

– Supporting Information (SI) –

# Ring-Closing Metathesis Approaches for the Solid-Phase Synthesis of Cyclic Peptoids

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## General methods

Chemical reagents were purchased from commercial sources and were used without further purification unless noted otherwise. Methylamine was used as 2 M solution in THF and glycine *tert*-butyl ester acetic acid was neutralized with aq. Na<sub>2</sub>CO<sub>3</sub> and extracted with DCM prior to use. Moisture sensitive reactions were performed under nitrogen or argon atmosphere. CH<sub>2</sub>Cl<sub>2</sub> was dried over calcium hydride. Grubbs Catalyst 1st generation (G1), Grubbs catalyst 2nd generation (G2) and Hoveyda-Grubbs Catalyst 2<sup>nd</sup> Generation (HG2) were purchased from Aldrich. Rink Amide AM resin LL (0.4 mmol/g) and TentaGel MB RAM (0.4 mmol/g) were purchased from Novabiochem. Reverse-phase HPLC experiments were conducted through an ACE 5 C18-HL (250 x 4.6mm) reverse phase column on a Shimadzu binary HPLC system equipped with a UV-visible detector at 220 nm. The typical flow rate for analytical HPLC was 1 mL/min. In all cases, a gradient elution of water/acetonitrile with 0.05% TFA was used. MALDI-TOF MS was performed on a Voyager-DE STR biospectrometry workstation (Applied Biosystems) with  $\alpha$ -hydroxy cinnamic acid as a matrix. The peptoids were synthesized in an incubator shaker (JEIO TECH, model SI-600R) or in a microwave oven (Daewoo, model KR-B200R). The microwave reactions were performed at a power of 100 W for peptoid synthesis and at a power of 300 W for RCM reactions.

## Peptoid synthesis

Peptoids were synthesized on Rink amide (**1-5**, **7-10**, **15-16** and **19-23**) and TentaGel (**24-29**) resins by the conventional submonomer strategy.<sup>1</sup> Peptoid syntheses were performed in 25 mL standard glass peptide synthesis vessels. The resins were swelled in DMF at 25 °C for 1-2 h. Then DMF was drained, and the beads were incubated 20% piperidine in DMF for 1 h and washed thoroughly with DMF (8 x 3 mL). The beads were treated with 2 M bromoacetic acid (1.0-1.5 mL) and 3.2 M DIC (1-1.5 mL) and irradiated in a microwave oven (100 W) for 3 x 12 sec with shaking for 30 sec after each pulse. The beads were thoroughly washed with DMF (8 x 3 mL) and then treated with primary amines (1-2 M, 2 mL) in DMF in a microwave oven (100 W) for 3 x 12 sec with shaking for 30 sec after each pulse. Both acylation and displacement were successively repeated to form the desired peptoid sequences. To prevent inactivation of RCM catalyst from free amines, the peptoids were capped with Boc-anhydride (10 eq.) and triethylamine (1 mL) in DCM (5 mL) and the beads were shaken for 2 h before being washed with MeOH (8 x 3 mL) and DCM (8 x 3 mL), and left to dry under vacuum for 2 h before RCM.

## General procedure for RCM of peptoids under microwave conditions

The beads (15 mg) containing linear peptoids were swelled in DCB (1 mL) in reaction vessel for 30 min. Then 2 mol% RCM catalysts were added and irradiated under microwave (300 W) for 4 x 30 sec (total 2 min) with shaking for 30 sec after each pulse. The resin was thoroughly washed with DCM (8 x 3 mL).

### General procedure for RCM of peptoids at 40 °C

The beads (15 mg) containing linear peptoids were charged in reaction flask and allowed to swell in anhydrous DCM (1 mL) for 30 min. Then 2 mol% RCM catalysts were added and refluxed at 40 °C under nitrogen atmosphere for 2 h. The resin was thoroughly washed with DCM (8 x 3 mL).

### General TFA cleavage procedure and reverse-phase HPLC

Peptoid-tethered resin was suspended in a cleavage cocktail (92% TFA/5% H<sub>2</sub>O/3% TIS) for 1-2 h. After cleavage solution was removed by blowing N<sub>2</sub> gas, 50% aq. acetonitrile containing 0.1% TFA was added and mixed uniformly. The mixture was filtered through 0.2 µm PTFE filter tip and the obtained solution was directly used for HPLC and MALDI-TOF analyses. Reverse-phase HPLC experiments were conducted through a C18 reverse phase column on a Shimadzu binary HPLC system by using a gradient elution of water/acetonitrile with 0.1% TFA. RCM products were produced as a mixture of *E/Z* isomers.

### Ozonolysis of cyclic peptoid

The requisite cyclic peptoid was cleaved from resin by a cleavage cocktail, concentrated and dried. Then cyclic peptoid was dissolved in DCM and cooled to -78 °C. Ozone gas was purged through the cooled solution, which turned blue after a few minutes. Then ozone was bubbled further for 10 min. Dimethylsulfide was added to the reaction mixture which turned it into clear solution. The reaction mixture was warmed slowly to room temperature and the solvent was removed *in vacuo*.

### Abbreviations

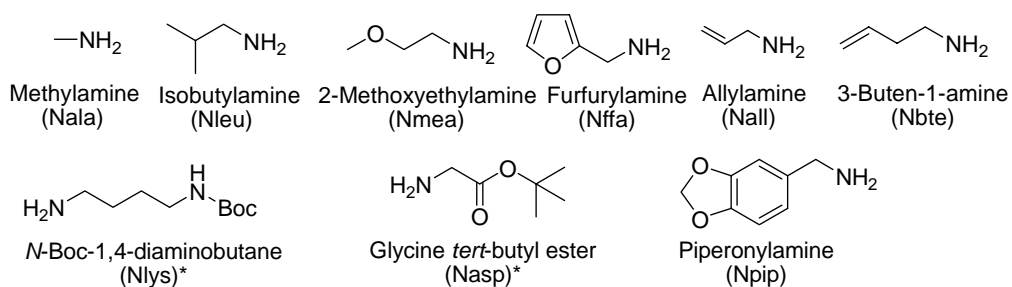
DMF: *N,N*-dimethylformamide, DCB: 1,2-dichlorobenzene, DCE: 1,2-dichloroethane, DCM: methylene chloride, TFA: trifluoroacetic acid, TIS: triisopropylsilane, DIC: *N,N'*-diisopropylcarbodiimide, G1: Grubbs Catalyst 1<sup>st</sup> generation [bis(tricyclohexylphosphine) benzylidene ruthenium(IV) chloride], G2: Grubbs catalyst 2<sup>nd</sup> generation [1,3-bis-(2,4,6-trimethylphenyl)-2-(imidazolidinylidene)(dichlorophenylmethylene)(tricyclohexylphosphine) ruthenium], HG2: Hoveyda-Grubbs Catalyst 2<sup>nd</sup> Generation [(1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium], RCM: ring-closing metathesis, HPLC: high-performance liquid chromatography, MALDI-TOF: matrix-assisted laser desorption/ionization-time of flight.

### References

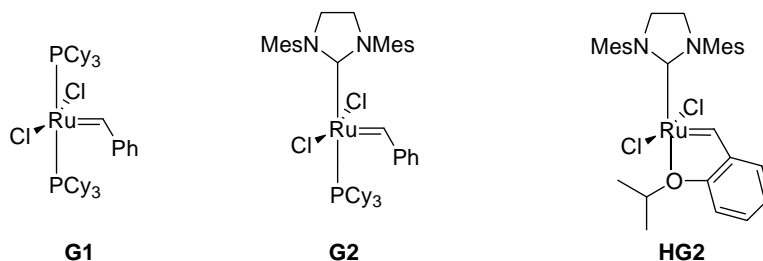
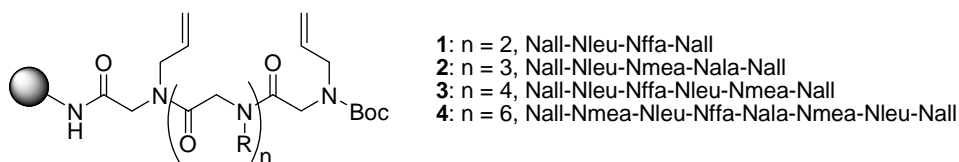
1. (a) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646-10647. (b) Olivos, H. J.; Alluri, P. G.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057-4509.

**Table S1** MALDI-MS data of synthesized peptoids

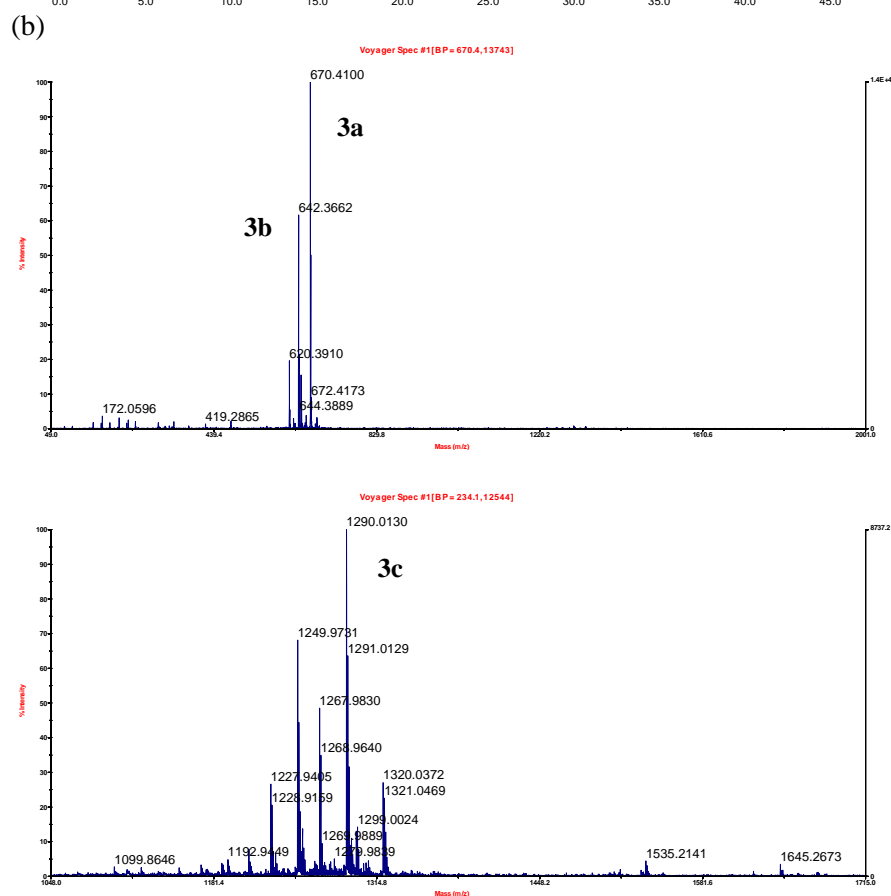
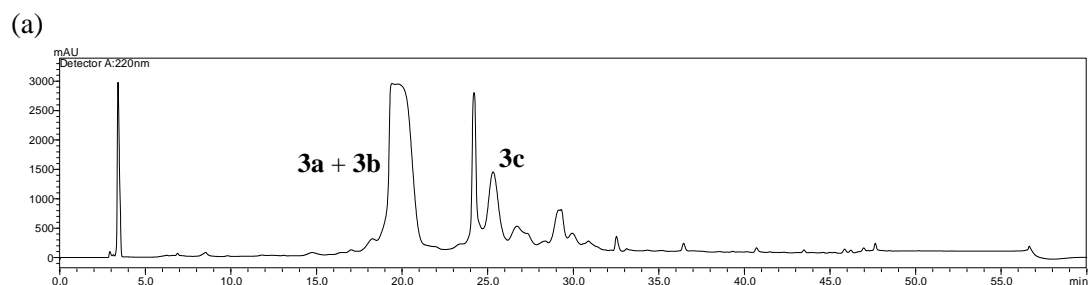
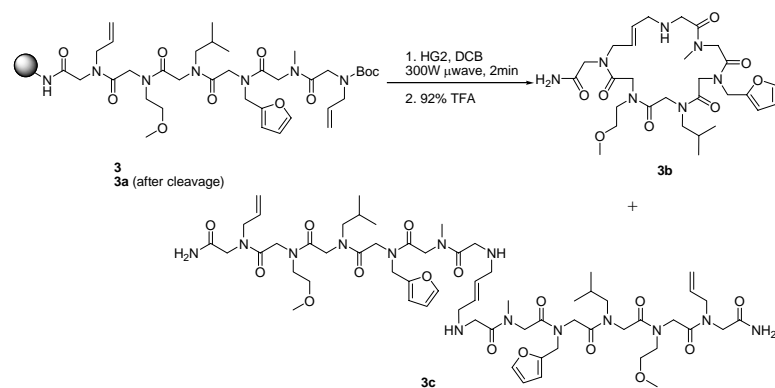
Entry	Peptoid	MS (calcd.)	MS (obs.)	
			[M+H] <sup>+</sup>	[M+Na] <sup>+</sup>
1	<b>3a</b>	647.36	-	670.41
2	<b>3b</b>	619.33	-	642.37
3	<b>3c</b>	1266.70	-	1290.01
4	<b>4a</b>	875.51	-	898.84
5	<b>4b</b>	847.48	-	870.79
6	<b>4c</b>	1722.99	-	1746.65
7	<b>5a</b>	682.40	-	705.34
8	<b>6</b>	654.37	655.32	677.33
9	<b>6a</b>	1308.74	1309.78	1331.80
10	<b>7a</b>	381.24	382.09	404.06
11	<b>8a</b>	452.27	453.13	475.12
12	<b>9a</b>	523.31	524.27	546.26
13	<b>10a</b>	594.35	595.22	617.20
14	<b>11</b>	353.21	354.18	376.24
15	<b>12</b>	424.24	425.41	447.41
16	<b>13</b>	495.28	496.18	518.16
17	<b>14</b>	566.32	567.43	589.44
18	<b>15a</b>	438.26	439.09	461.07
19	<b>16a</b>	509.30	510.20	532.18
20	<b>17</b>	410.22	411.26	433.23
21	<b>18</b>	481.26	482.40	504.40
22	<b>19a</b>	491.27	492.20	514.22
23	<b>20a</b>	604.36	605.48	627.50
24	<b>21a</b>	719.42	720.09	742.08
25	<b>22a</b>	734.40	735.20	757.21
26	<b>23a</b>	832.51	833.36	855.36
27	<b>24a</b>	504.31	505.34	527.31
28	<b>25a</b>	682.33	683.34	705.36
29	<b>26a</b>	682.30	683.38	705.37
30	<b>27a</b>	795.42	-	818.60
31	<b>28a</b>	734.40	735.21	757.21
32	<b>29a</b>	847.40	848.75	870.74
33	<b>30</b>	463.24	464.40	486.41
34	<b>31</b>	576.33	577.53	599.55
35	<b>32</b>	691.39	692.39	714.38
36	<b>33</b>	706.36	707.41	729.39
37	<b>34</b>	804.47	805.69	827.64
38	<b>35</b>	476.27	477.39	499.40
39	<b>36</b>	654.30	655.15	677.12
40	<b>37</b>	654.26	655.35	677.32
41	<b>38</b>	767.38	-	790.62
42	<b>39</b>	706.36	707.51	729.51
43	<b>40</b>	819.45	820.68	842.69
44	<b>41</b>	723.38	724.17	746.17

