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Nanosecond Generation of Tyrosyl Radicals via Laser-Initiated Decaging of Oxalate-Modified Amino Acids

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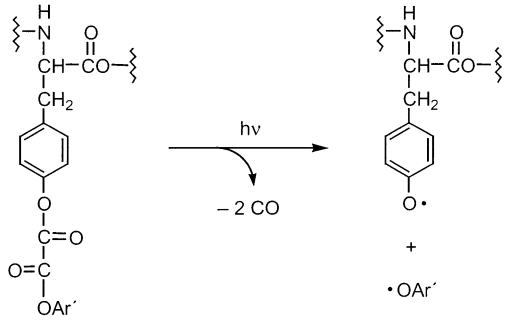
Abstract: We describe a general method for the unimolecular photochemical generation of tyrosyl radicals from a diaryl oxalate ester platform on the nanosecond time scale. Symmetric and asymmetric tyrosine oxalate esters have been prepared in gram quantities. Direct photocleavage of the oxalate linkage by laser flash photolysis affords tyrosyl radicals within 50 ns. This approach provides unnatural caged amino acids that may be incorporated into model and biological systems for the study of proton-coupled electron transfer in enzymatic catalysis.

Interest in the role of protein radicals in enzymatic catalysis has steadily escalated as stable and transient radicals have been increasingly identified in Nature.[1] Oxidized tyrosines and their derivatives, in particular, are ubiquitous in a variety of biological systems such as the Class I ribonucleotide reductases,[2] Photosystem II,[3] galactose oxidase[4] and prostaglandin H synthase.[5] Although radical-based catalysis is now being widely recognized as a paradigm for biochemical reactivity,[1,6] there are few time-resolved methods available to date for the investigation of reaction mechanisms enabled by the generation of amino acid radicals, especially on fast time scales. In this regard, laser flash photolysis techniques have led to significant advances in studies of biochemical reactions that require information at time scales shorter than the millisecond resolution provided by stopped flow experiments.[7] To this end, the development of techniques to "turn on" tyranosyl radicals quickly by light will open new avenues for the study of radical chemistry in biology.

One approach for fast tyrosyl radical generation is the linkage of tyrosine to transition-metal complexes that can undergo photoinduced electron transfer with the pendant amino acid. Systems based on covalently tethered ruthenium(II)[8] or rhenium(I)[9] and polypyridine complexes can generate tyrosyl radicals, within 20 μs for the former, using a bimolecular flash-quench technique. However, these derivatives may be cumbersome for biochemical studies because of their steric bulk and the requirement for high concentrations of external quencher. Thus, we sought a simple photochemical method that could cleanly deliver the tyrosyl radical on a sub-microsecond time scale with chemically benign byproducts and no added chemical quencher. We became interested in the advances made by Lahti and co-workers, who were able to generate aryloxyl radicals in solid state and solution environments by photolysis of diaryl oxalate esters with a quantum yield of ~0.1.[10] Inspired by this work, we have utilized this strategy for the preparation and study of oxalate-based unnatural amino acid derivatives for fast tyrosyl radical generation. We now report the synthesis and characterization of tyrosine-based derivatives that may be linked through the carboxy terminus.

Both symmetric and asymmetric caged tyranosyl radicals are readily prepared in gram-scale quantities by adaptation of literature procedures as outlined in Scheme 1.[10] Symmetric bis(*N*-acetyl tyrosyl methyl ester) oxalate ester **1** is prepared in good yield (65%) by addition of *N*-acetyltyrosyl methyl ester and triethylamine to oxalyl chloride at −78 °C in THF followed by gradual warming to room temperature. Similarly, asymmetric diaryl oxalate esters **2** and **3** are obtained in 42% and 41% yield, respectively, by reaction of the corresponding aryl oxalyl acid chloride with a protected tyrosine derivative and triethylamine. Deprotection of **3** by hydrogenation over 10% Pd/C gives carboxylic acid **4** in high yield (92%), without loss of the oxalate ester moiety. All compounds were fully characterized by ^{1}H NMR spectroscopy (available in Supporting Information) and high-resolution mass spectrometry.

