with ϵ = 29 000 at 300 nm. The results are expressed in Table V.

Acknowledgment. This work was supported by grants from the Cancer Research Campaign and Medical Research Council. We thank M. Baker for determining the mass spectra. J. Hawkes and F. Gall of King's College kindly provided NMR spectra under the auspices of the University of London Intercollegiate Research Service. Donna Nichols expertly typed the manuscript. We thank Dr. D. A. Matthews for permission to reproduce the drawing in Chart II.

Registry No. **2**, 2941-78-8; **3**, 19181-53-4; **4**, 106585-52-8; **5**, 106585-53-9; **6a**, 13726-52-8; **6b**, 2378-95-2; **6c**, 70280-71-6; **6d**, 76858-71-4; **6e**, 76858-72-5; **7a**, 106585-62-0; **7b**, 106585-59-5; **7c**, 106585-60-8; **7d**, 106585-61-9; **7e**, 106585-54-0; **8a**, 106585-70-0; **8b**, 106585-67-5; **8c**, 106585-68-6; **8d**, 106585-69-7; **8e**, 106585-65-3; TS, 9031-61-2; DHFR, 9002-03-3; $HCONH_2$, 75-12-7; *t*-$BuCOOCH_2Cl$, 18997-19-8; MeI, 74-88-4; EtI, 75-03-6.

Comparison of the Biological Effects of Selected 5,8-Dideazafolate Analogues with Their 2-Desamino Counterparts[1]

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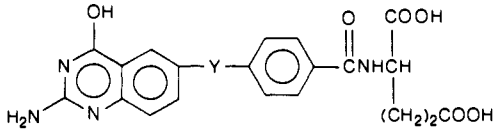
Three new 5,8-dideaza analogues of folic acid devoid of an amino group at position 2 have been prepared by using synthetic routes patterned after earlier methodologies. They were 2-desamino-5,8-dideazaisofolic acid, **2b**, 2-desamino-10-thia-5,8-dideazafolic acid, **2c**, and 2-desamino-10-oxa-5,8-dideazafolic acid, **2d**. These compounds were found to be 4–6-fold more cytoxic toward L1210 leukemia cells than their 2-NH_2 counterparts and to be poor inhibitors of mammalian thymidylate synthase. However, they were only 1.5–3-fold less inhibitory toward dihydrofolate reductase than the analogous compounds containing a 2-NH_2 group. The known thymidylate synthase inhibitors 2-desamino-10-propargyl-5,8-dideazafolic acid and 10-propargyl-5,8-dideazafolic acid were included in this study for purposes of comparison.

Efforts to discover a folate analogue having potent inhibitory activity toward mammalian thymidylate synthase, TS, culminated in the development of 10-propargyl-5,8-dideazafolic acid, **1a**, which became referred to as CB3717.[2] On the basis of its high degree of efficacy against L1210 leukemia in mice, CB3717 was introduced into clinical trials.[3] However, renal toxicity, apparently resulting from poor aqueous solubility, caused the cessation of human studies.[4] Subsequently, it was found that the removal of the 2-amino group of **1a** and related compounds resulted in improved solubility and cytotoxicity. For example, 2-desamino-10-propargyl-5,8-dideazafolic acid, **2a**, was found to be approximately 10-fold more cytotoxic toward L1210 leukemia cells in culture but 8-fold less inhibitory toward TS from L1210 leukemia cells than **1a**.[5,6]



1a: Y = $CH_2N(CH_2C{\equiv}CH)$
b: Y = $NHCH_2$
c: Y = CH_2S
d: Y = CH_2O

The folate analogue 5,8-dideazaisofolic acid, **1b** (IAHQ), has demonstrated activity against the colon tumor 38 in mice, the CX-1 human colon tumor implant in the nude mouse, a human osteogenic sarcoma xenograft in hamsters, as well as a variety of established human colon cell lines in culture.[7-11] However, high doses of IAHQ were required for activity in animal models, apparently due to its slow rate of influx into target cells as recently demonstrated with use of the human colon adenocarcinoma cell line HCT-8.[12]

In spite of the fact that IAHQ has greatly reduced affinity for TS as compared to CB 3717, it appeared to exert its antitumor action by inhibition of TS, since its cytotoxicity toward HCT-8 cells was largely reversed by thymidine.[7] It was of interest, therefore, to prepare the 2-desamino analogue of IAHQ, **2b**, in an effort to achieve enhanced antitumor efficacy. Also, prepared were 2-desamino-10-thia-5,8-dideazafolic acid, **2c**, and 2-desamino-10-oxa-5,8-dideazafolic acid, **2d**, in order to test the generality of cytotoxicity enhancement caused by removal of the 2-NH_2 group. The parent compound of the former, **1c**, was shown to be a reasonably effective inhibitor

† Medical University of South Carolina.
‡ Medical College of Ohio.

