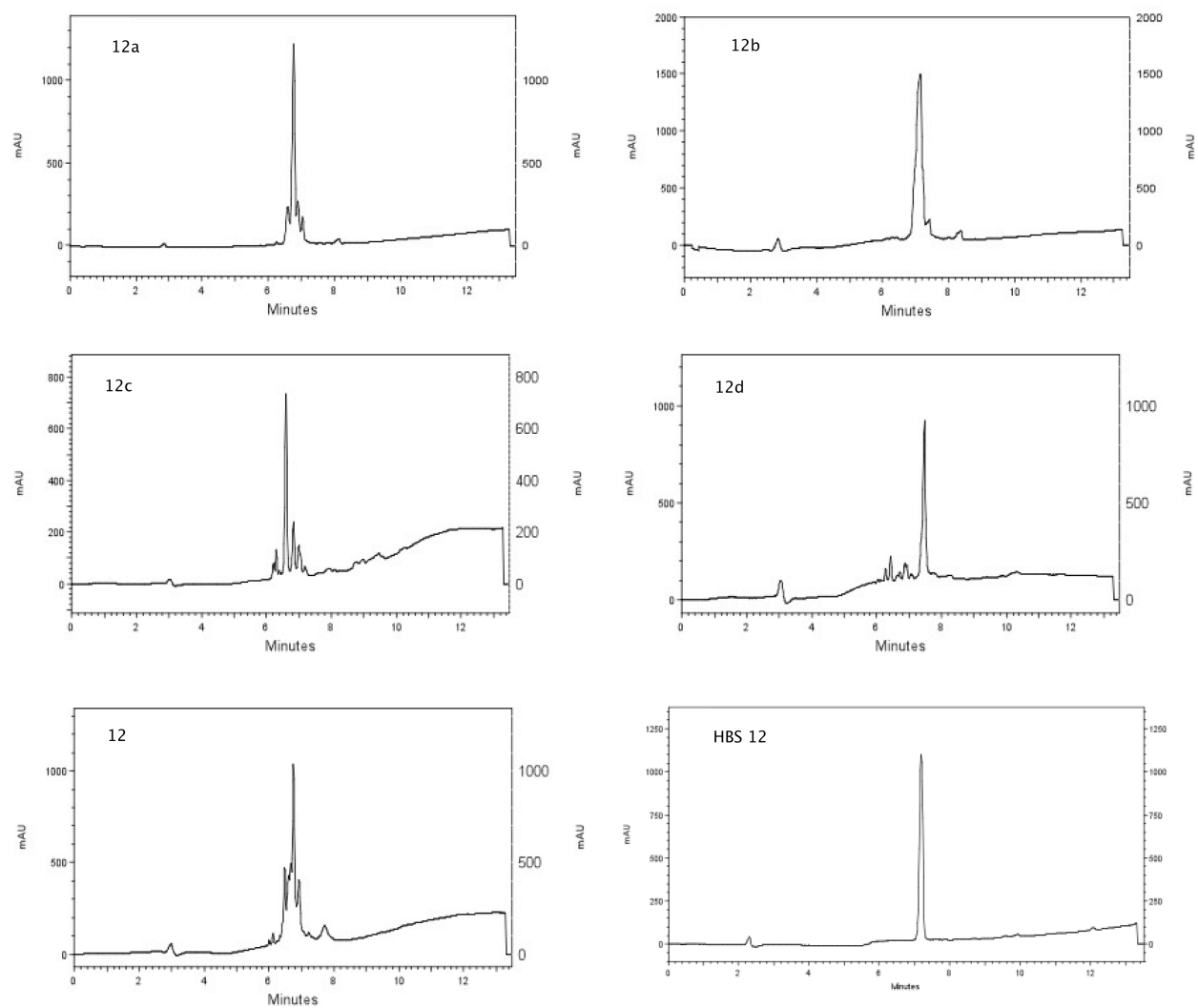


Figure S5. Analytical HPLC traces of crude peptides **12a**, **12b**, **12c**, **12d**, **12** and purified **HBS 12** (monitored at 220 nm).

