and Method N78 for triglycerides. Liver lipid was determined on $CHCl_3$–MeOH extracts of pooled liver samples.[11] Treated groups were compared with controls using Student's t test. Inhibition in vitro of the synthesis of digitonin-precipitable sterols in liver slices from sodium [1-^{14}C] acetate (The Radiochemical Centre, Amersham, U.K.) was measured according to the method of Fears and Morgan.[12] Thin-layer chromatography on total lipid extracts of the livers of rats treated with several compounds described in this paper gave a semiquantitative estimate of the concentration of compound achieved in vivo. Therefore, for experiments in vitro, compounds were added to the incubation mixtures, in propylene glycol, to give a final concentration in the assay approximately equivalent to that obtained in vivo. Fatty acid biosynthesis was measured in the same samples, the fatty acid being extracted with petroleum ether (bp 40–60 °C), after acidification of the sample.

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Synthesis of Quinazoline Analogues of Folic Acid Modified at Position 10[1]

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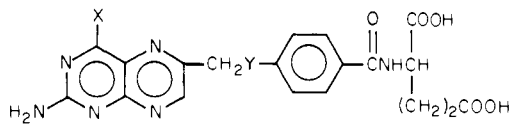
The quinazoline couterpart of folic acid (5,8-deazafolic acid) as well as its 10-methyl analogue has been shown to be an effective inhibitor of thymidylate synthetase from several different sources. This paper describes the synthesis of modifications in which the nitrogen atom at position 10 is replaced by sulfur, oxygen, or methylene affording 10-thia-5,8-deazafolic acid, 10-oxa-5,8-deazafolic acid, and 5,8,10-deazafolic acid, respectively. In preliminary testing, each of the target compounds displayed marginal activity against L1210 leukemia in mice.

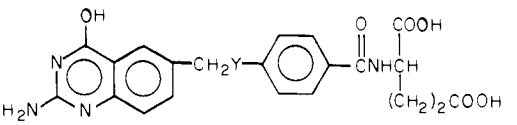
A number of investigations have focused attention on the synthesis of modifications of folic acid (**1a**) in which the nitrogen atom at position 10 is replaced by a sulfur, oxygen, or methylene group. For example, 10-thiafolic acid (**1b**) was prepared concurrently by two research groups and found to display antibacterial activity as well as modest



1a, X = OH; Y = NH
b, X = OH; Y = S
c, X = NH_2; Y = S
d, X = OH; Y = O
1e, X = NH_2; Y = O
f, X = OH; Y = CH_2
g, X = NH_2; Y = CH_2
h, X = NH_2; Y = N (CH_3)

activity against L1210 leukemia in mice.[2,3] Its 4-amino counterpart, 10-thioaminopterin (**1c**), has also been synthesized and was found to rival methotrexate (**1h**) in inhibitory potency against the dihydrofolate reductase from *Lactobacillus casei*.[4] The unequivocal synthesis of 10-oxafolic acid (**1d**) and 10-oxaaminopterin (**1e**) was also recently reported.[5] The latter compound was shown to be an excellent inhibitor of bacterial dihydrofolate reductase, while the former as well as its 7,8-dihydro modification failed to demonstrate substrate activity for this enzyme. The carbon isosteres, N^{10}-deazafolic acid (**1f**) and N^{10}-deazaminopterin (**1g**), resulted from additional elegant synthetic work.[6,7] Compound **1g**, as well as its 7,8-dihydro and 5,6,7,8-tetrahydro derivatives, exhibited remarkable potency against several folate-requiring bacteria.[7]

In view of the aforementioned observations, it was decided that quinazoline counterparts of folic acid analogues modified at position 10 were logical potential antitumor agents. This report is concerned with the synthesis of 4-OH modifications, **2a–c**, which became accessible by virtue of intermediates prepared earlier in this laboratory.[8,9]

