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PAPER

A novel D-ring modified taxoid: synthesis and biological evaluation of a γ-lactone analogue of docetaxel†

Feng Gao,[a,b] Zhan-Kun Yang,[a] Qiao-Hong Chen,[a] Xiao-Guang Chen[c] and Feng-Peng Wang*[a]

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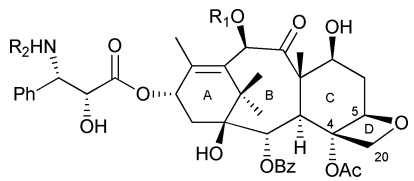
The synthesis of a novel D-ring modified docetaxel analogue, in which the oxetane ring is replaced with a γ-lactone, was achieved from 10-deacetylbaccatin III. The key steps of the synthesis include the direct acetylation of the secondary hydroxyl group at C-5 and D-ring opening and intramolecular aldol reaction to form the γ-lactone. In MTT assays, this analogue proved to have equipotent cytotoxicity relative to paclitaxel towards HCT8, HePG2 and BGC23 cancer cell lines, and be more potent than paclitaxel against A549 and A375. It represents the first example of D-ring modified taxoids with significant cytotoxicity.

Introduction

The anticancer drug paclitaxel (Taxol, **1**) and its semisynthetic analogue docetaxel (Taxotere, **2**) (Fig. 1), being clinically used in the treatment of ovarian cancer, breast cancer, and non-small-cell lung cancer, have contributed significantly to human health.[1]



Paclitaxel (**1**) R_1 = Ac; R_2 = Bz
Docetaxel (**2**) R_1 = H, R_2 = Boc

Fig. 1 Structures of paclitaxel and docetaxel.

The early studies of their structure–activity relationships established that the oxetane D-ring is essential for biological activity. This was concluded from the fact that all of the synthesized D-*seco* analogues at that time, such as **3** (Fig. 2),[2] did not show activity in both cytotoxicity and tubulin assembly assays. It was also proposed that the oxetane oxygen is very important for biological activity, according to the observations that the replacement of the oxetane oxygen with other atoms, such as analogues **4**,[3] **5**,[4] and **6**,[5] diminishes greatly the cytotoxic activity and the interaction with microtubules. With respect to microtubule binding, the oxetane D-ring in paclitaxel was proposed to serve two functions by the NMR study[6] and the Taxol-epothilone minireceptor model:[7] (i) the oxetane oxygen might serve as a hydrogen-bond acceptor; and (ii) the four-membered ring might operate to rigidify the paclitaxel

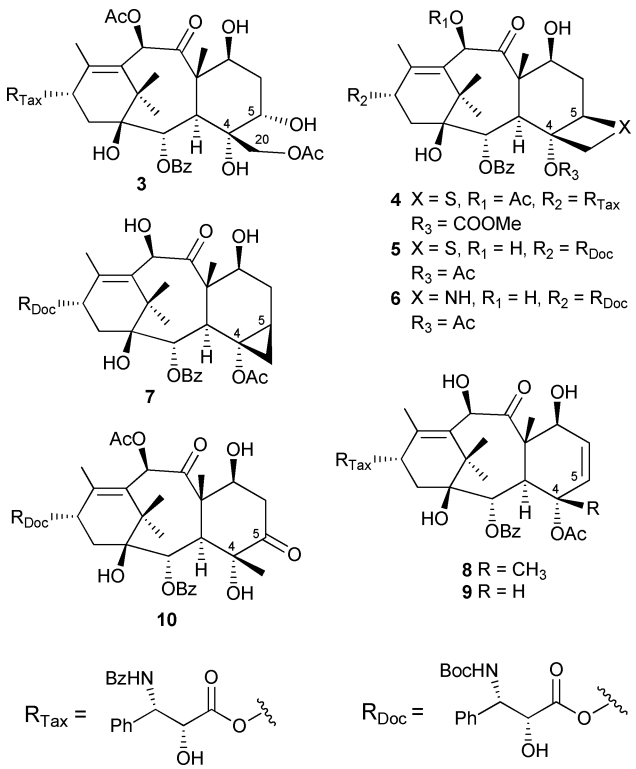


Fig. 2 Representatives of D-ring modified analogues of paclitaxel and docetaxel.

[a]*Department of Chemistry of Medicinal Natural Products, Sichuan University, No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China. E-mail: wfp@scu.edu.cn; Fax: +86-28-85501368; Tel: +86-28-85501368*
[b]*Department of Chinese Traditional Medicine, Agronomy College, Sichuan Agricultural University, No. 221, Huiming Road, Wenjing Region, Chengdu 611130, P. R. China*
[c]*Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, P. R. China*

† Electronic supplementary information (ESI) available: Key NOE relationships of compounds **15** and **16**, and NMR spectra for all new compounds. See DOI: 10.1039/c1ob06535a

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core and thereby enforce a favorable conformational bias on the side chains at C-2, C-4, and C-13.

