catalysts

MDPI

Article

Chemoenzymatic One-Pot Process for the Synthesis of Tetrahydroisoquinolines

Andreas Sebastian Klein [1], Anna Christina Albrecht [1] and Jörg Pietruszka [1,2,*]

[1] Institute of Bioorganic Chemistry, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich, Stetternicher Forst, Building 15.8, 52426 Jülich, Germany; Andreas.Sebastian.Klein@hhu.de (A.S.K.); a.albrecht@fz-juelich.de (A.C.A.)

[2] Institute of Bio- and Geosciences (IBG-1: Bioorganic Chemistry), Forschungszentrum Jülich, 52428 Jülich, Germany

* Correspondence: j.pietruszka@fz-juelich.de; Tel.: +49-2461-614158

Abstract: 1,2,3,4-Tetrahydyroisoquinolines form a valuable scaffold for a variety of bioactive secondary metabolites and commercial pharmaceuticals. Due to the harsh or complex conditions of the conventional chemical synthesis of this molecular motif, alternative mild reaction pathways are in demand. Here we present an easy-to-operate chemoenzymatic one-pot process for the synthesis of tetrahydroisoquinolines starting from benzylic alcohols and an amino alcohol. We initially demonstrate the oxidation of 12 benzylic alcohols by a laccase/TEMPO system to the corresponding aldehydes, which are subsequently integrated in a phosphate salt mediated *Pictet–Spengler* reaction with *m*-tyramine. The reaction conditions of both individual reactions were analyzed separately, adapted to each other, and a straightforward one-pot process was developed. This enables the production of 12 1,2,3,4-tetrahydyroisoquinolines with yields of up to 87% with constant reaction conditions in phosphate buffer and common laboratory glass bottles without the supplementation of any additives.

Keywords: laccase; TEMPO; chemoenzymatic cascade; pharmaceutical scaffold; heterogeneous catalysis; homogeneous catalysis; *Pictet–Spengler* reaction; biocatalysis

Citation: Klein, A.S.; Albrecht, A.C.; Pietruszka, J. Chemoenzymatic One-Pot Process for the Synthesis of Tetrahydroisoquinolines. *Catalysts* **2021**, *11*, 1389. https://doi.org/10.3390/catal11111389

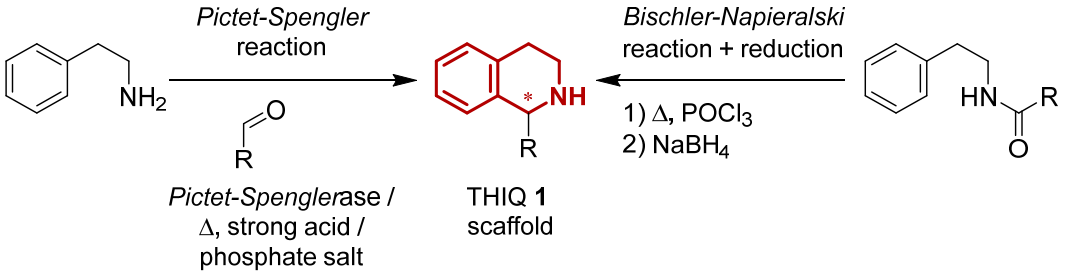
Academic Editors: Nicholas Harmer

Received: 27 October 2021
Accepted: 11 November 2021
Published: 17 November 2021

1. Introduction

1,2,3,4-Tetrahydyroisoquinolines (**1**, THIQs) form the backbone of numerous plant-derived and mammalian bioactive alkaloids and their derivatives [1,2]. Their potential pharmacological applications include their use as antidepressants, antitumor, anti-HIV and antimalarial drugs [3–6]. THIQs **1** available on the market include the antihypertensive quinapril and the antitussive noscapine [7,8]. The pharmacological and structural diversity of natural and synthetic THIQs **1** makes them a relevant motive for the synthesis of compound libraries in drug development (Scheme 1) [9].

Different strategies for obtaining THIQs **1** have been developed. In addition to the extraction of alkaloids from higher plants, which provides insufficient amounts of the target compounds and complex product mixtures, application of biocatalysts (*Pictet–Spengler*ases) of the corresponding biosynthetic pathways is the focus of current research [10]. One of the best-studied examples is norcoclaurine synthases (NCS) catalyzing the formation of a THIQ **1** between dopamine and 4-hydroxyphenylacetaldehyde in plants [11–16]. A major limitation of the use of these biocatalysts, however, is their narrow substrate spectrum, which is constantly being expanded by examining the protein structures and reaction mechanisms coupled with enzyme engineering [17–22].



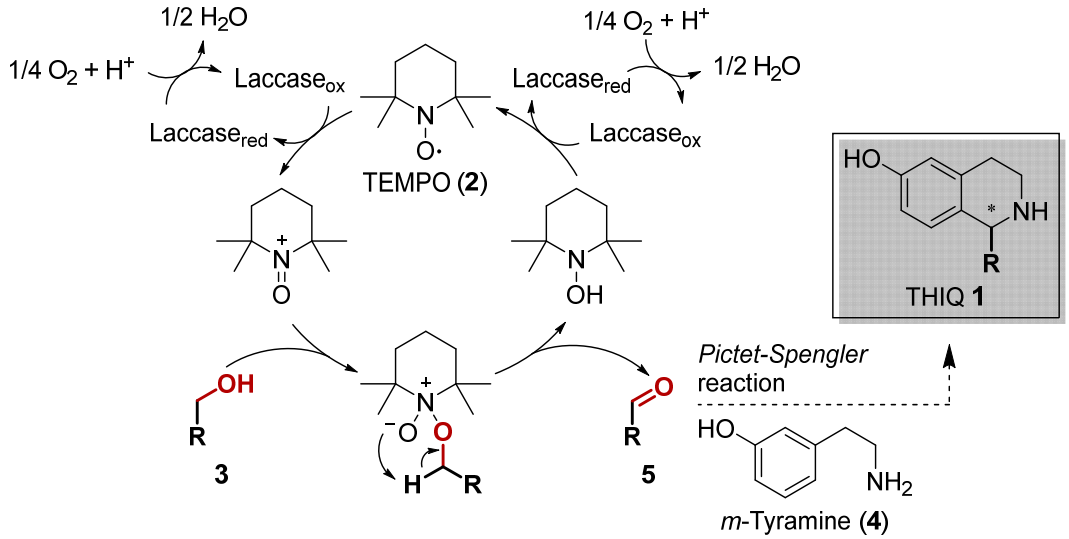
Scheme 1. Access to 1,2,3,4-Tetrahydyroisoquinolines **1** by the *Pictet–Spengler* reaction or *Bischler-Napieralski* reaction with subsequent reduction.

In classical synthetic processes, either the *Pictet–Spengler* reaction, starting from β-arylethylamines and aldehydes, or the *Bischler-Napieralski* reaction combined with a subsequent reduction provide access to the corresponding THIQ **1** scaffolds (Scheme 1) [23,24]. However, the use of these reactions is accompanied by harsh reaction conditions such as high reaction temperatures (> 100 °C) and the use of strong acids as well as *Lewis* acids [25–28]. They are therefore not suitable for use in chemoenzymatic cascades. Another alternative is the synthetic application of calcium hexafluoroisopropoxide complexes, which is a mild variant for the synthesis of THIQs **1** from β-arylethylamines and aldehydes, but these catalysts are not commercially available, and the reactions can only be carried out under inert conditions [29].