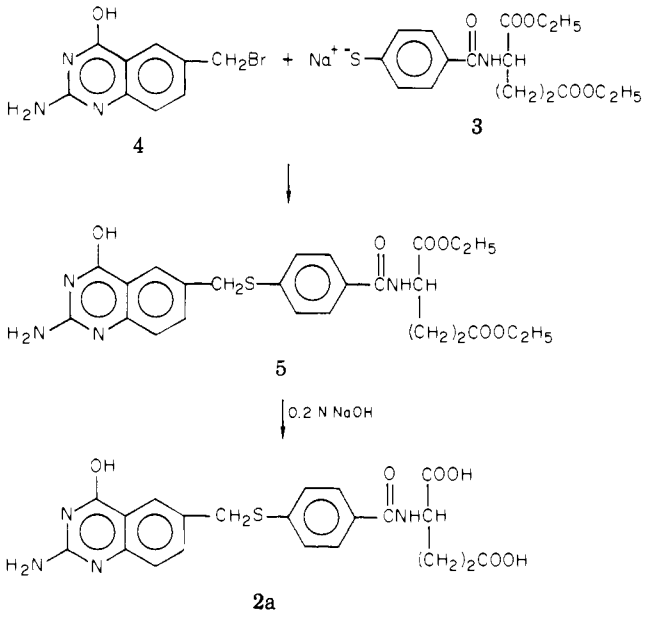


2a, Y = S
b, Y = O
c, Y = CH_2
d, Y = NH
e, Y = N(CH_3)

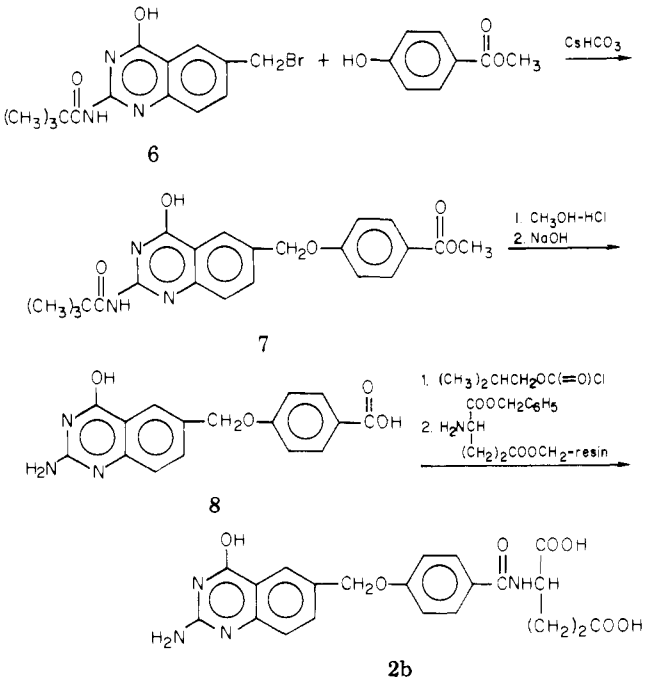
It will be recalled that the 5,8-deazafolates, **2d** and **2e**, were shown to be effective inhibitors of thymidylate synthetase from *Escherichia coli*,[10] *Diplococcus pneumoniae*,[11] and mouse neuroblastoma cells.[12] Moreover, it has been suggested that a selective inhibitor of this enzyme could prove to be of value in producing "thymineless death" in cancer cells.[13] Therefore, the new isosteric modifications should help to define the contribution of the basic nitrogen atom at position 10 to inhibitor potency against this enzyme. In the present study, the amino acid moiety was restricted to L-glutamyl since earlier studies have shown that the L-aspartyl analogues of **2d** and **2e** were substantially less potent as inhibitors of thymidylate synthetase.[10]

Chemistry. The target compound, 10-thia-5,8-deazafolic acid (**2a**), was prepared as shown in Scheme I. The sodium salt of diethyl 4-mercaptobenzoyl-L-glutamate (**3**) was generated according to the literature procedure[2] and

Scheme I



Scheme II



then reacted with 2-amino-6-bromomethyl-4-hydroxyquinazoline (4).[8] The resulting diethyl ester **5** was purified by column chromatography and then saponified in dilute NaOH to yield the free acid **2a**.

