Practical Synthesis of (*S*)-7-(2-Isopropylamino)ethylcamptothecin Hydrochloride, Potent Topoisomerase I Inhibitor

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A practical semi-synthetic method of (*S*)-7-(2-isopropylamino)ethylcamptothecin hydrochloride has been developed. The Mannich reaction of (*S*)-7-methylcamptothecin with isopropylamine hydrochloride in dimethyl sulfoxide as a formaldehyde source gave the desired product in moderate yield.

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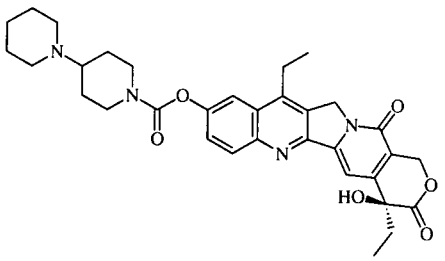
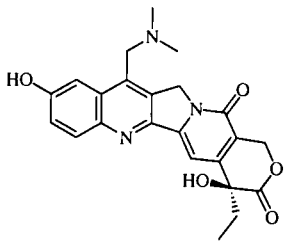
With their unique topoisomerase I inhibitory activity, camptothecin analogues have recently become one of the most actively studied anticancer agents [1]. To date numerous camptothecin analogues have been synthesized to improve the water solubility [2]. So far, irinotecan (**1**) [3] and topotecan (**2**) [4] are already on the market and 9-aminocamptothecin (**3**) [5], lurtotecan (**4**) [6] and DX-8951 (**5**) [7] are undergoing clinical trials.

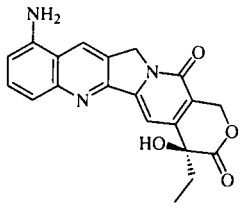
In our efforts to develop novel camptothecin derivatives with increased potency and water solubility, we have synthesized several 7-(2-alkylamino)ethylcamptothecin derivatives using a total synthetic method and have evaluated their anticancer activity [8], [9]. Among them, (*S*)-7-(2-isopropylamino)ethylcamptothecin hydrochloride (**6**) demonstrated greater activities and higher safeties than topotecan or 7-(2-methylamino)ethylcamptothecin [4] in



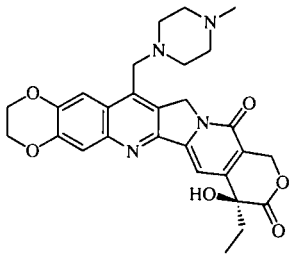
irinotecan **1**



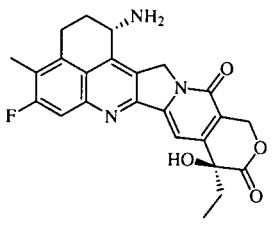
topotecan **2**



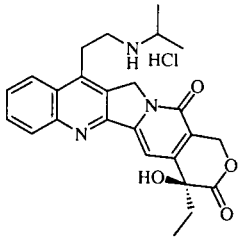
9-aminocamptothecin **3**



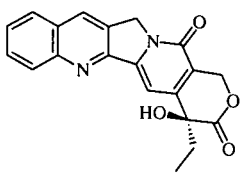
lurtotecan **4**



DX-8951 **5**



7-(2-isopropylamino)-ethylcamptothecin **6**

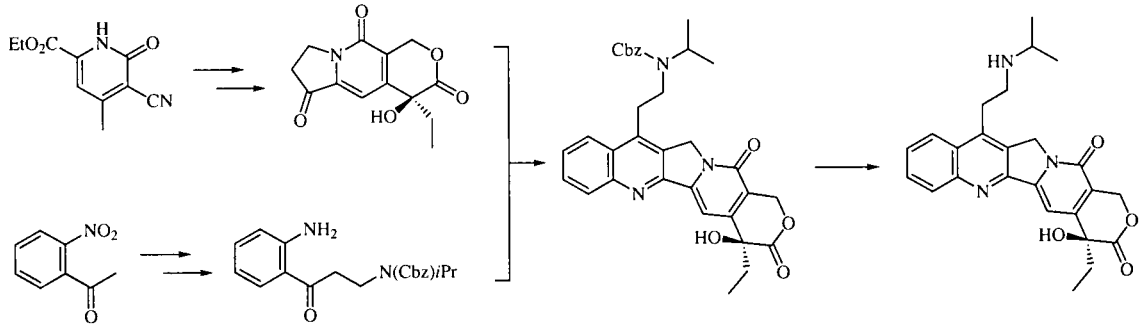


(*S*)-camptothecin **7**

preclinical tumor models [10], [11], [12]. As a result, (*S*)-7-(2-isopropylamino)ethylcamptothecin hydrochloride (**6**) was selected as a candidate for further development and is currently undergoing phase I clinical trials.

