Analogues of Methotrexate in Rheumatoid Arthritis. 2. Effects of 5-Deazaaminopterin, 5,10-Dideazaaminopterin, and Analogues on Type II Collagen-Induced Arthritis in Mice

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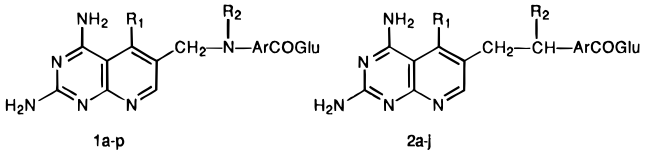
Twenty-six compounds derived from the 5-deaza- and 5,10-dideazaaminopterin series of aminopterin analogues were evaluated for antiarthritic activity in the mouse type II collagen model. New compounds in the 5-deaza series were prepared by alkylation of an appropriate *N*-substituted (4-aminobenzoyl)-L-glutamic acid dialkyl ester or *N*-(5-amino-2-thenoyl)-L-glutamate diester with a 2,4-diamino-5-alkyl-6-(bromomethyl)-5-deazapteridine. The resultant 5-deazaaminopterin diesters were saponified to provide the target 5-deaza analogues. 5,10-Dideazaaminopterins were synthesized by similar alkylation of the carbanions of appropriate 4-carboxyphenylacetic, (5-carboxy-2-thienyl)acetic, or (5-carboxy-2-pyridyl)acetic acid dimethyl esters. The diesters of the 2,4-diamino-4-deoxy-10-carboxy-5,10-dideazapteroic acid types so obtained were saponified and then readily decarboxylated by heating in Me_2SO solution to provide the 2,4-diamino-5,10-dideazapteroic acid-type intermediates. Peptide coupling with diethyl L-glutamate followed by ester hydrolysis at room temperature afforded the new 5,10-dideazaaminopterin analogues. 5-Deazaaminopterins bearing an alkyl substituent at the 5-position were generally quite effective as antiinflammatory agents. Thus 5-propyl-5-deazaaminopterin, 5-methyl-10-propargyl-5-deazaaminopterin, 5-methyl-10-allyl-5-deazaaminopterin, 5-ethyl-5-deazamethotrexate, and 2,5-disubstituted thiophene analogue of 5-methyl-5-deazaaminopterin showed potencies greater than methotrexate by intraperitoneal or oral administration and were active over a considerably broader dose range. Useful activity in the 5,10-dideaza series was only observed for 5,10-dideazaaminopterin and its 10-methyl analogue. Alkyl substitution at C-5 or C-10 was generally detrimental to antiinflammatory activity in this series.

In the previous paper of this series[1] on the effect of methotrexate (MTX) analogues against an animal model of rheumatoid arthritis, we reviewed the background in this area. We also reported the synthesis and evaluation of some 10-deazaaminopterin analogues in the mouse type II collagen model. 10-Ethyl-10-deazaaminopterin (edatrexate) and its analogue in which the 1,4-disubstituted benzene ring is replaced by the 2,5-disubstituted thiophene ring were found to be the most effective agents of the compounds evaluated from that series. We also conducted a cursory examination of 8,10-dideazaaminopterin and four 10-substituted analogues,[2] including 10-methyl, -ethyl, -propyl, and -allyl compounds, in the mouse model. These highly cytotoxic compounds showed weak to moderate activity with little separation between effective and toxic dose levels.[3] As a continuation of this broad investigation of deazaaminopterin analogues, we have prepared and evaluated a series of 5-deazaaminopterins (**1a**−**p**) and 5,10-dideazaaminopterins (**2a**−**j**) (Chart 1). The evaluation was conducted on 5-H- and 5-alkyl-5-deazaaminopterins including those with N^{10}-substituents. Analogous compounds were evaluated in the 5,10-dideazaaminopterin series. Compounds with heteroaroyl side chains are included in both series. We report herein the methodology for synthesis of new analogues and biological data

Chart 1



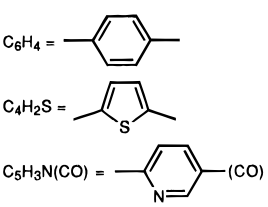
1a-p

a: $R_1 = R_2 = H$, Ar = C_6H_4
b: $R_1 = Me$, $R_2 = H$, Ar = C_6H_4
c: $R_1 = Et$, $R_2 = H$, Ar = C_6H_4
d: $R_1 = Pr$, $R_2 = H$, Ar = C_6H_4
e: $R_1 = Bu$, $R_2 = H$, Ar = C_6H_4
f: $R_1 = H$, $R_2 = prgl$, Ar = C_6H_4
g: $R_1 = R_2 = Me$, Ar = C_6H_4
h: $R_1 = Et$, $R_2 = Me$, Ar = C_6H_4
i: $R_1 = Me$, $R_2 = Pr$, Ar = C_6H_4
j: $R_1 = Me$, $R_2 = Allyl$, Ar = C_6H_4
k: $R_1 = Me$, $R_2 = Prgl$, Ar = C_6H_4
l: $R_1 = Et$, $R_2 = Allyl$, Ar = C_6H_4
m: $R_1 = Et$, $R_2 = Prgl$, Ar = C_6H_4
n: $R_1 = Me$, $R_2 = H$, Ar = C_4H_2S
o: $R_1 = R_2 = Me$, Ar = C_4H_2S
p: $R_1 = Et$, $R_2 = Me$, Ar = C_4H_2S

2a-j

a: $R_1 = R_2 = H$, Ar = C_6H_4
b: $R_1 = H$, $R_2 = Me$, Ar = C_6H_4
c: $R_1 = H$, $R_2 = Et$, Ar = C_6H_4
d: $R_1 = Me$, $R_2 = H$, Ar = C_6H_4
e: $R_1 = Me$, $R_2 = Et$, Ar = C_6H_4
f: $R_1 = Me$, $R_2 = Allyl$, Ar = C_6H_4
g: $R_1 = Me$, $R_2 = Prgl$, Ar = C_6H_4
h: $R_1 = R_2 = H$, Ar = C_4H_2S
i: $R_1 = R_2 = H$, Ar = C_5H_3N
j: $R_1 = Me$, $R_2 = H$, Ar = C_5H_3N

Glu = $NHCH(CO_2H)(CH_2)_2CO_2H$
Prgl = $-CH_2C{\equiv}CH$



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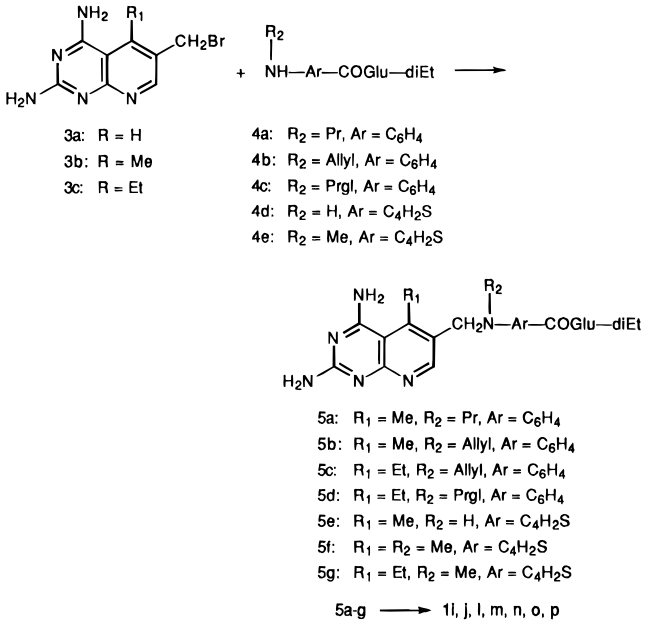
Scheme 1



for these and compounds previously reported from our laboratories.

