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Reduction of various ketones by red algae

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Abstract—The reduction of acetophenone derivatives, (+)- and (−)-camphorquinones and steroidal ketones using red algae (*Cyanidioschyzon merolae* 10D and *Cyanidium caldarium*) was investigated. It was found that fluoro, chloro and bromo acetophenone derivatives **1a**–**i** were reduced with good enantioselectivity. On the contrary, reduction of methyl and methoxy acetophenone **1j**–**o** showed low enantioselectivity. The reduction followed Prelog's rule, giving the (*S*)-alcohols in all cases. Moreover, (+)- camphorquinone **5a** was reduced to give (−)-3*S*- *exo*-hydroxycamphor **5d** as the major product with high stereoselectivity in high yield. In addition, it was found that reduction of 5α-androstane-3,17-dione **8a** gave the 3α-OH isomer (3α-OH/3β-OH = 76/24) with high stereoselectivity. Overall it was found that *C. merolae* and *C. caldarium* were able to reduce various substrates.

1. Introduction

Optically active compounds are important building blocks for the synthesis of pharmaceuticals, pesticides, pheromones, flavors, fragrances and advanced materials such as liquid crystals. It is known that the synthesis of chiral alcohols can be achieved from the corresponding prochiral ketones by asymmetric reduction. Recently, many biocatalytic reductions of ketones have been reported.[1–6] Asymmetric reduction by chemical methods involves the use of expensive reagents, and heavy metals are often employed.[7] Acetophenone derivatives are very interesting model compounds as foreign substrates for biotransformation, because either enantiomer may be formed, which may be determined easily. These compounds have been effectively used as a building block for the asymmetric synthesis of drugs.[8] Reduction of the carbonyl group is performed using various biocatalysts such as cultured cells[9–11] and fresh-cut vegetables.[12]

Recently, we have reported that the biotransformation of a synthetic substance into a more useful substance by plant cultured cells is an important reaction in synthetic chemistry.[13–17] During the course of our studies, it was revealed that the biotransformation of (+)-camphorquinone by cyanobacteria gives (−)-3*S*-*exo*-hydroxycamphor with high stereoselectivity.[3] Moreover, we reported the biotransformation of (+)- and (−)-camphorquinones by *Nicotiana tabacum* and *Catharanthus roseus*, and it was found that (+)-camphorquinone was reduced with moderate stereoselectivity by *C. roseus* to provide (−)-(3*S*)-*exo*-hydroxycamphor.[9] It is known that the plant cultured cells are capable of regio- and stereoselective hydroxylation, oxidation, reduction, hydrogenation, glycosylation and hydrolysis for various organic compounds.[18] Furthermore, *Synechococcus elongatus* PCC 7942 (cyanobacteria) reduced both the endocyclic C=C of *s-trans* enones and the exocyclic C=C of *s-cis* enones with high enantioselectivity to afford optically active (*S*)-α-substituted ketones.[19,20]

Here, we report a method for the asymmetric reduction with photosynthetic microbes (*Cyanidioschyzon merolae* (*C. merolae*) and *Cyanidium caldarium* (*C. caldarium*)).

2. Results and discussion

2.1. Reduction of acetophenone derivatives

Recently, a report by Kuroiwa et al. on unicellular red algae, *C. merolae*, provided information on the basic and essential genes.[21] The cells of *C. merolae* offer unique

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advantages for the studies of mitochondrial and plastid divisions[22,23] because they do not have a rigid cell wall and contain just one nucleus, one mitochondrion and one plastid, the divisions of which can be highly synchronized by light/dark cycles.[24] On the other hand, unicellular red algae, *C. caldarium*, is considered to be one of the most primitive algae.[25] This algae has a simple cellular structure, with each cell having one plastid and one nucleus plus other organelles, such as Golgi bodies, endoplasmic reticulum and microbodies.[26] *C. merolae* and *C. caldarium* are extremely characteristic algae inhabiting sulfate-rich hot springs.