However, during the large-scale total synthesis of compound **6**, we encountered many problems including the long reaction steps, the low overall yields and the unsatisfactory optical purities (Scheme 1). Also, the total synthetic method of racemic 7-(methylamino)ethylcamptothecin derivatives [4] was impractical for us.

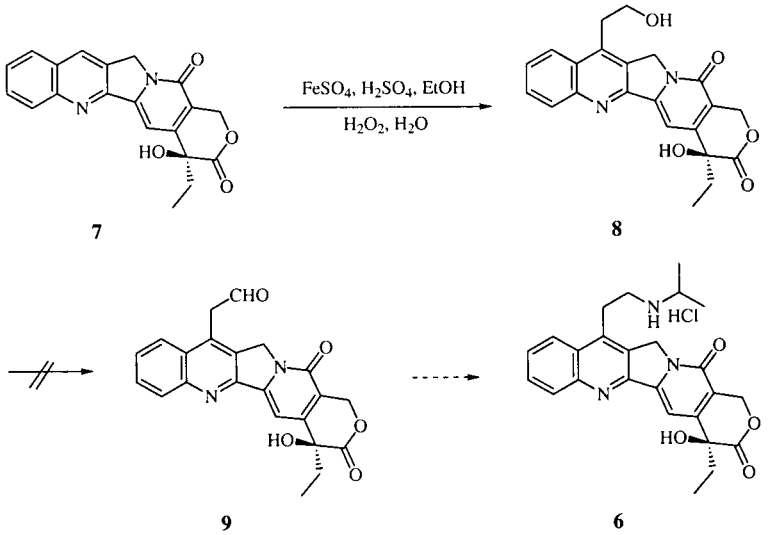
Scheme 1



We therefore envisaged the semi-synthetic preparation of compound **6** from commercially available (*S*)-camptothecin (**7**). Our initial attempts to prepare compound **6** from (*S*)-7-(2-hydroxyethyl)camptothecin (**8**) [13] *via* oxidation followed by reductive alkylation were unsuccessful (Scheme 2). Oxidation of (*S*)-7-(2-hydroxyethyl)camptothecin with pyridinium dichromate, pyridinium chlorochromate or Swern reagents failed to give us aldehyde **9.**

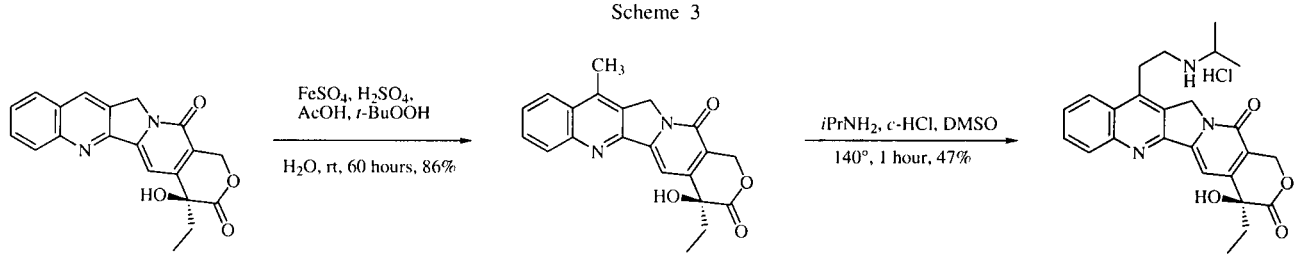
Therefore, we pursued the Mannich reaction of (*S*)-7-methylcamptothecin (**10**) [13]. However, the Mannich reaction of (*S*)-7-methylcamptothecin with formaldehyde and isopropylamine hydrochloride in various solvents failed to give the desired product. The reasons for this failure might include the notorious insolubility of camptothecin derivatives in organic solvents and the low reactivity of (*S*)-7-methylcamptothecin. When we tried the above reaction in acetic acid in the presence of formalin, the desired product was obtained in 5% yield. When the Mannich reaction was carried out in dimethyl sulfoxide in the presence of paraformaldehyde or formalin, the desired product was obtained in moderate yield. In addition, the desired product was obtained in even higher yield without adding any formaldehyde (Scheme 3). Dimethyl sulfoxide might work as a formaldehyde source in acidic conditions at high temperature [14], [15]. Compound **6** was obtained in a maximum yield (47%) by heating the solution of 7-methylcamptothecin (**10**) in excess dimethyl sulfoxide for 1 hour at 140° in the presence of concentrated hydrochloric acid (7 equivalents) and isopropylamine (6 equivalents). Longer reaction time, higher reaction temperature or other acids such as concentrated sulfuric acid or zinc chloride gave lower yields and more unidentified by-products. To our best knowledge, this result is the first synthetic application of the Mannich reaction using

Scheme 2



7 **8** **9** **6**

Scheme 3



7 10 6

dimethyl sulfoxide as a formaldehyde source [16].

