19.47 (q), 29.05 (q), 35.81 (s), 45.39 (d), 59.47 (s), 60.47 (t), 128.13 (s), 128.57 (d), 129.17 (d), 137.99 (d), 174.28 (s); MS *m/e* (relative intensity) 342 (M^+, 4), 340 (2) and 338 (1); exact mass calcd for $C_{17}H_{26}O_2Se$ *m/e* 342.1097, 340.1108, found *m/e* 342.1112 and 340.1148, respectively.

***rel*-(1*R*(*E*),5*R*,8*R*)-Ethyl 5-(8-Iodo-7-oxo-6-oxabicyclo-[3.2.1]oct-2-en-1-yl)-2-pentenoate (53).** To a solution of 432 mg (1.4 mmol) of the appropriate aldehyde[58] in 70 mL of dry benzene under Ar was added 541 mg (1.55 mmol) of 1-(carbethoxy)methylidenetriphenylphosphorane in one portion. The resulting solution was stirred at 75 °C for 2 h and solvent was removed in vacuo. The residue was chromatographed over 100 g of silica gel (eluted with EtOAc–petroleum ether (1:10)) to give 412 mg (77%) of a mixture of **53** and its geometrical isomer as a colorless oil. This material was chromatographed (MPLC, lobar size A column, eluted with EtOAc–petroleum ether (1:5)) to give 25 mg of the *Z* isomer of **53** and 372 mg of ester **53**. *Z* isomer of **53**: IR (neat) 1781, 1713 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.3 (t, *J* = 7.1 Hz, 3 H, CH_3), 1.8 (m, 1 H, CH), 2.05 (m, 1 H, CH), 2.5–3.0 (m, 4 H, CH_2 and CH), 4.15 (q, *J* = 7.1 Hz, 2 H, OCH_2), 4.55 (dd, *J* = 5.4, 1.6 Hz, 1 H, CHI), 4.75 (m, 1 H, HCO), 5.4 (dq, *J* = 9.5, 2.0 Hz, 1 H, =CH), 5.7–5.8 (m, 2 H, =CH), 6.2 (dt, *J* = 11.5, 7.4 Hz, 1 H, =CH); ^{13}C-NMR ($CDCl_3$) δ 14.28 (q), 23.35 (d), 23.35 (t), 28.96 (t), 30.10 (t), 48.43 (s), 60.01 (t), 76.27 (d), 120.76 (d), 126.82 (d), 129.83 (d), 147.78 (d), 166.21 (s), 171.51 (s); exact mass calcd. for $C_{14}H_{17}O_4I$ *m/e* 376.0172, found *m/e* 376.0113, respectively. Ester **53**: IR (neat) 1779, 1715 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.2 (d, *J* = 7.1 Hz, 3 H, CH_3), 1.7 (m, 1 H, CH), 2.0 (m, 1 H, CH), 2.2 (m, 2 H, =CCH_2), 2.5 (dm, *J* = 19.6 Hz, 1 H, =CCH), 2.75 (dm, *J* = 19.6 Hz, 1 H, =CCH), 4.1 (q, *J* = 7.1 Hz, 2 H, OCH_2), 4.4 (dd, *J* = 5.4, 1.5 Hz, 1 H, CHI), 4.7 (m, 1 H, CHO), 5.35 (dm, *J* = 9.5 Hz, 1 H, =CH), 5.8 (m, 2 H, =CH), 6.9 (dt, *J* = 15.6, 6.7 Hz, 1 H, =CH); ^{13}C-NMR ($CDCl_3$) δ 14.09 (q), 23.19 (d), 25.89 (t), 28.15 (t), 29.84 (t), 48.05 (s), 60.06 (t), 76.01 (d), 122.09 (d), 126.91 (d), 129.18 (d), 146.78 (d), 166.02 (s), 171.06 (s).

***rel*-(1*S*,3a*S*,7*S*,7a*S*)-Ethyl 1,2,3,6,7,7a-Hexahydro-α(*S*)-deuterio-7,3a-(epoxymethano)-3a*H*-indene-1-acetate (55) and *rel*-(1*S*,3a*S*,7*S*,7a*S*)-Ethyl 1,2,3,6,7,7a-Hexahydro-α(*R*)-deuterio-7,3a-(epoxymethano)-3a*H*-indene-1-acetate (56).** To a solution of 0.15 g (0.34 mmol) of iodo ester **53** and 1 mg of AIBN in 5 mL of dry benzene was added 175 mg (0.59 mmol) of *n*-Bu_3SnD in one portion. The reaction mixture was stirred under Ar at 60 °C for 5 h. The reaction mixture was then partitioned between 25 mL of acetonitrile and 25 mL of hexanes. The hexanes layer was extracted with 25 mL of acetonitrile, and the combined acetonitrile layers were washed once with 25 mL of hexanes and concentrated in vacuo. The residue was dissolved in 20 mL of petroleum ether and stirred with 5 mL of saturated aqueous KF for 30 min. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over 20 g of silica gel (eluted with EtOAc–petroleum ether (1:6)) to give 86 mg (86%) of a 92:8 mixture (^{1}H-NMR) of esters **55** + **56** and the respective C(1) diastereomers: IR (neat) 1772, 1730.7 cm^{-1}; ^{1}H-NMR (signals due to **55**, $CDCl_3$) δ 1.18 (t, *J* = 7.2 Hz, 3 H, CH_3), 1.29 (m, 1 H, CH), 1.59 (m, 1 H, CH), 2.06 (m, 1 H, CH), 2.25 (m, 1 H, CH), 2.34 (m, 1 H, CHD), 2.38 (d, *J* = 10.2 Hz, 1 H, CH), 2.45 (m, 2 H, CH_2), 2.6 (m, 1 H, CH), 4.05 (q, *J* = 7.2 Hz, 2 H, OCH_2), 5.6 (dm, *J* = 9.2 Hz, =CH), 6.0 (dt, *J* = 9.2, 1.8 Hz, =CH); diagnostic ^{1}H-NMR (**56**, $CDCl_3$) δ 2.21 (dm, *J* = 7.6 Hz, CHD); ^{13}C-NMR ($CDCl_3$) δ 14.08 (q), 28.18 (t), 33.63 (d), 34.26 (t), 35.44 (t), 36.17 (d, CHD), 52.74 (d), 54.07 (s), 60.39 (t), 75.89 (d), 126.94 (d), 130.02 (d), 172.83 (s), 178.96 (s); ^{2}H-NMR (C_6H_6) δ 1.92 (bs, CHD of C(1) diastereomers), 2.04 (bs, CHD of **55**), 2.21 (bs, CHD of **56**); MS *m/e* (relative intensity) 252 (M^+ + 1, 2), 22 (3), 207 (10). Diagnostic ^{1}H-NMR peaks for the C(1) diastereomers of **55** and **56** appeared at δ 5.89 (dt, *J* = 9.2, 1.8 Hz, =CH). The ratio of the diastereomers **55** to **56** was 82:18 by integration of peaks at δ 2.04 and δ 2.21 in the 76.8-MHz ^{2}H-NMR spectrum of the mixture.

Acknowledgment. We thank the National Science Foundation for generous support of this research and one of the reviewers for helpful comments.

Supplementary Material Available: Full experimental details and selected ^{1}H, ^{2}H, and ^{13}C NMR spectra (163 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Synthesis of Tetrahydropteridine C6-Stereoisomers, Including N^5-Formyl-(6*S*)-tetrahydrofolic Acid

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Chiral N1-protected vicinal diamines derived from amino acids were condensed with 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone, the nitro group reduced, and the amine deprotected. Oxidative cyclization of the resulting triaminopyrimidinone via quinoid pyrimidine intermediates gave a quinoid dihydropteridine, which was then reduced to a tetrahydropteridine C6-stereoisomer. Thus, 6(*R*)- and 6(*S*)-propyltetrahydropterin were stereospecifically synthesized (99% enantiomeric purity) in good yield from D- and L-norvaline, respectively. Reductive alkylation of (*p*-aminobenzoyl)-L-glutamate with a nitropyrimidine aldehyde derived from D- or L-serine similarly afforded, after cyclization and reduction, (6*R*)- or (6*S*)-tetrahydrofolic acid. The latter was then converted to the natural isomer of leucovorin by regioselective N5-formylation with carbonyl diimidazole/formic acid without loss of enantiomeric purity.

Introduction

There currently is no method for the stereospecific synthesis of the reduced pteridine cofactors tetrahydrofolic acid (**1a**) or tetrahydrobiopterin (**1b**). These are important not only for the investigation of enzymatic one-carbon transfer and aromatic amino acid hydroxylation[1] but also clinically. The former (**1a**), as the N5-formyl derivative (leucovorin), is used to potentiate the effects of 5-fluorouracil and in rescue therapy after high-dose methotrexate in the treatment of several forms of cancer. Leucovorin is also coadministered with trimetrexate for treatment of pneumonia caused by *Pneumocystis carinii*. The latter (**1b**) is used in replacement therapy for patients with genetic defects in the tetrahydrobiopterin biosynthetic pathway and is in clinical trials for the treatment of other neurological disorders.[2]

(1) *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; Wiley-Interscience: New York, 1985; Vols. 1-2.

