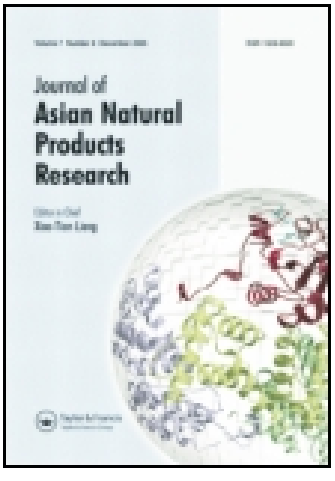


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Synthesis of three OSW-1 analogs with maltose side chains bearing different protection groups

Yu-Yao Guan[b], Chao Song[b] & Ping-Sheng Lei[a]

[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Sciences, Peking, 100050, China

[b] Pharmacy Department, Traffic Hospital of Shandong Province, Jinan, 250031, China

Published online: 06 Dec 2013.

To cite this article: Yu-Yao Guan, Chao Song & Ping-Sheng Lei (2014) Synthesis of three OSW-1 analogs with maltose side chains bearing different protection groups, Journal of Asian Natural Products Research, 16:1, 43-52, DOI: 10.1080/10286020.2013.863185

To link to this article: http://dx.doi.org/10.1080/10286020.2013.863185

Journal of Asian Natural Products Research, 2014
Vol. 16, No. 1, 43–52, http://dx.doi.org/10.1080/10286020.2013.863185

Synthesis of three OSW-1 analogs with maltose side chains bearing different protection groups

Yu-Yao Guan[b], Chao Song[b] and Ping-Sheng Lei[a]*

[a]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Sciences, Peking 100050, China;* [b]*Pharmacy Department, Traffic Hospital of Shandong Province, Jinan 250031, China*

(*Received 3 September 2013; final version received 4 November 2013*)

In order to simplify the synthesis of OSW-1's disaccharide side chain and explore the structure–activity relationship of OSW-1, three 16α-*O*-maltose OSW-1 analogs carrying three maltose side chains bearing different protections were designed and synthesized.

Keywords: OSW-1; antitumor; synthesis; maltose

1. Introduction

OSW-1 with considerable antitumor and antiproliferative activities has a potential for clinical use as a new antitumor drug [1]. It was isolated from *Ornithogalum saundersiae* bulbs in 1992 by Sashida and co-workers [2] and found to possess a cholestane aglycone and a disaccharide moiety. *In vitro* assay showed that it was considerably toxic to a broad spectrum of malignant cancer cells with a mean IC_{50} of 0.7 nM [3]. Because of its excellent antitumor activities and low toxicities to normal cells, the synthesis of OSW-1 has been studied by many groups [4–6]. In addition, a series of analogs have been synthesized and tested for antitumor activities in recent years [7–15,24]. According to the previous structure–activity relationship (SAR) work, it is interesting to note the removal of the acetyl (Ac) and the 4-methoxy benzoyl (MBz) groups on the disaccharide moiety which diminished the cytotoxicity significantly (about 1000 times less potent).

Many research groups worldwide have made much effort to study the action mechanism of OSW-1. Fuchs and co-workers [16] speculated that structurally the 22-oxocarbenium ions might be the active intermediate for the anticancer activity. Yohda and co-workers [17] indicated that the three-dimensional (3D) structure of OSW-1 was related to its good activity. The hydrophobic cluster constructed by the C20-C27 side chain and the C2′-acetyl, C2″-*p*-MBz groups in disaccharide was a biologically required functionality as well as of the overall conformation.

The NIH (National Institutes of Health) pointed out that the antiapoptosis protein Bcl-2 was the target of the study [18]. Zhou et al. [19] showed that OSW-1 can damage the membrane and cristae of cancer cell's mitochondria, which plays an important role in mediating the anticancer activities. Yu and co-workers [20] pointed that the target was caspase-8 which caused the cleavage of Bcl-2. Shair and co-workers [21] made breakthrough progress in finding the exact target. Shair revealed that the target of OSW-1 was oxysterol-binding protein (OSBP) and its closest

*Corresponding author. Email: lei@imm.ac.cn

paralog, OSBP-related protein 4L (ORP4L). OSBP and ORP4L were much more expressed in tumor cells than in normal cells. The antiproliferative activity of OSW-1 was mediated by OSBP. The cytotoxicity was mediated by ORP4L which caused apoptosis. Nevertheless, the previous study about the target was meaningful too. They were the subsequent processes of the apoptosis resulted from binding to ORP4L.

In order to simplify the synthesis of OSW-1's disaccharide side chain and explore the SAR of OSW-1, we designed and synthesized 16α-*O*-maltose OSW-1 analogs carrying three maltose side chains bearing different protections including [4′,6′-*O*-benzylidene-6-*O*-benzyl-α-D-maltose], [4′,6′-*O*-benzylidene-6-*O*-benzoyl-α-D-maltose], and [2,3,2′,3′-tetra-*O*-acetyl-α-D-maltose]. The synthesis of the original β-D-Xyl*p*-(1-3)-α-L-Ara*p* disaccharide was complicated in 14 steps [4]. The synthesis of the chosen protected maltose chain was simple with eight steps [22]. The protected maltose glycoside showed a potent antiproliferative activity against smooth muscle cells with an IC_{50} of 0.023–0.001 μM [22]. According to Shair's work, OSBP was the target which mediated the antiproliferative activity of OSW-1 and recognized steroids. Therefore, the coupling of aglycone of OSW-1 to the maltose was hoped to be recognized by OSBP and shows antiproliferative activity against the tumor cell. The protected maltose has similar polarity distribution to the disaccharide of OSW-1. One side of the disaccharide was distributed by lipophilic groups, and the other side was distributed by the hydrophilic hydroxyl groups. The lipophilic groups in maltose might form the biologically required hydrophobic cluster.

