Synthesis and Enzyme-Specific Activation of Carbohydrate−Geldanamycin Conjugates with Potent Anticancer Activity

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Geldanamycin (GA) is a potent anticancer antibiotic that inhibits Hsp90. Its potential clinical utility is hampered by its severe toxicity. To alleviate this problem, we synthesized a series of carbohydrate−geldanamycin conjugates for enzyme-specific activation to increase tumor selectivity. The conjugation was carried out at the C-17-position of GA. Their anticancer activity was tested in a number of cancer cell lines. The enzyme-specific activation of these conjugates was evaluated with β-galactosidase and β-glucosidase. Evidently, glycosylation of C-17-position converted GA to an inactive prodrug before enzyme cleavage. Glucose−GA, as positive control, showed anticancer activity with IC_{50} of 70.2−380.9 nM in various cancer cells by β-glucosidase activation inside of the tumor cells, which was confirmed by 3-fold inhibition using β-glucosidase specific inhibitor [2,5-dihydroxymethy-3,4-dihydroxypyrrolidine (DMDP)]. Compared to glucose−GA, galactose− and lactose−GA conjugates exhibited much less activity with IC_{50} greater than 8000−25 000 nM. However, when galactose− and lactose−GA were incubated with β-galactosidase in the cells, their anticancer activity was enhanced by 3- to 40-fold. The results suggest that GA can be inactivated by glycosylation of C-17-position and reactivated for anticancer activity by β-galactosidase. Therefore, galactose−GA can be exploited in antibody-directed enzyme prodrug therapy (ADEPT) with β-galactosidase for enzyme-specific activation in tumors to increase tumor selectivity.

Introduction

Geldanamycin (GA) is an anticancer antibiotic isolated from a *Streptomyces hygroscopicus*; it is a benzoquinoid ansamycin related to herbimycin A and macbecin (Figure 1). Geldanamycin was initially thought to be a nonspecific kinase inhibitor, but recently found to target the heat shock protein 90 (Hsp90).[1,2] Hsp90 is a molecular chaperon that modulates protein kinase activity (such as p60, p185, raf-1, cdk4/cdk6, FAK, and MAK, v-Src, Akt, Bcr-Abl, mutant P53, HIF-1α, and ErbB2) in cells. Hsp90 has been observed with 2- to10-fold higher expressions in various human cancer cells compared to normal.[3,4] The crystal structure showed that GA binds to Hsp90 and inhibits Hsp90-mediated protein conformation/refolding, resulting in a depletion of oncogenic kinases through the proteosomal degradation of immature protein.[2,5] This process subsequently down-regulates expression of many oncogenes in cancer cells.[2] While the antitumor potential of GA has long been recognized, clinical evaluation of GA has not been pursued due to its severe toxicity and poor water solubility.[6,7] For this reason, efforts have been made to modify GA, generating a number of analogues in attempts to increase clinical efficacy and water solubility,[8−13] including modifications of the C-17-position.[8,9] Among the successful analogues is 17-allylaminogeldanamycin (17-AAG), which is currently in phase II clinical trials at the National Cancer Institute.[14,15] Although 17-AAG showed improved efficacy and relatively low toxicity, it appears that the hepatotoxicity and the low water solubility may be still limiting factors for its clinical application.[16,17]

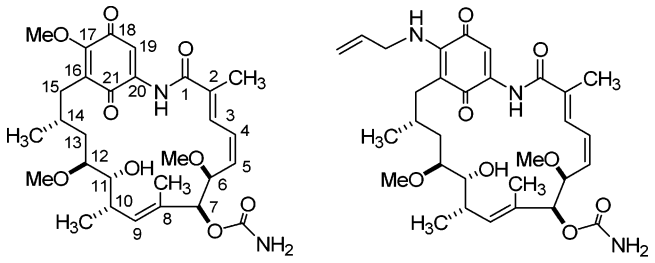

Figure 1.

Most existing chemotherapeutic compounds lack tumor selectivity and are associated with dose-limiting side effects. In some cases, such cytotoxic compounds are linked to monoclonal antibodies against tumor-specific antigen in an attempt to increase specific activity. However, this approach is limited by the amount of drug that could be linked to antibodies, slow internalization of drug conjugates into cancer cells, and heterogeneous antigen expression in solid tumors.[18,19]

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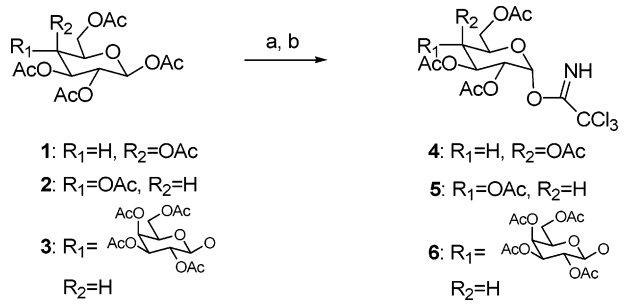
To circumvent these problems, a two-step strategy called antibody-directed enzyme prodrug therapy (ADEPT) [20,21] was recently emerged. In this approach, a monoclonal antibody is employed to selectively deliver an enzyme, such as β-galactosidase,[22] to the tumor cells, which subsequently converts an inactive prodrug to active drug at tumor site. Thus, the selective activation of the prodrug at the tumor site would result in improved antitumor activity with minimal side effects.[23] In this regard, we synthesized six carbohydrate−GA conjugates for possible delivery through ADEPT with β-galacosidase.

Crystal structure showed that GA binding to Hsp90 is highly compact with more than 85% surface area of GA buried in the complex. The benzoquinone group of GA binds near the entrance of Hsp90 binding pocket, whereas the ansamycin ring, which is similar to a five amino acid polypeptide in dimensions, inserts into the binding pocket. Carbamate group of C-7 is at the tip of GA and forms H-bond with Asp93 at the bottom of the binding pocket, while the flanked C-23 methoxyl and C-25 and C-26 methyl groups make high-density van der Waals contacts with the bottom and sides of the pocket.[2] SAR studies revealed that the C-7-postion and C-11-postion of GA are critical for its activity. Modifications of these two positions results in inactive compounds. Thus, modification at C-7- or C-11-position offered a good choice for synthesis of carbohydrate conjugates as inactive prodrugs. However, the X-ray structure of GA showed that the free C-11-OH group is buried inside the ansa ring.[2] The limited space around the C-11-OH group makes it hardly accessible for bulky sugar donors. The low reactivity at the C-7-position makes it difficult for modification. Previous results on GA derivatives revealed that modifications at the C-17-position on the quinone ring maintained anticancer activity within a nanomolar range.[8,9] However, carbohydrate−GA conjugates have never been reported. In this regard, we embarked on a synthetic program to produce carbohydrate−GA conjugates. Our hypothesis is that the bulky sugar structure might be able to affect GA binding to Hsp90 and thus make it an inactive prodrug. If the carbohydrate moiety is cleaved at the tumor site by a specifically delivered enzyme, the active drug will be released and become active at site of tumor growth.

It is known that β-glucosidase is ubiquitously expressed in many cell types; therefore, we designed glucose−GA conjugate, as a positive control, that could be activated by β-glucosidase inside of the cancer cells. β-Galatosidase is very low in the serum (almost undetectable), which makes this enzyme a very attractive enzyme for activation of these conjugates if it is delivered to tumors. The low levels of this enzyme would minimize the premature release of activate drug in blood in ADEPT study. Indeed, β-galatosidase has been applied to prodrug activation in animal models.[24] Therefore, we designed and synthesized galactose−GA conjugates with different spacers for enzyme-specific activation. If these conjugates are delivered with β-galactosidase in ADEPT, the conjugates will be specifically activated at tumor sites to exhibit antitumor activity. In addition, the sugar moiety is also expected to provide better water solubility for GA.

