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A concise preparation of the non-proteinogenic amino acid L-kynurenine

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ABSTRACT

A concise and practical preparation of the non-proteinogenic amino acid L-kynurenine is reported. The synthetic approach is scalable and provides ready access to this valuable amino acid in either L- or D-stereochemistry starting from L- or D-tryptophan, respectively. In the optimized procedure, two discreet oxidation steps are applied sequentially to convert the tryptophan indole ring into the keto-aniline moiety contained within the kynurenine side chain.

Kynurenine (Fig. 1) is a non-proteinogenic amino acid with roles in a number of biochemical signaling pathways and disease states.[1,2] In mammals, kynurenine is produced as the major metabolite of tryptophan via the self-named kynurenine pathway (~99% of dietary tryptophan is metabolized by this pathway).[1] Historically, study of the kynurenine pathway focused on its role in the endogenous production of nicotinamide (NAD, vitamin B3). Additional roles for kynurenine were subsequently brought to light when it was shown that the kynurenine pathway can also lead to formation of compounds with either neuroprotective or neurotoxic properties. These neuroactive metabolites are increasingly recognized as contributors to the pathogenesis of Alzheimer's[3–5] and Huntington's[6–8] diseases, depression,[9,10] schizophrenia,[11,12] and other neuroinflammatory processes. As a signaling molecule, kynurenine has recently been shown to elicit smooth muscle relaxation via activation of guanylate cyclase, the target of nitric oxide.[13] In addition, kynurenine was also demonstrated to activate the aryl hydrocarbon (AH) receptor leading to the generation of regulatory T cells.[14,15] Apart from its various roles as a discrete species, kynurenine is also found as a building block in certain non-ribosomal peptides, most notably the clinically used lipopeptide antibiotic daptomycin.[16]

While the majority of investigations into the biological effects of kynurenine have focused on the L-enantiomer, it has also been shown that the kynurenine pathway (in mice, rats, and humans) can also process D-kynurenine.[17,18] Given the wide range of interest in kynurenine, we sought to develop a practical and economical route for its synthetic preparation (commercial access to L-kynurenine is expensive while the D-enantiomer is available only in milligram quantities at even greater expense).

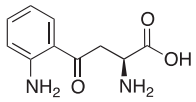

Figure 1. Structure of L-kynurenine.

Traditionally, two main approaches have been employed for the synthesis of kynurenine and its analogues. Among the earliest syntheses reported was the condensation of the sodium salt of diethyl acetamidomalonate with *o*-nitrophenacyl bromide followed by hydrolysis, decarboxylation, and reduction to yield kynurenine as the racemate (Scheme 1A).[19] Acetylation of the racemic kynurenine thus obtained, followed by treatment with acylase has been described as a means of resolving the enantiomers.[20,21] This multistep approach toward L- or D-kynurenine is, however, limited due to the low yields obtained for the overall process as well as variability in the activity of the enzyme used in the resolving step. As an alternative, ozonolysis of *N*-acetyl-tryptophan has been reported to give stereochemically pure material (Scheme 1B).[22,23] While providing a more direct access to kynurenine, the ozonolysis approach is also limited due to variable yields as well as the generation of a number of impurities making subsequent purifications challenging.[24,25] In addition to these approaches, the photocatalytic decomposition of tryptophan has also been reported to produce kynurenine, albeit on a scale not suitable for the preparation of multi-gram quantities of material.[26]

In pursuing an alternative route for the preparation of kynurenine, we initially drew upon the results of Evano and coworkers who described an unexpected indole ring cleavage upon treatment of a protected tryptophan-containing dipeptide with *m*-chloroperbenzoic acid (Scheme 2A).[27] The resulting *N*-formyl substituent on the kynurenine aniline unit can be subsequently removed under