Structural superimposition analysis was implemented in our study with Accelrys Discovery Studio program package. The minimum energy conformation of one of the target compounds was calculated and aligned as shown in Figure 1. The conformation of the target compound was similar to the 3D structure of OSW-1 which has the hydrophobic cluster constructed by the C20-C-27 side chain and the C2′-acetyl, C2″-*p*-MBz groups in disaccharide. OSW-1's conformation was believed to be important to the anticancer activity of OSW-1 [24].

2. Results and discussion

Three 16α-*O*-maltose-OSW-1 analogs **11**, **22**, and **23** were synthesized in a facile way. The key intermediate **4** was synthesized from the starting material (D)-maltose through four-step reactions with an overall yield of 64.4% (Scheme 1). After **4** in hand, we prepared three different protected maltose donors **8**, **15**, and **19** through relative protection strategy (Schemes 1–3). Aglycone **9** (Scheme 4) was prepared according to the procedures developed by our research group [23]. Coupling aglycone **9** and thioglycoside **8** in the presence of NIS/AgOTf (*N*-iodosuccinimide/silver triflate) provided the desired glycoside **10** (Scheme 4). The resulting saponin bearing acetyl protections was removed by 1 M MeONa/MeOH, providing the expected target compound **11** (Scheme 4). The ^{1}H NMR spectrum of **11** showed the signal for the 1′-β-anomeric proton at δ 5.17 (d, 1H, $J = 3.6$ Hz, H-1′). The ^{13}C NMR spectrum of **11** showed the signal for the anomeric carbon at δ 98.21. The glycosylation of aglycone **9** with the maltose trichloroacetimidates (**15** and **19**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.10 equiv.) provided the desired glycosides (**20** and **21**) (Scheme 5). The resulting saponin **20** bearing TES (triethylsilyl) protections was deprotected using HF·pyridine in pyridine, providing the desired product **22** (Scheme 5). The resulting saponin **21** bearing silyl and benzylidene protection groups was deprotected using HF·pyridine in pyridine and followed by $NaHSO_4{\cdot}SiO_2$ in CH_2Cl_2/

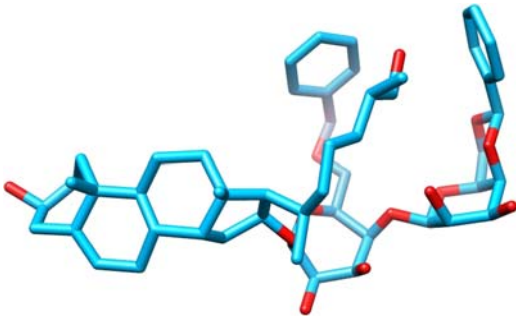
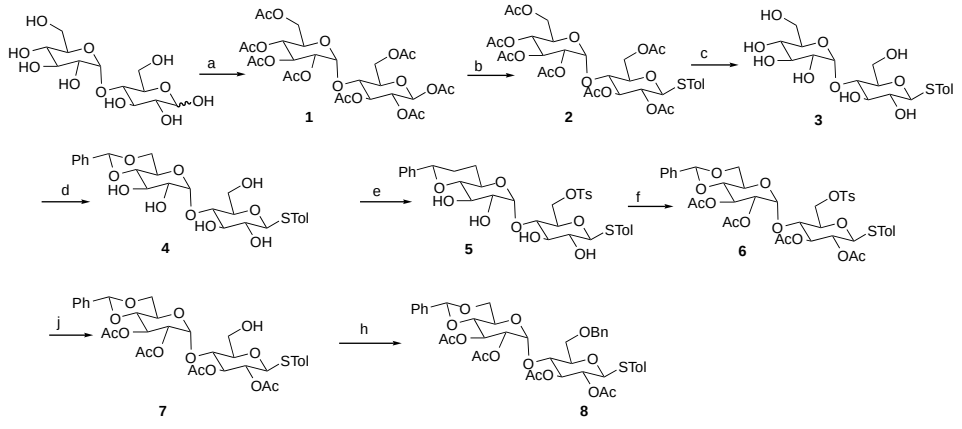


Figure 1. The minimum energy conformation of the target compound **11.**

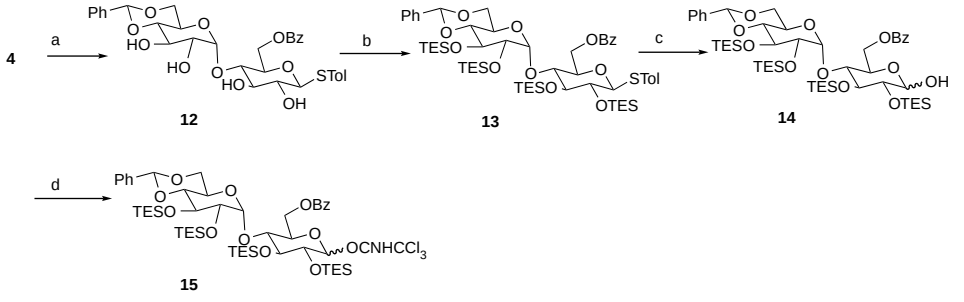
MeOH, providing the desired product **23** (Scheme 5). The ^{1}H NMR spectrum of **22** showed the signal for the 1′-β-anomeric proton at δ 5.12 (d, 1H, $J = 3.6$ Hz, H-1′). The ^{13}C NMR spectrum of **22** showed the signal for the anomeric carbon at δ 94.26. The ^{1}H NMR spectrum of **23** showed the signal for the 1′-β-anomeric proton at δ 5.32 (d, 1H, $J = 3.3$ Hz, H-1′). The ^{13}C NMR spectrum of **23** showed the signal for the anomeric carbon at δ 94.5.

