Inversion of Diastereoselectivity Depending on Substrate Concentration in Baker's Yeast Catalyzed Reduction of σ-Symmetrical 1,3-Cyclopentadiones and 1,3-Cyclohexadiones

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Abstract: Inversion of diastereoselectivity caused by a change in substrate concentration was first observed in baker's yeast catalyzed reduction of σ-symmetrical 2,2-dialkylated cyclopentan-1,3-diones **1**,**2** and cyclohexan-1,3-dione **20**. The selectivity altered by degrees depending on the substrate concentration from 8 mM to 40 mM. Meanwhile, application of the yeast-catalyzed reduction to the hydrogenated compound (**5**) of **1** afforded a single diastereomer in good yield when the reaction was conducted in high substrate concentration (40 mM).

Key words: baker's yeast catalyzed asymmetric reduction, σ-symmetrical cyclopentane-1,3-dione, substrate concentration

Asymmetric reductions with biocatalysts have been widely utilized to provide chiral synthons in organic synthesis since the reaction can be conducted under mild conditions, for example, in aqueous media and at moderate temperature.[1] Baker's yeast is one of the best-used biocatalysts among them mainly because of high potency for the reduction of ketones and availability in large amount.[2]

During our study on the application of the yeast-catalyzed reduction to σ-symmetrical cyclic 1,3-diketones, which afforded optically active cyclic 3-hydroxyketones, we encountered an interesting phenomenon that the diastereomeric ratio of products was inverted by changing the concentration of a substrate in a couple of cases while keeping Prelog's rule where newly generated secondary alcohols had the *S*-configuration.[3] Here we would like to report the details of this phenomenon.

Many studies on asymmetric reduction of 2,2-dialkylated cyclohexan-1,3-diones have been published and some of them reported practically excellent results,[4–6] and it is known that ratios of diastereomers obtained as products varied depending on chemical properties of alkyl groups on the C-2 quaternary carbon of the cyclohexan-1,3-diones.[7] However, almost nothing has been reported to date on the reduction of 2,2-dialkylated cyclopentan-1,3-diones with baker's yeast, except for the reduction of 2,2-dimethylcyclopentan-1,3-dione.[8] Moreover, σ-symmetrical cyclohexan-1,3-diones carrying a long alkyl chain on the C-2 position have been scarcely adopted as substrates in the asymmetric reduction using baker's yeast. Therefore, we embarked on study of the application of baker's yeast catalyzed reduction to σ-symmetrical derivatives of cyclopentan-1,3-dione and cyclohexan-1,3-dione, which carried a long alkyl chain and an ester on the terminal carbon.

We started with the preparation of substrates **1**–**6** having methyl and another alkyl group on the C-2 position of cyclopentan-1,3-dione. The substrates **1** and **2** having an ethylene unit between the cyclic ketone and α,β-unsaturated ester were synthesized in good overall yield by Michael addition of carbanion generated from 2-methylcyclopenta-1,3-dione (**7**) to acrolein, followed by Wittig reaction. On the other hand, the substrates **3** and **4** having a methylene unit between the cyclic ketone and α,β-unsaturated ester were synthesized in three steps, i.e., allylation of **7**, ozonolysis to afford aldehyde **8**, and successive Wittig reaction. Then, hydrogenation of the double bond of **1** and **3** afforded **5** and **6**, respectively (Scheme 1).

Next, the reduction of **1**–**6** with baker's yeast (Sigma Co. Ltd., Type II) was tried under normal conditions, i.e., 40 mM of the substrate was subjected to the reaction in an aqueous medium containing 10% DMSO, and in the presence of sucrose in the same medium (Table 1). Addition of sucrose tended to slightly increase the chemical yield while an undesired effect was observed on the de of the product (Table 1, entries 8–11), and the reactions of **3** and **4** that have one methylene between the cyclopentane ring and the olefinic carbon proceeded in better yield with satisfactory de than those of **1** and **2** having two methylene groups. Herein, it is noteworthy that the reactions of **5** and **6** having no carbon–carbon double bond proceeded to give satisfactory yield with excellent de (Table 1, entries 6 and 7).[9] Moreover, it was found that the diastereoselectivity of the products in the reaction of **1** was inverted when the reduction was conducted in lower substrate concentration (8.0 mM; Table 1, entries 1, 2).[10]

