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Facile Solid-Phase Parallel Synthesis of Linear and Cyclic Peptoids for Comparative Studies of Biological Activity

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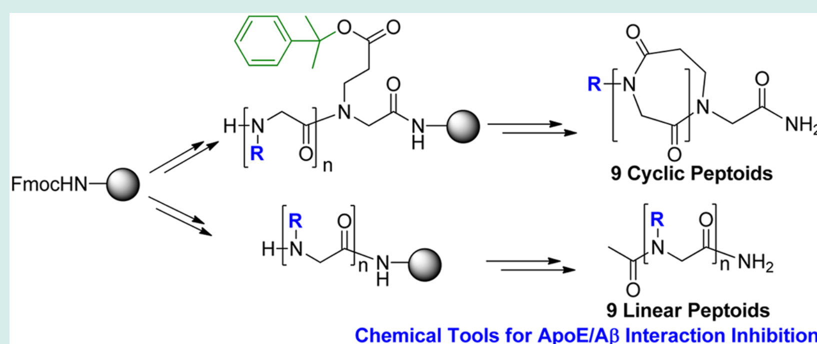
Supporting Information



ABSTRACT: A series of linear and cyclic peptoids, which were expected to possess better pharmacokinetic properties and biological activities for blocking the interaction between apolipoprotein E and amyloid-β, were designed and synthesized as possible therapeutic agents. Peptoids were easily synthesized on solid-phase by the submonomer strategy and polar side chain-containing amines were effectively introduced under the modified reaction conditions. For the synthesis of cyclic peptoids, β-alanine protected with the 2-phenylisopropyl group, which could be selectively removed by 2% TFA, was used as a primary amine to afford a complete peptoid unit. The macrolactamization between the carboxylic acid of β-alanine moiety and terminal amine of peptoids was successfully performed in the presence of the PyAOP coupling agent on solid-phase in all the cases, providing various sizes of cyclic peptoids. In particular, some cyclic peptoids prepared in this study are the largest in size among cyclic peptoids reported to date. The synthetic strategy which was adopted in this study can also provide a robust platform for solid-phase construction of cyclic peptoid libraries. Currently, synthetic peptoids have been used to test interesting biological activities including the ApoE/Aβ interaction inhibition, nontoxicity, the blood-brain barrier permeability, etc.

KEYWORDS: *cyclic peptoids, macrocyclization, macrolactamization, peptoid synthesis*

INTRODUCTION

Peptoids, N-substituted oligoglycines whose side chains are attached to the amide backbone nitrogen atom instead of the α-carbon of peptides (Figure 1), are a fascinating class of peptidomimetics because of their advantageous characteristics including tremendous chemical diversity,[1] proteolytic resistance,[2] and improved cell permeability.[3] In general, peptides are sensitive to proteases and have poor cell permeability and poor bioavailability. Thus, many different types of peptidomimetics with improved pharmacokinetic characteristics have been developed.[4] Peptidomimetics could be potential inhibitors for the protein−protein interaction (PPI) with shallow binding pockets, because many peptides are known to be natural protein ligands. In addition, cyclic peptides and cyclic compounds exhibit an enhanced cell permeability[5] and improved resistance to proteolytic degradation.[6] Moreover, cyclic compounds might be presumed to bind more tightly to their target proteins, because of their conformational restriction.[7]

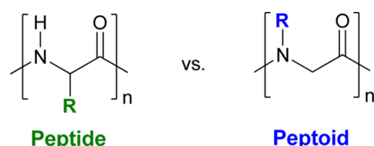


Figure 1. Chemical structures of peptide and peptoid.

Cyclic peptoids have been synthesized by several different methods[8] including macrolactamization,[8b] ring-closing metathesis,[8c] thioaryl-mediated cyclization,[8d] cycloaddition,[8e] and formation of boronate esters.[8f] Macrolactamization of peptoids was achieved by a head-to-tail cyclization in the solution phase[8b] or demonstrated on solid-phase[9] using glutamic acid as a carboxylic acid source forming a peptide bond. In this study, we report a modified macrolactamization strategy for the synthesis of cyclic peptoids composed of complete peptoid units on solid-phase as well as the efficient incorporation

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methods of polar side chain-containing amine submonomers. The examples employed in the study were derived from linear and cyclic sequences expected to be useful tools for the development of therapeutic agents for Alzheimer's disease by inhibition of the interaction between apolipoprotein E (ApoE) and amyloid-β (Aβ).[10]

