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Synthesis of Paclitaxel–BGL Conjugates

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ABSTRACT

Four kinds of symmetrically branched oligoglyceryl trimeric (BGL003)–paclitaxel conjugates and a corresponding heptameric (BGL007) conjugate were synthesized. Molecular weights of all the compounds were less than two times that of paclitaxel. The anti-tumor activity of the most water-soluble BGL003 conjugate was examined and found to be preserved in spite of the chemical modification that is displacement of the N3′-debenzoyl residue with the BGL003 succinyl residue.

1. Introduction

Paclitaxel (**1**)[1] is one of the most well known and commonly used anti-tumor agents via injection (Fig. 1).[2] Because **1** is very poorly water-soluble (0.25 μg/mL),[3] and **1** via oral administration is clinically ineffective, **1** must be administered via injection as an aqueous solution containing a surfactant and ethanol, which often cause undesired clinical side effects.[4]

Thus, several syntheses of chemically modified paclitaxel derivatives have been reported.[5] For example, Battaglia et al. reported a succinic derivative, although water-solubility was not specifically mentioned.[5a] Greenwald et al. reported a derivative possessing a polyethylene glycol region, with a distributed molecular weight of more than 5–50 times that of **1**. Therefore, appropriate doses of the entire drug package would be nonsensically increased if this derivative were to be clinically used.[5b] Mandai et al. reported derivatives of a diastereomeric mixture of sugars with a glucolate linker,[5c] which may be prohibited due to guidelines adopted by governments of advanced countries. Although inclusion complexes of **1** by a protein,[6] a micelle,[7] or a liposome[8] have also been developed, use of a chemically single molecule is preferable because quality control of a single molecule is generally simple and highly reproducible.

Our developed symmetrically branched oligoglycerols (BGL)[9] have no asymmetric centers when the target medicinal molecule is connected at the apex of BGL. Using BGL003 (**2**) (the trimer of BGL),[10] some medicinal molecules were converted to the corresponding water-soluble derivatives, in which water-solubility was increased 1000–5000 times, [9a,12,13] and molecular weight was increased only 1.5–2 times. [9b,12,13] The water-solubilizing effect is independent of pH because a number of neutral polar functionalities that is primary hydroxyl groups are available for water-solubilization. These characteristic features are attributed to the molecular design and synthetic method of BGL. Accordingly, we started to examine the covalent bond formation of BGL (BGLation) for paclitaxel with a linker possessing two carboxylates such as succinate or glutarate to synthesize advanced and potentially water-soluble derivatives, which would be diastereomerically and enantiomerically pure.

2. Results and discussion

In this paper, we report the synthesis of paclitaxel–BGL conjugates. The three linking points are illustrated using white arrows in Fig. 1. Chemical derivatization of free hydroxyl groups on **1** has been well studied.[14,15] For example, the hydroxyl group at C2′ is most reactive for acylation or silylation, that at C7 is the second most reactive, and that at C1 is hardly reactive. Panchagnula reported that activity as an anti-tumor agent was similar even if the C3′-benzoyl moiety was substituted with other large acyl

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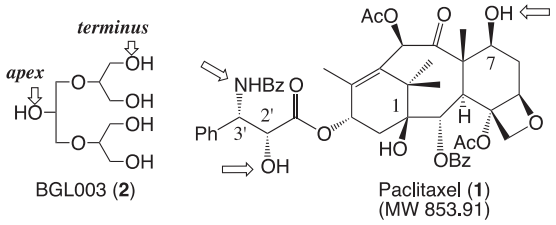


Figure 1. Structure of BGL003 and Paclitaxel (1) with examined BGLating points.

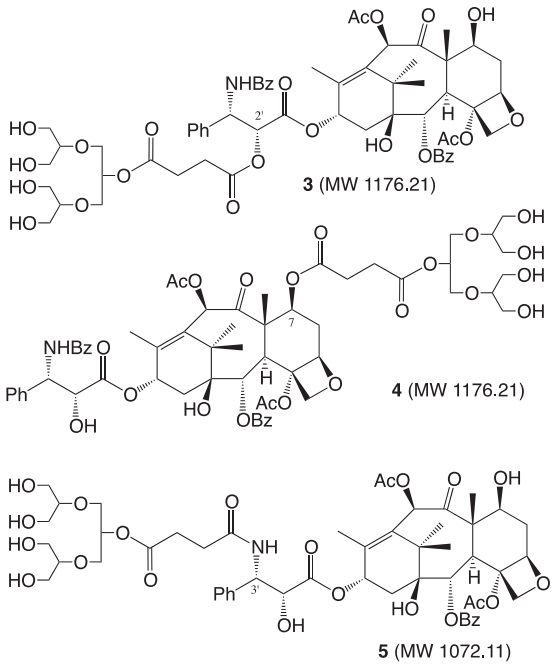


Figure 2. Synthesized paclitaxel derivatives via BGLation at C2′, C7 and C3′.

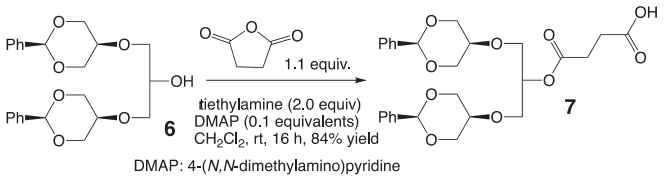
groups,[16] and a method for the preparation of C3′-debenzoylated paclitaxel is known.[17] Based on this information, **3**, **4** and **5** were chosen as reasonable targets (Fig. 2). It is noted that the molecular weights of **3**–**5** are only 1.26–1.38 times greater than that of **1**, which would minimize the dosage increase of the entire drug package given equivalent clinical activities of **3**–**5** to that of **1**.

For BGLation to synthesize **3** and **4**, the succinylated BGL003 possessing benzylidene (Bnd) groups **7** was prepared from **6**[9c,18] using succinic anhydride with triethylamine in the presence of a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in dichloromethane (CH_2Cl_2) at room temperature for 16 h in 84% yield (Scheme 1).

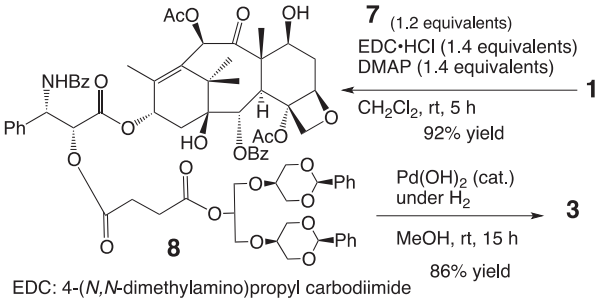
The first target **3** was synthesized in 79% overall yield from **1** via the condensation reaction of **1** and **7** using DMAP and 4-(*N*,*N*-dimethylamino)propyl carbodiimide (EDC) hydrochloride (92% yield), followed by hydrogenolysis of the two Bnd groups of the resulting **8** using palladium hydroxide in methanol under hydrogen atmosphere (86% yield).

The second target **4** was synthesized from the known silylated derivative **9**[14] in 77% overall yield. The condensation reaction of **9** and **7** using diisopropylcarbodiimide and 4-dimethylaminopyridinium p-toluenesulfonate afforded **10** in 93% yield. Treatment of HF·pyridine in THF gave **11** in 95% yield. Finally, **4** was successfully synthesized in 88% yield from **11** via similar hydrogenolysis as for **3**.