Fascinated by this phenomenon, the inversion of stereoselectivity depending on the substrate concentration, substrates **2**–**4** were subjected to the enzymatic reduction in two different concentrations, i.e., 40 mM and 8 mM of the substrate in a medium (Table 2). The difference of substrates affected the reactions; while the inversion of de occurred on the substrate **2**, only the decrease in the

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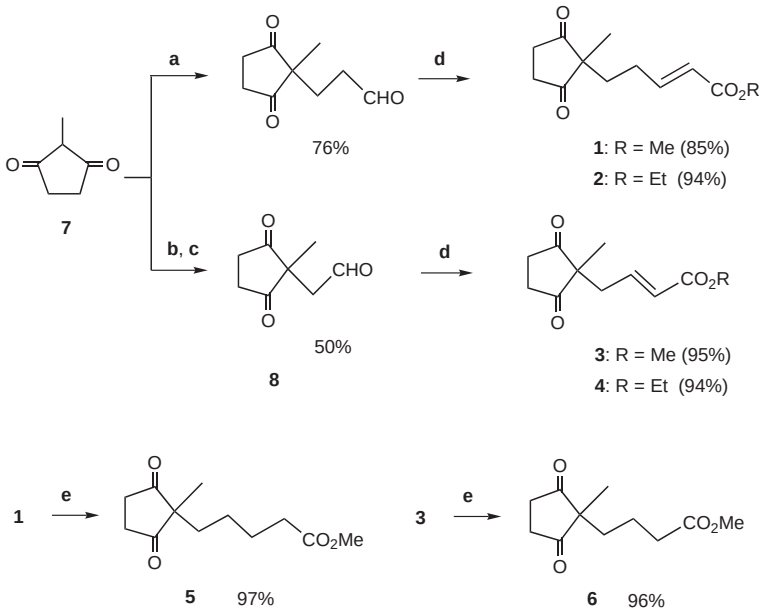
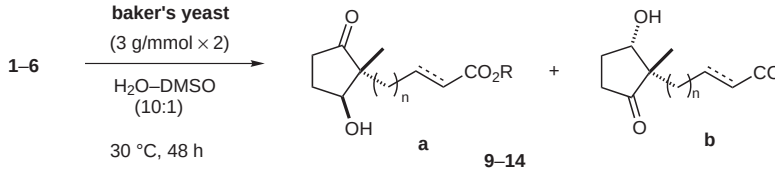


Scheme 1 *Reagents and conditions*: (a) acrolein; (b) allyl bromide; (c) O_3, then PPh_3; (d) $Ph_3P{=}CHCO_2R$; (e) Pd/C, H_2.

Table 1 Reduction of **1**–**6** with Baker's Yeast



Entry	Substrate[a]	Sucrose[b]	Yield (%)	Product			
					n	R	**a**:**b**
1	**1**	–	37	**9**	2	Me	12:**88**[d]
2[c]	**1**	–	30	**9**	2	Me	**89**[e]:11
3	**2**	–	30	**10**	2	Et	5:95
4	**3**	–	72	**11**	1	Me	16:84
5	**4**	–	70	**12**	1	Et	19:81
6	**5**	–	57	**13**	2	Me	1:99[f]
7	**6**	–	68	**14**	1	Me	0:100[g]
8	**1**	+	42	**9**	2	Me	26:74
9	**2**	+	27	**10**	2	Et	22:78
10	**3**	+	81	**11**	1	Me	21:79
11	**4**	+	73	**12**	1	Et	19:81

[a] 40 mM.
[b] Sucrose (yeast × 2) g.
[c] The reaction was carried out on 8 mM of substrate.
[d] 99% ee.
[e] 99% ee.[11]
[f] 97% ee.
[g] 98% ee.[12]