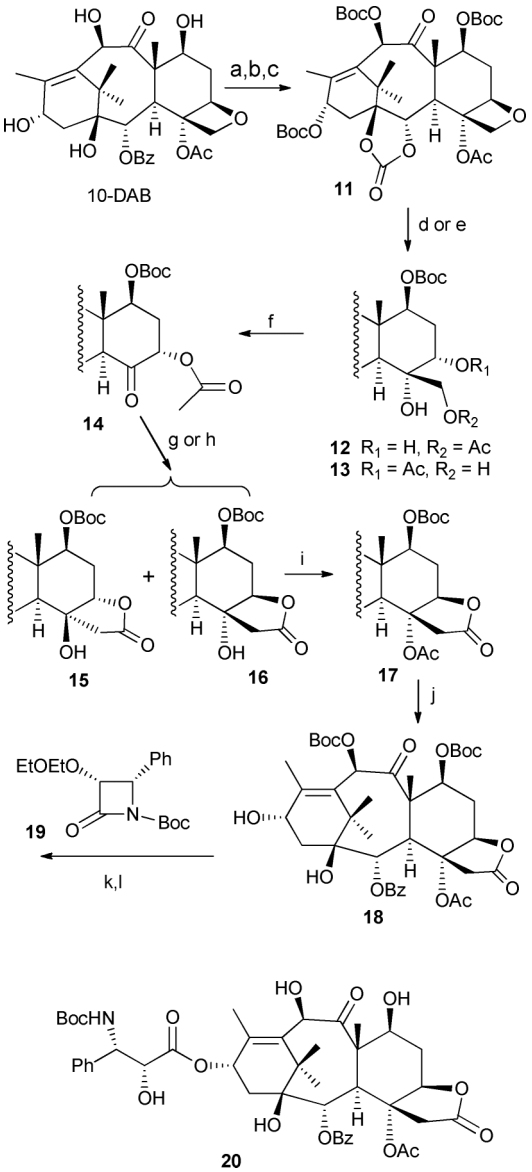
Later, the fact that cyclopropane analogue **7**[8] and D-seco analogues **8–10**[9–11] showed significant activities at microtubule stabilization demonstrated that neither the intact oxetane ring nor an oxygen on C-5 are necessary for efficient binding of taxanes to tubulin. For example, the analogues **7**[8] and **8**[9] are equipotent with paclitaxel in promoting the polymerization of tubulin to microtubules. The ratios IC_{50}(**7**)/IC_{50}(paclitaxel) and IC_{50}(**8**)/IC_{50}(paclitaxel) are 1.2 and 1.0, respectively. However, all of the reported D-ring modified analogues of paclitaxel and docetaxel have very low or no activity in cytotoxicity assays. It is envisioned that the oxetane oxygen might play an important role in cytotoxicity based on the lack of cytotoxicity of the microtubule stabilizer 5(20)-deoxydocetaxel. Consequently, we designed a new lactone analogue of docetaxel (**20**), in which an additional oxygen atom was incorporated into the D-ring and the γ-lactone was expected to play a similar role to the oxetane ring.

Results and discussion

Our synthetic strategy was to reconstruct the D-ring as a γ-lactone starting from commercially available 10-deacetylbaccatin III (10-DBA). The construction of the γ-lactone was envisioned with an intramolecular aldol reaction (Scheme 1) by analogy with Wender's successful construction of the oxetane D-ring through the photolysis of α-methoxy ketone.[12] For the aldol reaction, an acetoxy group at C-5α and a carbonyl group at C-4 are prerequisites. This functionality could be introduced through an intramolecular transacylation between C-4 and C-5, while the oxetane D-ring opening could be mediated by Lewis acid.

Starting from 10-DBA, the hydroxyl groups at C-7, C-10, and C-13 were protected with Boc_2O using DMAP as base. These hydroxyl groups in 10-DBA were mostly protected as TES ethers in the literature. However, we observed that the TES ethers are sensitive to Lewis acid during the opening of the oxetane ring. After screening the various protecting groups, Boc was selected as the optimized one. Selectively reductive deacylation of the benzoate at C-2 was completed with Red-Al at low temperature according to the procedure described in the literature.[13] Formation of the C-1, C-2 cyclic carbonate was easily achieved by addition of triphosgene to yield compound **11** (Scheme 1).

And then, Lewis acid-promoted opening of the oxetane ring was explored on compound **11**. The mechanism of the oxetane ring opening catalyzed by Lewis acid involves the formation of an orthoester between C-4, C-5, and C-20. The hydrolysis of this orthoester afforded the C-5 and C-20 acetoxy derivatives,[5] with C-20 acetoxy derivative as the major product. Unsurprisingly, treatment of **11** with $BF_3{\cdot}OEt_3$ at 0 °C generated **12** with a C-20 acetoxy group in 85% yield, as well as trace amounts of our desired product **13** (10%). To increase the yield of expected product **13**, we examined various Lewis acids, such as $AlCl_3$, $SnCl_4$, $TiCl_4$ *etc.*, in varied solvents, concentrations, and reaction temperature. Gratifyingly, it was found that treatment of a highly diluted solution of **11** (50 mg) in DCM (100 mL) with 1 equivalent of $TiCl_4$ at 25 °C for 10 min could produce the expected product **13** with an acetoxy group at C5 in 93% yield. The subsequent oxidation of the vicinal glycol at C-4 and C-20 with lead tetraacetate yielded the α-acetoxy ketone **14** in excellent yield.



Scheme 1 Synthesis of lactone analogue of docetaxel: Reagents and conditions: (a) $(Boc)_2O$, DMAP, CH_2Cl_2–THF (1 : 1), (95%); (b) Red-Al (4 equiv), THF, −15 °C (85%); (c) triphosgene, CH_2Cl_2–pyridine (80 : 20), −10 °C (92%); (d) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −5 °C; (**12**, 64%; **13**, 10%); (e) $TiCl_4$ (1 equiv), CH_2Cl_2 (2 mL for each mg of substrate) (**13**: 93%); (f) LTA, CH_3CN (85%); (g) LDA, HMPA, THF, −78 °C (**15**: 23%; **16**: 42%); (h) KO*t*Bu, HMPA, THF, −78 °C (**16**: 65%); (i) Ac_2O, DMAP (65%); (j) PhLi, THF, −78 °C (35%); (k) NaH (30 equiv), THF, rt (74%); (l) 1 M HCl, THF, 40 °C (60%).

