

Article

# Synthesis of Pelorol and Its Analogs and Their Inhibitory Effects on Phosphatidylinositol 3-Kinase

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**Abstract:** There are numerous biologically active substances with novel structures and unique physiological functions in marine organisms. These substances are important sources of new lead compounds. Pelorol is a natural product isolated from marine organisms that possesses a novel structure with high bioactivity. In this paper, the synthesis of pelorol has been completed, and the synthesis of some intermediates has been optimized and scaled up. Five pelorol analogs have also been prepared. Preliminary biological activity testing demonstrated that compounds **5** and **6** might be potential lead compounds for cancer therapy.

**Keywords:** marine organisms; pelorol; total synthesis; PI3K inhibitor; anti-tumor activity

## 1. Introduction

Natural products play significant roles in drug discovery and development [1]. Marine organisms have been extensively investigated because they produce natural substances; for instance, 245 furanosesterterpenoids from marine organisms were described in at least 133 articles from 1996 to 2006 [2]. The number of publications may be higher if halogenated monoterpenes, sesquiterpenes, and polyterpenes extracted from the sponges are considered [3]. Labdane-type diterpenes are excellent examples of natural products with important pharmaceutical activities (Figure 1). Most of these derivatives possess significant biological properties such as anti-tumor, anti-HIV, anti-inflammatory, antifungal, and antibacterial activities [4–8].

Pelorol is a labdane-type diterpene isolated from the sponge [9,10]. It exhibits promising activity against trypanosome and *Plasmodium*. *In vitro* tests and mouse models reveals that pelorol is a src homology 2-containing inositol 5-phosphatase (SHIP) activators.

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking. The targeting of the PI3K pathway is one of the most promising approaches for the treatment of cancers. Moreover, SHIP can negatively regulate the PI3K pathway. The increased activity of SHIP can reduce the anti-apoptotic effect of the PI3K-Akt signaling pathway [11,12]. Thus, pelorol might also be a potential PI3K inhibitor.

However, natural pelorol is difficult to extract, and the synthetic of pelorol is rarely described. As far as we know, Andersen and co-workers [13] described a new strategy to synthesize pelorol with 5% overall yield in 2005. Their route is difficult to scale up because of numerous reaction steps and strict reaction conditions. In 2012, Baran and co-workers [14] developed another efficient method for the synthesis of pelorol, and they realized the gram-scale preparation of the key intermediate “borono-sclareolide”. Hence, new methods and technologies are still highly desirable for the synthesis,

semi-synthesis, chemical modification and bioactivity assay of pelorol and related marine natural products, in order to provide technical support for the development of marine drug.

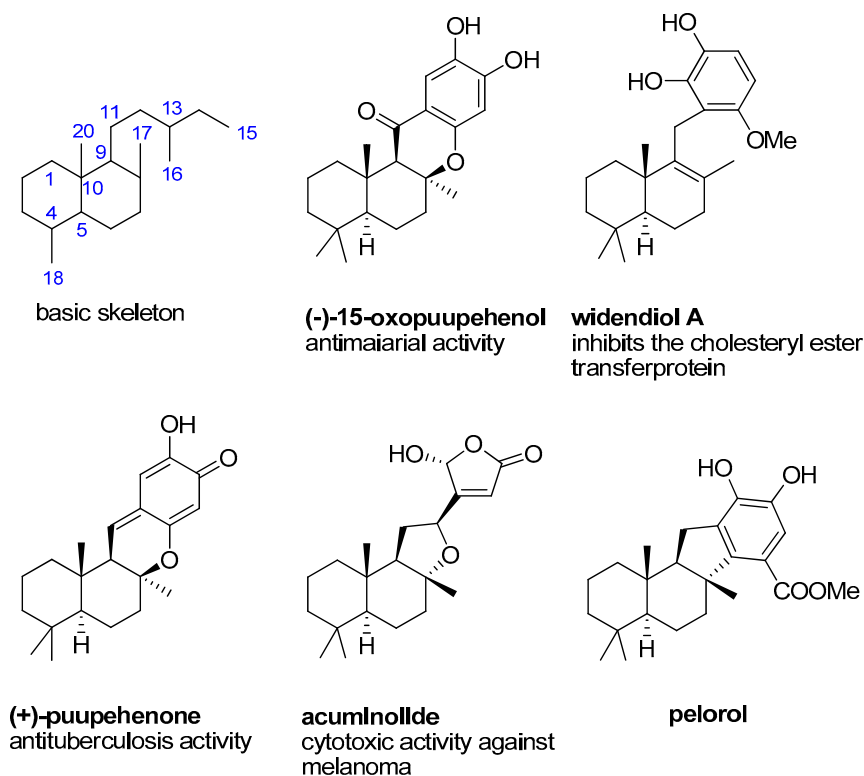


Figure 1. Representative bioactive compounds of labdane-type diterpenes.

## 2. Results and Discussion

### 2.1. Chemistry

In this paper, the synthesis of pelorol has been completed, optimized and scaled up based on Baran's method (Scheme 1). Five pelorol analogs 2–6 have also been prepared (Figure 2).

Commercially available terpenoid (+)-sclareolide (7), which exhibits the same absolute configurations at C-5, C-9, and C-10 with pelorol (1), was used as starting material. 7 was reduced to a mixture of 8 and 9 (8/9 = 4.59) with an excellent yield (99%); this mixture was used for the subsequent transformation without additional separation. Benzene is commonly used for iodination. Considering its toxicity, we replaced benzene with toluene and obtained product 10 with 78% yield. A sequential dehydroiodination/hydrolysis reaction (AgF in pyridine followed by  $K_2CO_3$  in methanol) produced intermediate 11 with 89% overall yield. AgF is a precious metal salt and 1.5 equiv. of fresh AgF was essential in this reaction. To reduce the production costs, recycled AgF catalyst can be used, and in this case, 2.0 equiv. of catalyst was needed to ensure complete conversion. Hydroboration of 11 resulted in a mixture of diastereomers in 95% combined yield, and borono-sclareolide (12) can be separated by column chromatography in 50% yield.

