Synthesis of the Pyrimido[5,4-*e*]-*as*-triazine Antibiotics Fervenulin and 2-Methylfervenulone[1,2]

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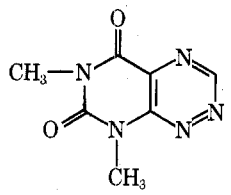
The antibiotic fervenulin (6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione, 1) has been synthesized by two different methods starting with 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil (3). In the first, 3 was cyclized with sodium ethoxide to fervenulone (6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione, 5), which was then converted to 1 by a sequence of reactions involving chlorination to 3-chloro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (6), reaction with hydrazine to give the 3-hydrazino derivative (7), and oxidation with mercuric oxide. In the second, 3 was converted to 1 *in a single step* by heating with a mixture of dimethylformamide and phosphorus oxychloride. The mechanism of this remarkable transformation is discussed. Treatment of 3 with diethyl azodicarboxylate or with lead tetraacetate gave 4-carbethoxyamino-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(4*H*,6*H*,8*H*)-trione ethanolate (15). The structure of 15 was unequivocally established by hydrogenolysis to 1,2-dihydro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(4*H*,6*H*,8*H*)-trione ethanolate (16), which was then synthesized independently from 1,3-dimethyl-6-(2-carbethoxyhydrazino)uracil by nitrosation, reduction of the resulting 5-nitroso derivative, and finally base-catalyzed intramolecular cyclization. Treatment of 16 with DDQ or, less efficiently, by exposure of solutions of 16 to air, provided a second synthesis of fervenulone (5). The antibiotic MSD-92 (2-methylfervenulone, 2,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione, 17) has been synthesized by methylation of fervenulone (5) with methyl iodide or diazomethane, as well as by an unequivocal total synthesis. Thus, reaction of 1,3-dimethyl-5-carbethoxyaminobarbituric acid with phosphorus oxychloride gave 1,3-dimethyl-5-isocyanato-6-chlorouracil (29). Addition of methylhydrazine to 29 then gave 32, which was cyclized to 17 with aqueous sodium acetate in the presence of a stream of air. A number of chemical transformations of these compounds and the preparation of the remaining monomethyl derivatives of fervenulone (18 and 20) are described.

Fervenulin. Fervenulin (1), also known as planomycin, is a crystalline, broad-spectrum antibiotic, isolated from cultures of *Streptomyces fervens* n. Sp.[3] and from *Streptomyces rubrireticuli*,[4] which possesses an interesting spectrum of biological activities.[5] It has been identified[6] as 6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (1),[7] and is a representative of a unique family of naturally occurring pyrimido[5,4-*e*]-*as*-triazine (7-azapteridine) antibiotics which includes toxoflavin (2)[3b,8] and 2-methylfervenulone (MSD-92, 17) (vide infra).[2b,9] There has been considerable recent interest in the development of synthetic approaches to fervenulin;[10-15] in this paper we present a full account of our own investigations.[2a]



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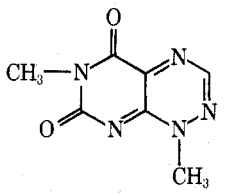

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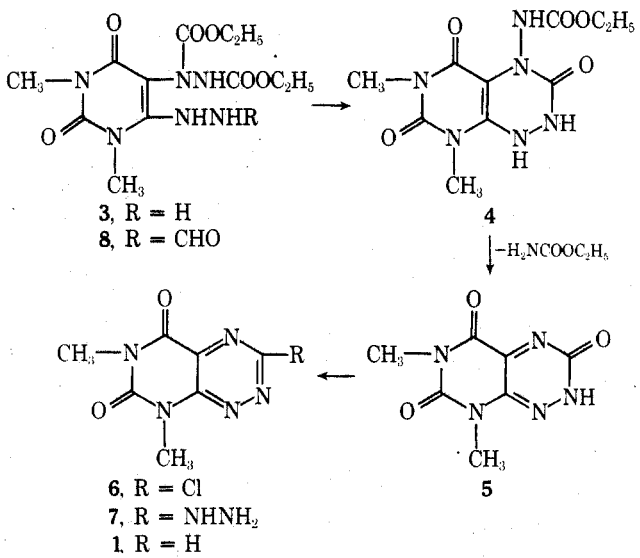
We have recently reported[16] that 5-(1,2-dicarbethoxyhydrazino)pyrimidines are readily formed by the reaction of 6-amino- or 6-hydrazinopyrimidines with diethyl azodicarboxylate. Thus, 1,3-dimethyl-6-hydrazinouracil reacts with diethyl azodicarboxylate to give 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil (3) in 71% yield. We now report the conversion of this readily available intermediate by two different routes to the antibiotic fervenulin.

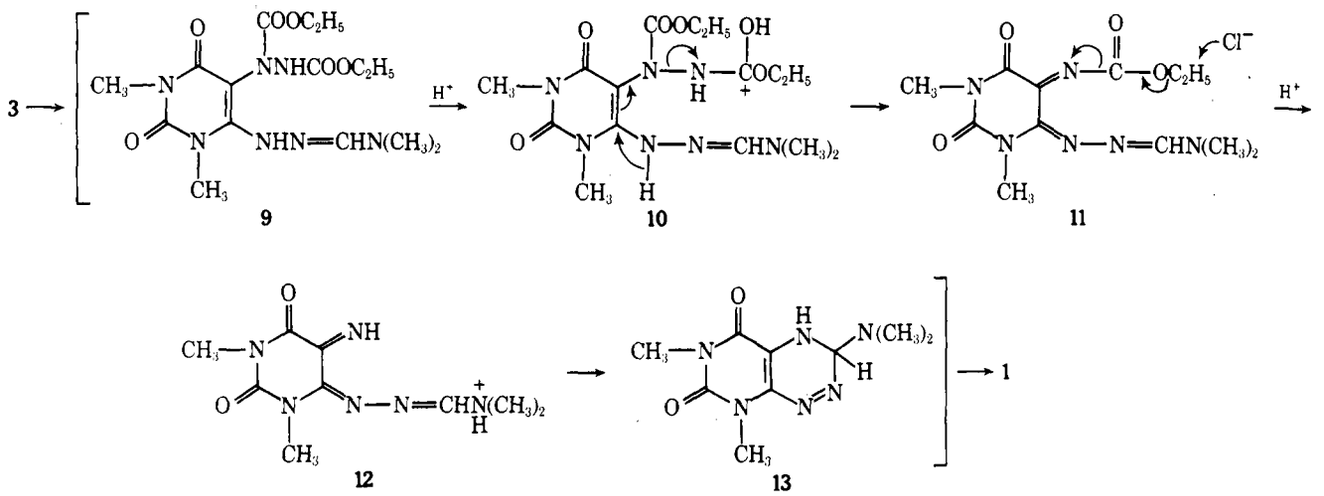
In the first of these routes, treatment of the Michael adduct 3 with sodium ethoxide in absolute ethanol (under scrupulously anhydrous conditions) led directly to the formation of 6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7-(2*H*,6*H*,8*H*)-trione (5), for which, in view of its role as a crucial intermediate for the synthesis of both fervenulin and 2-methylfervenulone and its structural relationship to both of these antibiotics, we propose the name *fervenulone*. It seems reasonable to assume an initial cyclization of 3 to 1,4-dihydro-4-carbethoxyamino-6,8- dimethylpyrimido[5,4-*e*]- *as*-triazine-3,5,7(2*H*,6*H*,8*H*)- trione (4), which then aromatizes by base-catalyzed 1,4 elimination of ethyl carbamate (see Scheme I). Fervenulone (5) could alternately be prepared in somewhat better overall yield by initial formylation of 3 to give 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-(2-formylhydrazino)uracil (8), followed by cyclization with sodium ethoxide in absolute ethanol. Fervenulone (5) forms stable solvates which, in contrast to the anhydrous, unsolvated compound, are elegantly crystalline. Thus, recrystallization of 5 from ethanol gave a crystalline ethanolate, which upon recrystallization from water then gave a hydrate; the cycle can also be reversed.