With the critical intermediate **14** in hand, we started to explore the possibility of its intramolecular aldol reaction. Initially, α-acetoxy ketone **14** was subjected to aldol reaction with LDA and HMPA at −78 °C to generate a pair of diastereoisomers **15** and **16**. The structures of **15** and **16** were established by careful interpretation of their 1D and 2D NMR spectra. The configurations at C-4 and C-5 in **15** were determined based on the critical NOE correlations between H-5β and H-2β, and between H_2-20 and H-3 (Fig. 3). Similarly, the key NOE correlations between H-5α/H-3α, and H-5α/H-7α could suggest the β-orientation of the γ-lactone (Fig. 4). The distinguishable configurations at C-4 and C-5 in **15**

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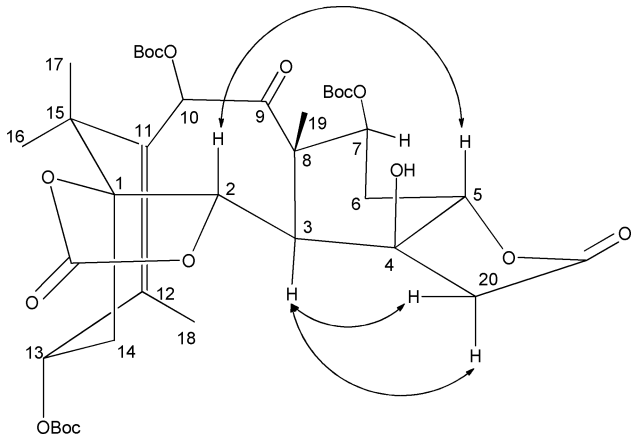

Fig. 3 Key NOE correlations of compound **15**.

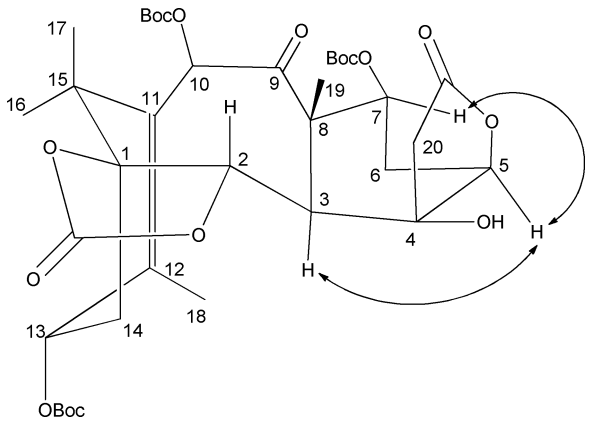

Fig. 4 Key NOE correlations of compound **16**.

and **16** could be explained by the enolization of C-4 ketone during the aldol reaction. We also observed that the acetoxy group at C-5α in **14** could be epimerized to the C-5β position during the chromatography process through a silica gel column. Encouraged by the successful synthesis of the γ-lactone D-ring, we began our optimization by testing the effect of several bases, such as KO^{t}Bu, LiHMDS, LDA *etc.* Finally, it was found that employing KO^{t}Bu as base could lead to the exclusive generation of the expected product.

The docetaxel analogue **20** was synthesized from the key intermediate **16** employing the Holton–Ojima method.[14] The hydroxyl group at C-4 in **16** was acetylated by reacting with acetic anhydride in the presence of DMAP. The regioselective opening of the C-1, C-2 carbonate and the benzoylation at C-2 in **17** were achieved simultaneously by reacting with phenyllithium. Luckily, the *t*-butyloxycarbonyl group at C-13 in the key intermediate **18** obtained in the above-mentioned step was selectively removed. The docetaxel side chain was then introduced to C-13 of compound **18** through coupling with commercially available β-lactam **19** in the presence of excess sodium hydride. Finally, the global deprotection of the product obtained from the above-mentioned step with 1 M hydrochloric acid furnished the expected docetaxel analogue **20**.

The cytotoxicity of docetaxel analogue **20** was evaluated against a small panel of human cancer cell lines[15] As shown in Table 1, compound **20** showed equipotent cytotoxicity relative to paclitaxel

Table 1 Cytotoxic data of compound **20** and paclitaxel

	IC_{50}, (μM)				
Cmpd	HCT8	HePG2	BGC823	A549	A375
Paclitaxel	4.49 ± 0.83	0.12 ± 0.47	2.42 ± 0.76	2.76 ± 1.53	2.6 ± 2.0
20	3.74 ± 1.90	0.11 ± 0.006	3.6 ± 2.26	0.13 ± 0.04	0.15 ± 0.04

Note: HCT8: human colon cancer cell line; HePG2: human liver cancer cell line; BGC-823: human stomach cancer cell line; A549: human lung cancer cell line; A375: human melanoma cell line.

