# ANTITUMOR AGENTS. VI.[1] SYNTHESIS AND ANTITUMOR ACTIVITY OF RING A-, RING B-, AND RING C-MODIFIED DERIVATIVES OF CAMPTOTHECIN

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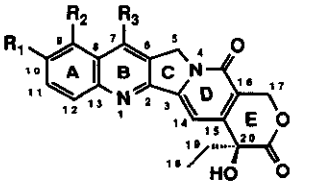
Abstract---Eleven ring A-, ring B-, and ring C-modified analogues of the antitumor alkaloid camptothecin (**1**) were prepared and evaluated for cytotoxicity and antitumor activity against P388 mouse leukemia. Among the six ring A-modified analogues, hexacyclic compound (**14**) retained the same order of activity as **1**. Most of the ring B- and ring C-modified analogues displayed greatly reduced activity, whereas compound (**39**), which has an alkylidene group at position 5, was found to be as active as **1**. These results confirmed the necessity of the intact rings A, B, and C of **1** for antitumor activity. Further, the higher activity of **14** and **39** suggest that the "northern" part of the camptothecin molecule may be a suitable site for functionalization to obtain more potent analogues of **1**.

Since the initial isolation and structure determination of the antitumor alkaloid camptothecin (**1**) in 1966,[2] numerous studies have shown the clinical value of this compound as an anticancer agent.[3-5] The important finding by Liu and colleagues[6] in 1985 that camptothecin induces single-strand DNA breaks by stabilizing a topoisomerase I-DNA cleavable complex has accelerated the pace of research in the camptothecin field. Results of these efforts have produced CPT-11 (**2**)[7] and topotecan (**3**),[8] both semisynthetic analogues of natural camptothecin which have exhibited significant efficacy in on-going clinical trials and are expected to prove effective antitumor agents.[9]




**1** $R_1=R_2=R_3=H$

**2** $R_1$= OCO-N⟨ ⟩-N⟨ ⟩ · HCl, $R_2=H$, $R_3=Et$

**3** $R_1=OH$, $R_2=CH_2NMe_2$ · HCl, $R_3=H$

**Figure 1**

Previously, we reported the synthesis and antitumor activity of various ring E-modified analogues of camptothecin.[1] The results of this study, coupled with previous reports,[4c] indicated the highly restricted structural requirements of ring E for the biological activity in camptothecin. Further, it has been also reported that the pyridone ring D is essential for antitumor activity.[10] The goal of our current research has been to define the optimal ring system in rings A, B, and C for antitumor activity. This paper describes some structural modifications of the ring system of camptothecin and their antitumor activity.

**Chemistry.** All compounds except **39** were prepared by total synthesis and were racemic. The synthesis of ring A-modified compounds (**11**-**14**) was performed by Friedländer condensation of the appropriate amino aldehydes (**4**),[11] (**5**),[12] (**8**), and (**9**) with racemic tricyclic ketone (**10**). Bicyclic amino aldehydes (**8**) and (**9**) were prepared from **6**[13] and **7**,[14] respectively, in three steps involving the processes of esterification, reduction, and oxidation. In the case of the condensation reaction of **8** and **9** with **10**, acetic acid was an effective catalyst and solvent as well, whereas the use of *p*-toluenesulfonic acid as a catalyst resulted in no reaction.

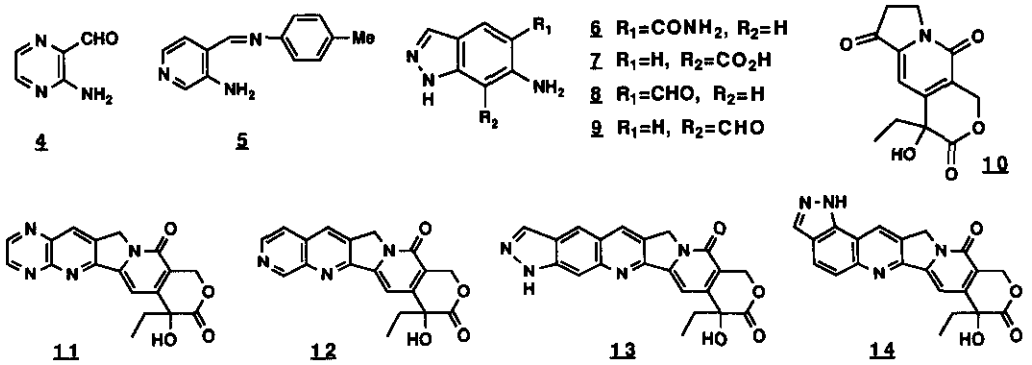



**Figure 2**

For the synthesis of compounds (**19**) and (**20**), we applied the procedure described by Breitmaier[15] for the preparation of pyridine ring (Figure 3). Thus, the reaction of heterocyclic amines (**17**) and (**18**) with tricyclic enaminone (**15**), which was derived from **10** by treatment with *N*,*N*-dimethylformamide dimethyl acetal,[16] provided pentacyclic compounds (**19**) and (**20**), respectively. Furthermore, tetracyclic compound (**21**) was also obtained by reaction of **15** with formamidine acetate, although the yield was low. The conversion of the quinoline ring system in rings A and B into a quinoxaline ring system was performed by reaction of 1,2-phenylenediamine (**22**) with dibromo tricyclic ketone (**16**), which was readily prepared by bromination of **10**. In addition, we prepared a unique pentacyclic compound involving the indole ring system in rings A and B by application of the procedure of Fischer indole synthesis. Thus, the reaction of phenylhydrazine with **10** provided phenylhydrazone (**24**), which was readily converted to a novel pentacyclic compound (**25**) upon heating with a mixture of acetic acid and hydrochloric acid. To investigate the role of ring C in antitumor activity, we prepared a compound in which ring C is a 6-membered ring (Figure 4). Alkylation of **26**[17] with ethyl bromopropionate gave a mixture of *O*-alkylated compound (**27**) and *N*-alkylated compound (**28**) in an approximate ratio of 8:2. Minor *N*-alkylated compound (**28**) was separated using column chromatography and subjected to Dieckmann condensation to give bicyclic compound (**29**). Treatment of **29** in a mixture of refluxing acetic acid and hydrochloric acid followed by protection of the carbonyl group with ethylene glycol provided bicyclic ketal (**30**) in 86% yield. At this stage, we applied the method for the preparation of $\delta$-lactone ring described by Wall and co-workers.[18] Thus, treatment of **30** with diethyl carbonate in the presence of sodium hydride followed by further reaction with ethyl iodide in the presence of potassium tertiary butoxide (*tert*-BuOK) gave ester (**31**) in 76% yield. Catalytic hydrogenation of **31** in the presence of Raney Ni in a mixture of acetic anhydride and acetic acid followed by the addition of sodium nitrite and heating gave diester (**32**), which was lactonized by successive treatment with lithium hydroxide and

