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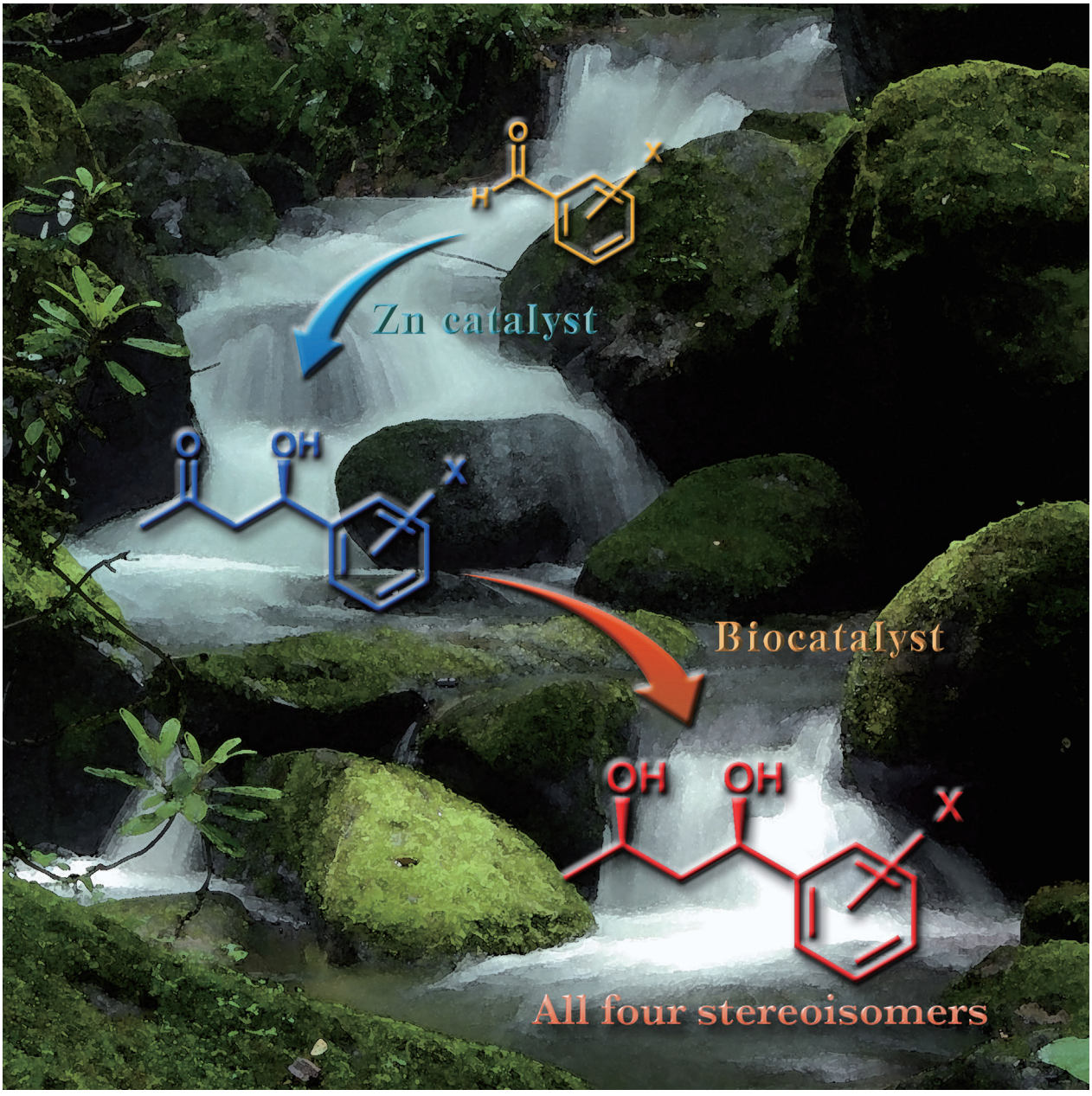
One-pot Chemoenzymatic Synthesis of Chiral 1,3-Diols Using an Enantioselective Aldol Reaction with Chiral Zn^{2+} Complex Catalysts and Enzymatic Reduction Using Oxidoreductases with Cofactor Regeneration

Shotaro Sonoike,[a] Toshinari Itakura,[a] Masanori Kitamura,[a, b] and Shin Aoki*[a, b]



Abstract: We previously reported on enantioselective aldol reactions of acetone and some aldehydes catalyzed by chiral Zn^{2+} complexes of L-prolyl-pendant [12]aneN$_4$ (L-ZnL^1) and L-valyl-pendant [12]aneN$_4$ (L-ZnL^2) in aqueous solution. Here, we report on the one-pot chemoenzymatic synthesis of chiral 1,3-diols in an aqueous solvent system at room temperature by a combination of enantioselective aldol reactions catalyzed by Zn^{2+} complexes of L- and D-phenylalanyl-pendant [12]aneN$_4$ (L-ZnL^3 and D-ZnL^3) and the successive enantioselective reduction of the aldol products using oxidoreductases with the regeneration of the NADH (reduced form of nicotinamine adenine dinucleotide) cofactor. The findings indicate that all four stereoisomers of 1,3-diols can be produced by appropriate selection of a chiral Zn^{2+}-complex and an oxidoreductase commercially available from the "Chiralscreen OH" kit.

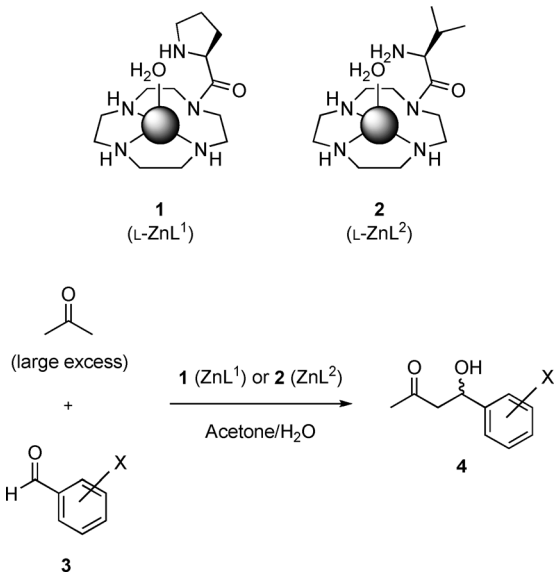
Keywords: aldol reaction · asymmetric catalysis · biocatalysis · water chemistry · zinc

Introduction

Chiral 1,3-diols are important intermediates in the synthesis of natural products, pharmaceutically active compounds, and related materials.[1] To date, numerous publications have appeared dealing with the stereoselective synthesis of 1,3-diols that contain two stereogenic centers,[2] involving asymmetric homogeneous and heterogeneous hydrogenation and diastereoselective reduction,[2,3] radical chain elongation,[4] enzymatic and non-enzymatic asymmetrization,[5] dynamic kinetic resolution,[6] and stereoselective aldol-Tishchenko reactions.[7] However, there is still substantial demand for stereoselective synthetic methods to produce all possible stereoisomers of chiral 1,3-diols.

We previously reported on chiral catalysts that are dually functionalized with chiral amino acids and achiral Zn^{2+} complexes of 1,4,7,10-tetraazacyclododecane ([12]aneN$_4$ or cyclen) such as L-prolyl-pendant [12]aneN$_4$ **1** (L-ZnL^1) and L-valyl-pendant [12]aneN$_4$ **2** (L-ZnL^2).[8] The findings indicate that **1** and **2** catalyze aldol reactions of acetone (and derivatives thereof) with benzaldehydes **3** such as 2-chlorobenzaldehyde in aqueous solution to give the corresponding aldol adducts **4** in good chemical and optical yields (Scheme 1). A mechanistic study strongly suggested that the amino acid portion and the Zn^{2+} ion of the catalysts act as a base and a Lewis acid, respectively, to generate a Zn^{2+}-enolate intermediate,[9] which is different from the enamine intermediate that is formed by organocatalysts such as L-proline.[10,11] Moreover, it was suggested that an enolate of acetone complexed with ZnL is more reactive than the enamine intermediate formed from ketone and L-proline.[12]

