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A Dde-based carboxy linker for solid-phase synthesis

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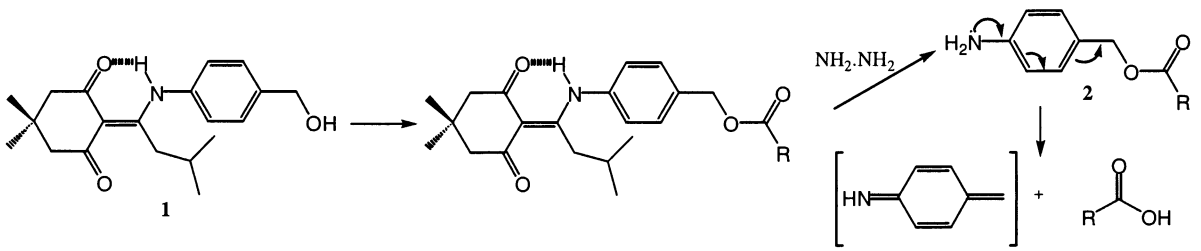
Abstract—The Dde-derived carboxy protecting group, 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl ester (*O*Dmab) has been developed into a carboxy functional linker. The stability of the linker to standard acid and base conditions employed in Fmoc/tBu SPPS has been demonstrated and its utility illustrated by the construction of model peptides, Leu-enkephalin and Human Angiotensin II. © 2001 Elsevier Science Ltd. All rights reserved.

Over the past decade the initial stages of the drug discovery process have been revolutionised by the advances in high-throughput screening and combinatorial chemistry.[1,2] The rapid progression of the latter from small peptides to complex compound libraries has hinged largely on the development, in solid-phase synthesis (SPS), of new chemistry, more robust and sophisticated solid supports, versatile linkers and new protecting groups.[3] In general, linker strategies have evolved from protecting groups,[4] and we now report such a development for a carboxy linker based on the protecting group *O*Dmab **1**.[5] This linker, which is stable to acid and base conditions but readily cleaved using 2% *v*/*v* hydrazine monohydrate in DMF, has been specifically developed to be compatible with other protecting groups in relation to some of our interests with atypical and glyco-peptides.

Although a variety of carboxy linkers are available, the majority are either acid (Wang[6]) or *hyper* acid (HAL,[7] SASRIN,[8] trityl[9]) labile. Linkers such as HMB[10] and glycolamido[11] are stable to acid, but release carboxylic acids on treatment with $NaOH_{(aq)}$ or $Bu_4NOH_{(aq)}$. Effective silicon based linkers which are stable to base only, yet cleaved by fluoride ion, have been described by Chao[12] and Ramage,[13] but they limit the use of silyl protecting groups in SPS, particularly for carbohydrate manipulation. A phenanthridine derived linker with similar acid/base stability has been reported recently,[14] but the oxidative cleavage conditions were unsuitable for a number of our purposes. Other carboxy linkers, requiring mild photochemical,[15] enzymatic[16] or Pd(0)[17] cleavage conditions to release the product are also available and have specific stabilities and applications. Further methodology, which has additional advantages, now offers the opportunity to extend the range of compound types amenable to SPS.

*O*Dmab **1**, synthesised by the condensation of Ddiv[18] and 4-aminobenzyl alcohol serves as a carboxy-protecting group. This group, like Dde[19] and Nde,[20] displays inherent stability towards the base-deprotection conditions required for Fmoc/tBu SPPS and the acid conditions required for side-chain deprotection. Cleavage is achieved via hydrazinolysis, followed by elimination of the resulting *p*-aminobenzyl group via a 1,6-electron shift (Scheme 1).



Scheme 1. Hydrazinolysis followed by 1→6 elimination of an *O*Dmab protected carboxylic acid.

Keywords: carboxylic acids; linkers; solid-phase synthesis; supported reagents/reactions.

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We envisaged that by modification and functionalisation of the alkyl side chain of **1**, in a manner akin to our development of a Dde-based amine linker,[21] the product could be successfully anchored to a solid-support. The synthetic approach followed two distinct routes and is outlined in Scheme 2.

