Synthesis and anticancer activity of dimeric podophyllotoxin derivatives

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Introduction

Podophyllotoxin 1 (Figure 1) is the most abundant naturally occurring cyclolignan mainly isolated from podophyllum species and shows strong cytotoxic activity against various cancer cell lines by inhibiting microtubule assembly.1–3 Podophyllotoxin is not a clinically useful anticancer drug because of its high toxicity; however, several semisynthetic derivatives, such as etoposide (2) (Figure 1), are clinically used chemotherapeutic agents.
for a number of cancers, including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi’s sarcoma.4–6

Earlier reports indicated that the β-configuration at C-4 of podophyllotoxin scaffold is not favorable for tubulin polymerization inhibition activity.7 However, the comparison of the crystal structures of tubulin-DMEP (4′-demethyllepipodophyllotoxin) and tubulin-podophyllotoxin suggests that the C-4 β-configuration does not show any disadvantage for tubulin binding.8 For podophyllotoxin derivatives as topoisomerase-II inhibitors, structure–activity relationship (SAR) data show that 4β-substitution is essential for the anticancer activity.9,10

In the attempt to discover less toxic and more effective anticancer agents, many podophyllotoxin derivatives have been synthesized for biological studies.11,12 4β-1,2,3-Triazole derivatives of podophyllotoxin have been shown to exhibit more potent anticancer activity and better binding to topoisomerase-II than etoposide.13–15 Recently, we also reported a group of podophyllotoxin glycoconjugates linked via 4β-1,2,3-triazole functionality as potential antitumor agents.16–18 Our studies showed that podophyllotoxin derivatives with a perbutyrylated sugar residue displayed higher activity than their counterparts lacking butyryl groups.16,17

There have also been reports on the synthesis of dimeric podophyllotoxin derivatives19,20 which exhibited promising in vitro anticancer activity against different human tumour cell lines. In the present study, a group of dimeric podophyllotoxin derivatives 4 (Figure 1), with different linkers have been prepared using the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.21,22 Their synthesis and anticancer activity against five cancer cell lines are described.

Results and discussion
Chemical synthesis

The click reaction of copper(I)-catalyzed Huisgen 1,3-dipolar azide-alkyne cycloaddition (CuAAC) provides 1,4-disubstituted 1,2,3-triazaoles, which is a powerful tool for the generation of novel pharmacophores.21,22 To access dimeric podophyllotoxin derivatives 4 (Figure 1), di-propargyl functionized linkers are required. Scheme 1 depicts the synthesis of symmetric 1,3-di-O-propargyl glycerol (10) and its glycosylated derivatives (12–14). Initially, we tried direct propargylation of glycerol with propargyl bromide in the presence of sodium hydride (NaH) to prepare 10. However, the reaction provided complicated products and the strategy was abandoned. Thus, Solketal 5 was treated with propargyl bromide and sodium hydride, and then with HCl in methanol solution to give diol 6 as previously described.23 The diol 6 was first converted to bis-silyl ether 7 by treatment with tert-butylimethylsilyle chloride (TBSCl) and imidazole in anhydrous N,N-dimethylformamide (DMF). The more labile primary TBS ether in 7 was then

Figure 1 Structures of podophyllotoxin (1), etoposide (2), 4β-1,2,3-triazolyl-podophyllotoxin derivatives (3), and dimeric 4β-1,2,3-triazolyl-podophyllotoxin derivatives (4).
selectively cleaved with pyridine-HF in pyridine.\textsuperscript{24} Etherification of the primary alcohol 8 with propargyl bromide gave nine in 56\% yield. Treatment of 9 with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave alcohol 10.\textsuperscript{25} Next, 10 was allowed to react with the α-glucose trichloroacetimidate derivative 11\textsuperscript{26} in the presence of BF\textsubscript{3}·Et\textsubscript{2}O at −78°C to provide, as expected, only the β-glycoside 12 in 63\% yield. Then, removal of acetyl groups with CH\textsubscript{3}ONa in CH\textsubscript{3}OH produced 13, which was subjected to perbutyrylation with butyric anhydride in the presence of pyridine to give the perbutyrylated product 14 in good yield. Interestingly, the anomeric proton of the glucose residue was found significantly downfield and shifted in the 1\textsuperscript{H}-NMR spectra of the peracetylated derivative 14 (δ 6.45 ppm, d, J=8.0 Hz) for the anomeric proton confirms a non-acylated linkage in 12−tritylated derivative 14. The synthesis of dipropargyl functionalized linkers based on glucose scaffold is described in Scheme 2. The readily available 6-O-tritylated 15\textsuperscript{27} was treated with TBSCI and imidazole in anhydrous DMF to yield 16 in 80\% yield. Acid catalyzed removal of the trityl group yielded 17 in 93\% yield. Compound 17 was then treated with propargyl bromide and NaH to provide 18 (50\%), which was treated with TBAF in the presence of acetic acid in THF to give triol 19 in 80\% yield. Compound 19 was then subjected to benzylation, acetylation, or butyrylation by treatment with benzyl bromide/NaH, acetic anhydride/pyridine, or butyric anhydride/pyridine, respectively, to give 20, 21, or 22 in good to excellent yield.

The azido-substituted podophyllotoxin derivatives needed for the click reaction, 4β-azido-4-deoxypodophyllotoxin 23 and 4β-azido-4-deoxy-4′-demethylpodophyllotoxin 24, can be readily prepared from podophyllotoxin according to a previous report.\textsuperscript{29} The azides 23 and 24 were allowed to react with dipropargyl functionalized linkers 10, 12−14, and 19−22 in the presence of CuSO\textsubscript{4}·5H\textsubscript{2}O, sodium ascorbate in t-butyl alcohol and water (1:1) at room temperature to obtain symmetric (25−32) and unsymmetric (33−40) dimeric podophyllotoxin derivatives in very good yield (Scheme 3).

All the products were characterized by 1\textsuperscript{H}-NMR, 13\textsuperscript{C}-NMR, ESI-MS, and HRESI-MS data. The presence of the triazole ring in these dimeric podophyllotoxin derivatives

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**Scheme 1** Reagents and reaction conditions: a. NaH, THF, propargyl bromide, reflux, overnight, 56\%; b. HCl, CH\textsubscript{3}OH, rt, 8 hours, 90\%; c. TBSCI, imidazole, DMF, overnight, 80\%; d. HF-pyridine, pyridine, THF, rt, 22 hours, 74\%; e. TBAF, HOAc, THF, rt, 18 hours, 70\%; f. BF\textsubscript{3}·Et\textsubscript{2}O, CH\textsubscript{3}Cl\textsubscript{2}, −78°C, N\textsubscript{2}, 63\%; g. CH\textsubscript{3}ONa, CH\textsubscript{3}OH, 12 hours, 72\%; h. (CH\textsubscript{3}CH\textsubscript{2}CH\textsubscript{2}CO)\textsubscript{2}O, pyridine, 0°C–rt, 12 hours, 90\%.

