agent 1 h prior to the injection of carrageenan into one of the hind paws. The rats were sacrificed 4 h after administration of the drug, at which time both of the hind paws were excised and weighed separately. The potencies of the test agents relative to phenylbutazone were determined from dose–response plots of the percent increase in weight of the inflamed paw over the noninflamed paw. Usually at least three doses, using 6 rats/dose, were employed in constructing the plots.

(c) Inhibition of Phenylquinone-Induced Writhing. The assay was performed according to the procedure described in a publication[23] from these laboratories. Thus, 18–20-g male mice were given the test substance orally 20 min prior to an intraperitoneal injection of phenylquinone. The mice were observed for the next 10 min for writhing, and the potencies, relative to aspirin, were determined as in (b) using 8–10 mice/dose.

Acknowledgment. We thank W. H. Rooks II and Al Tomolonis for providing the antiinflammatory and analgesic data and D. M. Green for the synthesis of some of the intermediates.

Registry No. 1 (R_1 = OCH_3, X = CH_2, R_2 = H, methyl ester), 104156-24-3; **1a**, 54225-54-6; **1b**, 104156-09-4; **1c**, 72568-22-0; **1d**, 104156-10-7; **1e**, 104156-11-8; **1e** (ethyl ester), 104156-27-6; **1e** (R_1 = 7-OCH_3), 104156-28-7; **1e** (diazo ketone), 104156-26-5; **1f**, 104156-12-9; **1g**, 55689-65-1; **1h**, 61220-69-7; **1i**, 104156-13-0; **2a**, 58452-78-1; **2b**, 58452-80-5; **2c**, 72568-23-1; **2d**, 104156-14-1; **3**, 104156-15-2; **5a**, 104156-16-3; **5b**, 104156-17-4; **6a**, 104156-31-2; **6a** (stilbene, isomer 1), 104156-29-8; **6a** (stilbene, isomer 2), 104156-30-1; **6b**, 104156-22-1; **6b** (stilbene), 104156-20-9; **6b** (R_2 = CH_3), 104156-21-0; **6b** (diacid chloride), 104156-23-2; **6c**, 104156-18-5; 3-$OHCC_6H_4CH_2CO_2H$-Li, 104156-19-6; 2-$H_3CO_2CC_6H_4CH_2Br$, 2417-73-4; 2-$H_3CO_2CC_6H_4CH_2PPh_3^+Br^-$, 60494-73-7; 2-$HOC_6H_4CH_2CO_2CH_2CH_3$, 41873-65-8; 2-$H_3CC_6H_4CO_2CH_3$, 89-71-4; (2-carbomethoxy-3-methoxy benzyl)triphenylphosphonium bromide, 104172-27-2; methyl 2-methoxy-6-methylbenzoate, 79383-44-1; 7-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid, 64717-08-4; 7-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid, 104156-25-4; 8-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-2-carboxylic acid, 64453-89-0; aldose reductase, 9028-31-3.

Furanose–Pyranose Isomerization of Reduced Pyrimidine and Cyclic Urea Ribosides

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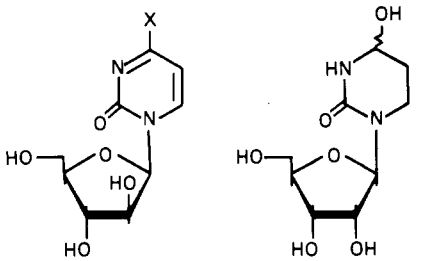
Tetrahydrouridine (THU, **2**) and other fully reduced cyclic urea ribofuranosyl nucleosides undergo a rapid, acid-catalyzed isomerization to their more stable ribopyranosyl form. This isomerization is characterized by a change in spectral properties and by a greater than 10-fold decrease in potency for those nucleosides that act as potent inhibitors of cytidine deaminase in their ribofuranose form. 1-(β-D-Ribopyranosyl)hexahydropyrimidin-2-one (**7**) was synthesized and used in conjuction with its furanose isomer **6** as a model compound for more extensive ^1H and ^{13}C NMR, mass spectral, and kinetic studies of this isomerization. The 0.4 δ upfield shift and 4-Hz increase in the $J_{1',2'}$ coupling constant for the pyranose anomeric proton in the ^1H NMR spectrum is indicative of a pyranose β-CI conformation in which the aglycon and C-2′ and C-4′ hydroxyls are equatorial. The mass spectra of trimethylsilylated pyranose nucleosides also show a characteristic large shift in the *m/z* 204–217 abundance and the appearance of two new rearrangement ions at M − 133 and M − 206. For furanose **6** the rate of isomerization is pH and temperature dependent with pyranose **7** predominating by a factor of 6–9 at equilibrium. At pH 1 and 37 °C, furanose **6** has an initial half-life of less than 12 min. Accordingly, this isomerization may explain the observed lack of enhanced *ara*-C levels in studies evaluating the oral administration of an *ara*-C and THU combination to species with an acidic stomach content.

Efficient in vivo inhibition of cytidine deaminase (CDA) has long been sought as a means of improving the therapeutic activity of the antitumor agent *ara*-C (**1a**) by overcoming its rapid deamination to inactive *ara*-U (**1b**).[1] The widespread distribution of CDA in many of the body's tissues, among them the liver, kidney, small intestine, and blood, ensures this rapid catabolism of *ara*-C and other cytidine analogues.[2] Moreover, significant CDA activity is encountered in human hematopoietic tissue as well as in acute and chronic myelogenous leukemia cells.[3,4]

One of the most potent and well-studied CDA inhibitors has been tetrahydrouridine (THU, **2**).[5] However, use of THU in combination with *ara*-C, both in animals and humans, has produced disappointing results for the most part. There has been little therapeutic advantage observed for this combination because of the parallel increase in toxicity associated with the resulting high plasma levels of *ara*-C.[6,7]

Use of intraperitoneal (ip) combinations of THU and *ara*-C against several in vivo mouse tumors, which were selected for their high levels of CDA activity, produced a significant increase in lifespan (ILS) against only the ascites form of S180J cells.[8] Results with other tumor lines



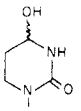
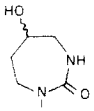
1a, X = NH_2
b, X = OH

2

†National Institutes of Health.
‡University of Vermont.

