# Synthesis and Antitumor Evaluation of Some 1,3-Disubstituted Tetrahydropyrimido[4,5-*c*]isoquinolines [1]



Aleem Gangjee* and Kwasi A. Ohemeng

Department of Pharmaceutical Chemistry and Pharmaceutics, School of Pharmacy, Duquesne University, Pittsburgh, PA 15282

Fu-Tyan Lin

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260

Arthur A. Katoh

Department of Pathology, Mercy Hospital, Pittsburgh, PA 15219

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Cyclocondensation of 6-amino-2,4-dioxopyrimidine or 2,4,6-triaminopyrimidine with 1-cyclohexenecarboxaldehyde **13** afforded regiospecifically, tricyclic, angular 1,3-disubstituted tetrahydropyrimido[4,5-*c*]isoquinolines **5** and **6** respectively. In addition, 2,4,6-triaminopyrimidine when condensed with 2-chloro-1-cyclohexenecarboxaldehyde **14**, regiospecifically afforded the angular isomer **6**. However, the cyclocondensation of 2,6-diamino-4-oxopyrimidine with **13** was regioselective and afforded a mixture of the linear and angular tetrahydropyrimidoisoquinolines **2** and **4**. The growth of leukemia L-1210 cells in culture were inhibited 50% by **6** at $9 \times 10^{-8}$ *M*. Compounds **4** and **5** were not significantly active.

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A number of bicyclic, 6-substituted pyrido[2,3-*d*]pyrimidines [2] and quinazolines [3] possess significant antitumor activity which, in some cases, is enhanced by substitution in the 5-position of the heterocyclic system. Our interest in the synthesis and antitumor activity of classical and nonclassical 5-deaza, tricyclic folates related to the cofactor 5,10-methylenetetrahydrofolate **1** prompted the synthesis of 1,3-disubstituted-7,8,9,10-tetrahydropyrimido-[4,5-*c*]isoquinolines as potential antitumor agents.

In our previous work [4] we have reported the synthesis of the linear isomers, 2-amino-4-oxo and 2,4-dioxo-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolines **2** and **3**. The compounds in this report are the new angular isomers **4** and **5** and the angular isomer **6** for which two new syntheses are reported.

A search of the literature revealed that the angular 1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinoline (**6**) had been first reported by Hitchings and coworkers [5]. The synthesis was carried out by the condensation of 2,4,6-triaminopyrimidine (**9**) with 2-carbethoxycyclohexanone, followed by chlorination with phosphorus oxychloride, thiation with sodium hydrosulfide and dethiation with Raney nickel to give **6** in very low over all yield. In another report Rosowsky and Papathanasopoulos [6] built the pyridine and pyrimidine rings in a synthetic sequence starting from cyclohexanone to afford **6**. The ultraviolet spectrum was the only spectral data reported for **6** [6].

The 3-amino-1-oxo-7,8,9,10-tetrahydro-2*H*-pyrimido-[4,5-*c*]isoquinoline (**4**) and the 1,3-dioxo-7,8,9,10-tetrahydro-2*H*,4*H*-pyrimido[4,5-*c*]isoquinoline (**5**) had purportedly been synthesized by Junek and Wrtilek [7] using a simple cyclocondensation of 4-amino-2,6-dihydroxypyrimidine (**8**) and 2-aminomethylenecyclohexanone as the 1,3-bis electrophile. Stark and Breitmaier [8] expanded on the work of Junek and Wrtilek [7] using similar reaction conditions to afford **4** and **5** among a variety of other bicyclic and tricyclic compounds. Rosowsky and Papathanasopoulos [6] first questioned the direction of cyclization reported by Junek and Wrtilek [7]. Subsequently, Broom and coworkers [9] reassigned the carbon chemical shifts of the dioxo-substituted bicyclic pyrido[2,3-*d*]pyrimidines synthesized by Stark and Breitmaier [8] base on a reassignment of the $^{13}C$ nmr spectral data. We [4] have recently reported the synthesis of the tricyclic, linear isomers **2** and **3** and shown that the direction of ring closure and indeed the angular structure of the tricyclic products reported by Junek and Wrtilek [7] and consequently by Stark and Breitmaier [8] were erroneously assigned.

