Anal. Calcd for C_7H_7N: C, 80.02; H, 6.66; N, 13.30. Found: C, 79.97; H, 6.83; N, 13.02.

A 15% solution of **8** in sulfolane yielded polymer when subjected to uv radiation for 8 hr at room temperature.

3-Vinyl-1-cyanocyclobut-2-ene (9) 3-(β-Chloroethylidene)cyanocyclobutane, 1.0 g (71 mmol), was dissolved in 15 ml of anhydrous ethyl ether. The resulting solution was placed in a 50-ml three-necked round-bottomed flask fitted with a nitrogen gas inlet adaptor and a 0.5-in. magnetic stirring bar (Teflon coated). The flask and contents were then cooled to −7° in an ice–methanol bath. At this point, 1.0 g (88 mmol) of potassium *tert*-butoxide was introduced into the flask all at once. The color of the solution turned dark brownish purple as the temperature rose slightly above 0°. After 10–12 min the temperature began to fall and the reaction mixture was worked up by adding *ca.* 0.5 g of powdered CO_2 and 1–2 ml of saturated KCl solution. The resulting slurry of salts was filtered and the filtrate was dried over a little $MgSO_4$ and distilled. The distillate (0.8 g), collected over a temperature range of 37–63° (0.1 mm), was found to contain, in addition to 20% unreacted starting material, a 55:45 (glc) mixture of two new lower boiling components. These two substances were separated from the unreacted chloride and their nmr spectrum was taken. When the spectrum (nmr) of pure 3-vinylbicyclobutanecarbonitrile was compared with that of the mixture of products of this reaction, it was evident that the conjugated diene **9** was present along with the bicyclic isomer. The yield of diene based on a 45:55 diene to bicyclic ratio was about 29%: nmr ($CDCl_3$) τ 6.5 (broad multiplet, 1 H, α to cyano), 7.0 (multiplet, 2 H, methylene), 3.5–4.8 (multiplet, 4 H, olefinic protons) (absorptions caused by 3-vinylbicyclobutane-1-carbonitrile not mentioned).

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Pyrimido[5,4-*e*]-*as*-triazines. VII. Synthesis of 7-Aza Analogs of Pteroic and Folic Acids[1]

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An investigation of the preparation and stability of dihydropyrimido[5,4-*e*]-*as*-triazines indicated that electron-donating groups in the dihydro-*as*-triazine ring promoted the air oxidation of these compounds to the corresponding heterocyclic derivatives. Work was also carried out in the preparation and reactions of a number of substituted anilinoacetonitriles. Conversion of [*p*-(ethoxycarbonyl)anilino]acetonitrile (**6a**) and diethyl *p*-[(cyanomethyl)amino]benzoyl-L-glutamate (**6c**) to the corresponding ethyl imidates and condensation of the latter with 2,5-diamino-4-(benzylthio)-6-hydrazinopyrimidine (**7**) provided directly ethyl *p*-[[[7-amino-5-(benzylthio)-pyrimido[5,4-*e*]-*as*-triazin-3-yl]methyl]amino]benzoate (**10a**) and the corresponding diethyl L-glutamate (**10b**), respectively. Nucleophilic replacement of the benzylthio group of **10a** with the appropriate reagent gave the 5-oxo (**11a,** ethyl 7-azapteroate), the 5-thione (**12a**), and the 5-amino (**13a**) derivatives. Similarly, **10b** was converted to the 5-oxo (**11c,** 7-azafolic acid) and 5-thione (**12c**) derivatives. Reaction of **10b** with sodium azide lead to the corresponding diethyl ester of the 5-amino derivative (**13b**).

Previously, we reported the development of synthetic methods for the preparation of 4-substituted 2-amino-7-azapteridines (5-substituted 7-aminopyrimido[5,4-*e*]-*as*-triazines).[2] Further modification of these procedures have now provided methods for the preparation of 7-azapteroic and 7-azafolic acids and related compounds.[3] These compounds, especially 7-azafolic acid and the corresponding 4-thio compound, are of interest as potential substrates for the folic reductase enzyme, which on interaction might produce biologically interesting 7-aza derivatives that are analogs of the tetrahydrofolate coenzymes.[4]

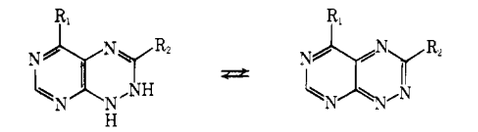
Earlier we showed that reaction of **2a** with NaSH not

only replaced the benzylthio group but also reduced the *as*-triazine ring to give the dihydro derivative **1a.**[5] To obtain information on the preparation and stability of dihydro-7-azapteridines containing a methylene function in the triazine ring, the reactions of some simple derivatives were investigated. The cyclization of **3**[6] in hot HOAc gave a mixture of products in which the desired **1b** was shown to be the major component by its pmr spectrum. Treatment of **1b** with NaSH gave a product which, after reprecipitation from a basic solution with acid, analyzed correctly for **1c** but was shown by its pmr spectrum to be a 7:3 mixture of **1c** and **2b.** Alkylation of this mixture with benzyl chloride gave both **1d** and **2c,** which were separated by recrystallization. Reaction of **2c** with NaSH and isolation of the product directly from the reaction medium by acidification gave only the dihydro derivative **1c** as determined by its pmr spectrum. However, reprecipitation of this sample from a basic solution with acid gave a 7:3 mixture of **1c** and **2b,** the same as that obtained in the reaction of **1b** with NaSH described above. These results indicated that the presence of the methyl group does not interfere with the reduction of the *as*-triazine ring but does promote the air oxidation of **1c** when compared with **1a,** which can be reprecipitated unchanged from a basic solution with acid.[5]

