Asymmetric Synthesis of Lometrexol ((6*R*)-5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid)

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An enantioselective synthesis of lometrexol (**1**) which utilizes (*5R*)-2-piperidone **18** as a key intermediate is described. Lipase-catalyzed enantioselective esterification of 1,3-propanediol derivative **5** provided (*R*)-(+)-**6**, the absolute configuration of which was established by X-ray analysis of the (*S*)-(α-methylbenzyl)carbamate derivative **8**. By suitable choice of functional group protection strategies, (*R*)-(+)-**6** could be converted to either enantiomer of azido alcohol **11**. The *S* isomer of **11** was utilized to prepare **18** in three steps. Conversion of **18** to the thiolactam and cyclization with guanidine provided (*6R*)-5-deaza-5,6,7,8-tetrahydropterin **20**. Cyanation of **20** (cuprous cyanide) followed by hydrolysis of the resulting nitrile **21** gave (*6R*)-5,10-dideaza-5,6,7,8-tetrahydropteroic acid (**22**). The synthesis of **1** was completed by reaction of **22** with diethyl glutamate via an active ester coupling procedure followed by hydrolysis of the resulting diester.

There has been considerable recent interest in development of improved folic acid antagonists as potential antitumor drugs.[3] The synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, (6*RS*)-**1**) by E. C. Taylor and co-workers[4] must be regarded as a milestone of progress toward this goal. DDATHF has been shown to possess potent, broad spectrum antitumor activity, and is the first clinical candidate from the series of folic acid analogs to derive its cytotoxic activity from specific inhibition of glycinamide ribonucleotide formyltransferase, a folate dependent enzyme on the purine de novo biosynthetic pathway.[5] Thus DDATHF can be mechanistically distinguished from methotrexate and its congeners which are inhibitors of dihydrofolate reductase (Figure 1).

DDATHF was initially prepared and biologically evaluated as an equal mixture of C-6 epimers (arbitrarily designated A and B). These diastereomers were eventually separated via fractional crystallization of the (+)-camphorsulfonate salts of the diethyl esters of (6*RS*)-**1** and found to possess remarkably similar activity as inhibitors of de novo purine synthesis,[6] although certain differences in antitumor spectra[7] have been observed. Isomer B, now designated as lometrexol,[8] was chosen for clinical evaluation, and is presently undergoing phase II clinical trials. We present herein a detailed account of an enantioselective synthesis of lometrexol (**1**) and consequent assignment of the *R* configuration to C-6, which places lometrexol in the same stereochemical series as naturally occurring (6*S*)-tetrahydrofolic acid.[9]

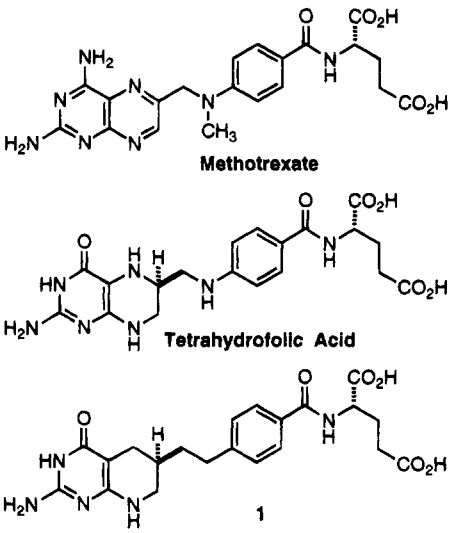

Figure 1.

The reported syntheses of lometrexol by Taylor and others[4,10] have involved construction of 5-deazapterin intermediates as precursors to the 5-deaza-5,6,7,8-tetrahydropterin chromophore. While this strategy readily accommodated powerful carbon–carbon bond forming methods for the construction of the required carbon framework of the molecule, such as the Wittig olefination and palladium(0)-catalyzed coupling processes, the issue

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(1) Lilly Summer Science Intern, 1990.

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(3) For a recent overview of the status of antifolate research related to antitumor therapy see: Berman, E. M.; Werbel, L. M. *J. Med. Chem.* **1991**, *34*, 479–485 and references cited therein.

(4) Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1985**, *28*, 914–921.

(5) Beardsley, G. P.; Taylor, E. C.; Shih, C.; Poore, G. A.; Grindey, G. B.; Moran, R. G. *Proc. Am. Assoc. Cancer Res.* **1986**, *27*, Abstr. No. 1027.

(6) (a) Baldwin, S. W.; Tse, A.; Gossett, L. S.; Taylor, E. C.; Rosowski, A.; Shih, C.; Moran, R. G. *Biochemistry* **1991**, *30*, 1997–2006. (b) Moran, R. G.; Baldwin, S. W.; Taylor, E. C.; Shih, C. *J. Biol. Chem.* **1989**, *264*, 21047–21051. (c) Taylor, E. C.; Wong, G. S. K.; Fletcher, S. R.; Harrington, P. J.; Beardsley, G. P.; Shih, C. In *Chemistry and Biology of Pteridines*; Cooper, B. A., Whitehead, V. M., Eds.; Walter de Gruyter: Berlin, 1986; pp 61–64.

(7) Moran, R. G.; Taylor, E. C.; Shih, C.; Beardsley, G. P.; Shih, C.; Grindey, G. B. *Proc. Am. Assoc. Cancer. Res.* **1987**, *28*, Abstr. No. 1084. Houghton, P. J.; Houghton, J. A. *Proc. Am. Assoc. Cancer. Res.* **1988**, *29*, Abstr. No. 1125.

(8) Name approved by the U.S.A.N.

(9) A preliminary account of this work has been published. Barnett, C. J.; Wilson, T. M. *Tetrahedron Lett.* **1989**, *30*, 6291–6294.

(10) (a) Taylor, E. C.; Harrington, P. M.; Warner, J. C. *Heterocycles* **1988**, *27*, 1925–1928. (b) Taylor, E. C.; Wong, G. S. K. *J. Org. Chem.* **1989**, *54*, 3618–3624. (c) Taylor, E. C.; Harrington, P. M. *J. Org. Chem.* **1990**, *55*, 3222–3227. (d) Boschelli, D. H.; Webber, S.; Whiteley, J. M.; Oronski, A. L.; Kerwar, S. S. *Arch. Biochem. Biophys.* **1988**, *265*, 43–49. (e) Piper, J. R.; McCaleb, G. S.; Montgomery, J. A.; Kisliuk, R. L.; Gaumont, Y.; Thorndike, J.; Sirotnak, F. M. *J. Med. Chem.* **1988**, *31*, 2164–2169.

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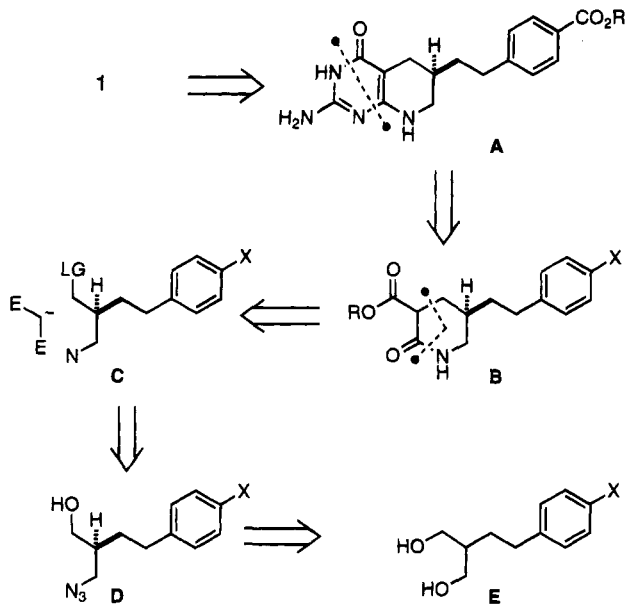
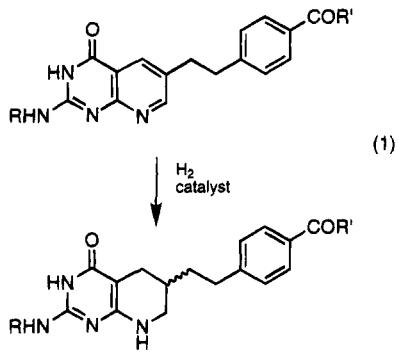


Figure 2.

of rational introduction of the stereogenic center at C-6 was not addressed. All previous approaches to **1** have relied on stereorandom (heterogeneous) catalytic hydrogenation for adjustment of the oxidation state of the 5-deazapterin moiety (eq 1).

