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# Application of Time-of-flight Secondary Ion Mass Spectrometry to *In Situ* Monitoring of Solid-phase Peptide Synthesis on the Multipin™ System

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**In the rapidly growing field of combinatorial chemistry, the Multipin approach has been used for rapid and efficient multiple parallel syntheses of organic compounds. This strategy is particularly well adapted for the optimization of reaction conditions prior to chemical library syntheses, for the preparation of a wide range of compounds in relation to a lead structure or for the generation of parallel libraries involving complex multistep chemistries. In all cases, direct *in situ* monitoring of support-bound products would be highly valuable. In this work, time-of-flight secondary ion mass spectrometry was applied to the analysis of support-bound intermediates. The analytical method was applied to a range of crowns loaded with various dipeptides. The solid support was subjected to primary ion bombardment and characteristic ions indicative of both the peptide chain and the polymeric support were unambiguously identified. Analysis could be performed at any stage of the synthesis, and as the method is effectively non-destructive, the analyzed crowns could be further used to prepare target compounds.** © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: pin; peptide; time-of-flight secondary ion mass spectrometry; combinatorial chemistry; supported synthesis

## INTRODUCTION

Combinatorial chemistry provides sets of compounds, so-called libraries, for bioscreening purposes.[1–3] Libraries exhibiting a positive response must be thereafter deconvoluted to find precisely among the millions of structures which is the active one. This methodology has then evolved to the simultaneous multiple preparations of spatially isolated compounds,[4] such parallel syntheses being carried out easily and efficiently on polymeric support such as plastic pins[5,6] and resin beads.[7] Under these conditions, each structure was assayed either bound to the support or after release from the polymer and analyzed when a positive response was obtained.

In theory, only the designed compound should be present, but side reactions could never be excluded. In the case of peptide libraries, deleted and partially deprotected chains produced by incomplete coupling and deprotection reactions, respectively, and also alkylated, acylated or oxidized materials have been encountered.[8,9] Thus, precise structure identification was required but such analysis was not straightforward when the active molecule was still linked to the solid support. Standard spectroscopic techniques such as $^{1}H$, $^{13}C$ NMR and fast atom bombardment (FAB) mass spectrometry require prior dissolution of the compound in an organic solvent and thus cannot be applied. Even with specific methods such as IR[10–12] and cross polarization magic angle spinning (CP-MAS) NMR,[13] the signals attributed to the sample are very weak and may be masked by the polymer. In mass spectrometry, which studies ions in the gaseous phase, cleavage of the product–polymer covalent bond must be achieved either before or during ionization. The monitoring of organic reactions in the solid-phase by mass spectrometric analysis following *in situ* release of support-bound intermediates has been reported for peptides,[14] cyclopeptides,[15] peptoids[16] and non-peptide structures.[17] For example, peptides anchored via a methionine residue were released by chemical treatment with CNBr[18,19] or, more generally, peptides built on polymer functionalized either by acid-labile or photolabile linkers were released by trifluoroacetic acid vapor[20] and UV irradiation,[21] respectively. In each case, the

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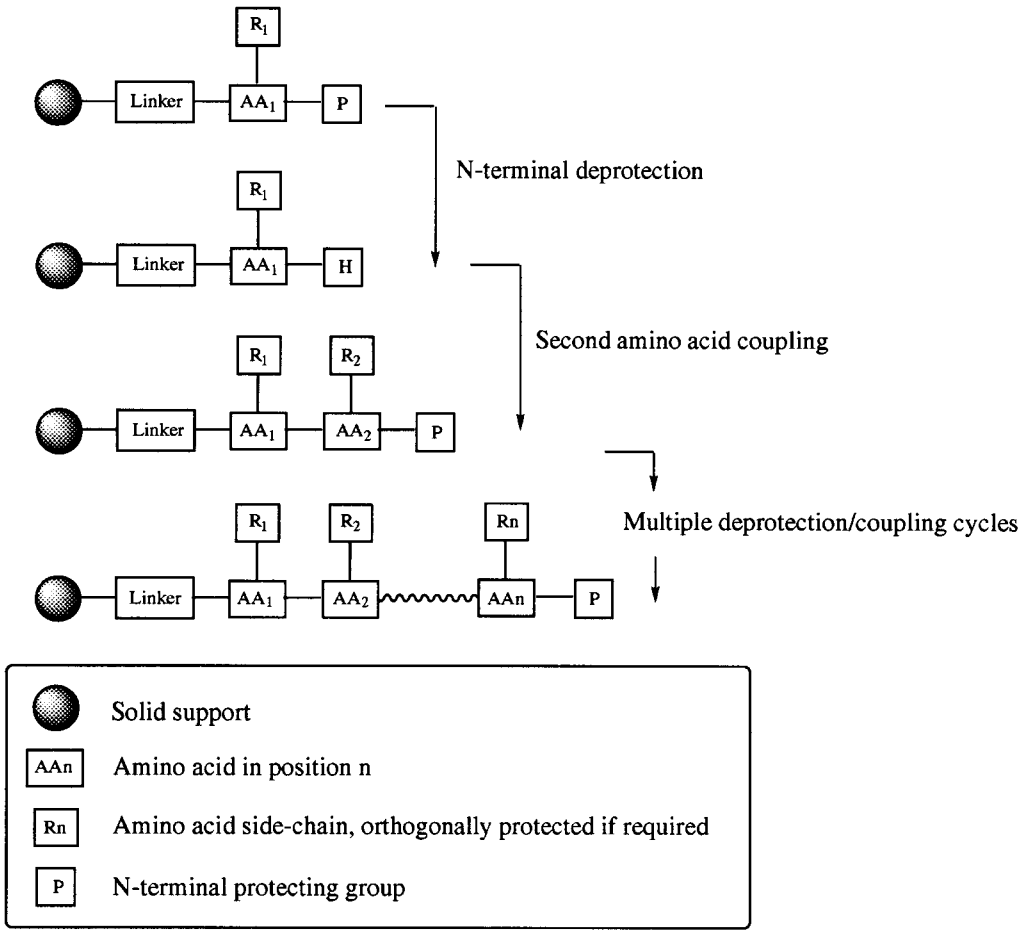



