the Krebs cycle (Figure 3B). The stimulation of Q_{O_2} (G) is thus not due to a reversal of the Crabtree effect, but is presumably associated with the large stimulation of the HMP pathway. Previously we have observed that 2,2′-dithiodipyridine also stimulates the HMP pathway,[8] although to a lesser extent than VIII. With both disulfides, the initial stimulation is followed by an inhibition.

Disulfides IX and X, at the low concentrations permitted by their limited solubility (about 10^{-5} M), inhibit Q_{O_2} (only in the absence of added glucose), and have no effect on glycolysis.

Compound XII is a strong inhibitor of Q_{O_2} and of $Q_{CO_2}^{N_2}$, while Q_{O_2} (G) and $Q_{CO_2}^{O_2}$ are relatively unaffected.

In general, the results obtained with disulfides confirm our previous observations of metabolic effects with structurally related analogs.[3]

Among the thiones studied in this report, 5-cyano-(2-thiopyridone) (IV), which appears to prevent utilization of exogenous glucose *via* the Krebs cycle and the HMP pathway, is unique. This effect is probably not due to any metabolic conversion to its corresponding disulfide X, or to hydrolysis of CN to COOH, in either the thione or the disulfide, since these have been shown not to cause such effects.[3] Thione V, which reverses the Crabtree effect, presumably acts as the thione rather than the disulfide, because the solubility of the disulfide XI is too low to elicit metabolic effects.

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Cofactor Inhibitors of Thymidylate Synthetase. Piperidine and Tetrahydroquinoline Analogs of Tetrahydrofolic Acid[1,2]

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The inhibition of the enzymes thymidylate synthetase and dihydrofolate reductase was examined with a series of pyridine and quinoline compounds and their reduced derivatives. Condensation of ethyl *p*-aminobenzoate or ethyl *p*-aminobenzoylglutamate with pyridine-2-carboxaldehyde followed by reduction of the Schiff base gave the corresponding secondary amines, **3a** and **3b**. Catalytic reduction of **3a** and **3b** yielded ethyl *p*-*N*-(2-piperidylmethyl)aminobenzoate **4a** and the glutamate **4b**. Condensation of **4a** or **4b** with 5-formyluracil gave 2-*p*-carbethoxyphenyl-3-(5-uracil)octahydroimidazo[1,5-*a*]pyridine **5a** and the corresponding glutamate analog **5b**. The synthesis of analogous series (**11–13**) starting from quinoline-2-carboxyaldehyde utilized the same procedure. Similarly, starting with pyridine-3-carboxyaldehyde, ethyl *p*-*N*-(3-pyridylmethyl)aminobenzoate (**7a**), the glutamate **7b**, and the 3-piperidyl analog **8** were prepared. Enzyme inhibition studies revealed the saponification product of **7b** to have highest activity against the synthetase [(**7b** salt)/(DL-THFA) = 0.12 for 50% inhibition] while **12** was most inhibitory against the reductase [(**12**)/(DHFA) = 8 for 50% inhibition].

Thymidylate synthetase catalyzes the reductive methylation of 2′-deoxyuridine 5′-phosphate using N_5,N_{10}-methylenetetrahydrofolic acid as the cofactor in the 1-C transfer.[3] This observation has led to the investigation of analogs of this cofactor for inhibition of the enzyme.[2,4,5] These studies have examined the structural features essential for binding of folate analogs to dihydrofolate reductase and thymidylate synthetase.

Previous studies from these laboratories have shown that the pyrimidine ring moiety of the pteridine system is not essential for binding to thymidylate synthetase and in fact may contribute little to binding.[2a,c] The common features for inhibition were found in models containing the analogous N_5, N_8, and N_{10} of the cofactor.

The purpose of this study is the design and synthesis of models to examine the requirements for both the analogous N_5 and N_8 in the cofactor. Therefore 2- and 3-substituted pyridines, piperidines, quinolines, and tetrahydroquinolines were prepared containing the methylaminobenzoate or methylaminobenzoylglutamate groups. In addition, the 2-piperidyl (**4**) and 2-tetrahydroquinolyl (**12**) derivatives were condensed to give analogs (**5** and **13**) of the proposed intermediate in the enzymatic reaction.[6]

