Pyridopyrimidines. 11. Synthesis of 5-Deaza-5-oxoaminopterin and Related Compounds

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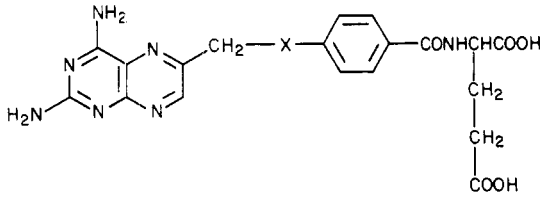
The syntheses of 5-deaza-5-oxo analogues of aminopterin, methotrexate, and 10-thiaaminopterin are described. The sequence involves, as a key step, the preparation of 2,4-diamino-6-(hydroxymethyl)-5-oxopyrido[2,3-*d*]pyrimidine from readily accessible pyridopyrimidine derivatives.

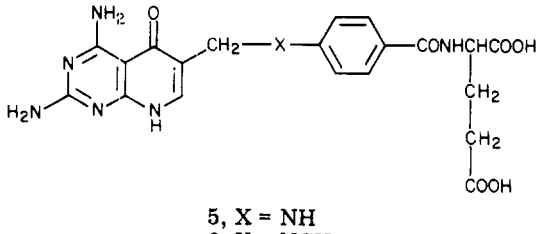
The use of powerful dihydrofolate reductase inhibitors, aminopterin (1) and its N^{10}-methyl derivative methotrexate (2), in the treatment of tumors is well-known. Various



1, X = NH
2, X = NCH_3
3, X = O
4, X = S

modifications have been made in an attempt to increase the potency and lessen the toxicity of these compounds. They include variations in the side chain to give, for example, 10-oxaaminopterin (3)[1] and 10-thiaaminopterin (4).[2] The former was shown to be an excellent inhibitor of bacterial dihydrofolate reductase, while the latter was found to be as active as methotrexate in inhibiting dihydrofolate reductase from *Lactobacillus casei*.

This present report is concerned with the synthesis of 5-deaza-5-oxoaminopterin (5) and related derivatives 6 and 7. The model of methotrexate–dihydrofolate reductase

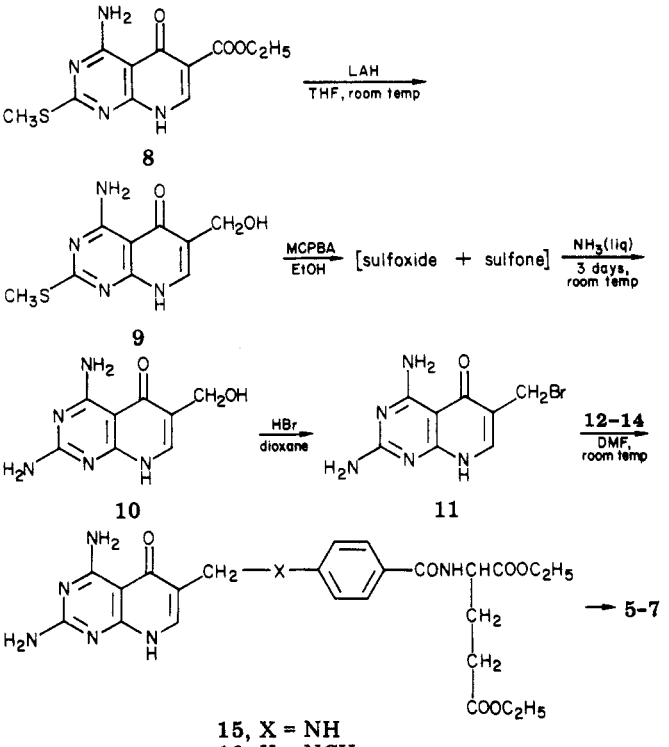


5, X = NH
6, X = NCH_3
7, X = S

interaction proposed by Matthews et al.[3] from their X-ray crystallographic studies suggests that these molecules may fit nicely into the dihydrofolate reductase active site and may shed additional light on the nature of that site in the region about the pteridine N^5.

