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Facile Method To Sequence Cyclic Peptides/Peptoids via One-Pot Ring-Opening/Cleavage Reaction

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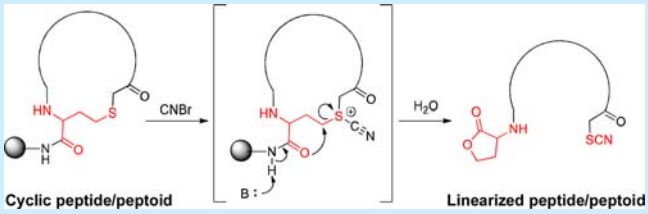
Supporting Information

ABSTRACT: A facile method for sequence determination of cyclic peptides/peptoids is described. Macrocyclic peptides/peptoids of 3−10 residues were efficiently synthesized through thioether formation. One-pot reaction of thioether-embedded cyclic peptides/peptoids involving cyanogen bromide-mediated ring-opening and cleavage provides linearized molecules, which can be efficiently sequenced by tandem mass spectrometry.



Macrocyclic peptides and peptidomimetics (e.g., cyclic peptoids) are of considerable interest as a promising class of potential protein capture agents.[1] Compared to their linear counterparts, cyclic peptides and cyclic peptidomimetics have increased conformational rigidity and relatively preorganized structures and thus could bind more tightly to the target proteins without a major entropy loss. Moreover, they often have improved cell permeability and proteolytic stability. Owing to their relatively large size and conformational constraint, macrocyclic peptides and peptidomimetics might be well-suited molecules to target many challenging protein targets such as protein−protein interactions, which cannot be easily modulated by traditional drug-like small molecules.

Despite many favorable features, the utility of macrocyclic peptides and peptidomimetics is limited by challenges in their sequence determination when using them in high-throughput screening (i.e., on-bead screening). Cyclic peptides and peptidomimetics from one-bead one-compound (OBOC) combinatorial libraries[2] are not generally sequenced by Edman sequencing and tandem mass spectrometry. When constructing a combinatorial OBOC library of nonlinear peptide molecules, it is generally needed to employ encoding tags to record synthesis history.[3] Pei and co-workers developed a one-bead two-compound strategy, in which each bead has two components, a cyclic peptide on the bead surface for screening and a linear peptide with the same sequence inside the bead for sequence analysis.[4] Recently, we have developed an alternate method for sequencing cyclic peptoids.[5] In this method, a cyclic peptoid on a single microbead can be converted into a sequenceable linearized peptoid through a site-specific ring-opening reaction. A thioether linker group incorporated in the cyclic peptoid sequence is oxidized by mCPBA, and the resulting sulfone group as a leaving group is readily attacked by a hydroxide ion, providing a linearized peptoid, which is sequenced by tandem mass spectrometry (MS/MS). While our mCPBA-mediated ring-opening strategy is useful, we found that this method has a drawback. Because of its strong oxidizing ability, mCBPA was shown to affect some other functional groups besides the thioether. For instance, reactive functional groups in several peptoid side chains such as guanidines and primary amines can also be oxidized during oxidative ring-opening reaction, causing complicated MS results.

More recently, the Kodadek and Biron groups developed a similar ring-opening strategy for sequence analysis of cyclic peptoids and cyclic peptides.[6] In these methods, they employed two methionine (Met) residues, one incorporated in the peptoid or peptide sequence and the other one tethered to the bead as a linker. Upon treatment of cyanogen bromide (CNBr), which is known to selectively cleave peptide bonds at the C-terminal to Met residues,[7] cyclic peptides or cyclic peptoids bearing the two Met residues are not only linearized but also cleaved from the bead. Herein we report a facile sequencing strategy of cyclic peptides/peptoids via one-pot ring-opening/cleavage reaction. We also describe a highly efficient solid-phase synthesis of cyclic peptides/peptoids via thioether formation.

Inspired by the Met-based methods,[6] we designed a different cyclic system, which has a thioether moiety embedded in the backbone of cyclic peptides/peptoids (Figure 1). We hypothesized that the thioether linkage could be cleaved via a five-membered ring intermediate by treating with CNBr through the similar mechanism by which CNBr hydrolyzes Met-containing peptide sequences. Concomitant hydrolysis of the intermediate iminium salt would result in cleavage of the linearized peptides/peptoids from the bead. The sequence of the resultant linear peptides/peptoids can be analyzed by MS/MS.