These results allowed us to develop a one-pot chemoenzymatic synthesis by the combined use of enantioselective aldol reactions catalyzed by chiral Zn^{2+} catalysts and stereoselective reduction by an oxidoreductase in an aqueous solvent. Despite the remarkable progress achieved in one-pot multistep synthetic methodologies,[13–15] only a few attempts have been made to combine a chemical catalyst and a biocatalyst in a one-pot multistep process.[16] For example, Gröger et al. reported on the synthesis of chiral biaryl-containing alcohols,[16e] the transformation of aromatic aldehydes into 1,3-diols[16c], and an enantioselective synthesis of ethyl (*S*)-3-aminobutanoate by means of combinations of chemical (enantioselective aldol reactions catalyzed by organocatalysts and cross-coupling reactions catalyzed by palladium catalyst)[16b,d] and biocatalytic reactions (enantioselective reduction and aminolysis).[16a]

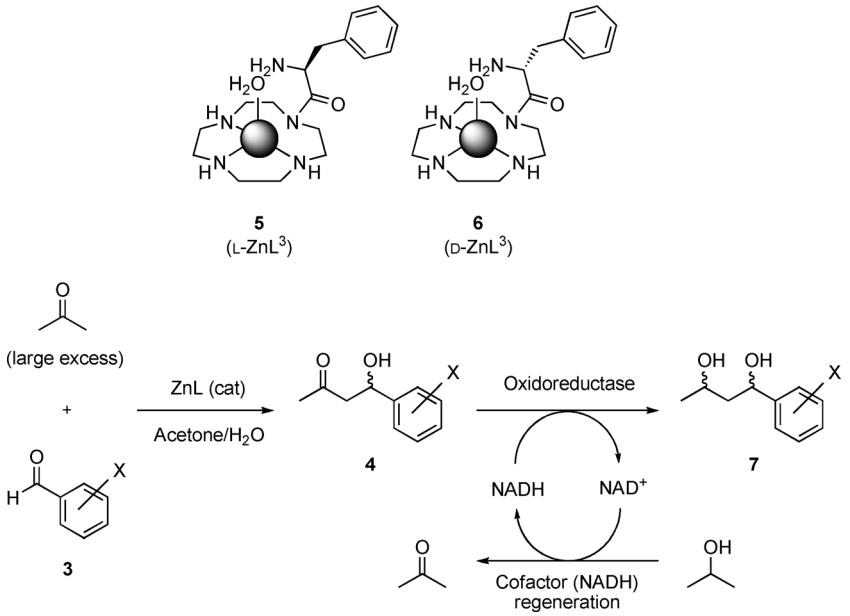


Scheme 1. Enantioselective aldol reaction of acetone with benzaldehyde catalyzed by chiral Zn^{2+} complexes.

Herein, we report on the selective synthesis of all possible stereoisomers of 1,3-diols **7** in a one-pot manipulation involving enantioselective aldol reactions of acetone with **3** to give **4** using chiral ZnL complexes, L-phenylalanyl- and D-phenylalanyl-pendant [12]aneN$_4$ (**5** (L-ZnL^3) and **6** (D-ZnL^3)), and the successive enantioselective reduction of **4** to give **7** using oxidoreductases, with the regeneration of the NADH (reduced form of nicotinamine adenine dinucleotide) cofactor (Scheme 2).[15a,17]

[a] S. Sonoike, T. Itakura, Dr. M. Kitamura, Prof. S. Aoki
Faculty of Pharmaceutical Sciences
Tokyo University of Science
2641 Yamazaki, Noda 278-8510 (Japan)
Fax: (+81) 4-7121-3670
E-mail: shinaoki@rs.noda.tus.ac.jp

[b] Dr. M. Kitamura, Prof. S. Aoki
Center for Technologies against Cancer
Tokyo University of Science
2641 Yamazaki, Noda 278-8510 (Japan)



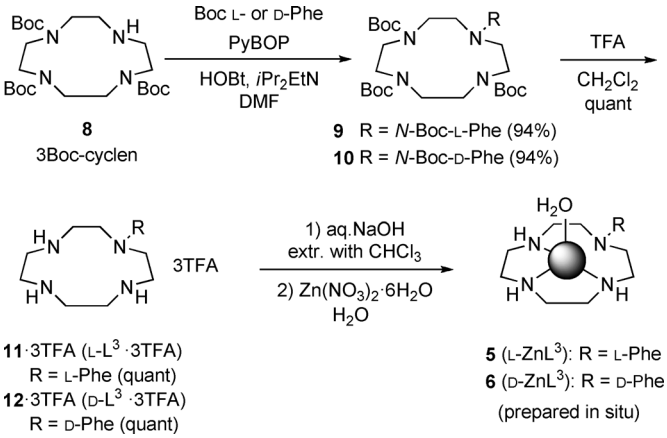
Scheme 2. One-pot synthesis of optically active 1,3-diols **7** by chemoenzymatic synthesis in an aqueous solvent, involving an enantioselective aldol reaction of acetone with **3** catalyzed by chiral Zn^{2+} complexes (ZnL) and the successive enzymatic reduction of **4** with regeneration of the NADH cofactor.

Results and Discussion

Synthesis of Phenylalanyl-Ligands (L-L^3 and D-L^3) and Their Deprotonation Constants and Zn^{2+} Complexation Behavior

We previously reported that **1** (L-ZnL^1) and **2** (L-ZnL^2) catalyze asymmetric aldol reactions of acetone with 2-chlorobenzaldehyde (**3a**) in aqueous solution to give the corresponding aldol adduct **4a** (X = *ortho*-Cl) in 72–73% yield with 80% to 89% *ee* (*R*). In this study, new chiral ligands, **11** (L-L^3) and **12** (D-L^3), were synthesized from 1,4,7-tris(-*tert*-butyloxycarbonyl)cyclen **8** (3Boc-cyclen)[18] via **9** or **10**, as shown in Scheme 3, to evaluate the effect of an aromatic ring on the side chain of the amino acid portion. The Zn^{2+} complexes of these ligands, **5** (L-ZnL^3) and **6** (D-ZnL^3), were prepared in situ by reacting acid-free **11** (L-L^3) or **12** (D-L^3) with $Zn(NO_3)_2$ (1.5 equiv vs. L^3), immediately before use.

The Zn^{2+} complexation properties of both enantiomers of L^3, **11**(L-L^3), and **12**(D-L^3), were studied by potentiometric pH titration. A typical potentiometric pH titration curve at 25 °C for 1 mM H_4(L-L^3) (prepared from 1 mM L-L^3·3TFA (TFA = trifluoroacetic acid) and 1 mM HNO_3) with 0.1 M NaOH in aqueous solution (with $I = 0.1$ ($NaNO_3$)) is shown as a dashed curve in Figure 1a. Analysis of this curve using the "BEST" program according to acid–base equilibrium, Equations (1)–(4), gave pK_a values of <3 (pK_{a1}), 5.5 ± 0.1 (pK_{a2}), 7.2 ± 0.1 (pK_{a3}), and 10.3 ± 0.1 (pK_{a4}), as summarized in Scheme 4 and Table 1.[8] An intrinsic complexation constant, log K_s(ZnL^3), and the deprotonation constant for the Zn^{2+}-bound H_2O of L-ZnL^3, pK_a(ZnL), defined by Equations (5) and (6) were determined to be 8.2 and 8.4, respectively, by an analysis of the pH titration curves for a mixture of H_4(L-L^3) and 1 mM $ZnSO_4$ (solid line curve in Figure 1a). The distribution diagram for a mixture of 50 mM **11** (L-L^3) and 50 mM Zn^{2+} in water is shown in Figure 1b. The pK_a, log K_s, and pK_a(ZnL) values of **11** (L-L^3) and **12** (D-L^3) were almost identical, as listed in Table 1. The apparent complexation constants, defined by Equations (7) and (8), log K_{app}(ZnL) for **11** (L-L^3) and **12** (D-L^3), at pH 7.4 were determined to be 5.6 and 5.7, which are almost the same as those of **1** (L^1) and **2** (L^2).



