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A New Method for the Preparation of Unprotected Peptides on Biocompatible Resins with Application in Combinatorial Chemistry†

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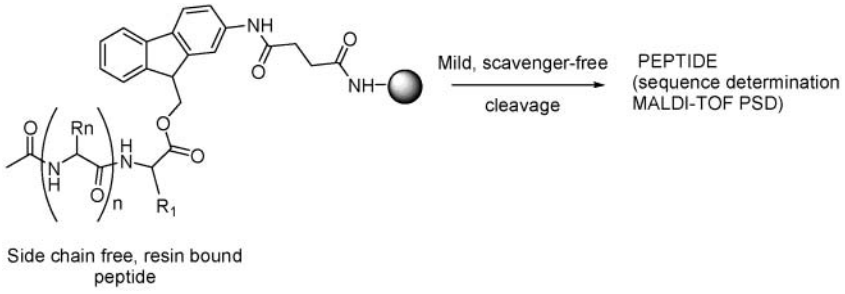
ABSTRACT



A synthetic strategy for the preparation of side chain free peptides on biocompatible solid supports is described. Final peptide detachment is afforded in mild basic conditions with no presence of scavengers or other additives, thus allowing single peptide–resin beads to be cleaved in mass spectrometry sample plates for direct sequencing using MALDI-TOF post-source decay. This methodology offers clear advantages for the development of one-bead–one-compound combinatorial libraries in addition to parallel and regular synthesis of peptides.

The successful application of combinatorial chemistry techniques relies in the development of optimal synthetic schemes, together with effective screening tests and deconvolution methods. The "one-bead−one-compound" combinatorial methodology has been successfully applied for the discovery of many ligands corresponding to biologically important targets.[1] This support-based method is highly efficient when used with consistent on-bead assays. Many of these assays involve the use of antibodies or enzymes in aqueous milieu, and thus resins must be biocompatible.

When applied to peptides, the availability of an optimal synthetic strategy to prepare unprotected peptides on resin in combination with an efficient sequence deconvolution method is likewise essential to the combinatorial approach. Regarding sequence determination, deconvolution of positive peptide beads obtained after screening is usually performed by Edman degradation,[2] although tagging[3] and ladder sequencing[4] are also commonly used. However, all these methods suffer from evident limitations. Tagging requires orthogonal protecting strategies that greatly increase the

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† Abbreviations: CHCA, α-cyano-4-hydroxycinnamic acid; ESI-MS, electrospray mass spectrometry; HMFS, *N*-[(9-hydroxymethyl)-2-fluorenyl] succinamic acid; HPLC, high-performance liquid cromatography; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MS, mass spectrometry; PSD, post-source decay.

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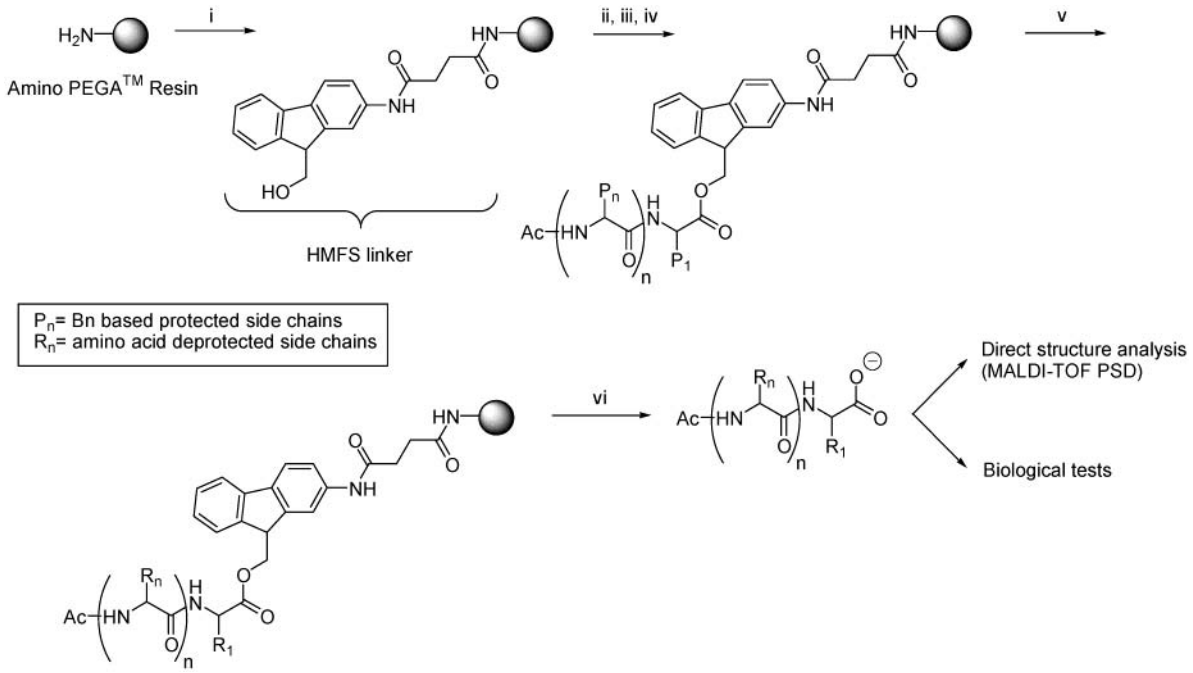