In an attempt to avoid tedious multistep synthetic procedures for the synthesis of the desired compounds, we looked to the literature and found that the classical Doebner-von Miller synthesis of quinolines involving the cyclocondensation of anilines and vinyl aldehydes had been extended to the synthesis of substituted pyrido-[2,3-*d*]pyrimidines by Wood *et al.*, [10]. These workers have reported that the acid catalyzed condensation of **8** with 2-methylbut-2-enal (**10**) affords, regiospecifically, the 5,6-dimethylpyrido[2,3-*d*]pyrimidine-2(1*H*),4(3*H*)-dione (**11**) rather than the 6,7-dimethyl isomer. Compound **11** had a single aromatic resonance peak in its $^1H$ nmr spectrum at $\delta$ 8.53 in deuterated trifluoroacetic acid. This signal was assigned to the $H_7$ proton of **11**. In the same study

Wood *et al.*, [10] also synthesized the 7-methyl isomer, **12** and assigned the $H_5$ proton at δ 9.05 which is about 0.5 ppm downfield from $H_7$ of **11**.

The work of Wood *et al.*, [10] suggested a simple regiospecific synthesis of the desired tricyclic pyrimido[4,5-*c*]isoquinoline system which could be achieved *via* the cyclocondensation of an appropriately substituted 6-aminopyrimidine with a vinyl aldehyde such as 1-cyclohexenecarboxaldehyde (**13**). Compound **13** was synthesized from the Vilsmeier chloroformylation of cyclohexanone to afford 2-chloro-1-cyclohexenecarboxaldehyde (**14**) according to a literature method [11]. Selective dehalogenation in the presence of the double bond was achieved by hydrogenation at atmospheric pressure using 5% palladium on charcoal as catalyst in a modification of a literature procedure for the debromination of bromovinyl esters to vinyl esters [12].

Having synthesized the required vinyl aldehyde **13** we turned our attention to the cyclocondensation reactions. In their report Wood *et al.*, [10] had used 20% hydrochloric acid as their solvent system for the cyclocondensation leading to **11**. However, in view of the possible deamination of the aminopyrimidines **7** and **9** and/or of their cyclocondensed products in the presence of 20% hydrochloric acid [13] we elected to employ glacial acetic acid as the reaction solvent for the synthesis of **4** and **6**.

Cyclocondensation of 4-amino-2,6-dihydroxypyrimidine (**8**) with **13** was carried out by the gradual addition of **13** to a solution of **8** in 20% hydrochloric acid followed by reflux to afford, 1,3-dioxo-7,8,9,10-tetrahydro-2*H*,4*H*-pyrimido[4,5-*c*]isoquinoline (**5**), as the only product.

The angular structure of the product **5** was established by $^1H$ nmr and $^{13}C$ nmr spectral data and a comparison of $^1H$ nmr spectral data with similar systems known in the literature. Rizkalla and Broom [14] reported the synthesis of 2,4-dioxo-6-methylpyrido[2,3-*d*]pyrimidine (**16**) and correctly assigned the more downfield of the two aromatic protons, in deuterated dimethylsulfoxide to the $H_7$ proton. Wood *et al.*, [10] in their synthesis of methyl substituted 2,4-dioxopyrido[2,3-*d*]pyrimidines **11** and **12** reported the $H_5$ proton of **12** at δ 9.05, about 0.5 ppm downfield compared to the $H_7$ proton of **11**. However, these $^1H$ nmr spectra reported by Wood *et al.*, [10] were recorded in deuterated trifluoroacetic acid and not in dimethylsulfoxide. It is well known that in non-acidic solvents the $H_7$ proton of 2-oxo and 2,4-dioxopyrido[2,3-*d*]pyrimidines occur more downfield than the $H_5$ proton. In acidic solvents such as deuterated trifluoracetic acid both protons are shifted downfield, however in this case, the $H_5$ proton occurs more downfield than the $H_7$ proton. This reversal of the relative chemical shifts in acidic media has been attributed to the protonation of the pyridine nitrogen [15]. The only aromatic proton signal for **5** in deuterated dimethylsulfoxide occurred at δ 8.23. This chemical shift position is about 0.4 ppm upfield compared to that reported by Stark and Breitmaier [8] for their purported compound **5** which was shown by Gangjee *et al.*, [4] to be the linear isomer **3**. In deuterated trifluoroacetic acid this aromatic proton of **5** shifted downfield, as expected, and occurred at δ 8.50 which compares favorably with the chemical shift of the $H_7$ proton of **11** at δ 8.53 [10], strongly supporting the angular structure for **5**. Further this proton position of $H_6$ of **5** was about 0.43 ppm upfield compared to the chemical shift of the aromatic proton $H_5$ of the linear isomer **3**. The structure of **3** has been unequivocally established in our previous report [4]. On comparing the chemical shifts of the aromatic protons $H_5$ of **3** and $H_6$ of **5** we have observed that in deuterated dimethylsulfoxide the positions are δ 7.87 and 8.23 respectively and in deuterated trifluoroacetic acid, δ 8.93 and 8.50 respectively. Clearly the aromatic proton $H_5$ of the linear isomer **3** shifts 1.06 ppm downfield in acid, while $H_6$ of the angular isomer **5** shifts only 0.27 ppm downfield in acid. Thus in deuterated dimethylsulfoxide the aromatic proton of the angular isomer is further downfield while in deuterated trifluoroacetic acid, it is the aromatic proton of the linear isomer that is further downfield.