■ RESULTS AND DISCUSSION

For the rational design of possible inhibitors of the ApoE/Aβ interaction, we planned to examine both linear and cyclic versions of the same peptoid sequences derived from residues 12–28 of Aβ, known to be the binding motif for ApoE (Aβ12–28: VHHQKLVFFAEDVGSNK).[10a] However, since Aβ12–28 is fibrillogenic and associated with toxicity, the Aβ12–28P (VHHQKLPFFAEDVGSNK) sequence was used, in which valine at residue 18 was replaced by proline. This peptide is a blood-brain-barrier-permeable, nontoxic, and nonfibrillogenic binding inhibitor, resulting in significant reduction of Aβ plaques in vivo.[10a,b] Therefore, we first designed peptoid derivatives of the minimal effective sequences [Aβ17–21(P)] as well as the longer Aβ15–24(P) and Aβ12–28P sequences in both linear and cyclic forms.[10d] Moreover, we hypothesized that it would be advantageous to test peptoid derivatives with two blocking domains of Aβ17–21. Since four permutations of Aβ17–21 and Aβ17–21P with a GSG spacer are possible, nine different peptoid sequences in both linear and cyclic forms were designed for the development of the ApoE/Aβ interaction inhibitors (Figure 2).

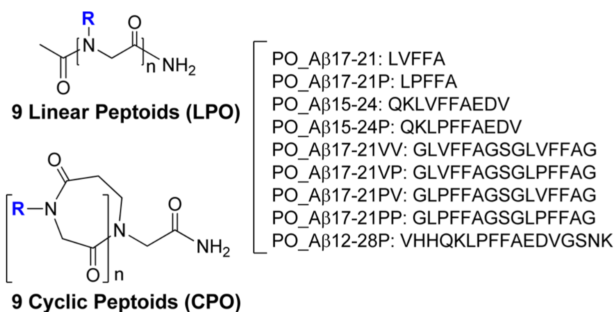


Figure 2. Peptoid-based chemical tools for ApoE/Aβ binding inhibition.

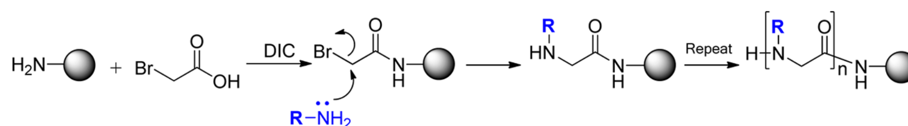
Peptoids can be easily synthesized by the well-known solid-phase submonomer strategy, which employs acylation using bromoacetic acid/*N,N*′-diisopropylcarbodiimide (DIC) and subsequent displacement by primary amines to afford the N-substituted glycine residues (Scheme 1).[1a] Microwave-assisted conditions were also well developed.[11] By repeating acylation and displacement steps, linear peptoids can be synthesized with unprecedented chemical diversity.

In this study, for the synthesis of linear peptoid derivatives, we also took advantage of the submonomer strategy. The terminal amino function of linear peptoids was capped with the acetyl group to prevent its ionization, and thus improving their cell permeability. To construct the desired linear peptoid sequences, 11 primary amines and two amino acids were necessary (Scheme 2). First, aliphatic or protected primary amines were directly used for peptoid synthesis under microwave conditions or at room temperature. However, for the introduction of amines such as histamine (H), glycinamide (N), and 3-aminopropanamide (Q) containing additional free nitrogen together with the primary amino functionality,[1b] a slightly modified strategy was adopted. The presence of the extra heteroatoms in side chains could result in a drastic decrease in the reaction yield. To reduce the extent of side product formation and increase the overall synthetic yield, our strategy relied on the interplay of reactivity and selectivity. Bromoacetic acid in the presence of DIC is a commonly used acylating agent in the submonomer strategy. In the case of peptoids containing unprotected amino heterocyclic or amide side chains, activated bromoacetic acid may acylate other nitrogen in side chains indiscriminately. However, these acylations are readily reversible in the presence of base.[12] Thus, although some acylated side products are temporarily formed, they may be deacylated in the second displacement step by plenty of amines. Instead, the irreversible N-alkylation of the nitrogen in side chains of peptoids by the activated bromoacetic acid at the acylation step can occur. However, this N-alkylation may compete with the N-acylation of the nitrogen in side chains which is normally much faster (about 1000 times) than the N-alkylation in the peptoid synthesis.[12] Thus, we hypothesized that the N-alkylation of the nitrogen in side chains might not be a serious problem at the acylation step although peptoids contain free amino heterocyclic or amide side chains.

