Plant Antitumor Agents. 25.[1] Total Synthesis and Antileukemic Activity of Ring A Substituted Camptothecin Analogues. Structure–Activity Correlations

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Nineteen racemic ring A substituted analogues of the antitumor agent 20(*S*)-camptothecin were prepared by total synthesis and evaluated for in vitro cytotoxic activity against KB cell culture and in vivo antileukemic activity against L1210. These compounds bore a wide variety of substitutents at C_{11} designed to confer upon the ring system a broad range of combinations of electronic, steric, and lipophilic effects. A few C_{10}-substituted derivatives as well as C_{10},C_{11}-disubstituted analogues prepared as part of a concurrent study have also been included for general comparison. With the notable exception of the cyano derivative, the 11-substituted compounds displayed only modest in vitro and in vivo activities, and there was a remarkable insensitivity toward the nature of the substituent. In contrast, the 9- and 10-substituted compounds exhibited a considerably higher level of dose potency and activity both in vitro and in vivo.

The potent antitumor agent 20(*S*)-camptothecin (1) was first isolated in this laboratory 20 years ago.[2] Since that time we have been engaged in studies of the structural features required for the antitumor activity of 1[1a,3,4] and studies of the total synthesis of 1 and analogues with particular emphasis on the antitumor activities of new analogues.[1a,5,6] Previous studies have shown absolute activity requirements for the α-hydroxy lactone moiety of 1 and analogues[3] and for the need of the aromatic ring ABCD nucleus.[4] We have shown that substitution of hydroxyl groups at C_{10} or C_{11} leads to increased antitumor activity[3,6] and that substitution of amino groups at C_9 or C_{10} leads to greatly increased activity and potency.[1a] On the other hand, substitution of an amino group at C_{12} led to inactivation, and disubstitution of bulky groups such as 10,11-dimethoxy also led to inactivation.[1a] Encouraged by the fact that 11-hydroxycamptothecin shows high activity against L-1210 in vivo mouse leukemia, we prepared a large number of 11-substituted 20(*RS*)-camptothecin analogues.

This paper describes the synthesis of a new series of racemic, totally synthetic 11-substituted camptothecin analogues displaying a full range of steric and electronic factors. The results of in vitro cytotoxicity (KB) and in vivo antileukemic (L1210) studies are presented. The inclusion of some new 10,11-disubstituted and 10-substituted derivatives serves as a comparison study for structure–activity relationship (SAR) discussion. A few previously reported examples (e.g., the 9-amino-20(*S*) analogue **4** and the 10-nitro-20(*RS*) analogue **9**) have also been assembled to further assist the discussion.

Chemistry. Acid-catalyzed Friedlander condensation of the racemic oxytricyclic ketone **5** with the substituted *o*-aminoaldehydes (or the corresponding acetals) **10c**,[7] **10d**,[8] **11b**, **12b**, **12d**,[9] **13b**, **14b**, **14d**, and **15c** (Chart I) afforded the nine 20(*RS*) analogues **16–21** and **31–33**. Hydrolysis of the ethylene acetal in **21** gave aldehyde **22**, which was further elaborated to yield compounds **23–30**. Thus, reaction of **22** with potassium cyanide and manganese dioxide gave an intermediate acyl cyanide,[10] which gave the 11-carbomethoxy analogue **23** upon methanolysis. Compound **23** was subjected to acid hydrolysis to give the 11-carboxy derivative **24**, which was not readily obtained by direct oxidation of **22**. The cyano group of **27** was introduced by a procedure[11] employing reaction of the formyl group in **22**, with hydroxylamine and formic acid. An overall amino–carbonyl exchange-type reaction[12] of **22** with 2-aminoisobutyric acid gave the aminomethyl hydrochloride **28**, which upon neutralization afforded free base **29**. Catalytic hydrogenation of the formyl group in **22** led to the hydroxymethyl derivative **30**. The dibenzyl groups in **33** were cleaved in refluxing aqueous hydrogen bromide to give the unstable 10,11-dihydroxy hydrobromide salt **34**, which by neutralization led to free base **35**. Compound **34** represents the first example of an isolated, albeit unstable, salt formed at the quinoline nitrogen in a camptothecin derivative. The somewhat increased basicity in this example presumably results from increased electron density in the A ring. Analogue **34** readily decomposed upon attempted chromatography or treatment with warm water.

In several cases, the substituted *o*-aminoaldehydes were new compounds that often presented stability problems. Many of these synthons, especially those containing "electron-releasing" substituents, were light, air and/or temperature sensitive, making purification attempts self-defeating. As a matter of general practice, the synthons were therefore prepared immediately prior to the Friedlander reaction and were employed as unpurified materials. Fortunately, this same instability led to more rapid Friedlander condensations with moderately good yields of the camptothecin analogues. In contrast, 4-nitro-2-aminobenzaldehyde (**10c**), which is quite stable, required prolonged reaction times under strongly acidic conditions to generate a poor yield of 11-nitro derivative **17**.

Biological Testing. Assays in the 9KB in vitro cell system were carried out at the Research Triangle Institute, and L-1210 leukemia in vivo assays were conducted by contractors for the National Cancer Institute by standard

(1) (a) For the preceding paper of the series, see: Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med Chem.* **1986**, *29*, 2358. (b) Wall, M. E.; Taylor, H.; Wani, M. C. *J. Nat. Prod.*, in press.
(2) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *94*, 3888.
(3) Wall, M. E. In *Biochemie und Physiologie der Alkaloide*; Mothes, K., Schreiber, K., Eds.; Akademie-Verlag: Berlin, 1969; pp 77–87.
(4) Wall, M. E.; Wani, M. C. In *Natural Products and Drug Development*; Larsen, P. K., Christensen, H. K., Eds.; Munksgaard: Copenhagen, 1984; pp 253–266.
(5) Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. *J. Med. Chem.* **1980**, *23*, 554.
(6) Wall, M. E.; Wani, M. C.; Natschke, S. M.; Nicholas, A. W. *J. Med. Chem.* **1986**, *29*, 1553.
(7) Lehmstedt, K. *Chem. Ber.* **1938**, 808.
(8) Ricci, A. *Ann. Chim. (Rome)* **1958**, *48*, 985; *Chem. Abstr.* **1959**, *53*, 8132*d*.
(9) Shanmugan, P.; Palaniappan, R.; Soundararajan, N.; Thiruvengadam, T. K.; Kanakarajan, K. *Monatsh. Chem.* **1976**, *107*, 259.
(10) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.
(11) Van Es, T. *J. Chem. Soc.* **1964**, 1564.
(12) Rizzi, G. P. *J. Org. Chem.* **1971**, *12*, 1710.

