Enzymatic Oxidative Cyclisation Reactions Leading to Dibenzoazocanes

Francesco Tozzi, Steven V. Ley, Matthew O. Kitching, Ian R. Baxendale

Innovative Technology Centre, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK
Fax +44(1223)336442; E-mail: theitc@ch.cam.ac.uk

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Abstract: From simple *N*-isovanillyltyramine derivatives double oxidative biotransformations can be achieved using tyrosinase leading to the corresponding hydroxylated dibenzoazocanes.

Key words: enzymes, oxidation, biotransformation, heterocycles

The development of new oxidation methods for phenolic compounds remains an enduring area of research interest. Not only are these methods desirable because of their potential similarity to biological processes,[1] the iterative application of such oxidations can rapidly lead to ever more complex structures. Unfortunately, current chemical approaches to these individual oxidation steps usually require stoichiometric reagents and can suffer from problems of chemoselectivity,[2] harsh conditions, high reagent costs, and the generation of toxic byproducts. By comparison enzymatic methods address many of these concerns but can often only be applied to very specific or simple substrates.[3] Having developed some expertise with enzymatic oxidation methods for organic synthesis[4] we decided to investigate the potential of enzyme-mediated oxidative cyclisation reactions of biaryl compounds containing phenolic groups, in the hope they would lead to new natural-product-like scaffolds.

Previous work using enzymes to effect inter- and intramolecular hetero-bond formation is known although carbon–carbon bond-forming events are rare.[5] Our aim therefore was to devise suitable substrates and investigate their selective oxidative biotransformations to architecturally more elaborate products. For the initial phase of these studies reported here, we prepared the norbelladine derivative, *N*-isovanillyltyramine (**1**) as a model substrate since the products could be expected to mimic natural products from the amaryllidaceae family[6] and are of particular interest to our group.[7]

The secondary amine **1** was readily obtained via a reductive amination procedure using isovanillin (**2**) and tyramine (**3**) in methanol to afford **1**[8] in 86% isolated yield following crystallisation from methanol (Scheme 1). The oxidase enzymes selected for screening were horseradish peroxidase (HRP, EC 1.11.1.7) and tyrosinase (Tyr, EC 1.14.18.1) as these two had shown good substrate scope in other studies.[9] Since our focus was on the reactivity of the substrate no specific enzymatic activity study was conducted, rather the enzymes were used as supplied without further purification although an SDS PAGE analysis of the commercial extracts gave us some idea of their purity (Figure 1).

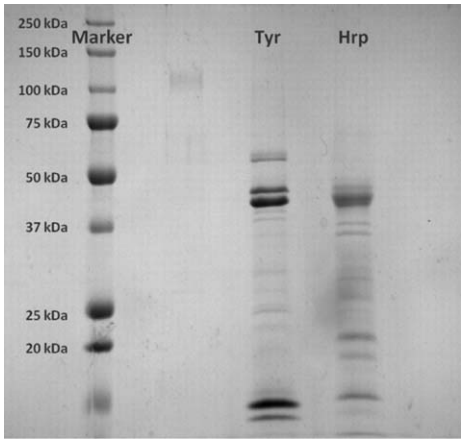
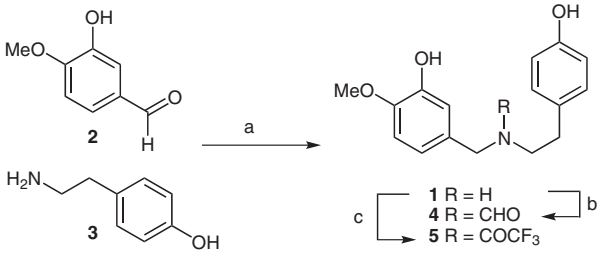


Figure 1 SDS-PAGE analysis of extracts containing tyrosinase (Tyr) and horseradish peroxidase (HRP)