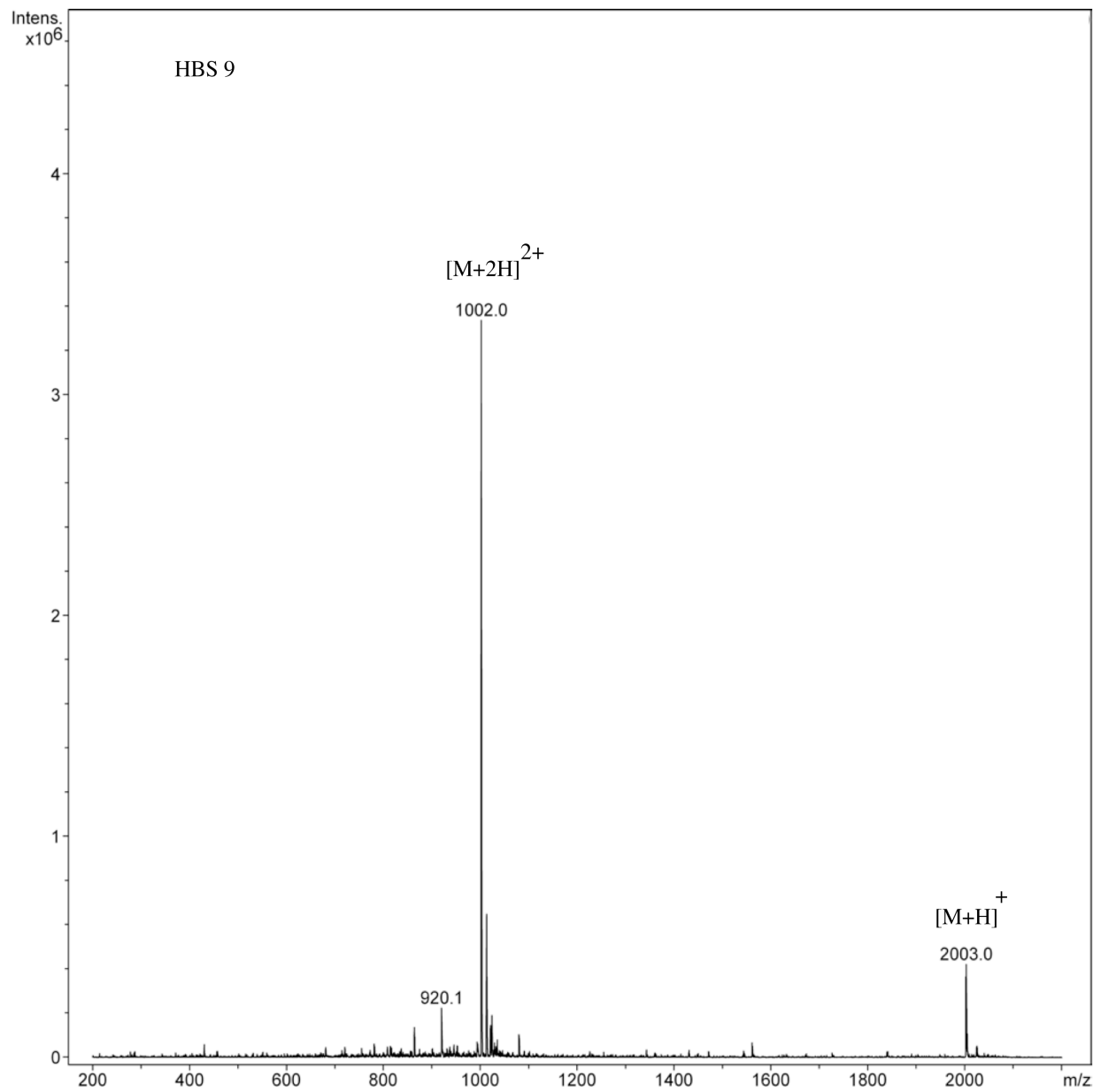


Figure S6. ESIMS spectrum of HBS 9.

