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Enhanced Biocatalytic Performance of Bacterial Laccase from *Streptomyces sviceus*: Application in the Michael Addition Sequence Towards 3-Arylated 4-Oxochromanes

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A fast and efficient laccase-catalysed oxidation/Michael addition sequence is performed using the bacterial laccase Ssl 1 from *Streptomyces sviceus* under basic conditions to provide a new class of 3-arylated 4-oxochromanes. This approach has advantages compared to previous biocatalytic arylation protocols that use fungal laccases under slightly acidic conditions to allow a significant decrease in reaction time with improved yields and maintained regio- and diastereoselectivity. Furthermore, a successful diastereoselective, consecutive one-pot approach with the use of a hydrogenation flow system combined with the laccase-catalysed arylation was performed. Finally, the general utility of this enzyme as a superior biocatalyst for Michael additions using several nucleophiles was demonstrated. The corresponding starting material was obtained in a straightforward esterification/hydrogenation process with the latter accomplished by using the flow system.

Introduction

Laccases (benzenediol: oxygen oxidoreductase EC 1.10.3.2.) belong to the family of multi-copper oxidases that contain four copper ions. They catalyse the one-electron oxidation of the substrate (through the T1 copper ion) and transfer the abstracted electrons to molecular oxygen, which is reduced to water (at the trinuclear cluster of one T2 copper and two T3 copper ions).[1] Suitable substrates such as phenols, catechols, hydroquinones or aromatic amines are oxidised readily by laccases in the presence of atmospheric oxygen.[2] As a result of their compatibility towards organic solvents, commercial availability and mild reaction conditions, fungal laccases represent a green alternative as an oxidative catalyst for arylation reactions in organic synthesis.[3] Despite their high activities caused by redox potentials between 0.5–0.8 mV versus the normal hydrogen electrode (NHE),[4] the use of fungal laccases requires neutral or acidic conditions (depending on the substrate used).[5a] In basic media, fungal laccases usually suffer from a strong inhibition of the electron transfer between the copper centres essential for its biocatalytic activity caused by the binding of hydroxide ions at the trinuclear cluster.[5b] However, alkalophilic fungal laccases are highly desirable biocatalysts in, for example, the pulp and paper industry and organic synthesis,[5c] and examples of laccases adapted successfully to high pH values by directed evolution are well documented.[5d,e]

In our previous protocols, laccase-catalysed oxidation/arylation sequences were performed successfully under acidic conditions. However, one question that needs to be asked is whether we could significantly reduce general reaction times for these conversions that vary from 18–72 h[3c,6] while maintaining yields and selectivities. The circumstance that Michael additions require a base or basic reaction conditions to activate the reaction donor to proceed well and the fact that biocatalytic performances under basic reaction conditions are still elusive leaves a gap for the optimisation of this type of reaction.[7]

Recently, we cloned and characterised a laccase from *Streptomyces sviceus* (Ssl 1) with an activity profile towards the oxidation of several substrates at different pH values. Ssl 1 showed an outstanding activity for the oxidation of 2,6-dimethoxyphenol (**1**) at pH 9, guaiacol (**2**) at pH 8 and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (**3**) at pH 4. Although it originates from a mesophilic organism, Ssl 1 demonstrates high stability at elevated temperatures ($t_{1/2,60\,^{\circ}C} = 88$ min) and in a wide pH range (pH 5.0–11.0). A number of detergents and organic co-solvents do not affect Ssl 1 stability.[8] As a result of the electronic and steric similarity of catechol (**4**) to **1** and **2**, a basic pH value seems to be promising for its oxidation and possible subsequent 1,4-addition using the new laccase. As *o*-quinone, the corresponding oxidised analogue of **4**, is not commercially available because of its high reactivity, the laccase-catalysed in situ formation allows its utilisation as an attractive arylating agent (Figure 1).

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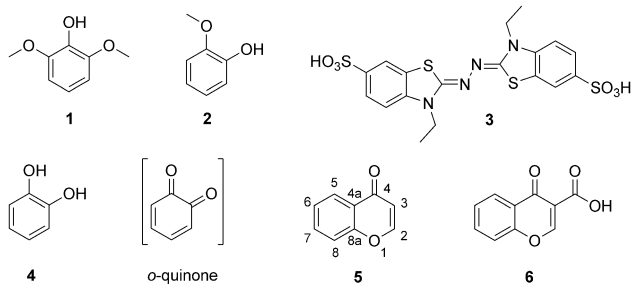
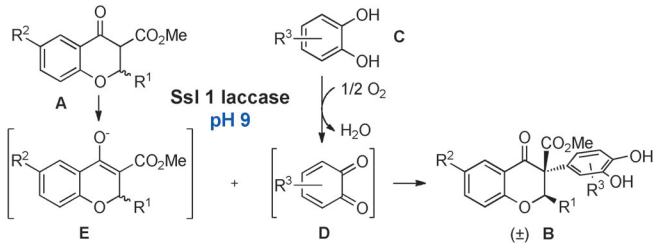


Figure 1. Suitable substrates for the Ssl 1-catalysed oxidation (**1**–**4**) as well as pharmacologically promising chromone scaffolds (**5** and **6**).