The preparation of the desired analogs were attempted by two routes. One involved the alkylation of a *p*-aminobenzoyl side-chain moiety with a preformed halomethyl-7-azapteridine intermediate. The products of these reactions have been difficult to characterize, and the results of this work will be reported at a later date. The successful route involved the condensation of a 5-amino-4-hydrazinopyrimidine with the imidate derived from an anilinoacetonitrile type intermediate. The known **6a**[7] was prepared in good yield by a new method, which involved the alkylation of **4a** with cyanomethyl *p*-toluenesulfonate in EtOAc. Similarly, treatment of methyl *p*-(methylamino)benzoate (**4b**)[8] and diethyl *p*-aminobenzoyl-L-glutamate (**4c**)[9] with this reagent in refluxing dioxane gave, respectively, **6b** and **6c.** The latter was purified by column chromatography and recrystallization and was obtained in low yield. The preparation of **6c** by treatment of **4c** successively with $HOCH_2SO_3Na$ ($NaHSO_3$ and CH_2O) and KCN was unsuccessful.[7]

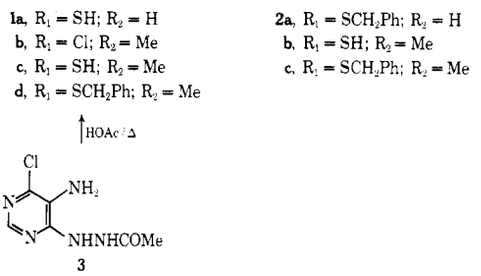
Initially, we considered the conversion of the cyanomethyl compounds to the corresponding ortho esters, which would be used as intermediates in the cyclization of 5-amino-4-hydrazinopyrimidines to 7-azapteridines. Treatment of the simple cyanomethyl compound **6d**[10] in Et_2O with ethanolic HCl gave only its hydrochloride salt rather than the imidate **5a.** In contrast, the conversion of **6d** to the acyl derivative **6e** and treatment of this product with ethanolic HCl gave **5b.** Under the same conditions that were successful for the preparation of **6e,** acylation of **6a** to give **6f** was unsuccessful, apparently because of the reduced basicity of the amino group. However, treatment of **6a** in Et_2O with ethanolic HCl provided directly the hydrochloride salt of the imidate **5c.** Conversion of this salt to the corresponding ortho ester was attempted in EtOH, but this reaction appeared to give mainly the hydrochloride salt of the corresponding acetic acid derivative based on elemental analysis. To eliminate from consideration the presence of HCl in **5c,** the method of Kim and McKee for the preparation of this compound was investigated.[7] This method involved the base-catalyzed addition of EtOH to **6a** to give **5c.**[11] After neutralization with acetic acid, refluxing the solution of **5c** evolved NH_3, presumably to give the ortho ester, which was successfully condensed with **7.** Further investigation showed that the condensation of **7** with the *in situ* formed imidate **5c** also gave the desired product (see below). Unexpectedly, treatment of **6b** under similar conditions to give **5d** and condensation of the latter *in situ* with **7** gave no 7-azapteridine. The isolated product analyzed closely for a ring-opened derivative **9,** which could not be cyclized in an acid medium. Similarly, reaction of 5-amino-4-chloro-6-hydrazinopyrimidine[5] with **5c** apparently gave a ring-opened amidrazone intermediate that also could not be cyclized under acidic, neutral, and basic conditions. Although not enough work was carried out to determine the cause of these unsuccessful reactions, the imidate intermediate can react with the hydrazino group either by replacement of the alkoxy group[12] or by exchange amination with the amino group.[13] However, both types of pyrimidine intermediates produced by these reactions should undergo cyclization although the product resulting from exchange amination might do so more readily.[14]

A solution of **5c** prepared as described above was condensed with **7** at room temperature to give **10a,** no doubt formed by air oxidation of the intermediate dihydro derivative **8a.** Reaction of **10a** with $KHCO_3$ in aqueous DMSO at 90° gave ethyl 7-azapteroate (**11a**). Under these conditions, no hydrolysis of the benzoate ester function was observed. The interaction of **10a** with NaSH in aqueous DMF at 80° gave the thione **12a.** The isolation of the heteroaromatic compound from this reducing medium is attributed to the

