# Coupling to the Pyrimido[4,5-*b*][1,4]oxazine Ring System: Synthesis of Potential Antifolates (1)



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The ability of 2-amino-4-hydroxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine derivatives to inhibit dihydrofolate reductase led to a search for means of synthesizing new side chain substituted analogs of this marginally stable pyrimidooxazine system. A study of the synthesis and use of 6-functionalized pyrimido[4,5-*b*][1,4]oxazines for coupling side chains was begun and has now revealed methods for coupling *p*-aminobenzoic acid with 2-amino-4-hydroxy-6-carboxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine and hydrolyzed 2-amino-4-hydroxy-6-carbethoxymethylene-6,7-dihdyro-5*H*-pyrimido[4,5-*b*][1,4]oxazine. The products are of interest for evaluation as potential antifolates.

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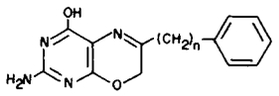
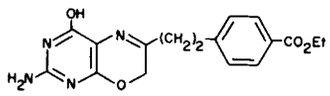
Steric and electronic similarities between the 7*H*-pyrimido[4,5-*b*][1,4]oxazine and 7,8-dihydropteridine ring systems suggested that pyrimidooxazine derivatives might exhibit antifolate activity. We studied the biological activity of 2-amino-4-hydroxy-6-substituted-7*H*-pyrimido-[4,5-*b*][1,4]oxazines and found that they did, indeed, exert an inhibitory effect in a pigeon liver dihydrofolate reductase enzyme system (2). 2-Amino-4-hydroxy-6-phenylethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**1**) and 2-amino-4-hydroxy-6-phenylpropyl-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**2**) were among the most biologically active compounds assayed. These data encouraged us to seek methods for the



**1** n = 2
**2** n = 3

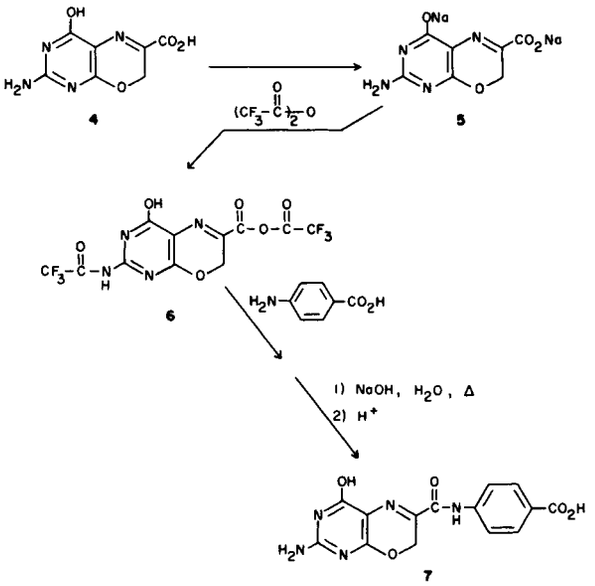


**3**

synthesis of pyrimidooxazines with varied side chains, especially those containing an aryl moiety attached by a two or three atom link to the pyrimidooxazine ring system.

A synthetic approach of condensing an α-haloketone with 2,5-diamino-4,6-dihydroxypyrimidine had sufficed for the synthesis of 2-amino-4-hydroxy-7*H*-pyrimido[4,5-*b*]-[1,4]oxazines substituted with relatively simple alkyl, phenyl, and phenylalkyl side chains (2,3). This approach was applied, also, to the synthesis of 2-amino-4-hydroxy-6-(4-carbethoxyphenylethyl)-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**3**) but failed when applied to the synthesis of derivatives with side chains containing nitrogen even though nitrogen was protected (4). Therefore, an investigation into the synthesis of pyrimido[4,5-*b*][1,4]oxazines with functional 6-substituents and their use for coupling side chains was begun (5). This study has now revealed two methods for coupling *p*-aminobenzoic acid to the pyrimidooxazine ring system which are described here.

2,5-Diamino-4,6-dihydroxypyrimidine condenses with ethyl bromopyruvate to give 2-amino-4-hydroxy-6-carbethoxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine which can be hydrolyzed to the 6-carboxy derivative **4** (5). Attempts to activate this carboxylic acid for coupling with amine derivatives were hampered initially by the insolubility of **4** in most solvents. Finally, a mixed anhydride technique applied originally to the coupling of 2-amino-4-hydroxypteridine-6-carboxylic acid with amines (6) was adapted and used successfully. Carboxylic acid **4** was reacted with an excess of 1*N* sodium hydroxide, and sodium analysis of the product indicated a disodium salt for which structure **5** was presumed. Reaction of the salt with trifluoroacetic anhydride gave intermediate **6** that was assigned a




**4** **5** **6** **7**

mixed anhydride identity and a single trifluoroacetyl protecting group by analogy to the intermediate proposed for the pteridine analog (6). The assignment of the single trifluoroacetyl protecting group also agreed with the resistance of 4-hydroxypyrimidines towards acylation (7).