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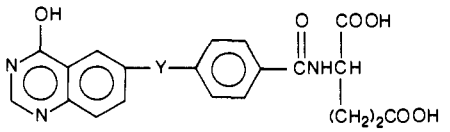


2a: Y = $CH_2N(CH_2C{\equiv}CH)$
b: Y = $NHCH_2$
c: Y = CH_2S
d: Y = CH_2O

of mammalian TS,[13] while 10-oxa-5,8-dideazafolic acid, **1d**, was found to be the most effective inhibitor of avian 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR Tfase) of a series of 5,8-dideazafolates tested.[14]

Chemistry

The preparation of 2-desamino-5,8-dideazaisofolic acid (**2b**) was facilitated by the observation that the reaction of 5-nitroisatoic anhydride (6-nitro-2*H*-3,1-benzoxazine-2,4(1*H*)-dione) (**3**) with formamide gave 4-hydroxy-6-nitroquinazoline (**4**) in excellent yield (Scheme I). Catalytic hydrogenation of **4** using the procedure described earlier afforded amine **5**.[15] Subsequent reductive condensation with di-*tert*-butyl *N*-(4-formylbenzoyl)-L-glutamate (**6**)[10] gave di-*tert*-butyl ester **7** in modest yield. Treatment of **7** with anhydrous trifluoroacetic acid then afforded the target compound **2b**.

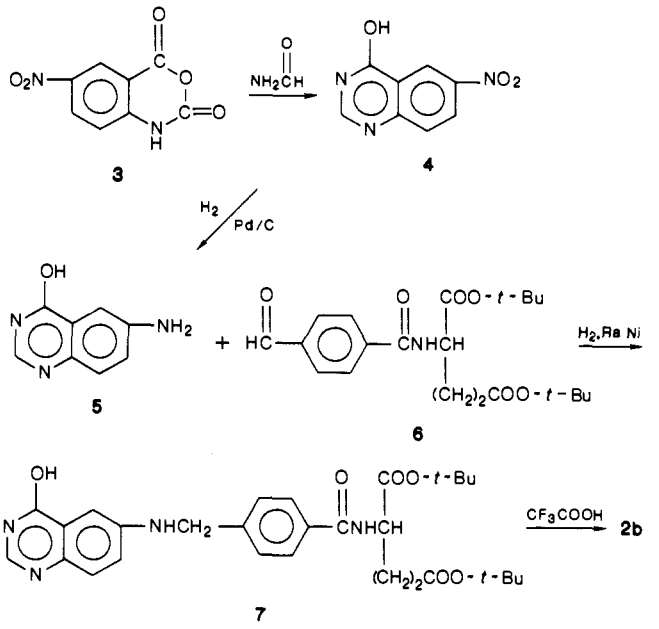
The access to 2-desamino analogues modified at position 10 was facilitated by the recent synthesis of 6-(bromomethyl)-3,4-dihydro-4-oxo-3-[(pivaloyloxy)methyl]-quinazoline **8**.[5] The sodium salt of diethyl (4-mercaptobenzoyl)-L-glutamate (**9**) was generated according to the literature procedure[16] and then reacted with **5** to yield the protected diethyl ester **10** (Scheme II). Subsequent treatment with base removed the ester as well as the (pivaloyloxy)methyl groups to afford 2-desamino-10-thia-5,8-dideazafolic acid (**2c**) in respectable overall yield.

On the basis of earlier experience with the preparation of 10-oxa-5,8-dideazafolic acid (**1d**),[17] a stepwise approach was employed for the preparation of its 2-desamino counterpart **2d** as shown in Scheme III. The 6-bromomethyl compound **8** reacted with methyl 4-hydroxybenzoate in the presence of cesium bicarbonate to yield the fully protected intermediate **11**, which in the presence of dilute sodium hydroxide gave 2-desamino-10-oxa-5,8-dideazapteroic acid (**12**) in good yield. Coupling of this key intermediate to di-*tert*-butyl L-glutamate using diethyl phosphorocyanidate afforded the di-*tert*-butyl intermediate **13**. Upon treatment with trifluoroacetic acid, **13** was converted into 2-desamino-10-oxa-5,8-dideazafolic acid (**2d**).

Biological Evaluation

The three new target compounds **2b–d** together with **2a** were evaluated as inhibitors of human WIL2/DHFR[18] and L1210 leukemia TS.[19] The results obtained are presented