Figure 1. Scheme showing the use of the HMFS linker to obtain deprotected peptides on resin. Reagents and conditions: (i) amino PEGA resin (300 mg, 0.4 mequiv/g), HMFS (2 equiv), DIPCDI (2 equiv), HOBt (2 equiv), DMF, 24 h; (ii) first amino acid attachment with Boc/Bn-protected amino acid (5 equiv), DIPCDI (5 equiv), DMAP (0.5 equiv), DMF, 2 × 1 h; (iii) standard Boc/Bn chain assembly (5 equiv of protected amino acid, 5 equiv of DCC, 5 equiv of HOBt, DMF, 1 h); (iv) N-terminal acetylation with acetic anhydride (250 μl), pyridine (250 μl), DMF, 20 min; (v) side chain deprotection with anhydrous 9:1 HF/*p*-cresol (5 mL), 0 °C, 1 h; (vi) cleavage with 1:4 morpholine/DMF, 1 h.

number of synthetic steps. Furthermore, the determination of the sequence is indirect, and thus the real composition and purity of the compounds are not really known. Generation of ladder sequences progressively reduces the amount of sample available on the solid support along with the number of synthetic steps. It may also induce interferences between full-length and capped peptides. Finally, Edman sequencing can only be applied to free N-terminal peptides composed of natural amino acids and is expensive and time-consuming (30−60 min/amino acid).

To overcome both synthetic and analytical problems, we report in this article an efficient methodology for the preparation of unprotected peptides on biocompatible resins that enables peptide cleavage to be carried out directly in MALDI sample plates for subsequent deconvolution using MALDI-TOF PSD.[5] The synthetic strategy (Figure 1) is based on the use of a fluorenylmethyl bifunctional linker (HMFS)[6] that allows peptides to be cleaved under mild basic conditions (morpholine in DMF; pK_a of morpholine = 8.3) with no presence of potentially interfering additives or byproducts (salts, thiol scavengers, etc). The fact that the cleavage is performed directly on the MALDI plate ensures that no losses of positive compounds occur. The synthetic strategy involves the assembly of peptides using a Boc/Bn general scheme of protection on a PEGA biocompatible resin.[6d,7] Once the sequence is built up, protecting groups are cleaved by acidolysis with 9:1 HF/*p*-cresol at 0 °C for 1 h (Figure 1).[8] Side chain-deprotected peptides remained bound to the HMFS derivatized resin as the ester bond proved to be stable to the anhydrous HF conditions as expected.[9]

The usefulness of this strategy is illustrated with the synthesis of two model peptides corresponding to the 38−59 natural sequence of the B domain of the protein A[10] and

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(8) Anhydrous HF has proven to be a useful reagent for the final side chain deprotection in the synthesis of peptide libraries (Lebl et al. *J. Comb. Chem.* **1999**, *1* (6), 474−479). TFMSA acid has also been successfully applied as an alternative reagent for this purpose (Bourne et al. *J. Org. Chem.* **2001**, *66*, 7706−7713).

(9) Quantitative amino acid analysis of an Ac−Phe−HMFS−PEGA resin model before and after the treatment proved that the amino acid loss was less than 5%.

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1 Ac-AspProSerGlnSerAlaAsnLeuLeuAlaGluAla
LysLysLeuAsnAspAlaGlnAlaProLys-OH

2 Ac-AlaGlnLysGlnAlaSerProAspAlaLeuGluLys
AsnLeuProLysAlaAspAsnAlaSerLeu-OH

Figure 2. Sequences of model peptides used in this study. Amino terminal groups are acetylated.

a scrambled version of the same fragment (Figure 2). After assembly and side chain deprotection, peptide detachment of the resin was carried out with 1:4 morpholine/DMF for 1 h at room temperature. HPLC of the peptide crudes show peptide purities above 80% (Figure 3). Mass spectra (ESI-MS) and amino acid analysis confirmed the identity of the products.[11]

To prove that the synthetic strategy can be applied in combinatorial chemistry, a single peptide−HMFS−resin bead of each peptide was cleaved on a MALDI sample plate and after MALDI-TOF PSD analysis, the peptide sequence was determined from the pattern of peaks in the spectrum (Supporting Information).

