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Incorporation of Heterocycles into the Backbone of Peptoids to Generate Diverse Peptoid-Inspired One Bead One Compound Libraries

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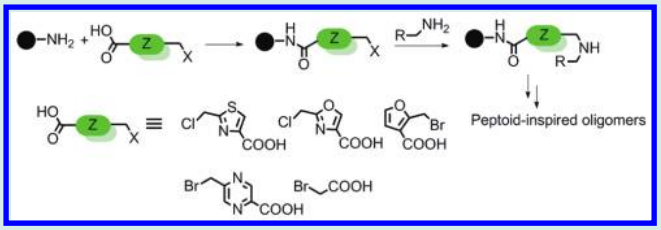
Supporting Information

ABSTRACT: Combinatorial libraries of peptoids (oligo-N-substituted glycines) have proven to be useful sources of protein ligands. Each unit of the peptoid oligomer is derived from 2-haloacetic acid and a primary amine. To increase the chemical diversity available in peptoid libraries, we demonstrate here that heterocyclic halomethyl carboxylic acids can be employed as backbone building blocks in the synthesis of peptoid-based oligomers. Optimized conditions are reported that allow the creation of large, high quality combinatorial libraries containing these units.



KEYWORDS: *peptoids, one bead one compound libraries, heterocycles, solid-phase synthesis, peptidomimetics*

INTRODUCTION

There is great interest in the discovery of potent and selective bioactive compounds for use in medicine and biology. In recent years, high-throughput screening has been a major contributor to the discovery of such species. These screening campaigns are carried out in a completely unbiased fashion if no structural information for the target protein is available to guide library design. In such cases, the more chemically diverse the library one screens, the better. One source of highly diverse peptidomimetic compounds is libraries of peptoids (N-substituted oligo-glycines).[1] Peptoids have certain advantages over native peptides, including protease insensitivity and improved cell permeability.[2,3]

Each unit of a peptoid is put together in two steps,[4] the first being acylation of an existing amine with an activated ester of 2-bromo (or chloro[5]) acetic acid, followed by displacement of the halide with a primary amine. These are almost ideal molecules for combinatorial chemistry,[6] because the source of diversity is a primary amine, of which thousands are readily available. In addition, the structures of peptoids can be determined by tandem mass spectrometry, meaning that split and pool strategies[7] can be used to create peptoid libraries without the requirement for encoding.

It would be desirable to develop peptoid analogues that retain the many favorable characteristics of this class of compounds but offer increased chemical diversity and greater conformational constraints. One strategy to accomplish this goal would be to replace the two-carbon haloacetic acid-derived fragment of peptoids with a more elaborate building block.[8,9] Aromatic heterocycles are of particular interest as main chain building blocks. In addition to expanding the functionality in the main chain relative to a peptoid, these units would be expected to introduce conformational constraints in the oligomer. Indeed, two previous reports indicate that incorporation of a 1,5-disubstituted-1,2,3-triazole[10] and a 1,3,5-triazine unit[11] in the peptoid backbone resulted in conformationally biased oligomers. Moreover, heterocycles can also increase the aqueous solubility of oligomers relative to all-carbon units.[8,12] The critical role of heterocycles in drug discovery is underscored by the fact that more than half of known small molecule drugs contain at least one heterocycle. Here we report novel heterocyclic halomethyl carboxylic acids that can be used in place of the halo acetate, allowing the creation of peptoid analogues in which a heterocycle is incorporated into the main chain. Optimized conditions for the creation of high quality combinatorial libraries are presented. Furthermore, we also show that molecules derived from a single bead of a one bead one compound library of heterocycle-modified peptoids can be characterized by mass spectrometry.

RESULTS

Our initial efforts were focused toward incorporation of oxazole and thiazole units in the peptoid backbone, deriving inspiration from variety of natural products that display these entities. Biosynthetic assembly of these heterocycles occurs via various post-translational modifications on serine, threonine, and cysteine residues in their peptide precursors. Synthetic routes have been reported for the generation of oxazole and thiazole amino acid building blocks for combinatorial synthesis,[13−15] and several strategies to assemble these scaffolds on solid phase have also been reported.[16] However, none of these strategies

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are applicable to a submonomer, peptoid-like approach to combinatorial library synthesis.

