nitrogen, at room temperature for 24 h, the mixture was treated according to the same procedure as in the case of **3a** to give 387 mg (26%) of the imidazolidinone compound **14** as colorless prisms (from cyclohexane): mp 209–210 °C; IR 1695 cm^{-1}; ^1H NMR δ 0.51, 1.19, 1.30 (each s, 3 H), 1.64–2.52 (m, 4 H), 6.91–8.26 (m, 15 H); ^{13}C NMR δ 27.0 (q), 28.4 (q), 31.6 (q), 37.5 (t), 43.1 (t), 63.6 (s), 73.4 (s), 87.1 (s), 126.8, 126.9, 127.3, 127.5, 128.2, 129.1, 132.7, 137.2, 140.3, 145.7, 175.1 (s); MS, *m/e* 396 (M^+). Anal. Calcd for $C_{27}H_{28}N_2O$: C, 81.78; H, 7.12; N, 7.07. Found: C, 81.59; H, 7.06; N, 7.06.

Registry No. 1, 14016-34-3; **2a**, 1137-96-8; **2b**, 3585-93-1; **2c**, 5909-74-0; **2d**, 3585-90-8; **2e**, 37056-75-0; **3a**, 3317-61-1; **3b**, 3146-84-7; **4**, 14181-84-1; **7a**, 71871-80-2; **7b**, 71871-81-3; **7c**, 71871-82-4; **7d**, 71871-83-5; **7e**, 71871-84-6; **8**, 19155-24-9; **10a**, 71871-85-7; **10b**, 71871-86-8; 11, 71871-87-9; **12**, 71871-88-0; **13**, 5554-37-0; **14**, 71871-89-1; benzophenone, 119-61-9; 1,2,3,5,5-pentaphenylimidazolidin-4-one, 71871-90-4.

Nucleosides. 112. Synthesis of Some New Pyrazolo[1,5-*a*]-1,3,5-triazines and Their *C*-Nucleosides[1,2]

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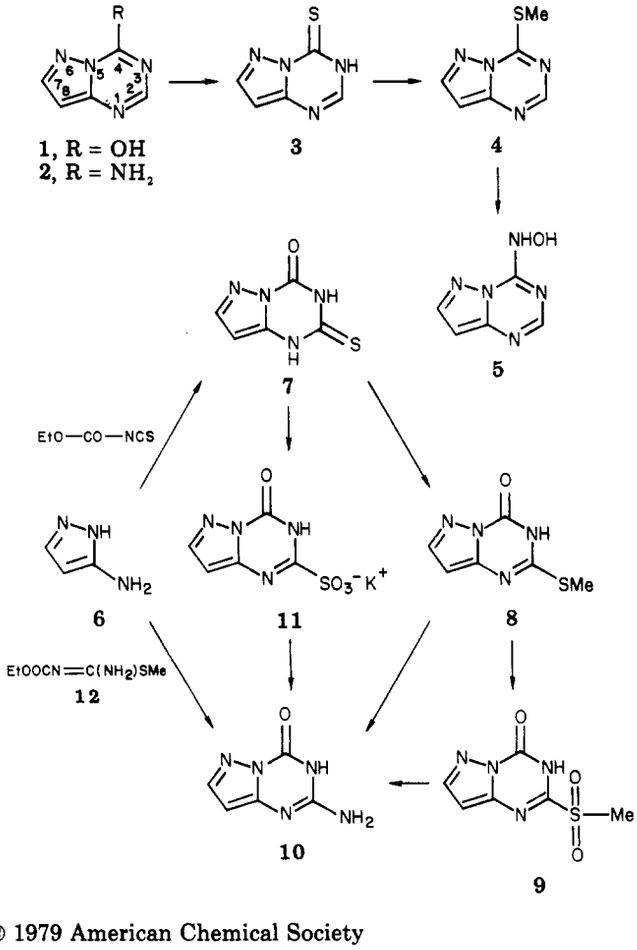
The synthesis of the new C-7 ribosylated pyrazolo[1,5-*a*]-1,3,5-triazine *C*-nucleosides **18**, **19**, and **20** is described. A key step in the conversion **17** → **18** → **19** → **20** involves direct substitution of the 4-amino group of unblocked aminopyrazolotriazine riboside **17** (APTR) with H_2S. The β configuration at C-1′ is retained throughout this sequence. Synthesis of the corresponding and as yet unknown pyrazolotriazine bases **3**, **4**, and **5** is also described.

As part of our program concerned with the synthesis and biological testing of *C*-nucleoside analogues of the natural purine nucleosides, we reported recently the synthesis of 4-amino-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (**17**, APTR) (see Scheme II) and of 4-oxo-3*H*-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (OPTR).[3] These are isosteres of adenosine and inosine, respectively. Preliminary in vitro and in vivo testings[2,4,5] have shown that these compounds possess antileukemic activities which are significantly better than those of the corresponding formycins[6,7] with which they are also isosteric.

We have now extended our investigation of the APTR class of *C*-nucleosides and wish to report here synthetic studies which have led to the 4-thioxo, 4-methylthio, and 4-hydroxylamino derivatives **18**, **19**, and **20** (Scheme III). Such derivatives are of potential biomedical interest in view of the known anticancer activity of the nucleosides of 6-mercaptopurine and its methylthio derivative and of

Scheme I



(1) This investigation was supported by funds from the National Cancer Institute, DHEW (Grants CA-08748, 18856, and 24634).

(2) Presented in part at the joint CIC–ACS meeting, Montreal, Canada, May, 1977, Abstract MEDI 14.

(3) S. Y.-K. Tam, J. S. Hwang, F. G. De Las Heras, R. S. Klein, and J. J. Fox, *J. Heterocycl. Chem.*, **13**, 1305 (1976).

(4) J. H. Burchenal, K. Kalaher, J. Chisholm, R. S. Klein, S. Y.-K. Tam, and J. J. Fox, 68th Meeting of the American Association for Cancer Research, Denver, CO, 1977, Abstract AACR 899.

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(6) For review on Formycins, see R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, 1970.

(7) C. A. Nichol in "Antineoplastic and Immunosuppressive Agents", Part II, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, New York, Heidelberg, Berlin, 1975.

