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Aziridine-Mediated Ligation and Site-Specific Modification of Unprotected Peptides

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Supporting Information

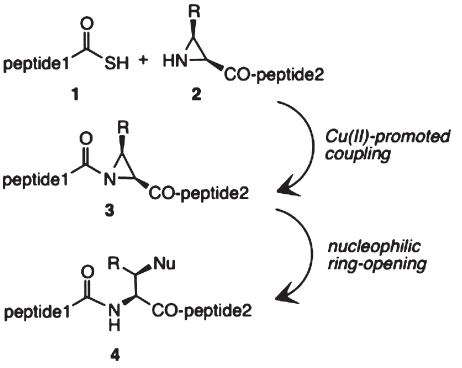
ABSTRACT: A synthesis of aziridine-containing peptides via the Cu(II)-promoted coupling of unprotected peptide thioacids and N−H aziridine-2-carbonyl peptides is reported. The unique reactivity of the resulting N-acylated aziridine-2-carbonyl peptides facilitates their subsequent regioselective and stereoselective nucleophilic ring-opening to give unprotected peptides that are specifically modified at the ligation site. The aziridine-mediated peptide ligation concept is exemplified using H_2O as the nucleophile, producing a Xaa−Thr linkage (where Xaa can be an epimerizable and hindered amino acid). The overall process is compatible with a variety of unprotected amino acid functionality, most notably the N-terminal and Lys side chain amines.

Native chemical ligation (NCL)[1] enables the convergent synthesis of peptides and proteins under mild reaction conditions without the need for protecting groups. The NCL process is characterized by the chemoselective coupling of unprotected peptide thioesters (peptide1-Xaa-SR) and unprotected cysteinyl peptides (H-Cys-peptide2) to give ligation products peptide1-Xaa-Cys-peptide2. Since an N-terminal Cys residue is required,[2] the general application of NCL to peptide/protein synthesis is limited to ligation at peptide linkages Xaa−Cys, where Xaa is preferably an unhindered amino acid. It follows that the incorporation of post-translationally modified or unnatural amino acids at the ligation site is not feasible with NCL.

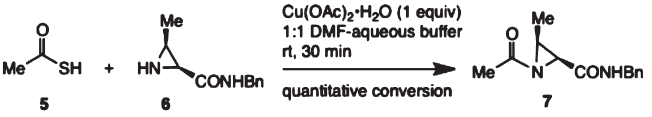
We now disclose a ligation protocol that combines the convergent synthesis of unprotected aziridine-2-carbonyl containing peptides with their controlled site-specific chemical modification. The key reaction (Scheme 1) involves chemoselective Cu(II)-promoted coupling of a peptide thioacid **1**[3] with an aziridine-2-carbonyl (Azy) peptide **2** to give the initial ligation product **3** under native conditions. The unique properties of the chemical species involved, a moderately acidic thioacid combined with a moderately basic aziridine, enable the aziridine-mediated peptide ligation to be performed without peptide protecting groups. The Azy-containing peptide **3** may be converted to a site-specifically modified peptide **4** through regioselective opening of the aziridine ring by a nucleophile. Modifications may be introduced via the aziridine substituent ("R") and/or the nucleophilic species ("Nu").

The potential utility of using an aziridine embedded in the backbone of a peptide as an electrophilic handle for the site-specific introduction of modifications has been recognized for some time.[4−6] The structural uniqueness of this unit has also been noted.[7]

Scheme 1. Aziridine-Mediated Peptide Ligation Concept



Scheme 2. Initial Coupling Reaction Optimization



However, the difficulty associated with the synthesis and manipulation of unprotected Azy-containing peptides has limited the full exploration and exploitation of their properties. In this communication, the aziridine-mediated peptide ligation concept is illustrated with a methyl-substituted aziridine-2-carbonyl moiety using water as the nucleophile, with the net result being ligation at a threonine site.

Our study began with thioacetic acid (**5**) serving as a model for **1** and the known[6] aziridine H-Azy(Me)-NHBn (**6**, Azy(Me) = (2*S*,3*S*) 3-methylaziridine-2-carbonyl) as a model for **2**. In the presence of $Cu(OAc)_2 \cdot H_2O$, thioacid **5** reacted rapidly with aziridine **6** in mixtures of DMF and phosphate-citrate buffer (pH range 4.2−7.2) to give **7** (Scheme 2). Stoichiometric Cu(II) was found to be essential for clean and efficient coupling. In its absence, a complex mixture of products was observed, emanating from nonregioselective ring-opening of **6** by **5** and, presumably, *S*- to *N*-acyl transfer.[8,9] The use of AgOAc instead of $Cu(OAc)_2 \cdot H_2O$ resulted in a much slower reaction.[10] The subpar performance with $K_3Fe(CN)_6$ suggested that the Cu(II)-mediated reaction does not simply involve an oxidative mechanism.[11] These experiments established the critical role that Cu(II) plays in the aziridine-mediated coupling process as well as the reaction's compatibility with aqueous conditions.