A series of aromatic ketones were targeted for biotransformation using *C. merolae* and *C. caldarium* in Allen's medium. *ortho*-, *meta*- and *para*-substituted fluoro, chloro, bromo, methyl and methoxy acetophenones **1a**–**o** were reduced to the corresponding (*S*)-alcohols in all cases (Table 1).[27] The alcohol products had the (*S*)-configuration, which is consistent with Prelog's rule. Reduction of fluoro, chloro and bromo acetophenones provided good enantioselectivity (67–99%). The yields increased with increasing size of the halogens. In contrast, reduction of methyl and methoxy acetophenones was observed in low yields (7–38%) and enantioselectivity (2–41%). In the case of methoxy groups, there was a substantial drop in the enantioselectivity of the product (Table 1, entries 10–12). In these cases, it seems that the −M effect of the substituents was superior to their −I effect. The enantioselectivity of the halogen-substituted acetophenones **1a**–**i** was high due to the −I effect, except at the *meta*-position. The enantioselectivity of the reduction depends on the position of the substituent. The minor role of the −I effect was indicated by the reduction of methyl acetophenones **1m**–**o**. It was found that the electronic effect of the substituents on the aromatic ring has a defined role in the enantioselectivity of the reaction.

The reduction of *C. merolae* 10D and *C. caldarium* of several aromatic ketones having alkyl chains of different length shown in Table 2. It was found that compounds **3a** and **3b** were reduced after 3 days incubation in good enantioselectivity, while longer alkanones, **3c**–**f**, could not be reduced. This result is apparently due to the size of the alkyl side chains and their lower solubility in the reaction medium.

2.2. Effect of XAD on reduction of acetophenone derivatives

Recently, the hydrophobic polymer, Amberlite™ XAD, has been used to reduce the substrate in microbial reduction, and increased the chemical yield of the reduction.[28] Amberlite™ XAD, a hydrophobic polymer such as polystyrene or polyacryl ester, is a commercially available adsorbent, and has a large surface area. The hydrophobic polymer, Amberlite™ XAD, can control the stereoselectivity of the reduction of ketones by the fungus, *Geotrichum candidam*.[29] Previously, Nakamura et al. reported that the use of XAD-7 increases the enantioselectivity in the reduction

Table 1. Reduction of aryl ketones by red algae

Entry	Substrate	R	Product	*C. merolae* 10D			*C. caldarium*		
				Yield (%)	ee (%)	Config.	Yield (%)	ee (%)	Config.
1	**1a**	*o*-F	**2a**	40	91	*S*	34	92	*S*
2	**1b**	*m*-F	**2b**	54	71	*S*	58	75	*S*
3	**1c**	*p*-F	**2c**	50	74	*S*	50	67	*S*
4	**1d**	*o*-Cl	**2d**	70	98	*S*	70	98	*S*
5	**1e**	*m*-Cl	**2e**	78	73	*S*	68	75	*S*
6	**1f**	*p*-Cl	**2f**	71	94	*S*	79	96	*S*
7	**1g**	*o*-Br	**2g**	87	99	*S*	80	99	*S*
8	**1h**	*m*-Br	**2h**	84	77	*S*	90	75	*S*
9	**1i**	*p*-Br	**2i**	92	98	*S*	85	98	*S*
10	**1j**	*o*-OCH_3	**2j**	30	2	*S*	38	4	*S*
11	**1k**	*m*-OCH_3	**2k**	32	6	*S*	29	7	*S*
12	**1l**	*p*-OCH_3	**2l**	13	6	*S*	9	12	*S*
13	**1m**	*o*-CH_3	**2m**	16	37	*S*	28	27	*S*
14	**1n**	*m*-CH_3	**2n**	7	39	*S*	9	41	*S*
15	**1o**	*p*-CH_3	**2o**	36	16	*S*	31	16	*S*

Reaction conditions: Substrate (20 mg), red algae (dry weight 1 g/L) and Allen's medium (100 mL) were employed for 3 days (42 °C, pH 2.5, and 2000 lx).