Scheme 3. Synthesis of chiral ligands, **11** (L-L^3) and **12** (D-L^3), and the corresponding Zn^{2+} complexes, **5** (L-ZnL^3) and **6** (D-ZnL^3).

$$H_4L \rightleftharpoons H_3L + H^+,\ K_{a1} = [H_3L][H^+]/[H_4L] \quad (1)$$

$$H_3L \rightleftharpoons H_2L + H^+,\ K_{a2} = [H_2L][H^+]/[H_3L] \quad (2)$$

$$H_2L \rightleftharpoons HL + H^+,\ K_{a3} = [HL][H^+]/[H_2L] \quad (3)$$

Abstract in Japanese:

我々はキラル亜鉛錯体が室温・水溶液中でエナンチオ選択的アルドール反応を触媒することを報告している。この水溶液中での機能を生かし、亜鉛錯体によるアセトンとベンズアルデヒド誘導体の不斉アルドール反応と、酸化還元酵素（Chiralscreen OH）を用いたNADHの再生を伴う不斉還元反応を組み合わせたone-pot合成の検討を行った。条件検討の結果、one-pot、2-stepで反応を行い、高収率・高立体選択的に1,3-diolを合成することができた。また、最適なキラル亜鉛錯体・酵素の選択によって1,3-diolの立体異性体４種類すべてを作り分けることに成功した。

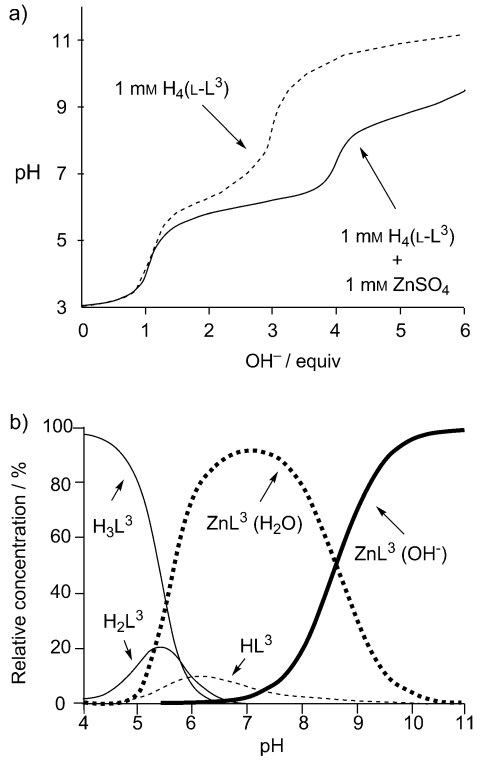
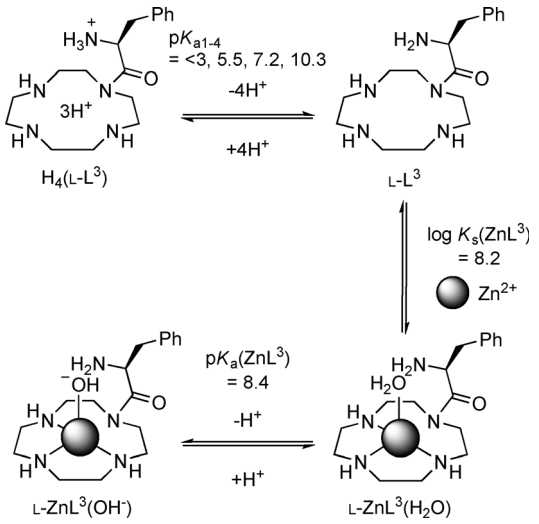


Figure 1. a) Typical potentiometric pH titration curves for a 1 mM **11** (H_4(L-L^3)) (dashed curve) and for a mixture of 1 mM H_4(L-L^3) and 1 mM $ZnSO_4$ (solid curve) with $I=0.1$ ($NaNO_3$) at 25 °C. b) Speciation diagram for a mixture of 50 mM **11** (H_4(L-L^3)) and 50 mM $ZnSO_4$ as a function of pH at 25 °C with $I=0.1$ ($NaNO_3$).



Scheme 4. Equilibria for deprotonation and Zn^{2+} complexation of **11** (L-L^3) and **12** (D-L^3).

$$HL \rightleftharpoons L+H^+,\ K_{a4} = [L][H^+]/[H_2L] \quad (4)$$

$$L+Zn^{2+} \rightleftharpoons ZnL\ (H_2O),\ K_s = [ZnL\ (H_2O)]/[L][Zn^{2+}] \quad (5)$$

Table 1. Deprotonation constants (pK_{ai}) and complexation constants [log K_s(ZnL)] of L-L^1, L-L^2, L-L^3, and D-L^3 with $I=0.1$ ($NaNO_3$) at 25 °C.[a]

	L-L^{1}[b]	L-L^{2}[b]	L-L^3	D-L^3
pK_{a1}	<3	<3	<3	<3
pK_{a2}	5.7	5.6	5.5	5.6
pK_{a3}	8.8	7.6	7.2	7.0
pK_{a4}	9.7	10.9	10.3	10.6
log K_s(ZnL)	7.6	9.6	8.2	8.5
	(for ZnL^1)	(for ZnL^2)	(for L-ZnL^3)	(for D-ZnL^3)
pK_a(ZnL)	8.2	8.6	8.4	8.7
log K_{app}(ZnL)	5.1	5.7	5.6	5.7

[a] See the text for definitions of pK_a, log K_s (ZnL), and pK_a (ZnL). [b] From ref. [8].

$$ZnL\ (H_2O) \rightleftharpoons ZnL\ (OH^-)+H^+,\quad K_a\ (ZnL) = [ZnL\ (OH^-)][H^+]/[ZnL\ (H_2O)] \quad (6)$$

$$K_{app}(ZnL) = [(ZnL\ (H_2O)+ZnL\ (OH^-))]/[L]_{free}[Zn^{2+}]_{free} \quad (7)$$

$$[L]_{free} = [H_4L]+[H_3L]+[H_2L]+[HL]+[L] \quad (8)$$

Enantioselective Aldol Reactions in Aqueous Media Catalyzed by Chiral Zn^{2+} Complexes

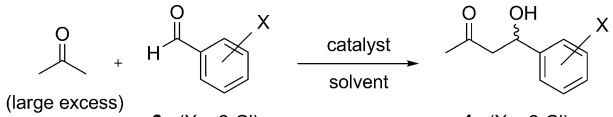
On the basis of the aforementioned Zn^{2+} complexation behavior, we carried out aldol reactions of acetone and 2-chlorobenzaldehyde (**3a**), 4-chlorobenzaldehyde (**3b**), or 4-nitrobenzaldehyde (**3c**) in acetone/H_2O in the presence of 50 mM ZnL catalysts (it is considered that ZnL is formed with >95% at this concentration). The results are summarized in Table 2.

As reported by List, Lerner, and Barbas III, L-proline (20 mol% relative to the aldehyde) gave **4a** in good chemical yield (85%) and enantioselectivity (67% *ee* (*R*)) in dimethyl sulfoxide (DMSO; Table 2, entry 1),[8] while its chemical and optical yields were lowered when an acetone/H_2O mixture was used (Table 2, entry 2). As previously reported, **1** (L-ZnL^1) and **2** (L-ZnL^2) gave **4a** with moderate enantioselectivity (80–89% *ee*) in acetone/H_2O (Table 2, entries 3 and 4).[8] The new catalysts **5** (L-ZnL^3) and **6** (D-ZnL^3) (5 mol% vs. **3a**) gave **4a** in somehow higher chemical yields than those for **1** and **2** (Table 2, entries 3 and 4) with almost the same enantioselectivity (91% *ee* (*R*) with **5** (L-ZnL^3) and 91% *ee* (*S*) with **6** (D-ZnL^3)) (Table 2, entries 5 and 7). When the number of equivalents of **5** and **6** were increased to 10 mol%, the product **4a** was obtained in almost quantitative yield (Table 2, entries 6 and 8). Similarly, 4-chlorobenzaldehyde **3b** and 4-nitrobenzaldehyde **3c** gave the corresponding 1,2-adducts **4b** and **4c**, with almost identical chemical and optical yields as **3a** (Table 2, entries 9–12).

Enantioselective Reductions of β-Hydroxyketones 4a–c using Oxidoreductase

For the reduction of β-hydroxyketones **4a–c**, we first chose Baker's yeast alcohol dehydrogenase (ADH) and oxidore-

Table 2. The results of asymmetric aldol reactions of acetone with benzaldehydes (**3a–c**) catalyzed by **1**, **2**, **5**, and **6**.