In recent years, the use of phosphate salts and buffers for the production of THIQs **1** by the *Pictet–Spengler* reaction involving a wide range of substrates including aliphatic, aromatic, and heterocyclic aldehydes and ketones has been reported (Scheme 1) [20,30–33]. These low-cost phosphate salts allow mild reaction conditions and are also compatible with chemoenzymatic reaction cascades. In the recent literature, there is a considerable number of reviews focusing on *in vitro* chemoenzymatic and biocatalytic cascades, their great benefits in synthesis routes of complex molecules, and the potential for their optimization [34–36].

Another critical aspect in the synthesis of THIQs **1** is the dependence on aldehydes. This functional group is characterized by its reactivity and tends to oxidize into the corresponding carboxylic acid when stored; one of the best-known examples is the autoxidation of benzaldehyde to benzoic acid [37]. Therefore, the *in situ* generation of aldehydes from their corresponding primary alcohols could provide an appropriate solution in cascade reactions. Several methods for this kind of oxidation, such as the application of inorganic metal salts, are known in chemistry, but they still cause problems due to environmental pollution or safety aspects.

As a biocompatible method, the use of the multi-copper enzyme laccase together with the air-stable organocatalyst (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (**2**, TEMPO) has been described in literature in recent years and was also implemented in cascade reactions [38–51]. The oxidation of the primary alcohol is catalyzed by the mediator TEMPO (**2**), which is in turn recycled in a second catalytic cycle by the primary oxidant oxygen and the multicopper oxidase (Scheme 2).



Scheme 2. Catalytic cycles of a laccase/TEMPO (**2**) system for the oxidation of primary alcohols to their corresponding aldehydes (adapted from Tromp et al.) [41]. THIQs **1** are formed by the consecutive *Pictet–Spengler* reaction with the amino alcohol *m*-tyramine (**4**).

Here, we develop a straightforward one-pot chemoenzymatic process for the production of 1-phenyl-1,2,3,4-THIQs **1** using benzylic alcohols **3** and the ß-arylethylamine *m*-tyramine (**4**). Based on the reaction conditions for the phosphate salt-mediated *Pictet–Spengler* reaction, first a suitable laccase/TEMPO (**2**) system was investigated for the oxidation of benzylic alcohols **3** to their corresponding benzaldehydes **5**. Subsequently, the two reactions were combined in a one-pot process.

2. Results and Discussion

For the development of an easy-to-operate one-pot chemoenzymatic process towards THIQs **1**, the following objectives were initially defined: The entire process should be performed in a standard glass vessel in potassium phosphate (KP_i) buffer with minimal or no supplementation of additives (no co-solvents and antioxidants) at constant reaction conditions (temperature, constant pH and ionic strength of the buffer) in a common device (incubator shaker, oxygen atmosphere).

2.1. Oxidation of Benzylic Alcohols 3 via the Laccase/TEMPO (2) System

Studies on the laccase-catalyzed oxidation of alcohols to the corresponding aldehydes or ketones using TEMPO (**2**) as a mediator have been published in recent years. Common to most publications is the use of the fungal laccase from *Trametes versicolor*, which has a pH optimum at pH 4.5–5.0 in acetate buffer (usually with the addition of co-solvents like toluene, acetonitrile or MTBE) [40–46]. As the subsequent phosphate salt-mediated *Pictet–Spengler* reaction was to take place in KP_i buffer, the acetate buffer was not compatible. In addition, computer-based DFT and MP2 calculations of the phosphate-mediated reaction with formaldehyde and 3-hydroxyphenylethylamines showed that an alkaline pH value of 8–9 represents an energetic minimum for the reaction. In this pH range both HPO_4^{2-} and $H_2PO_4^-$ ions are present, which are necessary for the abstraction of protons (for a suggested mechanism see S4.2.3, Scheme S1) [32]. Therefore, the two-domain laccase Ssl1 (*Streptomyces sviceus* laccase 1) from a mesophilic source organism was introduced as an alternative. Depending on suitable substrates, this laccase shows an alkaline activity profile with a pH optimum around pH 8–9 and also exhibits high stability over a wide pH range (pH 5–11) and elevated temperatures [52–54].

The heterologous expression of the laccase gene *ssl1* was performed in *Escherichia coli* BL21 (DE3) at 25 °C in Terrific Broth (TB) medium supplemented with 2 mM copper sulfate and 40 µM IPTG for induction. Higher concentrations of IPTG led to a reduction of the active enzyme amount. The cell lysates (either from frozen cell pellets or freeze-dried cells) were treated with heat at 65 °C for 20 min to deactivate non-specific enzyme activities. The determination of the laccase activity of the clarified cell supernatants was then performed in a colorimetric assay using 2,6-dimethoxyphenol (2,6-DMP) as the substrate (for further information on expression, preparation and activity determination see S3.1–S3.3). As a result of the heat treatment, the clarified cell supernatants could be directly used in the oxidation reactions without the requirement of time-consuming enzyme purification.

The substrate benzyl alcohol (**3a**), which was initially chosen for the standard reaction, proved to be unsuitable for closer analysis of the oxidation reaction towards benzaldehyde (**5a**), as no variation of the reaction conditions allowed a complete conversion to be achieved. Therefore, 2-bromobenzyl alcohol (**3b**) and its corresponding aldehyde 2-bromobenzaldehyde (**5b**) were applied as alternative substrates for all analyses of the laccase/TEMPO (**2**) system and the subsequent *Pictet–Spengler* reaction. Furthermore, the use of a halogenated substrate allows for a future entry point of chemical derivatizations.

The oxidation was carried out in KP_i buffer with a concentration of 0.2 M and a pH value of 8 (activity optimum of laccase) at 37 °C without any addition of a co-solvent. These reaction conditions represented a compromise between previously published parameters: studies on the laccase Ssl1 were mainly performed at room temperature, whereas for the phosphate salt-mediated *Pictet–Spengler* reaction temperatures ranging from 50 to 70 °C and phosphate concentrations between 0.1 and 1.0 M were reported [20,30,33,52,55]. In analytical approaches in glass vials, the oxidation of 2-bromobenzyl alcohol (**3b**, c = 0.12 M; batch size 1 mL) was initially investigated as a function of the volumetric activity of the laccase Ssl1 and the equivalents of TEMPO (**2**) as the mediator (Figure 1).

