Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 60 (2004) 5439–5451

Total synthesis of (±) maculalactone A, maculalactone B and maculalactone C and the determination of the absolute configuration of natural (+) maculalactone A by asymmetric synthesis

Geoffrey D. Brown[a,b,*] and Ho-Fai Wong[b]

[a]*School of Chemistry, The University of Reading, Whiteknights Rd., Reading RG6 6AD, UK*
[b]*The Department of Chemistry, The University of Hong Kong, Pokfulam Rd., Hong Kong, People's Republic of China*

Received 7 November 2003; revised 2 April 2004; accepted 22 April 2004

Abstract—Maculalactones A, B and C from the marine cyanobacterium *Kyrtuthrix maculans* are amongst the only compounds based on the tribenzylbutyrolactone skeleton known in nature and (+) maculalactone A from the natural source possesses significant biological activity against various marine herbivores and marine settlers. We now report a concise synthesis of racemic maculalactone A in five steps from inexpensive starting materials. Maculalactones B and C were synthesized by a minor modification to this procedure, and the synthetic design also permitted an asymmetric synthesis of maculalactone A to be achieved in around 85% ee. The (+) and (−) enantiomers of maculalactone A were assigned, respectively, to the *S* and *R* configurations on the basis of the chiral selectivity expected for catecholborane reduction of an unsymmetrical ketone in the presence of Corey's oxazoborolidine catalyst. Surprisingly, it appeared that natural (+) maculalactone A was biosynthesized in *K. maculans* in a partially racemic form, comprising ca. 90–95% of the (*S*) enantiomer and 5–10% of its (*R*) enantiomer. Coincidentally therefore, the percentage enantiomeric excess of the product obtained from asymmetric synthesis almost exactly matched that found in nature.

1. Introduction

Kyrtuthrix maculans Umezaki (*Brachytrichia maculans* Gomont; Stigonemataceae)[1] is a marine cyanobacterium found growing on moderately exposed rocky shores in Hong Kong and elsewhere in the world.[2,3] We first became interested in investigating the chemistry of *K. maculans* following the observation that this species normally survived as pure colonies, which were uncontaminated by other marine bacteria and were also apparently exempt from settling of barnacles and foraging by marine herbivores.

Maculalactone A (**1**),[4] a secondary metabolite with the highly unusual tribenzylbutyrolactone skeleton, was the first natural product to be reported from a preliminary chemical investigation of *K. maculans* made in 1996. Compound **1** was by far the most abundant secondary metabolite to be found in this species, although—following subsequent more extensive investigations—we have since described three further tribenzylbutyrolactones, maculalactones B (**2**),[5] C (**3**)[5] and L,[6] as well as the dibenzyldiphenyl-4,5,6,7-tetrahydrobenzofuranones,[5] maculalactones D (**4**), E, F, G, H, I, J and K, and the *seco*-dibenzyldiphenyl-4,5,6,7-tetrahydrobenzofuranone, maculalactone M,[7] as minor secondary metabolites (Fig. 1). *K. maculans* is still the only known natural source for all three classes of secondary metabolite. Our suspicions that some at least of these unusual metabolites might be responsible for providing the chemical defense which *K. maculans* seemed to enjoy against other marine organisms had been recently confirmed by experiments, in which samples of maculalactone A from the natural source were tested against a generalist marine herbivore (*Chlorostoma argyrostoma*) and the larvae of several barnacle species (*Balanus amphitrite*, *Ibla cumingii* and *Tetraclita japonica*). These experiments clearly demonstrated that compound **1** was a potent inhibitor of both herbivory and barnacle settling.[8–10]

Thus, there were now reasonable grounds to suppose that maculalactone A (**1**) might have some potential for development as a novel marine anti-fouling agent;[9,10] and also that the biological activity of some of the other unusual metabolites from *K. maculans* might be worthy of further investigation. Accordingly, we set out to devise a concise and inexpensive synthesis of compound **1** which would allow its preparation on a multi-gram scale for future

Keywords: Marine metabolites; Asymmetric synthesis; Grignard reactions/reagents; NMR.
* Corresponding author. Tel.: +44-118-3787418; fax: +44-118-9316331; e-mail address: g.d.brown@reading.ac.uk

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.060

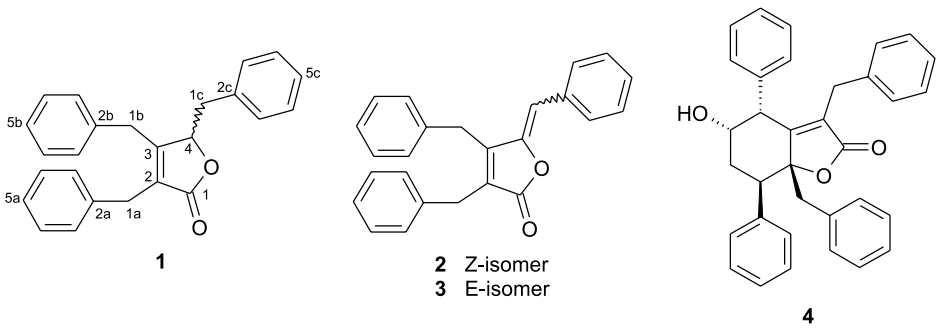


Figure 1. Maculalactones A, B, C and D (**1**–**4**), which have been reported as natural products from *Kyrtuthrix maculans* (the absolute configuration at the 4-position of compound **1** was not determined in the original report of this secondary metabolite; only relative stereochemistry is shown for compound **4**).

evaluation in field trials, when formulated as a paint[9,10] (the extracted yield of maculalactone A from *K. maculans* is of the order of 1 mg/g and it is very tedious and time-consuming to scrape even small amounts of the biological source material from the rocks on which it grows, making it almost impossible to obtain sufficiently large amounts of this compound from the natural source to permit further biological evaluation). A secondary goal of this project was to synthesize some of the other tribenzylbutyrolactones from *K. maculans*, which have not-as yet-been subjected to biological evaluation, due to their low natural abundance. Finally, we also sought to determine the absolute configuration of maculalactone A at the 4-position, an issue which was not addressed in the original report of this compound as a natural product from *K. maculans*.[4] The synthetic strategy described below, which is based on a well known route to the dibenzylbutyrolactone lignans that are commonly found in terrestial plants,[11] has resulted in the realization of all three of these goals.

2. Results and discussion

2.1. Synthesis of racemic maculalactone A ((±)-1)

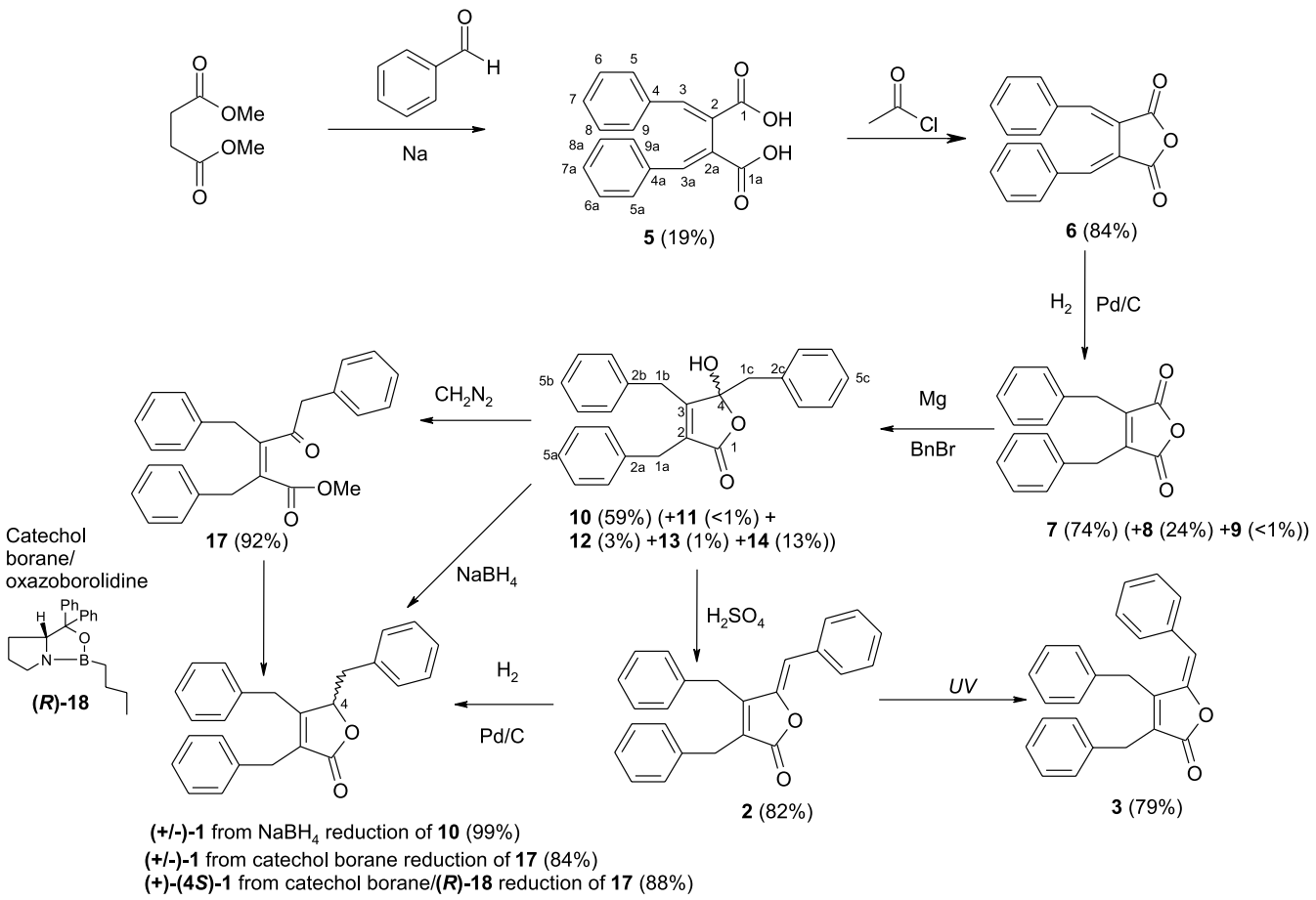
Our synthesis of the tribenzylbutyrolactone skeleton of maculalactone A (**1**) (Scheme 1) was based on previously reported syntheses of the 2,3-aryl di-substituted butyrolactone ring system, from the acid anhydride **7**.[11] 2,3-Dibenzylidenesuccinic acid (**5**), which was employed as the starting material for this synthesis, is commercially available (Section 3.2.1) although this source is rather costly for a large-scale synthesis of maculalactone A; alternatively, it can be prepared much more economically (albeit in low yield) from the Stobbe condensation of dimethyl succinate with benzaldehyde, as is shown in Scheme 1.[12,13] Treatment of the dicarboxylic acid **5** with acetyl chloride resulted in cyclization to the symmetrical acid anhydride **6**,[14,15] and the conjugated diene functional group in **6** then underwent preferential 1,4-hydrogenation to yield the tetra-substituted alkene **7**. Compound **8**, a minor side-product from this reaction, in which the diene has been completely reduced, probably arose via an initial 1,2-reduction, forming intermediate **9** (Fig. 2).† The ratio of the desired product **7** to the unwanted product **8** increased as the loading of palladium catalyst was reduced in the ethyl acetate solution, although the rate of reaction also became slower: a 5% catalyst loading was found to provide the best compromise for obtaining good yields of **7** (74%) in a reasonable time (18 h). Fully assigned NMR data for all of the compounds **5**–**9** (assigned using the 2D NMR techniques HSQC, HMBC, $^{1}H-^{1}H$ COSY and NOESY) are reported in Table 1; all other NMR data that is reported in Table 2 was also rigorously assigned by these same 2D NMR techniques.

