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Total synthesis and cytotoxic activity of dechlorogreensporones A and D

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ABSTRACT

The first and convergent total syntheses of polyketide natural products dechlorogreensporones A and D have been accomplished in 17 longest linear steps with 2.8% and 5.4% overall yields, respectively, starting from known methyl 2-(2-formyl-3,5-dihydroxyphenyl)acetate and commercially available *R*-(+)-propylene oxide and 1,2-epoxy-5-hexene. Our synthesis exploited key Mitsunobu esterification and (*E*)-selective ring-closing metathesis (RCM) to assemble the macrocycles as well as a Jacobsen hydrolytic kinetic resolution to install the stereogenic centers. Both synthetic compounds were found to display significant cytotoxic activity against seven human cancer cell lines with the IC_{50} ranges of 6.66–17.25 μM.

1. Introduction

The well-known 14-membered β-resorcylic acid lactones (RALs) are a group of fungal polyketide metabolites that possess a multitude of biological and pharmacological activities [1]. A subclass of RALs are those containing an α,β-unsaturated ketone at the 8–10 positions, which are derivatives of radicicol [2]. The major examples of this subclass of RALs are the pochonins [3] and the monocillins [4] (Fig. 1). This group of metabolites has been shown to exhibit various interesting biological activities e.g. antiviral activity against Herpes Simplex Virus 1 (HSV 1) [3a], antifungal activity (against *Mucorflavas* IFO 9560) [5], HSP-90 inhibitory activity [6], and latent HIV-1 reactivation activity [3c]. In consequence of their diverse and promising biological properties and structural features, this class of macrolides has been synthetic targets for many synthetic organic research groups worldwide [7]. Precedented strategies to construct the macrocyclic cores of RALs possessing similar core skeleton mainly relied on esterification reaction [7] and ring-closing metathesis [7c,f-k] (Fig. 2). Other key bond formations included Pd-catalyzed cross coupling/elimination [7a,b,d,e], substitution by dithiane anion [7c] and nucleophilic addition to Weinreb amide (acylation) [7f-i].

Dechlorogreensporones A (**5**) and D (**6**) are new 14-membered β-RALs, which were isolated, along with other 12 new RALs from a culture of a freshwater fungus *Halenospora* sp. by Oberlies and co-workers in 2014 (Fig. 3) [8]. Compounds **5** and **6** are radicicol analogues possessing a methoxy group at the 16-position, which represent rare examples of RALs containing β-resorcylic acid monomethyl ethers. Dechlorogreensporones A and D have the same planar structure which includes a stereogenic center at the 2-position. However, the minor structural difference is that **5** contains a keto group at the 5-position, whereas **6** bears an alcohol stereogenic center. In addition, dechlorogreensporone A (**5**) is structurally very similar to the previously reported natural product cryptosporiopsin A [9]. The absolute configuration of the C-2 asymmetric carbon in macrolactones **5** and **6** and other co-metabolites was proposed by the isolation group to be *S* by the evidence of X-ray diffraction analysis of the bromobenzoyl derivative of one of the metabolites in the series. The absolute configuration of the C-5 in **6** and co-metabolites containing C-5 alcohol stereogenic center was assigned to be *S* via a Mosher's ester method. Interestingly, the assigned C-2 absolute configuration of **5** and **6** and other analogues

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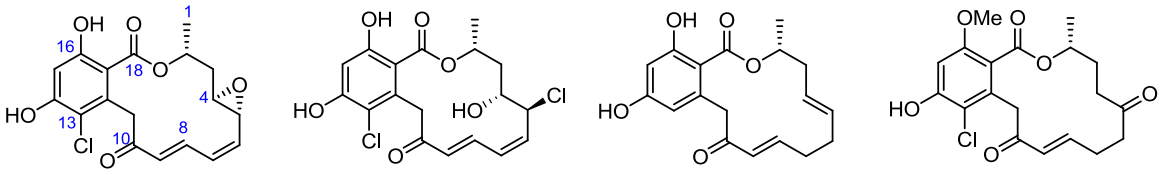

Fig. 1. Structures of radicicol and selected examples of its analogues.

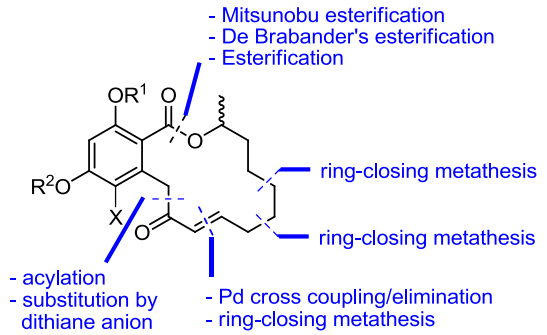

Fig. 2. Key bond formation strategies in previous syntheses of radicicol and its analogues.

in the series is opposite to that of cryptosporiopsin A, which was assigned by analogy to the known RAL pochonin D. Dechlorogreensporones A and D were tested for cytotoxic activities against two human cancer cell lines and were found to exhibit cytotoxicity against the MDA-MB-435 (melanoma) cancer cell line with IC_{50} values of 14.1 and 11.2 μM, respectively. They also exhibited cytotoxicity against the HT-29 (colon) cancer cell line with IC_{50} values of >20 and 25.4 μM, respectively. Due to promising biological activities of this subclass of RALs and our ongoing program for anticancer drug discovery, our research group has been focusing on a synthetic program of selected compounds of this class. Herein, we report the first total synthesis of both **5** and **6** as well as evaluation of their cytotoxic activity against seven human cancer cell lines.

2. Results and discussion

Our retrosynthetic approach toward dechlorogreensporones A (**5**) and D (**6**) would utilize similar disconnection strategy to Mohapatra and Thirupathi's [7j] and our previous report [7k] via ring-closing metathesis (RCM) as a key macrocyclization protocol and to concomitantly establish the (*E*) geometry of C8–C9 olefin. We would also rely on the Mitsunobu esterification to construct the ester functional group of the diene RCM precursor (Scheme 1). Although the targets **5** and **6** only differ by the functional groups at the 5-position and could be ideally synthesized from the same

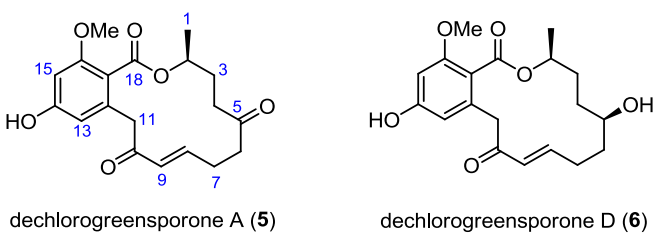

Fig. 3. Structures of dechlorogreensporones A (**5**) and D (**6**).

intermediate, the alcohol stereogenic center at the 5-position in **6** posed a challenge to the synthesis. Thus, we employed two different routes for the synthesis of the requisite alcohol fragments in conjunction with protecting group manipulation. The diene RCM precursor **9** (for **5**) or **10** (for **6**) would be assembled by Mitsunobu esterification between the common benzoic acid intermediate **11** and chiral alcohol intermediate **12** or **13**. The common benzoic acid intermediate **11** would be elaborated from the known phenol **14** using our previously described approach. The requisite chiral alcohol **12** for the synthesis of **5** would be obtained from *R*-(+)-propylene oxide (**15**) via double allylation, whereas enantioenriched alcohol **13** would be prepared from 1,2-epoxy-5-hexene (**16**) using Jacobsen hydrolytic kinetic resolution to construct both chiral centers [10].

