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

Gianni R. Lorello [a,b,1], Marc C.B. Legault [a,b,1], Bojana Rakić [a,b], Kathrine Bisgaard [a], John Paul Pezacki [a,b,*]

[a] *Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ont., Canada K1A 0R6*
[b] *Department of Chemistry, University of Ottawa, Ottawa, Canada*

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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

* Corresponding author. Address: Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ont., Canada K1A 0R6. Fax: +1 613 952 0068.
E-mail address: John.Pezacki@nrc.ca (J.P. Pezacki).
[1] These authors contributed equally to this work.

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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

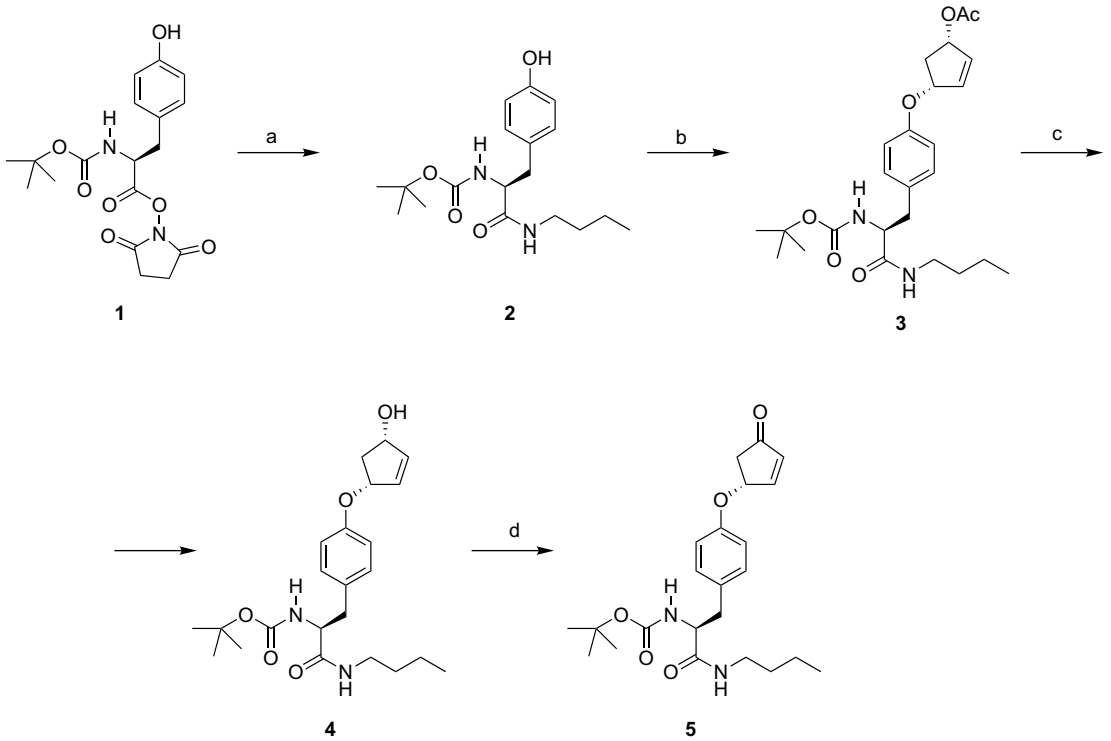

Fig. 1. Synthesis of unnatural *O*-alkylated Tyr derivative **5**. (a) Butyl amine, DCM, reflux, 1 h; (b) (1*R*,4*S*)-*cis*-4-acetoxy-2-cylcopenten-1-ol, DIAD, PPh_3, THF, rt, 24 h; (c) 1 M LiOH, THF, rt, 24 h; (d) PDC, DCM, rt, 7 h.

the modified Tyr alkene **5** was performed *in situ*. The desired reaction product **6** was observed, however only 13% of the product could be successfully isolated after 2 days of stirring at 37 °C (Fig. 2). We determined that **5** was completely recoverable from the latter reaction and thus was stable to the reaction conditions.