Therefore, we created heterocycles-containing haloacids **1** and **2** (Figure 1). These heterocycles were synthesized using a

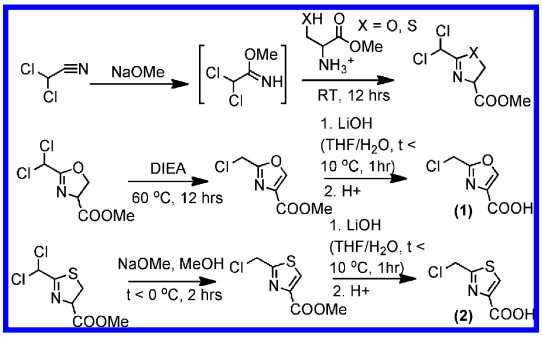

Figure 1. Synthesis of azole building blocks: (**1**) 2-chloromethyloxazole-4-carboxylic acid, (**2**) 2-chloromethylthiazole-4-carboxylic acid.

modification of a previously published procedure[17] as described in Figure 1. Briefly, serine or cysteine methyl ester hydrochloride was condensed with dichloroacetonitrile in the presence of sodium methoxide. The resulting oxazoline or thiazoline intermediate was allowed to undergo base-catalyzed elimination to yield the corresponding chloromethyl-azole methyl esters. Saponification at low temperature followed by acidification yielded the respective carboxylic acids. Synthesis of these heterocycles was performed on a 45 mmol scale with ~85% and ~45% overall yields for the thiazole and the oxazole building blocks, respectively.

With these building blocks in hand, we turned to deriving optimized conditions for inserting them into a peptoid chain. A five-residue peptoid (Figure 2) was assembled on polystyrene resin functionalized with the rink amide linker. We examined several conditions for carboxylic acid coupling and amine displacement (summarized in Table 1). In each case, the resin beads were subjected to a chloranil test[18] to determine the completeness of the coupling reaction. Coupling of halomethyl oxazole and thiazole carboxylic acids at room temperature for 3 h with *N,N'*-diisopropylcarbodiimide (DIC) as the coupling agent, both in the absence and presence of additives such as HOAt were found to be inefficient. Subsequently, we tested the utility of standard microwave-assisted peptoid synthesis conditions[19] (1 M acid +1 M DIC in dimethylformamide (DMF) with irradiation at 100 W for 15 s) for the coupling of azole heterocycles **1** and **2** to the N-terminus of this chain. Unfortunately, these conditions also resulted in inefficient coupling as indicated by the chloranil test. The sluggish nature of this coupling can be attributed to the much lower reactivity of the activated esters of these carboxylic acids relative to those obtained from bromo- or chloroacetic acid. Optimal coupling of these heterocyclic carboxylic acids could only be achieved by performing the DIC-mediated couplings at 70 °C, employing 10 equiv each of **1** or **2** along with 12 equiv of DIC for a period of 5 h.

