P1Supporting Information for

Amyloid β-Sheet Mimics that Antagonize Amyloid Aggregation and Reduce Amyloid Toxicity

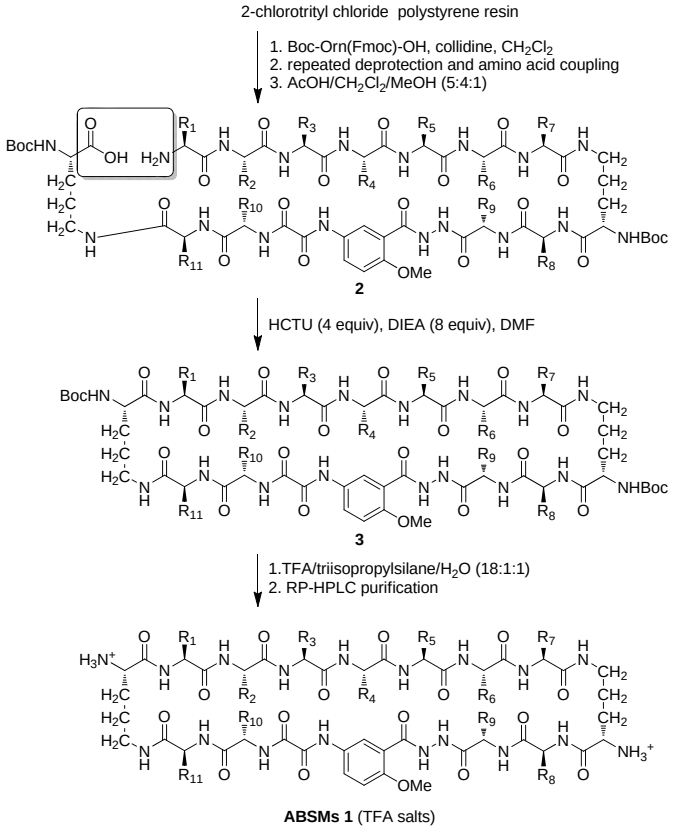
Pin-Nan Cheng[a,c], Cong Liu[b,c], Minglei Zhao[b], David Eisenberg[b*], James S. Nowick[a*]

[a] Department of Chemistry, University of California, Irvine, Irvine, California CA 92697-2025.
[b] UCLA-DOE Institute for Genomics and Proteomics, Howard Hughes Medical Institute, Molecular Biology Institute, University of California, Los Angeles, Los Angeles, California, CA 90095-1570.
[c] P.-N.C. and C.L. contributed equally to this work.

*To whom correspondence should be addressed: david@mib.ucla.edu and jsnowick@uci.edu.

Contents

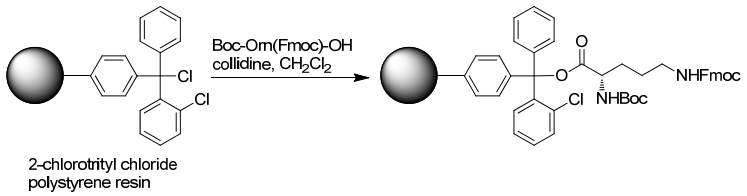
Synthesis of ABSMs 1.



ABSMs **1** was easily synthesized by standard Fmoc solid-phase peptide synthesis in which 2-chlorotrityl chloride resin was used as the solid support, followed by solution-phase cyclization, deprotection, and purification. Scheme 1 summarizes the synthesis of ABSMs **1**. Initially, Boc-Orn(Fmoc)-OH was attached onto 2-chlorotrityl chloride resin and the linear peptide was elongated by standard automated Fmoc solid-phase peptide synthesis, followed by cleavage from the resin under mildly acidic conditions. Then, the linear peptide was cyclized to the corresponding protected cyclic peptide by slow addition to HCTU and DIEA in dilute (ca. 0.5 mM) DMF solution. The cyclization condition used here efficiently avoids problematic epimerization, because the C-terminus of the protected linear peptide comprises an α-amino acid carbamate (Boc-NH-CHR-COOH). The final deprotection with TFA solution followed by RP-

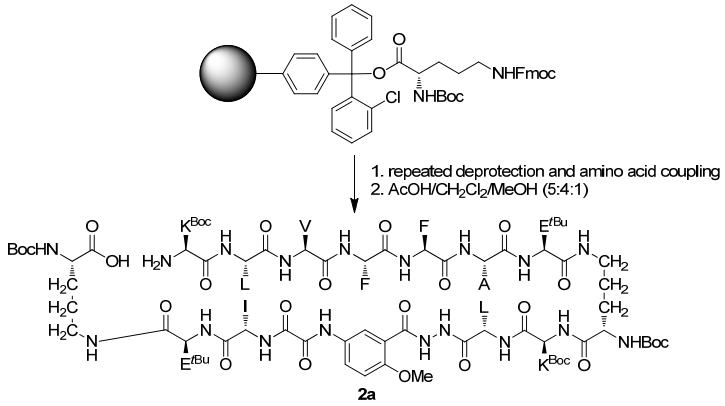
HPLC purification yielded ABSMs **1** in 20-30% overall yield, based on the loading of Boc-Orn(Fmoc)-OH attached onto the resin.

Representative Synthesis of ABSM 1a. Loading of Boc-Orn(Fmoc)-OH to 2-Chlorotrityl Chloride Polystyrene Resin for Solid-Phase Peptide Synthesis.



2-Chlorotrityl chloride resin (200 mg, 1.55 mmol/g) was added to a Bio-Rad Poly-Prep chromatography column (10 mL, 0.8 × 4.0 cm). The resin was washed with dry CH_2Cl_2 and suspended in 10 mL of dry CH_2Cl_2 to swell the resin. A solution of Boc-Orn(Fmoc)-OH (0.33 equiv, 46 mg, 0.1 mmol), 2,4,6-collidine (4.6 equiv, 0.16 mL, 175 mg), and 1.6 mL of dry CH_2Cl_2 was added directly to the resin, and the mixture was gently agitated for 4 h. The solution was then drained and the resin was washed with dry CH_2Cl_2 (5 × 7 mL). A mixture of CH_2Cl_2/MeOH/DIEA (17:2:1, 10 mL) was added to the resin, and the mixture was gently agitated for 1 h to cap the unreacted 2-chlorotrityl chloride sites. The capping step was repeated to achieve complete capping. The resin was then washed with dry CH_2Cl_2 followed by DMF. The resin was dried by passing nitrogen through the vessel. The resin loading was determined to be 0.41 mmol/g (80% based on Boc-Orn(Fmoc)-OH) by UV analysis of the Fmoc cleavage product.[1]

Representative Synthesis of Protected Linear Peptides 2a.

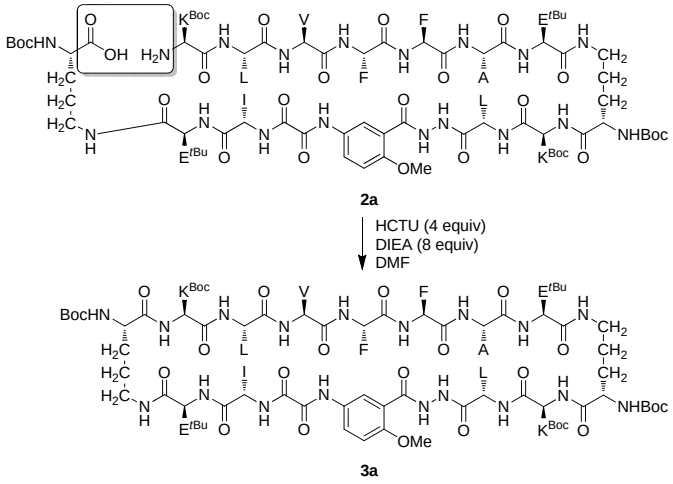


2a

The PS-2-chlorotrityl-Orn(Fmoc)-Boc generated from the previous step was washed with DMF (3 × 5 mL) and submitted to cycles of Fmoc solid-phase peptide synthesis on an automated peptide synthesizer using amino acid building blocks: Fmoc-Hao-OH, Fmoc-Orn(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(*t*Bu)-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, and Fmoc-Leu-OH. Automated solid-phase peptide synthesis was carried out on a PS3^{TM} Peptide Synthesizer (Protein Technologies, Inc.).[1] Fmoc-Hao-OH was used to introduce the Hao residue during solid-phase peptide synthesis. Fmoc-Hao-OH was prepared according to published procedures.[2] The protected linear peptide was elongated from the C-terminus to the N-terminus. Each coupling consisted of: (1) Fmoc-deprotection with 20% piperidine in DMF for 3 min; (2) washing with DMF (3 times); (3) coupling of the amino acid (0.5 mmol, 4 equiv) in the presence of HCTU; (4) washing with DMF (6 times). Each amino acid coupling step took 20 min for natural amino acids and 1 h for Hao. Because of the sluggish coupling of Fmoc-Hao-OH into the growing peptide, the Hao coupling reaction was carried out twice without Fmoc deprotection in between. After the last amino acid was coupled onto the growing peptide, the terminal Fmoc group was removed with 20% piperidine in DMF.

The resin with the protected linear peptide was transferred from the reaction vessel of the peptide synthesizer to a Bio-Rad Poly-Prep chromatography column and washed with DMF (3 × 5 mL) followed by CH_2Cl_2 (3 × 5 mL). A mixture of AcOH/CH_2Cl_2/MeOH (5:4:1, 20 mL) was added to the resin, and the suspension was agitated for 1 h. This cleavage solution was collected into a 250-mL round-bottomed flask and the resin was washed with CH_2Cl_2 (3 × 10 mL). The combined solutions were concentrated by rotary evaporation under reduced pressure. Hexanes (ca. 100 mL) were added to the flask and then removed by rotary evaporation to azeotropically remove residual AcOH. The resulting yellowish oil was dissolved CH_2Cl_2 (ca. 5 mL), diluted with hexanes (ca. 100 mL), and concentrated to dryness. The addition of CH_2Cl_2 and hexanes, followed by concentration, was repeated two additional times to remove the residual AcOH. The residue was dried under vacuum to give crude protected linear peptide **2a** as a white solid (130 mg, 68% crude yield based on resin loading).

Representative Synthesis of Protected Cyclic Peptides 3a.



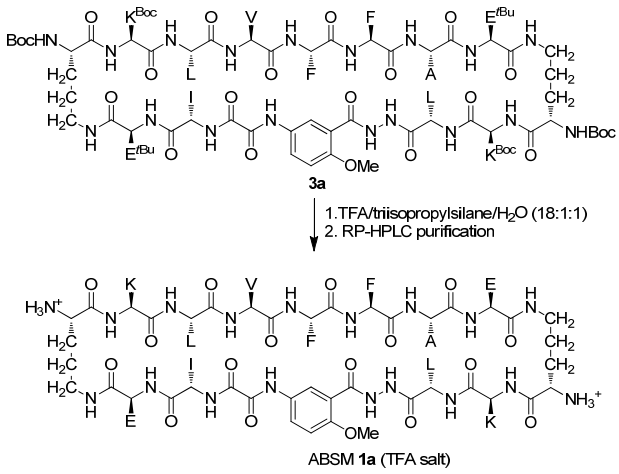
2a

3a

Crude protected linear peptide **2a** (130 mg, 0.05 mmol) was dissolved in DMF (50 mL). The peptide solution was added in drops via a 250-mL pressure-equalizing addition funnel to a 250-mL round-bottom flask containing a magnetic stirring bar, HCTU (82 mg, 0.20 mmol, 4

equiv), and DIEA (94 μL, 0.40 mmol, 8 equiv) in DMF (50 mL). (The final peptide concentration was 0.5 mM.) The reaction mixture was then stirred under nitrogen for 24 h. DMF was then removed by rotary evaporation under reduced pressure to give crude protected cyclic peptide **3a** as a yellowish waxy solid. The crude product was used in final deprotection step without further purification.

Representative Synthesis of ABSM 1a.



Protected cyclic peptide **3a** was dissolved in TFA/triisopropylsilane/H_2O (18:1:1, 20 mL) in a 50-mL round-bottom flask equipped with a nitrogen-inlet adaptor. The solution was magnetically stirred for 2 h. The reaction mixture was then concentrated by rotary evaporation under reduced pressure to give a yellowish oil. The resulting crude peptide **1a** was purified by reverse-phase HPLC (gradient elution with 10–50% CH_3CN over 60 min). The pure fractions were lyophilized to give 37 mg of peptide **1a** (22% yield, based on resin loading).

HPLC of ABSM 1a. Analytical reverse-phase HPLC was performed on an Agilent Zorbax SB-C18 column (50 mm × 4.6 mm) with a gradient of 5–100% CH_3CN in H_2O with 0.1% TFA and a flow of 1.0 mL/min over 20 minutes. Preparative reverse-phase HPLC purification was carried out on a 21.2 x 250 mm Zorbax SB-C18 PrepHT (7-μm particle size) column from Agilent on a

Rainin Dynamax system with a flow of 10.0 mL/min. UV detection (214 or 254 nm) was used for analytical and preparative HPLC. Water and acetonitrile were used as the solvents. Both solvents contained 0.1% biochemical grade TFA.

^{1}H NMR Spectroscopic Studies of ABSMs 1. ^{1}H NMR experiments of ABSMs **1** were performed in D_2O with the internal standard DSA[3] at 500 MHz at 298K. Solutions of ABSMs **1** were prepared gravimetrically by dissolving an appropriate weight of peptides in an appropriate volume of solvent. In calculating molecular weights, all amino groups were assumed to be protonated as the TFA salts. All 2D TOCSY and 2D ROESY spectra were collected with 2048 data points in the f_2 domain and 512 data points in the f_1 domain. Data were processed to a 1024 × 1024 real matrix with a Qsine weighting function and with forward linear prediction in the f_1 domain. The data were processed with the Brüker XwinNMR software.

Crystallization, Data Collection, and Structure Determination. Crystals of ABSM **1r** were grown from a 10 mg/mL aqueous stock solution using 35% (v/v) 2-methyl-2,4-pentanediol and pH 6.2 Na/K phosphate buffer at 18 °C using hanging drop vapor diffusion method. X-ray diffraction data were collected at the Advanced Photon Source (APS) and UCLA Crystallization Facility (Table S1). Reflections were integrated and scaled using XDS/XSCALE program packages.[4] Statistical analysis of the reflection intensities showed that the crystals are nearly perfectly twinned with an estimated twin fraction number close to 0.5.[5] Nevertheless, experimental phases were obtained with the apparent space group P4232 by SIRAS method using HKL2MAP.[6,7] The model was built using COOT and was refined using REFMAC in space group P23.[8,9] Coordinates and structure factor amplitudes have been deposited in the Protein Data Bank with accession code 3T4G.

Table S1. Statistics of Refinement

	ABSM **1r** (refinement)	ABSM **1r** (native)	ABSM **1r** (iodide)
Crystal parameters			
Space group	P23	$P4_232$	$P4_232$
Cell dimensions			
a, b, c (Å)	44.4, 44.4, 44.4	44.6, 44.6, 44.6	44.0, 44.0, 44.0
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Molecules in A.U.[a]	2	1	1
Data collection			
X-ray source	In-House[b]	APS (24-ID-C)[c]	APS (24-ID-C)[c]
Wavelength (Å)	1.542	0.9196	1.8443
Resolution (Å)	1.70	1.70	2.04
Reflections observed / unique	29228 / 3384	21581 / 3124	12251 / 1779
Completeness (%)	99.5 (95.9)[d]	99.1 (95.0)	99.6 (98.4)
R_{merge} (%)[e]	3.5 (51.3)	5.2 (31.4)	5.6 (51.6)
$\langle I/\sigma I \rangle$	29.7 (3.1)	19.9 (3.9)	21.4 (3.4)
Refinement			
Resolution (Å)	18.1 – 1.70		
R_{work} (%)[f]	23.6 (24.1)		
R_{free} (%)[g]	27.4 (37.7)		
No. of non-H atoms			
macrocycle	240		
ligand/ion	56		
Overall B-factors	15.7		
R.m.s. deviation			
Bond length (Å)	0.022		
Bond angle (°)	2.729		
PDB accession code	3T4G		

a. A.U. = Asymmetric Unit
b. Data was collected using an FRD rotating anode generator with R-AXIS HTC imaging plate detector.
c. Synchrotron beamline.
d. Values in parentheses correspond to the highest resolution shell.
e. $R_{merge} = \Sigma \mid I - \langle I \rangle \mid / \Sigma I$.
f. $R_{work} = \Sigma \mid F_o - F_c \mid / \Sigma F_o$.
g. $R_{free} = \Sigma \mid F_o - F_c \mid / \Sigma F_o$, calculated using a random set containing 5% reflections that were not included throughout structure refinement.

Expression of $A\beta_{42}$, $h\beta_2M$, and $h\alpha Syn_{1\text{-}100}$. Human wild-type $A\beta_{42}$ were expressed in *E.coli* and purified as described by Finder, Vodopivec, Nitsch, and Glockshuber.[10] The fusion construct was provided as a gift by Prof. Glockshuber and contains a His tag, linkers, soluble polypeptide segment ($NANP_{19}$), and TEV protease recognition site. Briefly, The fusion construct was

expressed into inclusion bodies in *E.coli* BL21(DE3). 8 M urea was used to solubilize the inclusion bodies. HisTrap™ HP Columns was used to purify the denatured Aβ fusion proteins, followed by further purification via reversed-phase high-performance liquid chromatography (RP-HPLC). The fusion proteins were lyophilized before TEV protease cleavage. The cleavage reaction was performed at 4 degree in reducing buffer (50 μM Tris-HCL, pH 8.0, 0.5 mM EDTA, and 1 mM DTT) for 30 h with the fusion protein concentration of 50 μM and TEV concentration of 3 μM. $A\beta_{42}$ was purified from the cleavage solution by RP-HPLC. The final product was verified by matrix-assisted laser desorption ionization spectrometry (MALDI-TOF MS).

