system. All of the compounds were run in 30% EtOH–H_2O.

Partition Coefficients. Partition coefficients were obtained by equilibrating the test compound between octanol and 0.1 ionic strength pH 7.4 phosphate buffer. The concentration in each phase was determined by UV spectrophotometry.

Acknowledgment. We are indebted to Dr. D. W. Cochran and J. S. Murphy for ^1H NMR and ^1H NMR chiral shift reagent studies, to D. Weitz and P. Arbegast for the in vitro determinations using guinea pig tissue, to C. L. Ludden for the ISA studies in reserpinized rats, to Y. C. Lee and J. B. Moreau for analytical determinations, and to M. Banker for preparation of this manuscript. A special thanks goes to Dr. D. F. Reinhold, R. B. Jobson, N. L. Abramson, T. Utne, and M. A. Kozlowski of Process Research for the development of the oxazolidone procedure (method I).

Syntheses and Antifolate Activity of 5-Methyl-5-deaza Analogues of Aminopterin, Methotrexate, Folic Acid, and N^{10}-Methylfolic Acid

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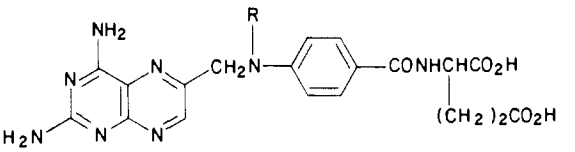
Evidence indicating that modifications at the 5- and 10-positions of classical folic acid antimetabolites lead to compounds with favorable differential membrane transport in tumor vs. normal proliferative tissue prompted an investigation of 5-alkyl-5-deaza analogues. 2-Amino-4-methyl-3,5-pyridinedicarbonitrile, prepared by hydrogenolysis of its known 6-chloro precursor, was treated with guanidine to give 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carbonitrile (**8**) which was converted via the corresponding aldehyde and hydroxymethyl compound to 6-(bromomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine (**15**). Reductive condensation of the nitrile **8** with diethyl *N*-(4-aminobenzoyl)-L-glutamate followed by ester hydrolysis gave 5-methyl-5-deazaaminopterin (**12**). Treatment of **12** with formaldehyde and $Na(CN)BH_3$ afforded 5-methyl-5-deazamethotrexate (**20**), which was also prepared from **15** and dimethyl *N*-[(4-methylamino)benzoyl]-L-glutamate followed by ester hydrolysis. 5-Methyl-10-ethyl-5-deazaaminopterin (**21**) was similarly prepared from **15**. Biological evaluation of the 5-methyl-5-deaza analogues together with previously reported 5-deazaaminopterin and 5-deazamethotrexate for inhibition of dihydrofolate reductase (DHFR) isolated from L1210 cells and for their effect on cell growth inhibition, transport characteristics, and net accumulation of polyglutamate forms in L1210 cells revealed the analogues to have essentially the same properties as the appropriate parent compound, aminopterin or methotrexate (MTX), except that **20** and **21** were approximately 10 times more growth inhibitory than MTX. In in vivo tests against P388/0 and P388/MTX leukemia in mice, the analogues showed activity comparable to that of MTX, with the more potent **20** producing the same response in the P388/0 test as MTX but at one-fourth the dose; none showed activity against P388/MTX. Hydrolytic deamination of **12** and **20** produced 5-methyl-5-deazafolic acid (**22**) and 5,10-dimethyl-5-deazafolic acid (**23**), respectively. In bacterial studies on the 2-amino-4-oxo analogues, 5-deazafolic acid proved to be a potent inhibitor of *Lactobacillus casei* DHFR and also the growth of both *L. casei* ATCC 7469 and *Streptococcus faecium* ATCC 8043. Its 5-methyl congener **22** is also inhibitory toward *L. casei*, but its IC_{50} for growth inhibition is much lower than its IC_{50} values for inhibition of DHFR or thymidylate synthase from *L. casei*, suggesting an alternate site of action.

Methotrexate (MTX) remains the only classical antifolate in established clinical use, and its use has continued to expand as new methods of administering the drug have been introduced and as other tumor types have been added to the list of those now being treated. MTX usage, however, suffers major limitations due to its toxic side effects and the development of resistance by tumor cells.[1] Some tumors are naturally resistant to MTX while others acquire resistance after a period of response.[2] Three factors known to contribute to drug resistance are (a) loss of the active-transport system by which MTX enters cells,[3-6] (b) increased levels of dihydrofolate reductase (DHFR),[7,8] the intracellular target of MTX, and (c) the presence of structurally altered DHFR having lower affinity for MTX.[9-11] Another explanation of resistance may be offered in the recent description of a structurally altered DHFR from a MTX-resistant cell line with unaltered affinity for MTX but with greater capacity to reduce dihydrofolate than the DHFR from the MTX-sensitive cell line.[12]

As part of a program aimed toward the identification of new antifolate agents that exert greater therapeutic



AMT, R = H
MTX, R = CH_3

effectiveness against a broader spectrum of tumors than agents now available, we continue to seek antifolates

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(1) These aspects of MTX are reviewed in: Albrecht, A. M.; Beidler, J. M. *Folate Antagonists Ther. Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. D., Montgomery, J. A., Eds.; Academic: Orlando, FL, 1982; Vol. 1, pp 317–353. Ensminger, W. D. *Folate Antagonists Ther. Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. D., Montgomery, J. A., Eds.; Academic: Orlando, FL, 1982; Vol. 2, pp 133–163. Tattersall, M. H. N. *Folate Antagonists Ther. Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. D., Montgomery, J. A., Eds.; Academic: Orlando, FL, 1982; Vol. 2, pp 165–189.
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having favorably altered transport characteristics but still possessing tight-binding affinity for DHFR. In studies aimed toward greater understanding of transport properties, Sirotnak et al. have observed differences between tumor and normal proliferative tissue in mediated cellular membrane transport of antifolates and in the intracellular γ-polyglutamylation of the agents. These biochemical parameters appear to be critical determinants for selective antitumor activity. In studies that document these differences, Sirotnak et al.[13,14] identified positions 5 and 10 on the classical antifolate-type molecular structure as sites where modification does not reduce binding to DHFR but does influence transport efficacy to favor inward flux into tumor cells and also intracellular γ-polyglutamylation resulting in greater accumulation in tumor cells than in normal proliferative cells. Sirotnak and DeGraw were mindful of these differences when they designed and synthesized the 10-deazaaminopterin series. Two members of that series showed increased therapeutic selectivity[15-18] and are undergoing clinical trials. Opportunity to search for similar therapeutic benefits in the 5-deaza series recently became available with the development of synthetic approaches to these types of compounds.

