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A Simple Linker for the Attachment of Aldehydes to the Solid Phase. Application to Solid Phase Synthesis by the Multipin™ Method.

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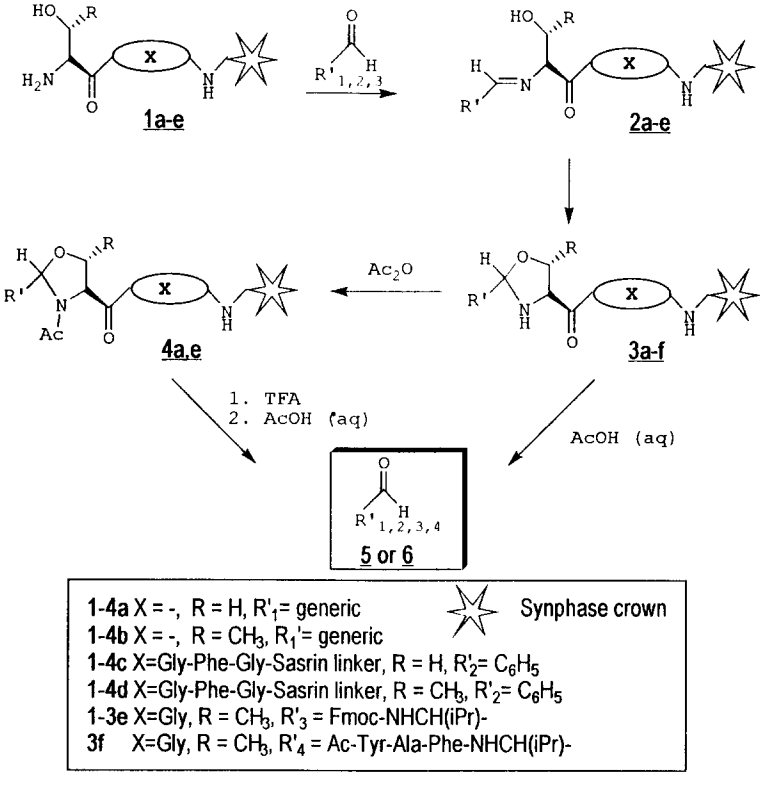
Abstract. *A simple linker has been developed for the attachment of aldehyde functionality. The aldehyde substrate is condensed with a support-bound serine or threonine to give an oxazolidine. The oxazolidine linker is stable to the conditions of Fmoc peptide synthesis, including TFA treatment, but is cleaved by mild aqueous acid at 60°C. The method is used, in conjunction with the Multipin method, to prepare a set of peptide aldehydes.*

The synthesis of diverse small molecule libraries for drug lead discovery through combinatorial synthesis techniques has resulted in a diverse range of chemistries being transferred to the solid phase[1,2]. Much of this chemistry has drawn on the experience of peptide and DNA chemistry for solid phase compatible reaction conditions but more importantly, for the linkers which form the basis of all solid phase chemistries. Most of the available linkers, however, afford carboxylic acids or amides upon cleavage. While linkers such as the trityl[3] and Barany[4] linkers have recently extended the reportoire of functionalities available on cleavage, there is still a lack of linkers allowing straightforward and direct attachment of aldehyde and ketone functionality to the solid phase.

Several methods for the solid phase synthesis of peptide aldehydes have been reported[5,6]. Murphy *et al.*[5] described a linker based on the semicarbazone derivative of a protected amino acid aldehyde. As the linker must be preformed, and is cleaved by formaldehyde, it is not well suited to multiple synthesis. Linkers based on the Weinreb amide[7] have found applications for both peptide[6] and small molecule aldehydes[8]. Since the target aldehyde is derived from a carboxylic acid starting material, this method allows a wide variety of aldehydes to be synthesized. Cleavage yields, however, are generally low and cleavage products are contaminated with $LiAlH_4$ byproducts, which may be toxic to sensitive biological assays. A further alternative is to link the aldehyde to the solid phase via acetal functionality[9]. Formation of acetals requires high concentrations of aldehyde and strongly acidic conditions to drive the protection reaction to completion and so this class of linker is usually preformed in solution.

