[CONTRIBUTION FROM LIFE SCIENCES RESEARCH, STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIFORNIA]

Synthesis of a Pyrimidine Analog of Tetrahydrofolic Acid and Its 7,10-Methenyl Derivative[1]

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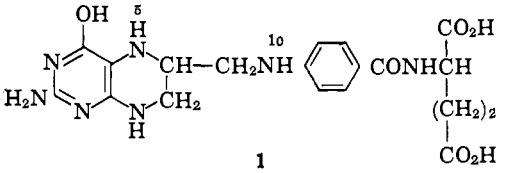
The synthesis of N-{*p*-[N-[2-(N-[2'-amino-4'-hydroxy-5'-pyrimidinyl]amino)ethyl]amino)benzoyl}-L-glutamic acid (**3**), an analog of tetrahydrofolic acid, is described. Reductive alkylation of 2,2-diethoxyacetaldehyde with dimethyl N-(*p*-aminobenzoyl)-L-glutamate afforded dimethyl N-[*p*-(2,2-diethoxyethylamino)benzoyl]-L-glutamate. Formylation with formic–acetic anhydride in pyridine followed by careful hydrolysis of the acetal afforded an aldehyde which was reductively alkylated wi h 2-acetamido-5-amino-4-hydroxypyrimidine. Formylation of the product afforded the crystalline dimethyl N-{*p*-[N-(2-[N-(2'-acetamido-4'-hydroxy-5'-pyrimidinyl)formamido]ethyl)formamido]benzoyl}-L-glutamate (**9**). A two-step hydrolysis with methanolic and aqueous hydrochloric acid afforded **3**. However, treatment with aqueous hydrochloric acid alone removed all blocking groups except one formyl group and caused cyclization to a methenyl derivative (**2b**) whose chemistry paralleled that of N^5,N^{10}-methenyltetrahydrofolic acid in many respects. Both **2b** and **3** are striking inhibitors of *Streptococcus faecalis*, with **3** being the more potent.

The vitamin, folic acid, generally as its tetrahydro derivative (fH_4, **1**), serves as a one-carbon transfer agent in a variety of biological systems. In these transfers, at either the formyl or the hydroxymethyl oxidation level, five-membered cyclic compounds involving the one-carbon fragment and the N^5 and N^{10} atoms of fH_4 are important intermediates.[2] Thus N^5,-N^{10}-methenyltetrahydrofolate (**2a**) serves as the cyclic intermediate in a number of one-carbon transfers at the formyl level.

As part of a continuing program in folic acid antagonists,[3] we became interested in preparing an analog of fH_4 which lacked the tetrahydropyrazine ring but otherwise contained the elements necessary for one-carbon transfer that are present in fH_4. Compound **3** fulfills these requirements and, as a consequence of its structure, possesses less rigidity and one less assymmetric carbon atom than fH_4. This paper reports the synthesis of **3**, its methenyl derivative **2b**, and some observations on their chemistry (see Chart I).

Synthesis of **3** by using mild reductive alkylation conditions to join together the properly blocked fragments of isocytosine, glyoxal, and *p*-aminobenzoyl-L-glutamic acid was an attractive approach. Reductive alkylation of dimethyl *p*-aminobenzoyl-L-glutamate[4] and glyoxal semiacetal[5] with hydrogen over palladium-on-charcoal afforded **4** as a homogeneous and analytically pure sirup in high yield. Several attempts to formylate **4** in

CHART I



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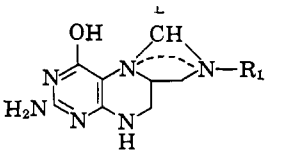
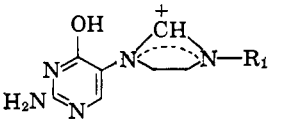
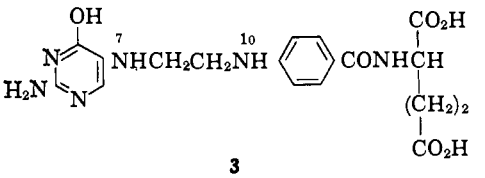


2a, $R_1 = C_6H_4CONHCH(CO_2H)(CH_2)_2CO_2H$



2b, $R_1 = C_6H_4CONHCH(CO_2H)(CH_2)_2CO_2H$



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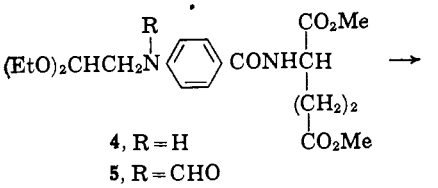
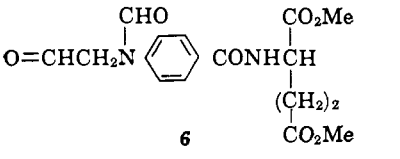


4, R = H
5, R = CHO



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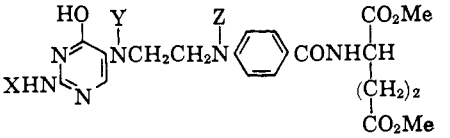


7, X = Ac, Y = H, Z = CHO
8, X = Ac, Y = H, Z = H
9, X = Ac, Y = CHO, Z = CHO
10, X = H, Y = H, Z = H

(1) (a) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH43-64-500. The opinions expressed are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. (b) Presented in part at the Fourth Annual Meeting-in-Miniature of the California Section, American Chemical Society, Berkeley, Calif., Dec. 18, 1963.

