

Asymmetric Transformation of Enones with *Synechococcus* sp. PCC 7942

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Asymmetric transformation of enones was investigated with cultured cells of *Synechococcus* sp. PCC 7942 (a cyanobacterium). The cells reduced both the endocyclic C–C double bond of *s-trans* enones and the exocyclic C–C double bond of *s-cis* enones with high enantioselectivity to afford optically active α-substituted (*S*)-ketones under illumination. In addition, the reduction of the double bond of these enones was accompanied by the formation of saturated alcohols. The cells preferentially reduced simple aliphatic ketones rather than cyclic ones to the corresponding (*S*)-alcohols with excellent enantioselectivity.

Optically active ketone derivatives are useful chiral building blocks for asymmetric synthesis.[1] Enantioselective reduction of the C–C double bond of enones by living organisms such as microorganisms and plants is an attractive method for the synthesis of optically enriched α-substituted ketones.[2–6] Recent studies on the cell-mediated reduction of *s-trans* enones with the endocyclic C–C double bond have disclosed that the resulting ketones have an *R*-configuration at the asymmetric carbon α to the carbonyl group.[2–4] However, a cell-mediated process in which *s-trans* enones are reduced into (*S*)-ketones is still unavailable. On the other hand, it has been reported that the yeast-mediated reduction of *s-cis* enones with the exocyclic C–C double bond afforded the chiral ketones possessing an *S*-configuration at the α-position to the carbonyl group.[3] In the course of developing a new asymmetric reduction, we have applied cultured cells of *Synechococcus* sp. PCC 7942 for the enantiofacially selective hydrogenation of the C–C double bond of enones.[7] This paper describes in detail the enantioselective formation of *S*-configurated α-chiral ketones by reduction of both the endocyclic and the exocyclic C–C double bond of a series of *s-trans* and *s-cis* enones with *Synechococcus* sp. PCC 7942 as biocatalyst.

Results and Discussion

Several *s-trans* enones such as 2-alkyl-2-cyclopenten-1-ones **1** and **2** and 2-alkyl-2-cyclohexen-1-ones **3–5** (Fig. 1) with alkyl substituents at the C-2 position were subjected to the reduction by cultured cells of *Synechococcus* sp. PCC 7942. When 2-methyl-2-cyclopenten-1-one (**1**) was used as a substrate, the reduction effectively occurred to give (*S*)-2-methylcyclopentanone (**17**) in over 99% yield with the highest enantioselectivity of 98% e.e., as shown in Table 1. In the case of 2-methyl-2-cyclohexen-1-one (**3**), the reduction of the C–C double bond was accompanied by the formation of saturated alcohols (9% yield) (Scheme 1). The saturated alcohols obtained were the mixture of (1*S*,2*S*)-2-methylcyclohexanol (**19b**) (>99% e.e.) and (1*S*,2*R*)-2-methylcyclohexanol (**19c**) (>99% e.e.) (7:2). In the case of the substrate **4** with ethyl group at the α-position to the carbonyl group, (*S*)-2-ethylcyclohexanone (**20**) was obtained as product; however, the corresponding saturated alcohols could not be detected. On the other hand, no reduction occurred in the case of substrate **5** that has a propyl group as the α-substituent. To clarify the effect of the alkyl substitutents at the other positions on the stereoselectivity, reductions of monoterpene enone derivatives, (*R*)-carvone (**6**) and (*S*)-carvone (**7**), and of 2,5,5-trimethyl-2-cyclohexen-1-one (**8**) were examined. During three days of incubation, substrates **6–8** were smoothly reduced to the corresponding (*S*)-ketones. Interestingly, no further reduction of the carbonyl group of the resulting ketones **22**, **23**, or **24** was found during the incubation time examined. *Synechococcus* sp. PCC 7942 cells could not reduce β-substituted substrates, 3,5,5-trimethyl-2-cyclohexen-1-one (**9**) and 3-methyl-2-cyclohexen-1-one (**10**). This may be explained by a steric hindrance of the β-methyl group to the enzyme. Nevertheless, *s-trans* aliphatic enone such as *trans*-2-octenal **11** was slightly reduced to the corresponding ketone **25a** (5%) with formation of major saturated alcohol **25b** (94%).

