Experimental Section

NMR spectra were obtained using a Bruker HX-60 spectrometer. All peak positions are in δ (parts per million) from internal tetramethylsilane reference. Pyridine was dried over calcium hydride before use. All evaporations were performed under vacuum below 40°.

Cyclopropanemethanol, obtained commercially (Aldrich Chemical Co.), was fractionated using a Vigreux column. The fraction boiling at 125° was used for syntheses below. Microanalysis was by Galbraith Laboratories, Knoxville, Tenn.

Dibenzyl Cyclopropylmethyl Phosphate (2). Dibenzyl phosphonate was prepared according to the literature[20] and was used undistilled (but free of benzyl chloride) to prepare bis(benzyl) phosphorochloridate by the *N*-chlorosuccinimide method.[19] A solution of 20.1 g of bis(benzyl) phosphorochloridate (68.6 mmol) in 75 ml of ether was added dropwise over a 15-min period to 5.44 g (70 mmol) of cyclopropanemethanol and 5.93 (75 mmol) of pyridine in 100 ml of ether at 0°. Pyridine hydrochloride precipitated from the solution immediately. The mixture was stirred at room temperature for 18 hr. Filtration of the pyridine hydrochloride and evaporation of the ether left 19.9 g of a clear oil (88%): NMR (CCl_4) δ 0.33 (m, 4 H, CH_2CH_2), 1.03 (m, 1 H, CH), 3.73 (dd, 2 H, CH_2), 4.97 (d, 4 H, $ArCH_2$), 7.25 (s, 10 H, ArH). Attempted distillation of **2** at reduced pressure resulted in decomposition.

Cyclopropylmethyl Dihydrogen Phosphate (1). A solution of 1.20 g (3.6 mmol) of **2** was dissolved in 30 ml of dry absolute ethanol and 0.5 g of 10% palladium on charcoal was added. The mixture was hydrogenated for 30 min at 20–30 psi using a Parr hydrogenator. The catalyst was filtered and the solvent evaporated, affording 0.24 g of an oil (91%): NMR (Me_2SO-d_6) δ 0.46 (m, 4 H, CH_2CH_2), 1.17 (m, 1 H, CH), 3.77 (m, 2 H, CH_2), 11.1 (broad s, 2 H, OH).

Treatment of the solution obtained after hydrogenolysis and filtration followed by treatment with ammonia gave the ammonium salt, mp 160–164°.

Anal. Calcd for $C_4H_{12}NO_4P$: C, 28.41; H, 7.15; N, 8.28; P, 18.32. Found: C, 28.57; H, 7.33; N, 8.42; P, 18.40.

Uridine 5′-Phosphate.[21] Cyclopropylmethyl dihydrogen phosphate (from 1.61 g of **2**) was converted to the pyridinium salt by addition of 5 ml of pyridine to the filtered ethanol solution from above. The solution was evaporated and the residue dissolved in 10 ml of pyridine. After addition of 0.284 g (1.0 mmol) of 2′,3′-*O*-isopropylideneuridine, the solution was treated with 2.06 g (10 mmol) of DCC. The mixture was kept at room temperature for 2 days, followed by treatment with 2 ml of water. The mixture was allowed to stand for an additional 1 hr. The solvents were evaporated and the residue treated with 10 ml of water and evaporated to dryness. The residue was treated with 75 ml of water and the mixture was filtered. The filter cake was washed with 50 ml of water. The filtrate and washings were poured through an Amberlite 120 H^+ column. The column was washed with water until the effluent was neutral. The final volume of solution was adjusted to 500 ml and the pH was 2.6. The solution was refluxed for 3 hr. The cooled solution was then reduced to a volume of 50 ml and the pH was adjusted to 7.5–8.0 with saturated barium hydroxide solution. The barium phosphate was removed by centrifugation. The salt was washed well with water and the filtrate and washings (150 ml) were treated with 300 ml of ethanol to precipitate the barium salt of uridine 5′-phosphate. The solid was collected using a centrifuge, washed with water–ethanol, 1:2 (v/v), ethanol, and ether, and dried over P_2O_5 at 0.1 mm for 4 hr. The dry powder was calculated to be the hexahydrate of UMP using uv analysis at 262 nm of a sample dissolved in 0.01 *N* HCl. The product weighed 0.345 g (61%). Chromatographic analysis was performed as reported previously.[13]

Registry No.—**1**, 56599-14-5; 1 NH_3, 56599-15-6; **2**, 56599-16-7; UMP, 58-97-9; AMP, 61-19-8; GMP, 85-32-5; bis(benzyl) phosphorochloridate, 538-37-4; cyclopropanemethanol, 2516-33-8.