Chemistry

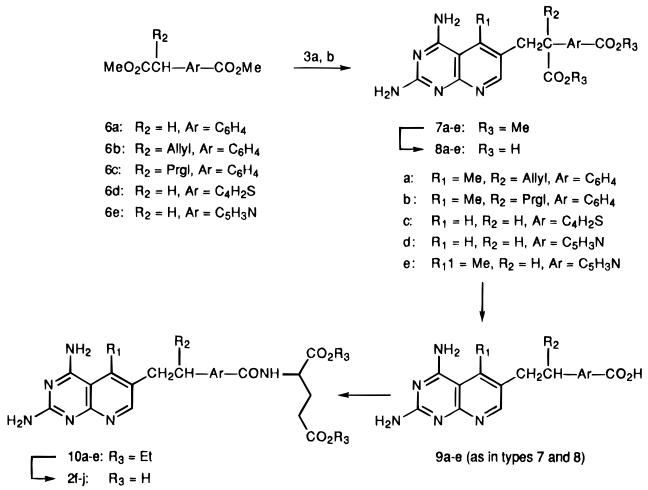
Synthetic procedures for 5-deazaaminopterin and its N^{10}-alkyl and 5-alkyl analogues have been previously reported.[4−9] The most convenient processes featured reductive alkylation of diethyl *N*-(4-aminobenzoyl)-L-glutamate with an appropriate 2,4-diamino-5-deazapteridine-6-carbonitrile or direct alkylation with 2,4-diamino-6-(bromomethyl)-5-deazapteridine or a 5-alkyl derivative.[6−9] Following purification by column chromatography, the glutamate esters were saponified by dilute alkali at room temperature to provide the target 5-deazaaminopterins.

We have used the direct alkylation process for synthesis of the new analogues 5-methyl-10-propyl- (**1i**), 5-methyl-10-allyl- (**1j**), 5-ethyl-10-allyl- (**1l**), and 5-ethyl-10-propargyl- (**1m**) 5-deazaaminopterins. Diethyl *N*-(4-aminobenzoyl)-L-glutamate was alkylated with allyl or propargyl bromide to afford the known allyl and propargyl side chain precursors **4b,c**[7,8,10] (Scheme 1), respectively. The corresponding propyl compound **4a** was available from previous work in these laboratories.[7] Subsequent alkylation of **4a**−**c** with the (bromomethyl)-5-deazapteridines **3b,c**[8,9] yielded the N^{10}-substituted 5-alkyl-5-deazaaminopterin esters **5a**−**d** which were then hydrolyzed to the targets **1i,j,l,m**.

The 2,5-disubstituted thiophene analogue **1n** was synthesized by a similar procedure from diethyl *N*-(5-amino-2-thenoyl)-L-glutamate[11] (**4d**) *via N*-alkylation with **3b** and hydrolysis of the intermediate glutamate diester **5e**. Treatment of **4d** with dimethyl sulfate yielded the methylamino compound **4e** which was in turn alkylated with **3b** and the 5-ethyl-6-bromomethyl reagent **3c** to afford the diesters **5f**,**g**, which were subsequently saponified to targets **1o**,**p**.

5,10-Dideazaaminopterin (**2a**)[12,13] and its 10-alkyl analogues **2b**,**c**[14,15] have been previously reported. Piper and co-workers have prepared 5-methyl-5,10-dideazaaminopterin (**2d**)[16] and 5-methyl-10-ethyl-5,10-dideazaaminopterin (**2e**).[17] Application of the synthetic methodology described in the preceding paper of this series[1] allowed us to conveniently prepare 5-methyl-10-allyl- (**2f**) and 5-methyl-10-propargyl- (**2g**) 5,10-dideazaaminopterins. Alkylation of the anions of α-allyl-, or α-propargylhomoterephthalate dimethyl esters **6b**,**c** with 2,4-diamino-6-(bromomethyl)-5-methyl-5-deazapteridine (**3b**) afforded the diesters **7a**,**b**. Subsequent saponification to the 10-carboxy-10-deaza compounds **8a**,**b** followed by facile decarboxylation provided the 2,4-diamino-4-deoxy-5-methyl-5,10-dideazapteroic acids **9a**,**b**. These pteroic acids were coupled with diethyl L-glutamate, and the resultant glutamate diesters **10a**,**b** were saponified at room temperature to give the final 5,10-dideazaaminopterin analogues **2f**,**g**. Similarly the dimethyl esters of (5-carboxy-2-thienyl)acetic (**6d**)[1] and (5-carboxy-2-pyridyl)acetic (**6e**)[1] acids were alkylated with the bromomethyl reagents **3a**[8],**b** to afford the diesters **7c**−**e**. Hydrolysis, decarboxylation, glutamate coupling, and final hydrolysis yielded the 5,10-dideazaheteroaminopterins **2h**−**j**.

Scheme 2



Biological Evaluation

The analogues in both series were evaluated for their ability to suppress inflammation in the mouse type II collagen assay as described in the preceding paper of this series.[1] DBA/1 mice were injected with bovine II collagen to induce inflammation followed by a booster dose at day 21. The analogues were injected intraperitoneally 2 days before the initial dose of the collagen II challenge. Dosing was continued every 2 days thereafter until day 42.

As shown in Tables 1 and 2 several 5-deazaaminopterin compounds were quite effective in delaying the onset of visual disease and reduction of swelling in affected footpads. The simplest members of the series, such as 5-deazaaminopterin (**1a**), 5-methyl-5-deazaaminopterin (**1b**), and 5-methyl-5-deazamethotrexate (**1g**), were not very active as antiinflammatory agents. 5-Ethyl-5-deazaaminopterin (**1c**) was moderately effective at a dose of 1.0 mg/kg but caused deaths at 1.5 mg/kg. However, 5-propyl-5-deazaaminopterin (**1d**) was a very active compound over a dose range of 0.75−6.0 mg/kg. At the optimum dose of 1.5 mg/kg, the mice showed no evidence of disease and swelling was held to baseline values even through day 44. Methotrexate was notably less efficacious by comparison. Extension of the 5-alkyl

Table 1. Effects of 5-Deaza Analogues of Methotrexate on the Incidence of Type II Collagen Arthritis[a]

compd	dose (mg/kg, ip)	onset and incidence of disease (% of animals) day 23	day 30	day 37	day 44
untreated		0	38	75	100
MTX	9.0	0	0	12	25
1a[b]	1.5	0	12	12	25
	0.75	0	12	38	62
1b[b]	6.0	0	0	100	100
	3.0	12	0	40	60
	1.5	0	0	25	75
1c[c,g]	1.0	12	25	62	62
	0.50	43	57	86	71
	0.25	38	0	50	62
1d[c]	6.0	0	25	25	0
	3.0	0	14	14	29
	1.5	0	0	0	0
	0.75	0	0	0	50
	0.38	0	0	75	88
1e[c]	6.0	0	0	50	50
	3.0	0	0	12	38
	1.5	0	0	38	38
1f[d]	18.0	0	0	38	38
	12.0	0	0	50	75
	6.0	0	38	88	100
1g[b]	3.0	0	50	100	100
	1.5	0	12	50	62
1h[c,g]	0.75	0	14	14	14
	0.38	0	0	0	0
	0.19	0	12	12	12
	0.094	0	12	25	25
	0.047	0	12	50	88
	0.024	0	0	0	25
1i[e]	6.0	0	12	12	25
	3.0	0	0	0	0
	1.5	0	0	12	25
1j[e]	6.0[f]	0	0	0	0
	3.0	0	0	0	0
	1.5	0	12	25	50
	0.75	0	25	38	50
	0.38	0	0	38	62
1k[d]	6.0	12	25	62	62
	3.0	0	25	50	62
	1.5	0	25	50	50
1l[e]	6.0	0	25	50	88
	3.0	0	29	57	86
	1.5	0	0	38	62
1m[e]	12.0	12	12	25	25
	6.0	0	12	25	25
	3.0	0	12	25	25
1n[e]	9.0	0	0	0	25
	6.0	12	12	12	12
1o[e]	6.0	0	0	12	38
	3.0	0	0	0	57
	1.5	0	0	12	50
1p[e]	6.0	0	0	12	50
	3.0	0	0	12	88
	1.5	25	12	38	50

[a] Methods described in ref 1. [b] Reported in ref 6. [c] Reported in ref 9. [d] Reported in ref 8. [e] Reported in this paper. [f] One death observed at this dose. [g] Deaths observed at 1.5 mg/kg.

substituent to an *n*-butyl group (**1e**) caused a serious decrease in activity. 5-Ethyl-5-deazamethotrexate (**1h**) was a very potent agent showing pronounced activity over a broad dose range from 0.75 down to 0.024 mg/kg, although measurements at the 0.047 mg/kg dose gave an anomalous result. Compound **1h** caused deaths at 1.5 mg/kg.

