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Total Synthesis of the G2/M DNA Damage Checkpoint Inhibitor Psilostachyin C

Chao Li,[†] Shanghui Tu,[‡] Shuguang Wen,[‡] Shan Li,[‡] Junbiao Chang,[§] Feng Shao,[‡] and Xiaoguang Lei*[,†,‡]

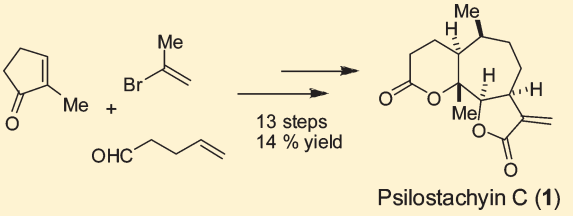
[†]School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China

[‡]National Institute of Biological Sciences (NIBS), Beijing 102206, China

[§]Zhengzhou University, Zhengzhou 450001, China

Supporting Information

ABSTRACT: A concise total synthesis of the G2/M DNA damage checkpoint inhibitor psilostachyin C is reported using a 1,4-addition-aldol condensation-ring-closing metathesis (RCM) strategy. Initial biological studies indicate that psilostachyin C could enhance the sensitivity of the HeLa cell toward camptothecin (CPT) treatment via the activation of the caspase-3 mediated apoptosis pathway.



DNA damage activates cell cycle checkpoints, which arrest cell cycle progression in order to allow for DNA repair.[1] In cancer cells, the G1/S checkpoint is normally inactive due to its dependence on p53, which is mutated in the variety of cancers.[2] Therefore, the mainly p53-independent G2/M checkpoint is likely to play a vital role in tumor cell sensitivity toward many conventional anticancer treatments (including: ionizing radiation, hyperthermia, DNA alkylating agents, and DNA topoisomerase inhibitors, etc.). Small-molecule inhibitors of G2/M DNA damage checkpoint may thus find therapeutical applications in combination with other anticancer agents.[3] Recently, the novel naturally occurring inhibitor of the G2/M checkpoint, psilostachyin C (**1**), was identified by Roberge and co-workers.[4] This molecule falls into a general class of sesquiterpene lactone natural products, which was originally isolated from *Ambrosia psilostachya* DC.[5] This family of natural products has attracted substantial attention from the scientific community for its interesting biological activity as well as complex molecular architecture.[6] Herein, we report a concise strategy for the total synthesis of psilostachyin C.

Our retrosynthetic analysis for psilostachyin C is depicted in Figure 1. According to the previously reported method, psilostachyin C should be prepared from the structurally related natural product damsin **2** through direct Baeyer−Villiger oxidation.[5,6a] Damsin **2** may be derived from a highly functionalized 5,7-bicyclic intermediate **3**. The construction of the seven-membered ring should be achieved by ring-closing metathesis (RCM), which has been proved to be a powerful method for the synthesis of a midsized ring.[7] We envisioned the key intermediate **4** could be rapidly and efficiently prepared from three readily available starting materials through an intermolecular tandem 1,4-addition-aldol condensation.[8]

Initially, we planned to develop a one-pot protocol for the 3-component tandem 1,4-addition-aldol condensation; however, the results were not encouraging.[9] Therefore, we shifted our focus to the stepwise sequence. The synthesis commenced with the commercially available 2-methylcyclopent-2-enone **5** (Scheme 1). Treatment of **5** with freshly prepared isopropenyl magnesium bromide and anhydrous cuprous iodide generated the 1,4-addition intermediate which was effectively trapped with TMSCl to afford trimethylsilyl enol ether **8**.[10] Mukaiyama-aldol condensation between compound **8** and pent-4-enal **7** using $BF_3 \cdot Et_2O$ as an optimal Lewis acid afforded alcohol **4** in 69% yield as a mixture of 4:1 diastereomers.[11] The diastereoselectivity is not important for our synthesis, since we will oxidize the hydroxyl group in the latter step. Ring-closing metathesis (RCM) of compound **4** using Grubbs second-generation catalyst cleanly provided the desired seven-membered ring intermediate **3** in 92% yield.[12] Hydrogenation of the double bond using Adam's catalyst afforded compound **9** in excellent yield and diastereoselectivity (95%, >20:1).

