within the test period were counted as having amnesia or having forgotten the training. The equation used to calculate the percent of amnesia reversal activity reported in Figure 3 was that employed by Butler et al.;[18] that is, percent amnesia reversal = [(drug group - base-line control group)/(ceiling control group - base-line control group)] × 100; data were analyzed according to the screening criteria and statistical constraints used by the same authors.

(C) Water-Maze Procedure. Four groups of 10 male Sprague–Dawley rats weighing about 250–300 g were used. The animals were housed in the same conditions reported for test A. The experiments were carried out in 5 consecutive days in an apparatus similar to that described by Batting et al.[28] In the first day, rats unable to find the exit of the water maze within 5 min were selected. On the second, third, and fourth day the selected rats were treated twice a day with the same dose (equimolar to 100 mg/kg ip of piracetam) of the compounds under study. On the fifth day the same two doses were administered in the morning in 30-min intervals and then the animals were subjected to transtemporal ECS (60 mA/0.2 s) that produced the classical tonic and clonic convulsions. After the shock the animals were observed in three subsequent sessions in the water maze after 15, 60, and 240 min. The time spent to go out of the water maze (speed of performance) and the number of errors, calculated as the sum of reversals and wrong entrances, were recorded.

(D) Neuropsychopharmacological Effects at High Doses.[29] Male and female Swiss inbred mice (Charles River) weighing 22 ± 2 g were used. Animals were housed in the same standardized environmental conditions described in test A. The compounds were injected intraperitoneally after solubilization in 1% Tween 80. Different doses up to 1000 mg/kg were used. Every single dose was administered to a group of 10 mice (five males and five females). Control groups of mice were treated only with vehicle.

(1) **Behavior in Free Conditions.** (a) Activity in the open field. This activity was determined by recording the total number of movements performed in 5 min by every animal.[33,34]

(b) Exploration activity in the hole board. The test was performed by using the Boissier and Simon technique.[35] The number of explorations was recorded automatically by an infrared device. During the experiment the total number of holes explored by each animal during 5 min was recorded.

(2) **Anticonvulsant Activity.** This was tested with use of the maximal electroshock which induced death in 50% of the control animals. The Model U. Basile ECT-Unit 7800 used for the test was adjusted as follows: 200-mA frequency pulses/s; 60-mA current adjustment; 0.4-s shock duration; 0.6-ms pulse width. We considered the animals protected when they did not show seizures.

(3) **Action on Motor Coordination.** This was examined by using the rota-rod test.[36] The number of animal falls, during a 100-s observation period, was recorded.

(4) **Myorelaxant Action.** This was analyzed by using the Boissier and Simon traction test.[37] We recorded how long the animals stayed on an horizontal wire with their forelegs. All tests were performed on each animal as in the following succession (time point, minutes): 0, ip injection; 15, open field activity; 20, exploration activity; 25, traction test; 30, rota-rod test; 60, effect of electroshock. Significance levels were obtained by the Student's *t* test.

Acknowledgment. A partial financial support to this research from NCNS (Nuovo Consorzio Sanitario Nazionale) is gratefully acknowledged.

Registry No. **2**, 116079-14-2; **3**, 116102-36-4; **4**, 116079-15-3; **5**, 116079-16-4; **6**, 116079-17-5; **7**, 116079-18-6; **8**, 116079-19-7; **9**, 116079-20-0; **10**, 116079-21-1; fumaronitrile, 764-42-1; ethyl malonate, 105-53-3.

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Synthesis and Structure–Activity Relationships of a New Series of Antiarrhythmic Agents: Monobasic Derivatives of Disobutamide[1]

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Analogues of the dibasic antiarrhythmic agent disobutamide (**2**) were prepared and evaluated for antiarrhythmic efficacy, myocardial depression, and anticholinergic activity. The replacement of an isopropyl group in disobutamide by an acetyl group led to the monobasic analogue SC-40230, **7a**, which demonstrated good antiarrhythmic activity accompanied by less myocardial depressant and anticholinergic activities. In addition, it did not induce clear cytoplasmic vacuoles as did the parent compound. SC-40230 was chosen from among other analogues as a candidate for clinical evaluation. Other compounds prepared and evaluated included indolizidinones and a secondary amine isomer of disobutamide.

We have aimed recent efforts of our antiarrhythmic program at the identification of effective class I antiarrhythmic agents[2] with an absence or diminution of the side effects most frequently associated with drugs used to treat chronic ventricular arrhythmias.[3] Emerging from this investigation was the bis[(dialkylamino)alkyl]phenylacetamide series **1**, which included compounds possessing good antiarrhythmic potency and diminished undesirable properties.[4] A representative from this series, disobutamide (**2**), was identified as a candidate for clinical evaluation.[5]

Disobutamide was withdrawn early in the course of clinical testing when clear cytoplasmic vacuoles were found

(1) Presented in part at the 17th Northeast Regional American Chemical Society Meeting, November, 1987, Rochester, NY.
(2) Vaughan Williams, E. M. *Prog. Pharmacol.* **1979**, *2*, 13 and references therein.
(3) Schwarz, J. B.; Keefe, D.; Harrison, D. C. *Drugs* **1981**, *21*, 23.
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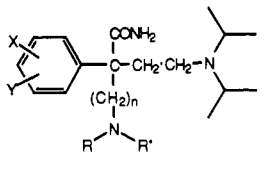
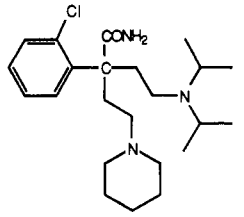
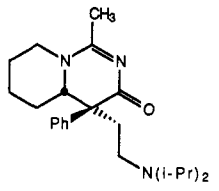


1



2

(±)-Disobutamide



(±)-SC-36602

in many types of cells in the rat and dog[6] during long-term toxicity studies. Such a phenomenon has been described by Levine and Sowinski for a wide variety of dibasic amines[7] and is well known for a number of other drugs including piperamide, chloroquine, and tilorone.[7] Subsequent work using cell culture systems confirmed that the presence of two strongly basic tertiary amine functions in our series is responsible for the induction of vacuoles.[8]

We reasoned that decreased basicity of one of these amine centers through the introduction of an adjacent carbonyl group would prevent the induction of vacuoles but maintain antiarrhythmic activity. Previous experience showed that other monobasic compounds were useful antiarrhythmic agents, and a bicyclic derivative, SC-36602, has undergone clinical evaluation.[9] We have identified SC-40230, **7a**, as a candidate for Phase I clinical trials on the basis of its efficacy against supraventricular[10] and ventricular[11,12] arrhythmias, as well as its lack of significant negative inotropic and anticholinergic activities.[13]

The compounds reported below are modifications of the disobutamide structure where the basicity of one of the tertiary amino groups has been diminished by the presence of an adjacent carbonyl group. Additionally, a less basic secondary amine isomer of disobutamide (which does not induce vacuoles[8]) and some bicyclic indolizidinones containing a single tertiary amine function were also prepared.