Initial attempts to prepare the oxygen analogue **2b**, by direct alkylation of diethyl 4-hydroxybenzoyl-L-glutamate with **4** or its *N*-trimethylacetyl derivative **6**, yielded complex reaction mixtures. Therefore, the route shown in Scheme II was employed to synthesize this target compound. Methyl 4-hydroxybenzoate reacted smoothly with 6-bromomethyl-4-hydroxy-2-trimethylacetamidoquinazoline (6)[8] in the presence of cesium bicarbonate[5] to yield compound **7**. Stepwise deprotection, first with acid and then with base, afforded the key intermediate, 2-amino-6-(4-carboxyphenoxymethyl)-4-hydroxyquinazoline (**8**). The direct conversion of **7** to **8** in base resulted in a substantially reduced yield. The glutamic acid moiety was then introduced via the solid-phase peptide synthesis technique[14,15] yielding 10-oxa-5,8-deazafolic acid (**2b**).

Scheme III

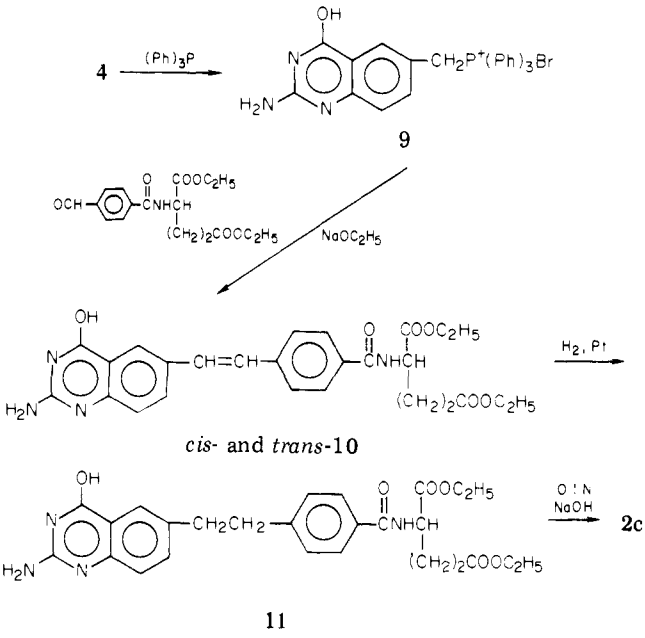
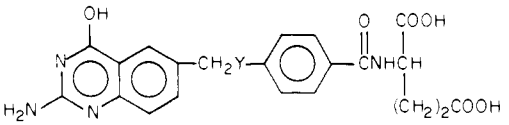


Table I. Activity against L1210 Leukemia in Mice



		% increase in survival after single ip dose, mg/kg[a]					
No.	Y	25	50	75	100	150	200
2a	S				9	22	
2b	O			6		28	14
2c	CH_2		14	18		23	
Methotrexate		68	61	48	81 (5T)		

[a] Testing was conducted under the direction of Dr. Glen R. Gale, Veteran's Administration Hospital, Charleston, S.C., using 10^6 rather than 10^5 cells for inoculum.[15] Compounds were administered on day 1 following infection and were dissolved in Me_2SO. Each test group consisted of six animals; controls died in an average of 6.5 days.

The synthesis of 5,8,10-deazafolic acid (**2c**) commenced with compound **4**, which was converted to its triphenylphosphonium salt **9** with triphenylphosphine as shown in Scheme III. Generation of the corresponding ylide with sodium ethoxide followed by coupling with diethyl 4-formylbenzoyl-L-glutamate (**8**) yielded approximately equal amounts of the cis and trans olefins **10** in high yield. This mixture was then reduced catalytically to give diethyl 5,8,10-deazafolate (**11**). Finally, hydrolysis in dilute NaOH yielded the desired compound, **2c**.