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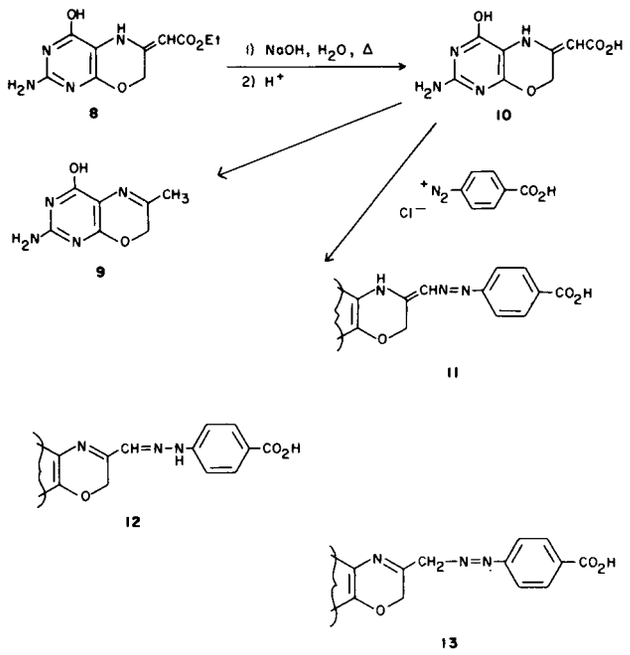
Reaction of **6** with *p*-aminobenzoic acid and careful hydrolysis of the product under basic conditions completed the synthesis. Purification was achieved through chromatography giving 9-oxo-8-oxadihydropteroic acid (**7**) (8).

Comparison of the uv curve shape and absorption maxima of **7** in 1*N* sodium hydroxide solution with the spectrum (5) of 2-amino-4-hydroxy-6-carbethoxy-7*H*-pyrimido-[4,5-*b*][1,4]oxazine revealed that the pyrimido-oxazine ring system had survived the coupling process. The pmr spectra of **7** and *p*-acetamidobenzoic acid were compared in two solvents to establish that *p*-aminobenzoic acid was bound through the nitrogen atom. The aromatic protons of **7** were represented by a singlet in dimethylsulfoxide-$d_6$ solution. This agreed with the spectrum of *p*-acetamidobenzoic acid in which the aromatic protons appeared in a highly distorted AB pattern that was about to coalesce to a single peak. In trifluoroacetic acid solution, less distorted AB patterns appeared for both substances with the shift difference between the doublets of one pattern similar to that of the other.

2-Amino-4-hydroxy-6-carbethoxymethylene-6,7-dihydro-5*H*-pyrimido[4,5-*b*][1,4]oxazine (**8**) (5) upon hydrolysis gives a product that has become one of the most interesting and useful of the recently synthesized 6-functionalized pyrimidooxazines for coupling purposes. This hydrolysis product converted easily to 2-amino-4-hydroxy-6-methyl-7*H*-pyrimido[4,5-*b*][1,4]oxazine (**9**) and was difficult to characterize (5); however, it could be inferred from analogy to **8** that in a suitable medium at least a portion would exist in the enamine form **10**. The reaction of **8** with bromine in glacial acetic acid (5), the susceptibility of enamines towards electrophilic attack (9), the rearrangement potential of **10**, and the observation that β-keto acids and β-imino acids can decarboxylate upon nitrosation (10) led to the prediction that diazotized *p*-aminobenzoic acid would react with **10** at C-9 with decarboxylation to give isomer **11** or **12**. Coupling at C-9 and decarboxylation did, infact, proceed as confirmed by the proton singlet at 7.57 ppm in the pmr spectrum in dimethylsulfoxide-$d_6$ solution and elemental analysis.




The coupled molecule could exist in a number of tautomeric and geometrical modifications. Pmr spectra did not give enough information to define the exact isomer or isomers present. The failure of the C-9 proton to exchange in the presence of deuterium oxide argued, however, against any contribution in pure dimethylsulfoxide-$d_6$ solution from a tautomer with two methylene protons such as **13** (11,12).

