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Hydrazinolysis of Dde: Complete Orthogonality with Aloc Protecting Groups[1]

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Abstract: Hydrazinolysis of Dde group is troublesome in the presence of Aloc protected peptides. We elucidate that reduction of the Aloc double bond occurs both in solution and on solid support preventing the subsequent Aloc deprotection and resulting in mixtures difficult to purify. We report here that addition of allyl alcohol as scavenger circumvents this side reaction and provides a complete orthogonality between Dde and Aloc in solution and solid phase peptide synthesis.

The increasing number of orthogonal protecting groups available for amino acids led to the development of new strategies for the synthesis of more and more complex peptides in solution as well as on the solid support under mild conditions. For instance, head to tail cyclisation[2]or side-chain bridges[3] of peptides can now be performed directly under SPPS conditions using three-dimensional orthogonal strategy. Additionally, combination of protecting groups represents also a prerequisite to regioselectively addressable peptides for the construction of libraries with positional scanning format[4] as well as for new functional scaffold peptides[5]. Our laboratory has concentrated on the development of peptides carrying various combinations of protecting groups cleavable by acid (Boc), base (Fmoc), Pd (Aloc[6]), or nucleophiles (Dde[7]). These associations provide us an unique opportunity to assess the real orthogonality between these protecting groups while deprotecting one in the presence of all the others. We describe in this communication a side reaction (hydrogenation of the Aloc alkene function) occurring during the hydrazinolysis of the Dde group in the presence of Aloc protecting group and elaborate a convenient procedure for the orthogonal use of these protecting groups in peptide synthesis.

During the removal of Dde protecting group (2% hydrazine solution in DMF[7]) on Aloc and Dde protected peptides, the formation of a side product (Figure 1) of mass two units greater than the expected free peptide[8] was observed (Scheme 1) presumably arinsing from partial reduction of the Aloc double bond. In accordance with this hypothesis, subsequent acylation of the mixture afforded products retaining this difference while conditions used to remove the Aloc group were found to leave the by-product unaffected. Complications with large protected peptides arise due to the difficulty for separating the two products by HPLC. These observations together with the increasing requirement of orthogonal protection techniques prompt us to study this critical step using the model peptide Ac-Lys(Dde)-Ala-Lys(Aloc)-NH_2 **1**.

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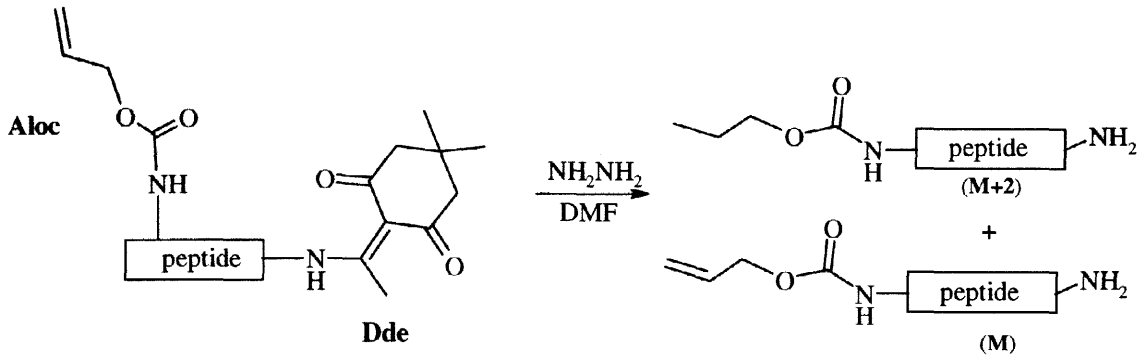
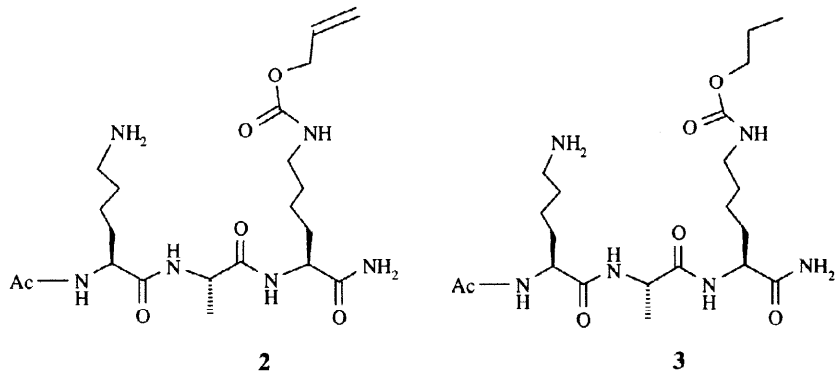


Scheme 1.

Peptide **1** was obtained in 93% yield using standard N-Fmoc strategy[9]. The desired Dde-free peptide **2** (Scheme 2) was synthesised by treating **1** with 10% hydrazine solution in the presence of allyl alcohol (see below).



Scheme 2. Structure of the expected Dde-free peptide **2** and by product **3**.

The by product **3** (Scheme 2) was obtained from **1** by treatment with 10% hydrazine solution for 3 hours and HPLC purification. Compound **3** corresponds to the N-propyloxycarbonyl-ε-lysine as clearly inferred by mass spectroscopy (**2** : M=472 and **3** : M= 474 $g.mol^{-1}$) and by NMR spectroscopy[10]. The kinetics of the hydrazinolysis of **1** were performed in solution and monitored by HPLC (Figure 1) as well as mass spectroscopy. The formation of the desired Dde-free peptide **2** occurred rapidly within 5 min although a small extent (5%) of **3** is already present in the mixture (Figure 1-a).