Biological Results. Each of the new free glutamates was subjected to preliminary evaluation for activity against L1210 leukemia in mice and the results are summarized in Table I. It will be noted that at the 150 mg/kg dose level each showed marginal activity with no evidence of acute toxicity. However, the new compounds are much less potent than methotrexate (**1h**), which is the standard of comparison for folate antagonists having potential antineoplastic activity. Based upon the marginal activities of the target compounds, additional testing, especially against methotrexate-resistant tumor lines, appears warranted. Further synthetic modifications in our laboratories and thymidylate synthetase inhibition studies in collaboration with other laboratories are in progress and

will be the subject of future publications.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Analytical samples were dried in vacuo at 100 °C and were free of significant impurities on TLC (Gelman SAF). All intermediates and target compounds had NMR (Varian T-60) spectra in accord with their assigned structures. Values for chemical shifts are presented in parts per million downfield from Me_4Si as an internal standard. High-pressure liquid chromatographic separations were done on a Waters Associates Model 400 liquid chromatograph equipped with a UV detector (365 nm) and an A6000 pump using a μ Bondapak-CN column of 30-cm length and 4-mm inside diameter.

Tetraethyl 4,4'-Dithiobis(*N*-benzoyl-L-glutamate). 4,4'-Dithiobis(benzoic acid) was prepared in 89% yield according to the procedure of Campaigne and Meyer.[16] The acid was esterified with ethanol–HCl according to the procedure of Mautner et al.[2] (yield 59%). To a solution of 15.6 g (51 mmol) of 4,4'-dithiobisbenzoic acid protected from moisture by a $CaCl_2$ drying tube in 250 mL of pyridine (freshly distilled from BaO) and 24.0 g (100 mmol) of diethyl L-glutamate hydrochloride was added 24.2 g (112 mmol) of DCC. The solution was stirred at ambient temperature for 7 days. After removal of the pyridine at reduced pressure, the oily residue was dissolved in 500 mL of $CHCl_3$. The dicyclohexylurea was removed by filtration and discarded. The filtrate was washed successively with two portions of 250 mL of 10% $NaHCO_3$, 250 mL of 0.1 N HCl, and 250 mL of H_2O. After drying over $MgSO_4$ and treatment with charcoal, the solvent was removed under reduced pressure. The oily residue was redissolved in 500 mL of warm CH_3CN and the insoluble material discarded. Concentration of the filtrate followed by cooling at 4 °C gave 6.80 g (20.5%), mp 97–99 °C (lit.[2] 97–99 °C).

Diethyl 5,8-Deaza-10-thiafolate (5). Tetraethyl 4,4'-Dithiobis(*N*-benzoyl-L-glutamate) (2.84 g, 4.2 mmol) was suspended in 40 mL of EtOH and reduced with 0.48 g (12.6 mmol) of $NaBH_4$. The reaction was complete in 5 min as examined by the bathochromic shift of the UV spectrum from λ_{max} (EtOH) 273 nm to λ_{max} (EtOH) 323 nm. The above solution was added to 2.03 g (8 mmol) of 2-amino-6-bromomethyl-4-hydroxyquinazoline (**4**)[8] suspended in 100 mL of DMF, and the resulting mixture was stirred at ambient temperature for 18 h, after which time the solution gave a negative active halogen test.[17] The EtOH was removed at reduced pressure and the residue poured into 500 mL of H_2O. After cooling at 4 °C for 3 h, the solid was isolated on a filter, washed with H_2O, and dried to give 2.53 g of tan solid, mp 175–178 °C dec. This material was dissolved in $CHCl_3$ and poured onto a silica gel (60–100 mesh) column (50 g of packing/g of product). The column was washed with $CHCl_3$, and then the product was eluted with 850 mL of 5% MeOH in $CHCl_3$. After removing the solvents at reduced pressure, 1.35 g of **10** (33%) was obtained: mp 183–188 °C dec; NMR (CF_3COOD) δ 1.40 (m, 6, CH_3), 2.7 [m, 4, CH_2(COOH)CH_2], 4.4 (m, CH_2S, CH_2CH_3), 5.0 (m, 1, NHC*H*), 7.2–8.5 (m, 7, aromatic). Anal. ($C_{25}H_{28}N_4O_6S$) C, H, N.

5,8-Deaza-10-thiafolic Acid (2a). A mixture of **10** (0.85 g, 1.63 mmol) was suspended in 40 mL of 0.2 N NaOH and stirred at 24 °C for 29 h. The cloudy solution was acidified to pH 4.5 with 1 N HCl. The white solid was isolated by filtration, washed with water, acetone, and ether, and then dried to yield 0.42 g (57%) of **11**: mp >220 dec; NMR (CF_3COOD) δ 2.7 [m, 4, CH_2(COOH)CH_2], 4.4 (s, 2, CH_2S), 5.1 (m, 1, NHC*H*), 7–8.5 (m, 7, aromatic). Anal. ($C_{21}H_{20}N_4O_6S\cdot1.5H_2O$) C, H, N.

