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Synthesis of Trifunctional Thiazolyl Amino Acids And Their Use for the Solid-Phase Synthesis of Small Molecule Compounds and Cyclic Peptidomimetics

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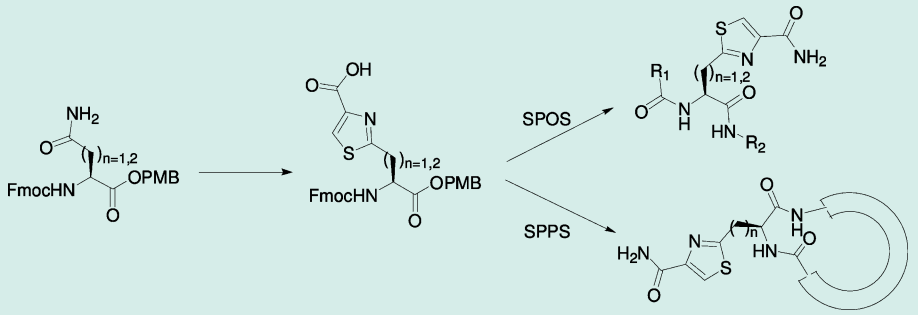
Supporting Information



ABSTRACT: Chiral thiazolyl amino acid building blocks for the solid-phase synthesis of small molecules, peptides, and cyclic peptides have been designed and synthesized starting from Fmoc protected asparagine and glutamine. In efforts to demonstrate the usefulness and validity of such building blocks, a small library of 16 new thiazole containing small molecules has been prepared and characterized. Additionally, we report the use of the newly prepared trifunctional thiazolyl glutamine for the on-resin, head-to-tail synthesis of cyclic peptides.

KEYWORDS: *unnatural amino acid, thiazole, solid-phase synthesis, peptide, heterocycle, pharmacophore*

Unnatural amino acids, particularly synthetic α-amino acids, have played a significant role in the area of peptide research and drug discovery.[1−4] They have been used extensively in peptide analogs to limit conformational flexibility, enhance enzymatic stability, and improve pharmacodynamics and bioavailability. Because of their structural diversity and functional versatility, they are widely used as chiral building blocks and molecular scaffolds in constructing combinatorial libraries.[5−7] Many of these unnatural amino acids are also critical components in pharmaceuticals and developmental drugs.

Additionally, the thiazole ring is present in many natural and synthetic products[8] with a wide range of pharmacological activities that can be well illustrated by the large number of drugs on the market containing this functional group. Small molecules containing a thiazole moiety have been demonstrated to possess drug like properties against varieties of diseases[9] resulting in, so far, 17 FDA-approved drugs containing the thiazole ring. The usefulness of thiazoles is evident by the wide scope of disease to which thiazole derivatives are prescribed. These include asthma (cinalukast), bacterial infections (ceftizoxime), diarrhea (nitazoxanide) myelogenous leukemia (dasatinib), pain (meloxicam), duodenal ulcers (famotidine), anthelmintics (thiabendazole), CNS disorders (riluzole), and as vitamin supplements (thiamine).[10] Herein, we report the side chain manipulation of asparagine and glutamine for the synthesis of thiazolyl amino acids and their use as building blocks for the parallel synthesis of small molecule libraries and for the on resin head-to-tail synthesis of cyclic peptides. Such building blocks are also useful for the synthesis of branched linear peptides.[11]

Our approach toward the synthesis of orthogonally protected trifunctional thiazolyl amino acids is outlined in Scheme 1. The thioamide (**1**) of both asparagine and glutamine was prepared by literature procedures.[12] Starting from Fmoc α-amino protected glutamine or asparagine, the carboxylic acid was orthogonally protected with 4-methoxybenzyl (PMB) to afford intermediate **1**. Upon sonication of **1** in the presence of phosphorus pentasulfide, the amide was selectively converted into the corresponding thioamide **2** with no evidence of the protected C-terminus being affected. The thioamidation was also successfully achieved using Lawesson's Reagent.[13,14] The generated thioamide was treated in refluxing conditions with a small excess of bromopyruvic acid to afford, following Hantzsch cyclocondensation, the desired enantiomerically pure thiazolyl amino acids in good yields. The orthogonal deprotection of both the amine and the acid allows for 2 sites of diversification, which allows for the parallel synthesis of diverse thiazole based small molecule compounds.