Photolysis of the oxalate-modified tyrosine analogue results in the homolysis of the carbon−oxygen bond with the concomitant loss of 2 equiv of carbon monoxide and the appearance of a tyrosyl and aryloxyl radical pair:



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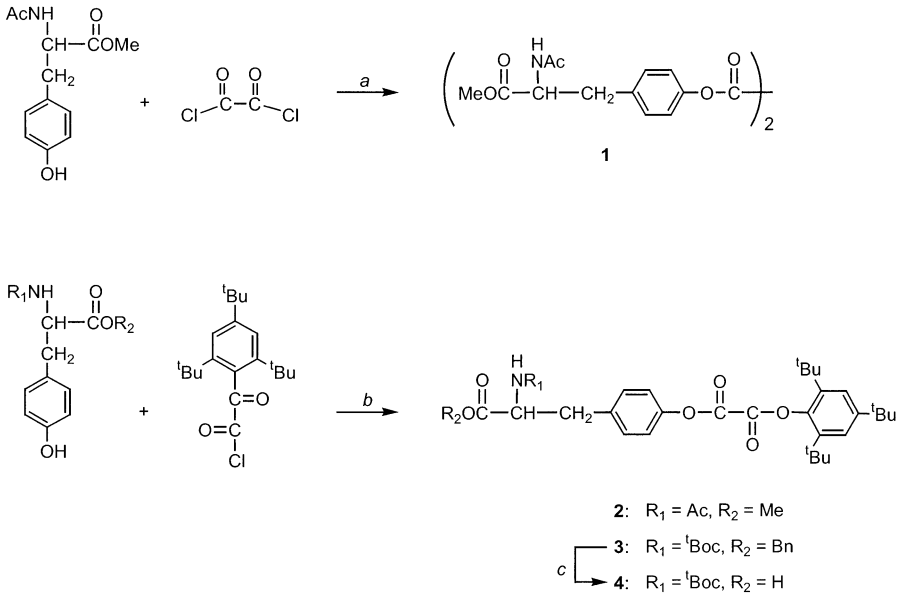
SCHEME 1[a]



[a] Reagents and conditions: (a) NEt_3, THF, N_2, −78 °C; (b) NEt_3, THF, N_2; (c) 10% Pd/C, THF, H_2.

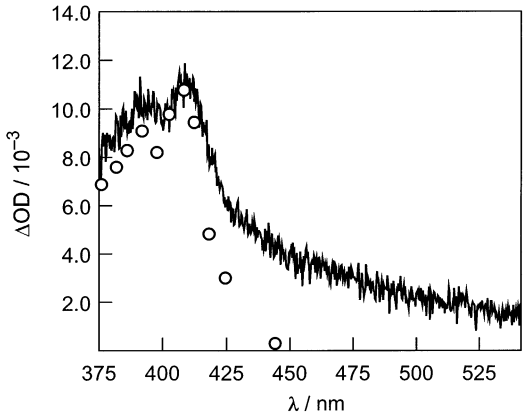


FIGURE 1. Transient absorption spectrum of the tyrosyl radical formed from laser flash photolysis of **1** overlaid on a spectrum of a tyrosyl radical (○) taken from ref 11. The two spectra are normalized with respect to each other.

In the case in which Ar′O• is a more stable species, such as the sterically protected 2,4,6-tri-*tert*-butylphenoxyl radical, the tyrosyl radical alone will be the reactive species on the time scale of interest.

Tyrosyl radical generation from either **1** or **2** was investigated by time-resolved absorption spectrocopy. Direct laser excitation of **1** (λ_{exc} = 270−300 nm) produces a species that has a transient difference spectrum exhibiting positive absorption maxima at 394 and 407 nm; the spectrum obtained is characteristic of a tyrosyl radical (Figure 1).[11] The signal intensity maximizes within the 50 ns integration time of the gated CCD detector and the tyrosyl radical subsequently decays after 200 ns. Spin-trapping by DMPO confirms the formation of a radical species from **1** by excimer laser photolysis at 308 nm. Compound **2** undergoes a similar photoinduced decaging to deliver a tyrosyl radical on the nanosecond time scale. The hydrogenation of the benzyl ester moiety of **3** to yield free carboxylic acid **4** provides a functional group to couple these amino acid derivatives through the C-terminus to form amide or ester linkages. A wide variety of other N-terminal protecting groups, such as Fmoc or acetyl, are compatible with the deprotection and can be facilely integrated into this class of compounds.