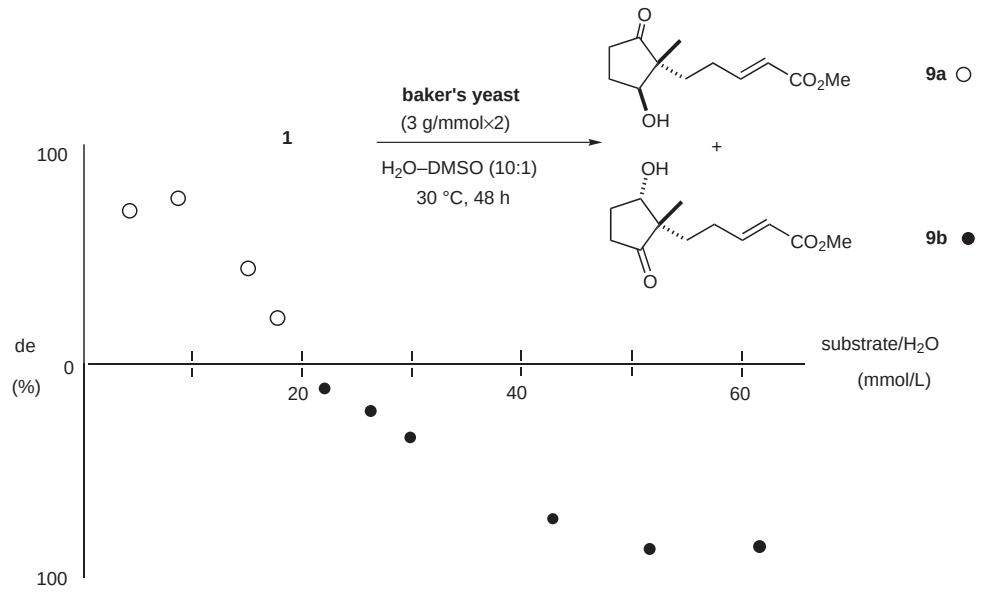


Figure 1

selectivity was observed with lower concentration of the substrates **3** and **4**.

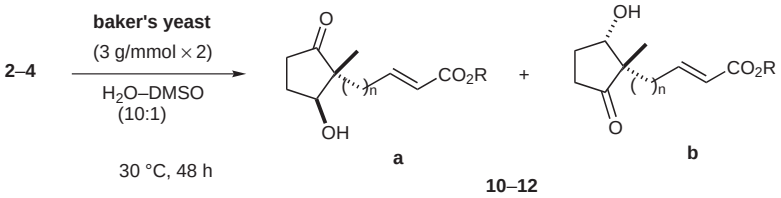
Thus, dependency of the diastereoselectivity in the reduction of **1** was scrutinized and resulted in obtaining a smooth sigmoid curve when the concentration of the substrate was changed from 4 mM to 60 mM (Figure 1).

Unfortunately, we cannot explain the mechanism which caused selectivity inversion of the products in the reduction of **1**, and why the substrate **1** behaved in this way.