It is not surprising that protection of the sterically hindered ketone moiety on compound **9** is difficult. After screening a number of conditions, we found the desired ketal **10** could be smoothly formed using a combination of 2-methoxy-5,5-dimethyl-1,3-dioxane and 2,2-dimethylpropane-1,3-diol in the presence of a catalytic amount of *p*-TsOH (Scheme 2).[13] Oxidation of alcohol **10** by Dess-Martin periodinane afforded ketone **11**, which was subjected to LDA and α-bromoethylacetate to generate ester **12** (92%).[6b] Hydrolysis and epimerization of **12** under basic conditions afforded acid **13**. The relative stereochemistry of **13** was unambiguously confirmed by the X-ray crystal structure

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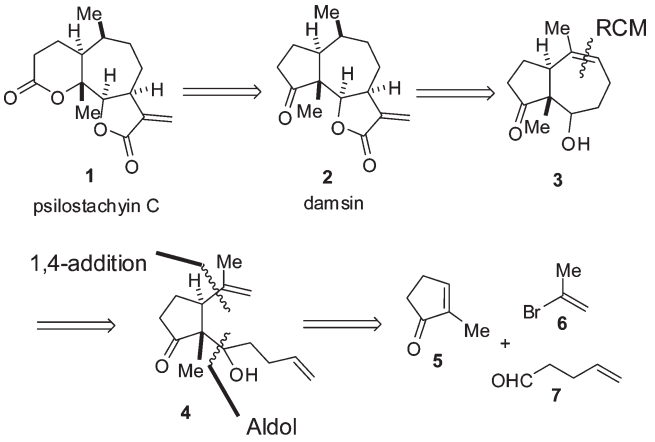
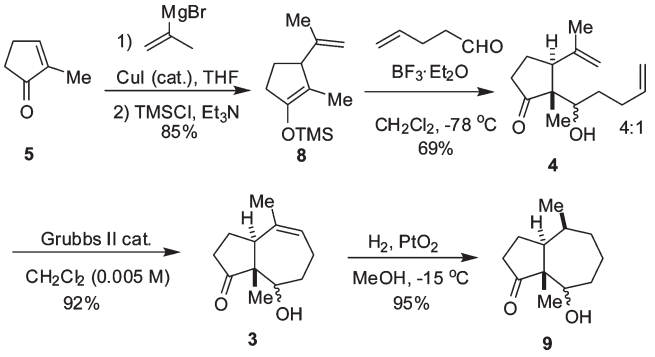


Figure 1. Retrosynthetic analysis for psilostachyin C.

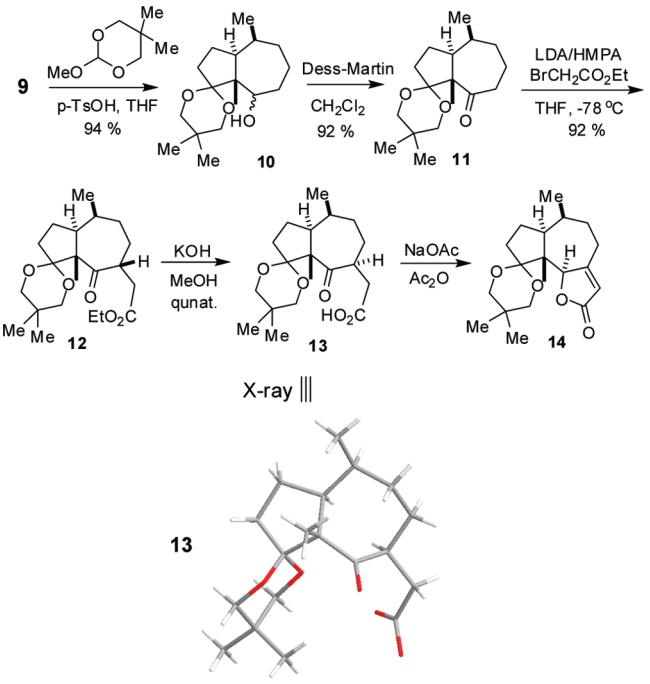
Scheme 1. Synthesis of 5,7-Bicyclic Ketone 9



analysis (see the Supporting Information). Treatment of acid **13** with NaOAc in Ac_2O smoothly afforded the desired lactone **14** which was directly used in the next step.[14]

