

Full Paper

## Synthesis and Molecular Structure of Ethyl [N-tosyl-(R)-prolyloxy]-2(S)-[4-cyano-8,8-ethylenedioxy-5-oxo-5,6,7,8-tetrahydroindolizin-3-yl] Acetate, a Key Intermediate in the Total Synthesis of (20S)-Camptothecins

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**Abstract:** The synthesis of optically active [N-tosyl-(R)-prolyloxy]-2(S)-[4-cyano-8,8-ethylenedioxy-5-oxo-5,6,7,8-tetrahydroindolizin-3-yl] acetate (**4a**), a key intermediate for the total asymmetric synthesis of 20(S)-camptothecin anticancer drugs, is described. Its structure was characterized by 2D-NMR techniques and the absolute configuration was further confirmed for the first time by X-ray crystal structure analysis.

**Keywords:** Ethyl [N-tosyl-(R)-prolyloxy]-2(S)-[4-cyano-8, 8-ethylenedioxy-5-oxo-5, 6, 7, 8-tetrahydroindolizin-3-yl] acetate; 2D-NMR techniques; X-ray crystal structure; configurational analysis.

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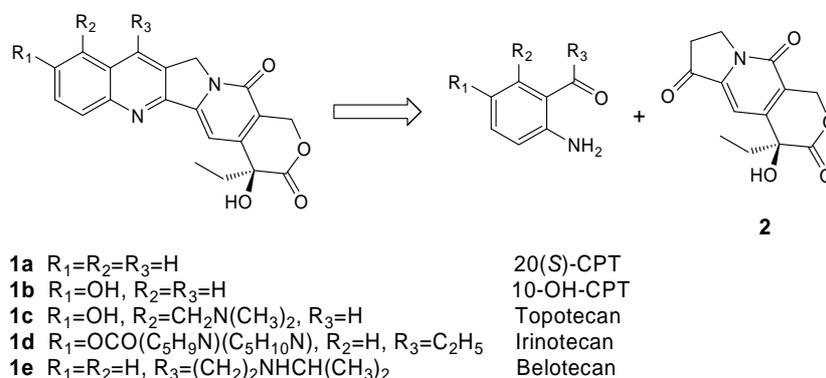
### Introduction

20(S)-Camptothecins [20(S)-CPTs] (Figure 1) have attracted lasting interest in organic synthetic chemistry due to their unique chemical structures and potent antitumor activities, and considerable effort has been devoted over the last thirty years to the development of elegant methods for the total synthesis of 20(S)-CPTs [1]. Among the numerous asymmetric synthesis approaches to these alkaloids described, the strategy utilizing the (S)-configuration tricyclic hydroxylactone **2** as a key chiral intermediate promises the most efficient industrial scale synthesis to 20(S)-CPTs. To date three

synthetic methods for the preparation of compound **2** have been reported using a chiral auxiliary to induce asymmetry [2], a chemocatalyst to catalyze asymmetric synthesis [3] and optical resolution [4]. We were especially interested in the asymmetric synthesis of compound **2** developed by Tagawa *et al.* [2] for its use of commercially available starting materials, mild reaction conditions and simple manipulations.

As a part of our continuing program [5] to explore a practical and commercial route of 20(*S*)-CPTs, herein we report the preparation and structural analysis of [*N*-tosyl-(*R*)-prolyloxy]-2(*S*)-[4-cyano-8,8-ethylenedioxy-5-oxo-5,6,7,8-tetrahydroindolizin-3-yl] acetate (**4a**), which is the key intermediate in the synthesis of lactone **2**.

