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Synthesis of Potential Anticancer Agents. Preparation of Some 1-Deazapurines and Pyrimidines†

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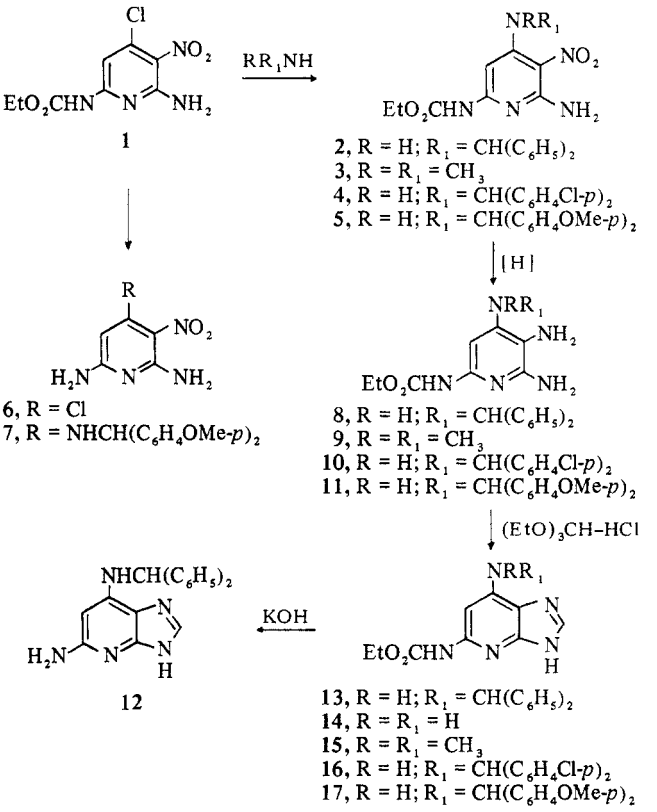
During an investigation of the preparation of 1-deazapurines (imidazo[4,5-*b*]pyridine) from pyridine precursors, several intermediates were found to possess activity in the leukemia L1210 test system. We report the preparation of these compounds and the search for activity in some pyrimidine and purine analogs.

Reaction of **1** with diphenylmethylamine gave **2**.[1] Hydrogenation of **2** in the presence of Raney Ni gave the 5,6-diaminopyridine **8**, which was cyclized with the triethyl orthoformate-concentrated HCl reagent[2] to give **13**. That cyclization of **8** occurred between the primary amino groups rather than between the diphenylmethylamino and the 5-amino groups was established by the pmr spectrum of the product, which showed spin-spin coupling between the NH and CH of the $(C_6H_5)_2CHNH$ moiety [δ_{TMS} 9.04 d, 6.02 d (J = 8.0 Hz)]. After the addition of D_2O the NH doublet collapsed and the CH doublet appeared as a singlet. Analogous ring closures have been observed in the pyrimidine series.[3] Removal of the diphenylmethyl group of **13** with HBr-HOAc gave **14**, and hydrolysis of the urethane group of **13** with KOH-EtOH gave **12**. Reaction of **1** with dimethylamine and bis(*p*-chlorophenyl)methylamine, respectively, gave **3** and **4**, which were converted to **15** and **16** *via* **9** and **10**. Treatment of **1** with bis(*p*-methoxyphenyl)methylamine gave a mixture of **5**, **6**, and **7**, which was separated to give each pure compound. Hydrogenation of **5** gave **11**, but the condensation of the latter with triethyl orthoformate to give **17** resulted in a mixture from which no pure compounds were isolated. Apparently the increased nucleophilicity of the NH of the 4-[bis(*p*-methoxyphenyl)methylamino] group directed some cyclization to this nitrogen atom. The deamino derivatives **20** and **21** of **13** and **8** were prepared by similar procedures from the known pyridine intermediate **18**[4] *via* **19** (Scheme I).

