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Development of Tyrosinase Labile Protecting Groups for Amines

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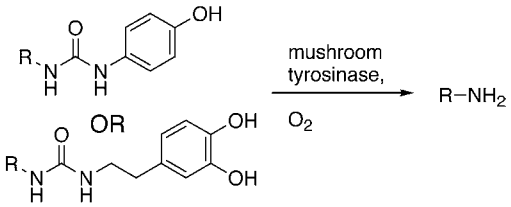
ABSTRACT



The development of two novel protecting groups for amines is described. Thus, a range of amines have been converted to ureas, and the deprotection of these upon exposure to mushroom tyrosinase (E.C. 1.14.18.1) has been demonstrated.

The need for efficient protection/deprotection protocols within synthetic strategies is well recognized, and the selection of protecting groups that will be compatible with specific synthetic strategies plays a fundamental part in the design of synthetic pathways. Although a large number of protecting groups for amines are already known and exploited,[1] relatively few amine protecting groups that can be removed using enzymes are known.[2] Enzyme labile protecting groups are particularly attractive due to the highly chemo- and stereoselective reactions and mild reaction conditions required for their removal. We have recently demonstrated that mushroom tyrosinase (E.C. 1.14.18.1) can promote release of cytotoxic agents from prodrugs by an oxidation/cyclization pathway analogous to that documented for the synthesis of melanin from L-tyrosine.[3] We now report a development of this strategy that has led to the identification of two classes of enzyme labile protecting groups for amines. The mushroom tyrosinase enzyme is a particularly attractive reagent for deprotection protocols since it is commercially available or can be readily isolated from the regular commercially available mushrooms, *Agaricus bisporus*.[4] Moreover, its use for the efficient cleavage of amino acid and peptide phenyl hydrazides has recently been demonstrated.[5]

The first stage of this study necessitated the selection of tyrosinase substrates for attachment to the amines, to serve as both protecting groups for the amines and triggers for tyrosinase-mediated deprotection. From the knowledge that the tyrosinase enzyme exhibits phenolase and catecholase activity,[6] and our previous oximetry investigations,[3] 4-aminophenol and 3-hydroxytyramine were selected as the tyrosinase substrates. It was decided to attach these to the amines via a urea linkage, which would serve to form urea-based protecting groups for the amines. Upon exposure to tyrosinase, it was hypothesised that these ureas would produce intermediates that were unstable to aqueous conditions, regenerating the deprotected amines. The mechanisms by which these deprotections are postulated to occur are illustrated in Schemes 1 and 2.

A range of amines was selected for protection, and these encompassed aromatic and aliphatic amines, and the methyl

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(6) For a recent review, see: Land, E. J.; Ramsden, C. A.; Riley, P. A. *Acc. Chem. Res.* **2003**, *36*, 300−308.

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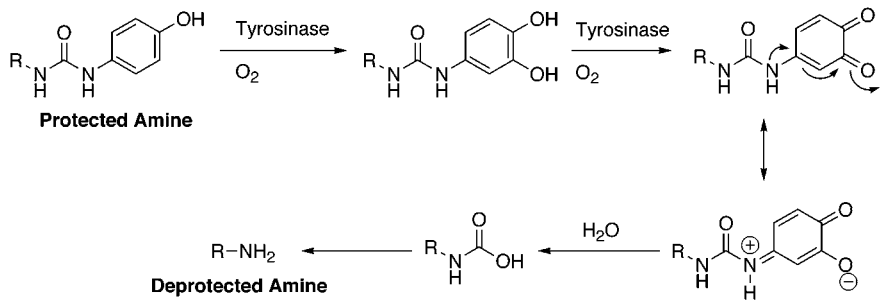
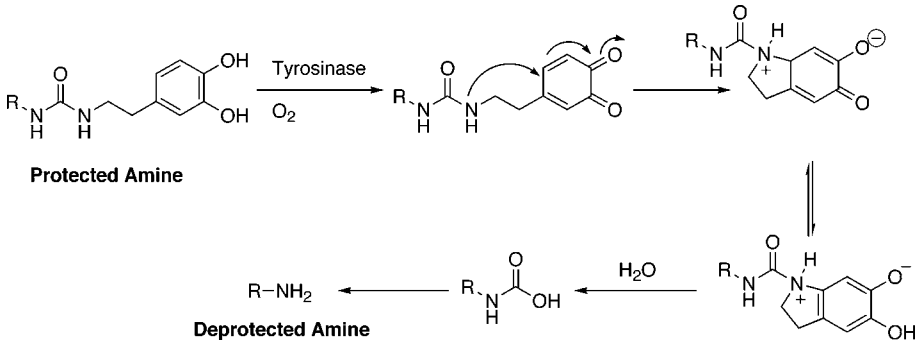
Scheme 1