References and Notes

(1) E. Cherbuliez in "Organic Phosphorus Compounds", Vol. 6, G. M. Kosalapoff and L. Maier, Ed., Wiley-Interscience, New York, N.Y., 1973, Chapter 15.
(2) (a) M. Yoshikawa, T. Kato, and T. Takenishi, *Bull. Chem. Soc. Jpn.*, **42**, 3505 (1969); (b) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **33**, 2583 (1968).
(3) (a) Y. Fujimoto and Teranishi, *Methods Carbohydr. Chem.*, **6**, 451 (1972); (b) "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Wiley-Interscience, New York, N.Y., 1968, pp 501, 523.
(4) (a) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961); (b) P. T. Gilham and G. M. Tener, *Chem. Ind. (London)*, 542 (1959).
(5) C. A. Bunton, *Acc. Chem. Res.*, **3**, 275 (1970).
(6) J. R. Cox and O. B. Ramsay, *Chem. Rev.*, **64**, 317 (1964).
(7) R. F. Hudson and M. Green, *Angew. Chem., Int. Ed. Engl.*, **2**, 11 (1963).
(8) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest", Wiley, New York, N.Y., 1961, pp 26–33.
(9) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides", Academic Press, New York, N.Y., 1963, pp 110–118.
(10) See, however, T. Hata and K.-J. Chong, *Bull. Chem. Soc. Jpn.*, **45**, 654 (1972).
(11) R. W. Chambers, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957).
(12) E. Cherbuliez and J. Rabinowitz, *Helv. Chim. Acta*, **39**, 1461 (1956).
(13) A. M. Schoffstall and H. Tieckelmann, *Tetrahedron*, **22**, 399 (1966).
(14) C. G. Bergstrom and S. Siegel, *J. Am. Chem. Soc.*, **74**, 145, 254 (1952).
(15) E. Tommila and M. Lindholm, *Acta Chem. Scand.*, **5**, 647 (1951).
(16) J. D. Roberts and R. H. Mazur, *J. Am. Chem. Soc.*, **73**, 2509 (1951).
(17) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6204 (1958).
(18) W. Weiss and L. Gladstone, *J. Am. Chem. Soc.*, **81**, 4118 (1959).
(19) G. W. Kenner, A. R. Todd, and F. J. Weymouth, *J. Chem. Soc.*, 3675 (1952).
(20) F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 382 (1945).
(21) AMP and GMP were prepared similarly except that hydrolysis times were different (see Table I).

Use of Hydrazides of Heterocyclic Carboxylic Acids for the Resolution of Z-DL-Alanine during Papain Catalysis

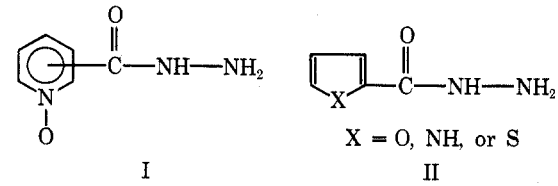
John Leo Abernethy,* David Srulevitch, and Marvin J. Ordway, Jr.

Department of Chemistry, California State Polytechnic University, Pomona, California 91768

Received June 10, 1975

Numerous arenecarboxylic hydrazides have been demonstrated to be effective amino bases for papain-catalyzed reactions with N-blocked amino acids.[1] We have now focused attention on the behavior of a few hydrazides which incorporate a heterocyclic nucleus and a single hydrazide function toward Z-amino acids under papain catalysis.[2] A substantial number of such hydrazides have been prepared in conjunction with a systematic investigation of their antituberculin activity.[3]

The first hydrazides used in the current study contained a pyridine nucleus. These were picolinic hydrazide, nicotinic hydrazide, and isonicotinic hydrazide. When subjected to proper conditions for papain catalysis of reactions with *N*-acylamino acids, all three failed to respond. With the conjecture that the difficulty might be attributed to the basic nature of the heterocyclic nitrogen, this nitrogen was blocked with oxygen. Picolinic *N*-oxide, nicotinic *N*-oxide, and isonicotinic *N*-oxide hydrazides (I) were then examined. In addition, the study was extended to three compounds with representative five-membered heterocycles, namely, 2-furoic, 2-pyrrolecarboxylic, and 2-thiophenecarboxylic hydrazides (II).