In addition the signals for the two methylene pairs of protons on $C_7$ and $C_{10}$ in **5** were distinctly separated by 0.70 ppm even on a 60 MHz instrument. While in the linear isomer **3** the signals for the methylene pairs of protons on $C_6$ and $C_9$ coalesced, on both a 60 MHz and a 300 MHz instrument, as part of a multiplet centered at δ 3.18 [4]. The methylene pair attached to $C_{10}$ in **5** was assigned the more downfield position at δ 3.73 and those on $C_7$ were assigned the upfield signal at δ 3.03. From Dreiding models it is apparent that the methylene protons on $C_{10}$ of **5** lie in the X-Z plane of the carbonyl at $C_1$ and due to their proximity to the oxygen a *peri* effect of deshielding [16] accounts for their downfield shift compared to the protons at $C_7$. Such a *peri* deshielding effect of the carbonyl would be absent in the linear isomer **3**, thus allowing for the coalesing of the signals as indeed was observed [4].

In addition to the chemical shifts of the aromatic proton and the nature of the signals from the methylene pairs of protons, support for the angular structure of **5** as designated was provided from $^{13}C$ nmr using the one bond coupling constant ($^1J_{C\text{-}H}$) of 173.34 Hz, the magnitude of this value indicates that it must arise from a $C_6$-$H_6$ coupling in the angular compound rather than from a $C_5$-$H_5$ coupling from the linear isomer which is about 15 Hz less [4,9].

For comparison purposes we have also synthesized 2,4-dioxo-6-methyl-1*H*,3*H*-pyrido[2,3-*d*]pyrimidine (**16**) using literature methods [8] by condensation of **8** and 3-amino-2-methylacrolein in acetic acid-water to afford the pro-

duct **16**. In deuterated trifluoroacetic acid the $H_5$ proton of **16** occurred at $\delta$ 9.13 and the $H_7$ proton was assigned at $\delta$ 8.83. These assignments are consistent with the discussions presented above with respect to the 2,4-dioxo substituted products. This compound was prepared since there was no $^1$H nmr spectral data in deuterated trifluoroacetic acid for it available in the literature.

Cyclocondensation of 2,6-diamino-4-hydroxypyrimidine (**7**) with 1-cyclohexenecarboxaldehyde **13** in acetic acid at reflux gave two products, (tlc, $R_f$ 0.61 and 0.43). The $^1$H nmr spectrum of the mixture in deuterated trifluoroacetic acid gave two signals in the aromatic region at $\delta$ 8.83 and $\delta$ 8.50 in a ratio of 5:8 (Fig. I,A). Addition of a small amount of the pure linear isomer **2** [4] to the $^1$H nmr tube containing the mixture, led to an enhancement of the signals at $\delta$ 8.83, 3.00 and 2.03 (Fig. I,B). The $R_f$ value of one