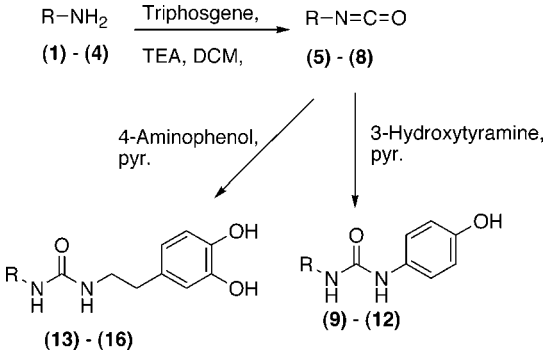


Scheme 2



ester of an amino acid. Amines **1**−**4** were easily converted into the urea derivatives by initial reaction with triphosgene, in anhydrous dichloromethane, to afford isocyanate derivatives **5**−**8** in quantitative yield. These could either be isolated and purified by column chromatography on silica gel or used directly in coupling reactions with either 4-aminophenol or 3-hydroxytyramine to afford the two classes of protected amines. Couplings were performed in anhydrous pyridine under an inert atmosphere for 2−6 h, and the progress of the reaction could be conveniently monitored by IR analysis of the reaction mixture, for complete disappearance of the NCO stretch. Urea products **9**−**16** were purified by column chromatography on silica gel and obtained in synthetically useful yields (Scheme 3, Table 1).[7]

Scheme 3



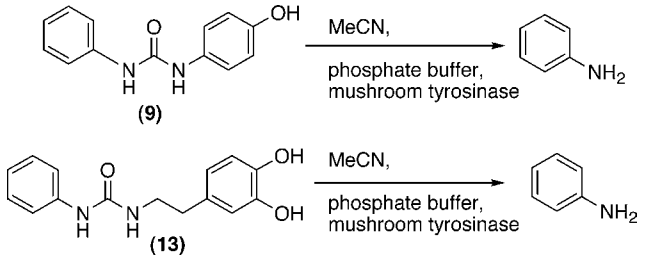
Before the tyrosinase-mediated deprotections were investigated, the stabilities of the two classes of ureas to some reaction conditions commonly used for the removal of other alcohol and amine protecting groups were investigated. Thus, ureas **12** and **16** derived from cyclohexylamine were treated, in turn, with neat TFA, NaOH (1 M), and K_2CO_3/MeOH for 8 h at room temperature. TLC and ^{1}H NMR spectroscopic analysis of the materials obtained after this time illustrated that no deprotection of the ureas occurred, with starting material being recovered in quantitative yields in all cases.

Our studies next probed the utility of tyrosinase for effecting deprotection of the ureas, to re-form the amines. Ureas **9** and **13** were dissolved in a mixture of acetonitrile and phosphate buffer (1:5, 100 mg of urea per 72 mL of solvent), and after complete dissolution, tyrosinase in phosphate buffer was added. Deprotections were performed

Table 1.

amine	tyrosinase substrate	urea, %
aniline (**1**)	4-aminophenol	**9**, 92
benzylamine (**2**)	4-aminophenol	**10**, 83
L-phenyl alanine methyl ester (**3**)	4-aminophenol	**11**, 86
cyclohexylamine (**4**)	4-aminophenol	**12**, 99
aniline (**1**)	3-hydroxytyramine	**13**, 78
benzylamine (**2**)	3-hydroxytyramine	**14**, 75
L-phenyl alanine methyl ester (**3**)	3-hydroxytyramine	**15**, 72
cyclohexylamine (**4**)	3-hydroxytyramine	**16**, 78

Scheme 4



with and without the bubbling of oxygen through the solutions and with two different quantities of the tyrosinase enzyme (0.005 equiv by mass and 0.05 equiv by mass). The reaction mixtures were analyzed by TLC for complete disappearance of starting material, and then the tyrosinase was removed by filtration. Extraction of the aqueous solution with dichloromethane, and purification of the crude reaction products by filtration through silica, allowed isolation of the deprotected aniline in pure form in excellent synthetic yields (Scheme 4, Table 2).[8]