Unfortunately, despite extensive screening of the reaction conditions no identifiable products could be isolated from the incubation of substrate **1** with these enzymes. In all cases, only unreacted starting material **1** or highly insoluble polymeric byproducts were observed. We therefore decided to protect the amine functionality due to its suspected role in inhibiting the desired oxidative cyclisation reaction. Formylation of **1** was achieved under microwave irradiation in neat ethylformamide with a catalytic amount of formic acid which gave 87% yield of the crystalline product **4** on standing.[8] Similarly, we also prepared the trifluoroacetyl derivative **5**[10] by standard methods in 78% isolated yield following column chromatography (Scheme 1).



Scheme 1 Synthetic route to the starting materials. *Reagents and conditions*: (a) MeOH, r.t., 3 h, then $NaBH_4$, 0 °C to r.t., 3 h, 86%; (b) HCOOEt, HCO_2H (cat.), MW 120 °C, 30 min, 87%; (c) TFAA (1.5 equiv), pyridine, 0 °C to r.t., 2 h, 78%.

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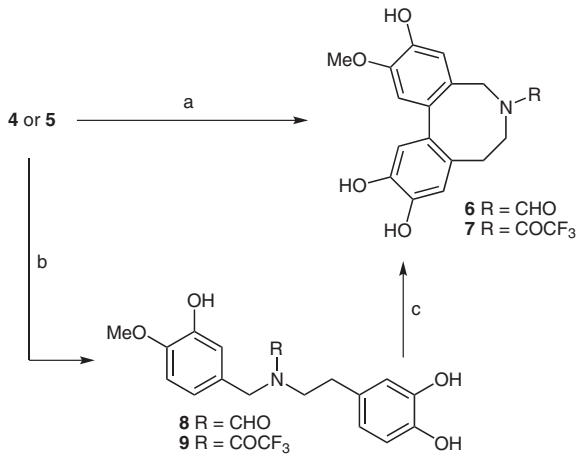
The *N*-formyl-protected amine **4** was then treated with HRP but afforded only decomposition products, however, more promising results were obtained using the tyrosinase enzyme system. A basic set of test conditions using a 4:1 mixture of buffer and acetone (to solubilise the substrate) under an oxygen atmosphere at ambient temperature was employed. Conversion to a single product was observed which, following chromatographic purification, could be isolated in 17% yield and its structure determined to be that of compound **6**.[11] Further optimisation experiments using lower enzyme loadings proceeded well without deleteriously impacting on the rate of conversion while higher dilution led to faster reaction times and increased yields of around 50% (Scheme 2). Moreover, we discovered it was possible to use air as the co-oxidant in these experiments, although necessitating extended reaction times, pleasingly gave no reduction in the final yields.

This result suggests that the enzyme initially induces a very specific chemo- and regioselective *ortho* oxidation of the mono phenolic ring. This is then followed by a second oxidation step presumably via an unstable *ortho*-quinone which itself then undergoes rapid intramolecular nucleophilic attack by the second electron-rich aryl ring eventually yielding the substituted dibenzoazocane **6** (Scheme 2). Under optimised conditions the reaction proceeded to full conversion in 1.5 hours using 1% bulk weight of enzyme (14 KU/mmol). The product was readily isolated by direct quenching of the reaction mixture with 1 M hydrochloric acid, followed by extraction of the solution with EtOAc, and finally purification of the residue by column chromatography (Scheme 2).[12]



Scheme 2 Oxidative enzymatic reactions. *Reagents and conditions*: (a) **4**, Tyr (14 KU/mmol), O_2, buffer–acetone (4:1), 1.5 h, 50%; **5**, Tyr (28 KU/mmol), O_2, buffer–acetone (3:1), 2 h, 53%; (b) **4**, Tyr (28 KU/mmol), O_2, buffer–acetone (4:1), sodium ascorbate (10 equiv), 4 h, 68%; **5**, Tyr (56 KU/mmol), O_2, buffer–acetone (3:1), sodium ascorbate (10 equiv), r.t., 6 h, 68%; (c) **8**, Tyr (14 KU/mmol), O_2, buffer–acetone (4:1), 1 h, 58%; **9**, Tyr (28 KU/mmol), O_2, buffer–acetone (3:1), 1 h, 56%.