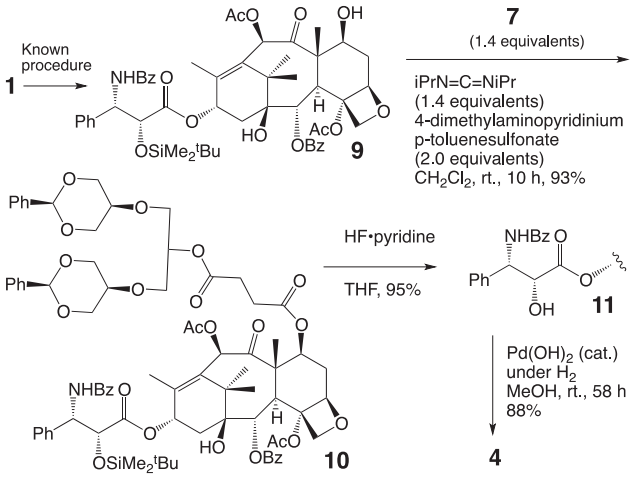
In the syntheses illustrated in Schemes 2 and 3, the Bnd groups on BGL were removed after BGLation of the target molecules, to avoid competition between the condensation of the hydroxyl group on the target molecule with those on the BGL terminus if the Bnd groups were absent. In contrast, in the synthesis of **5**,



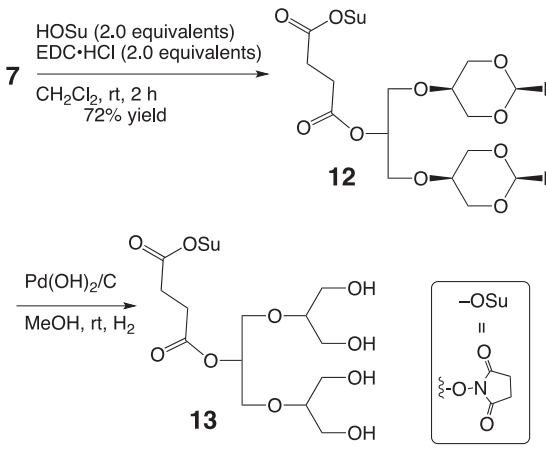
Scheme 1. Preparation of a BGLating agent for **3** and **4**.



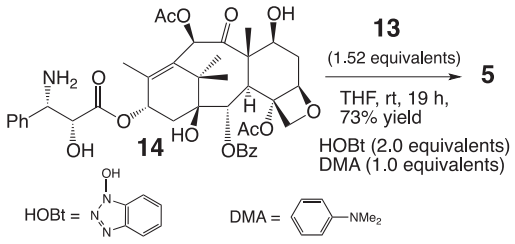
Scheme 2. Synthesis of **3**.



Scheme 3. Synthesis of **4**.



Scheme 4. Preparation of a BGLating agent for **5**.



Scheme 5. Synthesis of **5**.

the protecting groups on BGL were removed prior to BGLation, because amino groups are generally acylated much faster than hydroxyl groups, and several kinds of activated esters, which are reactive only toward amino groups but not hydroxyl groups, are available. Therefore, *N*-succinimidyl ester **13** was prepared from **7** via **12** as follows (Scheme 4). Condensation of **7** and *N*-hydroxysuccinimide (HOSu) using EDC gave **12** in 72% yield. Hydrogenolysis of **12** to remove the two Bnd groups afforded **13** in quantitative yield. The activated ester **13** was used for BGLation immediately after isolation via filtration to remove the palladium catalyst and subsequent concentration to remove methanol. The N–O bond in the *N*-succinimidyl ester moiety of **13** was preserved during the hydrogenolysis.[19] Purification of **13** by silica gel column chromatography was unsuccessful although NMR data of the crude product could be obtained. When **13** was left for more than half a day at room temperature, a non-negligible amount of unidentified byproducts and HOSu were observed, probably due to intra- or intermolecular alcoholysis of the *N*-succinimidyl ester moiety.

The simplest conditions [C3′-debenzoylated paclitaxel (**14**)[17] with 1.1 equivalents of **13** at room temperature for more than 20 h without any additive in various solvents such as THF, DMF and DMSO] gave **5** in unsatisfactory (10–30%) yield (Scheme 5). After several attempts, reaction conditions for **5** were found, in which the minimum amount of BGLating agent **13** among all our attempts (1.52 equiv vs **14**) was used, with N-hydroxybenzotriazole (HOBt) and *N*,*N*-dimethylaniline (DMA) as additives.

To determine the water-solubility, partition coefficients of **1**, **3**, **4** and **5** were measured using a water/1-octanol system (Table 1). Although their o/w ratios indicated that **4** and **5** can be significantly more water-soluble than **1** as expected from our previous reports, [11–13] **3** was surprisingly as water-insoluble as **1**. It is interesting that the location of BGLation can dramatically influence the water-solubility, probably due to the overall dipole moment of the entrie molecule.

Since the water-solubility of **5** was higher than those of **3** and **4**, the anti-tumor activity of **5** (3.51 μmol/kg) was examined using male nude mice (6-8 weeks old, BALB/c), inoculated subcutaneously with A549 human lung cancer cell, by intraperitoneal injection at the 1st, 4th and 7th days (indicated by black triangles at the bottom of Fig. 3). A solution of each compound in DMSO/saline (1:1, 100 μL) was intraabdominally administrated via injection. The area (mm^2) of tumor tissue was calculated by longest diameter × shortest diameter. It was found that the size of the tumor tissue by both **1** and **5** was significantly smaller than in the control (vehicle), suggesting that anti-tumor activity was certainly

Table 1
Partition Coefficient of **1**, **3**, **4** and **5**

Compound	Water layer (μM)	1-Octanol layer (μM)	O/W	log *P* o/w
1	0.55	97.3	177	2.25
3	0.51	92.4	181	2.26
4	1.00	98.2	98	1.99
5	3.44	99.1	29	1.46

($p < 0.001$).

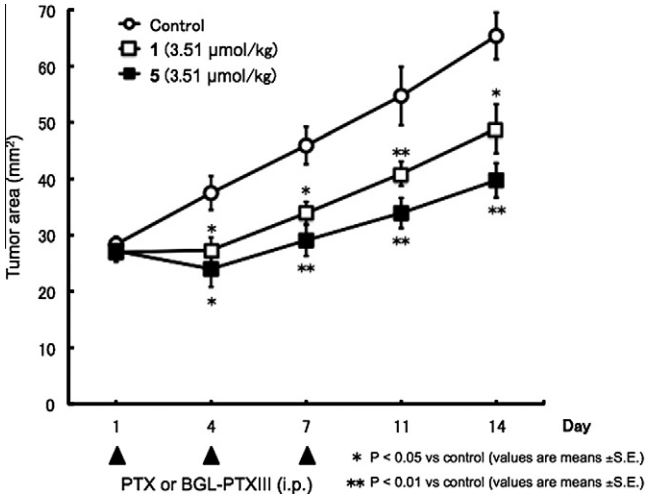


Figure 3. Anti-tumor activity of **5**.

preserved in spite of the chemical modification that is debenzoylation and BGLation.

Base on these promising results, synthesis of other BGL–paclitaxel conjugates based on the structure of **5** was carried out. The *N*-succinimidyl ester possessing amide bond **20** was prepared as illustrated in Scheme 6. The alcohol **6**[9c] was converted to azide **16** in 88% yield via sulfonate **15**. Reduction of the azide functionality of **16**, acylation of the resulting amino group of **17** with glutaric anhydride,[20] followed by condensation with HOSu gave **19** in 70% yield. Finally, hydrogenolysis of **19** afforded **20**, which was immediately used for the reaction with **14**.[17]

The *N*-succinimidyl ester possessing BGL007 **22** was prepared from **21**,[9c] via hydrogenolysis to remove the four Bnd groups (Scheme 7), and immediately used for the condensation with **14**.[17]

For the synthesis of two additional BGL conjugates **23**[21] and **24**,[22] the reaction was examined without using additives such as HOBt and DMA to simplify purification of **23** and **24** (Scheme 8). It was contrastive that the use of as small an amount of BGLating agent as possible was prioritized in the case of **5**. With 4.0 equiv of **20** for 48 h, **14** disappeared by thin layer chromatography to afford **23** in 68% yield. With 20 equiv of **22** for 40 h, **24** was afforded in 80% yield. Even when BGLation was carried out using glyceryl heptamers (BGL007), the molecular weight of **24** is less than two times that of **1**.