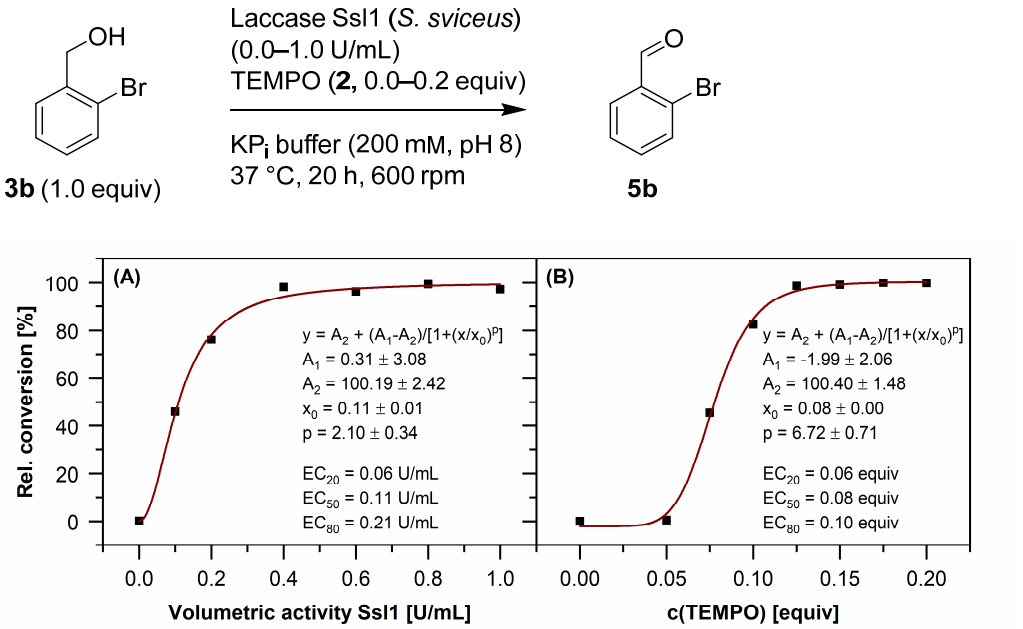
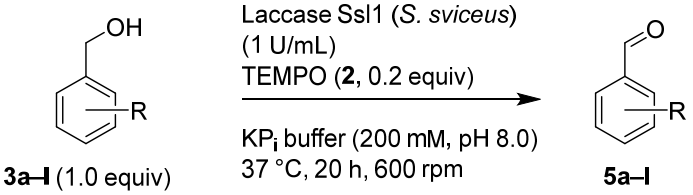


Figure 1. Analysis of the laccase/TEMPO (**2**) system for the oxidation of 2-bromobenzyl alcohol (**3b**, c = 0.12 M) to the corresponding 2-bromobenzaldehyde (**5b**). (**A**) To determine the conversion for individual volumetric activities of Ssl1 a constant amount of TEMPO (**2**, 0.175 equiv) was chosen; (**B**) in order to determine the conversion for individual amounts of TEMPO (**2**), a constant volumetric activity of Ssl1 of 1 U/mL was chosen. (The rel. conversion is derived from the ratio (benzylic alcohol:benzaldehyde) by GC; the data was analyzed by a logistic regression.).

It could be observed that complete conversion of the starting material was achieved at a laccase activity of > 0.5 U/mL and a TEMPO (**2**) quantity of > 0.15 equivalents (referring to 1 equiv of 2-bromobenzyl alcohol (**3b**)). A subsequent upscaling on a semi-preparative batch with 2-bromobenzyl alcohol (**3b**, c = 0.12 M; batch size 10 mL) also showed complete conversion under these conditions without the formation of the possible over-oxidized product 2-bromobenzoic acid. In contrast, in control studies without TEMPO (**2**) or laccase (buffer only or heat-treated clarified cell supernatants of *E. coli* bearing an empty vector) no conversion could be detected. This proved that a specific purification of the laccase via protein tags is therefore not essential for the preparative application in biocatalytic reactions. Likewise, no co-solvents are obligatory, despite the reaction being a heterogeneous mixture. Regarding different molarities of the KP_i buffer, an increase of the concentration had a negative effect on conversion. A significant reduction to only 50% is apparent at the concentration of 1 M, as previously applied in the literature for the subsequent phosphate salt-mediated *Pictet–Spengler* reaction with aldehydes.

The conditions of the analytical approaches with 2-bromobenzyl alcohol (**3b**) were transferred to benzyl alcohol (**3a**) and 10 additional benzyl alcohol derivatives **3c–l** containing substitutions at the *ortho* and *para* positions with electron withdrawing (EWG) and electron donating (EDG) groups (Scheme 3 and Table 1). While hydroxylated benzylic alcohols (compounds not shown; R=OH) were also tested, there was—as expected—a considerable formation of side product as seen from the discoloration of the reaction solution to dark brown, hinting to the formation of oligo-/ polymers. As a trend, a slightly adverse effect of activating EDG groups was observed in both positions. Except for benzyl alcohol (**3a**), all substrates applied in the laccase/TEMPO (**2**) system demonstrated a conversion of at least 72%—a considerable number of them even a complete conversion. For consistency, the conditions of the oxidation of benzylic alcohols **3** were applied unchanged to the subsequent cascade development.



Scheme 3. Oxidation of benzylic alcohols **3a–l** towards their corresponding benzaldehydes **5a–l** in the laccase/TEMPO (**2**) system; for results see Table 1.

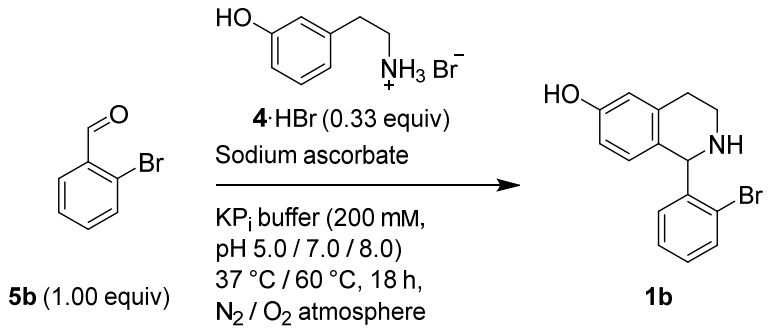
Table 1. Conversion of laccase-mediated oxidation.

Benzylic Alcohol	Benzaldehyde	R	Conversion [%] [1]
3a	5a	H	28
3b	5b	2-Br	100
3c	5c	2-F	73
3d	5d	2-NO_2	100
3e	5e	2-OMe	72
3f	5f	4-Br	100
3g	5g	4-Cl	92
3h	5h	4-F	98
3i	5i	4-CF_3	100
3j	5j	4-NO_2	84
3k	5k	4-Me	100
3l	5l	4-OMe	90

[1] The conversion was derived from the ratio (benzylic alcohol; benzaldehyde) by GC.

2.2. Phosphate Salt-Mediated Pictet–Spengler Reaction

In recent years it has been shown that phosphate compounds (KH_2PO_4, NaH_2PO_4, $Na_4P_2O_7$, glucose-1-phosphate, uridine monophosphate) can be used as mediators in the *Pictet–Spengler* reaction [30,33]. The application of the low-cost KP_i buffer avoids the aforementioned harsh reaction conditions and the use of metals as *Lewis* acids or complex catalysts. For the evaluation of the parameters of the selected standard reaction with 2-bromobenzaldehyde (**5b**) and *m*-tyramine hydrobromide (**4**·HBr), the previously published reactions of *Erdmann* et al. were referred to, in which the phosphate salt-mediated *Pictet–Spengler* reaction between 2-bromobenzaldehyde (**5b**) and metaraminol—an *m*-tyramine (**4**) derivative—was performed [20]. Erdmann *et al.* implemented their reaction in a 1:1 (*v*/*v*) mixture of KP_i buffer (0.2 M or 1.0 M, pH 7) and the co-solvent DMSO supplemented with the antioxidant sodium ascorbate (0.5 eq.) under an inert gas atmosphere at 60 °C for 18 h (54% yield). Taking the previous optimizations for the oxidation of the benzylic alcohols **3a–l** in the laccase/TEMPO (**2**) system into account, KP_i buffer (0.2 M) was chosen as the reaction medium without the addition of a co-solvent for the *Pictet–Spengler* reaction. By omitting the co-solvent (beside DMSO, acetonitrile, ethanol, and methanol are mentioned in literature) the product precipitates during the reaction and is therefore removed from a possible equilibrium. With the chosen reaction parameters, the corresponding THIQ **1b** was formed in 74% yield as a racemic mixture at pH 7 and 60 °C (Scheme 4 and Table 2).