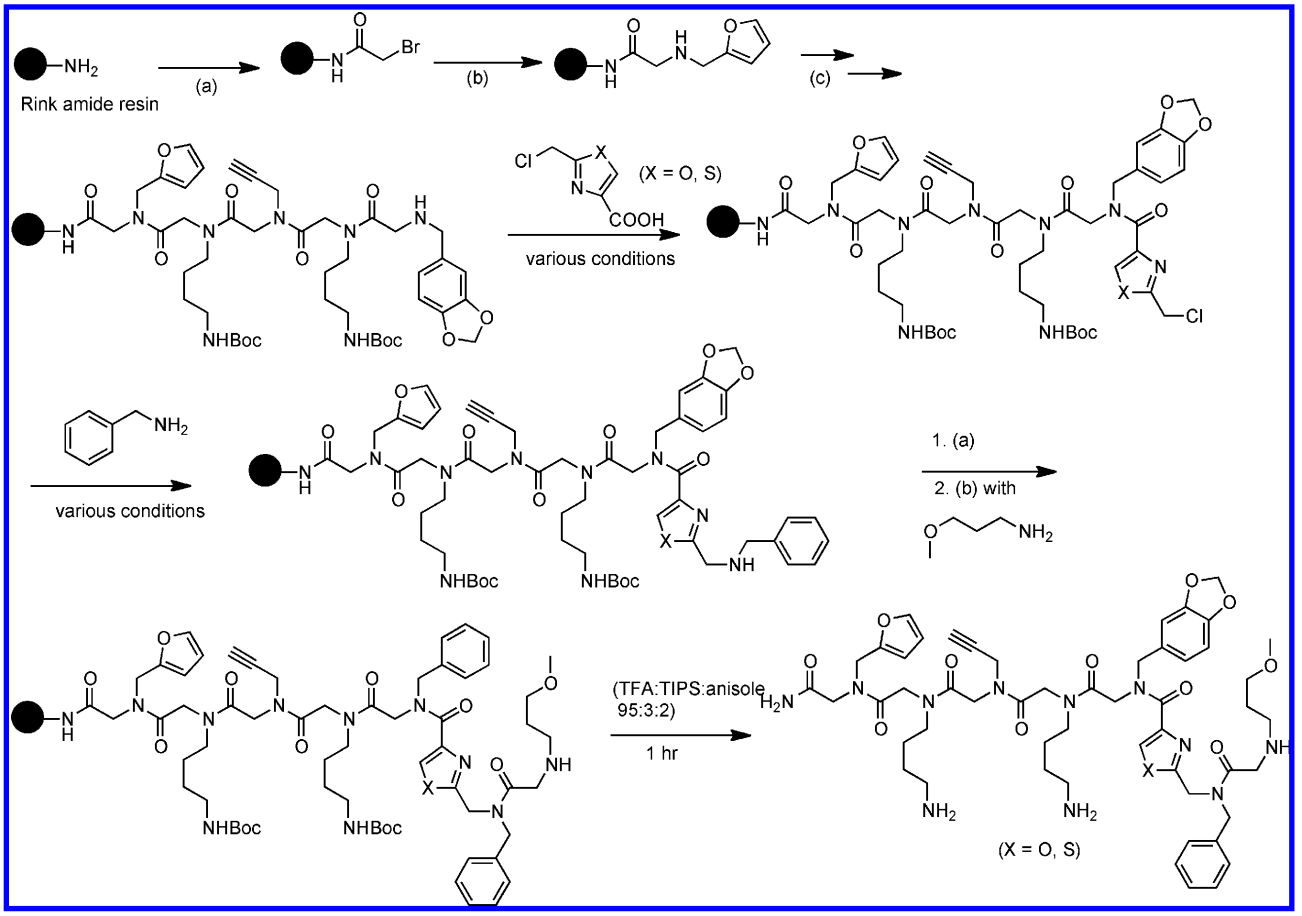

Figure 2. Incorporation of azole heterocycles to generate a backbone modified peptoid (a) $BrCH_2COOH$ (2 M in DMF), DIC (2 M in DMF), MW 15 s, 10% power (100 W) repeated twice. (b) Primary amine (2 M in DMF), MW 30 s, 10% power (100 W) repeated three times. (c) Steps (a) and (b) repeated four times with various amines. Different conditions for acylation with the heterocycle and subsequent displacement with the amine are described in Table 1.

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Table 1. Optimization of Acylation and Amine Displacement Conditions to Incorporate Azole Heterocycles into the Peptoid Backbone[a]

X	acylation conditions	amine displacement conditions	purity[b]	comments[f]
S	**2** (10 equiv) + DIC (10 equiv) in DMF, RT, 3 h.			0.5 M effective concentration of reagents, incomplete coupling
S	**2** (10 equiv) + DIC (10 equiv) + HOAt (10 equiv) in DMF, RT, 3 h.			0.5 M effective concentration of reagents, incomplete coupling
S	**2** (2 M in DMF) + DIC (2 M in DMF) 1:1, MW, 15 s, 100 W (10%) power[c], repeated 3 times			1 M effective concentration of reagents, incomplete coupling
S	**2** (10 equiv) + DIC (12 equiv) in DMF, 70 °C, 5 h	benzylamine (1 M in DMF), 80 °C, 12 h	91%	0.5 M effective concentration of reagents during acylation
O	**1** (10 equiv) + DIC (12 equiv) in DMF, 70 °C, 5 h	benzylamine (1 M in DMF), 80 °C, 12 h	73%	0.5 M effective concentration of reagents during acylation
S	**2** (10 equiv) + DIC (12 equiv) in DMF, MW[d], 80 °C, 20 min	benzylamine (1 M in DMF), MW[d] 100 °C, 30 min	96%	1 M effective concentration of reagents
O	**1** (10 equiv) + DIC (12 equiv) in DMF, MW[d], 80 °C, 20 min	benzylamine (1 M in DMF), MW[d] 100 °C, 30 min	91%	1 M effective concentration of reagents

