Linkers for Combinatorial Chemistry and Reaction Analysis using Solid Phase *In Situ* Mass Spectrometry†

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A method for the analysis of new solid phase reactions which is exceptionally time and material efficient and utilises a wide range of traditional peptide type linkers to generate a number of different termini is described.

Most efforts directed towards the synthesis of combinatorial libraries have utilised a variety of peptide or peptide related functionalities (*e.g.* peptoids, ureas and carbonates).[1] Their preparation has been highly efficient due in the main to the utilisation of a variety of sensitive ninhydrin and UV based assays. Attempts are now being made to augment these chemistries by a variety of C–C bond forming reactions.[2] However this process is severely hampered by the inability to directly determine the fate of a particular reaction when carried out on the solid phase. New methods for the analysis of resin-bound compounds by NMR have recently been reported and offer a very attractive approach to the analysis of solid phase reactions, although requiring relatively large samples of resin bound material.[3] IR spectroscopy has also been utilised,[4] however neither of these techniques are ideal for optimising combinatorial reactions. We have recently published a procedure where reactions on peptides attached to the resin by the acid labile Rink linker,[5] and hence released as primary amides, were directly monitored from single beads using matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry,[5] a technique many orders of magnitude more sensitive than NMR.

Here we report the use of a much wider range of linkers suitable for these direct monitoring studies which upon cleavage produce a variety of different termini (amines, carboxylates and homoserine lactones). This increase in number of released termini is an important feature if the method is to be of general use for the analysis of combinatorial reactions. We illustrate the method firstly with peptides containing residues which are often known to be problematic during cleavage, secondly we show the use of the method for the analysis of non oligomeric compounds suitable for small molecule non-peptide library generation, including the analysis of a Wittig reaction on the solid phase and finally we report on a novel method for the cleavage of methionine residues using cyanogen bromide (CNBr) vapour.

Peptide **1** was prepared in the normal Fmoc manner[6] using the 4-hydroxymethylphenoxy (Wang) linker.[7] N-terminal deprotection and subsequent dansylation gave peptide **2**. The Wang linker is one of the most widely utilised linkers in Fmoc chemistry and it was a good test of the applicability of the method due to the relatively poor acid liability of the Wang linker compared to the highly labile Rink and 4-(4-hydroxymethyl-3-methoxyphenoxybutyryl) (HMPB) linkers.[8] In addition peptide **2** contains a tryptophan in close proximity to the linker which is often a problem due to the susceptibility of tryptophan to alkylation. However as shown in Fig. 1 [spectrum (*a*)] no problems were encountered upon MS‡ analysis with the clean and clear observation of the protonated (MH^+ 1006.0) and sodiated (Na^+ 1028.0) pseudo-molecular ions corresponding to peptide **2a** by MALDI-TOF MS. A small peak is also observable corresponding to the undansylated peptide **2b**. Thus the very widely utilised and relatively acid stable Wang Linker is suitable for direct MALDI-TOF MS analysis using the TFA vapour cleavage method even for compounds which contain alkylation sensitive functionalities. This will dramatically simplify the ability to analyse reactions, both peptide and non-peptide based which utilise this linker.[9]

Super sensitive acid labile linkers have been used for a variety of peptide[8] and recently non-peptide studies.[10] The HMPB linker was therefore utilised to look at the synthesis of a small peptide containing two cysteine residues. These would

Peptide **1**; Fmoc-Ile-Thr(OBu^t)-Pro-Gln-Trp-Lys(Boc)-Wang Linker–Resin
Peptide **2**; Dansyl-Ile-Thr(OBu^t)-Pro-Gln-Trp-Lys(Boc)-Wang Linker–Resin
Peptide **2a**; Dansyl-Ile-Thr-Pro-Gln-Trp-Lys-OH
Peptide **2b**; Ile-Thr-Pro-Gln-Trp-Lys-OH
Peptide **3**; Fmoc-Cys(Trt)-Lys(Boc)-Ile-HMPB Linker–Resin
Peptide **4**; Fmoc-Asn-Cys(Trt)-Lys(Boc)-Ile-HMPB Linker–Resin
Peptide **5**; Fmoc-Cys(Trt)-Asn-Cys(Trt)-Lys(Boc)-Ile-HMPB Linker–Resin
Peptide **6**; Fmoc-Cys-Asn-Cys-Lys(Boc)-Ile-HMPB Linker–Resin disulphide
Peptide **6a**; Fmoc-Cys-Asn-Cys-Lys-Ile-OH disulphide.
Olefin **7**; EtOOC(Me)C=CH-Ph-4-CO-NH-$(CH_2)_4$-NH-chlorotrityl-Resin
Peptide **8**; Ser(OBu^t)-Asp(OBu^t)-Ile-Phe-Lys(Boc)-Met–Resin

