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Preparation and photophysical properties of a caged kynurenine

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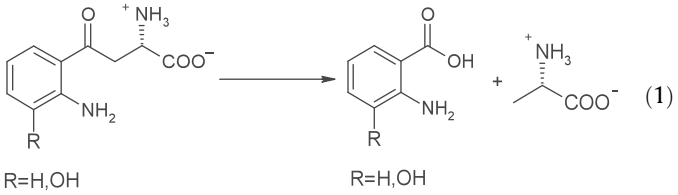
ABSTRACT

We have prepared L-kyurenine 4-hydroxyphenacyl ester, a caged derivative of L-kynurenine. N^{α}-*t*BOC-L-tryptophan was reacted with 4-hydroxyphenacyl bromide in DMF with K_2CO_3 as the base to give the N^{α}-*t*BOC 4-hydroxyphenacyl ester. The ester was then treated with O_3 in MeOH at −20 °C, followed by trifluoroacetic acid in CH_2Cl_2, then aqueous HCl to obtain the caged kynurenine as the dihydrochloride salt. The caged kynurenine is stable as a dry solid in the dark at −78 °C, but in aqueous solutions in phosphate buffer at pH 7–8 hydrolyzes rapidly ($t_{1/2}$ ~5 min). Solutions in Tris at pH 7 are more stable ($t_{1/2}$ >30 min), and solutions in 1 mM HCl are stable for several hours. As expected, the ester is cleaved in microseconds with laser pulses at 355 nm. The caged kynurenine may be useful for preparation of substrate complexes for crystallography or in biological studies on kynurenine.

Kynurenine is an intermediate in the eponymous metabolic pathway of tryptophan in mammals and some fungi and bacteria. The kynurenine pathway has been found to be involved in regulation of the immune response[1] and in neurodegenerative diseases.[2–4] Furthermore, kynurenine has been shown recently to have endothelial relaxing properties due to activation of guanylate cyclase, the target of NO.[5] Recently, kynurenine has been shown to be an endogenous ligand for the Aryl Hydrocarbon (AH) receptor.[6] Thus, enzymes in the kynurenine pathway are of interest as possible drug targets. Kynureninase, one of the enzymes in the pathway, catalyzes the hydrolytic cleavage of kynurenine in bacteria, or 3-hydroxykynurenine in mammals, to give L-alanine and anthranilate or 3-hydroxyanthranilate, respectively (Eq. 1). Previously, we have determined the structures of *Pseudomonas fluorescens*[7] and human kynureninase[8], and a complex of human kynureninase with 3-hydroxyhippurate,[9] by X-ray crystallography. We attempted to soak crystals of human kynureninase with substrate to obtain a structure of the complex, but the crystals dissolved upon substrate addition. In addition, in stopped-flow experiments, we found that the quinonoid intermediate, absorbing at 494 nm, formed within the dead time of the instrument,[10] precluding measurement of the rate constant for its formation. Thus for determination of a substrate structure by kinetic crystallography, as well as for mechanistic experiments, it would be useful to have a caged derivative of L-kynurenine.



(1)

Caged compounds, which contain a protecting group that can be rapidly removed by irradiation with light, have been prepared for over three decades. The first generation caged compounds used *o*-nitrobenzyl groups as the cage,[11–16] but they suffer from relatively slow uncaging (milliseconds) and potentially deleterious nitrosobenzaldehyde products. 7-Nitroindoline derivatives[17] overcome some of these problems, as do 4-methylcoumarins.[18–25] More recently, Givens popularized the 4-hydroxyphenacyl group as a cage,[26,27] which has fast photochemistry and releases relatively innocuous 4-hydroxyphenylacetate by a photochemical Favorskii rearrangement. The 4-hydroxyphenacyl group has been used previously to cage carboxylic acids, thiols and phosphates.[28–30] Hence, we decided to use the 4-hydroxyphenacyl group to prepare a caged L-kynurenine 4-hydrophenacyl ester.

