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An Appraisal of New Variants of Dde Amine Protecting Group for Solid Phase Peptide Synthesis

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Abstract: A series of new variants of Dde, substituted at the exocyclic alkene position have been developed and their application in solid phase peptide synthesis assessed. The new derivatives offer complete resistance towards 20% piperidine in DMF and provide a considerable barrier towards *N*→*N'*-intramolecular migration. © 1998 Elsevier Science Ltd. All rights reserved.

Since its introduction four years ago, the primary amine protecting group 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde)[1] has become a valuable tool for the construction of, *e.g.* branched, cyclic and side-chain modified peptides by Fmoc/^{t}Bu solid phase approach.[2] This is due to the ease with which it is removed with 2% *v/v* hydrazine in DMF and its relative stability to TFA and piperidine–DMF.[2] Furthermore, the selective primary amine protecting properties of Dde have been successfully exploited for the solid phase synthesis of polyamines such as spider toxins[3] and trypanothione[4].

Whilst the stability that *N*-Dde displays towards 20% *v/v* piperidine–DMF is acceptable for most applications, a small loss of Dde does occur during each deprotection cycle, which can seriously compromise the purity of large peptides. Dde has also been reported to undergo intramolecular *N*→*N'*-migration from a side-chain or α-amino group to the ε-amino function of lysine,[5] resulting in the scrambling of the group within the peptide chain. These limitations have led us to evaluate a series of Dde variants substituted at the exocyclic alkene position. We wish to report here the preparation of these new derivatives and the assessment of their utility in solid phase peptide synthesis.

The Dde derivatives (**1a-d**) were readily synthesised by acylation of dimedone with the appropriate DCC–DMAP activated carboxylic acids,[6] followed by condensation with Fmoc-Lys-OH in refluxing ethanol for 60 h to afford the orthogonally protected lysine derivatives Fmoc-Lys(DdX)-OH (**2a-d**).[7] In order to assess the relative stabilities of each of the *N*-DdX variants, a series of resin bound model dipeptides Boc-Arg(Mts)-Lys(DdX)-O-Clt PS were assembled using standard techniques[8] and then subjected to treatment with 20% *v/v* piperidine–DMF in a continuous-flow manner (Scheme 1).