**Scheme 1**

recovered solution was mixed with the appropriate matrix for matrix-assisted laser desorption/ionization (MALDI) analysis[18–20] or directly analyzed by electrospray ionization (ESI) analysis.[21]

A more elegant strategy involved the use of a photolabile handle which was cleaved upon the MALDI irradiation excluding any extra chemical treatment.[22,23] A single laser shot targeted on a single resin bead provided simultaneous cleavage of the peptide–support covalent bond and ion desorption into the gaseous phase. Although the analyzed bead suffered minor alterations, it could be used for subsequent synthesis. Peptides showing poor ionization efficiency were derivatized with a so-called 'ionization tag' to provide enhanced signals. At the completion of the synthesis, the inserted tag was chemically cleaved to yield the expected product.

Time-of-flight secondary ion mass spectrometry (TOF-SIMS)[24] has also been used to monitor organic reactions on solid supports.[25] This technique presents several advantages. First, the TOF analyzer provides accurate and sensitive mass measurements. Second, the weak intensity of the incident primary ions in static SIMS means that the bombarded surface per scan is small (100 × 100 μm), hence allowing the possibility that a single bead could be analyzed but also used afterwards for subsequent reaction steps. Although MALDI/TOF and ESI techniques are sensitive enough to allow single bead characterization, they do not allow further experiments on the analyzed bead. The method was successfully used to examine an amino acid residue covalently linked to a polymer support.[25] The loaded beads were either analyzed directly or submitted to trifluoroacetic acid treatment to release the residue before subsequent analysis. In both cases, the TOF-SIMS spectrum exhibited characteristic ions of the amino acid residue (protonated molecular ion, immonium ion).[26,27] It was shown that the acid pretreatment was not required in order to observe the expected ions.

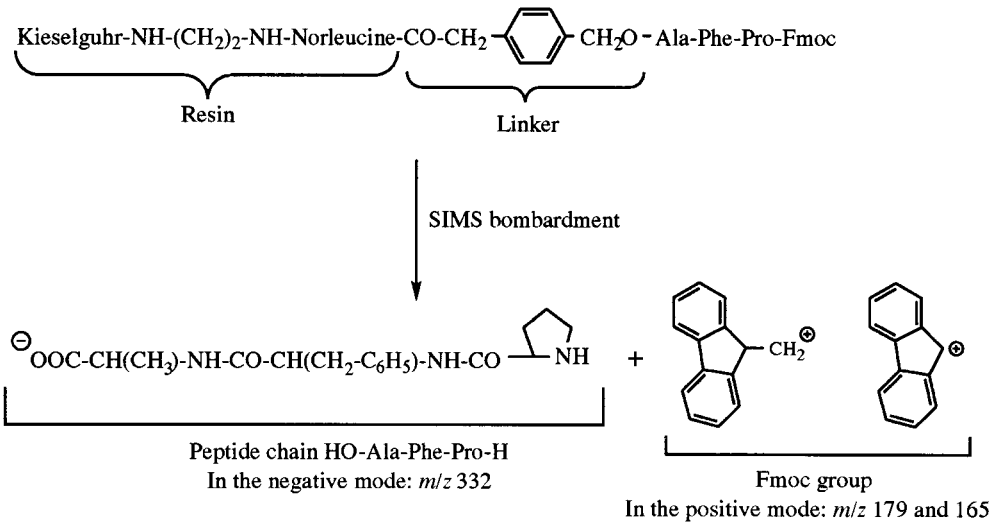
The efficiencies of ESI, MALDI and TOF-SIMS techniques to follow polymer-supported reactions have been compared.[28] It is worth noting that only MALDI and TOF-SIMS methods allowed *in situ* product release and analysis without the need for chemical pretreatment, which is potentially time consuming and could, in some cases, affect product integrity.[8,9] However, whereas the MALDI experiment required a specific resin functionalized with an appropriate photolabile linker, the TOF-SIMS analysis could be carried out on a number of handle structures, the choice of the support not being directed by analysis requirements but only by synthetic considerations. Another advantage of the TOF-SIMS technique resides in the possibility of imaging studies. Hence the superiority of TOF-SIMS prompted a detailed study of the analytical information provided by TOF-SIMS analysis of various supported peptides.

Our project was initiated by several published results concerning TOF-SIMS analyses of biopolymers.[29–31] Amino acids which were covalently linked to a solid support have been characterized by TOF-SIMS;[29,30] homopolymers were anchored directly to the support[29]

or a single residue was loaded on the support through a spacer to avoid any steric hindrance.[30] In both cases, the recovered spectra contained characteristic ions. In particular, immonium ions which are commonly used for structural identification of peptides by FABMS[25,26] were detected in the positive mode whereas a stable carboxylate ion was produced in the negative mode featuring the whole organic chain linked to the support. Finally, an esterification reaction of a primary alcohol function by propionyl chloride was also evidenced by static SIMS.[31]

