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Total synthesis of Palmarumycin BGs, C₁ and Guignardin E†

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The first total synthesis of Palmarumycin BG1–3, BG5–6, C₁ and Guignardin E (1–7) were achieved by the same intermediate Palmarumycin C₂ through a *N*-benzyl cinchoninium chloride-catalyzed epoxidation, an organoselenium-mediated reduction, and a cerium(III) chloride hydrate-promoted regioselective ring-opening and elimination of cyclic α,β -epoxy ketone as the key steps via 6–7 step routes using 1,8-dihydroxynaphthalene (DHN) and 5-methoxytetralone as the starting materials in overall yields of 1.0–17.4%, respectively. Their structures and absolute configurations were characterized and determined by ¹H, ¹³C NMR, IR, HR-ESI-MS and X-ray diffraction data. These compounds displayed significant inhibition activities against HCT116, U87-MG, HepG2, BGC823 and PC9 cell lines.

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Introduction

The members of the spirobisnaphthalene family showed broad bioactivities, such as antifungal, antimicrobial, antitumor, anticancer, antiparasitic, anti-inflammatory, and cytotoxic activities.^{1–4} Palmarumycin BG1–3, and BG5–6 (Fig. 1) were previously isolated from the leaves and stems of the *Bruguiera gymnorrhiza* plant, which were collected from the Zhanjiang mangrove national nature protection area.⁵ Guignardins A–F and Palmarumycin C₁ were isolated from the endophytic fungus *Guignardia* sp. KcF8, and the mangrove unidentified fungus BCC 25903, respectively.^{6,7} Among these compounds, Palmarumycin BG5 showed excellent cytotoxicities against human breast carcinoma MCF-7 with IC₅₀ 7.6 μ M and promyelocytic leukemia HL60 with IC₅₀ 1.9 μ M, Guignardins E, F and Palmarumycin C₁ exhibited significant cytotoxicities against 10 human tumor cell lines, such as MCF-7 with LD₅₀ 8.79, 6.48 and 3.08 μ M, HL60 with LD₅₀ 2.94, 3.06 and 2.90 μ M, and HeLa with LD₅₀ 1.32, 0.38 and 1.24 μ M *et al.*⁶ Many reports related to the total synthesis, structure modification, and biological activity evaluation of the spirobisnaphthalene natural products have appeared in recent years.^{8–20} The synthesis of Palmarumycin CP₁₇ and its analogues were completed in our laboratory, and the bioassay results showed that they have antifungal activity against several phytopathogens.²¹ Because of the interesting larvicidal activity of Palmarumycin B₆ against *Aedes albopictus*,

its limited access in the fermentation extract of the endophytic fungus *Berkleasmium* sp., and the importance of the halogen atom in the A-ring,^{22,23} the total synthesis and structure revision of Palmarumycin B₆ were achieved, and found that the location of the chlorine atom plays a crucial role for the larvicidal activity against *A. albopictus* in the previous report.²⁴ The antitumor activities of Palmarumycin BG5, Guignardin E and Palmarumycin C₁ aroused our enthusiasm to gain insights into the structure–activity relationships of these spirobisnaphthalene compounds and explore their mode of action. As such, the first total synthesis of Palmarumycin BGs, Guignardin E and Palmarumycin C₁ (1–7) were carried out, and the results would be presented in this paper.

Results and discussion

The retro-synthetic analysis of Palmarumycin BGs and Guignardin E (1–6) is shown in Scheme 1. Palmarumycin BG2, BG3, BG5 and BG6 can be obtained through reduction of Palmarumycin BG1 and Guignardin E. Palmarumycin BG1 and Guignardin E can be synthesized by the selective reduction and ring-opening of Palmarumycin C₂, which was easily prepared by the stereoselectivity epoxidation of Palmarumycin CP₁. Palmarumycin CP₁ could be obtained from a ketalization of 5-methoxytetralone with 1,8-dihydroxynaphthalene (DHN) in a similar process, such as that used in the synthesis of Palmarumycins CP₁₇ and B₆.^{21,24}

The synthesis of Palmarumycin BG1–6 and Guignardin E (1–6) started with 5-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and 1,8-dihydroxynaphthalene (DHN), as shown in Scheme 2. Compound 8 was obtained *via* the direct ketalization of 5-methoxytetralone with 1,8-dihydroxynaphthalene (DHN). Oxidation of compound 8 with pyridinium dichromate (PDC)

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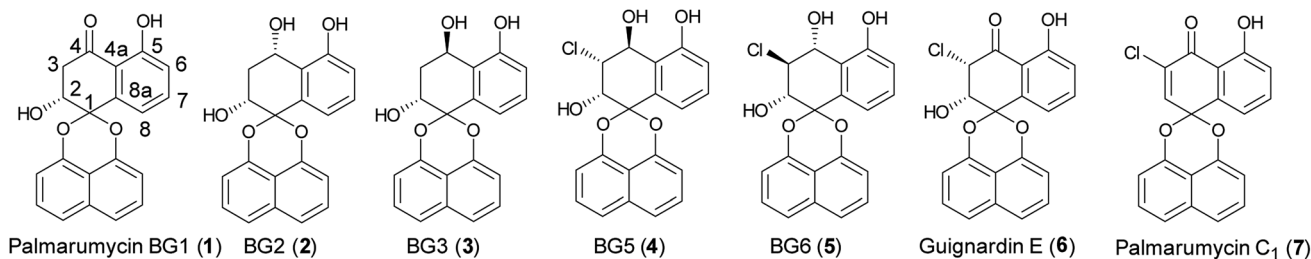
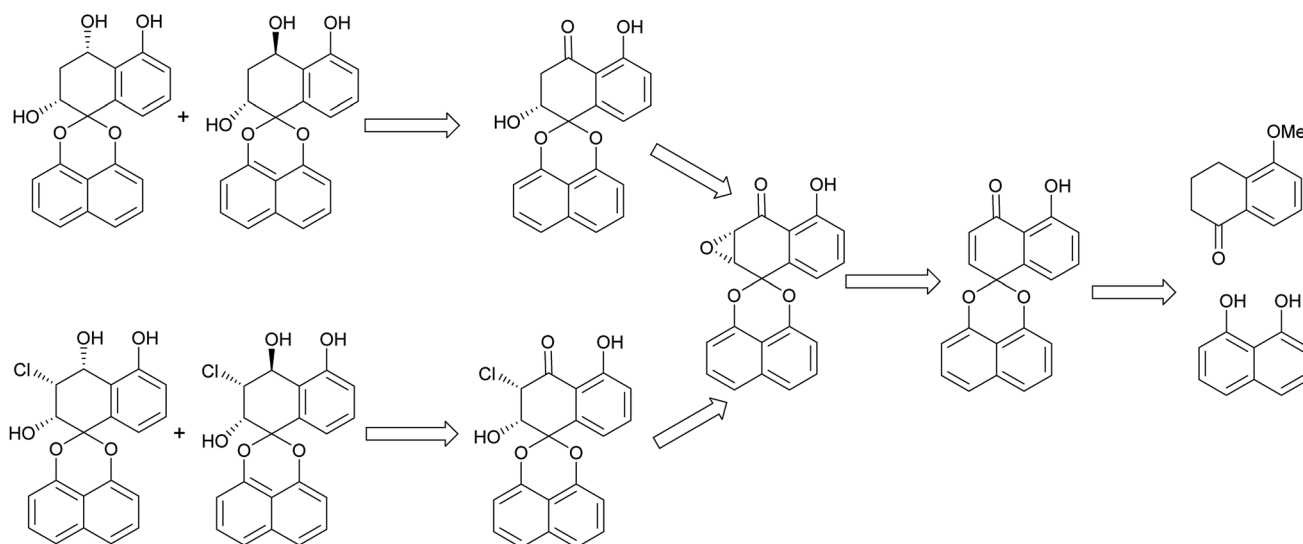