**Scheme 2** Reagents and reaction conditions: a. TBSCI, imidazole, DMF, overnight, 80\%; b. formic acid-ether (1:1), 93\%; c. NaH, THF, propargyl bromide, reflux, 50\%; d. TBAF, HOAc, THF, 18 hours, 80\%; e. Br\textsubscript{2}, NaH, DMF, 0°C–rt, 70\%; f. Ac\textsubscript{2}O, pyridine, 0°C–rt, 12 hours, 91\%; g. (CH\textsubscript{3}CH\textsubscript{2}CH\textsubscript{2}CO)\textsubscript{2}O, pyridine, 0°C–rt, 12 hours, 93\%.
was confirmed by the proton signal at around δ 7.27–7.96 ppm (C-5-H of the triazole ring) in the aromatic region of the 1H-NMR spectrum, as well as by a pair of carbon signals at around 145 ppm and 124 ppm in the 13C-NMR spectrum. The proton at C-4 of the podophyllotoxin scaffold of these derivatives appears to be doublet at 5.85–6.22 ppm, typically having J3,4,5,0 Hz due to a cis relationship between H-3 and H-4. The two podophyllotoxin moieties in symmetric dimeric derivatives (25–32) are identical and give one set of NMR signals. On the other hand, the two podophyllotoxin moieties in unsymmetric dimeric derivatives (33–40) are not identical and produce two sets of NMR signals very close in chemical shifts. ESI-MS and HRESI-MS of all compounds showed the [M+Na]+ or [M+H]+ adduct as the molecular ion.

Proton and carbon-13 NMR spectra for compounds 25–40 are available in the Supplementary Materials.

Anticancer activity
The in vitro anticancer activity of the synthesized dimeric podophyllotoxin derivatives 25–40 was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay against five human cancer cell lines, including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer). Etoposide (2) and cisplatin were taken as reference compounds and the IC50 (inhibition concentration with 50% cell growth relative to the control) of all compounds are presented in Table 1. Their IC50 values reveal that most of

Scheme 3 Reagents and reaction conditions: a. CuSO4·5H2O, sodium ascorbate, t-BuOH:H2O (1:1), 4 hours, rt. 74%–86%.
these derivatives are not active (IC\textsubscript{50}>40 \mu M). However, compound 29 is very active against all five cancer cell lines tested, with IC\textsubscript{50} values ranging from 0.43 to 3.50 \mu M, which is significantly more potent than etoposide and cisplatin. Among the compounds based on a glucose linker (33–40), only 39 and 40 show moderate activity with IC\textsubscript{50} values in the range of 14.00–30.45 \mu M.

The data in Table 1 indicate that the linking spacer between the two podophyllotoxin moieties can largely affect the anticancer activity of these compounds. Compound 29 which carries a perbutyrylated glucose residue displays much higher potency than those lacking a glucose residue (25), having a free glucose residue (27) or having a peracetylated glucose residue (28). This observation agrees with our earlier reports that several podophyllotoxin glycoconjugates containing perbutyrylated sugar residues show higher anticancer activity than those without butyryl groups. In comparison to 29, the 4’-demethylated analog 32 loses its activity, confirming the earlier observation that the substitution group on the 4’-position of podophyllotoxin scaffold can significantly affect the anticancer potency of podophyllotoxin derivatives.

Table 1 In vitro anticancer activity (IC\textsubscript{50} \mu M) of dimeric podophyllotoxin derivatives 25–40 against human tumor cell lines

| Compounds | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW480 | BEAS-2B |
|-----------|-------|-----------|-------|-------|-------|---------|
| 25        | >40   | >40       | >40   | >40   | >40   | >40     |
| 26        | >40   | >40       | >40   | >40   | >40   | >40     |
| 27        | >40   | >40       | >40   | >40   | >40   | >40     |
| 28        | >40   | >40       | >40   | >40   | >40   | >40     |
| 29        | 0.43±0.14 | 1.52±0.48 | 0.89±0.33 | 1.54±0.41 | 3.50±0.45 | 15.38±0.14 |
| 30        | >40   | >40       | >40   | >40   | >40   | >40     |
| 31        | >40   | >40       | >40   | >40   | >40   | >40     |
| 32        | >40   | >40       | >40   | >40   | >40   | >40     |
| 33        | >40   | >40       | >40   | >40   | >40   | >40     |
| 34        | 20.11±0.57 | >40       | >40   | >40   | >40   | >40     |
| 35        | >40   | >40       | >40   | >40   | >40   | >40     |
| 36        | >40   | >40       | >40   | >40   | >40   | >40     |
| 37        | >40   | >40       | >40   | >40   | >40   | >40     |
| 38        | >40   | >40       | >40   | >40   | >40   | >40     |
| 39        | 16.47±0.44 | 15.20±0.56 | 18.02±0.47 | 30.46±0.99 | 27.39±0.78 |
| 40        | 14.00±0.34 | 15.03±0.38 | 19.17±0.56 | 29.13±1.03 | 29.07±0.94 |
| Etoposide (2) | 0.31±0.24 | 8.12±0.72 | 11.92±0.12 | 32.82±0.44 | 17.11±0.67 | 11.17±0.56 |
| Cisplatin | 1.67±0.44 | 6.93±0.28 | 7.42±0.12 | 10.85±0.51 | 9.89±0.53 | 12.86±0.25 |

One major drawback of cancer chemotherapy is associated with the low-/non-selective nature of cytotoxic drugs, which attack cancer cells as well as normal cells, leading to serious side effects. To evaluate the degree of selectivity of 29, its growth inhibitory effect on a normal human bronchial epithelial cell line, BEAS-2B, was measured (Table 1). The selectivity index (SI) was expressed as the ratio of the IC\textsubscript{50} value of the compound in normal BEAS-2B cell line over that in cancer cell line. A greater SI value indicates that the drug molecule displays higher selectivity towards cancer cells as compared with normal cells. As shown in Table 2, compound 29 has SI values ranging from 4.4 to 35.7 in all five cancer cell lines tested. Literature papers have considered that an SI value greater than 2.0\textsuperscript{29} or 3.0\textsuperscript{30,31} is an interesting selectivity index. Importantly, the selectivity indexes of 29 are much greater than those observed for both clinically used anticancer drugs, etoposide and cisplatin.

Table 2 Selectivity of the cytotoxicity of 29, etoposide (2) and cisplatin to cancer cells as compared with BEAS-2B normal cells

| Compound | Selectivity index (SI) |
|----------|-----------------------|
|          | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW480 |
| 29       | 35.7  | 10.1      | 17.3  | 10.0  | 4.4   |
| Etoposide (2) | 36.0  | 1.4       | 0.9   | 0.3   | 0.7   |
| Cisplatin | 7.7   | 1.9       | 1.7   | 1.2   | 1.3   |

Note: Selectivity index (SI) = IC\textsubscript{50} of the compound in BEAS-2B cell line/IC\textsubscript{50} of the compound in cancer cell line.
except in the case of HL-60 cells where 29 and etoposide have similar SI values. These data suggest that 29 is significantly more cytotoxic to the cancer cell lines as compared with the normal cell line.

**Conclusion**

This paper describes the preparation of a group of dimeric podophyllotoxin derivatives linked via 1,2,3-triazole functional groups. 4β-Azido-podophyllotoxin/4′-demethylpodophyllotoxin reacts with various dipropargyl functionalized linkers by copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction to produce novel dimeric podophyllotoxin derivatives. MTT assay was used to evaluate the in vitro anticancer activity of these compounds against a panel of five human cancer cell lines including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer). Most of the synthesized compounds do not show anticancer activity. Notably, compound 29, which bears a perbutyrylated glucose residue on the glycerol linker and is 4′-O-methylated on the E ring, is highly active against all five tested cancer cell lines with IC_{50} values ranging from 0.43 to 3.50 μM. As compared with the normal BEAS-2B (lung) cell line, compound 29 is significantly more selective towards all five tested cancer cell lines with selectivity indexes in the range of 4.4–35.7. Taken together, compound 29 is significantly more cytotoxic and selective towards cancer cells than the clinically used drug etoposide or cisplatin. Further studies are required to study the promising antitumor agent.