Chemistry. In an earlier report from these laboratories, a synthesis of the pyrido[2,3-*d*]pyrimidine (5-deazapteridine) analogue of aminopterin (AMT) was reported.[19] 5-Deazaaminopterin (**11**, 5-dAMT) was in turn converted to 5-deazamethotrexate (5-dMTX) and 5-deazafolic acid. The key intermediate in the synthesis was 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde. Taylor et al. developed another synthesis of the key 6-carboxaldehyde intermediate and also converted it to 5-dAMT.[20]

Our search for an approach that would allow general and unequivocal access to 5-alkyl congeners of 5-dAMT and 5-dMTX led us to a report on improved syntheses of 4-substituted 2-amino-6-chloropyridine-3,5-dicarbonitriles from ortho esters and malononitrile via tetracyanopropenide salts as indicated in Scheme I.[21] The ready accessibility of 2-amino-6-chloropyridine-3,5-dicarbonitrile (**3**) and its methyl-substituted congener **4**, plus a report on the straightforward hydrogenolysis of **3** to 2-aminopyridine-3,5-dicarbonitrile,[22] clearly suggested that a relatively simple synthetic route was available since the annelation of the 2,4-diaminopyrimidine ring to systems bearing the 1,2-aminonitrile arrangement is an established method.[23] Our decision to pursue first the 5-methyl-5-deaza analogues was based in part on their structural relationship with the plasma folate, 5-methyltetrahydrofolate. We prepared **3** and **4** by the reported procedures, which proved to be readily adaptable to large-scale preparations. The reductive dechlorination steps to give **5** and **6** were done in DMF–MeOH solution containing 5% Pd on $BaCO_3$. This method differs slightly from the reported conversion of **3** to **5**, which was done in DMF with $PdCl_2$ with Et_3N serving as the HCl scavenger.[22] Annelation of the 2,4-diaminopyrimidine moiety by treatment of **5** and **6** with guanidine in refluxing EtOH followed. 2,4-Diaminopyrido[2,3-*d*]pyrimidine-6-carbonitrile (**7**) was obtained in 95% yield; starting **5** was no longer present in the reaction mixture after 24 h according to TLC. The 5-methyl congener **8** formed less readily. Even after 5 days, some **6** was still present, but the unchanged material was easily removed during isolation of **8** in acceptable (58%) yield. Reductive condensation of **7** with diethyl *N*-(4-aminobenzoyl)-L-glutamate in 70% AcOH solution in the presence of Raney Ni was done using a procedure similar to that reported for the reductive amination of the corresponding aldehyde.[19] The procedure was adapted from the reported general method for the preparation of quinazoline (5,8-dideaza) analogues of AMT.[24] The diethyl ester **9** thus formed proved to be identical with the sample previously reported.[19] The reductive condensation was then applied to **8** to give the diethyl ester of the corresponding 5-methyl compound **10**. The esters **9** and **10** were then hydrolyzed to give 5-dAMT (**11**) and 5-Me-5-dAMT (**12**). The methyl compound **12** was methylated at N^{10} to give 5-methyl-5-deazamethotrexate (5-Me-5-dMTX, **20**) by the reductive alkylation procedure used earlier for the methylation of 5-dAMT.[19]

After this phase of the work as outlined in Scheme I had been completed, we learned that an account of a synthesis of **11** by essentially the approach just described was included in a symposium lecture by Elslager and Davoll,[25] but experimental details have not been published.

In order to extend the usefulness of this facile method, the nitrile **8** was converted by treatment with Raney Ni in aqueous HCO_2H to the corresponding aldehyde **13**, which was then reduced by $NaBH_4$ to give the hydroxymethyl compound **14**. Methods that we developed earlier in the pteridine series then became applicable. The 6-(bromomethyl) compound **15** was prepared by adaptation of the procedure used to prepare 6-(bromomethyl)-2,4-pteridinediamine hydrobromide.[26] The bromomethyl compound **15** was not obtained in pure form as was the pteridine analogue, but it proved to be suitable for effective synthetic use. Alkylation of dimethyl *N*-[4-(methylamino)benzoyl]-L-glutamate (**16**) with **15** in Me_2NAc afforded the ester **18**, which was hydrolyzed to give the

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Table I. Biochemical Properties of AMT, MTX, and 5-Deaza and 5-Methyl-5-deaza Analogues in L1210 Cells

compd	DHFR inhibn:[a] K_i (n = 3) ± SE, pM	cell growth inhibn:[a,b] IC_{50} (n = 4) ± SE, nM	mediated transport[a] (n = 3–4)		rate of net polyglutamate formn,[c] pM/min/g dry wt
			influx	efflux	
			K_m, μM ± SE	k, min^{-1} ± SE	
AMT	3.55 ± 0.4	0.72 ± 0.1	1.2 ± 0.3	0.31 ± 0.04	165
5-dAMT (11)[d]	3.65 ± 0.7	0.63 ± 0.07	1.1 ± 0.2	0.30 ± 0.04	84.2
5-Me-5-dAMT (12)	2.93 ± 0.1	0.13 ± 0.02	1.2 ± 0.2	0.26 ± 0.05	98.3
MTX	5.48 ± 0.6	2.55 ± 0.3	3.3 ± 0.7	0.33 ± 0.06	56.3
5-dMTX[d]	5.26 ± 0.7	2.85 ± 0.3	4.0 ± 0.5	0.28 ± 0.04	48.8
5-Me-5-dMTX (20)	2.12 ± 0.4	0.24 ± 0.03	3.2 ± 0.4	0.31 ± 0.05	42.9
5-Me-10-Et-5-dAMT (21)	2.64 ± 0.3	0.26 ± 0.02	3.3 ± 0.5	0.29 ± 0.04	

[a] Methods used are described in ref 28–30. [b] IC_{50} values (nM) for the 2-amino-4-oxo compds: 5-dFA, 3200 ± 400; **22**, 285 ± 40; **23**, 2350 ± 300. [c] See ref 14. [d] See ref 27.

Table II. Comparison of the Response of Murine P388/0 and P388/MTX to Treatment with MTX and 5-Deaza and 5-Me-5-deaza Analogues of AMT and MTX[a]

compd	P388/0[b]			P388/MTX[b]		
	opt dose, mg/kg	survival time, days	% T/C	opt dose, mg/kg	survival time, days	% T/C
control		10.8			17.2	
MTX	4	19.7	182	3.5	20.3	118
9[c]	1	16.2	151	1	20.8	120
11[c]	0.5	15.8	146	2	21.8	126
12	0.5	16.4	151	0.5	20.8	120
5-dMTX[c]	4	18.0	166	4	20.3	118
20	1	19.7	182	0.25	20.8	120

[a] Treatment: ip; qd 1–5. [b] Implant: ip, 10^6 cells; method described in ref 32. [c] See ref 27.

Table III. Summary of Bacterial Studies on Folate Analogues 22, 23, and 5-dFA Compared with MTX

compd	IC_{50}, ng/mL				*L. casei* enzyme:[b] IC_{50}, M	
	S. faecium[a]		*L. casei*[a]			
	ATCC 8043	/MTX	ATCC 7469	/MTX	DHFR	TS
5-dFA	0.09	49	0.054	3500	7.0×10^{-8}	1.7×10^{-5}
5-Me-5-dFA (22)	380	>10000	0.57	>10000	1×10^{-5}	1.2×10^{-4}
5,10-Me_2-5-dFA (23)	30	>10000	4.6	>10000	2.8×10^{-5}	1.3×10^{-5}
MTX	0.26	3000	0.012	>500000	1.6×10^{-8}	1.2×10^{-4}

[a] Methods described in ref 33. [b] See ref 34.