(2) For recent reviews of folic acid metabolism see (a) M. Friedkin in "Annual Reviews of Biochemistry," Vol. 32, E. E. Snell, J. M. Luck, F. W. Allen, and G. MacKinney, Ed., Annual Reviews, Inc., Palo Alto, Calif., 1963, p. 185; (b) J. C. Rabinowitz in "The Enzymes," Vol. 2, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, Inc., New York, N. Y., 1960, p. 185. For the chemistry of N^5,N^{10}-methenyltetrahydrofolic acid, see (c) D. B. Cosculich, B. Roth, J. M. Smith, Jr., M. E. Hultquist, and R. P. Parker, *J. Am. Chem. Soc.*, **74**, 3252 (1952), and (d) M. May, T. J. Bardos, F. L. Barger, M. Lansford, J. M. Ravel, G. L. Sutherland, and W. Shive, *ibid.*, **73**, 3067 (1951).

(3) For preceding papers see (a) L. Goodman, J. DeGraw, R. L. Kisliuk, M. L. Friedkin, E. J. Pastore, E. J. Crawford, L. T. Plante, A. Al-Nahas, J. F. Morningstar, Jr., G. Kwok, L. Wilson, E. F. Donovan, and J. Ratzan, *ibid.*, **86**, 308 (1964); (b) J. DeGraw, L. Goodman, B. Weinstein, and B. R. Baker, *J. Org. Chem.*, **27**, 576 (1962).

(4) R. Koehler, L. Goodman, J. DeGraw, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5779 (1958).

(5) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **18**, 514 (1935). This procedure for glyoxal semiacetal (2,2-diethoxyacetaldehyde) was slightly modified to use the more convenient reagent, sodium periodate, rather than lead tetraacetate.

acidic media gave incomplete formylation accompanied by hydrolysis of the acetal as indicated by changes in the ultraviolet and infrared spectra. Finally, formylation without acetal hydrolysis was achieved by treating a pyridine solution of **4** with preformed formic–acetic anhydride[6] at 50°. The product, **5,** was a sirup, homogeneous on paper and possessing satisfactory spectral properties.

Hydrolysis of the acetal **5** to the aldehyde **6** without concomitant loss of the formyl group proved exceedingly difficult. The N-formyl group was very labile once the acetal was hydrolyzed, probably because of the close proximity of the aldehyde and formamido groups.[7] After considerable experimentation, it was found that use of 98% formic acid permitted hydrolysis of the acetal **5** with minimum loss of the formyl group to give the best yields of **6.** All attempts to prepare a crystalline derivative of **6** failed.

Reductive alkylation of the aldehyde **6** with 2-acetamido-5-amino-4-hydroxypyrimidine (**17**) (see Chart III) in N,N-dimethylformamide over hydrogen and palladium-on-charcoal gave a solid with a broad melting range. The ultraviolet spectrum of this had a peak at 298 mμ, suggesting that it was not the expected **7** but rather **8,** or some mixture of the two.

A crystalline, readily purified intermediate, **9,** was obtained by reformylating the product obtained from the reductive alkylation of the aldehyde **6** and the aminopyrimidine **17.** This diformyl derivative **9** was partially deblocked to the dimethyl ester **10** by reaction with 1 *N* methanolic hydrochloric acid at room temperature for 2 or 3 days.[8] The ester **10** was hydrolyzed to the desired acid, **3,** by heating in 12 *N* hydrochloric acid for 90 minutes at 37°.[9] Compound **3,** initially obtained as the trihydrochloride, could be recrystallized from water to give **3** as a hydrate in analytical purity. However, this form was less stable and slowly turned pink even when stored in the dark. It was best analyzed and stored as the trihydrochloride, **3**·(3HCl). The ultraviolet spectra of **3** is given in Fig. 1.

Interestingly, the direct hydrolysis of the diformamido ester **9** to **3** by heating with 12 *N* hydrochloric acid at 37° for 90 min. failed; all the blocking groups were removed except one formyl group. The product was identified as the N^7,N^{10}-methenyl derivative (**2b**) of **3** on the basis of analysis and ultraviolet spectral changes. The crystalline methenyl derivative of **3,** like that of fH_4,[2c] could be obtained with different amounts of water and hydrogen chloride, depending on the method of drying.

The cyclic methenyl compounds derived from fH_4 (**2a**) and from **3** (**2b**) show strikingly parallel behavior with changes in acidity as outlined in Chart II. The transformations for the fH_4 series,[2c,d] have been summarized by Rabinowitz.[2b] Briefly, both the N^5- (**11a**) or the N^{10}-formyl derivative (**12a**) of fH_4 are cyclized to the methenyl compound (**2a**) (ultraviolet maximum 355 mμ in 0.1 *N* acid) by acid, with the conversion from the N^{10}-formyl **12a** (maximum 253 mμ in 0.1 *N* base) being more rapid. In neutral or slightly alkaline solution, **2a** is hydrolyzed to the N^{10}-formyl