[a]**1** and **2** refer to azole building blocks 2-chloromethyloxazole-4-carboxylic acid and 2-chloromethylthiazole-4-carboxylic acid, respectively. [b]Area under major peak obtained in the HPLC analysis of the crude test peptoids shown in Figure 2. [c]Microwave reaction performed in a 1000 W house microwave apparatus. [d]Microwave reaction performed in a microwave reactor (Biotage Inc.) set to fixed temperature and variable power settings. [f]Completeness of coupling was determined by a chloranil test after acylation where beads turned blue if acylation was incomplete.

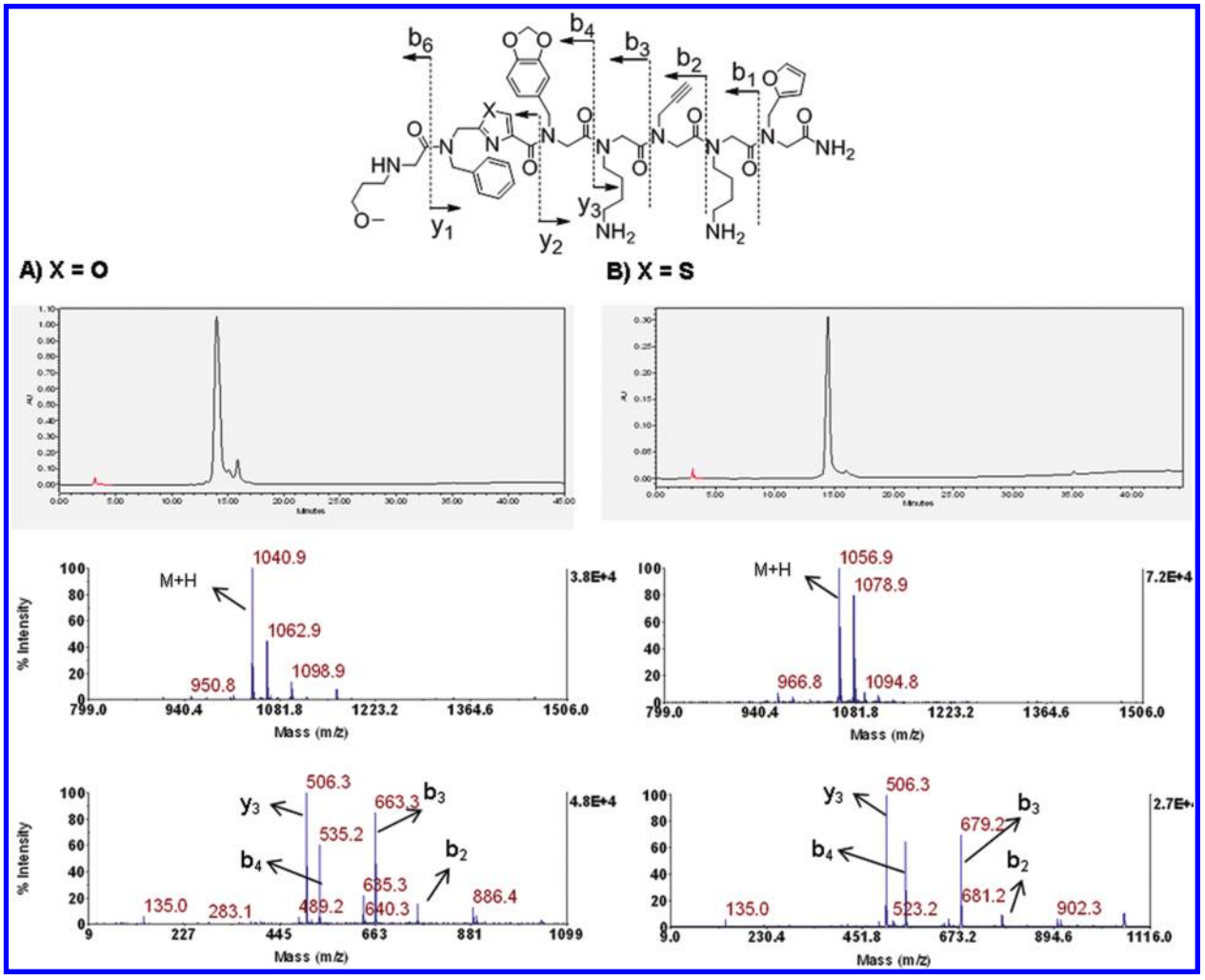

Figure 3. HPLC and MALDI-MS profiles of test peptoids containing azole building blocks synthesized by microwave-assisted coupling of heterocyclic building blocks. (A) A test peptoid containing an oxazole building block. (B) Test peptoid containing a thiazole building block. Magnified versions of these spectra are provided in the Supporting Information.

Performing the same reaction within a temperature-controlled microwave reactor (Biotage Inc.) at 80 °C for 20 min with 2 min of preactivation was found to decrease the reaction time considerably.

The amine coupling reaction also proved to be more difficult than is the case with standard peptoid synthesis. This is not surprising given the reduced electrophilicity of the chloro-substituted

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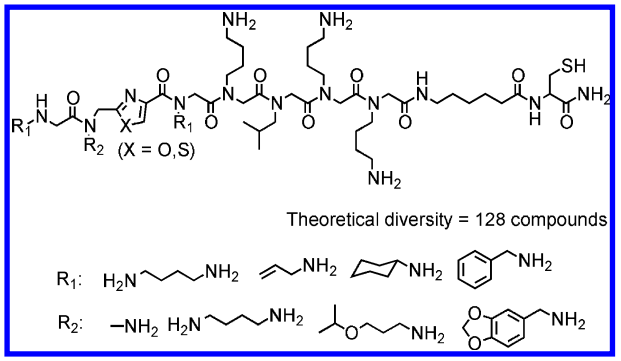

Figure 4. Design of peptoid library with three diversity positions incorporating the azole building block in the backbone. This library was assembled on polystyrene-Rink amide resin (500 μm beads) by utilizing microwave assisted peptoid synthesis conditions.

carbons in the heterocyles relative to a carbon adjacent to carbonyl center. Nonetheless, we found that essentially quantitative displacement of the chloride could be achieved by treating the beads with primary amine (1 M in DMF) at 80 °C for 12 h or at 100 °C for 30 min in the microwave reactor. In general, microwave-assisted couplings and amine displacement reactions generated the resulting oligomers with a greater purity in comparison with thermal conditions. After an additional standard peptoid residue was added to the chain as shown in Figure 2, the molecules were cleaved from the resin and analyzed by HPLC and MALDI mass spectrometry (Figure 3).

