Green Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c6gc02050j

Eco-friendly synthesis of indo dyes mediated by a bacterial laccase†

Ana Catarina Sousa,[a,b] M. Fátima M. M. Piedade,[b,c] Lígia O. Martins[d] and M. Paula Robalo*[a,b]

Received 25th July 2016,
Accepted 16th August 2016

DOI: 10.1039/c6gc02050j

www.rsc.org/greenchem

Several aminoindamine and indoaniline dyes were obtained in good to excellent yields (64–98%) by oxidative cross-coupling between 1,4-phenylenediamine (**1**), 4-aminophenol (**2**) or 2,5-diaminotoluene (**3**) and several *meta*- and *meta*,*para*-substituted couplers (**4a–j**) using a green biocatalyst, the bacterial enzyme CotA-laccase from *Bacillus subtilis*, in water and under mild conditions of pH and temperature. Our results show that the enzymatic route described represents an efficient and sustainable alternative to the chemical synthesis of indo dyes, with a potentially high impact in the cosmetic and hair dye industries.

Introduction

Oxidative dyeing of human hair has been practiced for over 100 years and as a result of increasing aging and thus greying, the global population demand for these products has been increasing rapidly, a trend that will likely continue in the future.[1] Oxidative hair dyes require three major components in the chemical process. The first step is the oxidation of a primary intermediate, an oxidation base or a developer, an *o*- or *p*-substituted phenol or aniline derivative; 1,4-phenylenediamine (1,4-PDA) and other related derivatives have been extensively used as oxidation bases in permanent hair colouration processes.[1–3] The second component, the coupler, is commonly a *meta*-disubstituted aromatic compound with electron donor groups, including *m*-phenylenediamines, resorcinol and naphthols and their derivatives; these compounds by themselves will not produce colour when oxidised, but they can react with the products from oxidation of the primary intermediates to form dyes. The final component is the oxidant, almost exclusively hydrogen peroxide under alkaline conditions provided usually by ammonia.[1] Structures like aminoindamine and indoaniline dyes have been suggested as oxidation products, based on the kinetic studies using the chemical oxidant ferricyanide.[3a,4–6]

The oxidative process is expected to remain dominant and the development of biotechnological systems, such as the exploitation of enzymatic oxidation processes to replace conventional oxidative processes is viewed as an attractive means of addressing potential environmental concerns.[7,8]

Laccases (benzenodiol : oxygen oxidoreductases, EC 1.10.3.2) belong to the multi-copper family of oxidases, with four copper ions in two catalytic centers and oxidise a wide range of electron-rich aromatic substrates using oxygen as an electron acceptor.[9] Considering that they operate under mild reaction conditions and water is the only by-product formed, they are the ideal biocatalysts for sustainable chemical and oxidoreductive biotechnological applications.[10] Although the use of laccases from different origins has been extensively studied with phenols, the substrates of excellence for these enzymes, their use in the oxidation of aniline derivatives and other aromatic amines has been much less explored.[11,12]

Enzymes particularly of the laccase type have been claimed to be well suited for use in cosmetic applications. Although the laccase-based hair dyes have been considered less irritant and easier to handle than current hair dyes,[13,14] most of the proposed ready-to-use formulations, using laccases of plant or fungi origins, produce colours which do not fulfill the requirements concerning the issues such as the homogeneity of colour distribution in the hair fibres and the chromaticity of the dyeing powder.[14]

Recently, we reported the use of CotA-laccase for the oxidation of aromatic amines looking forward to the sustainable synthesis of dyes[11] and the formation of different heterocyclic cores.[15] The recombinant bacterial CotA-laccase from *Bacillus subtilis*, an intrinsically highly thermostable enzyme,[16] showed

[a]*Área Departamental de Engenharia Química, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal. E-mail: mprobalo@deq.isel.ipl.pt; Fax: +351218317267; Tel: +351218317163*

[b]*Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal*

[c]*Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

[d]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-15 Oeiras, Portugal*

†Electronic supplementary information (ESI) available: X-ray crystallographic data, NMR and MS spectra. CCDC 1476627. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6gc02050j

optimal pH values around neutrality for the substrates tested,[11] illustrating the potentiality of this enzyme to use aromatic amines as substrates. The bacterial origin of the enzyme, the broad substrate range and its ability to operate at the alkaline pH range, which promotes hair swelling and the penetration of colour pigments into the fibres, prompted us to extend our studies to cross coupling reactions involving these aromatic derivatives.

We report herein the successful use of 1 U ml^{-1} of CotA-laccase, a lower value than the usually reported,[14] to mediate the oxidation of selected aromatic amines, 1,4-phenylenediamine (1,4-PDA), 4-aminophenol (4-AP) and 2,5-diaminotoluene (2,5-DAT) into the corresponding benzoquinoneimines, which will be further involved in hetero-coupling reactions with selected couplers. These cross coupling reactions were directed towards the production of indo dyes, widely used in permanent hair and leather colouration.[1,2]

The proposed enzymatic oxidation process leads to the synthesis of a wide range of indo dye structures in good to excellent yields, showing a comparable performance to the traditional chemical routes concerning colour development and the production of indo dyes. Our approach, avoiding the use of hydrogen peroxide and alkali, which due to their bleaching effect degrade the hair fibers, rendering them coarse and fragile,[1] can be viewed as a more environmentally friendly alternative synthetic route.

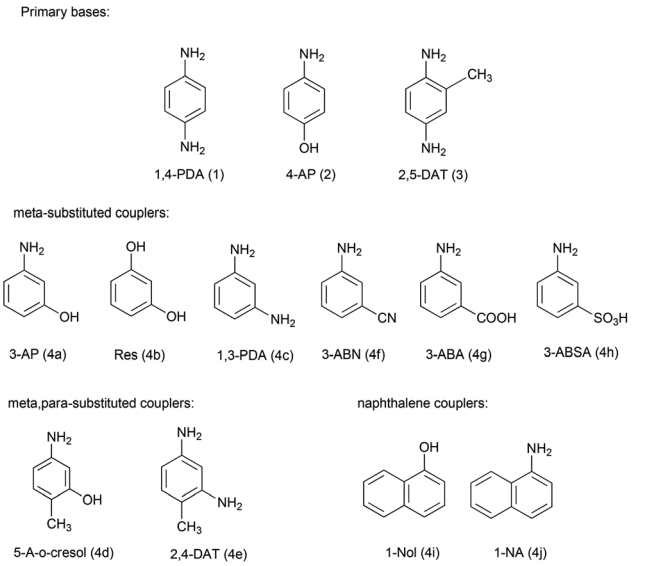


Fig. 1 Chemical structures of the aromatic amines and phenols used in the heterocoupling reactions mediated by CotA-laccase. (3-Aminophenol (3-AP), resorcinol (Res), 1,3-phenylenediamine (1,3-PDA), 3-aminobenzonitrile (3-ABN), 3-aminobenzoic acid (3-ABA), 3-aminosulfonic acid (3-ABSA), 1-naphthol (1-Nol), 1-naphthylamine (1-NA), 5-amino-*o*-cresol (5-A-*o*-cresol), 2,4-diaminotoluene (2,4-DAT).)