The *in vitro* antitumor activities of compounds **11**, **22**, and **23** against A2780, BEL-7402, HCT-8, KB, BGC-823, HeLa, and A549 were evaluated by the standard MTT assay using dioscin as a positive control. The results are listed in Table 1. The IC_{50} values of dioscin against these seven cell lines used in our assays are consistent with those determined by others [24].

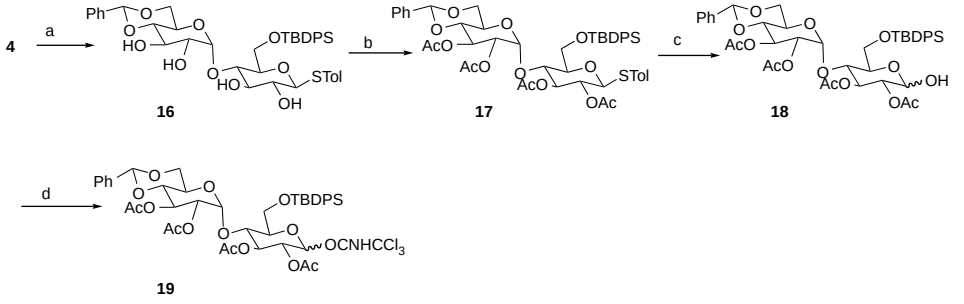
The bioassay results showed that the 16α-*O*-maltose OSW-1 analogs showed no cytotoxicity against the seven tested tumor cells. The consequences proved that the introduction of protected maltose did not improve the cytotoxicities of the whole molecule. However, the antitumor activity of OSW-1 was not only cytotoxic but also antiproliferative, mediated by target ORP4L and OSBP, respectively. Therefore, we planned to test the antiproliferative activity of compounds **11**, **22**, and **23** and the interaction between OSBP and



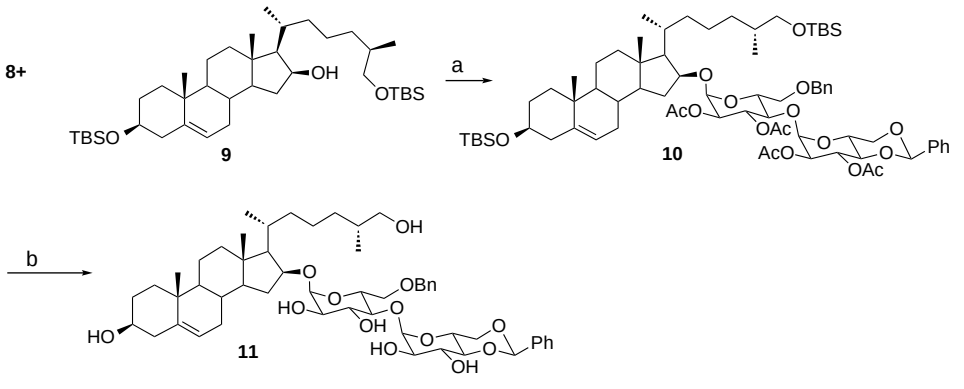
Scheme 1. Reagents and conditions: (a) Ac_2O, AcONa, 100°C, 92%; (b) *p*-thiocresol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, r.t., 73%; (c) 1 M MeONa/MeOH, CH_2Cl_2/MeOH, r.t., 99%; (d) PhCH $(OMe)_2$, $NaHSO_4{\cdot}SiO_2$, DMF/CH_3CN, r.t., 97%; (e) *p*-TsCl, pyridine/CH_2Cl_2, r.t., 64%; (f) Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t., 94%; (j) HCOONa, AcOEt/MeOH, 70°C, 75%; (h) BnBr, NaH, DMF, 0°C, 50%.



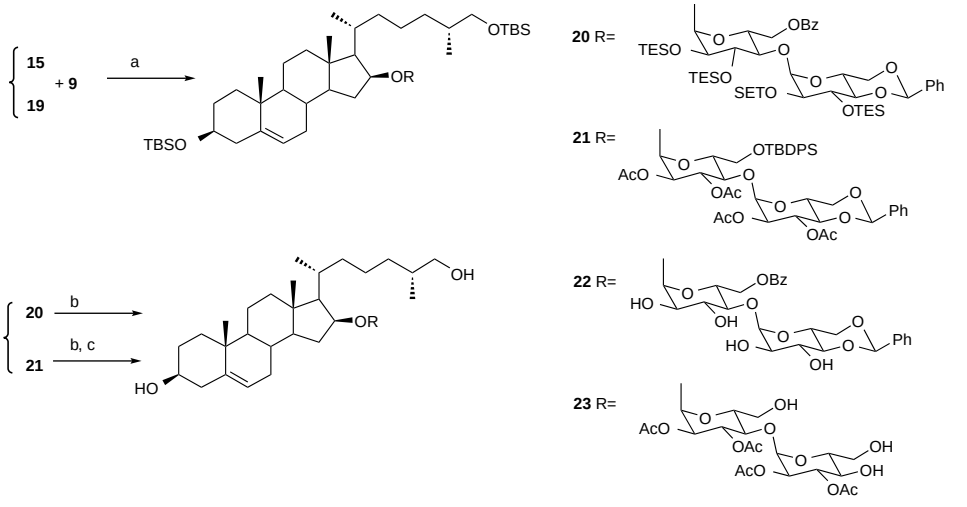
Scheme 2. Reagents and conditions: (a) BzCl, CH_2Cl_2/pyridine, r.t., 60%; (b) TESOTf (triethylsilyl trifluoromethanesulfonate), 2,6-lutidine, CH_2Cl_2, 0°C, r.t., 80%; (c) NBS (*N*-bromosuccinimide), TMSOTf, CH_2Cl_2/H_2O, r.t., 80%; (d) CCl_3CN, DBU (1,8-diazabicyclo[5.4.0] undec-7-ene), CH_2Cl_2, r.t., 90%.



