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A Versatile Scaffold for Site-Specific Modification of Cyclic Tetrapeptides

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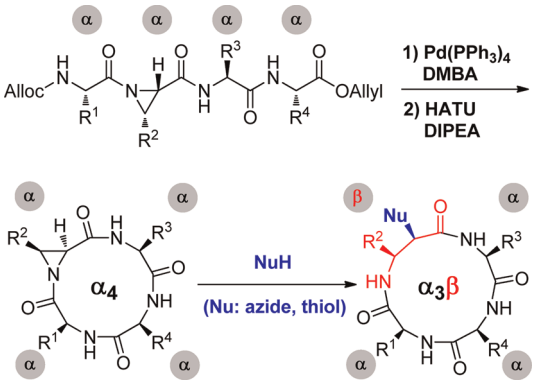
ABSTRACT



A novel scaffold that can be used to prepare conformationally homogeneous cyclic tetrapeptides equipped with a β-amino acid residue is disclosed. It is shown that regioselective structural modification can be accomplished using thiols and azide nucleophiles, commonly associated with rich downstream chemistry. The method should find application in efforts to constrain privileged tripeptide sequences in rigid molecular scaffolds.

Cyclic tetrapeptides belong to a privileged class of structures due to their capacity to mimic reverse turns.[1] Reverse turns are located at the surfaces of globular proteins and are common participants in a range of macromolecular recognition events, especially those mediated by G-protein coupled receptors (GPCRs).[2] In fact, it is estimated that over 100 peptide-activated GPCRs recognize ligands with reverse turn structure.[3] It is also well established that the minimal peptide recognition sequence depends on the number of effective molecular interactions that is required between a ligand and a protein receptor. Recent studies have ascribed optimal "ligand affinity" to a three amino acid residue motif.[4] Cyclic tetrapeptides act as the simplest means of presenting the coveted three amino acid based conformational epitopes in a rigid manner. In addition, cyclic tetrapeptides cannot adopt extended conformations and so resist cleavage by endopeptidases.[5]

Due to their inherent strain, cyclic tetrapeptides are notoriously difficult to synthesize.[6] For a linear tetrapeptide precursor to preorganize its reactive ends in close spatial proximity prior to ring closure, one of the internal amide bonds needs to adopt the unfavorable *cis* conformation. In addition to cyclization challenges, there is no current strategy for the site-specific modification of cyclic tetrapeptides at a late stage of synthesis.

Several methods focused toward cyclic tetrapeptides have been developed in recent years.[7] These include the incorporation of pseudoprolines as powerful *cis*-amide

(1) Che, Y.; Marshall, G. R. *J. Med. Chem.* **2006**, *49*, 111.
(2) Arbor, S.; Marshall, G. R. *J. Comput. Aided Mol. Des.* **2009**, *23*, 87.
(3) Tyndall, J. D. A.; Pfieffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793.
(4) Ung, P.; Winkler, D. A. *J. Med. Chem.* **2011**, *54*, 1111.
(5) Madala, P. K.; Tyndall, J. D. A.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, PR1.
(6) Cavelier-Frontin, F.; Achmad, S.; Verducci, J.; Jacquier, R.; Pèpe, G. *J. Mol. Struct.: THEOCHEM* **1993**, *286*, 125.
(7) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509.
(8) Fairweather, K. A.; Sayyadi, N.; Luck, I. J.; Clegg, J. K.; Jolliffe, K. A. *Org. Lett.* **2010**, *12*, 3136.
(9) Meutermans, W. D. F.; Bourne, G. T.; Golding, S. W.; Horton, D. A.; Campitelli, M. R.; Craik, D.; Scanlon, M.; Smythe, M. L. *Org. Lett.* **2003**, *5*, 2711.
(10) (a) Hili, R.; Rai, V.; Yudin, A. K. *J. Am. Chem. Soc.* **2010**, *132*, 2889. (b) Rotstein, B. H.; Rai, V.; Hili, R.; Yudin, A. K. *Nat. Protoc.* **2010**, *5*, 1813. (c) Jebrail, M. J; Ng, A. H. C.; Rai, V.; Yudin, A. K.; Wheeler, A. R. *Angew. Chem. Int. Ed.* **2010**, *49*, 8625. (d) Rotstein, B. H.; Mourtada, R.; Kelley, S. O.; Yudin, A. K. *Chem. Eur. J.* **2011**, *17*, 12257. (e) Rotstein, B. H.; Winternheimer, D. J.; Yin, L. M.; Deber, C. M.; Yudin, A. K. *Chem. Commun.* **2012**, *48*, 3775.