Results and discussion

The heterocoupling reactions mediated by the bacterial CotA-laccase were conducted using 1,4-PDA (**1**), 4-AP (**2**) or 2,5-DAT (**3**) as the oxidation bases and a range of aromatic compounds as couplers (**4a–j**) (Fig. 1). The selection of couplers was based on their use for indo dye synthesis and also on their oxidation potentials at the reaction pH.

Thus, the knowledge of the redox potential of the compounds involved in the enzyme-catalysed reaction provides useful information in the prediction and understanding of the reaction pathway. The electrochemical behaviour of the couplers presented in Fig. 1 was studied by cyclic voltammetry (Table 1).

Table 1 Electrochemical data of substituted aromatic amines and phenols used as couplers *vs.* NHE in buffered solutions at a scan rate of 100 mV s^{-1}

	E_{pa} (V)			
Compound	pH = 4	pH = 5	pH = 6	pH = 7
4a (3-AP)	1.09	0.92	0.87	0.80
4b (Res)	1.06	1.06	1.06	1.00
4c (1,3-DA)	0.91	0.89	0.80	0.79
4d (5A-*o*-cresol)	0.91	0.79	0.76	0.71
4e (2,4-DAT)	0.84	0.83	0.79	0.77
4f (3-ABN)	1.33	1.28	1.24	1.22
4g (3-ABA)	1.23	1.21	1.19	1.12
4h (3-ABSA)	1.19	1.16	1.14	1.07
4i (1-Nol)	0.93	0.92	0.87	0.85
4j (1-NA)	0.80	0.78	0.79	0.77

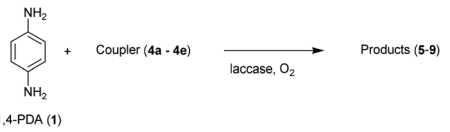
Experiments were performed at different scan rates and pH values in (1 : 9) MeOH : aqueous buffer media (pH range 4–7) using a platinum disk as the working electrode and the Ag/AgCl electrode as the reference electrode and corrected by +0.197 V to the NHE. All the tested couplers showed an irreversible oxidation at positive potentials and the determined E_{pa} values (Table 1) decrease as the pH increases, in accordance with our previous results using a diverse group of aromatic amines.[11,15a]

As expected, the redox potentials for *meta*-donor substituted aromatic amines (**4a** and **4c**) are higher than those previously determined for the equivalent *para*-isomers,[11] most likely due to the ring activation effect in the *para*-isomers.[17] In contrast, the introduction of a methyl group in the *ortho*-position (**4d** and **4e**) relative to the corresponding disubstituted compound (**4a** and **4c**) slightly decreases the oxidation potential in the tested pH range.

The obtained results indicate that all the studied compounds are most likely resistant to a direct monoelectronic oxidation by CotA-laccase, since the E_{pa} values (from +0.71 V to +1.33 V) are higher than the redox potential of the T1 copper centre of CotA-laccase (0.55 V).[18]

The cross coupling reactions between 1,4-PDA (**1**) and couplers **4a–4e** were performed at 37 °C in Britton & Robinson

Table 2 Heterocoupling reactions between 1,4-PDA and couplers (**4a–4e**) mediated by CotA-laccase (1 U mL^{-1})[a]



1,4-PDA (**1**)

Entry	Coupler	Conditions (pH, coupler)	Yield[b] (%)	Product
1	**4a**	6, 1.0 eq.	40	**5**
2	**4a**	7, 1.0 eq.	50	**5**
3	**4a**	7, 0.5 eq.	80	**5**
4	**4a**	8, 0.5 eq.	93[c]	**5**
5	**4b**	7, 1.0 eq.	20	**6**
6	**4b**	7, 0.5 eq.	27	**6**
7	**4b**	8, 0.5 eq.	64[c]	**6**
8	**4c**	8, 0.5 eq.	82[c]	**7**
9	**4d**	7, 1.0 eq.	83[c]	**8**
10	**4e**	7, 1.0 eq.	98[c]	**9**

[a] Reaction conditions: phosphate buffer (10% of ethanol); substrate concentration: 5 mM; reaction time: 24 h; temperature: 37 °C. [b] Yield of products was determined by ^{1}H NMR spectroscopy. [c] Isolated yields.

(B&R) or phosphate buffer (10 mM, in the pH range between 6 and 8) containing 10% of ethanol, using 0.5 or 1.0 equivalent of the coupler and 1 U mL^{-1} of CotA-laccase (Table 2). The use of 10% of the organic solvent increases the solubility of substrates and intermediates leading to improved results.

The formation of the coloured products occurs immediately after the addition of the CotA-laccase. For most of the reactions, the product formation is complete within 30 min to 1 h (data not shown). The yields presented in Table 2 were determined after 24 h; for prolonged reactions a considerable amount of by-products, most probably in the form of polymeric compounds, were observed. Control reactions confirmed that only small amounts of products (0–20%) were detected in the absence of the enzyme.

The heterocoupling reaction between 1,4-PDA (**1**) and 3-AP (**4a**) results in the trinuclear indo dye (**5**) (Table 2, Fig. 2). To increase the efficiency of the process, experiments were conducted at different pHs and in the presence of different

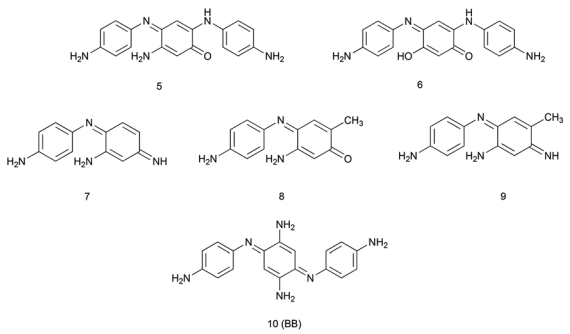


Fig. 2 Dinuclear and trinuclear compounds obtained in the biocatalysed reactions of 1,4-PDA and couplers (**4a–e** and **4f–h**) and Bandrowski's base (BB).

equivalents of the coupler. The increase of pH and the reduction of the number of equivalents of the coupler led to enhanced yields (Table 2, entries 1–4), with the highest yield (95%) achieved at pH 8, using 0.5 equivalents of the coupler (Table 2, entry 4). The alkaline conditions most likely promote the deprotonation of the hydroxyl group in 3-AP (pK_a 9.9), leading to the formation of a phenolate ion and consequently promoting the electrophilic attack on the *para*-position.[4b]

The commercial enzyme TvL from *Trametes versicolor* was also used in the heterocoupling between 1,4-PDA (**1**) and 3-AP (**4a**) resulting in significantly lower yields for the indo dye (**5**) when compared with CotA-laccase under all the conditions tested (data not shown) and for this reason it was not further used in this study.