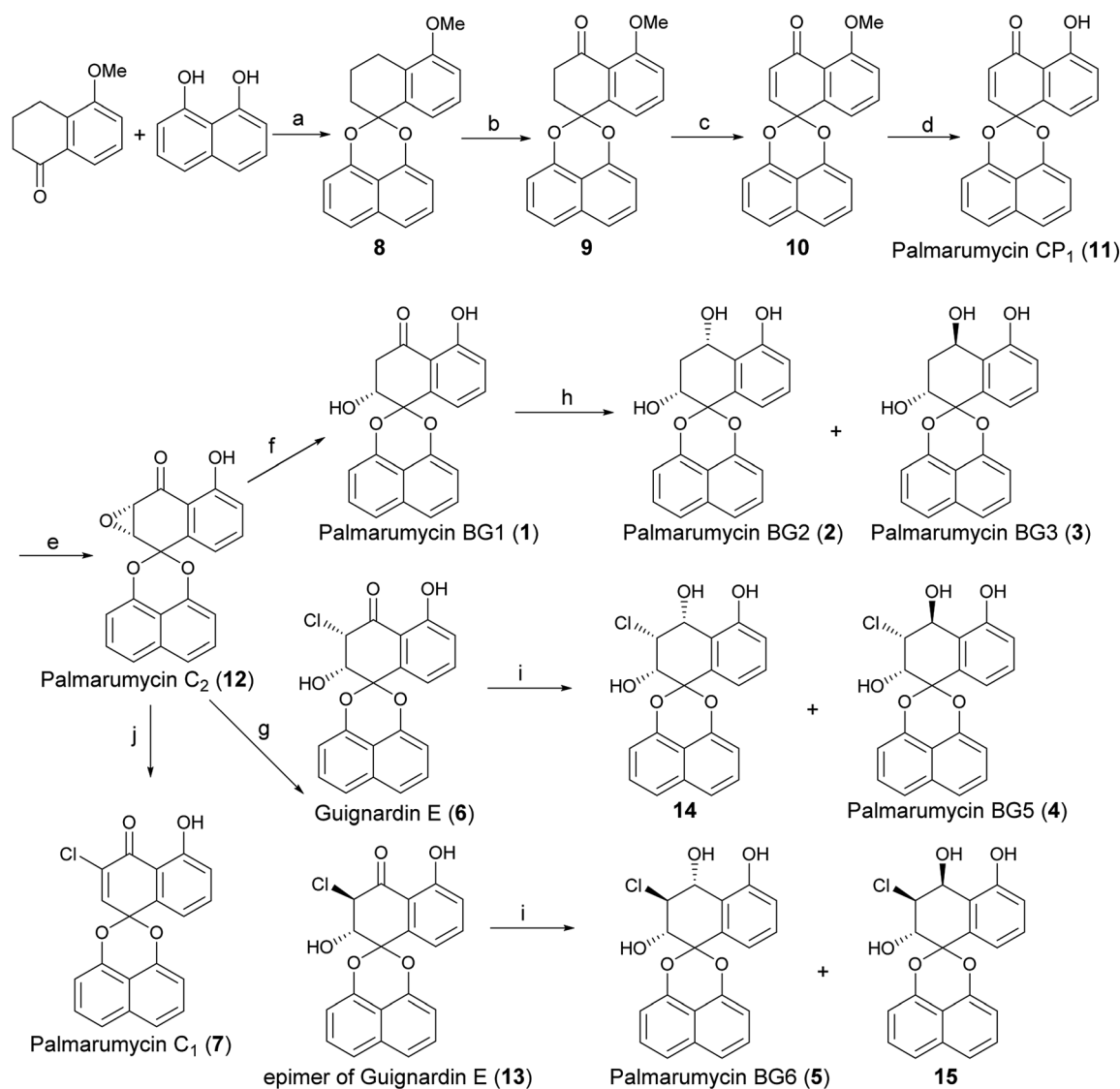
Fig. 1 Structures of Palmarumycin BG1–3, BG5–6, Guignardin E, and Palmarumycin C₁ (1–7).

and *t*-BuOOH in benzene gave compound **9**, which was further oxidized with DDQ to produce **10**. Then, **10** was demethylated with B-bromocatecholborane to afford the target compound Palmarumycin CP₁ (**11**) following the literature procedures.^{24–27} Next, the stereoselective epoxidation with *t*-BuOOH of Palmarumycin CP₁ catalyzed with *N*-benzyleinchoninium chloride at 25 °C yielded Palmarumycin C₂ (**12**) in 90.7% ee value. The ee value was improved to be 97.9% at 0 °C.²⁸ The organoselenium-mediated reduction of Palmarumycin C₂ afforded Palmarumycin BG1 (**1**) as the sole product in 92% yield, and the configuration of C-2 did not change.^{29–31} The NaBH₄ reduction of **1** resulted in a mixture of Palmarumycin BG2 (**2**) and BG3 (**3**), which were isolated in silica gel to give Palmarumycin BG2 (**2**) and BG3 (**3**) in 56% and 30% yields, respectively. Treatment of Palmarumycin C₂ (**12**) with the diluted hydrogen chloride at 25 °C afforded a mixture of Guignardin E (**6**) and its C-3 epimer (**13**) in 95% yield (dr value: 5.7 : 1).³² When this transformation was performed at 10 °C, the dr value was improved to 25 : 1. Recrystallization of this mixture yielded pure Guignardin E (**6**). Guignardin E as the unnatural product was mentioned in mixture form of Guignardin E and its epimer.^{32,33} but it was first isolated as the natural product from the fungus *Guignardia* sp. KcF8.⁶ The NaBH₄ reduction of Guignardin E (**6**) afforded Palmarumycin BG5 (**4**) and the C-4 epimer (**14**) of **4** in 22% and