However, the N-alkylation of additional free nitrogen over the primary amino functionality was possible at the displacement step even though the primary amino functionality of amine submonomers is much more nucleophilic than their free nitrogen in the side chains. To decrease the possibility of N-alkylation on nitrogen-containing side chains of amine submonomers at the displacement step, less reactive reagent chloroacetic acid was used instead of bromoacetic acid when additional free nitrogen-containing amine submonomers, such as histamine (H), glycinamide (N), and 3-aminopropanamide (Q) were introduced. We actually expected that the less reactive chloro-moiety of acylated peptoids may preferentially react with more nucleophilic primary amino functionality over less nucleophilic amino heterocyclic or amide functionalities of amine submonomers at the displacement step, increasing the regioselectivity of the N-alkylation of the primary amino functionality. This hypothesis was also demonstrated by Zuckermann et al.[12] In this case, the reaction time at the displacement should be extended up to 3 h to balance lower reactivity of the chloride.

Amine submonomers as their hydrochloride salt such as glycinamide and 3-aminopropanamide hardly undergo the displacement reaction under normal conditions. These amines were introduced into peptoids using *N,N*-diisopropylethyl-

Scheme 1. Solid-Phase Synthesis of Peptoids by the Submonomer Strategy



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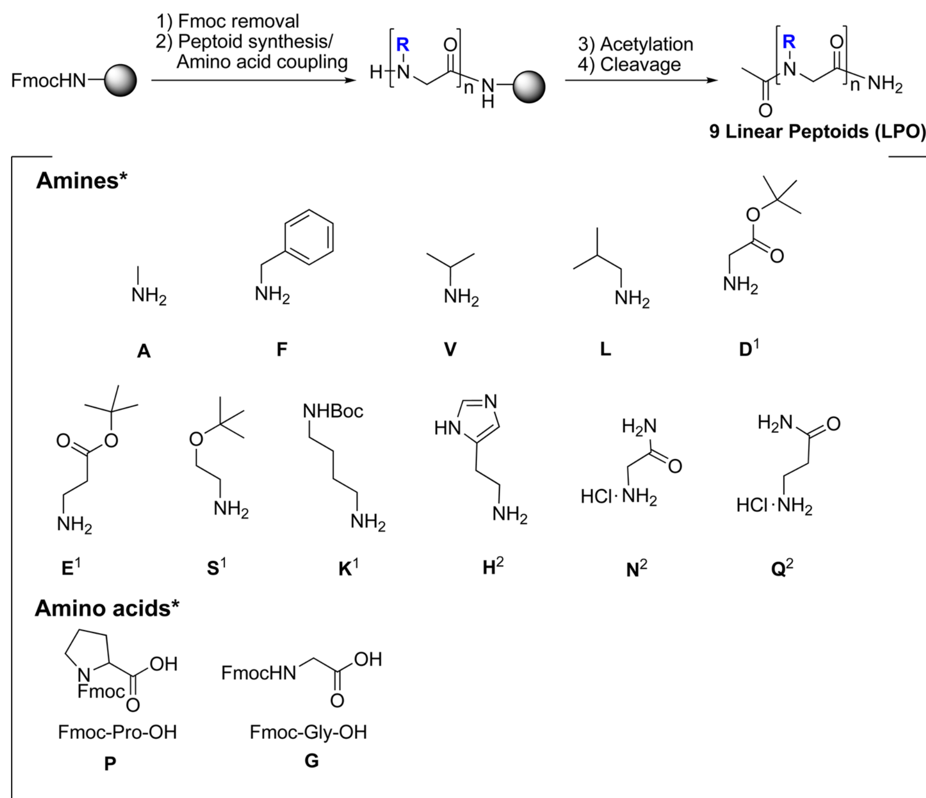
Scheme 2. Solid-Phase Synthesis of Linear Peptoids[a]



