

# Synthesis and anticancer activity of dimeric podophyllotoxin derivatives

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**Background:** Podophyllotoxin is a potent cytotoxic agent and serves as a useful lead compound for the development of antitumor drugs. Several podophyllotoxin-derived antitumor agents, including etoposide, are currently in clinical use; however, their therapeutic efficacy is often limited due to side effects and the development of resistance by cancer cells. Previous studies have shown that 4 $\beta$ -1,2,3-triazole derivatives of podophyllotoxin exhibit more potent anticancer activity and better binding to topoisomerase-II than etoposide. The effect of dimerization of such derivatives on the anticancer activity has not been studied.

**Methods:** Two moieties of podophyllotoxin were linked at the C-4 position via 1,2,3-triazole rings to give a series of novel dimeric podophyllotoxin derivatives. 4 $\beta$ -Azido-substituted podophyllotoxin derivatives (**23** and **24**) were coupled with various dipropargyl functionalized linkers by utilizing the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction to provide dimeric products in very good yield. The in vitro anticancer activity of the synthesized compounds was evaluated by MTT assay against a panel of five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480). The normal BEAS-2B (lung) cell line was also included for study in order to evaluate the cancer selectivity of the most active compound as compared with normal cells.

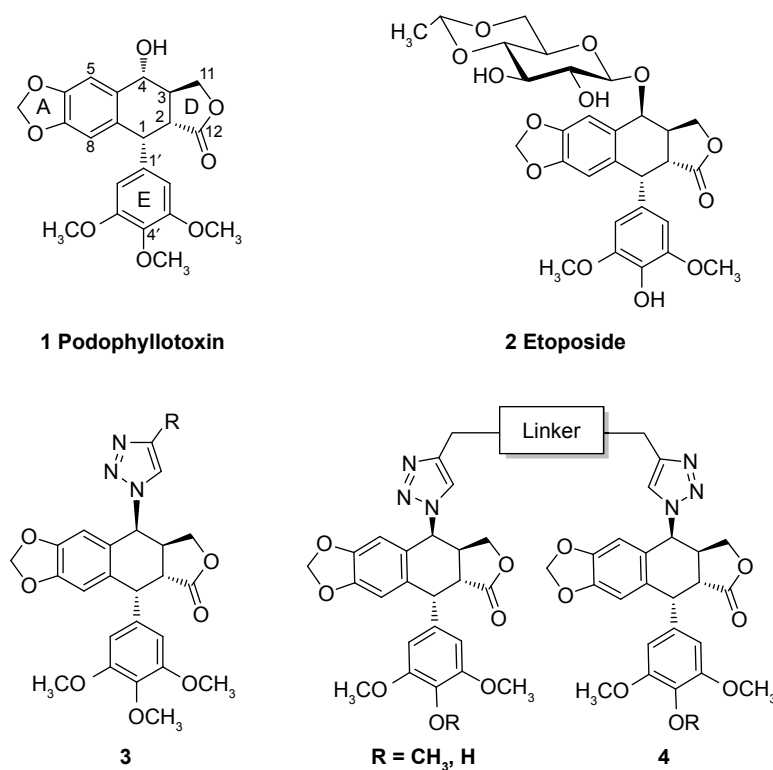
**Results:** A group of 16 dimeric podophyllotoxin derivatives with different linkers were synthesized and structurally characterized. Most compounds do not show significant cytotoxicity (IC<sub>50</sub> > 40  $\mu$ M) against all five cancer cell lines. However, one compound (**29**) which bears a perbutyrylated glucose residue on the glycerol linker is highly potent against all five cancer cell lines tested, with IC<sub>50</sub> values ranging from 0.43 to 3.50  $\mu$ M. This compound (**29**) also shows good selectivity towards cancer cell lines as compared with the normal BEAS-2B (lung) cell line, showing selectivity indexes from 4.4 to 35.7.

**Conclusion:** The anticancer activity of dimeric podophyllotoxin derivatives is generally speaking not improved as compared to their monomeric counterparts, and the potency of these dimeric derivatives can be largely affected by the nature of the linker between the two moieties. Among the synthesized derivatives, compound **29** is significantly more cytotoxic and selective towards cancer cells than etoposide and cisplatin, which are currently in clinical use. Compound **29** is a promising anticancer drug and needs further studies.

**Keywords:** podophyllotoxin, dimeric podophyllotoxin derivatives, CuAAC reaction, perbutyrylated glucose, antitumor, synthesis

## 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.<sup>1-3</sup> 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



**Figure 1** Structures of podophyllotoxin (1), etoposide (2), 4 $\beta$ -1,2,3-triazolyl-podophyllotoxin derivatives (3), and dimeric 4 $\beta$ -1,2,3-triazolyl-podophyllotoxin derivatives (4).

for a number of cancers, including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma.<sup>4-6</sup>

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

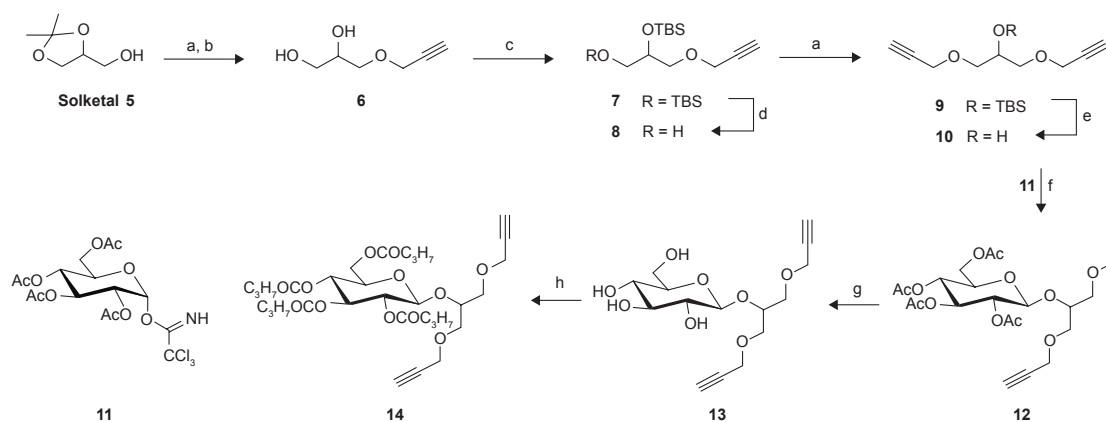
In the attempt to discover less toxic and more effective anticancer agents, many podophyllotoxin derivatives have been synthesized for biological studies.<sup>11,12</sup> 4 $\beta$ -1,2,3-Triazole derivatives of podophyllotoxin have been shown to exhibit more potent anticancer activity and better binding to topoisomerase-II than etoposide.<sup>13-15</sup> Recently, we also reported a group of podophyllotoxin glycoconjugates linked via 4 $\beta$ -1,2,3-triazole functionality as potential antitumor agents.<sup>16-18</sup> Our studies showed that podophyllotoxin derivatives with a perbutyrylated sugar residue displayed higher activity than their counterparts lacking butyryl groups.<sup>16,17</sup> There have also been reports on the synthesis of dimeric podophyllotoxin derivatives<sup>19,20</sup> 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.<sup>21,22</sup> 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-triazoles, which is a powerful tool for the generation of novel pharmacophores.<sup>21,22</sup> 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.<sup>23</sup> The diol **6** was first converted to bis-silyl ether **7** by treatment with *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole in anhydrous *N,N*-dimethylformamide (DMF). The more labile primary TBS ether in **7** was then