Scheme I



Conversion of fervenulone (5) to fervenulin (1) was accomplished as follows. Treatment of 5 with phosphorus oxychloride (or, less satisfactorily, with phosphorus pentachloride) gave 3-chloro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (6). Reductive removal of the 3-chloro substituent was then carried out in two steps (direct

Scheme II



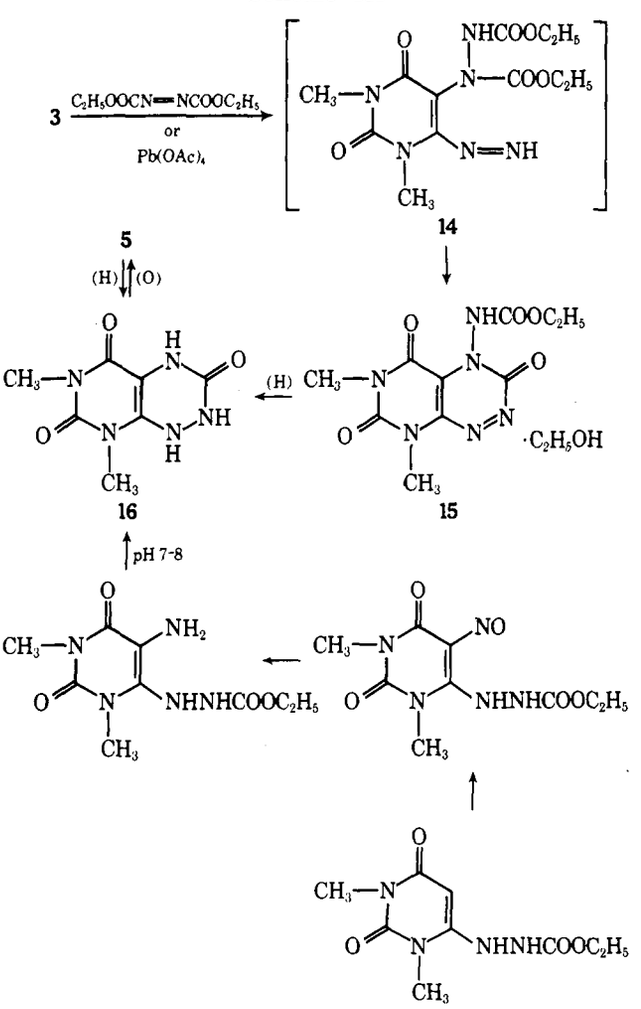
reduction was avoided because of the presence of the sensitive azo linkage in 1) by (a) reaction with hydrazine to give the 3-hydrazino derivative **7**, and (b) oxidation of **7** with mercuric oxide.[17] The product was identical in every respect with authentic fervenulin.[18]

The second method for the conversion of **3** to fervenulin (1) is, because of its extraordinary simplicity, without question the method of choice for the preparation of this antibiotic. Thus, fervenulin was formed in a single step by treatment of **3** with phosphorus oxychloride–dimethylformamide. When the reaction was carried out at 60° rather than at 125°, 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-dimethylaminomethylenehydrazinouracil (**9**)[19] separated directly from the reaction mixture, suggesting that a possible reaction course for this remarkable ring annelation might be as depicted in Scheme II. Key steps in the overall conversion are the loss of ethyl carbamate from **10** (for which the aromatization of **4** to **5** under basic conditions provides a precedent), loss of the carbethoxy grouping from **11** (for which there is ample precedent in carbamate chemistry),[20] and the cyclization step (**12** → **13** → **1**) involving the loss of dimethylamine (for which the Fischer indole synthesis provides a parallel,[21] although a concerted, electrocyclic reaction followed by aromatization with loss of dimethylamine cannot be excluded).

In the preparation of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil (**3**) from 1,3-dimethyl-6-hydrazinouracil and diethyl azodicarboxylate, a very small amount of a by-product could be isolated from the mother liquors which has been identified as 4-carbethoxyamino-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(4*H*,6*H*,8*H*)-trione ethanolate (**15**). This compound proved to be the major product of the reaction of 1,3-dimethyl-6-hydrazinouracil with 2 mol of diethyl azodicarboxylate, and we suggest that it is probably formed by the sequence of reactions outlined in Scheme III. Since **3** does not cyclize in the absence of base, the initial reaction of the Michael adduct **3** with excess diethyl azodicarboxylate may well be oxidation of the hydrazino substituent to a diimide (**14**),[22] which then ring closes with loss of ethanol to **15**.[23,24] Some support for this suggestion comes from the observation that the conversion of **3** to **15** could also be effected with lead tetraacetate, a reagent known to oxidize arylhydrazines to aryldiimides.[25]

The structure of **15** was confirmed by reduction to 1,4-dihydro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (dihydrofervenulone, **16**), identical

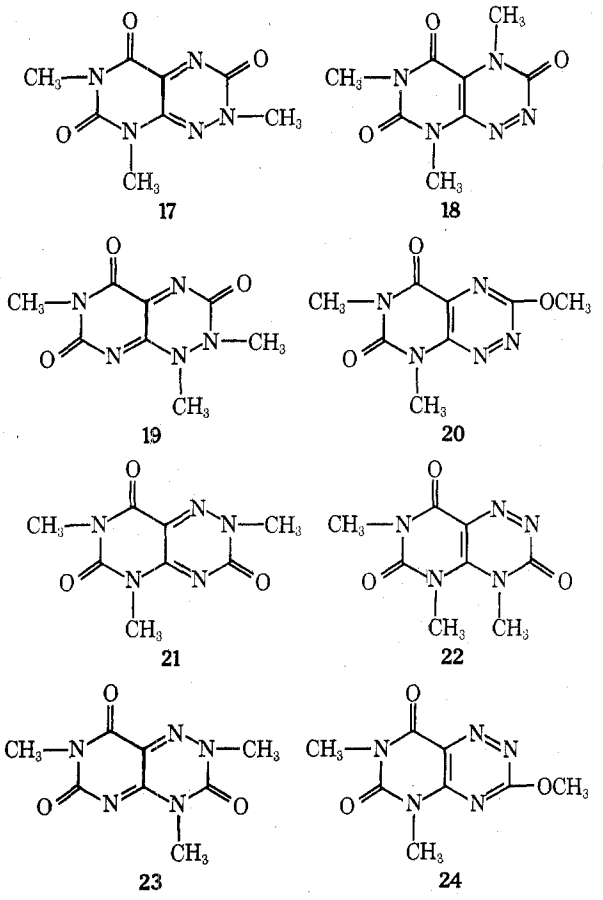
Scheme III



with an authentic sample prepared by the sequence of reactions depicted in Scheme III, as well as by reduction of fervenulone (**5**) itself. Conversion of **16** to fervenulone (**5**) could be readily accomplished by dehydrogenation with DDQ in chloroform solution, or even by repeated recrystallization from ethanol.

2-Methylfervenulone (MSD-92). The antibiotic MSD-92 was isolated from the fermentation broth of an unidentified actinomycete as a fluorescent, bright yellow,

crystalline compound possessing broad in vitro antibiotic activity.[9] On the basis of spectral and microanalytical data, this antibiotic was correctly identified by the Merck workers as a trimethylpyrimidotriazinetrione in either the [5,4-*e*] or [4,5-*e*] series. Of the eight possible structures for MSD-92 (structures **17–24**), only structure **20** (3-methoxyfervenulin) could be positively rejected; the Merck workers suggested structures **18, 19, 22,** and **23** as the most plausible. We present below several independent syntheses of this antibiotic which unequivocally support the assignment of structure **17** to MSD-92.



We were immediately able to eliminate structures **19–24** by the observation that methylation of fervenulone (**5**) with methyl iodide gave a trimethylpyrimido[5,4-*e*]-*as*-triazinetrione which was identical in every respect with the naturally occurring antibiotic. Thus MSD-92 must possess either structure **17** or **18**, and a decision in favor of **17** was readily made on the basis of the following unambiguous synthesis.

Hydrolysis[26] of the readily available 1,3-dimethyl-5-nitroso-6-aminouracil (**25**) provided a convenient route to 1,3-dimethylvioluric acid (**26**),[27] which on catalytic reduction gave 1,3-dimethyluramil (**27**).[28] Treatment of **27** in alkaline solution with ethyl chloroformate gave the 5-carbethoxyamino derivative **28**,[29] which was then converted in a single operation by reaction with phosphorus oxychloride to 1,3-dimethyl-5-isocyanato-6-chlorouracil (**29**).[30] Rapid work-up and drying of **29** proved to be essential, since relaxation of such precautions led to a mixture of **30** and the sym urea **31**. Addition of 1 equiv of methylhydrazine to an acetonitrile or chloroform solution of **29** then gave the semicarbazide **32**, whose structure was readily established by microanalysis and by conversion with benzaldehyde to the semicarbazone **33**. No evidence could be found for the formation of the isomeric semicarbazide which would have resulted from the alternate mode of addition of methylhydrazine to the isocyanate **29**.

An attempt to bring about cyclization of **32** with sodium ethoxide was unsuccessful, and led only to displacement of the 6-chloro substituent by an ethoxy group to give **34**. However, treatment of **32** with aqueous sodium acetate at 65–75° while passing a vigorous stream of air through the reaction solution resulted in the formation of 2,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (2-methylfervenulone, **17**), identical in every respect (melting point, mixture melting point, TLC, uv, ir, NMR, and microbiological assay)[31] with the naturally occurring antibiotic. These reactions are summarized in Scheme IV.

