A chemoenzymatic synthesis of the 12-membered macrolide (−)-cladospolide A

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The enantiomerically pure *cis*-1,2-dihydrocatechol 7, which is obtained by microbial oxidation of chlorobenzene, has been converted, *via* a sequence of reactions including ring-closing metathesis and Yamaguchi lactonisation steps, into the natural product (−)-cladospolide A (1).

Cladospolides A (**1**)[1] and B (**2**)[2] were first isolated from the culture filtrate of the fungus *Cladosporium cladosporioides* FI-113 by Isogai and co-workers. Subsequently Fukuda *et al.* reported[3] the isolation of cladospolide C (**3**), as well as congeners **1** and **2**, from *Cladosporium tenuissimum*. Very recently, Omura *et al.* detailed[4] the extraction of cladospolide D (**4**, stereochemistry undefined) from the fermentation broth of *Cladosporium* sp. FT-0012. Macrolide **1** has been shown to inhibit root growth in lettuce seedlings, whilst isomer **2** displays the opposite effect.[2] Interestingly, compound **2** also appears to inhibit gibberellin biosynthesis as evidenced by its capacity to constrain shoot elongation of rice seedlings (*Oryza sativa L.*) without causing necrosis.[3] Such interesting plant growth regulating properties have prompted several synthetic studies although these have all been focussed on compound **1**. The first total synthesis of (−)-cladospolide A was described in 1987[5] by Mori and Maemoto who employed ethyl (*R*)-3-hydroxybutyrate as starting material and the stereogenic centre associated with this synthon was parlayed into C-11 of the target **1**. The hydroxy groups at C-4 and C-5 were introduced *via* a Katsuki–Sharpless asymmetric epoxidation reaction while the macrolide ring of (−)-cladospolide A (**1**) was established using the Yamaguchi macrolactonisation procedure. A closely related synthesis was reported in the same year by Ichimoto *et al.*[6] The third and most recent synthesis of (−)-cladospolide A was detailed in 1994 by Solladié and Almario[7] who established all the stereogenic centers associated with the target *via* diastereoselective reduction of enantiomerically pure β-ketosulfoxides. Once again, the macrocyclic ring within target **1** was constructed *via* a late-stage Yamaguchi lactonisation step.
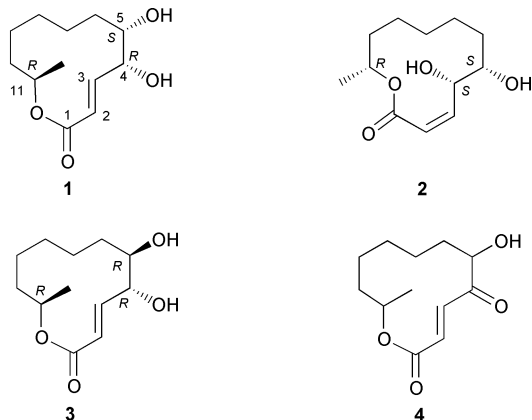
We now report a rather efficient synthesis of (−)-cladospolide A that employs, *inter alia*, three enzyme-mediated transformations and a ring-closing metathesis (RCM)[8] step. The original plan, shown in retrosynthetic form in Fig. 1, was
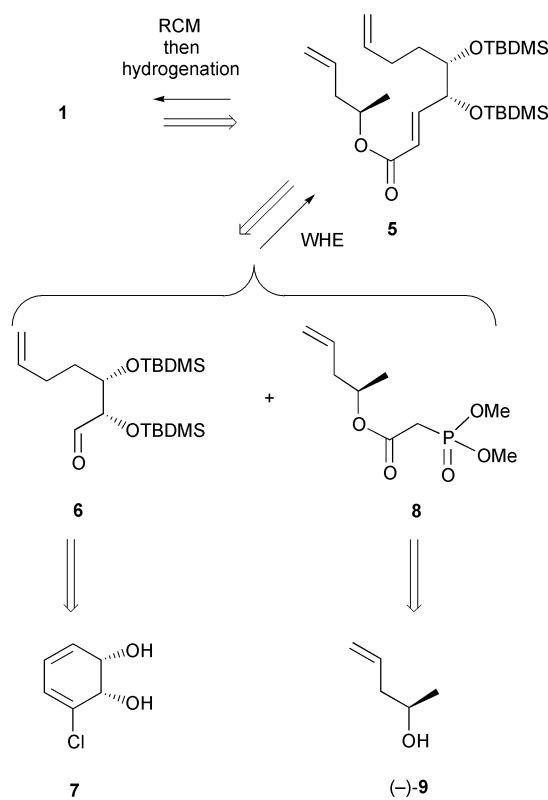
Fig. 1