Next, *s-cis* enones such as 2-alkylidenecyclohexanones **12** and **13** with the exocyclic C–C double bond were also reduced using the same reduction system. 2-Methylidenecyclohexanone (**12**) was smoothly reduced to give (*S*)-2-methylcyclohexanone (**19a**) in 82% yield; the hydrogenation at the α-position showed relatively low enantioselectivity (71% e.e.). The

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Table 1. Reduction of Enones by *Synechococcus* sp. PCC 7942

Substrate	Product	Time/day	Conversion/%	Ee/%	Configuration[a)]	$[\alpha]_D^{25}$/deg	$[\theta]_{288}$/deg
1	**17**	1	>99	98	*S*	+114.9 ($c = 0.52$, $CHCl_3$)	—
2	**18**	3	24	91	*S*	+127.7 ($c = 0.55$, $CHCl_3$)	—
3	**19a**	1	86	85	*S*	—	+887 ($c = 0.75$, MeOH)
4	**20**	3	17	83	*S*	—	+1914 ($c = 0.32$, MeOH)
5	—	3	0	—	—	—	—
6	**22**	3	>99	80[b)]	*S*	+30.2 ($c = 0.39$, $CHCl_3$)	—
7	**23**	3	>99	81[b)]	*S*	−19.5 ($c = 0.34$, $CHCl_3$)	—
8	**24**	3	15	86	*S*	—	+995 ($c = 0.14$, MeOH)
9	—	3	0	—	—	—	—
10	—	3	0	—	—	—	—
11	**25a**	3	5	—	—	—	—
12	**19a**	1	82	71	*S*	—	+701 ($c = 0.68$, MeOH)
13	**21**	1	7	72	*S*	—	+1860 ($c = 0.15$, MeOH)
14	—	3	0	—	—	—	—
15	**26a**	3	10	—	—	—	—
16	**30a**	3	62	—	—	—	—

a) Preferred configuration at the α-position to the carbonyl group of the products. b) Diastereomeric excess.

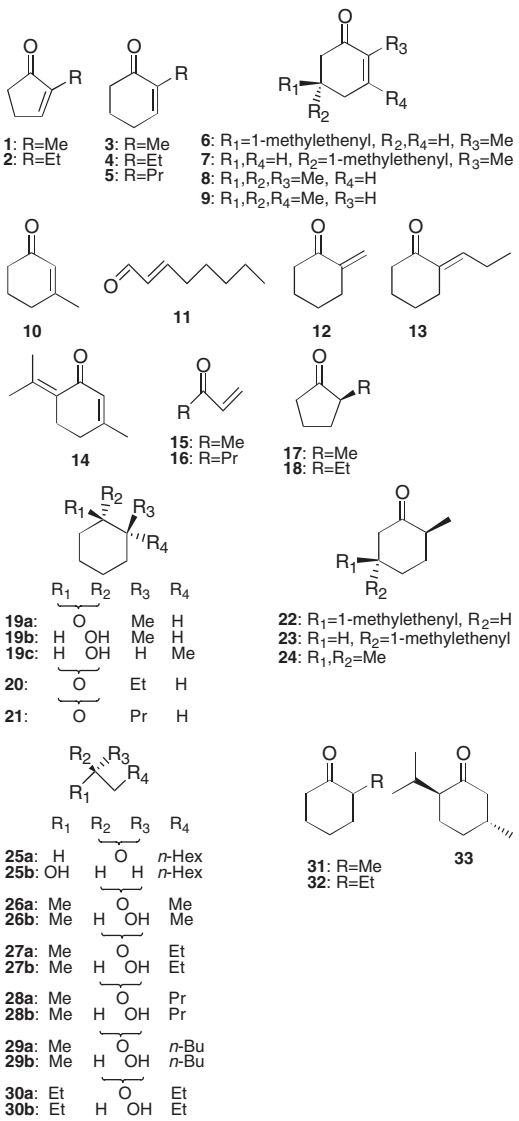
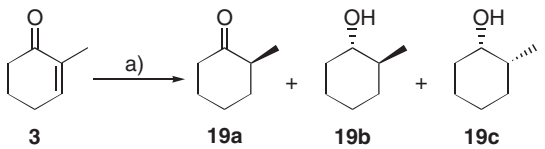
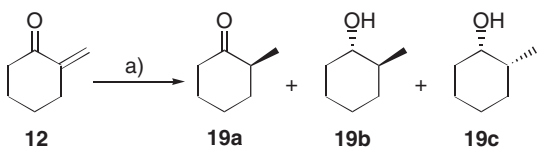


Fig. 1.



a) *Synechococcus* sp. PCC 7942

Scheme 1.



