The Synthesis of Functionalized Derivatives of Pyrimido[4,5-*b*][1,4]oxazines and Side Chain Attachment

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The synthesis and reactions of 2-amino-4-hydroxypyrimido[4,5-*b*][1,4]oxazines with functionalities in the six position are described (1). The use of functionalities in the six position for coupling side chains to the pyrimido-oxazine system is discussed and a successful coupling described.

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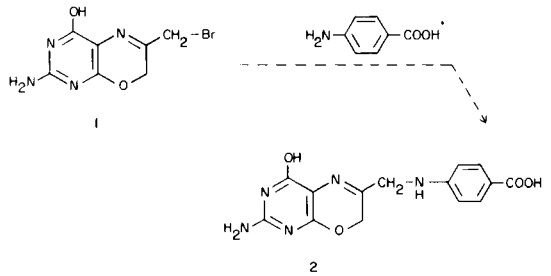
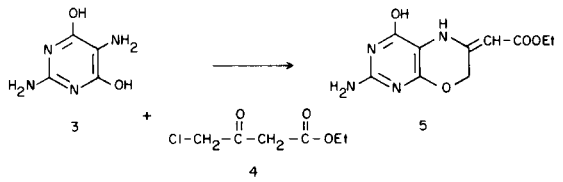
Our interest and study of pyrimido-oxazines has continued since discovering that 7*H*-pyrimido[4,5-*b*][1,4]oxazines could be synthesized by the condensation of 2,5-diamino-4,6-dihydroxypyrimidine with α-haloketones (2). The pyrimido-oxazine ring system is an analog of the pteridine nucleus derived by homeosteric (3) replacement of the N_8 nitrogen by oxygen. Since the pteridine nucleus is found in folic acid and antimetabolites of folic acid such as methotrexate (4,5), we have postulated that suitably substituted compounds possessing the pyrimido-oxazine moiety may exhibit antifolate activity.

Of particular interest to us at the present time is the synthesis of pyrimido-oxazines such as 8-oxadihydropteroic acid (**2**) with a N_{10} nitrogen in the side chain. Accordingly, we have studied the synthesis and chemistry of pyrimido-oxazines substituted with reactive functionalities in the six position with the goal in mind of reacting these functionalities with the terminal amino groups of suitable side chains.

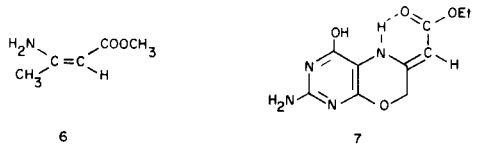
Pyrimido-oxazine **1** was the original target molecule as the 6-bromomethyl group would be ideal for reaction with *p*-aminobenzoic acid. However, the direct synthesis of **1** through bromination of 2-amino-4-hydroxy-6-methyl-7*H*-pyrimido[4,5-*b*][1,4]oxazine failed. Consequently, work



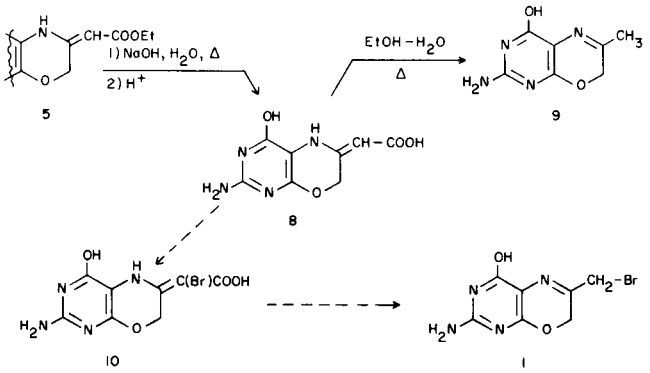
was done on an alternate approach to **1** involving the synthesis and reactions of 2-amino-4-hydroxy-6-carbethoxymethylene-6,7-dihydro-5*H*-pyrimido[4,5-*b*][1,4]oxazine (**5**). 2,5-Diamino-4,6-dihydroxypyrimidine (**3**) was found to condense with ethyl 4-chloroacetoacetate (**4**) to give **5**. Insight into the structure of **5** was gained from nmr spectroscopy in dimethylsulfoxide-d_6 solution. The chemical shift of the vinyl proton agreed well with that in the



Varian spectrum (6) of the model compound **6**. Therefore, the *exo* rather than an *endo* double bond was indicated, and the N_5 hydrogen atom was assigned to the five position. Because the vinyl proton and the methylene protons of the oxazine ring both appeared as sharp singlets in the nmr spectrum, it was possible that only one of the two possible *cis-trans* olefinic isomers was present. Isomer **7** is attractive in that hydrogen bonding of the N_5 hydrogen atom to the carbonyl of the carbethoxy group could exist.