predicated on our recent success in using related chemistry to prepare the 18-membered macrolide (+)-aspicilin.[9] Thus, we envisaged the 12-membered lactone ring of target **1** could be created *via* RCM of the terminal double-bonds within triene **5**.[8] Hydrogenation of the resulting olefin followed by removal of the TBDMS-protecting groups was then expected to deliver target **1**. Sub-target **5** should be accessible *via* an *E*-selective Wadsworth–Horner–Emmons (WHE)-type coupling of aldehyde **6** with phosphonoacetate **8**. The seven-carbon building block **6** was to be constructed from *cis*-1,2-dihydrocatechol **7**, a material available in large quantity and enantiomerically pure form *via* toluene dioxygenase-mediated dihydroxylation of chlorobenzene.[10] On the other hand, compound **8** was to be prepared by transesterification[11] of readily available trimethyl phosphonoacetate with (*R*)-(−)-pent-4-en-2-ol [(−)-(**9**)].

The route used to assemble compound **6** is shown in Scheme 1 and follows from earlier work.[9,12] Thus, diol **7** was

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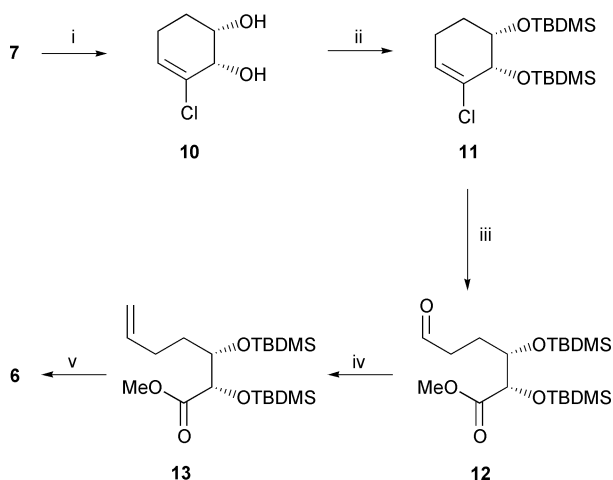
Scheme 1 *Reagents and conditions*: (i) H_2 (1 atm), 5% Rh on alumina, EtOH, 18 °C, 16 h; (ii) TBDMS-Cl (3.5 mol equiv.), imidazole (12.4 mol equiv.), DMF, 18 °C, 24 h; (iii) O_3, MeOH, -78 °C, 0.5 h then $NaBH_4$ (2.0 mol equiv.), 0 °C, 1 h; (iv) $Ph_3P=CH_2$ (1.5 mol equiv.), THF, 0–18 °C, 6.5 h; (v) DIBAL-H (1.1 mol equiv.), hexane, -78 °C, 0.75 h.

selectively reduced, with hydrogen in the presence of rhodium on alumina,[12] to the dihydro analogue **10**[13] (75%), which was, in turn, converted under standard conditions into the corresponding bis-TBDMS-ether **11**† {95%, $[a]_D$ -13 (c 7.4)‡}. When ozonolytic cleavage of alkene **11** was carried out in methanol at -78 °C and the ensuing peroxidic material subjected to reductive work-up with sodium borohydride, then the ester aldehyde **12** was obtained. The unstable nature of this last compound meant that it was best to effect its rapid methylenation, which was achieved with the Wittig reagent at 0 °C, and in this manner the unsaturated ester **13** {$[a]_D$ -9.1 (c 0.3)} was produced. This two step sequence was readily carried out on a multi-gram scale and compound **13** obtained in 65% overall yield from alkene **11**. Direct reduction of ester **13** to the target, but unstable, aldehyde **6** could be achieved by reaction of the former compound with essentially equimolar quantities of diisobutylaluminium hydride (DIBAL-H) in hexane at -78 °C.

