Benzyl Ether-Linked Glucuronide Derivative of 10-Hydroxycamptothecin Designed for Selective Camptothecin-Based Anticancer Therapy

Yu-Ling Leu,*[,†,||] Chien-Shu Chen,[‡,||] Yih-Jang Wu,[‡] and Ji-Wang Chern*[,‡]

Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan, and School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan

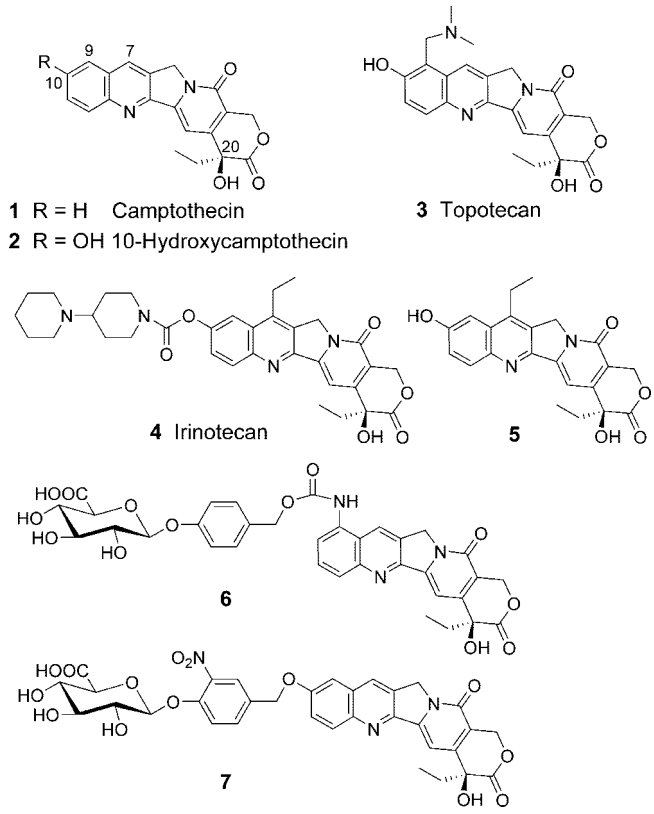
Received September 14, 2007

A β-glucuronidase-activated prodrug approach was applied to 10-hydroxycamptothecin, a *Camptotheca* alkaloid with promising antitumor activity but poor water solubility. We synthesized a glucuronide prodrug of 10-hydroxycamptothecin (**7**) in which glucuronic acid is connected via a self-immolative 3-nitrobenzyl ether linker to the 10-OH group of 10-hydroxycamptothecin. Compound **7** was 80 times more soluble than 10-hydroxycamptothecin in aqueous solution at pH 4.0 and was stable in human plasma. Prodrug **7** was 10- to 15-fold less toxic than the parent drug to four human tumor cell lines. In the presence of β-glucuronidase, prodrug **7** could be activated to elicit similar cytotoxicity to the parent drug in tumor cells. Enzyme kinetic studies showed that *Escherichia coli* β-glucuronidase had a quite low K_m of 0.18 μM for compound **7** and exhibited 520 times higher catalytic efficiency for **7** than for **6** (a glucuronide prodrug of 9-aminocamptothecin). Molecular modeling studies predicted that compound **7** would have a higher binding affinity to human β-glucuronidase than compound **6**. Prodrug **7** may be useful for selective cancer chemotherapy by a prodrug monotherapy (PMT) or antibody-directed enzyme prodrug therapy (ADEPT) strategy.

Introduction

(20*S*)-Camptothecin (**1**, Chart 1), first discovered in 1966 from *Camptotheca acuminata* (*Nyssaceae*),[1] displays antitumor activity by inhibiting DNA topoisomerase I.[2] Clinical use of camptothecin, however, was limited by its extremely poor water solubility. Efforts in developing water-soluble derivatives of camptothecin led to the synthesis of topotecan (**3**)[3] and irinotecan (CPT-11, **4**),[4] which are derivatives of 10-hydroxycamptothecin (**2**)[5,6] and have been approved for clinical use. Irinotecan is a prodrug, which is activated by carboxylesterases to generate the more potent metabolite, compound **5** (SN-38).[7] However, carboxylesterases are ubiquitous enzymes, resulting in a low therapeutic index of the prodrug.[8] We have first employed a β-glucuronidase-activated prodrug approach to more tumor-selective chemotherapy as well as increased water solubility for camptothecins.[9] In normal tissues, β-glucuronidase is localized primarily in lysosomes[10] and thereby not available for activation of glucuronide prodrugs because these prodrugs are generally hydrophilic, thus rendering them impermeable to cell membranes. In contrast, β-glucuronidase is found to accumulate extracellularly at the tumor site, mainly in necrotic areas.[11] Therefore, glucuronide-based prodrugs can be used in prodrug monotherapy (PMT[a]) for selective cancer chemotherapy.[11,12] It has also been shown that the glucuronide prodrugs are useful for application in the antibody-directed enzyme prodrug therapy (ADEPT) strategy such that β-glucuronidase can be targeted to tumor cells by administration of antibody−β-glucuronidase conjugates.[13] The recent development of β-glucuronidase-activated prodrugs includes elaborations of paclitaxel[14,15] and two potent DNA repair inactivators, O^6-benzylguanine and O^6-benzyl-2′-deoxyguanonsine.[16]

Chart 1



We previously designed and synthesized a glucuronide derivative of 9-aminocamptothecin (**6**) as a β-glucuronidase-cleavable prodrug in which glucuronic acid is connected via a self-immolative carbamate linker to the 9-amino group of 9-aminocamptothecin.[9] Compound **6** is water soluble, is stable

* To whom correspondence should be addressed. For Y.-L.L.: phone, 886-6-2664911, extension 200; fax, 886-6-2667318; e-mail, yulin@mail.chna.edu.tw. For J.-W.C.: phone, 886-2-2393-9462; fax, 886-2-2393-4221; e-mail, chern@jwc.mc.ntu.edu.tw.