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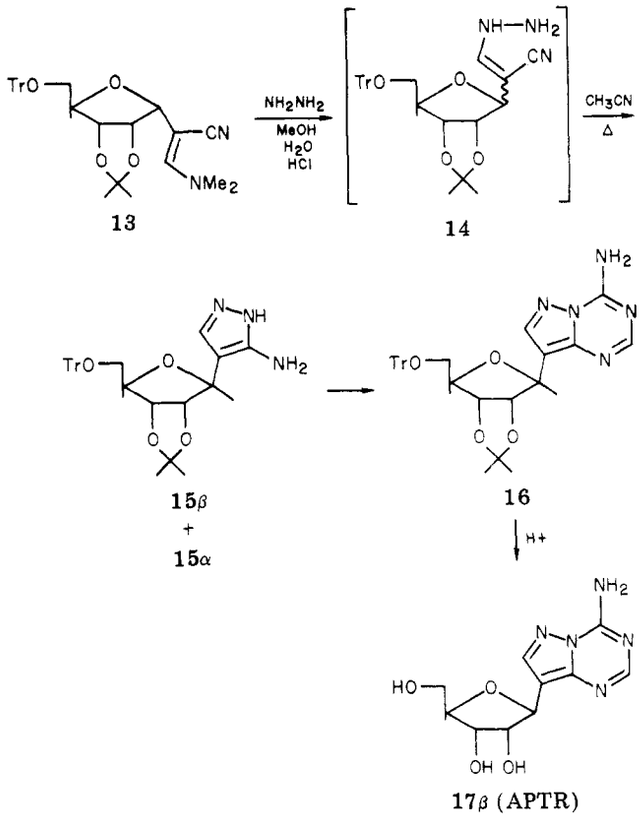
N_6-hydroxyadenine.[8] We also report the synthesis of the corresponding 2-amino-4-oxo-pyrazolotriazine **24**, a new analogue of guanosine. Since aminopyrazole riboside **15** is a key intermediate in our synthetic pathway to all the members of this series, we have developed and report here a new method for its large-scale preparation which affords better yields than the procedures we had described earlier.[9]

Although the pyrazolo[1,5-*a*]-1,3,5-triazine system has been studied extensively,[10,11] the particular compounds **3**, **4**, and **5** (Scheme I) which are the heterocyclic bases of the desired *C*-nucleosides **18**, **19**, and **20**, respectively, had never been described prior to this work. It was necessary, therefore, to first investigate conditions for their synthesis which would be compatible also with the presence of a ribosyl moiety substituted at C_8. Thiation of 2,7-dimethyl-4-oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine with phosphorus pentasulfide in pyridine has been reported to afford the corresponding 4-thioxo derivative in fair yields.[11] The thiation of 4-oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**1**) under these and other conditions, however, afforded **3** in disappointingly poor yields. In a lesser known method, a thione function had been derived from an amino group by reaction with hydrogen sulfide in aqueous pyridine as in the sulfhydrolysis of cytidine[12] and 1-methyladenosine.[13] This procedure seemed particularly well suited for our purpose because of the high susceptibility of triazine derivatives to undergo nucleophilic substitution and because it could in principle make direct use of an unblocked nucleoside, thus avoiding loss in yields in additional blocking and unblocking steps. Furthermore, should the method prove to be applicable to aminopyrazolotriazines, the starting material APTR (**17**) is readily available.[3] Treatment of 4-aminopyrazolotriazine **2** with hydrogen sulfide in aqueous pyridine readily afforded the 4-thioxo derivative **3** in good yield, which was readily methylated with methyl iodide in methanol to **4**. The ^1H NMR spectra of the latter exhibited an S–CH_3 signal at δ 2.75, thus confirming the structures of both **3** and **4**. Finally, compound **4** was converted smoothly to its corresponding hydroxylamino derivative **5**, thus completing the desired sequence.

In principle, the most direct route to the guanine analogue **10** would be by direct ammonolysis of the previously described[10] 2-thioxo-4-oxopyrazolotriazine **7** or of some suitable derivative thereof. One of its main advantages was the accessibility of the C_8 ribosylated derivative of **7** which would serve as starting material and had already been prepared in our laboratory.[9] In our hands, treatment of **7** with ammonia under various conditions (including liquid ammonia at 55 °C) was unsuccessful, and in every instance starting material was completely recovered. Methylation to derivative **8** and its ammonolysis was investigated next. Treatment of **8** with concentrated ammonium hydroxide afforded the desired product **10** only in modest yield. In view of the known increased susceptibility of methylsulfonyl groups of pyrimidines to nucleophilic displacement, compound **8** was converted to the 2-methylsulfonyl derivative **9** by oxidation with potassium permanganate. The latter, upon treatment with liquid ammonia at 50 °C, afforded the desired material in poor yields. Oxidation of **7** with potassium permanganate,[14] on the other hand, afforded sulfonate salt **11**, which upon treatment with concentrated ammonium hydroxide at reflux afforded a fair yield of **10**.

Scheme II



In an alternate approach, aminopyrazole **6**, itself a synthetic precursor of **7**, was treated with *N*-carbethoxy-*S*-methylisothiourea (**12**) in hot acetonitrile. The major product which crystallized in very good yields directly from the reaction mixture was found to be identical in all respects with **10** obtained from sulfonate **11**. Reagent **12** was obtained by the reaction of commercially available carbethoxy isothiocyanate with ammonia to give a thioureido intermediate which was S-methylated with methyl iodide. The experimental method used is a modification of an existing procedure[15] but has the added advantage of providing **12** readily in crystalline form and high purity. This synthesis of **12** is more practical than earlier methods utilizing the reaction of 3-alkylisothiourea with alkyl chloroformates[16,17] and also is adaptable to the preparation of N-alkylated derivatives as well.

Of direct relevance to our search for an improved preparation of the key intermediate *C*-nucleoside **15** was the recently reported elegant synthesis of oxazinomycin via conversion of (dimethylamino)acrylonitrile **13** (Scheme II) to the isoxazole analogue of **15**.[18] It seemed likely that reaction of **13** with hydrazine should afford similarly the corresponding aminopyrazoles **15**. An attractive feature of intermediate **13** was its facile preparation in reportedly good yields. We have found, furthermore, that the α iso-

(8) J. A. Montgomery in "Antineoplastic and Immunosuppressive Agents", Part I, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, New York, Heidelberg, Berlin, 1974.