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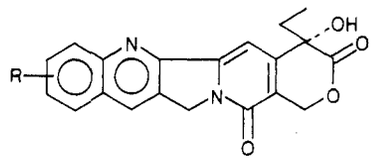
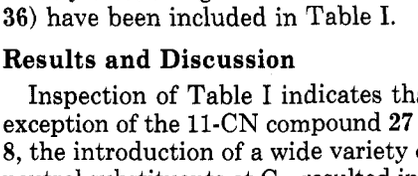
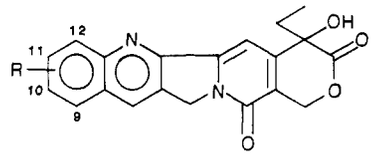
Chart I



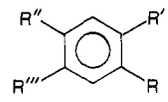
1: R = H
2: R = 10-OH
3: R = 10-OMe
4: R = 9-NH_2



5



6: R = 10-NH_2
7: R = 10,11-OCH_2O
8: R = 11-OH
9: R = 10-NO_2
16: R = 11-NMe_2
17: R = 11-NO_2
18: R = 11-NH_2
19: R = 11-CF_3
20: R = 11-CH_3
21: R = 11-formyl ethylene acetal
22: R = 11-CHO
23: R = 11-CO_2Me
24: R = 11-CO_2H
25: R = 11-CO_2Na
26: R = 11-formyl guanylhydrazone tosylate
27: R = 11-CN
28: R = 11-$NH_2CH_2 \cdot HCl$
29: R = 11-NH_2CH_2
30: R = 11-$OHCH_2$
31: R = 10-Cl
32: R = 10-Me
33: R = 10,11-$(C_6H_5CH_2O)_2$
34: R = 10,11-$(OH)_2 \cdot HBr$
35: R = 10,11-$(OH)_2$
36: R = 10,11-$(MeO)_2$



10a: R = CHO, R′ = NO_2, R″ = NMe_2, R‴ = H
b: R = CHO, R′ = R″ = NO_2, R‴ = H
c: R = CHO, R′ = NH_2, R″ = NO_2, R‴ = H
d: R = CHO, R′ = R″ = NH_2, R‴ = H
e: R = CHO, R′ = NO_2, R″ = NH_2, R‴ = H
11a: R = $HCO(CH_2)_2O$, R′ = NO_2, R″ = NMe_2, R‴ = H
b: R = $HCO(CH_2)_2O$, R′ = NH_2, R″ = NMe_2, R‴ = H
12a: R = CHO, R′ = NO_2, R″ = CF_3, R‴ = H
b: R = CHO, R′ = NH_2, R″ = CF_3, R‴ = H
c: R = CHO, R′ = NO_2, R″ = CH_3, R‴ = H
d: R = CHO, R′ = NH_2, R″ = CH_3, R‴ = H
13a: R = R″ = $HCO(CH_2)_2O$, R′ = NO_2, R‴ = H
b: R = R″ = $HCO(CH_2)_2O$, R′ = NH_2, R‴ = H
14a: R = CHO, R′ = NO_2, R″ = H, R‴ = Cl
b: R = CHO, R′ = NH_2, R″ = H, R‴ = Cl
c: R = CHO, R′ = NO_2, R″ = H, R‴ = Me
d: R = CHO, R′ = NH_2, R″ = H, R‴ = Me
15a: R = CHO, R′ = NO_2, R″ = R‴ = $OCH_2C_6H_5$
b: R = $HCO(CH_2)_2O$, R′ = NO_2, R″ = R‴ = $OCH_2C_6H_5$
c: R = $HCO(CH_2)_2O$, R′ = NH_2, R″ = R‴ = $OCH_2C_6H_5$

procedures.[13] The biological test results for 15 of the new racemic camptothecin analogues are presented in Table I. In order that more meaningful comparisons of relative (and absolute) activities could be obtained, 20(*S*)-camptothecin (**1**) or 10-hydroxy-20(*S*)-camptothecin (**2**), which have well-defined activity, were tested concurrently. Where appropriate for comparison purposes, some previously tested ring A substituted analogues (**4**, **6**–**9**, and **36**) have been included in Table I.

Results and Discussion

Inspection of Table I indicates that with the notable exception of the 11-CN compound **27** and the 11-hydroxy **8**, the introduction of a wide variety of basic, acidic, and neutral substituents at C_{11} resulted in formation of either inactive or relatively weakly active camptothecin analogues. In general there was reasonable agreement between 9KB cytotoxicity and "in vivo" activity in P-388 or L-1210 mouse leukemia assays. Camptothecin usually gives ED_{50} values of the order of 10^{-2}–10^{-3} μg/mL. In general camptothecin analogues in this cytotoxicity range will show activity of the order of % T/C = 200 or greater. Analogues that are inactive in 9KB or show cytotoxicity ED_{50} values in the 10^{0}–10^{-1} μg/mL ranges were usually inactive or weakly active. With the exception of 11-hydroxy-20-(*RS*)-camptothecin, $ED_{50} = 2\times10^{-2}$ μg/mL all of the 20-(*RS*)-11-substituted analogues showed ED_{50} values $\geq 10^{-1}$ μg/mL, and all except the 11-cyano analogue were either inactive or weakly inactive in the L-1210 assay. The 11-cyano analogue **27** is certainly anomalous and is being retested. Possibly the activity of **27** is due to the compact linear cyano unit. In general, however, substitution at C_{11} whether the moieties were neutral, acidic, or basic and bulky or nonbulky and regardless of the electronic nature of the substituent leads to inactivation or at best weak activity. In view of the high activity of the 20(*S*)-9-amino- and 20(*RS*)-10-aminocamptothecin analogues (**4** and **6**, respectively) reported previously,[1a] the relatively weak activity of the 11-amino analogue **18** was surprising. However, we previously found that the corresponding 12-amino analogue was inactive.[1a]

The various structural features required for maximal activity of camptothecin and its analogues may have a bearing on the action of camptothecin on the DNA–topoisomerase I complex.[14] Although camptothecin does not interact with purified DNA, when such DNA is in the presence of purified mammalian DNA topoisomerase I, camptothecin rapidly induces extensive single strand DNA breaks.[14] It has been suggested that there is a correlation between suppression of tumor growth and ability to cause fragmentation of DNA.[15] If so, then many of the structural features that we have shown to have increased or decreased effects on 9KB cytotoxicity and life prolongation in P-388 or L-1210 mouse leukemia may be involved in the increasingly important effects of camptothecin on topoisomerase I and DNA. It is evident from our current study and previous reports[1a,3–6] that, in general, substitution in ring A at C_{11} or C_{12} decreases the activity relative to camptothecin, e.g., 11- and 12-aminocamptothecin, whereas substitution at C_9 or C_{10} leads to compounds at least as active as camptothecin and frequently with much greater activity and potency, e.g., 9- and 10-amino analogues.

Experimental Section

Melting points were determined on a Kofler hot-stage melting point apparatus and are uncorrected. Thin-layer chromatography was conducted on precoated 0.25-mm layers of silica gel 60F-254 (Merck). The locations of components were determined by viewing under long-wave UV and short-wave UV light and by observation of charring after heating the plates sprayed with standard ceric sulfate and phosphomolybdic acid reagents. ^1H NMR spectra were obtained on a Bruker 250 250-MHz nuclear

(13) These compounds were evaluated at the Southern Research Institute under contract by the National Cancer Institute according to standard procedure of Geran et al.: Geran, R. I.; Greenberg, N. H.; McDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.

(14) Hsiang, Y. H.; Herzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.

(15) Horwitz, S. B. In *Antibiotics*; Corcoran, J. W., Hahn, F. E., Eds.; Springer-Verlag: New York, 1975; Vol. III, pp 48–57.