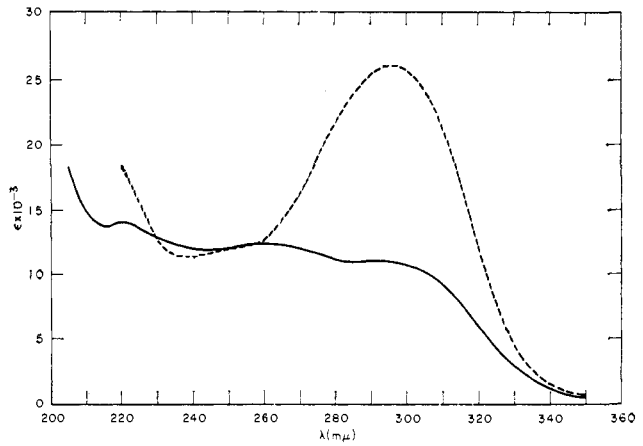
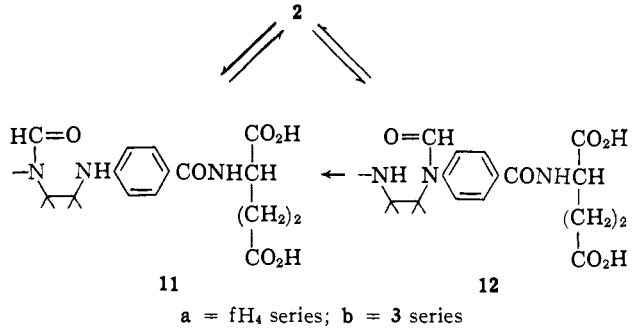


Fig. 1.—Ultraviolet absorption spectra of N-[*p*-(N-[2-(N-[2′-amino-4′-hydroxy-5′-pyrimidyl]amino)ethyl]amino)benzoyl]-L-glutamic acid (**3**): ——— pH 1; – – – – pH 13.

CHART II



a = fH_4 series; b = **3** series

derivative **12a** which changes to the more stable N^5-formyl derivative **11a** (maximum 282 mμ in 0.1 *N* base) upon standing in alkaline solution.

As expected, the maximum for the methenyl derivative **2b** in 0.1 *N* hydrochloric acid occurred at a longer wave length than that of **3** (compare Fig. 1 and 2). When **2b** is dissolved in 0.1 *N* base, the maximum appears at 253 mμ, suggesting immediate cleavage of **2b** to the N^{10}-formyl derivative **12b.** On standing, the maximum at 253 mμ gradually disappears and is replaced by one at 286 mμ, as expected for the N^7-formyl derivative **11b** (see Fig. 2). Acidification of these 0.1 *N* sodium hydroxide solutions that had been standing 9 min. (mostly N^{10}-formyl) or 23 hr. (mostly N^7-formyl) caused recyclization to **2b** as indicated by reappearance of its maximum.[10] The recyclization of the N^{10}-formyl was less rapid than that of the N^7-formyl as shown in Table I.

The shift of the formyl group from N^{10} to N^7 demonstrates how closely **3** mimics fH_4. Such shifts from one nitrogen to the other could not be observed in the earlier described model studies[2d,12] that employed N-formyl

(6) J. Žemlička, J. Beránek, and J. Smrt, *Collection Czech. Chem. Commun.*, **27,** 2784 (1962), have used formic–acetic anhydride in pyridine solutions for O-formylations.

(7) In our work (unpublished) toward the synthesis of the next higher homolog of **8,** it was observed that when one more carbon atom is inserted between the acetal and formamido functions of **5,** the formyl group is not lost as readily during acetal hydrolysis.

(8) J. C. Sheenan and D. H. Yang, *J. Am. Chem. Soc.*, **80,** 1154 (1958).

(9) R. B. Merrifield and D. W. Woolley, *ibid.*, **78,** 4646 (1956).

(10) In the recyclizations to **2b,** the maximum appears at 313 mμ rather than at 317 mμ as observed for spectrum taken immediately in acid (see Fig. 2). This 317 mμ maximum of **2b** shifts gradually to about 313 mμ when the acid solutions were left standing a few hours. Similar slight shifts have been observed for (a) various isomeric forms of **2a**[11] and (b) **2a** at different acidities (pH 0.1 to 3).[2b]

(11) F. M. Huennekens and M. J. Osborn in "Advances in Enzymology," Vol. 21, F. F. Nord, Ed., Interscience Publishers, Inc., New York, N. Y., 1959, p. 369.

(12) L. Jaenicke and E. Brode, *Ann.*, **624,** 120 (1959).

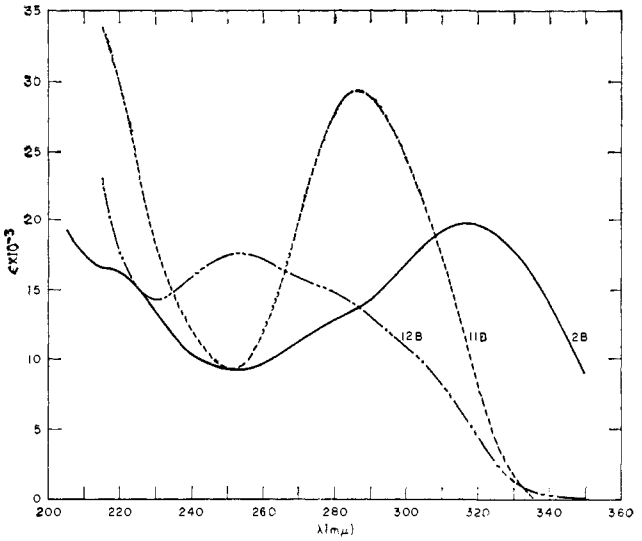


Fig. 2.—Ultraviolet absorption spectra of **2b** and its interconversion products: ——— **2b** in 0.1 *N* HCl; – – – – – **12b** from **2b** in 0.1 *N* NaOH, spectra taken immediately; – – – – **11b** from **2b** in 0.1 *N* NaOH, spectra after 22 hr.