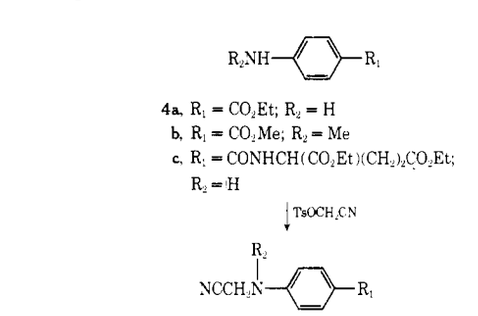


1a, R_1 = SH; R_2 = H
b, R_1 = Cl; R_2 = Me
c, R_1 = SH; R_2 = Me
d, R_1 = SCH_2Ph; R_2 = Me

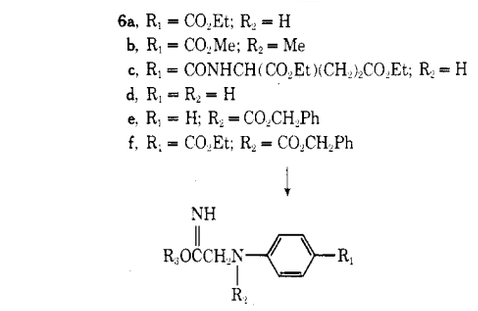
2a, R_1 = SCH_2Ph; R_2 = H
b, R_1 = SH; R_2 = Me
c, R_1 = SCH_2Ph; R_2 = Me



4a, R_1 = CO_2Et; R_2 = H
b, R_1 = CO_2Me; R_2 = Me
c, R_1 = $CONHCH(CO_2Et)(CH_2)_2CO_2Et$; R_2 = H



6a, R_1 = CO_2Et; R_2 = H
b, R_1 = CO_2Me; R_2 = Me
c, R_1 = $CONHCH(CO_2Et)(CH_2)_2CO_2Et$; R_2 = H
d, R_1 = R_2 = H
e, R_1 = H; R_2 = CO_2CH_2Ph
f, R_1 = CO_2Et; R_2 = CO_2CH_2Ph



5a, R_1 = R_2 = H; R_3 = Et
b, R_1 = H; R_2 = CO_2CH_2Ph; R_3 = Et
c, R_1 = CO_2Et; R_2 = H; R_3 = Et
d, R_1 = CO_2Me; R_2 = R_3 = Me
e, R_1 = $CONHCH(CO_2Et)(CH_2)_2CO_2Et$; R_2 = H; R_3 = Et

Table I

Compd	Uv absorption[a] spectra at pH 7, λ_{max}, nm ($\epsilon \times 10^{-3}$)	Ir absorption[b] spectra in KBr, selected bands, cm^{-1}	Pmr spectral assignments,[c] chemical shifts, δ (rel area)	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
1c	254 (10.4), 298 (5.16), 339 (6.96), 415 (3.62)[d,e]	1645	1.59 (3, CH_3), 6.47, 8.77 (1, 1, NH),[f] 7.47 (1, CH), ~13 br (NH)[f,g]	$C_6H_7N_5S$	39.77	3.89	38.65	39.66	3.69	38.50
1d	357 (5.34)[d,e]	1660	1.53 (3, CH_3), 3.26 (H_2O), 4.29 (2, CH_2), 7.27 (6, C_6H_5, NH[f]), 7.61 (1, CH), 8.62 (1, NH)[f]	$C_{13}H_{13}N_5S \cdot 0.33H_2O$	56.30	4.97	25.25	56.27	5.06	25.29
2c	240 (11.1), 263 sh (5.88), 391 (6.40)[d,e]	1595, 1540	3.10 (3, CH_3), 4.66 (2, CH_2), 7.40 (5, C_6H_5), 9.28 (1, CH)	$C_{13}H_{11}N_5S$	57.98	4.12	26.00	58.05	4.13	25.95
10a	242 (14.3), 281 sh (26.2), 299 (29.8), 420 (7.68)[h]	1695, 1640	1.25 (3, CH_3), 4.20 q (2, CH_2O), 4.52 (2, CH_2S), 4.78 d [2, CH_2N (J = 6.0 Hz)], 7.18, 7.37, 7.87 [12, C_6H_4 (C_6H_5, NH[f]), NH_2[f]]	$C_{22}H_{21}N_7O_2S$	59.05	4.73	21.91	58.94	4.85	21.95
10b	232 (26.4), 289 (19.8), 373 (8.85)[d,e]	1725, 1630	1.17 (6, CH_3), 2.04, 2.41 (CH_2-CH_2), 4.06 (4, CH_2Me), 4.39 (1, CHN), 4.52 (2, CH_2S), 4.77 d [2, CH_2N (J = 5.6 Hz)], 6.95 t (1, HNC), 7.17, 7.38 (9, C_6H_4, C_6H_5), 7.88 br (2, NH_2),[f] 8.17 d [1, HNCO (J = 7.0 Hz)][f]	$C_{29}H_{32}N_8O_5S$	57.60	5.33	18.53	57.22	5.40	18.61
11a	266 (24.7), 287 sh (21.7), 387 (4.87)[i]	1690, 1640	1.25 (3, CH_3), 4.20 q (2, CH_2O), 4.79 (2, CH_2N), 7.53 br (NH, NH_2),[f] 7.22 (C_6H_5)	$C_{15}H_{15}N_7O_3 \cdot HCl$	47.69	4.27	25.92	47.31	3.98	26.09
11c	272 (22.2), 377 (4.35)[d]	1700, 1650	2.04, 2.34 (CH_2CH_2), 3.35 (H_2O), 4.36, 4.74 br (3, CHN, CH_2N), 7.18, 7.32 br, 8.08 d [9, C_6H_4, (NH_2, NH, NH), NHCO (J = 8.0 Hz)], ~12 br (2, OH)	$C_{18}H_{18}N_8O_6 \cdot 1.5H_2O$	46.06	4.51	23.87	45.85	4.31	24.10
12a	299 (26.9), 378 sh (4.18), 443 (6.18)[d]	1685, 1635	1.23 (CH_3), 4.17 q (CH_2O), 4.71 (CH_2N), 7.15 (C_6H_5), 7.3 br (NH_2),[f] 7.67 br (NH)[f,j]	$C_{15}H_{15}N_7O_2S \cdot H_2O$	47.99	4.56	26.12	48.23	4.84	26.00
12b	291 (24.8), 360 (4.67), 440 (5.17)[d]	1725, 1640	1.17 (CH_3), 2.04, 2.51 (CH_2-CH_2), 4.06 (CH_2Me), 4.38 (CHN), 4.74 (CH_2N), 7.17 (C_6H_5), 7.27 br, 8.15 br (NH, NH_2)[f,j]	$C_{22}H_{26}N_8O_5S \cdot HCl$	47.95	4.94	20.34	47.58	5.02	20.16