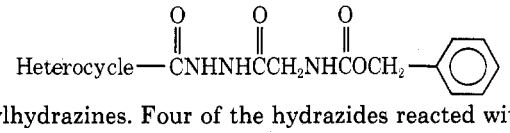
I II

When Z-glycine was the *N*-acylamino acid reactant, all six hydrazides yielded the unsymmetrical, achiral N^1,N^2-

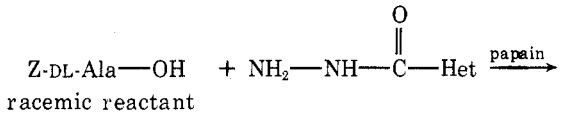
Table I
Properties of Unsymmetrical N^1,N^2-Diacylhydrazines Formed by Papain Catalysis

Name of product		pH optimum for reaction	% N Calcd	% N Found	$[\alpha]^{25}D$ in pyridine	Mp, °C	% L enantiomer in product
N^1-(2-Furoyl)-							
N^2-(Z-glycyl)hydrazine[a]	FGH[b]	3.75	13.24	13.46	Achiral	219–220	Achiral
N^2-(Z-L-alanyl)hydrazine	FLH		12.68	12.62	−50.9°	248–249	100
N^2-(Z-alanyl)hydrazine[c]	FAH		12.68[e]		−50.3°	248–252	99.4
N^1-(2-Thiophenecarboxylyl)-							
N^2-(Z-glycyl)hydrazine	TGH	4.25	12.61	12.88	Achiral	145–146	Achiral
N^2-(Z-L-alanyl)hydrazine	TLH		12.09	12.19	−55.4°	215–216	100
N^2-(Z-alanyl)hydrazine[c]	TAH		12.09[e]		−52.3°	219–220	97.2
N^1-(2-Pyrrolecarboxylyl)-							
N^2-(Z-glycyl)hydrazine	PyGH	4.00[d]	12.71	12.40	Achiral	194.5–195.5	Achiral
N^2-(Z-L-alanyl)hydrazine	PyLH		12.96	12.90	−51.1°	191–193	100
N^2-(Z-alanyl)hydrazine[c]	PyAH		12.96[e]		−49.1°	191–193	98.0
N^1-(Picolinyl N-oxide)-							
N^2-(Z-glycyl)hydrazine	PiGH	4.25	16.18	16.24	Achiral	199–200	Achiral
N^1-(Nicotinyl N-oxide)-							
N^2-(Z-glycyl)hydrazine	NGH	4.00	16.18	15.98	Achiral	216–217	Achiral
N^1-(Isonicotinyl N-oxide)-							
N^2-(Z-glycyl)hydrazine	IGH	4.25	16.18	16.18	Achiral	218–220	Achiral
N^2-(Z-L-alanyl)hydrazine	ILH		15.63	15.45	−51.0°	239–244	100
N^2-(Z-alanyl)hydrazine[c]	IAH		15.63	15.79	−30.8°	240–242	80.2

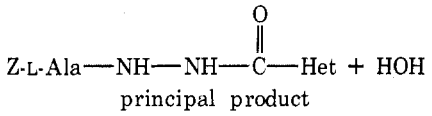
[a] Z is the accepted abbreviation for N-(benzyloxycarbonyl). [b] Abbreviations for products used in the Experimental Section. [c] Products from Z-DL-alanine contain some D enantiomer; hence Z-L-alanyl cannot be used. [d] Median pH of all other reactions with Z-glycine was used here. [e] Mixture melting point with product from Z-L-alanine showed no change or depression.



diacylhydrazines. Four of the hydrazides reacted with Z-L-alanine, and these same four hydrazides effectively resolved Z-DL-alanine. This represents another example of



racemic reactant



principal product

the power of papain to exert stereochemical preference during catalyzed reactions.[4] Results of these experiments are itemized in Table I. The extent of resolution, as calculated from optical rotations of products, varied from about 80 to 99%, also shown in Table I. Neither picolinic N-oxide hydrazide nor nicotinic N-oxide hydrazide underwent reactions with the Z-alanines.

The pH dependence of yield was determined for reactions between Z-glycine and five of the hydrazides; pH optima are given in Table I. The median optimum pH was 4.00. This was used satisfactorily for all reactions of the sixth hydrazide, 2-pyrrolecarboxylic hydrazide.

