Synthesis and Antifolate Properties of 5,10-Ethano-5,10-dideazaaminopterin

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2-Carbomethoxy-4-(*p*-carbomethoxyphenyl)cyclohexanone was prepared in a four-step process and thermally condensed with 2,4,6-triaminopyrimidine to afford methyl 2,4-diamino-4-deoxy-7-hydroxy-5,10-ethano-5,10-dideazapteroate. Reduction of the 7-oxo function with borane gave the 7,8-dihydro pterin which was subsequently oxidized to the fully aromatic pteroate ester with dicyanodichlorobenzoquinone. Saponification of the benzoate ester, coupling with diethyl glutamate and final ester hydrolysis afforded the title compound. This novel deazaaminopterin analogue was approximately as potent as methotrexate in vitro in terms of DHFR and L1210 cell growth inhibition. There are indications of diastereomeric differences in the enzyme inhibition measurements. A significant transport advantage over MTX for influx into L1210 cells was observed. The compound was active against the E 0771 murine mammary solid tumor, but further investigation with individual diastereomers is required to define the ED_{50}.

Previous research from our laboratories has been concerned with structure–activity studies with analogues of the powerful antifolate drugs aminopterin and methotrexate (MTX) bearing alterations in the pterin ring system and the bridge region joining the pterin at C_6 to a benzoylglutamate moiety. These studies have encompassed compounds of the 10-deazaaminopterin,[1] 8,10-dideazaaminopterin,[2a,b] and 5,10-dideazaaminopterin[3a,b] series. By suitable variations of alkyl substituents in the C_9–C_{10} bridge region, we have been able to modulate the strong inhibition of the dihydrofolate reductase enzyme (DHFR) and achieve selective transport and accumulation in tumor cells while sparing sensitive host tissue from cytotoxic attack.[4] A prime example of these investigations has been 10-ethyl-10-deazaaminopterin, which has shown remarkable activity against a variety of murine and human tumor models[5,6] and non-small cell lung cancer in clinical studies.[7,8] In an effort to further understand the effects on transport properties and enzyme inhibition by apparently subtle changes in alkyl substituents, we have continued to pursue the 5,10-dideazaaminopterin series in particular. This structural class allows further variation in substitution at the C_5 position as well as the C_{9-10} bridge region.

Initial investigations with 5-deaza analogues of aminopterin and MTX were reported by Montgomery and coworkers[9a,b] and Taylor et al.[10] These compounds, including 5-methyl analogues, were found to be potent inhibitors of DHFR from L1210 and chicken liver sources and were potent growth inhibitors of L1210 cells in culture. Effects against L1210 in mice were similar to or less than MTX for the 5-H compounds[9b] but were increased for 5-alkyl analogues.[11] 5,10-Dideazaaminopterin has been reported by the Taylor group[12] as well as our own.[3a] 5,10-Dideazaaminopterins substituted with lower alkyl groups at the 10-position[3b,13] and at the 5-position[14] were subsequently investigated by these same groups. Little antitumor advantage was gained by the former alteration in terms of cytotoxicity or transport. The 5-alkyl variations likewise did not show cytotoxic activity significantly greater than MTX but offered improvement in transport influx.

Piper et al.[15] have carried the investigation further with preparation of 5-methyl-10-ethyl-5,10-dideazaaminopterin but did not report biological results. They did, however, report 5-methyl-10-propargyl-5-deazaaminopterin to be 30 times more cytotoxic to L1210 cells than MTX and with a 9-fold influx advantage. A dose of 36 mg/kg per day for 5 days showed about a 90% reduction of tumor volume in the E0771 murine mammary tumor assay. These interesting in vitro and in vivo results suggested that further research in this area of 5,10-doubly substituted analogues