**Figure 1.** Structures of 20(*S*)-camptothecin and its clinic drugs.

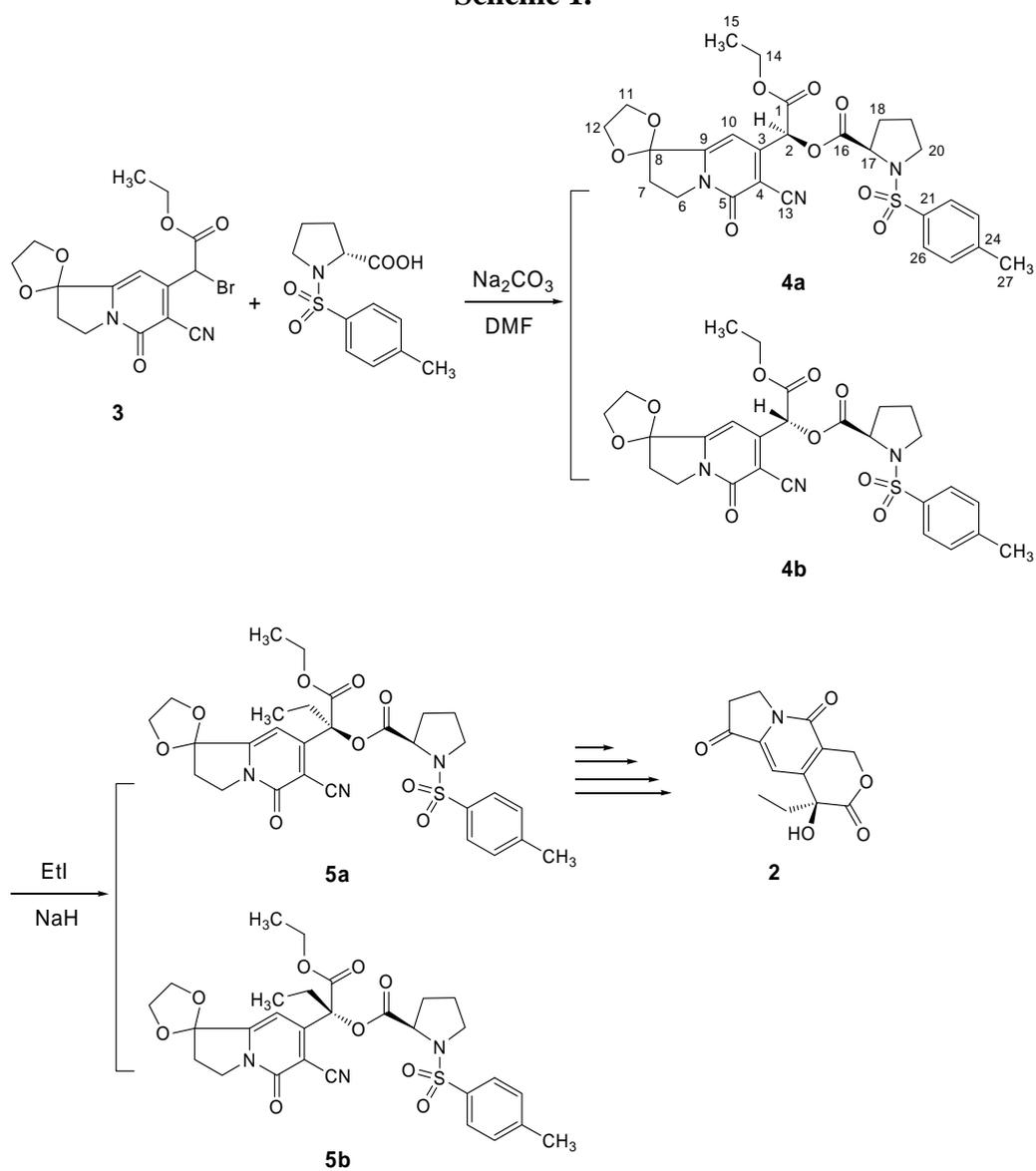
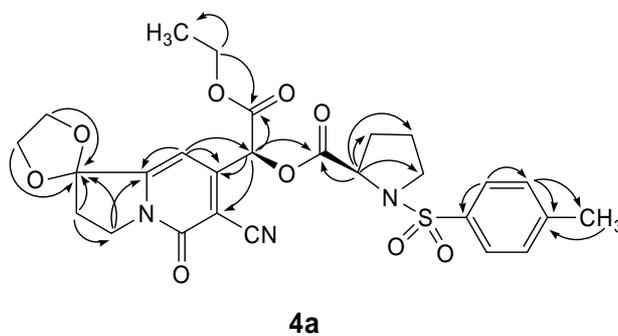


## Results and Discussion

The synthesis of optically active hydroxyl lactone **2** is depicted in Scheme 1. Firstly, 2-bromo-indolizine analogue **3**, prepared by the reported methods [6], was condensed with *N*-tosyl-(*R*)-proline to give a diastereoisomeric mixture **4a** and **4b**. Subsequently, the mixture afforded the appropriate optical active compound **5a** under the asymmetric induction of the chiral auxiliary reagent *N*-tosyl-(*R*)-proline *via* the diastereoselective ethylation and fractional crystallization from isopropanol. Finally, compound **5a** was treated by similar processes [2] to produce the key intermediate tricyclic hydroxyl lactone **2**.