Chemistry

The oxo analogues **7** were prepared as outlined in Scheme I. These compounds and all others in this paper are racemic mixtures or mixtures of diastereomers. The α-*N*-piperidinylethyl derivative of 2-chlorophenylacetonitrile **3**[4] was alkylated with *N*-benzyl-*N*-isopropylaminoethyl chloride[14] with sodamide in toluene to afford **4**. Hydration of the nitrile **4** with sulfuric acid to the carboxamide **5** and catalytic hydrogenolysis of the benzyl group yielded the amino carboxamide **6**. Acylation of **6** with acetyl chloride or benzoyl chloride in chloroform in the presence of triethylamine at 0 °C yielded the acetamides **7a** and benzamide **7b**, respectively. The use of trifluoroacetyl chloride or trifluoroacetic anhydride to acylate **6** under the above conditions produced the trifluoroacetamide nitrile **7c** as the main product along with a small amount of the corresponding carboxamide compound. (Since modification of the carboxamido group does not necessarily eliminate or diminish antiarrhythmic activity, this change and those shown in Scheme II provide functionality compatible with antiarrhythmic activity.[15,16])

Compound **7a** exists as a mixture of rotamers (in about a 2:1 ratio) resulting from hindered rotation about the N–CO bond. This mixture is indicated in the ^{1}H NMR spectrum by the presence of two acetyl methyl resonances at 1.88 and 2.15 ppm as well as two methine multiplets centered at 3.87 and 4.71 ppm. An elevated temperature NMR study of this compound (DMSO-d_6) showed that these peaks coalesced to positions between those of the two rotamers at about 100 °C and reappeared as the sample returned to ambient temperature. No obvious indication of rotamers was evident in the ^{1}H NMR spectra of compounds **7b** or **7c**.

Scheme II presents the synthesis of analogues of disobutamide containing a carbonyl on one of the quaternary carbon–basic nitrogen linkages. The (*N*,*N*-diisopropylamino)ethyl derivatives of 2-chlorophenylacetonitrile (**8a**),[4] ethyl 2-chlorophenylacetate (**8b**), or 2-chloro-*N*,*N*-dimethylphenylacetamide (**8c**) were alkylated with 1-(chloroacetyl)piperidine in the presence of NaH in DMF (in the case of nitriles) or LDA in THF (in the cases of ester and amide) to afford **9a**, **9b**, or **9c**, respectively. In addition, α-chloro-*N*,*N*-diisopropylacetamide was also used to alkylate the piperidinoethyl derivative **3** with NaH in DMF to provide the amide **9d**.

The preparation of monobasic bicyclic systems is described in Scheme III. The nitriles **10a–c**[4] were alkylated by 2-picolyl chloride in the presence of KH in toluene at 70 °C to produce the pyridylmethyl derivatives **11a–c**. Nitrile hydration using KOH in *t*-BuOH led to the amides **12a–c** and subsequent catalytic hydrogenation of the pyridine ring over platinum oxide in acetic acid afforded the substituted indolizidinones **13a–c** or mixtures of **13a–c** with uncyclized amides. Heating these uncyclized amides in refluxing acetonitrile converted them to the bicyclic compounds.

In the cases of **13a** and **13c** the bicyclic materials were mixtures of diastereomers that were not separated. The diastereomers **13b** and **13b′** did show significant differences in polarity by thin-layer chromatography and were isolated as individual diastereomers through column chromatography.

Scheme IV outlines the synthesis of the 2-piperidyl isomer of disobutamide. The anion generated from the nitrile **3** added in conjugate fashion in THF solution at ambient temperature to the vinyl group of 2-vinylpyridine.

(6) (a) Rorig, K. J.; Ruben, Z.; Dodd, D. C.; Semler, D. E. *Pharmacologist* **1986**, *28*, 122 (abstract). (b) Ruben, Z.; Dodd, D. C. *Pharmacologist* **1986**, *28*, 122 (abstract).

(7) Levine, S.; Sowinski, R. *Toxicol. Appl. Pharmacol.* **1977**, *40*, 147.

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(14) Prepared by treatment of ethylene oxide with 1 equiv of *N*-benzyl-*N*-isopropylamine in EtOH at 60 °C in a pressure vessel (bp 65 °C, 0.6 mmHg) followed by chlorination with thionyl chloride.

