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Aziridine-Mediated Ligation at Phenylalanine and Tryptophan Sites

Kiran Bajaj,*[a] and Rajeev Sakhuja[a]

This work is dedicated to Late Prof. Alan Roy Katritzky

Abstract: An efficient approach towards peptide synthesis has been described that allows an easy access to variety of small peptides via one-pot aziridine-mediated ligation-desulfurization strategy. The present protocol afforded a library of phenylalanine and tryptophan containing *α*-peptides in good yields by regioselective ring-opening of aziridine-3-aryl-2-carboxylates with peptide thioacids followed by desulfurization.

Amino acids and peptides are the natural building block of life that have attracted immense attention as therapeutics due to their high selectivity, potency and low toxicity towards biological systems.[1-4] A large number of commercialized peptides have proved their validity as active pharmaceutical ingredients and are in various clinical phases.[5] Due to the tremendous applicability of naturally-occurring and synthetic peptides in bio-chemical interactions,[6] considerable progress have been monitored towards the development of newer synthetic methodologies for peptides in the past two decades.[7] However, due to complex peptide multifunctional architecture, and their increasing demands in emerging medicinal and material chemistry, formulation of advance peptide bond-forming strategies remains a challenging area. One of the major breakthrough in peptide synthesis after Merrifield solid phase peptide synthesis (SPPS)[8] was the introduction of Native Chemical Ligation (NCL) by Kent's group,[9] that illustrated a regio- and chemoselective strategy for longer peptide syntheses by the reaction of a peptide-thioester with cysteine containing peptide *via* rapid *S*- to *N*-acyl transfer. However, requirement of an *N*-terminus cysteine residue restricted the applicability of NCL at all naturally-occurring amino acid sites. Thereafter modified ligation strategy involving post-ligation desulfurization has been first introduced by Yan and Dawson[10] and further explored by Wan and Danishefsky[11] that illustrated an excellent application of converting the pivotal cysteine to an alanine residue after ligation. This strategy have been well exemplified to various suitably placed thiol and selenol-substituted unnatural amino acids, and thus expanded the scope of NCL at arginine,[12] aspartic acid,[13] glutamic acid,[14] glutamine,[15] phenylalanine,[16a-c] valine,[17] lysine,[18] leucine,[19] tryptophan[20] and proline[21] junctions. However, the major challenges for implication of these modified NCL strategies are the multi-step syntheses required for preparation of thiol (or selanol)-functionalized amino acid auxiliaries and their selective installation into different peptide units. Apart from this, other modified ligation strategies such as *α*-ketoacid-hydroxylamine ligation (KAHA),[22] salicylaldehyde ester-mediated ligation,[23] oxime ligation,[24] and Staudinger ligation[25a-b] have also been developed to eliminate the cysteine requirement for NCL.

As per the significance of all naturally-occurring amino acids, phenylalanine (Phe) and tryptophan (Trp) are recognized for their vibrant pharmacological profiles[26a-c] and their profound applications in developing intelligent organic materials.[27] Phenylalanine constitute an integral part of numerous naturally-occurring peptides including *β*-casomorphin, Casoxin D, *α*-lactalbumin, *β*-lactoglobulin and *β*-amaloid.[28] On the other hand tryptophan although have low relative abundance in peptide and protein sequences (≈1% of the amino acids), yet its presence is critical for their activity.[29] Thus, the development of new synthetic methodologies for synthesis of Phe- and Trp based peptides is highly significant. Post ligation-desulfurization and other modified NCL strategies including *S*- (or *Se*-) to *N*-peptidyl migration-desulfurization method for Phe-ligation,[16a-c] and *S*- (or *N*-) to *N*-peptidyl migration[20,30] for Trp-ligation have been reported to generate Phe-and Trp-containing peptides. In spite of significant advancements in ligation strategies, there is always a scope for alternative synthetic protocols for accessing the peptides of choice under mild reaction conditions.