We have outlined a novel method for the fast photochemical generation of tyrosyl radicals by direct photocleavage of organic tyrosyl derivatives based on diaryl oxalate esters. The time scale for generation of radical product (<50 ns) from these unnatural amino acids is over 500 times faster than the ruthenium(II) polypyridine system without the need for an external chemical quencher. Moreover, the preparation of **4** contributes to methods aimed at generating tyrosyl radicals on peptides.[12] Future studies will focus on the incorporation of these unnatural amino acids into model and biological systems for the study of proton-coupled electron-transfer reactions[13,14] in enzyme catalysis.

Experimental Section

Materials. Silica gel 60 (70−230 and 230−400 mesh) was used for column chromatography. Analytical thin-layer chromatography was performed using F254 silica gel (precoated sheets, 0.2 mm thick). Solvents for synthesis were of reagent grade or better and were dried according to standard methods. Triethylamine was distilled over calcium hydride and stored at 4 °C under nitrogen. 2,4,6-Tri-*tert*-butylphenyl oxalyl chloride was prepared according to literature procedures.[10] The material was further purified by dissolution in dichloromethane and filtration to remove any remaining LiCl. Phenol starting materials were dried in vacuo for 24 h before use. Spectroscopic

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experiments employed tetrahydrofuran (spectroscopic grade, Burdick & Jackson), which was stored over sodium/benzophenone under high vacuum. Mass spectral analyses were performed in the MIT Department of Chemistry Instrument Facility. All manipulations were carried out under a nitrogen atmosphere using standard Schlenk and glovebox techniques unless otherwise noted.

Bis(*N*-acetyltyrosyl methyl ester) Oxalate Ester (1). In a 100-mL Schlenk flask was dissolved *N*-acetyltyrosyl methyl ester (4.0 g, 15.6 mmol) in dry THF (30 mL). The solution was stirred overnight over activated 4 Å molecular sieves, after which triethylamine (2.17 mL, 15.6 mmol) was added. A solution of oxalyl chloride (0.64 mL, 7.3 mmol) in THF (10 mL) was prepared in a separate 250-mL Schlenk flask. Both flasks were cooled to −78 °C, and the solution of *N*-acetyltyrosyl methyl ester was transferred dropwise via cannula to the solution of oxalyl chloride. The solution was stirred at −78 °C for 1 h and then allowed to warm to room temperature and stirred for an additional hour. The resulting white precipitate was collected by filtration in air and redissolved in dichloromethane containing a small amount of methanol. The organic layer was washed with HCl (3 × 25 mL, 3.7% v/v) and dried over Na_2SO_4. The solution was evaporated to dryness to yield pure **1** as a white powder (2.5 g, 65% yield). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.20 (m, 8H, ArH), 5.96 (d, J = 8 Hz, 2H, NH), 4.92 (dd, J_1 = 9.5 Hz, J_2 = 6 Hz, 2H, CH), 3.75 (s, 6H, methyl ester), 3.17 (ddd, J_1 = 29.5 Hz, J_2 = 14 Hz, J_3 = 6 Hz, 2H, CH_2), 2.02 (s, 3H, acetyl). HRFABMS (MH^+) *m*/*z*: calcd for $C_{26}H_{29}N_2O_{10}$ 529.182, found 529.181.

(*N*-Acetyltyrosyl methyl ester)(2,4,6-tri-*tert*-butylphenyl) Oxalate Ester (2). *N*-Acetyl tyrosyl methyl ester (1.5 g, 5.9 mmol) was dissolved in THF (30 mL) containing pyridine (0.475 mL, 5.9 mmol). The solution was transferred via cannula to a solution of 2,4,6-tri-*tert*-butylphenyl oxalyl chloride (2.8 g, 8.0 mmol) in THF (20 mL) and stirred overnight at room temperature. Pyridine hydrochloride was removed by filtration, and the filtrate was dried to a yellow oil that was triturated with hexanes. The resulting white precipitate was collected and redissolved in dichloromethane. Ether was added to precipitate unreacted tyrosine. The remaining solution was collected, washed with saturated aqueous Na_2CO_3 (1 × 50 mL), and dried over Na_2SO_4. The solvent was removed to deliver pure **2** as a white solid (1.37 g, 42% yield). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.38 (s, 2H, ArH), 7.21 (dd, J_1 = 23 Hz, J_2 = 9 Hz, 4H, ArH), 5.96 (d, J = 7.5 Hz, 1H, NH), 4.92 (dd, J_1 = 13.5 Hz, J_2 = 6 Hz, 1H, CH), 3.76 (s, 3H, methyl ester), 3.18 (ddd, J_1 = 30 Hz, J_2 = 14 Hz, J_3 = 5.5 Hz, 2H, CH_2), 2.03 (s, 3H, Acetyl), 1.41 (s, 18H, ^{t}Bu), 1.35 (s, 9H, ^{t}Bu). HRFABMS (MH^+) *m*/*z*: calcd for $C_{32}H_{44}NO_7$ 554.312, found 554.312.