a) *Synechococcus* sp. PCC 7942

Scheme 2.

saturated alcohols **19b** (>99% e.e.) and **19c** (>99% e.e.) were formed as minor products in 7% yield (4:1) (Scheme 2). The reduction of 2-propylidenecyclohexanone (**13**) gave (*S*)-2-propylcyclohexanone (**21**) of 72% e.e. in 7% yield without formation of saturated alcohols. The substrate, piperitenone (**14**), that has both endocyclic and exocyclic C–C double bonds was not reduced by the cells, probably due to the existence of the β-methyl group as mentioned above. However, *s-cis* aliphatic enones such as 3-buten-2-one (**15**) and 1-hexen-3-one (**16**) were reduced to the corresponding ketones **26a** (10%) and **30a** (62%) with formation of two saturated (*S*)-alcohols: **26b** in 89% yield with e.e. of 80% and **30b** in 32% yield with e.e. of 98%.

To examine the enantioselectivity and substrate specificity of the reduction of ketones by *Synechococcus* sp. PCC 7942, we subjected aliphatic ketones **26a**–**30a** and cyclic ketones **22**, **23**, and **31**–**33** to the same reduction system. The reduction of 2-butanone (**26a**) smoothly proceeded to give (*S*)-2-butanol (**26b**) in 90% yield with e.e. of 81% (Table 2). In this reduction system, the cells seem to differentiate clearly between the

Table 2. Reduction of Ketones by *Synechococcus* sp. PCC 7942

Substrate	Product	Conversion/%	Ee/%	Configuration[a)]	$[\alpha]_D^{25}$/deg
26a	**26b**	90	81	*S*	+13.5 ($c = 0.37$, $CHCl_3$)
27a	**27b**	88	98	*S*	+13.1 ($c = 0.33$, $CHCl_3$)
28a	**28b**	87	>99	*S*	+10.9 ($c = 0.45$, $CHCl_3$)
29a	**29b**	88	>99	*S*	+10.4 ($c = 0.52$, $CHCl_3$)
30a	**30b**	35	98	*S*	+8.7 ($c = 0.50$, $CHCl_3$)
31	**19b**	5	>99	*S*	+50.4 ($c = 0.26$, $CHCl_3$)
	19c	5	>99	*S*	+25.2 ($c = 0.24$, $CHCl_3$)
32	—	tr.	—	—	—
22	—	0	—	—	—
23	—	0	—	—	—
33	—	0	—	—	—

a) Preferred configuration at the α-position to the hydroxy group of the products.

methyl and ethyl groups. When the chain length of substrates was increased to C_6 and C_7 (**28a** and **29a**), the enantioselectivity of the reduction was improved to e.e. > 99%. 3-Hexanone (**30a**) was also reduced by *Synechococcus* sp. PCC 7942 to give (*S*)-3-hexanol (**30b**); the enantioselectivity of this reduction was 98% e.e., suggesting that the cells could distinguish between the ethyl and propyl groups. On the other hand, (*RS*)-2-methylcyclohexanone (**31**) was reduced to give (1*S*,2*S*)- and (1*S*,2*R*)-2-methylcyclohexanol (**19b** and **19c**). Although the enantioselectivities of the reduction were very high (>99% e.e.), the conversion was drastically reduced (10% in total). The reaction with (*RS*)-2-ethylcyclohexanone (**32**) resulted in a trace amount of product. When monoterpene ketones (1*S*,4*S*)-isodihydrocarvone (**22**), (1*S*,4*R*)-dihydrocarvone (**23**), and (1*R*,4*S*)-menthone (**33**) were used as substrates, no reduction occurred. These results demonstrate that *Synechococcus* sp. PCC 7942 cells preferentially reduce aliphatic ketones rather than cyclohexanone derivatives to the corresponding (*S*)-alcohols with excellent enantioselectivity and that they discriminate between two small alkyl groups with a difference of a methylene unit. It has been reported that *Geotrichum candidum* reduced simple aliphatic ketones with high enantioselectivity to the corresponding (*S*)-alcohols, showing excellent discriminating ability between two small alkyl groups.[6] Such results suggest that the reduction system of aliphatic ketones by *Synechococcus* sp. PCC 7942 will be very useful for practical preparation of (*S*)-alcohols like *G. candidum*.