The third aromatic substituent in the completed tribenzylbutyrolactone skeleton of **1** was introduced by the addition of a Grignard reagent derived from benzyl bromide (BnBr) to the symmetrically-substituted anhydride **7**. The optimum conditions for forming the key intermediate, tribenzyl-substituted compound **10**, were somewhat unusual and considerable experimentation was required in order to arrive at them. First of all, reflux should be avoided as this leads to dimerization of the Grignard reagent, forming stilbene (**11**). At lower temperatures (0 °C), concentrated solutions of the anhydride **7** and the ethereal Grignard reagent reacted rapidly to give the tetra-substituted addition compounds **12** and **13** (Fig. 2) as the favored products, in which 2 equiv. of Grignard reagent have participated in both 1,2- and 1,4-additions to the carbonyl group of the anhydride. Approximately half of the starting material **7** was recovered unchanged when these reactions were performed under stoichiometric conditions, which suggested that the desired compound **10** was perhaps being formed as an intermediate, but was then preferentially participating in the second addition of a Grignard reagent to yield the corresponding tetra-substituted products (this situation is analogous to the alkylation of an ester by a Grignard reagent, which, as is well known, commonly results in a di-substituted tertiary alcohol product).‡

The optimum conditions for formation of the desired addition product, compound **10**, required that both reactants were present as very dilute solutions, and in order to make compound **10** the dominant product over compound **12**, it

† Compound **9** was isolated in substantial amounts when the duration of the hydrogenation reaction was decreased from 18 to 2 h (see Section 3.2.3).

‡ The lactol group in the intermediate **10** arising from the first nucleophilic addition can reversibly interconvert in situ with its ring-opened keto/acid tautomer; the reactivity of the ketone group in this tautomer towards a second nucleophilic attack would then be greater than that of the starting material, acid anhydride **7**, thereby favoring a second Grignard addition reaction.



Scheme 1. Synthesis of maculalactones A–C (**1**–**3**) from 2,3-dibenzylidenesuccinic acid (**5**).

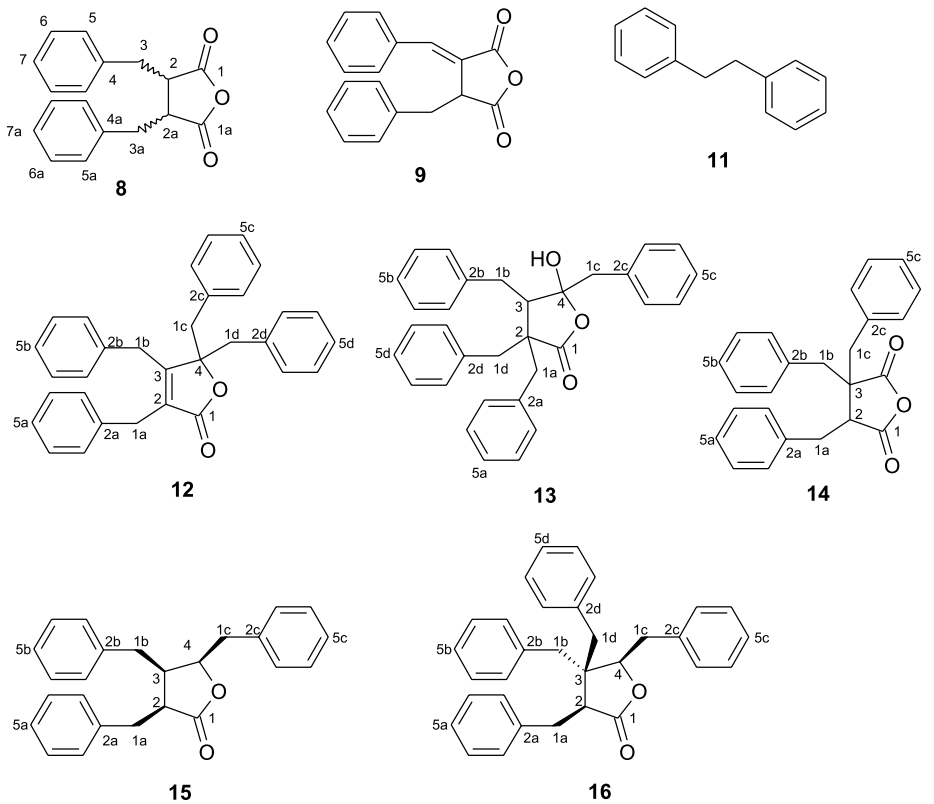


Figure 2. Minor products (**8**, **9** and **11**–**16**) obtained during the syntheses of maculalactones A–C which are described in Scheme 1.

Table 1. ^{13}C and ^{1}H NMR assignments ($CDCl_3$) for compounds **5**–**9**

		δ_C					δ_H				
Position	Mult.[a]	**5**[b]	**6**	**7**	**8**	**9**	**5**[b]	**6**	**7**	**8**	**9**
1	C	170.4	166.1	165.8	171.7	165.7	—	—	—	—	—
2	C	129.6	119.5	143.0	45.7 (CH)	123.5	—	—	—	3.50	—
3	CH	143.9	139.7	30.3 (CH_2)	32.0 (CH_2)	141.1	7.91	7.94	3.79, 3.79	3.10, 3.12	7.80
4	C	136.5	134.0	135.0	136.3	132.7	—	—	—	—	—
5=9	CH	130.9	130.1	129.1	129.0	130.4	7.35	6.83	7.13	7.14	7.58
6=8	CH	129.8	127.3	128.8	128.5	129.5	7.50	6.85	7.28	7.33	7.56
7	CH	130.8	130.8	127.5	127.4	131.4	7.35	7.15	7.31	7.28	7.53
1a	C	170.4	166.1	165.8	171.7	171.7	—	—	—	—	—
2a	C	129.6	119.5	143.0	45.7 (CH)	45.6 (CH)	—	—	—	3.50	4.38
3a	CH	143.9	139.7	30.3 (CH_2)	32.0 (CH_2)	33.7 (CH_2)	7.91	7.94	3.79, 3.79	3.10, 3.12	3.35, 3.28
4a	C	136.5	134.0	135.0	136.3	134.8	—	—	—	—	—
5a=9a	CH	130.9	130.1	129.1	129.0	129.3	7.35	6.83	7.13	7.14	6.89
6a=8a	CH	129.8	127.3	128.8	128.5	128.7	7.50	6.85	7.28	7.33	7.19
7a	CH	130.8	130.8	127.5	127.4	127.7	7.35	7.15	7.31	7.28	7.19

[a] Multiplicity determined from DEPT.
[b] Values recorded in d_4-MeOH solution.

was necessary to transfer the Grignard reagent to the solution of anhydride via a canula, rather than to perform the addition in the reverse sense (as is conventional and was practiced in the earlier trials). This procedure resulted in yields of up to 65% of **10** in the crude product (as judged by ^{1}H NMR analysis); its success may be the result of always maintaining the concentration of benzyl magnesium bromide in solution at a low level, thereby allowing compound **10**, which is the initial product of the Grignard reaction, sufficient time to precipitate out of solution before the lactol functional group undergoes a second Grignard reaction. The 1,4-adduct, compound **14**, consistently accounted for around 15% of the reaction product under all conditions that were assayed, and even under optimized conditions it was still necessary to separate compounds **10** and **14** chromatographically before proceeding to the next step.

The final step in the synthesis of maculalactone A, reduction of the lactol functionality in **10** by sodium borohydride, was straightforward, resulting in the preparation of compound **1** as a racemic mixture in almost quantitative yield. It is worth noting that although sodium borohydride was found to be a very effective reducing agent for converting compound **10** into compound **1**, reducing agents derived from neutral boranes (such as catechol borane) were completely unreactive. This may be a consequence of the ability of sodium borohydride to act also as a base, first removing the proton of the lactol hydroxy group in **10**, and thereby promoting ring opening to a keto/carboxylate tautomer, in which the carbonyl group of the ketone is more readily reduced to a secondary alcohol (this alcohol then condenses with the carboxylic acid group to recyclize the butyrolactone ring of **1**). The spectral properties (NMR, IR and MS) of (±)-**1** obtained from synthesis were identical with those of the natural product, (+) maculalactone A.[4] The two enantiomers of racemic **1** could be separated by HPLC using a semi-preparative Daicel chiral OD-column (Fig. 3). This procedure was then routinely automated so as to yield several 10 s of milligrams of each optical antipode, which were then used in making the further investigations into the absolute stereochemistry of **1** that are described at the start of Section 2.3.

2.2. Synthesis of maculalactones B (2) and C (3)

By making a slight modification to the above procedure, it was found that the key intermediate **10** could be quantitatively converted into compound **2**, which had identical physical properties to those of the natural product maculalactone B from *K. maculans*.[5] Since only the *Z*-geometric isomer was obtained from the dehydration of **10** in the presence of sulfuric acid[16] it appeared that maculalactone B must therefore be the thermodynamically more stable product (perhaps as a result of the decreased steric interaction between the benzyl and benzylidene substituents at the 3- and 4-positions in this isomer). However, synthetic maculalactone B (**2**) could be simply converted into the thermodynamically less favored *E*-isomer, with physical properties identical to those of maculalactone C (**3**) from nature,[5] by irradiation with UV light.[17] This reaction proceeded in good yield, always producing a mixture which consisted predominantly of compound **3** together with some unreacted starting material (**2**), that could only be separated by preparative normal phase HPLC. The ratio of maculalactone C (**3**) to maculalactone B (**2**) (approximately 4:1) that was obtained remained quite constant when UV irradiation was performed in a variety of solvents (e.g. benzene, dichloromethane or tetrahydrofuran) and it seems therefore to represent a photochemical equilibrium state.