We settled on the following criteria for a generic aldehyde linker: (i) it must be cheap and easy to construct, (ii) it must be chemically stable (long shelf life), (iii) it must not require preformation (i.e. the aldehyde should couple directly to the solid phase), (iv) aldehyde attachment must be generic and (v) it must cleave under mild conditions which leave no residue upon evaporation. Scheme 1 presents our solution to this problem. A support-bound serine or threonine **1a,b** is treated with a dilute solution of aldehyde to give an imine intermediate **2a,b**, which spontaneously cyclizes to give a stable oxazolidine **3a,b**. Following solid phase synthesis, the

aldehyde target **5** is liberated by treatment with mild aqueous acid. The "capture" of the aldehyde via imine formation is highly efficient, allowing for the direct coupling of aldehydes onto the solid phase, even when low concentrations are used. This concept is similar to the so called thiol capture ligation strategy for the synthesis of large peptides[10,11]. Mutter and coworkers[12] have previously described the use of serine and threonine oxazolidines for the disruption of secondary structure formation during peptide synthesis.



Scheme 1. Oxazolidine formation and cleavage

To optimise the attachment of an aldehyde (benzaldehyde) to the solid phase via oxazolidine formation, cleavable templates **1c,d** were constructed on Sasrin linker[13] derivatized MA/DMA grafted Synphase crowns[14,15]. The Sasrin linker cleaves under mild acid treatment (1% TFA/CH_2Cl_2) hence avoiding premature oxazolidine cleavage within **3c,d**. The ability to simultaneously synthesise many copies of a given compound on the Multipin system, while varying the reaction parameters, allows for rapid optimization of reaction conditions on the solid phase[16,17]. In optimising benzaldehyde attachment, six parameters were simultaneously varied: linker **1c,d**, (Ser or Thr), solvent (MeOH or DMF), additive (1% DIEA or 1% AcOH), temperature (25° or 60°C), time (2 h or 18 h), and benzaldehyde concentration (0.1M or 2M); a total of 64 conditions were concurrently examined. Prior to cleavage, **3c,d** were acetylated (Ac_2O/DIEA/DMF; (5:1:50), 1h, 25°C) to yield **4c,d**. Following simultaneous multiple cleavage (1% TFA/CH_2Cl_2), the products were analysed by ES MS and relative conversions determined. The best conversion was obtained by treatment with 0.1M benzaldehyde in MeOH (1% DIEA) for 2 h at 60°C (95% conversion). Later experiments demonstrated that the DIEA additive was not

required. Overnight reaction in MeOH at 60°C resulted in premature cleavage of model compound from the Sasrin linker. For all experiments conducted, the degree of acetylation of the oxazolidine varied and thus the % conversion sums both cleaved **3c,d** and **4c,d** (ratios were in the order of 60:40; full acetylation is obtained by the addition of 1% DMAP catalyst). A sample of the putative oxazolidine **3c,d** was treated with $NaBH_3CN$ to confirm that the product was **3c,d** rather than the analogous linear imine **2c,d**: ES MS analysis of the cleaved product demonstrated that no reduction had taken place, thus eliminating the possibility that linear imine had been isolated. Results of the serine template **1c** study mirrored those of threonine **1d** although the threonine template gave overall higher conversions (ca. 10% higher on average).

The kinetics of ring opening and thus liberation of the aldehyde is strongly dependent on the acid/solvent system. Threonine and serine oxazolidines derived from acetone or formaldehyde were reported to cleave with 95% TFA/water or 5% TFA/CH_2Cl_2[12]. The benzaldehyde derivatives **3c,d** and **4c,d**, however, gave less than 10% cleavage (of the oxazolidine) under these conditions. In contrast, treatment with AcOH/water at 60°C for 30 min gave 100% cleavage of **3c,d** but interestingly, only cleaved 10% of the acetylated oxazolidines **4c,d**. Acetic acid concentrations of 5%, 15%, 25% and 50% were equally effective but no cleavage occurs at 25°C to either **3c,d** or **4c,d**. The lack of cleavage of **4c,d** can be circumvented by capping with a TFA labile protecting group such as Boc since a pre-cleavage treatment with TFA is possible with little loss of product. The relative stability of the oxazolidines to TFA mixtures is advantageous since peptide aldehydes could be side-chain deprotected before cleavage from the solid phase.