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(10) (a) J. Kobe, R. K. Robins, and D. E. O'Brien, *J. Heterocyl. Chem.*, 11, 199 (1974); (b) K. Senga, R. K. Robins, and D. E. O'Brien, *ibid.*, **12**, 899 (1975), and references therein.

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(13) K. Miura and T. Ueda, *Chem. Pharm. Bull.*, **23**, 2064 (1975).

(14) I. L. Doerr, I. Wempen, D. A. Clarke, and J. J. Fox, *J. Org. Chem.*, **26**, 3401 (1961).

(15) D. Takiguchi, Japan Kokai 75 32 175 (1975).

(16) K. Gaetzi, German Offen. 2 212 827 (1972).

(17) J. J. Fuchs and K. Lim, German Offen. 2 245 449 (1973).

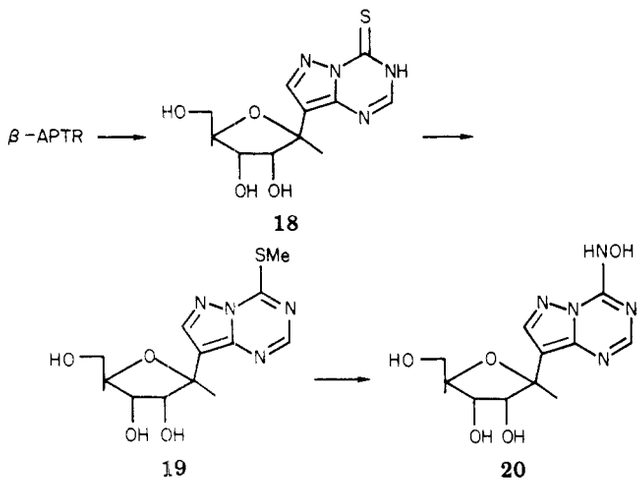
(18) S. De Bernardo and M. Weigele, *J. Org. Chem.*, **42**, 109 (1977).

Table I. 100-MHz ^{1}H NMR Parameters for Pyrazolo[1,5-a]-1,3,5-triazines

compd	solvent[a]	chemical shifts, ppm H-2	H-7	H-8	other	$J_{7,8}$, Hz
1	A	8.02 (s)	8.06 (d)	6.54 (d)	12.43 (br s, NH)	2.0
2	A	8.08 (s)	8.15 (d)	6.44 (d)	8.40 and 8.64 (2 br s, NH_2)	2.2
3	A	8.04 (s)	8.26 (d)	6.69 (d)	10.53 (br s, NH)	2.1
4	B	8.43 (s)	8.17 (d)	6.63 (d)	2.75 (s, SMe)	2.2
4	A	8.54 (s)	8.36 (d)	6.78 (d)	2.72 (s, SMe)	2.1
5	A	7.65 (s)	7.83 (d)	6.34 (d)	10.07 (s, OH) 11.59 (br s, NH)	1.9
7	A		7.88 (d)	5.90 (d)	13.06 (br s, 2 NH)	1.9
8	A		7.98 (d)	6.36 (d)	2.54 (s, SMe) 12.90 (s, NH)	2.1
10	A		7.76 (d)	5.81 (d)	6.63 (br s, NH_2) 11.19 (br s, NH)	1.9
11	A		8.06 (d)	6.56 (d)	11.70 (br s, NH)	2.0

[a] A = Me_2SO-d_6; B = $CDCl_3$.

Scheme III



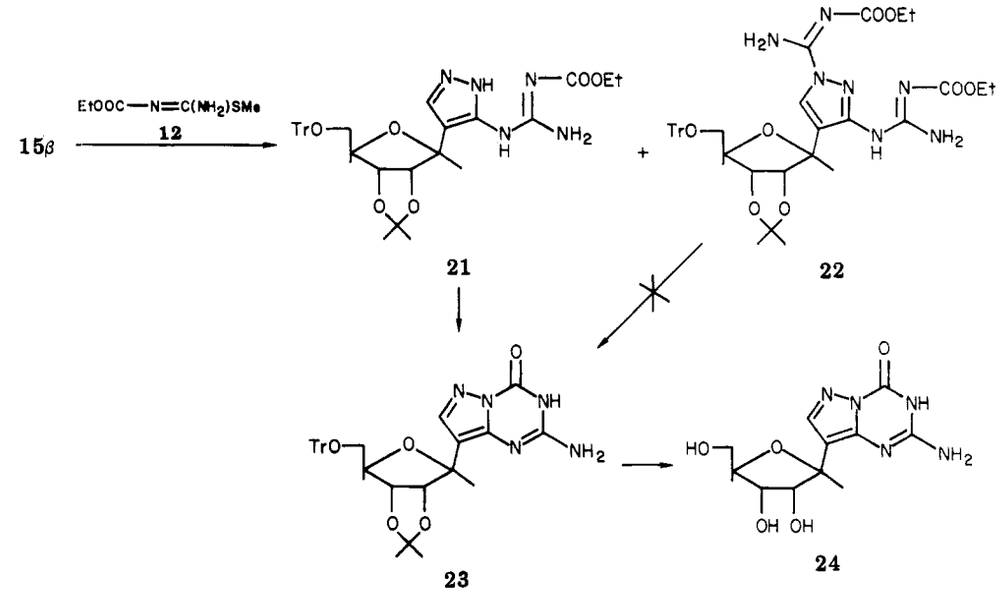
mer of **13** could be isolated conveniently by direct crystallization from the crude mixture of products in ethanol, thus avoiding a tedious chromatographic purification. Additional crops could be obtained from the stored mother liquor presumably due to a slow isomerization of the β isomer still in solution to its less soluble α form.

Preliminary studies indicated that reaction of **13α** with hydrazine, under conditions similar to those reported for the synthesis of the corresponding isoxazole, afforded aminopyrazole **15** in low yields together with many other products. Several modifications led to the finding that treatment of **13α** with a mixture of hydrazine and hydrazine hydrochloride in aqueous ethanol at refluxing temperature afforded a major product (presumably **14**) of lower mobility on TLC, together with small amounts of the desired aminopyrazoles **15**. Attempts to drive the conversion of **14** to **15** to completion by using longer reaction times were not successful. This cyclization was finally accomplished after freeing **14** of excess reagent and reheating the partially purified hydrazone intermediate in boiling acetonitrile overnight. This procedure afforded a 60% overall yield of **15** as a mixture of α and β isomers (α:β ~1). After chromatographic separation, **15β** could be readily converted to unblocked APTR (**17**) in two steps, as we reported earlier.[3] Utilizing conditions similar to those developed in the model studies described above, we have successfully converted APTR to the 4-thioxo derivative **18** (Scheme III), the 4-(methylthio)pyrazolotriazine **19**, and the 4-hydroxylamino derivative **20**. All of these compounds were obtained in good yields, and the β configuration was retained throughout these conversions.