Diastereoisomers **4a** and **4b** were obtained as a low melting (49.6–61°C) 1:1 mixture inseparable by chromatographic purification. However, it is interesting to note that the crude **4a/4b** mixture could be converted into isomer **4a** with a high melting point of 172.6–174.3°C in 70% yield by crystallization from methanol. This result was evident from the changes in their respective <sup>1</sup>H-NMR spectra. In the spectrum of the mixture there were two sets of signals ( $\delta_{\text{H}}$  6.65, 6.63 and  $\delta_{\text{H}}$  6.30, 6.19), representing the H-C(10) and H-C(2) protons of the two diastereoisomers **4a** and **4b**, respectively. After crystallization, the <sup>1</sup>H-NMR spectrum only showed the  $\delta_{\text{H}}$  6.65 and 6.30 signals of one isomer. The complete proton and carbon chemical shift assignment for the structure of isomer **4a** was established by the combination of 1D and 2D NMR experiments such as DEPT, H-H COSY, as well as HMQC and HMBC (see Table 1 and Figure 2). By comparing <sup>1</sup>H-NMR spectra data with those of similar compounds [4c], we elucidated the new chiral carbon atom C(2) to be (*S*)-configuration.

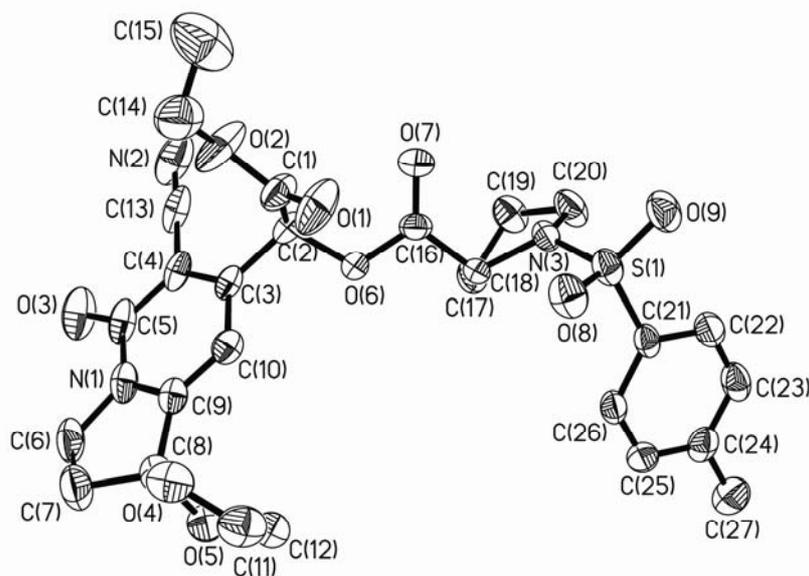
Scheme 1.

Figure 2. Important HMBC ( $\rightarrow$ ) correlations of compound **4a**.

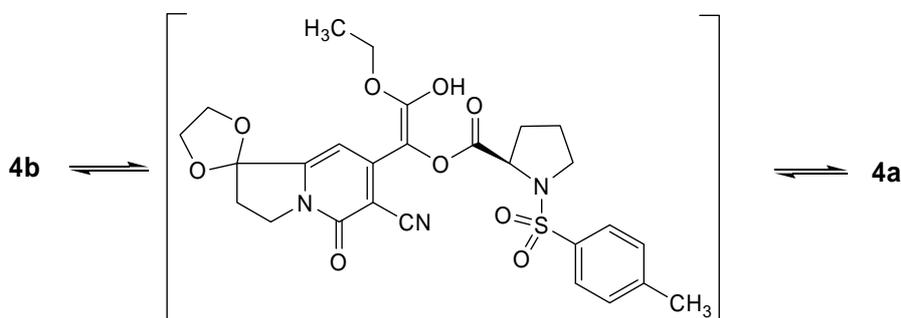
**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data for **4a** in  $\text{CDCl}_3$ ;  $\delta$  in ppm,  $J$  in Hz.

