行政院國家科學委員會補助專題研究計畫成果報告

設計合成喜樹鹼之葡萄糖醛酸的前驅物用於治療癌

計畫類別：√個別型計畫　　□整合型計畫

計畫編號：NSC90－2320－B－041－005－

執行期間：90 年 08 月 31 日至 91 年 07 月 31 日

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中　華　民　國　91 年　10 月　8 日

Design and synthesis of 10-hydroxycamptothecin glucuronide for antibody-directed enzyme prodrug therapy (ADEPT)

中文摘要

本計劃我們設計合成 10-羥基喜樹鹼之葡萄糖醛酸的前驅藥物, 此前驅藥物是將喜樹鹼與葡萄糖醛酸間以一個芳香環的空間捧利用醚鏈的方式連接而成, 此前驅藥可藉β-glucuronidase 催化水解葡萄糖醛酸, 游離的電子藉 1,6-離去反應後, 釋放出活性的 10-羥基喜樹鹼, 空間捧的芳香環之鄰位有一個好的離去基, 可以加速 1,6-離去反應. 本計劃我們証實 glucuronide benzyl ether 可以藉 1,6-離去反應自動分解, 而釋放出活性的藥物, 這是一個新穎的藥物釋出策略.

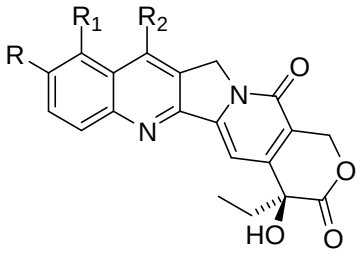
Abstract

We designed and synthesized of 10-hydroxycamptothecin glucuronide (**10-HCG**), in which 10-hydroxycamptothecin was connected to glucuronic acid by an aromatic spacer via ether linkage. **10-HCG** is activated by β-glucuronidase-mediated cleavage, leading to a 1,6-elimination reaction that release 10-hydroxycamptothecin and quinone methide. A good withdrawing group (-NO_2) in ortho position of the aromatic ring may accelerate electron transfer in 1,6-elimination reaction. Here we describe a novel drug release strategy involving the fragmentation of glucuronide benzyl ether derivates.

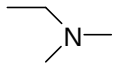
INTRODUCTION

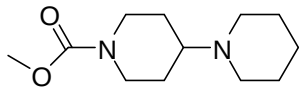
Chemotherapy plays an important role in cancer therapy. A major limiting factor in cancer chemotherapy is the toxicity of cytotoxic agents to normal tissues (1). Attempt to solve this problem have led to tumor targeting approach. The development of prodrugs can be activated selectively in tumor tissue (2). Prodrugs can be transformed to form the pharmacologically active species either by metabolism or by enzymatic hydrolysis after administration (3). Ideally, the activation of a prodrug should be restricted in the site of treatment. Antibodies to tumor-associated proteins have been used in the development of antibody-directed enzyme prodrug therapy (ADEPT). This concept was developed and described by Bagshawe and Senter (4- 7). In this approach, monoclonal antibodies are employed to target an enzyme to cancer cells. ADEPT can activate subsequently administered prodrug. Selective activation of prodrugs at neoplastic cells can increase the concentration of active drug in tumors (8-9), reduce systemic toxicity (10), and allow bystander killing of antigen-negative cancer cells (11-12).