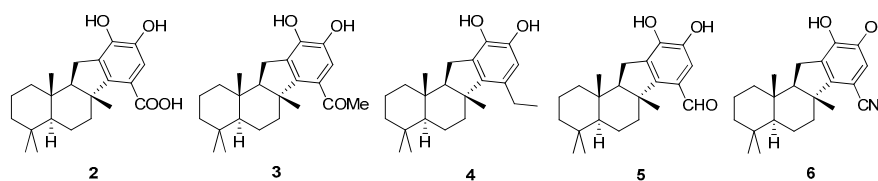
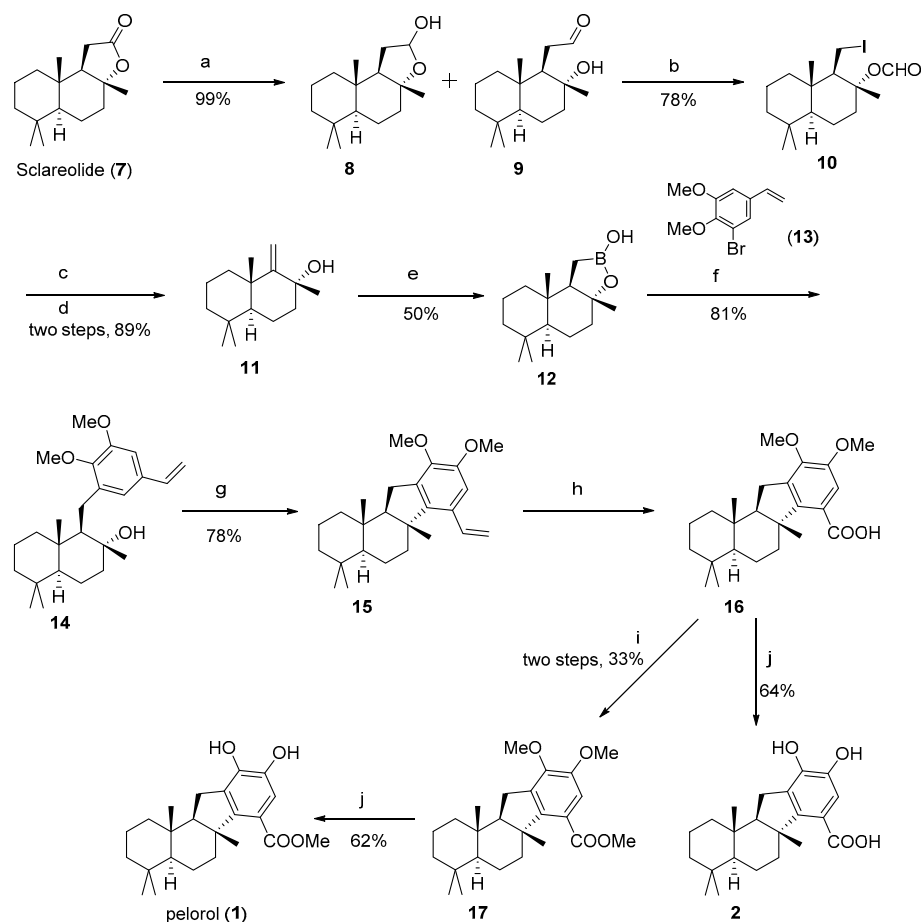


Figure 2. Pelorol analogs 2–6 in our work.



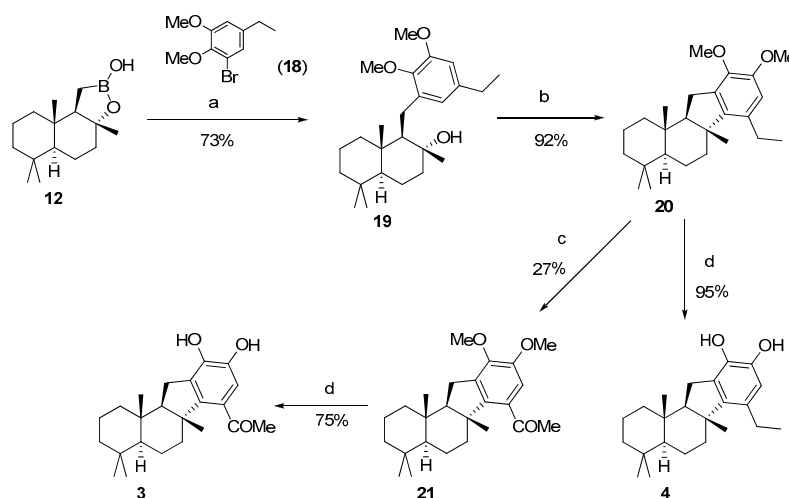
**Scheme 1.** Synthesis of **1** and **2**. Reagents and conditions: (a) DIBAL,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ , 1 h; (b) PIDA,  $\text{I}_2$ , toluene,  $h\nu$ ,  $80^\circ\text{C}$ , 1 h; (c) AgF, pyridine, room temperature (RT), 12 h; (d)  $\text{K}_2\text{CO}_3$ , MeOH,  $0^\circ\text{C}$ –RT, 2 h; (e)  $\text{BH}_3 \cdot \text{THF}$ , THF,  $0^\circ\text{C}$ –RT, 12 h; (f) S-Phos, CsF, Pd(OAc) $_2$ , 1,4-dioxane,  $50^\circ\text{C}$ , 18 h; (g)  $\text{SnCl}_4$ ,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ , 1 h; (h)  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , TBHP, NaOH, CTAB,  $\text{H}_2\text{O}$ ,  $80^\circ\text{C}$ , 48 h; (i) MeI,  $\text{K}_2\text{CO}_3$ , DMF, RT, 12 h; (j)  $\text{BI}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ , 1 h.

At first, we followed Baran's method and used aryl bromide with ethyl substituent (**18**) to couple with **12**, but the subsequent oxidation of intermediate **20** suffered from low yield. We have tried several different oxidants, such as DCC, PDC, ethylene oxide and potassium permanganate, but failed to improve the yield. Furthermore, subsequent reaction systems were quite complicated. Considering these problems, we changed the aryl bromide substrate to vinyl-substituted **13**, and found that the coupling reaction proceeded smoothly with up to 81% yield. What is more, this modification could remove two steps for the total synthesis of pelorol. The subsequent cyclization of **14** was performed at  $-78^\circ\text{C}$  to avoid the secondary reaction of the vinyl functional group. This cyclization provided the tetracyclic intermediate **15** in high yield (78%). Compounds **14** and **15** can also be prepared in gram-scale with comparable yields.

The oxidation of **15** via TBHP can generate **16** [15]. The synthesis of pelorol (**1**) was completed by the esterification of **16** with MeI and the selective cleavage of aryl methyl ether with  $\text{BI}_3$ . The total yield of this route was 4.4%, which was similar to that of Andersen *et al.* [13]. The direct demethylation of **16** afforded compound **2** in 62% yield.

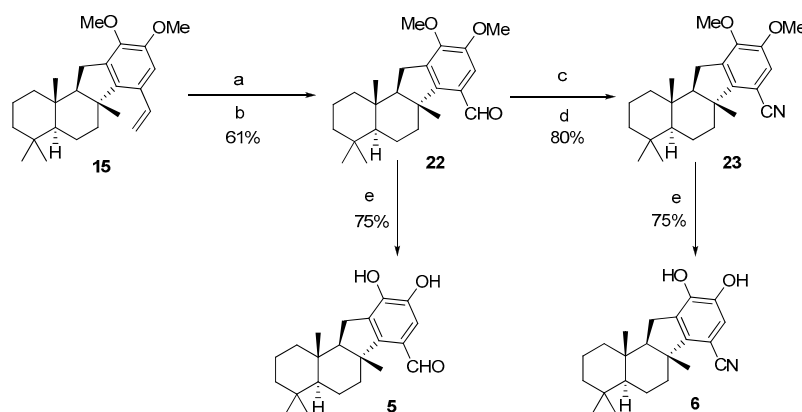
As we mentioned before, borono-sclareolide (**12**) could couple with ethyl-substituted **18** under Suzuki Coupling conditions developed by Buchwald [16]. A favorable yield of the coupling product **19** (73%) was obtained. The subsequent cyclization afforded **20** in excellent yield (92%). **20** was then oxidized by PCC to produce **21** in 27% yield; here the low yield might be due to the existence of two electron-donating methoxy groups on the phenyl ring of **20**. The demethylation of **20** and **21**

provided **4** (95% yield) and **3** (75% yield), respectively (Scheme 2). The low yield in the latter reaction might be due to side reactions between ketone and  $\text{BI}_3$ .