Wild-type human β_2-microglobulin ($h\beta_2M$) was expressed as inclusion bodies in *E.coli*. The $h\beta_2M$ monomer was refolded as described previously.[11] Briefly, the $h\beta_2M$ inclusion body pellets were washed by 0.1% Triton X-100, 1 M NaCl sequentially and solubilized in 8 M urea with 50 mM Tris-HCl, pH 8.0. Then gradient dialysis was performed for $h\beta_2M$ refolding. The refolded $h\beta_2M$ protein was loaded on Superdex™ 75 column for size exclusion purification. Monomeric $h\beta_2M$ was pooled for $h\beta_2M$ fibrillation inhibition studies.

Truncated human α-synuclein (residues 1-100) was expressed in *E. coli* strain BL21-Gold (DE3) (Agilent Technologies, Santa Clara, CA) at 37 °C through induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 h. The cells were resuspended in a lysis buffer containing 100 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM ethylenediaminetetraacetic acid (EDTA), and phenylmethylsulfonyl fluoride (PMSF) was also added to make 1 mM concentration. The cell lysate was centrifuged and the supernatant was incubated in 70 °C for 10 minutes. After centrifugation, the supernatant was dialyzed overnight in a buffer containing 10 mM HEPES, pH 7.4 and then loaded to a 5 mL HiTrap SP column (GE Healthcare, Piscataway,

NJ) for cation exchange. The column was washed with 10 mM HEPES (pH 7.4) and eluted with a linear gradient of NaCl. The peak fractions were collected, and then concentrated using an Amicon Ultra-15 concentrator (3 kDa MW cutoff; Millipore, Billerica, MA). The concentrated elution was subsequently applied to a Superdex S-75 column (GE Healthcare, Piscataway, NJ). The SEC running buffer contained 20 mM Tris-HCl (pH 8.0). The peak fractions were pooled and concentrated to about 3.5 mg/mL.

ThT Fluorescence Assays.

Effect of ABSM 1a on Inhibition of $A\beta_{40}$ Aggregation. ThT fluorescence assays were conducted in 96-well plates (black with flat optic bottom) with shaking in a Gemini XPS fluorescence plate reader (Molecular Devices, 442 nm excitation, 482 nm emission) at 37 °C. Experiments were run in quadruplicate or greater with 20 μM $A\beta_{40}$, 10 mM PBS buffer (pH 7.4), and 20 μM ThT.

Preparation of Buffered ThT Solution (Stock Solution A): A ThT solution was freshly prepared before use. Approximately 4 mg of thioflavin T was dissolved in ca. 20 mL of Nanopure water in a 25 mL Erlenmeyer flask. 10 mL of this solution was filtered through a 0.22 micron filter into a 15-mL plastic centrifuge tube. The concentration of a 1/25 dilution of this solution was determined by UV (ε = 36000 M^{-1} cm^{-1} at 412 nm). An aliquot of this stock solution was combined with H_2O and 5x PBS buffer containing 0.02% NaN_3 (the amount of ThT stock and H_2O are based on the determined concentration of the stock) to obtain 100 μM ThT solution.

Preparation of Peptide Inhibitor Solution (Stock Solution B): A stock solution of ABSM **1a** was freshly prepared before use by adding Nanopure water to each tube to obtain 200 μM peptide solutions.

Preparation of $A\beta_{40}$ solution (Stock Solution C): $A\beta_{40}$ (GL Biochem, Shanghai, China) was dissolved in in hexafluoroisopropanol (HFIP) to disrupt preformed aggregates and the HFIP was then evaporated in the hood for 12 h and dried under vacuum for 12 h. The $A\beta_{40}$ solution was freshly prepared by dissolving $A\beta_{40}$ in 100 mM NaOH in a 1.5 mL Epindorf tube. The mixture was sonicated for 30 seconds. The solution was diluted to 100 μM by addition of Nanopure water. The solution was filtered through 100 kDa Centricon filters that were previously washed 3 times with Nanopure water (10000 rpm for 3 minutes). The $A\beta_{40}$ solution was diluted with Nanopure water to 40 μM.

Preparation of the Wells of the 96-Well Plate. Each well contained 200 μL reaction solution containing 20 μM $A\beta_{40}$, 10 mM PBS buffer (pH 7.4), 20 μM ThT, and various amounts of ABSM **1a**. The preparation of each well is summarized in Table S2. The stock solution C was added last to all of the wells, and the plate was immediately inserted into the plate reader. The wells were prepared in quadruplicate or greater. (In the example shown in Figure S1, well were prepared in septaplicate.) Representative data were used for further analysis. (In the example shown in Figure S1, the five most representative traces are shown and two least representative traces are omitted.) The ThT assay was conducted for 41 h at 37 ºC. Readings were collected every 10 minutes with 5 seconds shaking before first reading and 575 seconds shaking between readings.

Table S2. Preparation of the Wells of the 96-Well Plate.

Wells	Water (μL)	Stock Solution A (μL)	Stock Solution B (μL)	Stock Solution C (μL)
ThT control	160	40	0	0
$A\beta_{40}$ control	60	40	0	100
0.2 equiv of ABSM **1a**	56	40	4	100
0.5 equiv of ABSM **1a**	50	40	10	100
1.0 equiv of ABSM **1a**	40	40	20	100

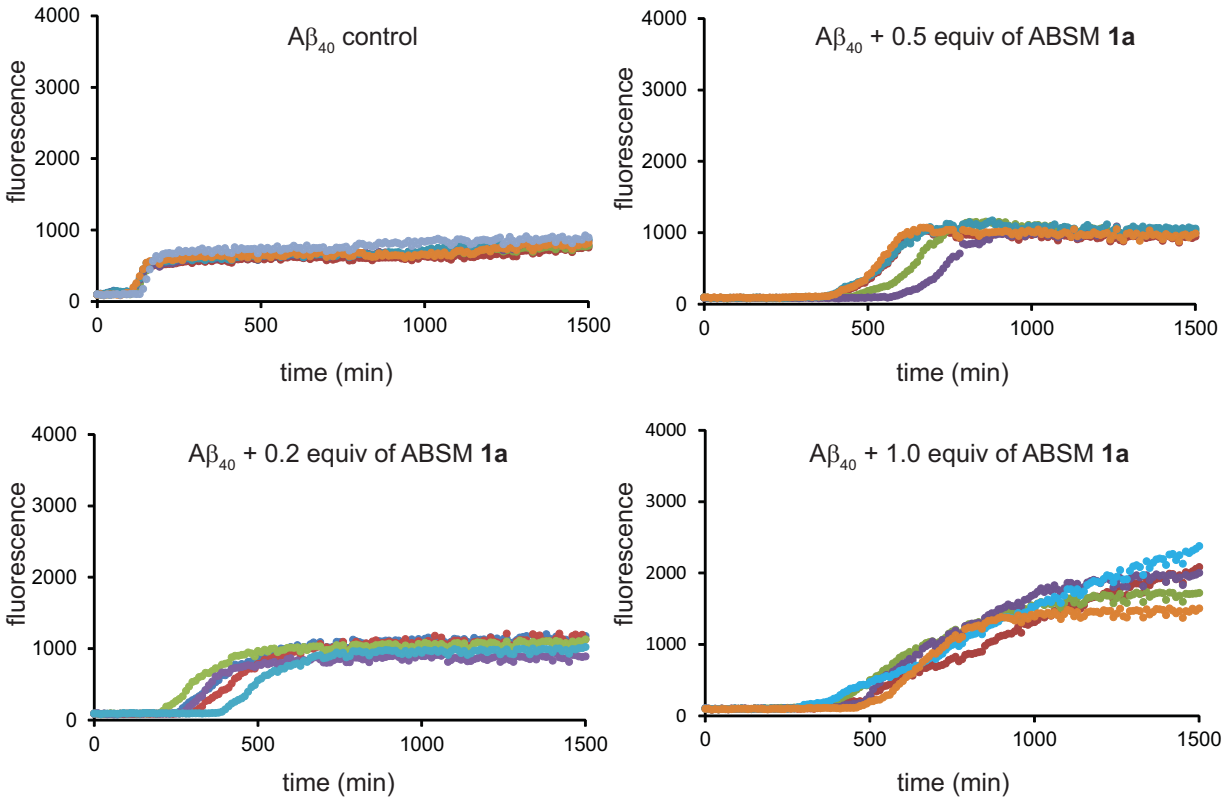


Figure S1. Effect of ABSM **1a** on inhibition of $A\beta_{40}$ aggregation monitored by ThT fluorescence assay. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

Effect of ABSM 1a on Inhibition of $A\beta_{42}$ Aggregation. ThT fluorescence assays were conducted in 96-well plates (black with flat optic bottom) without shaking in a Varioskan fluorescence plate reader (Thermo Scientific, 444 nm excitation, 484 nm emission) at 37 °C. Experiments were run in quadruplicate or greater with 20 μM $A\beta_{42}$, 10 mM PBS buffer (pH 7.4), and 10 μM ThT.

Preparation of Reaction Solutions and the Wells of the 96-Well Plate. The ThT assay of $A\beta_{42}$ aggregation was performed by preparing reaction solutions containing 20 μM $A\beta_{42}$, 10 μM ThT, and 1, 2, 4, 10, or 20 μM ABSM **1a** and then placing 150 μL portions of these solutions into wells in a 96-well plate. For pre-treatment to disaggregate pre-formed $A\beta_{42}$ aggregates: Prior

to the assay, lyophilized $A\beta_{42}$ was dissovled in HFIP and evaporated in vaccum, $A\beta_{42}$ was freshly dissoved in 10mM NaOH, followed by sonication. $A\beta_{42}$ was further diluted into PBS at the final concentration of 200uM as a stock. The reaction solution were prepared by combining a 2.0 mM ABSM **1a** solution (stock solution A) with a solution of 20 μM $A\beta_{42}$ and 10 μM ThT in PBS (pH 7.4) buffer (stock solution B). Stock solution B was prepared by diluting 1 mM ThT solution and 200 μM $A\beta_{42}$ solution in 10 mM PBS buffer (pH 7.4). Stock solution B was also used as $A\beta_{42}$ control. All solutions used for the assay were spin filtered through a 0.22 micron filter prior to the assay.

Table S3. Preparation of Reaction Solutions with Various Concentrations of ABSM **1a**.

Reaction Solution (900 μL)	Stock Solution A (μL)	Stock Solution B (μL)
0.05 equiv of ABSM **1a**	0.45	899.5
0.1 equiv of ABSM **1a**	0.9	899.1
0.2 equiv of ABSM **1a**	1.8	898.2
0.5 equiv of ABSM **1a**	4.5	895.5
1.0 equiv of ABSM **1a**	9.0	891

Table S3 summarizes the preparation of the reaction solutions. 150 μL portions of each reaction solution were pipetted into the wells of a 96-well plate. 150 μL portions of 10 μM ThT and 20 μM $A\beta_{42}$ were pipetted into additional wells as controls. The wells were prepared in quadruplicate or greater. Representative data were used for further analysis. The ThT assay was conducted for 24 h at 37 ºC and readings were collected every 10 minutes.

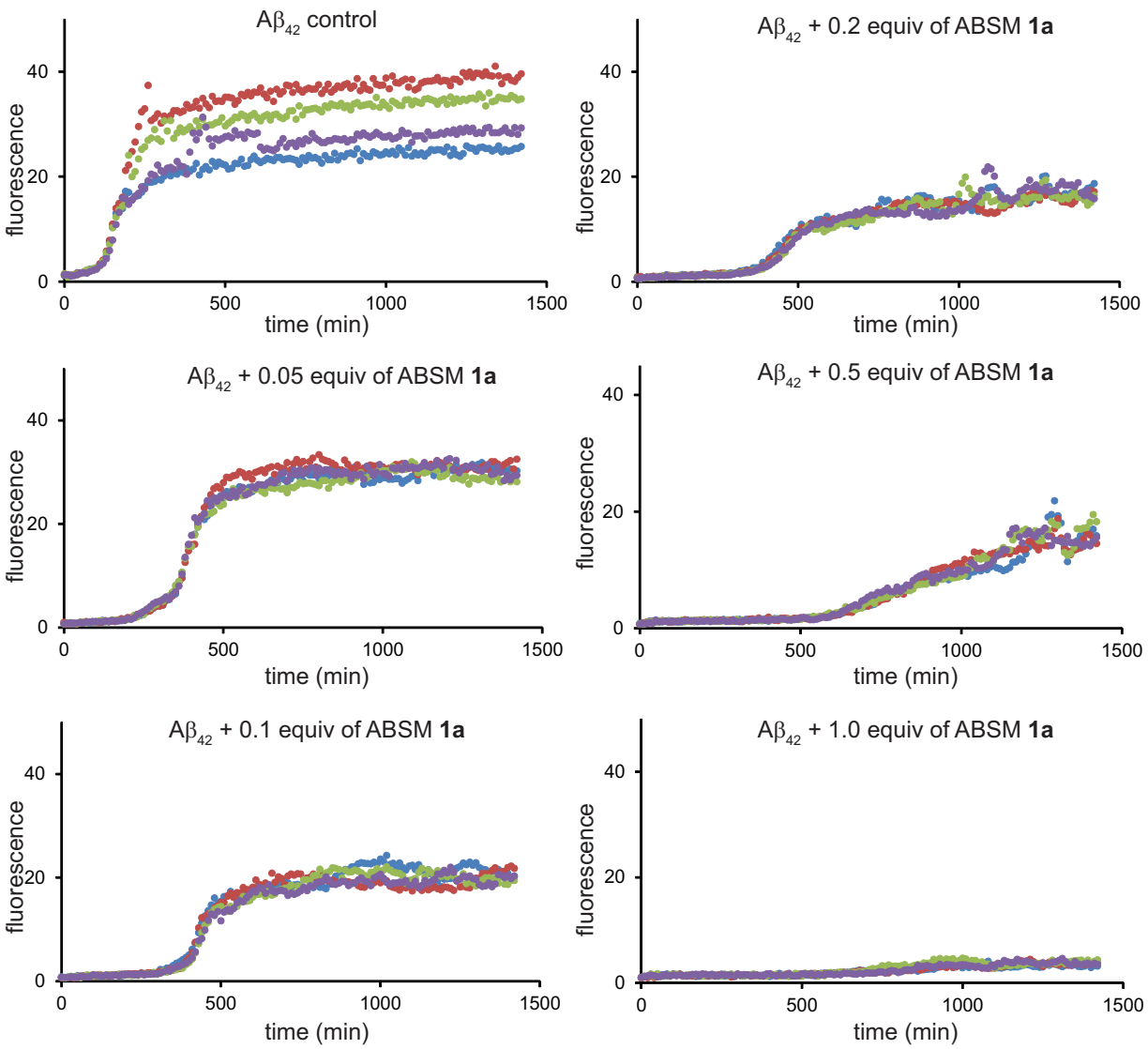


Figure S2. Effect of ABSM **1a** on inhibition of $A\beta_{42}$ aggregation monitored by ThT fluorescence assay. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

Effect of ABSM 1m on Inhibition of hβ_2M Aggregation. ThT fluorescence assays were conducted in 96-well plates (black with flat optic bottom) with continuous shaking at 900 rpm in a Varioskan fluorescence plate reader (Thermo Scientific, 444 nm excitation, 484 nm emission) at 37 °C. Experiments were run in quadruplicate or greater with 30 μM hβ_2M, 50 mM phosphate buffer (pH 2), and 10 μM ThT.

Preparation of Reaction Solutions and the Wells of the 96-Well Plate. The ThT assay of hβ_2M aggregation was performed by preparing reaction solution containing 30 μM hβ_2M, 10 μM ThT, and 6, 15, or 30 μM ABSM **1m** and then placing 150 μL portions of these solutions into wells in a 96-well plate. The reaction solution were prepared by combining a 2.0 mM ABSM **1m** solution (stock solution A) with a solution of 30 μM hβ_2M and 10 μM ThT in 50 mM phosphate (pH 2) buffer (stock solution B). Stock solution B was prepared by diluting 1 mM ThT solution and 5 mM hβ_2M solution in 50 mM phosphate buffer (pH 2). Stock solution B was also used as hβ_2M control. All solutions used for the assay were spin filtered through a 0.22 micron filter prior to the assay.

Table S4. Preparation of Reaction Solutions with Various Concentrations of ABSM **1m**.

Reaction Solution (1000 μL)	Stock Solution A (μL)	Stock Solution B (μL)
0.2 equiv of ABSM **1m**	3	997
0.5 equiv of ABSM **1m**	7.5	992.5
1.0 equiv of ABSM **1m**	15	985

Table S4 summarizes the preparation of the reaction solutions. 150 μL portions of each reaction solution were pipetted into the wells of a 96-well plate. 150 μL portions of 10 μM ThT and 30 μM hβ_2M were pipetted into additional wells as controls. The wells were prepared in quadruplicate or greater. Representative data were used for further analysis. The ThT assay was conducted for 24 h at 37 ºC and readings were collected every 10 minutes.

