# New Synthesis of *N*-[4-[[(2-Amino-4(3*H*)-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (8-Deazafolic Acid) and the Preparation of Some 5,6,7,8-Tetrahydro Derivatives

Carroll Temple, Jr.,* C. L. Kussner, J. D. Rose, D. L. Smithers, L. L. Bennett, Jr., and J. A. Montgomery

*Southern Research Institute, Birmingham, Alabama 35255.* *Received March 9, 1981*

Previously, 8-deazafolic acid (17) was shown to be a potent inhibitor of the folate-dependent bacteria, *Streptococcus faecium* (ATCC 8043) and *Lactobacillus casei* (ATCC 7469), and to have activity against lymphoid leukemia L1210 in mice. To examine the 5,6,7,8-tetrahydro derivatives, a new synthesis of 17 was developed from 8-deaza-2,4-dichloro-6-methylpteridine. Treatment of the latter with aqueous base gave the corresponding pteridin-4(3*H*)-one, which was aminated with ammonia to give 8-deaza-6-methylpterin (9). Bromination of 9 gave mainly 8-deaza-6-(tribromomethyl)pterin, which on reaction with *p*-aminobenzoyl-L-glutamic acid resulted in the formation of the 9-oxo derivative of 17. In contrast, bromination of the 2-acetyl derivative of 9 gave mainly the corresponding 6-(bromomethyl)pterin, which was converted to 17 in 23% yield (from 9). Hydrogenation of 17 at atmospheric pressure and room temperature was unsuccessful either in a basic medium or formic acid. In trifluoroacetic acid, overreduction occurred to give a mixture containing 8-deaza-5,6,7,8-tetrahydro-6-methylpterin and the 5,6,7,8-tetrahydro derivative of 17. The latter was characterized by conversion to the methenyl analogue 21, which was also prepared by hydrogenation of the 10-formyl derivative of 17. Treatment of 21 with hydroxide gave 8-deaza-10-formyl-5,6,7,8-tetrahydrofolic acid. Compound 21 showed cytotoxicity to cultured H.Ep.-2 cells and was tested as an inhibitor of bovine dihydrofolic reductase. Lineweaver–Burk analysis indicated inhibition competitive with dihydrofolate.

The requirement for 5,6,7,8-tetrahydrofolic acid in the metabolism of one carbon units is well established.[1] The six biologically active cofactor forms of THF, with the carbon unit at varying levels of oxidation, are substrates for at least 15 enzymes. Two enzymes, 5,10-methenyltetrahydrofolate:1-amino-*N*-ribosylacetamide-5′-phosphate transformylase (EC 2.1.2.2) and 10-formyltetrahydrofolate:5-amino-1-ribosyl-4-imidazolecarboxamide-5′-phosphate transformylase (EC 2.1.2.3), are important in purine biosynthesis catalyzing reactions that result in the incorporation of carbons 8 and 2 of inosinic acid. Two other enzymes, L-serine:tetrahydrofolate 10-hydroxymethyltransferase (EC 2.1.2.1) [serine transhydroxymethylase] and methylenetetrahydrofolate:deoxyuridine-5′-phosphate C-methyltransferase (EC 2.1.1.b) [thymidylate synthetase], catalyze the formation and utilization of 5,10-methylenetetrahydrofolate, which is the source of the 5-methyl group of thymidylic acid. The preparation of inhibitors of these enzymes will cause a deficiency of THF cofactors, which will result in blocks in the synthesis of pyrimidines, purines, protein, and lipid. Consequently, these blocks will arrest both DNA synthesis and cell division.[1]

The synthesis of tetrahydro derivatives of *N*-[4-[[(2-amino-4(3*H*)-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (8-deazafolic acid; 17) was investigated as part of a program involving the development of new agents that inhibit folate enzymes either as analogues of the cofactor forms of THF or as multisubstrate analogues when enzyme-bound substrates and analogues of the cofactor forms of THF interact to form covalent linkages. In this paper, we report a new method for the preparation of 17, the conversion of the latter to cofactor analogues of THF, and the characterization and biological activity of these derivatives (Scheme I).

The preparation and hydrolysis of the 4-amino group of 4-[*N*-[(2,4-diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoic acid to give 8-deazapteroic acid has been described by Oakes.[2] The coupling of 8-deazapteroic acid and glutamate to give 17 was reported by DeGraw et al.,[3] and the conversion of 8-deaza-6-(hydroxymethyl)pterin to 17 was reported by Srinivasan and Broom.[4] Both 17 and its di- and tetrahydro derivatives were antagonists of folates when tested against folate-dependent bacteria.[3] Also, DeGraw and collaborators have shown that 17 gave a 51% increase in life span against lymphoid leukemia L1210 in mice.[3] In contrast, these compounds were ineffective inhibitors of dihydrofolate reductase and thymidylate synthetase,[3] and recently Benkovic has reported that the 10-formyl derivative of 17 served as a cofactor for both glycinamide ribonucleotide and 5-amino-4-imidazolecarboxamide ribonucleotide transformylase.[5]