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

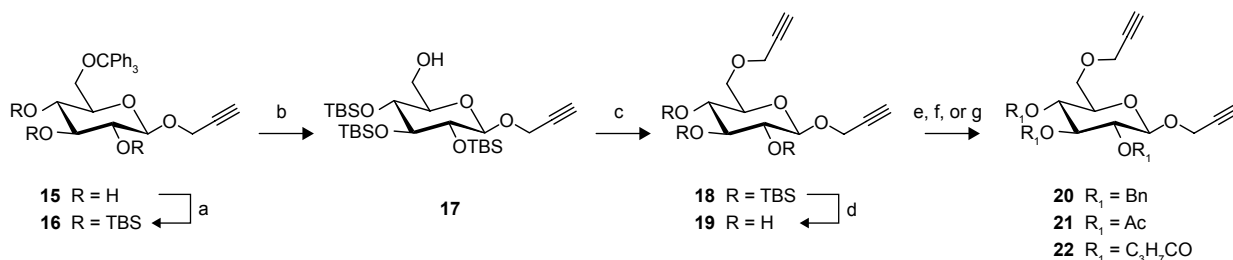
selectively cleaved with pyridine-HF in pyridine.<sup>24</sup> Etherification of the primary alcohol **8** with propargyl bromide gave **9** in 56% yield. Treatment of **9** with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave alcohol **10**.<sup>25</sup> Next, **10** was allowed to react with the  $\alpha$ -glucose trichloroacetimidate derivative **11**<sup>26</sup> in the presence of BF<sub>3</sub>·Et<sub>2</sub>O at -78°C to provide, as expected, only the  $\beta$ -glycoside **12** in 63% yield. Then, removal of acetyl groups with CH<sub>3</sub>ONa in CH<sub>3</sub>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 <sup>1</sup>H-NMR spectra of the peracetylated derivative **12** ( $\delta$  6.45 ppm, d,  $J=8.0$  Hz) and perbutyrylated derivative **14** ( $\delta$  6.14 ppm, d,  $J=8.0$  Hz), while it appears to be normal in non-acylated **13** ( $\delta$  4.35 ppm, d,  $J=8.0$  Hz). The coupling constant of 8.0 Hz for the anomeric proton confirms a  $\beta$ -glycosidic linkage in **12–14**.

The synthesis of dipropargyl functionalized linkers based on glucose scaffold is described in Scheme 2. The readily available 6-*O*-tritylated **15**<sup>27</sup> was treated with TBSCl and imidazole in anhydrous DMF to yield **16** in 80% yield. Acid

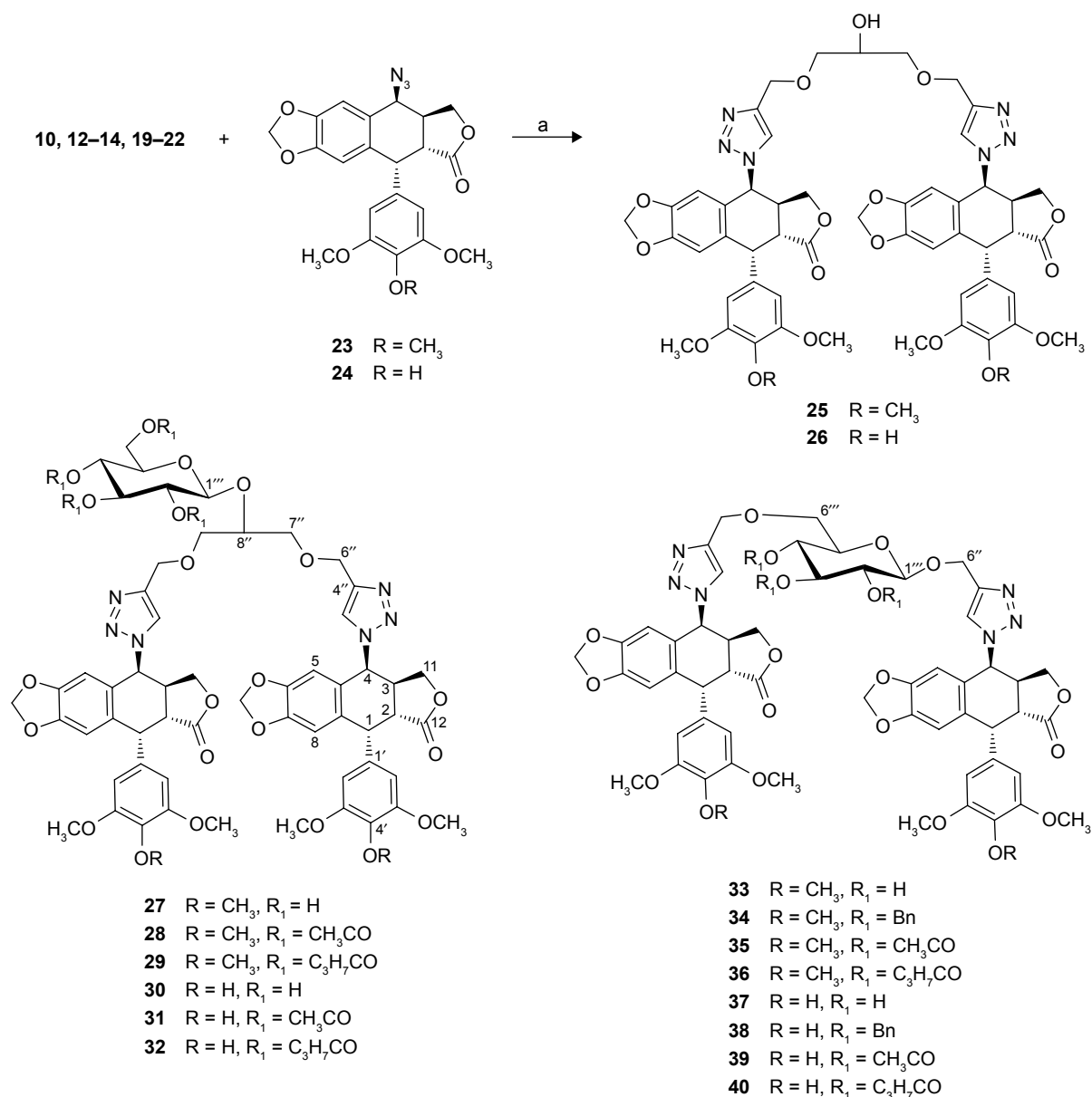
catalyzed removal of the trityl group yielded **17** in 93% yield. Compound **17** was then treated with propargyl bromide and NaH to provide **18** (50%), which was treated with TBAF in the presence of acetic acid in THF to give triol **19** in 80% yield. Compound **19** was then subjected to benzylation, 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, 4 $\beta$ -azido-4-deoxypodophyllotoxin **23** and 4 $\beta$ -azido-4-deoxy-4'-demethylpodophyllotoxin **24**, can be readily prepared from podophyllotoxin according to a previous report.<sup>28</sup> The azides **23** and **24** were allowed to react with dipropargyl functionalized linkers **10**, **12–14**, and **19–22** in the presence of CuSO<sub>4</sub>·5H<sub>2</sub>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 <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, ESI-MS, and HRESI-MS data. The presence of the triazole ring in these dimeric podophyllotoxin derivatives



**Scheme 2** Reagents and reaction conditions: a. TBSCl, 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. BnBr, NaH, DMF, 0°C–rt, 70%; f. Ac<sub>2</sub>O, pyridine, 0°C–rt, 12 hours, 91%; g. (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CO)<sub>2</sub>O, pyridine, 0°C–rt, 12 hours, 93%.