† Chia-Nan University of Pharmacy and Science.

|| These authors contributed equally to this work.

‡ National Taiwan University.

[a] Abbreviations: PMT, prodrug monotherapy; ADEPT, antibody-directed enzyme prodrug therapy; PBS, phosphate-buffered saline; *E. coli*, *Escherichia coli*.

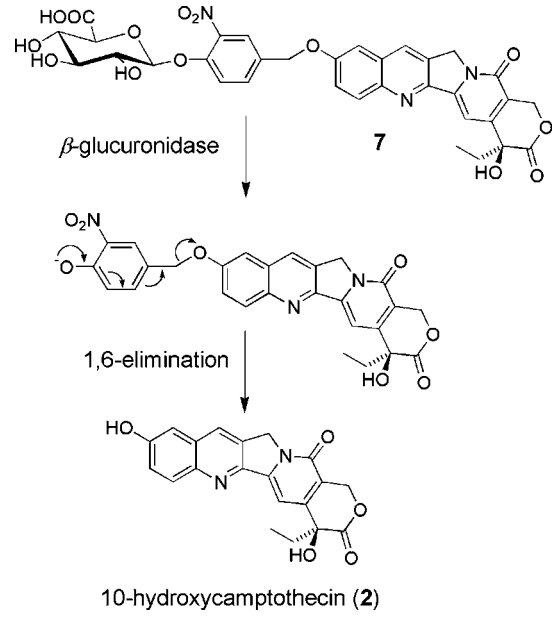
10.1021/jm701151c CCC: $40.75
Published on Web 03/05/2008

in human plasma, and is a substrate for β-glucuronidase. This prodrug is less toxic than 9-aminocamptothecin toward human tumor cell lines and upon enzyme activation can display cytotoxicity similar to that of the parent drug. Prodrug **6** was also shown to display potent in vivo antitumor activity against human tumor xenografts in nude mice.[17] More studies of prodrug **6** have been conducted to evaluate its potential clinical utility for selective cancer therapy.[18–20]

In the synthesis of compound **6**, the required material 9-aminocamptothecin was prepared from 10-hydroxycamptothecin via three synthetic steps. In fact, 10-hydroxycamptothecin is a promising antitumor alkaloid that is also produced by the roots of *Camptotheca acuminata* as a minor component. In topoisomerase I inhibition assays, 10-hydroxycamptothecin was found to be more potent than camptothecin and topotecan.[21] 10-Hydroxycamptothecin exhibited a broad spectrum of anticancer activity against various cancer cell lines and animal tumor models.[6,22–25] Moreover, 10-hydroxycamptothecin has been shown to be effective against multidrug-resistant cancer cell lines, whereas other camptothecin analogues such as topotecan, compound **5**, and 9-aminocamptothecin were relatively ineffective.[26] 10-Hydroxycamptothecin has entered clinical trials, mainly in China. To date, studies on prodrugs of 10-hydroxycamptothecin have been reported, including its fatty acid,[27,28] poly(ethylene glycol) (PEG),[29] and alkyl carbonate[30] derivatives.

In light of the promising prodrug properties of **6**, we wished to apply the same approach to 10-hydroxycamptothecin. The phenolic hydroxyl group of 10-hydroxycamptothecin was considered to be derivatized according to the work of Toki et al. where the peptide derivatives, containing a benzyl ether linker, of some phenolic anticancer drugs were studied in the protease-mediated prodrug activation strategy.[31] The glucuronide prodrug of 10-hydroxycamptothecin (**7**) was designed using a self-immolative 3-nitrobenzyl spacer which was ether-linked to the 10-OH group of 10-hydroxycamptothecin. The use of a spacer between the drug and glucuronic acid would allow superior enzymatic hydrolysis of prodrugs.[32] In addition, for the aromatic spacer of **7**, the strong electron-withdrawing nitro group (ortho to the glucuronyl moiety) might facilitate enzymatic cleavage of the glycosidic bond.[33] The enzymatic activation of prodrug **7** to liberate 10-hydroxycamptothecin through a 1,6-elimination reaction was expected as shown in Chart 2. Herein, we describe the synthesis and biological evaluation of the glucuronide prodrug **7**.

Chart 2. Release of 10-Hydroxycamptothecin (**2**) from Prodrug **7** by β-Glucuronidase Activation