12c	270 sh (22.3), 284 (22.8), 377 (4.78), 432 (4.45)[d]	1710, 1660, 1645	2.01, 2.32 (CH_2CH_2), 3.4 (H_2O), 4.34, 4.72 (3, CHN, CH_2N), 7.14, 8.05 d [9 (C_6H_4, NH_2,[f] NH,[f] NH^f), NHCO (J = 8.0 Hz)], ~11 (2, OH)[f]	$C_{18}H_{18}N_8O_5S\cdot H_2O$	45.38	4.23	23.52[k]	45.18	4.10	23.57[k]
13a	275 sh (26.4), 298 (31.7), 403 (5.03)[h]	1685, 1640	1.27 (3, CH_3), 3.27 (H_2O), 4.22 q (2, CH_2O), 4.77 d [2, CH_2N (J = 6.0 Hz)], 6.92, 7.04, 7.30 (7, NH,[f] NH_2,[f] C_6H_4), 8.19 br (2, NH_2)[f]	$C_{15}H_{16}N_8O_2\cdot H_2O$	50.28	5.06	31.27	50.63	4.61	31.05
13b	267 (27.3), 287 sh (24.6), 393 (5.90)[d]	1730, 1640	1.18 (6, CH_3), 2.05, 2.43 (CH_2-CH_2), 3.3 (H_2O), 4.19 q, 4.35, 4.76 (7, CH_2O, CHN, CH_2N), 7.25, 8.25 [10 (C_6H_4, NH_2,[f] NH^f), NH^f]	$C_{22}H_{27}N_9O_5\cdot 0.75H_2O$	51.71	5.62	24.67	51.96	5.29	24.28
14b	280 (22.4), 364 (4.81)[d]	1725, 1665, 1640		$C_{22}H_{25}N_{11}O_5\cdot 1.75H_2O$	47.61	5.18	27.76	47.70	4.86	27.66

[a] Cary Model 14 and 17 spectrophotometers. [b] Perkin-Elmer Model 521 and 621 spectrophotometers. [c] Pmr spectra of samples were determined on DMSO-d_6 solutions (3–6% w/v) on a Varian A-60A and XL-100-15 spectrometers with TMS as an internal reference; peak positions quoted in the case of multiples are measured from the approximate center, and the relative peak areas are given to the nearest whole number. [d] The sample was dissolved in a mixture containing 8% DMSO and 92% MeOH. [e] Determined in 0.1 *N* HCl. [f] Exchanged for deuterium on addition of D_2O. [g] This product was a 7:3 mixture of **1c** and **2b** in which **2b** showed δ 2.99 (CH_3), 8.32 (CH). [h] Determined in a mixture of 8% DMSO and 92% MeOH. [i] The sample was dissolved in 0.1 *N* NaOH and diluted with pH 7 buffer. [j] Relative peak areas were inconsistent. [k] Calcd: S, 6.73. Found: S, 6.90.

presence of the electron-releasing aminomethyl function in the triazine ring since the corresponding compound without this moiety gave only a dihydro derivative.[2] Incomplete reaction was obtained when **10a** was treated with NaSH in H_2O at 80°. The product obtained under these conditions was identified by the pmr spectrum as the benzoic acid derivative of **12a.** Replacement of the benzylthio group of **10a** to give **13a** was effected with 10% ethanolic NH_3 in warm DMAC. Treatment of **10a** with NaN_3 in aqueous DMSO at room temperature appeared to give the tetrazolo compound **14a.** This compound was not obtained pure, and the conversion of **14a** *via* reduction of the corresponding azido tautomer to **13a** was not attempted.