The synthesis of the caged kynurenine was performed as shown in Scheme 1. First, commercially available N^{α}-*t*BOC-L-tryptophan was reacted with 4-hydroxyphenacyl bromide in DMF with K_2CO_3 as the base to give the N^{α}-*t*BOC 4-hydroxyphenacyl ester in 98% isolated yield.[31] Treatment of 4-hydroxyacetophenone under the standard conditions of refluxing $CHCl_3$ and EtOAc[32] in our hands gave a product contaminated with the α,α-dibromide. However, we found that 4-hydroxyphenacyl bromide was cleanly

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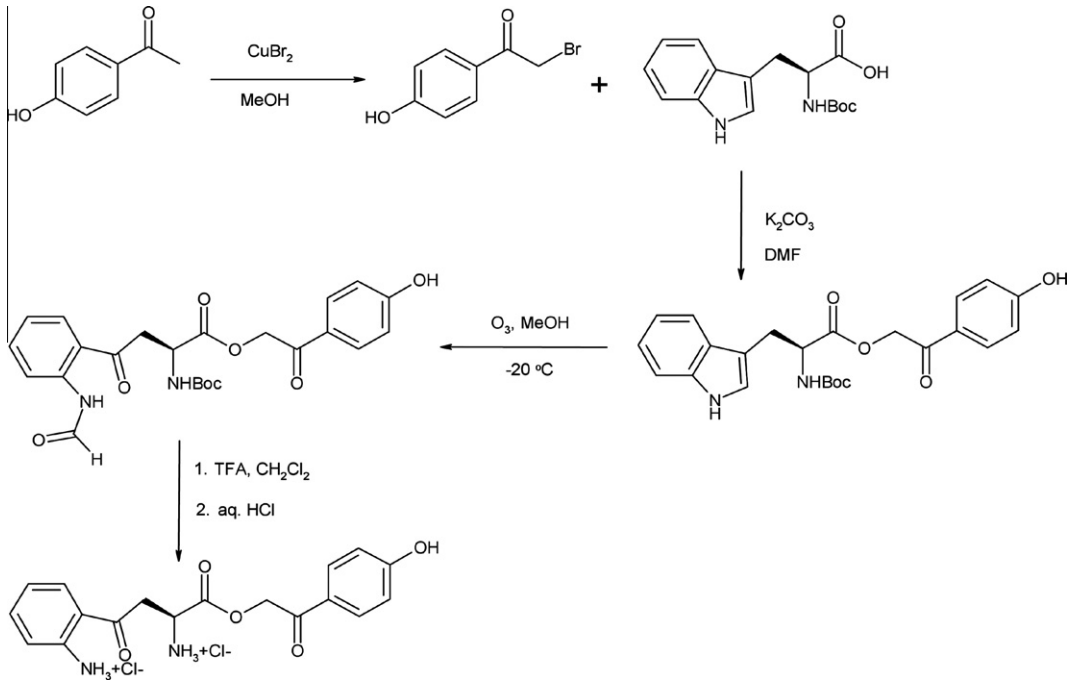


Scheme 1. Synthesis of L-kynurenine 4-hydroxyphenacyl ester dihydrochloride.

prepared in high yield by the reaction of 4-hydroxyacetophenone with $CuBr_2$ in MeOH for 4 h at room temperature. The product, *N*-*t*BOC-L-tryptophan 4-hydroxyphenacyl ester, was then subjected to ozonolysis at −20 °C in MeOH to provide the *t*BOC-protected *N*-formyl kynurenine. The *t*BOC and formyl groups were removed with TFA in CH_2Cl_2, and treatment of the residue after evaporation with aqueous HCl, followed by lyophilization, gave the caged kynurenine as the hygroscopic dihydrochloride salt. High resolution MS of the product gave a mass of 343.1294 (M+1, $C_{18}H_{19}N_2O_5$).[33]

The caged kynurenine is stable indefinitely as the dry solid at −78 °C in the dark, and at least for 15 days as a solution in dry DMSO in the dark. Aqueous solutions are much less stable, releasing L-kynurenine by hydrolysis with a $t_{1/2}$ of less than 5 min in phosphate buffer, pH 7 or 8, at room temperature. Solutions in 1 mM HCl at room temperature or below are much more stable, showing no decomposition for up to 3 h. We also checked the stability of the caged compound in pH 7 Tris buffer containing 55 mM $MgCl_2$ and 25% w/v polyethylene glycol (PEG), the buffer used in crystallization of human kynureninase.[8,9] We have found that the caged compound shows no sign of decomposition for about 30 min in this buffer, which should allow sufficient time to soak and freeze enzyme crystals.

