Enantioselective N-Dealkylation

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Enantioselective Oxidative Aerobic Dealkylation of *N*-Ethyl Benzylisoquinolines by Employing the Berberine Bridge Enzyme

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Abstract: *N-Dealkylation methods are well described for organic chemistry and the reaction is known in nature and drug metabolism; however, to our knowledge, enantioselective N-dealkylation has not been yet reported. In this study, exclusively the (S)-enantiomers of racemic N-ethyl tertiary amines (1-benzyl-N-ethyl-1,2,3,4-tetrahydroisoquinolines) were dealkylated to give the corresponding secondary (S)-amines in an enantioselective fashion at the expense of molecular oxygen. The reaction is catalyzed by the berberine bridge enzyme, which is known for C–C bond formation. The dealkylation was demonstrated on a 100 mg scale and gave optically pure dealkylated products (ee > 99 %).*

N-Dealkylation has been exploited for organic synthesis,[1] for example, by employing oxometal (M = O) complexes[2] or low-valence transition metal complexes,[2a,3] generally to transform *N*,*N*-dialkylaniline or tetrahydroisoquinoline derivatives. High-valence transition-metal complexes (non-oxo) have also been investigated for this transformation,[4] as has acylative dealkylation of tertiary amines with carbon chloridates (chloroformates) as reagents.[5]

In nature, oxidative N-dealkylation is catalyzed by P450 monoxygenases,[6] peroxidases,[6a,7] and FAD-dependent enzymes such as trimethylamine dehydrogenase,[8] sarcosine oxidase[9] and dimethylglycine dehydrogenase.[9a] N-Dealkylation has been reported in drug metabolism studies, for example, for fenproporex[10] and verapamil,[11] where the reported enantioselectivity (E) was not significant (E < 2). Recently, a cascade was reported for the dealkylation of amines that requires two enzymes, namely a monoamine oxidase (MAO-N) and an ω-transaminase.[12]

Despite the many chemical as well as enzymatic methods reported, none of them enable enantioselective transformation. An enantioselective N-dealkylation would thus represent a novel method for organic chemistry.

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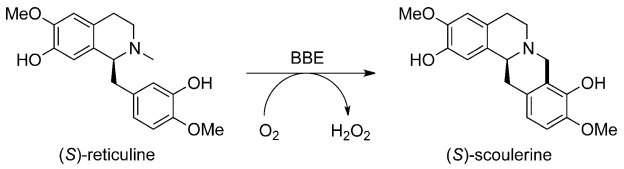
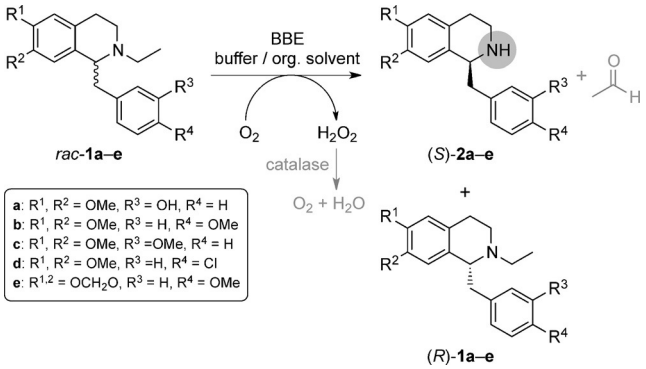
In an initial study, the aim was to extend the substrate scope of the C–C bond forming enzyme 'berberine bridge enzyme' (BBE; EC 1.21.3.3). In nature, BBE catalyzes the conversion of (*S*)-reticuline into (*S*)-scoulerine through oxidative C–C bond formation between the N–CH_3 and the phenol moiety at the expense of molecular oxygen (Scheme 1).[13] The oxidized bicovalently bound FAD cofactor abstracts a hydride from the *N*-methyl group, with concomitant attack of the phenol.[14]



Scheme 1. The natural reaction of BBE. The berberine bridge is formed through oxidative C–C coupling at the expense of molecular oxygen.

Since BBE, which originates from *Eschscholzia californica* (California poppy),[15] has been shown to enantioselectively transform various *N*-methyl reticuline derivatives into the corresponding berbines through C–C bond formation,[16] it was expected that *N*-ethyl derivatives **1**[17] might also be substrates for C–C coupling. To our surprise, the *N*-ethyl-1-benzyl-1,2,3,4-tetrahydroisoquinoline derivative **1a** did not lead to the expected enzymatic formation of a new C–C bond. In contrast, enzyme-catalyzed N-dealkylation to the corresponding secondary amine **2a** was observed (Scheme 2).