The scope of TOF-SIMS applied to biomolecules was extended further in our group to the analysis of peptides synthesized by solid-phase methodology. The first *N*-terminal urethane-protected amino acid was loaded on the chosen resin via a linker and submitted thereafter to selective *N*-terminal deprotection followed by the coupling of the second residue. The deprotection–coupling cycles were repeated until the required structure was reached as summarized in Scheme 1. The step-by-step control of several peptide-supported syntheses was established in the laboratory using both Boc/Merrifield[32] and Fmoc/Sheppard[33] strategies. The solid supports were, respectively, polystyrene and polyamide resins which were used in combination with Boc and Fmoc *N*-terminal protecting groups. In both cases, the following structural features were found by TOF-SIMS. *N*-Terminal protecting groups were clearly identified by abundant ions at *m/z* 57 ($[C_4H_9]^+$) for Boc protection and at *m/z* 165 ($[C_{13}H_9]^+$, $[C_{13}H_9]^-$) and *m/z* 179 ($[C_{14}H_{11}]^+$) for the Fmoc moiety. Deprotection reactions were easily followed by the disappearance of the aforementioned ions. In addition, a carboxylate ion present in the negative spectra enabled us to identify the whole peptide chain built on the polystyrene or polyamide resin. A small difference was observed in the identification of *N*-protected peptides assembled on Merrifield and Sheppard resins. Rupture of the peptide–resin bond occurred in the negative mode leading to the previously mentioned carboxylate for the two types of resin whereas an extra bond cleavage leading to the loss of the Fmoc protection was observed in the case of the polyamide support, as shown in Scheme 2. The Fmoc group was, however, detected by the ions at *m/z* 165 and 165 plus 179 in the negative and positive modes, respectively. Finally, immonium ions were also observed in the positive mode. Their abundances were a function of their position in the peptide chain. The *N*-terminal amino acid exhibited a stronger immonium ion than the previously coupled residues. Thus, at any stage of the synthesis, the resin could be sampled, subjected to TOF-SIMS analysis and the molecular mass of the bound peptide unambiguously checked in the negative mode by its released carboxylate or only the last deprotection–coupling cycle could be monitored in the positive mode by the presence or absence of the required ions related to the *N*-terminal protection or to the residue itself.

We were interested in verifying whether the above ions were detected using various solid supports, linkers, residues and protecting groups (Scheme 1). In particular, we wanted to assess if this analytical technique could be applied to plastic pins. Conventional resins used in solid-phase organic preparations are beaded cross-linked polymers and thus present different physico-chemical properties in different chemical environments. In contrast, the pins are constituted by polymer chains grafted at one end to stable plastic crowns themselves attached to removable stems. The use of pins overcomes some limitations of resins such as prior swelling before synthesis, time-consuming washing and filtration steps, individual weighing and production of particulates from bead breakdown. Further, in combinatorial chemistry, libraries synthesized on resins by the mix and split methodology exhibit a single compound per bead but the beads are all mixed and the nature of the anchored structure can only be known after release and analysis. In contrast, pins which can be mounted in an 8 × 12 format in a holder to fit exactly into the wells of ELISA plates are spatially differentiated, each position in the holder corresponding to a designed structure. The use of pins simplifies parallel synthesis and permits the simultaneous handling of thousands of isolated and traceable compounds.[34] Thus, the multipin approach is particularly well adapted for the rapid optimization of reaction conditions[35] and for the generation of parallel libraries of products involving complex multistep chemistries.[36]




**Scheme 2**

**Table 1. Derivatized crown samples studied by TOF-SIMS**

| No. | Protecting group | Peptide | Linker | Spacer | Polymer |
|---|---|---|---|---|---|
| **1** | — | — | — | — | HEMA |
| **2** | Fmoc | — | — | β-Alanine | HEMA |
| **3** | — | — | — | β-Alanine | HEMA |
| **4** | Fmoc | — | Rink | β-Alanine | HEMA |
| **5** | Fmoc | Ala–Phe | Rink | β-Alanine | HEMA |
| **6** | Fmoc | Leu–Phe | Rink | β-Alanine | HEMA |
| **7** | Fmoc | Phe–Glu–OtBu[a] | Rink | β-Alanine | HEMA |
| **8** | Boc | Ala–Phe | Rink | β-Alanine | HEMA |
| **9** | Boc | Lys(Z)–Phe | Rink | β-Alanine | HEMA |
| **10** | Fmoc | Phe–Ala | HMPA | β-Alanine | HEMA |
| **11** | Fmoc | Phe–Leu | HMPA | β-Alanine | HEMA |
| **12** | Fmoc | Phe–Glu–OtBu[a] | HMPA | β-Alanine | HEMA |
| **13** | Fmoc | Phe–Pro | Kiso | β-Alanine | HEMA |
| **14** | — | — | — | — | PS |
| **15** | Pht | — | — | —$CH_2NH$— | PS |
| **16** | $CF_3COO^-$ | — | — | —$CH_2NH_3^+$ | PS |
| **17** | Fmoc | — | Rink | —$CH_2NH$— | PS |
| **18** | — | — | — | — | MA–DMA |
| **19** | Boc | — | — | —$NH(CH_2)_6NH$— | MA–DMA |
| **20** | $CF_3COO^-$ | — | — | —$NH(CH_2)_6NH_3^+$ | MA–DMA |
| **21** | Fmoc | Gly | — | —$NH(CH_2)_6NH$— | MA–DMA |

[a] Peptides anchored to the linker through their side-chain carboxylic acid via an ester bond.

However, under these circumstances, rapid and efficient control of supported intermediate products is very valuable.

We report in this paper our preliminary results obtained in the direct monitoring by TOF-SIMS of solid-phase peptide synthesis on the Multipin system. Only pure products were analyzed to assess the validity of our analytical technique.