**Experimental**

**General**

Melting points were uncorrected. Mass spectroscopy (MS) data were obtained in the ESI mode using API Qstar Pulsar instrument. HRMS data were obtained in the ESI mode using LCMS-IT-TOF (Shimadzu, Kyoto, Japan). NMR spectra were acquired using Bruker AV400 or DRX-500 or Bruker AVANCE III-600 (Bruker BioSpin GmbH, Rheinstetten, Germany) instruments, where tetramethylsilane (TMS) was used as an internal standard. Column chromatography (CC) was performed with flash silica gel (200–300 mesh; Qingdao Makall Group Co., Ltd; Qingdao; China). All reactions were monitored by thin-layer chromatography (TLC) and spots were visualized by spraying 10% H_2SO_4 in ethanol (EtOH) on warm silica gel plates. The human cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480, and the normal BEAS-2B cell line were purchased from the American Type Culture Collection (ATCC).

**1,2-Di-O-tert-butyldimethylsilyl-3-(prop-2-yn-1-yloxy)propan-1,2-diol (7)**

Imidazole (3.8 g, 55.2 mmol) and TBSCl (7.2 g, 47.8 mmol) were added to a solution of diol 6 (2.4 g, 18.5 mmol) in dimethylformamide (DMF) (60 mL). The reaction mixture was stirred overnight, and then diluted with H_2O (300 mL). The solution was extracted with ether (Et_2O) (3×150 mL) and the combined organic layer was dried over sodium sulfate (Na_2SO_4). After removing the solvent in vacuo, the residue was purified by CC (RF=0.20, petroleum ether: ethyl acetate=40:1) to give 7 (5.2 g, 80%) as a colorless oil. ^1H-NMR (CDCl_3, 400 MHz) δ4.12 (d, 2 H, J=2.4 Hz, O-CH_2), 3.84–3.78 (m, 1 H), 3.60–3.52 (m, 3 H), 3.45–3.41 (m, 1 H), 2.37 (t, 1 H, J=2.4 Hz, C=CH), 0.87–0.86 (m, 18 H), 0.06–0.03 (m, 12 H); ^13C-NMR (CDCl_3, 100 MHz) δ79.7 (C=CH), 74.2 (C=CH), 72.5 (O-CH), 64.8 (O-CH), 58.4 (CH_2=C=CH), 25.9 (C-CH_3), 25.8 (C-CH_3), 25.6 (C-CH_3), 18.2 (Si-C), 18.1 (Si-C), –4.7 (Si-CH), –4.8 (Si-CH), –5.4 (Si-CH), –5.5 (Si-CH); ESIMS was calculated for C_{18}H_{38}O_5Si_2Na [M+Na]^+ 381 and found to be 381.

**2-O-tert-butylidethylsilyl-3-(prop-2-yn-1-yloxy)propan-1,2-diol (8)**

To a solution of 7 (6.8 g, 19.0 mmol) in dry THF (60 mL), the HF-pyridine complex (1.7 mL) and pyridine (10 mL) were added. The reaction mixture was stirred for 20 hours. After completion of the reaction (TLC monitoring), the solution was diluted with diethyl ether (100 mL), washed with 0.5 M HCl (2×50 mL) and saturated copper sulfate solution (50 mL), and dried over anhydrous Na_2SO_4. After removal of the solvents, the residue was purified by chromatography (RF=0.50, petroleum ether: ethyl acetate=4:1) to give 8 (3.4 g, 74%) as a colorless liquid. ^1H-NMR (CDCl_3, 400 MHz) δ4.16 (d, 2 H, J=2.4 Hz, O-CH_2), 3.93–3.88 (m, 1 H), 3.67–3.63 (m, 1 H), 3.60–3.53 (m, 3 H), 2.44 (t, 1 H, J=2.4 Hz), 0.89 (s, 9 H) 0.10 (s, 6 H); ^13C-NMR (CDCl_3, 100 MHz) δ79.7 (C=CH), 74.2 (C=CH), 72.5 (O-CH), 71.6 (O-CH), 58.4 (CH_2-C=CH), 64.8 (HO-CH), 58.4 (CH_2-C=CH), 25.9 (C-CH_3), 18.2 (Si-C), 5.44 (Si-CH); ESIMS was calculated for C_{18}H_{38}O_5Si_2Na [M+Na]^+ 267 and found to be 267.

**2-O-tert-butylidethylsilyl-1,3-di-(prop-2-yn-1-yloxy)propan-1,2-diol (9)**

Suspension of NaH (253.2 mg, 6.3 mmol) in dry THF (5 mL) under N_2 was added to a solution of 8 (1.0 g, 4.2 mmol) in dry tetrahydrofuran (THF) (15 mL) at 0°C. The mixture was stirred at room temperature for 0.5 hour, and then propargyl bromide (0.3 mL, 4.2 mmol) in THF (15 mL) was quickly
added and the reaction was refluxed overnight. The reaction mixture was quenched with water, and then THF was removed in vacuo. The residue was extracted with CH₂Cl₂ (2x50 mL), and the organic layer was washed with brine (100 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by CC (RF=0.40, petroleum ether: ethyl acetate=10:1) to obtain 9 (1.2 g, 56%). ¹H-NMR (CDCl₃, 400 MHz) δ 4.33–4.32 (m, 2 H, O-CH₂), 4.19–4.18 (m, 2 H, O-CH₂), 3.79–3.75 (m, 1 H), 3.73–3.68 (m, 3 H), 3.62–3.58 (m, 1 H), 2.43–2.41 (m, 2 H, 2×C=CH), 0.89 (s, 9 H), 0.06 (s, 9 H); ¹³C-NMR (CDCl₃, 100 MHz) δ 77.7 (C=CH), 74.9 (C=CH), 69.5 (O-CH₂), 62.4 (O-CH₂), 58.7 (O-CH₂), 25.9 (C-CH₂), 18.2 (Si-C), 5.44 (Si-CH₃); ESIMS was calculated for C₁₉H₂₀O₄Na [M+Na]+ 305 and was found to be 305.

1,3-Di-(prop-2-yn-1-xyloxy)propan-2-ol (10)
Acetic acid (0.3 mL, 5.4 mmol) and tetra-butylammonium fluoride trihydrate (1.5 mL, 5.4 mmol) were added to a solution of 9 (384.3 mg, 1.4 mmol) in dry THF (20 mL) at room temperature. The mixture was stirred for 18 hours, and then the solvent was evaporated under reduced pressure. The crude product was purified by passing through a short column (RF=0.30, petroleum ether: ethyl acetate=2:1) to obtain 10 (160 mg, 70%) as a colorless liquid. ¹H-NMR (CDCl₃, 400 MHz) δ 4.40–4.28 (m, 2 H, O-CH₂), 4.18–4.23 (m, 2 H, O-CH₂), 3.83–3.76 (m, 1 H), 3.70–3.66 (m, 4 H), 2.48–2.45 (m, 2 H, 2×C=CH); ¹³C-NMR (CDCl₃, 100 MHz) δ 77.7 (C=CH), 74.7 (C=CH), 69.5 (O-CH₂), 62.4 (O-CH₂), 58.7 (O-CH₂); ESIMS was calculated for C₁₉H₂₂O₄Na [M+Na]+ 191 and was found to be 191. The ¹H-NMR and ¹³C-NMR data are in full agreement with those reported in the literature.⁵