During the course of the reaction, the concentration of dealkylated product **2a** increased up to 25 % conversion before



Scheme 2. Biocatalytic enantioselective N-dealkylation of non-natural benzylisoquinoline alkaloids by BBE to give optically pure (*S*)-**2a**–**e** and (*R*)-**1a**–**f** as the main products through kinetic resolution.

additional follow-up products were formed, which were identified as the four stereo- and regioisomeric products of a spontaneous Pictet–Spengler reaction of **2a** with acetaldehyde,[18] a side product of the dealkylation. The formation of acetaldehyde was verified by GC and supported by a Purpald-based colorimetric assay (see the Supporting Information). From these data, we conclude that BBE catalyzes oxidative dealkylation.

To prevent the formation of the side products, an alternative substrate **1b**, which lacks the phenolic alcohol moiety essential for the spontaneous Pictet–Spengler reaction, was tested for N-dealkylation. Indeed, the transformation of **1b** led exclusively to the dealkylated product **2b**.

In a next step, the reaction conditions were optimized. The most suitable buffer was found to be 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) at pH 9 and containing 10 mM $MgCl_2$. The hydrogen peroxide formed as a byproduct was disproportionated to water and molecular oxygen by a catalase. Reactions were performed in the dark and the temperature was set to 40 °C. Since the non-natural substrates were barely soluble in buffer, various water-miscible and water-immiscible organic solvents (10 % *v*/*v*) were tested for substrate solubilization. In general DMSO (10 % *v*/*v*) turned out to be preferred at a substrate concentration of 10 mM.

Under optimized conditions, substrate *rac*-**1b** (10 mM) was transformed into **2b**, with 45 % conversion within 24 h.[19] Analysis of the optical purity revealed that the *ee* of the remaining substrate (**1b**) was 74 % in favor of the (*R*)-enantiomer and that the obtained product (*S*)-**2b** was optically pure (*ee* > 98 %). This corresponds to a kinetic resolution with an enantioselectivity of $E > 100$ (Table 1). Out of the racemic mixture, exclusively the (*S*)-enantiomer was transformed, while the (*R*)-isomer remained untouched.

Substrate *rac*-**1c**, which bears the methoxy substituent in the *meta* position of the benzyl group, led to clear conversion into the dealkylated product **2c**, although it was converted slowly, most likely due to steric hindrance in the active site by the methoxy group. In contrast, *N*-ethyl substituted derivatives bearing a chlorine atom at the *para* position of the benzyl group (*rac*-**1d**) or a methylenedioxy bridge at the isoquinoline core (*rac*-**1e**) were readily accepted. Both substrates were dealkylated with excellent stereoselectivity ($E > 100$), leading to optically pure secondary amines (*S*)-**2d** and (*S*)-**2e** (*ee* > 98 %; Table 1). Again, exclusively the (*S*)-enantiomer was transformed. These results also indicate that both electron-donating and electron-withdrawing substituents in the *para* position of the benzyl moiety in substrate **1** are readily accepted.

For comparison, related substrates possessing a *N*-methyl instead of the *N*-ethyl group were tested, although the compounds orientaline and isoorientaline (Figure 1) had already been reported not to be accepted by BBE.[20] Laudanosine, which bears two methoxy substituents on the isoquinoline core, was previously shown to be oxidized slowly at the C1–N bond to give a double bond in conjugation to the aromatic ring system, but no dealkylation was detected.[14a] Only for (*S*)-*N*-methylcoclaurine, 2 % dealkylation was reported within 6 h at 10 μM substrate concentration.[20] Consequently, it was unexpected that *rac*-**3a**, the *N*-methyl homologue to **1b**, was readily converted and enantioselectively dealkylated. Within 24 h, 47 % conversion was reached, giving (*S*)-**2b** in optically pure form (*ee* > 98 %) and the remaining substrate (*R*)-**3a** in 87 % *ee* ($E > 100$).

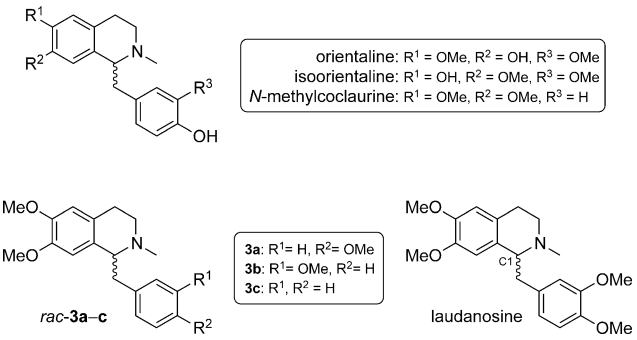


Figure 1. The 1-Benzyl-*N*-methyl-1,2,3,4-tetrahydroisoquinolines (**3**) investigated in the present study.