(1) For a recent review, see: Marquez, V. E. In *Developments in Cancer Chemotherapy*; Glazer, R. I., Ed.; CRC: Boca Raton, FL, 1984; pp 91–114.
(2) Caminier, G. W.; Smith, C. G. *Biochem. Pharmacol.* **1965**, *14*, 1405.
(3) Ho, D. H. W. *Cancer Res.* **1973**, *33*, 2816.
(4) Fanucchi, M.; Phillips, F.; Chou, T. C. *Proc. Am. Assoc. Cancer Res.* **1984**, *25*, 20.
(5) Hanze, A. R. *J. Am. Chem. Soc.* **1967**, *89*, 6720.

Table I. Anomeric Proton Signals of the Starting β-D-Ribofuranosides (RF) and the Corresponding β-D-Ribopyranosides (RP) Formed After Acid Catalysis

	B–R							
			H-1′				H-1′	
B	compd	R	$\delta_{1'}$	$J_{1'2'}$, Hz	compd	R	$\delta_{1'}$	$J_{1'2'}$, Hz
	**2**	RF	5.80	6.0	**3**	RP	5.40	10.0
	**4**	RF	5.40	6.0	**5**	RP	5.00	10.0
	**6**	RF	5.80	6.0	**7**	RP	5.40	9.6
	**8**	RF	5.40	7.0	**9**	RP	5.10	10.5
	**10**	RF	5.40	7.0	**11**	RP	5.10	10.0

possessing comparable or higher CDA levels were negative.[8] The only study that reported a therapeutic advantage for the above combination over *ara*-C alone was that of Neil et al., where both drugs were administered orally (po) to L1210 leukemic mice.[9] In the same study ip administration of both drugs produced no advantage over ip *ara*-C alone, possibly because L1210 cells lack CDA activity. Thus, it was hypothesized that the inhibition of intestinal and bacterial CDA in the lumen permitted a more regular and reproducible absorption of *ara*-C from the gastrointestinal (GI) tract.[9,10] A significant increase in therapeutic index was observed when the oral combination was given either as a single dose or on a daily × 5 schedule.[9] These two schedules using the oral combination produced ILS values of 81% (31% with po *ara*-C alone) and 130% (50% with po *ara*-C alone), respectively.[9] In some cases it was even found that the therapeutic results with the po combination were superior to those obtained with ip *ara*-C by itself.[9] The unique feature of the oral combination was the maintenance of lower but effective therapeutic plasma levels of *ara*-C (1 μg/mL) for a longer period of time than with the administration of ip *ara*-C alone.[9] Since *ara*-C is a cell-cycle phase-specific agent, the length of time to which tumor cells are exposed to a minimum effective drug level is of paramount importance. Also effective, but to a somewhat lesser degree, was the addition of THU to oral 5-azacytidine therapy.[11]

Despite such encouraging results with the oral combination in mice, similar studies in monkeys and humans have been very discouraging.[6,7,10] In monkeys, simultaneous oral doses of THU up to 1 g/kg failed to increase the serum levels of *ara*-C given orally at 50 mg/kg.[6,10] Later, when more accurate measurements were made using orally administered [^{14}C]THU in humans, the blood levels of the drug measured by an enzymatic assay indicated that only 23% of the radioactive species in plasma retained CDA inhibitory activity.[12] No explanation was given to account for this result except for the formation of an inactive, but uncharacterized, THU dimeric species.[10]

We report here the existence of an acid-catalyzed rearrangement and inactivation of THU and several other nucleosides that are CDA inhibitors. It is possible that this pH-dependent rearrangement might account for the aforementioned disparate results and the inability of THU to enhance oral activity of *ara*-C in monkeys and humans.

Chemistry. During the synthesis and evaluation of a series of saturated cyclic urea nucleosides (Table I, compounds **4**, **6**, **8**, and **10**), which functioned as very potent CDA inhibitors,[13] unusual mass spectral and NMR results were observed whenever these compounds were exposed to acidic conditions or were kept as aqueous solutions for extended periods of time. Gas chromatography/mass spectrometry (GC/MS) analysis of these nucleosides as their per-trimethylsilyl (Me_3Si) derivatives did not give the anticipated mass spectra,[14] although the apparent molecular weight and degree of trimethylsilylation were as expected. Fragment ions derived from the nucleoside sugar were not consistent with a ribofuranosyl sugar. The base peak was no longer m/z 217; m/z 204 was now a major peak, and the M − 103 and m/z 103 peaks, sometimes indicative of a 5′-silyloxy moiety,[14] were of reduced intensity. In addition, two new peaks appeared at masses corresponding to M − 133 and M − 206.

When D_2O solutions of these ribofuranosyl nucleosides were kept at room temperature for more than 24 h, the NMR spectra also showed the progressive appearance of a second compound. This newly formed nucleoside exhibited a different anomeric proton as judged by a change in chemical shift as well as coupling constant. This slow

(6) El Dareer, S. M.; Mulligan, L. T., Jr.; White, V.; Tillery, K.; Mellet, L. B.; Hill, D. L. *Cancer Treat. Rep.* **1977**, *61*, 395.
(7) Wong, P. P.; Currie, V. E., Mackey, R. W.; Krakoff, I. H.; Tan, C. T. C.; Burchenal, J. H.; Young, C. W. *Cancer Treat. Rep.* **1979**, *63*, 1245.
(8) Kreis, W.; Hession, C.; Soricelli, A.; Scully, K. *Cancer Treat. Rep.* **1977**, *61*, 1355.
(9) Neil, G. L.; Moxley, T. E.; Manak, R. C. *Cancer Res.* **1970**, *30*, 2166.
(10) Kreis, W.; Woodcock, T. M.; Gordon, C. S.; Krakoff, I. H. *Cancer Treat. Rep.* **1977**, *61*, 1347.
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(12) Ho, D. H. W.; Bodey, G. P., Sr.; Hall, S. W.; Benjamin, R. S.; Brown, N. S.; Freireich, E. J.; Loo, T. L. *J. Clin. Pharmacol.* **1978**, *18*, 259.
(13) Liu, P. S.; Marquez, V. E.; Driscoll, J. S.; Fuller, R. W.; McCormack, J. J. *J. Med. Chem.* **1981**, *24*, 662.
(14) Pang, H.; Schram, K. H.; Smith, D. L.; Gupta, S. P.; Townsend, L. B.; McCloskey, J. A. *J. Org. Chem.* **1982**, *47*, 3923.

