ethanol, and 100 ml of THF was stirred overnight at room temperature and filtered. The filtrate was concentrated to a brown gum which was dissolved in 40 ml of 1:1 EtOH–Et_2O. The solution was decolorized with Nuchar, concentrated to a small volume, diluted with EtOAc–heptane, and stored at 0°. The separated solid was collected and triturated with acetone giving 3.3 g (55%) of **15**.

In a later preparation the gummy reaction product was triturated with acetone to give comparable product in 73% yield.

Q. L-3,3′-Dithiobis(2-benzamidopropionamide) (22).—Benzoyl chloride (15.6 g, 0.11 mole) was added slowly at 5–10° to a mixture of 15.6 g (0.05 mole) of **19**, 16.4 g (0.2 mole) of anhydrous NaOAc, 150 ml of H_2O, and 10 ml of toluene. After the mixture was stirred for 3 days at room temperature, it was filtered to separate **22**; yield 17 g (76%).

R. L-3-(Benzylthio)-2-formamidopropionamide (10).—A solution of 20.5 g (0.098 mole) of **8** in 200 ml of 97–100% HCO_2H was treated dropwise at 5–10° with 70 ml (0.74 mole) of Ac_2O. After being warmed slowly to room temperature, the mixture was diluted with 1 l. of EtOAc, and filtered. The filtrate was concentrated at reduced pressure to a small volume and diluted with 600 ml of H_2O. The white solid which separated was collected, washed with H_2O, and dried; yield 11.5 g of crude material. Recrystallization from 50% EtOH gave 2 g (8%) of **10**.

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Synthesis and Reactions of Some Pyrimidylethyl Isocyanates

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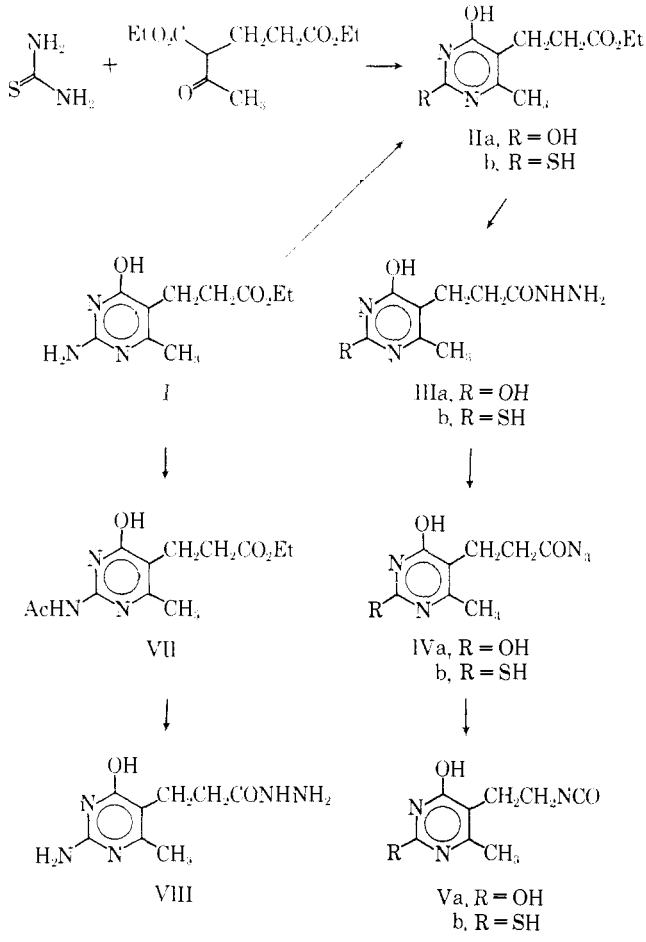
The synthesis of the *p*-aminobenzoyl-L-glutamic acid derivatives of pyrimidylethyl isocyanates was prompted by earlier work on nonclassical antimetabolites by Baker which demonstrated that drastic alterations in the tetrahydrofolic acid molecule brought forth related compounds with antimetabolite activity.[3]

The reactions outlined in Chart I illustrate the synthetic scheme followed for the acquisition of the intermediate isocyanates. Rearrangement of the azides was accompanied by a shift in infrared absorption from 2130–2140 (azide) to 2280 cm^{-1} (isocyanate).[4]

Acetylation of the 2-amino group of I to give ethyl 3-(2-acetamido-4-hydroxy-6-methyl-5-pyrimidyl)-propionate (VII) was undertaken to protect this active group in subsequent reactions. In spite of the fact that the acetyl group was lost on reaction of VII with hydrazine, giving the 2-amino hydrazide VIII, reaction of VIII with nitrous acid appeared to give 3-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl) propionyl azide which showed an infrared peak at 2160 cm^{-1}.

