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Selective Phenolic Acylation of 10-Hydroxycamptothecin Using Poly (Ethylene Glycol) Carboxylic Acid

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Abstract—Selective acylation of the phenolic hydroxyl group of 10-hydroxycamptothecin has been accomplished using phenyl dichlorophosphate. Additional modification of the 10-OH as an ether permits a 20-acyl derivative to be synthesized. This result along with data from a 6-hydroxyquinoline model strongly suggests that powerful intermolecular hydrogen bonding exists in the parent molecule.

10-Hydroxycamptothecin (10-HCPT)[1] is a naturally-occurring A-ring oxygenated derivative of camptothecin and a minor component found in extracts of *Camptotheca acuminata*. 10-HCPT (**1**) has been reported to be effective against certain human cancers, and has been shown to be more active and less toxic than other camptotheca alkaloids.[2] Potent synthetic water soluble 10-HCPT analogues, CPT-11,[3] a piperazine carbamate derivative of 10-hydroxy-7-ethyl-camptothecin, and topotecan,[4] the 9-dimethylaminomethyl derivative of 10-HCPT, have recently been approved for clinical use. Previously, we reported the preparation of a poly (ethylene glycol) (PEG) ester prodrug of camptothecin (CPT) by acylating the 20-OH moiety.[5] The resulting water soluble polymeric prodrugs (transport forms) showed increased efficacy in a P388/0 mouse model, and HPLC evidence[5] strongly implied that these 20-acyl derivatives are locked into the highly active lactone form. More recent chemical, NMR and UV studies have further validated this conclusion.[6] The intriguing possibility of preparing a 10-PEG ester transport form in which the 20-OH group, necessary for activity, has not been disabled, prompted us to explore the acylation of this bifunctional alkaloid. Although 10-HCPT contains two hydroxyls, one aromatic and one aliphatic, acylation of the phenolic moiety should be straightforward since the aliphatic 20-hydroxyl is a hindered 3° alcohol and accordingly, less reactive. Curiously, only a low yield synthesis of CPT-11 and analogues resulted from the direct acylation of 10-HCPT with *N*-piperazinyl carbamoyl chloride.[3] Perhaps that is why Takayama et al.[7] synthesized a series of potent anticancer long-chain fatty acid esters of 10-HCPT by a lengthy construction of the entire molecule and not by direct acylation. We now wish to report a direct, efficient, and high yield preparation of the PEG ester of 10-HCPT that we believe will prove to be a general procedure for similar bifunctional molecules. In addition we offer evidence that both the 10- and 20-OH groups appear to influence the reactivity of the other.

Chemistry

Acylation of the phenolic hydroxy moiety is generally accomplished with carboxylic acids using standard coupling agents such as DIPC or EDC.[8] Acylation reactions of **1** itself have been reported using only simple acid chlorides or acid anhydrides in pyridine.[9,10] Since **1** also has low solubility in most organic solvents, further variation of reaction conditions is limited. Direct acylation of **1** with PEG was first attempted by employing the acid chloride derived from mPEG-COOH (M_w 5000) and resulted in incomplete conversion, while employing the mPEG-NHS ester did not produce any product at all. Using PEG acid per se with various coupling reagents (DIPC, DCC, Mukaiyama reagent[11]), and different bases (pyridine, Et_3N, DMAP, K_2CO_3) continued to result in low yields of 10-position acylated products. Interestingly, no indication of 20-*O*-acylation (^{1}H and

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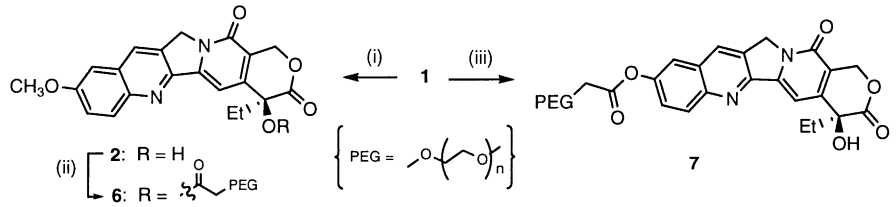


Scheme 1. (i) MeI, K_2CO_3, acetone; (ii) PEG-dicarboxylic acid (M_w 40,000, **3**), 2-chloro-1-methylpyridinium iodide (**4**), DMAP, DCM; (iii) **3**, phenyldichlophosphate (**5**), pyridine, $CHCl_3$.