In quest to explore the newer ligation approach for phenylalanine and tryptophan based peptides, we envisaged to extent the scope and reactivity of aziridine ring towards developing a new ligation approach for accessing phenylalanine and tryptophan based peptides. In this context, van der Donk and Gin investigated site selective ring opening of aziridine-2-carboxylic acid-containing peptides by various thiol nucleophiles including carbohydrate thiols, farnesyl thiol, and biochemical tags, both in solution and on solid support.[31a-b]

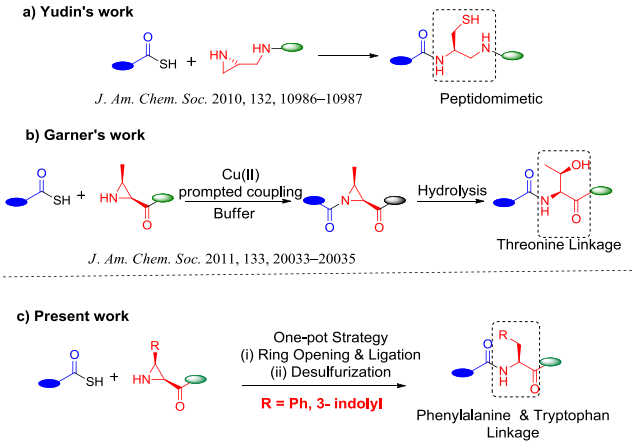

Figure 1. Previous and present work on aziridine-mediated ligation.

[a] Dr Kiran Bajaj, Dr Rajeev Sakhuja
Department of Chemistry
Birla Institute of Technology and Sciences
Pilani 333031, India
E-mail: kbajaj26@gmail.com
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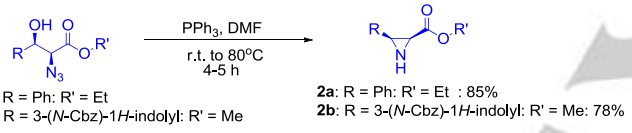
Yudin's group[32] has displayed aziridine ring-opening approach for peptidomimetic synthesis by incorporating the (2-aziridin)methyl group at the *N*-side of a peptide chain (Figure 1a). Garner's group[33] have reported a two-step ligation strategy yielding threonine-containing peptides *via* a Cu(II)-promoted coupling of unprotected peptide with N-H aziridine-2-carbonyl peptides followed by regioselective and stereoselective ring-opening by water (Figure 1b). In present work, we have explored phenyl and indolyl substituted aziridine-2-carboxylates as synthetic electrophilic handles and displayed an aziridine-mediated one-pot ligation-desulfurization strategy to afford phenylalanine and tryptophan appended α-peptides in a regio- and stereoselective manner (Figure 1c).

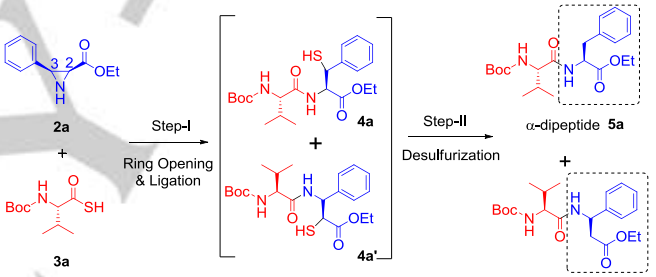
Our exploration of aziridine-mediated ligation at phenylalanine and tryptophan sites commenced with the synthesis of 3-phenyl- and 3-indolyl-substituted aziridin-2-carboxylates derivatives. For this purpose, (2*S*,3*R*) β-hydroxy–α-azidophenylalanine[34] (1a) and *N*-protected (2*S*,3*R*) β-hydroxy–α-azidotryptophan[35] (1b) derivatives were synthesized using reported protocols. Cyclization[36] of **1a** and **1b** using triphenylphosphine in DMF comfortably afforded (2*S*,3*S*)-ethyl 3-phenylaziridine-2-carboxylate (**2a**) and *N*-protected (2*S*,3*S*)-methyl 3-(1*H*-indol-3-yl)aziridine-2-carboxylate (**2b**) in 85% and 78% yields, respectively with high diastereoselectivity (up to 95% as evident from ^{1}H NMR) (Scheme 1).



Scheme 1. Synthesis of aziridin-2-carboxylates (**2a-b**).