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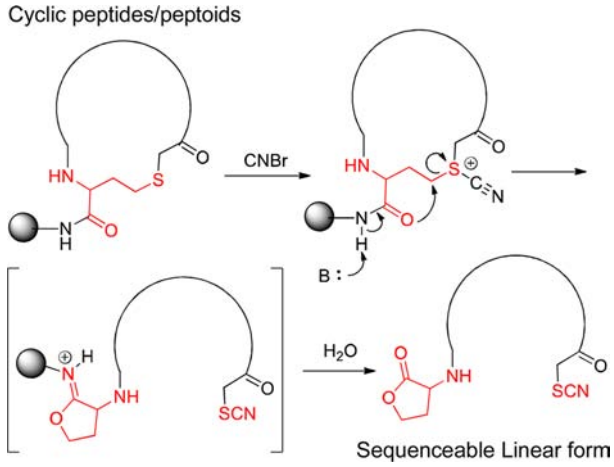
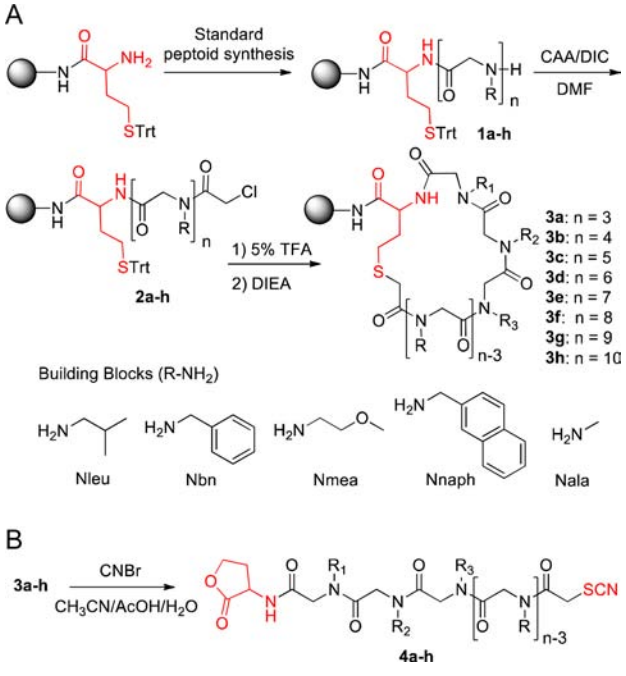


Figure 1. CNBr-mediated one-pot ring-opening/cleavage reaction for sequence determination of cyclic peptides/peptoids.

To test this idea, we first synthesized a series of thioether-containing cyclic peptoids having 3−10 peptoid residues (Scheme 1A). Trityl-protected homocysteine (Fmoc-hCys-(Trt)-OH) was coupled to TentaGel S NH_2 resin. After Fmoc deprotection, the peptoid sequence was incorporated by a submonomer synthesis method[8] using bromoacetic acid and primary amines such as benzylamine, isobutylamine, 2-methoxyethylamine, methylamine, and 1-methylnaphthylamine. The free amine on the N-terminus of peptoids **1a−h** was chloroacetylated,[9] and the trityl group on the homocysteine was removed with 5% TFA. Macrocyclization of linear peptoids **2a−h** was accomplished via the nucleophilic attack of the thiol group on the chloride group.[10] We then examined whether the cyclic peptoids **3a−h** can be linearized by CNBr-mediated ring-opening. The resin was treated with CNBr in AcCN/AcOH/H_2O solution, and the resulting solution was subjected to LC/MS and MALDI-TOF analysis. If these ring-opening/cleavage reactions do not occur, no product would be observed. Gratifyingly, the desired linearized peptoids **4a−h** were detected as a major product without significant amounts of byproducts (Figure 2 and Figure S1), demonstrating that

Scheme 1[a]



[a](A) Solid-phase synthesis of cyclic peptoids **3a−h** and primary amines used (RNH_2). (B) Ring-opening/cleavage reaction of cyclic peptoids **3a−h** into linearized peptoids **4a−h**.