derivatives of symmetrical N,N′-diarylethylenediamines to demonstrate the ready formation of cyclic methenyl derivatives. Both **3** and **2b** are undergoing biological

TABLE I

RATE OF REFORMATION OF **2b** UPON ACIDIFICATION OF BASIC SOLUTIONS

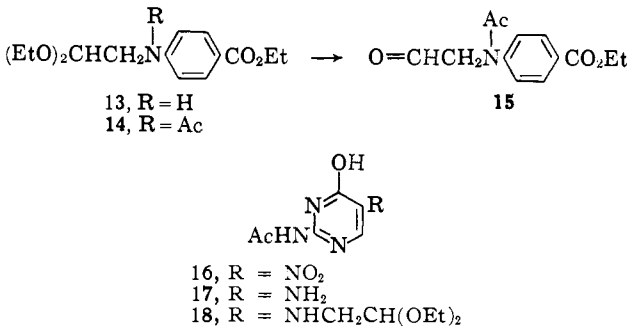
Time,[a] min.	Absorption, ε × 10⁻³, at 313 mμ — Solution B	Solution C
1	10.7	17.4
23	16.1	
31		18.1
71	17.6	18.3
121		17.8
179	17.8	

[a] Solution A was prepared by dissolving **2b** in 0.1 *N* sodium hydroxide solution. After solution A had stood for 9 min. and 23 hr., respectively, aliquots were acidified to give solutions B and C, respectively. The ultraviolet spectra of solutions B and C were determined at intervals.

testing in other laboratories. Against *Streptococcus faecalis* they are both striking inhibitors (about as good as aminopterin). They also inhibit the growth of *Lactobacillus casei*, with **3** being three times as potent as **2b** for both these organisms. **3** Is also more potent than **2b** as an inhibitor of thymidylate synthetase (3×10^{-5} and 7×10^{-4} *M* are required for half-maximal inhibition, respectively). They are equal in their ability to inhibit dihydrofolate reductase (4×10^{-5} *M* required for half-maximal inhibition).[13]

Preliminary experiments with ethyl *p*-aminobenzoate established the conditions for reductive alkylation with glyoxal semiacetal that were employed for dimethyl N-(*p*-aminobenzoyl)-L-glutamate also. Thus the preferred catalyst for the reductive alkylations with glyoxal semiacetal was palladium-on-charcoal. There was no advantage in first isolating the Schiff's base before hydrogenation.[14] The reductive alkylation of ethyl *p*-aminobenzoate and glyoxal semiacetal gave a liquid acetal, **13**, that was homogeneous. However, **13** could not be converted to a crystalline derivative except by acetylation to the sirup **14** followed by acetal hydrolysis to the crystalline acetamidoacetaldehyde **15**, in 34% over-all yield from ethyl *p*-aminobenzoate. Conceivably, **13** could be transformed by a number of steps to **3**.

CHART III



16, R = NO_2
17, R = NH_2
18, R = $NHCH_2CH(OEt)_2$

Nitration of isocytosine by a modification of the literature procedure[15] at or below room temperature afforded 5-nitroisocytosine. Acetylation gave **16**; catalytic hydrogenation then afforded the amine **17**. This was found to undergo reductive alkylation, although more slowly than ethyl *p*-aminobenzoate or dimethyl N-(*p*-aminobenzoyl)-L-glutamate, with glyoxal semiacetal. Heating the reaction mixture to 65–70° was necessary in the preparation of the acetal **18**. From this experiment it was anticipated that the reductive alkylation of **6** and **17** to give **7** was feasible. Conceivably, the formation of **3** from **18** was also feasible, but this order of joining the fragments of **3** was not pursued.

Experimental[16]

Dimethyl N-[*p*-(2,2-Diethoxyethylamino)benzoyl]-L-glutamate (4).—A mixture of 16.0 g. (54.3 mmoles) of dimethyl *p*-aminobenzoyl-L-glutamate,[4] 9.10 g. (68 mmoles) of glyoxal semiacetal,[5] and 8.0 g. of 5% palladium-on-carbon in 350 ml. of absolute ethanol was stirred under hydrogen at 1 atm. and room temperature for 15 hr., by which time 1.2 molar equiv. of hydrogen had been absorbed. The catalyst was removed and the solution was evaporated *in vacuo* to a sirup. This was dissolved in 300 ml. of benzene. The solution was washed with 100 ml. of 10% sodium bisulfite solution and two 100-ml. portions of water, dried, filtered, and evaporated *in vacuo* to afford 21.9 g. (99%) of **4** as a pale yellow sirup; $\lambda_{max}^{film}(\mu)$ 2.96 (NH), 5.72, 6.08 (ester, amide C=O), and 9.40 (C—O—C); $\lambda_{max}^{EtOH}(m\mu)$ 215 shoulder (ε 11,200), 298 (ε 20,900). It moved as a single spot, distinguishable from starting material, in solvents A with R_f 0.66 and B with R_f 0.76.

Anal. Calcd. for $C_{20}H_{30}N_2O_7$: C, 58.5; H, 7.31; N, 6.81. Found: C, 58.6; H, 7.48; N, 6.83.