We also improved the reported preparation method of (*S*)-7-methylcamptothecin from camptothecin. The reported preparation method of (*S*)-7-methylcamptothecin includes the extraction of the reaction mixture with large amounts of solvent and the tedious chromatographic separation step. By diluting the reaction mixture with cold water, we could get (*S*)-7-methylcamptothecin by a simple filtration of the precipitated solid.

In summary, we developed a short and efficient practical synthetic method for the preparation of (*S*)-7-(2-isopropylamino)ethylcamptothecin hydrochloride from (*S*)-7-methylcamptothecin *via* the Mannich reaction using dimethyl sulfoxide as a formaldehyde source.

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The ^1H and ^{13}C nmr spectra were recorded on a Bruker DPX-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. The ir spectra were recorded on a Bruker Vector 22 infrared spectrophotometer. Optical rotation was recorded on a Rudolph AUTOPOL-IV polarimeter. The electron impact mass spectrum was determined with a Hewlett-Packard 5989B instrument at 70eV. The high resolution mass spectrometry was determined on a Hewlett-Packard 5890-JMS AX505WA spectrometer. Elemental analysis was performed on a Perkin-Elmer 240 instrument. (*S*)-Camptothecin was purchased from Wenzhou Pharmaceutical Factory in the People's Republic of China.

(*S*)-7-Methylcamptothecin (**10**).

To a suspension of 100 g (0.28 mole) of (*S*)-camptothecin (**7**) in 3 l of water containing 40 g of ferrous sulfate heptahydrate (0.14 mole) and 300 ml of acetic acid (5.24 mole), was added 800 ml of concentrated sulfuric acid (15.01 mole) dropwise in an ice-salt bath. And then 450 ml of 70% *tert*-butylhydroperoxide in water (3.28 mole) was added dropwise at the same temperature. The mixture was stirred at room temperature for 60 hours. To the reaction solution was added 4.5 kg of crushed ice and the mixture was stirred until the temperature of the reaction mixture reached 0°. The precipitate was collected by filtration, and the filter cake was washed with water until the pH of the filtrate reached 6~7 and dried to afford 90 g (86% yield) of **10** as a yellow powder, mp 280~281°; ir (potassium bromide): ν 3410, 1755, 1650, 1600 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 0.91 (t, 3H, J = 8 Hz), 1.88 (q, 2H, J = 8 Hz), 2.79 (s, 3H), 5.26 (s, 2H), 5.41 (s, 2H), 6.43 (s, 1H), 7.34 (s, 1H), 7.57~8.32 (m, 4H); ms: (EI) m/z 362 (M^+).

(*S*)-7-(2-Isopropylamino)ethylcamptothecin hydrochloride (**6**).

To a solution of 20 g (0.055 mole) of (*S*)-7-methylcamptothecin (**10**) and 28 ml (0.328 mole) of isopropylamine in 400 ml of dimethyl sulfoxide was added 32 ml (0.389 mole) of concentrated hydrochloric acid and the mixture was heated for 1 hour at 140°. Water (2 l) was added to the reaction mixture and then filtered. After the filtrate was loaded on to 2 l of activated HP-20 resin, it was washed with water until the pH of the eluent reached 6. It was then eluted with 20% methanol-dichloromethane solution to obtain the product. The solvent was evaporated and the residue was purified through a silica gel column (dichloromethane:methanol = 20:1) to give 12.1 g (47% yield) of (*S*)-7-(2-isopropylamino)ethylcamptothecin hydrochloride (**6**), mp 267-268° dec; $[\alpha]_D$ = +53.49° (c 0.1, H_2O); ir (potassium bromide): ν 3410, 1753, 1661, 1597 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 0.87 (t, 3H, J = 8.0 Hz), 1.26 (d, 6H, J = 6.4 Hz), 1.83-1.90 (m, 2H), 3.13-3.19 (m, 2H), 3.34-3.45 (m, 1H), 3.60-3.64 (m, 2H), 5.37 (s, 2H), 5.43 (s, 2H), 6.52 (s, 1H), 7.32 (s, 1H), 7.74 (dd, 1H, J = 8.4, 8.0 Hz), 7.86 (dd, 1H, J = 8.4, 8.0 Hz), 8.17 (d, 1H, J = 8.4 Hz), 8.42 (d, 1H, J = 8.4 Hz), 9.30 (broad s, 1H); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 173.3, 157.7, 152.8, 150.9, 149.3, 146.5, 139.7, 131.0, 130.8, 130.5, 128.8, 127.6, 124.9, 120.0, 97.6, 73.2, 66.1, 50.7, 50.5, 43.7, 31.2, 26.9, 19.6, 19.5, 8.6; High resolution ms: (FAB) m/z (MH^+) calcd. 434.2080, obsd. 434.2073.

Anal. Calcd. for $C_{25}H_{27}ClN_3O_4 0.5H_2O$: C, 62.59; H, 6.09; N, 8.76. Found: C, 62.57; H, 5.92; N, 8.63.

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