Scheme I



Reaction of **22**[5] with diphenylmethylamine and its bis(*p*-chlorophenyl) and bis(*p*-methoxyphenyl) derivatives, respectively, gave **23**, **24**, and **25**. Reduction of the nitro group of **23** with Raney Ni and hydrogen gave **29**. The hydrogenation of **24** and **25** was attempted, but the isolation of a pure sample of the corresponding 5-aminopyrimidines was unsuccessful. The product from **25** was identified as 2,4,5,6-tetraaminopyrimidine, resulting from reductive removal of the bis(*p*-methoxyphenyl)methyl group (Scheme II).

Amination of **26** with diphenylmethylamine and its bis(*p*-chlorophenyl) derivative, respectively, gave **27** and **28**. The properties of the new compounds are summarized in Table I and typical procedures are given in the Experimental Section.

Compounds were tested against L1210 leukemia cells implanted intraperitoneally in mice[6] on single dose and chronic schedules; the test results indicated that single-dose treatment was superior in all cases. The diaminopyridine **8** showed a 22% ILS at 200 mg/kg on the single-dose schedule but no activity on the chronic schedule.‡ In the latter test toxicity was observed at 400 mg/kg/day, qd 1-15. The corresponding bis(*p*-chlorophenyl) and bis(*p*-methoxyphenyl) derivatives **10** and **11**, the deaminopyridine **21**, and the pyridine analog **29** appeared to be inactive. The most active compound, **13**, gave a 59% ILS at a dose of 40 mg/kg on the single-dose schedule (toxic 200 mg/kg) and a 24% ILS at a dose of 20 mg/kg/day on the chronic schedule (toxic 45 mg/kg/day, qd 1-9).§ The corresponding dimethylamino and

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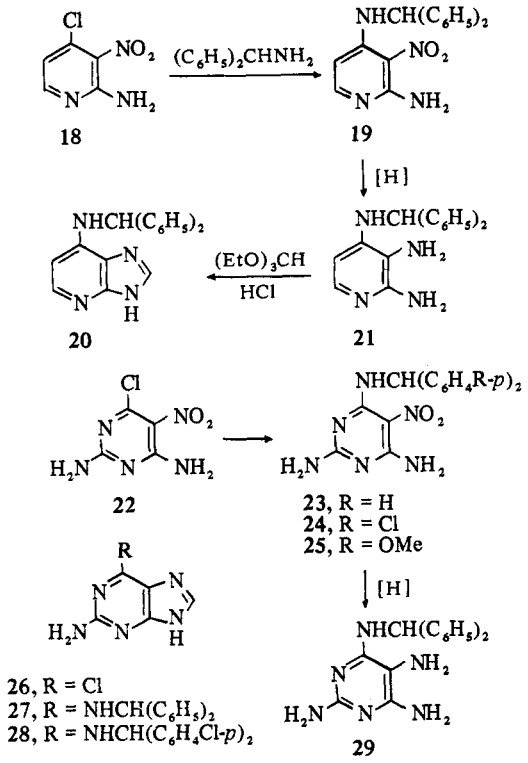
‡Against Walker carcinosarcoma 256 (im) in the rat at 200 mg/kg (qd 3-6); tumor weight of treated animals was 9% of controls.

§For comparison, 6-mercaptopurine at 15 mg/kg, qd 1-15, gave a 53% ILS.[7]

Scheme II



bis(*p*-chlorophenyl) derivatives **15** and **16** and the deamino compound **20** were less active. The ILS was 24% (400 mg/kg) for **15**, 25% (150 mg/kg) for **16**, and 8% (25 mg/kg) for **20** on the single-dose schedule. Inactive compounds resulted from the removal of the amino blocking groups of **13** to give either **12** or **14**. The purine analogs (**27** and **28**) of **12** were also inactive. Presumably the mode of action of **13** is dissimilar to that of the active purine ring analog, thioguanine. The more closely related purine ring analog, 2,6-diaminopurine, is inactive in the L1210 system.[8] In fact, the test results obtained on **12-14** indicated that both the ethoxycarbonyl and diphenylmethyl groups of **13** are necessary for activity. Although urethane itself gave a 25% ILS in the L1210 system, this activity is considerably less than that of **13** (59%).[9,10] Furthermore, urethane gave less active compounds when substituted on the primary amino group.[11] Next, the association of **13** with a macromolecule such as DNA was considered. However, the insolubility of **13** in saline-citrate buffer precluded the determination of its uv spectrum in the presence of DNA.[12]