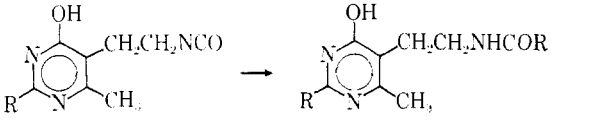
CHART I



However, rearrangement to the corresponding isocyanate was unsuccessful.

The presence of the isocyanate group in Va was demonstrated by its conversion to the isobutylcarbamate VIa. Also prepared were the *p*-aminobenzoic acid and *p*-aminobenzoyl-L-glutamic acid derivatives of Va and Vb, which are illustrated in Chart II.

CHART II



VIa, R = OH; R′ = $(CH_3)_2CHCH_2O$
b, R = OH; R′ = *p*-$HO_2CC_6H_4NH$
c, R = OH; R′ = $HO_2C(CH_2)_2CH(CO_2H)NHCOC_6H_4NH$
d, R = SH; R′ = *p*-$HO_2CC_6H_4NH$
e, R = SH; R′ = $HO_2C(CH_2)_2CH(CO_2H)NHCOC_6H_4NH$

Compounds VIb and VId were not inhibitory to the growth of *Streptococcus faecalis* (ATCC 8043) when tested at concentrations ranging from 10^{-3} to 10^{-8} *M*.[5] Compounds VIb, VIc, VId, and VIe also showed no inhibition when 10^{-3} *M* solutions were tested on the enzyme folic reductase.[6,7]

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(2) Taken from the thesis of K. Eskins, which was submitted as partial fulfillment of the requirements for the Ph.D. degree.

(3) (a) B. R. Baker and C. E. Morreal, *J. Pharm. Sci.*, **52**, 849 (1963); (b) B. R. Baker, C. E. Morreal, and B. T. Ho, *J. Med. Chem.*, **6**, 658 (1963); (c) B. R. Baker and H. S. Shapiro, *ibid.*, **6**, 664 (1963).

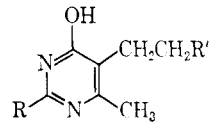
(4) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, p 263.

(5) S. F. Zakrzewski and C. A. Nichol, *J. Biol. Chem.*, **205**, 361 (1953).

(6) We are indebted to Dr. C. A. Nichol and Dr. W. C. Werkheiser, both of Roswell Park Memorial Institute, for the *Streptococcus faecalis* and folic reductase assays, respectively.

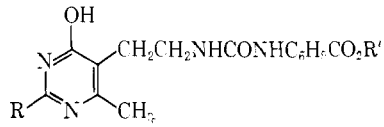
(7) W. C. Werkheiser, *J. Biol. Chem.*, **236**, 888 (1961).

TABLE I



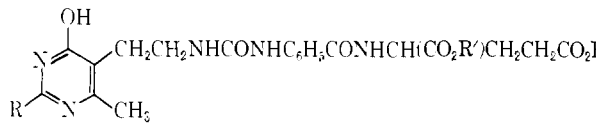
No.	R	R′	Yield, %	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
IIIa	OH	$CONHNH_2$	59	268–270	$C_8H_{12}N_4O_3$	45.27	5.67	26.40		45.16	5.95	26.30	
IIIb	SH	$CONHNH_2$	69	276–280	$C_8H_{12}N_4O_2S$	42.09	5.30	24.54	14.05	41.92	5.30	24.76	14.16
VIII	NH_2	$CONHNH_2$	73	>270	$C_8H_{13}N_5O_2$	45.49	6.20	33.16		45.50	6.12	33.32	
IVa	OH	CON_3	74	100–110 dec									
IVb	SH	CON_3	76	100–110 dec									
Va	OH	NCO	70	240–244 dec	$C_8H_9N_3O_3$	49.23	4.65	21.53		48.96	4.73	21.80	
Vb	SH	NCO	76	207–209	$C_8H_9N_3O_2S$	45.48	4.29	19.89	15.18	45.91	4.24	19.85	14.89

