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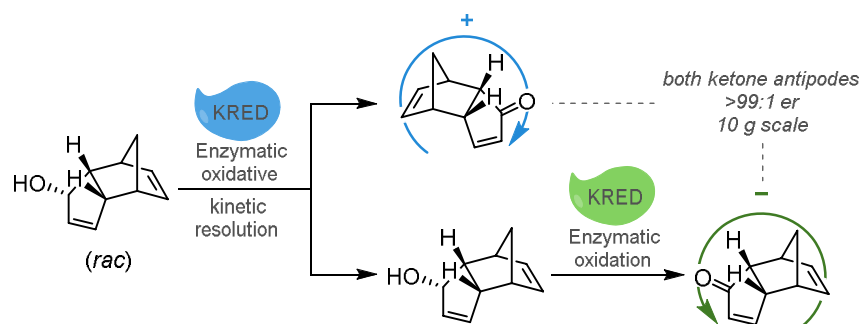
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Enzymatic Resolution of 3-Oxodicyclopentadiene in Decagram Scale

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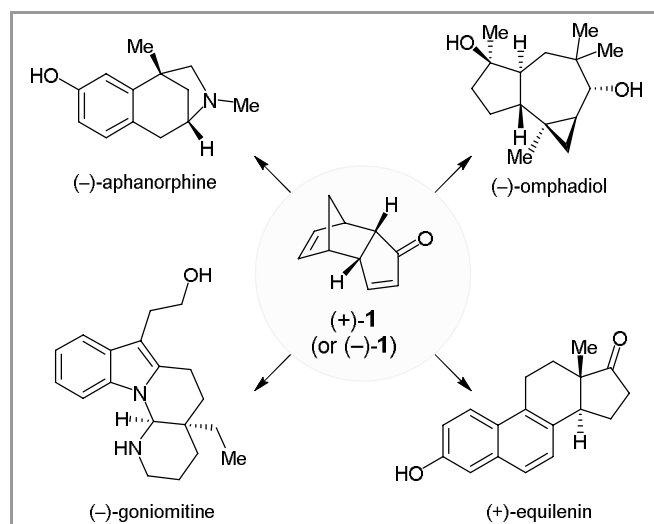
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Abstract The chiral building block 3-oxodicyclopentadiene (**1**) can be readily resolved in decagram scale by a short sequence consisting of 1) reduction to the corresponding endo-alcohol (\pm)-**4**, 2) enzymatic oxidative resolution with a ketoreductase enzyme to give (-)-**1** and (+)-**4**, and 3) reoxidation of (+)-**4** with another ketoreductase to give (+)-**1**. With a selectivity factor of 310, the enantiomeric ratios of the resolved (+)-endo-alcohol and (+)-ketone are both >99:1. Both enzymatic oxidations could be performed with 500:1 substrate:catalyst ratio (w/w).

Key words enzymes, oxidation, chiral resolution, alcohols, gram-scale, steric hindrance

3-Oxodicyclopentadiene (**1**) has been used as a starting material in number of natural product syntheses. Terpenes and terpenoids, such as (-)-herbertene, (+)- α -cuparenone, capnellene, (-)-omphadiol, and epi- β -santalene, steroids, such as (+)-estrone and (+)-equilenin, and alkaloids, including (-)-physostigmine, (-)-aphanorphine, and (-)-goniomitine have all been synthesized using either (+)- or (-)-**1** as a starting material (Scheme 1).^{1a-d} Despite the wide range of uses, preparation of enantiopure 3-oxodicyclopentadiene **1** remains cumbersome.

The synthesis and resolution of (\pm)-**1** has been described by Ogasawara, Takano and co-workers.² Their protocol involves resolution of either exo-alcohol **3** or the corresponding endo-alcohol **4** with a lipase, followed by saponification of the ester and oxidation of either alcohol enantiomer (+)-**4** and (-)-**4** with MnO₂. Although this protocol can be used to provide multigram quantities of either enantiomer of **1**, the original procedure involves up to seven steps starting from dicyclopentadiene (Scheme 2 a). An alternative route patented by Ogasawara, based on lipase-catalyzed resolution of acyloin, also involves seven steps from dicyclopentadiene.³ In 2003, Lucchini and coworkers disclosed a shorter route to the racemic ketone (\pm)-**1** via singlet oxygen oxidation of dicyclopentadiene **2** (Scheme 2 b), but for its resolution, they also used the Takano–Ogasawara route.⁴

