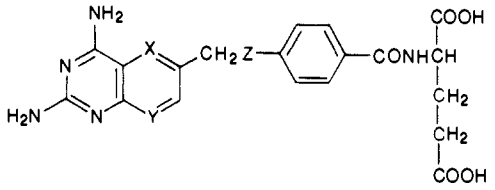
Synthesis and Antifolate Properties of 5,10-Methylenetetrahydro-8,10-dideazaminopterin

J. I. DeGraw,*† W. T. Colwell,† R. L. Kisliuk,‡ Y. Gaumont,‡ and F. M. Sirotnak§

Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025, Department of Biochemistry, Tufts University Medical School, Boston, Massachusetts 02111, and Laboratory for Molecular Therapeutics, Sloan Kettering Cancer Center, New York, New York 10021. *Received December 12, 1985*

The synthesis of the 5,10-methylene analogue of 5,6,7,8-tetrahydro-8,10-dideazaminopterin, a potential dual inhibitor of dihydrofolate reductase (DHFR) and thymidylate synthase (TS) enzymes, is described. The dimethyl ester of 10-carboxy-4-amino-4-deoxy-8,10-dideazapteroic acid was converted to the tetrahydro derivative by hydrogenation. Thermally induced cyclization of the 10-carbomethoxy and the 5-NH groups afforded the 5,10-carbonyl analogue. Reduction of the lactam with borane readily yielded the key 5,10-methylene-4-amino-4-deoxy-8,10-dideazatetrahydropteroic acid methyl ester. Saponification of the benzoate ester and coupling with L-glutamate concluded the synthesis. The title compound was a modest inhibitor of growth in folate-dependent bacteria, *Streptococcus faecium* and *Lactobacillus casei*, but inhibition of DHFR or TS derived from *L. casei* was poor. The compound was also a weak inhibitor of DHFR derived from L1210 murine leukemia and was a weak inhibitor of L1210 growth in culture.

The potent inhibitory action of aminopterin[1] (Ia), methotrexate (MTX)[2] (Ib), and their 10-deaza analogues[3] (Ic) on dihydrofolate reductase is well-established. More recently 5,10[4]- (Id) and 8,10-dideaza[5] (Ie) analogues of these powerful antifolates were also shown to be good inhibitors. The 8,10-dideaza series including 10-alkyl compounds[6] were exceptionally potent inhibitors of *Lactobacillus casei* and *Streptococcus faecium* growth in vitro and L1210 leukemia in mice. Fully aromatic compounds (Ia–e) are



Ia, X = Y = N; Z = NH
b, X = Y = N; Z = NCH_3
c, X = Y = N; Z = CH_2, $CHCH_3$
d, X = CH; Y = N; Z = CH_2
e, X = N, Y = CH; Z = CH_2

generally poor inhibitors of thymidylate synthase (TS) with values in the 100–300 μM range. However, it has been noted that conversion to the 5,6,7,8-tetrahydro derivatives can increase potency against TS by a factor of 10 or more in the aminopterin series.[7] The mechanism of action of TS and structure–activity effects of inhibitors have been reviewed by Jackman et al.[8]

With the above considerations in mind we considered it to be of interest to learn whether 5,10-methylene-8,10-dideazatetrahydroaminopterin (1) could function as a dual inhibitor of TS and DHFR and perhaps exert a synergistic antifolate effect. This compound is of the highly potent 8,10-dideaza series suggesting that one could accept some loss of potency insofar as DHFR inhibition is concerned. While being a 2,4-diamino compound reduces its affinity for TS, the 5,6,7,8-tetrahydro system and a 5,10-methylene moiety are in its favor for interaction with TS. The methylene unit is sp^3 and unreactive, but if oxidatively metabolized the carbon–carbon link at C-10 would prevent any one-carbon transfer.