3a (X = 2-Cl)
3b (X = 4-Cl)
3c (X = 4-NO_2)

4a (X = 2-Cl)
4b (X = 4-Cl)
4c (X = 4-NO_2)

Entry	Aldehydes	Catalyst [mM][a]	mol %[b]	Solvent	Conditions	Product (yield [%][c])	CTN[d]	*ee* [%][e]
1	**3a**	L-proline (50)[f]	20	DMSO/acetone (4:1)	4 h, 25 °C	**4a** (85)	4	67 (*R*)
2	**3a**	L-proline (50)[f]	5	acetone/H_2O (4:1)	20 h, 25 °C	**4a** (22)	4	48 (*R*)
3	**3a**	**1** (L-ZnL^1) (50)[g]	5	acetone/H_2O (4:1)	24 h, 25 °C	**4a** (73)	14	80 (*R*)
4	**3a**	**2** (L-ZnL^2) (50)[g]	5	acetone/H_2O (4:1)	24 h, 25 °C	**4a** (72)	14	89 (*R*)
5	**3a**	**5** (L-ZnL^3) (50)[g]	5	acetone/H_2O (4:1)	24 h, 25 °C	**4a** (85)	17	91 (*R*)
6	**3a**	**5** (L-ZnL^3) (50)[g]	10	acetone/H_2O (4:1)	24 h, 30 °C	**4a** (quant)	10	90 (*R*)
7	**3a**	**6** (D-ZnL^3) (50)[g]	5	acetone/H_2O (4:1)	24 h, 25 °C	**4a** (85)	17	91 (*S*)
8	**3a**	**6** (D-ZnL^3) (50)[g]	10	acetone/H_2O (4:1)	24 h, 30 °C	**4a** (quant)	10	90 (*S*)
9	**3b**	**5** (L-ZnL^3) (50)[g]	10	acetone/H_2O (4:1)	72 h, 30 °C	**4b** (quant)	10	90 (*R*)
10	**3b**	**6** (D-ZnL^3) (50)[g]	10	acetone/H_2O (4:1)	72 h, 30 °C	**4b** (quant)	10	90 (*S*)
11	**3c**	**5** (L-ZnL^3) (50)[g]	10	acetone/H_2O (4:1)	24 h, 30 °C	**4c** (quant)	10	90 (*R*)
12	**3c**	**6** (D-ZnL^3) (50)[g]	10	acetone/H_2O (4:1)	24 h, 30 °C	**4c** (quant)	10	90 (*S*)

[a] Numbers in parentheses are the concentrations of catalyst in the solvent system (acetone/H_2O). [b] Mol % of catalyst vs. aldehyde. [c] Isolated yield. [d] Catalytic turnover number (=chemical yield/equivalents of catalyst). [e] Determined by HPLC analysis using a chiral column (Chiralpak AD-H for **4a**, Chiralpak AD-H for **4b**, and Chiralcel OJ-H for **4c**). [f] From ref. [8]. [g] Chiral ligands were extracted with $CHCl_3$ from an aq. NaOH (pH > 12) solution prior to use and mixed with $Zn(NO_3)_2$ in situ.

ductases from *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Lactobacillus kefir* (*L. kefir*) because these enzymes have been reported to catalyze the enantioselective reduction of 4-phenyl-4-hydroxy-2-butanone (**4d**).[5a,16c,19] However, Baker's yeast ADH and *S. cerevisiae* ADH were not very effective for the reduction of **4a** (Table 3, entries 1 and 2). In entries 3 and 4, it was found that the ADH from *L. kefir* is effective for the stereoselective reduction of **4a** and **4b**, albeit this enzyme produces only the (*R*)-form of **7a** and **7b** (Table 3, entries 3 and 4).

We thus decided to test the Chiralscreen OH kit, which is available from Daicel Co., Ltd, Japan,[20] and contains a library of recombinant NADH-dependent oxidoreductases. Generally, oxidoreductases require an equivalent amount of NAD(P)H (reduced form) for activity. The reductases in Chiralscreen OH themselves can reduce NAD^+ (oxidized form) to NADH using 2-propanol as a hydride source, so that the concentration of NADH can be reduced to a catalytic amount.[17,20] It has been also reported that Chiralscreen OH can be used to catalyze the reduction of a variety of ketones even if the solubility of the substrate is low in aqueous solution.[20a]

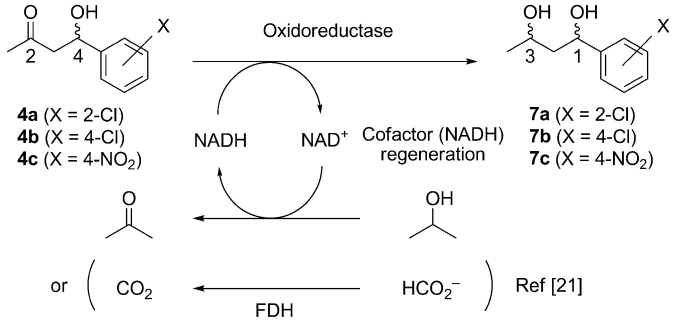
The results of the stereoselective reduction of racemic **4a–c** using Chiralscreen OH enzymes in 100 mM phosphate buffer (pH 7.2) at 30 °C are summarized in Table 3. Among the nine enzymes of Chiralscreen OH tested (E001, 021, 031, 039, 041, 051, 057, 092, 119), four enzymes such as E001, E031, E039, and E092, were found to be effective for the reduction of **4a** (Table 3, entries 5–13). In general, E001 and E039 gave better chemical yields than E031 and E092 (Table 3, entries 5 and 8 vs. entries 7 and 12).[21] Interestingly, it was found that E001 and E039 gave (*S*)- and (*R*)-forms of **7a** (99 % *ee*), respectively, with respect to the stereogenic center (position 3) in **7a**.[22] It should also be noted that the *syn*/*anti* ratios of **7a** were almost 1:1, thus indicating that kinetic resolution negligibly occurred (except for E092). It was also found that **4b** and **4c** are converted into **7b** and **7c** by E001 and E039, respectively (Table 3, entries 14–17),[22] and the reduction of (*S*)-**4a** (90 % *ee*) with E001 exclusively gave *anti*-**7a** ((1*S*,3*S*)-**7a**) as the main product (Table 3, entry 18).[23]

One-pot Chemoenzymatic Synthesis of Optically Active 1,3-Diols 7a–c from Acetone and Benzaldehydes 3a–c

Based on the aforementioned results dealing with enantioselective chemical aldol reactions and enzymatic reductions, we carried out the one-pot synthesis of 1,3-diols **7a–c** from acetone and benzaldehydes **3a–c**. The enantioselective aldol reaction of acetone and **3** with ZnL^3 (L-ZnL^3 or D-ZnL^3) (10 mol % relative to **2**) was conducted in acetone/H_2O to give **4**. The reaction mixture was then diluted with phosphate buffer (100 mM, pH 7.2), and the enzyme, NAD^+, and 2-propanol were added for the reduction of the aldol product **4**.

The results are summarized in Table 4. The aldol reaction of **3a** with acetone in the presence of **5** (L-ZnL^3) and successive reduction by E001 gave **7a** in 88 % yield with a *syn*/*anti* ratio of approximately 4:96 ((1*R*,3*S*)-**7a** is the major isomer; as listed in Table 4, entry 1). Employing E039 instead of E001 switched the product to (1*R*, 3*R*)-**7a** (Table 4, entry 2). The use of **6** (D-ZnL^3) with E001 and E039 gave (1*S*,3*S*)-**7a** and (1*S*,3*R*)-**7a**, respectively, with >99 % *ee* (Table 4, entries 3 and 4). These results suggest that all four stereoiso-

Table 3. Results for the asymmetric reduction of β-hydroxyketones **4a–c** catalyzed by oxidoreductase with NADH regeneration in 100 mM phosphate buffer (pH 7.2) at 30 °C for 1 day.