In order to investigate the proposed one-pot ligation-desulfurization strategy at phenylalanine site, aziridine ring-opening reaction of **2a** with thioacid, Boc-L-Val-SH (**3a**) (Table 1) was performed. The reaction of **2a** and **3a** in DMF at room temperature leads to the complete consumption of the starting materials and eventually formation of a new spot on TLC in 6 h. The ^{1}H NMR of the crude mixture indicated the formation of a thiol-substituted dipeptide (Boc-L-Val-Phe(SH)-OEt) (**4a**) in major amounts. Further reduction of this crude reaction mixture using $NiCl_2.6H_2O/NaBH_4$ in methanol at -10 °C yielded the phenylalanine containing *α*-dipeptide (Boc-L-Val-Phe-OEt) (**5a**) in 50% overall yield (entry 1). It is worth noticing that about 32% of *β*-dipeptide (**5a'**) was also obtained during this two-step one-pot strategy (entry 1). A change of solvent to DCM and THF in ring-opening-ligation step followed by reduction in methanol leads to reduction in the yields of **5a** (entries 2-3). However, ethanol was found to be best solvent for the ligation step (entry 4), and an additive effect of performing the two steps in ethanol was found to be most delightful reaction condition, yielding **5a** and **5a'** in 62% and 28% yields respectively (entry 5). To further increase the yield of C-3 regioisomer, the aziridine ring opening was attempted with K_2CO_3 and $BF_3.OEt_2$ separately, however, discouraging results were obtained as messy reaction mixtures were observed on TLC in step-I only (entries 6-7). Performing desulfurization in ethanol at 0 °C for slightly longer times (30 min.) also lead to decline in the yield of **5a** due to its further reduction to *α*-dipeptidyl alcohol (entry 8). Formation of major amount of **5a** (over **5a'**) (entry 5) clearly indicates that aziridine ring opening by thioacid preferred C-3 attack (over C-2), thereby triggering *S*-to-*N* aminoacyl group transfer *via* 5-membered cyclic transition state which followed by desulfurization afforded *α*-dipeptide regioselectively. To elucidate the stereochemistry of the chiral centre at phenylalanine site in the synthesized *α*-dipeptide (Boc-L-Val-Phe-OEt) (**5a**), its ^{1}H NMR spectrum was compared with dipeptides Boc-L-Val-L-Phe-OEt and racemic Boc-L-Val-DL-Phe-OEt, that were obtained by coupling Boc-L-Val-OH with L-Phe-OEt and DL-Phe-OEt, respectively using EDC coupling strategy. ^{1}H NMR of dipeptide **5a** was in exact agreement with the stereoisomer Boc-L-Val-L-Phe-OEt suggesting that the aziridine ring-opening proceeded with the retention of configuration at C-2 position, thereby generating an L-phenylalanine residue at ligation junction (see supporting information). The structure of *β*-phenylalanine peptide (**5a'**) was confirmed by its ^{1}H-^{1}H correlation spectrum (see supporting information).