The synthesis of compounds in the 2- and 3-piperidine

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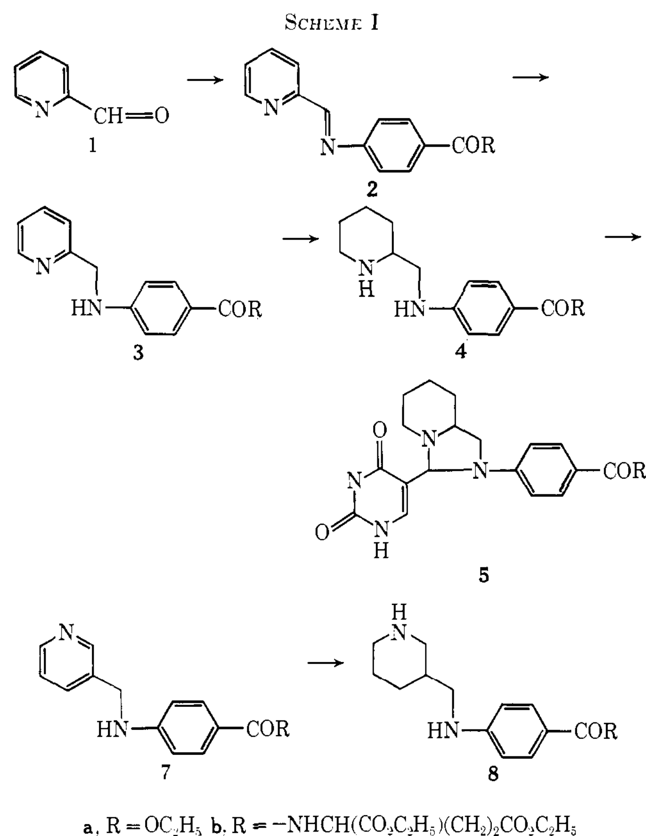
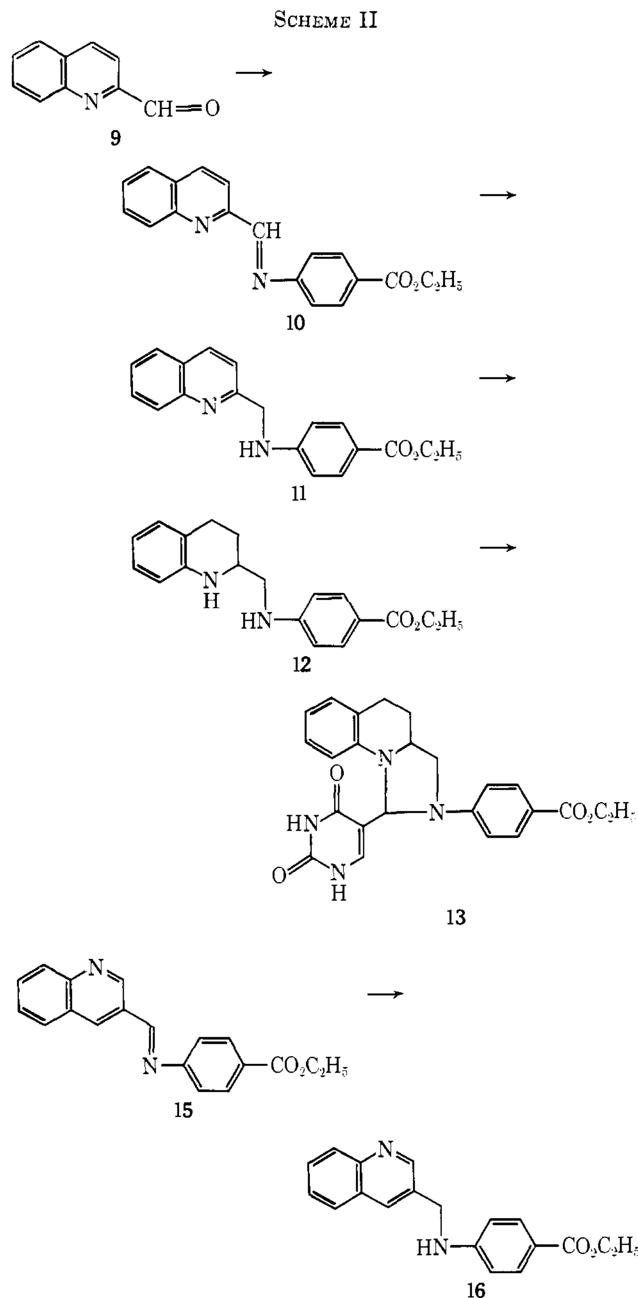
SCHEME I