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inducing elements by Jolliffe and co-workers[8] and the use of photolabile auxiliaries by Smythe and co-workers.[9] Recently, our lab reported a method to convert linear peptides into macrocycles through the use of aziridine aldehydes and isocyanides.[10] The products of this transformation are heterodetic cyclic peptides incorporating an *N*-acyl aziridine moiety in their backbones. Since then, we became interested in the incorporation of the nonstandard amino acid, aziridine-2-carboxylic acid (Azy), into cyclic peptides. We became intrigued by the physical properties of the aziridine−amide bond and, as part of an ongoing program, wanted to take advantage of a powerful one-step transformation of an aziridine into an amino acid residue.[11] Herein we report a straightforward synthesis of aziridine-containing cyclic peptide templates which allow for the late-stage modification. A significant feature of this new method is the facility with which α-substituted *β*-amino acid residues can be introduced into tetrapeptide scaffolds.

The outcome of the end-to-end cyclization of tetrapeptides often depends on the site of ring closure.[12] For a given cyclic tetrapeptide (**1**) containing an Azy residue, there exists four retrosynthetic disconnections for end-to-end cyclization (Figure 1). We decided to synthesize and cyclize linear Azy-containing tetrapeptides of the general structure **3** for the following reasons. Peptides of the general structure **2**, with an *N*-terminal aziridine and free carboxylic acid, have been studied by Moroder and coworkers as promising irreversible inhibitors of cysteine proteases.[13] However, many of these compounds were shown to exhibit sequence-dependent instability in both reaction and purification steps, as well as on storage. This is caused by self-protonation to generate a reactive aziridinium species that subsequently decomposes.

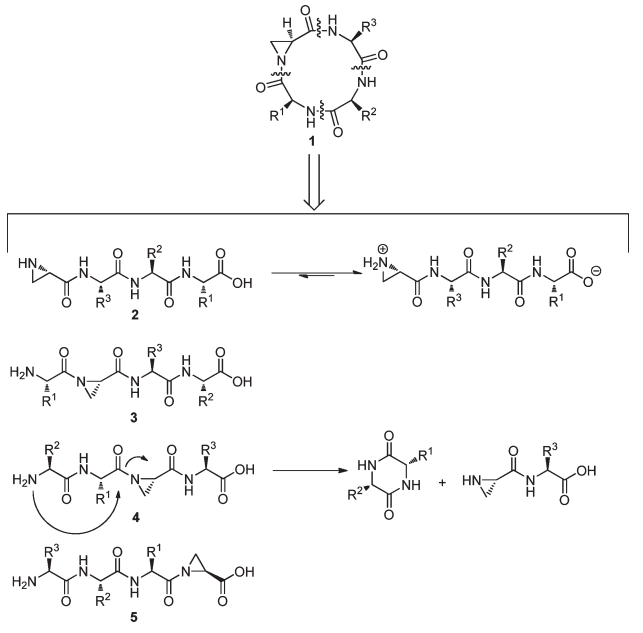


Figure 1. Possible ring disconnections for an Azy-containing cyclic tetrapeptide.

Furthermore, tetrapeptides of the general structure **4** were shown by Gin and co-workers to be unstable due to an intramolecular acyl transfer as a result of the N-terminus "biting back" and reacting with the distorted aziridine amide, forming the corresponding diketopiperazinone.[14] This left **3** and **5** as viable options for a linear precursor and we chose **3** as the centerpiece of our approach.

In order to synthesize the Azy-containing tetrapeptide **3** in solution phase, an appropriate protecting group strategy had to be designed. Acylated aziridines are very reactive moieties that readily undergo ring-opening or acyl-transfer reactions with a wide range of nucleophiles.[15] Since aziridines are unstable to acid, a Boc-based strategy was immediately ruled out. We also ruled out an Fmoc strategy since the secondary amines commonly used for Fmoc-deprotection would react with the acyl aziridine functionality. Furthermore, since activated aziridines are known to take part in hydrogenolysis reactions,[16] we decided that a Cbz/benzyl-based protecting group strategy was not a viable option. We therefore decided to pursue an allyl-based strategy since these protecting groups can be removed mildly in the presence of catalytic Pd(0) and an appropriate scavenger. No premature aziridine ring-opening was observed under these conditions.[17] Our course of action and results are shown in Scheme 1.