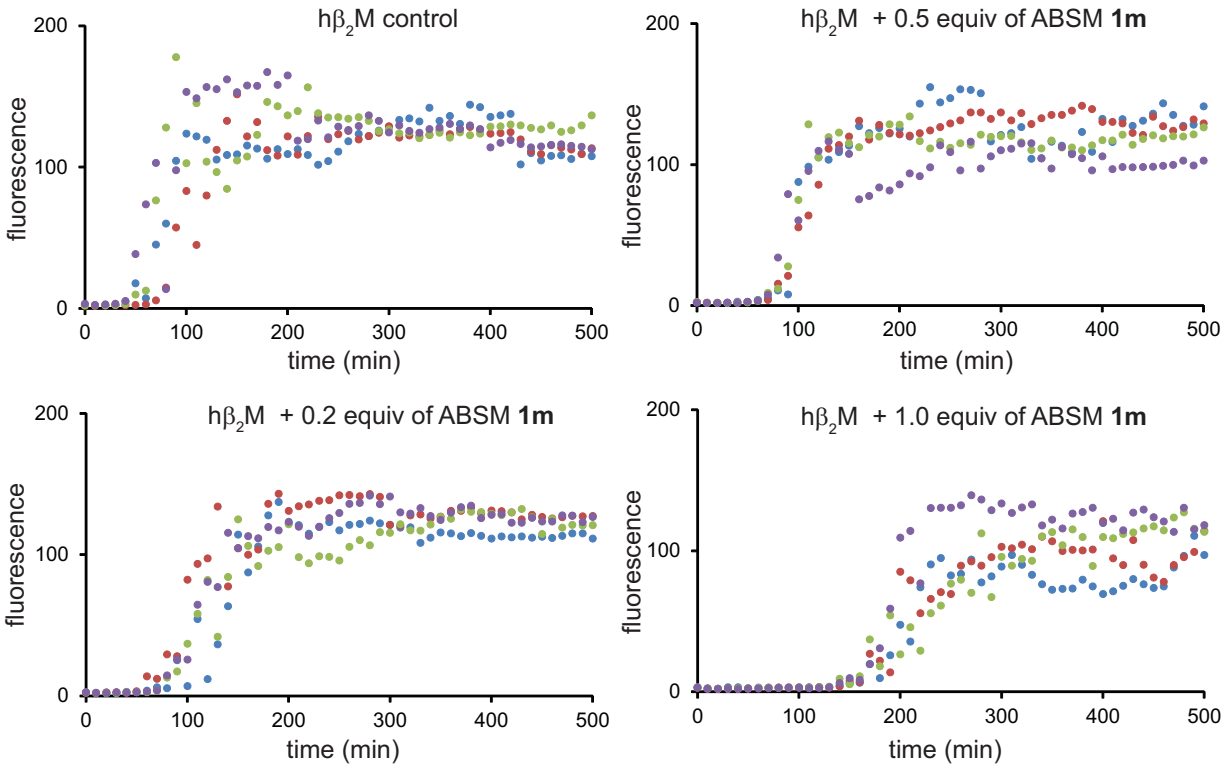


Figure S3. Effect of ABSM **1m** on inhibition of hβ$_2$M aggregation monitored by ThT fluorescence assay. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

Effect of ABSM 1o on Inhibition of hαSyn$_{1\text{-}100}$ Aggregation. ThT fluorescence assays were conducted in 96-well plates (black with flat optic bottom) with continuous shaking at 900 rpm in a Varioskan fluorescence plate reader (Thermo Scientific, 444 nm excitation, 484 nm emission) at 37 °C. Experiments were run in quadruplicate or greater with 50 μM hαSyn$_{1\text{-}100}$, 20 mM phosphate buffer (pH 2) with 100 mM NaCl, and 10 μM ThT.

Preparation of Reaction Solutions and the Wells of the 96-Well Plate. The ThT assay of hαSyn$_{1\text{-}100}$ aggregation was performed by preparing reaction solution containing 50 μM hαSyn$_{1\text{-}100}$, 10 μM ThT, and 10, 25, or 50 μM ABSM **1o** and then placing 150 μL portions of these solutions into wells in a 96-well plate. The reaction solutions were prepared by combining a 2.0 mM ABSM **1o** solution (stock solution A) with a 1 mM ThT (stock solution B), a 340 μM hαSyn$_{1\text{-}100}$ solution (stock solution C) and the phosphate buffer. hαSyn$_{1\text{-}100}$ control was prepared

by combining a 340 μM hαSyn$_{1-100}$ solution (stock solution C) with a 1mM ThT (stock solution B) and the phosphate buffer. All solutions used for the assay were spin filtered through a 0.22 micron filter prior to the assay.

Table S5. Preparation of Reaction Solutions with Various Concentrations of ABSM **1o**.

Reaction Solution (1200 μL)	Stock Solution A (μL)	Stock Solution B (μL)	Stock Solution C (μL)	Phosphate Buffer
ThT control	0	12	0	1188
hαSyn$_{1-100}$ control	0	12	177	1011
0.2 equiv of ABSM **1o**	6	12	177	1005
0.5 equiv of ABSM **1o**	15	12	177	996
1.0 equiv of ABSM **1o**	30	12	177	981

Table S5 summarizes the preparation of the reaction solutions and hαSyn$_{1-100}$ control. 150 μL portions of each reaction solution were pipetted into the wells of a 96-well plate. 150 μL portions of 10 μM ThT and 50 μM hαSyn$_{1-100}$ were pipetted into additional wells as controls. The wells were prepared in quadruplicate or greater. Representative data were used for further analysis. The ThT assay was conducted for 51 h at 37 ºC and readings were collected every 10 minutes.

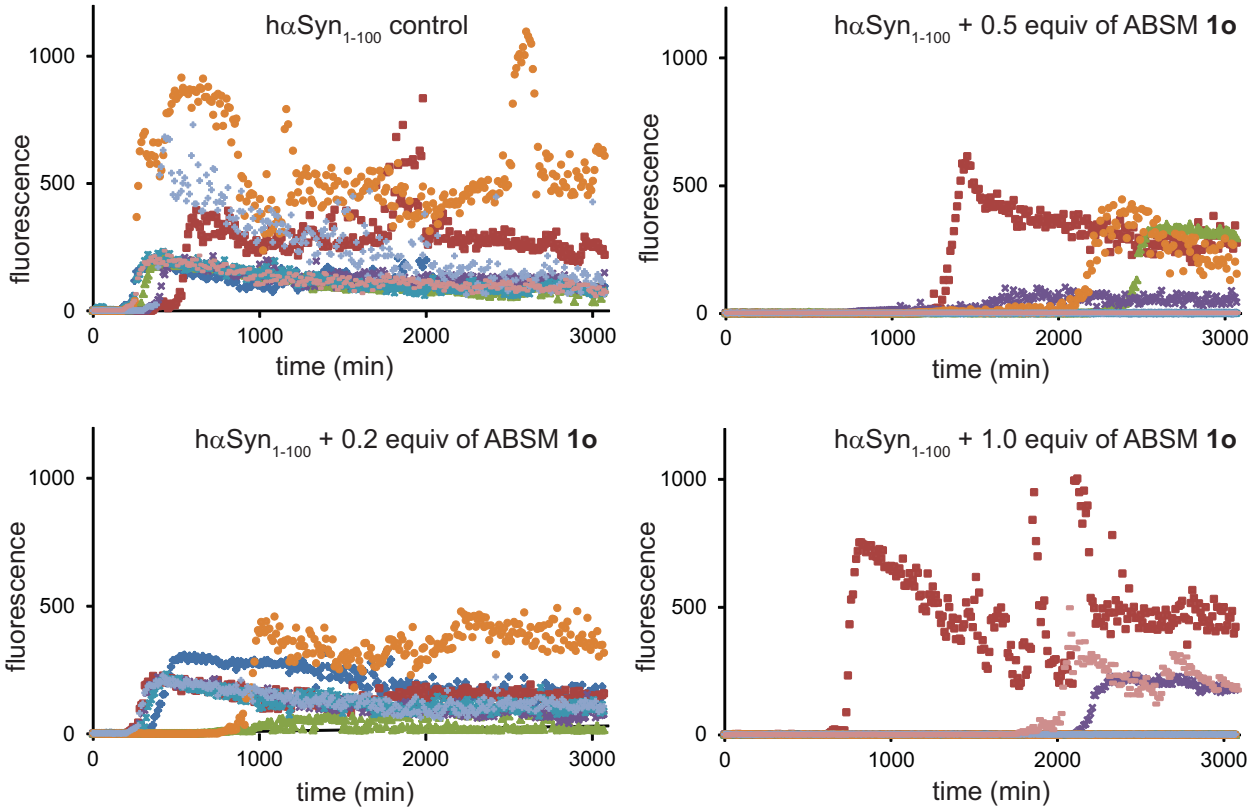


Figure S4. Effect of ABSM **1o** on inhibition of hαSyn$_{1\text{-}100}$ aggregation monitored by ThT fluorescence assay. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

Cross-Interaction between Aβ and ABSMs 1m and 1o. Aβ has been shown to cross-interact with different amyloidogenic proteins containing similar primary sequences. To investigate the cross-interaction of Aβ with ABSMs, we compared interaction of Aβ with ABSM **1a** to that with ABSM **1m** and to ABSM **1o**. ABSMs **1m** and **1a** have closely homologous sequences in which residues at positions 2, 7, and 8 are identical and residues at positions 3, 4, 5, 9, and 10 are similar, while ABSM **1o** and **1a** do not (Figure S5a). These similarities and differences raised the intriguing possibility different cross-interactions of ABSMs **1m** and **1o** with Aβ.

ThT fluorescence assays show that ABSM **1m**, like ABSM **1a**, inhibits Aβ aggregation, while ABSM **1o** has little or no inhibitory effect (Figure S5b). Although ABSM **1a** and **1m** delay Aβ aggregation in a dose-dependent manner (Figures S1 and S5c), ABSM **1a** is more effective at lower doses. ABSM **1a** delays $A\beta_{40}$ aggregation by 280% and 430% at 0.2 and 0.5 equivalents respectively (Figures S1), while ABSM **1m** shows little or no inhibition at 0.2 equivalents and delays $A\beta_{40}$ aggregation by 240% at 0.5 equivalents. ABSM **1a** delays $A\beta_{40}$ aggregation by 430% at 1 equivalent, while ABSM **1m** delays by 900%, which indicates that ABSM **1m** is more effective at higher doses. ThT fluorescence assays also show that ABSM **1m** effectively delays $A\beta_{42}$ aggregation, while ABSM **1o** causes little or no inhibition (Figure S5d). These results suggest that structurally homologous ABSMs can not only interact with their parent amyloid proteins but can also cross interact with different amyloid proteins.

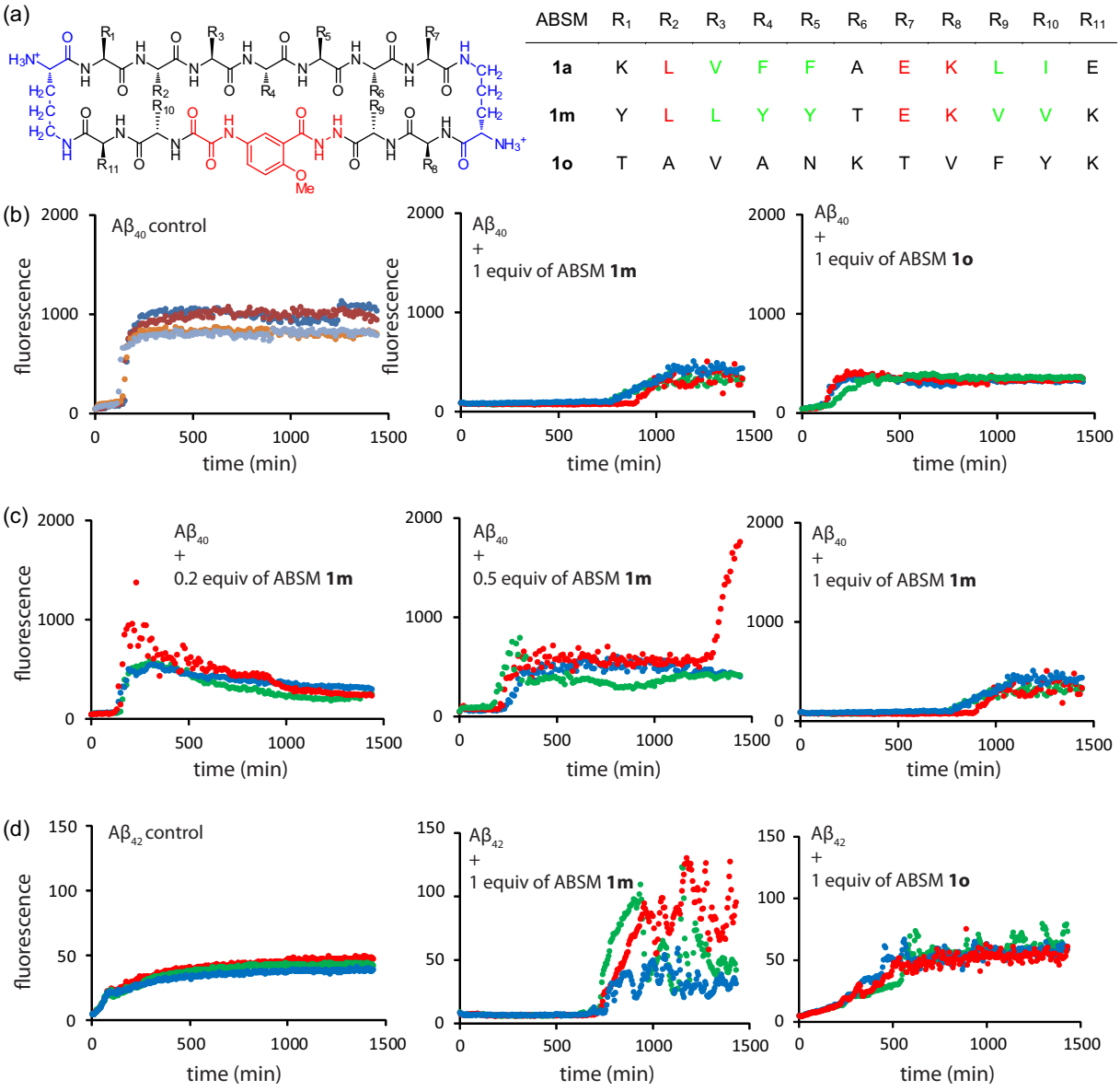


Figure S5. (a) Sequence comparison among ABSMs **1a**, **1m**, and **1o**. Identical residues among ABSM **1a** and **1m** are highlighted in red and similar residues are highlighted in green. (b) Effect of ABSMs **1m** and **1o** on inhibition of $A\beta_{40}$ aggregation monitored by ThT fluorescence assay. (c) Dose-dependent inhibition of ABSM **1m** on Aβ aggregation. (d) Effect of ABSMs **1m** and **1o** on inhibition of $A\beta_{42}$ aggregation monitored by ThT fluorescence assay. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

Interaction between Aβ and ABSMs 1b, 1c, 1d, and 1f. To investigate the effect of heptapeptide sequences within ABSMs **1** derived from $A\beta_{16–23}$ and $A\beta_{29–40}$ on interaction with Aβ, we compared the interaction of $A\beta_{40}$ with **ABSMs 1b**, **1c**, **1d**, and **1f** to that with ABSM **1a**. ThT fluorescence assays show that ABSM **1b**, like ABSM **1a**, is effective against $A\beta_{40}$ aggregation, while ABSMs **1c**, **1d**, and **1f** has little or no inhibitory effect (Figure S6). ABSM **1b** delays $A\beta_{40}$ aggregation by 290% at 1 equivalent, while ABSM **1a** delays by 430%. While ABSMs containing heptapeptide sequences from the *N*-terminal $A\beta_{16–23}$ inhibit $A\beta_{40}$ aggregation, ABSMs from the *C*-terminal $A\beta_{29–40}$ do not. ABSMs **1d** and **1f**, instead, accelerate $A\beta_{40}$ aggregation, reducing the duration of the lag phase. This acceleration by ABSM **1d** is reminiscent of the seeding effect in $A\beta_{40}$ aggregation.

