Supporting Information for

A Simple Strategy for the Construction of Combinatorial Cyclic Peptoid Libraries

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Materials and General Methods:

All commercial reagents were purchased from commercial suppliers and used without further purification. 3-N^{α}-Fmoc-Amino-3-(2-nitrophenyl)propionic acid was purchased from Advanced Chemtech. Fmoc-protected amino acids and *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Novabiochem. TentaGel S NH_2 resin (90 μm, 0.26 mmol/g, 80~100 pmol/bead) was purchased from Rapp Polymer. Analytical HPLC and LC/MS was performed on Agilent systems with a C18 reversed phase HPLC column (Agilent, 5 μM, 4.6 mm x 125 mm). A gradient elution of 100% A in 4 min followed by 90% B in 20 min was used at flow rate of 1 mL/min (solvent A: H_2O, 0.01% TFA; B: acetonitrile, 0.01% TFA). MALDI-TOF MS was performed on ABI 4800 mass spectrometer (Applied Biosystems) using α -cyano-4-hydroxycinnamic acid as a matrix. Peptoid synthesis under microwave conditions was performed in a 1000 W Whirlpool microwave oven (model MT4155SPT) with 10% power.

Synthesis of Cyclic Peptoids:

TentaGel S NH_2 resins (200 mg, 58 μmol) were swollen with DMF (2 mL) in a 5 mL fritted syringe for 2 h. To conjugate the linker sequence, the beads were treated with Fmoc-6-aminohexanoic acid or Fmoc-4-aminobutyric acid (5 equiv) in the presence of HOBt (5 equiv), HBTU (5 equiv) and DIPEA (10 equiv) in DMF (2 mL) at room temperature. After shaking for 2 h, the reaction mixture was drained and the resins were washed with DMF (3×), CH_2Cl_2 (2×), MeOH (2×), and DMF (3×). The Fmoc protecting group was removed by treating with 20 % piperidine in DMF (2 x 10 min). This process was repeated three times to couple four linker residues. Fmoc-Cys(Mmt)-OH (5 equiv) was then conjugated to the resin using the same reaction conditions. To the resins, peptoids were added by a standard submonomer route using a microwave-assisted protocol [S1]. For the synthesis of the 5-mer cyclic peptoid library, a conventional split-and-pool method was employed [S2]. At the end of the peptoid synthesis, the N-termini of the peptoids were capped with triazine by treating with cyanuric chloride (5 equiv.) and DIPEA (6 equiv) in anhydrous THF overnight at room temperature. After a thorough washing cycle, the Mmt protecting group of the cysteine was removed by treatment with 1% TFA and 5% triisopropylsilane in CH_2Cl_2 (2 mL) for 30 min. For macrocyclization, the beads were treated with 10% DIPEA in DMF (2 mL) at room temperature overnight

and then thoroughly washed. The remaining chloride group on the triazine was replaced by treating the beads with various amines (methoxyethylamine, NH_4OH or Boc protected 1,4-diaminobutane, 20 equiv each) and DIPEA (20 equiv.) in DMF at 65^oC overnight. The beads were washed with DMF (3×), CH_2Cl_2 (2×), MeOH (2×), and DMF (3×). For photocleavage of the ANP linker, the beads were placed in 1.5 ml microcentrifuge tubes with MeOH:H_2O (1:1). The tubes were then irradiated for 1 hour with light at 365 nm with a hand-held UV lamp. The cyclic peptoids were confirmed by LC/MS and MALDI-TOF mass spectrometry.

Ring Opening Reaction:

To a solution of *m*-CPBA (balanced with 3-chlorobenzoic acid, 77%) (10 equiv) in THF was added an aqueous solution of 1N NaOH (30 equiv.). This solution was added to the resin, and gently shaken for 12 h at room temperature. The solution was then drained, and the resins were washed with DMF (3×), CH_2Cl_2 (2×), MeOH (2×) and DMF (3×).

Library Screening:

The cyclic peptoid library beads (~150,000 beads) were swollen in DMF for 2 hr and washed with H_2O and 1×TBST (50 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween-20). The beads were equilibrated with 1×TBST at 4^oC overnight. The beads were placed in a 15 mL conical tube and blocked with 0.5 % bovine serum albumin (BSA) in 1×TBST at room temperature for 1 h. 10 μL of streptavidin conjugated Dynabeads (Invitrogen) slurry was added to the tube, and the beads were incubated for 1 hr at room temperature. Using a strong magnet (DynaMag-15, Invitrogen), positive beads were isolated. From this first round of screening, approximately 100 beads were selected. To find more tightly binding beads, second round of screening was carried out. The selected beads were boiled with 1% SDS for 20 min. Subsequently the beads were incubated with 2 μL of streptavidin-conjugated Dynabeads slurry for 30 min at room temperature (more stringent condition than the first screening conditions). The second screening resulted in the isolation of 3 beads. The individual beads were treated with m-CPBA in THF in the presence of 1N NaOH at room temperature overnight. After thorough washing and UV irradiation for 1 hr, the cleaved cyclic peptoids were

subjected to MS/MS.

On-Bead Fluorescence Assay:

A hit cyclic peptoid and a negative cyclic peptoid were re-synthesized on TentaGel S NH_2 resins (10 mg for each) as described above (Figure S14). After washing with DMF, water, and 1×TBST, the beads were equilibrated in 1×TBST at 4^{o}C overnight. After draining, the beads were blocked with 1% BSA in 1×TBST buffer for 1 hour at room temperature. Next, the beads were incubated with different concentrations (300 µL volume each) of Qdot®-streptavidin conjugates (100 nM, 50 nM, 25 nM, 10 nM, and no streptavidin) at room temperature for 1h. The beads were then washed with 1×TBST (3×5 min) and visualized under fluorescence microscope (Nikon) equipped with a DAPI filter.

Fluorescence Polarization (FP) Assay:

The fluorescein-labeled cyclic peptoids were synthesized on 50 mg of Knorr amide-MBHA Resin (Novabiochem, 0.69 mmol/g) in a similar manner to that used for the cyclic peptoid synthesis, except that propargyl amine residue was incorporated using a standard peptoid synthesis route. After the cyclization, followed by the addition of mono-Boc-protected diaminobutane, 3-azidopropan-1-amine (5 eq.) was coupled by treating with Na ascorbate (5 eq.), CuBr (5 eq.), DIPEA (10 eq.), and 2,6-lutidine (10 eq.) in DMF at room temperature overnight [S3]. The free amino group was coupled with 5(6)-carboxyfluorescein in the presence of HBTU, HOBt and DIPEA in DMF at room temperature for 2 h. The resulting conjugate was cleaved from the resin by 95% TFA, 2.5% water, and 2.5% triisopropylsilane at room temperature for 2 h. After evaporating TFA, the crude product was purified by C18 RP-HPLC to afford the fluoresein-labeled cyclic peptoid. The identity of the compound was confirmed by MALDI-TOF analysis. Observed 1853.9, expected 1853.8 for $C_{93}H_{120}N_{20}O_{19}S$ + H.

The fluoresceinated cyclic peptoids (50 nM) were incubated with indicated concentrations of streptavidin in the presence of 1.0 µM BSA in 1×PBS (pH 7.4) in a final volume of 60 µL in black Costar 384-well plates at room temperature for 1 hr in the dark. Note that fluorescence polarization was not observed at lower concentration of the fluoresceinated cyclic peptoids (e.g. 10 and 30 nM) presumably because of the low sensitivity of our instrument. The fluorescence polarization values were measured on a SpectraMax® M5

Multi-Mode Microplate Reader (Molecular Device). Excitation wavelength was 485 nm, and emission was detected at 535 nm. The K_D was calculated using GraphPad Prism® 4 software.

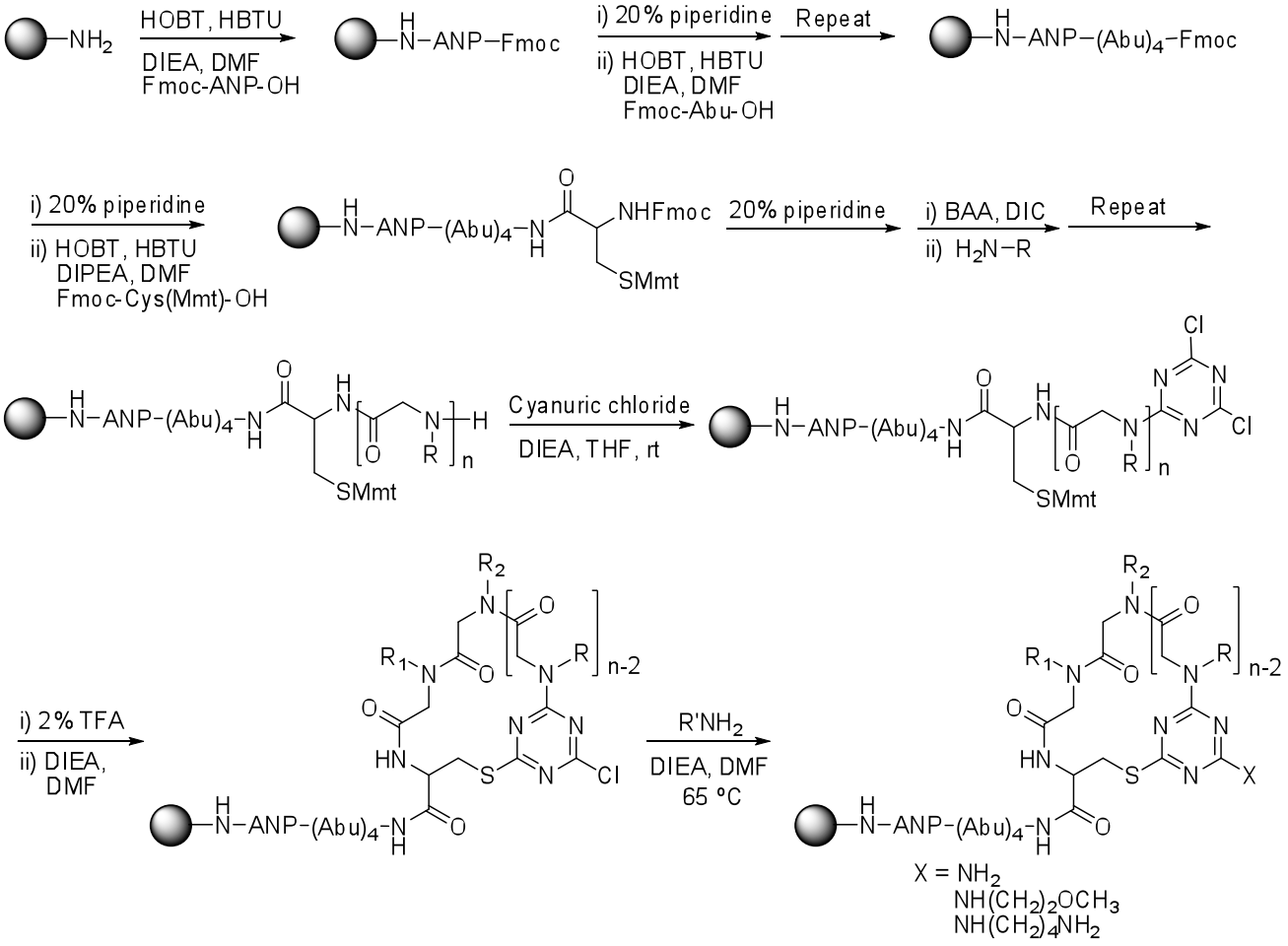
References:

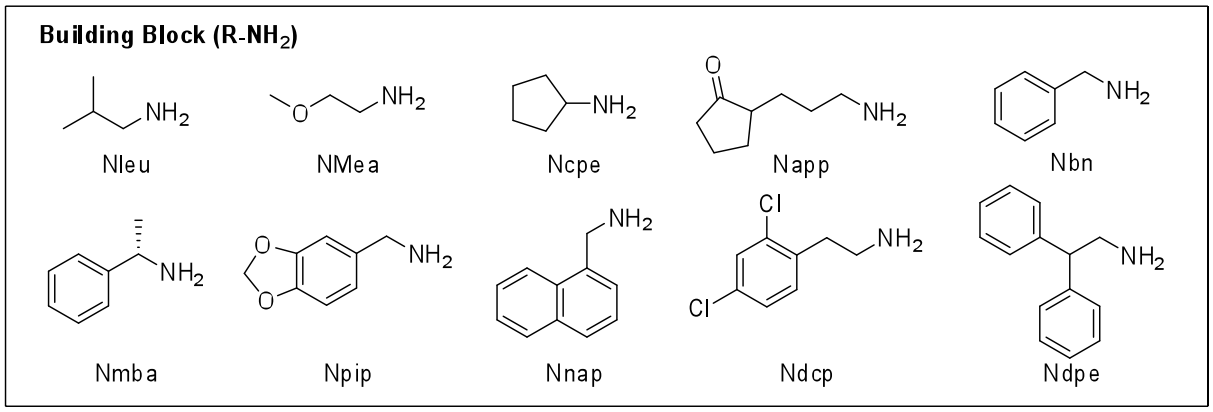
S1. Olivos, H. J.; Alluri, P. G.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057.

S2. Figliozzi, G. M.; Goldsmith, R.; Ng, S. C.; Banville, S.C.; Zuckermann, R. N. *Methods Enzymol.* **1996**, *267*, 437.

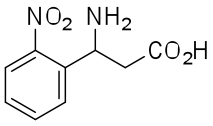
S3. Agnew, H. D.; Rohde, R. D.; Millward, S. W.; Nag, A.; Yeo, W.S.; Hein, J. E.; Pitram, S. M.; Tariq, A. A.; Burns, V. M.; Krom, R. J.; Fokin, V. V.; Sharpless, K. B.; Heath, J. R. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 4944.

Scheme S1. Synthetic scheme of cyclic peptoids and structures of the amines used.

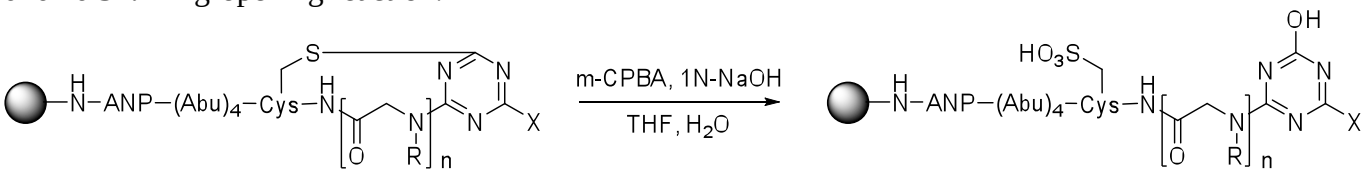




ANP (3-amino-3-(2-nitrophenyl)propionic acid)



Scheme S2. Ring-opening reaction.



Scheme S3. Synthesis of fluorescently-labeled cyclic peptoids.