N^{10}-methyl analogue **20**, identical with the sample prepared from **12** by reductive alkylation. Similarly, the N^{10}-ethyl analogue **21** was prepared via its diethyl ester **19** from **15** and diethyl *N*-[4-(ethylamino)benzoyl]-L-glutamate (**17**), thereby demonstrating the potential of **15** to prepare congeners modified in the C^9–N^{10} bridge and presumably in the remainder of the *N*-benzoyl-L-glutamic acid moiety.

It has been shown through unequivocal synthesis that **11** undergoes hydrolytic deamination at its 4-amino group in refluxing 1 N NaOH to produce 5-deazafolic acid.[19] These conditions were applied to convert 2,4-diamino compounds **12** and **20** to 5-methyl-5-deazafolic acid (5-Me-5-dFA, **22**) and 5,10-dimethyl-5-deazafolic acid (5,10-diMe-5-dFA, **23**) in order to test them for possible activity at sites other than DHFR.

Biological Studies. The inhibitory effects of **11**, **12**, **20**, **21**, and 5-dMTX[27] on DHFR from L1210 cells, their effect on growth of L1210 cells in culture, and their transport characteristics with respect to these cells are listed in Table I with results from AMT and MTX. Each analogue is at least as potent in the inhibition of DHFR as the parent compound. 5-Me-5-dMTX (**20**) and 5-Me-10-Et-5-dAMT (**21**) are 10 times as potent in the L1210 cell growth inhibition test as MTX. The remaining 2,4-diamino types gave essentially the same IC_{50} values as the respective parent compounds. In the transport studies (Table I), each analogue tested showed essentially the same influx and efflux characteristics as the respective parent compounds.

Initial rates of polyglutamate accumulation in L1210 cells of the analogue listed in Table I (except 5-Me-10-Et-5-dAMT, **21**) were determined under conditions of comparable rates of drug entry.[14] There appeared to be no correlation between the rate of anabolism to polyglutamate forms of 5-Me-5-dMTX (**20**), and its greater cytotoxicity compared with MTX. Among the analogues, the rates varied in the order AMT > 5-Me-5-dAMT > 5-dAMT > MTX > 5-dMTX > 5-Me-5-dMTX.

Results from analogue comparison studies against P388/0 and P388/MTX in vivo are summarized in Table II. Against P388/0, each of the five analogues tested showed significant activity comparable to that of MTX. 5-Me-5-dMTX appeared somewhat superior to MTX in that it produced similar T/C values at approximately one-fourth the dose level of MTX. The greater molar

(27) Samples of the 5-deaza compounds 5-dAMT diethyl ester (**9**), 5-dAMT (**11**), and 5-dMTX included in the biological studies summarized in Tables I and II were provided by: C. Temple, Jr., and R. D. Elliott (see ref 19). 5-Deazafolic acid (5-dFA) was prepared from **9** as reported in ref 19.

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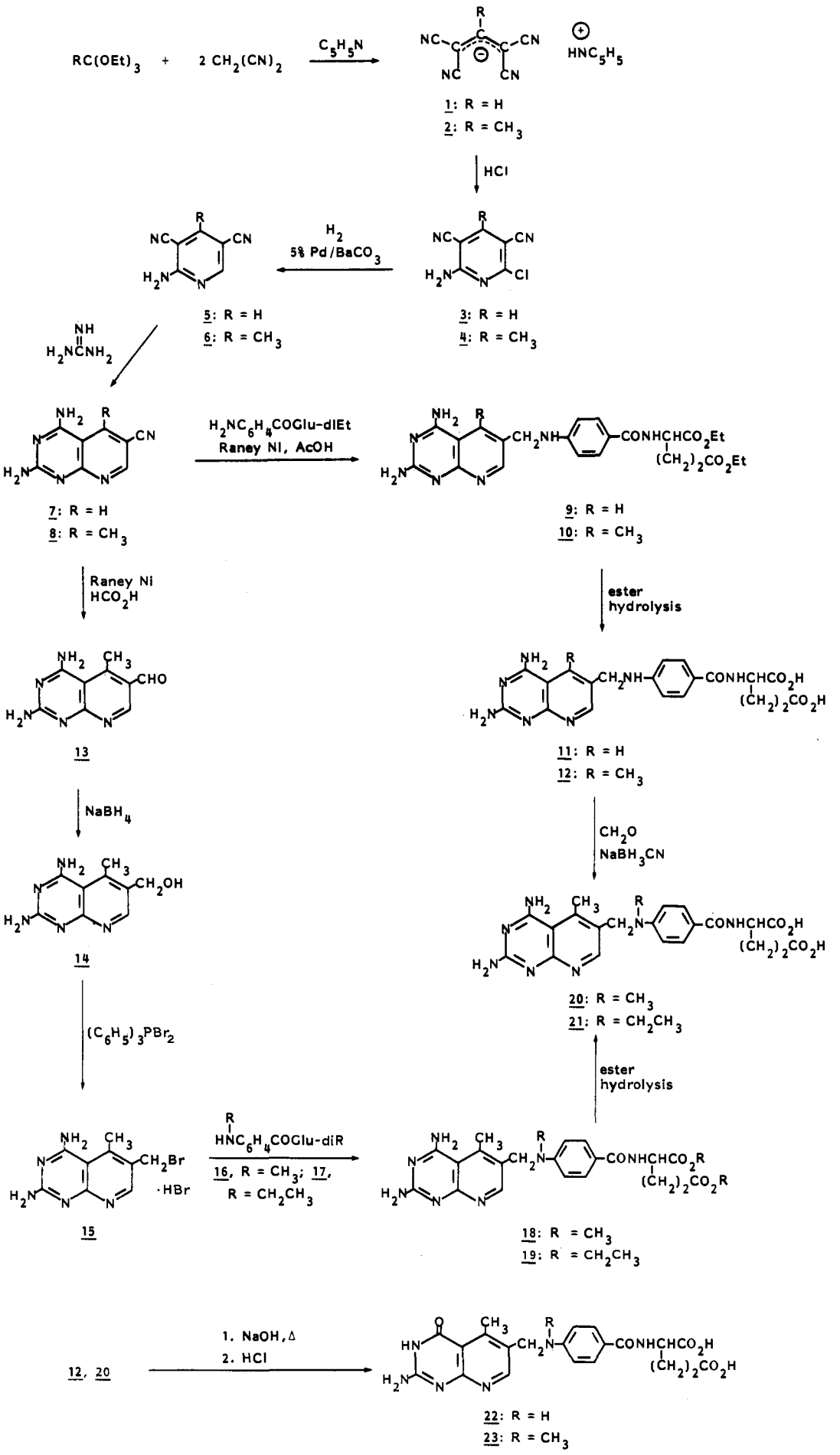
Scheme I


RC(OEt)3 + 2 CH2(CN)2
C5H5N
HNC5H5
1: R = H
2: R = CH3
HCl
H2
5% Pd/BaCO3
3: R = H
4: R = CH3
5: R = H
6: R = CH3
NH
H2NCNH2
H2NC6H4COGlu-dlEt
Raney Ni, AcOH
7: R = H
8: R = CH3
9: R = H
10: R = CH3
Raney Ni
HCO2H
ester
hydrolysis
13
11: R = H
12: R = CH3
NaBH4
CH2O
NaBH3CN
14
20: R = CH3
21: R = CH2CH3
(C6H5)3PBr2
ester
hydrolysis
15
HNC6H4COGlu-diR
16, R = CH3; 17,
R = CH2CH3
18: R = CH3
19: R = CH2CH3
12, 20
1. NaOH, Δ
2. HCl
22: R = H
23: R = CH3

potency of 5-Me-5-dMTX appears to correlate with the IC_{50} values from the cell growth inhibition assay. In the parallel comparison tests vs. MTX-resistant P388, none of the analogues showed activity greater than that of MTX.