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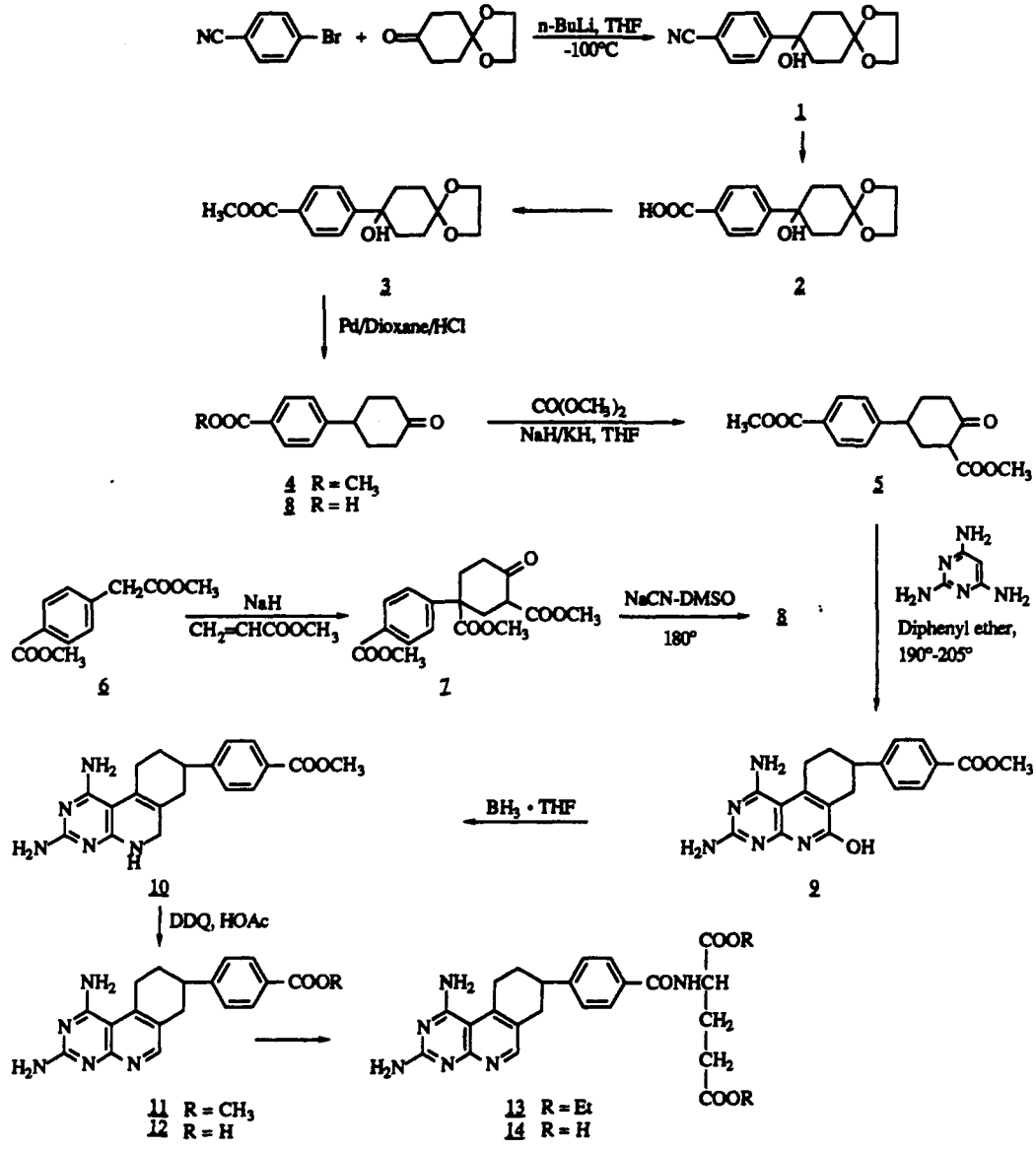
Scheme I. Synthesis of 5,10-Ethano-5,10-dideazaaminopterin



may be rewarding. In this paper we report the synthesis and biological activity of 5,10-ethano-5,10-dideazaaminopterin (14). This compound also embodies the elements of alkyl substitution at C_5 and C_{10}, but the rigidity introduced could affect both spatial and electronic properties vis-a-vis interaction with the DHFR and membrane transport protein.

Chemistry

The fundamental approach to the synthesis of the title compound (14) is based on the general observation of Hurlbert et al.[16] and Gangee et al.[17a,b] that condensation of 2,4,6-triaminopyrimidine with appropriate β-keto esters affords 2,4-diamino-7-hydroxy-5-deazapteridines substituted at the 5,6-positions. Subsequent removal of oxygen at C-7 by reductive means yielded the desired antifolate compounds. In Scheme I our procedure for the synthesis

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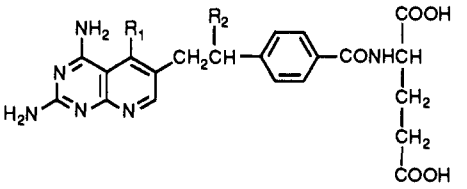
of 14 proceeding through the key intermediate, 4-(*p*-carbomethoxyphenyl)-2-carbomethoxycyclohexanone (5) is outlined.