Chemistry. The route used for the synthesis of the target compound 1 is shown in Scheme I. Dimethyl 4-amino-4-deoxy-10-carboxy-8,10-dideazapteroate (2) was prepared by alkylation of dimethyl homoterephthalate with 2,4-diamino-6-(bromomethyl)-8-deazapteridine as previously described.[6] Hydrogenation of 2 over PtO_2 in MeOH containing trifluoroacetic acid (TFA) cleanly afforded the 5,6,7,8-tetrahydro derivative (3) as the TFA salt. The salt was surprisingly stable to chromatographic purification on silica gel and was resistant to attempts to force ring closure to the lactam ester (4). Conversion to the free base was effected by treatment with 10% K_2CO_3; however, the base was also resistant to lactam formation, and required heating at 170–180 °C to cause ring closure to give 4.

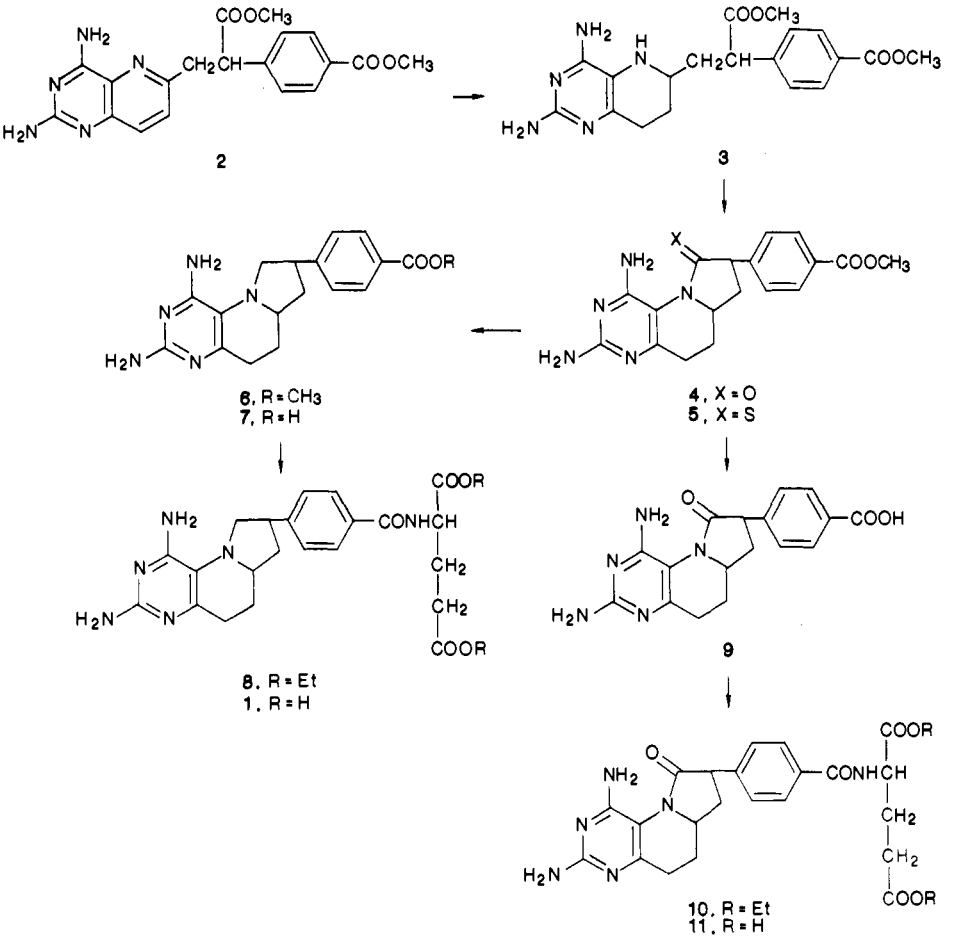
Our initial approach to reduction of the lactam 4 to the 5,10-methylene 8,10-dideazapteroate ester (6) proceeded through the thiolactam ester (5). Compound 5 was obtained in poor (10%) yield by reaction of 4 with P_2S_5 in pyridine. Most of the material so obtained appeared to be "phosphate" complexes of the product that underwent considerable decomposition when treated with strong acids to break up the complexes. It is interesting that Lawesson's reagent,[9] which normally effects facile conversion of lactam to thiolactam, also required refluxing pyridine to promote any reaction, but also caused attack at the benzoate ester. Desulfurization of 5 could only be achieved by treatment with Raney nickel in 2-methoxyethanol at 100 °C. The low yields and difficulty in purification ob-

† Bio-Organic Chemistry Laboratory.
‡ Department of Biochemistry.
§ Laboratory for Molecular Therapeutics.