Scheme 3. Reagents and conditions: (a) TBDPSiCl, DMAP, pyridine (99%), r.t., 78%; (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t., 99%; (c) NIS, AgOTf, H_2O, CH_2Cl_2, − 15°C, r.t.; (d) CCl_3CN, DBU, CH_2Cl_2, r.t., 70% for two steps.



Scheme 4. Reagents and conditions: (a) NIS, AgOTf, CH_2Cl_2, − 50°C, 45%; (b) (i) HF·pyridine, pyridine (99%), r.t.; (ii) 1 M MeONa/MeOH, CH_2Cl_2/MeOH, reflux, 76% for two steps.



Scheme 5. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −30°C, 55% for **20**, 56% for **82**; (b) HF·pyridine, pyridine (99%), r.t.; (c) $NaHSO_4{\cdot}SiO_2$, CH_2Cl_2/MeOH, r.t.

target compounds. These bioactive results and further synthesis of analogs with the maltose bearing various protection groups will be reported in future.

3. Experimental

3.1 General experimental procedures

All NMR spectra were recorded on Mercury-300 spectrometers (Varian Co., Palo Alto, CA, USA) in $CDCl_3$, dimethylsulfoxide-d_6 (DMSO-d_6), or pyridine-d_5 as solution. The chemical shifts are reported in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. HR-MS experiments were carried out using an Agilent 1100 series LC/MSD TOF (Agilent, Santa Clara, CA, USA). Analytical thin layer chromatography (TLC) was carried out on TLC plates coated with silica gel $HSGF_{254}$ (Marine Chemical Group Co., Qingdao, China). Chromatography was performed with silica gel H (HG/T2354-92) (Marine Chemical Group Co., Qingdao, China). Dried solvents used as reaction media were purified in the usual way. The major chemicals were purchased from Alfa Chemical Corporation (Massachusetts, USA). All other chemicals were of analytical grade.

Table 1. The *in vitro* cytotoxicities (IC_{50}, μM) of synthetic 16α-*O*-maltose OSW-1 analogs and dioscin.[a]

Cell lines	**11**	**22**	**23**	Dioscin
A2780	>10	>10	>10	0.87
BEL-7402	>10	>10	>10	0.81
HCT-8	>10	>10	>10	0.34
KB	>10	>10	>10	0.53
BGC-823	>10	>10	>10	1.08
HeLa	>10	>10	>10	0.50
A549	>10	>10	>10	0.81

[a]The *in vitro* cytotoxic activities against A2780 (ovarian cancer), BEL-7402 (liver cancer), HCT-8 (colon cancer), KB (nasopharyngeal carcinoma), BGC-823 (stomach cancer), HeLa (cervical cancer), and A549 (lung cancer) cell lines were evaluated by the standard MTT assay using dioscin as a positive control.

3.2 General procedures for the synthetic compounds

3.2.1 Synthesis of compound **4**

Catalyst $NaHSO_4{\cdot}SiO_2$:$NaHSO_4{\cdot}H_2O$ (4.14 g), H_2O (20 ml), and silica gel (200–300 mesh, 10 g) were mixed together and evaporated under reduced

pressure. The mixture was activated at 105°C in drying oven for 10 h.

Compound **3** (290 mg, 0.647 mmol) was dissolved in CH_3CN (15 ml) and dimethylformamide (DMF) (15 ml), followed by the addition of $NaHSO_4{\cdot}SiO_2$ (580 mg) and PhCH $(OMe)_2$ (0.49 ml, 3.235 mmol) at room temperature. The reaction mixture was stirred for 2 h and quenched with Et_3N. The solid was filtered, and the solvent was removed. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 20:1, v/v) to afford the desired compound **4** (349 mg, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.50–7.42 (m, 2H), 7.39 (d, $J = 7.8$ Hz, 2H), 7.35–7.25 (m, 3H), 7.06 (d, $J = 7.8$ Hz, 2H), 5.43 (s, 1H), 5.08 (brs, 1H), 4.50 (d, $J = 4.0$ Hz, 1H), 4.24–4.14 (m, 1H), 4.01–3.90 (m, 1H), 3.88–3.73 (m, 2H), 3.72–3.48 (m, 4H), 3.46–3.32 (m, 2H), 3.30–3.18 (m, 1H), and 2.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.1, 137.1, 132.6 (2C), 129.8 (2C), 19.2, 128.8, 128.3 (2C), 126.4 (2C), 102.0, 101.7, 87.9, 80.5, 79.7, 78.4, 73.2, 71.9, 70.8, 68.6, 63.7, 61.4, and 21.1. LC-HR-ESI-MS: *m/z* 537.1782 $[M + H]^+$ (calcd for $C_{26}H_{33}O_{10}S$, 537.1789).

