Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Encoded combinatorial libraries for the construction of cyclic peptoid microarrays

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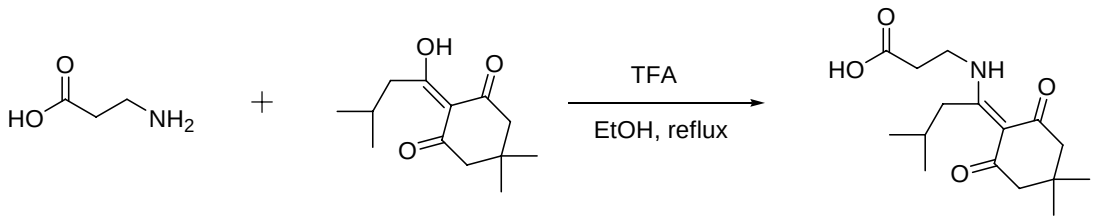
Materials and equipments. All commercial reagents were used as obtained without further purification. *O*-tert-Butyl-2-amino ethanol was purchased from CSPS Pharmaceuticals. Methylamine was used as 2 M solution in THF. Polystyrene AM RAM macrobead (500-560 μm; 0.52 mmol/g; 80-110 nmol/bead) and Rink Amide AM LL (100-200 mesh, 0.35 mmol/g) resins were obtained from Rapp Polymere and Novabiochem, respectively. NMR spectra were recorded on a Varian 300 MHz spectrometer. Preparative HPLC was performed on a Waters binary HPLC system with a C18 reverse-phase column with the gradient elution of water/acetonitrile with 0.1 % TFA. MS and tandem MS (MALDI-TOF) were performed on a Voyager-DE PRO biospectrometry workstation and 4700 Proteomics Analyzer (Applied Biosystems) with α-cyano-4-hydroxycinnamic acid as a matrix, respectively. The synthesis of peptides was performed in a New Brunswick Scientific Innova 4000 incubator shaker. The synthesis of peptoids under microwave conditions was performed in a 1000 W Whirlpool microwave oven (model MT1130SG) with 10% power. Standard glass peptide synthesis vessels (Chemglass) were used for the synthesis in the incubator shaker and in the microwave oven. Microarrays were prepared on maleimide-functionalized glass slides by using SpotArray 72 Microarray Printing System (PerkinElmer). Hybridized microarrays were scanned with a GenePix 4000B scanner.



Synthesis of ivDde-β-Ala-OH. To a stirred suspension of H-β-Ala-OH (1.02 g, 11.4 mmol) and ivDde-OH (5 ml, 22.9 mmol) in EtOH was added TFA (88 μL, 1 mmol) at room temperature.[1] The mixture was then refluxed for 24 hours. After the solvent was evaporated *in vacuo*, a crude product was purified by column chromatography with CH_3OH/CH_2Cl_2 (0.1% TFA) gradient to afford ivDde-β-Ala-OH (3.3 g, 97.6%). ^{1}H NMR ($CDCl_3$) δ 1.02 (m, 12H), 1.90-2.03 (m, 1H), 2.39(s, 4H), 2.75 (t, J = 6.0 Hz, 2H), 3.06 (br d, J = 6.0 Hz, 2H), 3.78 (q, J = 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 22.8, 28.4, 29.4, 30.2, 34.1, 37.4, 39.6, 52.9, 107.4, 173.0, 177.2; MS (MALDI): *m/z*: calcd for $C_{16}H_{26}NO_4$ 296.2; found 296.5 $[M + H]^+$.

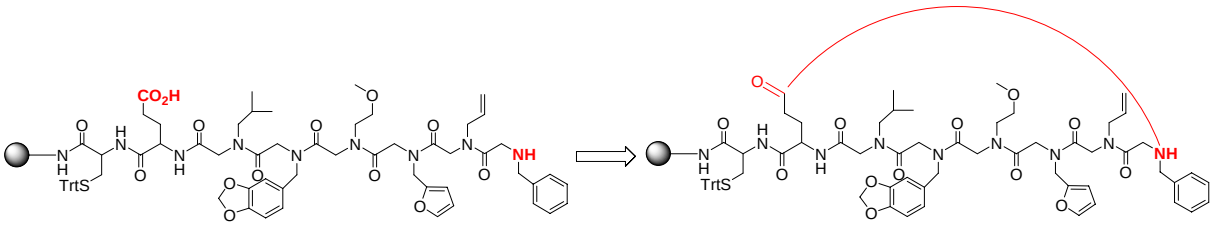


Figure S1. Cyclization reaction of model peptoid on bead.

Cyclization reactions of peptoids on bead. Preliminary cyclization reactions of peptoids on bead were tested under various conditions. The typical procedure with PyBOP which gave the best results is as follows. The cyclization yields also depended on the length of the peptoid with high yields requiring at least six monomeric units. Fmoc-Cys(Trt)-OH and Fmoc-Glu(*O*-2-PhiPr)-OH were coupled to the Rink Amide AM resin sequentially by using Fmoc chemistry. The synthesis of peptoids was performed by employing a microwave-assisted submonomer protocol.[2] 2-PhiPr group was deprotected with 1% TFA and 2% triisopropylsilane in DCM twice (for 30 min each time). After the resins were thoroughly washed with 5% DIPEA in DCM and DCM, cyclization was carried out under the conditions of PyBOP (3 eq.), HOBt (3eq.) and DIPEA (10 eq.) in DMF twice (for 10 h each time). Cyclic peptoids were confirmed by MALDI-MS and HPLC after cleavage from beads.

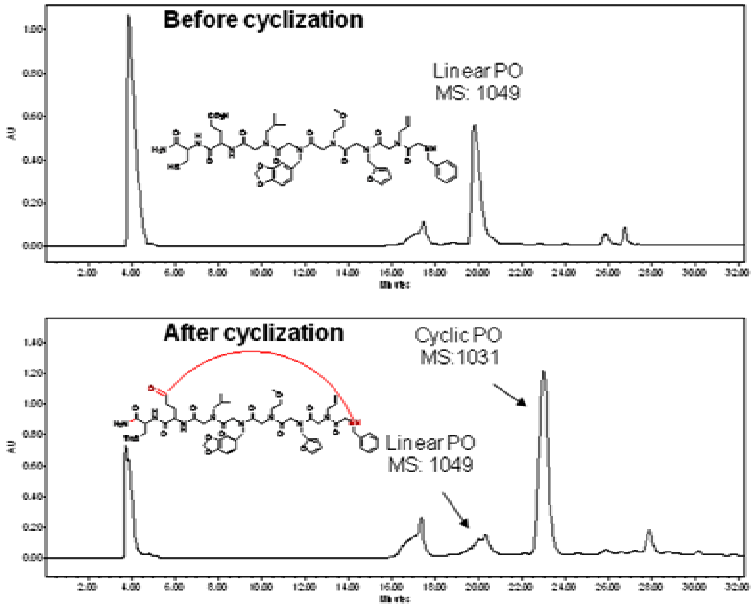


Figure S2. RP-HPLC traces of peptoids before cyclization and after cyclization by using PyBOP.

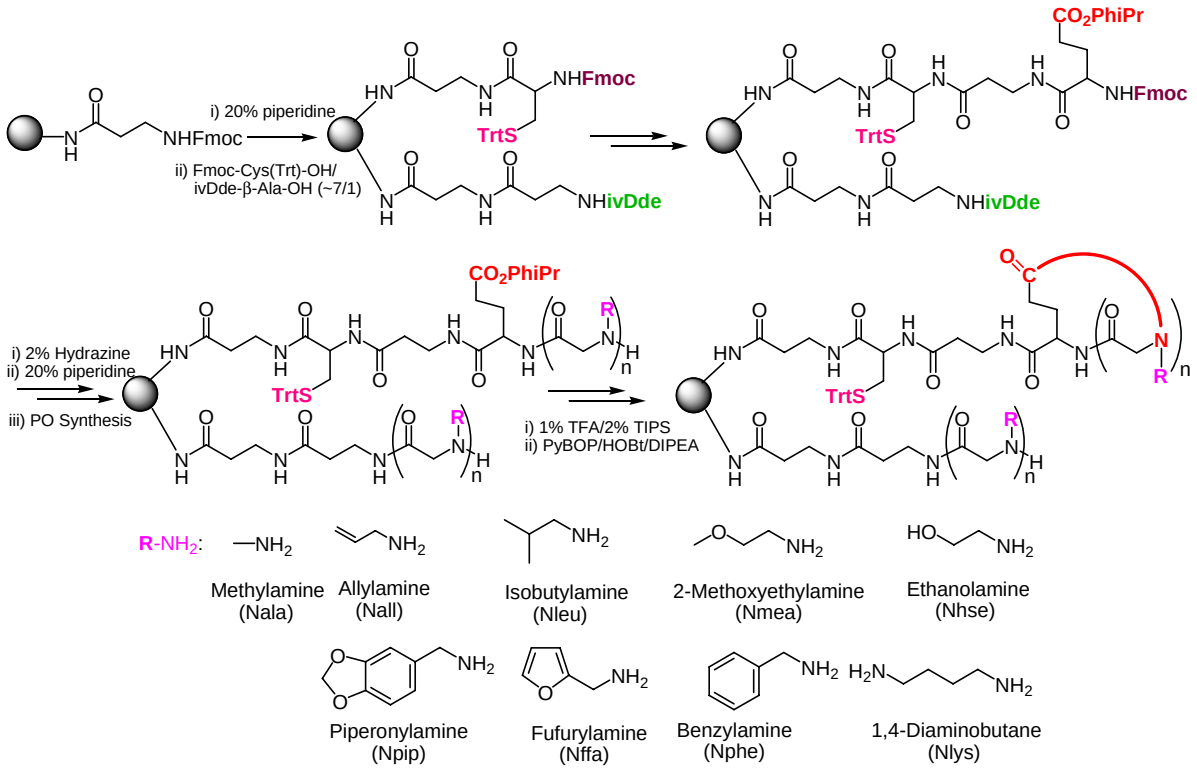


Figure S3. Synthesis of encoded cyclic peptoid libraries.