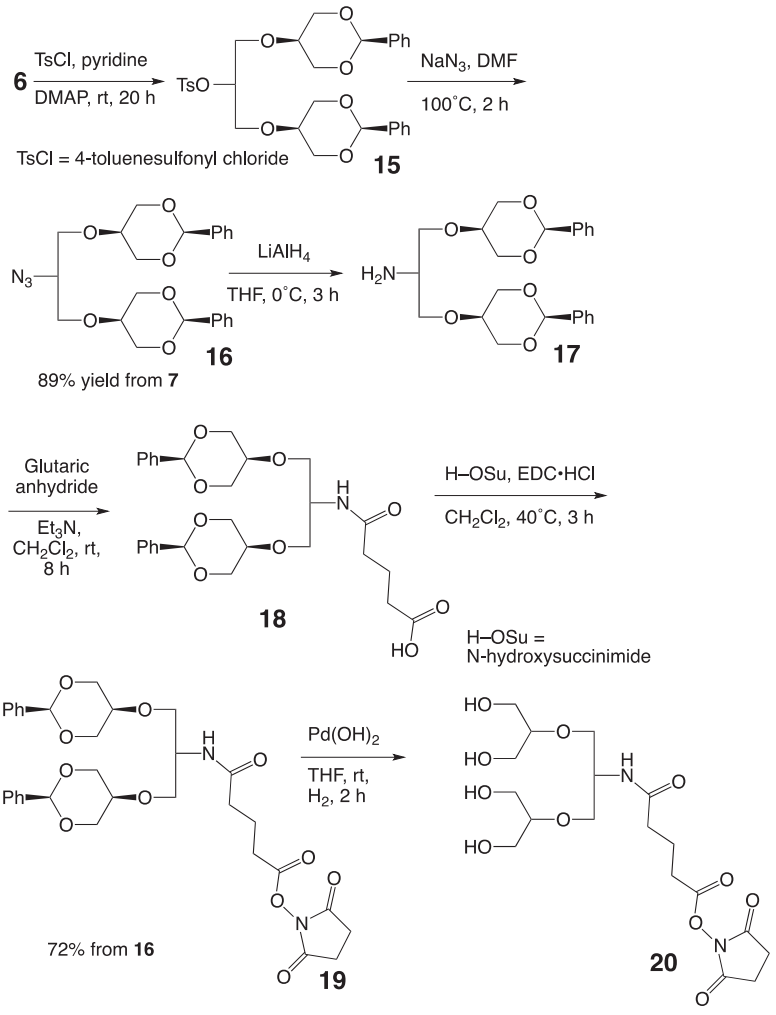
3. Conclusion

Five kinds of paclitaxel–BGL conjugates, each of which is a chemically single compound of less than two times the molecular weight of paclitaxel (**1**), were synthesized starting from **1**. Two procedures, deprotection after BGLation and deprotection prior to BGLation, were demonstrated. Significantly, the BGLation location unexpectedly influenced the water-solubilization. The anti-tumor activity of 3′-debenzoylated paclitaxel possessing BGL003 was examined and found to be preserved in spite of chemical modification. Further synthetic and evaluation studies are underway in our laboratories.

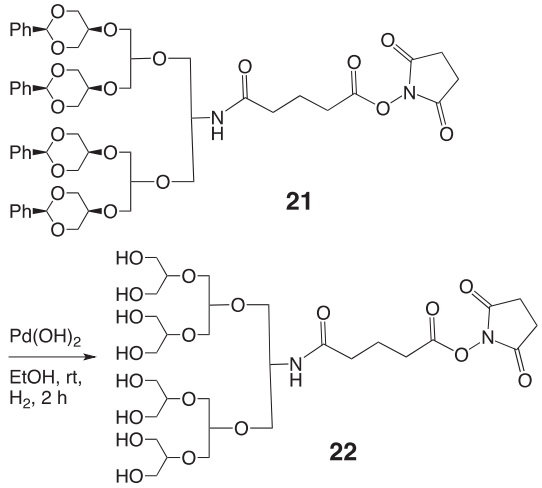
4. Experimental section

4.1. General

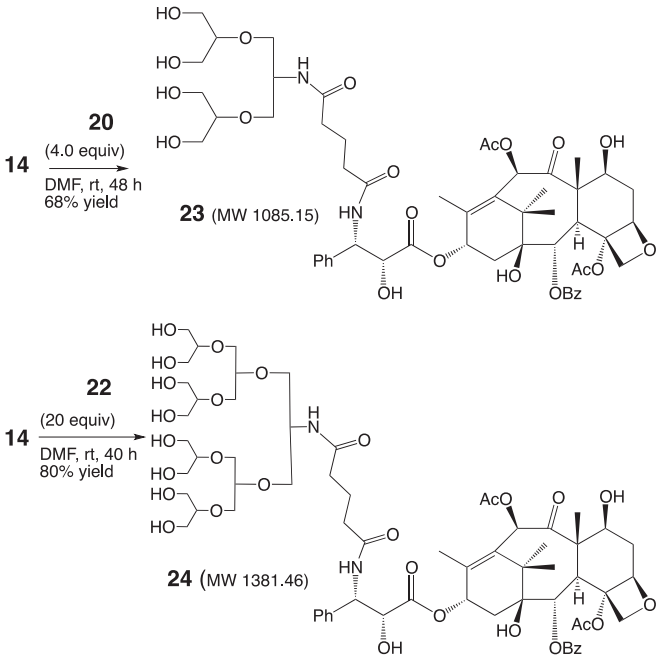
IR spctra were measured by Nihon Bunko FT-IR 6200 spectrometer. ^1H and ^{13}C NMR spectra were measured by JEOL JMN-AL400



Scheme 6. Preparation of **20**.



Scheme 7. Preparation of **22**.



Scheme 8. Syntheses of **23** and **24**.

at 400 MHz, and JEOL JMN-AL300 at 75 MHz, respectively in deutriolized chloroform or methanol ($CDCl_3$ or CD_3OD). Chemical shifts were indicated by δ value with tetramethylsilane as an internal standard. Multiplicity is abbreviated as s: singlet, d: doublet, t: triplet, q: quartet, quint: quintet, m: multiplet. High resolution

mass spectra (HRMS) were measured by Waters LCT PRIMIER using Electronically Sprayed Injection–Time-of-Fight (ESI-TOF). All the reactions were carried out under argon atmosphere unless otherwise noted. Reactions were monitored by thin layer chromatography of Merck Silicagel 60 F_{254} (0.25 mm) when it was applicable. Purifications were performed with Silica gel 60 N purchased from KANTO unless otherwise noted. Dichloromethane (CH_2Cl_2) was distilled over phosphorus pentoxide. Pyridine was distilled from potassium hydroxide. Triethylamine (TEA) and *N,N*-dimethylformaide (DMF) was distilled over calcium hydride. Anhydrous tetrahydrofuran (THF) was purchased from KANTO.

4.1.1. 4-(1,3-Bis(2-phenyl-1,3-dioxan-5-yloxy)propan-2-yloxy)-4-oxobutanoic acid (7)

To a solution of **6**[9c] (1.23 g, 2.95 mmol) in CH_2Cl_2 (10 mL) were added 4-(*N,N*-dimethylamino)pyridine (DMAP) (0.040 g, 0.29 mmol), TEA (0.84 mL, 6.0 mmol) and succinic anhydride (0.33 g, 3.29 mmol), and the mixture was stirred for 16 h at room temperature. The resulting solution was poured into a saturated aqueous solution of copper(II) sulfate ($CuSO_4$aq) (200 mL) and extracted with ethyl acetate (300 mL × 1, 50 mL × 2). The combined organic layers were washed with brine (200 mL), dried over anhydrous sodium sulfate (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with ethyl acetate to afford **7** as a colorless oil (1.27 g, 2.46 mmol, 84% yield). FT-IR (neat): 2863, 1735, 1453, 1392, 1344, 1238, 1215, 1155, 1091, 1009, 982, 915, 759, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.52–7.47 (m, 4H), 7.39–7.31 (m, 6H), 5.53 (s, 2H), 5.23 (quint, *J* = 5.0 Hz, 1H), 4.39–4.32 (m, 4H), 4.04–3.98 (m, 4H), 3.86–3.74 (m, 4H), 3.33 (quint, *J* = 1.5 Hz, 2H), 2.68–2.63 (m, 2H), 2.59–2.54 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 176.7 (C), 171.7 (C), 138.0 (C × 2), 128.8 (CH × 2), 128.1 (CH × 4), 126.0 (CH × 4), 101.0 (CH × 2), 71.8 (CH), 70.9 (CH × 2), 68.8 (CH_2 × 2), 68.4 (CH_2 × 2), 66.4 (CH_2 × 2), 29.0 (CH_2), 28.6 (CH_2); HRMS (ESI-TOF) *m/z* calcd for $C_{27}H_{32}O_{10}Na$ [M+Na]+ 539.1893, found 539.1901.