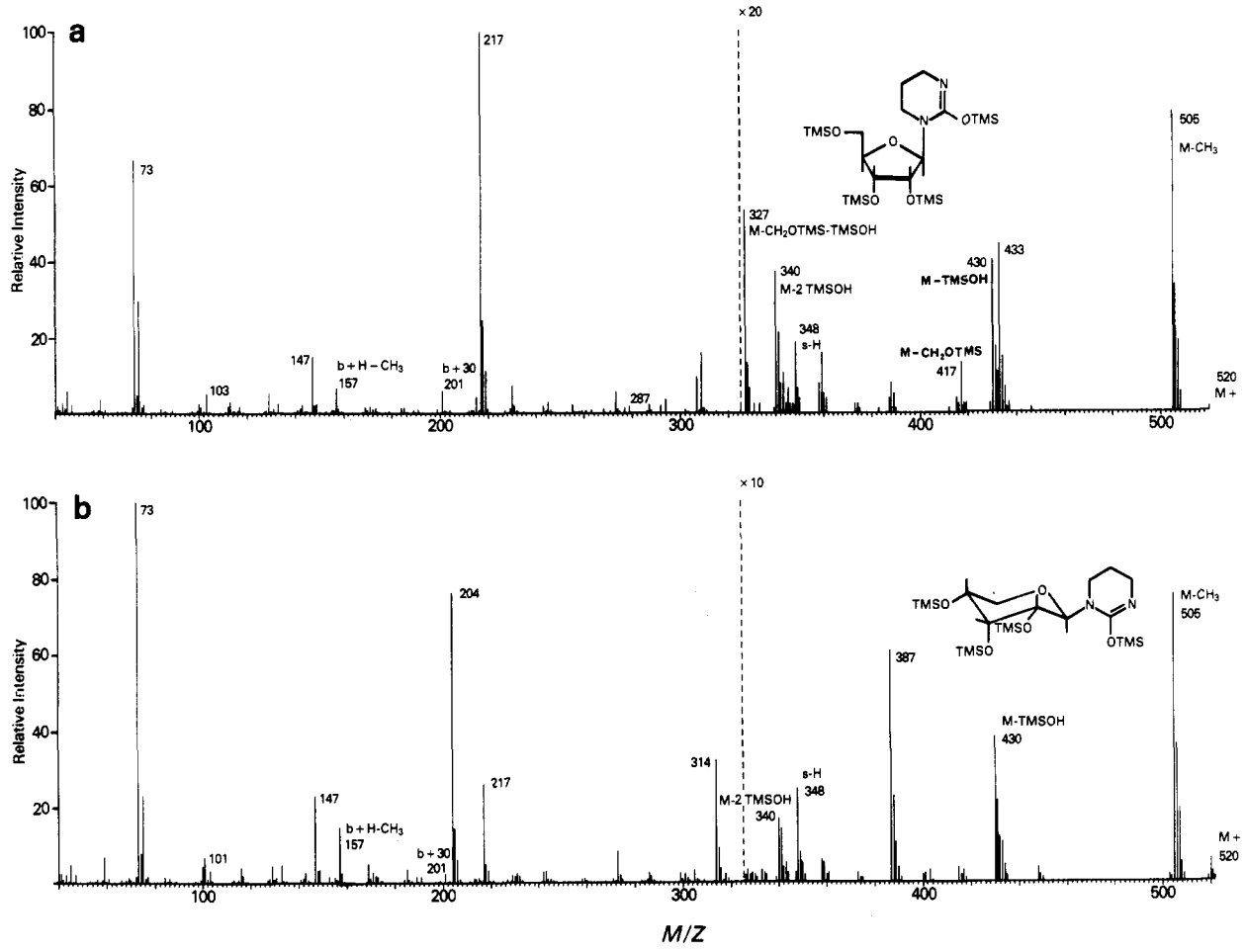
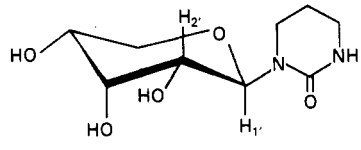


Figure 1. Electron impact mass spectra of (a) per-silylated ribofuranoside **6** and (b) persilylated ribopyranoside **7**. Mass spectra are the weighted average of at least three scans over the GC peak with background being computer subtracted.

transformation in aqueous solution could be dramatically accelerated by trace amounts of acid either from a dilute HCl solution or by a few beads of strongly acidic cation exchange resin. Under such conditions, NMR spectra indicated an equilibrium was established predominantly in favor of the newly formed nucleoside. This equilibrium mixture was maintained indefinitely and was only slightly changed by increased amounts of acid catalyst (vide infra). More importantly, when these samples were lyophilized and tested, CDA inhibitory potencies had dropped several orders of magnitude (i.e., $K_i > 10^{-5}$ M) when compared to the parent compounds ($K_i = 10^{-7}$ to 10^{-8} M).

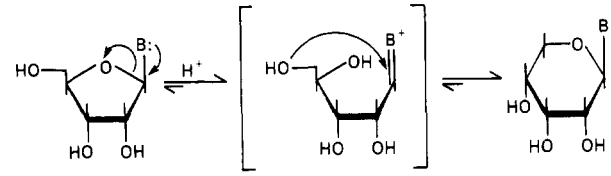
The above mass spectral and NMR observations are consistent with an acid-catalyzed ribofuranoside to ribopyranoside isomerization as depicted in Scheme I. The changes in chemical shift and coupling constants of several of the starting materials and their rearranged products are shown in Table I. These compounds, which include THU and other nucleosides with aglycons of varied ring size, were originally prepared as CDA inhibitors.[13]

The pyranose anomeric proton exhibited an upfield shift of 0.3–0.4 δ, and its $J_{1',2'}$ coupling constant increased by ca. 4 Hz relative to that of the furanose. Furthermore, as judged from these NMR spectral changes, all the compounds studied rearranged to the preferred β-CI pyranose conformation where the glycosidic bond is in an equatorial position to allow the H-1′ and H-2′ protons to be in the antiperiplanar relationship responsible for the large coupling constant observed.[15] No other compounds were detected by NMR analysis.



β - C I

Scheme I



GC/MS analysis of the fully trimethylsilylated β-D-ribofuranosides of Table I and their acid-treated products gave both chromatographic and mass spectral evidence of a change in molecular structure (Table II). The electron impact mass spectrum of the Me_3Si derivative of the acid-rearranged product dramatically indicated that the sugar moiety of the nucleoside had been altered (e.g., Figure 1b). As mentioned above, the base peak was no longer m/z 217 as expected for a silylated ribofuranoside (Figure 1a), but now m/z 204 was several times more intense than m/z 217. It is well-known that in a six-membered ring trimethylsilylated sugar, an intense m/z 204 peak is indicative of multiple vicinal diols.[16] Circum-

(15) Townsend, L. B. *Synth. Proced. Nucleic Acid Chem.* **1973**, 2, 330.

