Pyrimido[5,4-*e*]-*as*-triazines. VIII. Synthesis of 7-Azaaminopterin[1]

Carroll Temple, Jr.,* Conrad L. Kussner, and John A. Montgomery

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

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7-Azaaminopterin (**25**) was prepared as a potential inhibitor of dihydrofolic reductase and the enzymes involved in the interconversions of tetrahydrofolates. The projected route for the preparation of **25** required an investigation of the preparation and reactions of 3-(halomethyl)pyrimido[5,4-*e*]-*as*-triazines. Amination of both 3-(chloromethyl)-5-methoxy- and 7-amino-5-(benzylthio)-3-(chloromethyl)pyrimido[5,4-*e*]-*as*-triazines (**1** and **19**), respectively, with ethyl *p*-aminobenzoate resulted in displacement of the 5 substituent to give ring-substituted products. Similarly, oxidative amination of 5-chloro-3-(chloromethyl)-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine (**12**) with NH_3 in the presence of Ag_2O gave the corresponding 5-amino-3-(chloromethyl) derivative **7**. In contrast to the results obtained with **1** and **19**, treatment of **7** with ethyl *p*-aminobenzoate displaced the chloro group to give **9**. In the presence of KI, reaction of **19** with NaN_3 and *p*-aminobenzoyl-L-glutamic acid, respectively, replaced the chloro group to give the corresponding 3-methyl substituted products **22** and **23**. The interaction of **23** with NaN_3 resulted in displacement of the benzylthio group followed by conversion of the intermediate azido substituent to an amino group during the reaction to give 7-azaaminopterin (**25**). The latter showed no significant activity either against dihydrofolic reductase (pigeon liver) or in the KB cell culture screen, but was an inhibitor of *Streptococcus faecium* ATCC 8043.

The triazine ring of 7-azapteridines is easily reduced chemically, suggesting that the enzymatic reduction of appropriate derivatives of this ring system might also occur readily.[2-4] Previously, 7-azafolic acid (**24**) was prepared with the expectation that this compound would be a substrate for dihydrofolic reductase and produce hydro derivatives of **24** in vivo.[5] **24** was found not to be a substrate for dihydrofolic reductase from pigeon liver, but this lack of interaction was attributed to the electronic nature rather than to a structural effect of the 7-azapteridine ring. To increase the basicity of this ring system 7-azaaminopterin was prepared, which is not only a potential inhibitor of dihydrofolic reductase, but if a substrate for this enzyme, the resulting hydro derivatives might inhibit thymidylate synthetase[6] or other enzymes that utilize the reduced forms of folic acid.[7a] Earlier the preparation of 7-azafolic acid and the diethyl ester of 7-azaaminopterin from the corresponding diethyl glutamate derivative of a 4-(benzylthio)-7-azapteridine intermediate was described.[5] Simultaneously with the above investigation, work was carried out on the reactions of 6-(halomethyl)-7-azapteridines, which eventually led to the synthesis of 7-azaaminopterin [*N*-[*p*-[[(5,7-diaminopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoyl]-L-glutamic acid] (**25**).

In nucleophilic displacement reactions, benzyl-type halides and haloheterocyclics are known to be more reactive than methoxyheterocyclics.[8] Previously, we reported the preparation of **1**,[9] which was chosen initially for the investigation of the preferred reaction site in the displacement of groups from the 4 position of the ring and from the 6-methyl group. Although treatment of **1** with ethyl *p*-aminobenzoate in refluxing dioxane was expected to give **2**, the only identified product obtained from this reaction was a 26% yield of **3**. This result indicated that the 4-methoxy group was activated more by the ring than the benzyl-type chloromethyl group. The amino group was chosen to circumvent this problem as it should be less susceptible to nucleophilic displacement from the ring than other heteroatom-containing groups. To prepare this type of compound, **4**[10] was treated with Br_2 in HOAc at reflux and appeared to be converted to some extent to the bromomethyl compound **5** (^1H NMR), but TLC data showed that the reaction product was a complex mixture containing unreacted **4**. Previously, reaction of **6** with Br_2 in $CHCl_3$ at room temperature was found to give a low yield of an α-bromoacetic acid derivative.[9] In an effort to increase the yield of this product, this reaction was repeated at 55°, but the crude product that precipitated was identified as the dibromo compound **10** by reaction with NaN_3, which gave a low yield of the diazido derivative **11**. This result suggested that the preparation of the monobromo derivative of **6** in high yield might be difficult. The successful route to the desired type of compound involved the oxidative amination of **12**.[9] Treatment of **12** with Ag_2O in a mixture of NH_3 and dioxane gave a good yield of **7**, presumably formed via the intermediate **8**. The structure of **7** was confirmed by the similarity of its uv spectrum with that of **4**. Since intermediate **8** contains the same leaving group in the ring and side chain, the obtainment of **7** provided additional support for the greater reactivity of substituents at C-4 compared to the chlorine of the chloromethyl group. However, the 4-amino group is not preferentially displaced and the amination of **7** with ethyl *p*-aminobenzoate gave **9** identified by its uv and ^1H NMR spectra.

