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Letter

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

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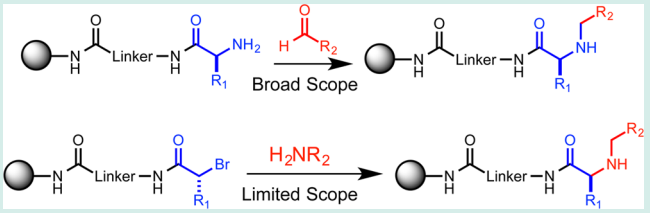
Supporting Information

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 a peptide tertiary amide (PTA). PTAs are conformationally constrained because of 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*

One bead one compound (OBOC) libraries created via split and pool solid-phase synthesis[1] 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 peptoids.[2] *N*-Methyl peptides have long been known to be highly constrained molecules because of 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 (Figure 1a).[5] This chemistry mirrors the submonomer route developed by Zuckermann and co-workers for the synthesis of peptoids.[6]

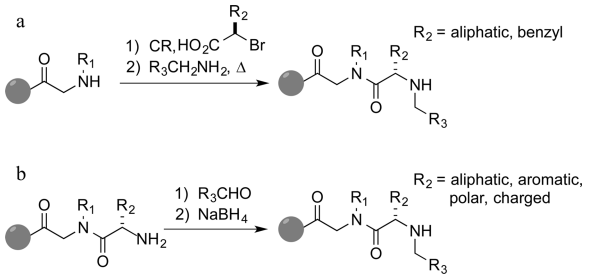


Figure 1. Submonomer syntheses of peptoid and peptide tertiary amide (PTA) on the solid phase. (a) Haloacid submonomer route and (b) reductive amination route (this work). CR = coupling reagent.

Certain 2-bromocarboxylic acids can be 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.

Twelve Fmoc-protected amino acids that are poor substrates for the synthesis of the corresponding chiral bromide[5] were coupled onto a presynthesized 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 (Figure 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 side chain-protected threonine substrate, showing that even molecules with bulky groups near the chiral center are good substrates. Tertiary amine products were not observed, showing

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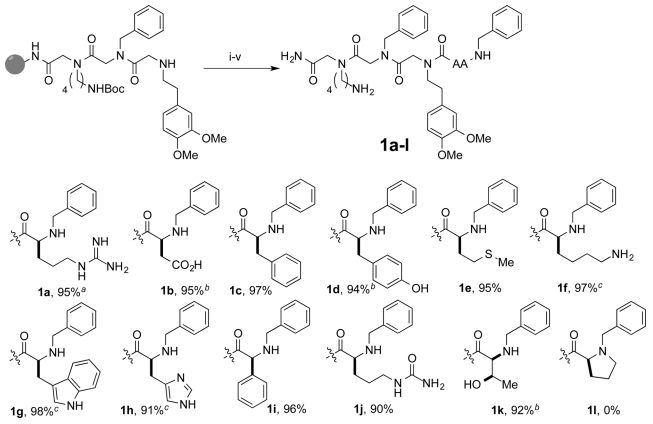

Figure 2. Purities of the reductive amination products of amino acid-terminated oligomers with benzaldehyde and sodium borohydride. Purity was determined from the HPLCs of crude cleaved material. [a]Pbf protected side chain, [b]*t*-butyl protected side chain, and [c]Boc-protected side chain. (i) Fmoc-AA-OH, oxyma, DIC (4 equiv each) DMF, rt 40 min; (ii) 20% piperidine DMF 10 min 2×; (iii) 1 M benzaldehyde DMF, rt 1 h; (iv) excess $NaBH_4$ DCM/MeOH, rt 30 min; (v) 95/2.5/2.5 TFA/H_2O/TIPS, rt 2.5 h.

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 diasteromers of CH_3CH_2NH-Phg-Ala-NH_2 were synthesized on polystyrene-RAM resin using reductive amination with acetaldehyde and $NaBH_4$ to add the ethyl group to the nitrogen of Phg. The pK_a of the α proton of Phg is 14.9[9] and racemization of this residue is often encountered in peptide synthesis.[10] The peptides were cleaved from the resin and analyzed by LC-MS without purification. The chromatograms (Figure 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 ^{1}H 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 (Figure 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-1*H*-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**;