Table 2 Comparison of key coupling constants (in Hz) of **20** with those of paclitaxel, docetaxel and representatives of D-ring modified analogues

Comp.	$J_{13\text{-}14a}$	$J_{13\text{-}14b}$	$J_{2\text{-}3}$
Paclitaxel	9.0	9.0	7.0
Docetaxel	8.0	8.0	7.0
3[13]	4.4	10.3	5.3
8[9]	7.3	9.4	7.4
9[10]	6.0	10.0	5.5
10[11]	8.0	8.0	7.0
20	9.6	9.6	7.2

towards HCT8, HePG2 and BGC23 cancer cell lines; it is more potent than paclitaxel against A549 and A375 cancer cell lines. These results suggested that the incorporation of an additional oxygen atom on D-ring could improve the cytotoxicity towards certain kinds of cancer cell lines. It is important to note that lactone **20** is the first D-ring modified analogue of paclitaxel and docetaxel that showed superior cytotoxic activity. After careful analysis of the coupling constants between H-13 and H-14a/H-14b, and between H-2 and H-3 in the D-ring modified analogues of paclitaxel (Table 2), it was found that these coupling constants of compound **20** are very close to those of paclitaxel. Compounds **8** and **10**, possessing microtubule disassembly activity comparable to paclitaxel but lacking cytotoxicity, are the compounds next to compound **20** according to the similarity of their coupling constants at these positions to paclitaxel. The coupling constants might reflect, at least in part, the conformation of the whole molecule.[9,16] Accordingly, the above-mentioned research results might suggest: (i) that the oxetane D-ring is not necessary for the cytotoxicity of paclitaxel; (ii) that the oxygen of the D-ring seems to be important to the cytotoxicity; and (iii) the conformation of the paclitaxel core structure remains after replacement of the oxetane D-ring with a γ-lactone ring.

Conclusions

In summary, we have successfully synthesized a novel D-ring modified docetaxel analogue, in which the oxetane ring is replaced with a γ-lactone, in 10 steps and 6% overall yield from the commercially available 10-deacetylbaccatin III (10-DBA). The critical reactions include the direct regioselective acetylation of the secondary hydroxyl group at C-5 and D-ring opening and an intramolecular aldol reaction to form the γ-lactone. Compound **20** represents the first example of the D-ring modified taxoids with significant cytotoxicity.

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Experimental

General procedures

NMR spectra were obtained on a Varian Unity INOVA 400/54 NMR spectrometer in $CDCl_3$ with TMS as the internal standard. Mass spectra were obtained on a VG Auto spec 3000 or on a Finnigan MAT 90 instrument. Optical rotations were measured on a Perkin–Elmer 341. Silica gel H (Qingdao Sea Chemical Factory, Qingdao, People's Republic of China) was used for column chromatography. Spots on TLC (silica gel G) were detected by spraying with H_2SO_4–EtOH. Commercially available reagents and solvents were directly used without further purification. All anhydrous reactions were performed under argon. Dichloromethane was distilled from calcium hydride, and THF was distilled from sodium–benzophenone. Cytotoxicity were carried out according to the protocols described in the literature. The "standard workup" means: the reaction mixture was extracted with EtOAc, the combined extracts were washed with water or brine and dried over anhydrous Na_2SO_4, and the organic solvent was removed under reduced pressure.

Preparation of 7,10,13-triBoc-10-DAB (a). To a stirred solution of 10-DAB (100 mg, 0.18 mmol) in anhydrous CH_2Cl_2–THF (1 : 1) at room temperature were added $(Boc)_2O$ (240 mg, 1.1 mmol) and DMAP (30 mg). The reaction mixture was stirred under argon for 12 h (monitored by TLC, cyclohexane–acetone 4 : 1) prior to the addition of water. After standard workup, the residue was subjected to column chromatography over silica gel (cyclohexane–acetone 8 : 1) to yield 7,10,13-triBoc-10-DAB (147 mg, 95%) as an amorphous solid: $[\alpha]_D^{25}$ +9.6 ($CHCl_3$, *c* 1.2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31–8.11 (10H, m), 6.27 (1H, s), 5.86 (1H, t, J = 8.0 Hz), 5.64 (1H, d, J = 6.8 Hz), 5.40 (1H, dd, J_1 = 6.8 Hz, J_2 = 10.4 Hz), 4.97 (1H, br.d, J = 8.0 Hz), 4.13, 4.32 (each 1H, ABq, J = 8.8 Hz), 3.96 (1H, d, J = 6.8 Hz), 1.50, 1.49, 1.48 (each 9H, s); MS (ESI, MeOH) m/z 845 $[M+H]^+$.

Preparation of 7,10,13-TriBoc-2-debenzoate-10-DAB (b). To a stirred solution of 7,10,13-TriBoc-10-DAB (147 mg, 0.175 mmol) in anhydrous THF (10 mL) at −15 °C was added $NaAlH_2(OCH_2CH_2OCH_3)_2$ (Red-Al, 3.5 M in toluene, 0.5 mL), and the reaction mixture was stirred at −15 °C for 24 h prior to addition of water. 1% Hydrochloric acid was added to adjust pH to 3. After standard workup, the residue was purified on column chromatography over silica gel (cyclohexane–acetone 6 : 1) to obtained 7,10,13-triBoc-2-debenzoate-10-DAB (110 mg, 85%) as an amorphous solid: $[\alpha]_D^{25}$ +15.2 ($CHCl_3$, *c* 1.1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.19 (1H, s), 5.83 (1H, t, J = 8.0 Hz), 5.36 (1H, dd, J = 10.4, 6.8 Hz), 4.97 (1H, br.d, J = 8.0 Hz), 4.63, 3.59 (each 1H, ABq, J = 8.8 Hz), 3.90 (1H, d, J = 6.8 Hz), 3.59 (1H, d, J = 6.8 Hz), 2.22 (3H, s), 2.02, (3H, s), 1.75 (3H, s), 1.50, 1.49, 1.46 (each 9H, s), 1.16 (3H, s), 1.05 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.9 s, 170.3 s, 139.4 s, 137.3 s, 85.1 d, 83.8 s, 82.8 s, 78.4 d, 75.9 s, 73.5 t, 73.1 d, 73.1 d, 72.3 d, 54.5 s, 53.6 d, 42.2 s, 35.5 t, 33.7 t, 27.6 q, 25.3 q, 21.5 q, 18.8 q, 15.0 q, 14.3 q; MS (ESI, MeOH) m/z 741 $[M+H]^+$; HR-ESI-MS: 741.3612 $[M+H]^+$, calc. for $C_{37}H_{57}O_{15}$ 741.3698.

