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Synthesis and bioorthogonal coupling chemistry of a novel cyclopentenone-containing unnatural tyrosine analogue

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Abstract

Herein we report the synthesis of a novel amino acid with orthogonal functionality to the natural amino acid side chains. Tyrosine was *O*-alkylated with a cyclic 5-membered α,β-unsaturated ketone ring (**5**). We have established that this amino acid analogue can undergo cycloaddition reactions in aqueous media with *in situ* generated nitrones. Nitrone formation occurred by micellar catalysis can undergo aqueous 1,3-dipolar cycloaddition reactions with the unnatural Tyr. We also performed a linear free energy analysis of the one pot bioconjugation reaction in water using cyclopentenone as a model for the Tyr analogue and seven different aryl nitrones. We found that the Hammett ρ value was −0.94, suggesting that the reaction occurs in a concerted fashion with a slight positive charge buildup in the transition state. The Hammett ρ value also suggests that the bioconjugation reaction is tolerant of different substituents and thus may be useful for introducing novel functionality into peptides and proteins containing the Tyr analogue **5**. The aqueous 1,3-dipolar cycloaddition reactions, that use nitrones to trap the *O*-alkylated Tyr **5**, establish a novel strategy for rapid, water compatible bioconjugation reactions.

Keywords: Tyrosine analogue; Nitrone; Micellar catalysis; Bioconjugation reaction; [3+2] cycloadditon reaction

1. Introduction

Highly selective chemical reactions that occur rapidly and with high yield and do so in complex biological media are important for a number of applications including the chemical synthesis of natural and unnatural peptides and proteins [1–5]. Some examples include the bioconjugation of proteomic tags to target proteins within extracted proteomes as well as in living systems [6,7], the addition of unnatural functionality to biomolecules such as proteins [8–16], and for the systematic alteration of structural and functional properties of different molecular scaffolds [17–20]. Ideally, these reactions are bioorthogonal in that no side reactions occur with the endogenous functional groups in the biological system. To date, there are several existing bioconjugation reactions such as Huisgen cycloaddition and Staudinger ligation, each suited to specific substrates and applications [2]. Nucleophilic catalysis of an oxime ligation of peptides has also been demonstrated recently [3]. The reactive groups involved in bioorthogonal chemical reactions of this type can be incorporated into a system of interest either by *in vitro* methods or by metabolic labeling strategies [4]. One common method for the latter is the use of unnatural amino acids [19,20]. The addition of unnatural amino acids (UAAs) into peptides and proteins has enabled the generation of biomolecules with novel properties and additional chemical functionality. Site specific incorporation of UAAs has been successfully achieved

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using *in vitro* translation systems as well as using orthogonal aminoacyl tRNA synthetases/tRNA pairs expressed in bacteria [16–19] and eukaryotes [20].

Recently it has been shown that the unnatural amino acids such as *O*-methyl-L-tyrosine [21,22], and *O*-allyl-L-tyrosine [15], as well as a host of other UAAs can be site-specifically incorporated into proteins with high efficiency and fidelity [19]. Here we report the synthesis of a novel *R*-(4-oxocyclopent-2-enyloxy)-L-tyrosine derivative containing an α,β-unsaturated ketone functionality. We show that this UAA can be trapped under physiological conditions with aryl nitrones generated *in situ* utilizing micellar catalysis [24]. This establishes a novel method for site specific functionalization of peptides and proteins *in vitro* that is complementary to existing methods used for this purpose.

2. Results and discussion

Previous studies have established that [3+2] cycloadditions involving organic nitrones and electron deficient olefins occur efficiently in aqueous solutions with the aid of micellar catalysis [23]. In order to adapt this reaction to bioconjugate chemistry, we first sought to develop a suitable UAA containing an electron deficient olefin that would be reactive enough to efficiently undergo cycloaddition reactions with nitrones but not so reactive that it would undergo addition reactions with the side chains of cystiene residues. We chose to make *R*-(4-oxocyclopent-2-enyloxy)-L-tyrosine **5** and the approach that we took for the synthesis of **5** is depicted in Fig. 1. We chose to demonstrate micellar catalyzed reactions with the unnatural tyrosine analog **5** because of its increased solubility in micelles, rather than work with the free amino acid. The *O*-activated *N*-tBoc-protected Tyr was reacted with butyl amine to produce amide **2** in 99% yield. The Mitsunobu coupling reaction between **2** and (1*R*,4*S*)-*cis*-4-acetoxy-2-cyclopenten-1-ol afforded olefin **3** in 43% yield. Ethanolic basic hydrolysis of the acetal was executed to form the secondary alcohol **4** in 70% yield. Finally, PDC oxidation of **4** afforded **5** in 87% yield (Fig. 1).