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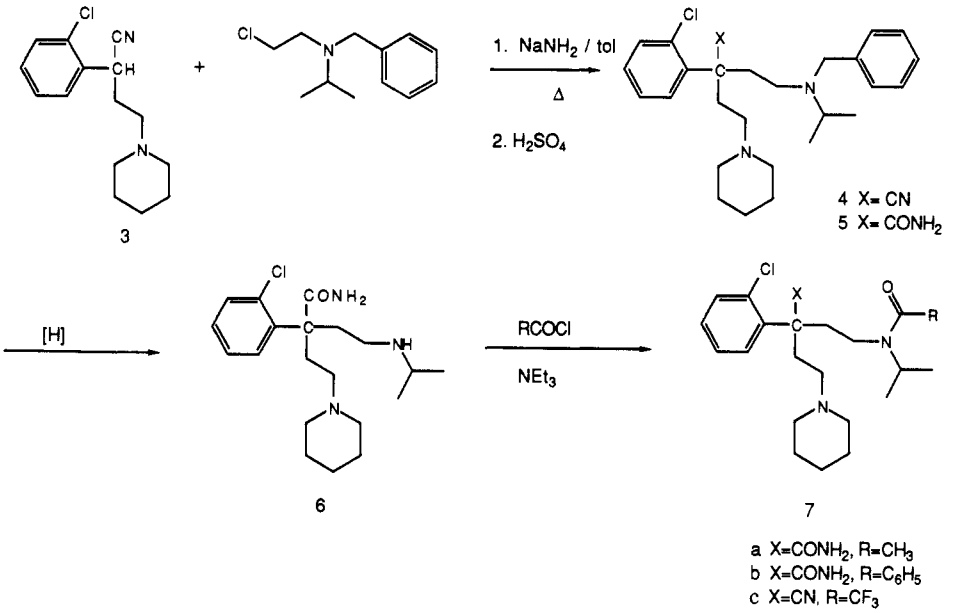
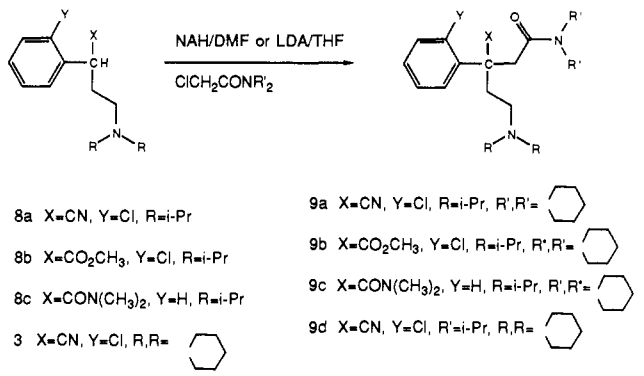
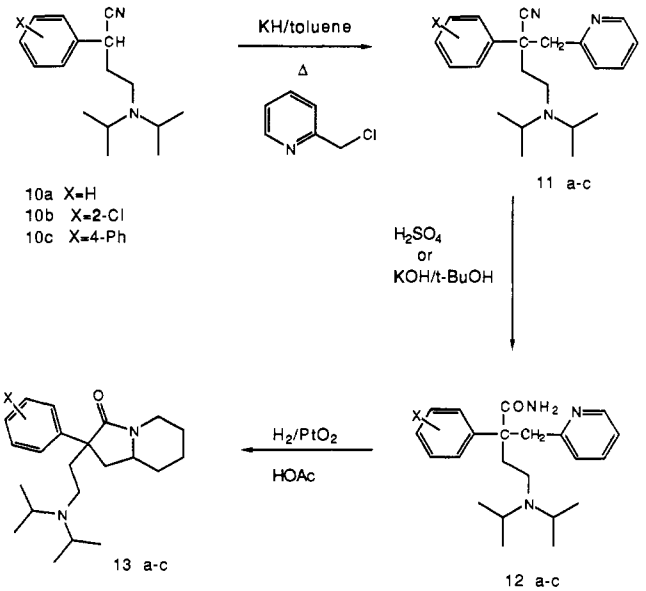
Scheme I



Scheme II



Scheme III



(The reaction was subsequently found to proceed much more efficiently in DMF.) Sulfuric acid hydration of the intermediate nitrile **14** led to the pyridyl amide **15**. Catalytic reduction of the pyridine ring afforded diastereomeric mixture **16**, which is isomeric with disobutamide but possesses a secondary rather than a tertiary basic piperidine nitrogen.

Biology

Compounds were evaluated for antiarrhythmic activity by using the unanesthetized 24-h Harris dog model.[17] A two-stage ligation of the left anterior descending coronary artery was performed on an anesthetized animal approximately 24 h before a test compound was administered. Criteria for acceptable test animals, compound administration, and dose regimens were as previously described.[9]

Displacement of the muscarinic radioligand [^{3}H]-quinuclidinylbenzilate (QNB) in a rat brain homogenate was used to assess muscarinic receptor binding affinity[18] and potential anticholinergic liability. The muscarinic binding affinity of disopyramide was the standard to which the binding of the test compounds was compared. Relative affinities were determined by calculating the following ratio: IC_{50}(test compound)/IC_{50}(disopyramide).

Myocardial depressant activity was assessed with the closed-chest anesthetized dog. Doses, methods of administration, and measurement were as previously described.[9] Compounds that depressed maximum $dP/dt \leq 25\%$ at the mean effective antiarrhythmic dose (MED) were arbitrarily considered to possess an acceptable degree of myocardial depressant activity.

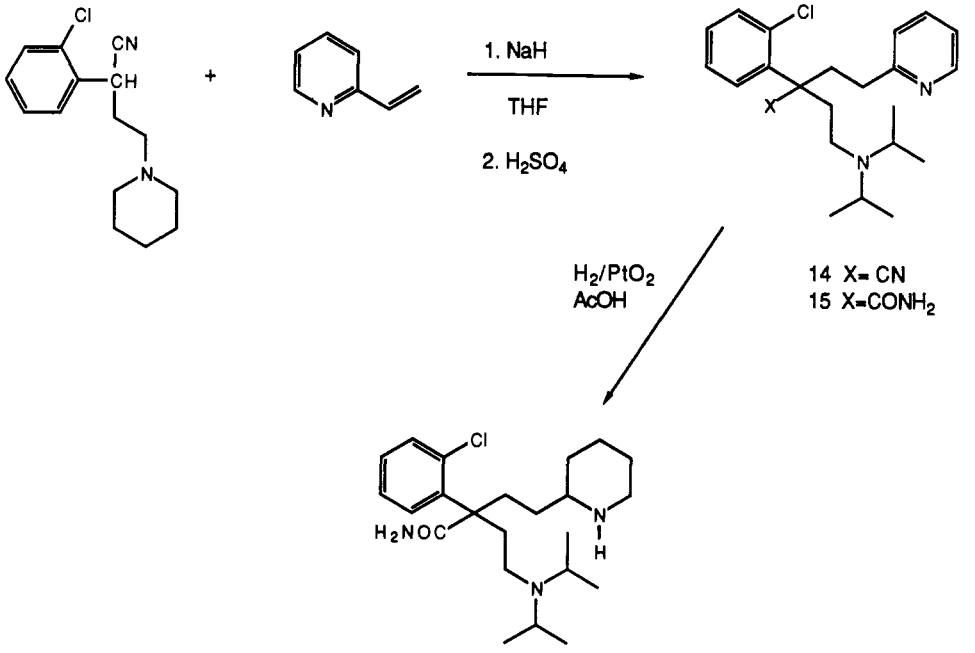
Comparisons among the compounds were made between the dose required to decrease mean arterial pressure 50% and the mean antiarrhythmic dose in order to assess general cardiovascular safety. If the mean antiarrhythmic dose was greater than 5 mg/kg, compounds were administered intravenously to anesthetized dogs in consecutive doses of 5 mg/kg for 5 min, every 15 min until mean arterial blood pressure was depressed 50% or up to a cumulative dose of 50 mg/kg. If the mean antiarrhythmic dose was 5 mg/kg or less, a compound was administered in consecutive doses of 1 mg/kg per min every 5 min until the depressor end point was reached or a maximum cumulative dose of 10 mg/kg was administered.