Scheme 1[a]



[a] Reagents and conditions: (a) NH_2NH_2/AcOH, DMF, 60 °C; (b) CCl_3CN/DBU, CH_2Cl_2, 0 °C, 60 min.

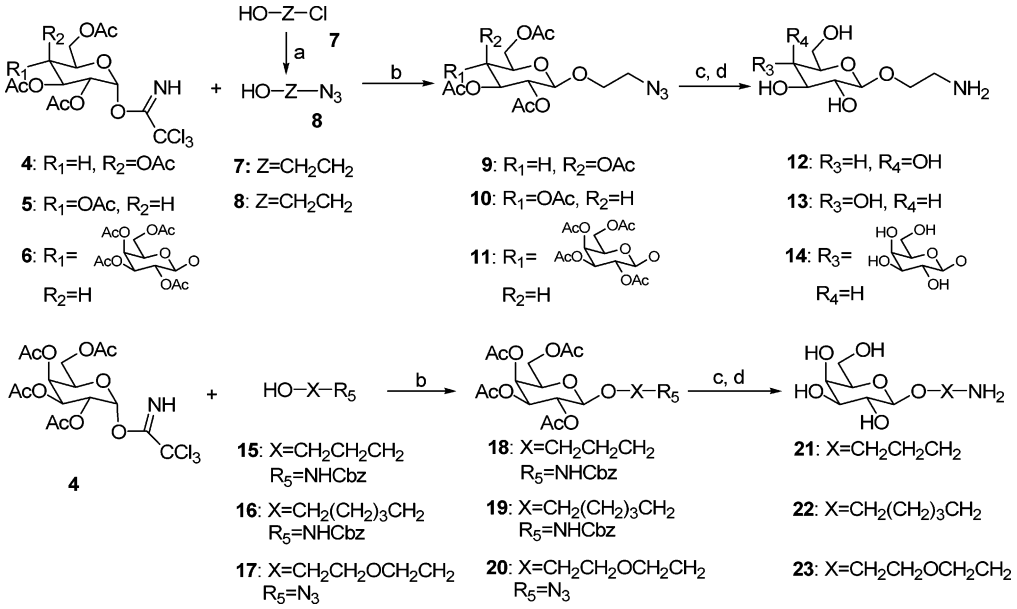
Results and Discussion

Chemistry. The crystal structure of Hsp90−GA complex indicates that 85% of the surface area of GA is buried in the binding pocket on the surface of Hsp90, while benzoquinone is positioned near the entrance of the pocket.[2] The carbamate group at the C-7-position is crucial in forming a hydrogen bond to Hsp90. Removal of the carbamate or attachment of additional atom abolishes GA activity. An amino group substitution at C-17-position of the benzoquinone may improve cellular activity indirectly by stabilizing the quinone over the reduced hydroquinone. Our hypothesis was that conjugating a bulky sugar at C-17-position of GA would affect the GA binding to Hsp90 and makes it inactive. Our first design was to synthesize glucose−GA conjugate as positive control for enzyme-specific activation by β-glucosidase since β-glucosidase is ubiquitously expressed in the cells. In addition, galactose−GA conjugates were designed by glycosylation of the C-17-position of GA with galactose through an amine linker with varied lengths. Since β-galactosidase activity is undetectable in cancer cells, these conjugates should not have anticancer activity. However, when the galactose−GA conjugates are incubated with exogenous β-galactosidase, they are expected to show enhanced antitumor activity. Thus, such conjugates may be applied in ADEPT when β-galactosidase is delivered into the tumor sites by a specific antibody.[25]

Synthesis. As shown in Scheme 1, three trichloroacetimidate derivatives (**4**, **5**, **6**) of sugar (galactose, glucose and lactose) were prepared as glycosyl donors from corresponding peracetylated sugars in two steps with high yield. Selective removal of the acetyl group at anomeric position was achieved under mild basic condition. Treatment of the free sugar with trichloroacetonitrile in the presence of DBU afforded the anomerically pure, stable α-trichloroacetimidates. As shown in Scheme 2, treatment of α-trichloroacetimidates (**4**, **5**, **6**) with different alchohol-linkers (**8**, **15**, **16**, **17**) in the presence of TMSOTf afforded a series of protected sugar primary amine derivatives (**9**, **10**, **11**, **18**, **19**, **20**) in β-configuration. The free sugar primary amine derivatives (**12**, **13**, **14**, **21**, **22**, **23**) were obtained in high yields by two steps of deprotection.

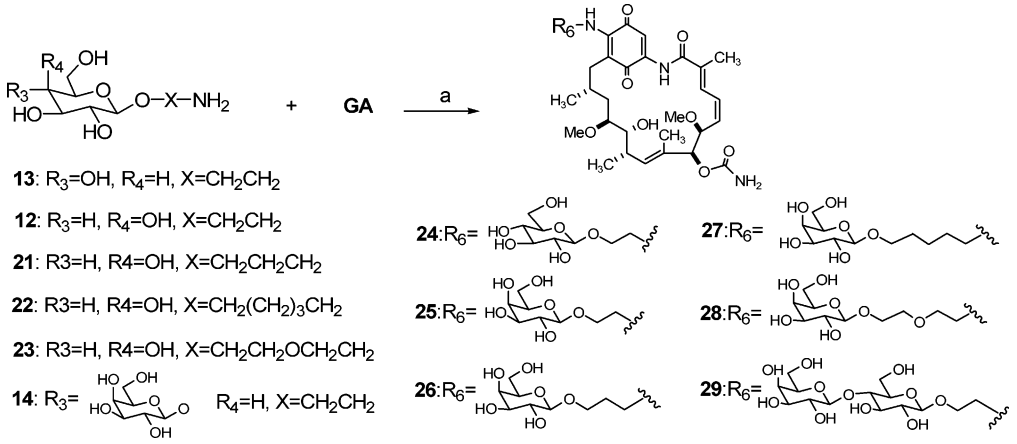
Coupling sugar-amine derivatives (**12**, **13**, **14**, **21**, **22**, **23**) with GA in DMF at room-temperature resulted in the formation of sugar−GA conjugates with good yield (70−90%). GA was readily losing its 17-methoxy group in such a mild condition and forming 17-amino-17-demethoxygeldanamycin derivatives. As shown in

Scheme 2[a]



[a] Reagents and conditions: (a) NaN_3, $NaOH/H_2O$, rt, 3 days; (b) TMSOTf,CH_2Cl_2, 4 Å MS, −30 °C−0 °C, overnight; (c) NaOMe (1 M), MeOH; (d) 10% Pd−C/AcOH, EtOH, H_2 50 psi, 20 h.

Scheme 3[a]



[a] Reagents and conditions: (a) Et_3N/DMF, rt, 24 h.

Scheme 3, six conjugates (**24**−**29**) with different sugar moieties or linkers have been prepared, respectively. The structures of synthesized compounds are confirmed by NMR and high-resolution mass spectrum (HRMS). The purity is confirmed with HPLC.

A series of methods were used in attempts for direct glycosylation of geldanamycin at C-11-position by treatment of the phenylthioglycosides of the protected sugars with mCPBA followed by GA and various Lewis acids (Tf_2O, TMSOTf, AgOTf, Ag_2CO_3, and PH_3P/HBr). The results, however, were quite disappointing. All these glycosylation conditions failed to yield the corresponding glycosides. Two reasons might account for the failure: (1) the solubility of GA was low in organic solvents that were used in glycosylation (such as methylene chloride and toluene). (2) As can be seen from the X-ray structure of GA, the C-11-OH group is buried inside the ansa ring. The limited space around C-11-OH group makes it hardly accessible for bulky and rigid glycosyl donors for direct glycosylation. Intramolecular hydrogen bond may also exist in this molecule, thus making this OH group less active.