**Figure S1.** Amines and RCM catalysts employed in this study

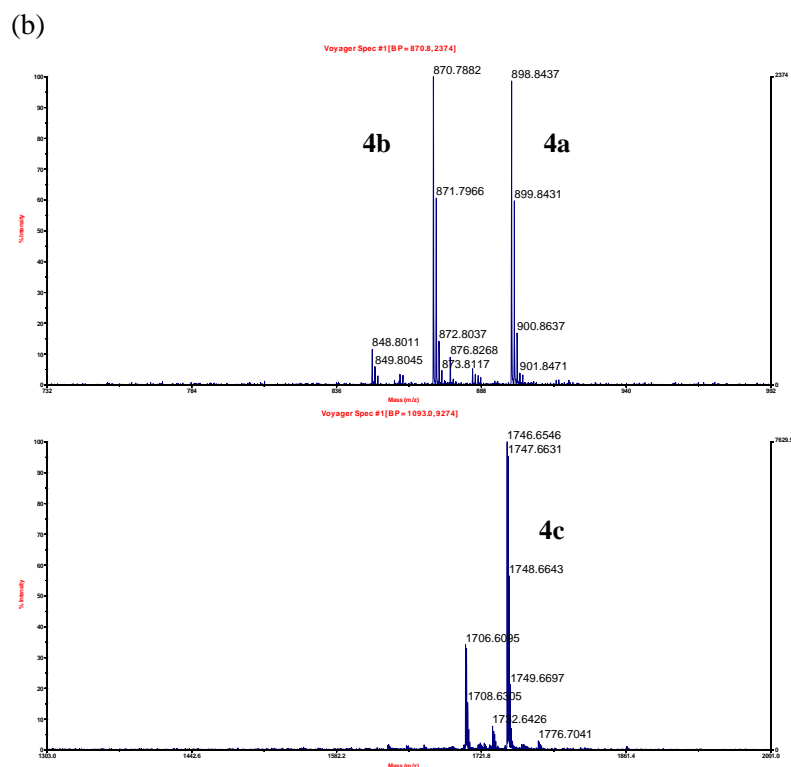
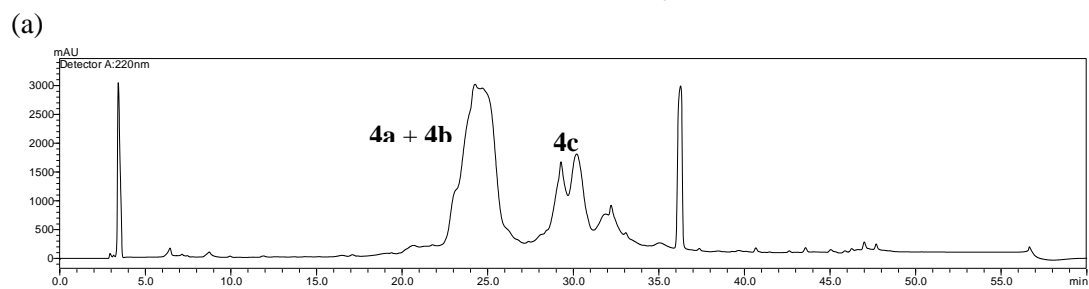
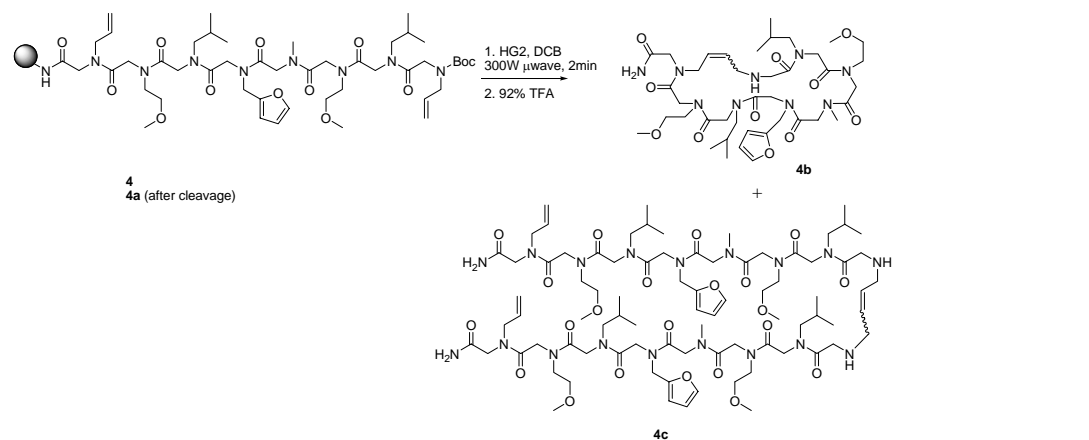
\* Nlys and Nasp: protecting groups (Boc and *tert*-butyl) were removed with the treatment of 92% TFA at the cleavage step.

**Ring-closing metathesis catalysts****Table S2.** RCM of peptoids (**1-4**) containing allylamines

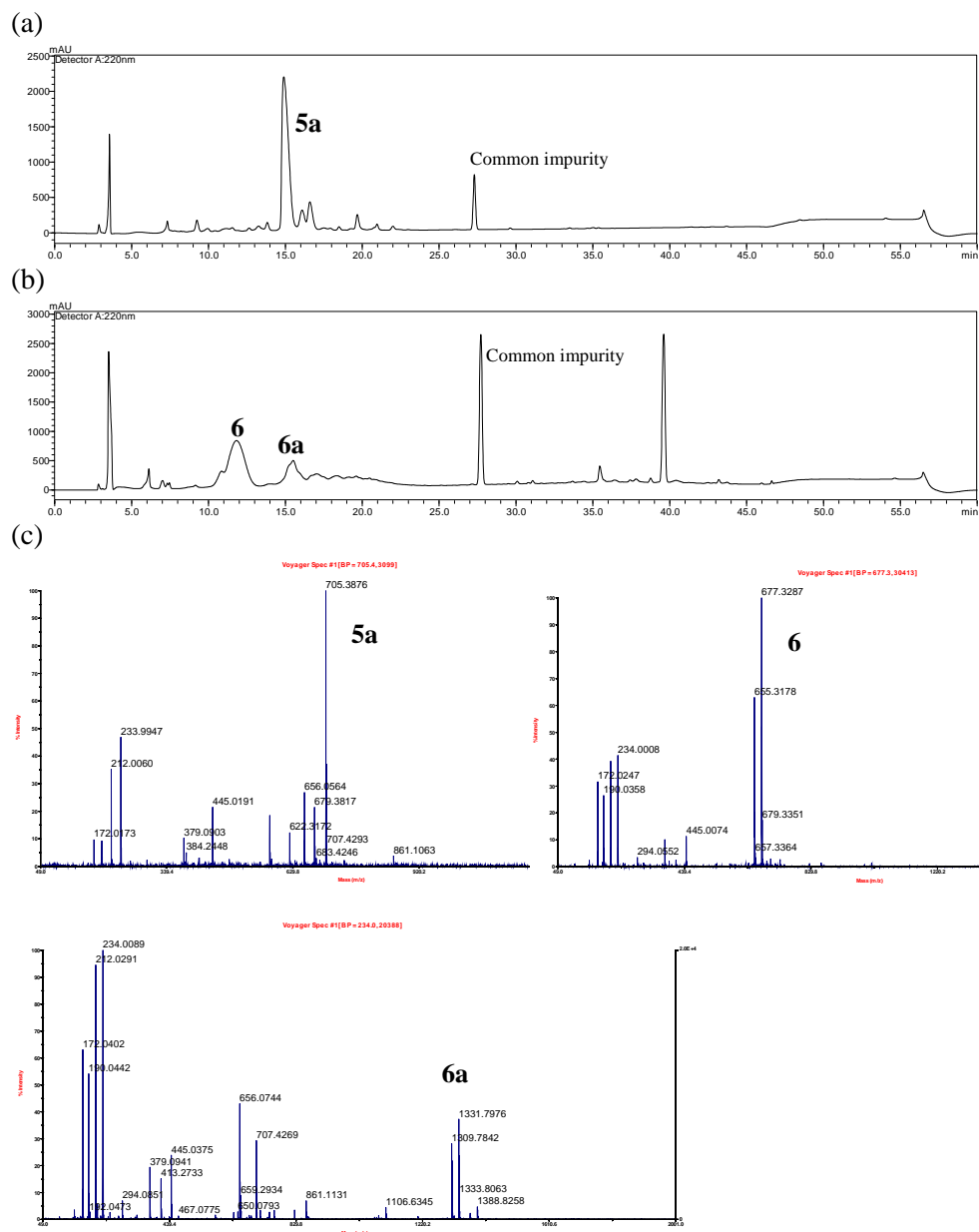
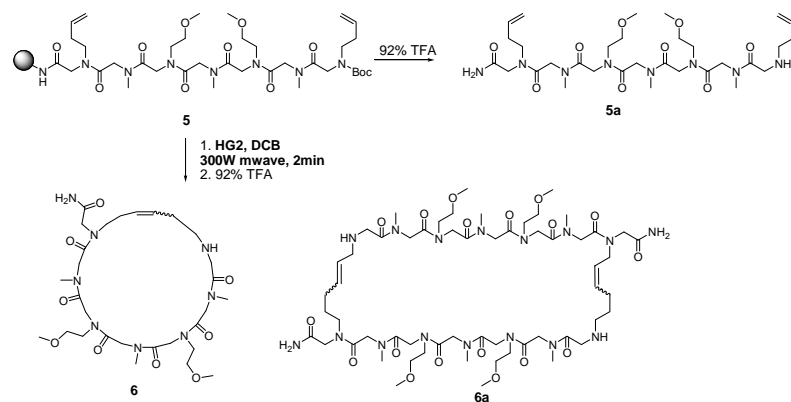
Entry	Peptoid	Catalyst	Solvent	Temp.	Time (h)	Remarks
1	4-mer ( <b>1</b> )	G1	DCM	25	12	No RCM
2	4-mer ( <b>1</b> )	G2	DCM	25	12	No RCM
3	5-mer ( <b>2</b> )	G1	DCM	25	12	No RCM
4	5-mer ( <b>2</b> )	G2	DCM	25	12	No RCM
5	5-mer ( <b>2</b> )	G2	DCB	80	12-24	No RCM
6	5-mer ( <b>2</b> )	G2	DCE	80	12-24	No RCM
7	5-mer ( <b>2</b> )	G2	DCM	MW	3 min	No RCM
8	6-mer ( <b>3</b> )	G2	DCM	25	24	No RCM
9	6-mer ( <b>3</b> )	G2	DCM	40	48	No RCM
10	6-mer ( <b>3</b> )	G2	DCB	80	24	No RCM
11	6-mer ( <b>3</b> )	G2	DCE	80	24	No RCM
<b>12</b>	<b>6-mer (3)</b>	<b>HG2</b>	<b>DCB</b>	<b>MW</b>	<b>2 min</b>	<b>10-20%</b>
12	8-mer ( <b>4</b> )	G1	DCM	25	12	No RCM
13	8-mer ( <b>4</b> )	G2	DCM	25	12	No RCM
14	8-mer ( <b>4</b> )	G2	DCM	MW	3 min	No RCM
<b>15</b>	<b>8-mer (4)</b>	<b>HG2</b>	<b>DCB</b>	<b>MW</b>	<b>2 min</b>	<b>10-20%</b>