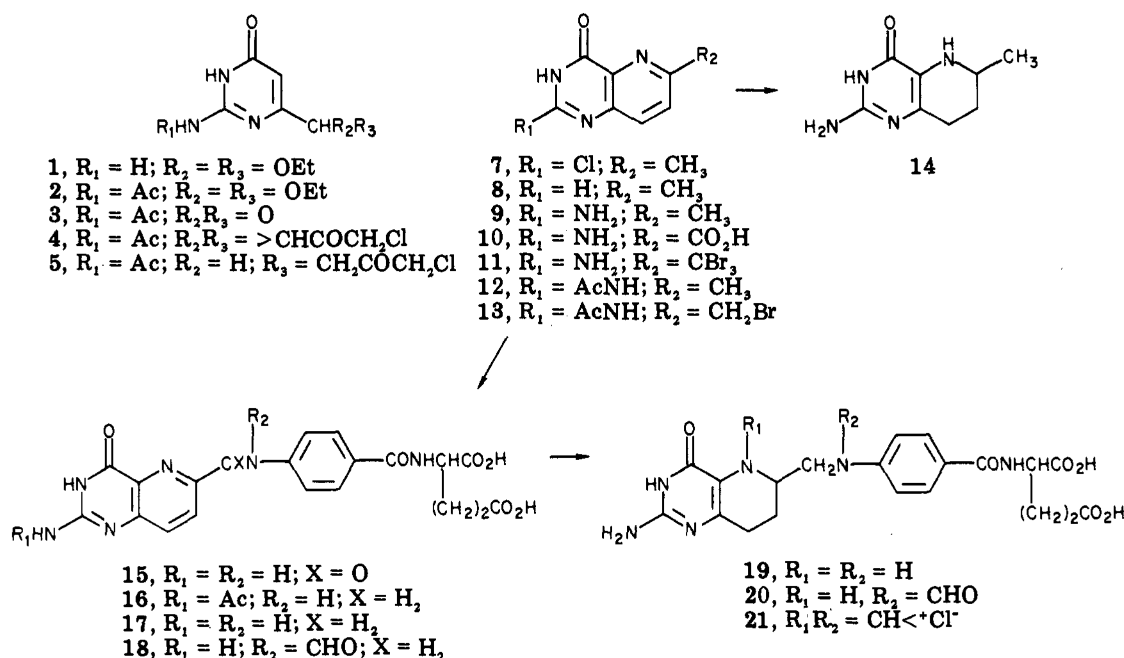
Two approaches were investigated for the synthesis of 17. Acetylation of the acetal 1[6] with acetic anhydride gave 2, which was converted with formic acid to the aldehyde 3, an intermediate that has been reported previously.[7] The condensation of 3 with 1-chloro-3-(triphenylphosphoranylidene)-2-propanone[8] gave the buten-3-one 4, which was reduced with diimide to give the butan-2-one 5. Because of the low overall yield of these two reactions, no further work was carried out on the introduction of a 5-amino group and ring closure to give a pyridopyrimidine intermediate.

The successful route to 17 used the known intermediate 2,4-dichloropyrido[3,2-*d*]pyrimidine (6).[9,10] The greater reactivity toward nucleophilic reagents of the 4-chloro atom

(1) R. L. Blakley, *Front. Biol.* **13**, (1969).
(2) V. Oakes, H. N. Rydon, and K. Undheim, *J. Chem. Soc.*, 4678 (1962).
(3) (a) J. I. DeGraw, R. L. Kisliuk, Y. Gaumont, and C. M. Baugh, *J. Med. Chem.*, 17, 470 (1974); (b) J. I. DeGraw, V. H. Brown, R. L. Kisliuk, Y. Gaumont, and F. M. Sirotnak, in "Chemistry and Biology of pteridines", Proceedings of the International Symposium, 6th, La Jolla, CA, 1978, Elsevier/North Holland: New York, 1979, p 229.
(4) A. Srinivasan and A. D. Broom, *J. Org. Chem.*, **46**, 1777 (1981).
(5) G. K. Smith, W. T. Mueller, P. A. Benkovic, and S. J. Benkovic, *Biochemistry*, **20**, 1241 (1981).
(6) W. Braker, E. J. Pribyl, J. T. Sheehan, E. R. Spitzmiller, and W. A. Lott, *J. Am. Chem. Soc.*, **69**, 3072 (1947).
(7) J. I. DeGraw and V. H. Brown, *J. Heterocycl. Chem.*, **13**, 439 (1976).
(8) R. F. Hudson and P. A. Chopard, *J. Org. Chem.*, **28**, 2446 (1963).
(9) V. Oakes and H. N. Rydon, *J. Chem. Soc.*, 4433 (1956).
(10) J. Davoll and D. H. Laney, British Patent 829 276 (1960); W. J. Irwin and D. G. Wibberly, *J. Chem. Soc. C*, 1745 (1967).

0022-2623/81/1824-1254$01.25/0 © 1981 American Chemical Society

Scheme I



of 6 as compared with that of the 2-chloro atoms has been demonstrated both by theoretical calculations and by chemical conversions.[9,11] Treatment of 6 with aqueous NaOH gave the pyrido[3,2-*d*]pyrimidin-4(3*H*)-one 7, which was confirmed by removal of the chloro atom to give the known pyrido[3,2-*d*]pyrimidin-4(3*H*)-one (8).[9]