Table I. Comparison of Activities and Potencies of Ring A Substituted Camptothecin Analogues in L-1210 Mouse Leukemia Assays[a] and KB Cell Cultures[b]

compound	max % T/C[c] (dose, mg/kg)	no. cures out of 6	K_E[d] at max % T/C	active dose range, mg/kg	toxic dose, mg/kg	ED_{50} for KB,[b] μg/mL
11-NMe_2-20(*RS*)-**16**[e]	inactive					inactive
11-NO_2-20(*RS*)-**17**[e]	147 (80.0)	0	0.29	20.0–80.0[f]	>80.0	3×10^0
11-NH_2-20(*RS*)-**18**[e]	147 (40.0)	0	0.29	10.0[g]–80.0[f]	>80.0	2×10^{-1}
11-CF_3-20(*RS*)-**19**[e]	inactive					9×10^{-1}
11-Me-20(*RS*)-**20**[e]	136 (40.0)	0	−0.68	20.0–80.0[f]	>80.0	9×10^{-1}
11-CO_2Me-20(*RS*)-**23**[h]	inactive					5×10^0
11-CO_2H-20(*RS*)-**24**[h]	inactive					inactive
11-CO_2Na-20(*RS*)-**25**[h]	inactive					3×10^{-1}
11-CN-20(*RS*)-**27**[h]	365 (10.0)	3	≥5.79	≤10.0[g]–20.0	20.0	3×10^{-1}
11-CH_2NH_2·HCl-20(*RS*)-**28**[i]	inactive					4×10^0
11-CH_2NH_2-20(*RS*)-**29**[i]	inactive					2×10^0
11-CH_2OH-20(*RS*)-**30**[j]	inactive					3×10^{-1}
11-CHO-20(*RS*)-**22**[j]	inactive					5×10^{-1}
11-OH-20(*RS*)-**8**[k]	357 (60.0)	3	≥5.68	7.5[g]–60.0[f]	>60.0	2×10^{-2}
11-formyl guanylhydrazone·OTs-20(*RS*)-**26**[i]	inactive					inactive
10,11-$(OMe)_2$-20(*RS*)-**36**[e]	inactive					9
10,11-$(OH)_2$·HBr-20(*RS*)-**34**[i]	173 (80.0)	0	2.62	10.0[g]–80.0[f]	>80.0	3
10,11-$(OH)_2$-20(*RS*)-**35**[i]	153 (10.0)	1	0.87	10.0[g]–80.0[f]	>80.0	1
10,11-OCH_2O-20(*RS*)-**7**[l]	325 (2.0)	2	≥5.97	2.0[g]–4.0	>8.0	7×10^{-3}
10-Cl-20(*RS*)-**31**[i]	280 (10.0)	2	≥5.97	10.0[g]–20.0[f]	40.0	1×10^{-2}
10-Me-20(*RS*)-**32**[i]	207 (10.0)	0	4.66	10.0[g]–80.0[f]	>80.0	8×10^{-2}
10-NH_2-20(*RS*)-**6**[m]	365 (3.75)	3	≥5.97	2.0[g]–15.5	32.0	3×10^{-2}
10-NO_2-20(*RS*)-**9**[m]	219 (15.5)	1	≥5.86	7.5[g]–15.5	31.0	3×10^{-1}
9-NH_2-20(*S*)-**4**[n]	361 (2.5)	4	≥5.97	0.25–5.0	10.0	2×10^{-2}

[a] Treatment schedule Q04D×02; ip using Klucel emulsifier. [b] Expressed as the μg/mL concentration resulting in a 50% cell number reduction (ED_{50}). [c] % T/C = (median survival time of treated/control animals) × 100. [d] $\log_{10}$ of initial tumor cell population minus $\log_{10}$ of tumor cell population at end of treatment. [e] 10-Hydroxy-20(*S*)-camptothecin (**2**) was used as a comparison standard: % T/C 183 (6.0). [f] Highest dose administered. [g] Lowest dose administered. [h] Compound **2** and 20(*S*)-camptothecin (**1**) were used as comparison standards: for **2**, % T/C 180 (12.0); for **1**, % T/C 195 (5.0). [i] Compound **1** was used as a comparison standard: % T/C 215 (5.0). [j] Compound **1** was used as a comparison standard: % T/C 186 (10.0). [k] Compound **1** was used as a comparison standard: % T/C 164 (8.0). [l] Compound **1** was used as a comparison standard: % T/C 166 (5.0). [m] Compound **1** and **2** were used as a comparison standard: for **1**, % T/C 197 (8.0); for **2**, % T/C 230 (24.0). [n] Compound **1** was used as a comparison standard: % T/C 197 (4.0).

magnetic resonance spectrometer, and infrared spectra were determined on a Perkin-Elmer 267 spectrophotometer in solid phase (KBr) or solution ($CHCl_3$). Exact mass measurements were made on a MS-902 mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and Galbraith Laboratories, Inc., Knoxville, TN; analyses were correct within ±0.4% for variously hydrated species. In this latter connection we have observed that many camptothecin analogues suffer decomposition upon stringent conditions of drying. Homogeneity of camptothecin analogues was established by HPLC with a Waters system and Whatman Partisil PXS 10/25 PAC column in the normal-phase mode (MeOH/$CHCl_3$) with detection at 254 nm.

11-(Dimethylamino)-20(*RS*)-camptothecin (16). *N,N*-Dimethyl-*m*-nitroaniline (Aldrich) was subjected to a standard Vilsmeier reaction[16] to give known 4-(dimethylamino)-2-nitrobenzaldehyde (**10a**) in 32% yield.

The aldehyde **10a** (0.35 g, 1.80 mmol) was treated with ethylene glycol (1 mL) in refluxing toluene (20 mL) containing *p*-TsOH·H_2O (10 mg). After 3 h, the dark orange solution was cooled and diluted with H_2O and EtOAc, and the organic phase was separated. Drying of the organic phase (Na_2SO_4) and evaporation afforded the crude acetal **11a** as a red-orange syrup (420 mg, 98%). Compound **11a** was purified by column chromatography (SiO_2, 5% EtOAc in hexanes) to give an orange oil; ^1H NMR ($CDCl_3$) δ 2.94 (6, NMe_2), 3.96 (s, 4, OCH_2CH_2O), 6.17 (s, 1, OCHO), 6.67 (dd, 1, *J* = 2, 8 Hz, H-5), 6.96 (d, 1, *J* = 2 Hz, H-3), 7.43 (d, 1, *J* = 8 Hz, H-6). Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

The nitro aldehyde ethylene acetal **11a** (650 mg, 2.73 mmol) was refluxed with $Na_2S·9H_2O$ (2.0 g, 8.33 mmol) in H_2O (3 mL) and EtOH (12 mL) for 30 min. The red-brown mixture was diluted with $CHCl_3$ and H_2O and the organic layer was reserved. The aqueous portion was reextracted with $CHCl_3$ and the combined extract was washed with H_2O, dried (Na_2SO_4), and evaporated to give the crude amino acetal **11b** as a red gum. Compound **11b** showed a lack of characteristic NO_2 absorption in the IR spectrum ($CHCl_3$) and the appearance of NH_2 absorption at 3450 cm^{-1}. Attempts to purify crude **11b** by chromatography only led to further darkening and decomposition, and thus further characterization was not possible.