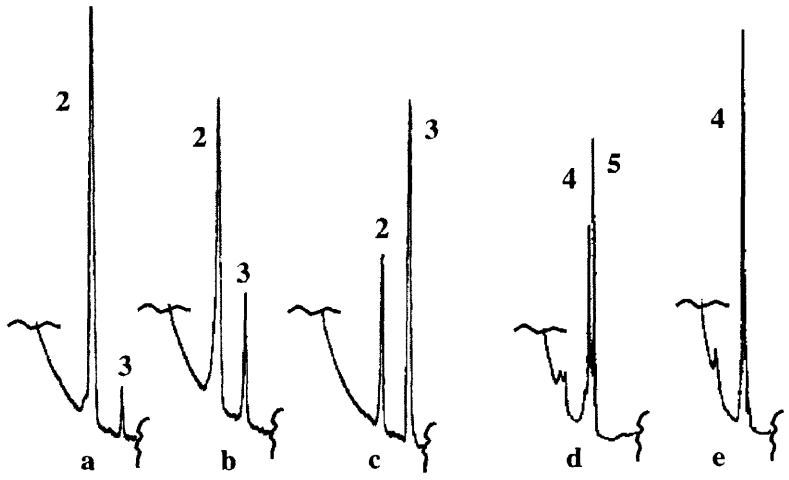


Figure 1. HPLC profile during hydrazinolysis of peptide **1** (**a**, **b** and **c**) and **4** (**d** and **e**).

It worth noticing that at this stage the starting peptide **1** has reacted to completion and hence the additional reaction time results in formation of side product **3** (Figure 1-b : 25 min 22% **3** and 1-c : 225 min 75% **3**). This is supported by the longer reaction time required to increase the content of **3** and the fact that **3** is obtained upon hydrazine treatment of pure **2**. These observations indicate that the hydrogenation of the Aloc alkene moiety occurs probably via diimide formation. Diimide is commonly formed *in situ* by dismutation of hydrazine which can originate from traces of oxydizing agents or from basic conditions as observed for the decomposition of tosylhydrazine derivatives[11]. However, the use of carefully degassed solutions did results in major improvements.

The utilisation of a scavenger appeared advantageous compared to the use of short reaction time for minimizing the sideproduct, being more general[12] and easy to handle in solid phase and solution synthesis. In sharp contrast to the standart conditions proposed for the removal of Dde[7], we found that the reaction in the presence of allyl alcohol proceeds cleanly and moreover, the deprotected peptide **2** is completely stable to hydrazine (10% NH_2NH_2, 200 equiv allyl-OH) even for prolonged reaction time[13]. The method is readily adaptable under solid phase peptide synthesis conditions. For example, whereas treatment of the resin-bound peptide **1** by 2% hydrazine in DMF afforded 34% of the sideproduct, the addition of allyl alcohol provided the expected peptide **2** in pure form after cleavage from the resin.

Finally, we successfully exploited these reaction conditions for the removal of Dde groups on a cyclodecapeptide protected by Boc, Aloc and Dde groups[5]. The formation of the hydrogenated-peptide **5** (Figure 1-d : **5** 55%) did not allow preparative purification of the expected product **4** by HPLC. As depicted in Figure 1-e, the addition of allyl alcohol (200 equiv) completely suppressed the formation of **5** and the desired peptide **4** was obtained in pure form whithout the need of further purification[9].

In summary, removal of Dde group by hydrazine results in side reaction in peptide containing Aloc protecting group. The addition of allyl alcohol as scavenger prevents entirely the hydrogenation of the Aloc

protecting group. Under these conditions, Dde and Aloc groups can be used as fully orthogonal protection techniques in solution and solid phase peptide synthesis.

Acknowledgements: The authors gratefully thank the Swiss National Science Foundation for financial support.

References and Notes:

1. Abbrevations used in this article: Aloc, allyloxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; Boc, *t*-butoxycarbonyl; Dde, 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl.
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8. ESI-MS analysis have been performed on a HPLC-ESI-MS coupled apparatus.
9. A typical procedure was as follows: Using N-Fmoc-Rink-amide MBHA® resin (1.0g, 0.45 mmol), the linear protected tripeptide was assembled using N^{α}-Fmoc-protected amino acids (1.0 mmol), PyBOP (2.5 mmol) and diisopropylethylamine (DIPEA) (4.0 mmol) in DMF for 30 min. The completeness of each coupling was confirmed by the Kaiser test. The N^{α}-Fmoc-protecting groups were removed by treatment with piperidine (20% v/v in DMF, 5 min, 3 cycles); the completeness of each deprotection being verified by the UV absorption of the piperidine washings at 302 nm. The protected peptide was then cleaved from the resin with 95% TFA in DCM (15 ml, 5 cycles of 5 min). The solvent was removed under reduced pressure and the residue taken in a water/acetonitrile and lyophilised to afford peptide **1** as a white powder (234 mg, 93%). ESI-MS : m/z=635.6 $[M+H]^+$. HPLC (C18 Vidac Nucleosil 300Å, 5µm; 214 nm; (A : H_2O/TFA 1%, B : H_2O/CH_3CN/TFA 9/90/1) 40% B to 100% B in 30 min) t=37.2 min.
10. The product was characterised by ^1H NMR on an ARX-400 Brucker spectrometer. The absence of the allyl spin system (5.9, 5.3, 5.2 and 4.4 ppm from **1** and **2**) and the presence of new signals due to the propyl system (1.0 ppm, 3H t ^3J = 4.3Hz; 1.71, 2H m; 4.02ppm, 2H t, 6.6 Hz) are consistent with attribution of **3**.
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12. The side reaction have been observed with larger molecules as well as with peptides containing several Dde and / or Aloc protected lysine side-chain. For each of them a different reaction rates is observed.
13. The product was stable during 24h as inferred by HPLC.