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A Photolabile Carbamate Based Dual Linker Analytical Construct for Facile Monitoring of Solid Phase Chemistry: 'TLC' for Solid Phase?

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Abstract: A dual linker analytical construct based on a photolabile carbamate is described. Photochemical cleavage from the solid support can be effected to afford an analytical fragment, containing the substrate, which is sensitised to electrospray mass spectrometry. We believe this simple construct now renders all substrates visible to high throughput mass specroscopic analysis.

The synthesis of combinatorial libraries using solid phase chemistry has now become a routine strategy in the practice of drug discovery.[1] However, powerful and successful as it has proved to be, there are still a number of shortcomings associated with the use of solid phase chemistry in this manner, particularly in its analysis.[2] Typically the high throughput of solid phase chemistry is not matched by universally applicable analytical techniques. For example, whilst affording excellent information, NMR analysis, both on and off bead, does not currently offer high throughput. In contrast, whilst Mass Spectroscopic (MS) techniques can offer high throughput, too many molecules do not have appropriate and common ionisation properties for this technique to be universally applicable. In an effort to address these problems of analysis Geysen and co-workers[3,4] have developed the concept of analytical constructs, in which the solid supported substrate and linker are attached to the support through a MS sensitiser, peak splitter and a second orthogonal linker. Cleavage at this second linker affords an analytical fragment containing the substrate, together with the first linker, the peak splitter and an MS sensitiser. The Geysen approach relies on the use of a protected amine as the sensitiser for electrospray MS. Similarly, Carrasco and co-workers[5] have also reported a powerful orthogonal double linker construct approach to the analysis of solid phase chemistries which uses a quaternary ammonium group as a sensitiser for MALDI MS.

Herein we report our development of a dual linker analytical construct (**1**, Figure 1), based on a photolabile carbamate, which releases a MS sensitising amine without the potential problems of linker protection/orthogonality and chemical compatability inherent in the above mentioned existing approaches.

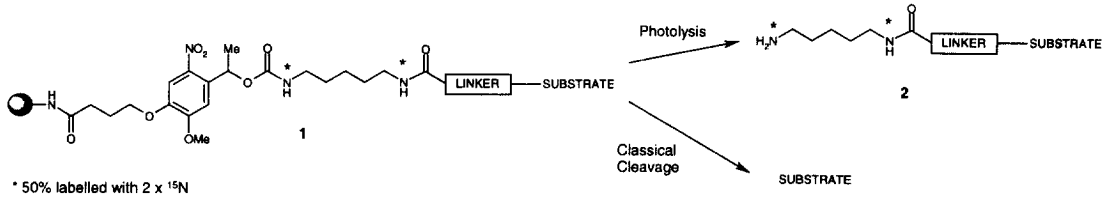

Figure 1: Alternative cleavages of the photolabile analytical construct

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The construct (**1**) can be cleaved either in the classical manner to afford simply the substrate, or cleaved photochemically to afford an analytical fragment (**2**) containing the substrate, the classical linker, and a free amine. The amine, revealed by photocleavage, sensitises the entire fragment to electrospray MS., now guaranteeing that all substrates are readily visible by high throughput MS when cleaved in this fashion. Moreover, the diamine from which the sensitiser portion is constructed also contains isotopically labelled atoms, a 1:1 mixture of $^{14}N_2$-diamine and doubly labelled $^{15}N_2$. This hallmarks all MS signals derived from construct resin as characteristic doublets distinguishing them from extraneous signals and background noise.[3,4a] The properties of the sensitiser are such that excellent MS resolution is achieved even from samples cleaved off single resin beads. The incorporation of the sensitising amine in a chemically benign form into the backbone of the construct eliminates the need for protection of the sensitising amine (which would cause problems of orthogonality between linkers and protecting groups), or incorporation of less chemically inert sensitising groups such as quaternary amines[5]. However, whilst the construct **1** protects and liberates the sensitising group in a novel and effective manner, it should be acknowledged that it does in fact exploit a similar photocleavage strategy to that employed by Carrasco and co-workers[5].