Synthesis of benzoic acid **11** which was required as a Mitsunobu coupling partner for syntheses of both **5** and **6** commenced with selective protection of known phenol **14** [11] with 4-methoxybenzyl ether (PMB) group [12] to afford PMB ether **17** in 82% yield. Subsequent methylation of the remaining phenol moiety with iodomethane and K_2CO_3 in DMF furnished methyl ether **18** in 94% yield. Following our previously established sequence [7k], benzaldehyde **18** was further elaborated to the requisite benzoic acid **11** in 10 steps and 31% overall yield (Scheme 2).

Synthesis of alcohol **12** required for the synthesis of **5** was achieved in a concise sequence of 6 steps as illustrated in Scheme 3. Regioselective ring opening of commercially available *R*-(+)-propylene oxide (**16**) (>99% ee) by allylmagnesium bromide in the presence of catalytic CuI provided the corresponding chiral secondary alcohol [13], which was instantaneously protected with TBDPS group to afford TBDPS ether **19** in 78% yield over 2 steps. Subsequent epoxidation of alkene **19** with *m*-CPBA afforded racemic epoxide **20**, which was then subjected to another regioselective ring opening by allylmagnesium bromide to give racemic alcohol **21** in 89% yield [14]. Protection of the secondary alcohol of **21** with ethoxymethyl (EOM) group provided EOM ether, which after TBDPS deprotection with TBAF furnished the desired chiral alcohol **12** in 95% yield. The absolute configuration of the alcohol stereogenic center was confirmed to be *R* based on Mosher ester analysis.

Having successfully synthesized both key fragments **11** and **12**, we continued to complete the synthesis of dechlorogreensporone A (Scheme 4). Benzoic acid **11** was subjected to esterification with (*R*)-alcohol **12** under Mitsunobu conditions using diisopropyl azodicarboxylate (DIAD) and PPh_3 in toluene at room temperature to smoothly furnish the ester RCM diene precursor **9** in 72% yield. This step was expected to provide the correct stereochemistry of the C-2 stereogenic center. With diene **9** in hand, the stage was then set for the key ring-closing metathesis. We and the Mohapatra group have previously demonstrated that the second-generation Grubbs catalyst is a remarkable RCM catalyst for this type of substrate [7j,k]. However, in this case the second-generation Grubbs catalyst proved to be less reactive and led to incomplete consumption of the starting diene. To our delight, RCM of **9** using 10 mol% of second-

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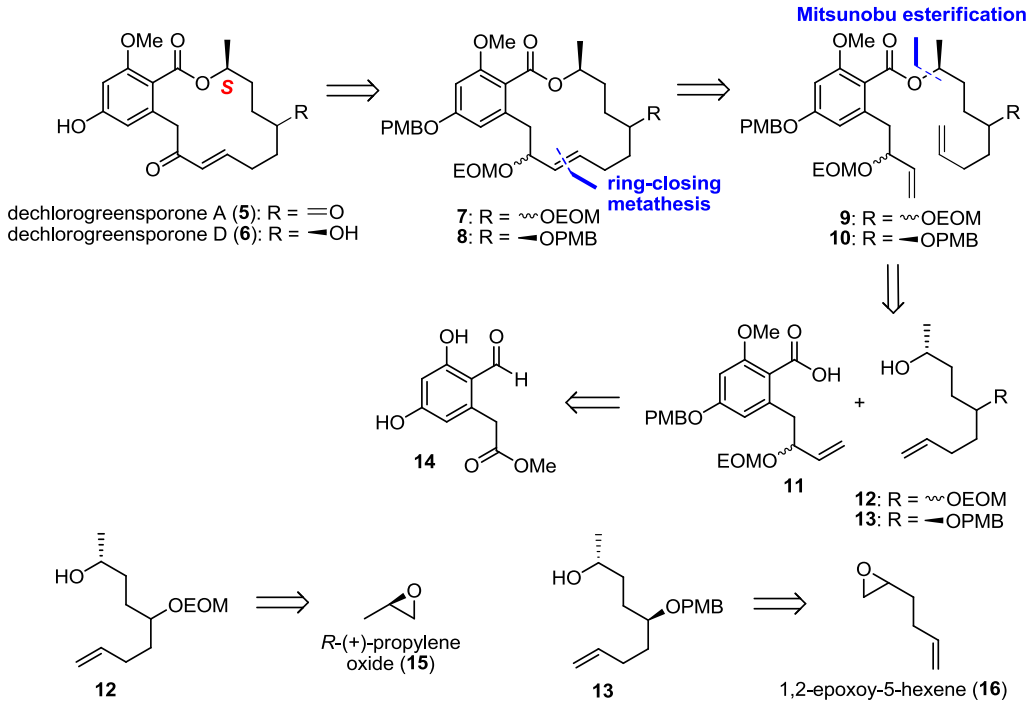
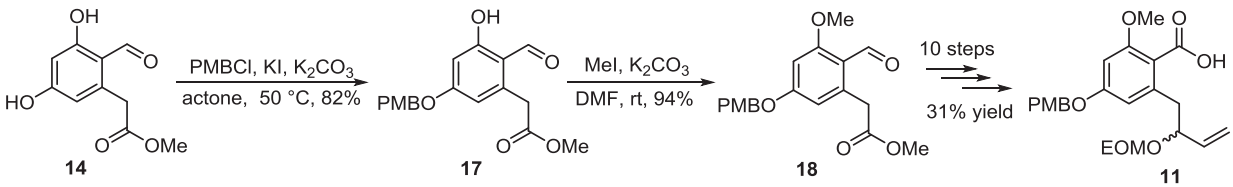
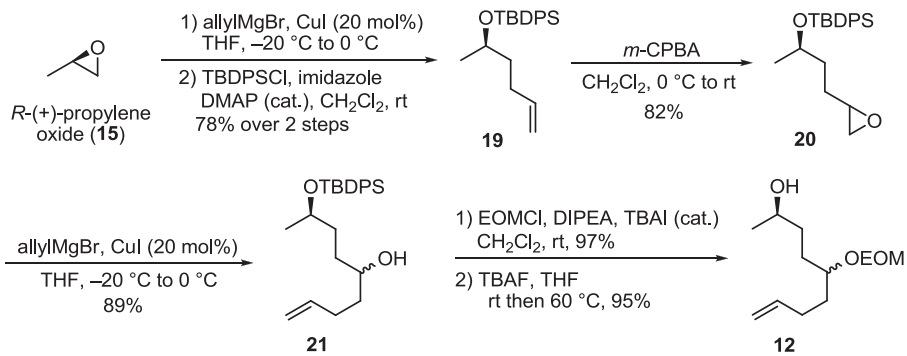


Scheme 1. Retrosynthesis of dechlorogreensporones A (**5**) and D (**6**).



Scheme 2. Synthesis of the common benzoic acid intermediate **11**.