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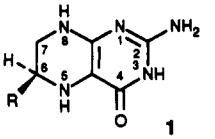
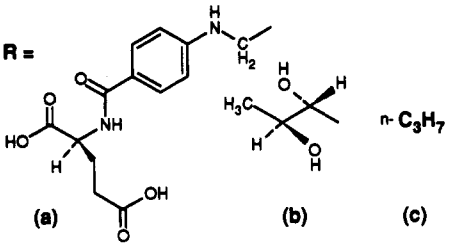
Pteridines are most often produced from pyrimidine precursors,[3] but very useful routes from pyrazine starting materials have also been developed.[4] Although the stereochemistry in the (*p*-aminobenzoyl)-L-glutamate (PABA-glu) or L-*erythro*-dihydroxypropyl side chains of these cofactors is easily introduced, the final products of almost all methods possess a sp^2 6-carbon. Consequently, subsequent reduction to tetrahydropteridine results in a mixture of C6-enantiomers. The natural isomers of **1a**



1

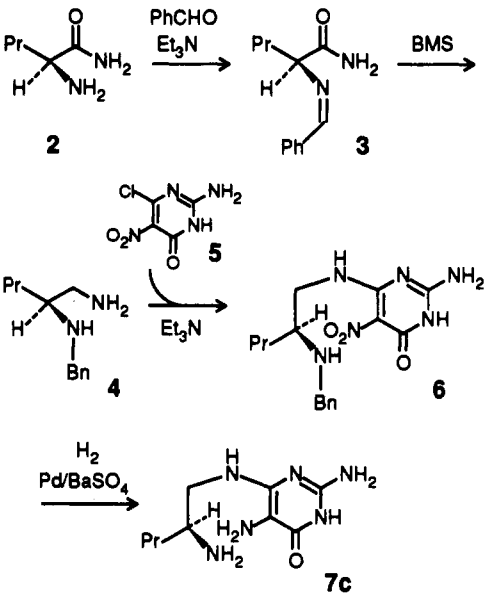
R =



(a) (b) (c)

and **1b** (both identically configured as 6*S* and 6*R*, respectively) have been previously obtained (in some cases as derivatives) by chromatographic separation,[3a,5] fractional crystallization,[6] stereoselective reduction,[5a,7] or enzymatic reduction.[3a,5a,8] The single example of a stereospecific synthesis yielded a mixture of 6(*S*)- and 7(*S*)-methyltetrahydropterin[9] starting from a 5-bromopyrimidine.[10] However, another attempt to utilize displacement of a 5-bromo group for generation of chiral **1a** was unsuccessful.[11] Our goal, therefore, was to develop a general regiospecific and C6-stereospecific synthesis of tetrahydropteridines. The result is exemplified by the production of both the 6*S* and 6*R* isomers of **1a** and 6-propyltetrahydropterin (**1c**). Additionally, a new method for the convenient N5-formylation of **1a** to leucovorin is presented.

Scheme I



The total C6-stereospecific synthesis of tetrahydropterins described in this paper utilizes quinoid pyrimidine and quinoid dihydropteridine intermediates.[12] 4,5-Diaminopyrimidines, especially those containing at least one other electron-donating substituent, are sensitive to oxidation. When substituted with a β-aminoethylamine moiety (e.g., **7**), this susceptibility to quinone formation can be used for pteridine ring closure. The 5-imine of the oxidized pyrimidine provides a latent carbonyl that is revealed by acid hydrolysis. This can be seen in the ready conversion of 2,5,6-triamino-4(3*H*)-pyrimidinone to 2,6-diamino-5-hydroxy-4(3*H*)-pyrimidinone (divicine) by an oxidation–hydrolysis–reduction cycle.[13] With the ethanediamine derivative **9**, the carbonyl can undergo Schiff's base condensation with the 2′-amino group. The initial result is the C4a-hydroxy adduct **11** which itself is of interest as the cofactor product of tetrahydropterin-dependent aromatic amino acid hydroxylases.[13,14] Dehydration of the carbinolamine provides the quinoid dihydropterin **12** which can be reduced to the tetrahydropterin. Although quinoid dihydropteridine intermediates have previously been utilized in the synthesis of 6,6-disubstituted tetrahydropterins,[12] these are relatively stable to rearrangement. 6-Monosubstituted quinoid dihydropteridines, however, are labile to tautomerization to nonchiral 7,8-dihydropteridines. A synthesis of enantiomerically pure derivatives of **1** from chiral precursors, therefore, required developing conditions that allow survival of the initial stereochemistry both upon incorporation as the β-carbon of the pyrimidine side chain as well as during oxidative cyclization and subsequent reduction.

Results and Discussion

Chiral 6-Alkyltetrahydropteridines. Several routes to the central triaminopyrimidinone **7c** are possible. However, for the current work condensation of a chiral diamine **4** with 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**5**) (as in the Boon–Leigh procedure[15]) was used as

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(5) (a) Bailey, S. W.; Ayling, J. E. *J. Biol. Chem.* **1978**, *253*, 1598. (b) Matsuura, S.; Sugimoto, T.; Murata, S.; Sugawara, Y.; Iwasaki, H. *J. Biochem.* **1985**, *98*, 1341.

(6) (a) Armarego, W. L. F. In *Chemistry and Biology of Pteridines*; Kisliuk, R. L., Brown, G. M., Eds.; Elsevier North-Holland: New York, 1979; p 1. (b) Ganguly, S. N.; Viscontini, M. *Helv. Chim. Acta* **1982**, *65*, 1090.

(7) (a) Boyle, P. H.; Keating, M. T. *J. Chem. Soc., Chem. Commun.* **1974**, *10*, 375. (b) Kwee, S.; Lund, H. *Bioelectrochem. Bioeng.* **1980**, *7*, 693. (c) Matsuura, S.; Murata, S.; Sugimoto, T. *Heterocycles* **1985**, *23*, 3115.

(8) (a) Mathews, C. K.; Huennekens, F. M. *J. Biol. Chem.* **1960**, *235*, 3304. (b) Ahmed, F.; Dunlap, R. B. *Anal. Biochem.* **1984**, *141*, 147. (c) Charlton, P. A.; Young, D. W.; Birdsall, B.; Feeney, J.; Roberts, G. C. K. *J. Chem. Soc., Perkin Trans. 1* **1985**, *7*, 1349.

(9) pterin = 2-amino-4(3*H*)pteridinone

(10) Matsuura, S.; Sugimoto, T. *J. Chem. Soc. Jpn.* **1981**, *54*, 2543.

(11) Doyle, P.; Szakolcai, A.; Young, D. W. In *Chemistry and Biology of Pteridines*; Blair, J. A., Ed.; Walter de Gruyter: Berlin, 1983; p 85.

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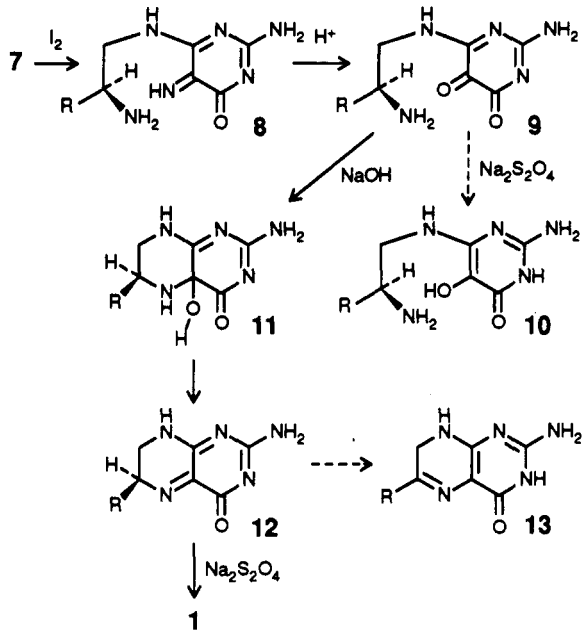
(13) Bailey, S. W.; Weintraub, S. T.; Hamilton, S. M.; Ayling, J. E. *J. Biol. Chem.* **1982**, *257*, 8253.

(14) (a) Kaufman, S.; Fisher, D. B. In *Molecular Mechanisms of Oxygen Activation*; Hayaishi, O., Ed.; Academic Press: New York, 1974; p 285. (b) Lazarus, R. A.; DeBrosse, C. W.; Benkovic, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 6869.

