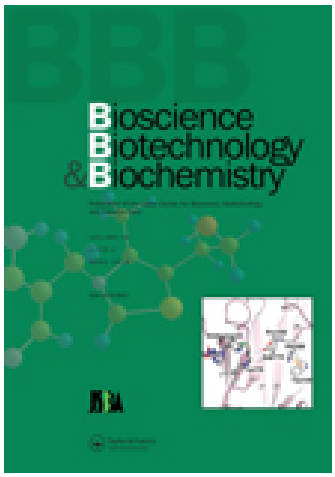

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Asymmetric synthesis of tetrahydroisoquinolines by enzymatic Pictet–Spengler reaction

Masakatsu Nishihachijo[1,*], Yoshinori Hirai[1], Shigeru Kawano[1], Akira Nishiyama[1], Hiromichi Minami[3], Takane Katayama[3], Yoshihiko Yasohara[2], Fumihiko Sato[4] and Hidehiko Kumagai[3]

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Norcoclaurine synthase (NCS) catalyzes the stereoselective Pictet–Spengler reaction between dopamine and 4-hydroxyphenylacetaldehyde as the first step of benzylisoquinoline alkaloid synthesis in plants. Recent studies suggested that NCS shows relatively relaxed substrate specificity toward aldehydes, and thus, the enzyme can serve as a tool to synthesize unnatural, optically active tetrahydroisoquinolines. In this study, using an *N*-terminally truncated NCS from *Coptis japonica* expressed in *Escherichia coli*, we examined the aldehyde substrate specificity of the enzyme. Herein, we demonstrate the versatility of the enzyme by synthesizing 6,7-dihydroxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline and 6,7-dihydroxy-1-propyl-1,2,3,4-tetrahydroisoquinoline in molar yields of 86.0 and 99.6% and in enantiomer excess of 95.3 and 98.0%, respectively. The results revealed the enzyme is a promising catalyst that functions to stereoselectively produce various 1-substituted-1,2,3,4-tetrahydroisoquinolines.

Key words: norcoclaurine synthase; Pictet–Spengler reaction; isoquinoline alkaloid

Isoquinoline alkaloids constitute a large group of secondary metabolites of higher plants. They include pharmaceutically important molecules such as the analgesic compound morphine, the antibacterial agent berberine, and the antispasmodic drug papaberine (Fig. 1). Although these compounds show variable structures, the synthetic pathway essentially involves (*S*)-norcoclaurine as a common intermediate (Fig. 1).[1] (*S*)-Norcoclaurine is produced by norcoclaurine synthase (NCS) (EC 4.2.1.78) that catalyzes the condensation and cyclization between dopamine (amine part) and 4-hydroxyphenylacetaldehyde (4-HPAA) (aldehyde part).[2] This reaction is the so-called Pictet–Spengler reaction, and in contrast to the chemical reaction, the NCS-catalyzed enzymatic reaction proceeds stereoselectively (Scheme 1). Therefore, if NCS can accommodate various aldehyde molecules as the substrates, the enzyme could serve as a promising biocatalyst for the production of unnatural, optically active 1-substituted-1,2,3,4-tetrahydroisoquinolines that are important starting materials in the pharmaceutical and agricultural fields.

Recently, synthesis of tetrahydroisoquinolines using NCS from *Thalictrum flavum* (*Tf*NCS) was reported.[3,4] *Tf*NCS was shown to recognize phenylacetaldehydes that are substituted with various electron-withdrawing or donating groups, heteroaromatic moieties, aromatic bicyclics, aliphatic cycles, and aliphatic open-chained compounds. In that study, however, the stereoselectivity of the reactions was not established, for example, enantiomeric excess was not determined (note that the Pictet–Spengler reaction occurs spontaneously in aqueous solution).

We have previously cloned the NCS gene (*Cj*PR10A; GenBank Accession No. AB267399) from *Coptis japonica* and used the enzyme to construct a bacterial platform for fermentative production of (*S*)-reticuline.[5–7] Herein, to further demonstrate the versatility of the enzymatic Pictet–Spengler reaction, we examined the aldehyde substrate specificity of *Cj*NCS and established an efficient method for synthesizing "optically active" 1-substituted-1,2,3,4-tetrahydroisoquinolines.