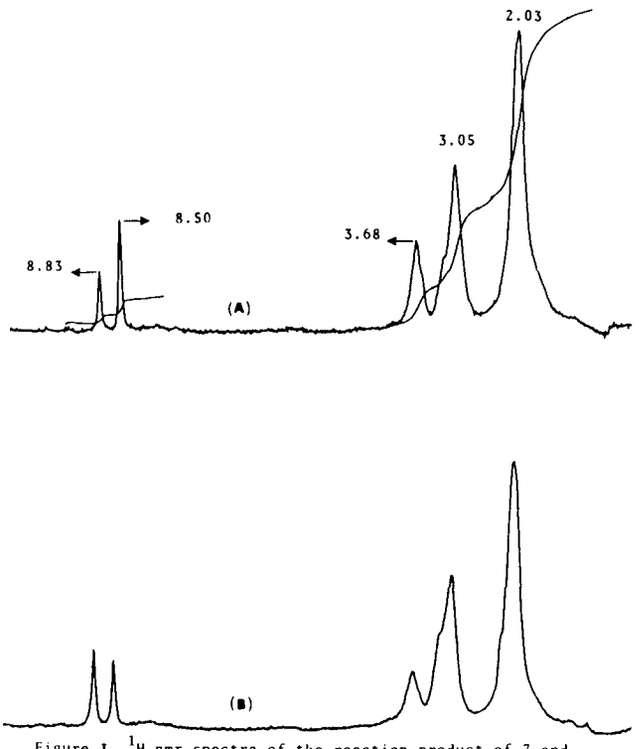



Figure I. $^1$H nmr spectra of the reaction product of 7 and 13 (A) and the products spiked with pure 2 (B).

of the spots (0.43) obtained for the mixture on tlc coincided with that for the linear isomer **2**. The tlc and $^1$H nmr data confirmed the presence of the linear isomer **2** as one of the components of the mixture. Due to the insolubility of the linear isomer **2** in acetic acid-water (3:2), it was possible to separate the other compound, uncontaminated with **2**, from the mixture. The mixture was stirred with hot acetic acid-water, filtered and basified to *p*H of 6 to precipitate any dissolved **2**. Filtration and tlc of the filtrate showed a single component, which was precipitated by basification to a *p*H of 7 to 8. The $^1$H nmr spectrum of the precipitate isolated at a *p*H 7-8 was different from **2** (Fig. II). This product was considered to be the angular isomer 3-amino-1-oxo-7,8,9,10-tetrahydro-2*H*-pyrimido[4,5-*c*]isoquinoline (**4**). In the $^1$H nmr spectrum the aromatic proton $H_6$ of **4** occurred at $\delta$ 8.40 in deuterated trifluoroacetic acid. This was in close agreement with the aromatic proton $H_6$ for the dioxo angular isomer **5**. The aromatic proton $H_5$ for the linear isomer **2** occurred at $\delta$ 8.93 [4]. The characteristic separation of the four methylene protons on $C_{10}$ and $C_7$ as described above for **5** was also observed with the angular compound **4** and was absent in the linear compound **2** (Fig. II).

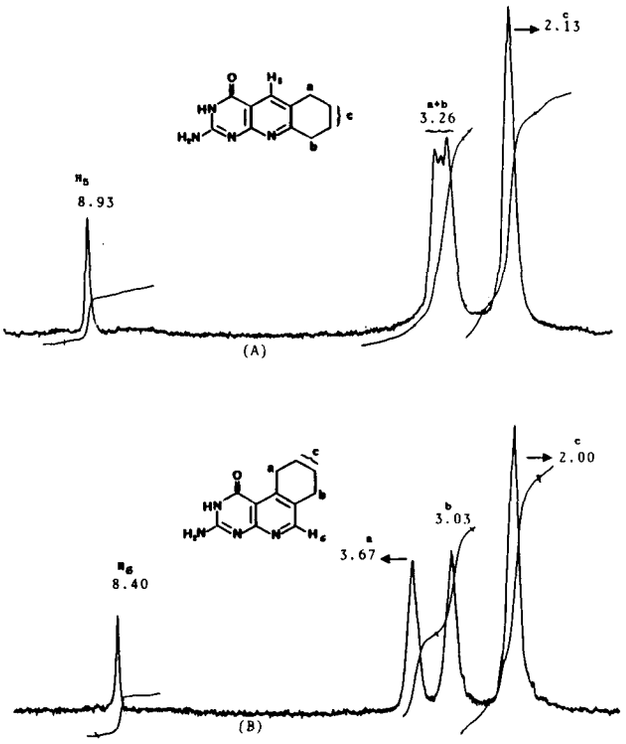



Figure II. $^1$H nmr spectra of 2 (A) and 4 (B)

Again, for comparison purposes we synthesized 2-amino-6-methyl-4-oxo-3*H*-pyrido[2,3-*d*]pyrimidine (**15**) as reported in the literature [8]. The $^1$H nmr in deuterated trifluoroacetic acid showed two aromatic protons which were assigned as $H_5$ at $\delta$ 9.06 and $H_7$ at $\delta$ 8.76.

The reaction of the dioxopyrimidine **8** with **13** was regiospecific affording only the angular isomer **5**. A similar reaction with the aminooxopyrimidine **7** was regioselective yielding a mixture of the angular and linear isomers in a ratio of 8:5. Thus it can be concluded that the nature of the aminopyrimidine is one of the factors which controls the direction(s) of ring closure and consequently the structure of the products.