[a]The asterisk (*) indicates that abbreviations are for the corresponding peptoid units of amines or amino acids. [1]Protecting groups (Boc and tert-butyl) were removed by the treatment with 70% TFA at the cleavage step. [2]Peptoid synthesis: For histamine (H) coupling, chloroacetic acid was used instead of bromoacetic acide, while for amine-HCl salts (N and Q) coupling, conditions of (a) chloroacetic acid (0.4 M), DIC (2 M), 35 °C, 5 min and (b) amine (2 M), H_2O/DIPEA/DMF (1:4:5), 35 °C, 3 h were used. Reagents and conditions: (1) 20% piperidine; (2) peptoid synthesis (a) BrAcOH (2 M)/DIC (3.2 M) [or ClAcOH (0.4 M)/DIC (2 M)], (b) amine (2 M); amino acid coupling amino acid (5 equiv), HATU (5 equiv), HOAt (5 equiv), DIPEA (10 equiv); (3) acetic anhydride (10 equiv), DIPEA (10 equiv); (4) 70%TFA/3%TIS/5%H_2O/22%DCM.

Table 1. HPLC and Mass Analysis Data of Linear and Cyclic Peptoids

	linear peptoid			cyclic peptoid		
		mass (*m*/*z*)			mass (*m*/*z*)	
peptoid sequence	HPLC retention time[a] (min)	calcd	found[c]	HPLC retention time[a] (min)	calcd	found[c]
LVFFA	22.3	636.4	659.4 (+Na)	23.5	705.4	727.9 (+Na)
LPFFA	20.2	634.4	657.2 (+Na)	21.0	703.4	725.7 (+Na)
QKLVFFAEDV	16.7	1235.7	1236.9 (+H)	18.0	1304.7	1305.1 (+H)
			1258.0 (+Na)			1327.2 (+Na)
QKLPFFAEDV	15.8	1233.6	1234.4 (+H)	17.3	1302.7	1303.9 (+H)
			1256.1 (+Na)			1325.9 (+Na)
GLVFFAGSGLVFFAG	25.1	1542.8	1565.2 (+Na)	27.5	1611.8	1634.3 (+Na)
GLVFFAGSGLPFFAG	23.7	1540.8	1563.6 (+Na)	26.1	1609.9	1632.5 (+Na)
GLPFFAGSGLVFFAG	23.7	1540.8	1563.9 (+Na)	26.1	1609.9	1632.7 (+Na)
GLPFFAGSGLPFFAG	22.8	1538.8	1561.7 (+Na)	24.8	1607.8	1630.1 (+Na)
VHHQKLPFFAEDVGSNK	3.5	2035.1	2035.7 (+H)	5.1	2106.1	2107.6 (+H)
	(22.8)[b]		2058.8 (+Na)			

[a]20% aq. ACN to 80% aq. ACN for 40 min. [b]10% aq. ACN to 50% aq. ACN for 40 min. [c]Measured by MALDI-TOF.

amine (DIPEA) as the base in water/DMF. This variation improved the reactivity as well as the solubility of amines. For the incorporation of amino acids such as proline and glycine into peptoids, the conventional amino acid coupling conditions with HATU, HOAt, and DIPEA in DMF were used. Actually, these amino acids were exactly equivalent to the peptoid unit (N-substituted glycine). Finally, the necessary peptoid sequences were efficiently constructed under the above-mentioned conditions (see Supporting Information), and the terminal of peptoids was acetylated with acetic anhydride and DIPEA at the late step to afford nine linear peptoid derivatives on solid-phase. The desired peptoids were released from the resins together with the removal of acid-labile protecting groups by the treatment of the trifluoroacetic acid (TFA) cleavage cocktail, purified by reverse-phase HPLC, and lyophilized in a

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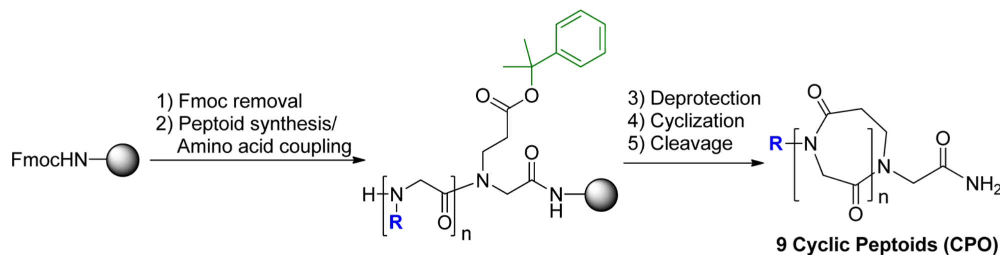
Scheme 3. Solid-Phase Synthesis of Cyclic Peptoids[a]