As we are also interested in the development of active scaffolds for medicinal purposes by establishing biocatalytic methods for the synthesis of new C–C bonds, the chromones[9] caught our attention. Chromones **5** (4*H*-chromen-4-one, 4*H*-1-benzopyran-4-one) are heterocyclic planar compounds that are highly distributed in nature, especially in the plant kingdom (Figure 1).[10] They regularly possess interesting biological activities and were used, for example, as antidiabetics and cardiovascular agents,[11] calcium antagonists,[12] diuretics[13] and antioxidants.[14] One basic scaffold of this class is 4-oxo-4*H*-chromene-3-carboxylic acid (**6**), which acts as an irreversible selective human monoamine oxidase-B (*h*MAO-B) inhibitor for the treatment of Parkinson's disease. In general, structure–activity relationship (SAR) studies performed by Borges and co-workers[15] showed that chromone derivatives with substituents in the 3-position act as MAO-B inhibitors, with IC_{50} values down to the nanomolar range (Figure 1).

Although examples of alkalophilic bacterial laccases and "engineered" fungal laccases are known,[5d–h] to the best of our knowledge, biocatalytic Michael-type reactions that use alkalophilic laccases under basic conditions have not been investigated yet.

Herein, we report the biocatalytic performance of the laccase Ssl 1 towards the 3-arylation of 3-substituted 4-oxochromanes (**A**), which leads to potentially pharmacologically interesting entities (**B**) that bear an all-carbon quaternary stereogenic centre. As the key step, the laccase-catalysed oxidation of commercially available catechols (**C**) to form the highly reactive *o*-quinone analogue (**D**) was performed at pH 9 followed by a regio- and diastereoselective 1,4-addition with the activated donor (**E**) as a soft nucleophile (Scheme 1).

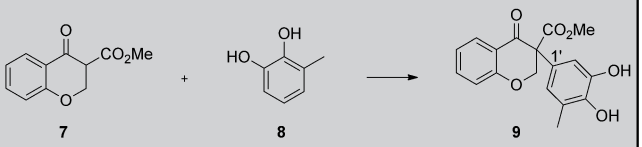


Scheme 1. Laccase-catalysed 3-arylation of 3-substituted 4-oxochromanes **A**.

Results and Discussion

To demonstrate the utility of the laccase Ssl 1 for oxidative C–C couplings in organic synthesis, we started our investigation by performing an arylation sequence that employed the chromanone ester **7** prepared previously by esterification[16] and flow hydrogenation[17] from the corresponding chromone (Supporting Information). As the initial model reaction, this ester **7** and 3-methylcatechol (**8**) as the arylating agent were chosen as substrates. The oxidation of catechol **8** with the new laccase was expected to furnish the Michael acceptor, and the chromanone **7** acted as the nucleophile (Table 1). Compared to a previous approach that used Pb-mediated arylations of chromanone motifs,[18] we present an environmentally benign alternative. In a first attempt to prove the general suitability of the chromanone ester **7**, the optimised reaction conditions from a previous protocol were considered in which the commercially available fungal laccase from *Agaricus bisporus* was used under slightly acidic conditions.[6b] Under acidic conditions, we could instantly isolate the desired product as a single regioisomer as determined by ^{1}H NMR spectroscopy (62%, Table 1, entry 1). However, full conversion was not achieved even after 48 h of reaction time. As anticipated, if the fungal laccase is used under basic conditions, its activity towards the oxidation of **8** decreased significantly, which was indicated by a poor yield (31%, entry 2). Even with an increased nucleophilicity of the nucleophile under basic conditions, the limiting factor seemed to be the low efficiency of catechol oxidation. We started the investigation with the bacterial laccase Ssl 1 from *Streptomyceus svi-*

Table 1. Optimisation of the laccase-catalysed synthesis of chromanone **9**.[a]



