Synthesis of Substituted Tetrahydropyrimido-[4,5-*b*][1,7]naphthyridines As Potential Antitumor Agents [1]

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The synthesis of two linear trisubstituted 6,7,8,9-tetrahydropyrimido[4,5-*b*][1,7]naphthyridines **1** and **3** were accomplished by the regiospecific cyclocondensation of two disubstituted 6-aminopyrimidines **10** and **11** with the ketoaldehyde 1-benzyl-4-hydroxymethylene-3-piperidone. Catalytic debenzylation of **1** afforded the disubstituted compound **2**. The linear structures of **1-3** were established by ^{1}H nmr and ^{13}C nmr.

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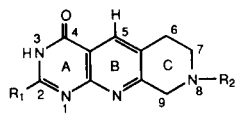
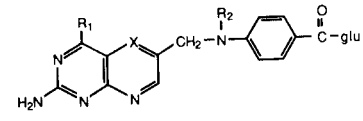
As part of our interest in angular and linear classical and nonclassical tricyclic, 5-deaza, folate analogues and homologues as potential antitumor agents [2,3,4], we have synthesized three new substituted tetrahydropyrimido-[4,5-*b*][1,7]naphthyridines **1-3**. Stone *et al.* [5] have reported that 5-deazafolic acid **4**, a 2-amino-4-oxo substituted analogue showed significant inhibitory activity against dihydrofolate reductases. The 2-amino-4-oxo substituted compounds **1** and **2** are nonclassical tricyclic homologues of **4**. In addition the debenzylated analogue **2** serves as a key intermediate for the synthesis of other classical and nonclassical antifolates related to the clinically useful agent methotrexate **5**. Compound **3** is a pyridine annulated analogue of 2,4-dioxo[2,3-*d*]pyrimidine **6** which has been reported to possess antitumor activity [6].

A search of the literature revealed that the parent tricyclic ring system pyrimido[4,5-*b*][1,7]naphthyridine had been reported by Decosmeille *et al.* [7] in 1975. These workers synthesized the tricyclic system by using a multistep synthetic sequence commencing from the synthesis of an appropriately substituted aminopicolinaldehyde which was condensed with an appropriate active methylene compound to afford 1-amino-2-carboxamido-1,7-naphthyridine which in turn was condensed with formamide to afford 1-oxo-pyrimido[4,5-*b*][1,7]naphthyridine. The elemental analysis was the only evidence cited, and no spectral proof of structure was presented.

We have had considerable success in the cyclocondensation of biselectrophiles with aminopyrimidines in the synthesis of tricyclic linear isomers of substituted tetrahydropyrimido[4,5-*b*]quinolines **7** [8] and tetrahydropyrimido-[4,5-*b*][1,6]naphthyridines **8** [4]. The use of ketoaldehydes as the biselectrophile regiospecifically affords the linear isomers **7** and **8** rather than the angular isomers.

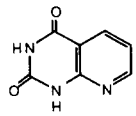
The appropriate ketoaldehyde for the synthesis of **1, 2** and **3** was 1-benzyl-4-hydroxymethylene-3-piperidone (**9**), which could be obtained from the 3-piperidone by a modified formylation as we had reported earlier for cyclohexanone and 4-piperidone [4,8]. However, the





	R_1	R_2
1	NH_2	$CH_2C_6H_5$
2	NH_2	H
3	OH	$CH_2C_6H_5$

	R_1	R_2	X
4	OH	H	CH
5	NH_2	CH_3	N



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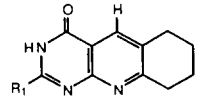

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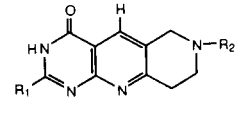

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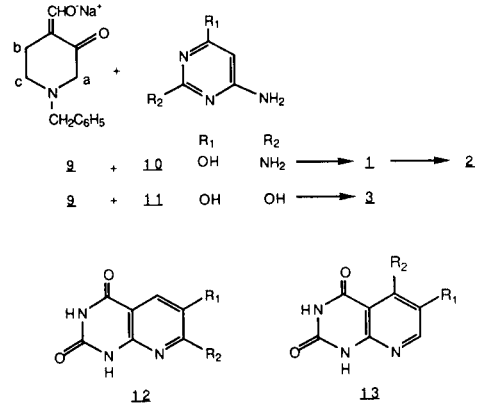