SCHEME II



series is straightforward starting with the pyridine carboxaldehydes. Compound **1** (Scheme I and Table I) condensed smoothly to give **2a** in good yield. Reduction of **2a** with $NaBH_4$ according to the procedure reported by Billman and Diesing[7] gave the corresponding secondary amine **3a**. Catalytic reduction of **3a** yielded the piperidine derivative **4a**. Similarly, treatment of pyridine 2-carboxaldehyde (**1**) with diethyl *p*-aminobenzoylglutamate gave the corresponding Schiff base **2b**. However, $NaBH_4$ reduction of **2b** under the same conditions used for the reduction **2a** gave a noncrystalline gum. Compound **3b** was eventually obtained by catalytic reduction of **2b**, using 5% Pd–C as catalyst at room temperature and atmospheric pressure. Further catalytic reduction of **3b** gave the piperidine analog **4b**. Treatment of **4a** or **4b** with 5-formyluracil[8] gave the respective condensation products **5a** or 2-(*p*-ethyl benzoylglutamate)-3-(5-uracil)octahydroimidazo[1,5a]-pyridine (**5b**).

The synthesis in the 3-substituted piperidine series followed the same procedure from pyridine-3-carboxaldehyde. Condensation with the amine to the Schiff bases, ethyl *p*-*N*-(3-pyridylmethylene)aminobenzoate (**6a**), and the glutamate **6b** was followed by $NaBH_4$ reduction to the substituted 3-aminomethylpyridines **7a** and **7b**. Reduction of **7a** to ethyl *p*-*N*-(3-piperidylmethyl)aminobenzoate (**8a**) was accomplished in low yield; however, the glutamate **7b** failed to give **8b** because of hydrogenolysis. Condensation of **8a** with 5-formyluracil[8] to give the desired bicyclic system was not successful.

The methods used for the synthesis of the pyridine and piperidine derivatives, **3** and **4**, were successfully applied to the synthesis of the quinolines. The starting materials, quinoline-2-aldehyde[9] (**9**) and quinoline-3-aldehyde[10] (**14**), were obtained by SeO_2 oxidation of 2- and 3-methylquinoline which in turn were prepared by modified Skraup quinoline synthesis.[11] α-Methylacrolein diacetate used for the synthesis of 3-methylquinoline was synthesized according to the method used by Tamura.[12]

Condensation of the aldehyde **9** with ethyl *p*-aminobenzoate gave ethyl *p*-*N*-(2-quinolinylmethylene)aminobenzoate (**10**) in good yield (Scheme II and Table II). Reduction of **10** with $NaBH_4$ to the secondary amine **11** followed by catalytic reduction (PtO_2) of **11** in AcOH gave ethyl *p*-*N*-[2-(1,2,3,4-tetrahydroquinolinylmethyl)]aminobenzoate (**12**). Treatment of the tetrahydroquinoline **12** with 5-formyluracil gave 2-*p*-

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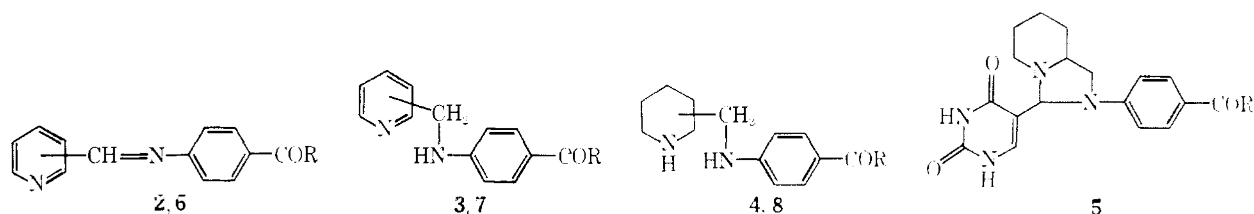
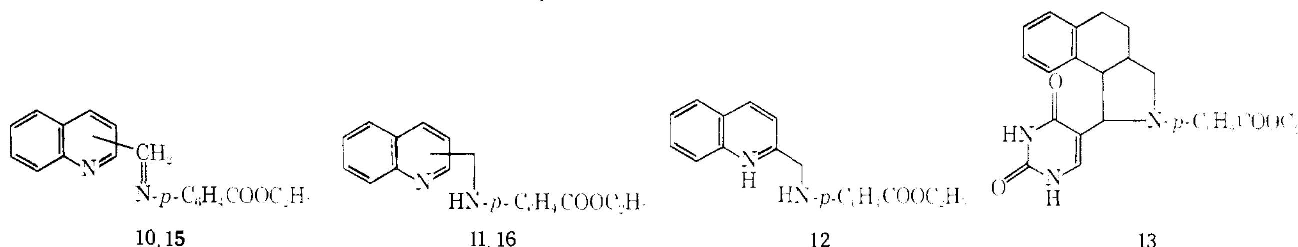
TABLE I
PHYSICAL CONSTANTS FOR PYRIDINE AND PIPERIDINE DERIVATIVES