Results and Discussion

Evaluation of compounds in the Harris dog assay (Table I) determined that **7b** caused prolongation of QRS interval at the efficacious dose of 2 mpk and was lethal at 5 mpk.

(17) Harris, A. S. *Circulation* **1950**, *1*, 318.
(18) Yamamura, H. I.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1725.

Scheme IV



16

Compound **13b'** manifested side effects before the mean antiarrhythmic dose was determined. Since such changes in behavior (e.g., vocalization, excessive limb movement) of conscious dogs during antiarrhythmia testing have been seen with compounds such as aprindine and mexiletine (unreported observations), which have been reported to show CNS activity in humans,[3] this compound was disregarded from further consideration. Two compounds (**7c** and **9d**) showed no antiarrhythmic efficacy up to 20 mg/kg and were also dropped from further consideration.

Of the compounds showing desirable antiarrhythmic efficacy, four (**9a**, **9c**, **13a**, **13c**) were shown to decrease maximum dP/dt more than the 25% that was considered acceptable. Thus, these were also rejected for further evaluation.

Of the remaining compounds (**7a**, **9b**, **13b**, and **16**), **13b** showed the best antiarrhythmic activity in the Harris dog. However, the muscarinic binding affinity of **13b** was comparable to that of disopyramide. This property is indicative of the undesirable anticholinergic effects that accompany disopyramide therapy.[3] Disobutamide isomer **16** was rejected because unlike disobutamide it was found to induce dense cytoplasmic vacuoles in cultured cells though it did not induce clear cytoplasmic vacuoles.[8] Thus, we favored the two compounds **7a** and **9b** as agents that would provide a second-generation drug with high efficacy and an absence or minimization of the side effects most frequently associated with class I antiarrhythmic agents.

These two compounds possessed the efficacy, absence of appreciable myocardial depression, and muscarinic binding properties we sought in an antiarrhythmic agent. We chose to pursue **7a**, SC-40230, on the basis of a slightly more favorable pharmacologic and pharmacokinetic profile. This compound is orally active in the Harris dog at a dose of 15 mpk[11] and there was no difference in the degree of sodium channel blockade between its enantiomers as determined by cellular electrophysiology[10]. It is currently undergoing Phase I clinical evaluation.

Experimental Section

Materials. Melting points were determined in a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra in $CDCl_3$ were obtained on a Varian T-60 or FT-80 instrument or a GE QE-300 spectrometer. Elemental analyses were determined by E. Zielinski and associates of our laboratories and are within ±0.4% of calculated values, unless otherwise noted. Hydrogenations were performed by M. Scaros and associates. Chromatography was carried out by the group of S. Dugar.

(±)-α-(2-Chlorophenyl)-1-piperidinebutanenitrile (3). A solution of 8.0 g (0.20 mol) of sodamide in 400 mL of toluene was treated with 30 g (0.20 mol) of 2-chlorophenylacetonitrile under

Table I. Antiarrhythmic and Myocardial Depressant Activity and Muscarinic Binding Affinity of Monobasic Compounds and Standards

compound[a]	MED, iv, mg/kg[b] (no. of active/ no. of dogs)	decrease in max dP/dt, % (no. of dogs; dose, mg/kg)[c]	muscarinic receptor affinity[d] (disopyramide = 1)
7a	9 (10/10)	20 ± 2 (5, 9)	1/24
7b	2 (2/2)[e]	NT[f]	NT
7c	inact[g]	NT	NT
9a	5 (3/5)	38 ± 2 (3, 10)	1/7
9b	5 (4/5)	23 ± 8 (5, 4.5)	1/3
9c	8 (5/6)	28 ± 6 (5, 8)	NT
9d	inact[g]	NT	NT
13a (HCl)	10 (5/5)	34 ± 4 (5, 10)	1/6
13b	3.5 (2/2)	NT	1
13b'	side effects	NT	1/2
13c	3 (4/5)	35 ± 5 (5, 3)	1/16
16	12.5 (2/2)	NT	1/4
disopyramide	10 (5/5)	56 ± 2 (5, 10)	1
disobutamide	3.5 (5/5)	42 ± 1 (3, 5)	1/4

[a] Compounds are racemic or mixtures of diastereomers (see text). [b] Mean effective dose required to suppress ventricular ectopic rate ≥ 25% for a minimum duration of 10 min. Number of active/total number of dogs in parentheses. [c] Myocardial depression determined by monitoring the maximum rate of rise of left ventricular pressure. Values given are mean percent decrease ± standard error of the mean. [d] Ratio of muscarinic receptor affinity (IC_{50}) of test compound to that of disopyramide. IC_{50}'s were determined by log probit analysis after measuring the displacement of [^{3}H]QNB by test compound at three to six different concentrations in triplicate.[18] [e] While this compound was effective at 2 mg/kg, it was lethal at 5 mg/kg; since prolongation of the QRS interval was observed at the efficacious dose, **7b** was dropped from further consideration. [f] Not tested. [g] Inactive up to 20 mg/kg dose.

nitrogen at room temperature. The mixture was heated to 80 °C for a period of $^1/_2$ h before the addition of 29.2 g (0.198 mol) of 1-(2-chloroethyl)piperidine over 10–15 min. The reaction mixture was maintained at 80 °C for 1 h, cooled to room temperature, and diluted with 300 mL of water. The layers were separated, and the aqueous layer was extracted once with toluene. The combined extracts were washed with water and dried (Na_2SO_4) and concentrated to provide the crude product as an oil. Distillation at 155–160 °C (0.3 mm) provided 20.0 g (38%) of nitrile **3**. Anal. ($C_{15}H_{19}ClN_2$) C, H, N.