Entry	Substrate	Oxidoreductase[a]	product[c] (*syn*/*anti*)	Yield [%][b]	*ee* [%][c] (*syn*/*anti*)	3*R*/3*S*[c]
1	*rac*-**4a**[d]	Baker's yeast	–	trace	–	–
2	*rac*-**4a**[d]	ADH from *S. cerevisiae*	–	trace	–	–
3	*rac*-**4a**[d]	ADH from *L. Kefir*	**7a** (52/48)	quant	>99 % (1*R*, 3*R*)/>99 % (1*S*, 3*R*)	>99/<1
4	*rac*-**4b**[d]	ADH from *L. Kefir*	**7b** (49/51)	quant	>99 % (1*R*, 3*R*)/>99 % (1*S*, 3*R*)	>99/<1
5	*rac*-**4a**[d]	E001[e]	**7a** (52/48)	quant	>99 % (1*S*, 3*S*)/>99 % (1*R*, 3*S*)	<1/>99
6	*rac*-**4a**[d]	E021[e]	–	trace	–	–
7	*rac*-**4a**[d]	E031[e]	**7a** (47/53)	50	95 % (1*S*, 3*S*)/93 % (1*R*, 3*S*)	3/97
8	*rac*-**4a**[d]	E039[e]	**7a** (48/52)	quant	>99 % (1*R*, 3*R*)/>99 % (1*S*, 3*R*)	>99/<1
9	*rac*-**4a**[d]	E041[e]	–	trace	–	–
10	*rac*-**4a**[d]	E051[e]	–	trace	–	–
11	*rac*-**4a**[d]	E057[e]	–	trace	–	–
12	*rac*-**4a**[d]	E092[e]	**7a** (78/22)	64	>99 % (1*S*, 3*S*)/94 % (1*R*, 3*S*)	1/99
13	*rac*-**4a**[d]	E119[e]	–	trace	–	–
14	*rac*-**4b**[d]	E001[e]	**7b** (50/50)	quant	>99 % (1*S*, 3*S*)/>99 % (1*R*, 3*S*)	<1/>99
15	*rac*-**4b**[d]	E039[e]	**7b** (49/51)	quant	>99 % (1*R*, 3*R*)/>99 % (1*S*, 3*R*)	>99/<1
16	*rac*-**4c**[d]	E001[e]	**7c** (50/50)	quant	>99 % (1*S*, 3*S*)/>99 % (1*R*, 3*S*)	<1/>99
17	*rac*-**4c**[d]	E039[e]	**7c** (48/52)	quant	>99 % (1*R*, 3*R*)/>99 % (1*S*, 3*R*)	>99/<1
18	**4a** (90 % *ee* (*S*))	E001[e]	**7a** (95/5)	quant	>99 % (1*S*, 3*S*)/>99 % (1*R*, 3*S*)	<1/>99

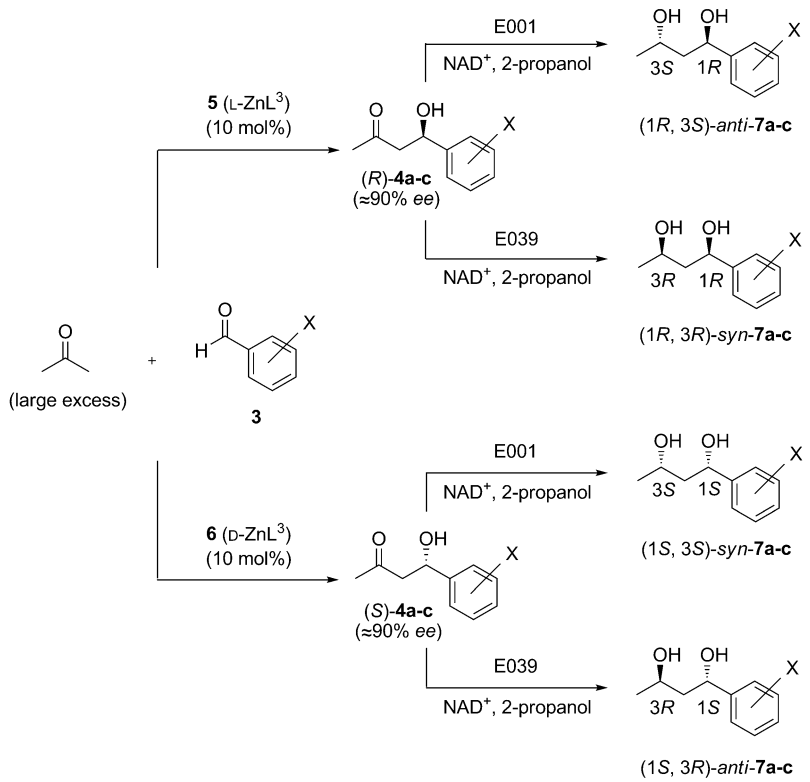
[a] Conditions for the enzymatic reductions: [ketone] = 10 mM in 100 mM phosphate buffer, 24 h, 30 °C in the presence of 2-propanol (100 mM) and NAD^+ (2 mM). [b] Yield of isolated product. [c] Determined by ^1H NMR and HPLC analysis using a chiral column (Chiralcel OD-H for **7a**, Chiralcel OJ-H for **7b**, and Chiralpak AD-H for **7c**). [d] Racemate of **4a** was used as the substrate. [e] Enzyme of Chiralscreen purchased from Daicel Co., Ltd.

Table 4. Results for the one-pot chemoenzymatic synthesis of chiral 1,3-diols **7a–c** in aqueous solvent at room temperature by the combined use of an enantioselective aldol reaction catalyzed by chiral Zn^{2+}-complex catalysts and an enantioselective reduction using Chiralscreen OH.

Entry	Substrate	ZnL[a]	Oxidoreductase[b] (Chiralscreen OH)	Product	Yield [%][c]	Product ratio[d]			
						(1*R*, 3*R*)	(1*S*, 3*R*)	(1*R*, 3*S*)	(1*S*, 3*S*)
1	**3a**	**5** (L-ZnL^3)	E001	**7a**	88	<1	<1	96	4
2	**3a**	**5** (L-ZnL^3)	E039	**7a**	88	95	5	<1	<1
3	**3a**	**6** (D-ZnL^3)	E001	**7a**	84	<1	<1	4	96
4	**3a**	**6** (D-ZnL^3)	E039	**7a**	92	5	95	<1	<1
5	**3b**	**5** (L-ZnL^3)	E001	**7b**	68	<1	<1	96	4
6	**3b**	**5** (L-ZnL^3)	E039	**7b**	60	96	4	<1	<1
7	**3b**	**6** (D-ZnL^3)	E001	**7b**	60	<1	<1	5	95
8	**3b**	**6** (D-ZnL^3)	E039	**7b**	48	4	96	<1	<1
9	**3c**	**5** (L-ZnL^3)	E001	**7c**	83	<1	<1	93	7
10	**3c**	**5** (L-ZnL^3)	E039	**7c**	87	94	6	<1	<1
11	**3c**	**6** (D-ZnL^3)	E001	**7c**	91	<1	<1	4	96
12	**3c**	**6** (D-ZnL^3)	E039	**7c**	80	4	96	<1	<1

[a] ZnL complexes were formed in situ. [b] Conditions for reductions by enzymes: [ketone] = 10 mM in 100 mM phosphate buffer (pH 7.2), 24 h, 30 °C in the presence of 2-propanol (100 mM) and NAD^+ (2 mM). [c] Yield of isolated product. [d] Determined by HPLC analysis of a mixture of all stereoisomers by column chromatography using a chiral column (Chiralcel OD-H for **7a**, Chiralpak AD-H for **7b**, and Chiralcel OJ-H for **7c**).

mers of 1,3-diols can be prepared by selecting the appropriate chiral Zn^{2+}-complexes and dehydrogenases. Similarly, entries 5–8 and 9–12 indicate that 4-chlorobenzaldehyde **3b** and 4-nitrobenzaldehyde **3c** can be converted into all possi-



Scheme 5. Summary of the one-pot chemoenzymatic synthesis of 1,3-diols **7a–c** from acetone and benzaldehydes **3a–c**.

ble stereoisomers of the corresponding 1,3-diols, **7b** and **7c** (Scheme 5).[22]

Conclusions

In this work, we report on the one-pot synthesis of optically active 1,3-diols **7** by enantioselective aldol reactions of acetone with **3** catalyzed by chiral Zn^{2+} complexes, **5** and **6**, to afford the 1,2-adducts **4**, and the successive reduction of **4** by a recombinant oxidoreductase from Chiralscreen OH. For example, a one-pot chemoenzymatic synthesis from acetone and **3a** with **5** (L-ZnL^3) and E039 afforded (1*R*, 3*R*)-**7a** in 88% yield with 99% *ee*. Using these methodologies, all possible stereoisomers of **7a–c** were obtained by the appropriate selection of the ZnL aldol catalyst and dehydrogenase. These results outline a strategy that can be useful for the design of new one-pot methodologies for stereoselective organic reactions in aqueous solutions.