Treatment of 4-bromobenzonitrile with *n*-BuLi at −100 °C in tetrahydrofuran formed the (4-cyanophenyl)lithium reagent[18] which was condensed with the monoethylene ketal of 1,4-cyclohexanedione to give the hydroxy nitrile 1 in 70% yield. The nitrile was saponified with hot 10% NaOH to afford the hydroxy ketal acid 2 as a very insoluble, highly crystalline solid. The acid was esterified by treatment with MeOH–HCl at reflux, and the resulting hydroxy ester 3 was subjected to hydrogenolysis over powdered palladium (100%) in dioxane containing 1% by volume of concentrated HCl. It was found necessary to heat the hydrogenation medium at 45 °C for 20 h to effect reduction. The acid catalysis was also critical to achieving successful hydrogenolysis. Addition of water to the solution promoted hydrolysis of the ketal moiety to ultimately afford 4-(*p*-carbomethoxyphenyl)cyclohexanone (4) in 37% yield from 1. When the anion of the keto ester (as formed by NaH) was condensed with dimethyl carbonate, the key 2-carbomethoxycyclohexanone (5) was obtained in 63% yield. In an alternate process, dimethyl homoterephthalate (6) was alkylated via Michael reaction with methyl acrylate with concomitant Dieckmann ring closure to give the triester 7. Decarboxylation–deesterification with NaCN–DMSO at 180 °C afforded 4-(*p*-carboxyphenyl)cyclohexanone (8), which was readily esterified to 4.

Condensation of the keto ester 5 with triaminopyrimidine in diphenyl ether at approximately 200 °C via the Hurlbert process[16] gave the 2,4-diamino-7-hydroxy-5-deazapteridine (9). It was interesting to note that 9 existed as a stable monohydrate as evidenced by its mass spectrum (M + 18) and elemental analysis. The UV and NMR spectra were consistent for structure 9. Attempts to deoxygenate 9 at C_7 via conversion to the 7-chloride followed by hydrogenolysis or formation of the 7-SH compound followed by desulfurization with Raney nickel at 100 °C were unrewarding. We then investigated the reduction of 9 with borane in THF. Previous work in our laboratory had shown that amido carbonyl reduction with BH_3 could be achieved in folate analogues without deleterious effect on heterocyclic or benzoate ester moieties.[19] Although some 7-H compound (11) was observed in the crude product from this direct reduction, the major product was the 7,8-dihydro intermediate (10). The UV spectrum as run in glacial HOAc (for solubility purposes) showed a shift from 302 nm in 9 to a value of 283 nm for 10. One or two retreatments with BH_3 were necessary as the initial reduction products showed UV maxima at about 290–295 nm, indicative of incomplete reduction.

Several oxidizing agents were investigated for conversion of 10 to the fully aromatic compound (11). Varying degrees of success were achieved, but the use of dicyanodichlorobenzoquinone in HOAc was found to be optimal for this process, affording 11 in about 35% overall yield from 9. Saponification of the benzoate ester with 10% NaOH in 2-methoxyethanol at room temperature gave the acid 12, which was coupled with diethyl L-glutamate via the mixed anhydride as prepared via the i-BuOCOCl/Et_3N reagent. The resulting diester 13 was purified by chromatography on silica gel and hydrolyzed with 1 N NaOH in methoxyethanol to yield the target analogue 14.

Table I. 5,10-Dideazaaminopterins Cell Growth and Enzyme Inhibition



		L1210[a]		
R_1	R_2	DHFR inhib K_i, nM	growth inhib IC_{50}, nM	L1210[b] relative influx
H	Me	0.007	23.9	1.43
H	Et	0.013	12.1	1.32
$-CH_2CH_2-$		0.017	4.5	3.3
MTX		0.006	3.9	1.0

[a] See ref 1 for methods. [b] Ratio versus MTX.