General procedure for the construction of encoded cyclic peptoid libraries. Polystyrene AM RAM macrobeads (80-110 nmol/bead, Rapp Polymere) in DMF were allowed to swell at room temperature for 1 h. After DMF was drained, the beads were incubated with 20 % piperidine for 30 min. The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with Fmoc-β-Ala-OH (5 eq.) by using HBTU (5 eq.), HOBt (5 eq.) and DIPEA (10 eq.) in DMF for 2 h. After The beads were thoroughly washed with DMF (8 × 3 mL) and incubated with 20 % piperidine for 30 min, they were thoroughly washed with DMF (8 × 3 mL) and then treated with ivDde-β-Ala-OH (0.6 eq.) and Fmoc-Cys(Trt)-OH (4 eq.) by using HBTU (4.6 eq.) and NMM (10 eq.) in DMF. After 2 h, the beads were thoroughly washed with DMF (8 × 3 mL) and then treated with Ac_2O (10 eq.) and DIPEA (10 eq.) in DMF for 1 h to block possible unreacted amines. After the beads were thoroughly washed with DMF (8 × 3 mL) and Fmoc group was selectively removed with the treatment of 20 % piperidine for 30 min, they were again coupled with Fmoc-β-Ala-OH (5 eq.) by using HBTU (5 eq.), HOBt (5 eq.) and DIPEA (10 eq.) in DMF

for 2 h. After the beads were thoroughly washed with DMF (8 × 3 mL) and incubated with 20 % piperidine for 30 min, they were treated with Fmoc-Glu(O-2-PhiPr)-OH (3 eq.) by using HATU (3 eq.), HOBt (3 eq.) and DIPEA (10 eq.) in DMF. After 2 h, the beads were thoroughly washed with DMF (8 × 3 mL) and then treated with Ac_2O (10 eq.) and DIPEA (10 eq.) in DMF for 1 h to block possible unreacted amines. ivDde and Fmoc groups were removed with the successive treatments of 2.5% hydrazine twice (for 10 min each time) and 20 % piperidine for 30 min. After the beads were thoroughly washed with DMF (8 × 3 mL), split-and-mix linear peptoid libraries consisting of 7-mer peptoids were prepared by using bromoacetic acid and primary amines such as methylamine, allylamine, 2-methoxyethylamine, *O*-tert-butyl-2-amino ethanol, piperonylamine, fufurylamine, benzylamine, 1-*N*-tert-butyloxycarbonyl-1,4-diaminobutane based on a microwave-assisted submonomer protocol.[2] 2-PhiPr group was selectively deprotected with 1% TFA and 2% triisopropylsilane (TIS) in DCM twice (for 30 min each time). After the resins were thoroughly washed with 5% DIPEA in DCM and DCM, cyclization was carried out under the conditions of PyBOP (3 eq., ~30 mM), HOBt (3eq. ~30 mM) and DIPEA (10 eq.) in DMF twice (for 10 h each time). Cyclization yields depended on the residues at N-terminal. Cyclic peptoid libraries consisting of Nmea at the N-terminal afforded much better results with almost complete cyclization. Cyclic peptoids were confirmed by MS, tandem MS (MALDI) or HPLC after cleavage from the resin under the conditions of 95% TFA and 5% TIS for 1.5 h. The sequence of each cyclic peptoid was successfully analyzed by tandem MS data of encoded linear peptoid on the same bead. At first, MALDI-MS data on a mixture consisting of the cyclic peptoid and encoded linear peptoid were obtained with the typical mass difference (m/z: 214). Then, the encoded linear peptoid was sequenced by analysis of b and y ions of fragments at tandem MS, revealing the sequence of cyclic peptoid.

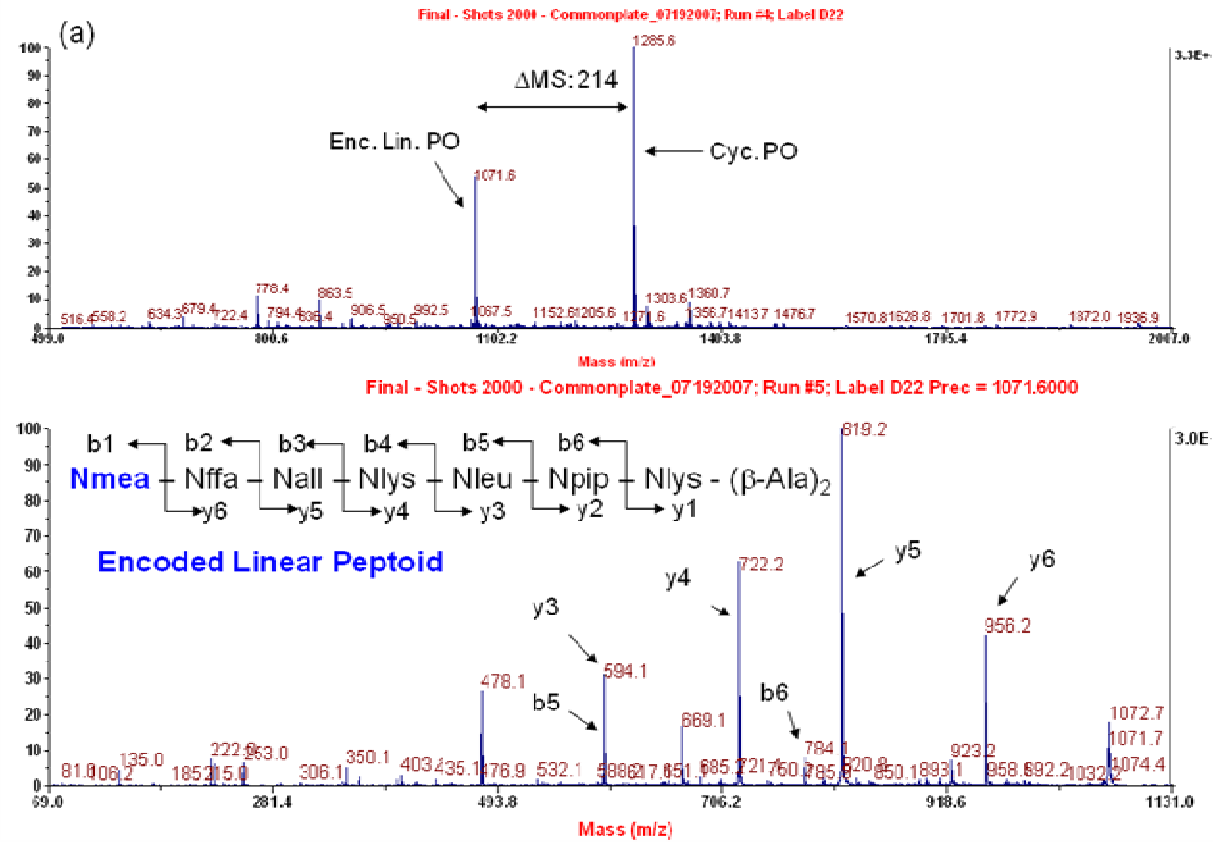


Figure S4. Sequence analysis for random members from cyclic peptoids library with Nmea at N-terminal: (a) Nmea-Nffa-Nall-Nlys-Nleu-Npip-Nlys.