Crude **11b** (356 mg, <1.7 mmol) and the ketone **5** (300 mg, 1.14 mmol) were brought to reflux in toluene (30 mL). After 1 min, P-TsOH·H_2O (65 mg) was added to the homogeneous orange solution, and heating at reflux was continued for 2 h. After cooling, the red mixture was treated with aqueous $NaHCO_3$ (10 mL) and EtOAc (50 mL). The organic phase was reserved, and the aqueous phase was reextracted with $CHCl_3$/MeOH (4:1, 2 × 75 mL). The combined extract was dried (Na_2SO_4) and evaporated to give a brown semisolid. The material was chromatographed (SiO_2, 40 g, 2% MeOH in $CHCl_3$) as a solid adsorbed on Celite (2.5 g). Evaporation of the appropriate fractions gave 11-(dimethylamino)-20(*RS*)-camptothecin (**16**) containing highly colored impurities. Trituration with $CHCl_3$ gave **16** as an orange-yellow solid (120 mg, 27%). Recrystallization from 13% MeOH in $CHCl_3$ afforded analytically pure **16** as an orange-yellow powder: mp 261–264 °C; IR (KBr) 3400 (OH), 1750 (lactone), 1658 (pyridone), 1620, 1612, 1592 (aromatic), 1162 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, *J* = 7 Hz, H-18), 1.86 (q, 2, *J* = 7 Hz, H-19), 3.10 (s, 6, NMe_2), 5.18 (s, 2, H-17), 5.41 (s, 2, H-5), 6.49 (s, 1, OH), 7.10 (d, 1, *J* = 2.5 Hz, H-12), 7.25 ($CHCl_3$), 7.27 (s, 1, H-14), 7.39 (dd, 1, *J* = 2.5, 9 Hz, H-10), 7.89 (d, 1, *J* = 9 Hz, H-10), 7.89 (d, 1, *J* = 9 Hz, H-9), 8.42 (s, 1, H-7) (H-12 was observed to exchange for deuterium in TFA-d_1). Anal. Calcd for $C_{22}H_{21}N_3O_4$: 391.1532. Found: 391.1534. ($C_{22}H_{21}N_3O_4$·0.25$CHCl_3$) C, H. N.

11-Nitro-20(*RS*)-camptothecin (17). 2,4-Dinitrobenzaldehyde (**10b**) was prepared by the standard method[17] from 2,4-dinitrotoluene. Reaction of **10b** with aqueous $TiCl_3$ in acidified ethanol gave the known 2-amino-4-nitrobenzaldehyde (**10c**).[7]

Compound **10c** (1.38 g, 8.31 mmol) and the tricyclic ketone **5** (1.00 g, 3.80 mmol) were brought to reflux in toluene (100 mL).

(16) Baumann, H.; Grychtol, K.; Oberlinner, A. Ger. Offen. 2 363 458, July 3, 1975; *Chem. Abstr.* **1976**, *84*, 4662z.

(17) Bennett, G. M.; Bell, E. V. *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. II, p 223.

The tan suspension was treated with *p*-TsOH·H_2O (250 mg), and the darkening suspension was refluxed for an additional 36 h. The toluene was evaporated and the black gummy solid was partitioned between $CHCl_3$ and H_2O. The aqueous phase was extracted with additional $CHCl_3$ and the combined organic phase was filtered and evaporated to give a brown gummy solid. Column chromatography (SiO_2, 75 g, gradient of 200 mL of $CHCl_3$, 300 mL of 2% MeOH in $CHCl_3$, 500 mL of 4% MeOH in $CHCl_3$, and 500 mL of 20% MeOH in $CHCl_3$) of the crude material adsorbed on powdered Na_2SO_4 (25 g) gave pure 11-nitro-20(*RS*)-camptothecin (**17**) as an orange powder (135 mg, 9%). Recrystallization from 13% MeOH in $CHCl_3$ afforded **17** as an orange-yellow powder: mp 176–180 °C [lit.[18] mp 246 °C for 20*S* isomer]; IR (KBr) 3440 (OH), 1742 (lactone), 1660 (pyridone), 1610 (aromatic), 1531 (NO_2), 1343, 1157 cm^{-1}; ^1H NMR (TFA-d_1) δ 1.16 (t, 3, J = 7 Hz, H-18), 2.18 (q, 2, J = 7 Hz, H-19), 5. 80 (AB q, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.90 (s, 2, H-5), 8.46 (s, 1, H-14), 8.61 (d, 1, J = 9 Hz, H-9), 8.83 (d, 1, J = 9 Hz, H-10), 9.44 (s, 1, H-12), 9.48 (s, 1, H-7) (the spectrum recorded in Me_2SO-d_6 matched that in the literature[18] and showed a coalescence of H-9 and H-10 at δ 8.42). Anal. Calcd for $C_{20}H_{15}N_3O_6$: 393.0960. Found: 393.0965. ($C_{20}H_{15}N_3O_6$·0.4$CHCl_3$) C, H, N.

11-Amino-20(*RS*)-camptothecin (18). The diamino aldehyde **10d** was prepared from **10c** by ferrous sulfate reduction in hot aqueous EtOH containing NH_4OH. The procedure was analogous to that described in the literature for the preparation of **10d** from the isomeric amino nitro aldehyde **10e**.[8]

The diamino aldehyde **10d** (512 mg, 3.765 mmol) and ketone **5** (350 mg, 1.331 mmol) were refluxed in toluene (75 mL). Glacial HOAc (2 mL) was added, and heating at reflux was continued for 4 h. Evaporation of the solvent left a dark brown solid, which was chromatographed as a dispersion on Celite (4.0 g) through SiO_2 (100 g, 200 mL of $CHCl_3$, 750 mL of 3% MeOH in $CHCl_3$, and 1 L of 5% MeOH in $CHCl_3$) to give 11-amino-20(*RS*)-camptothecin (**18**) as an orange solid (117 mg, 24%). Product **18** was obtained as a pale tan-green powder when recrystallized from 13% MeOH in $CHCl_3$: mp 186–190 °C dec; IR (KBr) 3390 (OH), 3330, 3240 (NH_2), 1750 (lactone), 1660 (pyridone), 1615–1570 (aromatic), 1512, 1380, 1234, 1162, 1045 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.86 (q, 2, J = 7 Hz, H-19), 5.15 (s, 2, H-17), 5.25–5.55 (br s, 2, NH_2), 5.41 (s, 2, H-5), 6.49 (s, 1, OH), 7.03 (d, 1, J = 2.5 Hz, H-12), 7.09 (dd, J = 2.5, 9 Hz, H-10), 7.24 (s, 1, H-14), 7.75 (d, 1, J = 9 Hz, H-9), 8.34 (s, 1, H-7). Anal. Calcd for $C_{20}H_{17}N_3O_4$: 363.1219. Found: 363.1216. ($C_{22}H_{17}N_3O_4$·2.0H_2O) C, H, N.