In comparison with other chemical methodologies for the preparation of peptide libraries, the use of the HMFS linker offers clear advantages over others described in the literature, namely, photolabile linkers, nucleophile-cleavable linkers, safety-catch linkers, and methionine-based linkers.[12] Generally speaking, all these linkers suffer from limitations such as moderate yields and long cleavage times (photolabile linkers), incompatibility with peptide sequences susceptible to oxidation or electrophilic attack (safety-catch linkers), and the presence of cleavage reagents in the final mixture that could be incompatible with biological assays and, hence, should be removed, introducing an additional purification step to the synthesis (nucleophile-cleavable and methionine linkers). In this sense, the HMFS methodology involves a mild, clean, nonacidolytic or electrophilic cleavage that renders peptides in good yields and ready for laser ionization. In conclusion, the HMFS-based synthetic strategy in combination with the MALDI-TOF PSD sequence elucidation is an efficient methodology for the preparation of detachable unprotected peptides on solid supports and a useful tool to be applied to one-bead−one-compound combinatorial libraries with direct deconvolution of positive hits. In addition, one may envisage that the clean reaction conditions involved in the HMFS linker cleavage could be useful for the direct screening in solution of peptides obtained by parallel or even regular syntheses. The HMFS methodology is currently being used in our laboratory for the preparation of one-bead−one-compound peptide libraries with successful results.

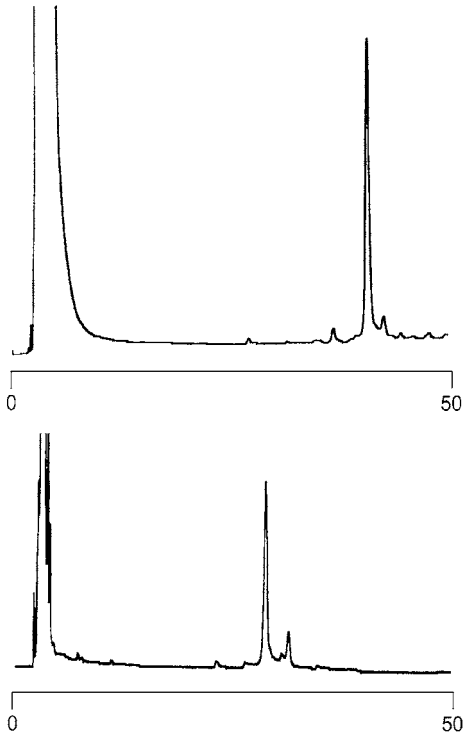

Figure 3. HPLC traces of crude peptides **1** (top) and **2** (bottom). Purities of crude peptides (calculated by area integration) were 85 (**1**) and 80% (**2**). HPLC elution conditions: (A) H_2O/0.045% TFA; (B) CH_3CN/0.036% TFA; gradient 10−35% B over 50 min.

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Supporting Information Available: Figure containing MALDI-TOF PSD spectra of peptides **1** and **2**, showing the identification of peptide fragments and explaining the methodology of sequence determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) **Analysis of Amino Acids:** [**1**] Asp = 4.13 (4), Ser = 0.78 (2), Glu = 3.16 (3), Ala = 4.92 (5), Lys = 3.17 (3), Leu = 2.92 (3), Pro = 1.87 (2); [**2**] Asp = 3.97 (4), Ser = 0.83 (2), Glu = 3.09 (3), Ala = 4.78 (5), Lys = 2.91 (3), Leu = 2.96 (3), Pro = 2.20 (2). Amino acids are shown in the three letter code. **ESI-MS:** [**1**] $(M + 3H)^{3+}$ = 785.6 *m*/*z* (calcd 784.4 *m*/*z*), $(M + 2H)^{2+}$ = 1176.2 *m*/*z* (calcd 1176.1 *m*/*z*); [**2**] $(M + 3H)^{3+}$ = 784.6 *m*/*z* (calcd 784.4 *m*/*z*), $(M + 2H)^{2+}$ = 1176.2 *m*/*z* (calcd 1176.1 *m*/*z*).

(12) For review of linkers used in solid-phase organic chemistry, see: Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091−2157.