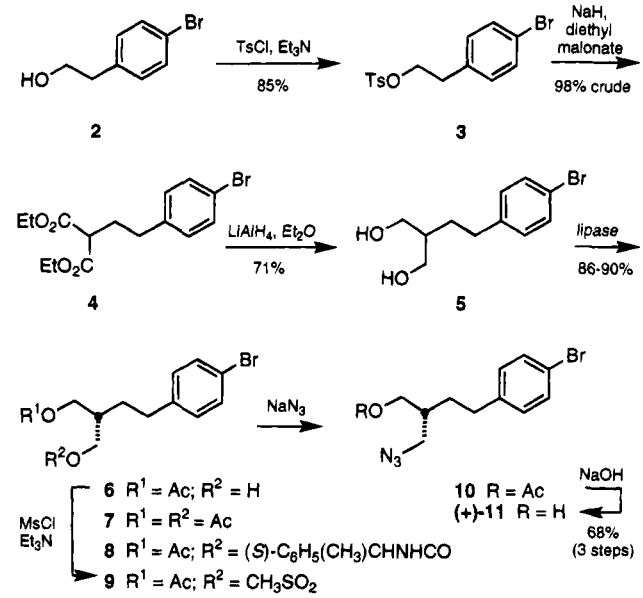


(1)

In our approach, shown in retrosynthetic format (Figure 2), we planned to construct the penultimate tetrahydrodideazapteroic acid **A** from nonracemic piperidone **B**, thus avoiding the issue of pyridine ring reduction and permitting unambiguous introduction of C-6 stereochemistry at an earlier point.

Disconnection of guanidine, leading to the carboxypiperidone **B** subgoal, was based on the well-established guanidine–malonate synthesis[11] of 2-aminopyrimidines. Documented examples of the employment of malonamides or carboxypiperidones in this mode of pyrimidine synthesis are, however, surprisingly rare. The reaction has been reported to proceed successfully in the case of carboxypiperidones, however, when the lactam carbonyl is activated as the imido ether.[12] Piperidone **B** was viewed, in turn, as derivable from a malonic ester and an intermediate **C** which possessed a leaving group and an amino group equivalent in proper stereochemical relationship. We planned to derive **C** from an azido alcohol (**D**)[13] which we would prepare from the *meso* diol

Scheme 1



E after the prochiral hydroxyl groups had been differentiated by precedented lipase-catalyzed[14] processes. The stereochemical versatility of the enzymatic process was an important consideration at the outset since the absolute configuration of the C-6 center in lometrexol (**1**) had not been determined. We further planned to use bromine as the aryl X substituent in **A**–**E** to act as a surrogate for the carboxyl group, so as to avoid potential problems of carboxyl group differentiation.

Our first objective was to prepare the *meso* diol **5** and to establish the sense of asymmetric induction of the lipase-mediated transesterification and related ester hydrolysis processes in this case. As depicted in Scheme 1, activation of 4-bromophenethyl alcohol (**2**)[15] as the tosylate **3** followed by displacement with sodium diethyl malonate provided substituted malonate **4**. Lithium aluminum hydride reduction of **4** afforded diol **5**.

In the absence of an absolute stereochemical assignment for C-6 in lometrexol (**1**), we arbitrarily decided to initially investigate the lipase-catalyzed partial transesterification of **5** to, for example, **6**, as a means of distinguishing the enantiotopic hydroxyl groups. Proceeding according to the procedure of Ramos-Tombo,[16] reaction of **5** with methyl acetate (neat) in the presence of porcine pancreatic lipase (PPL) immobilized on Hyflo Super Cel[17] afforded (+)-**6** in about 90% yield but with significant variation in enantiomeric purity (80–98% ee over several runs) and about 10% of the diester **7** which could be separated by silica chromatography. A modification of the procedure of Wong[18] which utilizes vinyl

(11) Brown, D. J. *The Pyrimidines*; Interscience: New York, 1962; Chapter 2.

(12) Payatin, B. M.; Glushkov, R. G. *Khim. Pharm. Zh.* **1968**, *2*, 17–20; *Chem. Abstr.* **1969**, *70*, 28887p.

(13) The corresponding amino alcohol may seem to be a more obvious consequence of the malonate disconnection. In the context of the plan, however, the most logical precursor to the amino alcohol would be the azido alcohol **D**. We avoided a number of ambiguous tactical situations by postponing reduction of the azido group until after the malonate unit had been introduced by alkylation.

(14) For a general discussion of the use of enzymes as asymmetric catalysts in nonaqueous media see: Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114–120.

(15) Aldrich Chemical Co., Milwaukee, WI 53233.

(16) Ramos Tombo, G. M.; Schar, H. P.; Fernandez I Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *27*, 5707–5710. Ramos Tombo, G. M.; Schar, H. P.; Fernandez I Busquets, X.; Ghisalba, O. In *Biocatalysis in Organic Media*; Laane, C., Tramper, J., Lilly, M. D., Eds.; Studies in Organic Chemistry; Elsevier: Amsterdam, 1987; Vol. 29, pp 43–50.

(17) Celite Corp., Lompoc, CA 93436.

esters in inert solvents as esterification reagents in the presence of lipases proved to be more stereochemically reproducible. Thus reaction of **5** with vinyl acetate in the presence of PPL in THF–hexane 1:1 provided an 84:16 mixture (mol ratio) of (+)-**6** and **7** at complete conversion of **5** (86% yield of (+)-**6** based on **5**). The ee of the monoacetate thus obtained was reproducibly about 94%.

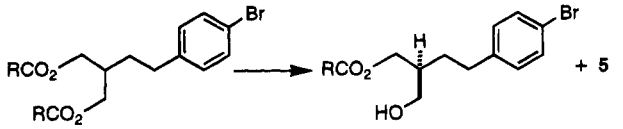
Unambiguous assignment of the absolute configuration of (+)-**6** was secured as follows. Acylation of (+)-**6** with (*S*)-(−)-α-methylbenzyl isocyanate gave the crystalline urethane derivative **8** as a single diastereomer. The absolute configuration of the unknown center in **8** and, therefore, (+)-**6**, was determined to be R by single crystal X-ray analysis. Stereochemical preference for the *pro*-R hydroxyl has also been observed in the PPL-catalyzed acylation of 2-*N*-(benzyloxycarbonyl)serinol by vinyl valerate in THF.[18]

The free hydroxyl group in (+)-**6** was replaced with azide via activation with methanesulfonyl chloride, providing mesylate **9**, followed by displacement with sodium azide in dimethylformamide. The resulting azido acetate **10** was hydrolyzed under basic conditions, giving rise to azido alcohol (*R*)-(+)-**11** in 68% overall yield from (+)-**6**. Determination of enantiomeric purity of (+)-**11** by HPLC of the naphthyl urethane derivative (chiral column) indicated that no racemization had occurred in this sequence.

The enantiomeric azido alcohol should be available, in principle, from the corresponding diol monoester, for example, (−)-**6**, derived from lipase-catalyzed partial hydrolysis of the corresponding diester. We have found, however, that there is apparently not sufficient hydrolytic discrimination between the starting diester and the monoacetate product by the PPL so as to provide practical yields of the desired monoacetate. Typically, 1:1 mixtures of diol **5** and monoacetate were obtained at complete conversion of starting diester. The ee of the monoester fraction of the products remained substantially constant at about 80% over the course of the reaction. Table 1 shows the results of our unsuccessful efforts to optimize the reaction by using various combinations of diesters of **5** and enzymes of both mammalian and bacterial origin.[19] For all enzymes examined the monoesters from partial hydrolysis of the diester substrates were themselves good substrates for enzymatic cleavage to the corresponding diols. Increasing the chain length of the ester residues in the substrate had a variable, enzyme-dependent effect on product distribution as expected, but no useful combinations were found.

The PPL used in our initial experiments was a crude preparation stipulated by the supplier to contain amylase and protease activity. We considered the possibility that a contaminating enzyme might have been responsible for the lower chemoselectivity observed in the hydrolysis mode as compared to the selective esterification of **5** in organic solvents.[20] As a control experiment, the hydrolysis of diacetate **7** was carried out with highly purified PPL both as obtained from a literature procedure[21] and from a commercial source,[22] but the chemo- and enantioselectivity of the hydrolysis in both experiments was essentially the same as in the case of the crude PPL-mediated hydrolyses.