**Scheme 3** Reagents and reaction conditions: a. CuSO<sub>4</sub>·5H<sub>2</sub>O, sodium ascorbate, t-BuOH:H<sub>2</sub>O (1:1), 4 hours, rt. 74%–86%.

was confirmed by the proton signal at around  $\delta$  7.27–7.96 ppm (C<sup>5</sup>-H of the triazole ring) in the aromatic region of the <sup>1</sup>H-NMR spectrum, as well as by a pair of carbon signals at around 145 ppm and 124 ppm in the <sup>13</sup>C-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  $J_{3,4} < 5.0$  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]<sup>+</sup> or [M+H]<sup>+</sup> 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 IC<sub>50</sub> (inhibition concentration with 50% cell growth relative to the control) of all compounds are presented in Table 1. Their IC<sub>50</sub> values reveal that most of

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

Compounds	$IC_{50} \pm SD$ ( $\mu M$ )					
	HL-60	SMMC-7721	A-549	MCF-7	SW480	BEAS-2B
<b>25</b>	>40	>40	>40	>40	>40	
<b>26</b>	>40	>40	>40	>40	>40	
<b>27</b>	>40	>40	>40	>40	>40	
<b>28</b>	>40	>40	>40	>40	>40	
<b>29</b>	0.43±0.14	1.52±0.48	0.89±0.33	1.54±0.41	3.50±0.45	15.38±0.14
<b>30</b>	>40	>40	>40	>40	>40	
<b>31</b>	>40	>40	>40	>40	>40	
<b>32</b>	>40	>40	>40	>40	>40	
<b>33</b>	>40	>40	>40	>40	>40	
<b>34</b>	20.11±0.57	>40	>40	>40	>40	
<b>35</b>	>40	>40	>40	>40	>40	
<b>36</b>	>40	>40	>40	>40	>40	
<b>37</b>	>40	>40	>40	>40	>40	
<b>38</b>	>40	>40	>40	>40	>40	
<b>39</b>	16.47±0.44	15.20±0.56	18.02±0.47	30.46±0.99	27.39±0.78	
<b>40</b>	14.00±0.34	15.03±0.38	19.17±0.56	29.13±1.03	29.07±0.94	
<b>Etoposide (2)</b>	0.31±0.24	8.12±0.72	11.92±0.12	32.82±0.44	17.11±0.67	11.17±0.56
<b>Cisplatin</b>	1.67±0.44	6.93±0.28	7.42±0.12	10.85±0.51	9.89±0.53	12.86±0.25

these derivatives are not active ( $IC_{50} > 40 \mu M$ ). However, compound **29** is very active against all five cancer cell lines tested, with  $IC_{50}$  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  $IC_{50}$  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 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.<sup>16,17</sup> 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.<sup>29</sup> Previously, we reported several groups of monomeric podophyllotoxin derivatives bearing similar structure elements as those dimeric ones in the present study.<sup>16,17,29</sup> Some of those monomeric derivatives show good anticancer activity with  $IC_{50}$  values in low  $\mu M$  range. Since most of the dimeric podophyllotoxin derivatives in this study display weak anticancer activity, dimerization

might not be a good strategy for improving the potency of podophyllotoxin derivatives.

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  $IC_{50}$  value of the compound in normal BEAS-2B cell line over that in cancer cell line.<sup>30,31</sup> 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<sup>32</sup> or 3.0<sup>33</sup> 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) <sup>a</sup>				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
<b>29</b>	35.7	10.1	17.3	10.0	4.4
<b>Etoposide (2)</b>	36.0	1.4	0.9	0.3	0.7
<b>Cisplatin</b>	7.7	1.9	1.7	1.2	1.3

**Note:** <sup>a</sup>Selectivity index (SI) =  $IC_{50}$  of the compound in BEAS-2B cell line/ $IC_{50}$  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. 4 $\beta$ -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<sub>50</sub> values ranging from 0.43 to 3.50  $\mu$ M. 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 AV-400 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<sub>2</sub>SO<sub>4</sub> 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-butyl dimethylsilyl-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<sub>2</sub>O (300 mL). The solution was extracted with ether (Et<sub>2</sub>O) (3 $\times$ 150 mL) and the combined organic layer was dried over sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>). 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. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  4.12 (d, 2 H, *J*=2.4 Hz, O-CH<sub>2</sub>), 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 $\equiv$ CH), 0.87–0.86 (m, 18 H), 0.06–0.03 (m, 12 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  79.7 (C $\equiv$ CH), 74.2 (C $\equiv$ CH), 72.5 (O-CH), 71.6 (O-CH<sub>2</sub>), 64.8 (O-CH<sub>2</sub>), 58.4 (CH<sub>2</sub>-C $\equiv$ CH), 25.9 (C-CH<sub>3</sub>), 25.8 (C-CH<sub>3</sub>), 25.6 (C-CH<sub>3</sub>), 18.2 (Si-C), 18.1 (Si-C), -4.7 (Si-CH<sub>3</sub>), -4.8 (Si-CH<sub>3</sub>), -5.4 (Si-CH<sub>3</sub>), -5.5 (Si-CH<sub>3</sub>); ESIMS was calculated for C<sub>18</sub>H<sub>38</sub>O<sub>3</sub>Si<sub>2</sub>Na [M+Na]<sup>+</sup> 381 and was found to be 381.

### 2-*O*-tert-butyl dimethylsilyl-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 $\times$ 50 mL) and saturated copper sulfate solution (50 mL), and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. 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. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  4.16 (d, 2 H, *J*=2.4 Hz, O-CH<sub>2</sub>), 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); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  79.7 (C $\equiv$ CH), 74.2 (C $\equiv$ CH), 72.5 (O-CH), 71.6 (O-CH<sub>2</sub>), 64.8 (HO-CH<sub>2</sub>), 58.4 (CH<sub>2</sub>-C $\equiv$ CH), 25.9 (C-CH<sub>3</sub>), 18.2 (Si-C), 5.44 (Si-CH<sub>3</sub>); ESIMS was calculated for C<sub>12</sub>H<sub>24</sub>O<sub>3</sub>SiNa [M+Na]<sup>+</sup> 267 and found to be 267.

### 2-*O*-tert-butyl dimethylsilyl-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<sub>2</sub> 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  $\text{CH}_2\text{Cl}_2$  (2×50 mL), and the organic layer was washed with brine (100 mL), dried over  $\text{Na}_2\text{SO}_4$ , 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%).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.33–4.32 (m, 2 H, O- $\text{CH}_2$ ), 4.19–4.18 (m, 2 H, O- $\text{CH}_2$ ), 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 $\equiv$ CH), 0.89 (s, 9 H), 0.06 (s, 9 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  77.7 (C $\equiv$ CH), 74.9 (C $\equiv$ CH), 69.5 (O- $\text{CH}_2$ ), 62.4 (O-CH), 58.7 (O- $\text{CH}_2$ ), 25.9 (C- $\text{CH}_3$ ), 18.2 (Si-C), 5.44 (Si- $\text{CH}_3$ ); ESIMS was calculated for  $\text{C}_{15}\text{H}_{26}\text{O}_3\text{SiNa}$  [M+Na] $^+$  305 and was found to be 305.