The target compounds 5–7 were prepared according to Scheme I. Lithium aluminum hydride reduction of 4-amino-6-carbethoxy-2-(methylthio)-5-oxopyrido[2,3-*d*]pyrimidine (8)[4] in dry THF gave the corresponding carbinol 9. The structure of 9 was established by its mass spectrum (*m/e* 238) and by the presence of a doublet at δ 4.46 (J = 4.0 Hz) and a triplet at δ 5.13 in the ^{1}H NMR for the

Scheme I



15, X = NH
16, X = NCH_3
17, X = S

methylene and hydroxyl protons, respectively. Oxidation of 9 with *m*-chloroperoxybenzoic acid to a mixture of a sulfoxide and sulfone[5] followed by treatment with liquid ammonia in a sealed vessel gave 2,4-diamino-6-(hydroxymethyl)-5-oxopyrido[2,3-*d*]pyrimidine (10). Treatment of a suspension of 10 in anhydrous dioxane with HBr gave the bromomethyl derivative 11 which was treated with diethyl (*p*-aminobenzoyl)-L-glutamate,[6] diethyl *N*-methyl-(*p*-aminobenzoyl)-L-glutamate,[7] or the sodium salt of diethyl (*p*-mercaptobenzoyl)-L-glutamate[8] in anhydrous DMF. After chromatographic purification, 15–17, respectively, were obtained. Finally, saponification in dilute base afforded the desired aminopterin analogues 5–7.

Examination of the ^{1}H NMR spectra of 5, 6, 15, and 16 revealed an interesting difference in otherwise very similar spectra. The proton at C^7 of each of the aminopterin analogues 5 and 15 exhibits a chemical shift of δ 7.56, approximately 0.3 ppm downfield from the signal for the C^7–H of methotrexate analogues 6 and 16. Although other

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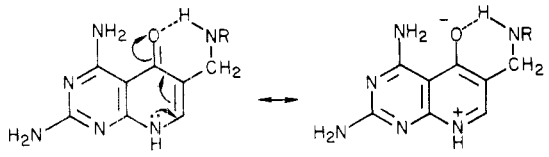
explanations may be made, the most likely reason for this phenomenon is the formation of an N^{10}–H to O^5 hydrogen bond with resultant alterations in the positive character at C^7, as shown by the resonance structures I and II.



I II

Precedent for such a relatively stable conformation (at ambient temperature in the NMR probe) exists in earlier work with the 4-amino-6-carboxamido-5-oxo analogues,[4,5] in which a strong hydrogen bond is formed between the amide N–H and the carbonyl at C^5.

Experimental Section

The ^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer in dimethyl-d_6 sulfoxide with DSS as the internal standard. Exchangeable protons were detected by the addition of D_2O. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Mass spectra were taken on a Varian 112-S spectrometer. Permethylations were carried out in dimethyl sulfoxide by using NaH and methyl iodide. UV spectra were taken on a Beckman Acta CIII or Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories.

4-Amino-6-(hydroxymethyl)-2-(methylthio)-5-oxopyrido[2,3-*d*]pyrimidine (9). To a stirred suspension of 2.28 g (0.06 mol) of lithium aluminum hydride in 175 mL of anhydrous THF was added 5.6 g (0.02 mol) of 4-amino-6-carbethoxy-2-(methylthio)-5-oxopyrido[2,3-*d*]pyrimidine (8) in small portions over a period of 30 min. After the mixture was stirred for 3 h at room temperature, excess LAH was destroyed by careful addition of water, and the suspension was filtered. The filtrate was made acidic with 6 N acetic acid and concentrated to about 50 mL. After the mixture had been allowed to stand overnight, the crystallized solid was filtered to give 2.76 g (58%) of **9**. An analytical sample was prepared by crystallization from water: mp 224–26 °C; mass spectrum, m/e 238 (M^+); ^{1}H NMR δ 2.6 (s, 3, SCH_3), 4.46 (d, J = 4.0 Hz, 2, CH_2), 5.13 (t, J = 4.0 Hz, 1, OH), 7.83 (s, 1, C^7H), 8.28 and 9.8 (d, 1 each, 4-NH_2), 12.3 (br, 1, 8-NH); UV λ_{max} (ϵ_{max}) (pH 1) 268 nm (16400), 295 (5400); (pH 7) 265 (15100), 312 (3300); (pH 11) 265 (14500), 302 (3300).

Anal. Calcd for $C_9H_{10}N_4O_2S{\cdot}0.5H_2O$: C, 43.71; H, 4.48; N, 22.65. Found: C, 43.42; H, 4.44; N, 22.35.