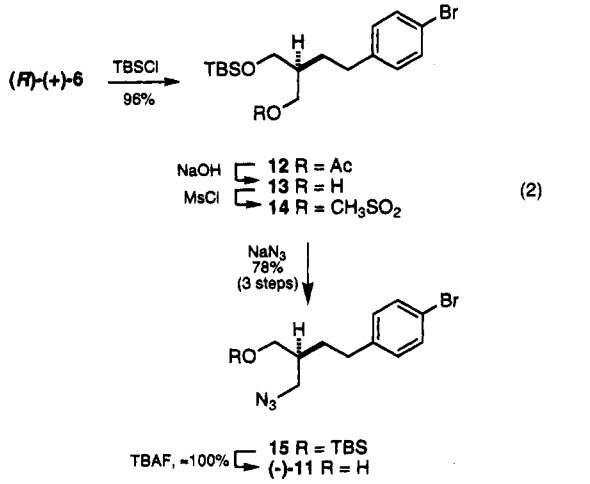
Table 1. Product Ratios for Enzymatic Partial Hydrolysis of Symmetrical Diesters of Diol 5 as a Function of Conversion



			ratio of monoester to diol **5** at indicated percent conversion of diester		
entry	R	enzyme[a]	25%	50%	75%
1	CH_3	PPL	1.3	1.2	1.2[b]
2	CH_3	lipase *Ps. fl.*	1.0	0.33	0.2
3	CH_3	lipase *Cd. cyl.*	1.5	1.0	0.5
4	CH_3	lipase *Asp.* sp.	0.05	0.03	ND
5	CH_3	PLE	0.33	0.33	0.2
6	C_2H_5	PPL	1.2	1.0	0.5
7	C_2H_5	lipase *Ps. fl.*	0.33	0.2	0.1
8	C_2H_5	lipase *Cd. cyl.*	0.5	0.33	ND
9	C_2H_5	lipase Asp. sp.	1.9	1.0	ND
10	C_2H_5	PLE	1.3	ND	ND
11	n-C_7H_{15}	PPL	1.0	0.5	ND
12	n-C_7H_{15}	lipase *Ps. fl.*	–	–	0.2
13	n-C_7H_{15}	lipase *Cd. cyl.*	–	0.5	0.5
14	n-C_7H_{15}	lipase *Asp.* sp.	1.0	0.5	0.5
15	n-C_7H_{15}	PLE	0.2	ND	ND

[a] Enzymes utilized: PPL-porcine pancreatic lipase (Sigma type II) immobilized on Hyflo Super Cel; Lipase *Ps. fl.*-lipase from *Pseudomonas fluorescens*, (Enzymatix); Lipase *Cd. cyl.*-lipase from *Candida cylindracea* (Enzymatix); Lipase *Asp. sp.*-lipase from *Aspergillus* sp. (Enzymatix); PLE-procine liver esterase. [b] ee of monoacetate product was 80% at 75% conversion of diester. ND: not determined.

It proved more practical to prepare the enantiomeric azido alcohol (*S*)-(−)-**11** from (*R*)-(+)-**6**, available from the nonaqueous esterification mode of the lipase process, by appropriate manipulation of functionality at the two oxygenated carbons of **6** as indicated in eq 2.



(18) Wang, Y.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.

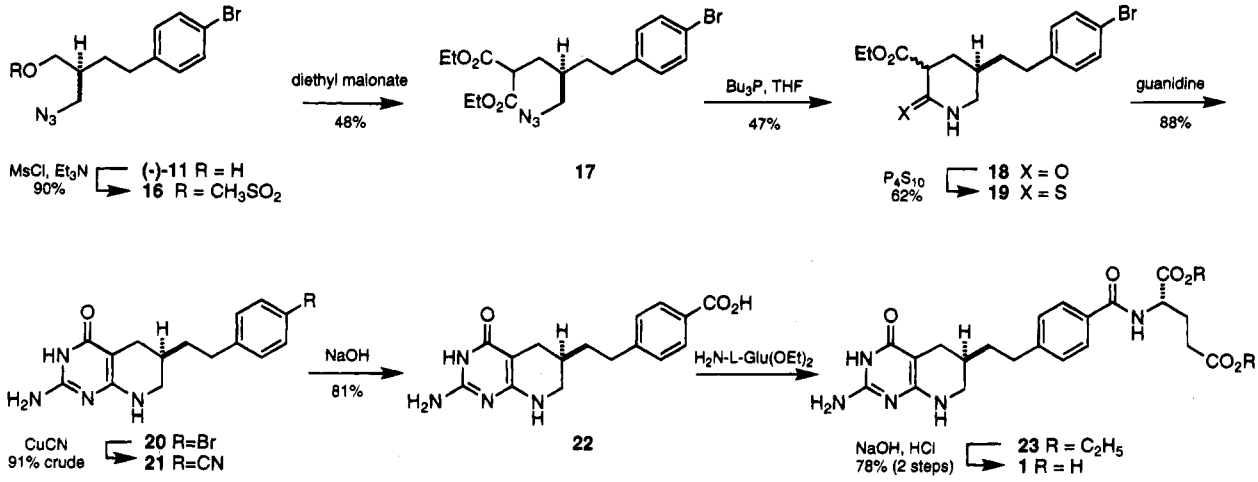
(19) For further examples of the substrate specificity of porcine pancreatic lipase toward 2-substituted 1,3-diol diesters see: Guanti, G.; Narisano, E.; Podgorgski, T.; Thea, S.; Williams, A. *Tetrahedron* **1990**, *46*, 7081–7092. Guanti, G.; Banfi, L.; Narisano, E. *J. Org. Chem.* **1992**, *57*, 1540–1554.

(20) Interestingly, highly purified PPL has been found to be ineffective in catalyzing the transesterification of certain 2-aralkyl- and alkenyl-substituted 1,3-diols in organic solvents, thus implicating a minor enzymatic component of the crude PPL as the true catalytic entity in these cases. See ref 16.

(21) Garner, C.; Smith, L. *Arch. Biochem. Biophys.* **1970**, *140*, 503–507.

(22) Sigma Porcine Pancreatic Lipase Type VI-S, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

Scheme 2



Thus silylation of the free hydroxyl group in (+)-**6** (94% ee) with *tert*-butyldimethylsilyl (TBS) chloride afforded **12** which was hydrolyzed in aqueous sodium hydroxide to **13**, thereby effecting an indirect inversion of configuration. Activation of the unprotected hydroxyl in **13** as the methanesulfonate (**14**) and reaction with sodium azide provided azide **15** which was desilylated to give the desired azido alcohol (−)-**11**, with complete retention of stereochemical integrity (94% ee) in 75% overall yield from (+)-**6**. The synthesis of (−)-**11** from (+)-**6** required one additional step as compared to the preparation of (+)-**11** from the same intermediate.

With both enantiomers of the key azido alcohol intermediate **11** in hand, we turned our attention to elaboration of the tetrahydrodeazapterin moiety required for lometrexol. As we have previously reported,[9] (*R*)-(+)-**11** was correlated by synthesis with (6*S*)-DDATHF which proved to be the C-6 epimer of lometrexol. We describe here the synthesis of lometrexol itself from (*S*)-(−)-**11**. Thus, (−)-**11** was converted to the mesylate **16** followed by reaction with sodium diethyl malonate, giving rise to diester **17** (Scheme 2). Reduction of the azido group in **17** with tributylphosphine in THF containing aqueous hydrochloric acid was accompanied by spontaneous cyclization, affording piperidone **18** in 47% yield (as a mixture of epimers at the carboethoxy-bearing C-3). The successful preparation of **18** thus established the piperidine ring of the target structure with defined configuration at C-6 and bearing suitable functionality for elaboration of the required fused aminopyrimidinone ring.

The piperidone carbonyl and adjacent carboxylate groups in **18** were utilized for the synthesis of the reduced pterin chromophore by a method which has been successfully applied to the preparation of dihydropyrrolo-[2,3-*d*]pyrimidines from the corresponding 3-carboalkoxy-2-pyrrolidinones in this laboratory.[23] Thus reaction of **18** with phosphorus pentasulfide provided the thiolactam **19** in 62% yield. Treatment of **19** with hot excess guanidine gave rise to the requisite tetrahydrodeazapterin **20** in 88% yield.[24]

At this point the aromatic bromine substituent in **20** was utilized to introduce the required aromatic carboxyl group. Reaction of **20** with cuprous cyanide in refluxing *N*-methylpyrrolidinone[25] provided the cyano derivative **21** in 91% crude yield. Basic hydrolysis of **21** gave rise to (−)-5,10-dideazatetrahydropteroic acid (**22**) which could be assigned the *R* configuration by virtue of its derivation from (*S*)-(−)-**11**.

The synthesis of lometrexol was completed by condensation of the tetrahydrodideazapteroic acid **22** with diethyl L-glutamate. An active ester method of peptide coupling based on esterification with 2-chloro-4,6-dimethoxytriazine[26] was found to be a particularly efficient and selective procedure in this case. Importantly, protection of the amino group at C-2 was not required. Thus reaction of **22** with the chlorodimethoxytriazine reagent in the presence of *N*-methylmorpholine and exposure of the resulting active ester with diethyl L-glutamate provided the diester **23** in 85% yield. Saponification of **23** in aqueous sodium hydroxide followed by acidification of the mixture with hydrochloric acid afforded **1** as an amorphous solid in 78% yield (based on **22**). The material thus obtained was shown to be identical with an authentic sample of lometrexol, the "B" diastereomer of DDATHF, by β-cyclodextrin inclusion chromatography,[27a] thus establishing the *6R* configuration for lometrexol. The HPLC results further indicated that no significant racemization (<1%) at the glutamate stereogenic carbon had occurred in the coupling step.