**Scheme 2.** Synthesis of **3** and **4**. Reagents and conditions: (a) *S*-Phos, CsF,  $\text{Pd}(\text{OAc})_2$ , 1,4-dioxane, 50 °C, 12 h; (b)  $\text{SnCl}_4$ ,  $\text{CH}_2\text{Cl}_2$ , −20 °C, 1 h; (c) PCC, 40 °C, 20 h; (d)  $\text{BI}_3$ ,  $\text{CH}_2\text{Cl}_2$ , −78 °C, 1 h.

Intermediate **15** can be further oxidized to aryl aldehyde **22** under mild conditions [17,18]. Subsequent transformation of **22** via the formation of oxime and dehydration could yield **23** (80%) [19,20]. The demethylation of **22** and **23** afforded **5** (75% yield) and **6** (75% yield), respectively (Scheme 3). In this way, we have obtained five pelorol analogs **2–6**.



**Scheme 3.** Synthesis of **5** and **6**. Reagents and conditions: (a) NMO,  $\text{H}_4\text{K}_2\text{O}_6\text{Os}$ , THF:acetone: $\text{H}_2\text{O}$ =2:1:1, RT, 8 h; (b)  $\text{NaIO}_4$ ,  $\text{CH}_2\text{Cl}_2$ , 0 °C–RT, 3 h; (c)  $\text{NH}_2\text{OH} \cdot \text{HCl}$ ,  $\text{Et}_3\text{N}$ , RT, 4 h; (d)  $\text{Cl}_3\text{COCOCCl}_3$ ,  $\text{CH}_2\text{Cl}_2$ , RT, 8 h; (e)  $\text{BI}_3$ ,  $\text{CH}_2\text{Cl}_2$ , −78 °C, 1 h.

## 2.2. PI3K Enzymatic Activity Assay

All the newly prepared pelorol and analogs have been assessed *in vitro* in terms of biological activity against  $\text{PI3K}\alpha$  kinase, and the results are summarized in Table 1. Under the assay condition,  $\text{IC}_{50}$  value of pelorol was 38.17  $\mu\text{M}$ . The carboxylic acid-substituted compound **2** showed slightly increased activity (20.70  $\mu\text{M}$ ). Among all these analogs, **3** (with acetyl substituent) and **5** (with aldehyde substituent) were the best  $\text{PI3K}$  inhibitors with  $\text{IC}_{50}$  of 8.76  $\mu\text{M}$  and 5.06  $\mu\text{M}$ , respectively [13]. Cyano-substituted compound **6** showed a significant decrease in activity ( $\text{IC}_{50}$  = 49.51  $\mu\text{M}$ ), while compound **4** with ethyl substituent on the phenyl ring exhibited poor performance ( $\text{IC}_{50}$  = 83.69  $\mu\text{M}$ ). We assumed that electronic effects might influence the inhibitory activity of compounds against  $\text{PI3K}\alpha$ . We have also found that dimethoxy-substituted compound **20** displayed weaker inhibitory activity

when comparing with corresponding phenolic hydroxy-substituted compound **4**. The most efficient compound **5** was also subjected to PI3K $\beta$ , PI3K $\gamma$ , PI3K $\delta$  tests, and it exhibited significant inhibitory activity against PI3K $\beta$  (IC<sub>50</sub> = 8.90  $\mu$ M).

**Table 1.** Inhibition of pelorol and analogs against PI3K $\alpha$  ( $\mu$ M)<sup>a</sup>.

| Compound         | 1     | 2     | 3    | 4     | 5 <sup>b</sup> | 6     | 20   |
|------------------|-------|-------|------|-------|----------------|-------|------|
| IC <sub>50</sub> | 38.17 | 20.70 | 8.76 | 83.69 | 5.06           | 49.51 | >100 |

<sup>a</sup> Average of three replicates; <sup>b</sup> For compound **5**, IC<sub>50</sub> value for PI3K $\beta$  is 8.90  $\mu$ M, for PI3K $\gamma$  is >100  $\mu$ M, for PI3K $\delta$  is >100  $\mu$ M.

### 2.3. Antiproliferative Assays in Vitro

Eight human cancer cell lines were used in the antiproliferative bioassay of pelorol and its analogs, the results were summarized in Table 2. The inhibitory activities of pelorol were poor. Compounds **2** and **4** also exhibited poor antiproliferative activities in these cancer cells, albeit **2** showed reasonable activity against PI3K $\alpha$ . In contrast, compound **5** displayed the highest inhibitory activities and suppressed most of the cancer cells, especially MOLT-4 (leukemia cell), U937 (leukemia cell), and DU145 (prostate cancer cell), with IC<sub>50</sub> at micromolar concentration levels. Although cyano-substituted compound **6** demonstrated moderate activity in kinase test, **6** could efficiently suppress a few tumor cells, especially MOLT-4 and DU145. Based on the above analysis, pelorol analogs **5** and **6** might be potential lead compounds for cancer therapy.

**Table 2.** Relative IC<sub>50</sub> values ( $\mu$ M) of pelorol and analogs against tumor cell lines<sup>a</sup>.

| Compound | 1     | 2                 | 3    | 4   | 5     | 6     |
|----------|-------|-------------------|------|-----|-------|-------|
| MCF-7    | >30   | >30               | >30  | >30 | 38.9  | 16.5  |
| K562     | >30   | >30               | >30  | >30 | 16.11 | >30   |
| Hela     | >30   | N.D. <sup>b</sup> | >30  | >30 | 13.15 | 12.05 |
| DU145    | 72.69 | >30               | >30  | >30 | 9.01  | 8.13  |
| MOLT-4   | >30   | N.D.              | 22.3 | >30 | 4.27  | 2.89  |
| SK-BR-3  | >30   | N.D.              | >30  | >30 | 19.14 | >30   |
| U937     | >30   | N.D.              | >30  | >30 | 5.99  | 12.85 |
| PC3      | >30   | >30               | >30  | >30 | 18.36 | >30   |

<sup>a</sup> Average of three replicates; <sup>b</sup> N.D.: not determined.

## 3. Materials and Methods

All commercial reagents and anhydrous solvents were obtained from commercial sources and were distilled from standard drying agents unless otherwise specified. <sup>1</sup>H NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer (Bruker Corp., Billerica, MA, USA) and referenced to tetramethylsilane in appropriate organic solutions. Chemical shifts were expressed as  $\delta$  units using tetramethylsilane as internal standard (in NMR description: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad signal; J, joules; Hz, hertz). Mass spectra were measured with an Agilent 6120 spectrometer (Agilent Tech., Santa Clara, CA, USA) using an ESI+APCI source coupled to an Agilent 1200 HPLC system (Agilent Tech.) operating in reverse mode. High-resolution mass spectra were recorded on an ESI-ion trap mass spectrometer (Shimadzu LCMS-IT-TOF, Shimadzu, Kyoto, Japan), and the results agreed with the theoretical values to within 5 ppm. Purity of all biologically evaluated compounds was greater than 95%.