## EXPERIMENTAL

### Abbreviations

Abbreviations are in accord with IUPAC–IUB: TFA = trifluoroacetic acid; DMF = dimethylformamide; Fmoc = 9-fluorenyloxycarbonyl; Z = benzyloxycarbonyl; Boc = *tert*-butoxycarbonyl; tBu = *tert*-butyl; Pht = phthalyl; Ala = alanine; Glu = glutamic acid; Gln = glutamine; Gly = glycine; Leu = leucine; Lys = lysine; Phe = phenylalanine; Pro = proline.

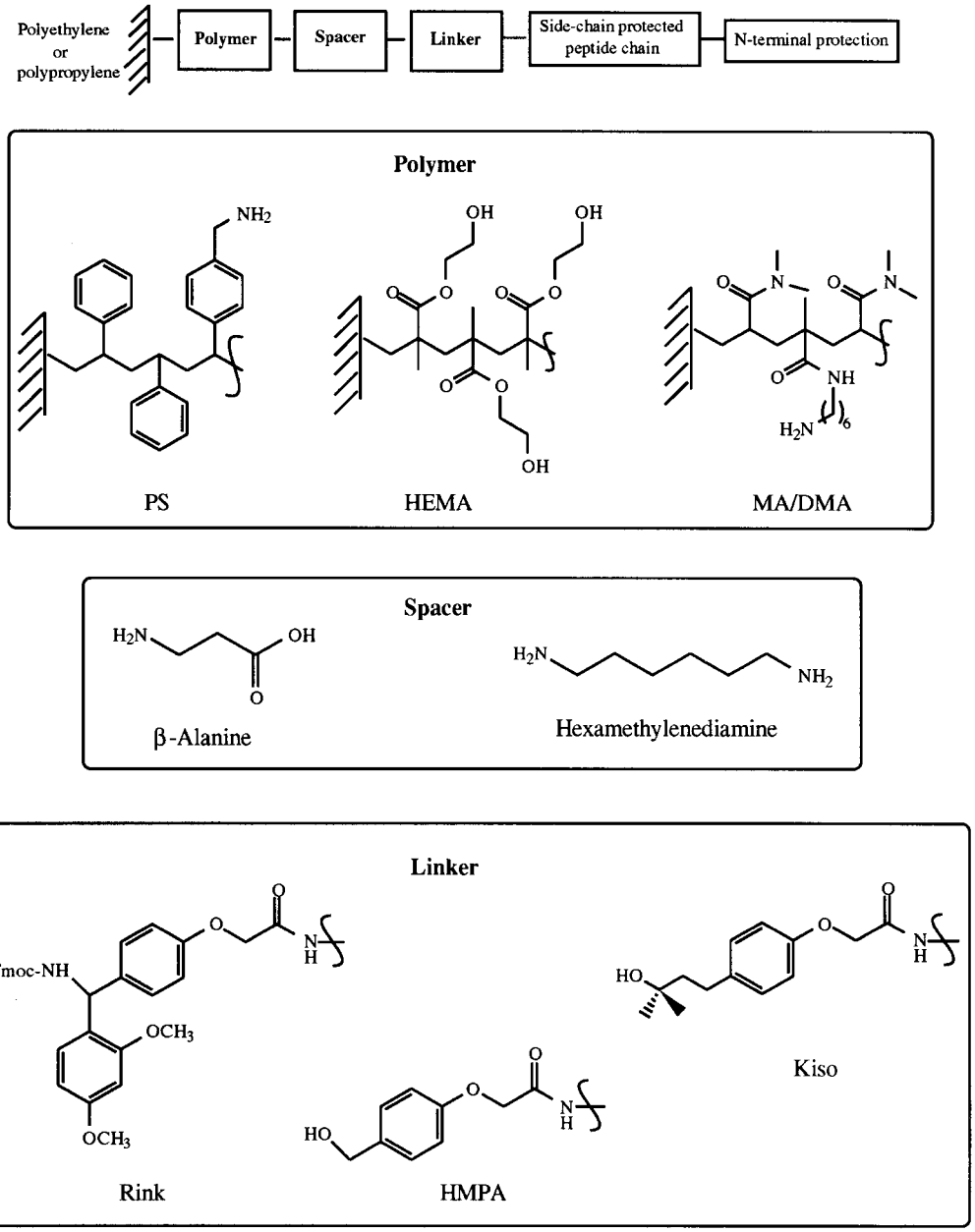
### Chemicals

Protected amino acids were purchased from Novabiochem (Switzerland) and Bachem (Switzerland). The Multipin system consists of a derivatized crown, upon which synthesis is performed, and an inert polypropylene stem. The assembled unit is a pin. Hydroxyethyl acrylate-grafted (HEMA) SynPhase crowns were obtained from Chiron Technologies (Australia).[37] The aminomethylated polystyrene-grafted and methacrylic acid/dimethylacrylamide-grafted crowns were also supplied by Chiron Technologies.[37] The graft surface was derivatized with the Rink amide-forming handle[38] (Rink), 4-(hydroxymethyl)phenoxyacetic acid handle[39] (HMPA) or the hindered handle[40] (Kiso) using methods described elsewhere.[6] The $N^{\alpha}$-Fmoc-protected amino acids coupled to the 4-(hydroxymethyl)phenoxyacetyl handle were Ala, Phe, Gly and Leu. Glu-OtBu was coupled via its side-chain to both the 4-(hydroxymethyl)phenoxyacetyl handle and to the Rink handle, to give Glu and Gln residues, respectively, upon cleavage. Fmoc-Pro was coupled to the hindered handle. Fmoc-Phe was coupled to the Rink handle. The Fmoc protecting group was removed by treating the crowns with 20% (v/v) piperidine–DMF for 30 min at 20 °C, followed by two washes with DMF (5 min) and one wash with methanol (5 min).