No.	Isomer	R	Method	Recrystn solvent	Yield, %	Mp, °C	Formula[c]
2a	2	OC_2H_5	A	Hexane	87	67–68	$C_{15}H_{14}N_2O_2$
3a	2	OC_2H_5	B	EtOH–H_2O	90	69–70	$C_{15}H_{16}N_2O_2$
4a[a]	2	CO_2H_5	C	EtOAc	95	159–161	$C_{17}H_{26}N_2O_4$
5a		OC_2H_5	D	C_5H_5N–H_2O	45	247–248	$C_{20}H_{24}N_4O_4$
2b	2	*N*-Glutamate	A	EtOAc	75	124–125	$C_{22}H_{25}N_3O_5$
3b	2	*N*-Glutamate	E	Et_2O	75	111–112	$C_{22}H_{27}N_3O_5$
4b	2	*N*-Glutamate	C	Et_2O	30	84–85	$C_{22}H_{33}N_3O_5$
5b		*N*-Glutamate	D	MeOH–H_2O	24	135–140	$C_{27}H_{35}N_5O_7$
6a	3	OC_2H_5	A	Hexane	80	66–68	$C_{15}H_{14}N_2O_2$
7a	3	OC_2H_5	B	MeOH–H_2O	90	104–105	$C_{15}H_{16}N_2O_2$
8a[b]	3	OC_2H_5	C		70	180–182	$C_{22}H_{28}N_2O_4S$
6b	3	*N*-Glutamate	A	Hexane–EtOAc	62	90–91	$C_{22}H_{25}N_3O_3$
7b	3	*N*-Glutamate	B	Et_2O–$CHCl_3$	44	105–107	$C_{22}H_{27}N_3O_3$

[a] Characterized as the monoacetate. [b] Characterized as the monotosylate. [c] All compounds were analyzed for C, H, N.

TABLE II
PHYSICAL CONSTANTS FOR QUINOLINE AND TETRAHYDROQUINOLINE DERIVATIVES



Compound	Isomer	Method	Recrystn solvent	Yield, %	Mp, °C	Formula[a]
10	2	A	Hexane–EtOAc	73	84–85	$C_{19}H_{16}N_2O_2$
11	2	B	Hexane	85	78–80	$C_{19}H_{18}N_2O_2$
12	2	C	Hexane–Et_2O	50	94–96	$C_{19}H_{22}N_2O_2$
13		D	C_5H_5N–H_2O	71	275–277	$C_{24}H_{24}N_4O_4$
15	3	A	Hexane–EtOAc	81	101–102	$C_{19}H_{16}N_2O_2$
16	3	B	EtOH–H_2O	75	132–134	$C_{19}H_{18}N_2O_2$

[a] See footnote *c*, Table I.

carbethoxyphenyl 3-(5-uracil)hexahydroimidazo[1,5-*a*]-quinoline (**13**) in good yield.

The C-3 derivative, **16**, was obtained by the same route; quinoline-3-aldehyde (**14**) was treated with ethyl *p*-aminobenzoate in hot PhH to give the Schiff base **15** which was reduced by $NaBH_4$ to ethyl *p*-*N*-(3-quinolinylmethyl)aminobenzoate (**16**). Unfortunately, the catalytic hydrogenation of **16** under the conditions used previously gave only hydrogenolysis products instead of the desired tetrahydroquinoline. Similar results were observed when 10% Pd–C or 5% Rh–C was used as catalyst and HOAc used as the solvent.

Biological Results.—The compounds in this series were examined for inhibition of thymidylate synthetase and dihydrofolate reductase;[2a] both the ester and the saponification product were assayed. The results in Table III show the inhibitor:substrate (DL-tetrahydrofolic acid or dihydrofolic acid) ratio required for 50% inhibition of the rate of the enzymatic reaction.