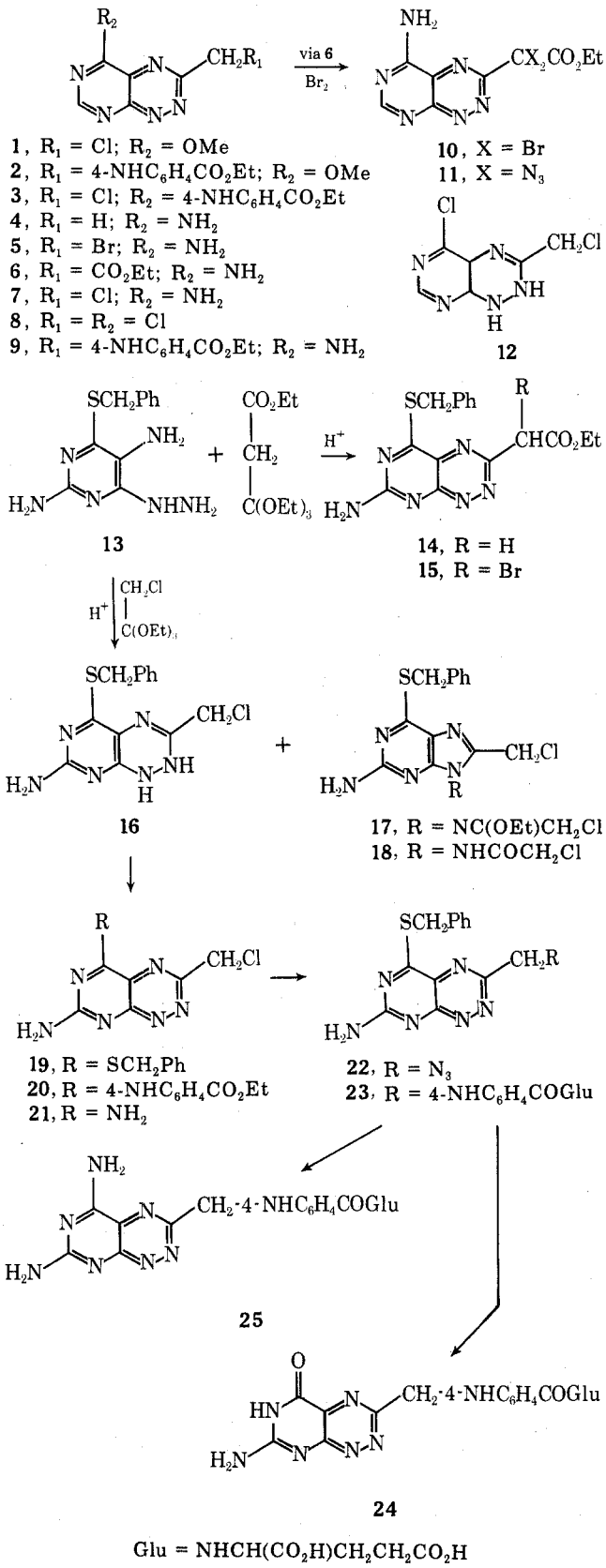
Simultaneously with the work on **6** described above, the bromination of **14**, prepared by the condensation of **13** with ethyl ortho(ethoxycarbonyl)acetate,[11] presumably via the air oxidation of the corresponding dihydro derivative, to give **15** was attempted. However, the conversion of **14** to **15** was unsuccessful, apparently because of the loss of the benzylthio group during the reaction. In another approach, the condensation of **13** with ethyl ortho(chloro)acetate[12] gave two products, **16** and **17**. The latter was identified by its uv spectrum and by conversion with dilute acid to the amide **18**. Apparently the air oxidation of **16** to **19** was prevented by the precipitation of **16** from the reaction mixture as its HCl salt. However, the oxidation of **16** to give **19** was effected with Ag_2O in dioxane.