2-Trimethylacetamido-6-(4-carbomethoxyphenoxymethyl)-4-hydroxyquinazoline (7). Methyl *p*-hydroxybenzoate (6.1 g, 40 mmol) and $CsHCO_3$ (6.8 g, 35 mmol) were suspended in 150 mL of anhydrous DMF and warmed at 60–70 °C for 10 min. To this mixture was added 6-bromomethyl-4-hydroxy-2-trimethylacetamidoquinazoline (**6**) (10.1 g, 30 mmol) and heating was continued for 1.5 h, at which time the reaction mixture gave a negative active halogen test.[17] After cooling to room temperature, it was poured into 250 mL of H_2O. Cooling at 4 °C for 2 h followed by filtration and washing with H_2O gave 9.2 g (75%) of **7** as a fine white powder, mp 190–200 °C dec. The product could be used without further purification. For analysis **7** was recrystallized from THF: mp 255–256 °C dec; NMR (CF_3COOD) δ 1.5 [s, 9, (CH_3)$_3$C], 4.0 (s, 3, $COCH_3$), 5.4 (s, 2, CH_2O), 6.8–8.8 (m, 7, aromatic). Anal. ($C_{22}H_{23}N_3O_5$) C, H, N.

2-Amino-6-(4-carboxyphenoxymethyl)-4-hydroxyquinazoline (8). Compound **7** (3.52 g, 8.6 mmol) was suspended in 80 mL of warm THF and 2.8 mL of H_2O was added followed by 50 mL of methanolic HCl. The resulting clear solution was refluxed for 4 h, during which time a white solid precipitated. The solvent was removed at reduced pressure and the residue diluted with 50 mL of H_2O. The pH was adjusted to 6 with 2 N NaOH. The white solid was separated by filtration and washed with H_2O, acetone, and ether to give 2.50 g (89.5%) of 2-amino-6-(4-carbomethyoxyphenoxymethyl)-4-hydroxyquinazoline as a white solid: mp >270 °C dec; NMR (CF_3COOD) δ 4.1 (s, 3, OCH_3), 5.4 (s, 2, OCH_2), 6.9–8.7 (m, 7, aromatic).

A 2.72-g (8.4 mmol) sample of this ester was suspended in 170 mL of 0.2 N NaOH and stirred at room temperature for 20 h. The slightly cloudy solution was filtered through Celite and the filtrate was acidified to pH 5 with 1 N HCl. The product was separated by filtration and washed with H_2O, acetone, and ether to give 1.42 g (62.3%) of white solid: mp >280 °C dec; NMR (CF_3COOD) δ 5.4 (s, 2, CH_2O), 6.9–8.7 (m, 7, aromatic). Anal. ($C_{16}H_{13}N_3O_4\cdot0.5H_2O$) C, H, N.

10-Oxa-5,8-deazafolic Acid (2b). The pH of a solution of *N-tert*-butoxycarbonyl-L-glutamic acid α-benzyl ester (2.49 g, 6.9 mmol) dissolved in 23 mL of 95% EtOH and 11.5 mL of H_2O was adjusted to 7.00 with an aqueous solution of $CsHCO_3$. The solvent was removed at room temperature on a rotoevaporator. The residue was dissolved twice in benzene and then the solvent removed. The resulting glassy solid of cesium *N-tert*-butoxycarbonyl-L-glutamate α-benzyl ester was dried in vacuo over P_2O_5 for 18 h.

The cesium salt of the amino acid prepared above was esterified to the 1% cross-linked chloromethylated Merrifield resin (6.70 g, 6.9 mequiv of Cl) by stirring in 46 mL of dry DMF at 60 °C for 15 h. The resin was filtered and washed with 3 × 25 mL of DMF, 25 mL of DMF–H_2O (10:1), 2 × 25 mL of DMF, and 2 × 25 mL of EtOH and then dried at room temperature for 18 h in vacuo (P_2O_5).