Heptaeptide Sequence from Aβ		
1a	$A\beta_{16-22}$	KLVFFAE
1b	$A\beta_{17-23}$	LVFFAED
1c	$A\beta_{29-35}$	GAIIGLM
1d	$A\beta_{30-36}$	AIIGLMV
1f	$A\beta_{34-40}$	LMVGGVV

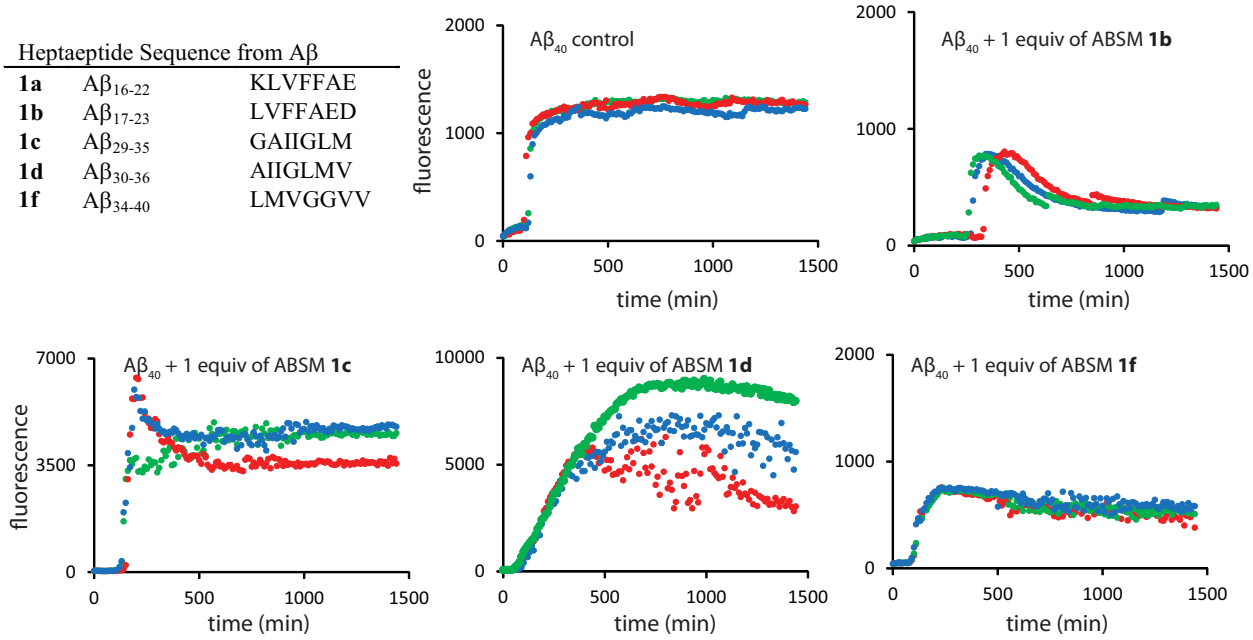
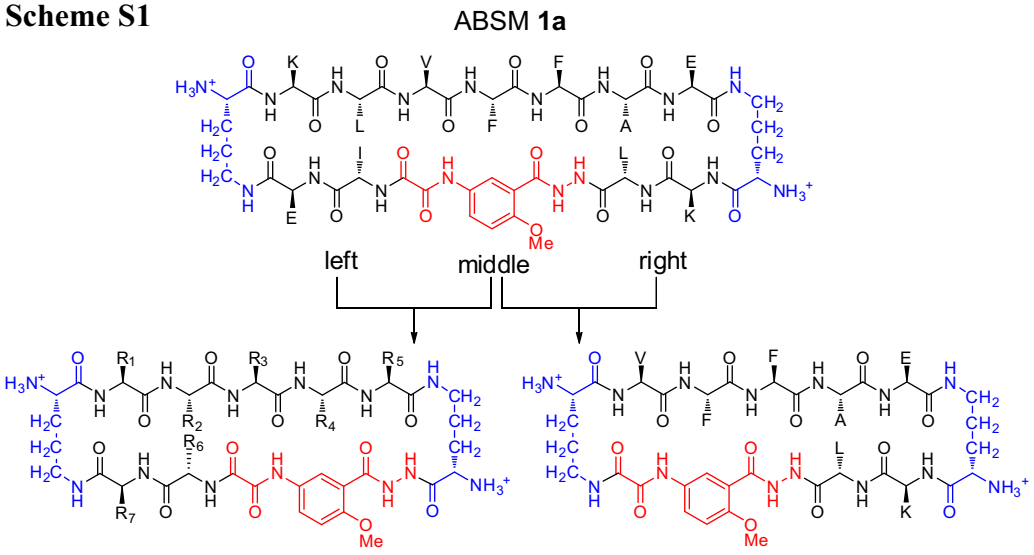


Figure S6. Interaction between $A\beta_{40}$ and ABSMs **1b**, **1c**, **1d**, and **1f** monitored by ThT fluorescence assay. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

The inhibition of $A\beta_{40}$ aggregation by both ABSMs **1a** and **1b** indicates that the central hydrophobic sequence $A\beta_{17–21}$ is critical to the activity of ABSMs against $A\beta_{40}$ aggregation. This result supports the role of $A\beta_{17-21}$ in Aβ aggregation and suggests that strong interaction of this sequence in these ABSMs with that of the Aβ oligomers delays the lag phase of Aβ aggregation.

Comparison of the Effect of 42- and 54-Membered Ring Macrocyclic β-Sheets on Aβ Aggregation. We previously introduced 42-membered ring macrocyclic β-sheets **4** that contain a pentapeptide in the upper strand. Macrocyclic β-sheets **4** encompass the left and middle portions of ABSMs **1** (Scheme S1). To investigate the effect of the size of macrocyclic β-sheets on amyloid aggregation, we divided the heptapeptide $A\beta_{16-22}$ β-strand of ABSM **1a** into three overlapping pentapeptide sequences ($A\beta_{16-20}$, $A\beta_{17-21}$, and $A\beta_{18-22}$). We incorporated these sequences into the smaller macrocycles **4a-c** and compared their effect on Aβ aggregation with that of ABSM **1a**. We anticipated that ABSM **1a** should be more effective against Aβ aggregation because it displays a longer β-strand and provides bigger hydrophobic surface to interact with Aβ.

Scheme S1



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
4a	K	L	V	F	F	I	E
4b	L	V	F	F	A	L	K
4c	V	F	F	A	E	I	E

ThT fluorescence assays show that **4b** inhibits $A\beta_{40}$ aggregation to a much lesser extent than ABSM **1a**, while **4a** and **4c** have little or no inhibitory effect (Figure S7). To further explore

the greater activity of ABSM **1a**, we designed and synthesized the new 42-membered ring macrocyclic β-sheet **5**. Macrocyclic β-sheet **5** encompasses the middle and right portions of ABSM **1a** (Scheme S1) and contains a pentapeptide in the upper strand. We incorporated amyloidogenic pentapeptide sequence $A\beta_{18\text{-}22}$ into **5**. ThT fluorescence assays show that **5** inhibits $A\beta_{40}$ aggregation by 250% at 1 equivalent, while **4a**, which encompasses the left and middle portions of ABSM **1a**, has little or no inhibitory effect. This result indicates that the right portion of ABSM **1a** is crucial to its greater activity against Aβ aggregation. A direct comparison of the effects of **1a**, **4a**, and **5** on $A\beta_{40}$ aggregation shows that ABSM **1a** is more effective against Aβ aggregation. This comparison establishes that ABSMs displaying longer β-strands and providing bigger hydrophobic surfaces are an effective design for inhibitors of amyloid aggregation.

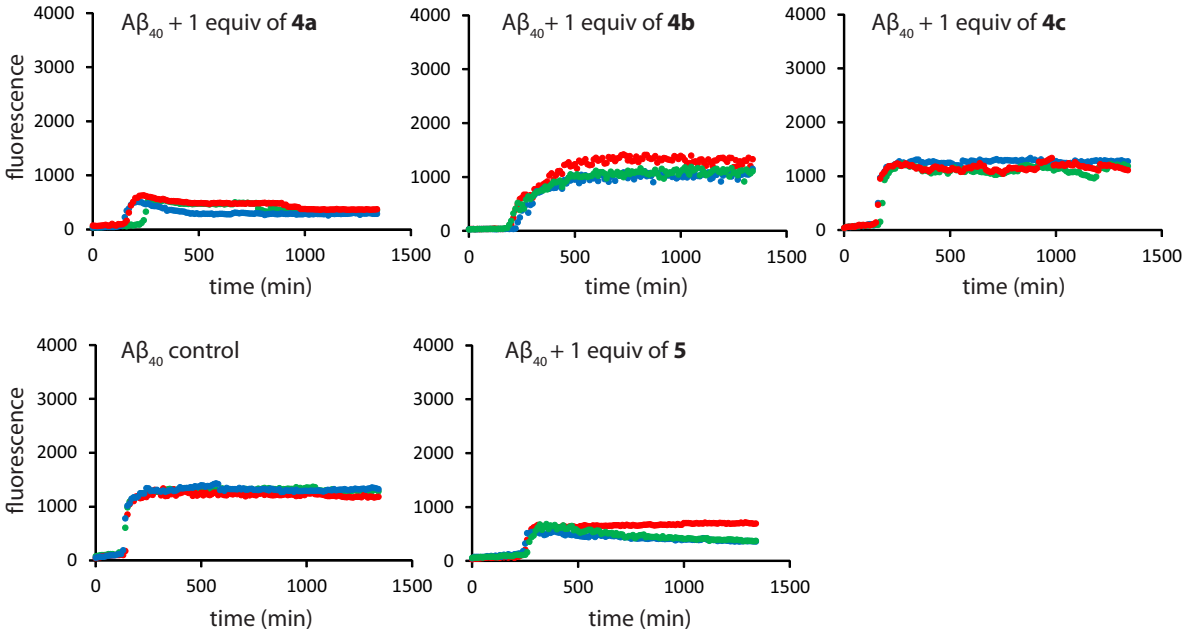


Figure S7. Effect of 42-membered ring macrocycles containing pentapeptide sequences from the amyloidogenic region of Aβ, residues 16-22, on inhibition of $A\beta_{40}$ aggregation. Different color data points correspond to data from multiple experiments run under identical conditions in the same plate.

Self-Association of ABSM 1a. To study the self-association of ABSM **1a** in water, we measured diffusion coefficients (*D*) of ABSM **1a** at different concentrations using DOSY (diffusion-ordered spectroscopy) experiments.[12] ABSM **1a** exhibits a *D* value of 19.5 ± 0.2 × 10^{-11} m^2/s at concentrations up to 1.3 mM in D_2O at 298 K. This value is comparable to that which we have seen similarly sized macrocycles in the monomeric state.[13] At higher concentrations, the *D* drops precipitously: 17.8 × 10^{-11} m^2/s at 2.0 mM, 17.1× 10^{-11} m^2/s at 3.3 mM, and 15.7 × 10^{-11} m^2/s at 5.0 mM. Figure S8 illustrates these data. These data demonstrate that the self-association of ABSM **1a** is cooperative. The self-association does not involve the formation of simple dimers, or even trimers, but rather involves the formation of higher-order oligomers. The formation of these higher-order structures characteristically shows a plateau at low concentrations, followed by the onset of substantial self-association above a higher *critical* concentration, as in the *critical micelle concentration* in micellar self-association.[14] In ABSM **1a**, the critical concentration is approximately 1.5 mM in D_2O at 298 K.

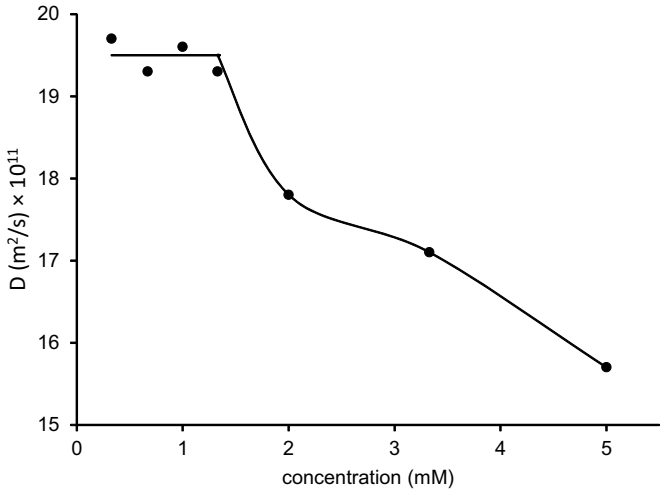


Figure S8. Diffusion coefficient of ABSM **1a** as a function of concentration. The diffusion coefficient was measured by 600 MHz DOSY experiments in D_2O at 298 K.

TEM Studies. Samples for TEM studies were taken from the ThT assays above during the delayed lag phase. Samples of $A\beta_{40}$ were prepared by applying aliquots from the wells with 1.0 equivalent of ABSM **1a** and without ABSM **1a** to TEM grids at 6 h. Samples of $A\beta_{42}$ were prepared by applying aliquots from the wells with 1.0 equivalent of ABSM **1a** and without ABSM **1a** to TEM grids at 7 h. Samples of $h\beta_2M$ were prepared by applying aliquots from the wells with 1.0 equivalent of ABSM **1m** and without ABSM **1m** to TEM grids at 2 h. Samples of $h\alpha Syn_{1\text{-}100}$ were prepared by applying aliquots from the wells with 1.0 equivalent of ABSM **1o** and without ABSM **1o** to TEM grids at 72 h. The samples were pipetted onto formvar and carbon coated electron microscopy grids (Ted Pella, catalog No. 01754-F) and incubated for 3 min. The grids were rinsed with distilled water and stained with 1% (w/v) uranyl acetate solution. Samples were imaged with a Philips CM120 electron microscope at an accelerating voltage of 120 kV. At least three independent experiments were carried out for each sample.

Lack of Toxicity of Amyloid β-Sheet Mimics 1a, 1m, and 1o. Cell viability (MTT) assays show that ABSMs **1a**, **1m**, and **1o** exhibit little or no toxicity at concentrations up to 50 μM. We incubated HeLa, HEK-293, and PC-12 cells with and without ABSMs **1a**, **1m**, and **1o**, and all cells showed comparable survival (Figure S9).

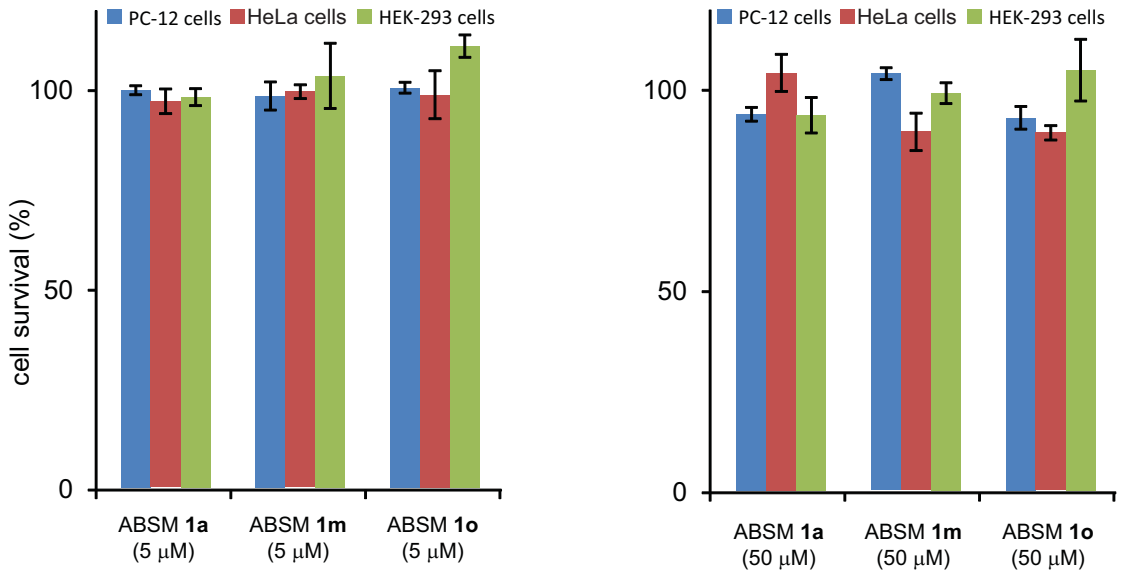


Figure S9. Toxicity of ABSM **1a**, **1m**, and **1o** towards PC-12, HeLa, and HEK-293 cells. The cell survival of the PBS controls is taken to be 100%.

Cell Viability Assays. PC-12 (ATCC, catalog No. CRL-1721), HeLa, and HEK-293 cells were used to evaluate the cytotoxic effect of the ABSMs and Aβ by MTT assays. A CellTiter 96 non-radioactive cell proliferation assay kit (Promega) was used in these MTT assays. HeLa and HEK-293 cells were cultured in DMEM medium with 10% fetal bovine serum, and PC-12 cells in ATCC-formulated RPMI 1640 medium (catalog No. 30-2001) containing 10% heat-inactivated horse serum and 5% fetal bovine serum. All cells were maintained at 37 °C in 5% CO_2.

*Toxicity of ABSM **1a**, **1m**, and **1o** towards PC-12, HeLa, and HEK-293 cells.* PC-12, HeLa, and HEK-293 cells were plated out at 10,000 cells/well in 96-well plates (Costar, catalog

No. 3596). The cells were cultured for 20 h at 37 °C in 5% CO_2 before the samples were added. ABSMs **1a**, **1m**, and **1o** were added to each well containing medium. (The total volume is 100 μL and the final concentration is 5 μM or 50 μM.) After cells were incubated with and without ABSMs for 24 h, the dye solution (15 μL, Promega, catalog No. G4000) was added to each well and incubation continued for additional 4 h at 37 °C in 5% CO_2. The solubilization Solution/Stop Mix (100 μL, Promega, catalog No. G4000) was then added to each well. After incubation for 12 h at room temperature, the absorbance was measured at 570 nm. The background absorbance was recorded at 700 nm. Each of the samples was repeated with 4 replicates in three independent experiments. The results were normalized by setting the cell survival of the PBS controls to be 100% and that of the SDS (0.2%) controls to be 0%.

*Detoxification of Amyloid-β by ABSM **1a**.* PC-12 cells were plated out at 10,000 cells/well in 96-well plates (Costar, catalog No. 3596). The cells were cultured 20 h at 37 °C in 5% CO_2 prior to addition of the samples. $A\beta_{40}$ and $A\beta_{42}$ monomers (5 μM) with and without ABSM **1a** were incubated at 37 °C with and without shaking for 16 h respectively prior to the addition to the cells. The preincubated Aβ solutions with and without ABSM **1a** were added to the cells. (The final concentration of the preincubated Aβ solutions is 0.5 μM.) The resulting mixtures were incubated for additional 24 h at 37 °C in 5% CO_2. The absorbance was then measured at 570 nm. The background absorbance was recorded at 700 nm. Each of the samples was repeated with 4 replicates in three independent experiments. The results were normalized by setting the cell survival of the PBS controls to be 100% and that of the SDS (0.2%) controls to be 0%.

---

1 Most standard peptide synthesis procedures followed those in the "Synthesis Notes" section of the **2009**/**2010** Novabiochem catalog.