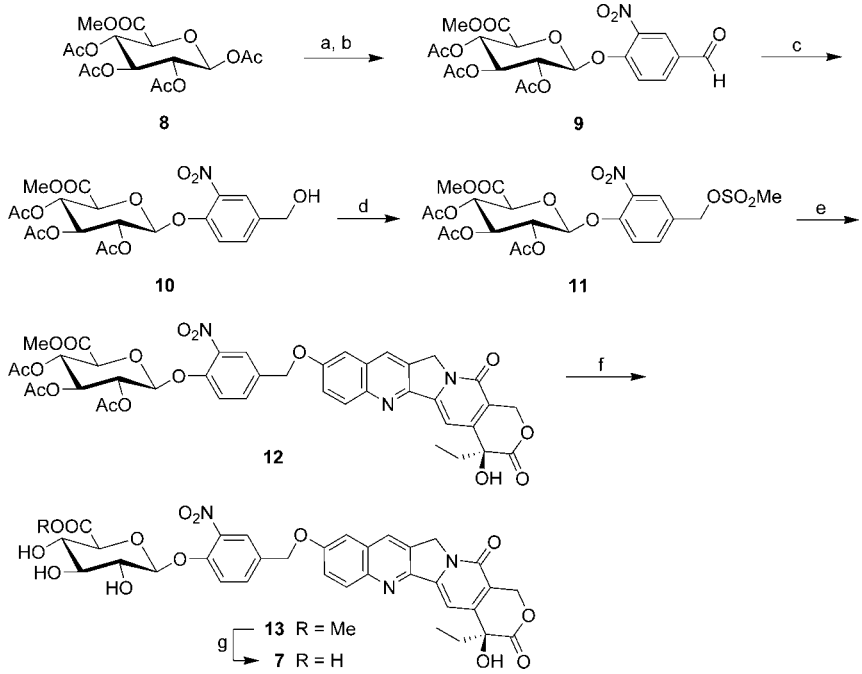
Chemistry

The glucuronide prodrug **7** was prepared as shown in Scheme 1. Methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**8**), prepared as described previously,[9] was brominated with $TiBr_4$ and then reacted with 4-hydroxy-3-nitrobenzaldehyde in the presence of Ag_2O to afford **9**. Reduction of the formyl group in **9** by treatment with $NaBH_4$ gave the alcohol **10**. To prepare compound **12**, we planned to utilize the Mitsunobu reaction[34] to couple alcohol **10** with 10-hydroxycamptothecin. At first a model study on the Mitsunobu reaction was carried out using 6-hydroxyquinoline, with the structure corresponding to the AB-ring part of 10-hydroxycamptothecin. Treatment of **10** with 6-hydroxyquinoline in DMF in the presence of diisopropyl azodicarboxylate (DIAD) and Ph_3P at room temperature for 4 days gave the coupling product in 40% yield. However, the reaction of **10** with 10-hydroxycamptothecin did not proceed at all under the same Mitsunobu conditions for 6 days, even at elevated temperature (up to 80 °C). This nonreactivity of 10-hydroxycamptothecin in the Mitsunobu reaction might be due to a relatively low electron density on the 10-OH oxygen atom of 10-hydroxycamptothecin containing a more extended π-conjugated system than 6-hydroxyquinoline. We then turned to the conversion of alcohol **10** to mesylate **11** by treatment with methanesulfonyl chloride. In the presence of Cs_2CO_3, the coupling reaction of mesylate **11** with 10-hydroxycamptothecin could be effected in DMF at room temperature for 2 h to afford **12** in 47% yield. It should be noted that prolonged reaction time (>2 h) would gradually lead to the formation of a byproduct. On the basis of NMR and mass spectral data, this byproduct was identified as the β-elimination product derived from **12** by loss of one molecule of acetic acid at positions C4 and C5 of the glucuronyl moiety. It has been reported in the literature that acetyl-protected glucuronic acids can undergo this type of elimination under basic conditions.[35,36] The *O*-acetyl groups of **12** were deprotected with NaOMe in MeOH to yield **13**. The methyl ester **13** was treated with potassium trimethylsilanolate ($KOSiMe_3$) in THF, acidified with 1 N HCl, and purified by reverse-phase column chromatography on silica gel to obtain the target compound **7**.

Results and Discussion

Camptothecins are generally poorly soluble in water and have a feature that the lactone E-ring is susceptible to nonenzymatic hydrolysis to form the carboxylate species at pH 7 or above. At neutral pH, the lactone−carboxylate equilibrium is shifted toward the hydrophilic carboxylate form.[37] Consequently, camptothecin compounds would display higher aqueous solubility at neutral pH than at acidic pH (existing in the hydrophobic lactone form). Unfortunately, the more soluble carboxylate form is biologically inactive. To circumvent the solubility problem, the elaboration of glucuronide derivatives of camptothecins may represent a potential approach based on the highly hydrophilic nature of the glucuronyl moiety with a carboxyl and three hydroxyl polar groups. Because of the water-solubilizing glucuronyl moiety, the synthesized prodrug **7** was expected to have greater aqueous solubility compared with the parent compound. As shown in Table 1, compound **7** was 80 and 19 times more water-soluble than 10-hydroxycamptothecin at pH 4.0 and 7.0, respectively. Formulation of camptothecins at acidic

Scheme 1[a]



[a] Reagents and conditions: (a) $TiBr_4$, CH_2Cl_2, room temp, 24 h; (b) 4-hydroxy-3-nitrobenzaldehyde, Ag_2O, CH_3CN, room temp, 15 h, 62%; (c) $NaBH_4$, silica gel, *i*-PrOH/$CHCl_3$, 0 °C, 1 h, 76%; (d) methanesulfonyl chloride, TEA, CH_2Cl_2, 0 °C, 1 h, 97%; (e) 10-hydroxycamptothecin (**2**), Cs_2CO_3, DMF, room temp, 2 h, 47%; (f) NaOMe, MeOH, room temp, 2 h, 46%; (g) $KOSiMe_3$, THF, room temp, 2 h, then acidified with 1 N HCl, 12%.

