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A cleavable scaffold strategy for the synthesis of one-bead one-compound cyclic peptoid libraries that can be sequenced by tandem mass spectrometry

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ABSTRACT

Many macrocyclic depsipeptides or related compounds have interesting medicinal properties and often display more favorable pharmacokinetic properties than linear analogues. Therefore, there is considerable interest in the development of large combinatorial libraries of macrocyclic peptidomimetic compounds. However, such molecules cannot be easily sequenced by tandem mass spectrometry, making it difficult to identify hits isolated from library screens using one bead one compound libraries. Here we report a strategy to solve this problem by placing a methionine in both the linker connecting the cyclic molecule to the bead as well as within the cycle itself. Treatment with CNBr both linearizes the molecule at a specific position and releases the molecule from the bead, making its characterization by tandem MALDI mass spectrometry straightforward.

Introduction

Many cyclic peptides and depsipeptides have been found to display potent biological activities and often have better pharmacokinetic properties than their linear counterparts.[1–5] This has prompted interest in the synthesis and screening of large libraries of cyclic peptides[6–11] or peptide-like compounds.[12,13] However, with the exception of genetically encoded cyclic peptides[7,14–16] a difficulty with screening such libraries is deducing the structure of hit compounds. Because cyclic peptides and peptide-like compounds lack a free N-terminus, Edman sequencing cannot be employed directly. In the mass spectrometer, cyclic oligomers fragment at multiple positions, making the interpretation of tandem MS-MS spectra difficult.[17] 'One bead-two compound' encoding strategies,[18] in which each bead contains both a cyclic and linear version of each compound have been used to mitigate this problem.[19,20] Another recent innovation is a combined Edman cleavage/mass spectrometric strategy for the identification of cyclic peptides and peptoids.[19,21]

Recently, Lim and co-workers reported a different strategy in which the macrocycle contained a group that could be cleaved selectively, thus linearizing the molecule, which could then be sequenced easily.[22,23] We describe here a different version of this strategy that incorporates a selectively-cleavable methionine residue into both the cyclic scaffold and the linker connecting the molecule to the bead. This allows for simultaneous site-specific linearization and release of the bead-bound molecule. The soluble species can then be sequenced by tandem mass spectrometry.

Results and discussion

'Dual methionine' libraries. The first strategy that we explored for the creation of macrocyclic peptoid libraries that can be sequenced by mass spectrometry is depicted in Figure 1. The design involves incorporation of a methionine amino acid unit into the macrocycle as well as the linker arm that tethers the molecule to the bead. When treated with cyanogen bromide, the anticipated cleavage at the C-terminal side of both methionines should provide a soluble, linear molecule that could be sequenced by tandem mass spectrometry.

The library was synthesized on 75 μm TentaGel beads, derivatized with an invariant linker comprised of Pro-Asp-Nffa-Met-Nlys-(3-aminopropanoic acid)-(2-phenyl isopropyl-protected Glu)-Nmea. The first three residues (Pro-Asp-Nffa) allow for mild acid cleavage of the molecules from the beads and subsequent spotting of the molecules onto maleimide-activated glass slides,[24] but this feature of the library is not relevant to the present study. The remaining residues provide a point of site-specific cleavage (Met), a positive charge to aid in ionization in the MALDI mass spectrometer (Nlys), two spacers (3-aminopropionic acid and Nmea), and a carboxylate side chain to support macrocyclization with N-terminal end of the molecule (differentially protected Glu). Following this invariant linker, split and pool synthesis using the standard 'sub-monomer' route to peptoids[25] was employed to construct a one-bead one-compound peptoid library[26,27] with a theoretical

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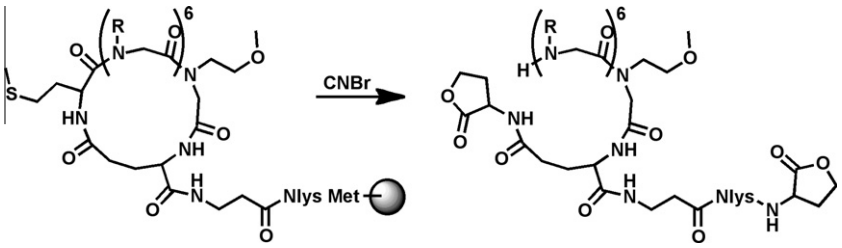

Figure 1. The dual methionine strategy for the creation of bead-displayed libraries of cyclic peptoids with a cleavable ring and linker.