4.1.2. 2′-[4-(1,3-Bis(cis-2-phenyl-1,3-dioxan-5-yloxy)propan-2-yloxy)-4-oxobutanoyl]-paclitaxel (8)

To a solution of **1** (70.0 mg, 0.0820 mmol) in CH_2Cl_2 (1.0 mL) were added **7** (50.8 mg, 0.0983 mmol), DMAP (12.0 mg, 0.0983 mmol) and 3-(*N,N*-dimethylamino)propyl carbodiimide hydrochloride (EDC) (18.9 mg, 0.0983 mmol), and the mixture was stirred for 5 h at room temperature. The resulting solution was poured into $CuSO_4$aq (30 mL) and extracted with ethyl acetate (50 mL × 1, 25 mL × 2). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/2) to afford **8** as a colorless gummy solid (102 mg, 0.0752 mmol, 92% yield). FT-IR (neat): 3502, 3065, 2975, 2250, 1732, 1660, 1603, 1580, 1522, 1488, 1453, 1372, 1240, 1153, 1093, 1016, 948, 912, 846, 799, 731, 648 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.15 (d, *J* = 7.4 Hz, 2H), 7.81 (d, *J* = 7.4 Hz, 2H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.56–7.31 (m, 20H), 7.17 (q, *J* = 9.1 Hz, 1H), 6.31 (s, 1H), 6.23 (t, *J* = 8.8 Hz, 1H), 5.98 (dd, *J* = 9.0, 3.2 Hz, 1H), 5.69 (d, *J* = 7.0 Hz, 1H), 5.51 (s, 1H), 5.48 (s, 2H), 5.15 (quint, *J* = 4.9 Hz, 1H), 4.97 (d, *J* = 8.7 Hz, 1H), 4.45 (m, 1H), 4.35–4.19 (m, 6H), 4.00–3.86 (m, 5H), 3.85–3.68 (m, 6H), 3.32 (s, 1H), 3.27 (s, 1H), 2.73 (m, 2H), 2.65 (m, 2H), 2.56 (m, 1H), 2.45 (s, 3H), 2.34 (dd, *J* = 15.3, 9.3 Hz, 1H), 2.22 (s, 3H), 2.19–2.11 (m, 1H), 1.94 (s, 3H), 1.91 (m, 1H), 1.69 (s, 3H), 1.24 (s, 3H), 1.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.6 (C), 171.6 (C), 171.0(C), 170.9(C), 169.7 (C), 167.8 (C), 167.0 (C), 166.8 (C), 142.6 (C), 138.0 (C), 137.9 (C), 136.8 (C), 133.5 (CH), 133.4 (C), 132.6 (C), 131.8 (CH), 130.1 (CH × 2), 129.0 (C), 128.9 (CH × 2), 128.7 (CH × 2), 128.6 (CH), 128.5 (CH × 2), 128.3 (CH), 128.0 (CH ×5), 127.1 (CH × 2), 126.5 (CH × 2), 125.9 (CH × 4), 100.9 (CH × 2), 84.3 (CH), 80.8 (C), 78.9 (C), 76.2 (CH_2), 75.4 (CH), 74.9 (CH), 74.2 (CH), 71.9 (CH × 2), 71.6 (CH), 71.0 (CH), 70.8 (CH), 68.9 (CH_2), 68.8 (CH_2), 68.4 (CH_2), 68.3 (CH_2), 66.3(CH_2), 66.1 (CH_2), 58.3 (C), 52.7 (CH), 45.4 (CH), 43.0 (C), 35.4 (CH_2 × 2), 29.1 (CH_2), 28.9 (CH_2), 26.6 (CH_3), 22.5 (CH_3), 22.0 (CH_3), 20.7 (CH_3), 14.6 (CH_3), 9.4 (CH_3); HRMS (ESI-TOF) *m/z* calcd for $C_{74}H_{81}NO_{23}Na$ $[M+Na]^+$ 1374.5097, found 1374.5150.

4.1.3. 2′-[4-(1,3-Bis(1,3-dihydroxypropan-2-yloxy)propan-2-yloxy)-4-oxobutanoyl]-paclitaxel (3)

To a solution of **8** (102 mg, 0.0752 mmol) in methanol (1.0 mL) was added palladium hydroxide (0.020 g, 0.014 mmol) and the suspension was stirred for 15 h under hydrogen atmosphere. After inflow of argon gas to turn hydrogen gas out, the resulting suspension was filtered. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with chloroform/methanol (9/1) to afford **3** as a colorless gummy solid (75.7 mg, 0.0643 mmol, 86% yield). FT-IR (neat): 3447, 2937, 1734, 1647, 1540, 1490, 1373, 1243, 1153, 1070, 909, 731 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.16 (d, *J* = 8.6 Hz, 2H), 7.80 (d, *J* = 7.4 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.54–7.49 (m, *J* = 9.6, 5.9 Hz, 3H), 7.45–7.38 (m, *J* = 3.0 Hz, 6H), 7.37–7.32 (m, 1H), 6.32 (s, 1H), 6.21 (t, *J* = 8.9 Hz, 1H), 5.96 (dd, *J* = 9.2, 3.4 Hz, 1H), 5.69 (d, *J* = 6.6 Hz, 1H), 5.49 (d, *J* = 3.6 Hz, 1H), 5.04 (quint, *J* = 5.2 Hz, 1H), 4.97 (dd, *J* = 9.6, 1.5 Hz, 1H), 4.43–4.41 (m, 1H), 4.31 (d, *J* = 8.4 Hz, 1H), 4.21 (d, *J* = 8.6 Hz, 1H), 3.81–3.56 (m, 14H), 3.49–3.41 (m, 2H), 2.86–2.61 (m, 6H), 2.57–2.50 (m, 1H), 2.45 (s, 3H), 2.35 (dd, *J* = 15.5, 9.4 Hz, 1H), 2.23 (s, 3H), 2.17–2.11 (m, 1H), 1.93 (s, 3H), 1.91–1.89 (m, 1H), 1.69 (s, 3H), 1.26 (s, 1H), 1.23 (s, 4H), 1.15 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.0 (C), 171.6 (C), 171.4 (C), 171.0(C), 169.9 (C), 168.1 (C), 167.3 (C), 166.7 (C), 142.0 (C), 136.7 (C), 133.5 (C), 132.8 (C), 131.8 (CH), 130.1 (CH × 2), 129.1 (C), 128.9 (CH × 2), 128.6 (CH × 2), 128.5 (CH × 3), 128.4 (CH), 127.2 (CH × 2), 126.6 (CH × 2), 84.3 (CH), 81.0 (CH × 2, C), 78.8 (C), 76.3 (CH_2), 75.5 (CH), 74.8 (CH), 74.4 (CH), 72.1 (CH), 71.9 (CH), 71.6 (CH), 67.8 (CH_2), 67.7 (CH_2), 61.9 (CH_2), 61.8 (CH_2 × 2), 61.7 (CH_2), 58.2 (C), 52.8 (CH), 45.7 (CH), 43.1 (C), 35.7 (CH_2), 35.3 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 26.5 (CH_3), 22.5 (CH_3), 21.9 (CH_3), 20.8 (CH_3), 14.6 (CH_3), 9.6 (CH_3); HRMS (ESI-TOF) *m/z* calcd for $C_{60}H_{73}NO_{23}Na$ $[M+Na]^+$ 1198.4471, found 1198.4474.