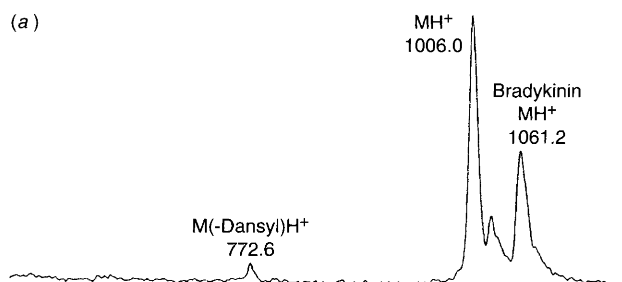
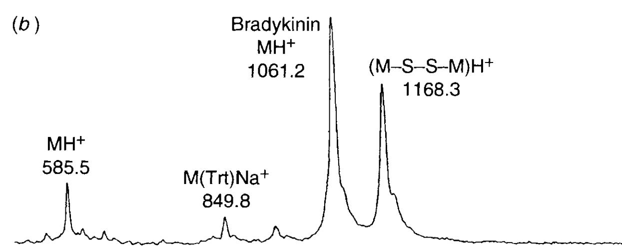


Fig. 1 (*a*) Analysis of Dansyl-Ile-Thr(OBu^t)-Pro-Gln-Trp-Lys(Boc)–Wang-linker–Resin **2**, giving peptide **2a**, Dansyl-Ile-Thr-Pro-Gln-Trp-Lys (MH^+, 1006.0). The peak at 772.6 represents a small amount of undansylated material.



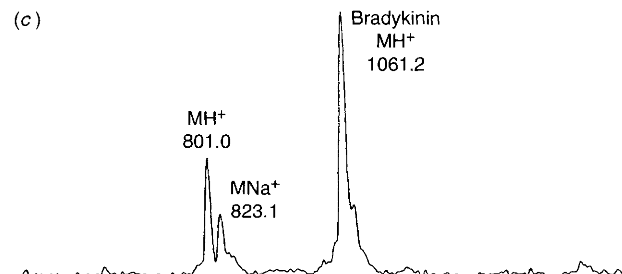


Fig. 2 (*b*) Analysis of Fmoc-Cys(Trt)-Lys(Boc)-Ile-HMPB–Linker–Resin **3**, giving Fmoc-Cys-Lys-Ile (MH^+, 585.5), Fmoc-Cys(Trt)-Lys-Ile (MH^+, 849.8), and Fmoc-Cys-Lys-Ile disulphide [$(M–S–S–M)H^+$, 1168.3]. (*c*) Analysis of thedisulphide of Fmoc-Cys-Asn-Cys-Lys(Boc)-Ile-HMPB–Linker–Resin **6** giving the disulphide of Fmoc-Cys-Asn-Cys-Lys-Ile (MH^+, 801.0).

not only be expected to complicate the analysis but would also be expected to be susceptible to alkylation. However even this peptide linker proved totally suitable for direct MS analysis. Thus the preparation of resin linked peptides **3**, **4**, **5** and **6** was monitored (Fig. 2). Analysis of **3** gave spectrum (*b*) which shows the protonated molecular ion (MH^+ 585.5) and the trityl protected peptide as its sodium adduct [M(Trt)Na^+ 849.8] as well as the disulphide dimer as its protonated ion [(M–S–S–M)H^+ 1168.3]. Similar molecular ions were obtained with **4** and **5**. Optimisation of the oxidation/deprotection procedure (I_2/DMF) gave **6** which upon mass spectrometry analysis [spectrum (*c*)] gave the protonated (MH^+ 801.0) and sodiated (MNa^+ 823.1) pseudo-molecular ions of the internal disulphide bridged peptide **6a**.

The 2-chlorotrityl chloride resin[11] was treated with 1,4-diaminobutane (putrescine)§ the free amine functionality coupled to 4-carboxybenzaldehyde. The resulting aldehyde was treated with $Ph_3PCMeCH_2CO_2Et$ to give the olefin **7**. Analysis of the reaction products gave the spectra as shown in Fig. 3. Spectrum (*d*) shows the result of the modified Wittig reaction following reaction optimisation with the product seen as its protonated molecular ion (MH^+ 305.5). This shows that the chlorotrityl linker is suitable firstly as an amine linking functionality in solid phase chemistry (the first time this has been reported) and secondly that it can be cleaved to allow direct MALDI-TOF MS analysis liberating a free amine function.