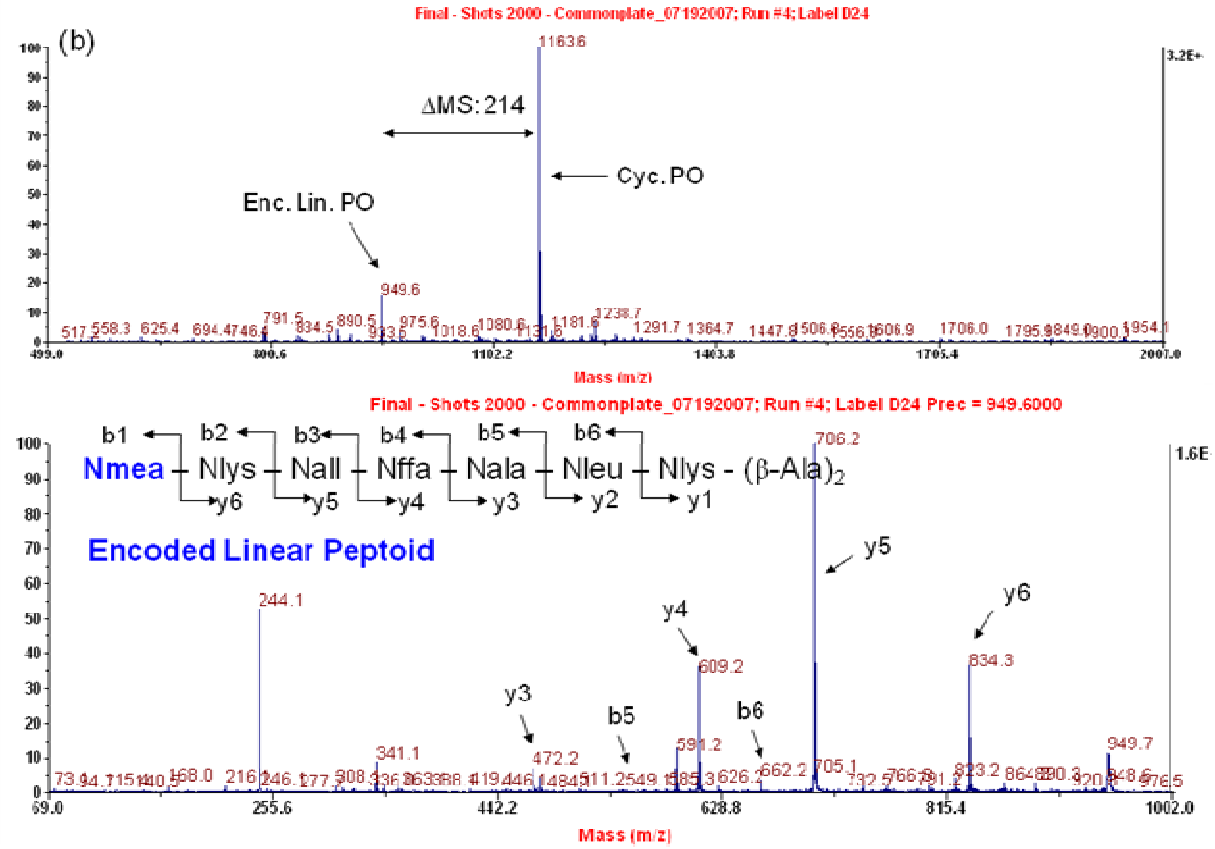


Figure S5. Sequence analysis for random members from cyclic peptoids library with Nmea at N-terminal: (b) Nmea-Nlys-Nall-Nffa-Nala-Nleu-Nlys.

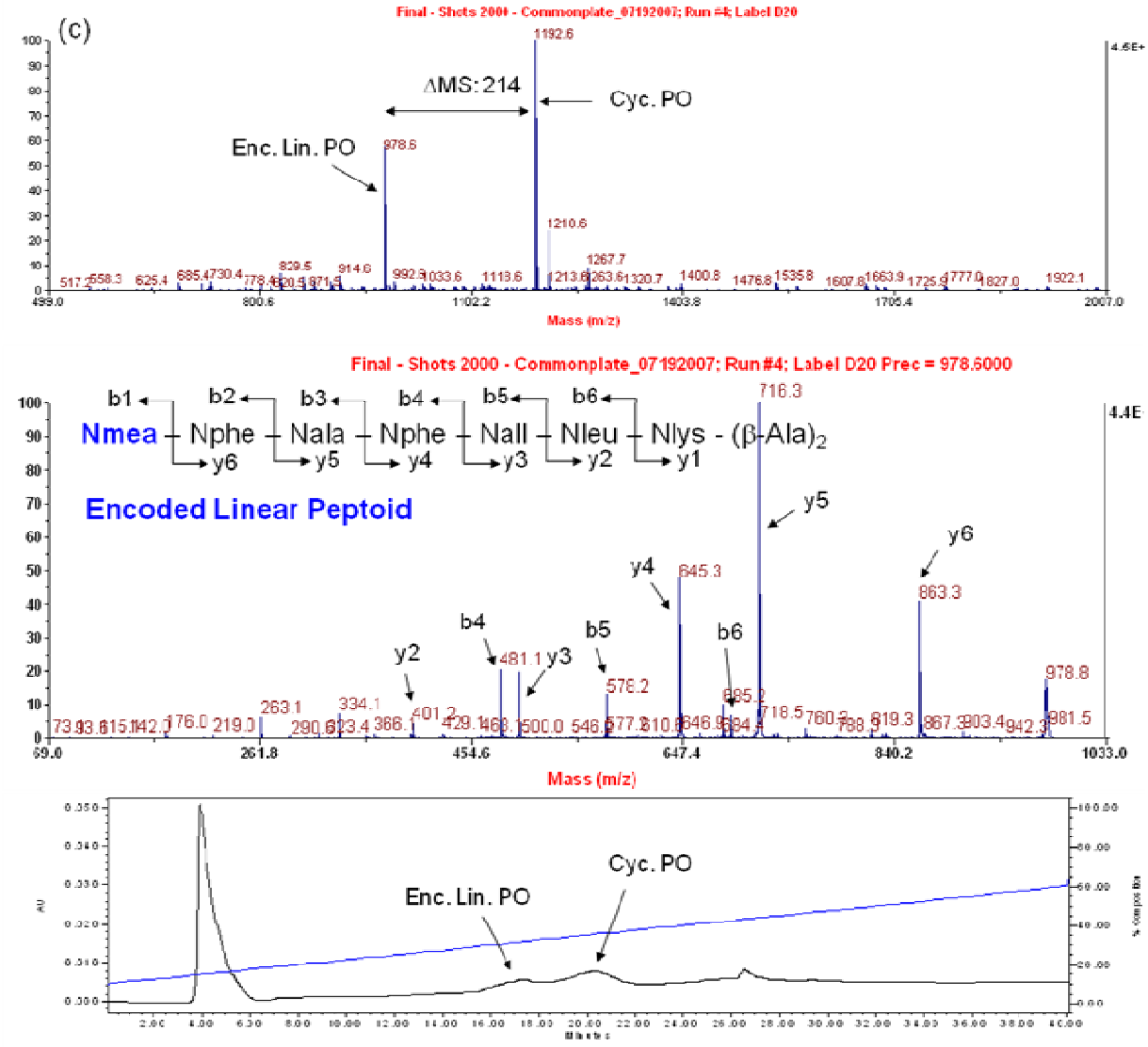


Figure S6. Sequence analysis for random members from cyclic peptoids library with Nmea at N-terminal: (c) Nmea-Nlphe-Nala-Nphe-Nall-Nleu-Nlys. RP-HPLC trace of cyclic peptoid and encoded linear peptoid are also shown.

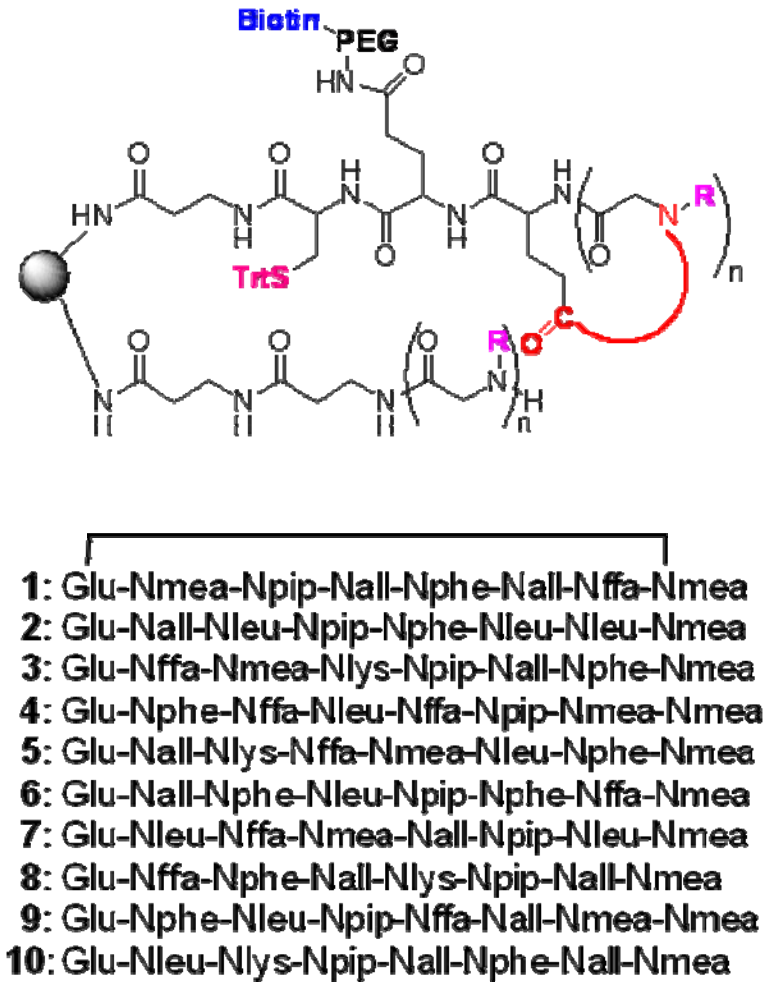


Figure S7. Synthesis of biotin-labeled cyclic peptoids with Nmea at N-terminal. To demonstrate the immobilization of the cyclic peptoids, ten different biotin-labeled cyclic peptoids with Nmea at N-terminal were synthesized similarly by introducing Fmoc-Glu(PEG-Biotin)-OH instead of Fmoc-β-Ala-OH at the second β-Ala coupling step. After cleavage of compounds from the resin, each solution in 50% aqueous acetonitrile was used for the decoding experiments by tandem MS and cyclic peptoids in DMSO were spotted robotically onto PEGylated, maleimide-activated glass microscope slides with 3-fold serial dilution of about 2 mM solution.