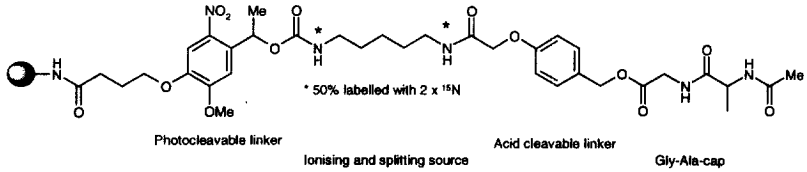
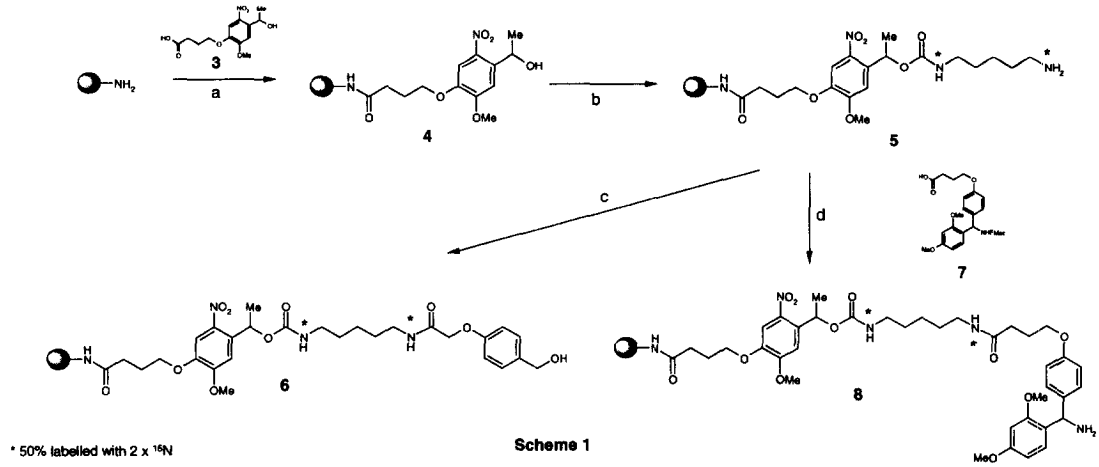


Figure 2. Structure of Ac-Ala-Gly attached to the analytical construct before cleavage

The power and utility of this photolabile carbamate analytical construct methodology is illustrated by a comparison of the MS analysis of a selection of N-acetylated dipeptides synthesised on the analytical construct (e.g. Fig. 2) with those prepared in the conventional manner. Constructs were prepared as shown in Scheme 1. Photolinker[6] **3** was coupled to commercially available amino Polystyrene-PEG resin with HOBT/DIC. Treatment of the resulting alcohol resin **4** with carbonyldiimidazole, subsequent displacement of the imidazole with a 1:1 mixture of $^{14}N_2$ / $^{15}N_2$ *N-tert*-butoxycarbonyl-diaminopentane and treatment with TFA afforded the amine resin **5**. The HMPA linker was then attached to amino resin **5** to give the alcohol resin **6** *via* 4-formylphenoxyacetic acid/DIC/HOBT with subsequent reduction of the aldehyde by tetrabutyl-ammonium borohydride. Modified Rink linker **7** was coupled to amino resin **5** with HOBT/DIC which, with subsequent Fmoc removal gave resin **8**. Ala-Gly, Gly-Val, and Val-Ala dipeptides were individually constructed on each resin (**6** and **8**) using standard HATU coupling/Fmoc procedures and then *N*-acylated with acetic anhydride/DMAP. These peptides were also similarly constructed on Polystyrene-PEG resin using conventional Rink and HMPA linkers without the incorporation of the analytical construct.

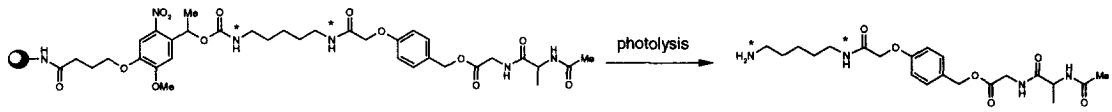


* 50% labelled with 2 x ^{15}N

Scheme 1

Reagents and Conditions: a. HOBT, DIC, DMF; b. i) CDI, CH_2Cl_2, 50ºC; ii) *N*-t-BOC-diaminopentane*, DMF, 50ºC; iii) 20% TFA, CH_2Cl_2, iv) DIPEA, DMF; c. i) 4-formylphenoxyacetic acid, HOBT, DIC, DMF; ii) Bu_4NBH_4,CH_2Cl_2; d. i) HATU, DIPEA, DMF; ii) 20% Piperidine/DMF.

The peptides built on the analytical construct resins **6** and **8** were cleaved by photolysis (Scheme 2) and analysed by electrospray ionisation MS.[7] The peptides built conventionally were cleaved using standard TFA conditions and analysed by the same technique. All samples were subject to electrospray MS analysis at the same nominal concentration. Figures 2a,b and 3a,b show typical mass spectra obtained.