Experimental Section

Ethyl 6-Amino-4-dimethylamino-5-nitropyridine-2-carbamate (3). A solution of **1** (2.0 g) and 67% aqueous Me_2NH (20 ml) was refluxed for 1 hr. The solid that deposited was collected by filtration and recrystallized to give **3**, yield 1.7 g.

Method A. Ethyl 6-Amino-4-{[bis(*p*-chlorophenyl)methyl]amino}-5-nitro-2-pyridinecarbamate (4). A mixture of **1** (10.0 g), Et_3N (7.8 g), and bis(*p*-chlorophenyl)methylamine [prepared from the HCl salt (12.2 g) and NaOEt (2.3 g)] in MeOH (150 ml) protected by $CaCl_2$ and NaOH tubes was refluxed for 35 hr. The solid that deposited was collected by filtration and washed with H_2O and MeOH, yield 8.8 g.

Reaction of 1 with Bis(*p*-methoxyphenyl)methylamine. A mixture of **1** (9.6 g), Et_3N (7.8 g), and bis(*p*-methoxyphenyl)methylamine [prepared from the HCl salt (10.6 g) and NaOMe (2.1 g)] in MeOH (150 ml) protected by $CaCl_2$ and NaOH tubes was refluxed for 48 hr. The solid that deposited was collected by filtration and washed with H_2O to give **6**, yield 2.7 g. The filtrate was evaporated to dryness, and the resulting gum was washed with H_2O and triturated with $CHCl_3$ (50 ml) to give an additional amount of crude **6**: yield 0.4 g; mp ~210 dec.

The $CHCl_3$ solution was absorbed on a previously prepared column (5 × 30 cm) containing silica gel H (Brinkmann Instruments, Inc.) and eluted with $CHCl_3$ to give two main bands. The solid obtained from the first band was recrystallized to give **5**, yield 2.1 g.

Table I. Deazapurines, Pyrimidines, and Related Compounds

Compd	Method	Reaction Time, hr	Reaction Temp, °C	Recrystn solvent[a]	Yield, %	Mp,[b] °C	Formula	Analyses[c]
3	*d*	1	>34	A	83	144-145	$C_{10}H_{15}N_5O_4$	C, H, N
4	A	35	65	B	48[e]	185-187	$C_{21}H_{19}Cl_2N_5O_4$	C, H, N
5	*d*	48	65	C	12[e]	104-106	$C_{23}H_{25}N_5O_6$	C, H, N
6	*d*	48	65	B	45[e]	268 dec[f]	$C_5H_5ClN_4O_2$	C, H, Cl, N
7	*d*	48	65	C	9[e]	118-120	$C_{20}H_{21}N_5O_4$	C, H, N
8[g]	B	7	*h*		96	107-109	$C_{21}H_{23}N_5O_2$	C, H, N
9	B	2	*h*	D	79[i]	125-126	$C_{10}H_{17}N_5O_2 \cdot 0.5H_2O$	C, H, N
10	B	12	*h*		99[j]	160-170	$C_{21}H_{21}Cl_2N_5O_2 \cdot 2HCl$	C, H, Cl, N
11	B	3.5	*h*		71[e]	96-98	$C_{23}H_{27}N_5O_3$	C, H, N
12	*d*	18	78	*d*	82[k]	210[l]	$C_{19}H_{17}N_5 \cdot HCl$	C, H, Cl, N
13[m]	C	120	*h*	E	61[e]	224-226	$C_{22}H_{21}N_5O_2 \cdot HCl$	C, H, N
14	*d*	2	*h*	*d*	76[n]	>300	$C_9H_{11}N_5O_2 \cdot 0.66H_2O$	C, H, N
15	C	24	*h*	*d*	86	300-305 dec	$C_{11}H_{15}N_5O_2 \cdot HCl$	C, H, Cl, N
16	C	72	*h*	*d*	90[e]	200-207 dec	$C_{22}H_{19}Cl_2N_5O_2 \cdot HCl$	C, H, Cl, N
19	A	18	78	E	80[e]	171-172	$C_{18}H_{16}N_4O_2$	C, H, N
20	C	18	*h*	*d*	82[e]	208-210	$C_{19}H_{16}N_4 \cdot HCl \cdot 0.5H_2O$	C, H, Cl, N
21	B	6	*h*		96[j]	72-74	$C_{18}H_{18}N_4$	C, H, N
23	D	18	65	B	76[j]	187-189	$C_{17}H_{16}N_6O_2$	C, H, N[o]
24	D	24	65	B	70[e]	205-207	$C_{17}H_{14}Cl_2N_6O_2$	C, H, N
25	D	20	65	B	79[j]	101-103	$C_{19}H_{20}N_6O_4$	C, H, N
27	E	150	118	F	43[e]	244-246	$C_{18}H_{16}N_6$	C, H, N
28	E	150	118	G	57[e]	147-148	$C_{18}H_{14}Cl_2N_6$	C, H, N
29	B	96	*h*		93[e]	148-152 dec	$C_{17}H_{18}N_6 \cdot 2HCl$	C, H, N