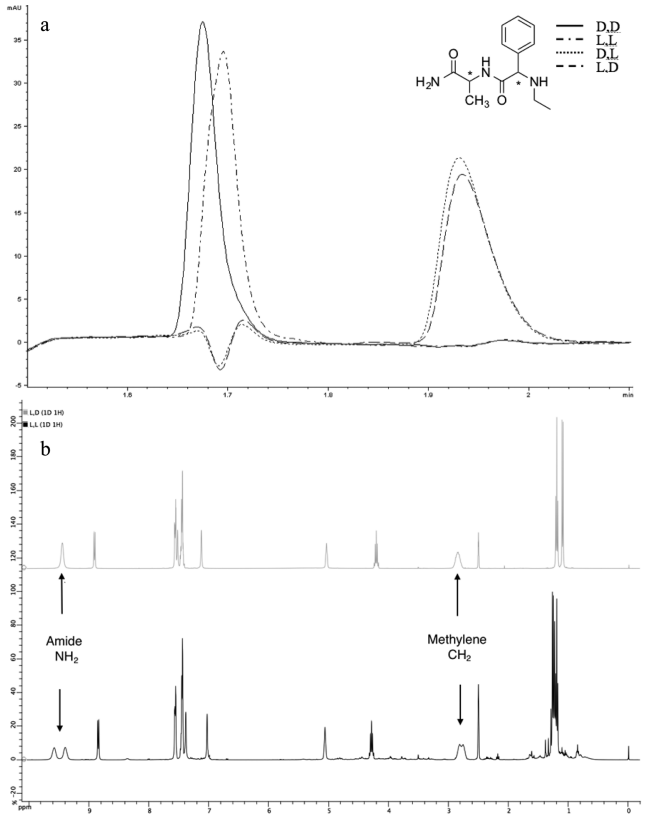

Figure 3. No racemization is observed at the α-chiral center. (a) LCMS overlay of four diastereomeric preparations of dipeptide CH_3CH_2NH-Phg-Ala-NH_2 show *cis* (D,L and L,D) and *trans* (D,D and L,L) stereoisomers elute differentially. (b) Crude NMR of two diastereomers (L,L in black, L,D in gray).

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, isoqunioline, 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 Figure 5 was undertaken by solid-phase split and pool synthesis. Diversity at R_3 derives from reductive amination, while nucleophilic displacement with primary amines provided diversity at R_4. R_1 diversity comes from both. The reductive amination on the N-terminus to provide R_5 diversity was performed under acidic conditions as reported previously,[9,10] which allows for efficient reductive amination of secondary amines. Tertiary amines can be 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 × 4 × 5 × 10 × 5 = 10 000 compounds. Note that because of the difficulty of acylating the nitrogen of an alkylated amino acid at the N-terminus of the chain, the following residue in the library is a peptoid, allowing

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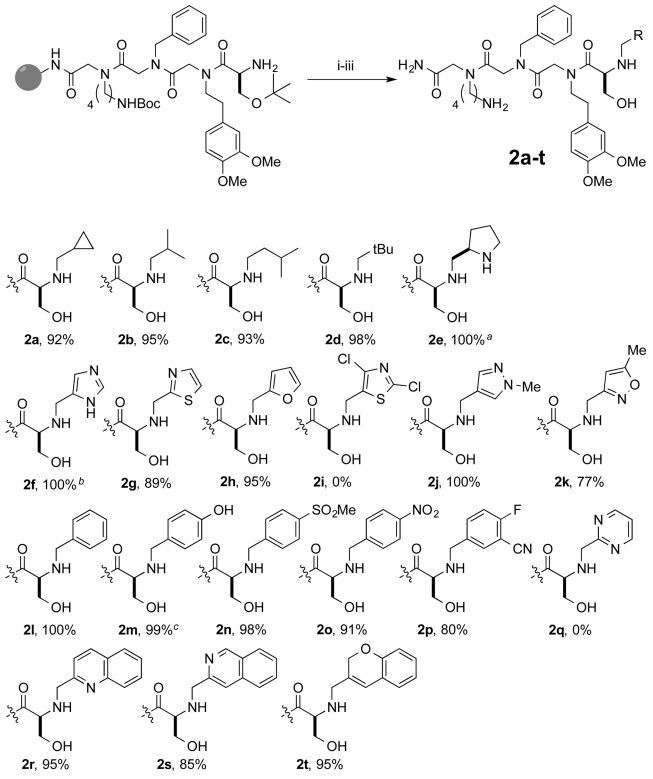

Figure 4. Aldehyde substrate scope for reductive amination of resin-bound primary amines. Purity determined by LCMS trace of crude cleaved material. [a]Boc protected side chain, [b]Trt protected side chain, and [c]*t*-butyl protected side chain. (i) 1 M RCHO (10−15 equiv) DMF, rt 1 h; (ii) excess $NaBH_4$ DCM/MeOH, rt 30 min; (iii) 95/2.5/2.5 TFA/H_2O/TIPS, rt 2.5 h.