1,3-Di-(prop-2-yn-1-xyloxy)prop-2-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (12)
A solution of BF₃·Et₂O (87.2 μL, 0.7 mmol) in dichloromethane (1 mL) at −78°C was added dropwise to a solution of 11²⁴ (225 mg, 0.5 mmol) and 10 (77 mg, 0.5 mmol) in dry CH₂Cl₂ (2 mL). The reaction mixture was brought to room temperature and stirred for 1 hour, and then Et₂N (0.1 mL) was added to the mixture, followed by addition of AcOH (0.1 mL). The solvent was evaporated and the residue was purified by CC (RF=0.60, petroleum ether: ethyl acetate=1:1) to obtain 12 (143.7 mg, 63%). ¹H-NMR (CDCl₃, 400 MHz) δ 6.45 (d, 1 H, J=8.0 Hz, C₁-H), 5.20 (t, 1 H, J=10.0 Hz), 5.08 (t, 1 H, J=10.0 Hz), 5.03–4.97 (m, 1 H), 4.57–4.55 (m, 1 H), 4.29–4.28 (m, 2 H), 4.25–4.24 (m, 1 H), 4.18–4.15 (m, 2 H), 3.99–3.95 (m, 1 H), 3.89–3.86 (m, 1 H), 3.72–3.60 (m, 5 H), 2.45–2.43 (m, 2 H, 2×C=CH₂), 2.09–2.00 (m, 12 H, 4×COCH₃); ¹³C-NMR (CDCl₃, 100 MHz) δ 173.3

1,3-Di-(prop-2-yn-1-xyloxy)prop-2-yl β-D-glucopyanoside (13)
To a solution of 12 (115 mg, 0.2 mmol) in CH₃OH (2 mL) sodium methoxide (4.2 mg, 0.07 mmol) was added. The resulting mixture was stirred for 24 hours (reaction monitored by TLC) and then the pH of the medium was adjusted to 7.0 by addition of HCl solution (1 M, H₂O). The solvent was concentrated and the residue was purified by CC (RF=0.20, CHCl₃; CHCl₃:OH=9:1) to obtain the desired product 13 (54.9 mg, 72%). ¹H-NMR (CDCl₃, 400 MHz) δ 4.35 (d, 1 H, J=8.0 Hz, C₁-H), 4.30–4.27 (m, 1 H), 4.19 (t, 4 H, J=2.4 Hz, 2×O-CH₂), 4.00–3.86 (m, 3 H), 3.72–3.64 (m, 4 H), 3.35–3.27 (m, 2 H), 3.20–3.16 (m, 1 H), 2.87–2.85 (m, 2 H, 2×C=CH); ¹³C-NMR (CDCl₃, 100 MHz) δ 104.7 (C-1), 81.0 (C=CH), 77.8 (C=CH), 76.1, 75.9, 75.1, 75.0, 71.5 (O-CH₂), 62.7 (C-6), 59.3 (O-CH₂); ESIMS was calculated for C₁₉H₂₂O₄Na [M+Na]+ 353 and was found to be 353.

1,3-Di-(prop-2-yn-1-xyloxy)prop-2-yl 2,3,4,6-tetra-O-butyl-β-D-glucopyranoside (14)
Butyric anhydride (0.3 mL, 2 mmol) was added to a solution of 13 (66.0 mg, 0.2 mmol) in pyridine (2 mL) at 0°C. The reaction mixture was stirred for 12 hours and then was diluted with water (5 mL) and extracted with ethyl acetate (3×5 mL). The organic layer was washed with 10% aqueous hydrochloric acid (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate and evaporated to give a residue, which was purified by CC (RF=0.20, petroleum ether: ethyl acetate=9:1) to obtain 14 (109.8 mg, 90%). ¹H-NMR (CDCl₃, 400 MHz) δ 6.14 (d, 1 H, J=8.0 Hz, C₁-H), 5.23 (t, 1 H, J=9.2 Hz), 5.10 (t, 1 H, J=9.2 Hz), 5.05–4.99 (m, 1 H), 4.58–4.55 (m, 1 H), 4.28 (d, 2 H, J=2.4 Hz, O-CH₂), 4.21–4.19 (m, 1 H), 4.16 (d, 2 H, J=2.4 Hz, O-CH₂), 3.98–3.85 (m, 2 H), 3.72–3.70 (m, 4 H), 3.63–3.60 (m, 2 H, 2×C=CH₂), 2.44–2.41 (m, 2 H, O-CH₂), 2.33–2.20 (m, 6 H, 3×COCH₃), 1.66–1.54 (m, 8 H, 4×CH₂CH₂), 0.95–0.88 (m, 12 H, 4×CH₂CH₂); ¹³C-NMR (CDCl₃, 100 MHz) δ
(C=O), 172.7 (C=O), 172.0 (C=O), 171.9 (C=O), 101.0 (C-1), 76.0 (C=CH), 74.8 (O-CH), 74.4 (C=CH), 71.9, 70.9, 70.1, 69.3, 68.0 (O-CH), 68.0 (O-CH), 61.7 (C-6), 58.6 (O-CH), 35.9 (COCH), 35.8 (COCH), 35.8 (COCH), 35.8 (COCH), 18.2 (CH2CH), 18.2 (CH2CH), 18.2 (CH2CH), 18.2 (CH2CH), 13.6 (CH2CH), 13.5 (CH2CH), 13.5 (CH2CH); ESIMS was calculated for C31H40O2Na [M+Na]+ 633 and was found to be 633.

Prop-2-yn-1-yl 2,3,4-tri-O-tert-butyldimethylsilyl-6-O-trityl-β-D-glucopyranoside (16)

Imidazole (2.5 g, 36.0 mmol) and TBSCI (4.9 g, 32.5 mmol) were added to a solution of 15 (4.2 g, 9.0 mmol) in DMF (50 mL). The reaction mixture was stirred overnight, and then diluted with H2O (200 mL). The solution was extracted with CH2Cl2, and the organic layer dried over Na2SO4. After removing the solvent in vacuo, the residue was purified by CC (Rf = 0.30, petroleum ether: ethyl acetate=30:1) to give 16 (5.9 g, 80%) as a colorless oil. 1H-NMR (CDCl3, 400 MHz) δ 77.52–7.24 (m, 15 H, Ar-H), 4.57 (d, 1 H, J = 6.8 Hz, C1=H), 4.54 (d, 2 H, J = 2.4 Hz, O-CH), 3.56–3.52 (m, 1 H), 3.45–3.38 (m, 4 H), 3.25–3.21 (m, 1 H), 2.49 (t, 1 H, J = 2.4 Hz, C=CH), 0.95–0.65 (s, 27 H), 0.20–0.05 (s, 18 H); 13C-NMR (CDCl3, 100 MHz) δ 144.1, 128.8, 128.7, 126.8, 103.0 (C-1), 86.3, 78.7 (C=CH), 78.7 (C=CH), 76.2, 75.3, 74.9, 71.5, 63.8 (C-6), 55.3 (O-CH), 26.0 (Si-C), 25.9 (Si-C), 25.8 (Si-C), 18.3 (C-CH3), 18.2 (C-CH3), 17.9 (C-CH3), 3.8 (Si-CH3), 3.9 (Si-CH3), 4.0 (Si-CH3), 4.7 (Si-CH3), 5.2 (Si-CH3); ESIMS was calculated for C31H40O2Si4Na [M+Na]+ 825 and was found to be 825.