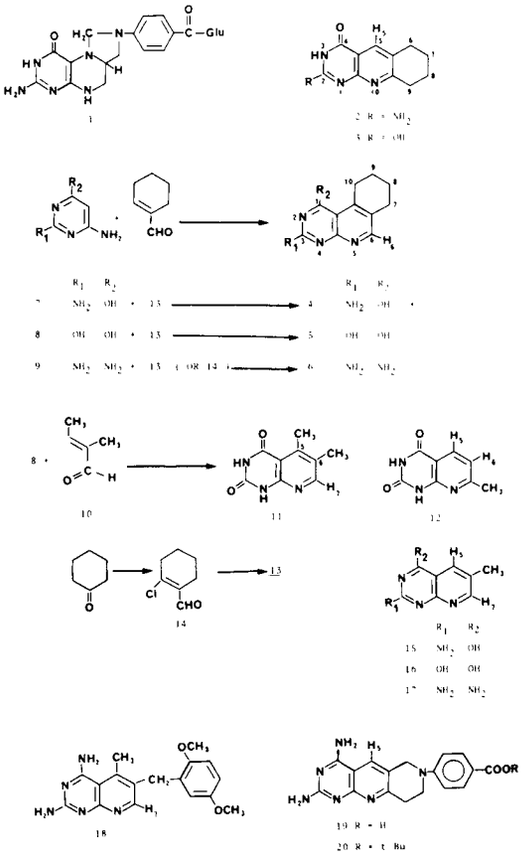
The synthesis of the diamino angular isomer 1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinoline (**6**) was accomplished by the cyclocondensation of 2,4,6-triaminopyrimidine (**9**) with **13** in glacial acetic acid. The reaction was regiospecific in that only one product was identifiable on tlc. In addition, the cyclocondensation product isolated from the reaction of **9** with **14** gave a product identical in all respects to **6** obtained from the reaction of **9** and **13**. The angular structure of compound **6** was confirmed by comparison of its $^1$H nmr spectrum with other 2,4-diamino systems. Grivsky and coworkers [2] have synthesized compound **18**. In deuterated dimethylsulfoxide this compound showed a signal for the aromatic proton $H_7$ at $\delta$ 8.45 [17]. For compound **6** the aromatic proton $H_6$ occured at $\delta$ 8.43 in deuterated dimethylsulfoxide. This chemical shift is in close agreement with that of **18**. In deuterated trifluoroacetic acid the signal for the aromatic proton $H_6$ is shifted downfield, as expected, to $\delta$ 9.17. The 2,4-diamino linear pyrimido[4,5-*b*][1,6]naphthyridines **19** and **20** have been recently synthesized *via* a multistep synthetic procedure [18]. The $^1$H nmr spectra, in deuterated trifluoroacetic acid, of these linear isomers **19** and **20** showed an aromatic proton at $\delta$ 8.90 and 8.85 respectively. These values are upfield compared to $H_6$ of **6**, and indirectly confirm the angular nature of **6**.

For comparison purposes we have also synthesized 2,4-diamino-6-methylpyrido[2,3-*d*]pyrimidine (**17**). This was accomplished by the cyclocondensation of **9** with 3-amino-2-methylacrolein, which in turn was obtained from the aminolysis of 3-ethoxy-2-methylacrolein [19,20]. It was previously reported [8] that the reaction was unsuccessful when acetic acid-water (3:2, v/v) is used as the reaction solvent. This was true in our hands also. However, at 140° in glacial acetic acid with piperidine acetate as catalyst [21], we obtained a moderate yield (57%) of **17**. The $^1$H nmr spectrum of **17** further substantiated the angular assignment of **6**. In deuterated trifluoroacetic acid the two aromatic protons $H_5$ and $H_7$ of **17** occurred at $\delta$ 8.88 and 9.17. These values are in accord with those obtained for the linear and angular compounds **19** and **6** respectively. It is noteworthy that in contrast to the 2,4-dioxo and the 2-amino-4-oxo series, the 2,4-diamino series has the aromatic proton of the angular isomer further downfield than that of the linear isomer in deuterated trifluoroacetic acid.

Both **7** and **8** when cyclocondensed with the chlorovinyl aldehyde **14** afforded regiospecifically the linear pyrimido[4,5-*b*]quinolines **2** and **3** respectively [4]. However, the cyclocondensation of **9** with **14** afforded a product identical in all respects (tlc, $^1$H nmr and ir) to **6** obtained as described above. Thus **9**, unlike **7** and **8**, affords the angular isomer when condensed with the chlorovinyl aldehyde **14**.