The photophysics of the caged kynurenine were examined in a combined laser flash photolysis and stopped-flow system (Applied Photophyhsics LKS.60 with SX.1 accessory and Quantel Brilliant B Nd:YAG laser). Solutions of caged kynurenine were prepared in 1 mM HCl and kept on ice until use. The compound was then mixed with pH 8.0 phosphate buffer in the stopped-flow instrument, and immediately flashed (within 1 s of mixing) with a laser pulse at 355 nm (6–8 ns pulse; ~200 mJ). Uncaging was monitored by the decrease in absorbance at 320 nm (Fig. 1A), the absorption maximum (ε ~10,000 M^{-1} cm^{-1} at pH 8.0) of the phenolate ion of the 4-hydroxyphenacyl cage. Absorbance decreases occurred within 1 μs of the laser pulse, showing that the uncaging kinetics is rapid and that about 1.7 μM of un-caged kynurenine is liberated in each pulse (Fig. 1B). Consistent with this, HPLC measurements after 100 pulses of laser irradiation showed the quantitative formation of kynurenine and 4-hydroxyphenylacetate in equimolar

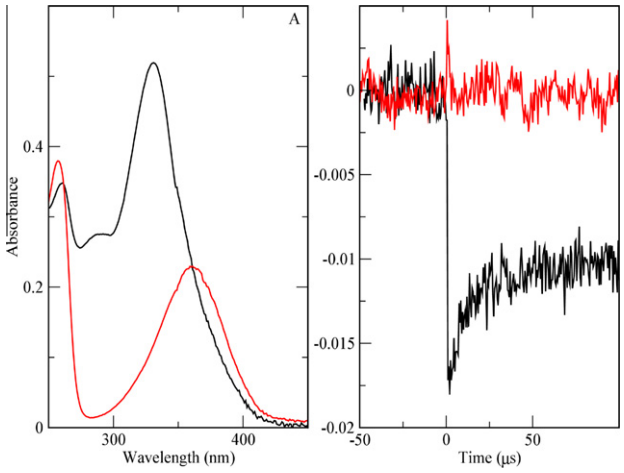
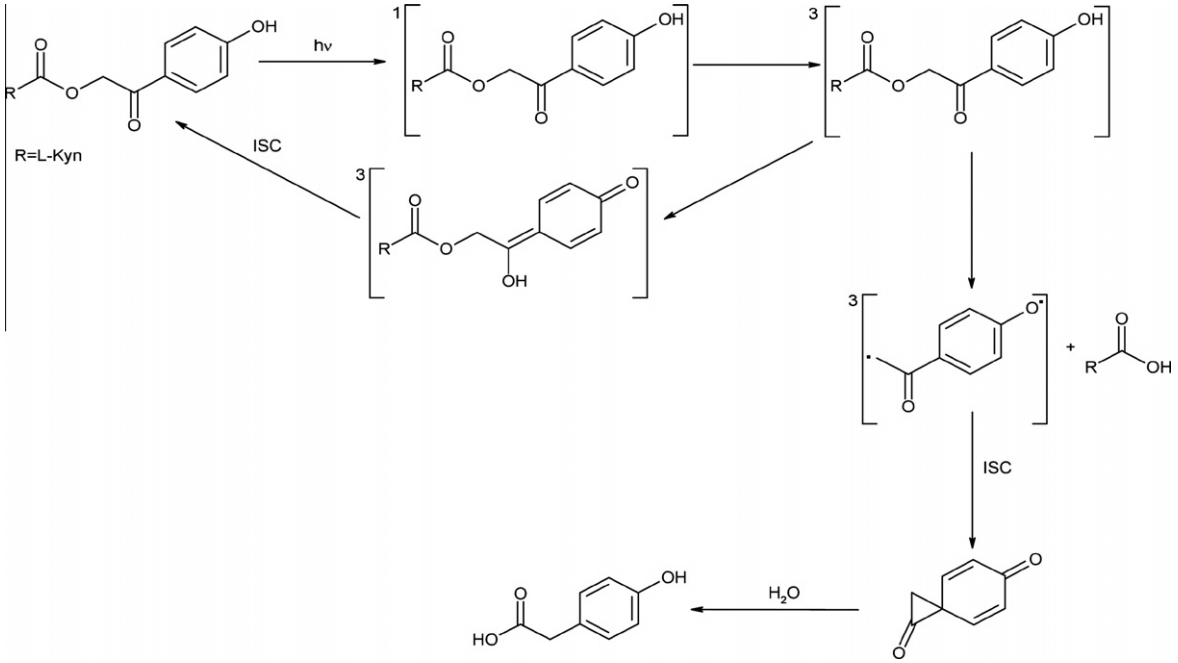


