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A New Tri-Orthogonal Strategy for Peptide Cyclization

Joseph T. Lundquist, IV* and Jeffrey C. Pelletier

Division of Discovery Chemistry, Wyeth Research, 500 Arcola Road, Collegeville, Pennsylvania 19426

Lundquj@WAR.Wyeth.com

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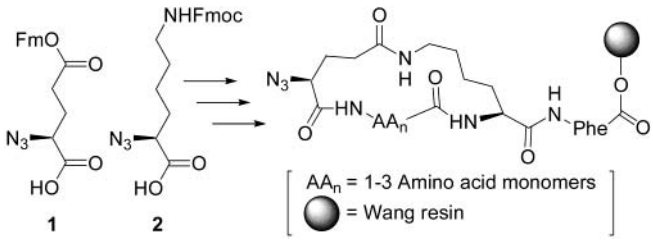
ABSTRACT



A solid phase tri-orthogonal protection/cleavage strategy that uses acidic, basic, and neutral conditions is described. Strategically protected α-azido-γ-9-fluorenylmethyl-L-glutamate (1) and α-azido-ε-*N*-Fmoc-L-lysine (2) were incorporated into growing peptides on Wang resin using a novel azide protection strategy. These residues, separated by 1–3 monomers, were deprotected at the side chains and cyclized via lactam formation. The N-terminus was further functionalized to extend the chain. This method represents a straightforward protocol for peptide cyclization on solid support.

With more than forty synthetic peptides on the market and eighty candidates in phase II or phase III clinical studies,[1] peptide-based drugs are predicted to be an attractive research area for industry and academia for decades to come.[2] In this field there remains an unmet need for optimized methods to synthesize various analogues for structure−activity relationship studies (SAR). Specifically, cyclization of the critical binding motifs of peptides has proven to be a successful strategy to gain information about the three-dimensional structure of a peptide ligand while bound to its receptor.[3] For example, cyclization can stabilize a β-turn,[4] one turn of an α-helix,[5] and two turns of an α-helix[6] providing clues to conformational requirements. Listings in the *Physicians' Desk Reference* reveal that several such compounds have found applications as drugs due to a combination of the following: enhanced binding affinity/selectivity, a decrease in molecular weight, enhanced stability, and improved pharmacokinetic properties. Cyclic peptides have been prepared by formation of disulfide-,[4−7] thioether-,[8] ether-,[9] and amide-linked bridges of side chain functionality.[10] Lactams are particularly attractive due to their chemical stability and ease of synthesis due to a number of readily

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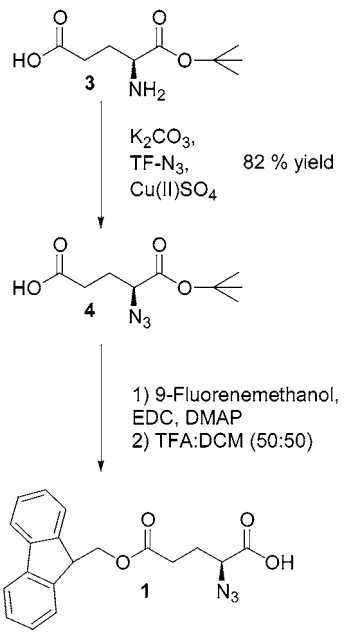
available starting amino acids that contain side chains suitable for cyclization.

Solid-phase peptide synthesis (SPPS) is the method of choice for the preparation of small to mid-sized linear oligomers. Cyclization of linear resin-bound peptides can prove to be cumbersome due to the lack of orthogonal protecting groups that can be cleanly removed in the presence of the linker as well as side chain and N-terminal α-amine protecting groups.[11] One approach to overcome this difficulty has been the removal of the linear sequence from the resin with subsequent C-terminal carboxy protection followed by cyclization. The method is limited due to the extra protection step and oligomer formation during the cyclization process. Conversely, amide bond side-chain cyclizations have been performed on Merrifield resin.[10g,12] The disadvantage to this is the requirement for hydrogen fluoride cleavage, which is too harsh to be carried out in most laboratory settings. Recently, milder orthogonal routes using allyl and alloc groups for the protection of the side chains of acid and amine have been used more frequently for on-resin lactamizations.[10d,e,11] Linear peptides were prepared using Fmoc or Boc temporary protection of the α-amino functionality in combination with selected orthogonal resin linkages. The allyl and alloc groups were subsequently removed under neutral conditions with Pd(0) leaving the acid and amine for solid-phase lactamization.