The resin was transferred to a stoppered 500-mL Erlenmeyer flask and all subsequent manipulations were carried out in that flask. The esterified resin was allowed to swell by shaking with three successive 75-mL portions of CH_2Cl_2 (3 min each). The *tert*-butoxycarbonyl function was removed by shaking for 30 min with 100 mL of 20% CF_3COOH in CH_2Cl_2.

The aminoacyl resin was then washed by shaking 3 min with each of the following, 3 × 75 mL of EtOH and 3 × 75 mL of DMF, and for 10 min with 85 mL of 18% Et_3N in DMF, after which the resin was washed with two additional 75-mL poritions of DMF.

The mixed anhydride was prepared by dissolving **8** (2.75 g, 8.6 mmol) in 75 mL of warm anhydrous Me_2SO followed by cooling to room temperature and adding 75 mL of dry THF (distilled from $LiAlH_4$). After cooling the reaction mixture to 0 °C in an ice bath, *N*-methylmorpholine (0.82 g, 8.12 mmol) was added and the solution stirred for additional 15 min. Isobutyl chloroformate (1.1 g, 8.07 mmol) was added and stirring was continued for another 0.5 h.

The solution of the mixed anhydride was added to the aminoacyl resin and the mixture shaken at room temperature for 21 h. After this period, the resin-bound product was filtered, washed with 4 × 25 mL of Me_2SO–THF (1:1) and 4 × 25 mL of dioxane, and suspended in 125 mL of dioxane–2 N NaOH (1:1). After shaking for 18 h at room temperature, the resin was filtered and the pH of the filtrate was adjusted to 4 with 1 N HCl. The resulting precipitate was cooled at 4 °C overnight and then filtered, washed with H_2O, and dried in vacuo over P_2O_5 to give 1.0 g of **2b** (60% yield based on reacted **8**) as a cream solid: mp 235–240 °C dec; NMR (CF_3COOD) δ 2.8 (m, 4, CH_2CH_2), 5.1 (m, 1 NCH), 5.4 (s, 2, CH_2O), 6.9–8.7 (m, 7, aromatic). Anal. ($C_{21}H_{20}N_4O_7\cdot1.25H_2O$) C, H, N.

2-Amino-4-hydroxyquinazol-6-ylmethylphosphonium Bromide (9). Compound **4** (2.54 g, 10 mmol) and triphenylphosphine (5.25 g, 20 mmol) were suspended in 50 mL of DMF and heated at 100 °C for 2 h. The resulting clear solution was cooled to room temperature and then poured into 350 mL of benzene. After cooling at 4 °C overnight, the white solid was

isolated by filtration, washed with benzene, and dried in vacuo over P_2O_5 to yield 5.32 g (quantitative): mp 300–320 °C dec; NMR (Me_2SO-d_6) δ 5.4 (d, $^2J_{^{31}P\text{-}H}$ = 16 Hz, 2, CH_2), 7.1–9.2 (m, 21, aromatic, OH, NH_2).

Diethyl 5,8,10-Deaza-9,10-dehydrofolate (10). In an oven-dried three-necked flask equipped with stirrer, gas inlet tube, and Hg pressure seal was placed **9** (2.58 g, 5 mmol), diethyl 4-formylbenzoyl-L-glutamate,[9] and 50 mL of dry DMF. After flushing the reaction flask with N_2 and maintaining a positive pressure, EtONa (prepared from 0.245 g, 10.06 mmol, of Na in 17.5 mL of EtOH) was added dropwise over a period of 1 h. The reaction mixture was then stirred overnight at room temperature. The pH of the solution was adjusted to 6 with 1 N HCl and the DMF removed at 30 °C in vacuo. The resulting fluorescent yellow oil was dissolved in 100 mL of $CHCl_3$ and dried over Na_2SO_4, and the solvent was removed in vacuo. The residual oil was dried in vacuo overnight and the resulting solid recrystallized from EtOH–H_2O (1:1.3, 110 mL/g) to yield 2.0 g (81%): mp 225–230 °C dec; NMR (CF_3COOD) δ 1.4 (m, 6, CH_3), 2.7 (m, 4, CH_2CH_2), 4.4 (m, 4, $COCH_2$), 5.0 (m, 1, NCH), 6.7–8.8 (m, 9, aromatic olefin). Anal. ($C_{26}H_{28}N_4O_6$) C, H, N [a sample of analytical purity could only be obtained after purification by HPLC on a μ Bondapak-CN column using hexane–EtOAc–MeOH (5:1:0.15) as eluent].