With the lactone **14** in hand, we evaluated a series of conditions for the installation of *exo*-methylene moiety. After considerable experimentation, we identified a highly efficient two-step protocol to achieve this goal (Scheme 3). Selective hydrogenation of **14** cleanly generated the *cis*-fused tricyclic lactone **15**. Treatment of γ-butyrolactone **15** with NaH and paraformaldehyde under 100 °C in THF in a sealed tube smoothly afforded the desired α-alkylidene-γ-butyrolactone **16**.[15] Removal of dimethylketal under mild acidic conditions furnished damsin **2** in 98% yield. Final transformation using direct Baeyer−Villiger oxidation of damsin to generate psilostachyin C turned out to be a great challenge. Attempted conditions including *m*-CPBA,[16] CH_3CO_3H (15%),[6b] $PhSeO_2H$-H_2O_2 (36%),[17] TFAA-H_2O_2,[18] H_2O_2-NaOH,[19] and Ph_3COOLi[20] led to either poor reactivity or excessive side reactions. After extensive screening of reaction conditions, we were pleased to identify an effective condition using bis(trimethylsilyl)-peroxide $(TMSO)_2$ and TMSOTf,[21] which allowed us to accomplish the total synthesis of racemic psilostachyin C in 60% yield. Synthetic **1** was confirmed to be identical to natural psilostachyin C by ^1H and ^{13}C NMR data and high-resolution mass spectrometry. The relative stereochemistry of psilostachyin C was further unambiguously confirmed by the X-ray crystal structure. Since natural damsin and psilostachyin C are chiral nonracemic substrates,[5]

Scheme 2. Synthesis of Tricyclic Compound 14



further development of an asymmetric strategy by which to gain access to optically active natural products is necessary.

It was previously reported that psilostachyin C could block the G2/M checkpoint upon DNA damage but did not show potentiation of the inhibition effect against cell proliferation in combination with γ irradiation.[4] The biological activity of the structurally related natural product damsin has not been studied to date. While evaluating the biological activity of our synthetic psilostachyin C, we failed to confirm the reported checkpoint inhibitor activity, at least in commonly used HeLa cells. As DNA damage agent is known to induce apoptosis as anticancer agents, we then tested whether psilostachyin C and damsin could sensitize DNA damage and facilitate cell apoptosis. Camptothecin (CPT), a classical DNA damage agent, can trigger basic caspase-3 activation in the damaged HeLa cells.[22] The in vivo substrate of caspase-3, poly(ADP-ribose) polymerase (PARP), can be partially cleaved upon CPT treatment. Herein, caspase-3 and PARP are both intrinsic apoptosis markers.[23] As shown in Figure 2, when HeLa cells were treated with CPT and damsin or psilostachyin C together for 4 h, more caspase-3 was activated than the basic level in DNA damaged cells. In combination with CPT treatment, damsin or psilostachyin C could promote the DNA damaged HeLa cells to apoptosis in a dose dependent manner, whereas the damsin or psilostachyin C alone did not activate caspase-3. Interestingly we found that at the concentration of 50 μM, damsin showed higher potency than psilostachyin C. These results suggest that damsin or psilostachyin C could sensitize cancer cell toward camptothecin (CPT) treatment by the activation of apoptosis. Therefore, damsin or psilostachyin C could serve as a very useful small molecule chemical probe for the identification of its cellular target(s).

In conclusion, the concise total synthesis of the G2/M DNA damage checkpoint inhibitor psilostachyin C has been achieved in only 13 steps with 14% overall yield. The synthesis relies on a

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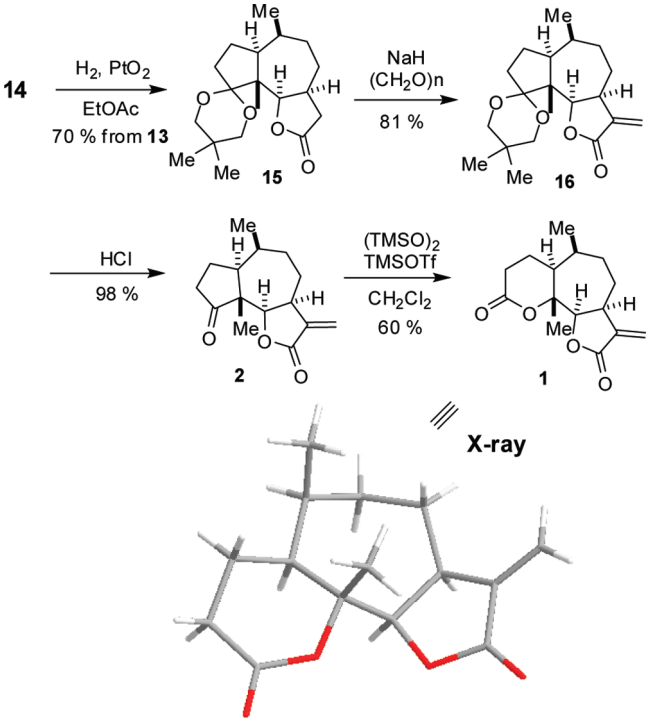
Scheme 3. Total Synthesis of Damsin and Psilostachyin C