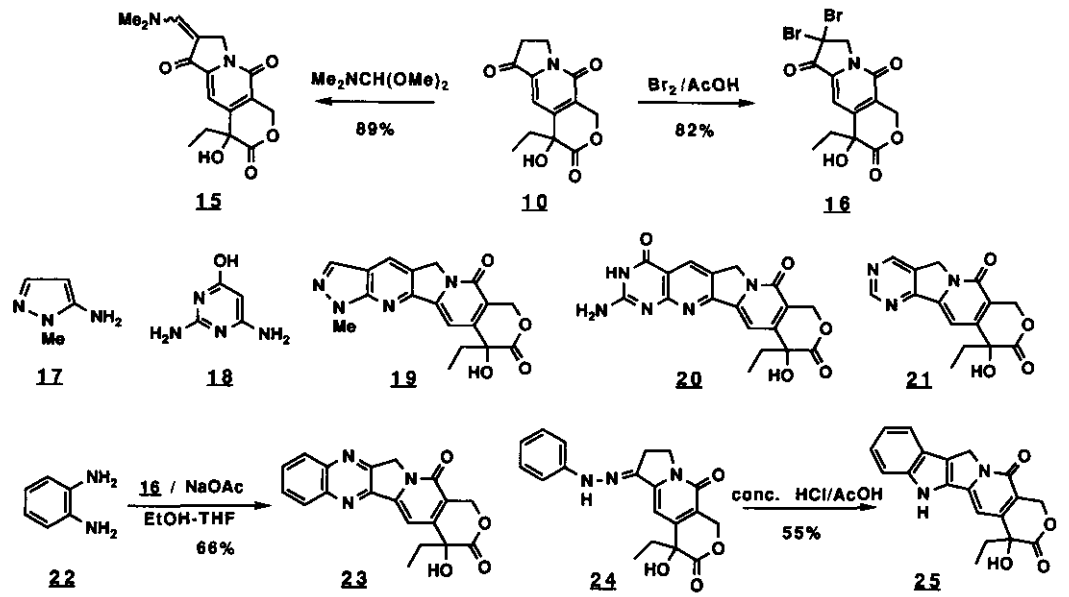



**Figure 3**

acetic acid to provide tricyclic ketal (**33**) in an overall 92% yield. Air oxidation of **33** in the presence of *tert*-BuOK and triethyl phosphite followed by deketalization with 80% aqueous trifluoroacetic acid gave tricyclic ketone (**34b**) in an overall 53% yield. Friedländer condensation of **34b** with *N*-(*o*-aminobenzylidene)-*p*-toluidine (**35**)[19] provided C-homo (20*RS*)-camptothecin (**36**) in 75% yield. Another modification of ring C was done by

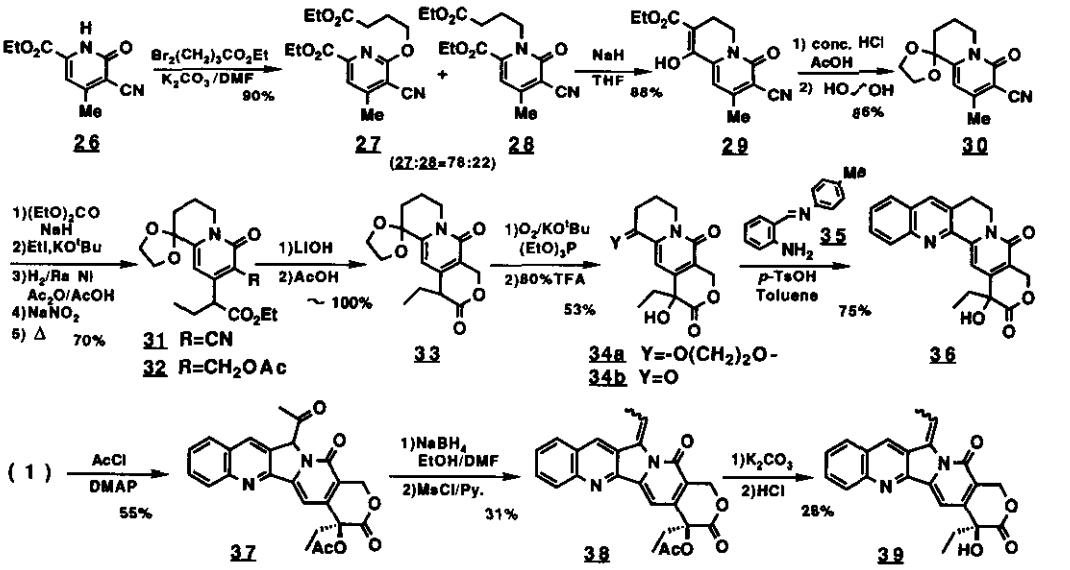



**Figure 4**

introduction of an alkylidene group at position 5 of natural (20*S*)-camptothecin (**1**). Thus, acetylation of **1** with acetyl chloride in the presence of 4-dimethylaminopyridine gave diacetyl derivative (**37**). Reduction of **37** with sodium borohydride followed by dehydration with methanesulfonyl chloride in the presence of pyridine provided compound (**38**). Hydrolysis of **38** with potassium carbonate gave (20*S*)-5-ethylidenecamptothecin (**39**), in which the stereochemistry of the ethylidene group cannot be clearly determined as *E* or *Z*.

**Biological Results and Discussion.** The results of biological tests for 11 of the new camptothecin analogues are presented in Table I. In all cases, natural (20*S*)-camptothecin (**1**) was also assayed at the same time as a positive control. Six ring A-modified analogues (**11-14, 19,** and **20**) were found to be inactive or only marginally active in *in vitro* P388 assay. As an exception, however, compound (**14**) was found to be half as active as **1**. It should be noted that racemic camptothecin was about half as potent as natural (20*S*)-camptothecin.[18] Hence, compound (**14**) of (20*S*)-configuration would be expected to have the same order of cytotoxicity as **1**. Interestingly, pyrazolo[3,4-*j*]-(20*RS*)-camptothecin (**13**), which is isomeric with **14**, was about 3-fold less active than **14** in *in vitro* P388 assay. This result may be coincident with studies of the effects of substitution in ring A of the camptothecin chromophore, which showed that substitutions at positions 11 and 12 are unfavorable for activity, whereas positions 7, 9, and 10 are acceptable sites for functionalization.[4a, 20] It also suggests that the "northern" part of the camptothecin molecule involving positions 7, 9, and 10 may be on the "outside" of the binary topoisomerase I-DNA complex.[21] Few studies have investigated the role of ring B in camptothecin. In order to define the importance of the intact pyridine ring for activity, we synthesized two novel ring B-modified analogues (**23**) and (**25**). Unfortunately, both analogues exhibited little if any activity compared with **1**. As compound (**23**) possesses a conjugated planar area defined as rings ABCD which appears to be a critical factor in topoisomerase I inhibition, the low activity of **23** may be due to the disturbance of the electronic factor in ring B. Molecular modeling studies show that the shapes of the "bay" region of the molecule, which seem to recognize a surface on the binary enzyme-DNA complex, are considerably different between **1** and **25**. The difference in this steric factor may explain the inactivity of **25**. Ring enlargement of the 5-membered ring C to a 6-membered ring led to inactivation as shown in Table I, compound (**36**). Inactivity of **36** was not unexpected as molecular modeling studies showed that the plane of the molecule of **36** bends at ring C so that the planarity of rings ABCD is lost. This result provides additional support for the importance of the planarity of rings ABCD for activity. It has been reported that substituents on position 5 through $sp^3$ bond, such as methyl, methoxy or hydroxy groups, reduce the antitumor properties of camptothecin.[20a, 22] These studies suggest that the substituents of position 5 require the minimal steric bulk as they are perpendicular to the molecular plane of camptothecin. If the substituents on position 5 are on the same plane as the molecule, however, functionalization of this position may be tolerated. As expected, another ring C-modified compound (**39**) which has an ethylidene group at position 5, retained the same order of potency as **1** in *in vitro* P388 assay. This result suggests that the enzyme-DNA complex may have a certain degree of bulk tolerance for substituents at position 5, if they, or at least the first atom connected to ring C, are on the same plane as the camptothecin molecule. Tetracyclic compound (**21**) was found to be inactive, consistent with the requirement for the complete rings ABCD for antitumor activity. Compounds (**12**), (**14**), and (**23**) were evaluated in *in vivo* P388 assays. Compound (**12**) was found to be modestly active, but was less potent than **1** at a dose of 30 mg/kg. Compound (**23**) showed the same order of activity as **1** at a dose of 240 mg/kg. In contrast, hexacyclic compound (**14**) was found to be highly active at a dose of 480 mg/kg.