Representative results of other heterocoupling reactions between 1,4-PDA (**1**) and different couplers are also reported in Table 2 (entries 5–10) and Fig. 2. For *m*-substituted couplers (**4a–c** and **4f–h**), the efficiency in the oxidation towards heterocoupled dinuclear or trinuclear dyes strongly depends on the nature of the substituents on the aromatic ring. The use of *m*-donor substituted couplers (**4b–c**) led to the heterocoupling products (**6** and **7**) in good to excellent yields (Table 2, entries 7 and 8, Fig. 2), at pH 8 in the presence of 0.5 equivalents of the coupler. The reactions between the 1,4-PDA and *m*-substituted couplers with electron withdrawing groups (3-ABN (**4f**), 3-ABA (**4g**) and 3-ABSA (**4h**)) yielded in all cases the brown dye Bandrowski's base (BB) (**10**) (see Fig. 2) as the only reaction product (70–100%), independent of the reaction conditions, the pH variation and the stoichiometric quantity of the coupler (data not shown). The coupler was always recovered at the end of reactions and BB is known to result from the homocoupling reaction of the oxidation base 1,4-PDA,[11] which means that the couplers **4f**, **4g** and **4h** are most probably weaker nucleophiles than 1,4-PDA, thus preventing the electrophilic attack on the coupler.

From the heterocoupling reactions between 1,4-PDA (**1**) and the *m*,*p*-disubstituted couplers 5-A-*o*-cresol (**4d**) and 2,4-DAT (**4e**) the indoaniline dyes (**8**) and (**9**) were respectively obtained in good to excellent yields (Table 2, entries 9 and 10).

The favorable results obtained with *m*-substituted and *m*,*p*-disubstituted couplers prompted us to extend our studies to naphthalene couplers (1-Nol (**4i**) and 1-NA (**4j**)), where the presence of electron donor groups promotes the heterocoupling reactions with 1,4-PDA catalysed by CotA-laccase (Table 3, entries 1 and 2). Surprisingly, the main product isolated in both reactions was identified to be the naphthol-derived indoaniline **11** (Fig. 3). This could be explained by the hydrolysis of the terminal amino/imino group in accordance with the previous reports.[5,6,19] The cross-coupling reaction between 1,4-PDA and **4j** additionally leads to the secondary product **12** (Fig. 3).

To expand the scope of the biocatalysed route, additional oxidation bases 4-AP (**2**) and 2,5-DAT (**3**) were used with *m*,*p*-disubstituted couplers under optimised reaction conditions (Table 3, entries 3–5). The heterocoupling reactions of 4-AP with **4d** and **4e** resulted in the production of the respective

Table 3 Heterocoupling reactions between 1,4-PDA, 4-AP or 2,5-DAT and couplers mediated by CotA-laccase (1 U mL^{-1})[a]

Entry	Oxidation base	Coupler	Yield[b] (%)	Product
1	**1** (1,4-PDA)	**4j**[c]	74	**11**
2	**1** (1,4-PDA)	**4j**[c]	56 + 29	**11** + **12**
3	**2** (4-AP)	**4d**	98	**13**
4	**2** (4-AP)	**4e**	52 + 28	**14** + **15**
5	**3** (2,5-DAT)	**4d**	60 + 27	**16** + **17**

[a] Reaction conditions: phosphate buffer (10% of ethanol); pH 7; substrate concentration: 5 mM; 1 equivalent of coupler; reaction time: 24 h; temperature: 37 °C. [b] Isolated yields. [c] 0.5 equivalents.

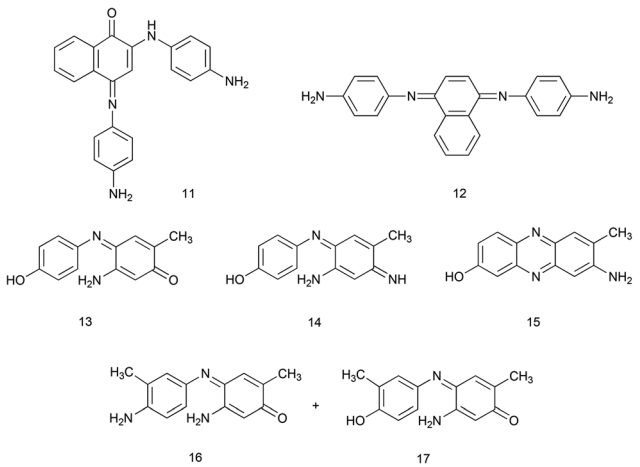


Fig. 3 Dinuclear and trinuclear compounds obtained in the cross-coupling reactions of 1,4-PDA (**1**), 4-AP (**2**) and 2,5-DAT (**3**) and couplers (**4d**, **4e**, **4i** and **4j**).

dinuclear indoaniline **13** and 2-aminoindamine **14** dyes, with the intramolecular cyclisation of the latter to an asymmetric phenazine core **15** (Table 3, entries 3 and 4, Fig. 3). The formation of heterocyclic phenazines was previously reported by Brown and Corbett[5,3a,19a] for similar oxidative reactions with 4-AP, using the chemical oxidant ferricyanide.

Substitution of the 1,4-PDA ring with a methyl group (2,5-DAT) and coupling with **4d** resulted in the production of the dimeric structures **16** and **17** but in lower yields (Table 3, entry 5, Fig. 3). This result can be explained by a lower oxidation rate for 2,5-DAT, when compared with 1,4-PDA. In fact, Corbett *et al.*[3b,6] showed that the *C*-methylation of the diamine 1,4-PDA decreases the rate of chemical oxidation with ferricyanide, due to the loss of basicity on the diamine, leading to a decreased amount of protonated species present at a specific pH. Compound **17** results from a hydrolysis process of the terminal amino group which could occur either during the oxidation, the heterocoupling reaction or the purification processes, as previously described.[5,15a,19,20]

The obtained products were isolated in good to excellent yields (64–98%) directly as crude solids and were fully characterised by FTIR, NMR (^{1}H, ^{13}C, COSY, HSQC and HMBC) and UV-Visible spectroscopy and mass spectrometry (see the Experimental section and the ESI†). Insoluble products were isolated from the reaction media by filtration, while the soluble products were extracted with acetyl acetate and isolated by solvent evaporation. For the reactions where two products were obtained, the isolation process includes a step of purification by silica gel preparative or column chromatography.

The structures proposed for the products **5–9** and **13–15** have been previously suggested by Corbett and coworkers,[3a,4–6] based on the chemical oxidation of several primary intermediates in the presence of different couplers. Nevertheless, with the exception of **5**, **8** and **13**, for which the proton NMR data were reported, the full spectroscopic characterization for these compounds was never reported.

The CotA-laccase mediated reactions between 1,4-PDA and the couplers 3-AP, 1,3-PDA and resorcinol yield the trimers **5** and **6** and the dimer **7** respectively (see Fig. 2). The presence of a quinone–imine central structure for the trimers and a quinonediimine pattern for the dimer **7** was confirmed by the combined analysis of the NMR and ESI/MS data. The ^{1}H and ^{13}C NMR data of trimers **11** and **12**, obtained from 1,4-PDA and naphthalene derived couplers, confirm the presence of a substituted naphthalene central core. The symmetric structure proposed for **12** points out the loss of the amino group of the naphthalene coupler during the course of the reaction.

