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Versatile Dde-based primary amine linkers for solid phase synthesis

Siri Ram Chhabra, Azra N. Khan and Barrie W. Bycroft*

School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, England

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Abstract

Linkers based on the Dde primary amine protecting strategy have been developed and their utility demonstrated in the solid phase synthesis of a naturally occurring spider toxin. The linkers are stable to both acid and base conditions and cleaved either with 2% v/v hydrazine hydrate or by transamination with a volatile primary alkylamine in a variety of organic solvents.

Keywords: Amines; solid-phase synthesis; supported reagents/reactions.

With the advent of combinatorial methodologies for the generation of compound libraries, solid phase chemistry hitherto confined predominantly to the synthesis of peptides and oligonucleotides has undergone a renaissance in recent years [1-3]. Amongst the many tools required, linkers occupy a key position. The ideal linker molecule is easily prepared, attached to the support, and allows the facile ligation of the starting material. In addition the latter linkage needs not only to be stable to a wide range of reaction conditions, but also permits the quantitative release of the end-product with mild reagents which can readily be removed.

In general linkers have been developed from protecting groups for various functionalities. The initial focus on carboxyl attachment, stemming out of peptide chemistry, has now widened to encompass alcohols [4-8], aldehydes [9], amidines [10] and amines [11-18]. In the main the latter have centred around carbamate chemistry, but more recently included anchors based on formamidines [17] and sulfonamides [18]. Although useful, all of these except sulfonamides are either stable towards acid or base conditions but not both. Recently a linker for primary amines based on the Dde protecting group [19] which is essentially stable to acid and base conditions has been described by Bannworth et al. [20]. Here we report an alternative and versatile approach based on Dde protection which addresses the major requirements for linker molecules.

1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) [19], developed in our laboratories, has shown promise in the assembly of atypical peptides and biomolecules [21-24]. The value of this new protecting group is in part due to its complete stability towards a

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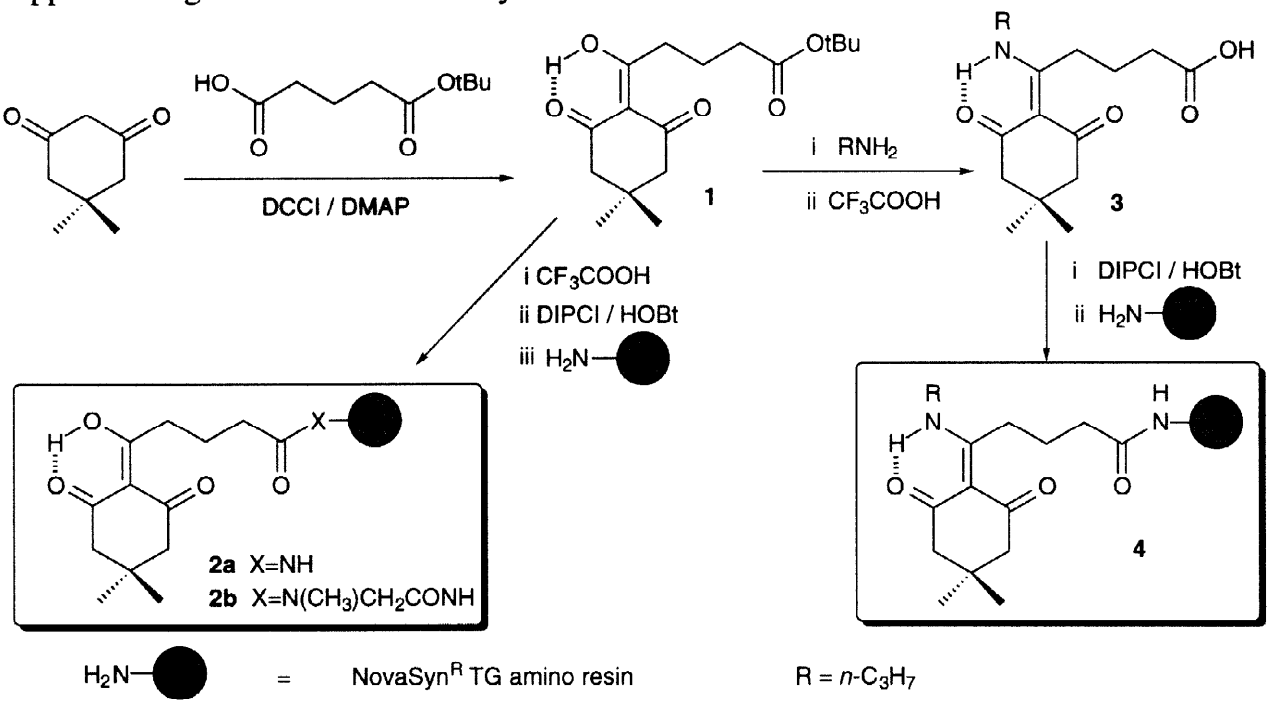
variety of acid conditions, compatibility with uronium type coupling reagents and ease of removal by 2% v/v hydrazine hydrate in DMF. It is also sufficiently stable to base for it to be used orthogonally with Fmoc protection in peptide synthesis. However it lacks complete stability to these conditions (*ca.* 12% loss after 6 h contact with 20% piperidine in DMF at room temperature) and as such required fine-tuning for application as a linker molecule.