We next determined the best sequence of reactions for the incorporation of the 4-amino group and the *p*-aminobenzoylglutamic acid side chain. As might be expected, treatment of **19** with ethyl *p*-aminobenzoate in refluxing dioxane gave a low yield of **20** as the only identifiable product. In contrast, under the same conditions, reaction of **19** with a hindered amine, methyl *p*-(methylamino)benzoate, replaced neither the benzylthio nor the chloro group.[10] Since previous observations indicated that 4-azido-7-azapteridines are converted by proton abstraction (from the solvent) to 4-amino-7-azapteridines,[5,9,13] treatment of **19** with NaN_3 was expected to give **21**. However, when this reaction was carried out in DMAC containing KI at room temperature, a good yield of the azidomethyl compound **22**

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}	^1H NMR spectral assignments,[c] chemical shifts, δ (rel area)	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
3	268 (21.2), 412 (11.3)	1700, 1600		$C_{15}H_{13}ClN_6O_2$[d]	52.26	3.80	24.38	52.04	3.63	24.07
7	256 (13.5), 290 sh (2.48), 375 (5.47)	1620, 1560	5.26 (2, CH_2), 8.68 (1, CH), 8.88 (2, NH_2)	$C_6H_5ClN_6$[e]	36.66	2.56	42.75	36.66	2.49	42.38
9	256 (15.2), 297 (21.4), 374 (5.52)	1685, 1640 1600	1.28 t (3, CH_3), 4.22 q, 4.98 (2, 2, CH_2), 7.11 (1, NH), 7.29 (4, C_6H_4), 8.65 (1, CH), 8.88 br (2, NH_2)	$C_{15}H_{15}N_7O_2 \cdot H_2O$	52.47	4.99	28.56	52.58	5.17	28.36
11	258 (12.7), 295 sh (2.65), 383 (5.01)	2120, 1750 1645	1.21 (3, CH_3), 4.37 (2, CH_2), 8.70, 8.92 br (3, CH, NH_2)	$C_9H_8N_{12}O_2$	34.18	2.55	53.15	34.23	2.58	52.97
14	272 (9.46), 349 (2.68), 422 (5.20)	1725, 1635	1.18 t (3, CH_3), 4.15 q, 4.26 (4, OCH_2, CH_2), 4.53 (2, CH_2), 7.43 (5, C_6H_5), 7.95 (2, NH_2)	$C_{16}H_{16}N_6O_2S$	53.92	4.53	23.58	53.94	4.63	23.57
16	252 sh (13.2), 337 (5.84), 403 (2.40)[f]	1655, 1600	3.98, 4.31 (2, 2, CH_2), 7.37, 7.55 br (7, C_6H_5, NH_2), 8.88, 10.87 (1, 1, NH), ~11.5 (HCl)	$C_{13}H_{13}ClN_6S$[g]·HCl	43.71	3.95	23.51	43.73	3.93	23.53
17	252 sh (14.4), 318 (16.3)	1650, 1630		$C_{17}H_{18}Cl_2N_6OS$·HCl	44.22	4.15	18.20	44.15	4.18	18.64
18	252 sh (13.6), 317 (15.2)	1680, 1615	4.37, 4.54, 4.71 (6, CH_2), 6.04 (NH_2), 7.33 (5, C_6H_5), 11.87 (1, NH)	$C_{15}H_{14}Cl_2N_6OS$	45.35	3.55	21.15	45.73	3.66	21.22
19	277 (13.5), 377 (3.16), 420 (5.85)	1635, 1560	4.52, 5.08 (CH_2), 7.33 (C_6H_5), 8.00 (NH_2)	$C_{13}H_{11}ClN_6S$	48.98	3.48	26.36	49.14	3.46	25.72
20	256 sh (19.2), 277 (24.0), 428 (9.03)[h]	1700, 1660 1620	1.34 t (3, CH_3), 4.32 q (2, CH_2O), 5.14 (2, CH_2Cl), 7.61 (2, NH_2), 8.13 (4, C_6H_4), 10.55 (1, NH)	$C_{15}H_{14}ClN_7O_2 \cdot 1.33H_2O$	46.94	4.38	25.55	47.05	4.50	25.17
22	273 (14.5), 345 sh (3.37), 421 (6.35)	2100, 1635 1620	4.57, 4.90 (CH_2), 7.43 m (C_6H_5, C_6H_6[i]), 8.04 br (NH_2)	$C_{13}H_{11}N_9S \cdot 0.25C_6H_6 \cdot 0.44H_2O$	49.35	3.82	35.72	49.36	3.91	35.78
23	274 (27.8), 4.22 (8.02)	1720, 1620	2.04, 2.33 (CH_2-CH_2), 4.39 (NCH), 4.52, 4.82 (CH_2), 7.20 (C_6H_4), 7.45 (C_6H_5)[j]	$C_{25}H_{23}N_8O_5SK$	51.18	3.95	19.10	50.92	4.11	19.04
25	266 (20.0), 293 sh (14.3), 348 br (8.15)[f]	1715, 1600	2.05, 2.36 (CH_2-CH_2), 4.39 (NCH), 4.78 (CH_2), 7.20 (C_6H_4)[j]	$C_{18}H_{19}N_9O_5 \cdot 0.15DMAC \cdot 1.33HCl$	44.42	4.35	25.48	44.71	3.88	25.22