In summary, we have successfully completed a stereochemically unambiguous synthesis of lometrexol which utilizes a readily available preparation of mammalian lipase as a catalyst for the key asymmetric transformation. This synthesis provides an unambiguous proof of the absolute configuration of lometrexol and related precursors via correlation with (*R*)-(+)-**6**, the configurational assignment of which was secured by the X-ray

(23) Barnett, C. J.; Wilson, T. M. *Heterocycles* **1993**, *36*, 925−936.
(24) In our initial work (ref 9) we followed literature precedent (ref 12) in utilizing the imido ether of **18** as an activated derivative of the lactam carbonyl for cyclization with guanidine. We now prefer the thiolactam approach described herein for its experimental simplicity. In the absence of activation of the lactam carbonyl the reaction of 3-carbethoxy-2-piperidones with guanidine proceeds in poor yield. See: Degraw, J.; Goodman, L. *Can. J. Chem.* **1963**, *41*, 3137−3139.
(25) For other examples of the use of *N*-methylpyrrolidinone as solvent in the Rosenmund−von Braun displacement see: Russell, H. F.; Harris, B. J.; Hood, D. B.; Thompson, E. G.; Watkins, A. D.; Williams, R. D. *Org. Prep. Proc. Int.* **1985**, *17*, 391−399. Zimmerman, H. E.; Swafford, R. L. *J. Org. Chem.* **1984**, *49*, 3069−3083.
(26) Kaminski, Z. J. *Tetrahedron Lett.* **1985**, *26*, 2901−2904.
(27) (a) Shih, C.; Wilson, G. M.; Osborne, L. M.; Harrington, P. M.; Gossett, L. S.; Snoddy, J. D. In *Chemistry and Biology of Pteridines 1989*; Curtius, H.-Ch., Ghisla, S., Blau, N., Eds.; Walter de Gruyter: Berlin, 1990; pp 177−180. (b) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

analysis of **8**.[27b] The methods described herein furthermore provide for the preparation of all four of the possible stereoisomers of lometrexol, given the ready availability of both D- and L-glutamic acid. The synthesis of (*6S*)-lometrexol from (+)-**11** has also been completed as reported previously,[9] but has not been described explicitly herein since all experimental procedures were the same as for the synthesis of lometrexol itself.

Experimental Section

General. Melting points and boiling points are uncorrected. Reactions were generally done in a nitrogen atmosphere. Column chromatography was carried out on columns of 230–400 mesh chromatography grade silica gel under 2–5 psig nitrogen pressure. Thin layer chromatography (TLC) was carried out on commercially available silica gel plates (E. Merck), used as supplied. All optical rotation measurements were conducted at 25 °C in a standard photoelectric polarimeter. Mass spectra are reported as *m/z* (relative intensity). Proton and carbon-13 NMR spectra were obtained on a General Electric QE-300 instrument at 300 and 75.5 MHz, respectively. The following conditions were used for high performance liquid chromatography (HPLC): method A, 25 cm Waters μ-Bondapak C18,[28] column acetonitrile–water 3:2, 2.0 mL/min flow rate, detection at 254 nm; method B (analysis of enantiomeric purity of nonracemic alcohols as their naphthyl urethane derivatives),[29] 25 cm Bakerbond Chiralcel OD[30] column, hexane–ethanol–*n*-propanol 2:2:1, 1.0 mL/min flow rate, detection at 280 nm; method C, 25 cm Waters μ-Bondapak C18 column, acetonitrile–0.025% aqueous trifluoroacetic acid 27:73, 2.0 mL/min flow rate, detection at 254 nm.

4-Bromobenzeneethanol 4-Methylbenzenesulfonate Ester (3). To a solution of 50.0 g (0.25 mol) of 4-bromobenzeneethanol (**2**) and 52 g (0.27 mol) of *p*-toluenesulfonyl chloride in 500 mL of methylene chloride at 18 °C was added dropwise with stirring 27 g (0.27 mol) of triethylamine in 100 mL of methylene chloride. The reaction was allowed to proceed at room temperature overnight. After addition of 100 mL of 1 M aqueous HCl the phases were separated, and the organic phase was washed with sodium bicarbonate and dried ($MgSO_4$). The solvent was removed under vacuum and the residue crystallized from 240 mL of 2-propanol, affording 75.2 g (85%) of **3** as a white solid: mp 92–93 °C; ^1H NMR ($CDCl_3$) δ 7.62 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.2 Hz, 2H), 6.94 (d, J = 8.2 Hz, 2H), 4.18 (t, J = 6.6 Hz, 2H), 2.87 (t, J = 6.6 Hz, 2H), 2.45 (s, 3H). Anal. Calcd for $C_{15}H_{15}BrO_3S$: C, 50.72; H, 4.26. Found: C, 50.79; H, 4.38.

2-[2-(4-Bromophenyl)ethyl]propanedioic Acid Diethyl Ester (4). To a solution of sodium diethyl malonate in THF, prepared from 27.4 g (0.171 mol) of diethyl malonate and 3.93 g (0.164 mol) of sodium hydride in 250 mL of anhydrous THF, were added 47.23 g (0.133 mol) of **3** and 4.27 g (0.028 mol) of sodium iodide. The mixture was heated under reflux for 4 h, cooled, and quenched by addition of 100 mL of water. Hexane (100 mL) was added, and the phases were separated. The organic phase was washed with brine and dried ($MgSO_4$). Evaporation of the solvent under vacuum provided 50.1 g of **4** as a yellow oil which was estimated to contain 4.6% (wt) of residual diethyl malonate (NMR). The corrected yield of **4** was 47.8 g (98%). The material was used in the next step without additional purification. A small sample was purified by chromatography (silica, hexanes–ethyl acetate 8:2) followed by distillation (kugelrohr) 200 °C (0.01 Torr): ^1H NMR ($CDCl_3$) δ 7.34 (d, J = 8.3 Hz, 2H), 7.02 (d, J = 8.3 Hz, 2H), 4.15 (q, J = 7.2 Hz, 4H), 3.28 (t, J = 7.4 Hz, 1H), 2.58 (t, J = 7.2 Hz, 1H), 2.14 (dt, J = 7.2, 7.4 Hz, 2H), 1.22 (t, J = 7.2 Hz, 6H). Anal. Calcd for $C_{15}H_{19}BrO_4$: C, 52.49; H, 5.58. Found: C, 52.29; H, 5.54.

2-[2-(4-Bromophenyl)ethyl]-1,3-propanediol (5). A solution of 113.7 g (0.33 mol) of crude **4** in 700 mL of anhydrous ether was added dropwise over about 30 min to a suspension of 15.7 g (0.414 mol) of lithium aluminum hydride in 800 mL of ether cooled to 0 °C (ice bath). The temperature was kept below 20 °C during the addition. The bath was removed and the mixture was allowed to stir for an additional 30 min. At this point the reaction was shown to be complete by HPLC analysis (method A; t_R 2.0 min). The mixture was cooled to 15 °C and excess reagent quenched by careful addition of 15 mL of water, followed by 800 mL of 6 M HCl. The phases were separated and the organic phase was dried ($MgSO_4$) and concentrated under vacuum, providing 89.3 g of crude **5** as a yellow solid. Recrystallization of the crude from toluene–hexane gave 61.2 g (71%) of **5** as a white solid: mp 74–76 °C; ^1H NMR ($CDCl_3$) δ 7.37 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 8.2 Hz, 2H), 3.79 (dd, J = 10.6, 3.7 Hz, 2H), 2.85 (s, 2H), 2.60 (dd, J = 7.7, 8.1 Hz, 2H), 1.74 (m, 1H), 1.56 (dt, J = 8.4, 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 141.0, 131.5, 130.1, 119.6, 65.7, 41.4, 32.8, 29.3; MS (EI) *m/z* 260 (28), 258 (29). Anal. Calcd for $C_{11}H_{15}BrO_2$: C, 50.98; H, 5.83. Found: C, 50.97, H, 5.81.

(*R*)-(+)-2-[2-(4-bromophenyl)ethyl]-1,3-propanediol Monoacetate (*R*)-(+)-(6). Methyl Acetate Method. A mixture of 4.60 g (17.7 mmol) of **5** and 33.1 g of an immobilized porcine pancreas lipase preparation[31] in 300 mL of methyl acetate was stirred at ambient temperature. The reaction was monitored by HPLC (method A). When consumption of **5** was complete the enzyme was immediately filtered and the filtrate concentrated *in vacuo* to an oil, HPLC (method A) t_R: 2.0 min (**5**, not detected), 3.0 min (**6**, 90.3%), 5.2 min (**7**, 9.7%). The crude product was purified by chromatography (silica, ethyl acetate–hexane 3:1) to give 4.82 g (90.4%) of (+)-**6** as an oil, TLC R_f 0.45 (silica, 3:1 ethyl acetate–hexane). A portion of the material thus obtained was converted to the 1-naphthylcarbamate with 1-naphthyl isocyanate for determination of enantiomeric purity by HPLC (method B) t_R: (+)-**6**, 9.4 min; (−)-**6**, 12.6 min. Enantiomeric purity varied unpredictably from 80 to 98% over several runs, most typically 85–88%. Optical rotation data for 98% ee product: $[\alpha]_{589}$ +10°; $[\alpha]_{365}$ +32° (*c* 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.39 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 4.21 (dd, J = 4.5, 11.3 Hz, 1H), 4.13 (d, J = 6.3, 11.3 Hz, 1H), 3.62 (dd, J = 4.6, 11.2 Hz, 1H), 3.56 (dd, J = 5.1, 11.2 Hz, 1H), 2.64 (t, J = 8.0 Hz, 2H), 2.07 (s, 3H), 1.94 (s, 1H), 1.83 (m, 1H), 1.64 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 171.3, 140.8, 131.3, 129.9, 119.4, 64.3, 62.2, 39.8, 32.4, 29.4, 20.6; IR ($CHCl_3$) 3635, 1727 cm^{-1}; MS (EI) *m/z* 302 (2), 300 (2), 184 (65), 182 (67), 171 (24), 169 (28), 90 (23), 43 (100). Anal. Calcd for $C_{13}H_{17}BrO_3$: C, 51.84; H, 5.67. Found: C, 51.86; H, 5.89.

