Design and Synthesis of Water-Soluble Glucuronide Derivatives of Camptothecin for Cancer Prodrug Monotherapy and Antibody-Directed Enzyme Prodrug Therapy (ADEPT)†

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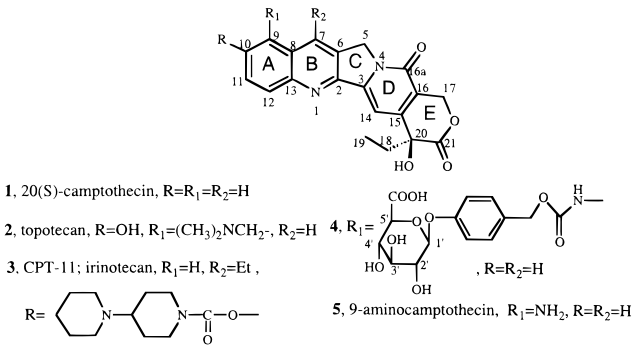
Glucuronide prodrugs of 9-aminocamptothecin were synthesized. Prodrug **4**, in which 9-aminocamptothecin was connected to glucuronic acid by an aromatic spacer via a carbamate linkage, was stable in both aqueous solution and human plasma. Prodrug **4** and its potassium salt **12** were 20−80-fold less toxic than 9-aminocamptothecin to human tumor cell lines. The simultaneous addition of β-glucuronidase and **4** or **12** to tumor cells resulted in a cytotoxic effect equal to that of 9-aminocamptothecin alone. Prodrugs **4** and **12** were over 80 and 4000 times more soluble than 9-aminocamptothecin in aqueous solutions at pH 4.0, respectively. Compounds **4** and **12** may be useful for prodrug monotherapy of tumors that accumulate extracellular lysosomal β-glucuronidase as well as for antibody-directed enzyme prodrug therapy (ADEPT) of cancer.

Introduction

20(*S*)-Camptothecin (**1**), an antitumor alkaloid first isolated from *Camptotheca acuminata* (Nyssaceae) by Wall and co-workers in 1966,[1] inhibits the activity of topoisomerase I and displays antitumor activity in various experimental tumor models.[2] Camptothecin, however, is difficult to formulate due to its poor water solubility. Several research teams have synthesized camptothecin derivatives aimed at preserving the antitumor properties of the parent compound while improving its safety and water solubility.[3−7] Two water soluble derivatives, topotecan (**2**) and irinotecan (CPT-11, **3**), are approved for clinical use.[8,9] We have employed an alternate strategy to improve the water solubility of camptothecin and increase its tumor cell selectivity based on the enzyme activation of prodrugs at tumor cells as pioneered by Senter[10,11] and Bagshawe.[12,13] In this approach, monoclonal antibodies are employed to target an enzyme to cancer cells which can activate subsequently administered prodrug. Selective activation of prodrugs at neoplastic cells can increase the concentration of active drug in tumors,[14,15] reduce systemic toxicity,[16] and allow bystander killing of antigen-negative cancer cells.[17,18] Prodrugs can also be designed to display increased water solubility for improved drug formulation.[19,20]

The design of a camptothecin prodrug suitable for targeted enzymatic activation at tumor cells was based upon four criteria: (1) improved water solubility, (2) stability in blood, (3) decreased cytotoxicity, and (4) susceptibility to defined enzymatic cleavage. Glucuronide prodrug **4** was selected for synthesis because of the hydrophilicity of this functional group,[21,22] the ability of glucuronide prodrugs to be preferentially activated at tumor cells targeted with β-glucuronidase-antibody conjugates,[21,23−28] and the strong antitumor activity observed after combined treatment with glucuronide prodrugs and β-glucuronidase-antibody conjugates[16,29] or fusion proteins[28] in animal models. In addition, glucuronide prodrugs may be useful for cancer prodrug monotherapy of tumors that overexpress[30] or accumulate lysosomal β-glucuronidase.[31]