Also noted in Table I are the effects on the growth of L1210 cells of the 2-amino-4-oxo compounds 5-dFA, 5-Me-5-dFA (**22**), and 5,10-Me_2-5-dFA (**23**). These compounds were, as expected, less inhibitory than the 2,4-diamino analogues, although **22** was much more inhibitory than the other two.

The bacterial studies on 5-dFA, **22**, and **23** summarized in Table III show that 5-dFA is the most inhibitory of the 2-amino-4-oxo types. It is 3 times more potent than MTX in inhibiting the growth of *Streptococcus faecium* ATCC 8043 and one-fifth as potent as MTX in inhibiting the growth of *Lactobacillus casei* ATCC 7469. 5-dFA is also a potent inhibitor of *L. casei* DHFR, being one-fourth as potent as MTX. Earlier work showed 5-dFA to be an inhibitor of DHFR derived from *Escherichia coli*,[31] chicken liver,[31] and beef liver.[20] In each instance 5-dFA is less inhibitory than MTX.

Compound **22** is a potent inhibitor of the growth of *L. casei* ATCC 7469, but it is a poor inhibitor of DHFR and thymidylate synthase (TS) derived from this organism. The finding that the IC_{50} for growth inhibition (0.57 ng/mL or 1.2×10^{-9} M) is 4–5 orders of magnitude lower than for enzyme inhibition suggests an alternate site of action. We plan further studies in this area and are presently pursuing tetrahydrofolate analogues corresponding to available **22** and **23** or 5-methyl-5,10-dideazafolic acid.

Experimental Section

Examinations by TLC were performed on Analtech precoated (250-μm) silica gel G(F) plates. HPLC assays were made with a Waters Associates ALC-242 liquid chromatograph equipped with a UV detector (254 nm) and an M-6000 pump using a 30 × 0.29 cm C_{18} μBondapak column. Purity assays were done by reversed-phase in the isocratic mode with a mobile phase consisting of CH_3CN (10 or 15% by volume) in 0.1 M NaOAc (pH 3.6) as described earlier.[35] Hydrolyses of esters were monitored with a 20-min linear gradient system with the combination CH_3CN–0.1 M NaOAc (pH 3.6) changing from 15% CH_3CN to 50%.[36] Melting points, unless indicated otherwise, were determined on a Mel-Temp apparatus and are uncorrected. Except where other conditions are specified, evaporations were performed with a rotary evaporator and a H_2O aspirator. Products were dried in vacuo (<1 mm) at 22–25 °C over P_2O_5 and NaOH pellets. Final products were dried and then allowed to equilibrate with ambient conditions of the laboratory. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Spectral determinations and some of the elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. Some elemental analyses were also performed at Galbraith Laboratories, Knoxville, TN, and also at Atlantic Microlab, Inc., Atlanta, GA.

The ^1H NMR spectral data reported were determined with a Nicolet NMC 300NB spectrometer (compound **20**) and with a Varian XL-100-15 spectrometer (all others) using Me_4Si as internal reference. Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded in the fast atom bombardment mode on a Varian MAT 311A mass spectrometer equipped with electron-impact field-ionization/field-desorption and fast atom bombardment ion sources. The UV spectra were determined with a Cary Model 17 spectrometer. Samples were first dissolved in 0.1 N NaOH, and the solutions were then diluted 10-fold with the medium given in the listings. Maxima are expressed in nanometers with the molar absorbance ($\epsilon \times 10^{-3}$) given in parentheses. Molecular weights used in all calculations conform with the compositions listed with elemental analysis results.

2-Amino-6-chloro-3,5-pyridinedicarbonitrile (3) was prepared by the procedure of Schmidt and Junek[21] in which a mixture of malononitrile, triethyl orthoformate, and pyridine in the molar ratio 2:1:1 was heated under reflux for 20 min to give crystalline pyridinium 1,1,3,3-tetracyanopropenide (**1**), which was then treated, without isolation, with concentrated HCl at 80 °C to give **3**. We obtained pure **3**, mp ~200 °C (sublimation), in 58% yield [23.1 g from a run using 30.0 g (0.454 mol) of malononitrile and 0.227 mol each of triethyl orthoformate and pyridine] after recrystallization from DMF (1 L)–H_2O (2 L) as described below for the recrystallization of methyl-substituted **4**. The **3** thus obtained was homogeneous according to TLC ($CHCl_3$–MeOH, 2:1). This compound has also been prepared via sodium 1,1,3,3-tetracyanopropenide.[37]

2-Amino-6-chloro-4-methyl-3,5-pyridinedicarbonitrile (4). A stirred solution of malononitrile (28.0 g, 0.424 mol), triethyl orthoacetate (35.0 g, 0.216 mol), and pyridine (86 mL) was refluxed for 35 min, cooled, and evaporated (bath to 50 °C). Treatment of the residue with concentrated HCl (240 mL) with stirring at 80 °C (bath temperature) for 45 min caused formation of **4** as an insoluble off-white solid. The mixture was cooled, and H_2O (400 mL) was added before the solid was collected and washed successively with H_2O, EtOH, and Et_2O. The solid was then dissolved in hot (80 °C) DMF (200 mL), and the clarified (Norit, Celite) solution was added to stirred H_2O (400 mL) to cause precipitation of **4**. The white solid was collected as before: yield 49% (19.8 g), homogeneous by TLC (cyclohexane–EtOAc, 1:1); IR (KBr) ν_{max} (cm^{-1}) 3351, 3329, 3175 (NH_2), 2230, 2222 (CN), 1652, 1567 (C=C, C=N). Anal. ($C_8H_5ClN_4$) C, H, N. Although **4** has been reported as having mp 210 °C,[21] the pure product that we obtained from three preparations did not have a distinct melting point; samples in capillaries were observed to undergo change in crystalline modification near 230 °C to form colorless rods that did not melt below 270 °C.