Preparation of (*R*)-(+)-6, Vinyl Acetate Method. To a mixture of 29.6 g (0.114 mol) of diol **5** and 49.3 g (0.572 mol) of vinyl acetate in 1 L of THF–hexanes 1:1 was added 44 g of an immobilized porcine pancreas lipase preparation[31] and the resulting mixture was stirred at room temperature. The progress of the reaction was monitored by HPLC (method A). After 90 min, analysis of an aliquot indicated complete conversion of starting **5** to monoacetate **6** and diacetate **7** (mol ratio 84:16). The mixture was immediately filtered and the solvent removed by vacuum evaporation, affording 35.3 g of crude (*R*)-(+)-**6** as a yellow oil. The yield of (+)-**6** was estimated to be 86% based on the final weight and HPLC ratio. Purification could be carried out as described above. Conversion of a sample to the 1-naphthylcarbamate and HPLC analysis as described above indicated an ee of 94% for (+)-**6** thus obtained.

2-[2-(4-Bromophenyl)ethyl]-1,3-propanediol Diacetate (7). To a solution of 7.77 g (30.0 mmol) of **5** in 40 mL of triethylamine cooled to 10 °C (ice bath) was added 8.2 mL (7.6 g, 75 mmol) of acetic anhydride. The mixture was allowed to warm to ambient temperature and stirred overnight. The mixture was concentrated under vacuum and the residue taken up in 20 mL of ethyl acetate. The solution was washed with 2 × 20 mL saturated $NaHCO_3$, 2 × 10 mL 1 N aqueous HCl and brine and dried ($MgSO_4$). Evaporation of the solvent

(28) Waters Division, Millipore Corp., Milford, MA 01757.
(29) Method developed by J. Kennedy, Lilly Research Laboratories.
(30) J. T. Baker, Inc., Phillipsburg, NJ 08865.
(31) Porcine pancreatic lipase (Type II, Sigma Chemical Co.) was partially purified and immobilized according to the method described in ref 16.

afforded 9.8 g of **7** as an oil which was distilled (kugelrohr) bp 200 °C (0.01 Torr), yielding 9.4 g (91%) of purified **7**, homogeneous by TLC: R_f 0.27 (silica, hexane-ethyl acetate 8:2); ^{1}H NMR ($CDCl_3$) δ 7.35 (d, J = 8.3 Hz, 2H), 7.02 (d, J = 8.3 Hz, 2H), 4.06 (dd, J = 1.4, 5.2 Hz, 4H), 2.60 (dd, J = 7.8, 9.8 Hz, 4H), 2.02 (s, 6H), 1.98 (m, 1H), 1.62 (dt, J = 7.0, 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 170.7, 140.4, 131.4, 129.9, 119.6, 63.9, 36.7, 32.3, 29.7, 20.7; IR ($CHCl_3$) 1735 cm^{-1}; MS (EI) m/z 344 (1), 342 (1), 184 (100), 182 (98), 171 (40), 169 (43). Anal. Calcd for $C_{15}H_{19}BrO_4$: C, 52.49; H, 5.58. Found: C, 52.28; H, 5.63.

(*S*)-α-Methylbenzyl Carbamate Derivative of (+)-6 (8). A mixture of 300 mg (1 mmol) of (+)-**6** and 147 mg (1 mmol) of (*S*)-(−)-α-methylbenzyl isocyanate and 3 drops of triethylamine in 1.5 mL of acetonitrile was stirred at room temperature overnight. The mixture was concentrated to an oil under vacuum and purified by chromatography (silica, ethyl acetate–hexane 3:2), affording 346 mg (77%) of **8**. Crystals suitable for X-ray analysis were obtained by slow crystallization from ethyl acetate–hexane: mp 57–59 °C; ^{1}H NMR ($CDCl_3$) δ 7.26–7.4 (m, 7H), 7.02 (d, J = 8.0 Hz, 2H), 4.94 (br m, 1H), 4.85 (br m, 1H), 4.10 (m, 4H), 2.61 (br m, 2H), 2.04 (s, 3H), 1.98 (br m, 1H), 1.61 (br m, 2H), 1.48 (d, J = 6.5 Hz, 3H).

(*S*)-(+)-2-[2-(4-bromophenyl)ethyl]-1,3-propanediol Acetate Methanesulfonate (9). To a solution of 15.5 g (50 mmol) of crude (+)-**6**, (82.5% pure by HPLC, method A, ee of monoacetate fraction 86%) prepared by the methyl acetate–PPL method described above, in 60 mL of chloroform (0 °C) containing 7.08 g (70 mmol) of triethylamine, was added 7.73 g (67.5 mmol) of methanesulfonic acid in 30 mL of chloroform. After addition was complete the mixture was stirred for 15 min and then partitioned with 100 mL of 1 N HCl. The layers were separated and the organic phase dried ($MgSO_4$). Concentration of the solution provided 19.6 g of crude mesylate **9** as a yellow oil which was taken to the next step without further purification. An analytical sample was prepared by chromatography of a sample obtained separately (silica, hexanes–ethyl acetate 1:1): $[\alpha]_{589}$ +2°, $[\alpha]_{365}$ +8° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.38 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 4.21 (d, J = 5.2 Hz, 2H), 4.14 (dd, J = 4.7, 11.3 Hz, 1H), 4.06 (dd, J = 6.7, 11.3 Hz, 1H), 2.97 (s, 3H), 2.63 (t, J = 8.0 Hz, 2H), 2.06 (m, 1H), 2.04 (s, 3H), 1.63 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.5, 140.0, 131.5, 130.0, 119.7, 68.8, 63.0, 37.2, 32.1, 29.1, 20.6; MS (EI) m/z 380 (2), 378 (3). Anal. Calcd for $C_{14}H_{19}BrO_5S$: C, 44.34; H, 5.05. Found: C, 44.42; H, 5.21.

(*R*)-(+)-4-Bromo-β-(azidomethyl)benzenebutanol Acetate (10). To a solution of 19.6 g of crude **9**, obtained as described above, in 150 mL of DMF was added 3.57 g (55 mmol) of sodium azide and the mixture was heated at 80 °C overnight. The mixture was cooled to room temp and diluted with 150 mL of ethyl acetate and 150 mL of water. The layers were separated and the aqueous phase extracted with an additional 150 mL of ethyl acetate. The combined organic layers were dried ($MgSO_4$) and concentrated to dryness, affording 16.0 g of crude **10** as an oil suitable for further processing. An analytical sample was obtained by chromatography of a sample obtained separately (silica, hexane–ethyl acetate 3:2): $[\alpha]_{589}$ +4°, $[\alpha]_{365}$ +13° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.37 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 4.10 (dd, J = 4.8, 11.3 Hz, 1H), 4.03 (dd, J = 6.6, 11.3 Hz, 1H), 3.36 (d, J = 5.7 Hz, 2H), 2.60 (t, J = 8.0 Hz, 2H), 2.05 (s, 3H), 1.89 (m, 1H), 1.65 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.6, 140.2, 131.4, 129.9, 119.7, 64.1, 52.4, 37.4, 32.2, 30.2, 20.6. Anal. Calcd for $C_{13}H_{16}BrN_3O_2$: C, 47.86; H, 4.94; N, 12.88. Found: C, 48.10; H, 5.03; N, 12.60.