Table 1 Optimization of the Reaction Conditions for Synthesizing **5a.**

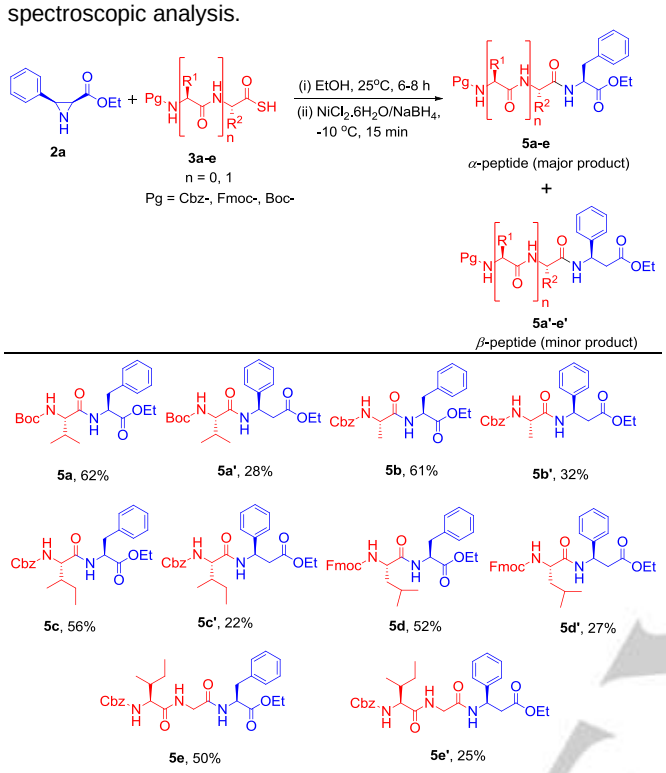


Entry	Aziridine Ring Opening & Ligation (Step-I)[a]		Desulfurization (Step-II)[b]		Overall Yield (%)
	Additive	Solvent	Reagent	Solvent	**5a**
1	-	DMF	$NaBH_4/NiCl_2.6H_2O$	MeOH	50
2	-	DCM	$NaBH_4/NiCl_2.6H_2O$	MeOH	25
3	-	THF	$NaBH_4/NiCl_2.6H_2O$	MeOH	40
4	-	EtOH	$NaBH_4/NiCl_2.6H_2O$	MeOH	60
5		**EtOH**	**$NaBH_4/NiCl_2.6H_2O$**	**EtOH**	**62**
6[d]	K_2CO_3	DMF	-	-	-
7[d]	$BF_3.OEt_2$	DMF	-	-	-
8[e]	-	EtOH	$NaBH_4/NiCl_2.6H_2O$	EtOH	-

[a] Reaction conditions: **2a** (0.52 mmol), **3a** (0.63 mmol), additive (0.52 mmol), solvent (10 mL). The reactions were performed at room temperature and monitored *via* TLC till completion for 6-8 h; [b] Reaction conditions: **4a+4a'** (crude mixture), $NaBH_4$ (4.70 mmol), $NiCl_2.6H_2O$ (1.56 mmol), solvent (10 mL). The reactions were performed at -10 °C and monitored *via* TLC till completion for 15 min; [c] Isolated yield over two steps; [d] messy mixture on TLC in Step I and thus step II was not performed; [e] (Step II) T = 0 °C for 30 min. Formation of peptidyl alcohol was observed in crude ^{1}H NMR spectra.

With optimized conditions in hand, the scope of the developed two-step one-pot transformation at phenylalanine site was exploited using a variety of peptide thioacids (**3b-e**). The reaction proceeded smoothly with *α*-amino thioacids (**3b-d**) and *α*-dipeptide thioacid (**3e**) affording phenylalanine containing *α*-

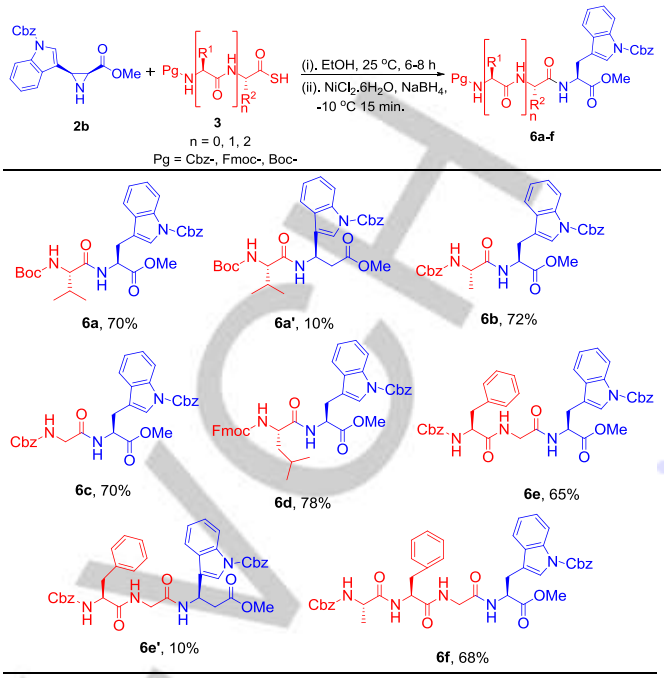
dipeptides (**5a-d**) and *α*-tripeptide (**5e**) in good yields. In all cases, a substantial amount of *β*-phenylalanine containing peptides (**5a'-e'**) were also obtained. The reaction tolerated a variety of protecting groups (Fmoc-, Boc- and Cbz) on thioacids (Scheme 2). All the synthesized compounds were purified by column chromatography and characterized by detailed spectroscopic analysis.