Presumably the initial product of cyclization of **32** is the dihydro derivative **35**, which then undergoes oxidation to **17** under the reaction conditions. The ready interconvertibility of **17** and **35** could be demonstrated independently by catalytic reduction of **17** to give the dihydro derivative **35**, which then could be reconverted to **17** by air oxidation (as above), or by repeated recrystallization from ethanol, or (in the highest yield) by dehydrogenation with DDQ.

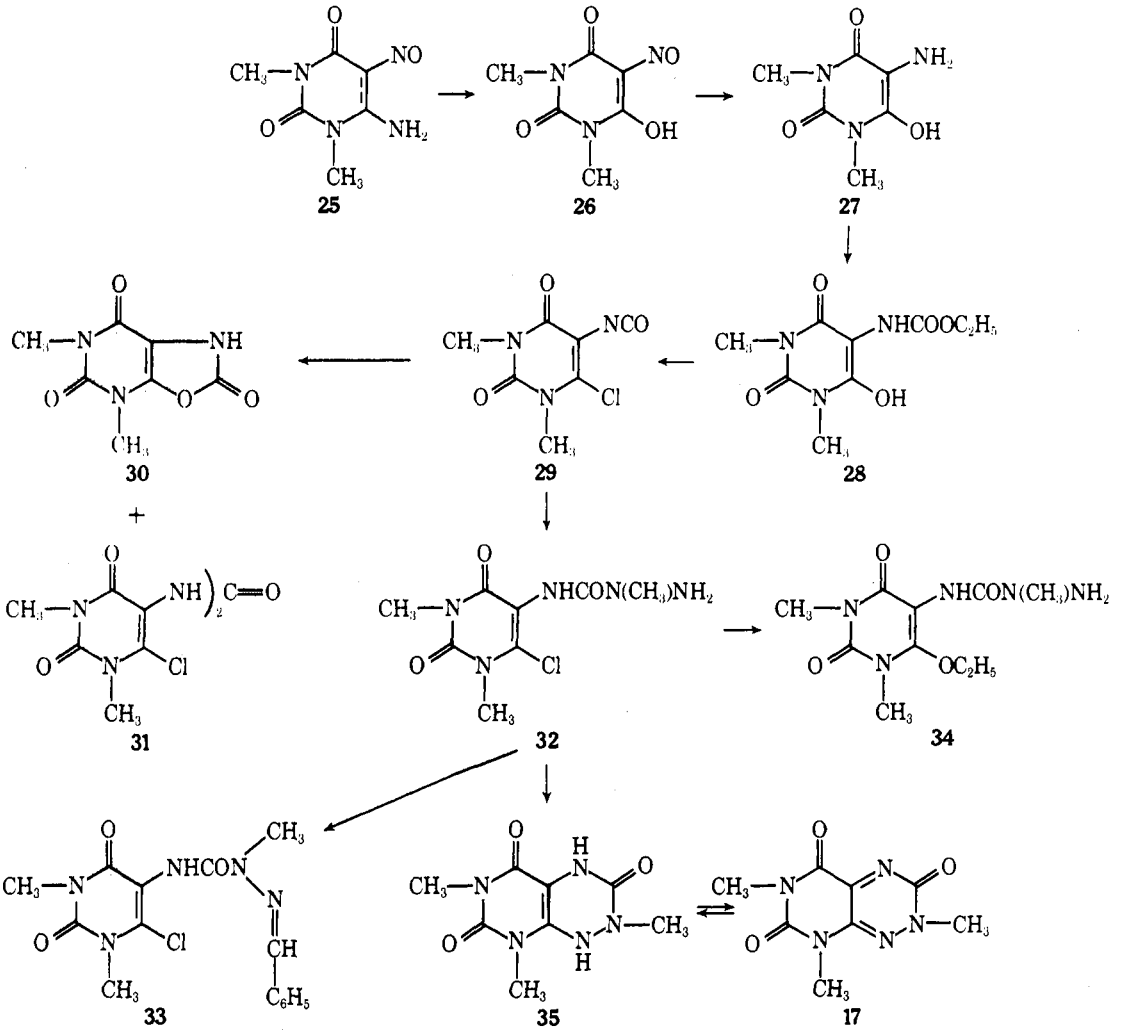
The identification and isolation of 2-methylfervenulone (MSD-92, **17**) was complicated by a number of polymorphic modifications whose formation depended upon the solvent employed in its purification. The form obtained upon recrystallization from ethyl acetate consisted of shining, thick yellow needles, mp 174–175°. Recrystallization of carefully dried **17** from ethanol gave thick, glistening plates melting at 181–182° dec; this was the form encountered by the Merck group, who reported that MSD-92 possessed mp 183–183.5° dec.[9] This same modification could be obtained by recrystallization from water, which initially gave **17** as small yellow plates, mp 92–93° dec, but which after prolonged drying at 100° then melted at 180–181° dec. This latter polymorphic form could be shown by mass spectroscopy to contain traces of water. Perhaps as a consequence of the presence of water, this latter form upon recrystallization from ethyl acetate gave small yellow plates, mp 157–158°, but which upon resolidification then remelted at 180–181° dec. Recrystallization of **17** from wet ethanol yielded a hemiethanolate, mp 155–156° dec, which tenaciously retained the solvent of recrystallization, even under prolonged drying in vacuo.

An attempt to convert **32** to **35** by pyrolysis led to a surprising result. The colorless needles which were formed were neither **35** nor **17**, but proved to be isomeric with **35**. However, although **35** (prepared independently as described above by catalytic reduction of **17**) could readily be dehydrogenated to **17** with DDQ, the fusion product, isomeric with **35**, could not be dehydrogenated under any conditions, and instead underwent extensive decomposition. We were thus led to suspect that this unexpected pyrolysis product was 1,4-dihydro-1,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (**36**), a structural assignment which was then verified by an independent but unequivocal synthesis of **36** (see Scheme V).

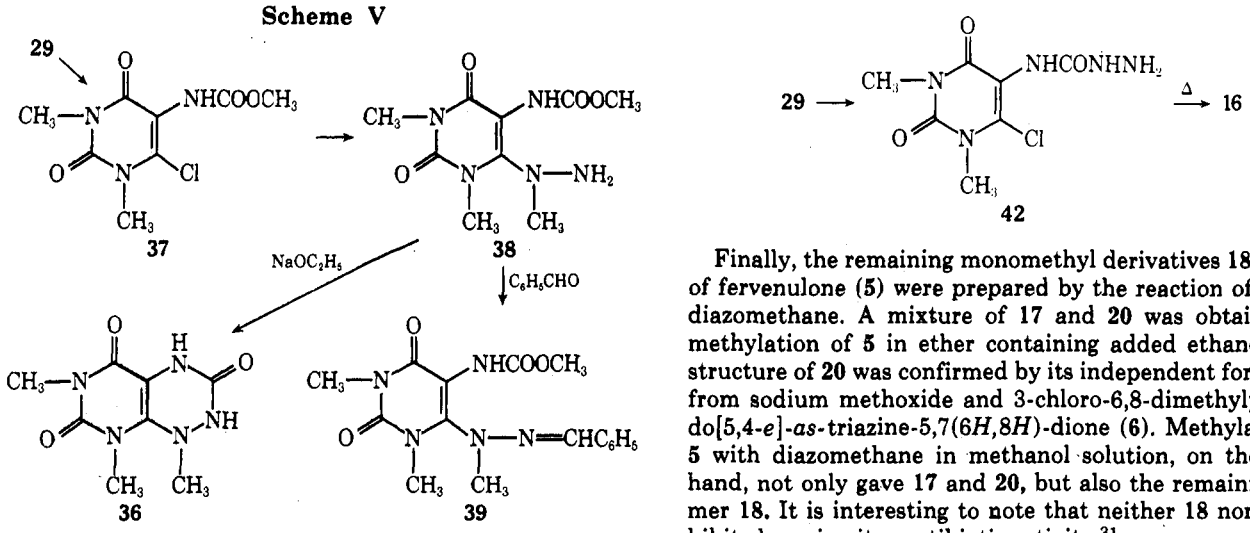
Thus, heating **29** in methanol solution gave 1,3-dimethyl-5-carbomethoxyamino-6-chlorouracil (**37**), which upon treatment in acetonitrile solution with methylhydrazine gave 1,3-dimethyl-5-carbomethoxyamino-6-(1-methylhydrazino)uracil (**38**). The structure of **38** was confirmed by its conversion with benzaldehyde to the benzylidene derivative **39**. Finally, treatment of **38** with sodium ethoxide resulted in cyclization to give **36**, identical in all respects with the product derived from pyrolysis of **32**.

