

Synthesis of a Large Library of Macrocyclic Peptides Containing Multiple and Diverse *N*-alkylated Residues

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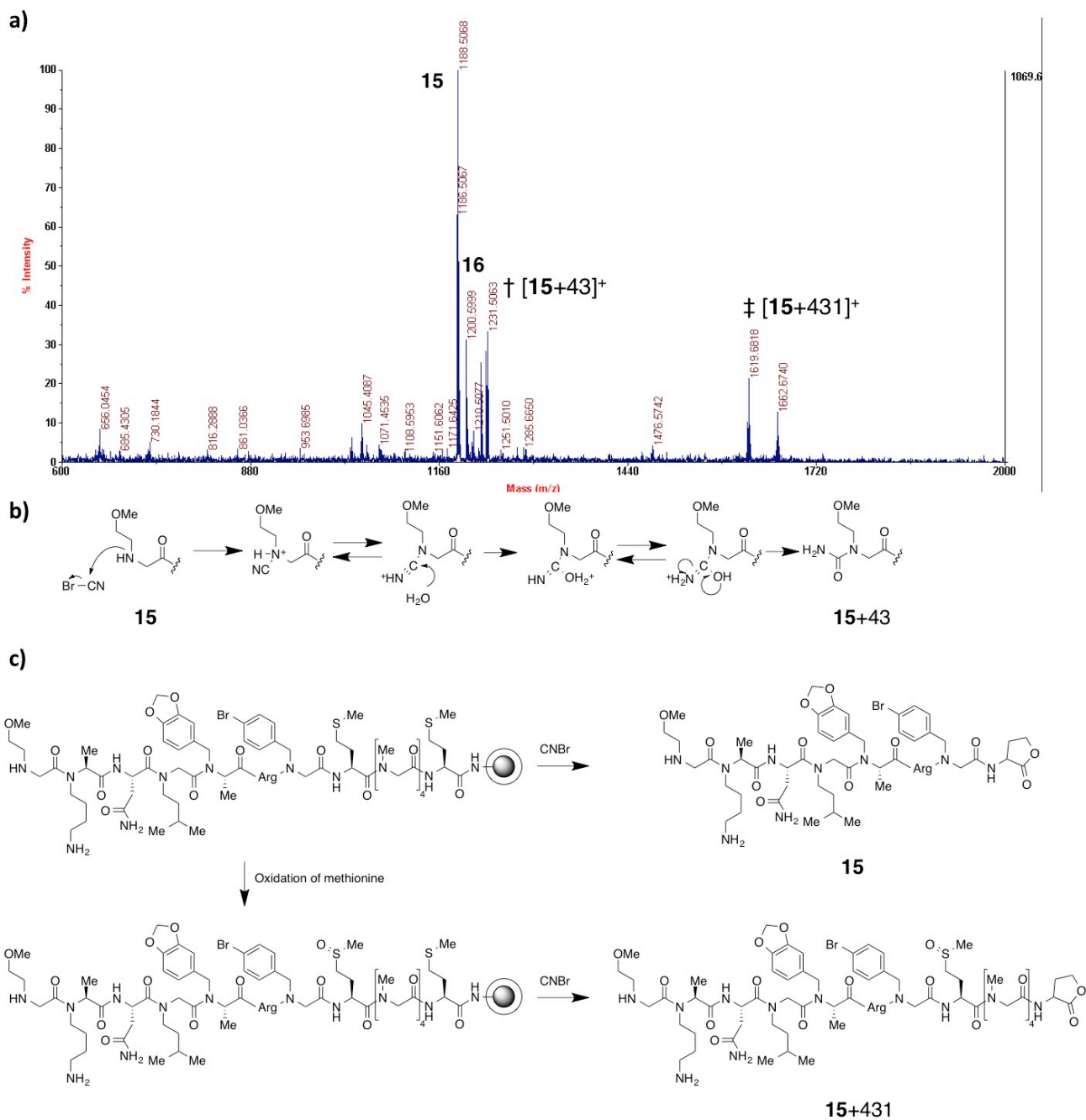


Fig. S1 Plausible mechanisms of byproduct peaks found on a mass spectrum in Fig. 5b. a) A mass spectrum is adapted from Fig. 5b. Two major byproduct peaks corresponding to $[15+43]^+$ and $[15+431]^+$ are labeled with † and ‡, respectively. b) A plausible mechanism of formation of byproduct peak $[15+43]^+$. During CNBr cleavage, a cyano group is added to the *N*-terminal secondary amine, which lead to terminal urea formation. c) A plausible mechanism of formation of byproduct peak $[15+431]^+$. As linear peptide has two methionines in the linker, if *N*-terminal side of methionine is oxidized during synthesis, the other one react with CNBr, which lead to formation of the 15+431 species.

Table S1 Sequences of oligomers cleaved from single bead in the constructed library.

Well	Cyclic MS	Linear MS	y1 Residue 1	y2 Residue 2	y3 Residue 3	y4 Residue 4	y5 Residue 5
A1	1263	1249	483 S-Ntbb	700 Nlys	828 D-Leu	941 R-Nace	1101 Npym
A2	1218	1204	483 R-Nibu	614 Nglu	743 L-His	880 R-Nthm	1051 Nchm
A3	1360	1346	483 R-Nthp	656 Ntmb	887 L-Pro	984 R-Npip	1193 Nchm
A4	1217	1203	483 R-Nthp	656 Nlys	784 L-Ser	871 S-Npip	1076 Nipt
A5	1333	1319	483 R-Nace	643 Ntmb	874 L-Pro	971 R-Ntbb	1192 Nipt
A6	1272	1258	483 S-Nthp	652 Nfur	789 D-Lys	917 S-Nfph	1110 Npym
A7	1291	1277	483 S-Nthm	650 Nipt	777 D-Tyr	940 S-Nthm	1107 Nmoe
A8	1276	1262	483 R-Nche	666 Nipt	793 L-His	930 S-Npip	1135 Nipt
A9	1265	1251	483 Nfph	676 Nchm	829 D-Leu	942 S-Nace	1098 Nchm
A11	1243	1229	483 R-Nace	643 Ntmb	874 L-Pro	971 R-Nibu	1102 Nipt
A12	1171	1157	483 S-Nace	639 Nlys	767 L-Pro	864 S-Nace	1020 Nfur
B2	1323	1309	483 R-Nace	643 Nipt	770 D-Lys	898 R-Nthm	1069 Nebs
B4	1296	1282	483 S-Npip	688 Nglu	817 L-Glu	946 R-Npip	1155 Nipt
B5	1300	1286	483 S-Nthm	650 Nglu	779 D-Tyr	942 S-Ntbb	1159 Nipt
B6	1255	1241	483 S-Nibu	610 Ntmb	841 D-Leu	954 R-Nace	1114 Nipt
B7	1193	1179	483 R-Nace	643 Nchm	796 D-Asn	910 S-Nlys	1052 Nipt
B8	1234	1220	483 S-Nthm	650 Nchm	803 L-Pro	900 S-Nfph	1093 Nipt
B9	1273	1259	483 R-Nibu	614 Nfur	751 D-Tyr	914 R-Nfph	1111 Npym
B10	1263	1249	483 S-Ntbb	700 Nchm	853 L-Pro	950 R-Nlys	1096 Nchm
B11	1162	1148	483 S-Nthm	650 Nipt	777 D-Leu	890 R-Nibu	1021 Nipt
B12	1246	1232	483 S-Nthp	652 Nfur	789 L-Pro	886 R-Npip	1095 Nfur
C1	1333	1319	483 R-Nthp	656 Nbpm	879 L-Ser	966 S-Npip	1171 Npym
C2	1256	1242	483 R-Nche	666 Nglu	795 L-Pro	892 R-Nfph	1089 Nchm
C3	1295	1281	483 S-Nthm	650 Nbpm	873 L-Ser	960 R-Nthp	1133 Npym
C4	1251	1237	483 R-Nthm	654 Nglu	783 L-His	920 S-Nthp	1089 Npym
C5	1264	1250	483 R-Npip	692 Nfur	829 D-Tyr	992 R-Nibu	1123 Nipt
C7	1165	1151	483 R-Nibu	614 Nipt	741 L-Pro	838 R-Nace	998 Nchm
C8	1208	1194	483 S-Nibu	610 Nglu	739 L-Pro	836 R-Ntbb	1057 Nfur
C9	1342	1328	483 S-Npip	688 Nfur	825 D-Lys	953 S-Npip	1158 Nmoe
C11	1220	1206	483 S-Nace	639 Nlys	767 D-Leu	880 R-Nthp	1053 Nchm
C12	1235	1221	483 R-Nche	666 Nlys	794 D-Tyr	957 S-Nibu	1084 Nfur
D2	1253	1239	483 R-Nace	643 Nlys	771 L-Pro	868 R-Nibu	999 Nebs
D3	1298	1284	483 S-Nthm	650 Nbpm	873 D-Asn	987 S-Nibu	1114 Nmoe
D4	1308	1294	483 S-Nace	639 Nbpm	862 L-His	999 S-Nlys	1141 Nchm
D5	1355	1341	483 S-Nche	662 Nchm	815 D-Leu	928 R-Nthp	1101 Nebs
D6	1223	1209	483 S-Nibu	610 Nfur	747 D-Lys	875 R-Nfph	1072 Nfur
D7	1168	1154	483 R-Nibu	614 Nfur	751 D-Lys	879 S-Nibu	1006 Npym
D8	1290	1276	483 R-Nthp	656 Ndmp	877 L-Ser	964 S-Nlys	1106 Nmoe
D9	1150	1136	483 R-Nthp	656 Nglu	785 L-Pro	882 S-Nibu	1009 Nipt
D11	1186	1172	483 R-Nace	643 Nipt	770 L-Glu	899 R-Nlys	1045 Nipt
D12	1205	1191	483 R-Nibu	614 Nipt	741 L-Ser	828 S-Nfph	1021 Nmoe

48 oligomers from the macrocyclic library were analyzed and 41 sequences were fully characterized. (85% sequensability).