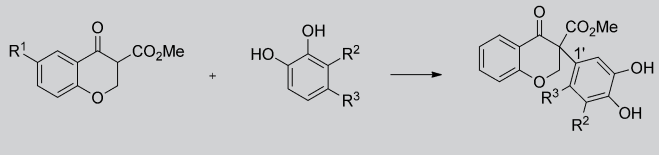
Entry	Laccase source	Buffer/solvent (ratio)	Unit [U]	Reaction time [h]	Yield [%][e]
1	*A. bisporus*[b]	buffer/MeCN (2:1)	15[c]	48	62
2	*A. bisporus*	buffer/MeCN (2:1)	15[c]	48	31
3	Ssl 1	buffer/MeCN (4:1)	16[d]	3	67
4	Ssl 1	buffer/MeCN (4:1)	8	4	86
5	Ssl 1	buffer/MeOH (4:1)	8	4	72
6	Ssl 1	buffer/MeCN (4:1)	4	3	39
7	Ssl 1	buffer	8	5	21
8	Ssl 1	buffer/MeCN (1:1)	8	24	70
9	Ssl 1	buffer/MeCN (2:1)	8	3	89
10	Ssl 1	buffer/MeOH (2:1)	8	3	71
11	Ssl 1	buffer/THF (2:1)	8	3	52

[a] Reaction conditions: 0.25 mmol **7**, 1.2 equiv. **8**, 3 mL solvent, 22 °C, buffer: 50 mM Gly/NaOH, pH 9.0. [b] Reaction performed in a KP_i-buffer of pH 6.0. [c] Activity as provided by the supplier with catechol **4** as substrate at pH 6 (6.8 U mg^{-1}). Activity determined with **3** at pH 5.5 (0.9 U mg^{-1}). [d] Activity determined with **3** at pH 4 (2.5 U mg^{-1}). [e] Yields of isolated products.

ceus[8] and used a basic pH of 9 in all cases for its optimal activity towards catechol oxidation. Acetonitrile was chosen as a co-solvent. The Ssl 1 was stored in 50 mM KP_i-buffer (potassium phosphate buffer: KH_2PO_4/K_2HPO_4) of pH 7.5. The determined volumetric activity of the laccase solution was 40 U/mL towards the oxidation of **3** at pH 4. For the first attempt, 16 U of Ssl 1 was used, and the desired product **7** was formed in a good yield (67%, entry 3). Again, as expected, only one regioisomer was isolated because of the favoured coupling at the 1′-position. Remarkably, the reaction time could be reduced significantly to 3 h for full conversion. In contrast to the first approaches that used the fungal laccase, both the oxidation of the catechol as well as the increase of nucleophilicity of the chromanone were addressed in the second approach that employed the bacterial laccase, which thus accelerated the desired reaction. If the amount of Ssl 1 was decreased to 8 U, the yield could be further increased and the reaction time was extended slightly to 4 h (86%, entry 4). We suggest that this outcome could be explained by a possible shift of the pH value in the reaction mixture. As we applied this laccase directly as a solution in 50 mM KP_i-buffer with a pH of 7.5 and diluted it afterwards with the Gly/NaOH-buffer (pH 9) to the desired ratio, a higher amount of laccase could lead to more acidic reaction conditions and consequently to a decrease of the rate. If we used methanol as a co-solvent under the same conditions, the outcome was similar to that of the previous approach. With the same ratio of buffer to co-solvent, full conversion was achieved after 4 h with a good yield (72%, entry 5). A further decrease of the amount of enzyme to 4 U did not have a positive effect; the reaction was incomplete after 3 h and the yield decreased significantly (39%, entry 6). As anticipated, if we used only buffer as the reaction medium, the amount of isolated product was very low (21%, entry 7). The solubility of **7** was very poor in water only. If we increased the amount of co-solvent to an equal ratio with the buffer, a good yield was achieved (70%, entry 8), but the reaction time until full conversion was increased to 24 h. However, the laccase showed a high tolerance to organic co-solvents even at high ratios. Fortunately, if we used a ratio of 2:1 buffer to acetonitrile we obtained full conversion after 3 h and a very good yield (89%, entry 9). If the ratio was shifted to 4:1 towards buffer, the outcome of the reaction was nearly the same in terms of yield (86%, entry 4), but the reaction time was increased slightly (4 h). Finally, with this optimised ratio of buffer to co-solvent in hand, we re-evaluated the organic solvent and used methanol and THF as alternatives. The result for methanol did not change compared to the previous approach (71%, entry 10 vs. 72%, entry 5), but the shift towards a higher ratio of buffer resulted in an increased reaction time of 4 h. With THF as a co-solvent, the yield of isolated product decreased further (52%, entry 11). In summary, the bacterial laccase Ssl 1 (8 U) and a ratio of 2:1 buffer/acetonitrile at a pH of 9 were proven to be the best reaction conditions for this arylation (89%, entry 9). The commercially available fungal laccase could not perform in this approach satisfactorily under acidic conditions. Moreover, Ssl 1 laccase showed an outstanding tolerance towards organic co-solvents as well as short reaction times and complete regioselectivity. As a result of solubility issues, the use of organic co-solvents was mandatory for the successful outcome of these approaches.

