Photolytic Mass Laddering for Fast Characterization of Oligomers on Single Resin Beads

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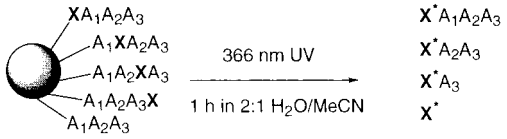
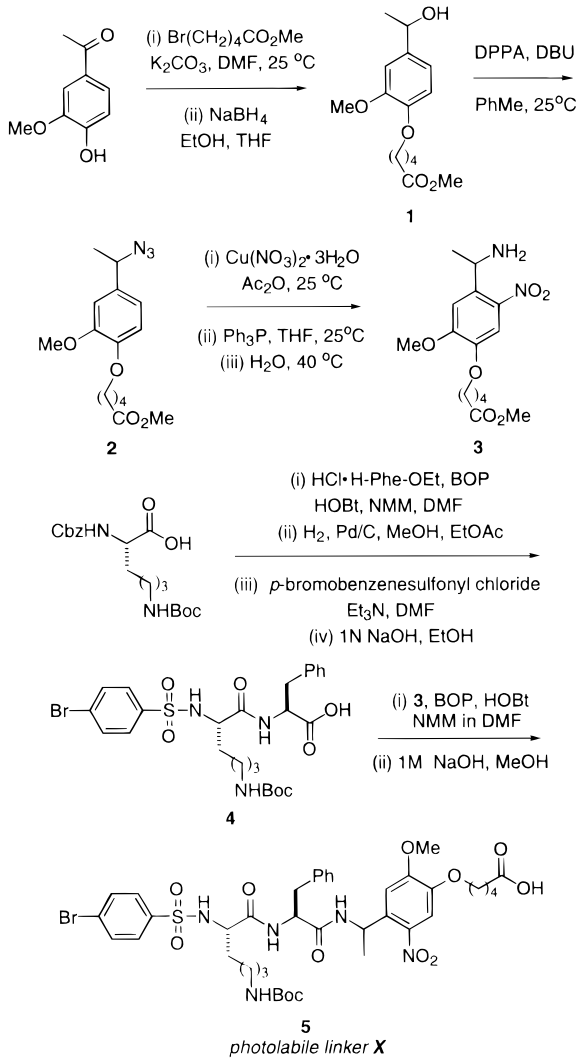
The divide/couple/recombine methodologies, also referred to as "split syntheses", represent a powerful combinatorial approach whereby a large number of supported compounds can be prepared in comparatively few synthetic operations. Originally, the technique was demonstrated for peptides,[1] and then coupled with a combinatorial screen.[2] Thus a library of approximately 2.47×10^6 different supported pentapeptides was mixed with a monoclonal antibody (mAb), and those ligands with highest affinities were identified via a marker on the mAb. The methodology is not restricted to peptides, however; split syntheses are conceptually applicable to any supported oligomers or collections of compounds that can be transformed via repetitive couplings.

Lead compound characterization is an obstacle that must be overcome whenever split syntheses are applied to generate libraries of oligomers that do not consist solely of the protein amino acids. In such cases, automated peptide/protein sequencers using Edman's degradation[3] chemistry are unsuitable. Mass spectrometric methods like matrix-assisted laser desorption/ionization (MALDI)[4] and electrospray ionization (ESI)[5] give data that can sometimes be used to characterize the product, but time-consuming interpretations are required and ambiguities can arise. Direct observation of the products by NMR is impractical unless exceptionally large and high-loading beads are used. Consequently, several innovative solutions to the problem of characterizing compounds on single resin beads have been reported.[6−11] This communication describes our own method which is one that may be described as a "photolytic mass-laddering technique". The split synthesis is performed such that most of the oligomer on any bead will consist of an unperturbed sequence, but a small fraction will have a photolabile group **X** inserted before each of the coupling steps. On irradiation, an isolated bead generates fragments of incrementally different molecular masses. MS analyses of the material liberated could be used to deduce the sequence from the molecular mass differences as shown below.



MALDI-TOF MS analyses

Scheme 1. Synthesis of a Photolabile Amino Acid



Many of our original experiments to test the photolytic mass-laddering concept were unsuccessful. Ultimately, it became apparent that upon swelling most polystyrene-containing beads (*e.g.* Rink and TentaGel resins) liberate small polymer fragments of less than approximately 550 Da into solution, and that these obscured low concentrations of any other materials liberated from the bead. Above 550 Da, however, the spectra are relatively clean. Consequently, it was necessary to prepare photocleavable groups that would leave relatively heavy fragments (**X***) on the liberated strand, which would generate mass ladders consisting of fragments larger than 550 Da.

Scheme 1 describes a synthesis of molecular fragment **X** that could be used for photolytic mass laddering. Quantitative alkylation of acetovanillone with methyl 5-bromopentanecarboxylate, followed by reduction with sodium borohydride gave **1** in 97% yield after two steps

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(no purification necessary). Conversion of the alcohol to the azide was then performed using a Mitsunobu type[12] displacement. The resulting azide **2** was nitrated with copper(2+) nitrate and subsequently reduced to the intermediate free amine **3**. This oxygenated nitrobenzyl system is desirable since such compounds cleave efficiently when irradiated at 360 nm.[13] In the second branch of this convergent approach to **X**, phenylalanine ethyl ester was coupled with Cbz-Lys(BOC). Hydrogenolysis of the Cbz group, reaction with bromobenzenesulfonyl chloride, and hydrolysis of the methyl ester yielded brominated, and relatively heavy, intermediate **4**. Finally, the free amine **3** was coupled to dipeptide **4** and hydrolyzed to give the photolabile amino acid **5** (**X**).

