THE SYNTHESIS OF CERTAIN 7-SUBSTITUTED TRIAZOLOPYRIMIDINES

by

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Typed by Steve Lower
Dedicated to

Mr. Charlie Frye

whose help contributed much

to the execution of this project.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>7</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>23</td>
</tr>
</tbody>
</table>

## TABLES AND FIGURES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I</td>
<td>Reaction Scheme</td>
<td>4</td>
</tr>
<tr>
<td>Table II</td>
<td>Ultraviolet Absorption Data</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Ultraviolet Absorption Curves</td>
<td>17</td>
</tr>
</tbody>
</table>
THE SYNTHESIS OF CERTAIN 7-SUBSTITUTED TRIAZOLOPYRIMIDINES

INTRODUCTION

From the time of their discovery, purines and related compounds have been under continual investigation as inhibitors, antimetabolites, bacteriostatic agents, carcinostatic- and carcinogenic agents, etc. In 1955, interest in these compounds was reintensified by the characterization and synthesis of 6-furfurylaminopurine (kinetin) by Miller, Skoog, and coworkers (12, p. 1392), and its identification as the active principle of the "growth factor" first described by Overbeek in 1941 (19, p. 350-351) and known for some time as the "coconut milk factor". The fact that kinetin activates the process of cell-division (and hence of growth) is of special interest to those engaged in cancer research, as carcinoma may be looked upon as essentially a problem of growth and its control.

Thus the elucidation of the nature of kinetin resulted in the preparation by many workers of kinetin analogs, principal attention having been given to 2,6-diaminopurine (3, p. 119-120), its N-alkylated derivatives (14, p. 404-408), 6-mercaptopurine (1, p. 766-769; 4, p. 7-8), 6-chloropurine and its 9-alkylated derivatives (15, p. 490-494), and a wide variety of six-substituted
amino purines (18, p. 2843-2846; 7, p. 411-414; 9, p. 2648; 17, p. 5097-5100). Other purines which have recently shown some evidence of cancer-inhibiting activity are 2-fluoroadenine (13, p. 4559) and the guanine analog of pyrazolo (3,4-d) pyrimidine (18, p. 2843-2846).

Interest in the 1-v-triazolo(d)pyrimidines began when Roblin and coworkers prepared several triazolo analogs of the common purines for bacteriostatic screening. The subsequent discovery of the carcinostatic properties of 8-azaguanine (8, p. 1030-1033) stimulated further interest in these and related compounds.

This Laboratory had previously prepared a series of 6-alkylaminopurines for carcinostatic screening, and it was decided to complement this with the preparation of an analogous series of 7-substituted triazolopyrimidines*. The general preparative scheme followed that employed earlier by Daly and Christensen (5, p. 177-179) (see Table I).

Pyrimidine-4,6-dione was prepared by the method of Hull (11, p. 2214), involving the condensation of malonodiamide with ethyl acetate.

The procedure originally given for the nitration of pyrimidine-4,6-dione (2, p. 96-102; 5, p. 177-179) was

* Because of the special numbering system employed for purines, a 6-substituted purine is an analog of a 7-substituted triazolopyrimidine.
TABLE I
REACTION SCHEME
found to be somewhat unreliable in that the reaction could not always be started and once started was frequently too rapid. The method finally devised consisted of adding the pyrimididine to the nitrating mixture (25 volume-percent fuming nitric acid in glacial acetic acid), keeping the temperature below 25°, and then adding excess fuming nitric acid and allowing the temperature to rise. This differed from the original procedure in that the twenty minute warming period following the addition of the pyrimididine was eliminated, the fuming nitric acid being added immediately, without any such waiting period.

The chlorination of the nitopyrimididine employed phosphoryl chloride and dimethylaniline, but the 85% yield claimed by Boon, Jones, and Ramage (2, p. 96-102) could not be reproduced; however, fairly consistent yields of approximately 20% were obtained several times. The trouble appears to be due possibly to the hydrolysis of the dichloride, which occurs very readily due to the activation of the chlorine atoms by the adjacent nitro group. In the original procedure the reaction mixture is poured on ice, liberating considerable local heat from the hydrolysis of undistilled phosphoryl chloride. Since the reaction mixture is a heavy sticky mass, it is difficult to achieve good mixing, severe local heating occurs, and considerable ice melts. This mixture of ice, water, and
product is then extracted with ether and the product recovered. In the modified version, the reaction mixture (after distillation of excess phosphoryl chloride) is extracted directly with ether. Evaporation of the ether and recrystallization result in a much cleaner product in more than twice the original yield.