A simple case study illuminating the use of the Asn scaffold to prepare thiazole based small molecules was carried out on solid

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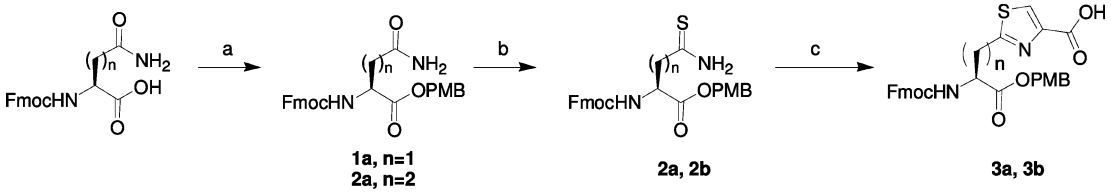
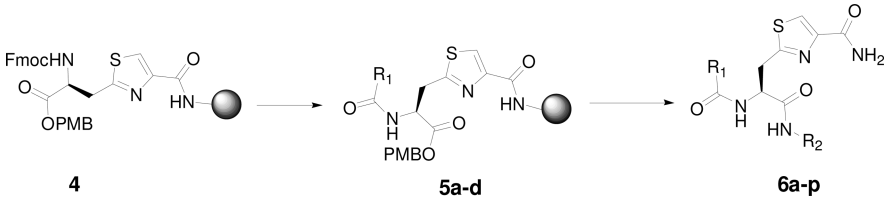
Scheme 1. Solution-Phase Organic Synthesis[a] of the Asparagine and Glutamine Thiazolyl Building Blocks

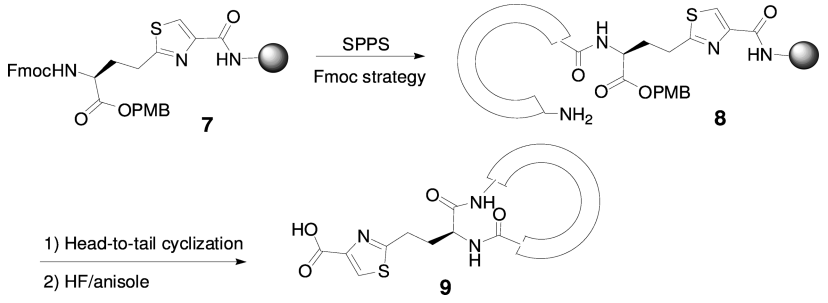


[a]Conditions: (a) NaI (1.5 equiv), 4-methoxybenzylchloride (1.2 equiv), DIEA (2 equiv), DMA. RT, 18 h. (b) P_4S_{10} (0.5 equiv), sonication, 2−4 h. (c) Bromopyruvic acid (1.1 equiv), THF, reflux, 18 h.

Scheme 2. Solid-Phase Organic Synthesis (SPOS) of Peptidomimetic Small Molecules Utilizing the Asparagine Scaffold



Scheme 3. Solid-Phase Peptide Synthesis (SPPS) of Hexa- and Pentacyclic Peptides Utilizing the Glutamine Scaffold



phase utilizing the tea-bag technique[15] and following the strategy outlined in Scheme 2. The coupling of thiazole **3a** (2 equiv) to *p*-methyl-benzhydrylamine (*p*MBHA) resin was carried out in the presence of 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (2 equiv) and diisopropylethylamine (DIEA) (2 equiv). Next, the resin-bound Fmoc-protected amino thiazole **4** was treated with piperidine (20% in DMF) to remove the Fmoc protecting group. The resulting amine was then acylated with different carboxylic acids. A second diversity was included following removal of the PMB group with TFA. The resulting acid was activated with acetic anhydride and then treated with different amines. The desired compounds **6** were obtained following the cleavage of the solid support with HF. Using 4 different carboxylic acids (adamantly acetic acid, cyclohexane carboxylic acid, propionic acid, and 3,5-bis-trifluoromethyl-phenyl acetic acid) and 4 different amines (morpholine, piperidine, benzylamine, and 1-naphthylmethyl-amine), we performed the parallel solid-phase synthesis of 16 different substituted thiazoles. All the compounds were obtained in good yield and high purity. This is an example of the utility of the proposed building blocks for the combinatorial synthesis of pharmacologically important small molecules. The identities of all compounds were confirmed by LC-MS, ^{1}H NMR, and ^{13}C NMR.