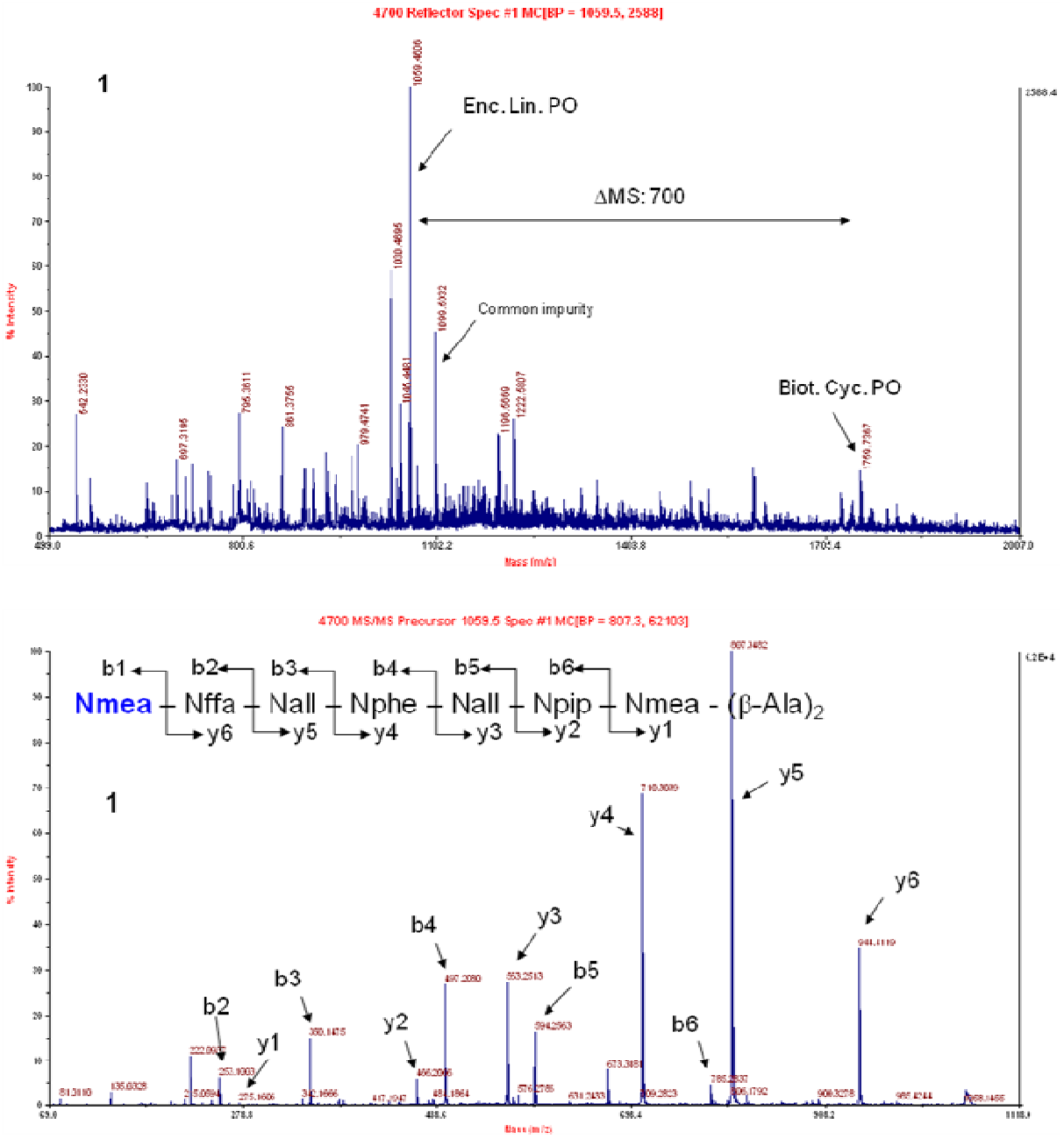


Figure S8. MS, MS/MS data for biotin-labeled cyclic peptoid (**1**) with Nmea at N-terminal.

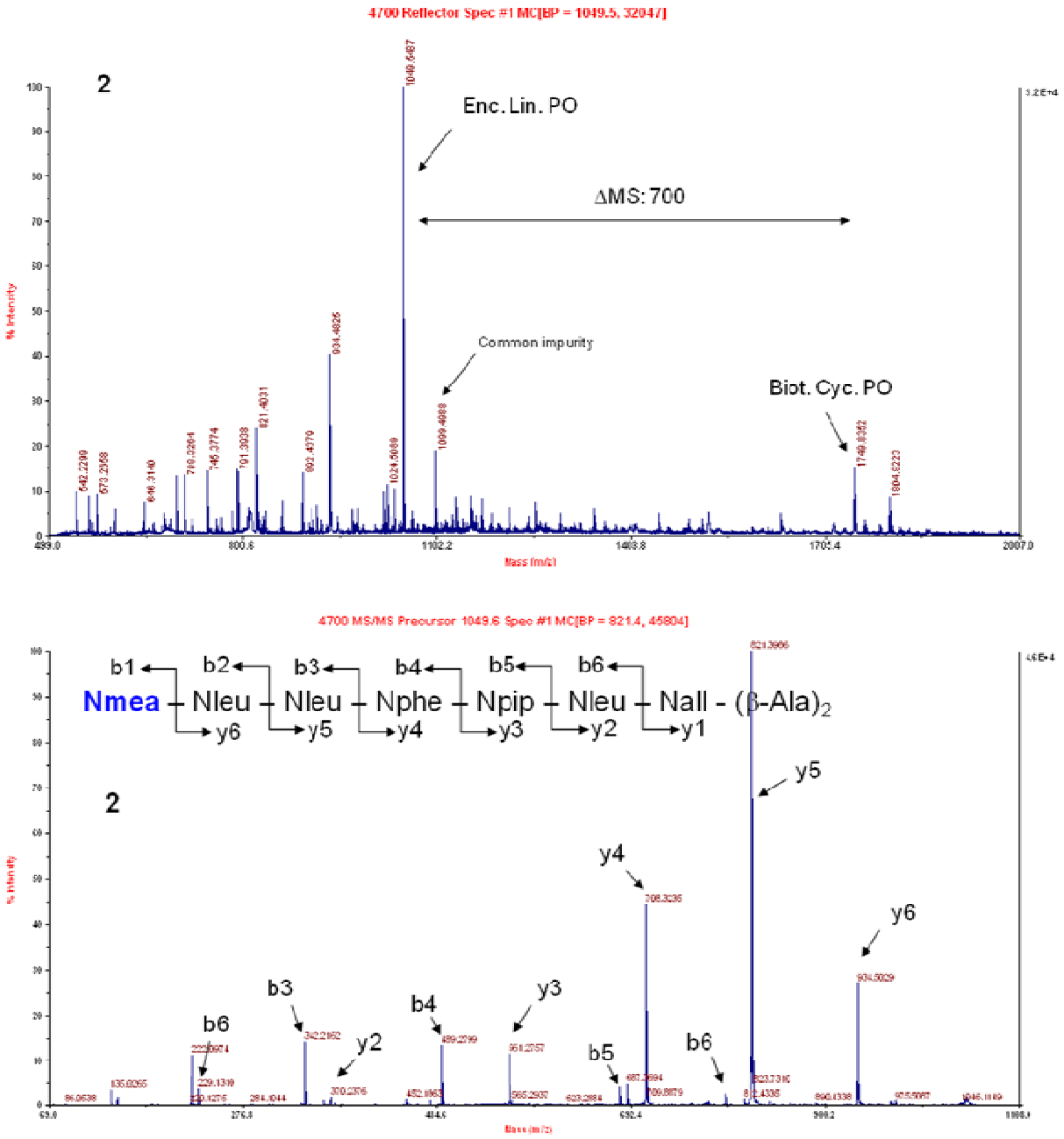


Figure S9. MS, MS/MS data for biotin-labeled cyclic peptoid (**2**) with Nmea at N-terminal.