Table 2.

urea	mass equiv of tyrosinase	oxygen bubbled through solution	time (h) for complete disappearance of urea	**1**, %
9	0.005	no	96	78
9	0.005	yes	72	75
9	0.05	no	60	75
9	0.05	yes	24	82
13	0.005	no	84	74
13	0.005	yes	48	82
13	0.05	no	24	71
13	0.05	yes	12	91

These studies illustrated that optimum results were obtained when larger mass equivalents of tyrosinase were utilized and when oxygen was continuously bubbled through the solution. Moreover, catechol-derived urea (**13**) was deprotected at a faster rate than phenol-derived urea (**9**), which correlates with a lag period for the oxidation of phenols to catcehols by tyrosinase.[6] Control experiments illustrated that bubbling oxygen through the solutions without adding tyrosinase did not effect deprotection of the ureas. The optimized deprotection conditions (0.05 equiv by mass of tyrosinase, and bubbling of oxygen through the reaction mixtures) were then utilized to effect deprotection of ureas **9**−**16**, to allow formation of the amines **1**−**4** in excellent yields (Scheme 5, Table 3). As expected, spectroscopic data for the products were identical to those for the starting material substrates **1**−**4**.

Scheme 5

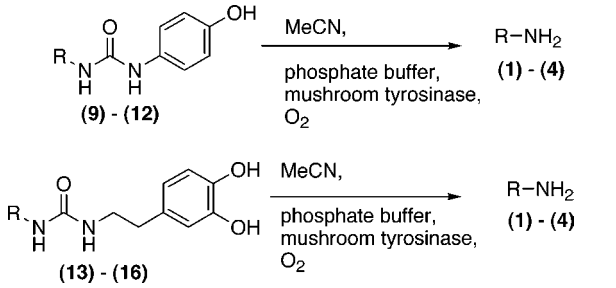


Table 3.

urea	deprotected amine, yield %
9	aniline, 82
10	benzylamine, 73
11	phenyl alanine, 87
12	cyclohexylamine, 80
13	aniline, 91
14	benzylamine, 76
15	phenylalanine, 93
16	cyclohexylamine, 74

Thus, the deprotection strategy proved to be equally effective for the two classes of ureas (**9**−**12** and **13**−**16**). This study has therefore illustrated the efficient synthesis of two classes of protected amines and has illustrated that both classes can be deprotected to regenerate the amines upon exposure to tyrosinase. In addition, preliminary studies have illustrated that the protected amines are stable to reaction conditions that are often used to remove other widely used alcohol and amine protecting groups. This protocol may also be applicable for the protection of alcohols as tyrosinase labile carbamates, and we are currently exploring the scope of this within our laboratory.

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(7) Typical Experimental Procedure for Formation of Protected Urea Derivatives: Step 1. The amine (1 equiv) and NEt_3 (2 equiv) were dissolved in anhydrous DCM and cooled in an ice bath at 0−5 °C. Triphosgene (2 equiv) was added, and the reaction was gradually brought to reflux under N_2 (g). The reaction was monitored by IR analysis until the formation of a strong NCO stretch (2−6 h) was observed. The reaction mixture was cooled and then filtered through a short pad of silica, and the filtrate was concentrated in vacuo to yield the pure isocyanate product (≥90%). Step 2. Either 3-hydroxytyramine (1 equiv) or 4-aminophenol (1 equiv) was dissolved in pyridine, and this was added to the isocyanate (1 equiv) in pyridine. The reaction mixture was then stirred under N_2 (g) for 6 h. The solvent was then removed in vacuo, and products were purified using chromatography on silica gel.

(8) Typical Experimental Procedure for Deprotection of Ureas to Generate the Amines. The protected amine was dissolved in MeCN and phosphate buffer (1:5, v:v) and sonicated for 20 min at rt. After complete dissolution, tyrosinase (0.05 equiv by mass) in phosphate buffer was added. After addition of the enzyme, oxygen was bubbled through the solution until the disappearance of starting material was evidenced by TLC analysis. The reaction was filtered through Celite and the MeCN removed by evaporation. The remaining solution was extracted with DCM, and the organic layers were combined and dried over $MgSO_4$; the solvents were removed in vacuo, and the residue was purified by chromatography on silica gel to afford the free amines.