Prop-2-yn-1-yl 2,3,4-tri-O-tert-butyldimethylsilyl-β-D-glucopyranoside (17)

A solution of formic acid in ether (30 mL: 30 mL) was added dropwise to a solution of 16 (1.2 g, 1.5 mmol) in diethyl ether (30 mL) at room temperature. The mixture was stirred for 3 hours, then diluted with water (30 mL), and quenched via careful addition of solid potassium carbonate (K2CO3). The layers were separated, and the aqueous layer was extracted with Et2O (3×30 mL), and the resulting organic layers were combined and concentrated in vacuo. The residue was dissolved in CH2OH (40 mL), treated with K2CO3 (1.1 g) and stirred for 5 minutes. The resulting mixture was concentrated, and the residue was purified by CC (RF=0.40, petroleum ether: ethyl acetate=9:1) to obtain 17 (779.3 mg, 93%). 1H-NMR (CDCl3, 400 MHz) δ 42.2 (d, 1 H, J = 7.6 Hz, C1=H), 4.35 (d, 2 H, J = 2.4 Hz, O-CH), 3.89–3.85 (m, 1 H), 3.71–3.66 (m, 1 H), 3.54–3.43 (m, 2 H), 3.36–3.30 (m, 2 H), 2.45 (t, 1 H, J = 4.2 Hz, C=CH), 0.90–0.88 (m, 27 H), 0.15–0.11 (m, 18 H); 13C-NMR (CDCl3, 100 MHz) δ 101.2 (C-1), 78.8 (C=CH), 78.0 (C=CH), 76.3, 75.1, 75.0, 70.7, 62.5 (C-6), 56.2 (O-CH), 25.9 (C-CH3), 25.9 (C-CH3), 25.8 (C-CH3), 18.2 (Si-C), 18.2 (Si-C), −3.9 (Si-CH3), 3.9 (Si-CH3), −4.0 (Si-CH3), −4.7 (Si-CH3), −5.0 (Si-CH3); ESIMS was calculated for C31H38O2Si3Na [M+Na]+ 779 and was found to be 821.

Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-2,3,4-tri-O-tert-butyldimethylsilyl-β-D-glucopyranoside (18)

A solution of 17 (536.3 mg, 1 mmol) in dry THF (10 mL) was added at 0°C to a suspension of NaH (60 mg, 2.5 mmol) in dry THF (3 mL) under N2. The mixture was stirred at room temperature for 0.5 hour, then propargyl bromide (1 mL, 0.2 mmol) in THF (5 mL) was quickly added and the reaction mixture was refluxed overnight. The reaction mixture was quenched with water (20 mL), and then THF was removed in vacuo. The residue was extracted with CH2Cl2 (2×20 mL), and the organic layer was washed with brine (20 mL) and dried over Na2SO4, concentrated under reduced pressure. The residue was purified by CC (RF=0.50, petroleum ether: ethyl acetate=30:1) to obtain 18 (299.2 mg, 50%). 1H-NMR (CDCl3, 400 MHz) δ 4.42 (d, 1 H, J = 7.6 Hz, C1=H), 4.37–4.35 (m, 1 H), 4.24–4.19 (m, 4 H, 2×O-CH), 3.87–3.84 (m, 1 H), 3.66–3.62 (m, 1 H), 3.55–3.50 (m, 1 H), 3.46–3.40 (m, 1 H), 3.36–3.32 (m, 1 H), 2.42–2.40 (m, 2 H, 2×O=C=CH), 0.90–0.89 (m, 27 H), 0.15–0.11 (m, 18 H); 13C-NMR (CDCl3, 100 MHz) δ 100.3 (C-1), 79.7 (C=CH), 78.8 (C=CH), 78.2 (C=CH), 76.0, 75.0, 74.8, 74.6 (C=CH), 70.7, 68.8 (C-6), 58.6 (CH2=C=CH), 55.6 (CH2=C=CH), 26.0 (C-CH3), 25.9 (C-CH3), 25.9 (C-CH3), 18.2 (Si-C), 18.2 (Si-C), 18.2 (Si-C), −3.8 (Si-CH3), −3.9 (Si-CH3), −4.9 (Si-CH3), −4.8 (Si-CH3), −5.0 (Si-CH3); ESIMS was calculated for C31H38O2Si3Na [M+Na]+ 779 and was found to be 821.

Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-β-D-glucopyranoside (19)

To a solution of 18 (119.7 mg, 0.2 mmol) in dry THF (5 mL), TBAF (0.2 mL, 0.7 mmol) was added at room temperature. The mixture was stirred for 18 hours, and then the solvent was evaporated under reduced pressure and the crude product was purified by CC (RF=0.50, CH2Cl2; CH3OH=20:1) to obtain the desired product 19 (41.0 mg, 80%) as a colorless syrup. 1H-NMR ((CD3)2SO, 600 MHz)
Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (20)

Suspension (60%) of NaH in paraffin (28.0 mg, 0.7 mmol) was added to a solution of 19 (51.2 mg, 0.2 mmol) in anhydrous DMF (3 mL) at 0°C under argon atmosphere. The resulting solution was stirred for 30 minutes at room temperature. Benzyl bromide (0.2 mL, 1.1 mmol) was added drop-wise at 0°C followed by a catalytic amount of tetra-n-butyl ammonium iodide (20 mg). The resulting reaction mixture was stirred at room temperature under argon for 10 hours. After completion of the reaction (as judged by TLC), excess NaH was quenched with methanol (0.5 mL) followed by ice water (10 mL) and extracted with ether (3×10 mL). The combined organic layer was washed with water, brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude residue was purified by CC (RF=0.20, petroleum ether: ethyl acetate=30:1) to obtain 20 (73.6 mg, 70%). ¹H-NMR (CDCl₃, 400 MHz) δ 7.38–7.26 (6, 1H, Ar-H), 4.99–4.92 (m, 1H), 4.88–4.79 (m, 1H), 4.70–4.64 (m, 6H, 3×O-CH₂), 4.45 (1H, J=6.8 Hz, C-1'-H), 4.43–4.40 (m, 1H), 4.27–4.15 (m, 1H), 3.86–3.82 (m, 1H), 3.78–3.75 (m, 1H), 3.70–3.61 (m, 2H, O-CH₂), 3.50–3.46 (m, 2H, O-CH₂), 2.47 (s, 1H, C=CH), 2.39 (s, 1H, C=CH), 13.6 ¹C-NMR (CDCl₃, 100 MHz) δ 138.6, 138.3, 138.1, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 101.4 (C-1), 84.5, 81.9, 79.6 (C=O), 79.0 (C=CH), 75.7, 75.0, 74.9, 74.8 (C=CH), 74.8 (C=CH), 68.1 (C=O), 58.6 (O-CH₂), 56.0 (O-CH₂), ESI-MS was calculated for C₂₁H₁₈O₉Na [M+Na]⁺ 549 and was found to be 549.

Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-2,3,4-tri-O-acetyl-β-D-glucopyranoside (21)

To a solution of 20 (51.2 mg, 0.2 mmol) in pyridine (2 mL) at 0°C, acetic anhydride (0.2 mL, 2 mmol) was added. The reaction mixture was stirred at room temperature for 12 hours (TLC monitoring). The reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (3×10 mL). The organic layer was washed with 10% aqueous hydrochloric acid (10 mL) and brine (10 mL). The organic layer was dried over Na₂SO₄ and evaporated to give a residue, which was purified by CC (RF=0.20, petroleum ether: ethyl acetate=4:1) to obtain 21 (69.5 mg, 91%). ¹H-NMR (CDCl₃, 400 MHz) δ 4.78 (t, 1H, J=9.4 Hz), 4.66 (d, 1H, J=8.0 Hz, C-1'-H), 4.37–4.36 (m, 2H), 4.24–4.20 (m, 2H), 3.87 (dd, 1H, J=1.7 Hz, 10.8 Hz), 3.68–3.43 (m, 4H, 2×O-CH₂), 2.43–2.42 (m, 2H, 2×C=CH), 2.15–2.10 (m, 9H, 3×COCH₃); ¹C-NMR (CDCl₃, 100 MHz) δ 171.0 (C=O), 169.9 (C=O), 169.8 (C=O), 100.4 (C-1), 79.5 (C=CH), 78.7 (C=CH), 76.2 (C=CH), 76.0 (C=CH), 74.9, 74.7, 73.9, 71.9, 68.5 (C-6), 58.7 (O-CH₂), 55.6 (O-CH₂), 25.9 (COCH₃), 25.9 (COCH₃), 25.8 (COCH₃); ESI-MS was calculated for C₁₂H₁₆O₇Na [M+Na]⁺ 405 and was found to be 405.