Scheme 3. Synthesis of alcohol **12**.

generation Hoveyda-Grubbs catalyst in toluene at high dilution (5 mM) at 85 °C proceeded to completion within 3.5 h to afford RCM products **7** in 59% yield as an inseparable mixture of diastereomers. No attempts were made to separate these diastereomeric products because they would eventually be transformed into the same diketo product via oxidation in the penultimate step. It should be noted that the geometry of the resulting olefin at C8–C9 could not be determined by NMR spectroscopy at this stage. We then carried this diastereomeric mixture on to the next step, which was removal of both EOM protecting groups of **7** using 4 M HCl in THF at ambient temperature to furnish diol **22** in 57% yield as, again, a mixture of diastereomers. Both hydroxyl groups of **22** were then simultaneously oxidized using a large excess Dess-Martin periodinane in CH_2Cl_2 to furnish diketone **23** in 62% yield. The geometry of the C8–C9 olefin of the macrocyclic products from RCM could then be verified to be (*E*) in this step on the basis of the coupling constant of 15.6 Hz between H-8 and H-9. Finally, following Mohapatra's protocol [7j], treatment of **23** with 1 M titanium tetrachloride in

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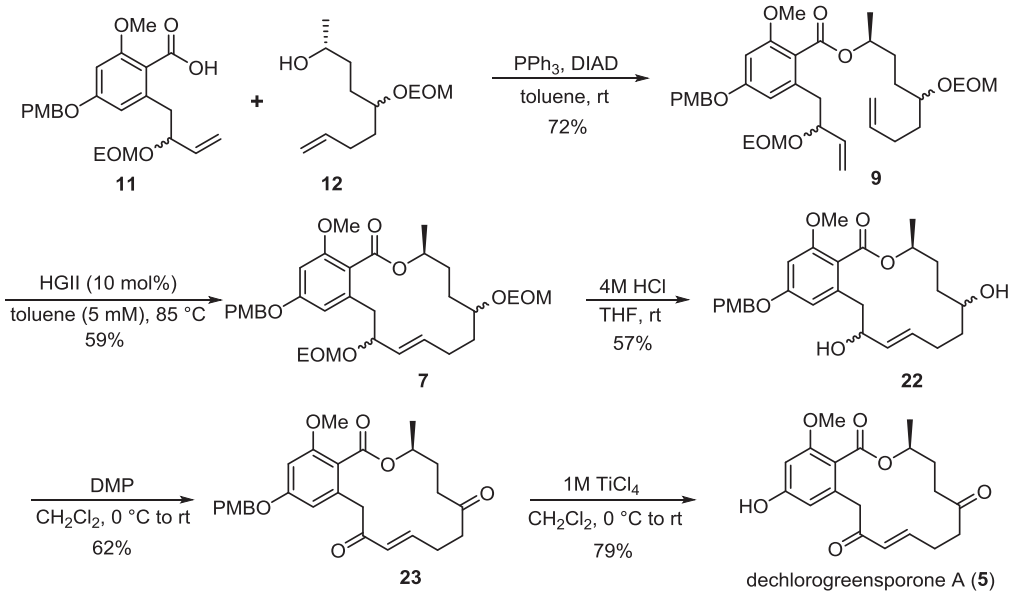
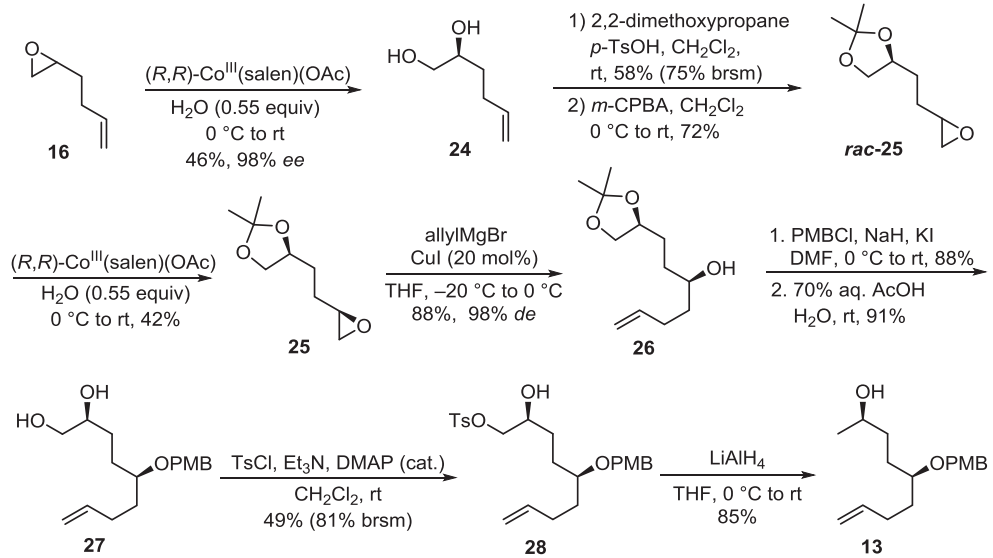


Scheme 4. Completion of the synthesis of dechlorogreensporone A (**5**).

CH_2Cl_2 at 0 °C furnished dechlorogreensporone A (**5**) in 79% yield. The ^1H and ^{13}C NMR spectroscopic and HRMS data of synthetic **5** were nearly identical to those reported for natural **5** (see Supplementary data). Additionally, the specific rotation of synthetic **5** ($[\alpha]_D^{26.4} = +66.02$ (*c* 0.10, MeOH)) was in excellent agreement with the reported value for natural **5** ($[\alpha]_D^{20} = +56.0$ (*c* 0.10, MeOH)) [8]. Our synthesis thus confirmed the absolute configuration of the natural product dechlorogreensporone A assigned by Oberlies and co-workers.

The synthesis of chiral alcohol **13** required for the synthesis of **6** is outlined in Scheme 5. Although we could in theory use the epoxide intermediate **20** for the synthesis of the desired chiral epoxide, the Jacobsen hydrolytic kinetic resolution of **20** was unsuccessful. Thus, we had to revise the synthesis of chiral alcohol **13** using a different starting material. Hydrolytic kinetic resolution of commercially available 1,2-epoxy-5-hexene (**16**) using Jacobsen's (*R*,*R*)-Co(III)(salen)(OAc) catalyst afforded (*S*)-diol **24** in 46% yield and 98% *ee* [10]. The enantiomeric excess of **24** was determined by chiral HPLC on the corresponding monobenzoate. Next, protection of diol **24** using 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid gave the corresponding acetonide in 75% yield based on the recovered diol **24** [15]. Subsequent epoxidation with *m*-CPBA furnished racemic epoxide ***rac*-25** in 72% yield [16]. Racemic epoxide **25** was then subjected to second hydrolytic kinetic resolution using (*R*,*R*)-Co(III)(salen)(OAc) as a catalyst to give (*R*)-epoxide **25** in 42% yield [16,17]. Regioselective ring-opening of epoxide **25** by allylmagnesium bromide in the presence of catalytic CuI yielded chiral alcohol **26** in 88% yield and 98% *de* (determined on the monobenzoate derivative by chiral HPLC). The absolute configuration of the newly generated alcohol stereogenic center was confirmed to be *S* based on Mosher ester analysis. We chose a PMB protecting group for this chiral alcohol for the purpose of



Scheme 5. Synthesis of chiral alcohol **13**.