Materials and methods

Genetic techniques. General DNA manipulations were carried out as described by Sambrook and Russell[8] Plasmid DNA was purified from *Escherichia coli* using a QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany). Restriction enzymes, a DNA ligation kit, and DNA polymerase (PrimeStar® HS) were purchased from Takara Bio (Shiga, Japan).

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Abbreviations: NCS, Norcoclaurine synthase; e.e., enantiomeric excess; 4-HPAA, 4-hydroxyphenylacetaldehyde.

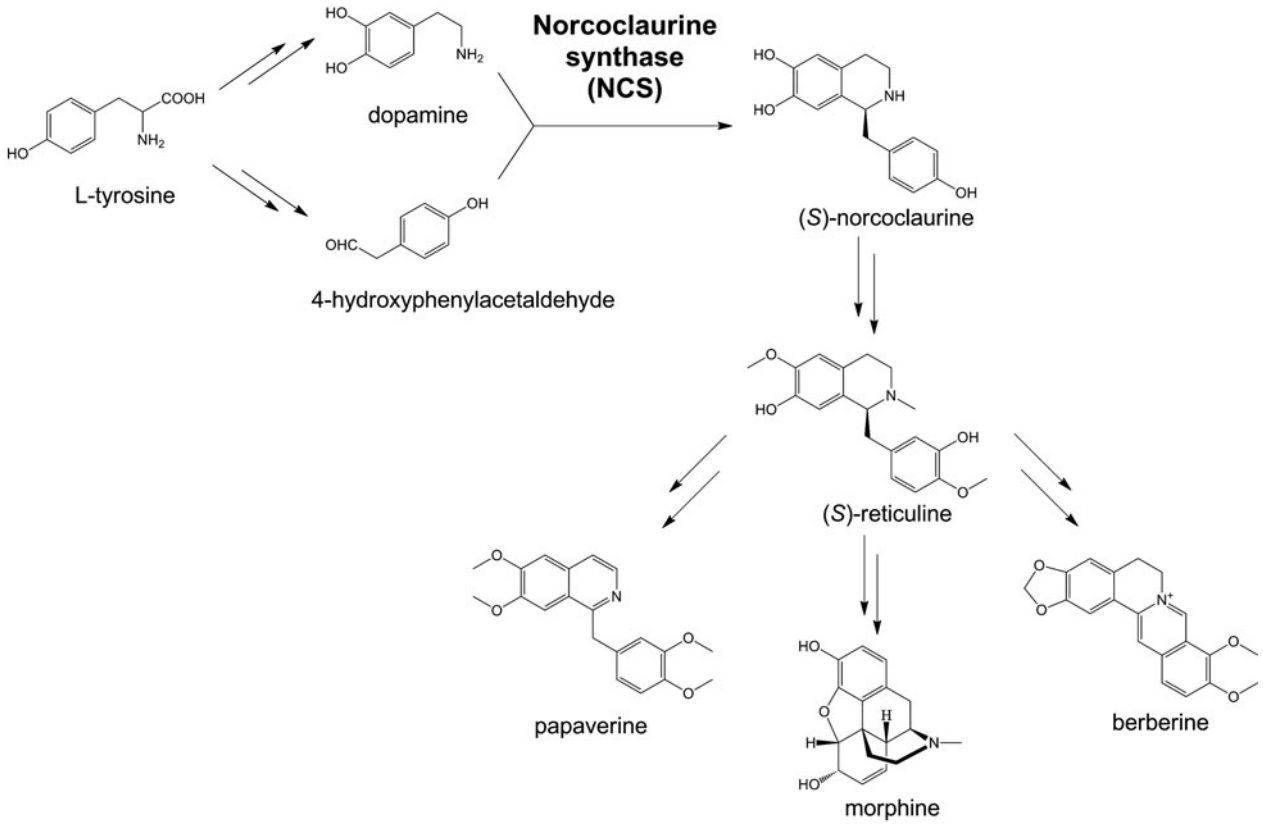


Fig. 1. Biosynthetic pathway of benzylisoquinoline alkaloids in plants.
Notes: Norcoclaurine synthase (NCS) is involved in the first committed step of benzylisoquinoline alkaloid synthesis. It catalyzes the condensation and cyclization between dopamine (amine part) and 4-hydroxyphenylacetaldehyde (aldehyde part), which is the so-called Enzymatic Pictet–Spengler reaction.