Table 1. Aqueous Solubility of Compounds[a]

	solubility (mM)	
compd	pH 4.0	pH 7.0
2	0.0137	0.137
7	1.10	2.60

[a] The solubility of 10-hydroxycamptothecin (**2**) and prodrug **7** was determined in PBS (pH 4.0 and 7.0) at 25 °C by a single determination.

pH is important to prevent formation of the inactive carboxylate form as a result of lactone ring opening.[37] Like 9-aminocamptothecin, 10-hydroxycamptothecin has poor aqueous solubility, especially at acidic pH (10 times lower for pH 4.0 compared with neutral pH). Thus, the 80-fold increased solubility at pH 4.0 of **7** (1.1 mM) is favorable for drug formulation. At pH 7.0, compound **7** had an aqueous solubility of 2.6 mM, which is less than that of **6**.[9] However, highly polar glucuronide prodrugs may have fast renal clearance, thus limiting their clinical applications.[38] Development of new glucuronide prodrugs with a less hydrophilic nature may be advantageous. Prediction of the octanol/water partition coefficient using the program XLOGP 2.0[39] gave the calculated log *P* values of 1.19 and 0.81 for prodrugs **7** and **6**, respectively. The relatively lower hydrophilicity of **7** may retard prodrug excretion and, hence, result in prolonged exposure at the tumor site.

The stability of **7** was studied in 95% human plasma at 37 °C. Glucuronide **7** proved to be completely stable for at least 3 h (Figure 1), indicating its high resistance to nonspecific enzymatic degradation in human plasma. Prior to this work, we attempted to synthesize a carbonate-linked analogue of **7**, 10-*O*-[4-(6-*O*-methyl-β-D-glucuronyloxy)benzyloxycarbonyl]-10-hydroxycamptothecin (designated here as 10-HCG-carbonate) and assess its stability. In contrast to prodrug **7**, 10-HCG-carbonate was unstable in human plasma with 70% degradation in 10 min (Figure 1). The rapid degradation of 10-HCG-carbonate in plasma was not likely due to chemical instability because this compound was much more stable in PBS with

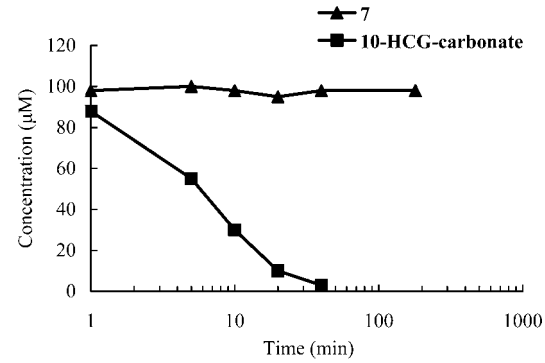


Figure 1. Stability of prodrug **7** and 10-HCG-carbonate studied in 95% human plasma: 10-HCG-carbonate, a carbonate-linked analogue of **7** with the chemical name 10-*O*-[4-(6-*O*-methyl-β-D-glucuronyloxy-)benzyloxycarbonyl]-10-hydroxycamptothecin.

~70% decomposition after 11 h (data not shown). As observed by TLC analysis, 10-HCG-carbonate was degraded in plasma to produce the parent drug **2**. This conversion presumably resulted from direct enzymatic cleavage of the carbonate linkage of the prodrug by esterases, which are abundantly present in blood. Previously, Toki et al. have described protease-activated prodrugs of the phenolic anticancer agent combretastatin A-4 with a stable benzyl ether or unstable benzyl carbonate linkage.[31] In contrast, nevertheless, 10-HCG-carbonate was much more unstable than the carbonate derivative of combretastatin A-4, which had a half-life of 45 h in human plasma. This indicates that a carbonate derivatization at the 10-OH group of 10-hydroxycamptothecin is not suitable for selective activation by β-glucuronidase.

10-Hydroxycamptothecin and prodrug **7** were evaluated for the cytotoxicity against four human tumor cell lines (HepG2, Colo 205, HT29, and H928) by measuring [^{3}H]thymidine incorporation into cellular DNA after 48 h of drug exposure. As indicated in Table 2, prodrug **7** was 10–15 times less toxic

Table 2. Cytotoxicity of Compounds to Human Tumor Cells

compd	IC_{50} (nM)[a]			
	HepG2	Colo 205	HT29	H928
2	5.4	9.1	8.8	6.9
7	56.5	94.2	97.8	91.1
7 + β-glucuronidase[b]	6.6	10.2	10.0	11.1

[a] Concentrations of compounds that inhibited incorporation of [^{3}H]thymidine into cellular DNA of human hepatocellular (HepG2), colorectal (Colo 205), colorectal (HT29), or lung (H928) carcinoma cells by 50% after 48 h are indicated. Values represent the mean of one to three experiments performed in triplicate with coefficients of variation of <10%. [b] β-Glucuronidase (5 μg/mL) was added with drugs.

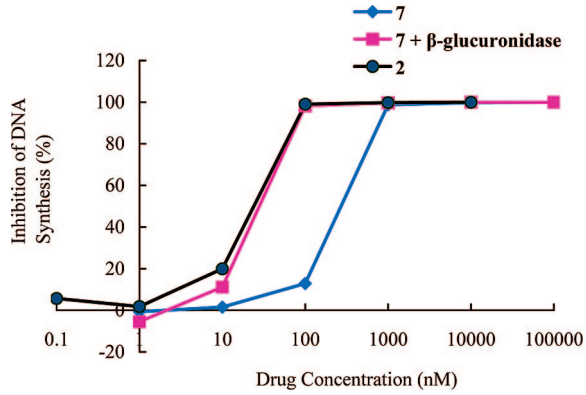


Figure 2. Cytotoxicity of compounds **2** and **7** to human colorectal carcinoma cells (HT29). HT29 cells were exposed to compounds with or without β-glucuronidase for 48 h before the incorporation of [^{3}H]thymidine into cellular DNA was measured. Results represent mean values of triplicate determinations.