78% yields, respectively. The NaBH₄ reduction of the mixture of Guignardin E (**6**) and its C-3 epimer (**13**) afforded Palmarumycin BG5 (**4**), BG6 (**5**) and the C-4 epimer (**14**) of **4** in 23%, 9% and 60% yields, respectively, but the C-4 epimer (**15**) of **5** was not obtained because of the limited quantity of **13** in the mixture and the hindrance effect in the NaBH₄ reduction. So we deduced rationally that Guignardin E (**6**) should be the biosynthetic precursor of Palmarumycin BG5 (**4**) and BG6 (**5**). Treatment of Palmarumycin C₂ (**11**) with cerium(III) chloride hydrate at reflux temperature afforded Palmarumycin C₁ (**7**) in 79% yield.³⁴ One possible mechanism is that cerium(III) chloride is hydrolyzed to produce hydrochloride *in situ*. Then, the regioselective epoxide ring-opening can occur between Palmarumycin C₂ and hydrochloride to give the mixture of chlorohydrins Guignardin E (**6**) and its C-3 epimer (**13**). A subsequent loss of water could then lead to obtain Palmarumycin C₁ at reflux temperature. This approach avoided the use of the strong acid, which removes the possibility of breaking the ketal bond.²⁰ When the mixture of **6** and **13** was treated with diluted hydrochloride in methanol or methanol/water at the reflux temperature, a complex mixture of products were obtained that could not be purified. Palmarumycin C₁, which was reported to exhibit antifungal activity against phytopathogen *Fusarium oxysporum* and cytotoxicities against 10 human tumor



Scheme 1 Retro-synthetic analysis of Palmarumycin BG1–6 and Guignardin E.



Scheme 2 Synthesis of Palmarumycin BG1–6, Guignardin E (1–6) and Palmarumycin C₁ (7). Reagents and conditions: (a) TsOH, toluene, reflux, 72 h, 57%; (b) PDC, Celite, *t*-BuOOH, benzene, rt, 24 h, 74%; (c) DDQ, benzene, reflux, 82%; (d) B-bromocatecholborane, DCM, 0 °C, 75%; (e) *N*-benzylcinchoninium chloride, *t*-BuOOH, NaOH (0.1 M), toluene/H₂O, 0 °C, 73%; (f) (PhSe)₂, NaBH₄, AcOH, EtOH, 0 °C, 92%; (g) HCl (1 M), THF/H₂O, 10 °C, 3 day, 96% for **6** + **13**, dr = 25 : 1; (h) NaBH₄, MeOH, rt, 56% for **2** and 30% for **3**, dr = 2 : 1; (i) NaBH₄, MeOH, rt, 23% for **4**, 9% for **5**, and 60% for **14**; (j) CeCl₃·7H₂O, MeOH/H₂O, reflux, 79%.

cell lines with LD₅₀ values in the range of 1.24–39.2 μM, was isolated from the fungus *Guignardia* sp. KcF8⁶, and *Coniothyrium* sp.³²

After finishing the synthesis of the desired compounds, all structures were characterized with the ¹H, ¹³C NMR, IR and HR-ESI-MS data. In order to confirm the absolute configuration, the X-ray diffraction analysis of **14** was performed using Cu Kα radiation and its structure was depicted in Fig. 2, which unambiguously showed that the absolute configuration of C-2, C-3 and C-4 were 2*S*, 3*R* and 4*R* in Flack parameter 0.006(16) (CCDC ID 1947664). Based on this result, the C-2 absolute configuration of Palmarumycin BG1 (**1**) was 2*R*, the configuration of C-2 and C-4 of Palmarumycin BG2 (**2**) were 2*R* and 4*S*, those of C-2 and C-4 of Palmarumycin BG3 (**3**) were 2*R* and 4*R*,

those of C-2, C-3 and C-4 of Palmarumycin BG5 (**4**) were 2*S*, 3*R* and 4*S*, those of C-2, C-3 and C-4 of Palmarumycin BG6 (**5**) were 2*S*, 3*S* and 4*R*, and those of C-2 and C-3 of Guignardin E (**6**) were 2*S* and 3*S*, all of these absolute configurations were consistent with those of the natural products.^{5,6} Because the organoselenium-mediated reduction of the α,β-epoxy ketone did not change the configuration of the β-carbon,^{29–31} so the absolute configurations of C-2 and C-3 of **12** would be 2*R* and 3*S*, which are in agreement with those of natural Palmarumycin C₂,^{32,33} and Guignardin F,⁶ implies that Palmarumycin C₂ and Guignardin F are the same compound.

The cytotoxic activities of **1–7** and **14** against the growth of tumor cell lines (HCT116, U87-MG, HepG2, BGC823 and PC9) were evaluated using a MTT assay²² and the results were shown



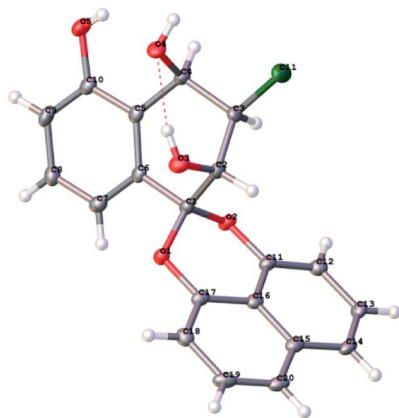


Fig. 2 ORTEP drawing of Palmarumycin BG5 C-4 epimer (**14**).