(1) Farber, S.; Diamond, L. K.; Dercer, R. D.; Sylvester, R. F. Wolff, J. A. *New Engl. J. Med.* **1948**, *238*, 787.
(2) Burchenal, J. H.; Kushida, M. N.; Johnston, S. F.; Cremer, M. A. *Proc. Soc. Exptl. Biol. Med.* **1949**, *71*, 559.
(3) DeGraw, J. I.; Brown, V. H.; Tagawa, H.; Kisliuk, R.; Gaumont, Y.; Sirotnak, F. M. *J. Med. Chem.* **1982**, *25*, 1227. DeGraw, J. I.; Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M.; Nair, M. G. *J. Med. Chem.* **1974**, *17*, 552.
(4) Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1985**, *28*, 914. DeGraw, J. I.; Tagawa, H.; Christie, P. H.; Lawson, J. A.; Brown, E. G.; Kisliuk, R. L.; Gaumont, Y. *J. Heterocycl. Chem.* **1986**, *23*, 1.
(5) DeGraw, J. I.; Kelly, L. F.; Kisliuk, R. L.; Gaumont, Y. *J. Heterocycl. Chem.* **1982**, *19*, 1587. DeGraw, J. I.; Kelly, L. F.; Kisliuk, R. L.; Gaumont, Y.; Sirotnak, F. M. *Chemistry and Biology of Pteridines*; DeGruyter: Berlin and New York, 1983; pp 457–461.
(6) DeGraw, J. I.; Christie, P. H.; Brown, E. G.; Kelly, L. F.; Kisliuk, R. L.; Gaumont, Y.; Sirotnak, F. M. *J. Med. Chem.* **1984**, *27*, 376.
(7) Kisliuk, R. L.; Levine, M. D. *J. Biol. Chem.* **1964**, *239*, 1900.
(8) Jackman, A. L.; Jones, T. R.; Calvert, A. H. In *Experimental and Clinical Progress in Cancer Chemotherapy*; Muggia, F. M., Ed.; Nijhoff, Hingam: Boston, MA, 1985; Vol. 24, pp 155–210.
(9) Scheibye, S.; Pedersen, B. S.; Lawesson, S. O. *Bull. Soc. Chem. Belg.* **1978**, *87*, 229.

0022-2623/86/1829-1786$01.50/0

Scheme I



viated further use of the thiolactam route.

Direct reduction of the lactam ester (4) was first attempted with disiamylborane, since this reagent is known to reduce tertiary amides with a low degree of reactivity toward ester functionality.[10] The reagent apparently reacted with the 2,4-diamino groups, since all starting material was converted to fast-moving, UV-absorbing spots on TLC examination. Digestion with dilute HCl only served to regenerate starting 4.

Reduction with 1 M borane solution in THF, however, caused smooth reduction of the lactam carbonyl to methylene without attack at the ester or pyrimidine ring to afford 6 in 76% yield. The product was confirmed as structure 6 on the basis of its UV, NMR, and mass spectra as well as elemental analysis. Saponification of the benzoate ester was conducted with 1 N NaOH in 2-methoxyethanol at room temperature to give the acid (7). Coupling of 7 with diethyl L-glutamate with carboxyl activation via the mixed anhydride with isobutyl chloroformate yielded the glutamate ester (8) as shown by mass spectral evidence. Saponification of 8 as above afforded the target compound 1.

The lactam ester (4) was also hydrolyzed to the acid (9) and coupled with glutamate. The diester intermediate (10) was likewise saponified to yield the 5,10-carbonyl analogue (11).