Amination of the dichloronitropyrimidine was by the method of Boon, Jones, and Ramage (2, p. 96-102) and involved the direct action of ammonia. The resulting 4-amino-5-nitro-6-chloropyrimidine was used as the starting material for the preparation of the various derivatives. The pyrimidine was treated directly with the desired amine and the aminolysis of the 6-chloro group proceeded without difficulty. The reduction of the 5-nitro group of the resulting compound was readily effected by direct hydrogenation, using Raney W-2 nickel catalyst (10, p. 180-183).

Cyclization of the 6-substituted diaminopyrimidines was easily accomplished by treatment with sodium nitrite in acid solution. As was noted previously (6, p. 171-177), a colored intermediate is usually formed which gradually becomes colorless as the reaction proceeds.

The derivatives prepared were the morpholino-, benzylamino-, anilino-, methylamino-, isobutylamino-, and
piperidino-triazolopyrimidines.

Physically, this series of triazolopyrimidines forms white crystals of an extremely light and fluffy nature, which begin to decompose above 270-300° but show no sharp melting or decomposition points. They show no tendency to explode on heating.

Unsubstituted triazolopyrimidine itself is very soluble in water, but as in many N-heteroaromatic molecules having a high N:C ratio, the addition of electron-donating groups (e.g., the sec-amino group) lowers the solubility greatly (16, p. 126); this series of derivatives is practically insoluble in water.

The ultraviolet spectra all show maximum absorptions at somewhat greater wavelengths than that of the unsubstituted parent compound (16, p. 134), which absorbs at 270 m over the pH range 3.5-6.
4,6-Pyrimidinedione. Malonodiamide (102 grams) was added to a solution of 46 grams of sodium metal in 1500 ml of absolute alcohol. To this mixture (still hot from the reaction with sodium) was added, over a period of an hour, 120 ml of ethyl formate. The mixture was heated by means of a water bath for about two hours and then allowed to stand for 12 hours, by which time a large quantity of white material had settled out. The white crystals were washed with absolute ethanol and redissolved in 500 ml of water. This aqueous solution was acidified with concentrated hydrochloric acid; after standing for several hours the yellow crystals were collected and washed with water and then with alcohol. The yields were fairly uniform and amounted to 40-45 grams (35-40% yield).

5-Nitro-pyrimidine-4,6-dione. Twenty-two grams of 4,6-pyrimidinedione was added in small portions to 100 ml of a nitrating mixture (25 volume-percent of fuming nitric acid in glacial acetic acid) cooled by an ice bath and agitated by a magnetic stirrer. The best rate of addition was judged by observing the rate of nitrogen dioxide evolution and the temperature rise, which was not permitted to exceed 25°. On completion of this stage the ice bath was
removed and 50 ml of fuming nitric acid rapidly added. The bright red mixture was allowed to react smoothly, while stirring was continued for several hours following the transition to a pale yellow color which denoted the completion of the reaction. The solid compound was collected, washed several times with cold water, then with ethanol, and finally air-dried. Yield, 70%.

4,6-Dichloro-5-nitropyrimidine. Dimethylaniline (82 ml) was added slowly to a suspension of 5-nitro-4,6-pyr-imidinedione (78 gm) in phosphoryl chloride (300 ml); the mixture was heated in an oil bath at 125-130° for one hour under nitrogen. The excess phosphoryl chloride was then removed under reduced pressure, and the reaction mixture extracted three times with 300 ml portions of ether. The extractions were performed as follows: the ether was slowly added to the rapidly agitated mixture, which was stirred for an hour following the final addition of ether. The extracts were evaporated to dryness and the solid product recrystallized from petroleum ether. The yields were around 50-55% and gave a much cleaner product than the method of Boon, et al.

4-Amino-5-nitro-6-chloropyrimidine. To 4,6-dichloro-5-nitropyrimidine (39 gm) dissolved in 300 ml of ether was added with stirring, over a period of one hour, a solution of 6.9 gm of ammonia in 30 ml of methanol. The
stirring was continued another hour and a solid, consisting of 4,6-diamino-5-nitropyrimidine, was removed, washed with ether, and extracted with two 100 ml portions of hot ethyl acetate. The united residues obtained by concentration of the ether-methanol filtrate and the ethyl acetate extracts were re-extracted with hot mixed heptanes to remove unchanged starting material. The remaining solid residue was crystallized from benzene, yielding 17 gm (54% yield) of product, mp 155-156°.

4-Amino-5-nitro-6-benzylaminopyrimidine. 4-Chloro-5-nitro-6-aminopyrimidine (8 gm) was added to a solution of 16 gm of benzylamine in 150 gm of butanol, and the mixture refluxed for two hours. The hot mixture was filtered and the rapidly-appearing crystals redissolved and recrystallized from fresh butanol. Yield, 5.25 gm, mp 197-199°.