Expression of recombinant CjNCS protein. The pUC19-derived expression vector pSNT-*Cj*NCS was constructed as follows. First, the sequence of the ribosome-binding site of pUCNT,[9] which was developed by Nanba et al. and carries the *lac* promoter and *rrnBT1* terminator for protein expression, was changed to TAAGGAGGTT from CACAGGAAAC by site-directed mutagenesis. The resulting plasmid pSNT was digested with *Nde*I and *Pst*I, and ligated with a PCR-amplified, *Nde*I- and *Pst*I-digested DNA fragment containing the *Cj*NCS (*Cj*PR10A) gene. The primers used were 5′-CCATATGCGTATGGAAGTGGTTCT-3′ (forward) and 5′-TACTGCAGTTATTCGGAAGATTTGTG-3′ (reverse), and the template used was a plasmid carrying the codon-optimized *Cj*NCS gene that was synthesized by GenScript (Piscataway, NJ, USA). *N*-Terminally truncated *Cj*NCS variants were also constructed, in which the first 10, 19, 29, and 42 amino acid residues were removed. The forward primers were 5′-TCCATATGCTGATGTTCATTGGCACC-3′ (Δ10), 5′-TCCATATGGAACGCCTGATTTTTAA-3′ (Δ19), 5′TCCATATGCTGCATCGCGTTACGAAA-3′ (Δ29), and 5′-TCCATATGCACGAACTGGAAGTGGCG-3′ (Δ42). The reverse primer was the same as described above. The PCR-amplified DNA fragments were sequenced by the dideoxy chain termination method[10] to ensure that no base changes other than those planned had occurred.

The expression plasmid was introduced into *E. coli* HB101, and the transformants were cultured at 20 or 30 °C for 24 h in 2 × YT medium (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Ampicillin was added to a final concentration of 0.1 mg/mL. The recombinant cells were harvested by centrifugation, suspended in 100 mM Tris-HCl buffer (pH 7.0), and disrupted by sonication to obtain the cell-free extracts.

Analytical methods. The concentrations of dopamine and isoquinoline alkaloids were determined using high-performance liquid chromatography (HPLC) equipped with a Finepak SIL C18T-5 (4.6 mm I.D. × 250 mm) column (JASCO, Tokyo, Japan). Elution was carried out by water/acetonitrile (6/4, by volume) containing 3.2 g/L KH_2PO_4 and 1.5 g/L SDS (adjusted to pH 3.5 with phosphoric acid) at a flow rate of 1 mL/min and was monitored at 230 nm. The column was kept at 30 °C. The optical purity of 6,7-dihydroxy-1-propyl-1,2,3,4-tetrahydroisoquinoline and 6,7-dihydroxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline was determined using an HPLC equipped with a Chiralpak AD-H (4.6 mm I.D. × 250 mm) column (Daicel, Osaka, Japan). The column was eluted by *n*-hexane/ethanol/diethylamine (80/20/0.1, by volume) as the mobile phase. The column temperature was kept at 30 °C, and the elution was carried out at a flow rate of 1 mL/min and monitored at 230 nm.

^{1}H-nuclear magnetic resonance (NMR) spectra were recorded in CD_3OD or $CDCl_3$ using a FT-NMR JNM-ECA500 spectrometer (500 MHz; JEOL, Tokyo, Japan). The content of *Cj*NCS variants in the cell-free extract

of *E. coli* was estimated by SDS-polyacrylamide gel electrophoresis (10%), followed by Coomassie Brilliant Blue staining.

Enzyme assays. Catalytic activities of wild-type and *N*-terminally truncated versions of *Cj*NCS were determined by measuring the consumption of dopamine. The reaction mixture contained 100 mM Tris-HCl buffer (pH 7.0), 10 mM dopamine-HCl, 10 mM aldehyde, and 1% dimethyl sulfoxide (DMSO) in a total volume of 1 mL. The reaction was initiated by adding the enzyme, and the mixture was incubated at 30 °C for 15 min. The reaction was terminated by the addition of the elution buffer used for the HPLC analysis (see above), and the products were injected onto the column. Spontaneous cyclization (non-enzymatic Pictet–Spengler reaction) occurred under the test conditions; therefore, the control experiment was carried out in the absence of enzyme, and the amount of dopamine consumed was subtracted. One unit of enzyme activity was defined as the amount of enzyme that consumes 1 μmol of dopamine per min. The aldehyde part of the substrate 3-(4-trifluoromethylphenyl)-1-propylaldehyde (**3**) was prepared as described below. All the other aldehydes used in this experiment were of analytical grade and are commercially available.