[a] A, aqueous EtOH; B, reaction solvent; C, MeOH; D, H_2O, E, EtOH; F, BuOH; G, MeCN. [b] Melting points were determined with a Mel-Temp apparatus. [c] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Except for a trace amount of impurity or artifact in compounds **9**, **15**, and **29**, all compounds were homogeneous on silica gel G plates (Analteck, Inc.), usually developed with mixtures of MeOH and $CHCl_3$. [d] See Experimental Section. [e] Dried at 78° *in vacuo* over P_2O_5. [f] With presublimation. [g] λ_{max} nm ($\epsilon \times 10^{-3}$) pH 7, 236 (34.5), 291 (7.89). [h] Room temperature. [i] Crude yield. [j] Dried at 56° *in vacuo* over P_2O_5. [k] Dried at 110° *in vacuo* over P_2O_5. [l] With presoftening. [m] λ_{max} nm ($\epsilon \times 10^{-3}$) pH 7, 233 (28.8), 278 (20.6). [n] Dried at 140° *in vacuo* over P_2O_5. [o] N: calcd, 24.98; found, 24.47.

The solid obtained from the second band was recrystallized to give 7, yield 1.3 g.

Method B. Ethyl 5,6-Diamino-4-{[bis(*p*-methoxyphenyl)methyl]amino}-2-pyridinecarbamate (11). A partial solution of **5** (2.2 g) in EtOH (225 ml) containing Raney Ni (~2.5 g, washed with EtOH) was hydrogenated at room temperature and atmospheric pressure. After 16 hr the mixture was heated to 60° under N_2 and filtered. The residue was extracted with an additional amount of hot EtOH (225 ml). The combined ethanol filtrate was evaporated to dryness, and the resulting solid was washed with petroleum ether (bp 60–80°), yield 1.4 g.

In the preparation of **10** and **29** concentrated HCl was added to the ethanol filtrate before evaporation.

5-Amino-7-[(diphenylmethyl)amino]-3*H*-imidazo[4,5-*b*]pyridine (12). A mixture of **13** (9.50 g), KOH (12.6 g), and EtOH (500 ml) protected with $CaCl_2$ and NaOH tubes was refluxed for 18 hr. After filtration the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in H_2O (25 ml) and acidified with concentrated HCl to precipitate the HCl of the product, yield 6.50 g.

Ethyl 7-Amino-3*H*-imidazo[4,5-*b*]pyridine-5-carbamate (14). A solution of **13** (2.0 g) in 8% HBr–HOAc (80 ml) was stirred at room temperature for 2 hr. The solid that deposited was collected by filtration, suspended in H_2O (35 ml), and treated with concentrated NH_4OH. After the solid dissolved, the resulting solution deposited the product as white needles, yield 0.79 g.