(±)-α-(2-Chlorophenyl)-α-[2-[(1-methylethyl)(phenylmethyl)amino]ethyl]-1-piperidinebutanenitrile (4). A solution of 106.7 g (0.43 mol) of *N*-benzyl-*N*-isopropylaminoethyl chloride hydrochloride[14] in 500 mL of H_2O was overlayed with 150 mL of toluene, and 25 mL of 50% NaOH solution (0.47 mol) was added. After the mixture was stirred for several minutes, the layers were separated, and the aqueous phase was extracted with two additional 100-mL portions of toluene. The combined extracts were washed with saturated NaCl solution and dried (Na_2SO_4) until used in the alkylation reaction.

A solution of 17.9 g (0.46 mol) of sodamide in 1.25 L of toluene was heated to about 90 °C under N_2 with stirring, and 97.2 g (0.37 mol) of nitrile **3** in 200 mL of toluene was added. The reaction mixture was maintained at 90 °C for 1 h before addition of the previously prepared amine solution over a 15–20-min period. After heating of the resultant mixture for 1 h at 90 °C, the solution was cooled and diluted with 600 mL of H_2O, and the layers were separated. After extraction of the aqueous portion once with toluene, the combined extracts were washed with H_2O and dried (Na_2SO_4). The solution was filtered through a cake of Celite and upon solvent removal from the filtrate, a quantitative recovery of oily product **4** remained (about 165 g containing some traces of toluene). TLC (77.5% cyclohexane, 20% 2-propanol, 2.5% NH_4OH solution on silica) indicated an absence of starting material and a single faster moving product, which was used without purification for the subsequent reaction: ^1H NMR ($CDCl_3$) δ 7.05–7.70 (m, 9 H, Ar), 3.5 (s, 2 H, $PhCH_2$), 0.90 (dd, 6 H, Me).

(±)-α-(2-Chlorophenyl)-α-[2-[(1-methylethyl)(phenylmethyl)amino]ethyl]-1-piperidinebutanamide (5). Nitrile **4** (163 g, 0.34 mol) was added neat to 200 mL (3.6 mol) of concentrated H_2SO_4 at room temperature. The temperature of the solution rose to about 95 °C and this temperature was maintained by the rate of addition of the remaining oil. The reaction mixture was then heated so that the temperature stayed at about 100 °C for 2.5 h. After cooling, the reaction solution was slowly added to 500 mL of ice water. The resulting aqueous solution was basified with 400 mL of 50% NaOH solution and the basic solution was extracted thrice with toluene. The combined extracts were washed once with H_2O and dried (Na_2SO_4). Solvent removal in vacuo gave about 172 g of thick oil. The oil was taken up into 650 mL of warmed hexane, and upon cooling 135 g (87%) of white crystalline **5** was obtained after washing with additional hexane and drying: mp 106–111 °C; ^1H NMR ($CDCl_3$) δ 7.1–7.4 (9 H, m, aryl), 3.5 (2 H, s, CH_2Ph), 0.96 (6 H, t, Me). Anal. ($C_{27}H_{38}ClN_3O$) C, H, N.

(±)-α-(2-Chlorophenyl)-α-[2-[(1-methylethyl)amino]ethyl]-1-piperidinebutanamide (6). A solution of 100 g (0.219 mol) of amide **5** in 1 L of EtOH containing 39.8 mL of concentrated HCl solution and 0.2 g of H_3PO_4 in a Parr shaker was exposed to hydrogen at 25 °C for 5 h over 50 g of 5% $Pd/BaSO_4$ catalyst. After this time 79.6 psi of hydrogen gas had been taken up (theory 76.7 psi). The catalyst was removed by filtration and the solvent was removed in vacuo. The crude product was taken up in water and was extracted with three 200-mL portions of ether. The aqueous extracts were made alkaline with 50% aqueous NaOH solution and extracted with three 250-mL portions of CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and concentrated in vacuo to afford an oily residue, which upon crystallization from toluene/hexane provided 73 g of **6** as a white crystalline solid (91%): mp 128–130 °C; ^1H NMR ($CDCl_3$) δ 7.2–7.5 (m, 4 H, Ar), 1.05 (d, 6 H, Me). Anal. ($C_{20}H_{32}ClN_3O$) C, H, N.

(±)-α-[2-[Acetyl(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-1-piperidinebutanamide (7a). A solution of 6.0 g (0.016 mol) of α-(2-chlorophenyl)-α-[2-[(1-methylethyl)(phenylmethyl)amino]ethyl]-1-piperidinebutanamide (**6**) and 1.71 g (0.017 mol) of triethylamine in 100 mL of chloroform at 0 °C under a nitrogen atmosphere was treated with 1.37 g (0.017 mol) of acetyl chloride. The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature and poured into water. The layers were separated, and the organic phase was washed with water and dried ($MgSO_4$). Solvent removal in vacuo provided the crude product, which was crystallized from ethyl acetate to afford 4.51 g (69.2%) of α-[2-[acetyl(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-1-piperidinebutanamide (**7a**): mp 140–141 °C; ^1H NMR ($CDCl_3$) δ 7.1–7.9 (4 H, m, Ar), 3.9 (1 H, m, NC*H*Me_2), 2.20 and 1.95 (3 H, 2 s, *N*-acetyl stereoisomers), 1.1 (6 H, d, Me). Anal. ($C_{22}H_{34}ClN_3O_2$) C, H, N.

(±)-α-[2-[Benzoyl(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-1-piperidinebutanamide (7b). Via the procedure described for the preparation of **7a**, the use of benzoyl chloride in place of acetyl chloride provided **7b** (60%): mp 130–131 °C; ^1H NMR ($CDCl_3$) δ 7.2–7.8 (9 H, m, Ar), 1.05 (6 H, d, Me). Anal. ($C_{27}H_{36}ClN_3O_2$) C, H, N.

(±)-α-[2-[(Trifluoroacetyl)(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-1-piperidinebutyronitrile (7c). Via the procedure described for the preparation of **7a**, the use of trifluoroacetic anhydride in place of acetyl chloride afforded nitrile **7c** as an oil as the result of amine acylation and concomitant dehydration of the carboxamide (41%): ^1H NMR ($CDCl_3$) δ 7.1–7.9 (4 H, m, Ar), 4.2 (1 H, m), 1.25 and 1.15 (6 H, 2 d, Me). Anal. ($C_{22}H_{29}ClF_3N_3O$) C, H, N.