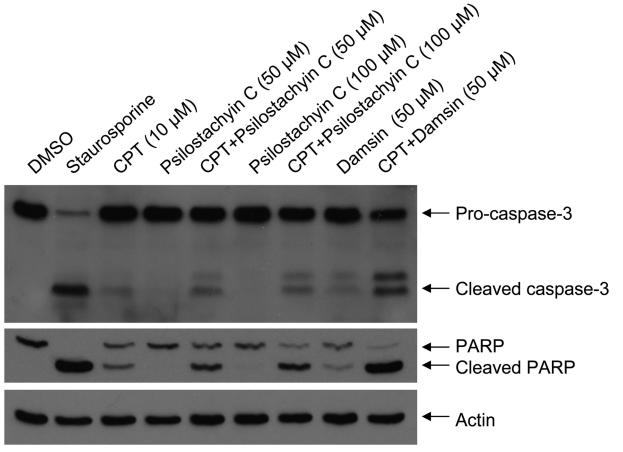


Figure 2. Biological evaluation of damsin and psilostachyin C.

1,4-addition-aldol condensation-RCM strategy to efficiently construct the key 5,7-bicyclic skeleton. Initial biological studies indicate that both damsin and psilostachyin C could enhance the sensitivity of HeLa cell toward camptothecin (CPT) treatment via the activation of caspase-3 mediated apoptosis pathway. Further studies toward the asymmetric synthesis and biological evaluation of damsin and psilostachyin C derivatives to identify suitable small molecule chemical probes for further understanding of the G2/M checkpoint pathway are in progress and will be reported in due course.

EXPERIMENTAL SECTION

(2-Methyl-3-(prop-1-en-2-yl)cyclopent-1-enyloxy)trimethylilane 8. To a solution of isopropenyl magnesium bromide (60.0 mmol, 60 mL) in anhydrous THF was added at −60 °C a mixture of 2-methylcyclopent-2-enone (3.94 mL, 40.0 mmol) and cuprous iodide (760 mg, 4.0 mmol) in anhydrous THF (20 mL). Stirring was continued for 3 h at −40 °C, and an equimolar mixture of trimethylsilylchloride (10.2 mL, 80.0 mmol) and triethylamine (free from triethylamine hydrochloride) (11.1 mL, 80.0 mmol) was added at −15 °C. The resulting mixture was allowed to warm to room temperature and stirred for 3 h. Then the reaction was quenched by addition of water and extracted with pentane. The combined organic phase was washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The enol ether was purified by flash chromatography (pentane) to provide **8** as a colorless oil (7.0 g, 85% yield). Physical properties were identical to those of a previous report.[10]

Diene 4. Boron trifluoride etherate (2.0 mL, 15.5 mmol, 1.0 equiv) was added dropwise to a 0.1 M solution of **8** (3.6 g, 17.1 mmol, 1.1 equiv) and pent-4-enal (1.3 g, 15.5 mmol, 1.0 equiv) in CH_2Cl_2 at −78 °C. The reaction was stirred for 3 h, quenched at −78 °C by addition of an equivalent volume of saturated aqueous $NaHCO_3$, and then warmed to ambient temperature. The mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The aqueous phase was extracted once with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel chromatography (PE/ EtOAc = 10:1) to afford diene **4** (2.3 g, 69%, 4:1 mixture) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer) 5.88−5.78 (m, 1H), 5.08−4.96 (m, 2H), 4.95 (s, 1H), 4.83 (s, 1H), 3.68−3.64 (m, 1H), 3.00 (dd, 1H, J_1 = 8.1 Hz, J_2 = 6.4 Hz), 2.43−2.19 (m, 3H), 2.16−2.08 (m, 2H), 2.04−1.96 (m, 1H), 1.94−1.83 (m, 1H), 1.81 (s, 3H), 1.80−1.72 (m, 1H), 1.63−1.54 (m, 1), 0.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, major isomer) δ 222.7, 144.9, 138.3, 115.1, 114.0, 75.1, 55.9, 48.8, 37.9, 31.3, 31.0, 24.1, 23.3, 14.3; IR (neat) ν_{max} 3461, 3077, 2963, 2918, 2850, 1728, 1641, 1451 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{14}H_{22}NaO_2$, 245.1512; found, 245.1506.