4 letter codes of amines and 3 letter codes of amino acids found in **Fig. 6** are used to describe each residue. For residue 1 and 4, configuration at α -carbon is shown by R or S. Note that R or S configuration in these oligomers are inverse of acid submonomers are inverted by amine displacement reaction.

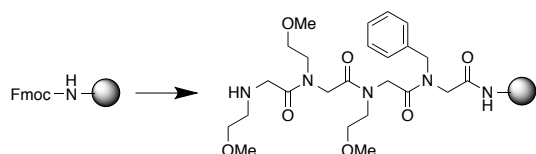
Mass spectra and chemical structures of compounds in well A2 and A4 are shown in **Fig. 7**.

Materials and methods

General: (*S*)-2-bromopropanoic acid- d_4 (S-BPA- d_4) was prepared according to the previously reported method¹. All other chemicals were purchased and used without further purification.

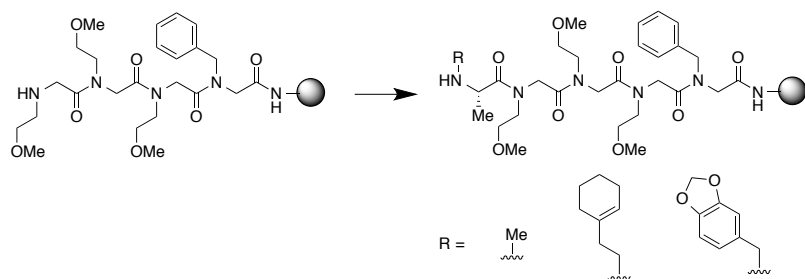
Synthesis of oligomers having *N*-terminal residues and extension of those oligomers with a peptoid residue (4–6): Each oligomer was synthesized on 50 mg of Rink Amide AM resin (0.69 mmol/g, 34.5 μ mol).

Step 1 Linker synthesis



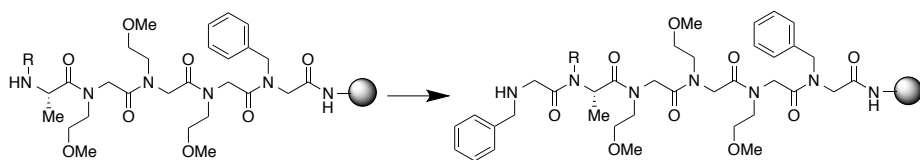
After resin was swollen in 1 mL of DMF for 15 min, Fmoc group was deprotected by treating resin with 1 mL of 20% piperidine in DMF for 3 min once and then 12 min once and resin was washed with DMF three times. Peptoid linker (Nmea-Nmea-Nmea-Nben) was synthesized following the standard submonomer synthetic method.

Step 2 PTA synthesis



Resin was washed with DCM three times and THF three times and incubated with 120 μ L of DIPEA (690 μ mol) in 3.1 mL of THF at RT for 10 min. 30.9 mg of triphosgene (104 μ mol) and 27.9 μ L of (R)-BPA (311 μ mol) in 3.1 mL of THF was cooled at -20 $^{\circ}$ C for 10 min. 113 μ L of TMP (863 μ mol) was added to the cooled triphosgene and (R)-BPA solution and the mixture was immediately applied to the resin incubated with DIPEA in THF. Resin was incubated with continuous shaking at 37 $^{\circ}$ C for 2 h and washed with THF three times and then with DMF three times. Resin was incubated with 1 M amine in DMF with continuous shaking at 60 $^{\circ}$ C overnight and washed with DMF three times.

Step 3 Peptoid synthesis after PTA residue

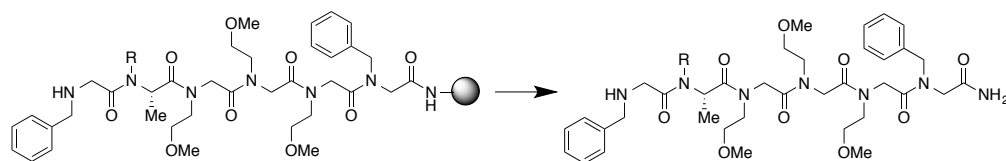


The PTA residue was chain extended with a peptoid residue using one of the following two conditions A or B.

A) Standard submonomer peptoid synthetic method: 345 μL of 2 M BAA (690 μmol) in DMF and 345 μL of 1 M DIC (345 μmol) in DMF were mixed and incubated at RT for 1 min and then applied to resin. Resin was incubated with continuous shaking at 37 $^{\circ}\text{C}$ for 2 h and resin was washed with DMF three times. This acylation procedures were repeated once more. Resin was incubated with 1 M amine in DMF with continuous shaking at 37 $^{\circ}\text{C}$ for 1 h and washed with DMF three times.

B) Chloroacetyl chloride method: 16.5 μL of chloroacetyl chloride (207 μmol) in 2.07 mL of DCM was cooled at -20°C for 10 min; Resin was incubated with 36.1 μL of DIPEA (207 μmol) in 2.07 mL of DCM at 4 $^{\circ}\text{C}$ for 10 min. 94.1 μL of TMP (311 μmol) was added to the cooled chloroacetyl chloride solution and the mixture was immediately applied to the resin. Resin was incubated with continuous shaking at 4 $^{\circ}\text{C}$ for 2 h and washed with DCM three times. These acylation procedures were repeated once more. Resin was incubated with 1 M amine in DMF at 60 $^{\circ}\text{C}$ overnight and washed with DMF three times.

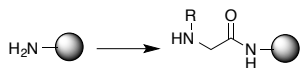
Step 4 Deprotection and cleavage of the synthesized oligomers from resin



Resin was washed with DCM three times and then synthesized oligomers were deprotected and cleaved from resin by treating resin with 2 mL of TFA/TIPS/thioanisole/ H_2O = 92.5/2.5/2.5/2.5 mixture with continuous shaking at RT for 3 h. Cleaved oligomer was recovered as a pellet by ether precipitation and the pellet was washed with 10 mL of ether twice. After drying the pellet under air, the oligomer was reconstituted in water:acetonitrile 5:1 solution containing 0.1 % TFA and used for HPLC analysis.

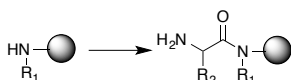
Synthesis and analysis of peptide and peptoid hybrid oligomers (7–12). Each oligomer was synthesized on 50 mg of Knorr Amide MBHA resin (0.69 mmol/g, 34.5 μmol). After resin was swollen in 1 mL of DMF for 15 min, Fmoc group was deprotected by treating resin with 1 mL of 20% piperidine in DMF for 3 min once and 12 min once and resin was washed with DMF three times. Peptide and peptoid hybrid oligomers were synthesized by repeating the following submonomer peptoid synthetic method and DIC/oxyma peptide synthetic method.

Submonomer peptoid synthetic method



First 345 μL of 2 M BAA (690 μmol) in DMF and 345 μL of 1 M DIC (345 μmol) in DMF were mixed and incubated at RT for 1 min and then applied to resin. Resin was incubated with continuous shaking at 37 $^{\circ}\text{C}$ for 10 min and resin was washed with DMF three times. Resin was incubated with 1 M amine in DMF with continuous shaking at 37 $^{\circ}\text{C}$ for 1 h and washed with DMF three times.

DIC/oxyma peptide synthetic method

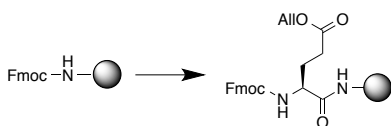


138 μmol each of Fmoc-amino acid, Oxyma (19.6 mg), and DIC (21.5 μL) were preincubated in 690 μL of DMF at RT for 10 min. The mixture was applied to resin and resin was incubated with continuous shaking at RT for 2 h and washed with DMF three times. The procedures were repeated once more.

After completion of the last amino acid coupling and Fmoc deprotection, resin was washed with DCM three times and then synthesized oligomers were deprotected and cleaved from resin by treating resin with 2 mL of TFA/TIPS/thioanisole/ H_2O = 92.5/2.5/2.5/2.5 mixture with continuous shaking at RT for 3 h. Cleaved oligomer was recovered as a pellet by ether precipitation and the pellet was washed with 10 mL of ether twice. After drying the pellet under air, the oligomer was reconstituted in 5 mL of water:acetonitrile 5:1 solution containing 0.1 % TFA. 20 μL of each oligomer solution was injected and analyzed on HPLC equipped with C18 reverse phase column.