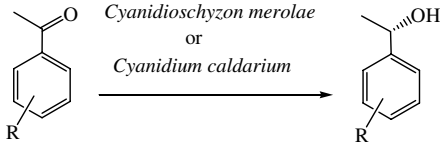
Table 2. Reduction of aryl ketones by red algae

Entry	Substrate	R	Product	*C. merolae* 10D			*C. caldarium*		
				Yield (%)	ee (%)	Config.	Yield (%)	ee (%)	Config.
1	**3a**	CH_3	**4a**	28	80	*S*	24	82	*S*
2	**3b**	CH_3CH_2	**4b**	53	78	*S*	46	74	*S*
3	**3c**	$CH_3(CH_2)_3$	—	0	—	—	0	—	—
4	**3d**	$CH_3(CH_2)_4$	—	0	—	—	0	—	—
5	**3e**	$CH_3(CH_2)_5$	—	0	—	—	0	—	—
6	**3f**	$CH_3(CH_2)_6$	—	0	—	—	0	—	—

Reaction conditions: Substrate (20 mg), red algae (dry weight 1 g/L) and Allen's medium (100 mL) were employed for 3 days (42 °C, pH 2.5, and 2000 lx).

of simple aliphatic and aromatic ketones.[30] Thus, we examined the efficiency of using XAD-7 in terms of the enantioselectivity.

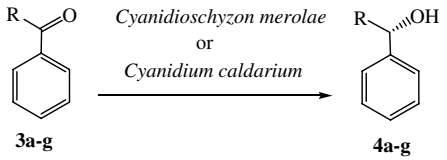
ortho-, *meta*- and *para*-substituted fluoro, chloro, bromo, methyl and methoxy acetophenones **1a**–**o** were reduced to the corresponding (*S*)-alcohols in all cases (Scheme 1). The yield and stereoselectivity did not change using XAD-7. Moreover, we investigated the effect of the size of the alkyl group for 1-phenylalkanones **3a**–**f** by *C. merolae* and *C. caldarium*. These results are shown in Table 3. From these results, it was found that increasing the length of the alkyl chain (Table 3, entries 3–6) decreased the enantioselectivity for the corresponding (*S*)-alcohols. In addition, the reduction of octanophenone **3f** is the first report of the use of biocatalysts for this substrate (Scheme 2).



1a-m **2a-m**

Substrate	R
1a	*o*-F
1b	*m*-F
1c	*p*-F
1d	*o*-Cl
1e	*m*-Cl
1f	*p*-Cl
1g	*o*-Br
1h	*m*-Br
1i	*p*-Br
1j	*o*-OCH_3
1k	*m*-OCH_3
1l	*p*-OCH_3
1m	*o*-CH_3
1n	*m*-CH_3
1o	*p*-CH_3

Scheme 1.



3a-g **4a-g**

Substrate	R
3a	CH_3
3b	CH_3CH_2
3c	$CH_3(CH_2)_3$
3d	$CH_3(CH_2)_4$
3e	$CH_3(CH_2)_5$
3f	$CH_3(CH_2)_6$

Scheme 2.

The reduction of asymmetrical ketones by cyanobacteria (*S. elongatus* PCC 7942) as biocatalysts under light conditions gave the corresponding (*S*)-alcohols with excellent enantioselectivities.[31–33] Such results suggest that the reduction of aliphatic ketones by red algae will be very useful for the practical preparation of (*S*)-alcohols with, for example, *S. elongatus* PCC 7942.

2.3. Reduction of (+)-and (−)-camphorquinones

Biotransformation of (+)-camphorquinone **5a** afforded a mixture of diastereoisomers of three α-keto alcohols: (+)-(2*R*)-*exo*-hydroxyepicamphor **5b**, (−)-(3*S*)-*exo*-hydroxycamphor **5d** and (+)-(3*R*)-*endo*-hydroxycamphor **5e**. However, (−)-(2*S*)-*endo*-hydroxyepicamphor **5c** could not be obtained from (+)-camphorquinone **5a** using *C. merolae* and *C. caldarium* (Scheme 3).