3.2.2 *Synthesis of compound* ***5***

To a solution of compound **4** (1.85 g, 3.457 mmol) in anhydrous pyridine (10 ml) was added *p*-toluenesulfonyl chloride (*p*-TsCl) (1.32 g, 6.915 mmol) in CH_2Cl_2 (7 ml) at room temperature. The reaction mixture was stirred for 2.5 h, quenched with MeOH (20 ml), and diluted with CH_2Cl_2 (200 ml). It was then washed with 1 M HCl (30 ml × 3), aqueous saturated $NaHCO_3$ (30 ml × 3), brine (30 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 40:1, v/v) to give compound **5** (1.52 g, 64%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.83 (d, $J = 8.1$ Hz, 2H), 7.50–7.42 (m, 2H), 7.40–7.30 (m, 5H), 7.18 (d, $J = 8.1$ Hz, 2H), 7.11 (d, $J = 7.8$ Hz, 2H), 5.49 (s, 1H), 5.37 (d, $J = 9.9$ Hz, 1H), 5.32 (d, $J = 3.9$ Hz, 1H), 5.21 (t, $J = 8.7$ Hz, 1H), 4.85 (dd, $J = 3.9$ and 10.2 Hz, 1H), 4.68–4.52 (m, 2H), 4.43 (d, $J = 5.4$ Hz, 1H), 4.36 (d, $J = 11.4$ Hz, 1H), 4.25 (dd, $J = 3.6$, 11.1 Hz, 1H), 3.93 (t, $J = 9.3$ Hz, 1H), 3.76–3.66 (m, 2H), 3.65–3.54 (m, 2H), 2.35 (s, H), 2.30 (s, 3H), 2.03 (s, 6H), 2.01 (s, 3H), and 1.96 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 145.0, 137.7, 136.7, 132.2, 131.5 (2C), 130.2 (2C), 129.6, 129.4 (2C), 128.9, 128.0 (2C), 127.5 (2C), 126.4 (2C), 101.6, 100.8, 86.5, 80.7, 79.6, 77.2, 74.9, 72.7, 71.5, 69.9, 69.5, 67.7, 63.4, 21.1, and 20.6; LC-HR-ESI-MS: *m/z* 691.1903 $[M + H]^+$ (calcd for $C_{33}H_{39}O_{12}S_2$ 691.1877).

3.2.3 *Synthesis of compound* ***7***

To a solution of compound **6** (1.5 g, 1.74 mmol) in EtOAc/MeOH (1:1, 60 ml) was added HCOONa (0.356 g, 5.24 mmol). The reaction mixture was stirred at 70°C for 24 h. It was then diluted with ethyl acetate (100 ml), washed with aqueous saturated $NaHCO_3$ (15 ml × 3), brine (15 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc 5:1, v/v) to give compound **7** (917 mg, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.48–7.39 (m, 2H), 7.38–7.30 (m, 5H), 7.07 (d, $J = 6.9$ Hz, 2H), 5.72 (d, $J = 4.8$ Hz, 1H), 5.56 (d, $J = 3.9$ Hz, 1H), 5.48 (s, 1H), 5.43 (d, $J = 9.6$ Hz, 1H), 4.97 (brs, 1H), 4.85 (dd, $J = 3.3$, 9.6 Hz, 1H), 4.31 (brs, 1H), 4.15 (dd, $J = 3.3$, 9.9 Hz, 1H), 3.91–3.78 (m, 2H), 3.74 (dd, $J = 9.0$, 10.5 Hz, 2H), 3.62 (dd, $J = 9.6$, 9.9 Hz, 1H), 2.99 (dd, $J = 6.9$, 13.5 Hz, 1H), 2.35 (s, 3H), 2.08 (s, 6H), 2.06 (s, 3H), and 1.60 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.6, 169.7, 169.5, 137.0, 136.9, 131.2 (2C), 129.9 (2C), 129.0, 128.1 (2C), 126.1 (2C), 121.9,

101.4, 96.9, 95.0, 78.8, 75.4, 72.4, 71.0, 68.8, 68.5, 67.8, 63.3, 50.9, 38.9, 20.9, 20.8 (2C), 20.7, and 19.6; LC-HR-ESI-MS: *m/z* 705.2216 $[M + H]^+$ (calcd for $C_{34}H_{41}O_{14}S$ 705.2212).

3.2.4 Synthesis of compound **10**

A solution of compound **8** (18.6 mg, 0.0234 mmol), compound **9** (30.3 mg, 0.0468 mmol), and dry 4 Å MS powder (about 20 mg) in dry CH_2Cl_2 (6 ml) was stirred at −50°C for 15 min under argon. TMSOTf (5% in CH_2Cl_2, v/v, 22 µl, 0.00585 mmol) was added. The reaction mixture was stirred at −50°C for 1 h and was quenched with 0.01 ml Et_3N. The solid was filtered, and the solvent was removed. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc 10:1, v/v) to afford compound **10** (14 mg, 45%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.44–7.40 (m, 2H), 7.36–7.29 (m, 6H), 7.05 (d, $J = 7.8$ Hz, 2H), 5.67 (d, $J = 5.4$ Hz, 1H), 5.47 (d, $J = 4.2$ Hz, 1H), 5.43 (s, 1H), 5.40 (dd, $J = 9.0$, 10.2 Hz, 1H), 5.30 (d, $J = 4.8$ Hz, 1H), 5.16 (d, $J = 2.4$ Hz, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.60 (d, $J = 12.6$ Hz, 1H), 4.38–4.32 (m, 1H), 4.31–4.27 (m, 1H), 4.14 (dd, $J = 4.8$, 10.2 Hz, 1H), 4.05–4.00 (m, 1H), 3.95–3.90 (m, 1H), 3.84–3.78 (m, 2H), 3.66 (dd, $J = 10.2$, 10.8 Hz, 1H), 3.61 (dd, $J = 3.6$, 10.2 Hz, 1H), 3.52 (t, $J = 9.6$ Hz, 1H), 3.50–3.41 (m, 2H), 3.38–3.30 (m, 2H), 3.00 (dd, $J = 6.6$, 13.8 Hz, 1H), 2.23 (s, 3H), 2.12 (s, 3H), 2.01 (s, 6H), 0.98 (d, $J = 6$ Hz, 3H), 0.92 (d, $J = 6.6$ Hz, 3H), 0.88 (s, 18H), 0.05 (s, 6H), 0.02 (s, 6H); LC-HR-ESI-MS: *m/z* 1317.7550 $[M + H]^+$ (calcd for $C_{73}H_{113}O_{17}Si_2$ 1317.7511).