**Figure S2.** RCM of peptoid **3** using HG2 under microwave: (a) HPLC chromatogram and (b) MALDI-TOF spectra.

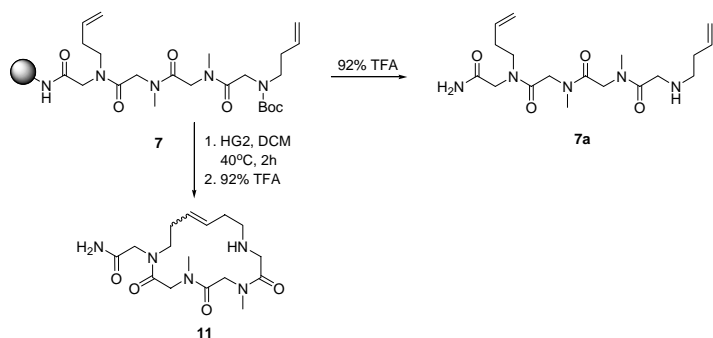


**Figure S3.** RCM of peptoid **4** using HG2 under microwave: (a) HPLC chromatogram and (b) MALDI-TOF spectra.

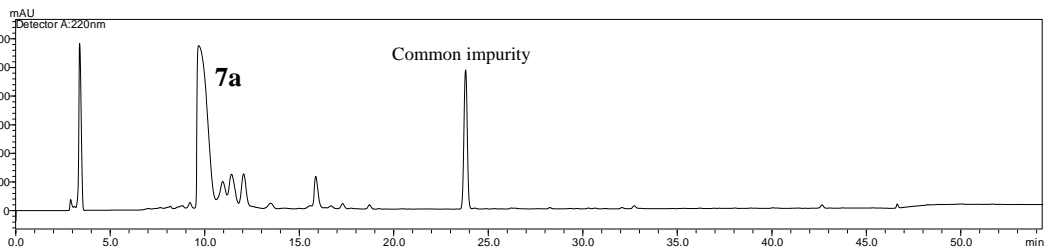


**Figure S4.** RCM of peptoid **5** using HG2 under microwave: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.

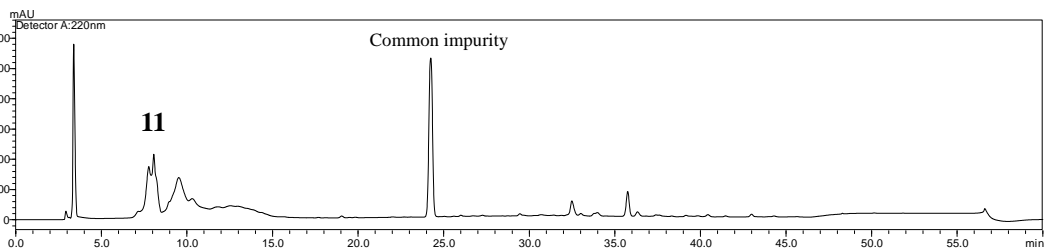




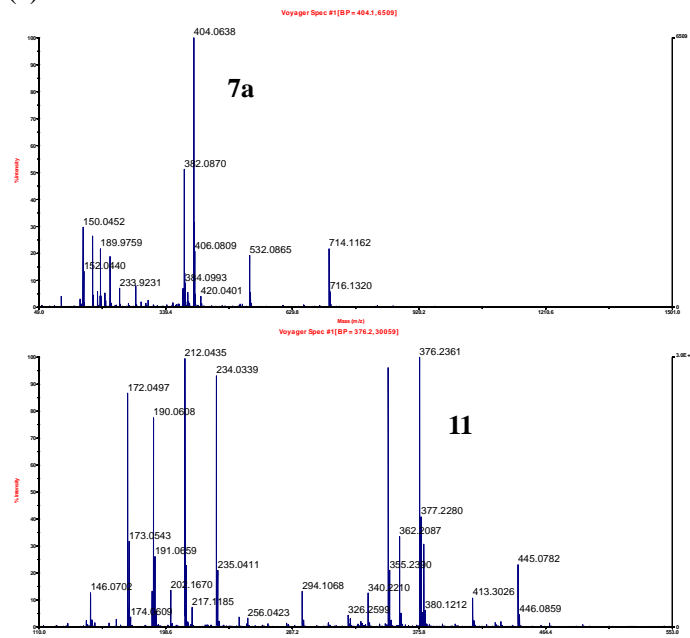
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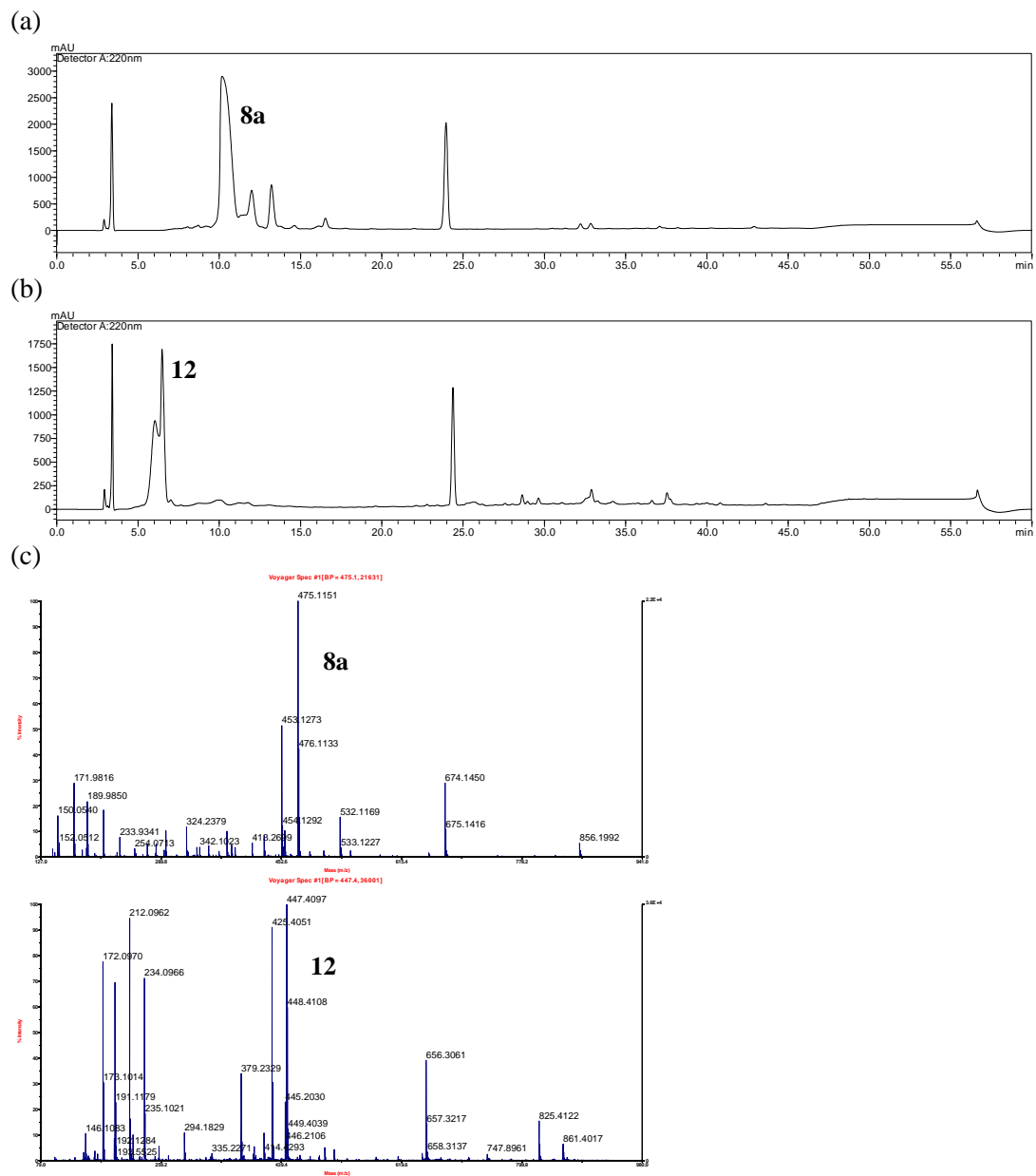
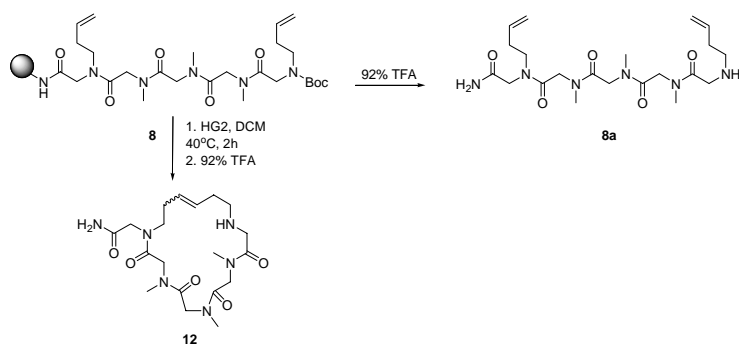
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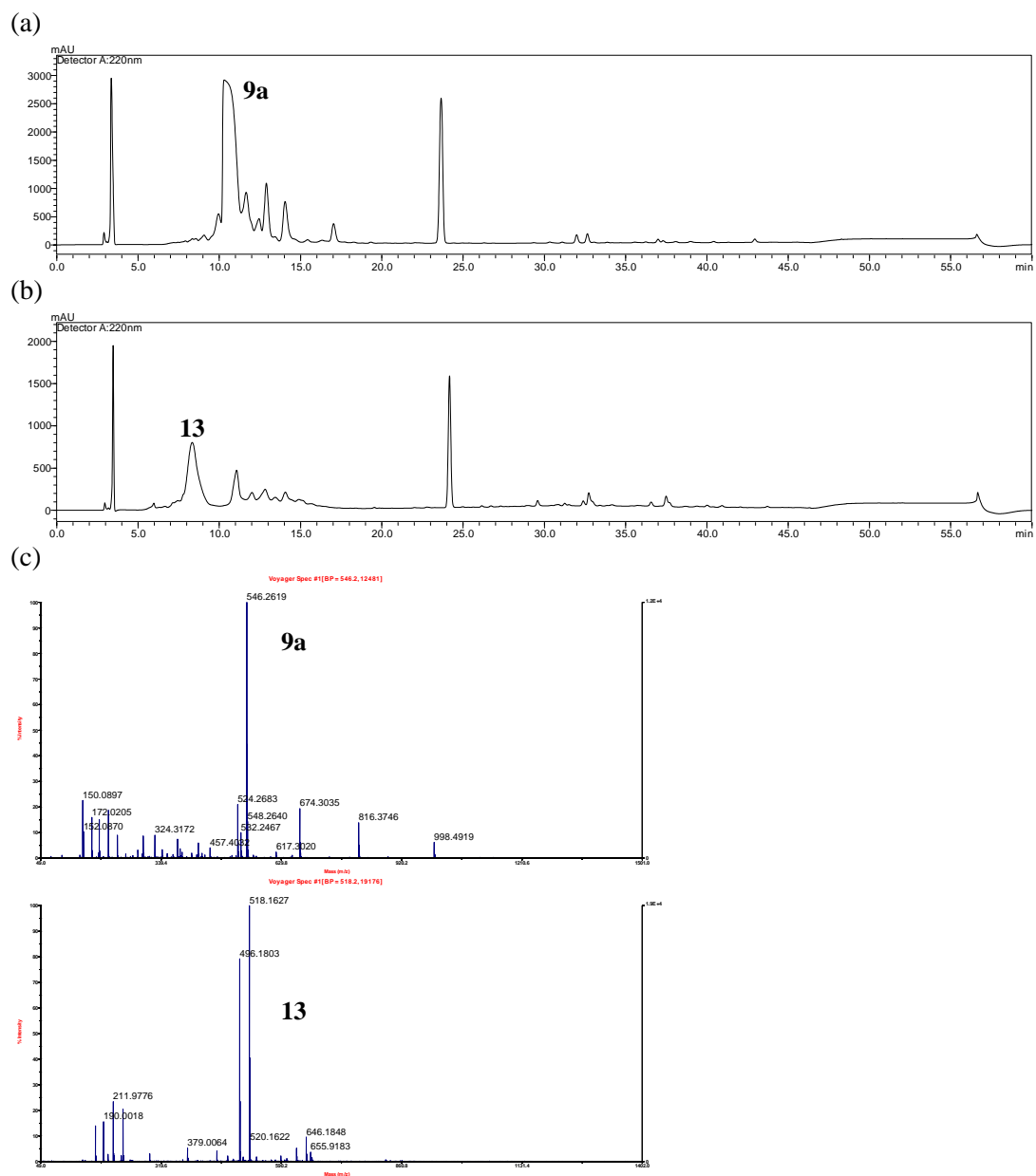
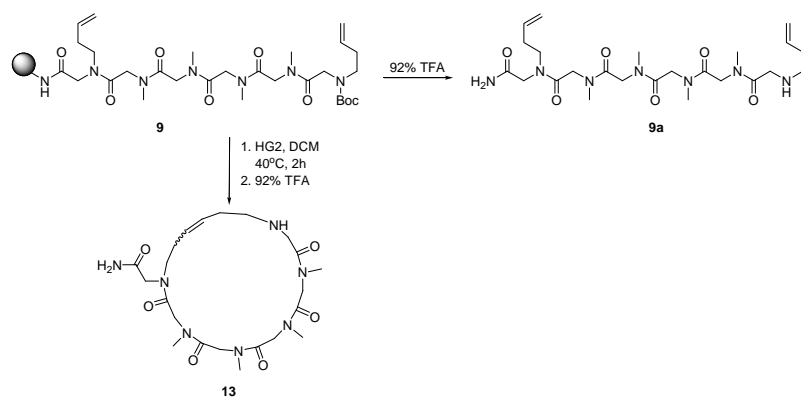
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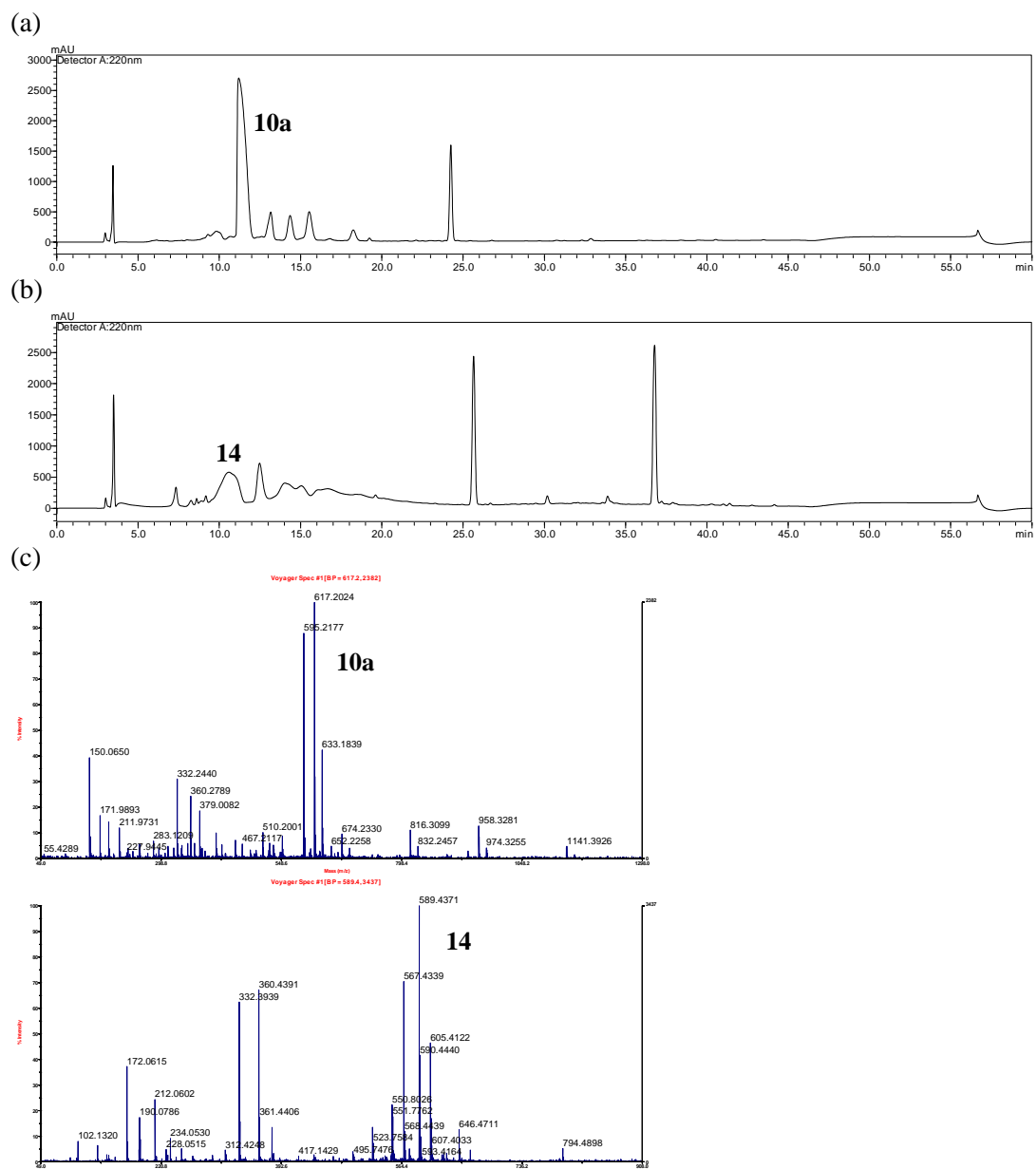
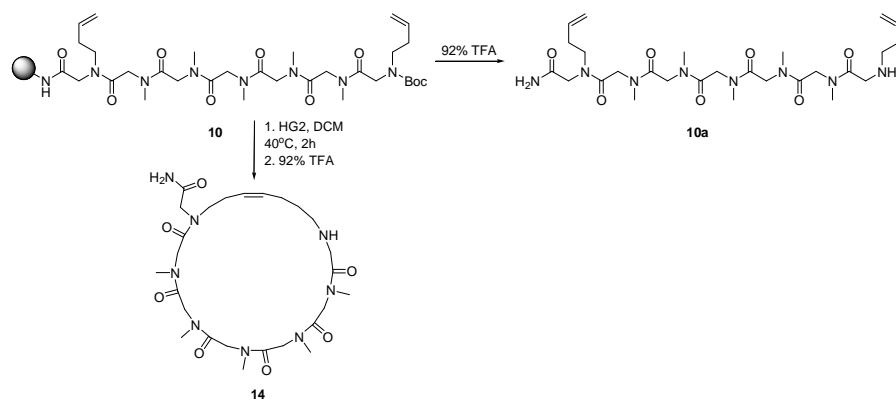
**Figure S5.** RCM of peptoid **7** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