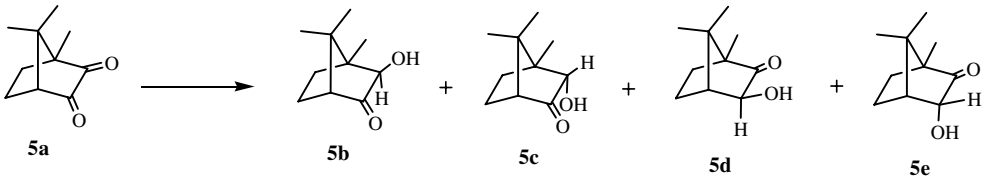
(−)-Camphorquinone **6a** was transformed to give the four corresponding isomers: (−)-(2*S*)-*exo*-hydroxyepicamphor **6b**, (+)-(2*R*)-*endo*-hydroxyepicamphor **6c**, (+)-(3*R*)-*exo*-hydroxycamphor **6d** and (−)-(3*S*)-*endo*-hydroxycamphor **6e** (Scheme 4).

From these results, it was found that only hydroxy ketones were formed and diols were not obtained. (+)-Camphorquinone **5a** was reduced stereoselectively by *C. merolae*

Table 3. Reduction of aryl ketones by red algae using XAD

Entry	Substrate	R	Product	*C. merolae* 10D			*C. caldarium*		
				Yield (%)	ee (%)	Config.	Yield (%)	ee (%)	Config.
1	**3a**	CH_3	**4a**	22	79	*S*	36	87	*S*
2	**3b**	CH_3CH_2	**4b**	48	82	*S*	50	75	*S*
3	**3c**	$CH_3(CH_2)_3$	**4c**	69	83	*S*	74	80	*S*
4	**3d**	$CH_3(CH_2)_4$	**4d**	69	54	*S*	66	59	*S*
5	**3e**	$CH_3(CH_2)_5$	**4e**	71	47	*S*	45	54	*S*
6	**3f**	$CH_3(CH_2)_6$	**4f**	44	42	*S*	55	37	*S*

Reaction conditions: Substrate (20 mg), red algae (dry weight 1 g/L), XAD-7 (250 mg) and Allen's medium (100 mL) were employed at 42 °C (5 days, pH 2.5 and 2000 lx).



Scheme 3. Reduction of (+)-camphorquinone **5a** by red algae.

10D and *C. caldarium* to give (−)-(3*S*)-*exo*-hydroxycamphor **5d** in 83% and 84% yield, respectively. These results indicate that the reduction activities are higher than those of plant cultured-cells *N. tabacum* and *C. roseus*.[9]

In the case of (−)-camphorquinone **6a** using *C. merolae* 10D and *C. caldarium*, (+)-(3*R*)-*exo*-hydroxycamphor **6d** was obtained in 64% yield, respectively (Table 4).

Table 4. Reduction of camphorquinone **5a** and **6a** by red algae[a]

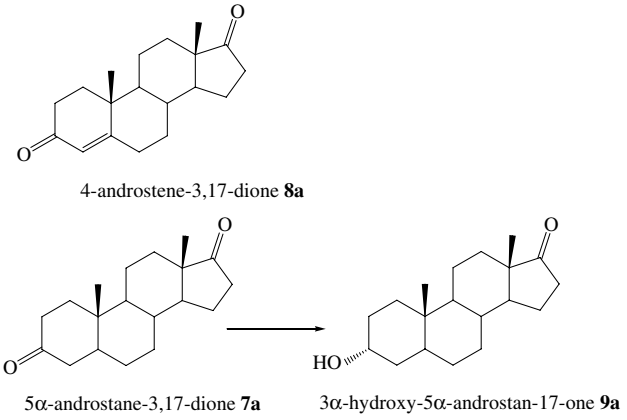
Run	Substrate	Red algae	Yield[b] (%)	Product ratio[c] (%)
1	**5a**	*C. merolae*	92	**5b** (16) **5c** (−) **5d** (83) **5e** (1)
2	**5a**	*C. caldarium*	91	**5b** (15) **5c** (−) **5d** (84) **5e** (1)
3	**6a**	*C. merolae*	93	**6b** (19) **6c** (12) **6d** (64) **6e** (5)
4	**6a**	*C. caldarium*	92	**6b** (18) **6c** (13) **6d** (64) **6e** (5)