Synthesis of a macrocyclic oligomer containing peptide, peptoid, and PTA units: Each oligomer was synthesized on 75 mg of 90 μm TentaGel R RAM beads (0.20 mmol/g, 15 μmol). Detailed procedures for synthesis of oligomer x are described below. Oligomer y is synthesized with the same procedures using different amines and an amino acid.

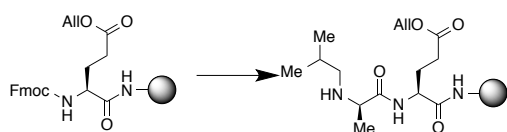
Step 1 Fmoc-Glu(OAll)-OH coupling



After resin was swollen in 1 mL of DMF for 15 min, Fmoc group was deprotected by treating resin with 1 mL of 20% piperidine in DMF for 3 min and then 12 min and resin was washed with DMF three times.

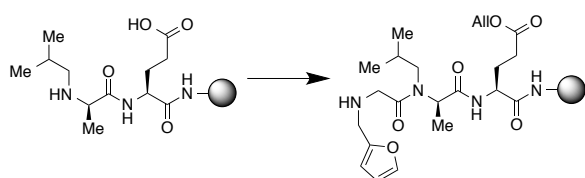
Fmoc-Glu(OAll)-OH was coupled using DIC/Oxyma coupling conditions at RT for 90 min.

Step 2 PTA synthesis



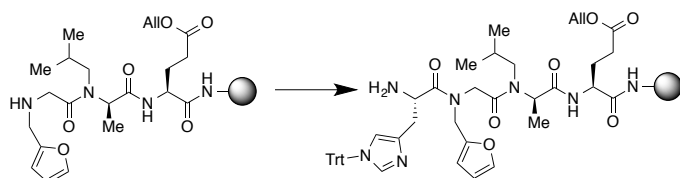
After Fmoc was deprotected, resin was washed with DMF and DCM three times each and with THF once. Resin was incubated with 52.3 μL of DIPEA (300 μmol) in 1.35 mL THF at RT for 10 min. 13.4 mg of triphosgene (45 μmol) and 12.2 μL of (S)-BPA (135 μmol) was dissolved in 1.35 mL of THF and cooled at $-20\text{ }^{\circ}\text{C}$ for 10 min. 49.3 μL of TMP (375 μmol) was added to the cooled triphosgene solution and the mixture was immediately applied to resin. Resin was incubated with continuous shaking at RT for 2 h and washed with DCM and DMF three times each. Amine displacement was performed with 1 mL of 1 M isobutylamine in DMF at $60\text{ }^{\circ}\text{C}$ overnight.

Step 3 Petpoid synthesis using chloroacetyl chloride



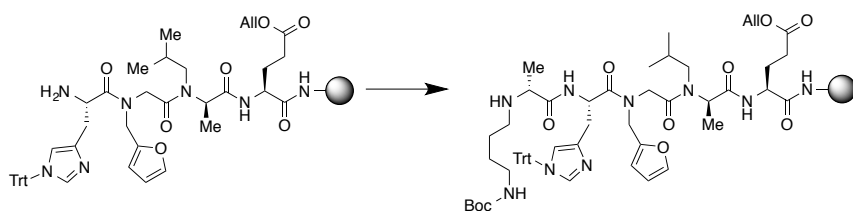
After washing with DMF and DCM three times each, resin was incubated with 13.1 μL of DIPEA (75 μmol) in 750 μL of DCM at $4\text{ }^{\circ}\text{C}$ for 10 min. 8.48 μL of chloroacetyl chloride (75 μmol) was dissolved in 750 μL of DCM and cooled at $-20\text{ }^{\circ}\text{C}$ for 10 min. 14.8 μL of TMP was added to the cooled chloroacetyl chloride solution and the mixture was immediately applied to resin. Resin was incubated with continuous shaking at $4\text{ }^{\circ}\text{C}$ for 2 h and washed with DCM three times. These acylation procedures were repeated once more. Amine displacement was performed with 1 mL of 1 M furfurylamine in DMF at $60\text{ }^{\circ}\text{C}$ overnight and resin was washed with DMF three times.

Step 4 Amino acid coupling



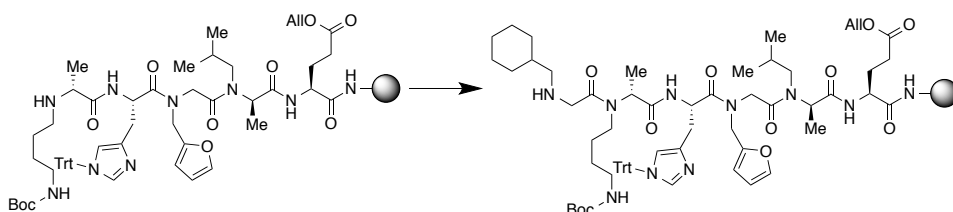
Fmoc-His(Trt)-OH (120 μmol), 17.0 mg of Oxyma (120 μmol), and 18.7 μL of DIC (120 μmol) were preincubated in 600 μL of DMF at RT for 10 min and applied to resin. Resin was incubated with continuous shaking at RT for 2 h and washed with DMF three times. These amino acid coupling procedures were repeated once more and Fmoc was deprotected.

Step 5 PTA synthesis



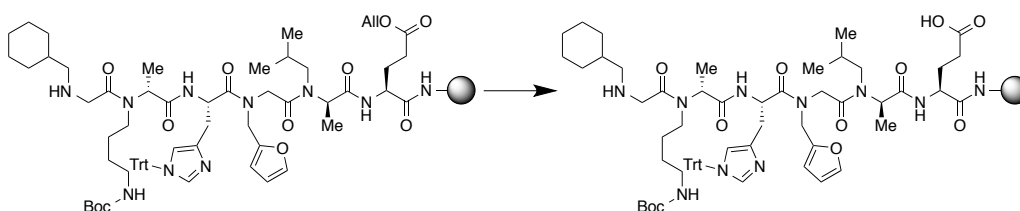
The BPA coupling and amine displacement were performed following the same procedures of step 2 using (S)-BPA and *N*-Boc-diaminobutane. BPA coupling was performed for 3 h.

Step 6 Petpoid synthesis using chloroacetyl chloride



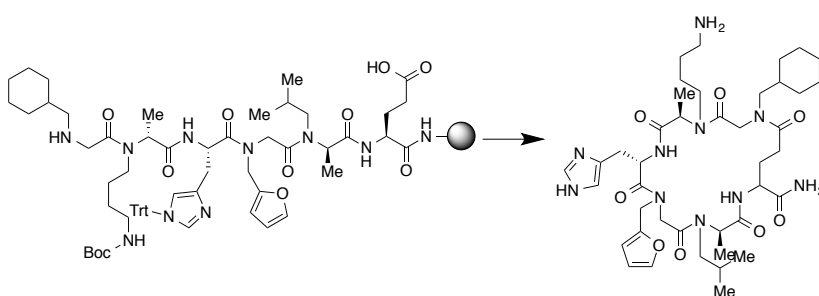
Chloroacetyl chloride reaction and amine displacement were performed following the same procedures of step 3 using cyclohexylmethylamine.

Step 7 Deprotection of allyl protecting group on Glu side chain



Allyl group was deprotected by incubating the resin with 52.0 mg of Pd(PPh₃)₄ (45 μmol) and 22.1 μL of PhSiH₃ (180 μmol) in 1.56 mL DCM with continuous shaking at RT for 30 min. Resin was washed with DCM, DMF, 0.5% DIPEA in DMF, and 0.5% sodium diethyldithiocarbamate trihydrate in DMF three times each and then with 0.5% DIPEA in DMF and DMF once each.

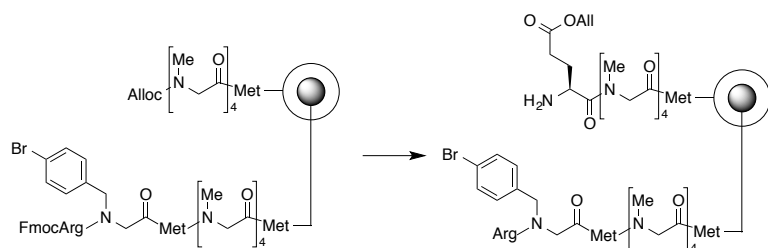
Step 8 Macrocyclization and cleavage



Resin was divided into two (7.5 μmol) each. One was cleaved without macrocyclization and the other one was macrocyclized. Macrocyclization was performed by incubating resin with 7.82 mg of PyAOP (15 μmol), 2.04

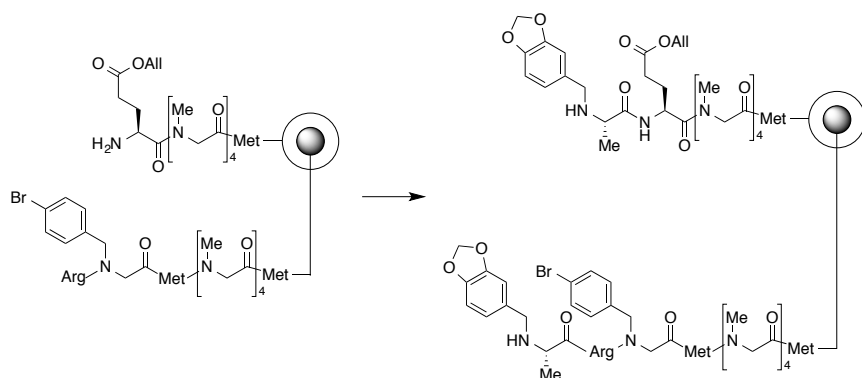
with 4-bromobenzyl side chain was synthesized with the standard peptoid submonomer synthetic method using 4-bromobenzylamine at amine displacement step. Fmoc-Arg(Pbf)-OH was coupled using DIC/Oxyma coupling conditions described above with 2 h reaction time.