TABLE II



No.	R	R′	Yield, %	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
VIb	OH	C_2H_5	18.5	>270	$C_{17}H_{20}N_4O_5$	56.65	5.59	15.54		56.21	5.79	15.38	
	OH	H	31.0	>270	$C_{15}H_{16}N_4O_5 \cdot H_2O$	51.42	5.17	15.99		51.16	5.63	16.05	
VId	SH	C_2H_5	44.4	>270	$C_{17}H_{20}N_4O_4S$	54.24	5.36	14.88	8.52	54.35	5.45	15.01	8.55
	SH	H	34.0	>270	$C_{15}H_{16}N_4O_4S \cdot H_2O$	49.17	4.95	15.29	8.75	49.70	5.00	15.46	8.86

TABLE III



No.	R	R′	Yield, %	Mp, °C	Formula	Calcd, % C	H	N	Found, % C	H	N
VIc	OH	C_2H_5	35.0	126–129	$C_{24}H_{31}N_5O_8 \cdot H_2O$	53.82	6.21	13.08	53.86	6.12	13.11
	OH	H	62.5	190–195	$C_{20}H_{33}N_5O_8 \cdot H_2O$	50.10	5.25	14.61	49.70	4.78	14.61
VIe	SH	C_2H_5	22.4	170–172	$C_{24}H_{31}N_5O_7S$	54.02	5.86	13.13	54.12	5.90	12.92
	SH	H	42.0	170–175	$C_{20}H_{25}N_5O_7S \cdot H_2O$	48.48	5.09	14.14	48.24	5.14	14.08

Experimental Section[8]

Ethyl 3-(2,4-Dihydroxy-6-methyl-5-pyrimidyl)propionate (IIa). Procedure A.—To a stirred solution of ethyl 3-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)propionate[9] (4.5 g, 0.02 mole) in 25% AcOH (50 ml) at 25° was added $NaNO_2$ (1.97 g, 0.03 mole) and the clear solution was heated on a steam bath for 1 hr. Overnight chilling gave 3.3 g (73%) of crude IIa, mp 201–205°. Recrystallization from 95% EtOH gave 2.7 g (60%) of a pale yellow solid, mp 204–205°.

Anal. Calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.39. Found: C, 53.14; H, 6.50; N, 12.62.

Procedure B.—To a stirred solution of 3-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)propionic acid[9] (6 g, 0.03 mole) in 40% AcOH (100 ml) at 25° was added $NaNO_2$ (3.5 g, 0.05 mole) and the solution heated on a steam bath for 1 hr. Chilling gave 4 g (66.5%) of 3-(2,4-dihydroxy-6-methyl-5-pyrimidyl)propionic acid, mp >270°. Refluxing the compound in absolute ethanol in the presence of H_2SO_4 gave the ester IIa as evidenced by mixture melting point and superimposable infrared spectra.

Ethyl 3-(2-Acetamido-4-hydroxy-6-methyl-5-pyrimidyl)propionate (VII).—A solution of ethyl 3-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)propionate (5 g, 0.022 mole) in Ac_2O (50 ml) was heated on a steam bath for 2 hr and evaporated *in vacuo*, and the solid was recrystallized from EtOAc to give 3.5 g (59%) VII, mp 135–137°.

Anal. Calcd for $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72. Found: C, 54.20; H, 6.27; N, 15.88.

Ethyl 3-(2-Mercapto-4-hydroxy-6-methyl-5-pyrimidyl)propionate (IIb).—A solution of $NaOCH_3$ (5.4 g, 0.10 mole), thiourea (7.6 g, 0.10 mole), and diethyl 2-acetoglutarate (23 g, 0.10 mole) in EtOH (200 ml) was refluxed for 2 hr, cooled to 25°, and poured into H_2O (500 ml). Neutralization with AcOH and recrystallization of the product from EtOH–H_2O gave 8.4 g (35%) of IIb, mp 195–196°. The infrared spectrum was as expected.

Anal. Calcd for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56; S, 13.23. Found: C, 49.00; H, 6.02; N, 11.91; S, 13.52.

Preparation of Hydrazides.—A solution of the substituted ethyl propionate (0.024 mole) in 95% hydrazine (15 ml) and 95% EtOH (50 ml) was heated on a steam bath for 1 hr and cooled to 25°, and the pH was adjusted to 8 ± 0.5 with AcOH and chilled to give the corresponding hydrazide. See Table I. Infrared spectra were as expected.