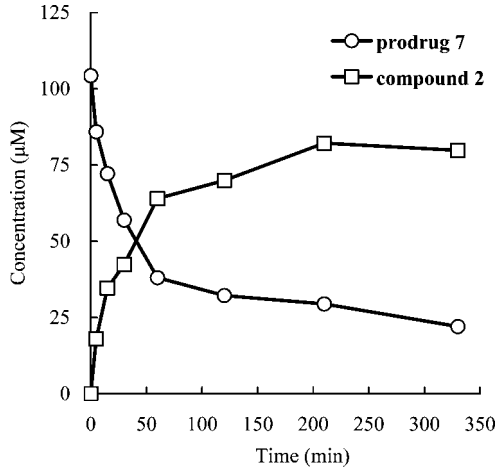


Figure 3. Hydrolysis of prodrug **7** to 10-hydroxycamptothecin (**2**) by *E. coli* β-glucuronidase. Prodrug **7** was incubated with 0.1 μg/mL β-glucuronidase at pH 7.0 and 37 °C. The concentrations of **7** and **2** in aliquots taken at the indicated times were determined by HPLC. Results are shown as mean values of triplicate determinations.

than 10-hydroxycamptothecin. In the presence of β-glucuronidase (5 μg/mL), prodrug **7** was as cytotoxic as 10-hydroxycamptothecin itself (Figure 2, Table 2), indicating efficient enzymatic cleavage with complete release of 10-hydroxycamptothecin.

Enzymatic cleavage of prodrug **7** was studied using *Escherichia coli* β-glucuronidase (0.1 μg/mL) in pH 7.0 phosphate buffer at 37 °C. Figure 3 shows the time course of hydrolysis of **7** on exposure to β-glucuronidase. The results reveal that prodrug **7** was readily cleaved by β-glucuronidase to release the active drug **2**. For a 20-min incubation with 0.125 μg/mL

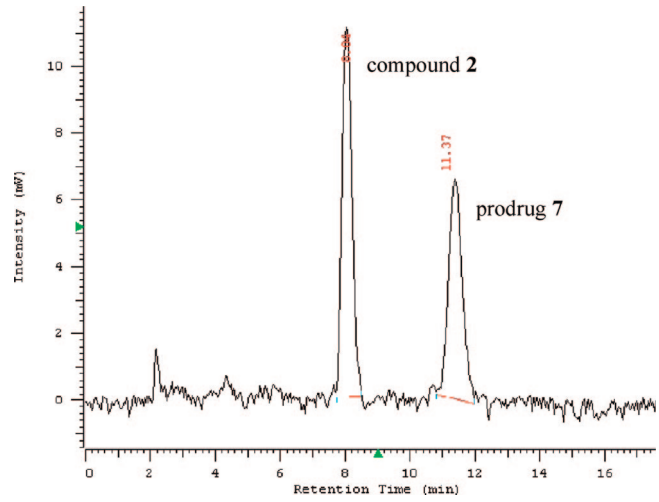


Figure 4. HPLC chromatogram for the hydrolysis of **7** by β-glucuronidase (0.125 μg/mL) at pH 7.0 after 20 min of incubation at 37 °C.

Table 3. Enzyme Kinetic Parameters for Prodrugs

prodrug	K_m (μM)	V_{max} (μM/min)	V_{max}/K_m
7	0.18	2.7	15
6	30	0.86	0.03

β-glucuronidase, HPLC analysis showed >50% conversion of **7** to 10-hydroxycamptothecin (**2**) with no spacer−drug intermediate detected (Figure 4). This indicates that prodrug **7** was susceptible to β-glucuronidase hydrolysis and that the spacer rapidly decomposed upon enzymatic activation. To our knowledge, this is the first example of a self-immolative glucuronide-based prodrug in which the spacer was ether-linked to the drug. Recently, a glucuronide prodrug of compound **5** was synthesized using an aromatic spacer connected via a carbamate linkage to the 10-OH group of **5**.[40] The enzyme kinetic parameters were determined for **7** as well as **6** (Table 3). As a substrate of β-glucuronidase, compound **7** showed a good K_m of 0.18 μM and a V_{max} of 2.7 μM/min. The data showed that β-glucuronidase exhibited 520 times higher catalytic efficiency for **7** than for **6**, as revealed by the V_{max}/K_m ratios (15 versus 0.029). According to the K_m values (0.18 versus 30 μM), compound **7** had an approximately 170 times higher affinity to β-glucuronidase than compound **6**. Thus, it appears that the large difference in catalytic efficiency between **7** and **6** can be attributed mainly to the considerably distinct binding affinities of these two prodrugs to the enzyme.

We were interested in understanding the interactions of compounds **7** and **6** with β-glucuronidase and carried out molecular docking studies to explore their probable binding modes within the active site. To date, the only available experimental three-dimensional structure for β-glucuronidase is the X-ray crystal structure of human β-glucuronidase disclosed by Jain et al.[41] The crystal structure of human β-glucuronidase was retrieved from the Protein Data Bank (PDB code 1BHG) for the present docking studies. In our molecular modeling process (details in Supporting Information), the known substrate molecule *p*-nitrophenyl β-glucuronide[42] was first docked into the active site of β-glucuronidase using the docking program GOLD 2.0.[43] The resulting complex structure was then optimized by energy minimization using the Tripos force field in the software package SYBYL 7.1 (Tripos, Inc., St. Louis, MO). The purpose of such initial modeling is to build a protein model whose active site exists in the probable substrate-bound