In addition, we also tried to directly link glycosylamine to C-17-position of GA. However, the reaction was inefficient; nearly half of the starting material (GA) was recovered even after 3 days. The major product obtained was 17-amine-17-demethoxy-GA.

Biology. Anticancer Activity. The anticancer activities of these six conjugates were tested by the MTS assay (MTS: tetrazolium [3-(4,5-dimethythiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) in four different cancer cell lines, including colorectal carcinoma cells (SW620, HT 29), breast cancer cells (MCF7), and leukemia cells (K562). The results are shown in Figures 2−4. As expected, only the glucose−GA (compound **24**) showed antitumor activity, while other four galactose−geldanamycin conjugates with different linker and lactose−GA were inactive. Table 1 summarizes the IC_{50} of all six geldanamycin−sugar conjugates for their anticancer activity. For example, in SW620 cell line, compound 24 showed anticancer activity with an IC_{50} of 70 nM, while the other conjugates[25,29] with the same spacer but differed in sugar moieties, showed low activity with IC_{50} > 8000 nM,

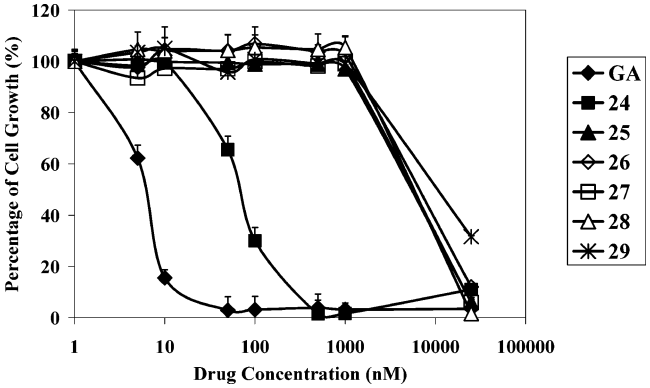


Figure 2. Anticancer activity of carbohydrate−geldanamycin conjugates **(24−29)** tested in SW620 cell line. Each compound was tested at concentration 1−25 000 nM. GA was also tested for comparison. The inhibition of cell growth was calculated compared to control cells without drug treatment. Each point represents the average of six experiments.

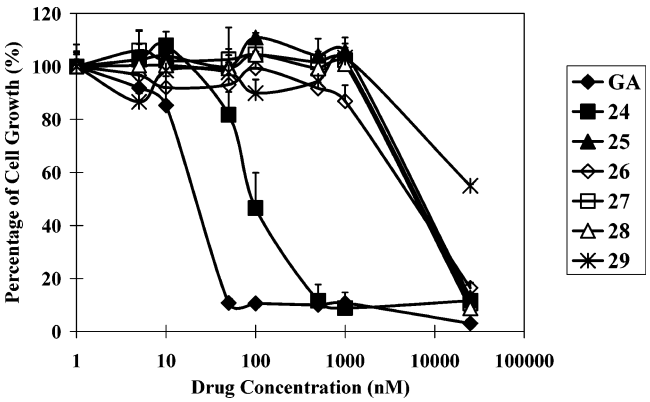


Figure 3. Anticancer activity of carbohydrate−geldanamycin conjugates **(24−29)** tested in HT29 cell line. Each compound was tested at concentration 1−25 000 nM. GA was also tested for comparison. The inhibition of cell growth was calculated compared to control cells without drug treatment. Each point represents the average of six experiments.

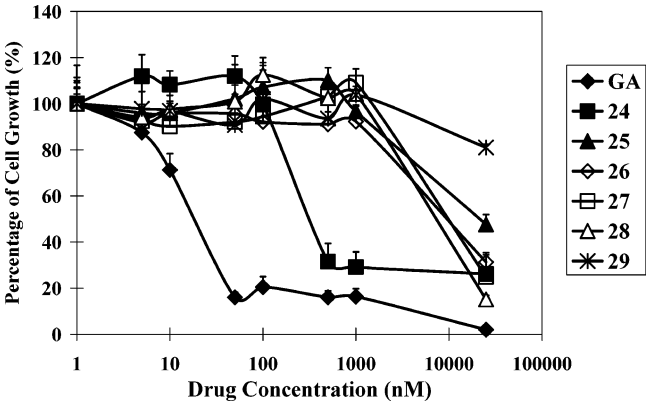


Figure 4. Anticancer activity of carbohydrate−geldanamycin conjugates **(24−29)** tested in K562 cell line. Each compound was tested at concentration 1−25 000 nM. GA was also tested for comparison. The inhibition of cell growth was calculated compared to control cells without drug treatment. Each point represents the average of six experiments.

20 000 nM, respectively. Similar results were obtained in other cell lines. It is possible that the glucose−GA conjugate is activated for anticancer activity through cleavage of glucose moiety by β-glucosidase inside of the cancer cells.

Table 1. Anticancer Activity (IC_{50}) of Carbohydrate−Geldanamycin in Four Different Cancer Cell Lines (nM)

	GA	**24**	**25**	**26**	**27**	**28**	**29**
SW620	6.2	70.2	>8000	>14000	>13000	>11000	>20000
HT29	24.5	104.7	>14000	>6000	>13000	>13000	>25000
MCF7	6.5	754.0	>25000	>12000	>17000	>22000	>22000
K562	22.1	380.9	>23000	>14000	>19000	>15000	>25000

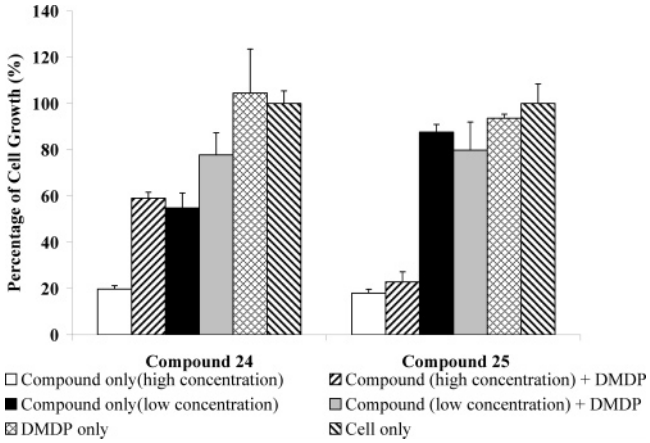


Figure 5. β-Glucosidase inhibition assay studied in K562 cell line. Two different concentrations were tested in this experiment. For compound **24**, the high concentration is 0.4 μM and the low concentration is 0.2μM. For compound **25**, the high concentration is 25μM and the low concentration is 12.5 μM. Percentages of cell growth were calculated compared to control cells in the absence of inhibitor and drug solution. Each data presented is the average of three experiments.