As a result of stability studies on a number of Dde derivatives [25], we have shown that the resistance to piperidine becomes effectively complete if the alkyl side-chain is extended. This also proved to be the case for the Dde based affinity tags which we have recently reported [26]. Following these observations, a simple strategy for stable amine linkers based on acylation of dimedone with mono-protected dicarboxylic acids was clearly apparent (**Scheme1**). This approach not only provides the Dde unit with a terminal carboxylic acid on the side-chain for loading onto the solid support, but also has an adjustable spacer arm which imparts complete base stability for the amine attachment. In this context, it is noteworthy that the Dde based linker previously described [20], was coupled onto the support through one of the 4-dimethyl substituents of the dimedone residue.



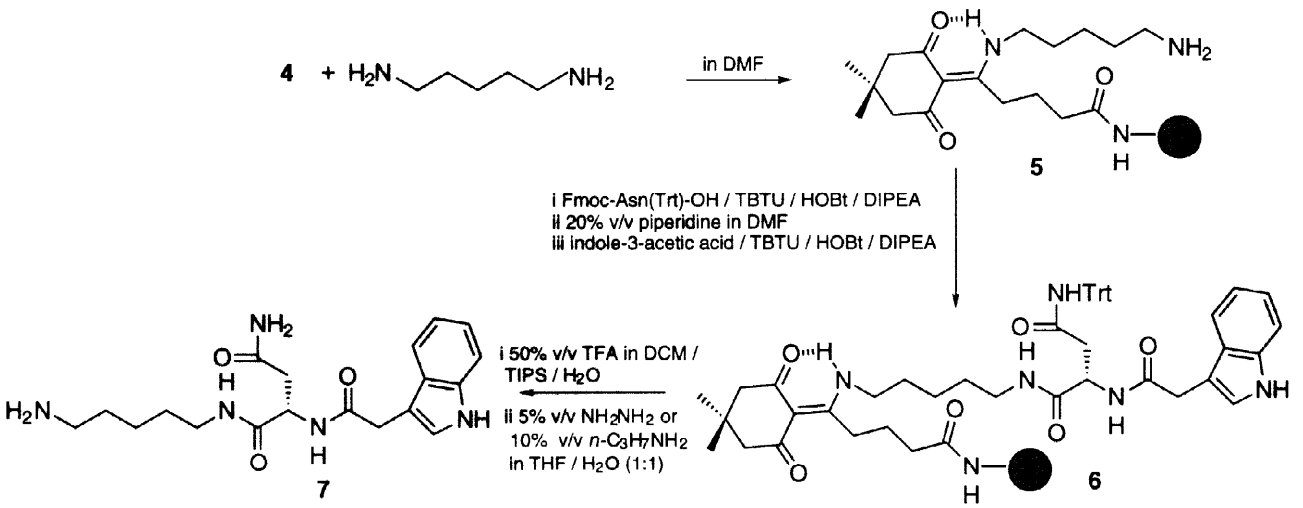
Scheme 1: Synthesis of amine linkers and their attachment to the resin