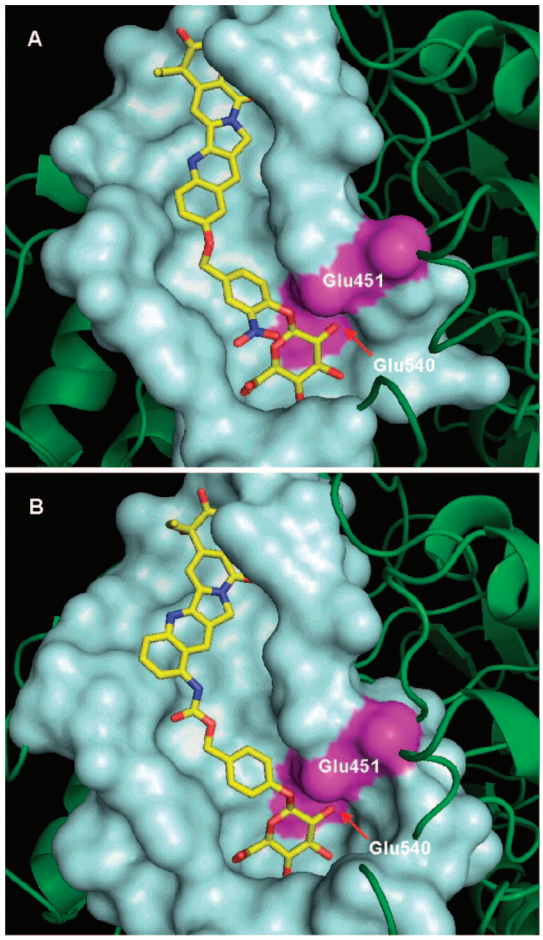


Figure 5. Docking models of (A) compound **7** and (B) compound **6** with human β-glucuronidase. The possible binding site of β-glucuronidase is depicted as molecular surface, and the catalytic residues Glu451 and Glu540 are colored magenta.

conformation. We chose *p*-nitrophenyl β-glucuronide for simulation because it has smaller molecular size and is also analogous to the spacer−glucuronyl parts of compounds **7** and **6**. The modeled substrate-bound structure of human β-glucuronidase showed that the glycosidic bond of *p*-nitrophenyl β-glucuronide was properly oriented toward the catalytic residues Glu451 and Glu540 (Figure S1 in Supporting Information). It has been proposed for human β-glucuronidase that in catalysis Glu451 acts as the acid/base catalyst and Glu540 as the nucleophilic residue.[44] This modeled protein structure was then used in the prediction of a favorable binding mode of **7** or **6** to β-glucuronidase by computational simulations as described above. The predicted binding models of compounds **7** and **6** to β-glucuronidase are shown in Figure 5 (see also Figures S2 and S3 in Supporting Information). In addition, for a more reliable comparison, the binding affinities of **7** and **6** were predicted using three programs that have different scoring functions for calculating the binding affinity of a ligand. According to the calculated data (Table 4), compound **7** possesses a lower binding free energy or a higher pK_d (or pK_i) value, which may allow more favorable binding to β-glucuronidase compared with compound **6**. Analysis of the predicted binding conformations revealed that compound **7** can adopt a linear conformation for better fitting to the straight binding groove of β-glucuronidase. In contrast, compound **6** has the spacer attached at the 9-position of camptothecin, thus being unable to adopt a linear overall molecular shape. The results from molecular modeling suggested

Table 4. Predicted Binding Affinities for Prodrugs **7** and **6** to β-Glucuronidase Based on the Docking Models

	predicted binding affinity[a]		
prodrug	AutoDock binding free energy (kcal/mol)	SCORE (pK_d)	LigScore (pK_i)
7	−10.65	9.18	7.28
6	−9.14	8.92	6.80

[a] For the scoring functions, see refs 5−7 in Supporting Information.

that compound **7** would bind to human β-glucuronidase more tightly than **6**. *E. coli* and human β-glucuronidases share 50% sequence homology with highly conserved active sites.[41,45] Accordingly, we believed that compound **7** could also adopt a more favorable shape complementary to *E. coli* β-glucuronidase than **6**, explaining the lower K_m value for **7**.

In summary, we have synthesized the glucuronide prodrug **7** that showed improved water solubility and reduced cytotoxicity relative to 10-hydroxycamptothecin. Prodrug **7** was stable in human plasma and could efficiently liberate the free drug by β-glucuronidase activation. Compared with prodrug **6** for 9-aminocamptothecin, prodrug **7** was much more susceptible to β-glucuronidase hydrolysis and possessed a less hydrophilic nature that should be considered in the development of new-generation glucuronide prodrugs with slower clearance. The requisite prodrug profiles of **7** suggest its potential application 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 on a Bruker DPX-200 spectrometer. FAB mass spectra were recorded on a Finnigan MAT 95S mass spectrometer. Elemental analyses for C, H, and N were carried out on a Heraeus VarioEL-III elemental analyzer. The thin-layer chromatographic analyses were performed using precoated silica gel (60 F_{254}, Merck) plates, and the spots were examined under UV light. Column chromatography was carried out on Merck silica gel 60 (70–230 mesh). Reverse phase column chromatography was performed on Merck LiChroprep RP-18 (40–63 μm). 10-Hydroxycamptothecin was purchased from China.