The rearrangement which had occurred in the conversion of **32** to **36** has been observed previously with substituted hydrazines,[32] and presumably involves a diaziridine inter-

Scheme IV



Scheme V



mediate (e.g., **40**). The unidirectional ring opening to give the isomeric semicarbazide **41** results from the greater basicity of the nitrogen bearing the methyl group (Scheme VI).

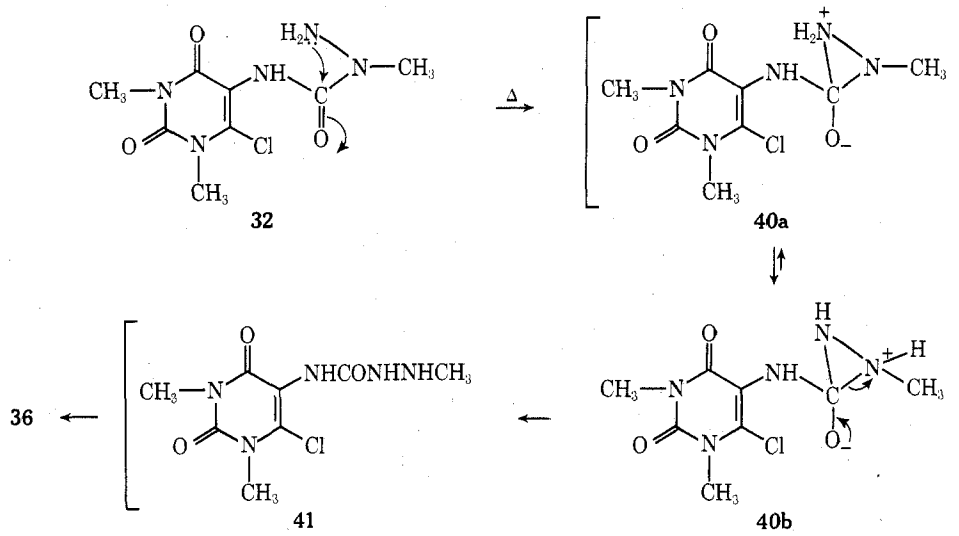
As would be expected, pyrolysis of the unsubstituted semicarbazide **42** (prepared from the isocyanate **29** and hydrazine) proceeded normally to give dihydrofervenulone (**16**). Since dehydrogenation of **16** was shown above to give fervenulone (**5**), this series of transformations constitutes a further useful preparation of this important synthetic precursor both to fervenulin and to MSD-92.

Finally, the remaining monomethyl derivatives **18** and **20** of fervenulone (**5**) were prepared by the reaction of **5** with diazomethane. A mixture of **17** and **20** was obtained by methylation of **5** in ether containing added ethanol. The structure of **20** was confirmed by its independent formation from sodium methoxide and 3-chloro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (**6**). Methylation of **5** with diazomethane in methanol solution, on the other hand, not only gave **17** and **20**, but also the remaining isomer **18**. It is interesting to note that neither **18** nor **20** exhibited any in vitro antibiotic activity.[31]

Experimental Section

6,8-Dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (Fervenulone, 5). Method A. To a stirred, ice-cooled solution of 0.115 g (0.005 mol) of sodium in 50 ml of dried, distilled absolute ethanol was added 1.72 g (0.005 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil (**3**),[16] and the mixture was stirred with continued cooling for 1.5 hr. The precipitate of the sodium salt of fervenulone which gradually separated (mp 317–319° dec) was collected by filtration, washed with absolute ethanol, and then dissolved in warm glacial acetic acid. Filtration removed a small amount of insoluble material; evaporation of the

Scheme VI



filtrate to a small volume and dilution with absolute ethanol resulted in the separation of 0.81 g (63%) of the ethanolate of fervenulone (**5**), mp 259–261° dec.

Anal. Calcd for $C_7H_7N_5O_3 \cdot C_2H_5OH$: C, 42.35; H, 5.14; N, 27.44. Found: C, 42.56; H, 5.15; N, 27.21.

The hydrate of fervenulone (**5**) was obtained by warming 0.75 g of the ethanolate in water for 30 min, concentrating to a small volume, and cooling. Filtration gave 0.62 g of yellow crystals,. mp 259–261° dec.

Anal. Calcd for $C_7H_7N_5O_3 \cdot H_2O$: C, 37.01; H, 4.00; N, 30.82. Found: C, 37.23; H, 4.24; N, 30.82.

Anhydrous fervenulone (**5**), mp 260–261° dec, was obtained by drying the hydrate in vacuo at 125° for 5 hr.

Anal. Calcd for $C_7H_7N_5O_3$: C, 40.19; H, 3.38; N, 33.48. Found: C, 39.98; H, 3.47; N, 33.30.

Method B. A stirred suspension of 1.86 g (0.005 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-(2-formylhydrazino)uracil (**8**) (see below) in a solution of 0.35 g (0.015 mol) of sodium in 25 ml of absolute ethanol was heated under reflux for 1 hr. The reaction mixture was then worked up as described above to give, after recrystallization from water, 0.92 g (81%) of deep yellow crystals, mp 260–261° dec, identical (ir spectrum) with fervenulone hydrate prepared as described above under method A. (See below for methods C and D.)

1,3-Dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-(2-formylhydrazino)uracil (8). A suspension of 3.44 g (0.01 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil in a formylation mixture prepared from 1.02 g (0.01 mol) of acetic anhydride and 10 ml of formic acid was heated at 50–60° for 15 min and then concentrated to dryness under reduced pressure. The residue was suspended in 10 ml of water and reconcentrated to a small volume. Cooling gave 2.91 g (78%) of **8**, mp 203–204° dec.

Anal. Calcd for $C_{13}H_{20}N_6O_7$: C, 41.94; H, 5.41; N, 22.57. Found: C, 41.93; H, 5.53; N, 22.37.

3-Chloro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (6). Method A. To a stirred solution of 3.47 g (0.024 mol) of diethylaniline and 100 ml of phosphorus oxychloride was added with cooling (in an ice bath) 5.0 g (0.024 mol) of 6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (fervenulone, **5**). The suspension was then heated gently under reflux in an oil bath maintained at 110–115° for 30 min, excess phosphorus oxychloride was removed by distillation under reduced pressure, and the residual syrup was poured over 25 g of crushed ice. Cooling of the resulting solution and filtering then gave 1.73 g of yellow crystals which were recrystallized from 20 ml of absolute ethanol to give 1.24 g (23%) of **6**, mp 146–147°.

Anal. Calcd for $C_7H_6N_5O_2Cl$: C, 36.94; H, 2.65; N, 30.77; Cl, 15.58. Found: C, 37.06; H, 2.76; N, 30.58; Cl, 15.29.

Method B. An intimate mixture of 5.10 g (0.002 mol) of fervenulone (**5**) ethanolate and 8.32 g (0.04 mol) of phosphorus pentachloride was gradually heated in an oil bath from an initial temperature of 110° to a final temperature of 140° over a period of 1 hr. The reaction mixture was then freed of volatile material by concentration under reduced pressure, the residue was stirred with ice, and the resulting solution was extracted with two 100-ml portions of chloroform. The combined, dried extracts were evaporated to a gummy red residue which was extracted with three 250-ml portions of ether. Concentration of the combined extracts and crystallization of the residue from absolute ethanol gave 2.06 g (45%) of **6**, identical with the material prepared by method A.

3-Hydrazino-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (7). A solution of 1.08 g (0.00475 mol) of 3-chloro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione in 50 ml of absolute ethanol cooled to 0° was added during 5 min to a solution of 4.69 g (0.0143 mol) of 97% hydrazine in 25 ml of absolute ethanol. The reaction mixture was then maintained at room temperature for 18 hr, cooled to 0°, and filtered to give bronze-colored platelets which were suspended in 30 ml of saturated sodium bicarbonate solution and stirred for 30 min. The suspended solid was collected by filtration, the filtrate was extracted with 50 ml of chloroform, the dried extract was evaporated to dryness, and the residue was combined with the above solid to give 1.05 g (99%) of crude **7**, mp 223–227° dec. Recrystallization from water afforded 0.51 g of pure **7**, mp 225–227° dec.

Anal. Calcd for $C_7H_9N_7O_2$: C, 37.67; H, 4.07; N, 43.93. Found: C, 37.82; H, 4.10; N, 44.04.

6,8-Dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (Fervenulin, 1). Method A. To a solution of 0.53 g (0.0024 mol) of 3-hydrazino-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (**7**) in 75 ml of water was added, in one portion, 1.04 g (0.0048 mol) of yellow mercuric oxide, and the mixture was stirred vigorously for 3 hr. The dark green reaction mixture was filtered through a mat of Celite and the filtrate was concentrated to dryness under reduced pressure. The residual yellow crystals were dissolved in 25 ml of hot water containing 0.35 g of concentrated hydrochloric acid, the solution was filtered and reconcentrated to dryness, and the pale yellow crystalline residue (0.29 g) was washed well with ice water. Recrystallization from 15 ml of water then gave 0.19 g (41%) of pure fervenulin, mp 177–178°, identical (melting point, mixture melting point, spectral comparisons) with authentic natural fervenulin: NMR (CF_3COOH) δ 3.18 (s, 3 H, NCH_3), 3.48 (s, 3 H, NCH_3), 9.48 (s, 1 H, C-3 proton).

