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Microchemical Structural Determination of a Peptoid Covalently Bound to a Polymeric Bead by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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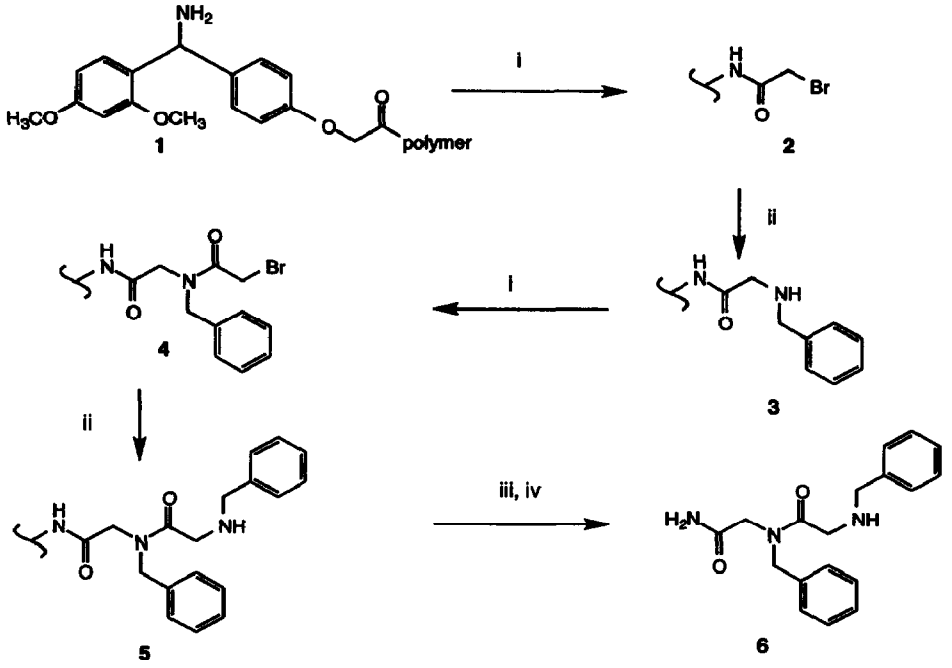
Abstract: New methodology utilizing matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF MS) for the direct identification of a ligand covalently attached to a polymeric bead is reported. The method allows for structure determination without relying on encoding strategies. Gaseous TFA cleavage of a peptoid from a single bead followed by mounting in MALDI matrix and irradiation with a beam at 337 nm gave abundant molecular ions and structurally informative fragment ions produced by post source decay (PSD).

A strategic difficulty associated with combinatorial synthesis on polymeric supports is the structural identification of the ligand covalently attached to the bead which has been affinity selected from the combinatorial mixture of beads.[1] Several strategies have emerged to address this problem. For example, a specific sequence of nucleotides,[2] amino acids[3] or halogenated hydrocarbons[4] encoded for a particular ligand may be orthogonally attached to the bead. If the bead is picked as a candidate, the encoding ligand may then be selectively cleaved and identified thereby providing the structure of the ligand of interest. While this strategy has achieved success, there are some obvious disadvantages. Attachment and chemical synthesis of the coding ligand adds complexity to the synthesis and may interfere with the biological assay. We report here an alternative strategy which requires no encoding ligand.

A peptoid[5] was synthesized on a polymeric bead[6] attached via a Rink amide handle[7] known to be very stable and cleavable only under highly acidic conditions (Scheme 1). Beads containing peptoid were subjected to high acid cleavage using gaseous TFA. A single bead was then analyzed by MALDI-TOF MS[8] (see experimental section).