Scheme I



Scheme II

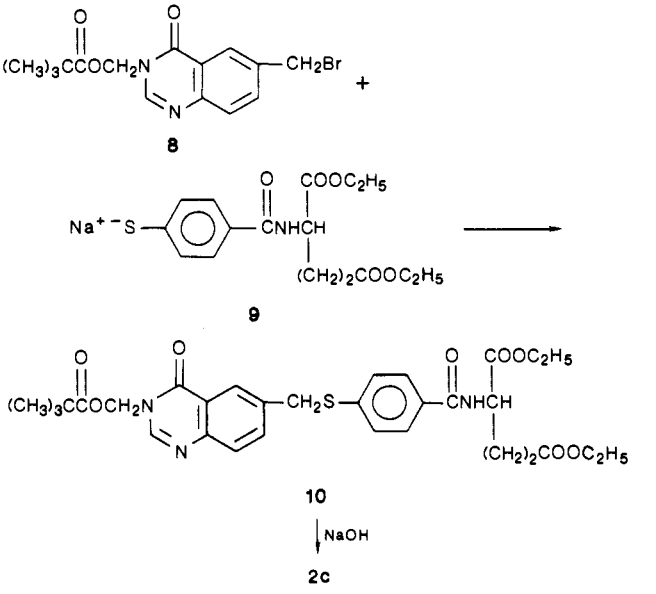


Table I. Comparison of the Biochemical and Antitumor Effects of Selected 5,8-Dideazafolates versus Their 2-Desamino Counterparts

no.	DHFR: I_{50},[a] μM	TS: I_{50},[a,b] μM	L1210/S: IC_{50},[b] μM	L1210/R81: IC_{50},[b] μM
1a	0.91[c]	0.014[c]	1.4	116
2a	7.0	0.13	0.27	75
1b	0.11[c]	1.3[c]	3.2	>200
2b	0.32	25	0.53	>200
1c	0.33[c]	0.22[c]	7.6	>200
2c	0.65	13.8	1.3	>200
1d	0.17[c]	12[c]	1.2	39
2d	0.26	49	0.28	>200
MTX	0.0043	20[c]	0.0044	186

[a] Limits of variability <±15%. [b] Average of three determinations. [c] Reported previously; cf. ref 13.

in Table I, which also contains values obtained for their 2-NH_2 counterparts. In addition, each of these compounds was evaluated as an inhibitor of the growth of methotrexate (MTX) sensitive L1210/S cells and MTX-resistant L1210/R81 cells in culture. The latter cell line is characterized by a 35-fold elevation of DHFR and completely

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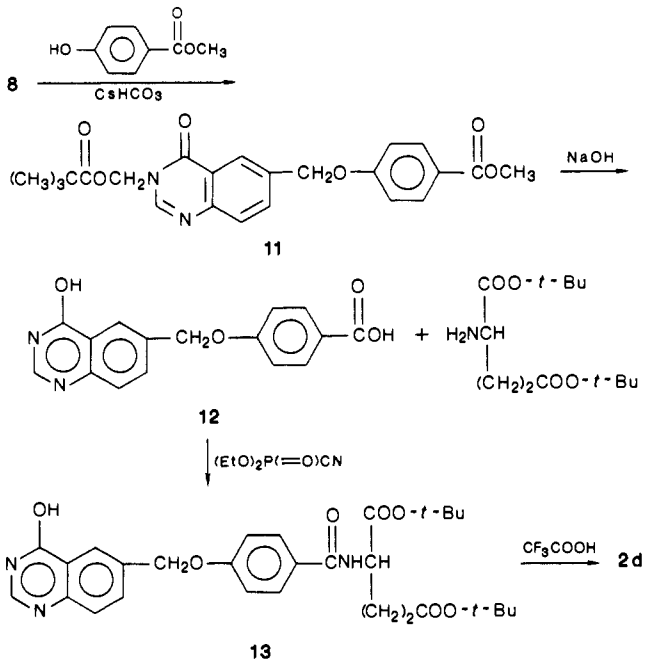
Scheme III



defective MTX transport.[20] Along with these results are values obtained for MTX, which are included for reference purposes.

Discussion

The initial report concerning 2-desamino-5,8-dideazafolates indicated that removal of the 2-NH_2 group caused a 15–30-fold decrease in affinity for mammalian DHFR based upon a comparison of K_i values.[5] The I_{50} values for **1a** and **2a** shown in Table I against human DHFR are in agreement with this observation. However, compounds **2b–d** are only 1.5–3-fold less inhibitory toward DHFR than their 2-NH_2 counterparts. This indicates that certain analogues not having a normal folate bridge at positions 9 and 10 attach to DHFR in a different conformation in which the 2-NH_2 group contributes less to the total binding energy of the enzyme–inhibitor complex.