Preparation of compound 11. To a stirred solution of 7,10,13-triBoc-2-debenzoate-10-DAB (50 mg, 0.067 mmol) in anhydrous CH_2Cl_2–pyridine (5 : 1) at −15 °C was added triphosgene (24 mg, 0.080 mmol), and the reaction mixture was stirred at −15 °C for 30 min prior to being quenched with saturated K_2CO_3 solution. After standard workup, the residue was subjected to column chromatography on silica gel (cyclohexane–acetone 6 : 1) to generate compound **11** (46 mg, 92%) as an amorphous solid: $[\alpha]_D^{25}$ +12.1 ($CHCl_3$, *c* 0.8); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.30 (1H, s), 5.92 (1H, t, J = 8.4 Hz), 5.37 (1H, dd, J = 10.0, 7.6 Hz), 5.00 (1H, br.d, J = 8.4 Hz), 4.66 (1H, d, J = 6.0 Hz), 4.64, 4.56 (each 1H, ABq, J = 8.8 Hz), 3.55 (1H, d, J = 6.8 Hz), 1.29 (3H, s), 2.23 (3H, s), 1.83 (3H, s), 1.50, 1.47, 1.45 (each 9H, s), 1.28 (3H, s), 2.12, (3H, s); MS (ESI, MeOH) m/z 767 $[M+H]^+$; HR-ESI-MS: 767.3462 $[M+H]^+$, calc. for $C_{38}H_{55}O_{16}$ 766.3490.

Preparation of compounds 12 and 13. To a solution of compound **11** (100 mg, 0.13 mmol) in anhydrous CH_2Cl_2 at −15 °C was added 3 drops of $BF_3{\cdot}Et_2O$, and the reaction mixture was stirred at −15 °C for 10 min under argon prior to being quenched with ammonium hydroxide. After standard workup, purification of the residue *via* column chromatography over silica gel (ether–acetone 6 : 1) yielded compounds **12** (65 mg, 64%) and **13** (10 mg, 10%) as amorphous powders. Compound **12**: $[\alpha]_D^{25}$ −7.5 ($CHCl_3$, *c* 2.3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.48 (1H, s), 5.72 (1H, dd, J = 10.0, 2.4 Hz), 5.44 (1H, dd, J = 11.2, 4.4 Hz), 4.52, 4.41 (each 1H, ABq, J = 12.0 Hz), 4.23 (1H, d, J = 4.4 Hz), 3.79 (1H, t, J = 2.8 Hz), 3.74 (1H, t, J = 4.4 Hz), 2.29 (3H, s), 2.13 (3H, s), 1.50, 1.47, 1.44 (each 9H, s), 1.35 (3H, s), 1.22 (3H, s), 1.15 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.0 s, 170.5 s, 151.8 s, 144.2 s, 132.0 s, 90.2 s, 82.8 s, 81.1 d, 78.3 d, 75.4 s, 72.1 d, 71.4 d, 69.8 d, 64.7 t, 59.1 s, 43.4 d, 40.6 s, 30.6 t, 32.5 t, 27.7 q, 27.1 q, 20.7 q, 18.8 q, 16.6 q, 12.7 q. MS (ESI, MeOH) m/z 785 $[M+H]^+$; HR-ESI-MS: 785.3567 $[M+H]^+$, calcd. for $C_{38}H_{57}O_{17}$ 785.3596. Compound **13**: $[\alpha]_D^{25}$ −13.2 ($CHCl_3$, *c* 2.0); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.44 (1H, s), 5.82 (1H, dd, J = 8.4, 5.6 Hz), 5.32 (1H, t, J = 2.8 Hz), 5.20 (1H, dd, J = 11.6, 4.8 Hz), 4.24 (1H, d, J = 4.8 Hz), 4.08, 3.57 (each 1H, ABq, J = 9.2 Hz), 3.50 (1H, t, J = 4.4 Hz), 2.31, (3H, s), 2.34 (3H, s), 1.49, 1.47, 1.44 (each 9H, s), 1.29 (3H, s), 1.28 (3H, s), 1.15 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.9 s, 171.0 s, 151.9 s, 143.8 s, 132.0 s, 90.1 s, 83.0 s, 81.7 d, 78.0 d, 74.0 s, 72.1 d, 71.6 d, 71.3 d, 62.9 t, 59.0 s, 45.3 d, 41.1 s, 32.7 t, 30.0 t, 27.6 q, 26.0 q, 21.3 q, 19.5 q, 16.1 q, 12.7 q; MS (ESI, MeOH) m/z 785 $[M+H]^+$; HR-ESI-MS: 785.3532 $[M+H]^+$, calcd. for $C_{38}H_{57}O_{17}$ 785.3596.

Regioselective preparation of compound 13. To a solution of compound **11** (100 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (200 mL) at 25 °C was added $TiCl_4$ (0.13 mL, 1 M in CH_2Cl_2) and the reaction was allowed to proceed with stirring at 25 °C for 5 min under argon before quenching with ammonium hydroxide. After standard workup, the residue was separated on silica gel (ether–acetone 6 : 1) to give compound **13** (94 mg, 93%) as an amorphous powder.