Ammonolysis of 7 with ethanolic ammonia gave the 2-amino derivative 9, which was hydrogenated over platinum in $CF_3CO_2H$ to give the 5,6,7,8-tetrahydro derivative 14. The structure of 14 was supported by the $^1H$ NMR spectrum, which showed the 6-methyl group as a doublet and the absence of proton peaks for the 7,8-CH=CH grouping of 9. In addition, the oxidation of the methyl group of 9 with permanganate in aqueous base gave the carboxylic acid 10. In contrast, the bromination of 9 in HOAc was complex in that 9 and an equimolar amount of $Br_2$ at 100 °C or excess $Br_2$ at 63 °C gave incomplete reaction. However, treatment of 9 with a 3-fold excess of $Br_2$ at 100 °C gave essentially complete reaction, but the mass spectrum of the product showed the presence of a mixture of mono-, di-, and tribromo derivatives. In the $^1H$ NMR spectrum the presence of minor proton peaks attributable to the 6-methyl group and its mono- and dibromo derivatives and of strong peaks assigned to the 7,8-CH=CH protons indicated that the 6-(tribromomethyl) derivative 11 was a major product of the reaction. Treatment of this crude product with *p*-aminobenzoyl-L-glutamic acid in $Me_2SO$ gave the 9-oxo derivative of 8-deazafolic acid (15). Presumably, 15 was formed by displacement of one bromo atom of 11 by the amino group of the side chain, followed by hydrolysis of the resulting dibromo intermediate. When 15 was heated in aqueous base, the carboxylic acid 10 and *p*-aminobenzoylglutamic acid were formed.

The bromination of the 2-(acetylamino) compound 12 gave different results in that monobromination occurred to give mainly 13. Apparently, deactivation of the 2-amino group reduced the electron density at the 6 position of the ring. Reaction of crude 13 with *p*-aminobenzoyl-L-glutamic acid in $Me_2SO$ gave 16, from which 17 (23% from 9) was prepared by removal of the 2-acetyl group with base.

(11) A. Srinivasan and A. D. Broom, *J. Org. Chem.*, **44**, 435 (1979).

Table I. Cell Culture Cytotoxicity Data[a]

| compd | $ED_{50}$,[b] μM |
|---|---|
| 15 | 100 |
| 17 | 23 |
| 18 | 32 |
| 20 | 28 |
| 21 | 8 |
| MTX | 0.001 |

[a] Human epidermoid carcinoma cell no. 2. [b] Concentration inhibiting colony formation by 50%.

Previous workers[3] have reported the catalytic hydrogenation of 17 to give a tetrahydro derivative, but no experimental details were given. Preliminary studies show that the hydrogenation of 17 in the presence of platinum was unsuccessful either in aqueous base or formic acid. In the latter, 17 was only converted to the 10-formyl derivative 18.[5] In contrast, the hydrogenation of 17 in $CF_3CO_2H$ was rapid, but overreduction occurred to generate a mixture that after isolation was shown to contain 14 (20%), *p*-aminobenzoylglutamic acid (38%), 19 (10%), and unidentified components. From the mixture, 19 was isolated in low yield and characterized by conversion with formic acid to the 5,10-methenyl derivative 21. The latter was also prepared by the catalytic hydrogenation of 18 in $CF_3CO_2H$ to give 20, which under the acidic conditions of the reaction was dehydrated to give 21. On treatment of 21 with aqueous base, the imidazolium ring was opened to give the 10-formyl derivative 20, which was isolated as its calcium salt. The position of the formyl group was confirmed by the $^1H$ NMR spectrum, which showed that one pair of the phenylene protons was considerably deshielded relative to one pair of the phenylene protons of 17.[12] The interconvertibility of 20 and 21 is similar with those reactions observed for the corresponding tetrahydrofolate derivatives.[13]

**Biological Evaluation.** The 8-deazafolates were initially assayed for cytotoxicity to cultured H.Ep.-2 cells, and

(12) C. Temple, Jr., R. D. Elliott, J. D. Rose, and J. A. Montgomery, in ref 3b, p 279.

(13) C. Temple, Jr., R. D. Elliott, J. D. Rose, and J. A. Montgomery, *J. Med. Chem.*, **22**, 731 (1979).

the results are listed in Table I.[14] Since only **21** showed an $ED_{50}$ of less than 10 μM,[15] this compound was given further examination. The determination of the capacities of various metabolites to prevent or reverse the cytotoxicity of **21** to H.Ep.-2 cells was carried out by a method previously described.[14] Colony formation of control cultures was 60–90%, whereas **21** inhibited colony formation by 65–100%; this inhibition was prevented or reversed completely by citrovorum factor (11 μM) and by a combination of hypoxanthine (147 μM) and thymidine (41 μM). Hypoxanthine (147 μM) gave partial protection, but thymidine (41 μM) alone was without effect. In addition, **21** was tested as an inhibitor of bovine dihydrofolic reductase and gave an $ID_{50}$ of $4.6 \times 10^{-6}$ M (MTX, $4.8 \times 10^{-9}$ M).[16] The actual value of the $ID_{50}$ is lower because HPLC studies showed that under the conditions of the assay (pH 7.5), **21** was slowly converted to a less cytotoxic compound, **20**. Lineweaver–Burk analysis indicated inhibition competitive with dihydrofolate. These results are consistent with inhibition of the reductase being responsible for the cytotoxicity of this compound.

These 8-deazafolic acids were considerably less cytotoxic than methotrexate, and no further testing was carried out with either **15** or **18**. Although **17** gave a 55% increase in life span against lymphocytic leukemia P388 cells implanted ip in mice, the dose (100 mg/kg) and schedule (days 1, 5, and 9) were toxic as determined by weight change.[15] In addition, the tetrahydro derivative **21** (200 mg/kg) was inactive on the day 1 and days 1, 5, and 9 schedules, as was the tetrahydro derivative **20** on the day 1 schedule. Although the in vivo screening data were disappointing, our results with **17** and **21** and the results of others with **17**[3] and **18**[5] indicate that the 8-deazafolates are capable of binding with the folate enzymes. This ability and the recently reported potent activity of the 10-propargylquinazolinyl analogue of folic acid against thymidylate synthetase[17] suggested that other derivatives of **17** and **19** might be prepared with greater biological activity.