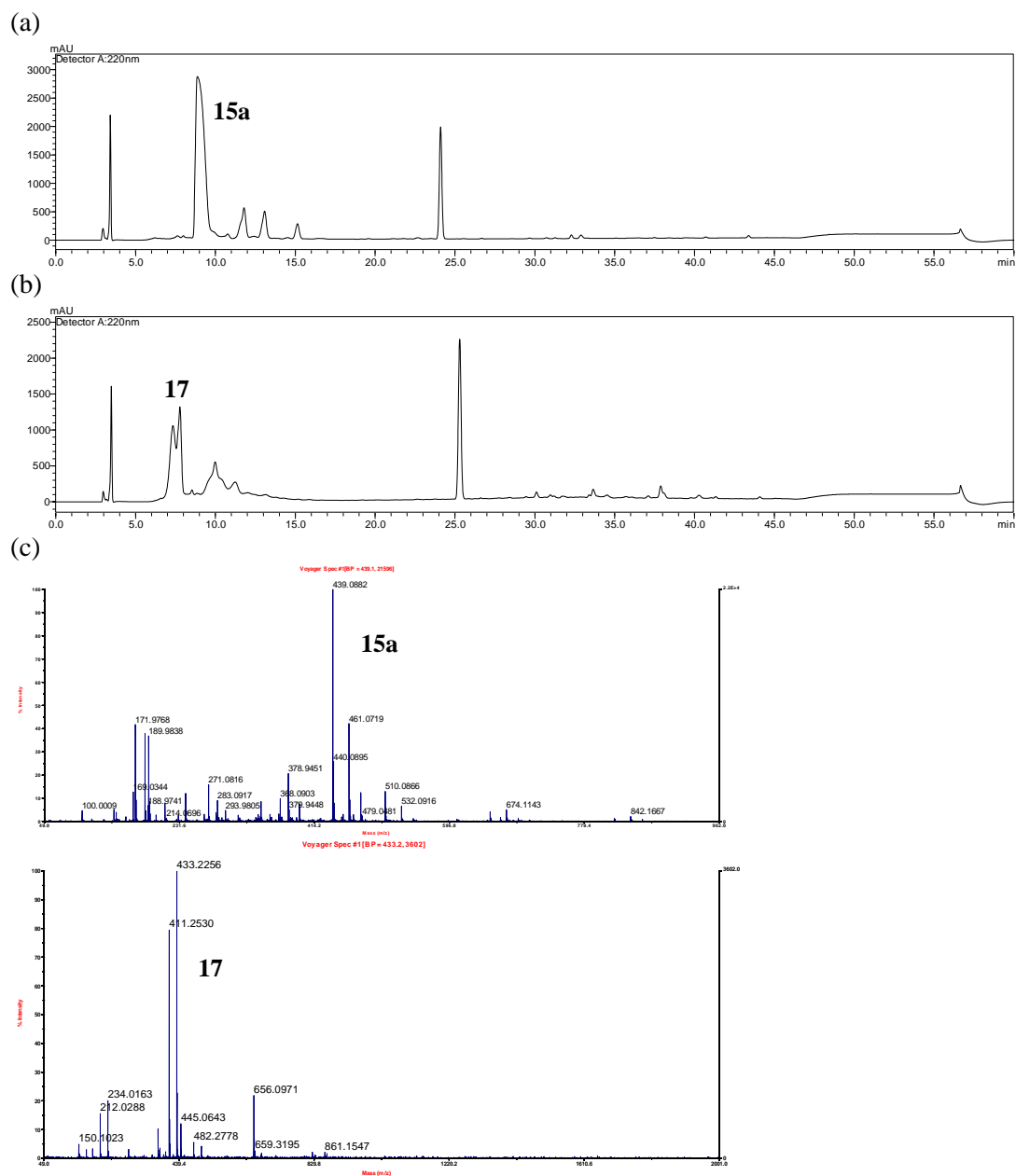
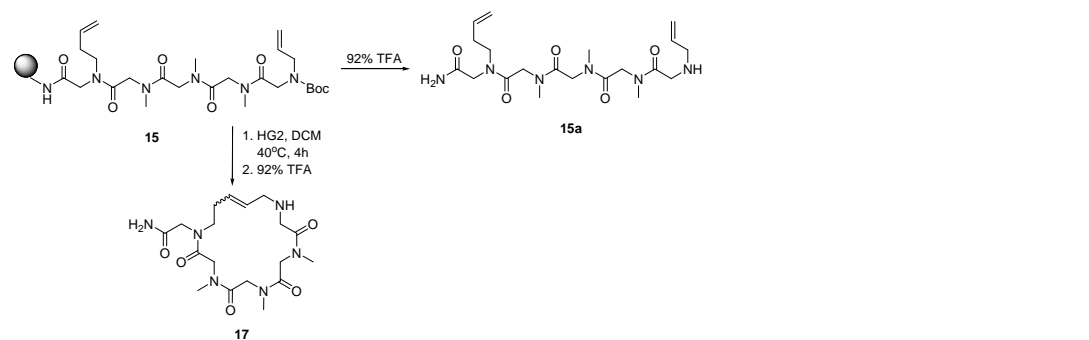
**Figure S6.** RCM of peptoid **8** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



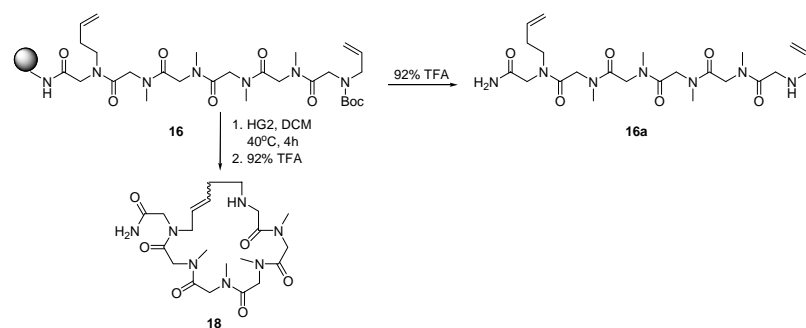
**Figure S7.** RCM of peptoid **9** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



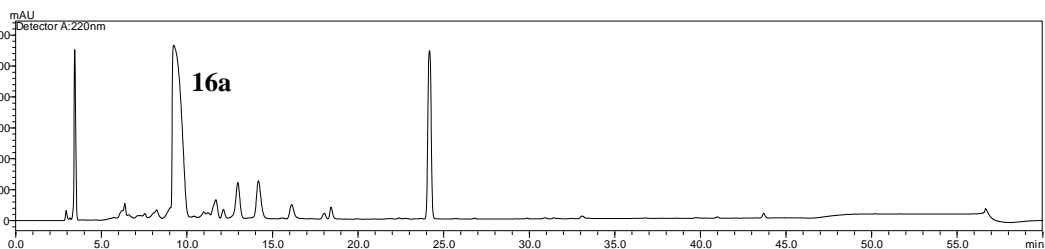
**Figure S8.** RCM of peptoid **10** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



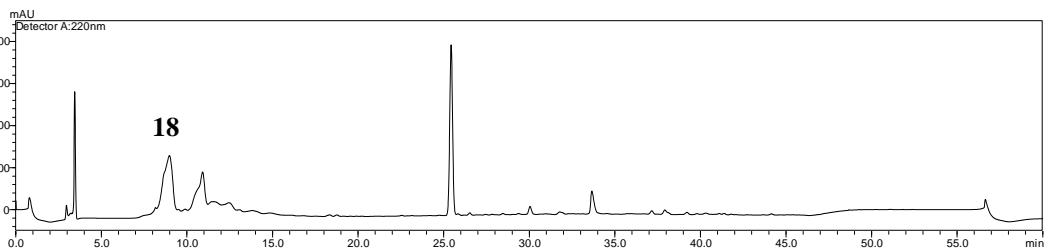
**Figure S9.** RCM of peptoid **15** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