Biological Studies

In Table I data are presented for the 5,10-ethano compound (14) as compared with the 10-methyl and 10-ethyl analogues and methotrexate in vitro. Compound 14 was an effective inhibitor of the DHFR derived from L1210 murine leukemia cells. The compound was about 35% as potent as MTX and the 10-methyl analogue and 75% as potent as the 10-ethyl analogue. There was an indication that the diastereomers present in 14 differed widely in their ability to inhibit the enzyme since a biphasic curve was obtained in the K_i plot of percent inhibition versus molar concentration of inhibitor. The plot suggested that the more potent isomer would have a potency similar to the 10-methyl analogue.

In the inhibition of growth of L1210 cells in culture, compound 14 was very similar to MTX in ability to inhibit cell growth. The 10-ethyl analogue was one-third as potent as MTX, and the 10-methyl compound had only one-sixth the potency of MTX. The compounds were also studied for their ability to be transported into L1210 cells via the folate active transport system. The relative influx of the 10-methyl and ethyl analogues was moderately greater than MTX, but the ethano compound was 3.3 times greater than MTX. These observations indicated that compound 14 was a potent antifolate agent in vitro with a strong potential for enhanced transport into tumor cells.

In vivo data obtained from the treatment of mice inoculated with E0771 cells were not as compelling as the in vitro data, however. At a dose of 48 mg/kg of 14, only 9% reduction in volume of the solid tumor was noted at the 7-day observation period as compared with untreated control, but at day 14 a 40% reduction was obtained. MTX at 6 mg/kg achieved a similar result after 14 days but was more pronounced at the 7-day point with a 64% reduction of tumor volume. When the dose of 14 was increased to 90 mg/kg, a 96% reduction was seen at 7 days, but lethal drug-induced toxicity occurred before day 14. It is possible that an optimally effective dose may lie between 50 and 90 mg/kg per day.

The DHFR inhibition curve suggested that one diastereomer may be inactive thus the effective dose of the active isomer could be less than 50 mg/kg. Nonetheless, the in vivo activity seems to be less than the potent in vitro inhibition and favored transport data would indicate. Further investigation of in vivo activity for 14 should await separation of the diastereomeric mixture. The results do suggest that substitution at both the 5- and 10-positions can be tolerated for retention of inhibitory potency against DHFR and cell growth in L1210. Transport into L1210

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cells was also enhanced but is probably related to substitution on C_5 since the C_{10} alkyl substituents above did not significantly enhance transport in this series. This is in contrast to the 10-deazaaminopterin series previously reported where transport was routinely enhanced by C-10 substitutions. Cytotoxic activity has also tended to be less than or about equal to MTX in the 5,10-dideaza series, whereas the 10-alkyl analogues in the 10-deaza and 8,10-dideaza series were significantly more effective than MTX against L1210 cells.

Experimental Section

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. The ^1H NMR spectra were taken on a JEOL FX90Q spectrometer. Mass spectra were run on a LKB 9000 GC–MS spectrometer or a Ribermag R10-10C MS system. Ultraviolet spectra were taken on a Perkin-Elmer 552 or Perkin-Elmer-Coleman 575. Melting points were determined on a Thomas-Hoover Unimelt apparatus.

4-(4′-Cyanophenyl)-4-hydroxycyclohexanone Ethylene Ketal (1). A solution of 4-bromobenzonitrile (10 g, 0.055 mol) in 260 mL of dry THF and 70 mL of dry hexane under argon was cooled to −100 °C in a liquid nitrogen–Et_2O bath. *n*-Butyllithium (34.3 mL, 0.055 mol, 1.6 M solution in hexane) was added rapidly dropwise so that the internal temperature did not exceed −95 °C. The orange solution was stirred an additional 10 min at −100 to −95 °C and then treated dropwise over 10 min with a solution of 1,4-cyclohexanedione monoethylene ketal (8.57 g, 0.055 mol) in 55 mL of dry THF, again carefully maintaining the temperature below −95 °C. The reaction mixture was stirred for 10 min at −100 to −95 °C, allowed to warm to 20 °C, and poured into ice water (400 mL). The organic layer was separated, and the aqueous layer was extracted twice with Et_2O (200 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated to give 14.1 g of a white crystalline solid. Trituration with Et_2O afforded 9.9 g (70% yield) of white crystals: mp 162–5 °C; IR (Nujol) ν 3436 (OH), 2222 cm^{-1} (CN); NMR ($CDCl_3$) δ 1.6–2.2 (8 H, m, cyclohexane), 3.97 (4 H, s, ketal), 7.63 (4 H, s, Ar). Anal. Calcd for $C_{15}H_{17}NO_3$: C, H, N.