4.1.4. 7-[4-(1,3-Bis(cis-2-phenyl-1,3-dioxan-5-yloxy)propan-2-yloxy)-4-oxobutanoyl]-2'-TBS-paclitaxel (10)

To a solution of **9**[14] (53.6 mg, 0.0554 mmol) in CH_2Cl_2 (2.0 mL) were added **7** (41.6 mg, 0.0804 mmol), 4-dimethylaminopyridinium p-toluenesulfonate (31.6 mg, 0.107 mmol) and diisoproyl carbodiimide (0.033 mL, 0.220 mmol), and the mixture was stirred for 12 h at room temperature. The resulting solution was poured into $CuSO_4$aq (30 mL) and extracted with ethyl acetate (50 mL × 1, 25 mL × 2). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/1) to afford **10** as a colorless gummy solid (75.5 mg, 0.0515 mmol, 93% yield). FT-IR (neat): 3440, 2930,1735, 1662, 1484, 1452, 1372, 1240, 1154, 1094, 1018, 982, 838, 757, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.14 (d, *J* = 7.2 Hz, 2H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.55–7.30 (m, 20H), 7.09 (d, *J* = 8.9 Hz, 1H), 6.29–6.21 (m, 2H), 5.77–5.69 (m, 2H), 5.61 (dd, *J* = 10.6, 7.1 Hz, 1H), 5.49 (s, 2H), 5.21 (quint, *J* = 5.0 Hz, 1H), 4.97 (d, *J* = 9.0 Hz, 1H), 4.67 (d, *J* = 2.0, 1H), 4.35–4.28 (m, 5H), 4.21 (d, *J* = 8.5 Hz, 1H), 3.99–3.95 (m, 5H), 3.84–3.77 (m, 4H), 3.40–3.36 (m, 2H), 2.77–2.55 (m, 8H), 2.46–2.37 (m, 1H), 2.20–2.11 (m, 4H), 1.97 (s, 3H), 1.90–1.85 (m, 1H), 1.81 (s, 3H), 1.20 (s, 3H), 1.16 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.9 (C), 172.1 (C), 171. 4(C × 2), 169.8 (C), 169.0

(C), 167.0 (C), 166.8 (C), 140.5 (C), 138.1 (C × 3), 134.0 (C), 133.6 (CH), 132.6 (CH), 131.7 (CH), 130.1 (CH × 2), 129.0 (C), 128.7 (CH × 2), 128.6 (CH × 4), 128.0 (CH × 5), 127.9 (CH), 126.9 (CH × 2), 126.3 (CH × 2), 126.0 (CH × 5), 100.9 (CH × 2) 83.8 (CH), 80.8 (C), 78.4 (C), 76.1 (CH_2), 75.0 (CH), 74.9 (CH), 74.3 (CH), 71.7 (CH), 71.3 (CH), 71.1 (CH), 70.9 (CH), 70.8 (CH), 68.9 (CH_2 × 2), 68.4 (CH_2), 68.3 (CH_2), 66.3 (CH_2 × 2), 55.7 (C), 55.4 (CH), 46.6 (CH), 43.1 (C), 35.3 (CH_2), 33.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.1 (CH_3), 25.2 (CH_3 × 3), 22.7 (CH_3), 21.1 (CH_3), 20.4 (CH_3), 17.8 (C), 14.3 (CH_3), 10.6 (CH_3), −5.53 (CH_3), −6.16 (CH_3); HRMS (ESI–TOF) *m*/*z* calcd for $C_{80}H_{95}NO_{23}SiNa$ $[M+Na]^+$ 1488.5962, found: 1488.5955.

4.1.5. 7-[4-(1,3-Bis(cis-2-phenyl-1,3-dioxan-5-yloxy)propan-2-yloxy)-4-oxobutanoyl]-paclitaxel (11)

To a solution of **10** (75.5 mg, 0.0515 mmol) in THF (1.0 mL) were added pyridinium fluoride (0.5 mL) and pyridine (1.5 mL), and the mixture was stirred for 20 h at room temperature. The resulting mixture was poured into $CuSO_4$aq (30 mL) and extracted with ethyl acetate (50 mL × 1, 25 mL × 2). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/3) to afford **11** as a colorless gummy solid (65.8 mg, 0.0487 mmol, 95% yield). FT–IR (neat): 3438, 3014, 1733, 1652, 1602, 1581, 1486, 1452, 1238, 845, 755, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.12 (d, *J* = 7.4 Hz, 2H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.53–7.31 (m, 20H), 7.07 (d, *J* = 8.9 Hz, 1H), 6.20–6.13 (m, 2H), 5.80 (dd, *J* = 9.0, 3.2 Hz, 1H), 5.67 (d, *J* = 6.9 Hz, 1H), 5.56 (dd, *J* = 10.4, 7.3 Hz, 1H), 5.50 (s, 1H), 5.49 (s, 1H), 5.19 (quint, *J* = 5.0 Hz, 1H), 4.92 (d, *J* = 9.0 Hz, 1H), 4.79 (dd, *J* = 4.7, 2.5 Hz, 1H), 4.35–4.26 (m, 6H), 4.18 (d, *J* = 8.5 Hz, 1H), 4.00–3.93 (m, 4H), 3.91 (d, *J* = 6.8 Hz, 1H), 3.85–3.76 (m, 4H), 3.62 (d, *J* = 4.8 Hz, 1H), 3.41–3.36 (m, 2H), 2.74–2.53 (m, 5H), 2.37 (s, 3H), 2.32 (dd, *J* = 8.9, 3.3 Hz, 1H), 2.15 (s, 3H), 1.86–1.78 (m, 7H), 1.20 (s, 3H), 1.16 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.8 (C), 172.4 (C), 172.2 (C), 171.4 (C), 170.3 (C), 169.0 (C), 167.1 (C), 166.8 (C), 140.3 (C), 138.1 (C × 2), 138.0 (C), 133.7 (CH), 133.6 (C), 132.8 (C), 131.8 (CH), 130.1 (CH × 2), 129.0 (C), 128.8 (CH × 2), 128.7 (CH × 2), 128.6 (CH × 3), 128.1 (CH × 5), 127.0 (CH × 4), 126.0 (CH × 5), 100.9 (CH × 2) 83.7 (CH), 80.8 (C), 78.2 (C), 76.2 (CH_2), 75.1 (CH), 74.1 (CH), 73.0 (CH), 71.8 (CH), 71.7 (CH), 71.4 (CH), 70.9 (CH × 2), 68.9 (CH_2 × 2), 68.4 (CH_2 × 2), 66.3 (CH_2 × 2), 55.9 (C), 54.7 (CH), 46.7 (CH), 43.0 (C), 35.3 (CH_2), 33.0 (CH_2), 28.8 (CH_2 × 2) 26.2 (CH_3), 22.3 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 14.3 (CH_3), 10.5 (CH_3); HRMS (ESI-TOF) *m*/*z* calcd for $C_{74}H_{81}NO_{23}Na$ $[M+Na]^+$ 1374.5097, found 1374.5088.

4.1.6. 7-[4-(1,3-Bis(1,3-dihydroxypropan-2-yloxy)propan-2-yloxy)-4-oxobutanoyl]-paclitaxel (4)