4,5-Diamino-6-benzylaminopyrimidine. Five grams of 4-amino-5-nitro-6-benzylaminopyrimidine was dissolved in 375 ml of methanol and hydrogenated at 32 psi overnight, using seven grams of Raney nickel W-2 catalyst. The mixture was acidified with dilute sulfuric acid, and the pure white crystals of the insoluble sulfate collected and washed with methanol.

7-Benzylamino-v-triazolopyrimidine. 4,5-Diamino-6-benzylaminopyrimidine sulfate (2.6 gm) was dissolved in 800 ml of warm water and the pH adjusted to 3 by addition
of ammonium hydroxide. The clear solution was allowed to cool at 4° overnight, by which time it had become yellowish in color. Sodium nitrite (1.7 gm, twice the theoretical amount) was added to the cold solution. Within one minute a fine white precipitate began to form, which became pink-grey in about three minutes. The mixture was stirred for an hour and cooled in the refrigerator overnight, and the crude product collected and dried. The fluffy precipitate was recrystallized from methanol-dioxane-water (1:9:3) and washed with water. Yield, 1.5 gm.

Anal. Calc'd. for C₁₁H₁₀N₆: C, 58.4; H, 4.45. Found: C, 58.0; H, 4.60.

4-Amino-5-nitro-6-morpholinopyrimidine. 4-Chloro-5-nitro-6-aminopyrimidine (8 gm) was added to a solution of 16 gm morpholine in 150 ml of butanol. After refluxing for 1½ hours and cooling, the crystals were collected and re-crystallized from butanol. Yield, 11 grams (86%).

4,5-Diamino-6-morpholinopyrimidine. Five grams of 4-amino-5-nitro-6-morpholinopyrimidine was added to 360 ml of methanol and hydrogenated at 30 lbs pressure for 20 hours, using Raney nickel, 7-8 grams. Since the sulfate salt of the morpholino compound is not isolable (5, p. 179), the methanol solution was diluted with water to 750 ml and used directly in the next step.
6-Morpholino-v-triazolopyrimidine. The aqueous-methenolic solution of the diamino compound from the last step was treated with two grams of sodium nitrite at 10°. A slight precipitate formed, and the solution was evaporated to 250 ml, filtered, and acidified with dilute sulfuric acid. A creamy yellowish precipitate formed, which was crystallized from dioxane-water. Yield, 1.2 grams.

Found: C, 46.5; H, 4.74.

4-Amino-5-nitro-6-anilinopyrimidine. Seven grams of 4-chloro-5-nitro-6-aminopyrimidine was added to a solution of 16 grams of aniline in 150 ml of butanol. An intense yellow precipitate immediately formed, which almost entirely dissolved after 1½ hours of refluxing, at which time the mixture was cooled and the crystals collected, washed and recrystallized from dioxane-water. Yield, 8.4 grams, 45%.

4,5-Diamino-6-anilinopyrimidine. Seven grams of 4-amino-5-nitro-6-anilinopyrimidine was hydrogenated in methanol at 35 psi for 12 hours, using Raney nickel catalyst. The medium was acidified with dilute sulfuric acid and the amine sulfate crystals collected and washed in cold methanol. Yield, 6.4 gm (95%) of 4,5-diamino-6-anilinopyrimidine sulfate.

6-Anilino-v-triazolopyrimidine. Three grams of
6-anilino-4,5-diaminopyrimidine sulfate was dissolved in approximately 1400 ml of water. Two grams of sodium nitrite was added to the acidic solution and a pink precipitate rapidly formed. After standing in the refrigerator for several days, the pink solid product was obtained (together with an intensely red mother liquor). Yield, 1.3 grams, which was recrystallized from methanol-dioxane-water to give pure white crystals.

Anal. Calc'd. for C_{10}H_{16}N_{8}: C, 56.7; H, 3.81. Found: C, 56.6; H, 3.68.

4-Amino-5-nitro-6-methylaminopyrimidine. Eight grams of 4-amino-5-nitro-6-chloropyrimidine was dissolved in 120 ml of dioxane, to which the theoretical amount of 40% aqueous methylamine was added. After stirring at room temperature for an hour and then refluxing briefly, the solution was cooled in the refrigerator overnight and the crystals collected and washed, then recrystallized from aqueous dioxane. Yield, 4 grams.

4,5-Diamino-6-methylaminopyrimidine. 4-Amino-5-nitro-6-methylaminopyrimidine (3.5 gm) was dissolved in 120 ml of methanol and reduced with Raney nickel (7-8 gm) and hydrogen at 35 psi over a period of 12 hours. The methanolic solution was then acidified with dilute sulfuric acid and the insoluble salt of the amine collected and washed
in cold methanol. Yield, 3.0 grams of colorless crystals.