The utility of the method was demonstrated by the synthesis of a selection of peptide aldehydes (including Ac-Tyr-Ala-Phe-Val-H, **6**). Freshly prepared aldehyde Fmoc-Val-H[18] was condensed onto threonine-derivatized Synphase crowns **1e** using the best conditions obtained from the optimisation experiment[19]. Since acetylation of the oxazolidine precludes cleavage, the Fmoc-Val-oxazolidine **3e** was not acetylated. Although the unprotected secondary amine within the oxazolidine ring is a potential source of side reaction during peptide synthesis, it is quite unreactive and highly reactive acyl fluorides are required for reaction[12]. DIC/HOBt activation was used to avoid acylation of the oxazolidine amine (including activation of acetic acid for N-terminal capping step). Figure 1 presents HPLC and ES MS data for peptide aldehyde **6** obtained from the cleavage of **3f**.

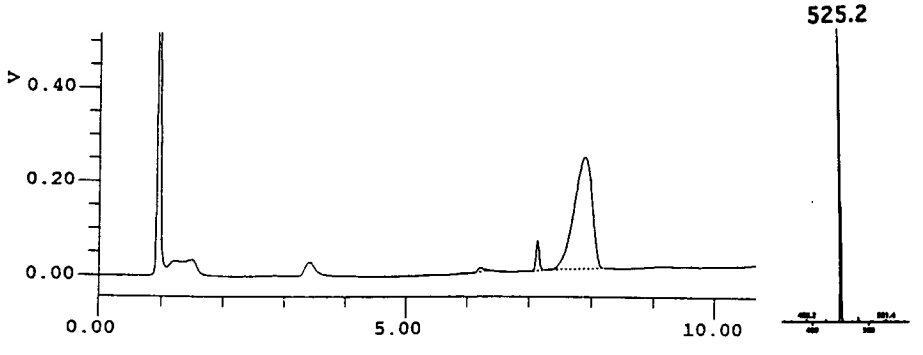


Figure 1. HPLC (214 nm) and positive ion ES MS analysis of Ac-Tyr-Ala-Phe-Val-H **6**.

In conclusion, a very simple and cheap linker for attaching aldehydes to the solid phase has been developed. The linker is chemically stable with a long shelf life and the aldehyde can be attached by a simple

procedure. Furthermore, the oxazolidine linker requires very mild cleavage conditions which leaves no residue which may affect sensitive biological assays. This last point is perhaps the most important as combinatorial techniques dealing with the synthesis of large numbers of compounds require optimised chemistries that negate the need for post synthesis purification.

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REFERENCES AND NOTES

Abbreviations: Ac_2O: acetic anhydride, AcOH: acetic acid, Boc: *t*-butoxycarbonyl, DIC: diisopropylcarbodiimide, DIEA: diisopropylethylamine, DMAP: dimethylaminopyridine, DMF: dimethylformamide, ES MS: electrospray mass spectrometry, Fmoc: 9-fluorenyl-methoxycarbonyl, HPLC: high performance liquid chromatography, HOBt: 1-hydroxybenzotriazole, TFA: trifluoroacetic acid.

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19. Fmoc-Val-H (0.1M) in 1% DIEA/MeOH was added to **1e** and reacted for 2 hrs at 60°C. Fmoc protected amino acids were coupled for 2h with DIC and HOBt in DMF (0.1M). Fmoc deprotection was performed by treatment with 20 % piperidine/DMF (30 min). The assembled support-bound peptide aldehydes were treated with 98% TFA/water for 3 min and washed with water, and then cleaved from the crowns with 5% AcOH/water for 30 min at 60°C. The crude products were analysed by HPLC (purity = 94 % at 214 nm) and the expected $[M+H]^+$ signal (525.4) was observed by ES MS. Analytical HPLC was performed on a Waters chromatography system using a Ranin microsorb-mv (#86-200-F3) RP-18 column (100Å, 3 µm). The following conditions were used: buffer A = water (0.1% H_3PO_4); buffer B = 90% CH_3CN/10% water (0.1% H_3PO_4); linear gradient A to B from 1 to 11 min; flow rate = 1.5 mL min^{-1}. Absorbances were recorded at 214nm. ES MS was performed on a Perkin Elmer Sciex API III ion spray mass spectrometer. The data were processed by software developed at Chiron Mimotopes Pty.Ltd[17].

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