11-(Trifluoromethyl)-20(*RS*)-camptothecin (19). The new benzaldehyde derivative 2-nitro-4-(trifluoromethyl)benzaldehyde (**12a**) was prepared in very poor yield by the formaldoxime displacement reaction on the corresponding diazonium salt.[19] Thus, the diazonium salt of 4-amino-3-nitrobenzotrifluoride (24.9 g, 0.120 mol) was prepared by stirring the amine in a mixture of concentrated HCl (27.6 mL), H_2O (24 mL), and ice (50 g) during which a solution of $NaNO_2$ (8.5 g, 0.123 mol) in H_2O (12 mL) was added over 10 min. After 30 min of stirring in the cold, most of the substrate remained undissolved. A solution of NaOAc (15 g) in H_2O (25 mL) was added, and the slurry was added in portions to a rapidly stirred formaldoxime solution (10% in H_2O, 0.23 mol) at 5 °C. The turbid gummy mixture was left for 1.5 h and acidified to pH 2 with concentrated HCl, and the solid was collected. The intermediate oxime (tarry brown material) was hydrolyzed by refluxing in concentrated HCl (75 mL) and H_2O (200 mL). The mixture was cooled and extracted with $CHCl_3$ (2 × 250 mL). Evaporation of $CHCl_3$ left a residue which was treated for 5 min with a refluxing solution of $Na_2S_2O_5$ (20 g) in H_2O (150 mL). The starting amine and other impurities were removed by extraction into $CHCl_3$ (2 × 200 mL). The aqueous phase was cooled and basified with 50% aqueous NaOH to pH 12, and the suspension of **12a** was extracted into $CHCl_3$ (4 × 100 mL). Drying (Na_2SO_4) and evaporation afforded the pure aldehyde **12a** as pale yellow prisms (0.42 g, 1.6%): mp 35 °C; IR ($CHCl_3$) 1706 (CHO), 1542, 1352, 1323, 1173, 1150, 1093 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.09 (m, 2, H-5 and H-6), 8.42 (s, 1, H-3), 10.49 (s, 1, CHO). Anal. ($C_8H_4NO_3F_3$) C, H, N, F.

The nitro aldehyde **12a** (160 mg, 0.731 mmol) was suspended in absolute EtOH (10 mL) and warmed to 70 °C. The yellow solution was diluted with H_2O (10 mL) and treated with $FeSO_4$·7H_2O (1.42 g, 5.11 mmol). Upon the dropwise addition of concentrated NH_4OH (2 mL) at 70 °C, the pale yellow mixture immediately became dark green-brown. The mixture was filtered (sintered glass) and the dark tan filtrate was extracted with $CHCl_3$ (3 × 25 mL). Drying (Na_2SO_4) and evaporation of the yellow extract gave 2-amino-4-(trifluoromethyl)benzaldehyde (**12b**) as a tan-yellow oil (115 mg, 83%). Compound **12b** could be further purified by simple filtration of its $CHCl_3$ solution through silica gel to remove traces of polar impurities. **12b**: IR ($CHCl_3$) 3505 and 3360 (NH_2), 1675 (CHO), 1630, 1585, 1550, 1440, 1330, 1282, 1180, 1150 (br), 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (1, d, J = 8.5 Hz, H-6), 7.93 (1, d, J = 8.5 Hz, H-5), 8.42 (1, s, H-3), 9.57 (1, s, CHO). Amino aldehyde **12b** darkened rapidly on storage and thus was used immediately after preparation.

Amino aldehyde **12b** (95 mg, 0.503 mmol) and tricyclic ketone **5** (110 mg, 0.418 mmol) were combined and refluxed in toluene (30 mL) for 5 min to give a clear yellow solution. Solid *p*-TsOH·H_2O (10 mg) was added and refluxing was continued for 2.5 h during which the solution became turbid and orange. After cooling, the solvent was evaporated and the residue was chromatographed as a $CHCl_3$ solution through SiO_2 (20 g, $CHCl_3$ eluent). The 11-trifluoromethyl analogue **19** resulted as a beige solid (90 mg, 52%). Recrystallization from $Et_2O/CHCl_3$ gave compound **19** as a beige powder: mp 172–175 °C; IR (KBr) 3500 (OH), 1755 (lactone), 1662 (pyridone), 1595 (aromatic), 1327, 1232, 1162, 1053, 950, and 848 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.98 (t, 3, J = 7 Hz, H-18), 1.89 (q, 2, J = 7 Hz, H-19), 5.33 (s, 2, H-17), 5.44 (s, 2, H-5), 6.58 (s, 1, OH), 7.39 (s, 1 H-14), 7.97 (dd, 1, J = 1.5, 8.5 Hz, H-10), 8.38 (d, 1, J = 8.5 Hz, H-9), 8.53 (d, 1, J = 1.5 Hz, H-12), 8.84 (s, 1, H-7). Anal. Calcd for $C_{21}H_{15}N_2O_4F_3$: 416.0984. Found: 416.0983: ($C_{21}H_{15}N_2O_4F_3$·2H_2O) C, H, N, F.

11-Methyl-20(*RS*)-camptothecin (20). 4-Methyl-2-nitrobenzaldehyde (**12c**)[20] was prepared from 4-methyl-2-nitroaniline via the diazonium salt in the same fashion as **12a** was prepared. Further reaction with $FeSO_4$·7H_2O and NH_4OH in hot EtOH gave the amino aldehyde **12d**.[9]

The amino aldehyde **12d** (520 mg, 3.852 mmol) and ketone **5** (250 mg, 0.951 mmol) were combined and brought to reflux in toluene (55 mL). Glacial HOAc (4 mL) was added to the clear yellow solution and after 2 h at reflux the mixture was red-brown. The solvent was evaporated, and the tan-red residue was triturated with hot Et_2O (20 mL). Recrystallization of the sample from $CHCl_3/Et_2O$ gave pure 11-methyl-20(*RS*)-camptothecin (**20**) as a beige solid (106 mg, 31%): mp 262–265 °C; IR (KBr) 3480 (OH), 1750 (lactone), 1655 (pyridone), 1622 and 1590 (aromatic), 1233, 1163, 1105, 1040 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, 7 Hz, H-18), 1.88 (m, 2, H-19), 2.58 (s, 11-CH_3), 5.27 (s, 2, H-17), 5.43 (s, 2, H-5), 6.54 (br s, 1, OH), 7.33 (s, 1, H-14), 7.56 (d, 1, J = 8.3 Hz, H-10), 7.96 (s, 1, H-12), 8.02 (d, 1, J = 8.3 Hz, H-9), 8.63 (s, 1, H-7). Anal. Calcd for $C_{21}H_{18}N_2O_4$: 362.1266. Found: 362.1264. ($C_{21}H_{18}N_2O_4$·0.3H_2O) C, H, N.

11-Formyl-20(*RS*)-camptothecin (22). 2-Nitroterephthaldicarboxaldehyde[21] was converted to the diacetal **13a** by conventional methods and reduced with Na_2S. Thus, a solution of the nitro diacetal **13a** (4.1 g, 17.5 mmol) and Na_2S (14 g) in 80% ethanol (65 mL) was refluxed for 1 h. Ethanol was removed in vacuo, and the reaction mixture was diluted with water (10 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 50 mL), and the organic phase was washed with water, dried ($MgSO_4$), and evaporated to give the amine **13b**, which was recrystallized from ethyl acetate/hexane (2.8 g, 78%): mp 76 °C; IR (KBr) 3480, 3395, 3000, 2960, 2900, 1625, 1445, 1395, 1085, 950 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.0 (m, 8, C-1 and C-4 OCH_2CH_2O), 5.6 (s, 1, C-4 $CH(OCH_2)_2$), 5.7 (s, 1, C-1, $CH(OCH_2)_2$), 6.6 (s, 1, 3-*H*), 6.65 (d, 1, J = 8 Hz, 5-H), 7.2 (d, 1, J = 8 Hz, 6-H). Anal. ($C_{12}H_{15}NO_4$) C, H, N.