[a]Reagents and conditions: (1) 20% piperidine; (2) peptoid synthesis (a) BrAcOH (2 M), DIC (3.2 M), (b) amine (2 M); amino acid coupling amino acid (5 equiv), HATU (5 equiv), HOAt (5 equiv), DIPEA (10 equiv); (3) 2%TFA/1%TIS; (4) PyAOP (5 equiv), HOAt (5 equiv), DIPEA (10 equiv); (5) 70%TFA/3%TIS/5%H_2O/22%DCM.

freeze-dryer. HPLC retention time and MALDI-TOF data are listed in Table 1.

Next, to synthesize cyclic peptoids for the development of the ApoE/Aβ interaction inhibitors, we employed the modified efficient macrolactamization strategy to afford cyclic peptoids composing complete peptoid units on solid-phase (Scheme 3). Therefore, β-alanine protected with the 2-phenylisopropyl (2-PhiPr) group instead of glutamic acid as the source of the carboxylic acid functionality, synthesized from Fmoc-β-alanine in two steps,[13] was first introduced to the Rink amide AM resins with low loading capacity (0.37 mmol/g) as the corresponding primary amine. Other necessary peptoid units were introduced by the same methods as used for the synthesis of linear peptoids. After the selective removal of 2-PhiPr group with 2% TFA and 1% triisopropylsilane (TIS) in dichloromethane (DCM),[9] the carboxylic acid functionality of β-alanine moiety was reacted with the terminal amino functionality of peptoids to afford cyclic peptoids in the last macrolactamization step. The efficiency of macrolactamization differs in various peptoids, depending on the size and sequences (mainly, the identity of last residue) of peptoids, activating agents, etc. In this study, macrocyclizations were successfully performed on solid-phase using PyAOP and HOAt at room temperature (see Supporting Information), although the structural motif of the proline moiety in the case of some proline-containing peptoids might help the efficient macrocyclization. In particular, the sizes of cyclic peptoids vary from 19 atomic members to 55 atomic members, although the macrolactamization of peptoids is generally known to be successful in moderate sizes. To the best of our knowledge, cyclic peptoids, CPO_Aβ12−28P, composed of 55 atomic members is the largest cyclic peptoid among the cyclic peptoid examples reported to date.[1b,8] The synthesized cyclic peptoids were cleaved from the resins with a cocktail of 70% TFA, 3% TIS, and 5% H_2O in DCM, purified by reverse-phase HPLC, lyophilized in a freeze-dryer and fully confirmed by MALDI-TOF MS analysis. As shown in Table 1, all the cyclic peptoids were generally more hydrophobic compared to the corresponding linear peptoids, based on the HPLC retention times. The interesting biological studies of peptoid derivatives in both linear and cyclic forms including the inhibition against ApoE/Aβ interaction, nontoxicity, etc., are currently under way.

■ CONCLUSIONS

In summary, we synthesized a series of linear and cyclic peptoids as possible therapeutic agents for AD, expecting them to possess better pharmacokinetic properties and biological activities for blocking the ApoE/Aβ interaction. The peptoids were easily synthesized on the solid-phase by the submonomer strategy, and the polar side chain-containing amines were effectively introduced under the modified reaction conditions. For the synthesis of cyclic peptoids, β-alanine protected with the 2-PhiPr group, which could be selectively removed by 2% TFA, was employed as a primary amine to afford a complete peptoid unit. The macrolactamization between the carboxylic acid of β-alanine moiety and terminal amine of peptoids was successfully performed in the presence of the PyAOP coupling agent on solid-phase in all the cases, providing various sizes of cyclic peptoids. In particular, to the best of our knowledge, some cyclic peptoids prepared in this study are the largest in size among cyclic peptoids reported to date. The synthetic strategy which was adopted in this study can also provide a robust platform for solid-phase construction of cyclic peptoid libraries. Currently, synthetic peptoids have been used to test interesting biological activities including the ApoE/Aβ interaction inhibition, nontoxicity, etc. and the vivo experiments using transgenic mice based on the in vitro results will also be performed. These results will be reported in due course.