Turning next to the inhibition of TS, the results presented in Table I show that the removal of the 2-NH_2 group decreases binding in each instance, but to widely varying degrees (4–63-fold for **2b–d**). On the other hand, for compounds having a normal folate bridge, the decrease in I_{50} due to 2-NH_2 removal falls into a considerably narrower range.[5] Each of the newly synthesized compounds is from 4–6-fold more cytotoxic toward L1210/S cells than its 2-NH_2-bearing counterpart. Similar results were reported earlier for compounds having a normal folate bridge such as **1a** and **2a**[5,6] against L1210 cells. None of the compounds tested was an effective inhibitor of the growth of the L1210/R81 cell line, which is defective in MTX transport and also possesses elevated levels of DHFR. The modest level of activity of **1d** against these cells relative to that of MTX may be associated with the ability of this compound to inhibit AICAR TFase.[14] The site of action of **2a** was shown to be TS by protection from cytotoxicity with thymidine despite the fact that **2a** is a considerably weaker inhibitor of TS than compound **1a**.[5,6] In view of the very high I_{50} values of **2b** and **2d** toward TS, it is improbable that the cytotoxicities of these analogues are due to the inhibition of this enzyme, in spite of the fact that they both have been shown to be effective substrates for mammalian folylpolyglutamate synthetase.[21] It will be recalled that the addition of γ-L-glutamyl residues to classical folate analogues can cause a significant enhancement in the inhibition of TS.[22,23] Whether the compounds **2b–d** are sufficiently potent inhibitors of DHFR to account for their interesting levels of cytoxicity has not been determined. However, metabolite reversal studies as well as their effects upon other folate requiring enzymes should shed light upon this interesting observation. The results of such efforts using these and structurally related compounds will be the subject of forthcoming communications from these laboratories.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. All analytical samples were dried under vacuum at 100 °C unless stated otherwise and gave combustion values of C, H, and N within ±0.4% of the theoretical values. Solvation due to water was confirmed by the presence of a broad peak centered at approximately 3.4 ppm in the ^1H NMR spectra, which was transformed into a sharp singlet (DOH) by the addition of D_2O. All intermediates were free of significant impurities on TLC using silica gel media (Baker 1B2-F). Free acids were assayed on Eastman 13254 cellulose (5% NH_4HCO_3). Column chromatographic separations were performed on Kieselgel 60 (70–230 mesh) obtained from E. Merck and Co. The UV spectra were determined with a Cary 219 spectrophotometer in 0.1 N phosphate buffer, pH 7.0. High-resolution ^1H NMR spectra were acquired on a Bruker AM-300 spectrometer at the Chemistry Department, University of South Carolina, Columbia, S.C. NMR values for chemical shifts are presented in parts per million downfield from Me_4Si as the internal standard, and the relative peak areas are given to the nearest whole number.

The FAB spectra were obtained with a VG 70SQ analytical spectrometer at the Chemistry Department, University of South Carolina, Columbia, SC, by Dr. Michael Walla.

Homogeneous DHFR was obtained from human WIL2 cells as described earlier.[18] It was assayed spectrophotometrically at 340 nm with 9 μM dihydrofolate, 30 μM NADPH, 0.15 M KCl in 0.05 M Tris buffer (pH 7.4); [DHFR] = 0.0086 μM by MTX titration. The final volume was 1 mL and the assay was performed at 22 °C after a preincubation period of 2 min. MTX was a gift from Dr. Suresh Kerwar, Lederle Laboratories, Pearl River, NY. Compound **2a** was generously provided by Dr. T. R. Jones, Agouron Pharmaceuticals, Inc., La Jolla, CA. TS was purified from an L1210 cell line resistant to **1a** by virtue of overproducing this enzyme by ca. 50-fold.[19] This cell line was a generous gift from Drs. A. L. Jackman and H. A. Calvert, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surry, U.K. TS was purified to homogeneity by affinity chromatography using 10-formyl-5,8-dideazafolic acid immobilized on aminoethyl-Sepharose as described previously.[24] The enzyme activity was estimated by a modification of ^3H-release method of Roberts.[25] The standard reaction mixture in a total volume of 80 μL contained: 3.6 nmol of [5-^3H]dUMP (ca. 3×10^7 cpm/μmol), 25 nmol of CH_2FAH_4, 1.6 μmol of 2-mercaptoethanol, 2.9 μmol of NaF, 2.9 μmol of sodium phosphate buffer, pH 7.5, 0.085% Triton X-100, and TS 0.24 pmol. Enzyme concentration was determined by titration with FdUMP. Enzyme was preincubated with [5-^3H]-dUMP plus or minus inhibitor for 10 min at 37 °C followed by the addition of *d,l*-L-5,10-methylenetetrahydrofolic acid, CH_2FAH_4, to start the reaction. Blank cpm were determined in the absence of CH_2FAH_4. The reaction was terminated after 1

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h of incubation at 37 °C by addition of 200 μL of a suspension of charcoal (Norit, 100 mg/mL) in 2% CCl_3COOH. The mixture was centrifuged at 16000g for 5 min. A 100-μL sample of the supernatant was added to 10 mL of Ecolume (ICN) and counted in a Beckman LS-100C liquid scintillation counter. All assays were performed in triplicate. Activity of the enzyme is expressed as a percent of control with no inhibitor present.