## Experimental Section

The ultraviolet absorption spectra were determined with a Carey Model 17 spectrophotometer, the mass spectra with a Varian MAT 311A spectrometer, the $^1H$ NMR spectra with a Varian XL-100-15 spectrometer with tetramethylsilane as an internal reference, and HPLC chromatograms with a Waters Associates ALC-242 liquid chromatograph equipped with a reversed-phase μBondapak $C_{18}$ column.

**2-Acetamido-6-(diethoxymethyl)pyrimidin-4(3*H*)-one (2).** A solution of **1** (15.0 g, 70.3 mmol)[6] in $Ac_2O$ (80 mL) was refluxed for 4 h and evaporated to dryness under reduced pressure. The gum was dissolved in EtOH and the solution was reevaporated to dryness to give a solid, which was recrystallized from EtOAc (150 mL): yield 10.5 g (58.5%); mp 120–121 °C. Anal. ($C_{11}H_{17}N_3O_4$) C, H, N.

Concentration of the EtOAc filtrate gave an additional amount of product: yield 1.92 g (10.7%); mp 112–116 °C.

**2-Acetamido-6-formylpyrimidin-4(3*H*)-one (3).** A solution of **2** (10.0 g, 39.2 mmol) in 98% formic acid (100 mL) was refluxed with stirring for 1 h. After cooling followed by filtration, the filtrate was evaporated to dryness. The residue was dissolved in EtOH and the solution was evaporated to dryness in vacuo: yield 6.70 g (94%); mp >260 °C dec (lit.[7] mp >300 °C). Recrystallization of a portion of this solid from EtOH–hexane gave the analytical sample. Anal. ($C_7H_7N_3O_3$) C, H, N.

**1-(2-Acetamido-4(1*H*)-oxopyrimidin-6-yl)-4-chloro-1-buten-3-one (4).** A suspension of **3** (14.7 g, 81.2 mmol) and 1-chloro-3-(triphenylphosphoranylidene)-2-propanone (30.0 g, 85.2 mmol)[8] in benzene (350 mL) was refluxed with stirring under $N_2$ for 20 h. The insoluble material was collected by filtration, refluxed in fresh benzene (250 mL), collected by filtration, and extracted in a Soxhlet apparatus for about 2 h with EtOAc (300 mL). The cooled EtOAc extract deposited the product (8.10 g), which was then recrystallized from fresh EtOAc: yield 7.52 g (36%); mp 193–194 °C dec; mass spectrum, $m/e$ 255 ($M^+$); UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 257 nm (27.3), 324 (6.49); $^1H$ NMR ($Me_2SO$-$d_6$, 5% w/v) δ 2.17 (s, 3, $CH_3$), 4.76 (s, 2, $CH_2$), 6.41 (s, 1, 5-CH), 7.14 and 7.43 (2 d, 1, 1, CH=CH, $J$ = 15 Hz). Anal. ($C_{10}H_{10}ClN_3O_3$) C, H, Cl, N.

**4-(2-Acetamido-4(1*H*)-oxopyrimidin-6-yl)-1-chlorobutan-2-one (5).** To a solution of **4** (4.59 g, 18.0 mmol) in dioxane (700 mL) containing potassium azodicarboxylate (6.39 g, 38.0 mmol)[18] was added dropwise with stirring under $N_2$ a solution of HOAc (2.28 g, 38.0 mmol) in dioxane. After 4 h, additional HOAc (2.28 g, 38.0 mmol) in dioxane was added dropwise, and the suspension was stirred at room temperature for 18 h. The insoluble material was removed by filtration and washed with dioxane, and the combined filtrate and wash was evaporated to dryness in vacuo. The resulting yellow solid (3.13 g) was eluted from a silica gel (300 g, 200–325 mesh) column with a mixture of $CHCl_3$–MeOH (97:3) to give **4** (0.9 g, 19.6% recovery) followed by **5**: yield 0.80 g (17.3%); mp 177–178 °C with prior sintering; mass spectrum, $m/e$ 257 ($M^+$); UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 220 nm (13.5), 236 (12.3), 279 (6.32); $^1H$ NMR ($Me_2SO$-$d_6$, 8% w/v) δ 2.17 nm (s, 3, $CH_3$), 2.75 (m, 4, $CH_2CH_2$), 4.56 (s, 2, $CH_2$), 5.92 (s, 1, 5-CH), 11.62 (2, NH). Anal. ($C_{10}H_{12}ClN_3O_3$) C, H, Cl, N.

A third fraction gave an additional amount of impure **5** (0.60 g) that was contaminated with the product resulting from hydrodechlorination of **5**.

**2-Chloro-6-methylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (7).** A suspension of **6** (12 g, 56 mmol) in $H_2O$ (570 mL) containing 1 N NaOH (114 mL) was stirred for 5 h at room temperature. The resulting dark red solution was neutralized with 1 N HCl (57 mL) and extracted with $CHCl_3$ (5 × 1500 mL), and the combined extracts were evaporated to dryness to give crude product: yield 11 g (100%); mp 207–214 °C dec. Recrystallization of this product from EtOAc gave the analytical sample: yield 7.9 g (72%); mp 232 °C dec; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 234 nm (23.1), 274 (6.72), 304 sh (4.72), 314 (5.57), 323 sh (4.32); $^1H$ NMR ($Me_2SO$-$d_6$, 5% w/v), δ 2.62 (s, 3, $CH_3$), 7.66 and 7.90 (2 d, 1, 1, CH=CH, $J$ = 8 Hz). Anal. ($C_8H_6ClN_3O$) C, H, Cl, N.