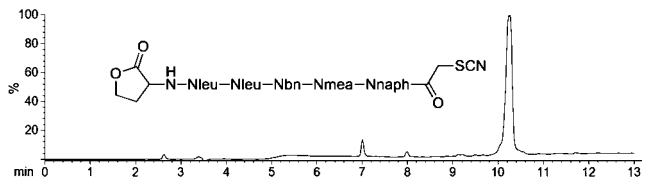


Figure 2. HPLC chromatogram of a crude linearized peptoid.

thioether-incorporated cyclic peptoids were indeed linearized and concomitantly cleaved from the bead. Note that homocysteine, rather than cysteine, is required for the formation of five-membered ring intermediates on the basis of the proposed mechanism (Figure 1). Because of its ring strain, a four-membered ring intermediate by cysteine is unlikely to be formed. To test this, we also synthesized a cysteine-incorporated cyclic peptoid instead of homocysteine (Scheme S1). As expected, no product was observed upon CNBr treatment. Next, we ascertained if the linearized peptoids can be sequenced. As shown in Figure 3 and Figure S1, the sequence of the linear peptoids was clearly determined by MS/MS. It is important to note that, to the best of our knowledge, this is the first example showing that a thioether linkage embedded in peptide or peptoid sequences can be specifically cleaved by CNBr treatment.

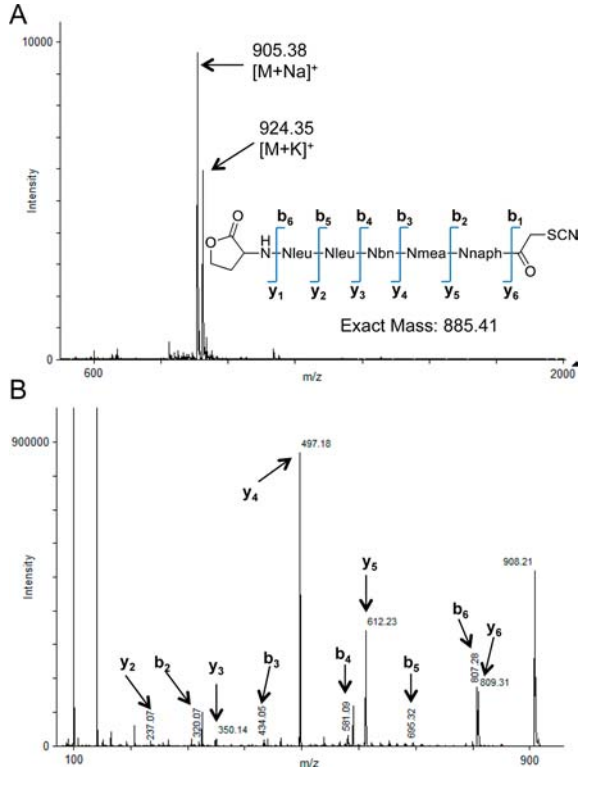


Figure 3. MS (A) and MS/MS (B) spectra of a linearized peptoid.

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Because linearized peptoids were observed as the only major product (Figure 2), solid-phase synthesis (Scheme 1) of cyclic peptoids **3a**–**h** appears to be highly efficient. However, the result would not precisely represent synthetic efficiency. Note that our one-pot ring-opening/cleavage reaction allows only cyclized peptoids to be released from the bead as linear forms, whereas uncyclized peptoids remain attached to the beads. To further assess macrocyclization efficiency, we prepared cyclic peptoids, which include a 3-amino-3-(2-nitrophenyl)propionic acid (ANP) residue as a photocleavable linker between bead and cyclic peptoids (Scheme S2). After the conjugation of ANP (Fmoc-ANP-OH), cyclic peptoids with 3-mer to 10-mer peptoid residues were synthesized under the same conditions described in Scheme 1 and then cleaved from the bead upon UV irradiation.[5a] The identity and purity of the crude products were analyzed by LC/MS and MALDI-TOF MS. As shown in Table 1 and Figure S2, macrocyclization was efficient, providing

Table 1. Purity Data for Cyclic Peptoids 3a–h with Various Ring Sizes (3-mer to 10-mer)

n	calculated mass (M)	observed mass $(M + Na)^+$	purity[a] (%)
3	547.28	570.25	91
4	662.35	685.31	89
5	859.43	882.42	92
6	1006.50	1029.5	94
7	1153.57	1176.5	92
8	1224.60	1247.5	95
9	1339.67	1362.6	96
10	1486.74	1509.7	95