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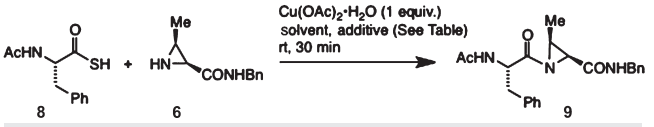
The next stage of reaction optimization focused on assessing the level of epimerization during the coupling of Ac-Phe-SH (**8**)[12] to **6** and defining conditions to minimize it (Table 1). This coupling reaction proceeded rapidly and cleanly in DMF-aqueous buffers to produce Ac-Phe-Azy(Me)-NHBn (**9**). However, 13−14% of the epimeric product Ac-phe-Azy(Me)-NHBn (**epi-9**) was also observed in these reactions (entries 1−3). The level of epimerization could be reduced to 6% when the reaction was performed in DMF alone but the crude yield was lowered (entry 4). The inclusion of 1-hydroxybenzotriazole (HOBt) in the reaction mixture was found to reduce the level of epimerization to 10% in DMF-aqueous buffer and 5% in DMF. HOBt also increased the yield of the ligation reaction. The optimal coupling conditions were thus defined as 1 equiv of $Cu(OAc)_2 \cdot H_2O$ and 2 equiv of HOBt in DMF.

We were now ready to combine the coupling reaction with an aziridine ring-opening reaction and address the chemoselectivity issue (Table 2). It was decided to use H_2O as the nucleophile converting the unprotected Azy(Me)-containing peptide to a Thr-containing peptide (**10** + **11** → [**12**] → **13**). First, the coupling of **8** and **6** was repeated but, rather than isolate **9**, the reaction mixture was treated directly with 10% TFA/H_2O. The hydrolysis product Ac-Phe-Thr-NHBn (**14**) was isolated in good overall yield after standard workup and purification (entry 1).[13] The formation of **14** is consistent with regioselective and stereoselective nucleophilic opening of the aziridine ring at C3 by H_2O. The reaction of diastereomerically pure dipeptide thioacid Fmoc-Phe-Ala-SH (**15**) and aziridine **6** produced Fmoc-Phe-Ala-Thr-NHBn (**16**) (entry 2).[14] Comparison with an authentic sample of Fmoc-Phe-ala-Thr-NHBn (**epi-16**) established the level of epimerization at 5%.

The coupling/ring-opening sequence was then performed with H-Lys-Tyr-Thr-SH (**17**)[15] and **6** to produce H-Lys-Tyr-Thr-Thr-NHBn (**18**) in good yield (entry 3). An analogous experiment using the peptide thioacid H-Glu-Tyr-Thr-SH (**19**) produced H-Glu-Tyr-Thr-Thr-NHBn (**20**) (entry 4). Compatibility of the coupling reaction with aqueous buffer and the denaturant urea was established (entries 5 and 6). The coupling/ring-opening protocol was also applied to a Cys-containing peptide thioacid **21** to afford a mixture of peptide **22** and its disulfide **23** (entry 7).[16] Finally, we extended the ligation to the union of thioacids **17** and **19** with the aziridine-containing tripeptide H-Azy(Me)-Phe-Gly-NH_2 (**24**)[17] to give hexapeptides **25** and **26**, respectively (entries 8 and 9). To summarize, the aziridine-mediated ligation is compatible with free NH_2, CO_2H, OH (aliphatic and aromatic), and SH functional groups (by virtue of in situ protection as a disulfide). The facility of ligation employing an equimolar quantity of relatively hindered thioacid (reaction complete within 1−2 h) is also noteworthy.

While much work remains to be done to develop the aziridine-mediated peptide ligation concept, these preliminary results are encouraging. The method disclosed herein enables one to synthesize unprotected aziridine-containing peptides and regioselectively hydrolyze the embedded aziridine moiety to give products corresponding to ligation at Xaa−Thr linkages.[18] It is anticipated that the aziridine ring-opening reaction will not be limited to the use of water as a nucleophile.[4,6]