Experimental Section

Preparation of Active Papain. Dried papaya latex, imported from the African Congo region, was donated by the Wallerstein Co., Deerfield, Ill. Activation, isolation, and drying of the papain over P_2O_5 have been described previously.[5]

Preparation of the Three Methyl Pyridinecarboxylate N-Oxides. Higher yields of the N-oxides than previously reported were obtained by using 40% peracetic acid in acetic acid. After the reactions, excess acetic acid was removed in a rotatory evaporator under very low pressure, with the use of an oil pump.

Picolinic acid N-oxide[6,7] was obtained in 57% yield as brown crystals, mp 158–160°. Dry HCl was passed into an absolute methanolic solution of the acid. After evaporation, treatment with Na_2CO_3 solution, extraction into chloroform, drying, and evaporation, recrystallization from toluene gave a 75% yield of colorless crystals of methyl picolinate N-oxide,[8] mp 73–74°.

Methyl nicotinate and methyl isonicotinate were converted into their N-oxides[3,9] by means of 40% peracetic acid. Recrystallization from 95% ethanol gave an 88% yield of methyl nicotinate N-oxide, mp 95–97°, and a 75% yield of methyl isonicotinate N-oxide, mp 118–120°.

Preparation of the Three Pyridinecarboxylic N-Oxide Hydrazides. More concentrated hydrazine than used in some of the earlier research was available for conversion of the esters to the hydrazides. Direct addition of methyl picolinate N-oxide to 95% hydrazine produced picolinic N-oxide hydrazide, mp 147–149°, in 82% yield (lit.[10] 148–148.5°).

Methyl nicotinate N-oxide was heated with 95% hydrazine in methanol. Rotatory evaporation was followed by treatment with activated charcoal in 95% ethanol. Subsequent work-up produced a 95% yield of the hydrazide as yellow needles, mp 225–227°. The literature[10] reported a substantially lower yield, with mp 222°. Methyl isonicotinate N-oxide and 95% hydrazine in absolute ethanol were refluxed for 2.5 hr on a steam bath and then cooled. Recrystallization from ethanol and carbon black yielded white needles of the hydrazide[3] in 75% yield, mp 228–229°. One literature value[10] was 227°.

2-Thiophenecarboxylic Hydrazide. Ethyl 2-thiophenecarboxylate was heated for 5 hr with 95% hydrazine in 95% ethanol. The hydrazide was treated with hot methanol and carbon black, and recrystallized in 75% yield, mp 134–136°. This melting point agrees with the early literature[9] but a more recent report[11] gives mp 138°.

2-Pyrrolecarboxylic Hydrazide. 2-Pyrrolecarboxylic acid was converted into the acid chloride with PCl_5, followed by treatment with methanol.[12] When the isolated ester was treated with 95% hydrazine in the refrigerator overnight, it yielded the crude hydrazide, mp 210–214°. The literature indicates mp 231–232°. It was used successfully without further purification for all papain-catalyzed reactions.

pH Dependence of Yield for Reactions between Z-Glycine and Hydrazides of Heterocyclic Carboxylic Acids. Furoic hydrazide was commercially available. The general procedure for determining the pH dependence of yield has been described previously.[4] Buffered solutions, 0.50 M buffer, were used at intervals of 0.25 pH units from pH 3.0 to 6.0. Solutions for a given hydrazide contained an equal molal quantity of Z-glycine, plus equal weights of L-cysteine·HCl·H_2O and activated papain from a common stock

solution. Adjustment of quantities was necessary for a specific hydrazide. Time of incubation at 40° was generally 24 hr, with the exception of 2-thiophenecarboxylic hydrazide, for which the time was reduced to 1.5 hr. Removal of products by suction filtration was followed by washing with water, drying, and weighing. For 2-pyrrolecarboxylic hydrazide, only the median optimum pH for the other five reactions, pH 4.00, was used to obtain the product from Z-glycine. pH optima are summarized in Table I. For nitrogen analyses and melting point determinations, products were dissolved in hot methanol, treated with decolorizing carbon, and suction filtered four or five times with thorough washing of any solid on the filter paper with fresh methanol into the filtrate each time to remove soluble solid. The final filtration involved a glass funnel with a fritted disk. The solvent was then removed by evaporation under the hood, with subsequent drying of solid.