[a] Spectra were determined on solutions obtained by dissolving the samples in a mixture of 8% DMSO and 92% MeOH on a Cary Model 17 spectrophotometer. [b] Perkin-Elmer Model 621 spectrophotometer. [c] Spectra were determined on DMSO-d_6 solutions (2–10% w/v) on a Varian A-60A and XL-100-15 spectrometers with Me_4Si 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] Calcd: Cl, 10.28. Found: Cl, 10.22. [e] Calcd: Cl, 18.03. Found: Cl, 18.18. [f] Determined in 0.1 *N* HCl. [g] Calcd: Cl; 19.85. Found: Cl, 19.64. [h] Determined in a mixture of 8% DMSO and 92% MeOH. [i] Recrystallization solvent. [j] The assigned peaks shown were poorly resolved and are based on the position of the corresponding peaks in the diethyl ester derivative.



was obtained. Presumably, in the presence of KI the chloro group of **19** underwent exchange with iodide, resulting in activation of this position. Under similar conditions, reaction of **19** with *p*-aminobenzoyl-L-glutamic acid gave **23** isolated as its monopotassium salt. The structure of this product was confirmed by treatment of **23** with base to give the known 7-azafolic acid (**24**).[5] Similarly, reaction of **23** with NaN_3 replaced the benzylthio group and resulted in the formation of 7-azaaminopterin (**25**).

The uv and ^1H NMR spectra and selected bands in the ir spectra for the new compounds are presented in Table I.

Against dihydrofolic reductase from pigeon liver, **25** (I_{50} 2.7×10^{-5} *M*) was less than one-thousandth as active as methotrexate (I_{50} 2×10^{-9} *M*).[14] Also, **25** was only slightly cytotoxic (ED_{50} 61 μg/ml) in the KB cell culture system.[15] Results similar to these were also observed with 7-azafolic acid, but surprisingly both **25** (ED_{50} 2.7×10^{-8} *M*) and **24** (ED_{50} 1.5×10^{-9} *M*) were good inhibitors of *Streptococcus faecium* ATCC 8043 (methotrexate, $ED_{50} < 10^{-10}$ *M*).[16] A possible explanation for the difference in activities observed in the *S. faecium* and KB tests is that the 7-azapteridines are reduced to hydro derivatives by the bacterium but not by mammalian cells.[7b]

Experimental Section[17]

Ethyl *p*-[[3-(Chloromethyl)pyrimido[5,4-*e*]-*as*-triazin-5-yl]amino]benzoate (3). A solution of 1 (500 mg)[9] and ethyl *p*-aminobenzoate (780 mg) in dioxane (25 ml) was refluxed for 4 hr. From the cooled reaction mixture, the precipitate was collected by filtration and extracted with $CHCl_3$; the residue obtained by evaporation of the extract was recrystallized from C_6H_6, yield 208 mg (26%), mp 245–246° dec.

5-Amino-3-(chloromethyl)pyrimido[5,4-*e*]-*as*-triazine (7). A mixture of **12** (500 mg)[9] and Ag_2O (1.35 g) in 3% anhydrous NH_3–dioxane (50 ml, v/v) was stirred at room temperature for 18 hr. The residue was removed by filtration, and the filtrate was evaporated to dryness in vacuo, yield 335 mg. For analyses a sample was recrystallized from THF, mp 212–213° dec.