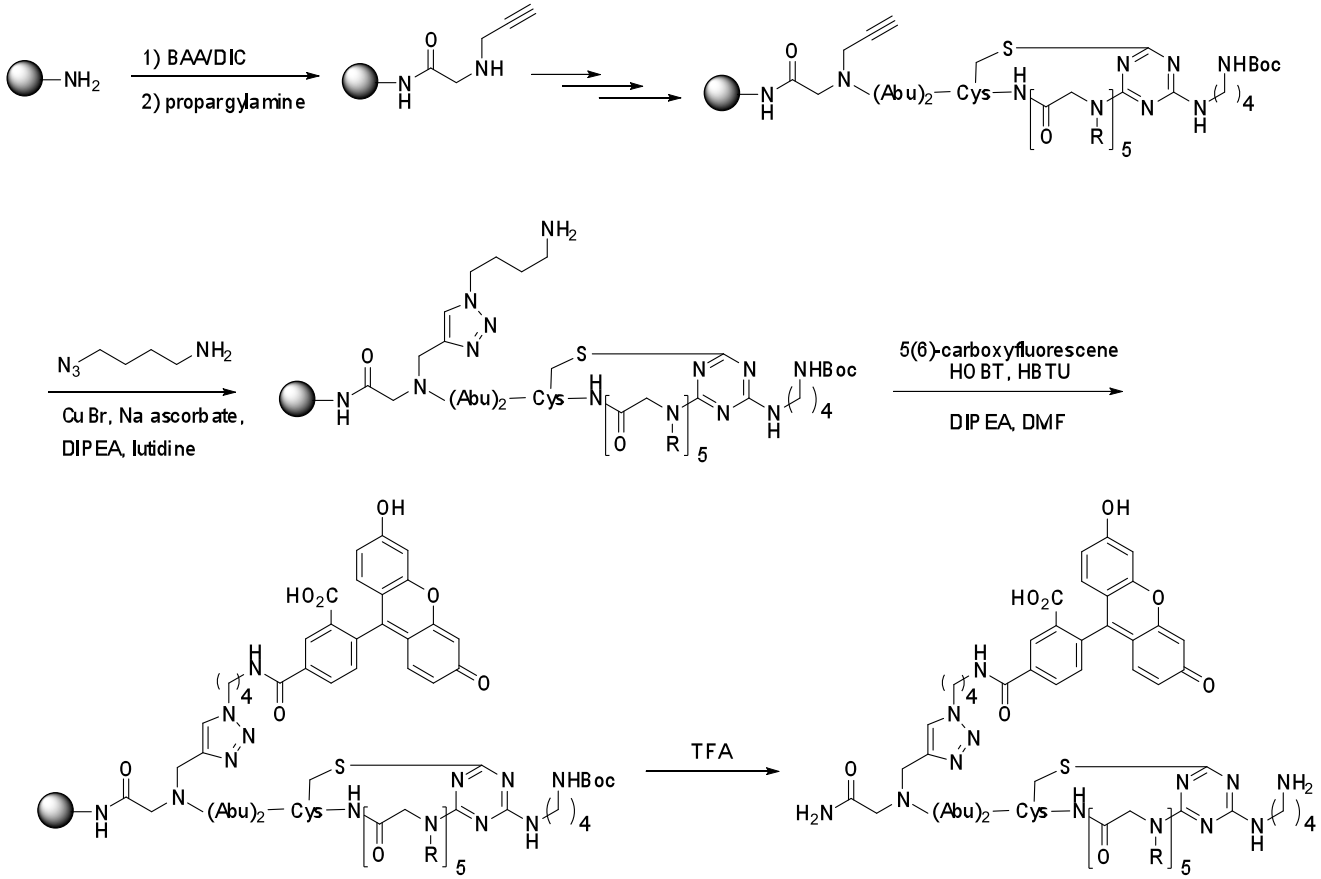
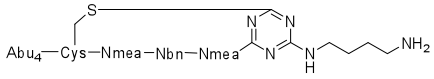


Table S1. Cyclization efficiency. The purity was accessed by HPLC of crude products.

n # of peptoid residues	Purity (%)	Calculated mass (M)	Observed mass $(M+H)^+$
3	88	1000.53	1001.44
4	85	1147.60	1148.52
5	82	1262.60	1263.58
6	84	1409.73	1410.54
7	80	1524.79	1525.70
10	77	1933.99	1934.99

Figure S1. RP-HPLC and MALDI-TOF MS spectra of peptoids after cyclization.



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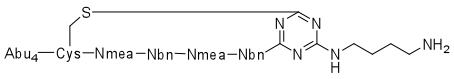
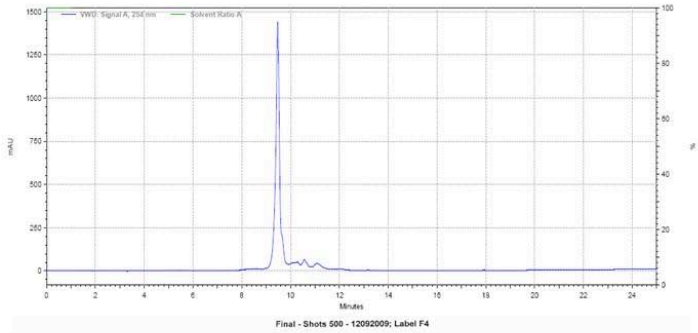
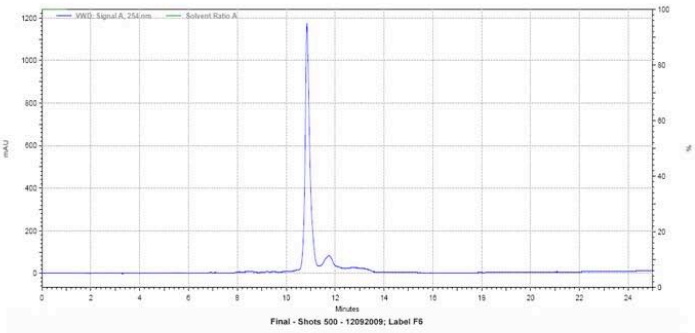
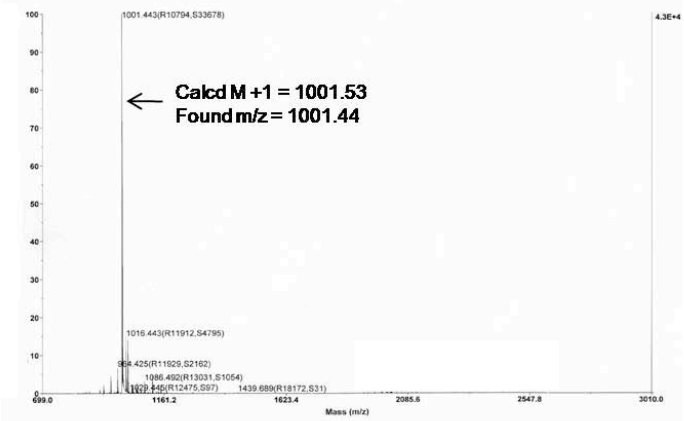
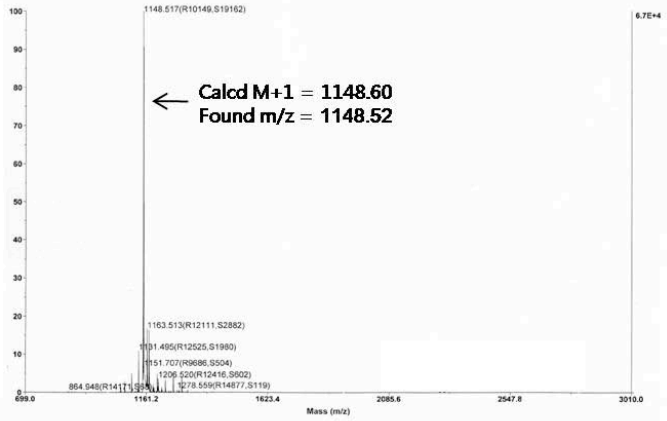


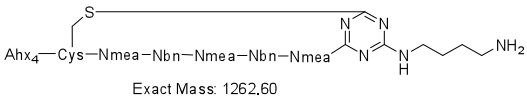
Exact Mass: 1147.60











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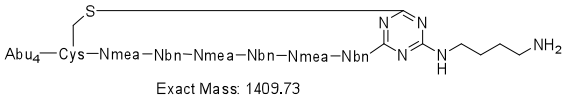

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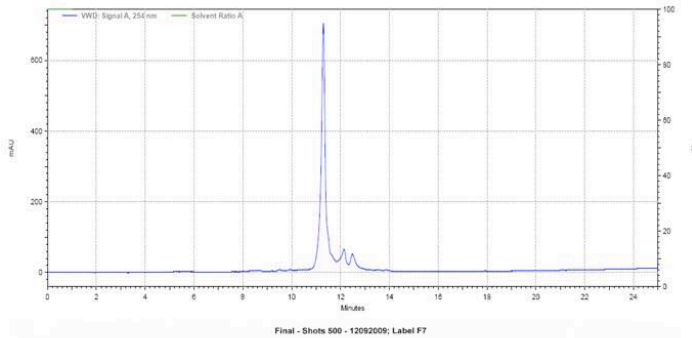
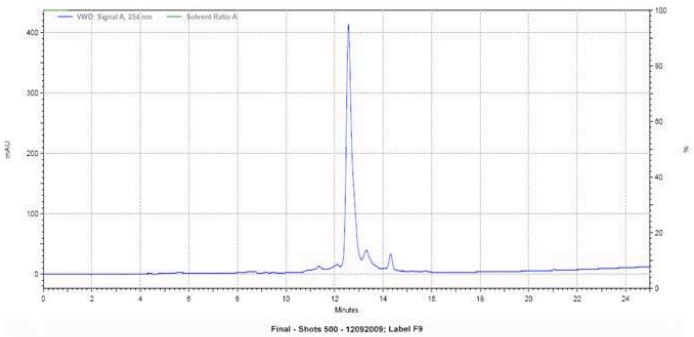
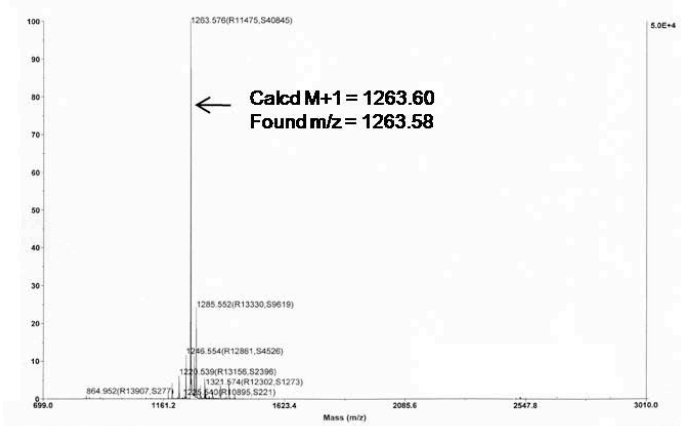






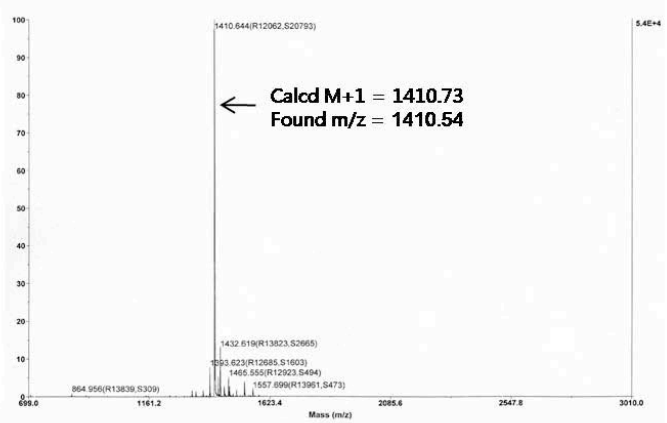


Figure S1. (Cont'd)

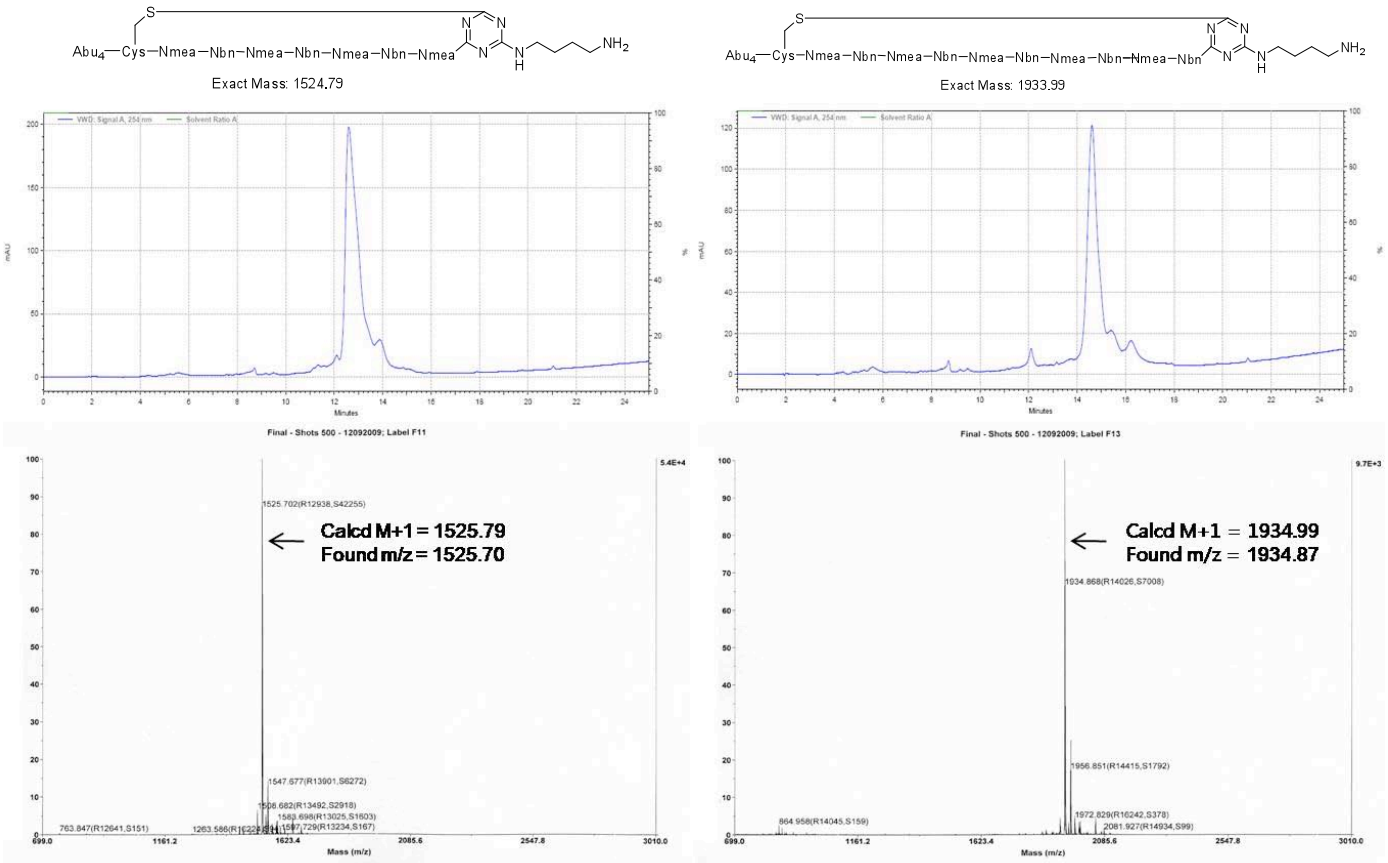


Figure S2. LC/MS traces of cyclic peptoids and linear peptoids.