To optimize the yield of **6** we systematically varied the reaction conditions including reaction time, temperature, and ratios of reactants and catalyst. While optimizing the yield of this reaction we discovered that, even after prolonged reaction times, only a maximum of ~38% yield was achievable even after 12–14 days with no decomposition of unreacted **5**. We hypothesized that product inhibition of the micelle catalyzed cycloaddition step was occurring and thus limiting the yield. Product inhibition did not occur for the micelle catalyzed nitrone formation step since we could observe full formation of the nitrone by LC–MS. To test this hypothesis we conducted reactions at 37 °C with a molar excess (0.025 M SDS and a ~100-fold reduction in the concentration of the other starting materials) of SDS and observed yields of ~84% of **6**.

The stereochemistry of **6** was elucidated using a combination of NOE experiments of resonances associated with the hydrogens of the 5-membered ring. High conversions could be achieved, however, the solubility of **5** within the SDS micelle is likely a limiting factor for the reaction times which typically required 48–72 h. The low reactivity of **5** towards diphenyl nitrone indicates that the functionalization of peptides and proteins containing **5** *via* [3+2] cycloaddition reactions with nitrones will likely be limited to *in vitro* synthesis. Peptide conjugates prepared *in vitro* using **5** can be performed for longer reaction times and at higher temperatures, however these conditions would be impractical for *in vivo* applications.

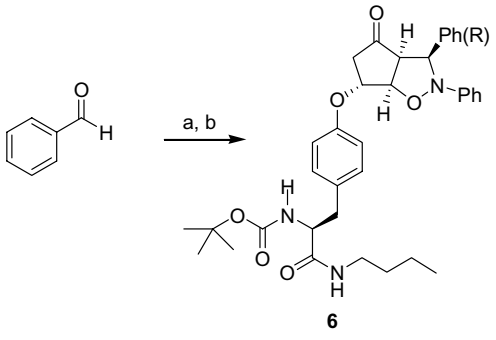

Fig. 2. Synthesis of 1,3-dipolar cycloaddition adduct **6**. (a) PhCHO, PhNHOH, 0.1 M SDS, sonication, rt, 2 h; (b) **5**, 37 °C, 2 days.

Although the L-tyrosine derivative **5** was observed to undergo cycloaddition reactions with nitrones slowly under physiological conditions, **5** is also a Michael acceptor that can potentially react with endogenous biological nucleophiles such as thiol groups via 1,4-nucleophilic addition. To determine if such addition reactions would occur competitively with nitrone cycloaddition, we performed competition experiments between modified Tyr substrate **5**, the diphenyl nitrone, and glutathione (up to 2 μM). We monitored the product formation by LC/MS. After 48 h stirring at 37 °C, this competitive study showed exclusively the [3+2] nitrone cycloaddition product with no thiol 1,4-addition product observed. This observation indicates that the unnatural tyrosine analogue **5** is suitable for bioconjugate chemistry because it displays reactivity that is orthogonal to the natural functionality of proteins and other cellular materials [6].

Secondly, we investigated substituent effects of aryl nitrones on the relative rates of 1,3-dipolar cycloaddition reaction with cyclopentenone, a model for **5**. We chose to perform this study with a model system rather than **5** because of the slower reaction times for **5** relative to those of cyclopentenone, probably because of differences in solubility in the micelles used for catalysis. Specifically, we performed competition reactions involving the cycloaddition of diphenyl nitrone and a series of nitrones substituted with substituent X at the para position on the phenyl ring with a limiting amount of cyclopentenone (Fig. 3), at 37 °C. In one pot, an excess amount of phenyl hydroxylamine was reacted with equimolar concentrations of benzaldehyde and the *para*-substituted benzaldehyde for 90 min in 0.1 M SDS. The excess phenyl hydroxylamine was used to ensure complete formation of both nitrones. For the [3+2] cycloaddition reaction, less than 1 equivalent of cyclopentenone was used. This reaction was performed for 48 h at 37 °C at which time complete conversion to products was observed. The reaction products were then analyzed by LC/MS. The relative rate constants for the competing cycloaddition reactions were determined from the relative peak heights or ion yields for the products in the LC/MS (see experimental section for further details). The seven substituted benzaldehydes (R = –OH; $–N(CH_3)_2$; $–NO_2$; $–CH_3$; –Cl; –CN; $–OCH_3$) were tested