Chemistry

Compound **4** in which 9-aminocamptothecin (**5**) and glucuronidic acid are linked via a self-immolative carbamate spacer[21,32] was selected as the target glucuronide prodrug because of preliminary results showing poor yield of prodrug in which glucuronic acid is directly linked to 10-hydroxycamptothecin and the superior enzymatic hydrolysis of prodrugs containing spacers.[21,32] 9-Aminocamptothecin (**5**) was prepared by published procedures.[33] 10-Hydroxycamptothecin was nitrated,

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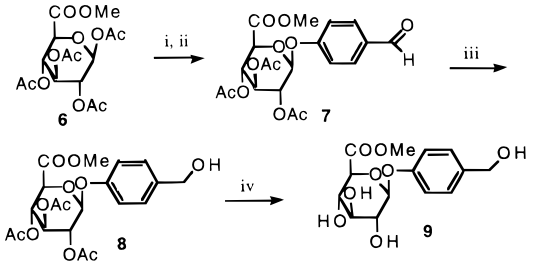
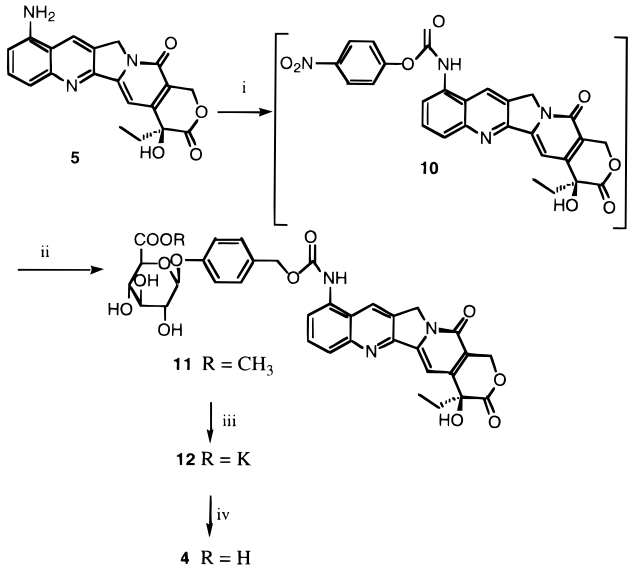
Scheme 1[a]



[a] Reagents and conditions: (i) $TiBr_4$, CH_2Cl_2, rt, 4 h; (ii) *p*-hydroxybenzaldehyde, Ag_2O, CH_3CN, rt, 2 h, 42%; (iii) $NaBH_4$, IPA, $CHCl_3$, silica gel, 0 °C, 1 h, 90%; (iv) CH_3ONa, MeOH, rt, 2 h, 60.2%.

Scheme 2[a]



[a] Reagents and conditions: (i) 4-nitrophenyl chloroformate, 1,4-dioxane, 60 °C, 1 h; (ii) **9**, DMAP, CH_3CN, 80 °C, 2 h, 32.3%; (iii) $KOSiMe_3$, THF, rt, 2 h, 62%; (iv) 1 N HCl, rt, 2 h, 50%.

and the resulting 9-nitro-10-hydroxycamptothecin was treated with tosyl chloride followed by palladium-catalyzed reduction to afford 9-aminocamptothecin (**5**) in an overall yield of 80%. Methyl 1,2,3,4-tetra-*O*-acetyl *β*-D-glucuronate (**6**), prepared as described,[34] was brominated with $TiBr_4$ and then reacted with *p*-hydroxybenzaldehyde in the presence of Ag_2O to afford **7** (Scheme 1). The aldehyde group in **7** was hydrogenated to benzyl alcohol by treatment with $NaBH_4$ to yield **8** in 90% yield. The *O*-acetyl groups of **8** were deprotected with CH_3ONa in MeOH to yield **9**. 9-Aminocamptothecin (**5**) was reacted with nitrophenyl chloroformate before addition of **9** for 1 day at 80 °C to afford **11** in an overall yield of 32.2% (Scheme 2). The methyl ester **11** was deprotected with 1 equiv of potassium trimethylsilanate in THF and purified by reverse phase MPLC to obtain the potassium salt **12**. Acidification with 1 N HCl yielded target compound **4**.