[a] Reaction conditions: Substrate (20 mg) and red algae (70 mL) were cultivated with shaking at 25 °C for 2 days.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR signals.

The growth rate of red algae is faster than that of other typical microbes. Some of the problems using plant cultured cells as biocatalysts are that they are usually grown very slowly and require a large amount of the catalyst. Reduction of (+)- and (−)-camphorquinones **5a** and **6a** by *N. tabacum* and *C. roseus* needs 15 g plant cultured cells to biotransform 20 mg of the substrates; however, only 100 mg (dry weight) of red algae is required to biotransform stereoselectively.

2.4. Reduction of steroidal ketones

Androstane-3,17-dione **7a** and Δ^4-androstene-3,17-dione **8a** are commercially available steroids which have been used for partial synthesis of many steroidal hormones, including testosterone. Steroidal ketones are known as important substrates for stereoselectivity and regioselectivity. It is known that the biotransformation for steroidal 3,17-dione gives the C_{17}-β hydroxy derivatives (Scheme 5).[34,35] In order to examine the stereoselectivity and regioselectivity in the reduction of steroidal ketones, androstane-3,17-dione **7a** and Δ^4-androstene-3,17-dione **8a** were investigated as model compounds. These results are shown in Table 5.



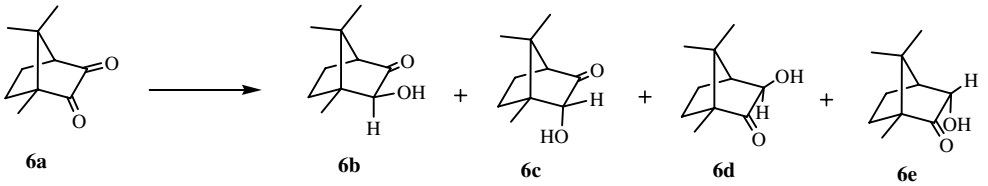
Scheme 5. Reduction of steroidal ketones **7a** and **8a** by red algae.

Table 5. Reduction of steroidal ketones **7a** and **8a** by red algae[a]

Run	Substrate	Red algae	Product ratio[b] (%)	3α-OH/ 3β-OH[c]	Conv.[b]
1	**7a**	*C. merolae*	**9a** (80)	75/25	80
2	**7a**	*C. caldarium*	**9a** (78)	76/24	78
3	**8a**	*C. merolae*	**7a** (27) **9a** (45)	77/23	72
4	**8a**	*C. caldarium*	**7a** (24) **9a** (42)	76/24	66

[a] Reaction conditions: Substrate (20 mg) and red algae (70 mL) were cultivated with shaking at 42 °C for 7 days.
[b] Determined by GC–MS peak ratio.
[c] Relative intensities by ^{1}H NMR signals.

The position of the hydroxyl group was established by chemical shifts in the ^{13}C NMR spectra. The stereochemistry of the secondary alcohols is due to the multiplicity of the relevant ^{1}H NMR signal (18-H and 19-H methyl signals). In addition, the 3β-H resonance showed a characteristic downfield shift ($\Delta\delta$ 0.4 ppm) compared to 3α-H for 3β-OH.



Scheme 4. Reduction of (−)-camphorquinone **6a** by red algae.