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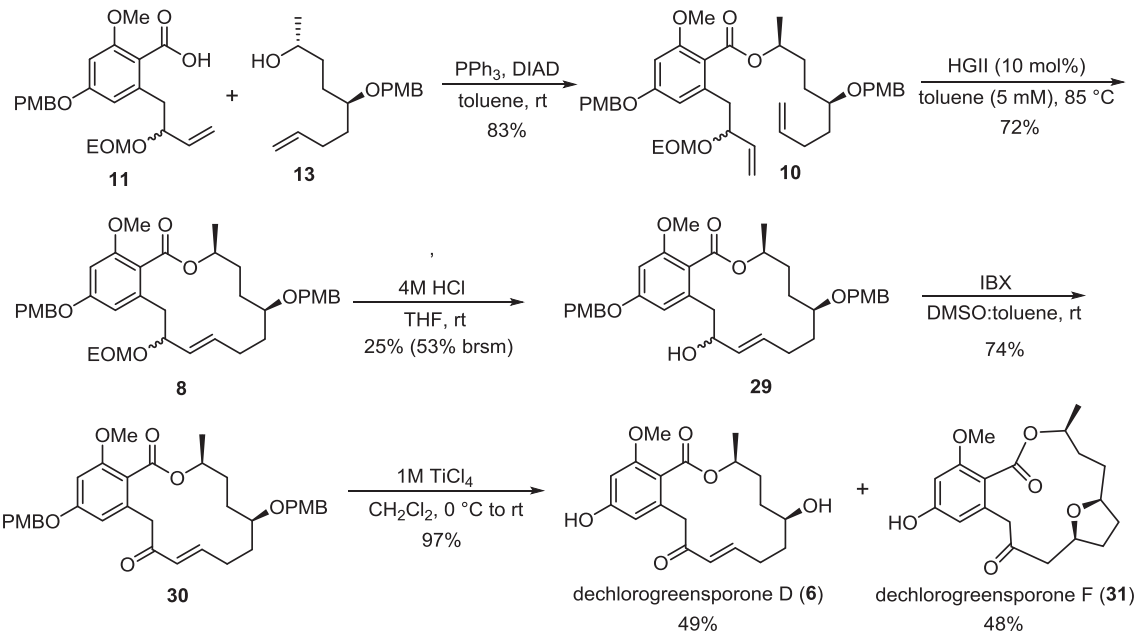
global deprotection in the final step. (*S*)-Alcohol **26** on protection using excess of both PMBCl and KI gave the corresponding PMB ether in 88% yield. The next task was to convert to the protected diol moiety to the chiral secondary alcohol which was accomplished in 3 steps. Removal of the acetonide protecting group with 70% AcOH smoothly gave diol **27** in 91% yield. Diol **27** on further monotosylation employing TsCl and Et_3N in the presence of catalytic DMAP in CH_2Cl_2, followed by reduction using $LiAlH_4$ in THF yielded the requisite (*R*)-alcohol **13** in 85% yield [17]. The absolute configuration of the alcohol stereogenic center was confirmed to be *R* via Mosher ester analysis.

With the requisite chiral alcohol **13** in hand, completion of the synthesis of dechlorogreensporone D (**6**) was achieved via the same synthetic approach as that of **5** (Scheme 6). Mitsunobu coupling of benzoic acid **11** and (*R*)-alcohol **13** under the same conditions previously described smoothly gave ester diene **10** in 83% yield. Ring-closing metathesis of diene **10** using second-generation Hoveyda-Grubbs catalyst (10 mol%) in toluene (5 mM) at 85 °C furnished macrocyclic product **8** in 72% yield as an inseparable mixture of diastereomers. The slightly higher yield of the RCM of **10** compared to **9** was ascribed to the better compatibility of the PMB protecting group under these RCM conditions. Similar to previous observation, the geometry of the newly formed C8–C9 olefin could be determined at a later stage of the synthesis. We proceeded to remove the EOM protecting group using 4 M HCl solution in THF at room temperature for 4 h to give **29** in 53% yield based on recovered starting EOM ether. Careful monitoring must be done in this step to prevent overdeprotection of the PMB groups. At this stage, the *trans* geometry of the double bond of **29** was confirmed based on the coupling constant (15.3 Hz) between H-8 and H-9. Oxidation of allylic alcohol **29** was achieved using excess 2-iodoxybenzoic acid (IBX) in a mixture of toluene and DMSO to afford macrocyclic enone **30** in 74% yield. Finally, both PMB protecting groups of **30** were removed using 6 equivalents of 1 M $TiCl_4$ in CH_2Cl_2 at 0 °C to deliver the requisite dechlorogreensporone D (**6**) in 49% yield along with unexpected analogue dechlorogreensporone F (**31**) in 48% yield. Byproduct **31** was a proposed artifact from a facile intramolecular cycloetherification of the parent **6** during the purification process by the Oberlies group. The spectroscopic and analytical properties of **6** and **31** (^1H and ^{13}C NMR, and HRMS) were identical to those of reported for the natural products **6** and **31** (see Supplementary data). The specific rotation of synthetic **6** was observed as $[\alpha]_D^{26.8} = +64.60$ (*c* 0.27, MeOH), which was in accordance with that of natural **6** ($[\alpha]_D^{20} = +116.0$ (*c* 0.27, MeOH)), yet in a lower magnitude [8]. In addition, the specific rotation of synthetic **31** was obtained as $[\alpha]_D^{27.3} = -38.48$ (*c* 0.11, MeOH), which was nearly identical to the reported value for natural **31** ($[\alpha]_D^{20} = -31.0$ (*c* 0.11, MeOH)) [8].

Synthetic compounds **5** and **6** were assessed for their cytotoxic activity by MTT assay against seven human cancer cell lines including two breast adenocarcinoma (MDA-MB-231 and MCF-7), one colorectal carcinoma (HCT116), one hepatoma (HepG2) and three cervical carcinoma (C33A, HeLa and SiHa) cells as well as one monkey kidney non-cancerous (Vero) cell line (Table 1) [18]. It was observed that both compounds could inhibit the proliferation of all cancer cell lines with the IC_{50} ranges of 6.94–17.25 μM for compound **5** and 6.66–11.84 μM for compound **6**, although in a significantly lower extent compared to a standard drug doxorubicin. Interestingly, however, both **5** and **6** showed more potent cytotoxic activity than a standard drug cisplatin against five cancer cell lines (MDA-MB-231, MCF-7, HCT116, HepG2 and SiHa) tested. Our results also revealed that dechlorogreensporone D (**6**) showed higher antiproliferative effect against most cancer cell lines tested than the

Table 1
Cytotoxic activity of synthetic **5** and **6** against seven cancer cell lines and Vero cells.

cell lines	cytotoxicity, IC_{50} (μM)			
	5	**6**	cisplatin	doxorubicin
MDA-MB-231	9.28 ± 0.13	6.97 ± 1.73	25.25	0.51
MCF-7	17.25 ± 0.71	11.84 ± 0.05	35.5	0.29
HCT116	7.53 ± 0.13	6.97 ± 0.05	35	0.81
HepG2	13.81 ± 0.27	7.88 ± 0.88	26	0.65
C33A	10.06 ± 0.53	10.41 ± 0.13	4.72	0.19
HeLa	15.5 ± 0	7.88 ± 1.06	8.98	0.16
SiHa	6.94 ± 1.06	6.66 ± 1.02	12.18	0.18
Vero	46.00 ± 3.18	10.13 ± 0.88	17.75	>1



Scheme 6. Completion of the synthesis of dechlorogreensporone D (**6**).