■ EXPERIMENTAL PROCEDURES

General. Chemical reagents were purchased from commercial sources and used as obtained without further purification unless otherwise noted. The primary amines used in this study were methylamine, benzylamine, isopropylamine, isobutylamine, glycine *tert*-butyl ester, β-alanine *tert*-butyl ester, β-alanine 2-phenylisopropyl ester, *O*-*tert*-butyl ethanolamine, *N*-Boc-1,4-diaminobutane, histamine, glycinamide hydrochloride, and 3-aminopropanamide hydrochloride. Glycine *tert*-butyl ester hydrochloride was neutralized with aq. Na_2CO_3 and extracted with DCM prior to use. β-Alanine *tert*-butyl ester,[14] β-alanine 2-phenylisopropyl ester,[13] and *O*-*tert*-butyl ethanolamine[15] were prepared according to the literatures. Polystyrene AM RAM resin (capacity: 0.60 mmol/g) and Rink Amide AM resin (capacity: 0.37 mmol/g) protected with Fmoc were purchased from RAPP POLYMERE and GL Biochem, respectively. Analytical or semipreparative HPLC experiments were conducted by ACE 5 C18-HL (250 × 4.6 mm) or ACE 10 C18 (250 × 10 mm) reverse-phase columns using a Shimadzu binary HPLC system equipped with a UV−visible detector at 220 nm. In all the cases, a gradient elution of water/acetonitrile (ACN) with 0.05% TFA was used. The typical flow rates for analytical and semipreparative HPLC were 1 and 5 mL/min, respectively. MALDI-TOF MS analysis was performed using an Axima Performance mass spectrometer (Shimadzu) with α-cyano-4-hydroxycinnamic acid as the matrix. The peptoids were synthesized in an incubator shaker (JEIO TECH, model: SI-

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600R) or in a microwave oven (Daewoo, model: KR-B200R) at a power of 100 W. Standard glass peptide synthesis vessels (25 mL) were used for both the syntheses in the incubator shaker and microwave oven.

General Procedure for the Solid-Phase Synthesis of Linear Peptoids. Peptoids were synthesized on Polystyrene AM RAM resin or Rink Amide AM resin by the conventional submonomer strategy. The resins were swelled in DMF at room temperature for 1−2 h. Then, DMF was drained, and the beads were incubated with 20% piperidine in DMF for 30 min and washed thoroughly with DMF (8 × 3 mL). The beads were treated with bromoacetic acid (2 M, 1.0−1.5 mL) and DIC (3.2 M, 1−1.5 mL), and irradiated in a microwave oven (100 W) for 3 × 12 s with shaking for 30 s after each pulse. The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with primary amines (2 M, 2 mL) in DMF in a microwave oven (100 W) for 3 × 12 s with shaking for 30 s after each pulse. Then the beads were thoroughly washed with DMF (8 × 3 mL). Both the acylation and displacement were successively repeated to form the desired peptoid sequences. Finally, the amino terminal groups of peptoids were acetylated with acetic anhydride (10 equiv) and DIPEA (10 equiv) in DMF (2 mL) at room temperature for 2 h. Then the beads were thoroughly washed with DMF (8 × 3 mL) and DCM (8 × 3 mL) before the TFA cleavage.

General Procedure for the Attachment of Additional Free Nitrogen-Containing Amines. For the introduction of amines, such as histamine, glycinamide, and 3-aminopropanamide, containing an additional free nitrogen together with the primary amino functionality, the slightly modified conditions were employed.[12] The resins were treated with chloroacetic acid (0.4 M, 850 μL) and DIC (2.0 M, 200 μL) in DMF, shaken at 35 °C for 5 min, drained, and then washed with DMF (8 × 3 mL). In the case of histamine, the resin-bound halogen was then displaced with the primary amine submonomer. To the resin-bound halogen was added a 2.0 M solution of the primary amine (0.85 μL, 1.7 mmol) in DMF and allowed to react at 35 °C for 3 h. In the case of glycinamide hydrochloride and 3-aminopropanamide hydrochloride, to the resin-bound halogen was added a 2.0 M solution of the primary amine containing DIPEA (0.8 mL) in H_2O/DMF (0.2/1.0 mL), and the reaction was allowed to react at 35 °C for 3 h. Then the beads were thoroughly washed with DMF (8 × 3 mL).