*Preparation of 3-(4-trifluoromethylphenyl)-1-propylaldehyde (**3**).* 3-(4-Trifluoromethylphenyl)-1-propanol (90.6%, 8.27 g, 36.7 mmol) synthesized as described by Sifferlen et al.[11] was dissolved in 80 mL of ethyl acetate and sodium hydrogen carbonate (9.38 g, 0.112 mol), NaBr (3.83 g, 37.2 mmol), 2,2,6,6-tetramethylpiperidine 1-oxyl (0.291 g, 1.86 mmol), and water (80 mL) were added to the mixture at 0 °C. Hypochlorous acid water (13.0%, 21.28 g, 37.2 mmol) was then added dropwise, and the mixture was further stirred for 1.5 h at 0 °C. The product was extracted with ethyl acetate. The organic layer was washed with water (2 × 40 mL) and concentrated *in vacuo* to give 8.08 g of 3-(4-trifluoromethylphenyl)-1-propylaldehyde as a light orange oil. NMR δ_H ($CDCl_3$): 2.81(2H, d, $J = 7.3$ Hz), 3.01(2H, t, $J = 7.6$ Hz), 7.30(2H, d, $J = 8.0$ Hz), 7.53 (2H, d, $J = 8.1$ Hz), 9.81(1H, s).

*Preparation of racemic 6,7-dihydroxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline (**1a**).* 1-Phenethyl-6,7-dimethoxy-3,4-dihydroisoquinoline (1.48 g, 5 mmol) synthesized as described by De Vries et al.[12] was dissolved in 10 mL of methanol and stirred at 5 °C. Sodium borohydride (189 mg, 5 mmol) was then added, and the mixture was further stirred for 3 h at 25 °C. The reaction mixture was poured slowly to cooled water (10 mL) together with concentrated HCl (2.61 g, 25 mmol), and the pH was adjusted to 12 with 7.5 M NaOH. After concentration *in vacuo*, the product was extracted with ethyl acetate (20 mL). The organic layer was washed with water (10 mL) and concentrated *in vacuo* to give 1.58 g of 6,7-dimethoxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline as a light yellow oil. Demethylation was performed by adding hydrobromic acid (48%, 10.0 g, 59.3 mmol) to 6,7-dimethoxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline (1.06 g, 3.37 mmol). The reaction mixture was stirred for 16 h at 90 °C, cooled to room temperature, and the pH was adjusted to 7 with 7.5 M NaOH. Water (20 mL) and ethyl acetate (10 mL) were then added to the mixture, and it was stirred for 30 min at 5 °C to achieve crystallization. The crystals were filtered out and washed with water and dried at 50 °C *in vacuo* to give 520 mg of a racemic product (**1a**) as a light brown solid. NMR δ_H (CD_3OD): 1.86(2H, m), 2.1–2.7(4H, m), 3.02(1H, m), 3.27 (1H, m), 3.5–4.4 (2H, brs), 3.91 (1H, m), 6.57 (1H, s), 6.60 (1H, s), 7.1–7.3 (5H, m).

*Preparation of racemic 6,7-dihydroxy-1-propyl-1,2,3,4-tetrahydroisoquinoline (**2a**).* Racemic (**2a**) was prepared as described by Pesnot et al.[13] Dopamine-HCl (194 mg, 1.0 mmol) and 1-butylaldehyde (111 mg, 1.5 mmol) were added to 1.94 mL of 100 mM potassium phosphate buffer (pH 6.5) and stirred for 2 h at 40 °C. The reaction products were then extracted with 1-butanol (2 × 4 mL), and the extracts were dehydrated with sodium sulfate. The solvent was removed *in vacuo* to give 241 mg racemic product (**2a**) as a yellow solid. NMR δ_H (CD_3OD): 1.03 (3H, t), 1.51–1.54 (2H, m), 1.84–1.86 (1H, m), 1.97–2.00 (1H, m), 2.90 (1H, m), 2.96 (1H, m), 3.29–3.30 (1H, m), 3.46–3.50 (1H, m), 4.32–4.35 (1H, dd), 6.59 (1H, s), 6.64 (1H, s).