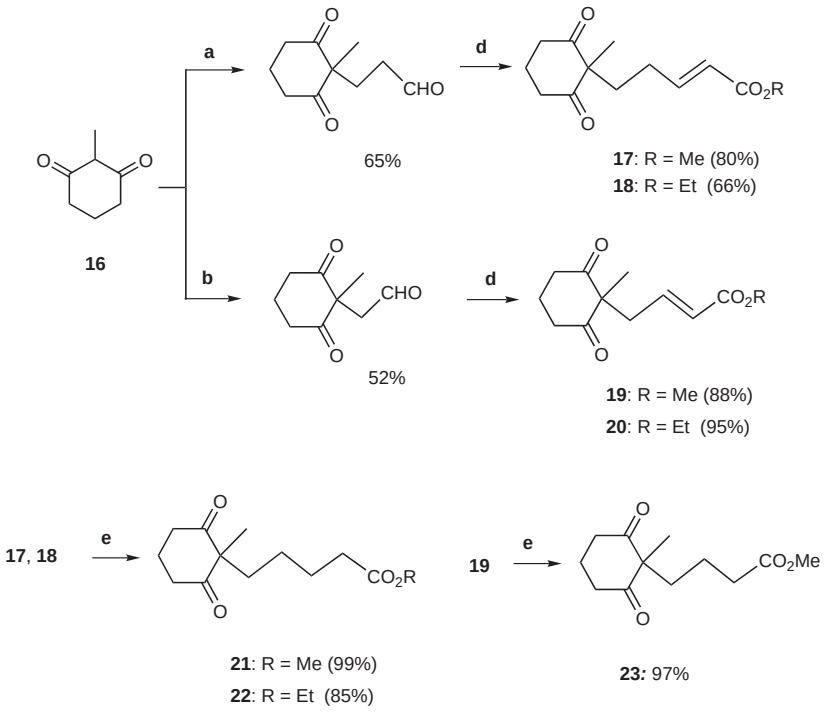
Finally, the absolute structure of the main product **9a** was determined by means of X-ray crystallography after deriving it to *p*-bromobenzoate (**15**), and the secondary alcohol and the quaternary carbon were determined to have the *S* and *R* configurations, respectively (Scheme 2, Figure 2). On the other hand, the absolute configuration of **9b** was determined, after hydrogenation, on the basis of identification with **13b**.[9] In addition, stereochemistry of **11b** was confirmed in a similar way, i.e., conversion to **14b** by hydrogenation and identification.[9] Moreover, comparison of ^{1}H NMR spectra between methyl esters (**11a**,**b**) and ethyl esters (**12a**,**b**) could suggest the stereochemistry of **12a** and **12b**, i.e., the signals assigned to the methyl groups on the cyclopentanone ring in **11a** and **12a** appeared in slightly lower field (0.05 ppm in both) than those of **11b** and **12b**, respectively.

We next investigated whether this phenomenon was observable in σ-symmetrical cyclohexan-1,3-dione or not. The substrates examined in the reaction were synthesized

Table 2 Enzymatic Reduction of Substrates **2**–**4**



Entry	Substrate	Conc. (mM)	Yield (%)	Product			
					n	R	**a**:**b**
1	**2**	40	30	**10**	2	Et	5:95
2	**2**	8	76	**10**	2	Et	79:21
3	**3**	40	72	**11**	1	Me	16:84
4	**3**	8	74	**11**	1	Me	42:58
5	**4**	40	70	**12**	1	Et	19:81
6	**4**	8	72	**12**	1	Et	44:56



Scheme 2

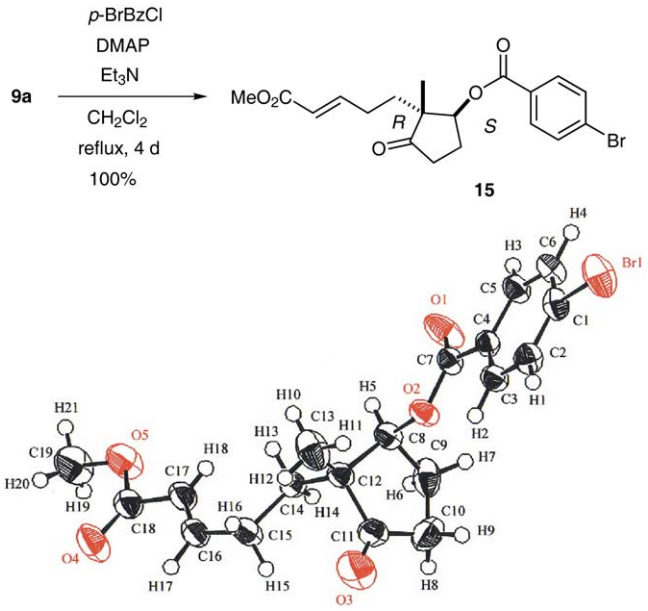


Figure 2

as the substrates **1**–**6**. Namely, elongation of a propionaldehyde or an acetaldehyde group from the C-2 position of cyclohexan-1,3-dione (**16**) was attained by Michael addition of acrolein and allylation followed by ozonolysis, respectively. Successive Wittig reaction of the aldehyde afforded **17**–**20**, of which the hydrogenation on Pd/C yielded **21**–**23** (Scheme 3).