To a solution of **11** (206 mg, 0.152 mmol) in methanol (2.0 mL) was added palladium hydroxide (0.011 g, 0.071 mmol) and the suspension was stirred for 58 h under hydrogen atmosphere. After inflow of argon gas to turn hydrogen gas out, the resulting suspension was filtered. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with chloroform/methanol (8/1) to afford **4** as a colorless gummy solid (158 mg, 0.134 mmol, 88% yield). FT–IR (neat): 3420, 2942, 2250, 1734, 1647, 1603, 1579, 1522, 1487, 1452, 1372, 1240, 1158, 1108, 1067, 979, 913, 846, 729 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.11 (d, *J* = 7.3 Hz, 2H), 7.79–7.76 (m, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.53–7.46 (m, 5H), 7.43–7.30 (m, 5H), 6.19–6.13 (m, 2H), 5.78 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.66 (d, *J* = 6.9 Hz, 1H), 5.56 (dd, *J* = 10.4, 7.2 Hz, 1H), 5.11 (quint, *J* = 4.8 Hz, 1H), 4.94 (d, *J* = 9.0 Hz, 1H), 4.80 (dd, *J* = 5.3, 2.7 Hz, 1H), 4.31 (d, *J* = 8.5 Hz, 1H), 4.18 (d, *J* = 8.5 Hz, 1H), 4.10 (d, *J* = 1.2 Hz, 1H), 3.88 (d, *J* = 6.8 Hz, 1H), 3.84–3.60 (m, 12H), 3.53–3.46 (m, 2H), 3.03–2.77 (m, 3H) 2.73–2.52 (m, 5H), 2.37 (s, 3H), 2.31 (d, *J* = 9.0 Hz, 2H), 2.16 (s, 3H), 1.87–1.78 (m, 9H), 1.20 (s, 3H), 1.15 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.7 (C), 172.5 (C × 2), 172.3(C), 171.7 (C), 170.5 (C), 169.1 (C), 167.4 (C), 166.5 (C), 140.5 (C), 138.0 (C), 133.6 (C, CH), 132.5(C), 131.7 (CH), 130.0 (CH × 2), 128.9 (C), 128.7 (CH × 3), 128.6 (CH), 128.5 (CH × 2), 127.9 (CH), 127.0 (CH × 2), 126.9 (CH × 2), 83.7 (CH), 81.0 (CH × 2), 80.8 (C), 78.2 (C), 76.2 (CH_2), 75.2 (CH), 74.1 (CH), 72.9 (CH), 71.8 (CH), 71.6 (CH), 71.5 (CH), 67.9 (CH_2 × 2), 61.7 (CH_2), 61.6 (CH_2 × 2), 61.5 (CH_2), 55.8 (C), 55.0 (CH), 46.9 (CH), 43.0 (C), 35.3 (CH_2), 33.0 (CH_2), 28.9 (CH_2 × 2), 26.3 (CH_3), 22.4 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 14.3 (CH_3), 10.7 (CH_3); HRMS (ESI–TOF) *m*/*z* calcd for $C_{60}H_{73}NO_{23}Na$ $[M+Na]^+$ 1198.4471, found 1198.4467.

4.1.7. 1,3-Bis(2-phenyl-1,3-dioxan-5-yloxy)propan-2-yl 2,5-dioxopyrrolidin-1-yl succinate (12)

To a solution of 7 (232 mg, 0.900 mmol) in CH_2Cl_2 (5.0 mL) were added HOSu (104 mg, 0.900 mmol) and EDC (172 mg, 0.900 mmol), and the mixture was stirred for 1 h at room temperature. The resulting mixture was poured into $CuSO_4$aq (50 mL) and extracted with ethyl acetate (50 mL × 1, 25 mL × 2). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/3) to afford **12** as a colorless gummy solid (237 mg, 0.403 mmol, 90% yield). FT–IR (neat): 2860, 1815, 1784, 1740, 1454, 1390, 1239, 1206, 1153, 1091, 1011, 915, 845, 800, 732, 701, 648 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.52–7.46 (m, 4H), 7.39–7.31 (m, 6H), 5.48 (s, 2H), 5.25 (quint, *J* = 5.2 Hz, 1H), 4.35–4.26 (m, 4H), 4.00–3.94 (m, 4H), 3.86–3.76 (m, 4H), 3.36 (quint, *J* = 1.5 Hz, 2H), 2.91 (t, *J* = 6.8 Hz, 2H), 2.80 (s, 2H), 2.76 (t, *J* = 6.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.5 (C), 168.9 (C × 2), 167.7 (C), 138.1 (C × 2), 128.8 (CH × 2), 128.1 (CH × 4), 126.0 (CH × 4), 100.9 (CH × 2), 72.0 (CH), 70.8 (CH × 2), 68.8 (CH_2 × 2), 68.3 (CH_2 × 2), 66.3 (CH_2 × 2), 28.5 (CH_2), 25.9 (CH_2), 25.2(CH_2 × 2); HRMS (ESI-TOF) *m*/*z* calcd for $C_{31}H_{35}NO_{12}Na$ $[M+Na]^+$ 636.2057, found 636.2040.

4.1.8. 1,3-Bis(1,3-dihydroxypropan-2-yloxy)propan-2-yl 2,5-dioxopyrrolidin-1-yl succinate (13)

To a solution of **12** (40.0 mg, 0.065 mmol) in ethanol (2.0 mL) was added palladium hydroxide (10.0 mg, 0.0071 mmol) and the suspension was stirred for 15 h under hydrogen atmosphere. After inflow of argon gas to turn hydrogen gas out, the resulting suspension was filtered. The filtrate was concentrated in vacuo. The residue, crude **13** (19.0 mg) was immediately used in the next reaction without further purification.

4.1.9. (1S,2S,4S,9S,10S,15S,3R,7R,12R)-4,12-Diacetyloxy-1,9-dihydroxy-10,14,17,17-tetramethyl-11-oxo-2- phenylcarbonyloxy-6-oxatetracyclo [11.3.1.0<3,10>.0<4,7>]heptadec-13-en-15-yl (3S,2R)-2-hydroxy-3-{3-[(2-[2-hydroxy-1-(hydroxymethyl)ethoxy]-1-{[2-hydroxy-1-(hydroxymethyl) ethoxy]methyl}ethyl)oxycarbonyl]propanoylamino}-3-phenylpropanoate (5)

To a solution of **14** (13.8 mg, 18.4 μmol) in THF (0.3 mL) were added **13** (12.0 mg, 28 μmol), 1-hydroxybenzotriazol monohydrate (0.60 mg, 37 μmol) and *N*,*N*-dimethylaniline (2.3 μL, 18.0 μmol), and the mixture was stirred for 19 h at room temperature. The resulting mixture was poured into $CuSO_4$aq (10 mL) and extracted with ethyl acetate (20 mL × 1, 10 mL × 2). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel

column chromatography eluted with chloroform/methanol (8/1) to afford **5** as a colorless gummy solid (14.0 mg, 13.0 μmol, 73% yield). FT–IR (neat): 3385, 2926, 2348, 2251, 1723, 1662, 1539, 1452, 1373, 1243, 1177, 1110, 1071, 1026, 979, 909, 854, 776, 731, 647 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ 8.11 (d, J = 7.7 Hz, 2H), 7.67 (t, J = 7.4 Hz, 1H), 7.57 (t, J = 7.6 Hz, 2H), 7.46–7.37 (m, 4H), 7.28 (t, J = 6.8 Hz, 1H), 6.47 (s, 1H), 6.16 (t, J = 8.9 Hz, 1H), 5.66 (d, J = 7.1 Hz, 1H), 5.45 (d, J = 4.1 Hz, 1H), 5.06 (t, J = 4.9 Hz, 1H), 4.99 (d, J = 9.4 Hz, 1H), 4.59 (q, J = 4.4 Hz, 1H), 4.32 (dd, J = 10.6, 6.8 Hz, 1H), 4.19 (s, 2H), 3.82 (d, J = 7.0 Hz, 1H), 3.78–3.47 (m, 13H), 3.31 (s, 4H), 2.61 (t, J = 14.0 Hz 4H), 2.51–2.42 (m, 1H), 2.34 (s, 3H), 2.25 (dd, J = 15.4, 9.4 Hz, 1H), 2.18 (s, 3H), 2.04 (dd, J = 15.4, 9.4 Hz, 1H), 1.93 (s, 3H), 1.85–1.76 (m, 1H), 1.66 (s, 3H), 1.19 (s, 4H), 1.17 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz): δ 205.4 (C), 174.6 (C), 174.3(C), 174.2 (C), 172.1 (C), 171.5 (C), 167.8 (C), 142.3 (C), 140.2 (C), 135.0 (C), 134.8 (CH), 131.5(C), 131.3 (CH × 2), 129.9 (CH × 2), 129.8 (CH × 2), 129.0 (CH), 128.6 (CH × 2), 85.9 (CH), 83.2 (CH × 2), 82.4 (C), 79.1 (C), 77.5 (CH_2) 76.9 (CH), 76.3 (CH), 74.9 (CH), 73.9 (CH), 72.5 (CH), 72.4 (CH), 69.6 (CH_2× 2), 62.5 (CH_2 × 4), 59.3 (C), 57.0 (CH), 47.9 (CH), 44.6 (C), 37.5 (CH_2), 36.6 (CH_2), 31.3 (CH_2), 30.5 (CH_2), 27.0 (CH_3), 23.2 (CH_3), 22.3 (CH_3), 20.8 (CH_3), 14.7 (CH_3), 10.4 (CH_3); HRMS (ESI–TOF) m/z calcd for $C_{53}H_{69}NO_{22}Na$ $[M+Na]^+$ 1094.4209, found 1094.4219.