It was found that the reduction of steroidal ketones **7a** and **8a** occurs at the C_3-position preferentially, rather than the C_{17}-ketone (Table 5).[36] Moreover, the more unstable C_3-α hydroxy compounds are obtained preferentially, rather than less hindered C_3-β hydroxy derivatives.

3. Conclusion

In summary, the above studies have demonstrated the biotransformation of a broad assortment of structurally different acetophenone derivatives, (+)- and (−)-camphorquinones and steroidal ketones. In the case of acetophenone derivatives **3a**–**f**, increasing the size of the flanking groups by incorporating hydrophobic groups (increasing the alkyl chain length) led to low enantioselectivity. The presence of electron-withdrawing substituents in the phenyl ring **1a**–**i** improved the enantioselectivity of the reduction product. Moreover, in the reduction of steroidal ketones **7a** and **8a**, the C_3-α hydroxy compounds are obtained preferentially, rather than C_3-β hydroxy derivatives. We have established a convenient and environmentally benign stereoselective reduction system employing red algae *C. merolae* 10D and *C. caldarium*.

4. Experimental

The racemic alcohols were obtained by the reduction of the corresponding ketones with sodium borohydride or authentic samples were purchased. XAD-7 was washed with 1 M hydrochloric acid, 1 M sodium hydroxide, water, methanol and ether. *C. caldarium* was obtained from the Institute of Applied Microbiology Culture Collection (IAMCC). Gas chromatographic analysis was performed using chiral GC-column (Chirasil-DEX CB; 25 m) equipped on Shimadzu GC-17A. Gas chromatography/mass spectrometry analysis was Shimadzu GCMS-QP5050 (EI-MS 70 eV) using DB1 (0.25 mm × 30 m, 0.25 μm) capillary column GC; GC: GC-17A. The IR spectra were measured on a Jasco FT-IR 230. The NMR spectra were measured on a JEOL GSX 400 spectrometer. $CDCl_3$ with tetramethylsilane as the internal standard was used.

4.1. Preparation of microbial culture

Allen's medium was prepared by mixing $(NH_4)_2SO_4$ (2.64 g), KH_2PO_4 (0.544 g), $MgSO_4$ (0.492 g), $CaCl_2$ (0.148 g) and P4 metal (4 mL) in distilled H_2O (1 L). The medium was adjusted to pH 2.2–2.5 and sterilized.

P4 metal solution was $FeCl_3$ (196 mg), $MnCl_2$ (36 mg), $ZnSO_4$ (22.2 mg), $CoCl_2$ (4 mg), Na_2MoO_4 (2.5 mg) and Na_2EDTA (1000 mg) dissolved in distilled H_2O (1 L).

4.2. General procedure of biotransformation conditions

Acetophenone derivatives (20 mg) with XAD-7 (250 mg) were added to the culture of *C. merolae* and *C. caldarium* (100 mL, 1 g/L as dry weight) in Allen's medium and shaken for the periods shown in the tables. The mixture was treated with a shaker (120 rpm) at 42 °C in the light (2000 lx). At the end of the reaction, XAD-7 was filtered from the medium. The filtrate was extracted with ether (2 × 50 mL), washed with water (2 × 20 mL) and dried. XAD-7 was washed with ether (50 mL) and the extract was concentrated into an oily residue. All the products were determined by IR, ^1H NMR and gas chromatographic analyses.

Compound **2c**.[39] Colourless oil; $[\alpha]_D^{27} = -40.0$ (*c* 0.9, $CHCl_3$); IR 3353, 1076 and 831 cm^{-1}; ^1H NMR: δ (ppm) 1.48 (d, $J = 6.0$ Hz, 3H), 1.83 (br s, 1H), 4.88 (q, $J = 6.0$ Hz, 1H) and 7.00–7.34 (m, 4H); MS *m*/*z*: 140 (M^+, 18.9), 125 (100), 122 (11.9), 101 (6.97), 96 (24.1), 83 (2.60), 75 (19.0), 63 (4.39), 57 (7.95) and 51 (21.3).