2-Amino-3,5-dicyanopyridine (5) was prepared from **3** by using the procedure described below for the preparation of **6** except that **5** was recrystallized from MeCN (instead of EtOH): yield 73% [7.00 g from 12.0 g (67.2 mmol) of **3**] of product, homogeneous on TLC (cyclohexane–EtOAc, 1:1); mp 220 °C dec; ^1H NMR (Me_2SO-d_6) δ 7.90 (s, NH_2), 8.40 and 8.58 (two d, 4-H and 6-H, J = 2 Hz). Anal. ($C_7H_4N_4$) C, H, N. This compound has also been prepared from **3** by hydrogenolysis ($PdCl_2$) in DMF containing Et_3N[22] and also by high-pressure hydrogenation of 3-amino-2-cyanoacrylonitrile in the presence of 10% Pd/C.[38]

2-Amino-4-methyl-3,5-pyridinedicarbonitrile (6). Hydrogenolysis of **4** (10.0 g, 52.0 mmol) in DMF (150 mL)–MeOH (75 mL) containing 5% Pd on $BaCO_3$ (10.0 g) was carried out in a Parr shaker with H_2 pressure kept near 3.5 kg/cm^2 (50 psi) for 6 h. The mixture was then filtered (Celite mat) and evaporated to dryness (final conditions <1 mm, bath to 45 °C). The residue containing Ba salts that separated during the evaporation was stirred with H_2O (100 mL) to give the H_2O-insoluble product (7.74 g). Recrystallization from EtOH (800 mL) then gave pure **6**: mp 222–223 °C; 86% yield (7.1 g), homogeneous by TLC (cyclohexane–EtOAc, 1:1); ^1H NMR (Me_2SO-d_6) δ 2.50 (s, CH_3), 7.82

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(s, NH_2), 8.50 (s, C^2-H). Anal. ($C_8H_6N_4$) C, H, N.

2,4-Diaminopyrido[2,3-*d*]pyrimidine-6-carbonitrile (7). Guanidine hydrochloride (2.66 g, 27.8 mmol) was added to a solution of NaOMe (1.50 g, 27.8 mmol) in absolute EtOH (180 mL). The mixture was stirred at 20–23 °C for 15 min before **5** (2.00 g, 13.9 mmol) was added. After a 24-h reflux period with rapid stirring, TLC (cyclohexane–EtOAc, 1:1) showed absence of **5**. The solid filtered from the cooled mixture was washed on the funnel with H_2O and EtOH; yield 95% (2.45 g). A sample of this material (1.0 g) was stirred with near-boiling Me_2SO (250 mL), and the slightly cloudy solution was filtered (Celite) to give a clear, pale yellow filtrate that was then concentrated by evaporation in vacuo (to about 60 mL). Addition of EtOH (200 mL) gave **7** as a light yellow solid: 880 mg; ^1H NMR (Me_2SO-d_6) δ 6.97 (s, NH_2), 7.84 (s, NH_2), 8.87 and 8.91 (two d, 5-H and 7-H, J = 2 Hz). Anal. ($C_8H_6N_6 \cdot 0.65H_2O$) C, H, N.

2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carbonitrile (8). Dried and pulverized guanidine hydrochloride (30.9 g, 0.323 mol) was added to a solution prepared by dissolving Na metal (7.44 g, 0.323 mol) in absolute EtOH (2.0 L). The mixture was stirred rapidly at 25 °C for 30 min before **6** (24.7 g, 0.156 mol) was added. The resulting mixture was refluxed with stirring for 5 days. The EtOH-insoluble product **8** and NaCl were filtered from the boiling mixture. The solid cake was then stirred with boiling EtOH (to ensure removal of unchanged **6**) before the EtOH-insoluble material was again collected and finally washed thoroughly with H_2O, EtOH, and Et_2O in that order to give pure **8**: yield 58% 18.0 g); mass spectrum, m/e 201 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 2.84 (s, CH_3), 6.84 (s, NH_2), 7.30 (s, NH_2), 8.74 (s, C^7-H). Anal. ($C_9H_8N_6$) C, H, N.

***N*-[4-[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid Diethyl Ester (9).** A mixture of **7** (580 mg, 3.12 mmol), diethyl *N*-(4-aminobenzoyl)-L-glutamate (1.11 g, 3.45 mmol), and Raney Ni (about 3 g damp) in 70% AcOH–H_2O (80 mL) contained in a pressure bottle was shaken on a Parr apparatus at 20–23 °C under a H_2 pressure maintained at 0.98–1.05 kg/cm^2 (14–15 psi) for 14 h. The mixture was treated with Norit and filtered (Celite), and AcOH was removed from the filtrate by evaporation under reduced pressure with the aid of added portions of EtOH. A stirred suspension of the residue in EtOH (20 mL) was treated with cold 2 N Na_2CO_3 (20 mL), and the mixture was stirred at 5 °C for 30 min before the solid was collected and washed on the funnel with H_2O, cold EtOH, and Et_2O. The dried solid (1.21 g) was stirred several hours with EtOH (50 mL) to remove diethyl *N*-(4-aminobenzoyl)-L-glutamate whose continued presence was shown by TLC ($CHCl_3$–MeOH, 95:5; UV and ninhydrin detection). The EtOH-insoluble material was collected and washed with Et_2O to give nearly pure product (700 mg), but TLC showed that diethyl *N*-(4-aminobenzoyl)-L-glutamate was still present to a slight extent. The sample was then dissolved in boiling EtOH (250 mL), and the filtered solution was evaporated to near dryness under reduced pressure. The residue was stirred with EtOH (40 mL), and the suspension was kept in a refrigerator for 2 days before it was collected and dried in vacuo at 65 °C. This material (217 mg, 14% yield) was homogeneous by TLC (MeOH). Spectral data: mass, m/e 496 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6), identical with the spectrum reported earlier from these laboratories for **7** prepared from 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde.[19] Anal. ($C_{24}H_{29}N_7O_5$) C, H, N.