Scheme 4. Analysis of the reaction parameters for the phosphate salt-mediated *Pictet–Spengler* reaction with 2-bromobenzaldehyde (**5b**) and *m*-tyramine hydrobromide (**4**·HBr) towards the THIQ **1b**; for results see Table 2.

Table 2. Parameters influencing the Pictet-Spengler reaction.

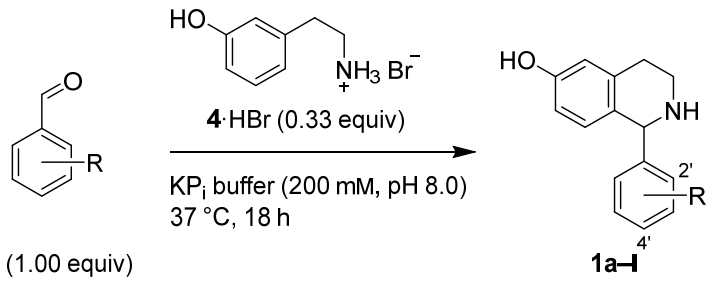
pH	T [°C]	Sodium Ascorbate	Atmosphere	Yield [%] [1]
5	60	+	N_2	2
7	60	+	N_2	74
7	37	+	N_2	65
8	37	+	N_2	67
8	37	-	N_2	65
8	37	-	O_2	68

[1] The yields are related to the quantity of *m*-tyramine hydrobromide (**4**·HBr) used.

With the envisioned goal of a straightforward one-pot process, the temperature and pH were adjusted to the established reaction conditions of the benzylic alcohol **3** oxidation in the laccase/TEMPO (**2**) system (37 °C, pH 8; yield 67%, Table 2). The slightly alkaline pH value thus also corresponds to the aforementioned optimum of the phosphate salt-mediated *Pictet–Spengler* reaction and the activity optimum of the laccase Ssl1 [32,52]. The high influence of an alkaline pH is also confirmed by a control experiment at pH 5 (optimum of the fungal laccase from *T. versicolor*), resulting in a yield of only 2%. By choosing

these reaction conditions the following predefined objectives of the one-pot process were already considered: constant temperature, pH, and ionic strength of the buffer; no co-solvents. In a second series of experiments, the requirement for the additive sodium ascorbate and reaction handling under inert gas atmosphere to avoid possible oxidation side reactions were evaluated. However, no significant influence of these parameters could be determined, enabling the reaction to be carried out under oxygen atmosphere and avoiding additives completely (Scheme 4). In addition, the reaction can be carried out with the same efficiency both in glass flasks with magnetic stirring bars and glass vials in an incubator shaker. Furthermore, the downstream process for the extraction and purification of THIQs **1** was also improved compared to the methods currently described in the literature for semi-preparative and preparative reaction scales [20,30,33]. The extracted raw material could be effectively purified from remaining aldehyde **5** by substituting aqueous HCl to HCl dissolved in diethyl ether resulting in the precipitation of only the final THIQ **1** and a single by-product (which will be discussed later).

The newly defined conditions for the phosphate salt-mediated *Pictet–Spengler* reaction with 2-bromobenzaldehyde (**5b**) and *m*-tyramine hydrobromide (**4**·HBr) were then implemented for benzaldehyde (**5a**) and the 10 additional derivatives **5c–l** already produced in the laccase/TEMPO (**2**) system. For the 1-phenyl-1,2,3,4-THIQs **1a,c–l**, yields between 52–93% were achieved, but no trend was observed for the substitutions in *ortho* and *para* position and their attributed electronic effects (Scheme 5 and Table 3). Due to the use of *m*-tyramine (**4**) as the amino alcohol, all products were obtained as racemic mixtures.



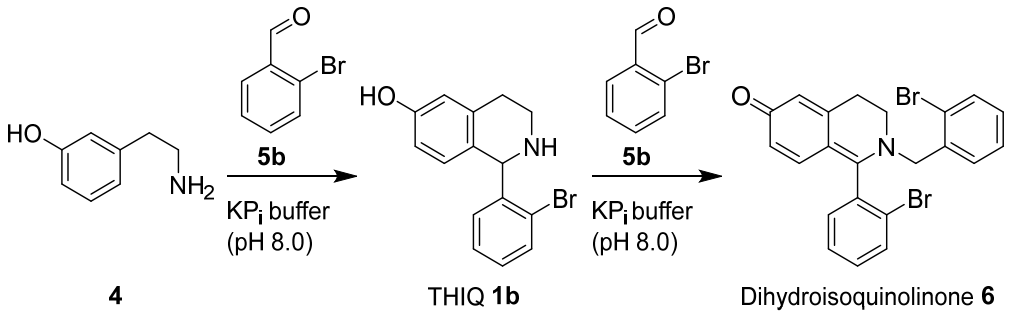
Scheme 5. Phosphate salt-mediated *Pictet–Spengler* reaction with benzaldehydes **5a–l** and *m*-tyramine hydrobromide (**4**·HBr) towards 1-phenyl-1,2,3,4-THIQs **1a–l**; for results see Table 3.

Table 3. Yields of phosphate salt-mediated *Pictet-Spengler* reaction.

THIQ	R	Yield [%] [1]
1a	H	93
1b	2′-Br	76
1c	2′-F	92
1d	2′-NO_2	76
1e	2′-OMe	86
1f	4′-Br	79
1g	4′-Cl	84
1h	4′-F	88
1i	4′-CF_3	52
1j	4′-NO_2	91
1k	4′-Me	60
1l	4′-OMe	65

[1] The yields are related to the quantity of *m*-tyramine hydrobromide (**4**·HBr) used.

In all reactions, the formation of a strongly yellow-colored and fluorescent under UV light (Figure S9) by-product was observed, which was analyzed in the standard reaction containing 2-bromobenzaldehyde (**5b**) and *m*-tyramine hydrobromide (**4**·HBr). The evaluation of mass and NMR data of the by-product **6** confirmed the *in situ* reaction of the produced THIQ **1b** with a second molecule of 2-bromobenzaldehyde (**5b**); under basic conditions the dihydroisoquinolinone **6** is formed via a contingent imine tautomerism (Scheme 6; for a detailed suggested reaction mechanism, see S4.2.4, Scheme S2).

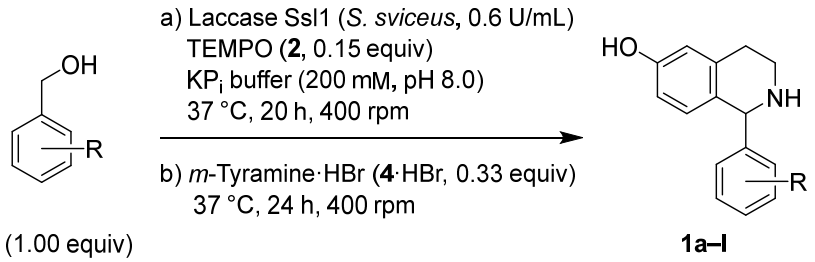


Scheme 6. Side reaction of the phosphate salt-mediated *Pictet–Spengler* reaction towards the by-product dihydroisoquinolinone **6** under alkaline conditions.