Anal. Calcd for $C_7H_7N_5O_2$: C, 43.52; H, 3.65; N, 36.26. Found: C, 43.48; H, 3.71; N, 36.49.

Recrystallization of fervenulin from ethanol gave a polymorphic form, mp 171–172°, mmp 171–172° with authentic fervenulin of mp 177–178°.

Anal. Found: C, 43.36; H, 3.59; N, 36.16.

Method B. To a stirred, ice-cooled suspension of 5.16 g (0.015 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil in 10 ml of dimethylformamide was added, over a period of 5 min, 2.53 g (0.0165 mol) of phosphorus oxychloride. After 10 min the ice bath was replaced by an oil bath maintained at 125–130°, and the reaction mixture was heated for 2 hr. It was then concentrated to dryness under reduced pressure, and the residue was triturated in ice water and filtered to give 2.30 g of bright yellow crystals of crude fervenulin (**1**), mp 169–171° slight dec. The crude product was dissolved in 100 ml of hot benzene and filtered from a small amount of insoluble material, and the filtrate was cooled and then chromatographed on a 25 × 450 mm column of Florisil, using

a 9:1 mixture of benzene and ethyl acetate as the eluent. Evaporation of the eluate to dryness and recrystallization of the residue from benzene then gave chunky yellow needles of pure fervenulin, mp 177–178°, identical with authentic fervenulin. Yields in three representative preparations were 1.37 (47%), 1.55 (53%), and 1.65 g (57%).

1,3-Dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-dimethylaminomethylenehydrazinouracil (9). To a suspension of 3.44 g (0.01 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil in 10 ml of dimethylformamide, cooled in an ice bath, was added over a period of 15 min 1.53 g (0.01 mol) of phosphorus oxychloride. The reaction mixture was then heated at 60° (oil bath) for 20 min. A copious precipitate of a colorless solid gradually separated during this period. The mixture was cooled, diluted with an equal volume of ethanol, and filtered to give 3.42 g (85%) of **9**, mp 183–184° dec. For analysis it was recrystallized from absolute ethanol and dried at 30° for 24 hr in vacuo.

Anal. Calcd for $C_{15}H_{25}N_7O_6 \cdot HCl \cdot \frac{1}{2}H_2O$: C, 40.49; H, 6.12; N, 22.04. Found: C, 40.33; H, 6.08; N, 22.07.

4-Carbethoxyamino-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7-(4*H*,6*H*,8*H*)-trione Ethanolate (15). Method A. A stirred suspension of 6.88 g (0.02 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil in 250 ml of anhydrous ether was treated during 15 min with 19.6 g (0.04 mol) of 90% lead tetraacetate, added in small portions. The mixture was then heated under reflux for 30 min and filtered (to remove lead acetate, 12.10 g, 95% of theory), and the ethereal filtrate was shaken with a solution of sodium bicarbonate and then dried over anhydrous magnesium sulfate. Evaporation of the ether gave a reddish oil which was dissolved in ethanol. Cooling resulted in the separation of 1.29 g (19%) of deep yellow crystals of **15**: mp 148–149°; NMR (DMSO-d_6) δ 1.04 (2 t, 6 H), 3.08 (s, 3 H, NCH_3), 3.18 (s, 3 H, NCH_3), 3.93 (m, 4 H).

Anal. Calcd for $C_{10}H_{12}N_6O_5 \cdot C_2H_5OH$: C, 42.10; H, 5.31; N, 24.56; mol wt, 342. Found: C, 42.34; H, 5.50; N, 24.68; mol wt, 346.

Method B. To a solution of 5.1 g (0.030 mol) of 1,3-dimethyl-6-hydrazinouracil in 25 ml of dimethylformamide was added dropwise, over a period of 30 min, 11.0 g (0.063 mol) of diethyl azodicarboxylate. The reaction mixture was stirred for an additional 30 min, heated at 90–100° for 1 hr, and then evaporated to dryness. The residue was triturated with ethanol, and the crystals which separated were collected by filtration and recrystallized from cyclohexane–benzene (4:1) to give 3.43 g (33%) of **15**, mp 131–133°.

A mixture melting point with a sample of **15** (mp 148–149°), prepared by method A, melted at 148–149°. When a cyclohexane–benzene solution of the lower melting polymorph was seeded with the higher melting material, the product which crystallized melted at 148–149°. These polymorphs were interconvertible; slow crystallization from a dilute solution favored formation of the higher melting material, whereas rapid recrystallization from concentrated solutions, particularly from nonpolar solvents, gave the lower melting material.

1,3-Dimethyl-6-(2-carbethoxyhydrazino)uracil. To a stirred slurry of 53.4 g (0.315 mol) of 1,3-dimethyl-6-hydrazinouracil in 300 ml of 50% aqueous dioxane at 50° was added dropwise, and simultaneously, a solution of 16.0 g (0.4 mol) of sodium hydroxide in 100 ml of water and 43.5 g (0.4 mol) of ethyl chloroformate. The reaction mixture was concentrated to one-half its volume under reduced pressure, and the crystals which separated were collected by filtration, air dried, and recrystallized from acetonitrile to give 22.9 g (30%) of large, shiny plates: mp 219–220° dec; NMR (DMSO-d_6) δ 1.11 (t, 3 H), 3.02 (s, 3 H, NCH_3), 3.20 (s, 5 H, NCH_3 and NHNH), 4.66 (s, 1 H, C_5H).

Anal. Calcd for $C_9H_{14}N_4O_4$: C, 44.62; H, 5.83; N, 23.13. Found: C, 44.78; H, 5.71; N, 23.34.

1,3-Dimethyl-5-nitroso-6-(2-carbethoxyhydrazino)uracil. A suspension of 18.13 g (0.076 mol) of 1,3-dimethyl-6-(2-carbethoxyhydrazino)uracil in 50 ml of absolute ethanol was treated with 15.4 g (0.15 mol) of isoamyl nitrite, 1 drop of concentrated hydrochloric acid was added, and the vigorously reacting mixture was maintained at 10–15° by immersion in an ice bath. After 15 min, the golden yellow plates which had separated were collected by filtration, washed with several small portions of absolute ethanol followed by ether, and dried to give 17.45 g (87%) of the pure 5-nitroso derivative, mp 134–135°. The compound was recrystallized from ethanol for analysis without change in the melting point: NMR (DMSO-d_6) δ 1.15 (t, 3 H, CH_3), 3.04 (s, 3 H, NCH_3), 3.19 (s, 3 H, NCH_3), 3.32 (s, 2 H, NHNH), 4.11 (q, 2 H, CH_2).

Anal. Calcd for $C_9H_{13}N_5O_5$: C, 39.85; H, 4.84; N, 25.83. Found: C, 39.74; H, 4.75; N, 25.54.

1,3-Dimethyl-5-amino-6-(2-carbethoxyhydrazino)uracil. A solution of 16.51 g (0.061 mol) of 1,3-dimethyl-5-nitroso-6-(2-carbethoxyhydrazino)uracil in 200 ml of absolute ethanol was hydrogenated at room temperature under 50 psi of hydrogen in the presence of 2.0 g of 10% Pd/C. The reaction mixture was heated to boiling to dissolve the partially separated product and filtered, and the filtrate was concentrated under reduced pressure to about ¼ its volume. Cooling and filtering then gave 9.76 g (62%) of small platelets, mp 159–160°.

Anal. Calcd for $C_9H_{15}N_5O_4$: C, 42.03; H, 5.88; N, 27.23. Found: C, 42.30; H, 5.79; N, 27.33.

1,3-Dimethyl-5-carbethoxyaminobarbituric Acid (28). 1,3-Dimethyluramil (1,3-dimethyl-5-aminobarbituric acid, **27**)[28] was prepared most conveniently from 1,3-dimethyl-5-nitroso-6-aminouracil by acid hydrolysis[26] to 1,3-dimethylvioluric acid (**26**),[27] followed by catalytic reduction in methanol solution over 5% Pd/C catalyst. To a stirred solution of 34.2 g of **27** in 100 ml of 2 *N* sodium hydroxide at 40° was added dropwise, and simultaneously, 26.1 g of ethyl chloroformate and 100 ml of 2 *N* sodium hydroxide over a period of 30 min. The reaction mixture was then stirred for an additional 30 min, cooled, and filtered, and the filtrate was acidified to give 38.7 g (80%) of colorless crystals of **28**, mp 135–136° (lit.[29] mp 134°).