4-(4′-Carbomethoxyphenyl)cyclohexanone (4). A mixture of 1 (15.09 g, 0.058 mol) in 190 mL of 2-methoxyethanol and 190 mL of 2.5 N NaOH was heated on the steam bath for 15 h. The solution was cooled in an ice bath, adjusted to pH 7–8 with concentrated HCl, and evaporated to dryness. Water (375 mL) was added, and the pH was adjusted to 2 with HCl. The tan solid was filtered off and washed with water to give 15.2 g (94% yield) of 4-(4′-carboxyphenyl)-4-hydroxycyclohexanone ethylene ketal (2): IR (Nujol) showed no CN at 2222 cm^{-1}; NMR ($CDCl_3$ + CD_3OD) δ 1.6–2.3 (8 H, m, cyclohexane), 4.00 (4 H, s, ketal), 7.60 (2 H, d, 2′,6′-ArH), 8.00 (2 H, d, 3′,5′-ArH). Elemental analysis was precluded by presence of 3–5% loss of ketal and extreme insolubility in organic solvents.

A mixture of 2 (20.2 g, 0.073 mol) in 750 mL of MeOH and 15 mL concentrated HCl was stirred at reflux for 4 h and then evaporated to dryness. The residue was treated with saturated $NaHCO_3$ (pH 8) and extracted three times with $CHCl_3$ (200 mL). The $CHCl_3$ extracts were dried over $MgSO_4$ and evaporated to dryness to give a quantitative yield of 4-(4′-carbomethoxyphenyl)-4-hydroxycyclohexanone ethylene ketal (3) as a syrup that contained some ketone: NMR ($CDCl_3$) δ 3.90 (3 H, s, $COOCH_3$), 3.97 (4 H, s, ketal). The remaining bands were a mixture of ketone and ketal.

A mixture of the crude ketal 3 (9.5 g), 350 mL of dioxane, 3.5 mL of concentrated HCl, and 950 mg of powdered Pd (100%) was stirred under an H_2 atmosphere at 40–48 °C for 20 h. The reaction mixture was filtered, and the filtrate was stirred with 950 mg of fresh catalyst under H_2 at 40–48 °C for 48 h. The mixture was filtered, water (100 mL) was added, and the solution was stirred at room temperature for 72 h to effect ketal removal. The solution was evaporated, and the residue was adjusted to pH 8 with saturated $NaHCO_3$ and then extracted three times with $CHCl_3$. The $CHCl_3$ extracts were dried over $MgSO_4$ and evaporated to leave 5.0 g of a solid. Chromatography on 155 g of silica gel (flash chromatography grade) with 50% Et_2O–hexane gave 2.92 g (39% yield) of white crystalline 4. An analytical sample was recrystallized from Et_2O: mp 93–4 °C; NMR ($CDCl_3$) δ 2.00 (4 H, 3,5-CH_2), 2.50 (4 H, m, 2,6-CH_2), 3.07 (1 H, m, 4-H), 3.90 (3 H, s, $COOCH_3$), 7.30 (2 H, d, 2′,6′-ArH) 7.95 (2 H, d, 3′,5′-ArH). Anal. Calcd for $C_{14}H_{16}O_3$: C, H.

2-Carbomethoxy-4-(4′-carbomethoxyphenyl)cyclohexanone (5). Freshly distilled (over CaH_2) dimethyl carbonate (3.6 mL, 0.043 mol) was added to a stirred suspension of 60% NaH in oil (2.15 g, 0.054 mol) in 120 mL of dry THF under argon. The reaction mixture was heated to reflux and treated with 0.7 mL of a solution of 4 (4.02 g, 0.017 mol) in 30 mL of dry THF, followed by 2 mL of 35% KH. The rest of the cyclohexanone solution was added dropwise. The reaction mixture was heated at reflux for 2 h and then cooled in an ice bath. HOAc (4 mL) was added followed by 200 mL of H_2O (initially dropwise). The mixture was extracted three times with Et_2O, and the Et_2O extracts were dried over $MgSO_4$ and evaporated to give 6.3 g of an oil. Chromatography on 193 g of silica gel with elution by 10% Et_2O in hexane afforded 4.5 g (63%) of 5. An analytical sample was recrystallized from Et_2O–pentane: mp 86–89 °C; NMR ($CDCl_3$) δ 1.90 (4 H, m, 3,5-CH_2), 2.40 (3 H, m, C-2 H + 6-CH_2), 2.80 (1 H, m, C-4H), 3.70 (3 H, s, $COOCH_3$), 3.85 (3 H, s, $ArCOOCH_3$), 7.23 (2 H, d, 3′,5′-ArH), 7.92 (2 H, d, 2′,6′-ArH). Anal. Calcd for $C_{16}H_{18}O_5$: C, H.