In addition, we used the orthogonally protected trifunctional thiazole derived from glutamine for the parallel head-to-tail synthesis of cyclic peptides. Conformational constraint by cyclization is a common approach used to restrict the flexibility of peptides, serving as a valuable approach to study topographical requirements of receptors.[16,17] Cyclization of peptides can also provide potent and selective ligands for receptors when appropriate conformational constraints are incorporated. Furthermore, cyclic peptides and peptidomimetics are often more stable to peptidases, potentially possessing improved pharmacokinetic profiles translating into promising lead compounds for further development.[18,19]

Our approach toward the use of the newly synthesized trifunctional thiazolyl amino acid for the synthesis of cyclic peptides is oulined in scheme 3. Following the coupling of thiazole **3b** (2 equiv) to *p*-methylbenzhydrylamine (*p*MBHA) resin in the presence of HBTU (2 equiv) and diisopropylethylamine (2 equiv), the Fmoc group was deprotected and the linear tetra- or pentapeptides were synthesized, following stepwise Fmoc deprotection and standard repetitive Fmoc-amino-acid couplings.[20] Upon the final Fmoc deprotection of the terminal amino acid of the N-terminus and subsequent deprotection of the PMB ester of the C-terminus of glutamine, the free amine and the acid were cyclized on solid phase in the presence of 3 equiv of HBTU, DIEA, and anhydrous DMF (Table 2). The cyclic peptides were obtained as the major product in good yields. The cyclic peptides were purified by HPLC and confirmed by LC-MS and NMR analysis. In all cases, the intramolecular lactamization reaction led to the desired cyclic monomers with negligible traces of dimerization.

In conclusion, we have successfully manipulated the side chain of asparagine and glutamine through solution-phase organic

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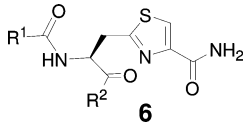
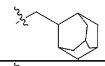
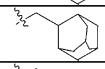
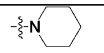
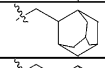
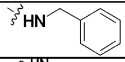
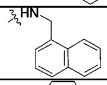
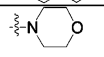
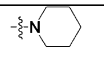
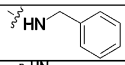
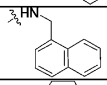
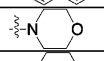
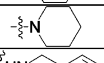
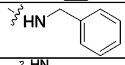
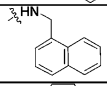
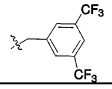
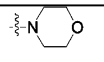
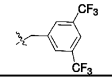
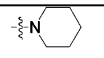
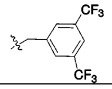
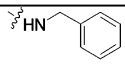
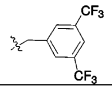
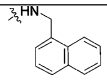
Table 1. Synthesis of Small Molecule Asparagine Derivatives from Solid-Phase Organic Synthesis



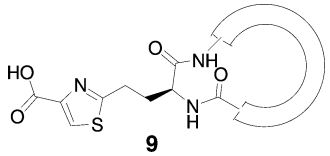
6

	R^1	R^2	Yield (mg)[a]	Purity[b]
6a			24	90 %
6b			18	70 %
6c			25	90 %
6d			24	80 %
6e			19	85 %
6f			16	70 %
6g			14	60 %
6h			16	60 %
6i	-CH_2CH_3		16	80 %
6j	-CH_2CH_3		14	70 %
6k	-CH_2CH_3		15	70 %
6l	-CH_2CH_3		17	70 %
6m			25	80 %
6n			14	45 %
6o			27	85 %
6p			21	60 %

[a]Based on crude yield. [b]Crude samples were analyzed on a Vydac column with a gradient of 5−95% formic acid in MeCN over 7 min. The purity was based on the analytical traces at λ = 214 and 254 nm.

chemistry for the synthesis of trifunctional orthogonally protected thiazolyl amino acids. The new amino acids were used as starting materials for the combinatorial libraries of thiazole-based small molecule compounds and for the head-to-tail synthesis of diverse cyclic peptides. The general nature of this approach permits large numbers of small molecules and cyclic peptides to be prepared. The chemistry described herein will be used to generate large libraries of thaizole based small molecules and cyclic peptides. The synthesis of the libraries and results from their screening for the identification of biologically active compounds will be reported elsewhere.

Table 2. Synthesis of Cyclic Peptides from the Glutamine Building Block via Solid-Phase Peptide Synthesis



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	AA[1c]	AA[2]	AA[3]	AA[4]	AA[5]	yield (mg)[a]	purity (%)[b]
9a	Phe	Leu	Pro	Ala		28	80
9b	Phe	Leu	Gly	Ala		38	80
9c	Val	Leu	Gly	Ala		22	70
9d	Phe	Leu	Pro	Ala	Phe	41	90
9e	Phe	Tyr	Gly	Ala		26	70
9f	Phe	Tyr	Gly	Ala	Phe	32	70

[a]On the basis of the crude yield. [b]The crude products were analyzed on a Vydac column with a gradient of 5−95% formic acid in MeCN over 7 min. The purity was estimated based on the analytical traces at λ = 214 and 254 nm. [c]AA^1 denotes the amino acid directly coupled to the N-terminus of the functionalized glutamine.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures used herein, as well as full ^1H and ^{13}C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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■ REFERENCES

(1) Sureshbabu, V.; Venkataramanarao, R.; Naik, S. A.; Chennakrishnareddy, G. Synthesis of tetrazole analogues of amino acids using Fmoc chemistry: Isolation of amino free tetrazoles and their incorporation into peptides. *Tetrahedron Lett.* **2007**, *48*, 7038−7041.