Next, we investigated the scope of the reaction. For this purpose, commercially available catechols substituted in the 3′- and 4′-position as well as chromanone esters prepared previously (Supporting Information) substituted in the 2′- and 6′-position were considered (Table 2). First, we used **7** and 3′-substituted catechols such as 3-methoxycatechol (**10**). The desired product **11** was obtained in a good yield after 4 h (64%, Table 2, entry 1). As expected, coupling only occurred in the 5′-position of the catechol because of the electron-donating group in the 3′-position that makes the *ortho* position unattractive for nucleophilic attack. If the unsubstituted catechol **4** was used, a slightly better yield was obtained after 8 h reaction time for full conversion (70%, entry 2). The use of 4-methylcatechol (**13**) gave the expected single regioisomer **14** after 7 h in a good yield (66%, entry 3). The 4′-position of the catechol **13** was clearly not available for the coupling. Next, the chromanone scaffold was varied, and 6′-methylchromanone ester **15** was used first. A very good yield was obtained after 8 h reaction time if unsubstituted catechol **4** was used as the acceptor (85%, entry 4). Last, 6′-fluorochromanone ester **17** was reacted selectively with **8**, and within 3 h regioisomer **18** was formed in good yield (74%, entry 5).

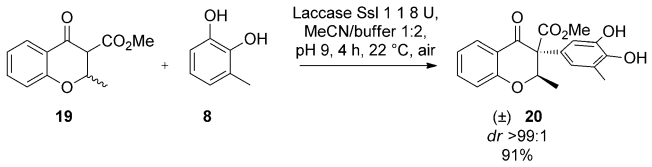
Table 2. Laccase-catalysed oxidation/Michael addition sequence.[a]



Entry	R^1	R^2	R^3	Ester	Catalyst	Product	Reaction time [h]	Yield [%][b]
1	H	MeO	H	**7**	**10**	**11**	4	64
2	H	H	H	**7**	**4**	**12**	8	70
3	H	H	Me	**7**	**13**	**14**	7	66
4	Me	H	H	**15**	**4**	**16**	8	85
5	F	Me	H	**17**	**8**	**18**	3	74

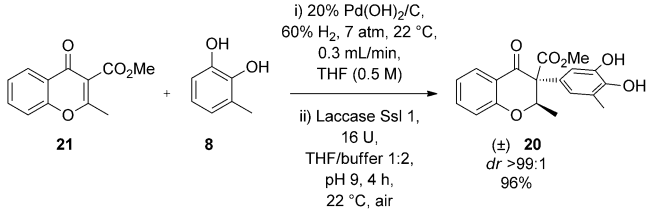
[a] Reaction conditions: 0.25 mmol ester, 1.2 equiv. catechol, 8 U (40 U mL^{-1}) laccase from *Streptomyces sviceus*, 3 mL solvent with MeCN/buffer 1:2, 22 °C, buffer: 50 mM Gly/NaOH, pH 9.0. [b] Yields of isolated products.

We were intrigued to investigate the simple diastereoselectivity of the coupling reaction and hence introduced the 2-methyl-substituted chromanone **19** (Supporting Information) to the reaction conditions. To our delight, if racemic ester **19** and **8** were used, complete regio- and diastereoselectivity of >99:1 (determined by chiral HPLC) was achieved with a very good yield of 91% (Scheme 2). For both racemic diastereoisomers **19** the arylation occurred in the *anti* position with respect to the methyl group in the 2′-position, which is in full agreement with a related report.[3c]



Scheme 2. Diastereoselective arylation of racemic 2′-methylchromanone **19**.

As both the reduction of 2-methylchromene **21** under flow conditions (95%, Table S2, entry 4) and the laccase-catalysed arylation of chromanone ester **7** (52%, Table 1, entry 11) could be performed using THF as a solvent, an additional consecutive one-pot approach to combine these two reaction steps was anticipated. Again 2-methylchromene **21** was reduced in under flow conditions and the formed ester **19** was introduced directly into a flask that contained Ssl 1 solution in buffer to which catechol **8** was added by using a syringe pump (Scheme 3), a set-up that was successfully established pre-

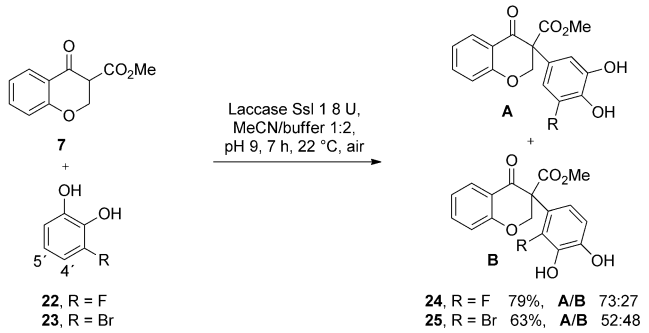


Scheme 3. Diastereoselective synthesis of ester **20** in a consecutive one-pot approach.