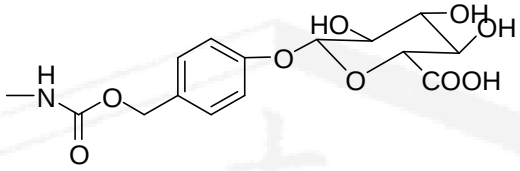
20(S)-Camptothecin (**1**), an antitumor alkaloid first isolated from Camptotheca acuminata (Nyssaceae) by Wall and co-workers in 1966 (13), inhibits the activity of topoisomerase I and displays antitumor activity in various experimential tumor models (14). Camptothecin, however, is difficult to formulate 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 (15-19). Two water soluble derivatives, topotecan (**2**) and irinotecan (CPT-11, **3**), are approved for clinical use. In previous study, we have been synthesized of 9-aminocamptothecin glucuronide (**4**, **9-ACG**)(20). **9-ACG** was stable in both aqueous solution and human plasma, 9-ACG was over 80 times more soluble than 9-aminocamptothecin in aqueous of solution at pH 4.0. **9-ACG** was 25-60 times less toxic than 9-aminocamptothecin to five human cell lines. The strong antitumor activity observed after combined treatment withβ-glucuronidase and **9-ACG** in human cell lines to produce similar cell killing as 9-aminocamptothecin. The *in vivo* toxicity of **9-ACG** in BALB/c mice was dose-, route-, sex-, and age-dependent (21). **9-ACG** was significantly less toxic to female than to male mice but the difference decrease with age. **9-ACG** cured a high percentage of CL1-5 human lung cancer xenogragt with efficacy that was similar to or greater than 9-aminocamptothecin. The potent anti-tumor activity of **9-ACG** suggests that this prodrug should be further evaluated for cancer treatment.



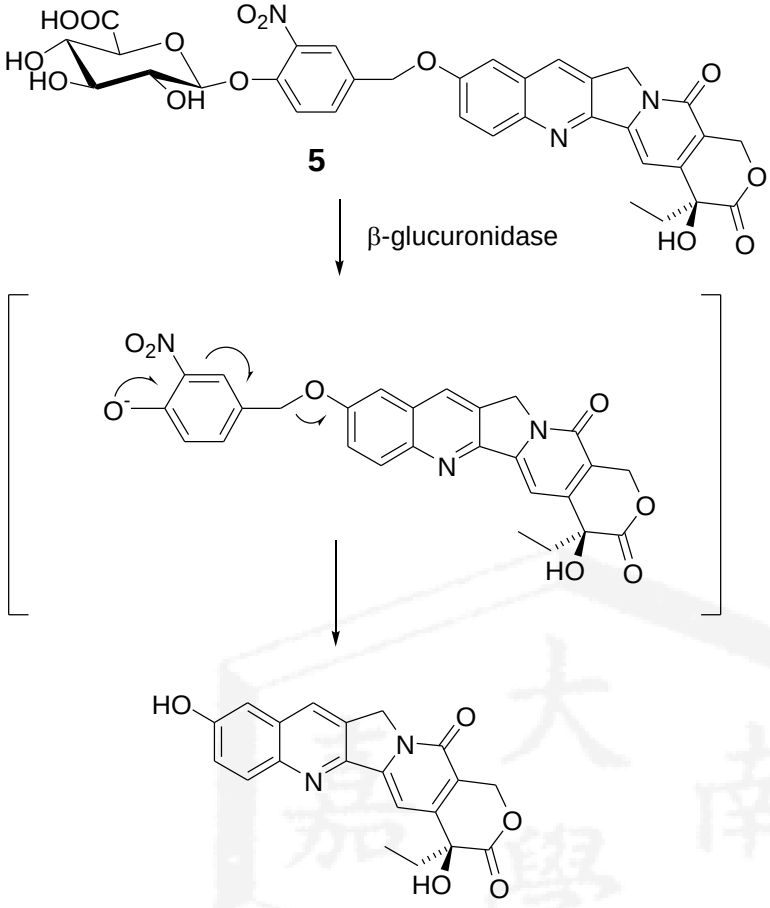
1, 20(S)-Camptothecin, R = R_1 = R_2 = H