A cytotoxicity assay based on a published method that uses a color reaction to measure the number of viable cells was used to obtain IC_{50} values for the compounds listed in Table I. The culture conditions for the L1210 and L1210/R81 cells used in the assay were those described earlier.[20] Serial dilutions of each compound in RPMI 1640 medium containing 10% fetal bovine serum were added in 50-μL aliquots to individual wells of a 96-well tissue culture plate (Costar 3596), leaving one row for controls (no drug) and one row for blanks (no cells). The cells, in log phase growth, were diluted to 1.2×10^5/mL, and 50 μL, or 6×10^3 cells, was added to each except in the blank row. After 48 h of incubation in an 8% CO_2 humidified atmosphere, 10 μL of a 5 mg/mL solution of the tetrazolium salt MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide, dissolved in 10 mM sodium phosphate buffer/150 mM NaCl, pH 7.4 and sterile filtered, was added to each well. During the ensuing 3-h incubation, the mitochondrial dehydrogenases in the viable cells cleaved the tetrazolium ring, resulting in formation of dark-blue formazan crystals. The crystals were dissolved by adding 100 μL of 0.04 N HCl in 2-propanol to each well, with thorough mixing as needed. The optical density of each well was then measured at 570 nm on a ELISA plate reader. The plates were read within 1 h following addition of the HCl and 2-propanol.

Synthetic methods for the preparation of compounds other than those described below have been reported earlier: 1a;[13] 1b;[10] 1c, 1d.[17]

4-Hydroxy-6-nitroquinazoline (6-Nitro-3,4-dihydro-4-oxoquinazoline) (4). A mixture of 50 g (0.24 mol) of 5-nitroisatoic anhydride and 320.2 g (7.11 mol) of formamide was heated at 160–165 °C for 6 h. After cooling, it was added to a large volume of cold H_2O and the resulting solid was separated by filtration, washed with H_2O, and dried under vacuum at 100 °C. There was obtained 41.7 g (91%) of white solid, mp 274–276 °C (lit.[26] mp 284 °C dec); TLC R_f 0.59 (EtOAc–MeOH, 4:1).

6-Amino-4-hydroxyquinazoline (6-Amino-3,4-dihydro-4-oxoquinazoline) (5). A solution of 14.0 g (73 mmol) of 4 in 275 mL of 2-methoxyethanol was hydrogenated at low pressure in the presence of 2.0 g of 10% Pd/C until H_2 uptake ceased. An additional 2.0 g of Pd/C was added and the procedure was repeated. After the addition of 300 mL of 2-methoxyethanol, the mixture was heated to ca. 95 °C, filtered through a Celite bed, and then evaporated to dryness under reduced pressure. The resulting solid was triturated in benzene, separated by filtration, and then dried under vacuum at 100 °C. There was obtained 10.86 g (92%) of a white crystalline solid, mp 302–303 °C dec (lit.[15] mp 302–304 °C dec); TLC R_f 0.49 ($CHCl_3$–MeOH, 4:1).

Di-*tert*-butyl 2-Desamino-5,8-dideazaisofolate (7). A mixture of 3.22 g (20 mmol) of 5 and 7.82 g (20 mmol) of di-*tert*-butyl (4-formylbenzoyl)-L-glutamate[10] in 160 mL of 70% AcOH was hydrogenated at low pressure in the presence of Raney Ni (ca. 1 g) until H_2 uptake ceased. The process was repeated with an additional ca. 1 g of Raney Ni. Charcoal was added and the reaction mixture was filtered through Celite and then basified to pH 8 with NH_4OH. The resulting solid was isolated by filtration, washed with H_2O and hexane, and then dried under vacuum. Next, it was dissolved in 200 mL of $CHCl_3$ and the solution washed successively with 2 × 100 mL of 10% $NaHSO_3$, 1 × 100 mL H_2O, 2 × 100 mL of 5% citric acid, and 2 × 100 mL H_2O and then dried over $MgSO_4$. After removal of the solvent at reduced pressure, the solid was recrystallized from EtOH–H_2O and dried under vacuum over P_2O_5 to yield 3.77 g (34%) of a white crystalline solid: mp 173–174 °C; TLC R_f 0.24 ($CHCl_3$–MeOH, 97:3); NMR (Me_2SO-d_6) δ 1.38 [s, 9, $C(CH_3)_3$], 1.40 [s, 9, $C(CH_3)_3$], 1.82–2.12 (m, 2, glu β-CH_2), 2.33 (t, 2, glu γ-CH_2, J = 7.44 Hz), 4.32 (m, 1, glu α-CH), 4.44 (app d, 2, $NHCH_2$, J = 5.61 Hz), 6.92 (t, 1, $NHCH_2$, J = 6.00 Hz), 7.02 (app d, 1, H_5, $J_{5,7}$ = 2.64 Hz), 7.18 (dd, 1, H_7, $J_{7,5}$ = 2.72 Hz, $J_{7,8}$ = 8.84 Hz), 7.42 (d, 1, H_8, $J_{8,7}$ = 8.79 Hz), 7.46 (d, 2, 3′, 5′, J_o = 8.16 Hz), 7.77 (s, 1, H_2), 7.83 (d, 2, 2′, 6′, J_o = 8.16 Hz), 8.54 (d, 1, CONH, J = 7.53 Hz), 11.86 (s, 1, lactam NH). Anal. ($C_{29}H_{36}N_4O_6 \cdot 0.85H_2O$) C, H, N.