Thus, asymmetric reductions of *s-trans* and *s-cis* enones have been effected and optically active α-substituted (*S*)-ketones were prepared by using *Synechococcus* sp. PCC 7942 as biocatalyst. It has been recently reported that there are two different types of enone reductase with respect to substrate specificity: one conducts the reduction of the endocyclic C–C double bond and the other catalyzes the reduction of the exocyclic C–C double bond of enones.[4,8,9] These findings lead us to the idea that at least two different enone reductases exist in cultured cells of *Synechococcus* sp. PCC 7942. Previous studies on the cell-mediated reduction of *s-trans* enones demonstrated that the reaction with microorganisms and plants occurred when the substituent at the β-position to the carbonyl group was hydrogen and when the α-substituent was not too bulky and that the resulting ketones had an *R*-configuration at the α-position to the carbonyl group.[2–4] *Synechococcus* sp. PCC 7942 cells have a similar substrate specificity in the reduction of *s-trans* enones to microorganisms and plants, despite the opposite enantioselectivity of the hydrogenation at the α-position to the carbonyl group, resulting in the production of (*S*)-ketones. On the other hand, it has been reported that yeast-mediated reduction of *s-cis* enones afforded (*S*)-ketones.[3] *Synechococcus* sp. PCC 7942 cells have the same enantioselectivity to the reduction of *s-cis* enones as the yeast. Further investigations using the enzyme preparation from *Synechococcus* sp. PCC 7942 are now in progress.

Experimental

General. Analytical and prep. TLC were carried out on glass sheets (0.25 mm and 0.5 mm in thickness) coated with silica gel (Merck silica gel 60; GF_{254}). GLC analyses were carried out with FID and a capillary column (0.25 mm × 25 m) coated with 0.25 μm CP cyclodextrin β 236M-19 (Chrompack) using N_2 as carrier gas (column temp: 100 °C, split ratio: 50, make up: 50 mL min^{-1}); GC–MS (Shimadzu) was carried out on a mass spectrometer equipped with an EI ion source (70 eV) and a gas chromatograph equipped with a capillary column (0.25 mm × 25 m) coated with 0.25 μm OV-101. ^1H NMR spectra were obtained on a JEOL GSX-500 spectrometer using tetramethylsilane as an internal standard in $CDCl_3$.

Chemicals such as enones, ketones, and optically active alcohols used as substrates or as authentic samples were purchased from Aldrich or Wako Pure Chemical Co. Ltd. unless otherwise indicated and were used without further purification. 2-Methyl-2-cyclohexen-1-one (**3**), 2-ethyl-2-cyclohexen-1-one (**4**), 2-propyl-2-cyclohexen-1-one (**5**), 2,5,5-trimethyl-2-cyclohexen-1-one (**8**), 2-methylidene-1-cyclohexanone (**12**), and 2-propylidene-1-cyclohexanone (**13**) were prepared according to the reported procedures.[3,10,11] The configuration of **13** at the propylidene site was assigned to be *E* based on 500 MHz NMR data; the chemical shift of the olefin proton signal moved comparatively downfield to δ 6.61 (1H, tt, $J = 7.4$ and 2.1 Hz) due to the placement of this proton in the deshielding region of the neighboring carbonyl group.[12] Piperitenone (**14**) was donated by Takasago Perfumery Co. Ltd.

Reduction of Enones with *Synechococcus* sp. PCC 7942. The suspension cells of *Synechococcus* sp. PCC 7942 were incubated in 500 mL conical flasks containing 300 mL of BG-11 medium for 3 weeks.[13] The grown cells (ca. 2 g of wet cells) were collected by centrifugation and added to a 100 mL conical flask containing 50 mL of 50 mM Na–phosphate buffer (pH 7.0) with 3% glucose. After the cells were resuspended in the flask, 10 mg of the substrate (water-solubilized with 1% Triton X-100)

