A New Base-labile Linker for Solid-phase Peptide Synthesis†

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A new base labile and orthogonally compatible linker for use in solid-phase peptide synthesis is described.

Solid-phase fragment condensation is the method of choice for chemical synthesis of large peptides and proteins.[1] This approach requires readily available protected peptide fragments. Many linkers have therefore, been developed for anchoring and selectively removing the peptide from the polymer support with the protecting groups intact.[2] These linkers have been designed specifically to suit either Fmoc-[3] or Boc-[4] chemistry, (Fmoc = fluoren-9-yl methoxycarbonyl; Boc = *tert*-butoxycarbonyl). Photolabile,[5] fluoride-labile[6] or Pd[0][7] cleavable linkers have been reported. However, some of these linkers although conceptually elegant, involve multistep synthesis, and are inexpedient while scaling-up. Here, we report a new base labile linker that is compatible with both Fmoc- and Boc- chemistry, the most commonly used approaches in solid-phase peptide synthesis. Our linker resembles the CASET(2) [2-(4-carboxyphenylsulfonyl)-ethanol] linker introduced by Schwyzer *et al.*,[4c] who have not demonstrated the application of their linker in peptide synthesis. Moreover, the synthesis of their linker is also difficult. Here, we demonstrate the simple synthesis and the utility of our linker by synthesising a few biologically active peptides.

The suitably protected linker has been synthesised in two steps (Scheme 1). Chloroacetic acid **1** was treated with phenacylbromide **2** to obtain the chloro intermediate **3** in quantitative yields. After filtering the hydrobromide salt from the above reaction, the ethyl acetate layer was concentrated under reduced pressure, and the chloro compound **3** was treated with 2-mercaptoethanol **4** in the presence of diisopropylethylamine. The reaction mixture was refluxed for 6 h and the hydrochloride salt was removed by filtration. The ethyl acetate layer was thoroughly extracted with water and concentrated to give the protected linker **5** in 98% yield.

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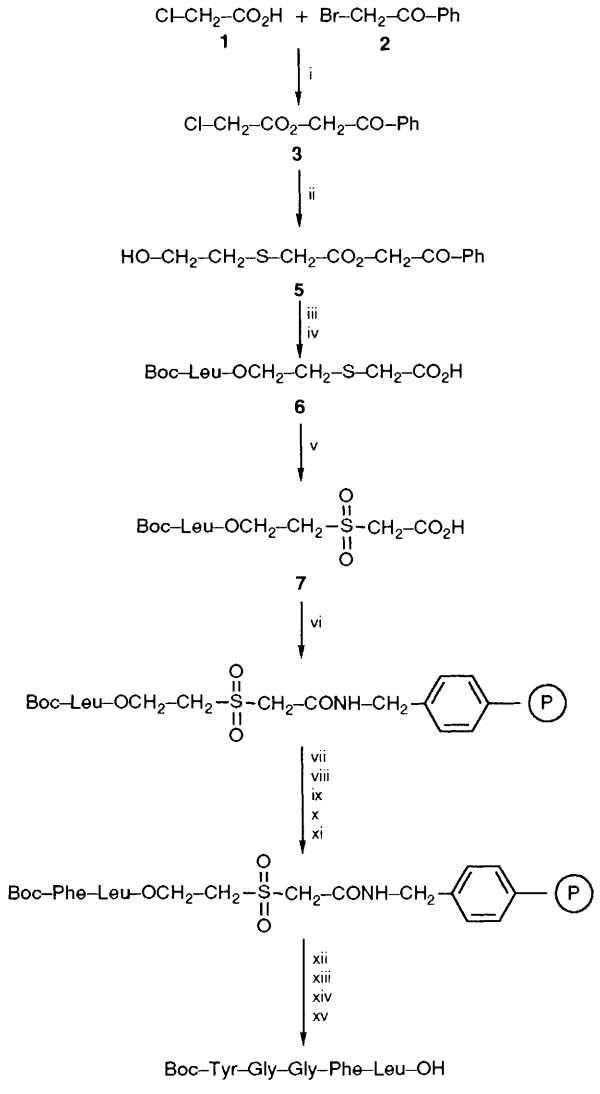