*N*-Terminal amino acids were coupled by treating the Fmoc-deprotected crowns with a 0.1 M solution of protected amino acid together with 0.1 M diisopropylcarbodiimide and 0.12 M *N*-hydroxybenzotriazole in DMF for 16 h at 25 °C. After coupling, the crowns were washed with DMF and methanol as outlined above. The $N^{\alpha}$-Fmoc-protected amino acids used were Ala, Phe and Leu and the $N^{\alpha}$-Boc-protected amino acids used were Ala and Lys(Z).

### Mass spectrometry

Crown samples analyzed by TOF-SIMS were cut out directly from the pins with a razor blade to obtain thin slices, which were then immediately placed on a stainless-steel grid. TOF-SIMS measurements were performed on a TRIFT I spectrometer (PHI-Evans).




**Scheme 3**

Spectra were recorded using a pulse (1 ns, 12 kHz) liquid metal source ($^{69}$Ga, 15 keV) operating in the bunched mode of operation in order to provide good mass resolution ($m/\Delta m = 2000$ measured at $m/z$ 43). Owing to large charge effects on such insulating materials, charge compensation was needed for all samples and was achieved by a pulsing electron flood ($E_k = 20$ eV) at a rate of one electron pulse per five ion pulses. The analyzed surfaces were squares of 100 × 100 μm. All positive and negative spectra were acquired in 10 min with a fluence of less than $10^{12}$ ions cm$^{-2}$, ensuring static conditions on the sample slices.

**Table 2. Ions characteristic of the underivatized grafted polymers**

| Sample No. | Polymer | *m/z* | Structure |
|---|---|---|---|
| **1** | HEMA | 45 | $[HOCH_2CH_2]^+$ |
| **14** | PS | 77 | $[C_6H_5]^+$ |
| | | 91 | $[C_7H_7]^+$ |
| | | 115 | $[C_9H_7]^+$ |
| **18** | MA–DMA | 72 | $[(CH_3)_2NCO]^+$ |

## RESULTS

Various analytical information was deduced from the positive and negative static TOF-SIMS mass spectra of differently derivatized pins. All studied samples are listed in Table 1. To assign every ion precisely, crowns consisting of only the naked grafted polymer were studied followed by crowns where the polymer had been derivatized by a spacer then by a linker and finally crowns bearing peptides anchored to the handle via their *C*-terminus (amide bond) or any side-chain functional group (amide or ester bond) (Scheme 3).