The reactions of 1,4-PDA, 4-AP and 2,5-DAT with the *m*,*p*-disubstituted couplers **4d** and **4e** yielded two different dyes: the indoaniline type dyes **8**, **13**, **16** and **17** and the aminoindamine dyes **9** and **14** (see Fig. 2 and 3). The position of the new N–C bonds and the presence of the quinoneimine (for indoanilines) and the quinonediimine (for aminoindamines) structures were unambiguously indicated by the characteristic resonances of carbonyl and imine quaternary carbons in the ^{13}C NMR spectra.

The structure of compound **8** was obtained by single X-ray diffraction. The compound crystallises in the monoclinic centrosymmetric space group $P2_1/n$, with one molecule per asymmetric unit. The molecular diagram (Mercury[21]) with the atomic numbering scheme is presented in Fig. 4.

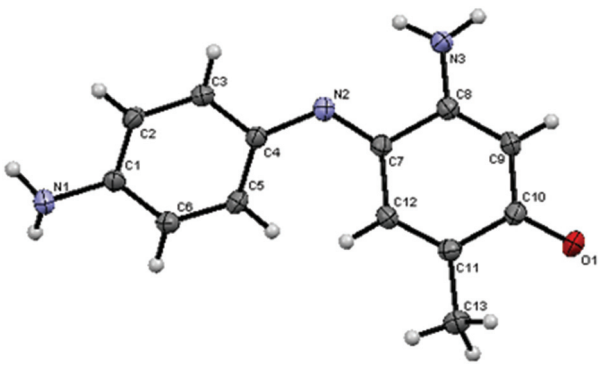


Fig. 4 Molecular diagram showing the crystallographic labeling scheme for compound **8**. Thermal ellipsoids are drawn at the 50% probability level.

The compound scaffold is composed of two 6-membered ring systems, a benzenoid and a quinoid ring. The bond distances [1.405(2) Å to 1.378(1) Å] and bond angles [120.6(1)° to 118.0(1)°] for aromatic carbons are consistent within the expected range for a benzene ring. The N1–(C1–C6)–N2 aromatic fragment is almost planar, with maximum deviations out-of-plane of 0.036(1) Å and 0.168(1) Å in the N1 and N2 atoms, respectively. In the quinoid fragment, the electronic delocalisation is observed with the short bond lengths C8–C9 and C11–C12 [1.368(2) Å and 1.344(1) Å], while C7–C8, C9–C10, C10–C11 and C12–C7 are larger [1.420(2) Å to 1.490(2) Å], in accordance with the values reported for similar compounds.[20,22] The connection between the benzenoid and quinoid fragments is established by the C4–N2–C7 single and double bonds with 1.400(1) Å and 1.295(1) Å, respectively. The dihedral angle formed between the planes of the two fragments is 47.42(3)°, denoting the distortion inside the molecule (see the ESI†).

The enzyme-catalysed formation of the aminoindamine and indoaniline dyes (see the ESI, Fig. S1†) follows a similar pathway to the one previously proposed for chemical oxidations and is reported in the literature.[3,23] The first step, the enzymatic oxidation of the primary intermediate (oxidation base or developer) promotes the formation of the benzoquinone-monoimine or -diimine intermediates, which are further involved in cross-coupling reactions with the aromatic or naphthalene couplers, yielding the dinuclear leuco derivatives and finally the dinuclear or trinuclear indoaniline or aminoindamine dyes.

Experimental

General procedures

Commercial reagents and solvents were used as received without additional purification. The aromatic compounds used as starting materials (Fig. 1) were commercially available (Sigma-Aldrich Co.). TLC: Merck 60 F254 plates. Preparative chromatography: Silicagel Fischer 60A (200 micron). The UV-Visible data for compounds were obtained in methanol on a Nicolet Evolution 300 spectrophotometer. The product characterization was performed by 1D-NMR (^{1}H, ^{13}C) and 2D-NMR (COSY, HSQC and HMBC) spectra, obtained in CD_3OD on a Bruker Advance 400 MHz spectrometer with a 5 mm probe. Chemical shifts are reported in ppm relative to the solvent peaks and coupling constants (J) are reported in hertz. FTIR spectra were obtained in KBr pellets on a Bruker Vertex 70 FT-IR spectrometer. LRESI mass spectrometry and tandem mass experiments were carried out on a LCQ Fleet mass spectrometer operated in the ESI positive/negative ion modes (Thermo Scientific). The optimised parameters were as following: ion spray voltage, ±4.5 kV; capillary voltage, +16 and −20 V; tube lens offset, −63 and +82 V; sheath gas (N_2), 80 arbitrary units; auxiliary gas, 5 arbitrary units; capillary temperature, 250 °C. The spectra were recorded in the range of 100–1000 Da. The spectrum typically corresponds to the average of 20–35 scans. Tandem mass spectra were obtained with an isolation window of 2 Da, a 30% relative collision energy and with an activation energy of 30 ms. HR ESI(+/−) mass spectra were obtained on a QTOF Impact II™ mass spectrometer (Bruker Daltonics, GMBH, Germany), operating in the high resolution ion mode. Calibration of the TOF mass analyser was performed with a lock mass calibrant.

The recombinant CotA-laccase from *Bacillus subtilis* (1 U defined as the amount of enzyme that transformed 1 μmol of ABTS per min at 37 °C) was produced and purified as described previously.[16,24] *Trametes versicolor* laccase (TvL, specific activity 20 U mg^{-1}; 1 U defined as the amount of enzyme that transformed 1 μmol of catechol per min) was purchased from Sigma-Aldrich. The protein concentration was measured by using the Bradford assay using bovine serum albumin as a standard.[16] Both laccases were stored frozen at −18 °C prior to use.

The redox potentials were measured by cyclic voltammetry using an EG&G Princeton Applied Research Model 273A potentiostat/galvanostat monitored with a personal computer loaded with Electrochemistry PowerSuite v2.51 software from Princeton Applied Research. Cyclic voltammograms were obtained using 1 mM of compounds in 9 : 1 buffer : MeOH (phosphate buffer for pH ≥ 6 and Britton–Robinson (B&R) buffer for pH 4 and 5 and 10 mM) using a three-electrode configuration cell with a home-made platinum-disk working electrode (1.0 mm diameter), a platinum wire counter electrode and an Ag/AgCl reference electrode (purchased from Radiometer analytical, SAS, France). The potential was scanned from −0.7 to 1.2 V at a scan rate of 100 mV s^{-1}. All measurements were performed at room temperature and the solutions were deaerated with dinitrogen before use. The measured potentials were corrected by +0.197 V to the normal hydrogen electrode (NHE).