Anal. Calcd for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.23; H, 5.37; N, 17.10.

1,3-Dimethyl-5-isocyanato-6-chlorouracil (29). To 150 ml of phosphorus oxychloride was added with stirring 24.3 g of 1,3-dimethyl-5-carbethoxyaminobarbituric acid, followed immediately by 6 ml of water (in small portions). As soon as the initial exothermic reaction had subsided, the reaction mixture was heated under reflux for 45 min, cooled, and filtered through a sintered glass funnel. The filtrate was concentrated under reduced pressure until all of the residual phosphorus oxychloride had been removed, and the residue was triturated with 100 g of crushed ice and then filtered. The very light green solid which was collected was washed thoroughly with water and then dried to give 13.9 g (65%) of **29**, mp 144–145°. The analytical sample, mp 146–147°, was prepared by recrystallization from acetonitrile followed by vacuum sublimation, ir (Nujol) 2225 cm^{-1} (NCO).

Anal. Calcd for $C_7H_6N_3O_3Cl$: C, 38.99; H, 2.80; N, 19.50. Found: C, 39.22; H, 2.88; N, 19.51.

1,4-Dihydro-4,6-dimethyloxazolo[5,4-*d*]pyrimidine-2,5,7(6*H*)-trione (30). A sample of 8.7 g of **29** was stored in a desiccator (atmospheric pressure) for 1 week. During this period the intensity of the isocyanate band (2225 cm^{-1}) decreased markedly, with a corresponding increase in the intensity of bands at 3060 and 3270 cm^{-1}. The material was then extracted with 250 ml of hot dioxane, and the extract was evaporated under reduced pressure, cooled, and filtered. Recrystallization of the residue from acetonitrile then gave 2.5 g (32%) of **30**, mp 201–202° dec, ir 1840 cm^{-1} (lactone).

Anal. Calcd for $C_7H_7N_3O_4$: C, 42.64; H, 3.58; N, 21.32. Found: C, 42.97; H, 3.48; N, 20.97.

Concentration of the dioxane filtrate above resulted in the separation of 1.0 g of unchanged **29**, mp 146–147°.

***sym*-(6-Chloro-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)urea (31).** The residue from the hot dioxane extraction above weighed 1.72 g (21%), mp 272–273° dec. The same compound could be prepared alternately from **29** by heating in DMF followed by cooling.

Anal. Calcd for $C_{13}H_{14}N_6O_5Cl_2$: C, 38.53; H, 3.48; N, 20.74; Cl, 17.51. Found: C, 38.72; H, 3.43; N, 20.64; Cl, 17.32.

4-(1,3-Dimethyl-6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methylsemicarbazide (32). To a solution of 15.1 g (0.07 mol) of 1,3-dimethyl-5-isocyanato-6-chlorouracil in 100 ml of acetonitrile was added with stirring a solution of 3.55 g (0.077 mol) of methylhydrazine in 100 ml of acetonitrile. A spontaneous, exothermic reaction resulted, and a voluminous mass of colorless crystals separated. The reaction mixture was stirred at room temperature for 24 hr, concentrated to 250 ml, and filtered, and the crude product (13.5 g, mp 183–185° dec) was recrystallized from 750 ml of methanol to give 8.4 g (46%) of **32** as colorless needles, mp 197–199° dec.

Anal. Calcd for $C_8H_{12}N_5O_3Cl$: C, 36.71; H, 4.62; N, 26.76; Cl, 13.55. Found: C, 36.75; H, 4.49; N, 26.69; Cl, 13.71.

This compound was converted into its benzylidene derivative (**33**) by heating with a slight excess of benzaldehyde in ethanol. Recrystallization of the crude product from ethanol gave colorless crystals (80%), mp 182–183°.

Anal. Calcd for $C_{15}H_{16}N_5O_3Cl$: C, 51.50; H, 4.61; N, 20.02; Cl,

10.14. Found: C, 51.34; H, 4.73; N, 20.00; Cl, 9.31.

4-(1,3-Dimethyl-6-ethoxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methylsemicarbazide (34). To a nitrogen-blanketed solution of sodium ethoxide in ethanol (prepared from 50 ml of absolute ethanol and 0.27 g of sodium) was added with stirring 3.1 g (0.012 mol) of carefully dried 4-(1,3-dimethyl-6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methylsemicarbazide (**32**). The clear solution was heated under reflux for 1.5 hr, filtered from the suspended sodium chloride, and cooled to give a flocculent precipitate which was collected by filtration, yield 2.8 g (89%), mp 199–200° dec. The analytical sample was prepared by recrystallization from ethanol.

Anal. Calcd for $C_{10}H_{17}N_5O_4$: C, 44.27; H, 6.32; N, 25.82. Found: C, 44.41; H, 6.23; N, 25.96.

2,6,8-Trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (2-Methylfervenulone, 17). Method A. To a well stirred, ice-cooled solution of 2.9 g (0.014 mol) of 6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (fervenulone, **5**) (dried in vacuo for 2 hr at 120° immediately prior to use) in 25 ml of dry DMF was added in small portions over a period of 15 min 0.84 g (0.018 mol) of a 50% dispersion of sodium hydride in mineral oil. The mixture was stirred for an additional 15 min and 2.8 g (0.02 mol) of methyl iodide was added to the partial suspension of the sodium salt of **5**. The ice bath was replaced by an oil bath (70°) for 3 hr, and the mixture was then stirred at room temperature for 40 hr. An atmosphere of dry nitrogen was maintained at a slight positive pressure over the reaction mixture during the entire reaction period. The neutral mixture was then filtered and concentrated to dryness under reduced pressure, and the residue was partitioned between 50 ml of chloroform and 25 ml of water. The chloroform solution was washed with a fresh portion of water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to give a thick, yellow syrup, which was thoroughly extracted with pentane. Trituration of this residual syrup with water then gave 0.68 g of yellow crystals, mp 162–163°, which were recrystallized from a small amount of water to give 0.35 g (11%), mp 179–180°. One further recrystallization from ethanol then gave shining, thick, yellow plates, mp 181–182°, identical with the material obtained by methods B and C below, and also with the naturally occurring antibiotic MSD-92.[31]

Anal. Calcd for $C_8H_9N_5O_3$: C, 43.05; H, 4.07; N, 31.38. Found: C, 43.18; H, 4.03; N, 31.22.

Method B. To a stirred, ice-cooled solution of 1.24 g (0.054 mol) of sodium in 150 ml of absolute ethanol, kept under a slight positive pressure of nitrogen, was added 15.9 g (0.046 mol) of 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-hydrazinouracil (**3**), and the reaction mixture was stirred for 2 hr. The thick slurry of pale yellow solid was then collected by filtration, washed with cold ethanol, and resuspended in 100 ml of absolute ethanol, and 14.2 g (0.10 mol) of methyl iodide was added. The mixture was then heated in an oil bath maintained at 50° for 2 hr. The resulting solution was concentrated to dryness under reduced pressure, and the residue was dissolved in 50 ml of water. Cooling then gave 3.9 g of yellow crystals; extraction of the aqueous filtrate with chloroform and evaporation of the chloroform extract gave an additional 6.8 g of product. Recrystallization from ethyl acetate then gave 6.2 g (60%) of the polymorphic form of **17** melting at 174–175°.

Anal. Calcd for $C_8H_9N_5O_3$: C, 43.05; H, 4.07; N, 31.38. Found: C, 43.07; H, 4.01; N, 31.27.

Recrystallization of a sample of these glistening yellow needles, mp 174–175°, from absolute ethanol gave yellow plates, mp 181–182°, identical with the material prepared by method A above, which upon subsequent recrystallization from ethyl acetate reverted to the yellow needles melting at 174–175°.

Recrystallization of a sample of either polymorphic form from water gave small, yellow plates, mp 92–93° dec. Drying this material for 48 hr under reduced pressure at 40° gave a pale yellow solid, mp 180–181°, identical with the material prepared above by method A.

Recrystallization from ethyl acetate of a sample of the material (mp 92–93°) obtained from water did not give the typical chunky, yellow needles usually obtained upon recrystallization of **17** from this solvent, but thick plates, mp 157–158° dec; the melting point did not change upon prolonged drying in vacuo.

Anal. Calcd for $C_8H_9N_5O_3$: C, 43.05; H, 4.07; N, 31.38. Found: C, 42.89; H, 3.88; N, 31.28.