***N*-[4-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid Diethyl Ester (10).** A stirred solution of **8** (3.00 g, 15.0 mmol) and diethyl *N*-(4-aminobenzoyl)-L-glutamate (5.33 g, 16.5 mmol) in glacial AcOH (600 mL) containing damp Raney Ni (about 20 g) was kept under H_2 at atmospheric pressure for 4 h (or 1 h after H_2 absorption from a gas buret had ceased at about 680 mL). The mixture was then treated with Norit and filtered (Celite mat). AcOH was removed from the filtrate by evaporation under reduced pressure (H_2O aspirator, bath to 45 °C) with the aid of added portions of EtOH. Then a solution of the residue in warm EtOH (20 mL) was added in a thin stream to stirred 1 N Na_2CO_3 (200 mL) at 0–5 °C. The mixture was stirred at about 5 °C for 20 min longer before the yellow precipitate that formed was collected, washed with H_2O, and dried. This crude product (6.1 g) contained unchanged diethyl *N*-(4-aminobenzoyl)-L-glutamate (TLC, MeOH), which was removed by repeated treatments with warm Et_2O. The Et_2O-insoluble material that remained (4.5 g) was dissolved in boiling EtOH (1.2 L). The cloudy solution was clarified (Celite), concentrated (to about 400 mL), and left several hours in a refrigerator while product separated as a yellow solid. This solid (1.79 g) was collected with the aid of EtOH and Et_2O. Examination by TLC ($CHCl_3$–MeOH, 2:1) showed a strongly dominant UV-absorbing spot of R_f about 0.5 with slight contamination by material that remained near the origin. The mass spectrum of this sample showed the expected peak of m/e 510 corresponding to $(M + 1)^+$ for **10**. The sample was dissolved in MeOH (200 mL), and dry-column grade silica (10 g, Silica Woelm TSC, Woelm Pharmaca) was added. Evaporation gave a uniform powdery mixture that was then spread evenly on the surface of a mat of dry Silica Woelm TSC of about 3-cm thickness in a 150-mL fritted-disk funnel (medium porosity). The mat was then eluted at ambient pressure with $CHCl_3$–MeOH (2:1), and portions of eluant of about 30 mL each were collected until TLC showed all the product had been eluted. Several portions contained only the component that gave the UV-absorbing spot of R_f about 0.5. These portions were combined (about 400 mL total) and evaporated to give pure **10** as a beige solid in 15% yield (1.15 g). Spectral data: mass, m/e 510 $(M + 1)^+$, weak peak m/e 496 (not present before the use of MeOH in the workup) attributable to a trace amount of transesterification not detectable in the ^1H NMR spectrum; ^1H NMR (Me_2SO-d_6) δ 1.14 (t, CH_3CH_2), 1.16 (t, CH_3CH_2), 2.05 (q, $CHCH_2CH_2$), 2.43 (t, CH_2CH_2CO), 2.68 (s, 5-CH_3), 3.9–4.2 (two q, CH_3CH_2O), 4.2–4.5 (overlapping m, $NHCHCO$, CH_2N), 6.24 (s, NH_2), 6.50 (t, CH_2NH), 6.68 and 7.70 (two d, C_6H_4), 7.00 (s, NH_2), 8.25 (d, $CONHCH$), 8.50 (s, 7-H). Anal. ($C_{25}H_{31}N_7O_5 \cdot 0.5H_2O$) C, H, N.

***N*-[4-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (12, 5-Methyl-5-deazaaminopterin).** A stirred solution of **10**·0.5H_2O (1.07 g, 2.6 mmol) in Me_2SO (30 mL) was treated under N_2 with 1 N NaOH (5.25 mL). The solution that formed was kept under N_2 in a stoppered flask at 20–23 °C for 6 h and was then evaporated to dryness in vacuo (about 0.1 mm, bath to 30 °C). The yellow solid that remained was dissolved in H_2O (40 mL), and the clear solution was immediately treated with 1 N HCl with stirring to produce pH 3.6 and cause precipitation of **12**. The mixture was kept several hours in an ice H_2O bath before the solid was collected. The ^1H NMR spectrum of this material was as expected for **12** except for retention of Me_2SO. An attempt to remove the Me_2SO by stirring the dried solid with Et_2O was ineffective. The Me_2SO was removed by reprecipitation as follows. The pulverized solid (0.91 g) was stirred with H_2O under N_2, and 1 N NaOH was added dropwise until the pH remained near 8. Solution occurred, and the product was then reprecipitated by careful addition of 1 N HCl to pH 3.6. After the mixture had been kept several hours at 0–5 °C, the beige solid was collected, washed with cold H_2O, and dried in vacuo; yield 82% (0.83 g). Spectral data: mass, m/e 454 $(M + 1)^+$; UV (0.1 N HCl) 224 nm (39.6), 298 (21.5), (pH 7) 222 (36.0), 282 (26.7), 0.1 N NaOH) 223 (34.1), 283 (27.2), 340 plateau (8.69); ^1H NMR (Me_2SO-d_6) δ 2.02 (m, $CHCH_2CH_2$), 2.30 (m, CH_2CO_2H), 2.72 (s, CH_3), 4.34 (m, $NHCHCO$, CH_2N), 6.53 (t, NH), 6.66 and 7.70 (two d, C_6H_4), 7.36 (s, NH_2), 8.02 (d, $CONHCH$), 8.54 (s, 7-H). Anal. ($C_{21}H_{23}N_7O_5 \cdot 2.2H_2O$) C, H, N. Analysis by high-performance LC showed a purity level of at least 98% with respect to UV-absorbing material.

2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (13). The procedure that follows is an adaptation of a reported general procedure.[39] A solution of **8** (2.00 g, 10.0 mmol) in HCO_2H (50 mL of 95–97%) was added in a thin stream with stirring to Raney Ni (about 14 g damp). The mixture was stirred at 75–80 °C (bath temperature) for 1.5 h and then filtered while hot, and the solid on the funnel was washed with small portions of warm HCO_2H until the washings were colorless. The filtrate was evaporated to dryness under reduced pressure with the aid of added portions of EtOH. The solid residue was then dissolved in hot H_2O (200 mL), and the solution was clarified (Norit, Celite) to give an orange filtrate, which, when neutralized with 1 N NaOH to pH 7, gave a yellow solid; yield 1.62 g. Spectral data: mass,

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dominant peak of m/e 204 $(M + 1)^+$ for **13**, lesser peak of m/e 206 $(M + 1)^+$ for the hydroxymethyl compound **14**; ^1H NMR (Me_2SO-d_6) δ 2.98 (s, CH_3), 8.90 (s, C^7-H), 10.23 (CHO). HPLC assay indicated the mixture to be at least 87% **13** and 4% **14** with remaining material unidentified. Another run (3.3 g of **8**) gave similar results (2.73 g of **13** suitable for conversion to **14** as described below).

2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-methanol (14). $NaBH_4$ (500 mg, 13.2 mmol) was added in portions during a 10-min interval to a stirred suspension of crude **13** (2.73 g) in MeOH (500 mL) at 20–23 °C. Stirring was continued for 2 h and then $NaBH_4$ (500 mg) was again added as before. After the mixture had been stirred overnight, HPLC (acetate buffer (pH 3.6)–MeOH, 9:1) showed the conversion of **13** to the more polar **14** was complete. After removal of the MeOH by evaporation, the residue was stirred with H_2O (90 mL) and treated with 1 N HCl to lower the pH to 8.0 (from pH 11.3 initially). This mixture was stirred while being heated at about 70 °C (bath temperature) for 20 min. The solid filtered from the cooled mixture amounted to 2.63 g and was used without further purification in the conversion to crude **15** described below. Spectral data: mass, m/e 206 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 2.68 (s, CH_3), 4.52 (s, CH_2), 8.48 (s, C^7-H).