General Procedure for the Attachment of Amino Acids. For the attachment of amino acids such as Fmoc-Pro-OH and Fmoc-Gly-OH into peptoids, the conventional amino acid coupling conditions with HATU, HOAt and DIPEA in DMF was used. The beads were treated with Fmoc-Pro-OH (5 equiv) or Fmoc-Gly-OH (5 equiv), HATU (5 equiv), HOAt (5 equiv), and DIPEA (10 equiv) in DMF (total 2 mL solution) in a shaker at room temperature for 2 h. The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with 20% piperidine to remove the Fmoc group. The other peptoid units were introduced by the above-mentioned submonomer strategy.

General Procedure for the Solid-Phase Synthesis of Cyclic Peptoids. For the synthesis of cyclic peptoids, Rink Amide AM resin with a low loading capacity of 0.37 mmol/g was used. The resins were swelled in DMF at room temperature for 1−2 h. Then, DMF was drained, and the beads were incubated with 20% piperidine in DMF for 30 min and washed thoroughly with DMF (8 × 3 mL). The beads were treated with bromoacetic acid (2 M, 1.0−1.5 mL) and DIC (3.2 M, 1−1.5 mL), and irradiated in a microwave oven (100 W) for 3 × 12 s with shaking for 30 s after each pulse. The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with *β*-alanine 2-phenylisopropyl ester (*β*-Ala-*O*-2-PhiPr)[13] (2 M, 2 mL) in DMF in a microwave oven (100 W) for 3 × 12 s with shaking for 30 s after each pulse. Then the beads were thoroughly washed with DMF (8 × 3 mL). Next, the desired sequences were constructed by the above-mentioned submonomer strategy or amino acid coupling method. Linear peptoid-bound resins were treated with a solution of 2% TFA, 1% TIS in DCM twice at room temperature for 30 min to selectively remove the 2-PhiPr group. Then, the resins were thoroughly washed with 5% DIPEA in DCM (3 × 3 mL), DCM (3 × 3 mL), and DMF (3 × 3 mL), macrolactamization was performed using PyAOP (5 equiv), HOAt (5 equiv), and DIPEA (10 equiv) in DMF at room temperature for 6 h. If necessary, the macrolactamization was repeated one more time. Then, the beads were thoroughly washed with DMF (8 × 3 mL) and DCM (8 × 3 mL) before the TFA cleavage.

General Procedure for TFA-Assisted Cleavage and Reverse-Phase HPLC. Peptoid-tethered resins which were thoroughly washed with DCM, were suspended in a cleavage cocktail of 70% TFA/3% TIS/5% H_2O/22% DCM for 2 h. After the cleavage solution was removed by blowing N_2 gas, 50% aq. ACN containing 0.05% TFA was added and mixed uniformly. The mixture was filtered through 0.2 μm PTFE filter tip, and the filtrate was directly used for HPLC and MALDI-TOF analyses. Reverse-phase HPLC experiments were conducted by a C18 reverse-phase column using a Shimadzu binary HPLC system equipped with a UV−visible detector at 220 nm by using a gradient elution of water/ACN with 0.05% TFA. The typical gradient elution conditions were from 20% aq. ACN to 80% aq. ACN for 40 min with the flow rates of 1 and 5 mL/min for analytical and semipreparative HPLC, respectively. The purified peptoids were lyophilized in a freeze-dryer and confirmed by MALDI-TOF analysis. The HPLC retention time and MALDI-TOF data of the synthesized peptoids are listed in Table 1.

■ ASSOCIATED CONTENT

Supporting Information

Additional data including chemical structures, HPLC chromatograms, MALDI-TOF spectra of target 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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■ ABBREVIATIONS

aa, amino acid; Aβ, amyloid-β; ACN, acetonitrile; AD, Alzheimer's disease; ApoE, apolipoprotein E; DCM, methylene chloride; DIC, *N,N′*-diisopropylcarbodiimide; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; Fmoc, fluorenylmethyloxycarbonyl; HATU, 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HPLC, high-performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; 2-PhiPr, 2-phenylisopropyl; PPI, protein−protein interaction; PyAOP, (3-hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]pyridinato-*O*)tri-1-pyrrolidinylphosphonium hexafluorophosphate; TFA, trifluoroacetic acid; TIS, triisopropylsilane

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