Position	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1		165.2 (s)
2	6.30 (s)	71.9 (d)
3		103.7 (s)
4		154.1 (s)
5		158.3 (s)
6	4.22 (m, H <sub>a</sub> ), 4.11 (m, H <sub>b</sub> )	45.7 (t)
7	2.44 (m)	33.5 (t)
8		112.9 (s)
9		154.4 (s)
10	6.65 (s)	97.9 (s)
11	4.31 (m, H <sub>a</sub> ), 4.11 (m, H <sub>b</sub> )	65.7 (t)
12	4.31 (m, H <sub>a</sub> ), 4.11 (m, H <sub>b</sub> )	66.3 (t)
13		113.7 (s)
14	4.31 (m, H <sub>a</sub> ), 4.22 (m, H <sub>b</sub> )	63.1 (t)
15	1.30 (t, $J = 7.1$ )	13.9 (q)
16		170.1 (s)
17	4.40 (dd, $J = 3.1, 5.6$ )	60.6 (d)
18	2.20 (m, H <sub>a</sub> ), 1.97 (m, H <sub>b</sub> )	30.9 (t)
19	2.10 (m, H <sub>a</sub> ), 1.80 (m, H <sub>b</sub> )	24.5 (t)
20	3.27 (m, H <sub>a</sub> ), 3.60 (m, H <sub>b</sub> )	48.6 (t)
21		143.9 (s)
22	7.72 (d, $J = 8.2$ )	127.3 (d)
23	7.31 (d, $J = 8.0$ )	129.8 (d)
24		134.7 (s)
25	7.31 (d, $J = 8.0$ )	129.8 (d)
26	7.72 (d, $J = 8.2$ )	127.3 (d)
27	2.43 (s)	21.5 (q)

To further confirm the absolute configuration of isomer **4a**, we performed an X-ray crystal diffraction analysis. A perspective view and the numbering scheme adopted for the **4a** molecule is depicted in Figure 3. The known (*R*)-configuration of the chiral atom C(17) of compound **4a** was characterized by the chemical correlation, and the O(6)-C(16)-C(17)-N(3) torsion angle of  $-163.7(3)^\circ$  and the O(6)-C(16)-C(17)-C(18) torsion angle of  $82.8(4)^\circ$ . As a result, the new stereogenic carbon C(2) is confirmed to be (*S*)-configuration by the C(3)-C(2)-O(6)-C(16) torsion angle of  $171.8(3)^\circ$  and the C(1)-C(2)-O(6)-C(16) torsion angle of  $-69.4(5)^\circ$ . The pyrrolidine ring of *N*-tosyl-(*R*)-proline segment is in an envelope conformation with the flap atom C18 lying  $0.553(7)\text{\AA}$  from the C(17)/N(3)/C(20)/C(19) plane. The phenyl ring almost locates at the right-angled position of the C(17)/N(3)/C(20)/C(19) plane with a dihedral angle of  $79.0(2)^\circ$ . Another pyrrolidine ring fused with pyridone ring is also in an envelope conformation with the flap atom C(7) lying  $0.442(8)\text{\AA}$  from the C(6)/N(1)/C(9)/C(8) plane.

**Figure 3.** ORTEP drawing and atom labelling scheme of compound **4a**.

In order to explain the (2*S*, 17*R*)-configuration preference of the title compound crystallized from methanol, the total energies of compounds (2*S*, 17*R*)-**4a** and (2*R*, 17*R*)-**4b** were calculated using the *HYPERCHEM* program [7] at the semi-empirical PM3 computational level, where a root-mean-square gradient of the forces acting on each atom of 0.05 kcal/molÅ as the convergence criterion was employed. The final energies of **4a** (-7332.73 a.u.) was slightly lower than that of **4b** (-7327.60 a.u.). Thus, qualitatively, there is energy advantage for the indolizine analogue **4a**. We conclude that there is a fast dynamic enol tautomerism equilibrium between compounds **4a** and **4b** (Figure 4). Upon changing the exterior condition such as heating, the equilibrium was destroyed and (2*R*, 17*R*)-**4b** was converted into the relatively stable (2*S*, 17*R*)-**4a** with lower energy.

**Figure 4.** Dynamic equilibrium of enol tautomerism between **4a** and **4b**

## Conclusions

For the first time we have prepared optically active [N-tosyl-(*R*)-prolyloxy]-2(*S*)-[4-cyano-8,8-ethylenedioxy-5-oxo-5,6,7,8-tetrahydroindolizin-3-yl] acetate (**4a**) and characterized it by 2D-NMR spectroscopy and X-ray crystallography. Theoretical calculations have further confirmed the configurational analysis. From the point of view of the synthetic strategy and atomic economy, it is

preferable if chiral centers are constructed as soon as possible in an asymmetric synthetic route. Use of optically active compound (2*S*, 17*R*)-**4a** as substrate instead of the usual mixture of diastereoisomers **4a** and **4b**, should help improve the stereoselectivity of the subsequent ethylation process. Further studies are under way to optimize the reaction conditions for this diastereoselective ethylation and to develop a more practical and scaled-up preparation of 20(*S*)-CPTs.