Reactions between Hydrazides of Heterocyclic Carboxylic Acids and Z-L-Alanine and Z-DL-Alanine. Buffer, 0.50 *M*, at the pH optimum for the reaction between Z-glycine and a given hydrazide was used for these reactions. For 2-pyrrolecarboxylic hydrazide, pH 4.00 was again used. Quantities of solutes are all relative to a total of 100 ml of resultant buffered solution. For reactions or attempted reactions involving Z-DL-alanine and a hydrazide, 5 ml of hexamethylphosphortriamide was added as a solubilizing agent, with the exception of isonicotinic *N*-oxide hydrazide and 2-furoic hydrazide. In recording results, first an abbreviation of a reaction product from Table I is given. Second, the weight of L-cysteine·$HCl·H_2O$ and therefore active papain is recorded. Third, the moles of hydrazide are immediately followed by the moles of *N*-acylamino acid. Fourth, the periods of incubation at 40° are given. Fifth, the weights of products obtained for each incubation period are indicated. Sixth, weights of recrystallized products from combined incubation periods that were dissolved in sufficient Eastman Spectrograde pyridine to produce 5.00 ml of solution at 25° precede the observed optical rotation at 25° in a Rudolph Model 80 high precision polarimeter, in a 2-dm polarimeter tube.

Recrystallized products, by means of essentially the same method as for products from Z-glycine, were used for nitrogen analyses, melting points, and mixture melting points, as well as optical rotations. Details are given in Table I.

FLH: 0.500 g; 0.0100 mol, 0.0100 mol; 0–24, 24–48 hr; 0.33 g, 0.075 g; 0.0901 g for α_{obsd} −1.834°.

FAH: 0.417 g; 0.0133 mol, 0.0133 mol; 0–24, 24–48 hr; 1.05 g, 0.100 g; 0.1090 g for α_{obsd} −2.194°.

TLH: 0.400 g; 0.0100 mol, 0.0100 mol; 0–24 hr; 1.31 g; 0.1691 g for α_{obsd} −3.750°.

TAH: 0.400 g; 0.0100 mol, 0.0200 mol; 0–24 hr; 1.29 g; 0.1000 g for α_{obsd} −2.092°.

PyLH: 0.500 g; 0.0100 mol, 0.0200 mol; 0–24 hr; 0.26 g; 0.1000 g for α_{obsd} −2.045°.

PyAH: 0.500 g; 0.0100 mol, 0.0200 mol; 0–24 hr; 0.25 g; 0.1000 g for α_{obsd} −1.962°.

ILH: 0.600 g; 0.0100 mol, 0.0100 mol; 0–72 hr; 0.35 g; 0.1103 g for α_{obsd} −2.250°.

IAH: 0.461 g; 0.0123 mol, 0.0123 mol; 0–72 hr; 0.31 g; 0.1126 g for α_{obsd} −1.386°.

Acknowledgments. The Squibb Institute for Medical Research supplied a sample of isonicotinic *N*-oxide hydrazide for preliminary experimentation. The Wallerstein Co., Deerfield, Ill., donated the dried papaya latex. This research was supported by grants from the Society of the Sigma Xi and a Federick Gardner Cottrell grant from the Research Corporation. Mr. C. F. Geiger, Ontario, Calif., ran the nitrogen analyses.

Registry No.—Papain, 9001-73-4; picolinic acid *N*-oxide, 824-40-8; picolinic acid, 98-98-6; peracetic acid, 79-21-0; methyl picolinate *N*-oxide, 38195-81-2; methyl nicotinate, 93-60-7; methyl isonicotinate, 2459-09-8; methyl nicotinate *N*-oxide, 15905-18-7; methyl isonicotinate *N*-oxide, 3783-38-8; hydrazine, 302-01-2; picolinic *N*-oxide hydrazide, 54633-17-9; nicotinic *N*-oxide hydrazide, 23597-85-5; isonicotinic *N*-oxide hydrazide, 6975-73-1; 2-thiophenecarboxylic hydrazide, 2361-27-5; ethyl 2-thiophenecarboxylate, 2810-04-0; 2-pyrrolecarboxylic hydrazide, 50269-95-9; 2-pyrrolecarboxylic acid, 634-97-9; Z-glycine, 1138-80-3; furoic hydrazide, 3326-71-4; Z-L-alanine, 1142-20-7; Z-DL-alanine, 4132-86-9; FGH, 56587-73-6; FLH, 56587-74-7; TGH, 56587-75-8; TLH, 56587-76-9; PyGH, 56587-77-0; PyLH, 56587-78-1; PiGH, 56587-79-2; NGH, 56587-80-5; IGH, 56587-81-6; ILH, 56587-82-7.