Experimental Section

General Procedures

All reagents and solvents were of the highest commercial quality and were used without further purification, unless otherwise noted. Acetone was dried over anhydrous $CaSO_4$ and distilled. 2-Chlorobenzaldehyde was washed with aqueous 10% Na_2CO_3 and then distilled. 4-Chlorobenzaldehyde and 4-nitrobenzaldehyde were purified by sublimation. $Zn(NO_3)_2 \cdot 6H_2O$ was purchased from Wako Chemical Co. Baker's yeast ADH was purchased from a nearby bakery and oxidoreductases from *S. cerevisiae* and *L. kefir* were purchased from Sigma. Chiralscreen OH (E001, 021, 031, 039, 041, 051, 057, 092, 119) was purchased from Daicel Co., Ltd, Japan. All aqueous solutions were prepared using deionized and distilled water. Buffer solutions (phosphate, pH 7.2 and HEPES, pH 7.4) were used. HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid, pK_a = 7.6 at 20 °C) was obtained from Dojindo. HPLC was carried out using an Intelligent UV/VIS detector (JASCO UV-2075), a quaternary gradient pump JASCO PU-2089, and a SHIMADZU Chromatopac C-R8A data processor. Optical rotations were measured with a JASCO P-1030 digital polarimeter in 50 mm cells using the D line of sodium (589 nm). ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a JEOL Always 300 spectrometer. Chemical shifts (δ) in $CDCl_3$ were determined relative to an internal reference of tetramethylsilane (TMS) (for ^{1}H NMR) or $CDCl_3$ (for ^{13}C NMR). Chemical shifts (δ) in D_2O were determined relative to an external reference of 2,2,3,3-[D_4]-3-(trimethylsilyl)propionic (TSP) sodium salt for ^{1}H NMR or [D_8]1,4-dioxane for ^{13}C NMR. TMS was used as an internal reference for ^{1}H NMR measurements in CD_3OD. Thin-layer chromatography (TLC) and silica-gel column chromatography were performed using aluminum-backed silica gel TLC plates (Merck-5554) and FL-100D silica gel (Fuji Silysia Chemical Ltd.), respectively. IR spectra were recorded at room temperature on a Spectrum 100 FT-IR spectrophotometer (Perkin–Elmer). MS measurements were performed on a JEOL JMX-SX102A and on a Varian 910 mass spectrometer.

*1,4,7-Tris(tert-butyloxycarbonyl)-10-(N-tert-butyloxycarbonyl-(S)-phenylalanyl-1,4,7,10-tetraazacyclododecane (**9**)*

1-benzotriazolyloxy-tris(pyrollidino)phosphonium (PyBop; 722 mg, 1.39 mmol) and *i*Pr_2NEt (356 mg, 2.76 mmol) were added to a solution of **8**[18] (437 mg, 0.92 mmol), *N*-tert-butyloxycarbonyl-L-phenylalanine (368 mg, 1.39 mmol), and HOBt (212 mg, 1.39 mmol) in anhydrous DMF (10 mL) at 0 °C, and the entire reaction mixture was stirred at room temperature for 72 h and then diluted with $CHCl_3$. The organic layer was washed with H_2O, sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica-gel column chromatography (hexane/AcOEt = 3:1) to give **9** as a colorless amorphous solid (519 mg, 83% yield). M.p. 109–111 °C; $[\alpha]_D^{23} = +55.0$ ($c = 1.00$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): $\delta = 1.38$–1.50 (m, 36H), 2.90–3.80 (br, 18H), 4.70 (d, $J = 7.6$ 1H), 5.28 (d, $J = 8.1$ 1H), 7.20–7.26 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$/TMS): $\delta = 28.2$, 28.3, 40.6, 49.0- 50.0 (br), 50.5, 50.9, 51.8, 79.2, 80.1, 80.2, 80.3, 126.8, 128.3, 129.3, 136.3, 154.3, 155.6, 156.7, 172.4 ppm; IR (ATR): $\tilde{\nu} = 3310$, 3107, 2977, 2933, 1690, 1642, 1466, 1404, 1365, 1248, 1158, 1130, 1105, 1048, 1037, 972, 862, 777, 701, 622, 506, 457, 209, 400, 393 cm^{-1}; HRMS (FAB+): calcd for $C_{37}H_{62}N_5O_9$, 720.4548; found, 720.4552.

*1-(S)-Phenylalanyl-1,4,7,10-tetraazacyclododecane·3 TFA salt (**11·3 TFA**)*

TFA (2 mL, 27 mmol) was added to a solution of **9** (519 mg, 0.72 mmol) in CH_2Cl_2 (8 mL), and the resulting solution was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and

the resulting solid was recrystallized from EtOH/Et_2O to afford **11**·3TFA salt (the number of TFA units was determined by potentiometric pH titration; see Figure 1a) (450 mg, 94% yield). M.p. 108–110 °C; $[\alpha]_D^{22}$ = +31.3 (c = 1.00, H_2O); ^{1}H NMR (300 MHz, D_2O/external TSP): δ = 3.21 (m, 18H), 4.00 (m, 1H), 4.80 (m, 2H), 7.33 (m, 2H), 7.45 ppm (m, 3H); ^{13}C NMR (75 MHz, D_2O): δ = 36.7, 43.1, 43.5, 44.0, 44.5, 45.9, 46.2, 46.9, 46.9, 51.9, 116.3 (q, J_{C-F} = 291.9), 128.3, 129.2, 129.5, 133.1 (q, J_{C-F} = 35.5), 171.3; IR (ATR): $\tilde{\nu}$ = 3014, 2860, 1661, 1499, 1457, 1429, 1360, 1175, 1120, 1016, 966, 835, 797, 762, 720, 702, 597, 548, 518, 469, 442, 409, 395, 386 cm^{-1}; HRMS (ESI+): calcd for $C_{17}H_{30}N_5O^{+1}$, 320.2444; found, 320.2445.

*1-(S)-Phenylalanyl-1,4,7,10-tetraazacyclododecane (**11**)*

Aqueous 1 N NaOH (1 mL) was added to a solution of **11·3TFA** (13 mg, 0.2 mmol). Subsequently, the solution was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give **11** as a colorless oil. $[\alpha]_D^{23}$ = +43.6 (c = 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, D_2O/external TSP): δ = 2.40–2.81 (m, 15H), 2.98 (dd, J = 13.0, 7.5 Hz, 1H), 3.13–3.40 (m, 3H), 3.51–3.67 (m, 1H), 3.94 (t, J = 7.1 Hz, 1H), 7.09–7.24 ppm (m, 5H); ^{13}C NMR (75 MHz, D_2O): δ = 42.6, 44.6, 45.7, 47.1, 47.5, 47.7, 48.0, 48.4, 49.5, 53.3, 126.4 128.2, 129.3, 137.9, 176.3 ppm; IR (ATR): $\tilde{\nu}$ = 3288, 2885, 2828, 1631, 1494, 1452, 1353, 1237, 1130, 1074, 1030, 895, 807, 744, 699, 661, 523, 419, 404, 398, 386 cm^{-1}. HRMS (ESI+): calcd for $C_{17}H_{30}N_5O^{+1}$, 320.2444; found, 320.2445.

*1,4,7-Tris(tert-butyloxycarbonyl)-10-(N-tert-butyloxycarbonyl-(R)-phenylalanyl-1,4,7,10-tetraazacyclododecane (**10**)*

This compound was prepared from **8** and *N*-tert-butyloxycarbonyl-D-phenylalanine following a method similar to that used for **9**. $[\alpha]_D^{22}$ = −52.5 (c = 1.00, $CHCl_3$); HRMS (FAB+): calcd for $C_{37}H_{62}N_5O_9$, 720.4548; found, 720.4542.

*1-(R)-Phenylalanyl-1,4,7,10-tetraazacyclododecane·3 TFA salt (**12·3 TFA**)*

This compound was prepared following a method similar to that used for **11·3TFA**. $[\alpha]_D^{23}$ = −30.3 (c = 1.00, H_2O); HRMS (ESI+): calcd for $C_{17}H_{30}N_5O^{+1}$, 320.2444; found, 320.2445.

*1-(3-(R)-Phenylalanyl-1,4,7,10-tetraazacyclododecane (**12**)*

This compound was prepared following a method similar to that used for **11**. $[\alpha]_D^{22}$ = −44.3 (c = 1.00, $CHCl_3$); HRMS (ESI+): calcd for $C_{17}H_{30}N_5O^{+1}$, 320.2444; found, 320.2445.

Potentiometric pH Titrations

The preparation of the test solutions and the calibration method for the electrode system (Potentiometric Automatic Titrator AT-400 and Auto Piston Buret APB-410, Kyoto Electronics Manufacturing, Co. Ltd.) with a Combination pH Electrode 98100C171 (Kyoto Electronics Manufacturing, Co.) were described in a previous report.[8] All test solutions (50 mL) were kept under an argon (>99.999% purity) atmosphere. Potentiometric pH titrations were performed with I = 0.10 ($NaNO_3$) at 25.0 ± 0.1 °C (0.1 N aq. NaOH was used as a base). Deprotonation constants of Zn^{2+}-bound water K'_2 (=[OH^--bound species] α_{H+}/[H_2O-bound species]) were determined by means of the software program BEST. All of the sigma fit values defined in the program were smaller than 0.2. The K_w (equivalent to $\alpha_{H+}\alpha_{OH-}$), K'_w (equivalent to $[H^+][OH^-]$), and f_{H+} values used at 25 °C were $10^{-14.00}$, $10^{-13.79}$, and 0.825, respectively. The corresponding mixed constants, K_2(=[OH^--bound species]α_{H+}/[H_2O-bound species]), were derived using $[H^+] = \alpha_{H+}/f_{H+}$. The species distribution values (%) against pH (= −log[H^+]+0.084) were obtained using the SPE software program.