### 3.1. Chemistry

(1*R*,2*R*,4*aS*,8*aS*)-1-(Iodoethyl)-2,5,5,8*a*-tetramethyldecahydronaphthalen-2-yl formate (**10**) [14]: To a solution of (+)-sclareolide (10.00 g, 40.0 mmol, 1.0 equiv.) in 200 mL CH<sub>2</sub>Cl<sub>2</sub> was added 1.0 M

solution of DIBAL in heptane (48.0 mL, 48.0 mmol, 1.2 equiv.) dropwise at  $-78\text{ }^{\circ}\text{C}$ . The reaction mixture was then stirred for another 60 min. Water was slowly added, the reaction mixture was warmed to room temperature and stirred overnight, extracted with  $\text{CH}_2\text{Cl}_2$  ( $4 \times 50\text{ mL}$ ), washed with saturated brine (50 mL), dried over  $\text{Na}_2\text{SO}_4$ . Evaporation of the solvent afforded a white solid, which was used immediately in the next step.

The white solid in 90 mL anhydrous toluene was treated with PIDA (18.04 g, 56 mmol, 1.4 equiv.) and  $\text{I}_2$  (12.18 g, 48 mmol, 1.2 equiv.). The purple reaction mixture was vigorously stirred at  $74\text{ }^{\circ}\text{C}$  for 60 min under the irradiation of flood lamp (150 W). The reaction mixture was cooled, concentrated under reduced pressure, diluted with saturated brine, and extracted with EtOAc. The organic layers were combined, washed with saturated  $\text{Na}_2\text{S}_2\text{O}_3$ , dried over  $\text{Na}_2\text{SO}_4$ . The solution was filtered and concentrated under reduced pressure. The residue was quickly diluted with anhydrous MeOH, stirred and cooled at  $-78\text{ }^{\circ}\text{C}$ . The resulting white solid **10** (7.87 g, 78% yield) was collected via filtration.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.02 (s, 1H), 3.36 (dd,  $J = 10.7, 2.9\text{ Hz}$ , 1H), 3.13 (dd,  $J = 10.7, 4.6\text{ Hz}$ , 1H), 2.55 (d,  $J = 12.7\text{ Hz}$ , 1H), 2.46 (d,  $J = 2.0\text{ Hz}$ , 1H), 1.93–1.85 (m, 2H), 1.69 (d,  $J = 3.5\text{ Hz}$ , 1H), 1.57 (s, 3H), 1.47 (d,  $J = 1.0\text{ Hz}$ , 3H), 1.38 (s, 1H), 1.25 (s, 2H), 1.07 (d,  $J = 2.3\text{ Hz}$ , 1H), 0.88 (s, 3H), 0.85 (d,  $J = 0.8\text{ Hz}$ , 3H), 0.79 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 160.3, 88.3, 62.1, 55.3, 41.6, 40.8, 39.6, 38.9, 33.2, 33.1, 21.4, 20.4, 19.7, 18.4, 14.8,  $-3.2$ . (Figure S1).

(+)-Drim-9(11)-en-8-ol (**11**) [14]: A solution of compound **10** (2.32 g, 6.13 mmol, 1.0 equiv.) in anhydrous pyridine (20 mL) was treated with silver (I) fluoride (1.16 g, 9.2 mmol, 1.5 equiv.) at room temperature. The reaction flask was covered with aluminum foil to prevent light. The solution was stirred at room temperature for 12 h, then concentrated *in vacuo*, diluted with  $\text{Et}_2\text{O}$  (20 mL), and filtered through celite pad. The dark grey solid (AgF) can be recycled. The filter cake was washed with  $\text{Et}_2\text{O}$  (20 mL). The filtrate was combined, washed with saturated  $\text{NaHCO}_3$  and saturated  $\text{Na}_2\text{S}_2\text{O}_3$  ( $V_{\text{NaHCO}_3}/V_{\text{Na}_2\text{S}_2\text{O}_3} = 5 : 1$ , 45 mL), then washed with brine (50 mL), dried over  $\text{Na}_2\text{SO}_4$ . The solution was filtered and concentrated under reduced pressure to afford unstable pale yellow syrup which was used immediately in the next step.

A solution of yellow syrup in 90 mL MeOH was treated with powdered, oven-dried  $\text{K}_2\text{CO}_3$  (1.01 g, 7.35 mmol, 1.2 equiv.) at  $0\text{ }^{\circ}\text{C}$ . The reaction was warmed to room temperature and vigorously stirred for 2 h. The mixture was concentrated under reduced pressure, diluted with  $\text{Et}_2\text{O}$  (20 mL), washed with  $\text{H}_2\text{O}$  (60 mL), and extracted with  $\text{Et}_2\text{O}$  ( $2 \times 20\text{ mL}$ ). The combined  $\text{Et}_2\text{O}$  extracts were dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to afford compound **11** as oyster white solid (1.18 g, 89% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.21 (s, 1H), 4.83 (s, 1H), 2.01 (d,  $J = 9.5\text{ Hz}$ , 1H), 1.79 (d,  $J = 12.6\text{ Hz}$ , 1H), 1.69 (t,  $J = 13.5\text{ Hz}$ , 2H), 1.53 (d,  $J = 14.8\text{ Hz}$ , 1H), 1.47 (s, 1H), 1.40 (s, 3H), 1.38–1.30 (m, 2H), 1.25 (s, 1H), 1.15 (d,  $J = 14.2\text{ Hz}$ , 1H), 1.08 (s, 3H), 0.98 (d,  $J = 11.0\text{ Hz}$ , 1H), 0.87 (s, 3H), 0.84 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.6, 103.7, 73.3, 53.5, 44.2, 41.8, 40.0, 39.0, 33.8, 33.2, 30.6, 29.68, 22.4, 21.6, 20.2, 19.1. (Figure S2).

Borono-sclareolide (**12**) [14]: Yield 50%, white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.62 (s, 1H), 1.95 (d,  $J = 11.6\text{ Hz}$ , 1H), 1.79–1.74 (m, 1H), 1.63 (d,  $J = 8.0\text{ Hz}$ , 2H), 1.56–1.50 (m, 1H), 1.42 (s, 3H), 1.38–1.34 (m, 1H), 1.33–1.23 (m, 1H), 1.17 (s, 4H), 0.98–0.90 (m, 2H), 0.85 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H), 0.75 (d,  $J = 7.6\text{ Hz}$ , 1H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 83.9, 61.6, 56.9, 42.5, 40.4, 39.5, 37.0, 33.3, 33.2, 23.1, 21.0, 20.9, 18.3, 14.6. (Figure S3).

