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Solid-Phase Synthesis of Diverse Peptide Tertiary Amides By Reductive Amination

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Abstract

The synthesis of libraries of conformationally-constrained peptide-like oligomers is an important goal in combinatorial chemistry. In this regard an attractive building block is the N-alkylated peptide, also known as peptide tertiary amide (PTA). PTAs are strongly biased conformationally due to allylic 1,3 strain interactions. We report here an improved synthesis of these species on solid supports through the use of reductive amination chemistry using amino acid-terminated, bead-displayed oligomers and diverse aldehydes. The utility of this chemistry is demonstrated by the synthesis of a library of 10,000 mixed peptoid-PTA oligomers.

Keywords

Peptoid; reductive amination; solid-phase synthesis; combinatorial chemistry; peptide

Introduction

One bead one compound (OBOC) libraries created via split and pool solid-phase synthesis¹ are a rich source of protein ligands. An important goal in this field is to create diverse libraries of conformationally constrained molecules that would likely bind to proteins with higher affinity than molecules lacking such constraints, such as linear peptides or peptoids². N-methyl peptides have long been known to be highly constrained molecules due to allylic 1,3 strain effects and often have better pharmacological properties than simple peptides.³ Therefore, we became interested in the creation of libraries of diverse N-alkylated peptides, also called peptide tertiary amides (PTAs). Some of these molecules can be accessed via Fukuyama-Mitsunobu reaction, though this process requires protection of most functional groups.⁴ We reported the synthesis of these molecules by extending a chain through acylation with a chiral 2-bromo acid followed by displacement of bromide with an amine (Fig. 1a).⁵ This chemistry mirrors the “sub-monomer” route developed by Zuckermann and co-workers for the synthesis of peptoids.⁶ Certain 2-bromocarboxylic acids can be

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Associated Content

Supporting Information. Detailed experimental protocols and data on compound characterization are provided. This material is available free of charge via the Internet at (placeholder).

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synthesized from amino acids in high enantiomeric purity but this conversion requires harsh acidic conditions that limits its scope with respect to amino acid starting materials.⁷

An alternative to the use of chiral bromides and amines to create PTAs would be to build them from amino acids and aldehydes using reductive amination (Figure 1b). Appella and co-workers have employed reductive amination successfully on TentaGel beads in the synthesis of combinatorial libraries of N-acylated polyamines (NAPAs),⁸ suggesting that this route to PTAs might proceed efficiently enough to support the creation of high quality PTA-containing OBOC libraries. Indeed, we report here that this is the case. Diverse, enantiomerically pure PTA units are accessible through solid-phase reductive amination reactions using many different amino acids and aldehydes without the need for protecting groups.

Results and Discussion

Twelve Fmoc-protected amino acids that are poor substrates for the synthesis of the corresponding chiral bromide⁵ were coupled onto a pre-synthesized linker on acid-cleavable polystyrene-RAM resin. After deprotection, each set of beads was incubated with benzaldehyde for an hour at room temperature, followed by reduction with sodium borohydride (Fig. 2). The compounds were then cleaved from the resin and analyzed by HPLC. Eleven of the molecules were monoalkylated efficiently to provide **1a-k** in high purity. This group included phenylglycine (Phg) and a *t*-butyl sidechain-protected threonine substrate, showing that even molecules with bulky groups near the chiral center are good substrates. Tertiary amine products were not observed, showing that resin-bound secondary amines were not substrates under these conditions. Consistent with this observation, the reductive amination of proline to provide **1l** failed. Only starting material was observed.

To determine if any racemization occurs during this process, the four diastereomers of CH₃CH₂NH-Phg-Ala were synthesized on polystyrene-RAM resin using reductive amination with acetaldehyde and NaBH₄ to add the ethyl group to the nitrogen of Phg. The pK_a of the α proton of Phg is 14.9⁹ and racemization of this residues is often encountered in peptide synthesis.¹⁰ The peptides were cleaved from the resin and analyzed by LC-MS without purification. The chromatograms (Fig. 3a) showed that the two *trans* and two *cis* stereoisomers separated well on the column, thus making it straightforward to observe the formation of the undesired diastereomer in all of the four chromatograms. There was no evidence of racemization.

The crude ¹H NMR spectra of the L,D and L,L products were also consistent with a stereochemically clean product. The spectrum of the L,L diastereomer preparation showed splitting of the C-terminal amide and N-ethyl methylene protons, suggesting a defined conformation, that were averaged in the L,D spectrum. Furthermore, there are multiple chemical shift differences that distinguish the diastereomers (Fig. 3b). Neither spectrum displayed detectable peaks indicative of formation of the undesired diastereomer through racemization of the Phg chiral center during reductive amination.