Color changes were observed during manipulation of the diazonium salt coupling product. When it was dissolved in dimethylsulfoxide and mixed with water, a yellow solid separated, but solution in 1*N* sodium hydroxide and strong acidification gave a red solid that upon collection and washing with water converted to yellow. In addition, substantial changes in the visible portion of the spectrum in dimethylsulfoxide solution occurred upon addition of 1*N* hydrochloric acid. Absorptions at 413 and 435 diminished and absorption appeared at 484 nm. These changes could be due to tautomeric and geometrical isomerism. An ability to isomerize easily might enhance this molecule's ability to conform to and associate with the enzyme's active site.

Of the two procedures for coupling to the pyrimido-oxazine ring system, the mixed anhydride approach is the least promising for extension to the coupling of more amino-terminated molecules. Laborius manipulation, low yield, and limited stability of **7** towards sodium hydroxide solution are hindering factors. In contrast, the diazonium salt procedure requires less manipulation, gives a considerably better yield, and should be a practical method for attaching more aryl amines with substituents stable to nitrous acid to the pyrimdooxazine ring system.

## EXPERIMENTAL

A Thomas Hoover apparatus was used for melting point determinations. A Beckman IR-33 was used for ir spectra, and some of the stronger absorptions are reported. Uv and visible spectra were obtained with a Beckman DB spectrophotometer. Pmr spectra were recorded with a Hitachi Perkin-Elmer R-24B nmr spectrometer. Solutions of tetramethylsilane in deuterochloroform were used for standardization. Microanalysis was performed by Midwest Microlab, Ltd., Indianapolis, Indiana or Galbraith Laboratories, Inc., Knoxville, Tennessee.

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

2-Amino-4-hydroxy-6-carboxy-7*H*-pyrimido[4,5-*b*][1,4]oxazine·X $H_2O$ (0.46 g.) prepared as previously described (5) was stirred with 1*N* sodium hydroxide (4.8 ml.) for 20 minutes. Then, absolute ethanol (6 ml.) was ad-

ded. After refrigerating 1 hour at 2°, the separated solid was collected, washed with portions of 95% ethanol, and oven dried at 100° for 23 hours.

*Anal.* Calcd. for $Na_2C_7H_4N_4O_4 \cdot 1.5\ H_2O$: Na, 16.36. Found: Na, 16.39.

2-Amino-4-hydroxy-*N*$^{10}$-(4-carboxyphenyl)-7*H*-pyrimido[4,5-*b*][1,4]oxazine-6-carboxamide (**7**).

a) Preparation of Intermediate **6**.

All of the dried disodium salt prepared above (powdered) was stirred, trifluoroacetic anhydride (10 ml.) was added in one portion (**caution**), and the reaction was allowed to proceed with stirring protected from moisture for 3.5 hours. Dry *N,N*-dimethylformamide (9 ml.) was added. Upon solution of the solid, dry benzene (50 ml.) was added and the solution reduced in volume *in vacuo* under anhydrous conditions below 40° to 10-15 ml. Adding benzene and evaporating to 10-15 ml. was repeated three more times.

b) Coupling and Hydrolysis.

*p*-Aminobenzoic acid (0.28 g.) was added to the concentrate and stirred 19 hours protected from moisture at room temperature. Cold water (80 ml.) was added and the mixture stirred 15 minutes. The crude solid was collected, washed with portions of water, and dried *in vacuo* at room temperature. This powdered coupling product combined with 0.2*N* sodium hydroxide (34 ml.) in a test tube was heated with steam and stirred for 7 minutes, cooled, kept in the refrigerator at 3° for 1 hour, and adjusted to a *p*H of 1-2 with 1*N* hydrochloric acid. The solid was collected, washed, and dried as before. Separation of the product was achieved in sodium salt form through chromatography on a cellulose-ECTEOLA column packed and eluted with 0.1*N* sodium hydroxide. The product fraction was adjusted to a *p*H of 1-2 with 1*N* hydrochloric acid, refrigerated at 3° for 1 hour, and the solid collected. Washing with water portions and *in vacuo* drying at room temperature and with heat gave the product, yield 0.06 g. Heating a capillary from room temperature did not give a definitive decomposition point; ir (potassium bromide): 3405 m, 3115 m, 1668 s, 1591 m, 1506 s, 1273 m, 1237 m, $cm^{-1}$; uv (1*N* sodium hydroxide): 400-230 range, spectrum changes with time, 252, 274 (shoulder), 368 nm; pmr (DMSO-$d_6$): 4.98 ppm (singlet, 2H), 7.10 (broadened singlet, 2H), 7.76 (singlet, 4H), 10.16 (broadened singlet, 1H), 10.88 (broad band, 1 or 2H), protons at 7.10, 10.16 and 10.88 exchanged with deuterium oxide; pmr (trifluoroacetic acid): 5.07 ppm (singlet, 2H), 7.22 (distorted doublet, 2H), 7.69 (distorted doublet partially overlapped by broad band at 7.82, approx. 4H total), 9.16 (broadened singlet, approx. 1H). The analytical specimen was prepared by chromatographing the product fraction from the first column on a second cellulose-ECTEOLA column.