(1*R*,2*R*,4*aS*,8*aS*)-1-(2,3-diMethoxy-5-vinylbenzyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (**14**): To the borono-sclareolide **12** (1.00 g, 4.0 mmol, 1.0 equiv.) was added bromide **13** (1.94 g, 8 mmol, 2.0 equiv.), *S*-Phos (0.25 g, 0.6 mmol, 0.15 equiv.), CsF (1.82 g, 12 mmol, 3.0 equiv.) and 50 mL anhydrous dioxane. The solution was degassed for 20 min by bubbling  $\text{N}_2$  under sonication.  $\text{Pd}(\text{OAc})_2$  (0.09 g, 0.4 mmol, 0.1 equiv.) was immediately added under  $\text{N}_2$ , then the reaction mixture was heated at  $50\text{ }^{\circ}\text{C}$  for 18 h. The solution was cooled to room temperature and filtered through a celite pad, concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether/EtOAc = 30:1) to afford **14** (1.22 g, 80.5% yield) as colorless oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.87 (d,  $J = 1.6\text{ Hz}$ , 1H), 6.81 (d,  $J = 1.7\text{ Hz}$ , 1H), 6.63 (dd,  $J = 17.5, 10.8\text{ Hz}$ , 1H),

5.63 (d,  $J = 17.5$  Hz, 1H), 5.18 (d,  $J = 10.9$  Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 2.84 (dd,  $J = 14.5, 5.1$  Hz, 1H), 2.66 (s, 1H), 2.60 (d,  $J = 4.9$  Hz, 1H), 1.86 (d,  $J = 12.5$  Hz, 1H), 1.75 (d,  $J = 12.8$  Hz, 1H), 1.62 (s, 3H), 1.41 (d,  $J = 14.0$  Hz, 2H), 1.33 (d,  $J = 16.6$  Hz, 2H), 1.28 (s, 3H), 1.10 (dd,  $J = 13.2, 3.6$  Hz, 1H), 0.91 (s, 3H), 0.85 (s, 3H), 0.83–0.81 (m, 1H), 0.80 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 152.6, 146.3, 138.1, 136.8, 133.5, 121.5, 112.9, 107.0, 73.7, 62.9, 60.7, 56.1, 55.7, 43.8, 41.8, 40.3, 39.5, 33.5, 33.3, 25.1, 24.2, 21.6, 20.3, 18.6, 15.5. (Figure S4).

(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diMethoxy-4,4,6*a*,11*b*-tetramethyl-7-vinyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene (**15**): To a stirred solution of **14** (232 mg, 0.6 mmol, 1.0 equiv.) in  $\text{CH}_2\text{Cl}_2$  (60 mL),  $\text{SnCl}_4$  (0.6 mL) was added dropwise at  $-78^\circ\text{C}$  under  $\text{N}_2$ . The resulting mixture was stirred for an additional 1 h. Upon the completion of the reaction (monitored by TLC), the solution was diluted with  $\text{CH}_2\text{Cl}_2$  (90 mL) and poured into ice. The aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 20$  mL), the combined  $\text{CH}_2\text{Cl}_2$  extracts were dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 15:1) to give **15** as yellow-green crystal (173 mg, 78.3% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.06 (dd,  $J = 17.4, 10.9$  Hz, 1H), 6.81 (s, 1H), 5.49 (dd,  $J = 17.3, 1.3$  Hz, 1H), 5.17 (dd,  $J = 10.9, 1.3$  Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 2.72 (dd,  $J = 14.7, 6.2$  Hz, 1H), 2.56–2.47 (m, 1H), 2.42–2.35 (m, 1H), 1.73 (s, 3H), 1.61 (s, 2H), 1.43 (s, 2H), 1.26 (s, 1H), 1.18 (d,  $J = 4.3$  Hz, 1H), 1.11 (s, 3H), 1.04 (s, 3H), 1.01–0.95 (m, 2H), 0.87 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 150.6, 145.5, 145.3, 135.8, 134.3, 128.3, 113.6, 107.7, 64.2, 60.4, 57.0, 56.0, 47.9, 42.5, 40.1, 39.5, 37.1, 33.4, 33.1, 25.3, 21.6, 21.1, 19.7, 18.4, 16.1. (Figure S5).

(4*aS*,6*aR*,11*aR*,11*bS*)-Methyl-9,10-dimethoxy-4,4,6*a*,11*b*-tetramethyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene-7-carboxylate (**17**): To a mixture of **15** (100 mg, 0.27 mmol, 1.0 equiv.) and ferric chloride hexahydrate (40 mg) was added TBHP (1.2 mL, 70% aqueous solution), CTAB (2 g) and 8 mL water. NaOH (40 mg, 1.0 mmol) was added after 3 h, and the mixture was heated at  $90^\circ\text{C}$  for 48 h. After the completion of the reaction, dilute HCl and crushed ice were added. The mixture was extracted with EtOAc ( $2 \times 50$  mL), washed with saturated brine, dried over  $\text{Na}_2\text{SO}_4$ , then concentrated to dryness. The residue was dissolved in 2 mL DMF, then potassium carbonate (350 mg) and MeI (0.4 mL) were added. The solution was stirred overnight, quenched with 10 mL brine. The mixture was extracted with  $\text{Et}_2\text{O}$  ( $2 \times 20$  mL), washed with brine, dried over  $\text{MgSO}_4$ , and evaporated to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 8:1) to give **17** as colorless oil (34 mg, 33.3% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.03 (s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 2.71 (dd,  $J = 14.7, 6.2$  Hz, 1H), 2.60–2.52 (m, 1H), 2.46 (dd,  $J = 9.1, 3.2$  Hz, 1H), 1.66 (s, 2H), 1.40 (d,  $J = 6.2$  Hz, 2H), 1.32 (s, 3H), 1.24 (s, 3H), 1.06 (s, 3H), 0.99 (d,  $J = 6.7$  Hz, 1H), 0.94 (s, 1H), 0.90 (d,  $J = 2.8$  Hz, 2H), 0.86 (s, 3H), 0.85 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.3, 149.8, 148.8, 148.3, 137.1, 121.2, 111.5, 64.8, 60.4, 57.1, 56.0, 51.8, 48.1, 42.5, 40.2, 37.1, 36.4, 33.4, 33.1, 25.3, 21.1, 20.0, 19.5, 18.35, 16.3. (Figure S6).