2,4-Diamino-6(hydroxymethyl)-5-oxopyrido[2,3-*d*]pyrimidine (10). To a suspension of 11.9 g (0.05 mol) of **9** in 200 mL of ethanol was added 30 g of *m*-chloroperoxybenzoic acid, and the mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo, and the residue was stirred with ether and filtered. The sulfone and sulfoxide mixture was stirred in a sealed vessel with ~200 mL of liquid ammonia for 3 days. The ammonia was allowed to evaporate, and addition of water to the residue and filtration gave **10**. Crystallization from water gave pure **10**: mp >280 °C; yield 6.8 g (64%); mass spectrum, m/e 207 (M^+); ^{1}H NMR δ 4.76 (s, 2, CH_2), 6.8 (br, 2, 2-NH_2), 7.76 (s, 1, C^7 H), 7.76 and 9.75 (d, 1 each, 4-NH_2), 10.73 (br, 1, 8-NH); UV λ_{max} (ϵ_{max}) (pH 1) 257 (19000), 292 (3500); (pH 7) 254 (16700), 287 (5300); (pH 11) 255 (15700), 287 (4300).

Anal. Calcd for $C_8H_9N_5O_2{\cdot}0.5H_2O$: C, 44.44; H, 4.66; N, 32.39. Found: C, 44.20; H, 4.60; N, 32.35.

Diethyl *N*-[*p*-[[(2,4-Diamino-5-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (15). A suspension of 2.07 g (10 mmol) of **10** in 75 mL of dry dioxane was saturated with gaseous hydrogen bromide, and the suspension was stirred overnight. The precipitated solid was filtered. The mass spectrum showed at peak at m/e 190 (M^+ – Br). TLC in $CHCl_3$–CH_3OH (80:20) gave a single spot with traces of minor impurities. The compound **11** was dried at 80 °C over P_2O_5 for 2–3 h. To a solution of this compound in 50 mL of dry DMF (distilled over P_2O_5) was added 6.4 g (20 mmol) of diethyl (*p*-aminobenzoyl)glutamate **12**.[6] The resulting solution was stirred for 12 h at room temperature and evaporated to dryness. The residue was suspended in water and stirred with 20 mL of 5% HCO_3^- solution and extracted with $CHCl_3$ (3 × 100 mL). The chloroform solution was dried, evaporated to about 50 mL, and poured onto a column (silica gel, 60–200 mesh). Elution with $CHCl_3$–CH_3OH (96:4) gave unreacted **12**. Elution with $CHCl_3$ (90:10) gave pure **15**: 2.8 g (54% based on **10** used); mp 128–131 °C; mass spectrum, m/e 609 (M^+) (heptamethyl derivative); ^{1}H NMR δ 6.81 and 7.9 (q, 4, C_6H_4), 7.56 (s, 1, C-7H), 8.48 (d, 1, CONHCH), 11.55 (br, 1, 8-NH); UV λ_{max} (ϵ_{max}) (pH 1) 255 (42250), 292 (12500); (pH 7) 252 (28100), 293 (29400); (pH 11) 252 (29100); 293 (28100).

Anal. Calcd for $C_{24}H_{29}N_7O_6{\cdot}0.5\ H_2O$: C, 55.37; H, 5.80; N, 18.83. Found: C, 55.54; H, 5.91; N, 18.73.

Diethyl *N*-[*p*-[[(2,4-Diamino-5-oxopyrido[2,3-*d*]-pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamate (16). This compound was prepared in a similar procedure by using **10** and diethyl [*N*-(methylamino)-*p*-aminobenzoyl]-L-glutamate (**13**)[7] in 62% yield: mp 99–101 °C with prior softening; mass spectrum, m/e 609 (M^+)(hexamethyl derivative); ^{1}H NMR δ 3.11 (s, 3, NCH_3), 6.93 and 7.99 (q, 4, C_6H_4), 7.26 (s, 1, C^7 H), 8.58 (d, 1, CONH); UV λ_{max} (ϵ_{max}) (pH 1) 258 (40000), 301 (8400); (pH 7) 253 (27500), 308 (34000); (pH 11) 253 (29000), 311 (34000).

Anal. Calcd for $C_{25}H_{31}N_7O_6{\cdot}1.0H_2O$: C, 55.22; H, 5.93; N, 18.03. Found: C, 55.63; H, 6.17; N, 18.09.

