Aziridine electrophiles in the functionalisation of peptide chains with amine nucleophiles

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Complete List of Authors:	Spork, Anatol; University of Oxford, Chemistry Donohoe, T; University of Oxford, Department of Chemistry

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Anatol P. Spork and Timothy J. Donohoe*

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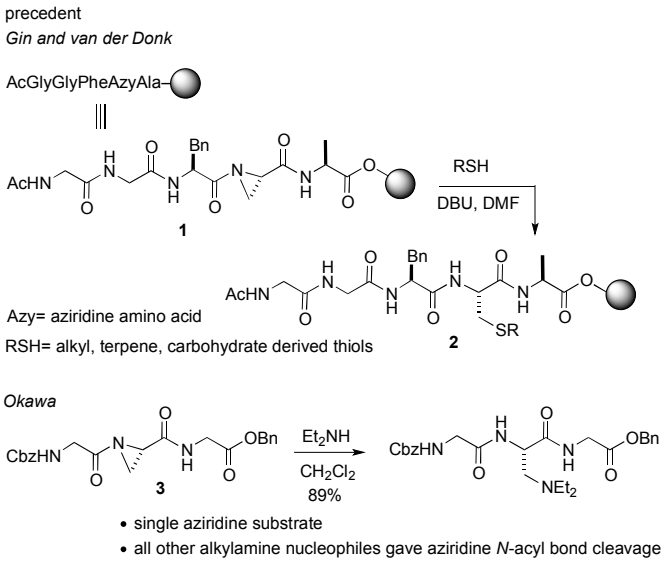
We describe herein the synthesis of aziridine-containing amino acids embedded within tripeptide structures. A range of amine nucleophiles have been shown to open the aziridine amino acid regioselectively at the β-position under mild conditions and without the requirement for a catalyst, forming new adducts in the process. Amino acid N-termini (or an N-containing sidechain) also served as effective nucleophiles for such aziridines and this concept could be extended to encompass a di- or tripeptide nitrogen as a nucleophile, thus providing new methodology for linking together peptide strands using an amine linker.

Introduction

The history of aziridines as electrophiles in organic synthesis is an interesting one, that shows how these heterocyclic rings can be very effective in allowing the formation of a variety of functionalised amines, depending on the nucleophile that is used to open them, and the nature of the activating/protecting group on nitrogen.[1] The reactions of serine derived aziridines are particularly interesting, and many differently substituted serine derivatives have been prepared by regioselective ring opening.[2] In particular, the role of oxygen[3] and sulfur[4] derived nucleophiles has been well explored, with amine nucleophiles also known to open single amino acid derived aziridines.[5] This body of work reveals an innate preference for addition to the least hindered β-carbon of the aziridine amino acid under both acidic and basic conditions.

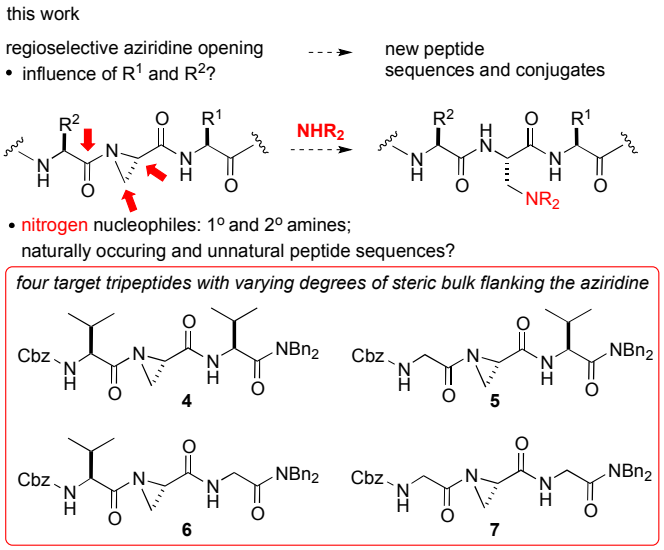
Seminal work by Gin and van der Donk has also revealed that the serine derived aziridine amino acid (Azy) could be embedded within a peptide chain with the nitrogen acyl activating group thus becoming part of the amino acid chain.[6] Gin also showed that, in this environment, the aziridine ring could be opened with a variety of nucleophiles, especially those based on sulfur, and form a set of differentially functionalised peptides, (**1**→**2**, Scheme 1).[6,7] This work is also important because it showed that aziridine derived amino acids can be incorporated into peptides by using solid-phase synthesis techniques. Note that in this context Garner has reported the preparation of threonine-derived aziridines within peptides and their nucleophilic ring opening by water[8] and Yudin has reported on the synthesis of cyclic peptides containing a methyl substituted aziridine and its regioselective ring opening by azide.[9]