(18) Yakult Honsha Co., Ltd. Jpn. Kokai Tokkyo Koho JP 5951287, March 24, 1984; *Chem. Abstr.* **1984**, *101*, 91320x.
(19) Beech, W. F. *J. Chem. Soc.* **1954**, 1297.
(20) Sidhu, V. G. S.; Thyagarajan, G.; Ansari, S. *Liebigs Ann. Chem.* **1959**, *627*, 218.
(21) Ruggli, P.; Preiswerk, E. *Helv. Chem. Acta* **1939**, *22*, 478.

A solution of oxytricyclic ketone **5** (265 mg, 1.0 mmol), amino diacetal **13b** (500 mg, 2.1 mmol; 300 mg initially, 100 mg each at intervals of 5 and 10 h) in toluene (70 mL) was refluxed for 0.5 h. Acetic acid (2 mL) was added and the mixture refluxed for 18 h. The solvent was evaporated in vacuo and the residue containing a mixture of **21** and **22** was taken up in 75% methanol (250 mL). Concentrated HCl (3 mL) was added and the solution was heated at 50–60 °C for 24 h. After cooling, the suspension was filtered, the residue was washed with water and recrystallized from $CHCl_3$/MeOH/EtOAC to give **22** (175 mg, 46%): mp 276–279 °C; IR (KBr) 3460, 1745 (lactone), 1690 (CHO), 1655 (pyridone), 1600, 1200, 1150, 1135 cm^{-1}; ^1H NMR (TFA-d_1) δ 1.16 (t, 3, J = 7 Hz, H-18), 2.16 (q, 2, J = 7 Hz, H-19), 5.78 (AB q, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.89 (s, 2, H-5), 8.43 (s, 1, H-14), 8.66 (d, 1, J = 8.5 Hz, H-10), 8.60 (d, 1, J = 8.5 Hz, H-9), 9.12 (s, 1, H-12), 9.49 (s, 1, H-7), 10.42 (s, 1, C*H*O). Anal. Calcd for $C_{21}H_{16}N_2O_5$: 376.1059. Found: 376.1059. ($C_{21}H_{16}N_2O_5 \cdot 1.0H_2O$) C, H, N.

11-Carbomethoxy-20(*RS*)-camptothecin (23). The Corey oxidation procedure[10] was applied in this preparation. Acetic acid (0.2 mL) was added to a suspension of KCN (200 mg) in MeOH (5 mL) and stirred for 5 min. 11-Formyl-20(*RS*)-camptothecin (**22**) (50 mg, 0.13 mmol) in MeOH (5 mL) was added and stirred for 5 min, followed by addition of CH_2Cl_2 (10 mL). After 15 min, active MnO_2 (800 mg) was added and the suspension was stirred vigorously for 24 h. After filtration the residue was thoroughly washed with CH_2Cl_2, and the combined filtrate and washings were carefully evaporated to dryness. The residue was taken up in CH_2Cl_2, washed with H_2O, dried ($NaSO_4$), and concentrated to a volume of 5 mL. The precipitate was collected and recrystallized from $CHCl_3$ to yield **23** (40 mg, 74%): mp 268 °C; IR (KBr) 1750 (lactone), 1720 (ester), 1650 (pyridone), 1595 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.89 (q, 2, H-19), 3.97 (s, 3, 11-C(O)$_2$OCH_3), 5.31 (s, 2, H-17), 5.44 (s, 2, H-5), 7.38 (s, 1, H-14), 8.15 (d, 1, J = 8.5 Hz, H-9), 8.26 (d, 1, J = 8.5 Hz, H-10), 8.70 (s, 1, H-12), 8.78 (s, 1, H-7). Anal. Calcd for $C_{22}H_{18}N_2O_6$: 406.1164. Found: 406.1163. ($C_{22}H_{18}N_2O_6$) C, H, N.

11-Carboxy-20(*RS*)-camptothecin (24). 11-Carbomethoxycamptothecin (**23**) (55 mg, 0.14 mmol) was heated at 90 °C for 3 h with acetic acid (4 mL) and aqueous HCl (5 mL, 3 N HCl). The solution was evaporated to dryness under vacuum. The residue was washed two times with CH_2Cl_2 to give nearly pure **24**. Recrystallization from dilute ethanol gave pure 11-carboxy-20(*RS*)-camptothecin (**24**) (45 mg, 85%): mp >300 °C; IR (KBr) 1750 (lactone), 1710 (acid), 1650 (pyridone), 1590 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.90 (t, 3, J = 7 Hz, H-18), 1.88 (q, 2, H-19), 5.30 (s, 2, H-17), 5.44 (s, 2, H-5), 7.38 (s, 1, H-14), 8.14 (d, 1, H-9), 8.23 (d, 1, H-10), 8.68 (s, 1, H-12), 8.77 (s, 1, H-7). Anal. Calcd for $C_{21}H_{16}N_2O_6$: 392.1008. Found: 392.1009. ($C_{21}H_{16}N_2O_6 \cdot 0.75H_2O$) C, H, N.

11-Carboxy-20(*RS*)-camptothecin Monosodium Salt (25). A mixture of 11-carboxycamptothecin (**24**) (56.0 mg, 1.4 mmol) and $NaHCO_3$ (12.0 mg) in aqueous MeOH (7:3) was refluxed gently for 2 h. The sample was evaporated to dryness, and the residue was recrystallized twice from EtOH/Et_2O (27 mg, 46%): mp >250 °C; IR (KBr) 1730 (lactone), 1655 (pyridone), 1590 cm^{-1}; ^1H NMR (Me_2SO-d_6/D_2O) δ 0.89 (t, 3, H-19), 2.1 (m, 2, H-18), 4.79 (AB q, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.23 (s, 2, H-5), 7.72 (s, 1, H-14), 8.15 (d, 1, J = 8.4 Hz, H-9), 8.18 (d, 1, J = 8.4 Hz, H-10), 8.59 (s, 1, H-12), 8.63 (s, 1, H-7). Anal. ($C_{21}H_{15}N_2O_6Na \cdot 3.3H_2O$) C, H, N, Na.

11-Formyl-20(*RS*)-camptothecin 11-Guanylhydrazone Tosylate (26). A mixture of 11-formyl-20(*RS*)-camptothecin (**22**) (57 mg, 0.15 mmol), aminoguanidine bicarbonate (22 mg), and p-TsOH·H_2O (35 mg) in dioxane (50 mL) was refluxed for 8 h. The solvent was removed and the residue was taken up in hot dilute EtOH. After concentration of the solution, addition of Et_2O yielded **26** as a yellow powder (69 mg, 74%): mp 267 °C; IR (KBr) 3380, 3170, 1660, 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.88 (m, 2, H-19), 2.29 (s, 3, CH_3), 5.28 (s, 2, H-17), 5.43 (s, 2, H-5), 7.13 (d, 2, J = 8.0 Hz, OTs), 7.33 (s, 1, H-14), 7.51 (d, 2, J = 8.0 Hz, OTs), 8.14 (d, 1, J = 8.5 Hz, H-9), 8.33 (d, 1, J = 8.5 Hz, H-10), 8.38 (s, 1, 11-C*H*), 8.49 (s, 1, H-12), 8.67 (s, 1, H-7). Anal. ($C_{29}H_{28}N_6SO_7$) C, H, N, S.