Work on the chemistry of **5** revealed that its hydrolysis led to a product which upon heating in an ethanol-water medium gave 2-amino-4-hydroxy-6-methyl-7*H*-pyrimido-[4,5-*b*][1,4]oxazine (**9**). The hydrolysis product was assumed to be carboxylic acid derivative **8** or a tautomeric form



of **8** with the imine double bond in the oxazine ring. Since **8** appeared to easily convert to **9**, a route to the target molecule **1** might exist. If bromination of **8** gave **10**, subse-

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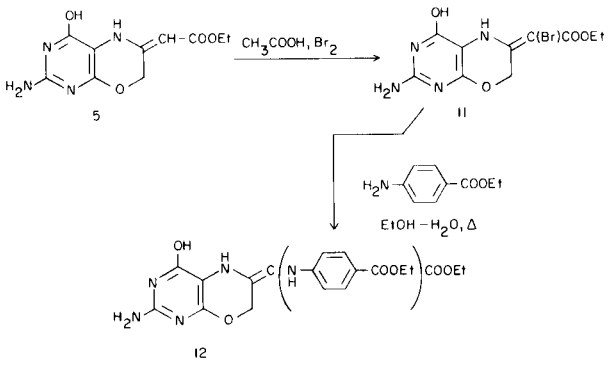
quent decarboxylation could give **1**. Accordingly, an attempt was made to brominate **8** suspended in glacial acetic acid, but the product of this attempt apparently degraded upon collection and washing with water. It was found, however, that bromination of **5** did give a product stable enough for isolation, and it was through the use of this bromination intermediate that our first successful attachment of a nitrogen containing side chain to the pyrimido[4,5-*b*][1,4]oxazine ring system was accomplished.

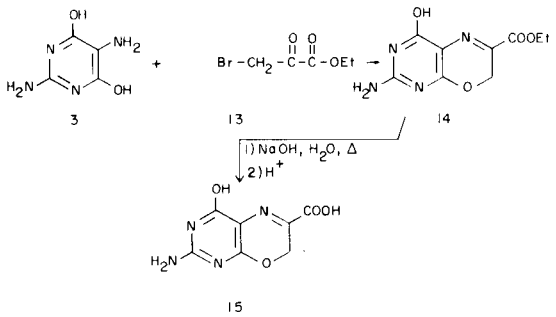
When **5** was reacted with bromine in glacial acetic acid, a product was obtained that elemental analysis indicated had a molecular weight corresponding to the monobromination product. This reactive product was treated as an intermediate and assumed to have structure **11**. Reaction of **11** with ethyl *p*-aminobenzoate was found to



proceed under mild conditions, and the product **12** was examined with nmr spectroscopy in dimethylsulfoxide-d_6 solution. The nmr spectrum and its similarities to the nmr of **5** indicated that the five position was the most likely position for the N_5 hydrogen atom and that a double bond *exo* to the oxazine ring was more likely than an *endo* double bond. Signals roughly resembling a doublet with unresolved fine structure that appeared at 4.85 ppm were assigned to the methylene protons of the oxazine ring. This pattern could arise from the presence of one of the *cis-trans* olefinic isomers of **12** but might also be an indication that both are present.

Compound **12** represents our first successful coupling of an amino-terminated side chain to the pyrimido-oxazine system. The possibility of hydrolyzing and decarboxylating **12** to obtain **2** is under study.