was added. The transformation was performed by incubating the mixture at 25 °C for 1 or 3 days on a rotary shaker (70 rpm) under illumination (4000 lux). The incubation product was extracted with ether and was subjected to chromatography on silica gel with pentane–ethyl acetate (95:5, v/v) to separate the products. The product ketones were identified by comparisons of their TLC, GLC, and GC–MS data with those of authentic samples.[3,9,14] The absolute configurations and optical purities of the resulting ketones **17**, **18**, **19a**, **20**, and **21** were determined by optical rotation or circular dichroism (CD) analyses and the peak area of the corresponding enantiomers by GLC analyses on CP cyclodextrin β 236M-19 column. In order to obtain the products adequate for identification by optical rotation or CD analysis, we scaled up the incubation of substrates with the cells by 10-fold in a similar manner to the standard biotransformation system. Diastereomeric excesses of **22** and **23** were also determined from the peak areas of the corresponding diastereomers by GLC analyses. Retention times for the products in the GLC were as follows: (*S*)- and (*R*)-**17**, 10.5 and 11.4 min; (*S*)- and (*R*)-**18**, 12.1 and 12.9 min; (*S*)- and (*R*)-**19a**, 11.8 and 12.8 min; (*S*)- and (*R*)-**20**, 12.7 and 12.9 min; (*S*)- and (*R*)-**21**, 27.7 and 27.9 min; (*S*)- and (*R*)-**24**, 50.1 and 51.9 min. The optical rotation data of the products obtained in the reduction of **1**, **2**, **6**, and **7** were the following. **17**: $[\alpha]_D^{25}$ +114.9 (*c* 0.52, $CHCl_3$) {lit.[15]: $[\alpha]_D^{25}$ −110.5 for (*R*)-enantiomer}; **18**: $[\alpha]_D^{25}$ +127.7 (*c* 0.55, $CHCl_3$); **22**: $[\alpha]_D^{25}$ +30.2 (*c* 0.39, $CHCl_3$) {lit.[2b]: $[\alpha]_D^{15}$ −23.7 for (1*R*,4*S*)-enantiomer}; **23**: $[\alpha]_D^{25}$ −19.5 (*c* 0.34, $CHCl_3$) {lit.[2b]: $[\alpha]_D^{15}$ +13.9 for (1*R*,4*R*)-enantiomer}. The CD data of the products obtained in the reduction of **3**–**5** and **8** were the following. **19a** converted from **3**: $[\theta]_{288}$ +887 (*c* 0.75, MeOH) {lit.[16]: $[\theta]_{288}$ −987 for (*R*)-enantiomer}; **19a** from **12**: $[\theta]_{288}$ +701 (*c* 0.68, MeOH); **20**: $[\theta]_{288}$ +1914 (*c* 0.32, MeOH) {lit.[17]: $[\theta]_{288}$ +2200}; **21**: $[\theta]_{288}$ +1860 (*c* 0.15, MeOH) {lit.[17]: $[\theta]_{288}$ +2480}; **24**: $[\theta]_{288}$ +995 (*c* 0.14, MeOH). The product alcohols produced in the reduction of **3**, **11**, **12**, **15**, and **16** were identified by direct comparisons of TLC, GLC, and GC–MS data with those of authentic samples.[6] The absolute configurations and enantiomeric purities of **19b** and **19c** were determined by ^{1}H NMR analyses of the corresponding MTPA esters, as described previously.[18] The absolute configurations of **26b** and **30b** after esterification with propionyl chloride and pyridine in CH_2Cl_2 were determined by comparing the GLC retention times on CP cyclodextrin β 236M-19 column with those of corresponding esters prepared from enantiomerically pure alcohols. The enantiomeric compositions of **26b** and **30b** were determined by the peak areas of their corresponding propionyl esters in the chiral GLC analyses. Retention times for the esterified enantiomers in the GLC were as follows: (*S*)- and (*R*)-*sec*-butyl propionate, 5.9 and 6.7 min; (*S*)- and (*R*)-1-ethylbutyl propionate, 7.1 and 7.8 min.

Reduction of Ketones with *Synechococcus* sp. PCC 7942. Substrate ketones were incubated with *Synechococcus* sp. PCC 7942 for 2 days in a manner similar to that described above. The product alcohols were identified by comparisons of their TLC, GLC, and GC–MS data with those of authentic samples. The absolute configurations and the enantiomeric compositions of the products were determined by chiral GLC analyses of their corresponding acetyl or propionyl esters on CP cyclodextrin β 236M-19 column. Retention times for the esterified enantiomers in the GLC were as follows: (*S*)- and (*R*)-1-methylbutyl acetate, 5.5 and 8.4 min; (*S*)- and (*R*)-1-methylpentyl propionate, 7.1 and 8.8 min; (*S*)- and (*R*)-1-methylhexyl propionate, 6.0 and 6.8 min. The optical rotation data of the products obtained in the reduction of **26a**–**30a** and **31** were the following. **26b**: $[\alpha]_D^{25}$ +13.5 (*c* 0.37, $CHCl_3$); **27b**: $[\alpha]_D^{25}$ +13.1 (*c* 0.33, $CHCl_3$) {lit.[19]: $[\alpha]_D^{20}$ +10.3}; **28b**: $[\alpha]_D^{25}$ +10.9 (*c* 0.45, $CHCl_3$) {lit.[19]: $[\alpha]_D^{20}$ +10.4}; **29b**: $[\alpha]_D^{25}$ +10.4 (*c* 0.52, $CHCl_3$) {lit.[19]: $[\alpha]_D^{20}$ +10.2}; **30b**: $[\alpha]_D^{25}$ +8.7 (*c* 0.50, $CHCl_3$) {lit.[19]: $[\alpha]_D^{20}$ +8.5}; **19b**: $[\alpha]_D^{25}$ +50.4 (*c* 0.26, $CHCl_3$) {lit.[20]: $[\alpha]_D^{20}$ +42.9}; **19c**: $[\alpha]_D^{25}$ +25.2 (*c* 0.24, $CHCl_3$) {lit.[20]: $[\alpha]_D^{20}$ +24.3}.