We proceeded to incorporate the Azy unit into the peptide chain through a coupling reaction of *N*-tritylaziridine-2-carboxylic acid **6**[18] with the corresponding dipeptide **7** containing an allyl ester to afford tripeptide **8**. A reductive trityl deprotection[19] yielded N−H aziridine tripeptide **9** in quantitative yield. A subsequent coupling to the Alloc-protected amino acid furnished the protected linear tetrapeptide **10**. The Alloc and allyl ester protecting groups were then removed by treating the protected tetrapeptide with a catalytic amount of $Pd(PPh_3)_4$ and 2 equiv of *N*,*N*-dimethylbarbituric acid (DMBA) as a scavenger to afford the deprotected tetrapeptide **11**. DMBA is a weak carbon-based nucleophile that was determined to be unreactive toward acylated aziridines in a control experiment.[20] Compound **11** was isolated by a simple filtration from the reaction solution in high yield and

(11) Sweeney, J. B. *Chem. Soc. Rev.* **2002**, *31*, 247.

(12) (a) Pastuszak, J.; Gardner, J. H.; Singh, J.; Rich, D. H. *J. Org. Chem.* **1982**, *47*, 2982–2987. (b) Schmidt, U.; Beutler, U.; Lieberknecht, A. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 333.

(13) Korn, A.; Rudolph-Böhner, S.; Moroder, L. *Tetrahedron* **1994**, *50*, 1717.

(14) Galonić, D. P.; van der Donk, W. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2004**, *126*, 12712.

(15) Nakajima, K.; Tanaka, T.; Morita, K.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 283.

(16) Davis, F. A.; Zhang, Y.; Rao, A.; Zhang, Z. *Tetrahedron* **2001**, *57*, 6345.

(17) For an example of Pd(0) insertion into aziridines, see: Wolfe, J. P.; Ney, J. E. *Org. Lett.* **2003**, *5*, 4607.

(18) For synthesis, see: (a) Tanaka, T.; Nakajima, K.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1352–1355. (b) Galonić, D. P.; Ide, N. D.; van der Donk, W. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2005**, *127*, 7359.

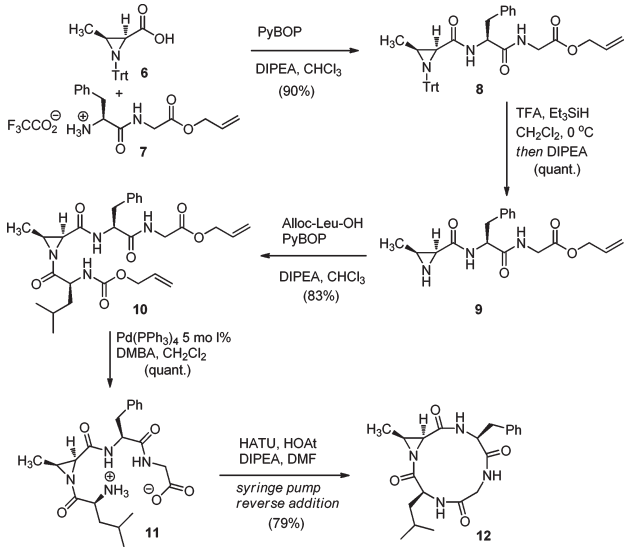
(19) Vedejs, E.; Klapars, A.; Warner, D. L.; Weiss, A. H. *J. Org. Chem.* **2001**, *66*, 7542.

(20) In a control experiment, Cbz-Gly-Azy-Gly-OBn was stirred with a variety of scavengers commonly used in allyl deprotections. After stirring with DMBA for 60 h, the tripeptide remained completely unreacted.

purity and was shown to be stable to prolonged storage at −20 °C, despite the possibility of transamidation.

With this, we began an investigation to optimize conditions for end-to-end cyclization that would suppress the formation of oligomeric side products. Reagents such as EDC/HOAt, HATU, COMU, DPPA, and FDPP yielded predominantly the product of cyclodimerization under dilute concentrations (1 mM). We therefore set out to perform this macrocyclization under pseudohigh dilution conditions,[21] in which the peptide and base are very slowly added to a solution of the coupling reagent. Our optimal conditions were realized with HATU and HOAt with DIPEA as base.

Scheme 1. Synthesis of a Homodetic Cyclic Tetrapeptide Containing the Azy Amino Acid[a]

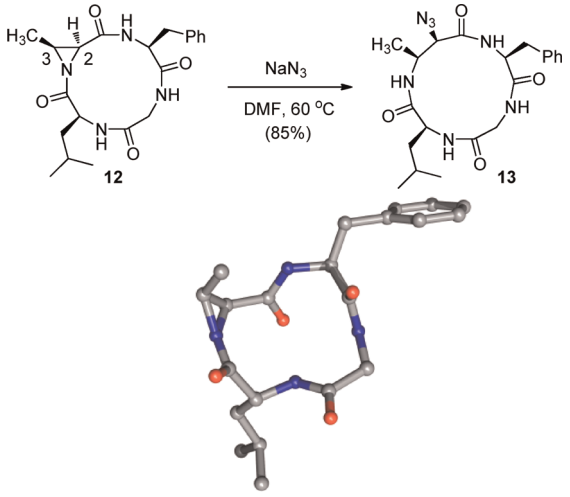


[a] Isolated yields reported.