We then wanted to evaluate our ability to insert these heterocyclic building blocks into oligomeric chains in the context of one bead one compound (OBOC) libraries created by split and pool synthesis where the library members can still be sequenced by tandem MS unambiguously. The MS sequencing profiles of representative azole-modified peptoids, shown in Figure 3, exhibit a fragmentation pattern somewhat different from the fragmentation trends observed with regular peptoids and peptides. We observed that fragmentation of the amide bond at the C-terminal side of the heterocycle was suppressed, resulting in a complete absence or a low intensity of the corresponding *y* and *b* fragments resulting from that bond breakage. This unusual fragmentation trend can potentially complicate the sequencing of these oligomers. This means that the heterocycle-containing monomer and the one on the C-terminal side of it must be read as a single unit in the MS/MS spectrum. Thus, one must employ a different set of amines with distinct molecular weights to functionalize the azole submonomer to assign unequivocally the side chains on the heterocycles unit and the one before it from the fragmentation spectrum. Bearing this in mind, we synthesized a library of heptamers containing a constant four-residue peptoid linker appended to a peptide dimer constructed by cysteine and 6-aminohexanoic acid at the C-terminal. The variable three residues were diversified by using four different amines where the sixth position was further diversified by substituting oxazole or thiazole submonomer in place of the usual bromoacetic acid, thus resulting in a library of 128 compounds (Figure 4). The oxazole and thiazole-containing units were inserted using the optimized conditions mentioned above while all of the peptoid residues were inserted using standard microwave-assisted conditions. Twenty beads were picked at random in a 96-well plate for quality analysis of this library, and each bead was subjected to cleavage with trifluoroacetic acid. The postcleavage MS analysis displayed clean mass spectra for all compounds. MS-MS fragmentation of the major peak obtained in each case enabled the sequencing of all oligomers obtained from the aforementioned 20 beads, where seven sequences appeared more than once. The design template, amine building blocks utilized for each variable position, and the mass spectral profile of representative members of this library are shown in Figures 4 and 5.