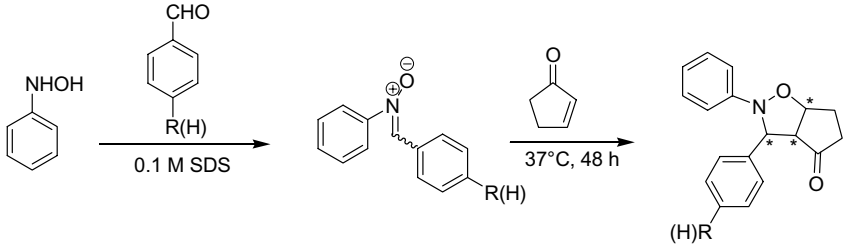

Fig. 3. General reaction scheme depicting nitrone synthesis and 1,3-dipolar cycloaddition reaction. R = –OH, –Me, –OMe, $–NO_2$, –Cl, –CN, $–N(CH_3)_2$.

in duplicates. The relation between the substituent on the phenyl ring and the rate of reaction with the [3+2] cycloaddition reaction is presented in the Hammet plot and plot fits the data best using σ_p parameters (see Fig. 4). The plot

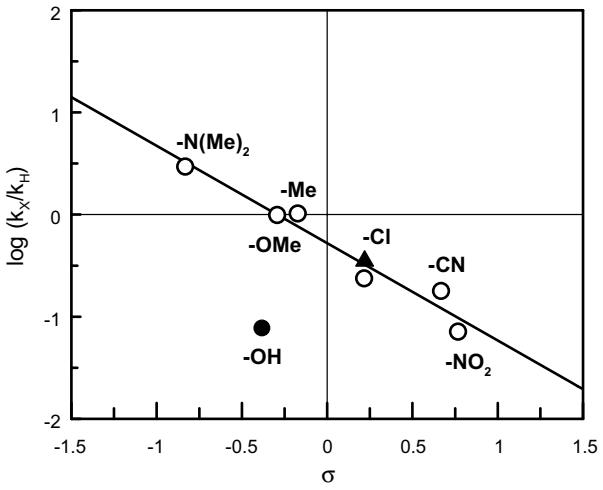

Fig. 4. Hammett plot depicting the substituent effect on the one pot 1,3-dipolar cycloaddition reaction. The open circles represent the data points obtained after a 2-h pre-incubation of the aldehydes with an excess of hydroxylamine followed by the addition of olefin. The solid triangle represents the data point for 4-chlorobenzaldehyde following only a 10 min pre-incubation of aldehydes with hydroxylamine followed by the addition of olefin. The solid circle represents the data point for 4-hydroxybenzaldehyde that deviates significantly from the linear free energy relationship. Extrapolation of points shows a ρ value of -0.94 ($r = 0.98$).

gives a ρ of -0.94. This value of ρ is consistent with a transition state that has modestly more localized positive charge than the reactant and is consistent with a concerted rather than a stepwise mechanism for the micelle catalyzed cycloaddition.

For the plot in Fig. 4, the cycloaddition products of two analogs did not agree well with the line of best fit. These were the products formed from reaction with 4-hydroxybenzaldehyde and 4-chlorobenzaldehyde. For the former, the pK_a of the hydroxyl group is ~7.6 which in aqueous solution is largely deprotonated. We interpret the lower than expected reactivity of 4-hydroxybenzaldehyde in the one pot reaction to be due to an unfavorable solubility of the charged species inside the non-polar interior of the micelle. This lowers the rate constant for the reaction with the olefin as indicated in Fig. 5. For the case of 4-chlorobenzaldehyde, significant dimerization was observed during the nitrone formation reaction [26–30] that led to a lower ratio of rate constants. However, when the olefin was added to the nitrone-forming reaction after 10 min, negligible dimer was observed and the ratio of rate constants for k_X/k_H fit the line in the plot in Fig. 4. The ρ value representing the slope of the line of best fit is small indicating that the micelle catalyzed [3+2] cycloaddition in water displays little sensitivity towards substituent effects. This indicates that the reaction can be used with a variety of substrates and that these reactions should proceed with similar efficiency.