Interestingly, apart from early work by Okawa using a single glycine-Azy-glycine peptide **3** and diethylamine as a nucleophile,[10] Scheme 1, there is very little literature precedent regarding the opening of amino acid derived aziridines with amine nucleophiles. Furthermore, these early reports are invariably limited to aziridines contained in a single amino acid and not within peptidic environments.



Scheme 1: Aziridine amino acids within peptides

We were attracted to this powerful methodology, and sought to gain more information about the types of amine nucleophile that might open aziridines within a peptide, and also whether the amino acid residues that flanked the aziridine would influence its reactivity. Our primary goal was a study of amine nucleophiles that might open peptidic aziridines and to use this information to develop the ability to conjugate peptide sequences, selectively, using a naturally occurring or unnatural amine, Scheme 2. This prospect would allow the formation of peptide sequences with differing structures and functionality that would ultimately allow studies in a biological setting, for example in the covalent modification of proteins.[11] In order to accomplish this study, the issues of aziridine ring opening (including the regioselectivity) versus attack on the exocyclic aziridine carbonyl group must be addressed and also the extent to which these factors relate to the residues on each end of the aziridine.

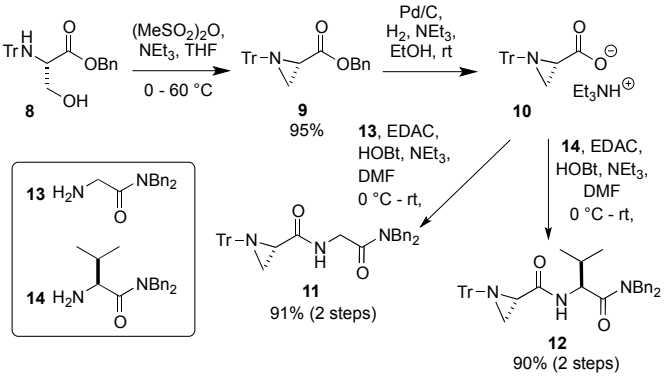


Scheme 2: Ring opening of aziridine amino acids in a peptide environment.

Results and Discussion

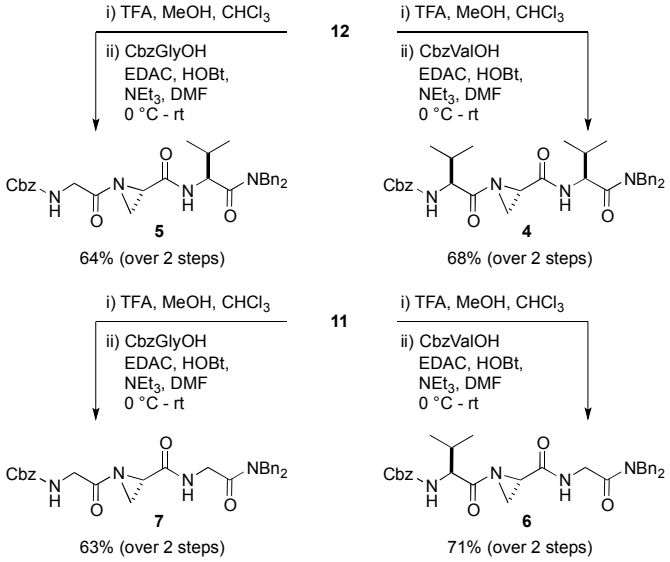
In the first instance we prepared four tripeptide derivatives (**4-7**), each bearing a hindered (Val) or unhindered (Gly) amino acid next to the aziridine at both the N and C-termini, Scheme 2. Note that the *N,N* dibenzylamide derivatives of both valine and glycine were chosen so as to enhance the solubility characteristics of the target tripeptides. We planned to expose these aziridines to reaction with a set of amines in order to probe the structural features that facilitated regioselective reaction with a nitrogen nucleophile.