General procedure for laccase mediated heterocoupling reactions

Procedure A – for compounds 5–9 and 11–17. Laccase (1 U mL^{-1}) was added to a stirred solution of the primary intermediate (1,4-PDA, 4-AP or 2,5-DAT) (0.5 mmol, 5 mM) in phosphate buffer : ethanol solution (9 : 1) (10 mL, 10 mM) at the selected pH. The solution was stirred at 37 °C in the presence of an adequate amount of the coupler and the reaction was monitored by TLC. After 24 h, for the soluble products, the aqueous solution was extracted with ethyl acetate. The collected organic phases were dried over Na_2SO_4, filtered, concentrated in a vacuum and characterised. In the cases of compounds **5**, **6** and **11**, insoluble in the reaction medium, the final aqueous phase was filtered and the residue was directly lyophilised and characterised. In the case of mixtures, the products were isolated by chromatography (column or preparative) using an acetyl acetate/ethanol 3 : 1 (v/v) solution as an eluent. Control reactions in the absence of the enzyme were also conducted to check for the auto-oxidation of substrates and after 24 h, the same work-up procedure was followed.

Procedure B. Laccase (1 U mL^{-1}) was added to a stirred solution of 1,4-PDA (0.5 mmol, 5 mM) in phosphate : ethanol solution (9 : 1) (10 mL, 10 mM) at the selected pH. The solution was stirred at 37 °C in the presence of an adequate amount of couplers (3-ABN, 3-ABA, and 3-ABSA) and the reaction was monitored by TLC. After 24 h, the dark blue solution was extracted with ethyl acetate. The collected organic phases were dried over Na_2SO_4, filtered, concentrated *in vacuo* and analysed by ^{1}H NMR spectroscopy. In all the cases, the coupler and the trinuclear dye BB were found to be the final products.

5: brown solid (6.8 mg, 93%); UV/Vis: λ_{max}(MeOH)/nm 400 (ε/dm^3 mol^{-1} cm^{-1} 4845); FTIR (KBr) ν_{max}/cm^{-1}: 3482, 3441, 3342, 3217, 1627, 1576, 1511, 1431, 1410, 1362, 1269, 1231, 1207, 1170, 1125, 1013, 831, 489; ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 6.90 (d, 2H, J = 8.7 Hz, H_8, H_{12}), 6.77 (d, 2H, J = 8.7 Hz, H_{15}, H_{17}), 6.71 (d, 2H, J = 8.7 Hz, H_{14}, H_{18}), 6.67 (d, 2H, J = 8.7 Hz, H_9, H_{11}), 6.01 (s, 1H, H_5), 5.63 (s, 1H, H_2); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 182.1 (C_1), 159.1 (C_3), 153.1 (C_4), 146.3 (C_{13}), 143.7 (C_6, C_{10}), 130.9 (C_7), 124.8 (C_8, C_{12}), 124.2 (C_{15}, C_{17}), 117.1 (C_9, C_{11}), 116.7 (C_{14}, C_{18}), 97.4 (C_2), 89.9 (C_5); ESI/MS positive mode: m/z 342 (34%, $[M + Na]^+$), 320 (100, $[M + H]^+$); MS^2 m/z 303 (27%, $[M + H - NH_3]^+$), 292 (100, $[M + H - CO]^+$), 212 (20, $[M + H - C_6H_8N_2]^+$); HR-ESI/MS: m/z calcd for $C_{18}H_{18}N_5O$ $[M + H]^+$: 320.1506; found 320.1505.

6: brown solid (4.6 mg, 64%); UV/Vis: λ_{max}(MeOH)/nm 420 (ε/dm^3 mol^{-1} cm^{-1} 3962); FTIR (KBr) ν_{max}/cm^{-1}: 3335, 3207, 1650, 1541, 1510, 1347, 1287, 1168, 826, 502; ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.06 (d, 4H, J = 8.6 Hz, H_8, H_{12}, H_{14}, H_{18}), 6.72 (d, 4H, J = 8.6 Hz, H_9, H_{11}, H_{15}, H_{17}), 6.68 (s, 1H, H_2), 6.03 (s, 1H, H_5), 5.42 (s, NH_2); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 176.1 (C_1), 155.2 (C_3, C_4), 149.6 (C_{10}, C_{16}), 127.1 (C_6), 126.9 (C_7, C_{13}), 126.3 (C_8, C_{12}, C_{14}, C_{18}), 118.8 (C_2), 116.2 (C_9, C_{11}, C_{15}, C_{17}), 85.8 (C_5); ESI/MS positive mode: m/z 321 (100%, $[M + H]^+$); MS^2 m/z 293 (15%, $[M + H - CO]^+$), 213 (100, $[M + H - C_6H_8N_2]^+$); HR-ESI/MS: m/z calcd for $C_{18}H_{17}N_4O_2$ $[M + H]^+$: 321.1346; found 321.1356.

7: purple solid (8.0 mg, 82%); UV/Vis: λ_{max}(MeOH)/nm 560 (ε/dm^3 mol^{-1} cm^{-1} 12 745); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.23 (d, 1H, J = 9.2 Hz, H_5), 7.17 (d, 2H, J = 8.9 Hz, H_8, H_{12}), 6.77 (d, 2H, J = 8.9 Hz, H_9, H_{11}), 6.65 (dd, 1H, J = 2.4 Hz, J = 9.2 Hz, H_6) and 5.99 (dd, 1H, J = 2.4 Hz, H_2); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 170.4 (C_1), 139.5 (C_7), 135.6 (C_{10}), 129.8 (C_8, C_{12}), 128.0 (C_5), 125.3 (C_6), 116.0 (C_9, C_{11}), 92.3 (C_2); ESI/MS positive mode: m/z 213 (100%, $[M + H]^+$); HR-ESI/MS: m/z calcd for $C_{12}H_{13}N_4$ $[M + H]^+$: 213.1135; found 213.1126.

8: brown solid (8.7 mg, 83%); UV/Vis: λ_{max}(MeOH)/nm 460 (ε/dm^3 mol^{-1} cm^{-1} 10 438); FTIR [KBr] ν_{max}/cm^{-1}: 3427, 3370, 3311, 3213, 3161, 2942, 2930, 1650, 1599, 1582, 1502, 1444, 1385, 1361, 1287, 1256, 1214, 1168, 1132, 1003, 885, 853, 819, 731, 706, 686, 636, 615, 550. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 6.87 (d, 2H, J = 8.7 Hz, H_8, H_{12}), 6.84 (s, 1H, H_5), 6.76 (d, 2H, J = 8.7 Hz, H_9, H_{11}), 5.65 (s, 1H, H_2), 1.91 (s, 3H, CH_3); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 188.5 (C_1), 156.7 (C_4), 151.3 (C_3), 149.2 (C_{10}), 142.4 (C_6), 140.1 (C_7), 125.6 (C_8, C_{12}), 123.2 (C_5), 116.2 (C_9, C_{11}), 100.1 (C_2), 16.6 (CH_3); ESI/MS positive mode: m/z 250 (37%, $[M + Na]^+$), 228 (100, $[M + H]^+$); MS^2 m/z 212 (100%, $[M + H - CH_4]^+$); HR-ESI/MS: m/z calcd for $C_{13}H_{14}N_3O$ $[M + H]^+$: 228.1131; found 228.1126.