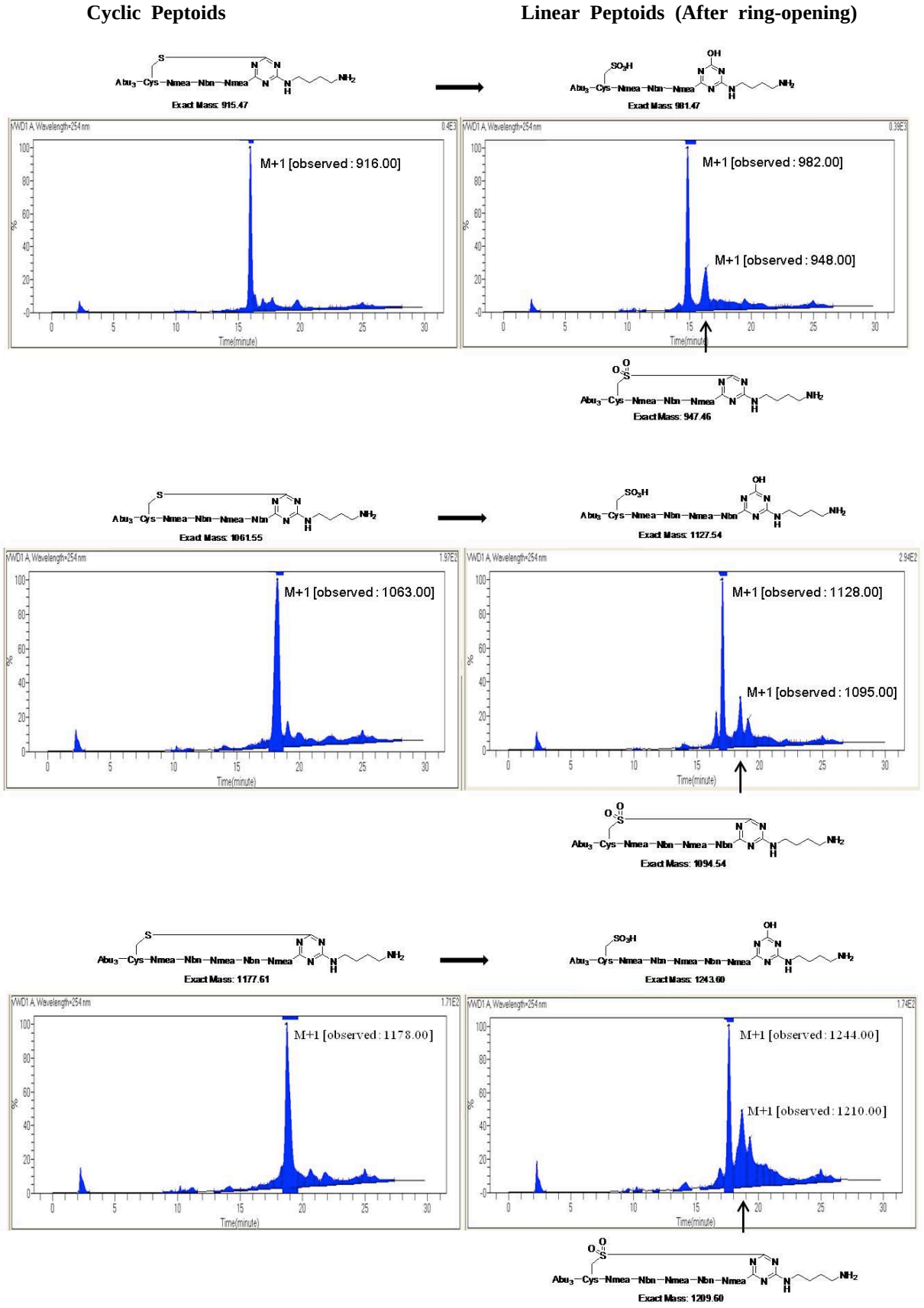
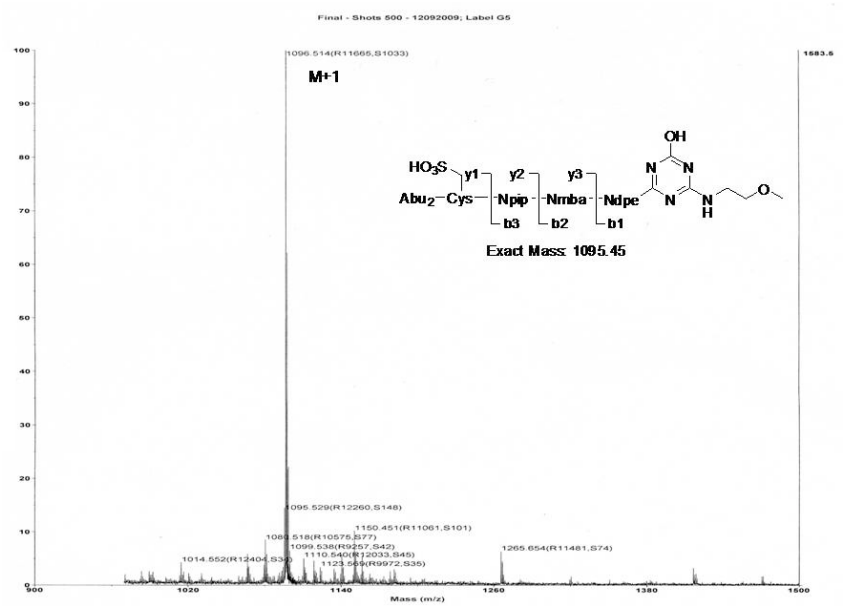


Figure S3. MS and sequence analysis of Abu_2-Cys(SO_3H)-Npip-Nmba-Ndpe-triazine-Nmea. (a) MS, (b) MS/MS.

(a)



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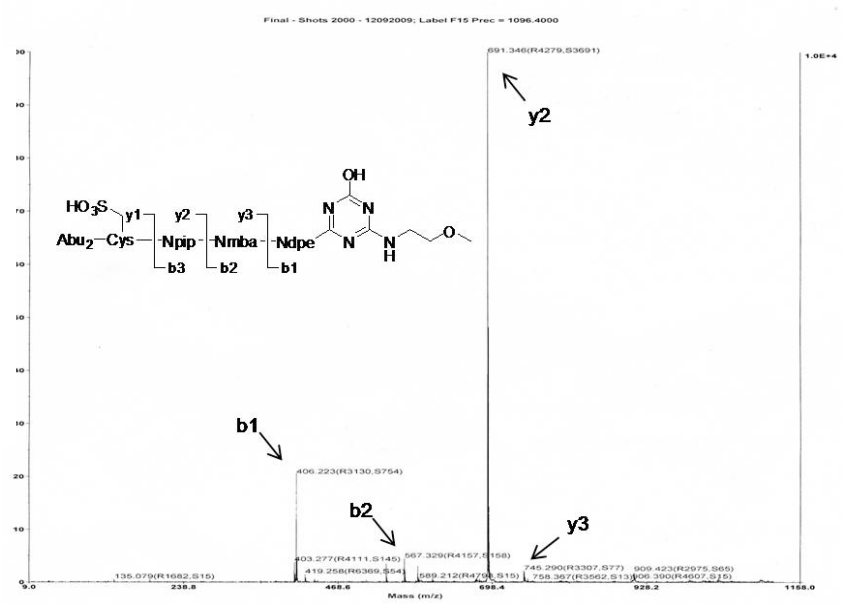

Figure S4. MS and sequence analysis of Abu_4-Cys(SO_3H)-Nmea-Nbn-Nmea-triazine-NH_2. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

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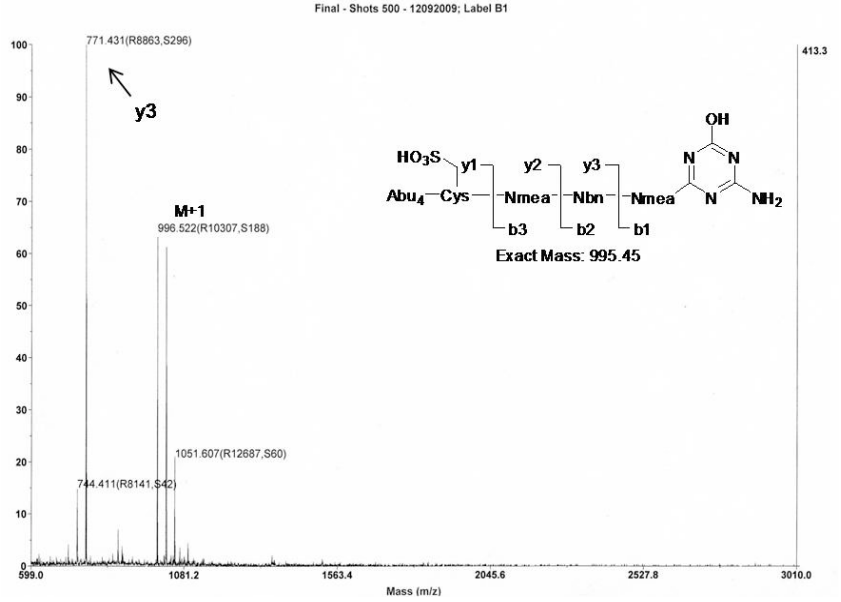

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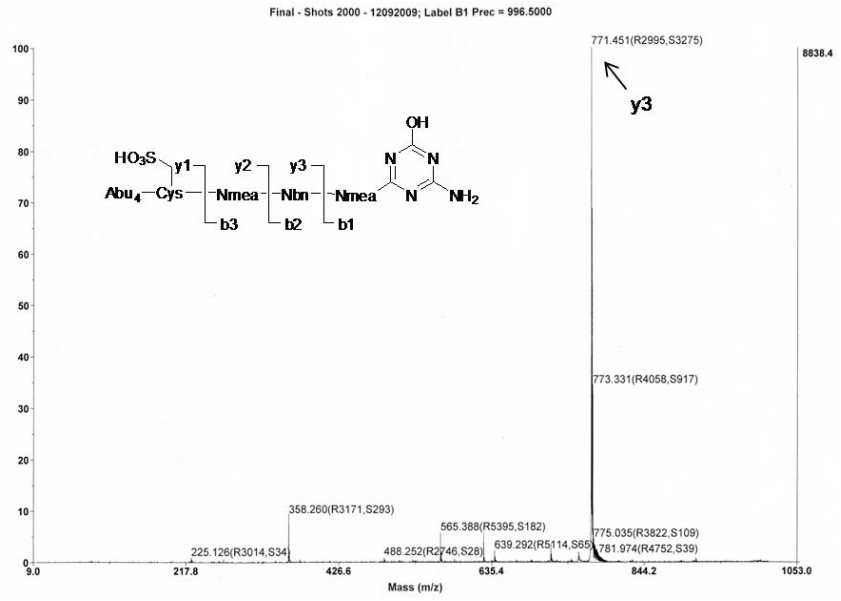

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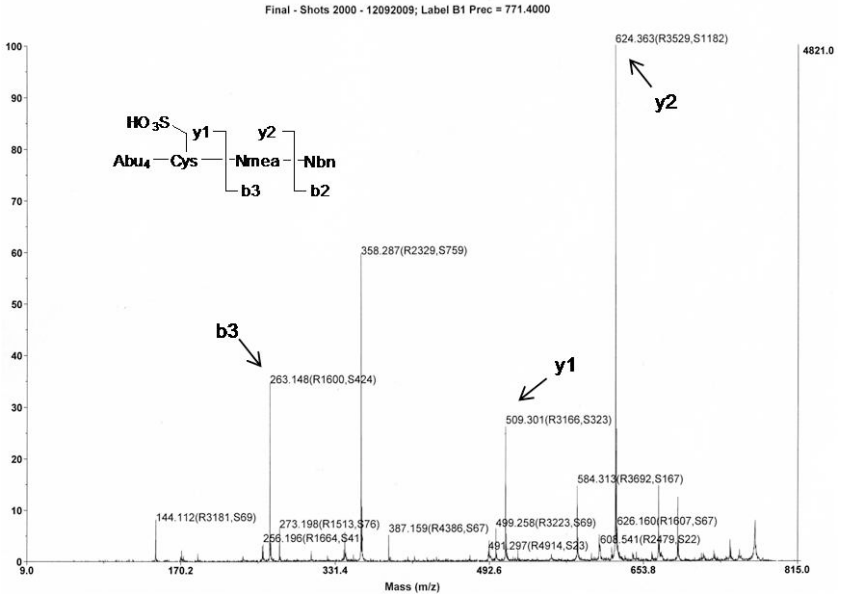
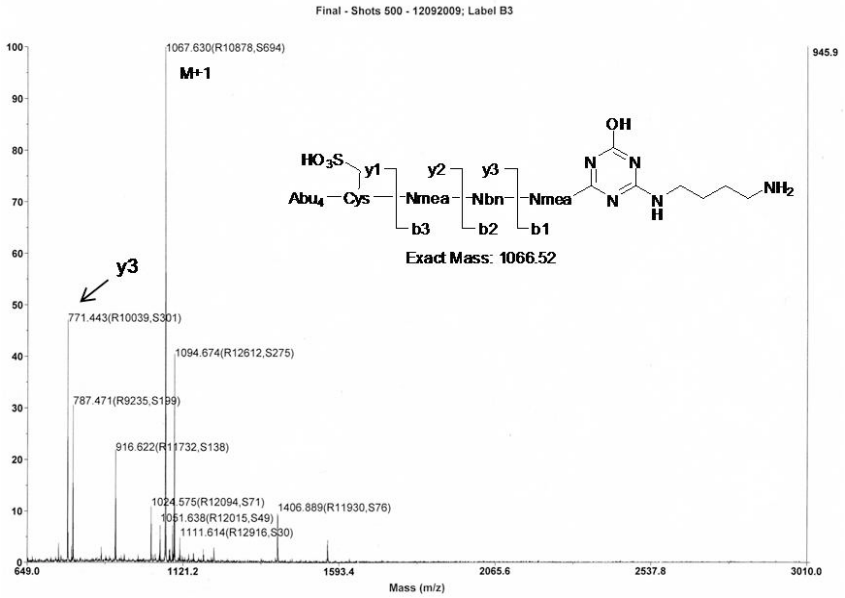


Figure S5. MS and sequence analysis of Abu_4-Cys(SO_3H)-Nmea-Nbn-Nmea-triazine-Nlys. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

(a)



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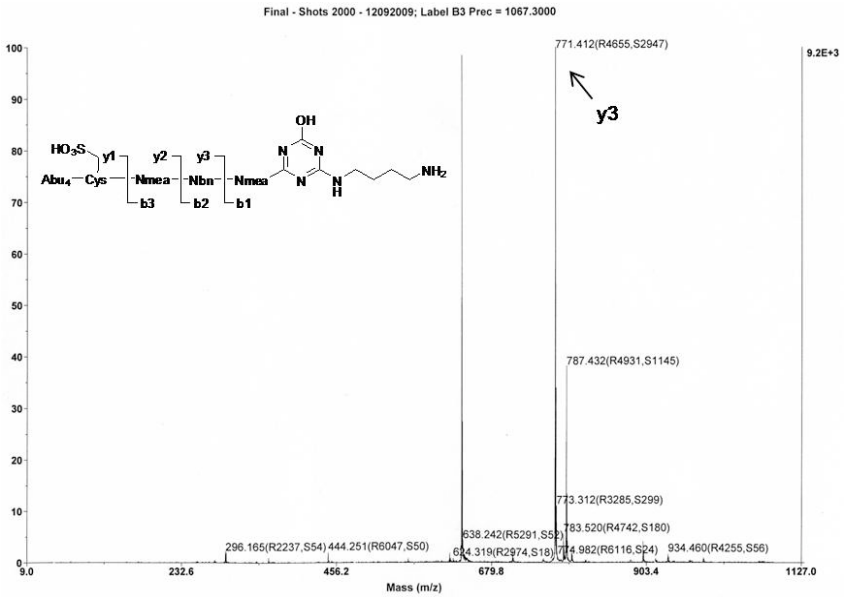