We envisioned a new solid-phase cyclization strategy that could be accomplished with relatively mild deprotection protocols. We recently described the utility of the azide functionality as a temporary α-amino protecting group for amino acids used in SPPS.[13] Azides are easily converted to amines under neutral conditions on Wang solid support, and they are stable to the usual acidic and basic conditions required for SPPS as well. Thus, we determined that utilization of the α-azides in combination with base-labile side chain protection on Wang resin would add a new dimension to the field of SPPS. Herein we describe the development of this tri-orthogonal protection/cleavage strategy through the straightforward preparation of lactam cyclic peptides.

For the side chain cyclization units, we chose glutamic acid and lysine. Hence, the preparation of α-azido glutamic acid (**1**) with the base-labile γ-fluorenylmethyl ester is shown in Scheme 1. Glutamic acid α-*tert*-butyl ester (**3**) was converted to the α-azide (**4**) by the diazo transfer method described previously.[13,14] The product was converted to the γ-fluorenylmethyl ester, and the α-*tert*-butyl ester was cleaved under standard conditions to provide the desired product (**1**) in 65% yield (53% overall yield). Likewise,

Scheme 1

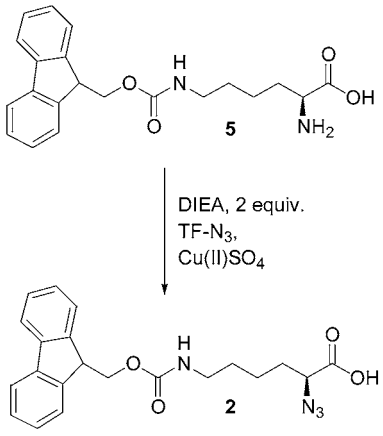


α-Azido-γ-9-fluorenylmethylester-L-glutamic acid

53% overall yield

commercially available *N*-ϵ-Fmoc-lysine (**5**) was converted to the α-azidolysine derivative (**2**) under weakly basic conditions to prevent Fmoc removal (Scheme 2, 58% yield).

Scheme 2



α-Azido-ε-*N*-Fmoc-L-lysine

58% yield

Preparation of many common α-azidoacid building blocks was described earlier.[13] The azidoacids incorporated into the cyclic peptide products were synthesized by the same method.

Peptide synthesis and cyclization is shown in Scheme 3. The strategically protected lysine (**2**) was coupled using a standard Fmoc-Wang protocol[15] to resin-bound phenylalanine