viously.[6b] However, as the outcome of the arylation in THF was not satisfactory in the first place (vide supra), the amount of enzyme was doubled (16 U instead of 8 U) and the flow rates were adjusted. We were pleased to find that despite the higher dilution (recommended concentration for the reduction in flow conditions: 0.5 M) for the enzyme-catalysed transformation (a factor of 6), the results matched the batch reactions. The regio- and diastereoselectivity was excellent as was the yield (96%, diastereomeric ratio (*dr*) > 99:1, 4 h; Scheme 3).

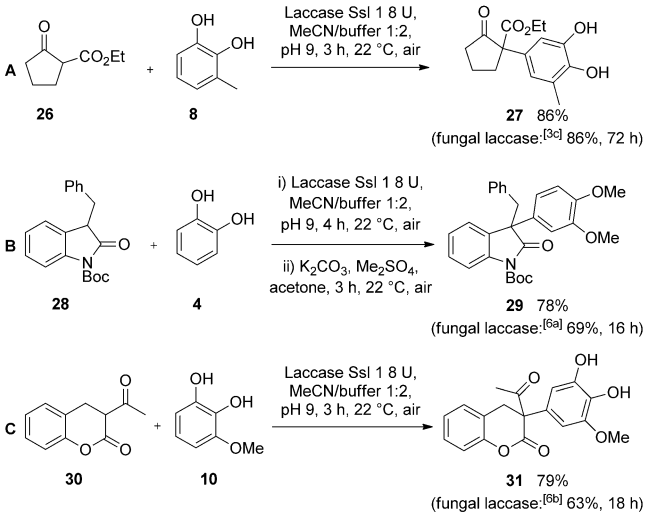
Finally, the substituents of the catechol moiety were altered. For this approach, 3-fluorocatechol (**22**) and 3-bromocatechol (**23**) were utilised as arylation agents for chromanone **7**. After 7 h reaction time, the desired products were isolated in good to very good yields of 79% for the fluorine derivative **24** and 63% for the bromine derivative **25** (Scheme 4). As expected, both possible regioisomers were obtained. In the case of the fluorine-containing scaffold **24**, the ratio of regioisomers was determined to be 73:27 in favour of **24 A** (arylation in the 5′-position). In contrast, if we used bromocatechol **23**, the ratio decreased to 52:48 albeit slightly in favour of **25 A**. Nevertheless, the outcome was surprising as laccase-catalysed arylations with **23** were described previously to provide coupling exclusively in the 4′-position.[19] Clearly, the choice of nucleophile also influences the outcome of the regioselectivity significantly.

To demonstrate the general superiority of the laccase Ssl 1 as a new biocatalyst for oxidative C–C couplings in organic



Scheme 4. Laccase-catalysed sequence using 3-halocatechols **22** and **23**.

synthesis, the scope was broadened by using alternative nucleophiles from our protocols developed previously in which commercially available fungal laccases were applied. As a first comparison, the β-ketoester **26** was employed as a suitable nucleophile again using **8** as the arylating agent. Previously, the reaction was performed successfully, and the desired product **27** was obtained in a very good yield using the fungal laccase from *Pleurotus ostreatus* (86% after 72 h). Almost complete regioselectivity was achieved with a ratio of 96:4 toward the arylated β-ketoester **27**.[3c] The use of the optimised reaction conditions and Ssl 1 gave a comparable yield and the selectivity was increased to provide the 5′-coupling product exclusively (86%; Scheme 5 A). However, the most pleasing



Scheme 5. Improved laccase-catalysed arylation protocols.

aspect was that the reaction time could be decreased drastically from 72 to 3 h. Scale-up was unproblematic, and to our delight, we were able to isolate the desired arylated β-ketoester **27** as major regioisomer on a 5.0 mmol scale with comparable yield and selectivity (85%; see Supporting Information). Similar results were obtained if the protected oxindole **28** was used as the coupling partner for **4** (Scheme 5 B). With the acidophilic fungal laccase, product **29** was synthesised in two steps in

69% yield; the oxidative laccase-catalysed coupling took 16 h.[6a] With the use of Ssl 1, not only was the reaction time reduced significantly to 4 h but also the yield could be increased to 78% over two steps towards oxindole **29** (Scheme 5B). Again, because of purification issues, the methylation step was necessary for this substrate. As in the last example, we considered the structural motif of coumarins as a suitable coupling partner and in particular the acetyl-substituted 3,4-dihydrocoumarin **30** because the lowest yield for this scaffold was observed previously (63% after 18 h).[6b] The use of Ssl 1 to synthesise derivative **31** again improved the yield and decreased the reaction time (79% after 3 h; Scheme 5C).