2-Desamino-5,8-dideazaisofolic Acid (2b). A 0.70 g (1.3 mmol) sample of 7 was stirred in 10 mL of CF_3COOH at ambient temperature for 1 h. The CF_3COOH was removed under reduced pressure and the residue triturated with Et_2O. The solid was separated by filtration, washed with Et_2O, and then dissolved in H_2O and basified to pH 8.5 with NH_4OH. Precipitation was effected by the addition of 0.5 N HCl to pH 3.0. The product was separated by filtration and then dried under vacuum at 65 °C. There was obtained 0.49 g (82%) of white powder: mp 175–177 °C; TLC R_f 0.58; UV λ_{max} 236 nm (ε 34.6×10^3), 296 (ε 14.7×10^3); NMR (Me_2SO-d_6) δ 1.84–2.20 (m, 2, glu β-CH_2), 2.35 (t, 2, glu γ-CH_2, J = 7.41 Hz), 4.42 (m, 3, $NHCH_2$ and glu α-CH), 6.94 (app t, 1, $NHCH_2$), 7.01 (d, 1, H_5, $J_{5,7}$ = 2.55 Hz), 7.18 (d, 1, H_7, $J_{7,8}$ = 8.85 Hz), 7.42 (d, 1, H_8, $J_{8,7}$ = 8.85 Hz), 7.46 (d, 2, 3′, 5′, J_o = 8.19 Hz), 7.77 (s, 1, H_2), 7.84 (d, 2, 2′, 6′, J_o = 8.16 Hz), 8.57 (d, 1, CONH, J = 7.65 Hz). 11.92 (br s, 1, lactam NH), 12.42 (br s, 1, COOH); FAB/MS 425 (M + 1). Anal. ($C_{21}H_{20}N_4O_6 \cdot 1.7H_2O$) C, H; N: calcd, 12.31; found, 13.02.

Diethyl *N*-[4-[[[3,4-Dihydro-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]thio]benzoyl]-L-glutamate (10). A solution of tetraethyl 4,4′-dithiobis(benzoyl-L-glutamate) (0.24 g, 0.36 mmol) in 5 mL of EtOH was treated with 0.27 g (0.71 mmol) of $NaBH_4$. After stirring for 0.25 h, this solution was added dropwise to a stirred mixture of 6-(bromomethyl)-3,4-dihydro-4-oxo-3-[(pivaloyloxy)methyl]quinazoline, 8[5] (0.25 g, 0.69 mmol) in 8 mL of DMF at ambient temperature. After 7 h the solvent was removed under reduced pressure and the residue partitioned between $CHCl_3$ and H_2O. The organic phase was dried over $MgSO_4$ and the solvent removed under vacuum. The residue was dissolved in $CHCl_3$ and applied to a silica gel column (24 × 2.4 cm). After washing with $CHCl_3$, the product was eluted with $CHCl_3$–MeOH; 99:1. After solvent removal and drying under vacuum at ambient temperature, there was obtained 0.34 g (80%) of product: mp 72–76 °C; TLC R_f 0.31 ($CHCl_3$–MeOH, 99:1); NMR (Me_2SO-d_6) δ 1.07–1.27 (m, 6, CH_2CH_3 superimposed on s, 9, $C(CH_3)_3$), 1.90–2.17 (m, 2, glu β-CH_2); 2.42 (t, 2, glu γ-CH_2, J = 7.53 Hz), 4.00–4.17 (m, 4, CH_2CH_3), 4.40 (m, 1, glu α-CH), 4.53 (s, 2, CH_2S), 5.89 (s, 2, $COOCH_2N$), 7.44 (d, 2, 3′, 5′, J_o = 8.43 Hz), 7.65 (d, 1, H_8, $J_{8,7}$ = 8.31 Hz), 7.78 (d, 2, 2′, 6′, J_o = 8.46 Hz), 7.90 (dd, 1, H_7, $J_{7,5}$ = 2.13 Hz, $J_{7,8}$ = 8.37 Hz), 8.23 (d, 1, H_5, $J_{5,7}$ = 2.04 Hz), 8.43 (s, 1, H_2), 8.67 (d, 1, CONH, J = 7.29 Hz); FAB/MS 612 (M + 1). Anal. ($C_{31}H_{37}N_3O_8S$) C, H, N.