Table 1. Optimization of Coupling Reaction Conditions

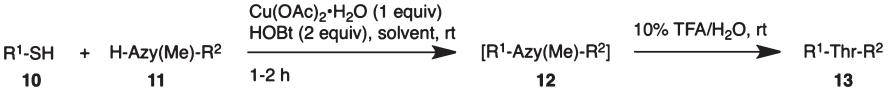


entry	solvent	HOBt (equiv)	9 + epi-9 (% yield)	epi-9 (mol %)
1	1:1 DMF-buffer (pH 7.2)	0	68	13
2	1:1 DMF-buffer (pH 6.2)	0	82	13
3	1:1 DMF-buffer (pH 5.2)	0	87	14
4	DMF	0	53	6
5	1:1 DMF-buffer (pH 7.2)	2	88	10
6	DMF	2	89	5

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and characterization data for all new compounds is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Table 2. Aziridine-Mediated Peptide Ligation at Xaa−Thr Sites



entry	thioacid (1.1 equiv)	aziridine	solvent	product	% yield
1	Ac-Phe-SH (**8**)	H-Azy(Me)-NHBn (**6**)	DMF	Ac-**Phe**-**Thr**-NHBn (**14**)	69
2	Fmoc-Phe-Ala-SH (**15**)	H-Azy(Me)-NHBn (**6**)	DMF	Fmoc-Phe-**Ala**-**Thr**-NHBn (**16**)	72
3	H-Lys-Tyr-Thr-SH (**17**)	H-Azy(Me)-NHBn (**6**)	DMF	H-Lys-Tyr-**Thr**-**Thr**-NHBn (**18**)	80
4	H-Glu-Tyr-Thr-SH (**19**)	H-Azy(Me)-NHBn (**6**)	DMF	H-Glu-Tyr-**Thr**-**Thr**-NHBn (**20**)	69
5	H-Glu-Tyr-Thr-SH (**19**)	H-Azy(Me)-NHBn (**6**)	0.2 M Pi-citrate buffer, pH 6.9	H-Glu-Tyr-**Thr**-**Thr**-NHBn (**20**)	71
6	H-Glu-Tyr-Thr-SH (**19**)	H-Azy(Me)-NHBn (**6**)	8 M urea in 0.1 M NaPi, pH 7.5	H-Glu-Tyr-**Thr**-**Thr**-NHBn (**20**)	88
7	H-Cys-Tyr-Ala-SH (**21**)	H-Azy(Me)-NHBn (**6**)	DMF	H-Cys-Tyr-**Ala**-**Thr**-NHBn (**22**)	43
				(H-Cys-Tyr-**Ala**-**Thr**-NHBn)$_2$ (**23**)	40
8	H-Lys-Tyr-Thr-SH (**17**)	H-Azy(Me)-Phe-Gly-NH_2 (**24**)	DMF	H-Lys-Tyr-**Thr**-**Thr**-Phe-Gly-NH_2 (**25**)	78
9	H-Glu-Tyr-Thr-SH (**19**)	H-Azy(Me)-Phe-Gly-NH_2 (**24**)	8 M urea in 0.1 M NaPi, pH 7.5	H-Glu-Tyr-**Thr**-**Thr**-Phe-Gly-NH_2 (**26**)	77

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(11) $K_3Fe(CN)_6$, is known to promote the N-acylation of primary amines via dithioacids: Liu, R.; Orgel, L. E. *Nature* **1997**, *389*, 52–54.

(12) Thioacid **8** was prepared from commercially available Ac-Phe-OH [(1) NHS, DCC, DCM, rt, 4 h; (2) NaHS, MeOH, 63% yield] using a known method: Goldstein, A. S.; Gelb, M. H. *Tetrahedron Lett.* **2000**, *41*, 2797–2800.

(13) The structure of **14** was confirmed through comparison with an authentic sample prepared using standard peptide coupling protocols.

(14) The Fmoc protecting group was retained in this example to facilitate quantitative determination of the epimer ratio.

(15) Peptide thioacids **17**, **19**, and **21** were prepared by deprotection (TFA, DCM, Et_3SiH, 0 °C) of their STmb thioester precursors in 73, 53, and 45% yields.

(16) MS analysis of this coupling reaction indicated predominant formation of a disulfide corresponding to intermediate **12**, which implies that the free thiol may be undergoing an in situ protection. Reductive disulfide cleavage likely occurs during the workup with aqueous NaSH, which can act as a reducing agent. Minor products emanating from perthioester intermediates were also detected. See: Liu, C. F.; Rao, C.; Tam, J. P. *Tetrahedron Lett.* **1996**, *37*, 933–936.

(17) The aziridine-containing tripeptide **24** was prepared from the union of Tr-Azy(Me)-OH and H-Phe-Gly-NH_2 (HATU, DIEA, DMF, rt, 48% yield) followed by deprotection (TFA, (1:1) $CHCl_3$-MeOH, 0 °C, 61% yield).

(18) A protocol for ligation at Thr via chemical ligation of a γ-thiol-substituted N-terminal Thr peptide followed by postligation desulfurization was recently reported. See ref 2m.