Pelorol (**1**): To a solution of methyl ester **17** (11 mg, 0.028 mmol, 1.0 equiv.) in 4 mL  $\text{CH}_2\text{Cl}_2$  was added  $\text{BI}_3$  (129 mg, 0.336 mmol, 12 equiv.) in 2 mL  $\text{CH}_2\text{Cl}_2$  dropwise at  $-78^\circ\text{C}$  under  $\text{N}_2$ . The mixture was stirred for an additional 0.5 h, then poured into  $\text{H}_2\text{O}$  and extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 50$  mL). The combined  $\text{CH}_2\text{Cl}_2$  extracts were dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 3:1) to provide **1** as colorless oil (6.5 mg, 61.6% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.09 (s, 1H), 5.59 (s, 2H), 3.83 (s, 3H), 2.61 (d,  $J = 6.2$  Hz, 1H), 2.54–2.47 (m, 2H), 1.66 (d,  $J = 6.2$  Hz, 2H), 1.58–1.53 (m, 2H), 1.41 (dd,  $J = 8.8, 3.4$  Hz, 2H), 1.25 (s, 3H), 1.23 (s, 3H), 1.05 (s, 3H), 0.98 (d,  $J = 4.5$  Hz, 1H), 0.96–0.93 (m, 1H), 0.85 (d,  $J = 5.6$  Hz, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.3, 149.4, 143.7, 140.6, 130.0, 118.0, 114.8, 65.2, 57.1, 51.7, 48.5, 42.5, 40.2, 37.2, 36.5, 33.3, 33.1, 24.3, 21.1, 19.9, 19.5, 18.4, 16.3. (Figure S12). HRMS (ESI):  $m/z$   $[\text{M}-\text{H}]^-$  calcd. for  $[\text{C}_{23}\text{H}_{32}\text{O}_4]^-$ : 371.2228, found: 371.2220.  $[\alpha]_D^{30} = -64.5$  ( $c = 0.41$ , MeOH).

(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diHydroxy-4,4,6*a*,11*b*-tetramethyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene-7-carboxylic acid (**2**): To a solution of **16** (13 mg, 0.033 mmol, 1.0 equiv.) in 8 mL



CH<sub>2</sub>Cl<sub>2</sub> was slowly added BI<sub>3</sub> (158 mg, 0.396 mmol, 12 equiv.) in 2 mL CH<sub>2</sub>Cl<sub>2</sub> at −78 °C under N<sub>2</sub>. The mixture was stirred for an additional 0.5 h, then poured into H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 50 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 1:1) to provide **2** as colorless oil (7.7 mg, 64.1% yield). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 12.15 (s, 1H), 8.98 (d, *J* = 113.4 Hz, 2H), 6.91 (s, 1H), 2.63 (d, *J* = 12.4 Hz, 1H), 2.34 (d, *J* = 13.2 Hz, 1H), 1.97 (s, 1H), 1.54 (s, 1H), 1.49–1.45 (m, 2H), 1.39–1.33 (m, 2H), 1.21 (s, 3H), 1.16 (d, *J* = 7.1 Hz, 2H), 1.11 (s, 3H), 0.99 (s, 3H), 0.90 (s, 1H), 0.81 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ: 169.1, 147.2, 145.2, 142.9, 130.3, 117.8, 115.1, 65.1, 60.2, 57.1, 48.1, 42.5, 37.1, 36.7, 33.6, 33.2, 24.9, 21.5, 20.1, 19.5, 18.4, 16.5. (Figure S13). HRMS (ESI): *m/z* [M-H]<sup>−</sup> calcd. for [C<sub>22</sub>H<sub>30</sub>O<sub>4</sub>]<sup>−</sup>: 357.2071, found: 357.2059. [α]<sub>D</sub><sup>30</sup> = −11.61 (*c* = 0.37, MeOH).

(1*R*,2*R*,4*aS*,8*aS*)-1-(5-Ethyl-2,3-diMethoxybenzyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (**19**) [14]: Yield 73%, colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.66 (s, 1H), 6.56 (s, 1H), 3.83 (s, 6H), 2.80 (s, 2H), 2.61–2.53 (m, 3H), 1.82 (s, 2H), 1.67 (s, 1H), 1.61 (s, 3H), 1.41 (d, *J* = 6.6 Hz, 2H), 1.33 (d, *J* = 15.8 Hz, 2H), 1.28 (s, 3H), 1.20 (t, *J* = 7.5 Hz, 3H), 1.09 (d, *J* = 3.4 Hz, 1H), 0.90 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ: 152.3, 144.3, 140.0, 137.6, 122.0, 109.6, 73.7, 62.8, 60.56, 56.1, 55.6, 43.7, 41.8, 40.3, 39.4, 33.5, 33.3, 28.8, 25.3, 24.3, 21.6, 20.3, 18.6, 15.7, 15.4. (Figure S7).

(4*aS*,6*aR*,11*aR*,11*bS*)-7-Ethyl-9,10-diMethoxy-4,4,6a,11b-tetramethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1*H*-benzo[*a*]fluorene (**20**) [14]: Yield 92%, colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.53 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.76–2.68 (m, 2H), 2.59 (dd, *J* = 30.3, 10.4 Hz, 2H), 2.41 (d, *J* = 11.8 Hz, 1H), 1.81 (s, 1H), 1.79–1.73 (m, 3H), 1.61 (d, *J* = 13.1 Hz, 3H), 1.44 (d, *J* = 10.6 Hz, 3H), 1.29–1.25 (m, 3H), 1.13 (s, 3H), 1.07 (s, 3H), 1.00 (s, 1H), 0.90 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ: 150.4, 144.8, 143.6, 135.8, 133.7, 111.1, 64.3, 60.3, 57.1, 56.0, 47.9, 42.6, 40.2, 39.3, 37.1, 33.4, 33.1, 25.3, 24.7, 21.4, 21.2, 19.7, 18.4, 16.2, 16.1. (Figure S8).

1-((4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diMethoxy-4,4,6a,11b-tetramethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1*H*-benzo[*a*]fluoren-7-yl)ethanone (**21**) [14]: Yield 27%, white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.81 (s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.64 (d, *J* = 4.3 Hz, 1H), 2.71 (dd, *J* = 14.7, 6.2 Hz, 1H), 2.58 (d, *J* = 13.0 Hz, 1H), 2.52 (s, 3H), 2.32 (d, *J* = 12.5 Hz, 1H), 1.62 (d, *J* = 5.9 Hz, 6H), 1.40 (d, *J* = 9.1 Hz, 2H), 1.25 (s, 3H), 1.14 (s, 1H), 1.05 (s, 3H), 0.92 (s, 1H), 0.85 (d, *J* = 5.6 Hz, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ: 201.9, 149.6, 147.9, 147.6, 137.4, 130.5, 110.0, 65.0, 60.5, 57.2, 56.2, 48.0, 42.5, 40.2, 37.1, 36.4, 33.4, 33.1, 30.3, 25.2, 21.1, 20.6, 19.4, 18.4, 16.3. (Figure S9).