Preparation of Azides.—A solution of the hydrazide (8.5 mmoles) in H_2O (25 ml) and 6 *N* HCl (3 ml) was added dropwise to a solution of $NaNO_2$ (17 mmoles) in H_2O at 0–5°. The resulting clear solution was left in the ice bath for 30 min after addition was complete, and the light yellow solid which crystallized was dried at 25° (vacuum) to give the azide. See Table I. Infrared spectra showed peaks at 2140 and 2130 cm^{-1} for IVa and IVb, respectively.

Reaction of VIII with nitrous acid as above gave 38% of a product which decomposed at 110–115°. However, attempted conversion to the isocyanate gave only polymeric materials. Decomposition in the presence of ethyl *p*-aminobenzoate in toluene or DMF failed to produce any recognizable products.

(8) Melting points were determined on the Thomas–Hoover capillary apparatus and are corrected. All infrared spectra were obtained from Nujol mulls on a Perkin-Elmer Infracord. Elemental analyses were conducted by Weiler and Strauss, Alfred Bernhardt, and Galbraith microanalytical laboratories. All carbamates and ureas were heated at 100° under vacuum prior to analysis.

(9) V. Rericha and M. Protiva, *Chem. Listy*, **44**, 232 (1950).

Preparation of Isocyanates.—A suspension of the azide (2.2 mmoles) in toluene (50 ml) was refluxed for 6 hr, chilled, and filtered to give the isocyanate. Recrystallization from toluene gave the analytical samples. See Table I. The isocyanates Va and Vb exhibited an infrared peak at 2280 cm^{-1}.

Isobutyl N-[2-(2,4-Dihydroxy-6-methyl-5-pyrimidyl)-1-ethyl]-carbamate (VIa).—A solution of the isocyanate Va (0.5 g, 2.6 mmoles) in *i*-BuOH (50 ml) was refluxed for 6 hr and cooled to give 0.3 g (45%) of VIa, mp 233–234°. The infrared spectrum was as expected.

Anal. Calcd for $C_{12}H_{19}N_3O_4$: C, 53.53; H, 7.11; N, 15.60. Found: C, 53.38; H, 7.33; N, 15.51.

Preparation of Ureas.—A solution of ethyl *p*-aminobenzoate or diethyl *p*-aminobenzoyl-L-glutamate (10 mmoles) and isocyanate (4.5 mmoles) in dry[10] DMF (25 ml) was heated on a steam bath for 20 hr, evaporated *in vacuo* to 5 ml, and poured into toluene (200 ml). The precipitated solid was recrystallized from dioxane–H_2O and EtOH–H_2O to give the substituted urea. See Table II.

The ethyl ester (1.4 mmoles) was hydrolyzed by 3 *N* NaOH (10 ml) at 25° for 5 hr. The chilled solution was neutralized with 6 *N* HCl to give the free acid. The crude material was purified by dissolving in 2% $NaHCO_3$, treatment with Norit, and precipitation with 6 *N* HCl. See Table III. The infrared spectra of the acids and their ethyl esters were as expected.

(10) G. R. Leader and J. F. Gormley, *J. Am. Chem. Soc.*, **73**, 5731 (1951).

Substituted 1-Benzyl-3-(N,N-diethylcarbamoyl)-piperidine Cholinesterase Inhibitors. Relationships between Molecular Constitution, pK_a' Values, and Partition Coefficients[1]

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Received March 16, 1967

During the past several years, appreciable efforts have been made to explore relationships between the molecular constitution of carbamoylpiperidines and their physicochemical properties, as well as between the latter two and the biochemical response of these entities in isolated cholinesterase systems.[2-4] In this specific instance, we have designed a series of compounds which would enable us to study the effect of electron-density changes around the heterocyclic nitrogen of the piperidine derivatives. In addition to anticipating the evaluation of their effect upon isolated butyrylcholinesterase,[5] we were interested in determining the influence our structural modifications would exert in terms of lipophilic–lipophobic characteristics.

More specifically, we have prepared a series of 1-benzyl-3-(N,N-diethylcarbamoyl)piperidine hydrobromides with methyl, methoxy, chloro, and nitro substituents located in the *meta* and *para* positions (see Table I). We have determined the pK_a' values and apparent partition coefficients ($CHCl_3$–water) of these compounds and of the unsubstituted benzyl derivatives with the expectation that the values would *reflect* the *relative influence* each substituent has upon electron density at the heterocyclic nitrogen and upon lipophilic–lipophobic characteristics, respectively.