Step 4 Introduction of Glu(OAll) on exterior layer



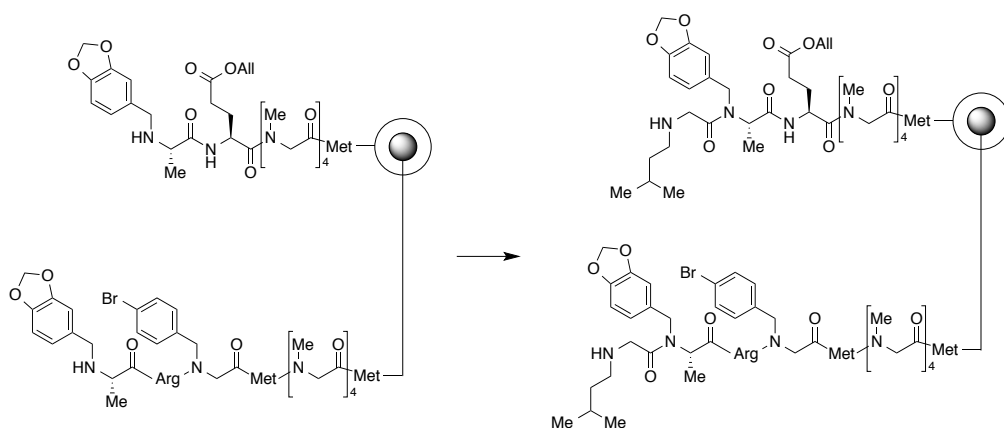
Alloc group on exterior amine was deprotected by incubating the resin with 76.3 mg of $\text{Pd}(\text{PPh}_3)_4$ (66 μmol) and 32.4 μL of PhSiH_3 (264 μmol) in 2.3 mL DCM with continuous shaking at RT for 30 min. Resin was washed with DCM, DMF, 0.5% DIPEA in DMF, and 0.5% sodium diethyldithiocarbamate trihydrate in DMF three times each and then with 0.5% DIPEA in DMF and DMF once each. Fmoc-Glu(OAll)-OH was coupled using DIC/Oxyma at RT overnight. Fmoc group on both exterior and interior layers of the beads were deprotected by treating the resin with 20% piperidine in DMF for 3 min and 12 min once each and beads were washed with DMF three times.

Step 5 PTA synthesis



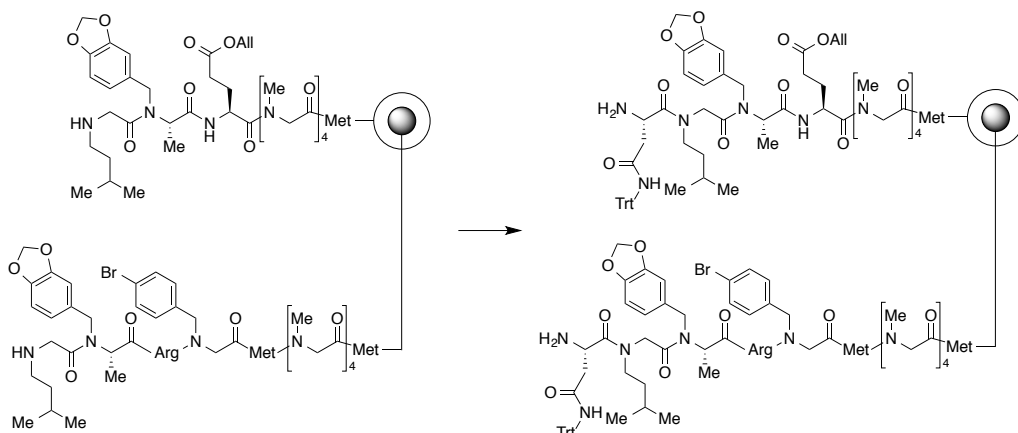
The resin was washed with DCM and THF three times each and incubated with 76.7 μL of DIPEA (440 μmol) in 1 mL THF at RT for 10 min. 19.6 mg of triphosgene (66 μmol) and 17.8 μL of (R)-BPA (198 μmol) was dissolved in 2 mL of THF and cooled at $-20\text{ }^\circ\text{C}$ for 10 min. 72.3 μL of TMP (550 μmol) was added to the cooled triphosgene solution and the mixture was immediately applied to resin. Resin was incubated with continuous shaking at RT for 90 min and washed with DCM and DMF three times each. Resin was incubated with 1 mL of 1 M piperonylamine (1 mmol) in DMF at $60\text{ }^\circ\text{C}$ overnight.

Step 6 Peptoid synthesis using chloroacetyl chloride



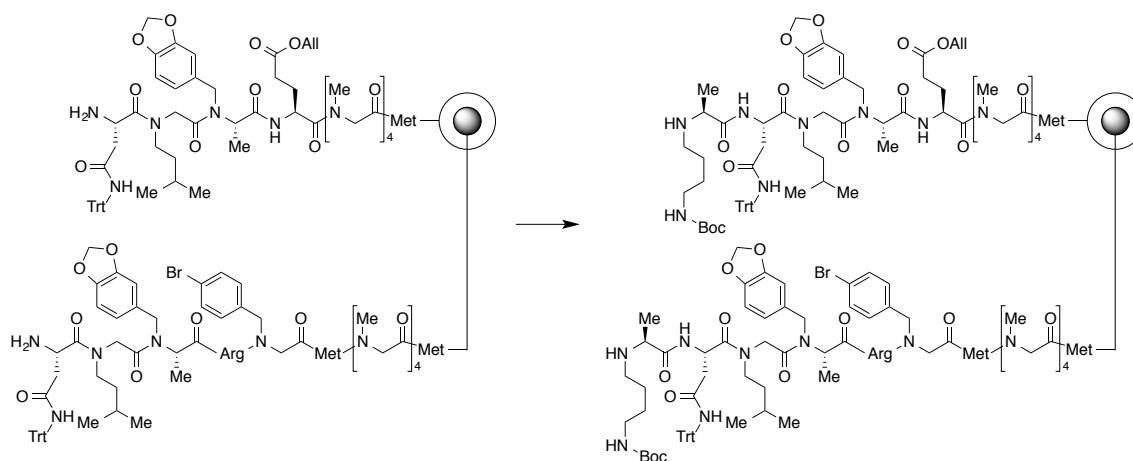
After washing with DMF and DCM three times each, resin was incubated with 23 μL of DIPEA (132 μmol) in 1.32 mL of DCM at 4 $^{\circ}\text{C}$ for 10 min. 10.5 μL of chloroacetyl chloride (132 μmol) was dissolved in 1.32 mL of DCM and cooled at -20°C for 10 min. 26.0 μL of TMP (198 μmol) was added to the cooled chloroacetyl chloride solution and the mixture was immediately applied to resin. Resin was incubated with continuous shaking at 4 $^{\circ}\text{C}$ for 2 h and washed with DCM three times. These acylation procedures were repeated once more and resin was washed with DMF three times. Amine displacement was performed with 1 mL of 1 M isopentylamine in DMF (1 mmol) at 60 $^{\circ}\text{C}$ overnight and resin was washed with DMF three times.

Step 7 Amino acid coupling



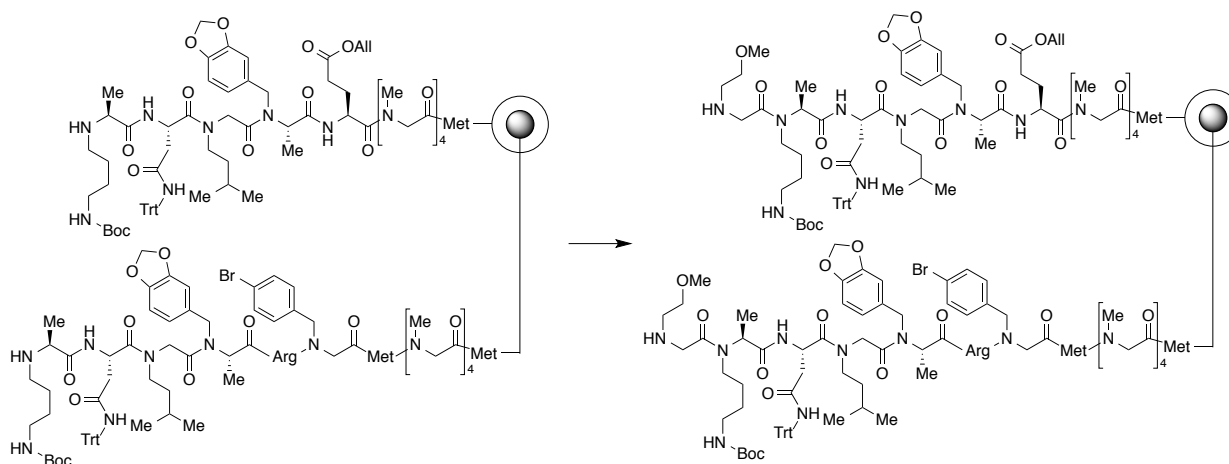
78.8 mg of Fmoc-Asn(Trt)-OH (132 μmol), 18.7 mg of Oxyma (132 μmol), and 20.5 μL of DIC (132 μmol) were preincubated in 660 μL of DMF at RT for 10 min and applied to resin. Resin was incubated with continuous shaking at RT for 2 h and washed with DMF three times. These amino acid coupling procedures were repeated once more and Fmoc was deprotected.