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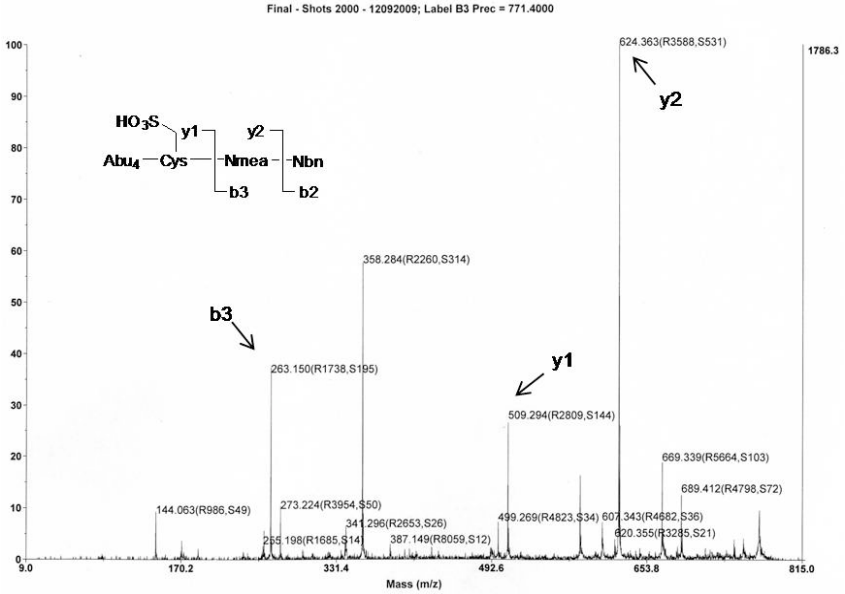
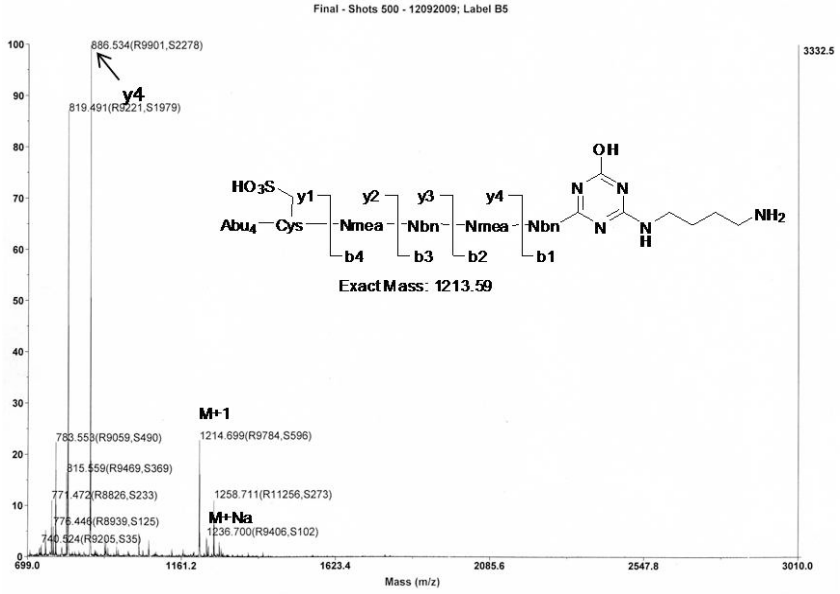


Figure S6. MS and sequence analysis of Abu_4-Cys(SO_3H)-Nmea-Nbn-Nmea-Nbn-triazine-Nlys. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

(a)



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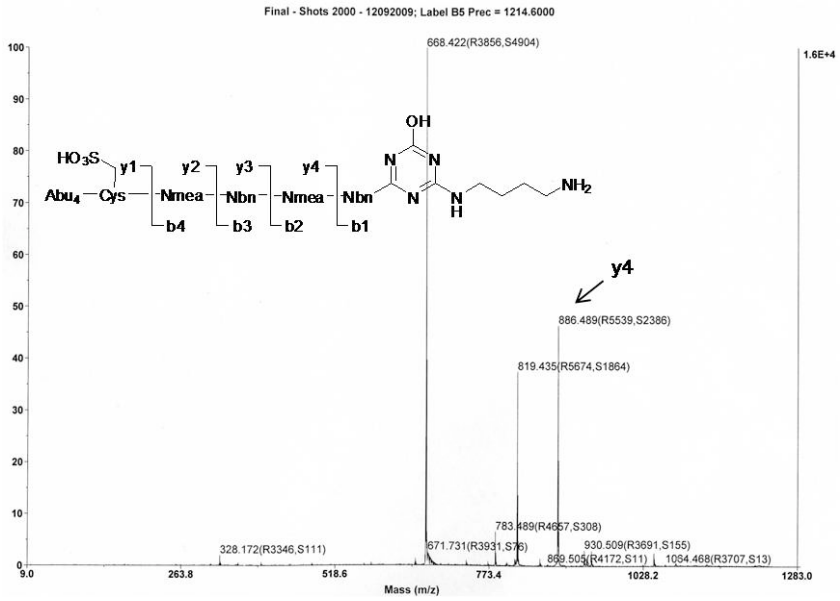

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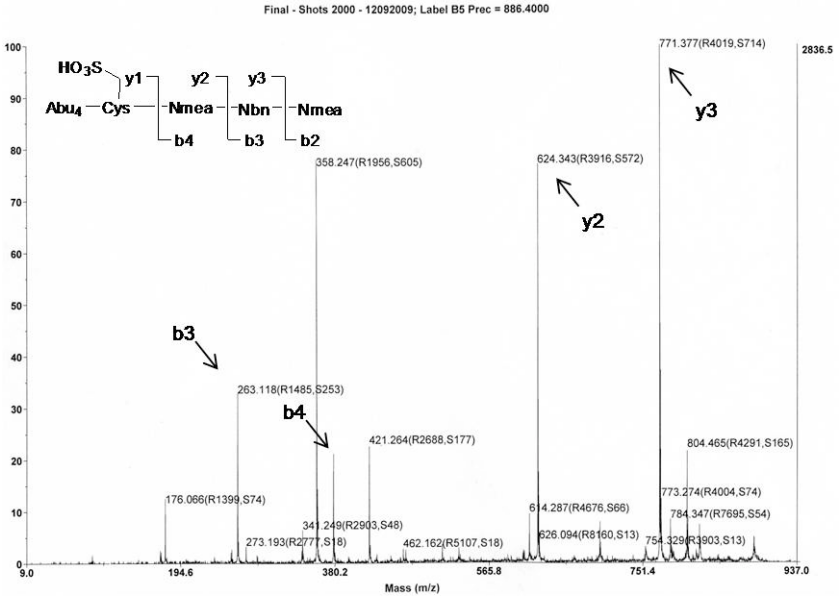
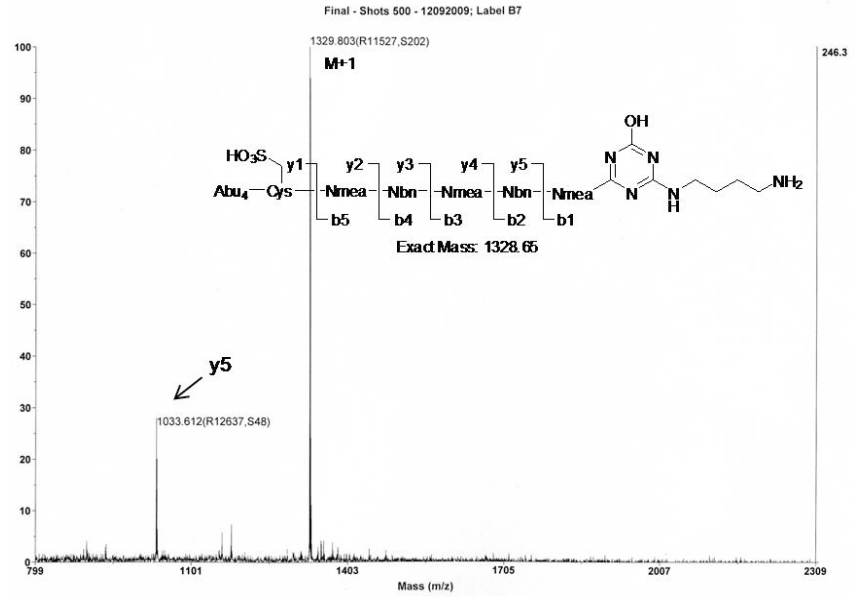


Figure S7. MS and sequence analysis of Abu_4-Cys(SO_3H)-Nmea-Nbn-Nmea-Nbn-Nmea-triazine-Nlys. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

(a)



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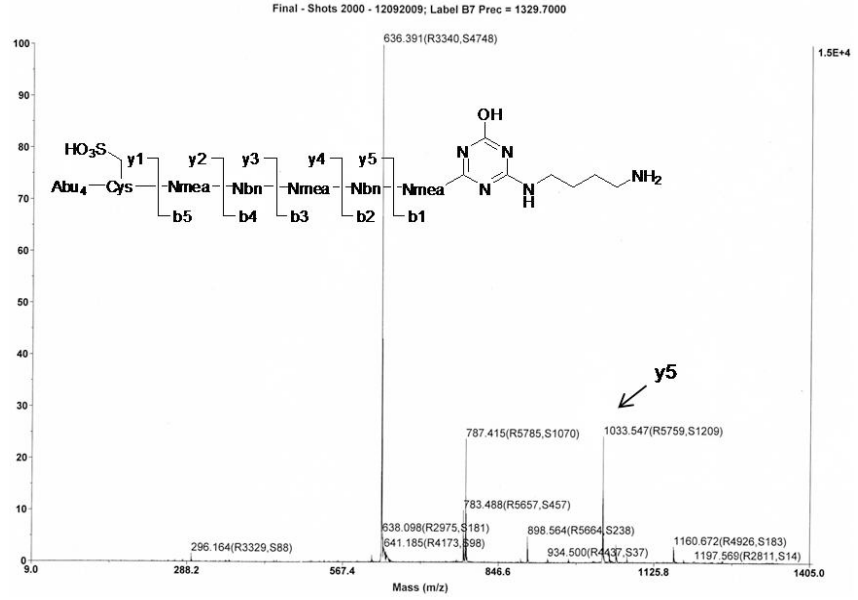

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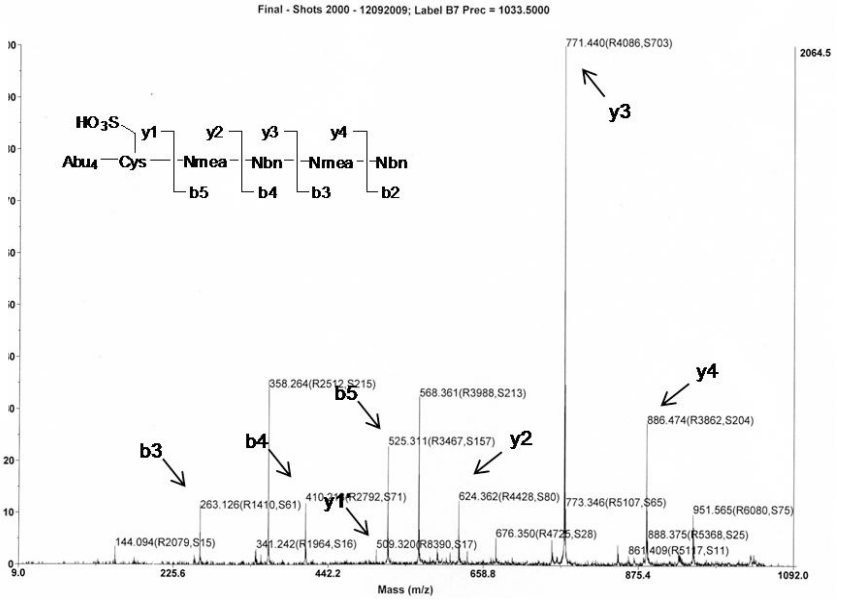

Figure S8. MS and sequence analysis of $(Abu)_4$-Cys(SO_3H)-Nmea-Nbn-Nmea-Nbn-Nmea-Nbn-triazine-Nlys. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

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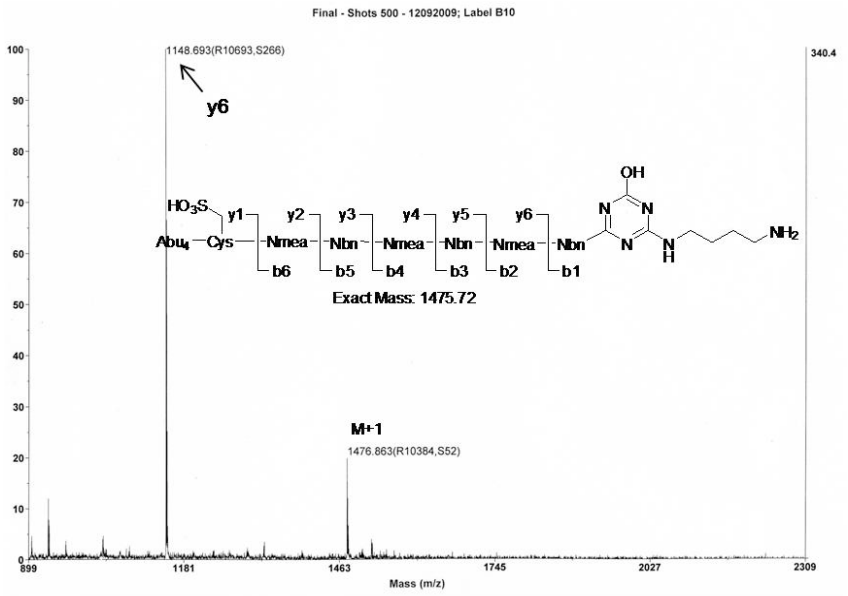

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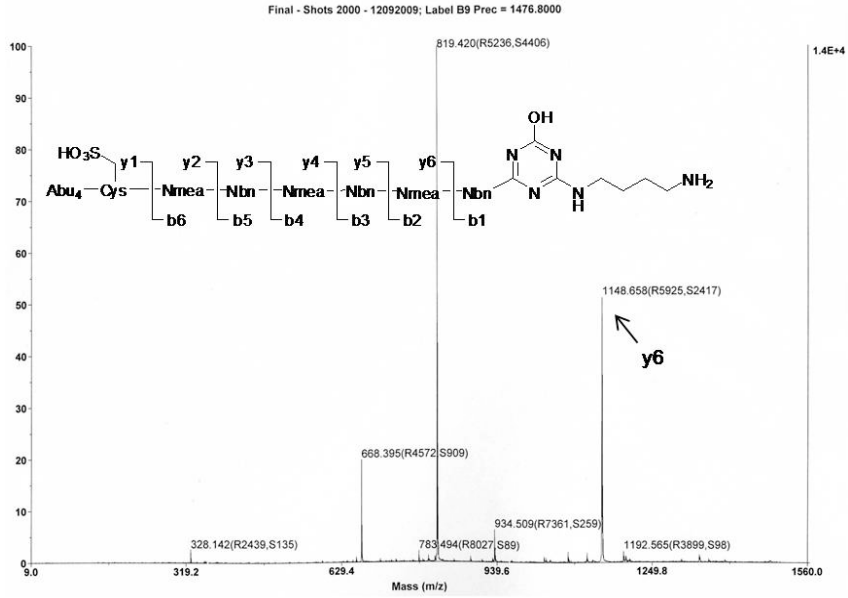