Preparation of compound 14. LTA (62 mg, 0.14 mmol) was added to a solution of the compound **13** (98 mg, 0.125 mmol) in CH_3CN (20 mL). The reaction mixture was stirred at room temperature for 12 h and then quenched with water (10 mL). After standard workup, the residue was separated on silica gel column (petroleum ether–acetone 6 : 1) to give compound **14** (80 mg) as an amorphous powder: $[\alpha]_D^{25}$ +23.6 ($CHCl_3$, *c* 1.0); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.39 (1H, s), 5.96 (1H, t, J = 8.0 Hz), 5.50 (1H, dd, J = 11.2, 5.2 Hz); 5.02 (1H, t, J = 2.8 Hz), 4.29 (1H, d, J = 6.0 Hz),

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4.11 (1H, d, J = 6.4 Hz), 1.18 (3H, s), 2.34 (3H, s), 2.32, (3H, s), 1.48, 1.48, 1.46 (each 9H, s), 1.32 (3H, s), 1.26 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.8 s, 195.1 s, 169.5 s, 151.9 s, 143.3 s, 131.3 s, 89.3 s, 82.6 s, 78.9 d, 77.7d, 75.3 d, 71.2 d, 70.9 d, 59.8 s, 49.8 d, 40.8 s, 33.9 t, 33.0 t, 27.6 q, 25.4 q, 21.1 q, 19.7 q, 16.0 q, 12.4 q; MS (ESI, MeOH) m/z 753 $[M+H]^+$; HR-ESI-MS 753.3345 $[M+H]^+$, calcd. for $C_{37}H_{53}O_{16}$ 753.3333.

Preparation of compounds 15 and 16. To a stirred solution of compound **14** (125 mg, 0.17 mmol) in anhydrous THF at −78 °C was added HMPA (0.05 mL), and the reaction mixture was stirred under argon at −78 °C for 15 min prior to addition of fresh LDA (0.2 mL, 1 M in THF). The reaction was allowed to proceed for an additional 2 h prior to being quenched with water. The mixture was extracted with CH_2Cl_2, and the combined extracts were washed successively with water, 1% HCl, and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel chromatography (cyclohexane–acetone 5 : 1) to give compounds **15** (28 mg, 23%) and **16** (52 mg, 42%). Their structures were established by 1D and 2D NMR including ^{1}H–^{1}H COSY, HMQC, HMBC and NOE (see ESI†).

Compound 15. $[\alpha]_D^{25}$ −13.5 ($CHCl_3$, c 0.8); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.37 (1H, s), 5.72 (1H, dd, J = 7.2, 3.6 Hz), 5.15 (1H, dd, J = 8.0, 6.4 Hz), 4.46 (1H, d, J = 6.8 Hz), 4.61 (1H, t, J = 5.2 Hz), 3.03, 2.79 (each 1H, ABq, J = 18.0 Hz), 2.70 (1H, t, J = 6.8 Hz), 2.19, (3H, s), 1.53 (3H, s), 1.48 (each 27H, s), 1.23 (3H, s), 1.20 (3H, s); ^{13}C NMR (100 MHz) δ 202.2, 170.6, 151.8, 142.9, 131.2, 90.6, 83.4, 81.8, 80.5, 77.8, 77.1, 71.5, 70.7, 57.8, 46.4, 46.1, 40.4, 34.1, 29.2, 27.6, 26.7, 19.0, 16.4, 13.5; MS (ESI, MeOH) m/z 753 $[M+H]^+$; HR-ESI-MS: 753.3351 $[M+H]^+$, calcd. for $C_{37}H_{53}O_{16}$ 753.3333.

Compound 16. $[\alpha]_D^{25}$ +5.2 ($CHCl_3$, c 1.0); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.30 (1H, s), 5.73 (1H, t, J = 7.2 Hz), 5.00 (1H, dd, J = 10.0, 4.8 Hz), 4.61 (1H, t, J = 3.6 Hz), 4.05 (1H, d, J = 6.4 Hz), 3.20, 2.73 (each 1H, ABq, J = 18.4 Hz), 2.84 (1H, t, J = 6.4 Hz), 2.05, (3H, s), 1.56 (3H, s), 1.49 (each 27H, s), 1.15 (3H, s), 1.08 (3H, s); ^{13}C NMR (100 MHz) δ 202.5 s, 172.4 s, 141.0 s, 132.4 s, 82.9 s, 81.6 d, 78.6 s, 77.1d, 76.9 s, 74.1 d, 72.3d, 71.5 s, 57.0 s, 48.3 t, 48.0 d, 42.2 s, 35.6 t, 27.4 q, 27.3 t, 26.3 q, 19.4 q, 15.2 q, 12.1 q; MS (ESI, MeOH) m/z 753 $[M+H]^+$; HR-ESI-MS: 753.3346 $[M+H]^+$, calcd. for $C_{37}H_{53}O_{16}$, 753.3333.

Selective preparation of compound 16. To a stirred solution of compound **14** (100 mg, 0.13 mmol) in anhydrous THF at −78 °C was added HMPA (0.05 mL), and the reaction mixture was stirred under argon at −78 °C for 15 min before addition of tBuOK (0.15 mL, 1 M in THF). The reaction was allowed to proceed for an additional 1 h prior to being quenched with water. The subsequent mixture was extracted with CH_2Cl_2, and the combined extracts were washed successively with water, 1% HCl and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane–acetone 5 : 1) to give compound **16** (65 mg, 65%).