Step 8 PTA synthesis



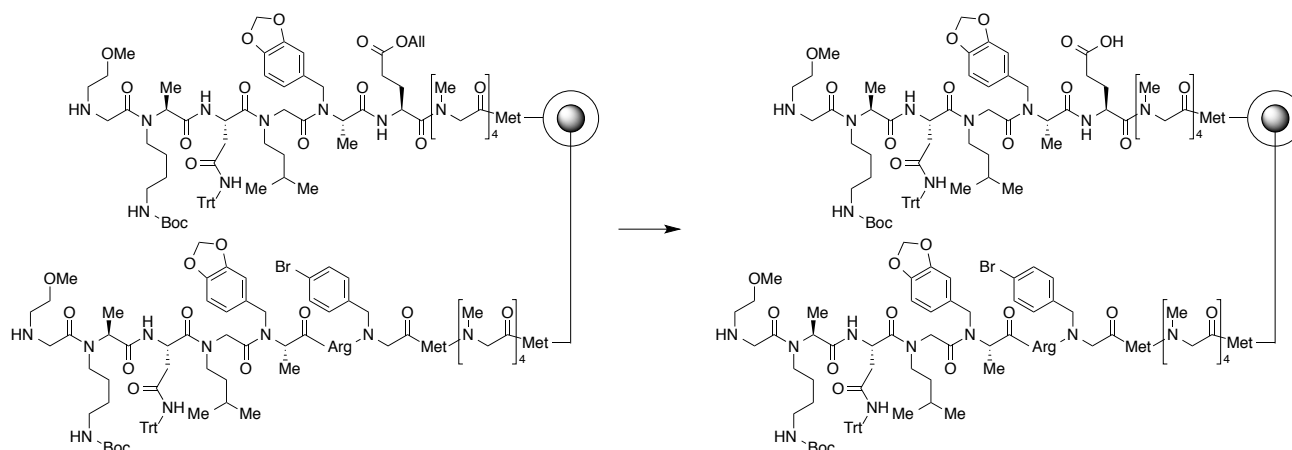
The BPA coupling and amine displacement were performed following the same procedures of step 5 using N-Boc-1,4-diaminobutane.

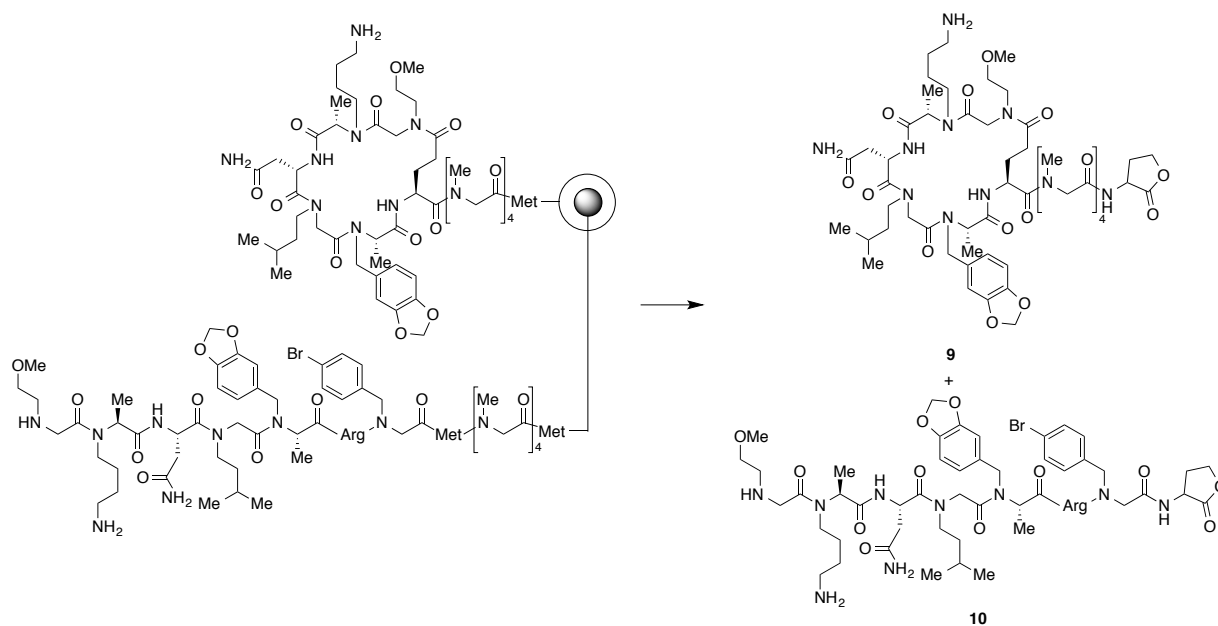
Step 9 Peptoid synthesis



Chloroacetyl chloride reaction and amine displacement were performed following the same procedures of step 6 using 2-methoxyethylamine.

Step 10 Deprotection of allyl group on glutamic acid side chain

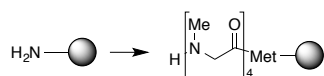




Single bead was isolated in 96-well plate and oligomers on the bead was cleaved by incubating the bead with 50 μL of 30 mg/mL CNBr in acetonitrile/acetic acid/water = 5/4/1 with gentle shaking at RT overnight. The solution was dried under vacuum and the cleaved oligomers were dissolved in 20 μL of 80% acetonitrile/water containing 0.1% TFA. 0.5 μL of the solution was spotted on MALDI plate, mixed with 0.5 μL of 5 mg/mL CHCA in 80% acetonitrile/water containing 0.1% TFA, dried, and analyzed by MALDI-TOF MS.

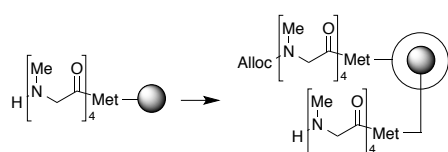
Synthesis of a macrocyclic oligomer library on physically segregated bilayer TentaGel beads with linear encoding chain: 1.2 g of 90 μm TentaGel R NH_2 beads (0.20 mmol/g, 240 μmol total) were swollen in DMF for 15 min.

Step 1 Sarcosine linker synthesis



A linker of methionine and trimer sarcosine was synthesized by the standard submonomer peptoid synthetic method. For amine displacement step in the synthesis, 2 M methylamine in THF was diluted with DMF to 1 M and used.

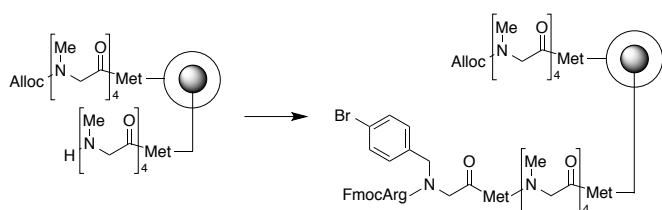
Step 2 Bead segregation



Beads were separated into 5 reaction vessels (240 mg, 48 μmol each). Beads were washed with water 10 times

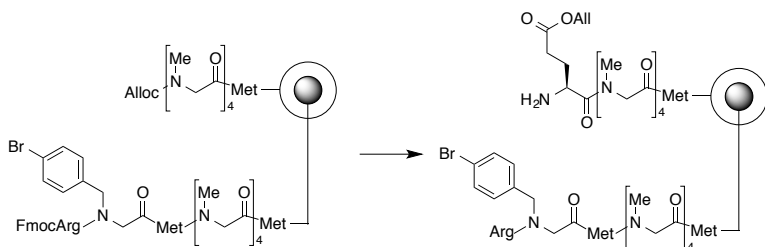
and incubated in water with continuous shaking at RT overnight. After water was drained, beads in each reaction vessel were briefly washed with 1 mL of DCM:Ether = 55:45 twice and incubated with 2.99 μL of *N*-(Allyloxycarbonyloxy)succinimide (19.2 μmol) and 6.69 μL of DIPEA (38.4 μmol) in 9.6 mL of DCM:Ether = 55:45 with vigorous shaking at RT for 30 min to cap amines on exterior layer of beads with alloc group. Resin was combined into one reaction vessel, washed with DMF three times, and incubated in DMF with continuous shaking at RT overnight.