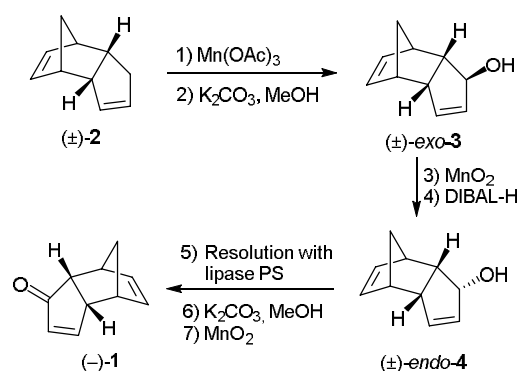


Scheme 1: Selected natural products accessible from (+)-**1** or (-)-**1**.

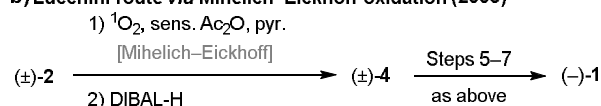
A considerably shorter route to both (+)-**1** and (-)-**1** would involve direct resolution of ketone **1** via reduction, or either alcohol **3** or **4** via oxidation (Scheme 2 c). However, the hindered nature of **1** and the relative ease by which it undergoes conjugate reduction might pose difficulties in the kinetic resolution of **1** by catalytic reduction.⁵ On the other hand, catalytic oxidation of **4** has been attempted with a Noyori Ru(II)-(S,S)-TsDPEN catalyst, but with unsatisfactory results (with selectivity factors $S < 10$).⁶ Herein, we report a scalable resolution of **1** by oxidative resolution of endo-alcohol **4** with a commercially available ketoreductase enzyme, providing a selectivity factor of 300 and allowing practical reaction times with only 0.2 wt-% of enzyme relative to the substrate.

We initially attempted the enzymatic reduction of ketone (\pm)-**1**. For the activity screens, we used a panel of 24 ketoreductase enzymes from Codexis, consisting of 5 natural enzymes (using glucose and glucose dehydrogenase for recycling) and 19

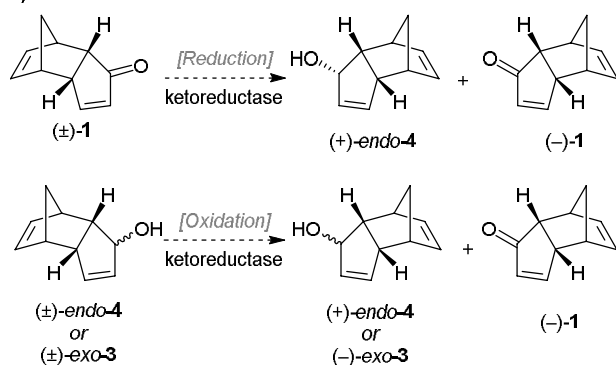
a) Takano–Ogasawara route (1994)



b) Lucchini route via Mihelich–Eickhoff oxidation (2003)



c) Direct resolution via reduction or oxidation?



Scheme 2: Current routes to enantiopure (-)-1 or (+)-1 rely on a lipase resolution (a, b) A proposed direct resolution via reduction or oxidation would be more convenient (c)

engineered enzymes (with excess i-PrOH for recycling).⁶ No conversion to either alcohol 3 or 4 could be detected over 24 hours. In addition, 6 ketoreductase (aldehyde dehydrogenase) enzymes from Johnson–Matthey were also screened, but without success.⁶

As a result, we then attempted the oxidative resolution of alcohol (±)-exo-3, using the same panel of 24 enzymes from Codexis. The best selectivity (*S* = 58) was obtained with KRED-P2-C02 (Table 1, entry 10), affording (+)-1 in 97:3 er at 38% conversion. In the screening phase, a relatively large amount of enzyme was used (1:1 or even 2:1 enzyme:substrate ratio by weight) to facilitate finding of hits. In preparative experiments, when more affordable 1:25 enzyme:substrate ratios were employed, it was very difficult to push the conversion beyond 40% with acetone as oxidant, even after prolonged reaction times. With ethyl acetoacetate as oxidant, conversions were higher, but the separation of the product from the byproduct, ethyl-3-hydroxybutyrate, turned out to be very difficult. Treatment of the crude reaction mixture with 1 M NaOH to saponify the hydroxybutyrate and acetoacetate esters allowed easier purification. However, the isolated yield of alcohol (+)-1 in a gram-scale experiment was only 32%, suggesting that side reactions were taking place either during the reaction or during the saponification step.