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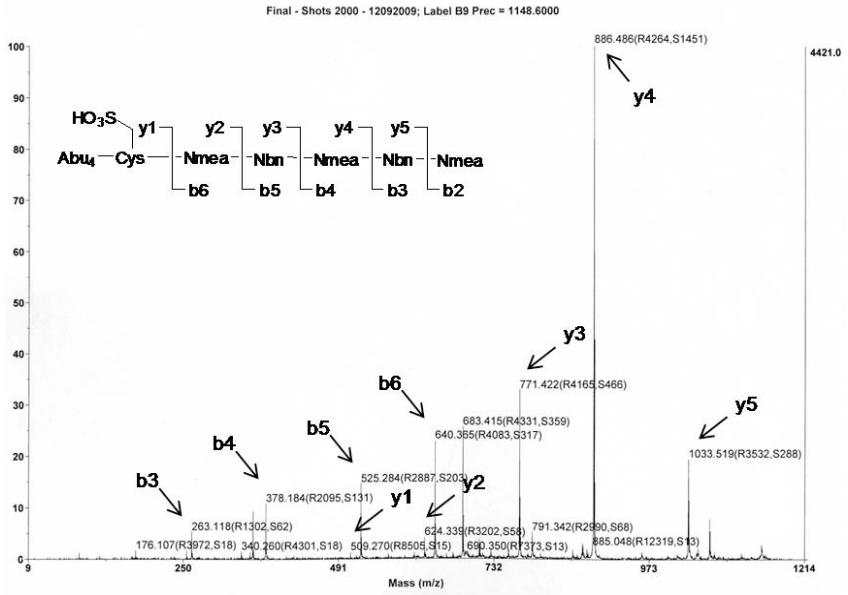
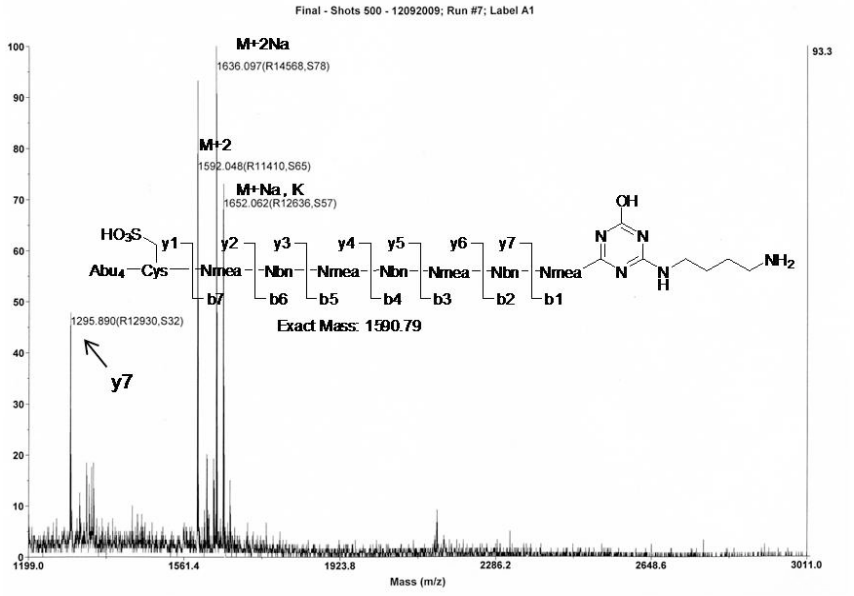


Figure S9. MS and sequence analysis of Abu_4-Cys(SO_3H)-Nmea-Nbn-Nmea-Nbn-Nmea-Nbn-Nmea-triazine-Nlys. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

(a)



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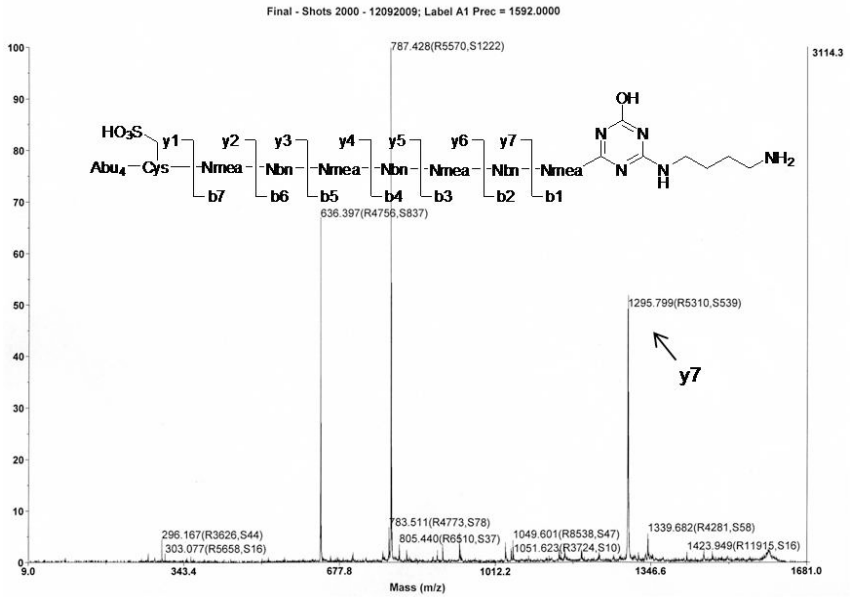


(c)

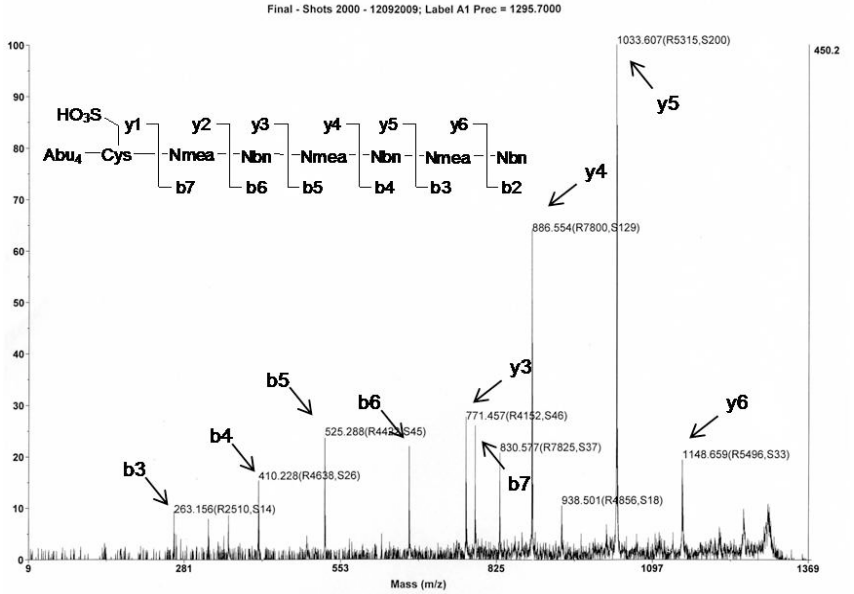


Figure S10. MS and sequence analysis of Abu_4-Cys(SO_3H)-Nmea-Nbn-Nmea-Nbn-Nmea-Nbn-Nmea-Nbn-Nmea-Nbn-triazine-Nlys. (a) MS, (b) MS/MS, (c) second MS/MS for b1 ion.

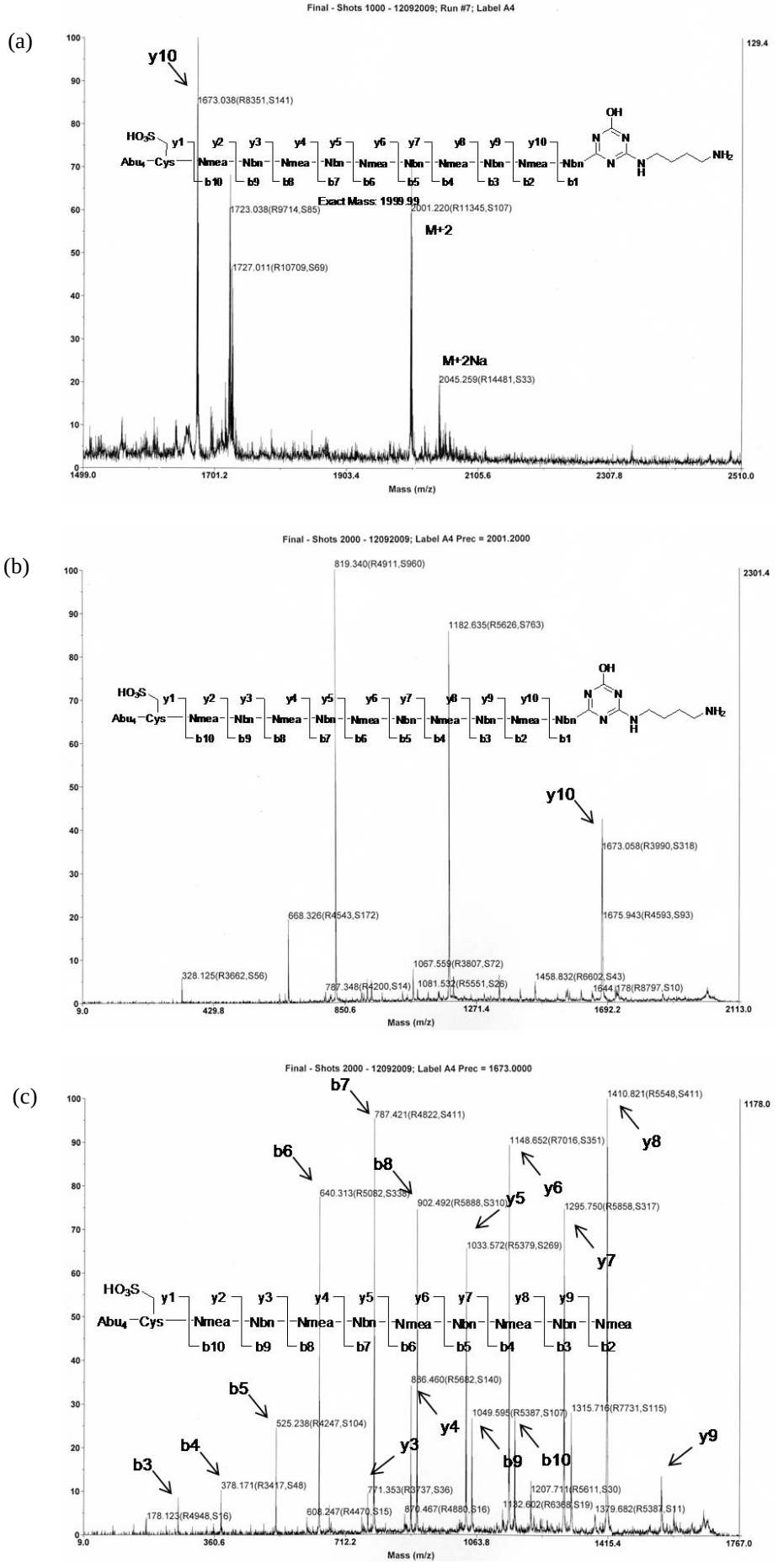


Figure S11. MALDI-TOF MS spectra of 24 randomly selected beads from the cyclic peptoid library.

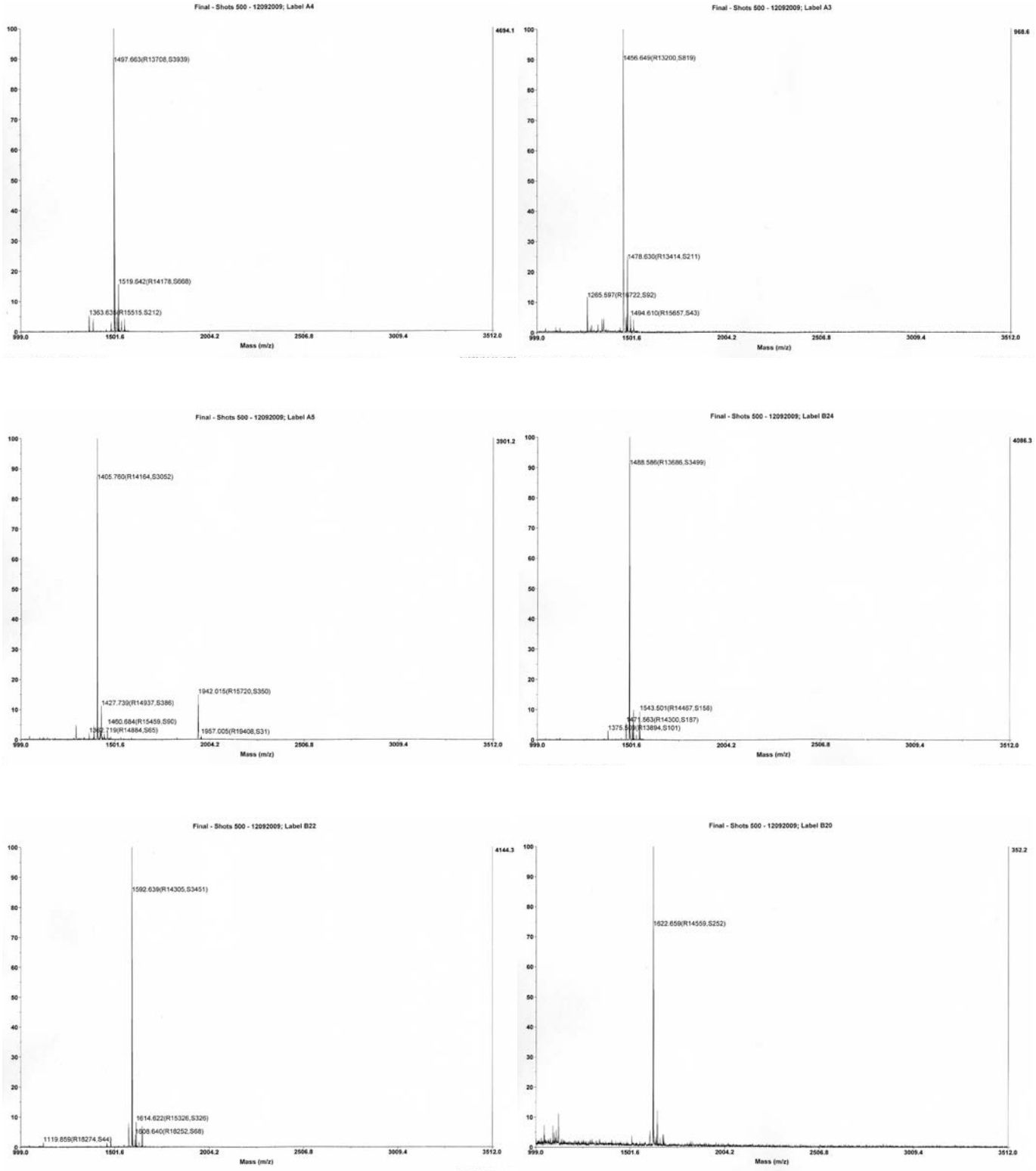


Figure S11. (Cont'd)