Table 1 Cytotoxic activities of compounds **1–7** and **14** (IC_{50} , μM)

Compounds	HCT116	U87-MG	HepG2	BGC823	PC9
1	9.14	19.40	20.36	19.15	14.87
2	30.08	>50	35.56	>50	30.50
3	29.12	26.10	28.81	24.55	22.10
4	6.71	9.62	4.21	6.25	5.58
5	>50	>50	>50	>50	>50
6	3.70	2.45	4.06	4.36	7.42
7	2.63	2.60	6.05	14.08	15.39
14	6.62	19.64	9.64	12.06	14.37
Taxol	0.000616	0.000245	0.00475	0.000642	0.000823

in Table 1. These results indicated that compounds **1–4**, **6–7** and **14** exhibited significant cytotoxicity against several tumor cell lines with an IC_{50} in the range of 2.45–30.50 μM , while compound **5** was inactive against above mentioned cancer cells ($IC_{50} > 50 \mu M$). When comparison the IC_{50} data of compounds **1–3** with compounds **4**, **6–7** and **14**, we found that the compounds bearing a chlorine atom at the C-3 position exhibited stronger cytotoxic activity than those compounds without the chlorine atom at C-3 position. In the other aspect, the reduction products of carbonyl group at C-4 such as **2** and **3**, **4**, **5** and **14** exhibited weaker cytotoxic activity than those retaining C-4 carbonyl compounds **1** and **6**. These results indicated that the chlorine atom at C-3 and the carbonyl at C-4 play a critical role for cytotoxicity.^{23,24}

Conclusions

In summary, Palmarumycin BG1–3, BG5–6, Guignardin E and Palmarumycin C₁ (**1–7**) were first synthesized in overall yields of 1.0–17.4% through a *N*-benzylcinchoninium chloride-catalyzed epoxidation, an organoselenium-mediated reduction of the α,β -epoxy ketone, and a cerium(III) chloride hydrate-promoted ring-opening and elimination as the key steps *via* **6–7** step routes using 1,8-dihydroxynaphthalene (DHN) and 5-methoxytetralone as the starting materials. Their structures and absolute configurations were characterized by 1H , ^{13}C NMR, IR, HR-ESI-MS data and X-ray crystallographic data (CCDC 1947664).

Compounds **1–4**, **6–7** and **14** displayed significant inhibition activity against HCT116, U87-MG, HepG2, BGC823 and PC9 cell lines.

Experimental procedures

General experimental procedures

Melting points (uncorrected) were measured on a WRX-4 microscopic melting point apparatus (Shanghai Yi Ce Instrument Factory). All 1H and ^{13}C NMR spectra were obtained on a Bruker DPX 300 spectrometer with $CDCl_3$ and CD_3OD as solvents and TMS as an internal standard. HR-ESI-MS spectra were analyzed on a Bruker Apex II mass spectrometer. IR spectra were recorded with a Shimadzu IRTracer 100 FT-IR spectrometer (KBr plate). Optical rotation values were measured with a JASCO P-2000 Polarimeter. The crystal structure was analyzed with a Thermo Fisher ESCALAB 250 four-circle X-ray diffractometer (Xcalibur, Eos, Gemini). Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Organic solutions were concentrated under reduced pressure using a rotary evaporator or oil pump. Flash column chromatography was performed using Qingdao Haiyang silica gel (200–300 mesh). HPLC analyses were performed on an Agilent 1100 instruments, UV detection was monitored at 254 and 220 nm, an IB N-5 column (5 μm , 4.6 \times 250 mm) was used as the chiral stationary phase, and hexane/*i*-PrOH (90 : 10) was used as the mobile phase at a flow rate of 1.0 mL min^{−1}.

Synthesis of intermediates **8–12**

The intermediates **8–11** were synthesized following the literature procedures, and their analytical data were consistent with the reported data.^{25–27}

Palmarumycin CP₁ (**11**, 120 mg, 0.38 mmol) was added into a toluene (10 mL) solution of *N*-benzylcinchoninium chloride (48 mg, 0.114 mmol, 0.3 eq.) in a 50 mL round-bottom flask. NaOH solution (0.1 M; 5.7 mL, 1.5 eq.) was added dropwise in the mixture, followed by the addition of *t*-BuOOH (0.524 mL, 7.2 M, 3.77 mmol) at 0 °C (the ice-water bath) and stirred for 6 h. After completion of the reaction, the solution was diluted with EtOAc (50 mL), washed with 0.2 M HCl solution (2 \times 20 mL), brine, and the organic phase was dried over anhydrous Na₂SO₄. The solvent was removed and the crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 20 : 1) to give Palmarumycin C₂ (**12**, 100 mg, 79%) as a yellow solid.²⁸ The ee value of the key intermediate **12** was analyzed by HPLC to be 97.9%. Compound **12**, a yellow solid, mp 218–220 °C (lit. 11 mp 219–221 °C; lit. 28 mp 225 °C; lit. 32 mp 228 °C); $[\alpha]_D^{20} = -335$ ($c = 0.66$, $CHCl_3$), (lit. 11 $[\alpha]_D^{20} = -340$, $c = 1.00$, $CHCl_3$; lit. 28 $[\alpha]_D^{20} = -300$, $c = 1.00$, CH_2Cl_2 ; lit. 32 $[\alpha]_D^{20} = -341$, $c = 1.00$, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$) δ : 11.37 (1H, s), 7.64 (1H, dd, $J = 8.4$, 8.0 Hz), 7.62–7.50 (3H, m), 7.45 (2H, dd, $J = 8.0$, 7.5 Hz), 7.19 (1H, d, $J = 7.5$ Hz), 7.14 (1H, d, $J = 8.4$ Hz), 6.92 (1H, d, $J = 7.5$ Hz), 4.09 (1H, d, $J = 4.0$ Hz), 3.68 (1H, d, $J = 4.0$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ : 196.65, 162.00, 147.07, 146.81, 137.77, 137.02, 134.31, 127.90, 127.76, 121.56,



121.46, 120.17, 119.18, 112.92, 112.41, 110.28, 109.45, 96.12, 53.40, 53.36; IR ν_{\max} 3426, 3051, 1651, 1612, 1454, 1412, 1381, 1269, 1238, 1177, 1115, 1065, 968, 876, 806, 752 cm^{-1} ; ESI-MS, m/z : 333 $[\text{M} + \text{H}]^+$. These data were identical with the published data.^{11,28,32}

Synthesis of (2*R*)-2,5-dihydroxy-2,3-dihydro-4*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxin]-4-one (Palmarumycin BG1, 1)