4-(4-Carbomethoxyphenyl)-2,4-dicarbomethoxycyclohexanone (7). A solution of dimethyl homoterephthalate (6) (31.97 g, 0.15 mol) in 158 mL of DMF was added dropwise to an ice-cold suspension of pentane-washed 50% NaH (7.39 g, 0.15 mol) in 800 mL of dry DMF. The reaction mixture was stirred at 0–5 °C for 20 min and then treated dropwise with freshly distilled methyl acrylate (34.5 mL, 0.38 mol). The red solution was stirred cold for 20 min and then at room temperature for 20 h. DMF was evaporated in vacuo, and the residue was acidified to pH 5 with AcOH. Water (300 mL) was added, and the mixture was extracted three times with $CHCl_3$ (200 mL). The $CHCl_3$ extracts were dried over $MgSO_4$ and evaporated to dryness. The residual oil was stirred at 0–5 °C in 100 mL of 70% MeOH. The white solid was collected and washed with 70% MeOH. The yield was 41.44 g (77%). An analytical sample was recrystallized from Et_2O–pentane: mp 99.5–100.5 °C; NMR ($CDCl_3$) δ 2.0–2.5 (5 H, m, 5-CH_2, 6-CH_2 C-2 H), 2.9 (2 H, q, 3-CH_2), 3.65 (3 H, s, 4-$COOCH_3$), 3.83 (3 H, s, 2-$COOCH_3$), 3.92 (3 H, s, benzoate), 7.45 (2 H, d, 3′,5′-ArH), 8.00 (2′,6′-ArH). Anal. Calcd for $C_{18}H_{20}O_7$: C, H.

Decarbomethoxylation of Triester 7. The triester 7, 41.4 g (0.12 mol), was dissolved in 520 mL of dry DMSO. Sodium cyanide (17.49 g, 0.36 mol) was added, and the mixture was stirred under argon at 180–185 °C for 2 h. DMSO was removed by distillation. H_2O (825 mL) was added to the residue. The solution was filtered and acidified to pH 3 with 6 N HCl. The gummy solid was collected by filtration and washed with water. The crude product (18.9 g) contained mostly (*p*-carboxyphenyl)cyclohexane: MS *m/e* 218. Esterification of crude 8 in hot 5% HCl in MeOH (15 h) followed by chromatography on silica gel ($CHCl_3$) gave 4.63 g of 4 (17% yield from 7).

Methyl 4-Amino-4-deoxy-5,10-ethano-7-hydroxy-5,10-dideazapteroate (9). A mixture of 5 (2.53 g, 8.71 mmol) and 2,4,6-triaminopyrimidine (1.09 g, 8.71 mmol) in 18 mL of diphenyl ether was stirred at 190–205 °C for 4 h. The reaction mixture was cooled to room temperature, treated with 85 mL of MeOH, stirred for 10 min, and filtered. The solid was washed with MeOH and Et_2O, followed by treatment with hot water and filtration. The damp solid was triturated with hot 90% aqueous DMF to give 2.61 g (82%) of 9 as an off-white solid: UV (HOAc) λ_{max} 250, 302 nm; NMR (CF_3COOD) δ 2.00 (2 H, m, bridge CH_2), 3.00 (5 H, m, CH_2-C_5, 9-CH_2, 10 H), 3.85 (3 H, s, $COOCH_3$), 7.20 (2 H, d, 3′,5′-ArH), 7.90 (2 H, d, 2′,6′-ArH); MS *m/e* 383 (hydrate), 365. Anal. Calcd for $C_{19}H_{19}N_5O_3 \cdot H_2O$: H, N; C: calcd, 59.5; found, 59.0.