A direct correlation between the amount of the by-product **6** and the increase in aldehyde **5b** concentration in the *Pictet–Spengler* reaction could be identified, providing a further opportunity for optimization with regard to increasing yields.

2.3. Chemoenzymatic One-Pot Process

The final easy-to-operate chemoenzymatic one-pot process is designed as a consecutive reaction sequence. First, the oxidation of the benzylic alcohols **3** to the corresponding aldehydes **5** takes place in the laccase/TEMPO (**2**) system and subsequently the formation of the THIQs **1** is initiated by adding the amino alcohol *m*-tyramine hydrobromide (**4**·HBr) (Scheme 7 and Table 4).



Scheme 7. Chemoenzymatic one-pot process with benzylic alcohols **3a–l** and *m*-tyramine hydrobromide (**4**·HBr) towards 1-phenyl-1,2,3,4-THIQs **1a–l**; for results see Table 4.

Table 4. Yields of sequential one-pot process.

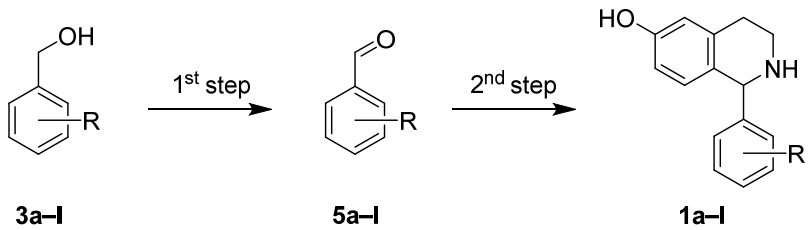
Benzylic Alcohol	R	THIQ	Yield [%][1]
3a	H	**1a**	32[2]
3b	2-Br	**1b**	49[3]
3c	2-F	**1c**	64
3d	2-NO_2	**1d**	47[3]
3e	2-OMe	**1e**	57[2]
3f	4-Br	**1f**	70
3g	4-Cl	**1g**	55[2]
3h	4-F	**1h**	71
3i	4-CF_3	**1i**	37
3j	4-NO_2	**1j**	87[2]
3k	4-Me	**1k**	58[3]
3l	4-OMe	**1l**	43

[1] The yields are related to the quantity of *m*-tyramine hydrobromide (**4**·HBr) used. [2] First reaction was shaken for 40–45 h. [3] 0.20 equiv TEMPO (**2**), Laccase SSL1 1 U/mL.

The results of first reaction setups containing 0.20 equiv of TEMPO (**2**) suggest that the components of the first reaction step of the cascade have an influence on the phosphate salt-mediated *Pictet–Spengler* reaction. Therefore, the second reaction step was investigated with 2-bromobenzaldehyde (**5b**) as substrate in the presence of different amounts of TEMPO (**2**). In the presence of 0.2 equiv TEMPO (**2**), a 57% yield of THIQ **1b** was obtained, whereas a reduction to 0.1 equiv increased the yield to 73%. Continued reduction of TEMPO (**2**) to 0.067 equiv did not result in a further increase in yield (71%).

As decreasing the amount of TEMPO (**2**) in the cascade reaction should simply lead to slightly increased reaction times in the chemoenzymatic oxidation step (see Figure 1), the equivalents were reduced to 0.15 in further cascade reactions and samples taken for GC measurement to follow the reaction progress. Following this reasoning, the volumetric activity of laccase was also reduced to 0.6 U/mL in an effort to facilitate workup with ethyl acetate. The cascade reaction starting with the oxidation of, e.g., **3e** was performed for 44 h, resulting in a conversion of 93%. For more detailed reaction times and conversions of the first step in the reaction cascade, see Supplementary Table S1. As expected based on the literature and confirmed by measuring the optical rotation, the presence of laccase during the *Pictet–Spengler* reaction did not result in any asymmetric induction [56].

It was found that in the consecutive cascade with respect to all benzyl alcohols **3** the intended THIQs **1** could be isolated after column chromatography with yields ranging from 32–87%. Again, no clear trend based on the electronic effects of the substituents in *ortho* and *para* position was observed. When compared to the theoretical yield (calculated by the multiplication of the conversion and yield of the individual steps), it was observed that the yield of all but three cascade reactions was within 16% of the theoretical yield. The cascades resulting in **1a** and **1j** even slightly exceeded expectations (Scheme 8 and Table 5).



Scheme 8. Comparison of obtained and theoretical yields of the chemoenzymatic one-pot process with benzylic alcohols **3a–l** and *m*-tyramine hydrobromide (**4**·HBr) towards 1-phenyl-1,2,3,4-THIQs **1a–l**; for results see Table 5.

Table 5. Obtained and theoretical yields of chemoenzymatic one-pot process.

R	Conversion [1] 1st step [%]	Yield [2] 2nd step	Theor. Yield [3] Cascade [%]	Yield [%] [1]	THIQ
H	28	93	26	32	**1a**
2-Br	100	76	76	49	**1b**
2-F	73	92	67	64	**1c**
2-NO_2	100	76	76	47	**1d**
2-OMe	72	86	62	57	**1e**
4-Br	100	79	79	70	**1f**
4-Cl	92	84	77	55	**1g**
4-F	98	88	86	71	**1h**
4-CF_3	100	52	52	37	**1i**
4-NO_2	84	91	76	87	**1j**
4-Me	100	60	60	58	**1k**
4-OMe	90	65	59	43	**1l**

[1] The conversion is derived from the ratio (benzylic alcohol:benzaldehyde) by GC. [2] The yields are related to the quantity of *m*-tyramine hydrobromide (**4**·HBr) used. [3] The theoretical yield is calculated by the multiplication of the conversion and yield of the individual steps.

The reaction sequence is carried out under constant conditions and can be performed using standard laboratory equipment, with the following parameters allowing an easily parallelized experiment setup:

- Laccase Ssl1 from *S. sviceus* (heat-treated clarified cell supernatant, prepared as a batch, aliquoted, thawed as needed).
- 1.00 equiv benzylic alcohol **3**, 0.15 equiv TEMPO (**2**), 0.33 equiv *m*-tyramine hydrobromide (**4**·HBr).
- KP_i buffer (0.2 M, pH 8).
- Glass bottle, constant shaking in incubator at 37 °C, oxygen atmosphere, no additives.

3. Materials and Methods

All materials and methods are given in detail in the Supplementary Materials. The procedure for the chemoenzymatic one-pot process as a combination of both single reaction steps is described in the following:

The chemoenzymatic one-pot process was performed in a 100 mL Schott® flask fitted with a PTFE/silicon septum perforated with a cannula for oxygen exchange under constant shaking at 400 rpm on an orbital shaker for culture flasks at 37 °C.