Step 3 Interior linker synthesis



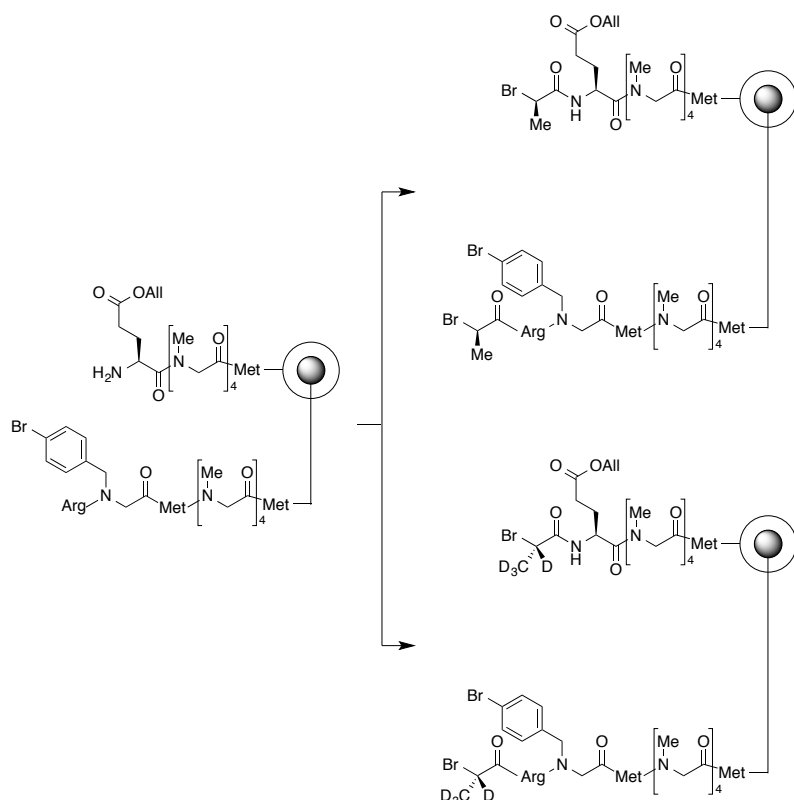
Fmoc-Met-OH was coupled using DIC/Oxyma coupling conditions with 2 h reaction time. A peptoid residue with 4-bromobenzyl side chain was synthesized with the standard peptoid submonomer synthetic method using 4-bromobenzylamine at amine displacement step. Fmoc-Arg(Pbf)-OH was coupled using DIC/Oxyma coupling conditions described above with overnight reaction.

Step 4 Introduction of Glu(OAll) on exterior layer



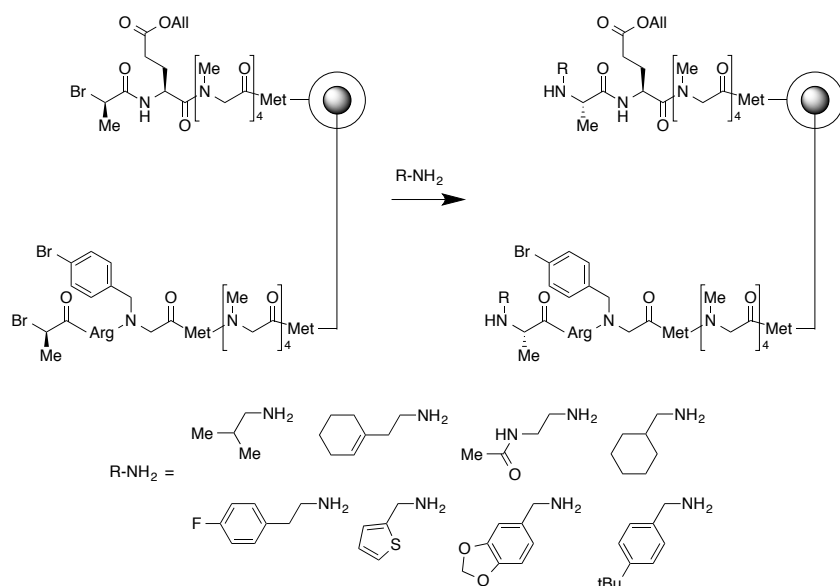
Alloc group on exterior amine was deprotected by incubating the resin with 555 mg of $\text{Pd}(\text{PPh}_3)_4$ (480 μmol) and 236 μL of PhSiH_3 (1.92 mmol) in 16.7 mL DCM with continuous shaking at RT for 1 h. Resin was washed with DCM, DMF, 0.5% DIPEA in DMF, and 0.5% sodium diethyldithiocarbamate trihydrate in DMF three times each and then with 0.5% DIPEA in DMF and DMF once each. Fmoc-Glu(OAll)-OH was coupled using DIC/Oxyma at RT for 4 h and Fmoc group on both exterior and interior layers of the beads were deprotected.

Step 5 BPA coupling



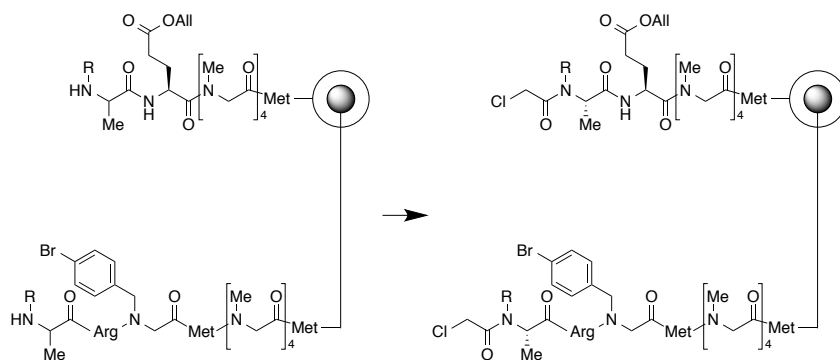
The resin was washed with DCM and THF three times each and split into 2 reaction vessels (600 mg, 120 μmol each). Each resin was incubated with 418 μL of DIPEA (2.40 mmol) in 5.4 mL THF at RT for 10 min. 107 mg of triphosgene (360 μmol) and 97.2 μL of (R)-BPA or (S)-BPA- d_4 (1.08 mmol) was dissolved in 5.4 mL of THF and cooled at $-20\text{ }^\circ\text{C}$ for 10 min. 395 μL of TMP (3.00 mmol) was added to the cooled triphosgene solution and the mixture was immediately applied to resin. Resin was incubated with continuous shaking at RT for 2 h and washed with DCM and DMF three times each. Resin was combined in one reaction vessel and mixed thoroughly.

Step 6 Amine displacement



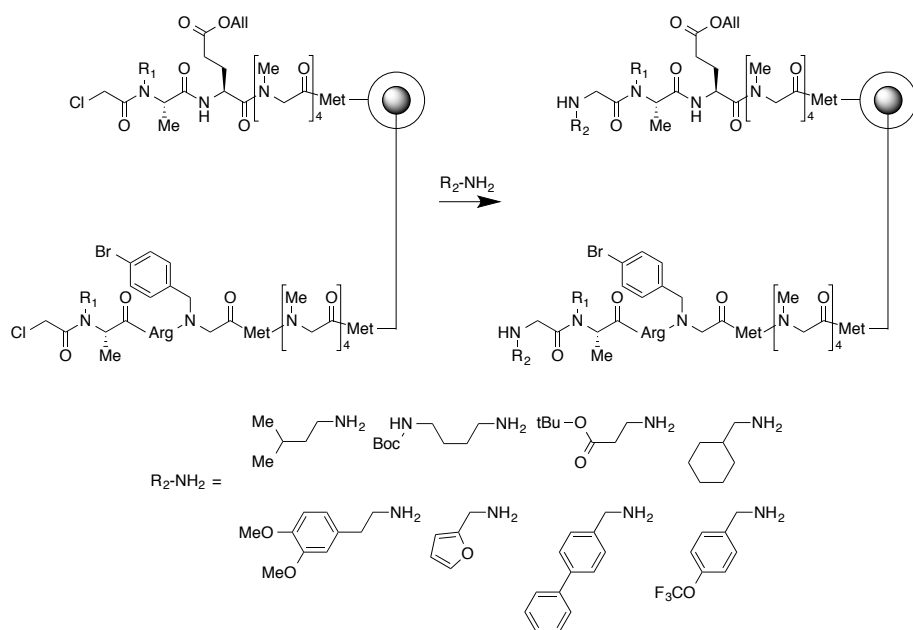
Resin was divided into 8 reaction vessels (150 mg, 30 μmol each) and each resin was incubated with 1.2 mL of 1 M solution of one of the 8 primary amines shown above in DMF with continuous shaking at 60 $^{\circ}\text{C}$ overnight. A reaction scheme with resin obtained from (R)-BPA coupling in step 5 is shown above as an example. Note that a stereocenter of α -carbon at the terminal residue is inverted during amine displacement reaction. Resin was washed with DMF three times, combined into one reaction vessel, and mixed thoroughly.