Conclusions

The synthesis of a new compound class of 3-arylated 4-oxochromanes was established successfully by an efficient three-step sequence, which gave excellent yields and regioselectivities. The key to success was the use of the bacterial Ssl 1 laccase from *S. sviceus* for the environmentally benign oxidation with aerial oxygen. It showed an outstanding performance in terms of reaction time, yield and tolerance towards organic solvents, which thus proved its superiority compared to fungal laccases. Moreover, complete simple diastereoselectivity was already induced by an additional methyl group. High yields for the single-step transformations also rendered a convenient consecutive one-pot approach possible, which also furnished the pharmacologically promising scaffold in excellent yield and diastereoselectivity. These findings consolidate the Ssl 1 laccase as a valuable biocatalyst for Michael additions. Its catalytic properties enabled us to perform these oxidative C–C couplings in a highly efficient manner under basic conditions for the first time.

Experimental Section

Representative procedures

Reductions under flow conditions: Methyl 4-oxochromane-3-carboxylate (7)

The H-Cube Pro flow system[17] by ThalesNano was equipped with a 20% $Pd(OH)_2/C$ (30 mm) catalyst cartridge and absolute THF and the conditions (7 atm, 22 °C, 40% H_2, 0.3 mL min^{-1}) were entered. Methyl 4-oxo-4*H*-chromene-3-carboxylate (1.20 g, 5.89 mmol) was dissolved in absolute THF (120 mL). After the flow system had stabilised the desired conditions, the reduction was started, and the dead volumes of the system (2.59 mL) and of the catalyst cartridge (0.19 mL) were taken into consideration. The crude product solution was evaporated and purified using flash column chromatography (PE/EtOAc 75:25). Compound **7** was obtained as a pale-rose solid (86%, 5.04 mmol, 1.04 g). Mp 58 °C; R_f = 0.60 (PE/EtOAc 60:40); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 11.95 (br s, 1H, OH)*, 7.93 (dd, 1H, $^3J_{5,6}$ = 7.9 Hz, $^4J_{5,7}$ = 1.7 Hz, 5-H), 7.66 (dd, 1H, $^3J_{5,6}$ = 7.7 Hz, $^4J_{5,7}$ = 1.7 Hz, 5-H)*, 7.51 (ddd, 1H, $^3J_{7,8}$ = 8.7 Hz, $^3J_{7,6}$ = 7.2 Hz, $^4J_{7,5}$ = 1.8 Hz, 7-H), 7.32 (ddd, 1H, $^3J_{7,8}$ = 8.2 Hz, $^3J_{7,6}$ = 7.3 Hz, $^4J_{7,5}$ = 1.7 Hz, 7-H)*, 7.06 (ddd, 1H, $^3J_{6,5}$ = 7.9 Hz, $^3J_{6,7}$ = 7.2 Hz, $^4J_{6,8}$ = 1.1 Hz, 6-H), 7.00 (ddd, 1H, $^3J_{6,5}$ = 7.7 Hz, $^3J_{6,7}$ = 7.3 Hz, $^4J_{6,8}$ = 1.1 Hz, 6-H)*, 6.99 (dd, 1H, $^3J_{8,7}$ = 8.7 Hz, $^4J_{8,6}$ = 1.1 Hz, 8-H), 6.87 (dd, 1H, $^3J_{8,7}$ = 8.2 Hz, $^4J_{8,6}$ = 1.1 Hz, 8-H)*, 4.96 (s, 2H, 2-H)*, 4.81 (dd, 1H, $^2J_{2a,2b}$ = 11.6 Hz, $^3J_{2a,3}$ = 8.2 Hz, 2a-H), 4.63 (dd, 1H, $^2J_{2b,2a}$ = 11.6 Hz, $^3J_{2b,3}$ = 4.4 Hz, 2b-H), 3.83 (s, 3H, CO_2CH_3)*, 3.79 (s, 3H, CO_2CH_3), 3.75 ppm (dd, 1H, $^3J_{3,2a}$ = 8.2 Hz, $^3J_{3,2b}$ = 4.4 Hz, 3-H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 186.87 (C-4), 167.66 (CO_2CH_3), 162.82 (CO_2CH_3)*, 161.36 (C-8a), 157.66 (C-4)*, 136.44 (C-7), 133.07 (C-7)*, 127.64 (C-5), 124.56 (C-5)*, 124.55 (C-8a)*, 121.86 (C-6), 121.48 (C-6)*, 120.43 (C-4a), 118.24 (C-4a)*, 117.92 (C-8), 116.45 (C-8)*, 91.73 (C-3)*, 68.15 (C-2), 63.76 (C-2)*, 52.79 (C-3), 52.46 (CO_2CH_3), 51.69 ppm (CO_2CH_3)* (*signals from the corresponding enol form): IR (ATR): $\tilde{\nu}$ = 2955, 2921, 1737, 1725, 1682, 1457, 1329, 761 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{11}H_{10}O_4Na^+$ 229.04713 [*M*+Na$^+$]; found 229.04710.