5-Methyl-10-allyl-5-deazaaminopterin (**1j**) was very active at doses of 3 and 6 mg/kg, but the higher dose was apparently the toxic threshold as one death was encountered. Dilution below 3 mg/kg caused a clear decline in activity. The 5-methyl-10-propyl analogue **1i** was quite effective over a dose range of 1.5−6.0 mg/kg, with the 3 mg/kg dose being especially efficacious. 5-Methyl-10-propargyl-5-deazaaminopterin (**1k**) was moderately effective over a broad dose range from 0.19 to 6.0 mg/kg. The 10-propargyl-5-deazaaminopterin analogue (**1f**) lacking the 5-methyl group showed a serious decline in activity. 5-Ethyl-10-allyl-5-deazaaminopterin (**1l**) was essentially inactive, but the 5-ethyl-10-propargyl analogue was moderately effective.

Substitution of the 2,5-disubstituted thiophene moiety in place of the 1,4-disubstituted benzene ring in the side chain, as in structures **1n**,**o**, afforded active compounds. However, the 5-ethyl-5-deazamethotrexate thiophene analogue **1p** was a less effective compound, especially when compared to its phenyl analogue **1h**.

In the 5,10-dideaza series, 5,10-dideazaaminopterin (**2a**) and its 10-methyl analogue **2b** showed strong activity (Tables 3 and 4). It was surprising that the 10-ethyl compound **2c** was quite ineffective as was the 5,10-dideazaaminopterin thiophene analogue **2h**. Likewise, the 3′-aza analogues **2i**,**j** (with the pyridine ring incorporated in the side chain) were also inactive. In contrast to the 5-deaza series, introduction of an alkyl substituent into the 5-position was very detrimental to antiinflammatory activity as seen in compounds **2d**−**g**.

Most of the 5-deazaaminopterin analogues were evaluated for general cytotoxicity in L1210 murine leukemia cell cultures and in the Chang liver cell line as reported in the accompanying paper.[1] The data shown in Table 5 indicate these compounds to be strongly cytotoxic in L1210 except for the 5-methylthienyl analogue **1n** which was moderately cytotoxic. All were moderately active to strongly active in their ability to suppress inflammation as determined by caliper measurement. Except for compounds **1b**,**c**,**g**, the analogues also were moderately to strongly active for counteractions of the visual onset of disease. MTX was moderately cytotoxic to L1210 and very effective against the disease.

In this series there is an indication that activity parallels cytotoxicity in L1210. However, many of the compounds were considerably less cytotoxic to the immortalized liver cells by factors of 1−2 magnitudes. Compound **1h** was efficacious over a broad dose range and 89-fold less cytotoxic to the liver cells. Compound **1k** was 370 times less cytotoxic to liver; however, its antiarthritic behavior was only moderate. The very effective analogue **1d** was highly cytotoxic to L1210 and the liver cells.

Some compounds in the 5,10-dideaza series were assayed for cytotoxicity as shown in Table 5. 5,10-Dideazaaminopterin (**2a**), its 10-methyl homologue **2b**, and the 5-methyl-10-allyl analogue **2f** were moderately to strongly cytotoxic to L1210. Compounds **2a**,**b** were efficacious against arthritis, but **2f** was ineffective. Compounds **2c**,**d**,**j** were not very cytotoxic and likewise ineffective against the disease. The limited results from this series still suggest that greater cytotoxicity portends activity and that poorly cytotoxic compounds are inactive. It was interesting that there was little separation of cytotoxic effect between L1210 and liver cells for the respective analogues in the 5,10-dideaza series.

We indicated in the accompanying paper[1] that construction of a true activity−cytotoxicity profile would require measurements with the target cell, were it

Table 2. Effects of 5-Deaza Analogues of Methotrexate on the Extent of Inflammation in Murine Type II Collagen Arthritis[a]

compd	dose (mg/kg, ip)	paw swelling (mean ± SD, mm) day 23	day 30	day 37	day 44
untreated		2.15 ± 0.03	2.33 ± 0.14	2.52 ± 0.18	2.87 ± 0.41
MTX	9	2.14 ± 0.04	2.16 ± 0.06	2.18 ± 0.02	2.24 ± 0.13
1a	1.50	2.10 ± 0.04	2.16 ± 0.09	2.17 ± 0.15	2.21 ± 0.14
	0.75	2.14 ± 0.04	2.19 ± 0.11	2.23 ± 0.15	2.28 ± 0.19
1b	6.0	2.12 ± 0.08	2.16 ± 0.06	2.34 ± 0.16	2.59 ± 0.44
	3.0	2.21 ± 0.13	2.18 ± 0.09	2.42 ± 0.19	2.53 ± 0.21
	1.5	2.13 ± 0.06	2.18 ± 0.08	2.31 ± 0.10	2.55 ± 0.23
1c	1.00	2.19 ± 0.11	2.19 ± 0.12	2.32 ± 0.18	2.29 ± 0.15
	0.50	2.31 ± 0.14	2.38 ± 0.17	2.42 ± 0.20	2.43 ± 0.14
	0.25	2.25 ± 0.16	2.22 ± 0.07	2.34 ± 0.22	2.33 ± 0.16
1d	6.0	2.13 ± 0.04	2.23 ± 0.15	2.22 ± 0.13	2.11 ± 0.05
	3.0	2.12 ± 0.05	2.24 ± 0.11	2.23 ± 0.14	2.20 ± 0.12
	1.5	2.12 ± 0.12	2.14 ± 0.13	2.19 ± 0.10	2.15 ± 0.15
	0.75	2.13 ± 0.04	2.17 ± 0.12	2.16 ± 0.09	2.22 ± 0.17
	0.38	2.11 ± 0.10	2.13 ± 0.12	2.22 ± 0.21	2.57 ± 0.61
1e	6.0	2.12 ± 0.03	2.15 ± 0.03	2.27 ± 0.19	2.29 ± 0.19
	3.0	2.16 ± 0.04	2.15 ± 0.04	2.20 ± 0.09	2.27 ± 0.16
	1.5	2.16 ± 0.05	2.17 ± 0.03	2.23 ± 0.13	2.27 ± 0.20
1f	18.0	2.16 ± 0.06	2.13 ± 0.06	2.23 ± 0.15	2.27 ± 0.18
	12.0	2.17 ± 0.04	2.16 ± 0.07	2.30 ± 0.19	2.49 ± 0.48
	6.0	2.12 ± 0.04	2.32 ± 0.32	2.46 ± 0.39	2.65 ± 0.57
1g	3.0	2.10 ± 0.05	2.26 ± 0.29	2.47 ± 0.01	2.48 ± 0.08
	1.5	2.26 ± 0.08	2.26 ± 0.21	2.42 ± 0.17	2.44 ± 0.14
1h	0.75	2.14 ± 0.04	2.20 ± 0.12	2.20 ± 0.09	2.18 ± 0.10
	0.38	2.15 ± 0.06	2.16 ± 0.05	2.17 ± 0.07	2.15 ± 0.05
	0.19	2.11 ± 0.07	2.14 ± 0.10	2.17 ± 0.20	2.20 ± 0.11
	0.094	2.15 ± 0.05	2.14 ± 0.13	2.20 ± 0.13	2.27 ± 0.37
	0.047	2.10 ± 0.07	2.21 ± 0.10	2.33 ± 0.18	2.57 ± 0.68
	0.024	2.12 ± 0.06	2.22 ± 0.10	2.18 ± 0.08	2.23 ± 0.15
1i	6.0	2.16 ± 0.03	2.19 ± 0.11	2.20 ± 0.12	2.24 ± 0.16
	3.0	2.12 ± 0.05	2.13 ± 0.04	2.17 ± 0.05	2.16 ± 0.03
	1.5	2.15 ± 0.03	2.15 ± 0.03	2.21 ± 0.13	2.22 ± 0.12
1j	6.0	2.09 ± 0.04	2.10 ± 0.04	2.15 ± 0.04	2.13 ± 0.06
	3.0	2.12 ± 0.03	2.12 ± 0.03	2.14 ± 0.02	2.16 ± 0.04
	1.5	2.16 ± 0.05	2.24 ± 0.10	2.26 ± 0.14	2.32 ± 0.16
	0.75	2.17 ± 0.04	2.22 ± 0.25	2.33 ± 0.16	2.36 ± 0.16
	0.38	2.17 ± 0.05	2.17 ± 0.05	2.30 ± 0.19	2.39 ± 0.17
1k	6.0	2.23 ± 0.15	2.21 ± 0.16	2.32 ± 0.17	2.46 ± 0.25
	3.0	2.12 ± 0.08	2.27 ± 0.11	2.29 ± 0.14	2.38 ± 0.15
	1.5	2.16 ± 0.10	2.31 ± 0.09	2.33 ± 0.18	2.37 ± 0.16
1l	6.0	2.15 ± 0.04	2.21 ± 0.12	2.28 ± 0.17	2.45 ± 0.26
	3.0	2.16 ± 0.05	2.27 ± 0.17	2.34 ± 0.24	2.55 ± 0.34
	1.5	2.14 ± 0.05	2.18 ± 0.06	2.26 ± 0.24	2.40 ± 0.25
1m	12.0	2.22 ± 0.14	2.20 ± 0.09	2.23 ± 0.26	2.20 ± 0.10
	6.0	2.17 ± 0.05	2.16 ± 0.13	2.21 ± 0.15	2.20 ± 0.14
	3.0	2.13 ± 0.10	2.16 ± 0.11	2.24 ± 0.17	2.39 ± 0.54
1n	9.0	2.09 ± 0.06	2.09 ± 0.05	2.11 ± 0.02	2.19 ± 0.18
	6.0	2.17 ± 0.11	2.12 ± 0.12	2.13 ± 0.09	2.19 ± 0.12
1o	6.0	2.11 ± 0.05	2.13 ± 0.06	2.19 ± 0.09	2.27 ± 0.20
	3.0	2.13 ± 0.07	2.16 ± 0.15	2.14 ± 0.10	2.32 ± 0.23
	1.5	2.12 ± 0.08	2.13 ± 0.11	2.21 ± 0.23	2.33 ± 0.23
1p	6.0	2.12 ± 0.08	2.13 ± 0.10	2.16 ± 0.12	2.32 ± 0.25
	3.0	2.14 ± 0.06	2.15 ± 0.05	2.20 ± 0.10	2.45 ± 0.28
	1.5	2.24 ± 0.12	2.20 ± 0.08	2.26 ± 0.16	2.37 ± 0.20