The [3+2] cycloadditions between the α,β-unsaturated ketone functionality in **5** and nitrones were then performed in aqueous media in the presence of SDS micelles [24]. Aryl nitrones were efficiently generated *in situ* from phenyl hydroxylamine and substituted benzaldehydes, inside SDS micelles, as previously reported [24]. Phenyl hydroxyl amine was prepared by reducing nitrobenzene with Zn following the previously reported procedure [25]. Freshly obtained phenyl hydroxylamine was then reacted with benzaldehyde in the presence of 0.1 M SDS with 5 min sonication and 2 h of stirring at room temperature. Sonication allowed faster formation of the nitrone which was monitored by TLC. We determined that a concentration of 0.1 M of SDS was sufficient to form micelles that concentrate the organic reagents preferentially in the hydrophobic core of the micelle. Water molecules formed during the reaction are expelled from micellar hydrophobic interior leaving the nitrone product inside the micelle and promoting further reaction. The [3+2] cycloaddition reaction with

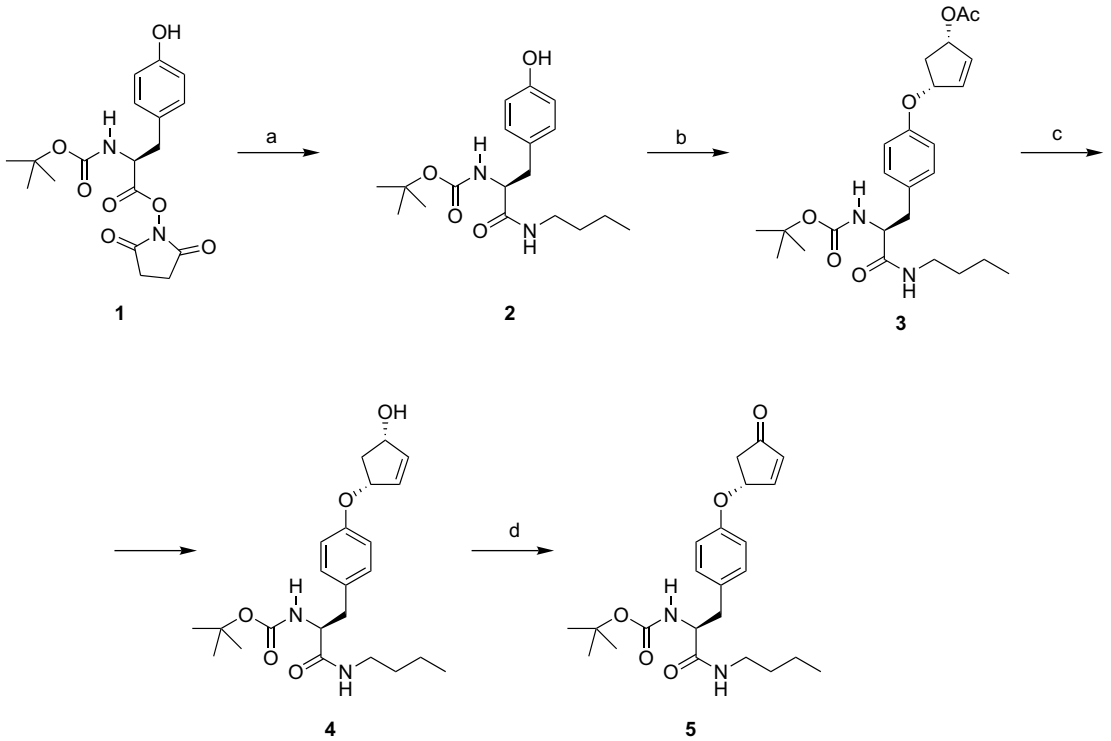

Fig. 1. Synthesis of unnatural *O*-alkylated Tyr derivative **5**. (a) Butyl amine, DCM, reflux, 1 h; (b) (1*R*,4*S*)-*cis*-4-acetoxy-2-cylcopenten-1-ol, DIAD, PPh_3, THF, rt, 24 h; (c) 1 M LiOH, THF, rt, 24 h; (d) PDC, DCM, rt, 7 h.