2 Cheng, P.-N.; Nowick, J. S. *J. Org.Chem.* **2011**, *76*, 3166–3173.

3 Nowick, J. S.; Khakshoor, O.; Hashemzadeh, M.; Brower, J. O. *Org. Lett.* **2003**, *5*, 3511–3513.

4 Kabsch, W. *J. Appl. Crystallogr.* **1993**, *26*, 795–800.

5 *Acta Crystallogr. D Biol. Crystallogr.***1994**, *50*, 760–763.

6 Sheldrick, G. M. *Acta Crystallogr. A* **2008**, *64*, 112–122.

7 Pape, T.; Schneider, T. R. *J. Appl. Crystallogr.* **2004**, *37*, 843–844.

8 Emsley, P.; Cowtan, K. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2126–2132.

9 Vagin, A. A.; Steiner, R. A.; Lebedev, A. A.; Potterton, L.; McNicholas, S.; Long, F.; Murshudov, G. N. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2184–2195.

10 Finder, V. H.; Vodopivec, I.; Nitsch, R. M.; Glockshuber, R. *J. Mol. Biol.* **2010**, *396*, 9–18.

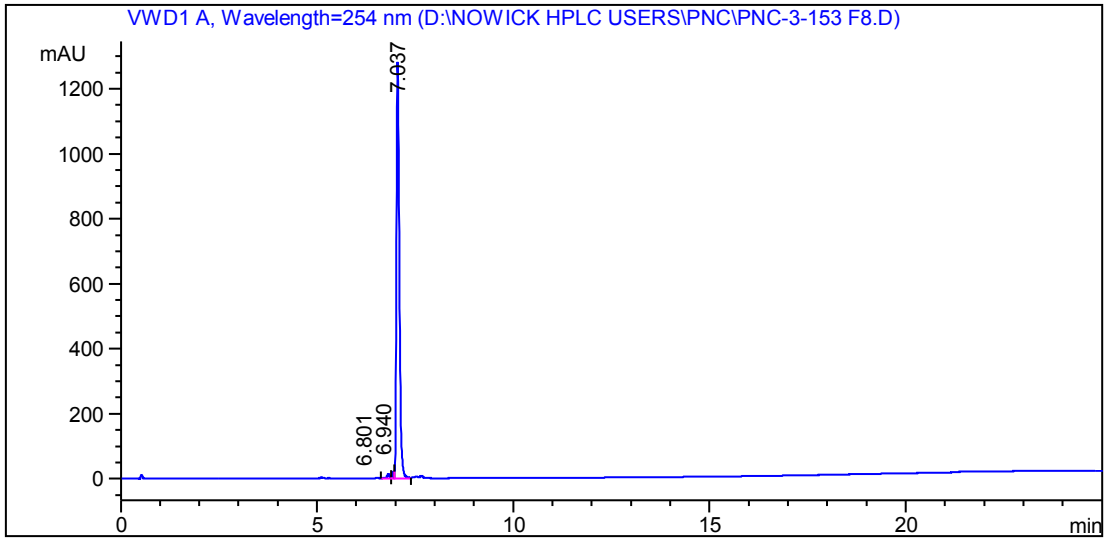
11 Liu, C.; Sawaya, M. R.; Eisenberg, D. *Nat. Struct. Mol. Biol*. **2011**, *18*, 49–55.

12 (a) Yao, S.; Howlett, G. J.; Norton, R. S., *J. Biomol. NMR* **2000**, *16*, 109–119. (b) Cohen, Y.; Avram, L.; Frish, L., *Angew. Chem., Int. Ed.* **2005**, *44*, 520–554.

13 Khakshoor, O.; Demeler, B.; Nowick, J. S., *J. Am. Chem. Soc.* **2007**, *129*, 5558**–**5569.

14 Nowick, J. S.; Cao, T.; Noronha, G., *J. Am. Chem. Soc.* **1994**, *116*, 3285–3289.

Representative Analytical RP-HPLC of ABSM **1a**



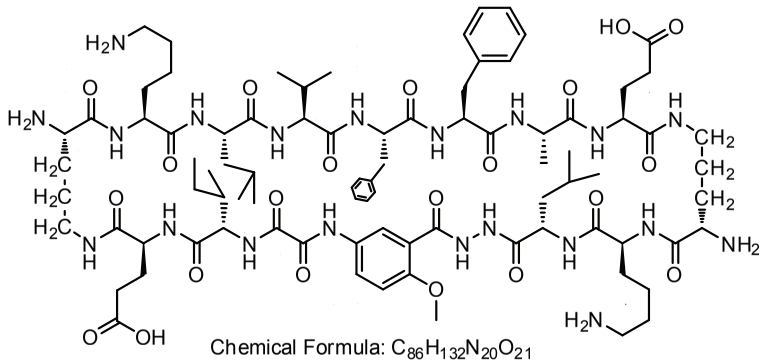
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	6.801	VV	0.0662	59.33563	13.04519	0.8863
2	6.940	VV	0.0447	65.37052	22.11021	0.9765
3	7.037	VV	0.0787	6569.83398	1281.51428	98.1372

PNC-3-153 f8-1 16 (0.294) Sm (SG, 2x3.00); Cm (2:54)

TOF MS ES+
2.49e4

MS (ESI) of ABSM **1a**
calculated *m/z*:
$[2M+3H]^{3+}$: 1188.3
$[M+2H]^{2+}$: 891.5
$[M+3H]^{3+}$: 594.6
$[M+4H]^{4+}$: 446.2

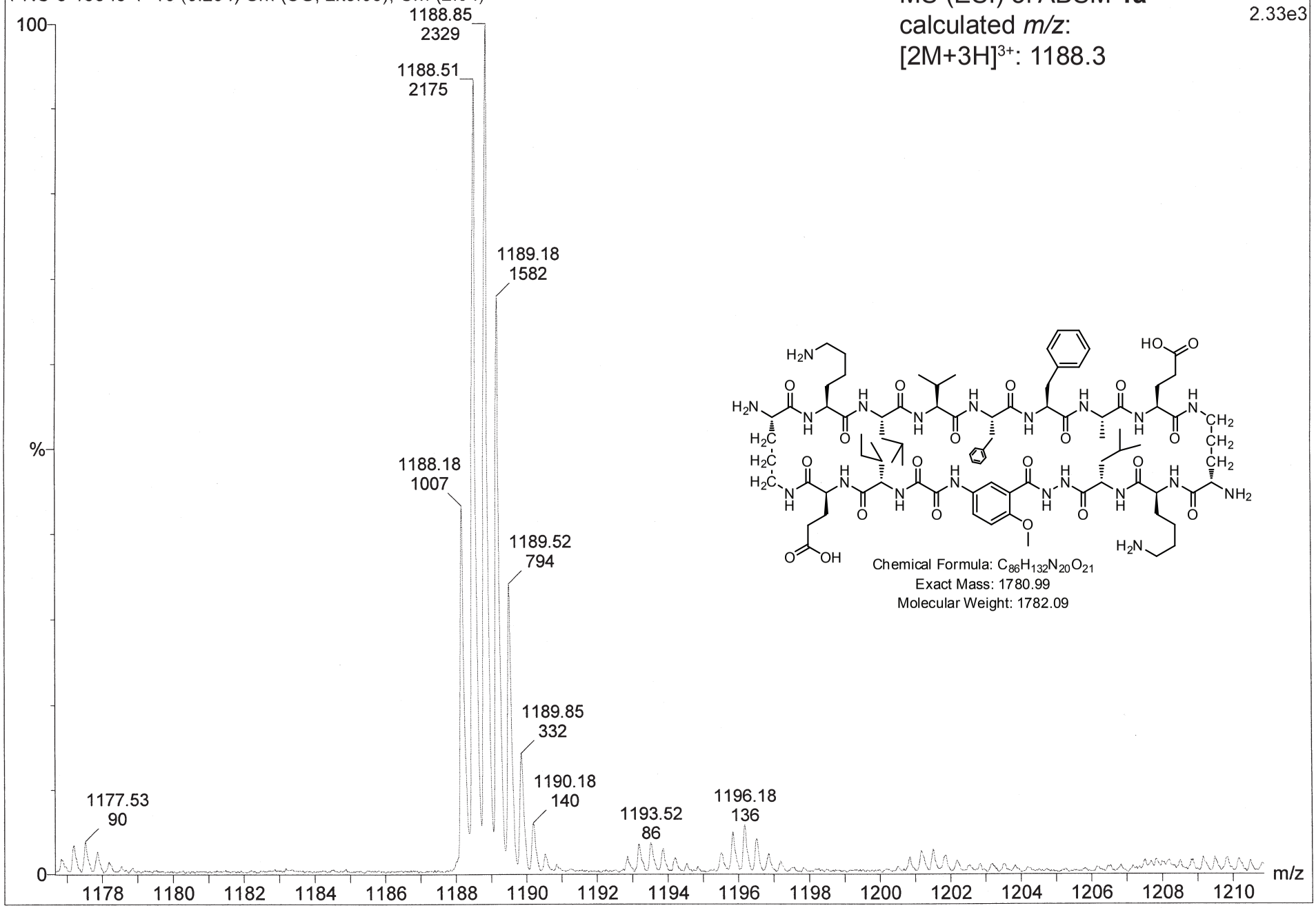


Chemical Formula: $C_{86}H_{132}N_{20}O_{21}$
Exact Mass: 1780.99
Molecular Weight: 1782.09

PNC-3-153 f8-1 16 (0.294) Sm (SG, 2x3.00); Cm (2:54)

MS (ESI) of ABSM **1a**
calculated *m/z*:
$[2M+3H]^{3+}$: 1188.3

TOF MS ES+
2.33e3

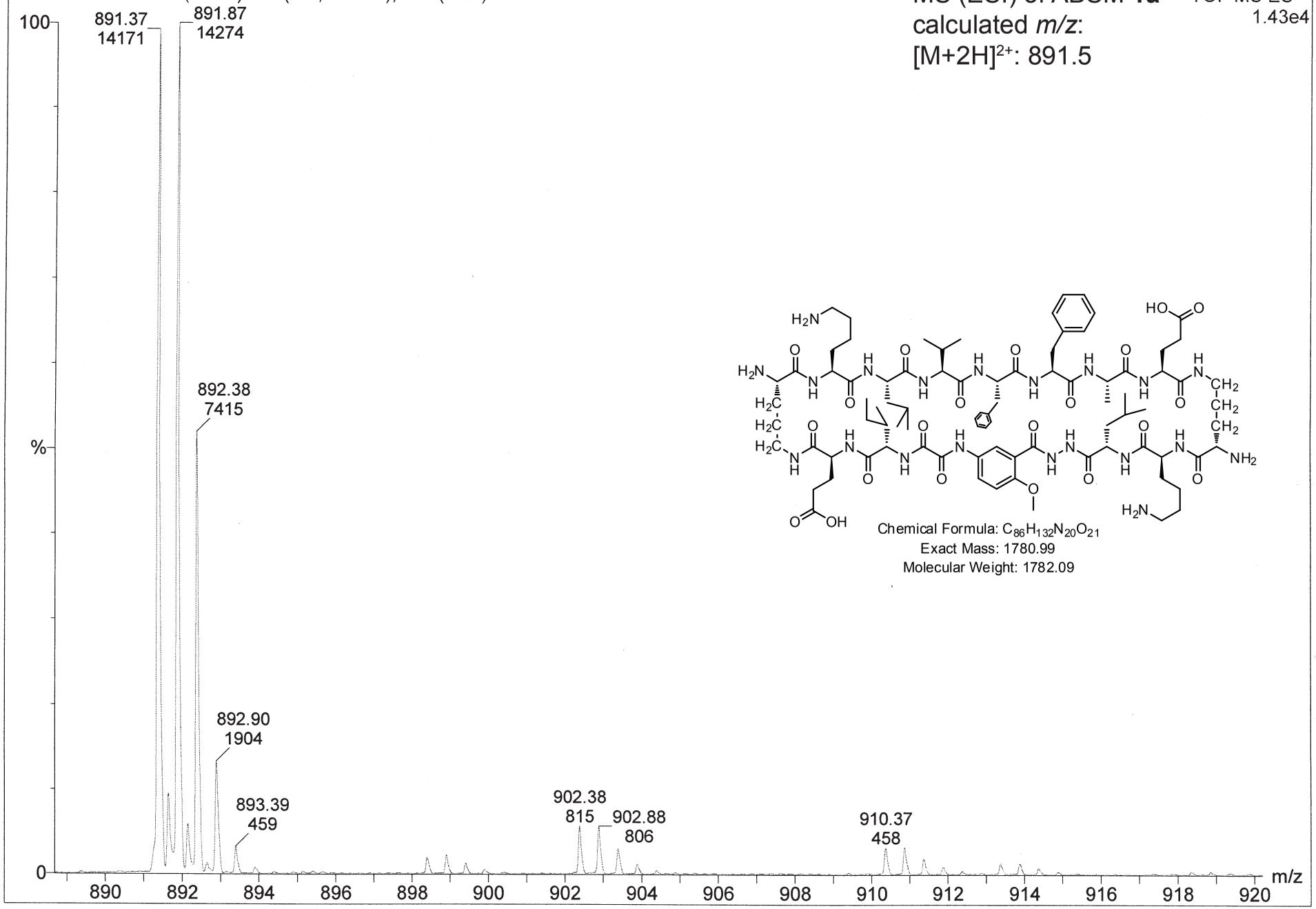


Chemical Formula: $C_{86}H_{132}N_{20}O_{21}$
Exact Mass: 1780.99
Molecular Weight: 1782.09

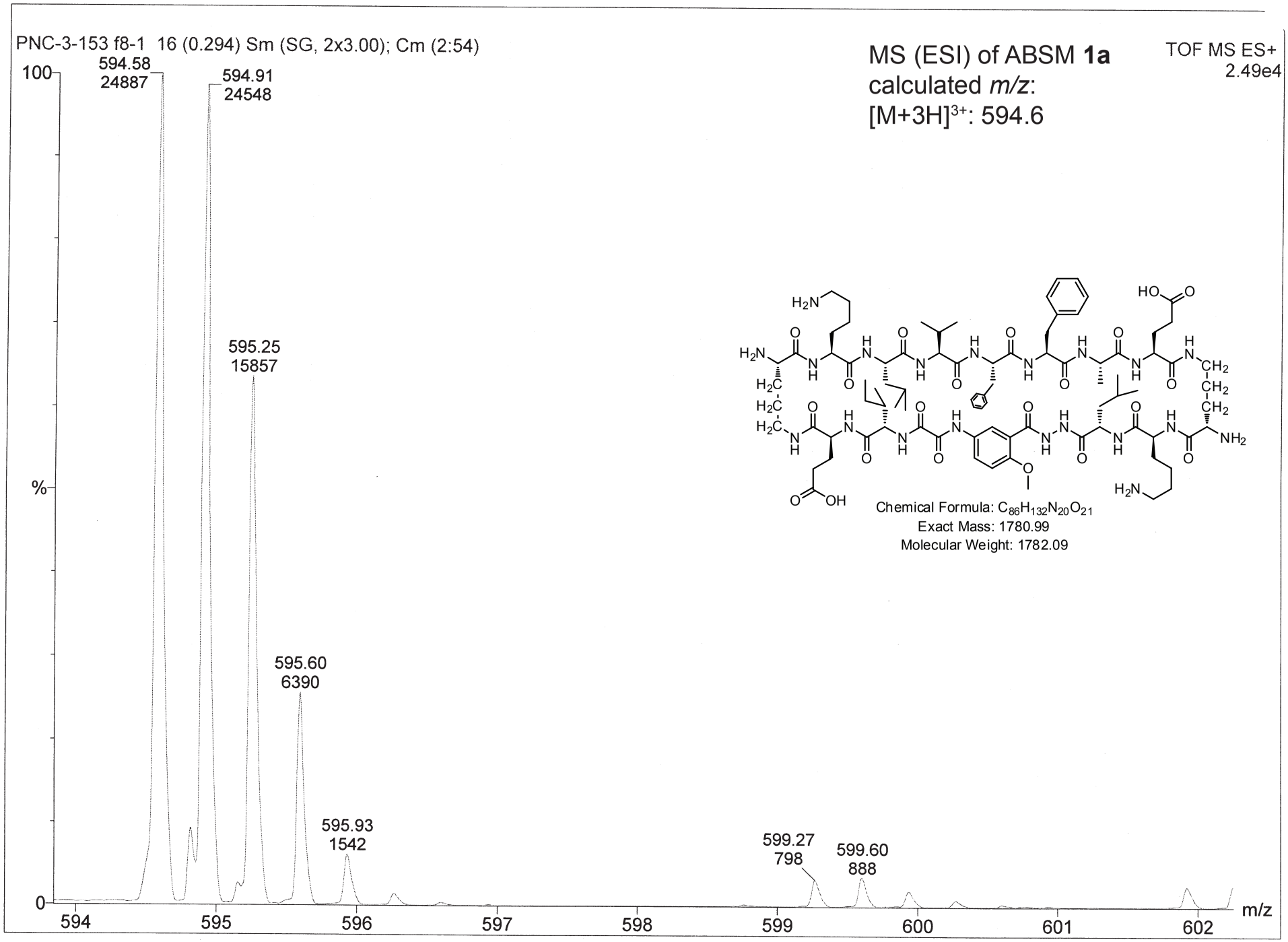
PNC-3-153 f8-1 16 (0.294) Sm (SG, 2x3.00); Cm (2:54)