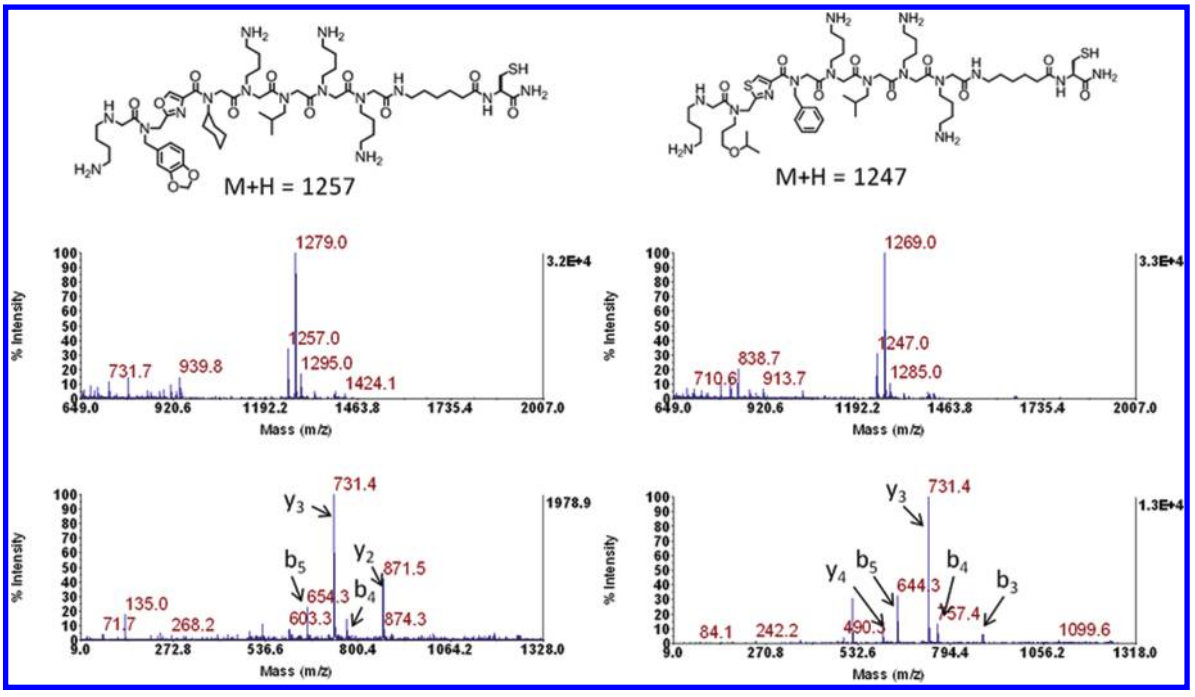

Figure 5. MALDI-MS and MS-MS profiles of representative members of library shown in Figure 4. These spectra were obtained from compounds cleaved from individual beads handpicked for quality control of this library. (The fragmentation scheme along with magnified traces of these spectra are reproduced in the Supporting Information along with spectral data for other members characterized for quality control of this library.).

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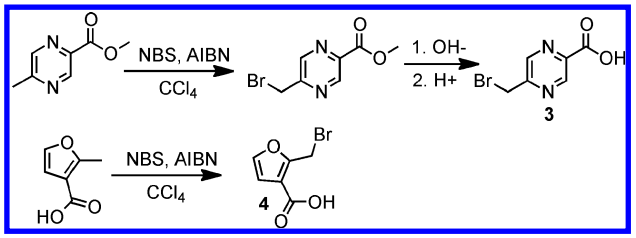

Figure 6. Synthesis of pyrazine and furan building blocks for incorporation into peptoid backbone.