Scheme II



this generally proceeds rapidly in high yield under mild conditions[16] (Scheme I). Chiral ethanediamines are available via a number of routes including asymmetric Strecker reactions,[17] as well as from amino acids.[11,18] Reduction of α-amino amides with retention of configuration with borane–THF has been previously reported.[18] With α-protected amino amides borane–Me_2S (BMS)[19] was found to give improved yields. Further, the ease of amide reduction with either reagent was considerably dependent on the nature of the protecting group, benzyl providing the most favorable result. In the case of norvalinamide, a benzylidene group was equally effective.[20]

Protection of the 2-amino group in diamine **4** regiospecifically orients condensation with **5** specifically toward the precursor of a 6-(rather than 7-)substituted tetrahydropterin. Although 1,1-disubstituted 1,2-diaminoethanes are sufficiently differentially hindered to give 99% regiospecific condensation without protection,[12] the product of 1,2-diaminopropane contains about 10% of the precursor to the 7-isomer.[21] However, the *N*-benzyl group was sufficient to suppress formation of the undesired regioisomer to less than 1%. Catalytic hydrogenation over palladium of **6** serves to first reduce the nitro group, and also on extended reaction to cleave the benzyl group.[22] Triaminopyrimidines were found to be especially sensitive to air oxidation in the presence of trace residual catalyst, a process evidenced by purple coloration.

When oxidation of **7c** is performed with iodine or bromine to initiate oxidative cyclization (Scheme II), an excess (2.4–3.6 equiv) was required in order to obtain a rapid reaction. With 2 equiv of halogen all of **7c** will eventually be oxidized, but at a rate inconsistent with the stability of the subsequent product **9c**. The observation of chlorine adducts with pyrimidine and quinoid dihydropterin nitrogens[23] suggests that a fraction of the halogen is not available immediately for iminoquinone formation. Hydrolysis of the resulting quinoid pyrimidine **8c** in a primarily aqueous solvent at pH 2 is complete within a few minutes at 27 °C. At this pH an optimal compromise was found between the rate of hydrolysis and the stability of **9c**. Completion of both oxidation and hydrolysis can be determined by addition of a reductant, e.g., 2-mercaptoethanol (2-ME), to a sample and HPLC analysis for residual **7c** vs the divicine derivative **10c**.

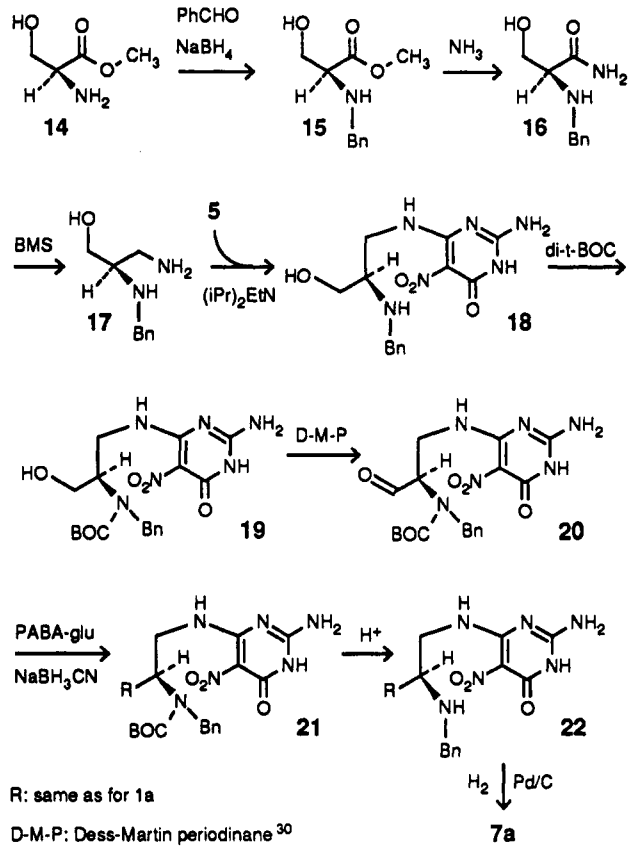
The rate of cyclization of **9** (R = CH_3) was determined as a function of pH by diluting hydrolyzed reaction mix into dilute buffers and then reducing aliquots at timed intervals. This showed that the rate of condensation to carbinolamine **11** is primarily governed by the lack of charge on the exocyclic 2′-amino group. The quinoid divicine **9** is also increasingly unstable to decomposition at alkaline pH.[24] However, as pH is increased from 7 to 10, yields rise (to a maximum of 90% in small-scale reactions) as cyclization outcompetes decomposition. Due to the poor yields at intermediate pH, titration of **9c** was done quickly while cooled.

A good yield of final reduction to **1c** was found to be quite dependent on optimal timing. Addition of reductant before completion of cyclization of **9c** results in contamination with the noncyclizable **10c**. Too late a reduction allows quinoid **12** to tautomerize to the 7,8-dihydropterin **13c**. It was found that this dilemma can be resolved by minimizing the presence of general acid and base catalysts that promote rearrangement of quinoid dihydropteridines.[25] Further, the observation that higher yields were obtained when the total concentration of intermediates was kept below about 0.05 M during cyclization suggests that tautomerization is autocatalytic. Both ascorbic acid and $Na_2S_2O_4$ were found to be suitable, since both can selectively reduce quinoid dihydropterins. There is some potential that any 7,8-dihydropterin byproduct could be reduced to racemic tetrahydropterin by dithionite, but this is minimized by the cold, nonacidic environment.[26] Addition of either reductant lowers the reaction pH somewhat, thus promoting acid-catalyzed dehydration of the carbinolamine **11c**.

Although C6-epimers of diastereomeric tetrahydropterins such as **1b** or leucovorin have been chromatographically resolved, simple enantiomers such as 6-alkyltetrahydropterins have not. A number of chiral HPLC columns failed to separate **1c** into its 6*R* and 6*S* isomers. However, derivatization with phenyl isothiocyanate cleanly produced a single compound (broad λ_{max} ~284 nm), presumably by reaction at N5, the enantiomers of which were well-resolved on a cyclodextrin-modified HPLC column.[27] This procedure showed the enantiomeric purity of the final

(15) Boon, W. R.; Leigh, T. *J. Chem. Soc.* **1951**, 1497.
(16) The chloropyrimidine **5** should be as free as possible of sulfuric acid remaining from nitration so as to minimize the amount of base required for completion of the condensation.
(17) (a) Stout, D. M.; Black, L. A.; Matier, W. L. *J. Org. Chem.* **1983**, *48*, 5369. (b) Subramanian, P.; Woodard, R. W. *Synth. Commun.* **1986**, *16*, 337. (c) Kunz, H.; Sager, W.; Pfrengle, W.; Schanzenbach, D. *Tetrahedron Lett.* **1988**, *29*, 4397.
(18) Yeh, S. M.; Sherman, D. G.; Meares, C. F. *Anal. Biochem.* **1979**, *100*, 152.
(19) Brown, H. C.; Choi, Y. M.; Narasimhan, S. *J. Org. Chem.* **1982**, *47*, 3153.
(20) Reduction of the amide by BMS appeared to be preceded by imine reduction as determined with limiting reagent.
(21) Pike, D. C.; Hora, M. T.; Bailey, S. W.; Ayling, J. E. *Biochemistry* **1986**, *25*, 4762.
(22) It was found that residual $(Et)_3NHCl$ from the condensation can inhibit reduction.
(23) (a) Hoyano, Y.; Bacon, V.; Summons, R. E.; Pereira, W. E.; Halpern, B.; Duffield, A. H. *Biochem. Biophys. Res. Commun.* **1973**, *53*, 1195. (b) Noar, J. B.; Venkataram, U. V.; Bruice, T. C.; Bollag, G.; Whittle, R.; Sammons, D.; Henry, R.; Benkovic, S. J. *Bioorg. Chem.* **1986**, *14*, 17.
(24) This can be seen in the pH dependence of decay of quinoid divicine itself or of quinoid 6-*N*-(3′-aminobutyl)divicine, which does not significantly cyclize to a pyrimidodiazepine.[21]
(25) Archer, M. C.; Scrimgeour, K. G. *Can. J. Biochem.* **1970**, *48*, 278.
(26) Kawai, M.; Scrimgeour, K. G. *Can. J. Biochem.* **1972**, *50*, 1191.
(27) Reaction with (*R*)-(+)-α-methyl benzyl isocyanate followed by resolution of the diastereomers by reversed-phase HPLC also worked well. However, PTIC was used to overcome potential differential reaction rates of the isomers.

Scheme III



1c to be 99%.[28] The possible contributions of an isomeric contaminant in the starting norvalinamide to the final 1% impurity has not yet been established.

Chiral Tetrahydrofolic Acid. Formulating a route to chiral **1a** and related compounds requires choosing whether to add the (*p*-aminobenzoyl)-L-glutamate chain before or after the central triaminopyrimidinone **7**. Reductive alkylation of 6-formyl-6-methyltetrahydropterin with PABA-glu has recently been demonstrated.[29] However, due to the likelihood of a moderate rate of tautomerization of a quinoid 6-(protected-formyl)dihydropterin, attention was focused on making this link early. Several paths to **7a** are feasible. The following describes a procedure starting with serine that led to a significant overall yield of **1a** possessing high C6 enantiomeric purity (Scheme III).