2, Topotecan, R = OH, R =  R_2 = H

3, CPT-11, irinotecan, R_1 = H, R_2 = Et, R = 

4, 9-ACG, R_1 = R = R_2 = H 

However, the cost of **9-ACG** synthesize was expensed, because 9-aminocamptothecin was prepare by three steps from 10-hydroxycamptothecin. To circumvent this shortcoming, we also designed and synthesized of 10-hydroxycamptothecin glucuronide (**10-HCG**), in which 10-hydroxycamptothecin was connected to glucuronic acid by an aromatic spacer via ether linkage. **10-HCG** is activated by β-glucuronidase-mediated cleavage, leading to a 1,6-elimination reaction that release 10-hydroxycamptothecin and quinone methide. A good withdrawing group (-NO_2) in ortho position of the aromatic ring may accelerate electron transfer in 1,6-elimination reaction. Here we describe a novel drug release strategy involving the fragmentation of glucuronide benzyl ether derivates.



6, 10-hydroxycamptothecin

MATERIALS AND METHODS

Materials

10-hydroxycamptothecin was obtained from china.

Chemistry

Melting points were determined with a Kofler apparatus and are uncorrected. Reaction courses were routinedly monitored by thin-layer chromatography (TLC) on silica gel precoated Durasil-25 UV254 Merck plates with detection under 254 nm UV lamp or heating. Nuclear magnetic resonance (1H-NMR) spectra were determined in DMSO-d6 or CDCl3 solution with a Bruker AC-200 spectrometer and chemical shifts are given in ppm from internal tetramethylsilane as a standard. Column chromatography was performed with Merck 70-230 mesh silica gel. Reverse column chromatography was performed with LiChroprep RP-18 (40-63 μm).

Preparation of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**7**). A mixture of D (+)-glucurono-3, 6-lactone (44.5 g, 249.83 mmol) and CH_3ONa (0.46 g, 8.52 mmol)

in methanol (300 mL) was stirred at room temperature for 11 h. The reaction mixture was concentrated under reduced pressure to give a yellow-orange oily residue. To the residue was added acetic anhydride (170 mL). A solution of perchloric acid (0.8 mL) in acetic anhydride (10 mL) was then added to the mixture drop-wise in an ice bath and stirred for 2 h. The resulting precipitate was collected by filtration to give **2** (43.2 g, 46%). mp 140 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.98 (t, J = 4.6 Hz, 9 H), 2.07 (s, 3 H), 3.62 (s, 3 H), 4.65 (d, J = 9.8 Hz, 1 H), 4.98 (q, J = 8.4 Hz, 2 H), 5.50 (t, J = 9.5 Hz, 1 H), 6.00 (d, J = 8.1 Hz, 1 H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.0, 21.1, 21.2, 21.3, 53.5, 69.7, 70.7, 71.7, 72.3, 91.5, 167.8, 169.6, 169.9, 170.2, 170.3.

Preparation of methyl 1-bromo-1-deoxy-2, 3, 4-tri-*O*-acetyl-α-D-glucopyranuronate (**8**). A solution of **7** (5.1 g, 13.31 mmol) and $TiBr_4$ (5.7 g, 13.79 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 24 h. The mixture was washed with ice water (50 mL) and saturated aqueous $NaHCO_3$ solution (50 mL), dried over Na_2SO_4, and evaporated to dryness to give **8** (5.1 g, 96%), which was used directly in the next step without further purification.

Preparation of methyl 1-*O*-(2-nitro-4-formylphenyl)-2, 3, 4-tri-*O*-acetyl-β-D-glucopyranuronate (**9**). A suspension of **8** (5.1 g, 12.84 mmol), 4-hydroxy-3-nitrobenzaldehyde (2.3 g, 13.76 mmol) and Ag_2O (3.2 g, 13.82 mmol) in CH_3CN (100 mL) was stirred at room temperature for 24 h. Ag_2O was filtered out. The solvent was removed under reduced pressure to give dark brown crude product, which was washed with methanol to give **9** (5.99 g, 96.5%). mp 180-182 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.02 (d, J = 3.6 Hz, 9 H), 3.64 (s, 3 H), 4.81 (d, J = 9.6 Hz, 1 H), 5.16 (q, J = 7.7 Hz, 2 H), 5.48 (t, J = 9.3 Hz, 1 H), 5.95 (d, J = 7.5 Hz, 1 H), 7.65 (d, J = 8.7 Hz, 1 H), 8.23 (d, J = 6.9 Hz, 1 H), 8.45 (d, J = 1.7 Hz, 1 H), 9.99 (s, 1 H); ^{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；EIMS *m/z* 424 (M^+ - $OCOCH_3$).