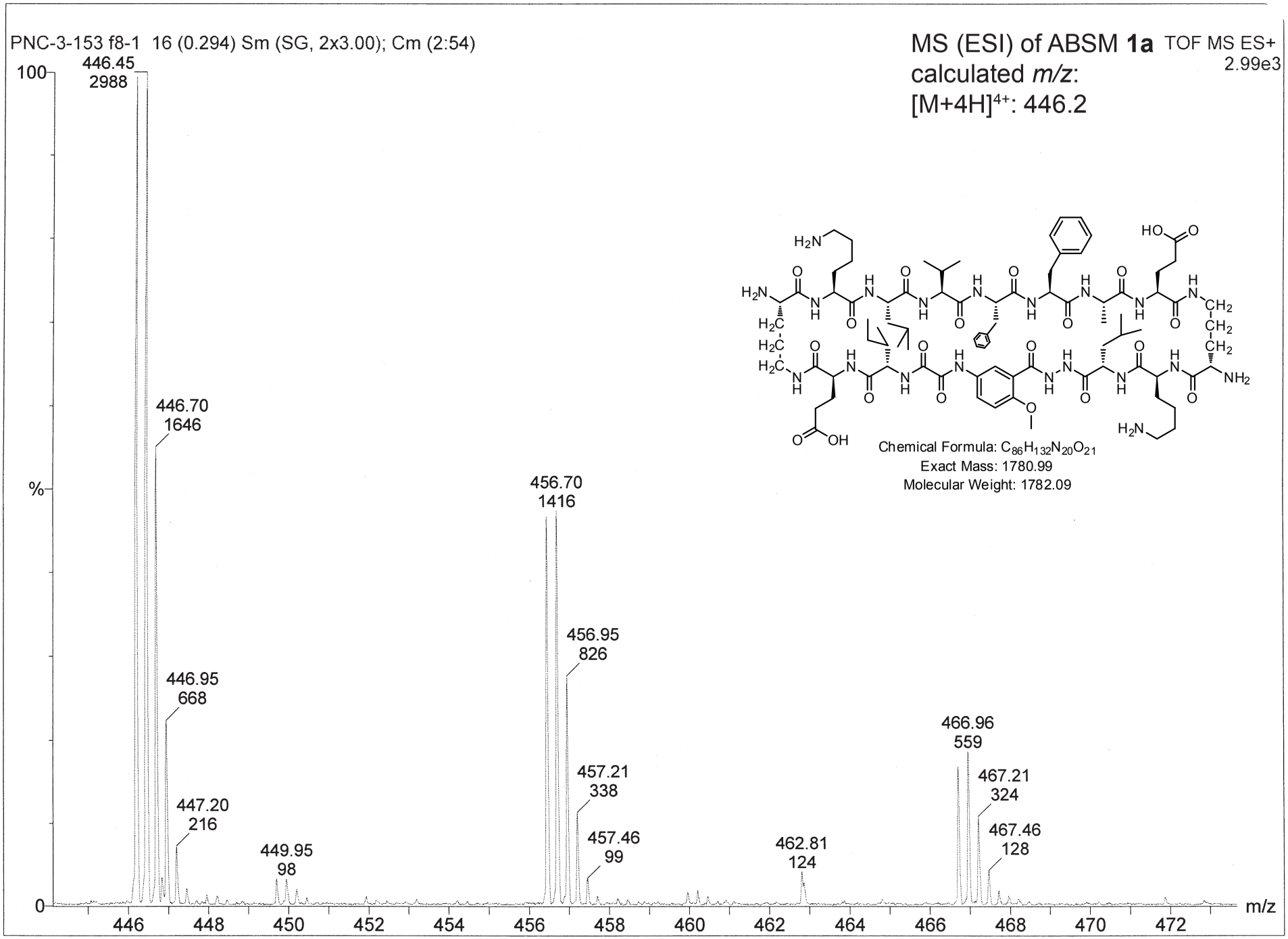
MS (ESI) of ABSM **1a**
calculated *m/z*:
$[M+2H]^{2+}$: 891.5

TOF MS ES+
1.43e4

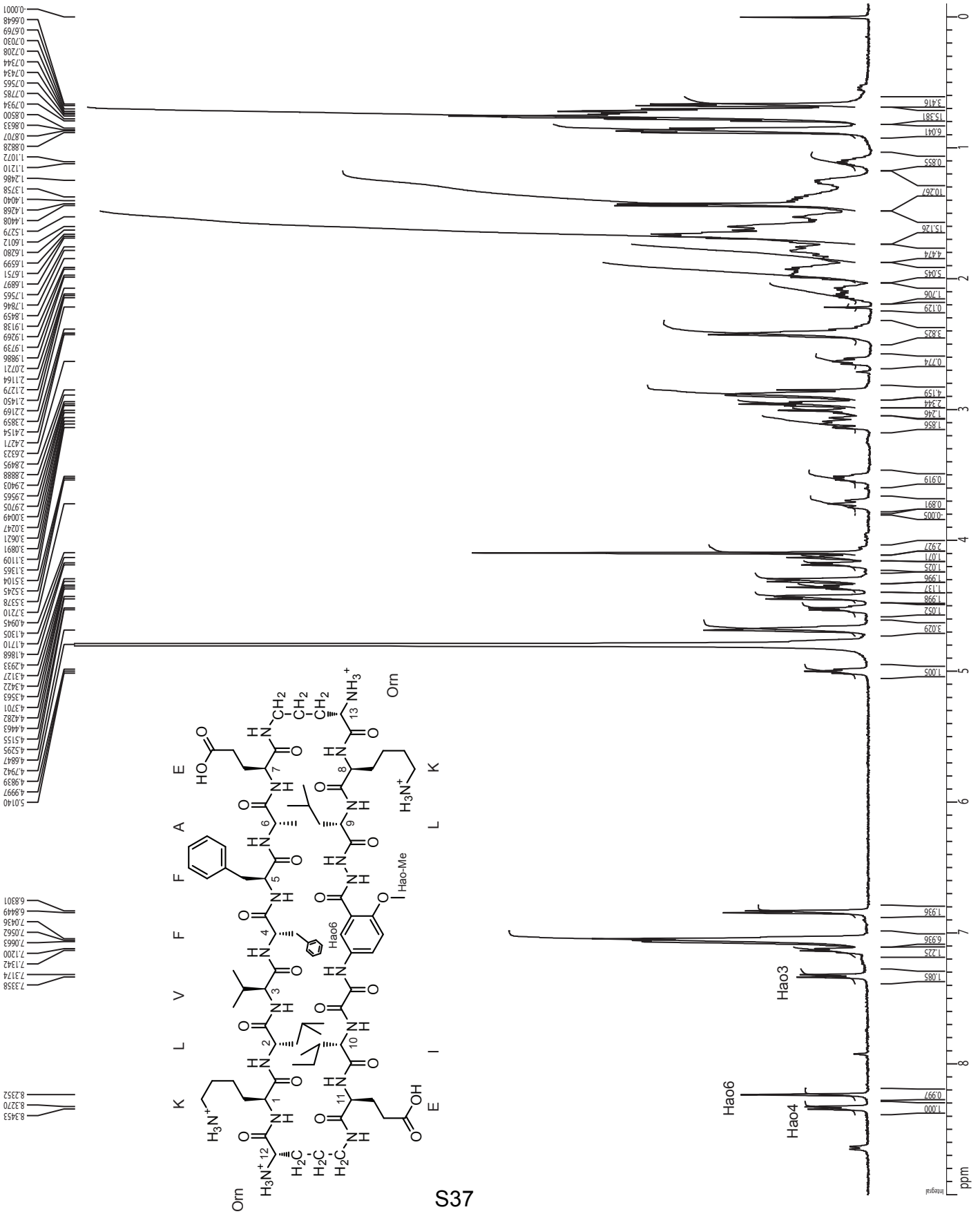


Chemical Formula: $C_{86}H_{132}N_{20}O_{21}$
Exact Mass: 1780.99
Molecular Weight: 1782.09

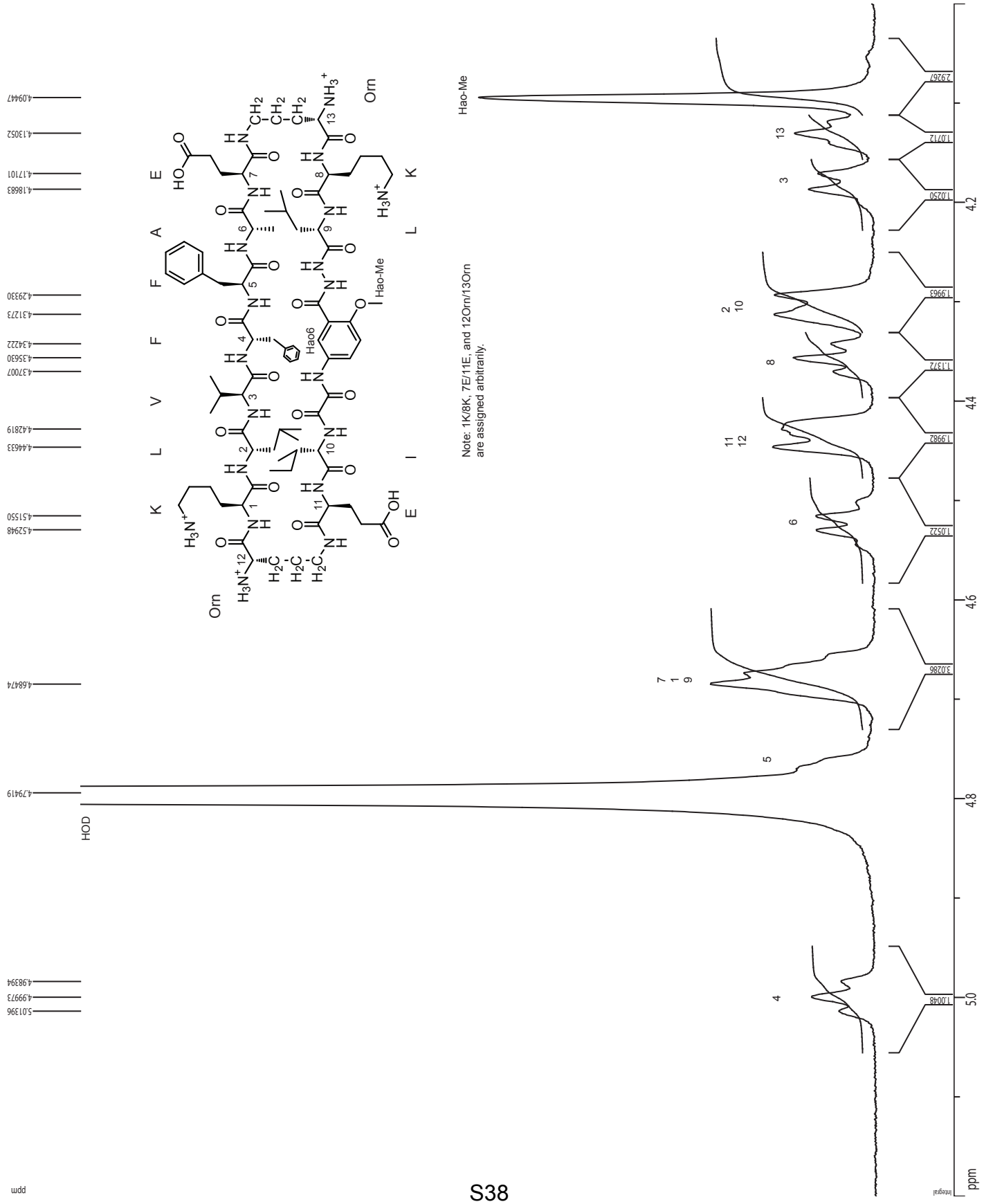

PNC-3-153 f8-1 16 (0.294) Sm (SG, 2x3.00); Cm (2:54)
MS (ESI) of ABSM **1a**
calculated *m/z*:
$[M+3H]^{3+}$: 594.6
TOF MS ES+
2.49e4
100
%
0
594.58
24887
594.91
24548
595.25
15857
595.60
6390
595.93
1542
599.27
798
599.60
888
594
595
596
597
598
599
600
601
602
m/z
Chemical Formula: $C_{86}H_{132}N_{20}O_{21}$
Exact Mass: 1780.99
Molecular Weight: 1782.09


PNC-3-153 f8-1 16 (0.294) Sm (SG, 2x3.00); Cm (2:54)
MS (ESI) of ABSM 1a TOF MS ES+
2.99e3
calculated m/z:
[M+4H]4+: 446.2
Chemical Formula: C86H132N20O21
Exact Mass: 1780.99
Molecular Weight: 1782.09
100
%
0
446.45
2988
446.70
1646
446.95
668
447.20
216
449.95
98
456.70
1416
456.95
826
457.21
338
457.46
99
462.81
124
466.96
559
467.21
324
467.46
128
m/z
446
448
450
452
454
456
458
460
462
464
466
468
470
472

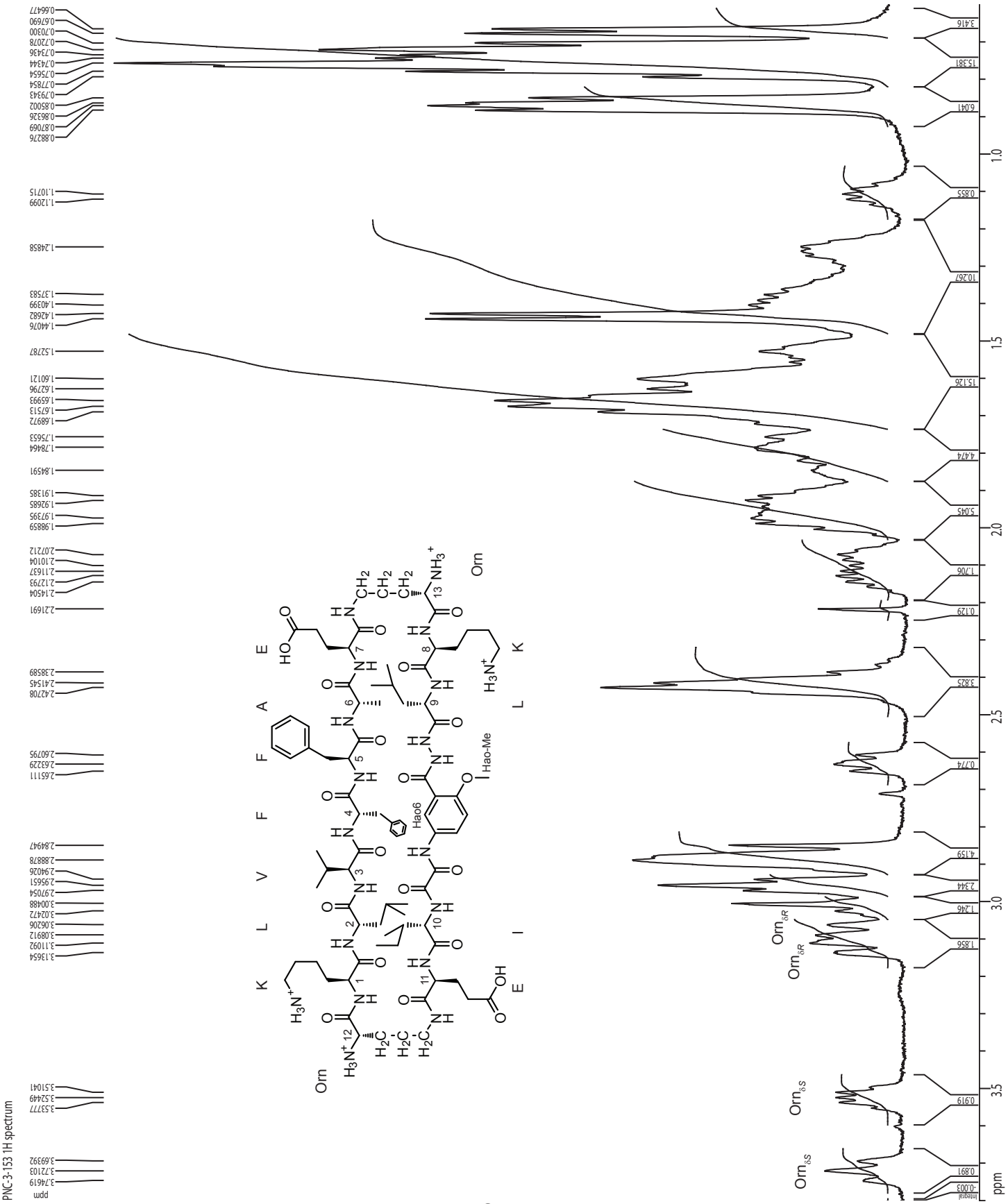
1D ^{1}H NMR spectrum of ABSM **1a**, 500 MHz, 2 mM in D_2O, 298K



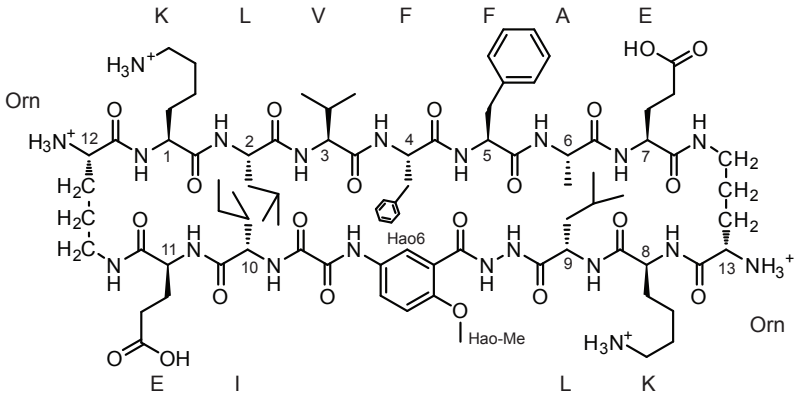
1D ^{1}H NMR spectrum of ABSM **1a**, 500 MHz, 2 mM in D_2O, 298K