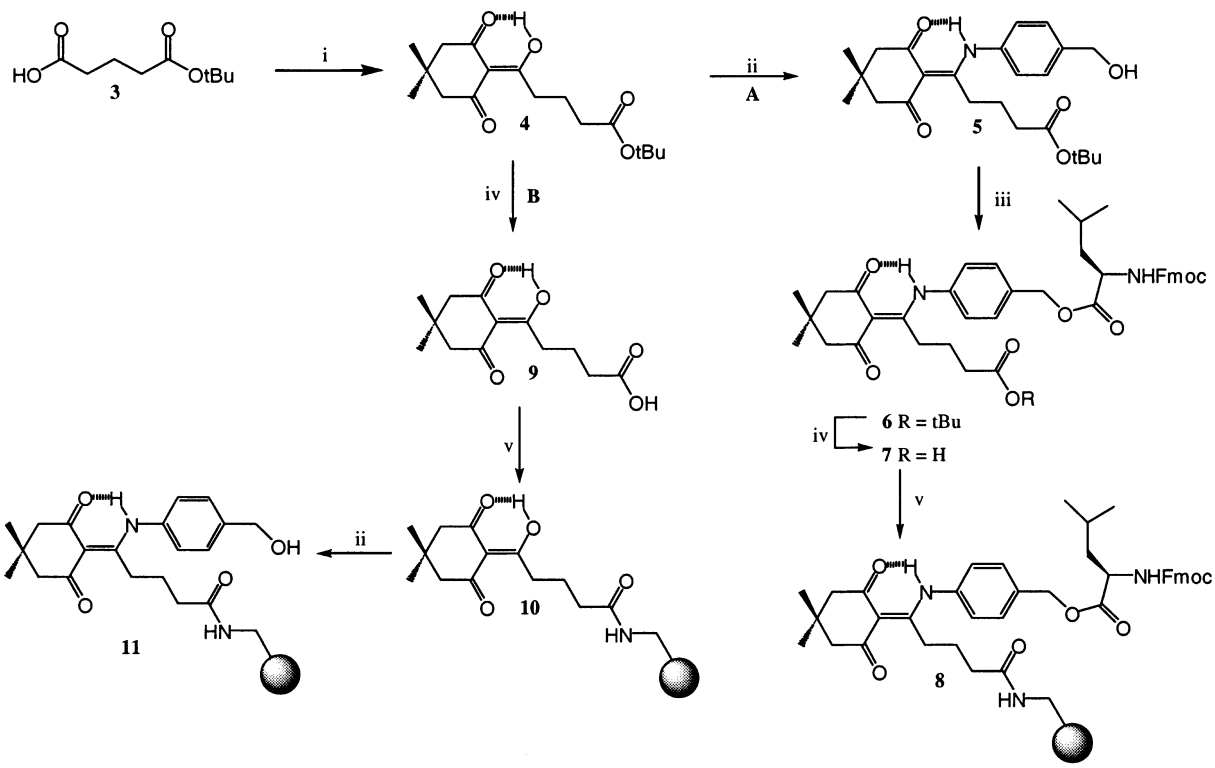
Route **A** was undertaken primarily in solution to highlight any potential problems in synthesis; since the reactions could be monitored using standard chromatographic techniques. This route however generated a linker with the first amino acid (e.g. Fmoc-Leu-OH) pre-attached. Therefore, to construct a 'generic linker' route **B** was developed in parallel to yield **11** with a free benzylic-OH functionality available for the attachment of any carboxylic acid.

DCCI/DMAP activated coupling of dimedone with mono *tert*-butyl glutarate **3** afforded the desired *C*-acylated product **4** in high yield. Amidation of **4** with 4-aminobenzyl alcohol (Route **A**) proved problematic; presumably due to the reduced basicity of the anilino nitrogen and the propensity of the 4-aminobenzyl alcohol to undergo polymerisation via 1,6-dehydration. These problems were circumvented by the addition of 4-aminobenzyl alcohol in aliquots over 72 hours to a refluxing solution of **4** in THF, yielding the amidated product **5**[22] as a crystalline solid in 87% yield. Attachment of a carboxylic acid was achieved via the corresponding acid fluoride to afford **6**.[23] Subsequent acidolysis afforded the free carboxylic acid **7**, which was immobilised onto aminomethyl polystyrene resin via DIPCDI/HOAt mediated coupling to furnish **8**. Complete acylation was confirmed by a negative TNBS test for primary amines.[24]

In route **B** the free acid **9** was first immobilised onto the solid support to afford **10**, again complete acylation was confirmed by a negative TNBS test. Condensation of **10** with 4-aminobenzylalcohol using the optimised conditions described in route **A** yielded the desired linker **11**. Coupling of the first residue was achieved via either the corresponding amino acid fluoride or the pre-formed symmetrical anhydride, the latter resulting in higher loading (>80%) as confirmed by Fmoc loading tests.[25]

The acid stability of the acylated linker was demonstrated by exposing **8** to 90% TFA in DMF over a period of 4 h. Fmoc loading remained unchanged. The base stability of **5** was assessed following initial acylation with Boc-Leu-OH. Exposing the resultant product to 20% *v*/*v* piperidine in DMF or 2% *v*/*v* DBU in DMF over a 3 h period resulted in no detectable decomposition or side reactions as determined by TLC, RP-HPLC and ^{1}H NMR analyses.