Preparation of methyl 1-*O*-[2-nitro-4-(hydroxymethyl)phenyl]-2, 3, 4-tri-*O*-acetyl–β-D-glucopyranuronate (**10**). A mixture of **9** (2.1 g, 4.14 mmol), $NaBH_4$ (0.5 g, 14.01 mmol) and silica gel (10.6 g) in *i*-PrOH/$CHCl_3$ (1:5) (200 mL) was stirred at 0 ℃ for 1 h. The reaction was quenched with water and filtered to remove silica gel. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a residue which was washed with EtOH to give **10** (1.39 g, 69%). mp 167-168 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.98 (d, J = 4.0 Hz, 9 H) 3.63 (s, 3 H), 4.49 (s, 2 H), 4.71 (d, J = 9.8 Hz, 1 H), 5.07 (t, J = 9.5 Hz, 2 H), 5.44 (t, J = 9.4 Hz, 2 H), 5.69 (d, J = 7.74 Hz, 1 H), 7.37 (d, J = 8.6 Hz, 1 H), 7.59 (d, J = 8.6 Hz, 1 H), 7.78 (s, 1 H); ^{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; FABMS *m/z* 426 (M^+ - $OCOCH_3$).

Preparation of 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) in CH_2Cl_2 (15 mL)was stirred with methanesulfonyl chloride (0.06 mL, 0.78 mmol) and triethylamine (0.1 mL, 0.71 mmol) at 0 ℃ for 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution, dried with anhydrous $MgSO_4$, and evaporated to dryness to give **11** (340 mg, 98%). mp 110-112 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.00 (s, 9 H), 3.27 (s, 3 H), 3.63 (s, 3 H), 4.74 (d, J = 9.8 Hz, 1 H), 5.10 (q, J = 7.48 Hz, 2 H), 5.29 (s, 2 H), 5.46 (t, J = 9.4 Hz, 1 H), 5.77 (d, J = 7.7 Hz, 1 H), 7.47 (d, J = 8.4 Hz, 1 H), 7.79 (d, J = 8.6 Hz, 1 H), 8.02 (s, 1 H); FABMS *m/z* 562 (M^+ - 1).

Preparation of 10- [4-*O*- (Methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate)-3-nitro benzyloxy]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 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.87 (t, J = 7.1 Hz, 3 H, CH_3) 1.80-2.00 (m, 2 H, CH_2), 2.01-2.03 (m, 9 H, CH_3), 3.63 (s, 3 H, OCH_3), 4.71 (d, J = 9.7 Hz, 1 H, sugar-H), 5.08-5.18 (m, 2 H, sugar-H), 5.25 (s, 2 H, CH_2), 5.31 (s, 2 H, CH_2), 5.40-5.52 (m, 1 H, sugar-H), 5.74 (d, J = 8.0 Hz, 1 H, sugar-H), 6.52 (s, 1 H, OH), 7.27 (s, 1 H, Ar-H), 7.35-7.71 (m, 3 H, Ar-H), 7.74-7.95 (m, 1 H, Ar-H), 8.10 (s, 2 H, Ar-H), 8.53 (s, 1 H, Ar-H); FABMS *m/z* 832 (M^+). Anal. ($C_{40}H_{37}N_3O_{17}$ • H_2O): Calcd, C, 56.54; H, 4.63; N, 4.95; Found, C, 56.65; H, 4.32; N, 4.86.