**6-Methylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (8).** A solution of **7** (505 mg, 2.59 mmol) in 0.1 N NaOH (129 mL) was hydrogenated for 1 h over 5% palladium on charcoal (250 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration (Celite), and the filtrate was acidified with concentrated HCl (1.1 mL) and evaporated to dryness in vacuo. The resulting residue was recrystallized twice from a small amount of $H_2O$: yield 114 mg (27%); mp 303–304 °C (lit.[9] mp 299 °C); mass spectrum, $m/e$ 161 ($M^+$); UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 262 nm (6.97), 294 sh (4.13), 304 (5.08), 317 (3.70); $^1H$ NMR ($Me_2SO$-$d_6$, 5% w/v) δ 2.63 (s, 3, $CH_3$), 7.68 and 7.88 (2 d, 1, 1, CH=CH, $J$ = 8 Hz). Anal. ($C_8H_7N_3O \cdot 0.6H_2O$) C, H, N.

Neutralization of the aqueous filtrate from the above sample with base gave an additional 76 mg (18%) of **8**: mp 301 °C with presoftening. The total yield was 190 mg (46%).

**2-Amino-6-methylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (9).** A solution of **7** (6.1 g, 31 mmol) in 3% ethanolic ammonia (400 mL) was heated in a bomb at 135 °C for 18 h. The insoluble material present in the bomb after cooling was collected by filtration, washed with $H_2O$ (100 mL), and dried in vacuo over $P_2O_5$ at 78 °C: yield 4.1 g (75%); mp >265 °C; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 262 nm (6.69), 3.15 (2.78), 324 (2.77); $^1H$ NMR ($Me_2SO$-$d_6$, 4% w/v), δ 2.50 (s, $CH_3$, $Me_2SO$-$d_5$), 6.63 (br s, 2, $NH_2$), 7.42 and

(14) L. L. Bennett, Jr., H. P. Schnetli, M. H. Vail, P. W. Allan and J. A. Montgomery, *Mol. Pharmacol.*, **2**, 432 (1966).
(15) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schemacher, and B. J. Abbott, *Cancer Chemother. Rep.*, 3(2) (1972).
(16) B. G. Stanley, G. E. Neal, and D. C. Williams, *Methods Enzymol.*, **18B**, 775 (1971).
(17) T. R. Jones, A. H. Calvert, A. L. Jackman, S. J. Brown, M. Jones, and K. R. Harrap, *Eur. J. Cancer*, 17, 11 (1981).
(18) J. Thiele, *Justus Liebigs Ann. Chem.*, **271**, 127 (1892).

7.54 (2 d, 1, 1, CH=CH, $J$ = 8 Hz). Anal. ($C_8H_8N_4O$) H, N; C: calcd, 54.54; found, 54.06.

**2-Amino-4(3*H*)-oxopyrido[3,2-*d*]pyrimidine-6-carboxylic Acid (10).** A solution of 9 (176 mg, 1.00 mmol) in 1 N NaOH (60 mL) at reflux was treated dropwise with 0.2 M aqueous $KMnO_4$ (~12 mL) over a period of 1 h. The hot, violet-colored reaction mixture was decolorized with $NaHSO_3$ and filtered through Celite, and the filtrate was acidified (pH 3) with HCl. After the mixture cooled to room temperature, the solid was collected by filtration and dissolved in warm 2 N NaOH. On cooling in an ice bath, the sodium salt of the acid was collected by filtration and dissolved in water, and the solution was acidified with HCl to deposit the product: yield 67 mg (29%); mp 264 °C; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) in 0.1 N NaOH 246 nm (23.1), 292 (10.9), 332 (7.36). Anal. ($C_8H_6N_4O_3$·0.6HCl) C, H, N.

The carboxylic acid 10 was also formed when a solution of 15 in aqueous NaOH was heated.

**2-Amino-5,6,7,8-tetrahydro-6-methylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (14).** A solution of 9 (529 mg, 3.00 mmol) in $CF_3CO_2H$ (20 mL) was added to a prereduced mixture of platinum oxide (100 mg)[1] in $CF_3CO_2H$ (20 mL), and the mixture was hydrogenated at room temperature and atmospheric pressure for 3 h. The residue was removed by filtration (Celite), and the filtrate was evaporated to dryness. The resulting oil was washed with $Et_2O$ to give a solid, which was recrystallized from isopropyl alcohol to give the trifluoroacetate salt: yield 625 mg (71%); mp 222 °C dec with presoftening from 210 °C. Anal. ($C_8H_{12}N_4O$·$CF_3CO_2H$) H, N; C: calcd, 40.82; found, 41.23.

A solution of the trifluoroacetate salt (200 mg) in $H_2O$ (5 mL) was neutralized with 1 N NaOH (0.7 mL), and the resulting precipitate was collected by filtration and dried in vacuo over $P_2O_5$: yield 100 mg, mp 334–335 °C dec; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 249 nm (7.50), 297 (5.17); $^1H$ NMR ($CF_3CO_2D$, 3% w/v), $\delta$ 1.70 (d, 3, $CH_3$, $J$ = 6 Hz), 2.36 (m, 2, 7-$CH_2$), 3.06 (m, 2, 8-$CH_2$), 3.93 (m, 1, 6-CH). Anal. ($C_8H_{12}N_4O$·0.44$H_2O$) C, H, N.