11-Cyano-20(*RS*)-camptothecin (27). A general literature procedure[11] was employed in this preparation. A mixture of 11-formyl-20(*RS*)-camptothecin (**22**) (225 mg, 0.6 mmol), hydroxylamine hydrochloride (50 mg, 0.72 mmol), sodium formate (90 mg, 1.3 mmol), and formic acid (6 mL) was refluxed for 1.5 h. The mixture was evaporated to dryness in vacuo, and the residue was washed with H_2O, dried ($MgSO_4$), and chromatographed (SiO_2, 0.5% MeOH/$CHCl_3$). Compound **27** was recrystallized from $CHCl_3$/EtOAc (65 mg, 29%): mp 288 °C; IR (KBr) 3400, 2235, 1735 (lactone), 1655 (pyridone), 1590, 1450, 1400, 1230, 1150, 1110, 1045 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.88 (m, 2, H-19), 5.32 (s, 2, H-17), 5.44 (s, 2, H-5), 7.37 (s, 1, H-14), 7.98 (d, 1, J = 8.5Hz, H-10), 8.32 (d, 1, J = 8.5 Hz, H-9), 8.74 (s, 1, H-12), 8.80 (s, 1 H-7). Anal. Calcd for $C_{21}H_{15}N_3O_4$: 373.1062. Found: 373.1065. ($C_{21}H_{15}N_3O_4 \cdot 1.5H_2O$) C, H, N.

11-(Aminomethyl)-20(*RS*)-camptothecin Hydrochloride (28). The following is based on a general literature[12] procedure. A solution consisting of 11-formylcamptothecin (**22**) (75 mg, 0.2 mmol) and 2-aminoisobutyric acid (20 mg) in DMF (5 mL) was refluxed for 1.5 h. The mixture was concentrated in vacuo and to the residue was added aqueous HCl (2 N, 5 mL). The resulting solution was gently refluxed for 2 h and filtered hot, and the filtrate was dried (Na_2SO_4). The residue upon evaporation was recrystallized twice from hot MeOH/Et_2O to give hydrochloride **28** (35 mg, 41%): mp >300 °C; IR (KBr) 3400 (br), 2980 (br), 1730 (lactone), 1655 (pyridone), 1585 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.88 (m, 2, H-19), 4.32 (s, 2, 11-CH_2NH$_2$), 5.29 (s, 2, H-17), 5.44 (s, 2, H-5), 7.35 (s, 1, H-14), 7.82 (d, 1, J = 8.5 Hz, H-9), 8.18 (d, 1, J = 8.5 Hz, H-10), 8.28 (s, 1, H-12), 8.70 (s, 1, H-7). Anal. ($C_{21}H_{20}N_3O_4Cl \cdot 0.5H_2O$) C, H, N, Cl.

11-(Aminomethyl)-20(*RS*)-camptothecin (29). To an aqueous solution of the amine hydrochloride **28** (83 mg, 0.2 mmol) was slowly added 10% aqueous $NaHCO_3$ solution until a pH of 8 was obtained. After centrifugation, the residue was dissolved in hot $CHCl_3$/MeOH (99:1). Upon reducing the volume and cooling, there resulted the free base **29** (59 mg, 78%): mp >250 °C; IR (KBr) 3400 (br), 2980, 1735 (lactone), 1655 (pyridone), 1585 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.84 (m, 2, H-19), 4.00 (s, 2, 11-CH_2NH$_2$), 5.23 (s, 2, H-17), 5.41 (s, 2, H-5), 7.31 (s, 1, H-14), 7.67 (d, 1, J = 8.5 Hz, H-10), 8.03 (d, 1, J = 8.5 Hz, H-9), 8.09 (s, 1, H-12), 8.61 (s, 1, H-7). Anal. Calcd for $C_{21}H_{19}N_3O_4$: 377.1375. Found: 377.1376. ($C_{21}H_{19}N_3O_4 \cdot 1.0H_2O$) C, H, N.

11-(Hydroxymethyl)-20(*RS*)-camptothecin (30). A suspension of 11-formylcamptothecin (**22**) (113 mg, 3.0 mmol) in EtOH (20 mL) and 5% Pd–C (40.0 mg) was hydrogenated at atmospheric pressure until the uptake of hydrogen was complete. The catalyst was filtered and washed with ethanol, the solvent was evaporated, and the residue was chromatographed (SiO_2, 3% MeOH/$CHCl_3$) to give a light yellow solid (33 mg, 28%), which was crystallized from $CHCl_3$/MeOH: mp 254–257 °C; IR (KBr) 3320, 2950, 1750 (lactone), 1650 (pyridone), 1585 (aromatic) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.86 (q, 2, J = 7 Hz), 4.76 (d, 2, CH_2OH), 5.27 (s, 2, H-17), 5.43 (s, 2, H-5), 5.51 (t, 1, CH$_2$O*H*), 6.53 (s, 1, H-14), 7.65 (d, 1, J = 8 Hz, H-9), 8.07 (d, 1, J = 8 Hz, H-10), 8.07 (s, 1, H-12), 8.65 (s, 1, H-7). Anal. ($C_{21}H_{18}N_2O_5 \cdot 0.25H_2O$) C, H, N.

10-Chloro-20(*RS*)-camptothecin (31). 5-Chloro-2-nitrobenzaldehyde (**14a**) was converted to 2-amino-5-chlorobenzaldehyde (**14b**) with $FeSO_4$ and NH_4OH by a literature procedure.[22] Recrystallization from hexane gave pure **14b**, mp 70 °C (lit.[22] mp 72 °C). A solution of the 2-amino-5-chlorobenzaldehyde (**14b**) (80 mg, 0.51 mmol) and oxytricyclic ketone **5** (100 mg, 0.38 mmol) in toluene (60 mL) was refluxed for 15 min. p-Toluenesulfonic acid (30 mg) was then added, and refluxing was continued for an additional 5 h. The solvent was removed in vacuo, and the residue was chromatographed (SiO_2, 2% MeOH/$CHCl_3$). The product obtained was recrystallized ($CHCl_3$/MeOH/EtOAC) to give pure **31** (60 mg, 41%): mp 270 °C; IR (KBr) 3430, 1745 (lactone), 1655 (pyridone), 1600, 1495, 1230, 1160 cm^{-1}; ^1H NMR (TFA-d_1) δ 1.15 (t, 3, J = 7 Hz, H-18), 2.16 (m, 2, H-19), 5.73 (AB q, 2 J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.84 (s, 2, H-5), 8.29 (d, 1, J = 9 Hz, H-11), 8.35 (s, 1, H-14), 8.40 (s, 1, H-9), 8.45 (d, 1, J

(22) Horner, J. K.; Henry, D. W. *J. Med. Chem.* **1968**, *11*, 946.

= 9 Hz, H-12), 9.31 (s, 1, H-7). Anal. ($C_{20}H_{15}N_2ClO_4 \cdot 0.5H_2O$) C, H, N. Cl.

10-Methyl-20(*RS*)-camptothecin (32). 5-Methyl-2-nitrobenzaldehyde (**14c**) was prepared by the oxidation of 5-methyl-2-nitrobenzyl alcohol.[23] The reduction of **14c** using $FeSO_4$ and NH_4OH in hot aqueous EtOH yielded the unstable amino aldehyde **14d**, which was used as such in the Friedlander condensation.

