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The development of solid phase protocols for a backbone amide linker and its application to the Boc-based assembly of linear peptides

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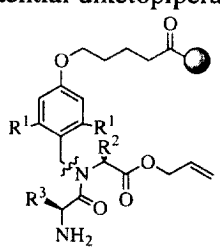
Abstract

Herein, we report on the solid phase synthesis of a backbone amide linker. The key step is the acylation of a secondary amide in quantitative yield. To show the application of this methodology we synthesised linear peptides CCK (25-33) and ACP (64-73) using Boc-based protocols in high yields and purity.

Keywords: secondary amine acylation; peptide synthesis; solid support.

The development of the backbone amide linker **1** (BAL)[1–3] has generated a new and versatile strategy for the Fmoc solid phase synthesis of C-terminal modified peptides and cyclic peptides. We have recently developed a TFA-stable backbone linker **2** for the solid phase synthesis of cyclic peptides.[4] The improved acid stability of this linker allows the use of in situ neutralisation Boc chemistry protocols[5] and therefore, contrary to linker **1**, minimises potential diketopiperazine formation during SPPS assembly.[3]



1 $R^1 = OCH_3$
2 $R^1 = H$

In the synthesis of cyclic peptides using linker **2** we generated dipeptide-linker units in solution which were then purified prior to being attached to the solid support. The synthesis involved a solution phase reductive amination and a difficult acylation of a secondary amine.[4] To increase the applicability of this backbone linker, especially towards development of combinatorial libraries, we have now adopted and

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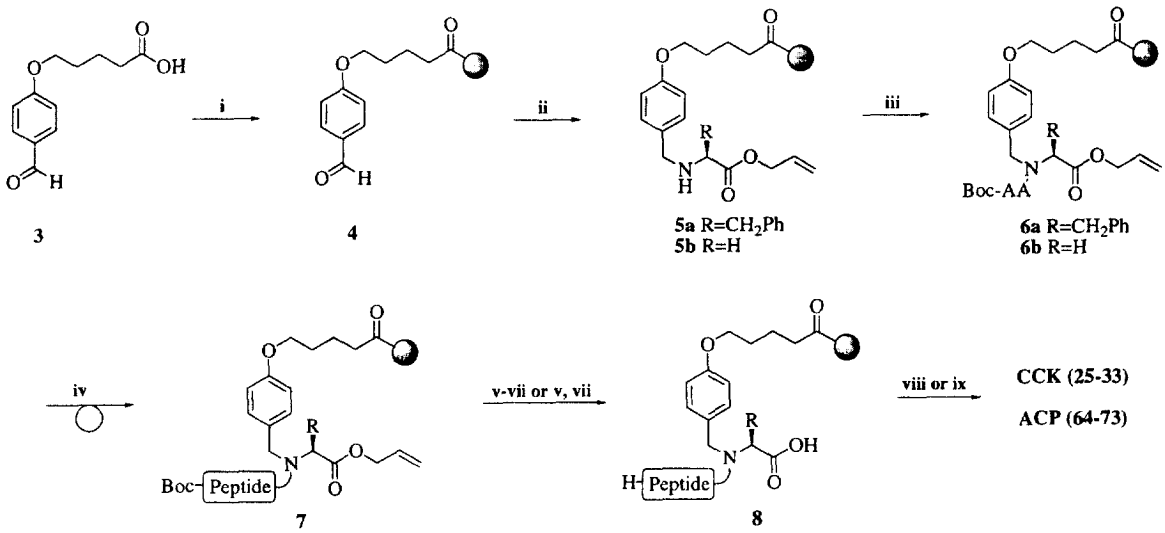
optimised these chemistries on solid support starting from the benzaldehyde linker **3** (Scheme 1).[6] To illustrate the improved utility of this linker, two peptide fragments; CCK (25-33)[7] containing a sterically hindered C-terminal sequence and ACP (64-73),[8] a widely used model peptide were synthesised.