The effect of a glutamate residue on binding is shown in comparing **3a**, **4a**, and **5a**, the 2-pyridine analogs having a *p*-carboxyl function, with **3b**, **4b**, and **5b** where the *p*-*N*-(carboxyglutamate) group is used. The glutamate does not enhance thymidylate synthetase binding significantly in the 2-pyridine analogs **3a** and **3b** and appears to be five–tenfold less active in the piperidine series **4** and **5**. A significant (200-fold) increase in activity in the 3-pyridine series is noted in the most active compound in the series, the glutamate salt **7b** [$(I)/(S)^{0.5} = 0.12$], compared with the carboxylate salt, **7a**.

The effect of reduction of the heterocyclic ring on enzymatic inhibition is difficult to evaluate; however, it appears that reduction of the 2-substituted pyridine, **3a** to the piperidine **4a** leads to tenfold increase in inhibition against thymidylate synthetase with little effect on dihydrofolate reductase. The opposite effect is noted in the quinoline **11** where the tetrahydroquinoline **12** is much less active against the synthetase and more active against the reductase.

Previous studies utilized pyrazine and quinoxaline

analogs and the reduced derivatives, piperazines and tetrahydroquinoxalines.[2a,c] The present series examines the differences in binding of analog models containing N corresponding to N_5 or N_8 of the cofactor. The N_5 analogs (2-substituted models **3**–**5** and **11**–**13**) were slightly more inhibitory against thymidylate synthetase than the N_8 analogs (3-substituted models, **7**, **8**, and **16**) with the exception of **7b**; inconsistent changes were noted against the reductase system. Overall comparison to the pyrazines and quinoxalines containing both the analogous N_5 and N_8 reveals the latter series to be more inhibitory against both enzymes[2c] than either the 2- or 3-substituted pyridine–quinoline series of this study. These results suggest that both the N_5 and N_8 are important binding sites to thymidylate synthetase and of the two, the N_5 may be more essential. Further studies on the nature of the inhibition are in progress.

This series of compounds was also examined for inhibition of growth of *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Filter paper disks were saturated with a 0.1 *M* solution of the compound, dried, and placed on innoculated agar plates. Compound **8** showed a slight inhibition of all cultures (~1 mm). Compound **4b** inhibited growth of *B. subtilis*, *S. aureus*, and *E. coli* (~1 mm). None of the other compounds were inhibitory.

TABLE III

ENZYME INHIBITION STUDIES[a]

Compound	$(I)/(S)_{0.5}$ thymidylate synthetase[b]		$(I)/(S)_{0.5}$ dihydrofolate reductase[c]	
	Ester[d]	Salt of the acid[e]	Ester[d]	Salt of the acid[e]
3a	26	>75	35	130
4a	2.8	28	72	182
5a	2.0	12.5	18	61
3b	8		120	49
4b	23		105	
5b	12	6		
7a	6	27	120	210
8a	5	15	31	290
7b	12	0.12	18	75
11		1.5	30	
12		50	8	
13		1.8	14	165
16	1.8	5	15	130

[a] For a description of the enzyme sources, isolation procedure, and assay, see ref 2a. [b] $(I)/(S)_{0.5}$ refers to the ratio of the molar concentrations of the inhibitor and substrate (cofactor) measured as DL-tetrahydrofolic acid, necessary for 50% inhibition. The assay solution was 2.8×10^{-4} *M* *dl*-L-tetrahydrofolic acid and 4.2×10^{-4} *M* deoxyuridine 5′-phosphate. The enzyme source was *E. coli B*. [c] $(I)/(S)_{0.5}$ refers to the ratio of the molar concentrations of the inhibitor and substrate, dihydrofolic acid, necessary for 50% inhibiion. The substrate was present in 3.3×10^{-4} *M* in the assay solution. The enzyme source was chicken livers. [d] The esters were assayed as DMSO solutions using an equivalent amount of DMSO as the control rate. [e] The esters were saponified by heating for several hours in 0.1 *M* KOH and assaying the salt of the acid as an aqueous solution.

Experimental Section[13]

Ethyl *p*-*N*-(2-Pyridylmethylene)aminobenzoate (2a). Method A.—Pyridine 2-carboxaldehyde (5 g, 0.046 mol) and ethyl *p*-aminobenzoate (7.70 g, 0.046 mol) were refluxed in 50 ml of C_6H_6 overnight, with a Dean–Stark H_2O trap to remove the H_2O formed. The solution was evaporated to dryness under reduced pressure and the residue was recrystallized from petroleum ether (bp 60–70°) to give **2a** as yellow needle crystals (10 g, 87%); mp 67–68°. *Anal.* ($C_{15}H_{14}N_2O_2$) C, H, N.