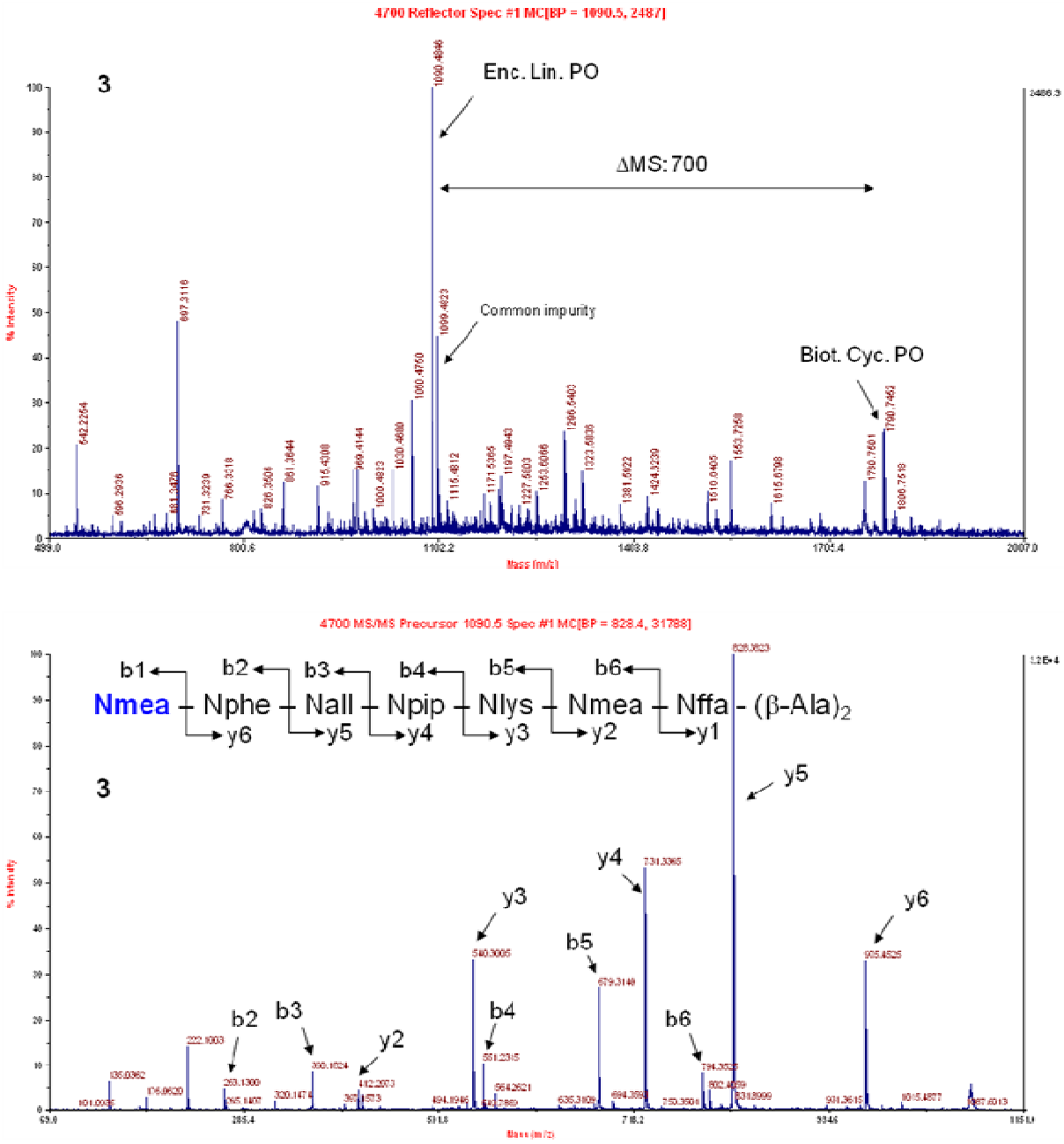


Figure S10. MS, MS/MS data for biotin-labeled cyclic peptoid (**3**) with Nmea at N-terminal.

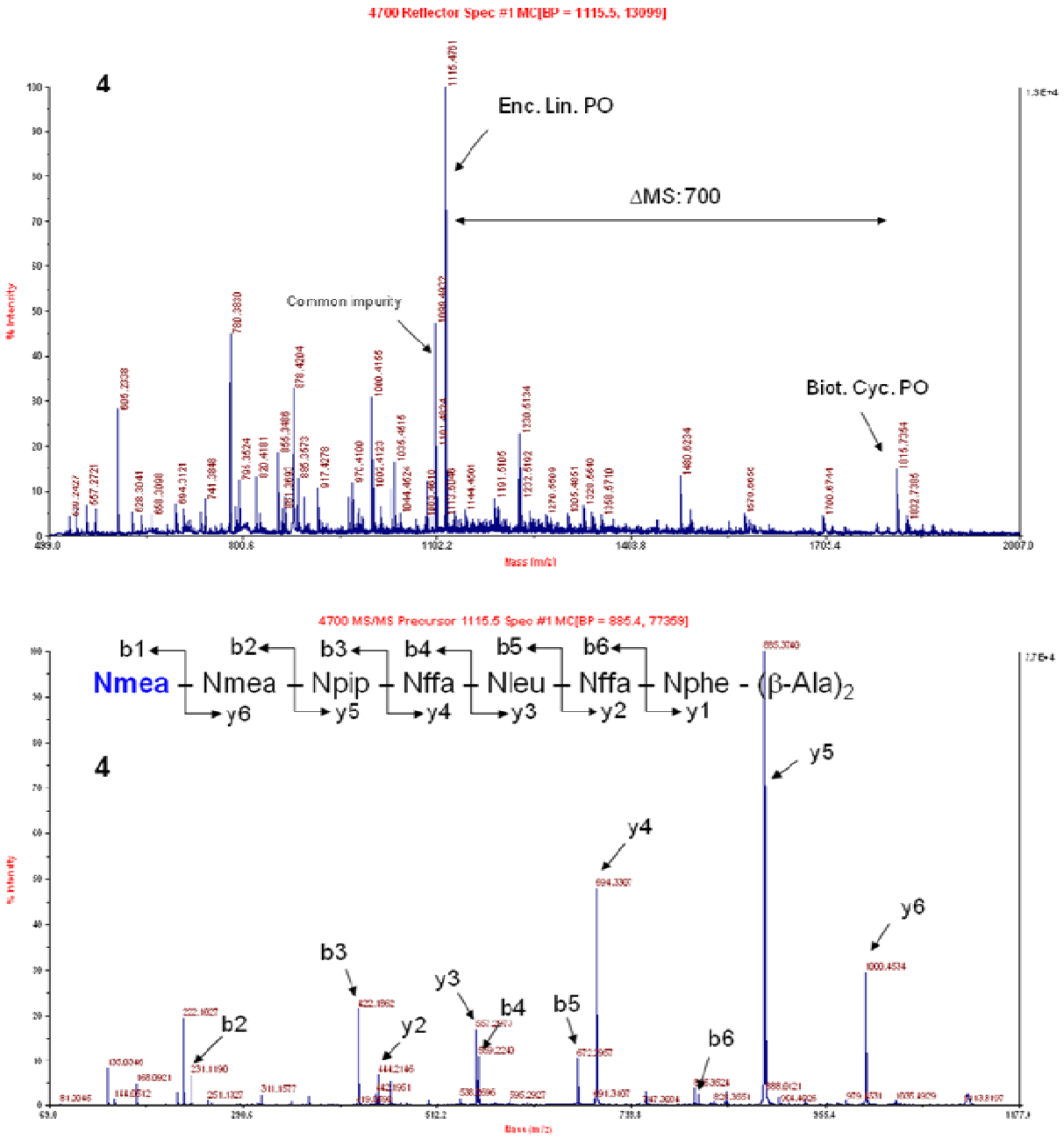


Figure S11. MS, MS/MS data for biotin-labeled cyclic peptoid (**4**) with Nmea at N-terminal.