It was formally possible to have obtained 4 or 6 as diastereomeric mixtures epimeric about the C-6 and C-10 positions. However, the NMR spectra of the target 1 and 4 and 6, as determined on a 400-MC instrument, strongly indicated a single diastereomer. The resolution-enhanced spectrum for 6 showed clear first-order signal patterns for the pertinent 3′,5′-benzoate, C-10, and benzoate methyl ester protons. Molecular models show significantly differing environments for those protons in the diastereomers; thus, more complex patterns would be expected from a mixture of isomers. The HPLC analyses of 1 and 7 and the sharp melting point for 6 also clearly reinforce this indication. We believe the stereochemistry of the newly created tricyclic compounds to be that of the more thermodynamically stable isomer as determined by the thermally induced ring closure of 3 to 4. The isomer with the hydrogen atoms at C-6 and C-10 in a trans-relationship is regarded as the more stable, since it is considerably more planar with fewer unfavorable interactions. The alternate cis-form would have the benzoate ring essentially perpendicular to and partially eclipsed by the 8-deazatetrahydropterin ring system.

Biological Results

The target compound (1) and the carbonyl analogue (11) were examined for their ability to inhibit growth in folate-dependent bacteria, *S. faecium* and *L. casei*, as shown in Table I. The 5,10-methylene compound (1) was about $^1/_{10}$ as potent as MTX for inhibition of *S. faecium*, but was only $^1/_{100}$ as potent as MTX against *L. casei*. Inhibition of DHFR derived from *L. casei* was also considerably less than that observed for MTX, falling into the micromolar range. Compound 1 was particularly unimpressive as an inhibitor of TS derived from the same organism.

Examination of 1 in L1210 murine leukemia cells produced a similar result. The compound was also ineffective ($K_i > 10^{-8}$ M) as an inhibitor of DHFR derived from L1210 cells and was only $^1/_{100}$ as potent as MTX as an inhibitor

(10) Brown, H. C.; Bigley, D. B.; Arora, S. K.; Yoon, N. M. *J. Am. Chem. Soc.* **1970**, *92*, 7161.

Table I. Growth and Enzyme Inhibition Data

compd	IC_{50},[b,e] ng/mL for inhibition of growth				IC_{50},[e] M		IC_{50},[c,d] M, for inhibition of growth, L1210
	Streptococcus faecium		*Lactobacillus casei*		dihydrofolate[a] reductase	thymidylate[a] synthase	
	ATCC 8043	MTX-resist	ATCC 7469	MTX-resist			
11	6.2	>10000	0.54	>10000	1.6×10^{-6}	$>2 \times 10^{-4}$	$>10^{-5}$
1	2.0	530	1.0	>10000	1.5×10^{-6}	$>5 \times 10^{-4}$	2.5×10^{-7}
MTX	0.2	3300	0.009	>500000	1.0×10^{-8}	1×10^{-4}	2.5×10^{-9}

[a] Derived from *Lactobacillus casei*. [b] Folate concentration = 1 ng/mL. [c] Compounds 1 and 11 were inactive ($K_i > 10^{-6}$ M) as L1210 DHFR inhibitors; MTX control was $K_i = 3.2 \times 10^{-12}$ M. [d] See Sirotnak, F. et al. *Biochem. Pharmacol.* **1979**, *28*, 2993 for methods. [e] See ref 3 for methods.

of growth for L1210 cells in culture. These observations in L1210 leave open the possibility that still another folate-related enzyme could be the locus of action for the modest growth inhibition shown by compound 1 both in L1210 and the bacterial cells. However, the compounds are essentially cross resistant with MTX in the resistant bacterial strains, suggesting a mechanism of action similar to MTX.

The carbonyl compound (11) was similarly ineffective as an inhibitor of DHFR or TS, but was actually twice as potent as a growth inhibitor of *L. casei* than was 1. This moderately potent inhibition of *L. casei* growth despite poor inhibition of DHFR and TS could also relate to inhibition of another folate enzyme. Compound 11 was inactive against growth of L1210 cells and also failed to inhibit DHFR derived therefrom.

Experimental Section

Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectra were obtained by using a LKB 9000 GC–MS. HPLC analyses utilized C_{18} Novapak columns eluting with 25:75 CH_3OH–0.1M NaH_2PO_4 at pH 6.7.