4.1.10. 5,5′-(2-Azidopropane-1,3-diyl)bis(oxy)bis(2-phenyl-1,3-dioxane) (16)

To a solution of **6**[9c] (2.35 g, 5.64 mmol) in pyridine (12 mL) were added p-toluenesulfonyl chloride (1.62 g, 8.46 mmol) and DMAP (690 mg, 0.56 mmol), and the mixture was stirred for 20 h. The resulting mixture was poured into $CuSO_4$aq (200 mL) and extracted with ethyl acetate (200 mL × 1, 100 mL × 2). The combined organic layers were washed with $NaHCO_3$aq (200 mL), brine (200 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue, crude **15** (3.49 g) was used for the next reaction without further purification. To a solution of **15** (3.49 g) in anhydrous DMF (20 mL) was added sodium azide (1.11 g, 16.9 mmol), and the mixture was stirred for 2 h at 100 °C. The resulting mixture was poured into $NaHCO_3$aq (200 mL) and extracted with ethyl acetate (200 mL × 1, 100 mL × 2). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/1) to afford **16** as a colorless gummy solid (2.21 g, 5.01 mmol, 89% yield). FT–IR (neat): 2099, 1154, 1092, 1010, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.52–7.47 (m, 4H), 7.39-7.31 (m, 6H), 5.52 (s, 2H), 4.38–4.31 (m, 4H), 4.04–3.98 (m, 4H), 3.85–3.75 (m, 5H), 5.15 (quint, J = 1.6 Hz, 2H), ^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.0 (C × 2), 128.7 (CH × 2), 128.0 (CH × 4), 125.9 (CH × 4), 100.9 (CH × 2), 71.1 (CH × 2), 68.5 (CH_2 × 2), 68.3 (CH_2 × 2), 67.5 (CH_2 × 2), 60.0 (CH); HRMS (ESI-TOF) m/z calcd for $C_{23}H_{27}N_3O_6Na$ $[M+Na]^+$ 464.1798, found 464.1797.

4.1.11. 2,5-Dioxopyrrolidin-1-yl 5-(1,3-bis(2-phenyl-1,3-dioxan-5-yloxy)propan-2-ylamino)-5-oxopentanoate (20)

To a solution of **16** (1.03 g, 2.33 mmol) in THF (20 mL) was added lithium aluminum hydride (265 mg, 6.99 mmol) in some portions at 0 °C, and the mixture was stirred for 30 min at 0 °C, and quenched with ethyl acetate (0.6 mL) and water (0.2 mL) at 0 °C. The resulting suspension was filtered through celite, and the filtrate was dried over Na_2SO_4, and concentrated in vacuo to afford the crude amine **17** (1.14 g), which was used in the next reaction without further purification. To a solution of **17** (1.14 g) in CH_2Cl_2 (15 mL) were added TEA (0.65 mL, 4.07 mmol) and glutaric anhydride (319 mg, 2.79 mmol), and the mixture was stirred for 8 h at room temperature. The resulting mixture was poured into $CuSO_4$aq (100 mL) and extracted with ethyl acetate (100 mL × 1, 50 mL × 2). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated in vacuo to afford crude **18** (1.31 g), which was used in the next reaction without further purification. To a solution of **18** (1.31 g) in CH_2Cl_2 (15 mL) were added H–OSu (536 mg, 4.66 mmol) and EDC (893 mg, 4.66 mmol), and the mixture was stirred for 3 h at 40 °C. The resulting mixture was quenched with water (50 mL), and extracted with ethyl acetate (50 mL × 1, 25 mL × 2). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residiue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/3) to afford **19** as a colorless gummy solid (1.02 g, 1.63 mmol, 70% yield). FT-IR (neat): 2866, 1783, 1738, 1661, 1530, 1454, 1388, 1209, 1154, 1091, 1010, 755, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.50–7.44 (m, 4H), 7.39–7.32 (m, 6H), 6.79 (d, J = 8.4, 2H), 5.50 (s, 2H), 4.37–4.27 (m, 5H), 4.00–3.93 (m, 4H), 3.80–3.75 (m, 2H), 3.71–3.65 (m, 2H), 3.35 (s, 2H), 2.60 (t, J = 6.8, 2H), 2.53 (s, 4H), 2.28 (t, J = 6.8, 2H), 2.06 (t, J = 6.8, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.4 (C), 169.4 (C × 2), 168.2 (C), 138.1 (C × 2), 128.7 (C × 2), 128.0 (C × 4), 125.8 (C × 2), 100.9 (CH × 2), 70.6 (CH × 2), 68.9 (CH_2 × 2), 68.3 (CH_2 × 2), 65.8 (CH_2 × 2), 48.6 (CH), 34.1 (CH_2), 29.6 (CH_2), 25.2 (CH_2 × 2), 20.9 (CH_2); HRMS (ESI-TOF) m/z calcd for $C_{32}H_{38}N_2O_{11}Na$ $[M+Na]^+$ 649.2373, found 649.2350.

4.1.12. 2,5-Dioxopyrrolidin-1-yl 5-((1,3-bis((1,3-dihydroxypropan-2-yl)oxy)propan-2-yl)amino)-5-oxopentanoate (20)

A solution of **19** (47.6 mg, 76 μmol) in THF (2 mL) suspended with palladium hydroxide (3.0 mg, 21 μmol) was stirred for 2 h at room temperature under hydrogen atmosphere. After inflow of argon gas to remove hydrogen gas, the resulting suspension was filtered. The filtrate was concentrated in vacuo, and the residue, crude **20** (34 mg, 76 μmol), was immediately used in the next reaction without further purification.

4.1.13. (1S,2S,4S,9S,10S,15S,3R,7R,12R)-4,12-Diacetyloxy-1,9-dihydroxy-10,14,17,17-tetramethyl-11-oxo-2-phenylcarbonyloxy-6-oxatetracyclo[11.3.1.0<3,10>.0<4,7>]heptadec-13-en-15-yl (3S,2R)-2-hydroxy-3-{4-[*N*-(2-[2-hydroxy-1- (hydroxymethyl) ethoxy]-1-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl} ethyl) carbamoyl]butanoylamino}-3-phenylpropanoate (23)

To a solution of **14**[17] (14.2 mg, 19.0 μmol) in DMF (0.2 mL) was added crude **20** (34 mg, 76 μmol), and the mixture was stirred for 48 h at room temperature. The resulting mixture was poured into $NaHCO_3$aq (20 mL) and extracted with ethyl acetate (30 mL × 1, 10 mL × 2). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluted with chloroform/methanol (7/1) to afford **23** as a colorless gummy solid (12.9 mg, 12.9 μmol, 68% yield). FT-IR (neat): 3365, 2924, 2853, 1721, 1648, 1542, 1458, 1374, 1276, 1121, 1072, 977, 748, 708 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ 8.13 (d, J = 7.3 Hz, 2H), 7.68 (t, J = 7.4 Hz, 1H), 7.58 (t, J = 7.6 Hz, 2H), 7.48–7.39 (m, 4H), 7.30 (t, J = 7.0 Hz, 1H), 6.48 (s, 1H), 6.17 (t, J = 8.8 Hz, 1H), 5.68 (d, J = 7.1 Hz, 1H), 5.48 (d, J = 4.5 Hz, 1H), 5.01 (d, J = 9.4 Hz, 1H), 4.61 (d, J = 4.6 Hz, 1H), 4.35 (dd, J = 10.8, 6.65 Hz, 1H), 4.21 (s, 2H), 4.15 (dt, J = 10.3, 5.2 Hz, 1H), 3.85 (d, J = 7.1 Hz, 1H), 3.79–3.71 (m, 2H), 3.69–3.52 (m, 10H), 3.46-3.38 (m, 2H), 3.34–3.31 (m, 1H), 2.53–2.42 (m, 1H), 2.37–2.21 (m, 8H), 2.20 (s, 3H), 2.08 (dd, J = 15.5, 9.0 Hz, 1H), 1.95 (s, 3H), 1.91–1.77 (m, 3H), 1.68 (s, 3H), 1.22-1.16 (m, 6H); ^{13}C NMR (CD_3OD, 75 MHz): δ 205.1 (C), 175.4 (C), 175.2(C), 174.5 (C), 171.8 (C), 171.3 (C), 167.6 (C), 142.1 (C), 140.0 (C), 135.0 (C), 134.6 (CH), 131.3(C), 131.1 (CH × 2), 129.7 (CH × 4), 128.9 (CH), 128.5 (CH × 2), 85.8 (CH), 83.1 (CH), 83.0 (CH), 82.3 (C), 79.0 (C), 77.5 (CH_2) 76.8 (CH), 76.2