3.2.5 Synthesis of compound **11**

To a solution of compound **10** (6 mg, 0.00455 mmol) in dry pyridine (2 ml) was added HF·pyridine (6.3 µl, 0.0455 mmol) at room temperature. The reaction mixture was stirred for 12 h and quenched with aqueous saturated $NaHCO_3$ (1 ml). It was then extracted with CH_2Cl_2 (15 ml × 3), and the combined extracts were washed with aqueous saturated $NaHCO_3$ (5 ml × 3), brine (5 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was dissolved in CH_2Cl_2/MeOH (1:1, 2 ml), followed by the addition of 10 equiv. 1 M MeONa/MeOH. The reaction was refluxed for 12 h and then cooled to room temperature. It was neutralized by ion exchange resin Amberlite IR-120(H^+), filtered, and concentrated to give yellow oil, which was purified by silica gel column chromatography (CH_2Cl_2/MeOH 20:1, v/v) to afford the desired compound **11** (3.2 mg, 76%). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.38 (d, $J = 7.2$ Hz, 2H), 7.30–7.24 (m, 6H), 6.99 (d, $J = 8.4$ Hz, 2H), 5.62 (d, $J = 4.8$ Hz, 1H), 5.28 (d, $J = 4.8$ Hz, 1H), 5.17 (d, $J = 3.6$ Hz, 1H), 4.71 (d, $J = 12.0$ Hz, 1H), 4.69 (d, $J = 11.4$ Hz, 1H), 4.29 (dd, $J = 3.6$, 4.2 Hz, 1H), 4.09–4.05 (m, 2H), 4.03–4.02 (m, 1H), 3.85 (dd, $J = 3.0$, 3.6 Hz, 1H), 3.80–3.67 (m, 1H), 3.72–3.59 (m, 4H), 3.45–3.42 (m, 2H), 3.38 (dd, $J = 5.4$, 10.8 Hz, 1H), 3.35–3.25 (m, 1H, H-3), 2.99 (dd, $J = 8.4$, 13.8 Hz, 1H), 0.98 (s, 3H), 0.90 (d, $J = 6.6$ Hz, 3H), 0.85 (d, $J = 6.6$ Hz, 3H), and 0.83 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz): δ 142.4, 139.7, 137.8, 129.9, 129.3 (2C), 129.1 (2C), 129.1 (2C), 128.6, 127.6 (2C), 122.3, 102.9, 98.7, 98.2, 82.6, 80.9, 79.6, 78.0, 73.9, 73.7, 72.4, 71.0, 70.7, 69.7, 68.7, 64.9, 62.6, 56.3, 51.7, 43.5, 43.0, 41.1, 39.3, 38.5, 37.7, 37.5, 36.9, 36.8, 34.8, 32.9, 32.3, 31.2, 25.5, 24.8, 21.9, 21.0, 19.9, 18.8, 17.3, and 13.4; LC-HR-ESI-MS: *m/z* 921.5340 $[M + H]^+$ (calcd for $C_{53}H_{77}O_{13}$ 921.5359).

3.2.6 Synthesis of compound **12**

Benzoyl chloride (BzCl, 1.7 ml, 15 mmol, 10 equiv.) was added several times to a stirred solution of compound **4** (833 mg,

1.553 mmol) in CH_2Cl_2/pyridine (3:1, v/v, 40 ml). The reaction mixture was stirred for 48 h and quenched with MeOH (2 ml). It was then concentrated *in vacuo* and diluted with CH_2Cl_2 (100 ml), then washed with 1 M HCl (10 ml × 3), aqueous saturated $NaHCO_3$ (10 ml × 3), brine (10 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 40:1, v/v) to give compound **12** (596 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.02 (d, $J = 7.6$ Hz, 2H), 7.57 (t, $J = 7.2$ Hz, 1H), 7.49–7.40 (m, 4H), 7.38 (d, $J = 7.6$ Hz, 2H), 7.32–7.26 (m, 3H), 6.89 (d, $J = 8.0$ Hz, 2H), 5.42 (s, 1H), 4.98 (d, $J = 2.8$ Hz, 1H), 4.68 (d, $J = 12.0$ Hz, 1H), 4.43 (d, $J = 9.6$ Hz, 1H), 4.34–4.28 (m, 2H), 3.96 (dd, $J = 9.2$, 9.6 Hz, 1H), 3.92–3.84 (m, 1H), 3.73 (dd, $J = 8.4$, 8.8 Hz, 1H), 3.63–3.42 (m, 4H), 3.35 (t, $J = 9.2$ Hz, 1H), and 2.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.1, 138.0, 137.0, 133.0, 133.0 (2C), 129.9, 129.7 (2C), 129.6 (2C), 129.2, 128.4 (2C), 128.3 (2C), 128.2, 126.4 (2C), 102.5, 101.8, 87.5, 81.2, 80.5, 77.4, 76.2, 73.2, 71.5, 70.9, 68.5, 63.7, and 21.1; LC-HR-ESI-MS: *m/z* 641.2052 $[M + H]^+$ (calcd for $C_{33}H_{37}O_{11}S$ 641.2051).

