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Published on Web 09/15/2004

Site-Selective Conjugation of Thiols with Aziridine-2-Carboxylic Acid-Containing Peptides

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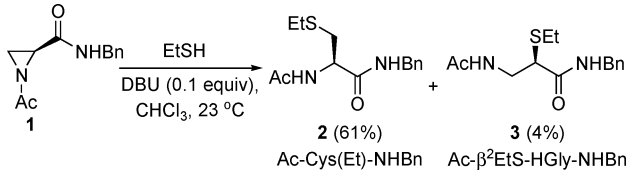
Biological roles of proteins are often determined by their posttranslational modifications that result in the introduction of various functionalities such as carbohydrates and lipids. The resulting conjugates play pivotal roles in numerous processes, including cell adhesion, signal transduction, and immune response.[1] As such, the development of methods for site-selective peptide and protein modifications has been a focus of extensive research.[2] Herein, we report a strategy for the site- and stereoselective conjugation of electrophilic aziridine-carboxylic acid-containing peptides with thiols promoted by DBU. The methodology is also adapted to solid-phase peptide synthesis (SPPS), in which incorporation of the aziridine-carboxylic acid residue into peptides and their ligation with thiols are effected on a solid support.

Aziridine-2-carboxylic acid (Azy)-containing peptides have the potential to function as useful precursors to modified peptide derivatives.[3] However, only short (di- and tri-) peptides of this type have been prepared, presumably as a result of the lability[4] of the Azy-containing peptide backbone toward consecutive amino acid couplings. Moreover, nucleophilic ring openings of the aziridine in these short peptides have employed only Lewis or protic acid promoters,[3b,d] which are likely to be rendered ineffective by the multiple Lewis basic functionalities in larger peptides.

To evaluate nonacidic conditions for the reaction of aziridine-containing peptides with thiol nucleophiles, *N*-acetylaziridine-2-carboxamide **1** (Scheme 1) was reacted with ethanethiol in the

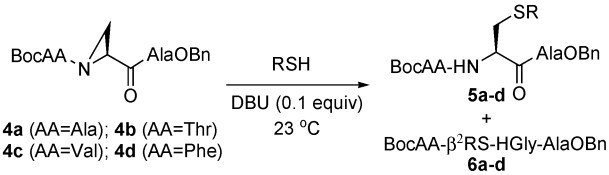
Scheme 1

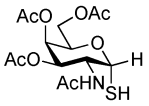


presence of DBU as a catalyst (0.1 equiv). Aziridine opening proceeded with high regioselectivity, providing cysteine derivative **2** (61%), accompanied by a small amount of the β^2-amino acid derivative **3** (4%).[5]

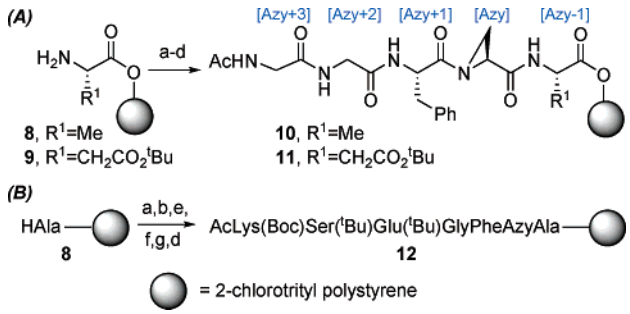
The application of this aziridine opening protocol to peptide substrates was established with the reaction of a series of tripeptides containing a central Azy moiety. Each of the peptides **4a**−**d** (Table 1) was prepared using a modification of Okawa's procedure.[3a] Reaction of either ethanethiol or the sterically demanding *tert*-butyl thiol with BocAlaAzyAlaOBn (**4a**) in the presence of DBU (0.1 equiv) proceeded smoothly at 23 °C to provide tripeptide−thiol conjugates in good yield, strongly favoring the α-amino acid product **5** over its β^2-isomer **6** (ratio ≥ 20:1, entries 1 and 2). The procedure is also amenable to carbohydrate thiols, exemplified by the coupling of the C1 thio analogue of the T_N-antigen **7** (Table 1, entries 3−6).[6] Importantly, these reactions proceed with retention of both the α-anomeric configuration of **7** and the L-configuration of the newly