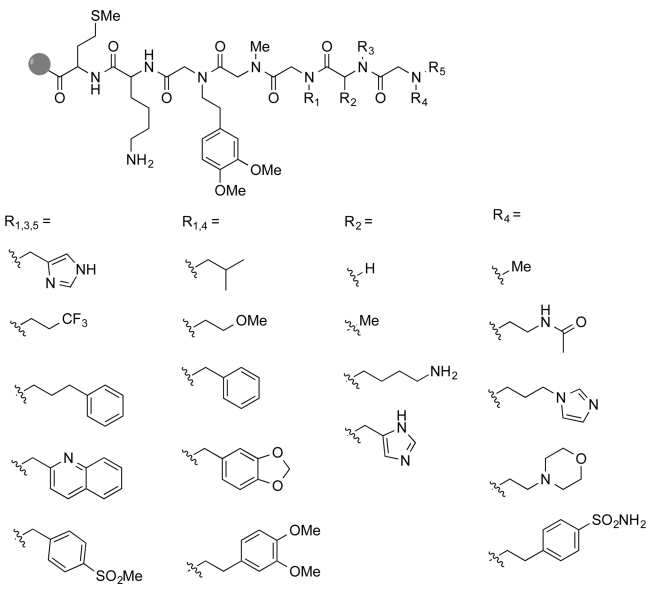

Figure 5. Peptoid−PTA−peptoid (tertiary amine) library synthesized by combining haloacid submonomer synthesis with reductive amination.

for use of the sterically undemanding and highly reactive chloroacetyl chloride.

After synthesis and deprotection, 60 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 subjected to tandem MS/MS to generate fragments for sequencing. The MS/MS of two representative compounds with their inlaid MS spectra are shown (Figure 6).[11] From the

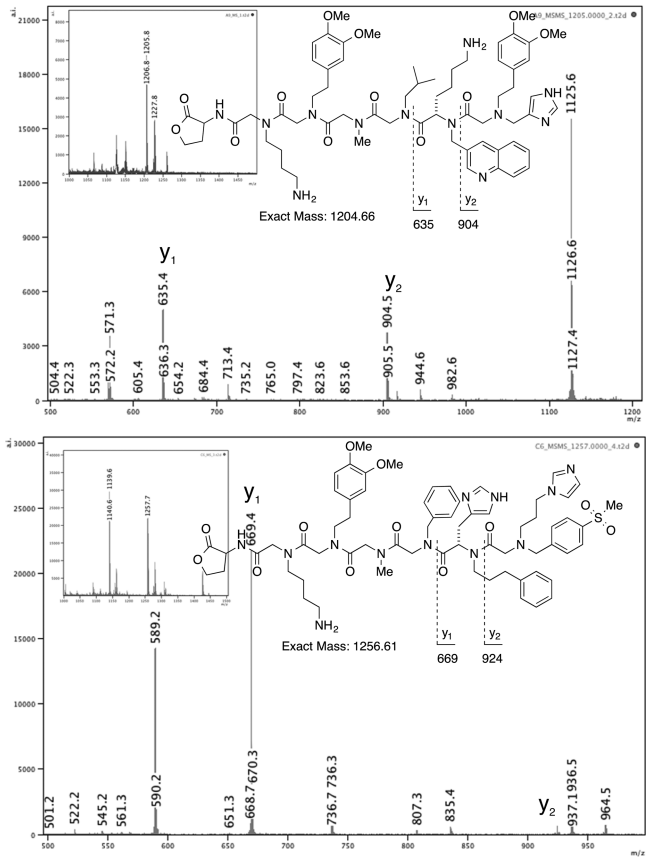

Figure 6. Sequencing of library compounds from MS/MS data obtained from crude material from single beads. MS experiment for identification of parental M+H ion is inlaid in the top left corner of each spectrum.

60 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.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental protocols and data on compound characterization. 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) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* **1991**, *354*, 82−84. (b) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. *Nature* **1991**, *354*, 84−86.

(2) (a) Figliozzi, G. M.; Goldsmith, R.; Ng, S. C.; Banville, S. C.; Zuckermann, R. N. Synthesis of N-substituted glycine peptoid libraries. *Methods Enzymol.* **1996**, *267*, 437−447. (b) Alluri, P. G.; Reddy, M. M.; Bachhawat-Sikder, K.; Olivos, H. J.; Kodadek, T. Isolation of protein ligands from large peptoid libraries. *J. Am. Chem. Soc.* **2003**, *125*, 13995−14004.