(±)-Methyl α-[2-[Bis(1-methylethyl)amino]ethyl]-2-chlorobenzeneacetate (8b). Concentrated H_2SO_4 (100 mL) was added to a stirred solution of 100 g (0.35 mol) of (±)-α-[2-[bis(1-methylethyl)amino]ethyl]-2-chlorophenylacetonitrile (**8a**)[4] in 200 mL of MeOH, and the reaction mixture was refluxed for 6 h. After cooling to room temperature, the solution was poured into ice water and was carefully neutralized with 50% NaOH solution with ice-bath cooling. The resulting basic solution was extracted with two 300-mL portions of ether, and the extracts were washed with water and dried ($MgSO_4$). Solvent removal in vacuo provided the crude product as an oil, which was distilled [142–148 °C (0.3 mm)] to provide 85.1 g (78%) of ester **8b**: IR (neat) 1735 cm^{-1}. Anal. ($C_{17}H_{26}ClNO_2$) C, H, N.

(±)-*N*,*N*-Dimethyl-α-[2-[bis(1-methylethyl)amino]ethyl]phenylacetamide (8c). A solution of 4.0 g (0.024 mol) of *N*,*N*-dimethylphenylacetamide in 20 mL of THF was added by motor-driven syringe over a period of 33 min to a suspension of KH (6.0 g, 35% in oil, 0.052 mol) in 20 mL of freshly distilled THF under argon atmosphere. To this solution was added 4.26 g (0.026 mol) of *N*,*N*-diisopropylaminoethyl chloride[4] in 6 mL of THF over a 33-min period, and the mixture was stirred at room temperature for 6h. The reaction mixture was poured onto ice water and extracted with three portions of ether. The ether extracts were washed with water and saturated NaCl and then dried ($MgSO_4$). Solvent removal in vacuo provided the crude product, which was taken up in 1 N hydrochloric acid solution. The acidic solution was washed with pentane and then made alkaline with 50% sodium hydroxide solution and extracted with three portions of ether. The ether extracts were dried ($MgSO_4$), and the solvent was removed in vacuo to give 3.77 g (54.6%) of **8c**, which was used without further purification: mp 47–50 °C; ^1H NMR ($CDCl_3$) δ 1.00 and 0.93 (2 d, 12 H, Me), 2.90 (s, 6 H, NMe), 3.85 (m, 1 H, CH), 7.32 (m, 5 H, Ar).

(±)-1-[5-[Bis(1-methylethyl)amino]-3-(2-chlorophenyl)-3-cyano-1-oxopentyl]piperidine (9a). To an oil-free suspension of NaH (2.0 g of 50% oil dispersion, 0.041 mol) in 25 mL of DMF was added a solution of 11.2 g (0.04 mol) α-[2-[bis(1-methylethyl)amino]ethyl]-2-chlorophenylacetonitrile (**8a**) in 50 mL of DMF dropwise over a 10-min period. The mixture was stirred at room temperature under N_2 atmosphere for 45 min before the addition of 6.4 g (0.04 mol) of 1-(chloroacetyl)piperidine[19] in 10 mL of DMF. The reaction mixture was stirred at room temperature for 1 h, poured into water, and extracted with three portions of ether. The ether extracts were washed with water and dried ($MgSO_4$). Solvent removal in vacuo provided a crude product, which was crystallized from hexane to give 9.2 g (57%) of **9a**: mp 87–89 °C; ^1H NMR ($CDCl_3$) δ 0.90 (d, 12 H, Me), 7.25–7.95 (m, 4 H, Ar). Anal. ($C_{23}H_{34}ClN_3O$) C, H, N.

(19) Bruce, W. F.; U.S. Patent 2456911, 1948.

(±)-Methyl α-[2-[Bis(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-γ-oxo-1-piperidinebutanoate (9b). A solution of lithium diisopropylamide was formed by the dropwise addition over a 10-min period at 0 °C of 37.5 mL (60 mmol) of 1.6 M *n*-BuLi to 8.4 mL (60 mmol) of diisopropylamine dissolved in 200 mL of freshly distilled THF under N_2 atmosphere. The mixture was stirred at 0 °C for 30 min and cooled to −50 °C and 18.6 g (60 mmol) of **8b** in 100 mL of THF was added dropwise over a 20-min period. The reaction mixture was stirred at −50 °C for 30 min before the dropwise addition of 8.5 g (60 mmol) of 1-(chloroacetyl)piperidine in 60 mL of THF at −50 °C. The reaction mixture was allowed to warm to room temperature where it was kept for 2 h and then poured into water. The aqueous mixture was extracted with three portions of ether, and the combined extracts were washed with water and dried ($MgSO_4$). Solvent removal in vacuo gave a residue, which was crystallized from hexane to provide 10.1 g (37%) of **9b**: mp 104–106 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (d, 12 H, Me), 3.70 (s, 3 H, CO_2Me), 7.12–7.6 (m, 4 H, Ar). Anal. ($C_{24}H_{37}ClN_2O_3$) C, H, N.

(±)-α-[2-[Bis(1-methylethyl)amino]ethyl]-*N*,*N*-dimethyl-γ-oxo-α-phenyl-1-piperidinebutanamide (9c). Via the procedure described above for the preparation of **9b** and with amide **8c** in place of ester **8b**, amide **9c** (47%) was obtained after recrystallization from ether/pentane: mp 142–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.0 (d, 12 H, Me), 2.85 (d, 6 H, $CONMe_2$), 7.25 (s, 5 H, Ar). Anal. ($C_{25}H_{41}N_3O_2$) C, H, N.

(±)-β-(2-Chlorophenyl)-β-cyano-*N*,*N*-bis(1-methylethyl)-1-piperidinepentanamide (9d). Via the procedure described for the preparation of **9a** and with substitution of nitrile **3** for **8a** and replacement of 1-(chloroacetyl)piperidine with 1-chloro-*N*,*N*-bis(1-methylethyl)acetamide, nitrile **9d** (46%) was obtained after recrystallization from hexane: mp 83–85 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (dd, 12 H, Me), 3.15 and 3.70 (d, AB, 2 H), 7.3–7.9 (m, 4 H, Ar). Anal. ($C_{23}H_{34}ClN_3O$) C, H, N.

General Procedure for the Preparation of the (±)-α-[(Diisopropylamino)ethyl]-α-(2-picolyl)phenylacetonitriles (11). Potassium carbonate (22.0 g, 0.159 mol) was added in portions to a solution of 26.0 g (0.159 mol) of 2-picolyl chloride hydrochloride in 50 mL of H_2O. After the carbonate was completely dissolved, the solution was extracted thrice with ether, and the extracts were washed with saturated NaCl solution and dried (Na_2SO_4). Solvent removal in vacuo gave 19.8 g (97%) of the picolyl chloride as a pink oil which was used for the subsequent reaction without further purification.