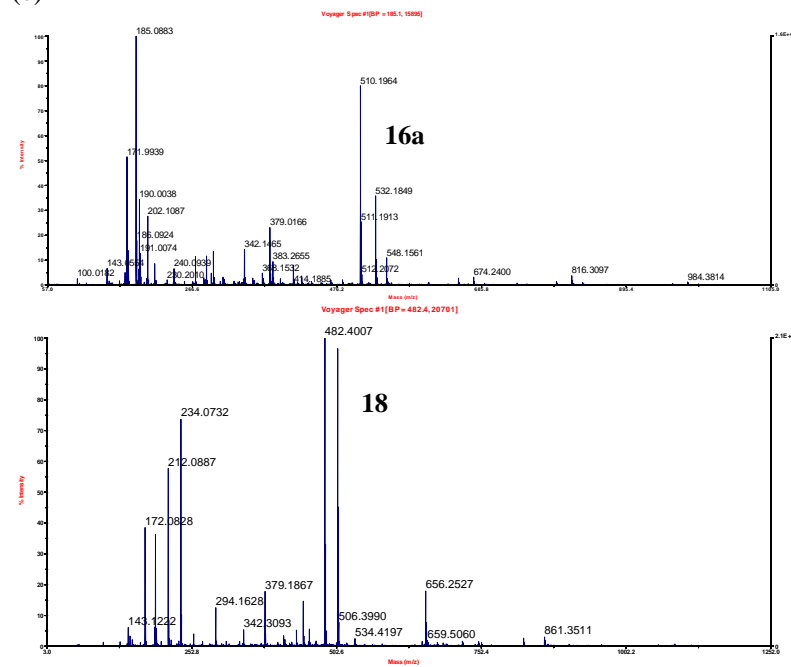
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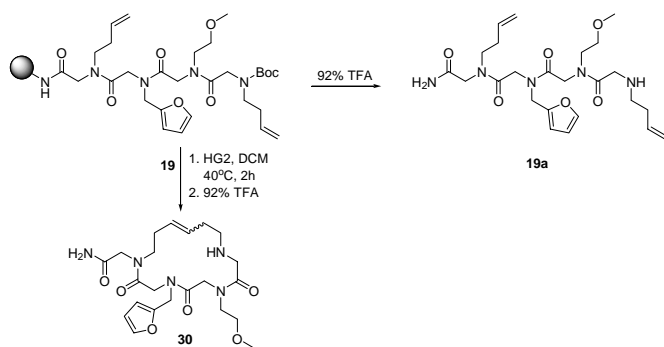
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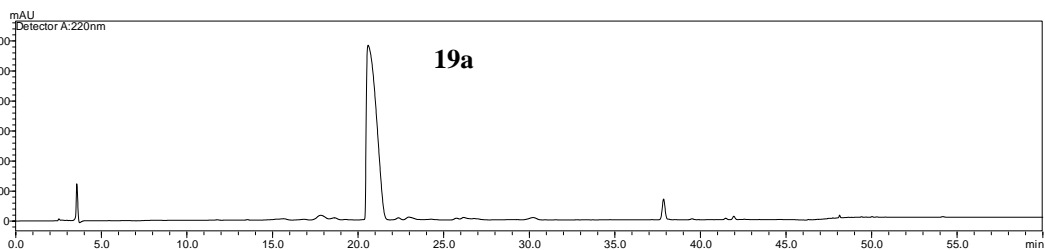
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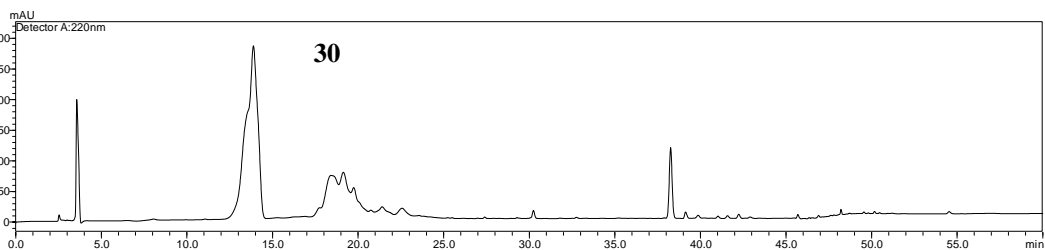
**Figure S10.** RCM of peptoid **16** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



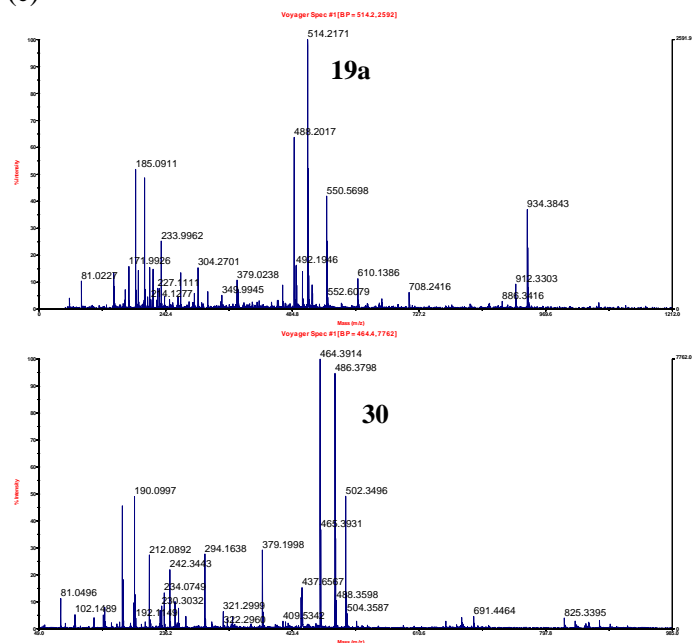
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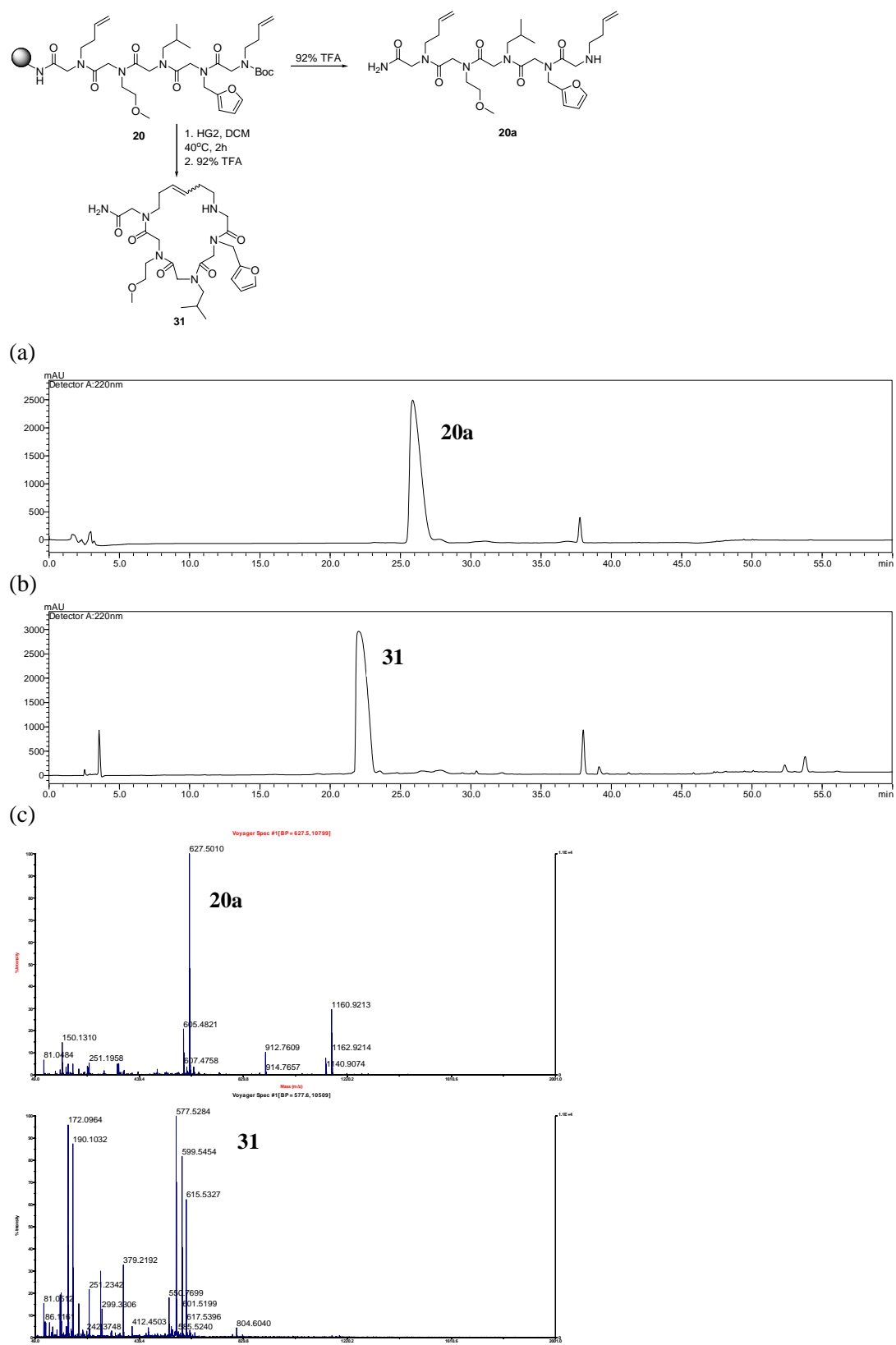
(b)



(c)

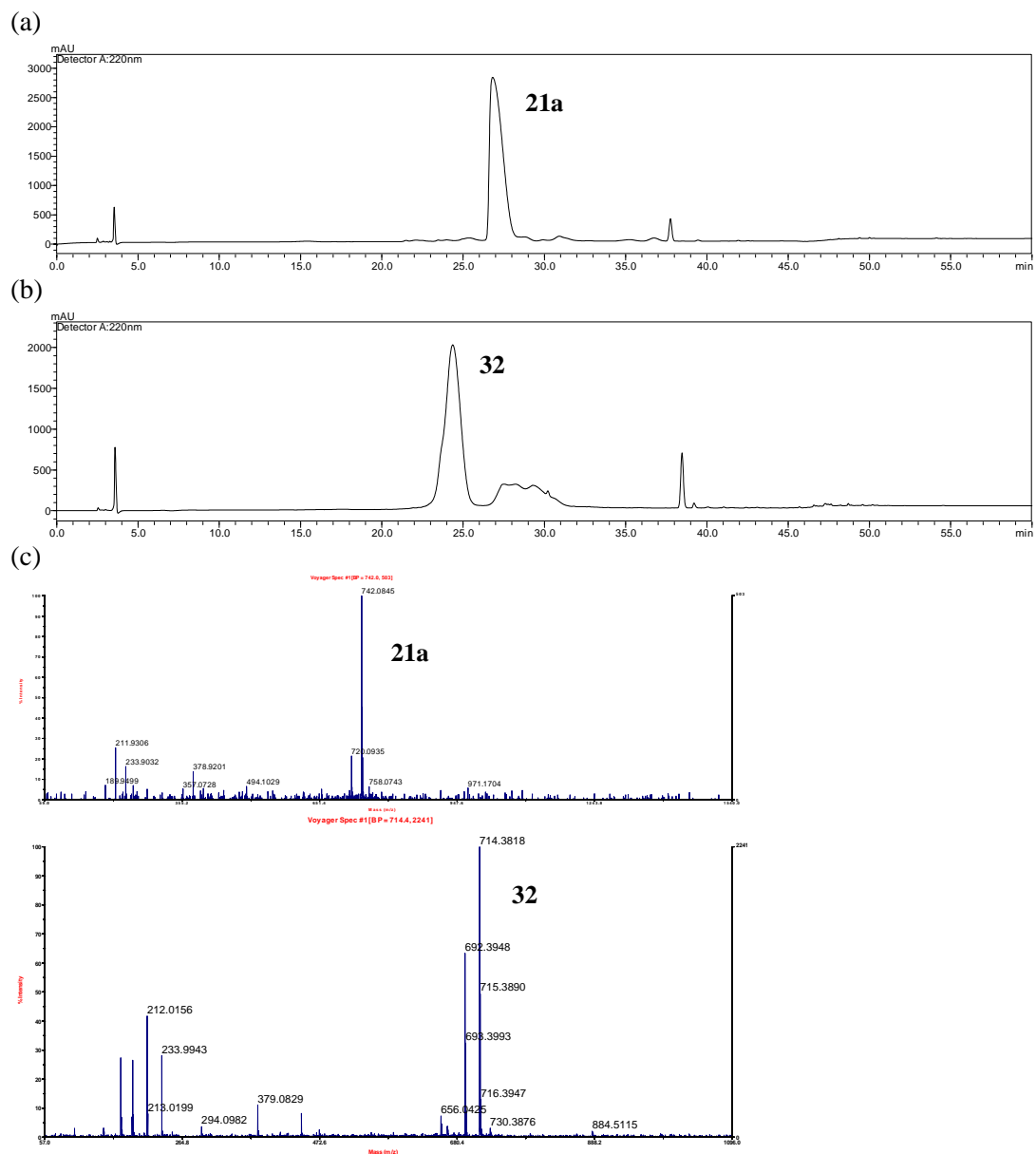
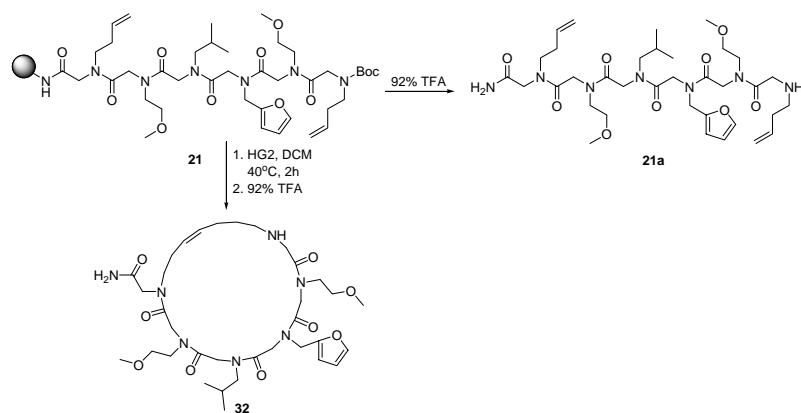