Laccase-catalysed arylation: Methyl 3-(3,4-dihydroxyphenyl)-4-oxochromane-3-carboxylate (12)

Chromanone **7** (51.6 mg, 0.25 mmol) and catechol **4** (33.1 mg, 0.30 mmol) were dissolved in acetonitrile (1.00 mL) and buffer (1.80 mL; 50 mmol Gly/NaOH, pH 9). The laccase Ssl 1 (0.20 mL, 8 U) was added dropwise to the vigorously stirred mixture, and stirring was continued for 8 h at 22 °C under air. The reaction was quenched by the addition of 1 M HCl (1–2 drops), EtOAc (5.00 mL) and water (5.00 mL). The layers were separated and the aqueous phase was extracted with EtOAc three times. The combined organic layer was washed with brine, dried with $MgSO_4$ and filtered, and the solvent was evaporated under reduced pressure. The crude product was purified using flash column chromatography (PE/EtOAc 70:30) to provide product **12** as a yellow-brown viscid solid (70%, 0.17 mmol, 54.7 mg). Mp 128 °C; R_f = 0.11 (PE/EtOAc 70:30); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.95 (dd, 1H, $^3J_{5,6}$ = 8.0 Hz, $^4J_{5,7}$ = 1.8 Hz, 5-H), 7.48 (ddd, 1H, $^3J_{7,8}$ = 8.6 Hz, $^3J_{7,6}$ = 7.1 Hz, $^4J_{7,5}$ = 1.8 Hz, 7-H), 7.03 (ddd, 1H, $^3J_{6,5}$ = 8.1 Hz, $^3J_{6,7}$ = 7.1 Hz, $^4J_{6,8}$ = 1.1 Hz, 6-H), 6.94 (dd, 1H, $^3J_{8,7}$ = 8.4 Hz, $^4J_{8,6}$ = 1.1 Hz, 8-H), 6.81 (d, 1H, $^4J_{2',6'}$ = 2.3 Hz, 2′-H), 6.79 (d, 1H, $^3J_{5',6'}$ = 8.4 Hz, 5′-H), 6.72 (dd, 1H, $^3J_{6',5'}$ = 8.4 Hz, $^4J_{6',2'}$ = 2.3 Hz, 6′-H), 5.83 (s, 1H, OH), 5.64 (s, 1H, OH), 5.04 (d, 1H, $^2J_{2a,2b}$ = 12.0 Hz, 2a-H), 4.87 (d, 1H, $^2J_{2b,2a}$ = 12.0 Hz, 2b-H), 3.76 ppm (s, 3H, CO_2CH_3); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 189.20 (C-4), 169.35 (CO_2CH_3), 160.86 (C-8a), 144.11 (C-3′), 143.73 (C-4′), 136.45 (C-7), 128.19 (C-5), 124.91 (C-1′), 121.91 (C-6), 120.18 (C-6′), 120.08 (C-4a), 117.83 (C-8), 115.46 (C-2′), 115.28 (C-5′), 71.83 (C-2), 61.64 (C-3), 53.32 ppm (CO_2CH_3); IR (ATR): $\tilde{\nu}$ = 3396, 2955, 1727, 1679, 1604, 1232, 1209, 1037, 757 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{17}H_{15}O_6^+$ [*M*+H$^+$] 315.08631; found 315.08629.

Consecutive one-pot approach: Methyl 3-(3,4-dihydroxy-5-methylphenyl)-2-methyl-4-oxochromane-3-carboxylate (20)