6-(Bromomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine Hydrobromide (15). Powdered **14** (205 mg, 1.0 mmol) was added to a stirred suspension of $(C_6H_5)_3PBr_2$[40] (1.39 g, 3.3 mmol) in Me_2NAc (4 mL). After the mixture had been stirred at 20–23 °C for 2 h, the clear red solution that had formed was treated with C_6H_6 (40 mL) to cause precipitation of a red solid. The clear liquid phase was removed by decantation, and the solid was stirred successively with portions of C_6H_6 and Et_2O (40 mL of each). Residual solvent was removed by evaporation, and the residue was dissolved in the minimum volume of 48% HBr at 20–23 °C. The solution was kept at that temperature for 30 min and was then added to MeCN (50 mL) to give a tan solid precipitate. The mixture was stirred in an ice H_2O bath for 30 min before the solid was collected with the aid of MeCN and Et_2O and then dried in vacuo to give the bromomethyl compound (177 mg) suitable for use in the preparations of **18** and **19** described below. Spectral data: mass, m/e 268 and 270 $(M + 1)^+$ for $C_9H_{10}BrN_5$.

***N*-[4-(Methylamino)benzoyl]-L-glutamic acid dimethyl ester (16)** was prepared in two steps as follows:

Step A. *N*-[4-[[(Benzyloxy)carbonyl]methylamino]-benzoyl]-L-glutamic acid dimethyl ester was prepared from 4-[[(benzyloxy)carbonyl]methylamino]benzoyl chloride[41] and L-Glu-Me_2·HCl by essentially the same procedure reported for the preparation of the corresponding diethyl ester from the in situ prepared aroyl chloride.[42] The yield of pure (benzyloxy)carbonyl derivative, mp 82 °C (Kofler Hiezbank), was 89% (5.9 g from a 15.0-mmol run), homogeneous by TLC ($CHCl_3$–MeOH, 95:5). Anal. ($C_{23}H_{26}N_2O_7$), C, H, N.

Step B. Conversion to 16. A solution of the (benzyloxy)-carbonyl derivative (5.00 g, 11.3 mmol) in MeOH (75 mL) containing 30% Pd on C (0.5 g) was stirred under H_2 (over H_2O in a gas buret) at ambient conditions for 2 h or until H_2 uptake had ceased (about 255 mL). The catalyst was removed by filtration, and the viscous oil that remained following evaporation was homogeneous by TLC ($CHCl_3$–MeOH, 95:5, detection by UV and ninhydrin); yield 90% (3.4 g). This material was used as such for the conversion to **18** described below. After about 2 months, the oil had mostly solidified. A sample for analysis after trituration with ligroin had mp 54–55 °C. Anal. ($C_{15}H_{20}N_2O_5$) C, H, N.

***N*-[4-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid Dimethyl Ester (18).** The crude bromomethyl compound **15** (175 mg) was added to a stirred solution of **16** (185 mg, 0.55 mmol) in Me_2NAc (3 mL). Solution occurred within 5 min, and a TLC ($CHCl_3$–MeOH, 3:1) spot of R_f about 0.4 due to a reaction product was strongly evident after 6 h. The solution was kept at 22–25 °C for 24 h, warmed at 50–55 °C for 4 h, and then left 16 h longer at 23–25 °C before it was added to EtOAc (40 mL) with stirring. The tan solid that formed was collected, dried (217 mg), and dissolved in H_2O (5 mL). This solution was added to 1 N Na_2CO_3 (5 mL) with stirring to give a brown solid (112 mg). This crude material was stirred in boiling MeOH (25 mL), and the mixture was clarified by filtration from a small portion of insoluble material. The filtrate was mixed with dry-column grade silica (500 mg, Silica Woelm TSC, Woelm Pharmaca), and the mixture was evaporated. The residue was distributed on a mat of the silica gel described above about 2.5 cm thick contained in a 15-mL funnel of medium porosity. The mat was then eluted at ambient pressure with $CHCl_3$–MeOH (2:1). Early fractions contained unchanged **16**. Intermediate fractions contained only **18** as evidenced by TLC examination. These fractions were evaporated to give **18** as a beige solid, yield 67 mg (approximately 27% if based on 0.50 mmol of **15**). Spectral data: mass, m/e 496 $(M + 1)^+$. Anal. ($C_{24}H_{29}N_7O_5$·0.75H_2O) C, H, N: calcd, 19.26; found, 18.66. This material was suitable for the conversion to pure **20** described below. Another run like that above but using 740 mg of crude **15** and left 5.5 days at 22–25 °C produced 407 mg of **18** homogeneous by TLC.

***N*-[4-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid (20, 5-Methyl-5-deazamethotrexate). A. From 18.** A solution of **18** (396 mg, 0.8 mmol) in MeOH (60 mL) containing 1 N NaOH (1.68 mL) was kept at 22–25 °C for 48 h, then at 45–50 °C for 1 h, and finally 17 h longer at 22–25 °C while the progress of the saponification was followed by HPLC.[36] The solution was evaporated to dryness, and the solid residue was dissolved in H_2O (20 mL). This solution was kept at 22–25 °C for 1 h and then at 45 °C for 30 min. HPLC showed ester hydrolysis to be complete. The solution was filtered (Norit, Celite) and then carefully treated with 1 N HCl to pH 3.8, giving **20** as a light yellow solid. After several hours in an ice H_2O bath, the precipitate was collected with the aid of cold H_2O and dried in vacuo; yield 74% (308 mg). Spectral data: mass, m/e 468 $(M + 1)^+$; UV (0.1 N HCl) 227 (39.7), 310 (22.4), (pH 7) 227 (37.5), 307 (28.6), (0.1 N NaOH) 228 (36.5), 306 (27.9); ^1H NMR (Me_2SO-d_6) δ 1.9–2.2 (br m, $CHCH_2CH_2$), 2.30 (m, CH_2CO_2H), 2.63 (s, CH_3C), 3.02 (s, CH_3N), 4.34 (m, $NHCHCO$), 4.66 (s, CH_2N), 6.73 and 7.75 (two d, C_6H_4), 8.10 (d, $CONHCH$), 8.13 (s, 7-H). Anal. ($C_{22}H_{25}N_7O_5$·3H_2O) C, H, N. Analysis by HPLC showed the purity level to be at least 98% with respect to UV-absorbing material.

B. From 12. A stirred suspension of **12**·2.2H_2O (150 mg, 0.304 mmol) in O_2-free H_2O (8 mL) was treated under N_2 with sufficient 1 N NaOH to cause solution. The pH was then adjusted to 6.4 using 1 N HCl. A solution of CH_2O in H_2O (93 μL of 37%, sp gr 1.08, 3.3 mmol) was added followed by $NaBH_3CN$ (31.2 g, 0.496 mmol). The pH of the solution was kept at 6.4 by addition of 1 N HCl as required during the next hour. The solution was kept under N_2 while the progress of the conversion was followed by HPLC[43] and observed to be complete after 21 h at 22–25 °C. The clarified (Norit, Celite) solution was acidified to pH 3.8 with 1 N HCl to give **20** (130 mg). A reprecipitation from a solution of pH 8.5 by addition of 1 N HCl to pH 3.8 afforded pure **20** in 64% yield (96 mg), identical (HPLC) with the sample described under method A above. Anal. ($C_{22}H_{25}N_7O_5$·1.65H_2O) C, H, N.