An alternative synthesis of racemic maculalactone A from maculactone B was also devised by effecting hydrogenation of the diene functional group in maculalactone B (**2**) over a palladium catalyst (Scheme 1). This resulted preferentially in reduction at the tri-substituted double bond of **2**, yielding racemic **1** together with small amounts of the fully reduced tribenzylbutyrolactone **15** (trace amounts of a second fully reduced tetrabenzyl-substituted butyrolactone, compound **16** (Fig. 2), which may have arisen from a very minor

Table 2. ^{13}C and ^{1}H NMR assignments ($CDCl_3$) for compounds **10** and **12**–**17**

		δ_c							δ_H						
Position	Mult.[a]	**10**	**12**	**13**	**14**	**15**	**16**	**17**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
1	C	171.0	172.7	176.9	171.8	177.3	177.6	167.6	—	—	—	—	—	—	—
2	C	130.4	130.2	52.4	47.6 (CH)	48.1 (CH)	50.3 (CH)	153.1	—	—	—	3.16	3.16	2.75	—
3	C	159.7	162.9	49.0 (CH)	56.9	42.8 (CH)	51.8	129.3	—	—	2.48	—	2.96	—	—
4	C	106.4	90.1	104.8	173.1	83.7 (CH)	84.7 (CH)	204.5	—	—	—	—	4.59	4.24	—
1a	CH_2	29.1	29.3	41.2	30.0	31.0	30.7	33.9	3.29, 3.48	3.23, 3.23	2.97, 3.56	3.03, 3.35	2.80, 3.28	2.65, 3.11	3.86, 3.86
2a	C	137.0	137.0	136.1	137.7	138.6	139.9	138.2	—	—	—	—	—	—	—
3a=7a	CH	128.4	127.8	131.0	129.2	128.5	129.0	128.2	6.69	6.25	7.22	7.28	7.09	7.21	7.14
4a=6a	CH	128.6	128.9	128.5	128.9	128.7	128.6[b]	128.7	7.11	6.98	7.31	7.37	7.27	7.26	7.30
5a	CH	126.2	125.8	126.8	128.1	126.4	126.5	126.5	7.11	7.04	[c]	7.35	7.19	[c]	7.22
1b	CH_2	32.3	33.2	30.4	39.4	29.6	39.6	37.3	3.65, 3.81	3.68, 3.68	2.91, 3.10	2.54, 3.42	2.82, 2.94	3.07, 3.07	3.70, 3.70
2b	C	135.9	135.3	139.4	135.1	139.4	135.5	135.9	—	—	—	—	—	—	—
3b=7b	CH	129.1	128.9	129.7	130.5	128.7	130.8	129.0	7.11	6.99	7.25	6.78	7.02	6.84	7.07
4b=6b	CH	128.8	128.2	128.7	128.7	128.6	128.6[b]	128.9	7.26	7.23	7.35	7.18	7.25	7.18	7.28
5b	CH	127.1	127.2	128.8	127.2	126.7	127.1	127.1	7.26	7.23	7.29	7.18	7.19	[c]	7.24
1c	CH_2	43.3	42.8	45.8	42.1	37.5	35.3	49.6	3.06, 3.23	2.90, 3.09	2.10, 2.25	3.11, 3.27	2.55, 2.96	2.86, 3.08	3.52, 3.52
2c	C	133.1	134.5	132.9	133.6	137.4	137.9	133.4	—	—	—	—	—	—	—
3c=7c	CH	130.5	130.3	130.8	130.3	129.0	128.9	130.0	7.17	7.16	6.85	7.22	7.06	7.23	7.03
4c=6c	CH	128.2	128.4	128.0	128.9	128.4	128.5[b]	128.3	7.26	7.23	7.18	7.35	7.25	7.28	7.26
5c	CH	127.6	127.2	127.5	127.6	126.6	126.8	126.9	7.35	7.23	7.23	7.31	7.19	[c]	7.20
1d	CH_2	—	42.8	40.1	—	—	37.6	—	—	2.90, 3.09	2.65, 3.26	—	—	3.11, 3.11	—
2d	C	—	134.5	136.9	—	—	137.1	—	—	—	—	—	—	—	—
3d=7d	CH	—	130.3	130.2	—	—	131.5	—	—	7.16	7.07	—	—	7.45	—
4d=6d	CH	—	128.4	128.5	—	—	128.4[b]	—	—	7.23	7.24	—	—	7.37	—
5d	CH	—	127.2	127.1	—	—	127.2	—	—	7.23	[c]	—	—	7.32	—
–OMe	CH_3	—	—	—	—	—	—	52.4	—	—	—	—	—	—	3.67

[a] Multiplicity determined from DEPT.
[b] Interchangeable within column.
[c] Not assigned.

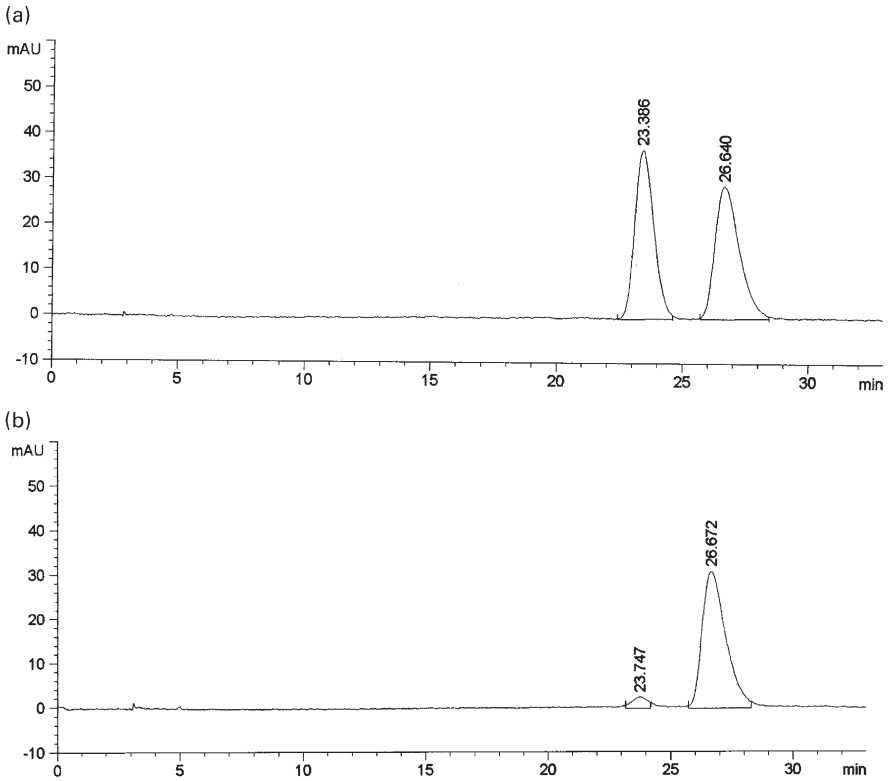


Figure 3. Analytical chiral HPLC chromatograms (Chiracel OD–H column) of: a) racemic (±) maculalactone A from synthesis; and b) natural (+) maculalactone A from *K. maculans*.

product carried through the synthesis from the preceding Grignard reaction, were also isolated).

2.3. Asymmetric synthesis of 1

Having developed two routes to racemic **1**—firstly, by borohydride reduction of the key intermediate **10** (Section 2.1) and, secondly, by hydrogenation of the diene **2** (Section 2.2)—we next attempted to perform an enantioselective synthesis of the 4*R* and 4*S* forms of **1**. As noted in the Introduction, the absolute stereochemistry of natural maculalactone A had yet to be determined, and the need to achieve an asymmetric synthesis became particularly pressing following on from the failure of all of the three most commonly used physical techniques: CD,[18] X-ray (Fig. 4) and NMR[19–21] (Mosher esterification of the secondary alcohol obtained from reductive opening of the butyrolactone ring of **1**) to define the absolute stereochemistry of the (+) and (−) enantiomorphs of **1** which had been obtained from semi-preparative chiral HPLC of the synthetic racemate (Section 2.1).

Accordingly, it was decided to modify the strategy for preparing **1** that has been reported in Sections 2.1 and 2.2 in order to effect an enantioselective synthesis of maculalacatone A. The high yield that had been recorded for the preparation of maculalactone A (**1**) from hydrogenation of maculalactone B (**2**) (Section 2.2) suggested that it might be possible to achieve an efficient enantioselective synthesis of maculalactone A by employing a chiral rhodium(I)-DuPhos catalyst[22–24] in place of the palladium-on-charcoal hydrogenation catalyst. In the event, although the overall yield of maculalactone A (**1**) from the reduction of **2** remained high

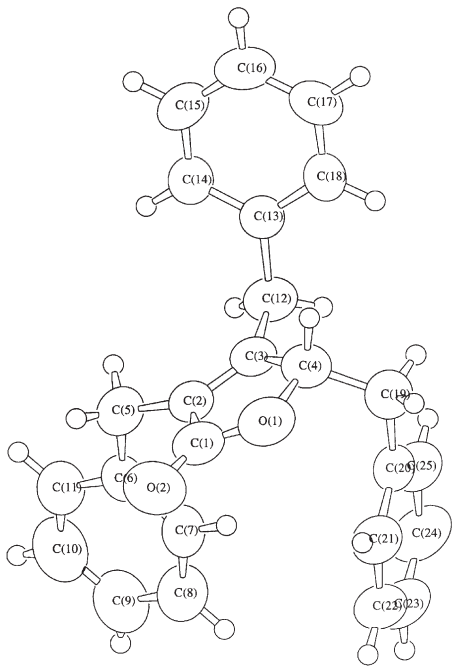


Figure 4. ORTEP diagram of the X-ray structure for synthetic (+) maculalctone A (which was separated from its enantiomer by semi-preparative chiral HPLC). The stereochemistry shown at the 4-position was suggested, but not conclusively established, by X-ray crystallographic analysis (similarly, the absolute stereochemistry could not be definitively established for synthetic (−) maculalactone A which was also obtained from semi-preparative chiral HPLC).

in the presence of this catalyst, this strategy yielded maculalactone A with an enantiomeric excess (% ee) very close to zero. However, it did prove possible to achieve a much more enantioselective synthesis of **1** by modifying the synthetic procedure that was described in Section 2.1 in order to enable reduction by a neutral borane-derivative rather than by a borohydride reducing agent. As noted in Section 2.1, the key lactol intermediate **10** was unaffected by borane reduction and it was therefore necessary to first convert this compound to its methyl ester derivative,[25] compound **17** (Scheme 1). Catecholborane reduction of the ketone group in this ring-opened substrate, in the presence of either the *R*- or *S*- enantiomer of the chiral oxazoborolidine catalyst **18** which has been developed by Corey,[26–31] then resulted in either (+) or (−) maculalactone A, respectively, in high chemical yield (85–90%) and with a reproducibly good-to-high enantiomeric excess (80–85% ee), as determined by analytical chiral HPLC (see Section 3.2.6 in the Experimental).