The hydrophobic product **12** can be easily precipitated and subsequently triturated with a 1:9 mixture of MeCN and water. The crude product was isolated in 79% yield in high purity. LC-MS analysis revealed that the product was devoid of any oligomeric side products. Compound **12** can be used without further purification; however an analytically pure sample may be obtained by preparative RP-HPLC purification.

We then set out to functionalize our aziridine-containing cyclic tetrapeptide scaffold through ring-opening reactions with nucleophiles. First we looked into ring opening with azides because the resulting product may be reduced to the corresponding amine or can partner with an alkyne in a click reaction.[22] To realize this, we treated compound **12** with NaN_3 in DMF at 60 °C and were pleased to observe full conversion after 24 h to one azide-containing product by HPLC. The reaction product was isolated by trituration with water and was found to be of high purity. In an interesting twist, a 2D NMR analysis revealed the structure to be that of **13**. A COSY correlation between the β-methine proton of the β-amino acid residue and the neighboring CH_3, NH, and α-methine protons confirmed the strucutre of **13**. Evidently, the azide nucleophile had attacked the aziridine at the C2 position to generate the corresponding β-amino acid in a 13-membered ring (Scheme 2). It is well-known that 13-membered cyclic tetrapeptides containing a β-amino acid residue ($\alpha_3\beta$-scaffold) exhibit greater conformational homogeneity than their 12-membered ring analog consisting of α-amino acid residues.[23] Our NMR studies indicate that **13** possesses a well-defined conformation in solution, which is in accord with Fairlie's studies.[24] Interest in the $\alpha_3\beta$ architecture has grown across various fields of chemical research. In fact,

Scheme 2. Regioselective Aziridine Ring Opening with Sodium Azide (Top) and Optimized Structure for Compound **12** (Bottom)[a]



[a] The starting geometry was calculated by the OPLS2005 force field in Macromodel.[25] The geometry was optimized by DFT using the B3LYP/6311G level of theory in Gaussian 09.[26]

several natural products are known to contain the cyclic $\alpha_3\beta$ tetrapeptide core. These include the rhodopeptins[27] which display antifungal activity and the azumamides[28] which are known HDAC inhibitors. Furthermore, Ghadiri and co-workers have utilized the $\alpha_3\beta$ architecture as a

(21) Malesevic, M.; Strijowski, U.; Bächle, D.; Sewald, N. *J. Biotech.* **2004**, *112*, 73.

(22) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(23) Glenn, M. P.; Kelso, M. J.; Tyndall, J. D. A.; Fairlie, D. P. *J. Am. Chem. Soc.* **2003**, *125*, 640.

(24) The Cα−H protons of **12** appear as broad singlets with no discernible coupling constants in d_6-DMSO whereas the Cα−H protons of **13** and **16** are better resolved.

(25) *MacroModel*, version 9.9; Schrödinger, LLC: New York, NY, 2011.

(26) Frisch, M. J., et al. *Gaussian 09*, revision A.1; Gaussian, Inc., Wallingford, CT, 2009 (see Supporting Information for full reference).

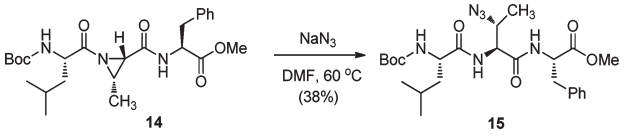
(27) (a) Chiba, H.; Agematu, H.; Kaneto, R.; Terasawa, T.; Sakai, K.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 695. (b) Chiba, H.; Agematu, H.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 700.