A suspension of 17.5 g of 35% KH oil dispersion (0.153 mol) in 250 mL of toluene was treated with 29.0 g (0.119 mol) of nitrile **10a** in 300 mL of toluene at a rapid rate, and the reaction mixture was then heated at 65–70 °C for 30 min. The crude 19.8 g (0.155 mol) of picolyl chloride in 400 mL of toluene was then added to the reaction mixture as a slow stream of solution, and the temperature was maintained at 70 °C for 30 min after addition. After cooling of the reaction mixture in an ice bath, 400 mL of H_2O was added, and the layers were separated. The toluene layer was extracted with two 150-mL portions of 10% HCl solution, and these were made alkaline with 25% NaOH solution. The aqueous basic solution was extracted thrice with CH_2Cl_2, and the combined extracts were washed with saturated NaCl solution and dried (Na_2SO_4). Solvent removal gave about 40 g (quantitative recovery) of orange oil **11a**, which could be used for the subsequent reaction without purification: ^{1}H NMR ($CDCl_3$) δ 0.85, 0.95 (2 d, 12 H, Me), 3.36 (dd, 2 H, pyr-CH_2), 8.42 (m, 1 H, CH=N).

In a similar manner **11b** was produced in near-quantitative recovery and used for the subsequent reaction without purification: ^{1}H NMR ($CDCl_3$) δ 0.90, 1.00 (d, 12 H, Me), 3.56 (d, 1 H, J = 14 Hz, pyr-CH_2), 3.88 (d, 1 H, J = 14 Hz, pyr-CH), 8.42 (m, 1 H, CH=N).

The procedure described for **11a** also gave a near-quantitative recovery of **11c** as an oil which was used for the subsequent reaction without purification: ^{1}H NMR ($CDCl_3$) δ 0.85, 0.97 (2 d, 12 H, Me), 3.37 (m, 2 H, pyr-CH_2), 8.42 (m, 1 H, CH=N).

General Procedure for the Hydration of Nitriles 11 to Carboxamides 12. To a solution of 23.0 g (0.0685 mol) of nitrile **11a** dissolved in 275 mL of *t*-BuOH was added 46 g (0.82 mol) of powdered KOH. The reaction mixture was heated at reflux overnight, cooled, and poured into water. The aqueous solution was extracted thrice with ether, and the combined extracts were washed with saturated NaCl solution and dried ($MgSO_4$). Solvent removal in vacuo gave an off-white solid, which upon trituration with Skellysolve B afforded 17.1 g (70.6%) of **12a** as a white solid: mp 123–125 °C; ^{1}H NMR ($CDCl_3$) δ 0.99, 1.07 (d, 6 H, Me), 3.42 (s, 2 H, pyr-CH_2), 8.38 (m, 1 H, CH=N). Anal. ($C_{22}H_{31}N_3O$) C, H, N.

In a similar manner **11b** afforded **12b** (47%): mp 119–121 °C; ^{1}H NMR ($CDCl_3$) δ 0.98, 1.08 (2 d, 12 H, Me), 3.60 (m, 2 H, pyr-CH_2), 8.33 (m, 1 H, CH=N). Anal. ($C_{22}H_{30}ClN_3O$) C, H, N.

Substituting **11c** in the procedure described for **11a** gave **12c** (48%): mp 140–142 °C; ^{1}H NMR ($CDCl_3$) δ 0.92, 1.03 (2 d, 12 H, Me), 3.50 (br s, 2 H, pyr-CH_2), 8.43 (m, 1 H, CH=N). Anal. ($C_{28}H_{35}N_3O$) C, H, N.

General Procedure for the Preparation of Indolizidinones 13 from Carboxamides 12. A solution of 4.0 g (9.3 mmol) of picolyl amide **12c** in 250 mL of EtOH containing 1 mL of concentrated HCl solution was exposed to 50 psi of H_2 gas at room temperature for 24 h over 0.4 g of PtO_2 catalyst. The catalyst was removed by filtration and the filtrate concentrated in vacuo to give a solid residue. The residue was partitioned between sodium hydroxide solution and ether, and the layers were separated. The basic solution was extracted with two additional portions of ether, and the combined extracts were washed with saturated NaCl solution and dried (Na_2SO_4). Solvent removal in vacuo gave 3.4 g of a white solid. Recrystallization from EtOH/H_2O gave 2.5 g (64%) of **13c**: mp 86–89 °C; ^{1}H NMR ($CDCl_3$) δ 0.88, 0.98 (2 d, 12 H, Me), 4.00–4.40 (br d, 1 H, CH), 7.20–7.80 ((H, Ar). Anal. ($C_{28}H_{38}N_2O$) C, H, N.

In an analogous manner **12b** was hydrogenated to provide a mixture of **13b**, **13b′**, and uncyclized material. Refluxing this mixture of CH_3CN (20 mL/g) for 8 h converted the uncyclized precursors to the indolizidinones **13** (70%). Chromatography of the crude mixture of diastereomers over silica gel with cyclohexane/*i*-PrOH/NH_4OH (70:28:2) as the eluent afforded the faster moving, less polar diastereomer **13b** (12%) as a waxy solid: mp 65–67 °C; ^{1}H NMR ($CDCl_3$) δ 0.94, 1.02 (2 d, 12 H, Me), 4.05–4.40 (br d, 1 H, CH), 6.95–7.45 (4 H, Ar). Anal. ($C_{22}H_{33}ClN_2O$) C, H, N.

The more polar diastereomer **13b′** was also obtained (22%) as a waxy solid: mp 57–58 °C; ^{1}H NMR ($CDCl_3$) δ 0.94, 1.02 (d, 12 H, Me), 4.0–4.40 (br d, 1 H, CH), 6.90–7.45 (3 H, Ar), 7.60–7.90 (1 H, Ar). Anal. ($C_{22}H_{33}ClN_2O$) C, H, N.

In a similar manner **13a** (55%) was produced from **12a** as a clear oil after chromatography of the crude product over silica with $CHCl_3$/EtOH/NH_4OH (92:7:1) as the eluent on a Waters Prep 500 chromatograph: ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 12 H, Me), 4.18 (m, 1 H, CH), 7.2–7.6 (4 H, Ar). Anal. ($C_{22}H_{34}N_2O$) C, H, N.