Ethyl *p*-*N*-(2-Pyridylmethyl)aminobenzoate (3a). Method B.—The Schiff base **2a** (1.0 g, 4 mmol) was dissolved in 30 ml of MeOH and cooled in an ice bath. $NaBH_4$ (0.3 g, 8 mmol) was added in small portions with stirring within 15–30 min. The solution was allowed to stir at room temperature for another hour and refluxed for 20 min. The solvent was evaporated to dryness and the residue was dissolved in $CHCl_3$ (60 ml) which was washed twice with 30 ml of H_2O. The $CHCl_3$ layer was separated, dried ($NaSO_4$), and evaporated to dryness to give **3a** as pale yellow crystals (0.9 g, 90%). Recrystallization from EtOH–H_2O mixed solvent gave white platelet crystals: mp 69–70°. *Anal.* ($C_{15}H_{16}N_2O_2$) C, H, N.

Ethyl *p*-*N*-(2-Piperidylmethyl)aminobenzoate (4a). Method C.—The amine **3a** (0.5 g, 2 mmol) was dissolved in 30 ml of HOAc and hydrogenated at room temperature and atmospheric pressure with 0.4 g of prereduced PtO_2 in 25 ml of HOAc. The reduction was stopped when 3 mol equiv of H_2 (*ca.* 135 ml) was absorbed. The catalyst was removed by filtration and the filtrate was lyophilized to give an oil which was dissolved in 20 ml of dry Et_2O. After standing at room temperature, white crystals of monoacetate (0.6 g, 95%) were formed. Recrystallization (EtOAc) gave **4a** as fine white crystals; mp 159–161°. *Anal.* ($C_{17}H_{26}N_2O_4$) C, H, N.

The free base which is an oil was obtained by dissolving the monoacetate in H_2O, neutralization with concentrated NaOH solution, and extraction with $CHCl_3$. The $CHCl_3$ extracts were combined, dried ($NaSO_4$), and evaporated to dryness under reduced pressure; nmr ($CDCl_3$) δ 1.32 (t, 3, J = 7.5 Hz, OCH_2CH_3), 1.85 (s, 1, R-NH), 1.10–2.0 (m, 6, aliphatic proton), 2.35–3.30 (m, 5, N-CH), 4.30 (q, 2, J = 7.5 Hz), 4.75 (t, 1, J = 7 Hz, Ar-NH), 6.53 (d, 2, J = 8 Hz, phenyl 3,5-H), 7.85 (d, 2, J = 8 Hz, phenyl 2,6-H).

2-*p*-Carbethoxyphenyl-3-(5-uracil)octahydroimidazo[1,5-*a*]-pyridine (5a). Method D.—The amine **4a** (0.75 g, 2.9 mmol) and 5-formyluracil (0.4 g, 2.9 mmol) were refluxed in 30 ml of MeOH overnight. The product precipitated as the reaction proceeded. The reaction mixture was cooled and the white precipitate was collected. Recrystallization from pyridine–H_2O mixed solvent gave **5a** as white crystals (0.5 g, 45%); mp 247–248° dec. *Anal.* ($C_{20}H_{24}N_4O_4$) C, H, N.

Ethyl *p*-*N*-(2-Pyridylmethyl)aminobenzoylglutamate (3b). Method E.—The Schiff base (**2b**) (0.5 g, 1.2 mmol) was dissolved in EtOH (50 ml), 5% Pd–C (0.1 g) was added, and the mixture was hydrogenated at room temperature and atmospheric pressure until 1 mol equiv of H_2 had been absorbed. The catalyst was removed by filtration and the filtrate evaporated to dryness under reduced pressure. The oily residue was dissolved in Et_2O (40 ml), filtered, and concentrated to 20 ml. After standing at room temperature, 0.35 g (75%) of **3b** as white crystals was obtained; mp 111–112°. *Anal.* ($C_{22}H_{27}N_3O_5$) C, H, N.

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(13) All melting points were taken on a calibrated Thomas-Hoover capillary melting point apparatus. Analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind., and on an F & M Model 185, University of Kansas. Spectral data were obtained using Beckman IR-8, IR-10, Varian A-60, and A-60A spectrometers. The latter used Me_4Si as an internal standard except in D_2O where 3-trimethylpropanesulfonic acid sodium salt was employed. The nmr and ir spectra were as expected. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements are within 0.4% of the theoretical values.