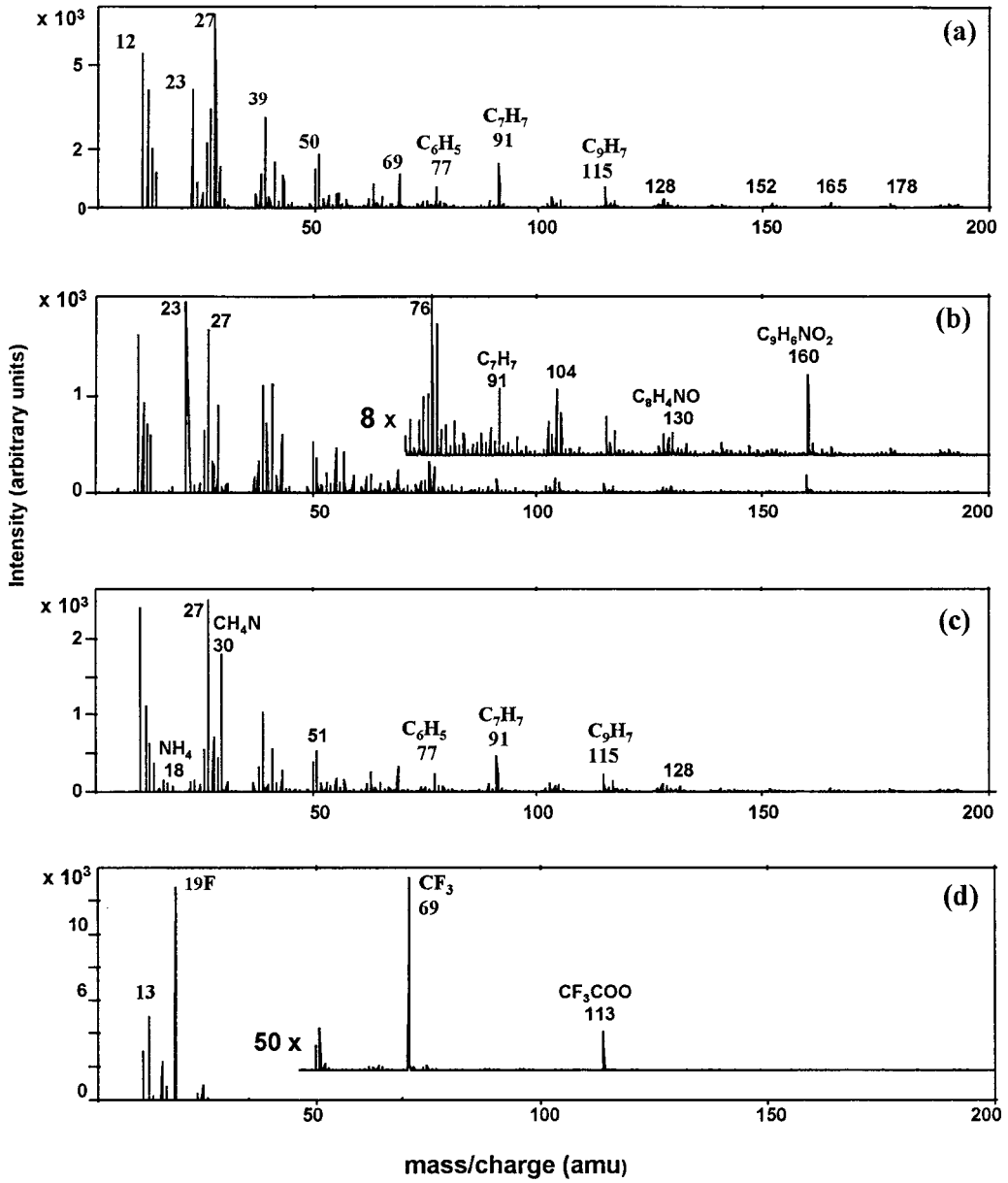


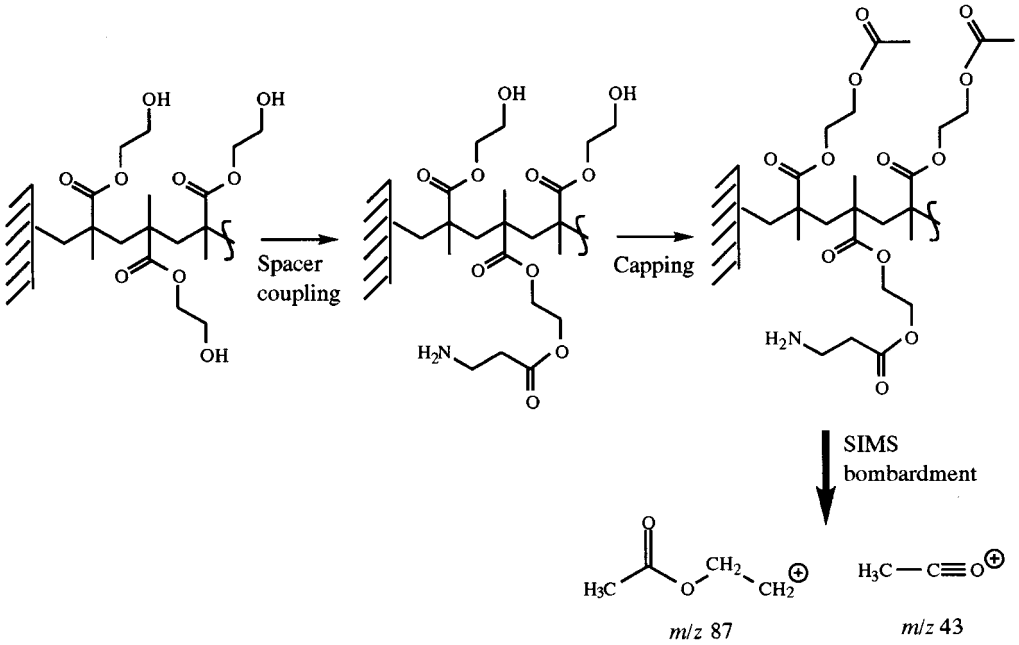

**Figure 1.** Positive ion spectra of (a) sample **14**, PS pin; (b) sample **15**, PS-$CH_2NH$-Pht; (c) sample **16**, PS-$CH_2$-$NH_3^+$ $CF_3COO^-$. (d) Negative ion spectrum of (c).

According to the nature of the polymer, three different underivatized crowns, samples **1**, **14** and **18**, based on polymer of hydroxyethylmethacrylic acid (HEMA), polystyrene (PS) or copolymer of methacrylic acid and dimethylacrylamide (MA–DMA), respectively, the structures of which are given in Scheme 3, were analyzed and exhibited characteristic ions listed in Table 2. As an illustration, the spectrum of sample **14** is reported in Fig. 1(a).

The second step involved functionalization of the polymer with a spacer ($\beta$-alanine or hexamethylenediamine). For instance, HEMA was coupled through its free hydroxyl group to $\beta$-alanine and the sites which had not been loaded were then acylated. The crown substitution level was therefore controlled by adjusting the respective quantities of spacer and acylating reagent. The spectra of samples **2**–**12** exhibited with noticeable abundances ions at $m/z$ 43 and 87 indicative of acylated positions (Scheme 4). The spectrum of sample **3** is reproduced in Fig. 2(a). Thus, the capped active sites were always detected by these specific ions irrespective of the structures on the adjacent sites. The fact that they were still accessible to the SIMS bombardment indicated that they were not buried in the polymer core.

At this stage the spacer can be functionalized by a specific handle which will direct the conditions for the final peptide release from the solid support. Rink amide (Rink), 4-(hydroxymethyl)phenoxyacetamido (HMPA) and the Kiso linkers were studied. Upon cleavage the Rink amide leads to peptide amides whereas the last two handles produce peptide acids. Only the Rink linker exhibited a specific noticeable ion at $m/z$ 166 corresponding to the stable immonium ion shown below in