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ketone analogue **5**. This observation was consistent with the report by the Oberlies group [8]. Nevertheless, **5** was approximately 5-fold less cytotoxic to Vero cells compared to **6**.

3. Conclusion

In conclusion, the first and convergent total syntheses of dechlorogreensporones A (**5**) and D (**6**) have been accomplished via a longest linear sequence of 17 steps in 2.8% and 5.4% overall yields, respectively, from known phenol **14** and commercially available *R*-(+)-propylene oxide and 1,2-epoxy-5-hexene. Our approach exploited key Mitsunobu esterification and ring-closing metathesis to assemble the macrocycles and construct the (*E*)-olefin. Jacobsen hydrolytic kinetic resolution was also utilized to install the C-2 and C-5 stereogenic centers. Our syntheses verified the absolute stereochemistry of the natural products proposed by the Oberlies group. Synthetic compounds **5** and **6** were found to display significant cytotoxic activity against seven human cancer cell lines with the IC_{50} ranges of 6.66–17.25 μM. In addition, dechlorogreensporone D (**6**) showed more potent antiproliferative activity compared to dechlorogreensprone A (**5**), although **5** was approximately 5-fold less cytotoxic to Vero cells compared to **6**.

4. Experimental

4.1. General

All reactions were performed under argon or nitrogen atmosphere in oven- or flamed-dried glassware unless otherwise noted. Solvents were used as received from suppliers or distilled prior to use using standard procedures. All other reagents were obtained from commercial sources and used without further purification. Column chromatography was performed on SiliaFlash® G60 Silica (60–200 μm, Silicycle) or Silica gel 60 (0.063–0.200 mm, Merck). Thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} (Merck). ^{1}H, ^{13}C and 2D NMR spectroscopic data were recorded on a 300 MHz Bruker FTNMR UltraShield spectrometer. ^{1}H NMR spectra are reported in ppm on the δ scale and referenced to the internal tetramethylsilane. The data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, br = broad, app = apparent), coupling constant(s) in hertz (Hz), and integration. Infrared (IR) spectra were recorded on a Perkin Elmer 783 FTS165 FT-IR spectrometer. High-resolution mass spectra were obtained on a liquid chromatograph-mass spectrometer (2690, LCT, Waters, Micromass) and on a SpiralTOF™ MALDI TOF Mass Spectrometer Revolutionary (Scientific and Technological Research Equipment Centre; STREC, Chulalongkorn University). The optical rotations were recorded on a JASCO P-2000 polarimeter. Melting points were measured using an Electrothermal IA9300 melting point apparatus and are uncorrected. Enantiopurity was determined using HPLC on an Agilent series 1200 equipped with a diode array UV detector using either CHIRALCEL® OD-H column (15 cm) or CHIRALPAK® AS-H column (15 cm) and a guard column (1 cm).

4.2. Synthesis of diene RCM precursor **9**

To a solution of benzoic acid **11** (245.3 mg, 0.59 mmol, 1.0 equiv) and (*R*)-alcohol **12** (122.3 mg, 0.57 mmol) in 5.9 mL of toluene at room temperature were added PPh_3 (314.9 mg, 1.20 mmol, 2.0 equiv), followed by diisopropyl azodicarboxylate (40% in toluene, 0.58 mL, 1.18 mmol, 2.0 equiv). The resultant yellow mixture was stirred at rt overnight before being concentrated *in vacuo*. Purification of the crude residue by column chromatography (5–10% EtOAc/hexanes) yielded ester diene **9** as a light yellow oil (259.1 mg, 72%): R_f = 0.63 (40% EtOAc/hexanes); $[\alpha]_D^{24.6}$ = +0.47 (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 6.49 (s, 1H), 6.40 (s, 1H), 5.81 (ddt, *J* = 17.1, 10.2, 6.6 Hz, 1H), 5.70 (ddd, *J* = 17.1, 9.9, 7.2 Hz, 1H), 5.21–5.12 (m, 3H), 5.04–4.93 (m, 4H), 4.69 (s, 2H), 4.61 (d, *J* = 6.6 Hz, 1H), 4.49 (d, *J* = 6.6 Hz, 1H), 4.29–4.23 (m, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 3.65–3.58 (m, 3H), 3.32 (qd, *J* = 6.9, 2.1 Hz, 2H), 2.95–2.73 (m, 2H), 2.16–2.07 (m, 2H), 1.70–1.57 (m, 6H), 1.36–1.30 (m, 3H), 1.12 (td, *J* = 6.9, 2.1 Hz, 3H), 1.05 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 160.0, 159.5, 157.9, 138.5, 138.3, 138.2, 137.8, 129.2, 128.6, 117.9, 117.2, 117.1, 114.6, 114.0, 108.2, 97.8, 93.9, 92.3, 77.4, 77.3, 76.7, 76.5, 72.0, 71.7, 69.9, 63.3, 63.0, 55.7, 55.3, 39.7, 33.7, 33.6, 31.8, 31.5, 30.2, 30.1, 29.9, 29.8, 29.5, 29.4, 29.3, 21.9, 21.7, 20.2, 20.1, 15.1, 14.9; IR (thin film) 2977, 2935, 1717, 1517, 1250, 1159, 1107 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{35}H_{50}NaO_9$ $(M + Na)^+$ 637.3347, found 637.3341.