The reduction reagent ($\text{Na}[\text{PhSeB}(\text{OEt})_3]$) was prepared following the protocol in the literature.^{29,30} (PhSe)₂ (245 mg, 0.79 mmol) and EtOH (15 mL) were added into a 100 mL round-bottom flask at 0 °C, then NaBH_4 (60 mg, 1.58 mmol) was gradually added into the mixture. After vigorous evolution of hydrogen ceased and NaBH_4 was thoroughly consumed, AcOH (0.108 mL, 1.89 mmol) was added and the mixture was stirred for 10 min to obtain the reduction reagent solution. Then a solution of Palmarumycin C₂ (**12**, 131 mg, 0.39 mmol) in EtOH/AcOH (1 : 1, 10 mL) was added into the reduction reagent solution, stirred for 10 min and removed from the ice-water bath. After completion of the reaction, the solution was diluted with EtOAc (100 mL), washed with brine, and the organic phase was dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 5 : 1) to give Palmarumycin BG1 (**1**, 121 mg, 92%) as a white solid. mp 73–75 °C; $[\alpha]_{\text{D}}^{20} = -152$ ($c = 0.60$, CHCl_3); (lit. 5 $[\alpha]_{\text{D}}^{17} = -151$, $c = 0.50$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ : 12.35 (1H, s), 7.57 (1H, d, $J = 8.0$ Hz), 7.54 (2H, d, $J = 8.4$ Hz), 7.49 (1H, dd, $J = 8.0$, 7.6 Hz), 7.44 (1H, dd, $J = 8.4$, 7.6 Hz), 7.34 (1H, dd, $J = 8.0$, 1.1 Hz), 7.08 (2H, d, $J = 8.4$ Hz), 6.91 (1H, d, $J = 7.6$ Hz), 4.59 (1H, s), 3.24 (1H, dd, $J = 17.8$, 4.0 Hz), 2.94 (1H, dd, $J = 17.8$, 4.0 Hz), 2.38 (1H, s); ^{13}C NMR (75 MHz, CDCl_3) δ : 201.12, 162.21, 147.24, 146.42, 138.03, 137.16, 134.30, 127.82, 127.78, 121.52, 121.22, 119.94, 118.10, 115.46, 113.26, 109.66, 108.97, 98.83, 67.36, 41.40; IR ν_{\max} 3433, 3059, 2924, 1643, 1608, 1585, 1454, 1412, 1381, 1346, 1269, 1234, 1165, 1119, 1069, 976, 891, 822, 756 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{14}\text{O}_5$ $[\text{M}-\text{H}]^-$, calcd. 333.0768, found: 333.0791. These data were consistent with the published data.^{5,6}

Synthesis of (2*R*,4*S*)-3,4-dihydro-2*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxine]-2,4,5-triol and (2*R*,4*R*)-2,3-dihydro-2*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxine]-2,4,5-triol (Palmarumycin BG2 and BG3, 2 and 3)

NaBH_4 (38 mg, 1.00 mmol) was added into a mixture of palmarumycin BG1 (**1**, 107 mg, 0.32 mmol) and MeOH (10 mL) in a 25 mL round-bottom flask at 0 °C. The mixture was stirred at room temperature for 2 h. The solution was extracted with EtOAc (2×30 mL). The organic phase was washed with brine, and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure, the residue was subjected to flash column chromatography on silica gel and eluted with petroleum ether/EtOAc (3 : 1) to afford palmarumycin BG2 (**2**, 61 mg, 56%) as a white solid and palmarumycin BG3 (**3**, 31 mg, 30%) as a white solid. Palmarumycin BG2 (**2**), mp 189–191 °C; $[\alpha]_{\text{D}}^{20} =$

-108 ($c = 0.64$, CHCl_3), (lit. 5 $[\alpha]_{\text{D}}^{16} = -40$, $c = 0.055$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ : 7.55 (1H, d, $J = 8.4$ Hz), 7.52 (1H, d, $J = 8.4$ Hz), 7.48 (1H, d, $J = 7.4$ Hz), 7.45 (1H, dd, $J = 7.6$, 1.5 Hz), 7.42 (1H, dd, $J = 8.4$, 7.5 Hz), 7.40 (1H, dd, $J = 8.0$, 7.6 Hz), 7.09 (1H, dd, $J = 7.6$, 1.5 Hz), 7.04 (1H, d, $J = 7.0$ Hz), 6.90 (1H, s), 6.87 (1H, dd, $J = 7.5$, 0.3 Hz), 5.0 (1H, dd, $J = 10.7$, 5.3 Hz), 4.43 (1H, d, $J = 3.7$ Hz), 3.40 (1H, d, $J = 11.2$ Hz), 2.55 (1H, dd, $J = 15.3$, 5.6 Hz), 2.51 (1H, s), 2.40 (1H, ddd, $J = 15.3$, 4.5, 1.7 Hz); ^{13}C NMR (75 MHz, CDCl_3) δ : 155.65, 147.33, 146.99, 134.34, 132.08, 130.30, 127.83, 127.67, 124.88, 121.40, 120.95, 119.65, 118.24, 113.47, 109.43, 109.10, 99.68, 65.86, 63.41, 31.89; IR ν_{\max} 3210, 3059, 2928, 1636, 1609, 1474, 1412, 1377, 1339, 1269, 1119, 1042, 972, 891, 822, 790, 756 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{16}\text{O}_5$ $[\text{M}-\text{H}]^-$, calcd. 335.0925, found: 335.0954. Palmarumycin BG3 (**3**), mp 145–147 °C; $[\alpha]_{\text{D}}^{20} = -262$ ($c = 0.56$, MeOH); (lit. 5 $[\alpha]_{\text{D}}^{17} = -261$, $c = 0.14$, acetone); ^1H NMR (300 MHz, CD_3OD) δ : 7.51 (1H, dd, $J = 8.4$, 1.2 Hz), 7.49 (1H, dd, $J = 8.4$, 1.2 Hz), 7.45 (1H, dd, $J = 8.4$, 7.3 Hz), 7.42 (1H, dd, $J = 8.4$, 7.3 Hz), 7.20 (2H, d, $J = 4.2$ Hz), 7.01 (1H, dd, $J = 7.2$, 1.3 Hz), 6.92–6.85 (2H, m), 5.30 (1H, dd, $J = 8.8$, 6.2 Hz), 4.31 (1H, dd, $J = 5.8$, 2.2 Hz), 2.43 (1H, ddd, $J = 13.4$, 6.1, 6.1 Hz), 2.28 (1H, ddd, $J = 13.5$, 8.8, 2.3 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ : 157.16, 149.40, 148.74, 135.89, 135.54, 129.94, 128.68, 128.42, 126.38, 121.38, 121.30, 119.77, 117.52, 114.69, 110.47, 109.22, 100.75, 67.62, 65.21, 36.18; IR ν_{\max} 3152, 2924, 2851, 1636, 1605, 1474, 1412, 1377, 1315, 1269, 1115, 1072, 972, 883, 822, 802, 756 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{16}\text{O}_5$ $[\text{M}-\text{H}]^-$, calcd. 335.0925, found: 335.0939. These data were identical with the published data.⁵