(*R*)-(+)-β-(Azidomethyl)-4-bromobenzenebutanol ((+)-11). The 16.0 g of crude **10** obtained as described above was dissolved in 150 mL of methanol and 100 mL of 1 N NaOH, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was extracted with 2 × 100 mL of chloroform. The combined organic phases were dried ($MgSO_4$) and concentrated to dryness, and the crude material thus obtained was purified by chromatography (silica, hexane–ethyl acetate 1:1), affording 9.73 g (68% based on (*R*)-(+)-**6**) of (*R*)-(+)-**11** as an oil: $[\alpha]_{589}$ +1°, $[\alpha]_{365}$ +4.5° (*c* 10.3, $CHCl_3$). Enantiomeric purity was determined to be 85.4% by HPLC of the 1-naphthylcarbamate derivative (method B): ^{1}H NMR ($CDCl_3$) δ 7.39 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 3.66 (dd, J = 4.4, 10.8 Hz, 1H), 3.59 (dd, J = 6.1, 10.8 Hz, 1H), 3.45 (dd, J = 5.1, 12.2 Hz, 1H), 3.41 (dd, J = 6.0, 12.2 Hz, 1H), 2.61 (t, J = 7.9 Hz, 2H), 2.08 (s, 1H), 1.76 (m, 1H), 1.65 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 140.6, 131.4, 130.0, 119.6, 63.1, 52.7, 40.1, 32.5, 30.1; IR ($CHCl_3$) 3625, 2930, 2102, 1480 cm^{-1}; MS (EI) m/z 256 (18, M − 29), 254 (20). Anal. Calcd for $C_{11}H_{14}BrN_3O$: C, 46.50; H, 4.97; N, 14.79. Found C, 46.48; H, 4.72; N, 14.90.

General Procedure for Enzymatic Partial Hydrolysis of *sym*-Diesters of 5. To a mixture of 75 mg of the test enzyme in 12 mL of 0.1 M phosphate buffer (pH 7) was added 100 mg of the diester, and the resulting mixture was stirred vigorously at room temperature. Aliquots were removed periodically to assess the progress of the reaction and product distribution by HPLC analysis (method A). The HPLC data were plotted and the ratio of monoester to diol **5** at various percent conversion of starting diester was found by extrapolation. The results are presented in Table 1.

(*S*)-(−)-4-Bromo-β-[[[(1,1-dimethylethyl)dimethylsilyl]-oxy]methyl]benzenebutanol Acetate (12). To a solution of 34.7 g of crude (*R*)-(+)-**6** (86% by HPLC, 0.097 mol, 94% ee) and 8.16 g (0.12 mol) of imidazole in 300 mL of dichloromethane was added 17.7 g (0.12 mol) of *tert*-butyldimethylsilyl chloride in 150 mL of dichloromethane. The reaction temperature was maintained at 17–20 °C with cooling as necessary. The reaction was stirred for 1 h, filtered, and concentrated *in vacuo*. Chromatography of the crude (silica, hexane–ethyl acetate 8:2) afforded 38.8 g (96%) of **12** as an oil: $[\alpha]_{589}$ −0.4°, $[\alpha]_{365}$ −0.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.39 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 4.08 (d, J = 6.0 Hz, 2H), 3.60 (d, J = 5.2 Hz, 2H), 2.61 (t, J = 8.0 Hz, 2H), 2.04 (s, 3H), 1.85 (m, 1H), 1.64 (m, 2H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.5, 141.1, 131.3, 129.9, 119.5, 64.4, 62.5, 39.8, 32.6, 29.6, 25.8, 20.6, 18.1, −5.6; IR ($CHCl_3$) 2951, 2922, 2848, 1728, 1485, 1471, 1254, 839 cm^{-1}; MS (EI) m/z 414 (1), 412 (1), 225 (20), 223 (22), 171 (19), 169 (18), 144 (26), 117 (100), 75 (64). Anal. Calcd for $C_{19}H_{31}BrO_3Si$: C, 54.93; H, 7.52. Found: C, 55.20; H, 7.31.

(*S*)-(−)-4-Bromo-β-[[[(1,1-dimethylethyl)dimethylsilyl]-oxy]methyl]benzenebutanol (13). A mixture of 38.4 g (0.092 mol) of **12**, 240 mL of MeOH, and 135 mL of 1 N NaOH was stirred overnight at ambient temperature. To the mixture was added 400 mL of *tert*-butyl methyl ether and the phases mixed and separated. The organic phase was washed twice with 2 × 100 mL of brine, 100 mL of saturated NH_4Cl, and 100 mL of brine, dried ($MgSO_4$), and concentrated *in vacuo*, affording 33.3 g (96%) of substantially pure (NMR) **13** as an oil. The crude material was taken to the next step without further purification. An analytical sample was prepared separately by chromatography (silica, ethyl acetate–hexane 4:1): $[\alpha]_{589}$ −6°, $[\alpha]_{365}$ −17° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.35 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 3.79–3.60 (m, 4H), 3.05 (s, 1H), 2.58 (t, J = 7.3 Hz, 2H), 1.70 (m, 1H), 1.56 (m, 2H), 0.89 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 141.3, 131.3, 129.9, 119.4, 65.9, 65.2, 41.6, 32.7, 29.2, 25.8, 18.0, −5.5; IR ($CHCl_3$) 2954, 2930, 2898, 2859, 1488, 1471, 1258, 837 cm^{-1}; MS (EI) m/z 373 (1), 225 (28), 223 (27), 171 (30), 169 (33), 144 (55), 129 (16), 105 (25), 75 (100). Anal. Calcd for $C_{17}H_{29}BrO_2Si$: C, 54.68; H, 7.83. Found: C, 54.48; H, 7.77.

(*R*)-(−)-4-Bromo-β-[[[(1,1-dimethylethyl)dimethylsilyl]-oxy]methyl]benzenebutanol Methanesulfonate (14). To a 0 °C solution of 32.6 g (0.087 mole) of **13** and 13.25 g (0.13 mole) of triethylamine in 225 mL of dichloromethane was added dropwise a solution of 15.0 g (0.13 mole) of methanesulfonyl chloride in 125 mL of dichloromethane so that the temperature was maintained at 4–7 °C. The cooling bath was removed and the reaction stirred for 35 min. After addition of 300 mL of water and separation of the layers, the organic phase was washed with 1 × 300 mL of water, dried ($MgSO_4$), and concentrated under vacuum, affording 40.3 g of crude **14** as an oil which was taken to the azide displacement step without further purification. An analytical sample was prepared separately by chromatography of crude material (silica, hexane–ethyl acetate 4:1): TLC R_f 0.41 (hexane–ethyl acetate 4:1); $[\alpha]_{589}$ −4°, $[\alpha]_{365}$ −9° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.39

(d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 4.26 (dd, J = 6.0, 9.6 Hz, 1H), 4.24 (dd, J = 5.1, 9.6 Hz, 1H), 3.65 (dd, J = 4.4, 10.2 Hz, 1H), 3.58 (dd, J = 6.1, 10.2 Hz, 2H), 2.98 (s, 3H), 2.62 (t, J = 8.0 Hz, 2H), 1.89 (m, 1H), 1.65 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 140.6, 131.5, 130.0, 119.7, 69.7, 61.6, 40.2, 37.0, 32.4, 28.9, 25.8, 18.2, −5.6; IR ($CHCl_3$) 2957, 2933, 2860, 1489, 1474, 1360, 838 cm^{-1}; MS (EI) m/z 225 (38), 223 (43), 171 (28), 169 (27), 153 (100), 144 (48), 129 (13), 75 (39). Anal. Calcd for $C_{18}H_{31}BrO_4SSi$: C, 47.89; H, 6.92. Found C, 48.16; H, 6.70.

(*S*)-(−)-[2-(Azidomethyl)-4-(4-bromophenyl)butoxy] (1,1-dimethylethyl)dimethylsilane (15). A mixture of 39.9 g of **14** and 6.18 g (0.095 mole) of sodium azide in 300 mL of DMF was heated to 80 °C overnight. After cooling, the mixture was diluted with 300 mL of hexane and 300 mL of water. The phases were separated and the organic phase was washed with 4 × 100 mL of brine, dried ($MgSO_4$), and concentrated under vacuum affording 33.0 g of an oil. The residue was purified by chromatography (silica, heptane−ethyl acetate 9:1) giving 27.8 g (78% based on **12**) of **15** as an oil: $[\alpha]_{589}$ −5°, $[\alpha]_{365}$ −18° (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 3.64 (dd, J = 4.2, 10.1 Hz, 1H) 3.57 (dd, J = 5.6, 10.1 Hz, 1H), 3.41 (dd, J = 5.9, 12.0 Hz, 1H), 3.37 (dd, J = 5.3, 12.0 Hz, 1H), 2.61 (t, J = 7.9, 2H), 1.71 (m, 1H), 1.63 (m, 1H), 0.92 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 141.0, 131.5, 130.0, 119.7, 62.8, 52.6, 40.7, 32.7, 30.3, 25.9, 18.3, −5.5; IR ($CHCl_3$) 2953, 2930, 2858, 2101, 1488, 838 cm^{-1}. Anal. Calcd for $C_{17}H_{28}BrN_3OSi$: C, 51.25; H, 7.08; N, 10.55. Found: C, 51,48; H, 7.11; N, 10.70.