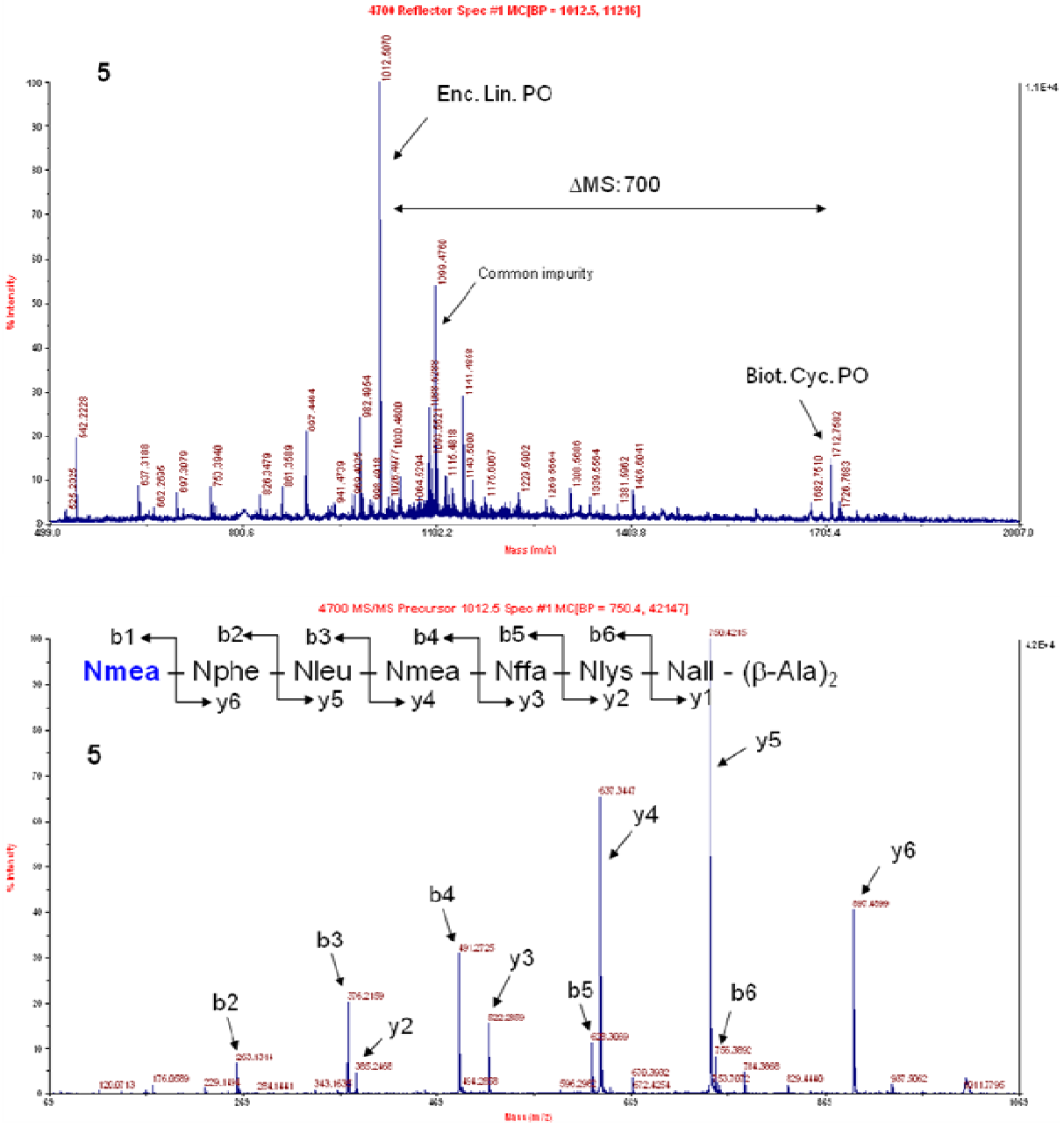


Figure S12. MS, MS/MS data for biotin-labeled cyclic peptoid (**5**) with Nmea at N-terminal.

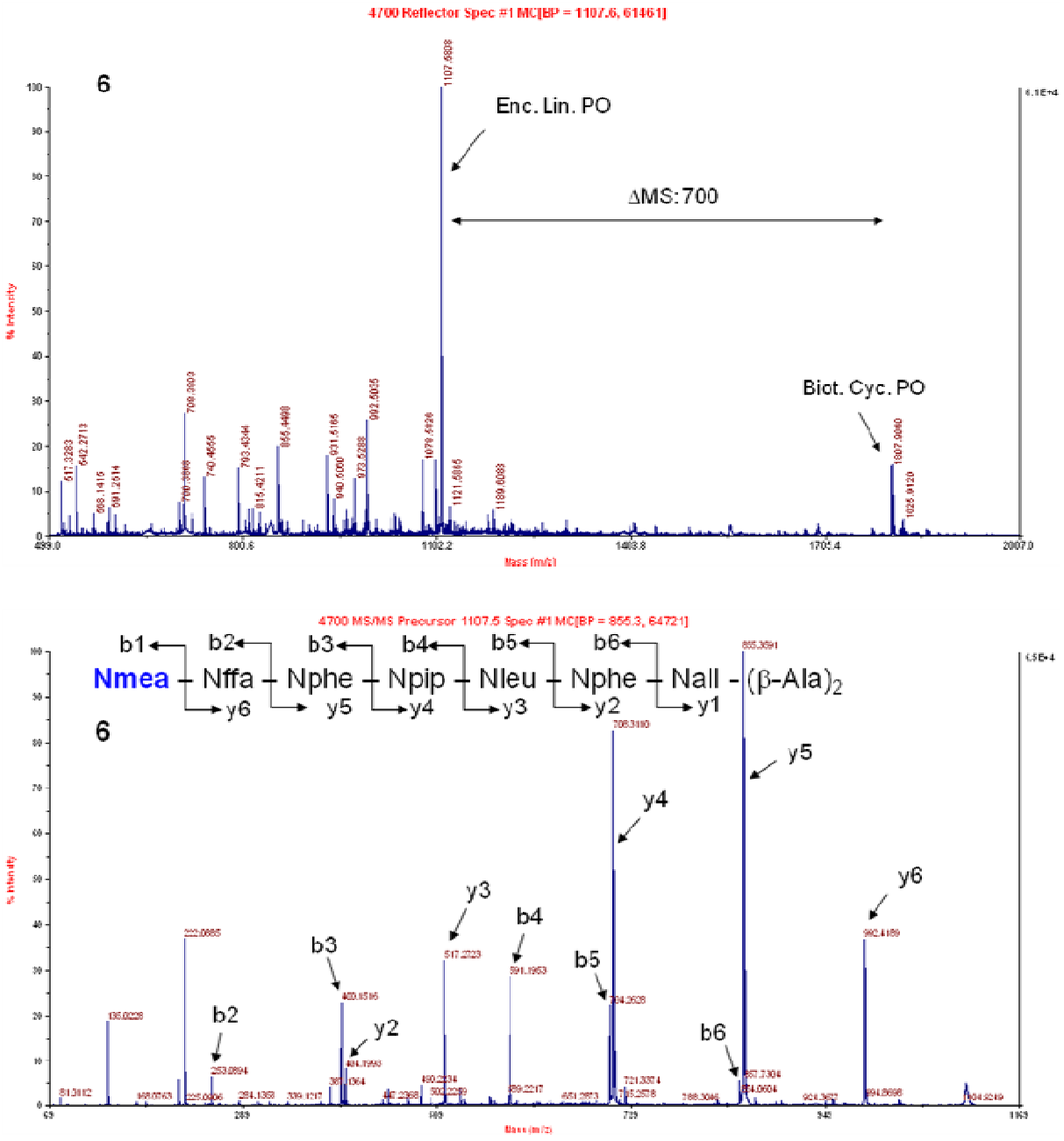


Figure S13. MS, MS/MS data for biotin-labeled cyclic peptoid (**6**) with Nmea at N-terminal.

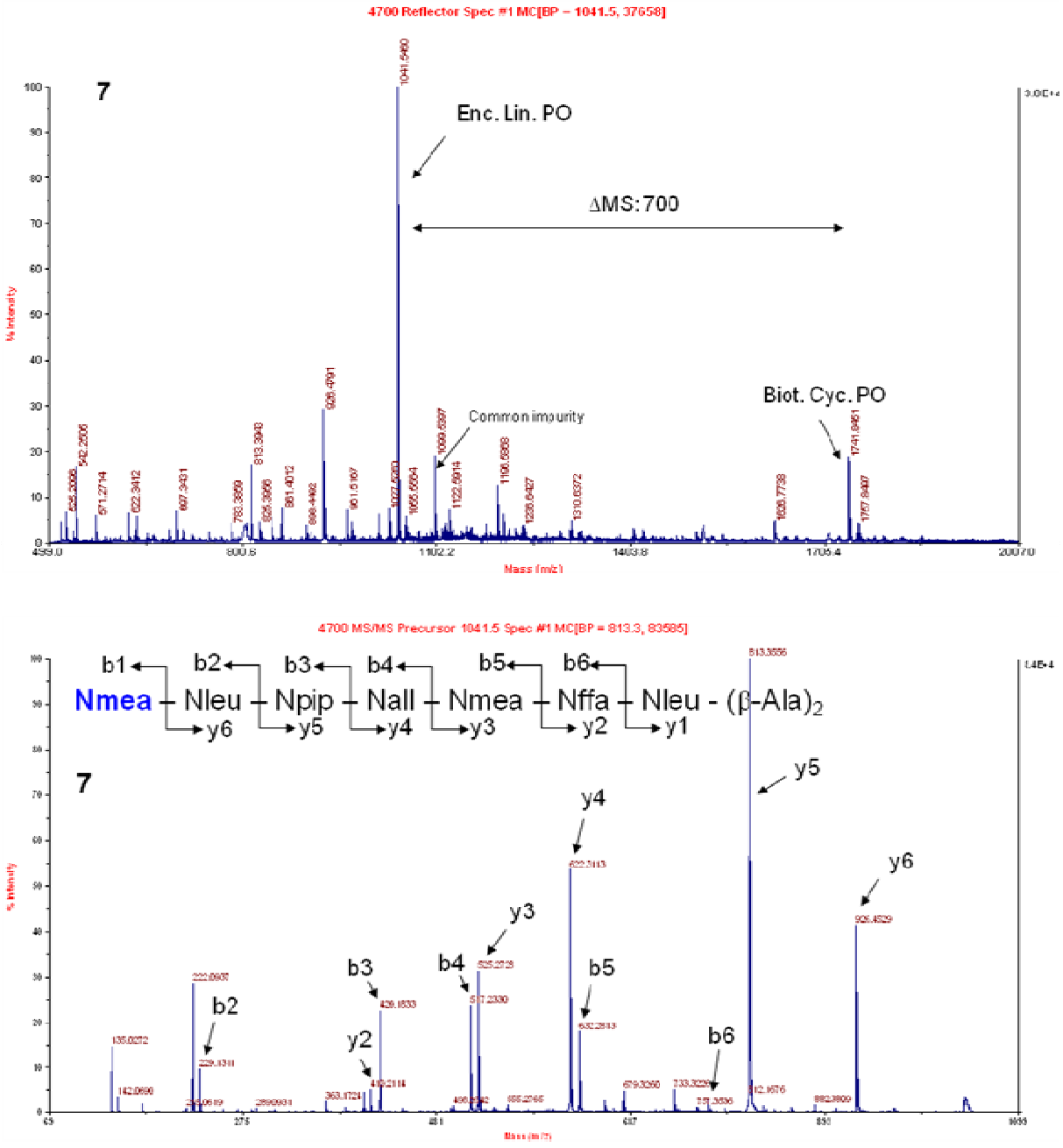


Figure S14. MS, MS/MS data for biotin-labeled cyclic peptoid (**7**) with Nmea at N-terminal.