[a]Purity of crude products assessed by HPLC.

cyclic peptoids in excellent purity (93% on average). It is known that head-to-tail cyclization of peptides having less than seven residues is challenging.[1a] It often gives unreacted linear peptides due to incomplete cyclization or unwanted byproducts (e.g., cyclodimerized or oligomerized products). In contrast, our cyclization method yields exclusively monocyclic products, even for small-sized cyclic peptoids with 3–4 peptoid residues. Note that the unexpected small peaks appearing next to the desired products in the HPLC chromatogram (Figure S2) were found to be a methyl ester form of cyclic peptoids based on mass analysis. It is known that such a methyl ester form is generated as a byproduct by methanol used in the photocleavage reaction.[11] To confirm this result, we replaced methanol with CD_3OD as a solvent during the photoreaction. As anticipated, deuterated products were produced as a major product (Figure S3). Because the methyl ester form, as well as the desired amide form, is produced from the same cyclic peptoids, purity described in Table 1 was calculated by combining both products.

We then examined if this method can be applied to cyclic peptides. A series of cyclic peptides was synthesized by the similar synthetic route described in Scheme 1 (Scheme S3). For cyclic peptide synthesis, we employed various amino acids, including amino acids with reactive side chains such as Arg, His, Trp, and Lys, which are protected with Pbf, Trt, or Boc protecting groups. After the macrocyclization reaction, beads were treated with 95% TFA for global deprotection. Upon CNBr treatment, the resulting cyclic peptides were cleaved from the beads as a linear form (Figure 4A). The mass spectrum typically showed two prominent peaks, which include a peak for linearized peptides and another one corresponding

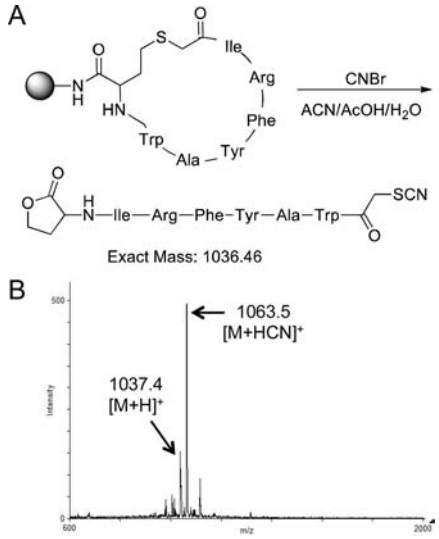

Figure 4. (A) Ring-opening/cleavage reaction for a cyclic peptide. (B) MALDI-TOF MS spectra of the linearized peptide.

to cyano adduct ($[M + HCN]^+$) (Figure 4B). Peptide sequences were unambiguously obtained by MS/MS analysis on either a molecular ion or a cyano adduct (Figure 4). Notably, amino acids with reactive side chains were not affected by CNBr-mediated ring-opening/cleavage reaction conditions. Next, we synthesized cyclic peptides involving an ANP linker to evaluate cyclization efficiency (Scheme S4). After global deprotection in 95% TFA, cyclic peptides were released by UV irradiation and analyzed by LC/MS. The purity of crude products was over 90% on average (Figure S5 and Table S1), showing excellent cyclization efficiency.

A main application of this ring-opening method should be sequence determination of cyclic peptides or cyclic peptidomimetics on a single bead isolated from high-throughput on-bead screening of a combinatorial library. To test this, we constructed a combinatorial OBOC library of 6-mer cyclic peptide/peptoid hybrids possessing a thioether linkage as a model system (Figure S6). Peptide/peptoid hybrids are a promising class of peptidomimetics, which are expected to have the advantages of both peptides and peptoids.[12] Using standard split-and-pool method,[2] a 729000-member library was synthesized by using 9 amino acids and 10 primary amines as monomer building blocks (Figure S6). After the completion of library synthesis, 20 beads were randomly chosen from the library. Each bead was placed in an individual tube and treated with CNBr. MS and MS/MS analyzed the resulting linearized peptide–peptoid hybrid molecules released from each single bead (Figure S7). MS spectra of each sample showed one major peak within the range of expected molecular weight, indicating the robustness of cyclization efficiency during combinatorial library synthesis. The molecular ion peaks were further analyzed by MS/MS. As expected, linearized peptide/peptoid hybrid molecules were successfully sequenced. Given the high quality and structural diversity, this cyclic peptoid/peptide hybrid library will be highly useful for on-bead screening against biologically important targets.