Diethyl 5,8,10-Deazafolate (11). Once recrystallized **10** (1.65 g, 3.36 mmol) dissolved in 45 mL of DMF and 0.66 g of CH_3SO_3H was hydrogenated over PtO_2 (23 mg). Additional PtO_2 (3 × 30–35 mg) was added at intervals when uptake of H_2 ceased due to poisoning of catalyst. After shaking overnight, fresh catalyst (25 mg) was added whereupon no net uptake of H_2 was observed. TLC ($CHCl_3$–MeOH, 5.6:1) showed the reaction to be complete. The catalyst was filtered off and the DMF removed in vacuo. Water was added and the resulting solid filtered and dried in vacuo over P_2O_5. Recrystallization from EtOH gave 1.4 g (84%): mp 123–126 °C; NMR (CF_3COOD) δ 1.4 (m, 6, CH_3), 2.7 (m, 4, $CHCH_2CH_2$), 3.2 (br s, 4, $CH_2CH_2C_6H_4$), 4.4 (m, 4, CH_2), 5.0 (m, 1, CHNH), 7–8.6 (m, 7, aromatic). Anal. ($C_{26}H_{30}N_4O_6 \cdot 0.5H_2O \cdot CH_3CHOHCH_3$) C, H, N [a sample of analytical purity could only be obtained after purification by HPLC on a μ Bondapak-CN column using $CHCl_3$–2-propanol (15.5:1) as eluent].

5,8,10-Deazafolic Acid (2c). Compound **11** (0.61 g, 1.23 mmol) was stirred overnight in 40 mL of 0.2 N NaOH. After filtering the reaction mixture through Celite, the filtrate was acidified to pH 4 with 1 N HCl. The resulting product was isolated by centrifugation, washed with H_2O, acetone, and ether, and filtered to yield 0.36 g (64.4%) of **2c** as a cream solid: mp 236–240 °C dec; NMR (CF_3COOD) δ 2.8 (m, 4, $CHCH_2CH_2$), 3.2 (br s, 4, $CH_2CH_2C_6H_4$), 5.1 (m, 1, NCH), 7–8.8 (m, 7, aromatic). Anal. ($C_{22}H_{22}N_4O_6 \cdot H_2O$) C, H, N.

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Synthesis of Prostaglandin Synthetase Substrate Analogues. 2. (8*Z*,11*Z*,14*Z*)-15-Methyl-8,11,14-eicosatrienoic Acid

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The regioselective convergent synthesis of (8*Z*,11*Z*,14*Z*)-15-methyl-8,11,14-eicosatrienoic acid is described. This compound exhibited prostaglandin synthetase inhibitory activity, although it was not metabolized by the enzyme.

The essential fatty acids, (8*Z*,11*Z*,14*Z*)-8,11,14-eicosatrienoic acid and arachidonic acid, are converted to the prostaglandins E_1 and E_2, respectively, by a multistage biosynthetic process catalyzed by the enzyme prostaglandin synthetase. As part of a program to prepare substrate analogues of these acids with potential prostaglandin synthetase inhibitory activity, we have synthesized (8*Z*,11*Z*,14*Z*)-15-methyl-8,11,14-eicosatrienoic acid (**1**).[1] This analogue was envisioned as a competitive, nonmetabolizable inhibitor, in which enzymatically catalyzed attack by oxygen at the C-15 position would be inhibited by the increased steric bulk of the methyl substituent.

The two intermediates selected for this 3 + 4 step convergent synthesis were (*Z*)-1-bromo-3-methyl-2-octene (**5**) and 11-chloro-1,4-undecadiyne (**7**), which were prepared from ethyl 2-octynoate (**2**) and 8-chloro-1-octyne (**6**), respectively.