Diethyl *N*-[*p*-[[(2,4-Diamino-5-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]thio]benzoyl]-L-glutamate (17). A suspension of 4.06 g (6 mmol) of tetraethyl 4,4′-dithiobis(*N*-benzoyl-L-glutamate) in 50 mL of ethanol was reduced with 0.68 g of sodium borohydride according to the procedure of Mautner et al.[8] The solution was concentrated to about 20 mL, to which a solution of **11** [prepared from 2.07 g (10 mmol) and HBr in dioxane as described earlier] was added. After the mixture was stirred for 6 h at room temperature, the solvents were removed in vacuo. The residue was stirred with water and extracted with 500 mL of ethyl acetate. The organic phase was washed with water, dried, and concentrated to about 100 mL. The crystallized solid was filtered and dried to give 3.1 g (58%) of **17**. An analytical sample was prepared by crystallization from ethyl acetate: mp 122–124 °C; mass spectrum, m/e 612 (M^+)(hexamethyl derivative); ^{1}H NMR δ 7.73 and 8.1 (q, 4, aromatic), 7.60 (s, 1, C^7 H), 8.93 (d, 1, CONH), 11.51 (br, 1, 8-NH); UV λ_{max} (ϵ_{max}) (pH 1) 258 (31000), 284 (14500); (pH 7) 255 (21000), 289 (20000), 259 (27800), 289 (18700).

Anal. Calcd for $C_{24}H_{28}N_6O_6S$: C, 54.33; H, 5.33; N, 15.90. Found: C, 54.44; H, 5.43; N, 15.91.

General Procedure for Alkaline Hydrolysis of 15–17. A solution of the ester in ethanol and 1 N NaOH (3 mL of ethanol and 3.5 mL of 1 N NaOH per 1 mmol of ester) was stirred for 48 h at room temperature. The solution was filtered and carefully acidified with 0.2 N HCl to pH 3. The precipitated solid was filtered and washed with water. The solid was dissolved in 2–3 mL of 5% HCO_3^- solution and made acidic to pH 3. The precipitated solid was filtered, washed with water, and dried in vacuo over P_2O_5.

***N*-[*p*-[[(2,4-Diamino-5-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (5)**: prepared from **15**; 61% yield; mp 199–203 °C; slowly turns yellow on standing; mass spectrum, m/e 595 (M^+) (nonamethyl derivative); ^{1}H NMR δ 4.03 (s, 2, $HNCH_2$), 6.78 and 7.88 (q, 4, aromatic), 7.56 (s, 1, C^7 H), 8.35 (d, 1, CHNH); UV λ_{max} (ϵ_{max}) (pH 1) 257 (40000), 295 (11000); (pH 7) 255 (19700), 293 (21000): (pH 11) 258 (22000), 291 (23000).

Anal. Calcd for $C_{20}H_{21}N_7O_6{\cdot}2.0H_2O$: C, 48.87; H, 5.12; N, 19.89. Found: C, 48.55; H, 5.15; N, 19.54.

***N*-[*p*-[[(2,4-Diamino-5-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic acid (6)**: prepared from **16**; 57% yield; mp 178–181 °C; mass spectrum, m/e 581 (M^+) (octamethyl derivative); ^{1}H NMR δ 3.14 (s, 3, NCH_3), 4.44 (s, 2, CH_2NCH_3), 6.91 and 7.97 (q, 4, aromatic), 7.24 (s, 1, C^7 H), 8.38 (d, 2, CONH); UV λ_{max} (ϵ_{max}) (pH 1) 257 (40000), 298 (7400); (pH 7) 253 (23500), 304 (29000); (pH 11) 257 (24000), 307 (28000).

Anal. Calcd for $C_{21}H_{23}N_7O_6{\cdot}2.25H_2O$: C, 49.45; H, 5.43; N, 19.22. Found: C, 49.22; H, 5.40; N, 18.96.

***N*-[*p*-[[(2,4-Diamino-5-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]thio]benzoyl]-L-glutamic acid (7)**: prepared from **17**;

57% yield; mp 199–202 °C; mass spectrum, m/e 584 (M^+) (octamethyl derivative); ^1H NMR δ 4.16 (s, 2, S-CH_2), 7.73 (s, 1, C^7 H), 7.6 and 8.06 (q, 4, C_6H_4), 8.8 (d, 1, CONH); UV λ_{max} (ϵ_{max}) (pH 1) 258 (35000), 281 (18000); (pH 7) 256 (31000), 281 (17600); (pH 11) 257 (26500), 289 (18000).

Anal. Calcd for $C_{20}H_{20}N_6O_6S\cdot 1.0H_2O$: C, 48.90; H, 4.52; N, 17.12. Found: C, 48.82; H, 4.76; N, 17.21.