Table I. Cytotoxicity and Antitumor Activity of Camptothecin Analogues on P388 Mouse Leukemia Cells[a]

| Compound | $IC_{50}$ (nM)[b] | T/C (%) (40-day survival)[c] Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 480 | 240 | 120 | 60 | 30 | 15 |
| **11** | >1000 | | | NT[f] | | | |
| **12**[d] | 41.9 | 58.2 | 57.4 | 201 | 163 | 144 | |
| **13** | 53.0 | | | NT | | | |
| ***14***[d] | *16.4* | *257(2/6)* | *175(1/6)* | *176* | *157* | *186(1/6)* | |
| **19** | >1000 | | | NT | | | |
| **20** | >1000 | | | NT | | | |
| **21** | >1000 | | | NT | | | |
| **23**[d] | 120 | 103 | 193 | 171 | 158 | | |
| **25** | >1000 | | | NT | | | |
| **36** | >1000 | | | NT | | | |
| **39** | 6.00 | | | NT | | | |
| ***1***[e] | *8.16* | | *144* | *176* | *142* | *130* | *128* |
| ***(RS)*-1**[e] | 22.2 | | 163 | 148 | 128 | 128 | 114 |

a) P388 cells ($1x10^6$) were transplanted intraperitoneally (i. p.) into CDF1 mice on day 0; compounds were administered i. p. on day 1. b) Concentration that inhibited the proliferation of P388 cells ($2x10^4$) by 50% on 72 h continuous exposure c) T/C (%) = (median survival time of treated/control animals) x 100. d) Injected as a suspension in $H_2O$ containing 0.9% NaCl, 0.9% benzyl alcohol, 0.4% Tween 80 and 0.5% carboxymethyl cellulose. e) Injected as an aqueous solution of the sodium salt f) Not tested.

In summary, studies on the ring modification described here have confirmed the necessity of the intact rings A, B, and C of camptothecin and have also suggested that there is some degree of bulk tolerance for substituents at positions 9 and 10, consitent with the findings reported previously. In addition, position 5 in ring C may be an acceptable site for functionalization if the substituents do not disturb the steric factor around position 5. The present study, taken together with previous observations of the structure-activity relationships of camptothecin derivatives, suggests that modifications of the northern part of the molecule involving positions 5, 7, 9, and 10 may provide more potent analogues than the parent compound, depending on the type and location of substituents at these positions.

## EXPERIMENTAL SECTION

Melting points were determined on a Yanagimoto apparatus and are uncorrected. Infrared (ir) spectra were recorded on a Hitachi 260-30 or 270-30 spectrophotometer. $^1$H-Nmr spectra were recorded on a JEOL JNM-FX90Q (90 MHz) or a JEOL GSX500 (500 MHz) instrument. Coupling constants are reported in hertz (Hz) and chemical shifts in ppm ($\delta$ units) downfield from internal tetramethylsilane. Mass spectra (ms) were recorded on a JEOL JMS-01SG-2 or a JMS-D300 mass spectrometer. High resolution mass spectra (hrms) was recorded on a JEOL JMS-HX110 mass spectrometer. Elemental analyses were made on a Heraeus instrument. Column chromatographies were performed with silica gel 60 $F_{254}$ (70-230 mesh) (Merck).

**6-Amino-5-indazolecarboxaldehyde (8)**: Dry HCl gas was bubbled through a stirred suspension of 6-amino-5-indazolecarboxamide[13] (3.2 g, 18.2 mmol) in EtOH (250 ml) at room temperature for 3 h. The reaction mixture was then heated to reflux for 18 h. After being cooled, dry HCl gas was again bubbled through and the

residual solution was heated to reflux for another 18 h. After the solvent was removed, the residue was diluted with $H_2O$ (50 ml) and neutralized by adding solid $Na_2CO_3$. The resulting mixture was extracted with $CHCl_3$ (20 ml x 3), and the combined organic layer was washed with $H_2O$ and dried over $Na_2SO_4$. Evaporation of the solvent gave ethyl 6-amino-5-indazolecarboxylate (980 mg, 26%) as a pale brown solid. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 1.34 (3H, t, $J$ = 8 Hz), 4.28 (2H, q, $J$ = 8 Hz), 6.48 (2H, br s), 6.66 (1H, s), 7.90 (1H, s), 8.29 (1H, s). To a stirred suspension of lithium aluminium hydride (LAH) (150 mg, 4 mmol) in tetrahydrofuran (THF) (20 ml), a solution of the above ethyl carboxylate (410 mg, 2 mmol) in THF (20 ml) was added dropwise at room temperature, and the reaction mixture was heated to reflux for 15 h. After being cooled, $H_2O$ (0.15 ml), 15% aqueous NaOH solution (0.15 ml), and $H_2O$ (0.45 ml) were added successively, and the residue was diluted with THF (50 ml). The mixture was filtered through Celite and the filtrate was concentrated *in vacuo* to give crude 6-amino-5-indazole-methanol (180 mg), which was used without purification in the next step. A mixture of crude 6-amino-5-inda-zolemethanol (180 mg) and freshly prepared $MnO_2$ (900 mg, 10 mmol) in THF (50 ml) was stirred at room temperature for 20 h. The suspension was filtered through Celite and the filtrate was concentrated to dryness *in vacuo* to give a yellow solid, which was triturated with $CHCl_3$ (3 ml). The solid separated was collected by filtration, washed with a small amount of $CHCl_3$ and dried to give **8** (85 mg, 48%) as a pale yellow powder. Ir (KBr) $\upsilon_{max}$: 3466, 1680, 1632, 1596 cm$^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 6.58 (1H, s), 8.02 (1H, s), 10.03 (1H, s).