Experimental Section

Synthetic Work.—Most of the substituted-benzyl halides required as intermediates were commercially available (Aldrich Chemical Co.). *p*-Methoxybenzyl bromide, *m*-methoxybenzyl bromide, and *p*-chlorobenzyl bromide were prepared from the corresponding alcohols by the method of Beard and Hauser.[6] In the latter instances, the crude products were used in the subsequent reactions.

The compounds listed in Table I were prepared by the following methods.

Method A. 1-Benzyl-3-(N,N-diethylcarbamoyl)piperidine Hydrobromide (I).—The procedure described is patterned after that employed by Beasley, *et al.*,[7] for the preparation of 1-alkylcarbamoylpiperidine derivatives. To a cold solution of N,N-diethylnicotinamide (61.6 g, 0.346 mole) in 300 ml of anhydrous benzene, a solution of benzyl chloride (50.0 g, 0.395 mole) in 50 ml of the latter was added slowly with stirring. The reaction mixture was refluxed for 59 hr;[8] after removal of the solvent by filtration (or decantation), the product was dissolved in 50% aqueous ethanol and subjected to hydrogenation *in* the presence of 2 g of PtO_2 at a maximum pressure of 3.51 kg/cm^2. The catalyst was removed by filtration, and solvent was removed by distillation under reduced pressure. The residue was treated with 100 ml of cold 40% NaOH, and the mixture was extracted with benzene. The combined benzene extracts were dried ($MgSO_4$) and filtered, and the benzene was removed by distillation under reduced pressure. The product was dissolved in anhydrous ether and converted to the hydrobromide by the addition of a solution of dry HBr in anhydrous ether. The salt was then recrystallized from absolute ethanol–anhydrous ether.

Method B. 1-(*p*-Nitrobenzyl)-3-(N,N-diethylcarbamoyl)-piperidine Hydrobromide.—The procedure described is patterned after that used by Quintana, *et al.*,[4] for the preparation of other N,N-diethylnipecotamide derivatives. To a cold, stirred mixture of N,N-diethylnipecotamide[3] (41.8 g, 0.227 mole), anhydrous K_2CO_3 (41.5 g, 0.300 mole), and 150 ml of anhydrous benzene, *p*-nitrobenzyl bromide (49.0 g, 0.227 mole) was added slowly. The reaction mixture was gradually warmed and was subsequently refluxed for 16 hr with stirring. After cooling, the mixture was treated with a total of 500 ml of water; the benzene layer was separated and the aqueous layer was extracted with benzene. The benzene solution was dried ($MgSO_4$) and filtered, and the solvent was removed by distillation under reduced pressure. The oily residue was dissolved in absolute ethanol–anhydrous ether and was treated with a solution of dry HBr in anhydrous ether. The salt was recrystallized from absolute ethanol.

Determination of pK_a' Values.—A Radiometer TTTlc automatic titrator equipped with a SBUla syringe buret, a K401 calomel electrode, and a G202c glass electrode was employed. The reaction vessel was thermostated at 25.00 ± 0.05° by water from a Hetotherm Model 623K circulating bath; it was provided, also, with a rotor for mechanical stirring. The instrument was calibrated with standard buffer (pH 6.50 ± 0.02 at 25°) prior to use.

(1) Portion of W. R. Smithfield's thesis submitted to the Graduate School of the University of Tennessee in partial fulfillment of the requirements for the degree Master of Science (Medicinal Chemistry). This investigation was supported, in part, by Grants GB-2381 and GB-4453 from the National Science Foundation.

(2) (a) R. P. Quintana and W. A. Shrader, *J. Pharm. Sci.*, **52**, 1186 (1963); (b) R. P. Quintana, *ibid.*, **53**, 1221 (1964); (c) *ibid.*, **54**, 462 (1965); (d) *ibid.*, **54**, 573 (1965).

(3) R. P. Quintana, T. D. Smith, and L. F. Lorenzen, *ibid.*, **54**, 785 (1965).

(4) W. P. Purcell, J. G. Beasley, R. P. Quintana, and J. A. Singer, *J. Med. Chem.*, **9**, 297 (1966).

(5) J. G. Beasley, S. T. Christian, W. R. Smithfield, and L. L. Williford, *ibid.*, **0**, 1003 (1967).

(6) W. Q. Beard, Jr., and C. R. Hauser, *J. Org. Chem.*, **25**, 334 (1960).

(7) J. G. Beasley, R. P. Quintana, and G. C. Nelms, *J. Med. Chem.*, **7**, 698 (1964).

(8) The reflux period employed for other compounds prepared by this method varied from 6 to 73 hr.