### 1,3-Di-(prop-2-yn-1-yloxy)propan-2-ol (**10**)

Acetic acid (0.3 mL, 5.4 mmol) and tetra-butylammoniumfluoride 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.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.40–4.28 (m, 2 H, O- $\text{CH}_2$ ), 4.18–4.23 (m, 2 H, O- $\text{CH}_2$ ), 3.83–3.76 (m, 1 H), 3.70–3.66 (m, 4 H), 2.48–2.45 (m, 2 H, 2× C $\equiv$ CH);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  77.7 (C $\equiv$ CH), 74.7 (C $\equiv$ CH), 69.5 (O- $\text{CH}_2$ ), 62.4 (O-CH), 58.7 (O- $\text{CH}_2$ ); ESIMS was calculated for  $\text{C}_9\text{H}_{12}\text{O}_3\text{Na}$  [M+Na] $^+$  191 and was found to be 191. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data are in full agreement with those reported in the literature.<sup>25</sup>

### 1,3-Di-(prop-2-yn-1-yloxy)prop-2-yl 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranoside (**12**)

A solution of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (87.2  $\mu\text{L}$ , 0.7 mmol) in dichloromethane (1 mL) at  $-78^\circ\text{C}$  was added dropwise to a solution of **11**<sup>26</sup> (225 mg, 0.5 mmol) and **10** (77 mg, 0.5 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (2 mL). The reaction mixture was brought to room temperature and stirred for 1 hour, and then  $\text{Et}_3\text{N}$  (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%).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.45 (d, 1 H,  $J=8.0$  Hz, C $^1$ -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 $\equiv$ CH), 2.09–2.00 (m, 12 H, 4× COCH $_3$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  170.7 (C=O), 170.3 (C=O), 170.2 (C=O), 169.4 (C=O), 101.0 (C-1), 76.2 (C $\equiv$ CH), 74.8 (O-CH), 74.4 (C $\equiv$ CH), 72.7, 71.8, 71.2, 69.2, 68.3 (O- $\text{CH}_2$ ), 61.8 (C-6), 58.6 (O- $\text{CH}_2$ ), 20.7 (COCH $_3$ ), 20.7 (COCH $_3$ ), 20.6 (COCH $_3$ ), 20.5 (COCH $_3$ ); ESIMS was calculated for  $\text{C}_{23}\text{H}_{30}\text{O}_{12}\text{Na}$  [M+Na] $^+$  521 and was found to be 521.

### 1,3-Di-(prop-2-yn-1-yloxy)prop-2-yl $\beta$ -D-glucopyranoside (**13**)

To a solution of **12** (115 mg, 0.2 mmol) in  $\text{CH}_3\text{OH}$  (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,  $\text{H}_2\text{O}$ ). The solvent was concentrated and the residue was purified by CC (Rf=0.20,  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$ =9:1) to obtain the desired product **13** (54.9 mg, 72%).  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  4.35 (d, 1 H,  $J=8.0$  Hz, C $^1$ -H), 4.30–4.27 (m, 1 H), 4.19 (t, 4 H,  $J=2.4$  Hz, 2× O- $\text{CH}_2$ ), 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 $\equiv$ CH);  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  104.7 (C-1), 81.0 (C $\equiv$ CH), 77.8 (C $\equiv$ CH), 76.1, 75.9, 75.1, 75.0, 71.5 (O- $\text{CH}_2$ ), 62.7 (C-6), 59.3 (O- $\text{CH}_2$ ); ESIMS was calculated for  $\text{C}_{15}\text{H}_{22}\text{O}_8\text{Na}$  [M+Na] $^+$  353 and was found to be 353.

### 1,3-Di-(prop-2-yn-1-yloxy)prop-2-yl 2,3,4,6-tetra-O-butyryl- $\beta$ -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  $0^\circ\text{C}$ . 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%).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.14 (d, 1 H,  $J=8.0$  Hz, C $^1$ -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- $\text{CH}_2$ ), 4.21–4.19 (m, 1 H), 4.16 (d, 2 H,  $J=2.4$  Hz, O- $\text{CH}_2$ ), 3.98–3.85 (m, 2 H), 3.72–3.70 (m, 4 H), 3.63–3.60 (m, 2 H, 2× C $\equiv$ CH), 2.44–2.41 (m, 2 H, COCH $_2$ ), 2.33–2.20 (m, 6 H, 3× COCH $_2$ ), 1.66–1.54 (m, 8 H, 4×  $\text{CH}_2\text{CH}_3$ ), 0.95–0.88 (m, 12 H, 4×  $\text{CH}_2\text{CH}_3$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  173.3

(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<sub>2</sub>), 68.0 (O-CH<sub>2</sub>), 61.7 (C-6), 58.6 (O-CH<sub>2</sub>), 35.9 (COCH<sub>2</sub>), 35.8 (COCH<sub>2</sub>), 35.8 (COCH<sub>2</sub>), 35.8 (COCH<sub>2</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>), 13.5 (CH<sub>2</sub>CH<sub>3</sub>), 13.5 (CH<sub>2</sub>CH<sub>3</sub>); ESIMS was calculated for C<sub>31</sub>H<sub>46</sub>O<sub>12</sub>Na [M+Na]<sup>+</sup> 633 and was found to be 633.

### Prop-2-yn-1-yl 2,3,4-tri-O-tert-butyltrimethylsilyl-β-D-glucopyranoside (**16**)

Imidazole (2.5 g, 36.0 mmol) and TBSCl (4.9 g, 32.5 mmol) were added to a solution of **15**<sup>27</sup> (4.2 g, 9.0 mmol) in DMF (50 mL). The reaction mixture was stirred overnight, and then diluted with H<sub>2</sub>O (200 mL). The solution was extracted with CH<sub>2</sub>Cl<sub>2</sub>, and the organic layer dried over Na<sub>2</sub>SO<sub>4</sub>. 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. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.52–7.24 (m, 15 H, Ar-H), 4.57 (d, 1 H, *J*=6.8 Hz, C<sup>1</sup>-H), 4.54 (d, 2 H, *J*=2.4 Hz, O-CH<sub>2</sub>), 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); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) δ 144.1, 128.8, 127.8, 126.9, 100.3 (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<sub>2</sub>), 26.0 (Si-C), 25.9 (Si-C), 25.8 (Si-C), 18.3 (C-CH<sub>3</sub>), 18.2 (C-CH<sub>3</sub>), 17.9 (C-CH<sub>3</sub>), –3.8 (Si-CH<sub>3</sub>), –3.9 (Si-CH<sub>3</sub>), –4.0 (Si-CH<sub>3</sub>), –4.7 (Si-CH<sub>3</sub>), –4.7 (Si-CH<sub>3</sub>), –5.2 (Si-CH<sub>3</sub>); ESIMS was calculated for C<sub>46</sub>H<sub>70</sub>O<sub>6</sub>Si<sub>3</sub>Na [M+Na]<sup>+</sup> 825 and was found to be 825.