**6-Amino-7-indazolecarboxamide (9)**: To a stirred suspension of 6-amino-7-indazolecarboxilic acid[14] (5 g, 28.2 mmol) in acetone (1.8 l), a solution of $CH_2N_2$ (prepared from 21.4 g of Diazald) in $Et_2O$ was added at 0 °C. After the reaction mixture was stirred at room temperature for 20 h, the solvent was concentrated *in vacuo* to dryness to give methyl 6-amino-7-indazolecarboxylate as a yellow powder (5.3 g, 98%). $^1$H-Nmr ($CDCl_3$) $\delta$: 4.04 (3H, s), 6.56 (1H, d, $J$ = 9 Hz), 7.68 (1H, d, $J$ = 9 Hz), 7.95 (1H, s). To a stirred suspension of LAH (300 mg, 7.9 mmol) in THF (20 ml), a solution of the above methyl carboxylate (1 g, 5.2 mmol) in THF (20 ml) was added dropwise at room temperature, and stirring was continued for 18 h at the same temperature. After the reaction mixture was cooled to 0 °C, $H_2O$ (0.3 ml), 15% aqueous solution (0.3 ml), and $H_2O$ (0.9 ml) were added successively. The suspension was filtered through Celite and the filtrate was concentrated *in vacuo* to give a gray solid, which was triturated with $CHCl_3$ (20 ml), and the solid separated was collected by filtration to give 6-amino-7-indazolemethanol (375 mg, 44%) as a pale green powder. Ir (KBr) $\upsilon_{max}$: 3406, 3150, 1626 cm$^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 4.72 (2H, s), 5.12 (1H, s) 6.56 (1H, d, $J$ = 9 Hz), 7.32 (1H, d, $J$ = 9 Hz), 7.75 (1H, s). A mixture of 6-amino-7-indazolemethanol (375 mg, 2.3 mmol) and freshly prepared $MnO_2$ (1.8 g, 8 mmol) in acetone was stirred at room temperature for 20 h. The resultant mixture was filtered through Celite and the filtrate was concentrated *in vacuo* to give a solid, which was triturated with ether (20 ml). and filtered to give **9** (300 mg, 81%) as a yellow powder. Ir (KBr) $\upsilon_{max}$: 3400, 3200, 1650, 1635, 1580 cm$^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 6.56 (1H, d, $J$ = 9 Hz), 7.62 (1H, d, $J$ = 9 Hz), 7.84 (1H, s), 10.36 (1H, s).

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*,11*H*-pyrano[3",4":6',7']indolizino[2',1':5,6]pyrido[2,3-*b*]pyrazine-3,14(4*H*,12*H*)-dione (11)**: A solution of 2-amino-3-pyrazinecarboxaldehyde (**4**) (664 mg, 5.4 mmol), the tricyclic ketone **10** (468 mg, 1.78 mmol), and AcOH (0.52 ml) in benzene (100 ml) was heated to reflux for 36 h using a Dean-Stark apparatus. The precipitate obtained after cooling was collected by filtration and

purified by column chromatography [$CHCl_3$-MeOH-AcOEt (100 : 2: 2, v/v)] to give **11** (190 mg, 31%). Ir (KBr) $\upsilon_{max}$: 3360, 1761, 1656, 1632, 1602 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$-MeOH-$d_4$) $\delta$: 1.04 (3H, t, $J$ = 7 Hz), 1.94 (2H, q, $J$ = 7 Hz), 5.36, 5.67 (2H, ABq, $J$ = 17 Hz), 5.42 (2H, br s), 7.88 (1H, s), 8.78 (1H, s), 9.07 (1H, d, $J$ = 2 Hz), 9.19 (1H, d, $J$ = 2 Hz). Ms $m/z$: 351 ($M^+$+1), 350 ($M^+$). *Anal.* Calcd for $C_{18}H_{14}N_4O_4 \cdot 1/2H_2O$: C, 60.17; H, 4.21; N, 15.59. Found: C, 60.14; H, 4.31; N, 15.48.

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*,11*H*-pyrano[3",4":6',7']indolizino[2',1':5,6]pyrido[2,3-*c*]pyridine-3,14(4*H*,12*H*)-dione (12)**: A solution of *N*-(3-amino-4-picolylidene)-*p*-toluidine **5**[12] (411 mg, 1.96 mmol) and **10** (430 mg, 1.63 mmol) in toluene (25 ml) was brought to reflux and then cooled before adding *p*-TsOH·$H_2O$ (30 mg). The mixture was heated to reflux for 7 h using a Dean-Stark apparatus and cooled. The precipitate separated was collected by filtration and was purified by column chromatography [$CHCl_3$-MeOH (100:1.4, v/v)] to give a yellow solid. Recrystalization from $CHCl_3$-MeOH (5:3, v/v) gave **12** (200 mg, 21%) as yellow scales, mp 285-295 °C (decomp.). Ir (KBr) $\upsilon_{max}$: 3466, 1743, 1656, 1602 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.05 (3H, t, $J$ = 7.4 Hz), 1.91 (2H, q, $J$ = 7.4 Hz), 3.8 (1H, br s), 5.34 (1H, s), 5.36 (1H, s), 5.32, 5.73 (2H, ABq, $J$ = 16.5 Hz), 7.71 (1H, s) 7.76 (1H, d, $J$ = 5.7 Hz), 8.39 (1H, s), 8.72 (1H, d, $J$ = 5.7 Hz), 9.64 (1H, s). FDms $m/z$ : 350 ($M^+$+1), 349 ($M^+$). *Anal.* Calcd for $C_{19}H_{15}N_3O_4 \cdot 1/6H_2O$: C, 64.77; H, 4.39; N, 11.93. Found: C, 64.68; H, 4.27; N, 11.94.

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*,8*H*-pyrano[3",4":6',7']indolizino[2',1':5,6]pyrido[3,2-*f*]indazole-3,15(4*H*,13*H*)-dione (13)**: A solution of **10** (44 mg, 0.17 mmol) and **8** (27 mg, 0.17 mmol) in AcOH (3 ml) was heated to reflux for 5 h in a nitrogen atmosphere. The precipitate obtained after cooling was collected by filtration and recrystalized from AcOH to give **13** (23 mg, 35%), mp >280 °C. Ir (KBr) $\upsilon_{max}$: 3280, 1746, 1653, 1590 $cm^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 0.92 (3H, t, $J$ = 7 Hz), 1.86 (2H, m), 5.28 (2H, s), 5.44 (2H, s), 7.45 (1H, s), 8.30 (1H, br s), 8.48 (1H, d, $J$ = 1.5 Hz), 8.66 (1H, s), 8.82 (1H, s). *Anal.* Calcd for $C_{21}H_{16}N_4O_4 \cdot 3/2H_2O$: C, 60.72; H, 4.61; N, 13.49. Found: C, 60.59; H, 4.40; N, 13.12.

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*,11*H*-pyrano[3",4":6',7']indolizino[2',1':5,6]pyrido[2,3-*g*]indazole-3,15(4*H*,13*H*)-dione (14)**: A solution of **10** (160 mg, 0.6 mmol) and **9** (100 mg, 0.6 mmol) in AcOH (12 ml) was heated to reflux for 4 h in a nitrogen atmosphere. The precipitate obtained after cooling was collected by filtration, washed with AcOH and $H_2O$, and dried to give **14** (100 mg, 43%) as a yellow powder, mp >300 °C. Ir (KBr) $\upsilon_{max}$: 3226, 1746, 1656, 1587 $cm^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 0.92 (3H, t, $J$ = 7 Hz), 1.89 (2H, m), 5.37 (2H, s), 5.44 (2H, s), 6.50 (1H, s), 7.36 (1H, s), 7.75 (1H, d, $J$ = 9 Hz), 8.08 (1H, d, $J$ = 9 Hz), 8.28 (1H, br s), 9.12 (1H, s). *Anal.* Calcd for $C_{21}H_{16}N_4O_4 \cdot 1/2H_2O$: C, 63.47; H, 4.31; N, 14.10. Found: C, 63.59; H, 4.27; N, 13.82.