The oxytricyclic ketone **5** (130 mg, 0.5 mmol) and the 5-methyl-2-aminobenzaldehyde (**14d**) (560 mg) in toluene (60 mL) were refluxed for 0.5 h. Acetic acid (1 mL) and *p*-TsOH·H_2O (35 mg) were added, and refluxing was continued for an additional 5 h. The solvent was removed in vacuo, and warm Et_2O (30 mL) was added. The collected residue was recrystallized from $CHCl_3$/MeOH/EtOAC to yield **32** (102 mg, 57%); mp 278–281 °C; IR (KBr) 3460, 2980, 1740 (lactone), 1655 (pyridone), 1590, 1550, 1470, 1450, 1370, 1260, 1240, 1160, 1050 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.87 (q, 2, H-19), 2.54 (s, 3, 10-CH_3), 5.24 (s, 2, H-17), 5.42 (s, 1, H-5), 7.31 (s, 1, H-14), 7.69 (d, 1, J = 8.6 Hz, H-11), 7.86 (s, 1, H-9), 8.05 (d, 1, J = 8.6 Hz, H-12), 8.55 (s, 1, H-7). Anal. ($C_{21}H_{18}N_2O_4 \cdot 0.25H_2O$) C, H, N.

10,11-Dihydroxy-20(*RS*)-camptothecin Hydrobromide (34) and 10,11-Dihydroxy-20(*RS*)-camptothecin (35). 4,5-Bis(benzyloxy)-2-nitrobenzaldehyde (**15a**) was converted to the nitro acetal **15b**, which was then reduced to the amino acetal **15c** with Na_2S by well-established procedures. Both of these intermediates could not be purified and were used as such for further reactions.

A solution of the crude bis(benzyloxy) amino acetal **15c** (400 mg) and oxytricyclic ketone **5** (132 mg, 0.5 mmol) in toluene (60 mL) was refluxed for 8 h. The mixture was filtered and the intermediate 10,11-bis(benzyloxy) product **33** was collected as pure material (220 mg, 81%): mp 276 °C; IR (KBr) 3440, 1740 (lactone), 1650 (pyridone), 1590, 1490, 1440, 1380, 1250, 1140, 1100 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.86 (m, 2, H-19), 5.22 (s, 2, H-17), 5.34 (s, 2, 10-OC$H_2C_5H_5$), 5.39 (s, 2, 11-OC$H_2C_6H_5$), 5.41 (s, 2, H-5), 6.50 (s, 1, OH), 7.25 (s, 1, H-14), 7.35-7.65 (m, 12, H-9, H-12, 10- and 11-OC$H_2C_6H_5$), 8.44 (s, 1, H-7).

The bis(benzyloxy)camptothecin derivative **33** (130 mg, 0.23 mmol) was gently refluxed for 2 h in 24% aqueous HBr (50 mL). The acid was removed in vacuo and the residue was dissolved in hot MeOH (50 mL). Ether (50 mL) was added and the powdery yellow 10,11-dihydroxy-20(*RS*)-camptothecin hydrobromide (**34**) was collected (122 mg, 77%): mp >300 °C. Anal. ($C_{20}H_{17}N_2O_6Br \cdot 0.5H_2O$) C, H, N, Br.

The dihydroxy hydrobromide salt **34** (110 mg, 0.23 mmol) was suspended in water (10 mL). Sodium hydroxide (0.1 N, 7.2 mL) was added, and the mixture was stirred until a clear solution resulted. Acidification to slightly acid pH using 5 *N* HCl gave a suspension, which was centrifuged after 1 h. The supernatant liquid was decanted and the process repeated with additional water (20 mL). The residue was dried to give free base **35** (78 mg, 74%): mp >300 °C; IR (KBr) 3490, 3000 (b), 1740 (lactone), 1645 (pyridone), 1590, 1460, 1385, 1265, 1190, 1150 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.87 (q, 2, H-19), 5.20 (s, 2, H-17), 5.42 (s, 2, H-5), 7.35 (s, 1, H-14), 7.44 (s, 1, H-9), 7.52 (s, 1, H-12), 8.51 (s, 1, H-7). Anal. Calcd for $C_{20}H_{16}N_2O_6$: 380.1008. Found: 380.1007. ($C_{20}H_{16}N_2O_6 \cdot 0.75H_2O$) C, H, N.

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Registry No. (±)-**5**, 102978-40-5; **10a**, 56670-20-3; **10c**, 109466-84-4; **10d**, 98276-57-4; **11d**, 109466-82-2; **11b**, 109466-83-3; **12a**, 109466-87-7; **12b**, 109466-88-8; **12d**, 59236-38-3; **13a**, 109466-91-3; **13b**, 109466-92-4; **14a**, 6228-86-0; **14b**, 20028-53-9; **14c**, 5858-28-6; **14d**, 109467-00-7; **15a**, 18002-41-0; **15b**, 109467-02-9; **15c**, 109467-03-0; (±)-**16**, 109581-95-5; (±)-**17**, 109581-96-6; (±)-**18**, 109581-97-7; (±)-**19**, 109466-89-9; (±)-**20**, 109466-90-2; (±)-**22**, 109466-93-5; (±)-**23**, 109466-94-6; (±)-**24**, 109466-95-7; (±)-**25**, 109466-96-8; (±)-**26**, 109466-97-9; (±)-**27**, 109466-98-0; (±)-**28**, 109494-80-6; (±)-**29**, 109494-81-7; (±)-**30**, 109466-99-1; (±)-**31**, 109581-98-8; (±)-**32**, 109467-01-8; (±)-**33**, 109467-04-1; (±)-**34**, 109467-05-2; (±)-**35**, 109494-82-8; (±)-**36**, 104155-88-6; 4-(trifluoromethyl)-2-nitrobenzenediazonium chloride, 109466-85-5; 4-amino-3-nitrobenzotrifluoride, 400-98-6; formaldoxime, 75-17-2; 4-(trifluoromethyl)-2-nitrobenzaldehyde oxime, 109466-86-6; aminoguanidine bicarbonate, 2582-30-1; 2-aminoisobutyric acid, 62-57-7.

(23) Miller, T. G.; Hollander, R. C. *J. Org. Chem.* **1980**, *45*, 1334.

Synthesis and Structure–Activity Relationships of 1-Substituted 4-(1,2-Diphenylethyl)piperazine Derivatives Having Narcotic Agonist and Antagonist Activity[1]

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Racemates and enantiomers of 1-substituted 4-[2-(3-hydroxyphenyl)-1-phenylethyl]piperazine derivatives (**3**–**18**) were synthesized, and their analgesic and other pharmacological activities and structure–activity relationships were investigated. The *S*-(+) enantiomers of **2a**, **5**, **7**, **9**, **10**, and **15**–**18** had a stronger analgesic activity than their *R*-(−) enantiomers; analgesic activity of the strongest one [(*S*)-(+)-**10**] was 105 times as potent as that of morphine. The *S*-(+) enantiomers of these compounds had the opposite configuration to that of morphine with respect to its (C-9) asymmetric center but the same configuration to that of the tyrosine residue of Met^5-enkephalin. The *R*-(−) enantiomers of **16** and **18** showed narcotic antagonist activity, but the *S*-(+) enantiomers did not. (*R*)-(−)-**18** had analgesic and narcotic antagonist activities comparable to pentazocine but showed no significant physical dependence liability. From these results, it is suggested that these compounds show an uncommon enantioselectivity in comparison with morphine and its surrogates, and belong to a new series of compounds having a potent analgesic activity.

Previously, this laboratory found that (±)-1-cyclohexyl-4-(1,2-diphenylethyl)piperazine (**1**) (MT-45) has a central analgesic activity comparable to that of morphine.[2] The analgesic activity of the compound is predominantly

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