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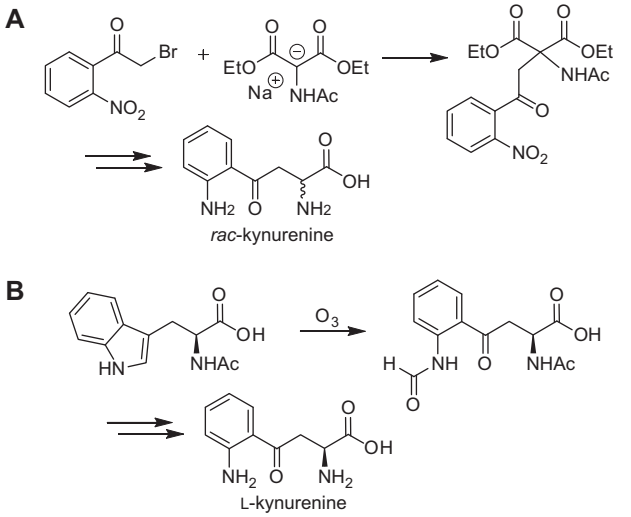
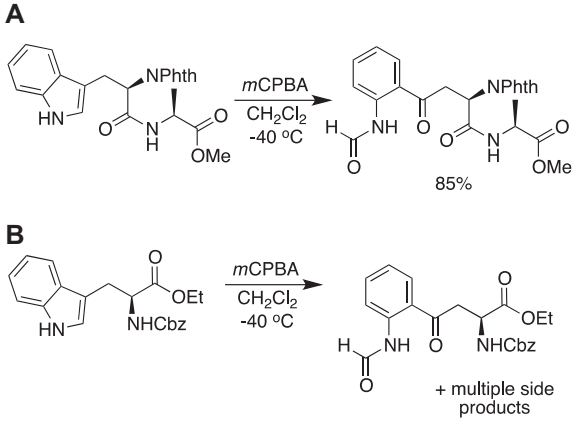


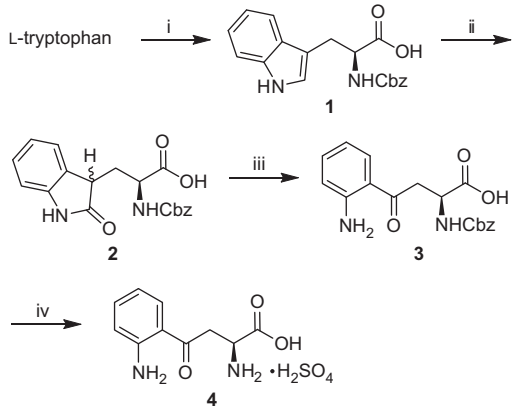
Scheme 1. Previously reported approaches for the synthesis of kynurenine; (A) preparation of *rac*-kynurenine via condensation of diethyl acetamidomalonate with *o*-nitrophenacyl bromide and (B) preparation of L-kynurenine by ozonolysis of *N*-acetyl-L-tryptophan.



Scheme 2. (A) *m*CPBA oxidation of a tryptophan-containing dipeptide as reported by Evano and co-workers yielding an *N*-formyl kynurenine unit and (B) attempted oxidation of protected tryptophan under similar conditions.

mild conditions using dilute acid.[20] As such, we chose to pursue an analogous approach wherein a suitably protected tryptophan was exposed to similar oxidizing conditions (Scheme 2B). While the expected *N*-formyl kynurenine species could be prepared in this manner, a large number of undesired side products (attributed to either under- or over-oxidation) were also formed. Unfortunately, attempts to perform this reaction under different conditions (varying time, temperature, or solvent) had little impact on the distribution of product and side-products formed.

As an alternative approach, a recent report by the group of Hoffman demonstrated that β-3-oxindolylalanine, itself an oxidation product of tryptophan, can be readily oxidized to kynurenine.[28] While providing for a concise route to kynurenine, we found the yields achieved via this procedure to be somewhat variable. Furthermore, the reported use of reverse phase chromatography for the purification of both the polar intermediate and final product is not readily amenable to scale-up. To address these issues we examined the use of Cbz-carbamate protected tryptophan **1** as a starting material to allow for a more convenient method of monitoring the subsequent conversions as well as product isolation. To this end, Cbz-tryptophan was prepared via standard approaches after which it was treated with an oxidizing mixture of DMSO/



Scheme 3. Reagents and conditions: (i) CbzCl, 1 M NaOH, H_2O, 99%; (ii) DMSO, concentrated HCl, AcOH, 62%; (iii) air, 1 M NaOH, H_2O, 52%; (iv) (a) H_2, 10% Pd/C, 1,4-dioxane/aq. HCl; (b) H_2SO_4, recrystallization from EtOH to H_2O, 65%.