As a necessary prelude to synthesizing phosphonoacetate **8**, access to the enantiomerically pure alcohol $(-)$-**9**[14] was required. In our hands the most effective route to gram quantities of this material involved initial reaction of the commercially available racemate $(\pm)$-**9** with isopropenyl acetate in the presence of *Candida antarctica* lipase B[15] (CALB, *ex* Novo Nordisk). Under appropriate conditions (Scheme 2) a chrom-
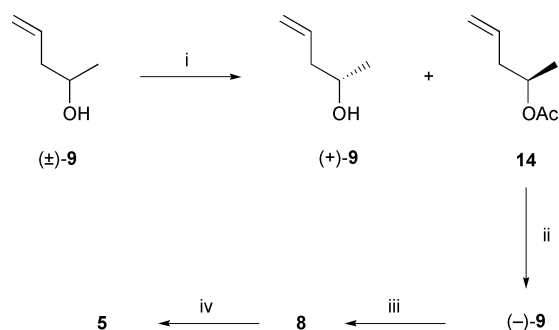
Scheme 2 *Reagents and conditions*: (i) CALB (10% by weight), isopropenyl acetate (solvent), 25 °C, 1 h; (ii) CALB (10% by weight), aqueous phosphate buffer (pH 7.6), 25 °C, 16 h; (iii) $(MeO)_2P(O)CH_2CO_2Me$ (1 mol equiv.), DMAP (12 mol equiv.), toluene, 111 °C, 6.5 h; (iv) NaH (1.0 mol equiv.), THF, 0–18 °C, 2 h then compound **6** (0.83 mol equiv.), 0–18 °C, 1.5 h.

atographically separable mixture of $(+)$-(S)-**9** (60% yield, 99% ee§) and acetate **14** (80% yield, *ca.* 75% ee) was obtained. Treatment of the latter compound with CALB in phosphate

buffer (pH 7.6) then provided enantiomerically pure samples of the target alcohol $(-)$-(R)-**9** (60% yield).§ Reaction of alcohol $(-)$-**9** with equimolar amounts of trimethyl phosphonoacetate and an excess of 4-(N,N-dimethylamino)pyridine (DMAP) in refluxing toluene for 6.5 h resulted in a smooth transesterification reaction[11] and formation of the target diester **8** {$[a]_D$ $+79$ (c 1.1)} in *ca.* 75% yield. WHE-type coupling of this latter compound with aldehyde **6** then afforded the target RCM substrate **5** {65% *ex* compound **13**, $[a]_D$ -3 (c 0.7)}. However, contrary to expectations,[9] when this substrate was treated with either the first[16] or the second[17] generation Grubbs' catalyst the desired 12-membered macrocycle **15** (Scheme 3) was
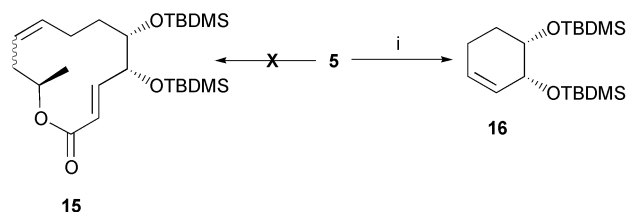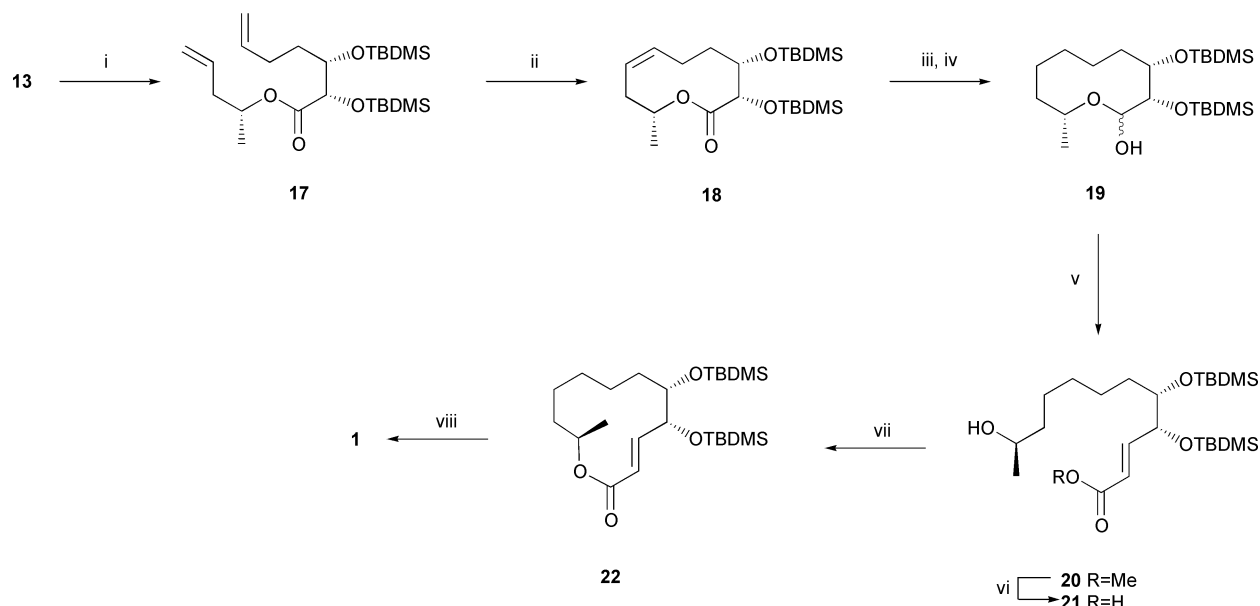