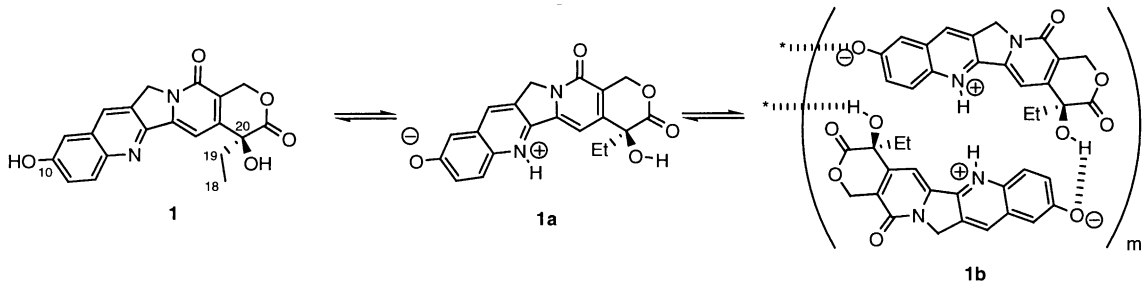
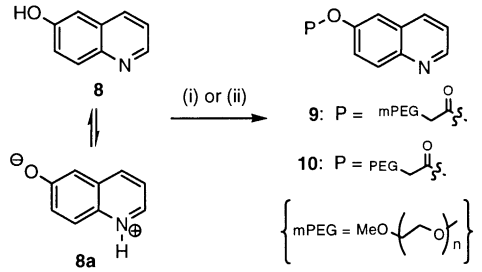


Figure 1.

^{13}C NMR analyses) could be detected although it has been established that camptothecin readily forms a PEG ester under these reaction conditions.[5] Alkylation of 10-HCPT with MeI and K_2CO_3 as base, on the other hand, gave good yields (80%) of the known ether, 10-methoxycamptothecin **2**.[1] Unlike **1**, and in similar fashion to native camptothecin, **2** underwent facile acylation at the 20-OH position employing the Mukaiyama reagent to yield PEG ester prodrug **6** (Scheme 1). In order to explain these results, we propose the existence of strong intermolecular hydrogen bonding between the 10-OH and the 20-OH moieties in the parent compound as depicted in Figure 1 (see **1b**). Methylation of **1** appears to interrupt the hydrogen bonding between species, thus allowing acylation to take place easily at the 20-OH position. In 1978 Liu et al.[12] reported that phenyl dichlorophosphate in dimethoxyethane, with just sufficient pyridine to accomplish neutralization of all acids, could effect esterification of carboxylic acids with various alcohols as well as phenol at room temperature under virtually neutral conditions. Later work[13] indicated that the reactive species in these acylations was probably the mixed phenylphosphoric–carboxylic anhydride or dicarboxylic acid anhydride. We also have chosen 6-hydroxy quinoline (6-HQ, **8**) as a model of the phenolic-quinoline portion of 10-HCPT since the phenolic 6-OH group cannot be influenced by an aliphatic OH as is the case for the 10-HCPT (Scheme 2). The results of several acylation experiments on 6-HQ using acid chloride or carboxylic acid with GOC or the Liu phosphorous reagents as condensing agent are summarized in Table 1. Two important features for acylation of this model can be gleaned from these results. First, phenyl dichlorophosphate and EDC give the best yields. Secondly, because under the essentially neutral conditions of the Liu reagent there is a sufficient concentration of the uncharged and/or non H-bonded species, **8** and **8a** in the equilibrium as shown in Scheme 2, which is reactive towards acylation. Perhaps more informative is the result that EDC/base now is an effective reagent for phenolic acylation, whereas in the presence of the 20-OH in 10-HCPT this reaction does not occur. Our earlier postulate that methylation of the 10-OH moiety interrupts intermolecular hydrogen bonding with the 20-OH position, which then allows acylation of 20-OH to take place is substantiated by the 6-HQ model where there is no possibility of intermolecular hydrogen bonding: acylation in the presence of EDC and pyridine now takes place easily. However, employing phenyl dichlorophosphate as coupling agent, 10-HCPT was now successfully and selectively acylated with PEG dicarboxylic acid[5] (**3**, M_w 40,000) in the presence of pyridine to produce the 10-PEG ester transport form **7** in 89% yield. The selective 10-acylation was also confirmed by NMR data. ^1H NMR of the 20-acylated camptothecin derivatives exhibited the C-19 protons as two quartets[14] (at 2.21 ppm for **6**) compared to the unacylated camptothecin as a single quartet. The PEG acylated product **7** showed the C-19 protons as one quartet at 1.83 ppm. Therefore, it appears that the 20-OH is still present and that selective 10-OH aromatic acylation was achieved. In vitro data for compound **7** is shown in Table 2 with an in vivo evaluation using a human colon tumor (LS174T) xenograft in nude mice in comparison with PEG-ala-camptocthecin[15] (Prothecan®) which currently is in clinical trial. The results show that the PEGylated version of 10-HCPT was statistically



Scheme 2. (i) mPEG carboxylic acid (**3a**, M_w 5000), EDC·HCl, DMAP, DCM; (ii) **3**, **5**, pyridine, $CHCl_3$.