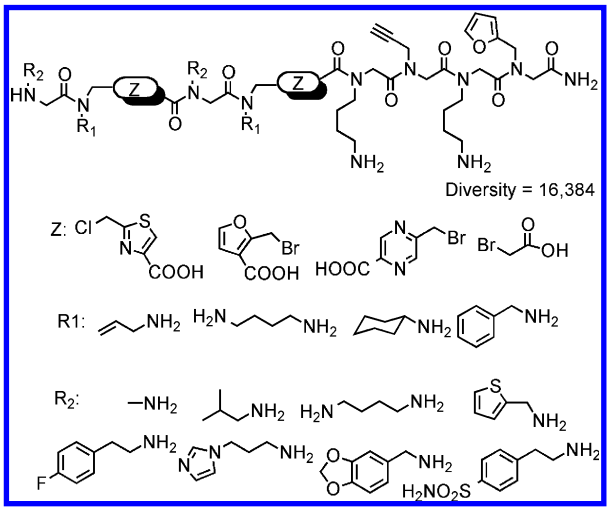

Figure 7. Design of a complex peptoid-based library where heterocyclic building blocks with various configurations are incorporated in the backbone.

This encouraging outcome of incorporation of oxazole and thiazole building blocks into the peptoid backbone prompted us to develop other heterocyclic scaffolds. With 2,4-disubstituted azoles in hand that orient the carboxamide and amine substitution in a 1,3 configuration in the peptoid backbone, we wanted to develop heterocyclic building blocks that can be used to exhibit 1,4 and 1,2 connectivities. To this end, we synthesized the pyrazine **3** and furan **4** building blocks as shown in Figure 6. These building blocks were synthesized from inexpensive commercially available methyl substituted carboxylic acids via *N*-bromosuccinimide (NBS)-mediated free radical bromination. Briefly, 5-methylpyrazine-2-carboxylic acid was converted to its corresponding methyl ester that was subsequently treated with NBS in presence of AIBN as the radical initiator. The corresponding bromomethyl carboxylate ester was saponified at low temperature to generate the required pyrazine building block **3** in an overall yield of ~43% from the starting carboxylic acid. We envisioned utilizing the same route for generating the furan building block **4**. However, methyl 2-bromomethyl-3-furoate[20] could not be saponified at ambient temperature, and we feared that this intermediate would be hydrolyzed to the corresponding alcohol at higher temperatures. Instead, this building block was obtained directly by radical bromination of 2-methyl-3-furoic acid with a yield of ~53%. The efficacy of these new building blocks as submonomers for incorporation into the peptoid backbone was investigated by utilizing the microwave-assisted conditions established for azoles earlier. The HPLC profiles of the resulting oligomers indicated that the microwave-assisted acylation and amine displacement conditions developed earlier for the azole building blocks can be utilized to incorporate the pyrazine and furan building blocks efficiently as well.

The synthesis of four heterocyclic halomethyl carboxylic acids and establishment of conditions that can be employed to append these building blocks onto a growing peptoid chain expands our ability to synthesize fairly diverse peptoid-derived libraries. Design of a complex library that showcases the incorporation of more than one thiazole, pyrazine, and furan building blocks in the backbone is shown in Figure 7. Bromoacetic