The synthesis of **1** proceeded smoothly by the method indicated, and following deprotection the product was readily coupled onto a NovaSyn^R TG amino resin to afford the immobilised linker system **2a**. Although the acylation of resin amine appears to be chemoselective, some amidation *via* the Dde centre could not be ruled out. In order to avoid this unwanted side reaction, sarcosine was first linked to the resin to provide a terminal secondary amine group which is incapable of reacting with the Dde unit [24]. The extended linker **2b** was formed attaching the free acid derived from **1** to the resin in the manner previously described. Alternatively the 2-acyldimedone derivative **1** was first masked with

n-propylamine and deprotected to yield **3**[1] which also could be secured onto the amine support only in the orientation shown **4**.

The loading capacity of all three systems **2a, 2b** and **4** was determined by individually loading them with ethylenediamine and derivatising the free amine function with Fmoc.OSu. Employing standard spectrophotometric measurements on the Fmoc-derived product, released upon treatment with 20% piperidine in DMF, the loadings were in all three cases >85% of the theoretical . The stability of the amine attachment towards 20% piperidine in DMF and 50% TFA in DCM was demonstrated by exposing the ethylenediamine charged linkers to these reagents over a period of 24 h. The resins were washed, derivatised with Fmoc.Su and the loading redetermined. Within the limits of experimental error these were essentially unchanged.

The utility of these linkers is illustrated by the synthesis of pseudoargiopinine III, a pharmacologically active component from the venom of the spider *Argiope lobata* [27] **Scheme 2.**



Scheme 2: Solid-phase synthesis of Pseudoargiopinine III (**7**)

Treatment of the resin **4** with 1,5-pentanediamine in DMF at room temperature overnight resulted in a smooth transamination to yield **5.** The usual cycles of solid phase peptide chemistry were employed to couple and deprotect Fmoc-L-Asn(Trt).OH and the synthesis was completed by acylating with indole-3-acetic acid. The efficiency of both coupling steps was monitored using the TNBS amine test. Acidolysis (50% TFA in DCM with TIPS and H_2O as scavengers) of **6** removed the trityl side chain protection and the

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[1]*Preparation of linker molecule* **3**: *n*-Propylamine (1.64 ml, 20 mmol) was added to a solution of **1** (0.62 g, 2 mmol) in DCM (10 ml) and the mixture stirred at room temperature for 2 h. Removal of solvent and excess amine in vacuo afforded the aminated product as an oil. The oil was redissolved in 50% TFA/DCM (10 ml) and the solution left at room temperature for 3 h. Evaporation of solvent and trituration of the residue with diethyl ether/hexane gave the desired free acid **3** as a white crystalline solid (0.196 g, 90%), m.p. 131-132 °C ; R_f 0.46 (SiO_2, 1% AcOH/EtOAc). ES-MS *m/z* 296.91 (M+H, $C_{16}H_{25}NO_4$ requires *m/z* 295.37); δ_H (250 MHz, $CDCl_3$) 1.04 (6 H, s, ring 2xCH_3), 1.05 (3 H, t, *J* 7.0 Hz, CH_3), 1.68-1.82 (2 H, m, CH_2CH_3), 1.81-1.93 (2 H, m, CH_2.CH_2.CO_2H), 2.39 (4 H, s, ring 2xCH_2), 2.59 (2 H, t, *J* 5.0 Hz, CH_2CO_2H), 3.01-3.08 (2 H, m, NH.CH_2) , 3.44-3.52 (2 H, m, =C.CH_2), 13.44 (1 H, bs, NH).

product was cleaved from the resin in THF-H_2O (1:1) containing either 5% hydrazine hydrate or 10% *n*-propylamine. RP-HPLC analysis of the crude product **7** showed the product to be > 90% pure and its identity following HPLC was confirmed by ^1H NMR and ES-MS which displayed *m/z* 374.35 (M+H, $C_{19}H_{27}N_5O_3$ requires *m/z* 373.45).

In summary, we have described the synthesis and applications of functionalised 2-acyldimedone derivatives which can be readily attached to a solid support and serve as primary amine linkers. The robust nature of these linkers allows diverse chemical reactions to be accomplished without significant leakage of material from the resin. A further notable feature is the alternative cleavage conditions using *n*-propylamine which, unlike hydrazine, is sufficiently volatile to be removed with the solvents. The opportunities for exploitation of these linkers for the preparation of small molecule libraries carrying both acid and base sensitive groups is self evident.

