

The utility of N^{ω} -Fmoc-Asp/Glu derived 5-oxazolidinones for side chain carboxyl group modifications

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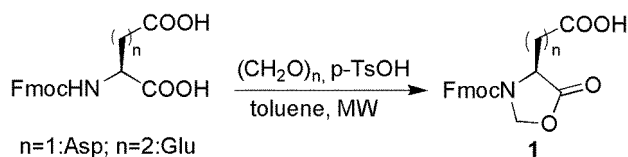
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Introduction

N -Fmoc-5-oxazolidinones **1** derived from amino acids are versatile synthons for N -methyl amino acids and also key intermediates of several biologically active molecules including angiotension II analogues. Earlier reports for the synthesis of 5-oxazolidinones used long hour reflux of the mixture of N -Fmoc amino acid, paraformaldehyde, p -TsOH in toluene and often required azeotropic removal of water [1,2]. Sureshbabu *et al.* have significantly improved the protocol for the preparation of **1** by using microwave accelerated synthesis. The method is high yielding and highly rapid with the reaction time of 3-6 min only (Scheme 1) [3].



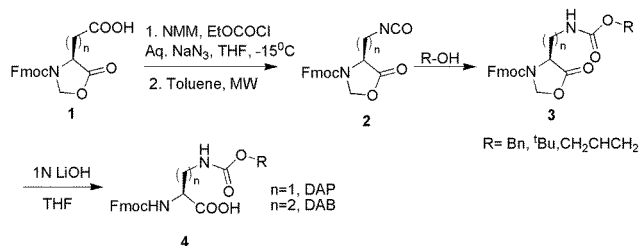
Scheme 1

An important application of 5-oxazolidinone in synthetic peptide chemistry is its use as internal bidentate protection for α -amino and $-\text{COOH}$ groups of amino acids. This allows for site selective chemical modification of ω - COOH group of Asp/Glu through practical and fewer synthetic steps. Herein we describe some of the applications of N -Fmoc-Asp/Glu derived 5-oxazolidinones **1** for side chain selective modification in the synthesis of peptidomimetics and glycopeptides.

Results and Discussion

1. Orthogonally protected DAP / DAB

2,3-Diaminopropionic acid (DAP)/2,4-diaminobutanoic acid (DAB) are lower homologues of lysine. They are constituents of peptide antibiotics like edeine, bleomycin, *etc.* The utilization of DAP/DAB in peptide synthesis requires orthogonal protection for the two amino groups. A simple two-step synthesis of DAP/DAB starting from **1** has been developed by our group [4]. The ω - COOH of **1** was initially converted to acyl azide and then subjected to Curtius rearrangement under microwave irradiation. The resulting isocyanate **2** was then trapped with allyl alcohol, tert-butanol or benzyl alcohol followed by hydrolysis to obtain N -Fmoc, N^1 -allyl, $-\text{Boc}$, $-\text{Cbz}$ protected DAP/DAB. (Scheme 2, Table 1)



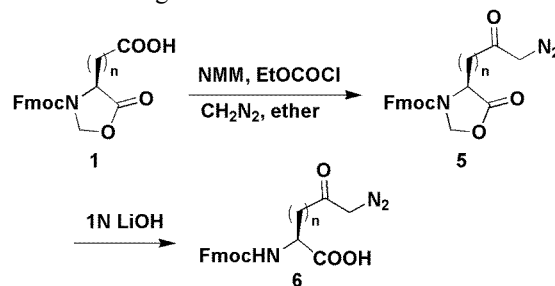
Scheme 2

Table 1. List of orthogonally protected DAP/DAB

Compd.	R	n	Yield (%)	m.p. (°C)
3a	tert-butyl	1	87	Oil
3b	tert-butyl	2	88	Oil
3c	benzyl	1	92	115
3d	alloc	1	93	105
4a	tert-butyl	1	70	63
4b	tert-butyl	2	73	112
4c	benzyl	1	83	119
4d	alloc	2	84	124
4e	alloc	1	86	165

2. α -Diazomethylketones

Diazomethylketones derived from N -protected amino acids are valuable precursors for various classes of pharmaceutically and synthetically important compounds such as halomethylketones and epoxides. The insertion of this group into amino acid side chains is key to introducing heterocycles. Starting with **1**, the ω - COOH was converted to diazoketone by treating its mixed anhydride with ethereal solution of diazomethane (Scheme 3) [5]. The alkaline hydrolysis with 1N LiOH cleaved the oxazolidinone ring to furnish the free α - COOH .