2-Desamino-5,8-dideaza-10-thiafolic Acid (2c). A mixture of 0.30 g (0.49 mmol) of 10 in 17 mL of 50% aqueous EtOH and 2.6 mL of 1 N NaOH was stirred at ambient temperature for 2 h. After filtration, the solution was neutralized to pH 2.5 with 1 N HCl and diluted with 12 mL of H_2O. After cooling, the precipitated solid was separated by centrifugation, washed 3 × 25 mL with H_2O, and then dried under vacuum over P_2O_5 at 65 °C to give 0.15 g (68%) of white powder: mp 200–201 °C dec (with preliminary softening); TLC R_f 0.56; UV λ_{max} 228 nm (ε 32.2×10^3), 276 (ε 16.9×10^3); NMR (Me_2SO-d_6) δ 1.76–2.20 (m, 2, glu β-CH_2), 2.33 (t, 2, glu γ-CH_2, J = 7.20 Hz), 4.37 (m, 1, glu α-CH), 4.51 (s, 2, CH_2S), 7.43 (d, 2, 3′, 5′, J_o = 8.43 Hz), 7.61 (d, 1, H_8, $J_{8,7}$ = 8.37 Hz), 7.79 (d, 2, 2′, 6′, J_o = 8.40 Hz), 7.85 (d, 1, H_7, $J_{7,8}$ = 8.49 Hz), 8.05 (s, 1, H_5), 8.16 (s, 1, H_2), 8.56 (d, 1, CONH, J = 7.44 Hz), 12.24 (br s, 1, superimposed on the peak for lactam NH, 2, COOH); FAB/MS 442 (M + 1). Anal. ($C_{21}H_{19}N_3O_6S \cdot 0.7H_2O$) C, H, N.

6-[(4-Carbomethoxyphenoxy)methyl]-3,4-dihydro-4-oxo-3-[(pivaloyloxy)methyl]quinazoline (11). A stirred mixture of 1.36 g (8.94 mmol) of methyl 4-hydroxybenzoate and 1.74 g (8.97 mmol) of $CsHCO_3$ in 35 mL of dry DMF was warmed to 65 °C. Next, a solution of 6-(bromomethyl)-3,4-dihydro-4-oxo-3-[(pivaloyloxy)methyl]quinazoline, 8 (3.16 g, 8.94 mmol) in 15 mL of DMF was added and the heating was continued for 2 h. The solvent was removed under vacuum and 50 mL of H_2O was added to the residue. This mixture was extracted with 5 × 50 mL of $CHCl_3$ and the organic layers were combined and dried over $MgSO_4$. After removal of the solvent at reduced pressure, the resulting oil was dissolved in ca. 10 mL of $CHCl_3$ and slurried with silica gel, and the mixture was applied to a silica gel column

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(26 × 2.8 cm). Elution with $CHCl_3$–MeOH, 99:1, removal of the solvent under vacuum, and drying under vacuum over P_2O_5 at 65 °C gave 2.55 g (67%) of 11: mp 107.5–113.5 °C; TLC R_f 0.17 (Whatman KC18F reverse phase, MeOH–H_2O, 8:2); NMR ($CDCl_3$) δ 1.18 [s, 9, $C(CH_3)_3$], 3.87 (s, 3, CH_3), 5.22 (s, 2, CH_2O), 5.94 (s, 2, $COOCH_2$), 6.98 (d, 2, 3′, 5′, J_o = 8.88 Hz), 7.77 (d, 1, H_8, $J_{8,7}$ = 8.37 Hz), 7.85 (dd, 1, H_7, $J_{7,8}$ = 8.37 Hz, $J_{7,5}$ = 1.95 Hz), 7.98 (d, 2, 2′, 6′, J_o = 8.85 Hz), 8.33 (s, 1, H_2), 8.37 (d, 1, H_5, $J_{5,7}$ = 1.71 Hz). Anal. ($C_{23}H_{24}N_2O_6$) C, H, N.

6-[(4-Carboxyphenoxy)methyl]-3,4-dihydro-4-oxoquinazoline (12). A mixture of 11 (2.4 g, 5.65 mmol), EtOH (65 mL), H_2O (65 mL), and 1 N NaOH (20 mL) was stirred at ambient temperature for 48 h. The solution was filtered and the filtrate acidified to pH 5.0 with 1 N HCl. The mixture was stirred and then cooled in the refrigerator for 1 h. The precipitated solid was separated by filtration, washed with water (75 mL), and dried under vacuum over P_2O_5 at 100 °C for 5 h. There was obtained 1.36 g (81%) of a white solid: mp >300 °C; TLC R_f 0.08; NMR (Me_2SO-d_6) δ 5.35 (s, 2, CH_2), 7.13 (d, 2, 3′, 5′, J_o = 8.28 Hz), 7.71 (d, 1, H_8, $J_{8,7}$ = 8.37 Hz), 7.90 (d, 2, 2′, 6′, J_o = 8.19 Hz, superimposed upon dd, 1, H_7), 8.11 (s, 1, H_5), 8.20 (s, 1, H_2), 12.31 (s, 1, lactam NH), 12.48 (br s, 1, COOH). Anal. ($C_{16}H_{12}N_2O_4 \cdot 0.15H_2O$) C, H, N.