In conclusion, we have prepared a novel *O*-alkylated tyrosine analogue with a cyclic 5-membered α,β-unsatu-

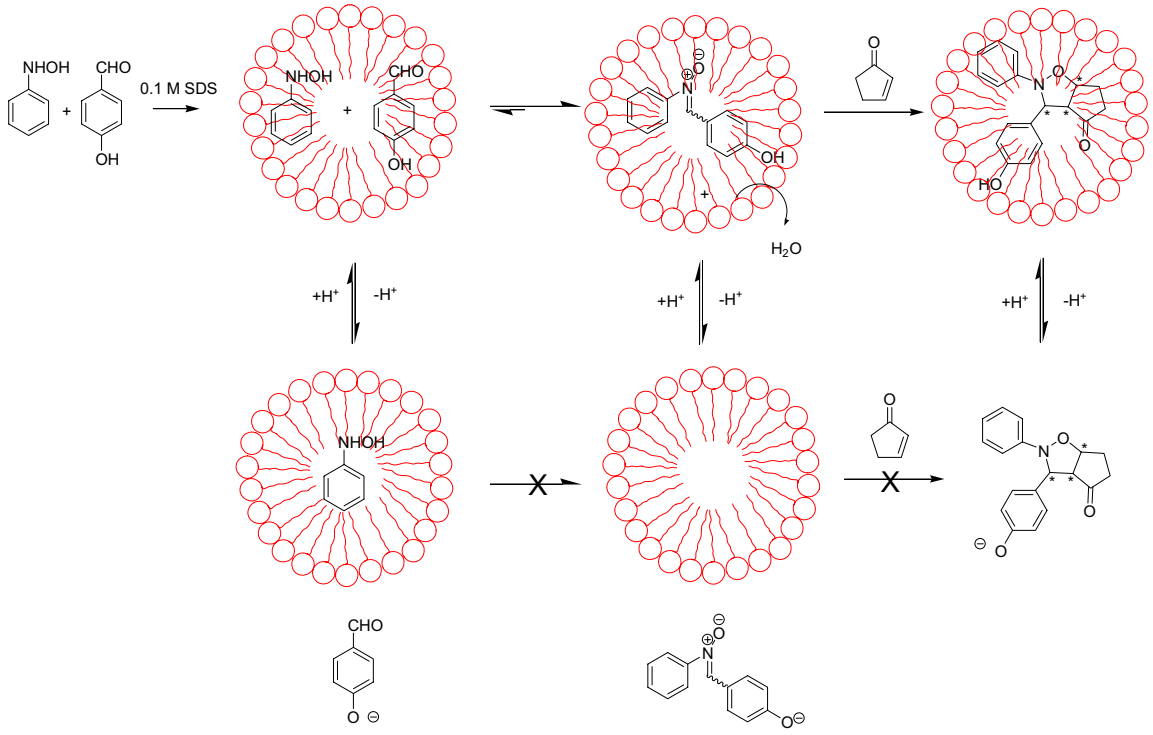

Fig. 5. General reaction scheme depicting the proposed mechanism by which nitrone synthesis and 1,3-dipolar cycloaddition reaction occur for the reaction involving 4-hydroxybenzaldehyde (R = –OH) that results in its lower reactivity.

rated ketone ring amino acid with orthogonal functionality to the natural amino acid side chains. We have shown this amino acid analogue can undergo cycloaddition reactions in aqueous media with *in situ* generated nitrones in one pot reactions and that these reactions may be useful in the chemical synthesis of unnatural proteins. We evaluated the linear free energy relationships of the one pot bioconjugation reaction and determined that the reaction is relatively insensitive to substituent indicating that a variety of substrates should be compatible with the reaction conditions described. We are currently exploring utility of this chemistry towards the systematic modifications of peptides and proteins.