(CH), 74.7 (CH), 72.4 (CH), 72.3 (CH), 69.8 ($CH_2 \times 2$), 62.6 (CH_2), 62.5 (CH_2), 62.4 ($CH_2 \times 2$), 59.3 (C), 56.8 (CH), 51.0 (CH), 47.9 (CH), 44.6 (C), 37.5 (CH_2), 36.6 (CH_2), 36.1 (CH_2), 36.0 (CH_2), 27.0 (CH_3), 23.3 (CH_2), 23.2 (CH_3), 22.4 (CH_3), 20.8 (CH_3), 14.7 (CH_3), 10.5 (CH_3); HRMS (ESI-TOF) *m*/*z* calcd for $C_{54}H_{72}N_2O_{21}Na$ $[M+Na]^+$ 1107.4525, found 1107.4520.

4.1.14. 2,5-Dioxopyrrolidin-1-yl 5-(5,11-bis((1,3-dihydroxypropan-2-yloxy)methyl)-1,15-dihydroxy-2,14-bis (hydroxymethyl)-3,6,10,13-tetraoxapentadecan-8-ylamino)-5-oxopentanoate (21)

A solution of **20**[9c] (28.0 mg, 0.025 mmol) in ethanol (2.5 mL) suspended with palladium hydroxide (10 mg, 0.071 mmol) was stirred for 2 h at room temperature under hydrogen atmosphere. The resulting suspension was filtered to remove palladium catalyst. The filtrate was concentrated in vacuo to afford **21** (17.0 mg, 0.022 mmol), which was used in the next reaction without further purification.

4.1.15. (1S,2S,4S,9S,10S,15S,3R,7R,12R)-4,12-Diacetyloxy-1,9-dihydroxy-10,14,17,17-tetramethyl-11-oxo-2-phenylcarbonyloxy-6-oxatetracyclo[11.3.1.0<3,10>. 0<4,7>]heptadec-13-en-15-yl (3S,2R)-2-hydroxy-3-[4-(N-{2-(2-[2-hydroxy-1-(hydroxymethyl)ethoxy]-1-{[2-hydroxy-1-(hydroxymethyl) ethoxy]methyl}ethoxy)-1-[(2-[2-hydroxy-1-(hydroxymethyl) ethoxy]-1-{[2-hydroxy-1-(hydroxymethyl) ethoxy]methyl} ethoxy)methyl]ethyl}carbamoyl) butanoylamino]-3-phenylpropanoate (24)

To a solution of **14**[17] (0.8 mg, 1.1 μmol) in DMF (0.5 mL) was added crude **22** (17.0 mg, 0.022 mmol), and the mixture was stirred for 40 h. The resulting mixture was concentrated in vacuo, and the residue was purified by HPLC [ODS-80Ts column, 2.0 mm id × 100 mm length, flow rate = 1.0 mL/min, linear gradient (water/acetonitrile = 95/5–5/95 with 0.1% TFA for 1 h), retention time of **24** = 30.5–38.5 min] to afford **24** as a colorless gummy solid (1.2 mg, 0.87 μmol, 80% yield). FT–IR (neat): 3357, 2940, 1719, 1677, 1544, 1452, 1372, 1245, 1203, 1070, 906, 837, 801, 719 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ 8.13 (d, J = 7.3 Hz, 2H), 7.69 (t, J = 7.4 Hz, 1H), 7.59 (t, J = 7.6 Hz, 2H), 7.48–7.39 (m, 4H), 7.30 (t, J = 6.8 Hz, 1H), 6.48 (s, 1H), 6.16 (t, J = 9.0 Hz, 1H), 5.67 (d, J = 7.2 Hz, 1H), 5.47 (dd, J = 8.6, 4.9 Hz, 1H), 5.00 (d, J = 9.4 Hz, 1H), 4.60 (d, J = 4.8 Hz, 1H), 4.34 (dd, J = 11.0, 6.7 Hz, 1H), 4.23–4.16 (m, 3H), 3.86-3.56 (m, 31H), 3.51–3.42 (m, 4H), 2.53-2.43 (m, 1H), 2.39–2.23 (m, 8H), 2.20 (s, 3H), 2.10–2.00 (m, 1H), 1.98–1.77 (m, 6H), 1.68 (s, 3H), 1.20 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz): δ 205.1 (C), 175.8 (C), 175.2(C), 174.5 (C), 171.8 (C), 171.3 (C), 167.6 (C), 142.1 (C), 140.0 (C), 134.9 (C), 134.6 (CH), 131.3(C), 131.2 (CH × 2), 129.7 (CH × 4), 129.0 (CH), 128.5 (CH × 2), 85.8 (CH), 82.9 (CH × 4), 82.3 (C), 80.1 (CH × 2), 79.0 (C), 77.5 (CH_2) 76.8 (CH), 76.2 (CH), 74.8 (CH), 72.4 (CH), 72.3 (CH), 70.9 ($CH_2 \times 2$), 70.7 ($CH_2 \times 2$), 70.6 ($CH_2 \times 2$), 62.4 ($CH_2 \times 8$), 59.3 (C), 57.0 (CH), 51.1 (CH), 47.9 (CH), 44.6 (C), 37.5 (CH_2), 36.6 (CH_2), 36.1 ($CH_2 \times 2$), 27.0 (CH_3), 23.3 (CH_2), 23.2 (CH_3), 22.3 (CH_3), 20.8 (CH_3), 14.8 (CH_3), 10.4 (CH_3); HRMS (ESI–TOF) *m*/*z* calcd for $C_{66}H_{96}N_2O_{29}Na$ $[M + Na]^+$ 1403.5996, found 1403.5996.

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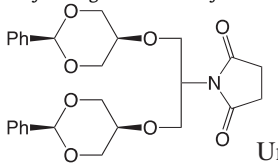
18. It is well known that hydrogenation of the bridged tetra-substituted C=C bond did not proceed at all under hydrogen atmosphere (1 atom), and skeleton of **1** was decomposed under strong acidic conditions.[5] Therefore, Bnd group was used for BGL protecting group. Additionally, because of the facility, the method for the synthesis of BGL possessing Bnd[9c] is more recommendable than that of BGL possessing Bn,[9a] as the inventor of BGL.[10a,b]
19. In our previous paper,[11f] hydrogenolysis of eight benzyl groups of BGL006 possessing *N*-hydroxysuccinimidyl ester was reported. In such a case, the N–O bond was preserved to smoothly carry out the BGLation of the lysine side chains of an artificial protein drug. The protection-free N-succinimidyl ester decomposed after a couple of days at room temperature.
20. When succinic anhydride was used for the primary amine, more than 20% of the cyclic imide illustrated below was obtained as an undesired byproduct. Glutaric anhydride gave no such cyclic imide.[9c]



Undesired byproduct

21. Either **5** or **23** was not pro-drug of paclitaxel any more, because even the selective cleavage of the amide bond at C3' generate no paclitaxel. Furthermore, ester bonds are generally cleaved faster than amide bonds in biological circumstance. Therefore, we believe that **5** itself shows the anti-

tumor activity but not some of the metabolites from **5** does not. Only one possible metabolite is the compound generated by the hydrolysis of ester bond between BGL and linker of **5**. Therefore, the amide bond was introduced instead of the ester bond in the case of **23**.

22. If a paclitaxel–BGL conjugate is commercially available, to reduce the total amount of water in the drug package will be desirable for patients because price of drug package will be reduced and the required time for injection will be shorten. From this point of view, **24** is more attractive than **5** or **23**.