Step 7 Acylation by chloroacetyl chloride



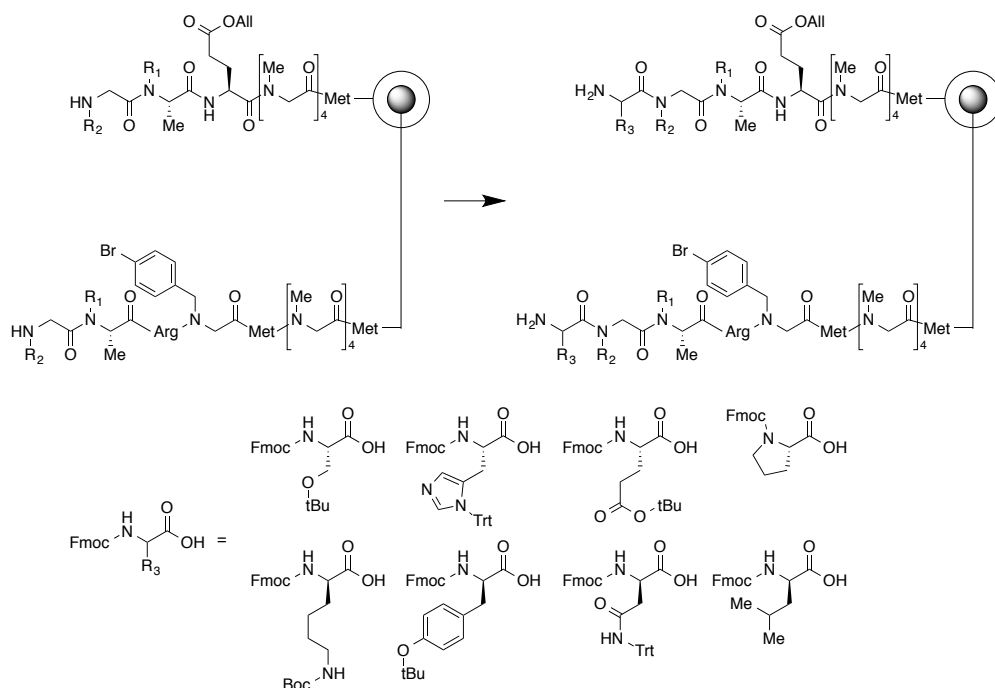
After washing with DCM three times, resin was incubated with 251 μL of DIPEA (1.44 mmol) in 14.4 mL of DCM at 4 $^{\circ}\text{C}$ for 10 min. 114 μL of chloroacetyl chloride (1.44 mmol) was dissolved in 14.4 mL of DCM and cooled at -20°C for 10 min. 284 μL of TMP (2.16 mmol) was added to the cooled chloroacetyl chloride solution and the mixture was immediately applied to resin. Resin was incubated with continuous shaking at 4 $^{\circ}\text{C}$ for 2 h and washed with DCM three times. These acylation procedures were repeated once more and resin was washed with DMF three times.

Step 8 Amine displacement



Resin was divided into 8 reaction vessels (150 mg, 30 μ mol each) and each resin was incubated with 1.2 mL of 1 M solution of one of the 8 primary amines shown above in DMF with continuous shaking at 60 $^{\circ}$ C overnight. Resin was washed with DMF three times, combined into one reaction vessel, and mixed thoroughly.

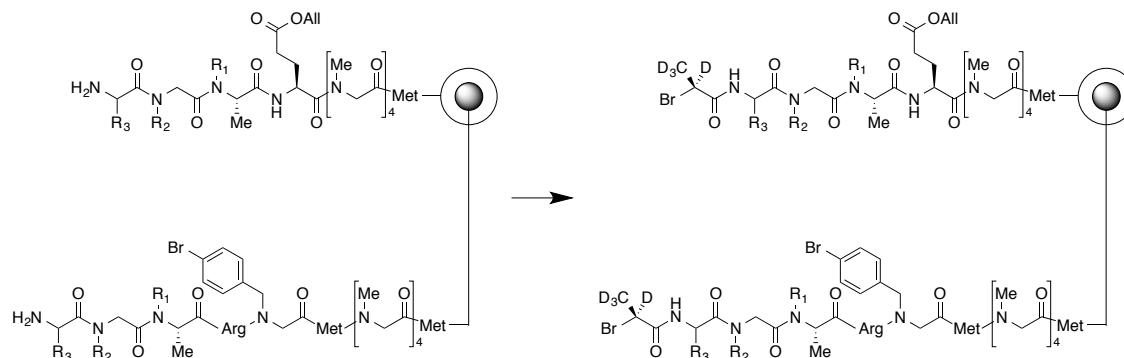
Step 9 Amino acid coupling



Resin was divided into 8 reaction vessels (150 mg, 30 μ mol each). Fmoc-amino acid (180 μ mol), 25.6 mg of Oxyma (180 μ mol), and 28.0 μ L of DIC (180 μ mol) were preincubated in 900 μ L of DMF at RT for 10 min and applied to resin. Resin was incubated with continuous shaking at RT for 2 h and washed with DMF three

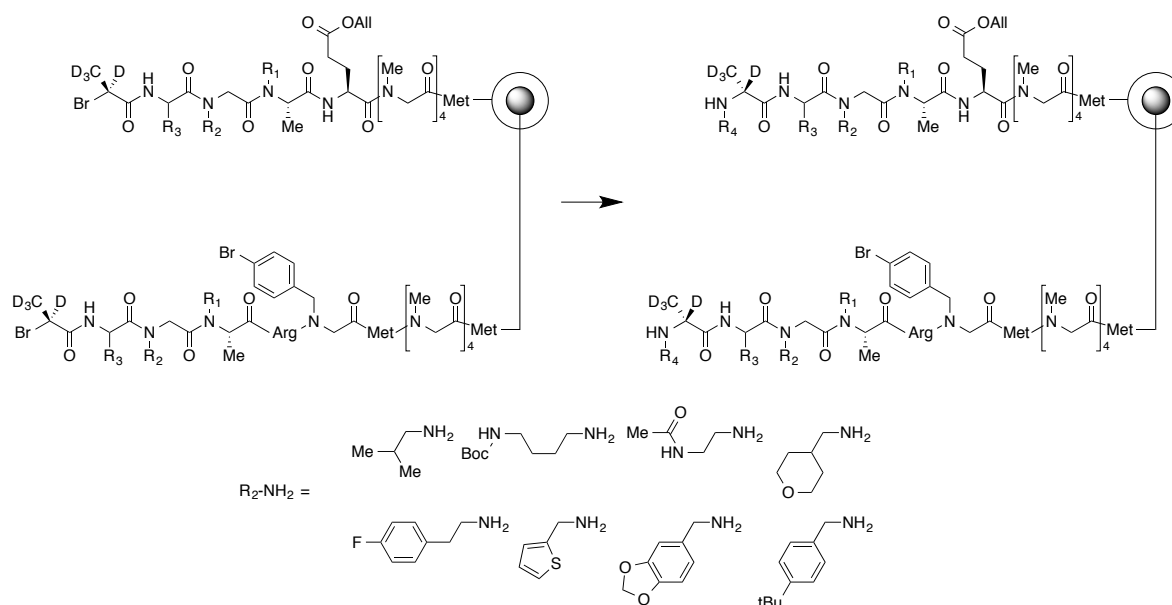
times. These amino acid coupling procedures were repeated once more and resin was combined in one reaction vessel. Fmoc was deprotected and beads were thoroughly mixed.

Step 10 BPA coupling



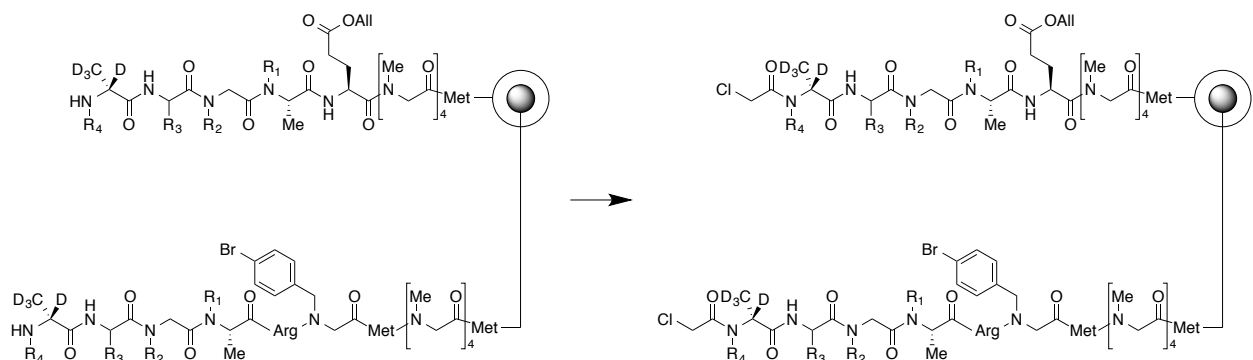
Beads were divided into two reaction vessels (600 mg, 120 μ mol each). One was acylated with (R)-BPA and the other was acylated with (S)-BPA-d₄ following the same procedures of step 5. A structure of oligomer coupled with (S)-BPA-d₄ is shown as a representative structure above and hereafter. Beads were combined in one reaction vessel and mixed thoroughly.

