# Reactions of Fervenulone. An Unprecedented Ring Contraction of a 7-Azapteridine Ring System[1]

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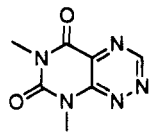
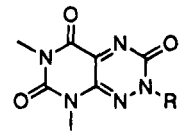
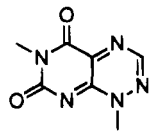
The 7-azapteridine (pyrimido[5,4-*e*]-*as*-triazine) ring system has received considerable chemical and medicinal interest primarily because of the potent, broad-spectrum antibacterial activity displayed by several members of this chemical class, including fervenulin (**1**), 2-methylfervenulone (MSD-92) (**2**), and toxoflavin (**4**).[2] However, their high toxicities ($LD_{50}$ values in mice 60 mg/kg, 2.5 mg/kg, and 3 mg/kg, respectively) and low therapeutic indices have impeded their clinical development.[3]



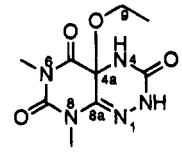
**1**



**2**, R = Me
**3**, R = H



**4**

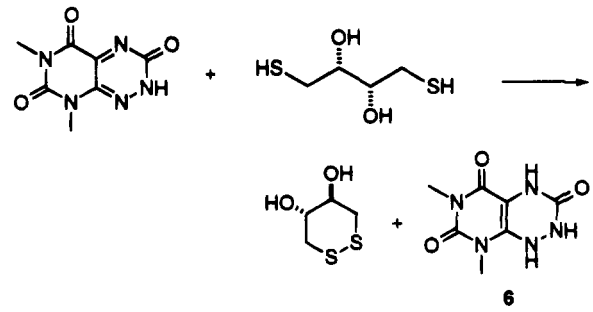


**5**

We became interested in the chemistry of this ring system because 2-methylfervenulone exhibited interesting biological activity in our high throughput screen for the inhibitors of Src-Homology 2 (SH2) domain-mediated protein–protein interactions. Chemically, this electron-deficient nitrogenous heteroaromatic system should be amenable to extensive SAR investigations. We examined the chemical reactivity of this class of compounds and report some of the interesting chemistry these compounds undergo.

Indeed, the nucleophilic susceptibility of this ring system became quite apparent early on in our studies. When a fervenulone (2,3,5,6,7,8-hexahydro-6,8-dimethylpyrimido[5,4-*e*](1,2,4)-triazine-3,5,7-trione) (**3**) sample was subjected to recrystallization from boiling ethanol, the only isolated product was its ethanol adduct **5**. The formation of a fervenulone–ethanol addition product has been observed by Taylor *et al.*[4] and Senga *et al.*,[5] but no structure has ever been proposed. Russian chemists, based solely on $^1H$ and $^{13}C$ proton-decoupled NMR data, have assigned it structures which were ambiguous with regard to the site of addition being at the 4a or 8a carbon.[6] We now report the unambiguous structure assignment of this adduct based on extensive NMR data (heteronuclear multiple bond correlation, HMBC; distortionless enhancement through polarization transfer, DEPT). The $^{13}C$-NMR spectrum of **5** shows C-4a shifted to 76 ppm from 146 ppm in fervenulone, indicating that it is a quaternary $sp^3$ carbon. In contrast, the chemical shift of C-8a (135 ppm) for **5**, which was identified by its coupling to N-8-$CH_3$ ($^1H$, 3.19 ppm) in the HMBC spectrum, remains almost unchanged ($\Delta = 3$ ppm) from that of the corresponding carbon resonance in fervenulone. Further evidence for the structure of **5** was garnered from the $^{13}C$ spectrum of the adduct formed with isotopically labeled ethanol {[1-$^{13}C$]ethyl alcohol}, which shows the labeled atom (C-9, 58 ppm) coupled to C-4a and C-5 (163 ppm) with $J$ values of 2.3 and 2.1 Hz, respectively.