(*S*)-(−)-β-(Azidomethyl)-4-bromobenzenebutanol ((−)-11). A mixture of 27.6 g (69.3 mmol) of **15** and 83 mL of tetrabutylammonium fluoride 1 M in THF was stirred at room temperature for 1 h, after which time the disappearance of starting material was confirmed by TLC. The mixture was diluted with 200 mL of *tert*-butyl methyl ether and the resulting solution was washed with 3 × 100 mL of 1 N HCl. The combined aqueous layers were back extracted with 100 mL of *tert*-butyl methyl ether and the combined organic phases were dried ($MgSO_4$) and concentrated to give 19.8 g (100%) of (*S*)-(−)-**11** as an oil suitable for further processing without further purification: TLC R_f 0.40 (silica, ethyl acetate−hexane 1:1); $[\alpha]_{589}$ −1°, $[\alpha]_{365}$ −3° (c 0.8, $CHCl_3$); HPLC enantiomeric purity (derivatized as the 1-naphthylcarbamate, method B) t_R: R, 15.5 min; S, 10.8 min, 90% ee. The ^{1}H NMR, IR, and mass spectral data were the same as described for (*R*)-(+)-**11** (see above).

(*S*)-(−)-β-(Azidomethyl)-4-bromobenzenebutanol Methanesulfonate (16). To a solution of 18.9 g (0.067 mol) of (−)-**11** prepared as described above and 10.1 g (0.1 mol) of triethylamine in 175 mL of dichloromethane cooled to 3 °C was added dropwise a solution of 11.4 g (0.1 mol) of methanesulfonyl chloride in 100 mL of dichloromethane so that the temperature was maintained at 3−5 °C. After 15 min of additional stirring TLC indicated complete conversion of starting material. The reaction was quenched by addition of 100 mL of water. The layers were separated and the organic layer was washed with 100 mL of 1 N HCl and 100 mL of saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated under vacuum. The crude product was purified by chromatography (silica, ethyl acetate−hexane 2:3) to give 21.6 g (90%) of purified **16** as an oil: TLC R_f 0.49 (silica, ethyl acetate−hexane 2:3); $[\alpha]_{589}$ −3°, $[\alpha]_{365}$ −7° (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.39 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 4.20 (m, 2H), 3.46 (dd, J = 5.1, 12.9 Hz, 1H), 3.40 (dd, J = 6.0, 12.9 Hz, 2H), 3.01 (s, 3H), 2.62 (t, J = 7.9 Hz, 2H), 1.96 (m, 1H), 1.67 (q, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 139.9, 131.5, 123.0, 119.8, 69.1, 51.4, 37.8, 37.2, 32.1, 29.0; IR ($CHCl_3$) 2939, 2104, 1489, 1362, 1176, 971 cm^{-1}. Anal. Calcd for $C_{12}H_{16}BrN_3O_3S$: C, 39.79; H, 4.45; N, 11.60. Found: C, 40.02; H, 4.53; N, 11.73.

(*R*)-(−)-[2-(Azidomethyl)-4-(4-bromophenyl)butyl]propanedioic Acid Diethyl Ester (17). A solution of 28.2 g (0.15 mole) of diethyl malonate in 100 mL of dry THF was added to a rapidly stirred suspension of 3.67 g (0.153 mol, oil free) of sodium hydride in 150 mL of dry THF. When hydrogen evolution had ceased, a solution of 21.3 g (0.059 mol) of **16** prepared as described above in 150 mL of dry THF and 1.76 g of sodium iodide were added. The mixture was heated under reflux for 18 h, cooled, and then partitioned by the addition of 400 mL of ethyl acetate and 300 mL of water. The organic phase was separated, washed with 2 × 300 mL of brine, dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by chromatography (silica, ethyl acetate−hexane 2:3), affording 12.0 g (48%) of **17** as an oil, pure by HPLC (method A) t_R: 14.0 min; TLC R_f 0.41 (silica, ethyl acetate−hexane 2:3); $[\alpha]_{589}$ −3°, $[\alpha]_{365}$ −5° (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.37 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 4.16 (m, 4H), 3.42 (t, J = 7.6 Hz, 1H), 3.33 (d, J = 4.7 Hz, 2H), 2.58 (t, J = 7.6 Hz, 2H), 1.95 (m, 2H), 1.67 (m, 3H), 1.24 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 169.0, 140.5, 131.5, 130.0, 119.6, 61.4, 54.7, 49.8, 35.9, 33.4, 32.1, 31.0, 13.9; IR ($CHCl_3$) 2102, 1744, 1726 cm^{-1}; MS (FD) m/z 428 (M + 1), 426, 420, 418, 397, 384. Anal. Calcd for $C_{18}H_{24}BrN_3O_4$: C, 50.71; H, 5.67; N, 9.86. Found: C, 50.75; H, 5.47; N, 9.94.

(3*RS*,5*R*)-5-[2-(4-Bromophenyl)ethyl]-2-oxo-3-piperidinecarboxylic Acid Ethyl Ester (18).[32] To a solution of 10.8 g (25.3 mmol) of **17** and 0.5 mL of 0.1 N HCl in 60 mL of THF was added dropwise 5.64 g (27.9 mmol) of tri-*n*-butylphosphine. An exotherm (with evolution of N_2) was observed. After 1 h the reaction mixture was concentrated under vacuum. The crude product was purified by chromatography (silica, CH_2Cl_2−ethanol 95:5) to afford 6.8 g of a yellow oil contaminated with tributylphosphine oxide. The oil was taken up in 100 mL of hexane at reflux, causing the product to crystallize. Filtration and drying afforded 4.20 g (47%) of **18** as a 2:1 mixture of epimers at C-3 (estimated by TLC) which was not separated: ^{1}H NMR ($CDCl_3$) δ 7.49, 7.45 (s, total 1H), 7.36, 7.35 (d, J = 8.4 Hz, total 2H), 7.00 (d, J = 8.4 Hz, 2H), 4.19, 4.15 (q, J = 7.8 Hz, total 2H), 3.34 (m, 2H), 2.98 (t, J = 11.0 Hz, 1H), 2.94 (t, J = 13.0 Hz, 1H), 2.55 (m, 2H), 2.15 (m, 1H), 1.93 (m, 2H), 1.59 (m, 2H), 1.25, 1.22 (t, J = 7.8 Hz, total 3H); ^{13}C NMR ($CDCl_3$) δ 170.4, 168.2, 167.9, 140.3, 140.2, 131.5, 131.4, 129.9, 129.8, 119.70, 119.66, 61.3, 61.1, 48.8, 47.3, 47.1, 46.9, 34.7, 34.1, 32.4, 32.3, 32.1, 31.1, 31.0, 30.3, 29.4, 14.0; IR (KBr) 3200, 1743, 1734, 1673 cm^{-1}; MS (EI) m/z 355 (53), 353 (51). Anal. Calcd for $C_{16}H_{20}BrNO_3$: C, 54.25; H, 5.69; N, 3.95; found: C, 54.16; H, 5.62; N, 3.92.

(3*RS*,5*R*)-2-Thioxo-3-(ethoxycarbonyl)-5-[2-(4-bromophenyl)ethyl]piperidine (19). A mixture of 1.6 g (4.5 mmol) of lactam **18** and 1.10 g (4.9 mmol) of phosphorus pentasulfide (calcd on P_2S_5) in 60 mL of THF was heated to 60 °C for 45 min, when the reaction was judged complete by TLC (silica, 1:1 heptane−ethyl acetate). The mixture was then cooled, and 100 mL of ethyl acetate was added. The resulting mixture was washed with 2 × 50 mL of saturated sodium bicarbonate and with brine. The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (silica, 1:1 heptane−ethyl acetate) affording 1.03 g (62%) of **19** as a mixture of C-3 epimers: mp 105−115 °C; ^{1}H NMR ($CDCl_3$) δ 9.47 (s, 1H), 7.41, 7.39 (d, J = 8.2 Hz, total 2H), 7.02 (d, J = 8.2 Hz, 2H), 4.20 (m, 2H), 3.94, 3.69 (m, total 1H), 3.45 (m, 1H), 2.99 (m, 1H), 2.60 (m, 2H), 2.13 (m, 2H), 1.5−1.87 (m, 3H), 1.31, 1.26 (t, J = 7.1 Hz, total 3H); IR ($CHCl_3$) 2983, 1731, 1540, 1373, 1073 cm^{-1}; MS (FD) m/z 371 (100), 369 (75); UV (EtOH) λ_{max} (ϵ) 219.8 (13 174), 281.4 (13 557) nm. Anal. Calcd for $C_{16}H_{20}BrNO_2S$: C, 51.90; H, 5.44; N, 3.78. Found: C, 51.61; H, 5.38; N, 3.88.