Addition of ethanol to the cyano group of **6c** in the presence of sodium ethoxide gave the imidate **5e.** The solution of **5e** was neutralized and condensed *in situ* with **7** to give **10b,** presumably formed *via* air oxidation of the corresponding dihydro derivative **8b.** Treatment of an aqueous DMSO solution of **10b** with $KHCO_3$ at 90° replaced the benzylthio group to give crude **11b.** Saponification of the ester groups of **11b** to give **11c** at room temperature with oxygen-free NaOH was slow and required two 18-hr treatments to complete the hydrolysis. Reaction of **10b** with NaSH in refluxing EtOH gave the diethyl ester of the thione **12b,** which was saponified in the absence of oxygen with NaOH to give **12c.** Thin layer chromatography data indicated that hydrolysis of the thioamide moiety of **12c** occurred slowly in dilute NH_4OH to give mainly 7-azafolic acid (**11c**). The preparation of **13b** from **10b** and ethanolic NH_3 was eliminated from consideration as this reagent might also convert the ester functions to amides. For this reason, **10b** was reacted with NaN_3 to give a mixture of **13b** and **14b** which were separated. The isolation of **13b** directly from the reaction mixture is in agreement with the observation that 4-azido-7-azapteridines are readily converted to 4-amino-7-azapteridines.[15] The structure of **14b** is based on the absence of an azido absorption band in its infrared spectrum. Hydrolysis of the ester groups of **13b** to give **13c** was attempted under the same conditions that were suc-

cessful for the preparation of **11c** and **12c**, but the pmr spectrum of the recovered material indicated that little or no saponification occurred.

Although the activities (ED_{50}) of the folic acid analogs (**11c** and **12c**) were similar to that of methotrexate when tested against *Streptococcus faecium* ATCC 8043, no activity was observed in preliminary tests against L1210 leukemia cells implanted intraperitoneally in mice.

The uv and pmr spectra and selected bands in the ir spectra for the new compounds are presented in Table I.

Experimental Section[16]

5-Chloro-1,2-dihydro-3-methylpyrimido[5,4-*e*]-*as*-triazine (1b). A solution of **3** (2.4 g) in glacial HOAc (50 ml) was refluxed for 2 hr and evaporated to dryness *in vacuo*. The resulting residue was washed with Et_2O and dried *in vacuo* over P_2O_5: yield, 2.2 g. A sample of the crude product was recrystallized three times from EtOAc. The pmr spectrum (DMSO-d_6) of the resulting sample showed a three-component mixture of which 74% was the desired product **1b**. In later experiments, the crude product first isolated was used in the preparation of **1c**.

1,2-Dihydro-3-methylpyrimido[5,4-*e*]-*as*-triazine-5(6*H*)-thione (1c). A. A mixture of crude **1b** (3.4 g) and hydrated sodium hydrosulfide (3.4 g) in EtOH (700 ml) was refluxed for 1 hr and evaporated to dryness *in vacuo*. This residue was dissolved in H_2O, and the resulting solution was acidified to pH 5 (paper) with dilute HCl. The solid that precipitated was collected by filtration and stirred for 1 hr in C_6H_6 (500 ml) and then for 30 min in EtOH (50 ml) to give practically pure **1c**: yield, 1.9 g (57%). Further purification was obtained by reprecipitation of a portion of this sample (0.90 g) from a NaOH solution with dilute HCl. The resulting product was dried *in vacuo* over P_2O_5 at 110°: yield, 0.65 g (72% recovery); mp >264°. The pmr spectrum indicated that this sample was a 7:3 mixture of **1c** and **2b**.

B. Treatment of **2c** (100 mg) as described above gave, after acidification to pH 5, only **1c**: yield, 51 mg. None of **2b** was observed in the pmr spectrum (DMSO-d_6) of this product. However, reprecipitation of a portion of this sample (32 mg) from a NaOH solution with dilute HCl gave a sample (20 mg) which was shown to be about a 7:3 mixture of **1c** and **2b** by its pmr spectrum (DMSO-d_6).

5-(Benzylthio)-1,2-dihydro-3-methylpyrimido[5,4-*e*]-*as*-triazine (1d) and 5-(Benzylthio)-3-methylpyrimido[5,4-*e*]-*as*-triazine (2c). A solution of **1c** (1.0 g) in 0.2 *N* NaOH (28 ml) containing benzyl chloride (0.65 ml) was stirred at room temperature for 5 hr. The solid that precipitated was collected by filtration, dried *in vacuo* over P_2O_5, and extracted with hot C_6H_6 (30 ml). On cooling, the extract deposited a small amount of solid that was removed by filtration. The filtrate was concentrated to about one-half volume and on standing deposited **1d**: yield, 0.30 g (20%); mp 145–146°.

The C_6H_6 filtrate from above was evaporated to dryness *in vacuo*, and the residue was washed with H_2O. The resulting solid was dried (P_2O_5) and recrystallized from hexane to give **2c**: yield, 0.46 g (31%); mp 125–126°.

Ethyl [*N*-Benzyloxycarbonyl)anilino]ethanimidate Hydrochloride (5b). A solution of **6e** (0.57 g) in Et_2O (35 ml) containing absolute EtOH (0.15 ml) and excess anhydrous HCl was cooled at 5° for 48 hr. The hygroscopic precipitate was collected by filtration and washed with fresh Et_2O: yield, 0.08 g (11%).