*Enzymatic synthesis of optically active 6,7-dihydroxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline (**1a**) and 6,7-dihydroxy-1-propyl-1,2,3,4-tetrahydroisoquinolines (**2a**).* The cell-free extract of *E. coli* strain expressing *Cj*NCS-Δ29 was incubated with 65.3 mM dopamine-HCl (10 mg/mL dopamine) and hydrocinnamaldehyde (added stepwise to a final 1.25 eq. of dopamine) at 30 °C for 16 h (for **1a**) or 1-butylaldehyde (added stepwise to a final 2.9 eq. of dopamine) at 30 °C for 1.5 h (for **2a**) in a total volume of 1 mL. In the control experiment, the cell-free extract was omitted. The reaction products were extracted with 1-butanol and the organic layer was concentrated *in vacuo*. The concentration and optical purity of the products were determined using HPLC as described above.

Results

*Expression of Cj*NCS in E. coli

In a previous study, we employed the T7 system for *Cj*NCS (*Cj*PR10A) expression; however, in this study, we newly constructed a pUC-derived expression vector because this expression system does not require an expensive inducer such as isopropyl-β-1-thiogalactopyranoside. This is important from the viewpoint of later commercially adopting the process.

NCS is predicted to be localized in a vesicular compartment.[5] SignalP server (http://www.cbs.dtu.dk/services/SignalP) predicted the presence of the signal peptide (first 19 amino acid resides) in the *Cj*NCS sequence. The *Cj*NCS protein possessing the signal peptide was not efficiently expressed in *E. coli* as

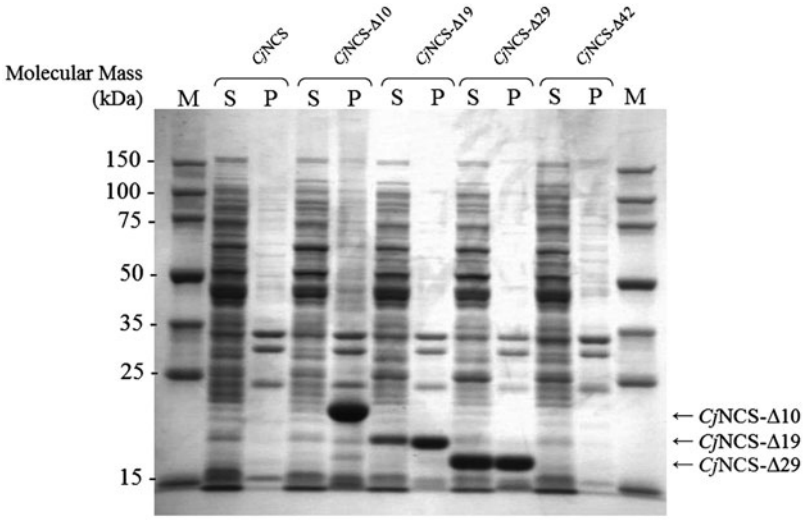


Fig. 2. SDS-polyacrylamide gel electrophoresis of the *E. coli* cell-free extracts expressing *Cj*NCS variants.
Notes: *N*-Terminally truncated versions of *Cj*NCS were constructed using a pUC-derived vector and expressed in *E. coli*. S: soluble fraction, P: precipitated fraction.

expected, and no corresponding band appeared in the SDS-polyacrylamide gel electrophoresis (Fig. 2). Reflecting this, quite low activity (0.03 U/mL) was detected in the cell-free extract when it was measured using dopamine and hydrocinnamaldehyde as the substrates. We then designed four *N*-terminally truncated *Cj*NCS variants to enhance the expression level in *E. coli*. Among the tested constructs (Δ10, Δ19, Δ29, and Δ42), *Cj*NCS-Δ29 was found to be highly expressed as a soluble form, although about half of the protein was still recovered in an insoluble fraction. The ratio of the soluble to insoluble fraction of recovered *Cj*NCS-Δ29 did not change when the cells were cultivated at low temperature (20 °C) and when the cells co-expressing chaperones (GroES and GroEL) were used as an expression host (data not shown). The activities of the *Cj*NCS variants apparently coincided with the amounts of proteins recovered in the soluble fractions (Table 1). The highest activity was obtained for *Cj*NCS-Δ29 (1.20 U/mL).