***β*-Glucosidase Inhibition Assay.** To verify if the cleavage of glucose moiety from compound **24** is crucial for the anticancer activity, we performed the β-glucosidase inhibition study in K562 cell line. The K562 cells were treated with β-glucosidase inhibitor (2,5-dihydroxymethy-3,4-dihydroxypyrrolidine, DMDP) solution (100 μM) for 10 min before adding carbohydrate−geldanamycin conjugates. The concentrations of glucose−GA were 0.4 μM and 0.2 μM, respectively. Interestingly, after treatment with DMDP, the activity of compound **24** decreased significantly by 3-fold, while DMDP did not affect the activity of compound **25** (Figure 5)**.** DMDP alone have no effect on cell growth. Since the only difference between compound **24** and **25** is the sugar moiety, which is glucose in compound **24** and galactose in compound **25**, the results suggest that the anticancer activity of compound **24** is achieved through cleavage of glucose moiety by β-glucosidase inside of the cells, while low activity of compound **25** is due to the lack of β-galactosidase for the cleavage of galactose moiety.

***β*-Galactosidase Cleavage Assay.** Since β-glucosidase failed to activate compound **25**, β-galactosidase was subsequently evaluated to activate galactose−GA and lactose−GA conjugates. SW620, HT29, and K562 cells were incubated with 2 units of β-galactosidase and then treated individually with 1 μM of compounds **25**−**29**. At this concentration, galactose−GA conjugates alone did not show anticancer activities. However, all five inactive galactose−GA conjugates and the lactose−GA conjugate were activated with 3- to 40-fold increase of their anticancer activity against HT29 and K562 after incubation with β-galactosidase (Figure 6−8), while β-galactosidase treatment did not affect the anticancer

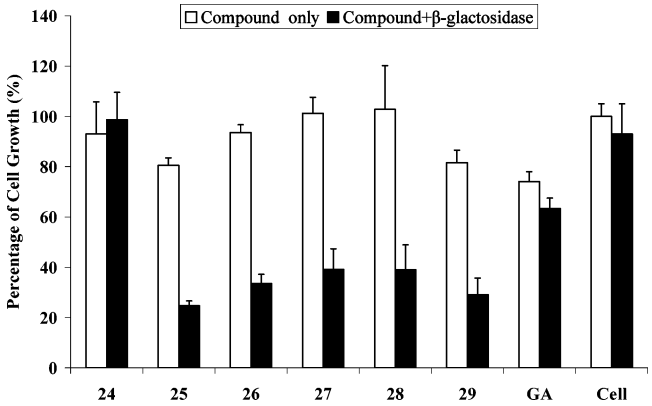


Figure 6. β-Galactosidase cleavage assay of compound **24**–**29** and GA in SW620 cell line. 0.5 μM each of compounds **25**–**29** was used. 0.005 μM GA, 0.1 μM compound 24 and 2 units β-galactosidase were used. Each data shown is the average of three experiments. Percentages of cell growth were calculated compared to control cells in the absence of β-galactosidase and drug solution.

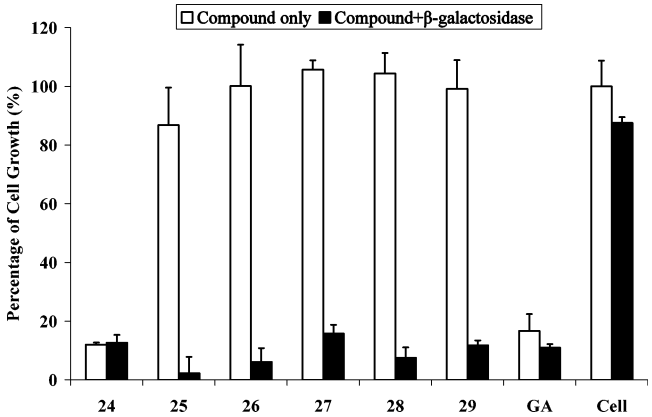


Figure 7. β-Galactosidase cleavage assay of compound **24**–**29** and GA in HT 29 cell line. 1 μM each of compounds **25**–**29** was used. 0.01 μM GA, 0.5 μM compound 24 and 2 units β-galactosidase were used. Each data shown is the average of three determinations. Percentages of cell growth were calculated compared to control cells in the absence of β-galactosidase and drug solution.

activity of glucose–GA conjugate **24** and GA. These results support our hypothesis that the bulky sugar moiety at C-17-position of GA can abolish its anticancer activity, and the inactive carbohydrate–GA prodrugs can be enzyme-specifically activated by β-glucosidase or β-galactosidase. Interestingly, compounds **25**–**29** showed nearly the same activity as GA after being cleaved by β-galactosidase. This indicates that the length of spacer has little effect on GA anticancer activity. In addition, although lactose is a disaccharide, its structure is galactose-β-1,4-glucose, the terminal galactose in the lactose–GA structure could be cleaved by β-galactosidase and thus expose the glucose moiety, which can be subsequently cleaved by the β-glucosidase inside the cells to exhibit anticancer activity.

Production of Geldanamycin. Geldanamycin was isolated from the fermentation broth of *Streptomyces hygroscopicus* NRRL3602. Fermentation and chemical isolation was carried out as described by DeBoer et al.[1]

Conclusion

In summary, a new series of carbohydrate–GA conjugates were synthesized through glycosylation of GA

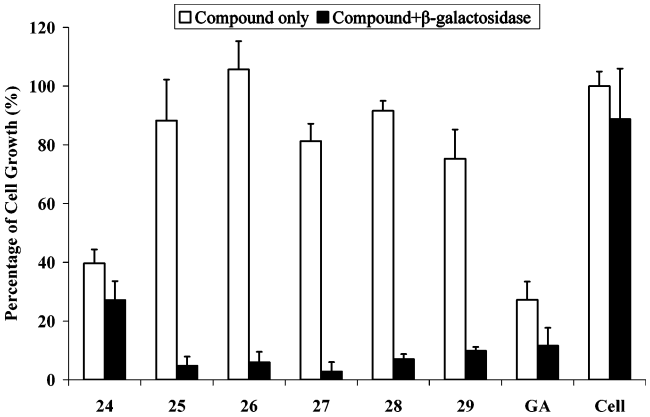


Figure 8. β-Galactosidase cleavage assay of compound **24**–**29** and GA in K562 cell line. 1 μM each of compounds **25**–**29** was used. 0.01 μM GA, 0.5 μM compound **24** and 2 units β-galactosidase were used. Each data shown is the mean of three determinations. Percentages of cell growth were calculated compared to control cells in the absence of β-galactosidase and drug solution.

at C-17-position. Glucose–GA conjugate showed anticancer activity through cleavage of glucose moiety by β-glucosidase inside of the cancer cells, which is confirmed by 3-fold inhibition with β-glucosidase inhibitor. Galactose–GA conjugates remained inactive in the absence of exogenous β-galactosidase. However, the anticancer activity of galactose–GA conjugate increased by 3- to 40-fold when incubated with β-galactosidase. These data support our hypothesis that carbohydrate conjugation of GA through the C-17-position produces inactive prodrugs. The inactive carbohydrate–GA prodrugs can be enzyme-specifically activated by β-glucosidase or β-galactosidase. In addition, the length of the spacer, between carbohydrate and GA in the conjugates, does not seem to play a significant role in the anticancer activity after enzyme cleavage.[1,2] These galactose–GA conjugates can be potentially used for antibody-directed enzyme prodrug therapy (ADEPT).

Experimental Section

The NMR data were recorded on a Varian-400 or -500 MHz spectrometer. MS spectra were obtained from a Kratos MS 80 spectrometer using electrospray ionization mode (ESI). The MTS assay was measured on a DYNEX MRX microplate reader. Silica gel F254 plates (Merck) and silica gel 60 (70–230 mesh) were used in analytical thin-layer chromatography (TLC) and column chromatography, respectively.