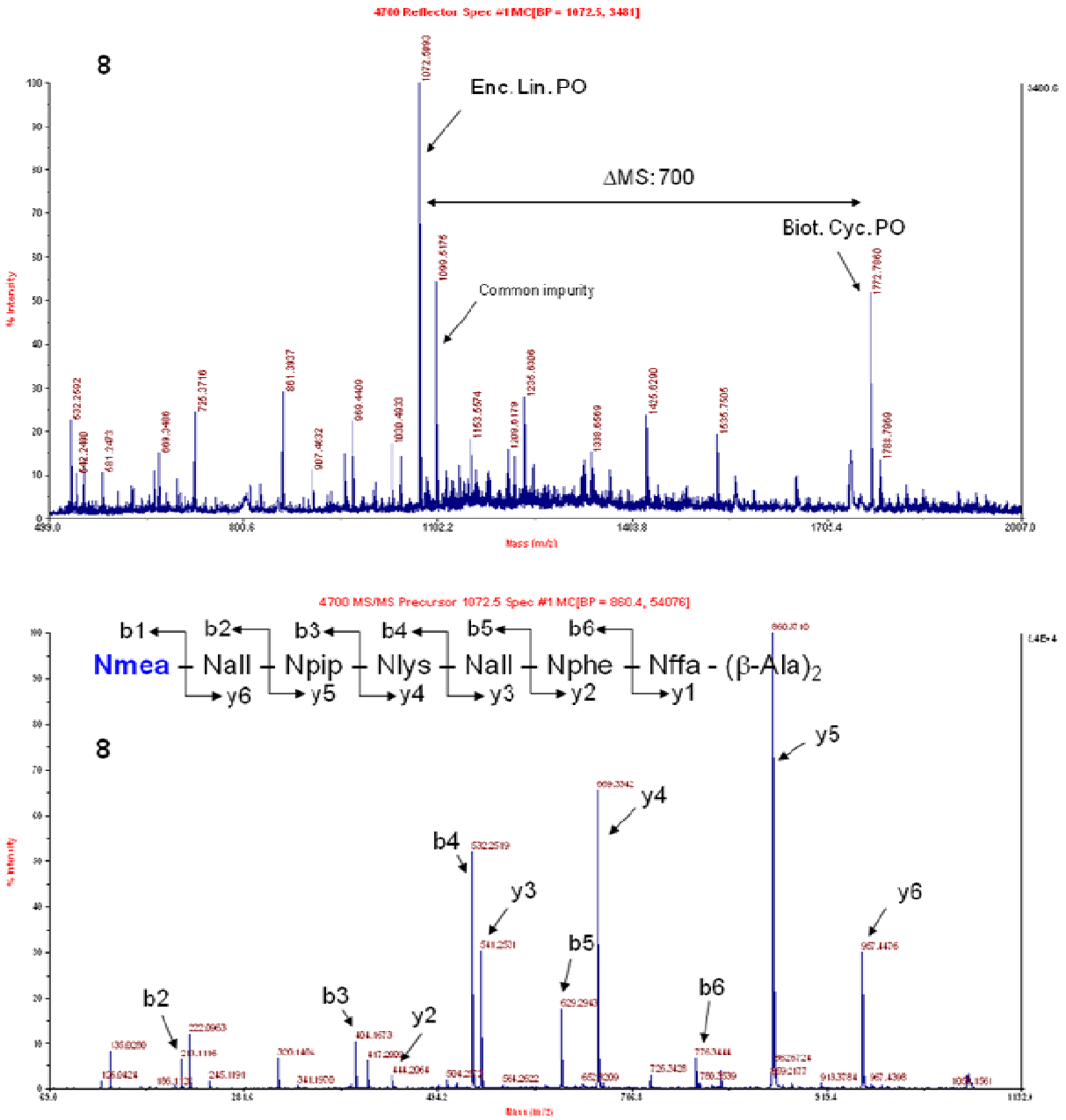


Figure S15. MS, MS/MS data for biotin-labeled cyclic peptoid (**8**) with Nmea at N-terminal.

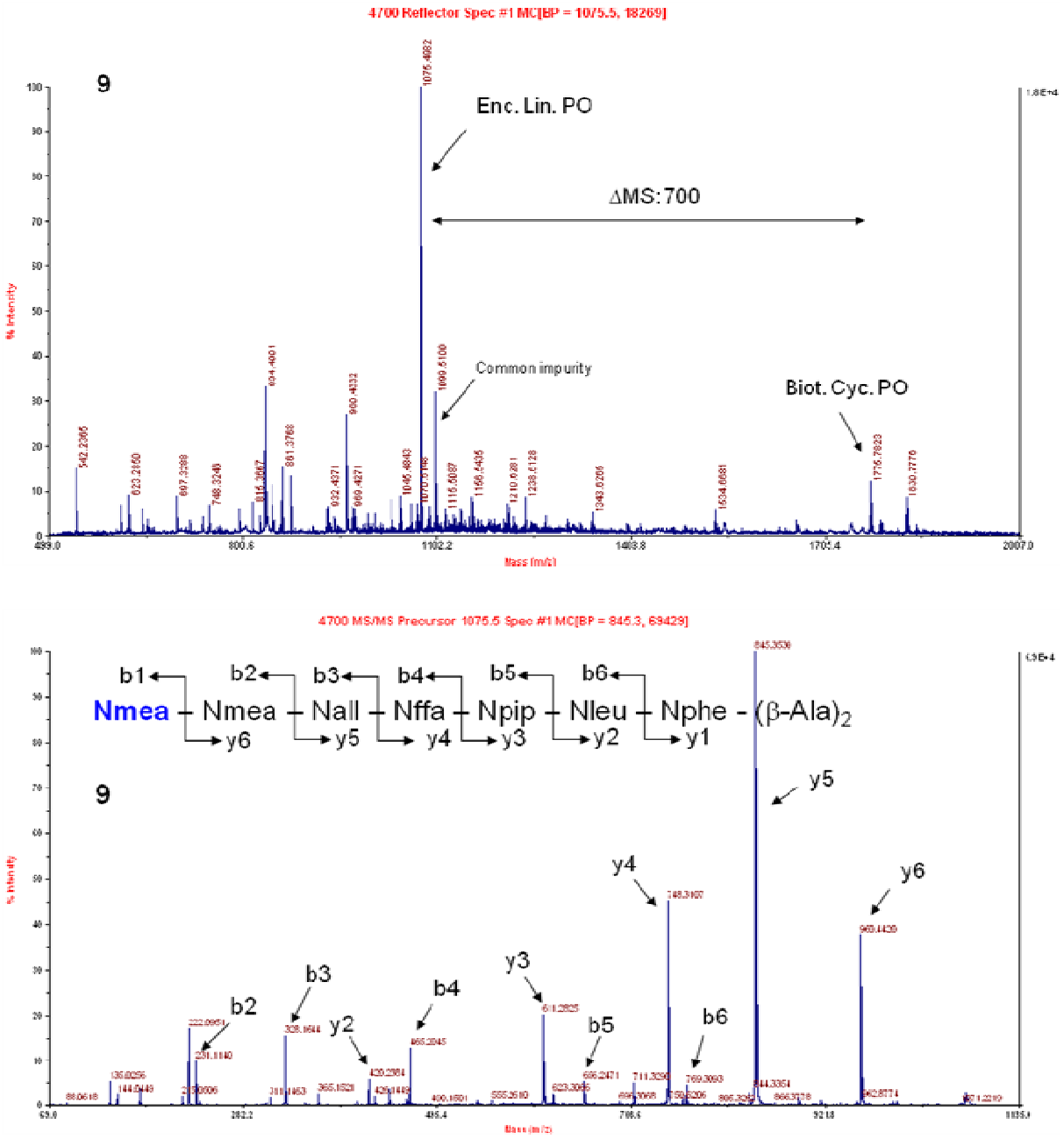


Figure S16. MS, MS/MS data for biotin-labeled cyclic peptoid (**9**) with Nmea at N-terminal.