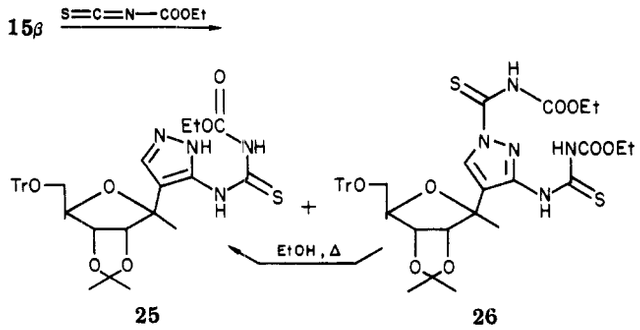
Unexpectedly, when aminopyrazole **15β** was treated with a slight excess of *N*-carbethoxy-*S*-methylisothiourea (**12**) under a variety of conditions, none of the products obtained corresponded to the desired blocked guanosine analogue **23**. Thus, when the reaction was carried out in acetonitrile at 80 °C or in dichloromethane at reflux (conditions known to convert **6** to **10**), a major product (**22**, Scheme IV) was isolated in good yields, which was shown by ^{1}H NMR to possess two carbethoxy groups. This indicates that 2 equiv of **12** had reacted at both nucleophilic positions of **13**, i.e., at the exocyclic amino group on C_3 and at either N_1 or N_3. These results parallel the reported isolation of *N*-carbethoxy-*N'*-[1-(carbethoxythiocarbamoyl)pyrazol-3-yl]thiourea from the reaction of 3-aminopyrazole with ethoxycarbonyl isothiocyanate.[10a] Whereas, however, that isolated product could be made to cyclize to 4-oxo-2-thioxo-1*H*,3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine in the presence of 2 N sodium hydroxide,[10a] **22** under similar conditions afforded only starting material **15β**. Earlier work in our laboratory had already shown[19] that treatment of **15β** with carbethoxy isothiocyanate (Scheme V) affords the expected monosubstituted derivative **25**, as well as a bis-substituted derivative **26**, and the latter could be readily converted to the former by heating it in boiling ethanol. Similar conditions, however, failed to convert **22** to **21**. Attempts to minimize formation of **22** by using less than 1 equiv of reagent **12** were also unsuccessful, leading only to mixtures of starting material **15β**, small amounts of **21**, and again predominant amounts of **22**. Formation of the latter was finally suppressed by carrying out the reaction of **15** with **12** at room temperature. Although these conditions afforded **21** as the major product, together with traces of **22**, completion of the reaction was reached after more than 10 days. To circumvent this difficulty, we took advantage of the very low solubility property of **21** in hot ether by carrying out the reaction in this solvent instead, so that as reaction of **15** with **12** proceeded, **21** precipitated from the mixture as it was first formed. It could thus be conveniently isolated by filtration in 60% yield and high purity with minimum amounts of **22** still detectable in the mother liquor. Structural assignment to **21** was made principally on the basis of the analytical data and signals in the ^{1}H NMR

(19) F. G. De Las Heras, S. Y.-K. Tam, R. S. Klein, and J. J. Fox, unpublished results.

Scheme IV



Scheme V



spectra which indicated the presence of one amino and of two distinct NH groups, all exchangeable with deuterium oxide. Treatment of **21** with sodium methoxide or sodium hydroxide in dimethylformamide at room temperature or simply by heating it at 100 °C in this solvent afforded the blocked β-2-amino-4-oxopyrazolotriazine *C*-nucleoside **23**. Several attempts to unblock this compound revealed that product **24** was exceedingly sensitive to acids and isomerized to mixtures of the α and β isomers under the mild acidic conditions which had been successfully used to convert **16** to **17**. Conversion of **23** to **24** was finally carried out in a mixture of acetyl chloride in *n*-butyl alcohol (a source of dilute hydrogen chloride) at room temperature.[20] The precipitated product, after purification, still contained a very small amount of the α anomer which could be removed by column chromatography.

Structural assignments to the heterocyclic bases of *C*-nucleosides **18**, **19**, **20**, and **24** were confirmed by (a) the similarity of their ultraviolet absorption spectra with those of the corresponding bases (i.e., **3**, **4**, **5**, and **10**, respectively) and (b) the close relationship that exists between the ^{1}H NMR chemical shifts of H_2 and H_7 of these *C*-nucleosides and those of the corresponding free bases, as shown on Tables I and II.

Convincing evidence for the β-anomeric configuration assigned to nucleosides **18**, **19**, **20**, and **24** can be obtained from a comparison of their ^{1}H NMR coupling constants $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ and the chemical shift of the H-1′ signal with those of the known isomers of APTR **17** and of 4-oxo-3*H*-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (**27**, OPTR) (see Tables II and III).

Preliminary in vitro inhibitory activities of some of the compounds synthesized here against mouse leukemias are summarized in Table IV.[21]

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. The ^{1}H NMR spectra were obtained with a JEOL PFT-100 spectrometer with Me_4Si as internal standard. Ultraviolet absorption spectra were obtained with a Cary recording spectrophotometer, Model 15. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. Column chromatography was performed on Woelm silica gel (70–230 mesh). Thin-layer chromatography was performed on 250-μm silica gel plates (Analtech, Inc.), and substances were visualized either by UV absorption, with iodine vapor, or by spraying with 20% ethanolic sulfuric acid and charring.

4-Thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (3). To a frozen solution of compound **1** (810 mg, 6 mmol) in water (15 mL) at −78 °C in a stainless-steel bomb was added a mixture of liquid hydrogen sulfide (50 mL) and pyridine (30 mL). The container was sealed and kept in an oven at 60 °C for 2 days. The hydrogen sulfide was allowed to escape into lead acetate solution traps, and the remaining solvent was removed in vacuo. The residue was treated with hot water, and the insoluble material was filtered. Evaporation of the filtrate afforded a white solid residue which was extracted with hot MeOH. After filtration, the methanolic solution on standing deposited 550 mg of pure **3** (61%): mp >240 °C; UV λ_{max} (pH 7–12) 269 nm, 316; λ_{min} (pH 7–12) 291 nm; λ_{max} (pH 1) 273 (sh, 295–315); λ_{min} (pH 1) 228 nm.