Preparation of 10-[4-*O*- (Methyl-β-D-glucopyranuronate)-3-nitrobenzyloxy] camptothecin (**13**). A suspension of **12** (360 mg, 0.43 mmol) and sodium methoxide (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 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, J = 7.2 Hz, 3 H, CH_3), 1.85-1.87 (m, 2 H, CH_2), 3.32-3.48 (m, 3 H, sugar-H), 3.64 (s, 3 H, OCH_3), 4.13 (d, J = 8.9 Hz, 1 H, sugar-H), 5.26-5.53 (m, 9 H), 6.51 (s, 1 H), 7.27 (s, 1 H), 7.47-7.62 (m, 3 H), 7.81 (d, J = 8.3 Hz, 1 H), 8.08 (d, J = 8.6 Hz, 2 H), 8.53 (s, 1 H); FABMS *m/z* 706 (M^+ - 1). Anal. ($C_{34}H_{31}N_3O_{14}$ • EtOH): Calc, C, 57.52; H, 4.96; N, 5.59; Found, C, 57.19; H, 4.97; N, 5.21.

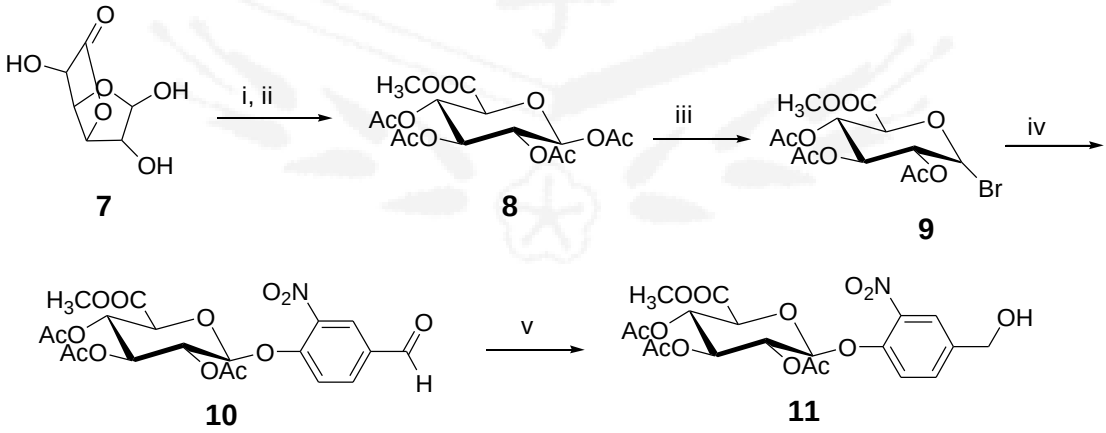
Preparation of 10-[4-*O*- (β-D-glucopyranuronate)-3-nitrobenzyloxy]camptothecin(**6**). A suspension of **13** (110 mg, 0.16 mmol) and potassium trimethylsilanolate (105 mg, 0.43 mmol) in anhydrous THF (30 mL) was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure to give a residue, which was dissolved with water, and 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 **6** (13 mg, 12%). mp 196-197 ℃, ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.88 (t, J = 7.2 Hz, 3 H, CH_3), 1.84-1.85 (m, 2 H, CH_2), 3.12-3.39 (m, 3 H), 3.96 (d, 3 H), 5.22-5.83 (m, 8 H), 6.54 (s, 1 H), 7.29 (s, 1 H), 7.42-7.89 (m, 4 H), 8.08 (d, J = 8.6 Hz, 2 H), 8.57 (s, 1 H); FABMS *m/z* 692 (M^+). Anal. ($C_{33}H_{29}N_3O_{14}$ • 2HCl • $6H_2O$): Calc, C, 45.42; H, 4.97; N, 4.82; Found, C, 45.34; H, 4.82; N, 4.64.