(a) R = CH_2Ph Peptide = Tyr(4-MeBz)-Met-Gln-Gly-Trp(CHO)-Met-Asp
(b) R = H Peptide = Val-Gln-Ala-Ala-Ile-Asp(Bz)-Tyr(4-MeBz)-Ile-Asn(Xan)

● = Gly-Leu-Leu-Resin

Scheme 1. Reagents and conditions. (i) H-●, HBTU, DMF, rt, 10 min; (ii) H-Phe-OAllyl or H-Gly-OAllyl, 1.5 equiv. $NaBH_3CN$, 5% AcOH/MeOH, rt, 3 h; (iii) $[Boc\text{-}AA]_2\text{-}O$, DCM, rt, 2×3 h; (iv) SPPS, rt; (v) 3 equiv. $Pd(PPh_3)_4$, $CHCl_3$:HOAc:NMM, (37:2:1), rt, 3 h; (vi) 10% ethanolamine/DMF, rt; (vii) TFA:DCM, (40:60), rt, 2×5 min; (viii) HF:*p*-cresol:*p*-thiocresol, 18:1:1, −5°C, 90 min; (ix) HBr/TFA, *p*-cresol, rt, 90 min. CCK (25-33)=Tyr-Met-Gln-Gly-Trp-Met-Asp-Phe; ACP (64-73)=Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly

The aldehyde **3** was initially linked to Wang resin containing a tripeptide spacer (Gly-Leu-Leu),[4,9] followed by addition of the C-terminal residue by reductive amination (Scheme 1). Thus excess of the allyl-protected amino acid, H-Phe-OAllyl, was mixed with the resin in MeOH prior to addition of $NaBH_3CN$[1,3,10] to give **5a**. TFA:DCM (40:60) cleavage of **5a** produced the expected amino acid-linker-tripeptide spacer unit with no trace of the corresponding aldehyde as determined by LCMS.

Acylation of the Wang-resin bound secondary amine **5a** with Boc-Phe-OH was examined in more detail by varying the activating reagent, solvent system and type and quantity of base. Products were characterised by LCMS after cleavage from the resin with TFA. An hour acylation with the symmetric anhydride of Boc-Phe-OH was inefficient in polar aprotic solvents such as DMF, DMSO and CH_3CN (5 to 10% yield). However in less polar solvents such as DCM or dioxane the reaction proceeded in 37 to 75% yield (Table 1). Activating agents such as BOP, HATU and PyBrop did not significantly enhance acylation yield, while addition of base impeded the reaction. The general trends we obtained were in agreement with the results reported by Jensen et al.[1,2] for acylation of linker **2**.

The symmetric anhydrides of Boc amino acids are more soluble in DCM when compared to their Fmoc analogues resulting in higher concentrations of the anhydrides, and negating the requirement for addition of DMF to improve solubility. The optimised protocols involved acylation of the secondary amine **5a** using 1 M symmetric anhydride (12 equivalents) in DCM or dioxane in the absence of base for 6 h. These protocols were applied to a range of amino acids and in the majority of cases the acylation yield

Table 1
Effect of solvent[#] on the yield of acylation of **5a** using 120 µl of 1 M $(BocPhe)_2$-O in DCM

Entry	Solvent	% Yield
1	DMF	4.7 %
2	DMSO	2.2 %
3	CH_3CN	9.7 %
4	Toluene	37.1 %
5	DCM	55.7 %
6	THF	41.5 %
7	Dioxane	74.8 %

[#]Resin was swollen in 500 µl of solvent.

Table 2
Yield of acylation of **5a** using 1 M $(BocAA)_2$-O in DCM (6 h)

Entry	Amino Acid	% Yield
1	Boc-Gly-OH	>99%
2	Boc-(β-Ala)-OH	>99%
3	Boc-Ala-OH	>99%
4	Boc-Phe-OH	>99%
5	Boc-Asp(Bz)-OH	>99%
6	Boc-Asn(Xan)-OH	76%
7	Boc-Val-OH	45%

was >99% (Table 2). For the more sterically hindered amino acids Asn and Val lower yields of acylated material were obtained. We observed by leaving these experiments for 72 h, >99% yield of acylation.