Peptide **8** was synthesised directly on the amino methyl resin using Fmoc chemistry. Treatment of the resin beads with CNBr/TFA vapour gave the expected protonated (MH^+ 692.8) and sodiated (MNa^+ 714.9) molecular ions [spectrum (*e*)] for the C-terminal homoserine lactone peptide (Fig. 4). The ring opened product was also observed as its sodiated molecular ion [M(H_2O)Na^+ 732.6].

In conclusion we have demonstrated the utility of a range of linkers suitable for direct monitoring using our MALDI-TOF mass spectrometry conditions and importantly have shown that a variety of different termini can be efficiently liberated (base labile linkers cleaved by ammonia/THF, light cleavable linkers and alcohols linked to the chlorotrityl resin should also be equally suitable for the described method), we have shown that the method, although exceptionally useful for peptide chemistry, will have major applications in non-peptide based solid phase chemistry where the efficiency of monitoring allows if necessary, reaction conditions to be altered to ensure high reaction yields all in a very time and material efficient manner.

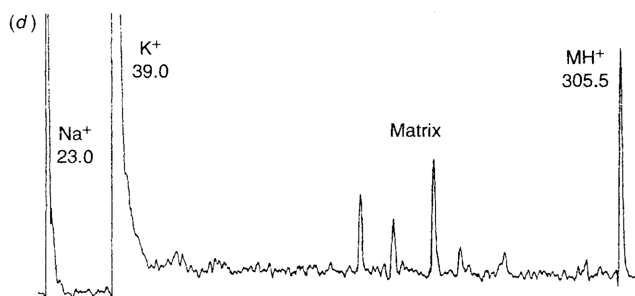


Fig. 3 (*d*) Analysis of olefin **7**, [M(H_2O)Na^+, 305.5]

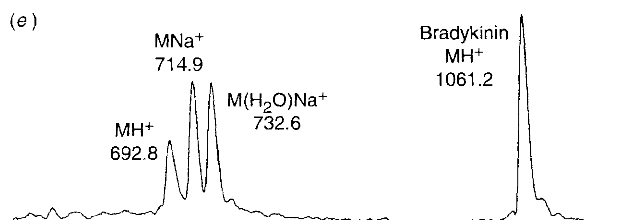


Fig. 4 (*e*) CNBr/TFA vapour cleavage and analysis of Ser(OBu^t)-Asp(OBu^t)-Ile-Phe-Lys(Boc)-Met–Resin **8** giving Ser-Asp-Ile-Phe-Lys-Homoserine lactone (MH^+ 692.8, MNa^+ 714.9) and Ser-Asp-Ile-Phe-Lys-Homoserine (MH^+ 732.6)

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Footnotes

† This method has been given the acronym SPIMS—Solid Phase *In Situ* Mass Spectrometry.

‡ A single bead or beads (approximately 10–100) were placed into a well of the sample plate and then placed onto a metal rack in a glass dish which contained 10 ml of slowly stirred TFA or TFA–CNBr (20 mg). The glass dish was sealed with a plate of glass and the sample plate was left in the TFA vapour for 5–30 mins or overnight with CNBr. Matrix solution and internal standards were added to each well [2 µl of a saturated 2,5-dihydrobenzoic acid solution (100 mg ml^{-1}) in water/acetonitrile/TFA (70/30/0.1) plus 1 µl of a solution of Bradykinin (10 µg ml^{-1} in water–TFA (100/0.1)]. These were left to cocrystallise around the bead(s) for 15–30 mins at room temp. before the plate was loaded into a GSG fOCUS Benchtop II linear-laser desorption time-of-flight mass spectrometer. The sample was irradiated with a N_2 laser (337 nm, 3 ns pulse width, 20 Hz) with an acceleration voltage of 20 kV. The spectra were typically generated from the sum of 10–50 scans with a laser power just above the threshold of the least ionisable component.

§ Chlorotrityl resin (substitution 1.4 mmol^{-1} g) (0.1 g) was shaken overnight with putrescine (4 equiv.) in CH_2Cl_2 (4 ml) before washing with CH_2Cl_2 (4 × 4 ml), DMF (4 × 4 ml), EtOH (4 × 4 ml) and dichloromethane (4 × 4 ml). The derivatised resin was shaken with 2 equiv. of [4-carboxybenzaldehyde + 1-hydroxybenzotriazole + diisopropylcarbodiimide in DMF–CH_2Cl_2 (1 : 2), 4 ml] for 2 h. A modified Wittig reaction on the aldehyde followed using 10 equiv. of $Ph_3PCMe–CH_2CO_2Et$ in CH_2Cl_2 overnight.

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