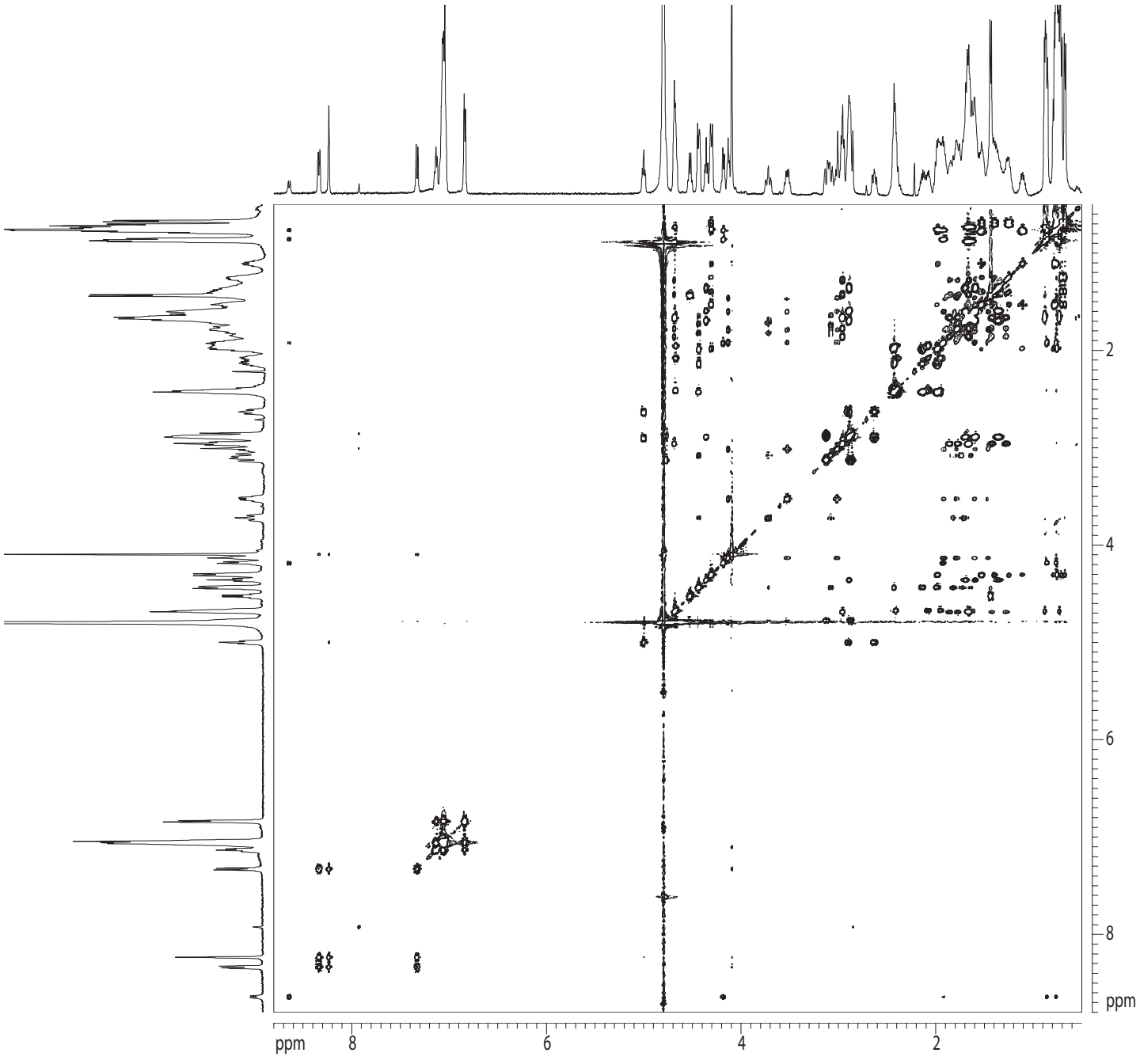


1D ^{1}H NMR spectrum of ABSM **1a**, 500 MHz, 2 mM in D_2O, 298K

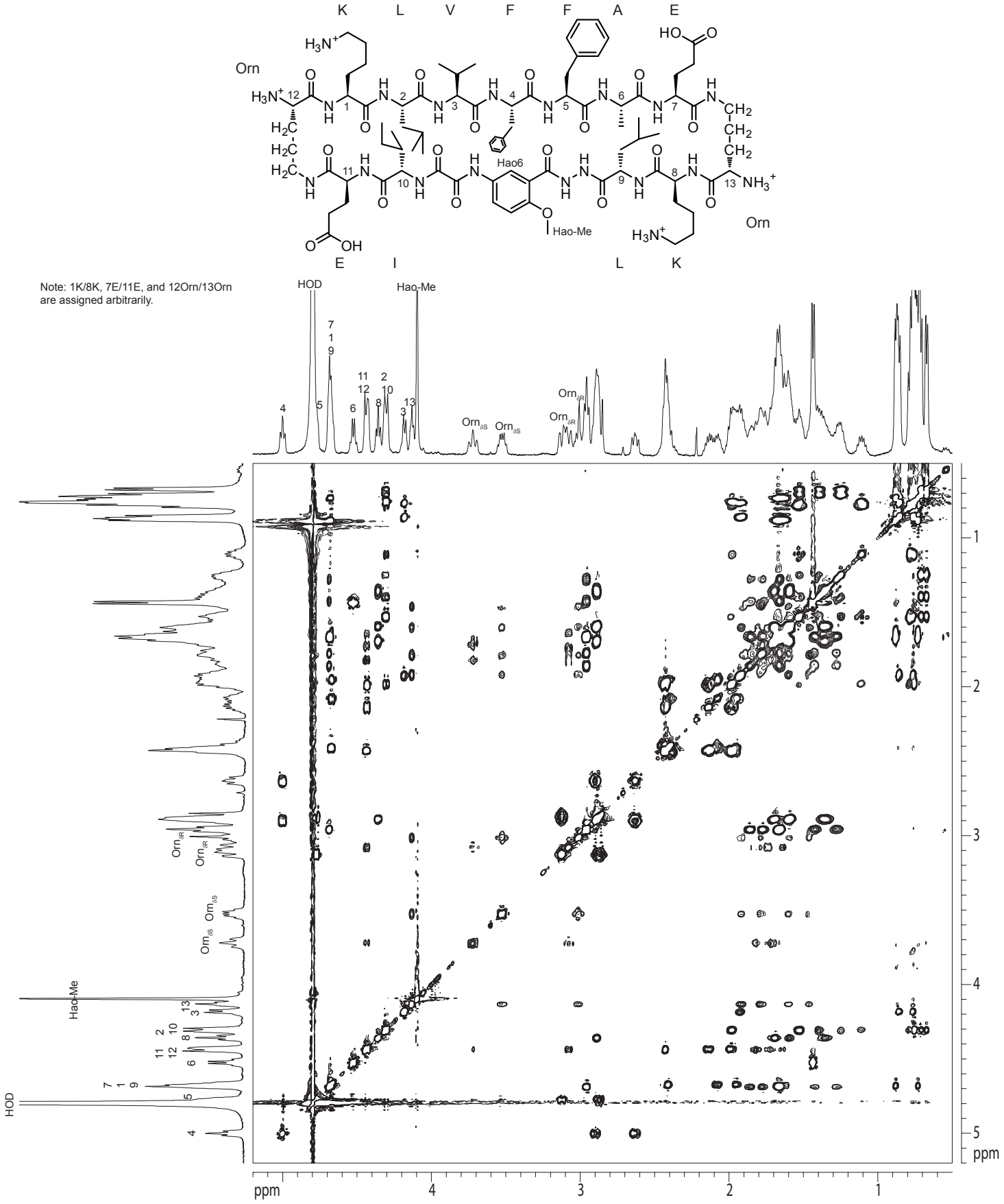


2D TOCSY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
150-ms spin-lock mixing time

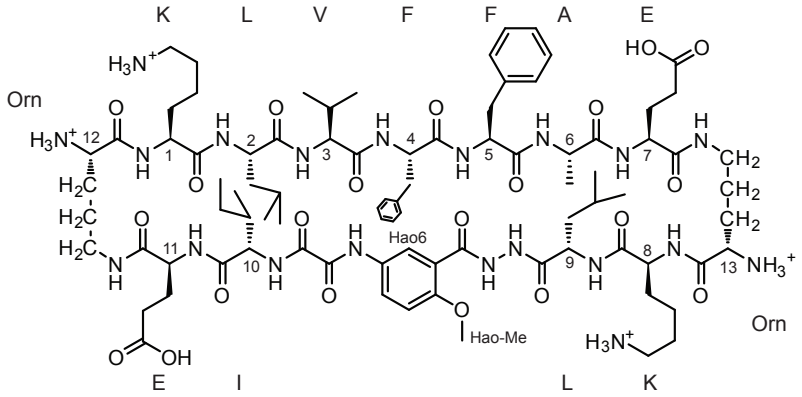


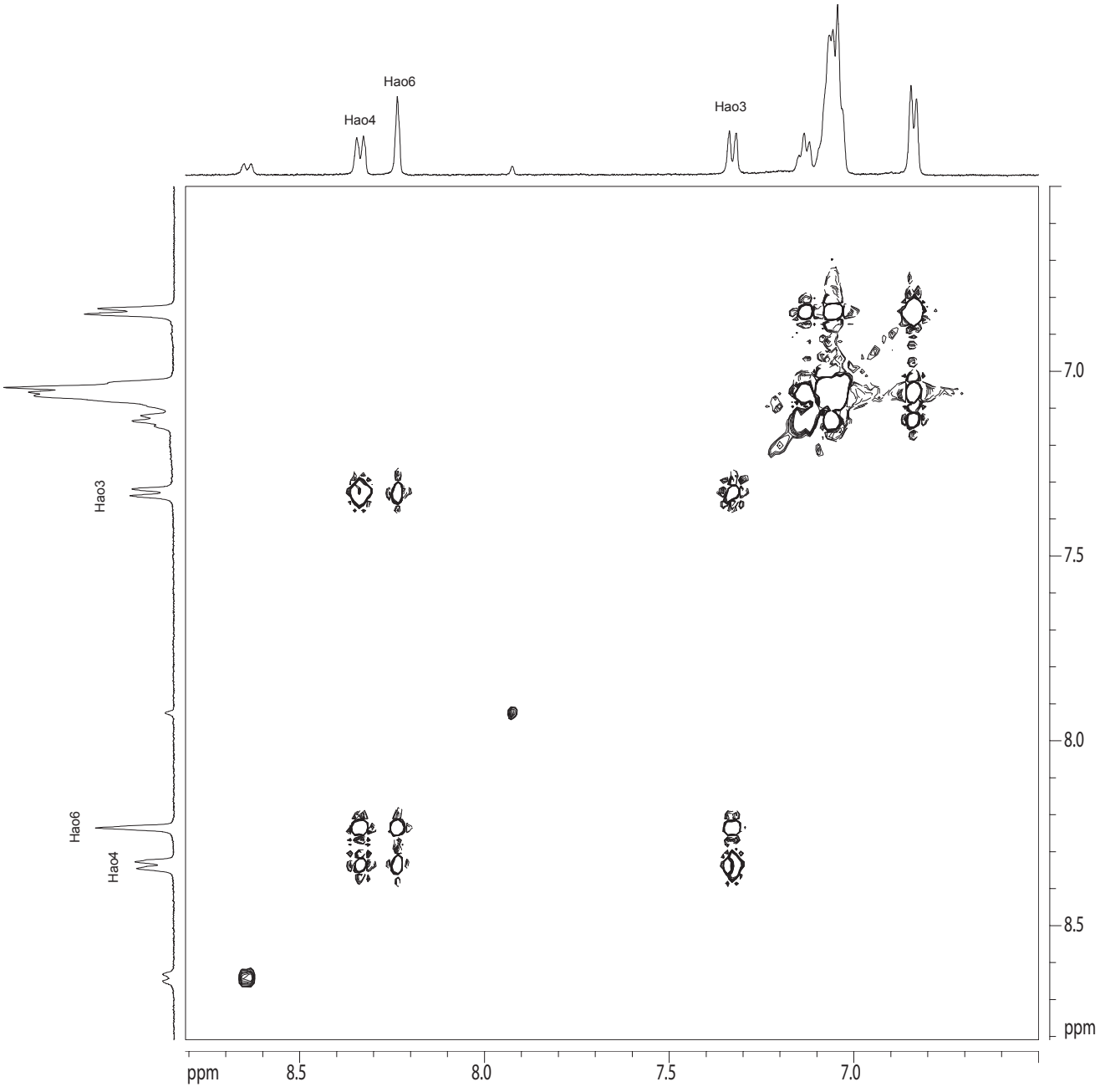


2D TOCSY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
150-ms spin-lock mixing time

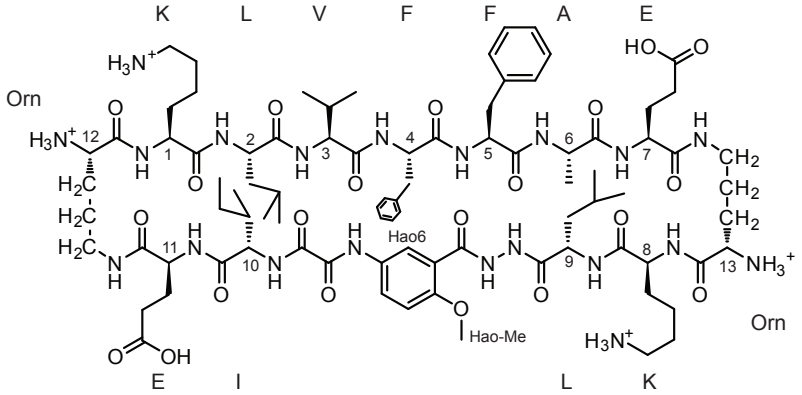


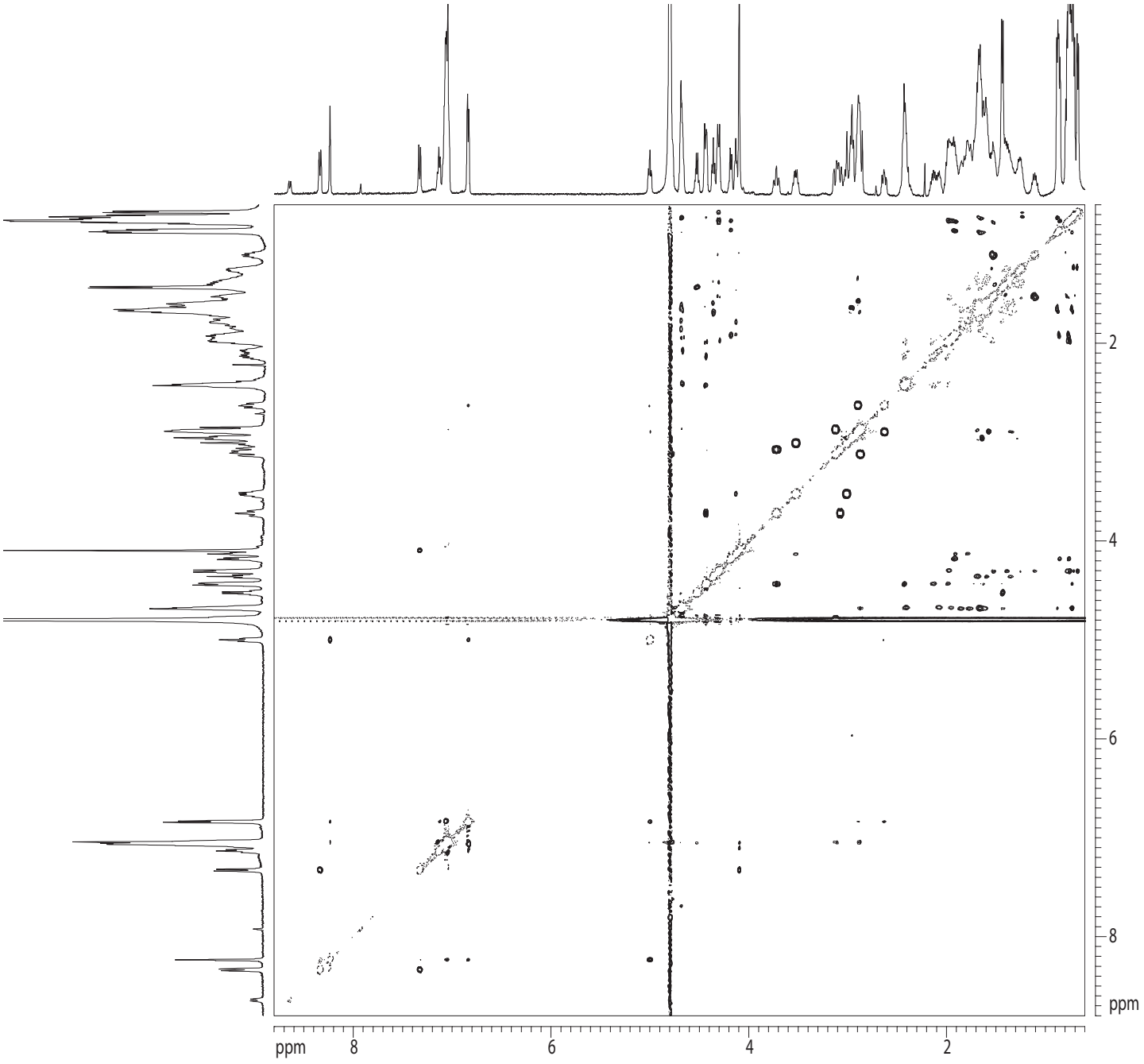
2D TOCSY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
150-ms spin-lock mixing time