### Prop-2-yn-1-yl 2,3,4-tri-O-tert-butyltrimethylsilyl-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 (K<sub>2</sub>CO<sub>3</sub>). The layers were separated, and the aqueous layer was extracted with Et<sub>2</sub>O (3×30 mL), and the resulting organic layers were combined and concentrated *in vacuo*. The residue was dissolved in CH<sub>3</sub>OH (40 mL), treated with K<sub>2</sub>CO<sub>3</sub> (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%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 4.57 (d, 1 H, *J*=7.6 Hz, C<sup>1</sup>-H), 4.35 (d, 2 H, *J*=2.4 Hz, O-CH<sub>2</sub>), 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*=2.4 Hz, C≡CH), 0.90–0.88 (m, 27 H), 0.15–0.11 (m, 18 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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<sub>2</sub>), 25.9 (C-CH<sub>3</sub>), 25.9 (C-CH<sub>3</sub>), 25.8 (C-CH<sub>3</sub>), 18.2 (Si-C), 18.2 (Si-C), 18.2 (Si-C), –3.9 (Si-CH<sub>3</sub>), –3.9 (Si-CH<sub>3</sub>), –4.0 (Si-CH<sub>3</sub>), –4.7 (Si-CH<sub>3</sub>), –4.7 (Si-CH<sub>3</sub>), –5.0 (Si-CH<sub>3</sub>); ESIMS was calculated for C<sub>27</sub>H<sub>56</sub>O<sub>6</sub>Si<sub>3</sub>Na [M+Na]<sup>+</sup> 583 and was found to be 583.

### Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-2,3,4-tri-O-tert-butyltrimethylsilyl-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 N<sub>2</sub>. 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 CH<sub>2</sub>Cl<sub>2</sub> (2×20 mL), and the organic layer was washed with brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>, 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%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) 4.42 (d, 1 H, *J*=7.6 Hz, C<sup>1</sup>-H), 4.37–4.35 (m, 1 H), 4.24–4.19 (m, 4 H, 2×O-CH<sub>2</sub>), 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×C≡CH), 0.90–0.89 (m, 27 H), 0.15–0.11 (m, 18 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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 (CH<sub>2</sub>-C≡CH), 55.6 (CH<sub>2</sub>-C≡CH), 26.0 (C-CH<sub>3</sub>), 25.9 (C-CH<sub>3</sub>), 25.9 (C-CH<sub>3</sub>), 18.2 (Si-C), 18.2 (Si-C), 18.2 (Si-C) –3.8 (Si-CH<sub>3</sub>), –3.9 (Si-CH<sub>3</sub>), –4.0 (Si-CH<sub>3</sub>), –4.8 (Si-CH<sub>3</sub>), –4.7 (Si-CH<sub>3</sub>), –5.0 (Si-CH<sub>3</sub>); ESIMS was calculated for C<sub>30</sub>H<sub>58</sub>O<sub>6</sub>Si<sub>3</sub>Na [M+Na]<sup>+</sup> 621 and was found to be 621.

### 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, CHCl<sub>3</sub>: CH<sub>3</sub>OH=20:1) to obtain the desired product **19** (41.0 mg, 80%) as a colorless syrup. <sup>1</sup>H-NMR ((CD<sub>3</sub>)<sub>2</sub>SO, 600 MHz)



5.97 (d, 1 H,  $J=7.6$  Hz, C<sup>1</sup>-H), 5.93–5.89 (m, 1 H), 5.13–4.91 (m, 2 H), 4.56 (dd, 1 H,  $J=1.8$  Hz, 11.4 Hz), 4.31–4.29 (m, 1 H), 4.00–3.97 (m, 4 H, 2× O-CH<sub>2</sub>), 3.85–3.76 (m, 1 H), 2.38–2.35 (m, 2 H, 2× C≡CH); <sup>13</sup>C-NMR ((CD<sub>3</sub>)<sub>2</sub>SO, 150 MHz)  $\delta$  100.8 (C-1), 80.5 (C≡CH), 79.8 (C≡CH), 77.4 (C≡CH), 77.1 (C≡CH), 76.5, 75.4, 73.1, 70.0, 69.1 (C-6), 57.6 (O-CH<sub>2</sub>), 57.5 (O-CH<sub>2</sub>); ESIMS was calculated for C<sub>12</sub>H<sub>16</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 279 and was found to be 279.

### Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-2,3,4-tri-O-benzyl- $\beta$ -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 Na<sub>2</sub>SO<sub>4</sub> 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%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.38–7.26 (m, 15 H, Ar-H), 4.99–4.92 (m, 1 H), 4.88–4.79 (m, 1 H), 4.70–4.64 (m, 6 H, 3× O-CH<sub>2</sub>), 4.45 (d, 1 H,  $J=6.8$  Hz, C<sup>1</sup>-H), 4.43–4.40 (m, 1 H), 4.27–4.15 (m, 1 H), 3.86–3.82 (m, 1 H), 3.78–3.75 (m, 1 H), 3.70–3.61 (m, 2 H, O-CH<sub>2</sub>), 3.50–3.46 (m, 2 H, O-CH<sub>2</sub>), 2.47 (s, 1 H, C≡CH), 2.39 (s, 1 H, C≡CH); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  138.6, 138.3, 138.1, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 101.4 (C-1), 84.5, 81.9, 79.6 (C≡CH), 79.0 (C≡CH), 75.7, 75.0, 74.9, 74.8 (C≡CH), 74.8 (C≡CH), 68.1 (C-6), 58.6 (O-CH<sub>2</sub>), 56.0 (O-CH<sub>2</sub>); ESIMS was calculated for C<sub>33</sub>H<sub>34</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 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- $\beta$ -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 Na<sub>2</sub>SO<sub>4</sub> 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%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  4.78 (t, 1 H,  $J=9.4$  Hz), 4.66 (d, 1 H,  $J=8.0$  Hz, C<sup>1</sup>-H), 4.37–4.36 (m, 2 H), 4.24–4.20 (m, 2 H), 3.87 (dd, 1 H,  $J=1.7$  Hz, 10.8 Hz), 3.68–3.43 (m, 4 H, 2× O-CH<sub>2</sub>), 2.43–2.42 (m, 2 H, 2× C≡CH), 2.15–2.10 (m, 9 H, 3× COCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  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-CH<sub>2</sub>), 55.6 (O-CH<sub>2</sub>), 25.9 (COCH<sub>3</sub>), 25.9 (COCH<sub>3</sub>), 25.8 (COCH<sub>3</sub>); ESIMS was calculated for C<sub>18</sub>H<sub>22</sub>O<sub>9</sub>Na [M+Na]<sup>+</sup> 405 and found to be 405.

### Prop-2-yn-1-yl 6-O-(prop-2-yn-1-yl)-2,3,4-tri-O-butyryl- $\beta$ -D-glucopyranoside (**22**)

Butyryl anhydride (0.3 mL, 2 mmol) was added to a solution of **20** (51.2 mg, 0.2 mmol) in pyridine (2 mL) at 0°C. The reaction mixture was stirred at room temperature for 12 hours. 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 Na<sub>2</sub>SO<sub>4</sub> and evaporated to give a residue, which was purified by CC (Rf=0.20, petroleum ether: ethyl acetate=10:1) to obtain **22** (86.7 mg, 93%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  4.92 (t, 1 H,  $J=9.3$  Hz), 4.47 (d, 1 H,  $J=8.0$  Hz, C<sup>1</sup>-H), 4.38–4.36 (m, 2 H), 4.19–4.17 (m, 3 H), 3.65–3.44 (m, 4 H, 2× O-CH<sub>2</sub>), 2.43–2.41 (m, 2 H, 2× C≡CH), 2.36–2.32 (m, 6 H, 3× COCH<sub>2</sub>), 1.70–1.63 (m, 6 H, 3× CH<sub>2</sub>CH<sub>3</sub>), 0.98–0.89 (m, 9 H, 3× CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  173.2 (C=O), 173.2 (C=O), 173.1 (C=O), 100.4 (C-1), 79.3 (C≡CH), 78.6 (C≡CH), 76.7 (C≡CH), 76.2 (C≡CH), 75.2, 74.9, 73.3, 71.9, 68.7 (C-6), 58.7 (O-CH<sub>2</sub>), 55.8 (O-CH<sub>2</sub>), 36.2 (COCH<sub>2</sub>), 36.2 (COCH<sub>2</sub>), 36.1 (COCH<sub>2</sub>), 18.4 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 13.7 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>); ESIMS was calculated for C<sub>24</sub>H<sub>34</sub>O<sub>9</sub>Na [M+Na]<sup>+</sup> 489 and was found to be 489.