3. Materials and methods

All chemicals were obtained from commercial sources and used without further purification unless indicated. Analytical thin-layer chromatography was performed on commercial glass plates pre-coated (250 μm layer thickness) with silica gel 60 F254 (E. Merck). The TLC spots were viewed under ultraviolet light (254 nm) and by heating the TLC plate after treatment with a solution of ammonium molybdate in 10% aqueous H_2SO_4. Conventional flash column chromatography, using Silicycle Ultra Pure Silica Gel (230–400 mesh), was performed to purify all compounds. Removal of organic solvents was performed by roto-evaporation on a Büchi R-114 Rotovapor using a Buchi B-178 vacuum system. Trace solvents were removed on a high vacuum pump. The ^1H and ^{13}C nuclear magnetic resonance (1H NMR) spectra were recorded at 400 MHz and at 100 MHz, respectively, on a Bruker DRX-400 spectrometer. The nuclear magnetic resonance spectra (NMR) of all compounds were measured in a solution of deuterated chloroform ($CDCl_3$). The chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane (δscale). The multiplicity, coupling constants (J in Hz), and number of protons were indicated in parentheses after each chemical shift. HPLC/MS analysis was performed with Waters Alliance 2795 liquid chromatograph equipped with Waters 996 PDA diode array detector and connected to Micromass ZQ2000 mass spectrometer equipped with pneumatically assisted electrospray ionization source, operating in positive mode. The Waters SunFire C18 (2.1 × 100 mm, 3.5 μm) column was used. Samples were run on a Waters Alliance 2795 LC Column, with the column temperature at 20 °C. HPLC was performed with gradient elution of acetonitrile/water both with 0.1% formic acid. Flow rate was 0.2 ml/min and all the eluent was directed first to diode array detector and then to mass spectrometer. The source temperature was set at 80 °C, desolvation gas temperature was set at 200 °C an electrospray capillary was set at 3.5 kV with a cone voltage set at 10 V. Data were collected in single ion recording (SIR) mode.

3.1. Synthesis

3.1.1. *N*-(Boc-L-tyrosyloxy)butylamine (**2**)

Butylamine (1.35 ml, 13.65 mmol) was added to a solution of *N*-(Boc-L-tyrosyloxy)succinimide (2.58 g, 6.82 mmol) in dry DCM (20 ml). The reaction mixture was refluxed for 1 h. Ten microliters of water was added and the resulting mixture was extracted with DCM, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography (heptane/EtOAc 1:1) afforded the desired product (2.30 g, 99.9%). TLC (heptane/EtOAc 1:1) R_f 0.18. ^1H NMR (400 MHz $CDCl_3$) 7.06 (d, $J = 8.4$ Hz, 2H), 6.77 (d, $J = 8.4$ Hz, 2H), 6.07 (br s, 1H), 5.22 (br s, 1H), 4.28 (s, 1H), 3.25–3.13 (m, 2H), 3.07–2.92 (m, 2H), 1.44 (s, 9H), 1.41–1.33 (m, 2H), 1.29–1.19 (m, 2H), 0.89 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 156.1, 155.7, 130.7, 128.3, 80.8, 60.9, 56.7, 39.7, 38.2, 31.7, 28.7, 21.5, 21.0, 20.3, 14.6, 14.0. MS (ESI^+) $m/z = 337.3$ $(MH)^+$.

3.1.2. (1*S*,4*S*)-4-Acetoxy-(4-(2-tert-butoxycarbonylamino-2-butylcarbamoyl-ethyl)-phenoxy)-2-cyclopentene (**3**)

N-(Boc-L-tyrosyloxy)butylamine **2** (0.79 g, 2.36 mmol) was dissolved in dry THF (12 ml) and (1*R*,4*S*)-*cis*-4-acetoxy-2-cyclopenten-1-ol (0.40 g, 2.84 mmol), DIAD (0.93 ml, 4.71 mmol) and PPh_3 (1.24, 4.71 mmol) were added successively at room temperature. The reaction mixture was stirred at room temperature under the argon atmosphere for 24 h. Water was added and the resulting mixture was extracted with EtOAc, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography (heptane/EtOAc 1:1) afforded the desired product (0.47 g, 43%). TLC (heptane/EtOAc 1:1) R_f 0.31. ^1H NMR (400 MHz, $CDCl_3$), δ 7.11 (d, $J = 8.4$ Hz, 2H), 6.81 (d, $J = 8.4$ Hz, 2H), 6.26–6.22 (m, 1H), 6.18–6.14 (m, 1H), 5.88–5.80 (m, 1H), 5.70 (br s, 1H), 5.47–5.42 (m, 1H), 5.07 (br s, 1H), 4.27–4.16 (m, 1H), 3.22–3.09 (m, 2H), 3.09–2.88 (m, 2H), 2.39–2.24 (m, 2H), 2.05 (s, 3H), 1.41 (s, 9H), 1.38–1.30 (m, 2H), 1.28–1.17 (m, 2H), 0.87 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 157.3, 136.5, 135.3, 130.9, 130.8, 81.4, 79.0, 60.8, 39.6, 38.3, 38.2, 31.8, 31.0, 28.7, 21.5, 21.4, 20.3, 19.5, 14.6, 14.0. MS (ESI^+) $m/z = 461.3$ $(MH)^+$.