Reductive benzylation of serine methyl ester (**14**) proved more productive than that of serinamide. To minimize potential racemization, the intermediate imine ester was not isolated. The rate of amidation of the *N*-benzyl ester **15** was slow in comparison to the unprotected amino acid ester. Reduction of amide **16** also was found to be optimal with benzyl protection and use of BMS with continuous distillation of Me_2S. Conensation of the diamine **17** with chloropyrimidine **5** proceeded with typical yield, though the solubility of the product **18** hindered complete recovery.

Many attempts to oxidize **18** directly to an aldehyde were unsuccessful. Most reagents simply left unreacted alcohol, but some (e.g., $(Pr)_4N$ perruthenate/morpholine *N*-oxide) generated significant cleavage of benzaldehyde. The use of Dess–Martin periodinane[30] in DMF produced the desired product, but in low yield. However, double protection of the exocyclic amine with the addition of BOC gave good solubility in CH_2Cl_2 and allowed oxidation by the periodinane in >93% yield. An interesting aspect of aldehyde **20** was its chromatographic profile, which suggested an on-column equilibration with an earlier eluting hydrate. The aldehyde was not isolated, but used directly for reductive coupling. A brief period was allowed for imine formation with PABA-glu in cold dry DMF, before addition of $NaBH_3CN$. Reformation of the alcohol **19** was minimized by an excess of PABA-glu, the residual reagent being extracted into water. Yield was found to be quite pH dependent and optimal at a value close to that established by the buffering effect of the free acid of the PABA-glu itself. Following deprotection, catalytic reduction of the nitro group of **22** and subsequent debenzylation were found to be promoted by dilute acid with DMF as cosolvent.

The factors involved in the cyclization of **7a** to **1a** are generally similar to those discussed above for the simpler alkyl derivative. However, some differences were noted. In comparison to **9c**, the quinoid divicine **9a** was somewhat less stable, and the reaction was quickly cooled after completion of hydrolysis. With the PABA-glu side-chain autocatalysis of tautomerization of quinoid **12a** appeared to be more significant than with the propyl analog. Production of 7,8-dihydrofolate during oxidative cyclization was difficult to suppress at concentrations higher than 0.03 M. In addition, decomposition of the quinoid **12a** by cleavage of the PABA-glu moiety[31] further increases the need for accurate timing of reduction. The water content of the reaction was also found to be relatively important. Despite these concerns, a yield of 40–59% (6*S*)-tetrahydrofolic acid could be obtained from **7a**.

The enantiomeric purity of the (6*S*)- and (6*R*)-tetrahydrofolic acid (FH_4) resulting from, respectively, L- and D-serine methyl esters was established by injection onto a cyclodextrin column eluted with a formaldehyde containing buffer which forces an equilibrium almost exclusively favoring the N^5,N^{10}-methylene derivatives during HPLC. This showed enantiomeric purity of 97% to 98% from several cyclizations (six (6*S*)-FH_4, one (6*R*)-FH_4). Only 0.5–1.0% of the enantiomeric impurity in the final (6*S*)-FH_4 could be accounted for by contamination of the starting ester. Although the potential exists for introduction of the undesired isomer by dithionite reduction, the use of ascorbic acid as reductant gave the same result. Racemization of the Schiff's base during reductive alkylation of **20** with PABA-glu seems unlikely, as extending the incubation period of imine formation from 5 min to 1 h before reduction also did not affect the enantiomeric purity of the end product.

Tetrahydrofolic acid can be purified from the cyclization reaction by DEAE chromatography with HCl/2-ME as eluant (overall 15% recovered yield from **17**). Alternatively, the crude reaction mixture of tetrahydrofolic acid was directly converted to the N5-formyl derivative using a new regioselective formylation with 1,1′-carbonyl dimidazole (CDI) and formic acid in DMF. This presumably proceeds via formation of *N*-formylimidazole in situ as judged by comparison to reactions using preformed reag-

(28) The propyl enantiomers have been used to elucidate the chiral specificities of phenylalanine and tyrosine hydrolyases toward cofactor: Bailey, S. W.; Dillard, S. B.; Ayling, J. E. *Biochemistry* **1991**, *30*, 10226.

(29) Bailey, S. W.; Chandrasekaran, R. Y.; Ayling, J. E. Unpublished results.

(30) 1,1,1-Trisacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one, from 2-iodobenzoic acid: Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4156; completion of 2-iodoxybenzoic acid acetylation was facilitated by continuous passage of dry inert gas through the reaction head space.

(31) (a) Blakley, R. L. *Biochem. J.* **1957**, *65*, 331. (b) Chippel, D.; Scrimgeour, K. G. *Can. J. Biochem.* **1970**, *48*, 999. (c) Reed, L. S.; Archer, M. C. *J. Agric. Food Chem.* **1980**, *28*, 801. When loss of PABA-glu occurs, formaldehyde is generated which can result in N^5,N^{10}-methylene-**1a**.

ent.[32] To avoid unproductive consumption of reagent, following addition of DMF, water is completely evaporated from the reaction. Less than 1% of N^{10}-formyl- or N^5,N^{10}-methenyl-FH_4 was formed. The enantiomeric purity of the resulting N^5-formyl-(6S)-FH_4 was found to be identical to that of the starting **1a**. This procedure was developed initially with pure commercial racemic tetrahydrofolic acid and modified for use with crude **1a** by inclusion of increased formic acid for solubility purposes.

Experimental Section

General. Unless otherwise specified, all pH values of primarily nonaqueous solutions were measured after 10-fold dilution in water. All solvents were spectrophotometric and/or HPLC grade. THF was dried by distillation under argon from $LiAlH_4$; CH_2Cl_2 and DMF were dried over 4-Å sieves; benzaldehyde was distilled under agon. D- and L-norvaline were purchased from Sigma, D- and L-serine methyl ester from Schweizerhal, and 10% Pd/C from EM Science. Unless otherwise specified, all reagents were ACS grade or the 99% purity grade available from Aldrich or Fluka and used as received. Solvents were removed from reaction mixtures using a rotary evaporator with a room-temperature bath and vacuum pump, and products were dried under high vacuum over P_2O_5. Whatman GFA 427 glass fiber pads were used for general filtration, and especially to remove catalyst from **7a** and **7c**. ^1H and ^{13}C NMR spectra were acquired in DMSO-d_6 at 300 and 75 MHz, respectively, and chemical shifts are referred to Me_4Si, unless otherwise specified. Ultraviolet extinction coefficients (ϵ) are in units of M^{-1} cm^{-1}.

Yields of crude or partially purified products were determined by comparison to purified materials by quantitative HPLC. The format for chromatographic conditions is as follows: stationary phase, mobile phase, flow rate (mL/min), detection wavelength(s) (nm), electrochemical voltage (vs Ag/AgCl). Except as noted, column dimensions are 25 × 0.46 cm, packing materials are 5 μm, and mobile-phase buffer molarities refer to the cation concentration, and elution was performed at ambient temperature. Columns used for HPLC analysis or purification of **7a,c** or **1a,c** were pretreated with fresh 1 M $Na_2S_2O_4$ to remove adsorbed oxygen.

The enantiomeric purity of the L-serine methyl ester was determined by hydrolysis of sample in 0.5 mL of 1 M HCl at 55 °C for 1 h. After removal of solvent, the resulting serine was treated with dansyl chloride and the derivative separated by reversed-phase HPLC (RoSil ODS (20 × 0.40 cm), 20 mM NH_4O_2CH, pH 3.0/MeOH (13:9), 1.0, 325). Chiral chromatography (Cyclobond I, 50 mM NaOAc, pH 5.5/MeCN (9:1), 0.6, 250) of the purified material indicated the presence of 0.5–1.0% of D-dansylserine.

1-Amino-2(*S*)-(benzylamino)pentane (4). Benzaldehyde (4.02 g, 37.9 mmol) was added to a slurry of 4.0 g of L-norvalinamide·HCl (26.2 mmol)[33] in 70 mL of 1-PrOH, followed by 4.08 mL of Et_3N (29.3 mmol). The mixture, which became clear after stirring at 23 °C for 70 min, was then evaporated to 15 mL and 175 mL of Et_2O added. After filtration, the precipitated salts were washed with additional Et_2O, solvent evaporated from the combined filtrate, and residual benzaldehyde removed under high vacuum to give 5.13 g of *N*-benzylidene-L-norvalinamide as a light yellow solid (UV λ_{max} = 249 nm in MeOH). The purity of this material was determined by HPLC (Spherisorb CN, 1-PrOH/heptane (1:3), 1.0, 245).