The utility of the linker was evaluated via the synthesis of model peptides Leucine-Enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) and Human Angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) using standard Fmoc chemistry. The first amino acid was attached to **11** via its preformed symmetrical anhydride and subsequent couplings were performed using standard TBTU/



Scheme 2. *Reagents and conditions*: (i) Dimedone, DCC/DMAP, DCM, rt, 4 h, 81%; (ii) 4-aminobenzyl alcohol, THF, reflux, 72 h, 87%; (iii) Fmoc-Leu-F, rt, 1.5 h, 88%; (iv) TFA:TIPS:H_2O (95:2.5:2.5), 3 h, 81%; (v) aminomethylpolystyrene resin (200–400 mesh), DIPCDI, HOAt, DMF.

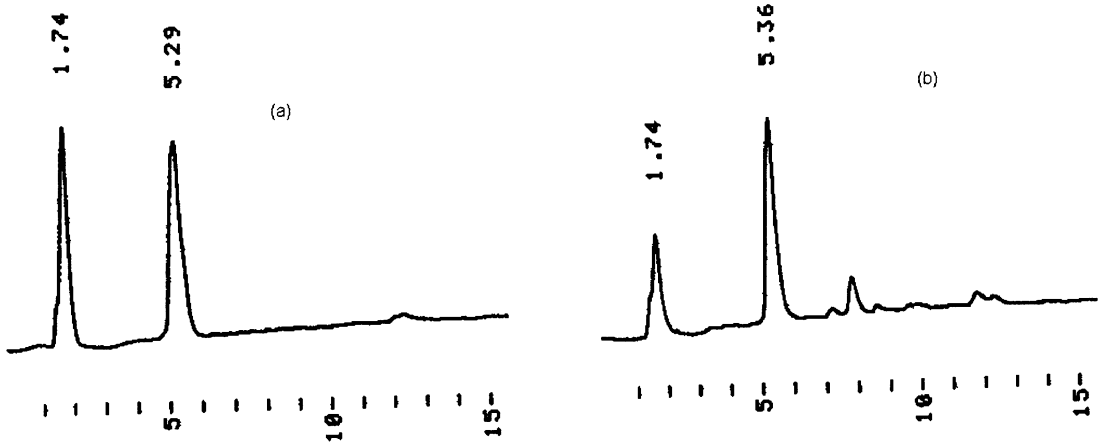


Figure 1. RP-HPLC profiles of the crude native peptides (a) Leu-enkephalin and (b) Angiotensin II.

HOBt/DIEA activation. Fmoc deprotection was carried out either with 20 % *v*/*v* piperidine or 2% *v*/*v* DBU in DMF. However, the crude peptides in the latter case displayed the highest purity as demonstrated by RPH-PLC. Peptide-resin cleavage was achieved using 2% *v*/*v* hydrazine·H_2O in DMF (2×20 min), releasing the protected fragments, which on treatment with TFA:TIPS:H_2O (95:2.5:2.5) afforded the native peptides (Fig. 1).[26] Upon cleavage, the Dde portion of the linker remains resin bound and is converted to the 6,6-dimethyl-4,5,6,7-tetrahydro-4(1*H*)-indazolone.[19]

In conclusion, we have successfully developed a new carboxy linker stable to acidic and basic environments, yet easily cleaved in 2% *v*/*v* hydrazine monohydrate. The application of the linker for the synthesis of model side-chain protected or unprotected peptide fragments by Fmoc/tBu SPPS has been demonstrated. The exploitation of the described linker for modified glycopeptide synthesis is currently under investigation in our laboratories.