**(4*RS*)-4-Ethyl-7,8-dihydro-4-hydroxy-7-dimethylaminomethylene-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione (15)**: A mixture of **10** (500 mg, 1.9 mmol) and *N*,*N*-dimethylformamide dimethylacetal (3 ml, 22.6 mmol) was heated at 110 °C for 10 min to give a purple solid, which was dissolved in $CHCl_3$ (50 ml) and treated with activated carbon (50 mg). The suspension was filtered through Celite and the filtrate was concentrated *in vacuo* to give a red powder, which was recrystallized from EtOH to give **15** (540 mg, 89%). Ir

(KBr) $\upsilon$ max: 3372, 1748, 1690, 1580 $cm^{-1}$. $^1$H-Nmr ($CHCl_3$) $\delta$: 0.98 (3H, t, $J$ = 7 Hz), 1.82 (2H, q, $J$ = 7 Hz), 3.26 (6H, s), 3.67 (1H, s), 4.99 (2H, s), 5.24, 5.66 (2H, ABq, $J$ = 17 Hz), 7.21 (1H, s), 7.68 (1H, s). *Anal.* Calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.21; H, 5.91; N, 8.69.

**(4*RS*)-7,7-Dibromo-4-ethyl-7,8-dihydro-4-hydroxy-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione (16)**: To a solution of **10** (430 mg, 1.6 mmol) in AcOH (10 ml), bromine (784 mg, 4.9 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 6 h. The separated precipitate was collected by filtration, washed successively with AcOH and $H_2O$, and dried to give **16** (560 mg, 82%) as a yellow powder, mp 162-165 °C (decomp.). Ir (KBr) $\upsilon$ max: 3300, 1760, 1740, 1650, 1600 $cm^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 0.83 (3H, t, $J$ = 7 Hz), 1.83 (2H, q, $J$ = 7 Hz), 4.97 (2H, s), 5.39 (2H, s), 7.17 (1H, s).

**(4*RS*)-4-Ethyl-4-hydroxy-7-methyl-1*H*-pyrano[3",4":6',7']indolizino[2',1':5,6]pyrido[2,3-*c*]pyrazole-3,13(4*H*,11*H*)-dione (19)**: A solution of enaminone **15** (100 mg, 0.31 mmol) and 1-methyl-5-aminopyrazole **(17)** (31 mg, 0.31 mmol) in AcOH (10 ml) was heated to reflux for 25 h in a nitrogen atmosphere. After evaporation of the solvent, the residue was purified by column chromatography [$CHCl_3$-MeOH (100:1, v/v)] to give a yellow solid. Recrystallization from EtOH gave **19** (25 mg, 23%) as a white crystalline powder, mp 249-253 °C (decomp.). Ir (KBr) $\upsilon$ max: 3400, 1745, 1660, 1620 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.06 (3H, t, $J$ = 7 Hz), 1.88 (2H, m), 4.25 (3H, s), 5.23 (2H, s), 5.32, 5.72 (2H, ABq, $J$ = 17 Hz), 7.58 (1H, s), 8.13 (1H, s), 8.27 (1H, s). Ms *m/z*: 352 ($M^+$). *Anal.* Calcd for $C_{18}H_{16}N_4O_4 \cdot 1/2H_2O$: C, 59.83; H, 4.74; N, 15.50. Found: C, 60.15; H, 4.78; N, 15.51.

**(4*RS*)-8-Amino-4-ethyl-4-hydroxy-1*H*-pyrano[3",4":6',7']indolizino[2',1':5,6]pyrido[2,3-*d*]pyrimidine-3,10,14(4*H*,9*H*,12*H*)-trione (20)**: A solution of enaminone **15** (100 mg, 0.31 mmol) and 2,4-diamino-6-hydroxypyrimidine **(18)** (40 mg, 0.31 mmol) in AcOH (10 ml) was heated to reflux for 20 h in a nitrogen atmosphere. The precipitate obtained after cooling was collected by filtration and washed successively with $H_2O$, EtOH and $Et_2O$ to give **20** (105 mg, 88%) as a yellow powder, mp >300 °C. Ir (KBr) $\upsilon$ max: 3350, 1750, 1660, 1620 $cm^{-1}$. $^1$H-Nmr (DMSO-*d6*) $\delta$: 0.86 (3H, t, $J$ = 7 Hz), 1.85 (2H, m), 5.11 (2H, s), 5.41 (2H, s), 6.50 (1H, s), 6.70-7.00 (2H, br s), 7.18 (1H, s), 8.57 (1H, s), 11.34 (1H, s). Ms *m/z*: 381 ($M^+$). *Anal.* Calcd for $C_{18}H_{15}N_5O_5 \cdot 1/2H_2O$: C, 55.39; H, 4.13; N, 17.94. Found: C, 55.04; H, 3.87; N, 18.30.

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*d*]pyrimidine-3,12(4*H*,10*H*)-dione (21)**: A solution of enaminone **15** (100 mg, 0.31 mmol) and formamidine acetate (64 mg, 0.62 mmol) in AcOH (10 ml) was heated to reflux for 15 h in a nitrogen atmosphere. After evaporation of the solvent, the residue was purified by column chromatography [$CHCl_3$-MeOH (100:1, v/v)] to give a yellow solid. Recrystallization from MeOH gave **21** (7 mg, 7%) as colorless needles, mp 267-269 °C (decomp.). Ir (KBr) $\upsilon$ max: 3406, 1746, 1665, 1617 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.03 (3H, t, $J$ = 7 Hz), 1.89 (2H, m), 5.25 (2H, s), 5.34, 5.72 (2H, ABq, $J$ = 17 Hz), 7.64 (1H, s), 9.11 (1H, s), 9.42 (1H, s). Hrms *m/z*: Calcd for $C_{15}H_{13}N_3O_4$ ($M^+$): 299.0906. Found: 299.0898.

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoxaline-3,14(4*H*,12*H*)-dione (23)**: To a stirred solution of dibromo tricyclic ketone **16** (100 mg, 0.24 mmol) in a mixture of EtOH (5 ml) and THF (5 ml), *o*-phenylenediamine (**22**) (78 mg, 0.72 mmol) and NaOAc (60 mg, 0.72 mmol) were added, and stirring was continued at room temperature for 48 h in a nitrogen atmosphere. The mixture was diluted with $CH_2Cl_2$ (70 ml) and washed successively with 10% aqueous HCl solution, $H_2O$, and brine. After being dried over $Na_2SO_4$ and freed of solvent, the residue was triturated with a small amount of MeOH to give a yellow solid, which was collected by filtration, washed with MeOH, and dried to give **23** (55 mg, 66%) as a yellow powder, mp 280-285 °C (decomp.). Ir (KBr) $\upsilon$ max: 3484, 1740, 1655, 1602 $cm^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 0.91 (3H, t, $J$ = 7 Hz), 1.90 (2H, q, $J$ = 7 Hz), 5.28 (2H, s), 5.45 (2H, s), 6.53 (1H, s), 7.40 (1H, s), 7.97 (2H, m), 8.24 (2H, m). Ms *m/z*: 349 ($M^+$). *Anal.* Calcd for $C_{19}H_{15}N_3O_4 \cdot 1/4H_2O$: C, 64.49; H, 4.41; N, 11.87. Found: C, 64.74; H, 4.46; N, 12.14.