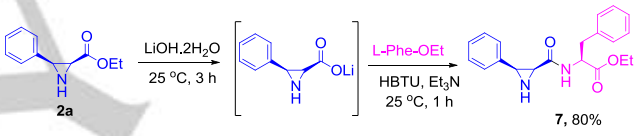
Scheme 2. Substrate scope for aziridine-mediated ligation at phenylalanine site.

After thriving results obtained in phenylalanine based aziridine ligation, we explored the scope of our developed one-pot ligation-desulfurization methodology at tryptophan site. Interestingly, the reaction of *N*-protected (2*S*,3*S*)-methyl 3-(1*H*-indol-3-yl)aziridine-2-carboxylate (**2b**) with various *α*-amino thioacids (**3**) were more regioselective affording their corresponding native tryptophan containing *α*-peptides (**6a-f**) in good yields (Scheme 3). In few cases, *β*-trytophan peptides (**6a'** & **6e'**) were also isolated in very low yields (~10%).

In order to exemplify the developed methodology towards amide bond formation between two peptidic fragments, the coupling between aziridine embedded dipeptide and a peptide thioacid was planned as a model reaction. Since Phe-Phe motif is found as a core component of *β*-amyloid peptide and have shown tremendous applications as a nano-material and pharmaceutical persuit[37], we planned to affix L-phenylalanine to 3-phenyl substituted aziridin-2-carboxylate (**2a**) *via* peptide bond. For this purpose, lithiated salt of **2a** was prepared by hydrolyzing **2a** using lithium hydroxide in MeOH-H_2O, which on further coupling with L-Phe-OEt using HBTU/Et_3N in DMF yielded the novel aziridine embedded dipeptide precursor **7** in 80% yield (Scheme 4).

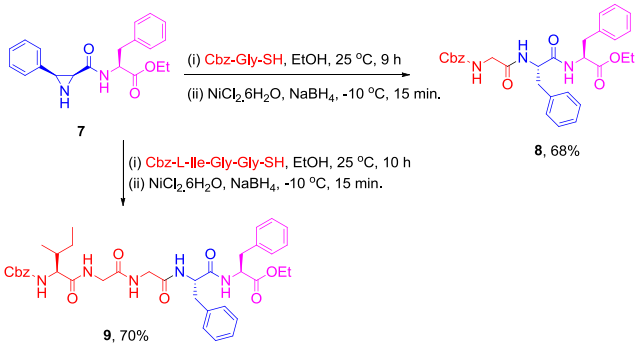


Scheme 3. Substrate scope for aziridine-mediated ligation at tryptophan site.



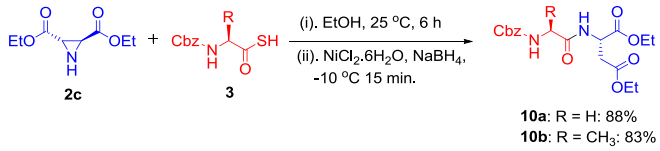
Scheme 4. Synthesis of aziridine based dipeptide precursor **7**.

Application of one-pot ligation-desulfurization strategy to aziridine embedded dipeptide precursor **7** and Cbz-Gly-SH gratifyingly afforded *α*-tripeptide, Cbz-Gly-L-Phe-L-Phe-OEt (**8**) in 68% yield (Scheme 5). The structure of *α*-tripeptide was confirmed by detailed spectroscopic data including ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY spectra (see supporting information). Another peptide-peptide bond coupling using ligation-desulfurization strategy successfully yielded *α*-pentapeptide (**9**) in 70% yield by reacting Cbz-L-Ile-Gly-Gly-SH with **7** (Scheme 5) Thus, the scope of aziridine-mediated ligation-desulfurization protocol could be effectively used to regioselectively generate tryptophan and phenylalanine containing *α*-peptides in high yields.