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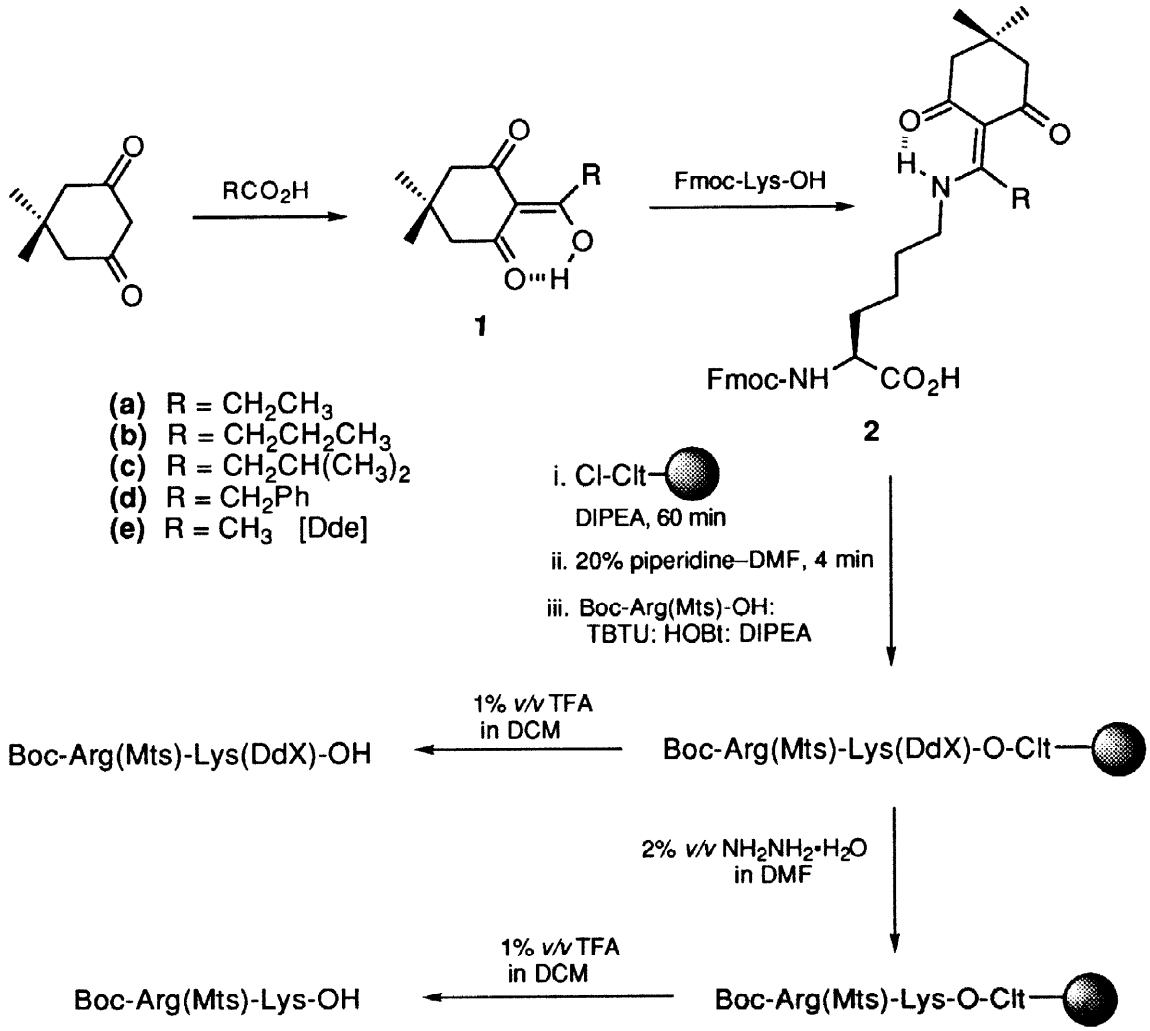
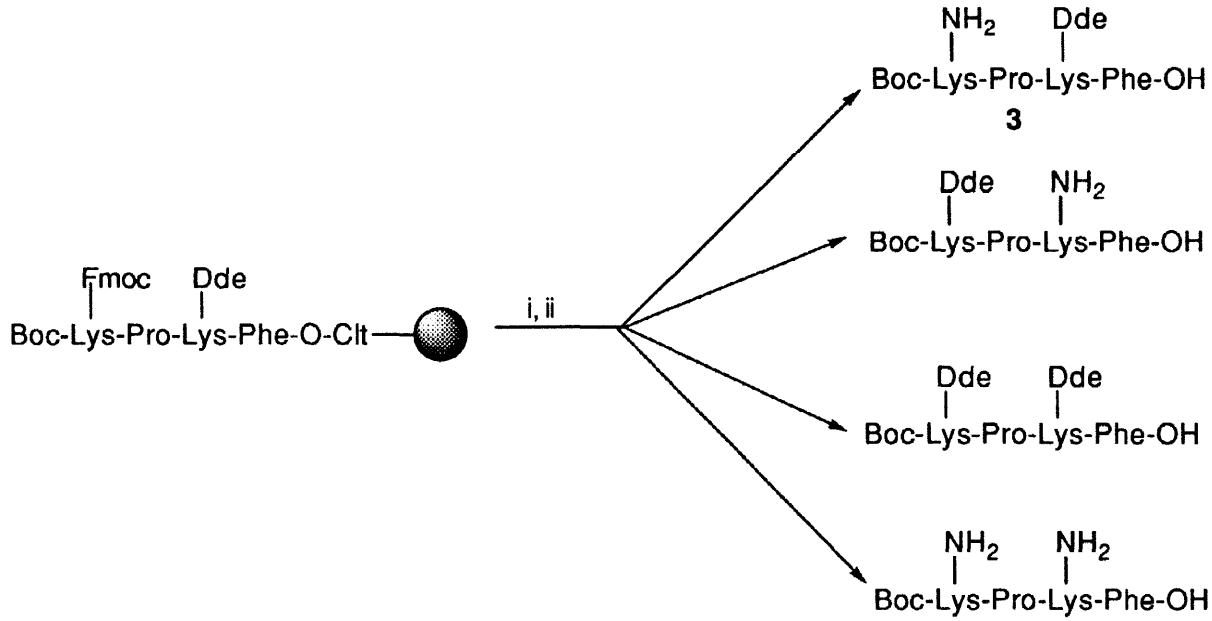


Scheme 1.

Resin samples were collected after 2 and 4 h, respectively and each treated with 1% TFA–DCM to release the crude dipeptides, which were analysed by RP-HPLC. In each case the elution profile showed exclusively a single peak, identified by ES-MS as Boc-Arg(Mts)-Lys(DdX)-OH; no trace (< ± 0.2%) of the premature deprotection product, Boc-Arg(Mts)-Lys-OH, was observed. The remaining portion of each of the peptidyl resins were then subjected to 20% *v/v* piperidine–DMF for a further 36 h. After cleavage from the resin, RP-HPLC analysis of the crude peptidic material obtained in each instance still indicated no loss of the protecting group, within the limits of the analytical methodology. In contrast, the corresponding *N*-Dde-protected model dipeptide gave 3% and 6.5% cleavage over 2 and 4 h, respectively.

In order to ascertain whether the increased stability to base (piperidine) affects the rate of deprotection with 2% *v/v* hydrazine•H_2O–DMF, the deprotection profile was monitored spectrophotometrically at 290 nm and demonstrated a smooth and efficient removal of the *N*-DdX groups within a 10 min period (flow rate at 2.5 ml min^{-1}).