*General Procedure for Catalytic Aldol Reactions of Acetone and Aldehydes **3a–c** (Table 2)*

A given chiral ligand for the Zn^{2+} complex (TFA salt) (12.5 μmol) was extracted from its 0.2 M NaOH aqueous solution with $CHCl_3$. After drying the combined organic layer over anhydrous Na_2SO_4, the solution was filtered and concentrated under reduced pressure. The remaining residue was added to a solution of a mixture of $Zn(NO_3)_2·6H_2O$ (18.7 μmol) in H_2O (50 μL) and acetone (0.2 mL), and the mixture was stirred for 10 min. Subsequently, aldehyde **3** (0.25 mmol) was added and the whole reaction mixture was stirred for 20–72 h at 25 °C or 30 °C. The reaction mixture was diluted with 6% aq. NH_4Cl (2.5 mL) and extracted with ethyl acetate (30 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica-gel column chromatography (hexane/AcOEt) to provide **4a–c**. The optical purities of the aldol products were determined by HPLC using a chiral HPLC column, as described below.

*General procedure for the enzymatic enantioselective reduction of β-hydroxyketone **4a–c** (Table 3)*

Cofactor NAD^+ (36 mg, 0.05 mmol) and enzyme (10 mg) were added to a mixture of **4** (50 mg, 0.25 mmol) in 100 mM phosphate buffer (pH 7.2, 25 mL) and 2-propanol (1 mL), and the resulting solution was stirred for 24 h at 30 °C. The entire reaction mixture was extracted with EtOAc (30 mL × 3), and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica-gel column chromatography (hexane/AcOEt) to provide the 1,3-diol **7**, the diastereo- and enantioselectivities of which were determined by NMR spectrocopy and HPLC with a chiral HPLC column, as described below.

*General Procedure for the One-Pot Chemoenzymatic Synthesis of 1,3-diols **7a–c** (Table 4)*

A solution of ZnL (12.5 μmol) in acetone/H_2O was prepared as described above. The substrate aldehyde (0.125 mmol) was added and the entire reaction mixture was stirred at 30 °C for 24–72 h. After confirming the completion of the aldol reaction by TLC, the reaction mixture was diluted with 12.5 mL of 100 mM phosphate buffer (pH 7.2), to which 2-propanol (0.5 mL), cofactor NAD^+ (18 mg, 25 μmol), and enzyme (10 mg) were added. After stirring the mixture at 30 °C for 24 h, the entire reaction mixture was extracted with ethyl acetate (30 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solution was concentrated under reduced pressure. The resulting residue was purified by silica-gel column chromatography (hexane/AcOEt) to provide the pure product. The optical purities of the thus obtained 1,3-diols were determined by NMR spectroscopy and HPLC using a chiral HPLC column.

*4-(2-Chlorophenyl)-4-hydroxybutan-2-one (**4a**)[24]*

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 2.23 (s, 3H), 2.64–3.01 (m, 2H), 3.61 (br, 1H), 5.51 (m, 1H), 7.18–7.63 ppm (m, 4H; ArH); HPLC (Daicel Chiralpak AD-H column, ∅ 0.46 cm × 25 cm, hexane/EtOH = 95:5, flow rate: 1 mL min^{-1}, λ = 254 nm, 25 °C): t_R (S) = 14.4 min, t_R (R) = 19.4 min.

*4-(4-Chlorophenyl)-4-hydroxybutan-2-one (**4b**)[24]*

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 2.20 (s, 3H), 2.80–2.85 (m, 2H), 3.37 (br, 1H), 5.10–5.16 (m, 1H), 7.26–7.35 ppm (m, 4H; ArH); HPLC (Daicel Chiralpak AD-H column (∅ 0.46 cm × 25 cm), hexane/2-propanol = 97:3, flow rate: 1 mL min^{-1}, λ = 254 nm, 25 °C): t_R (S) = 26.1 min, t_R (R) = 23.7 min.

*4-(4-Nitrophenyl)-4-hydroxybutan-2-one (**4c**)[24]*

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 2.23 (s, 3H), 2.84–2.90 (m, 2H), 3.59 (d, J = 3.2 Hz, 1H), 5.51 (m, 1H), 7.54 (d, J = 7.0 Hz, 2H; ArH) , 8.21 ppm (d, J = 7.0 Hz, 2H; ArH); HPLC (Daicel Chiralcel OJ-H column (∅ 0.46 cm × 25 cm), hexane/EtOH = 95:5, flow rate: 1 mL min^{-1}, λ = 254 nm, 25 °C): t_R (S) = 37.6 min, t_R (R) = 32.1 min.

*(1R, 3R)-1-(2-Chlorophenyl)-1,3-butanediol ((**1R,3R)-7a**)[22]*

$[\alpha]_D^{23}$ = +71.1 (c = 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 1.21 (d, J = 6.3 Hz, 3H), 1.56–1.69 (m, 1H), 1.80–1.91 (m, 1H), 3.55 (br, 1H), 4.12 (br, 1H), 4.15–4.24 (m, 1H), 5.25–5.34 (m, 1H), 7.15–7.62 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.9, 45.0, 69.0, 71.5, 126.9, 127.2, 128.3, 129.2, 131.2, 141.7 ppm; IR (ATR): $\tilde{\nu}$ = 3326, 3069, 2969, 2913, 1596, 1574, 1473, 1438, 1321, 1130, 1077, 1032, 929, 751, 703,

460 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{14}ClO_2^{+1}$, 201.0677; found, 201.0681; HPLC (Daicel Chiralcel OD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=98:2, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R= 26.5 min.

*(1S, 3R)-1-(2-Chlorophenyl)-1,3-butanediol (**(1S,3R)-7a**)*[22]

$[\alpha]_D^{23}=-91.7$ (c=0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 1.22 (d, J = 6.3 Hz, 3H), 1.79–1.93 (m, 2H), 3.26 (br, 1H), 3.97–4.07 (m, 1H), 4.21 (br, 1H), 5.37–5.42 (m, 1H), 7.15–7.62 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.1, 43.3, 65.7, 68.5, 126.9, 127.2, 128.2, 129.3, 131.1, 141.5 ppm; IR (ATR): $\tilde{\nu}$=3329, 3069, 2969, 2916, 1596, 1574, 1472, 1440, 1377, 1129, 1078, 1033, 975, 751, 703, 461 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{14}ClO_2^{+1}$, 201.0677; found, 201.0683; HPLC (Daicel Chiralcel OD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=98:2, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=34.0 min.

*(1R, 3S)-1-(2-Chlorophenyl)-1,3-butanediol (**(1R,3S)-7a**)*[22]

$[\alpha]_D^{23}=+86.3$ (c=0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 1.24 (d, J = 6.6 Hz, 3H), 1.81–1.95 (m, 2H), 2.95 (br, 1H), 3.95 (br, 1H), 3.99–4.09 (m, 1H), 5.37–5.46 (m, 1H), 7.16–7.63 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.2, 43.3, 65.8, 68.6, 126.9, 127.2, 128.3, 129.3, 131.1, 141.6 ppm; IR (ATR): $\tilde{\nu}$=3328, 3069, 2968, 2916, 1596, 1574, 1472, 1440, 1377, 1129, 1078, 1033, 975, 751, 703, 460 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{14}ClO_2^{+1}$, 201.0677; found, 201.0683; HPLC (Daicel Chiralcel OD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=98:2, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=28.4 min.

*(1S, 3S)-1-(2-Chlorophenyl)-1,3-butanediol (**(1S,3S)-7a**)*[22]

$[\alpha]_D^{23}=-81.5$ (c=0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 1.20 (d, J = 6.3 Hz, 3H), 1.57–1.70 (m, 1H), 1.80–1.92 (m, 1H), 3.34 (br, 1H), 3.95 (br, 1H), 4.15–4.25 (m, 1H), 5.25–5.33 (m, 1H), 7.15–7.63 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): 24.0, 45.1, 69.1, 71.5, 126.9, 127.2, 128.4, 129.2, 131.2, 141.7 ppm; IR (ATR): $\tilde{\nu}$=3323, 3069, 2969, 2912, 1596, 1574, 1473, 1438, 1320, 1130, 1076, 1033, 929, 751, 703, 460 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{14}ClO_2^{+1}$, 201.0677; found, 201.0681; HPLC (Daicel Chiralcel OD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=98:2, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=42.9 min.