**(4*RS*)-4-Ethyl-4-hydroxy-1*H*,*11H*-pyrano[3',4':6,7]indolizino[1,2-*b*]indole-3,13(4*H*,6*H*)-dione (25)**: A solution of **10** (500 mg, 1.9 mmol) and phenylhydrazine (226 mg, 2.1 mmol) in MeOH (50 ml) was stirred at room temperature for 16 h in a nitrogen atmosphere. The precipitate was collected by filtration, washed with cold MeOH, and dried to give phenylhydrazone (**24**) (420 mg, 63%) as a yellow powder, mp 240-260 °C (decomp.). Ir (KBr) $\upsilon$ max: 3310, 1734, 1650, 1557 $cm^{-1}$. $^1$H-Nmr (DMSO-$d_6$) $\delta$: 0.86 (3H, t, $J$ = 7 Hz), 1.76 (2H, q, $J$ = 7 Hz), 2.97 (2H, t, $J$ = 7 Hz), 4.17 (2H, t, $J$ = 7 Hz), 5.29 (1H, s), 6.30 (1H, s), 6.82 (1H, s), 7.25 (5H, s). Ms *m/z*: 353 ($M^+$). A suspension of **24** (210 mg, 0.59 mmol) in a mixture of AcOH (4 ml) and concentrated HCl (1 ml) was heated to reflux for 15 min in a nitrogen atmosphere. The precipitate obtained after cooling was collected by filtration, washed successively with $H_2O$ and EtOH, and dried to give a yellow powder. Recrystallization from *N*,*N*-dimethylformamide (DMF) gave **25** (110 mg, 55%) as a yellow crystalline powder, mp 260-265 °C (decomp.). $^1$H-Nmr (DMSO-$d_6$) $\delta$: 0.89 (3H, t, $J$ = 7 Hz), 1.85 (2H, q, $J$ = 7 Hz), 5.00 (2H, s), 5.37 (2H, s), 6.35 (1H, s), 6.92 (1H, s), 7.00-7.80 (4H, m), 11.94 (1H, s). Ms *m/z*: 336 ($M^+$). *Anal.* Calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.80; N, 8.33. Found: C, 67.61; H, 4.96; N, 8.22.

**Alkylation of 3-cyano-6-ethoxycarbonyl-4-methyl-2-pyridone (26)**: Powdered anhydrous $K_2CO_3$ (3.1 g, 22.4 mmol) was added to a stirred solution of **26** (2.08 g, 10.1 mmol) in DMF (40 ml) at 60 °C. After the formation of a yellow suspension, ethyl 4-bromobutyrate (5.6 ml, 39.1 mmol) was added and the reaction mixture was stirred at 60 °C for 3.5 h. After being cooled, the mixture was filtered and the filtrate was concentrated *in vacuo* to give an oil, which was purified by column chromatography [EtOAc-hexane (1:1, v/v)] to give **27** (640 mg, 20%) as an oil and **28** (2.25 g, 70%) as needles. **27** : Ir (neat) $\upsilon$ max: 2960, 2220, 1750, 1660, 1590 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.25 (3H, t, $J$ = 7 Hz), 1.42 (3H, t, $J$ = 7 Hz), 1.80-2.00 (4H, m), 2.46 (3H, s), 4.12 (2H, q, $J$ = 7 Hz), 4.10-4.40 (2H, m), 4.43 (2H, q, $J$ = 7 Hz), 6.59 (1H, s). **28**: mp 65-67 °C. Ir ($CHCl_3$) $\upsilon$ max: 3010, 2230, 1720, 1586, 1566 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.26 (3H, t, $J$ = 7 Hz), 1.41 (3H, t, $J$ = 7 Hz), 2.16 (2H, m), 2.53 (2H, t, $J$ = 7 Hz), 2.57 (3H, s), 4.14 (2H, q, $J$ = 7 Hz), 4.42 (2H, q, $J$ = 7 Hz), 4.55 (2H, t, $J$ = 6.2 Hz), 7.61 (1H, s).

**7-Cyano-2-ethoxycarbonyl-8-methyl-1,6-dioxo-$\Delta^{7(9)}$-tetrahydroquinolizine (29)**: A suspension of NaH (50% dispersion in mineral oil) (100 mg, 2.5 mmol) and **27** (114 mg, 0.37 mmol) in THF (15 ml) was

heated to reflux for 2.5 h and cooled. The mixture was poured into cold $H_2O$ (30 ml) and acidified to pH 1-2 with 1*N* aqueous HCl solution. The resulting mixture was extracted with $CHCl_3$ (20 ml x 4), and the combined organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated to dryness *in vacuo* to give **29** (86 mg, 86%) as yellow crystals. Ir (KBr) $\upsilon_{max}$: 3450, 2230, 1640, 1590 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.37 (3H, t, *J* = 7 Hz), 2.48 (3H, s), 2.68 (2H, t, *J* = 8 Hz), 4.14 (2H, t, *J* = 8 Hz), 4.34 (2H, q, *J* = 7 Hz), 6.81 (1H, s), 12.00 (1H, s).

**7-Cyano-1,1-ethylenedioxy-8-methyl-1,6-dioxo-$\Delta^{7(9)}$-tetrahydroquinolizine (30)**: A solution of **29** (520 mg, 1.9 mmol) in a mixture of AcOH (1 ml) and concentrated HCl (1 ml), was heated to reflux for 3 h. After being cooled, the mixture was extracted with $CH_2Cl_2$ (100 ml x 5), and the combined organic layer was washed with brine and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave 7-cyano-8-ethyl-1,6-dioxo-$\Delta^{7(9)}$-tetrahydroquinolizidine (350 ml, 91%), which was used without further purification in the next step. A solution of the above dioxo compound (320 mg, 1.58 mmol), ethylene glycol (3 ml) and *p*-TsOH•$H_2O$ (10 mg) in toluene (50 ml) was heated to reflux for 3.5 h using a Dean-Stark apparatus. The toluene layer was decanted and further toluene (30 ml) was added to the resulting ethylene glycol layer. The reaction mixture was heated to reflux for 1 h and the toluene layer was decanted. After repeating this procedure two more times, the combined toluene layer was washed successively with 5% aqueous $NaHCO_3$ and brine and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave a residue, which was purified by column chromatography [$CH_2Cl_2$-AcOEt (4:1, v/v)] to give **30** (370 mg, 95%), mp 114-115 °C. Ir (KBr) $\upsilon_{max}$: 3092, 2972, 2900, 2224, 1646, 1596 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.90-2.30 (4H, m), 2.45 (3H, s), 3.90-4.30 (6H, m), 6.40 (1H, s).

**7-Cyano-8-ethoxycarbonylmethyl-1,1-ethylenedioxy-6-oxo-$\Delta^{7(9)}$-tetrahydroquinolizine (31)**: To a stirred suspension of NaH (1.6 g, 33.3 mmol; the 50% dispersion of NaH in oil was washed twice with toluene before use) in toluene (10 ml), a solution of **30** (2.1 g, 8.4 mmol) in toluene (10 ml) was added. The reaction mixture was heated to reflux for 20 min before adding absolute EtOH (0.01 ml) and diethyl carbonate (2.8 ml, 23.1 mmol), then heated to reflux for a further 2 h. The resultant mixture was carefully poured into a mixture of AcOH (20 ml) and ice-water (40 ml), and the aqueous mixture was extracted with $CH_2Cl_2$ (70 ml x 5). The combined organic layer was washed with brine, and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave an oil, which was purified by column chromatography [$CH_2Cl_2$-AcOEt (3:1, v/v)] to give 7-cyano-8-ethoxycarbonylmethyl-1,1-ethylenedioxy-6-oxo-$\Delta^{7(9)}$-tetrahydroquinolizine (2.04 g, 76%). $^1$H-Nmr ($CDCl_3$) $\delta$: 1.29 (3H, t, *J* = 7 Hz), 2.03-2.30 (4H, m), 3.75 (2H, s), 3.90-4.30 (6H, m), 4.20 (2H, q, *J* = 7 Hz), 6.47 (1H, s). To a stirred solution of the above ester (1.99 g, 6.25 mmol) in dioxane (50 ml), *tert*-BuOK (840 mg, 7.4 mmol) was added, and the mixture was stirred at 60 °C for 15 min before adding EtI (2 ml, 25 mmol). After stirring at the same temperature for a further 4 h, the resultant mixture was poured into ice-water (200 ml), acidified to pH 1-2 with 1*N* aqueous HCl, and extracted with $CH_2Cl_2$ (60 ml x 5). The combined organic layer was washed successively with 5% aqueous $NaHCO_3$ and brine, dried over $Na_2SO_4$, and freed of solvent *in vacuo* to give **31** (2.16 g, quant.) as a red oil. Ir (neat) $\upsilon_{max}$: 3598, 2974, 2224, 1737, 1659 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 0.95 (3H, t, *J* = 7 Hz), 1.24 (3H, t, *J* = 7 Hz), 1.60-2.30 (6H, m), 3.95 (1H, t, *J* = 7 Hz), 4.00-4.40 (8H, m), 6.53 (1H, s).