Biological Data

9-Aminocamptothecin (**5**) was poorly soluble in aqueous solution at both acid and neutral pH (0.006 mg/mL at pH 4.0 and 0.06 mg/mL at pH 7.0). In contrast, **4** was 80 times more soluble at pH 4.0 and 1800 times more soluble at pH 7.0 (Table 1). The potassium salt **12** displayed excellent solubility even at pH 4.0. Incubation of prodrug **4** or the potassium salt **12** at 37 °C in PBS (phosphate-buffered saline, pH 7.0) or 90% human serum in PBS revealed that the prodrugs were stable for at least 48 h (results not shown).

Table 1. Aqueous Solubility of Drugs[a]

drug	solubility (mg/mL) pH 4.0	pH 7.0
5	0.006	0.06
4	0.5	110
12	25	ND[b]

[a] The solubility of 9-aminocamptothecin (**5**), prodrug **4,** and its potassium salt **12** were determined at pH 4.0 and 7.0. [b] Not determined.

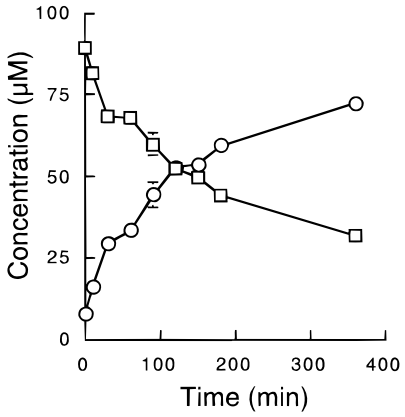


Figure 1. *β*-Glucuronidase hydrolysis of **4**. Prodrug **4** was incubated at pH 7.0 with 0.05 *μ*g/mL *β*-glucuronidase at 37 °C. The concentrations of **4** (□) and 9-aminocamptothecin (**5**) (○) in aliquots removed at the indicated times were determined by HPLC. The mean values of triplicate determinations are shown. Bars, SE.

Table 2. Hydrolysis of **4** by *β*-Glucuronidase[a]

β-glucuronidase (*μ*g/mL)	% conversion[b]
0.5	92
0.1	98
0.05	92
0.01	45

[a] Compound **4** (25 *μ*M) was incubated with the indicated concentrations of *β*-glucuronidase at pH 7.0 for 2 h at 37 °C. [b] The percentage of **4** converted to 9-aminocamptothecin (**5**).

Prodrug **4** (100 *μ*M) was readily cleaved by 0.05 *μ*g/mL *β*-glucuronidase (0.18 nM) at pH 7.0 (Figure 1). Near quantitative conversion of 25 *μ*M **4** to **5** was acheived in 2 h by addition of 0.05 *μ*g/mL or higher concentrations of *β*-glucuronidase (Table 2).

The cytotoxicity of **1**, **4**, **5**, and **12** to five human tumor cell lines was determined by measuring [^{3}H]thymine incorporation into cellular DNA after 72 h of drug exposure. Comparison of IC_{50} values revealed that prodrug **4** and its potassium salt **12** were 20−80-fold less toxic than **5** (Table 3). Simultaneous addition of *β*-glucuronidase (5 *μ*g/mL) and **4** or **12** to tumor cells resulted in a cytotoxic effect similar to **5** alone (Figure 2, Table 3), indicating efficient enzymatic cleavage of the glucuronide functional group and release of 9-aminocamptothecin (**5**).

Discussion

We attempted to synthesize a glucuronide prodrug of 9-aminocamptothecin. Prodrug **4**, in which glucuronic

Table 3. Cytotoxicity of Drugs to Human Tumor Cells

drug	IC_{50} (nM)[a]				
	Colo 205	CaSki	HepG2	HT29	H928
5	6.6	8.5	3.5	7.4	7.9
1	5.4	8.5	6.3	ND	7.8
4	200	190	110	150	300
4 + βG[b]	8.0	7.8	2.9	6.6	7.9
12	460	510	280	560	ND
12 + βG[b]	8.2	9.1	6.7	11	ND

[a] Concentrations of drugs that inhibited incorporation of [^{3}H]-thymidine into cellular DNA of human colorectal (Colo 205), cervical (CaSki), hepatocellular (HepG2), colorectal (HT29), or lung (H928) carcinoma cells by 50% after 72 h are indicated. Values represent means of 1−3 experiments performed in triplicate with coefficients of variation of <10%. [b] β-Glucuronidase (βG, 5 μg/mL) was added with drugs.