(2) de Graaf, A. J.; Kooijman, M.; Hennink, W. E.; Mastrobattista, E. Nonnatural amino acids for site-specific protein conjugation. *Bioconj. Chem.* **2009**, *20*, 1281−1295.

(3) Hicks, R. P.; Abercrombie, J. J.; Wong, R. K.; Leung, K. P. Antimicrobial peptides containing unnatural amino acid exhibit potent bactericidal activity against ESKAPE pathogens. *Bioorg. Med. Chem.* **2013**, *21*, 205−214.

(4) Daya, J. W.; Kima, C. H.; Smiderb, V. V.; Schultz, P. G. Identification of metal ion binding peptides containing unnatural amino acids by phage display. *J. Bioorg. Med. Chem. Lett.* **2013**, *23*, 2598−2600.

(5) Vagner, J.; Qu, H.; Hruby, V. J. Peptidomimetics, A synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292−296.

(6) Dolle, R. E.; Michaut, M.; Martinez-Teipel, B.; Belanger, S.; Graczyk, T. M.; DeHaven, R. N. Further studies of tyrosine surrogates in opiod receptor peptide ligands. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2656−2660.

(7) Ryder, T. R.; Hu, L.-Y.; Rafferty, M. F.; Lotarski, S. M.; Rock, D. M.; Stoehr, S. J.; Taylor, C. P.; Weber, M. L.; Miljanich, G. P.; Millerman, E.; Szoke, B. G. Synthesis and biological activity of 4-aminopiperidine derivatives as N-type calcium channel antagonists. *Med. Chem. Res.* **2000**, *10*, 11−18.
(8) Nefzi, A.; Arutyunyan, S.; Fenwick, J. E. Two-step Hantzsch-based macrocyclization approach for the synthesis of thiazole-containing cyclopeptides. *J. Org. Chem.* **2010**, *75*, 7939−7941.
(9) Siddiqui, N.; Arya, S. K.; Ahsan, W.; Azad, B. Diverse biological activities of thiazoles: A retrospect. *Int. J. Drug Dev. Res* **2011**, *3*, 55−61.
(10) Wishart, D. S.; Knox, C.; Guo, A. C.; Cheng, D.; Shrivastava, S.; Tzur, D.; Gautam, B.; Hassanali, M. DrugBank: A knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res.* **2008**, No. D901-6.
(11) Branched linear peptides derived from the Asn thiazolyl building block have been synthesized successfully in our lab, however the data will not be included herein.
(12) Sanderson, J. M.; Singh, P.; Fishwick, C. W. G.; Findlay, J. B. C. The synthesis and reactivity of optically pure amino acids bearing side-chain thioamides. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3227−3231.
(13) Brillon, D. In situ reagents for the thionation of amides, peptides and lactams. *Synth. Commun.* **1990**, *20*, 3085−3095.
(14) Pattenden, G.; Thompson, T. Design and synthesis of novel tubular and cage structures based on thiazole-containing macrolactams related to marine cyclopeptides. *Chem. Commun.* **2001**, 717−718.
(15) Houghten, R. A. General method for the rapid solid-phase synthesis of large numbers of peptides—Specificity of antigen−antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131.
(16) Kopple, K. D. Synthesis of cyclic peptides. *J. Pharm. Sci.* **1972**, *61*, 1345−1356.
(17) Gracia, S. R.; Gaus, K.; Sewald, N. Synthesis of chemically modified bioactive peptides: recent advances, challenges and developments for medicinal chemistry. *Future Med. Chem.* **2009**, *1*, 1289−1310.
(18) Li, P.; Roller, P. P.; Xu, J. Current synthetic approaches to peptide and peptidomimetic cyclization. *Curr. Org. Chem.* **2002**, *6*, 411−440.
(19) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. The synthesis of cyclic peptides. *J. Chem.Soc., Perkin Trans. 1* **2001**, 471−484.
(20) Fields, G. B.; Noble, R. L. Solid-phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino-acids. *Int. J. Peptide Protein Res.* **1990**, *35*, 161−214.