**Scheme 4**

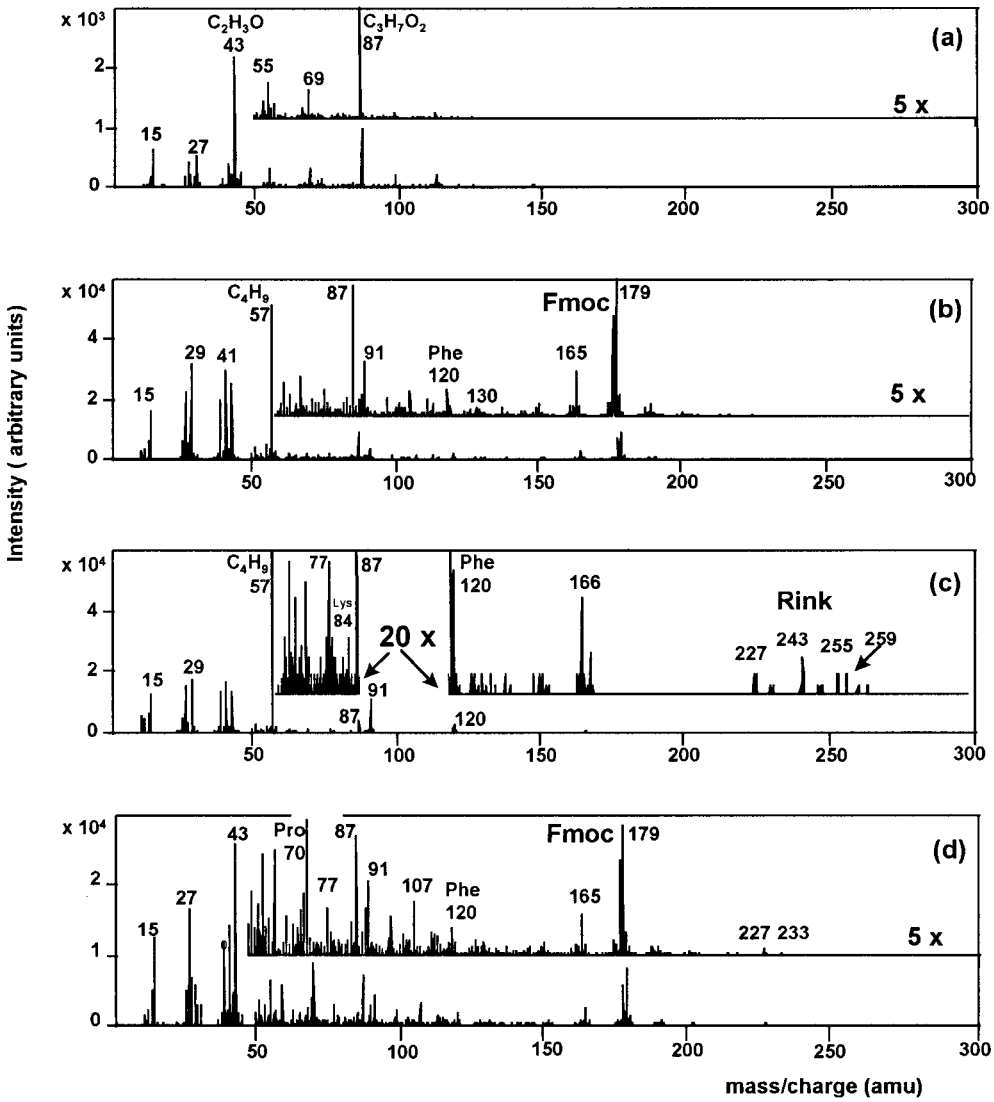
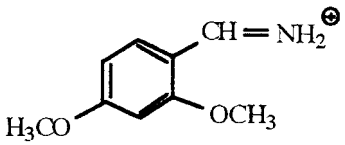



**Figure 2.** Positive ion spectra of (a) sample **3**, HEMA-βAla; (b) sample **12**, HEMA-βAla-HMPA-Glu(OtBu)-Phe-Fmoc; (c) sample **9**, HEMA-βAla-Rink-Phe-Lys(Z)-Boc; (d) sample **13**, HEMA-βAla-Kiso-Pro-Phe-Fmoc.

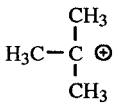
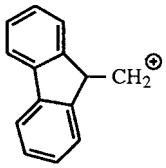
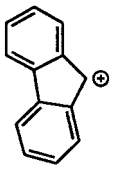
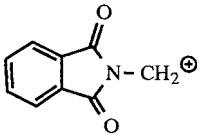
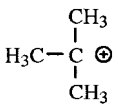
addition to a cluster of signals at *m/z* 227, 243, 255, 259 and 270.



Furthermore, all side-chain or *N*-terminal protecting groups studied including Boc, Fmoc, Z, phthalyl and *tert*-butyl, were evidenced by their corresponding characteristic ions listed in Table 3. The positive ion mass spectrum of sample **19** contained an ion signal at *m/z* 57, which is indicative of the Boc protecting group.[32] Similarly, the Fmoc moiety was always detected by the ions at *m/z* 165 and 179 in the positive mode [Table 3, Fig. 2(b), illustrating the mass spectrum of sample **12**]. Such results, which were established previously on a polyamide resin[33] (Sheppard methodology), were found to be general as the expected ions were observed irrespective of the crown graft polymer (PS, HEMA, MA–DMA) and the linker (Rink, HMPA). Z, phthalyl and *tert*-butyl protecting groups were studied for the first time by SIMS. The urethane Z protection exhibited a very abundant tropylium ion at *m/z* 91 [Fig. 2(c), sample **9**]; the phthalyl group was evidenced in the spectrum of sample **15** [Fig. 1(b)] by the ion at *m/z* 160, whereas the *tert*-butyl moiety was detected by the very abundant ion at *m/z* 57 [Fig. 2(b), sample **12**], assignable to $[C_4H_9]^+$. Hence it was not possible to discriminate between the Boc and tBu protecting groups.