Crystal structure analysis by X-ray methods of this new dibenzoazocane derivative **6**[13] (which is related to the apogalanthamine alkaloid family)[14] revealed a twisted boat-chair (TBC) conformation of the eight-membered ring existing as a racemic mixture of atropoisomers.[15] This result was confirmed by optical rotation measurements as well as ^{1}H NMR studies which supported the same observation in solution. Moreover, all the NMR signals were duplicated in a general ratio of between 7:3 and 8:2 depending on the exact deuterated solvent used. Even using VT-NMR analysis a complete coalescence of the peaks could not be achieved (DMSO, 140 °C). Separation of the species via preparative supercritical chromatography (CO_2–*i*-PrOH) and NMR analysis of the major fraction afforded again the same ratio, thus strongly suggesting the presence of a rotameric equilibrium of the formamide group. A favourable π-stacking interaction can be observed between the carbonyl and aryl ring leading to the high stability of these structural forms at least in the crystalline state.

To further investigate the mechanism of the reaction we found that the catechol intermediate **8** could be isolated in 68% yield if the reaction was conducted in the presence of excess reductant such as L-sodium ascorbate (Scheme 2, conditions b). Having this intermediate available allowed us to intercept the catalytic cycle and further investigate the documented lag time period that has been observed in many tyrosinase-mediated reactions.[16]

It has been established that the active site of tyrosinase is present in three forms: deoxy (Ty_{deoxy}), oxy (Ty_{oxy}), and met (Ty_{met}, Figure 3). The first of these is a free form that is able to bind molecular oxygen producing the oxy form, which is active in both the cresolase and the catecholase cycle.[17] The met form on the other hand is only able to catalyse oxidation to the dione[18] but is the predominant form found in the crude enzyme (Figure 3). The observed induction period (lag time) is due to an autocatalytic mechanism where the small amount of oxy form present facilitates a chemical oxidation producing an *ortho*-dihydroxy benzene. Once present, this intermediate can relay all of the met form to the corresponding deoxy state. The deoxy form is then capable of binding oxygen to generating the oxy form allowing the catalytic cycle to reach steady-state operation.[16]

This was exemplified by adding 5 mol% of the intermediate **8** to the standard reaction mixture. This yielded the desired product **6** in under 1 hour instead of the typical 1.5 hours. As a control, doping the reaction mixture with the product **6** or catechol as the exogenous bis-*ortho*-hydroxylated activator, respectively, had no influence at all, or in the case of catechol, produced secondary unwanted byproducts and deactivated the enzyme (Table 1).

Modification of the protecting group with a more labile trifluoroacetoxy moiety proved equally viable, and required only minor modifications (acetone/buffer 1:3, 28 KU/mmol) to the enzymatic reaction conditions (Scheme 2). The alternative product **7** could be isolated in almost identical yield (58%) after two hours. As in the case of compound **6**, product **7** was obtained as a racemic atropoisomeric mixture and in a TBC conformation of the