(3) (a) Doedens, L.; Opperer, F.; Cai, M.; Beck, J. G.; Dedek, M.; Palmer, E.; Hruby, V. J.; Kessler, H. Multiple N-methylation of MT-II backbone amide bonds leads to melanocortin receptor subtype hMC1R selectivity: pharmacological and conformational studies. *J. Am. Chem. Soc.* **2010**, *132*, 8115−8128. (b) Ovadia, O.; Greenberg, S.; Chatterjee, J.; Laufer, B.; Opperer, F.; Kessler, H.; Gilon, C.; Hoffman, A. The effect of multiple N-methylation on intestinal permeability of cyclic hexapeptides. *Mol. Pharmaceutics* **2011**, *8*, 479−487. (c) Kessler, H.; Chatterjee, J.; Doedens, L.; Operrer, F.; Biron, E.; Hoyer, D.; Schmid, H.; Gilon, C.; Hruby, V. J.; Mierkes, D. F. New perspective in peptide chemistry by N-alkylation. *Adv. Exp. Med. Biol.* **2009**, *611*, 229−231. (d) Rezai, T.; Yu, B.; Millhauser, G. L.; Jacobson, M. P.; Lokey, R. S. Testing the conformational hypothesis of passive membrane permeability using synthetic cyclic peptide diastereomers. *J. Am. Chem. Soc.* **2006**, *128*, 2510−2511. (e) Zhang, S.; Prabpai, S.; Kongsaeree, P.; Arvidsson, P. I. Poly-N-methylated alpha-peptides: Synthesis and X-ray structure determination of beta-strand forming foldamers. *Chem. Commun.* **2006**, *5*, 497−499.

(4) Demmer, O.; Dijkgraaf, I.; Schottelius, M.; Wester, H. J.; Kessler, H. Introduction of functional groups into peptides via N-alkylation. *Org. Lett.* **2008**, *10*, 2015−2018.

(5) Gao, Y.; Kodadek, T. Synthesis and screening of stereochemically diverse combinatorial libraries of peptide tertiary amides. *Chem. Biol.* **2013**, *20*, 360−369.

(6) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient method for the preparation of peptoids (oligo(N-substituted glycines)) by sub-monomer solid-phase synthesis. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.

(7) (a) Izumiya, N.; Nagamatsu, A. Walden invesrion of amino acids. VI. The synthesis of D-surinamine (*N*-methyl-D-tyrosine). *Bull. Chem. Soc. Jpn.* **1952**, *25*, 265−267. (b) Tanasova, M. T.; Yang, Q.; Olmsted, C. C.; Vasileiou, C.; Li, X.; Anyika, M.; Borhan, B. An unusual conformation of alpha-haloamides due to cooperative binding with zincated porphyrins. *Eur. J. Org. Chem.* **2009**, *25*, 4242−4253.

(8) (a) Hayashi, R.; Wang, D.; Hara, T.; Iera, J. A.; Durell, S. R.; Appella, D. H. *N*-Acylpolyamine inhibitors of HDM2 and HDMX binding to p53. *Bioorg. Med. Chem.* **2009**, *17*, 7884−7893. (b) Iera, J. A.; Jenkins, L. M.; Kajiyama, H.; Kopp, J. B.; Appella, D. H. Solid-phase synthesis and screening of N-acylated polyamine (NAPA) combinatorial libraries for protein binding. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6500−6503.

(9) Stroud, E. D.; Fife, D. J.; Smith, G. G. A method for the determination of the pKa of the alpha hydrogen in amino acids using racemization and exchange studies. *J. Org. Chem.* **1983**, *48*, 5368−5369.

(10) Elsawy, M. A.; Hewage, C.; Walker, B. Racemisation of N-Fmoc phenylglycine under mild microwave-SPPS and conventional stepwise SPPS conditions: Attempts to develop strategies for overcoming this. *J. Pept. Sci.* **2012**, *18*, 302−11.

(11) Vaisar, T.; Urban, J. Gas phase fragmentation of protonated mono-N-methylated peptides. Analogy with solution phase acid-catalyzed hydrolysis. *J. Mass Spectrom.* **1998**, *33*, 505−524.

DOI: 10.1021/acscombsci.5b00007
ACS Comb. Sci. 2015, 17, 152−155