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References

1 K. Tomioka and K. Koga, "Asymmetric Synthesis," ed by J. D. Morrison, Academic Press, New York (1983), Vol. 2, p. 201.

2 a) A. Kergomard, M. F. Renard, and H. Veschambre, *Tetrahedron Lett.*, **19**, 5197 (1978). b) A. Kergomard, M. F. Renard, and H. Veschambre, *J. Org. Chem.*, **47**, 792 (1982). c) T. Hirata, H. Hamada, T. Aoki, and T. Suga, *Phytochemistry*, **21**, 2209 (1982). d) M. Desrut, A. Kergomard, M. F. Renard, and H. Veschambre, *Biochem. Biophys. Res. Commun.*, **110**, 908 (1983). e) A. Kergomard, M. F. Renard, H. Veschambre, D. Courtois, and V. Petiard, *Phytochemistry*, **27**, 407 (1988). f) Y. Noma and Y. Asakawa, *Phytochemistry*, **31**, 2009 (1992).

3 K. Matsumoto, Y. Kawabata, J. Takahashi, Y. Fujita, and M. Hatanaka, *Chem. Lett.*, **1998**, 283.

4 K. Shimoda and T. Hirata, *J. Mol. Catal. B: Enzym.*, **8**, 255 (2000).

5 Y. Kawai, M. Hayashi, and N. Tokitoh, *Tetrahedron: Asymmetry*, **12**, 3007 (2001).

6 K. Nakamura, R. Yamanaka, T. Matsuda, and T. Harada, *Tetrahedron: Asymmetry*, **14**, 2659 (2003).

7 K. Shimoda, N. Kubota, H. Hamada, M. Kaji, and T. Hirata, *Tetrahedron: Asymmetry*, **15**, 1677 (2004).

8 K. Shimoda, T. Hirata, and Y. Noma, *Phytochemistry*, **49**, 49 (1998).

9 K. Shimoda, S. Izumi, and T. Hirata, *Bull. Chem. Soc. Jpn.*, **75**, 813 (2002).

10 F. Huet, M. Pellet, and J. M. Conia, *Tetrahedron Lett.*, **18**, 3505 (1977).

11 D. F. Taber, *J. Org. Chem.*, **41**, 2649 (1976).

12 J. K. Crandall, J. P. Arrington, and J. Hen, *J. Am. Chem. Soc.*, **89**, 6208 (1967).

13 K. Nakamura, R. Yamanaka, K. Tohi, and H. Hamada, *Tetrahedron Lett.*, **41**, 6799 (2000).

14 T. Hirata, Y. Tang, K. Okano, and T. Suga, *Phytochemistry*, **28**, 3331 (1989).

15 J. J. Partridge, N. K. Chada, and M. R. Vskokovic, *J. Am. Chem. Soc.*, **95**, 532 (1973).

16 C. J. Cheer and C. Djerassi, *Tetrahedron Lett.*, **17**, 3877 (1976).

17 A. I. Meyers, D. R. Williams, G. W. Erickson, S. White, and M. Druelinger, *J. Am. Chem. Soc.*, **193**, 3081 (1981).

18 T. Hirata, S. Izumi, K. Akita, H. Yoshida, and S. Gotoh, *Tetrahedron: Asymmetry*, **4**, 1465 (1993).

19 E. Keinan, E. K. Hafeli, K. K. Seth, and R. Lamed, *J. Am. Chem. Soc.*, **108**, 162 (1986).

20 R. Backstrom and B. Sjoberg, *Ark. Kemi*, **26**, 549 (1967).