The solid phase approach to the synthesis of the linker-dipeptide unit was then evaluated by the synthesis of two linear peptide fragments, the non-sulphated CCK (25-33) and ACP (64-73). The linear peptides CCK (25-33) and ACP (64-73) were assembled starting from aminomethylpolystyrene resin (0.21 mmol/g) using the above solid phase linker approach and HBTU activation protocols.[5] Following allyl[11] and Boc-deprotection, the peptides **8a** and **8b** were cleaved from the resin by HF or HBr/TFA treatment. HPLC analysis of the crude products are shown in Fig. 1. No racemisation or diketopiperazine formation was observed by LCMS. The linear peptides were isolated in excellent yields and purity.[12,13] The high yields of the model peptides obtained by this approach further demonstrate that the linker is relatively stable towards short treatments of TFA:DCM (40:60) within Boc SPPS protocols.[14]

The absence of the two *ortho*-methoxy groups in backbone linker **2** when compared to the original BAL **1** leads to a number of advantages in terms of practicality and quality of the product. Firstly, it improves acid stability and enables in situ neutralisation Boc protocols for peptide assembly that avoid diketopiperazine formation. Secondly, it improves acylation of the secondary amine, presumably due to a combination of reduced steric hindrance, and the improved solubility of symmetrical anhydrides of Boc amino acids over their Fmoc counterparts in DCM. The utility of this approach in the assembly of cyclic peptide libraries is under investigation.

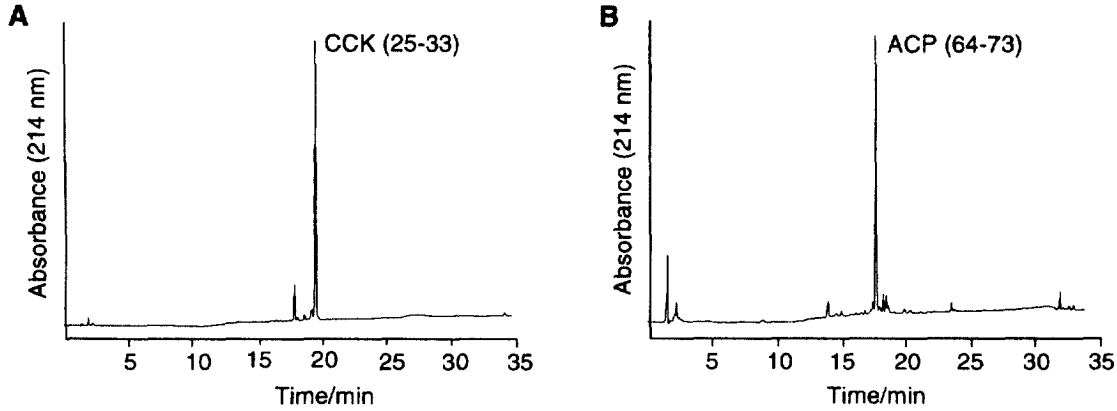


Figure 1. HPLC analysis of crude cleavage product: (**A**) CCK (25-33) (**B**) ACP (64-73)

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6. The linker **3** was prepared as follows: 4-Hydroxybenzaldehyde (12.2 g, 100 mmol), methyl 5-bromo valerate (22.6 g, 125 mmol) and K_2CO_3 (27.6 g, 200 mmol) were refluxed in acetone (250 mL) for 16 h. The solids were filtered, washed with acetone and volatiles removed in vacuo. The product was purified by flash column chromatography (hexane:EtOAc, 4:1) to yield a yellow oil (22.3 g, 94% yield). The oil was dissolved in THF:H_2O (1:1, 500 mL) and LiOH·H_2O (4.4 g, 110 mmol) was added portionwise to the solution at room temperature. The solution was stirred for 2 h, THF was removed in vacuo, and the pH of the aqueous phase lowered to 4 with dropwise addition of HCl (0.1 M). The aqueous solution was washed with EtOAc and the combined EtOAc washes were then washed with saturated brine and water before being dried over $MgSO_4$. Volatiles were removed in vacuo and the resulting solid recrystallised (hexane:EtOAc, 1:1) to yield a yellow solid (18.2 g, 90%), mp 99–101°C.
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12. CCK (25-33) Purified Yield: (20.0 mg, 52%); ES-MS Mr 1063.6 (calcd 1063.2).
13. ACP (64-73) Purified Yield: (18.0 mg, 46%); ES-MS Mr 1062.9 (calcd 1062.5).
14. TFA hydrolysis of *N*-substituted peptides (Urban, J.; Vaisar, T.; Shen, R.; Lee, M. S. *Int. J. Pept. Protein Res.* **1996**, *47*, 182–189) or dipeptide cleavage from a similar linker (Raju, B.; Kogan, T. P. *Tetrahedron Lett.* **1997**, *38*, 4965–4968) have recently been reported. In the systems we have investigated no hydrolysis or dipeptide cleavage have been detected after a 2 h treatment with TFA:DCM, (40:60).