**Figure S11.** RCM of peptoid **19** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.

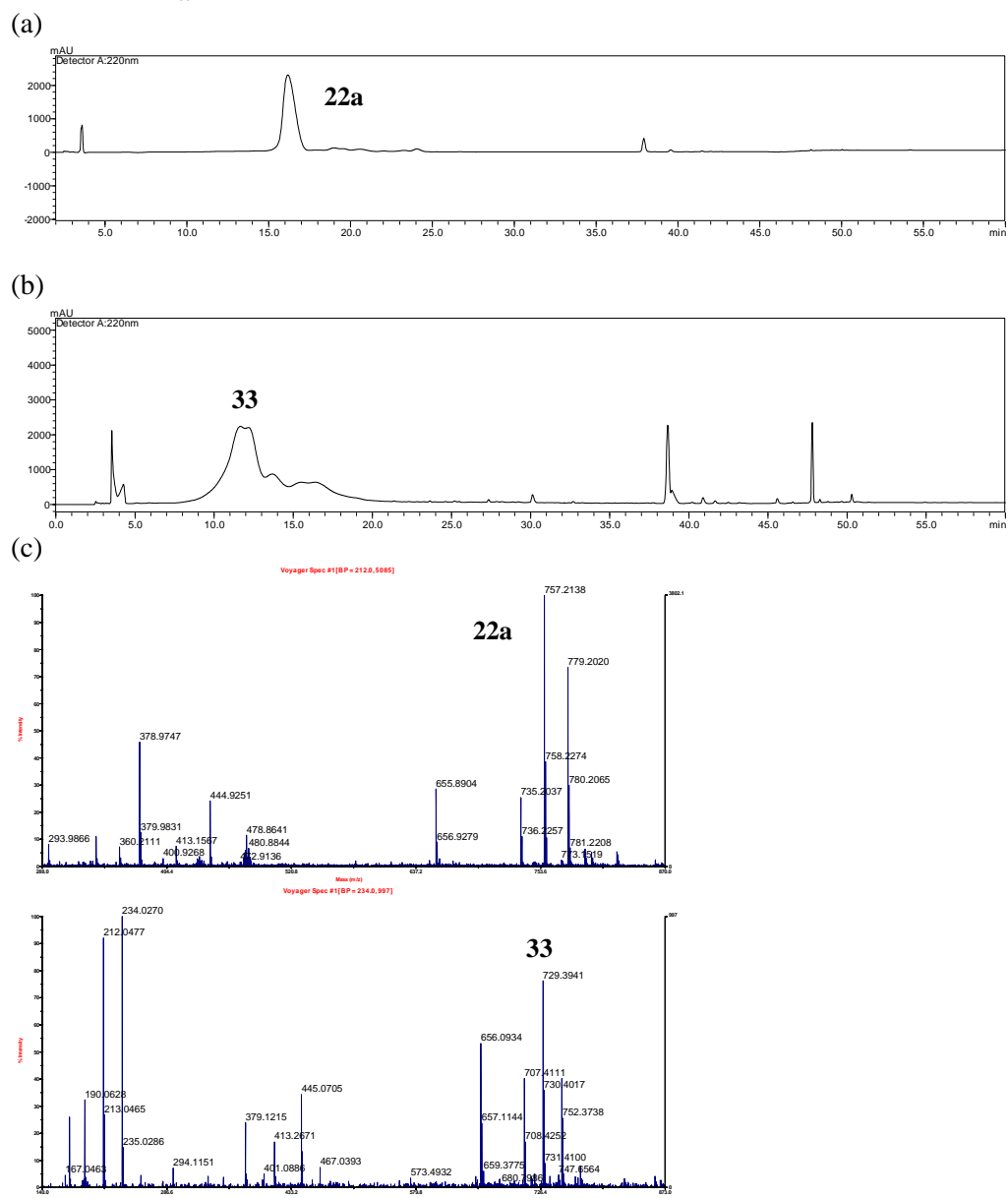
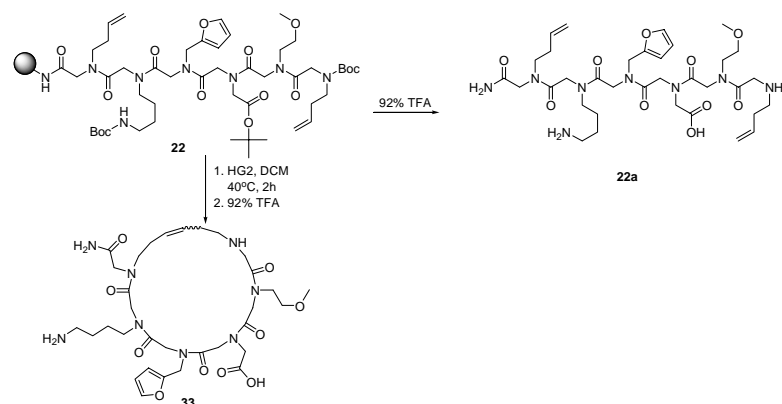


**Figure S12.** RCM of peptoid **20** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.

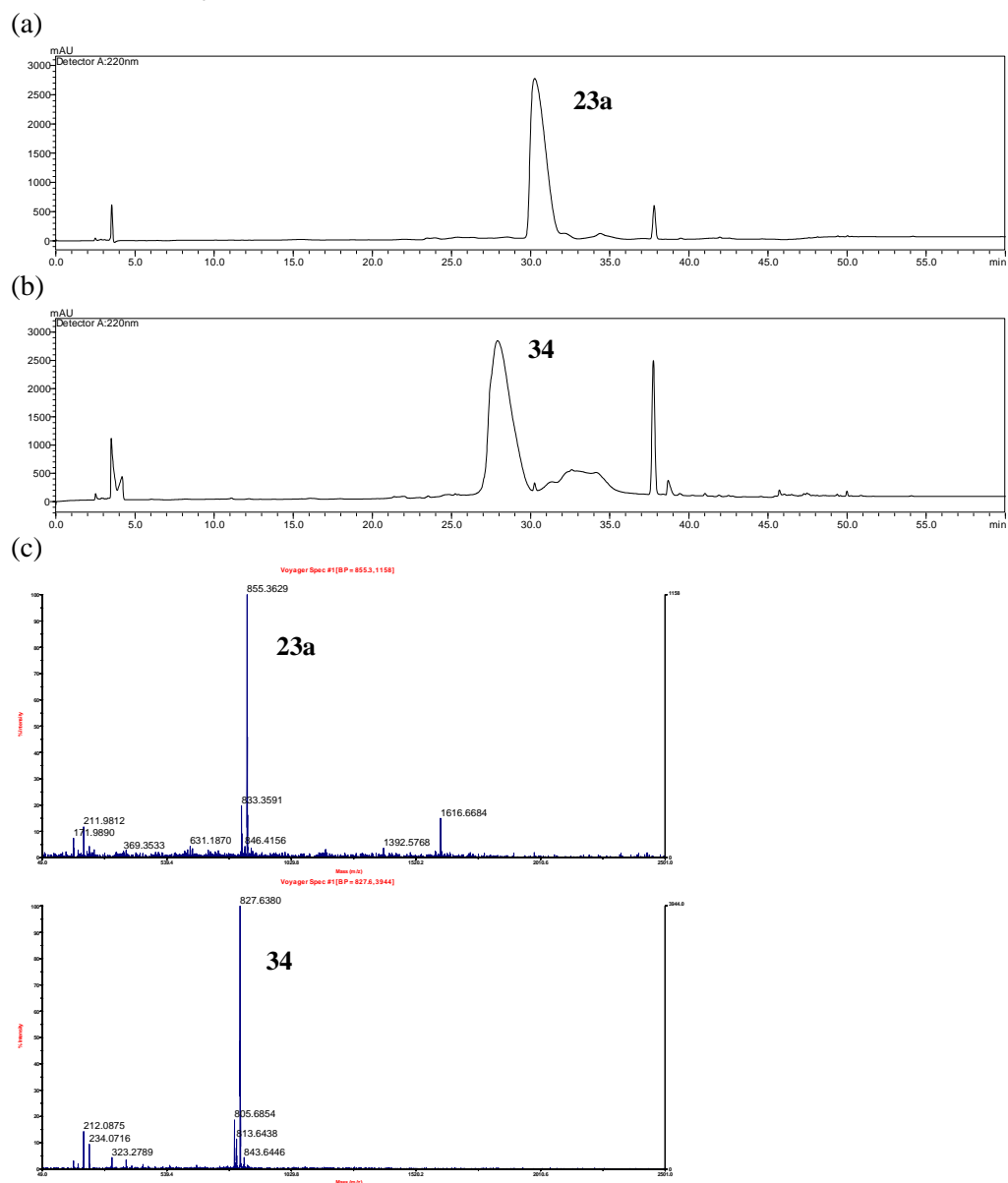
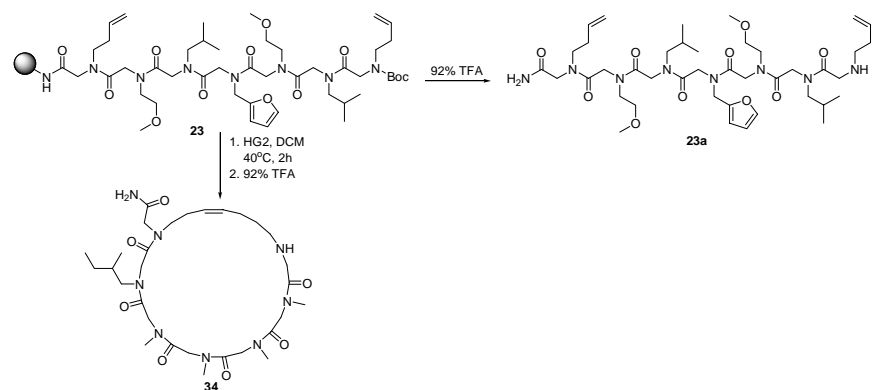




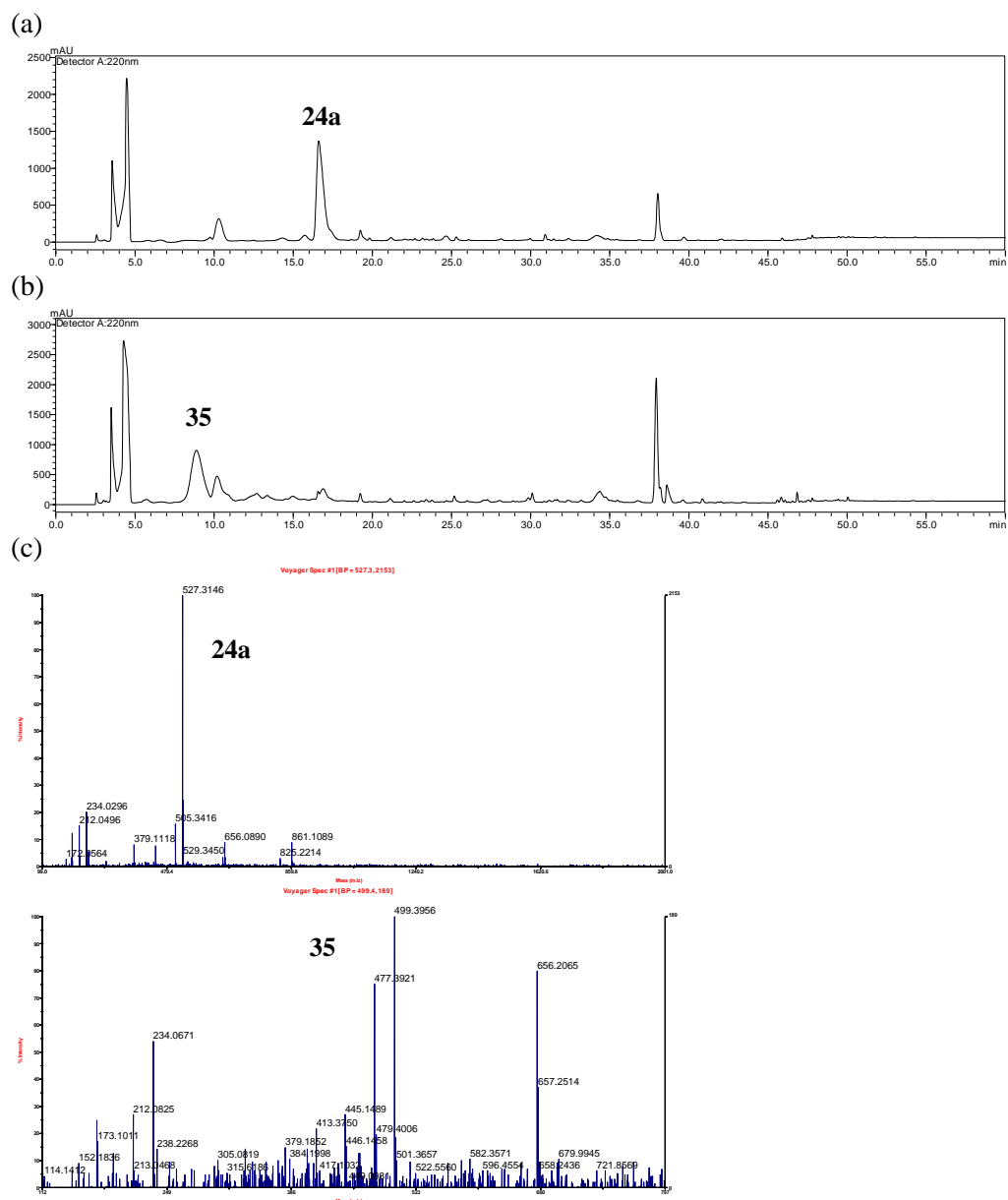
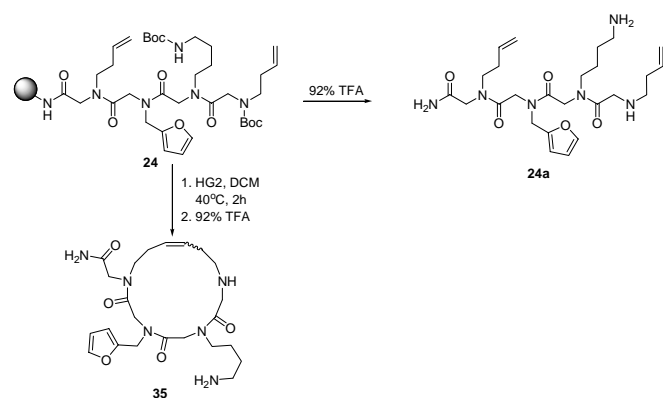
**Figure S13.** RCM of peptoid **21** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