(16) DeJongh, D. C.; Radford, T.; Hribar, J. D.; Hanessian, S.; Bieber, M.; Dawson, G.; Sweeley, C. C. *J. Am. Chem. Soc.* **1969**, *91*, 1728.

Table II. Mass Spectral and Gas Chromatographic Data for Trimethylsilylated Ribofuranosyl (RF) and Ribopyranosyl (RP) Nucleosides

compd	sugar	no. Me_3Si groups	MW	%I^a m/z 204	%I^a m/z 217	RI (OV-17)[b]
uridine	RF	4	532	2.7	100	2610
5,6-dihydrouridine	RF	4	534	1.1	100	2539
2	RF	5	608	1.2	100	2504
3	RP	5	608	80	39	2581
4	RF	4	506	0.9	100	2364
5	RP	4	506	66	58	2427
6	RF	4	520	1.1	100	2413
7	RP	4	520	76	26	2459
8	RF	4	534	2.5	100	2410
9	RP	4	534	64	27	2471
10	RF	5	622	1.8	100	2521
11	RP	5	622	79	23	2615

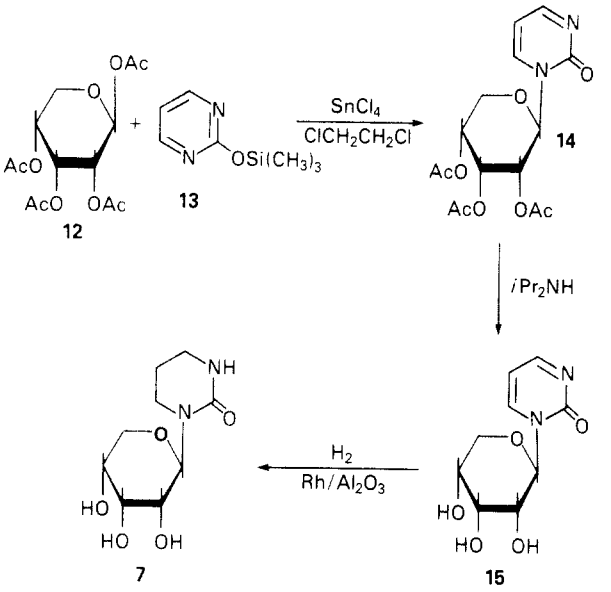
[a] Percent relative intensity. [b] Isothermal retention index on an OV-17 liquid phase.

stantial evidence for a pyranose nucleoside also came from the reduced relative intensity of the peaks at m/z 103 and M - 103, which suggested some modification of the 5′-hydroxyl (Me_3SiOCH_2 after derivatization). That this new compound was an isomer was suggested by fragment ions indicating that the molecular weight, the degree of silylation, and the silylated nucleoside base (b) had not changed. Mass spectral peaks corresponding to $M^{\cdot+}$, M - CH_3, M - Me_3SiOH, b + H - CH_3, and b + 30 were essentially identical to those expected for a trimethylsilylated β-ribofuranosyl nucleoside.[14] In addition, two new, very characteristic peaks of moderate intensity appeared at masses corresponding to M - 133 and M - 206.

In order to verify that this rearrangement was unique for the fully reduced cyclic urea ring system, uridine and dihydrouridine were treated under the same acidic conditions. However, their NMR spectra remained unchanged indefinitely. This would suggest that the rearrangement through an open-chain Schiff's base, as depicted in Scheme I, is favored in the fully reduced compounds by the ability of the electrons on the N-1 nitrogen to participate in the ring-opening process. The aromaticity of the uracil ring of uridine prevents this, while the partial double bond character between C-2 and N-3 in dihydrouridine must be able to provide sufficient conjugative interaction between the electron-withdrawing carbonyl at C-4 and N-1 to effectively prevent the ribofuranose to ribopyranose interconversion. This partial double bond character of amides has been recognized as an efficient transmitter of electronic and inductive effects.[17]

In order to investigate this rearrangement in more detail, we selected a model compound on which to perform spectral and chromatographic studies. The compound chosen was 1-(β-D-ribofuranosyl)hexahydropyrimidin-2-one (**6**), which had been prepared previously and was known to act as a moderate inhibitor of CDA.[5,13] The reason for selecting compound **6** rather than THU was the absence of the unstable carbinolamine moiety found in the latter compound. Thus, compound **6** permitted the study of the ribofuranose–ribopyranose rearrangement free of other possible carbinolamine-related reactions. The study was divided into three parts: first, chemical synthesis and characterization of the authentic rearranged product, 1-(β-D-ribopyranosyl)hexahydropyrimidin-2-one (**7**); second, a detailed NMR and MS study of both starting and rearranged products; and third, a preliminary kinetic investigation of the pH and temperature dependence of this rearrangement.

Scheme II

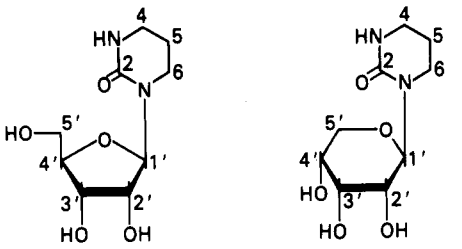


Synthesis of Authentic Ribopyranoside. 1-(β-D-Ribopyranosyl)hexahydropyrimidin-2-one (**7**) was prepared by the Vorbruggen modification of a method developed earlier by Holy et al.[18,19] As shown in Scheme II, the 2-trimethylsilyloxypyrimidine (**13**) was condensed with tetra-*O*-acetyl-D-ribopyranose (**12**) in the presence of $SnCl_4$.[20] The protected ribopyranoside **14** was obtained in 70% yield, and the correspondingly deblocked **15** proved to be identical in every respect to the compound previously reported by Holy et al.[18] The remaining step was the facile reduction of the aglycon to the desired hexahydropyrimidin-2-one ribopyranoside (**7**). This compound was fully characterized by ^1H and ^{13}C NMR, IR, MS, and elemental analysis. As expected, **7** proved to be identical to the material isolated from **6** after acid treatment. The identity of compound **7** with the isolated acid rearrangement product was also confirmed by HPLC analysis of a mixture of the two compounds.