7-Methylamino-v-triazolopyrimidine. Three grams of 4,5-diamino-6-methylaminopyrimidine was treated with 1.5 grams of sodium nitrite in 200 ml of ice water. Since the amine was in the form of its sulfate salt, the solution was acidic and a white precipitate appeared immediately, which soon became lavender, yielding 1.9 gm of light-brown product. Recrystallization from methanol-dioxane-water gave only 0.3 gm of pure white product. A brownish soluble residue was obtained from the mother liquor, which was not further investigated.

Anal. Calc'd. for C₅H₆N₆: C, 40.0; H, 4.00. Found: C, 39.9; H, 3.84.

4-Amino-5-nitro-6-isobutylaminopyrimidine. Five grams of 4-amino-5-nitro-6-chloropyrimidine was treated with excess isobutylamine in 120 ml of butanol. After 1½ hours of refluxing the mixture was cooled and the crystals collected. Yield, 4.6 grams, which, when recrystallized from butanol, gave pure white crystals melting sharply at 170°.

4,5-Diamino-6-isobutylaminopyrimidine. Four grams of 4-amino-5-nitro-6-isobutylaminopyrimidine was hydrogenated for 10 hours at 32 psi in 360 ml of methanol using six grams of Raney nickel W-2. The product was isolated as the sulfate salt by treatment of the methanolic reaction mixture
with dilute sulfuric acid. The diamine sulfate separated out as cream-colored crystals, 3.7 grams.

**Anal.** Calc'd. for \((C_8H_{15}N_5)_{2}SO_4\): C, 34.4; H, 6.12.

*Found:* C, 34.4; H, 6.00.

**7-Isobutylamino-5-triazolopyrimidine.** 4,5-Diamino-6-isobutylaminopyrimidine sulfate (3.5 gm) was treated with two grams of sodium nitrite in 400 ml of ice water, resulting in the rapid formation of a white precipitate. Yield, 1.7 grams of product which was recrystallized from dioxane-water.

**4-Amino-5-nitro-6-piperidinopyrimidine.** Five grams of 4-amino-5-nitro-6-chloropyrimidine in 125 ml of butanol was treated with excess piperidine. The reaction was unusually rapid; the mixture was therefore refluxed for only 30 minutes. The yellow crystalline product obtained by cooling in the deep-freeze was collected. Yield, 7.3 grams, mp 1380.

**Anal.** Calc'd. for \(C_9H_{13}O_2N_5\): C, 48.5; H, 5.88.

*Found:* C, 48.6; H, 5.87.

**4,5-Diamino-6-piperidinopyrimidine.** Five grams of 4-amino-5-nitro-6-piperidinopyrimidine was reduced in 350 ml of methanol for 24 hours with hydrogen at 32 psi and Raney nickel (6 gm). The diamine could not be isolated as its sulfate salt, an experience observed in the case of the corresponding morpholino compound.
7-Piperidino-ν-triazolopyrimidine. Since the diamino compound from the preceding step could not be isolated, the acidified (sulfuric acid) methanolic solution of the diaminopyrimidine was partially evaporated and diluted with water, cooled to 10°, and treated with two grams of sodium nitrite. Considerable trouble was experienced in achieving the correct pH for precipitation of the triazolo compound; the initial reaction mixture was too acidic and it was necessary to partially neutralize with ammonium hydroxide. Only 0.1 gram of product was obtained, which was recrystallized from dioxane-water.

Anal. Calc'd. for C₉H₁₂N₆: C, 53.0; H, 5.89. Found: C, 52.7; H, 5.76.

Ultraviolet absorption spectra. Ultraviolet absorption data were obtained for all the triazolopyrimidines prepared. All were run in water, pH 5, except in the case of the isobutylamino derivative, which, due to its insolubility, was run in 50% aqueous ethanol. The instrument employed was a Beckman model DU spectrophotometer using silica cells. Concentrations were 8 mg per liter of solution except in those cases where the solubility of the compound was less than this. The individual absorption spectra are shown in the following figures (where the abbreviation "T.A.P." stands for triazolopyrimidine) and
<table>
<thead>
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<th>7-substituted triazolopyrimidine</th>
<th>max</th>
<th>log ε</th>
<th>min</th>
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<tr>
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<tr>
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<td>278</td>
<td>-</td>
<td>236</td>
<td>-</td>
</tr>
<tr>
<td>piperidino-</td>
<td>295</td>
<td>-</td>
<td>240</td>
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Table II. Ultraviolet absorption maxima (in millimicrons) of the derivatives prepared.

The data are summarized in Table II; wavelengths of the maximum and minimum absorptions are given, and extinction coefficients are calculated where solubilities are known.
TRANSMITTANCE, %

7-MORPHOLINO-T.A.P.
7-BENZYLAMINO-T.A.P.
TRANSMITTANCE, %

7-ANILINO-T.A.P.

WAVELENGTH
TRANSMITTANCE, %

7-ISOBUTYLAMINO-T.A.P.