Preparation of compound 17. To a stirred solution of compound **16** (50 mg, 0.066 mmol) in CH_2Cl_2 was added DMAP (50 mg) and acetic anhydride (0.4 mL), and the reaction mixture was allowed to stir overnight at room temperature before being quenched with aqueous Na_2CO_3 solution. After standard workup, the residue was purified by column chromatography over silica gel (cyclohexane–acetone 8 : 1) to yield compound **17** (34 mg, 65%) as an amorphous powder: $[\alpha]_D^{25}$ +11.2 ($CHCl_3$, c 1.0); ^{1}H NMR (400 MHz) δ 6.45 (1H, s), 5.40 (1H, dd, J = 11.2, 4.8 Hz), 4.82 (1H, d, J = 7.6 Hz), 4.24 (1H, d, J = 4.8 Hz), 3.89 (1H, br.s), 3.77 (1H, d, J = 4.8 Hz), 3.24, 2.96 (each 1H, ABq, J = 11.6 Hz), 2.36 (3H, s), 2.12 (3H, s), 1.36, 1.18, 1.14 (each 3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.5, 198.1, 167.1, 152.2, 152.1, 151.1, 141.8, 132.5, 89.3, 83.2, 82.5, 78.1, 77.4, 77.0, 74.8, 72.2, 68.8, 57.9, 51.1, 40.0, 35.4, 33.7, 30.6, 29.4, 28.1, 27.5, 27.4, 26.6, 18.1, 18.0, 14.5; MS (ESI, MeOH) m/z 817 $[M+Na]^+$; HR-ESI-MS: 817.3250 $[M+Na]^+$, calcd. for $C_{39}H_{54}NaO_{17}$ 817.3258.

Preparation of compound 18. To a solution of compound **17** (85 mg, 0.107 mmol) in dry THF at −78 °C under argon was added a solution of phenyllithium (1 M, 0.4 mL, 4 equiv.) in hexane. The solution was stirred for 1 h at −78 °C and then poured onto a mixture of CH_2Cl_2 and saturated ammonium chloride, and the subsequent mixture was stirred vigorously for 5 min at room temperature. After standard workup, the residue was purified on silica gel chromatography (petroleum ether–acetone 10 : 1) to give compound **18** (29 mg, 35%) as an amorphous solid: $[\alpha]_D^{25}$ +23.6 ($CHCl_3$, c 1.0); ^{1}H NMR (400 MHz) δ 8.11–7.46 (5H, m), 6.39 (1H, s), 5.01 (1H, dd, J = 11.2, 4.4 Hz), 5.76 (1H, d, J = 7.2 Hz), 4.89 (1H, t, J = 8.4 Hz), 2.98 (2H, m), 2.90, 2.52 (each 1H, ABq, J = 11.6 Hz), 2.27 (3H, s), 2.25 (3H, s), 1.67, 1.22, 1.18 (each 3H, s), 1.50, 1.47 (each 9H, s); MS (ESI, MeOH) m/z 795 $[M+Na]^+$; HR-ESI-MS: 795.3201 $[M+Na]^+$, calcd. for $C_{40}H_{52}NaO_{15}$ 795.3204.

Preparation of compound 20. (1) The link of side chain. To a solution of compound **18** (120 mg, 0.155 mmol) in dry THF were added the docetaxel side chain (compound **19**, 160 mg, 3 equiv.) and NaH (40 mg, 10 equiv.). The reaction mixture was stirred at room temperature for 2 h, and then quenched by addition of water (10 mL). After standard workup, the residue was purified by preparative TLC (cyclohexane–acetone 2 : 1) to give the side chain linked product (128 mg, 74%) as an amorphous powder: $[\alpha]_D^{25}$ −3.5 ($CHCl_3$, c 1.0); ^{1}H NMR (400 MHz) δ 7.98–7.36 (10H, m), 6.39 (1H, s), 5.76 (1H, d, J = 7.2 Hz), 5.69 (1H, d, J = 9.2 Hz), 5.59 (1H, t, J = 8.4 Hz), 5.01 (1H, dd, J = 11.2, 4.4 Hz), 3.90 (1H, s), 2.90, 2.31 (each 1H, ABq, J = 11.6 Hz), 2.27 (3H, s), 2.25 (3H, s), 1.67 (3H, s), 1.51, 1.48 (each 9H, s), 1.22, 1.18 (each 3H, s); ^{13}C NMR (100 MHz) δ 202.8, 173.9, 170.5, 169.8, 167.5, 166.3, 152.2, 146.3, 132.4, 132.0, 129.2, 128.9, 128.2, 126.4, 88.7, 84.0, 81.2, 77.3, 77.1, 76.0, 76.0, 72.8, 72.4, 71.3, 71.3, 70.5, 70.3, 69.9, 69.1, 64.7, 64.5, 62.5, 57.2, 52.5, 43.0, 38.6, 32.9, 32.0, 29.6, 29.2, 21.3, 20.8, 20.3, 20.0, 13.9, 12.5, 11.7, 10.8; MS (ESI, MeOH) m/z 1130, $[M+Na]^+$; HR-ESI-MS: 1130.4914 $[M+Na]^+$, calcd. for $C_{58}H_{77}NO_{20}Na$ 1130.4937.