The photocleavable amino acid **X** was incorporated into a series of representative tetra- and pentapeptides of known sequence to provide substrates to demonstrate photolytic mass laddering. Thus, six different peptides were constructed on TentaGel amino resin (80−100 pmol/bead). The peptide syntheses were performed in such a way that a small mole fraction of **X** (5 mol %, except the initial example attempted, FFMF, 10 mol %) was coupled prior to each regular coupling step.

Single beads of each of the six test sequences were placed in small cuvettes with 5 μL of a 2:1 water/acetonitrile mixture and irradiated at a distance of approximately 1 cm from an 8 W 360 UV lamp. The rate of photochemical cleavage is proportional to the flux of radiation inputted to **X**. An irradiation time of 3 h was routinely used in these experiments, but the most recent studies have shown that this was longer than was actually necessary; for instance, only 5 min exposure of the sequence FFMF to a 8 W UV source was sufficient (data not shown).

MALDI spectra of the samples obtained from single beads are shown in Figure 1. Isotopic distribution of bromine isomers in the heavy part of the linker, **X***, gives 1:1 molecular mass distributions for peaks containing this fragment, allowing them to be easily differentiated from background signals (unfortunately these doublets do not show up well in this photoreduced figure, but they are clear on normal size spectra, see Supporting Information). Oxidation of methionine was observed for peptide **f**. This is probably due to the presence of radicals produced by the photolytic reaction.[14] However, this oxidation did not generate any problems with the assignment process.

In summary, this work demonstrates that photolytic mass laddering can be used for efficient sequence determination of peptides generated in split syntheses. The technique could also be used for other oligomer types by modifying the functionality of the photocleavable group **X** to accommodate the preferred coupling scheme. Isomeric residues could be encoded by appropriate use of two **X** groups of different masses. There is a concern that activity in the binding assays may be observed solely as a result of the linker system, but only a small fraction (*e.g.* 4 × 5% if 5 mol % of linker is used for a tetramer) of the sequences displayed on any given bead will contain a linker unit. Consequently, this phenomenom will probably not occur frequently. However, if it does occur, the false positives that result should be easily identified by further screens. Photolytic mass laddering has some advantages over other MS methodologies developed for the same purpose. For instance, it is compatible with methionine, whereas methods that involve cyanogen bromide cleavage are not.[11] It is appropriate for split syntheses involving more than three coupling cycles whereas isotopic encoding methodologies[15] would give very complicated mass spectra for libraries of the same composition. In conclusion, this paper demonstrates proof of concept for photolytic mass laddering of oligomers formed in split syntheses, a characterization technique which has potential advantages over some of the methods reported to date.

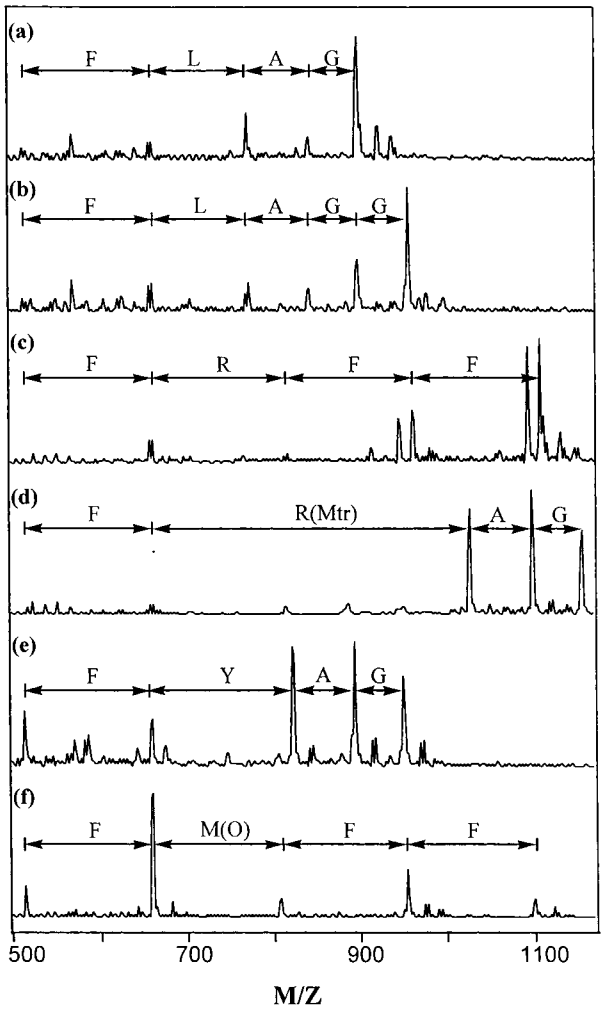

Figure 1. MALDI-MS of peptide samples/photocleavable tag sequences, liberated from single beads. The known peptide sequences were (a) GALF, (b) GGALF, (c) FFRF, (d) GAR(Mtr)F, (e) GAYF, and (f) FFMF. Matrix: α-cyano-4-hydroxycinnamic acid. Instrument: Voyager-Elite XL MALDI mass spectrometer in the linear mode with a stainless steel sample plate.

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Supporting Information Available: Procedures for synthesis of the photolabile linker, protocol for the peptide syntheses, method for analyte preparation and MALDI analysis, full size copies of the MALDI-MS spectra that were reduced to form Figure 1 (14 pages).

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