Anal. Calcd for $C_5H_4N_4S$: C, 39.48; H, 2.65; N, 36.83; S, 21.04. Found: C, 39.56; H, 2.73; N, 36.78; S, 21.00.

4-(Methylthio)pyrazolo[1,5-*a*]-1,3,5-triazine (4). Compound **3** (152 mg, 1 mmol) was dissolved in 5 mL of methanol containing 1 mmol of sodium methoxide and methyl iodide (0.15 mL). After 2 h, the pH of the reaction solution was adjusted to ~5 by addition of IRC 50 (H^+) resin. Filtration and evaporation of the filtrate afforded a white solid which was extracted with hot hexane. After decantation, the solution on standing afforded 200 mg of pure

(20) We wish to thank Drs. K. A. Watanabe and C. K. Chu for suggesting this new deblocking procedure.

(21) The authors are indebted to Dr. J. H. Burchenal of this Institute for these preliminary data.

Table II. 100-MHz Proton Chemical Shifts (ppm)

compd	solvent[a]	H-1′	H-2′	H-3′	H-4′	H-5′a	H-5′b	H-2	H-7	other
15α	A	5.15 (d)	——— 4.81 (m) ———		4.29 (m)	——— 3.28 (m) ———				1.34 and 1.53 (2 s, isopropylidene)
15β	A	——— 4.76 (m) ———			4.18 (m)	3.29 (dd)	3.45 (dd)			1.36 and 1.58 (2 s, isopropylidene)
16	A	5.11–5.26 (m)		4.78 (dd)	4.33 (m)	——— 3.24 (m) ———		8.06	8.14	1.38 and 1.62 (2 s, isopropylidene) 6.74 (br s, NH_2), 7.18–7.49 (m, Tr[e])
17	B	4.83 (d)	4.20 (dd)	3.98 (dd)	3.80 (m)	——— 3.54 (m) ———		8.06	8.19	8.48 (br s, NH_2)
18	B	4.82 (d)	4.13 (dd)	3.98 (dd)	3.80 (m)	——— 3.54 (m) ———		7.96	8.23	
19	B	4.92 (d)	4.20 (dd)	3.99 (dd)	3.82 (m)	——— 3.52 (m) ———		8.52	8.42	
20	B	4.74 (d)	4.09 (dd)	3.96 (dd)	3.80 (m)	——— 3.55 (m) ———		7.67	7.88	9.93 (br s, OH)
21	B	4.75–4.95 (m)		4.59 (m)	4.01 (m)	——— 3.10 (m) ———			7.78	1.10 (t, OCH_2CH_3), 1.26 and 1.48 (2 s, isopropylidene), 3.85 (q, OCH_2CH_3), 7.30 (m, Tr[e]), 8.72 (br s, NH_2), 10.52 and 11.95 (2 br s, NH)
22	A	———	4.73 (m) ———		4.22 (m)	——— 3.31 (m) ———			8.19	1.33 (t, OCH_2CH_3), 1.33 and 1.56 (2 s, isopropylidene), 4.08–4.36 (m, OCH_2CH_3), 8.91 (br s, NH)
23	B	4.84–5.02 (m)		4.67 (dd)	4.05 (m)	——— 3.05 (m) ———			7.80	1.23 and 1.47 (2 s, isopropylidene), 6.75 (br s, NH_2), 7.32 (m, Tr[e]) 11.34 (br s, NH)
24	B	4.63 (d)	4.03 (dd)	3.91 (dd)	3.70 (m)	——— 3.56 (m) ———			7.84	6.69 (br s, NH_2), 11.22 (br s, NH)
27[b]	B	4.79 (d)	4.10 (dd)	3.95 (dd)	3.77 (m)	——— 3.50 (m) ———		8.03	8.13	12.59 (br s, NH)
28[c]	B	5.21 (d)	3.95 (m)	4.16 (dd)	3.86 (m)	3.63 (dd)	3.45 (dd)	8.04	8.17	8.39 and 8.41 (2 br s, NH_2)
29[d]	B	5.13 (d)	3.75–3.96 (m)	4.14 (dd)	3.75–3.96 (m)	——— 3.54 (m) ———		7.98	8.06	12.66 (br s, NH)

[a] A = $CDCl_3$; B = Me_2SO-d_6. [b] 27 = 4-oxo-3*H*-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (OPTR, ref 3). [c] 28 = α isomer of APTR (ref 3). [d] 29 = α isomer of OPTR (ref 3). [e] Tr = trityl.

Table III. First-Order Coupling Constants (Hz)

compd	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
15α	*a*	*a*	*a*	*a*	*a*	*a*
15β	*a*	*a*	*a*	4.2	3.5	−10.3
16	*a*	5.5	3.4	*a*	*a*	*a*
17	7.0	5.2	3.6	*a*	*a*	*a*
18	6.7	5.2	3.7	*a*	*a*	*a*
19	6.4	5.5	3.9	*a*	*a*	*a*
20	7.0	5.5	3.4	*a*	*a*	*a*
21	*a*	*a*	*a*	*a*	*a*	*a*
22	*a*	*a*	*a*	*a*	*a*	*a*
23	*a*	5.8	3.7	*a*	*a*	*a*
24	6.4	5.2	3.7	*a*	*a*	*a*
27	6.4	5.2	3.6	*a*	*a*	*a*
28	3.1	4.3	7.7	4.9	2.5	−11.9
29	3.4	4.2	7.6	*a*	*a*	*a*

[a] Unresolved.

crystalline **4** (82%): mp 110–112 °C; UV λ_{max} (pH 0–10) 253 nm, 305; λ_{min} (pH 0–10) 227 nm, 287.

Anal. Calcd for $C_6H_6N_4S$: C, 43.38; H, 3.64; N, 33.72; S, 19.26. Found: C, 43.48; H, 3.65; N, 33.69; S, 19.23.