## Experimental Section

### General

Melting points were determined with a WRS-1B digital melting point apparatus and are uncorrected. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on a Bruker DMX (500 MHz) instrument in CDCl<sub>3</sub> solutions using TMS as an internal standard. Chemical shifts are given in the δ-scale (ppm). Mass spectra (MS) was performed on a MAT95 instrument [electronic impact (EI) at 70 eV]. Optical rotation was recorded on a JASCO P-1020 polarimeter. Diastereoisomeric excess (d.e.) was detected on an XTerra<sup>®</sup> MS C18 column (2.1×150 mm, Ø5 μm) using a Waters 2695 separations module (eluent: CH<sub>3</sub>CN/H<sub>2</sub>O=55:45; λ<sub>max</sub>=225 nm).

### Synthesis of ethyl [*N*-tosyl-(*R*)-prolyloxy]-2(*S*)-[4-cyano-8,8-ethylenedioxy-5-oxo-5,6,7,8-tetrahydro-indolizin-3-yl] acetate (**4a**)

The mixture of *N*-tosyl-(*R*)-proline (2.16 g, 8.04 mmol) and Na<sub>2</sub>CO<sub>3</sub> (0.34 g, 3.25 mmol) in anhydrous DMF (20 mL) was stirred for 10 minutes under nitrogen at room temperature. The solution of 2-bromoindolizine analogue **3** (1.4 g, 3.66 mmol) dissolved in anhydrous DMF (10 mL) was added to the above reaction mixture and stirred for another 1.5 hours at 70 °C. The reaction mixture was cooled, diluted with water (100 mL) and extracted with ethyl acetate (4 × 50 mL). The combined organic phase was successively washed with 5% NaHCO<sub>3</sub> solution (50 mL), water (50 mL), and brine (50 mL), then dried over MgSO<sub>4</sub> and evaporated to dryness. The residue was crystallized from methanol (45 mL) to give **4a** (1.46 g, 70%) with a melting point of 172.6-174.3 °C, [α]<sub>D</sub><sup>20</sup> +140.62° (*c* 1, CHCl<sub>3</sub>), d.e.>99%. <sup>1</sup>H- and <sup>13</sup>C-NMR data see Table 1.

### X-ray techniques

A block single crystal of the title compound **4a** suitable for X-ray diffraction was grown by slow evaporation from a methanol solution. Crystal and experimental data are summarized in Table 2. The data were collected with a Bruker SMART CCD area-detector diffractometer equipped with a graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å) at 293(2) K. The crystal structure data have been deposited at the Cambridge Crystallographic Data Center [8]. Absorption corrections were made with semi-empirical from equivalents. The crystal structure was resolved by direct methods using SHELXS-97 [9a] and refined by full-matrix least-squares methods on F<sup>2</sup> using SHELXL-97 [9b]. All non-H atoms were refined anisotropically.

**Table 2.** Crystal and experimental data for compound **4a**.

Formula	C <sub>27</sub> H <sub>29</sub> N <sub>3</sub> O <sub>9</sub> S
Formula weight	571.59
Crystal system	Trigonal
Space group	P3(2)
Unit-cell dimensions (Å)	<i>a</i> = 10.303(2) <i>b</i> = 10.303(2) <i>c</i> = 22.564(6)
Unit-cell volume, V (Å <sup>3</sup> )	2074.3(8)
Formula per unit cell, Z	3
<i>D</i> <sub>calcd</sub> (g/cm <sup>3</sup> )	1.373
Absorption coefficient, μ(mm <sup>-1</sup> )	0.175
<i>F</i> (000)	900
Crystal size (mm)	0.20 × 0.10 × 0.08
θ Range (°)	2.28-26.00
Index ranges	-12 ≤ <i>h</i> ≤ 12 -12 ≤ <i>k</i> ≤ 10 -27 ≤ <i>l</i> ≤ 27
Max. and min. transmission	0.9861 and 0.9658
Independent reflection	5345 ( <i>R</i> <sub>int</sub> = 0.0323)
Data/restraints/parameters	5345 / 1 / 362
Final <i>R</i> indices [ <i>I</i> > 2σ( <i>I</i> )]	<i>R</i> <sub>1</sub> = 0.0679, <i>wR</i> <sub>2</sub> = 0.1003
<i>R</i> indices (all data)	<i>R</i> <sub>1</sub> = 0.1067, <i>wR</i> <sub>2</sub> = 0.1119
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.090
Largest difference peak and hole (e/Å <sup>3</sup> )	0.231 and -0.146

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*Sample Availability:* Available from the authors.