Alcohol 3. A solution of diene **4** (2.3 g, 10.4 mmol) in anhydrous CH_2Cl_2 (2100 mL) was treated with Grubbs' II catalyst (90 mg, 0.104 mmol, 1 mol %). The mixture was stirred at reflux for 1 h and then cooled to room temperature. The mixture was stirred open to air overnight and concentrated in vacuo. Purification by flash chromatography (PE/EtOAc = 5:1) afforded alcohol **3** (1.83 g, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) 5.70−5.67 (m, 1H), 4.37 (s, 1H), 3.69 (dd, 1H, J_1 = 11.2 Hz, J_2 = 7.2 Hz), 2.78 (t, 1H, J = 8.8 Hz), 2.53−2.46 (m, 1H), 2.31−2.21 (m, 1H), 2.09−1.90 (m, 3H), 1.75, s, 3H), 1.71−1.64 (m, 1H), 1.36−1.23 (m, 1H), 0.89(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 227.1, 136.5, 126.4, 77.3, 51.7, 44.6, 37.0, 27.5, 22.8, 22.3, 22.2, 8.5; IR (neat) ν_{max} 3499, 2962, 2935, 2854, 1718, 1446, 1286, 1070, 1050 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{12}H_{18}NaO_2$, 217.1199; found, 217.1192.

Ketone 9. A solution of 1.41 g (7.3 mmol) of alcohol **3** and 100 mg (5 wt %) of platinum oxide in 100 mL of absolute methanol was stirred at −15 °C and under atmospheric hydrogen gas. After 2 h, the uptake of hydrogen ceased and the reaction mixture was filtered and concentrated to afford 1.35 g (95%) of the product **9** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) 4.55 (s, 1H), 3.55 (dd, 1H, J_1 = 11.2 Hz, J_2 = 4.8 Hz), 2.50−2.43 (m, 1H), 2.31−2.21 (m, 1H), 2.16−1.92 (m, 5H), 1.89−1.81 (m, 1H), 1.70−1.63 (m, 1H), 1.52−1.43 (m, 1H), 1.37−1.19 (m, 1H), 1.06 (s, 3H), 1.00 (d, 3H), J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 228.4, 76.7, 54.4, 43.6, 37.6, 36.3, 34.8, 32.9, 23.7, 20.9, 18.8, 11.4; IR (neat) ν_{max} 3490, 2959, 2929, 2860, 1719, 1467, 1411, 1057 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{12}H_{20}NaO_2$, 219.1356; found, 219.1349.

Ketal 10. Ketone **9** (1.18 g, 6.02 mmol) was combined with 2,2-dimethylpropane-1,3-diol (7.51 g, 72.24 mmol, 12 equiv) and crude 2-methoxy-5,5-dimethyl-1,3-dioxane (2.64 g, 18.06 mmol, 3 equiv) in 12 mL of anhydrous THF containing *p*-TsOH (58 mg, 0.33 mmol, 0.05 equiv) at 23 °C. The reaction was stirred at room temperature overnight and quenched with half-saturated aqueous $NaHCO_3$. Then the aqueous

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phase was extracted with EtOAc, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product. Purification by the silica gel chromatography (PE−EtOAc = 10:1) provided 1.6 g (94%) of ketal **10** as a white solid. Mp 70−71 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.14 (dd, 1H, J_1 = 11.2 Hz, J_2 = 4.8 Hz), 3.75−3.73 (m, 2H), 3.57−3.39 (m, 3H), 2.38−2.32 (m, 1H), 2.16−2.09 (m, 1H), 2.00−1.67(m, 5H), 1.63−1.52 (m, 2H), 1.47−1.40 (m, 1H), 1.36−1.22 (m, 2H), 1.21 (s, 3H), 1.01 (s, 3H), 0.97 (d, 3H, J = 5.1 Hz), 0.74 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.1, 74.7, 72.9, 70.6, 54.7, 40.3, 36.7, 35.0, 32.7, 30.0, 24.9, 23.5, 23.0, 22.3, 21.0, 19.0, 11.8; IR (neat) ν_{max} 3536, 2953, 2927, 2865, 1728, 1470, 1301, 1260, 1124 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{17}H_{30}NaO_3$, 305.2087; found, 305.2084.