**Scheme 1**



**6**

Since fervenulone formed an adduct with ethanol, we decided to investigate whether it reacted with dithiothreitol (DTT, Cleland's reagent), a reagent which is used at high concentrations in our high throughput ELISA (enzyme linked immunosuppressant assay) to insure the protein sulfhydryl groups remain in the reduced state.[7] We speculated that the active species might well be the DTT–fervenulone adduct and not fervenulone itself. Indeed, treatment of fervenulone with 1,4-dithio-L-threitol in DMSO-$d_6$ resulted in an instantaneous reaction, as judged by the disappearance of the fluorescent yellow color of fervenulone. An NMR analysis of the reaction showed that fervenulone had reacted with DTT not to form an adduct but rather to yield the reduction product which appeared to be **6**, and the corresponding oxidized product *trans*-1,2-dithiane-4,5-diol (Scheme 1).

Such a facile reduction of fervenulone is in good accord with the findings of Miller *et al.*[2] who noted an analogous reduction of 2-methylfervenulone with bisulfite and ascorbic acid, during its isolation and structure elucidation. In Miller's studies, however, no structure was assigned to any of the reduction products.

In order to synthesize N-2-substituted analogs of fervenulone, several alkylation reactions were performed with various electrophiles.[8] Treatment of fervenulone with *tert*-butyl bromoacetate (2.2 equiv) and potassium carbonate (2.1 equiv) in $CH_3CN$ at 90 °C surprisingly

(1) Dedicated to Professor E. J. Corey on the occasion of his 67th birthday.

(2) For a review on the chemistry of 7-azapteridines, see: Brown, D. J.; Lynn, R. K. In *Chemistry and Biology of Pteridines;* Pfleiderer, W., Ed.; Walter de Gruyter: New York, 1975; pp 575–601.

(3) Miller, T. W.; Chaiet, L.; Arison, B.; Walker, R. W.; Trenner, N. R.; Wolf, F. J. *Antimicrobial Agents and Chemotherapy*; Sylvester, J. C., Ed.; Medical Textbook Publishers: New York, 1963; p 58.

(4) Taylor, E. C.; Sowinski, F. *J. Org. Chem.* **1975**, *40*, 2321.

(5) Ichiba, M.; Nishigaki, S.; Senga, K. *J. Org. Chem.* **1978**, *43*, 469.

(6) Azev, Yu. A.; Mudretsova, I. I.; Didorov, E. O.; Pidemdkii, E. L.; Goleneva, A. F.; Aleksandrova, G. A. *Khim.-Farm. Zh.* **1987**, *21(7)*, 829. Azev, Yu. A.; Sidorov, E. O.; Mudretsova, I. I. *Khim. Geterotsikl. Soedin.* **1985**, 1692.

(7) Cleland, W. W. *Biochemistry* **1964**, *3*, 480. See also: *"Cleland's reagent"* a detailed brochure and bibliography published by Calbiochem, Los Angeles.

(8) Reaction with potassium carbonate and dimethyl sulfate in acetonitrile at reflux yielded 2-methylfervenulone as the sole product.

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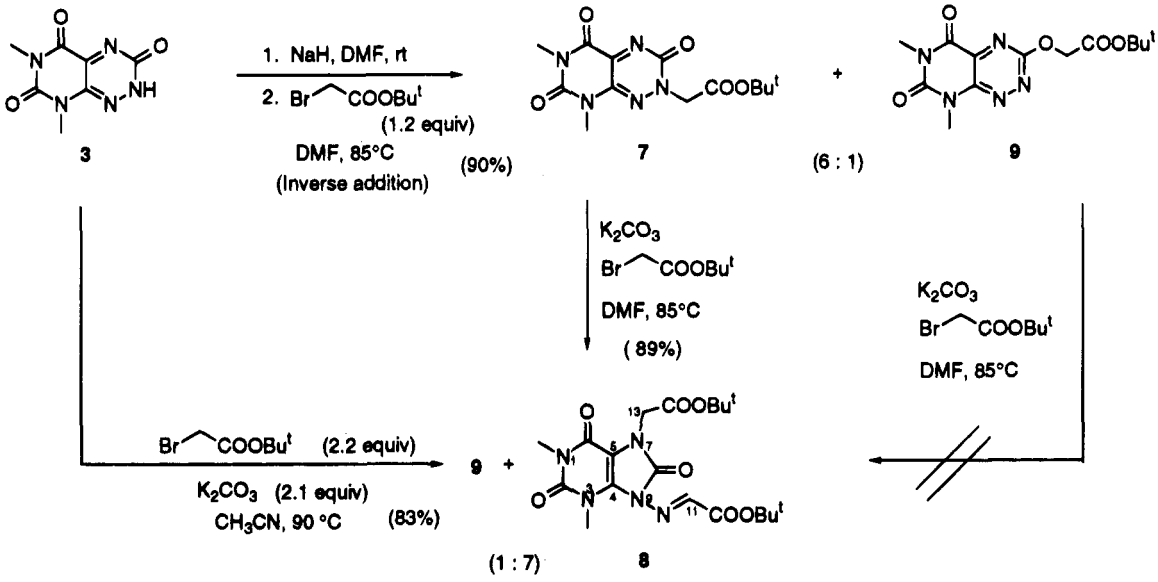
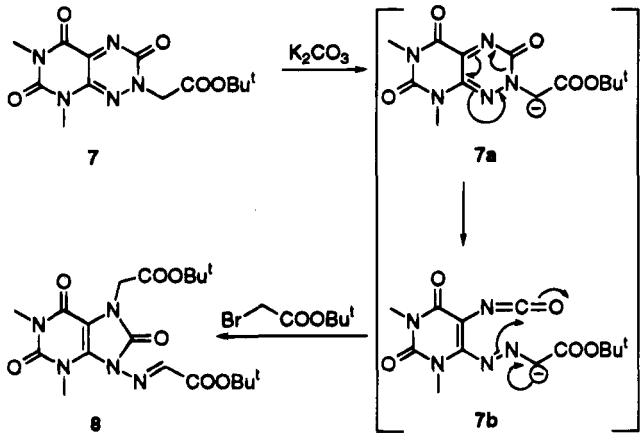
**Scheme 2**