Table 1: Enantioselective N-dealkylation with BBE.[a]

Substrate	*c*[b] [%]	*ee* (**1**)[c] [%]	*ee* (**2**)[c] [%]	*E*[d]
rac-**1b**[e]	45	74 (*R*)	> 98 (*S*)	> 100
rac-**1c**	4	n.d.[h]	n.d.[h]	n.a.[i]
rac-**1d**[f]	30	41 (*R*)	> 98 (*S*)	> 100
rac-**1d**	28	21 (*R*)	> 98 (*S*)	> 100
rac-**1e**[f]	46	80 (*R*)	> 98 (*S*)	> 100
rac-**1e**[g]	42	63 (*R*)	> 98 (*S*)	> 100

[a] Reaction conditions: substrate *rac*-**1** (10 mM), Tris-HCl buffer (50 mM, containing 10 mM $MgCl_2$), pH 9, 10 % v/v DMSO, crude catalase preparation (5 mg mL^{-1}), BBE (5 mg mL^{-1}), glass vials (4 mL) in vertical position while shaking, reactions performed in the dark, 40 °C, 24 h. [b] Conversion was measured by HPLC on a C18 stationary phase. [c] Enantiomeric excess was measured by HPLC on a chiral stationary phase. [d] *E* value calculated from the *ee* of the substrate and product by using the online tool: http://biocatalysis.uni-graz.at/enantio/cgi-bin/enantio.pl. [e] Glass vials in horizontal positon while shaking. [f] Substrate concentration 5 mM. [g] Substrate concentration 5 mM, enzyme concentration 3 mg mL^{-1}, 10 % *v*/*v* DMF. [h] Not determined due to low conversion. [i] Not applicable.

Finally, the enantioselective dealkylation was performed on a preparative scale for substrates **1b** and **1e** (171 and 81 mg, respectively). After 48 h, the products of the kinetic resolution (*S*)-**2b** and (*S*)-**2e** were isolated with good yield and excellent optical purity (*ee* > 98 %; Table 2).

Table 2: Preparative enantioselective N-dealkylation with BBE.[a]

Substrate	c[b] [%]	(*R*)-**1** [% (mg)]	*ee* (**1**)[c] [%]	(*S*)-**2** [% (mg)]	*ee* (**2**)[c] [%]	E[d]
rac-**1b**	33	47 (80)	54 (*R*)	16 (24)	>98 (*S*)	>100
rac-**1e**	39	39 (32)	68 (*R*)	24 (18)	>98 (*S*)	>100

[a] Reactions were performed in buffer/DMSO (90:10, *v*/*v*), pH 9, at a substrate concentration of 10 mM for **1b** and 5 mM for **1e**, 3 mg mL^{-1} BBE for **1b** and 5 mg mL^{-1} BBE for **1e**, 5 mg mL^{-1} catalase, 40 °C, in the dark, 48 h. [b] Conversion was measured by HPLC on a C18 stationary phase. [c] Enantiomeric excess was measured by HPLC on chiral stationary phase. [d] *E* value determined from the *ee* of the substrate and product.

Alkaloids[21] such as benzylisoquinolines and their derivatives show a broad range of biological activities.[22] For instance, 1-benzyl-1,2,3,4-tetrahydroisoquinolines have been found to act as antispasmodic[23] or hypotensive agents.[24] Various chemical asymmetric synthetic strategies have been reported to prepare benzylisoquinoline alkaloids. In general, these require many steps and result in limited overall yields, and optically pure compounds are obtained only in rare cases.[25] Enantioselective dealkylation may become an alternative strategy for selected cases. However, dealkylation is not the natural activity of BBE but represents a promiscuous activity.[26,27]

In summary, the reaction presented herein is, to the best of our knowledge, the first reported enantioselective N-dealkylation for transforming *N*-ethyl benzylisoquinoline alkaloids and one *N*-methyl derivative into optically pure NH-benzylisoquinoline derivatives (>99% *ee*). The metal-free biocatalytic dealkylation was successfully verified on a preparative scale and required only molecular oxygen as the oxidant and mild reaction conditions. It thus represents a step towards cleaner and more selective organic transformations. This enantioselective dealkylation may develop into an alternative method in asymmetric synthesis.

Experimental Section

Representative preparative enantioselective N-dealkylation: Substrate **1b** (170.6 mg, 0.50 mmol, final concentration: 10 mM) was dissolved in DMSO (5 mL, final concentration: 10% *v*/*v*) and buffer (41.2 mL, 50 mM Tris-HCl, pH 9, 10 mM $MgCl_2$) with BBE-W165F (3.79 mL enzyme solution, final concentration: 3 mg mL^{-1}) and catalase (206 mg crude preparation). The reaction mixture was shaken in a light-shielded Erlenmeyer flask (250 mL) in a thermoshaker at 40 °C and 850 rpm for 48 h. Then the mixture was extracted with ethyl acetate (4×50 mL) and the phases were separated after centrifugation (4×15 min at 4000 rpm). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by preparative TLC (silica gel 60; hexane/EtOAc = 1:1 + 0.1% NH_4OH). The purification yielded 80 mg (47%) of **1b** and 24.4 mg (16%) of **2b**. For full characterization (NMR spectra, HPLC chromatograms, optical rotation, HRMS, and CD spectra) see the Supporting Information.

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