Dimethyl N-{*p*-[N-(2,2-Diethoxyethyl)formamido]benzoyl}-L-glutamate (5).—To 25 ml. (0.27 mole) of cold (0°), stirred acetic anhydride was added slowly 10.4 ml. (0.27 mole) of 97–100% formic acid. The solution was allowed to warm to room temperature and then heated at 50–55° for 15 min. The solution of formic–acetic anhydride was cooled to 0°, and then added slowly with stirring to a cold (0°) solution of 10.6 g. (26 mmoles) of **4** in

(13) We express our appreciation to Drs. R. L. Kisliuk and M. L. Friedkin, Department of Pharmacology, Tufts University School of Medicine, who obtained the microbiological and enzymatic data mentioned here.

(14) J. I. DeGraw, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1156 (1961), found it advantageous to isolate the anil in their synthesis of *p*-[2-acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methylamino]benzoic acid.

(15) T. B. Johnson and C. O. Johns, *Am. Chem. J.*, **34**, 554 (1905), noted the violent reaction in their nitration of isocytosine.

(16) Melting points were determined with the Fisher-Johns apparatus and are uncorrected. Paper chromatography was done by the descending technique on Whatman No. 1 paper and the spots were detected by visual examination under ultraviolet light. When adenine was used as a standard, the spots were located relative to R_{Ad} 1.00. The solvent systems were: (A) 5% aqueous disodium hydrogen phosphate, pH 8.9; (B) water; (C) 1-butanol–acetic acid–water (5:2:3); (D) 2-methoxyethanol–water (9:1); (E) benzene–methanol–water (2:6:1); and (F) same as E except on Schleicher and Schuell No. 2496 acetylated paper. In all experiments, anhydrous magnesium sulfate was used as the drying agent.

75 ml. of dry pyridine. The mixture was allowed to warm to room temperature and then heated at 50–55° for 1 hr. After evaporation *in vacuo* at 35–40°, the residue was dissolved in 75 ml. of toluene and re-evaporated, this procedure being repeated once with toluene and twice with methanol to afford 10.7 g. (95%) of **5** as an orange-yellow sirup; $\lambda_{max}^{film}(\mu)$ 3.00 (NH), 5.72, 5.92, 6.00 (esters, amides), and 9.42 (C–O–C); $\lambda_{max}^{EtOH}(m\mu)$ 260 (ϵ 15,200). It moved as a single spot in solvent B with R_f 0.89. A sample of sirup was dried at 56° (0.1 mm.) for 2 hr. and analyzed.

Anal. Calcd. for $C_{21}H_{30}N_2O_8$: N, 6.38. Found: N, 6.53.

An identical product was obtained when the reaction of **4** with formic–acetic anhydride in pyridine was run overnight at room temperature.

Dimethyl N-{*p*-[N-(Formylmethyl)formamido]benzoyl}-L-glutamate (6).—A solution of 10.6 g. (24.1 mmoles) of the acetal **5** in 60 ml. of 97–100% formic acid was allowed to stand at room temperature for 15 min. and then evaporated *in vacuo* at 25–35° to leave 11.3 g. (128%) of **6** as an orange-red sirup; $\lambda_{max}^{film}(\mu)$ 2.98 (NH), 5.72, 5.95, 6.00 (C=O of esters, aldehyde, and amides), and loss of absorption at 9.40 (acetal); $\lambda_{max}^{EtOH}(m\mu)$ 262 (ϵ 13,200) and 303 shoulder (ϵ 3800); ϵ-values were corrected for residual solvent. This product was generally used immediately after preparation. No crystalline derivative of **6** could be prepared.

Other hydrolysis conditions were less satisfactory. Treatment for 15 min. with 95% instead of 97–100% formic acid caused more loss of N-formyl as indicated by increased absorption at 303 mμ.

Dimethyl N-{*p*-[N-(2-{N-[2'-Acetamido-4'-hydroxy-5'-pyrimidinyl]amino}ethyl)formamido]benzoyl}-L-glutamate (7).—The reductive alkylation by the general procedure (see preparation of **4**) of a mixture of 3.51 g. (20.9 mmoles) of the aminopyrimidine **17**, 8.73 g. (24 mmoles) of the aldehyde **6**, and 1.76 g. of 5% palladium-on-charcoal in 210 ml. of distilled N,N-dimethylformamide required 18 hr. (0.85 molar equiv. of hydrogen absorbed). After the work-up using chloroform instead of benzene, there was obtained 10.66 g. of foam. This was redissolved in 25 ml. of chloroform and added dropwise with stirring to 550 ml. of ether. The precipitate was triturated overnight to afford 9.21 g. (85%) of **7** as a gray powder, m.p. 86–95°; $\lambda_{max}^{Nujol}(\mu)$ 3.0–3.12 (NH), 5.73 and 6.0 (C=O of amides, esters); $\lambda_{max}^{EtOH}(m\mu)$ 223 shoulder (ϵ 19,400), 275 (ϵ 17,400), and 306 shoulder (ϵ 12,700). It moved as a single spot in solvents C and D with R_{Ad} 1.30 and 1.45, respectively. Compound **7** of this quality is used in the preparation of **9**. The height of the shoulder at 306 mμ is indicative of the amount of N-formyl loss. While **7** was a solid, it could not be purified by recrystallization; the reformylated product **9** was more tractable.[17]

Dimethyl N-{*p*-[N-(2-[N-(2'-Acetamido-4'-hydroxy-5'-pyrimidinyl)formamido]ethyl)formamido]benzoyl}-L-glutamate (9).—A solution of 1.85 g. (3.57 mmoles) of **7** in 15 ml. of dry pyridine was treated with formic–acetic anhydride, by the procedure used to prepare **5**, to obtain 1.19 g. (61%) of **9** as a yellowish tan powder, m.p. 201–204° (softens at 195°), homogeneous on paper chromatograms. A sample from an earlier run was recrystallized from 1,2-dimethoxyethane to afford the analytical sample of **9**, m.p. 205.5–207°; $\lambda_{max}^{Nujol}(\mu)$ 3.05 (NH), 5.71, 5.90, and 6.00 (C=O of esters, amides); $\lambda_{max}^{EtOH}(m\mu)$ 255 (ϵ 19,900) and 304 shoulder (ϵ 8100). It moved as a single spot in solvents C and D with R_{Ad} 1.28 and 1.50, respectively.