9: dark blue solid (10.3 mg, 98%); UV/Vis: λ_{max}(MeOH)/nm 600 (ε/dm^3 mol^{-1} cm^{-1} 22 899); FTIR (KBr) ν_{max}/cm^{-1}: 3461, 3343, 3202, 1634, 1603, 1544, 1498, 1455, 1327, 1282, 1170, 1081, 983, 866, 715, 610, 526. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.12 (d, 1H, J = 8.7 Hz, H_8, H_{12}), 7.00 (s, 1H, H_5), 6.78 (d, 2H, J = 8.7 Hz, H_9, H_{11}), 6.00 (s, 1H, H_2), 2.16 (s, 3H, CH_3); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 165.2 (C_1), 159.6 (C_3), 153.2 (C_{10}), 145.4 (C_4), 139.5 (C_7), 134.1 (C_6), 129.3 (C_8, C_{12}), 125.9 (C_5), 116.0 (C_9, C_{11}), 92.9 (C_2), 17.4 (CH_3); ESI/MS positive mode: m/z 227 (100%, $[M + H]^+$); MS^2 m/z 211 (100%, $[M + H - CH_4]^+$); HR-ESI/MS: m/z calcd for $C_{13}H_{15}N_4$ $[M + H]^+$: 227.1291; found 227.1297.

11: purple solid (1.2 mg, 74%); UV/Vis: λ_{max}(MeOH)/nm 505 (ε/dm^3 mol^{-1} cm^{-1} 8634); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 8.44 (dd, 1H, J = 7.1 Hz, J = 1.6 Hz, H_8), 8.19 (dd, 1H, J = 7.8, J = 1.6 Hz, H_5), 7.74 (t, 1H, J = 8.8 Hz, J = 1.6 Hz, H_7), 7.75 (dt, 1H, J = 8.8 Hz, J = 1.6 Hz, H_6), 6.96 (d, 2H, J = 8.8 Hz, H_{18}, H_{22}), 6.77 (m, 4H, H_{12}, H_{13}, H_{15}, H_{16}), 6.69 (d, 2H, J = 8.8 Hz, H_{19}, H_{21}), 6.54 (s, 1H, H_3); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 180.3 (C_1), 170.3 (C_4), 134.2 (C_7), 131.2 (C_6), 127.1 (C_5), 125.7 (C_8), 125.2 (C_{18}, C_{22}), 124.1 (C_{13}, C_{15}), 117.1 (C_{19}, C_{21}), 116.9 (C_{12}, C_{16}), 97.1 (C_3); ESI/MS negative mode: m/z 353 (100%, $[M - H]^-$); ESI/MS positive mode: m/z 377 (100%, $[M + Na]^+$), 355 (33, $[M + H]^+$); MS^2 m/z 237 (100%, $[M + H - C_7H_8N_2]^+$), 248 (18, $[M + H - C_6H_7N_2^{\bullet}]^{\bullet+}$), 262 (13, $[M + H - C_6H_7N]^+$), 328 (8, $[M + H - HCN]^+$); HR-ESI/MS: m/z calcd for $C_{22}H_{19}N_4O$ $[M + H]^+$: 355.1553; found 355.1539.

12: red solid (4.5 mg, 29%); UV/Vis: λ_{max}(MeOH)/nm 505 (ε/dm^3 mol^{-1} cm^{-1} 5780); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 8.44 (m, 2H, H_4, $H_{4'}$), 7.63 (m, 2H, H_5, $H_{5'}$), 7.04 (s, 2H, 2H, H_1, $H_{1'}$), 6.80 (m, 8H, H_7, H_8, H_{10}, H_{11}, $H_{7'}$, $H_{8'}$, $H_{10'}$, $H_{11'}$); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 180.3 (C_2, $C_{2'}$), 170.3 (C_9, $C_{9'}$), 147.0 (C_6, $C_{6'}$), 134.7 (C_3, $C_{3'}$), 131.2 (C_5, $C_{5'}$), 126.5 (C_1, $C_{1'}$), 125.7 (C_4, $C_{4'}$), 124.2 (C_7, C_{11}, $C_{7'}$, $C_{11'}$), 116.8 (C_8, C_{10}, $C_{8'}$, $C_{10'}$); ESI/MS positive mode: m/z 339 (100%, $[M + H]^+$); MS^2 m/z 322 (8%, $[M + H - NH_3]^+$), 233 (100, $[M + H - C_6H_6N_2]^+$); HR-ESI/MS: m/z calcd for $C_{22}H_{19}N_4$ $[M + H]^+$: 339.1604; found 339.1609.

13: dark orange solid (10.2 mg, 98%); UV/Vis: λ_{max}(MeOH)/nm 460 (ε/dm^3 mol^{-1} cm^{-1} 9340); FTIR (KBr) ν_{max}/cm^{-1}: 3548, 3475, 3417, 1639, 1618, 1573, 1540, 1498, 1434, 1384, 1268, 1236, 1101, 988, 879, 853, 795, 619, 528; ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 6.86 (m, 4H, H_8, H_9, H_{11}, H_{12}), 6.75 (s, 1H, H_5), 5.66 (s, 1H, H_2), 1.91 (s, 3H, CH_3); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 188.3 (C_1), 157.1 (C_{10}), 156.1 (C_3), 153.2 (C_4), 143.4 (C_6), 141.9 (C_7), 124.4 (C_8, C_{12}), 122.8 (C_5), 116.7 (C_9, C_{11}), 100.2 (C_2), 16.6 (CH_3); ESI/MS positive mode: m/z 229 (100%, $[M + H]^+$); MS^2 m/z 212 (100%, $[M + H - OH^{\bullet}]^{\bullet+}$); HR-ESI/MS: m/z calcd for $C_{13}H_{13}N_2O_2$ $[M + H]^+$: 229.0972; found 229.0964.

14: dark orange solid (5.4 mg, 52%); UV/Vis: λ_{max}(MeOH)/nm 320 (ε/dm^3 mol^{-1} cm^{-1} 24 459); FTIR (KBr) ν_{max}/cm^{-1}: 3428, 2923, 1639, 1581, 1391, 1138, 994, 855, 620, 534;

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.06 (d, 2H, J = 8.8 Hz, H_8, H_{12}), 6.91 (d, 2H, J = 8.6 Hz, H_9, H_{11}), 6.85 (s, 1H, H_5), 6.04 (s, 1H, H_2), 2.16 (s, 3H, CH_3); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 165.8 (C_1), 159.3 (C_{10}), 150.6 (C_4), 141.4 (C_7), 136.8 (C_6), 126.7 (C_8, C_{12}), 125.4 (C_5), 117.5 (C_9, C_{11}), 92.8 (C_2), 17.4 (CH_3); ESI/MS positive mode: m/z 228 (100%, $[M + H]^+$); MS^2 m/z 211 (100%, $[M + H - OH^{\bullet}]^{\bullet+}$).