[a] Methods described in ref 1.

known. However, the studies reported in these two papers suggest a general correlation of antiarthritic activity with cytotoxicity. The 5-deazaaminopterin series is particularly interesting with several active compounds with their activity spread over a broad dose range. This greater spread between effective and toxic doses as compared with MTX offers a better prospect for treatment of patients with greater individual susceptibilities to various toxic discomforts of MTX-like drugs.

Compound **1d** was an especially promising candidate for further development. In studies to be reported elsewhere, it was found to be very effective in the standard rat adjuvant assay (0.75 mg/kg, ip, less than 1.5 mg/kg, po). Its acute toxicity profile was excellent in several rodent species, being the least toxic compound of those studied including MTX. The compound had an LD_{10} of 96 mg/kg in the BD_2F mouse compared with MTX at 7.5 mg/kg. The powerful and prolonged effect of **1d** in mice is probably related to decrease of collagen II antibody levels. Compound **1d** was 10 times more potent than MTX for suppression of the antibody production *in vitro*. It would be premature to suggest that suppression of antibody response to an inherent antigen in humans constitutes the mode of action of **1d** or other analogues in these series.

Experimental Section

Examinations by TLC were performed on Analtech precoated (250 μm) silica gel G(F) plates. High-performance liquid chromatography (HPLC) assays were made with a Waters Associates ALC-242 liquid chromatograph equipped with an

Table 3. Effects of 5,10-Dideaza Analogues of Methotrexate on the Incidence of Type II Collagen Arthritis[a]

compd	dose (mg/kg, ip)	onset and incidence of disease (% of animals) day 23	day 30	day 37	day 44
untreated		0	38	75	100
MTX	9	0	0	12	25
2a[b]	18	12	0	0	0
	12	0	12	12	25
	6	12	25	25	38
2b[c]	10	20	0	20	20
2c[d]	6.0	12	100	88	100
	3.0	25	88	100	100
	1.5	25	62	88	88
2d[e]	12.0	50	75	88	100
	6.0	38	100	100	100
	3.0	25	100	100	100
2e[f]	9.0	0	50	88	88
	4.5	0	12	38	75
2f[g]	12.0	50	38	75	100
	6.0	0	25	62	100
	3.0	25	38	88	88
2g[g]	10.0	14	57	71	71
	5.0	12	12	62	88
2h[g]	8.0	0	25	88	88
2i[g]	12.0	0	62	88	88
	6.0	0	88	88	88
2j[g]	12.0	0	38	62	75
	6.0	0	25	38	75

[a] Methods described in ref 1. [b] Reported in refs 12 and 13. [c] Reported in ref 14. [d] Reported in refs 14 and 15. [e] Reported in ref 16. [f] Reported in ref 17. [g] Reported in this paper.

ultraviolet detector (254 nm) and a M-6000 pump using a 30 × 0.29 cm C18 μBondapak column. Purity assays were done in the reversed-phase isocratic mode with a mobile phase consisting of MeCN (10% or 15% by vol) in 0.1 M NaOAc (pH 3.6). Column chromatographic purifications were done with silica gel (Merck; 60 A, 230−400 mesh for flash chromatography). When solubility limitations made it necessary, crude products to be purified were dispersed in silica gel for application to the column. Dispersal was achieved by evaporating *in vacuo* a solution of the crude product in DMF containing suspended silica gel (3 g of 60−200 mesh/g of crude product). Evaporations were performed with a rotary evaporator; higher boiling solvents (DMF, Me_2NAc) were removed *in vacuo* (<1 mmHg, bath to 35 °C) and more volatile solvents with a H_2O aspirator. Products were dried *in vacuo* (<1 mmHg) at 22−25 °C over P_2O_5 and NaOH pellets. Final products were dried and then allowed to equilibrate with ambient conditions of the laboratory. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Spectral determinations and elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. The ^{1}H NMR spectral data reported were determined in Me_2SO-d_6 with a Nicolet NMC 300 NB spectrometer using Me_4Si as internal reference. Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast-atom-bombardment (FAB) mode. UV spectra were determined with a Perkin-Elmer Model Lambda 9 spectrometer. Samples were first dissolved in 0.1 N NaOH; then the solutions were diluted 10-fold with the medium given in the listings. Maxima are expressed in nanometers with the molar absorbance given in parentheses. Molecular weights used in all calculations conform with the compositions listed with the indicated elemental analyses.

Diethyl *N*-[4-(Allylamino)benzoyl]-L-glutamate (4b). **4b** was prepared essentially as reported for the propargyl compound **4c**.[7,8] Elution from the silica gel column by cyclohexane−EtOAc (3:1) was carried out until the front-running component had cleared the column. Elution with cyclohexane−EtOAc (2:1) followed to give fractions homogeneous in **4b**: yield 50%; mp 58−60 °C (lit.[10] mp 60−61 °C).

Diethyl *N*-[4-(propargylamino)benzoyl]-L-glutamate (4c): prepared as previously reported; yield 48%; mp 96−98 °C (lit.[7,8,10] mp 98−99 °C).

Diethyl *N*-[5-(Methylamino)-2-thenoyl]-L-glutamate (4e). A solution of diethyl *N*-(5-amino-2-thenoyl)-L-glutamate[11] (**4d**; 1.78 g, 5.42 mmol), *i*-Pr_2NEt (1.0 mL, 0.74 g, 5.7 mmol), and Me_2SO_4 (0.59 mL, 0.79 g, 6.2 mmol) in DMF (20 mL) was kept at 60−65 °C for 2 h and then left at 20−25 °C for 42 h. Following evaporation (<1 mmHg, bath 25−30 °C), the residue was dissolved in EtOAc−cyclohexane (1:1) for application to a silica gel column from which fractions were eluted by the same solvent. Fractions homogeneous in the product were combined and evaporated to afford pure **4e** in 16% yield (287 mg) as an amber oil: MS *m*/*e* 343, MH^+. Anal. ($C_{15}H_{22}N_2O_5S$) C, H, N. Marsham *et al.*[11] obtained **4e** by another route as a brown viscous oil.