Click reaction – general procedure for the preparation of dimeric podophyllotoxin derivatives 25–40

Copper II sulfate pentahydrate (0.01 mmol) and sodium ascorbate (1.0 M in H₂O, 3 d) were added to a solution of a terminal-alkyne 10, 12–14, or 19–22 (0.1 mmol) and a 4β-azido-podophyllotoxin analog 23 or 24 (0.1 mmol) in t-BuOH·H₂O (1:1, 1 mL) at room temperature. The reaction mixture was stirred at room temperature for 4 hours until the starting material disappeared as indicated by TLC. Then, the
mixture was diluted with water (10 mL) and extracted with ethyl acetate (3×10 mL), and the combined organic layer was dried over Na₂SO₄. The solvent was evaporated and the residue was purified by CC to obtain the cycloaddition product.

1,3-Di-[l-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-propan-2-ol (25)

Rf=0.60 (CHCl₃; CH₃OH=30:1). White amorphous powder, yield 86%; mp. 164°C–166°C (CH₂Cl₂); [α]D₂⁰ = −117.9 (c 0.28, Pyridine); ¹H-NMR (CDCl₃, 400 MHz) δ 7.35 (s, 1 H), 7.32 (s, 1 H), 6.62–6.61 (m, 4 H), 6.31 (s, 4 H), 6.08–5.99 (m, 6 H), 4.75–4.71 (m, 2 H), 4.68–4.60 (m, 6 H), 4.38–4.35 (m, 2 H), 3.81 (s, 6 H), 3.76 (s, 12 H), 3.70–3.69 (s, 1 H), 3.64–3.60 (m, 4 H), 3.24–3.22 (m, 2 H), 3.14–3.11 (m, 2 H); ¹³C-NMR (CDCl₃, 100 MHz) δ 173.2, 152.8, 149.4, 148.0, 145.4, 145.0, 137.5, 134.3, 133.2, 124.6, 123.0, 110.5, 108.8, 108.1, 102.0, 79.1, 70.3, 70.1, 67.4, 60.7, 58.7, 56.3, 43.6, 41.5, 37.1; ESIMS: m/z 1069 [M+Na]⁺, HRESIMS was calculated for C₄₇H₅₅N₂O₁₇ [M+Na]⁺ 1069.3443 and was found to be 1069.3437.

1,3-Di-[l-(4-deoxy-4′-demethylpodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-propan-2-ol (26)

Rf=0.40 (CHCl₃; CH₃OH=30:1). White amorphous powder, yield 82%; mp. 190°C–192°C (CH₂OH); [α]D₂⁰ = −200.6 (c 0.11, Pyridine); ¹H-NMR (CD₂SO, 400 MHz) δ 7.96 (s, 1 H), 7.95 (s, 1 H), 6.73 (s, 2 H), 6.64 (s, 2 H), 6.25 (s, 4 H), 6.22 (d, 2 H, J=5.2 Hz), 6.02–5.96 (m, 4 H), 4.67–4.64 (m, 2 H), 4.62–4.58 (m, 4 H), 4.48–4.47 (m, 2 H), 4.36–4.33 (m, 2 H), 3.63 (s, 12 H), 3.53–3.26 (m, 5 H), 3.24–3.18 (m, 2 H), 2.95–2.91 (m, 2 H); ¹³C-NMR (CD₂SO, 100 MHz) δ 173.7, 148.1, 147.3, 146.9, 144.5, 134.9, 133.5, 129.7, 126.1, 124.7, 124.6, 109.9, 108.7, 108.4, 101.6, 78.7, 69.6, 67.2, 60.7, 57.5, 56.0, 42.8, 40.9, 36.5; ESIMS: m/z 1041 [M+Na]⁺, HRESIMS was calculated for C₆₇H₅₇N₂O₁₇ [M+Na]⁺ 1041.3130 and was found to be 1041.3127.

1,3-Di-[l-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl 2,3,4,6-tera-0-acetyl-β-D-glucopyranoside (27)

Rf=0.50 (CHCl₃; CH₃OH=9:1). White amorphous powder, yield 76%; mp. 140°C–142°C (CH₂Cl₂); [α]D₂⁰ = −53.7 (c 0.19, CHCl₃); ¹H-NMR (CDCl₃, 400 MHz) δ 7.49 (s, 1 hour), 7.41 (s, 1 H), 6.59–6.58 (m, 2 H), 6.55 (m, 2 H), 6.30 (s, 4 H), 6.04–5.93 (m, 6 H), 4.71–4.67 (m, 5 H), 4.53–4.50 (m, 2 H), 4.33–4.29 (m, 4 H), 3.95–3.92 (m, 1 H), 3.77 (s, 6 H), 3.74 (s, 12 H), 3.65–3.52 (m, 9 H), 3.37–3.18 (m, 5 H); ¹³C-NMR (CDCl₃, 100 MHz) δ 173.6, 173.5, 152.7, 149.2, 147.9, 144.8, 144.5, 137.3, 134.4, 133.2, 124.8, 110.4, 108.8, 108.1, 103.2, 102.0, 77.2, 76.4, 75.9, 73.4, 69.7, 67.5, 64.3, 64.2, 62.6, 61.5, 60.7, 58.6, 56.3, 43.6, 41.4, 37.0; ESIMS: m/z 1209 [M+H]⁺, HRESIMS was calculated for C₉₅H₇₅N₂O₂₁ [M+Na]⁺ 1231.3971 and was found to be 1231.3962.

1,3-Di-[l-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl 2,3,4,6-tera-0-buteryl-β-D-glucopyranoside (28)

Rf=0.50 (CHCl₃; CH₃OH=30:1). White amorphous powder, yield 80%; mp. 140°C–142°C (CH₂Cl₂); [α]D₂⁰ = −42.9 (c 0.14, CHCl₃); ¹H-NMR (CDCl₃, 400 MHz) δ 7.44 (s, 1 H), 7.35 (s, 1 H), 6.63 (s, 2 H), 6.62–6.61 (m, 2 H), 6.32 (s, 4 H), 6.08 (d, 2 H, J=2.0 Hz), 6.01–6.00 (m, 4 H), 5.90 (d, 1 H, J=7.2 Hz), 5.22–5.17 (m, 1 H), 5.11–5.09 (m, 1 H), 5.00–4.96 (m, 1 H), 4.76–4.73 (m, 4 H), 4.71–4.66 (m, 2 H), 4.58–4.52 (m, 3 H), 4.39–4.25 (m, 3 H), 4.15–4.11 (m, 1 H), 3.94–3.91 (m, 1 H), 3.81 (s, 6 H), 3.77 (s, 12 H), 3.65–3.58 (m, 4 H), 3.31–3.21 (m, 4 H), 2.07–2.01 (m, 12 H, 4x COCH₃); ¹³C-NMR (CDCl₃, 100 MHz) δ 173.3, 173.2, 170.6, 170.2, 169.5, 169.5, 152.8, 149.3, 148.0, 145.2, 144.9, 137.5, 134.3, 133.2, 124.7, 123.2, 110.5, 108.8, 108.2, 101.9, 100.9, 77.2, 72.6, 71.8, 71.2, 70.2, 68.3, 67.4, 64.7, 61.8, 60.8, 58.6, 56.2, 43.4, 41.5, 37.1, 20.8 (COCH₃), 20.7 (COCH₃), 20.7 (COCH₃), 20.6 (COCH₃); ESIMS: m/z 1377 [M+H]⁺, HRESIMS was calculated for C₆₇H₅₇N₂O₂₁ [M+Na]⁺ 1399.4394 and was found to be 1399.4391.
1,3-Di-[1-(4-deoxy-4'-demethylpodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl β-D-glucopyranoside (30)