The benzylic alcohol **3** (1.38 mmol, 1.00 equiv) and TEMPO (**2**, 32 mg, 0.21 mmol, 0.15 equiv) were added to a freshly thawed laccase solution (0.6 U/mL) in KP_i buffer (10 mL, 200 mM, pH 8.0) with 0.3 mM $CuSO_4$. The reaction mixture was shaken for 20–45 h at 37 °C. Afterwards, *m*-tyramine hydrobromide (**4**·HBr, 100 mg, 0.46 mmol, 0.33 equiv) in KP_i buffer (10 mL, 200 mM, pH 8.0) was added to the reaction mixture and shaken for another 18–24 h. The solution was then cooled to room temperature (25 °C) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The THIQs **1a–l** were precipitated by adding small amounts of cold HCl solution in diethyl ether (1 M) to the residue. The resulting solid was washed with cold diethyl ether, filtered, and resuspended in MeOH. The solvent was removed under reduced pressure and the THIQs **1a–l** were purified via column (length = 10–16 cm, diameter = 3 cm) chromatography on silica with dichloromethane/MeOH [4% (*v*/*v*)]/ammonia in MeOH (7 N) [1% (*v*/*v*)] providing the THIQs **1a–l** as free amines and racemic mixtures. The yields are related to the quantity of *m*-tyramine hydrobromide (**4**·HBr) used.

4. Conclusions

In summary, we successfully developed an easy-to-operate chemoenzymatic one-pot process for the synthesis of tetrahydyroisoquinoline libraries with yields of up to 87% starting from benzylic alcohols and an amino alcohol. The reaction conditions of the individual processes, the oxidation of benzylic alcohols and the phosphate salt-mediated *Pictet–Spengler* reaction, were adapted to each other and, furthermore, a by-product of the latter reaction was identified. After demonstrating the feasibility of this cascade, future studies could focus on expanding the library by substituting *m*-tyramine with different β-arylethylamines or combining the chemoenzymatic oxidation with a stereoselective *Pictet–Spengler* reaction.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/catal11111389/s1, S1: Overview of compounds (including numbers); S2: General methods (materials and analytics); S3: Methods in biology [S3.1–S3.3: strains, vectors, protein production, laccase activity assay; S3.4: oxidation of benzylic alcohols **3a–l** using the laccase/TEMPO (**2**) system]; S4: Chemical syntheses [S4.1: synthesis and analysis (NMR, IR, LC-MS) of *m*-tyramine·HBr (**4**·HBr); S4.2: phosphate salt mediated *Pictet–Spengler* reaction + suggested mechanism (Scheme S1), analysis of by-product **6** (NMR, IR, HRMS) + suggested mechanism (Scheme S2)]; S5: Chemoenzymatic one-pot cascade towards THIQs **1a–l**; S6: Compound characterization of THIQs **1a–l** (NMR, IR, LC-MS, HRMS); S7: Reaction monitoring (Supplementary Table S1).

Author Contributions: Conceptualization, A.S.K. and J.P.; methodology, A.S.K.; validation, A.C.A., A.S.K. and J.P.; investigation, A.C.A., A.S.K.; writing—original draft preparation, A.S.K.; writing—review and editing, A.C.A., ASK, and J.P.; visualization, A.S.K.; supervision, A.S.K. and J.P.; project administration, J.P.; funding acquisition, J.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Culture and Science within the framework of the NRW-Strategieprojekt Bioeconomy Science Center (BioSC, no. 313/323-400-002 13).

Data Availability Statement: The data presented in this study are available in the Supplementary Materials of this article.

Acknowledgments: The authors thank Kevin Bochinsky (Institute of Biological Information Processing, Structural Biochemistry (IBI-7), Forschungszentrum Jülich) for his help with NMR analysis and Beatrix Paschold for her support concerning heterologous expression.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Stöckigt, J.; Antonchick, A.P.; Wu, F.; Waldmann, H. The Pictet-Spengler reaction in nature and in organic chemistry. *Angew. Chem. Int. Ed.* **2011**, *50*, 8538–8564, doi:10.1002/anie.201008071.
2. Dostert, P.; Strolin Benedetti, M.; Dordain, G. Dopamine-derived alkaloids in alcoholism and in Parkinson's and Huntington's diseases. *J. Neural Transm.* **1988**, *74*, 61–74, doi:10.1007/BF01245140.
3. Christinat, A.; Leyvraz, S. Role of trabectedin in the treatment of soft tissue sarcoma. *Onco Targets Ther.* **2009**, *2*, 105–113, doi:10.2147/ott.s4454.
4. Truax, V.M.; Zhao, H.; Katzman, B.M.; Prosser, A.R.; Alcaraz, A.A.; Saindane, M.T.; Howard, R.B.; Culver, D.; Arrendale, R.F.; Gruddanti, P.R.; et al. Discovery of Tetrahydroisoquinoline-Based CXCR4 Antagonists. *ACS Med. Chem. Lett.* **2013**, *4*, 1025–1030, doi:10.1021/ml400183q.
5. Antkiewicz-Michaluk, L.; Wąsik, A.; Możdżeń, E.; Romańska, I.; Michaluk, J. Antidepressant-like Effect of Tetrahydroisoquinoline Amines in the Animal Model of Depressive Disorder Induced by Repeated Administration of a Low Dose of Reserpine: Behavioral and Neurochemical Studies in the Rat. *Neurotox. Res.* **2014**, *26*, 85–98, doi:10.1007/s12640-013-9454-8.
6. Hanna, J.N.; Ntie-Kang, F.; Kaiser, M.; Brun, R.; Efange, S.M.N. 1-Aryl-1,2,3,4-tetrahydroisoquinolines as potential antimalarials: Synthesis, in vitro antiplasmodial activity and in silico pharmacokinetics evaluation. *RSC Adv.* **2014**, *4*, 22856–22865, doi:10.1039/C3RA46791K.
7. Wadworth, A.N.; Brogden, R.N. Quinapril. *Drugs* **1991**, *41*, 378–399, doi:10.2165/00003495-199141030-00006.
8. DeBono, A.; Capuano, B.; Scammells, P.J. Progress Toward the Development of Noscapine and Derivatives as Anticancer Agents. *J. Med. Chem.* **2015**, *58*, 5699–5727, doi:10.1021/jm501180v.
9. Welsch, M.E.; Snyder, S.A.; Stockwell, B.R. Privileged scaffolds for library design and drug discovery. *Curr. Opin. Chem. Biol.* **2010**, *14*, 347–361, doi:10.1016/j.cbpa.2010.02.018.