General Synthetic Procedure of α-Glycopyranosyl Trichloroacetimidate. Hydrazine acetate (0.89 g, 9.6 mmol) was added to a solution of 1,2,3,4,6-penta-*O*-acetylpyranose (8.1 mmol) in DMF (10 mL) at 60 °C under Ar. When TLC (1:1 hexanes–EtOAc) showed the formation of a new product and the disappearance of starting material, the mixture was diluted with EtOAc, washed with aqueous 5% NaCl (2×) and water, dried over Na_2SO_4, and precipitated with toluene to give the crude product. A solution of this crude product was then reacted with trichloroacetonitrile (10 equiv) and DBU (2 equiv) in CH_2Cl_2 (30 mL) for 60 min at 0 °C. When TLC (2:1 hexanes–EtOAc) showed the conversion to be complete, the solution was concentrated and purified by flash chromatography to yield the product.

2,3,4,6-Tetra-*O*-acetyl-α-galactopyranosyl trichloroacetimidate (4): ^{1}H NMR ($CDCl_3$): δ 8.67 (s, 1H), 6.61 (d, 1H, J = 3.4 Hz), 5.56, (dd, 1H, J = 3.0, 1.4 Hz), 5.43 (dd, 1H, J = 10.8, 3.0 Hz), 5.36 (dd, 1H, 10.8, 3.4 Hz), 4.44 (m, 1H), 4.17 (dd, 1H, J = 6.6, 11.3 Hz), 4.08 (dd, 1H, 6.7, 11.3 Hz),

2.17, 2.03, 2.02, 2.01 (4 s, each 3H). ^{13}C NMR ($CDCl_3$): δ 170.35−169.31 (4 *C*OCH$_3$), 160.82, 93.45, 68.85, 67.38, 67.32, 66.47, 61.10, 20.11−19.85 (4 CO*C*H$_3$). MS ES$^+$ *m/z*: 514.17 (M + Na). Yield: 79%.

2,3,4,6-Tetra-*O*-acetyl-α-glucopyranosyl trichloroacetimidate (5): ^{1}H NMR ($CDCl_3$) δ δ 8.70 (s, 1H), 6.57 (d, 1H, *J* = 3.6 Hz), 5.14 (dd, 1H, *J* = 3.7, 10.2 Hz), 2.08, 2.05, 2.04, 2.02 (4s, each 3H). ^{13}C NMR ($CDCl_3$): δ 169.95−169.02 (4 *C*OCH$_3$), 160.11, 92.55, 90.32, 69.65, 69.40, 69.31, 67.45, 61.08, 20.23−20.01 (4 CO*C*H$_3$). MS ES$^+$ *m/z* 514.13 (M + Na). Yield: 75%.

2,3,4,6-tetra-*O*-acetyl-α-lactopyranosyl trichloroacetimidate (6): ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1H), 6.48 (d, 1H, *J* = 3.6 Hz), 5.55 (t, 1H, *J* = 9.9 Hz), 5.35 (d, 1H, *J* = 3.0 Hz), 5.15−5.03 (m, 2H), 4.97−4.92 (dd, 1H, *J* = 3.6, 10.2 Hz), 4.52−4.46 (m, 2H), 4.18−4.05 (m, 4H), 3.89−3.83 (m, 2H), 2.15 (s, 3H), 2.10 (s, 3H), 2.06 (s, 6H), 2.04 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.31−169.28 (7 *C*OCH$_3$), 161.21, 101.47, 93.10, 76.15, 71.36, 71.13, 70.93, 70.18, 69.78, 69.33, 66.79, 61.72, 60.98, 21.11−20.74 (7 CO*C*H$_3$). MS ES$^+$ *m/z*: 802.19 (M + Na). Yield: 69%.

2-Azidoethanol (8). 2-Chloroethanol (25.2 mL, 375 mmol) was added to a solution of NaN_3 (30 g, 461 mmol) and NaOH (1.5 g, 37.5 mmol) in water (115 mL). The mixture was stirred at room temperature for 3 days, and sodium sulfate (35 g) was added. After 10 min, the mixture was extracted with CH_2Cl_2 (3 × 70 mL). The combined extracts were dried in Na_2SO_4 and concentrated. The residue was distilled to give 2-azidoethanol. ^{1}H NMR ($CDCl_3$): δ 3.79−3.76 (t, 2H, *J* = 5.1 Hz), 3.47−3.44 (t, 2H, *J* = 5.1 Hz), 2.02 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 61.76, 53.75. MS ES$^+$ *m/z*: 88.12 (M + H). Yield: 86%.

2-(2-Azidoethoxy)ethanol (17). NaN_3 (4.5 g, 69 mmol), tetrabutylammonium iodide (2.5 g, 6 mmol), and 18-crown-6 (10 mg) were added to a solution of 2-(2-chloroethoxy)ethanol (5 mL, 47 mmol) in 2-butanone (25 mL). The mixture was refluxed at 90 °C for 2 days. When ^{13}C NMR spectroscopy of the supernatant showed the absence of a signal at δ 42.7 and the presence of a strong signal at δ 50.9 ppm, the mixture was filtered. The precipitate was rinsed with acetone, and the combined solutions were concentrated. Distillation of the residue gave the pure product. ^{1}H NMR ($CDCl_3$): δ 3.74−3.71 (t, 2H, *J* = 4.5 Hz), 3.68−3.65 (t, 2H, *J* = 5.1 Hz), 3.59−3.56 (t, 2H, *J* = 4.2 Hz), 3.40−3.37 (t, 2H, *J* = 5.4 Hz), 2.41 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 72.65, 70.25, 61.94, 50.92. MS ES$^+$ *m/z*: 132.13 (M + H). Yield: 80%.

General Glycosylation Procedure of *O*-Acetylpyranosyl Trichloroacetimidate. The trichloroacetimidate (1.63 mmol) was dissolved in dry CH_2Cl_2 (10 mL) with glycosyl acceptor (the primary alcohols, 1.5 equiv) and 4 Å MS (100 mg). The mixture was stirred at room temperature for 1 h. After the mixtrue was cooled to −30 °C, TMSOTf (1 equiv) was added. The reaction was stirred at −30 °C for 1 h and then at room temperature overnight. The reaction was quenched with saturated $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified with chromatography (silica gel, hexane/EtOAc 7:3−1:1) to give the product.

2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-galactopyranoside (9): ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.40 (d, 1H, *J* = 3.5 Hz), 5.26−5.23 (dd, 1H, *J* = 8.0, 10.5 Hz), 5.04−5.01 (dd, 1H, *J* = 10.5, 3.5 Hz), 4.56 (d, 1H, *J* = 8.0 Hz), 4.21−4.11 (m, 2H), 4.07−4.03 (dt, 1H, *J* = 10.0, 4.5 Hz), 3.93−3.91 (t, 1H, *J* = 8.0 Hz), 3.72−3.67 (m, 1H), 3.54−3.49 (m, 1H), 3.32−3.28 (dt, 1H, *J* = 13.5, 3.5 Hz), 2.16, 2.07, 2.05, 1.99 (4 s, each 3H). ^{13}C NMR ($CDCl_3$): δ 170.41−169.68 (4 COCH$_3$), 101.38, 71.12, 71.03, 68.74, 68.65, 67.21, 61.47, 50.79, 21.03−20.83 (4 CO*C*H$_3$). MS ES$^+$ *m/z*: 440.23 (M + Na). Yield: 75%.