(*N*-α-Boc-tyrosyl benzyl ester)(2,4,6-Tri-*tert*-butylphenyl) Oxalate Ester (3). *N*-α-Boc-tyrosyl benzyl ester (2.0 g, 7.4 mmol) was dissolved in THF (15 mL) containing triethylamine (1.4 mL, 10 mmol). The solution was transferred dropwise via cannula into a solution of 2,4,6-tri-*tert*-butylphenyl oxalyl chloride (2.8 g, 8.0 mmol) in THF (20 mL), and the resulting solution was stirred overnight at room temperature. The solvent was removed in vacuo, and purification by flash column chromatography (silica gel, 98/2 dichloromethane/methanol) afforded pure **3** as a white powder (2.1 g, 41% yield). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 7.39 (s, 2H, ArH), 7.36 (m, 5H, benzyl-ArH), 7.12 (dd, J_1 = 14.1 Hz, J_2 = 8.4 Hz, 4H, ArH), 5.16 (dd, J_1 = 27.3 Hz, J_2 = 12 Hz, 2H, benzyl-CH_2), 5.03 (d, J = 8.7 Hz, 1H, NH), 4.65 (dd, J_1 = 14.7 Hz, J_2 = 7.2 Hz, 1H, CH), 3.13 (m, 2H, CH_2), 1.45 (s, 9H, ^{t}Boc), 1.42 (s, 18H, ^{t}Bu), 1.36 (s, 9H, ^{t}Bu). HRFABMS (MNa^+) *m*/*z*: calcd for $NaC_{41}H_{53}NO_8$ 710.367, found 710.368.

(*N*-α-Boc-tyrosyl)(2,4,6-Tri-*tert*-butylphenyl) Oxalate Ester (4). Ester **3** (1.0 g, 1.45 mmol) was dissolved in THF (50 mL) and stirred over 10% Pd/C (250 mg) for 6 h under a hydrogen atmosphere. The Pd/C was removed by filtration, and the solvent was evaporated to give pure **4** as a pale manila solid (0.8 g, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 7.38 (s, 2H, ArH), 7.27 (dd, J_1 = 15.9 Hz, J_2 = 9 Hz, 4H, ArH), 4.99 (d, J = 10.2 Hz, 1H, NH), 4.63 (m, 1H, CH), 3.20 (dd, J_1 = 45 Hz, J_2 = 6.9 Hz, 2H, CH_2), 1.45 (s, 9H, ^{t}Boc), 1.41 (s, 18H, ^{t}Bu), 1.36 (s, 9H, ^{t}Bu). HRFABMS (MNa^+) *m*/*z*: calcd for $NaC_{34}H_{47}NO_8$ 620.320, found 620.319.

Physical Measurements. ^{1}H NMR spectra were collected at 25 °C in $CDCl_3$ at the MIT Department of Chemistry Instrumentation Facility (DCIF). All chemical shifts are reported using the standard δ notation in parts-per-million; positive chemical shifts are to higher frequency from the given reference. Nanosecond transient absorption measurements were performed using a Nd:YAG laser with OPO running at 20 Hz to excite the samples at 270 nm after frequency doubling from 540 nm. A 50-W Xe arc lamp (unpulsed) provided the probe light. A shutter was used to block the probe light. The signal was dispersed and detected using an intensified gated CCD camera (ICCD). The timing of the ICCD, probe light shutter, and laser were controlled using two delay generators. Series of four spectra were taken: I (pump on/probe on), I_F (pump on/probe off), I_0 (pump off/probe on), and I_{0F} (pump off/probe off). Transient spectra corrected for fluorescence and laser signals were calculated from these spectra: $\Delta OD = -\log[(I_0 - I_{0F})/(I - I_F)]$. Spectra reported are the average of 250 of the four-spectra sequences. Instrument control and data analysis were performed using software written in LabView. Samples for photochemical measurements were contained within a cell equipped with a solvent reservoir and a 2-mm clear fused-quartz cell.

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Supporting Information Available: ^{1}H NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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