Scheme 3 *Reagents and conditions*: (i) tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene](benzylidene)... dichloride (18 mol%), CH_2Cl_2, 18 °C, 24 h.

not formed. Rather, varying yields (90–100%) of an alternate RCM product, *viz.* cyclohexene **16**, {$[a]_D$ -119 (c 0.5)} were observed.

On the basis of the foregoing observations it seemed clear that if a RCM-based strategy was to be employed in accessing the target macrolide **1** then the substrate to be used for such purposes should include just two C–C double bonds such that only one reaction pathway would be followed. To this end, various methods for temporarily masking the conjugated double bond within substrate **5** were pursued. However, all efforts along these lines proved fruitless, thus highlighting the need for a new approach to target **1**. The revised strategy that followed is shown in Scheme 4 and involved initial conversion of ester **13** into the corresponding acid (64%) which was immediately subject to Yonemitsu esterification[18] using alcohol $(-)$-**9**. The resulting ester **17** {100%, $[a]_D$ -5 (c 0.8)} readily engaged in an RCM reaction upon exposure to Grubbs' second generation catalyst[17] so as to afford the ten-membered lactone **18** (73% at 75% conversion) as a single geometric isomer for which the (Z)-configuration is tentatively assigned.[19] Hydrogenation of the latter compound and DIBAL-H reduction of the ensuing saturated lactone {89%, $[a]_D$ -2 (c 0.8)} then afforded the lactol **19** which reacted smoothly with the sodium salt of trimethyl phosphonoacetate to give the α,β-unsaturated ester **20** {43% from saturated lactone, $[a]_D$ -3 (c 0.4)}. Saponification of the last compound followed by acidification afforded the free acid **21** which was subject to a standard Yamaguchi lactonisation protocol[20] thereby providing the bis-TBDMS-ether **22** (89% from **20**) of $(-)$-cladospolide A (**1**). Initial attempts to effect desilylation of compound **22** involved the use of TBAF but only a complex mixture of products, some of which may arise *via* a retro-aldol process, was observed. After extensive experimentation with a wide range of fluoride ion sources, it was established that zinc tetrafluoroborate in water–acetonitrile[21] could circumvent such difficulties and $(+)$-cladospolide A (**1**) (73%) was obtained as a crystalline solid, mp 90–91 °C (lit.[1] mp 92–93 °C). The various spectroscopic data, including specific rotation {$[a]_D$ -53 (c 0.2); lit.[5] $[a]_D$ -49 (c 0.23 in $CHCl_3$)}, derived from the synthetic material were in agreement with those reported[5] previously.