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Abbreviations: Amino acids and peptides follow the IUPAC-IUB nomenclature where applicable (*Eur. J. Biochem.* **1984**, 9-37); DIPCI, *N,N'*-diisopropylcarbodiimide; DCCI, *N,N'*-dicyclohexylcarbodiimide; DCM, dichloromethane, DIPEA, *N,N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; ES-MS, electrospray mass spectrometry; Fmoc.OSu; *N*-(9-fluorenylmethoxycarbonyloxy)succinimide; HOBt, 1-hydroxybenzotriazole; TBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; Trt, trityl.

References

[1] Brown R. Contemp. Org. Synth. 1997;4:216-226.
[2] Hermkens PHH, Ottenheijm HCJ, Rees DC. Tetrahedron. 1997;53:5643-5678.
[3] Fruchtel JS, Jung G. Angew. Chem. Int. Ed. Engl. 1996;35:17-42.
[4] Liu GC, Ellman JA. J. Org. Chem. 1995;60:7712-7713.
[5] Chen CX, Randall LAA, Miller RB, Jones AD, Kurth MJ. J. Am. Chem. Soc.1994;116:2661-2662.
[6] Boojamra CG, Burow KM,, Ellman JA. J. Org. Chem. 1995;60:5742-5743.
[7] Chenera B, Finkelstein JA, Veber DF. J. Am. Chem. Soc.1995;117:11999-12000.
[8] Meyers HV, Dilley GJ, Durgin TL, Powers TS, Winssinger NA, Zhu H, Pavia MR. Molecular Diversity. 1995;1:13-20.
[9] Wang GT, Li S, Widenberg N, Kraft GA, Kempf DJ. J. Med. Chem.1995;38:2995-3002.
[10] Roussel P, Bradley M. Tetrahedron Lett. 1997;38:4861-4864.
[11] Hernandez AS, Hodges JC, J. Org. Chem. 1997;62:3153-3157.
[12] Dressman BA, Spangle LA, Kaldor SW. Tetrahedron Lett. 1996;37:937-940.
[13] Gouilleux L, Fehrentz JA, Winternitz F, Martinez J. Tetrahedron Lett. 1996;37:7031-7034.
[14] Alsina J, Rabanal F, Giralt E, Albericio F. Tetrahedron Lett. 1994;35:9633-9636.
[15] Kaljuste K, Unden A. Tetrahedron Lett. 1996;37:3031-3034.
[16] Ho CY, Kukla MJ. Tetrahedron Lett. 1997;38:2799-2802.
[17] Furth PS, Teitman MS, Cook AF. Tetrahedron Lett. 1997;38:5403-5406.
[18] Kay C, Murray PJ, Sandow L, Holmes AB Tetrahedron Lett. 1997;38:6941--6944.
[19] Bycroft BW, Chan WC, Chhabra SR, Hone ND. J. Chem. Soc., Chem. Commun. 1993:778-779.
[20] Bannworth W, Huebscher J, Barner R. Bioorg. Med. Chem. Lett. 1996;6:1525-1528.
[21] Bloomberg, GB, Askin D, Gargaro, AR, Tanner, MJA. Tetrahedron Lett. 1993;34:4709-4712.
[22] Eichler J, Lucka AW, Houghten RA.. Peptide Research. 1994;7:300-307.
[23] Bycroft BW, Chan WC, Hone ND, Millington S, Nash I. J. Am. Chem. Soc. 1994;116:7415-7416.
[24] Nash I, Bycroft BW, Chan WC. Tetrahedron Lett. 1996;37: 2625-2628.
[25] Chhabra SR, Hothi B, Evans DJ, White PD, Bycroft BW, Chan WC. Tetrahedron Lett. 1998;39:1603-1606.
[26] Kellam B, Chan WC, Chhabra SR, Bycroft BW. Tetrahedron Lett. 1997;38:5391-5394.
[27] Grishin EV, Volkova TM, Arseniev AS. Toxicon. 1989;27:541-549.