Based on the mechanism which has been proposed for the enantiomeric induction by the Corey catalyst (Fig. 5),[32] it is possible to infer that the (+) form of **1**-which was obtained when using the *R*- enantiomer of the oxazoborolidine catalyst (*R*)-**18**—should correspond to the (4*S*) enantiomer of maculalactone A, and that the (−)-form of maculalactone A must therefore be the (4*R*) enantiomer. Hence, it appeared that the first fraction obtained from chiral HPLC in Figure 3, i.e. (−) maculalactone A with $[\alpha]_D=-156.7$ [*c* 1.0], was the enantiomeric form of **1** with the *R* configuration at the 4-position, while the second fraction, (+) maculalactone A ($[\alpha]_D=+153.1$ [*c* 1.4]), corresponded to the 4*S* absolute configuration.

2.4. The absolute stereochemistry of natural maculalactone A from *K. maculans*

Intriguingly, although all the samples of natural maculalactone A that we had ever isolated[4,8–10] were dextroratory, none had ever exhibited such a large positive optical rotation as that recorded from the second fraction which had been obtained from semi-preparative chiral HPLC of the synthetic racemate, which should correspond to (+)-(4*S*)-**1**. Rather, samples of natural maculalactone A collected at different times from *K. maculans* growing in different localities seemed to vary quite substantially in their specific optical rotation, with an $[\alpha]_D$ averaging around +120–130. When we subjected samples of natural maculalactone A isolated from *K. maculans* to analytical chiral HPLC (as in Section 3.2.6), the chromatograms consistently demonstrated that the natural form of **1** was a mixture of two compounds, with retention times that were consistent with the contamination of the more predominant (4*S*) enantiomer by a little of the (4*R*) form of the metabolite (see Fig. 3(b)) for a representative chromatogram). These analytical HPLC studies typically indicated an 'enantiomeric excess' for naturally occurring (+) maculalactone A of the order of 85–95% ee.

Final confirmation that natural maculalactone A was, in fact, a partially racemic mixture of the two enantiomorphs of **1**, was obtained by performing ^{1}H NMR experiments with maculalactone A isolated from *K. maculans* in the presence of a chiral shift reagent (europium tris[3-heptafluoropropylhydroxymethylene]-(+)-camphorate). The effects of making successive additions of this shift reagent to a $CDCl_3$ solution of maculalactone A (**1**) from the natural

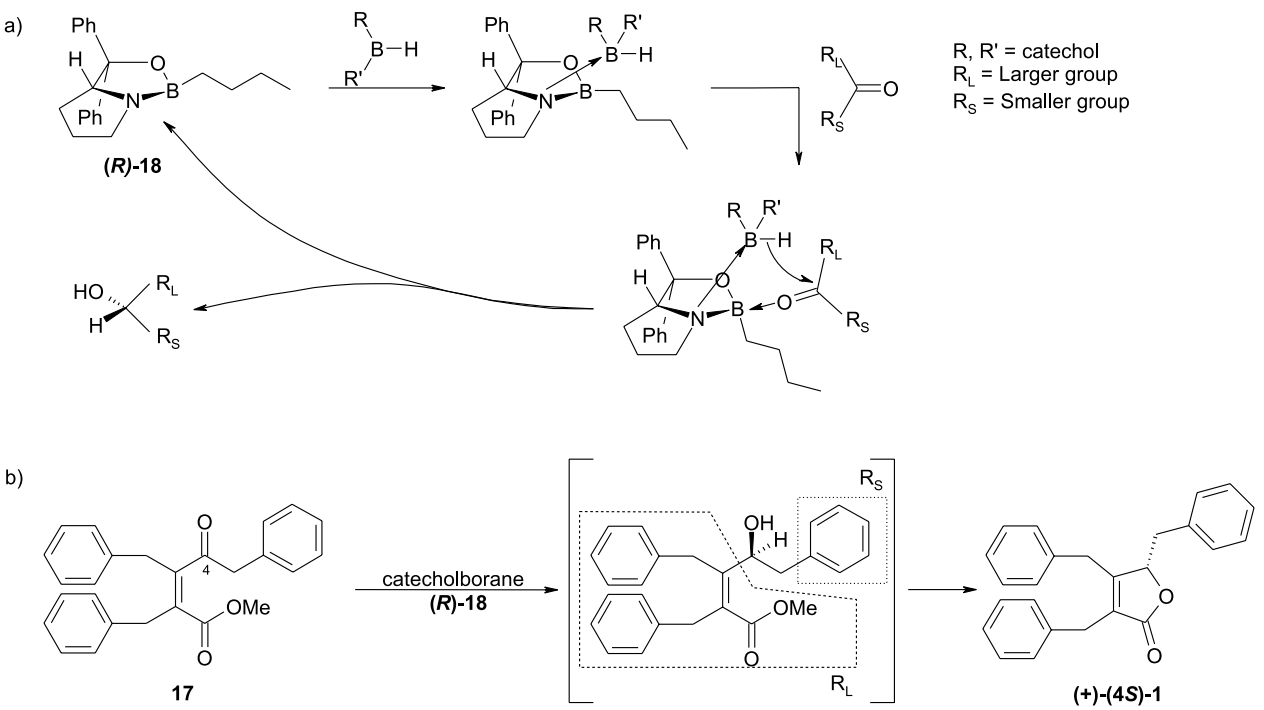


Figure 5. (a) The transition state assembly of the chiral oxazoborolidine (*R*)-**18** with a generalized prochiral ketone, and the resulting absolute configuration of the secondary alcohol product from catecholborane reduction, that has been proposed in the literature to account for the enantioselectivity of the Corey catalyst.[32] (b) The predicted absolute configuration of (+) maculalactone A (**1**) from the enantioselective reduction of the ketone **17** in the presence of (*R*)-**18**, based on this proposed mechanism.

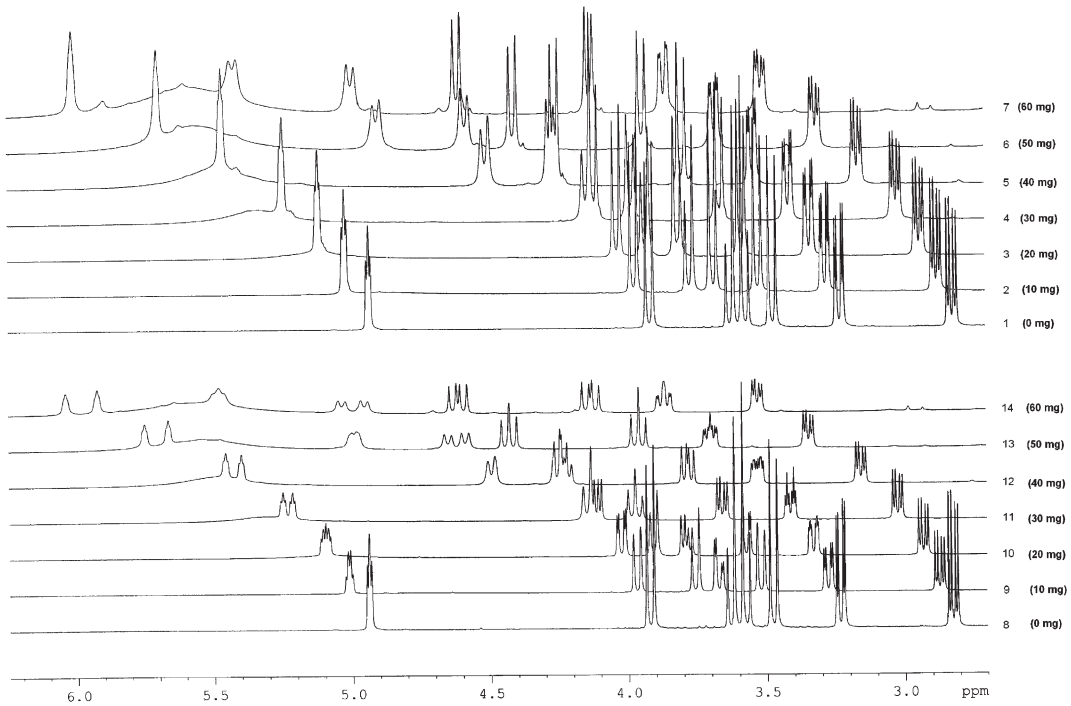


Figure 6. Expanded ^{1}H NMR spectra of natural (+)-maculalactone A from *K. maculans* (expts. 1–7); and racemic (±) maculalactone A from synthesis (Section 2.1; expts. 8–14) following successive additions (10 mg) of a europium tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorate] chiral shift reagent.

source are shown in the upper seven ^{1}H NMR spectra in Figure 6 (experiments 1–7). Several peaks (most obviously the H-4 proton at δ_H 4.94 ppm)[§] appeared to resolve into two components in an approximately 9:1 ratio in this experiment (this is particularly evident in the ^{1}H NMR spectrum shown in entry 7 of Figure 6, which contained the largest amount of added chiral shift reagent). As demonstrated by experiments 8–14 in Figure 6, the H-4 proton of synthetic (±)-**1** (Section 2.1) was also observed to separate into two components, in the same manner as for experiments 1–7, although in this case equal intensities were observed for the two sets of peaks due to the two enantiomers of **1**, as the sample was, of course, racemic. The inescapable conclusion is therefore that natural samples of (+)-**1** are, in fact, partially racemic at the 4-position.