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3-piperidone was an unsymmetrical ketone and formylation could afford the 4- and/or 2-formyl derivatives. Formylation of 1-benzyl-3-piperidone with ethylformate [4] in the presence of sodium formate afforded a single product **9** in 75% yield which was homogeneous on tlc. The proton nmr spectrum of this product in deuterated water indicated an aldehydic proton at δ 9.18, a five proton singlet at δ 7.48, assigned to the aromatic protons and two proton singlets at δ 3.58 and 3.00 which were assigned to the benzylic protons and to protons a, respectively. The other four protons formed a centro-symmetric pattern characteristic of an AA'BB' system and hence must be assigned to vicinal

protons b and c centered at δ 2.30 and 2.53, respectively. Clearly the proton splitting pattern confirmed that formylation of 1-benzyl-3-piperidone occurs exclusively at the 4-position. The mechanism of formylation requires the formation of an enol intermediate. With this ketone, the 4-enol intermediate is formed preferentially, in part because the 2-enol intermediate is destablized by the adjacent basic nitrogen.

Cyclocondensation of the sodium salt of the ketoaldehyde **9** with 2,6-diamino-4-hydroxypyrimidine (**10**) in phosphoric acid (generating the ketoaldehyde *in situ*) afforded a single product as indicated by tlc analysis. This product was the linear isomer 2-amino-8-benzyl-4-oxo-6,7,8,9-tetrahydro-3*H*-pyrimido[4,5-*b*][1,7]naphthyridine (**1**).

The linear structure of **1** was confirmed based on literature precedent that ketoaldehydes when condensed with aminopyrimidines afford regiospecifically 6,7-disubstituted pyrido[2,3-*d*]pyrimidines **12** rather than the 5,6-disubstituted isomers **13** [9]. In the tricyclic series **7** and **8** we have previously shown that linear rather than angular isomers were obtained [4,8]. In each case the most nucleophilic position of the pyrimidine (the C_5-carbon) attacks the more reactive of the two carbonyls (the aldehyde) of the ketoaldehyde, while the 6-amino group of the pyrimidine condenses with the ketone carbonyl. Catalytic hydrogenolysis of **1** with 10% palladium on carbon at room temperature and atmospheric pressure afforded the debenzylated, linear analogue 2-amino-4-oxo-6,7,8,9-tetrahydro-3*H*-pyrimido[4,5-*b*][1,7]naphthyridine **2** in 85% yield. Absence of the protons of the benzyl moiety in the ^{1}H nmr along with a homogenous tlc indicated that only debenzylation had occurred.

Spectral evidence for the linear structure of **1** and **2** was furnished from the ^{13}C nmr spectra. It is well established that the aromatic carbon *gamma* to the ring nitrogen atom in pyridines and pyrido[2,3-*d*]pyrimidines and similar multicyclic heteroaromatic systems resonates in the range 134-137 ppm with a one bond coupling constant ^{1}JC-H of 157-164 Hz while the aromatic carbon *alpha* to the ring nitrogen of the pyridine ring in similar heteroaromatics occurs at about 155 ppm with a one bond coupling constant ^{1}JC-H of 177-180 Hz [11,12]. The proton coupled ^{13}C nmr spectra of **1** and **2** showed doublets at 140.30 and 140.21 ppm respectively, with a one bond coupling constant of 169 Hz for both **1** and **2**. These values compare favorably with those in the literature for an aromatic carbon *gamma* to the nitrogen of the pyridine ring. These resonance positions were assigned to the C_5 of **1** and **2** which established their linear structures.