All of the Schiff's base was dissolved in 120 mL of dry THF and heated, and 15.2 mL of 10.1 M BMS (154 mmol) was added with stirring over 20 min while simultaneously distilling off Me_2S. The reaction was then refluxed another 2.5 h, cooled to ambient temperature, and acidified to pH 4 with concd HCl. After extraction once with 250 mL of Et_2O, the aqueous phase was taken to pH 11 with solid NaOH and extracted with 4 × 150 mL of Et_2O. The latter extract was concentrated and dried to give 3.09 g of thick oil containing 11.0 mmol of 4 (42%). Analysis of this material by HPLC (RoSil ODS (20 × 0.40 cm), 3 mM octane sulfonic acid (OSA), 10 mM $NaPO_4$, pH 2.85/MeCN (3:1), 0.8, 200) showed 93% of the absorbance area in the product peak. A sample was purified as the dihydrochloride salt by repeated crystallization from MeOH/Et_2O to give a white, highly hygroscopic semisolid: ^1H NMR δ 0.89 (t, 3 H, CH_3), 1.37 (m, 2 H, $-CH_2CH_3$), 1.80 (m, 2 H, $-CHCH_2-$), 3.2–3.55 (m, 3 H, $-CH_2NH_2$, CHNH), 4.26 (m, 2 H, benzyl), 7.4–7.7 (m, 5 H, Ar); ^{13}C NMR δ 13.5, 17.9, 29.2, 38.2, 47.3, 55.0, 128.4, 128.8, 130.2, 131.6; HRMS (+FAB, Xe, thioglycerol) m/z calcd for $C_{12}H_{21}N_2$ (MH^+) 193.170, found 193.171.

2-Amino-6-[[2′(*S*)-benzylamino)pentyl]amino]-5-nitro-4-(3*H*)-pyrimidinone (6). A solution of 5·H_2SO_4[12,34a] (3.7 g, 12.8 mmol) in 250 mL of hot absolute EtOH was adjusted to pH 9.5 with Et_3N, and taken to reflux, and 3.09 g of 4 (containing 11.0 mmol) was added. After 90 min of stirring, analysis by HPLC (RoSil ODS (20 × 0.40 cm), 3 mM OSA in 10 mM $NaPO_4$, pH 2.85/MeCN (7:3), 1.0, 200, and 340) indicated complete consumption of diamine. Half of the solvent was distilled off and the mixture cooled to ambient temperature. A first crop was collected by filtration, washed with a few mL of cold EtOH, and dried to give 4.04 g of light yellow powder containing 9.46 mmol of **6**. Another 0.082 mmol was found in the filtrate (93% total), from which 0.046 mmol (86% recovered) was collected after the filtrate was cooled to −20 °C. Greater than 98% of the 340-nm chromatographic absorbance resided in the product peak.

A sample was purified to a white powder by extraction into, and crystallization from, $CHCl_3$: UV λ_{max} (ϵ) (0.1 M HCl) 236 nm (12 800), 334 nm (13 500); (0.1 M NaOH) 235 nm (sh), 348 nm (14 800); ^1H NMR δ 0.93 (t, 3 H, $-CH_3$), 1.35–1.57 (m, 4 H, $-CH_2CH_2-$), 2.78 (m, 1 H, -NH-), 3.2–3.7 (m, 4 H, $-CHCH_2N-$, $-NH-$), 3.84 (m, 2 H, benzyl), 7.25–7.5 (m, 5 H, Ar); ^{13}C NMR δ 14.1, 18.5, 33.9, 43.2, 49.6, 54.6, 110.4, 126.5, 127.9, 128.0, 140.7, 154.2, 156.6, 159.0; HRMS (+FAB, Xe, thioglycerol) m/z calcd for $C_{16}H_{23}N_6O_3$ (MH^+) 347.183, found 347.177. Anal. Calcd for $C_{16}H_{22}N_6O_3$: C, 55.32; H, 6.67; N, 24.19. Found: C, 55.30; H, 6.64; N, 24.08.

6-[(2(*S*)-Aminopentyl)amino]-2,5-diamino-4(3*H*)-pyrimidinone (7c). To a suspension of 2.02 g of crude **6** (containing 4.73 mmol) in 200 mL MeOH was added 2.0 g of 5% Pd/$BaSO_4$ and the mixture stirred vigorously at room temperature under 45 psi of hydrogen. Analysis by HPLC (Partisil SCX 10 μm, 1 M NH_4O_2CH, pH 3.3/MeOH/1 mM EDTA (3:2:5), 1.0, 270, and 330, +0.3) showed complete reduction of the nitro group and debenzylation after 24 h. With the reaction still under an atmosphere of H_2, the mixture was pulled through an in-line filter (Cole-Parmer L-06621-05) using reduced pressure into an ice-cooled flask. Deaerated fresh MeOH (50 mL) was added to the hydrogenation vessel and pulled through the filter to wash remaining product from the catalyst. The clear light yellow filtrate was quickly sparged with argon, adjusted to pH 2 with 5 M HCl in MeOH, and concentrated to 60 mL. Analysis of this material by HPLC showed 3.60 mmol of **7c** (76%) with 92% of the total 270-nm absorbance area in the product peak, and no significant electrochemically active impurities. This solution of **7c** was used directly for the next step.

A sample was purified by addition of 98% H_2SO_4 and precipitation with Et_2O. After the sample was washed with fresh Et_2O and dried, a white powder was obtained that showed greater than 99% of the 270-nm chromatographic absorbance in a single peak. An extinction coefficient was determined by dichlorophenol indophenol (DCIP) titration: UV λ_{max} (ϵ) (0.1 N HCl) 217 nm (30 200), 270 nm (15 600); HRMS (+FAB, Xe, thioglycerol) m/z calcd for $C_9H_{19}N_6O$ (MH^+) 227.162, found 227.161.

6(*S*)-Propyl-5,6,7,8-tetrahydropterin (1c). A solution of crude **7c** (1.50 mmol) in 25 mL of MeOH was diluted with 25 mL of water, well sparged with argon, and warmed to 27 °C and 54 mL of 50 mM of I_2 in MeOH (2.7 mmol) added all at once with vigorous stirring. After 12 min the mixture was cooled for 7 min on ice to 4 °C and rapidly (<20 s) adjusted to pH 10 with 10 M NaOH. After 1.0 min, 6.0 mL of ascorbic acid (0.75 M in ice-cold water, 4.5 mmol) was added quickly with good stirring and continued bubbling with argon. Analysis after 5 min by HPLC (as

(32) Staab, H. A.; Polenski, B. *Ann. Chem.* **1962**, *655*, 95.

(33) From L-norvaline by the method of Smith et al. (Smith, E. L.; Polglase, W. J. *J. Biol. Chem.* **1949**, *180*, 1209) further recrystallized from H_2O/acetone.

(34) (a) Freshly prepared. (b) Solutions of aged material should be filtered, if necessary, to remove decomposed pyrimidine.

for 7c, except 30:5:65 solvent ratio) showed 0.97 mmol of product (65%) along with trace of 6-propyl-7,8-dihydropterin and 10c (λ_{max} = 285, pH 3.3). After acidification to pH 3.0 with 6 N HCl and addition of 78 μL of 2-ME (1.11 mmol), the reaction was evaporated to approximately 8 mL.

Crude product was purified on Bakerbond SCX (7046-00) 40 μm (37 × 2.1 cm) at 10 mL/min with detection by absorbance at 340 nm. Following elution of unretained material with 40 mM 2-ME, the mobile phase was changed to NH_4OAc, pH 6.0/40 mM 2-ME/MeOH (5:35:40). When UV spectra indicated appearance of product, the mobile phase was changed to 100% MeOH. The fractions not contaminated significantly with 6-propyl-7,8-dihydropterin typically contained 89% of the 1c applied to the column. Analytically pure colorless material was obtained by two crystallizations from 0.25 M H_2SO_4/MeCN: UV λ_{max} (ϵ) (0.1 M HCl) 215 nm (16 000), 265 nm (14 900); ^{1}H NMR (D_2O, TSP = 0 ppm) δ 0.95 (t, 3 H, CH_3), 1.48 (m, 2 H, CH_2), 1.75 (m, 2 H, CH_2), 3.35 (q, 1 H, H_{7a}), 3.59 (m, 1 H, H_6), 3.75 (q, 1 H, H_{7b}); (^{13}C NMR (D_2O, TSP = 0 ppm) δ 16.0, 21.0, 34.0, 44.2, 54.8, 88.0, 155.4, 155.9, 161.1; HRMS (+FAB, Xe, thioglycerol) m/z calcd for $C_9H_{16}N_5O$ (MH^+) 210.135, found 210.139. Anal. Calcd for $C_9H_{15}N_5O \cdot H_2SO_4 \cdot {}^1/_2H_2O$: C, 34.17; H, 5.74; N, 22.14; S, 10.13. Found: C, 34.29; H, 5.47; N, 22.11; S, 10.24.

Enantiomeric purity was determined by derivatization of 2 mg of 1c·H_2SO_4 in 200 μL of MeCN with 60 μL of phenyl isothiocyanate (PITC) (0.5 mmol) and 27 μL of Et_3N (0.2 mmol). After 15 min at room temperature, 50 μL of the mixture was purified by HPLC (RoSil ODS (20 × 0.40 cm), 0.5 M NH_4OAc, pH 4.8/MeOH (3:1), 0.8, 265). The PITC derivative was collected and analyzed by chiral HPLC (Cyclobond I, 55 mM NH_4OAc, pH 5.5/MeCN (91:9), 1.0, 285, +0.7). The two C6-enantiomers were well resolved (R_s = 1.8) and the enantiomeric purity found to be ≥99%.