4.3. RCM of **9** to afford macrolactones **7**

A solution of diene **9** (131.5 mg, 0.214 mmol) in toluene (42 mL, 5 mM) was degassed with Ar for 10 min and second-generation Hoveyda Grubbs catalyst (13.4 mg, 0.021 mmol, 10 mol%) was added. The reaction mixture was heated at 85 °C for 3.5 h, which the starting diene was completely consumed as judged by TLC. Solvent was then removed under reduced pressure. Purification of the crude residue by column chromatography (10–15% EtOAc/hexanes) yielded a mixture of macrolactone products **7** as a light yellow oil (74.2 mg, 59%): R_f = 0.50 (40% EtOAc/hexanes); $[\alpha]_D^{25.3}$ = −5.26 (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.4 Hz, 2H), 6.91 (dd, *J* = 8.4, 2.7 Hz, 2H), 6.81–6.58 (m, 1H), 6.41 (s, 1H), 5.66–5.54 (m, 1H), 5.29–5.04 (m, 2H), 4.98 (s, 2H), 4.75–4.58 (m, 4H), 4.29–4.17 (m, 1H), 3.80 (s, 3H), 3.77 (d, *J* = 2.1 Hz, 3H), 3.72–3.45 (m, 5H), 3.24–2.75 (m, 2H), 2.30–1.42 (m, 8H), 1.34 (t, *J* = 6.6 Hz, 3H), 1.30–1.14 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 167.9, 167.8, 160.3, 159.9, 159.5, 158.3, 158.2, 157.8, 157.7, 138.6, 138.4, 138.1, 137.9, 137.4, 136.7, 134.8, 134.6, 133.8, 130.7, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.4, 118.1, 114.1, 114.0, 109.3, 108.4, 107.1, 107.0, 98.1, 98.0, 97.8, 94.2, 94.0, 93.8, 93.5, 93.0, 92.4, 91.9, 91.7, 91.4, 79.3, 78.2, 77.9, 76.0, 75.5, 74.3, 73.0, 72.3, 70.9, 69.9, 69.8, 63.4, 63.3, 63.2, 63.1, 62.8, 55.9, 55.8, 55.3, 39.2, 38.9, 38.2, 37.2, 33.2, 32.8, 32.6, 32.1, 32.0, 31.1, 30.9, 30.5, 29.1, 28.8, 28.3, 27.8, 23.7, 21.9, 21.7, 20.7, 20.2, 20.1, 15.2, 15.1, 14.8; IR (thin film) 2971, 2932, 1718, 1603, 1458, 1252, 1159 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{33}H_{46}NaO_9$ $(M + Na)^+$ 609.3034, found 609.3036.

4.4. Removal of EOM protecting groups of **7** to give diol **22**

To a solution of EOM ether **7** (49.5 mg, 0.084 mmol) in THF (4.2 mL) at rt was added 2.4 mL of 4 M HCl. The mixture was stirred at rt overnight, then which was quenched with saturated aqueous $NaHCO_3$ (5 mL) and diluted with EtOAc (5 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (4 × 5 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification of the crude residue by column chromatography (40% EtOAc/hexanes) yielded diol **22** as a light yellow oil (22.5 mg, 57%): R_f = 0.34 (80% EtOAc/hexanes); $[\alpha]_D^{25.1}$ = −24.43 (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.4 Hz, 2H), 6.91 (dd, *J* = 8.4, 1.8 Hz, 2H), 6.75–6.52 (m, 1H), 6.43–6.40 (m, 1H), 5.60–5.51 (m, 1H), 5.38–5.02 (m, 2H), 4.98–4.97 (m, 2H), 4.46–4.34 (m, 1H), 3.81–3.75 (m, 6H), 3.72–3.57 (m, 1H), 3.21–2.74 (m, 2H), 2.17–1.54 (m, 8H), 1.36 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 167.9, 160.8, 160.3, 159.7, 158.9, 138.5, 138.0, 133.4, 133.0, 132.6, 132.2, 132.0, 131.6, 129.7, 129.5, 128.8, 128.6, 127.5, 118.1, 117.8, 114.2, 109.2, 108.4, 107.2, 106.9, 98.5, 98.1, 73.9, 73.8, 73.6, 73.2, 73.1, 72.9,

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70.3, 70.1, 70.0, 69.8, 67.7, 67.5, 56.1, 55.5, 41.7, 41.4, 39.1, 38.5, 36.6, 36.4, 35.5, 35.3, 34.5, 32.2, 31.9, 30.7, 30.6, 29.4, 29.1, 28.5, 27.9, 21.1, 20.9, 20.4, 20.3; IR (thin film) 3447, 2933, 2858, 1700, 1603, 1251, 1161 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{27}H_{34}NaO_7$ $(M + Na)^+$ 493.2202, found 493.2211.

4.5. Oxidation of diol **22** to give diketone **23**

To a solution of macrolactone diol **22** (112.2 mg, 0.24 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added Dess-Martin periodinane (808.8 mg, 1.90 mmol, 8.0 equiv). The reaction mixture was stirred from 0 °C to room temperature for 4 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (15 mL) and diluted with CH_2Cl_2 (10 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification of the crude residue by column chromatography (20–40% EtOAc/hexanes) provided diketone **23** as a light yellow oil (66.2 mg, 62%): $R_f = 0.21$ (40% EtOAc/hexanes); $[\alpha]_D^{25.3} = +2.80$ (*c* 0.50, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, $J = 8.7$ Hz, 2H), 6.92 (d, $J = 8.7$ Hz, 2H), 6.78 (dt, $J = 15.6$, 6.3 Hz, 1H), 6.55 (d, $J = 1.8$ Hz, 1H), 6.43 (d, $J = 1.8$ Hz, 1H), 6.05 (d, $J = 15.6$ Hz, 1H), 5.18 (m, 1H), 4.98 (d, $J = 2.4$ Hz, 1H), 4.32 (d, $J = 14.1$ Hz, 1H), 3.82 (s, 3H), 3.75 (s, 3H), 3.36 (d, $J = 14.1$ Hz, 1H), 2.73–2.38 (m, 6H), 2.07–1.97 (m, 1H), 1.82–1.68 (m, 1H), 1.37 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.5, 196.7, 167.9, 160.9, 159.7, 159.1, 146.1, 135.2, 130.6, 129.4, 128.4, 116.8, 114.1, 107.9, 99.0, 71.1, 70.0, 56.0, 55.3, 44.2, 40.5, 39.1, 28.6, 28.3, 20.3; IR (thin film) 3011, 2933, 2853, 1701, 1605, 1252, 1161 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{27}H_{30}NaO_7$ $(M + Na)^+$ 489.1884, found 489.1884.

4.6. Deprotection of PMB group of **23** to furnish dechlorogreensporone A (**5**)

To a solution of macrolactone **23** (66.2 mg, 0.14 mmol) in 15 mL of CH_2Cl_2 at 0 °C was added $TiCl_4$ (1.0 M solution in CH_2Cl_2, 450 μL, 0.140 mmol, 3.2 equiv). The brick orange cloudy mixture was stirred from 0 °C to room temperature for 30 min, which was then quenched with saturated aqueous $NaHCO_3$ (20 mL) and the orange color dissipated. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by column chromatography (30–40% EtOAc/hexanes) to give dechlorogreensporone A (**5**) as a light yellow solid (38.4 mg, 79%): $R_f = 0.37$ (60% EtOAc/hexanes); mp 142.9–146.4 °C; $[\alpha]_D^{26.4} = +66.02$ (*c* 0.10, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 6.82–6.75 (m, 1H), 6.43 (d, $J = 2.1$ Hz, 1H), 6.30 (d, $J = 2.1$ Hz, 1H), 6.05 (d, $J = 15.6$ Hz, 1H), 5.21–5.16 (m, 1H), 4.29 (d, $J = 14.1$ Hz, 1H), 3.72 (s, 3H), 3.35 (d, $J = 14.1$ Hz, 1H), 2.73–2.40 (m, 6H), 2.19–1.96 (m, 1H), 1.84–1.72 (m, 2H), 1.37 (d, $J = 6.0$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.1, 198.6, 168.4, 159.6, 159.1, 147.5, 134.9, 130.6, 115.7, 109.8, 98.9, 71.4, 56.0, 44.1, 40.6, 39.4, 28.7, 28.4, 20.5; IR (thin film) 3367, 2930, 2855, 1699, 1610, 1458, 1273 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{19}H_{22}NaO_6$ $(M + Na)^+$ 369.1314, found 369.1322.