Method C. A mixture of 1.05 g of 4-(1,3-dimethyl-6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methylsemicarbazide (**32**), 0.33 g of sodium acetate, and 50 ml of water was heated at 65–75° for 7 hr while a vigorous stream of air was passed through the solution. The reaction mixture (which exhibited a strong greenish-blue fluorescence) was then cooled and extracted with three 50-ml portions of chloroform. The combined extracts were dried ($MgSO_4$) and evaporated to give a gummy yellow residue (0.89 g). Trituration of this residue with ethyl acetate gave yellow crystals, which were collected by filtration; the filtrate was evaporated to dryness and the residue was triturated with water. The collected yellow solid was combined with the yellow solid above, dried thoroughly in vacuo, and recrystallized from ethyl acetate to give 0.37 g (41%) of yellow needles, mp 175–176°, identical in all respects with the material prepared above from ethyl acetate under method B.

Reduction of 2-Methylfervenulone (17) to 1,4-Dihydro-2,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (35). A solution of 1.12 g of 2-methylfervenulone (**17**) in 200 ml of warm water (50°) was reduced with 50 psi of hydrogen in the presence of 100 mg of Pd/C catalyst. After 30 min hydrogen uptake had ceased, and the reaction mixture was heated to boiling, filtered, concentrated, and cooled to give 0.67 g of a colorless solid, mp 245–246° dec. Attempted recrystallization of this material from ethyl acetate resulted in its gradual reconversion to bright yellow **17**, mp 174–175°.

Oxidation of 35 to 17. To a stirred solution of 0.28 g (0.00125 mol) of 2,3-dichloro-5,6-dicyanoquinone (DDQ) in 100 ml of chloroform was added 0.225 g (0.001 mol) of 1,4-dihydro-2,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (**35**), prepared as described above, and the mixture was heated under reflux for 30 min. It was then filtered to remove the precipitated hydroquinone, and the filtrate was concentrated to dryness under reduced pressure. Recrystallization of the residue from ethyl acetate gave 0.14 g (63%) of 2-methylfervenulone (**17**), mp 157–158°.

1,3-Dimethyl-5-carbomethoxyamino-6-chlorouracil (37). A solution of 6.3 g of 1,3-dimethyl-5-isocyanato-6-chlorouracil (**29**) in 100 ml of absolute methanol was heated under reflux for 1 hr, concentrated to a small volume under reduced pressure, and cooled. The yellow crystals which had separated were collected by filtration and recrystallized from methanol to give 5.8 g (80%) of **37**, mp 169–170° dec.

Anal. Calcd for $C_8H_{10}N_3O_4Cl$: C, 38.80; H, 4.07; N, 16.97. Found: C, 38.65; H, 3.99; N, 16.85.

1,3-Dimethyl-5-carbomethoxyamino-6-(1-methylhydrazino)uracil (38). A mixture of 2.5 g (0.01 mol) of 1,3-dimethyl-5-carbomethoxyamino-6-chlorouracil (**37**) and 0.9 g (0.02 mol) of methylhydrazine in 100 ml of acetonitrile was heated under reflux for 2 hr and filtered to remove a small amount of suspended solid, and the filtrate was concentrated to dryness under reduced pressure. The residual solid, consisting of methylhydrazine hydrochloride and **38**, was washed with acetonitrile and then extracted with 20 ml of warm absolute ethanol. Recrystallization of the residual solid from acetonitrile then gave 1.2 g (46%) of **38**, mp 179–180° dec.

Anal. Calcd for $C_9H_{15}N_5O_4$: C, 42.02; H, 5.88; N, 27.23. Found: C, 42.14; H, 5.80; N, 27.29.

This compound was converted to its benzylidene derivative (**39**) in 75% yield by heating with a slight excess of benzaldehyde in methanol solution for 1 hr, evaporation to a small volume, and addition of ether, mp 159–160°.

Anal. Calcd for $C_{16}H_{19}N_5O_4$: C, 55.64; H, 5.55; N, 20.28. Found: C, 55.71; H, 5.53; N, 20.24.

1,4-Dihydro-1,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (36). Method A. Pyrolysis of 3.0 g of 4-(1,3-dimethyl-6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methylsemicarbazide (**32**) at 135° (0.01 mm) for 3 hr gave a dark lavender mass which was dissolved in a small amount of hot acetonitrile. Cooling resulted in the separation of colorless crystals, which were then recrystallized from acetonitrile to give 1.7 g (66%) of **36**, mp 241–242°.

Anal. Calcd for $C_8H_{11}N_5O_3$: C, 42.66; H, 4.93; N, 31.10. Found: C, 42.64; H, 4.89; N, 31.24.

Method B. To a solution of sodium ethoxide in ethanol (prepared from 0.05 g of sodium and 50 ml of absolute ethanol) was added 0.51 g of 1,3-dimethyl-5-carbomethoxyamino-6-(1-methylhydrazino)uracil (**38**), and the golden solution was heated under reflux for 1 hr. It was then cooled, acidified by the addition of 1.2 ml of 2 *N* ethereal hydrogen chloride, and filtered, and the filtrate was evaporated to dryness. The dry residue was extracted with hot acetonitrile; evaporation of the extracts then gave crude **36**, which was recrystallized from acetonitrile, yield 0.17 g (38%), mp 241–242°. The product was identical in all respects with the material prepared by method A above.

4-(1,3-Dimethyl-6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)semicarbazide (42). To a stirred solution of 7.1 g (0.033 mol) of 1,3-dimethyl-5-isocyanato-6-chlorouracil (**29**) in 250 ml of chloroform was added a solution of 1.3 g (0.04 mol) of anhydrous hydrazine (97%) in 250 ml of chloroform. After 72 hr of stirring, the mixture was filtered to give 6.5 g of crude **42**, mp 178–179° dec. Two recrystallizations from water then gave 2.6 g (33%) of pure **42**, mp 194–195° dec.

Anal. Calcd for $C_7H_{10}N_5O_3Cl$: C, 33.95; H, 4.06; N, 28.28. Found: C, 34.13; H, 4.16; N, 28.53.

1,4-Dihydro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (16). Method A. To a stirred solution of 2.57 g of 1,3-dimethyl-5-amino-6-(2-carbethoxyhydrazino)uracil in 50 ml of water was added dropwise over a period of 20 min a total of 10 ml of 1 *N* NaOH. The pH was adjusted to 5, the deep orange solution was concentrated to dryness under reduced pressure, and the residue was extracted with 100 ml of chloroform. The extract was dried and evaporated to dryness, and the residue was triturated with water, dried, and recrystallized from absolute ethanol to give 0.51 g (20%) of the ethanolate of **16**, mp 167–168° dec.

Anal. Calcd for $C_7H_9N_5O_3 \cdot C_2H_5OH$: C, 42.03; H, 5.88; N, 27.23. Found: C, 41.92; H, 5.45; N, 27.12.

Recrystallization of the ethanolate of **16** from water gave **16**, mp 251–252° dec.

Anal. Calcd for $C_7H_9N_5O_3$: C, 39.80; H, 4.30; N, 33.16. Found: C, 39.81; H, 4.11; N, 33.07.

Method B. A solution of 0.21 g of 4-carbethoxyamino-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(4*H*,6*H*,8*H*)-trione ethanolate (**15**) in 25 ml of absolute ethanol containing 0.20 g of 10% Pd/C was hydrogenated under 50 psi of hydrogen at room temperature for a period of 2 hr. The mixture was then filtered, the filtrate was concentrated to 2–3 ml, and the crystals were collected by filtration, yield 0.06 g, mp 167–168° dec, identical in every respect (melting point, mixture melting point, ir and NMR spectra) with a sample of **16** ethanolate prepared by method A.

Method C. Pyrolysis of 1.0 g of 4-(1,3-dimethyl-6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)semicarbazide (**42**) at 175–180° for 2.5 hr gave a violet-colored glass which was cooled, crushed to a powder, and extracted with 25 ml of boiling water. Cooling of the extract resulted in the separation of colorless crystals which were collected by filtration, washed with water, and dried to give 0.23 g (27%) of **16**, mp 251–252° dec, identical with the material prepared as described above.

Method D. A solution of 2.35 g of 2-methylfervenulone (**5**) ethanolate in 200 ml of absolute ethanol was shaken with 50 psi of hydrogen over 0.12 g of 10% Pd/C catalyst. Hydrogen uptake ceased after 1 hr. The mixture was filtered and the collected solids were extracted with 250 ml of boiling water. Concentration of the plum-colored filtrate and cooling then gave 1.1 g (56%) of colorless crystals of **16**, mp 251–252° dec, identical with the material prepared by methods A, B, and C above.

6,8-Dimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2*H*,6*H*,8*H*)-trione (Fervenulone, 5). Method C. Repeated crystallization of a sample of **16** from ethanol gave fervenulone ethanolate, mp 259–260° dec, identical in every respect with authentic material.