Spectral Studies. In the high-resolution (250 MHz) proton NMR spectrum of **7**, the magnitude of the vicinal coupling constant of the anomeric proton indicated that this compound was the β isomer.[15] The anomeric coupling constant obtained for the starting ribofuranose **6** (Table I) was compatible with either α or β configurations. An

(17) Idoux, J. P.; Kiefer, G. E.; Baker, G. R.; Puckett, W. E.; Spence, F. J., Jr.; Simmons, K. S.; Constant, R. B.; Watlock, D. J.; Fuhrman, S. L. *J. Org. Chem.* **1980**, *45*, 411.
(18) Pischel, H.; Holy, A.; Wagner, G. *Collect. Czech. Chem. Commun.* **1972**, *37*, 3475.
(19) Holy, A. *Collect. Czech. Chem. Commun.* **1977**, *42*, 902.
(20) Neidballa, V.; Vorbruggen, H. *J. Org. Chem.* **1974**, *39*, 3668.

Table III. ^{13}C NMR Spectra of Ribofuranoside (6) and Ribopyranoside (7) Nucleosides of Hexahydropyrimidin-2-one



6 7

compd	carbons, ppm								
	2[a]	4	5	6	1′	2′	3′	4′	5′
6	155.27, s	b	22.03, t	b	87.69, d	69.90, d	70.48, d	82.71, d	62.00, t
7	156, s	b	22.07, t	b	79.53, d	67.30, d	71.82, d	66.53, d	64.73, t

[a] Multiplicity indicated below the value for ^{13}C chemical shifts. [b] Obscured by solvent (Me_2SO-d_6).

Table IV. Major Ions from Trimethylsilyated 1-(β-D-Ribopyranosyl)hexahydropyrimidin-2-one (7)

ions	mass, m/z		no. Si	obsd mass, m/z
	Me_3Si-d_0	Me_3Si-d_9		
$M^{\cdot+}$	520	556	4	520.260
M − CH_3	505	538	4	505.232
M − Me_3SiOH	430	457	3	430.209
M − $Me_3SiOCHCH_2OH$	387	414	3	387.191
S − H	348	375	3	348.157
M − $C_2H_4O_2(Me_3Si)_2$	314	332	2	314.145
$C_3H_3O_2(Me_3Si)_2$	217	235	2	217.099
$C_2H_2O_2(Me_3Si)_2$	204	222	2	204.091
b + H − CH_3	157	163	1	157.074
$Me_3SiO^+{=}Si(CH_3)_2$	147	162	2	147.057
$(CH_3)_3Si$	73	82	1	73.050

unequivocal chemical proof of the β configuration for this compound, however, was performed earlier in this laboratory.[13]

The ^{13}C NMR spectral assignments listed in Table III for carbons 5 and 5′ were based on the observed off-resonance coupling patterns. The assignments for C-1′ were based on literature data for similar compounds, which predicts that the anomeric carbon is the lowest field carbon in the sugar group.[21] Carbon 2 was distinguished by its intensity, indicative of the low nuclear Overhauser effect on the carbonyl carbon. Carbons 2′ and 3′ were assigned by comparison with the data of Gorin who used specifically labeled deuterated sugars.[22] The C-1′ carbon resonance was shifted upfield 8.16 ppm in ribopyranose 7, relative to ribofuranose 6. Likewise, the upfield shift (16.18 ppm) of the C-4′ absorbance in 7, as compared to that in 6, is another consequence of the sugar ring expansion, which moves the cyclic ether oxygen away from this carbon atom. These upfield shifts appear to be the salient characteristics in the transformation of 6 to 7 as observed in the ^{13}C NMR spectrum.

Additional mass spectral studies were also undertaken in order to characterize further the M − 133 and M − 206 fragment ions and to confirm assignments based on nominal mass data. Trimethylsilylation with bis(trimethylsilyl)acetamide-d_{18} was used to determine the extent of silylation and the number of Me_3Si groups in each fragment ion (Table IV). Accurate mass measurement was also employed to check the tentative structural assignments for the major fragment ions observed in the mass spectrum of silylated ribopyranose 7 (Figure 1b, Table IV). The M − 133 fragment (m/z 387) represents the loss of $C_2H_3O_2SiMe_3$ from the molecular ion, while M − 206 (m/z 314) is an odd-electron ion resulting from the formal loss of $C_2H_4O_2(SiMe_3)_2$. Both of these ions apparently arise by fragmentation of the silylated pyranose ring and they are, for all intents and purposes, absent (relative abundances less than 0.2% and not the most intense peak in a cluster) in silylated furanose isomer 6. Similar fragmentation patterns are observed in the mass spectra of the

Table V. Isomerization Kinetics of Ribofuranose 6

apparent pH	temp, °C	apparent $t_{1/2}$,[a] h	R_{eq}[b]
1.0	25	<0.2	0.12
2.0	25	2.23 ± 0.05	0.11
2.0	37	0.63	0.18
2.8[c]	37	5.2 ± 0.3	0.16

[a] Half-life of the initial pseudo-first-order isomerization. [b] Apparent ratio of 6 to 7 at equilibrium. [c] 0.1 M acetic acid.

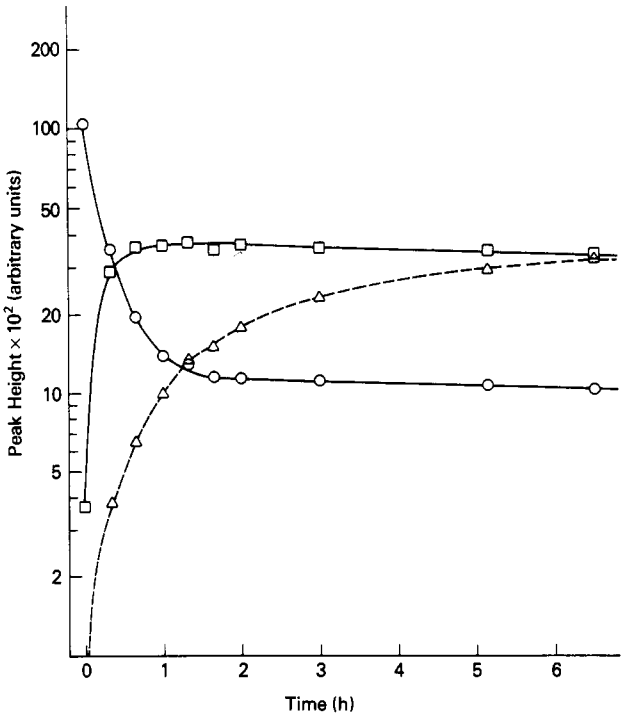


Figure 2. The furanose–pyranose isomerization of model compound 6 in pH 1 HCl buffer at 25 °C. Mixture components were separated by reverse-phase HPLC and monitored at 215 nm (see Experimental Section). Key: (○) ribofuranoside 6, (□) ribopyranoside 7, (△) ribopyranoside 16a.