1-((4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diHydroxy-4,4,6a,11b-tetramethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1*H*-benzo[*a*]fluoren-7-yl)ethanone (**3**): To a solution of **21** (34 mg, 0.088 mmol, 1.0 equiv.) in 5 mL CH<sub>2</sub>Cl<sub>2</sub> was slowly added BI<sub>3</sub> (413 mg, 1.05 mmol, 12 equiv.) in 2 mL CH<sub>2</sub>Cl<sub>2</sub> at −78 °C under N<sub>2</sub>. The mixture was stirred for an additional 0.5 h, then poured into H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 30 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 4:1) to provide **3** as colorless oil (23.5 mg, 75% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.94 (s, 1H), 5.80 (d, *J* = 157.3 Hz, 2H), 2.61 (dd, *J* = 14.2, 6.3 Hz, 1H), 2.51 (d, *J* = 12.9 Hz, 1H), 2.46 (s, 3H), 2.41 (d, *J* = 12.6 Hz, 1H), 1.64 (d, *J* = 5.9 Hz, 2H), 1.53 (dd, *J* = 9.7, 3.0 Hz, 2H), 1.64 (d, *J* = 5.9 Hz, 2H), 1.53 (dd, *J* = 9.7, 3.0 Hz, 2H), 1.42 (d, *J* = 4.8 Hz, 2H), 1.26 (s, 6H), 1.23 (s, 2H), 1.04 (s, 3H), 0.84 (d, *J* = 6.3 Hz, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ: 201.5, 148.3, 143.3, 140.8, 130.3, 127.3, 113.7, 65.3, 57.2, 48.5, 42.5, 40.3, 37.1, 36.3, 33.4, 33.1, 29.7, 24.3, 21.1, 20.3, 19.5, 18.4, 16.3. (Figure S14). HRMS (ESI): *m/z* [M-H]<sup>−</sup> calcd. for [C<sub>23</sub>H<sub>32</sub>O<sub>3</sub>]<sup>−</sup>: 355.2279, found: 355.2270. [α]<sub>D</sub><sup>30</sup> = −18.0 (*c* = 0.36, MeOH).

(4*aS*,6*aR*,11*aR*,11*bS*)-7-Ethyl-4,4,6a,11b-tetramethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1*H*-benzo[*a*]fluorene-9,10-diol (**4**): To a solution of **20** (34 mg, 0.092 mmol, 1.0 equiv.) in 5 mL CH<sub>2</sub>Cl<sub>2</sub> was slowly added BI<sub>3</sub> (431 mg, 1.10 mmol, 12 equiv.) in 2 mL CH<sub>2</sub>Cl<sub>2</sub> at −78 °C under N<sub>2</sub>. The mixture was stirred for an additional 0.5 h, then poured into H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 30 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 4:1) to provide **4** as colorless oil



(29.9 mg, 95% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.49 (s, 1H), 2.61 (s, 2H), 2.48 (s, 2H), 2.35 (s, 1H), 1.77 (s, 2H), 1.69 (s, 1H), 1.60–1.53 (m, 3H), 1.43 (d,  $J = 4.1$  Hz, 2H), 1.25 (s, 3H), 1.18 (s, 3H), 1.08 (s, 3H), 1.03 (s, 3H), 0.87 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 144.8, 141.4, 137.9, 130.9, 128.7, 113.7, 64.6, 57.1, 48.1, 42.5, 40.1, 39.3, 37.1, 33.4, 33.1, 24.5, 24.2, 21.4, 21.1, 19.7, 18.4, 16.2, 15.9. (Figure S15). HRMS (ESI):  $m/z$   $[\text{M}-\text{H}]^-$  calcd. for  $[\text{C}_{23}\text{H}_{34}\text{O}_2]^-$ : 341.2486, found: 341.2481.  $[\alpha]_D^{30} = -161.3$  ( $c = 0.54$ , MeOH).

(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diMethoxy-4,4,6*a*,11*b*-tetramethyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene-7-carbaldehyde (**22**): To compound **15** (60 mg, 0.16 mmol, 1.0 equiv.) in 4 mL solvent (THF:acetone:H<sub>2</sub>O = 2:1:1) at room temperature was added NMO (0.7 mL) and H<sub>4</sub>K<sub>2</sub>O<sub>6</sub>Os (1 mg). The reaction mixture was stirred at room temperature for 12 h. Then the mixture was quenched with 5 mL saturated NaHS<sub>2</sub>O<sub>3</sub>, extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 30 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was dissolved in 10 mL CH<sub>2</sub>Cl<sub>2</sub>, then NaIO<sub>4</sub> (68.4 mg in 2 mL H<sub>2</sub>O) was added at room temperature for 3 h. The mixture was extracted with EtOAc (2 × 30 mL), washed with saturated brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 8:1) to provide **22** as colorless oil (36.8 mg, 61% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 10.32 (s, 1H), 7.21 (s, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 2.70 (d,  $J = 6.2$  Hz, 1H), 2.53 (s, 1H), 2.42 (d,  $J = 12.1$  Hz, 1H), 1.87 (s, 1H), 1.73 (d,  $J = 12.8$  Hz, 2H), 1.63 (d,  $J = 3.3$  Hz, 1H), 1.56 (s, 1H), 1.52 (s, 1H), 1.46–1.40 (m, 1H), 1.37 (d,  $J = 13.3$  Hz, 2H), 1.19 (s, 2H), 1.14 (s, 1H), 1.02 (s, 3H), 0.96 (s, 2H), 0.83 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 189.9, 153.2, 150.7, 136.1, 127.0, 108.1, 63.9, 60.4, 56.7, 55.9, 48.7, 42.4, 40.8, 40.1, 37.1, 33.3, 33.0, 25.5, 23.7, 21.1, 19.8, 18.3, 16.1. (Figure S10).

(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diMethoxy-4,4,6*a*,11*b*-tetramethyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene-7-carbonitrile (**23**): To a solution of **22** (49 mg, 0.13 mmol, 1.0 equiv.) and Et<sub>3</sub>N (25.6 mg, 0.14 mmol, 1.1 equiv.) in 2 mL CH<sub>2</sub>Cl<sub>2</sub> was slowly added NH<sub>2</sub>OH·HCl (10 mg, 1.4 mmol, 10 equiv.) at 0 °C. The mixture was stirred for an additional 4 h at room temperature. Then Cl<sub>3</sub>COCOCCl<sub>3</sub> (43 mg, 0.15 mmol, 1.2 equiv.) was added, releasing a large number of bubbles. The reaction mixture was stirred at room temperature for 12 h, then extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 50 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 8:1) to provide **23** as colorless oil (38.7 mg, 79.6% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.86 (s, 1H), 3.91 (s, 3H), 3.83 (s, 3H), 2.71 (d,  $J = 6.2$  Hz, 1H), 2.62 (s, 1H), 2.53 (s, 1H), 1.82–1.76 (m, 1H), 1.72 (d,  $J = 6.5$  Hz, 2H), 1.61 (s, 2H), 1.47 (s, 2H), 1.25 (d,  $J = 5.4$  Hz, 2H), 1.14 (s, 3H), 1.04 (s, 3H), 0.99 (s, 1H), 0.95 (d,  $J = 10.3$  Hz, 1H), 0.88 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 151.8, 149.5, 148.8, 135.5, 117.0, 112.9, 98.2, 63.1, 59.5, 56.2, 55.2, 46.3, 41.5, 39.1, 36.1, 35.8, 32.4, 32.1, 24.4, 20.8, 20.1, 18.3, 17.2, 15.1. (Figure S11).