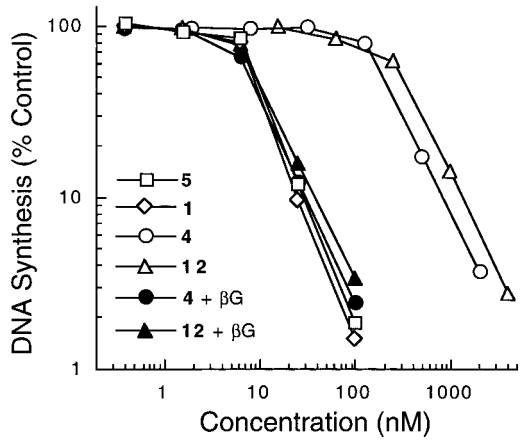


Figure 2. Cytotoxicity of drugs to CaSki human cervical carcinoma cells. CaSki cells were exposed to drugs with or without β-glucuronidase (βG) for 72 h before the incorporation of [^{3}H]thymidine into cellular DNA was measured. Results represent mean values of triplicate determinations. Bars, SE.

acid was coupled to 9-aminocamptothecin (**5**) via a self-immolative carbamate linker, as well as the potassium glucuronide salt **12**, displayed properties suitable for cancer prodrug monotherapy and ADEPT including resistance to nonspecific cleavage, differential toxicity between parent drug and prodrug, and susceptibility to β-glucuronidase hydrolysis. The prolonged stability of **4** in human serum indicates that the carbamate linker is resistant to enzymatic cleavage and confirms that β-glucuronidase activity is low in human serum at physiological pH values.[35] Prodrugs **4** and **12** displayed reduced toxicity compared with **5** to a variety of human tumor cells as found for other glucuronide prodrugs.[21,23,26,36−38] Reduced systemic toxicity of **4** or **12** may allow administration of curative doses of 9-aminocamptothecin (**5**), currently difficult due to the high sensitivity of human myeloid progenitor cells to camptothecin and its derivatives.[39]

The potassium salt **12** exhibited high aqueous solubility (25 mg/mL) at pH 4.0. Formulation of camptothecins at acidic pH is important to prevent opening of the lactone ring and formation of the inactive carboxylate form of camptothecin.[40] The solubitiy of **12** compares favorably with topotecan (1.02 mg/mL at pH 5).[3,6] The high solubility of the glucuronic acid prodrug **4** at pH 7.0 (109 mg/mL) indicates that the prodrug will not precipitate after intravenous administration.

9-Aminocamptothecin (**5**) has displayed promising activity in preclinical models and initial clinical trials.[41−43] In contrast to topotecin, 9-aminocamptothecin (**5**) is not a substrate of the *p*-glycoprotein transporter involved in multidrug resistance.[44] Conversion of the water-soluble glucuronides **4** or **12** to 9-aminocamptothecin (**5**) at tumor cells may thus possess advantages for the treatment of tumors that overexpress *p*-glycoprotein^{MDR}.

In summary, glucuronide prodrugs of 9-aminocamptothecin (**5**) have been synthesized and shown to possess the necessary prerequisites to be considered as candidates for cancer prodrug monotherapy and antibody-directed enzyme prodrug therapy of cancer.