Method D. Dehydrogenation of **16** to fervenulone could also be accomplished somewhat more efficiently by heating under reflux for 30 min a solution of 0.53 g of **16** and 0.68 g of DDQ in 50 ml of chloroform. The reaction mixture was filtered, the filtrate was concentrated under reduced pressure, and the residue was recrystallized from ethanol to give fervenulone ethanolate (0.56 g, 88%), mp 260–261° dec, identical with authentic material.

Reaction of Fervenulone (5) with Diazomethane. Formation of 2-Methylfervenulone (17). A stirred suspension of 1.28 g of fervenulone (**5**), solvated with ethanol, in 50 ml of a mixture of anhydrous ether and absolute ethanol (10:1) and 50 ml of 0.1 *M* ethereal diazomethane was stirred for 18 hr and then filtered. The filtrate was evaporated to dryness and the residue was dissolved in 10 ml of water and extracted with three 20-ml portions of chloroform. The combined, dried ($MgSO_4$) extracts were evaporated to dryness and the residue was crystallized from ethyl acetate to give 0.12 g (11%) of 2-methylfervenulone (**5**), mp 174–175°, identical with an authentic sample.

Formation of 3-Methoxy-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (20). Method A. The collected solid material from the reaction of **5** with diazomethane (above) was extracted with hot absolute ethanol, and the extracts were evaporated to a small volume, cooled, and filtered to give 0.12 g of yellow crystals. Evaporation of the filtrate and trituration of the residual gum with ethanol gave a further 0.22 g of the same material, combined yield 0.34 g (30%), mp 143–144°.

Anal. Calcd for $C_8H_9N_5O_3$: C, 43.05; H, 4.06; N, 31.38. Found: C, 42.83; H, 4.01; N, 31.16.

Method B. To a stirred solution of 1.14 g (0.005 mol) of 3-chloro-6,8-dimethylpyrimido[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (**6**) in 50 ml of absolute methanol, under a slight positive pressure of nitrogen, was added dropwise a solution of sodium methoxide in methanol (from 0.115 g, 0.005 mol, of sodium and 18 ml of methanol). The reaction mixture was then heated under reflux for 15 min and filtered, and the filtrate was concentrated under reduced pressure to ca. 5 ml. Cooling resulted in the separation of 0.95 g (85%) of yellow needles, mp 144–145°, identical in all respects with the material prepared by method A above.

Formation of 4,6,8-Trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(4*H*,6*H*,8*H*)-trione (18). To a stirred solution of 2.65 g (0.01 mol) of fervenulone (**5**), solvated with ethanol, in 200 ml of absolute methanol was added 100 ml of ethereal diazomethane (0.01 mol). Stirring was continued for 6 hr, and the yellow solution was then evaporated to dryness. Dissolution of the residual gum in hot ethanol followed by cooling gave 0.74 g of a pale yellow solid, mp 208–210°, which was then recrystallized three times from ethanol to give 0.12 g (5%) of **18**, mp 218–220°.

Anal. Calcd for $C_8H_9N_5O_3$: C, 43.05; H, 4.06; N, 31.38. Found: C, 42.92; H, 4.42; N, 31.05.

Registry No.—**1**, 483-57-8; **3**, 18969-82-9; **5**, 22712-37-4; **6**, 18969-84-1; **7**, 18969-85-2; **8**, 54632-29-0; **9**, 54632-30-3; **15**, 54667-56-0; **16**, 22712-36-3; **17**, 22712-32-9; **18**, 22712-42-1; **20**, 22712-41-0; **27**, 54632-31-4; **28**, 54632-32-5; **29**, 22712-33-0; **30**, 54632-33-6; **31**, 54632-34-7; **32**, 22712-34-1; **33**, 54632-35-8; **34**, 54632-36-9; **35**, 54632-37-0; **36**, 22712-38-5; **37**, 22712-39-6; **38**, 22712-40-9; **39**, 54632-38-1; **42**, 22712-35-2; 1,3-dimethyl-6-(2-carbethoxyhydrazino)uracil, 54632-39-2; 1,3-dimethyl-5-nitroso-6-(2-carbethoxyhydrazino)uracil, 54667-57-1; 1,3-dimethyl-5-amino-6-(2-carbethoxyhydrazino)uracil, 54632-40-5.

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Synthesis of Isofervenulin and 2-Methylisofervenulone[1]

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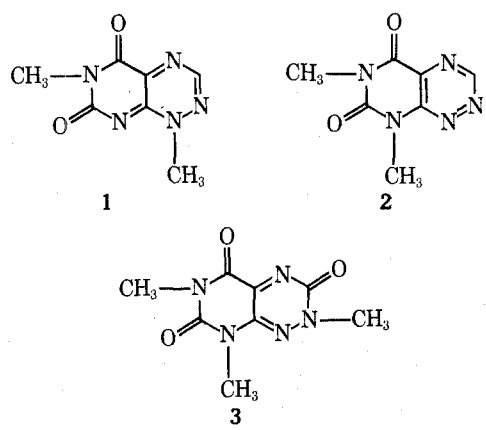
Syntheses of 5,7-dimethylpyrimido[4,5-*e*]-*as*-triazine-6,8(5*H*,7*H*)-dione (isofervenulin, **13**) and 2,5,7-trimethylpyrimido[4,5-*e*]-*as*-triazine-3,6,8(2*H*,5*H*,7*H*)-trione (2-methylisofervenulone, **10**) are described from a common intermediate, 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-aminouracil (**4**). Although these compounds are ring isomers of the naturally occurring antibiotics fervenulin (**2**) and 2-methylfervenulone (MSD-92, **3**), neither exhibited antibiotic activity.

Derivatives of the pyrimido[4,5-*e*]-*as*-triazine (6-azapteridine) ring system have received considerable recent attention,[2] primarily because of their demonstrated antiviral activity,[3] and as a consequence of their close structural relationship to the pteridines and their isomeric relationship with the pyrimido[5,4-*e*]-*as*-triazine ring system present in the naturally occurring antibiotics toxoflavin (**1**), fervenulin (**2**), and 2-methylfervenulone (MSD-92, **3**).[4] We report in the present paper the synthesis of 5,7-dimethylpyrimido[4,5-*e*]-*as*-triazine-6,8(5*H*,7*H*)-dione (isofervenulin, **13**) and 2,5,7-trimethylpyrimido[4,5-*e*]-*as*-triazine-3,6,8(2*H*,5*H*,7*H*)-trione (2-methylisofervenulone, **10**). Both of these compounds are of considerable potential interest as ring isomers of the antibiotics **2** and **3**, respectively.



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3

We have recently reported[5] a new method for C-5 functionalization of a variety of 6-amino- and 6-hydrazinopyrimidines which involves Michael addition to diethyl azodicarboxylate. This gives rise to a 5-(1,2-dicarbethoxyhydrazino) derivative which can then be converted (a) to a 5-carbethoxyamino derivative by Raney nickel reduction, or (b) to derivatives of the pyrimido[5,4-*e*]-*as*-triazine ring system (in the case of 6-hydrazino-substituted pyrimidines) by a variety of cyclization procedures.[4] We have now found that 1,3-dimethyl-5-(1,2-dicarbethoxyhydrazino)-6-aminouracil (**4**), readily available in high yield from 1,3-dimethyl-6-aminouracil and diethyl azodicarboxylate,[5] can be smoothly cyclized with sodium ethoxide in ethanol to **5a**, a derivative of the isomeric pyrimido[4,5-*e*]-*as*-triazine ring system. Although it might have been expected that base-catalyzed intramolecular cyclization of **4** would have led to compound **5b** or **5c**, we present below convincing evidence that the structure of this intramolecular cyclization product of **4** possesses structure **5a**, in which the carbethoxy group is attached to N-2 of the pyrimidotriazine ring.

Thus, compound **5a** could be dehydrogenated with either phosphorus oxychloride or thionyl chloride to 2-carbethoxy-5,7-dimethylpyrimido[4,5-*e*]-*as*-triazine-3,6,8(2*H*,5*H*,7*H*)-trione (**7**), which could then be reconverted to **5a** by catalytic reduction. This simple sequence of interconversions thus serves to eliminate both structures **5b** and **5c** from consideration. It seems reasonable to suggest that the 1-carbethoxy derivative **5b** is probably the initial product of intramolecular cyclization of **4**, but that a subsequent intramolecular acyl transfer of the carbethoxy group from N-1 to N-2 then ensues. Steric hindrance at N-1 in compound **5b** may well be responsible for this unidirectional rearrangement. The structure of **5a** was further confirmed as follows. Treatment with sodium hydride followed by addition of methyl iodide gave 2-carbethoxy-1,4-dihydro-4,5,7-trimethylpyrimido[4,5-*e*]-*as*-triazine-3,6,8(2*H*,5*H*,7*H*)-trione (**9**), whose structure was then firmly established by its independent synthesis by base-catalyzed cycli-