Method C. Ethyl 7-{[Bis(*p*-chlorophenyl)methyl]amino}-3*H*-imidazo[4,5-*b*]pyridine-5-carbamate (16). A suspension of **10** · 2HCl (3.5 g) in $(EtO)_3CH$ (150 ml) containing concentrated HCl (0.6 ml) was stirred at room temperature for 72 hr. The course of the reaction was followed by tlc of aliquot portions from the reaction mixture. The product was collected by filtration and washed with Et_2O, yield 3.0 g.

Method D. 2,4-Diamino-6-{[bis(*p*-chlorophenyl)methyl]amino}-5-nitropyrimidine (24). A mixture of **22** (2.0 g), bis(*p*-chlorophenyl)-methylamine · HCl (3.1 g), and Et_3N (2.1 g) in MeOH (250 ml) protected by $CaCl_2$ and NaOH tubes was refluxed for 24 hr. After filtration the filtrate was concentrated to about ¼ volume and refrigerated for 18 hr. The solid that deposited was collected by filtration and washed with H_2O, yield 3.0 g.

Method E. 2-Amino-6-(diphenylmethyl)aminopurine (27). A mixture of **26** (2.0 g), diphenylmethylamine (2.2 g), and NaOAc (0.97 g) in BuOH (50 ml) was refluxed for 150 hr and evaporated to dryness *in vacuo*. The resulting residue was washed with H_2O to give crude **27**, yield 2.2 g (59%).

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Preparation and Biological Activity of Various 3-Deazapyrimidines and Related Nucleosides†

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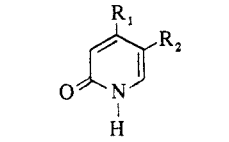
Among analogs of naturally occurring pyrimidine nucleosides modified in the heterocycle, the 5-aza and 6-aza analogs have demonstrated a broad spectrum of biological activity (see, for example, ref 1). The C^3 ribonucleoside of 6-hydroxy-2-pyridone (1-deazauridine) was rather unstable and showed little biological activity.[2] The 3-deazapyrimidines, substituted analogously to the naturally occurring pyrimidines, constitute another logical class of analogs with potential biological activity. Although a number of *N*-glycosides of pyridines have been prepared (see, for example, ref 3), no 3-deazapyrimidine nucleosides, as defined above, had been synthesized or biologically evaluated prior to our studies (for preliminary accounts, see ref 4).

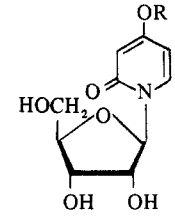
The syntheses of the nucleoside analogs evaluated in this study have been reported previously.[5] A modified decarboxylation of **2** to **1** and a simplified synthesis of 3-deazaC (**3**) from 4-amino-2-chloropyridine *via* the 2-benzyloxy derivative have been accomplished.

Due to the improved antitumor activity reported for arabinosyl cytosine upon 5'-esterification with the adamantoyl group,[6] a monoadamantoyl derivative **5** of 3-deazaUR (**4**) has been prepared.

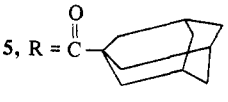
The growth inhibitory activity of these and other 3-deazapyrimidines has been evaluated in microbial and in tumor cell systems, and their effect against leukemia L-1210 *in vivo* has been determined. The results of these studies are reported in this paper. A preliminary account of some of the data has been provided earlier.[4]



1, R_1 = OH; R_2 = H
2, R_1 = OH; R_2 = CO_2H
3, R_1 = NH_2; R_2 = H



4, R = H



5, R = C(=O)–adamantyl

Results and Discussion

Chemical Results. The reported synthesis[7] of 3-deazaU (4-hydroxy-2-pyridone, **1**) employed decarboxylation of 4-hydroxy-2-pyridone-5-carboxylic acid (**2**) in concentrated

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