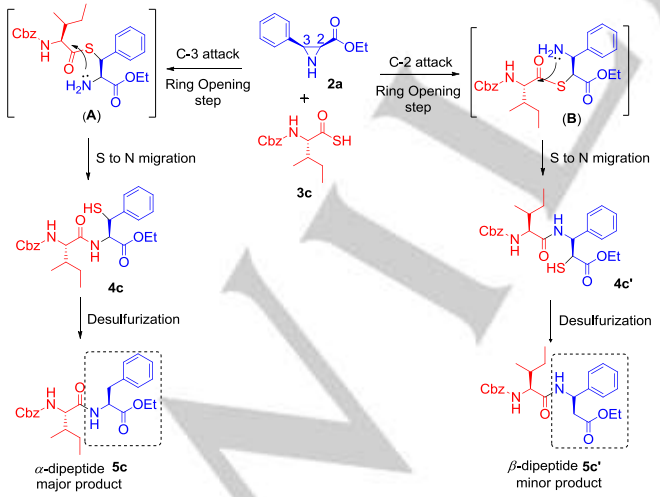
Scheme 5. Exemplified synthesis of *α*-tripeptide and *α*-pentapeptide *via* aziridine-mediated ligation-desulfurization strategy.

To further explore the generality of the methodology for studying ligation at a non-aromatic amino acid junction, (2*S*,3*S*)-diethyl aziridine-2,3-dicarboxylate (**2c**)[38] was allowed to react with peptide thioacids, Cbz-Gly-SH and Cbz-L-Ala-SH under our optimized conditions in a two-step one-pot fashion. Pleasingly Asp-based *α*-dipeptides, Cbz-Gly-L-Asp(OEt)-OEt (**10a**) and Cbz-L-Ala-L-Asp(OEt)-OEt (**10b**) were obtained in 88% and 83% yields respectively (Scheme 6). This clearly demonstrates the potential of aziridine-mediated ligation towards synthesizing peptides of choice using appropriate starting materials.



Scheme 6. Aziridine mediated ligation at aspartic acid junction.

To gain some insight Into the reaction mechanism, we successfully isolated one of the thiol intermediate (**4c/4c'**) obtained by the reaction of **2a** and Cbz-L-Ile-SH (**3c**). ^{1}H NMR of the isolated intermediate (**4c**+**4c'**) clearly inferred it to be a mixture of thiol substituted *α*- and *β*-regioisomers. (see supporting Information). In addition, LC-HRMS of intermediate (**4c**+**4c'**) further confirmed it to the major product, along with the presence of a small amount of oxidized (-S-S-) dimer (see supporting information). Thus, from the above experiment and literature reports,[32] the reaction could be believed to proceed by nucleophilic (C-2/C-3) ring-opening of aziridine ring by amino acyl thioacid to generate *α*- and *β*-aminoacyl thioesters (**A** & **B**), which undergoes *S*- to -*N* aminoacyl group transfer *via* 5-membered cyclic transition state to yield *α*- and β-thiol substituted intermediates (**4c** & **4c'**). Desulfurization of **4** afforded the ligated *α*-dipeptide & *β*-dipeptide (Scheme 7).



Scheme 7. Proposed ligation mechanism.

In summary, an epimerization free, regioselective route *via* aziridine-mediated ligation–desulfurization technique has been developed to synthesize native peptide through one-pot approach at trytophan and phenylalanine junctions. This strategy provided a new way to synthesize a series of tryptophan and phenylalanine containing *α*-peptides in highly regioselective manner. The inclusion of a derivatized aziridine to a peptide backbone, and its efficiency to react with various peptide thioacids illustrated a promising pathway to generate Phe- and Trp-containing peptides in solution phase. With the recent development of solid phase synthesis of long chain thioacids, this methodology could also be extended to solid phase synthesis of peptides and proteins of choice. We anticipate that this methodology could also be extended to other amino acid junctions.

Experimental Section

The synthetic procedures and characterization of the compounds are described in the Supporting Information.

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Keywords: Aziridine • Amino acids • Tryptophan • Phenylalanine • Ligation

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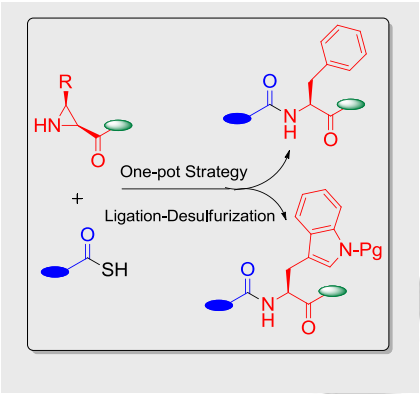
COMMUNICATION

- Aziridine-mediated Ligation
- Mild reaction conditions
- Facile access to Phe- and Trp- peptides



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