Anal. Calcd. for $C_{24}H_{28}N_6O_9$: C, 52.9; H, 5.14; N, 15.42. Found: C, 52.9; H, 5.18; N, 15.29.

Dimethyl N-{*p*-[N-(2-[N-(2'-Amino-4'-hydroxy-5'-pyrimidinyl)-amino]ethyl)amino]benzoyl}-L-glutamate (10).—A suspension of 1.23 g. (2.27 mmoles) of the diformamide **9** in a solution containing 3.7 ml. of 12 *N* hydrochloric acid and 40 ml. of absolute methanol was stirred at room temperature for 65 hr.; complete solution was attained in 0.5 hr. The solution, after treatment with Norit, was evaporated *in vacuo* to a residue which was twice redissolved in 50 ml. of methanol and re-evaporated. The residue was dissolved in 15 ml. of hot methanol, diluted with 20 ml. of hot benzene, and allowed to cool. The precipitate was collected, washed with methanol–benzene (3:4), and dried to afford 0.77 g. (60%) of **10** as a white crystalline powder, m.p. 132–154°; $\lambda_{max}^{Nujol}(\mu)$ 3.03 (NH), 3.6–4.2 (NH^+), 5.70 (C=O, ester), 5.90, and 6.05 (C=O of amide, pyrimidine); $\lambda_{max}^{EtOH}(m\mu)$ 215 shoulder (ϵ 19,200), 301 (ϵ 29,200); $\lambda_{max}^{pH1}(m\mu)$ 216 shoulder (ϵ 14,300), 265 (ϵ 12,800), and 296 (ϵ 12,700); $\lambda_{max}^{pH\ 13}(m\mu)$ 296 (ϵ 25,600). It moved as a single spot in solvents A, C, and E with R_{Ad} = 1.49 (fluorescent), 1.23, and 1.44, respectively. A sample dried at 25° (0.1 mm.) for 17 hr. over phosphorus pentoxide was analyzed.

Anal. Calcd. for $C_{20}H_{26}N_6O_6 \cdot 2HCl \cdot 0.5C_6H_6 \cdot CH_3OH$ (564.5): C, 50.0; H, 5.55; Cl, 12.58; N, 14.9. Found: C, 50.0; H, 6.19; Cl, 12.26; N, 14.8.

N-{*p*-[N^{10}-(2-[N^7-(2'-Amino-4'-hydroxy-5'-pyrimidinyl)amino]-ethyl)amino]-[N^7,N^{10}-methenyl]benzoyl}-L-glutamic Acid Chloride Hydrochloride Hydrate (2b; Cl·HCl·H$_2$O).—A solution of 2.00 g. (3.68 mmoles) of the diformamide **9** in 50 ml. of 12 *N* hydrochloric acid was kept under nitrogen at 37° for 1.5 hr., then evaporated *in vacuo* at 25° below 0.1 mm. to a yellow foam. This was dissolved in 10 ml. of 6 *N* hydrochloric acid; crystalline product immediately began to precipitate. After storing at 5° overnight, the crystals were collected, washed with two 2-ml. portions of cold 6 *N* hydrochloric acid, and dried at 25° below 0.1 mm. to afford 1.03 g. (54%) of product as yellowish green crystals. A 0.99-g. portion was dissolved in 5 ml. of 12 *N* hydrochloric acid, diluted with 5 ml. of water, and cooled overnight. The crystals were collected, washed as before, and dried at 25° below 1 mm. for 6 hr. over phosphorus pentoxide to afford 0.70 g. (37%) of greenish yellow crystals of product, m.p. 207–213° dec.; λ_{max}^{Nujol} (μ) 3.6–4.2 (NH^+, CO_2H), 5.73, 5.87 (C=O, acid), 6.00 (C=O, amide), 6.15, 6.27 (aryl), 6.45 (amide), and 11.75 (aryl) (for ultraviolet spectrum, see Fig. 2); it gave two spots in solvents A, R_{Ad} 0.0 (white) and 2.28 (deep blue), and C, R_{Ad} 0.0 (white) and 0.82 (white). The compound was analyzed at two different laboratories.

Anal. Calcd. for $C_{19}H_{21}ClN_6O_6 \cdot HCl \cdot H_2O$: C, 43.9; H, 4.65; Cl, 13.7; N, 16.2. Found: C, 44.06, 43.85; H, 4.61, 4.72; Cl, 13.5, 13.1; N, 16.0, 16.0.