Table 1. Acylation reactions of 6-hydroxyquinoline

Entry	Acid or acid chloride	Condition	% Acylation[a]
1	$BnOCH_2C(=O)Cl$	Pyridine, CH_2Cl_2	0
2	mPEG (5000) CO_2H	$PhOP(=O)Cl_2$, pyridine, $CHCl_3$	>95
3	PEG (40,000) CO_2H	$PhOP(=O)Cl_2$, pyridine, $CHCl_3$	>95
4	PEG (40,000) CO_2H	$Me_2NP(=O)Cl_2$, pyridine, $CHCl_3$	20%
5	mPEG (5000) CO_2H	EDC, pyridine	>95

[a]Estimated by NMR and HPLC.

Table 2. In vitro and in vivo data of PEG 10-HCPT derivative (**7**)

	$t_{1/2}$ (h, PBS)[a]	$t_{1/2}$ (h, rat plasma)[a]	IC_{50} (nM, P388/0)[b]	%TGI (LS174) solid tumor[c]
Camptothecin	—	—	7	4.9
10-HCPT (**1**)	—	—	12	36.5
Prothecan®	100	15	16	71.5
PEG 10-HCPT (**7**)	5	0.12	55	57.6

[a]All in vitro experiments were done at 37 °C in duplicate with standard deviation of ±10%.
[b]For method, see ref 5.
[c]Percent tumor growth inhibition (%TGI) was calculated from the quotient of the median tumor volume of the treatment group divided by the median tumor volume of the control group {(1-T/C)×100} when the latter reached 3000 mm^3. All compounds were administered at 12 mg/kg/inj (CPT content) intravenously qd7×3 in established (300 mm^3) colorectal LS174 subcutaneous tumors.

better than control and demonstrated enhanced activity over the unmodified drug as well in this model.

Conclusion

It is probable that 10-HCPT exists as a strongly H-bonded intermolecular species such as **1b** depicted in Figure 1, and does not easily undergo acylation at either position. On the other hand, compound **2** (10-methoxy-CPT), unlike10-HCPT (**1**), undergoes facile acylation at the 20-OH position clearly demonstrating the effect of blocking the phenolic OH and, in turn, intermolecular hydrogen bonding. Selective high yield acylation of the 10-OH position of **1** can be achieved with carboxylic acids under neutral conditions using phenyl dichlorophosphate (**5**) as condensing agent. It is not clear at this time whether **5** is superior for the acylation of **1** due to the neutrality of the conditions or due to the properties of the intermediates, but it is clear that **5** has the capability of acylation even in the presence of the H-bonding form (**1b**) compared to other acylating agents.

Experimental

General

All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. 10-Hydroxycamptothecin was obtained from Hande Tech USA, Inc. (Houston, TX, USA). ^1H spectra were obtained with a JEOL FT NMR System JNM GSX-270 instrument using deuteriochloroform as solvent. ^{13}C NMR spectra were obtained at 67.80 MHz on the JNM GSX-270. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and coupling constants (*J* values) are given in hertz (Hz).

Preparation of 10-methoxycamptothecin (2). A mixture of 10-hydroxycamptothecin (**1**, 50 mg, 0.14 mmol), potassium carbonate (100 mg, 0.72 mmol), and iodomethane (50 μL, 0.80 mmol) in acetone (8 mL) was refluxed overnight. The mixture was filtered and the filtrate concentrated. The residue was recrystallized from MeOH–Et_2O to give **2** (53 mg, 80%) as a yellow solid: ^1H NMR δ 1.04 (t, 3H, *J* = 13.5, H-18), 1.94 (q, 2H, *J* = 13.5, H-19), 3.99 (s, 3H, 10-OCH_3), 5.25 (s, 2H, H-5), 5.31 (d, 1H, *J* = 16.2, H-17), 5.64 (d, 1H, *J* = 16.2, H-17), 7.21 (d, 1H, *J* = 2.7, H-11), 7.48 (m, 1H, H-9), 7.66 (s, 1H, H-14), 8.04 (d, 1H, *J* = 8.1, H-12), 8.35 (s, 1H, H-7); ^{13}C NMR δ 7.10, 30.89, 49.79, 55.15, 65.37, 72.51, 97.79, 105.14, 117.90, 123.56, 128.82, 129.45, 129.71, 129.87, 144.24, 145.84, 149.20, 150.99, 157.49, 158.70, 173.33.