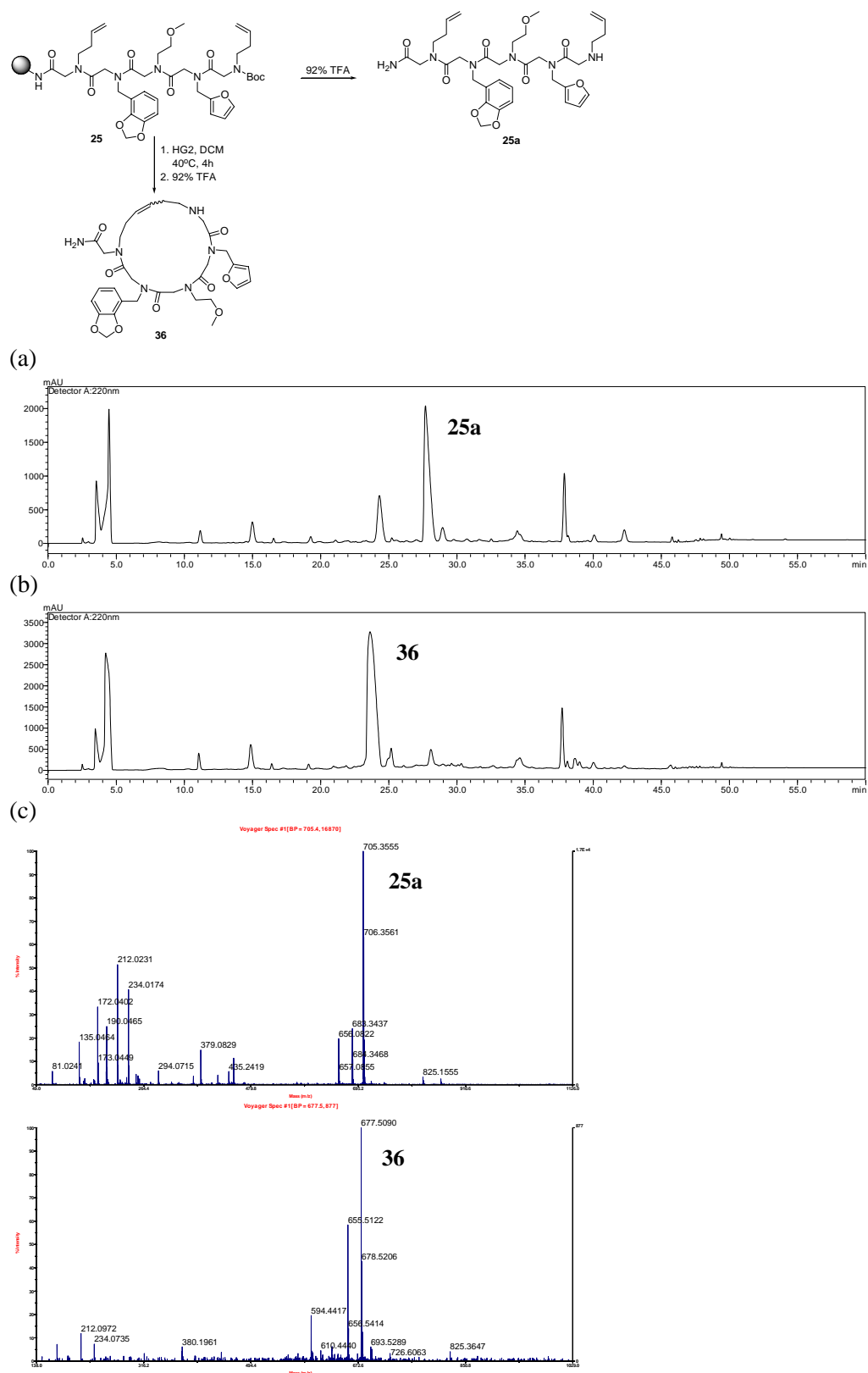
**Figure S14** RCM of peptoid **22** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



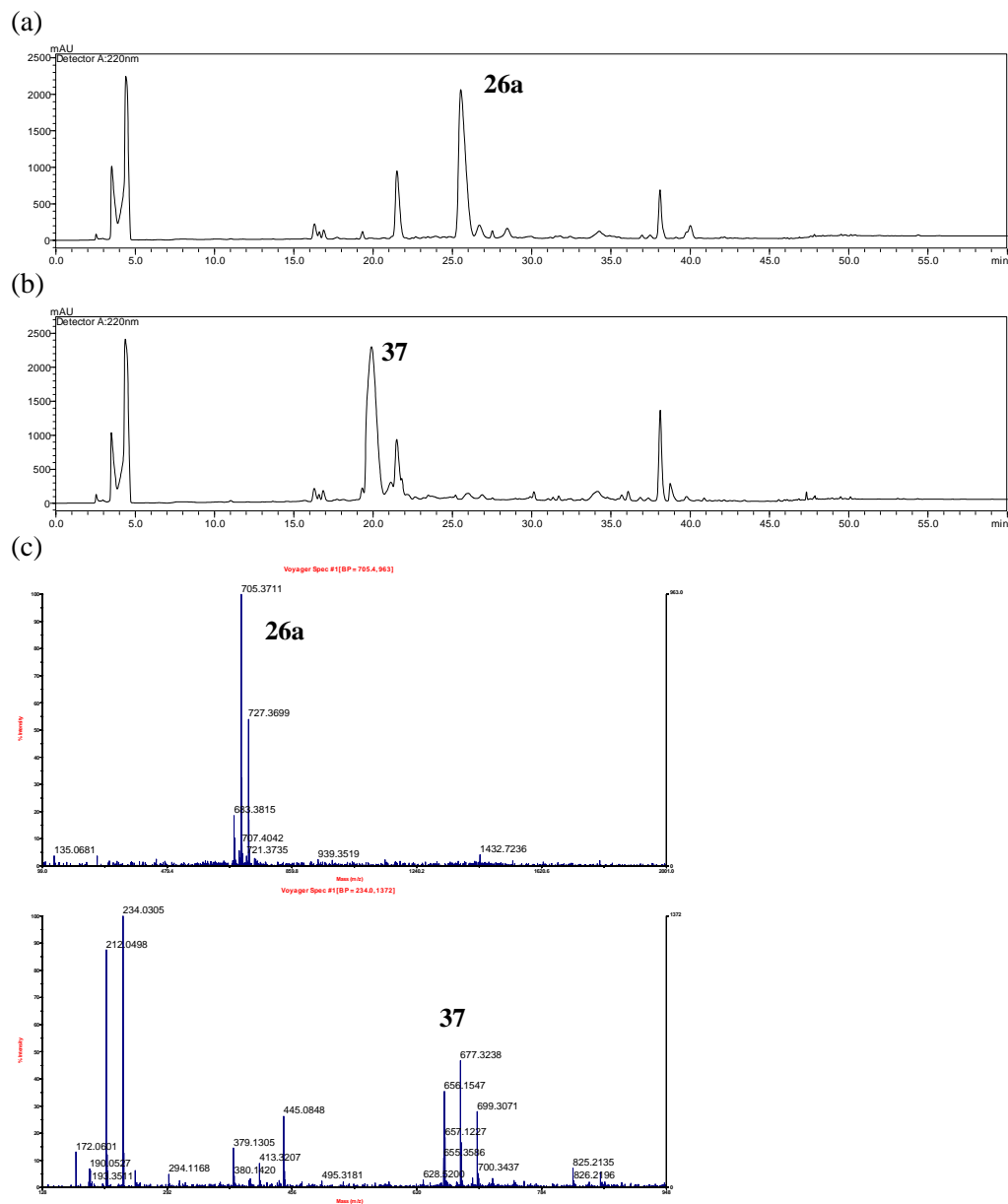
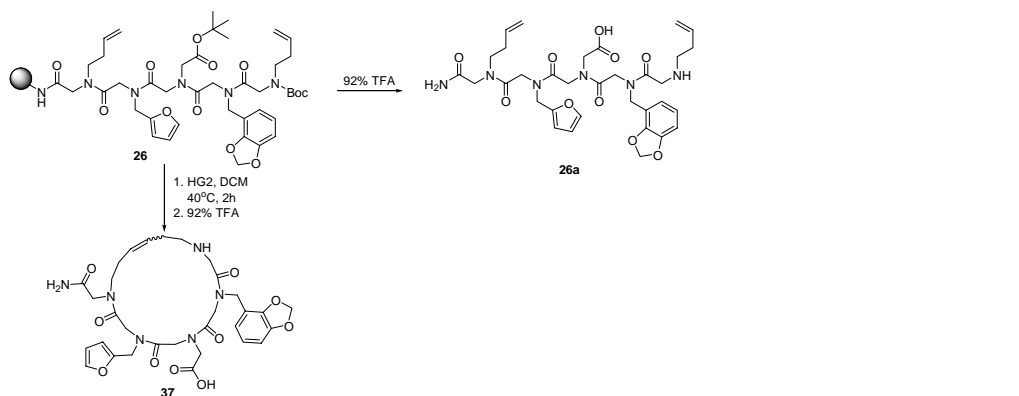
**Figure S15.** RCM of peptoid **22** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



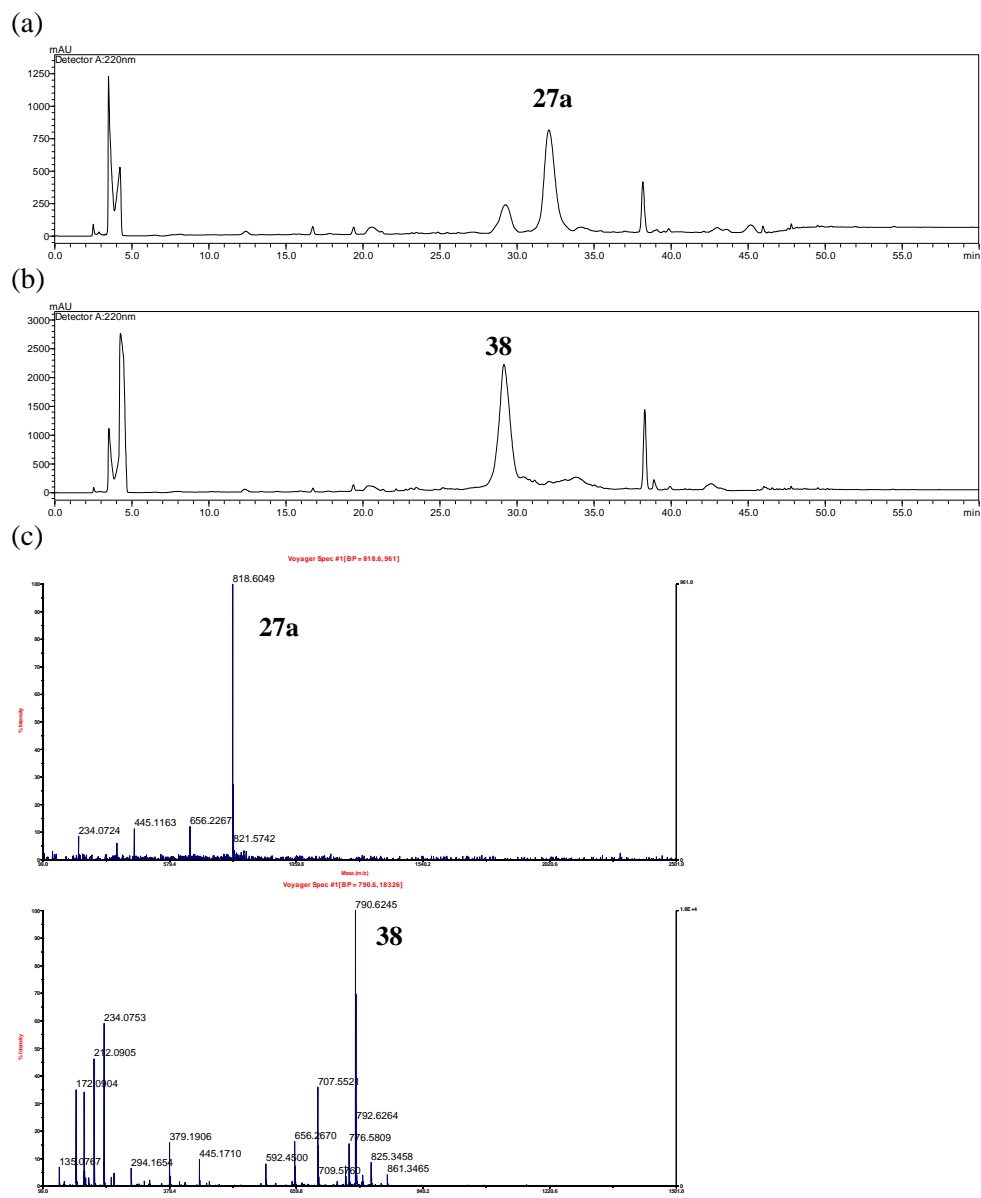
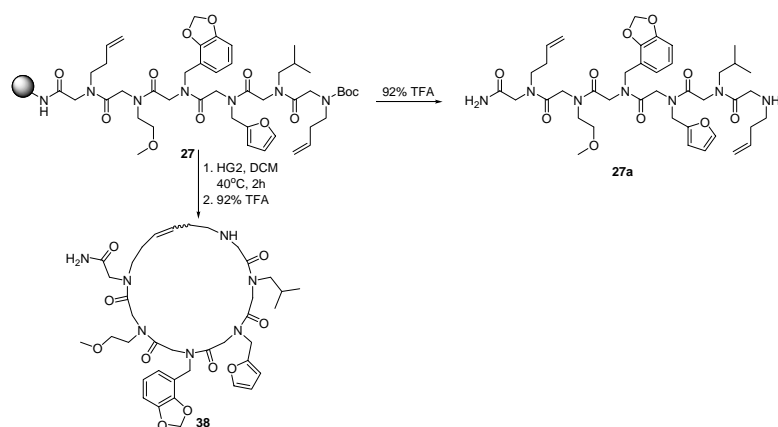
**Figure S16.** RCM of peptoid **24** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