Ketone 11. A solution of ketal **10** (1.47 g, 5.18 mmol) in 10 mL of anhydrous CH_2Cl_2 was added to a solution of Dess-Martin periodinane (3.3 g, 7.8 mmol, 1.5 equiv) in 10 mL of anhydrous CH_2Cl_2 with stirring. After 2 h, the reaction mixture was diluted with ether and the resulting suspension was added to 50 mL of 1.3 M NaOH aq. After the mixture was stirred for 10 min, the ether layer was washed with 50 mL of 1.3 M NaOH, 50 mL of water, and brine. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (PE−EtOAc = 10:1) provided 1.35 g (92%) of ketone **11** as a white solid. Mp 108−109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.57 (d, 1H, J = 11.2 Hz), 3.47−3.38 (m, 2H), 3.28−3.35 (m, 1H), 3.08−3.00 (m, 2H), 2.48−2.32 (m, 2H), 2.05−2.02 (m, 1H), 1.85−1.62 (m, 6H), 1.44−1.37 (m, 1H), 1.26 (s, 3H), 1.03 (s, 3H), 0.96 (d, 3H, J = 7.6 Hz), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.6, 110.6, 72.2, 70.5, 61.3, 43.1, 43.0, 35.0, 34.0, 30.2, 27.1, 23.9, 22.4, 21.9, 20.6, 16.9, 14.3; IR (thin film) ν_{max} 2957, 2916, 2861, 1703, 1473, 1308, 1175, 1133, 1070 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{17}H_{28}NaO_3$, 303.1931; found, 303.1927.

Keto Ester 12. To a stirred solution of diisopropylamine (233 μL, 1.65 mmol, 1.1 equiv) in 2 mL of anhydrous THF at −78 °C was added a solution of *n*-butyllithium (630 μL 2.5 M, 1.58 mmol, 1.05 equiv) in hexane. Then the reaction mixture was warmed to 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was cooled to −78 °C, and a solution of ketone **11** (423 mg, 1.5 mmol) in 3 mL of anhydrous THF was added. After 1 h, the reaction mixture was warmed to −20 °C slowly. Then the reaction mixture was cooled down to −78 °C again and a solution of ethyl bromoacetate (183 μL, 1.65 mmol, 1.1 equiv) and HMPA (287 μL, 1.65 mmol, 1.1 equiv) in 1 mL of anhydrous THF was added. The reaction mixture was warmed to room temperature over 2 h. Then the reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with water and brine subsequently, dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash column chromatography (PE−EtOAc = 5:1) to give keto ester **12** (498 mg, 92% yield) as a white solid. Mp 84−85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.12 (q, 2H, J = 7.2 Hz), 3.57 (d, 1H, J = 11.2 Hz), 3.48−3.38 (m, 2H), 3.32−3.28 (m, 1H), 3.16−3.10 (m, 1H), 3.06−3.00 (m, 1H), 2.61 (d, 2H, J = 8.0 Hz), 2.41−2.34 (m, 1H), 2.03−1.78 (m, 4H), 1.68−1.43 (m, 3H), 1.29−1.17 (m, 4H), 1.11 (s, 3H), 1.03 (s, 3H), 1.00 (d, 3H, J = 7.2 Hz), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.4, 172.4, 110.1, 72.0, 70.4, 62.7, 60.3, 52.1, 39.9, 38.8, 34.5, 32.3, 30.2, 26.6, 26.3, 22.6, 22.3, 21.8, 16.9, 16.3, 14.2; IR (neat) ν_{max} 2952, 2858, 1734, 1689, 1472, 1180, 1134, 1124 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{21}H_{34}NaO_5$, 389.2299; found, 389.2289.