3-Cbz-amino-1-propyl-2,3,4,6-tetra-*O*-acetyl-β-galactopyranoside (18): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35−7.30 (m, 5H), 5.36 (d, 1H, *J* = 3.6 Hz), 5.21−5.15 (dd, 1H, *J* = 10.2, 7.8 Hz), 5.07 (br, 3H), 5.01−4.96 (dd, 1H, *J* = 10.5, 3.9 Hz), 4.44 (d, 1H, *J* = 8.1 Hz), 4.14−4.11 (dd, 2H, *J* = 6.6, 3.0 Hz), 3.95−3.85 (m, 2H), 3.66−3.53 (m, 1H), 3.36−3.18 (m, 2H), 2.11, 2.02, 2.01, 1.96 (4 s, each 3H), 1.80−1.75 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 170.65, 170.49, 170.36, 169.80, 156.67, 136.87, 128.72, 128.33, 128.29, 101.39, 71.05, 70.93, 68.97, 67.88, 67.24, 67.02, 66.78, 61.54, 38.45, 29.75, 20.94, 20.87, 80.80. MS ES$^+$ *m/z*: 562.16 (M + Na).

5-Cbz-amino-1-pentyl-2,3,4,6-tetra-*O*-acetyl-β-galactopyranoside (19): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.30 (m, 5H), 5.35 (d, 1H, *J* = 3.6 Hz), 5.19−5.13 (dd, 1H, *J* = 10.2, 7.8 Hz), 5.0 (s, 2H), 5.01−4.96 (dd, 1H, *J* = 10.5, 3.6 Hz), 4.90 (br, 1H), 4.42 (d, 1H, *J* = 7.8 Hz), 4.18−4.06 (m, 2H), 3.88−3.82 (m, 2H), 3.48−3.40 (m, 1H), 3.17−3.11 (m, 2H), 2.10, 2.01, 2.00, 1.95 (4 s, each 3H), 1.59−1.43 (m, 4H), 1.37−1.30 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 170.62, 170.51, 170.38, 169.66, 156.64, 136.87, 128.70, 128.28, 101.48, 71.12, 70.79, 70.06, 69.13, 67.28, 66.75, 62.73, 61.49, 41.08, 32.43, 29.78, 29.17, 23.23, 23.08, 20.94, 20.86, 20.79. MS ES$^+$ *m/z*: 590.20 (M + Na).

5-Azido-3-oxapentyl-2,3,4,6-tetra-*O*-acetyl-β-galactopyranoside (20): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.35 (d, 1H, *J* = 3.2 Hz), 5.20−5.16 (dd, 1H, *J* = 10.4, 8.0 Hz), 5.01−4.97 (dd, 1H, *J* = 10.4, 3.2 Hz), 4.55 (d, 1H, *J* = 8.0 Hz), 4.17−4.07 (m, 2H), 3.96−3.87 (m, 2H), 3.76−3.71 (m, 1H), 3.65−3.61 (m, 4H), 3.35−3.32 (m, 2H), 2.12, 2.04, 2.01, 1.95 (4 s, each 3H). ^{13}C NMR ($CDCl_3$): δ 170.60, 170.46, 170.34, 169.71, 101.54, 71.10, 70.88, 70.64, 70.41, 69.25, 69.02, 67.25, 61.48, 50.98, 20.98, 20.88, 20.80. MS ES$^+$ *m/z*: 484.06 (M + Na).

2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-glucopyranoside (10): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21−5.17 (t, 1H, *J* = 9.6 Hz), 5.10−5.05 (t, 1H, *J* = 9.6 Hz), 5.02−4.98 (dd, 1H, *J* = 9.2, 8.0 Hz), 4.58 (d, 1H, *J* = 7.2 Hz), 4.25−4.21 (dd, 1H, *J* = 13.2, 4.4 Hz), 4.15−4.12 (dd, 1H, *J* = 12.0, 2.4 Hz), 4.04−3.99 (m, 1H), 3.71−3.64 (m, 2H), 3.51−3.45 (m, 1H), 3.29−3.23 (m, 1H), 2.07, 2.03, 2.01, 1.98 (4 s, each 3H). ^{13}C NMR ($CDCl_3$) δ 170.88, 170.50, 169.62, 100.86, 72.97, 72.10, 71.22, 68.82, 68.46, 61.70, 50.70, 20.97, 20.92, 20.83. MS ES$^+$ *m/z*: 440.22 (M + Na). Yield: 73%.

2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-lactopyranoside (11): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.29 (d, 1H, *J* = 3.2 Hz), 5.15 (t, 1H, *J* = 9.2 Hz), 5.05 (t, 1H, *J* = 9.2 Hz), 4.94−4.84 (m, 2H), 4.52−4.44 (m, 2H), 4.09−4.03 (m, 4H), 3.96−3.90 (m, 1H), 3.85−3.75 (m, 2H), 3.65−3.59, (m, 2H), 3.45−3.40 (m, 1H), 3.24−3.20 (m, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.99 (s, 9H), 1.91 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.55, 170.35, 170.26, 169.96, 169.26, 100.28, 100.62, 76.35, 72.98, 72.88, 71.64, 71.14, 70.83, 69.25, 68.91, 66.77, 61.97, 60.97, 50.67, 21.06, 20.99, 20.92, 20.84, 20.72. MS ES$^+$ *m/z*: 728.08 (M + Na). Yield: 61%.

General Deprotection Procedure of *O*-Acetylpyranoside. The protected pyranoside (200 mg) was dissolved in dry MeOH (10 mL). NaOMe solution (1 M in MeOH) was added until pH 9 was reached. Then the reaction mixture was stirred at room temperature until the deprotection reaction was complete (TLC iso-PrOH−water, 7:3, +1% NH_3). Then the mixture was neutralized with ion-exchange resin (Amberylst), filtered, and dried in a vacuum. The resulted residue was then dissolved in ethanol (10 mL), and then palladium catalyst (10% Pd−C, 50 mg) and a few drops of acetic acid were added. The reaction mixture was hydrogenated at 50 psi for 20 h. Then it was filtered through a Celite bed and washed with MeOH. The filtrate was concentrated under low pressure to give final product.

2-Aminoethyl-β-galactopyranoside acetate (12): ^{1}H NMR (500 MHz, CD_3OD): δ 4.30 (d, 1H, *J* = 7.5 Hz), 4.06−4.02 (m, 1H), 3.92−3.88 (m, 1H), 3.84 (d, 1H, *J* = 2.5 Hz), 3.78−3.70 (m, 2H), 3.60−3.50 (m, 3H), 3.18−3.15 (m, 1H), 1.93 (s, 3H). ^{13}C NMR (CD_3OD): δ 177.63, 103.66, 75.77, 73.55, 71.30, 69.09, 65.76, 61.37, 39.87, 21.99. MS ES$^+$ *m/z*: 246.35 (M + Na).

3-Aminopropyl-β-galactopyranoside acetate (21): ^{1}H NMR (400 MHz, CD_3OD): δ 4.27 (d, 1H, *J* = 7.2 Hz), 4.05−3.99 (m, 1H), 3.83 (d, 1H, *J* = 1.6 Hz), 3.80−3.73 (m, 3H), 3.56−3.46 (m, 3H), 3.14−3.08 (m, 2H), 1.97−1.95 (m, 2H), 1.93 (s, 3H). ^{13}C NMR (CD_3OD): δ 103.83, 75.68, 73.71, 71.25, 69.12, 67.61, 61.33, 38.03, 27.18, 21.66. MS ES$^+$ *m/z*: 260.08 (M + Na).