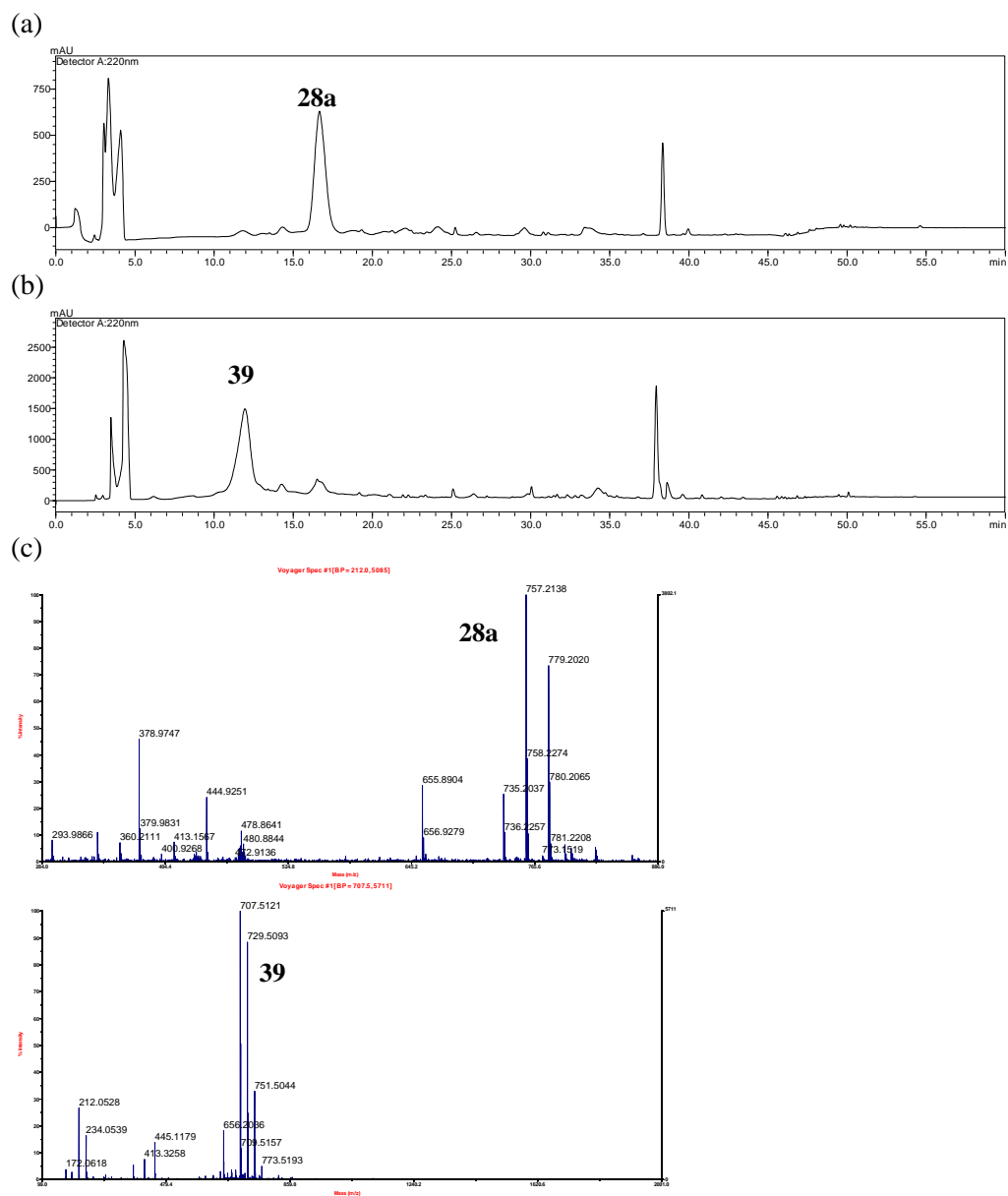
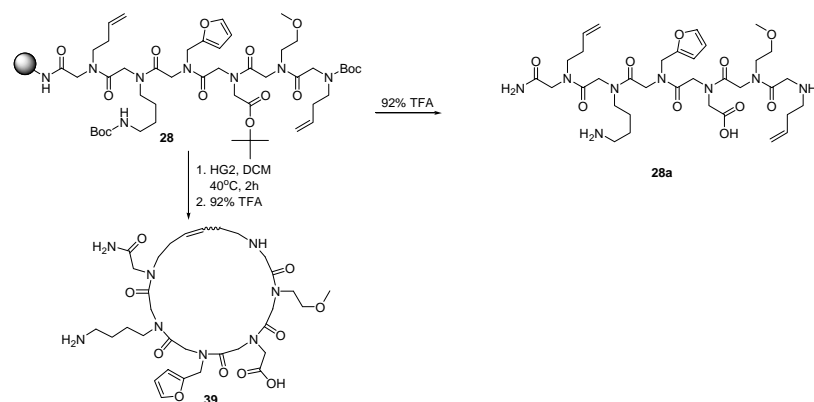
**Figure S17.** RCM of peptoid **25** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



**Figure S18.** RCM of peptoid **26** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.

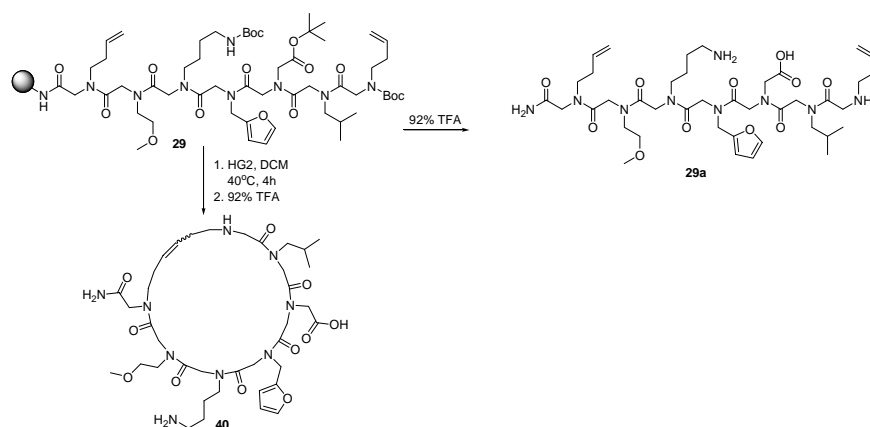


**Figure S19.** RCM of peptoid **27** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.

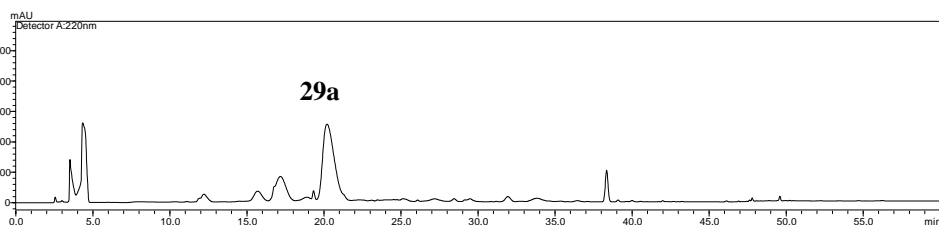


**Figure S20.** RCM of peptoid **28** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.

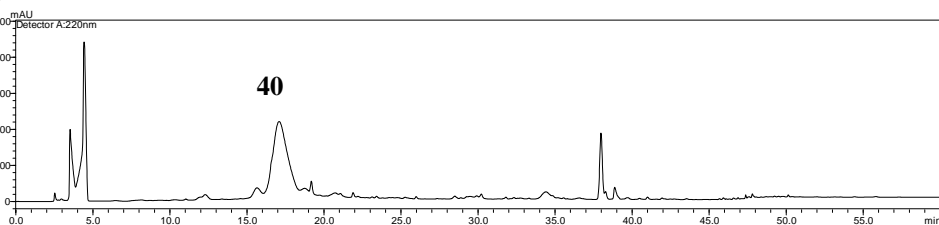




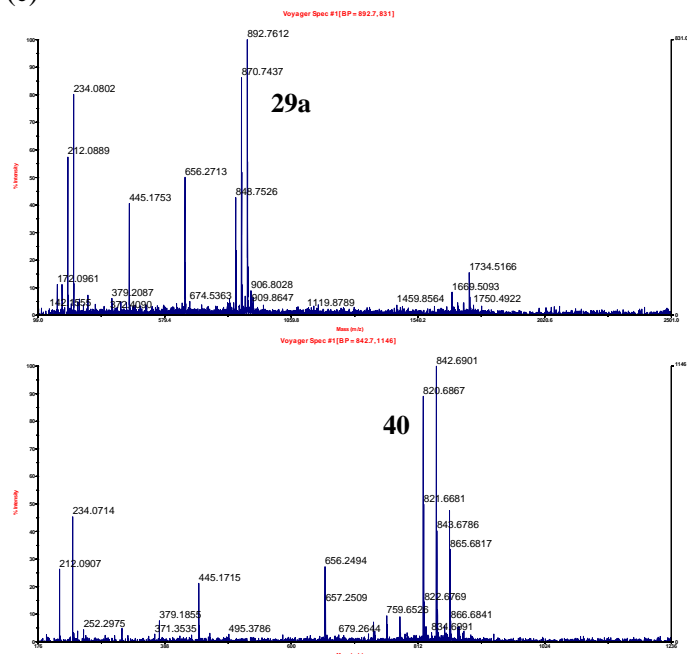
(a)



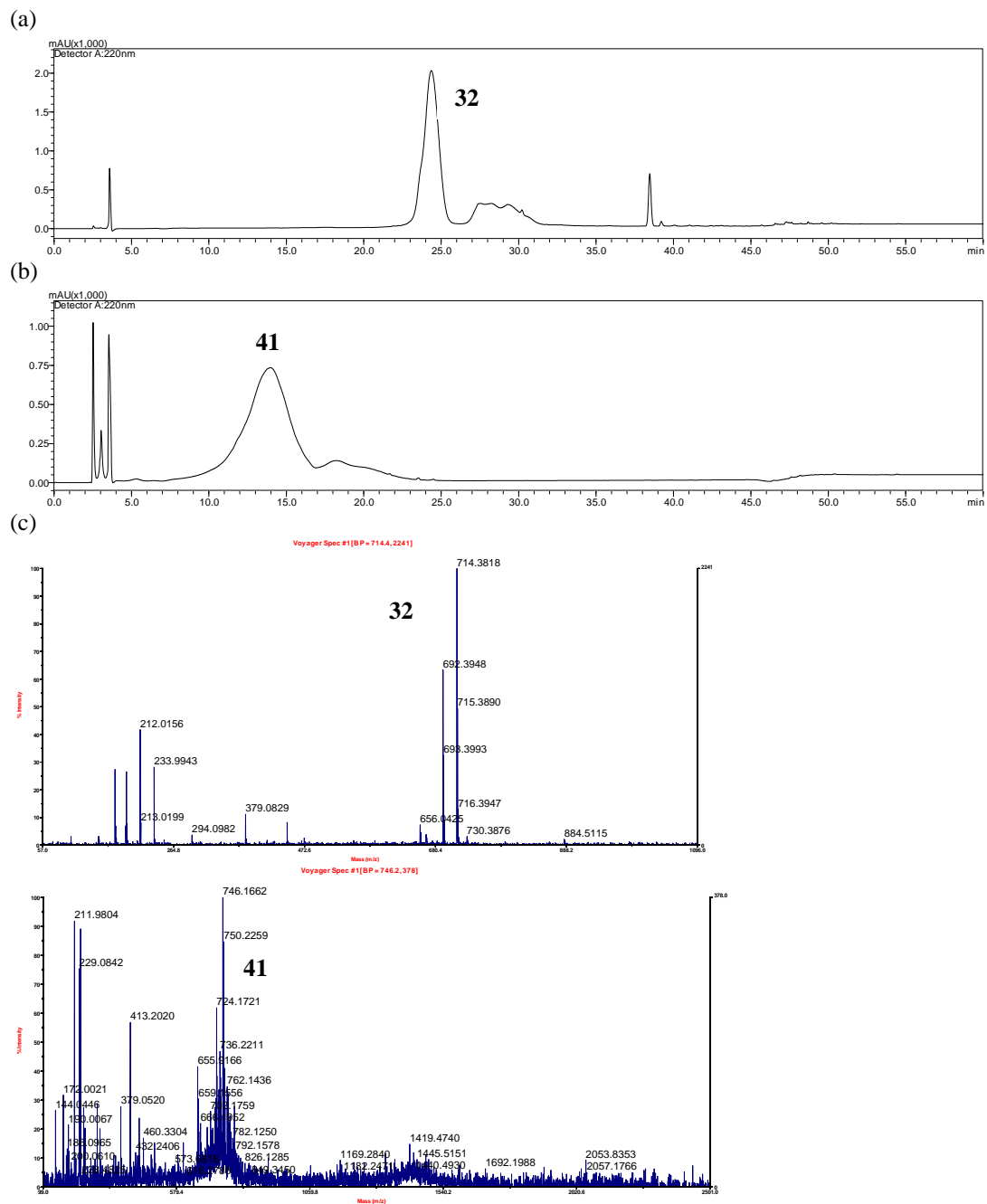
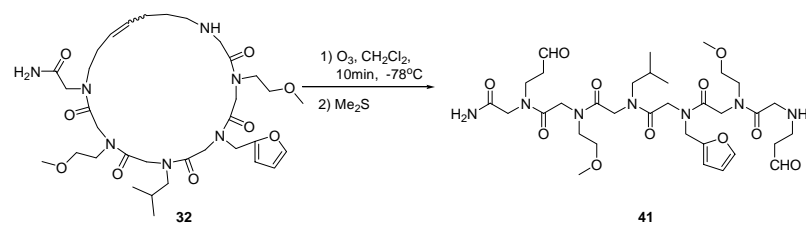
(b)



(c)



**Figure S21.** RCM of peptoid **29** using HG2 at 40 °C: HPLC chromatograms (a) before RCM and (b) after RCM, and (c) MALDI-TOF spectra.



**Figure S22.** Ozonolysis of cyclic peptoid **32**: HPLC chromatograms (a) before ozonolysis and (b) after ozonolysis, and (c) MALDI-TOF spectra.