Research has also been carried out on the feasibility of condensing molecules other than ethyl 4-chloroacetoacetate with 2,5-diamino-4,6-dihydroxypyrimidine (**3**) to obtain other suitably functionalized pyrimido[4,5-*b*][1,4]-oxazines for side chain attachment. This study has led to the synthesis of 2-amino-4-hydroxy-6-carbethoxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**14**) through the condensation of **3** and ethyl bromopyruvate (**13**). We have also found that ester **14** can be hydrolyzed to 2-amino-4-hydroxy-



6-carboxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**15**). Investigations are now in progress concerning the conversion of the carboxylic acid group to a reactive intermediate capable of reacting with amino-terminated molecules in order to bind side chains through an amide linkage.

The synthesis of pyrimido[4,5-*b*][1,4]oxazines with functionalities in the six position has given us intermediates which may be key to the successful synthesis of 8-oxadihydropteroic acid (**2**). Moreover, they may prove useful for the synthesis of a number of other side chain substituted pyrimido[4,5-*b*][1,4]oxazines with potential as folic acid antagonists.

EXPERIMENTAL (1)

Melting points were determined in a Thomas Hoover apparatus and are uncorrected. A Hitachi Perkin-Elmer R-24B nmr spectrometer was used for nuclear magnetic resonance spectra. Solutions of tetramethylsilane in deuteriochloroform were used as external standards. A Beckman IR-33 was used for ir spectra, and some of the stronger absorptions are reported. The wavelengths of ultraviolet absorption maxima occurring in the 240-400 nm range are given and were determined with a Beckman DB spectrophotometer. Microanalysis was performed by Midwest Microlab, Ltd., Indianapolis, Indiana.

2,5-Diamino-4,6-dihydroxypyrimidine (**3**).

Adaptions of a published procedure by Dunn and Skinner (2) were used. Elemental analysis on a sample of one such product after commercial drying indicated $C_4H_6N_4O_2 \cdot HCl \cdot H_2O$ as the formula. This product was the hydrochloride salt monohydrate.

2-Amino-4-hydroxy-6-carbethoxymethylene-6,7-dihydro-5*H*-pyrimido-[4,5-*b*][1,4]oxazine (**5**).

2,5-Diamino-4,6-dihydroxypyrimidine hydrochloride (approximately monohydrate, 3.94 g.) was suspended in 1*l*. of 50% aqueous ethanol with stirring and raised to reflux. Ethyl 4-chloroacetoacetate, 98% (6.58 g.) dissolved in 50 ml. of absolute ethanol was added and allowed to reflux another 5 minutes. Sodium bicarbonate (3.36 g.) in 50 ml. of water was added dropwise and refluxed for 15 minutes. The hot reaction mixture was refrigerated at 0° for a day. The solid which separated was collected, washed with water, and dried *in vacuo* at room temperature, yield 3.97 g.

A sample for spectral and analytical purposes was recrystallized by dissolving in hot aqueous ethanol, keeping in the cold, collecting, washing with water, and drying *in vacuo* at room temperature. A definite melting or decomposition point could not be obtained. The sample changed to brown-black upon heating to 305°; ir (potassium bromide): 3410 m cm^{-1}, 1665 s, 1620 s, 1290 m; uv (1*N* sodium hydroxide): 269 nm (log ϵ 3.90), 316 (log ϵ 3.96); nmr (DMSO-d_6): 1.16 ppm (triplet, 3H), 4.00 (quartet, 2H), 4.57 (singlet, 1H), 4.74 (singlet, 2H), 6.45 (singlet, approx. 2H), 9.39 (singlet, approx. 1H), 10.98 (broadened singlet, approx. 1H). The singlets at 6.45, 9.39 and 10.98 exchanged with deuterium oxide.

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.38; H, 4.85; N, 21.99.

2-Amino-4-hydroxy-6-methyl-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**9**).

a) Hydrolysis of 2-Amino-4-hydroxy-6-carbethoxymethylene-6,7-dihydro-5*H*-pyrimido[4,5-*b*][1,4]oxazine.