Baker's yeast (Sigma, Type II) catalyzed asymmetric reductions of **17**–**20** were attained under the conditions of low and high substrate concentrations (Table 3).

As the result, such drastic inversion as in the case of **1** was not observed; however, an inversion of diastereoselectivity in the product appeared in the reaction with **20** (Table 3, entries 7, 8).

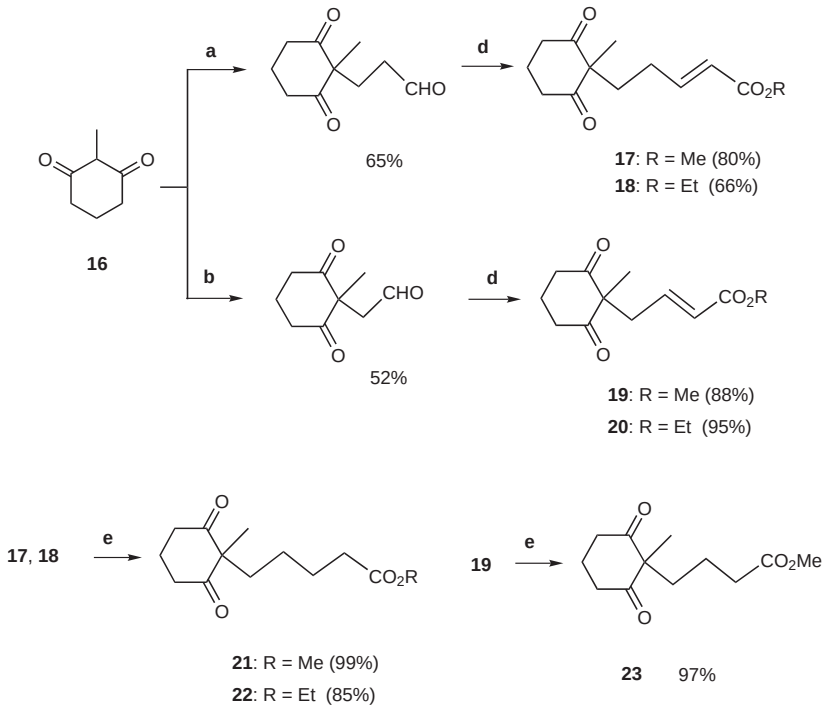
The absolute configuration of the major product **24a** in entry 7 was determined by X-ray crystallography after deriving to *p*-bromobenzoate.[13]

In comparison with the reaction of 1,3-cyclopentadiones **5** and **6**, 1,3-cyclohexandiones **21**–**23** were not converted to a single product but to a mixture of diastereomers with low selectivity in spite of a common structural feature, that is, the absence of the carbon–carbon double bond (Table 3, entries 9–11).

In conclusion, we have found an interesting phenomenon that the diastereoselectivity of the product in the reduction of **1** was inverted depending on the concentration of the substrate. Moreover, it was found that the baker's yeast catalyzed reduction of **5** that have no carbon–carbon double bond on the side-chain afforded a single diastereomer in satisfactory yield.

Our finding will provide a promising method for the synthesis of many biologically active compounds and naturally occurring compounds, and disclosed an important issue of the substrate concentration in baker's yeast catalyzed reaction as well as in other enzymatic reactions.

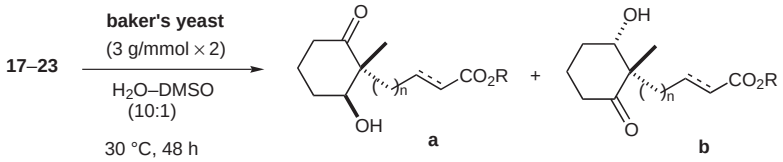
Further studies on the relationship between freshness of the baker's yeast and the potency of the inversion in diastereoselectivity[10] as well as those on the application of the baker's yeast catalyzed reduction for the synthesis of naturally occurring bioactive compounds are in progress.



a: acrolein, **b**: allyl bromide, **c**: O_3, then PPh_3, **d**: $Ph_3P{=}CHCO_2R$, **e**: Pd-C/H_2

Scheme 3 *Reagents and conditions*: (a) acrolein; (b) allyl bromide; (c) O_3, then PPh_3; (d) $Ph_3P{=}CHCO_2R$; (e) Pd/C, H_2.