The scope of the reaction with respect to aldehyde substrate was surveyed by performing reductive amination on resin-bound molecules presenting a side chain-protected serine at the N-terminus (Figure 4). Aliphatic aldehydes provided products **2a-e** nearly quantitatively, but results varied for aryl and heterocyclic aldehydes. The 1-Trityl-1H-imidazole, thiazole, and furan aldehydes gave products **2f-h** in high purity, but the more hindered 2,4-dichloro-5-formylthiazole did not provide any of the desired product **2i**. Conversely, methyl-substituted heterocyclic aldehydes provided **2j** and **2k** in high and modest purity, respectively. Various substituted benzaldehydes were successfully employed in the synthesis of **2l-p**. 2-Pyrimidinecarboxaldehyde provided none of the desired product **2q**; pyrrole and pyridine aldehydes reacted poorly as well (not shown). Given the lack of success with pyridine and pyrimidine, it was surprising that **2r-t** were accessible from quinoline, isoquinoline, and chromene aldehydes. Taken together the data show that a diverse set of PTAs can be constructed via this chemistry, but that it is important to test the reactivity of any new aldehyde empirically.

To test the utility of this reaction in the creation of large collections of PTA-containing molecules, the construction of the library shown in Fig. 5 was undertaken by solid-phase split and pool synthesis. Diversity at R₃ derives from reductive amination, while nucleophilic displacement with primary amines provided diversity at R₄. R₁ diversity comes from both. The reductive amination on the N-terminus to provide R₅ diversity was performed under acidic conditions as reported previously^{9,10}, which allows for efficient reductive amination of secondary amines. Essentially, tertiary amines are generated via the *in situ* reductive amination at a lower pH (1% AcOH) and a milder reducing agent, sodium cyanoborohydride. Four amino acids used at the interior position provided side chain diversity for the central PTA element, two of which have polar side chains that were not accessible via the PTA haloacid submonomer method. The library was designed carefully, such that all fragments have unique masses, allowing unequivocal identification from the MS/MS spectra. The total diversity of this library is $10 \times 4 \times 5 \times 10 \times 5 = 10,000$ compounds.

After synthesis and deprotection, sixty beads were selected and compounds were released from the resin with cyanogen bromide, which cleaves selectively at Met, in individual wells of a 96-well plate. The cleaved material from single beads was analyzed by MALDI-TOF mass spectrometry. The parental M+H ion detected from the initial experiment was then was subjected to tandem MS/MS to generate sequence-specific fragments for sequencing. The MS/MS of two representative compounds with their inlaid MS spectra are shown (Figure 6).¹¹ From the sixty crude samples, fifty-eight gave M+H peaks in the mass range of the library members, and fifty-three of these were sequenced unequivocally from the MS/MS of the parental ion. The ability to unambiguously assign structures to 91% of the observed parent ions reflects the effectiveness of the methods established in this work, and proves its suitability for use in the synthesis of high quality OBOC combinatorial libraries.

In summary, the reductive amination method has been optimized for selective monoalkylation of primary amine substrates on the solid phase, providing PTA units that were inaccessible using our previously reported method. The reaction is general for all

primary amine substrates tested, does not lead to racemization of adjacent chiral centers, and utilizes a variety of aliphatic, aromatic, and heterocyclic aldehyde reagents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Submonomer syntheses of peptoid and peptide tertiary amide (PTA) on the solid phase. (a) Haloacid submonomer route (b) Reductive amination route (this work) CR = coupling reagent.

Figure 2.

Purities of the reductive amination products of amino acid-terminated oligomers with benzaldehyde and sodium borohydride. Purity was determined from the HPLCs of crude cleaved material. ^aPbf protected sidechain. ^b*t*-Butyl protected sidechain. ^cBoc-protected sidechain. (i) Fmoc-AA-OH, oxyma, DIC (4 eq. each) DMF, rt 40 m (ii) 20% piperidine DMF 10 m 2× (iii) 1M benzaldehyde DMF, rt 1 h (iv) xs NaBH₄ DCM/MeOH, rt 30 m (v) 95/2.5/2.5 TFA/H₂O/TIPS, rt 2.5 h.

Figure 3.

No racemization is observed at the α -chiral center. a) LCMS overlay of four diastereomeric preparations of dipeptide Et-Phg-Ala-NH₂ show *cis* (D,L and L,D) and *trans* (D,D and L,L) stereoisomers elute differentially. b) Crude NMR of two diastereomers (L,L in black, L,D in grey).

Figure 4.

Aldehyde substrate scope for reductive amination of resin-bound primary amines. Purity determined by LCMS trace of crude cleaved material. ^aBoc protected sidechain. ^bTrt protected sidechain. ^ct-Butyl protected sidechain (i) 1M RCHO (10-15 eq.) DMF, rt 1 h (ii) xs NaBH₄ DCM/MeOH, rt 30 m (iii) 95/2.5/2.5 TFA/H₂O/TIPS, rt 2.5 h.

Figure 5.

Peptoid-PTA-peptoid(tertiary amine) library synthesized by combining haloacid submonomer synthesis with reductive amination.

Figure 6.

Sequencing of library compounds from MS/MS data obtained from crude material from single beads. MS experiment for identification of parental M+H ion is inlaid in the top left corner of each spectrum.