Thin-layer chromatographic analyses (TLC) were conducted on silica gel plates using CH_3OH–$CHCl_3$ (1:9) as eluent. The solvents dimethylformamide (DMF) and tetrahydrofuran (THF) were routinely dried over 4A molecular sieves before use. NMR spectra were taken on a Varian EM360A or XL400 spectrometer. UV spectra were run on a Perkin-Elmer 552 spectrophotometer.

4-Amino-4-deoxy-10-carboxytetrahydro-8,10-dideazapteroic Acid Dimethyl Ester (3). Compound 2 (12.9 g, 0.034 mol) was dispersed in 1 L of CH_3OH, and the stirred mixture was treated with 5.4 mL of CF_3COOH to yield a yellow solution. Two grams of crystalline PtO_2 was added, and the mixture was hydrogenated at atmospheric pressure. A quantitative uptake of hydrogen slowly occurred over 72 h. The catalyst was filtered and washed with CH_3OH, and the solvent was evaporated to yield 17.8 g of the CF_3CO_2H salt of the product as a pale-yellow foam. An aliquot was washed with CH_3OH and ether and dried for analysis. Anal. Calcd for $C_{19}H_{23}N_5O_4 \cdot CF_3CO_2H \cdot 1.5H_2O$: C, H, N.

The CF_3CO_2H salt was dissolved in 600 mL of $CHCl_3$ and stirred thoroughly with 200 mL of 10% K_2CO_3. The $CHCl_3$ solution was separated and filtered then evaporated to a constant weight of 11.5 g (83%) of a white solid. An infrared spectrum was free of absorptions for $CF_3CO_2^-$ at 1200, 1130, 840, and 800 cm^{-1}: UV max (pH 13) 290 nm, 235; (abs. EtOH) 312 nm, 235; NMR (Me_2SO-d_6) δ 1.5 (2 H, m, CH_2), 1.9 (2 H, m, CH_2), 2.4 (2 H, m, C-8H), 2.7 (H, m, C-6H), 3.55 (3 H, s, 10-$COOCH_3$), 3.80 (3 H, s, Ar$COOCH_3$), 4.20 (1 H, t, C-10H), 5.5 (2 H, br s, NH_2), 6.2 (2 H, br d, NH_2), 7.50 (2 H, d, 3′,5′-H), 7.90 (2 H, d, 2′,6′-H).

Methyl 4-Amino-4-deoxy-5,10-carbonyl-5,6,7,8-tetrahydro-8,10-dideazapteroate (4). Compound 3 (7.83 g, 0.020 mol) was placed in a short-neck distillation flask and heated under argon. At a bath temperature of 165 °C the solid began to melt. The heating was maintained at 175–180 °C for 45 min, at which time the melt resolidified and methanol evolution ceased. Upon cooling the resultant solid was powdered to yield a gray solid, 6.8 g (95%). TLC of the product indicated the formation of a single new compound. UV max (pH 13) 289 nm, 240; (abs. EtOH) 303 nm, 238; mass spectrum, *m/e* 353; NMR (Me_2SO-d_6) δ 1.5 (2 H, *m*, CH_2), 2.0 (2 H, m, CH_2), 2.4 (2 H, m, C-8H), 2.60 (1 H, m, C-6H), 3.85 (3 H, s, $COOCH_3$), 4.15 (1 H, t, C-10H), 6.0 (4 H, m, NH_2), 7.50 (2 H, d, 3′,5′-H), 7.90 (2 H, d, 2′,6′-H). Anal. Calcd for $C_{18}H_{19}N_5O_3$: C, H, N.

Treatment of 4 with an equivalent of P_2S_5 in pyridine at reflux for 4 h followed by evaporation and extraction into $CHCl_3$ afforded the thiolactam (5) in 10% yield after chromatography on silica gel: mass spectrum, *m/e* 369.