4.7. Synthesis of diene RCM precursor **10**

To a solution of benzoic acid **11** (272.5 mg, 0.65 mmol, 1.2 equiv) and (*R*)-alcohol **13** (148.1 mg, 0.53 mmol) in 6 mL of toluene at room temperature were added PPh_3 (351.9 mg, 1.34 mmol, 2.5 equiv), followed by diisopropyl azodicarboxylate (40% in toluene, 0.66 mL, 1.34 mmol, 2.5 equiv). The resultant yellow mixture was stirred at rt overnight before being concentrated *in vacuo*. Purification of the crude residue by column chromatography (5–10% EtOAc/hexanes) yielded ester diene **10** as a light yellow oil (297.8 mg, 83%): $R_f = 0.55$ (40% EtOAc/hexanes); $[\alpha]_D^{25.5} = -2.40$ (*c* 0.50, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, $J = 8.7$ Hz, 2H), 7.26 (d, $J = 8.7$ Hz, 2H), 6.92 (d, $J = 8.7$ Hz, 2H), 6.87 (d, $J = 8.7$ Hz, 2H), 6.49 (s, 1H), 6.39 (s, 1H), 5.81 (ddt, $J = 17.1$, 10.2, 6.3 Hz, 1H), 5.69 (ddd, $J = 17.4$, 9.9, 7.2 Hz, 1H), 5.20–5.18 (m, 3H), 5.03–4.92 (m, 4H), 4.61 (d, $J = 6.9$ Hz, 1H), 4.45 (d, $J = 6.9$ Hz, 1H), 4.43 (s, 2H), 4.29–4.22 (m, 1H) 3.82 (s, 3H), 3.80 (s, 3H), 3.74 (s, 3H), 3.46–3.42 (m, 1H), 3.33 (qd, $J = 7.2$, 3.0 Hz, 2H), 2.95–2.75 (m, 2H), 2.16–2.07 (m, 2H), 1.78–1.60 (m, 6H), 1.34–1.31 (m, 3H), 1.05 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 160.0, 159.6, 159.1, 158.0, 138.7, 138.3, 138.2, 137.8, 131.1, 129.3, 129.2, 128.7, 118.0, 117.0, 114.5, 114.1, 113.8, 108.4, 97.9, 92.4, 77.5, 77.4, 77.3, 71.7, 70.4, 69.9, 63.0, 55.8, 55.3, 39.7, 33.2, 31.5, 29.6, 29.4, 20.2, 20.1, 14.9; IR (thin film) 2933, 2862, 1716, 1516, 1250, 1159, 1034 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{40}H_{52}NaO_9$ $(M + Na)^+$ 699.3509, found 699.3533.

4.8. RCM of **10** to afford macrolactones **8**

To a solution of diene **10** (41.7 mg, 0.061 mmol) in toluene (12.3 mL, 5 mM) was degassed with Ar for 10 min and second-generation Hoveyda Grubbs catalyst (3.9 mg, 0.006 mmol, 10 mol %) was added. The reaction mixture was heated at 85 °C for 4 h, at which the starting diene was completely consumed as judged by TLC. Solvent was then removed under reduced pressure. Purification of the crude residue by column chromatography (10% EtOAc/hexanes) yielded a mixture of macrolactone products **8** as a light yellow oil (28.8 mg, 72%): $R_f = 0.48$ (40% EtOAc/hexanes); $[\alpha]_D^{24.7} = -2.53$ (*c* 0.50, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, $J = 8.4$ Hz, 4H), 7.26–7.21 (m, 4H), 6.92–6.86 (m, 8H), 6.79 (s, 1H), 6.59 (s, 1H), 6.39 (s, 2H), 5.66–5.46 (m, 2H), 5.36–5.13 (m, 2H), 5.13–4.90 (m, 6H), 4.76–4.71 (m, 2H), 4.68–4.60 (m, 2H), 4.53–4.46 (m, 2H), 4.38–4.25 (m, 4H), 3.79 (s, 6H), 3.74–3.62 (m, 8H), 3.62–3.47 (m, 2H), 3.46–3.21 (m, 2H), 3.21–3.07 (m, 1H), 3.01–2.95 (m, 2H), 2.85–2.64 (m, 1H), 2.51–1.41 (m, 16H), 1.40–1.29 (m, 6H), 1.25–1.18 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 167.9, 160.4, 159.9, 159.6, 159.2, 158.3, 138.6, 138.1, 134.8, 131.0, 129.3, 129.2, 128.7, 128.5, 118.2, 118.0, 114.0, 113.9, 109.2, 107.1, 98.0, 97.8, 93.1, 91.5, 77.4, 76.1, 76.0, 74.8, 70.8, 70.5, 70.2, 70.0, 63.4, 63.2, 55.9, 55.3, 39.9, 37.3, 31.5, 31.4, 30.7, 30.5, 28.8, 28.3, 28.2, 21.9, 21.7, 20.3, 20.2, 15.2; IR (thin film) 2933, 2875, 1716, 1603, 1516, 1250, 1160 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{38}H_{48}NaO_9$ $(M + Na)^+$ 671.3191, found 671.3157.

4.9. Removal of EOM protecting group of **8** to give allylic alcohol **29**

To a solution of EOM ether **8** (148.8 mg, 0.23 mmol) in THF (11 mL) at rt was added 6.5 mL of 4 M HCl. The mixture was stirred at rt for 4 h, which was then quenched with saturated aqueous $NaHCO_3$ (15 mL) and diluted with EtOAc (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification of the crude residue by column chromatography (10–20% EtOAc/hexanes) yielded the desired allylic alcohol **29** as a light yellow oil (34.2 mg, 25%, 53% based on 78.5 mg of recovered **8**): $R_f = 0.27$ (40% EtOAc/hexanes); $[\alpha]_D^{25.1} = -17.33$ (*c* 0.50, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, $J = 8.4$ Hz, 4H), 7.27–7.21 (m, 4H), 6.93–6.87 (m, 8H), 6.72 (s, 1H), 6.53 (s, 1H), 6.42–6.40 (m, 2H), 5.59–5.50 (m, 2H), 5.36 (dd, $J = 15.3$, 3.6 Hz, 1H), 5.26 (dd, $J = 15.3$, 8.4 Hz, 1H), 4.99 (s, 2H), 4.97 (s, 2H), 4.55–4.48 (m, 2H), 4.36–4.29 (m, 4H), 3.81 (s, 12H), 3.75 (s, 3H), 3.74 (s, 3H), 3.38–3.33 (m, 2H), 3.15 (dd, $J = 14.4$, 3.6 Hz, 1H), 3.03–2.94 (m, 2H), 2.81 (dd, $J = 12.9$, 9.6 Hz, 1H), 2.14–1.71 (m, 16H), 1.42–1.26 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 167.9, 160.5, 160.1, 159.6, 159.2, 158.7, 158.6,

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138.2, 137.7, 132.9, 132.0, 131.6, 131.0, 129.4, 128.6, 128.5, 127.9, 118.1, 117.9, 114.1, 114.0, 113.9, 108.7, 106.9, 98.2, 98.0, 75.3, 75.1, 73.3, 73.0, 70.5, 70.3, 69.9, 69.8, 55.9, 55.3, 55.3, 41.4, 38.6, 31.5, 30.7, 30.6, 28.5, 28.4, 28.0, 27.8, 20.2; IR (thin film) 3447, 2933, 2860, 1701, 1605, 1249, 1161 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{35}H_{42}NaO_8$ $(M + Na)^+$ 613.2772, found 613.2753.