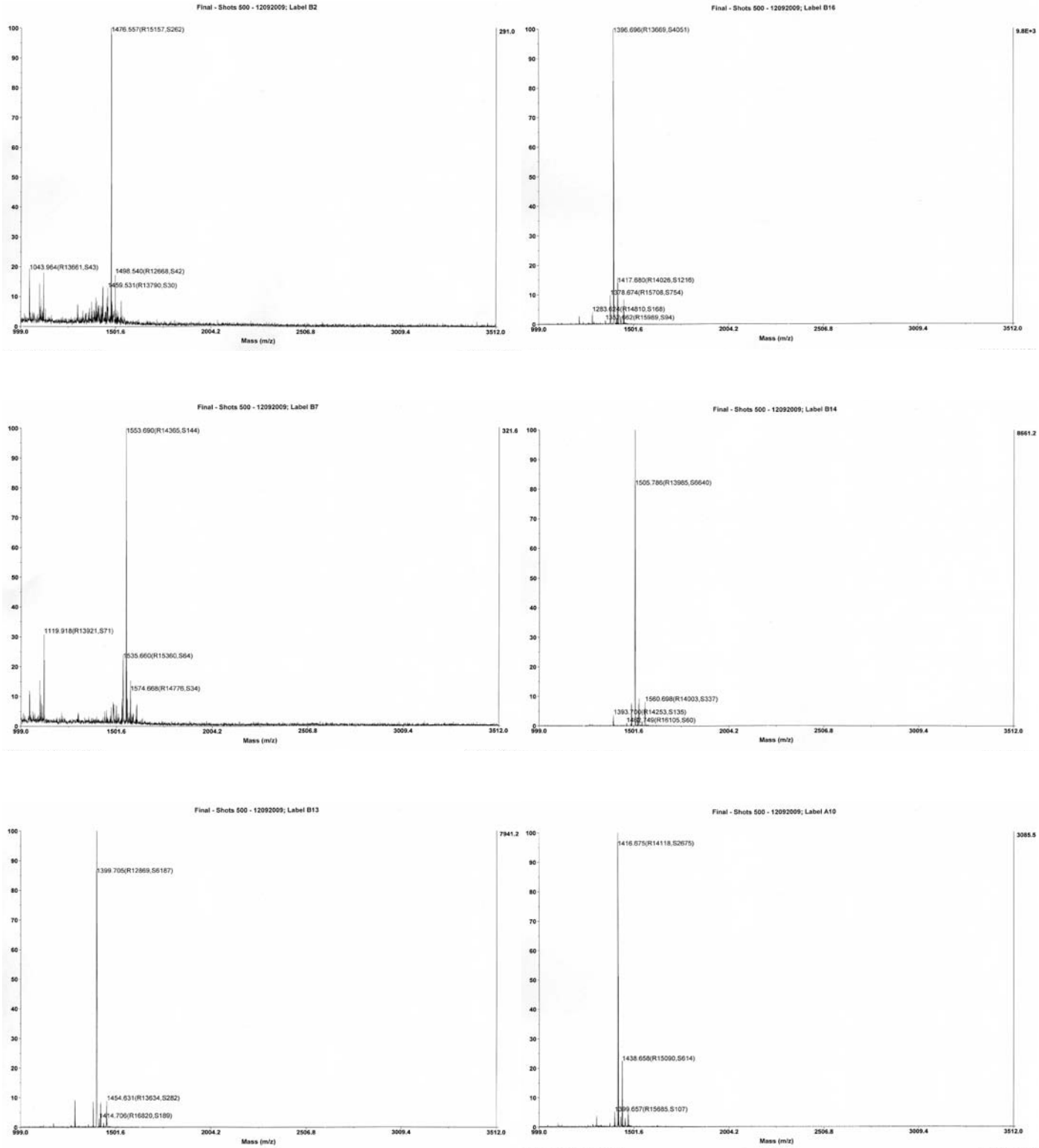


Figure S11. (Cont'd)

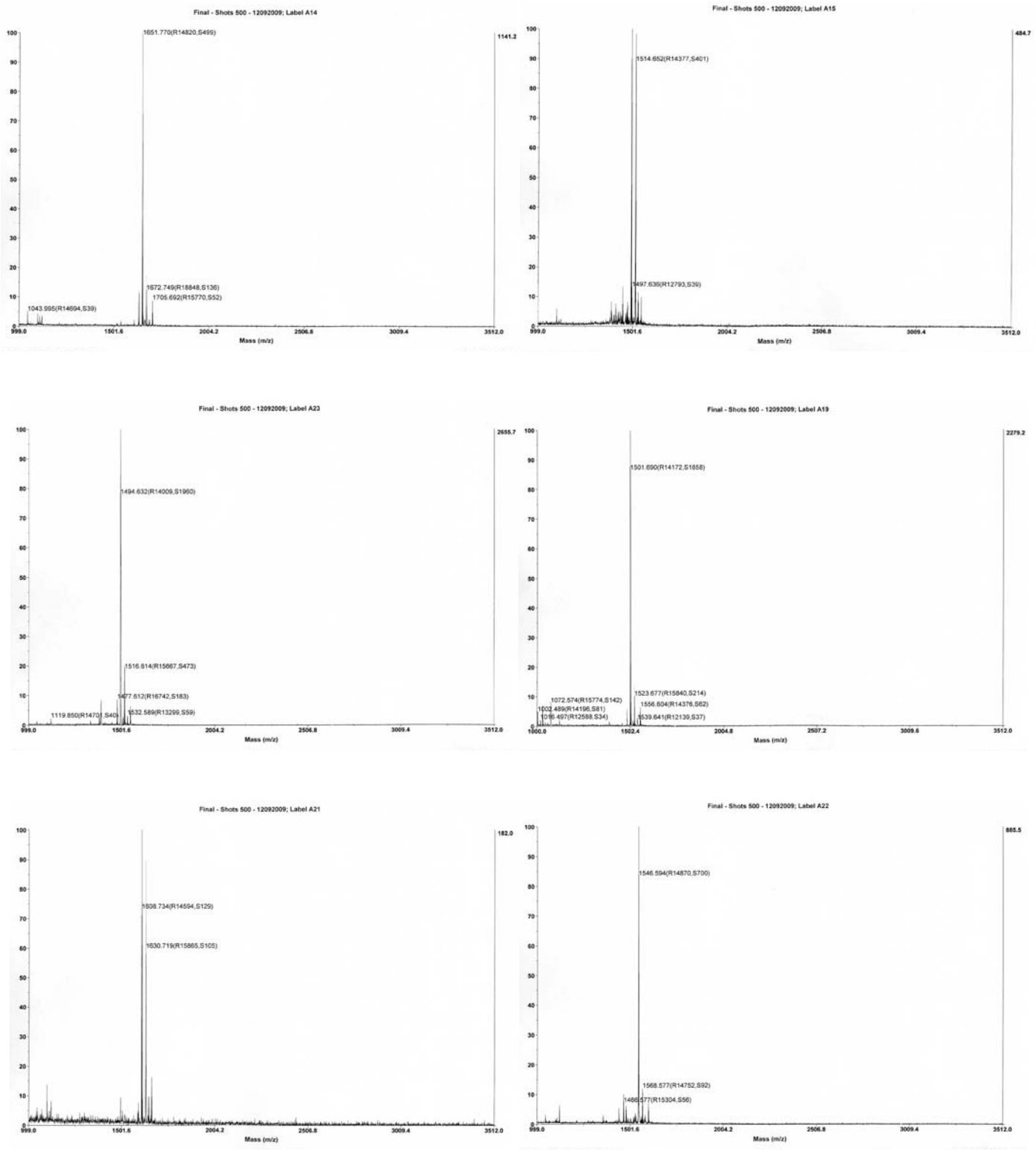


Figure S11. (Cont'd)

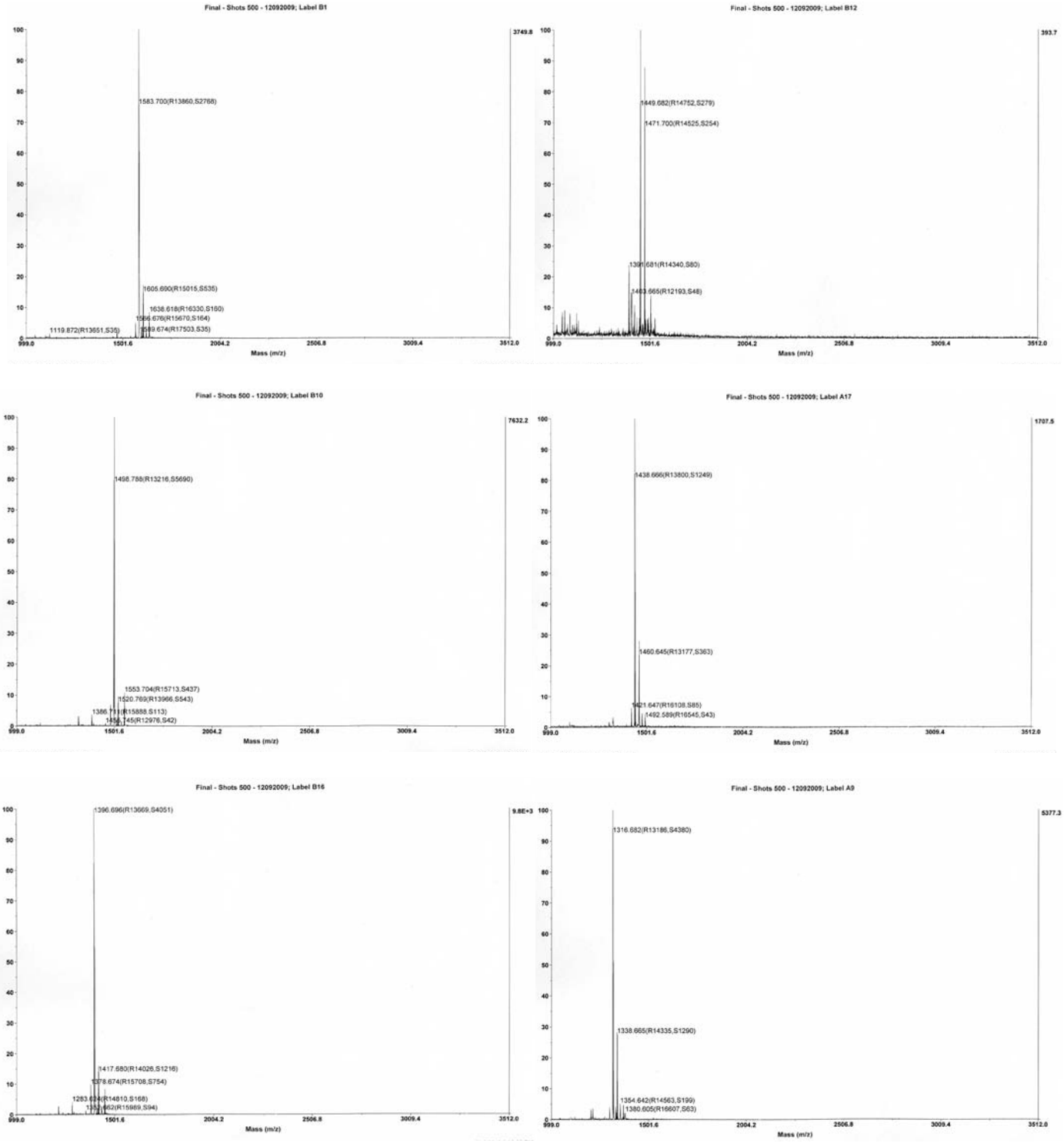


Figure S12. Schematic representation of on-bead screening using magnetic separation.

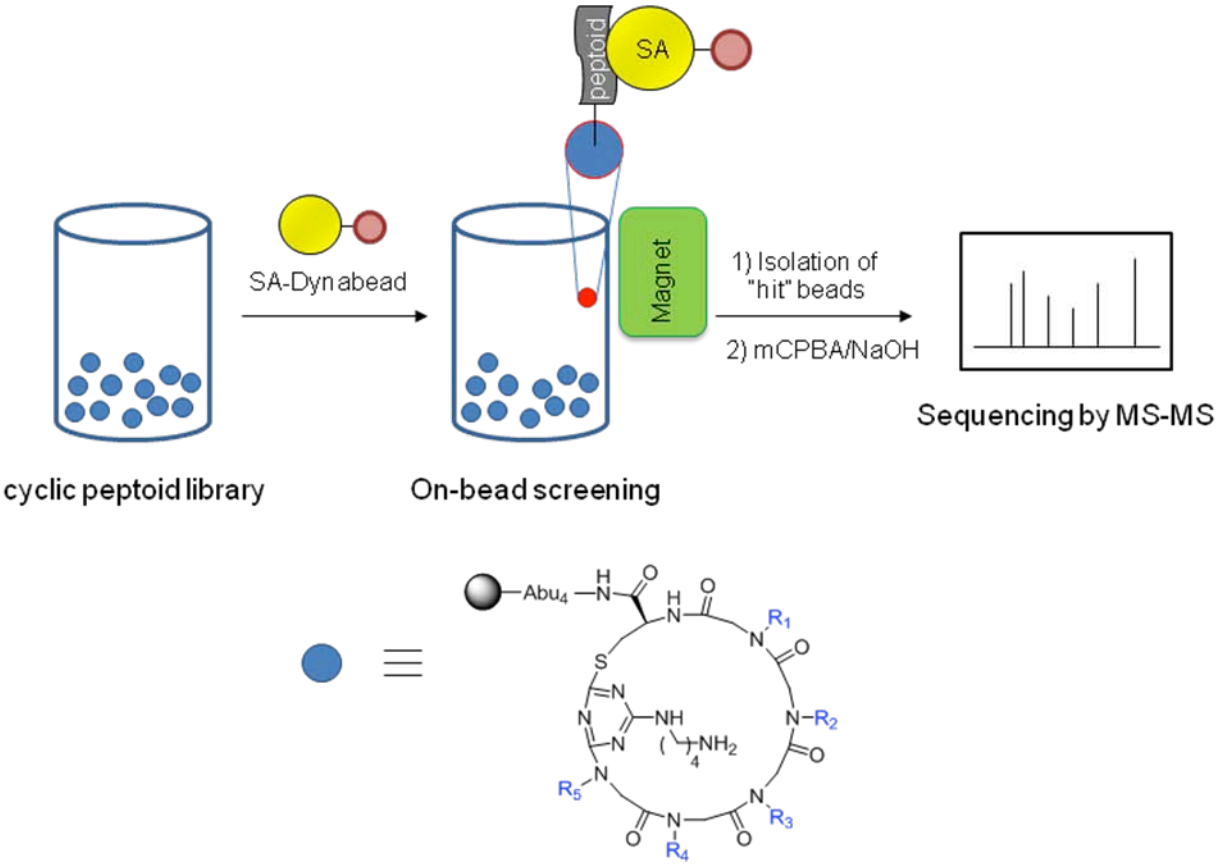
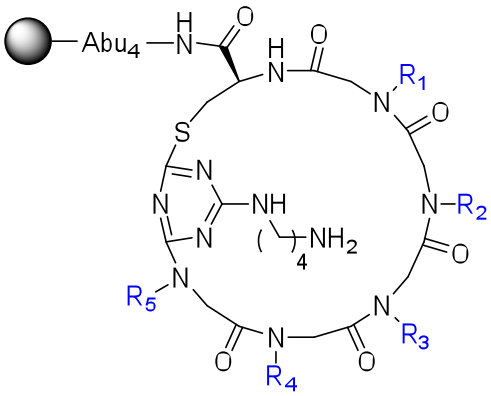


Figure S13. General structure of a pentameric cyclic peptoid library used for screening against streptavidin and sequences of the 14 cyclic peptoids identified from the screen.



