Supporting Information

For

Click Radicals: Rapid, Quantitative, and Site Specific Synthesis of Biomolecular Radicals by UV Photodissociation

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Experimental Section

Materials

Organic solvents and reagents were purchased from Sigma-Aldrich or Acros Organics and used without purification unless otherwise noted. Water was purified to 18.2 MΩ resistivity using a Millipore Direct-Q water purification system.

Methods

Synthesis of Covalent and Noncovalent Radical Precursors

Succinimidyl 4-iodobenzoate (3). 0.50mmol DCC in 5.0 mL dioxane was added to a 100 mL round bottom flask containing 0.50mmol of 4-iodobenzoic acid and 0.50mmol N-hydroxysuccinimide in 10.0 mL dioxane. After a 12 hour reaction period, a crystalline hair-like precipitate was observed. The precipitate was removed by filtration. The filtrate was then evaporated over nitrogen, leaving a white solid. Identical procedures were used for the other isomers.

2-(hydroxymethyl-4-iodobenzoate)-18-crown-6 ether (4). 0.50 mmol DCC in 5.0 mL dioxane was added to a 50 mL round bottom flask containing 0.50 mmol of 4-iodobenzoic acid and 0.50 mmol 2-hydroxymethyl-18-crown-6 ether. A catalytic amount of DMAP (~10 mg) was added. After a 12 hour reaction period, a crystalline hair-like precipitate was observed. The precipitate was removed by filtration. The filtrate was then passed through celite and evaporated over nitrogen. The product was recovered as a white solid.

Peptide Derivatization

Peptides (~10 nmol) were derivatized with **3** by reacting 2:1 1-NHS:peptide in 100 μL 1:1 borate buffer (pH 8.5):organic solvent (DMSO/dioxane) for 30 minutes at 37 °C. Relative yields were estimated to be 50-90% using the ratio of unmodified to modified peptide in the ESI-MS spectrum (see below for MS of RPPGFSPF and ubiquitin reactions). For gas phase UV experiments, a Michrom Peptide Trap was used to desalt the modified peptide or protein prior to mass spectrometry. For aqueous UV irradiation experiments, modified peptides were purified by high performance liquid chromatography, using a fast elution gradient (5% to 80% B in 20 minutes, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA) on an analytical C18 column.

Electrospray Mass Spectrometry

Solutions containing ~7 μM peptide or protein in 50/50 water/acetonitrile + 0.1% acetic acid were infused by syringe into the electrospray source of an LTQ linear ion trap mass spectrometer. The back of the instrument vacuum housing was modified with a quartz window to permit introduction of UV photons into the linear ion trap from a Continuum MiniLiteNd:YAG laser (4^{th} harmonic). Mass spectrometry was performed using a modified LTQ linear ion trap equipped with a Nd:YAG laser. The LTQ software is capable of emitting a voltage differential signal timed to the beginning of aMS^n experiment. This signal, after conditioning by a digital delay generator (Berkeley Nucleonics), triggers the laser to fire during a user-specified MS^n experiment.

UV Photodissociation in Aqueous Solvent

A 50 μL aliquot of a ~50 μM solution of **3**-modified peptide was transferred to a 300 μL thin quartz cuvette. The solution was gently degassed for 2 minutes with N_2, and then irradiated using a T8 15W Hg lamp (Phillips) in a hypoxic environment (constant N_2 flow). Irradiation was paused at 1, 2, 3, and 4 minutes to remove 2 μL aliquots for mass spectrometry. The 2 μL aliquots were loop-injected into a flow of 50/50 water/acetonitrile that is infused intothe electrospray source. Spectra were acquired for 1 minute. At the flow rate used (20 mL/min), injected samples were observed at ~0.5 min.