***N*-[4-[[(2-Amino-4(3*H*)-oxopyrido[3,2-*d*]pyrimidin-6-yl)carbonyl]amino]benzoyl]-L-glutamic Acid (15).** A mixture of 9 (885 mg, 5.00 mmol), sodium acetate (1.25 g, 15.0 mmol), and bromine (2.80 g, 17.5 mmol) in HOAc (250 mL) was heated at 98 °C for 4 h. The resulting solution was evaporated to dryness, and the residue was washed with $Et_2O$ and dried in vacuo over $P_2O_5$: yield 3.20 g. The mass spectrum of this sample showed peaks that could be assigned to mono-, di-, and tribromomethyl derivatives of 9, whereas the $^1H$ NMR spectrum suggested that 11 was the major product.

The above sample was added to a solution of *p*-aminobenzoyl-L-glutamic acid (11.3 g, 42.5 mmol) in $Me_2SO$ (63 mL). The mixture was stirred at room temperature for 72 h and evaporated to a small volume in vacuo, and the resulting gum was triturated with $CHCl_3$ (400 mL). The residue was then washed with $H_2O$ (125 mL), and the resulting solid was dried in vacuo over $P_2O_5$: yield, 2.08 g. This solid was suspended in $H_2O$ (70 mL) and dissolved by the addition of 1 N NaOH to pH 9. After filtration, the filtrate was adjusted to pH 7.2 with 1 N HCl and treated with a solution of $CaCl_2$ (700 mg) in water. The precipitate (1.34 g) was collected by centrifugation, suspended in $H_2O$ (70 mL), and heated to boiling, and the calcium salt of the product was collected by filtration: yield 576 mg (21%); mp >265 °C; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 214 nm (36.5), 242 sh (22.1), 303 (25.9), 315 sh (24.2); $^1H$ NMR ($CF_3CO_2D$, 4% w/v) $\delta$ 2.55 (m, 2, $CH_2CH_2CO_2$), 2.83 (m, 2, $CH_2CH_2CO_2$), 5.14 (m, 1, $CHCH_2$), 7.99 (s, 4, $C_6H_4$), 8.30 and 8.85 (2 d, 1, 1, CH=CH, $J$ = 9 Hz). Anal. ($C_{20}H_{16}N_6O_7$·Ca·2.7$H_2O$) C, H; N: calcd, 15.53; found, 15.97.

The aqueous filtrate from the above product contained mainly *p*-aminobenzoylglutamic acid.

In another experiment, acidification of the initial precipitate of the calcium salt in $H_2O$ with 1 N HCl to pH 3 gave the free acid contaminated with *p*-aminobenzoylglutamic acid and 10.

***N*-[4-[[(2-Amino-4(3*H*)-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (8-Deazafolic Acid; 17).** A mixture of 9 (7.50 g, 42.6 mmol) in acetic anhydride (850 mL) containing concentrated sulfuric acid (1.5 mL) was stirred in a preheated oil bath at 125 °C for 4 h. The hot solution was filtered, and the filtrate was evaporated to dryness in vacuo, washed with $Et_2O$, and dried in vacuo over $P_2O_5$: yield 10.0 g.

A mixture of this solid (12), sodium acetate (4.16 g, 50.7 mmol), and $Br_2$ (2.52 mL, 8.06 g, 50.4 mmol) in HOAc (4200 mL) was stirred in a preheated oil bath at 95 °C for 4 h. The solution was filtered hot, and the filtrate was evaporated to dryness in vacuo, washed with $Et_2O$, and dried in vacuo over $P_2O_5$: yield 18.7 g.

A solution of this solid (13) in $Me_2SO$ (470 mL) containing *p*-aminobenzoyl-L-glutamic acid (60.7 g, 229 mmol) was stirred at room temperature for 22 h and evaporated to a small volume in vacuo. The oil was stirred successively in $Et_2O$ (1400 mL), $CHCl_3$ (3 × 1100 mL), and $H_2O$ (1900 mL), and the resulting solid was collected by filtration and dried in vacuo over $P_2O_5$: yield 9.69 g. This sample was dissolved in $H_2O$ (325 mL) with stirring by the dropwise addition of 1 N NaOH to pH 9, and after filtration the filtrate was readjusted to pH 7.2 by the addition of 1 N HCl. After the addition of an aqueous solution of $CaCl_2$ (3.50 g, 31.5 mmol), the resulting precipitate of the calcium salt was collected by centrifugation and dried in vacuo over $P_2O_5$: yield 4.68 g. The $^1H$ NMR spectrum ($CF_3CO_2D$, 5% w/v) showed the methyl of the acetyl group at $\delta$ 2.57. In addition, a small portion (58 mg) of this material was suspended in $H_2O$ (5 mL) and neutralized to pH 3 with 1 N HCl to deposit the free acid (20 mg). The field-desorption mass spectrum of this solid showed a peak for 16 at $m/e$ 483 $(M + 1)^+$. The main portion of this sample was suspended in 1 N NaOH (188 mL), the mixture was heated with stirring at 50 °C for 1 h, and a trace amount of insoluble material was removed by filtration. The filtrate was neutralized to pH ~3 with 1 N HCl, and the precipitate of 17 was collected by centrifugation and washed by stirring with 0.1 N HCl (700 mL): yield 2.85 g (13%); mp indefinite with decomposition from 193 °C; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) in 0.1 N HCl 250 (15.1), 298 (19.4); at pH 7 280 (24.8), 292 sh (24.3), in 0.1 N NaOH 283 (25.0), 292 sh (24.0), 345 sh (6.19); $^1H$ NMR ($CF_3CO_2D$, 4% w/v) $\delta$ 2.52 (m, 2, $CH_2CH_2CO_2$), 2.81 (m, 2, $CH_2CH_2CO_2$), 5.11 and 5.18 (2 m, 3, $CHCO_2$, 6-$CH_2N$), 8.05 (m, 6, $C_6H_4$, 7,8-CH=CH); $^1H$ NMR ($Me_2SO$-$d_6$, 6% w/v), 2.04 (m, $CH_2CH_2CO_2$), 2.33 (m, $CH_2CH_2CO_2$), 4.39 (m, $CHCO_2$), 4.51 (br s, 9-$CH_2$), 6.61 and 7.65 (2 d, $C_6H_4$), 7.74 (m, 7,8-CH=CH), 8.09 (m, NH). Anal. ($C_{20}H_{20}N_6O_6$·2HCl) C, H, N.