Table 3 Baker's Yeast Catalyzed Asymmetric Reductions of **17**–**23**



24–**30**

Entry	Substrate	Conc. (mM)	Yield (%)	Product			
					n	R	**a**:**b**
1	**17**	40	67	24	2	Me	94:6
2	**17**	8	72	**24**	2	Me	93:7
3	**18**	40	26	**25**	2	Et	84:16
4	**18**	8	60	**25**	2	Et	86:14
5	**19**	40	35	**26**	1	Me	50:50
6	**19**	8	55	**26**	1	Me	49:51
7	**20**	40	71	**27**	1	Et	30:70
8	**20**	8	74	**27**	1	Et	66:34
9	**21**	40	68	**28**	2	Me	70:30
10	**22**	40	70	**29**	2	Et	72:28
11	**23**	40	58	**30**	1	Me	61:39

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References and Notes

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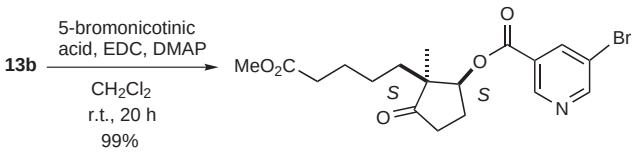
(9) The stereochemistry of **13b** (Scheme 4, Figure 3) and **14b** (Scheme 5, Figure 4) was confirmed by X-ray crystallography after deriving to bromonicotinates. Compound **13b**: colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.98 (s, 3 H), 1.24–1.35 (m, 1 H), 1.39–1.72 (m, 5 H), 1.92–2.00 (m, 1 H), 2.05 (br s, 1 H), 2.15–2.24 (m, 1 H), 2.26–2.37 (m, 3 H), 2.43–2.52 (m, 1 H), 3.67 (s, 3 H), 4.11 (br s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.2, 23.0, 25.3, 27.8, 29.2, 33.4, 33.9, 51.6, 53.2, 77.2, 174.3, 220.8. Compound **14b**: colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.97, (s, 3 H), 1.44–1.73 (m, 4 H), 2.00–2.07 (m, 1 H), 2.12–2.22 (m, 1 H), 2.27–2.37 (m, 2 H), 2.42–2.53 (m, 2 H), 3.14 (br s, 1 H), 3.69 (s, 3 H), 4.21 (br dt, *J* = 4.4, 2.2 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.7, 18.9, 27.2, 29.2, 33.1, 34.0, 51.9, 53.8, 76.8, 175.1, 221.3.

(10) In order to obtain high reproducibility in the reaction with 8 mM substrate, newly opened baker's yeast must be used. If long-term-stored yeast (longer than ca. 6 months at 4 °C) is used in the reaction, a reduced de is observed. The reproduced results were obtained with baker's yeast (Sigma, type II, lot. No. 125K0062).



Scheme 4

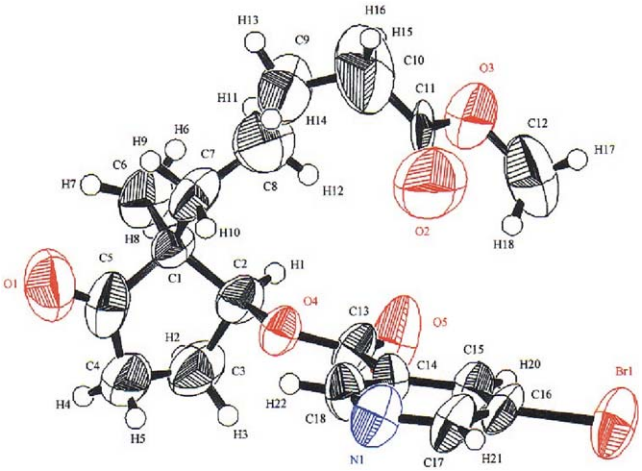
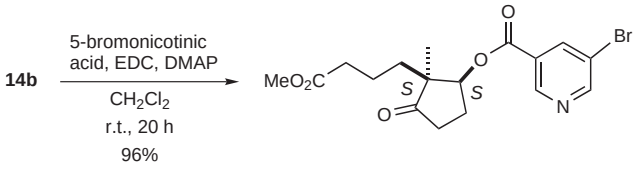