4-(Hydroxylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (5). Hydroxylamine hydrochloride (700 mg, 10 mmol) was dissolved in a minimum amount of warm ethanol. After the solution cooled to room temperature, a drop of ethanolic phenolphthalein was added followed by slow addition of a freshly prepared ethanolic sodium ethoxide solution until a faintly pink color persisted. The precipitated sodium chloride was removed by filtration, and the filtrate was added to a solution of compound **4** (166 mg, 1 mmol) in methanol (5 mL). The mixture was heated at 60 °C for 3 h. Evaporation of the reaction solution afforded a white solid which was crystallized from methanol to give 105 mg of **5** (69%): mp 198–200 °C dec; UV λ_{max} (pH 7) 246 nm; λ_{min} (pH 7) 280 nm; λ_{max} (pH 0) 233 nm; λ_{min} (pH 0) 275 nm; λ_{max} (pH 14) 265 nm.

Anal. Calcd for $C_5H_5N_5O$: C, 39.74; H, 3.33; N, 46.34. Found: C, 39.76; H, 3.24; N, 46.25.

4-Oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine-2-sulfonic Acid Potassium Salt (11). A solution of compound **7**[10a] (500 mg, 2.98 mmol) in 10 mL of 0.5 N KOH was treated by dropwise addition of a solution of potassium permanganate in 35 mL water. After addition was complete, the reaction mixture was stoppered and stirred at room temperature until the ratio of the ultraviolet absorption maxima (at 265/290 nm) approached 1.30. The excess permanganate was destroyed by careful addition of a 10% solution of sodium disulfite. The manganese dioxide was removed by filtration through a Celite pad which was thoroughly washed with water. The clear filtrate and washings were neutralized with IRC 50 (H^+) resin and evaporated in vacuo. The residue was taken up in hot water and filtered and, on cooling, deposited **11** as needles: 220 mg (30%), mp >250 °C. An aliquot for analysis was twice recrystallized from hot water and dried 2 h at 100 °C under high vacuum.

Anal. Calcd for $C_5H_3KN_4O_4S$: C, 23.62; H, 1.19; N, 22.03; S, 12.61. Found: C, 23.74; H, 1.51; N, 22.06; S, 12.61.

***N*-Carbethoxythiourea.** To a cold saturated solution of ethanolic ammonia (30 mL) was added carbethoxy isothiocyanate (5 mL) with efficient stirring. After 1 h and 20 min, the reaction solution was concentrated to ~5 mL, and the crystals were collected by filtration, washed with cold ethanol, and then dried to give the title compound (4.8 g), mp 137–139 °C.

Anal. Calcd for $C_4H_8N_2O_2S$: C, 32.42; H, 5.44; N, 18.90; S, 21.63. Found: C, 32.50; H, 5.40; N, 18.88; S, 21.61.

***N*-Carbethoxy-*S*-methylisothiourea (12).** *N*-Carbethoxythiourea (3.5 g, 23 mmol) was added to an ethanol solution (50 mL) containing sodium ethoxide (23 mmol) and methyl iodide (5 mL). After 1 h, the reaction solution was evaporated to dryness, and the residue was extracted with ether (100 mL). Filtration and evaporation of the filtrate afforded a clear syrup which crystallized slowly in the cold to give **12** (3.3 g, 86%), mp 44–46 °C.

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Table IV. Inhibitory Activity (ID_{50}, μg/mL) in Vitro against Mouse Leukemia Cell Lines L-1210 and P-815

cell line	17 (APTR)	27 (OPTR)	18	19	20	24	formycin	formycin B
L-1210	0.1	0.5	0.7	0.09	>10.0	4.9	2.6	5.5
P-815	0.2	0.4	1.0	0.07	0.3	>10.0	2.4	>10.0

Anal. Calcd for $C_5H_{10}N_2O_2S$: C, 37.02; H, 6.21; N, 17.27; S, 19.76. Found: C, 36.95; H, 6.13; N, 17.20; S, 19.71.

2-Amino-4-oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (10). Method A. From Aminopyrazole 6 and 12. Thioureido derivative **12** (3.2 g, 20 mmol) was dissolved in 30 mL of acetonitrile, and the solution was stirred and heated to reflux. To the slightly milky mixture was then added slowly 3-aminopyrazole **6** (424 mg, 5 mmol) dissolved in 10 mL of acetonitrile, and heating was continued for 16 h. (Shortly after the addition was completed, clearing of the reaction mixture occurred, followed in 2 h by precipitation.) The suspension was cooled, and the precipitate was removed by filtration. The nearly dried solid was resuspended in methanol and stirred for 1 h. The product was isolated by filtration and washed thoroughly with fresh methanol followed by ether and then dried at 100 °C in vacuo for 1.5 h: 573 mg (75%); mp >250 °C; UV λ_{max} (pH 7) 259 nm; λ_{min} (pH 7) 230 nm; λ_{max} (pH 0) 242 nm; λ_{min} (pH 0) 228 nm; λ_{max} (pH 14) 260 nm; λ_{min} (pH 14) 233 nm.

Method B. From the Ammonolysis of Sulfonate Salt 11. Compound **11** (750 mg, 2.95 mmol) was treated with 40 mL of concentrated ammonium hydroxide in a glass lined bomb tube overnight at 110 °C. After cooling in an ice bath, the mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in boiling water. The pH was adjusted to ~6 with glacial acetic acid and the suspension allowed to cool. The resulting precipitate was removed by filtration to afford 242 mg (54%) of crude **10** with chromatographic and spectroscopic properties identical with those of the sample obtained in method A. Several attempts to obtain a satisfactory analysis of **10** failed. Attempts to prepare a hydrochloride or picrate salt of **10** were also unsatisfactory. A (dimethylamino)methylene derivative was obtained, however, which afforded a correct elemental analysis (vide infra).

2-[((Dimethylamino)methylene)amino]-3-methyl-4-oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine. Compound **10** (28.7 mg, 0.19 mmol) in a mixture of 5 mL of DMF and 3 mL of dimethylformamide dimethyl acetal was stirred and heated at 90 °C overnight and then allowed to cool to room temperature, whereupon crystallization occurred. The precipitate was removed by filtration and recrystallized from hot ethanol as needles: mp 204–205.5 °C; ^1H NMR (Me_2SO-d_6) δ 3.10 and 3.22 (s, 3, $N(CH_3)_2$), 3.51 (s, 3, NCH_3), 6.00 (d, 1, $J_{7,8}$ = 2.0 Hz, H-8), 7.85 (d, 1, H-7), 8.63 (s, 1, vinyl CH).