(2): **The global deprotection**. To a solution of the previously prepared side chain linked product (100 mg, 0.097 mmol) in THF was added 1 M HCl (0.5 mL). The reaction mixture was stirred at 40 °C for 3 h before being quenched with 5% $NaHCO_3$ solution. After standard workup, the residue was purified by preparative TLC ($CHCl_3$–MeOH 10 : 1) to afford compound **20** (48 mg, 60%) as an amorphous powder: $[\alpha]_D^{25}$ −29.6 ($CHCl_3$, c 1.0); ^{1}H NMR (400 MHz) 8.13–7.31 (10H, m), 6.30 (1H, d, J = 8.0 Hz), 6.22 (1H,

t, J = 9.2 Hz), 5.78 (1H, t, J = 11.2 Hz), 5.70 (1H, d, J = 7.2 Hz), 5.30 (1H, br.s), 4.97 (1H, d, J = 8.0 Hz), 4.44 (1H, m), 3.80 (1H, d, J = 7.2 Hz), 2.85, 2.19 (each 1H, ABq, J = 11.2 Hz), 2.57 (1H, m, H-14), 2.42 (3H, d, J = 3.2 Hz), 2.36 (1H, m, H-14), 2.23 (3H, s, OAc), 1.95 (3H, s), 1.85 (1H, m, H-6), 1.45 (9H, s, Boc), 1.39 (1H, m, H-6), 1.26, 1.15 (each 3H, s); ^{13}C NMR (100 MHz) 203.8, 176.2, 176.1, 171.1, 169.8, 168.3, 166.9, 152.3, 142.8, 133.5, 132.7, 130.2, 129.2, 128.9, 128.6, 128.1, 128.1, 126.4, 84.4, 84.0, 81.0, 79.0, 76.4, 75.9, 75.6, 75.1, 71.9, 58.5, 51.9, 51.7, 45.5, 43.2, 43.1, 42.9, 35.5, 27.5, 27.1, 26.9, 26.6, 22.6, 20.7, 17.4, 14.7. MS (ESI, MeOH) m/z 858 $[M+Na]^+$; HR-ESI-MS: 858.3304 $[M+Na]^+$, calcd. for $C_{44}H_{53}NO_{15}Na$ 858.3313.

Determination of cell viability by MTT assay

Cells were plated in the RPMI 1640 with 10% fetal calf serum media on 96-well plates in a total volume of 100 µL with a density of 1×10^4 cells mL^{-1}. Triplicate wells were treated with media and tested compounds. The plates were incubated at 37 °C in 5% CO_2 for 72 h. Cell viability was determined based on mitochondrial conversion of 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) to formazan. The amount of MTT converted to formazan is a sign of the number of viable cells. Each well was supplemented with 50 µL of a 1 mg mL^{-1} solution of MTT in uncompleted media. The plates were incubated in 37 °C, 5% CO_2 for an additional 4 h. The media was carefully removed from each well and then 200 µL of DMSO was added. The plates were gently agitated until the reaction color was uniform and the OD_{570} was determined using a microplate reader (Wellscan MK3, Labsystems Dragon). Microsoft® Excel 2000 was used to analyze data. Media-only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay.

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Notes and references

1 D. G. I. Kingston, *Phytochemistry*, 2007, **68**, 1844 and the references cited therein.
2 G. Samaranayake, N. F. Magri, C. Jitrangeri and D. G. I. Kingston, *J. Org. Chem.*, 1991, **56**, 5114.
3 A. A. L. Gunatilaka, F. D. Ramdayal, M. H. Sarragiotto, D. G. I. Kingston, D. L. Sackett and E. Hamel, *J. Org. Chem.*, 1999, **64**, 2694.
4 L. Merckle, J. Dubois, E. Place, S. Thoret, F. Guéritte, D. Guenard, C. Poupat, A. Ahond and P. Potier, *J. Org. Chem.*, 2001, **66**, 5058.
5 R. Marder-Karsenti, J. Dubois, L. Bricard, D. Guénard and F. Guéritte-Voegelein, *J. Org. Chem.*, 1997, **62**, 6631.
6 T. C. Boge, M. Hepperle, D. G. V. Velde, C. W. Gunn, G. L. Grunewald and G. I. Georg, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 3041.
7 M. Wang, B. Cornett, J. Nettles, D. C. Liotta and J. P. Snyder, *J. Org. Chem.*, 2000, **65**, 1059.
8 J. Dubois, S. Thoret, F. Guéritte and D. Guénard, *Tetrahedron Lett.*, 2000, **41**, 3331.
9 L. Barboni, G. Giarlo, M. Ricciutelli, R. Ballini, G. I. Georg, D. G. VanderVelde, R. H. Himes, M. Wang, A. Lakdawala and J. P. Snyder, *Org. Lett.*, 2004, **6**, 461.
10 V. Deka, J. Dubois, S. Thoret, F. Guéritte and D. Guénard, *Org. Lett.*, 2003, **5**, 5031.
11 S. Thoret, F. Guéritte, D. Guénard and J. Dubois, *Org. Lett.*, 2006, **8**, 2301.
12 P. A. Wender and D. B. Rawlins, *Tetrahedron*, 1992, **48**, 7033.
13 S.-H. Chen, V. Farina, J.-M. Wei, B. Long, C. Fairchild, S. W. Mamver, J. F. Kadow, D. Vyas and T. Doyle, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 479.
14 R. A. Holton, US Patent (1991) 5,015,744; US Patent (1992) 5,136,060also see:; I. Ojima, C. M. Sun, M. Zucoo, Y. H. Park, O. Duclos and S. D. Kuduk, *Tetrahedron Lett.*, 1993, **34**, 4149.
15 A. D. Da Silva, A. S. Machado and C. Tempête, *Eur. J. Med. Chem.*, 1994, **29**, 149.
16 L. Barboni, A. Datta, D. Dutta, G. I. Georg, D. V. V. Velde, R. H. Himes, M. Wang and J. P. Snyder, *J. Org. Chem.*, 2001, **66**, 3321.

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