The synthesis was conducted using L-serine derivative **8** as a starting point for the aziridine amino acid **10**, via activation/displacement and benzyl ester deprotection.[12] The C-terminus residues were then attached using standard peptide coupling techniques to form aziridine dipeptides **11** and **12**, Scheme 3.



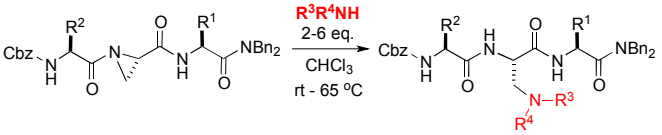
Scheme 3: Synthesis of aziridine amino acids within dipeptides

The two aziridine dipeptides **11** and **12** were separately N-deprotected with acid and then the aziridine nitrogen coupled to the free acid of N-Cbz L-valine and glycine using EDAC/HOBT as a coupling agent, Scheme 4. Thus, all four target tripeptides **4-7** were prepared in a short and high yielding sequence that was amenable to scale-up. Each of the four aziridine tripeptides was subject to thorough characterisation by NMR spectroscopy and in each case, both ^{1}H and ^{13}C NMR clearly indicated the presence of a single compound.



Scheme 4: Synthesis of four target tripeptides.

With the four key substrates in hand, we examined their reaction with primary and secondary amines, Scheme 5. Pleasingly, the aziridine underwent regioselective ring opening by the amine, under very mild conditions (temperatures between rt and 65 °C) without the need for catalysis, forming a wide range of adducts (**15-30**). While our early studies with volatile amines used an excess of the nucleophile (6 eq.), later work revealed that as little of 1.5 equivalents of the amine was a viable amount for the ring opening reaction, *vide infra*.



	4 R1= *i*Pr; R2= *i*Pr	5 R1= *i*Pr; R2= H	6 R1= H; R2= *i*Pr	7 R1= H; R2= H
Et_2NH	**15** (96%)	**16** (94%)	**17** (94%)	**18** (96%)
MeHN	**19** (92%)	**20** (78%)	**21** (93%)	**22** (73%)
*t*BuNH$_2$	**23** (92%)	**24** (94%)	**25** (94%)	**26** (91%)
BuNH$_2$	**27** (98%)	a	**28** (85%)	a
NH$_2$ BocHN NBn$_2$	**29** (85%)	–	**30** (95%)	–

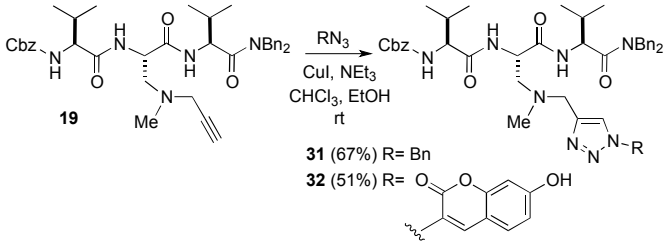
[a] Deacylation after attack on aziridine NCOR

Scheme 5: Aziridine opening with amines

Throughout this work, the identity of each new adduct was ascertained by detailed NMR studies which showed clearly the presence of the derivative formed by nucleophilic ring opening at the least hindered position of the aziridine. For example, the new amide NH proton was identified and showed to couple to a methine CH proton, and the chemical shift of the new CH_2NR_2 methylene protons were consistently around 3ppm, in agreement with the

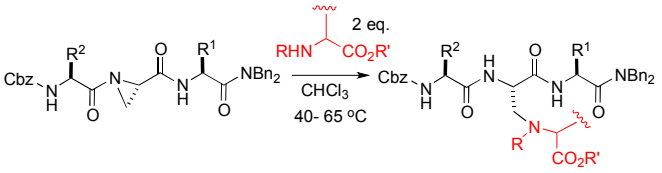
proposed structures. Scheme 5 shows that a trend emerged whereby secondary and hindered primary amines were excellent nucleophiles for all four aziridines. However, we found that unhindered primary amines such as *n*-butylamine was not a viable nucleophile for substrates **5** and **7** with a glycine amino acid on the aziridine nitrogen: in these cases the lack of steric protection of the acyl group lead to chain cleavage reactions rather than aziridine opening.