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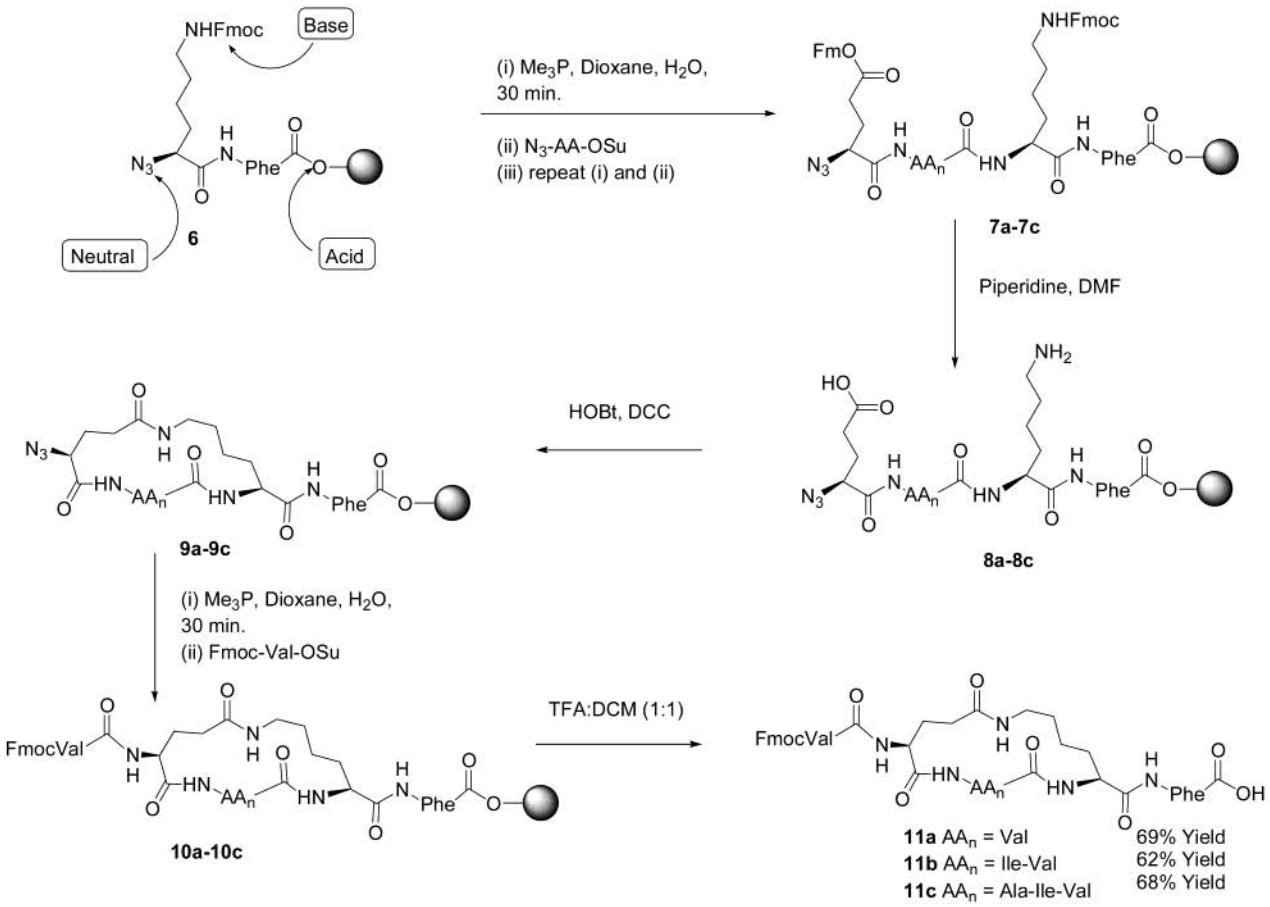
Scheme 3



giving the common precursor (**6**). The tri-orthogonal strategy is highlighted with flags on the functionalities of **6**. The labeling details conditions needed to deprotect the α-amino group, side chain functionalities, and resin linkage. The temporary α-amino protecting group (azide) was reduced under neutral conditions with trimethylphosphine in aqueous dioxane allowing for condensation with subsequent α-azidoacids. The reduction and coupling procedure was repeated until one to three monomers was incorporated between the lysine and glutamic acid residues. The resulting linear peptides (**7a**−**c**) were deprotected at the side chains of the lysine and glutamic acid with piperidine. The free acid and amino functionalities of **8a**−**c** were then condensed under typical activation conditions to give the resin bound cyclic intermediates (**9a**−**c**). The reactions were monitored with the Kaiser test.[16] Once cyclized, the N-terminal azide was converted to the amine as above, which in turn was coupled to an Fmoc-valine residue to provide resin-bound peptides **10a**−**c**. This last step allows for the return to standard Fmoc-Wang methodology and is a good indicator of the flexible nature of this strategy. Cyclic peptides (**11a**−**c**) were cleaved from the solid support with TFA and isolated in 62−69% yield and >90% purity.

In summary, a tri-orthogonal method using mild deprotection protocols was successfully developed for peptides cyclized through side chain lactamization. The methodology is centered around two strategically protected amino acids, α-azido-γ-9-fluorenylmethylester-L-glutamic acid (**1**) and α-azido-ϵ-*N*-Fmoc-L-lysine (**2**), which were synthesized in 53 and 58% yields, respectively, with minimal synthetic steps. Incorporation of the residues into the growing peptide chain allowed for facile cyclization on the solid phase. Incorporation of an Fmoc-valine residue at the N-terminus after cyclization displays the compatibility and interchangeability of utilizing the Fmoc group and azido functionality for α-amino protection on the Wang resin. It is apparent that multiple cyclized regions could be synthesized in tandem utilizing this methodology. The additional degree of orthogonality described in this paper offers another dimension to solid-phase methodology, which could prove useful in combination with other protecting groups to prepare more elaborate products.

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Supporting Information Available: Full experimental details and physical data for synthesized compounds.

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