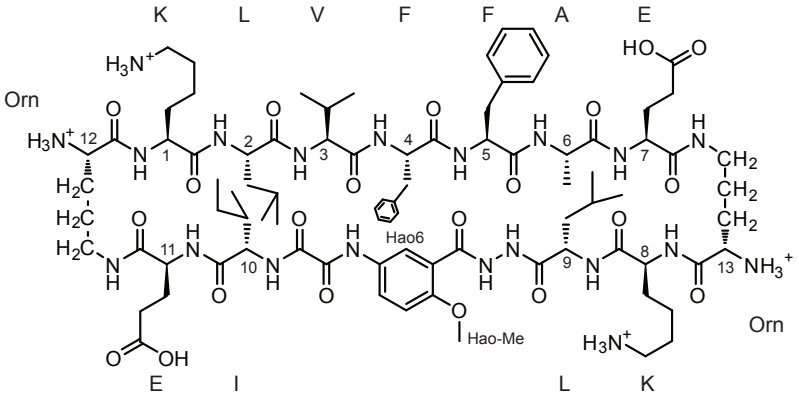


2D ROESY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
300-ms spin-lock mixing time

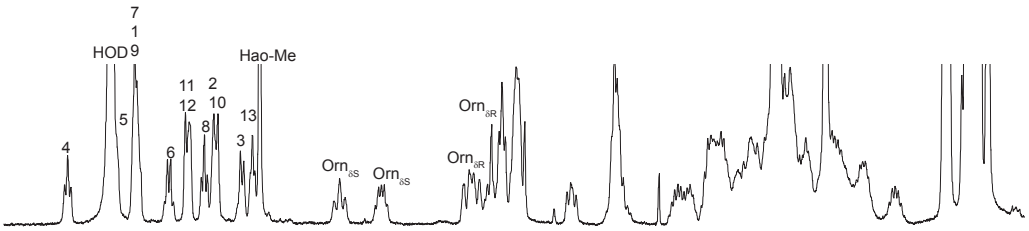


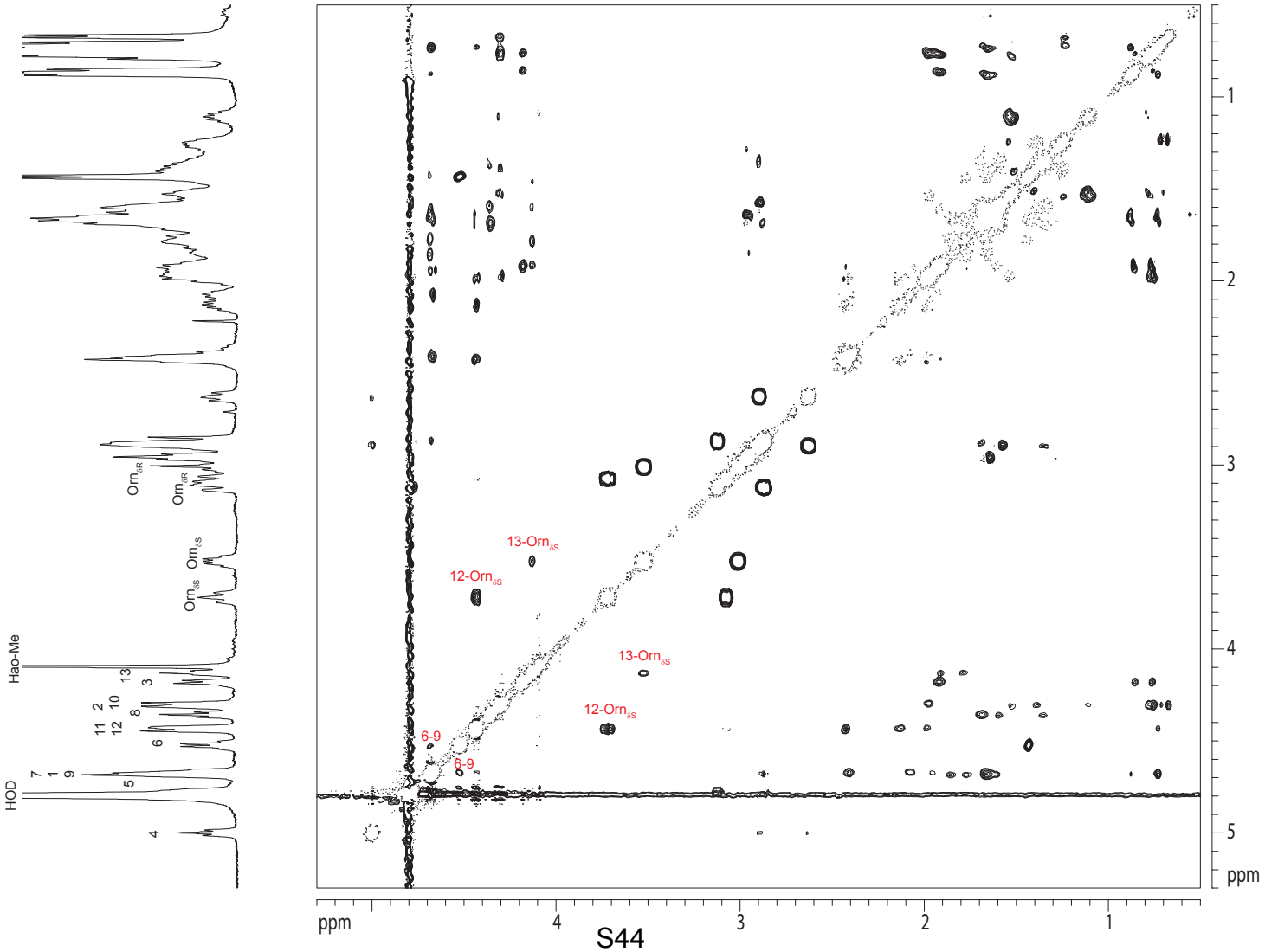


2D ROESY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
300-ms spin-lock mixing time

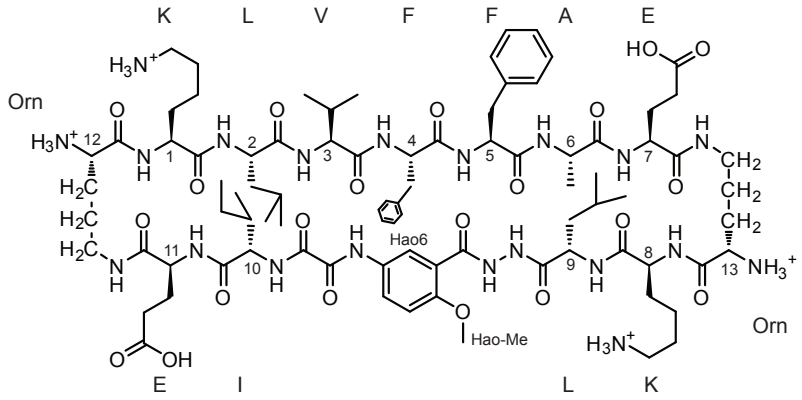


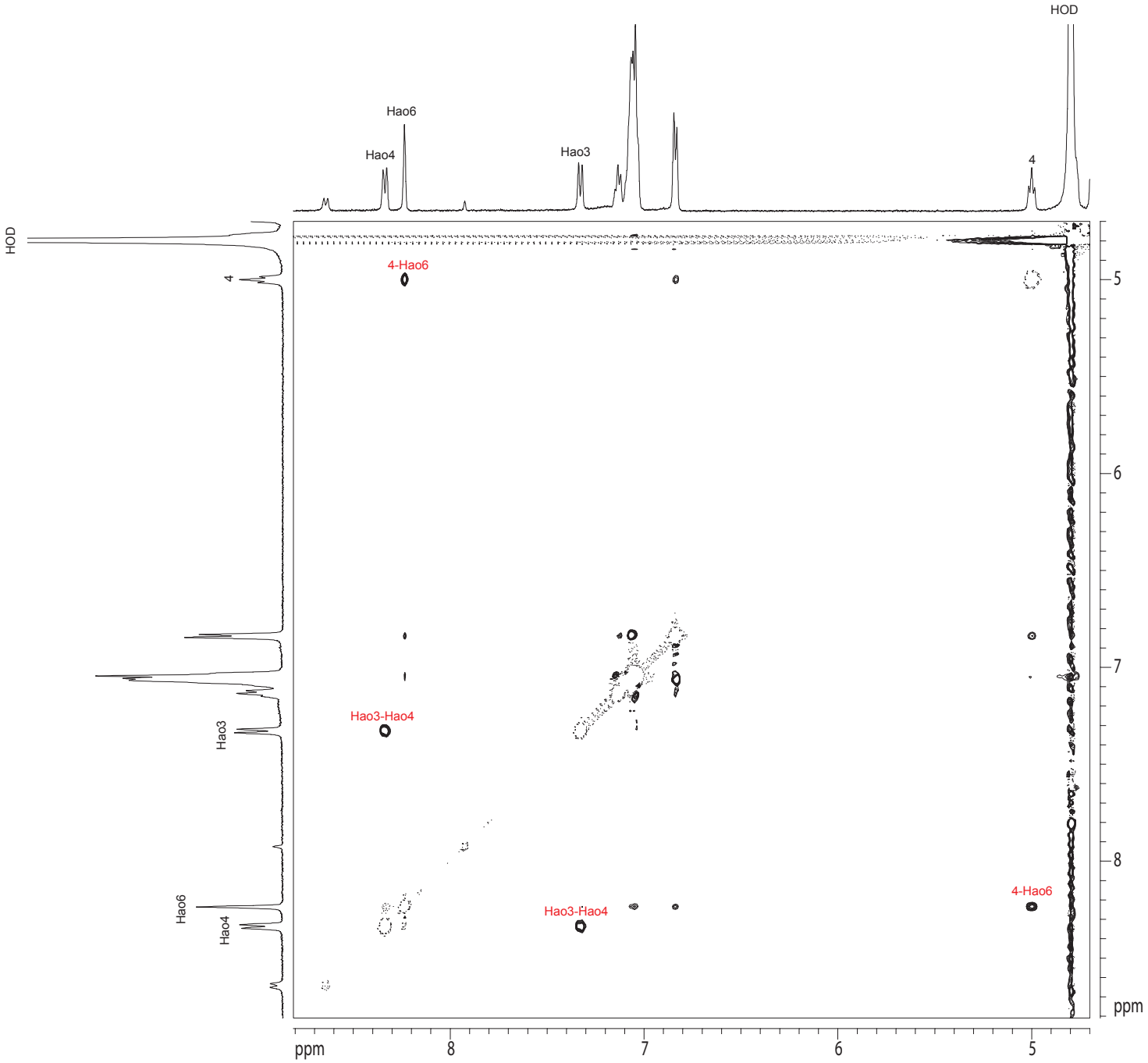
Note: 1K/8K, 7E/11E, and 12Orn/13Orn are assigned arbitrarily.



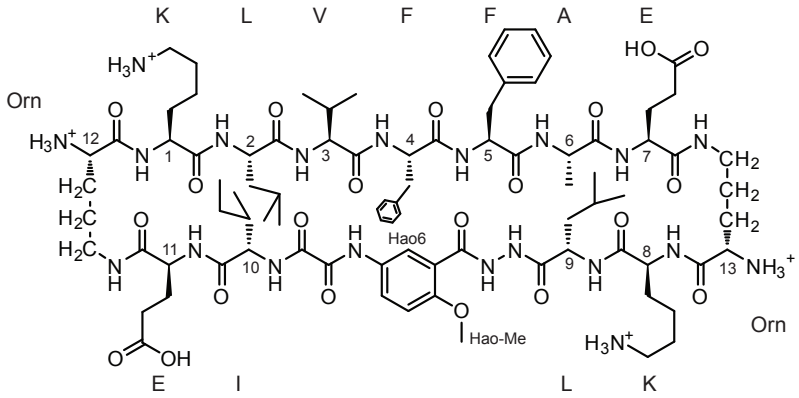


2D ROESY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
300-ms spin-lock mixing time

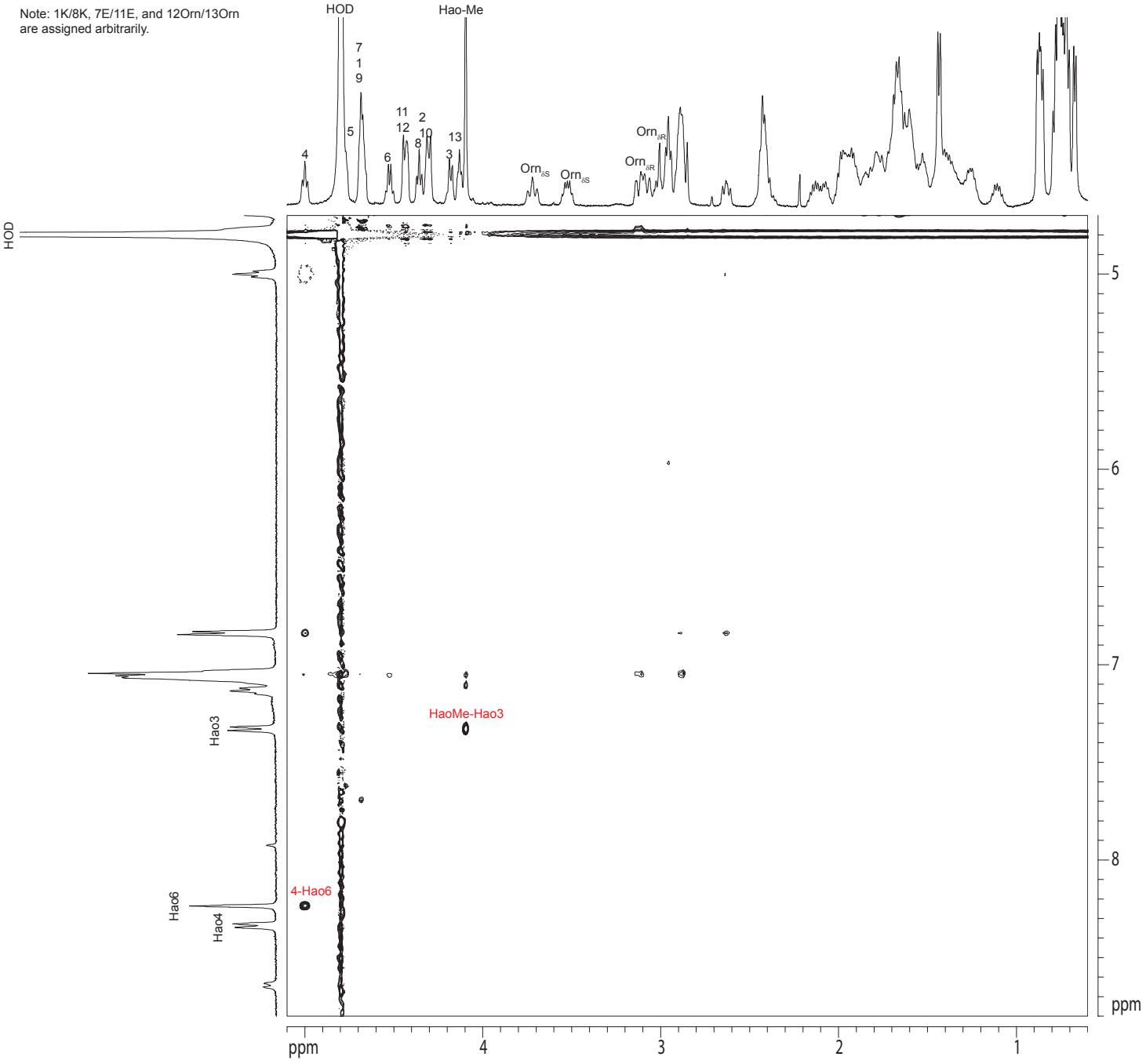




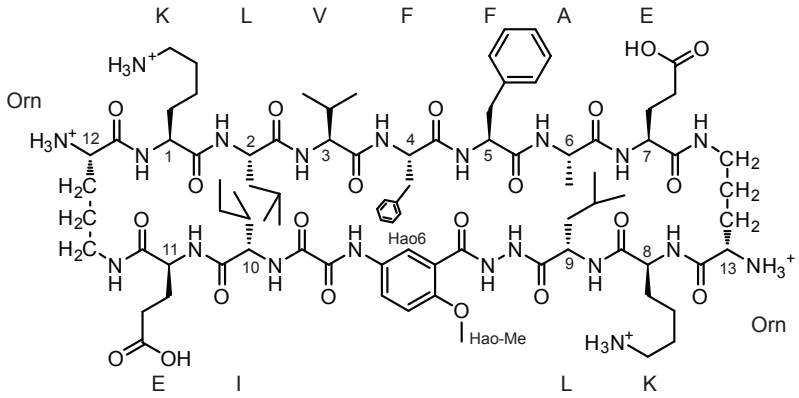
2D ROESY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
300-ms spin-lock mixing time



Note: 1K/8K, 7E/11E, and 12Orn/13Orn are assigned arbitrarily.



2D ROESY spectrum of ABSM **1a**, 2 mM in D_2O, 298K, 500 MHz
300-ms spin-lock mixing time



Note: 1K/8K, 7E/11E, and 12Orn/13Orn are assigned arbitrarily.