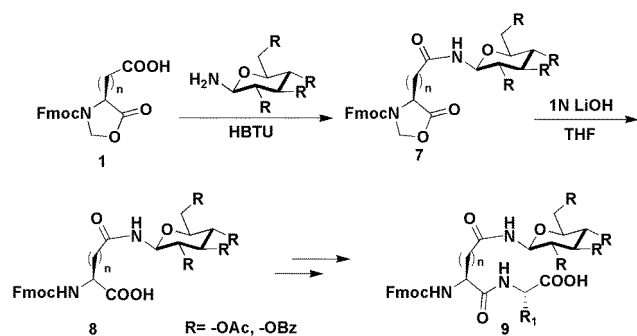


Scheme 3

3. Glycosylated amino acids

The glycopeptides constitute a prominent class of important biomolecules due to their involvement in biochemical processes like cell-cell recognition, adhesion and signaling. To meet the increasing demand for glycopeptides for biological studies, researchers have turned attention towards the use of synthetic and chemically modified glycopeptides.

An easier protocol consisting of fewer synthetic steps has been developed for the incorporation of amide bond between sugar and peptide segments (Scheme 4) [6]. Starting with **1**, the side chain COOH group was coupled to suitably protected sugar-1-amine using HBTU. The product after base hydrolysis produced the glycosylated amino acid with Fmoc-protected N-terminus and free α -COOH end (Table 2). The **8** was then coupled with bis-TMS derivative of an amino acid to incorporate the glycosylated amino acid moiety in to peptide chain and the resulting glycosylated peptide acid **9** was isolated as crystalline solids after a simple work up in high yield.



Scheme 4

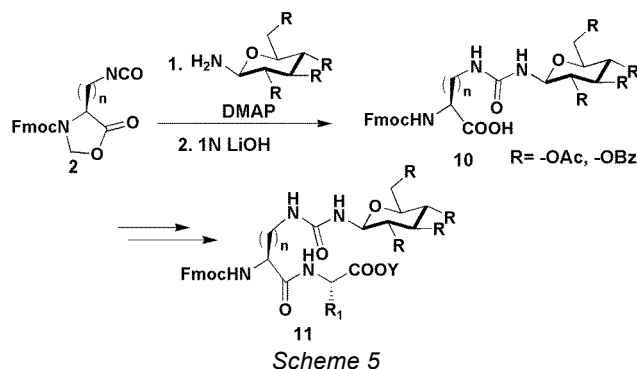
Table 2. List of glycosylated amino acids

Compd.	n	Sugar moiety	Yield (%)	m.p. (°C)
7a	1	tetra-O-acetyl- β -D-glucopyranosyl-1-amine	87	87
7b	2	tetra-O-acetyl- β -D-galactopyranosyl-1-amine	86	115
7c	1	tetra-O-benzoyl- β -D-glucopyranosyl-1-amine	85	108
7d	2	tetra-O-benzoyl- β -D-glucopyranosyl-1-amine	86	138
7e	1	tetra-O-acetyl- β -D-galactopyranosyl-1-amine	90	130
8a	1	tetra-O-acetyl- β -D-glucopyranosyl-1-amine	92	98
8b	2	tetra-O-acetyl- β -D-galactopyranosyl-1-amine	86	135
8c	1	hepta-O-acetyl- β -D-maltosyl-1-amine	82	110
8d	2	hepta-O-acetyl- β -D-maltosyl-1-amine	86	120
8e	1	tetra-O-acetyl- β -D-glucopyranosyl-1-amine	86	75

4. Urea linked glycosylated amino acids

Neoglycopeptides are the synthetic derivatives of glycopeptide wherein the covalent linkage between the carbohydrate and peptide is replaced by non-native bonds. They have attracted medicinal and organic chemists because of their ability to act as low molecular weight probes to understand glycopeptide roles in biochemical processes. The presence of unnatural linkage in their structure makes them resistant towards metabolic degradation. The interest towards incorporation of urea into glycopeptides is due to the strong hydrogen bonding capacity of urea linkage which makes the glycopeptides more hydrophilic thereby increasing bio-availability [7].

The earlier syntheses of urea linked glycopeptides have employed the reaction of sugar isocyanate with amino/peptidyl esters. An improved method has been developed by making use of Curtius rearrangement of the -COOH group of peptide segment. The protocol involves generation of isocyanate from the carboxyl group of **1** via the Curtius rearrangement of corresponding acyl azide [8,9] and coupling with O-acyl/benzoyl sugar-1-amine (Scheme 5) [10]. Treatment of the product with 1N LiOH has produced the required urea linked glycosylated amino acids which allows the peptide chain extension from either C or N-terminus (Table 3).