RESULTS

Chemistry

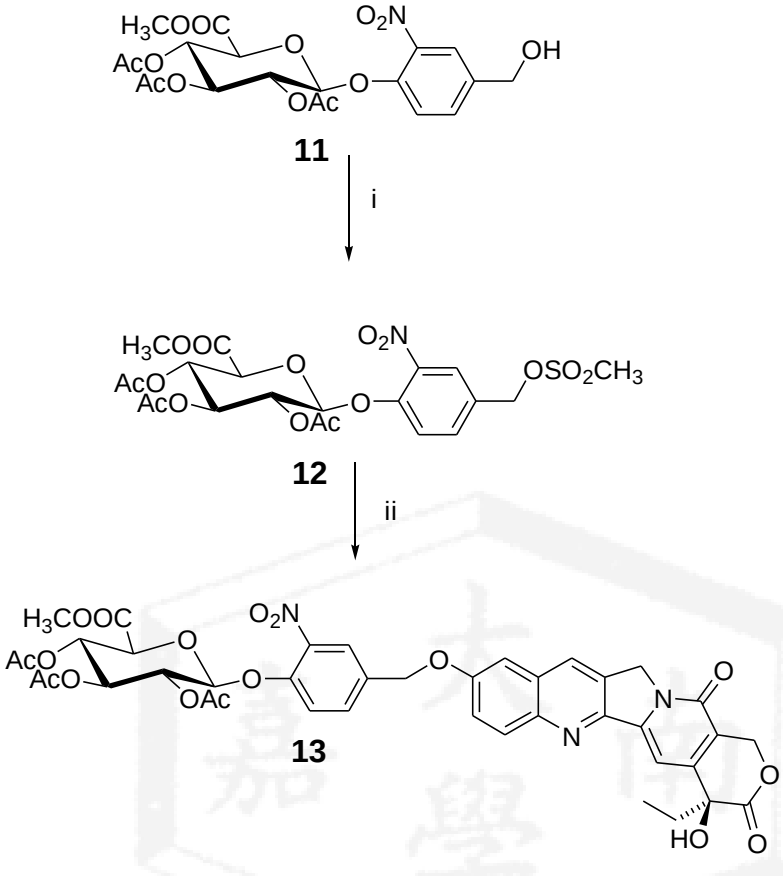
Methyl 1, 2, 3, 4-tetra-O-acetyl β-D-glucuronate (**7**) prepared as described, was brominated with $TiBr_4$, and then reacted with 4-hydroxy-3-nitrobenzaldehyde in the presence of Ag_2O to afford **9** in 96% yield. The aldehyde group in **9** was hydrogenated to benzyl alcohol by treatment with $NaBH_4$ to yield **10** in 70% yield. We have been coupling benzyl alcohol of **10** and hydroxy group of 10-hydroxycamptothecin by Mitsunobu reaction, but this reaction cannot achievable. So, compound **10** was reacted with mesyl chloride in dichloromethane to give compound **11** in 98% yield. 10-hydroxycamptothecin was treatment with cesium carbonate before addition of **11** at room temperature to afford **12** in 47% yield. We have been treated various base in this step, but that cannot contenting this ether linkage. The O-acetyl of **12** was deprotected with sodium methoxide in MeOH to yield **13**. The methyl ester **13** was deprotected with potassium trimethylsilanolate in THF to obtain the potassium salt of **6**. Accification of the potassium salt of **6** with 1N HCl and purified by reverse phase MPLC to yield target compound **6**. This synthetic strategy can provide the novel method to prepare of benzyl ether linkage. The prodrug is capable of undergoing 1,6-elimination reaction after β-glucuronidase exposure, which provide to release active 10-hydroxycamptothecin.