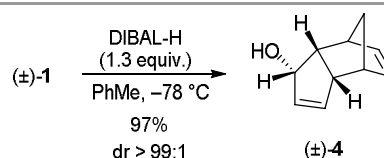
Table 1: Enzymatic oxidation of exo-alcohol 3^a

Entry	Enzyme	Enzyme (mg)	Conv. (%) ^c	er Major enantiomer ^d	Time (h)	Selectivity factor ^e
1	KRED-P1-A04 ^b	5.0	8.5	75:25 (-)	26	3.0
2	KRED-P1-B02 ^b	5.0	15	99:1 (+)	26	71
3	KRED-P1-B05 ^b	5.0	42	85:15 (+)	26	9.1
4	KRED-P1-B10 ^b	5.0	28	95:5 (+)	26	25
5	KRED-P1-B12 ^b	5.0	13	93:7 (+)	26	15
6	KRED-P1-C01 ^b	2.5	18	86:14 (+)	3	7.1
7	KRED-P1-H08 ^b	2.5	24	88:12 (+)	3	9.8
8	KRED-P1-H10 ^b	5.0	28	74:26 (-)	26	3.4
9	KRED-P2-B02 ^b	2.5	26	89:11 (+)	3	11
10	KRED-P2-C02 ^b	2.5	38	97:3 (+)	3	58
11	KRED-P2-C11 ^b	5.0	22	94:6 (+)	26	23
12	KRED-P2-D11 ^b	5.0	37	80:20 (+)	26	5.4
13	KRED-P2-D12 ^b	2.5	6.5	81:19 (+)	3	4.2
14	KRED-P2-G03 ^b	5.0	21	90:10 (-)	26	11

a) A full table is available in SI; b) 2.5 mg of (±)-exo-3, 25 μL of acetone, 475 μL of KRED Recycling Mix P, pH 7, 30 °C; c) determined by HPLC: Chiralpak IC, hexane/IPA 95:5, 1 mL/min, 210 nm; d) 230 nm; e) based on product, see the SI for further details.

We therefore turned to the resolution of endo-alcohol (±)-4. To obtain (±)-4 (Scheme 3), the DIBAL-H reduction of (±)-1 using the original procedure, with aq. ammonia quench, turned out to give variable yields of (±)-4.^{2a} Changing the solvent to toluene and quenching with MeOH followed by aqueous NaK tartrate gave consistently higher yields. In 10 g scale, 9.7 g of (±)-4 (97% yield) could be obtained from the DIBAL-H reduction.^{7,8}

The oxidation of 4 by the enzymes turned out to be more facile than that of 3. In nearly all cases, the (+)-ketone (+)-1 was again the major product. This was rationalized on the basis of the X-ray structures of (-)-exo-3 and (±)-endo-4: the hydrogen atom in C3 is far more accessible in endo-4 than in exo-3. In exo-3, the C3-H group was severely sterically hindered (Figure 1).^{8,9}



Scheme 3: DIBAL-H reduction of (±)-1 to (±)-endo-4

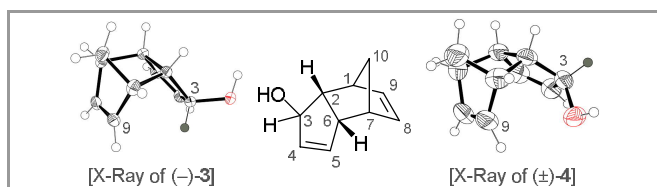


Figure 1 Comparison of the structures of exo-3 and endo-4. In exo-3, the hydrogen atom at C3 is blocked by the carbon backbone, whereas in endo-4 the hydrogen atom accessible as it is disposed on the concave face (C3 hydrogen atom highlighted in gray).⁸

Two highly selective enzymes could be identified: KRED-P1-B02 ($S = 310$, Table 2, entry 2) and KRED-P2-D12 ($S = 150$, entry 14). The higher selectivity obtained with KRED-P1-B02 prompted us to choose this enzyme for further exploration.