To summarize, the results from measurements of optical rotation, chiral HPLC and ^{1}H NMR in the presence of a chiral shift reagent all confirmed that natural maculalactone A (**1**) was present in *K. maculans* as a mixture of approximately 85–95% of the (4*S*) enantiomer with 5–15% of the (4*R*) enantiomer. This is a very unusual situation, as most natural products are normally isolated either in enantiomerically pure form (if their biosynthesis is entirely enzymic) or as completely racemic mixtures (if non-enzymic chemical processes are also involved in their biogenesis). It is rare to find intermediate cases, involving partially racemic metabolites, in natural product chemistry. Although this phenomenon has been reported on a few occasions for terpenoids (e.g. (+)-α-pinene, which is very often found as an admixture with lesser amounts of its (−)-enantiomer),[34] it seems to be extremely rare for lignans,[35] to which biogenetic class maculalactone A presumably belongs.[4,5] We were therefore rather concerned that the apparent partial racemization of **1** might be an artifact of the extraction and chromatographic procedures which were employed in the isolation of maculalactone A from *K. maculans*, especially as it is quite easy to propose a mechanism which would account for such racemization: if a trace of acid (or base) were present at either or both of the extraction and purification stages for the isolation of maculalactone A, then an extended enol could be generated by tautomerization, involving a movement of the H-4 proton to the carbonyl group; reprotonation from either side of the resulting 2-hydroxyfuran π-system would then result in racemization.

In order to test this possibility, the length of the extraction procedure that was routinely used for obtaining maculalactone A from *K. maculans*was varied from 20 h to 10 days. The effect of including a second column chromatographic step in the purification of **1** was also investigated. However, neither procedure appeared to affect the % ee of natural **1**, as determined by chiral analytical HPLC, to any significant extent. Additionally, when a sample of partially racemic **1** (from synthesis) was dissolved in organic solvent (1 mg/10 mL EtOAc) and shaken with HCl (1 M, 10 mL) for 15 min-in an attempt to induce racemization via the proposed enolic form of **1**-there was, once again, no

[§] The shifts induced by the chiral lanthanide for the proton resonances at H-1a (δ_H 3.56 and 3.64 ppm) in (±)-**1** showed the most significant changes in chemical shift as the amount of chiral shift reagent added to the $CDCl_3$ solution was gradually increased. These two protons are closest to the carbonyl functional group in **1**, suggesting that it is the non-bonding pairs of electrons in the carbonyl group which are donated to the europium ion of the shift reagent,[33] thereby producing the greatest downfield shifts at the 1a-position. The proton resonance at the 4-position (δ_H 4.94 ppm) exhibited the largest separation in chemical shifts between the 4*R*- and 4*S*- forms of **1**, as the amount of chiral shift reagent was increased, perhaps because it is this proton which is directly attached to the chiral center.

significant effect. It therefore appeared that the variation in the % ee found for (+)-**1** from the natural source was not an artifact that had been introduced by either the extraction or purification procedures. Hence, we are forced to conclude that maculalactone A is indeed naturally present in *K. maculans* in a partially racemized form.

In closing, it is worth pointing out that-quite by chance-the ratio of the two enantiomers found for natural maculalactone A (**1**) was almost identical with that which was obtained from the asymmetric synthesis described in Section 2.3. Perhaps uniquely in natural product chemistry, it appears that the asymmetric synthesis of **1** via the Corey procedure has yielded a target compound of similar enantiomeric composition to that of the natural material, and that even though the enantioselectivity of this synthetic procedure is perhaps relatively modest by the standards of the current day-at 85% ee-there is little incentive to attempt to improve the chiral selectivity of the enantiomeric step, as the product obtained from asymmetric synthesis is already almost identical with the natural product!

3. Experimental

3.1. General methods

General experimental procedures were similar to those described recently in: Brown, G.D.; Sy, L.-K., *Tetrahedron* **2004**, *60*, 1125–1138.

3.1.1. Isolation of natural (+) maculalactone A (2,3,4-tribenzyl-γ-butyro-2,3-en-lactone) (1) from *Kyrtuthrix maculans*. *K. maculans* (132.8 g wet wt., 78.6 g after freeze drying overnight) was collected from the shores at Shek Mei Tau, New Territories, Hong Kong in March. Taxonomic identification was made by Dr Williams of the Department of Ecology and Biodiversity, The University of Hong Kong. Following lyophilization, the sample was pulverized to a fine powder under liq. N_2, repeatedly extracted with CH_2Cl_2, dried ($MgSO_4$) and solvent was removed under reduced pressure to yield a dark brown gum (1.09 g; 0.82 w/w % [fresh wt]). The extract was subjected to gradient CC (*n*-hexane→EtOAc) followed by HPLC to obtain compound **1** (115.2 mg, 0.09% w/w [fresh wt.]; R_f 0.35 in 5% EtOAc/*n*-hexane) which was recrystallized from 10% *i*-PrOH/*n*-hexane, to obtain maculalactone A (**1**) as a solid, mp 88–89 °C; $[\alpha]_D$=+130.2 (*c*=2.4, $CHCl_3$)-see Ref. 4 for other physical properties.

3.2. Synthesis of (±) maculalactone A

3.2.1. Preparation of 2*E*,3*E*-Dibenzylidenesuccinic acid (5). A solution of dimethyl succinate (5.0 g, 34.2 mmol), benzaldehyde (7.3 g, 68.4 mmol) and Na (1.7 g, 75.3 mmol) in anhyd. Et_2O (50 mL) was stirred under N_2 at 0 °C and allowed to warm to room temperature over 12 h. MeOH (10 mL) was added to destroy any remaining excess Na, followed by H_2O (10 mL) and the mixture was then acidified with HCl (6 M, 10 mL), and extracted with Et_2O (2×10 mL). The combined organic layers were re-extracted with sat. $NaHCO_3$ (aq.) (3×20 mL) and the combined sat. $NaHCO_3$ (aq.) extracts were acidified and extracted with Et_2O (3×20 mL). The combined organic layers were dried ($MgSO_4$) and rotary evaporated to give a residue consisting predominantly of compound **5**, which was purified by washing with Et_2O.

2E,3E-Dibenzylidenesuccinic acid (**5**). Solid, mp 213–215 °C, (1.87 g, 6.4 mmol, 19%). ν_{max}/cm^{-1} (KBr disc): 3435 (v br), 3069, 1680, 1638, 1611; δ_H (CD_3OD)-7.91 (2H, s), 7.50 (4H, m), 7.35 (6H, m)—see also Table 1; ^{13}C NMR—see Table 1; ESI-MS *m/z* (rel. int.): 317 (M^+ $[C_{18}H_{14}O_4]+Na^+$) (100).

Note that compound **5** was also commercially available from the Aldrich Chemical Company (Cat. No. S-300187) but that this source was judged to be too costly as a starting material for performing a synthesis of maculalactone A on a gram scale, such as that described in Sections 3.2.1–3.2.5.

3.2.2. Conversion of 5 to 2*E*,3*E*-dibenzylidenesuccinic anhydride (6). To 2*E*,3*E*-dibenzylidene succinic acid (**5**) (1.5 g, 5.1 mmol) was added excess acetyl chloride (1.5 mL) and the reaction was refluxed for 2 h. After completion, as determined by TLC, the reaction mixture was cooled in an ice bath and the anhydride **6** was separated by filtration as yellow crystals, which were washed with a small amount of cold Et_2O to yield the pure product.

2E,3E-Dibenzylidenesuccinic anhydride (**6**). Solid, mp 202–204 °C (1.18 g, 4.3 mmol, 84%). ν_{max}/cm^{-1}: 3030, 1823, 1771, 1618, 1595; δ_H-7.94 (2H, s), 7.15 (2H, tt, *J*=7.1, 1.6 Hz), 6.85 (4H, dd, *J*=7.1, 7.1 Hz), 6.83 (4H, dd, *J*=7.1, 1.6 Hz)—see also Table 1; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.): 276.0786 (M^+, calcd 276.0786 for $C_{18}H_{12}O_3$) (60), 232 (40), 203 (80), 199 (100).

3.2.3. Reduction of 6 to 2,3-dibenzylbut-2-en-dioic anhydride (7) and 2*R*/*S*,3*S*/*R*-dibenzylbutanedioic anhydride (8). A solution of 2*E*,3*E*-dibenzylidene succinic anhydride (**6**) (1.0 g, 3.62 mmol) in EtOAc (10 mL) was charged with 5% (w/w) Pd/C (50 mg) and stirred under H_2 (1 atm) at room temperature for 18 h. The resultant colorless solution was filtered and rotary evaporated to give a crude product consisting of the anhydrides **7** and **8** in an approximately 3:1 ratio (as determined by ^1H NMR spectroscopy). Anhydride **8** precipitated out of solution after thoroughly mixing the crude product with 10% EtOAc/*n*-hexane, while compound **7** remained largely in the supernatant and was further purified by semi-preparative HPLC (10% EtOAc/*n*-hexane).

2,3-Dibenzylbut-2-en-dioic anhydride (**7**). Oil (R_t 19.1 min, 756 mg, 2.71 mmol, 74%). ν_{max}/cm^{-1}: 3032, 2928, 1825, 1767, 1603, 1497, 1456; δ_H 7.31 (2H, t, *J*=7.5 Hz), 7.28 (4H, dd, *J*=7.5, 7.5 Hz), 7.13 (4H, d, *J*=7.5 Hz) 3.79 (4H, s)—see also Table 1; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.): 278.0948 (M^+, calcd 278.0943 for $C_{18}H_{14}O_3$) (100), 260 (14), 233 (49), 205 (21).

2(R/S),3-Dibenzylbutanedioic anhydride (**8**). Solid, mp 105–106° C (R_t 30.0 min, 254 mg, 0.95 mmol, 24%). ν_{max}/cm^{-1}: 3032, 2926, 1863, 1786, 1605, 1496, 1456; δ_H-7.33 (4H, dd, *J*=7.3, 7.3 Hz), 7.28 (2H, t, *J*=7.3 Hz), 7.14 (4H, d, *J*=7.3 Hz), 3.50 (2H, m), 3.12 (2H, m), 3.10

(2H, m)—see also Table 1; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.): 280.1102 (M^+, calcd 208.1099 for $C_{18}H_{16}O_3$) (70), 125 (100).