Compound **2f**.[37,39] Colourless oil; $[\alpha]_D^{27} = -45.0$ (*c* 0.9, $CHCl_3$); IR 3360, 1050, 898 and 828 cm^{-1}; ^1H NMR: δ (ppm) 1.47 (d, $J = 6.0$ Hz, 3H), 1.88 (br s, 1H), 4.86 (q, $J = 6.0$ Hz, 1H) and 7.22–7.30 (m, 4H); MS *m*/*z*: 156 (M^+, 13.2), 141 (60.3), 138 (7.39), 121 (10.2), 113 (27.1), 103 (9.88), 87 (1.42), 77 (100), 63 (6.40) and 51 (41.8).

Compound **2i**.[39,41] Yellow oil; $[\alpha]_D^{27} = -37.3$ (*c* 1.1, $CHCl_3$); IR 3326, 1078, 896 and 825 cm^{-1}; ^1H NMR: δ (ppm) 1.47 (d, $J = 6.0$ Hz, 3H), 1.87 (br s, 1H), 4.86 (q, $J = 6.0$ Hz, 1H) and 7.23–7.45 (m, 4H); MS *m*/*z*: 200 (M^+, 10.7), 184 (7.46), 182 (6.05), 157 (20.7), 121 (17.1), 107 (0.58), 77 (100), 63 (7.93) and 51 (53.6).

Compound **2l**.[39,40] Colourless oil; $[\alpha]_D^{27} = -4.2$ (*c* 0.9, $CHCl_3$); IR 3378, 1079 and 831 cm^{-1}; ^1H NMR: δ (ppm) 1.47 (d, $J = 6.0$ Hz, 3H), 1.87 (br s, 1H), 3.80 (s, 3H), 4.85 (q, $J = 6.0$ Hz, 1H) and 6.9–7.3 (m, 4H); MS *m*/*z*: 152 (M^+, 15.6), 134 (100), 119 (64.2), 103 (5.69), 91 (77.5), 77 (38.1), 65 (63.6) and 51 (36.7).

Compound **2o**.[37] Colourless oil; $[\alpha]_D^{27} = -13.0$ (*c* 0.4, $CHCl_3$); IR 3367, 1078, 896 and 811 cm^{-1}; ^1H NMR: δ (ppm) 1.48 (d, $J = 6.0$ Hz, 3H), 1.80 (br s, 1H), 2.34 (s, 3H), 4.86 (q, $J = 6.0$ Hz, 1H) and 6.84–7.30 (m, 4H); MS *m*/*z*: 136 (M^+, 32.3), 121 (100), 118 (35.3), 117 (43.9), 115 (19.8), 103 (5.47), 93 (93.6), 91 (95.7), 79 (3.25), 77 (59.3), 65 (39.5), 63 (22.6) and 51 (34.4).

Compound **4a**.[37–39] Colourless oil; $[\alpha]_D^{27} = -43.7$ (*c* 0.9, $CHCl_3$); IR 3350, 1060, 749 and 732 cm^{-1}; ^1H NMR: δ (ppm) 1.48 (d, $J = 6.0$ Hz, 3H), 1.90 (br s, 1H), 4.88 (q, $J = 6.0$ Hz, 3H) and 7.2–7.4 (m, 5H); EIMS *m*/*z*: 122 (M^+, 27.0), 107 (77.3), 104 (7.31), 79 (100), 77 (61.0) and 51 (47.5).

Compound **4b**.[37–41] Colourless oil; $[\alpha]_D^{27} = -38.1$ (*c* 1.0, $CHCl_3$); IR 3355, 1093, 750 and 692 cm^{-1}; ^1H NMR: δ (ppm) 0.90 (t, $J = 6.2$ Hz, 3H), 1.7–1.9 (m, 2H), 1.95 (br s, 1H), 4.6 (t, $J = 6.0$ Hz, 1H) and 7.2–7.4 (m, 5H); EIMS *m*/*z*: 136 (M^+, 11.5), 118 (4.70), 117 (7.53), 107 (100), 91 (6.55), 79 (97.9), 77 (54.9) and 51 (47.5).