### 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 H<sub>2</sub>O, 3 d) were added to a solution of a terminal-alkyne **10**, **12–14**, or **19–22** (0.1 mmol) and a 4 $\beta$ -azido-podophyllotoxin analog **23** or **24**<sup>28</sup> (0.1 mmol) in *t*-BuOH-H<sub>2</sub>O (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 Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated and the residue was purified by CC to obtain the cycloaddition product.

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

Rf=0.60 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=30:1). White amorphous powder, yield 86%; mp. 164°C–166°C (CH<sub>2</sub>Cl<sub>2</sub>); [α]<sub>D</sub><sup>23.4</sup>: –117.9 (c 0.28, Pyridine); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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]<sup>+</sup>, HRESIMS was calculated for C<sub>33</sub>H<sub>34</sub>N<sub>6</sub>NaO<sub>17</sub> [M+Na]<sup>+</sup> 1069.3443 and was found to be 1069.3437.

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

Rf=0.40 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=30:1). White amorphous powder, yield 82%; mp. 190°C–192°C (CH<sub>3</sub>OH); [α]<sub>D</sub><sup>23.5</sup>: –200.6 (c 0.11, Pyridine); <sup>1</sup>H-NMR (C<sub>2</sub>D<sub>6</sub>SO, 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); <sup>13</sup>C-NMR (C<sub>2</sub>D<sub>6</sub>SO, 100 MHz) δ 173.7, 148.1, 147.3, 146.9, 144.5, 143.9, 134.9, 133.3, 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]<sup>+</sup>, HRESIMS was calculated for C<sub>51</sub>H<sub>50</sub>N<sub>6</sub>NaO<sub>17</sub> [M+Na]<sup>+</sup> 1041.3130 and was found to be 1041.3127.

### 1,3-Di-[1-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl β-D-glucopyranoside (27)

Rf=0.50 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=9:1). White amorphous powder, yield 76%; mp. 140°C–142°C (CH<sub>2</sub>Cl<sub>2</sub>); [α]<sub>D</sub><sup>23.6</sup>: –53.7 (c 0.19, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.49 (s, 1 H), 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); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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]<sup>+</sup>, HRESIMS was calculated for C<sub>59</sub>H<sub>64</sub>N<sub>6</sub>NaO<sub>22</sub> [M+Na]<sup>+</sup> 1231.3971 and was found to be 1231.3962.

### 1,3-Di-[1-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl 2,3,4,6-tera-O-acetyl-β-D-glucopyranoside (28)

Rf=0.50 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=30:1). White amorphous powder, yield 80%; mp. 140°C–142°C (CH<sub>2</sub>Cl<sub>2</sub>); [α]<sub>D</sub><sup>23.8</sup>: –42.9 (c 0.14, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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, 4× COCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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 (COCH<sub>3</sub>), 20.7 (COCH<sub>3</sub>), 20.7 (COCH<sub>3</sub>), 20.6 (COCH<sub>3</sub>); ESIMS: *m/z* 1377 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>67</sub>H<sub>72</sub>N<sub>6</sub>NaO<sub>26</sub> [M+Na]<sup>+</sup> 1399.4394 and was found to be 1399.4391.

### 1,3-Di-[1-(4-deoxypodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl 2,3,4,6-tera-O-butyryl-β-D-glucopyranoside (29)

Rf=0.30 (petroleum ether: ethyl acetate=1:3). White amorphous powder, yield 78%; mp 110°C–112°C (CH<sub>2</sub>Cl<sub>2</sub>); [α]<sub>D</sub><sup>23.8</sup>: –40.1 (c 0.22, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.44 (s, 1 H), 7.37 (s, 1 H), 6.63 (s, 2 H), 6.61 (s, 2 H), 6.32 (s, 4 H), 6.08–5.99 (m, 6 H), 5.88 (d, 1 H, *J*=3.2 Hz), 5.25–5.21 (m, 1 H), 5.14–5.08 (m, 1 H), 5.02–4.95 (m, 1 H), 4.79–4.76 (m, 4 H), 4.72–4.66 (m, 2 H), 4.57–4.51 (m, 3 H), 4.38–4.31 (m, 2 H), 4.21–4.17 (m, 2 H), 3.91–3.88 (m, 1 H), 3.81 (s, 6 H), 3.76 (s, 12 H), 3.63–3.57 (m, 4 H), 3.54–3.48 (m, 2 H), 3.31–3.21 (m, 2 H), 2.30–2.18 (m, 8 H, 4× COCH<sub>2</sub>), 1.63–1.53 (m, 8 H, 4× CH<sub>2</sub>CH<sub>3</sub>), 0.92–0.87 (m, 12 H,

4× CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.2 (2C), 172.6, 172.4, 172.0, 172.0, 152.7 (2C), 149.3, 148.0, 145.3, 145.2, 137.5, 134.3, 133.0, 124.7, 123.2, 110.5, 108.8, 108.2, 101.9, 100.7, 77.2, 72.2, 72.0, 71.0, 68.0, 67.3, 64.7, 61.7, 60.7, 56.3, 56.0, 43.6, 41.5, 37.1, 35.9 (COCH<sub>2</sub>), 35.8 (COCH<sub>2</sub>), 35.7 (COCH<sub>2</sub>), 18.3 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.1 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>), 13.5 (CH<sub>2</sub>CH<sub>3</sub>), 13.5 (CH<sub>2</sub>CH<sub>3</sub>); ESIMS: *m/z* 1489 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>75</sub>H<sub>88</sub>N<sub>6</sub>NaO<sub>26</sub> [M+Na]<sup>+</sup> 1511.5646 and was found to be 1511.5639.

### 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 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=9:1). White amorphous powder, yield 79%; mp 173°C–174°C (CHCl<sub>3</sub>); [α]<sub>D</sub><sup>23.3</sup>: –60.0 (c 0.14, CH<sub>3</sub>OH+CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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]<sup>+</sup>, HRESIMS was calculated for C<sub>57</sub>H<sub>60</sub>N<sub>6</sub>NaO<sub>22</sub> [M+Na]<sup>+</sup> 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 2,3,4,6-tera-O-acetyl-β-D-glucopyranoside (31)

Rf=0.50 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=15:1). White amorphous powder, yield 81%; mp 158°C–160°C (CHCl<sub>3</sub>); [α]<sub>D</sub><sup>23.2</sup>: –49.7 (c 0.15, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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, 4× COCH<sub>3</sub>); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 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, 43.5,

41.7, 37.1, 20.8 (COCH<sub>3</sub>), 20.7 (COCH<sub>3</sub>), 20.6 (COCH<sub>3</sub>), 20.6 (COCH<sub>3</sub>); ESIMS: *m/z* 1349 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>65</sub>H<sub>68</sub>N<sub>6</sub>NaO<sub>26</sub> [M+Na]<sup>+</sup> 1371.4081 and was found to be 1371.4072.