When incomplete reduction of 2*E*,3*E*-dibenzylidene-succinic anhydride (**6**) (20.0 mg, 0.07 mmol) was performed in an EtOAc (1 mL) solution charged with 10% Pd/C (1 mg, 5% w/w) and stirred under H_2 (1 atm) at room temperature for 2 h, the resulting crude product consisted of the three anhydrides **7**–**9**, which were separated by semi-preparative HPLC (10% EtOAc/*n*-hexane): **7** (4.1 mg, 0.015 mmol, 20%; R_t 19.1 min); **8** (3.3 mg, 0.012 mmol, 16%; R_t 30.0 min); and **9** (5.5 mg, 0.020 mmol, 27%; R_t 32.3 min).

2-Benzylidene,3(R/S)-benzyl-butanedioic anhydride (**9**). Solid, mp 138–140 °C. ν_{max}/cm^{-1}: 3030, 2930, 1771, 1709, 1636, 1603, 1496, 1454, 1447 cm^{-1}; δ_H-7.80 (1H, d, *J*=2.4 Hz), 7.58–7.53 (5H, m), 7.19 (3H, m), 6.89 (2H, m), 4.38 (1H, ddd, *J*=5.7, 4.1, 2.4 Hz), 3.35 (1H, dd, *J*=13.7, 5.7 Hz), 3.25 (1H, dd, *J*=13.7, 4.1 Hz)—see also Table 1; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.): 278.0944 (M^+, calcd 278.0943 for $C_{18}H_{14}O_3$) (50), 233 (20), 205 (25), 178 (15), 153 (100).

3.2.4. Synthesis of 2,3,4-tribenzyl-4(*R/S*)-hydroxy-γ-butyro-2-en-lactone (10). A Grignard reagent, freshly prepared from the addition of Mg (52.4 mg, 2.16 mmol) to benzyl bromide (0.26 mL, 2.16 mmol) in anhyd. Et_2O (50 mL) at room temperature for 1 h, was transferred to a solution of anhydride **7** (500 mg, 1.80 mmol) in anhyd. Et_2O (50 mL) at 0 °C via a cannula. The reaction mixture was stirred under N_2 at 0 °C for 30 min, quenched by H_2O (15 mL) and then acidified with HCl (1 M, 15 mL). The aqueous layer was extracted with Et_2O (2×50 mL) and the combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and rotary evaporated to give a crude product consisting predominantly of the butyrolactone **10** together with small amounts of **11**–**14**, which was purified by CC (10% EtOAc/*n*-hexane).

2,3,4-Tribenzyl-4(R/S)-hydroxy-γ-butyro-2-en-lactone (**10**). Solid, mp 107–109 °C (394 mg, 1.06 mmol, 59%; R_f 0.20). ν_{max}/cm^{-1}: 3527, 3392 (br), 3026, 2928, 1759, 1602, 1497, 1454; δ_H-7.35 (1H, m), 7.26 (5H, m), 7.17 (2H, m), 7.11 (5H, m), 6.69 (2H, dd, *J*=7.1, 1.8 Hz), 3.81 (1H, d, *J*=14.7 Hz), 3.65 (1H, d, *J*=14.7 Hz), 3.48 (1H, d, *J*=15.3 Hz), 3.29 (1H, d, *J*=15.3 Hz), 3.26 (s, –OH), 3.23 (1H, d, *J*=13.9 Hz), 3.06 (1H, d, *J*=13.9 Hz)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m/z* (rel. int.): 352.1461 (M^+-H_2O, calcd 352.1463 for $C_{25}H_{20}O_2$) (100), 279 (55).

2,3,4,4-Tetrabenzyl-γ-butyro-2-en-lactone (**12**). Solid, mp 135–137 °C (19 mg, 0.043 mmol, 3%; R_f 0.40). ν_{max}/cm^{-1}: 3030, 3013, 2926, 1747, 1496, 1454; δ_H-7.23 (9H, m), 7.16 (4H, dd, *J*=7.5, 1.8 Hz), 7.04 (1H, t, *J*=7.0 Hz), 6.99 (2H, d, *J*=7.0 Hz), 6.98 (2H, dd, *J*=7.0, 7.0 Hz), 6.25 (2H, d, *J*=7.0 Hz), 3.68 (2H, s), 3.23 (2H, s), 3.09 (2H, d, *J*=14.3 Hz), 2.90 (2H, d, *J*=14.3 Hz)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m/z* (rel. int.): 444.2089 (M^+, calcd 444.2089 for $C_{32}H_{28}O_2$) (30), 353 (100).

2,2,3(R/S),4(R/S)-Tetrabenzyl-4-hydroxy-γ-butyrolactone (**13**). Solid, mp 196–198 °C (7 mg, 0.016 mmol, 1%; R_f 0.31). ν_{max}/cm^{-1}: 3649, 3034, 2926, 1749, 1601, 1497, 1454; δ_H-7.35 (2H, dd, *J*=7.5, 7.5 Hz), 7.31 (2H, m), 7.29–7.14 (12H, m), 7.07 (2H, d, *J*=6.9 Hz), 6.85 (2H, dd, *J*=6.6, 1.6 Hz), 3.56 (1H, d, *J*=13.7 Hz), 3.26 (1H, d, *J*=13.9 Hz), 3.10 (1H, dd, *J*=13.9, 11.0 Hz), 3.02 (1H, s,–OH), 2.97 (1H, d, *J*=13.7 Hz), 2.91 (1H, dd, *J*=13.9, 4.2 Hz), 2.65 (1H, d, *J*=13.9 Hz), 2.48 (1H, dd, *J*=11.0, 4.2 Hz), 2.25 (1H, d, *J*=13.9 Hz), 2.10 (1H, d, *J*=13.9 Hz)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m/z* (rel. int.): 444.2092 (M^+-H_2O, calcd 444.2089 for $C_{32}H_{28}O_2$) (45), 353 (100).

2,2,3(R/S)-Tribenzylbutanedioic anhydride (**14**). Solid, mp 145–147 °C (85 mg, 0.23 mmol, 13%; R_f 0.49). ν_{max}/cm^{-1}: 3028, 2928, 2856, 1782, 1718, 1497, 1454; δ_H-7.37 (2H, dd, *J*=7.5, 7.5 Hz), 7.35 (3H, m) 7.31 (1H, t, *J*=7.5 Hz), 7.28 (2H, d, *J*=7.5 Hz), 7.22 (2H, d, *J*=7.5 Hz), 7.18 (3H, m), 6.78 (2H, dd, *J*=7.5, 1.8 Hz), 3.42 (1H, d, *J*=14.4 Hz), 3.35 (1H, dd, *J*=14.2, 6.6 Hz), 3.27 (1H, d, *J*=13.9 Hz), 3.16 (1H, t, *J*=6.4 Hz), 3.11 (1H, d, *J*=13.9 Hz), 3.03 (1H, dd, *J*=14.2, 6.6 Hz), 2.54 (1H, d, *J*=14.4 Hz)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m/z* (rel. int.): 370.1572 (M^+, calcd 370.1569 for $C_{25}H_{22}O_3$) (25), 352 (8), 279 (44), 251 (16), 222 (100).

3.2.5. Sodium borohydride reduction of 10 to racemic maculalactone A (4(*R/S*)-2,3,4-tribenzyl-γ-butyro-2-en-lactone) (±)-(1). To a solution of the γ-hydroxy-butyro-lactone **10** (250 mg, 0.68 mmol) in THF/H_2O (10 mL; 24:1) was added $NaBH_4$ (128 mg, 3.37 mmol) in portions at 0 °C with stirring for 2 h. The reaction mixture was quenched by HCl (1 M, 10 mL), concentrated by rotary evaporation and extracted with EtOAc (3×10 mL). The combined organic layers were dried ($MgSO_4$) and rotary evaporated to yield the butyrolactone **1** without the need for further purification.

4(R/S)-2,3,4-Tribenzyl-γ-butyro-2-en-lactone (**1**). Solid, mp 88–89 °C (237 mg, 0.67 mmol, 99%). ν_{max}/cm^{-1}: 3030, 3013, 2926, 1751, 1668, 1603, 1497, 1454; δ_H-7.30 (2H, m), 7.27 (1H, m), 7.25 (3H, m), 7.17 (1H, m), 7.15 (4H, m), 7.03 (2H, dd, *J*=6.6, 1.9 Hz), 6.88 (2H, dd, *J*=6.1, 2.3 Hz), 4.94 (1H, dd, *J*=6.1, 4.0 Hz), 3.92 (1H, d, *J*=15.6 Hz), 3.64 (1H, d, *J*=15.4 Hz), 3.56 (1H, d, *J*=15.4 Hz), 3.47 (1H, d, *J*=15.6 Hz), 3.23 (1H, dd, *J*=14.6, 4.0 Hz), 2.81 (1H, dd, *J*=14.6, 6.1 Hz)—see also Ref. 4; δ_C−173.5 C, 161.7 C, 137.7 C, 135.9 C, 134.8 C, 129.5 CH×2, 129.1 CH×2, 128.7 CH×2, 128.6 CH×4, 128.6 C, 128.2 CH×2, 127.3 CH, 127.2 CH, 126.4 CH, 81.6 CH, 38.0 CH_2, 33.2 CH_2, 29.4 CH_2—see also Ref. 4; HREIMS *m/z* (rel. int.): 354.1616 (M^+, calcd 354.1620 for $C_{25}H_{22}O_2$) (60), 263 (100).

3.2.6. Separation of racemic (±) maculalactone A (4(*R/S*)-2,3,4-tribenzyl-γ-butyro-2-en-lactone) (1) into its (+) and (−) enantiomers. Semi-preparative chiral HPLC separation of a sample of (±)-**1** (60 mg) was performed using a Waters chromatograph equipped with RI 410 detector and a Daicel chiral OD-column (10 mm×25 cm; 10 μm particle size; Cat. no. 14045; Chiral Technologies Inc., Exton, PA, USA) operating isocratically with 10% *i*-PrOH/ *n*-hexane at a flow rate of 8 mL min^{-1}. Typical recoveries per injection (1 mg of (±)-**1** in 100 μL) were ca. 0.35 mg of each enantiomer, yielding overall:

(−)-2,3,4-tribenzyl-γ-butyro-2-en-lactone ((−)-**1**) (15 mg, 25%; R_t=16.9 min; $[\alpha]_D$=−156.7 (*c*=1.0, $CHCl_3$); and *(+)-2,3,4-tribenzyl-γ-butyro-2-en-lactone* ((+)-**1**) (12 mg, 20%, R_t=18.3 min; $[\alpha]_D$=+153.1 (*c*=1.2, $CHCl_3$).