In summary, a concise synthesis of the title natural product has been achieved *via* a tandem RCM–Yamaguchi lactonisation sequence employing the enzymatically derived synthons **7** and $(-)$-**9**. The strategies and tactics reported herein should be applicable to the preparation of a range of related macrolides, especially compounds **2**, **3** and **4**, and studies directed toward this end are now underway in these laboratories.

Scheme 4 *Reagents and conditions*: (i) NaOH (3.7 mol equiv.), 9 : 1 v/v EtOH–H$_2$O, 60 °C, 2 h then 1 M aq. HCl then 2,4,6-trichlorobenzoyl chloride (0.95 mol equiv.), (−)-**9** (1.1 mol equiv.), Et$_3$N (5.0 mol equiv.), DMAP (1.0 mol equiv.), toluene, 18 °C, 1 h; (ii) tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene](benzylidene)ruthenium(IV) dichloride (15 mol%), CH$_2$Cl$_2$, 18 °C, 24 h; (iii) H$_2$ (1 atm), 10% Pd on C, EtOH, 18 °C, 6 h; (iv) DIBAL-H (2.0 mol equiv.), toluene, −78 °C, 10 min; (v) (MeO)$_2$P(O)CH$_2$CO$_2$Me, NaH (0.9 mol equiv.), THF, 0–18 °C, 3 h then compound **19** (0.5 mol equiv.), 0–18 °C, 1 h; (vi) NaOH (3.7 mol equiv.), 4 : 1 v/v EtOH–H$_2$O, 18 °C, 18 h then 1 M aq. HCl; (vii) 2,4,6-trichlorobenzoyl chloride (1.1 mol equiv.), Et$_3$N (1.2 mol equiv.), THF, 18 °C, 2 h then DMAP (4.0 mol equiv.), toluene, 111 °C, 1 h; (viii) Zn(BF$_4$)$_2$ (5.0 mol equiv. of a 5 M aq. solution), acetonitrile, 18 °C, 24 h.

Experimental

Compound 18

A magnetically stirred solution of diene **17** (125 mg, 0.27 mmol) in degassed CH$_2$Cl$_2$ (140 mL) and maintained under a nitrogen atmosphere was treated, *via* cannula, with a solution of tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene](benzylidene)ruthenium(IV) dichloride[17] (35 mg, 0.04 mmol, 15 mol%, *ex* Strem Chemicals Inc.) in CH$_2$Cl$_2$ (10 mL). The resulting mixture was stirred at 18 °C for 24 h then (HOCH$_2$)$_3$P[22] (~50 mg) and Merck silica gel 60 (~200 mg) were added to the reaction mixture. The resulting slurry was stirred at 18 °C for 1 h then filtered through a *ca.* 3 cm deep pad of Merck silica gel 60 which was washed with CH$_2$Cl$_2$ (*ca.* 10 mL). The combined filtrates were concentrated under reduced pressure and the light-yellow oil thus obtained was subject to flash chromatography (silica gel, 98 : 2 v/v hexane–Et$_2$O elution). In this manner two fractions, A (R_f 0.30 in 95 : 5 v/v hexane–EtOAc) and B (R_f 0.28 in 95 : 5 v/v hexane–EtOAc), were obtained.

Concentration of fraction A gave the starting diene **17** (32 mg, 25% recovery) which was identical, in all respects, with an authentic sample.