In conclusion, we have developed a facile method for sequence determination of cyclic peptides/peptoids by using CNBr-mediated one-pot ring-opening/cleavage reaction. While it is well-known that CNBr cleaves the peptide sequence at the C-terminus of a Met residue, we for the first time demonstrated

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that a thioether linkage embedded in a peptide sequence can also be cleaved by a similar mechanism (Figure 1). We believe that our novel one-pot ring-opening/cleavage strategy will serve as a powerful tool to screen a large OBOC combinatorial library of such thioether-bridged cyclic peptides and cyclic peptidomimetics. Further, we have developed an efficient macrocyclization method (Scheme 1 and Table 1). Importantly, our solid-phase synthesis affords monocyclic peptides/peptoids with various ring sizes (3-mer to 10-mer) without commonly observed byproducts such as cyclodimerization or cyclooligomerization products. Given the remarkable efficiency (even for highly strained small-sized ring systems), our on-resin macrocyclization method should be highly useful in a wide range of applications from drug discovery and chemical biology to material sciences.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures for the synthesis and characterization data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509. (b) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discovery* **2008**, *7*, 608. (c) Shin, S. B.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2007**, *129*, 3218.

(2) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82.

(3) (a) Liu, R.; Marik, J.; Lam, K. S. *J. Am. Chem. Soc.* **2002**, *124*, 7678. (b) Kwon, Y. U.; Kodadek, T. *Chem. Commun.* **2008**, 5704. (c) Oh, M.; Lee, J. H.; Wang, W.; Lee, H. S.; Lee, W. S.; Burlak, C.; Im, W.; Hoang, Q. Q.; Lim, H. S. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 11007. (d) Kim, J. H.; Kang, H.; Kim, S.; Jun, B. H.; Kang, T.; Chae, J.; Jeong, S.; Kim, J.; Jeong, D. H.; Lee, Y. S. *Chem. Commun.* **2011**, *47*, 2306.

(4) Joo, S. H.; Xiao, Q.; Ling, Y.; Gopishetty, B.; Pei, D. *J. Am. Chem. Soc.* **2006**, *128*, 13000.

(5) (a) Lee, J. H.; Meyer, A. M.; Lim, H. S. *Chem. Commun.* **2010**, *46*, 8615. (b) Lee, J. H.; Kim, H. S.; Lim, H. S. *Org. Lett.* **2011**, *13*, 5012.

(6) (a) Simpson, L. S.; Kodadek, T. *Tetrahedron Lett.* **2012**, *53*, 2341. (b) Liang, X.; Girard, A.; Biron, E. *ACS Comb. Sci.* **2013**, *15*, 535.

(7) Kappel, J. C.; Barany, G. *J. Comb. Chem.* **2005**, *7*, 78.

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

(9) Burkoth, T. S.; Fafarman, A. T.; Charych, D. H.; Connolly, M. D.; Zuckermann, R. N. *J. Am. Chem. Soc.* **2003**, *125*, 8841.

(10) Sako, Y.; Goto, Y.; Murakami, H.; Suga, H. *ACS Chem. Biol.* **2008**, *3*, 241.

(11) Peters, F. B.; Brock, A.; Wang, J.; Schultz, P. G. *Chem. Biol.* **2009**, *16*, 148.

(12) (a) Kawakami, T.; Murakami, H.; Suga, H. *J. Am. Chem. Soc.* **2008**, *130*, 16861. (b) Ruijtenbeek, R.; Kruijtzer, J. A.; van de Wiel, W.; Fischer, M. J.; Fluck, M.; Redegeld, F. A.; Liskamp, R. M.; Nijkamp, F. P. *ChemBioChem* **2001**, *2*, 171. (c) Olsen, C. A.; Montero, A.; Leman, L. J.; Ghadiri, M. R. *ACS Med. Chem. Lett.* **2012**, *3*, 749.