Step 11 Amine displacement reaction



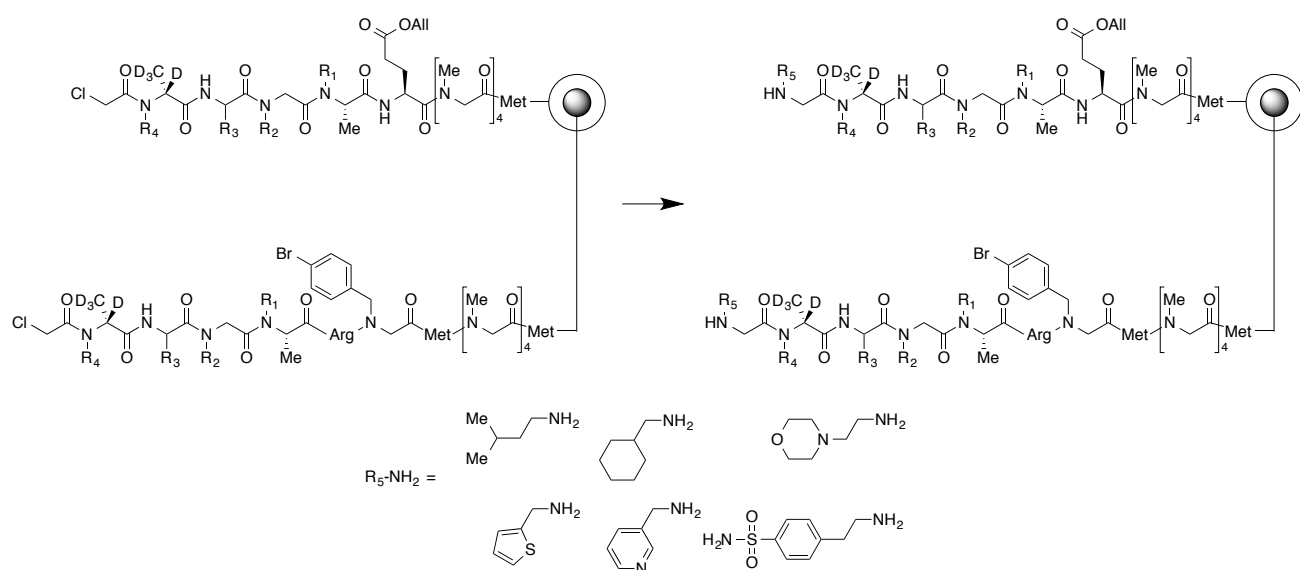
Resin was divided into 8 reaction vessels (150 mg, 30 μ mol each) and each resin was incubated with 1.2 mL of 1 M solution of one of the 8 primary amines shown above in DMF with continuous shaking at 60 °C overnight. Resin was washed with DMF three times, combined into one reaction vessel, and mixed thoroughly.

Step 12 Acylation by chloroacetyl chloride



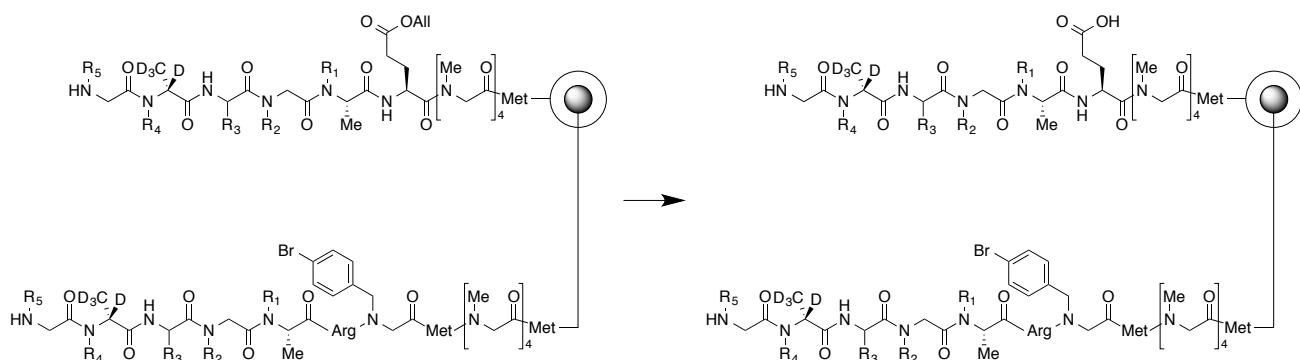
Chloroacetyl chloride reaction was performed following the same procedures of step 7.

Step 13 Amine displacement reaction



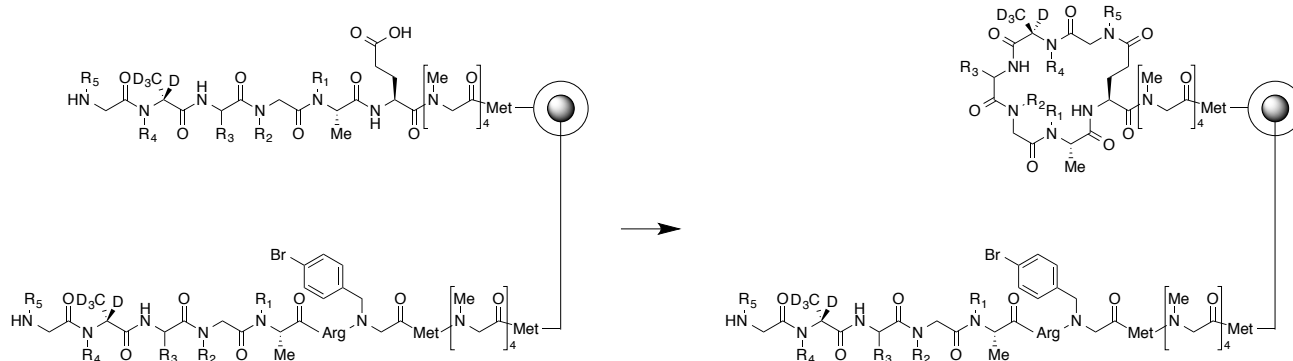
Resin was divided into 6 reaction vessels (200 mg, 40 μmol each) and each resin was incubated with 1.6 mL of 1 M solution of one of the 6 primary amines shown above in DMF with continuous shaking at 60 $^{\circ}\text{C}$ overnight. Resin was washed with DMF three times, combined into one reaction vessel, and mixed thoroughly.

Step 14 Deprotection of allyl group on glutamic acid side chain



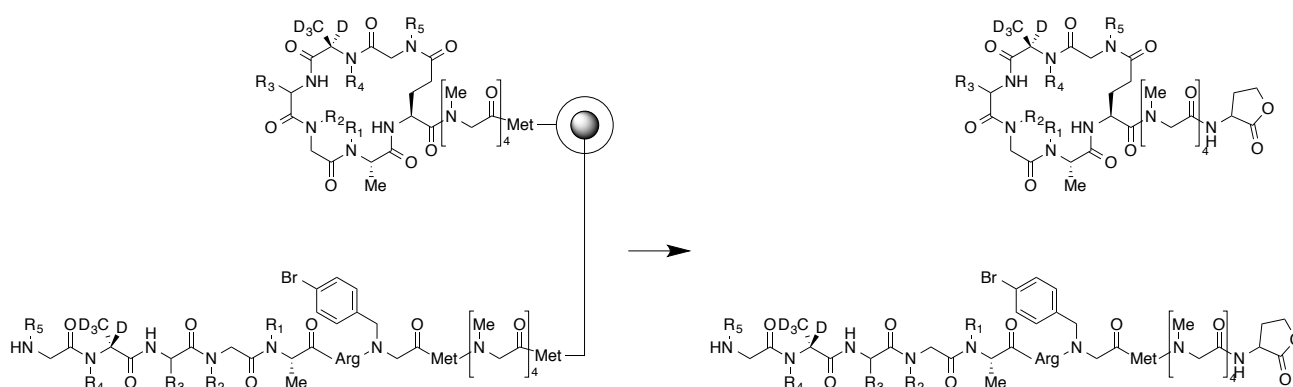
Allyl group was deprotected by incubating resin with 832 mg of Pd(PPh₃)₄ (720 μmol) and 353 μL of PhSiH₃ (2.88 mmol) in 25 mL DCM with continuous shaking at RT for 1 h. Resin was washed with DCM, DMF, 0.5% DIPEA in DMF, and 0.5% sodium diethyldithiocarbamate trihydrate in DMF three times each and then with 0.5% DIPEA in DMF and DMF once each.

Step 15 Macrocyclization and deprotection



Macrocyclization was performed by incubating resin with 250 mg of PyAOP (480 μmol), 65.3 mg of HOAt (480 μmol), and 167 μL of DIPEA (960 μmol) in 24 mL of DMF with rotation at RT overnight. Resin was washed with DMF and DCM three times each. Small aliquot of the beads were incubated with 1 mL of 50% TFA/DCM containing 2.5% TIPS and 2.5% thioanisole with continuous shaking at RT for 2 h to deprotect all the side chains, washed with DCM three times, and then used for single bead cleavage.

Step 16 Single bead cleavage and MALDI-TOF MS analysis of the synthesized linear and macrocyclic oligomers



48 beads were separated into microwells of a 96-well plate as one bead per well and oligomers on the beads were cleaved by incubating each bead with 50 μL of 30 mg/mL CNBr in acetonitrile/acetic acid/water = 5/4/1 with gentle shaking at RT overnight. The solution was dried under vacuum and the cleaved oligomers were dissolved in 20 μL of 80% acetonitrile/water containing 0.1% TFA. 0.5 μL of each oligomer solution was spotted on MALDI plate, mixed with 0.5 μL of 5 mg/mL CHCA in 80% acetonitrile/water containing 0.1% TFA, dried, and analyzed by MALDI-TOF MS.

References

- 1 Gao, Y.; Kodadek, T. *Chem. Biol.* **2013**, *20*, 360.