Methyl 1-*O*-(4-Formyl-2-nitrophenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate (9). A solution of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**8**)[9] (5 g, 13.3 mmol) and $TiBr_4$ (5.07 g, 13.8 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 24 h. The mixture was washed with ice-cold water (50 mL) and saturated aqueous $NaHCO_3$ (50 mL), dried over Na_2SO_4, and evaporated under reduced pressure to dryness to give 5.1 g of solid. The solid was dissolved in CH_3CN (100 mL). To this solution were added 4-hydroxy-3-nitrobenzaldehyde (2.3 g, 13.8 mmol) and Ag_2O (3.2 g, 13.8 mmol), and the mixture was stirred at room temperature for 15 h. The insoluble material was filtered off. The filtrate was evaporated under reduced pressure to give a dark-brown solid, which was washed with MeOH to give **9** (3.99 g, 62%): mp 180–182 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.00 (s, 9H, CH_3), 3.61 (s, 3H, OCH_3), 4.77 (d, J = 9.5 Hz, 1H, sugar-H), 5.07–5.18 (m, 2H, sugar-H), 5.45 (t, J = 9.2 Hz, 1H, sugar-H), 5.91 (d, J = 6.0 Hz, 1H, sugar-H), 7.62 (d, J = 8.7 Hz, 1H, ArH), 8.20 (d, J = 8.6 Hz, 1H, ArH), 8.42 (s, 1H, ArH), 9.95 (s, 1H, CHO); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.0, 21.1, 21.2, 53.5, 69.3, 70.5, 71.3, 72.1, 98.1, 118.5, 127.1, 131.9, 135.6, 141.1, 152.9, 167.7, 169.6, 170.2, 170.4, 191.4. Anal. ($C_{20}H_{21}NO_{13}$) C, H, N.

Methyl 1-*O*-(4-Hydroxymethyl-2-nitrophenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate (10). A mixture of **9** (2 g, 4.14 mmol), $NaBH_4$ (434 mg, 11.5 mmol), and silica gel (10 g) in *i*-PrOH/$CHCl_3$ (3:17) (200 mL) was stirred at 0 °C for 1 h. The reaction was quenched with water, and the mixture was filtered to remove silica gel. The organic layer was dried over $MgSO_4$ and

evaporated under reduced pressure to give a residue, which was washed with EtOH to give **10** (1.52 g, 76%): mp 167–168 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.98–2.00 (m, 9H, CH_3) 3.64 (s, 3H, OCH_3), 4.50 (d, J = 5.4 Hz, 2H, CH_2), 4.72 (d, J = 9.8 Hz, 1H, sugar-H), 5.03–5.14 (m, 2H, sugar-H), 5.37–5.49 (m, 2H, sugar-H and OH), 5.70 (d, J = 7.8 Hz, 1H, sugar-H), 7.37 (d, J = 8.6 Hz, 1H, ArH), 7.60 (d, J = 7.3 Hz, 1H, ArH), 7.79 (s, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.0, 21.1, 21.2, 53.5, 62.2, 69.6, 70.8, 71.7, 71.9, 98.9, 118.6, 123.2, 132.9, 139.4, 141.1, 147.8, 167.8, 169.6, 170.2, 170.4. Anal. ($C_{20}H_{23}NO_{13}$) C, H, N.

Methyl 1-*O*-(4-Methanesulfonyloxymethyl-2-nitrophenyl)-2,3,4-tri-*O*-acetyl-*β*-D-glucopyranuronate (11). A solution of **10** (300 mg, 0.62 mmol), methanesulfonyl chloride (0.06 mL, 0.78 mmol), and triethylamine (0.1 mL, 0.71 mmol) in CH_2Cl_2 (15 mL) was stirred at 0 °C for 1 h. The mixture was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated under reduced pressure to dryness to give **11** (340 mg, 97%): mp 110–112 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.00 (s, 9H, CH_3), 3.27 (s, 3H, SO_2CH_3), 3.63 (s, 3H, OCH_3), 4.74 (d, J = 9.8 Hz, 1H, sugar-H), 5.04–5.16 (m, 2H, sugar-H), 5.29 (s, 2H, CH_2), 5.46 (t, J = 9.4 Hz, 1H, sugar-H), 5.77 (d, J = 7.7 Hz, 1H, sugar-H), 7.47 (d, J = 8.4 Hz, 1H, ArH), 7.79 (d, J = 8.6 Hz, 1H, ArH), 8.02 (s, 1H, ArH); FAB-MS m/z 562 ($M^+ - 1$). Anal. ($C_{21}H_{25}NO_{15}S$) C, H, N.

10-[4-(2,3,4-Tri-*O*-acetyl-6-*O*-methyl-*β*-D-glucuronyloxy)-3-nitrobenzyloxy]camptothecin (12). To a suspension of 10-hydroxycamptothecin (600 mg, 1.65 mmol) and Cs_2CO_3 (600 mg, 1.84 mmol) in anhydrous DMF (30 mL) was added a solution of **11** (3 g, 5.32 mmol) in anhydrous DMF (30 mL). The mixture was stirred at room temperature for 2 h. The crude product was purified by column chromatography on silica gel (MeOH/$CHCl_3$ = 2:98) to give **12** (650 mg, 47%): mp 259–261 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.87 (t, J = 7.1 Hz, 3H, CH_3) 1.80–2.00 (m, 2H, CH_2), 2.01–2.03 (m, 9H, CH_3), 3.63 (s, 3H, OCH_3), 4.71 (d, J = 9.7 Hz, 1H, sugar-H), 5.08–5.18 (m, 2H, sugar-H), 5.25 (s, 2H, CH_2), 5.31 (s, 2H, CH_2), 5.40–5.52 (m, 1H, sugar-H), 5.74 (d, J = 8.0 Hz, 1H, sugar-H), 6.52 (s, 1H, OH-20), 7.27 (s, 1H, ArH), 7.35–7.71 (m, 3H, ArH), 7.74–7.95 (m, 1H, ArH), 8.10 (s, 2H, ArH), 8.53 (s, 1H, ArH); FAB-MS m/z 832 (M^+). Anal. ($C_{40}H_{37}N_3O_{17}\cdot H_2O$) C, H, N.