Experimental Section

Chemistry. Melting points were obtained on an Electrothermal apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded either on a JEOL JNM-EX400 spectrometer at the National Taiwan University or on a Bruker model AM 300 spectrometer at the National Defense Medical Center, Taipei, and are reported in parts per million on a δ scale with DMSO-d_6 as internal standard. Chemical shifts are reported as δ values in parts per million. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, dd = doublet doublet, dt = doublet triplet, t = triplet, br = broad, m = multiplet. EI mass spectra were recorded on a JEOL JMS-D100 mass spectrometer at the National Taiwan University. Elemental analysis for C, H, and N were carried out on a Heraeus elemental analyzer at the Cheng-Kong University, Tainan, or a Perkin-Elmer 240 elemental analyzer at the National Taiwan University, Taipei, and were within 0.4% of the theoretical values. The thin-layer chromatographic analyses were performed using precoated silica gel (60F254) plates, and the spots were examined with UV light and phosphomolybdic acid spray. Column chromatography was carried out on Merck silica gel (70−230 mesh).

9-Aminocamptothecin (5).[33] A solution of 9-nitro-10-tosylcamptothecin (1.45 g, 2.57 mmol) in dioxane (80 mL) was mixed with $Pd(OAc)_2$ (144 mg, 0.64 mmol) and $P(Ph)_3$ (674 mg, 2.57 mmol) at room temperature under an argon atmosphere. The temperature was raised to 90 °C, and 1 M triethylammonium formate in dioxane (30 mL) was added dropwise over a period of 1.5 h. The mixture was cooled to room temperature, diluted with $CHCl_3$, and washed with water. After the usual work up, the crude product was purified by column chromatography on silica gel (MeOH−$CHCl_3$, 5:95) to give compound **5** (884 mg, 94.5%): EI-MS *m*/*z* 363 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (t, 3H, J = 7.1 Hz, CH_3), 1.85 (m, 2H, CH_2), 5.28 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 6.13 (s, 2H, NH_2), 6.52 (s, 1H, OH), 6.81 (d, 1H, J = 9.0 Hz, Ar*H*), 7.30 (s, 1H, ArH), 7.34 (d, 1H, J = 8.4 Hz, ArH), 7.53 (m, 1H, ArH), 8.85 (s, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.2, 30.7, 50.6, 65.6, 72.8, 96.8, 108.9, 116.6, 117.9, 119.0, 126.9, 127.5, 131.6, 146.1, 149.6, 150.4, 152.2, 157.3, 163.3, 172.9. Anal. ($C_{20}H_{17}N_3O_4$) C, H, N.

Methyl 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranuronate (6). Glucuronic acid γ-lactone (88 g, 0.5 M) was added to MeOH (500 mL) that contained CH_3ONa (0.75 g). The mixture was stirred at room temperature for 60 min before MeOH was removed under reduced pressure. The syrup was dissolved in acetic anhydride (340 mL), and $HClO_4$ (1.5 mL) in acetic anhydride (10 mL) was added dropwise such that the reaction temperature never exceeded 40 °C. The mixture was stirred 24 h at room temperature and the solution stored overnight at 4 °C to yield 64.7 g (34.4%) of crystalline material. The mother liquor was poured onto 1 kg of crushed ice and neutralized with $NaHCO_3$. Excess $NaHCO_3$ was removed by filtration, and the filtate was extracted with $CHCl_3$. The $CHCl_3$ extract was dried with anhydrous Na_2SO_4 and concentrated to a syrup. Upon storage at 4 °C an additional 52 g of crude crystalline material was obtained, resulting in compound **6** in an overall yield of 62%. The crystals were recrystallized once from hot MeOH: mp 140 °C [lit.[34] 137−138 °C]; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.97 (s, 3H, CH_3), 2.00−2.01 (m, 6H, CH_3),

2.08 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 4.68 (d, 1H, $J = 9.8$ Hz, sugar-H), 4.94−5.04 (m, 2H, sugar-H), 5.52 (t, 1H, $J = 9.5$ Hz, sugar-H), 6.02 (d, 1H, $J = 8.1$ Hz, sugar-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.5, 20.6, 20.7, 20.8, 53.0, 69.1, 70.1, 71.2, 71.7, 90.9, 167.3, 169.1, 169.4, 169.7, 169.8. Anal. ($C_{15}H_{20}O_{11}$) C, H, N.