3.1.3. (1*R*,4*R*)-4-Hydroxy-(4-(2-tert-butoxycarbonylamino-2-butylcarbamoyl-ethyl)-phenoxy)-2-cyclopentene (**4**)

(1*R*,4*R*)-4-Acetoxy-(4-(2-*tert*-butoxycarbonylamino-2-butylcarbamoyl-ethyl)-phenoxy)-2-cyclopentene **3** (0.47 g, 1.02 mmol) was dissolved in THF (10 ml), followed by addition of 1 M LiOH (10 ml). The reaction mixture was stirred at room temperature for 24 h. The solvent was then evaporated under reduced pressure and the residue was dissolved in ethyl acetate, washed with water, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography (heptane/EtOAc 2:3) afforded the desired product (0.29 g, 70%). TLC (heptane/EtOAc 2:3) R_f 0.11. ^1H NMR (400 MHz, $CDCl_3$), δ

7.11 (d, $J = 8.4$ Hz, 2 H), 6.81 (d, $J = 8.4$ Hz, 2H), 6.18–6.12 (m, 2H), 5.68 (br s, 1H), 5.48–5.43 (m, 1H), 5.17–4.98 (m, 2H), 4.26–4.15 (m, 1H), 3.22–3.09 (m, 2H), 3.08–2.86 (m, 2H), 2.37–2.27 (m, 1H), 2.22–2.14 (m, 1H), 1.42 (s, 9H), 1.42–1.29 (m, 2H), 1.28–1.16 (m, 2H), 0.87 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 157.4, 139.6, 133.9, 130.8, 129.4, 115.8, 81.9, 80.6, 76.4, 70.5, 60.8, 56.5, 41.5, 39.6, 38.2, 31.8, 28.6, 22.5, 22.3, 20.3, 14.6, 14.0. MS (ESI^+) $m/z = 419.4$ $(MH)^+$.

3.1.4. (1R)-4-Oxo-(4-(2-tert-butoxycarbonylamino-2-butylcarbamoyl-ethyl)-phenoxy)-2-cyclopentene (5)

PDC (0.54 g, 1.43 mmol) was added to a room temperature solution of (1*R*,4*R*)-4-hydroxy-(4-(2-*tert*-butoxycarbonylamino-2-butylcarbamoyl-ethyl)-phenoxy)-2-cyclopentene **4** (0.29 g, 0.69 mmol) in dry DCM (25 ml). The resulting reaction mixture was stirred for 7 h and then diluted with Et_2O (50 ml) and filtered through celite. Purification by flash chromatography afforded the desired product (0.25 g, 87%). TLC (heptane/EtOAc 2:3) R_f 0.33. ^{1}H NMR (400 MHz, $CDCl_3$) 7.64 (dd, $J = 5.7$, 2.3 Hz, 1 H), 7.10 (d, $J = 8.6$ Hz, 2H), 6.79 (d, $J = 8.6$ Hz, 2H), 6.31 (dd, $J = 5.7$, 1.1 Hz, 1H), 6.19 (br s, 1H), 5.40–5.35 (m, 1H), 5.27 (br s, 1H), 4.25 (s, 1H), 3.25–3.03 (m, 2H), 3.00–2.88 (m, 2H), 2.82 (dd, $J = 18.4$, 6.0 Hz, 1H), 2.36 (dd, $J = 18.4$, 2.0 Hz, 1H), 1.36 (s, 9H), 1.34–1.28 (m, 2H), 1.27–1.10 (m, 2H), 0.83 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 205.4, 171.5,159.9, 156.7, 136.9, 131.1, 115.8, 75.6, 42.2, 39.6, 38.2, 31.8, 28.7, 20.4, 14.1. MS (ESI^+) $m/z = 417.3$ $(MH)^+$.