Cyclocondensation of **9** with 6-aminouracil (**11**) in phosphoric acid similarly afforded a single product homogeneous on tlc which was characterized as 8-benzyl-2,4-dioxo-6,7,8,9-tetrahydro-1*H*,3*H*-pyrimido[4,5-*b*][1,7]naphthyridine **3**, based on spectral data. The linear structure of **3** was also established from its ^{13}C nmr spectra. The coupled ^{13}C nmr of **3** showed a doublet centered at 141.94 ppm with a one bond coupling constant of 167 Hz. Both the chemical shift position and the coupling constant identifies this carbon as the C_5 carbon and consequently the linear structure as depicted in **3**.

In previous reports we have also utilized the chemical shift position of the H_5 proton to support the structural assignment of linear isomers [4,8]. In general the linear isomers such as **7** and **8** have their H_5 proton about 0.5 ppm downfield compared to the corresponding angular isomers. However, this chemical shift position is highly sensitive to the nature of the solvent and the substitution pattern on the C-ring. In acidic solvents such as trifluoroacetic acid the aromatic proton of tricyclic linear isomers are found further downfield than that of the angular isomer. In non acidic solvents such as dimethylsulfoxide, however, the aromatic protons of the angular tricyclic isomer are found further downfield. This difference is attributable, in part, to the protonation of the the ring nitogen in acidic solvents.

The H_5 aromatic proton of **1**, **2** and **3** occur at δ 8.47, 8.40 and 8.46, respectively in a mixture of deuterated trifluoroacetic acid and deuterated water (2:1). These chemical shift positions do not coincide with that expected in pure deuterated trifluoroacetic acid, where these protons would be anticipated closer to 9.00 ppm [4]. The presence of the deuterated water in the ^{1}H nmr solvent could, in part, be responsible for the upfield shift. Compounds **1**, **2** and **3** were insoluble in pure trifluoroacetic acid, hence the ^{1}H nmr spectra could not be determined without deuterated water. However, compound **2** was soluble in deuterated dimethylsulfoxide in which the aromatic proton H_5 occurred at δ 8.18, about 0.22 ppm upfield from that in deuterated trifluoroacetic acid/deuterated water. The methylene region of the ^{1}H nmr spectrum of these compounds deserves attention. Both **1** and **3** had very similar spectra. For compound **1** the C_9 methylene protons being flanked by the protonated N_8 nitrogen and the aromatic B-ring occur at δ 4.60. The two methylene protons on C_7 are in different environments and are differently affected by the phenyl ring of the benzyl moiety such that one of the C_7 protons is deshielded and shifts downfield as a multiplet at δ 4.01, while the other occurs as a multiplet at δ 3.48 overlapping the C_6 methylene. In compound **2**, which lacks the benzyl moiety, both the C_6 and C_7 methylene protons occur as pseudo triplets, characteristic of AA'XX' systems. The downfield triplet being at δ 3.69, assigned to the C_7-methylenes and the upfield triplet at δ 3.33 were assigned to the C_6-methylenes.

The benzyl moiety of **1** was chosen because the starting

piperidone was commercially available but more importantly because hydrogenolysis of the benzyl moiety would afford compound **2** which could function as a key intermediate in the synthesis of a variety of classical and nonclassical linear, tricyclic antifolates substituted at N_8. We are currently involved in the synthesis of classical and nonclassical analogues of **1** in particular of the 2,4-diamino compound with various N_8-substitutions as potential antitumor and antibacterial agents.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorected. Infrared spectra (ir) were recorded with a Perkin Elmer Model 1430, in Nujol mulls. Nuclear magnetic resonance spectra for proton (^{1}H nmr) were recorded on a Varian EM-360 (60 MHz) or Brucker WH-300 (300 MHz), and for carbon-13 (^{13}C nmr) on a Brucker WH-300 at 75.46 MHz; 90° pulse: 14 seconds. The data was accumulated by 16K size with 0.5 second delay time and 70° tip angle, with internal standard TMS; s = singlet, d = doublet, t = triplet, m = multiplet. Thin layer chromatography (tlc) was performed on silica gel plates and cellulose plates with fluorescent indicator or as otherwise indicated and were visualized with light at 254 nm and 366 nm. Elemental analysis was performed by Atlantic Microlabs Inc., Norcross, Georgia.

Sodium Salt of 1-Benzyl-4-Hydroxymethylene-3-piperidone (**9**).