Substrate specificity of CjNCS-Δ29

NCS requires two substrates to form a tetrahydroisoquinoline skeleton, one being amine and the other aldehyde. *Tf*NCS was shown to strictly recognize the amine part of the substrate (only the 3'-hydroxyphenyl-2-ethylamine derivatives are accepted), while the recognition of the aldehyde part is much more relaxed.[14] We examined the substrate specificity of *Cj*NCS-Δ29 using dopamine and various aldehydes as substrates (Table 2). *Cj*NCS-Δ29 showed activity toward aryl and aliphatic aldehydes such as hydrocinnamaldehyde (**2**), 3-(4-trifluoromethylphenyl)-1-propylaldehyde (**3**), 2-phenylpropylaldehyde (**4**), 3-(4-isopropylphenyl)-isobutyraldehyde (**5**), 1-butylaldehyde (**6**), isobutyraldehyde (**7**), 1-pentylaldehyde (**8**), 1-hexylaldehyde (**9**), 1-heputylaldehyde (**10**), and glutaraldehyde (**11**). Among these non-physiological substrates, phenylacetaldehyde and hydrocinnamaldehyde were almost equally accepted and the most suitable. *Cj*NCS-Δ29 efficiently accepts aromatics with longer alkyl side chains in addition to 4-HPAA (physiological substrate) as the aldehyde part (82−99% relative to phenylacetaldehyde). *n*-Alkyl aldehydes were also efficient substrates (27−68%). However, the enzyme is less active toward branched-chain alkyl groups. These results support the idea that *Cj*NCS-Δ29 catalyzes the condensation and cyclization reaction between dopamine and various alkyl and aromatic aldehydes with considerable efficiency.

Preparative synthesis of optically active 1-substituted tetrahydroisoquinolines

We synthesized two unnatural, 1-substituted tetrahydroisoquinolines (Table 3). Incubation of *E. coli* cell-free extracts expressing *Cj*NCS-Δ29 with dopamine and hydrocinnamaldehyde yielded 19.0 g/L of 6,7-dihydroxy-1-phenethyl-1,2,3,4-tetrahydroisoquinoline (**1a**). The molar yield and enantiomeric excess (e.e.) of **1a** were 86.0 and 95.3%, respectively (Fig. 3). In contrast, in the control experiment without the enzyme, non-stereoselective spontaneous condensation and cyclization (non-enzymatic Pictet–Spengler reaction) between dopamine and hydrocinnamaldehyde was observed. In this case, the molar yield and e.e. were 10.6 and 9.4%, respectively (Table 3).

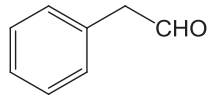
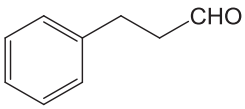
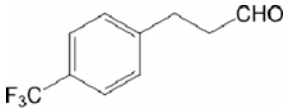
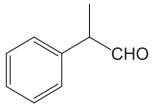
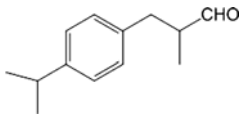
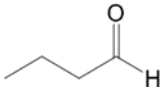
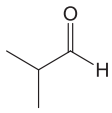
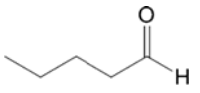
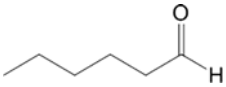
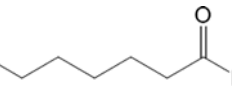
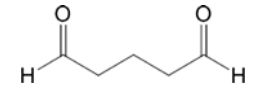
6,7-Dihydroxy-1-propyl-1,2,3,4-tetrahydroisoquinoline (**2a**) (13.5 g/L) was also synthesized using the

Table 1. Activity of *N*-terminally truncated *Cj*NCS.

Constructs	Estimated from SDS-PAGE[a]		Activity[b] (U/mL)
	Soluble form	Insoluble form	
*Cj*NCS	–	–	0.03
*Cj*NCS-Δ10	–	++++	0.10
*Cj*NCS-Δ19	+	++	0.30
*Cj*NCS-Δ29	+++	+++	1.20
*Cj*NCS-Δ42	–	–	0.09

[a]"–" indicates that the corresponding protein band was not observed, and "+" indicates the apparent quantity of the corresponding protein.
[b]Activity was measured using dopamine and hydrocinnamaldehyde as the substrates.