**Scheme 3**




yielded none of the desired 2-[(*tert*-butoxycarbonyl)-methyl]fervenulone (**7**) but rather gave rise to the rearranged and bis-alkylated compound **8** as the major product (70–75%), accompanied by a small amount (10–15%) of the O-alkylated compound **9** (Scheme 2). Reaction with 1 equiv of *tert*-butyl bromoacetate and 1 equiv of potassium carbonate under identical conditions produced **8** and **9** in the ratio of 5:1, with 60% of fervenulone being recovered.

We surmise that a reasonable mechanism for this transformation must proceed through the intermediacy of **7**, as under the above conditions pyridones are known to alkylate preferentially at nitrogen.[9] This N-alkylated compound could then rearrange and realkylate under the basic conditions to furnish **8**, most likely *via* the mechanism depicted in Scheme 3.

The proposed mechanism involves as the key step a sigmatropic rearrangement which is analogous to that of the anion-accelerated oxy-Cope rearrangement. Presumably, the formation of the anion **7a** α to the bond to be broken weakens it and facilitates its rupture. The increased resonance energy and low basicity of the α-ester, α-diazo anion **7b** thus formed provides the required thermodynamic stability and helps shift the equilibrium toward the product **8**.

To gain some evidence for the intermediacy of **7** in the formation of **8** from fervenulone, we sought to synthesize **7** and then determine if it could rearrange to **8**. We synthesized **7** by slowly adding the preformed sodium salt of fervenulone to *tert*-butyl bromoacetate in DMF at 85 °C, thus avoiding exposure of **7** to excess base. Using these conditions, **7** was isolated in 78% yield along with the O-alkylated compound **9** (12%). As expected, when **7** was treated with potassium carbonate and *tert*-butyl bromoacetate in DMF at 80 °C for 1.5 h, it rearranged quantitatively to **8**. Under identical conditions **9** remained unchanged.

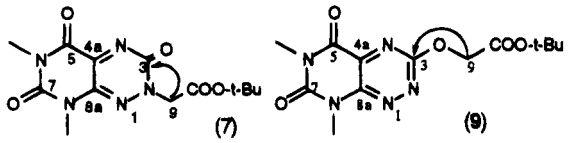
The structure of **8** was based on the $^{13}C$ chemical shifts and heteronuclear coupling information and was confirmed by single crystal X-ray crystallography. The two monoalkylated compounds **7** and **9** have very similar $^1H$ NMR spectra, and any assignment based solely upon this information was impossible. Their structures were assigned based on the distinct $^{13}C$ chemical shifts of C-3, C-4a, and C-9 carbon atoms and from the heteronuclear coupling information obtained from their HMBC spectra. The chemical shift of the α-ester carbon atom (C-9) of **9** (65.5 ppm) is approximately 10 ppm downfield compared to that of C-9 of **7** (56.1 ppm). Also, the isoureido carbon atom (C-3) of **9** (164.0 ppm) is shifted downfield by approximately 10 ppm relative to the ureido carbon atom (C-3) of **7** (153.3 ppm). The other noteworthy chemical shifts are tabulated in Table 1, along with the values predicted by the Specinfo database.[10] In the HMBC spectrum of **7**, the ureido carbon atom (C-3) is long-range coupled to the protons on C-9 ($^1H$, 4.80 ppm). Likewise, for **9**, an HMBC connectivity was observed between the isoureido carbon atom (C-3) and the protons on C-9 (δ $^1H$ 5.03 ppm).