(*R*)-(−)-2-Amino-6-[2-(4-bromophenyl)ethyl]-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (20). To a suspension of 4.64 g (48.6 mmol) of guanidine hydrochloride in 30 mL of dry ethanol was added 49 mL of 1 M potassium *tert*-butoxide in THF. After stirring 15 min the solution was filtered through Celite and added to a mixture of 4.50 g (12.15 mmol) of **19** in 30 mL of ethanol. The mixture was stirred briefly, concentrated *in vacuo* to remove most of the alcohol, and then heated to 100 °C for 3 h under reduced pressure (about 10 Torr). After cooling to ambient temperature, the

(32) This procedure was based on the well-known reduction of azides to amines with triphenylphosphine and water. See: Vaultier, M.; Knouzi, N.; Carrie, R. *Tetrahedron Lett.* **1983**, *24*, 763−764. We utilized tributylphosphine in this case in order to facilitate removal of the phosphine oxide from the product.

solid residue was heated to 100 °C and mixed thoroughly with 70 mL of water. The resulting suspension was cooled to rt, adjusted to pH 8 by addition of 1 N HCl, filtered, and dried under vacuum, affording 4.15 g (98%) of **20** as a light yellow solid. HPLC indicated that the crude material was 90% pure (normalized peak area, 88% corrected yield, t_R 2.0 min, method A). A 225 mg sample was purified by chromatography (silica, 1:1 ethyl acetate–2-propanol containing 1% triethylamine): mp 275–283 °C; $[\alpha]_{589}$ −42°, $[\alpha]_{365}$ −162° (*c* 1, DMSO); ^{1}H NMR (DMSO-d_6) δ 9.70 (br s, 1H), 7.40 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 6.23 (d, J = 2.4 Hz, 2H), 5.96 (s, 2H), 3.15 (br d, J = 11 Hz, 1H), 2.72 (m, 1H), 2.58 (m, 2H), 2.45 (m, 2H), 1.78 (dd, J = 8.6, 15.1 Hz, 1H), 1.50 (m, 3H); IR (KBr) 3400, 3343, 1678, 1642 cm^{-1}; MS (FD) m/z 350, 348. UV (EtOH) λ_{max} (ϵ) 221 (34 500), 278 (13 800) nm. Anal. Calcd for $C_{15}H_{17}BrN_4O$: C, 51.59; H, 4.91; N, 16.04. Found: C, 51.37; H, 4.93; N, 16.04.

(*R*)-(−) 4-[2-(2-Amino-1,4,5,6,7,8-hexahydro-4-oxopyrido-[2,3-*d*]pyrimidin-6-yl)ethyl]benzonitrile (21). A mixture of 750 mg (2.15 mmol) of **20** and 365 mg (4.07 mmol) of CuCN in 6.0 mL of 1-methyl-2-pyrrolidinone was heated under reflux (N_2 atmosphere) for 4 h. The reaction was cooled and concentrated *in vacuo*. To the resulting slurry was added 6 mL of 6 M HCl and the mixture stirred for 10 min. The product was collected by filtration, washed with methanol followed by ether and dried to provide 613 mg (96%) of **21** as a lightly colored solid. HPLC analysis (method A) showed the material to be 95% pure (normalized peak area). Material thus obtained (91% corrected crude yield) was taken to the next step without further purification.[33] An analytical sample was prepared by chromatography (silica, CH_2Cl_2–MeOH 9:1): mp 310–315 °C dec; $[\alpha]_{589}$ −42°, $[\alpha]_{365}$ −161° (*c* 1, DMSO); ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 7.69 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 6.20 (d, J = 1.2 Hz, 1H), 5.86 (s, 2H), 3.13 (dd, J = 1.2, 10.8 Hz, 1H), 2.71 (m, 3H), 2.41 (m, 1H), 1.77 (dd, J = 7.4, 14.9 Hz, 1H), 1.52 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 161.4, 158.5, 153.2, 148.4, 132.1, 131.8, 129.3, 119.0, 108.5, 45.1, 34.2, 32.6, 30.8, 25.9; HRMS (FAB) calcd for $C_{16}H_{18}N_5O$ m/z 296.1511 (MH^+). Found: 296.1515.[34]

(*R*)-(−)-4-[2-(2-Amino-1,4,5,6,7,8-hexahydro-4-oxopyrido-[2,3-*d*]pyrimidin-6-yl)ethyl]benzoic Acid (22). A mixture of 100 mg (0.339 mmol) of **21** and 10 mL of 2 N aqueous KOH was heated at 90 °C for 3 h and cooled, and the pH was adjusted to 3.4 by addition of 1 M HCl. The resulting suspension was filtered and the crude product was reslurried in 10 mL of water at 95 °C, for 1 h (to remove residual NaCl), cooled, filtered, and dried, affording 86.4 mg (81%) of (*S*)-(−)-**22** as an amorphous solid: $[\alpha]_{589}$ −52.6° (*c* 1, 1N NaOH); ^{1}H NMR (DMSO-d_6) δ 12.7 (br s 1H), 10.1 (br s, 1H), 7.31 (d, J = 8.1 Hz, 2H), 6.95 (br s, 1H), 6.50 (br s, 2H), 3.29 (d, J = 12.2 Hz, 1H), 2.85 (dd, J = 12.0, 8.2 Hz, 1H), 2.68 (m, 2H), 2.52 (m, obscured by DMSO absorption, 1H), 1.86 (dd, J = 8.4, 15.2 Hz, 1H), 1.57 (m, 3H), identical with the spectrum of an authentic sample of (*R*)-(−)-**22** prepared by acidic hydrolysis of lometrexol (see below).

Preparation of 22 by Acidic Hydrolysis of Lometrexol.[35] A 500 mg sample of lometrexol (96% de) was suspended in 15 mL of 6 M aqueous HCl and heated under reflux overnight. The flask was cooled to 5 °C and the precipitated product was filtered, washed thoroughly with water and acetone, and dried at 50 °C in a vacuum oven, affording 320 mg (81%) of **22** hydrochloride as an off white powder: mp 312 °C dec; $[\alpha]_{589}$ −48.3°, $[\alpha]_{365}$ −178° (*c* 1, 1 N NaOH). The free base could be obtained by dissolving the salt in dilute NaOH (pH 10–11) and adjusting the pH to 3.6–4.0 by addition of aqueous HCl, filtering the precipitated product; and drying (amorphous solid): mp > 330 °C; $[\alpha]_{589}$ −51° (*c* 1, 1N NaOH); ^{1}H NMR (DMSO-d_6) δ 12.7 (br s, 1H), 10.2 (br s, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 6.95 (br s, 1H), 6.50 (br s, 2H), 3.29 (d, J = 12.2 Hz, 1H), 2.85 (dd, J = 8.2, 12.0 Hz, 1H), 2.68 (m, 2H), 2.52 (d, obscured by DMSO, 1H), 1.86 (dd, J = 8.4, 15.2 Hz, 1H), 1.57 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 25.4, 30.7, 32.5, 34.3, 45.1, 81.9, 128.4, 129.4, 147.5, 153.3, 159.6, 161.6, 167.3; UV (80% aqueous EtOH) λ_{max} (ϵ) 224 (26 300), 279 (13 500) nm. Anal. (hydrochloride) Calcd for $C_{16}H_{19}ClN_4O_3$: C, 54.78; H, 5.46; N, 15.97; Cl, 10.11. Found: C, 54.62; H, 5.58; N, 16.03; Cl, 9.92.

(6*R*)-5,10-Dideazatetrahydrofolic Acid (1). To a suspension of 900 mg (2.56 mmol) of (*R*)-(−)-**22** and 770 mg (7.7 mmol) of *N*-methylmorpholine (NMM) in 13 mL of DMF at room temperature was added 450 mg (2.56 mmol) of 2-chloro-4,6-dimethoxy-1,3,5-triazine.[26] The reaction mixture was stirred for 30 min, and then 610 mg (2.56 mmol) of L-glutamic acid diethyl ester hydrochloride was added. The reaction was stirred for 30 min at room temperature and then filtered and concentrated under vacuum. The material thus obtained was triturated with saturated $NaHCO_3$ and then water, and dried under reduced pressure, affording 1.06 g (83%) of diester **23**. A 206 mg portion of the **23** thus obtained was saponified by dissolving in 2.0 mL of 1 N NaOH. The resulting diacid **1** was precipitated from the solution by acidification with 1 N HCl to pH 3.6. The product was isolated by filtration and air-dried to afford 173 mg (78% from **22**) of **1**, identical by ^{1}H NMR and HPLC with an authentic sample of lometrexol prepared by the camphorsulfonic acid salt method of Shih.[6b,c] The ratio of 6*R* to 6*S* diastereomers was determined to be 98:2 by β-cyclodextrin inclusion HPLC analysis.[27a] The 6*R* isomer containing a D-glutamate residue could be separated from the other two diastereomers by the cyclodextrin-based method. The amount of D-glutamate derived lometrexol in isolated **1** was estimated to be <1%.

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Supplementary Material Available: X-ray crystal structure for compound **8** and ^{1}H NMR spectrum of compound **21** (2 pages). This material is contained in 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.

(33) Chromatography of **21** on silica gel in several solvent systems provided only marginal purification (poor elemental analysis) while approximately half of the sample failed to elute from the column. It proved advantageous to postpone purification until after hydrolysis of the cyano group.

(34) Repeated attempts to obtain a satisfactory elemental analysis on compound **21** were unsuccessful. The material was amorphous.

(35) Shih, C., Lilly Research Laboratories, unpublished procedure. The antitumor activity of **22** has been previously reported (ref 6a). Since the details of the preparation and characterization of **22** were not disclosed in that reference, they are provided herein with Dr. Shih's permission.