Methyl 4-Amino-4-deoxy-5,10-ethano-5,10-dideazapteroate (11). Borane–THF (1 M, 49.6 mL, 49.6 mmol) was added to an ice-cold suspension of 9 (3.62 g, 9.9 mmol) in 173 mL of dry THF under argon. The reaction mixture was stirred at room temperature for 16 h, cooled in ice, and adjusted to pH 2 by dropwise addition of 6 N HCl. The mixture was evaporated and water (250 mL) was added. The pH was adjusted to 7–8 with 1 N NaOH. The white solid was collected, washed with H_2O, dried, and

triturated with 300 mL of hot MeOH. The mixture was filtered, and the MeOH filtrate was evaporated to give 1.03 g (30% yield) of **10**: UV (AcOH) λ_{max} 248, 283 nm.

The MeOH-insoluble solid (2.46 g; UV (HOAc) λ_{max} 250, 302 nm) was retreated with BH_3 to afford another 0.9 g of **10** for a total yield of 56%.

DDQ (0.67 g, 2.93 mmol) was added to a solution of **10** (1.03 g, 2.93 mmol) in 100 mL of HOAc. The reaction mixture was stirred at room temperature for 18 h, then filtered. The filtrate was evaporated and the residue triturated with THF. The insoluble solid was collected by filtration and extracted with 200 mL of hot MeOH. The MeOH was evaporated to leave 0.62 g (61%) of **11**. An analytical sample was obtained by trituration with hot DMF: UV (HOAc) λ_{max} 250, 270, 320 nm; NMR (DMSO-d_6) δ 2.00 (2 H, m, bridge CH_2), 3.05 (5 H, m, CH_2-C_5, 9-CH_2, 10-H), 3.88 (3 H, s, $COOCH_3$), 7.50 (2 H, d, 3',5'-ArH), 7.95 (2 H, d, 2',6'-ArH), 8.51 (1 H, s, 7-H); MS *m/e* 349. Anal. Calcd for $C_{19}H_{19}N_5O_2 \cdot 1.4$ HCl: C, H, N, Cl.

5,10-Ethano-5,10-dideazaaminopterin Diethyl Ester (13). A suspension of 462 mg (1.32 mmol) of ester **11** in 8 mL of 2-methoxyethanol was treated with 1.36 mL (3.4 mmol) of 10% NaOH. The mixture was stirred at room temperature for 18 h; 10% NaOH (0.1 mL, 0.25 mmol) was added, and stirring was continued for another 20 h. The mixture was diluted with 5.5 mL of H_2O, acidified to pH 5 with AcOH, and stirred for 2 h. The tan precipitate was collected by filtration, washed with H_2O, and dried to leave 353 mg (80%) of the pteroic acid (**12**): MS *m/e* tris(trimethylsilyl) derivative, 551. This material was very insoluble in organic solvents and was not further characterized.

To a suspension of 335 mg (1 mmol) of the pteroic acid (**12**) in 7 mL of Me_2SO was added 0.28 mL (2 mmol) of Et_3N and 0.26 mL (2 mmol) of isobutyl chloroformate. The mixture was stirred for 1.5 h, and then 0.28 mL (2 mmol) of Et_3N and 479 mg (2 mmol) of diethyl L-glutamate hydrochloride were added. The mixture was stirred under argon for 4 h. The sequence was repeated as above with half the respective quantities of reagents, and the mixture was stirred for 18 h at room temperature. Ice water (70 mL) was added, and the tan precipitate was collected by filtration followed by washing with H_2O. Chromatography on 25 g of silica gel with elution by $CHCl_3$-MeOH (97.5:2.5) afforded 218 mg (44%). Trituration with 2-propanol gave 160 mg (31%) of analytically pure tan solid: NMR ($CDCl_3$ + CD_3OD) δ 1.27 (6 H, m, CH_3), 1.8-2.6 (6 H, m, bridge CH_2, CH_2CH_2), 3.05 (5 H, m, CH_2-C_5, 9-CH_2, 10-H), 4.17 (4 H, m, OCH_2CH_3), 4.70 (1 H, m, *CH*NH), 7.30 (2 H, d, 3',5'-ArH), 7.78 (2 H, d, 2',6'-ArH), 8.42 (1 H, s, 7-H); MS *m/e* 520. Anal. Calcd for $C_{27}H_{32}N_6O_5 \cdot H_2O$: C, H, N.