Pyrimido-oxazine **5** (0.75 g.) was dissolved with stirring in 22.5 ml. of water containing 0.36 g. of sodium hydroxide and the solution was heated with stirring and continued at 95°, for another 2 minutes. Then the solution was allowed to stand for 2.75 hours. The *p*H of the solution was then adjusted to 0-1 with 1*N* hydrochloric acid. The solid which separated was collected and washed. The solid was dried *in vacuo* at room temperature, yield 0.52 g. This product was assumed to be carboxylic acid **8** or a tautomeric form of **8** with the imine double bond in the oxazine ring. The decomposition point was poorly defined. A black decomposed solid was left in the capillary upon reaching 285°; ir (potassium bromide): 3420 m cm^{-1}, 3120 m, 2920 m, 2760 m, 1660 s, 1570 s, 1265 s; uv (1*N* hydrochloric acid, undissolved solid noted): 265 nm, 347; uv (1*N* sodium hydroxide): 267 nm, 314.

b) Decarboxylation Procedure.

The hydrolysis product (0.36 g.) was combined with 32 ml. of aqueous ethanol, heated and stirred. Additional aqueous ethanol was added as heating continued. Eventually, a cloudy boiling solution was obtained (*ca.* 85 ml.) that was placed in the refrigerator at 1° for 26 hours. The solid which separated was collected and dried *in vacuo* at room temperature, yield 0.25 g. The nmr spectrum in trifluoroacetic acid indicated methyl and methylene singlets and a broad band attributed to the amino protons. The ir spectrum was comparable with that of crude 2-amino-4-hydroxy-6-methyl-7*H*-pyrimido[4,5-*b*][1,4]oxazine which had been prepared by an adaptation of a literature procedure, and the uv absorption maxima were also comparable with that reported in the literature (2); uv (2*N* hydrochloric acid): 264 nm, 346 (lit. 266, 347); uv (2*N* sodium hydroxide): 268 nm, 311 (lit. 267, 311); nmr (trifluoroacetic acid): 2.23 ppm (singlet, 3H), 5.17 (singlet, 2H), 7.22 (broad band, 1-2H).

2-Amino-4-hydroxy-6-[(carbethoxy)(4-carbethoxyphenylamino)methylene]-6,7-dihydro-5*H*-pyrimido[4,5-*b*][1,4]oxazine (**12**).

a) Bromination of 2-Amino-4-hydroxy-6-carbethoxymethylene-6,7-dihydro-5*H*-pyrimido[4,5-*b*][1,4]oxazine.

Pyrimido-oxazine **5** (3.55 g.) was stirred with 71 ml. of glacial acetic acid, and 0.72 ml. of bromine dissolved in 43 ml. of glacial acetic acid was added dropwise, after which stirring was continued for another 15 minutes. The solid was collected, washed with water, and dried *in vacuo* at room temperature, yield 4.28 g. This product was assumed to be **11**. A distinct decomposition point could not be obtained; ir (potassium bromide): 3440 m cm^{-1}, 1670 s, 1605 s, 1255 s, nmr (trifluoroacetic acid): 1.07 ppm (triplet, 3H), 4.05 (quartet, 2H), 5.06 (broadened singlet, approx. 1.5 H), 5.38 (broadened singlet, approx. 0.5H), 7.89 (swell, 1-2H).

Anal. Calcd. for $C_{10}H_{11}N_4O_4Br$: C, 36.27; H, 3.35; Br, 24.13. Found: C, 36.44; H, 3.62; Br, 23.84.

b) Reaction of **11** with Ethyl *p*-Aminobenzoate.

The bromination product **11** (3.50 g.) was combined with 325 ml. of absolute ethanol, 325 ml. of water, and 1.75 g. of ethyl *p*-aminobenzoate and stirred and heated at reflux for 10 minutes. The orange solution was kept in the refrigerator at 1° for one day. The solid which separated was collected, washed with water, and dried *in vacuo* at room temperature, yield 0.92 g. It was later found in a synthesis scaled down from the above that doubling the calculated weight of ethyl *p*-aminobenzoate used increased the precentage yield substantially. The ir spectrum of the product was essentially equivalent to that obtained in the lower yield synthesis.

An analytical specimen was prepared by two recrystallizations from aqueous ethanol and dried *in vacuo* at room temperature, m.p. 212-216° dec.; ir (potassium bromide): 3410 m cm^{-1}, 1670 s, 1610 s, 1290 s, 1255 s; nmr (DMSO-d_6): 1.25 ppm (multiplet, 6H), 4.18 (multiplet, 4H), 4.85 (roughly resembles doublet with unresolved fine structure, 2H), 6.54 (distorted doublet with 2H singlet overlapping doublet portion at 6.48, 4H total), 7.18 (singlet, 1H), 7.65 (distorted doublet, 2H), 9.82 (singlet, approx. 1H), 11.00 (singlet approx. 1H). The singlets at 6.48, 7.18, 9.82, and 11.00 exchanged with deuterium oxide.