15: dark orange solid (2.8 mg, 28%); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.90 (d, 1H, J = 9.4 Hz, H_9), 7.73 (s, 1H, H_6), 7.30 (d, 1H, J = 9.2 Hz, H_8), 7.16 (s, 1H, H_1), 7.00 (s, 1H, H_4), 2.44 (s, 3H, CH_3); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 131.0 (C_9), 129.3 (C_6), 123.8 (C_8), 107.0 (C_1), 102.1 (C_4), 18.2 (CH_3); ESI/MS positive mode: m/z 226 (100%, $[M + H]^+$); MS^2 m/z = 211 (45%, $[M + H - CH_3^{\bullet}]^{\bullet+}$), 198 (28%, $[M + H - CO]^+$); HR-ESI/MS: m/z calcd for $C_{13}H_{12}N_3O$ $[M + H]^+$: 226.0975; found 226.0965.

16: purple solid (7.0 mg, 60%); UV/Vis: λ_{max}(MeOH)/nm 510 (ε/dm^3 mol^{-1} cm^{-1} 21 217); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 6.84 (d, 1H, J = 7.6 Hz, H_8), 6.79 (s, 1H, H_{12}), 6.68 (d, 1H, J = 7.6 Hz, H_9), 6.55 (s, 1H, H_5), 5.66 (s, 1H, H_2), 2.17 (s, 3H, $CH_{3\ \text{aromatic ring}}$), 1.90 (s, 3H, $CH_{3\ \text{iminic ring}}$); ^{13}C{H} NMR (400 MHz, CD_3OD): δ (ppm) = 188.6 (C_1), 170.2 (C_{10}), 151.0 (C_4), 142.5 (C_3), 139.6 (C_7), 134.9 (C_8), 126.4 (C_6), 123.0 (C_{12}), 118.3 (C_9), 116.2 (C_{11}), 117.6 (C_5), 100.1 (C_2), 18.3 ($CH_{3\ \text{aromatic ring}}$), 16.5 ($CH_{3\ \text{iminic ring}}$); ESI/MS positive mode: m/z 242 (100%, $[M + H]^+$); MS^2 227 (100%, $[M + H - CH_3^{\bullet}]^{\bullet+}$); HR-ESI/MS: m/z calcd for $C_{14}H_{16}N_3O$ $[M + H]^+$: 242.1288; found 242.1285.

17: yellow solid (3.2 mg; 27%); UV/Vis: λ_{max}(MeOH)/nm 440 (ε/dm^3 mol^{-1} cm^{-1} 3962); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.10 (d, 1H, J = 8.1 Hz, H_8), 6.64 (s, 1H, H_{12}), 6.34 (s, 1H, H_5), 6.29 (d, 1H, J = 8.1 Hz, H_9), 5.66 (s, 1H, H_2), 2.20 (s, 3H, $CH_{3\ \text{aromatic ring}}$), 1.88 (s, 3H, $CH_{3\ \text{iminic ring}}$); ^{13}C{H} NMR (400 MHz, CD_3OD): δ(ppm) = 188.1 (C_1), 170.5 (C_{10}), 156.9 (C_4), 149.1 (C_3), 144.1 (C_7), 132.0 (C_8), 123.7 (C_6), 122.9 (C_{12}), 112.7 (C_9), 108.6 (C_{11}), 108.2 (C_5), 99.8 (C_2), 20.2 ($CH_{3\ \text{aromatic ring}}$), 15.9 ($CH_{3\ \text{iminic ring}}$); ESI/MS negative mode: m/z = 241 (100%, $[M - H]^-$); MS^2 m/z = 226 (100, $[M - H - CH_4]^-$), 223 (41, $[M - H - H_2O]^-$), 199 (29, $[M - H - CH_2O]^-$); ESI/MS positive mode: m/z = 243 (100%, $[M + H]^+$); HR-ESI/MS: m/z calcd for $C_{14}H_{15}N_2O_2$ $[M + H]^+$: 243.1128; found 243.1128.

Crystal structure determination of compound 8

Single crystals of compound **8** were obtained by slow evaporation of the phosphate buffer saturated solution.

Crystal data. $C_{13}H_{13}N_3O$, M = 227.26, monoclinic, a = 13.551(1) Å, b = 3.9004(3) Å, c = 20.986(2) Å, β = 94.725(4)°, V = 1105.5(2) Å^3, T = 150(2) K, space group $P2(1)/n$, Z = 4, 10 590 reflections measured, 2710 unique (R_{int} = 0.0295), which were used in all calculations. The final R and w$R(F^2)$ were 0.0399 and 0.1021, respectively.

The X-ray diffraction data were collected using a Bruker AXS-KAPPA APEX II diffractometer with graphite-monochromated radiation (Mo Kα, λ = 0.71069 Å) at 150 K. The X-ray data were collected using the omega scans of 0.5 per frame and a full sphere of data was obtained. All the data were corrected for Lorentzian, polarization, and absorption effects using SAINT[25] and SADABS[26] programs. SIR97[27] and SHELXL-97[28] were used for structure solution and refinement, respectively. Calculations were performed using the package of programs WINGX-Version 1.80.05.[29] A full-matrix least-squares refinement on F^2 was performed on the non-hydrogen atoms with anisotropic thermal parameters. The hydrogen atoms were inserted in the idealised positions (d(C_{methyl}–H) = 0.96 Å, d(C_{rings}–H) = 0.93 Å) and were allowed to refine riding on the parent carbon atom, with U_{iso}(H) = 1.5 $U_{eq.}$ (C_{methyl}) and 1.2 $U_{eq.}$ (other C atoms); an exception for H atoms bonded to nitrogen whose positions were found in the electronic density map and were allowed to refine freely. The graphics were made using MERCURY 3.0.[21] PLATON[30] was used for determining the hydrogen bond and intermolecular interactions. Crystallographic and experimental details are summarised in Table S1 in the ESI and in Tables S2 and S3,† the bond lengths, bond angles and torsion angles, respectively. CCDC 1476627 contains the cif file for this compound.

Conclusions

We have demonstrated the ability and efficacy of CotA-laccase to oxidise, in water, three oxidation bases (1,4-PDA, 4-AP and 2,5-DAT) to the corresponding benzoquinonediimine or benzoquinoneimine species and promote the selected cross-coupling reactions with different aromatic couplers, which resulted in the formation of dimeric and trimeric indo dyes. The dinuclear and trinuclear aminoindamine, indoaniline and indonaphthol compounds were obtained in good to excellent yields and the results showed that the electron character and position of the substituents in the coupler have fundamental importance in the occurrence of the hetero-coupling reactions.

The proposed mechanistic pathway for the dye formation involves an initial oxidation of the primary intermediates by laccase and further reactions with amine or phenolic couplers to give the leuco-indo dyes and, upon oxidation, the corresponding indamine or indoaniline derivatives.

