Synthesis of a Substituted Tetrahydropyrimido[4,5-*c*][2,7]naphthyridine as a Tricyclic 5-Deaza Nonclassical Folate

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Condensation of 2,6-diamino-4-hydroxypyrimidine with 1-benzoyl-1,2,3,6-tetrahydropyrimidine-5-carboxaldehyde (**4**) afforded a mixture of angular and linear tricyclic tetrahydropyrimidonaphthyridines. Separation of the mixture was affected by fractional precipitation. Condensation of 2,4,6-triaminopyrimidine with 1-benzoyl-4-chloro-1,2,3,6-tetrahydropyridine (**5**) was regiospecific and afforded only the substituted angular tetrahydropyrimido[4,5-*c*][2,7]naphthyridine (**2**). The vinyl aldehyde **4** and the chlorovinyl aldehyde **5** were prepared by modifications of literature procedures.

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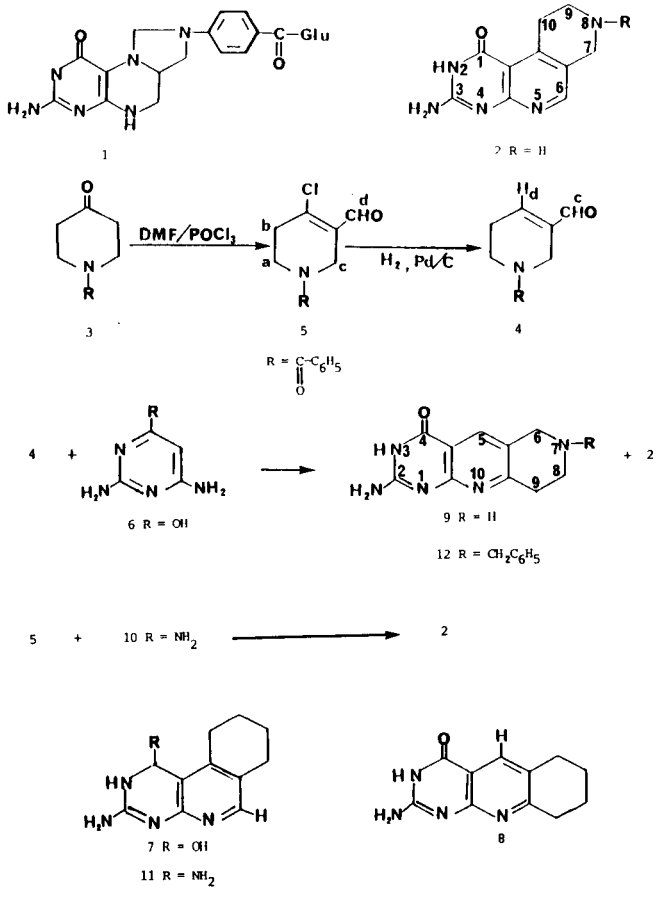
As part of our continuing effort towards the synthesis of stable tricyclic analogues and homologues of the folate cofactor **1** [1,2], we have synthesized an angular tricyclic pyrimido[4,5-*c*][2,7]naphthyridine **2** as a potential antitumor agent, and more importantly as a key precursor to a variety of non-classical and classical tricyclic, 5-deaza, folate antitumor and antibacterial agents substituted at N_8.