In summary, we have characterized some unexpected reaction products that are obtained when fervenulone is treated with ethanol, dithiothreitol, or *tert*-butyl bromoacetate/potassium carbonate. These studies have shown that fervenulone undergoes an unprecedented and facile ring contraction upon attempted N-alkylation with *tert*-butyl bromoacetate. The unusual chemical reactivity of fervenulone renders it an interesting heterocyclic system deserving of further study.

(9) Comins, D. L.; Jianhua, G. *Tetrahedron Lett.* **1994**, *35*, 2819, and references cited therein.

(10) Specinfo CNMR database, STN International, Cincinnati, OH.

**Table 1. $\delta$ $^{13}$C Values for 7 and 9, and Major HMBC Correlations (arrowhead represents carbon, and tail of arrow represents proton)**




(7) (9)

| atom no. | found (predicted) | found (predicted) |
|---|---|---|
| C-3 | 153.3(161) | 164.0(168) |
| C-4a | 146.8(162)[a] | 134.7(137) |
| C-5 | 158.4(161) | 160.1(161) |
| C-7 | 150.3(150) | 150.5(150) |
| C-8a | 138.7(150) | 149.6(150) |
| C-9 | 56.1(52) | 65.5(66) |

[a] The predicted value is interpolated and does not agree with the data obtained on many fervenulone analogs synthesized in our labs. The range of values which we observe for this carbon atom is from 134 to 150 ppm.

## Experimental Section[11]

**2-[(*tert*-Butoxycarbonyl)methyl]-6,8-dimethylpyrimido-[5,4-*e*]-*as*-triazine-5,7(6*H*,8*H*)-dione (2-[(*tert*-Butoxycarbonyl)methyl]fervenulone) (7), and 3-[(*tert*-Butoxycarbonyl)-methoxy]fervenulone (9).** A solution of fervenulone **2** (100 mg, 0.478 mmol) in DMF (4.0 mL) was added dropwise to sodium hydride (20 mg, 0.5 mmol, 60% dispersion in oil, prewashed with hexanes) in DMF (1 mL), at 0 °C. The resulting yellow slurry was stirred at room temperature for 1 h and then added in portions to a stirred solution of *tert*-butyl bromoacetate (90 $\mu$L, 0.52 mmol, 1.1 equiv) in DMF (1 mL) at 85 °C. The clear yellow solution was stirred at this temperature for 2 h. Water (5 mL) was added and the mixture diluted with chloroform (20 mL). The organic layer was separated and the aqueous layer re-extracted with chloroform (3 × 10 mL). The combined organic extract was dried ($Na_2SO_4$), concentrated, and chromatographed to give **7** (121 mg, 78%) as a pale yellow solid: TLC $R_f$ (20% $CH_3CN/CHCl_3$) = 0.24, and **9** (18 mg, 12%): TLC $R_f$ (20% $CH_3CN/CHCl_3$) = 0.62.

**7**: $^1$H NMR (acetone-$d_6$, $\delta$): 4.80 (s, 2H), 3.44 (s, 3H), 3.38 (s,3H), 1.47 (s, 9H). $^{13}$C NMR (acetone-$d_6$, $\delta$): 166.4, 158.4, 153.3, 150.3, 146.8, 138.7, 83.2, 56.1, 29.5, 29.3, 28.1. HMBC (H/C, acetone-$d_6$): N6-$CH_3$/5, 7 ; N8-$CH_3$/7; 9/3, 10. HRMS: calcd ($C_{13}H_{17}N_5O_5$) 324.1308, obsd 324.1307.