(±)-α-[2-[Bis(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-2-pyridinebutanenitrile (14). Sodium hydride (0.777 g of a 50% suspension in oil, 16.2 mmol) was washed with hexane to remove the oil and suspended in 10 mL of dry THF in a flame-dried flask. Nitrile **3** (3.00 g, 10.8 mmol) was dissolved in 7 mL of DMF and added rapidly by syringe, leading to H_2 evolution. After the reaction mixture had been stirred 1 h at room temperature, 2-vinylpyridine (1.2 mL, 11.1 mmol) was added by syringe. After stirring of the reaction mixture at room temperature for 1.5 h, it was poured cautiously into 100 mL of water. Extraction three times with ether, washing with water, and drying gave 3.64 g of residue after solvent removal. Purification by flash chromatography (95:4.7:0.3 $CHCl_3$/EtOH/NH_4OH, silica gel) afforded nitrile **14** as a clear oil (2.80 g, 68%), which was hydrated to the carboxamide without further purification: ^{1}H NMR ($CDCl_3$) δ 7.5 (m, 1 H, pyr), 8.0–6.95 (m, 7 H, aryl), 3.1–2.05 (cm, 10 H), 1.00 (d, 12 H, Me); IR ($CHCl_3$) 2940, 2220, 1590, 1520, 1470, 1430 cm^{-1}.

A subsequent reaction conducted on a 13-g scale in DMF instead of THF led to the quantitative formation of product in 98% crude yield, which was pure by NMR and suitable for further reaction without chromatography.

(±)-α-[2-[Bis(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-2-pyridinebutanamide (15). Nitrile **14** (236 mg, 0.615 mmol) was dissolved in 2 mL of concentrated H_2SO_4 and heated on a steam bath for 45 min. The mixture was diluted with ice water, made alkaline with 6 N KOH, and extracted three times with ether. The organic layers were washed with water and

saturated NaCl, dried, and concentrated in vacuo to give 0.25 g of a foamy gum. Crystallization from EtOAc/heptane afforded 124 mg (50%) of carboxamide **15** as a white powder: mp 113.5–115.5 °C; IR ($CHCl_3$) 3500, 3400, 2950, 1680 cm^{-1}. Anal. ($C_{23}H_{32}ClN_3O$) C, H, N.

(±)-α-[2-[Bis(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-2-piperidinebutanamide (16). Pyridyl carboxamide **15** (2.25 g, 5.62 mmol) was dissolved in 30 mL of acetic acid and reduced over 0.225 g of PtO_2 catalyst at a hydrogen pressure of 50 psi. After 45 min the theoretical amount of H_2 had been taken up, and the catalyst was filtered off and the solvent removed in vacuo. The residue was dissolved in water and the solution made alkaline with 6 N KOH. Extraction three times with ether, washing with water and saturated NaCl, drying, and solvent removal afforded the piperidyl carboxamide **16** (1.96 g, 85%) as a crunchy foam: ^1H NMR ($CDCl_3$) δ 7.3 (cm, 4 H), 5.60 (br s, 2 H), 3.2–1.0 (comp alkyl), 1.00 (d, 12 H, Me). Anal. ($C_{23}H_{38}N_3ClO$) C, H, N.

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Synthesis and Antifolate Activity of 5-Methyl-5,10-dideaza Analogues of Aminopterin and Folic Acid and an Alternative Synthesis of 5,10-Dideazatetrahydrofolic Acid, a Potent Inhibitor of Glycinamide Ribonucleotide Formyltransferase

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The title compounds were prepared in extensions of a general synthetic approach used earlier to prepare 5-alkyl-5-deaza analogues of classical antifolates. Wittig condensation of 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**2a**) and its 5-methyl analogue **2b** with [4-(methoxycarbonyl)benzylidene]triphenylphosphorane gave 9,10-ethenyl precursors **3a** and **3b**. Hydrogenation (DMF, ambient, 5% Pd/C) of the 9,10-ethenyl group of **3b** followed by ester hydrolysis led to 4-[2-(2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoic acid (**5**), which was converted to 5-methyl-5,10-dideazaaminopterin (**6**) via coupling with dimethyl L-glutamate (mixed-anhydride method using *i*-BuOCOCl) followed by ester hydrolysis. Standard hydrolytic deamination of **6** gave 5-methyl-5,10-dideazafolic acid (**7**). Intermediates **3a** and **3b** were converted through concomitant deamination and ester hydrolysis to **8a** and **8b**. Peptide coupling of **8a**,**b** (using $(EtO)_2POCN$) with diesters of L-glutamic acid gave intermediate esters **9a** and **9b**. Hydrogenation of both the 9,10 double bond and the pyrido ring of **9a** and **9b** (MeOH–0.1 N HCl, 3.5 atm, Pt) was followed by ester hydrolysis to give 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (**11a**) and the 5-methyl analogue **11b**. Biological evaluation of **6**, **7**, **11a**, and **11b** for inhibition of dihydrofolate reductase (DHFR) isolated from L1210 cells and for growth inhibition and transport characteristics toward L1210 cells revealed **6** to be less potent than methotrexate in the inhibition of DHFR and cell growth. Compounds **6**, **11a**, and **11b** were transported into cells more efficiently than methotrexate. Growth inhibition IC_{50} values for **11a** and **11b** were 57 and 490 nM, respectively; the value for **11a** is in good agreement with that previously reported (20–50 nM). In tests against other folate-utilizing enzymes, **11a** and **11b** were found to be inhibitors of glycinamide ribonucleotide formyltransferase (GAR formyltransferase) from one bacterial (*Lactobacillus casei*) and two mammalian (Manca and L1210) sources with **11a** being decidely more inhibitory than **11b**. Neither **11a** nor **11b** inhibited aminoimidazolecarboxamide ribonucleotide formyltransferase. These results support reported evidence that **11a** owes its observed antitumor activity to interference with the purine de novo pathway with the site of action being GAR formyltransferase.

In earlier reports we reviewed evidence that modifications of the classical antifolate structure at positions 5 and 10 might lead to new antifolates with enhanced selectivity of antitumor action.[1-3] In this connection, we recently described a versatile synthetic approach to 5-deaza analogues of aminopterin (AMT) and methotrexate (MTX).[3,4] The general route allows the introduction of 5-substituents

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