Table 4. Effects of 5,10-Dideaza Analogues of Methotrexate on the Extent of Inflammation in Murine Type II Collagen Arthritis[a]

compd	dose (mg/kg, ip)	paw swelling (mean ± SD, mm) day 23	day 30	day 37	day 44
untreated		2.15 ± 0.03	2.33 ± 0.14	2.52 ± 0.18	2.87 ± 0.41
MTX	9	2.14 ± 0.04	2.16 ± 0.06	2.18 ± 0.02	2.24 ± 0.13
2a	18	2.15 ± 0.13	2.16 ± 0.09	2.15 ± 0.05	2.20 ± 0.08
	12	2.12 ± 0.06	2.24 ± 0.13	2.24 ± 0.13	2.28 ± 0.13
	6	2.17 ± 0.17	2.28 ± 0.18	2.26 ± 0.14	2.29 ± 0.20
2b	10	2.24 ± 0.09	2.23 ± 0.09	2.22 ± 0.10	2.24 ± 0.10
2c	6.0	2.20 ± 0.12	2.57 ± 0.25	2.80 ± 0.66	2.76 ± 0.46
	3.0	2.26 ± 0.15	2.43 ± 0.15	2.67 ± 0.66	2.70 ± 0.47
	1.5	2.17 ± 0.14	2.31 ± 0.18	2.48 ± 0.25	2.59 ± 0.49
2d	12.0	2.32 ± 0.26	2.71 ± 0.58	3.01 ± 0.72	2.85 ± 0.33
	6.0	2.30 ± 0.12	2.77 ± 0.43	2.81 ± 0.53	2.97 ± 0.50
	3.0	2.21 ± 0.14	2.73 ± 0.46	3.09 ± 0.82	3.12 ± 0.42
2e	9.0	2.12 ± 0.06	2.45 ± 0.57	3.09 ± 0.85	2.99 ± 0.70
	4.5	2.10 ± 0.07	2.18 ± 0.14	2.32 ± 0.56	2.68 ± 0.62
2f	12.0	2.25 ± 0.15	2.24 ± 0.14	2.69 ± 0.61	3.15 ± 0.87
	6.0	2.15 ± 0.07	2.21 ± 0.15	2.66 ± 0.69	3.01 ± 0.69
	3.0	2.20 ± 0.16	2.24 ± 0.30	2.99 ± 0.95	2.99 ± 0.72
2g	10.0	2.19 ± 0.11	2.37 ± 0.35	2.65 ± 0.75	2.70 ± 0.57
	5.0	2.18 ± 0.13	2.17 ± 0.09	2.45 ± 0.48	2.64 ± 0.69
2h	8.0	2.13 ± 0.03	2.19 ± 0.11	2.60 ± 0.65	2.63 ± 0.57
2i	12.0	2.10 ± 0.04	2.28 ± 0.14	2.50 ± 0.46	2.67 ± 0.66
	6.0	2.12 ± 0.03	2.39 ± 0.19	2.61 ± 0.57	2.86 ± 0.76
2j	12.0	2.16 ± 0.05	2.33 ± 0.04	2.69 ± 0.80	2.66 ± 0.55
	6.0	2.16 ± 0.05	2.22 ± 0.19	2.59 ± 0.74	2.55 ± 0.42

[a] Methods described in ref 1.

Table 5. Cytotoxicity of 5-Deaza- and 5,10-Dideazaaminopterins in L1210 and Chang Liver Cells[a]

compd	growth inhibition IC_{50} (nM)[b]	
	L1210	Chang liver
1b	1.8	123
1c	1.9	126
1d	6.5	8.2
1f	13	6.3
1g	1.3	78
1h	1.0	89
1i	11	14
1j	3.4	30
1k	1.6	593
1n	25	34
2a	27	37
2b	7.5	78
2c	75	12
2d	379	334
2f	9	11
2j	629	4110
MTX	20	14

[a] See ref 20 for methods. [b] Average of three runs.

5-Methyl-10-propyl-5-deazaaminopterin Diethyl Ester (5a). A mixture of **3b**·2HBr[8] (645 mg, 1.50 mmol), **4a**[7] (583 mg, 1.60 mmol), and $CaCO_3$ (225 mg, 2.25 mmol) in Me_2NAc (10 mL) was stirred under N_2 in a stoppered flask wrapped in aluminum foil for 9 days. The mixture was filtered, and the filtrate was evaporated to dryness (<1 mmHg, bath to 30 °C). The residue was dissolved in $CHCl_3$−MeOH (1:1) and loaded onto a silica gel column. Elution with $CHCl_3$−MeOH (6:1) gave fractions homogeneous by TLC (R_f 0.55; $CHCl_3$−MeOH, 4:1), which when combined and evaporated gave pure **5a**: yield 30% (259 mg); MS *m/e* 552, MH^+. Anal. ($C_{28}H_{37}N_7O_5$·1.1H_2O) C, H, N.

Intermediate Esters 5b−f. These were prepared in a manner similar to that described above for **5a**. Results are as follows.

5b: prepared from **3b** and **4b**; yield 36%; MS *m/e* 550, MH^+. Anal. ($C_{28}H_{35}N_7O_5$·0.4H_2O) C, H, N.

5c: from **3c**[8,9] and **4b**; yield 23%; MS *m/e* 564, MH^+. Anal. ($C_{29}H_{37}N_7O_5$·1.4H_2O) C, H, N.

5d: from **3c** and **4e**; yield 26%; MS *m/e* 562, MH^+ for $C_{29}H_{35}N_7O_5$.

5e: from **3b** and **4d**; 29% yield; MS *m/e* 516, MH^+. Anal. ($C_{23}H_{29}N_7O_5S$·2.8H_2O) C, H, N.

5f: from **3b** and **4e**; 28% yield; MS *m/e* 530, MH^+. Anal. ($C_{24}H_{31}N_7O_5S$·1.8H_2O) C, H; N: calcd, 17.44; found, 16.79.

5g: from **3c** and **4c**; 27% yield; MS *m/e* 544, MH^+ for $C_{25}H_{33}N_7O_5S$.

5-Methyl-10-propyl-5-deazaaminopterin (1i). A solution of diethyl ester **5a** (175 mg, 0.31 mmol) in MeOH (10 mL) containing NaOH (0.7 mL of 1 N) was kept for 4 days at 20−25 °C and then evaporated to remove MeOH, and the residue was dissolved in H_2O (5 mL). More NaOH (0.35 mL of 1 N) was added, and the solution was kept for 2 days longer at 20−25 °C. The solution was then treated with stirring with 1 N HCl to produce pH 3.8−4.0 and cause precipitation of **1i**. After overnight refrigeration, the solid was collected and washed with cold H_2O: yield 92% (153 mg), homogeneous by HPLC; MS *m/e* 496, MH^+; UV (0.1 N HCl) λ_{max} 313 nm (ϵ 21 100), 228 (39 700); (0.1 N NaOH) 309 (28 900), 229 (36 700); ^{1}H NMR (Me_2SO-d_6) δ 0.88 (t, 3, $CH_2CH_2CH_3$), 1.60 (m, 2, $CH_2CH_2CH_3$), 1.92 and 2.02 (2m, 2, Glu-3-CH_2), 2.31 (t, 2, CH_2CH_2CO), 2.65 (s, 3, C^5-CH_3), 3.38 (t, 2, $CH_2CH_2CH_3$), 4.32 (m, 1, CONHC*H*), 4.64 (s, 2, CH_2N), 6.69 and 7.70 (2d, 4, C_6H_4), 7.02 and 7.52 (2 br s, 4, NH_2), 8.06−8.12 (d, 2, overlap of C^7-H and CON*H*). Anal. ($C_{24}H_{29}N_7O_5$·2.3H_2O) C, H, N.