A facile entry into the desired tricyclic ring system was envisioned through the cyclocondensation of an appropriately substituted aminopyrimidine and a biselectrophile derived from *N*-benzoylpiperidone. Wood *et al.*, [3] had reported the regiospecific cyclocondensation of 6-aminopyrimidines with vinyl aldehydes to afford appropriate 5,6-substituted pyrido[2,3-*d*]pyrimidines. This suggested that the vinyl aldehyde, 1-benzoyl-1,2,3,6-tetrahydropyrimidine-5-carboxaldehyde (**4**) could serve as the biselectrophile in the synthesis of **2**. Compound **4** was synthesized from *N*-benzoyl-4-piperidone (**3**) by the Vilsmeier chloroformylation with dimethylformamide and phosphorus oxychloride using a modification of the method of Benson and Pohland [4]. The initial product of the chloroformylation, 1-benzoyl-4-chloro-1,2,3,6-tetrahydropyridine-5-carboxaldehyde (**5**) gave a single spot on tlc (silica gel: methanol-chloroform 1:20, R_f 0.88). The ir spectrum gave a distinct peak for the α,β-unsaturated aldehydic carbonyl at 1672 cm^{-1}. The ^1H nmr in deuterated chloroform gave a multiplet centered at δ 3.73 which integrated for four protons and was assigned to the methylene protons *a* and *b* on **5**. A singlet for two protons at δ 4.1 was assigned to the methylene protons at *c*. The aromatic protons occurred at δ 7.4 as a singlet integrating for five protons. The most downfield of the signals was a singlet at δ 10.08 and was characteristic of chlorovinyl aldehydic protons. Further purification by vacuum distillation of the liquid **5** was unsuccessful owing to the instability of the compound. The crude yield obtained for the reaction was 86% and a homogeneous tlc coupled with the infrared and ^1H nmr confirmed the structure of **5**. Selective reduction of the β-chloro moiety of **5** with zinc in 96.5% ethanol gave poor yields. However a modification of a method [5] for the selective dehalogenation of 2-bromovinyl esters to vinyl esters using hydrogen and 10% palladium on charcoal at atmospheric pressure in the presence of excess triethylamine, which served to inhibit double bond reduction, was successful for the synthesis of **4**. The modifications included a reduction of the strength of the catalyst from 10% to



5% palladium on charcoal and the use of exactly one equivalent of hydrogen. The structure of **4** was confirmed by the appearance of a signal at δ 9.40 assigned to the vinyl aldehydic proton H_c and a broad multiplet at about δ 6.75 which was assigned to the vinylic proton H_d. In addition the disappearance of the signal at δ 10.08 for the chlorovinyl aldehydic proton H_d of **5** indicated that dechlorination had occurred. The ^{1}H nmr spectrum of **4** showed the presence of ethanol and triethylamine as contaminants. However, attempts to remove these solvents under reduced pressure without heating were unsuccessful. Heating the vinyl aldehyde **4** led to polymeric products. The crude material was therefore used for the condensation reaction without further purification.

Cyclocondensation of 2,6-diamino-4-hydroxypyrimidine (**6**) with **4** in 20% hydrochloric acid (v/v) afforded on work up a mixture of two products as determined by tlc. The mixture could not be separated by fractional crystallization or by chromatography (cellulose, 3% ammonium chloride or 50% ethanol). A similar cyclocondensation carried out in this laboratory [6], of **6** with 1-cyclohexenecarboxaldehyde was regioselective giving a mixture of the angular and linear products, **7** and **8** respectively, from which the angular isomer was separated. We therefore, suspected that the present cyclocondensation had also afforded a mixture of angular and linear products. Since separation with the benzoyl group present had not been possible, perhaps due to very similar solubility characteristics, we decided to debenzoylate the mixture. This was accomplished by acid hydrolysis using hydrochloric acid-water, 15:4 (v/v). The debenzoylated mixture was isolated and showed two spots on tlc. This indicated that the mixture initially formed was not due to benzoylated and debenzoylated products. Addition of absolute ethanol to the hydrolyzed mixture allowed the precipitation of a product, which was identified as the pure linear isomer **9** on comparison with a product previously reported from this laboratory [7]. This left the pure angular isomer **2**, as indicated by one spot on tlc, in solution from where it was isolated uncontaminated with the linear isomer.

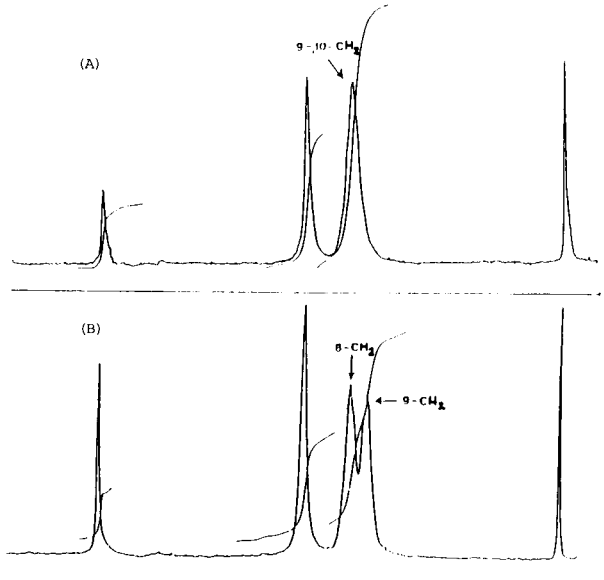


Figure 1. ^{1}H-NMR (60 MHz) of **2** (A) and **9** (B) in deuteriotrifluoroacetic acid.