Table 2. Aldehyde substrate specificity of *Cj*NCS-Δ29.[a]

Entry	Substrate	Relative activity (%)
1		100
2		99
3		82
4		8
5		2
6		35
7		6
8		67
9		27
10		68
11		10

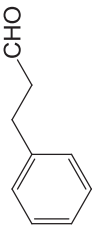
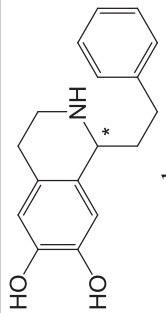
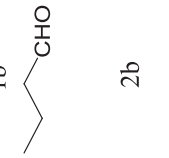
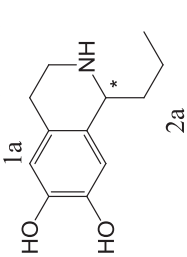
[a]Activity was determined by measuring the consumption of dopamine. In the control experiment, the enzyme was omitted from the reaction mixture (see Materials and Methods).

E. coli cell-free extracts expressing *Cj*NCS-Δ29 from dopamine and 1-butylaldehyde. The molar yield and e.e. of **2a** were determined to be 99.6 and 98.0%, respectively. This reaction took only 1.5 h to complete. The non-enzymatic reaction produced racemic **2a** with a molar yield and e.e. of 5 and 0.4%, respectively (Table 3).

Discussion

Chemical methods for preparing optically active 1-substituted tetrahydroisoquinolines have been developed, but they are not very efficient.[15–17] The theoretical maximum yield of the optical resolution of

Table 3. Enzymatic synthesis of optically active 1-substituted 1,2,3,4-tetrahydroisoquinolines.[a]

Entry	Substrate: Aldehyde	(eq.)	Product	Reaction time (h)	Enzyme	Molar yield (%)	Optical purity (% e.e.)	Accumulation (g/L)
1	 1b	1.25	 1a	16	*Cj*NCS-Δ29	86.0	95.3	19.0
2					none	10.6	9.4	–
3	 2b	2.90	 2a	1.5	*Cj*NCS-Δ29	99.6	98.0	13.5
4					none	5.0	0.4	–

[a]The reactions were carried out as described in the Materials and Methods. See also Fig. 4.

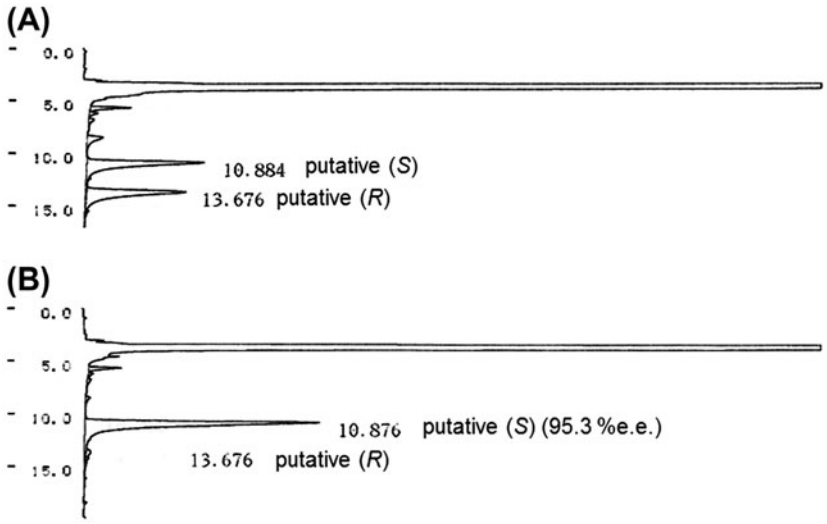


Fig. 3. Chiral HPLC analysis of the reaction products catalyzed by *Cj*NCS-Δ29.
Notes: Non-enzymatic (A) and enzymatic (B) Pictet–Spengler reactions catalyzed by NCS. Dopamine and hydrocinnamaldehyde were used as the substrates. Chiralpak AD-H column was used as described in the Materials and Methods.