(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diHydroxy-4,4,6*a*,11*b*-tetramethyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene-7-carbaldehyde (**5**): To a solution of **22** (34 mg, 0.092 mmol, 1.0 equiv.) in 5 mL CH<sub>2</sub>Cl<sub>2</sub> was slowly added BI<sub>3</sub> (431 mg, 1.10 mmol, 12 equiv.) in 2 mL CH<sub>2</sub>Cl<sub>2</sub> at –78 °C under N<sub>2</sub>. The mixture was stirred for an additional 0.5 h, then poured into H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 30 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 4:1) to provide **5** as colorless oil (23.5 mg, 75% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 10.26 (s, 1H), 7.37 (s, 1H), 6.80 (s, 1H), 6.14 (s, 1H), 2.72 (dd,  $J = 14.7, 5.9$  Hz, 1H), 2.54 (d,  $J = 13.4$  Hz, 2H), 1.92 (s, 1H), 1.76 (s, 3H), 1.58 (d,  $J = 12.1$  Hz, 1H), 1.43 (d,  $J = 11.4$  Hz, 2H), 1.26 (s, 2H), 1.22 (s, 3H), 1.07 (s, 3H), 1.00 (d,  $J = 13.8$  Hz, 2H), 0.88 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 190.5, 154.5, 147.3, 142.0, 129.2, 124.3, 111.2, 64.2, 56.8, 49.0, 42.4, 40.7, 40.1, 37.1, 33.3, 33.1, 24.6, 23.8, 21.1, 19.8, 18.3, 16.1. (Figure S16). HRMS (ESI):  $m/z$   $[\text{M}-\text{H}]^-$  calcd. for  $[\text{C}_{22}\text{H}_{30}\text{O}_3]^-$ : 341.2122, found: 341.2107.  $[\alpha]_D^{30} = 48.6$  ( $c = 0.23$ , MeOH).

(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-diHydroxy-4,4,6*a*,11*b*-tetramethyl-2,3,4,4*a*,5,6,6*a*,11,11*a*,11*b*-decahydro-1*H*-benzo[*a*]fluorene-7-carbonitrile (**6**): To a solution of **23** (11 mg, 0.03 mmol, 1.0 equiv.) in 5 mL CH<sub>2</sub>Cl<sub>2</sub> was slowly added BI<sub>3</sub> (140 mg, 0.36 mmol, 12 equiv.) in 1 mL CH<sub>2</sub>Cl<sub>2</sub> at –78 °C under N<sub>2</sub>. The mixture was stirred for an additional 0.5 h, then poured into H<sub>2</sub>O. The solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 30 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered

and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 3:1) to provide **6** as colorless oil (7.6 mg, 75% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.89 (s, 1H), 4.13 (d,  $J = 7.2$  Hz, 2H), 2.60 (dd,  $J = 20.5, 8.7$  Hz, 2H), 2.45 (s, 1H), 2.06 (s, 1H), 1.73 (d,  $J = 12.9$  Hz, 3H), 1.40 (s, 1H), 1.25 (s, 3H), 1.10 (s, 2H), 1.02 (s, 2H), 0.86 (s, 9H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ : 152.9, 145.6, 141.8, 129.8, 118.3, 116.7, 95.3, 64.4, 57.2, 47.6, 42.5, 40.1, 37.0, 36.8, 33.4, 33.1, 24.5, 21.9, 21.1, 19.4, 18.3, 16.2. (Figure S17). HRMS (ESI):  $m/z$   $[\text{M}-\text{H}]^-$  calcd. for  $[\text{C}_{22}\text{H}_{29}\text{NO}_2]^-$ : 338.2126, found: 338.2119.  $[\alpha]_D^{30} = 10.2$  ( $c = 0.53$ , MeOH).

### 3.2. Kinase Assays of PI3K

PI3K reactions were performed in 50 mM HEPES at pH 7.5 with 1 mM EGTA, 100 mM NaCl, 3 mM  $\text{MgCl}_2$ , 2 mM DTT, and 0.03% CHAPS. All tested compounds were dissolved in 100% DMSO. PIP2 and ATP were used as substrates, and the final reaction volume was 10  $\mu\text{L}$ . To evaluate the PI3K $\alpha$  inhibitors, 1.65 nM enzyme, 50  $\mu\text{M}$  PIP2, and 25  $\mu\text{M}$  ATP were used for every 10  $\mu\text{L}$  reaction volume with inhibitor concentrations ranging from 0.5 nM to 10  $\mu\text{M}$ . After incubating for 1 h at room temperature, the reactions were quenched by adding 10  $\mu\text{L}$  of Kinase-Glo reagent. Raw data were collected from Flexstation program.

### 3.3. Antiproliferative Assays in Vitro

The following cell lines, MCF-7, SK-BR-3, K562, HeLa, MOLT-4, DU145, U937, and PC3 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained at 37  $^\circ\text{C}$  in a 5%  $\text{CO}_2$  incubator in DMEM (Gibco, Invitrogen, Carlsbad, CA, USA) or RPMI1640 (Gibco, Invitrogen) containing 10% FBS. Cell proliferation was determined by CCK8 assay (DOjinDo, Kyoto, Japan). Cells were seeded at a density of 800–1000 cells/well in 384 well plates and treated with solvent control or various concentration of compounds. After 72 h incubation, CCK8 reagent was added and absorbance was measured at 450 nm by using Envision 2104 multi-label Reader (Perkin Elmer, Waltham, MA, USA). Dose-response curves were plotted to determine the  $\text{IC}_{50}$  values using Prism 5.0 (Graph Pad software Inc., San Diego, CA, USA).

## 4. Conclusions

Pelorol is natural product isolated from marine organisms, which possesses novel structure with high bioactivity. To solve the source problem of pelorol, we have developed an efficient and practical synthetic route for the preparation of pelorol in large scale. Moreover, the synthesis of some key intermediates has been optimized and scaled up. Five pelorol analogs have been prepared, their PI3K and tumor cell inhibitory activities have also been evaluated. This work is useful for further route optimization, structural modifications, activity assays and large-scale preparation of pelorol and related analogs.

**Supplementary Materials:** The following are available online at [www.mdpi.com/link](http://www.mdpi.com/link), Figures S1–S17:  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compounds.

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**Author Contributions:** Y.L and H.C. performed experiments for the synthesis, structure confirmation, PI3K enzymatic activity assay and antiproliferative assays of the compounds; G.L. supervised the research work; and all authors contributed to writing the paper.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|        |  |
|--------|--|
| CHAPS  | 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate |
| CTAB   | Hexadecyl trimethyl ammonium bromide                       |
| DCC    | Dicyclimide  |
| DIBAL  | Diisobutylaluminium hydride                                |
| DMEM   | Dulbecco minimum essential medium                          |
| DTT    | Dithiothreitol in Ethyl acetate                            |
| EGTA   | Ethylenebis(oxyethylenitrilo)tetraacetic acid              |
| FBS    | Fetal calf serum   |
| HEPES  | 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid    |
| NMO    | 4-Methylmorpholine N-oxide                                 |
| PCC    | Pyridinium chlorochromate                                  |
| PDC    | Pyridinium dichromate                                      |
| PIDA   | Iodobenzene diacetate                                      |
| TBHP   | <i>tert</i> -Butylhydroperoxide                            |
| TLC    | Thin layer chromatography                                  |
| S-Phos | 2-Dicyclohexylphosphino-2',6'-dimethoxy-1,1'-biphenyl      |

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