6(*R*)-Propyl-5,6,7,8-tetrahydropterin. The above series of reactions was also repeated starting with D-norvaline with similar results.

***N*-Benzyl-L-serine Methyl Ester Hydrochloride (15).** A solution of 20 g (0.129 mol) of L-serine methyl ester hydrochloride in 100 mL of MeOH was adjusted to pH 8 with 12.9 mL of 10 M NaOH, thus precipitating NaCl. Benzaldehyde (15.0 g, 0.141 mol), was added and stirred well for 30 min. After the solution was cooled to 4 °C, 2.43 g (0.064 mol) of $NaBH_4$ was added in parts over 1 h. The reaction was then warmed to ambient temperature and stirred for another hour. Analysis by HPLC (as for 4) showed that aside from the desired product, identified by coelution with material obtained by esterification of *N*-benzyl-L-serine, the only other product observed was benzyl alcohol. The reaction was filtered, concentrated, and dried. The thick residue was dissolved in 120 mL of acetone, refiltered to remove more salt, adjusted to between pH 1 and 1.5 with HCl gas, 750 mL of Et_2O added, and the product collected by centrifugation and dried, giving 28.1 g of colorless hygroscopic solid. This was shown to contain 91.6 mmol (71%) by chromatographic comparison to purified material:[35] MS (EI direct insertion) m/z (relative abundance) 210 (MH^+) (5), 178 (55), 150 (64), 118 (6), 106 (29), 91 (100); HRMS (+DCI, CH_4) m/z calcd for $C_{11}H_{16}NO_3$ (MH^+) 210.113, found 210.112.

***N*-Benzyl-L-serinamide (16).** *N*-Benzyl-L-serine methyl ester·HCl (28.1 g, 80% pure, 91.5 mmol) was dissolved in 450 mL of MeOH saturated with NH_3 at 0 °C. The solution was then kept at room temperature in a pressure bottle. The flask was resaturated with NH_3 at 2-day intervals. Analysis by HPLC (as for 4) indicated 86% conversion to product after 6 days, at which time solvent was removed and product dried. The resulting 29.0 g of crude material was extracted into 500 mL of ethyl acetate/MeOH (3:2), filtered, and evaporated to a gum. This was reextracted with 200 mL of 1-PrOH, 50 mL ethyl acetate added, and the suspension centrifuged. Removing the solvent from the supernate and drying gave 24.3 g of thick oil of crude 16 hydrochloride salt (74 mmol, 81%). A sample was purified by crystallization from MeOH/acetone/$(Et)_2O$: ^{1}H NMR δ 3.70 (m, 1 H, C*H*CH_2), 3.87 (m, 2 H, -CH_2-), 4.15 (s, 2 H, benzyl), 5.58 (br s, 1 H, OH), 7.3–7.6 (m, 5 H, Ar), 7.67 (s, 1 H, $CONH_a$), 8.03 (s, 1 H, $CONH_b$); MS (EI, direct insertion) m/z (relative abundance) 195 (MH^+) (2), 163 (7), 150 (51), 106 (17), 91 (100); HRMS (+DCI, CH_4) m/z calcd for $C_{10}H_{15}N_2O_2$ (MH^+) 195.113, found 195.113.

3-Amino-2(*R*)-(benzylamino)propan-1-ol (17). *N*-Benzyl-L-serinamide·HCl (45.7 mmol, 15.0 g of crude material) was mostly dissolved in 800 mL of dry THF under argon. The mixture was heated, 46 mL of 10.1 M BMS (0.465 mol) was added via syringe with stirring over 20 min and simultaneous distillation of Me_2S, and then the resulting mixture was refluxed for 2 h. After the mixture was cooled to room temperature, 11 mL of 6 N HCl was added dropwise to give pH 4.5–5.0 and the reaction stirred for 30 min. Water was added to produce a clear solution which was extracted once with 300 mL of Et_2O. The aqueous layer was adjusted to pH 12 with solid NaOH, product extracted with 3.8 L of Et_2O, and the extract dried (Na_2SO_4). After solvent was removed, drying under vacuum over NaOH pellets gave an oil weighing 8.35 g containing 25.6 mmol of 17 (56%) (HPLC, as for 4). Another 6.3 mmol remained in the aqueous layer. A chromatogram (Spherisorb ODS2, 10 mM KPO_4, pH 6.5/MeOH (3:7), 1.0, fluorescence 320ex, 460em) of the *o*-phthalaldehyde derivative[36] of crude product showed greater than 99.5% of the fluorescence response in a single peak.

A sample of the oil was further purified by distillation at 95 °C and approximately 50 Torr pressure and precipitation of the dihydrochloride salt from MeOH/HCl with Et_2O: ^{1}H NMR δ 3.2–3.7 (m, 3 H, -CH_2NH_2, C*H*NH), 3.86 (m, 2 H, OCH_2), 4.29 (m, 2 H, benzyl), 7.4–7.8 (m, 5 H, Ar); ^{13}C NMR δ ~39 (submersed under DMSO), 47.7, 55.7, 56.5, 128.5, 128.8, 130.2, 131.7; MS (EI, direct insertion) m/z (relative abundance) 181 (MH^+) (1), 164 (1), 150 (87), 106 (6), 91 (100); HRMS (+DCI, CH_4) m/z calcd for $C_{10}H_{17}N_2O$ (MH^+) 181.134, found 181.135.

2-Amino-6-[[2′(*R*)-(benzylamino)-3′-hydroxypropyl]amino]-5-nitro-4(3*H*)-pyrimidinone (18). Chloropyrimidine 5[12,34b] (14 g) was partially dissolved in 900 mL of hot absolute EtOH and filtered. To the filtrate, determined to contain 40 mmol of the pyrimidine, was added *N*,*N*-diisopropylethylamine to achieve pH 8 and the mixture taken to reflux. A solution of 38 mmol of 17 (12.3 g crude material) in 100 mL of EtOH was added all at once and refluxed for 2 h with stirring while being monitored by HPLC (Partisil SCX 10μ, 0.1 M NH_4OAc, pH 4.8/MeCN (9:1), 0.8, 290, and 330). Solvent was then distilled from the mixture until 700 mL remained, which was then refrigerated overnight. A first crop collected by filtration contained, after drying, 9.56 g of crude 18 (containing 21.3 mmol), with 12.8 mmol remaining in the filtrate (90% total). Further concentration of the filtrate produced two more crops with a combined weight of 4.44 g (containing 6.06 mmol 18). All three crops were purified by suspension in ice-cold water. The collected precipitates were dried to give 12.86 g (containing 26.5 mmol) of light yellow powder (70%).

Crude product was purified by suspension in water at 0 °C and then filtered. The precipitate was partially dissolved in boiling EtOH (300 mL/g), filtered while still warm, and dried to give a light yellow powder: UV λ_{max} (ϵ) (0.1 N HCl, 0.1 M KPO_4, pH 6.5) 334 nm (15 100), 286 nm (sh), 236 nm (13 400); (0.1 N NaOH) 347 nm (16 600), 232 nm (sh); ^{1}H NMR δ 2.85 (m, 1 H, -NH-), 3.3–3.75 (m, 5 H, OCH_2CHNCH_2N-), 3.85 (m, 2 H, benzyl), 7.1–7.5 (m, 7, Ar, NH_2), 9.83 (br, 1 H, CONH); ^{13}C NMR δ 41.3, 49.8, 56.9, 60.7, 110.5, 126.8, 128.0, 128.2, 139.5, 154.0, 156.3, 159.0; MS (EI, direct insertion) m/z (relative abundance) 335 (MH^+) (0.5), 303 (2), 167 (8), 150 (75), 91 (100); HRMS (+DCI, CH_4) m/z calcd for $C_{14}H_{19}N_6O_4$ (MH^+) 335.147, found 335.146.

2-Amino-6-[[2′(*R*)-[*N*-(*tert*-butoxycarbonyl)-*N*-benzylamino]-3′-hydroxypropyl]amino]-5-nitro-4(3*H*)-pyrimidinone (19). A slurry of 10.3 mmol of 18 (5 g crude material) in 200 mL of dioxane was cooled on ice and 1 M NaOH added slowly to give pH 9.5 (26 mL). Di-*tert*-butyl dicarbonate 97% (2.5 g, 11.1 mmol) was added and the clear solution warmed to room temperature. At 3, 5.5, and 23 h the pH was readjusted with 1 M NaOH and 0.3 g more dicarbonate added. HPLC (R-Sil SCX 10 μm, 0.1 M NH_4OAc, pH 4.8/MeCN (4:1), 1.0, 330) showed 4% 18 remaining at 46 h, and the reaction was then filtered and evaporated to remove the dioxane. The aqueous solution was

(35) Hardegger, E.; Szabo, F.; Liechti, P.; Rostetter, C.; Zankowska-Jasinska, W. *Helv. Chim. Acta* **1968**, *51*, 78.