In gram-scale experiments with endo-alcohol (±)-4, the enzyme loading could be lowered to 2000:1 substrate:enzyme ratio by weight. Thus, 2.0 g of (±)-4 was readily resolved to give 930 mg of (+)-4 (46%, 99.5:0.5 er) and 850 mg of (+)-1 (43%, 99.5:0.5 er) with only 3 days of reaction time in 10 mL of buffer containing 30% acetone by volume. Finally, in a decagram scale, 23.7 g of (±)-4 could be resolved in 14 hours with only 60 mg of KRED-P1-B02, giving, after chromatographic separation, alcohol (+)-4 in 42% yield (>99.5:0.5 er) and ketone (+)-1 in 41% yield (99:1 er).⁹ It should be noted that only very pure fractions of both compounds were collected, to avoid contaminating the alcohol (+)-4 with (+)-1 which would lead to a loss of enantiopurity after the oxidation step.

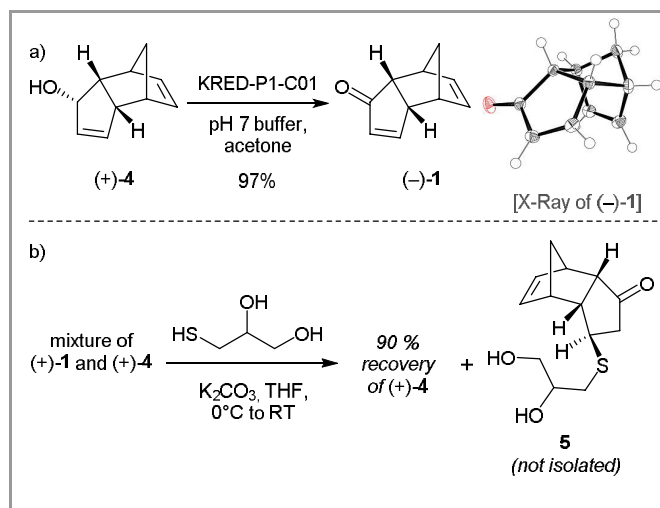
To obtain the other ketone enantiomer, (–)-1, the resolved alcohol (+)-4 was reoxidized (Scheme 4). Initial experiments with various sources MnO_2 or IBX gave variable and low yields of ketone 1. Instead, the fastest enzyme of the resolution screens, KRED-P1-C01, turned out to be ideal for reoxidation of (+)-4. KRED-P1-C01 was not only fast catalyst, but it also had a relatively low preference for either enantiomer of 4 ($S = 6.0$). As such it could also readily oxidize (+)-4, the enantiomer that reacted only very slowly with more selective KRED enzymes. In preparative scale experiments, 9.7 g of resolved (+)-4 in a total reaction volume of 80 mL of 30:70 acetone:buffer mixture containing only 34 mg of KRED-P1-C01 gave, after purification, 9.3 g (97%) of (–)-1 (99.5:0.5 er).^{10,11} The ease of the operation, very low enzyme consumption, and high yield renders the enzymatic oxidation extremely attractive.

Finally, as the resolved alcohol (+)-4 and ketone (+)-1 have very similar R_f values, an alternative method for separation of (+)-4 from (+)-1 was developed. Michael trapping of (+)-1 to generate a water soluble adduct would allow easy purification of 4 by simple extraction. Towards this end, attempted reaction of ketone (+)-1 with cysteine led to full consumption of (+)-1 on TLC. However, upon extraction, the cysteine adduct hydrolyzed back to 1 and 4. In search of more stable adducts, thioglycerol turned out to be a better choice, and it readily reacted with (+)-1 in the presence of K_2CO_3 fully consuming the ketone (+)-1 based on TLC (Scheme 4). Filtration of the reaction mixture through a short pad of silica gel retained the thioglycerol adduct 5 while alcohol (+)-4 was recovered (90% recovery) containing only 0.5% of (+)-1 by HPLC – the same level of purity that was attained by chromatographic separation of (+)-1 and (+)-4 directly.