Scheme 2

The spectra obtained for the compounds generated by conventional synthesis are largely uninformative (Figs. 2a and 3a). The compounds, despite their excellent purity (nmr, >95%), have poor ionisation properties and it is difficult to distinguish the molecular ions from noise and extraneous peaks.

Figure 2a

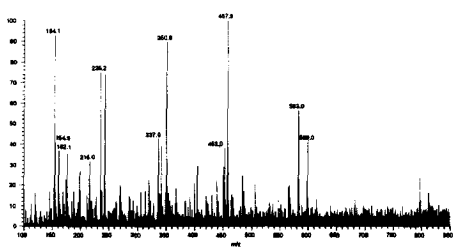


Figure 2b

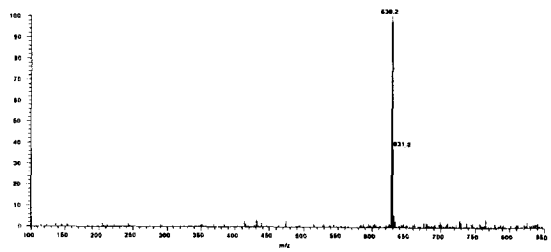


Figure 2a and 2b: Electrospray MS[7] of Ac-Gly-Val (from Rink linker) using a) conventional synthesis and cleavage (MH^+ = 216) and b) using construct resin and photolytic cleavage[8] (MH^+ = 628/630, this was the spectrum obtained from a single bead).

In contrast, figures 2b and 3b are the spectra obtained from the molecules prepared on the constuct. The molecular ions are readily distinguished, the sensitising group is highly effective, and the isotopic peak splitter

Figure 3a

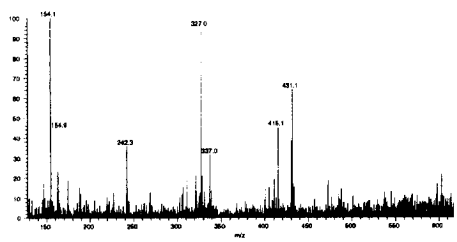


Figure 3b

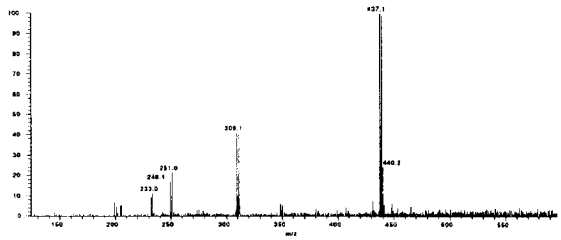


Figure 3a and 3b: Electrospray MS^7 of Ac-Ala-Gly (from HMPA linker) using a) conventional synthesis and cleavage (MH+ = 189) and b) using construct resin and photolytic cleavage[8] (MH^+ = 437/439).

distinguishes all construct-derived signals as doublets. The sensitivity confered by the sensitising amine is illustrated by the spectrum of fig. 2b, which was obtained from material cleaved from a single resin bead. [8] One example particularly illustrates the power of using this construct methodology. Figure 3b clearly shows a strong peak split signal corresponding to the desired substrate/construct at MH^+ = 437/439, however there is also a second peak split signal at MH^+ = 309/311. This signal corresponds to acetylation of the HMPA linker where the amino acid couplings have failed (MS^2 analysis reveals that the 309/311 signal is a genuine molecular species and not a fragment ion, but that the doublet at 249/251 are fragment ions of the 437/439 parent). This failure of the amino acid couplings could highlight synthesis breakdowns and in this case would not have been revealed by analysis after conventional cleavage. The quantitation of these techniques is currently being investigated.[3]

In summary, a powerful photolabile based dual linker, analytical construct has been developed. This construct should overcome many of the difficulties of analysing solid phase chemistries. We believe that this construct renders essentially any solid phase chemistry amenable to high throughput analysis by electrospray mass spectroscopy, provided that the constituents of the chemistry are stable to the photochemical cleavage. This methodology potentially offers to solid phase what thin layer chromatography gives to solution phase chemistry and also has powerful applications in the monitoring and analysis of chemistries and libraries synthesised on solid phase.

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References and Notes

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7 The MS analysis was performed on a Finnigan MAT LCQ Ion Trap Mass Spectrometer in positive electrospray mode.

8 Typical photolysis procedure: A 160μm resin bead in 10 μl of DMSO is irradiated at 365nm, 8-10mW/cm^2 (or ca. 0.1mg resin in 50μl DMSO) for 30 min and the solution subjected to electrospray MS.