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Registry No. **5**, 74332-03-9; **6**, 74346-17-1; **7**, 74332-04-0; **8**, 36707-45-6; **9**, 74332-05-1; **9** sulfene, 74332-10-8; **9** sulfoxide, 74332-11-9; **10**, 74332-06-2; **11**, 74332-07-3; **12**, 13726-52-8; **13**, 2378-95-2; **15**, 74332-08-4; **16**, 74332-09-5; **17**, 74346-18-2; tetraethyl 4,4-dithiobis(*N*-benzoyl-L-glutamate), 56527-28-7.

Heterocyclic Ring-Closure Reactions. 6.[1] Preparation and Further Cyclization Reactions of 5-Imino-1,3-diphenyl-4-thioxo-2-imidazolidinone and 5-Imino-1,3-diphenyl-2,4-imidazolidinedithione

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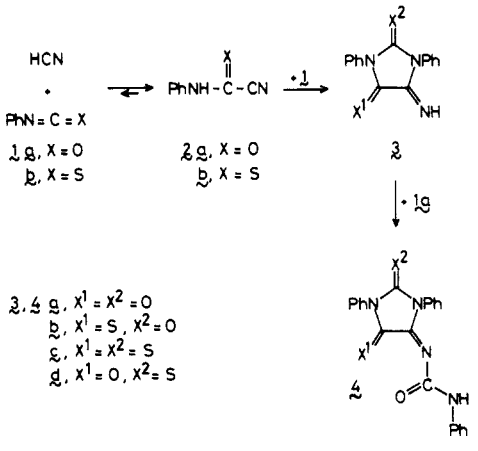
Phenyl isocyanate (**1a**) and phenyl isothiocyanate (**1b**) react with cyanothioformanilide (**2b**) to give the title compounds **3b** and **3c**. With additional **1a**, **3b,c** provide the 5-(*N*-phenylcarbamoyl) derivatives **4b,c**. With hydrochloric acid, **3b,c** afford the 4-oxo compounds **5a,b**, whereas excess hydrochloric acid converts **3c** into the 2,5-thiazolidinedione **6**. With hydrogen sulfide, **3c** gives phenyldithiooxamide (**7**) and **3b** the 4-thiohydantoin **8**. With benzaldehyde, **3b,c** furnish 4,6-dihydro-2,4,6-triphenyl-5*H*-imidazo[4,5-*d*]thiazol-5-one (**9a**) and -5-thione (**9b**). When boiled in phenol at 180 °C, **3b,c** cyclize to 5,7-dihydro-1,3,5,7-tetraphenyldiimidazo[4,5-*b*:4′,5′-*e*]-pyrazine-2,6(1*H*,3*H*)-dione (**10a**) and -dithione (**10b**).

Our interest in the ring-closure reactions of oxalic acid derivatives such as dithiooxamide[1,4] and cyanogen[5] on the one hand and of heterocumulenes[6] on the other led us to investigate the possible cyclization reactions of cyanothioformanilide (**2b**) with phenyl isocyanate (**1a**) and phenyl isothiocyanate (**1b**). The feasibility of such reactions was anticipated from the earlier work of Dieckmann and Kämmerer on the base-catalyzed reactions of **1a** with hydrogen cyanide to give **2a**, 1,3-diphenyl-5-imino-2,4-imidazolidindione (**3a**), and 1,3-diphenyl-5-(*N*-phenylcarbamoyl)-imino-2,4-imidazolidindione (**4a**), the 1:1, 1:2, and 1:3 products of hydrogen cyanide with **1a**[7] (Scheme I).

Scheme I



Of particular interest were the reaction products **3b** and **3c** which possess a thiocarbonyl group at C_4 and an imino function at C_5 since they might be capable of further cyclization reactions in which these sulfur and/or nitrogen functions could be incorporated into an additional fused ring.

The cyanothioamide **2b** reacts rapidly with **1a**[8] to give **3b** in high yield under a variety of conditions. Not unexpectedly, at elevated temperatures in the absence of a base, the only product is **4b**. With phenyl isothiocyanate (**1b**) and **2b** formation of **3b** proceeds more slowly, and deeply colored side products reduce the yield. It was not

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(2) On leave from the University of California. Work done at University of Hamburg. Supported in part by grants from the Academic Senate, University of California, and the German Academic Exchange Service.

(3) Inquiries may be directed to either author.

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