*(1R, 3R)-1-(4-Chlorophenyl)-1,3-butanediol (**(1R,3R)-7b**)*[16c]

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 1.20 (d, J = 6.3 Hz, 3H), 1.57–1.70 (m, 1H), 1.80–1.92 (m, 1H), 3.34 (br, 1H), 3.95 (br, 1H), 4.15–4.25 (m, 1H), 5.25–5.33 (m, 1H), 7.15–7.63 ppm (m, 4H); HPLC (Daicel Chiralpak AD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=97:3, flow rate 0.8 mL min^{-1}, λ=254 nm): t_R=35.7 min.

*(1S, 3R)-1-(4-Chlorophenyl)-1,3-butanediol (**(1S, 3R)-7b**)*[16c]

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ=1.23 (d, J=6.2 Hz, 3H; CH_3), 1.80–1.92 (m, 2H; CH_2), 2.14 (s, 1H; OH), 3.11 (s, 1H; OH), 4.03–4.09 (m, 1H; C*H*CH_3), 5.04 (dd, J=6.9 Hz, J=4.1 Hz, 1H; C*H*CH_2), 7.25–7.34 ppm (m, 4H; ArH); HPLC (Daicel Chiralpak AD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=97:3, flow rate 0.8 mL min^{-1}, λ=254 nm): t_R=46.5 min.

*(1R, 3S)-1-(4-Chlorophenyl)-1,3-butanediol (**(1R,3S)-7b**)*[16c]

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ=1.23 (d, J=6.2 Hz, 3H; CH_3), 1.80–1.92 (m, 2H; CH_2), 2.16 (s, 1H; OH), 3.13 (s, 1H; OH), 4.03–4.09 (m, 1H; C*H*CH_3), 5.04 (dd, J=6.9 Hz, J=4.1 Hz, 1H; C*H*CH_2), 7.25–7.34 ppm (m, 4H; ArH); HPLC (Daicel Chiralpak AD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=97:3, flow rate 0.8 mL min^{-1}, λ=254 nm): t_R=43.5 min.

*(1S, 3S)-1-(4-Chlorophenyl)-1,3-butanediol (**(1S,3S)-7b**)*[16c]

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ=1.24 (d, J=6.3 Hz, 3H; CH_3), 1.68–1.87 (m, 2H; CH_2), 2.67 (s, 1H; OH), 3.42 (s, 1H; OH), 4.12–4.20 (m, 1H; C*H*CH_3), 4.92 (dd, J=9.5 Hz, J=3.3 Hz, 1H; C*H*CH_2), 7.25–7.34 ppm (m, 4H; ArH); HPLC (Daicel Chiralpak AD-H column (⌀0.46 cm×25 cm), hexane/2-propanol=97:3, flow rate 0.8 mL min^{-1}, λ=254 nm): t_R=38.7 min.

*(1R, 3R)-1-(4-Nitrophenyl)-1,3-butanediol (**(1R,3R)-7c**)*[22]

$[\alpha]_D^{23}=+38.6$ (c=0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ= 1.26 (d, J=6.1 Hz, 3H; CH_3), 1.75–1.86 (m, 2H; CH_2), 2.50 (s, 1H; OH), 4.10 (s, 1H; OH), 4.14–4.24 (m, 1H; C*H*CH_3), 5.06 (t, J=5.9 Hz, 1H; C*H*CH_2), 7.53 (d, J=8.3 Hz, 2H; ArH), 8.20 ppm (d, J=10.2 Hz, 2H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$): 24.5, 46.8, 69.2, 74.2, 123.7, 126.4, 146.9, 151.7 ppm; IR (ATR): $\tilde{\nu}$=3351, 3117, 2971, 2954, 2908, 2879, 1602, 1514, 1336, 1124, 1074, 864, 749, 698 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{13}NO_4^{+1}$, 211.0845; found, 211.0763; HPLC (Daicel Chiralcel OJ-H column (⌀0.46 cm×25 cm), hexane/EtOH=95:5, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=32.7 min.

*(1S, 3R)-1-(4-Nitrophenyl)-1,3-butanediol (**(1S,3R)-7c**)*[22]

$[\alpha]_D^{23}=-54.9$ (c=0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ= 1.27 (d, 3H, J=6.1 Hz), 1.75–1.79 (m, 2H), 2.11 (br, 1H), 3.65 (br, 1H), 3.95–4.13 (m, 1H), 5.10–5.21 (m, 2H), 7.53 (d, J=8.4 Hz, 2H), 8.21 ppm (d, J=8.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.7, 45.5, 65.6, 71.0, 123.6, 126.3, 147.1, 152.0 ppm; IR (ATR): $\tilde{\nu}$=3339, 2969, 2932, 1601, 1513, 1343, 1290, 1107, 1074, 1052, 853, 749, 698 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{13}NO_4^{+1}$, 211.0845; found, 211.0770; HPLC (Daicel Chiralcel OJ-H column (⌀0.46 cm×25 cm), hexane/EtOH=95:5, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=30.5 min.

*(1R, 3S)-1-(4-Nitrophenyl)-1,3-butanediol (**(1R,3S)-7c**)*[16c]

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ=1.27 (d, 3H, J=6.1 Hz), 1.75–1.79 (m, 2H), 2.01 (br, 1H), 3.61 (br, 1H), 4.14–4.24 (m, 1H), 5.04–5.07 (m, 1H), 7.53 (d, J=8.3 Hz, 2H), 8.18 ppm (d, J=8.3 Hz, 2H); HPLC (Daicel Chiralcel OJ-H column (⌀0.46 mm×25 cm), hexane/EtOH= 95:5, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=28.4 min.

*(1S, 3S)-1-(4-Nitrophenyl)-1,3-butanediol (**(1S,3S)-7c**)*[22]

$[\alpha]_D^{23}=-30.5$ (c=0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ= 1.26 (d, J=6.1 Hz, 3H; CH_3), 1.75–1.91 (m, 2H; CH_2), 2.57 (s, 1H; OH), 4.10 (s, 1H; OH), 4.14–4.27 (m, 1H; C*H*CH_3), 5.06 (t, J=5.9 Hz, 1H; C*H*CH_2), 7.53 (d, J=8.3 Hz, 2H; ArH), 8.20 ppm (d, J=10.2 Hz, 2H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=24.5, 46.8, 69.2, 74.2, 123.7, 126.4, 146.9, 151.7 ppm; IR (ATR): $\tilde{\nu}$=3351, 3117, 2971, 2954, 2908, 2879, 1602, 1514, 1336, 1124, 1074, 864, 749, 698 cm^{-1}; HRMS (FAB+): calcd for $C_{10}H_{13}NO_4^{+1}$, 211.0845; found, 211.0763; HPLC (Daicel Chiralcel OJ-H column (⌀0.46 mm×25 cm), hexane/EtOH=95:5, flow rate 1.0 mL min^{-1}, λ=254 nm): t_R=35.0 min.

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[21] Chiralscreen OH contains formate dehydrogenases (FDH) mutants, which are more stable than wild type, for the regeneration of NADH (reduced form) from NAD^+ (oxidized form) using formate or D-glucose as a hydride source (see Scheme in Table 3). When sodium formate was used as a reductant of NAD^+, the results were

almost identical (>99% yield, 3*R*/3*S* = <1/>99 for E001) as those when 2-propanol was used.

[22] The relative configuration of **7a** and **7c** (*syn*/*anti*) was determined by ^{1}H NMR spectroscopy and HPLC (Daicel Chiralcel OD-H column and Chiralcel OJ-H column, respectively) by comparing with the products of the *syn*-selective reduction of racemic **4a** and **4c** with $NaBH_4$ (in MeOH) and DIBAL-H (in $CHCl_3$) (see, D. L. Boger, *Modern Organic Synthesis: Lecture Notes*, The Scripps Research Institute Press, San Diego, CA, **1999**). The absolute configuration of (1*S*,3*S*)- and (1*S*,3*R*)-**7a** and **7c** was determined by HPLC (Daicel Chiralcel OD-H column and Chiralcel OJ-H column, respectively) by comparing with the *syn*-selective reduction of optically active **4a** (91% *ee* (*S*)) and **4c** (90% *ee* (*S*)) with $NaBH_4$.

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