Rf=0.40 (CHCl₃; CH₂OH=9:1). White amorphous powder, yield 79%; mp 173°C–174°C (CHCl₃); [α]₂₃³: −60.0 (c 0.14, CH₂OH+CHCl₃); ¹H-NMR (CDCl₃, 400 MHz) δ 7.44 (s, 1 H), 7.36 (s, 1 H), 6.56–6.55 (m, 2 H), 6.54 (s, 2 H), 6.26 (s, 4 H), 6.03–5.92 (m, 6 H), 4.69–4.66 (m, 5 H), 4.52 (d, 2 H), J=4.0 Hz), 4.31–4.27 (m, 2 H), 4.24–4.19 (m, 2 H), 3.93–3.90 (m, 1 H), 3.80–3.77 (m, 2 H), 3.72 (s, 12 H), 3.64–3.54 (m, 7 H), 3.36–3.18 (m, 5 H); ¹³C-NMR (CDCl₃, 100 MHz) δ 173.9, 173.8, 149.3, 147.9, 146.7, 144.8, 144.6, 134.4, 133.4, 129.6, 124.6, 123.6, 110.4, 108.76, 107.8, 101.3, 101.9, 77.2, 76.2, 75.9, 73.4, 73.3, 69.9, 67.4, 64.4, 63.2, 58.7, 56.4, 43.3, 41.5, 36.9; ESIMS: m/z 1181 [M+H]+, HRESIMS was calculated for C₅₅H₆₆N₆O₂₆ [M+Na]+ 1203.3658 and was found to be 1203.3637.

1,3-Di-[1-(4-deoxy-4'-demethylpodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl β-D-glucopyranoside (31)

Rf=0.50 (CHCl₃; CH₂OH=15:1). White amorphous powder, yield 81%; mp 158°C–160°C (CHCl₃); [α]₂₃³: −49.7 (c 0.15, CHCl₃); ¹H-NMR (CDCl₃, 400 MHz) δ 7.52 (s, 1 H), 7.41 (s, 1 H), 6.65–6.64 (m, 2 H), 6.37–6.35 (m, 6 H), 6.10 (d, 2 H, J=4.2 Hz), 6.04–6.03 (m, 4 H), 6.00 (d, 1 H, J=7.2 Hz), 5.24–5.20 (m, 1 H), 5.15–5.00 (m, 1 H), 5.02–4.96 (m, 1 H), 4.79–4.70 (m, 6 H), 4.60–4.55 (m, 3 H), 4.40–4.37 (m, 1 H), 4.32–4.27 (m, 2 H), 4.17–4.15 (m, 1 H), 3.96–3.92 (m, 1 H), 3.78–3.70 (m, 2 H), 3.66–3.60 (m, 2 H), 3.27–3.20 (m, 4 H), 2.09–2.03 (m, 12 H, 4x COCH₃); ¹³C-NMR (CDOD, 100 MHz) δ 173.4, 173.3, 170.7, 170.3, 169.5, 169.5, 149.3, 147.9, 146.6, 145.2, 144.9, 134.4, 133.4, 130.0, 124.7, 123.3, 110.5, 108.8, 107.8, 101.9, 100.9, 77.0, 72.6, 71.9, 71.2, 70.2, 70.1, 68.3, 67.4, 64.8, 61.8, 58.7, 56.6, 34.5, 41.7, 37.1, 20.8 (COCH₃), 20.7 (COCH₃), 20.6 (COCH₃); ESIMS: m/z 1349 [M+H]+, HRESIMS was calculated for C₆₅H₅₈N₆NaO₂₆ [M+Na]+ 1371.4081 and was found to be 1371.4072.

1,6-Di-O-[1-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methyl]-β-D-glucopyranoside (33)

Rf=0.60 (CHCl₃; CH₂OH=10:1). White amorphous powder, yield 72%; mp 200°C–202°C (CHCl₃-CH₂OH); [α]₂₃³: −52.7 (c 0.18, CHCl₃); ¹H-NMR (CDCl₃, 600 MHz) δ 76.50 (s, 1 H), 7.40 (s, 1 H), 6.60 (s, 2 H), 6.59–6.57 (m, 2 H), 6.33 (s, 4 H), 6.06–5.96 (m, 6 H), 4.89 (d, 1 H, J=8.0 Hz), 4.74–4.70 (m, 4 H), 4.64–4.56 (m, 2 H), 4.44–4.42 (m, 2 H), 4.16–4.12 (m, 2 H), 3.81 (s, 6 H), 3.77 (s, 12 H), 3.73–3.71 (m, 2 H), 3.49–3.36 (m, 4 H), 3.20–3.18 (m, 4 H); ¹³C-NMR (CDCl₃, 150 MHz) δ 173.5, 173.5, 152.8, 149.2, 147.9, 144.7, 144.3, 137.5, 134.4, 133.2, 124.8, 123.5, 110.4, 108.8, 108.2, 102.4, 101.9, 76.4, 75.2, 73.3, 69.8, 69.6, 67.4, 64.4, 62.5, 60.7, 58.6, 56.3, 43.6, 41.5, 37.0; ESIMS: m/z 1135 [M+H]+, HRESIMS was calculated for C₅₅H₅₈N₆NaO₂₆ [M+Na]+ 1157.3604 and was found to be 1157.3592.
1,6-Di-O-[1-(4-deoxypodophyllotoxin-4 β-y1)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-benzyl-β-D-glucopyranose (34)

Rf=0.50 (CHCl₃; CH₂OH:30:1). White amorphous powder, yield 86%; mp 150°C–153°C (CHCl₃); [α]D¹ = –48.2 (c 0.14, CHCl₃); ¹H-NMR (CDCl₃, 400 MHz) δ 7.32–7.24 (m, 17 H), 6.60 (s, 2 H), 6.57–6.55 (m, 2 H), 6.61 (s, 4 H), 6.07–5.88 (m, 6 H), 4.85–4.79 (m, 7 H), 4.73–4.62 (m, 6 H), 4.57–4.48 (m, 2 H), 4.38–4.34 (m, 1 H), 4.29–4.26 (m, 1 H), 3.81 (s, 6 H), 3.76 (s, 12 H), 3.67–3.59 (m, 2 H), 3.51–3.43 (m, 3 H), 3.23–3.12 (m, 4 H); ¹³C-NMR (CDCl₃, 100 MHz) δ 173.2, 153.6, 152.8, 149.3, 149.2, 148.0, 140.8, 138.4, 138.3, 137.9, 137.5, 134.2, 133.1, 128.5, 128.4, 128.4, 127.9, 127.9, 127.7, 124.7, 124.7, 110.5, 108.8, 108.2, 102.0, 100.3, 73.4, 72.3, 71.0, 69.3, 68.3, 67.3, 65.3, 63.0, 60.8, 58.6, 53.6, 43.6, 37.1, 35.9; ESIMS: m/z 1405 [M+Na]+. HRESIMS was calculated for C₆₄H₆₆NaO₃₂ [M+Na]+ 1367.4860 and was found to be 1367.4844.