**1,1-Ethylenedioxy-6-oxo(5'-ethyl-2'*H*,5'*H*,6'*H*-6-oxopyrano)[3',4'-*g*]-$\Delta^{7(9)}$-tetrahydroquinolizine (33)**: A suspension of **31** (2.27 g, 6.6 mmol) and Raney Nickel R-100 (NIKKO RIKA Corp.) (3 ml; prewashed with AcOH) in $Ac_2O$ (35 ml) and AcOH (10 ml) was hydrogenated at room temperature for 7 h under irradiation with a 300-W tungsten lamp. The suspension was filtered and the filtrate was cooled to 0 °C before adding $NaNO_2$ (2 g, 29 mmol) over a period of 30 min. After stirring for 2 h, the mixture was filtered and the filtrate was heated at 80 °C for 1.5 h. Evaporation of the solvent *in vacuo* at 80-90 °C gave an oil, to which MeOH (50 ml), $H_2O$ (23 ml) and $LiOH{\cdot}H_2O$ (2.6 g, 62 mmol) were added. After the reaction mixture was stirred at room temperature for 2 h, most of the MeOH was removed *in vacuo* to leave a residue, to which $H_2O$ (20 ml) and AcOH (30 ml) were added and the resultant mixture was stirred at room temperature for 17 h. The mixture was extracted with $CH_2Cl_2$ (100 ml x 4) and the combined organic layer was washed with brine, and dried over $Na_2SO_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography ($CHCl_3$) to give **33** (1.85 g, 92%). A small sample was recrystallized from EtOAc-hexane (1:1, v/v) for elemental analysis to give colorless needles, mp 117-119 °C. Ir (KBr) $\upsilon_{max}$: 3070, 2944, 1734, 1656 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.01 (3H, t, $J$ = 7 Hz), 1.70-2.20 (6H, m), 3.41 (1H, t, $J$ = 6 Hz), 4.00-4.40 (6H, m), 5.20 (1H, dd, $J$ = 1 and 15 Hz), 5.47 (1H, dd, $J$ = 1 and 15 Hz), 6.25 (1H, s). *Anal.* Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.93; H, 6.33; N, 4.67.

**4-Ethyl-6,6-ethylenedioxy-6,7,8,9-tetrahydro-4-hydroxy-1*H*-pyrano[4,3-*b*]quinolizine-3,11(4*H*)-dione (34a)**: To a stirred solution of **33** (1.38 g, 4.5 mmol) in DMF (20 ml), *tert*-BuOK (660 mg, 5.9 mmol) was added at -40 °C, before adding triethyl phosphite (2.8 ml, 16.3 mmol). After dry oxygen was bubbled through the solution at the same temperature for 3.5 h, concentrated HCl (2 ml) was added and the reaction mixture was stirred for 20 min. Concentrated $NH_4OH$ (1 ml) was added and the solvent was removed *in vacuo* to give a residue, which was extracted with $CH_2Cl_2$ (150 ml x 3). The combined organic layer was washed successively with $H_2O$ and brine, dried over $Na_2SO_4$. Removal of the solvent *in vacuo* gave **34a** as a colorless powder (926 mg, 64%). A small sample was recrystallized from EtOAc-hexane (1:1, v/v) for elemental analysis to give colorless needles, mp 137-138 °C. Ir (KBr) $\upsilon_{max}$: 3508, 2974, 1740, 1662, 1608 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 0.98 (3H, t, $J$ = 7 Hz), 1.80 (2H, q, $J$ = 7 Hz), 1.90-2.20 (4H, m), 3.64 (1H, s), 4.00-4.30 (6H, m), 5.15 (1H, d, $J$ = 16.5 Hz), 5.60 (1H, d, $J$ = 16.5 Hz), 6.71 (1H, s). *Anal.* Calcd for $C_{16}H_{19}NO_6$: C, 59.81; H, 5.96; N, 4.36. Found: C, 59.84; H, 5.85; N, 4.39.

**4-Ethyl-6,7,8,9-tetrahydro-4-hydroxy-1*H*-pyrano[4,3-*b*]quinolizine-3,6,11(4*H*)-trione (34b)**: A solution of **34a** (449 mg, 1.4 mmol) in 80% aqueous trifluoroacetic acid (5 ml) was stirred at room temperature for 1 h. Evaporation of the solvent *in vacuo* gave a residue, which was extracted with $CH_2Cl_2$ (30 ml x 5) and the combined organic layer was washed with brine and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave an orange oil, which was purified by column chromatography [$CHCl_3$-acetone-MeOH (80:19:1, v/v)] to give **34b** (320 mg, 83%). A small sample was recrystallised from EtOAc-hexane (1:1, v/v) for elemental analysis to give colorless needles, mp 142-143 °C. Ir (KBr) $\upsilon_{max}$: 3424, 1756, 1714, 1644, 1580 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 0.97 (3H, t, $J$ = 7 Hz), 1.81 (2H, q, $J$ = 7 Hz), 2.10-2.40 (2H, m), 2.77 (2H, t, $J$ = 6 Hz), 3.63 (1H, s), 4.19 (2H, t, $J$ = 6 Hz), 5.19 (1H, d, $J$ = 17 Hz), 5.64 (1H, d, $J$ = 17 Hz), 7.64 (1H, s). *Anal.* Calcd for $C_{14}H_{15}NO_5{\cdot}1/3H_2O$: C, 59.36; H, 5.57; N, 4.94. Found: C, 59.43; H, 5.44; N, 4.99.

**(1*RS*)-1-Ethyl-7,8-dihydro-1-hydroxy-4*H*,5*H*-pyrano[3',4':7,8]quinolizino[1,2-*b*]quinoline-2,5(1*H*)-dione (36)**: A solution of *N*-(*o*-aminobenzylidene)-*p*-toluidine **(35)**[19] (582 mg, 2.77 mmol) and **34b** (384 mg, 1.39 mmol) in toluene (60 ml) was heated to reflux for 1 h and then cooled before adding *p*-TsOH•$H_2O$ (20 mg). The mixture was heated to reflux for a further 6 h using a Dean-Stark apparatus and cooled. After evaporation of the solvent *in vacuo*, the residue was triturated with acetone. The separated solid was collected by filtration and was purified by recrystalization from $CHCl_3$-acetone to give **36** (375 mg, 75%) as yellow scales, mp 264-266 °C (decomp.). Ir (KBr) $\upsilon_{max}$: 3292, 2932, 1755, 1647, 1620 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.05 (3H, t, $J$ = 7.4 Hz), 1.92 (2H, q, $J$ = 7.4 Hz), 3.26 (2H, t, $J$ = 6 Hz), 3.77 (1H, s), 4.44 (2H, q, $J$ = 6 Hz), 5.25 (1H, d, $J$ = 17 Hz), 5.68 (1H, d, $J$ = 17 Hz), 7.50-8.30 (6H, m). *Anal.* Calcd for $C_{21}H_{18}N_2O_4$•1/4$H_2O$: C, 68.83; H, 5.02; N, 7.64. Found: C, 69.03; H, 5.05; N, 7.67.