### 1,3-Di-[1-(4-deoxy-4'-demethylpodophyllotoxin-4β-yl)-1,2,3-triazol-4-yl-methoxy]-prop-2-yl 2,3,4,6-tera-O-butyryl-β-D-glucopyranoside (32)

Rf=0.20 (petroleum ether: ethyl acetate=1:3). White amorphous powder, yield 78%; mp 138°C–139°C (CH<sub>2</sub>Cl<sub>2</sub>); [α]<sub>D</sub><sup>23.8</sup>: –50.8 (c 0.14, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.52 (s, 1 H), 7.40 (s, 1 H), 6.62 (s, 2 H), 6.61 (s, 2 H), 6.33 (s, 4 H), 6.07–5.97 (m, 7 H), 5.26–5.21 (m, 1 H), 5.16–5.12 (m, 1 H), 5.02–4.96 (m, 1 H), 4.78–4.73 (m, 4 H), 4.70–4.68 (m, 2 H), 4.57–4.51 (m, 3 H), 4.36–4.28 (m, 2 H), 4.21–4.17 (m, 2 H), 3.92–3.88 (m, 1 H), 3.80 (s, 12 H), 3.74–3.69 (m, 2 H), 3.61–3.58 (m, 2 H), 3.54–3.47 (m, 2 H), 3.32–3.27 (m, 2 H), 2.31–2.20 (m, 8 H, 4× COCH<sub>2</sub>), 1.65–1.53 (m, 8 H, 4× CH<sub>2</sub>CH<sub>3</sub>), 0.92–0.87 (m, 12 H, 4× CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.2, 172.3, 172.1, 172.0, 172.0, 148.2, 148.0, 146.6, 145.1, 145.0, 134.3, 133.4, 129.8, 124.7, 123.2, 110.5, 108.8, 107.8, 101.9, 100.4, 77.2, 72.2, 72.1, 71.0, 68.0, 67.3, 65.2, 64.7, 61.7, 58.6, 56.5, 43.5, 41.6, 37.0, 36.5, 36.0 (COCH<sub>2</sub>), 35.9 (COCH<sub>2</sub>), 35.9 (COCH<sub>2</sub>), 18.3 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.1 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>), 13.5 (CH<sub>2</sub>CH<sub>3</sub>); ESIMS: *m/z* 1461 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>73</sub>H<sub>84</sub>N<sub>6</sub>NaO<sub>26</sub> [M+Na]<sup>+</sup> 1483.5333 was found to be 1483.5317.

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

Rf=0.60 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=10:1). White amorphous powder, yield 72%; mp 200°C–202°C (CHCl<sub>3</sub>-CH<sub>3</sub>OH); [α]<sub>D</sub><sup>22.9</sup>: –52.7 (c 0.18, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz) δ 7.50 (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); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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]<sup>+</sup>, HRESIMS was calculated for C<sub>56</sub>H<sub>58</sub>N<sub>6</sub>NaO<sub>20</sub> [M+Na]<sup>+</sup> 1157.3604 was found to be 1157.3592.

### 1,6-Di-O-[1-(4-deoxypodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-benzyl- $\beta$ -D-glucopyranose (34)

Rf=0.50 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=30:1). White amorphous powder, yield 86%; mp 150°C–153°C (CHCl<sub>3</sub>); [ $\alpha$ ]<sub>D</sub><sup>23.4</sup>: –48.2 (c 0.14, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.32–7.24 (m, 17 H), 6.60 (s, 2 H), 6.57–6.55 (m, 2 H), 6.31 (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), 3.67–3.61 (m, 2 H), 3.51–3.43 (m, 3 H), 3.23–3.12 (m, 4 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  173.2, 153.6, 152.8, 149.3, 149.2, 148.0, 148.0, 138.4, 138.3, 137.9, 137.5, 134.2, 133.1, 128.5, 128.4, 128.4, 127.9, 127.9, 127.9, 127.7, 124.7, 124.7, 110.5, 108.8, 108.2, 103.0, 101.9, 84.5, 82.0, 75.7, 75.7, 74.9, 69.6, 67.3, 64.9, 60.8, 58.6, 56.3, 43.5, 41.5, 37.1; ESIMS: *m/z* 1405 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>77</sub>H<sub>76</sub>N<sub>6</sub>NaO<sub>20</sub> [M+Na]<sup>+</sup> 1427.5012 and was found to be 1427.4997.

### 1,6-Di-O-[1-(4-deoxypodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranose (35)

Rf=0.30 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=15:1). White amorphous powder, yield 80%; mp 140°C–141°C (CH<sub>2</sub>Cl<sub>2</sub>); [ $\alpha$ ]<sub>D</sub><sup>23.1</sup>: –7.2 (c 0.12, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  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 $\times$ COCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  177.6, 177.4, 170.2, 169.5, 169.4, 153.7, 148.5, 147.8, 145.0, 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, 45.3, 44.7, 38.3, 20.7 (COCH<sub>3</sub>), 20.6 (COCH<sub>3</sub>), 20.6 (COCH<sub>3</sub>); ESIMS: *m/z* 1261 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>62</sub>H<sub>64</sub>N<sub>6</sub>NaO<sub>23</sub> [M+Na]<sup>+</sup> 1283.3921 and was found to be 1283.3918.

### 1,6-Di-O-[1-(4-deoxypodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-butyryl- $\beta$ -D-glucopyranose (36)

Rf=0.40 (petroleum ether: ethyl acetate=10:1). White amorphous powder, yield 75%; mp 151°C–153°C (CHCl<sub>3</sub>); [ $\alpha$ ]<sub>D</sub><sup>22.8</sup>: –55.3 (c 0.17, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  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, 6 H, 3 $\times$ COCH<sub>2</sub>), 1.59–1.54 (m, 6 H, 3 $\times$ CH<sub>2</sub>CH<sub>3</sub>), 0.92–0.88 (m, 9 H, 3 $\times$ CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  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, 56.3, 43.6, 41.5, 37.1, 35.9 (COCH<sub>2</sub>), 35.9 (COCH<sub>2</sub>), 35.8 (COCH<sub>2</sub>), 18.3 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 18.2 (CH<sub>2</sub>CH<sub>3</sub>), 13.6 (CH<sub>2</sub>CH<sub>3</sub>), 13.5 (CH<sub>2</sub>CH<sub>3</sub>), 13.4 (CH<sub>2</sub>CH<sub>3</sub>); ESIMS: *m/z* 1345 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>68</sub>H<sub>76</sub>N<sub>6</sub>NaO<sub>23</sub> [M+Na]<sup>+</sup> 1367.4860 and was found to be 1367.4844.