Anal. Calcd for $C_{18}H_{20}N_2O_3$·HCl: C, 61.98; H, 6.07; N, 8.03. Found: C, 61.70; H, 6.30; N, 8.32.

Ethyl [*p*-(Ethoxycarbonyl)anilino]ethanimidate Hydrochloride (5c). A solution of **6a** (1.0 g) in Et_2O (100 ml) containing 10 ml of 22% ethanolic HCl was cooled at 5° for 18 hr. The resulting hydrochloride salt was collected by filtration and washed with fresh Et_2O: yield, 0.32 g (21%); mp ~95° with presoftening (capillary tube).

Anal. Calcd for $C_{13}H_{18}N_2O_3$·HCl: C, 54.45; H, 6.68; N, 9.77. Found: C, 54.46; H, 6.40; N, 10.00.

[*p*-(Ethoxycarbonyl)anilino]acetonitrile (6a). A mixture of **4a** (36 g) and cyanomethyl *p*-toluenesulfonate (21 g) in ethyl acetate was refluxed with stirring for 48 hr. The cooled mixture was filtered, the residue was washed with ethyl acetate, and the combined filtrate and wash was evaporated to dryness *in vacuo*. The resulting oil was extracted with ether (500 ml), and the solid obtained by evaporation of the extract was recrystallized from CCl_4: yield, 16 g (79% based on cyanomethyl *p*-toluenesulfonate); mp 92–93° (lit.[7] mp 92–93.5°); ν_{max} 2235 cm^{-1} (CN).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.70; H, 5.91; N, 13.57.

[*p*-(Methoxycarbonyl)-*N*-methylanilino]acetonitrile (6b). A solution of **4b** (2.0 g) and cyanomethyl *p*-toluenesulfonate (2.5 g) in dioxane (30 ml) was refluxed for 72 hr. The cooled reaction mixture was filtered to remove some solid material (0.17 g), the filtrate was evaporated to dryness *in vacuo*, and the resulting oil was extracted by stirring with Et_2O. After the separation of the solid (1.4 g), the Et_2O solution was washed with 1 *N* NaOH (3 × 20 ml portions), dried ($MgSO_4$), and evaporated to dryness *in vacuo* to yield an oil (2.5 g). A solution of the oil in Et_2O (25 ml) was diluted with hexane (25 ml) and refrigerated to deposit crude **6b**: yield, 1.1 g; mp 77° with presoftening from 32°. Two additional reprecipitations of the product from Et_2O–hexane gave pure **6b**: yield, 0.23 g (18.6%); mp 82°; ν_{max} 2230 cm^{-1} (CN); pmr (DMSO-d_6) δ 3.05 (3, CH_3N), 3.81 (3, CH_3O), 4.65 (2, CH_2), 7.47 (4, C_6H_4).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.36; H, 5.92; N, 13.73.

Dilution of the combined Et_2O–hexane filtration from above with hexane followed by refrigeration gave recovered crude **6b**: yield, 0.61 g; mp ~33–49°.

Diethyl *p*-[(Cyanomethyl)amino]benzoyl-L-glutamate (6c). A mixture of **4c** (45 g) and cyanomethyl *p*-toluenesulfonate (27 g) in dioxane (350 ml) was refluxed for 120 hr and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and poured onto a 3-l. fritted-glass funnel containing silica gel H (375 g). The funnel was washed with $CHCl_3$ to give fractions I (17 g, 1500 ml) and II (19 g, 8500 ml) and with EtOAc to give fraction III (7 g, 2000 ml). Fractions I and II were combined and eluted ($CHCl_3$) from a silica gel H (350 g) column to give a crude oily product (6.6 g), which was washed with Et_2O to give a solid: yield, 3.6 g; mp 90–92°. For analyses a sample (124 mg) was recrystallized from a mixture of C_6H_6 and hexane: mp 95°; ν_{max} 2250 cm^{-1} (CN); pmr (DMSO-d_6) δ 1.18 (6, CH_3), 2.09, 2.43 (CH_2CH_2), 4.07, 4.32 [7, CH_2O (CHN, CH_2N)], 6.7 (br, 1, NH), 7.24 (4, C_6H_4), 8.32 [d, 1, NH (J = 8.0 Hz)].

Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.58; H, 6.37; N, 11.37.

Fraction III was eluted with $CHCl_3$ from a coarse silica gel 60 column to give an oily product which was then washed with Et_2O to give a solid: yield, 2.8 g. This sample melted with softening from 80°.

The residue obtained from evaporation of the combined Et_2O washes was eluted with $CHCl_3$ from a silica gel H (80 g) column to give an additional amount of crude oily product: yield, 1.0 g.