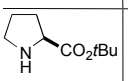
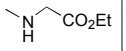
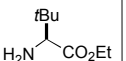
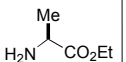
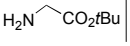
The amines produced by this sequence have potential for derivatisation, and this was illustrated by the reaction of alkyne **19** with two different azides under typical click reaction conditions;[13] both reactions proceeded smoothly producing the 1,2,3-triazole products **31**-**32** in reasonable yields, Scheme 6. The regiochemical outcome of the cycloaddition was assigned for compound **31** by analysis of the chemical shift of the triazole C-H carbon,[14] and that of compound **32** was assigned by analogy. The coumarin functionalised triazole was chosen for its optical properties, and it imparted fluorescent behaviour onto the peptide chain in compound **32**.[15]



Scheme 6: Click reactions on aziridine derived alkynes

With the information gained during the reaction of simple amines in aziridine ring opening, we then moved to examine amino acid amine residues as nucleophiles in the same reaction, Scheme 7. A similar trend of reactivity was observed, with the secondary amines derived from proline and sarcosine being effective in opening each of the four substrates, and all primary amino acids (even hindered *tert*-leucine) opening the aziridine when it did not bear a glycine residue on the N-terminus (see **33**-**46**, Scheme 7). The yield for this process were good, and in each case, NMR studies on the products were completely consistent with the structures shown in the scheme which were formed as single compounds, indicating that a lack of regioselectivity or epimerisation were not a problem in this reaction.

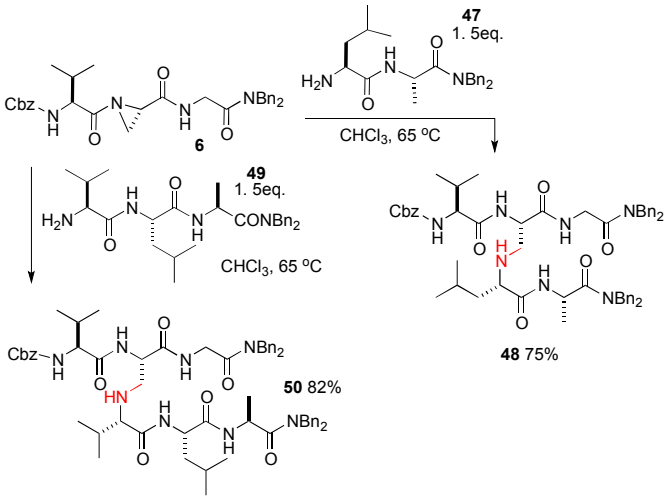


	4 R^1= *i*Pr; R^2= *i*Pr	**5** R^1= *i*Pr; R^2= H	**6** R^1= H; R^2= *i*Pr	**7** R^1= H; R^2= H
	**33** (98%)	**34** (93%)	**35** (93%)	**36** (95%)
	**37** (98%)	**38** (93%)	**39** (98%)	**40** (95%)
	**41** (78%)	a	**42** (75%)	a
	**43** (95%)	–	**44** (75%)	–
	**45** (90%)	–	**46** (80%)	–