5-Methyl-10-allyl-5-deazaaminopterin (1j). Ester **5b** was hydrolyzed as described above (for the conversion of **5a** to **1i**) to give pure **1j** in 86% yield: MS *m/e* 494, MH^+; UV (0.1 N HCl) λ_{max} 308 nm (ϵ 28 300); (0.1 N NaOH) 305 (29 200), 229 (36 000); ^{1}H NMR (Me_2SO-d_6) δ 1.92 and 2.03 (2m, 2, Glu-3-CH_2), 2.30 (t, 2, CH_2CH_2CO), 2.64 (s, 3, CH_3), 4.06 (br s, 2, -CH_2CH=CH_2) 4.33 (m, 1, CONHC*H*), 4.64 (s, 2, CH_2N), 5.14 (m, 2, -CH_2CH=CH_2), 5.85 (m, 1, CH_2CH=CH_2), 6.72 and 7.70 (2d, 4, C_6H_4), 6.92 and 7.48 (2 br s, 4, NH_2), 8.12 (d, 1, CON*H*), 8.17 (s, 1, C^7-H). Anal. ($C_{24}H_{27}N_7O_5$·1.8H_2O) C, H, N.

5-Ethyl-10-allyl-5-deazaaminopterin (1l): obtained in 85% yield from **5c** as described for the preparation of **1i**; MS *m/e* 508, MH^+; ^{1}H NMR δ 1.21 (t, 3, CH_3), 1.86−2.10 (m, 2, Glu-3-CH_2), 2.32 (t, 2, CH_2CH_2CO), 3.05 (m, 2, CH_2CH_3), 4.08 (br s, 2, -CH_2CH=CH_2), 4.35 (m, CONHC*H*), 4.68 (s, 2, CH_2N), 5.17 (m, 2, -CH_2CH=CH_2), 5.87 (m, 1, CH_2CH=CH_2), 6.49 and 7.19 (2br s, 4, NH_2), 6.74 and 7.72 (2d, 4, C_6H_4), 8.15 (d, CON*H*), 8.22 (s, 1, C^7-H). Anal. ($C_{25}H_{29}N_7O_5$·2.15H_2O) C, H, N.

5-Ethyl-10-propargyl-5-deazaaminopterin (1m). Hydrolysis of **5d** (as previously reported[8] for conversions of homologous ester precursors to **1f**,**k**) afforded pure **1m** in 77% yield: UV (0.1 N HCl) λ_{max} 299 nm (27 600), 228 (41 700); (0.1 N NaOH) 346 (8170), 294 (27 000), 227 (35 400); ^{1}H NMR δ 1.20 (t, 3, CH_3), 1.93 and 2.04 (2m, 2, Glu-3-CH_2), 2.32 (t, 2, CH_2CH_2CO), 3.07 (m, 2, CH_2CH_3), 3.20 (s, CH_2C≡CH), 4.20 (s, 2, CH_2C≡CH), 4.35 (q, CONHC*H*), 4.68 (s, 2, CH_2N), 6.90 (d, 4, overlap of NH_2 and $C^{2'}$-H and $C^{6'}$-H), 7.36 (br s, 2, NH_2), 7.78 (d, 2, $C^{3'}$-H and $C^{5'}$-H), 8.19 (d, 1, CONH), 8.35 (s, 1, C^7-H). Anal. ($C_{25}H_{27}N_7O_5$·1.1H_2O) C, H, N.

***N*-[5-[[2,4-Diamino-5-methyl-5-deaza-6-pteridinyl)-methyl]amino]-2-thenoyl]-L-glutamic Acid (1n).** The diethyl ester **5e** (470 mg, 0.91 mmol) was stirred with 1 N NaOH (9 mL) at 20−25 °C for 5 h. (Solution occurred during the first 2 h.) Acidification with glacial AcOH to pH 4.2 followed, and the pH was finally adjusted to 4.0 with 1 N H_2SO_4. After overnight refrigeration, the pale yellow solid was collected, washed with cold H_2O (until the filtrate no longer gave a precipitate with $BaCl_2$), and dried to afford pure **1n**: yield 80% (329 mg); MS *m/e* 460, MH^+; UV (0.1 N HCl) λ_{max} 332 (ϵ 23 500), 226 (37 800); (0.1 N NaOH) 341 (25 500), 230 (33 600); ^{1}H NMR δ 1.88 and 1.98 (2m, 2, Glu-3-CH_2), 2.30 (t, 2, CH_2-CO), 2.66 (s, 3, CH_3), 4.25 (m, 1, CONHC*H*), 5.94 (d, 4-ArH), 6.68 (s, 2, NH_2), 7.2−7.35 (m, 3, NH_2 overlapping 5-ArNH), 7.48 (d, 1, 3-ArH), 7.98 (d, 1, CONH), 8.52 (s, 1, C^7-H). Anal. ($C_{19}H_{21}N_7O_5S$·1.5H_2O) C, H, N.

***N*-[5-[[(2,4-Diamino-5-methyl-5-deaza-6-pteridinyl)-methyl]methylamino]-2-thenoyl]-L-glutamic Acid (1o).** Hydrolysis of **5f** as described above for the conversion of **5e** to **1n** led to pure **1o** in 80% yield: MS *m/e* 474, MH^+; ^{1}H NMR δ 1.90 and 2.00 (2m, 2, Glu-3-CH_2), 2.32 (t, 2, CH_2CO), 2.60 (s, 3, 5-CH_3), 2.87 (s, 3, CH_3N), 4.30 (m, 1, CONHC*H*), 4.50 (s, 2, CH_2N), 6.05 (d, 1, 4-ArH), 6.68 (s, 2, NH_2), 7.32 (s, 2, NH_2), 7.58 (d, 1, 3-ArH), 8.06 (d, 1, CONH), 8.35 (s, 1, C^7-H). Anal. ($C_{20}H_{23}N_7O_5S$·3H_2O) C, H, N.

***N*-[5-[[(2,4-Diamino-5-ethyl-5-deaza-6-pteridinyl)methyl]methylamino]-2-thenoyl]-L-glutamic acid (1p)**: prepared from **5g** as described for the preparation of **1n**, yield 82%; MS *m/e* 488, MH^+; ^{1}H NMR (Me_2SO-d_6) δ 1.16 (t, CH_3-CH_2), 1.90 and 2.00 (2m, 2, Glu-3-CH_2), 2.30 (t, 2, CH_2CO), 2.86 (s, 3, CH_3N), 3.00 (q, 2, CH_3CH_2), 4.28 (m, 1, CONHC*H*), 4.52 (s, 2, CH_2N), 6.05 (d, 1, 4-ArH), 6.60 (s, 2, NH_2), 7.22 (s, 2, NH_2), 7.61 (d, 1, 3-ArH), 8.06 (d, 1, CONH), 8.40 (s, 1, C7-H). Anal. ($C_{21}H_{25}N_7O_5S$·3H_2O).

Homoterephthalic Acid Dimethyl Ester (6a). This compound was prepared *via* a three-step sequence as follows.

(a) (4-Carboxyphenyl)acetonitrile. A solution of 4-(chloromethyl)benzoic acid (40.9 g, 0.240 mol) in tetrahydrofuran (225 mL) was carefully treated with stirring with saturated $NaHCO_3$ solution (200 mL). Solid NaCN (69.3 g, 1.41 mol) was then added followed by H_2O (225 mL). The solution was kept at 20−25 °C for 48 h before it was chilled in an ice bath and carefully acidified (HOOD!) with concentrated HCl to produce pH 4.0. THF was removed by evaporation (in the hood) under reduced pressure; then the residual solution was treated with more concentrated HCl to pH 2. The brown solid which had formed was collected, washed with H_2O, and then dissolved in 1 N NaOH (about 400 mL required). Acidification of the Norit-treated and filtered solution afforded the expected product as a tan solid in essentially theoretical yield (38.6 g): MS (negative FAB mode) *m/e* 160 $(M - H)^-$ for $C_9H_7NO_2$.

(b) Homoterephthalic Acid. A portion of the crude product (22.9 g, 0.142 mol) from the preceding preparation was

added to the solution which resulted when concentrated H_2SO_4 (133 mL) was combined with H_2O (160 mL) in a 3-L three-necked flask equipped with a mechanical stirrer. The stirred mixture was refluxed for 6 h and then allowed to cool to 25 °C. Cold H_2O (400 mL) was added, and after overnight refrigeration, the product, mp 234−240 °C (lit.[19] mp 237−238 °C), was collected, washed with H_2O, and dried: yield 90% (23.1 g); MS *m/e* 181, MH^+ for $C_9H_8O_4$.