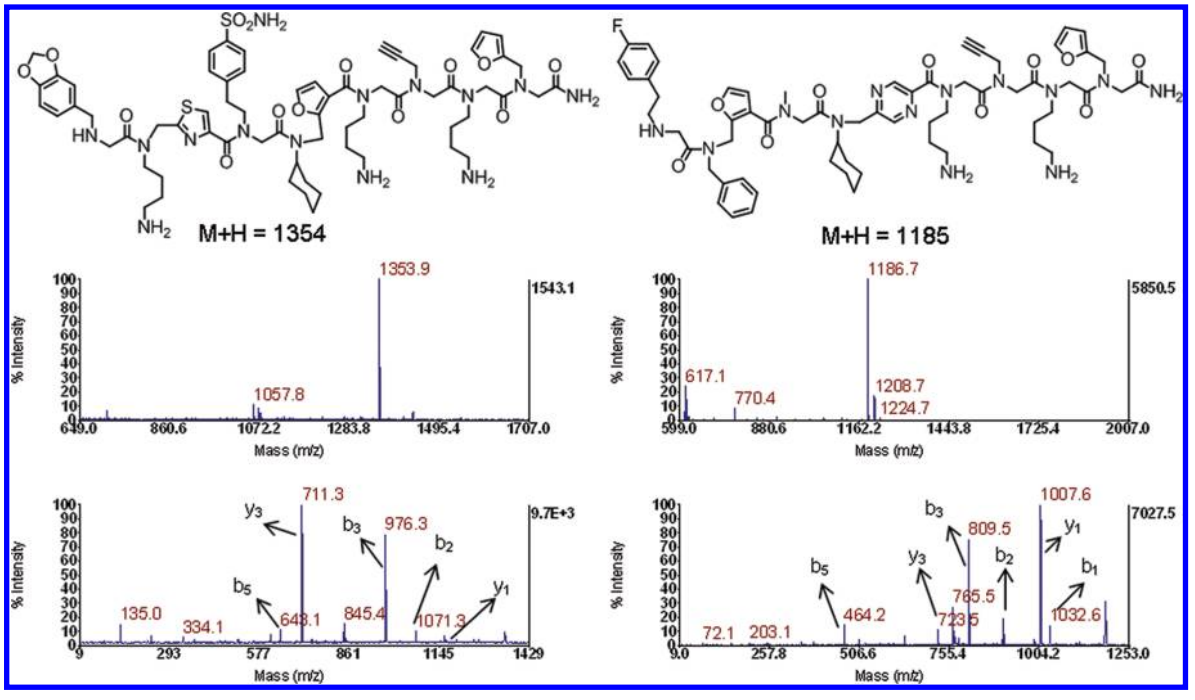

Figure 8. MALDI-MS and tandem MS profiles of selected members of the library shown in Figure 7. These spectra were obtained from compounds cleaved from individual beads handpicked for quality control of this library. (The y and b fragments in the tandem MS spectra are indicated according to the fragmentation shown in the Supporting Information.).

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acid was also included as a building block at positions where heterocycles were incorporated to increase the overall theoretical diversity to 16,384 compounds in this library. This library was assembled on polystyrene Rink amide resin (500 μm, Rapp Polymere), and the overall quality of this library was assessed by cleaving the oligomers from 24 beads in a 96-well format as described earlier. Out of these, compounds obtained from 21 beads were sequenced unambiguously, indicating that roughly 87% of this library will yield the desired sequences. The MS spectra for representative members are shown in Figure 8.

Diverse oligomeric libraries that display a variety of backbone scaffolds and side-chain appendages are a promising source of bioactive ligands. Generation of such libraries in high quality is often challenging, primarily because the chemical reactions utilized to generate diverse scaffolds are unable to generate the resulting oligomers in high purity.[21] In this paper, we have reported the development of heterocyclic halomethyl carboxylic acids as building blocks for generation of diverse backbone scaffolds in peptoid-derived oligomers. We have also demonstrated that these halomethyl heterocyclic carboxylic acids can be conveniently utilized to construct peptoid-based libraries via a submonomer approach through microwave-assisted amide bond formation and subsequent nucleophilic substitution with primary amines. Furthermore, this strategy has been utilized to generate peptoid-derived libraries in the OBOC format where the library members were sequenced by tandem mass spectrometry. To expand the scope of this approach, development of other heterocyclic building blocks is currently in progress that can further enhance the backbone diversity of oligomeric libraries. These OBOC libraries will be screened with different biological targets to mine for structurally diverse ligands.

MATERIAL AND METHODS

A full description of the materials and methods can be found in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Further details on the synthesis of halomethyl heterocyclic carboxylic acids, synthesis and HPLC profiles of test peptoids, synthesis and MS data of OBOC library with one heterocycle and at most two heterocycles in the backbone, and on probing for N-acylation/alkylation of nitrogen-containing heterocycles. 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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