Redrying of the sample was necessary between weighings. The effect of drying *in vacuo* (below 1 mm.) over phosphorus pentoxide under different conditions was studied with product from an earlier run. *Anal.* Calcd. for $C_{19}H_{21}ClN_6O_6 \cdot 0.9HCl$: C, 45.9; H, 4.46; Cl, 13.5; N, 16.9. Found (for 4 hr. at 56°): C, 43.6; H, 4.68; Cl, 13.8; N, 15.9. Found (for 6 hr. at 100°): C, 45.6; H, 4.54; Cl, 13.5; N, 16.7.

N-{*p*-[N-(2-[N-(2'-Amino-4'-hydroxy-5'-pyrimidinyl)amino]-ethyl)amino]benzoyl}-L-glutamic Acid (3).—A solution of 0.51 g. (0.98 mmole) of the amino ester **10** in 15 ml. of 12 *N* hydrochloric acid was heated at 37° under a nitrogen atmosphere for 90 min., then evaporated *in vacuo* at 25° below 1 mm. to afford 0.47 g. (91%) of analytically pure **3**·3HCl, m.p. 156–165° dec. (softens at 150°); $\lambda_{max}^{Nujol}(\mu)$ 2.97 (NH) 3.65–4.15 (NH^+, CO_2H), 5.80 and 6.01 (C=O, acid and amide); ultraviolet spectrum is shown in Fig. 1; $[\alpha]^{23}D$ −8.7 ± 1.0° (*c* 0.99, 1 *N* HCl). It moved as a single blue fluorescent spot in solvent E with R_{Ad} 1.00; in solvent A, it had R_{Ad} 0.00 and 1.97, both blue fluorescence.

Anal. Calcd. for $C_{18}H_{22}N_6O_6 \cdot 3HCl$: C, 40.9; H, 4.77; Cl, 20.2; N, 15.9. Found: C, 40.7; H, 4.82; Cl, 20.1; N, 15.6.

A sample (0.265 g.) of **3**·3HCl was recrystallized from 10 ml. of water to afford 0.166 g. of the hemihydrate, **3**·0.5H$_2$O, m.p. 192.5–196.5° (color change from white to pink, *ca.* 140–190°; to yellow, 195.5–196.5°); $\lambda_{max}^{Nujol}(\mu)$ 2.95 (NH), 3.5–4.2 (CO_2H; much less absorption than the HCl salt above), 5.80 (C=O, acid), and 6.20 (aryl); ultraviolet spectrum, same as before. It moved as a single blue fluorescent spot in solvent E with R_{Ad} 1.03; as a blue fluorescent spot with a tail in solvents A and C with R_{Ad} 1.95 and 0.98, respectively.

Anal. Calcd. for $C_{18}H_{22}N_6O_6 \cdot 0.5H_2O$: C, 50.6; H, 5.41; N, 19.7. Found: C, 51.0; N, 5.60; Cl, 0.0; N, 19.4.

This hemihydrate changed from white to pink on standing in a closed vial at room temperature overnight. The trihydrochloride has been stored in the dark over calcium sulfate for several months with no apparent change. Preparation of 3·3HCl by this procedure is reliable and reproducible.

Ethyl *p*-(2,2-Diethoxyethylamino)benzoate (13).—Reductive alkylation, by the procedure used to prepare **4**, of the suspension formed by adding 1.24 g. of 5% palladium-on-charcoal to a premixed solution of 2.97 g. (22.5 mmoles) of glyoxal semiacetal and 2.48 g. (15 mmoles) of ethyl *p*-aminobenzoate in 50 ml. of absolute ethanol was complete after 25 hr. (1.07 mole equiv. uptake of hydrogen). This was worked up as for **4** to obtain 4.12 g. (98%) of **13** as a pale yellow oil; $\lambda_{max}^{film}(\mu)$ 2.95 (NH), 5.88 (C=O), 9.05, and 9.4 (C—O—C); $\lambda_{max}^{EtOH}(m\mu)$ 225 (ϵ 6960) and 304 (ϵ 23,400). It moved as one main spot in solvent F, R_f 0.50, with a trace at R_f 0.39, which corresponds to ethyl *p*-aminobenzoate.

(17) In one experiment **7** was used without reformylation; hydrolysis with base and recrystallization from acetic acid gave a small amount of **3** as the acetic acid solvate, m.p. 183–187°. *Anal.* Calcd. for $C_{18}H_{22}N_6O_6 \cdot 0.5CH_3CO_2H$: C, 50.9; H, 5.39; N, 18.73. Found: C, 50.6; H, 5.55; N, 18.72. This had the same paper chromatographic behavior and ultraviolet absorption spectrum as **3**·3HCl.

Attempts to prepare a crystalline sulfonamide or carboxamide derivative failed.

Ethyl *p*-[N-(2,2'-Diethoxyethyl)acetamido]benzoate (14).—A solution of 3.73 g. (13.3 mmoles) of **13** in 20 ml. (0.21 mole) of acetic anhydride was refluxed for 1 hr. and evaporated *in vacuo* to leave a yellow oil. A solution of this in 50 ml. of benzene was washed successively with 25-ml. portions of saturated sodium bicarbonate solution and of water (two portions), dried, treated with Norit, and evaporated *in vacuo* to leave 3.81 g. (89%) of **14** as a yellow oil; $\lambda_{max}^{film}(\mu)$ 5.81, 5.98 (ester, amide), 9.45, 9.80 (C–O–C), and no NH absorption at 3 μ; $\lambda_{max}^{EtOH}(m\mu)$ 256 (ϵ 7250). It moved as a single spot in solvent A, R_f 0.66.