3.2.7 *Synthesis of compound **16***

Compound **4** (302 mg, 0.563 mmol) was dissolved in dry pyridine (5 ml), followed by the addition of 4-dimethylaminopyridine (DMAP; 13.7 mg, 0.112 mmol) and tert-butyldiphenylchlorosilane (TBDPSiCl) (0.57 ml, 2.253 mmol) at room temperature. The mixture was stirred for 5 h and diluted with EtOAc (50 ml). Then it was washed with aqueous saturated $NaHCO_3$ (5 ml × 3), brine (5 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc 5:1, v/v) to give compound **16** (340 mg, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.81 (d, $J = 6.9$ Hz, 2H), 7.73 (d, $J = 6.9$ Hz, 2H), 7.49–7.41 (m, 4H), 7.40–7.31 (m, 5H), 7.30–7.22 (m, 4H), 6.98 (d, $J = 7.5$ Hz, 2H), 5.43 (s, 1H), 5.05 (d, $J = 3.0$ Hz, 1H), 5.39 (d, $J = 3.9$ Hz, 1H), 4.42 (t, $J = 9.6$ Hz, 1H), 4.20–4.16 (m, 1H), 3.96–3.84 (m, 2H), 3.83–3.75 (m, 2H), 3.74–3.60 (m, 2H), 3.59–3.44 (m, 2H), 3.43–3.30 (m, 2H), 3.29–3.20 (m, 2H), 2.26 (s, 3H), and 1.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 137.9, 137.1, 135.9 (2C), 135.7 (2C), 133.4, 132.9, 132.7 (2C), 129.7 (4C), 129.2, 128.7, 128.3 (2C), 127.6 (4C), 126.4 (2C), 102.3, 101.8, 87.8, 80.6, 80.5, 79.2, 73.7, 71.7, 71.0, 68.6, 63.6, 62.4, 26.78 (3C), 21.07, and 19.21; LC-HR-ESI-MS: *m/z* 775.2964 $[M + H]^+$ (calcd for $C_{42}H_{51}IO_{10}SSi$ 775.2967).

3.2.8 *Synthesis of compound **22***

To a solution of compound **20** (11 mg, 0.010 mmol) in pyridine, HF·pyridine (9.0 μl, 0.100 mmol) was added at room temperature. The mixture was stirred for 12 h and quenched with aqueous saturated $NaHCO_3$ and then extracted with CH_2Cl_2 (15 ml × 3). The combined extracts were washed with aqueous saturated $NaHCO_3$ (5 ml × 3), brine (5 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc 5:1, v/v) to give compound **22** (5.4 mg, 58%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.03 (d, $J = 7.8$ Hz, 2H), 7.57 (t, $J = 7.2$ Hz, 2H), 7.46 (d, $J = 6.0$ Hz, 2H), 7.43 (d, $J = 6.6$ Hz, 2H), 7.34 (d, $J = 6.0$ Hz, 3H), 5.49 (s, 1H), 5.32 (d, $J = 3.6$ Hz, 1H), 5.12 (d, $J = 3.6$ Hz, 2H), 5.01 (t, $J = 3.6$ Hz, 1H), 4.64 (d, $J = 12.0$ Hz, 1H), 4.48–4.38 (m, 2H), 4.33 (dd, $J = 4.2$, 10.2 Hz, 1H), 4.02–3.94 (m, 2H), 3.93–3.85 (m, 2H), 3.68 (dd, $J = 3.9$, 9.9 Hz, 1H), 3.66–3.58 (m, 2H), 3.56–3.47 (m, 1H), 3.44 (dd, $J = 9.0$, 9.6 Hz, 1H), 3.40 (dd, $J = 6.0$, 10.2 Hz, 1H), 3.28 (dd, $J = 6.0$, 10.2 Hz, 1H), and 0.96 (d,

$J = 6.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.2, 140.8, 136.9, 135.2, 133.1, 129.6 (2C), 128.4 (2C), 128.3 (2C), 126.2 (2C), 125.0, 121.3, 102.5, 101.7, 94.3, 81.8, 80.8, 75.5, 74.6, 73.4, 71.8, 70.7, 70.8, 69.6, 68.6, 68.0, 63.9, 63.6, 61.1, 54.2, 49.9, 42.3, 37.1, 36.4, 35.4, 33.3, 32.4, 32.2, 31.6, 31.4, 30.2, 29.7, 26.4, 23.9, 23.4, 20.7, 19.4, 18.1, 16.3, and 13.2; LC-HR-ESI-MS: *m/z* 935.5162 $[M + H]^+$ (calcd for $C_{53}H_{75}O_{14}$ 935.5151).

3.2.9 *Synthesis of compound* ***23***

To a solution of compound **21** (15 mg, 0.0102 mmol) in pyridine, HF·pyridine (9.2 μl, 0.102 mmol) was added at room temperature. The mixture was stirred for 12 h and quenched with aqueous saturated $NaHCO_3$ and then extracted with CH_2Cl_2 (15 ml × 3). The combined extracts were washed with aqueous saturated $NaHCO_3$ (5 ml × 3), brine (5 ml × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was resolved in CH_2Cl_2/MeOH (2:1, v/v, 5 ml), followed by the addition of $NaHSO_4{\cdot}SiO_2$ (30 mg) at room temperature. The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc 5:1, v/v) to give compound **23** (6 mg, 64.6%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.41 (d, $J = 3.6$ Hz, 1H), 5.32 (d, $J = 3.3$ Hz, 1H), 5.20 (d, $J = 9.9$ Hz, 1H), 5.18–5.07 (m, 1H), 4.95 (d, $J = 3.3$ Hz, 1H), 4.78 (dd, $J = 3.9$, 10.2 Hz, 1H), 4.46–4.34 (m, 1H), 4.10–3.92 (m, 3H), 3.90–3.66 (m, 4H), 3.62–3.38 (m, 5H), 2.10 (s, 3H), 2.08 (s, 6H), 2.04 (s, 3H), 0.89 (d, $J = 6.9$ Hz, 3H), and 0.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 171.8, 171.6, 170.6, 170.4, 140.9, 121.2, 95.8, 94.5, 75.99, 75.67, 72.92, 72.65, 71.71, 71.45, 71.37, 70.39, 70.20, 68.20, 62.57, 61.06, 60.73, 49.97, 42.3, 39.7, 37.2, 36.5, 36.3, 34.8, 32.8, 32.2, 31.9, 31.6, 31.5, 30.2, 29.7, 23.4, 23.1, 21.3, 20.9, 20.7, 20.6, 19.3, 18.2, 15.8, 14.1, and 10.9; LC-HR-ESI-MS: *m/z* 911.4989 $[M + H]^+$ (calcd for $C_{47}H_{75}O_{17}$ 911.4999).