5-Aminopentyl-β-galactopyranoside acetate (22): ^{1}H NMR (500 MHz, CD_3OD): δ 4.22 (d, 1H, J = 7.2 Hz), 3.94−3.89 (m, 1H), 3.84 (d, 1H, J = 2.4 Hz), 3.72 (d, 2H, J = 5.6 Hz), 3.61−3.55 (m, 1H), 3.52−3.46 (m, 3H), 2.94−2.90 (m, 2H), 1.93 (s, 3H), 1.73−1.63 (m, 4H), 1.59−1.42 (m, 2H). ^{13}C NMR (CD_3OD): δ 103.79, 75.43, 73.83, 71.42, 69.09, 69.04, 61.27, 39.41, 28.86, 27.20, 27.01, 22.81, 22.70, 21.71. MS ES$^+$ *m/z*: 266.10 (M + H).

5-Amino-3-oxapentyl-β-galactopyranoside acetate (23): ^{1}H NMR (500 MHz, CD_3OD): δ 4.30−4.28 (d, 1H, J = 7.5 Hz), 4.05−4.01 (m, 1H), 3.84−3.83 (dd, 1H, J = 3.0, 1.0 Hz), 3.80−3.76 (m, 1H), 3.75−3.71 (m, 6H), 3.54−3.47 (m, 3H), 3.13−3.11 (m, 2H), 1.95 (s, 3H). ^{13}C NMR (CD_3OD): δ 176.37, 103.82, 75.61, 73.81, 71.36, 69.97, 69.14, 68.90, 66.63, 61.35, 39.37, 21.13. MS ES$^+$ *m/z*: 268.19 (M + H).

2-Aminoethyl-β-glucopyranoside acetate (13): ^{1}H NMR (400 MHz, CD_3OD): δ 4.34 (d, 1H, J = 8.0 Hz), 4.08−4.03 (dt, 1H, J = 12.0, 4.8 Hz), 3.90−3.84 (m, 2H), 3.69−3.65 (dd, 1H, J = 12.0, 5.6 Hz), 3.41−3.22 (m, 4H), 3.17−3.15 (t, 1H, J = 6.0 Hz), 1.94 (s, 3H). ^{13}C NMR (CD_3OD): δ 176.63, 103.08, 76.91, 76.59, 73.74, 70.27, 65.86, 61.33, 39.73, 21.60. MS ES$^+$ *m/z*: 246.05 (M + Na). Yield: 95%.

2-Aminoethyl-β-lactopyranoside acetate (14): ^{1}H NMR (300 MHz, CD_3OD): δ 4.39−4.37 (d, 1H, J = 8.4 Hz), 4.36−4.35 (d, 1H, J = 7.2 Hz), 4.07−4.04 (m, 1H), 3.94−3.68 (m, 6H), 3.62−3.46 (m, 7H), 3.34−3.30 (m, 1H), 3.18−3.16 (t, 1H, J = 4.8 Hz), 1.96 (s, 3H). ^{13}C NMR (CD_3OD): δ 175.62, 103.92, 102.78, 79.16, 75.93, 75.40, 75.02, 73.59, 73.42, 71.33, 69.09, 65.76, 61.34, 60.45, 39.70, 20.65. MS ES$^+$ *m/z*: 408.01 (M + Na). Yield: 95%.

General Procedure for the Synthesis of Sugar−GA Conjugate through C-17 Linkage of Geldanamycin. GA (30 mg, 0.54 mmol), sugar-amine derivative (4 equiv), and 100 μL of Et_3N were dissolved in 0.5 mL of dry DMF. The mixture was stirred at room temperature in dark for 24 h. Then the mixture was directly subjected to flash chromatograph (silica gel, hexane/EtOAc system, then EtOAc/MeOH 9:1) to give pure carbohydrate−GA conjugate.

17-Demethoxy-17-[(2-β-glucopyranosylethyl)amino]-geldanamycin (24): ^{1}H NMR (400 MHz, CD_3OD): δ 7.13 (d, 1H, J = 12.4 Hz), 7.05 (s, 1H), 6.62, (t, 1H, J = 12.0 Hz), 5.87 (t, 1H, J = 8.8 Hz), 5.58 (d, 1H, J = 9.6 Hz), 5.20 (s, 1H), 4.53 (d, 1H, J = 8.4 Hz), 4.34 (d, 1H, J = 8.0 Hz), 4.07−4.01 (m, 1H), 3.92−3.89 (m, 2H), 3.83−3.78 (m, 1H), 3.76−3.70 (m, 2H), 3.60−3.55 (m, 1H), 3.46 (br, 1H), 3.34−3.29 (m, 9H), 3.24−3.19 (t, 1H, J = 8.0 Hz), 2.75−2.68 (m, 2H), 2.37−2.32 (dd, 1H, J = 14.4, 8.8 Hz), 1.99−1.98 (s, 3H), 1.79 (br, 1H), 1.73 (s, 3H), 1.65 (br, 1H), 1.60−1.56 (m, 1H), 0.99−0.96 (t, 6H, J = 7.2 Hz). HRMS (M + Na$^+$) (ESI$^+$) calcd for $C_{36}H_{53}N_3O_{14}Na^+$ 774.3420, found 774.3434. Yield: 83%. Purity > 99%.

17-Demethoxy-17-[(2-β-galactopyranosylethyl)amino]-geldanamycin (25): ^{1}H NMR (500 MHz, CD_3OD) δ 7.12 (d, 1H, J = 11.5 Hz), 7.04 (s, 1H), 6.62, (t, 1H, J = 12.0 Hz), 5.86 (t, 1H, J = 9.0 Hz), 5.58 (d, 1H, J = 10.0 Hz), 5.20 (s, 1H), 4.53 (d, 1H, J = 8.5 Hz), 4.30 (d, 1H, J = 8.0 Hz), 4.06−4.02 (m, 1H), 3.91−3.87 (m, 1H), 3.84−3.79 (m, 3H), 3.76−3.69 (m, 2H), 3.60−3.55 (m, 3H), 3.50−3.44 (m, 2H), 3.34 (s, 3H), 3.29 (s, 3H), 2.74−2.68 (m, 2H), 2.36−2.31 (dd, 1H, J = 14.5, 9.0 Hz), 1.99 (s, 3H), 1.79 (bs, 1H), 1.73 (s, 3H), 1.68−1.65 (br, 1H), 1.60−1.56 (m, 1H), 0.99−0.96 (t, 6H, J = 8.5 Hz). HRMS (M + Na$^+$) (ESI$^+$) calcd for $C_{36}H_{53}N_3O_{14}Na^+$ 774.3420, found 774.3425. Yield: 85%. Purity > 99%.

17-Demethoxy-17-[(2-β-galactopyranosylpropyl)amino]-geldanamycin (26): ^{1}H NMR (500 MHz, CD_3OD) δ 7.13 (d, 1H, J = 12.0 Hz), 7.05 (s, 1H), 6.65−6.60 (t, 1H, J = 12.0 Hz), 5.89−5.85 (t, 1H, J = 11.0 Hz), 5.58 (d, 1H, J = 10.0 Hz), 5.22 (s, 1H), 4.54 (d, 1H, J = 8.0 Hz), 4.23 (d, 1H, J = 7.5 Hz), 4.03−3.98 (m, 1H), 3.84−3.83 (d, 1H, J = 3.5 Hz), 3.78−3.67 (m, 5H), 3.60−3.56 (m, 2H), 3.53−3.45 (m, 3H), 3.34 (s, 3H), 3.29 (s, 3H), 2.77−2.68 (m, 2H), 2.39−2.35 (dd, 1H, J = 14.0, 9.0 Hz), 1.99 (s, 3H), 1.96−1.93, (m, 2H), 1.79 (br, 1H), 1.73 (s, 3H), 1.70−1.68 (br, 1H), 1.67 (br, 1H), 1.00−0.97 (t, 6H, J = 6.5 Hz). HRMS (M + Na$^+$) (ESI$^+$) calcd for $C_{37}H_{55}N_3O_{14}Na^+$ 788.3576, found 788.3564. Yield: 90%. Purity > 99%.