5,10-Ethano-5,10-dideazaaminopterin (14). A solution of 139 mg (0.27 mmol) of diester **13** in 2 mL of 2-methoxyethanol was treated with 2 mL of 1 N NaOH. The mixture was stirred at room temperature for 4 h. The addition of 3 mL of H_2O gave complete solution. The pH was adjusted to 5 with HOAc, and the mixture was evaporated to dryness in vacuo (0.5 mm) without application of heat. The residue was treated with 4 mL of H_2O, and the product was collected to afford 91 mg (73%): UV (pH 13) λ_{max} 240 (ϵ 43 876), 343 nm (ϵ 5809); NMR (CF_3COOD) δ 2.35 (1 H, m, bridge CH_2), 2.57 (1 H, m, CH_2CH_2COOH), 2.68 (1 H, m, bridge CH_2), 2.75 (1 H, m, CH_2CH_2COOH), 2.98 (2 H, m, CH_2COOH), 3.36 (2 H, m, CH_2-C-5), 3.55 (1 H, m, C-10), 3.83 (2 H, m, C-9), 5.24 (1 H, m, *CH*NH), 7.62 (2 H, d, 3',5'-ArH), 8.04 (2 H, d, 2',6'-ArH), 8.87 (1 H, s, 7-H); MS *m/e* 752 (TMS_4), 680 (TMS_3), 608 (TMS_2). Anal. Calcd for $C_{23}H_{24}N_6O_5 \cdot H_2O$: C, H, N.

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Registry No. **1**, 137464-95-0; **2**, 137464-96-1; **3**, 137464-97-2; **4**, 137464-98-3; **5**, 137464-99-4; **6**, 52787-14-1; **7**, 137465-00-0; **8**, 137465-01-1; **9**, 137465-02-2; **10**, 137465-03-3; **11**, 137465-04-4; **12**, 137465-05-5; **13**, 137465-06-6; **14**, 137465-07-7; DHFR, 9002-03-3; $CO(OCH_3)_2$, 616-38-6; CH_2=$CHCOOCH_3$, 96-33-3; 4-bromobenzonitrile, 623-00-7; 1,4-cyclohexanedione monoethylene ketal, 4746-97-8; diethyl L-glutamate hydrochloride, 1118-89-4; 2,4,6-triaminopyridine, 1004-38-2.

New Neplanocin Analogues. 1. Synthesis of 6'-Modified Neplanocin A Derivatives as Broad-Spectrum Antiviral Agents

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Novel neplanocin A analogues modified at the 6'-position, i.e., 6'-deoxy analogues (**2**, **3**, **6**, **9**, **20**), 6'-*O*-methylneplanocin A (**15**), and 6'-*C*-methylneplanocin A's (**22a** and **22b**) have been synthesized and evaluated for their antiviral activity in a wide variety of DNA and RNA virus systems. These compounds showed an activity spectrum that conforms to that of *S*-adenosylhomocysteine hydrolase inhibitors. They were particularly active against pox- (vaccinia), paramyxo- (parainfluenza, measles, respiratory syncytial), arena- (Junin, Tacaribe), rhabdo- (vesicular stomatitis), reo-, and cytomegalovirus. In order of (increasing) antiviral activity, the compounds ranked as follows: **3** < **15** ~ **20** < **6** < **9** ~ **2** < **22a**. Of the two diastereomeric forms of **22**, only **22a** was active; **22a** surpassed neplanocin A both in antiviral potency and selectivity. Compound **22a** appears to be a promising candidate drug for the treatment of pox-, paramyxo-, arena-, rhabdo-, reo-, and cytomegalovirus infections.

Introduction

The enzyme *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase, SAH hydrolase), which is responsible for the (reversible) hydrolysis of *S*-adenosyl-L-homocysteine to adenosine (Ado) and L-homocysteine (Hcy), has been recognized as an important target for broad-spectrum antiviral agents.[1,2] AdoHcy hydrolase is a key enzyme in transmethylation reactions using *S*-adenosyl-L-methionine (AdoMet, SAM) as the methyl donor. Such transmethylation reactions are involved in the maturation of viral mRNAs and hence play a critical role in the virus replicative cycle. Several adenosine analogues of both the acyclic and carbocyclic type are assumed to achieve their

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