Scheme 1 *Reagents and conditions*: i, TEA, EtOAc, heat, 3 h; ii, $HOCH_2CH_2SH$ **4**, DIEA, EtOAc, heat, 6 h; iii, Boc-Leu, DCC–DMAP, DMF/CH_2Cl_2; iv, Zn/AcOH; v, oxone, MeOH; vi, H_2N-CH_2-(C_6H_4)-Ⓟ, DCC–MOBt, DMF/CH_2Cl_2; vii, HCl (5 mol dm^{-3})–dioxane, viii, wash with CH_2Cl_2, DMF, ix, DIEA (DIEA = diisopropylethylamine); x, wash with CH_2Cl_2, DMF; xi, Boc-Phe, DCC–HOBt, DMF/CH_2Cl_2; xii, repeat above cycle up to 4th amino acid; xiii, Boc-Tyr-OPfp, HOBt, DMF/CH_2Cl_2; xiv, wash with CH_2Cl_2, DMF; xv, NaOH (0.1 mol dm^{-3}), 30 min

Esterification of Boc-protected amino acid with the linker **5** was carried out by DCC/DMAP (1,3-dicyclohexylcarbodiimide/4-dimethylaminopyridine) followed by deblocking of the phenacyl moiety under reducing conditions to obtain **6** in excellent yields. It was subsequently oxidised to sulfone‡ by oxone. The linker carrying the first amino acid **7** was tethered to the aminomethyl polystyrene resin by the DCC/HOBt (HOBt = 1 hydroxybenztriazole) method. The unconverted amino groups on the solid support were capped by Ac_2O/DMAP.

To demonstrate the utility of this linker, synthesis of leu-enkephalin and its [D-Ala]^2 analogue was carried out by the Boc-chemistry using DCC/HOBt coupling method (Scheme 1). In order to achieve high yields, coupling reactions were repeated and capping was dispensed with. At each step, coupling efficiency was more than 90% as monitored by the picric acid and ninhydrin methods. Cleavage of the final peptide from the solid support was carried out by a mixture of dioxane : methanol : 4 mol dm^{-3} NaOH (30 : 9 : 1).§ It has previously been shown that no racemization is caused during detachment of peptides from the solid support under these conditions.[8] The removal of protected peptide was selective and more than 90% cleavage was achieved in 30 min. The crude product was dissolved in water and extracted with ethyl acetate. After acidifying the aqueous layer, the protected peptide was extracted in ethyl acetate. The product was purified by crystallisation and the Boc-protected peptide was obtained in 54% overall yield. Furthermore, the suitability of this linker in Fmoc-chemistry has been shown by synthesising leu-enkephalin using Fmoc-protected amino acids and the DCC/HOBt coupling method, except the last amino acid, which was used as Boc-Tyr-OPfp ester. Similar results were obtained as in case of Boc strategy and the protected pentapeptide was obtained in 60% yield after crystallisation. Finally Boc group was deblocked to obtain HCl·leu-enkephalin and its [D-Ala]^2 analogue.¶

In summary, the new linker is compatible with both, Boc- and Fmoc-chemistry and can be conveniently synthesised in the laboratory. Protected peptides can be synthesised for use in segment condensation approach. However, the linker cannot be used for peptides containing amino acids sensitive to oxidation at the C-terminal, we are currently investigating this difficulty.

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‡ All intermediate compounds gave satisfactory spectral and elemental analysis data.

§ Small aliquots of H-leu-linker-(P) were treated with the following solutions: (*a*) TFA; (*b*) 5 mol dm^{-3} HCl/dioxane; (*c*) 20% piperidine/DMF; (*d*) TEA/DMF. After 24 h the solutions were filtered and the filtrate collected. The amount of leucine was analysed in the filtrate and on the resin. Most of the amino acid was bound to the resin, thereby suggesting that the linker is stable to Fmoc- and Boc-chemistries. (TFA = trifluoroacetic acid, DMF = dimethylformamide and TEA = triethylamine).

¶ The spectroscopic and elemental analyses data of the pentapeptides obtained by the solid-phase method correspond with the samples prepared by the solution phase method and the results are comparable with the literature data.