17-Demethoxy-17-[(2-β-galactopyranosylpentyl)amino]-geldanamycin (27): ^{1}H NMR (500 MHz, CD_3OD) δ 7.13 (d, 1H, J = 11.5 Hz), 7.05 (s, 1H), 6.64−6.60 (t, 1H, J = 11.5 Hz), 5.89−5.85 (t, 1H, J = 11.0 Hz), 5.58 (d, 1H, J = 9.5 Hz), 5.21 (s, 1H), 4.54 (d, 1H, J = 8.5 Hz), 4.21 (d, 1H, J = 7.5 Hz), 3.93−3.90 (m, 1H), 3.82 (d, 1H, 3.5 Hz), 3.74−3.71 (m, 2H), 3.60−3.44 (m, 8H), 3.34 (s, 3H), 3.29 (s, 3H), 2.75−2.73 (m, 2H), 2.35−2.30 (dd, 1H, J = 14.0, 9.0 Hz), 1.99 (s, 3H), 1.81 (bs, 1H), 1.73−1.49 (m, 11H), 0.99−0.96 (m, 6H). HRMS (M + Na$^+$) (ESI$^+$) calcd for $C_{39}H_{59}N_3O_{14}Na^+$ 816.3890, found 816.3926. Yield: 88%. Purity > 99%.

17-Demethoxy-17-{[2-(2-β-galactopyranosylethyl)ethyl]-amino}geldanamycin (28): ^{1}H NMR (500 MHz, CD_3OD) δ 7.14−7.12 (d, 1H, J = 11.5 Hz), 7.06 (s, 1H), 6.64−6.60, (t, 1H, J = 12.0 Hz), 5.87−5.85 (t, 1H, J = 9.0 Hz), 5.60−5.58 (d, 1H, J = 10.0 Hz), 5.20 (s, 1H), 4.54−4.53 (d, 1H, J = 9.0 Hz), 4.29−4.28 (d, 1H, J = 7.5 Hz), 4.05−4.01 (m, 1H), 3.83−3.82 (m, 1H), 3.78−3.70 (m, 9H), 3.61−3.28 (t, 1H, J = 5.5 Hz), 3.56−3.45 (m, 4H), 3.34 (s, 3H), 3.29 (s, 3H), 2.75−2.69 (m, 2H), 2.36−2.32 (dd, 1H, J = 14.5, 9.5 Hz), 1.99 (s, 3H), 1.81 (bs, 1H), 1.73 (s, 3H), 1.68−1.65 (br, 1H), 1.60−1.56 (m, 1H), 1.00−0.98 (d, 3H, J = 6.5 Hz), 0.98−0.96 (t, 3H, J = 7.0 Hz). HRMS (M + Na$^+$) (ESI$^+$) calcd for $C_{38}H_{57}N_3O_{15}Na^+$ 818.3682, found 818.3677. Yield: 78%. Purity > 99%.

17-Demethoxy-17-[(2-β-lactopyranosylethyl)amino]-geldanamycin (29): ^{1}H NMR (500 MHz, CD_3OD): δ 7.12 (d, 1H, J = 11.5 Hz), 7.05 (s, 1H), 6.64−6.60 (t, 1H, J = 12.0 Hz), 5.89−5.85 (t, 1H, J = 9.0 Hz), 5.58 (d, 1H, J = 10.0 Hz), 5.21 (s, 1H), 4.53 (d, 1H, J = 8.0 Hz), 4.39−4.37 (m, 3H), 4.04−3.99 (m, 1H), 3.94−3.87 (m, 3H), 3.83−3.73 (m, 3H), 3.71−3.66 (m, 2H), 3.61−3.52 (m, 4H), 3.50−3.44 (m, 4H), 3.34 (s, 3H), 3.29 (s, 3H), 2.74−2.68 (m, 2H), 2.36−2.32 (dd, 1H, J = 14.0, 8.5 Hz), 1.99 (s, 3H), 1.80 (br, 1H), 1.73 (s, 3H), 1.68−1.65 (br, 1H), 1.60−1.56 (m, 1H), 0.99−0.96 (t, 6H, J = 8.5 Hz). HRMS (M + Na$^+$) (ESI$^+$) calcd for $C_{42}H_{63}N_3O_{19}Na^+$ 936.3948, found 936.3977. Yield: 70%. Purity > 99%.

HPLC Determination of Purity of Synthesized Compound 24−29. The purity of synthesized compounds was determined with HPLC by applying the sample to a prepacked BDS hypersil column (C18 5μm, 100 × 4.6 mm, Keystone Scientific, Inc.), using a Shimadzu LC-10AD HPLC system equipped with a SPD-M10A Diode Array Detector. The sample was prepared by adding 1 μL of DMSO stock solution of synthesized conjugates to 1 mL of acetonitrile/H_2O (5/95). Fifty microliter sample was subjected to HPLC system. The chromatographic analyses were performed at room temperature while the samples were kept in the autosampler at 4 °C. Mobile phase A is 5%ACN in water while mobile phase B is 5% water in ACN. The mobile phase was run in a gradient fashion in which phase A was decreased from 100% to 0% while phase B was increased from 0% to 100% at a flow rate of 0.5 mL/min. The carbohydrate−GA conjugates were detected from 250 to 600 nm with maximum absorbance at 330 nm.

Cell Culture. Cell lines SW620, HT29, MCF7, and K562 were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acid, and penicillin (100 μg/mL)/streptomycin (100 μg/mL) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The culture medium was changed every 2−3 days.

Anticancer Activity of Compound (24−29) (MTS assay). Cells (2000−10 000) were seeded in 96-well plates in RPMI-1640 and incubated for 24 h. The exponentially growing cancer cells were incubated with various concentrations of compounds for 72 h at 37 °C (5% CO_2, 95% humidity). After 72 h incubation, tetrazolium [3-(4,5-dimethythiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, 2 mg/ml) and phenazine methosulfate (PMS, 25 μM) were mixed and added directly to the cells. After incubated for 3 h at 37 °C, the absorbance of formazan (the metabolite of MTS by viable cells) was measured at 490 nm. The IC_{50} values of the carbohydrate−drug conjugates for antiproliferation were calculated by the dose−response curves of percentage of cell growth vs control (no compound added).

β-Glucosidase Inhibition Assay for Compound 24, 25. K562 Cells (2000cells/80 μL RPMI-1640) were seeded in 96-well plates and incubated for 24 h. The exponentially growing cancer cells were incubated in three different conditions (cells with different concentration of test compounds and DMDP, cells with different concentration of test compounds only, and cells with DMDP only) for 72 h at 37 °C (5% CO_2, 100% humidity). The control cells were exposed to fresh medium only. Then, the anticancer activity of compound **24**, **25** was measured with MTS assay as described above. The percentages of cell growth for tested compounds vs control cells were calculated by the absorbance at 490 nm.

β-Galactosidase Cleavage Assay. Cells (2000−10 000) were seeded in 96-well plates in RPMI-1640 and incubated for 24 h. The exponentially growing cancer cells were incubated with 2 units of β-galactosidase for 10 min. The same concentration solutions of various compounds (**25**−**29**) were added to each well. The control cells were exposed to fresh medium only or to β-galactosidase alone. After incubated for 72 h at 37 °C (5% CO_2, 100% humidity), the cell growth was measured with MTS assay as described above. The percentages of cell growth of tested compounds vs control were calculated by the absorbance at 490 nm.

Supporting Information Available: Additional experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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