4-Amino-4-deoxy-5,10-carbonyl-5,6,7,8-tetrahydro-8,10-dideazapteroic Acid (9). Compound 4 (1.2 g 0.0034 mol) was dissolved in 17 mL of methoxyethanol and 9 mL of H_2O. The resulting solution was treated with 4.3 mL of 2.5 N NaOH (0.011 mol) and stirred under argon for 24 h. The solution was neutralized with HOAc and evaporated at 2 mmHg. The residue was stirred thorougly with 20 mL of H_2O (pH 5) and filtered, and the filtrate was washed with H_2O to yield, after drying, 1.005 (87%) of a tan solid: Anal. Calcd for $C_{17}H_{17}N_5O_3 \cdot 1.25\ H_2O$: N; C, 56.4. Found C, 56.9. UV max (pH 13) 298 nm, 230; mass spectrum, *m/e* 339.

Methyl 4-Amino-4-deoxy-5,10-methylene-5,6,7,8-tetrahydro-8,10-dideazapteroate (6). The lactam ester (4); 4.8 g 0.014 mol) was dissolved in 190 mL of THF under argon atmosphere. Commercial 1 M BH_3 solution (56 mL, 0.056 mol) was added dropwise, and the reaction was stirred for 22 h at 22 °C. The reaction was then treated with CH_3OH at 0 °C and stirred until H_2 evolution ceased. Gaseous HCl was introduced at 0 °C, and a white precipitate formed. The solvents were removed in vacuo and the residue treated with 300 mL of $CHCl_3$ and 150 mL of 10% K_2CO_3. A gummy residue formed and slowly dissolved. The $CHCl_3$ layer was dried over $MgSO_4$ and filtered, and the $CHCl_3$ was removed in vacuo to yield 3.48 g (76%) of product as a pale-tan solid. A sample was crystallized from warm benzene: mp 235–235.5 °C; UV max (abs. EtOH) 240 nm, 308; mass spectrum, *m/e* 339; NMR ($CDCl_3$, 400 mHz) δ 1.75 (1 H, m, C-7H), 1.90 (1 H, m, C-7H), 2.17 (1 H, m, C-9), 2.34 (1 H, m, C-9H), 2.67 (2 H, m, C-8H), 3.05 (1 H, t, C-10H), 3.41 (1 H, m, C-6H), 3.58 (2 H, m, 5,10-CH_2), 3.91 (3 H, s, $COOCH_3$), 4.46 (2 H, br s, NH_2), 4.60 (2 H, br s, NH_2), 7.41 (2 H, d, 3′,5′-H), 8.00 (2 H, d, 2′,6′-H). Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, N; H, 6.24. Found H, 6.73.

4-Amino-4-deoxy-5,10-methylene-5,6,7,8-tetrahydro-8,10-dideazapteroic Acid (7). The ester (6; 3.48 g, 0.0103 mol) was suspended in a mixture of 51 mL of methoxyethanol and 27 mL of H_2O and treated, under argon, with 0.88 g of NaOH (0.022 mol) in 12 mL of H_2O. The resulting solution was stirred at 22 °C for 6 h then stored at 5 °C for 15 h. The mixture was filtered then taken to pH 7 by addition of HOAc. The resulting mixture was taken to dryness at 2 mm pressure, and the residue was stirred thoroughly with 40 mL of H_2O. The mixture was filtered and the filtrate dried to yield a pale-tan solid, 2.5 g (75%). HPLC analysis showed only a single peak: UV max (pH 13) 290 nm, 235; mass spectrum, *m/e* 325. Anal. Calcd for $C_{17}H_{19}N_5O_2 \cdot H_2O$: C, H, N.

5,10-Carbonyl-5,6,7,8-tetrahydro-8,10-dideazaminopterin (11). Lactam acid (9; 1.0 g, 0.003 mol) was stirred in 14 mL of DMF under argon. To the suspension was added 0.92 mL (0.0066 mol) of triethylamine. The suspension was stirred for 25 min; then 0.86 mL (0.0066 mol) of isobutyl chloroformate was added to yield a deep-blue solution. After 30 min a mixture of 1.58 g 0.0066 mol) of diethyl L-glutamic acid hydrochloride and 0.92 g (0.0066 mol) of triethylamine in 7 mL of DMF was added and the mixture stirred for 20 h. The volatile materials were removed at 2 mmHg, and the residue was washed with 2 × 10 mL of H_2O. Saturated $NaHCO_3$ solution, 15 mL, and 30 mL of $CHCl_3$ were added and stirred thoroughly. The $CHCl_3$ layer was separated,