Synthesis of (2*S*,3*S*)-3-chloro-2,5-dihydroxy-2,3-dihydro-4*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxin]-4-one (Guignardin E, 6)

A solution of HCl (1 M, 10 mL) was added into a mixture of Palmarumycin C₂ (**12**, 32 mg 0.10 mmol) and THF (12 mL) in a 50 mL round-bottom flask at 25 °C, and the mixture was stirred at room temperature for 24 h. The solution was diluted with EtOAc (50 mL), washed with brine, and the organic phase was dried over anhydrous Na_2SO_4 . The solvent was removed, and the crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 2 : 1) to give the mixture of Guignardin E and its C-3 epimer (**6** and **13**, 34 mg, 95%) as a yellow solid (dr = 5.7 : 1).

A solution of HCl (1 M, 10 mL) was added into a palmarumycin C₂ (**12**, 91 mg, 0.27 mmol) and THF (20 mL) in a 100 mL round-bottom flask at 0 °C, and the mixture was stirred at 10 °C for 72 h. Workup as routine, and the crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 2 : 1) to afford the mixture of Guignardin E and its C-3 epimer (98 mg, 96%) as a yellow solid (dr = 25 : 1). The mixture (dr = 25 : 1) was recrystallized repeatedly with methanol to provide Guignardin E (**6**, 61 mg, 60%) as a yellow solid, mp 178–180 °C; $[\alpha]_{\text{D}}^{20} = -191$ ($c = 0.53$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ : 11.84 (1H, s), 7.66 (1H, t, $J = 8.1$ Hz), 7.61 (1H, d, $J = 8.1$ Hz), 7.58 (1H, d, $J = 8.1$ Hz), 7.52 (1H, dd, $J = 8.5$, 7.4 Hz), 7.48 (1H, d, $J = 8.5$ Hz), 7.46 (1H, dd, $J = 8.5$, 8.4 Hz), 7.16 (1H, dd, $J = 7.4$, 1.0 Hz), 7.15 (1H, dd, $J = 7.4$, 1.0 Hz), 6.93 (1H, dd, $J =$



= 7.5, 0.8 Hz), 5.31 (1H, d, $J = 2.2$ Hz), 4.70 (1H, dd, $J = 2.8, 2.7$ Hz), 2.68 (1H, d, $J = 2.7$ Hz); ^{13}C NMR (75 MHz, CDCl_3) δ : 194.14, 162.17, 146.74, 146.10, 138.03, 137.48, 134.34, 127.93, 127.84, 121.85, 121.70, 120.46, 118.83, 114.28, 113.06, 110.13, 109.24, 98.69, 72.34, 62.55; IR ν_{max} 3471, 1663, 1609, 1585, 1458, 1412, 1377, 1265, 1196, 1172, 1115, 1065, 987, 894, 818, 760, 694 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{13}\text{ClO}_5$ $[\text{M}-\text{H}]^-$, calcd. 367.0379, found: 367.0397. These data were consistent with the published data.⁶

Synthesis of (2*S*,3*R*,4*S*)-3-chloro-3,4-dihydro-2*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxine]-2,4,5-triol, (2*S*,3*R*,4*R*)-3-chloro-3,4-dihydro-2*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxine]-2,4,5-triol and (2*S*,3*S*,4*R*)-3-chloro-3,4-dihydro-2*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxine]-2,4,5-triol (Palmarumycin BG5, its C-4 epimer and BG6, 4, 14 and 5)

NaBH_4 (7 mg, 0.18 mmol) was added into a solution of Guignardin E (6, 23 mg, 0.06 mmol) in MeOH (1.5 mL) in a 10 mL round-bottom flask. The mixture was stirred at room temperature for 2 h. The solution was extracted with EtOAc (2 \times 10 mL). The organic phase was washed with brine, and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure, the residue was subjected to flash column chromatography on silica gel and eluted with petroleum ether/EtOAc (2 : 1) to afford Palmarumycin BG5 (4, 5 mg, 22%) as a white solid and its C-4 epimer (14, 18 mg, 78%) as a white solid. Palmarumycin BG5 (4), mp 108–110 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{20} = -316$ ($c = 0.96$, CHCl_3), (lit. 5 $[\alpha]_{\text{D}}^{16} = -314.7$, $c = 0.75$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ : 8.38 (1H, s), 7.59 (1H, d, $J = 8.5$ Hz), 7.55 (1H, dd, $J = 8.5, 0.9$ Hz), 7.49 (1H, dd, $J = 8.3, 7.5$ Hz), 7.45 (1H, dd, $J = 7.6, 7.6$ Hz), 7.43–7.37 (2H, m), 7.09 (1H, dd, $J = 7.4, 0.9$ Hz), 7.07 (1H, dd, $J = 7.0, 1.5$ Hz), 6.92 (1H, dd, $J = 7.5, 1.0$ Hz), 5.47 (1H, d, $J = 9.1$ Hz), 4.68 (1H, dd, $J = 9.1, 1.9$ Hz), 4.47 (1H, d, $J = 1.9$ Hz), 3.23 (1H, s), 2.40 (1H, s); ^{13}C NMR (75 MHz, CDCl_3) δ : 155.95, 147.08, 146.35, 134.36, 132.68, 130.90, 127.85, 127.80, 121.73, 121.25, 120.03, 119.88, 119.19, 113.19, 109.75, 109.33, 99.82, 71.77, 70.86, 63.95; IR ν_{max} 3325, 2963, 2920, 2851, 1609, 1462, 1412, 1381, 1342, 1265, 1107, 1022, 891, 799, 756 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{15}\text{ClO}_5$ $[\text{M}-\text{H}]^-$, calcd. 369.0535, found: 369.0548. These data were consistent with the published data.⁵ The C-4 epimer of Palmarumycin BG5 (14), mp 200–202 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{20} = -96$ ($c = 0.58$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ : 7.58 (1H, d, $J = 7.6$ Hz), 7.56 (1H, d, $J = 8.5$ Hz), 7.51–7.42 (4H, m), 7.12 (1H, dd, $J = 7.3, 2.0$ Hz), 7.07 (1H, dd, $J = 7.6, 0.9$ Hz), 6.91 (1H, dd, $J = 7.5, 1.0$ Hz), 6.38 (1H, s), 5.08 (1H, s), 4.77 (1H, dd, $J = 4.8, 1.7$ Hz), 4.48 (1H, s), 3.43 (1H, d, $J = 9.8$ Hz), 2.86 (1H, s); ^{13}C NMR (75 MHz, CDCl_3) δ : 155.22, 146.90, 146.61, 134.39, 131.59, 131.15, 127.90, 127.79, 123.53, 121.73, 121.37, 120.29, 118.79, 109.72, 109.32, 100.26, 100.17, 71.59, 66.25, 57.59; IR ν_{max} 3464, 2928, 1609, 1585, 1466, 1416, 1377, 1323, 1180, 1115, 1057, 1038, 984, 887, 822, 791, 764, 691 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{15}\text{ClO}_5$ $[\text{M}-\text{H}]^-$, calcd. 369.0535, found: 369.0547.