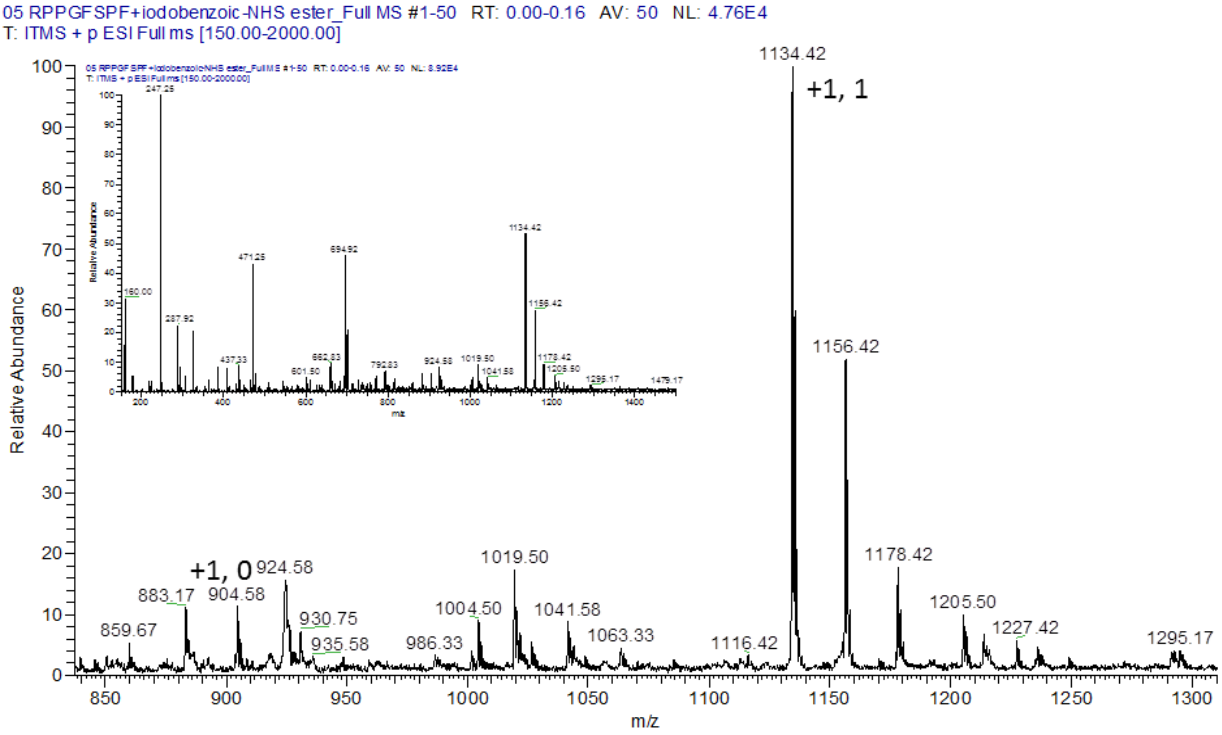
High resolution mass spectrometry

Compounds **1**-**4** were analysed by high resolution mass spectrometry to verify composition. **4** was dissolved in 50/50 water methanol and ionized in a mixed ESI / CI source. The exact mass of the**4** + NH_4^+ adduct was determined with an LC/TOF (Agilent 6210, Santa Clara, CA).Compounds **1**-**3** were dissolved in acetonitrile and analyzed by GC/MS. The compounds were ionized by 70eV EI and exact mass was determined with a Waters (Milford, MA)GCT Premiere GC/TOF mass analyzer.

The results are reported for each compound below:

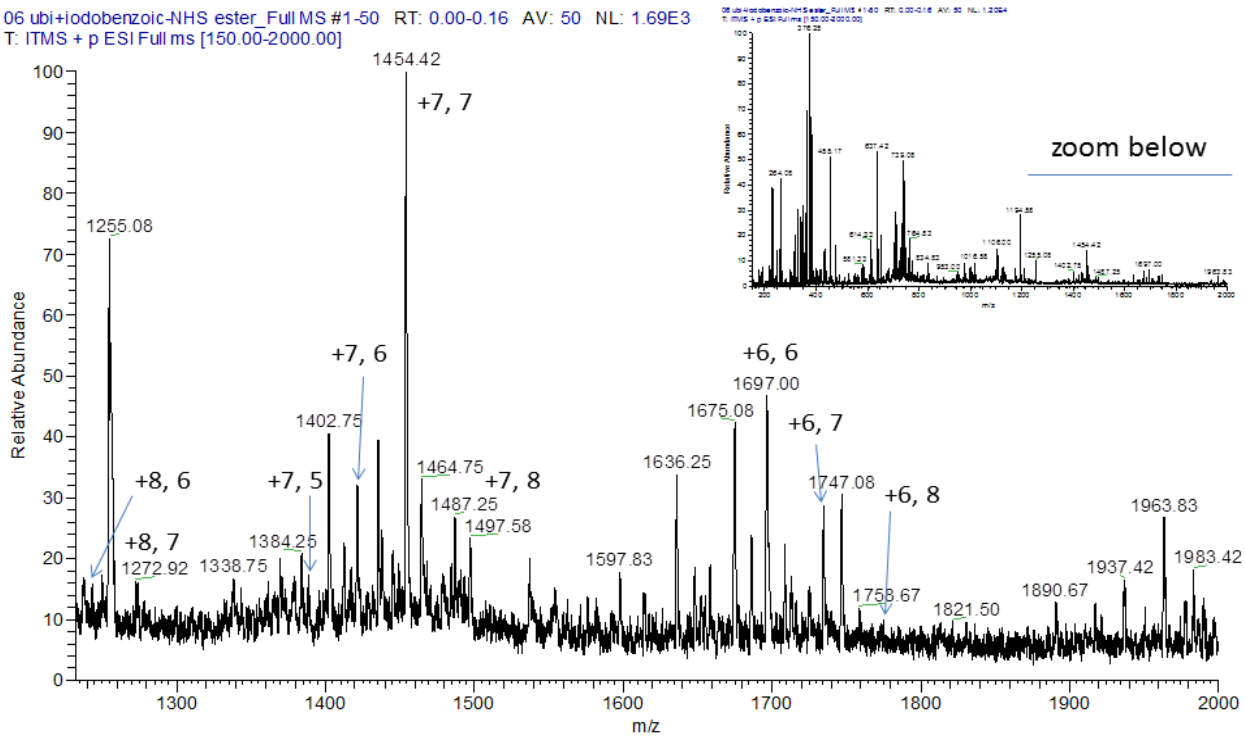
Compound	**Mass**	**Error**
4+ NH_4^+	542.12509	2.7 ppm
1	344.9493	-0.6 ppm
2	344.9493	0.9 ppm
3	344.9493	-3.2 ppm

MS of product mixture after reacting RPPGFSPF and 3



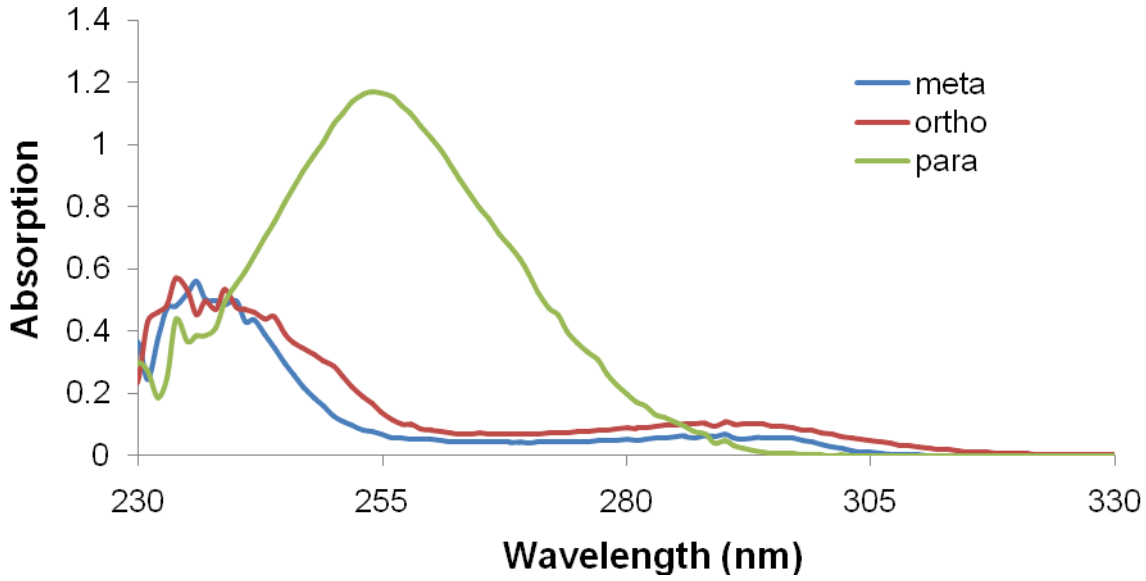
Peak label = charge state, number of modifications