(c) Dimethyl Homoterephthalate (6a). The product from part b above (23.0 g, 0.128 mol) was dissolved in MeOH (1.37 L) to which concentrated H_2SO_4 (2.7 mL) had been added. The resulting solution was left at 20−25 °C for 3 days and then boiled under reflux for 4.5 h. The solution was concentrated (H_2O aspirator, bath 20−25 °C) to about one-third the original volume. The remaining solution was treated with stirring with 5% Na_2CO_3 to neutralize remaining acid. The rest of the MeOH was then removed, and the remaining mixture was stirred with Et_2O (500 mL). The Et_2O solution was washed with 5% Na_2CO_3 solution followed by H_2O, dried (Na_2SO_4), and evaporated to give **6a** as a pale yellow oil, homogeneous by TLC (R_f 0.5; cyclohexane−EtOAc, 3:1): 91% yield (23.3 g); MS *m/e* 209 (MH^+), 177 (M − OCH_3), 149 (208 − CO_2CH_3), 417 ($2M + H^+$). Anal. ($C_{11}H_{12}O_4$) C, H. The oil crystallized readily when left in a refrigerator but melted when allowed to warm to room temperature.

Dimethyl α-allylhomoterephthalate (6b): prepared by KH-promoted alkylation of **6a** with allyl bromide by the procedure reported for the preparation of its propargyl analogue **6c**,[18] pale yellow oil, yield 89%; ^{1}H NMR ($CDCl_3$) δ 2.75 (m, 2, C*H*$_2$CHAr), 3.8 (m, 7, both CH_3O and ArC*H* overlapping), 5.09 (m, 2, C*H*$_2$=CH), 5.64 (m, 1, C*H*=CH_2), 7.69 (q, 4, C_6H_4). Another run in which NaH was used in place of KH led to pure **6b** (TLC R_f 0.70; cyclohexane−EtOAc, 3:1) in 83% yield: MS *m/e* 249 (MH^+), 217 (M − OCH_3), 189 (M − CO_2CH_3). Anal. ($C_{14}H_{16}O_4$) C, H.

4-Amino-4-deoxy-5-methyl-10-allyl-10-carboxy-5,10-dideazapteroic Acid Dimethyl Ester (7a). The procedure that follows is adapted from that described earlier for the preparation of analogous 8,10-dideaza analogues.[2] NaH (0.49 g of 60% in oil dispersion, 12.3 mmol) was stirred with DMF (9 mL). The suspension was chilled to 0 °C and then treated with a solution of **6b** (3.05 g, 12.3 mmol) in DMF (9 mL). After 0.5 h at 0 °C, the stirred mixture was chilled to −25 °C and then treated with a solution of **3b**·2HBr (1.76 g, 4.09 mmol). The temperature was allowed to rise to −10 °C, kept near −10 °C for 2.5 h, and then allowed to rise to room temperature. The stirred mixture was left overnight at 20−25 °C and then evaporated (<1 mmHg, bath to 30 °C). The residual solid was triturated with Et_2O, collected, air-dried, and then washed with H_2O. After being dried, the solid was dispersed in $CHCl_3$−MeOH (7:1) and chromatographed on silica gel with elution by $CHCl_3$−MeOH (7:1). Evaporation of collected fractions homogeneous in the product gave **7a** in 23% yield (400 mg): MS *m/e* 436, MH^+ for $C_{23}H_{25}N_5O_4$.

The following 4-amino-4-deoxy-10-carboxy-5,10-dideazapteroic acid diesters **7b**−**e** were prepared essentially as described above for **7a**.

7b: obtained in 15% yield by alkylation of **6c**[18] with **3b**; MS *m/e* 434, MH^+ for $C_{23}H_{23}N_5O_4$.

7c: obtained in 23% yield after alkylation of **6d**[1] with **3a**;[8] MS *m/e* 388, MH^+ for $C_{17}H_{17}N_5O_4S$.

7d: was obtained after alkylation of **6e**[1] with **3a**; precipitated within the silica gel column and successful elution required use of MeOH alone; eluted product (MS *m/e* 383, MH^+ for $C_{18}H_{18}N_6O_4$), obtained in an apparent yield of 90%, was of suitable purity for subsequent conversions leading to target compound **2i**.

7e: alkylation of **6e**[1] with **3b** led to **7e**, homogeneous on TLC, in 43% yield; MS *m/e* 397, MH^+ for $C_{19}H_{20}N_6O_4$.

4-Amino-4-deoxy-5-methyl-10-allyl-10-carboxy-5,10-dideazapteroic Acid (8a). Compound **7a** (400 mg, 0.92 mmol) was dissolved in Me_2SO (10 mL) containing 4 N NaOH (0.55 mL), and the solution was stirred under N_2 at 20−25 °C for 18 h. HPLC monitoring showed incomplete hydrolysis. More 4 N NaOH (0.27 mL) was added, and the solution was warmed under N_2 at 50−60 °C (bath temperature) for 3.25 h. The solution was then left for 3 days under N_2 and protected from light, at 20−25 °C. The Me_2SO was removed by short-path distillation *in vacuo*, and the residue was taken up in H_2O (15 mL). The clear solution was acidified with glacial AcOH to pH 5.0 to cause precipitation. After refrigeration, the solid was collected and dried; yield 254 mg. A second crop (42 mg) was obtained when the filtrate was acidified further to pH 4.0. Both crops gave the expected mass spectral results: MS *m/e* 408, MH^+. The combined and homogenized crops (72% yield) gave satisfactory elemental analysis results. Anal. ($C_{21}H_{21}N_5O_4$·$2.2H_2O$) C, H, N.

The following 4-amino-4-deoxy-10-carboxy-5,10-dideazapteroic acids **8b**−**e** were prepared by saponification of their respective diesters as described above for **8a**.

4-Amino-4-deoxy-5-methyl-10-propargyl-10-carboxy-5,10-dideazapteroic acid (8b): from saponification of **7b** in 74% yield; MS *m/e* 406, MH^+ for $C_{21}H_{19}N_5O_4$.

5-[1-Carboxy-2-(2,4-diamino-5-deaza-6-pteridinyl)ethyl]-2-thenoic acid (8c): from saponification of **7c** in 85% yield; MS *m/e* 360, MH^+ for $C_{15}H_{13}N_5O_4S$.

4-Amino-4-deoxy-10-carboxy-5,10-dideaza-3′-azapteroic acid (8d): from saponification of **7d** in 95% yield; MS *m/e* 355, MH^+ for $C_{16}H_{14}N_6O_4$.

4-Amino-4-deoxy-5-methyl-10-carboxy-5,10-dideaza-3′-azapteroic acid (8e): from saponification of **7e** in 97% yield; MS *m/e* 369, MH^+ for $C_{17}H_{16}N_6O_4$.

4-Amino-4-deoxy-5-methyl-10-allyl-5,10-dideazapteroic Acid (9a). Compound **8a** (291 mg, 0.65 mmol) was suspended in Me_2SO (10 mL) under N_2, and the stirred mixture was heated for 9 min at 140−145 °C. (The progress of the reaction was monitored by both CO_2 evolution and HPLC.) After removal of Me_2SO *in vacuo*, the residue was dissolved in 1 N NaOH, treated with Norit, and filtered. The filtrate was acidified with 1 N HCl to pH 4.5−5.0, and the precipitate was collected, washed with H_2O, and dried: yield 95% (225 mg); MS *m/e* 364, MH^+ for $C_{20}H_{21}N_5O_2$.

4-Amino-4-deoxy-5-methyl-10-propargyl-5,10-dideazapteroic acid (9b): prepared in 89% yield by decarboxylation of **8b** in Me_2SO solution at 105−110 °C for 50 min; MS *m/e* 362, MH^+ for $C_{20}H_{19}N_5O_2$.

5-[2-(2,4-Diamino-5-deaza-6-pteridinyl)ethyl]-2-thenoic acid (9c): prepared in 99% yield by decarboxylation of **8c** in Me_2SO at 120−125 °C for 20 min; MS *m/e* 316, MH^+ for $C_{14}H_{13}N_5O_2S$.

4-Amino-4-deoxy-5,10-dideaza-3′-azapteroic acid (9d): prepared in 84% yield by decarboxylation of **8d** in DMF solution at 75−80 °C for 10 min; MS *m/e* 311, MH^+ for $C_{15}H_{14}N_6O_2$.