Recently, the *N*-Dde protecting group has been reported, under certain conditions, to undergo both intra- and intermolecular *N*→*N'*-migration, resulting in a number of resin-bound sequences as illustrated for the example shown in Scheme 2.[5]



Reagents: i. 50% *v/v* piperidine in DMF; ii. 1%TFA in DCM

Scheme 2. Scrambling of Dde between ε-NH_2 functions of different lysines

To establish the extent of *N*-DdX migration, the following study was conducted using the sterically hindered isovaleryl dimedone derivative, *N*-Ddiv (**2c**). The peptidyl resin Boc-Lys(Fmoc)-Pro-Lys(Ddiv)-Phe-O-Clt PS was assembled in the usual manner and treated with 50% *v/v* piperidine–DMF for 4 and 9 h. After cleavage from the solid support, RP-HPLC analysis of the crude products indicated, even under such forcing conditions, the presence of *ca.* 94 and 84%, respectively of Boc-Lys-Pro-Lys(Ddiv)-Phe-OH. In comparison, similar treatment (50% piperidine–DMF for 4 h) of the corresponding Dde-protected resin-bound peptide gave only 33% of Boc-Lys-Pro-Lys(Dde)-Phe-OH (**3**).[5] Since during the routine construction of branched peptides, the *N*-Ddiv protection in the presence of a side-chain unprotected lysine residue would normally be exposed to 20% v/v piperidine–DMF for only one deprotection cycle, negligible *N*→*N'*-migration of Ddiv is expected.

In conclusion, we anticipate that these new derivatives, in particular the *N*-Ddiv protecting group, will offer complete stability towards the conditions necessary for the removal of *N*-Fmoc and thus will serve as ideal orthogonal amine protecting groups in solid phase peptide synthesis. Furthermore, these amine protecting groups have obvious application for the assembly of polyamine libraries.

Acknowledgements

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References and Notes

Abbreviations: Amino acids and peptides follow the IUPAC-IUB nomenclature where applicable (*Eur. J. Biochem.* **1984**, 9-37); Boc, *tert*-butoxycarbonyl; Clt, 2-chlorotrityl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCM, dichloromethane, DIPEA, *N,N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; ES-MS, electrospray mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; Mts, mesitylene 2-sulphonyl; PS, polystyrene; TBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid.

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7. *Typical procedure for preparation of Fmoc-Lys(Ddiv)-OH* (**2c**)

   TFA (15 µl, 0.195 mmoles) was added to a stirred suspension of Fmoc-Lys-OH (0.72 g, 1.95 mmoles) and **1c** (0.874 g, 3.9 mmoles) in ethanol (15 ml) at room temperature. The mixture was then refluxed for 60 h and the reaction monitored by analytical TLC. The solvent was rotary-evaporated, and the orange residue redissolved in ethyl acetate (50 ml). The organic solution was washed with 1 M aqueous $KHSO_4$ (2 x 40 ml), dried and concentrated *in vacuo* to afford a yellow oil. The oil was triturated with hexane to remove any unreacted **1c** and then crystallised from ethyl acetate–hexane to afford the desired product **2c** as a white crystalline solid (64%); m.p. 69-71°C.

   ^{1}H NMR (250 MHz ,$CDCl_3$): δ 0.98 (d, 6 H, *J* 6.82 Hz, $(CH_3)_2CH$), 1.02 (s, 6 H, 2 x CH_3), 1.56 (m, 2 H, Lys C$^\gamma$H$_2$), 1.75 (m, 2 H, Lys C$^\delta$H$_2$), 1.94 (m, 1 H, $CH(CH_3)_2$), 2.34 (s, 4 H, 2 x CH_2), 2.96 (m, 2 H, CH_2CH), 3.47 (m, 2 H, Lys C$^\varepsilon$H$_2$), 4.21 (t, 1 H, *J* 7.04 Hz, Fmoc CH), 4.38 (d, 2 H, *J* 7.07 Hz, Fmoc CH_2), 4.47 (m, 1 H, Lys C$^\alpha$H), 5.88 (d, 1 H, *J* 8.01 Hz, Lys N$^\alpha$H), 7.30-7.76 (m, 8 H, Fmoc ArH), 7.95 (br s, 1 H, Lys CO_2H), 13.64 (s, 1 H, Lys N$^\varepsilon$H).

   m/z (+ve ES-MS) 574.5 (MH^+, $C_{34}H_{42}N_2O_6$ requires 574.70).
8. Compounds **2a-d** were attached to 2-chlorotrityl chloride polystyrene by stirring the protected amino acid (1 eq.) and resin in DCM for 1 h in the presence of 2 eq. of DIPEA. After this time, unreacted chloride sites were "eliminated" by adding methanol (1 ml) and leaving the mixture to stir for a further 10 min. The resulting resins exhibited loadings which were typically > 0.7 mmol g^{-1}.

   N-Fmoc removal was effected by treatment with 20% *v/v* piperidine in DMF for 7 min (flow rate 2.5 ml min^{-1}). Acylation reactions were carried out using a 4-fold excess of the appropriate protected amino acid, activated with TBTU (1 eq.)–DIPEA (2 eq.)–HOBt (1 eq.) in DMF.

   Boc-Arg(Mts)-Lys(Ddiv)-OH, ES-MS MH^+ found 791.3, requires 791.3; Boc-Arg(Mts)-Lys-OH, MH^+ found 585.3, requires 585.8.