Neutralization of the acidic filtrate to pH 3.5 with 1 N NaOH gave an additional amount of practically pure 17: yield 0.26 g (1.2%).

The aqueous filtrate resulting from the isolation of the calcium salt of 16 was diluted with 2 vol of ethanol, and the resulting precipitate was collected by filtration and dried in vacuo over $P_2O_5$: yield 3.60 g. This sample was a mixture of the calcium salts of 16 and *p*-aminobenzoylglutamic acid (TLC) and was treated as described above for the major crop to give 17 contaminated with *p*-aminobenzoylglutamic acid: yield 1.91 g (9%). The total yield was 5.0 g (23%).

***N*-[4-[[(2-Amino-10-formyl-4(3*H*)-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (10-Formyl-8-deazafolic Acid; 18).** A solution 17·2HCl (224 mg, 0.437 mmol) in 95% $HCO_2H$ (10 mL) was heated with stirring at 57 °C for 1 h and diluted to 100 mL with ether. The solid that deposited was collected by filtration, washed with ether, and dried in vacuo over $P_2O_5$: yield 190 mg. A portion of this sample (146 mg) was heated to reflux with vigorous stirring in water (75 mL), and some dark material was removed by filtration. After standing at room temperature for 3 days, the resulting mixture was concentrated in vacuo to a small volume, and the solid was collected by filtration and dried in vacuo over $P_2O_5$: yield 90 mg; mp 253–255 °C dec; field-desorption mass spectrum, $m/e$ 469 $(M + 1)^+$, 491 $(M + Na)^+$; UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) in 0.1 N HCl 252 nm (27.9), 307 (6.16), 321 sh (4.89); at pH 7 263 (26.2), 314 (5.35), 322 sh (5.28), 345 (2.57); in 0.1 N NaOH 244 (35.2), 270 sh (21.5), 335 (6.12). Anal. ($C_{21}H_{20}N_6O_7$·1.8$H_2O$) C, H, N.

***N*-[4-[[(2-Amino-10-formyl-5,6,7,8-tetrahydro-4(3*H*)-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (8-Deaza-10-formyl-5,6,7,8-tetrahydrofolic Acid; 20).** A solution of 21 (200 mg, 0.333 mmol) in oxygen-free 0.1 N NaOH (20 mL) was stirred at room temperature for 30 min and adjusted to pH 8–9 (paper) with 1 N HCl. After the addition of a solution of $CaCl_2$ (50 mg) in water, the solution was diluted with EtOH (40 mL) to deposit the calcium salt of 17: yield 72 mg. HPLC [pH 3.6, $NH_4OAc$–MeCN (9:1)] of a solution of this sample in 1% $NH_4OAc$ (pH 6.9) showed the presence of 21 (3.5%)

and **20** (90%). Dilution of the filtrate from above with additional EtOH (40 mL) and refrigeration (5 °C) of the resulting mixture for 18 h gave a second crop of **20**: yield 89 mg; mp 350 °C; HPLC showed the presence of **21** (~3%) and **20** (93%); total yield 161 mg (~81%). Anal. ($C_{21}H_{21}N_6O_7$·1.5Ca·0.5$C_2H_6O$·2.5$H_2O$) C, H, N.

Spectral data were determined on a sample obtained in another experiment in which the calcium salt of **20** was precipitated from an aqueous solution with 3 vol of EtOH: UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) at pH 7 254 nm (21.0), 312 br sh (4.67); in 0.1 N NaOH 253 (21.2), 310 br sh (5.28); $^1$H NMR ($D_2O$, 3% w/v), $\delta$ 7.67 (d of d, $C_6H_4$), 8.50 (CHO). Anal. ($C_{21}H_{21}N_6O_7$·1.4Ca·5$H_2O$) C, H, Ca, N.