3.1.5. {1-Butylcarbamoyl-2-[4-(4-oxo-2,3-diphenyl-hexahydro-cyclopenta[d]isoxazol-6-yloxy)-phenyl]-ethyl}-carbamic acid tert-butyl ester (6)

Benzaldehyde (0.12 ml, 1.20 mmol) and phenylhydroxylamine (0.13 g, 1.20 mmol) were dissolved in 0.1 M SDS (1 ml). The reaction mixture was sonicated for 5 min and then stirred at room temperature for 2 h before compound **5** (0.10 g, 0.24 mmol) was added. The resulting reaction mixture was stirred for 2 days at 37 °C. Saturated aqueous NaCl solution was added and the product was extracted into EtOAc, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by preparative HPLC (NovaPak C18 19 × 300 mm) gradient 10–95% acetonitrile in water, afforded the desired product (19 mg, 13%). The stereochemistry of **6** was determined using NOE experiments involving the hydrogens of the 5-membered ring. ^{1}H NMR (400 MHz, $CDCl_3$) 7.45–7.38 (m, 2H), 7.39–7.33 (m, 2H), 7.32–7.23 (m, 3H), 7.18–7.12 (m, 2H), 7.10–7.05 (m, 2H), 7.05–6.99 (m, 1H), 6.88–6.82 (m, 2H), 5.75 (br s, 1H), 5.16–5.03 (m, 3H), 5.00 (d, $J = 6.7$ Hz, 1H), 4.27–4.15 (m, 1H), 3.67–3.60 (m, 1H), 3.24–3.08 (m, 2H), 3.06–2.93 (m, 2H), 2.58 (dd, $J = 18.2$, 6.5 Hz, 1H), 2.42–2.33 (m, 1H), 1.41 (s, 9H), 1.39–1.30 (m, 2H), 1.27–1.16 (m, 2H), 0.86 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 211.34, 171.37, 155.84, 149.76, 136.62, 131.09, 129.73, 129.50, 129.04, 128.49, 127.81, 123.79, 116.44, 115.81, 84.86, 75.14, 73.47, 60.93, 45.40, 39.58, 38.19, 31.85, 28.71, 20.32, 14.10. MS (ESI^+) $m/z = 614.4$ $(MH)^+$.

3.2. General procedure for [3+2] cycloaddition reactions under micellar catalysis

5.0 equivalents (0.618 mmol) of the variable aldehyde were added to a vial containing 5.0 equivalents of benzaldehyde (63 μl, 0.618 mmol) and phenylhydroxylamine (162 mg, 1.483 mmol, 12 equiv). A 0.1 M solution of SDS (2 mL, 0.2 mmol) was then added [22]. The mixture was sonicated for 5 min at room temperature. A Teflon coated magnetic stirring bar was added and the mixture was stirred for 1.5 h to permit nitrone formation. At this point; 1.0 equivalent of 2-cyclopentene-1-one was added and the reaction was allowed to stir for 48 h at 37 °C. The products were extracted with ethyl acetate, and the organic layer was washed twice with brine. The organic phase was then dried over magnesium sulfate and filtered, and the ratio of products was then analyzed by LC–MS.

3.3. Inlet methods for monitoring kinetics in [3+2] cycloaddition reactions by LC/MS

para-substituent on benzaldehyde: $-N(CH_3)_2$ and $-OCH_3$. Total run time: 30 min. Mobile phase consisting of 60% water/0.1% formic acid [A] and 40% MeCN/0.1% formic acid [B] for 10 min followed by a gradual change in gradient over the next 10 min to 45% [A] 55% [B], followed by a change to 5% [A] 95% [B] at 21 min and run at this ratio for 9 min.

para-substituent on benzaldehyde: $-NO_2$. Total run time: 30 min. Mobile phase consisting of 50% water/0.1% formic acid [A] and 50% MeCN/0.1% formic acid [B]. Gradient is changed over a 20 min period to 45% [A] 55% [B]. Gradient is then changed to 5% [A] 95% [B] over a 5 min period and run for another 5 min using this gradient.

para-substituent on benzaldehyde: $-CH_3$, –Cl, –OH, –CN. Total run time: 35 min. Mobile phase consisting of 49% water/0.1% formic acid [A] and 51% MeCN/0.1% formic acid [B] for 10 min. Gradient is then changed over 10 min to 42% [A] 58% [B]. Gradient is then changed to 5% [A] 95% [B] over 1 min and sample is run for 9 min at this gradient. The gradient is then changed to 90% [A] 10% [B] over a 1 min period and the sample is run for another 5 min. The plots were generated using sigma values from the literature [29,30] using GraFit version 4.0 (Erithacus Software Ltd., Surrey, UK).

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