HCl in AcOH to yield the expected diastereomeric mixture of Cbz-protected β-3-oxindolylalanines **2** (Scheme 3). A basic aqueous solution of **2** was then aerated, leading to formation of the protected kynurenine species **3** (conversion can be directly monitored by TLC). While the isolated yield of **3** was somewhat moderate (52%), its purification by conventional silica gel chromatography was straightforward and allowed for the reliable preparation of this material on large scale. Removal of the Cbz group by routine hydrogenation, followed by addition of an equimolar quantity of sulfuric acid and recrystallization from EtOH–H_2O yielded pure kynurenine sulfate **4**. In this manner, multi-gram quantities of both L- and D-kynurenine were successfully prepared.

A mechanistic rationale for the conversion of Cbz-protected tryptophan into kynurenine via this two-step oxidation process can be provided based upon literature precedents (Scheme 4). The first oxidation step, wherein the indole moiety is converted into oxindole species **2** via the action of a DMSO/HCl mixture, has been previously described by Savige and Fontana.[29–31] The second oxidation process in which the oxindole is converted into the keto-aniline moiety by simple treatment with air under basic conditions is a less commonly encountered manipulation. A mechanistic explanation for this step can be offered based upon the analogous reaction of indoles with superoxide anion.[32,33] As depicted in Scheme 4, hydrogen abstraction by molecular oxygen leads to formation of the oxindole radical which upon recombination leads to formation of the hydroperoxide species. Under the basic conditions employed, an intramolecular nucleophilic addition then leads to formation of the dioxetane which in turn opens to yield the corresponding ketone and carbamic acid functionalities. Decarboxylation then leads to formation of the keto-aniline unit comprising the kynurenine side chain.

In summary, a concise and practical synthesis of the non-proteinogenic amino acid kynurenine is reported. This approach allows for the preparation of both L- and D-kynurenine from the corresponding readily available and inexpensive L- or D-tryptophan. The process developed is operationally straightforward and can be scaled up to provide multi-gram quantities of material. Given the increasing level of interest in kynurenine due to its many biochemical roles, this methodology serves to greatly improve access to this unique amino acid.

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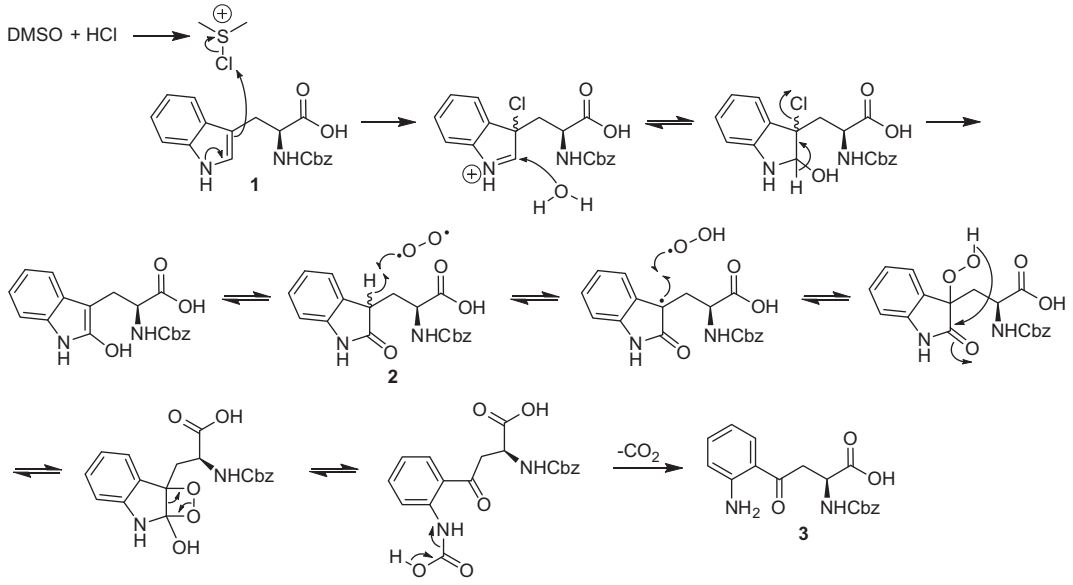

Scheme 4. Proposed mechanism for the two-step oxidative transformation of Cbz-protected tryptophan **1** into Cbz-protected kynurenine **3** via oxindole **2**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.09.055.

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