From the above discussions and our previous report [4] regarding the structure(s) of the cyclocondensed products, we conclude that differently substituted aminopyrimidines afford different products when condensed with 1,3-biselectrophiles and that a generalization for the product(s) from the cyclocondensations of aminopyrimidines is not possible even when the same vinyl aldehdye is used. Thus reactions of the dioxoaminopyrimidine or the 2,6-diamino-4-oxopyrimidine with the chlorovinyl aldehyde **14** affords regiospecifically the linear pyrimido[4,5-*b*]quinolines. However, the 2,4,6-triaminopyrimidine with the chlorovinyl aldehyde **14** affords the angular pyrimido[4,5-*c*]isoquinoline. With the vinyl aldehyde **13**, the dioxoaminopyrimidine and the 2,4,6-triaminopyrimidine affords regiospecifically the angular pyrimido[4,5-*c*]isoquinolines. However, the 2,6-diamino-4-oxopyrimidine with the vinyl aldehyde **13** affords both the linear and angular tricyclic isomers and the cyclocondensation in this case is regioselective. This regioselectivity has recently been confirmed by us in the synthesis of substituted pyrimido[4,5-*c*]-[2,7]naphthyridines [22]. Clearly the nature of the acidic solvent may also play an important role in determining the nature of the product(s).

The growth of leukemia L-1210 cells in culture [23] was inhibited 50% by **6** at $9 \times 10^{-8}$ *M*, 28% by **4** at $10^{-4}$ *M* and 16% by **5** at $10^{-4}$ *M*. We are currently involved in the synthesis of analogs of **4** and **6** in an attempt to increase antitumor activity.



## EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded with a Perkin-Elmer Model 337 in Nujol mulls. Nuclear magnetic resonance spectra for proton ($^1$H nmr) were recorded on a Varian EM-360 and for carbon-13 ($^{13}$C nmr) on a Brucker WH-300 at 75.46 MHz; 90° pulse: 14 μsec. The data was accumulated by 16 K size with 0.5 sec delay time and 70° tip angle, with internal standard TMS; s = singlet, d = doublet and m = multiplet. Thin-layer chromatography (tlc) was performed on cellulose plates, or as indicated, with fluorescent indicator and were visualized with light at 254 nm. The elemental analysis were performed by Atlantic Microlabs, Inc., Atlanta, Georgia.

1-Cyclohexenecarboxaldehyde (**13**).

Palladium on charcoal (5.4 g) was suspended in 300 ml of 10% potassium hydroxide for 5 minutes, filtered and washed successively with 20 ml of ethanol, 20 ml of water, 20 ml of ethanol and 50 ml of anhydrous ether, and dried under reduced pressure (15 mm Hg) for 2 hours. To a mixture of the catalyst and 112.5 g (1.11 moles) of triethylamine in 540 ml of absolute ethanol saturated with hydrogen, was added 27 g (0.19 mole) of 2-chloro-1-cyclohexencarboxaldehyde **14**. The mixture was reduced with hydrogen for 45 minutes (4190 ml, 0.19 mole of hydrogen consumed). The catalyst was filtered and the filtrate concentrated to 25% of the original volume (white crystals of triethylamine hydrochloride precipitated out). The mixture was poured into 50 ml of methylene chloride, washed successively with 100 ml of 10% hydrochloric acid twice, and 50 ml of water twice, dried (magnesium sulfate) and concentrated under reduced pressure (water aspirator). The resulting syrup was distilled and redistilled under reduced pressure to give 13 g (63%) of **13**, bp 68-70° (11 mm Hg) (lit 63-67°, 11 mm Hg) [24]; $^1$H nmr (carbon tetrachloride): δ 1.70 (m, 4H, $CH_2$-$CH_2$), 1.97 (m, 4H, $CH_2$-C=C, and $CH_2$-C-C=O), 6.77 (broad s, 1H, HC=C), 9.40 (s, 1H, HC=O).

3-Amino-1-oxo-7,8,9,10-tetrahydro-2*H*-pyrimido[4,5-*c*]isoquinoline (**4**).