Into a three-necked flask fitted with a drying tube was placed sodium metal 0.56 g (25.5 mmoles) which had been cut into small pieces (about 1 cm^3). Anhydrous ether (50 ml) was added followed by 2.8 g (38 mmoles) of ethyl formate (dried with potassium carbonate). To this mixture was added 4.8 g (25.5 mmoles) of 1-benzyl-3-piperidone. The mixture was cooled to 5° using an ice bath and the reaction was initiated by the addition of 0.12 ml of absolute ethanol. Stirring was continued for 6 hours and stopped. The reaction mixture was left to stand for a further 6 hours. An additional 0.16 ml of absolute ethanol was added to the mixture which was stirred for an additional 1 hour. The pale brown precipitate that formed was collected by filtration, washed with anhydrous ether and dried under reduced pressure with phosphorus pentoxide for 6 hours to give 4.5 g (75%) of **9**. Due to the nature of the product, further purification was not possible; tlc, (silica gel, methanol-chloroform, 3:17 v/v), R_f 0.52; (cellulose, 1-butanol-water-acetic acid, 3:3:1 v/v) R_f 0.86; ir (nujol) 1725 (C=O), 1600 cm^{-1}; ^{1}H nmr (deuterium oxide): (60 MHz) δ 2.30 (pseudo t, 2H, 5-CH_2), 2.53 (pseudo t, 2H, 6-CH_2), 3.00 (s, 2H, 2-CH_2), 3.58 (s, 2H, $CH_2C_6H_5$), 7.48 (s, 5H, C_6H_5), 9.18 (s, 1H, HC-ONa).

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

2,6-Diamino-4-hydroxypyrimidine 0.79 g (6.28 mmoles) was dissolved in 10 ml of 85% phosphoric acid, to this was added 1.5 g (6.28 mmoles) of the powdered sodium salt of 1-benzyl-4-hydroxymethylene-3-piperidone (**9**) and the mixture heated at 100° for 3 hours. The mixture was then cooled and poured into 100 ml of water, and cooled to 5° in an ice bath and neutralized to *p*H 7 with concentrated ammonium hydroxide solution while maintaining the temperature below 20°. The dark brown precipitate that separated was filtered, washed with water until neutral and dried under reduced pressure with phosphorous pentoxide for 24 hours. The dried solid was suspended in absolute ethanol, boiled for 10 minutes and filtered. The residue was air dried and dissolved in 95% ethanol with heating and the addition of drops of concentrated hydrochloric acid until a solution resulted. This solution was stored at 5° to deposit 1.5 g (75%) of a brown solid; tlc, (cellulose, 1-butanol-water-acetic acid, 3:3:1 v/v) R_f 0.85; mp >300°; ir (nujol): 3320 (-NH_2), 3130 (—NH), 1685 cm^{-1}; ^{1}H nmr (deuteriotrifluoroacetic acid-deuterium oxide, 2:1 v/v): (300 MHz) δ 3.48 (m, 3H, 6-CH_2 and one of 7-CH_2), 4.01 (m, 1H, one of 7-CH_2), 4.50 (d, 1H, J = 17.1 Hz, C_6H_5-CH_2), 4.60 (s, 2H, 9-CH_2), 4.74 (d, 1H, J = 17.1 Hz, C_6H_5-CH_2), 7.58 (s, 5H, C_6H_5), 8.47 (s, 1H, 5-CH); ^{13}C nmr (deuteriotrifluoroacetic acid-deuterium oxide, 2:1 v/v): (75.46 MHz) 140.30 ppm, J = 169 Hz (C-5 aromatic carbon *gamma* to the nitrogen of the pyridine ring).

Anal. Calcd. for $C_{17}H_{17}N_5O\cdot1.7HCl\cdot1.7H_2O$: C, 51.05; H, 5.57; N, 17.51; Cl, 15.07. Found: C, 51.24; H, 5.27; N, 17.67; Cl, 15.03.