When counterions were present on the surface, their abundances were fairly high, as expected under ionization by desorption. Indeed, preformed ions in the condensed phase were ejected into the gas phase without the need for the ionization step. Samples **16** and **20** contained the trifluoroacetate ion bound to an ammonium cation. In the negative mode, the ion at *m/z* 113 ($CF_3COO^-$) was very abundant [Fig. 1(d)]. Such counterion characterization suggested that ion exchange could be evidenced directly by SIMS. Nevertheless, in addition to such surface detection, immonium ions were also recorded, showing that information about less accessible constituents of the peptide chains could be obtained by static TOF-SIMS. The residue identification from immonium ions produced in SIMS was compared with FAB experiments, where it has been established[41] that some amino acids such as phenylalanine, tyrosine, alanine, proline, valine, leucine, isoleucine and lysine exhibited abundant ions. The previous studies[41] established that other amino acid residues produced corresponding immonium ions that were too weak (or even absent) to constitute an analytical criterion. We observed the same trends in all our SIMS experiments.[25,26,32,33] For instance, proline, leucine and phenylalanine were characterized by the ions at *m/z* 70, 86 and 120, respectively, and it should be noted that the ion at *m/z* 70 was very abundant in the spectrum of sample **13**, which contains the Phe–Pro sequence [Fig. 2(d)], the ion at *m/z* 120 being weak but not suppressed. Similarly, the nature of the protecting groups (sample **5** *vs.* **8**) and the linker system (sample **5** *vs.* **10**) did not affect the amino acid signal intensities. The immonium ion abundances were found not to depend upon the order of the residues as positive ion SIM spectra of samples **5** and **10** and samples **6** and **11** were similar.

**Table 3. Characteristic ions observed by TOF-SIMS analysis of protecting groups**

| Sample No. | Protecting group | Ion (*m/z*) | Structure |
|---|---|---|---|
| **8, 9, 19** | Boc | 57 |  |
| **3–7, 10–13, 17, 21** | Fmoc | 179 |  |
| | | 165 |  |
| **9** | Z | 91 |  |
| **15** | Pht | 160 |  |
| **7, 12** | tBu | 57 |  |

## DISCUSSION

Our main concern was to develop a direct and non-destructive analytical method in which the pin can be subjected to mass spectrometric analyses and used again for further reaction steps without the need for special derivatization (i.e. photolabile linker) or specific chemical treatment (TFA, etc.). Although in principle the whole crown could be subjected to SIMS analysis provided that a special holder has been designed, we chose to cut off a thin slice of the studied crown which was subjected to the analysis while the remaining part was mounted on the stem for subsequent syntheses. It was found that the yields of post-analysis reactions were not significantly reduced, showing that the slice was thin enough to leave sufficient reactive sites on the crown. It was found, however, that the crowns were not homogeneous as slices cut from the top exhibited higher ion abundances than slices generated from the sides.

Another parameter to consider was the crown substitution level. All the reported results were obtained with pins possessing a loading of 7 μM per crown. Never-

theless, satisfactory ion abundances were also recovered with crowns only loaded at 2.0, 1.8 and 1.3 μM.

These points having been established, a systematic survey of structural parameters was undertaken by varying the nature of the crown, spacer, linker, dipeptides and $N^{\alpha}$-protecting group. For each type of crown (HEMA, PS, MA–DMA), successive TOF-SIMS analyses were carried out in the positive and negative modes to allow the unambiguous assignment of each observed ion. The graft-only crown was studied in order to identify ions directly related to the polymer core. The spacer, linker and protected dipeptide were then added successively (see Table 1).

Each grafted polymer type exhibited characteristic ions; however, only ions at *m/z* 91 and 77 from the benzyl moiety of PS interfered with substrate ions of phenylalanine and of benzyl-based protecting groups such as the Z group. At this stage, PS and HEMA were found to be better polymers for SIMS analysis than MA–DMA, which led to weaker ion abundances.

Only the Rink handle showed an abundant immonium ion at *m/z* 166 which was clearly differentiated from any other detected ions. Further, the nature of the linker did not influence the abundances of the ions related to the anchored chain.

The amino acids exhibiting immonium ions under positive FAB conditions were found to behave similarly in SIMS analysis, allowing the identification of Ala, Leu, Lys, Phe and Pro residues. The glutamic acid residue was not detected and in this case only components of the structures were identified.

The Fmoc, Boc, Z and tBu ester protecting groups showed very abundant ions in positive ion SIMS. The phthalyl protecting group gave significant, although less intense, characteristic ion signals. However, Boc and tBu and also Z and Phe could not be differentiated as they were detected as the *tert*-butyl cation (*m/z* 57) and the tropylium ion (*m/z* 91), respectively.

Spectra obtained in the negative mode only contained ions featuring the Fmoc protection or the trifluoroacetic acid counterion when present. These results differed from those obtained previously with resins,[32,33] where the whole anchored chain was detected as a carboxylate. Thus, the negative ion mode could be used to detect counterions such as TFA whereas the positive mode is the most useful tool as it gave most of the relevant characterization data.

## CONCLUSION

Our method to analyze *in situ* solid-phase peptide synthesis established with resins was validated with plastic pin supports. This technique is direct without the need for any deleterious chemical treatment, thus providing real anchored product assessment. It can also be used to examine counterions in the solid phase. It has proved to be highly reproducible as long as the experimental parameters are kept constant. Its low throughput with current instrumentation could be a drawback for automated analyses but the use of a specific holder designed to maintain several samples would greatly accelerate the analysis rate. Sample surface heterogeneity can be detected by bombarding and acquiring spectra from different areas. Nevertheless, no quantitative studies have been undertaken so far in the case of mixtures; in particular, incomplete deprotection reactions or side products were deliberately avoided. The scope of this work could be extended to the study of mixtures, longer chains or more generally small organic molecules in the context of non-peptide supported library characterization. The SIMS technique is sensitive enough to obtain structural data without the need for large quantities of material and the method allows direct identification, thus, on the one hand, avoiding the use of specific linkers or chemical treatments to release the structures in solution which could be problematic in the case of non-peptide libraries and, on the other, allowing subsequent syntheses on the analyzed support after intermediate structure characterization.

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