To a solution of 6.6 g (0.053 mole) of 2,6-diamino-4-hydroxypyrimidine **7** in 330 ml of glacial acetic acid at reflux was added the light-blue solution of 5.8 g (0.053 mole) of **13** in 30 ml of glacial acetic acid over a 20 minute period with continuous stirring. The mixture was refluxed for an additional 17 hours, cooled and poured into 200 ml of water kept at 15°, followed by neutralization with concentrated ammonium hydroxide to *p*H 7, with the temperature maintained at below 20°. The precipitate formed was collected, washed with excess amounts of water and dried under reduced pressure with phosphorus pentoxide to give 10.0 g (87%) of a mixture of **2** and **4**. Tlc (cellulose; butanol-acetic acid-water, 3:1:3) $R_f$ 0.82; (cellulose, 3% ammonium chloride), two spots $R_f$ 0.61 and 0.43. The $^1$H nmr (deuteriotrifluoroacetic acid) gave two signals in the aromatic region, δ 8.83 and 8.50 in a ratio of 5:8. Spiking of the nmr tube with pure **2** led to an enhancement of the signals at δ 8.83, 3.0 and 2.03. The mixture (2.5 g) was suspended in 10 ml of acetic acid-water (3:2), boiled for 15 minutes and left to cool to 25°. This was filtered and the filtrate basified to *p*H 6 with concentrated ammonium hydroxide, the precipitate thus formed was filtered and the filtrate basified further to *p*H 8, to give a yellow precipitate. This was filtered, washed with water and air-dried, then dissolved in 20 ml of 20% hydrochloric acid, followed by evaporation of the water under reduced pressure which gave a solid. Recrystallization of the solid from butanol-hexane (9:1) gave 500 mg of **4**; tlc (cellulose, butanol-acetic acid-water, 3:1:3) $R_f$ 0.83, (cellulose, 3% ammonium chloride) $R_f$ 0.66; mp >300°; ir (Nujol): 3279 cm$^{-1}$ ($NH_2$), 3125 (NH), 1700, 1645 (C=O); $^1$H nmr (deuteriotrifluoroacetic acid): δ 2.00 (s, 4H, 8 and 9-$CH_2$), 3.03 (s, 2H, 7-$CH_2$), 3.67 (s, 2H, 10-$CH_2$), 8.40 (s, 1H, 6-CH).

*Anal.* Calcd. for $C_{11}H_{12}N_4O \cdot 0.7HCl$): C, 54.65; H, 5.29; N, 23.17. Found: C, 55.02; H, 5.40; N, 22.97.

1,3-Dioxo-7,8,9,10-tetrahydro-2*H*,4*H*-pyrimido[4,5-*c*]isoquinoline (**5**).

A solution of 1.5 g (0.01 mole) of **8** was prepared in 40 ml of 20% (v/v) hydrochloric acid by warming and the mixture cooled to 25°. To this solution was added 1.7 g (0.015 mole) of 1-cyclohexenecarboxaldehyde **13**, dropwise over a 1 hour period with continuous stirring. The reaction was allowed to proceed at 25° for a further 17 hours, then refluxed for 3 hours, cooled and chilled in an ice bath to 5°. A white precipitate formed which was not identified and was discarded on filtration. Tlc (cellulose, butanol-acetic acid-water, 3:1:3), of the filtrate showed three spots, $R_f$ 0.90, 0.84 and 0.78. The filtrate was neutralized (*p*H 7) with concentrated ammonium hydroxide at a temperature maintained below 10° to afford a brown precipitate, which was filtered, washed with water and dried at reduced pressure with phosphorus pentoxide to yield 0.9 g (35%) of **5**. Tlc (cellulose, butanol-acetic acid-water, 3:1:3) $R_f$ 0.88; (cellulose, 3% ammonium chloride) $R_f$ 0.20. An analytical sample was obtained by recrystallization from ethanol-water (1:1) with a few drops of acetic acid. The first crop obtained from the recrystallization in 50% yield were yellow needlelike crystals. Additional crops obtained from the mother liquor were yellowish amorphorus powders which were found to be degraded product, mp >300°; ir (Nujol): 3185 cm$^{-1}$ (NH), 1748, 1724 (C=O); $^1$H nmr (DMSO-$d_6$): δ 8.23 (s, 1H, 5-CH), 11.16 (s, 1H, 2-NH or 4-NH), 11.40 (s, 1H, 2-NH or 4-NH); (deuteriotrifluoroacetic acid): δ 2.00 (s, 4H, 8 and 9-$CH_2$), 3.03 (s, 2H, 7-$CH_2$), 3.73 (s, 2H, 10-$CH_2$), 8.5 (s, 1H, 6CH).