[a] Deacylation after attack on aziridine NCOR

Scheme 7: Aziridine opening with amino acids

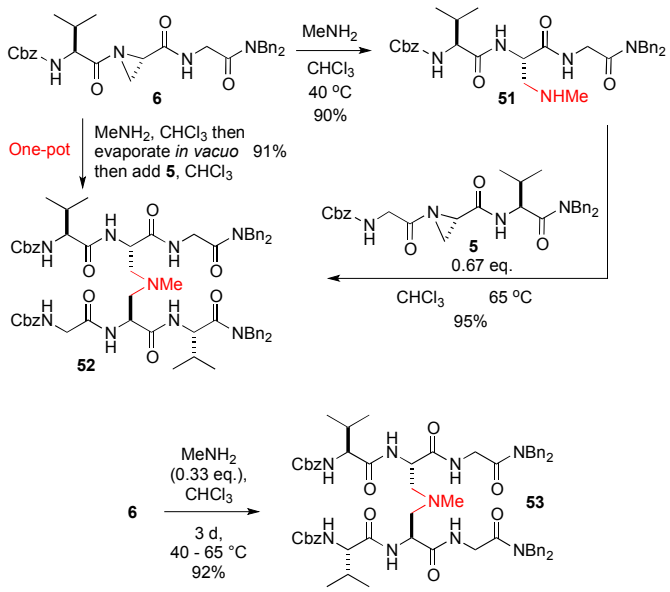
We then selected tripeptide aziridine **6** as a model substrate and examined its reaction with a set of more complex amino acid derivatives, Scheme 8. Reaction of **6** with dipeptide derived amine **47** (1.5 eq.) was successful and generated pseudo-pentapeptide **48** in 75% yield, again formed as a single compound as proven by NMR spectroscopy. Extending this reactivity pattern we then opened aziridine **6** using tripeptide amine **49**, forming peptide **50** (82% yield) in the process. These results indicate that there is considerable prospect for extending the complexity of the peptidic amine nucleophile in this sequence.



Scheme 8: Aziridine opening with di- and tripeptides

Finally, two different aziridine containing tripeptides could also be cross linked by sequential ring opening of **6** with methylamine to form **51**, Scheme 9, followed by treatment of the peptide thus formed with another aziridine peptide (here **5** with a glycine residue on the aziridine nitrogen) to generate the dimer **52** in 86% yield over two steps. The fact that nucleophile **51** contains a secondary amine explains why the *N*-glycine derived aziridine **5** does not suffer acyl bond cleavage and instead undergoes smooth ring opening. Moreover, the reaction sequence from **6**→**52** can also

be performed in one-pot simply by removing the excess of methylamine from **51** by evaporation before adding solvent and amine **5** (91% overall yield of **52**). Taking this idea further, we discovered that aziridine **6** could be dimerised efficiently by simply adding 0.33 eq. of methylamine, with the amine performing a double ring opening to form symmetrical dimer **53** in 92% yield (based on methylamine).



Scheme 9: Aziridine dimerization sequences

The efficiency of these studies bode well for a future extension of this methodology to incorporate Azy amino acids in more complex peptide sequences and selective ring opening reactions with longer peptide derived nucleophiles.

Note that all of the ring opening reactions reported herein produced a single stereoisomeric product because the stereogenic centre on the aziridine was not affected during nucleophilic displacement. This S_N2 mechanism was also supported by a control experiment whereby a tripeptide with a dehydroalanine residue[16] in place of the Azy amino acid (prepared separately, see ESI) was subjected to reaction with an amine. Under all conditions examined the reaction failed to give any of the β-substituted amine as was observed during aziridine opening. This observation rules out a possible mechanism which involves base promoted rearrangment of the Azy to a dehydroalanine,[17] followed by conjugate addition by the nitrogen.

Conclusions

We have prepared four tripeptide derivatives, incorporating an aziridine amino acid (Azy) in the central position. Each peptide was then subjected to ring opening reactions with a battery of different amines, and regioselective ring opening at the β-position was observed in each case. The presence of a glycine residue on the aziridine nitrogen restricted the nature of the nucleophile to secondary amines. Nevertheless, an ever increasingly complex range of amines were able to open these aziridines under mild reaction conditions; showing high regioselectivity and yields. Finally, peptide linking processes were described whereby the aziridine containing peptides could be opened by nucleophiles embedded within both di and tri-peptides. Further work on increasing the complexity of both the aziridine-containing peptides and the nitrogen nucleophiles is currently underway.

Acknowlegements

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Notes and references

[a] Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, United Kingdom.

Electronic Supplementary Information (ESI) available: [experimental procedures for all compounds and copies of ^{1}H and ^{13}C NMR spectra for all novel compounds]. See DOI: 10.1039/c000000x/

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