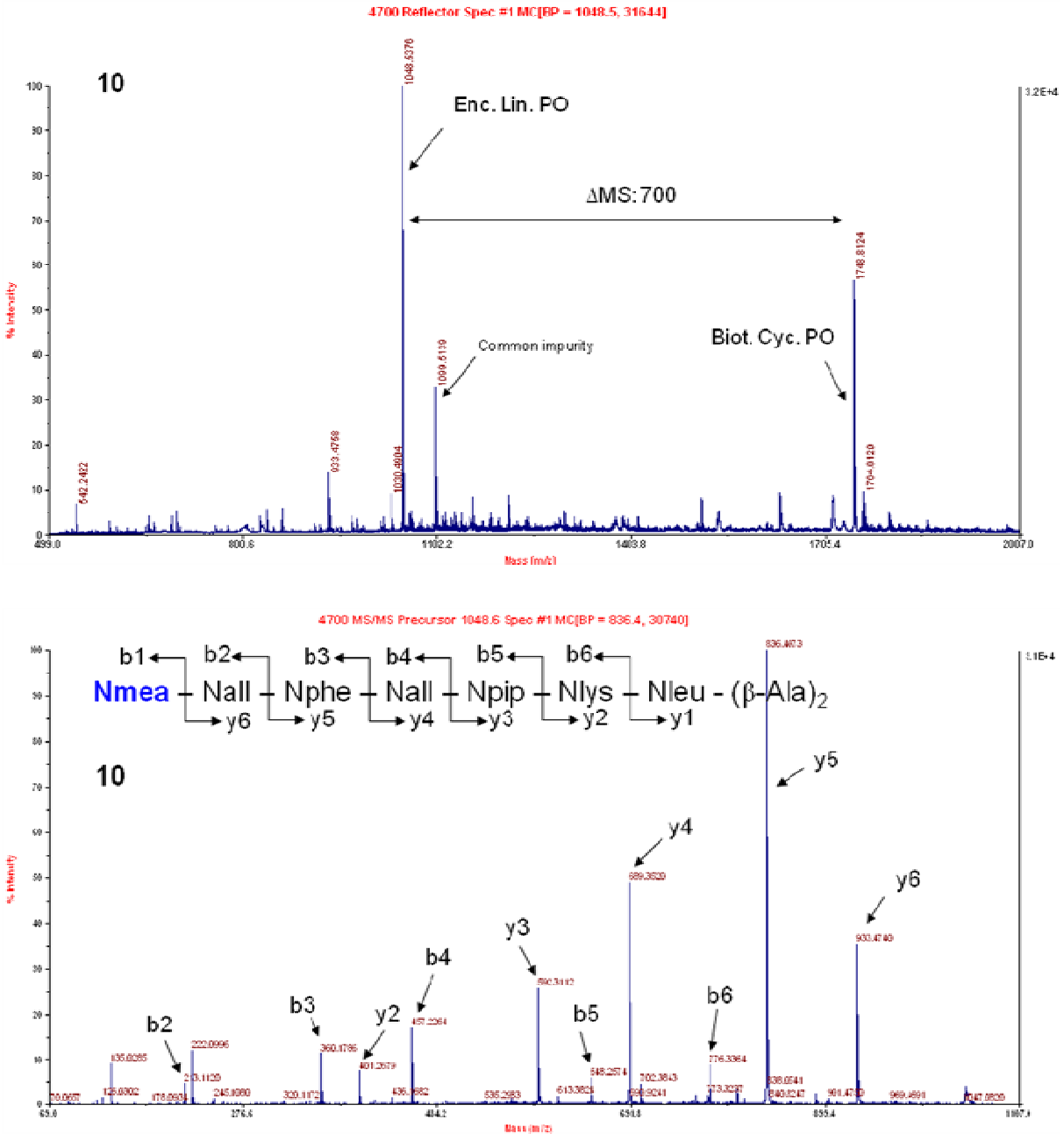


Figure S17. MS, MS/MS data for biotin-labeled cyclic peptoid (**10**) with Nmea at N-terminal.

Hybridization of biotin-labeled cyclic peptoids microarray and Streptavidin-Cy3. Microarrays consisting of biotin-labeled cyclic peptoids with Nmea at the N-terminal were prepared as described in ref. 3. Biotin-labeled cyclic peptoids were spotted onto maleimide-functionalized glass slides with 3-fold serial dilution of about 2 mM solution in DMSO. Microarrays were equilibrated with 1× TBST (50 mM Tris/150 mM NaCl/0.1 % Tween 20, pH 8.0) for 30 min at 4 °C. Microarray slides were incubated with Streptavidin-Cy3 (10 µL, Sigma) and BSA (50 µL of 2 mg/mL) in 1× TBST (total 1 mL solution) with gentle shaking for 45 min at 4 °C. The slides were washed with 1× TBST (3 × 5 min) at 4 °C, and then dried by centrifugation. Hybridized microarrays were scanned with a GenePix 4000B scanner.

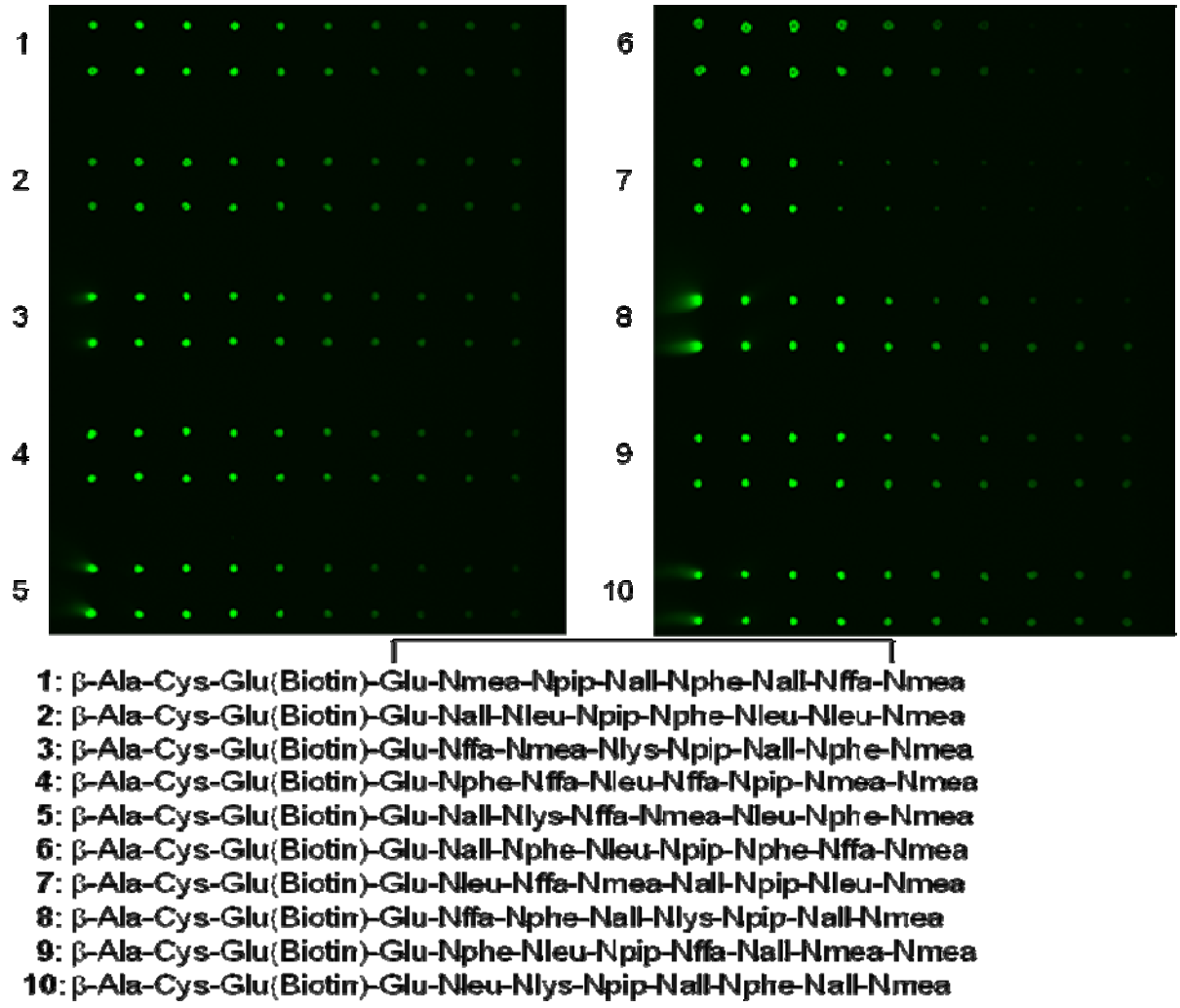


Figure S18. Fluorescence image from hybridization of biotin-labeled cyclic peptoids microarray and Streptavidin-Cy3.

References

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(3) Reddy, M. M.; Kodadek, T. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12672-12677.