*Anal.* Calcd. for $C_{14}H_{11}N_5O_5 \cdot 0.75\ H_2O$: C, 49.05; H, 3.68; N, 20.43. Found: C, 48.93; H, 3.67; N, 20.44.

Coupling Diazotized *p*-Aminobenzoic Acid with Hydrolyzed 2-Amino-4-hydroxy-6-carbethoxymethylene-6,7-dihydro-5*H*-pyrimido[4,5-*b*][1,4]oxazine.

Sodium nitrite (0.16 g.) dissolved in water (2.7 ml.) was added dropwise to a stirring mixture prepared by dissolving *p*-aminobenzoic acid (0.29 g.) in 1*N* hydrochloric acid (6.4 ml.) and cooling to 0°. Temperature was kept between 0 and 5° during addition. After 5 minutes more stirring and cooling, the diazonium salt solution at 1° was transferred in portions that were added dropwise before they could warm significantly to a stirring suspension of hydrolysis product (0.48 g.) and glacial acetic acid (12.2 ml.) at room temperature. The hydrolysis product had been prepared from ester **8** as previously described (5). After a 25 minute stirring period, the suspended solid was collected and washed with portions of water. It was dissolved in 1*N* sodium hydroxide (25 ml.) and the solution filtered and adjusted to a *p*H of 0-1 with 1*N* hydrochloric acid. Collection, washing with water portions, and *in vacuo* drying at room temperature and 100° gave the coupling product, yield 0.36 g. A definitive decomposition point was not obtained; ir (potassium bromide): 3287 m, 3087 m, 1668 s, 1646 s, 1603 s, 1552 s, 1505 m-s, 1260 s, 1160 s, 1098 m, $cm^{-1}$; uv (DMSO): 740-272 range, 315, 413, 435 nm; uv (DMSO and drop 1*N* hydrochloric acid): 309, 414, 436, 484 nm; pmr (DMSO-$d_6$): 5.04 ppm (singlet, 2H), 7.02 (distorted doublet partially overlapped with broadened singlet at 6.89, 4H total), 7.73 (distorted doublet partially overlapped by singlet at 7.57, 3H total), 11.02 (broadened singlet, approx. 1H), remaining protons were difficult to discern from the base line, protons at 6.89 and 11.02 exchanged with deuterium oxide.

*Anal.* Calcd. for $C_{14}H_{12}N_6O_4 \cdot 0.75\ H_2O$: C, 49.20; H, 3.98; N, 24.59. Found: C, 49.60; H, 4.38; N, 24.24.

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## REFERENCES AND NOTES

(1) Presented in part at the 35th Southwest Regional Meeting of the American Chemical Society, Austin, Texas, Dec. 1979.

(2) Shwu-C. Lin, G. P. Holmes, D. L. Dunn and C. G. Skinner, *J. Med. Chem.*, **22**, 741 (1979).

(3) D. L. Dunn and C. G. Skinner, *J. Org. Chem.*, **40**, 3713 (1975).

(4) D. L. Dunn, Dissertation, "Homeosteric Analogues of Folic Acid, 8-Oxadihydropteridines", *Chem. Abstr.*, **86**, 43659q (1977).

(5) M. J. Winchester, *J. Heterocyclic Chem.*, **16**, 1455 (1979).

(6) M. G. Nair and C. M. Baugh, *J. Org. Chem.*, **38**, 2185 (1973).

(7) D. J. Brown and S. F. Mason, "The Pyrimidines", Interscience Publishers, New York, N.Y., 1962, pp. 252-253.

(8) All structures throughout have been depicted as 4-hydroxy tautomers for convenience. The hydroxy forms are not necessarily favored.

(9) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *J. Am. Chem. Soc.*, **85**, 207 (1963).

(10) O. Touster, in "Organic Reactions", Vol. VII, R. Adams, Ed., John Wiley and Sons, Inc., New York, N.Y., 1953, pp. 337-338.

(11) Y. Iwanami, *Bull. Chem. Soc. Japan*, **44**, 1311 (1971).

(12) Y. Iwanami, T. Seki and T. Inagaki, *ibid.*, **44**, 1316 (1971).