**(5*RS*,20*S*)-5-Acetyl-20-*O*-acetylcamptothecin (37)**: To a stirred solution of camptothecin **(1)**[23] (875 mg, 2.5 mmol) in pyridine (120 ml), acetyl chloride (0.9 ml, 12.6 mmol) and 4-dimethylaminopyridine (2.2 g, 18 mmol) were added and the reaction mixture was stirred at room temperature for 2 h. The reaction temperature was then raised to 100 °C and stirring was continued for 6 h. Acetyl chloride (0.4 ml, 5.6 mmol) was added again and the reaction mixture was heated at 100 °C for a further 5 h with stirring. The mixture was poured into 10% aqueous HCl (100 ml) and the resulting aqueous solution was extracted with $CH_2Cl_2$ (100 ml x 5). The combined organic layer was washed successively with 5% aqueous HCl, $H_2O$, 5% aqueous $NaHCO_3$, and brine, and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave a yellow oil, which was purified by column chromatography [benzene-AcOEt (3:1, v/v), then hexane-AcOEt (4:3, v/v)] to give **37** (596 mg, 55%). Ir (KBr) $\upsilon_{max}$: 1740, 1660, 1610 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 0.99 and 1.03 (each 3/2H, each t, $J$ = 7 Hz), 2.00-2.40 (2H, m), 2.10 and 2.23 (each 3/2H, each s), 2.25 and 2.28 (each 3/2H, each s), 5.40 (1H, d, $J$ = 18 Hz), 5.61 and 5.65 (each 1/2H, each d, $J$ = 18 Hz), 6.00 and 6.06 (each 1/2H, each d, $J$ = 1 Hz), 7.22 and 7.25 (each 1/2H, each s), 7.55-8.35 (4H, m), 8.40 (1H, br s). FDms $m/z$: 432 ($M^+$). *Anal.* Calcd for $C_{24}H_{20}N_2O_6$•$H_2O$: C, 64.00; H, 4.92; N, 6.22. Found: C, 64.14; H, 4.79; N, 5.92.

**(20*S*)-5-Ethylidene-20-*O*-acetylcamptothecin (38)**: To a stirred solution of **37** (443 mg, 1.03 mmol) in a mixture of DMF (5 ml) and EtOH (0.5 ml), sodium borohydride (39 mg, 1.03 mmol) was added at 0 °C. Stirring was continued at 0 °C for 50 min and then at room temperature for 2 h. The mixture was poured into $H_2O$ (100 ml), acidified to pH 2 with 10% aqueous HCl, and the resultant aqueous solution was extracted with $CH_2Cl_2$ (40 ml x 5). The combined organic layer was washed successively with $H_2O$, 5% aqueous $NaHCO_3$ and brine, and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave a red oil, which was purified by column chromatography [$CHCl_3$-MeOH, (100:1, v/v)] to give (5*RS*,20*S*)-5-[1-(*RS*)-hydroxyethyl]-20-*O*-acetylcamptothecin (278 mg, 63%). Ir (KBr) $\upsilon_{max}$: 3412, 1746, 1665, 1596 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 0.79 and 0.82 (each 3/2H, each d, $J$ = 7 Hz), 0.97 (3H, br t, $J$ = 7 Hz), 1.90-2.45 (2H, m), 2.22 (3H, s), 5.00-6.20 (4H, m), 7.23 and 7.34 (each 1/2H, each s), 7.55-8.30 (4H, m), 8.34 (1H, s). FDms $m/z$: 434 ($M^+$). To a stirred solution of the above hydroxyethyl compound (30 mg, 0.07 mmol) in a mixture of DMF (2 ml) and pyridine (1 ml, 12.4 mmol), methanesulfonyl chloride (0.05 ml, 0.65 mmol) was added at room temperature. Stirring was continued at the same temperature for 1 h and then at 70 °C for 12 h. The precipitate separated was collected by filtration, washed with $H_2O$, and dried to give **38** (9 mg). Another crop was obtained from the

filtrate. The filtrate was poured into water (10 ml) and the aqueous mixture was extracted with $CHCl_3$ (20 ml x 4), washed successively with 10% aqueous HCl, $H_2O$, and brine, and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave a residue, which was purified by preparative thin-layer chromatography [silica gel 60 $F_{254}$ (Merck), $CHCl_3$-MeOH, (100:2, v/v)] to give **38** (5 mg, total 49%). Ir (KBr) $\upsilon_{max}$: 1746, 1674, 1623 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$-MeOH-$d_4$) $\delta$: 1.01 (3H, t, $J$ = 7.3 Hz), 2.23 (2H, q, $J$ = 7.3 Hz), 2.26 (3H, s), 2.50 (3H, dd, $J$ = 7.9 Hz), 5.45, 5.70 (2H, ABq, $J$ = 17.6 Hz), 7.41 (1H, s), 7.65-8.30 (4H, m), 8.45 (1H, q, $J$ = 7.9 Hz), 8.77 (1H, s). FDms *m/z*: 416 ($M^+$).

**(20*S*)-5-Ethylidenecamptothecin (39)**: A solution of **38** (88 mg, 0.21 mmol) and $K_2CO_3$ (240 mg, 1.74 mmol) in a mixture of $CHCl_3$ (21 ml), MeOH (15 ml), and $H_2O$ (3 ml) was stirred at 50 °C for 10 h. The mixture was poured into $H_2O$ (50 ml), and the aqueous residue was washed with $CHCl_3$ (20 ml x 2) and filtered. The filtrate was cooled to 0 °C and acidified to pH 2 with 10% aqueous HCl. The solid separated was collected by filtration to give a yellow solid, which was purified by column chromatography [$CHCl_3$-MeOH (400:1, v/v)] and recrystallization from $CHCl_3$-MeOH to give **39** (22 mg, 28%) as a yellow crystalline powder, mp 240-250 °C (decomp.). Ir (KBr) $\upsilon_{max}$: 3450, 1730, 1650, 1600 $cm^{-1}$. $^1$H-Nmr ($CDCl_3$) $\delta$: 1.05 (3H, t, $J$ = 7.0 Hz), 1.90 (2H, q, $J$ = 7.0 Hz), 2.47 (3H, d, $J$ = 7.9 Hz), 5.32, 5.78 (2H, ABq, $J$ = 16.7 Hz), 7.72 (1H, s), 7.50-8.30 (4H, m), 8.48 (1H, q, $J$ = 7.9 Hz), 8.65 (1H, s). FDms *m/z*: 374 ($M^+$). *Anal.* Calcd for $C_{22}H_{18}N_2O_4 \cdot H_2O$: C, 67.34; H, 5.13; N, 7.14. Found: C, 67.17; H, 4.78; N, 7.29.

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