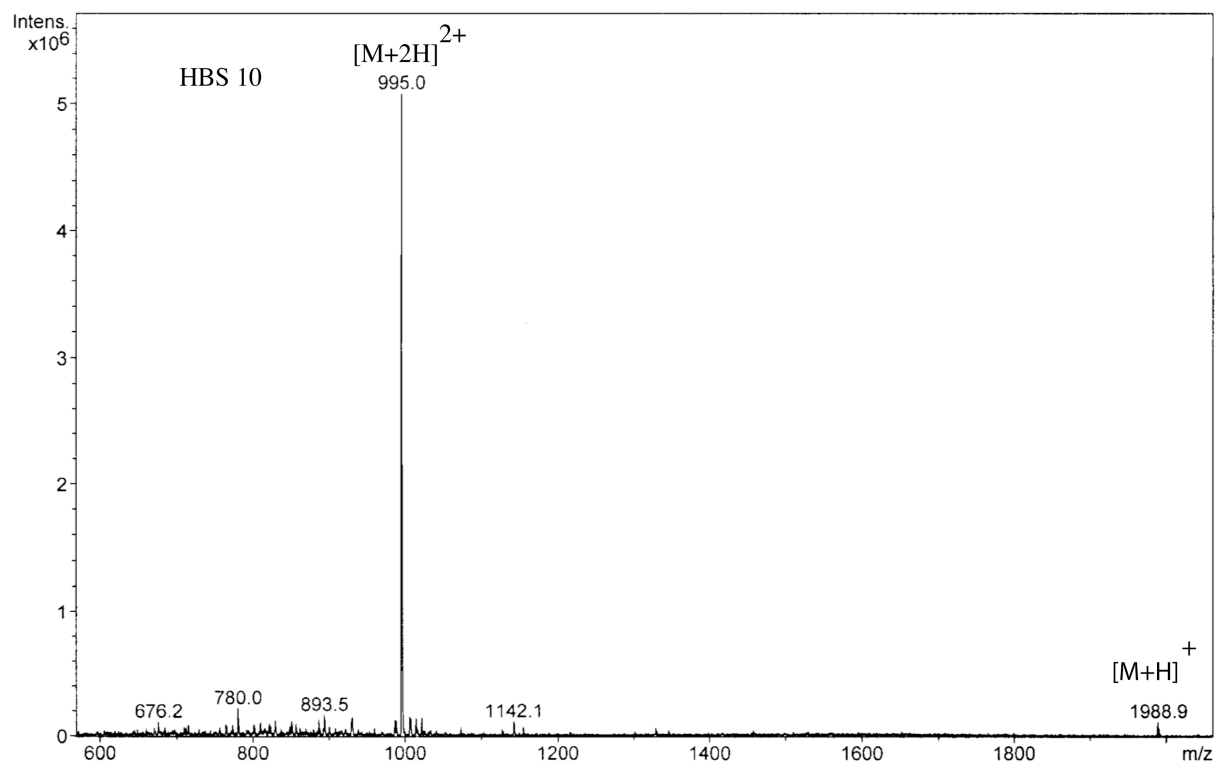


Figure S7. ESIMS spectrum of HBS 10.