**3-Amino-8-[4-[[(1,3-dicarboxypropyl)amino]carbonyl]-phenyl]-2,5,6,6a,7,8-hexahydro-1-oxo-1*H*-imidazo[1′,5′:6,1]-pyrido[3,2-*d*]pyrimidin-10-ium Chloride (8-Deaza-5,6,7,8-tetrahydro-*N*⁵,*N*¹⁰-methenylfolic Acid Chloride; 21).** A solution of **17** (900 mg, 1.75 mmol) in 95% formic acid (30 mL) was heated with stirring at 60 °C for 1 h and evaporated to dryness in vacuo. The residue was dried in vacuo over $P_2O_5$ and dissolved in $CF_3CO_2H$ (50 mL). The resulting solution was mixed with a prereduced suspension of $PtO_2$ (600 mg)[1] in $CF_3CO_2H$ (40 mL) and hydrogenated at room temperature and atmospheric pressure. The catalyst was removed by filtration (Celite) and washed with $CF_3CO_2H$. The combined filtrate and wash was evaporated to dryness, and the residue was dissolved in 3 N HCl (90 mL). This solution was stirred for 30 min and evaporated to dryness, and the product was washed with $Et_2O$: yield 932 mg (89%); mp 196 °C foamed; field-desorption mass spectrum, *m/e* 455 ($M^+$); UV $\lambda_{max}$ ($\epsilon \times 10^{-3}$) in 0.1 N HCl 220 nm (15.8), 331 (28.5); at pH 7 342 (26.7, unstable); in 0.1 N NaOH 253 (20.6), 300 sh (8.19); $^1$H NMR ($CF_3CO_2D$, 6% w/v): $\delta$ 2.6 (br), 2.8 (br, $CH_2$), 5.1 (br, $CHCH_2$), 7.7 (br), 8.1 (br), ($C_6H_4$, methenyl CH); HPLC [pH 3.6, $NH_4OAc$–MeCN (9:1)] chromatograms showed that a solution of **21** in Tris buffer (pH 7.5) was slowly converted to **20**: 41% (1.5 h), 53% (3.5 h), 57% (5 h), and 100% (20 h). Anal. ($[C_{21}H_{23}N_6O_6]^+Cl^-$·2HCl·2$H_2O$) C, H, Cl, N.

**Acknowledgment.** This investigation was supported by Grant CA-23141 from the National Cancer Institute, National Institutes of Health. The authors are indebted to Dr. W. C. Coburn, Jr., and Mrs. M. C. Thorpe who interpreted NMR data and to other members of the Molecular Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations.

# Synthesis and Adrenoceptor Affinity of Some Highly Polar β-Substituted Catecholamines

James G. Henkel,* Neil Sikand, Alexandros Makriyannis,

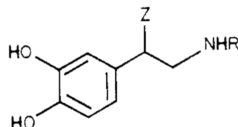
*Section of Medicinal Chemistry and Pharmacognosy and Institute for Materials Science*

and Gerald Gianutsos

*Section of Pharmacology and Toxicology, School of Pharmacy, The University of Connecticut, Storrs, Connecticut 06268.*
*Received March 30, 1981*

In order to assess the potential for sympathomimetic or sympatholytic activity within the series of catecholamine β-sulfonates **3a–c**, α- and β-adrenoceptor binding affinities were determined using rat brain homogenate preparations. Furthermore, their potential for indirect activity was assessed by measurement of blockade of norepinephrine uptake into rat synaptosomal preparations. Activity was uniformly low or nonexistent throughout the series. The possibility of unfavorable solution conformational distribution within the series was investigated by examination of the side chain vicinal $^1$H NMR coupling constants, but no differences that could account for the lack of affinity were found. The observed behavior may be due to receptor intolerance of the bulky β-sulfonate substituent or an electronic mismatch in which normal H bonding is significantly altered.

The catecholamines norepinephrine (**1a**) and epinephrine (**1b**), in addition to being endogenous neurotransmitters, have found widespread use as therapeutic agents. A number of synthetic congeners have also been developed that exhibit enhanced or more selective sympathomimetic activity, including isoproterenol (**1c**) and, most recently, dobutamine (**1d**). A large body of knowledge has been




1a, R = H; Z = OH
b, R = $CH_3$; Z = OH
c, R = $CH(CH_3)_2$; Z = OH
d, R = $CH(CH_3)CH_2CH_2C_6H_4OH$; Z = H

accumulated over the years concerning the structure–activity relationships at every position of the phenethylamine nucleus, with the notable exception of the benzylic (Z) β position. Activity within the catecholamines is known to be enhanced by the presence of the β-hydroxyl group (Z = OH), but very few other substituents have been investigated. In the norepinephrine series, several sulfur analogues of **1a** were reported by Rachlin and Enemark,[1] some of which (Z = SH, $SCH_3$, $SSC_3H$, R = H) had weak pressor activity in the anesthetized cat. Larger thioethers in this series were essentially inactive, possibly indicating that bulk tolerance in this region of the receptor may be limited. No investigations of N-alkylated congeners of this series have been reported. Recently, Chavdarian et al.[2] also found very weak pressor activity in rats for the congeners of the catecholamines β-methyldopamine (1, R = H; Z = $CH_3$), β-methylepinine (1, R = $CH_3$; Z = $CH_3$), and β-methoxyepinine (1, R = $CH_3$; Z = $OCH_3$). Again, the pressor activities of these analogues were well below therapeutic significance.

A third set of β-substituted catecholamine derivatives is represented by the amino acids **3a–c**. The analogues have potential pharmacologic interest, since they are known to be formed as degradation products of **1a–1c** in bisulfite-stabilized parenteral dosage forms.[1,3,4] A qui-

(1) Rachlin, S.; Enemark, J. *J. Med. Chem.* **1969**, *12*, 1089.
(2) Chavdarian, C. G.; Karashima, D.; Castagnoli, N., Jr.; Hundley, H. K. *J. Med. Chem.*, **1978**, *21*, 548.

0022-2623/81/1824-1258$01.25/0 © 1981 American Chemical Society