Figure 3



Scheme 5

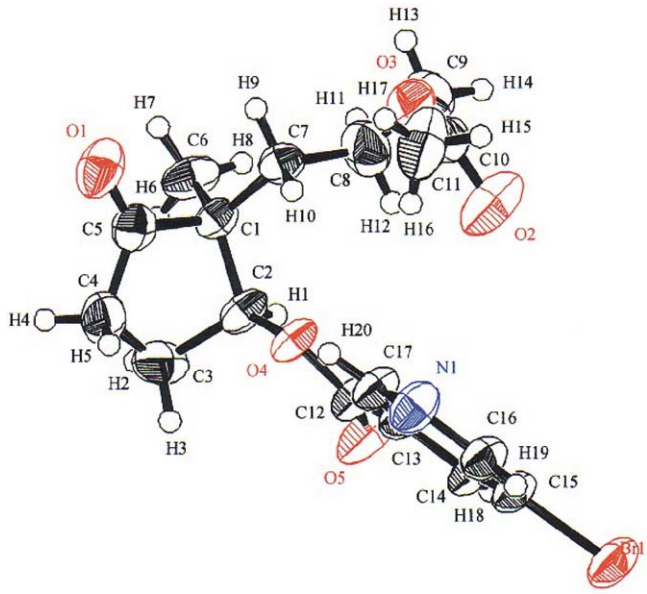


Figure 4

Compound **9a**: colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.03 (s, 3 H), 1.51–1.64 (m, 2 H), 1.76 (br s, 1 H), 1.84–1.93 (m, 1 H), 2.16–2.31 (m, 4 H), 2.44–2.52 (m, 1 H), 3.72 (s, 3 H), 4.17–4.20 (m, 1 H), 5.83 (td, *J* = 1.6, 15.7 Hz, 1 H), 6.92 (td, *J* = 6.8, 15.7 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.7, 26.8, 27.9, 33.3, 34.9, 51.5, 52.6, 75.8, 121.2, 148.7, 166.9, 219.6.
Compound **9b**: colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.02 (s, 3 H), 1.67–1.76 (m, 3 H), 1.90–1.97 (m, 1 H), 2.18–2.35 (m, 4 H), 2.44–2.53 (m, 1 H), 3.72 (s, 3 H), 4.13 (br dt, *J* = 3.7, 4.1 Hz, 1 H), 5.86 (td, *J* = 1.6, 15.7 Hz), 6.99 (td, *J* = 6.8, 15.7 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.2, 26.7, 28.2, 28.4, 33.9, 51.4, 52.7, 77.3, 121.0, 149.2, 167.1, 220.0.

(11) Chiral HPLC analysis of **9b** was performed with a Shimadzu LC-10A Liquid Chromatograph series using a SHISEIDO Ceramospher Chiral RU-1 (0.46 ϕ × 250 mm). Two enantiomers of **9b** were detected at the retention time of 10.7 and 11.7 min by eluting with MeOH, flow rate: 0.5 mL/min, at 50 °C. The enantiomer predominantly obtained in the reaction (entry 1, Table 1) appeared at 11.7 min. Meanwhile, two enantiomers of **9a** were detected using Daicel CHIRALCEL OJ-H (0.46 ϕ × 250 mm) at 43.2 and 47.5 min by eluting with a mixed solvent of *n*-hexane and 2-PrOH (9:1), flow rate: 0.5 mL/min, at 25 °C. The predominant enantiomer in the reaction (entry 2, Table 1) was detected at 47.5 min.
(12) Chiral HPLC analyses of **13b** and **14b** were performed with Daicel CHIRALCEL OD-H (*n*-hexane–*i*-PrOH = 96:4).
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