10. Roddan, R.; Ward, J.; Keep, N.; Hailes, H.C. Pictet–Spenglerases in alkaloid biosynthesis: Future applications in biocatalysis. *Curr. Opin. Chem. Biol.* **2020**, *55*, 69–76, doi:10.1016/j.cbpa.2019.12.003.
11. Samanani, N.; Liscombe, D.K.; Facchini, P.J. Molecular cloning and characterization of norcoclaurine synthase, an enzyme catalyzing the first committed step in benzylisoquinoline alkaloid biosynthesis. *Plant. J.* **2004**, *40*, 302–313, doi:10.1111/j.1365-313X.2004.02210.x.
12. Pasquo, A.; Bonamore, A.; Franceschini, S.; Macone, A.; Boffi, A.; Ilari, A. Cloning, expression, crystallization and preliminary X-ray data analysis of norcoclaurine synthase from Thalictrum flavum. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2008**, *64*, 281–283, doi:10.1107/S1744309108005678.
13. Pesnot, T.; Gershater, M.C.; Ward, J.M.; Hailes, H.C. The Catalytic Potential of Coptis japonica NCS2 Revealed-Development and Utilisation of a Fluorescamine-Based Assay. *Adv. Synth. Catal.* **2012**, *354*, 2997–3008, doi:10.1002/adsc.201200641.
14. Ruff, B.M.; Bräse, S.; O'Connor, S.E. Biocatalytic production of tetrahydroisoquinolines. *Tetrahedron Lett* **2012**, *53*, 1071–1074, doi:10.1016/j.tetlet.2011.12.089.
15. Lechner, H.; Soriano, P.; Poschner, R.; Hailes, H.C.; Ward, J.M.; Kroutil, W. Library of Norcoclaurine Synthases and Their Immobilization for Biocatalytic Transformations. *Biotechnol. J.* **2018**, *13*, e1700542, doi:10.1002/biot.201700542.
16. Luk, L.Y.P.; Bunn, S.; Liscombe, D.K.; Facchini, P.J.; Tanner, M.E. Mechanistic Studies on Norcoclaurine Synthase of Benzylisoquinoline Alkaloid Biosynthesis: An Enzymatic Pictet–Spengler Reaction. *Biochemistry* **2007**, *46*, 10153–10161, doi:10.1021/bi700752n.
17. Lichman, B.R.; Zhao, J.; Hailes, H.C.; Ward, J.M. Enzyme catalysed Pictet-Spengler formation of chiral 1,1′-disubstituted- and spiro-tetrahydroisoquinolines. *Nat. Commun.* **2017**, *8*, 14883, doi:10.1038/ncomms14883.
18. Sheng, X.; Himo, F. Enzymatic Pictet–Spengler Reaction: Computational Study of the Mechanism and Enantioselectivity of Norcoclaurine Synthase. *J. Am. Chem. Soc.* **2019**, *141*, 11230–11238, doi:10.1021/jacs.9b04591.
19. Lichman, B.R.; Sula, A.; Pesnot, T.; Hailes, H.C.; Ward, J.M.; Keep, N.H. Structural Evidence for the Dopamine-First Mechanism of Norcoclaurine Synthase. *Biochemistry* **2017**, *56*, 5274–5277, doi:10.1021/acs.biochem.7b00769.
20. Erdmann, V.; Lichman, B.R.; Zhao, J.; Simon, R.C.; Kroutil, W.; Ward, J.M.; Hailes, H.C.; Rother, D. Enzymatic and Chemoenzymatic Three-Step Cascades for the Synthesis of Stereochemically Complementary Trisubstituted Tetrahydroisoquinolines. *Angew. Chem. Int. Ed.* **2017**, *56*, 12503–12507, doi:10.1002/anie.201705855.
21. Roddan, R.; Gygli, G.; Sula, A.; Méndez-Sánchez, D.; Pleiss, J.; Ward, J.M.; Keep, N.H.; Hailes, H.C. Acceptance and Kinetic Resolution of α-Methyl-Substituted Aldehydes by Norcoclaurine Synthases. *ACS Catal* **2019**, *9*, 9640–9649, doi:10.1021/acscatal.9b02699.
22. Roddan, R.; Sula, A.; Méndez-Sánchez, D.; Subrizi, F.; Lichman, B.R.; Broomfield, J.; Richter, M.; Andexer, J.N.; Ward, J.M.; Keep, N.H.; et al. Single step syntheses of (1S)-aryl-tetrahydroisoquinolines by norcoclaurine synthases. *Commun. Chem.* **2020**, *3*, 170, doi:10.1038/s42004-020-00416-8.
23. Pictet, A.; Spengler, T. Über die Bildung von Isochinolin-derivaten durch Einwirkung von Methylal auf Phenyl-äthylamin, Phenyl-alanin und Tyrosin. *Ber. Dtsch. Chem. Ges.* **1911**, *44*, 2030–2036, doi:10.1002/cber.19110440309.
24. Nicoletti, M.; O'Hagan, D.; Slawin, A.M.Z. The asymmetric Bischler–Napieralski reaction: Preparation of 1,3,4-trisubstituted 1,2,3,4-tetrahydroisoquinolines. *J. Chem. Soc. Perkin Trans. 1* **2002**, 116–121, doi:10.1039/B106942J.
25. Youn, S.W. The Pictet-Spengler reaction: Efficient carbon-carbon bond forming reaction in heterocyclic synthesis. *Org. Prep. Proced. Int.* **2006**, *38*, 505–591, doi:10.1080/00304940609356447.
26. Awuah, E.; Capretta, A. Strategies and Synthetic Methods Directed Toward the Preparation of Libraries of Substituted Isoquinolines. *J. Org. Chem.* **2010**, *75*, 5627–5634, doi:10.1021/jo100980p.
27. Calcaterra, A.; Mangiardi, L.; Delle Monache, G.; Quaglio, D.; Balducci, S.; Berardozzi, S.; Iazzetti, A.; Franzini, R.; Botta, B.; Ghirga, F. The Pictet-Spengler Reaction Updates Its Habits. *Molecules* **2020**, *25*, 414, doi:10.3390/molecules25020414.
28. Manabe, K.; Nobutou, D.; Kobayashi, S. Catalytic Pictet–Spengler reactions using Yb(OTf)$_3$. *Bioorg. Med. Chem* **2005**, *13*, 5154–5158, doi:10.1016/j.bmc.2005.05.018.
29. Eynden, M.J.V.; Stambuli, J.P. Calcium-Catalyzed Pictet–Spengler Reactions. *Org. Lett.* **2008**, *10*, 5289–5291, doi:10.1021/ol802173r.
30. Pesnot, T.; Gershater, M.C.; Ward, J.M.; Hailes, H.C. Phosphate mediated biomimetic synthesis of tetrahydroisoquinoline alkaloids. *Chem. Commun.* **2011**, *47*, 3242–3244, doi:10.1039/C0CC05282E.
31. Maresh, J.J.; Crowe, S.O.; Ralko, A.A.; Aparece, M.D.; Murphy, C.M.; Krzeszowiec, M.; Mullowney, M.W. Facile one-pot synthesis of tetrahydroisoquinolines from amino acids via hypochlorite-mediated decarboxylation and Pictet–Spengler condensation. *Tetrahedron Lett.* **2014**, *55*, 5047–5051, doi:10.1016/j.tetlet.2014.07.043.
32. Parra, R.D.; Maresh, J. Structural and energetics aspects of a proposed mechanism for the phosphate-mediated Pictet–Spengler cyclization reaction: A computational study. *Comput. Theor. Chem.* **2016**, *1082*, 1–10, doi:10.1016/j.comptc.2016.02.014.
33. Zhao, J.; Méndez-Sánchez, D.; Ward, J.; Hailes, H.C. Biomimetic Phosphate-Catalyzed Pictet–Spengler Reaction for the Synthesis of 1,1′-Disubstituted and Spiro-Tetrahydroisoquinoline Alkaloids. *J. Org. Chem.* **2019**, *84*, 7702–7710, doi:10.1021/acs.joc.9b00527.
34. Siedentop, R.; Claaßen, C.; Rother, D.; Lütz, S.; Rosenthal, K. Getting the Most Out of Enzyme Cascades: Strategies to Optimize In Vitro Multi-Enzymatic Reactions. *Catalysts* **2021**, *11*, 1183, doi:10.3390/catal11101183.
35. Classen, T.; Pietruszka, J. Complex molecules, clever solutions—Enzymatic approaches towards natural product and active agent syntheses. *Bioorg. Med. Chem.* **2018**, *26*, 1285–1303, doi:10.1016/j.bmc.2017.06.045.