Thus, the laccase-based approach developed herein depicts a greener alternative route for the preparation of indo dyes and contributes to improved sustainability for the synthesis of this important class of dyes.

Acknowledgements

This work was supported by FCT- Fundação para a Ciência e Tecnologia through projects PTDC/BIO/72108/2006, RECI/QEQ-QIN/0189/2012 and REM2013 and R&D units UID/QUI/00100/2013 and UID/Multi/04551/2013 (GreenIT). The authors thank Joana Bernardo for help in preliminary studies and Dr M. Conceição Oliveira for the helpful discussions about the ESI-MS results.

Notes and references

1 O. J. X. Morel and R. M. Christie, *Chem. Rev.*, 2011, **111**, 2537 and references cited therein.
2 J. F. Corbett, *Dyes Pigm.*, 1999, **41**, 127.
3 (*a*) J. F. Corbett, *J. Soc. Cosmet. Chem.*, 1973, **24**, 103 and references cited therein; (*b*) K. C. Brown and J. F. Corbett, *J. Soc. Cosmet. Chem.*, 1979, **30**, 191 and references cited therein.
4 (*a*) J. F. Corbett, *J. Chem. Soc., Perkin Trans. 2*, 1972, 539; (*b*) K. C. Brown, J. F. Corbett and R. Labinson, *J. Chem. Soc., Perkin Trans. 2*, 1978, 1292.
5 K. C. Brown and J. F. Corbett, *J. Chem. Soc., Perkin Trans. 2*, 1979, 304.
6 J. F. Corbett, *J. Chem. Soc. B*, 1969, 827.
7 (*a*) Y. Tsujino, Y. Yokoo and K. Sakato, *J. Soc. Cosmet. Chem.*, 1991, **42**, 273; (*b*) J. Fu, G. S. Nyanhongo, G. M. Gubitz, A. Cavaco Paulo and S. Kim, *Biocatal. Biotransform.*, 2012, **30**, 125 and references cited herein.
8 (*a*) J. R. Jeon, E. J. Kim, K. Murugesan, H. K. Park, Y. M. Kim, J. H. Kwon, W. G. Kim, J. Y. Lee and Y. S. Chang, *Microbiol. Biotechnol.*, 2010, **3**, 324; (*b*) J. Polak and A. Jarosz-Wilkolazka, *Process Biochem.*, 2012, **47**, 1295 and references cited herein.
9 For a review on laccases see: (*a*) S. Witayakran and A. J. Ragauskas, *Adv. Synth. Catal.*, 2009, **351**, 1187; (*b*) L. Rulisek and U. Ryde, *Coord. Chem. Rev.*, 2013, **257**, 445 and references therein.
10 (*a*) C. M. Clouthier and J. N. Pelletier, *Chem. Soc. Rev.*, 2012, **41**, 1585; (*b*) J. R. Jeon and Y. S. Chang, *Trends Biotechnol.*, 2013, **31**, 335; (*c*) A. Mikolasch and F. Schauer, *Appl. Microbiol. Biotechnol.*, 2009, **82**, 605.
11 A. C. Sousa, L. O. Martins and M. P. Robalo, *Adv. Synth. Catal.*, 2013, **355**, 2908.
12 (*a*) P. Galletti, F. Funiciello, R. Soldati and D. Giacomini, *Adv. Synth. Catal.*, 2015, **357**, 1840; (*b*) A. Wells, M. Teria and T. Eve, *Biochem. Soc. Trans.*, 2006, **34**, 304; (*c*) A. Kollmann, F.-D. Boyer, P.-H. Ducrot, L. Kerhoas, C. Jolivalt, I. Touton, J. Einhorn and C. Mougin, *Appl. Microbiol. Biotechnol.*, 2005, **68**, 251.
13 A. Kunamneni, F. J. Plou, A. Ballesteros and M. Alcalde, *Recent Pat. Biotechnol.*, 2008, **2**, 10 and references cited herein.
14 (*a*) B. Lalleman, P. Choisy and A. Lagrange, (L'Oreal), *WO* 2010057854A2, 2010; (*b*) G. Lang and J. Cotteret, (L'Oreal), *US* 006471730B1, 2002; (*c*) G. Lang and J. Cotteret, (L'Oreal), *US* 7175673B2, 2007; (*d*) G. Lang and J. Cotteret, (L'Oreal), *US* 65513159B1, 2003.
15 (*a*) A. C. Sousa, M. C. Oliveira, L. O. Martins and M. P. Robalo, *Green Chem.*, 2014, **16**, 4127; (*b*) A. C. Sousa, M. F. M. M. Piedade, L. O. Martins and M. P. Robalo, *Green Chem.*, 2015, **17**, 1429.
16 L. O. Martins, C. M. Soares, M. M. Pereira, M. Teixeira, T. Costa, G. H. Jones and A. O. Henriques, *J. Biol. Chem.*, 2002, **277**, 18849.
17 T. A. Enache and A. M. Oliveira-Brett, *J. Electroanal. Chem.*, 2011, **655**, 9.
18 P. Durão, Z. Chen, A. T. Fernandes, P. Hildebrandt, D. H. Murgida, S. Todorovic, M. M. Pereira, E. P. Melo and L. O. Martins, *J. Biol. Inorg. Chem.*, 2008, **13**, 183.
19 (*a*) J. F. Corbett, S. Pohl and I. Rodriguez, *J. Chem. Soc., Perkin Trans. 2*, 1975, 728; (*b*) J. F. Corbett, *J. Chem. Soc. B*, 1969, 213.
20 F. Bruyneel, O. Payen, A. Rescigno, B. Tinant and J. Marchand-Brynaert, *Chem. – Eur. J.*, 2009, **15**, 8283.
21 C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, *J. Appl. Crystallogr.*, 2008, **41**, 466.
22 A. P. Avdeenko, S. A. Konovalova, A. G. Sergeeva, R. I. Zubatyuk, G. V. Palamarchuk and O. V. Shishkin, *Russ. J. Org. Chem.*, 2009, **44**, 1765.
23 (*a*) J. F. Corbett, *J. Chem. Soc. B*, 1969, 818; (*b*) J. F. Corbett, *J. Chem. Soc. B*, 1969, 823.
24 L. Pereira, A. V. Coelho, C. A. Viegas, M. M. Santos, M. P. Robalo and L. O. Martins, *J. Biotechnol.*, 2009, **139**, 68.
25 *SAINT+, release 6.22*, Bruker Analytical Systems, Madison, WI, 2005.
26 *SADABS*, Bruker Analytical Systems, Madison, WI, 2005.
27 A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori and R. Spagna, *J. Appl. Crystallogr.*, 1999, **32**, 115.
28 G. M. Sheldrick, *Acta Crystallogr., Sect. A: Fundam. Crystallogr.*, 2008, **64**, 112.
29 L. J. Farrugia, *J. Appl. Crystallogr.*, 1999, **32**, 837.
30 A. L. Spek, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2009, **65**, 148.