Table 2: Enzymatic oxidative resolution of endo-alcohol 4^a

Entry	Enzyme	Conv. (%) ^d	er, Major enantiomer ^c	Time (min)	Selectivity factor ^f
1	KRED-P1-A04 ^b	42	96:4 (+)	30	84
2	KRED-P1-B02 ^b	18	>99:1 (+)	5	310
3	KRED-P1-B05 ^b	26	93:7 (+)	30	14
4	KRED-P1-B10 ^b	47	88:12 (+)	30	14
5	KRED-P1-B12 ^b	40	94:6 (+)	30	22
6	KRED-P1-C01 ^b	57	76:24 (+)	2	6.2
7	KRED-P1-H08 ^b	61	72:28 (+)	10	5.0
8	KRED-P1-H10 ^b	48	92:8 (+)	10	25
9	KRED-P2-B02 ^b	31	89:11 (+)	10	11
10	KRED-P2-C02 ^b	16	88:12 (+)	10	9.1
11	KRED-P2-C11 ^b	45	91:9 (+)	10	19
12	KRED-P2-D03 ^b	46	90:10 (+)	10	19
13	KRED-P2-D11 ^b	54	82:18 (+)	10	10
14	KRED-P2-D12 ^b	20	>99:1 (+)	10	150
15	KRED-P2-G03 ^b	59	72:28 (+)	10	4.9
16	KRED-P2-H07 ^b	44	94:6 (+)	30	36
17	KRED-P3-B03 ^b	37	68:32 (+)	30	2.5
18	KRED-P3-G09 ^b	10	69:31 (–)	30	2.6
19	KRED-P3-H12 ^b	51	70:30 (–)	30	3.5
20	KRED-101 ^b	17	69:31 (+)	30	2.5
21	KRED-119 ^b	17	92:8 (–)	30	16
22	KRED-130 ^b	9.1	89:11 (–)	30	9.8
23	KRED-NADH-101 ^b	27	89:11 (–)	30	11
24	KRED-NADH-110 ^b	45	92:8 (+)	10	22
25	ADH-101 ^a	40	95:5 (+)	30	33
26	ADH-110 ^a	38	97:3 (+)	30	58
27	ADH-105 ^c	21	76:24 (–)	30	3.7
28	ADH-112 ^c	5.8	58:42 (+)	30	1.4
29	ADH-104 ^c	3.1	90:10 (+)	30	13
30	ADH-132 ^b	1.7	59:41 (+)	30	1.6

a) A full table is available in SI; b) 5.0 mg of (±)-endo-4, 2.5 mg of enzyme, 25 μL of acetone, 475 μL of KRED Recycling Mix P, pH 7, rt; c) NADH (0.4 mg) added to KRED Recycling Mix P; d) determined by HPLC: Chiralpak IC, hexane/IPA 95:5, 1 mL/min, 210 nm; e) 230 nm; f) based on product, see the SI for further details.



Scheme 4. a) Reoxidation of (+)-4 to (-)-1. b) Michael trapping of (-)-1 with thioglycerol to ease the purification of the mixture of (+)-4 to (-)-1.

In conclusion, using a sequence of DIBAL-H reduction, ketoreductase-catalyzed oxidative resolution, and reoxidation with another ketoreductase, racemic ketone (\pm)-1 could be readily resolved in over 20 g scale using a minimal amount of enzyme (down to 1:2000 enzyme:substrate ratio for the resolution, and 1:300 ratio for the oxidation step). The successful oxidation with enzymes suggests that enzymatic oxidations of alcohols to ketones should be more widely considered in synthesis, not only for resolution but also for preparative oxidations.

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Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

References and Notes

- (1) Terpenes and terpenoids: (a) (-)-herbertene, (+)- β -cuparenone, (-)-debromoaplysin, and (-)-aplysin: Takano, S.; Moriya, M.; Ogasawara, K. *Tetrahedron Lett.* 1992, 33, 329. (b) cuparenone: Takano, S.; Inomata, K.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* 1989, 271. (c) epi- β -santalene: Takano, S.; Inomata, K.; Kurotaki, A.; Ohkawa, T.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* 1987, 1720. (d) pentenocin B: Suguhara, T.; Fukuda, H.; Iwabuchi, Y. *J. Org. Chem.* 2004, 69, 1744. (e) omphadiol: Parthasarathy, G.; Eggert, U.; Kalesse, M. *Org. Lett.* 2016, 18, 2320. (f) capnellene: Tanaka K.; Ogasawara, K. *Chem. Commun.* 1996, 1839. (g) sordaricin: Mander L. N.; Thomson, R. J. *J. Org. Chem.* 2004, 70, 1654. (h) guanacastepene A core: Mehta, G.; Umarye, J. D. *Org. Lett.* 2002, 4, 1063. Steroids: (i) (+)-equilenin: Takano, S.; Inomata, K.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* 1990, 1544; (j) estrone, formal: Tanaka, K.; Nakashima, H.; Taniguchi, T.