Hit #	R_1	R_2	R_3	R_4	R_5
1	Nmba	Napp	Nmea	Nmba	Nleu
2	Nmea	Napp	Nnap	Npip	Nleu
3	Nnap	Napp	Ncpe	Nleu	Napp
4	Npip	Nmea	Nnap	Nlue	Napp
5	Nmba	Nmea	Nmea	Nmba	Napp
6	Ndpe	Napp	Napp	Npip	Ncpe
7	Nleu	Nnap	Napp	Napp	Nmba
8	Nnap	Napp	Npip	Ndpe	Napp
9	Ndpe	Napp	Napp	Npip	Ncpe
10	Ncpe	Napp	Ndpe	Nmea	Napp
11	Npip	Ncpe	Nmea	Nbn	Nbn
12	Nmea	Nnap	Napp	Nbn	Npip
13	Ndcp	Nmea	Napp	Napp	Nmea
14	Nmea	Nbn	Nbn	Nleu	Napp
15	Ncpe	Npip	Napp	Napp	Nbn
16	Ncpe	Napp	Nmea	Ndpe	Napp

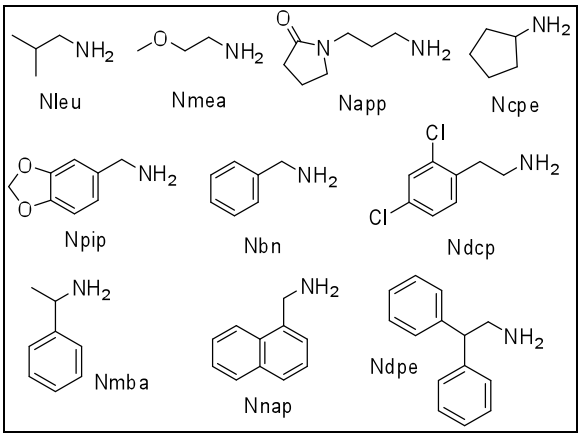
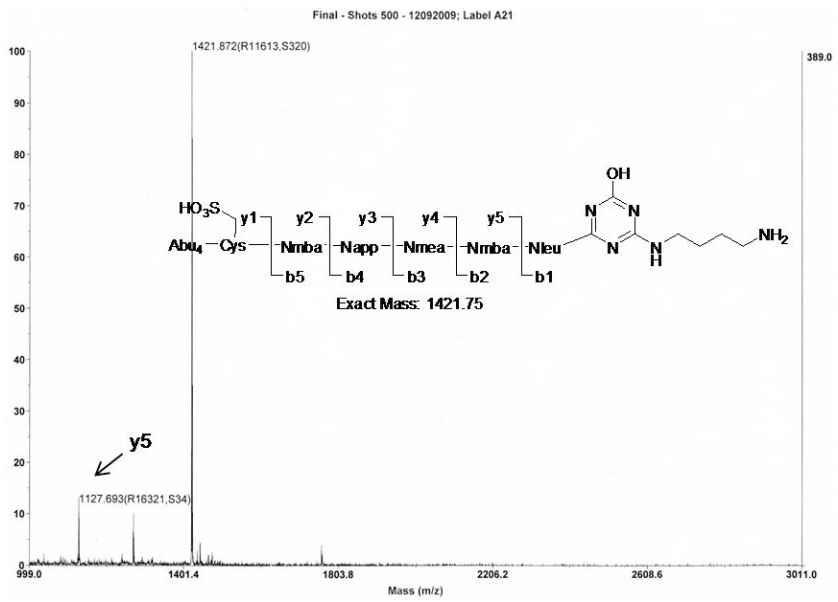
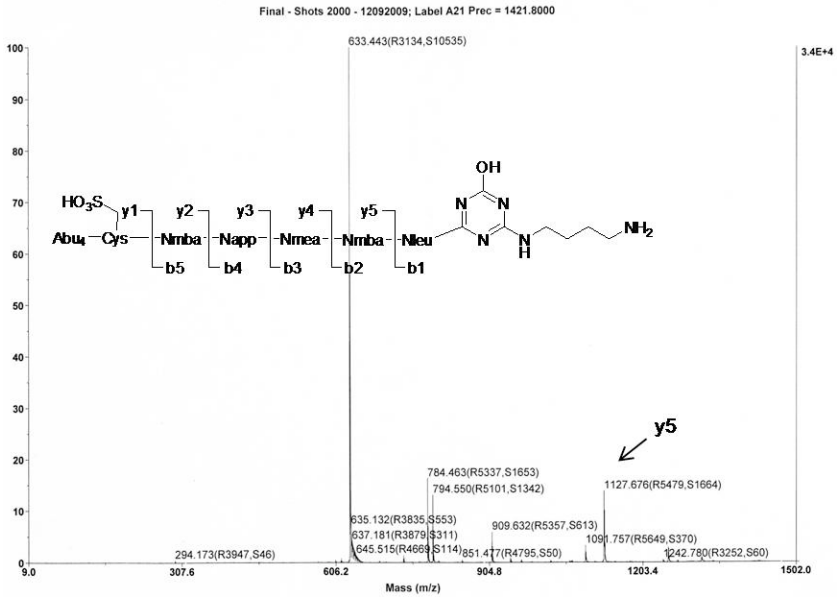


Figure S14. MS and MS/MS data of streptavidin-binding cyclic peptoids.

Hit 1





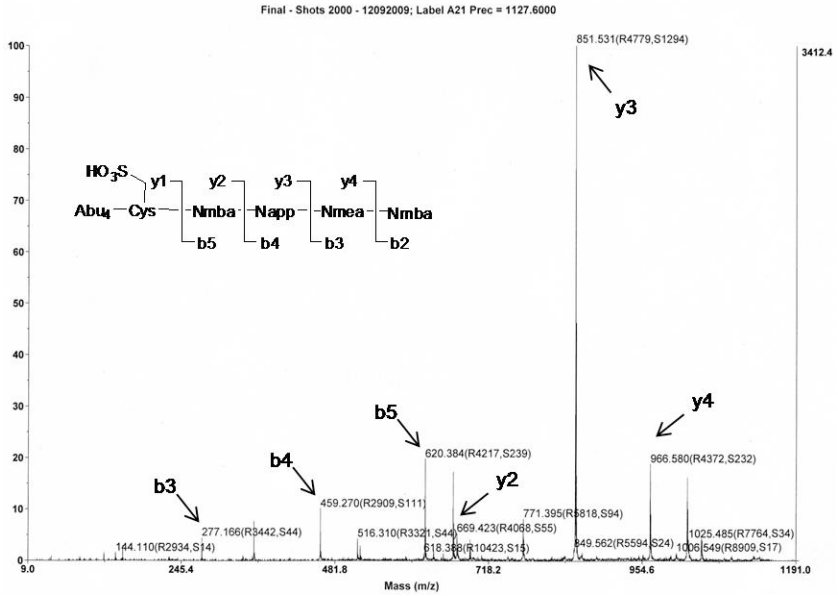
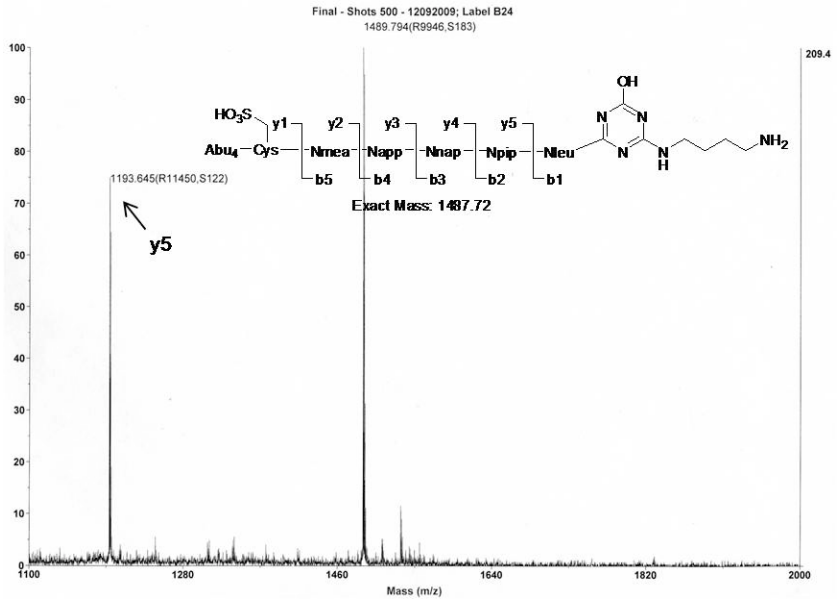
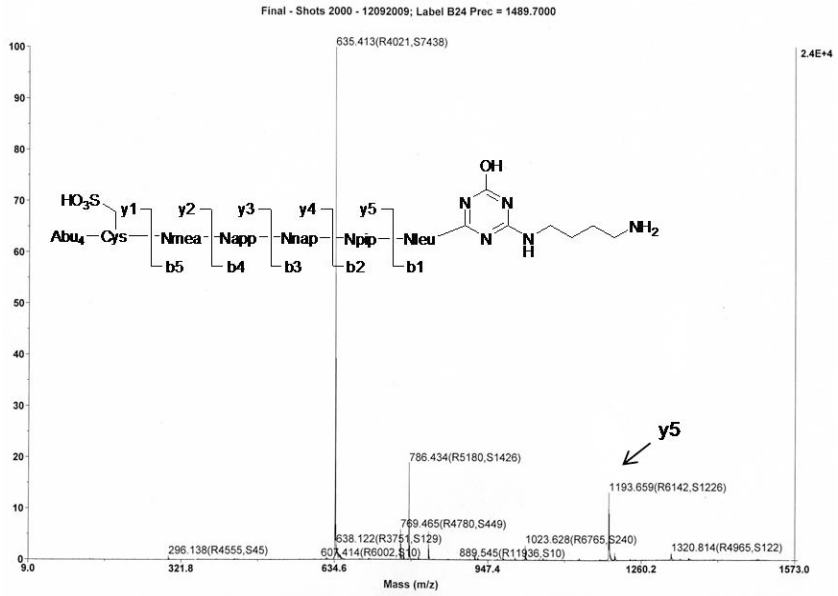


Figure S14. (Cont'd)

Hit 2





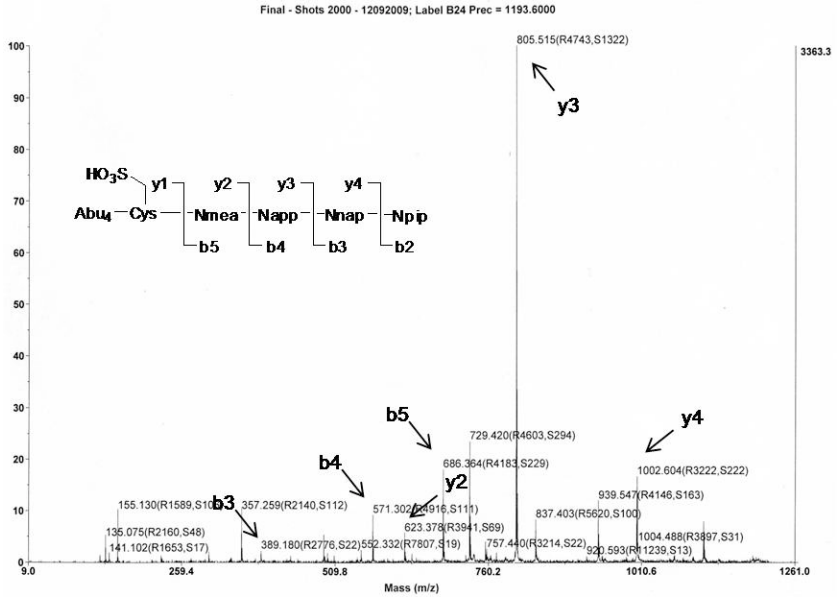


Figure S14. (Cont'd)

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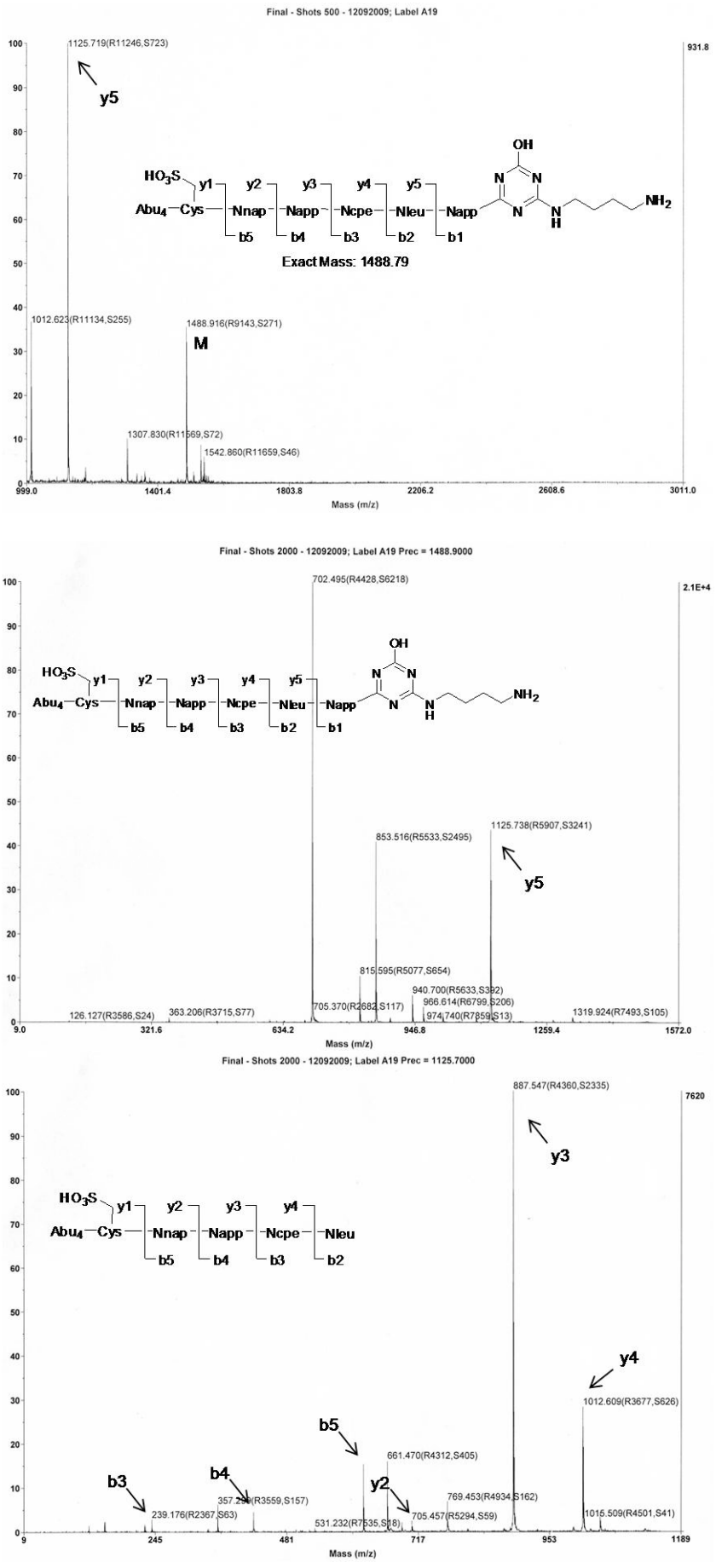