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

Compound **1** 0.75 g (2.19 mmoles) was dissolved in a mixture of 21 ml of 2-propanol, 24 ml of water and 69 ml of concentrated hydrochloric acid. To this solution was added 1.5 g of 10% Pd/C and hydrogenolysis was carried out at room temperature and atmosphere pressure for 4 hours (hydrogen consumption was 80 ml). The catalyst was filtered and washed with 150 ml of hydrochloric acid. The washings were concentrated to dryness under reduced pressure and the dark brown solid obtained was suspended in 45 ml of absolute ethanol and stirred for 20 minutes, filtered and dried over phosphorus pentoxide to afford 0.48 g (85%) of **2** as the hydrochloride salt; tlc [a. cellulose; 1-butanol-water-acetic acid (3:3:1 v/v), R_f 0.55; b. cellulose, 2-propanol-ammonium hydroxide-water (6:3:1 v/v), R_f 0.33], mp >300°; ir (nujol): 3410 (-NH_2), 3250 (-NH), 1675 cm^{-1}; ^{1}H nmr (deuteriotrifluoroacetic acid-deuterium oxide 2:1 v/v): (300 MHz) δ 3.33 (t, 2H, 6-CH_2), 3.69 (t, 2H, 7-CH_2), 4.60 (s, 2H, 9-CH_2), 8.46 (s, 1H, 5-CH); ^{13}C nmr (deuteriotrifluoroacetic acid-deuterium oxide, 2:1 v/v): (75.46 MHz) 140.21 ppm, J = 169 Hz (C-5 aromatic carbon *gamma* to the nitrogen of the pyridine ring).

Anal. Calcd. for $C_{10}H_{11}N_5\cdot2HCl$: C, 41.40; H, 4.52; N, 24.14; Cl, 24.44; Found: C, 41.29; H, 4.24; N, 24.00; Cl, 24.29.

8-Benzyl-2,4-dioxo-6,7,8,9-tetrahydro-1*H*,3*H*-pyrimido[4,5-*b*][1,7]-naphthyridine (**3**).

6-Aminouracil, 0.8 g (6.28 mmoles) was dissolved in 10 ml of 85% phosphoric acid by warming to 60°, then cooled to 25°. To this solution was added 1.5 g (6.28 mmoles) of the powdered sodium salt of 1-benzyl-4-hydroxymethylene-3-piperidone **9**. The mixture was refluxed for 3 hours, cooled and poured into 50 ml of water, filtered and the filtrate basified to *p*H 8 with concentrated ammonium hydroxide while maintaining the temperature below 20°. The brown precipitate that separated was filtered, washed with water until the washings were neutral and suspended in an ethanol-water-concentrated hydrochloric acid (6:2:1) mixture, heated to boiling and filtered while hot to afford 1.2 g (80%) of a white solid as a residue. An analytical sample was obtained by recrystallization from ethanol-water; tlc [a. cellulose, 1-butanol-water-acetic acid (3:3:1 v/v), R_f 0.81; b. cellulose, 2-propanol-ammonium hydroxide-water (6:3:1 v/v), R_f 0.72], mp >300°; ir (nujol): 3290, 1830, 1770, 1640 cm^{-1}, ^{1}H nmr (deuteriotri-

fluoroacetic acid-deuterium oxide, 2:1 v/v); (300 MHz) δ 3.33-3.46 (m, 3H, 6-CH_2 and one of 7-CH_2), 4.01 (m, 1H, one of 7-CH_2), 4.44 (d, 1H, J = 16.9 Hz, C_6H_5-CH_2), 4.58 (s, 2H, 9-CH_2), 4.66 (d, 1H, J = 16.8 Hz, C_6H_5-CH_2), 7.58 (s, 5H, C_6H_5), 8.40 (s, 1H, 5-CH); ^{13}C nmr (deuteriotrifluoroacetic acid-deuterium oxide, 2:1 v/v): (75.46 MHz) 141.94 ppm, J = 167 Hz (C_5 aromatic carbon *gamma* to the pyridine nitrogen.)

Anal. Calcd. for $C_{17}H_{16}N_4O_2 \cdot 1.1HCl$: C, 59.39; H, 4.69; N, 16.30; Cl, 10.31. Found: C, 59.01; H, 5.06; N, 16.20; Cl, 10.08.

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