***N*-[4-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]ethylamino]benzoyl]-L-glutamic Acid Diethyl Ester (19).** Alkylation of diethyl *N*-[4-(ethylamino)benzoyl]-L-glutamate (**17**)[44] (648 mg, 1.85 mmol) with the crude bromomethyl compound **15** (636 mg, prepared as described above) in Me_2NAc (11 mL) during 6 days at 22–25 °C followed by a workup like that described for **18** (except that 9:1 $CHCl_3$–MeOH was used in the

(40) Prepared by dropwise addition of Br_2 (1.92 g, 12.0 mmol) in MeCN (10 mL) to a stirred partial solution of $(C_6H_5)_3P$ (3.14 g, 12.0 mmol) in MeCN (40 mL) at 10–15 °C. The crystalline $(C_6H_5)_3PBr_2$ that formed was collected and subsequently handled under N_2.

(41) Piper, J. R.; Montgomery, J. A.; Sirotnak, F. M.; Chello, P. L. *J. Med. Chem.* **1982**, *25*, 182.

(42) Fu, S.-C. J.; Reiner, M.; Loo, T. L. *J. Med. Chem.* **1975**, *30*, 1277.

(43) Assayed at intervals using a 20-min linear gradient with acetate buffer (pH 3.6)–MeCN changing in volume proportions from 9:1 to 1:1. During the conversion, the peak due to **12** was observed near 9.2 min and that due to **20** near 10.6 min.

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elution from silica gel) led to a sample of **19** (67 mg, about 8% yield) homogeneous by TLC; mass spectrum, m/e 538 $(M + 1)^+$. Anal. ($C_{27}H_{35}N_7O_5 \cdot 0.6H_2O$) C, H, N.

***N*-[4-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]ethylamino]benzoyl]-L-glutamic acid (21, 5-methyl-5-deaza-10-ethylaminopterin)** was prepared by saponification of **19** as described for the conversion of **18** to **20**; yield 97% (56 mg from 60 mg (0.109 mmol) of **19**·$0.6H_2O$), homogeneous by HPLC. Spectral data: mass, m/e 482 $(M + 1)^+$; UV (0.1 N HCl) 228 (40.8), 313 (17.1), (pH 7) 227 (39.2), 308 (30.2), (0.1 N NaOH) 228 (36.0), 308 (29.0). Anal. ($C_{23}H_{27}N_7O_5 \cdot 2.8H_2O$) C, H, N.

***N*-[4-[[(2-Amino-5-methyl-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (22, 5-Methyl-5-deazafolic Acid)**. Hydrolysis of **12**·$2.65H_2O$ (300 mg, 0.600 mmol) in boiling 1 N NaOH (15 mL) under N_2 during 4.25 h (as previously reported for the conversion of 5-deazaaminopterin to 5-deazafolic acid[19]) followed by acidification to pH 3.1 afforded material whose assay by HPLC revealed the presence of about 4% unchanged **12**. This sample (250 mg) was again dissolved in 1 N NaOH (10 mL), and the solution was boiled as before for 1 h. Subsequent acidification to pH 3.5 gave **22** (235 mg, 79% yield), homogeneous by HPLC. Spectral data: mass, m/e 455 $(M + 1)^+$; UV (0.1 N HCl) 220 (32.8), 280 (26.4), 295 sh (23.4), 328–340 plateau (10.5), (pH 7) 223 (39.8), 280 (25.9), 287–293 plateau (25.6), (0.1 N NaOH) 221 (34.1), 283 (25.4), 295 sh (24.8). Anal. ($C_{21}H_{22}N_6O_6 \cdot 1.25H_2O$) C, H, N.

***N*-[4-[[(2-Amino-5-methyl-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid (23, 5,10-Dimethyl-5-deazafolic Acid)**. Treatment of **20**·$3H_2O$ (100 mg, 0.192 mmol) with refluxing 1 N NaOH (5 mL) under N_2 for 4.25 h followed by acidification to pH 3.1 afforded **23** in 83% yield (80 mg). Assay by HPLC [acetate buffer (pH 3.6)–MeCN (85:15)] indicated 99% purity. Spectral data: mass, m/e 469 $(M + 1)^+$; UV (0.1 N HCl) 223 (32.1), 278 (21.3), 306 (24.9), (pH 7) 224 (40.8), 307 (29.9), 275 sh (17.9), (0.1 N NaOH) 226 (34.9), 309 (28.0). Anal. ($C_{22}H_{24}N_6O_6 \cdot 1.75H_2O$) C, H, N.

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Probes for Narcotic Receptor Mediated Phenomena. 12.[1] *cis*-(+)-3-Methylfentanyl Isothiocyanate, a Potent Site-Directed Acylating Agent for δ Opioid Receptors. Synthesis, Absolute Configuration, and Receptor Enantioselectivity

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The first enantiomeric pair of irreversible opioid ligands [(+)- and (−)-4] were synthesized in >99.6% optical purity as determined by HPLC analysis of diastereoisomeric derivatives of the intermediate 3-methyl-*N*-phenyl-4-piperidinamine (**9**) enantiomers. Single-crystal X-ray analysis of the (*R*,*R*)-L-(+)-tartaric acid salt of (−)-**9** revealed the absolute configuration to be 3*S*,4*R*. The absolute configuration of (−)-**3** [*cis*-(−)-3-methylfentanyl] and (−)-4 derived from (−)-**9** is thus 3*S*,4*R* and that of (+)-**3** and (+)-4 is 3*R*,4*S*. The (+) enantiomer of **4** (SUPERFIT) was shown to be highly potent and specific for acylation of δ opioid receptors (to the exclusion of μ) in rat brain membranes like its achiral prototype FIT (**2**) and was about 10 times as potent as the latter in this assay. The (+)-4 was about 5 times as potent as FIT in acylation of δ receptors in NG108-15 neuroblastoma × glioma hybrid cells and about 50 times as potent as its enantiomer. Both FIT and (+)-4 behaved as partial agonists in inhibition of δ receptor coupled adenylate cyclase in NG108-15 membranes and (+)-4 was 5–10 times more potent than FIT and about 100 times more potent than its enantiomer in this assay. Dibromination of amine **12**, catalytic exchange of bromine with tritium gas, and reaction of the labeled amine with thiophosgene afforded [^{3}H]-(+)-4 with a specific activity of 13 Ci/mmol. Previous experiments indicated (+)-4 acylates the same 58 000-dalton glycoprotein previously shown to be acylated by FIT but with less nonspecific labeling. In view of the high potency and specificity of (+)-4 and the availability of its enantiomer, it seems likely that these compounds will prove to be valuable tools for study of the opioid receptor complex.

Opioid drugs possess a wide spectrum of activity, and there has been great effort expended trying to understand the function of these agents at the molecular level. A variety of structurally diverse opioids have been used to provide evidence of a multiplicity of opioid receptors, with the three major types commonly referred to as μ, δ, and κ.[2] Presently a number of researchers are attempting to isolate and characterize individual receptor subtypes, and numerous reports of synthesis of opioid receptor irreversible ligands as tools for such studies have appeared.[3-7] We recently described the μ-specific acylating drug benzimidazole isothiocyanate (BIT) based on etonitazene, and

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