Preparation of 10-methoxy-20-PEG-camptothecin ester (6). PEG carboxylic acid (**3**, M_w 40,000, 0.5 g, 0.0124 mmol) was dissolved in anhydrous methylene chloride (20 mL) at room temperature followed by the addition of **2** (14 mg, 0.04 mmol). The reaction mixture was cooled to 0 °C in an ice bath. 2-Chloro-1-methylpyridinium iodide (**4**, 17 mg, 0.067 mmol) and DMAP (17 mg, 0.14 mmol) were added to the mixture at 0 °C. The reaction mixture was allowed to warm to room temperature and left for 24 h. The solution was washed with 0.1 N HCl (10 mL×2), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a light yellow syrup. The crude product was crystallized from 2-propanol to give **6** as a white solid (0.39 g, 80%). The amount of **1** in **6** measure by UV assay[14] was 1.8% (wt/wt): ^1H NMR δ 0.98 (t, 3H, *J* = 5.4, H-18), 2.21 (m, 2H, H-19), 3.37–3.93 (m, PEG) 3.99 (s, 3H, 10-OCH_3), 4.36 (s, 2H, PEG–CH_2CO_2–), 5.28 (s, 2H, H-5), 5.41 (d, 1H, *J* = 16.2, H-17), 5.68 (d, 1H, *J* = 16.2, H-17), 7.14 (s, 1H, H-11), 7.41 (s, 1H, H-9), 7.51 (s, 1H, H-14), 8.09 (d, 1H, *J* = 8.1, H-12), 8.33 (s, 1H, H-7); ^{13}C NMR δ 7.15, 31.28, 49.58, 55.33, 66.70–70.77 (PEG), 94.66, 105.15,

119.08, 123.33, 128.64, 129.25, 130.50, 144.56, 144.97, 146.33, 169.61, 156.87, 158.56, 166.80, 169.18.

Preparation of 10-PEG-camptothecin ester (7). A mixture of **3** (M_w 40,000, 1.0 g, 0.025 mmol) and **1** (35 mg, 0.096 mmol) in toluene (30 mL) was dried by azeotropic distillation of 15 mL of toluene. The mixture was cooled to room temperature and the solvent removed by distillation in vacuo. Anhydrous chloroform (30 mL) was added to the mixture followed by addition of pyridine (1.0 mL, 12.4 mmol) and phenyl dichlorophosphate (**5**, 0.15 mL, 1.0 mmol). The reaction mixture was stirred at room temperature for 18 h. The solution was washed with ice-cold 1 N HCl (25 mL×2), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the product as a light yellow solid. After recrystallization from 2-propanol **7** was obtained as a white solid (0.8918 g, 89% yield). The amount of **1** in **7** measured by UV assay[14] was 1.7% (wt/wt): ^{1}H NMR δ 0.98 (t, 3H, J = 11.2 Hz, H-18), 1.83 (q, 2H, J = 8.6, H-19), 2.94 (s, H_2O in PEG), 3.38–3.90 (bs, PEG), 4.53 (s, PEG–CH_2–CO_2–), 5.31 (s, 2H, H-5), 5.19–5.69 (ABq, 2H, J = 108.21 & 27.06, H-17), 7.2 (d, 1H, J = 11.21, H-11), 7.64 (s, 1H, H-9), 7.82 (s, 1H, H-14), 8.18 (d, 1H, J = 11.88, H-12), 8.44 (s, 1H, H-7); ^{13}C NMR δ 7.33, 31.10, 49.53, 65.65, 68.13, 70.03–70.97 (PEG), 77.92, 97.45, 118.24, 118.42, 125.05, 127.94, 128.85, 130.45, 130.78, 145.57, 146.35, 148.55, 149.64, 152.07, 156.97, 168.30, 172.98.

Preparation of 6-mPEG-hydroxyquinoline ester (9). A mixture of 6-Hydroxyquinoline (**8**, 129 mg, 0.89 mmol), mPEG acid (**3a**, M_w 5,000, 1.5 g, 0.3 mmol), and EDC·HCl (228 mg, 1.19 mmol) in anhydrous pyridine (20 mL) was stirred at room temperature overnight. The mixture was concentrated in vacuo followed by crystallization of the residue from 2-propanol to give **9** (1.2 g, 80%): ^{13}C NMR δ 57.44, 66.80–71.81 (PEG), 117.19, 120.53, 123.22, 127.13, 129.63, 134.54, 144.84, 146.72, 149.05, 167.59.

Preparation of 6-PEG-hydroxyquinoline ester (10). Compound **8** was subjected to the same conditions for the conversion of **1** to **7** to give **10** in 89%: ^{13}C NMR δ 67.92–72.85 (PEG), 118.21, 120.38, 123.32, 128.42, 130.69, 135.65, 146.13, 148.09, 150.15, 168.35.

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