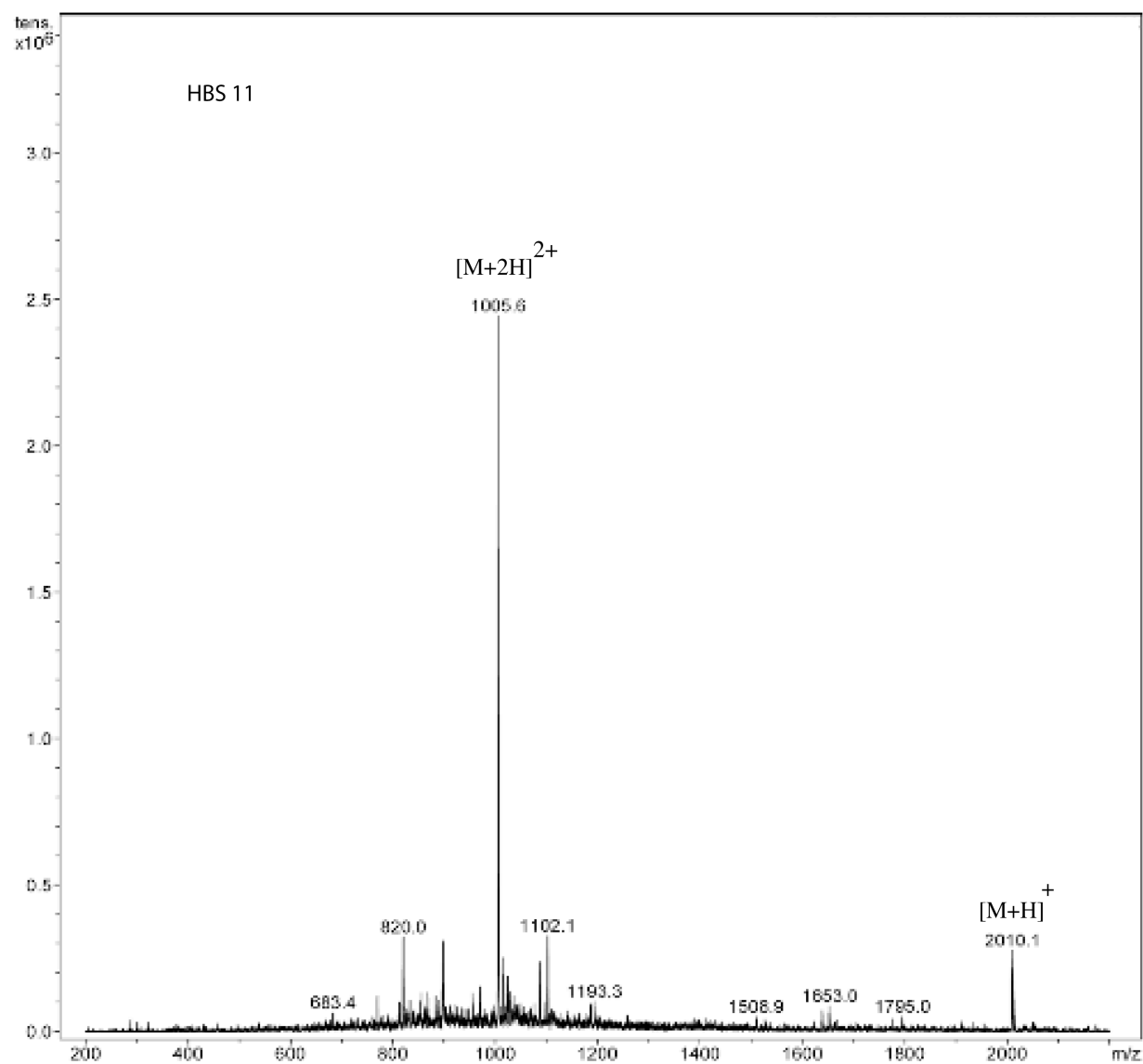


Figure S8. ESIMS spectrum of HBS 11.

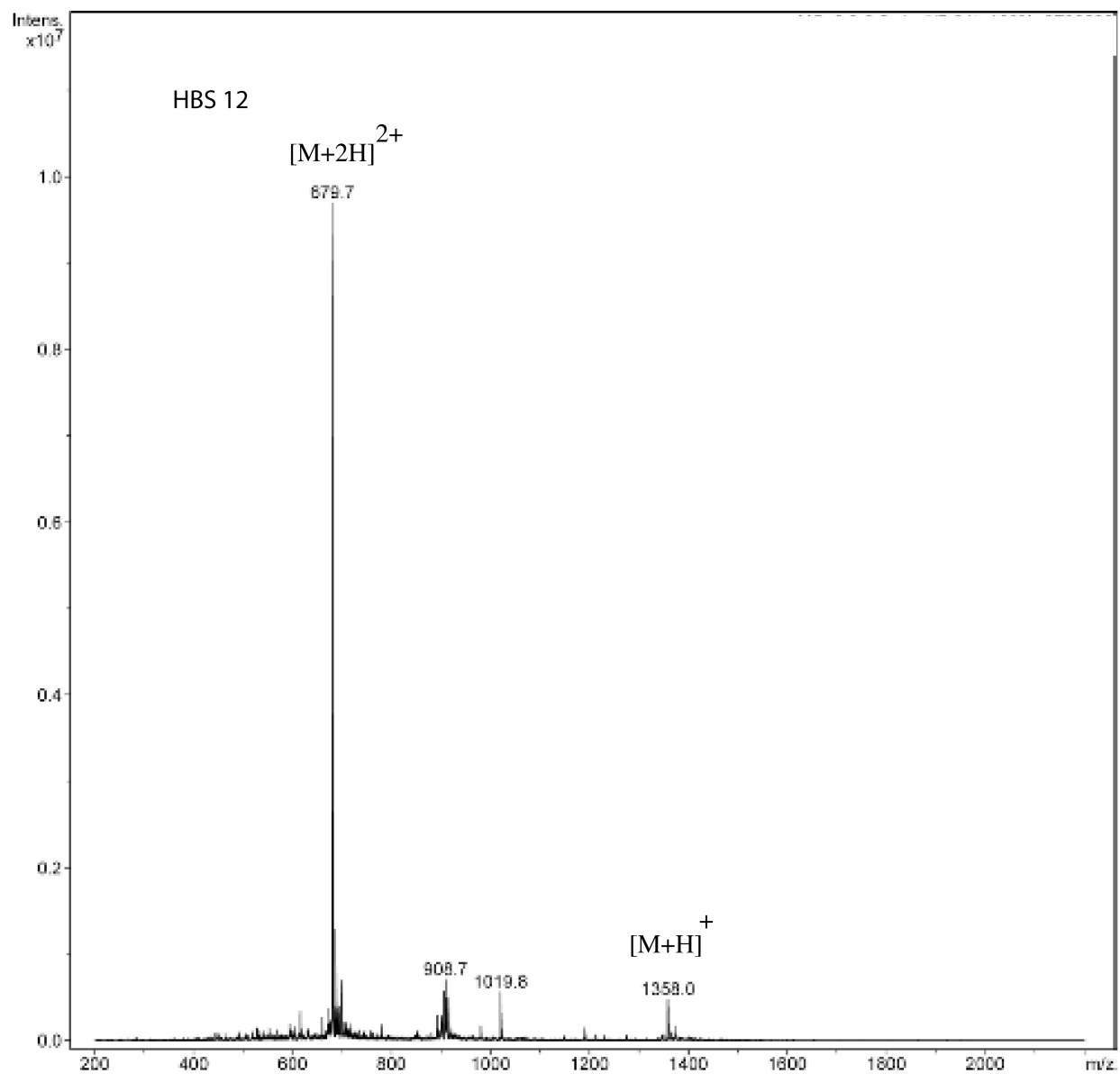


Figure S9. ESIMS spectrum of HBS 12.