Anal. Calcd for $C_9H_{12}N_6O$: C, 48.90; H, 5.84; N, 38.02. Found: C, 48.72; H, 5.70; N, 37.71.

Large-Scale Preparation of 3-(Dimethylamino)-2-(2,3-*O*-isopropylidene-5-*O*-trityl-β-D-ribofuranosyl)acrylonitrile (13α). To a solution of 2′,3′-*O*-isopropylidene-5′-*O*-trityl-α-D-ribofuranosylacetonitrile[22] (160 g, 0.35 mol) in 1 L of dimethylformamide (sieve dried) was added bis(dimethylamino)-*tert*-butoxymethane (306 g, 1.76 mol), and the reaction mixture was stirred at room temperature overnight or until TLC in $CHCl_3$–MeOH (50:1) showed complete absence of starting material. The solvent was evaporated in vacuo, and the residue was partitioned between methylene chloride and water; the organic layer was dried over sodium sulfate and evaporated to dryness in vacuo. The residual syrup was stirred with ethanol (~250 mL) and the precipitated crystalline material removed by filtration to afford 85 g (48%) of crude **13α**. The ethanolic mother liquor contained mostly the β isomer plus reaction byproducts. (When the mother liquor was allowed to stand at room temperature several months, more of the α isomer (~20 g) could be obtained.) The isolated product was utilized without further purification.

Large-Scale Preparation of 3-amino-4-(2,3-*O*-isopropylidiene-5-*O*-trityl-β-D-ribofuranosyl)pyrazole (15). A mixture of dimethylaminoacrylonitrile **13α** (crystalline α isomer, 50 g), MeOH (250 mL), anhydrous hydrazine (75 mL), H_2O (12.5 mL), and hydrazine hydrochloride (10 g) was heated to reflux for 19 h. The solution was cooled to 20 °C and evaporated in vacuo (high-vacuum pump), and the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was twice washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness to afford crude hydrazone **14** (58 g) as a slightly yellow syrup.

All of **14** obtained above was dissolved in 500 mL of CH_3CN, and the solution was heated to reflux for 16 h when TLC ($CHCl_3$–MeOH, 10:1) indicated that cyclization was completed. The mixture was evaporated to dryness to afford a crude isomeric α–β mixture of **15** (53.3 g).

Partial prepurification of 2.0 g of this mixture by dry silica gel column chromatography (EtOAc–petroleum ether, 4:1) followed by preparative high-pressure LC (Lichroprep Si 60, 3% MeOH in CH_2Cl_2) afforded two analytically pure samples. **15β**: 0.535 g, 29% from **13α**. Anal. Calcd for $C_{30}H_{31}N_3O_4$: C, 72.41; H, 6.28; N, 8.44. Found: C, 72.26; H, 6.22; N, 8.41. **15α**: 0.400 g, 21% from **13α**. Anal. Calcd for $C_{30}H_{31}N_3O_4$: C, 72.41; H, 6.28; N, 8.44. Found: C, 72.19; H, 6.17; N, 8.28.

8-(β-D-Ribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (18). To a frozen solution of APTR[3] (**17**; 1 g, 3.7 mmol) in water (10 mL) at −70 °C in a stainless-steel container was added a solution of liquid hydrogen sulfide (15 mL) and methanol (10 mL). The container was then sealed and stored at 55–60 °C for 20 h. After the excess hydrogen sulfide was allowed to escape (lead acetate solution trap), the reaction solution was evaporated to dryness to give a white solid residue. This was warmed with methanol, and the insoluble material was removed by filtration. Evaporation of the filtrate afforded a solid residue which was purified by cellulose column chromatography with 50% aqueous ethanol as eluant to give 680 mg of pure **18** (64% yield): mp 206–208 °C (aqueous methanol); UV λ_{max} (pH 7–12) 273 nm (ε 10 720), 320 (11 340); λ_{min} (pH 7–12) 295 nm (ε 7 110); λ_{max} (pH 1) 277 nm (ε 19 060) (sh 305–315); λ_{min} (pH 1) 238 nm (ε 3 810).

Anal. Calcd for $C_{10}H_{12}N_4O_4S{\cdot}0.5H_2O$: C, 40.95; H, 4.46; N, 19.10; S, 10.93. Found: C, 40.64; H, 4.29; N, 18.94; S, 11.21.

4-(Methylthio)-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (19). Compound **18** (200 mg, 0.7 mmol) was dissolved in methanol (3 mL) containing 1.4 mmol of sodium methoxide and then treated with methyl iodide (0.2 mL). After 0.5 h, the pH of the reaction solution was adjusted to ~6 with the addition of IRC 50 (H^+) resin. Filtration and evaporation of the filtrate afforded a white solid which crystallized from methanol to give 170 mg of **19** (81%): mp 194–195 °C; UV λ_{max} (pH 0–10) 314 nm (ε 3 800), 257 (12 110); λ_{min} (pH 0–10) 294 nm (ε 3 190), 237 (3770). This compound slowly decomposed at pH > 13.

Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.30; H, 4.73; N, 18.78; S, 10.73. Found: C, 44.26; H, 4.88; N, 18.65; S, 10.71.

4-(Hydroxylamino)-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (20). Hydroxylamine hydrochloride (140 mg, 2 mmol) was dissolved in a minimum amount of warm ethanol. After the solution cooled to room temperature, a drop of dilute ethanolic phenolphthalein was added followed by ethanolic sodium ethoxide until a faint color persisted. The precipitated sodium chloride was filtered and the filtrate added to a solution of compound **19** (60 mg, 0.2 mmol) in methanol (1.5 mL). After being heated at 60 °C for 45 min, the reaction solution was evaporated to dryness in vacuo to give a white solid which crystallized from ethanol to give 46 mg (81%) of **20**: mp 175–177 °C dec; UV λ_{max} (pH 7) 248 nm (ε 12 600) (sh 285–325); λ_{min} (pH 7) 282 nm (ε 3 840); λ_{max} (pH 0) 235 nm (ε 11 730) (sh 250 and slight sh 275–325); λ_{min} (pH 0) 275 nm (ε 4850); λ_{max} (pH 14) 267 nm (ε 10 120).

Anal. Calcd for $C_{10}H_{13}N_5O_5{\cdot}0.5H_2O$: C, 41.10; H, 4.83; N, 23.96. Found: C, 41.33; H, 4.79; N, 23.83.