In view of the fact that this cyclocondensation yielded both angular and linear isomers, an alternate regeospecific route was proposed. It had been found in our previous report [6] that 2,4,6-triaminopyrimidine (**10**) afforded regiospecifically the angular isomer **11** with both 1-cyclohexenecarboxaldehyde and 2-chloro-1-cyclohexenecarboxaldehyde. With this in mind **10** was condensed with **5** in glacial acetic acid and refluxed for 14 hours. Basification to *p*H 8 afforded a yellow-green precipitate which was washed until the washings were neutral. This yellow-green product showed two spots on tlc and was suspected to be a mixture of benzoylated and debenzoylated products. This was confirmed when, on treatment with dilute hydrochloric acid under reflux to allow for selective deamination of the 4-amino moiety and for debenzoylation, a single product was obtained which was homogenous on tlc. This confirmed the fact that unlike the regioselective condensation of **4** and **6**, the condensation of **5** and **10** was regiospecific. Addition of absolute ethanol to the hydrolyzed mixture and recrystallization from absolute ethanol-hydrochloric acid afforded a yellow product **2** as the hydrochloride salt. This product was identical in all respects (tlc, ^{1}H nmr and ir) to compound **2** obtained as described above.

The structure of **2** was established based on the ^{1}H nmr and comparison with the previously synthesized linear isomer **9**. The ^{1}H nmr spectra of the two compounds were distinctly different (Figure I). In the ^{1}H nmr spectrum in deuteriotrifluoroacetic acid, the aromatic proton H_5 of the linear isomer **9** occurred at δ 8.93 while H_7 of the angular isomer **2** occurred at δ 8.73. Further the chemical shifts of the methylene protons, of the linear isomer, at C_8 and C_9 appeared with different chemical shifts at δ 4.06 and δ 3.70 respectively. The C_8 methylene pair being further downfield due to their proximity to the protonated (deuterated) N_7 which causes them to be deshielded compared to the C_9 methylene pair. The methylene pair on C_6 is the most deshielded owing to the fact that they are flanked by both a heteroaromatic ring and a protonated N_7. The methylene protons on C_9 and C_{10} of the angular isomer **2**, on the other hand, coalesced as a broad singlet centered at δ 4.08. In this case the "peri" deshielding effect [8] of the C_1 carbonyl on the C_{10} methylene pair causes them to shift downfield such that they overlap with the C_9 methylene pair which is downfield due to its proximity to the protonated N_8. The C_7 methylene pair is once again the further-

est downfield at δ 4.97 owing to its being flanked by a heteroaromatic ring and a protonated nitrogen, N_8. Further support for the structure of the angular compound **2** comes indirectly from the fact that the linear isomer **9** was obtained by the debenzylation of **12**. Compound **12** in turn was obtained *via* a regiospecific synthesis [7] involving the cyclocondensation of **6** with 1-benzyl-3-hydroxymethylene-4-piperidone (a keto aldehyde) according to a modification of Robins and Hitchings [9].

The growth of leukemia L-1210 cells in culture [10] was inhibited 36% by **2** at $10^{-4}M$. However, the importance of compound **2** lies in its ability to serve as a key precursor for a variety of N_8-substituted non-classical and classical tricyclic, 5-deaza folate analogues. We are currently pursuing these compounds in an attempt to develop potential antitumor and antibacterial agents.

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 run on a Varian EM-360, with internal standard TMS; s = singlet, d = doublet and m = multiplet. Thin-layer chromatography (tlc) was performed on cellulose plates with fluorescent indicator and were visualized with light at 254 nm. The elemental analysis was performed by Atlantic Microlabs, Inc., Atlanta, Georgia.