Figure 1. Caged kynurenine photophysics. A. The absorbance spectrum of 50 μM L-kynurenine 4-hydroxyphenacyl ester in 0.05 M potassium phosphate, pH 8.0 (black line). The red line is the absorbance spectrum of 50 μM L-kynurenine in 0.05 M potassium phosphate, pH 8.0. B. The black trace shows photolysis of a 100 μM solution of caged kynurenine at 320 nm. The red trace was measured at 494 nm in the presence of kynureninase. In both cases, single traces are shown.

amounts. However, ~40% of the transient absorbance decrease at 320 nm is recovered with a lifetime of 14 μs (Fig. 1B), suggesting that a reversible process is competitive with the uncaging reaction. When *P. fluorescens* kynureninase was added to the buffer, no evidence for reaction on this time-scale was observed at 494 nm (where reaction of the enzyme with substrate forms a quinonoid intermediate[9]) upon laser flashing, suggesting that the ~1–2 μM concentration of kynurenine released after a single flash was not sufficient to trigger an observable amount of enzymatic reaction. The enzyme absorption at 355 nm in this experiment was less than 0.1, so the lack of reaction could not be due to an inner filter effect.

A possible mechanism for the uncaging of kynurenine 4-hydroxyphenacyl ester is shown in Scheme 2. Absorption of a photon



Scheme 2. Mechanism for uncaging of L-kynurenine 4-hydroxyphenacyl ester.

results in excitation of the phenolate to a singlet state, which rapidly crosses over to a triplet state. It is possible that the kynurenine chromophore, with λ_{max} at 360 nm (Fig. 1A), can act as an antenna to assist in the excitation, since kynurenine is only weakly fluorescent.[34] The triplet state of the cage has been shown to undergo elimination of the leaving group,[35–37] resulting in the Favorskii intermediate, which undergoes rapid hydrolysis to give the product, 4-hydroxyphenylacetate. Tautomerization of the triplet state may also occur, giving the quinone methide, which converts back to the ester by intersystem crossing and tautomerization rather than release of the amino acid (Scheme 2). This latter process may account for the apparent reversibility of uncaging seen in Figure 1B. The quantum yield for photolysis of 4-hydroxyphenacyl esters decreases dramatically at pH values above the pK_a of the phenol, ca. 7.5, so we expect the efficiency of uncaging to be 10% or less under our reaction conditions at pH 8.

Conclusions

We have successfully synthesized a caged form of L-kynurenine, the 4-hydroxyphenacyl ester, and demonstrated that it can be uncaged in microseconds with 355 nm laser pulses. Although the stopped-flow-flash experiments did not appear to liberate sufficient quantities of uncaged kynurenine to observe formation of reaction intermediates of kynureninase, the compound still may be useful where continuous illumination can be used, or to prepare substrate complexes of kynureninase for crystallography. In addition, it may find application in studies of the biological effects of kynurenine.

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31. Mp 195–198 °C; ^{1}H NMR: $CDCl_3$, 7.85 (d, 2H), 7.75 (d, 1H), 7.54 (d, 1H), 7.32 (t, 1H), 7.25 (t, 1H), 7.1 (d, 2H), 7.05 (s, 1H), 5.62 (s, 2H), 4.57 (m, 1H), 3.57 (dd, 2H), 2.95 (s, 9H); ^{13}C NMR: $CDCl_3$, 27.6, 28.9, 55.3, 67.1, 78.9, 110.8, 112.2, 116.2, 118.7, 119.2, 121.7, 124.6,126.2, 127.8, 131.1, 136.9, 156.2, 163.4, 172.9, 191.3.
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