3-[*N*′-(Ethoxycarbonyl)guanidino]-4-(2,3-*O*-isopropylidene-5-*O*-trityl-β-D-ribofuranosyl)-1*H*-pyrazole (21). Compound **12** (650 mg, 4 mmol) in methylene chloride (2 mL) was added slowly to a stirred solution of aminopyrazole **15β** (1

g, 2 mmol) in boiling anhydrous ether (20 mL). The mixture was heated to reflux for 16 h and then cooled to 20 °C, and the precipitated product was collected by filtration, dried, and crystallized from acetonitrile to give 475 mg (39%) of **21**, mp 215–217 °C dec. On being allowed to stand, the mother reaction solution deposited another 40 mg of **21**.

Anal. Calcd for $C_{34}H_{37}N_5O_6$: C, 66.76; H, 6.10; N, 11.45. Found: C, 66.61; H, 6.09; N, 11.50.

2-Amino-8-(2,3-*O*-isopropylidene-5-*O*-trityl-β-D-ribofuranosyl)-4-oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (23). Compound **21** (475 mg, 0.84 mmol) was dissolved in dimethylformamide (15 mL) containing 1.5 mL of 1 N NaOH. After being stirred at room temperature for 6 h, the reaction solution was made neutral with the addition of 1 N HCl and then evaporated to dryness. The residue was stirred with water, and the solid material was collected by filtration. Crystallization of this material from methanol afforded 400 mg (91%) of **23**, mp 245–247 °C dec.

Anal. Calcd for $C_{32}H_{31}N_5O_5$: C, 67.95; H, 5.52; N, 12.38. Found: C, 67.86; H, 5.53; N, 12.27.

2-Amino-4-oxo-3*H*-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (24). A mixture of acetyl chloride (0.5 mL) and *n*-butyl alcohol (10 mL) was stirred at room temperature for 1 h. To this was added compound **23** (500 mg, 0.88 mmol), and the suspension was stirred at room temperature for another hour. The precipitate formed was collected by filtration, washed immediately with anhydrous ether, and then added to methanol (5 mL). The magnetically stirred mixture was carefully neutralized with 5 M KOH/MeOH. After evaporation of the methanol, the solid residue was purified by silica gel column chromatography with ethyl acetate/acetone/methanol/water (6:1:1:1). Fractions containing **24** were evaporated in vacuo. the solid residue was redissolved in hot methanol, filtered through a 0.5-μm filter and evaporated to dryness to give analytically pure **24** (133 mg, 53%): mp >250 °C; UV λ_{max} (pH 7) 263 nm (ε 9150); λ_{min} (pH 7) 232 nm (ε 2910); λ_{max} (pH 0) 246 nm (ε 8450); λ_{min} (pH 0) 231 nm (ε 5760); λ_{max} (pH 14) 264 nm (ε 9840); λ_{min} (pH 14) 234 nm (ε 2570).

Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot H_2O$: C, 39.87; H, 5.02; N, 23.25. Found: C, 40.27; H, 4.62; N, 23.12.

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Registry No. **1**, 54346-27-9; **2**, 54346-29-1; **3**, 55458-17-8; **4**, 55522-55-9; **5**, 71774-62-4; **6**, 1820-80-0; **7**, 34682-99-0; **8**, 54346-18-8; **10**, 71774-63-5; **11**, 71774-64-6; **12**, 62946-44-5; **13α**, 60526-02-5; **14**, 71774-65-7; **15α**, 71774-66-8; **15β**, 71774-67-9; **16**, 62156-20-1; **17**, 62156-19-8; **18**, 71774-68-0; **19**, 71774-69-1; **20**, 71774-70-4; **21**, 71774-71-5; **22**, 71774-72-6; **23**, 71774-73-7; **24**, 71774-74-8; **27**, 62156-06-3; **28**, 62156-21-2; **29**, 62156-08-5; hydroxylamine hydrochloride, 5470-11-1; *N*-carbethoxythiourea, 3673-38-9; carbethoxy isothiocyanate, 16182-04-0; 2-[((dimethylamino)methylene)amino]-3-methyl-4-oxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine, 71774-75-9; dimethylformamide dimethyl acetal, 4637-24-5; 2′,3′-*O*-isopropylidene-5′-*O*-trityl-α-D-ribofuranosylacetonitrile, 56703-40-3; dimethylformamide, 68-12-2; bis(dimethylamino)-*tert*-butoxymethane, 5815-08-7; hydrazine, 302-01-2.

(±)-Carpesiolin: Total Synthesis and Structural Determination

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The total synthesis of the helenanolide (±)-carpesiolin starting from the key trans-fused hydroazulenic ketone **1** is described. Salient features of the sequence involve the introduction of the α configuration at C-10 via catalytic hydrogenation (**9**), the stereoselective reduction of cycloheptenone **14**, obtained from **9** in five steps, to yield exclusively the α-oriented allylic alcohol **16** and its conversion into lactone **19** via stereodirected epoxidation, regioselective epoxide ring opening by dilithioacetate, and selective ring closure to the C-7, C-8 oriented γ-lactone. **19** is further transformed into the title compound. This total synthesis also enables the full structural determination of (±)-carpesiolin as **6**.

In connection with our continuing efforts directed toward pseudoguaianolides we have reported the short and efficient synthesis of ketone **1** (50% overall yield, 6 steps) from 2-methylcyclopentenone,[2,3] its conversion into several ambrosanolides, characterized by a β-oriented C-10 methyl group (damsin (**2**),[3] neoambrosin, parthenin (**3**) and hymenin),[4] and recently its transformation into intermediate **4**,[5] possessing the α orientation for the methyl group at C-10, which is a characteristic feature of the helenanolides.[6] In this paper we describe the conversion of **1** into (±)-carpesiolin, an antibacterial helenanolide recently isolated from *Carpesium abrotanoides* L by Maruyama and Omura;[7] its structure was tentatively assigned as **5** on spectroscopic and biogenetic grounds. Our synthesis of the title compound not only unequivocally establishes the relative stereochemistry of carpesiolin as depicted in **6** but also clearly demonstrates the general potentiality of our approach[8] based on ketone **1** for the synthesis of both ambrosanolides[9] and helenanolides.[10]

In view of the systematic structural features displayed

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