Table 1. Azy-Tripeptide Conjugation with Thiols



Entry	RSH	Peptide	Solvent (Time)	Yield (**5**:**6**)
1	EtSH	**4a**	CH_3CN (4 h)	85% (20:1)
2	^{t}BuSH	**4a**	CH_3CN (8 h)	84% (**5a** only)
3		**4a**	$CHCl_3$ (11 h)	83% (6.3:1)
4		**4b**	$CHCl_3$ (3 h)	93% (5.3:1)
5		**4c**	$CHCl_3$ (46 h)	89% (13:1)
6		**4d**	$CHCl_3$ (19 h)	90% (16:1)

Scheme 2 [a]



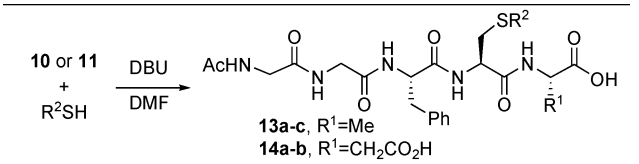
[a] Reagents and conditions: (a) FmocAzyOH, HBTU, NMM; DBU; (b) FmocPheOH, HBTU, NMM; DBU; (c) FmocGlyGlyOH, HBTU, NMM; DBU; (d) Ac_2O, pyridine; (e) FmocGlu(^{t}Bu)GlyOH, HBTU, NMM; DBU; (f) FmocSer(^{t}Bu)OH, HBTU, NMM; DBU; (g) FmocLys(Boc)OH, HBTU, NMM; DBU.

generated cysteine derivative, providing *S*-mucin isosteres from the coupling of preformed Azy-peptides with carbohydrates.[7]

Further expansion of the versatility of the aziridine-peptide conjugation was realized through the rapid construction of Azy-containing peptides via SPPS. Due to the known acid sensitivity of acylated aziridines,[3a] Fmoc-based solid-phase synthesis was chosen over Boc-based SPPS. Likewise, polystyrene with a 2-chlorotrityl linker was selected as the polymer support as it can be cleaved under weakly acidic conditions.[8] Adhering to these criteria, several peptides were prepared on solid support using FmocAzyOH as a novel amino acid monomer,[9] HBTU as the coupling agent, and 1% DBU as the Fmoc deblocking agent (Scheme 2).[10] Thus, the solid-supported Azy-pentapeptides **10** and **11** (Scheme 2A), as well as the Azy-heptapeptide **12** (Scheme 2B), incorporating additional functionalized residues such as glutamic acid, serine, and lysine, were readily accessed. It is important to note that attempts to extend Azy-containing peptides by sequential

10.1021/ja046793x CCC: $27.50

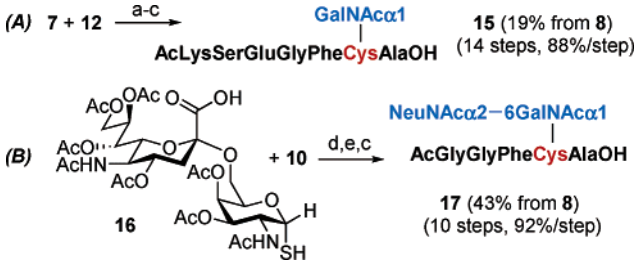
Table 2. On-Bead Conjugation of Azy-Tripeptides with Thiols[a]



entry	peptide	R^2SH	time (h), temp (°C)	peptide conjugate; yield from **8** or **9**, % (yield/step, %)
1	**10**	EtSH	16, 23	**13a**; 52% (93%)[a]
2	**10**	^{t}BuSH	28, 23	**13b**; 40% (90%)[a]
3	**11**	EtSH	22, 23	**14a**; 35% (89%)[b]
4	**10**	**7**	22, 60	**13c**; 21% (86%)[a,c]
5	**11**	**7**	22, 60	**14b**; 17% (84%)[b,c]

[a] Reagents and conditions: (a) resin cleavage: $(CF_3)_2CHOH$:CH_2Cl_2 (20% v/v). [b] Resin and ^{t}Bu-ester removal: TFA:CH_2Cl_2:^{i}Pr$_3$SiH:HS$(CH_2)_2$SH = 10:10:1:1. [c] Acetate removal: NaOMe, MeOH, pH 8.5.