*Anal.* Calcd. for $C_{11}H_{11}N_3O_2 \cdot 0.3H_2O$: C, 59.35; H, 5.25; N, 18.87. Found: C, 59.33; H, 5.23; N, 19.00.

1,3-Diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinoline (**6**).

Method A.

To a solution of 3.4 g (0.027 mole) of 2,4,6-triaminopyrimidine **9** in 100 ml of glacial acetic acid, was added 2.9 g (0.027 mole) of **13** at 40° over a 45 minute period and stirred for an additional 2 hours. The mixture was then refluxed for 17 hours and poured into 70 ml of water, cooled to 15° and basified with concentrated ammonium hydroxide to *p*H 8 with the temperature maintained at below 20°. The precipitate thus formed was filtered, washed with water until neutral, air-dried, dissolved in absolute ethanol containing a small amount of sodium hydroxide and left at 5° to deposit 1.9 g (33%) of **6**. Tlc (cellulose, butanol-acetic acid-water, 3:1:3) $R_f$ 0.73. The hydrochloride salt was formed by recrystallization from ethanol-water-hydrochloric acid (3:3:1) mp >300°; ir: 3289 cm$^{-1}$ ($NH_2$); $^1$H nmr (DMSO-$d_6$): δ 1.8 (s, 4H, 8 and 9-$CH_2$), 2.8 (s, 4H, 7 and 10-$CH_2$), 8.2 (s, 2H, $NH_2$), 8.43 (s, 1H, 6-CH), 8.81 (s, 1H, NH), 9.13 (s, 1H, NH); (deuteriotrifluoroacetic acid): δ 2.13 (s, 4H, 8 and 9-$CH_2$), 3.26 (broad d, 4H, 7 and 10-$CH_2$), 9.17 (s, 1H, 6-CH).

*Anal.* Calcd. for $C_{11}H_{13}N_5 \cdot 1HCl \cdot 1.2H_2O$: C, 48.34; H, 6.05; N, 25.62. Found: C, 47.97; H, 6.06; N, 25.64.

Method B.

To a solution of 6 g (0.048 mole) of **9** in 200 ml of glacial acetic acid was added dropwise 6.9 g (0.048 mole) of 2-chloro-1-cyclohexenecarboxaldehyde **14** at 40° over a 1 hour period with continuous stirring and then refluxed for 4 hours. The solution was cooled to 15° and made basic with concentrated ammonium hydroxide solution at temperature below 20° to *p*H 7, to afford a yellow-brown precipitate which was filtered and washed with water until neutral. The precipitate was suspended in absolute ethanol and boiled for 10 minutes, cooled and filtered to give a yellow-brown solid which was dried under reduced pressure with phosphorus pentoxide to give 4.1 g (40%) of a compound which was identical to **6** (tlc, $^1$H nmr and ir) obtained by method A.

2,4-Diamino-6-methylpyrido[2,3-*d*]pyrimidine (**17**).

To a solution of 12.5 g (0.1 mole) of 2,4,6-triaminopyrimidine **9** in glacial acetic acid containing 0.3 g of piperidine acetate catalyst was added 8.8 g (0.1 mole) of powdered 3-amino-2-methylacrolein at 60°. The mixture was refluxed with continuous stirring for 24 hours and then poured into 450 ml of water, and cooled to 15°. The solution was neutralized to *p*H 7 with concentrated ammonium hydroxide and the temperature maintained below 15°. This afforded a yellow precipitate which was filtered, washed with water, air-dried and suspended in 60 ml of warm water and made basic to *p*H 10 with sodium hydroxide pellets. The mixture was stirred at 60° for 20 minutes, filtered, washed with water, air-dried and re-

crystallized from ethanol-water (3:7) to give 9.9 g (57%) of **17** mp >300°; ir (Nujol): 3449 $cm^{-1}$, 3289, ($NH_2$); $^1H$ nmr (DMSO-$d_6$): δ 2.33 (s, 3H, $CH_3$), 6.33 (s, 2H, 4-$NH_2$), 7.56 (s, 2H, 2-$NH_2$), 8.23 (d, 1H, 5-CH), 8.56 (d, 1H, 7-CH); (deuteriotrifluoroacetic acid): δ 2.67 (s, 3H, $CH_3$), 8.88 (s, 1H, 5-CH), 9.17 (s, 1H, 7-CH).

*Anal.* Calcd. for $C_8H_9N_5 \cdot 0.125\ H_2O$: C, 54.19; H, 5.15; N, 39.52. Found: C, 53.93; H, 5.22; N, 39.12.

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