4-Amino-4-deoxy-5-methyl-5,10-dideaza-3′-azapteroic acid (9e): prepared in 77% yield by decarboxylation of **8e** in DMF solution at 80−85 °C for 40 min; MS *m/e* 325, MH^+. Anal. ($C_{16}H_{16}N_6O_2$·$1.4H_2O$) C, H; N: calcd, 24.04; found, 24.47.

5-Methyl-10-allyl-5,10-dideazaaminopterin (2f) *via* Diethyl Ester 10a. A mixture of **9a** (225 mg, 0.62 mmol), Et_3N (254 mg, 2.51 mmol), and *i*-BuOCOCl (84 mg, 0.62 mmol) was stirred at 20−25 °C in DMF (22 mL) for 15 min. Consecutively smaller additions of diethyl L-glutamate·HCl (149 mg, 0.62 mmol; 74 mg, 0.31 mmol; 37 mg, 0.16 mmol) were made every 15 min with each followed 1 min later by an addition of *i*-BuOCOCl (respectively 42 mg, 0.31 mmol; 21 mg, 0.16 mmol; 21 mg, 0.16 mmol). After 15 min, a final amount of diethyl L-glutamate·HCl (37 mg, 0.16 mmol) was added, and the reaction mixture was stirred for 30 min. The DMF was removed *in vacuo* (<1 mmHg, bath 20−25 °C), and the residue was dissolved in $CHCl_3$−MeOH (6:1) and then chromatographed on a silica gel column. Fractions containing the diethyl ester **10a** were eluted with $CHCl_3$−MeOH (6:1), pooled, and evaporated to yield 179 mg (51% yield): MS *m/e* 549, MH^+. Anal. ($C_{29}H_{36}N_6O_5$·$0.9H_2O$) C, H, N.

This sample of **10a** (174 mg, 0.308 mmol) was hydrolyzed in a typical manner as described for **1f**, and pure **2f** was obtained in 78% yield (128 mg): MS *m/e* 493, MH^+; UV (0.1 N HCl) λ_{max} 321 nm (ϵ 7670), 233 (43 200); (0.1 N NaOH) 345 (6550), 237 (41 000); ^{1}H NMR δ 1.93 and 203 (2m, 2, Glu-3-CH_2), 2.32 (t, 2, CH_2CH_2CO), 2.40−2.54 (m, 2, -CH_2CH=CH_2), 2.62 (s, 3, CH_3), 2.80−3.00 (m, 2, overlap of C^9-H and C^{10}-H),

3.06−3.16 (m, 1, C^9-H), 4.34 (q, 1, CONHC*H*), 4.88−5.04 (m, 2, -$CH_2CH{=}CH_2$), 5.58−5.74 (m, 1, $CH_2CH{=}CH_2$), 6.87 (br s, 2, NH_2), 7.24 and 7.73 (2d, 4, C_6H_4), 7.40 (br s, 2, NH_2), 8.02 (d, 1, C7-H), 8.42 (q, 1, CONH). Multiple resonances by protons of the 9,10-bridge, the allyl group, CONH, and C^7-H indicate a diastereomeric mixture. Anal. ($C_{25}H_{28}N_6O_5{\cdot}2H_2O$) C, H, N.

The remaining 5,10-dideazaaminopterin analogues **2g−j** were prepared by similar coupling of the corresponding pteroic acid-type precursors **9b−e** with diethyl L-glutamate followed by hydrolysis of the resulting diesters **10b−e** (Scheme 2).

5-Methyl-10-propargyl-5,10-dideazaaminopterin (2g). The peptide coupling of **9b** (175 mg, 0.48 mmol) with diethyl L-glutamate·HCl was conducted as described for **10a** to give diethyl ester **10b** (106 mg, 40% yield): MS *m/e* 547, MH^+. Hydrolysis of **10b** (102 mg, 0.187 mmol) afforded 80 mg of **2g** (81% yield): MS *m/e* 491, MH^+. Anal. ($C_{25}H_{26}N_6O_5{\cdot}2H_2O$) C, H, N.

***N*-[5-[2-(2,4-Diamino-5-deaza-6-pteridinyl)ethyl]-2-thenoyl]-L-glutamic Acid (2h).** Peptide coupling of **9c** with diethyl L-glutamate mediated by *i*-BuOCOCl in DMF containing Et_3N afforded diethyl ester **10c** (120 mg, 36% yield) after purification on silica gel (TLC R_f 0.5; $CHCl_3$−MeOH, 5:1): MS *m/e* 501, MH^+ for $C_{23}H_{28}N_6O_5S$. Ester **10c** was then subjected to the same ester hydrolysis procedure as described under the preparation of **2f** to give **2h** in 37% yield (43 mg): MS *m/e* 445, MH^+. Anal. ($C_{19}H_{20}N_6O_5S{\cdot}2H_2O$) C, H, N.

5,10-Dideaza-3′-azaaminopterin (2i). Coupling of **9d** with diethyl L-glutamate·HCl to give the diethyl ester **10d** was done as described above. The yield of **10d**, homogeneous by TLC ($CHCl_3$−MeOH, 5:1; R_f 0.5), was 18% (215 mg from 740 mg, 2.38 mmol, of **10d**). The yield was lowered by solubility problems in the chromatographic purification system: MS *m/e* 496, MH^+ for $C_{24}H_{29}N_7O_5$. Hydrolysis of the ester groups of this product (175 mg, 0.353 mmol) led to pure **2i** in 77% yield (119 mg): MS *m/e* 440; ^{1}H NMR δ 1.95, 2.08 (2m, 2, Glu-3-CH_2), 2.34 (t, 2, CH_2CO), 3.06 (m, 2, C_9H_2), 3.17 (m, 2, $C_{10}H_2$), 4.38 (m, 1, CONHC*H*), 6.85 (s, 2, NH_2), 7.36 (d, 1, pyridyl-3-H), 7.84 (s, 2, NH_2), 8.12 (m, 1, pyridyl-4-H), 8.37 (d, 1, C^5-H), 8.54 (d, 1, C^7-H), 8.68 (d, 1, CONH), 8.97 (d, 1, pyridyl-6-H, between N and carboxamide). Anal. ($C_{20}H_{21}N_7O_5{\cdot}3H_2O$) C, H, N.

5-Methyl-5,10-dideaza-3′-azaaminopterin (2j). Compound **9e** (407 mg, 1.16 mmol) was coupled with diethyl L-glutamate as described above. After the DMF had been removed *in vacuo*, the residue was taken up in CH_3OH and the solution was treated with 2 g of silica gel. The mixture was evaporated to dryness, and the residual dispersion was pulverized and applied to a column of silica gel in $CHCl_3$−CH_3OH (5:1). The column was eluted with the same solvent system, and the fractions homogeneous by TLC (R_f 0.31; $CHCl_3$−CH_3OH, 3:1) were pooled and evaporated. The residue was stirred with Et_2O, collected, washed with H_2O, and dried to give the diester **10e**: yield 44% (281 mg); MS *m/e* 510, MH^+. Anal. ($C_{25}H_{31}N_7O_5{\cdot}2H_2O$) C, H, N.

Hydrolysis of **10e** (261 mg, 0.478 mmol) as above afforded **2j**: yield 88% (204 mg); MS *m/e* 454, MH^+; UV (0.1 N HCl) λ_{max} 319 nm (ϵ 7960), 270 (17 600), 228 (38 600); (0.1 N NaOH) 345 (6720), 269 (18 100), 233 (37 400); ^{1}H NMR δ 1.96 and 2.05 (2m, 2, Glu-3-CH_2), 2.34 (t, 2, CH_2CH_2CO), 2.68 (s, 3, CH_3), 3.05 (m, 4, $C_9H_2C_{10}H_2$), 4.38 (q, 1, CONHC*H*), 6.90 (br s, 2, NH_2), 7.34 (d, 3, overlap of NH_2 and pyridyl-3-H), 8.11 (q, 1, pyridyl-4-H), 8.31 (s, 1, C^7-H), 8.63 (d, 1, CONH), 8.96 (d, 1, pyridyl-6-H between N and carboxamide. Anal. ($C_{21}H_{23}N_7O_5{\cdot}1.6H_2O$) C, H, N.

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