Figure S14. (Cont'd)

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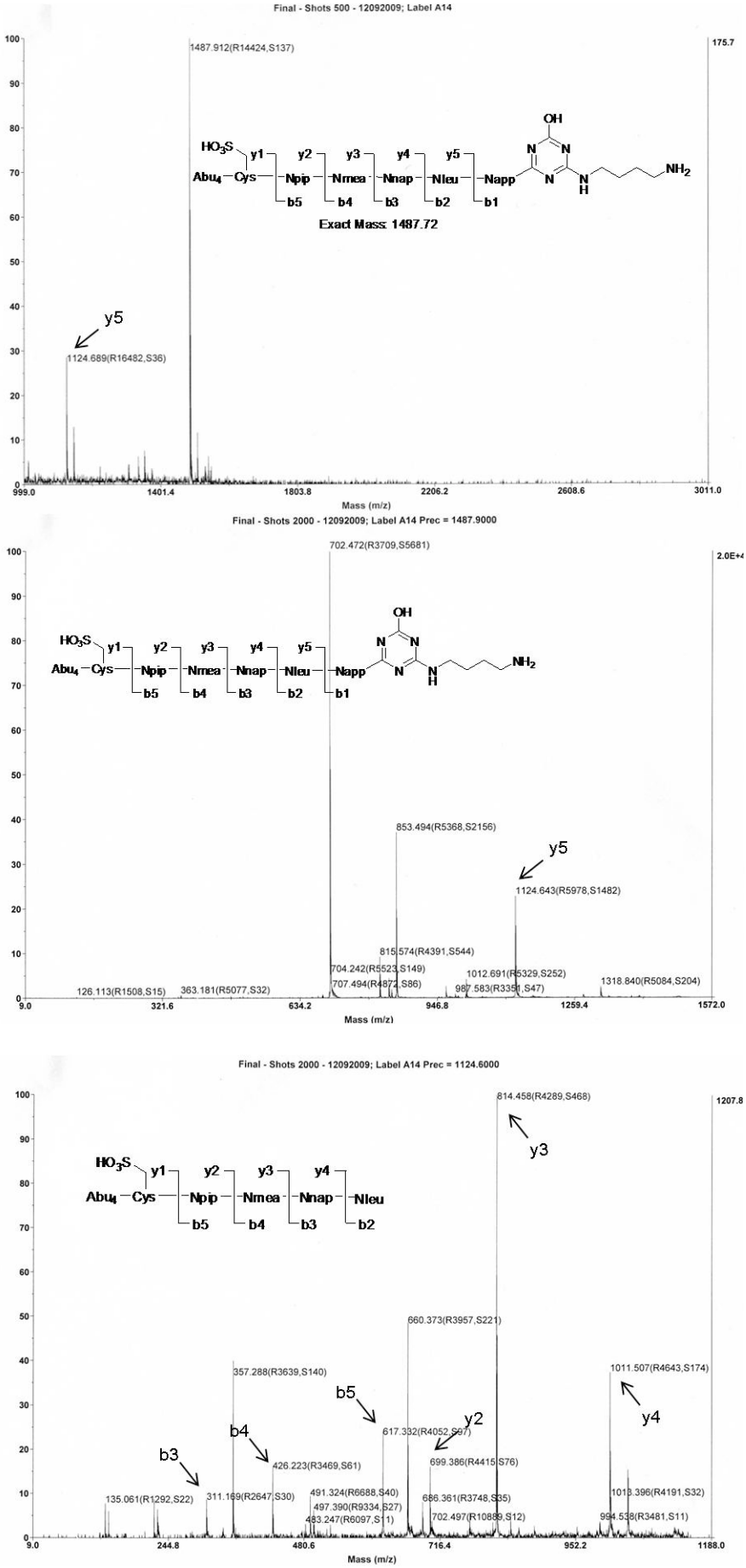

Figure S14. (Cont'd)

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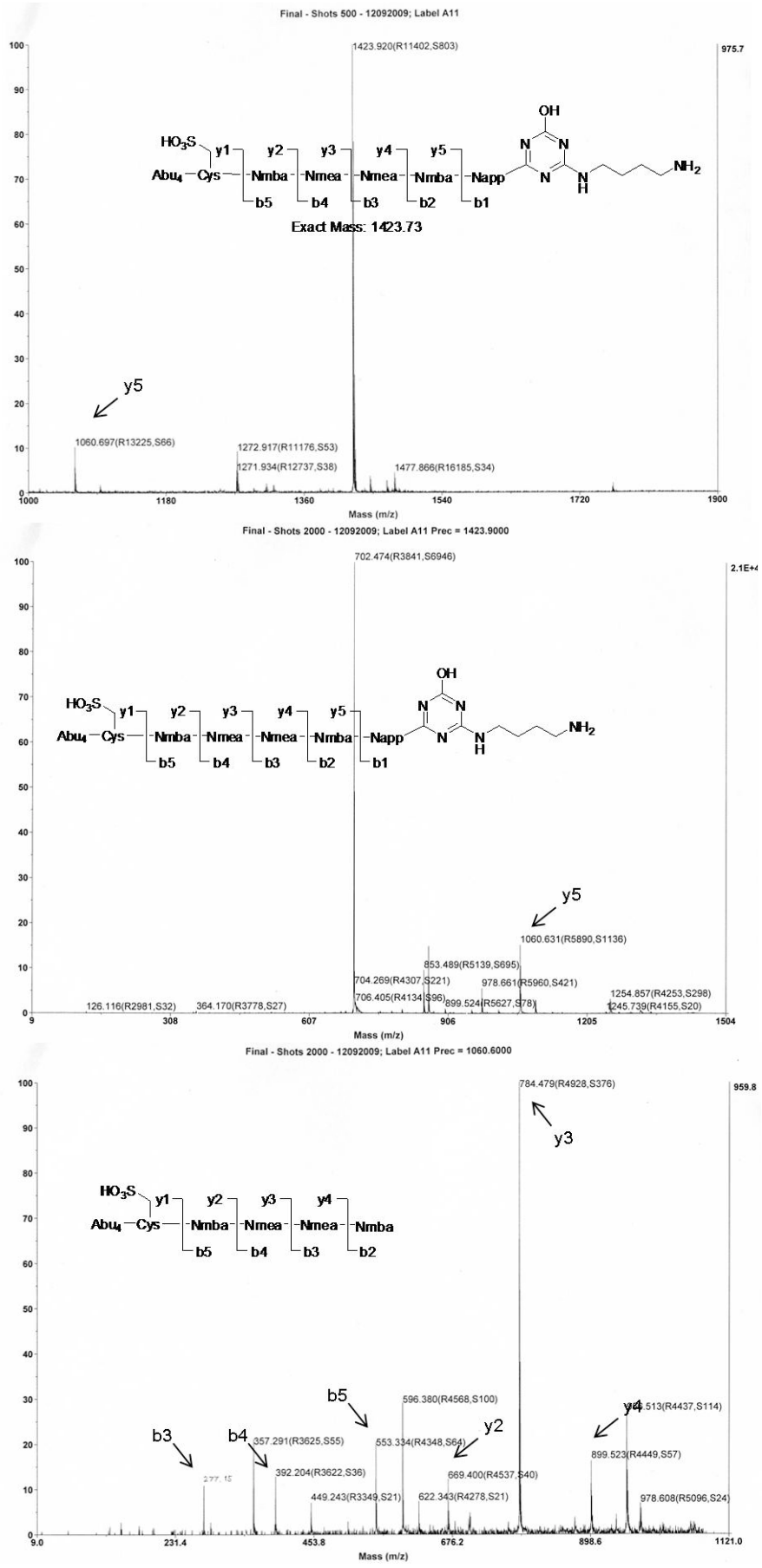

Figure S14. (Cont'd)

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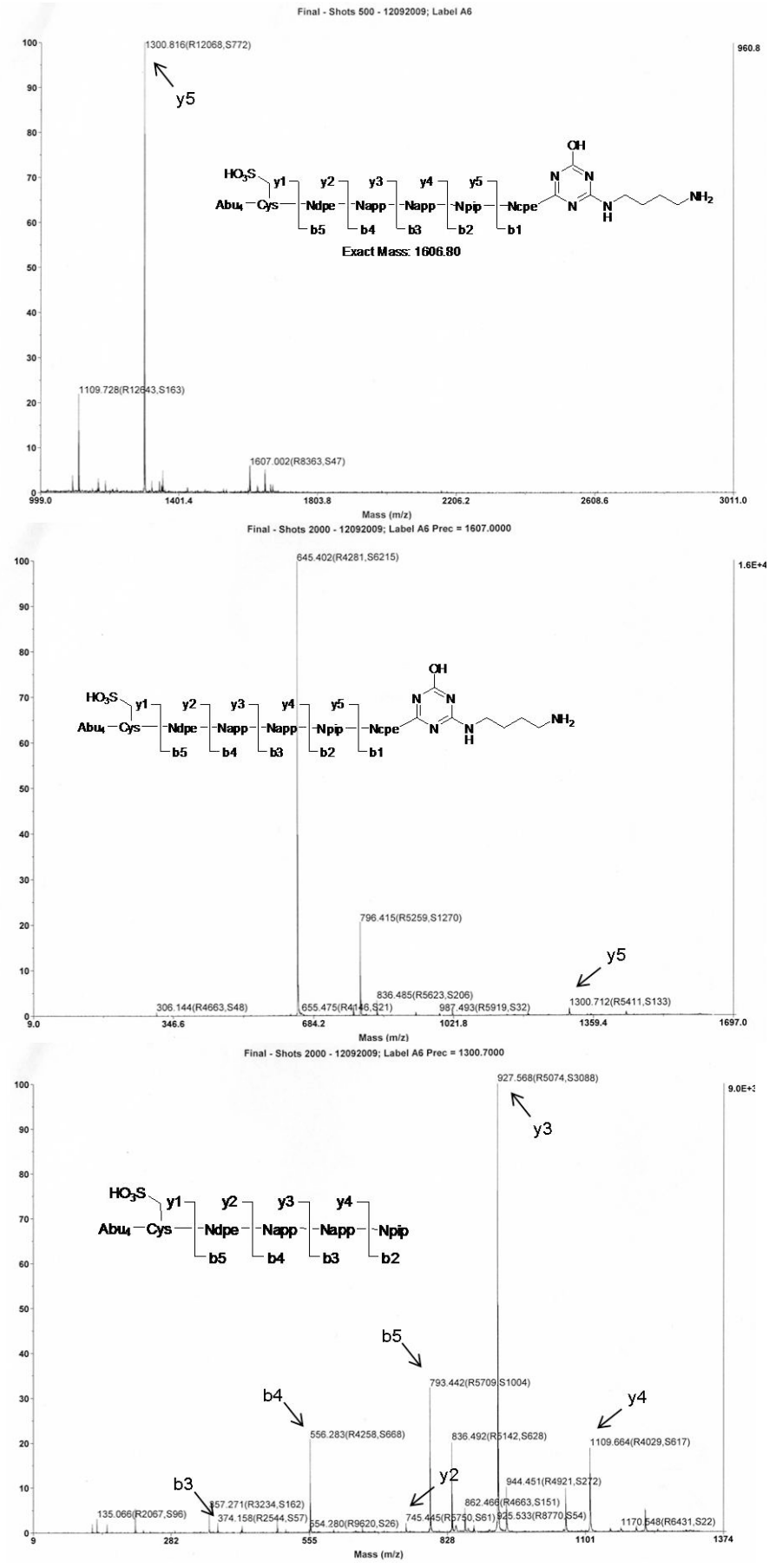
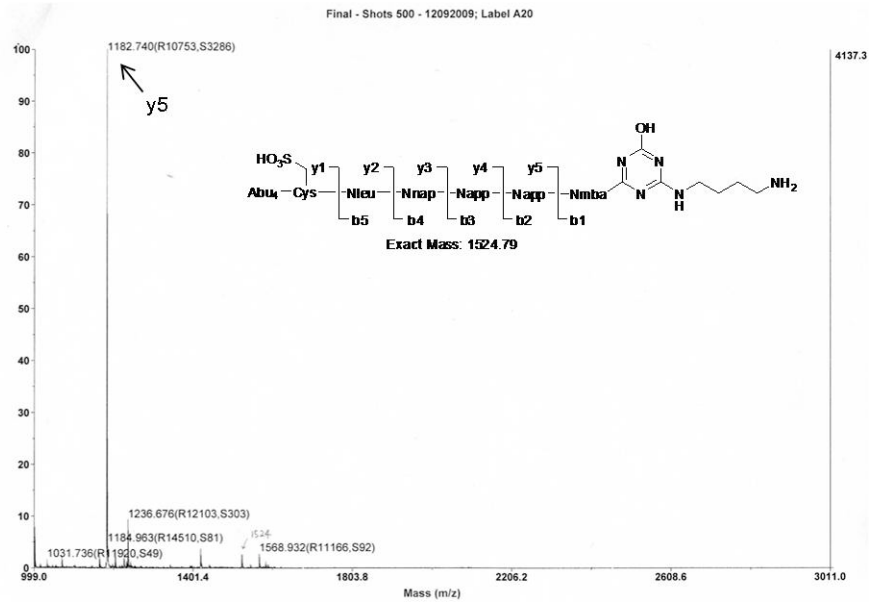


Figure S14. (Cont'd)

Hit 7



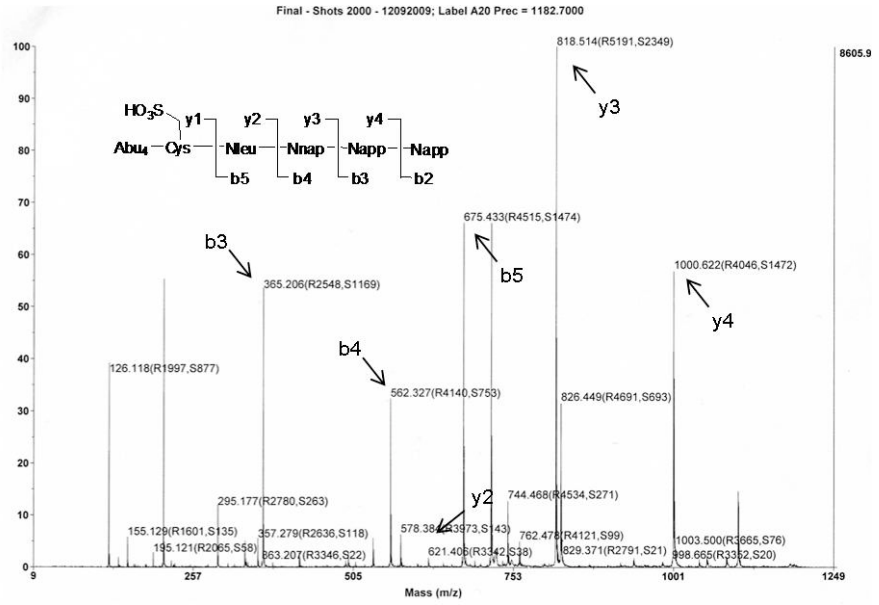


Figure S15. Binding of fluorescein-conjugated cyclic peptoids, Hit 1 and Hit 2, (Figure S13, Scheme S3) to streptavidin monitored by fluorescence polarization. Error bars are mean ± SEM from triplicate samples.