**9**: $^1$H NMR (acetone-$d_6$, $\delta$): 5.03 (s, 2H), 3.74 (s, 3H), 3.40 (s, 3H), 1.45 (s, 9H). $^{13}$C NMR (acetone-$d_6$, $\delta$): 167.5, 164.0, 160.1, 150.5, 149.6, 134.7, 82.6, 65.5, 29.8, 28.1. HMBC (H/C, acetone-$d_6$): N6-$CH_3$/5, 7 ; N8-$CH_3$/7, 8a; 9/3, 10. HRMS: calcd ($C_{13}H_{17}N_5O_5$) 324.1308, obsd 324.1307.

**Synthesis of 8 from 7** (ring contraction and alkylation of **7**). To a solution of **7** (20 mg, 0.0618 mmol) and *tert*-butyl bromoacetate (13 $\mu$L, 0.075 mmol) in DMF (400 $\mu$l) was added $K_2CO_3$ (8 mg, 0.065 mmol). The mixture was heated at 80 °C for 1.5 h, cooled to rt, diluted with $CHCl_3$, and washed with water and brine. The organic layer was dried ($Na_2SO_4$), filtered, concentrated, and chromatographed to afford **8** (24 mg, 89%): TLC $R_f$ (10% $CH_3CN/CHCl_3$) = 0.51. $^1$H NMR ($CDCl_3$, $\delta$): 9.10 (s, 1H), 4.68 (s, 2H), 3.81 (s, 3H), 3.34 (s, 3H), 1.52 (s, 9H), 1.45 (s, 9H). $^{13}$C NMR ($CDCl_3$, $\delta$): 166.7, 161.3, 153.7, 150.7, 148.2, 146.1, 132.6, 97.8, 83.1, 43.6, 32.6, 28.3, 28.0, 27.9. HMBC (H/C, $CDCl_3$): N6-$CH_3$/5, 7; N8-$CH_3$/7, 8a; 9/3, 4a, 10; 11/12. HRMS: calcd ($C_{19}H_{27}N_5O_7$) 437.1910, obsd 437.1910. Anal. Calcd for $C_{19}H_{27}N_5O_7$: C, 52.17; H, 6.22; N, 16.10. Found: C, 52.08; H, 6.25; N, 16.10.

**[9-[[(*tert*-Butoxycarbonyl)methylene]amino]-1,3-dimethyl-2,6,8-trioxo-1,2,3,6,8,9-hexahydropurin-7-yl]acetic Acid *tert*-Butyl Ester (8)[12] from Fervenulone (3).** To a mixture of fervenulone (100 mg, 0.478 mmol) and *tert*-butyl bromoacetate (150 $\mu$L, 1.016 mmol) in $CH_3CN$ (2.0 mL) was added $K_2CO_3$ (138 mg, 1.0 mmol). The mixture was heated at 90 °C for 1.5 h, cooled to rt, diluted with $CHCl_3$ and washed with water and brine. The organic layer was dried ($Na_2SO_4$), filtered, concentrated, and chromatographed to afford **8** (153 mg, 73%), and **9** (17 mg, 11%).

**Acknowledgment.** We thank Drs. Francis G. Fang and Jeffrey A. Stafford for many helpful discussions, and Larry Shampine and Roderick Davis for recording mass spectra. The X-ray crystallographic data were obtained by Dr. Peter White of the University of North Carolina at Chapel Hill.

**Supporting Information Available:** $^1$H and $^{13}$C NMR spectra of compounds **2**, **3**, **5**, and **7**–**9**; and HMBC spectra of **5** and **7**–**9** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(11) All starting materials were obtained from commercial suppliers and used without purification. All reactions involving oxygen- or moisture-sensitive compounds were performed under a dry nitrogen atmosphere. All reactions and chromatography fractions were analyzed by thin-layer chromatography on 2.5 × 7.5 silica gel plates (250-$\mu$m $SiO_2$ thickness), visualized with UV light and $I_2$ stain. Flash column chromatography was carried out using Merck silica gel 60 (230–400 mesh). Evaporation of solvents was accomplished with a rotary evaporator. $^1$H NMR spectra were measured in $CDCl_3$ using either a Varian VXR-300 or a Varian Unity-300 instrument unless otherwise indicated. *J* values are reported in hertz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Apparent multiplicities are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. All mass spectra were taken in the positive ion mode by fast atom bombardment (FAB). Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

(12) This name was generated by the Autonom program.