1-Benzoyl-4-chloro-1,2,3,6-tetrahydropyridine-5-carboxaldehyde (**5**).

Into a three-necked flask fitted with a drying tube, a thermometer and a nitrogen inlet, was placed dimethylformamide 14.6 g (200 mmol) and cooled to 0-5°. To this was added phosphorus oxychloride 24.52 g (160 mmoles) dropwise, with continuous stirring. The rate of addition was controlled such that the temperature remained below 20°. After 30 minutes the reaction mixture became turbid and 50 ml of methylene chloride was added. After the addition of phosphorus oxychloride was completed (about 45 minutes), the reaction was continued at 27° for 2 hours. A solution of 1-benzoyl-4-piperidone **3**, 20.32 g (100 mmoles) in 20 ml of methylene chloride was added dropwise to the mixture at a rate such that temperature did not exceed 30°. After the addition (45 minutes), the reaction was continued at 27° for 2 hours. After this 150 g of crushed ice was added and the mixture stirred until all the ice had dissolved. Solid sodium acetate (70 g) was added and the mixture stirred for 15 minutes and the methylene chloride fraction separated. The aqueous portion was extracted with 50 ml of methylene chloride. The combined methylene chloride extracts were poured into 100 ml of a saturated sodium bicarbonate solution and the mixture stirred vigorously for 15 minutes. The methylene chloride fraction was then separated, washed twice with water (50 ml), dried (magnesium sulfate) and evaporated under reduced pressure (water aspirator) to give 21.5 g (86%) of **5**; tlc (silica gel, methanol-chloroform, 1:20) R_f 0.88; ir (neat): 1672 cm^{-1} (CHO); ^{1}H nmr (deuteriochloroform): δ 3.73 (m, 4H, CH_2-CH_2), 4.1 (s, 2H, CH_2-C-C=O), 7.4 (s, 5H, C_6H_5), 10.08 (s, 1H, CHO). This liquid was used without further purification in the preparation of **4** and for the cyclocondensation with **10**.

1-Benzoyl-1,2,3,6-tetrahydropyridine-5-carboxaldehyde (**4**).

Palladium on charcoal (5%, 1.2 g) was suspended in 66.5 ml of 10% potassium hydroxide for 5 minutes and the catalyst filtered, washed successively with 20 ml of ethanol, 20 ml of water and 50 ml of anhydrous ether and then dried under reduced pressure for 2 hours. To a mixture of the catalyst and 4.5 ml of triethylamine in 120 ml of ethanol saturated with hydrogen, was added 7.5 g (30 mmoles) of **5**. The mixture was reduced with hydrogen at atmospheric pressure until exactly one equivalent of hydrogen was consumed. The reaction was stopped, the catalyst filtered and the filtrate concentrated to 25% of the original volume. (During concentration a white precipitate assumed to be triethylamine hydrochloride was obtained.) The mixture was poured into 15 ml of methylene chloride, washed successively with 2 × 25 ml of 10% hydrochloric acid and 2 × 15 ml of water. The methylene chloride layer was separated, dried (magnesium sulfate) and concentrated under reduced pressure to afford a syrup. The ^{1}H nmr of this syrup showed the presence of ethanol and triethylamine. Attempts to remove these solvent contaminants under reduced pressure were not successful without heating. However, heating above 30° led to polymeric products. Thus the syrup was not further purified due to its instability and was used directly in the synthesis of **2**. Two ^{1}H nmr signals were assigned, a multiplet centered at about δ 6.75 (m, 1H, *H*-C=C), and a singlet at δ 9.40 (s, 1H, -C=C-C*H*O).