The H-Cube Pro flow system[17] by ThalesNano was equipped with a 20% $Pd(OH)_2/C$ (30 mm) catalyst cartridge and absolute THF. The reduction conditions (7 atm, 24 °C, 60% H_2, 0.3 mL min^{-1}) were entered. Methyl 2-methyl-4-oxo-4*H*-chromene-3-carboxylate (**21**) (54.5 mg, 0.25 mmol) was dissolved in absolute THF (5.00 mL). 3-Methylcatechol (**8**) (38.1 mg, 0.30 mmol) was dissolved in absolute THF (1.00 mL) and taken up into a syringe (to a volume of 3.00 mL). A syringe pump was prepared and equipped with the syringe. Its flow rate was adjusted to that of the flow system (0.07 mL min^{-1}). The laccase Ssl 1 (0.40 mL, 16 U) was dissolved in buffer (12.0 mL; 50 mmol Gly/NaOH, pH 9) in a round-bottomed flask (100 mL) equipped with a stirring bar. After the flow system had stabilised the desired conditions, the reduction was started. The dead volumes of the system (2.59 mL) and of the catalyst cartridge (0.19 mL) were considered. Simultaneously, the syringe pump was started, and catechol **8** and the crude reagent solution from the flow system were introduced into the flask that contained

the laccase. The reaction was stirred vigorously for 4 h at 22 °C under air. The mixture was acidified with 1 M HCl (1–2 drops) and quenched with EtOAc (10.0 mL) and water (10.0 mL). The layers were separated, and the aqueous phase was extracted with EtOAc three times. The combined organic layer was washed with brine and dried with $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, and the crude product was purified using flash column chromatography (PE/EtOAc 80:20). An orange viscid solid (96%, 0.24 mmol, 78.8 mg) was obtained for the *anti* diastereoisomer **20**.[3c] $R_f = 0.10$ (PE/EtOAc 80:20); $R_f = 0.22$ (PE/EtOAc 70:30); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.95$ (dd, 1H, $^3J_{5,6} = 8.0$ Hz, $^4J_{5,7} = 1.7$ Hz, 5-H), 7.49 (ddd, 1H, $^3J_{7,8} = 8.7$ Hz, $^3J_{7,6} = 7.1$ Hz, $^4J_{7,5} = 1.8$ Hz, 7-H), 7.04 (ddd, 1H, $^3J_{6,5} = 8.1$ Hz, $^3J_{6,7} = 7.2$ Hz, $^4J_{6,8} = 1.1$ Hz, 6-H), 6.98 (dd, 1H, $^3J_{8,7} = 8.5$ Hz, $^4J_{8,6} = 1.1$ Hz, 8-H), 6.53 (d, 1H, $^4J_{2',6'} = 2.3$ Hz, 2′-H), 6.50 (d, 1H, $^4J_{6',2'} = 2.4$ Hz, 6′-H), 5.94 (s, 1H, OH), 5.44 (s, 1H, OH), 4.90 (q, 1H, $^3J_{2,2\text{–}CH_3} = 6.5$ Hz, 2-H), 3.69 (s, 3H, CO_2CH_3), 2.21 (s, 3H, 5′-CH_3), 1.54 ppm (d, 3H, $^3J_{2\text{–}CH_3,2} = 6.5$ Hz, 2-CH_3); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 190.73$ (C-4), 168.43 (CO_2CH_3), 160.66 (C-8a), 143.20 (C-3′), 142.38 (C-4′), 136.12 (C-7), 128.29 (C-5), 125.77 (C-5′), 124.70 (C-1′), 122.41 (C-6′), 121.73 (C-6), 120.64 (C-4a), 117.83 (C-8), 113.16 (C-2′), 80.43 (C-2), 65.74 (C-3), 52.72 (CO_2CH_3), 16.24 (2-CH_3), 15.80 ppm (5′-CH_3); IR (ATR): $\tilde{\nu} = 3419$, 2924, 1727, 1688, 1606, 1462, 1295, 1222, 762 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{19}H_{19}O_6^+$ [*M*+H$^+$] 343.11761; found 343.11760.

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Keywords: enzyme catalysis · hydrogenation · Michael addition · multicomponent reactions · oxidation

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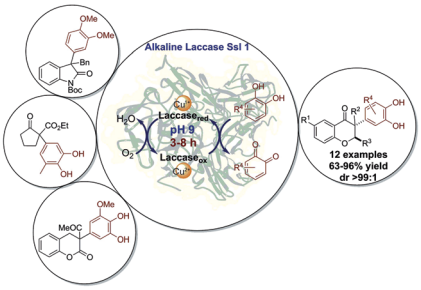
It's all in the laccase: A laccase-catalyzed oxidative regio- and diastereoselective Michael addition sequence towards a new compound class of 3-arylated 4-oxochromanes, with the creation of a quaternary carbon stereogenic center, is described. Reactions are performed in basic media using an alkalophilic laccase from *Streptomyces sviceus*. Its general utility as a biocatalyst with several different nucleophiles is demonstrated. Bn = Benzyl; Boc = *tert*-butoxycarbonyl.



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Enhanced Biocatalytic Performance of Bacterial Laccase from *Streptomyces sviceus*: Application in the Michael Addition Sequence Towards 3-Arylated 4-Oxochromanes