washed with 10 mL of H_2O, dried over $MgSO_4$, filtered, and evaporated. The resulting solid was chromatographed on Baker "flash" SiO_2 eluting with 5% MeOH–$CHCl_3$. Fractions with TLC R_f 0.5 were combined to yield 170 mg of light-yellow solid (11%): mass spectrum, m/e 524. The diester **10** (0.17 g, 0.000 32 mol) was dissolved in 2.5 mL of methoxyethanol and treated with 2.5 mL of 1.0 N NaOH solution (0.0025 mol). After 3 h at 22 °C the solution was taken to pH 7 by HOAc addition. The solvents were then removed at 2 mmHg. The residue was stirred with 10 mL of H_2O and filtered to yield the product, 50 mg (33%), as a white solid: UV max (pH 13) 246 nm (ε 21 360), 297 (10 030); mass spectrum, m/e 468 (bis trimethyl derivative). Anal. Calcd for $C_{22}H_{24}N_6O_6$: C, H, N.

5,10-Methylene-5,6,7,8-tetrahydro-8,10-dideazaminopterin (1). This reaction was conducted on 1.5 g of **7** in the same manner as described for the preparation of compound **10** to yield 1.7 g of crude product. Chromatography was conducted in the same manner as previously described with fractions that showed a TLC R_f = 0.4 being combined to yield the product as a tan solid (20%): mass spectrum, m/e 510. Anal. Calcd for $C_{22}H_{26}N_6O_5$: C; H; N, 16.9. Found N, 16.4.

The diester (**8**) was saponified in the manner described for the preparation of compound **11** to yield the product as a white solid (68%): UV max (pH 13) 305 nm (ε 5100), 242 (18 800), 225 sh (16 700), (pH 1) 310 nm (ε 4000), 232 (29 200); HPLC 97% pure; mass spectrum, m/e 454; NMR (Me_2SO-d_6, 400 MHz) δ 1.70 (1 H, m, C-7H), 1.80 (1 H, m, C-7H), 2.00 (1 H, m, CH), 2.05 (1 H, m, CH both of glutamate CH_2), 2.15 (2 H, m, C-9H), 2.35 (2 H, m, CH_2COOH), 2.54 (2 H, m, C-8H), 2.95 (1 H, t, C-10H), 3.47 (1 H, m, C-6H), 3.65 (2 H, m, 5,10-CH_2), 4.35 (1 H, m, CHNH), 6.25 (2 H, br s, NH_2), 6.40 (2 H, br s, NH_2), 7.50 (2 H, d, 3′,5′-H), 7.85 (2 H, d, 2′,6′-H), 8.42 (1 H, d, NH). Anal. Calcd for $C_{22}H_{26}N_6O_5$: C, H, N.

Acknowledgment. This work was supported by NIH Grants Ca-28783 (JID), Ca-08748, Ca-18856 (FMS), and Ca-10914 (RLK). We thank Dr. David Thomas, SRI International, for mass spectrometric analyses.

Registry No. 1, 103003-96-9; **2**, 88392-93-2; **3**, 103003-97-0; **3** (TFA salt), 103024-65-3; **4**, 103003-98-1; **5**, 103003-99-2; **6**, 103004-00-8; **7**, 103004-01-9; **8**, 103004-02-0; **9**, 103004-03-1; **10**, 103004-04-2; 11, 103004-05-3; diethyl L-glutamate hydrochloride, 1118-89-4; diethyl L-glutamate, 16450-41-2; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2; folic acid, 59-30-3.