The analytical chiral separation shown in Figure 3 was made using a chiral stationary phase with comparable properties (Chiracel OD-H column, 4.6 mm×25 cm; 5 μm particle size; Cat. no. 14325; Chiral Technologies Inc., Exton, PA, USA). Experiments were performed with either an analytical HP 1100 series chromatogram or with an HP 2200 HPLC instrument equipped with HP 2210 UV detector (λ=245 nm), using a 20 μL injection loop (sample concentration typically 1 mg/mL) and operating isocratically with 10% *i*-PrOH/*n*-hexane at a flow rate of 1 mL min^{-1}. Under these conditions, the R_t for (−)-**1** was 23.7 min and that for (+)-**1** was 26.6 min.

3.3. Syntheses of other tribenzylbutyrolactone natural products

3.3.1. Dehydration of 10 to maculalactone B (2,3-dibenzyl-4*Z*-benzylidene-γ-butyro-2-en-lactone) (2). To a solution of the γ-hydroxy-butyrolactone **10** (100 mg, 0.27 mmol) in toluene (4 mL) was added a powder of silica gel onto which had been adsorbed H_2SO_4 (30 mg; prepared by suspending silica [100 g] in acetone [100 mL] containing H_2SO_4 [conc.; 10 g] and removing the solvent on a rotary evaporator). The mixture was heated at reflux for 5 h, and after completion of the reaction, as determined by TLC, the mixture was filtered and the solvent was removed on a rotary evaporator to yield the butyrolactone **2** without the need for further purification.

2,3-Dibenzyl-4Z-benzylidene-γ-butyro-2-en-lactone (**2**). Solid, mp 114–115 °C (78 mg, 0.21 mmol, 82%). ν_{max}/cm^{-1}: 3030, 3015, 2920, 1755, 1651, 1603, 1494, 1454; δ_H-7.70 (2H, d, *J*=7.5 Hz), 7.33 (2H, dd, *J*=7.5, 7.5 Hz), 7.28–7.20 (7H, m), 7.17 (2H, d, *J*=7.1 Hz), 7.10 (2H, d, *J*=7.4 Hz), 5.96 (1H, s), 3.92 (2H, s), 3.73 (2H, s)—see also Ref. 5; δ_C−170.3 C, 150.8 C, 148.2 C, 137.4 C, 136.6 C, 133.0 C, 130.5 CH×2, 128.9 CH×2, 128.8 CH, 128.7 CH×2, 128.6 CH×4, 128.2 CH×2, 127.8 C, 127.0 CH, 126.7 CH, 110.5 CH, 30.6 CH_2, 29.8 CH_2—see also Ref. 5; HREIMS *m/z* (rel. int.): 352.1457 (M^+, calcd 352.1463 for $C_{25}H_{20}O_2$) (100), 261 (15).

3.3.2. Photo-isomerization of maculalactone B (2) to maculalactone C (2,3-dibenzyl-4*E*-benzylidene-γ-butyro-2-en-lactone) (3). A degassed solution of the butyrolactone **2** (20 mg, 0.06 mmol) in anhyd. C_6H_6 (10 mL) was irradiated by UV light (450 W medium-pressure Hg arc lamp) at room temperature under N_2 for 1 h. Solvent was removed by rotary evaporation to give a mixture containing the butyrolactones **3** and **2**, in an approximately 4:1 ratio (by ^1H NMR of the crude product) which were separated by preparative HPLC (10% EtOAc/*n*-hexane).

2,3-Dibenzyl-4Z-benzylidene-γ-butyro-2-en-lactone (**2**). (4.0 mg, 0.011 mol, 20%; R_t 19.8 min). See Section 3.3.1 for physical properties.

2,3-Dibenzyl-4E-benzylidene-γ-butyro-2-en-lactone (**3**). Oil (15.8 mg, 0.045 mol, 79%; R_t 18.7 min). ν_{max}/cm^{-1}: 3032, 3013, 2928, 1756, 1603, 1494, 1454; δ_H-7.24–7.10 (11H, m), 7.01 (2H, d, *J*=7.3 Hz), 6.84 (1H, s), 6.60 (2H, dd, *J*=7.8, 1.7 Hz), 3.69 (2H, s), 3.66 (2H, s)—see also Ref. 5; δ_C−169.7 C, 149.3 C, 148.3 C, 137.2 C, 136.2 C, 133.0 C, 132.6 C, 129.3 CH×2, 128.7 CH×4, 128.4 CH×2, 128.2 CH×2, 128.2 CH, 127.7 CH×2, 126.7 CH, 126.4 CH, 115.3 CH, 31.5 CH_2, 29.8 CH_2—see also Ref. 5; HREIMS *m/z* (rel. int.): 352.1465 (M^+, calcd 352.1463 for $C_{25}H_{20}O_2$) (100), 261 (17).

3.3.3. Hydrogenation of maculalactone B (2) to maculalactone A (1). A solution of the butyrolactone **2** (30 mg, 0.09 mmol) in EtOAc (3 mL) was charged with 10% Pd/C (8 mg) and stirred under H_2 (1 atm) at room temperature for 18 h. The resultant mixture was filtered and rotary evaporated to give a crude product consisting predominantly of the butyrolactone **1**, which was purified from the alternative hydrogenation products **15** and **16** by preparative HPLC (10% EtOAc/*n*-hexane).

2,3,4(R/S)-Tribenzyl-γ-butyro-2-en-lactone (**1**). Solid mp 88–89 °C (23.7 mg, 0.07 mmol, 79%, R_t 31.4 min)-see Section 3.2.5 for physical properties.

2(R/S),3(S/R),4(R/S)-Tribenzyl-γ-butyrolactone (**15**). Solid, mp 145–147 °C (3.7 mg, 0.01 mmol, 12%, R_t 26.9 min). ν_{max}/cm^{-1}: 3028, 2957, 1767, 1603, 1497, 1454; δ_H-7.27 (2H, m), 7.25 (4H, m), 7.19 (3H, m), 7.09 (2H, d, *J*=7.3 Hz), 7.06 (2H, d, *J*=7.0 Hz), 7.02 (2H, d, *J*=7.3 Hz), 4.59 (1H, ddd, *J*=9.6, 4.0, 3.9 Hz), 3.28 (1H, dd, *J*=15.0, 4.8 Hz), 3.16 (1H, ddd, *J*=10.7, 6.4, 4.6 Hz), 2.96 (2H, m), 2.94 (1H, m), 2.82 (1H, dd, *J*=15.5, 4.8 Hz), 2.80 (1H, dd, *J*=15.0, 6.4 Hz), 2.55 (1H, dd, *J*=14.6, 3.9 Hz)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m/z* (rel. int.): 356.1779 (M^+, calcd 356.1776 for $C_{25}H_{24}O_2$) (15), 265 (25), 247 (30), 229 (18), 219 (20), 208 (100).

2(R/S),3,3,4(R/S)-Tetrabenzyl-γ-butyrolactone (**16**). Solid, mp 240–242 °C (0.5 mg, 0.001 mmol, 1%; R_t 24.8 min). ν_{max}/cm^{-1}: 3026, 2926, 1773, 1497, 1454; δ_H-7.45 (2H, d, *J*=7.5 Hz), 7.37 (2H, dd, *J*=7.5 Hz), 7.32 (1H, t, *J*=7.5 Hz), 7.30–7.15 (13H, m), 6.84 (2H, m), 4.24 (1H, dd, *J*=10.7, 1.8 Hz), 3.11 (1H, dd, *J*=14.9, 10.0 Hz), 3.11 (2H, s), 3.08 (1H, m), 3.07 (2H, s), 2.86 (1H, dd, *J*=13.5, 1.8 Hz), 2.75 (1H, dd, *J*=10.0, 4.1 Hz), 2.65 (1H, dd, *J*=14.9, 4.1 Hz)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m/z* (rel. int.): 446.2245 (M^+, calcd 446.2246 for $C_{32}H_{30}O_2$) (20), 355 (30), 337 (50), 319 (10), 309 (15), 298 (35), 207 (100).

3.4. Asymmetric synthesis of (+) maculalactone A ((+)-1)

3.4.1. Attempted enantioselective hydrogenation of the trisubstituted olefin group in maculalactone B (2) in the presence of (+)-1,2-bis[(2*S*,5*S*)-2,5-diethylphospholano]-benzene-(cyclooctadiene)rhodium (I) trifluoromethanesulfonate (Et-DuPhos) catalyst. A 25 mL Schlenk tube was charged with compound **2** (25 mg, 0.071 mmol) in anhyd. MeOH (5 mL), and the solution was degassed by three freeze-thaw cycles under an atmosphere of Ar. The solution was then transferred to another 25 mL Schlenk

tube, which was pre-charged with the catalyst Rh(I)-(*S*,*S*)-Et-DuPhos (0.05 mg, 0.1 mol %; Strem chemical company, Cat. no. 45–0151), via a cannula and further degassed by two more freeze-thaw cycles. H_2 (1 atm) was introduced to the system and the reaction mixture was allowed to stir at room temperature for 3 days, after which complete conversion to product was indicated by TLC. The resulting mixture was concentrated to yield an extract consisting mostly of product **1** (22.5 mg, 0.063 mmol, 89%; R_f 0.22), following purification by CC (10% EtOAc/*n*-hexane). The enantiomeric excess (% ee) was determined as 0.5 by measurement of the optical rotation ($[\alpha]_D$=+0.70 [*c*=2.2, $CHCl_3$]), assuming $[\alpha]_D$ (+)-**1**=+153.1 and $[\alpha]_D$ (−)-**1**=−153.1.

When the same experimental procedure was employed using the catalyst Rh(I)-(*R*,*R*)-Et-DuPhos (Strem Chemical Company, Cat. no. 45–0150) in place of its (*S*) enantiomer, compound **1** (18.4 mg, 0.052 mmol, 73%; R_f 0.22) was obtained with 0.4% ee ($[\alpha]_D$=−0.67, [*c*=1.8, $CHCl_3$]).

3.4.2. Methyl esterification of compound 10 to 2,3-dibenzyl-4-oxo-5-phenyl-pent-2-en-oic acid methyl ester (17). Compound **10** (500 mg, 1.35 mmol) was dissolved in Et_2O (20 mL) and then treated with excess of a solution of CH_2N_2 in Et_2O (100 mL) at room temperature for 4 h. After the reaction was complete, excess CH_2N_2 and Et_2O were removed by gently passing N_2 gas through the solution, leaving a product consisting entirely of compound **17** (477 mg, 1.24 mmol, 92%), which could be used without the need for any further purification.