(36) Lindroth, P.; Mopper, K. *Anal. Chem.* **1979**, *51*, 1667.

adjusted to pH 7 with glacial acetic acid, and kept overnight on ice. The precipitate was collected by centrifugation, resuspended in 10 mL of ice-cold water, recentrifuged, and dried to give 5.64 g of a light yellow powder (containing 9.6 mmol **19**, 93%), with an additional 0.3 mmol remaining in the aqueous supernates.

A sample in DMF was purified by preparative HPLC on Bakerbond High Hydrophobicity (C_{18}), 40 μm (37 × 2.1 cm), equilibrated with distilled H_2O, and eluted at 10 mL/min with H_2O (40 mL), 1 mM formic acid/MeOH (52:48) (270 mL), and finally H_2O/MeOH (30:70) to elute product, which on reanalysis showed 98% of the 330-nm absorbance in a single peak: UV λ_{max} (ϵ) (0.1 M HCl, 0.1 M NH_4PO_4, pH 2.8, 0.1 M KPO_4, pH 6.5) 335 nm (13 300), ~288 nm (sh), 260 nm (min), ~233 nm (sh); (0.1 M NaOH) 346 nm (15 600); ^{1}H NMR (90 MHz) δ 1.33 (br s, 9 H, $C(CH_3)_3$), 3.0–3.9 (5 H, C*H*N, OCH_2, NCH_2), 4.39 (s, 2 H, benzyl), 7.8–8.4 (br s, 7 H, Ar, NH_2), 10.4 (br, 1 H, CONH); MS (EI, direct insertion) m/z (relative abundance) 435 (MH^+) (0.25), 410 (0.4), 404 (2), 360 (4), 303 (1), 250 (9), 194 (15), 167 (12), 150 (75), 91 (100); HRMS (+DCI, CH_4) m/z calcd for $C_{19}H_{27}N_6O_6$ (MH^+) 435.199, found 435.197.

***N*-[4-[[2(*S*)-[*N*-(*tert*-Butoxycarbonyl)-*N*-benzylamino]-3-[(2-amino-5-nitro-4(3*H*)-oxopyrimidin-6-yl)amino]propyl]amino]benzoyl]-L-glutamic Acid (21).** To a solution of 5.0 g of crude **19** (8.51 mmol) in 125 mL of dry CH_2Cl_2 was added 8.49 g of Dess–Martin periodinane[30] (19.2 mmol + some HOAc) in 32 mL of dry CH_2Cl_2 while stirring at room temperature under argon. The alcohol **19** was completely consumed by 70 min as determined by HPLC (RoSil ODS (20 × 0.40 cm), H_2O + formic acid to pH 2.7/MeCN (7:3), 1.0, 330), and the reaction was then poured into a mixture of 1 M $NaHCO_3$ (76 mL) and 0.125 M $Na_2S_2O_3$ (500 mL) and stirred for 15 min. The organic layer was washed with 250 mL of H_2O and dried briefly (Na_2SO_4), solvent removed, and the residue dried to give 5.01 g of yellow semisolid. A minimum yield of the aldehyde **20** was determined by reduction of a small sample with $NaBH_4$ in DMF/H_2O (1:1) which gave 93% of the expected alcohol **19**. Stopped-flow UV in pH 2.7 eluant showed λ_{max} 335 nm, 288 nm (sh), 260 nm (min), 233 nm (sh). The product of this reaction, which is free of reagent byproducts, was utilized immediately for reductive alkylation.

Crude aldehyde **20** (7.91 mmol) was dissolved in 28.5 mL of dry DMF and cooled to 0 °C. To this was rapidly added with stirring an ice-cold solution of 12 g of PABA-glu (45 mmol) dissolved in 40 mL of DMF. After 5 min, a cold solution of 0.57 g of $NaBH_3CN$ (95%, 8.6 mmol) in 8.2 mL of DMF was quickly added and the mixture stirred for 1 h. Analysis of the reaction by HPLC (as for **20**) showed 5.92 mmol of **21** (75%), no aldehyde **20**, and 0.45 mmol of alcohol **19**. Solvent was removed and ice-cold water (90 mL) added. The resulting slurry was adjusted to pH 2.5 with concd HCl and centrifuged and the precipitate dried. The supernate contained 0.45 mmol of product and the bulk of the excess PABA-glu. The resulting 6.27 g of yellow powder was mostly dissolved in 350 mL of absolute EtOH at 60 °C and filtered. The filtrate was concentrated and rewarmed and 2.5 volumes of water added. After being cooled, the precipitate was collected by centrifugation and dried, giving 4.56 g of a light yellow powder containing 4.35 mmol of **21** (51% from **19**).

A sample was purified by semipreparative HPLC on Spherisorb ODS2, 5 μm (25 × 1 cm), eluted with H_2O/formic acid pH 2.7, followed by this mixed with MeCN (75:25) at 6 mL/min. Analytical HPLC of the collected product showed that greater than 99% of the UV absorbance area at either 295 or 330 nm was contained in a single peak: UV λ_{max} (ϵ) (0.1 M NH_4PO_4, pH 2.8) 301 nm (23 900), 335 nm (sh); (0.1 M KPO_4, pH 6.5) 297 nm (23 300), 335 nm (sh); (0.1 M NaOH) 299 nm (23 200), 347 nm (16 000); ^{1}H NMR (90 MHz) δ 1.29 (br s, 9 H, $C(CH_3)_3$), 2.00 (m, 2 H, β-CH_2), 2.33 (t, 2 H, γ-CH_2), 3.0–4.0 (5 H, C*H*NH, PyrNHCH_2, PhNHCH_2), 4.0–4.7 (3 H, benzyl, α-CH), 6.53 (d, 2 H, 3′,5′-H), 7.22 (br s, 5 H, Ar), 7.66 (d, 2 H, 2′,6′-H), 8.10 (d, 1 H, CONHCH), 9.45 (br s, 1 H, CONH); HRMS (FAB, Xe, glycerol/KTFA) m/z calcd for $C_{31}H_{36}N_8O_{10}K$ (MK^+) 719.219, found 719.233.

***N*-[4-[[2(*S*)-(Benzylamino)-3-[(2-amino-5-nitro-4(3*H*)-oxopyrimidin-6-yl)amino]propyl]amino]benzoyl]-L-glutamic Acid (22).** A slurry of 3.87 g of crude **21** (3.69 mmol) in 39 mL of THF plus 156 mL of 1 M HCl was heated to 58 °C with good stirring, resulting after 45 min in a clear solution. Analysis by HPLC (as for **21**, but 295-nm detection) showed the $t_{1/2}$ of hydrolysis to be 19 min. At 150 min (with 99.5% conversion) the reaction was cooled, solvent removed, and the residue dried to give 3.51 g (containing 3.65 mmol **22**). This material was extracted at 60 °C with water (110 mL), leaving a residue containing 0.57 mmol of product. The extract was taken to pH 3.1 with 10 M NaOH and cooled for 18 h at 4 °C. The resulting precipitate was collected and dried to give 2.17 g of light yellow powder (containing 3.04 mmol **22**) (3.61 mmol total, 98%). HPLC analysis of both the unextracted residue and the pH 3.1 precipitate showed more than 98% of the absorbance area at 295 nm within a single peak.

A sample was further purified by semipreparative HPLC on Spherisorb ODS2, 5 μm (25 × 1 cm) eluted at 6 mL/min initially with water/formic acid pH 3.0, followed by this mixed with MeCN (65:35): UV λ_{max} (ϵ) (0.1 M NH_4PO_4, pH 2.8) 292 nm, 332 nm; (0.1 M KPO_4, pH 6.5) 290 nm, 332 nm; (0.1 M NaOH) 300 nm (22 300), 346 nm (16 500); ^{1}H NMR δ 2.02 (m, 2 H, β-CH_2), 2.34 (t, 2 H, γ-CH_2), 2.8–3.8 (5 H, C*H*NH, PyrNHCH_2, PhNHCH_2), 3.90 (s, 2 H, benzyl), 4.32 (m, 1 H, α-CH), 6.60 (d, 2 H, 3′,5′-H), 7.15–7.5 (5 H, Ar), 7.67 (d, 2 H, 2′,6′-H), 8.14 (d, 1 H, CONHCH), 9.88 (br s, 1 H, CONH); HRMS (FAB, Xe, glycerol/PEG400) calcd for $C_{26}H_{31}N_8O_8$ (MH^+) 583.226, found 583.228.