Scheme 3 [a]



[a] Reagents and conditions: (a) DBU, DMF, 60 °C; (b) TFA:CH_2Cl_2:^{i}Pr$_3$SiH:HS$(CH_2)_2$SH = 10:10:1:1; (c) NaOMe, MeOH, pH 8.5; (d) DBU, DMF, 23 °C; (e) $(CF_3)_2CHOH$:CH_2Cl_2 (20% v/v).

coupling of single Fmoc-amino acids were unsuccessful, presumably due to intramolecular aziridine *N*-deacylation by the liberated amine upon Fmoc removal at the [Azy+2] position (Scheme 2). Fortunately, employing a dipeptide building block for simultaneous incorporation of the [Azy+2] and [Azy+3] residues avoided aziridine deacylation, allowing for further sequential peptide elongation.[11]

After successful incorporation of the Azy into peptides via SPPS, each was used for on-bead ligation with thiols (Table 2). The polymer-supported peptides were treated with the thiols (5−10 equiv) in the presence of DBU (1 equiv) in DMF. After 16−28 h, the products were removed from the solid support with concomitant peptide deprotection ($(CF_3)_2CHOH$ or TFA). Preparative RP-HPLC purification provided the deprotected peptide conjugates in good overall isolated yields (calculated from **8** and **9**). For example, preparation of pentapeptides **10** and **11** and ligation with EtSH or ^{t}BuSH provided peptide conjugates **13a**−**b** and **14a**, each in a nine-step sequence from **8** or **9** with an average yield of at least 89% per step (Table 2, entries 1−3).

In like manner, the C1 thio analogue of the T_N-antigen **7** was reacted with polymer-supported pentapeptides **10** and **11** (Table 2, entries 4−5). Release of thioglycopeptides from the resin, followed by acetate removal provided, after RP-HPLC purification, the thioglycopeptides **13c** and **14b** in 21 and 17% yield, respectively, each over a 10-step sequence (≥84%/step). Finally, on-bead synthesis and ligation of heptapeptide **12** with thiol **7** (Scheme 3A), as well as peptide **10** with the more complex thio-ST_N-antigen **16**[7h] (Scheme 3B), proceeded with equally high efficiency providing, after release from solid support, deprotection and RP-HPLC, glycoconjugates **15** and **17** (19% over 14 steps and 43% over 10 steps, respectively) from **8** (≥88%/step).

This work describes the synthesis and convergent site-selective conjugation of aziridine-2-carboxylic acid-containing peptides with thiols, both in solution and on solid support. The synthesis and use of FmocAzyOH in this capacity demonstrate both the efficient incorporation and tolerance of the Azy moiety in multistep Fmoc-SPPS, as well as the competence of solution and on-bead ligation through a highly regioselective base-promoted aziridine ring-opening process. The strategy presents prospects not only for the convergent preparation of complex oligosaccharyl−peptide conjugates and multivalent glycopeptide displays but also for the rapid assembly of Azy-containing peptides for functional proteomics studies.[12]

Acknowledgment. This research was supported by the NIH (GM58833 and GM58822). A Procter and Gamble Fellowship to D.P.G. is acknowledged. We thank Jennifer Showerman for useful suggestions on SPPS, and Kate Lindley for initial exploratory work in base-promoted aziridine thiolysis.

Supporting Information Available: Experimental details . This information is available free of charge via the Internet at http://pubs.acs.org.

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JA046793X