NaBH_4 (50 mg, 1.32 mmol) was added into a mixture of Guignardin E and its C-3 epimer (6 and 13, 161 mg, 0.44 mmol, dr = 5.7 : 1), and MeOH (10 mL) in a 25 mL round-bottom flask. The mixture was stirred at room temperature for 2 h. The

solution was extracted with EtOAc (2 \times 30 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure, the residue was subjected to flash column chromatography on silica gel and eluted with petroleum ether/EtOAc (2 : 1) to afford Palmarumycin BG5 (4, 37 mg, 23%), the C-4 epimer of Palmarumycin BG5 (14, 96 mg, 60%), and Palmarumycin BG6 (5, 15 mg, 9%) as a yellow solid. Palmarumycin BG6 (5), mp 164–166 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{20} = +56$ ($c = 0.50$, CHCl_3), (lit. 5 $[\alpha]_{\text{D}}^{20} = +60$, $c = 0.04$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ : 8.13 (1H, s), 7.51 (1H, d, $J = 8.1$ Hz), 7.50 (1H, d, $J = 8.3$ Hz), 7.45 (1H, dd, $J = 8.2, 7.5$ Hz), 7.41 (1H, dd, $J = 8.1, 7.7$ Hz), 7.18 (1H, dd, $J = 8.0, 8.0$ Hz), 7.03 (1H, d, $J = 7.9$ Hz), 6.96 (2H, d, $J = 8.0$ Hz), 6.90 (1H, d, $J = 7.4$ Hz), 5.32 (1H, d, $J = 7.7$ Hz), 4.63 (1H, dd, $J = 8.7, 7.8$ Hz), 4.38 (1H, d, $J = 8.7$ Hz), 3.51 (1H, brs), 2.64 (1H, brs); ^{13}C NMR (75 MHz, CDCl_3) δ : 156.04, 148.06, 147.12, 134.08, 134.04, 130.56, 127.68, 127.66, 120.93, 120.76, 119.21, 118.87, 118.53, 112.85, 108.72, 108.49, 99.32, 74.58, 73.73, 64.35; IR ν_{max} 3319, 2933, 1609, 1585, 1465, 1415, 1381, 1344, 1266, 1181, 1115, 1062, 986, 893, 796, 756 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{15}\text{ClO}_5$ $[\text{M}-\text{H}]^-$, calcd. 369.0535, found: 369.0547. These data were in agreement with the reported data.⁵

Synthesis of 3-chloro-5-hydroxy-4*H*-spiro[naphthalene-1,2'-naphtho[1,8-de][1,3]dioxin]-4-one (Palmarumycin C1, 7)

$\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (64 mg, 0.172 mmol), Palmarumycin C₂ (12, 52 mg, 0.157 mmol), MeOH (9 mL), and H_2O (3 mL) were added in a 25 mL round-bottom flask. The mixture was stirred at reflux temperature for 16 h, cooled to room temperature and the mixture was diluted with EtOAc (50 mL). The organic phase was washed with brine, and dried over anhydrous Na_2SO_4 . The solution was concentrated under vacuum. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 20 : 1) to give palmarumycin C₁ (7, 41 mg, 79%) as a yellow solid. mp 272–273 $^\circ\text{C}$ (lit. 32 mp > 280 $^\circ\text{C}$); ^1H NMR (300 MHz, CDCl_3) δ : 11.87 (1H, s), 7.69 (1H, dd, $J = 8.4, 7.5$ Hz), 7.61 (2H, dd, $J = 8.4, 1.0$ Hz), 7.49 (2H, dd, $J = 8.4, 7.5$ Hz), 7.47 (1H, dd, $J = 7.5, 1.0$ Hz), 7.18 (1H, dd, $J = 8.4, 1.0$ Hz), 7.17 (1H, s), 7.00 (2H, dd, $J = 7.5, 1.0$ Hz); ^{13}C NMR (75 MHz, CDCl_3) δ : 182.09, 162.32, 146.93, 138.63, 137.40, 135.84, 135.18, 134.34, 127.84, 121.81, 120.28, 119.85, 113.30, 112.95, 110.24, 93.70; IR ν_{max} 3063, 2924, 1651, 1628, 1612, 1497, 1458, 1412, 1381, 1269, 1231, 1169, 1119, 1069, 941, 903, 845, 826, 802, 756 cm^{-1} ; HR-ESI-MS, m/z : $\text{C}_{20}\text{H}_{10}\text{ClO}_4$ $[\text{M}-\text{H}]^-$, calcd. 349.0273, found: 349.0276. These data were identical with the published data.^{6,32,33}

X-ray diffraction analysis of Palmarumycin BG5 C-4 epimer (14)

Colorless plate-like crystals of compound 14 were obtained from a slowly evaporating methanol solution. A $0.35 \times 0.24 \times 0.03$ mm³ crystal was selected for analysis. The parameters and structure information for compound 14 have been deposited at the Cambridge Crystallographic Data Centre. CCDC ID 1947664 contains the supplementary crystallographic data for this paper.