2,3-Dibenzyl-4-oxo-5-phenyl-pent-2-en-oic acid methyl ester (**17**). Solid, mp 65–67° C; ν_{max}/cm^{-1}: 3030, 3019, 2954, 1710, 1603, 1496, 1454 cm^{-1}; δ_H-7.30 (2H, dd, *J*=6.9, 6.9 Hz), 7.28 (2H, dd, *J*=7.9, 7.9 Hz), 7.26–7.20 (5H, m), 7.14 (2H, dd, *J*=8.2, 1.1 Hz), 7.07 (2H, dd, *J*=7.9, 1.1 Hz), 7.03 (2H, dd, *J*=8.1, 1.5 Hz), 3.86 (2H, s), 3.70 (2H, s), 3.67 (3H, s), 3.52 (2H, s)—see also Table 2; ^{13}C NMR—see Table 2; HREIMS *m*/*z* (rel. int.): 352.1471 (M^+−MeOH, calcd 352.1463 for $C_{25}H_{20}O_2$) (100), 293 (25), 261 (20).

3.4.3. Reduction of compound 17 to racemic maculalactone A (±)-1 by catecholborane. To a solution of **17** (10 mg, 0.026 mmol) in anhyd. toluene (0.26 mL) was added a solution of catecholborane (5.5 μL, 0.052 mmol) under an atmosphere of N_2 at −78 °C. The mixture was stirred for 6 h and then left in the freezer (at −18 °C) for 15 h. After the reaction was complete, as indicated by TLC, H_2O (1 mL) was added and the resulting mixture was extracted with EtOAc (3×5 mL). The combined organic layers were washed with sat. $NaHCO_3$ (aq.) (5 mL), dried ($MgSO_4$) and rotary evaporated to obtain a crude product, which was purified by CC (5% EtOAc/*n*-hexane), to yield racemic (±)-**1** (7.7 mg, 0.022 mmol, 84%; R_f 0.35).

3.4.4. Asymmetric reduction of 17 by catecholborane in the presence of the chiral oxazaborolidine catalyst (*R*)-18. The oxazoborolidine catalyst (*R*)-**18** was prepared in four steps from (*R*)-proline (the more expensive unnatural enantiomer of this amino acid), according to Corey's procedure.[26,30] To a solution of (*R*)-**18** (20 μL, 0.01 mmol, 0.5 M in toluene) and compound **17** (19.2 mg, 0.05 mmol) in anhyd. toluene (0.5 mL) at −78 °C was added a solution of catecholborane (21.3 μL, 0.2 mmol) dropwise under an atmosphere of N_2. The reaction mixture was stirred at −78 °C for 6 h and then kept in the freezer (−18 °C) for 15 h. H_2O (3 mL) was added, and the mixture was extracted with EtOAc (3×5 mL). The combined organic layers were washed with sat. $NaHCO_3$ (aq.) (10 mL), dried ($MgSO_4$) and rotary evaporated to obtain a crude product, which was purified by CC (5% EtOAc/*n*-hexane) to yield **1** (15.5 mg, 0.043 mmol, 88%; R_f 0.35).

The enantiomeric composition of the product was estimated at 90.7% of (+)-**1** and 9.3% of (−)-**1** by chiral HPLC analysis (Section 3.2.6), and the % ee of the reaction was therefore 81.4%.

3.4.5. Asymmetric reduction of 17 by catecholborane in the presence of the chiral oxazaborolidine catalyst (*S*)-18. The oxazoborolidine catalyst (*S*)-**18** was prepared in four steps from (*S*)-proline, according to Corey's procedure.[26,30] The same experimental procedure was employed as for the enantioselective reduction of **17** in the previous section, except that (*S*)-**18** was substituted for (*R*)-**18**. The product (15.7 mg, 0.044 mmol, 89%) consisted of 92.2% of (−)-**1**, and 7.8% of (+)-**1**, and the % ee of the reaction was therefore 84.4%.

3.5. Investigation of the extent of partial racemization in (+) maculalactone A isolated from *K. maculans*

3.5.1. Determination of the enantiomeric purity of naturally-occurring (+) maculalactone A (1) by analytical HPLC using a chiral column. See Section 3.1.1 for details of the extraction and isolation procedure for obtaining maculalactone A from *K. maculans* and Section 3.2.6 for details of the analytical chiral HPLC analysis.

3.5.2. Determination of the enantiomeric purity of naturally-occurring (+) maculalactone A (1) by ^1H NMR spectroscopy in the presence of a chiral shift reagent. The enantiomeric composition of **1** which had been isolated from *K. maculans* was determined by ^1H NMR spectroscopy in the presence of the chiral shift reagent, europium *tris*[3-(heptafluoropropylhydroxy-methylene)-(+)-camphorate]. A sample of **1** (10 mg) from the natural source was dissolved in $CDCl_3$ (0.5 mL, containing 0.03% v/v TMS) and ^1H NMR spectra were recorded after successive additions of the chiral shift reagent (10 mg per addition) into the sample (expts. 1–7 in Fig. 6). The experiment was stopped when sufficient chiral shift reagent had been added to obtain a clear baseline separation of ^1H resonances which were considered characteristic of the two enantiomeric forms of **1**. Expts. 8–14 in Figure 6 were recorded in a similar way using racemic (±)-**1** which had been obtained from synthesis (see Section 3.2.5) in place of natural maculalactone A.

Acknowledgements

We thank the Chemistry Department of the University of Hong Kong for providing a postgraduate studentship to Mr.

Wong. This project was funded by a grant from the Committee on Research and Conference Grants (CRCG) of The University of Hong Kong.

References and notes

1. Kaehler, S. *Asian Mar. Biol.* **1994**, *11*, 41–54.
2. Kamura, S.; Choohabandit, S. *Galaxea* **1986**, *5*, 97–114.
3. Kaehler, S.; Williams, G. A. *Mar. Biol.* **1996**, *125*, 177–187.
4. Tsui, W.-Y.; Williams, G. A.; Brown, G. D. *Phytochemistry* **1996**, *43*, 1083–1085.
5. Lee, S.-C.; Brown, G. D. *J. Nat. Prod.* **1998**, *61*, 29–33.
6. Lee, S.-C.; Williams, G. A.; Brown, G. D. *Phytochemistry* **1999**, *52*, 537–540.
7. Wong, H.-F.; Williams, G. A.; Brown, G. D. *Phytochemistry* **2002**, *60*, 425–429.
8. Lee, S.-C. Chemical ecology of algae and the cyanobacterium *Kyrtuthrix maculans* on Hong Kong rocky shores. Ph.D. Thesis, The University of Hong Kong, 2001.
9. Brown, G. D.; Wong, H.-F.; Hutchinson, N.; Lee, S.-C.; Williams, G. A. Chemistry and biology of maculalactone A from the marine cyanobacterium *Kyrtuthrix maculans*, International Symposium Chemistry and Biology of Marine Organisms, Kolympari, Crete., 21–26 September 2003; pp. 60–61.
10. Wong, H.-F. Applications of high field nuclear magnetic resonance spectroscopy to the structure elucidation, conformational analysis and asymmetric synthesis of natural products. Ph.D. Thesis, The University of Hong Kong, 2002.
11. Ward, R. S.; Pelter, A.; Edwards, M. I.; Gilmore, J. *Tetrahedron* **1996**, *52*, 12799–12814.
12. Johnson, W. S.; Daub, G. H. *Organic Reactions*; Wiley: New York, 1951; Vol. 6. pp. 1–73.
13. Andersson, J. *Acta Chem. Scand. B* **1977**, *B31*, 340–341.
14. Fieser, L. F.; Martin, E. L. Succinic Anhydride. *Organic Synthesis*; Blatt, A. H., Ed.; Wiley: London, 1948; Collective Vol. 2, pp 560–562.
15. Davidse, P. A.; Dillen, J. L. M.; Heyns, A. M.; Modro, T. A.; van Rooyen, P. H. *Can. J. Chem.* **1990**, *68*, 741–746.
16. Chavez, F.; Suarez, S.; Diaz, M. A. *Synth. Commun.* **1994**, *24*, 2325–2339.
17. Bushan, K. M.; Rao, G. V.; Soujanya, T.; Rao, V. J.; Saha, S.; Samanta, A. *J. Org. Chem.* **2001**, *66*, 681–688.
18. Allenmark, S. G. *Nat. Prod. Rep.* **2000**, *17*, 145–155.
19. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
20. Seco, J. M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.
21. Seco, J. M.; Quiñoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2000**, 2781–2791.
22. Burk, M. J. *J. Am. Chem. Soc.* **1991**, *113*, 8518–8519.
23. Burk, M. J.; Feaster, J. E.; Harlow, R. L. *Tetrahedron: Asymmetry* **1991**, *2*, 569–592.
24. Burk, M. J.; Feaster, J. E.; Harlow, R. L. *Organometallics* **1990**, *9*, 2653–2655.
25. Thompson, H. W.; Wong, J. K.; Lalancette, R. A.; Boyko, J. A.; Robertiello, A. M. *J. Org. Chem.* **1985**, *50*, 2115–2121.
26. Corey, E. J.; Shibata, S.; Bakshi, R. K. *J. Org. Chem.* **1988**, *53*, 2861–2863.
27. Corey, E. J.; Da Silva Jardine, P.; Mohri, T. *Tetrahedron Lett.* **1988**, *29*, 6409–6412.
28. Corey, E. J.; Da Silva Jardine, P.; Rohloff, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 3672–3673.
29. Corey, E. J.; Reichard, G. A. *Tetrahedron Lett.* **1989**, *30*, 5207–5210.
30. Corey, E. J.; Bakshi, R. K. *Tetrahedron Lett.* **1990**, *31*, 611–614.
31. Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C.-P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925–7926.
32. Corey, E. J.; Helal, C. J. *Angew. Chem. Int. Ed.* **1998**, *37*, 1986–2012.
33. Kemp, W. *Organic Spectroscopy*; MacMillan Press: New York, 1991; pp. 169–170.
34. Charlwood, B. V.; Banthorpe, D. V. Methods in Plant Biochemistry. In *Terpenoids*; Dey, P. M., Harborne, J. B., Eds.; Academic: New York; Vol. 7, pp. 33–34.
35. Sy, L.-K.; Brown, G. D. *J. Nat. Prod.* **1998**, *61*, 987–992.