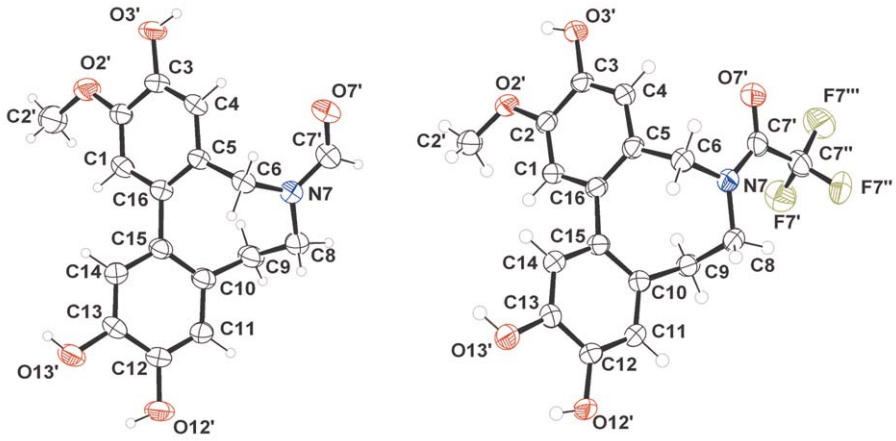


Figure 2 Crystal structures of **6** and **7**, respectively

dibenzoazocane ring as confirmed by its X-ray analysis (Figure 2). However, no rotameric forms were observed in the ^{1}H NMR at ambient conditions.

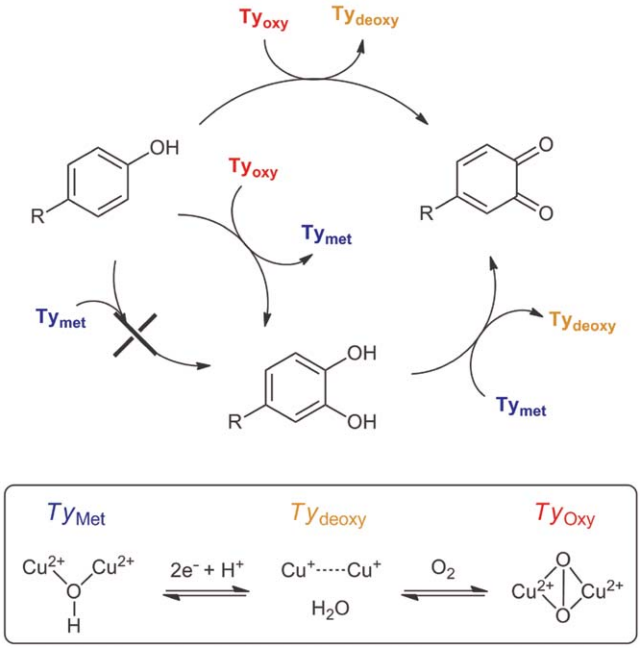


Figure 3 Catalytic active forms of tyrosinase and associated catalytic cycle

Table 1 Investigation of Lag Time

Entry	Activator[a]	Reaction time (h)	Conversion (%)[b]
1	none	1.5	99
2	8	1	99
3	6	1.5	99
4	catechol	3.5	80[c]

[a] Conditions: 5 mmol%.
[b] As determined by LC-MS analysis.
[c] Additional products with no additional turnover after 1.5 h.

In conclusion, we have demonstrated the power of enzyme oxidases to bring about selective late tailoring oxidation processes on less functionalised substrates to afford new natural-product-like structures.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(11) Preparation data X-ray structure file reference: CCDC 770222. See Supporting Information for full experimental details and characterisation.

(12) Alumina (neutral and basic) and Florisil were also used as well as several basic ion exchange resins with a catch and release procedure although these showed no benefits in terms of isolated yield or purities.

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(15) In one of the rotamers H-6a and the H-6b are, respectively, located at $\delta = 4.80$ and 2.99 ppm are in an AB system with a coupling constant of 13.5 Hz. The high shift of H-6a is due to its position in the plane of the carbonyl. In the minor rotamer they are in the same system with a coupling constant of 14.5 Hz but at $\delta = 4.40$ and 3.40 ppm, respectively. Moreover, COSY experiment showed that the protons H-9a and H-8b, and the H-9b and H-8a do not, respectively, couple because of their ca. 90° dihedral angle, thus confirming the TBC conformation. See also: Landais, Y.; Robin, J. *Tetrahedron* **1992**, *48*, 7185.

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