Methyl 1-(4-Formylphenyl)-2,3,4-tri-*O*-acetyl-*β*-D-glucopyranuronate (7). A solution of compound **6** (1 g, 2.66 mmol) and $TiBr_4$ (1.17 g, 3.19 mmol) in CH_2Cl_2 (25 mL) was stirred at room temperature for 24 h. The mixture was washed with ice−water and $NaHCO_3$ solution, dried with Na_2SO_4, and evaporated to dryness to give 0.82 g of solid. The solid was dissolved in CH_3CN (100 mL) before adding *p*-hydroxybenzaldehyde (275 mg, 2.55 mmol) and Ag_2O (712 mg, 3.07 mmol) at room temperature for 2 h. The material was separated by column chromatography on silica gel (EtOAc−hexane, 2:3) to give compound **7** (528 mg, 42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.02 (s, 9H, CH_3), 3.63, (s, 3H, OCH_3), 4.76 (d, 1H, $J =$ 9.9 Hz, sugar-H), 5.05−5.18 (m, 2H, sugar-H), 5.48 (t, 1H, J = 9.6 Hz, sugar-H), 5.85 (d, 1H, $J = 7.7$ Hz, sugar-H), 7.19 (d, 2H, $J = 7.4$ Hz, ArH), 7.91 (d, 2H, $J = 7.4$ Hz, ArH), 9.91 (s, 1H, COH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6, 20.7, 20.8, 53.0, 69.2, 70.7, 71.3, 71.4, 96.5, 116.8, 131.9, 132.2, 161.0, 167.3, 169.4, 169.7, 169.9, 191.9. Anal. ($C_{20}H_{22}O_{11}$) C, H, N.

Methyl 1-(4-Hydroxymethylphenyl)-2,3,4-tri-*O*-acetyl-*β*-D-glucopyronuronate (8). A solution of compound **7** (528 mg, 1.2 mmol) in IPA/$CHCl_3$ (1:5) (100 mL) was stirred with $NaBH_4$ (123 mg, 2.76 mmol) and silica gel (5 g) at 0 °C for 1 h. The reaction was quenched with water and filtered to remove silica gel. The organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure to give a residue which was washed with EtOH to produce compound **8** (475 mg, 90%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.99−2.02 (m, 9H, CH_3), 3.63 (s, 3H, OCH_3), 4.43 (d, 2H, $J = 5.7$ Hz, CH_2), 4.69 (d, 1H, $J = 10$ Hz, sugar-H), 5.02−5.16 (m, 3H, sugar-H & OH), 5.47 (t, 1H, $J = 9.6$ Hz, sugar-H), 5.62 (d, 1H, $J = 8.0$ Hz, sugar-H), 6.94 (d, 2H, $J = 8.3$ Hz, ArH), 7.26 (d, 2H, $J =$ 8.5 Hz, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6, 20.7, 20.8, 53.0, 69.4, 70.9, 71.3, 71.4, 97.6, 106.6, 116.5, 128.3, 137.0, 155.5, 167.5, 169.4, 169.7, 169.9. Anal. ($C_{20}H_{24}O_{11}$) C, H, N.

Methyl 1-(4-Hydroxymethylphenyl)-*β*-D-glucopyranuronate (9). Compound **8** (1.37 g, 3.11 mmol) was dissolved in anhydrous MeOH (100 mL). CH_3ONa (168 mg,3.11 mmol) was added at 0 °C for 30 min and at room temperature for 2 h. The mixture was quenched with water, extracted with CH_2Cl_2, and purified by column chromatography on silica gel (EtOAc) to give compound **9** (590 mg, 60.2%): MS *m*/*z* 314.255 (FAB^+); ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.22−3.42 (m, 3H, sugar-H), 3.61 (s, 3H, OCH_3), 4.09 (d, 1H, $J = 8.6$ Hz, sugar-H), 4.37 (d, 2H, $J = 5.2$ Hz, CH_2), 5.0 (d, 1H, $J = 7.1$ Hz, sugar-H), 5.04 (s, 1H, OH), 5.24 (s, 1H, OH), 5.38−5.43 (m, 2H, OH), 6.92 (d, 2H, $J = 8.0$ Hz, ArH), 7.19 (d, 2H, $J = 8.0$ Hz, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 53.0, 63.0, 72.3, 73.9, 76.0, 76.5, 101.2, 117.5, 129.2, 137.3, 156.4, 170.2.