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22. tert-*Butyl 5-(4-hydroxymethylanilino)-5-(4,4-dimethylcyclohexylidene-2,6-dioxo)pentanoate* **5**: Oil, R_f 0.64 (80% EtOAc/hexane); selected spectral data: *m*/*z* (ES-MS) 416 (M+H); δ_H (250 MHz, $CDCl_3$) 1.08 (6H, s, $(CH_3)_2$), 1.34 (9H, s, $(CH_3)_3$), 1.55–1.85 (2H, m, 3-H_2), 2.20 (2H, t, 2-CH_2), 2.39 and 2.48 (4H, s, Dde 2-CH_2), 2.85–2.95 (2H, m, 4-H_2), 3.48 (1H, br s, OH), 4.73 (2H, s, CH_2OH), 7.12 and 7.45 (4H, 2×d, ArH), 14.97 (1H, s, NH); δ_C (62.9 MHz, $CDCl_3$) 24.59 (3-CH_2), 28.27 ($(CH_3)_2$), 28.59 ($(CH_3)_3$), 30.30 (Dde 4-C), 30.41 (4-CH_2), 35.86 (Dde 3,5-CH_2), 53.98 (2-CH_2), 64.40 (CH_2OH), 80.14 ($C(CH_3)_3$), 107.49 (Dde 1-C), 126.47 (Ar 3,5-C), 128.12 (Ar 2,6-C), 135.52 (Ar 4-C), 141.84 (Ar 1-C), 172.61 (COOBu^t), 176.25 (5-C), 196.79 (Dde C=O (H bonded)), 200.92 (Dde C=O).

23. tert-*Butyl 5-(4-(*N*-9-fluorenylmethoxycarbonylleucinyloxymethyl)anilino)-5-(4,4-dimethylcyclohexylidene-2,6-dioxo)pentanoate* **6**: Oil R_f 0.52 (40% EtOAc/hexane); selected spectral data: *m*/*z* (ES-MS) 751 (M+H); δ_H (250 MHz, $CDCl_3$) 0.95 (6H, d, *J* 4.15, Leu γ-Me_2), 1.08 (6H, s, Dde-Me_2), 1.34 (9H, s, $(CH_3)_3$), 1.44–1.78 (5H, br m, Leu γ-H, β-H_2 and 3-H_2), 2.22 (2H, t, *J* 7.55, 2-H_2), 2.40 and 2.49 (4H, s, Dde 2×CH_2), 2.85–2.95 (2H, m, 4-H_2), 4.21 (1H, t, *J* 8, Fmoc-CH), 4.39–4.51 (3H, br m, Fmoc-CH_2, Leu α-H), 5.13 (2H, s, PABA CH_2), 5.30 (1H, d, *J* 8.6, Leu-NH), 7.15 (2H, d, Fmoc-ArH), 7.26–7.41 (6H, br m, Fmoc-ArH), 7.12 and 7.45 (4H, 2×d, ArH), 15.08 (1H, s, NH); δ_C (62.9 MHz, $CDCl_3$) 21.77 and 22.85 (Leu γ-Me_2), 24.28 (3-CH_2), 24.75 (Leu α-CH), 28.00 ($(CH_3)_3$), 28.27 (Dde Me_2), 29.95 (Dde 4-C), 35.47 (Dde 3,5-CH_2), 41.53 (Leu β-CH_2), 47.17 (Leu α-CH), 52.34 (Fmoc CH), 53.79 (2-CH_2), 66.08 (benzyl alcohol CH_2), 66.98 (Fmoc CH), 80.14 ($C(CH_3)_3$), 107.25 (Dde 1-C), 119.99, 125.04, 127.70 and 129.16 (Fmoc ArCH), 126.39 and 127.04 (ArCH), 135.31 (aromatic 1-C), 136.71 (aromatic 4-C), 141.29 and 143.70 (Fmoc-C), 156.00 (NH·C=O), 172.16 (COOBu^t), 172.96 (Leu C=O), 175.62 (5-C), 196.28 (Dde C=O (H bonded)), 200.57 (Dde C=O).

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26. HPLC conditions: Vydac C_8 column:208TP5415; 4.6 mm (id)×150 mm. Eluents: A: 0.06% $TFA_{(aq)}$. B: 90% MeCN/H_2O *v*/*v* (0.06% TFA). Gradient: 20–100% B in 30 min linearly. Leu-enkephalin protected fragment: R_t 9.22 min; *m*/*z* (ES-MS) 612.4 [MH^+]. Leu-enkephalin: R_t 5.29 min; *m*/*z* (ES-MS) 556.3 [MH^+]. Angiotensin II protected fragment R_t 19.46 min; *m*/*z* (ES-MS) 1652.6 [M^+], Angiotensin II: R_t 5.36 min; *m*/*z* (ES-MS) 1047.0 [MH^+]. From the HPLC profiles obtained (cf. Fig. 1), it is apparent that the *p*-iminomethine quinoid species, or its hydrazine adduct, is either removed during peptide workup or elutes with the solvent front and therefore does not hinder the isolation or purification of the crude cleavage products.