Ethyl *p*-[[(5-Aminopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoate (9). A solution of **7** (391 mg) and ethyl *p*-aminobenzoate (706 mg) in DMAC (25 ml) was heated at 120° for 4 hr and evaporated to dryness in vacuo. The resulting residue was washed with Et_2O, recrystallized from EtOH, and dried at 78° in vacuo over P_2O_5, yield 95 mg, mp 160° dec with presoftening.

The ethanol filtrate provided an additional 208 mg of crude **9**.

Ethyl 5-Amino-α,α-diazidopyrimido[5,4-*e*]-*as*-triazine-3-acetate (11). A solution of **6** (1.0 g)[9] in $CHCl_3$ (150 ml) containing Br_2 (0.25 ml) was heated at 55° for 6 hr. The cooled reaction mixture was filtered, and the resulting residue (0.96 g) was treated with NaN_3 (0.40 g) in 5:1 EtOH–H_2O (30 ml) at room temperature for 60 hr. This solution was evaporated to dryness, and the residue was washed with H_2O and recrystallized from C_6H_6, yield 0.11 g, mp 154°.

Ethyl 7-Amino-5-(benzylthio)pyrimido[5,4-*e*]-*as*-triazine-3-acetate (14). To a mixture of **13** (0.50 g)[3] and 1 *N* HCl (0.25 ml) in H_2O (25 ml) was added ethyl ortho(ethoxycarbonyl)acetate[11] (5.0 ml) with vigorous stirring. After 18 hr the orange product was collected by filtration, washed with H_2O, and dried in vacuo over P_2O_5, yield 0.31 g (46%), mp 201°.

7-Amino-5-(benzylthio)-3-(chloromethyl)-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine (16). To a mixture of **13** (2.0 g)[3] and ethyl ortho(chloro)acetate (20 ml)[12] was added concentrated HCl (0.71 ml) with stirring. After 15 min the solid was collected by filtration and washed with Et_2O to give crude **16**, yield 1.5 g, mp 213° dec with presoftening. A portion of this solid (0.24 g) was recrystallized from MeOH and then MeCN to give pure **16**, yield 0.08 g, mp 219° dec.

The reaction filtrate from the first crop was stirred for an additional 15 min to deposit a second crop, which was collected by filtration and washed with Et_2O, yield 0.83 g, mp 139° dec with presoftening. The product was mainly the purine **17**, identified by its ultraviolet spectrum.

A portion of the above solid (0.5 g) was stirred in 0.1 *N* HCl for 18 hr, the solid was collected by filtration and recrystallized from EtOH–hexane to give **18**, yield 0.1 g, mp 217° dec with presoftening.

7-Amino-5-(benzylthio)-3-(chloromethyl)pyrimido[5,4-*e*]-*as*-triazine (19). A mixture of crude **16** HCl (0.50 g) and Ag_2O in dioxane was stirred at room temperature for 72 hr. After filtration the filtrate was evaporated to dryness, and the resulting residue was extracted with hot C_6H_6 (300 ml). Concentration of the extract deposited **19**, yield 0.03 g, mp 243° dec.

An additional amount of crude **19** was obtained by evaporation of the C_6H_6 filtrate to dryness in vacuo, yield 0.28 g, mp 215–217° dec.

Ethyl [*p*-[7-Amino-3-(chloromethyl)pyrimido[5,4-*e*]-*as*-

triazin-5-yl]amino]benzoate (20). A solution of **19** (150 mg) and ethyl *p*-aminobenzoate (155 mg) in dioxane (20 ml) was refluxed for 72 hr and evaporated to dryness in vacuo. The residue was triturated with Et_2O, and the resulting solid was reprecipitated from a DMSO solution by the addition of H_2O, yield 46 mg (22%), mp 157° dec taken rapidly. A sample was dried in vacuo over P_2O_5 at 78° for analysis. The ^1H NMR spectrum indicated that this sample was contaminated with a trace amount of an unidentified material.

7-Amino-3-(azidomethyl)-5-(benzylthio)pyrimido[5,4-*e*]-*as*-triazine (22). A mixture of **19** (100 mg), NaN_3 (25 mg), and KI (55 mg) in DMAC (2 ml) was stirred at room temperature for 18 hr and diluted with H_2O (10 ml), and the resulting precipitate was collected by filtration and recrystallized from C_6H_6: yield, 42 mg (60.5%); mp 210° dec; M^+ *m/e* 325. The ^1H NMR spectrum of this sample showed the presence of C_6H_6.