Methyl *N*-[9-(*β*-D-Glucuronyl)benzyloxycarbonyl]aminocamptothecin (11). A solution of 9-aminocamptothecin (200 mg, 0.55 mmol) in 1,4-dioxane (100 mL) that contained pyridine (5 drops) and 4-nitrophenyl chloroformate (135 mg, 0.66 mmol) was stirred at 60 °C for 1 h. The mixture was then stirred at 80 °C for 1 h before addition of compound **9** (350 mg, 1.16 mmol). After being stirred at 80 °C for an additional 1 h, the mixture was cooled to room temperature, quenched with water, and extracted with $CHCl_3$. The crude product was purified by column chromatography on silica gel (MeOH−$CHCl_3$, 1:9) to give compound **11** (125 mg, 32.3%): LC-MS *m*/*z* 353 ($M^+ + 1$); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 7.0 Hz, CH_3), 1.86 (m, 2H, CH_2), 3.23−3.50 (m, 3H, sugar-H), 3.66 (s, 3H, OCH_3), 4.08 (d, 1H, $J = 8.6$ Hz, sugar-H), 5.16 (s, 2H, CH_2), 5.30 (s, 2H, CH_2), 5.45−5.52 (m, 3H, CH_2 & sugar-*H*), 6.56 (s, 1H, OH), 7.06 (d, 2H, $J = 8.4$ Hz, ArH), 7.36 (s, 1H, ArH), 7.43 (d, 2H, $J = 8.2$ Hz, ArH), 7.81 (m, 1H, ArH), 8.00 (m, 1H, ArH), 8.83 (s, 1H, ArH), 9.93 (s, 1H, ArH), 11.14 (br s, 1H, NH).

Potassium *N*-[9-(*β*-D-Glucuronyl)benzyloxycarbonyl]-aminocamptothecin (12). Compound **11** (125 mg, 0.18 mmol) was mixed with potassium trimethylsilanolate (60 mg, 0.45 mmol) in anhydrous THF (25 mL) at ambient temperature under nitrogen for 2 h. The yellow solid was filtered under nitrogen, dissolved in water, and washed with $CHCl_3$. Purification of the water layer by reverse phase column chromatography on silica gel (CH_3CN-H_2O, 3:1) gave compound **12** (80 mg, 62%): LC-MS, *m*/*z* 728 (M^+), 746 ($M^+ + H_2O$), 690 ($M^+ - K^+ + 1$), 708 ($M^+ - K^+ + 1 + H_2O$); ^{1}H NMR (200 MHz, D_2O) δ 0.89 (t, 3H, $J = 7.0$ Hz, CH_3), 1.99−2.05 (m, 1H, CH_2), 2.15−2.26 (m, 1H, CH_2), 3.40−3.45 (m, 3H, sugar-H), 3.68 (d, 1H, J = 8.5 Hz, sugar-H), 4.39 (s, 2H, CH_2), 4.90 (s, 2H, CH_2), 5.01 (m, 3H, CH_2 & sugar-H), 6.97 (d, 2H, $J = 8.2$ Hz, ArH). 7.26−7.35 (m, 6H, ArH), 7.89 (s, 1H, ArH). Anal. ($C_{34}H_{31}N_3O_{14}K_2 + 7H_2O$) C, H, N.