[*N*-(Benzyloxycarbonyl)anilino]acetonitrile (6e). A solution of **6d** (1.0 g)[10] in dioxane (25 ml) containing pyridine (0.61 ml) and benzyloxycarbonyl chloride (1.3 g) was stirred at room temperature for 5 hr. After filtration the filtrate was evaporated to dryness, and the resulting residue was extracted with ether. Addition of anhydrous HCl in Et_2O to the filtrate gave a precipitate that was discarded. The filtrate was washed (H_2O), dried ($MgSO_4$), and evaporated *in vacuo* in a tared flask: yield, 0.67 g (33%); pmr (DMSO-d_6) δ 4.78 and 5.17 (CH_2), 7.35 (C_6H_5).

Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.17; H, 5.30; N, 10.52. Found: C, 72.22; H, 5.29; N, 10.34.

Ethyl *p*-[[[7-Amino-5-(benzylthio)pyrimido[5,4-*e*]-*as*-triazin-3-yl]methyl]amino]benzoate (10a). A solution of **6a** (4.0 g) in absolute EtOH (40 ml) containing NaOEt (0.17 g) was stirred at room temperature for 18 hr. followed by neutralization with glacial HOAc (0.16 ml). This solution was added to a suspension of **7** (4.0 g)[2] in dioxane (200 ml). The resulting dark solution was stirred in an open flask (cotton plug) at room temperature for 18 hr to deposit **10a**, which was collected by filtration and washed with $CHCl_3$ (1500 ml): yield, 2.8 g (42%). For analyses a sample was reprecipitated from a DMSO solution with H_2O and dried *in vacuo* over P_2O_5 at 78°, mp >264°.

Diethyl *N*-[*p*-[[[7-Amino-5-(benzylthio)pyrimido[5,4-*e*]-*as*-triazin-3-yl]methyl]amino]benzoyl]-L-glutamate (10b). A solution of **6c** (3.61 g) in EtOH (50 ml) containing NaOEt (0.1 g) was stirred at room temperature for 18 hr followed by neutralization with HOAc (0.1 ml). This solution was added to a solution of **7** (2.62 g)[2] in dioxane (200 ml) and whole was stirred in an unstoppered flask (cotton plug) at room temperature for 72 hr. After the solution was evaporated to dryness, the resulting residue was washed successively by stirring with H_2O (3 × 500 ml portions) and Et_2O (100 ml) to give crude **10b**: yield, 4.9 g (81%). A portion (500 mg) of this sample was recrystallized twice from EtOH to give the pure product: yield, 85 mg; mp 151°. From the filtrates 353 mg of crude **10b** was recovered.

Ethyl *p*-[[(7-Amino-5(6*H*)-oxopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoate Hydrochloride (Ethyl 7-Azapteroate) (11a). A solution of **10a** (250 mg) in DMSO (25 ml) containing $KHCO_3$ (250 mg) and H_2O (0.5 ml) was heated with stirring at 90° for 18 hr. The cooled mixture was diluted with 1 *N* HCl (2.5 ml) and H_2O (400 ml) and chilled for 18 hr. The resulting precipitate was collected by filtration, dissolved in 0.1 *N* NaOH (10 ml), and acidified to pH 3 (paper) with 0.1 *N* HCl. The product that precipitated was collected by filtration and dried *in vacuo* over P_2O_5 at 110°: yield, 77 mg (37%); mp >264°. Chlorine analysis indicated that this sample was a partial hydrochloride salt. A portion of the sample was stirred in 1 *N* HCl for 1 hr to give the monohydrochloride salt, mp >264°.

***N*-[*p*-[[7-Amino-5(6*H*)-oxopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoyl]-L-glutamic Acid Sesquihydrate (7-Azafolic Acid) (11c).** A solution of crude **10b** (1.68 g) in DMSO (30 ml) containing $KHCO_3$ (3.37 g) and H_2O (17 ml) was heated with stirring at 90° for 18 hr and diluted with 1 *N* HCl (51 ml) and H_2O (750 ml). The precipitate (0.75 g) was collected by filtration, washed with Et_2O (100 ml), and dissolved in oxygen free 0.2 *N* NaOH (100 ml). After 18 hr at room temperature, the solution was acidified with dilute HCl (~pH 3, paper) and the precipitate (0.55 g) was collected under N_2. The pmr spectrum of this product showed that ester hydrolysis was about 85% complete. Retreatment of this product with 0.2 *N* NaOH for 18 hr gave **11c:** yield, 0.40 g (30.5%). This sample soften at about 240° but melted >264°. Elemental analysis showed the absence of chloride.

Ethyl *p*-[[(7-Amino-5(6*H*)-thioxopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoate (12a). A solution of 10a (0.54 g) in DMF (20 ml) containing hydrated NaSH (0.54 g) and H_2O (3.5 ml) was heated with stirring at 80° for 2 hr and evaporated to dryness *in vacuo*. This residue was washed with H_2O (85 ml), and the wash was extracted with Et_2O (3 × 35 ml portions) and acidified with 1 *N* HCl. The product (0.40 g) was collected by filtration and reprecipitated from 1 *N* NH_4OH by the addition of dilute HCl (pH ~3, paper). The solid was collected by filtration, extracted successively by stirring with C_6H_6 (50 ml) and EtOH (5 ml), and dried *in vacuo* over P_2O_5 at 78°: yield, 0.18 g (40%); mp ~264°. Elemental analysis indicated the absence of chlorine.