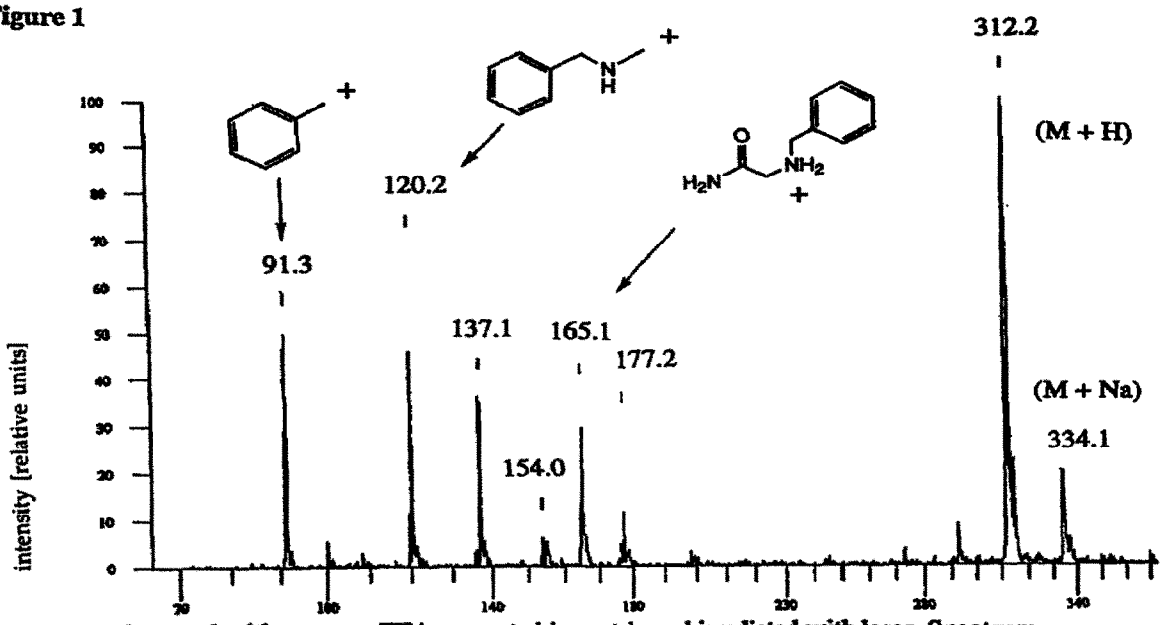
Scheme I



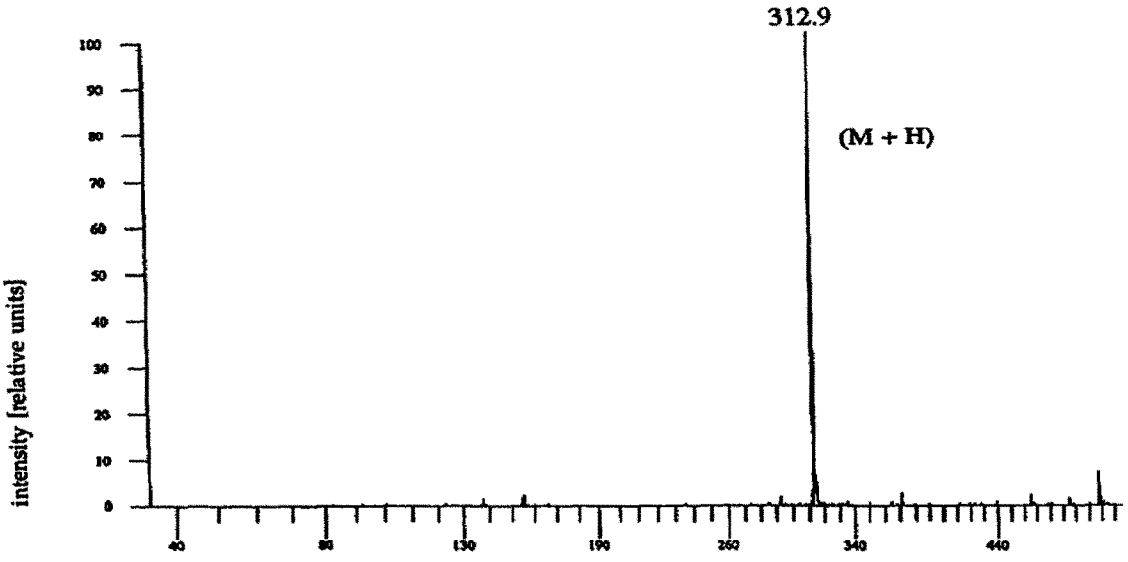
(i) Bromoacetic acid, diisopropylcarbodiimide, N,N-dimethylacetamide. (ii) Benzylamine, N,N-dimethylacetamide. (iii) gaseous TFA. (iv) Mounting on stainless-steel sample target with laser probe and 3,5-dihydroxybenzoic acid.

Irradiation of the bead with a 337 nm beam produced abundant molecular ions of the parent peptoid (Figure 1). Fragment ions generated by post source decay[9] were also observed in the mass spectrum thus providing a direct structural readout of the ligand on the bead. Since the diameter of the laser beam is between 10-20 microns, this technique consumes less than 1% of the sample on the bead allowing additional experiments to be performed on a single bead. Irradiation of the matrix surrounding the bead produced the parent ion but no fragmentation ions were observed. Analysis of a bead containing the same peptoid without acid cleavage afforded no molecular ions by MALDI-TOF. A 50 micron bead with the peptoid adsorbed onto the surface but not covalently attached was also analyzed. We found that in the absence of MALDI matrix no signal could be produced; however, when the same bead was mounted with the MALDI matrix abundant parent and fragment ions were observed. These results clearly demonstrate that direct structural determination of a ligand on a bead is possible without the use of encoding strategies.

Figure 1



Bead treated with gaseous TFA, mounted in matrix and irradiated with laser. Spectrum shows M+H, M+Na and daughter ion fragments. Matrix peaks are 137.1, 154.0 and 177.2.



Irradiation of matrix surrounding the TFA treated bead.

Experimental Section

A single bead was placed in the reaction chamber of a gas phase protein sequencer and subjected to a stream of gaseous TFA for 5 minutes. The TFA gas was generated by sparging a vessel of TFA with nitrogen gas and directing the flow to the reaction chamber. Mass spectra were recorded on a Finnigan MAT (San Jose, CA) Vision 2000 matrix-assisted laser desorption ionization time-of-flight mass spectrometer or MALDI-TOF MS. The Vision 2000 is equipped with a CCD high resolution micro camera with zoom optics and monitor to aid in the location of polymer bead on the sample target. Beads were affixed to the stainless-steel sample target surface (12 mm diameter) by deposition of 0.5 ml of a solution of dihydroxybenzoic acid (Aldrich; saturated, ca. 50 mM in dH_2O). Crystallization of DHB during solvent evaporation immobilizes the polymer bead to the target. Mass spectra were generated as follows. Ionization was accomplished with a 337 nm beam from a LSI VSL-337 ND nitrogen laser (5ns pulse width, 20 Hz). Ions were then accelerated to 5 kV, reflected using a high resolution gridless ion mirror and detected with a secondary electron multiplier with 30 kV post acceleration. Total flight path was 1.7 m. Signal from the detector was digitized at a sampling rate of 100 MHz using an 8 bit / 64 K high speed static RAM transient recorder. Data processing was accomplished using a Gateway 386 PC running Finnigan MAT software. Typically, spectra were generated from the sum of 5-15 laser shots. Spectra were calibrated internally using matrix ions as calibrants.

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