10-[4-(6-*O*-Methyl-*β*-D-glucuronyloxy)-3-nitrobenzyloxy]camptothecin (13). A suspension of **12** (360 mg, 0.43 mmol) and NaOMe (290 mg, 5.37 mmol) in anhydrous MeOH (160 mL) was stirred at room temperature for 2 h. The crude product was purified by column chromatography on silica gel (MeOH/$CHCl_3$ = 1:9) to give **13** (140 mg, 46%): mp 207–209 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, J = 7.2 Hz, 3H, CH_3), 1.85–1.87 (m, 2H, CH_2), 3.32–3.48 (m, 3H, sugar-H), 3.64 (s, 3H, OCH_3), 4.13 (d, J = 8.9 Hz, 1H, sugar-H), 5.26–5.53 (m, 9H, CH_2, sugar-H and OH), 6.51 (s, 1H, OH-20), 7.27 (s, 1H, ArH), 7.47–7.62 (m, 3H, ArH), 7.81 (d, J = 8.3 Hz, 1H, ArH), 8.08 (d, J = 8.6 Hz, 2H, ArH), 8.53 (s, 1H, ArH); FAB-MS m/z 706 (M^+). Anal. ($C_{34}H_{31}N_3O_{14}\cdot CH_3OH$) C, H, N.

10-[4-(*β*-D-Glucuronyloxy)-3-nitrobenzyloxy]camptothecin (7). A suspension of **13** (110 mg, 0.16 mmol) and $KOSiMe_3$ (105 mg, 0.82 mmol) in anhydrous THF (30 mL) was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the resulting residue was dissolved in water and then washed with $CHCl_3$. The aqueous layer was acidified with 1 N HCl and purified by reverse phase column chromatography on silica gel (CH_3CN/H_2O = 1:5) to give **7** (13 mg, 12%): mp 196–197 °C; ^{1}H NMR (200 MHz, DMSO-d_6 + D_2O) δ 0.88 (t, J = 6.6 Hz, 3H, CH_3), 1.85–1.88 (m, 2H, CH_2), 3.16–3.25 (m, 3H, sugar-H), 3.72 (m, 1H, sugar-H), 5.23 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 5.41 (s, 2H, CH_2), 5.73 (s, 1H, sugar-H), 7.28 (s, 1H, ArH), 7.54–7.81 (m, 4H, ArH), 8.04 (s, 1H, ArH), 8.08 (s, 1H, ArH), 8.52 (s, 1H, ArH); FAB-MS m/z 692 ($M^+ + 1$). HRMS (FAB) calcd for $C_{33}H_{30}N_3O_{14}$ ($M^+ + 1$) 692.1728, found 692.1731. Anal. Calcd for $C_{33}H_{29}N_3O_{14}\cdot 10H_2O$: C, 45.47; H, 5.67; N, 4.82. Found: C, 45.34; H, 4.82; N, 4.64.

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 (LiChroCART RP-18, 4 mm inside diameter, 250 mm length, 5 μm particle size) using a mobile phase (1 mL/min) of 50% MeOH and 50 mM phosphate buffer (pH 2.5). Eluted compounds were detected on a Hitachi L-7480 fluorometer (excitation, 397 nm; emission, 482 nm). Peak areas were analyzed with Hitachi D-7000 chromatography data station software. Calibration curves were obtained by plotting the peak area of standards as a function of drug concentration. The retention times of 10-hydroxycamptothecin and prodrug **7** were 8.0 and 11.4 min, respectively.

2. Drug Solubility. Drug solubilities were determined in phosphate buffer (100 mM, 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 0.2 μm Millipore filter, diluted in HPLC mobile phase, and analyzed by HPLC.

3. Prodrug Stability in 95% Human Serum. Prodrug stability was determined in 95% human plasma at 37 °C for 6 h. Aliquots were taken, neutralized with 12 mM phosphoric acid, and extracted three times with an equal volume of ethyl acetate. The ethyl acetate layer was evaporated under reduced pressure to dryness. This residue was dissolved in HPLC mobile phase, filtered through a 0.2 μm Millipore filter, and analyzed by HPLC.

4. In Vitro Cytotoxicity. Exponentially growing tumor cells at a density of 5×10^3 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. 10-Hydroxycamptothecin and prodrug **7** were dissolved in DMSO such that the final concentration of DMSO in wells did not exceed 0.5%. Control wells consisted of cells exposed to 0.5% DMSO in medium. β-Glucuronidase, added at 1 μg/well in some experiments, was not toxic by itself to cells. Triplicate wells were prepared for each drug concentration and for the controls. After 48 h, cells were pulsed for 12 h with [^{3}H]thymidine (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.

5. Enzyme Kinetic Analysis. A solution of various concentration of prodrug **7** was incubated with 0.125 μg/mL *E. coli* β-glucuronidase at pH 7.0 for 20 min at 37 °C. The reaction was stopped by addition of 900 μL of ice-cold MeOH to precipitate proteins. The samples were centrifuged at 12 000 rpm for 5 min. The supernatants were evaporated in vacuo to dryness, and the resulting residues were dissolved in 5% H_3PO_4−MeOH (1:6) for HPLC analysis. For the time-course assay of hydrolysis of **7** to the parent compound **2**, prodrug **7** was incubated with 0.1 μg/mL β-glucuronidase at pH 7.0 and 37 °C. The concentrations of **7** and **2** in aliquots taken at the indicated times were determined by HPLC.

Acknowledgment. The authors thank Dr. Steve R. Roffler of Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, for providing helpful experimental facilities and the National Center for High-Performance Computing (NCHC), Hsinchu, Taiwan, for providing computing resources. We also thank Dr. Shin-Yu Lai and Hsiang-Ling Huang for their technical assistance.

Supporting Information Available: Elemental analysis results for synthesized compounds, HPLC analysis results and NMR spectra for final compound **7**, and molecular modeling details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM701151C