Scheme 1. Enzymatic synthesis of unnatural 1-substituted 1,2,3,4-tetrahydroisoquinolines.

a racemic compound is 50%. The asymmetric reduction of 3,4-dihydroisoquinolines (prochiral imines) and the chemical asymmetric Pictet–Spengler reaction require expensive ligands and/or rare metals as catalysts or chiral auxiliaries. In contrast, an enzymatic Pictet–Spengler reaction catalyzed by NCS is capable of producing optically active 1-substituted tetrahydroisoquinolines in one step without using expensive reagents.

Functional expression of CjNCS-Δ29 in E. coli

From a practical viewpoint, it is important to prepare "a catalyst" easily and cost-effectively. In this study, we succeeded in overexpressing the *Cj*NCS protein in *E. coli* by deleting the first 29 amino acid residues. The expression system did not require an expensive inducer. The cell-free extract of *E. coli* expressing *Cj*NCS-Δ29 exhibited 40-fold higher activity than that expressing intact *Cj*NCS protein. Increase in the NCS expression level and the rate of its recovery in a soluble fraction by deleting the N-terminus has also been observed for *Tf*NCS, although the specific activity was not compared between the two variants.[18] Our results suggest that the N-terminal 29 amino acid residues of *Cj*NCS are dispensable for its functional expression. Interestingly, in the crystal structure of *Tf*NCS, the first 39 amino acid residues of the B chain in the asymmetric unit are not visible (PDB ID: 2VNE, 2VQ5).[19]

Aldehyde substrate specificity of CjNCS

*Cj*NCS-Δ29 efficiently recognized aromatic aldehydes and *n*-alkyl aldehydes as substrates (as judged from the consumption of dopamine). It also accepted a branched-chain alkyl group (α-substituted aldehydes, entries **4**, **5,** and **7** in Table 2), although the activity was significantly lower than that toward an *n*-alkyl group. These results agree well with a recent report by Pesnot et al., in which they showed using the intact, His-tagged form of *Cj*NCS that dopamine was consumed when incubated with aliphatic and aromatic aldehydes with a longer alkyl side chain. Isobutyraldehyde (**7**) was also recognized by the enzyme, albeit poorly.[20] On the contrary, Ruff et al. have reported that the condensation product between dopamine and an α-substituted aldehyde was not detectable when *Tf*NCS was used as a catalyst.[4] This apparent discrepancy might be caused by the difference in the enzyme source (*T. flavum* or *C. japonica*) or by the difference in the assay method (detection of dopamine consumption or detection of product). Therefore, in order to confirm whether *Cj*NCS can accept α-substituted aldehydes as substrates, it is necessary to isolate the condensation product of α-substituted aldehydes and dopamine.

Preparative synthesis of tetrahydroisoquinolines

Two optically active, 1-substituted 1,2,3,4-tetrahydroisoquinolines, **1a** and **2a**, were synthesized in high optical purity and high yield. Although we did not determine their steric configurations, the products are likely the (*S*)-enantiomer according to the proposed catalytic mechanism.[20] Production of **1a** is especially significant because its derivatives are in high demand as pharmaceutical intermediates.[21]

Interestingly, despite the relative activity of *Cj*NCS toward butylaldehyde (Table 2, entry 6) being lower than that for hydrocinnamaldehyde (entry 2), the yield of **2a** was higher than that of **1a** from the preparative synthesis. In addition, the reaction time required for the synthesis of **2a** was significantly shorter than that required for **1a**. We do not have a clear answer for this discrepancy; however, it might be a result of the different reaction conditions employed in the experiments, including substrate concentrations, ratio of dopamine to aldehyde, and the presence of DMSO.

Physiological implications

Phenethyl isoquinoline alkaloids such as dysoxyline and colchicine are rare species of plant secondary metabolites.[22,23] In an earlier work, Battersby et al. suggested that the synthetic pathway of these compounds involves condensation between dopamine and cinnamaldehyde[24]; however, cinnamaldehyde was not found to be accepted by *Cj*NCS.[20] In this report, we demonstrated that *Cj*NCS can synthesize 1-phenethyl tetrahydroisoquinoline from dopamine and hydrocinnamaldehyde. These results suggest that plants may use hydrocinnamaldehyde to synthesize phenethyl isoquinoline alkaloids via the involvement of NCS.

In summary, we succeeded in developing an efficient method for practically (industrially) producing unnatural, optically active 1-substituted tetrahydroisoquinolines, which should serve as useful chemicals for drug discovery purposes.

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