Supplementary data

The synthetic procedure, ^{1}H NMR, ^{13}C NMR, HR-MS data of compounds **1**–**3**, **6**, **8**–**9**, **13**–**15**, **17**–**19**, and **21**, and experimental procedures of these compounds are available online in supporting information.

Acknowledgments

Financial support of this research by the National Natural Science Foundation of China (NNSFC 30772632) and the National Program of New Drug Innovation and Production (2009ZX09301-003-9-1) is gratefully acknowledged by the authors.

References

[1] K. Tamura, H. Honda, Y. Mimaki, Y. Sashida, and H. Kogo, *Br. J. Pharmacol.* **121**, 1796 (1997).
[2] S. Kubo, Y. Mimaki, M. Terao, Y. Sashida, T. Nikaida, and T. Ohmoto, *Phytochemistry* **31**, 3969 (1992).
[3] Y. Mimaki, M. Kuroda, A. Kameyama, Y. Sashida, T. Hirano, K. Oka, R. Maekawa, T. Wada, K. Sugita, and A. Beutler, *J. Bioorg. Med. Chem. Lett.* **7**, 633 (1997).
[4] W.S. Yu and Z.D. Jin, *J. Am. Chem. Soc.* **124**, 6576 (2002).
[5] Q.H. Xu, X.W. Peng, and W.S. Tian, *Tetrahedron Lett.* **44**, 9375 (2003).
[6] J. Xue, P. Liu, Y.B. Pan, and Z.W. Guo, *J. Org. Chem.* **73**, 157 (2008).
[7] J.W. Morzycki, A. Wojtkielewicz, and S. Wolczynski, *Bioorg. Med. Chem. Lett.* **14**, 3323 (2004).
[8] A. Wojtkielewicz, A. Długosz, J. Maj, J. W. Morzycki, M. Nowakowski, J. Renkiewicz, M. Strnad, J. Swaczynova, A.Z. Wilczewska, and J. Wojcik, *J. Med. Chem.* **50**, 3667 (2007).
[9] L.H. Deng, H. Wu, B. Yu, M.R. Jiang, and J.R. Wu, *Bioorg. Med. Chem. Lett.* **17**, 278 (2004).
[10] B.F. Shi, P.P. Tang, X.Y. Hu, J.O. Liu, and B.J. Yu, *Org. Chem.* **70**, 10354 (2005).
[11] P.P. Tang, F. Mamdani, X.Y. Hu, J.O. Liu, and B. Yu, *Bioorg. Med. Chem. Lett.* **17**, 1003 (2007).

[12] W.J. Peng, P. Tang, X.Y. Hu, J.O. Liu, and B. Yu, *Bioorg. Med. Chem. Lett.* **17**, 5506 (2007).
[13] T. Tschamber, S. Adam, Y. Matsuya, S. Masuda, N. Ohsawa, S. Maruyama, K. Kamoshita, H. Nemoto, and J. Eustache, *Bioorg. Med. Chem. Lett.* **17**, 5101 (2007).
[14] D. Zheng, L. Zhou, Y.Y. Guan, and P.S. Lei, *Bioorg. Med. Chem. Lett.* **20**, 5439 (2010).
[15] Y.Y. Guan, D. Zheng, and P.S. Lei, *Bioorg. Med. Chem. Lett.* **21**, 2921 (2011).
[16] C. Guo, T.G. LaCour, and P.L. Fuchs, *Bioorg. Med. Chem. Lett.* **9**, 419 (1999).
[17] K. Sakurai, T. Fukumoto, K. Noguchi, N. Sato, H. Asaka, N. Moriyama, and M. Yohda, *Org. Lett.* **12**, 5732 (2010).
[18] I.M. Muller, V.M. Dirsch, A. Rudy, A.N. Lopez, G.R. Pettit, and A.M. Vollmar, *Mol. Pharmacol.* **67**, 1684 (2005).
[19] Y. Zhou, C. Garcia-Prieto, D.A. Carney, R. Xu, H. Pelicano, Y. Kang, W. Yu, C. Lou, S. Kondo, J. Liu, D.M. Harris, Z. Estrov, M.J. Keating, Z. Jin, and P. Huang, *J. Natl. Cancer. Inst.* **97**, 1781 (2005).
[20] J. Zhu, L. Xiong, B. Yu, and J. Wu, *Mol. Pharm.* **68**, 1831 (2005).
[21] A.W.G. Burgett, T.B. Poulsen, K. Wangkanont, D.R. Anderson, C. Kikuchi, K. Shimada, S. Okubo, K.C. Fortner, Y. Mimaki, M. Kuroda, J.P. Murphy, D.J. Schwalb, E.C. Petrella, I. Cornella-Taracido, M. Schirle, J.A. Tallarico, and M. D. Shair, *Nat. Chem. Biol.* **7**, 639 (2011).
[22] S.C. Mayer, P.I. Dollings, R.E. Medevitt, CN 1328566A, CA2350066A1. EP1133509 A1, WO2000031096A1.
[23] D. Zheng, Y.Y. Guan, and P.S. Lei, *Bioorg. Med. Chem. Lett.* **21**, 3257 (2011).
[24] Z. Wang, J. Zhou, Y. Ju, H. Zhang, M. Liu, and X. Li, *Biol. Pharm. Bull.* **24**, 159 (2001).