Ethyl *p*-[N-(Formylmethyl)acetamido]benzoate (15).—The clear yellow solution obtained by adding 1.17 g. (3.62 mmoles) of the acetal **14** to 4 ml. of 90% formic acid was allowed to stand at room temperature for 15 min., and then slowly poured with stirring into 20 ml. of ice–water. The precipitate was extracted from the water with two 25-ml. portions of benzene. The benzene solution was washed successively with two 25-ml. portions each of saturated sodium bicarbonate solution and water, dried, and evaporated *in vacuo* to afford 0.82 g. (91%) of a clear yellow oil which solidified to a waxy solid. Recrystallization from ether afforded 0.36 g. (40%) of **9**, m.p. 80.5–83.5°. A second recrystallization from ether afforded the analytical sample of **9**, m.p. 82.5–83.5.°; $\lambda_{max}^{Nujol}(\mu)$ 5.80, 5.84, and 5.98 (ester, aldehyde, and amide C=O); $\lambda_{max}^{EtOH}(m\mu)$ 256 (ϵ 8850). It moved as a single spot with R_f 0.68 in solvent F.

Anal. Calcd. for $C_{13}H_{15}NO_4$: C, 62.6; H, 6.06; N, 5.62. Found: C, 62.5; H, 6.11; N, 5.47, 5.72.

From crude **15** was obtained a 66% yield of the *p*-nitrophenylhydrazone, m.p. 181–187°. A double recrystallization from ethanol gave the hydrazone as pale orange crystals, m.p. 189–192.5° (softens at 180°); $\lambda_{max}^{Nujol}(\mu)$ 3.10 (NH), 5.80 (ester), 6.25, 6.65 (C=N, aryl), and 7.53 (NO_2).

Anal. Calcd. for $C_{19}H_{20}N_4O_5$: C, 59.3; H, 5.24; N, 14.6. Found: C, 59.0; H, 5.31; N, 14.9.

2-Acetamido-4-hydroxy-5-nitropyrimidine (16).—A stirred suspension of 10.1 g. (65 mmoles) of 2-amino-4-hydroxy-5-nitropyrimidine (5-nitroisocytosine)[15] in 100 ml. of acetic anhydride was heated at reflux for 4 hr., then kept overnight at 5°. The precipitate was collected and washed with ether to afford 12.3 g. (95%) of tan crystalline **16**, m.p. 289–295° dec. Recrystallization from N,N-dimethylformamide afforded analytically pure **16**, white needles, m.p. 294–296° dec.; $\lambda_{max}^{Nujol}(\mu)$ 3.20 (NH), 5.85 (C=O), 6.35 and 7.5 (NO_2). It moved as a single spot with R_f 0.57 (starting material, R_f 0.41) in solvent A.

Anal. Calcd. for $C_6H_6N_4O_4$: C, 36.4; H, 3.03; N, 28.3. Found: C, 36.4; H, 2.93; N, 28.1.

2-Acetamido-5-amino-4-hydroxypyrimidine (17).—A suspension of 6.10 g. (30.8 mmoles) of the nitropyrimidine **16** and 0.61 g. of 5% palladium-on-charcoal in 300 ml. of N,N-dimethylformamide was stirred under hydrogen at 1 atm., room temperature, until 3 molar equiv. of hydrogen was absorbed (about 3.5 hr.). The catalyst was removed by filtration and the clear, yellow-brown filtrate was evaporated to dryness at 55° (0.1 mm.). The residue was triturated and washed with ether (three portions totaling 100 ml.) to afford 5.20 g. (100%) of **17** as a light brown powder m.p. 232.5–236.5° dec., resolidifying at 240° and not remelting at 300°. Recrystallization from N,N-dimethylformamide afforded the analytical sample of **17**, m.p. 230–234°, resolidifying at 236°, not melting at 300°; $\lambda_{max}^{Nujol}(\mu)$ 3.15 (NH), 6.0 and 6.15 (C=O). It gave one main spot with a trace of a tail in solvent A with R_f 0.68, C with R_f 0.61, and B with R_f 0.78.

Anal. Calcd. for $C_6H_8N_4O_2$: C, 42.9; H, 4.80; N, 33.4. Found: C, 43.1; H, 4.73; N, 33.0.

2-Acetamido-4-hydroxy-5-[2',2'-(diethoxy)ethylamino]pyrimidine (18).—The reductive alkylation, by the procedure for **4**, of a suspension of 3.36 g. (20 mmoles) of the aminopyrimidine **17**, 3.96 g. (30 mmoles) of glyoxal semiacetal, and 1.68 g. of 5% palladium-on-charcoal in 50 ml. of dry N,N-dimethylformamide at 65–70°, and 1 atm. required 46 hr. (hydrogen uptake 0.74 molar equiv.). Filtration followed by evaporation of the filtrate afforded a dark green gum. After work-up, using chloroform instead of benzene, there was obtained 5.54 g. (97%) of **18**, a yellowish brown solid. Slow recrystallization from methylene chloride and Skellysolve C afforded 2.63 g. (46%) of **18**, m.p. 139–142.5° (softens at 135°); $\lambda_{max}^{Nujol}(\mu)$ 2.93 (NH), 9.25 and 9.42 (C–O–C). It moved as a single spot in solvent B with R_f 0.85 and D with R_f 0.86.

Anal. Calcd. for $C_{12}H_{20}N_4O_4$: C, 50.7; H, 7.09; N, 19.7. Found: C, 50.7; H, 7.31; N, 20.1.

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