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Table VI. CDA Inhibitory Activity of Ribofuranoside and Ribopyranoside Forms of Various Cyclic Urea Nucleoside Inhibitors

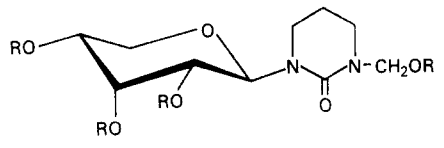
compd pairs	K_i, M, mouse kidney CDA
2 (ribofuranose)	1.5×10^{-7}
3[a] (ribopyranose)	2×10^{-5}
6 (ribofuranose)	4×10^{-6}
7 (ribopyranose)	$>5 \times 10^{-5}$
8 (ribofuranose)	3×10^{-7}
9[a] (ribopyranose)	6.6×10^{-6}

[a] Results from a mixture of ribofuranose and ribopyranose formed after acid treatment.

trimethylsilyl derivatives of pyranoses **3**, **5**, **9**, and **11**.

Kinetics. The rate of isomerization of ribofuranose **6** to ribopyranose **7** as a function of pH was determined by HPLC analysis of solutions of **6** buffered in the pH 1–3 range and maintained at either 25 or 37 °C. As expected, lowering the pH or raising the temperature accelerated the rate of isomerization (Table V). At pH 1 and 25 °C the rate of isomerization was so rapid that the apparent half-life for **6** could not be accurately determined (Figure 2). However, at pH 2 and 25 °C the disapperance of **6** with respect to time initially followed pseudo-first-order kinetics with an apparent half-life of 2.23 ± 0.05 h. In all cases a constant ratio of **6** to **7** was eventually reached with the ribopyranose form predominating by factors of 6–9. That this isomerization results in an equilibrium was further demonstrated by facile conversion of synthetic **7** to **6** at pH 1 and 25 °C to give an identical ratio of **6** to **7** as that observed in Figure 2.

The slow increase of an additional, longer retained peak with a $k' = 5.16$ was observed during HPLC analysis of **6** and **7** in acid buffer (dashed line, Figure 2). The formation of this material may account for the gradual and parallel decrease in both **6** and **7**; however, nothing can be said about the relative amount of this compound since its extinction coefficient at 215 nm is not known. Semipreparative HPLC was used to isolate this material for further characterization by NMR and mass spectrometry. Proton NMR indicated a nucleoside with the anomeric proton being a doublet centered at 5.55 δ and with $J_{1',2'}$ equal to 10 Hz. The positive ion FAB mass spectrum showed an apparent protonated molecular ion at m/z 263, indicating an increase of 30 daltons over either **6** or **7**. GC/MS analysis of this material after trimethylsilylation was especially revealing. A ribopyranose sugar was clearly indicated by the m/z 204 base peak and other diagnostic ions (e.g., M − 133, M − 206, S − H at m/z 348) in the electron impact spectrum. The site of modification was identified as the nucleoside base, which now possessed a moiety that could be readily trimethylsilylated and was 30 daltons more than the base in **6** or **7**. Based on this evidence, this derivative was tentatively assigned structure **16b**. Thus, compound **16a** probably arises from reductive N-formylation of **7** because of the 200 ppm formaldehyde that is added to the commerical buffer as a preservative.



16a, R = H
b, R = $Si(CH_3)_3$

Biology. As observed in Table VI, CDA inhibitory activity was dramatically reduced for the ribopyranoside nucleosides. This was particulary noteworthy in the case of compound **7**, which was studied in its pure form. In the other cases (i.e., compounds **3** and **9**) the assay mixtures obtained after acid treatment contained greater than 90% of the ribopyranoside form as evidenced from NMR. These samples were obtained from the aqueous solutions of the pure ribofuranosides after treatment with excess acidic resin for 15 min and subsequent lyophilization. It is possible that the residual ribofuranoside contributed a significant portion of the activity seen in these equilibrium mixtures.

Discussion

Ribofuranoside to ribopyranoside rearrangements have been described previously. The very simple 1-(β-D-ribofuranosyl)urea has been reported to convert slowly to the ribopyranoside form in aqueous solution at room temperature.[23,24] Likewise, a furanoid glycosyl derivative of 5,5-dibromo-5,6-dihydro-6-hydroxyuracil underwent anomerization as well as ring expansion of the sugar moiety to give the corresponding pyranoside form.[25] Some aminopyrimidine nucleosides, derived from the opening of the imidazole ring in certain purines, also underwent rearrangement to the more stable ribopyranoside form after acid treatment.[26] Finally, the deblocking of *N*-(2′,3′,5′-tri-*O*-acetylribofuranosyl) anthranilonitrile with methanolic ammonia resulted in the formation of the more thermodynamically favored β-pyranoside isomer.[27]

The THU literature does not address the problem of ribofuranose to ribopyranose rearrangement in the cases where this drug has been given orally. Information regarding the acid stability of THU, however, was reported by chemists at Stanford Research Institute who analyzed different lots of THU for the NCI. They considered the material to be highly unstable under acidic conditions as judged by NMR and optical rotation measurements.[28] At that time, however, it was not realized that there was a concomitant loss of biological potency associated with the acid instability of the drug. This finding is of particular importance when one observes that patients who received oral THU had fasted for 12–18 h and that the solution of the drug was often given with an equal volume of orange juice. Human gastric fluid has a pH of 1.2–1.7[29] and orange juice can be as low as pH 3.[30] This combination of circumstances makes it very likely that THU was being rapidly inactivated and therefore rendered ineffective in protecting *ara*-C from deamination. This would also explain the discrepancy between the radioactive and enzymatic assays in determining THU plasma levels.[12] Since the study of Neil et al.[9] showed a definite advantage with orally administered THU in mice, while the work of Kreis[10] in humans and El Dareer[6] in monkeys did not, it is possible in light of the present discussion that this is simply the result of pH differences between the stomachs of the experimental subjects. In agreement with this suggestion, the reported values for the pH of the stomach contents in

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mice fluctuates between 2.2 and 4.8.[31] Since the acid stability and the kinetics of isomerization of THU are similar to **6**,[32] THU in its active ribofuranose form would have been inactivated to a great extent during oral administration to humans.