***N*-[4-[[2(*S*)-Amino-3-[(2,5-diamino-4(3*H*)-oxopyrimidin-6-yl)amino]propyl]amino]benzoyl]-L-glutamic Acid (7a).** To a clear solution of 1.05 g of partially purified **22** (1.47 mmol) in 45 mL of DMF and 180 mL of 0.1 M HCl was added 1.05 g of 10% Pd/C and the mixture stirred vigorously under 45 psi of H_2. Analysis at 25 h by HPLC (R-Sil SCX 10 μm, 0.2 M NH_4O_2CH, pH 3.0/MeCN (9:1), 1.0, 275, +0.3) showed complete consumption of the starting material and less than 1% of the benzyl derivative of **7a**. With the reaction still under an atmosphere of hydrogen, the mixture was filtered as described for **7c** and deaerated 0.01 M HCl (10 mL) used to further wash product from the catalyst. The clear light yellow filtrate was quickly sparged with argon. Solvent was removed, and product redissolved in 30 mL of deaerated 0.01 M HCl. HPLC analysis of this material showed that 99% of the electrochemical response in the chromatogram was located in a single peak, and titration with DCIP indicated 1.34 mmol of product. This 91% yield was confirmed by UV spectra in 0.1 M HCl.

A sample was purified semipreparatively on Spherisorb ODS2, 5 μm (25 × 1.0 cm), eluted initially at 3 mL/min with water and, after 100 mL, with H_2O/MeCN (98:2). The collected fraction was acidified to pH 1 with HCl, evaporated to remove MeCN, and lyophilized: UV λ_{max} (ϵ) (0.1 M HCl) 214 nm, 274 nm (23 500), 293 nm (sh); HRMS (FAB, Xe, glycerol/PEG400/DMF) calcd for $C_{19}H_{27}N_8O_6$ (MH^+) 463.205, found 463.208.

(6*S*)-Tetrahydrofolic Acid (1a). A solution of crude **7a** (1.34 mmol) in 30 mL of 0.01 M HCl was cooled to 18 °C, and 33 mL of 50 mM I_2 in MeOH (1.65 mmol) was added over 10 s with good stirring, with a consequent increase in temperature to 27 °C. After 2.0 min, the mixture was cooled within 2.7 min to −20 °C. This temperature was maintained during subsequent titration to pH 9.2 (measured without dilution) with 10 M NaOH over a period of 1.5 min. The reaction was rapidly warmed to 0 °C and at the same time sparged vigorously with argon. After 3.0 min at 0 °C, 58 mL of 0.1 M $Na_2S_2O_4$ (freshly dissolved in deaerated water) was added all at once with stirring, followed 2.0 min later by 0.82 mL of 2-ME (11.7 mmol). Analysis by HPLC (R-Sil SCX 10 μm, 80 mM NH_4O_2CH, pH 3.0 + 1 mM EDTA, 1.0, 285, +0.3) indicated 0.74 mmol **1a** (55%). Contaminating impurities included small amounts of 7,8-dihydrofolic acid (**13a**), **10a** (λ_{max} = 290 nm, pH 3.0), PABA-glu, and trace N^5,N^{10}-methylene-**1a**, with 74% of the absorbance area located in the peak of desired product.

The enantiomeric purity of this material was established by collecting the entire peak of **1a** from the above HPLC system and reinjection onto a chiral column (Cyclobond I, 1 M NaOAc, pH 5.5/MeCN/3 mM HCHO (1:3:16), 1.0, 285, +0.3). Comparison of the resulting N^5,N^{10}-methylene derivative with the peaks produced by racemic (6*R*,*S*)-L-tetrahydrofolic acid (R_s = 1.5) showed 97.0–97.5% enantiomeric purity.

For purification from salts and most byproducts, all of the crude product from a reaction of the scale described above was pumped directly onto a column of Whatman DE52 (24- × 10-cm) preequilibrated with deaerated 0.1 M 2-ME neutralized to pH 7 with NaOH. The column was then eluted at 10 mL/min with the same

solution (500 mL), 0.1 M 2-ME in 0.01 M HCl (200 mL), and then 0.1 M 2-ME in 0.05 M HCl. Analysis by HPLC showed 90% recovery of tetrahydrofolic acid along with a small amount of 7,8-dihydrofolic acid. Eluant was removed after addition of 5 mL DMF by evaporation to a volume less than 5 mL and precipitation of product with Et_2O. The chromatographic elution and mass spectra of this material were found to be identical to authentic tetrahydrofolic acid. A sample was purified further by precipitation from aqueous solution by adjustment to pH 3.4 with NaOH, followed by recrystallization from distilled water: UV λ_{max} (ϵ) (1.0 M HCl) 269 nm (25100), 292 nm (20600); (0.05 M KPO_4, pH 7.0) 218 nm (31900), 297 nm (30100). Anal. Calcd for $C_{19}H_{23}N_7O_6 \cdot 1.3H_2O$: C, 48.67; H, 5.50; N, 20.91. Found: C, 48.71; H, 5.52; N, 20.89.

(6*R*)-Tetrahydrofolic Acid. The above series of experiments was repeated starting with D-serine methyl ester with similar results yielding the unnatural (6*R*)-epimer of tetrahydrofolic acid (97.5 ± 0.5% enantiomeric purity).

N^5-Formyl-(6*S*)-tetrahydrofolic Acid. A crude reaction mixture containing 0.056 mmol of (6*S*)-tetrahydrofolic acid was evaporated together with 40 mL of DMF to a final volume of 10.4 mL. The resulting slurry was mostly solubilized by addition of 1% v/v of 98% formic acid. After centrifugation, the supernate was found to contain 0.042 mmol of **1a**, the remainder being associated with the undissolved salts. The clear supernate was sparged with argon and 13.6 mg (0.084 mmol) CDI dissolved in dry DMF added in two aliquots with vigorous stirring. Analysis by HPLC (Spherisorb ODS2, 5 mM Bu_4NPO_4, pH 5.7/MeOH (7:3), 1.0, 285, +0.5) showed 0.035 mmol of N^5-formyl-(6*S*)-tetrahydrofolic acid with 8% of starting material remaining. The chromatographic properties and UV spectra were identical to authentic material.

Enantiomeric purity was established after an initial HPLC purification (as for **1a** above, but without electrochemical detection). The collected fraction was then analyzed by HPLC (Nucleosil-HSA,[37] 50 mM KPO_4, pH 7.1/2-PrOH (24:1), 30 °C, 0.7, 285, +0.8), which showed 97.0–97.5% enantiomeric purity, identical to the starting **1c**.

Pure tetrahydrofolic acid can be similarly formylated, but in 91% initial yield. In this case only 5 mol of formic acid is required per mole of **1c**. No 7,8-dihydrofolate or N^5,N^{10}-methylenetetrahydrofolate was detected, and N^5,N^{10}-methenyl- and N^{10}-formyltetrahydrofolate were both less than 1% of the total absorbance of the chromatogram.

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Remote Participation during Photooxidation at Sulfur. Evidence for Sulfurane Intermediates

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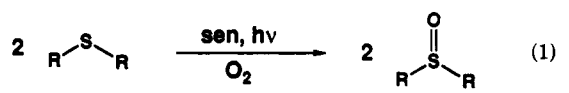
The photooxidations of geminally substituted γ-hydroxy sulfides results in formation of unusual oxidative elimination products. Detailed spectral data and the independent synthesis of a close analogue provide compelling evidence for the structures of these olefins. The formation of the olefins is attributed to decomposition of sulfurane intermediates. This conclusion is supported by a detailed kinetic study which separated the chemical, k_r, and physical, k_q, components to the overall deactivation of singlet oxygen. Those sulfides with the best geometry for sulfide–hydroxyl interaction are also the substrates which react most rapidly with singlet oxygen to give oxidation products. In addition, sulfone yields are in excess of 50% for the hydroxy-substituted sulfides but less than 5% for their hydrocarbon analogues. Several mechanisms that provide explanations for these unusually high sulfone yields are presented.

Sulfides (R_2S) are ubiquitous in the biosphere, and as a result their chemistry has been extensively investigated. Oxidation reactions, in particular, have been thoroughly examined with the realization that sulfides act as antioxidants and are also easily converted into the more highly oxidized sulfoxides (R_2SO) and sulfones (R_2SO_2). Both chemical and photosensitized oxidations are effective in these interconversions. For example, photodynamic destruction of the enzyme α-chymotrypsin occurs by oxidation of methonine-192,[1] and sodium metaperiodate chemically converts penicillin into its *S*-oxide.[2]

The photosensitized oxidations of simple dialkyl sulfides (eq 1) were first reported by Schenck and Krausch[3] in 1962 as a new synthetic method for the formation of sulfoxides. Sulfones (R_2SO_2) are formed in appreciable quantities in



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these reactions only at very low (10^{-3} M) sulfide concentrations and at very low (−78 °C) temperatures. Photooxidation of the sulfoxide product is a very slow reaction and can be eliminated as a viable mechanism for sulfone formation under these reaction conditions.

Foote and co-workers[4] examined the photooxidative formation of diethyl sulfoxide in detail in both aprotic and protic solvents. A mechanism for sulfoxide formation which summarized a large amount of experimental work

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