Acid 13. The solution of 200 mg (0.55 mmol) of keto ester **12** and 150 mg of potassium hydroxide in 3 mL of methanol was heated at reflux for 2 h. The solution was cooled, poured into water, and washed with ether. The aqueous layer was carefully acidified with 2 M HCl, and the product was isolated with ether. After concentration in vacuo, the crude product was purified by flash column chromatography (PE−EtOAc = 3:1) to give the acid **13** (185 mg, 100%) as a white solid. Mp 148−149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.72 (m, 1H), 3.54, d, J = 11.2 Hz, 3.44−3.36 (m, 2H), 3.27−3.23 (m, 1H), 3.19−3.13 (m, 1H), 2.70 (dd, 1H, J_1 = 16 Hz, J_2 = 6 Hz), 2.41−2.18 (m, 2H), 2.06−1.91 (m, 2H), 1.86−1.66 (m, 4H), 1.46−1.32 (m, 2H), 1.12 (s, 3H), 0.96−0.94 (m, 6H), 0.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.9, 178.2, 110.7, 72.2, 70.4, 61.4, 45.8, 42.3, 36.9, 34.2, 33.1, 30.1, 29.0, 27.4, 23.8, 22.1, 21.9, 17.3, 13.8; IR (neat) ν_{max} 2958, 2866, 1739, 1711, 1474, 1306, 1124, 1067 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{19}H_{30}NaO_5$, 361.1986; found, 361.1983.

Lactone 15. A mixture of 75 mg (0.22 mmol) of acid **13** and 273 mg (3.3 mmol) of sodium acetate in 5 mL of acetic anhydride was heated at 150 °C for 1 h. The resulting mixture was cooled to 0 °C, and 2 mL of EtOAc was added followed by filtration. The filtrate was concentrated in vacuo to provide 53 mg (75%) of butenolide **14** as a colorless oil. The crude product **14** was directly used for the next step. To the solution of **14** (53 mg, 0.17 mmol) in 4.0 mL of anhydrous EtOAc was added 8 mg of PtO_2. The reaction mixture was placed under atmospheric hydrogen gas and stirred at 0 °C for 12 h. The resulting mixture was filtered through Celite, and the filtrate was then concentrated in vacuo and purified by flash column chromatography (PE−EtOAc = 10:1) to give 48 mg (93%) as a white solid. Mp 136−137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 5.17 (d, 1H, J = 8.4 Hz), 3.67 (d, 1H, J = 11.6 Hz), 3.42 (m, 3H), 2.80 (m, 1H), 2.67 (dd, 1H, J_1 = 17.2 Hz, J_2 = 9.2 Hz), 2.43−2.34 (m, 1H), 2.24 (dd, 1H, J_1 = 17.6 Hz, J_2 = 8.4 Hz), 2.16−2.09 (m, 1H), 2.04−1.99 (m, 1H), 1.89−1.53 (m, 6H), 1.25 (s, 3H), 1.08 (s, 3H), 1.06 (d, 3H, J = 7.6 Hz), 0.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 109.7, 83.3, 72.1, 71.0, 54.7, 44.9, 40.3, 37.6, 34.3, 33.7, 30.2, 25.3, 24.9, 24.2, 23.0, 22.1, 15.2, 13.6; IR (neat) ν_{max} 2950, 2862, 1772, 1471, 1130, 1112 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{19}H_{30}NaO_4$, 345.2036; found, 345.2030.

α-Methylene Lactone 16. To a solution of lactone **15** (44 mg, 0.14 mmol) in 2 mL of anhydrous THF in a sealed tube was added dry paraformaldehyde (130 mg, 4.3 mmol) and NaH (19 mg, 60 wt %, 0.46 mmol). The resulting mixture was stirred for 15 min at 100 °C before the resulting brown solution was cooled to 0 °C. The reaction mixture was diluted with EtOAc, washed by water. The aqueous layer was extracted twice with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (EA/PE = 1:9) to give the product **16** (40 mg, 81%) as a white solid. Mp 141−142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 6.16 (d, 1H, J = 3.6 Hz), 5.42 (d, 1H, J = 3.6 Hz), 5.24 (d, 1H, J = 9.2 Hz), 3.67 (d, 1H, J = 13.2 Hz), 3.43−3.40 (m, 3H), 3.35−3.28 (m, 1H), 2.44−2.38 (m, 1H), 2.16−2.09 (m, 1H), 2.05−2.00 (m, 1H), 1.95−1.90 (m, 2H), 1.86−1.81 (m, 2H), 1.65−1.57 (m, 3H), 1.28 (s, 3H), 1.03 (d, 3H, J = 8.0 Hz), 0.93 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 141.7, 118.6, 109.6, 81.5, 72.2, 71.1, 55.1, 45.1, 43.7, 33.6, 33.3, 30.3, 25.2, 24.4, 24.2, 23.0, 22.2, 14.8, 12.5; IR (neat) ν_{max} 2949, 2866, 1758, 1474, 1152, 1112 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{20}H_{30}NaO_4$, 357.2036; found, 357.2031.