Full MS of product mixture after reacting ubiquitin and 3

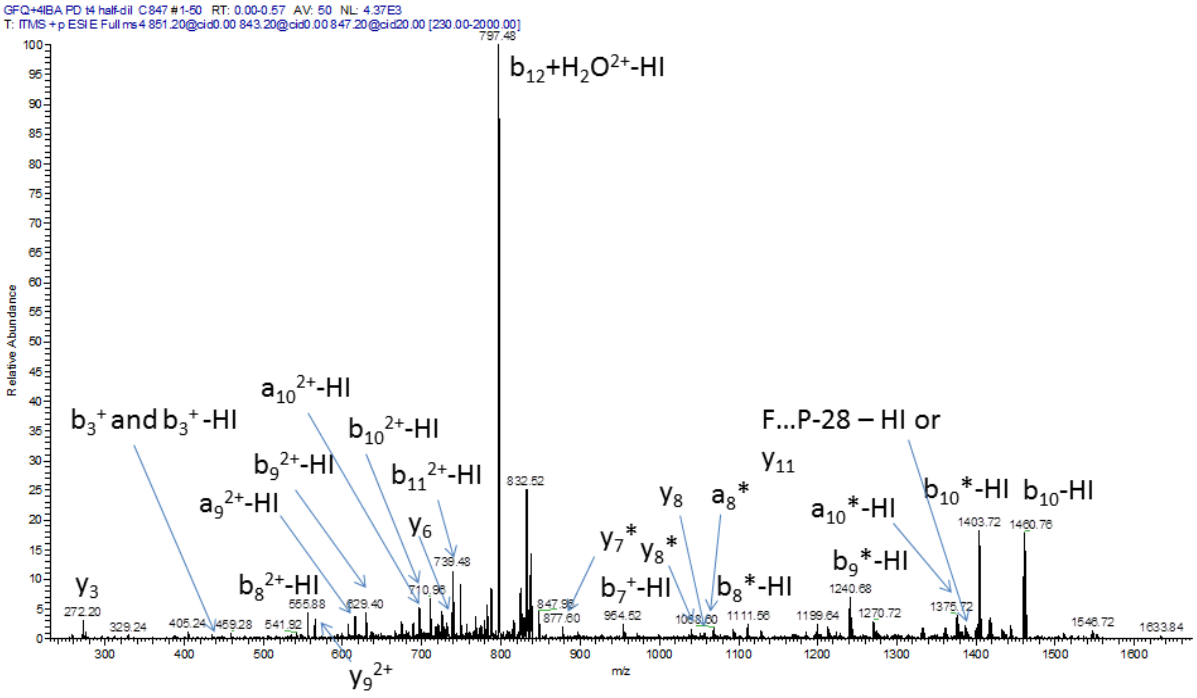


Peak label = charge state, number of modifications

Absorption spectra of 67 mM*meta*-, *ortho*-, and*para*- iodobenzoic acids in BHT-stabilized dioxane



CID spectrum of $[3GFQ + 2H - HI]^{2+}$



Photodissociation of [Ubiquitin + 5H + 4]$^{5+}$ yields loss of iodine atom