It can be concluded, therefore, that stomach acidity might be a determining factor in controlling the concentration of THU that is available to block deamination of *ara*-C in the GI tract. As discussed earlier, inhibition of the deamination reaction is an important prerequisite for a gradual absorption of *ara*-C. This situation has probably never been achieved in the animal experiments or during the THU clinical trials where *ara*-C and THU were used concomitantly in species with an acidic stomach content.

Experimental Section

General Methods. ^{1}H NMR spectra for routine samples were recorded on Varian T-60 instrument. For high resolution, a Brucher WM 250 model was used. High-resolution ^{1}H NMR and ^{13}C NMR studies were performed using Me_2SO-d_6 as solvent. IR spectra were obtained on a Perkin-Elmer 727B infrared spectrophotometer with the samples as Nujol mulls. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Cytidine deaminase was measured by using a mouse kidney preparation with the same methodology as reported previously by us.[13]

Microscale trimethylsilylation of nucleosides (1–2 mg) and the corresponding isomerized mixtures was conducted at room temperature with 0.45 mL of a 1:2 (v/v) solution of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and redistilled acetonitrile. The trimethylsilyl-d_9 derivatives of compounds **6** and **7** were also prepared. About 1 mg of each isomer was individually reacted with 0.15 mL of a 1:2 (v/v) mixture of bis(trimethylsilyl-d_9)-acetamide and redistilled acetonitrile for 15 min at 70 °C. Gas chromatography (GC) of these derivatives was accomplished with a Varian 2740 GC equipped with a flame ionization detector and interfaced to a Spectra-Physics 4100 computing integrator. A 1.83-m × 2-mm-i.d. glass column packed with 3% OV-17 on 100/120 mesh Gas-Chrom Q was either operated isothermally at 230 °C or temperature programmed from 170 to 250 °C at 4 °C/min after being held at the initial temperature for 2 min. Typical GC operating conditions employed an injector and detector temperature of 250 °C, a 30 mL/min flow rate for both helium carrier gas and hydrogen, and a 300 mL/min flow rate for air. Isothermal retention indexes (Table II) were determined for selected trimethylsilyl derivatives by using the appropriate *n*-alkanes as internal references.[33]

Electron impact mass spectra were obtained on either a DuPont Instruments 21-492B or on a VG Analytical 7070E GC/MS system; both instruments were interfaced to a VG 2035 data system. Samples were introduced via a Varian 2740 GC (492) or a Hewlett-Packard 5710 GC (7070), operated as previously described, and interfaced to their respective mass spectrometers by a single-stage glass jet separator. For the DuPont 21-492B typical operating conditions were as follows: jet separator, 210 °C; transfer line, 230 °C; ion source, 245 °C; accelerating voltage, 1.6 kV; resolution, 1000; electron energy, 75 eV; ionizing current, 250 μA; scan speed, 2 s/decade. The VG 7070E employed the following operating conditions: jet separator, 240 °C; transfer line, 240 °C; ion source, 250 °C; accelerating voltage, 6.0 kV; resolution, 2000; electron energy, 70 eV; ionizing current, 200 μA; scan speed, 2 s/decade. Accurate masses were determined for trimethylsilylated **6** and **7** on the VG 7070E mass spectrometer by using tetraiodoethylene as an internal reference and standard VG software. A mass measurement accuracy of ±4 mmu was observed for ions of at least 5% relative abundance, which were not isobaric. Positive ion fast atom bombardment (FAB) mass spectra were obtained on the VG 7070E mass spectrometer, which was also equipped with a VG FAB ion source. Glycerol was used as the sample matrix, and ionization was effected by a beam of xenon atoms derived by charge exchange neutralization of a 1-mA beam of xenon ions accelerated through 8.2–8.8 kV. FAB spectra were acquired at a scan speed of 10 s/decade, and the background due to the glycerol matrix was automatically subtracted by the data system.

Kinetic Studies. HPLC analyses were conducted on a Waters Associates Model 204 W liquid chromatograph consisting of a U6K injector, a M6000A pump, and a Perkin-elmer LC-85 variable wavelength detector, which was connected to a Spectra-Physics 4100 computing integrator. A 250-mm × 4.6-mm-i.d. 5-μm Spherisorb S5 ODS II column, preceded by a Waters guard column packed with C_{18}/Corasil, was isocratically eluted with a mobile phase of 1% CH_3CN/H_2O (v/v) at a flow rate of 1.0 mL/min. A 25-μL aliquot of standard solutions or reaction mixtures was injected, and compounds of interest were detected by their UV end adsorption at 215 nm. In this system ribofuranose **6** had a capacity factor ($k' = (V_r - V_0)/V_0$) of 1.78, and the corresponding ribopyranose **7** possessed a $k' = 3.95$. The kinetics of the furanose–pyranose isomerization were determined by adding compound **6** to buffer of the appropriate pH such that the final concentration of **6** was 2–3 mg/mL. The buffer was preheated in a Dubnoff metabolic shaking incubator at either 25 or 37 °C and maintained at this temperature after mixing. Aliquots of the reaction mixture were then taken for direct HPLC analysis at predetermined times. The HPLC fractions corresponding to **7** and to an additional product with $k' = 5.16$ were collected on dry ice and lyophilized for subsequent analysis by NMR and MS. The change of the absolute peak area or peak height of **6** and **7** with respect to time was kinetically analyzed using MLAB, an on-line mathematical modeling program employing an iterative least-squares routine.[34]