Di-*tert*-butyl 2-Desamino-5,8-dideaza-10-oxafolate (13). A solution of Et_3N (0.10 g, 0.99 mmol) in 2 mL of dry DMF was added to a stirred solution of 12 (0.15 g, 0.5 mmol) in 20 mL of DMF under a N_2 atmosphere. This was followed by the addition of a solution of diethyl phosphorocyanidate (0.16 g, 0.98 mmol) in 2 mL of DMF. The clear colorless solution was stirred at ambient temperature for 3 h when it attained a yellow color. To this solution was added a solution of di-*tert*-butyl L-glutamate hydrochloride (Sigma Chemical Co.) (0.16 g, 0.54 mmol) in 2 mL of DMF. The resulting mixture was then stirred at ambient temperature for 3 h. The solvent was removed under reduced pressure and the residue dissolved in $CHCl_3$ (30 mL). The solution was washed with 15 mL of 10% $NaHCO_3$, saturated NaCl, and dried over $MgSO_4$. Removal of the solvent at reduced pressure gave a yellow oil which was dissolved in $CHCl_3$ (0.6 mL) and applied to a silica gel column (24.5 × 0.9 cm). The column was eluted first with $CHCl_3$–MeOH, 99:1, followed by elution of the product with $CHCl_3$–MeOH, 98:2. Removal of the solvent gave a solid which was dried under vacuum over P_2O_5 at 65 °C for 20 h. There was obtained 0.21 g (79%) of a white solid: mp 172–173.5 °C dec (with preliminary softening); TLC R_f 0.38 ($CHCl_3$–MeOH, 95:5); NMR (Me_2SO-d_6) δ 1.38 [s, 9, $C(CH_3)_3$], 1.40 [s, 9, $C(CH_3)_3$], 1.84–2.12 (m, 2, glu β-CH_2), 2.33 (t, 2, glu γ-CH_2, J = 9.0 Hz), 4.20–4.36 (m, 1, glu α-CH), 5.35 (s, 2, CH_2O), 7.13 (d, 2, 3′, 5′, J_o = 8.70 Hz), 7.70 (d, 1, H_8, $J_{7,8}$ = 8.34 Hz), 7.86 (d, 2, 2′, 6′, J_o = 8.67 Hz, superimposed upon d, 1, H_7), 8.11 (s, 1, H_5), 8.21 (s, 1, H_2), 8.45 (d, 1, CONH, J = 7.44 Hz), 12.32 (s, 1, lactam NH). Anal. ($C_{29}H_{35}N_3O_7 \cdot 0.5H_2O$) C, H, N.

2-Desamino-5,8-dideaza-10-oxafolic Acid (2d). A 0.10 g (0.18 mmol) sample of 13 was dissolved in 2 mL of CF_3COOH and stirred at ambient temperature for 2 h. The CF_3COOH was removed under vacuum and the residue triturated three times with Et_2O. The dried solid was suspended in 3 mL of H_2O and basified to pH 11.0 with 15% NH_4OH. Insoluble material was removed by filtration and the filtrate acidified to pH 3.0 with 5 N HCl. After refrigeration, the precipitate was separated by filtration, washed with H_2O, and dried under vacuum over P_2O_5 at 50 °C for 17 h to yield 49 mg (59%) of a white powder: mp 222–228 °C dec (with preliminary softening); TLC R_f 0.32; UV λ_{max} 230 nm (ε 30.3 × 10^3), 256 (ε 22.5 × 10^3); NMR (Me_2SO-d_6) δ 1.80–2.20 (m, 2, glu β-CH_2), 2.35 (t, 2, glu γ-CH_2, J = 7.32 Hz), 4.38 (m, 1, glu α-CH), 5.35 (s, 2, CH_2O), 7.13 (d, 2, 3′, 5′, J_o = 8.55 Hz), 7.70 (d, 1, H_8, $J_{8,7}$ = 8.25 Hz), 7.87 (d, 2, 2′, 6′, superimposed upon dd, 1, H_7), 8.11 (app d, 1, H_5), 8.21 (s, 1, H_2), 8.47 (d, 1, CONH, J = 7.41 Hz), 12.34 (two partially overlapping broad singlets, 3, 2 COOH and lactam NH); FAB/MS 426 (M + 1). Anal. ($C_{21}H_{19}N_3O_7 \cdot 1.95H_2O$) C, H; N: calcd, 9.12; found, 8.71.

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Synthesis and Evaluation of the Pharmacological Activity of Rigid Analogues of Sympathomimetic Catecholamines Derived from Bicyclo[2.2.1]heptane[1]

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endo-3-Amino-*exo*-2-(3,4-dihydroxyphenyl)-2-hydroxybicyclo[2.2.1]heptane (**4a**) and its *N*-isopropyl derivative (**4b**) were synthesized and assayed for their adrenergic activity on various isolated preparations. Compounds **4a** and **4b**, tested up to a dose of 10^{-4} M, did not reveal any activity, either stimulant or blocking, on the α- and β-adrenoceptors. Possible rationalizations of the results obtained, however, are suggested.

Natural catecholamines are flexible molecules that can exist in several conformations. The free energy differences between the various conformers are not high enough to make it possible to define a priori the precise molecular shape (i.e., conformation) required for effective direct interaction with α- and β-adrenoceptors. The problem of the identification of the conformation(s) of these compounds that is(are) active at the receptor site has been faced in various ways;[3] one of these consists of the comparative study of the adrenergic properties of a variety of cyclic compounds in which certain portions of the cathe-

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