References and Notes

(1) J. L. Abernethy, M. Klentz, R. Johnson and R. Johnson, *J. Am. Chem. Soc.*, **81**, 3944 (1959).
(2) Z is the currently accepted abbreviation for *N*-(benzyloxycarbonyl).
(3) (a) H. L. Yale, K. Losee, J. Martins, M. Holsing, F. M. Perry and M. Bernstein, *J. Am. Chem. Soc.*, **75**, 1933 (1953); (b) N. P. Buu-Hoi, N. D. Zuang, N. H. Nam, F. Binon and R. Boyer, *J. Chem. Soc.*, 1358 (1953); (c) R. I. Meltzer, A. D. Lewis, F. H. McMillan, J. D. Genzer, F. Leonard, and J. A. King, *J. Am. Pharm. Assoc.*, **42**, 594 (1953).
(4) J. L. Abernethy, R. Boebeck, A. Ledesma, and R. Kemp, *J. Org. Chem.*, **38**, 1286 (1973).
(5) (a) W. Grassmann, *Biochem. Z.*, **279**, 131 (1935); (b) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **72**, 1798 (1950).
(6) F. Diels and H. Alder, *Justus Liebigs Ann. Chem.*, **16**, 493 (1932).
(7) E. Profft and W. Steinke, *J. Prakt. Chem.*, **13**, 58 (1961).
(8) G. T. Newbold and F. S. Spring, *J. Chem. Soc., C*, 133 (1949).
(9) T. Curtius and J. Thyssen, *J. Prakt. Chem.*, **65**, 7 (1902).
(10) M. Shimazu, T. Naito, G. Ohta, T. Yoshihawa, and R. Dohmori, *J. Pharm. Soc. Jpn.*, **72**, 1474 (1952).
(11) D. Liberman, N. Rist, F. Grumbach, M. Moyeux, B. Gauthier, A. Rouaix, J. Maillard, J. Himbert, and S. Cals, *Bull. Soc. Chim. Fr.*, 1430 (1954).
(12) E. Fischer and D. D. van Slyke, *Ber.*, **44**, 3166 (1911).

The Circular Dichroism Spectra of Folic Acid and 10-Thiafolic Acid and the Problem of Racemization in the Synthesis of Analogs of Folic Acid through the Cyclization of Substituted 2-Amino-3-cyanopyrazines[1]

Henry G. Mautner* and Young-Ho Kim

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111

Received May 30, 1975

In view of the central role of derivatives of folic acid in cellular metabolism[2] and the usefulness of analogs of folic acid in the treatment of neoplastic disease,[3,4] a great deal of effort has been expended on searching for improved syntheses of molecules related to folic acid.

A relatively simple synthesis of 6-substituted pteridines was introduced by Taylor and his coworkers.[5-7] The reaction of aminomalononitrile with α-ketoaldoximes yields 2-amino-3-cyano-5-substituted pyrazine 1-oxides, deoxygenation and guanidine cyclization of which yields pteridines. This procedure, like some older but more cumbersome syntheses,[8,9] has the advantage of avoiding ambiguity in the positioning of the side chain and, in addition, delays the problems introduced by the extreme insolubility of pteridines until the final stages of the synthetic sequence. Recently, this synthesis was applied to the preparation of analogs of the antineoplastic agent methotrexate by Chaykovsky and his coworkers,[10] while our laboratory explored the usefulness of this approach in synthesizing 10-thiafolic acid, 10-thiapteroic acid, and related compounds.[11]

To prepare the latter group of compounds two synthetic routes were followed (Figure 1). In the first approach, reaction of 2-amino-3-cyano-5-chloromethylpyrazine[6] with ethyl 4-thiobenzoate, followed by cyclization with guanidine, yielded the ethyl ester of the 4-amino derivative of 10-thiapteroic acid. Mild hydrolysis led to the formation of 10-thiapteroic acid from which 10-thiafolic acid could be prepared using condensation with diethyl L-glutamate via the mixed anhydride method.

Alternatively, the complete side chain could be formed before addition to the pyrazine ring. In this approach, diethyl 4-thio-*N*-benzoyl-L-glutamate[11] was permitted to react with 2-amino-3-cyano-5-chloromethylpyrazine. Cyclization with guanidine, followed by mild hydrolysis, led to the formation of 4-amino-4-deoxy-10-thia-10-deazafolic acid or 10-thiaaminopterin.