Diethyl *N*-[*p*-[[(7-Amino-5(6*H*)-thioxopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoyl]-L-glutamate Monohydrochloride (12b). A mixture of **10b** (2.1 g) and hydrated sodium hydrosulfide (1.0 g) in ethanol (200 ml) was refluxed for 3 hr, cooled to room temperature, and filtered through a Celite pad. The filtrate was evaporated to dryness *in vacuo*, the residue was stirred with water (650 ml), and the resulting mixture was filtered through a Celite pad. The filtrate was adjusted to pH 3.5 (paper) with 1 *N* hydrochloric acid, and the resulting gelatinous precipitate was collected by filtration, washed successively with ether (120 ml) and benzene (120 ml) and dried *in vacuo* over P_2O_5: yield, 0.92 g (48%); melting point indefinite. This sample underwent softening and decomposition from about 164°.

Concentration of the filtrate from above to ~¼ volume gave an additional crop of product: yield, 0.18 g (yield, 9.4%). The total yield was 57.4%.

***N*-[*p*-[[(7-Amino-5(6*H*)-thioxopyrimido[5,4-*e*-]-*as*-triazin-3-yl)methyl]amino]benzoyl]-L-glutamic Acid Monohydrate (12c).** Sodium hydroxide (0.2 *N*, 94 ml) was boiled to expel oxygen and cooled to room temperature under N_2. A solution of **12b** (0.91 g) in this medium was stirred at room temperature for 24 hr, filtered, and acidified to pH 3.4 (paper) with 1 *N* HCl. After the gelatinous precipitate was allowed to stand in the medium for 3 hr, the solid was collected by filtration and stirred with a 1:1 mixture of ethanol and water (120 ml) for 30 min. The solid was collected by filtration and dried *in vacuo* over P_2O_5: yield, 0.51 g (65%); melting point indefinite. This sample underwent softening and decomposition from 255°.

A sample of the product dissolved in dilute NH_4OH showed on tlc after 2 weeks mainly **11c** and several minor spots, one of which corresponded to *p*-aminobenzoic acid.

Ethyl *p*-[[(5,7-Diaminopyrimido[5,4-*e*]-*as*-triazin-3-yl)-methyl]amino]benzoate (13a). A solution of **10a** (223 mg) in DMAc (20 ml) containing 10% ethanolic NH_3 (10 ml) was heated at 60° for 5 hr. The resulting mixture was cooled, and the product was collected by filtration and dried *in vacuo* over P_2O_5 at 140°. This sample analyzed correctly for a partial hydrate of **13a:** yield, 105 mg (61%); mp >264°. The monohydrate was obtained by reprecipitation of this sample from DMSO with H_2O, which was dried *in vacuo* over P_2O_5 at 78°.

The reaction filtrate gave an additional 65 mg of crude **13a.**

Diethyl *N*-[*p*-[[(5,7-Diaminopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoyl]-L-glutamate Hydrate (4:3) (13b) and Diethyl *N*-[*p*-[[(5-Aminotetrazolo[5′,1′:6,1]pyrimido[5,4-*e*]-*as*-triazin-8-yl)methyl]amino]benzoyl]-L-glutamate Hydrate (4:7) (14b). A solution of **10b** (0.80 g) in DMSO (10 ml) containing H_2O (1 ml) and sodium azide (0.20 g) was heated at 80° for 2 hr and diluted with H_2O (90 ml). The resulting mixture was cooled; the solid was collected by filtration, washed with Et_2O (20 ml) to remove benzyl disulfide, and then with 1:1 EtOH–H_2O (10 ml), and dried *in vacuo* over P_2O_5 at 78° to give **13b:** yield, 0.20 g (29.6%); melting point indefinite. This sample underwent softening and decomposition from 140°.

The reaction filtrate was evaporated to dryness *in vacuo*, and the resulting residue was washed with Et_2O and hot MeCN to give a solid. From the MeCN wash crude **10b** (0.11 g) was recovered. The MeCN insoluble residue was dissolved in H_2O and adjusted to pH 3–4 (paper) with 1 *N* HCl to give a precipitate of **14b:** yield, 0.10 g (13.6%); melting point indefinite. This sample underwent softening and decomposition from ~200°.

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Registry No.—**1b,** 51934-15-7; **1c,** 51934-16-8; **1d,** 51934-17-9; **2b,** 51934-18-0; **2c,** 51934-19-1; **3,** 7597-91-3; **4a,** 94-09-7; **4b,** 18358-63-9; **4c,** 51934-20-4; **5b** HCl, 51934-21-5; **5c** HCl, 51934-22-6; **6a,** 22433-08-5; **6b,** 51934-23-7; **6c,** 51043-64-2; **6d,** 3009-97-0; **6e,** 51934-24-8; **10a,** 35171-06-3; **10b,** 51043-66-4; **11a,** 35171-24-5; **11a** HCl, 51934-25-9; **11c,** 51043-68-6; **12a,** 35171-08-5; **12b** HCl, 51934-26-0; **12c,** 51934-27-1; **13a,** 35171-07-4; **13b,** 51934-28-2; **14b,** 51934-29-3.

References and Notes

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