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- (5) Brown, H. C.; Hess, H. M., *J. Org. Chem.* 1969, 34, 2206.
- (6) Iura, Y.; Sugahara, T.; Ogasawara, K. *Tetrahedron Lett.* 1999, 40, 5735.
- (7) (\pm)-endo-Alcohol ((\pm)-4): To a solution of (\pm)-1 (10.0 g, 68 mmol, 1.0 equiv.) in toluene (200 mL) was added DIBAL-H (1.0 M in toluene, 89 mL, 89 mmol, 1.3 equiv.) dropwise at -78°C . After stirring for 20 min at the same temperature, the reaction was quenched with MeOH (40 mL), and aq. sat. NaK tartrate (120 mL) was added. The mixture was stirred until clear solution formed and layers were separated. The aqueous layer was extracted with DCM (2×200 mL) and the combined organic layers dried with Na_2SO_4 and concentrated to afford (\pm)-endo-4 (9.7 g, 97%) as a yellowish crystalline solid. mp = 54.4 – 55.5°C ; ^1H NMR (300 MHz, CDCl_3): δ = 6.16 (1H, dd, J = 5.6, 2.3 Hz), 5.82 (1H, dd, J = 5.6, 3.1 Hz), 5.59 (2H, s), 4.67 (1H, d, J = 7.4 Hz), 3.30 (1H, dd, J = 7.3, 3.6 Hz), 2.99–2.91 (3H, m), 1.57 (1H, d, J = 8.0 Hz), 1.46 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, CDCl_3): δ = 135.4, 135.3, 134.8, 133.5, 76.1, 54.2, 52.7, 47.2, 47.0, 44.9.
- (8) Crystallographic data for (+)-1, (-)-1, (-)-exo-3 and (\pm)-endo-4 have been deposited with the accession numbers CCDC 1827649, 1827648, 1831369 and 1827647 respectively, and can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.
- (9) The distance between the C3 hydrogen atom and C9 was measured from the X-ray structure of (-)-exo-3 to be 2.58 Å on average, and the distance between C3 oxygen atom and C9 was measured from (\pm)-4 to be 2.86 Å on average.
- (10) Another 10.7 g batch of (+)-4 was oxidized under the following conditions: total reaction volume of 75 mL, initial loading of 27 mg of KRED-P1-C01, with additional 10 mg of KRED-P1-C01 added after 73 h. In this way, the total reaction time was reduced to 5 days, affording a quantitative yield (10.6 g) of (-)-1.
- (11) Oxidative enzymatic kinetic resolution of (\pm)-4: To a solution of (\pm)-endo-4 (23.7 g, 160 mmol) in acetone (47 mL) and KRED Recycle Mix P (110 mL) was added KRED-P1-B02 (60 mg) in KRED Recycle Mix P (3 mL). The mixture was stirred for 14 h at rt, extracted with Et_2O (2×100 mL), washed with brine (50 mL), dried with Na_2SO_4 and concentrated. Purification by Combiflash (100% hexane to 20% EtOAc/hexane) gave alcohol (+)-endo-4 (9.9 g, 42%, er > 99:1) and ketone (+)-1 (9.7 g, 41%, er > 99.5:0.5) as white crystalline solids. (+)-endo-4: mp = 66.6 – 68.2°C (lit. 81 – 82.5°C); $[\alpha]_D^{20}$ = $+120^\circ$ (c = 1.0, DCM); ^1H NMR (300 MHz, CDCl_3): δ = 6.16 (1H, dd, J = 5.6, 2.3 Hz), 5.82 (1H, dd, J = 5.6, 3.1 Hz), 5.59 (2H, s), 4.67 (1H, d, J = 7.4 Hz), 3.30 (1H, dd, J = 7.3, 3.6 Hz), 2.99–2.91 (3H, m), 1.57 (1H, d, J = 8.0 Hz), 1.46 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, CDCl_3): δ = 135.4, 135.3, 134.8, 133.5, 76.1, 54.2, 52.7, 47.2, 47.0, 44.9. Enzymatic oxidation of alcohol (+)-4 to ketone (-)-1: To a solution of resolved alcohol (+)-4 (9.7 g, 65 mmol, ee > 99%, containing <0.5% ketone (+)-1) in acetone (24 mL) and KRED Recycle Mix P (45 mL) was added KRED-P1-C01 (34 mg) in KRED Recycle Mix P (1 mL). After stirred for 7 days at 30°C , the mixture was extracted with Et_2O , washed with brine, dried with Na_2SO_4 and concentrated to afford (-)-1 (9.3 g, 97%, er > 99:1) as a white crystalline solid.

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