### 1,6-Di-O-[1-(4-deoxy-4'-demethylpodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]- $\beta$ -D-glucopyranose (37)

Rf=0.20 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=9:1). White amorphous powder, yield 76%; mp 195°C–196°C (CHCl<sub>3</sub>-CH<sub>3</sub>OH); [ $\alpha$ ]<sub>D</sub><sup>23.5</sup>: –124.6 (c 0.14, Pyridine); <sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N, 600 MHz)  $\delta$  8.20 (s, 1 H), 8.17 (s, 1 H), 6.85 (m, 4 H), 6.80–6.79 (m, 4 H), 6.55 (d, 1 H, *J*=4.8 Hz), 6.50 (d, 1 H, *J*=4.8 Hz), 5.95 (s, 4 H), 5.93 (d, 1 H, *J*=7.8 Hz), 5.33–5.31 (m, 1 H), 5.14–5.12 (m, 1 H), 4.99–4.94 (m, 5 H), 4.41–4.37 (m, 1 H), 4.15–4.10 (m, 1 H), 4.00–3.95 (m, 1 H), 3.83–3.76 (m, 3 H), 3.73 (s, 12 H), 3.67–3.61 (m, 3 H), 3.44–3.36 (m, 4 H); <sup>13</sup>C-NMR (C<sub>5</sub>D<sub>5</sub>N, 150 MHz)  $\delta$  174.0, 173.9, 149.0, 148.6, 148.0, 145.6, 145.4, 137.3, 134.3, 129.9, 126.3, 124.5, 110.6, 109.6, 109.1, 104.3, 102.2, 78.3, 77.0, 74.7, 71.4, 71.2, 67.8, 65.3, 63.2, 58.6, 56.4, 44.0, 41.9, 37.8; ESIMS: *m/z* 1107 [M+H]<sup>+</sup>, HRESIMS was calculated for C<sub>54</sub>H<sub>54</sub>N<sub>6</sub>NaO<sub>20</sub> [M+Na]<sup>+</sup> 1129.3291 and was found to be 1129.3281.

### 1,6-Di-O-[1-(4-deoxy-4'-demethylpodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-benzyl- $\beta$ -D-glucopyranose (38)

Rf=0.20 (CHCl<sub>3</sub>: CH<sub>3</sub>OH=30:1). White amorphous powder, yield 85%; mp 174°C–176°C (CHCl<sub>3</sub>); [ $\alpha$ ]<sub>D</sub><sup>23.5</sup>: –58.8 (c 0.20, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.32–7.24 (m, 17 H), 6.59 (s, 2 H), 6.56–6.54 (m, 2 H), 6.32 (s, 4 H), 6.06 (d, 1 H, *J*=4.0 Hz), 6.01 (d, 1 H, *J*=4.0 Hz), 5.97–5.87 (m, 4 H), 4.91–4.79 (m, 7 H), 4.72–4.67 (m, 4 H), 4.63 (d, 2 H, *J*=4.0 Hz), 4.57–4.55 (m, 1 H), 4.50–4.48 (m, 1 H), 4.34–4.31 (m, 1 H), 4.26–4.24 (m, 1 H), 4.14–4.09 (m, 1 H), 3.78 (s, 12 H), 3.69–3.61 (m, 2 H), 3.51–3.40 (m, 3 H), 3.22–3.11



(m, 4 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  173.3, 149.3, 148.0, 147.9, 146.6, 144.8, 138.5, 138.3, 137.9, 134.4, 129.8, 128.5, 128.4, 128.4, 127.9, 127.9, 127.9, 127.7, 124.8, 123.0, 110.5, 110.4, 107.8, 103.0, 101.8, 84.5, 81.9, 75.7, 74.9, 74.7, 69.6, 67.4, 65.0, 63.2, 58.5, 56.5, 43.4, 41.6, 37.0; ESIMS:  $m/z$  1377  $[\text{M}+\text{H}]^+$ , HRESIMS was calculated for  $\text{C}_{75}\text{H}_{72}\text{N}_6\text{NaO}_{20}$   $[\text{M}+\text{Na}]^+$  1399.4699 was found to be 1399.4683.

### 1,6-Di-O-[1-(4-deoxy-4'-demethylpodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-acetyl- $\beta$ -D-glucopyranose (39)

Rf=0.50 ( $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$ =15:1). White amorphous powder, yield 84%; mp 183°C–184°C ( $\text{CHCl}_3$ );  $[\alpha]_D^{23.3}$ : –55.9 (c 0.18,  $\text{CHCl}_3$ );  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.33 (s, 1 H), 7.27 (s, 1 H), 6.62–6.59 (m, 4 H), 6.32–6.31 (m, 4 H), 6.09–6.07 (m, 2 H), 6.01–5.98 (m, 4 H), 5.16 (t, 1 H,  $J$ =9.6 Hz), 5.04 (t, 1 H,  $J$ =9.6 Hz), 4.96–4.92 (m, 1 H), 4.80 (d, 1 H,  $J$ =8.0 Hz), 4.76–4.72 (m, 2 H), 4.64–4.54 (m, 7 H), 4.36–4.32 (m, 2 H), 3.78 (s, 12 H), 3.70–3.67 (m, 1 H), 3.60–3.56 (m, 1 H), 3.26–3.19 (m, 2 H), 1.98–1.93 (m, 9 H,  $3\times\text{COCH}_3$ );  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  173.3, 173.1, 170.2, 169.4, 169.4, 149.3, 148.0, 146.6, 144.8, 144.4, 134.3, 133.4, 129.8, 124.6, 123.3, 110.5, 108.8, 107.8, 101.9, 100.0, 73.1, 72.7, 71.2, 69.1, 68.6, 67.3, 65.0, 62.8, 58.6, 56.5, 43.4, 42.1, 41.6, 37.0, 20.6 ( $\text{COCH}_3$ ), 20.6 ( $\text{COCH}_3$ ), 20.5 ( $\text{COCH}_3$ ); ESIMS:  $m/z$  1317  $[\text{M}+\text{H}]^+$ , HRESIMS was calculated for  $\text{C}_{66}\text{H}_{72}\text{N}_6\text{NaO}_{23}$   $[\text{M}+\text{Na}]^+$  1339.4547 and was found to be 1339.4526.

### 1,6-Di-O-[1-(4-deoxy-4'-demethylpodophyllotoxin-4 $\beta$ -yl)-1,2,3-triazol-4-yl-methyl]-2,3,4-tri-O-butyryl- $\beta$ -D-glucopyranose (40)

Rf=0.20 (petroleum ether: ethyl acetate=10:1). White amorphous powder, yield 82%; mp 184°C–186°C ( $\text{CHCl}_3$ );  $[\alpha]_D^{23.4}$ : –56.4 (c 0.28,  $\text{CHCl}_3$ );  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  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,  $3\times\text{COCH}_2$ ), 1.56–1.50 (m, 6 H,  $3\times\text{CH}_2\text{CH}_3$ ), 0.89–0.81 (m, 9 H,  $3\times\text{CH}_2\text{CH}_3$ );  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  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 ( $\text{COCH}_2$ ), 35.8 ( $\text{COCH}_2$ ), 35.8 ( $\text{COCH}_2$ ), 18.3 ( $\text{CH}_2\text{CH}_3$ ), 18.2 ( $\text{CH}_2\text{CH}_3$ ), 18.2 ( $\text{CH}_2\text{CH}_3$ ), 13.6 ( $\text{CH}_2\text{CH}_3$ ), 13.5 ( $\text{CH}_2\text{CH}_3$ ), 13.4 ( $\text{CH}_2\text{CH}_3$ ); ESIMS:  $m/z$  1233  $[\text{M}+\text{H}]^+$ , HRESIMS was calculated for  $\text{C}_{60}\text{H}_{60}\text{N}_6\text{NaO}_{23}$   $[\text{M}+\text{Na}]^+$  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 RMPI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37°C in a humidified atmosphere with 5%  $\text{CO}_2$ . 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  $\mu\text{L}$ ) 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  $1\times 10^5$  cells/mL in 100  $\mu\text{L}$  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  $\mu\text{g}$ ) was added to each well, and the incubation continued for 4 hours at 37°C. The cells lysed with SDS (200  $\mu\text{L}$ ) after removal of 100  $\mu\text{L}$  of medium. The OD of lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680).

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### Disclosure

The authors report no conflicts of interest in this work.

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