(28) Nakao, Y.; Yoshida, S.; Matsunaga, S.; Shindoh, N.; Terada, Y.; Nagai, K.; Yamashita, J. K.; Ganesan, A.; van Soest, R. W. M.; Fusetani, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 7553.

means of HDAC inhibitor development.[29] Through a regioselective aziridine ring-opening reaction, our system provides access to the $\alpha_3\beta$ scaffold, in which the β-amino acid can be functionalized at the challenging α-position through aziridine ring-opening. Given the well-known difficulties associated with the synthesis of enantiopure α-substituted β-amino acids[30] our method offers an attractive alternative to those already in the literature.[31]

We were intrigued by the exceptionally high regioselectivity observed in the azide reaction. This was particularly suprising given that *N*-acyl-aziridine-2-carbonyl compounds are known to give mixtures of ring-opened regioisomers when reacted with the azide nucleophile.[32] Aside from this and other examples of azides opening aziridine rings,[33] there exists, to the best of our knowledge, no reports in the literature pertaining to the ring opening of Azy-containing peptides with metal azides. Because of this, we investigated the regioselectivity of ring opening performed on the linear precursor. Therefore, we synthesized Boc-Leu-3MeAzy-Phe-OMe,[34] a linear tripeptide with identical substitution around the aziridine ring. When **14** was subjected to the same conditions as those to afford **13**, an interesting result was obtained (Scheme 3). Azide-induced aziridine ring-opening proceeded with high regioselectivity (>20:1) but gave compound **15**, the opposite ring-opened isomer to **13**. The low yield (38%) was attributed to various side reactions that led to the decomposition of **14**, mainly through the formation of the corresponding dehydroalanine products. No dehydroalanine byproduct was detected in the ring opening of **12**, likely due to the difficulty in attaining the required *anti*-periplanar geometry.

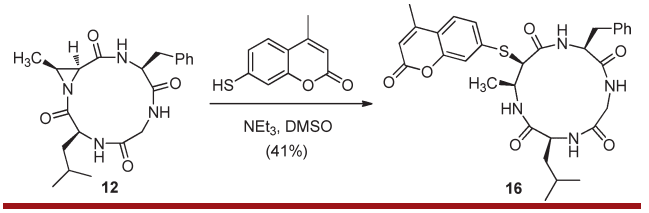
Scheme 3. Ring Opening of a Linear Azy-Containing Peptide with Sodium Azide[a]



[a] Isolated yield after column chromatography.

With these results, we wondered how the aziridine ring in cyclic peptide **12** would react with other nucleophiles. Thiols are known to readily open aziridine rings, and these reactions have even been applied to the modification of linear Azy-containing peptides.[14,17b18b] To demonstrate that fluorescent tags can be easily appended to our novel scaffolds, we reacted **12** with the widely used fluorescent tag 7-mercapto-4-methyl-coumarin (Scheme 4). Again, we observed the formation of a single regioisomer by HPLC. Upon isolation, the product was determined to have structure **16**. Since it has previously been documented that thiols typically open up Azy-containing peptides to generate the corresponding α-amino acid,[14,17b18b] this example again demonstrates an intriguing reversal of regioselectivity of addition of nucleophiles to cyclic versus linear Azy-containing peptide scaffolds.

Scheme 4. Fluorescent Labelling of a Cyclic Tetrapeptide



In summary, we have demonstrated that not only the site-selective modification but also the selective incorporation of β-amino acids into cyclic tetrapeptides can be readily achieved through the cyclization and subsequent ring-opening of tetrapeptides containing the nonstandard Azy amino acid. Of note is the facility with which α-substituted β-amino acid derivatives can be introduced into cyclic tetrapeptides using our method. Given the well-known conformational rigidity imparted by the presence of a β-amino acid residue,[23] our method offers an effective way to constrain the highly coveted tripeptide motif in a rigidified and metabolically stable fashion. Efforts toward elucidating the mechanism of the reversal of regioselectivity in our system as well as toward the synthetic utility of other cyclic tetrapeptides and those derived from precursors of structure **5** are current under analysis in our laboratory and will be reported in due course.

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Supporting Information Available. Experimental details, HPLC and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

(29) (a) Montero, A.; Beierle, J. M.; Olsen, C. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2009**, *131*, 3033. (b) Olsen, C. A.; Ghadiri, M. R. *J. Med. Chem.* **2009**, *52*, 7836.

(30) Juaristi, E.; Soloshonok, V. A., Eds. *Enantioselective Synthesis of β-Amino Acids*; Wiley-VCH: New Jersey, 2005.

(31) For an example, see: Jerphagnon, T.; Pizzuti, M. G.; Minnaard, A. J.; Feringa, B. L. *Chem. Soc. Rev.* **2009**, *38*, 1039 and references therein.

(32) Davoli, P.; Forni, A.; Moretti, I.; Prati, F. *Tetrahedron: Asymmetry* **1995**, *6*, 2011.

(33) Hu, X. E. *Tetrahedron* **2004**, *60*, 2701.

(34) See Supporting Information for the synthesis of **14**.

The authors declare no competing financial interest.