36. Schrittwieser, J.H.; Velikogne, S.; Hall, M.; Kroutil, W. Artificial Biocatalytic Linear Cascades for Preparation of Organic Molecules. *Chem. Rev.* **2018**, *118*, 270–348, doi:10.1021/acs.chemrev.7b00033.
37. Sankar, M.; Nowicka, E.; Carter, E.; Murphy, D.M.; Knight, D.W.; Bethell, D.; Hutchings, G.J. The benzaldehyde oxidation paradox explained by the interception of peroxy radical by benzyl alcohol. *Nat. Commun.* **2014**, *5*, 3332, doi:10.1038/ncomms4332.
38. Fabbrini, M.; Galli, C.; Gentili, P.; Macchitella, D. An oxidation of alcohols by oxygen with the enzyme laccase and mediation by TEMPO. *Tetrahedron Lett.* **2001**, *42*, 7551–7553, doi:10.1016/S0040-4039(01)01463-0.
39. d'Acunzo, F.; Baiocco, P.; Fabbrini, M.; Galli, C.; Gentili, P. A Mechanistic Survey of the Oxidation of Alcohols and Ethers with the Enzyme Laccase and Its Mediation by TEMPO. *Eur. J. Org. Chem.* **2002**, *2002*, 4195–4201, doi:10.1002/1099-0690(200212)2002:24<4195::AID-EJOC4195>3.0.CO;2-X.
40. Arends, I.W.C.E.; Li, Y.-X.; Ausan, R.; Sheldon, R.A. Comparison of TEMPO and its derivatives as mediators in laccase catalysed oxidation of alcohols. *Tetrahedron* **2006**, *62*, 6659–6665, doi:10.1016/j.tet.2005.12.076.
41. Tromp, S.A.; Matijošytė, I.; Sheldon, R.A.; Arends, I.W.C.E.; Mul, G.; Kreutzer, M.T.; Moulijn, J.A.; de Vries, S. Mechanism of Laccase–TEMPO-Catalyzed Oxidation of Benzyl Alcohol. *ChemCatChem* **2010**, *2*, 827–833, doi:10.1002/cctc.201000068.
42. Larson, T.M.; Anderson, A.M.; Rich, J.O. Combinatorial evaluation of laccase-mediator system in the oxidation of veratryl alcohol. *Biotechnol. Lett.* **2013**, *35*, 225–231, doi:10.1007/s10529-012-1078-1.
43. Albarrán-Velo, J.; Gotor-Fernández, V.; Lavandera, I. One-pot two-step chemoenzymatic deracemization of allylic alcohols using laccases and alcohol dehydrogenases. *Mol. Catal.* **2020**, *493*, 111087, doi:10.1016/j.mcat.2020.111087.
44. Albarrán-Velo, J.; Lavandera, I.; Gotor-Fernández, V. Sequential Two-Step Stereoselective Amination of Allylic Alcohols through the Combination of Laccases and Amine Transaminases. *ChemBioChem* **2020**, *21*, 200–211, doi:10.1002/cbic.201900473.
45. Arends, I.W.C.E.; Li, Y.-X.; Sheldon, R.A. Stabilities and rates in the laccase/TEMPO-catalyzed oxidation of alcohols. *Biocatal. Biotransform.* **2006**, *24*, 443–448, doi:10.1080/10242420601040683.
46. Gross, J.; Tauber, K.; Fuchs, M.; Schmidt, N.G.; Rajagopalan, A.; Faber, K.; Fabian, W.M.F.; Pfeffer, J.; Haas, T.; Kroutil, W. Aerobic oxidation of isosorbide and isomannide employing TEMPO/laccase. *Green Chem.* **2014**, *16*, 2117–2121, doi:10.1039/C3GC41855C.
47. Jiang, J.; Chen, H.; Yu, J.; Liu, L.; Fan, Y.; Saito, T.; Isogai, A. Rate-Limited Reaction in TEMPO/Laccase/O2 Oxidation of Cellulose. *Macromol. Rapid Commun.* **2021**, *42*, 2000501, doi:10.1002/marc.202000501.
48. Cheng, A.-D.; Zong, M.-H.; Lu, G.-H.; Li, N. Solvent-Promoted Oxidation of Aromatic Alcohols/Aldehydes to Carboxylic Acids by a Laccase-TEMPO System: Efficient Access to 2,5-Furandicarboxylic Acid and 5-Methyl-2-Pyrazinecarboxylic Acid. *Adv. Sustain. Syst.* **2021**, *5*, 2000297, doi:10.1002/adsu.202000297.
49. Risi, C.; Zhao, F.; Castagnolo, D. Chemo-Enzymatic Metathesis/Aromatization Cascades for the Synthesis of Furans: Disclosing the Aromatizing Activity of Laccase/TEMPO in Oxygen-Containing Heterocycles. *ACS Catal.* **2019**, *9*, 7264–7269, doi:10.1021/acscatal.9b02452.
50. Zippilli, C.; Botta, L.; Bizzarri, B.M.; Baratto, M.C.; Pogni, R.; Saladino, R. Biomimetic synthesis of galantamine via laccase/TEMPO mediated oxidative coupling. *RSC Adv.* **2020**, *10*, 10897–10903, doi:10.1039/D0RA00935K.
51. Bassanini, I.; Ferrandi, E.E.; Riva, S.; Monti, D. Biocatalysis with Laccases: An Updated Overview. *Catalysts* **2021**, *11*, 26, doi:10.3390/catal11010026.
52. Gunne, M.; Urlacher, V.B. Characterization of the Alkaline Laccase Ssl1 from Streptomyces sviceus with Unusual Properties Discovered by Genome Mining. *PLoS ONE* **2012**, *7*, e52360, doi:10.1371/journal.pone.0052360.
53. Gunne, M.; Höppner, A.; Hagedoorn, P.-L.; Urlacher, V.B. Structural and redox properties of the small laccase Ssl1 from Streptomyces sviceus. *FEBS J.* **2014**, *281*, 4307–4318, doi:10.1111/febs.12755.
54. Suljić, S.; Mortzfeld, F.B.; Gunne, M.; Urlacher, V.B.; Pietruszka, J. Enhanced Biocatalytic Performance of Bacterial Laccase from *Streptomyces sviceus*: Application in the Michael Addition Sequence Towards 3-Arylated 4-Oxochromanes. *ChemCatChem* **2015**, *7*, 1380–1385, doi:10.1002/cctc.201500142.
55. Zhao, J.; Lichman, B.R.; Ward, J.M.; Hailes, H.C. One-pot chemoenzymatic synthesis of trolline and tetrahydroisoquinoline analogues. *Chem. Commun.* **2018**, *54*, 1323–1326, doi:10.1039/C7CC08024G.
56. Suljić, S.; Pietruszka, J. Synthesis of 3-Arylated 3,4-Dihydrocoumarins: Combining Continuous Flow Hydrogenation with Laccase-Catalysed Oxidation. *Adv. Synth. Catal.* **2014**, *356*, 1007–1020, doi:10.1002/adsc.201300990.