Scheme 5

Table 3. List of urea linked glycosylated amino acids

Compd.	n	Sugar moiety	Yield (%)	m.p. (°C)
10a	1	tetra-O-acetyl- β -D-glucopyranosyl-1-amine	82	123
10b	2	tetra-O-acetyl- β -D-galactopyranosyl-1-amine	85	135
10c	2	tetra-O-benzoyl- β -D-galactopyranosyl-1-amine	85	129
10d	1	tetra-O-benzoyl- β -D-glucopyranosyl-1-amine	82	127
10e	1	hepta-O-acetyl- β -D-maltosyl-1-amine	89	105
10f	2	tetra-O-acetyl- β -D-glucopyranosyl-1-amine	85	99
10g	2	hepta-O-acetyl- β -D-maltosyl-1-amine	86	107

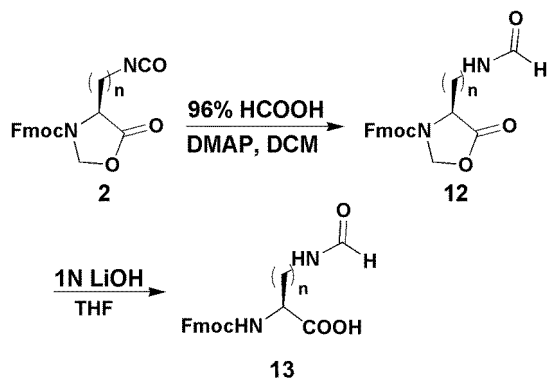
Further, the incorporation of these urea linked glycosyl amino acids in to peptides was demonstrated by coupling them with amino acid methyl ester using DCC/HOBt method. The resulting neoglycopeptides were obtained as analytically pure ones in 80-92% yield after simple purification (Table 4).

Table. 4 List of urea linked glycopeptides

Entry	R ₁	Sugar moiety	Yield (%)
11a (n=1)	CH(CH ₃) ₂	tetra-O-acetyl-β-D-glucopyranosyl-1-amine	82
11b (n=1)	CH ₃	tetra-O-benzoyl-β-D-galactopyranosyl-1-amine	83
11c (n=2)	CH(CH ₃) ₂	tetra-O-acetyl-β-D-glucopyranosyl-1-amine	85
11d (n=2)	CH ₃	tetra-O-benzoyl-β-D-galactopyranosyl-1-amine	86

5. N-Formamides

N-Formamides exhibit wide range of applications as starting materials for compounds such as formamidines, isonitriles, etc. and also as useful reagents in Vilsmeier formylation and asymmetric allylation. They are the precursors to an important class of compounds, the isonitriles which are the components of Pessirani and Ugi's multicomponent reactions. In peptide chemistry, the reported formylation protocols have aimed at converting the α-amine into N-formamide using reagents like formic acid, ZnO, KF-Al₂O₃, chloral and carbodiimide mediated formylation. On the other hand, we have recently reported the novel conversion of the carboxyl group of N-Fmoc-α-amino acid into N-formamide. The procedure involves the formylation of N-Fmoc amino acid derived isocyanate using 96 % formic acid and catalytic DMAP under Goldsmith-Wick condition. The reaction has been extended for the α-COOH group of **1** to append the formamide group into the side chain. (Scheme 6) [11].



Conclusions

In conclusion, the 5-oxazolidinones derived from aspartic and glutamic acids have been employed as efficient intermediates for a variety of site selective chemical modifications of the ω-COOH group. Adopting the Fmoc-oxazolidinone approach has conferred the following synthetic merits:

- Fmoc-5-oxazolidinones can be prepared rapidly and easily in higher yields than the Z and Boc counterparts.
- The use of bidentate reagent for simultaneous protection for α-amine and 1-carboxyl group avoids multi-step synthesis as required in conventional orthogonal protection and deprotection strategy.
- The isocyanates of α-amino acids are easily accessible as there will be no internal urea formation through the trapping of isocyanate by the nitrogen of the 5-oxazolidinone ring.
- The regeneration of the 1-carboxyl group employable in peptide synthesis can be achieved through site selective alkaline hydrolysis of the oxazolidinone moiety.

Acknowledgments

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