1,6-Di-O-[1-(4-deoxypodophyllotoxin-4 β-y1)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-acetyl-β-D-glucopyranose (35)

Rf=0.30 (CHCl₃; CH₂OH:15:1). White amorphous powder, yield 80%; mp 140°C–141°C (CH₂Cl₂); [α]D = –7.2 (c 0.12, CHCl₃); ¹H-NMR (CDCl₃, 600 MHz) δ 7.51 (s, 1 H), 7.41 (s, 1 H), 6.72–6.71 (m, 2 H), 6.39–6.38 (m, 4 H), 6.25–6.24 (m, 2 H), 6.01–5.98 (m, 4 H), 5.87 (d, 1 H, J=4.8 Hz), 5.85 (d, 1 H, J=4.8 Hz), 5.20 (t, 1 H, J=9.6 Hz), 5.11 (t, 1 H, J=9.6 Hz), 4.99–4.96 (m, 1 H), 4.91 (d, 1 H, J=8.0 Hz), 4.80–4.78 (m, 1 H), 4.71–4.69 (m, 1 H), 4.65–4.61 (m, 5 H), 4.56–4.49 (m, 2 H), 4.41–4.38 (m, 2 H), 3.85 (s, 6 H), 3.82 (s, 12 H), 3.67–3.59 (m, 2 H), 3.55–3.50 (m, 2 H), 2.01–1.96 (m, 9 H, 3 × COCH₃); ¹³C-NMR (CDCl₃, 150 MHz) δ 177.6, 177.4, 170.2, 169.5, 169.4, 153.7, 148.5, 147.8, 145.7, 144.3, 138.1, 137.2, 130.3, 125.9, 123.9, 110.2, 106.6, 104.7, 101.6, 99.5, 73.1, 72.9, 71.2, 69.1, 68.0, 65.0, 62.4, 60.9, 59.2, 56.3, 54.3, 47.7, 38.3, 20.7 (COCH₃), 20.6 (COCH₃); ESIMS: m/z 1261 [M+H]+. HRESIMS was calculated for C₆₄H₆₄NaO₃₂ [M+Na]+ 1283.3921 and was found to be 1283.3918.

1,6-Di-O-[1-(4-deoxypodophyllotoxin-4 β-y1)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-butyryl-β-D-glucopyranose (36)

Rf=0.40 (petroleum ether: ethyl acetate:10:1). White amorphous powder, yield 75%; mp 151°C–153°C (CHCl₃); [α]D = –55.3 (c 0.17, CHCl₃); ¹H-NMR (CDCl₃, 600 MHz) δ 7.38 (s, 1 H), 7.30 (s, 1 H), 6.65–6.62 (m, 4 H), 6.35–6.34 (m, 4 H), 6.00 (d, 2 H, J=4.2 Hz), 6.03–6.01 (m, 4 H), 5.24 (t, 1 H, J=9.6 Hz), 5.13–5.08 (m, 1 H), 5.04–5.00 (m, 1 H), 4.97–4.87 (m, 2 H), 4.81–4.76 (m, 2 H), 4.72–4.65 (m, 4 H), 4.59–4.57 (m, 2 H), 4.39–4.36 (m, 2 H), 3.84 (s, 6 H), 3.79 (s, 12 H), 3.74–3.69 (m, 1 H), 3.61–3.58 (m, 1 H), 3.25–3.21 (m, 1 H), 3.14–3.11 (m, 1 H), 2.23–2.18 (m, 1 H, 3 × COCH₂), 1.59–1.54 (m, 6 H, 3 × CH₂CH₃), 0.92–0.88 (m, 9 H, 3 × CH₂CH₃); ¹³C-NMR (CDCl₃, 150 MHz) δ 173.2, 173.1, 172.6, 172.1, 172.0, 152.8, 149.4, 148.1, 137.8, 134.3, 133.2, 124.7, 123.2, 110.5, 108.8, 108.2, 102.0, 100.3, 73.4, 72.3, 71.0, 69.3, 68.3, 67.3, 65.3, 63.0, 60.8, 58.6, 53.6, 43.6, 37.1, 35.9 (COCH₃), 35.9 (COCH₃), 35.9 (COCH₃), 18.3 (CH₂CH₃), 18.2 (CH₂CH₃), 18.2 (CH₂CH₃), 13.6 (CH₂CH₃), 13.5 (CH₂CH₃), 13.4 (CH₂CH₃), ESIMS: m/z 1345 [M+H]+. HRESIMS was calculated for C₆₄H₆₄NaO₃₂ [M+Na]+ 1367.4860 and was found to be 1367.4844.
1,6-Di-O-[1-(4-deoxy-4'-demethylpodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-acetyl-β-D-gluco.pyranose (39)

RF = 0.50 (petroleum ether: ethyl acetate = 10:1). White amorphous powder, yield 82%; mp 184°C–186°C (CHCl3); [α]D234: −56.4 (c 0.28, CHCl3); 1H-NMR (CDCl3, 400 MHz) δ 7.35 (s, 1 H), 7.28 (s, 1 H), 6.60 (s, 2 H), 6.59–6.58 (m, 2 H), 6.32–6.31 (m, 4 H), 6.06–5.97 (m, 6 H), 5.21 (t, 1 H, J = 9.6 Hz), 5.05 (t, 1 H, J = 9.6 Hz), 5.00–4.95 (m, 1 H), 4.82–4.78 (m, 2 H), 4.74–4.71 (m, 2 H), 4.65–4.62 (m, 4 H), 4.56–4.52 (m, 2 H), 4.33–4.30 (m, 2 H), 3.77 (s, 12 H), 3.71–3.65 (m, 1 H), 3.59–3.53 (m, 1 H), 3.20–3.16 (m, 1 H), 3.09–3.04 (m, 1 H), 2.20–2.13 (m, 6 H, 3× COCH3), 1.56–1.50 (m, 6 H, 3× CH2CH3), 0.89–0.81 (m, 9 H, 3× CH3CH2), 13C-NMR (CDCl3, 100 MHz) δ 173.3, 173.2, 172.6, 172.0, 171.9, 149.3, 149.3, 147.9, 144.9, 144.5, 134.3, 133.3, 129.7, 124.6, 123.3, 100.4, 108.7, 107.8, 101.9, 100.2, 73.3, 72.3, 70.9, 68.3, 67.3, 65.2, 62.9, 58.6, 56.5, 43.4, 41.5, 37.0, 35.9 (COCH3), 35.8 (COCH3), 35.8 (COCH3), 18.3 (CH2CH3), 18.2 (CH2CH3), 18.2 (CH2CH3), 13.6 (CH2CH3), 13.5 (CH2CH3), 13.4 (CH2CH3); ESIMS: m/z 1377 [M+H]+, HRESIMS was calculated for C25H22N6O29 [M+Na]+ 1255.3608 and was found to be 1255.3595.

Cell culture and cytotoxicity assay

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW480. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37°C in a humidified atmosphere with 5% CO2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich Co., St Louis, MO, USA). Briefly, adherent cells (100 µL) were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 hours before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×104 cells/mL in 100 µL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 hours. After the incubation, MTT (100 µg) was added to each well, and the incubation continued for 4 hours at 37°C. The cells lysed with SDS (200 µL) after removal of 100 µL of medium. The OD of lystate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680).

Acknowledgments

This work was financially supported by Yunnan province (grant nos 2015HB093 and 2015FB168) and the National Key Research and Development Program of China (grant no 2017YFD0201402). The authors thank the staff of analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measurements of all spectra.

Disclosure

The authors report no conflicts of interest in this work.

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