Concentration of fraction B gave the *title compound* **18** (61 mg, 73% yield at 75% conversion) as clear colourless oil, $[a]_D$ +0.8 (*c* 0.7). v_{max} (KBr) 2931, 2857, 1729, 1465, 1255, 1155, 1073, 957, 837 and 777 cm^{-1}; δ_H (300 MHz, CDCl$_3$) 5.45–5.35 (2H, complex m), 5.10 (1H, m), 4.28 (1H, br s), 3.98 (1H, dd, *J* 10.9 and 4.7 Hz), 2.72 (1H, br m), 2.40 (1H, br m), 2.06–1.76 (3H, complex m), 1.60 (1H, m), 1.32 (3H, d, *J* 6.5 Hz), 0.93 (9H, s), 0.89 (9H, s), 0.09 (3H, s), 0.07 (3H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C (75 MHz, CDCl$_3$) 172.6, 133.7, 124.7, 78.4, 74.5, 70.5, 34.6, 32.1, 25.8, 25.6, 22.7, 20.2, 18.3, 18.0, −4.8, −4.9, −5.1, −5.4; *m/z* (EI) 428.2777 (C$_{22}$H$_{44}$O$_4$Si$_2$ requires 428.2778, M$^{+\bullet}$, 18%), 413 (28), 371 (76), 239 (81), 147 (69), 73 (100).

Compound 22

A magnetically stirred solution of methyl ester **20** (28 mg, 0.057 mmol) in EtOH (3 mL) was treated with NaOH (1 mL of a 2.5 M aqueous solution). The ensuing mixture was allowed to stir at 18 °C for 18 h, acidified to pH 3 (with 1 M aqueous HCl) then extracted with EtOAc (5 × 12 mL). The combined organic

phases were washed with brine (1 × 5 mL) then dried (MgSO$_4$), filtered and concentrated under reduced pressure to give acid **21** (26 mg, 95%) as a light-yellow oil. The crude acid was dissolved in THF (1 mL) containing Et$_3$N (10 μL, 0.07 mmol). The resulting and magnetically stirred solution was treated with 2,4,6-trichlorobenzoyl chloride (9 μL, 0.06 mmol) and, after 2 h, diluted with toluene (70 mL). The ensuing mixture was then added, over *ca.* 10 minutes and *via* cannula, to a solution of DMAP (37 mg, 0.3 mmol) in refluxing toluene (10 mL). The resulting mixture was heated at reflux for 1 h then cooled, quenched with NaHCO$_3$ (5 mL of a saturated aqueous solution) and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine (1 × 5 mL) then dried (MgSO$_4$), filtered and concentrated under reduced pressure. The light-yellow oil thus obtained was subject to flash chromatography (silica gel, 95 : 5 v/v hexane–Et$_2$O elution) and concentration of the appropriate fractions (R_f 0.3) then gave macrolactone **22** (22.5 mg, 89% from **20**) as a clear colourless oil, $[a]_D$ −23 (*c* 0.2). v_{max} (KBr) 2932, 2858, 1723, 1649, 1466, 1364, 1254, 1159, 1074, 1003, 835, 777 cm^{-1}; δ_H (300 MHz, CDCl$_3$) 6.76 (1H, dd, *J* 15.8 and 4.7 Hz), 6.15 (1H, dd, *J* 15.8 and 1.6 Hz), 5.08 (1H, m), 4.43 (1H, m), 3.53 (1H, dm, *J* 8.9 Hz), 1.90–1.05 (10H, complex m), 1.28 (3H, d, *J* 6.6 Hz), 0.92 (9H, s), 0.90 (9H, s), 0.09 (3H, s), 0.06 (3H, s), 0.05(2) (3H, s), 0.04(8) (3H, s); δ_C (75 MHz, CDCl$_3$) 168.4, 147.8, 121.7, 76.6, 75.7, 72.9, 32.2, 30.7, 27.7, 26.1, 26.0, 25.8, 23.4, 19.4, 18.5, 18.4, −4.4 (two signals superimposed), −4.6(7), −4.6(9); *m/z* (EI) 456.3090 (C$_{24}$H$_{48}$O$_4$Si$_2$ requires 456.3091, M$^{+\bullet}$, 3%), 399 (28), 371 (13), 241 (34), 216 (51), 198 (62), 147 (88), 73 (100).

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† All new and stable compounds had spectroscopic data (IR, NMR, mass spectrum) consistent with the assigned structure. Satisfactory combustion and/or high-resolution mass spectral analytical data were obtained for new compounds and/or suitable derivatives.

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