Anal. Calcd. for $C_{19}H_{21}N_5O_6 \cdot \frac{1}{2}H_2O$: C, 53.77; H, 5.23; N, 16.50. Found (commercial drying): C, 53.77; H, 5.13; N, 16.75.

2-Amino-4-hydroxy-6-carbethoxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**14**).

2,5-Diamino-4,6-dihydroxypyrimidine hydrochloride (approx. monohydrate, 3.94 g.) was reacted with 7.80 g. of ethyl bromopyruvate, tech., 90% as described in the synthesis of **5**. The reflux period was 25 minutes. Then, the reaction mixture was allowed to stand at 6° for one day. The solid which separated was collected, washed with water, and dried *in vacuo* at room temperature, yield 3.45 g.

An analytical sample was prepared as described for **5**. It was difficult to obtain a distinct decomposition point for the recrystallized product. In the 240-245° range, a tan color began to develop, and at 257-260° the sample rapidly darkened. At 260°, the sample was reddish brown in color; ir (potassium bromide): 3440 m cm^{-1}, 3140 m, 1680 s, 1515 s, 1275 s; uv (1*N* hydrochloric acid, spectrum changes in time): 271 nm, 364; uv (1*N* sodium hydroxide): 246 nm, 273 (log ϵ 3.73), 357 (log ϵ 4.01); uv (DMSO, window from 400-268 nm): 277 nm, 380; nmr (DMSO-d_6): 1.27 ppm (triplet, 3H), 4.17 (quartet, 2H), 4.88 (singlet, 2H), 7.14 (broadened singlet, 2H), 10.76 (broadened singlet, 1H).

Anal. Calcd. for $C_9H_{10}N_4O_4$: C, 45.38; H, 4.23; N, 23.52. Found: C, 45.55; H, 4.33; N, 23.36.

Evidently, **14** can also recrystallize in alternate form. It was observed that a sample of product recrystallized from aqueous ethanol in a manner differing from that used for **5** had upon washing and drying *in vacuo* at room temperature an ir spectrum that differed from that described for **14** above. A medium to strong absorption appeared above 1700 cm^{-1}, and other shifts and changes were in evidence. After long storage, another ir was obtained which was now essentially equivalent to that described for **14**.

2-Amino-4-hydroxy-6-carboxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**15**).

Pyrimido-oxazine **14** (3.52 g.) was stirred with a solution prepared from 2.11 g. of sodium hydroxide and 470 ml. of water. Once the solid dissolved, the solution was refluxed for 15 minutes and allowed to cool with stirring to 40°. The solution was filtered and acidified to a *p*H of 0-1 with 1*N* hydrochloric acid. The solid which separated was collected, washed with water and dried *in vacuo* at room temperature, yield 3.17 g. This substance did not exhibit a well defined decomposition point; ir (potassium bromide): 3420 s, 3100 m, 1665 s, 1505 s, 1480 s, 1290 s; uv 1*N* hydrochloric acid, undissolved solid noted, spectrum changes in time): 270 nm, 362; uv (1*N* sodium hydroxide): 245 nm, 273, 355; uv (DMSO, window from 400-268 nm, spectrum changes in time): 276 nm, 376; nmr (trifluoroacetic acid): 5.10 ppm (singlet), 8.12 (swell).

Anal. Calcd. for $C_7H_6N_4O_4 \cdot 0.92\ H_2O$: C, 37.08; H, 3.49; N, 24.71. Found: C, 36.82; H, 3.60; N, 24.97.

An anhydrous specimen was prepared by drying *in vacuo*.

Anal. Calcd. for $C_7H_6N_4O_4$: C, 40.01; H, 2.88; N, 26.66. Found: C, 39.70; H, 2.80; N, 26.40.

Acknowledgment.

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REFERENCES AND NOTES

(1) Structures, for convenience, have been depicted as 4-hydroxy tautomers. The hydroxy form is not necessarily favored.

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