***N*-[9-(*β*-D-Glucuronyl)benzyloxycarbonyl]aminocamptothecin (4).** The water layer of compound **12** (80 mg, 0.11 mmol) was acidified with 1 N HCl, and compound **4** was precipitated in an ice bath and collected by filtration. The crude product was purified by reverse phase column chromatography on silica gel (20% CH_3CN-H_2O) to give compound **4** (38 mg, 50%): LC-MS, *m*/*z* 690.3 (M^+, lactone form), 706.3 (M^+, carboxylate form); ^{1}H NMR (200 MHz, $CD_3CN + D_2O$) δ 0.89 (t, 3H, $J = 7.2$ Hz, 19-CH_3), 1.86−1.90 (m, 2H, 18-CH_2), 3.45−3.47 (m, 3H, H-2′,4′,5′), 3.84−3.90 (m, 3H, ArCH_2 & H-3′), 4.98 (d, 1H, $J = 6.7$ Hz, H-1′), 5.11 (s, 2H, 5-CH_2), 5.15 (s, 2H, 17-CH_2), 7.05 (d, 2H, $J = 8.4$ Hz, ArH), 7.34 (d, 2H, $J = 8.4$ Hz, ArH), 7.49 (s, 1H, C_{14}−H), 7.71−7.75 (m, 2H, $C_{11,12}$−H), 7.94 (d, 1H, $J = 7.7$ Hz, C_{10}−H), 8.59 (s, 1H, C_7−H). Anal. ($C_{34}H_{31}N_3O_{13} + 5.5\ H_2O$) C, H, N.

Biological Tests. 1. HPLC Analysis. Drugs were analyzed by high-pressure liquid chromatography (HPLC). Briefly, 20 μL of sample was injected onto a reversed phase column (Hypersil C18, 4.6 mm inside diameter, 250 mm length, 5 μm particule size) using a moble phase (1 mL/min) of 45% MeOH and 25 mM phosphate buffer (pH 2.55) for compounds **4**, **5**, and **12**. Eluted compounds were detected on a Gilson model 121 fluorometer (excitation: 397 nm, emission: 482 nm). Peak areas were analyzed with Beckman System Gold software. Calibration curves were obtained by plotting the peak area of standards as a function of drug concentration. The retention times of **5** and **4** were 9.5 and 15.8 min, respectively. The recoveries of **4** and **5** from 90% human plasma were greater than 90%.

2. Drug Solubility. Drug solubilities were determined in βG buffer (100 mM acetic acid, 50 mM bis-tris, 50 mM triethanolamine, pH 7.0) or phosphate buffer (100 mM, pH 4.0) by equilibrating an excess of solid compound in 0.25 mL of buffer at 25 °C for 24 h. The samples were filtered through a 0.22 μm Millipore filter, diluted in HPLC mobile phase, and analyzed by HPLC.

3. Enzymatic Cleavage of Prodrugs. Compound **4** (100 μM or 25 μM) was incubated with the indicated concentration of βG in 30 μL of βG buffer containing 0.1 μg/mL bovine serum albumin at 37 °C for 2 h. The reaction was stopped by addition of 700 μL of ice-cold MeOH/5% H_3PO_4 (6/1) to precipitate proteins. The samples were centrifuged at 14 000 rpm for 5 min, and the supernatants were analyzed by HPLC.

4. In Vitro Cytotoxicity. Exponentially growing tumor cells at a density of $1-1.5 \times 10^4$ cells/well in RPMI medium containing 10% bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin were incubated in a 96-well microtiter plate for 72 h (37 °C, 5% CO_2, humidity) with various concentrations of drug. Camptothecin and 9-aminocamptothecin were dissolved in DMSO such that the final concentration of DMSO in wells did not exceed 0.5%. Compounds **12** and **4** were dissolved in medium. Control wells consisted of cells exposed to either medium or 0.5% DMSO in medium. βG, added at 5 μg/mL in some experiments, was not toxic by itself to cells. Triplicate wells were prepared for each drug concentration and for the controls. After 72 h, cells were washed once with sterile PBS, and then pulsed for 12 h with [^{3}H]thymine (1 μCi/well) in complete medium. Medium was removed, and

the wells were washed once with PBS before trypsinized cells were harvested and counted for radioactivity in a Topcount liquid scintillation counter. The coefficient of variation for triplicate determinations was <10%. IC_{50} values were calculated from interpolation of logarithmic dose−response curves.

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