1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-1,2-dihydropyrimidin-2-one (14). The starting 1,2-dihydropyrimidin-2-one (0.321 g, 3.34 mmol) was suspended in 7 mL of dry acetonitrile and treated with 4 mL of BSTFA. The reaction mixture was stirred at room temperature for 1 h and then concentrated immediately in vacuo at room temperature. The oily residue (**13**) was dissolved in dry 1,2-dichloroethane (15 mL) and added to a solution of tetra-*O*-acetyl-D-ribopyranose (0.63 g, 1.98 mmol) in 15 mL of 1,2-dichloroethane. To this solution, 0.5 mL of freshly distilled $SnCl_4$ was quickly added, and the mixture was stirred under anhydrous conditions for 1 h. After the addition of 50 mL of a saturated solution of $NaHCO_3$, the reaction mixture was filtered through a bed of celite and the organic layer separated and dried (Na_2SO_4). The solvent was removed in vacuo to afford 0.46 g (69%) of a nearly white solid. The solid material was dissolved in 20 mL of acetone, treated with charcoal, and filtered. The filtrate was treated with petroleum ether until cloudiness appeared. After standing at room temperature the compound crystallized as long needles: mp 224 °C; IR (Nujol) 1720, 1640 cm^{-1}; NMR ($CDCl_3$) δ 8.50 (t, 1, J = 2 Hz, H-6), 7.80 (dd, 1, J = 8 Hz, J' = 3 Hz, H-4), 6.40 (m, 2, H-5), 6.30 (d, 1, J = 9 Hz, H-1′), 5.75 (m, 1, H-4′), 5.10 (m, 2, H-2′, H-3′), 4.00 (m, 2, H-5′, H-5′a), 2.20 (s, 3, $COCH_3$), 2.00 (s, 3, $COCH_3$), 1.90 (s, 3, $COCH_3$); MS, m/z (rel intensity), 294 (M – AcOH, 0.2), 259 (3), 216 (10), 97 (32), 96 (36). Anal. ($C_{15}H_{18}N_2O_5$) C, H, N.

1-(β-D-Ribopyranosyl)-1,2-dihydropyrimidin-2-one (15). The protected nucleoside **14** (0.450 g, 1.27 mmol) was dissolved in 50 mL of methanol and treated with 2 mL of diisopropylamine. The solution was refluxed for 2 h and then reduced to dryness in vacuo. The residue was treated with 40 mL of water and the aqueous mixture extracted five times with 20 mL of chloroform. The aqueous solution was then treated with charcoal, filtered, and lyophilized to give a white powder, which was recrystallized from ethanol to give 0.24 g (83%) of **15** as off-white crystals: mp 207–208 °C, dec begins at 191 °C [lit.[18] mp 190–191 °C]; IR (Nujol) 1615 cm^{-1}; NMR (D_2O) δ 8.67 (dd, 1, J = 7 Hz, J' = 3 Hz, H-6), 8.30 (dd, 1, J = 7 Hz, J' = 3 Hz, H-4), 6.80 (dd, 1, J = 7 Hz, J' = 7 Hz, H-5), 5.98 (d, 1, J = 10 Hz, H-1′), 4.40 (m,1), 4.00 (m,4); MS (as 3 Me_3Si), m/z (rel intensity), 429 (M – CH_3, 0.1), 348 (S

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– H, 7.4), 232 (12), 217 (100), 169 (8.4), 153 (b + Me_3Si – CH_3, 21), 147 (26), 96 (b + H, 5.0), 73 (74).

1-(β-D-Ribopyranosyl)hexahydropyrimidin-2-one (7). The nucleoside **15** (0.118 g, 0.52 mmol) was dissolved in water (12 mL) and hydrogenated at 30 psi in the presence of 50 mg of 5% Rh/Al_2O_3. After 12 h the catalyst was filtered off and the aqueous solution lyophilized to give 0.1 g (83%) of a white solid: mp ~110 °C. Anal. ($C_9H_{16}N_2O_5 \cdot 0.6H_2O$) C, H, N, H_2O.

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Plant Antitumor Agents. 23.[1] Synthesis and Antileukemic Activity of Camptothecin Analogues

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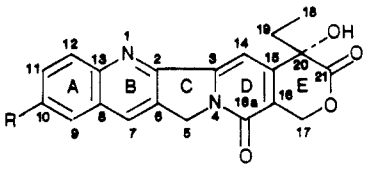
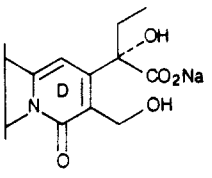
Eight optically active and nine racemic ring A modified analogues of 20(*S*)-camptothecin were prepared and evaluated for antitumor activity in the L-1210 leukemia system. The ring A mono- and disubstituted analogues displayed a wide variance in activity and potency. It was found that monosubstitution by NH_2 or OH at positions 9, 10, or 11 yielded compounds with activity much higher than the parent compound, camptothecin, whereas substitution at position 12 greatly reduced activity. In general, disubstitution in ring A greatly reduced antileukemic activity. Replacement of ring A by heterocyclic rings (thiophene or pyridine) leads to analogues with only moderate activity.

The discoveries of the naturally occurring compound 20(*S*)-camptothecin (**1**) in 1966[2] and the corresponding 10-hydroxy-20(*S*)-camptothecin (**2**) and 10-methoxy-20-(*S*)-camptothecin (**3**) a few years later[3] have led to much interest in this type of pentacyclic system because of the marked activity of **1** and **2** in a number of experimental rodent leukemia and solid tumor systems.[4] The search for analogues of **1**, such as the water-soluble sodium salt **4**, was undertaken with the practical goal of finding a clinically useful anticancer drug, and, to this end, our laboratory has directed considerable efforts.





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Previously, we have reported the synthesis and biological activity of various camptothecin analogues.[5,6] These analogues were obtained by modifications of the natural alkaloids or by total synthesis.[5,6,7] The former approach yields optically active 20*S* analogues; the latter is more versatile and capable of generating a wider variety of analogues but suffers from yielding only racemic 20*RS* compounds with half the potency of the corresponding 20*S* analogue. More recently we have described the isolation of 11-hydroxy-20(*S*)-camptothecin and the total synthesis and biological activity of the corresponding racemic compound **13**.[1]

We have found that monohydroxylation in ring A at positions 10 or 11 results in analogues with significant increase in the activity against L1210 or P-388 mouse leukemia.[1,5] The rationale behind our current efforts has been to further define the optimal substituent and position in ring A for maximal antitumor activity. In this paper we wish to report the synthesis and mouse antileukemic activity of a new series of totally synthetic and semisynthetic analogues of camptothecin.

Chemistry. The compounds **10–18a** were prepared by total synthesis and were racemic (20*RS*). The formation of the final pentacyclic ring system involved the Friedlander condensation of the amino aldehydes (or protected aldehydes) **5a**,[8] **5b**,[9] **5c**,[10] **5d**,[11] **6**,[12] and **7**[13] with the key racemic oxytricyclic ketone **8**. Details of the preparation

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