***N*-[*p*-[[[7-Amino-5-(benzylthio)pyrimido[5,4-*e*]-*as*-triazin-3-yl]methyl]amino]benzoyl]-L-glutamic Acid (23).** A mixture of **19** (500 mg, 1.57 mmol), *p*-aminobenzoyl-L-glutamic acid (425 mg, 1.59 mmol), and KI (250 mg) in DMAC (10 ml) was stirred at room temperature for 40 hr and diluted with H_2O (100 ml). The resulting precipitate was collected by filtration, washed with H_2O and Et_2O, and dried in vacuo over P_2O_5, yield 648 mg. This material appeared to decompose at 203°. A solution of this product in ethanolic HCl showed several spots on TLC (9:1 $CHCl_3$–MeOH), one of which was identical with that of the diethyl ester of **23**.[5]

When a portion of this sample was treated with 0.1 *N* HCl to obtain the free acid, elemental analyses indicated partial loss of the benzylthio group in the recovered material.

7-Azafolic Acid (24).[5] A mixture of **23** (50 mg) in oxygen-free 0.1 *N* NaOH (10 ml) was stirred at room temperature for 18 hr, neutralized with 1 *N* HCl, and centrifuged. The resulting residue was washed with Et_2O and identified as **24** by TLC [BuOH (5)–HOAc (2)–H_2O (3)] and by its uv and ir spectra, yield 19 mg.

Also, this compound was prepared by treatment of a solution of **23** (50 mg) in DMSO (2 ml) containing $KHCO_3$ (100 mg) and H_2O (1 ml) at 90° for 18 hr, yield 10 mg.

***N*-[*p*-[[(5,7-Diaminopyrimido[5,4-*e*]-*as*-triazin-3-yl)methyl]amino]benzoyl]-L-glutamic Acid (7-Azaaminopterin 25).** A mixture of **23** (200 mg) and NaN_3 (100 mg) in DMSO (2 ml) was heated with stirring at 90° for 4 hr and diluted with H_2O (20 ml), and the resulting solution was adjusted to pH 2 (paper) with 1 *N* HCl and centrifuged. The residue was washed successively with 0.1 *N* HCl, Et_2O, 10% aqueous DMAC, and H_2O and dried in vacuo over P_2O_5, yield 95 mg, mp >270°. A solution of this product in ethanolic HCl was shown to contain the diethyl ester of **25**[5] by TLC (EtOH). Another spot in the TLC of this solution was identified as the diethyl ester of **24**, presumably formed via acidic hydrolysis of the 5-amino group of **25** or its diethyl ester.

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References and Notes

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Synthesis of 3,3a-Dihydro-8*H*-pyrazolo[5,1-*a*]isoindol-8-ones and 8*H*-Pyrazolo[5,1-*a*]isoindol-8-ones

E. W. Bousquet, M. D. Moran, J. Harmon, A. L. Johnson,* and J. C. Summers

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3-(4-Methoxyphenacyl)phthalide (**3**) arises from the base-catalyzed condensation of phthalaldehydic acid (**1**) with 4-methoxyacetophenone (**2**), and readily undergoes cyclization with hydrazine to form 2-(4-methoxyphenyl)-3,3a-dihydro-8*H*-pyrazolo[5,1-*a*]isoindol-8-one (**4**). Dehydrogenation of **4** produces 2-(4-methoxyphenyl)-8*H*-pyrazolo[5,1-*a*]isoindol-8-one (**5**). This synthetic sequence is completely general, and may be used to prepare numerous analogs of structures **3–5**.

The condensation of 3,4-dimethoxyphthalaldehydic acid (opianic acid) with acetone and acetophenone under Claisen conditions to give 1:1 and 1:2 products was described in 1891 by Goldschmiedt,[1] elaborated by Hemmelmayr[2,3] shortly thereafter, and extended to phthalaldehydic acid (**1**) by Hamburger[4] in 1898. We have found their structural assignments of these reaction products as ketonic phthalide derivatives to be essentially correct,[5] and have investigated their further reaction products with hydrazine.[6,7] Hamburger[4] described the reaction products of 3-phenacylphthalide with hydroxylamine and phenylhydrazine, but his structural conclusions were uncertain, and the present work establishes the reaction course of carbonyl reagents with these interesting compounds.

Our exploratory work was done with the condensation product of **1** and 4-methoxyacetophenone (**2**) (Scheme I).