Studies on Ca^{2+} Channel Antagonists. 5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylpentyl Isothiocyanate, a Chemoaffinity Ligand Derived from Verapamil

Louis J. Theodore,[†] Wendel L. Nelson,*[†] Ray H. Zobrist,[‡] Kathleen M. Giacomini,[§] and John C. Giacomini*[‡]

Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, Washington 98195, Stanford University Medical School, Veterans Administration Hospital, Palo Alto, California 94305, and Department of Pharmacy, School of Pharmacy, University of California, San Francisco, California 94143. *Received December 9, 1985*

Reduction of **1** (verapamil) afforded amine **2**, which was converted with thiophosgene to isothiocyanate **3**, a chemoaffinity ligand for Ca^{2+} channels. Compound **3** showed concentration-dependent negative inotropic effects in rat right myocardial ventricular strips, EC_{50} = (4.56 ± 3.40) × 10^{-6} M (mean ± SD), being slightly less potent than **4** (gallopamil), EC_{50} = (1.95 ± 1.22) × 10^{-6} M. It displaced [^{3}H]gallopamil in rat myocardial membranes, IC_{50} = (3.42 ± 2.51) × 10^{-7} M, approximately equipotent with **1**. It showed irreversible antagonism of [^{3}H]gallopamil binding when preincubated at 10^{-5} M; only 25% of [^{3}H]gallopamil binding vs. control was observed. This agent may be a useful chemoaffinity ligand to aid in characterization of Ca^{2+} channels.

Influx of extracellular Ca^{2+} through ion channels is thought to be an important process in the excitation coupling of contraction of cardiac and smooth muscle.[1-4] Ca^{2+} channel antagonists like verapamil (**1**), nifedipine, diltiazem, prenylamine, and their congeners have provided important therapeutic advances in the treatment of a variety of cardiovascular diseases.[5-8] Study of a large number of these chemically diverse agents has provided significant advances in our understanding of the processes involved in Ca^{2+} mediated contraction of excitable tissues. Ligand binding studies have provided evidence indicating allosteric regulation of multiple sites by different classes of ligands.[9-13]

The use of chemoaffinity and photoaffinity ligands has aided our understanding of the biochemistry and pharmacology of these agents, especially the dihydropyridine Ca^{2+} channel antagonists.[14-16] In this paper, we report preparation of an isothiocyanate chemoaffinity ligand, **3**, derived from verapamil, **1**, and the characterization of its interaction with verapamil binding sites in rat myocardium membrane homogenates.

[†] Department of Medicinal Chemistry.
[‡] Stanford University Medical School.
[§] Department of Pharmacy.

(1) Kohlhardt, M. *Basic Res. Cardiol.* **1981**, *76*, 589–601.
(2) Bolton, T. B. *Physiol. Rev.* **1979**, *59*, 607–718.
(3) Reuter, H. *Nature (London)* **1983**, *301*, 569–574.
(4) Fleckenstein, A. *Cir. Res. Suppl. I* **1983**, *52*, 3–16.
(5) Henry, p. D. *Am. J. Cardiol.* **1980**, *46*, 1047–1058.
(6) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 776–785.
(7) *Calcium Blockers—Mechanism of Action and Clinical Implications*; Flaim, S. F., Zelis R., Eds.; Urban and Schwarzenberg: Baltimore, MD, 1982.
(8) Stone, P. H.; Antman, E. M.; Muller, J. E.; Braunwald, E. *Ann. Int. Med.* **1980**, *93*, 886–904.
(9) Murphy, K. M. M.; Gould, R. J.; Largent, B. L.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 860–864.
(10) Ehlert, F. J.; Roeske, W. R.; Itoga, E.; Yamamura, H. I. *Life Sci.* **1982**, *30*, 2191–2202.
(11) Glossman, H.; Ferry, D. R.; Goll, A.; Streissnig, J.; Schober, M. *J. Cardiovasc. Pharmacol. Suppl. 6* **1985**, *7*, S20–S30.
(12) De Pover, A.; Matlib, M. A.; Lee, S. W.; Dubē, G. P.; Grupp, I. L.; Grupp, G.; Schwartz, A. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 110–117.
(13) Janis, R. A.; Scriabine, A. *Biochem. Pharmacol.* **1983**, *32*, 3499–3507.

0022-2623/86/1829-1789$01.50/0