4.10. Oxidation of allylic alcohol **29** to give ketone **30**

To a solution of **29** (75.0 mg, 0.127 mmol) in 3 mL of DMSO:toluene (1:1) was added IBX (178.1 mg, 0.636 mmol, 5.0 equiv). The reaction mixture was stirred at room temperature for 2 h before being added IBX (106.9 mg, 0.382 mmol, 3.0 equiv), and stirred at room temperature for 1 h. The reaction mixture was quenched with H_2O (3 mL), and diluted with EtOAc (3 mL). The mixture was filtered through a pad of Celite and washed with EtOAc. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification of the crude residue by column chromatography (20% EtOAc/hexanes) provided ketone **30** as a white solid (55.4 mg, 74%): $R_f = 0.48$ (40% EtOAc/hexanes); mp 141.3–144.5 °C; $[\alpha]_D^{24.5} = +10.6$ (*c* 0.50, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, $J = 8.4$ Hz, 2H), 7.23 (d, $J = 8.4$ Hz, 2H), 6.91 (d, $J = 8.4$ Hz, 2H), 6.89 (d, $J = 8.4$ Hz, 2H), 6.72 (dt, $J = 15.6$, 7.8 Hz, 1H), 6.47 (s, 1H), 6.45 (s, 1H), 6.07 (d, $J = 15.6$ Hz, 1H), 4.96 (s, 2H), 4.90–4.85 (m, 1H), 4.51 (d, $J = 11.1$ Hz, 1H), 4.32 (d, $J = 11.1$ Hz, 1H), 4.27 (d, $J = 15.6$ Hz, 1H), 3.81 (s, 6H), 3.76 (s, 3H), 3.45 (d, $J = 15.6$ Hz, 1H), 3.37–3.31 (m, 1H), 2.29–2.22 (m, 2H), 1.82–1.35 (m, 6H), 1.29 (d, $J = 6.0$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 167.7, 160.9, 159.6, 159.4, 159.3, 147.6, 135.8, 130.6, 129.5, 129.4, 129.2, 128.3, 116.8, 114.1, 113.9, 107.8, 98.9, 75.0, 70.5, 70.0, 56.0, 55.3, 45.7, 30.8, 30.4, 28.7, 27.9, 20.2; IR (thin film) 2926, 2856, 1700, 1521, 1251, 1162, 1034 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{35}H_{40}NaO_8$ $(M + Na)^+$ 611.2621, found 611.2640.

4.11. Deprotection of PMB groups of **30** to furnish dechlorogreensporone D (**6**)

A solution of macrolactone **30** (55.4 mg, 0.094 mmol) in 9.5 mL of CH_2Cl_2 at 0 °C was added 1.0 M $TiCl_4$ (565 μL, 0.565 mmol, 6.0 equiv). The brick orange cloudy mixture was stirred from 0 °C to room temperature for 30 min, which was then quenched with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by column chromatography (20–30% EtOAc/hexanes) to yield dechlorogreensporone D (**6**) as a light yellow solid (16.1 mg, 49%) and dechlorogreensporone F (**31**) as a light yellow oil (15.6 mg, 48%).

*Dechlorogreensporone D (**6**).* 16.1 mg, 49%; $R_f = 0.23$ (60% EtOAc/hexanes); mp 182.7–185.8 °C; $[\alpha]_D^{26.8} = +64.60$ (*c* 0.27, MeOH); ^1H NMR (300 MHz, DMSO-d_6) δ 10.0 (br s, 1H), 6.68 (dt, $J = 15.9$, 7.5 Hz, 1H), 6.39 (s, 1H), 6.29 (s, 1H), 5.98 (d, $J = 15.9$ Hz, 1H), 4.94–4.87 (m, 1H), 4.54 (br s, 1H), 4.06 (d, $J = 15.9$ Hz, 1H), 3.72–3.62 (m, 4H), 3.42–3.36 (m, 1H), 2.20–2.08 (m, 2H), 1.79–1.40 (m, 5H), 1.23 (d, $J = 5.7$ Hz, 3H), 1.19–1.10 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 196.0, 167.3, 159.8, 159.2, 148.3, 135.6, 128.5, 114.2, 109.7, 98.5, 69.4, 66.2, 55.9, 44.6, 34.6, 30.3, 29.2, 28.3, 20.2; IR (thin film) 3446, 2926, 2857, 1695, 1685, 1523, 1089 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{19}H_{24}NaO_6$ $(M + Na)^+$ 371.1471, found 371.1478.

*Dechlorogreensporone F (**31**).* 15.6 mg, 48%; $R_f = 0.33$ (60% EtOAc/hexanes); $[\alpha]_D^{27.3} = -38.48$ (*c* 0.11, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 6.32 (d, $J = 1.8$ Hz, 1H), 6.24 (d, $J = 1.8$ Hz, 1H), 5.28–5.25 (m, 1H), 4.22–4.14 (m, 1H), 4.01 (d, $J = 17.1$ Hz, 1H), 3.92 (d, $J = 17.1$ Hz, 1H), 3.89–3.79 (m, 1H), 3.73 (s, 3H), 2.65 (dd, $J = 13.8$, 3.9 Hz, 1H), 2.56 (dd, $J = 13.8$, 8.1 Hz, 1H), 2.02–1.43 (m, 8H), 1.31 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.6, 167.9, 159.0, 158.4, 133.8, 116.5, 109.4, 98.5, 79.3, 76.0, 72.6, 55.8, 49.1, 47.8, 33.5, 32.9, 31.2, 30.4, 20.8; IR (thin film) 3366, 2927, 2855, 1716, 1608, 1458, 1269 cm^{-1}; HRMS (MALDI-TOF) *m/z* calcd for $C_{19}H_{24}NaO_6$ $(M + Na)^+$ 371.1471, found 371.1464.

4.12. Cytotoxicity assay

Cytotoxic activity of synthetic **5** and **6** were evaluated against seven human cancer cell lines including two breast adenocarcinoma (MDA-MB-231 and MCF-7), one colorectal carcinoma (HCT116), one hepatoma (HepG2) and three cervical carcinoma (C33A, HeLa and SiHa) cells as well as one monkey kidney non-cancerous (Vero) cell line by MTT assay using the general procedure previously described [18]. Cancer cells were exposed to various concentrations of compounds **5** and **6** (0–25 μM; 0.2% (v/v) DMSO). Vero cells were exposed to 0–50 μM of **5** and **6**. Each experiment was performed in triplicate and was repeated three times. Data was expressed as IC_{50}values (the concentration needed for 50% cell growth inhibition) relative to the untreated cells (0.2% (v/v) DMSO) (means ± SD). Cisplatin (0–50 μM) and doxorubicin (0–1 μM) (Pfizer, Australia) were used as positive controls.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2018.07.025.

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