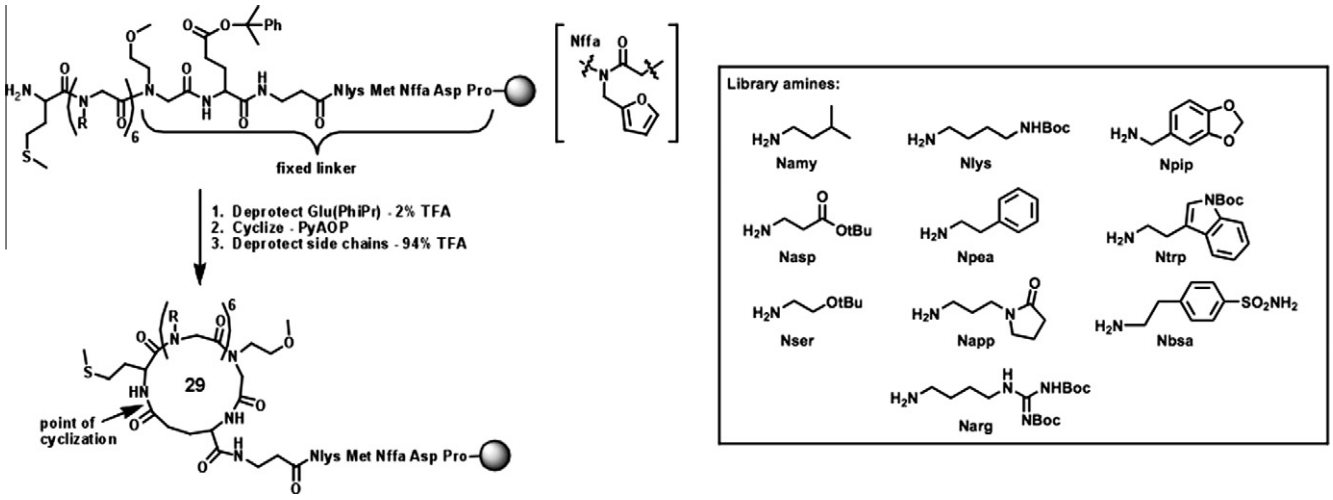

Figure 2. General structure and synthetic route to the combinatorial library of macrocyclic peptoids. The ten amines shown at the bottom of the figure were employed in the split and pool synthesis. The Boc and OtBu protecting groups shown on the amines were removed on resin after completion of the library.

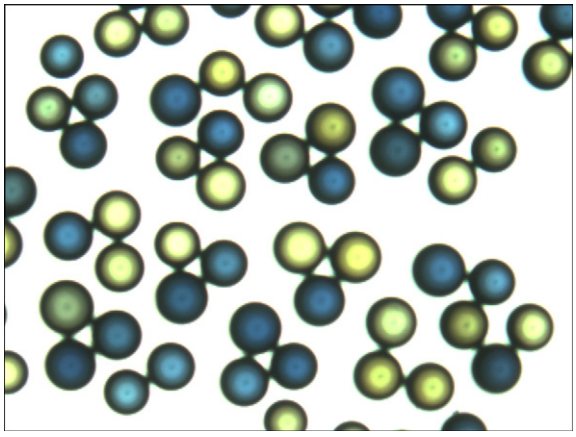

Figure 3. Photomicrograph of beads from the cyclic peptoid library after subjecting them to the ninhydrin test.

diversity of one million compounds by incorporating the ten amines shown in Figure 2. The library was completed by capping the peptoid chain with methionine, selectively deblocking the phenyl isopropyl group with 2% TFA and, finally, effecting cyclization with PyAOP.

A critical issue is to assess the cyclization efficiency for each molecule, since the chemical nature of the intervening residues can have significant effects on cyclization rates. To do so, we employed a ninhydrin test, which reveals unreacted terminal amines from uncyclized N-terminal methionine residues. To calibrate the assay, aliquots of beads removed from the library prior to the cyclization reaction were counted and analyzed. Kaiser test reagents (equal parts: 5% ninhydrin in ethanol, 80% phenol in ethanol, and 2% 0.001 M aqueous KCN in pyridine; 25 μl total volume) were added to each tube and the tubes were heated to 95 °C for 7 min. Aqueous ethanol (60% v/v, 225 μl) was added to each tube. The optical density of the resulting solutions was measured at 590 nm (see Table 1). This provided a benchmark for the intensity of color that would be obtained if no cyclization at all had occurred. The same protocol was then employed to analyze batches of beads that had been subjected to cyclization conditions. As can be seen from Table 1, the average value obtained in these experiments was approximately 30% of that observed from uncyclized beads, suggesting that the average overall cyclization efficiency was approximately 70%.

To assess the level of bead-to-bead variability of the yield, a portion of the beads from the cyclization reaction that had undergone the ninhydrin test were examined under a low power microscope.

Table 1
Results of the ninhydrin tests to determine cyclization efficiency

	# Beads	OD (590 nm)	Corrected OD	OD/ bead	Averages
Blank	0	0.0585			
Uncyclized	90	0.3539	0.2954	3.28	
	110	0.4072	0.3487	3.17	
	140	0.5263	0.4678	3.34	3.26
Cyclized	120	0.1845	0.1260	1.05	
	115	0.1603	0.1018	0.89	
	150	0.2103	0.1518	1.01	0.98
					30% Uncyclized