Scheme I



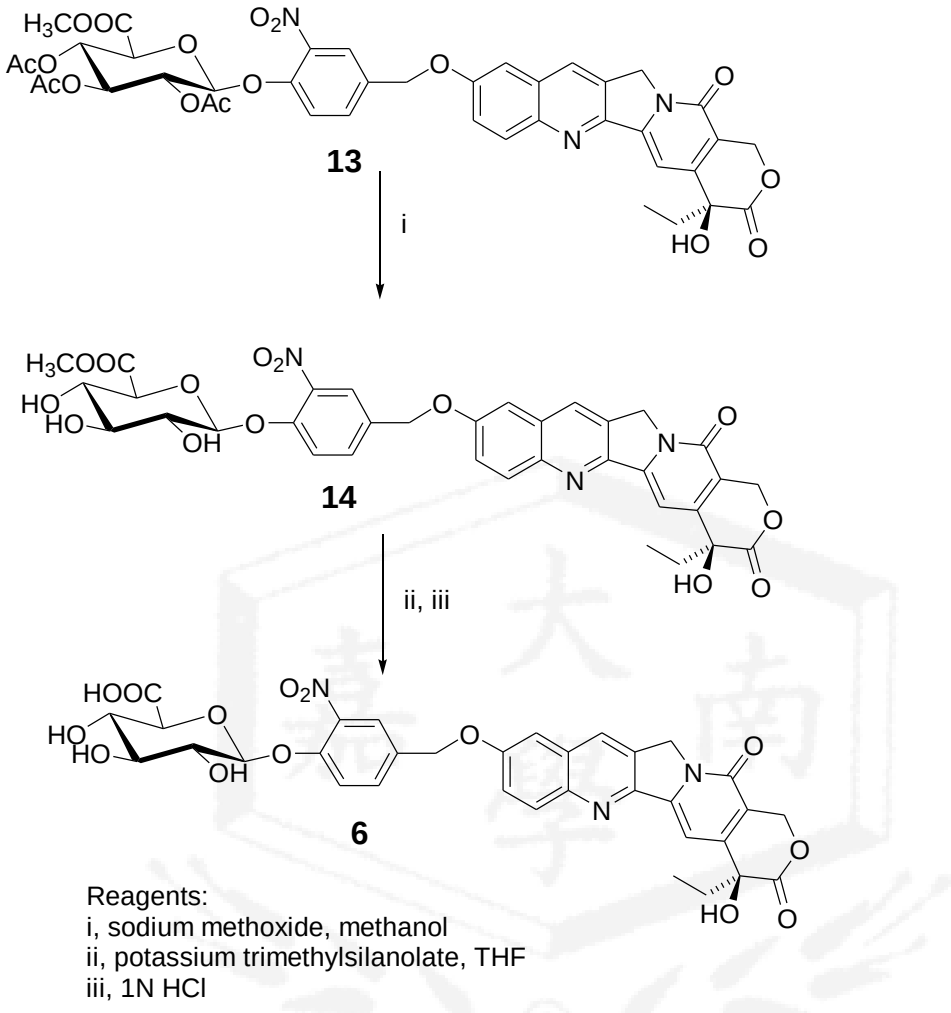
Reagents:
i, sodium methoxide, MeOH
ii, $HClO_4$, acetic anhydride
iii, $TiBr_4$, CH_2Cl_2
iv, 4-hydroxy-3-nitrobenzaldehyde, Ag_2O, CH_3CN
v, $NaBH_4$, silica gel, i-PrOH, $CHCl_3$

Scheme II



Reagents:
i, mesyl chloride, CH_2Cl_2, TEA
ii, 10-hydroxycamptothecin, Cs_2CO_3, DMF

Scheme III



DISCUSSION

We designed and synthesized of 10-hydroxycamptothecin glucuronide (**10-HCG**). **10-HCG** was connected 10-Hydroxycamptothecin and glucuronic acid by an aromatic spacer via ether linkage. In this synthesized strategy has high yield in all steps, the benzyl alcohol was coupling with mesyl chloride to create a leaving groups, compound **12** was connected to the hydroxy group of 10-hydroxycamptothecin by cesium carbonate. **10-HCG** is activated by β-glucuronidase- mediated cleavage glucuronic acid, leading to a 1, 6-elimination reaction that release 10-hydroxycamptothecin and a quinone methide. Here we describe a novel drug release strategy involving the fragmentation of glucuronide benzyl ether derivates.

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