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

To a solution of 6.62 g (53 mmoles) of 2,4,6-triaminopyrimidine (**10**) in 300 ml of glacial acetic acid was added a solution of 13.22 g (53 mmoles) of **5** in 30 ml of glacial acetic acid at reflux over a period of 20 minutes and refluxed for 14 hours. Water (100 ml) was added and the mixture cooled to 25° and made basic with concentrated ammonium hydroxide to *p*H 8. The yellow-green precipitate which formed was filtered, washed with water until neutral and air-dried. The product obtained (7.0 g) was dissolved in a mixture of 20 ml of concentrated hydrochloric acid and 4 ml of water and refluxed for 4 hours. To the cooled solution was added 25 ml of absolute ethanol and left at 5° to deposit 5.0 g of a light yellow solid which was suspended in ethanol, boiled and dissolved by the dropwise addition of concentrated hydrochloric acid. The solution was filtered and left to recrystallize to afford 4.5 g (27%) of **2** as the hydrochloride salt, mp >300°. The compound was homogenous on tlc (cellulose, butanol-acetic acid-water, 3:1:3) R_f 0.61; ir (Nujol): 3390, 3300 (NH_2), 3150 (NH), 1700, 1666 cm^{-1} (C=O); ^{1}H nmr (deuteriotrifluoroacetic acid): δ 4.08 (s, 4H, 9 and 10-CH_2), 4.97 (s, 2H, 7-CH_2), 8.73 (s, 1H, 6-CH).

Anal. Calcd. for $C_{10}H_{11}N_5O \cdot 2HCl \cdot 0.8H_2O$: C, 39.44; H, 4.83; N, 22.99. Found: C, 39.61; H, 4.85; N, 22.97.

Method B.

To a solution of 3.15 g (52.5 mmoles) of **6** in 100 ml of 20% hydrochloric acid at 25°, was added a solution of 20 g of **4** (assumed 65% yield), in 10 ml of ethanol over a 2 hour period with continuous stirring. The reaction was continued at 25° for 18 hours and refluxed for an additional 2 hours. The solution was chilled to 5°, filtered and the filtrate made basic with concentrated ammonium hydroxide to *p*H 8. The precipitate formed was filtered, washed with water until neutral, air-dried and boiled in absolute ethanol for 15 minutes. After cooling to 25°, the mixture was filtered and dried to give 5.6 g of a yellow powder; tlc (cellulose, butanol-acetic acid-water, 3:1:3) showed two spots R_f 0.75 and 0.57 (which were suspected to be a mixture of **2** and its 7-benzoyl derivative).

The mixture was dissolved in a solution of 30 ml of concentrated hydrochloric acid and 8 ml of water and refluxed for 3 hours, (white needle-like crystals of benzoic acid were found condensed inside the reflux condenser). To this was added 50 ml of absolute ethanol and the mixture cooled and filtered. The filtrate was evaporated to dryness under reduced pressure and the thick syrupy material so obtained was crystallized from absolute ethanol to give 1.2 g (16%) (based on amount of **6**) of a compound that was identical with **2** (tlc, ^{1}H nmr and ir) obtained by Method A.

2-Amino-4-oxo-6,7,8,9-3*H*-tetrahydropyrimido[4,5-*b*][1,6]naphthyridine (**9**).

Compound **12**, 307 mg (1.0 mmole) [7] was dissolved in a mixture of 70 ml of isopropyl alcohol, 50 ml of water and 120 ml of concentrated hydrochloric acid by warming to 60°. After cooling to 25°, 2 g of 5% palladium on charcoal was added to the solution. The mixture was then hydrogenated at 25° at atmospheric pressure for 5 hours. The catalyst

was filtered, and the solvents evaporated under reduced pressure to afford a solid which was recrystallized from ethanol-water-hydrochloric acid, 8:2:1 (v/v) to give 250 mg (85%) of **9** as the hydrochloride salt; tlc (cellulose, butanol-acetic acid-water, 3:1:3) R_f 0.75, mp >300°; ir (Nujol): 3322 cm^{-1} (NH_2), 3135 (NH), 1695 (C=O); ^1H nmr (deuteriotrifluoroacetic acid): δ 3.7 (broad s, 2H, 9-CH_2), 4.06 (broad s, 2H, 8-CH_2), 4.9 (s, 2H, 6-CH_2), 8.93 (s, 1H, 5-CH).

Anal. Calcd. for $C_{10}H_{11}N_5O \cdot 2HCl \cdot 0.3H_2O$: C, 40.64; H, 4.64; N, 23.70. Found: C, 40.76; H, 4.91; N, 23.41.

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