Compound **4c**.[37] Colourless oil; $[\alpha]_D^{27} = -39.3$ (*c* 0.5, $CHCl_3$); IR 3361, 1110, 754 and 694 cm^{-1}; ^1H NMR: δ (ppm) 0.90 (t, $J = 7.0$ Hz, 3H), 1.2–1.8 (m, 6H), 2.10 (br

s, 1H), 4.60 (t, $J = 6.0$ Hz, 1H) and 7.2–7.4 (m, 5H); EIMS m/z: 164 (M^+, 0.59), 146 (2.20), 128 (0.50), 117 (8.56), 115 (4.73), 107 (100), 104 (3.79), 91 (5.87), 79 (65.4), 77 (32.5) and 51 (15.5).

Compound **4d**.[4] Colourless oil; $[\alpha]_D^{27} = -15.6$ (c 1.2, $CHCl_3$); IR 3349, 1060, 755 and 694 cm^{-1}; ^1H NMR: δ (ppm) 0.88 (t, $J = 7.0$ Hz, 3H), 1.4–1.8 (m, 8H), 1.9 (br s, 1H), 4.62 (t, $J = 6.0$ Hz, 1H) and 7.2–7.4 (m, 5H); EIMS m/z: 178 (M^+, 2.84), 160 (1.91), 131 (0.68), 117 (9.47), 115 (4.77), 107 (100), 104 (6.74), 91 (6.64), 79 (53.6), 77 (27.2) and 51 (11.1).

Compound **4e**.[4] Colourless oil; $[\alpha]_D^{27} = -17.6$ (c 1.2, $CHCl_3$); IR 3343, 1035, 755 and 694 cm^{-1}; ^1H NMR: δ (ppm) 0.90 (t, $J = 6.2$ Hz, 3H), 1.2–1.8 (m, 10H), 2.0 (br s, 1H), 4.62 (t, $J = 6.0$ Hz, 1H) and 7.2–7.4 (m, 5H); EIMS m/z: 192 (M^+, 1.71), 174 (1.99), 145 (0.16), 131 (0.73), 117 (10.2), 115 (4.97), 107 (100), 104 (10.0), 91 (7.57), 79 (46.7), 77 (23.1) and 51 (8.58).

Compound **4f**.[42] Colourless oil; $[\alpha]_D^{27} = -18.3$ (c 0.8, $CHCl_3$); IR 3347, 1060, 757 and 698 cm^{-1}; ^1H NMR: δ (ppm) 0.87 (t, $J = 6.2$ Hz, 3H), 1.2–1.8 (m, 10H), 2.0 (br s, 1H), 4.62 (t, $J = 6.0$ Hz, 1H) and 7.2–7.4 (m, 5H); EIMS m/z: 206 (M^+, 1.39), 188 (2.06), 131 (0.85), 117 (11.9), 115 (5.48), 107 (100), 104 (14.4), 91 (8.76), 79 (40.6), 77 (20.2) and 51 (6.78).

Compound **5d**.[43] White crystal; $[\alpha]_D^{27} = -78.4$ (c 1.5, $CHCl_3$); IR 3448, 1751 and 1123 cm^{-1}; ^1H NMR: δ (ppm) 0.94 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H), 1.20–2.00 (m, 4H), 2.10 (d, $J = 5.0$ Hz, 1H), 2.59 (br s, 1H) and 3.75 (s, 1H); EIMS m/z: 168 (M^+, 13.2), 153 (1.21), 140 (4.02), 125 (22.8), 107 (6.47), 100 (11.6), 83 (86.9), 69 (39.8), 55 (100) and 53 (15.1).

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