Cytotoxic activity evaluation of title compounds

The cytotoxicity of the title compounds was evaluated against HCT116, U87-MG, HepG2, BGC823 and PC9 cell lines using a microculture tetrazolium (MTT) assay as in the previous report.²² Taxol was used as the positive control. All of human carcinoma cell lines (colon cancer cell HCT116, glioblastoma cell U87-MG, hepatocellular carcinoma cell HepG2, gastric cancer cell BGC823 and lung carcinoma cell PC9) were obtained from the American Type Culture Collection (ATCC) and purchased from the Cell Culture Center of Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences. These cell lines were cultured in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin (100 U mL⁻¹)-streptomycin (100 µg mL⁻¹). The cell culture medium, serum and antibiotics were purchased from Invitrogen. All the cells were maintained at 37 °C in 5% CO₂.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- 1 K. Miyashita and T. Imanishi, *Chem. Rev.*, 2005, **105**, 4515–4536.
- 2 L. Zhou, J. Zhao, T. Shao, X. Cai and Y. Peng, *Mini-Rev. Med. Chem.*, 2010, **10**, 977–989.
- 3 Y. S. Cai, Y. W. Guo and K. Krohn, *Nat. Prod. Rep.*, 2010, **27**, 1840–1870.
- 4 X. Liu, Y. Zhao, W. Wang, M. Wang and L. Zhou, *Chin. J. Org. Chem.*, 2017, **37**, 2883–2894.
- 5 Y. Cai, T. Kurtan, Z. Miao, A. Mandi, I. Komaromi, H. Liu, J. Ding and Y. Guo, *J. Org. Chem.*, 2011, **76**, 1821–1830.
- 6 W. Ai, X. Wei, X. Lin, L. Sheng, Z. Wang, Z. Tu, X. Yang, X. Zhou, J. Li and Y. Liu, *Tetrahedron*, 2014, **70**, 5806–5814.
- 7 T. Bunyapaiboonsri, S. Yoiprommarat, R. Nopgason, K. Intereya, R. Suvannakad and J. Sakayaroj, *Tetrahedron*, 2015, **71**, 5572–5578.
- 8 E. E. Erika, Synthetic progress toward parvistemonine, spiroxins A and B, and generation of palmarumycin analogues, PhD. dissertation, University of Pittsburgh, USA, 2008.
- 9 A. C. Murphy, S. R. A. Devenish, A. C. Muscroft-Taylor, J. W. Blunt and M. H. G. Munro, *Org. Biomol. Chem.*, 2008, **6**, 3854–3862.
- 10 K. Krohn, S. Wang, I. Ahmed, S. Altun, A. Aslan, U. Florke, I. Kock and S. Schlummer, *Eur. J. Org. Chem.*, 2010, 4476–4481.
- 11 A. Aslan, S. Altun, I. Ahmed, U. Florke, B. Schulz and K. Krohn, *Eur. J. Org. Chem.*, 2011, 1176–1188.
- 12 H. Tsukamoto, S. Hanada, K. Kumasaka, N. Kagaya, M. Izumikawa, K. Shinya and T. Doi, *Org. Lett.*, 2016, **18**, 4848–4851.
- 13 H. Tsukamoto, Y. Nomura, K. Fujiwara, S. Hanada and T. Doi, *Org. Lett.*, 2018, **20**, 3140–3143.
- 14 H. Tsukamoto, S. Hanada, Y. Nomura and T. Doi, *J. Org. Chem.*, 2018, **83**, 9430–9441.
- 15 T. Doi, H. Tsukamoto and Y. Nomura, *Heterocycles*, 2019, **99**, 549–565.
- 16 Y. Ando, A. Hanaki, R. Sasaki, K. Ohmori and K. Suzuki, *Angew. Chem., Int. Ed.*, 2017, **56**, 11460–11465.
- 17 B. S. Chinta and B. Baire, *Eur. J. Org. Chem.*, 2017, 3457–3460.
- 18 S. Martinez-Luis, G. Della-Togna, P. Coley, T. Kursar, W. H. Gerwick and L. Cubilla-Rios, *J. Nat. Prod.*, 2008, **71**, 2011–2014.
- 19 J. Dong, H. Song, J. Li, Y. Tang, R. Sun and L. Wang, *J. Nat. Prod.*, 2008, **71**, 952–956.
- 20 M. L. Macías-Rubalcava, B. E. Hernández-Bautista, M. Jemenez-Estrada, M. C. Gonzalez, A. E. Glenn, R. T. Hanlin, S. Hernández-Ortega, A. Saucedo-Garcia, J. M. Muria-Gonzalez and A. L. Anaya, *Phytochemistry*, 2008, **69**, 1185–1196.
- 21 R. Wang, G. Liu, M. Yang, M. Wang and L. Zhou, *Molecules*, 2016, **21**, 600.
- 22 T. Shan, J. Tian, X. Wang, Y. Mou, Z. Mao, D. Lai, J. Dai, Y. Peng, L. Zhou and M. Wang, *J. Nat. Prod.*, 2014, **77**, 2151–2160.
- 23 J. Tian, X. Liu, Z. Liu, D. Lai and L. Zhou, *Pest Manag. Sci.*, 2016, **72**, 961–965.
- 24 X. Liu, W. Wang, Y. Zhao, D. Lai, L. Zhou, Z. Liu and M. Wang, *J. Nat. Prod.*, 2018, **81**, 1803–1809.
- 25 J. Ragot, M. Alcaraz and R. Taylor, *Tetrahedron Lett.*, 1998, **39**, 4921–4924.
- 26 J. Ragot, C. Steeneck, M. Alcaraz and R. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1999, **8**, 1073–1082.
- 27 A. G. M. Barrett, D. Hamprecht and T. Meyer, *Chem. Commun.*, 1998, **8**, 809–810.
- 28 A. Barrett, F. Blaney, A. Campbell, D. Hamprecht, T. Meyer, A. White, D. Witty and D. Williams, *J. Org. Chem.*, 2002, **67**, 2735–2750.
- 29 M. Miyashita, T. Suzuki, M. Hoshino and A. Yoshikoshi, *Tetrahedron*, 1997, **53**, 12469–12486.
- 30 T. Suzuki and M. Miyashita, *Yuki Gosei Kagaku Kyokaiishi*, 1998, **56**, 736–744.
- 31 Y. Yamashita, Y. Hirano, A. Takada, H. Takikawa and K. Suzuki, *Angew. Chem., Int. Ed.*, 2013, **52**, 6658–6661.
- 32 K. Krohn, A. Michel, U. Flouke, H.-J. Aust, S. Draeger and B. Schulz, *Liebigs Ann. Chem.*, 1994, **11**, 1099–1108.
- 33 G. Bringmann, S. Busemann, K. Krohn and K. Beckman, *Tetrahedron*, 1997, **53**, 1655–1664.
- 34 A. G. Montalban, L.-O. Wittenberg and A. Mckillop, *Tetrahedron Lett.*, 1999, **40**, 5893–5896.