Damsin 2. A solution of **16** (37 mg, 0.111 mmol) and HCl (0.3 mL, 0.2 M) in THF (1.5 mL) was stirred at room temperature for 20 h and poured into a saturated aqueous solution of $NaHCO_3$ (20 mL). The reaction mixture was extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (PE−EtOAc = 3:2) to give 27 mg (98%) of damsin **2** as a white solid. Mp 107−108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 6.26 (d, 1H, J = 3.2 Hz), 5.54 (d, 1H, J = 3.2 Hz), 4.53 (d, 1H, J = 8.8 Hz), 3.30−3.28 (m, 1H), 2.50−2.43 (m, 1H), 2.29−2.20 (m, 2H), 2.10−1.96 (m, 3H), 1.90−1.80 (m, 3H), 1.78−1.69 (m, 1H), 1.09 (s, 3H), 1.08 (d, 3H, J = 12.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.7, 170.1, 139.6, 120.8, 81.7, 54.9, 46.1, 44.4, 36.0, 34.3, 33.4, 25.7, 23.9, 15.8, 13.8; IR (neat) ν_{max} 2923, 2872, 1755, 1738, 1270, 1160, 1014 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{15}H_{20}NaO_3$, 271.1305; found, 271.1300.

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Psilotachyin C 1. To a solution of the TMSOTf (1 mg, 0.0044 mmol, 0.1 equiv) in 0.5 mL of anhydrous CH_2Cl_2 was slowly added bis(trimethylsilyl)peroxide $(TMSO)_2$ (40 mg, 0.22 mmol, 5 equiv) at −78 °C, followed by a solution of damsin **2** (11 mg, 0.044 mmol) in 0.2 mL of anhydrous CH_2Cl_2. The reaction mixture was warmed to −50 °C and stirred at this temperature for 24 h, before quenched by an ice cooled, saturated $NaHCO_3$ aqueous solution (5 mL). The mixture was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by reverse phase HPLC [(50% water−50% methanol → 0% water−100% methanol (10 min), (15 mL/min)] to give 7 mg (60%) of psilotachyin C **1** as a white solid. Mp 223−225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 6.26 (d, 1H, J = 3.2 Hz), 5.51 (d, 1H, J = 3.2 Hz), 4.65 (d, 1H, J = 9.6 Hz), 3.42−3.39 (m, 1H), 2.72−2.65 (m, 1H), 2.52−2.42 (m, 1H), 2.21−2.18 (m, 1H), 2.17−2.02 (m, 2H), 1.97−1.92 (m, 1H), 1.87−1.67 (m, 4H), 1.31 (s, 3H), 1.02 (d, 3H, J = 15.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 168.9, 138.4, 120.4, 89.8, 86.2, 77.3, 43.2, 41.4, 35.3, 31.7, 30.9, 24.0, 22.5, 18.9, 14.4; IR (thin film) ν_{max} 2918, 2882, 1761, 1729, 1259, 1235, 1145, 984 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{15}H_{20}NaO_4$, 287.1254; found, 287.1255.

CCDC-806549 (**1**) and CCDC-760919 (**13**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retriev-ing.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax, (+44) 1223−336−033; or deposit@ccdc.cam.ac.uk).

Biological Evaluation of Psilostachyin C (1) and Damsin (2). *Materials and Methods.* HeLa cells were grown in modified Eagle's medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37° in a 5% CO_2 incubator. To induce DNA damage, camptothecin (CPT) was added into cell culture medium at 10 μM for 4 h. Damsin and psilostachyin C at indicated concentrations were also added for 4 h alone or together with CPT. Staurosporine was used at 2 mM for 4 h to induce apoptosis. Following drug treatment, cells were collected and lysed with cell lysis buffer. Proteins in cell lysates were separated on a 4−20%-gradient sodium dodecyl sulfate polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat milk buffer, first probed with antibodies recognizing caspase-3, PARP, or actin, and subsequently probed with horseradish peroxidase-conjugated secondary antibodies. Antibody-recognized protein bands on the membrane were visualized by chemiluminescence resulting from peroxidase-catalyzed substrate oxidation.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of all new compounds and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: leixiaoguan@nibs.ac.cn

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