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ASYMMETRIC SYNTHESIS AND ABSOLUTE CONFIGURATION OF 5,10-DIDEAZA-5,6,7,8-TETRAHYDROPTEROIC ACID AND 5,10-DIDEAZA-5,6,7,8-TETRAHYDROFOLIC ACID (DDATHF).

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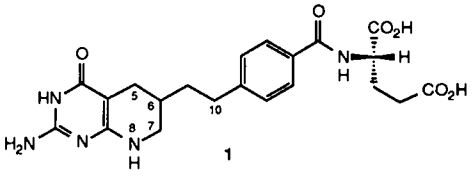
Summary: Lipase-catalyzed enantioselective esterification of 2-substituted 1,3-diols has been utilized in the asymmetric synthesis and consequent configurational assignments of the title compounds.

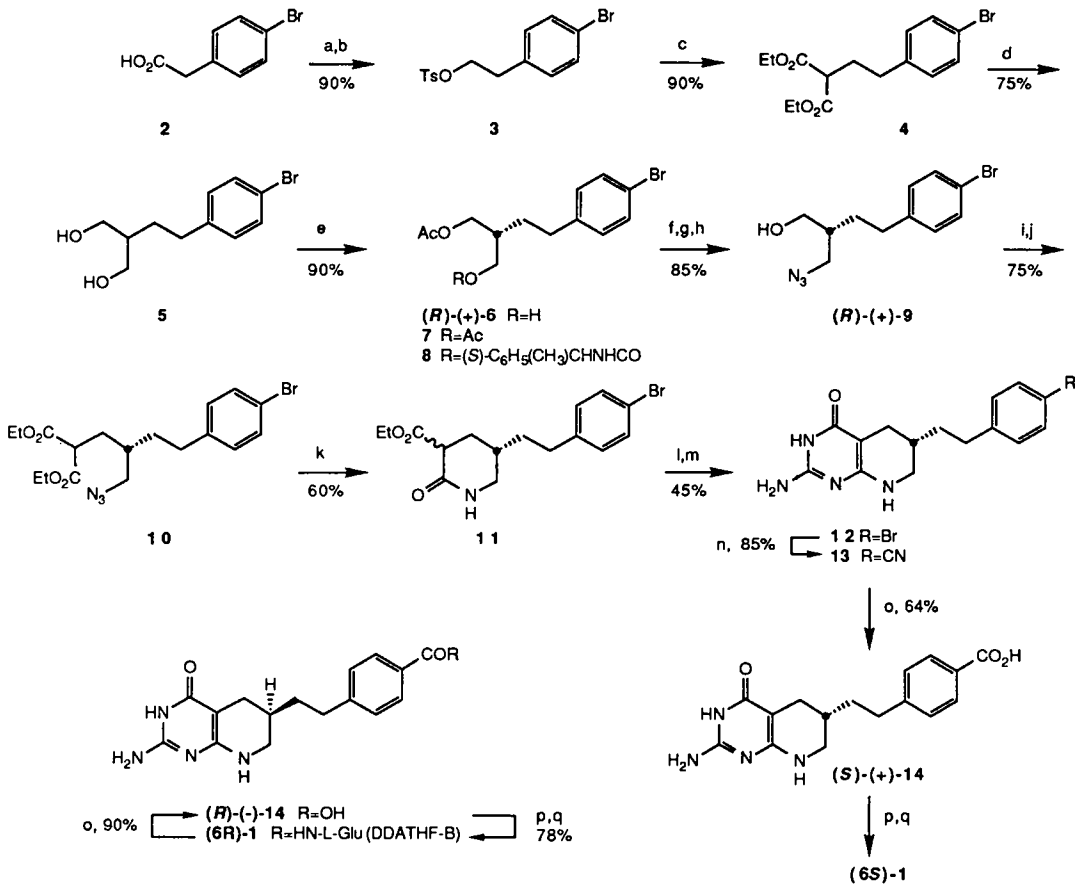
The deaza analogs of tetrahydrofolic acid represent an important class of folate antimetabolites of interest as potential oncolytic agents.[1] Within this series, 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, **1**) has shown exceptionally high activity in a variety of animal models of malignancy.[2] Published syntheses of DDATHF[3,4] have provided mixtures of C-6 epimers arising from nonselective reduction of 5,10-dideazafolate precursors. The epimers have been separated by HPLC and by fractional crystallization of the (+)-camphorsulfonates of the corresponding DDATHF diethyl esters,[4] and the separated diastereomers (arbitrarily designated DDATHF-A and B) have been evaluated separately.[5] DDATHF-B (LY264618) was selected for clinical evaluation and is currently undergoing phase I trials. We wish to report the asymmetric synthesis of DDATHF (**1**) and assignment of absolute configuration to the A and B isomers and the related 5,10-dideaza-5,6,7,8-tetrahydropteroic acids (**14**).



1

Our synthetic plan was designed to utilize enzymatic enantiodifferentiation of prochiral 1,3-diol **5** (Scheme I) as a means of establishing the configuration of C-6 in the target structure. Thus reduction of 4-bromophenylacetic acid (**2**) and tosylation of the resulting alcohol provided **3** which was converted to the diol **5** by reaction with sodium diethyl malonate and reduction of the resulting diester **4**[6] (Scheme I). Reaction of **5** with methyl acetate in the presence of porcine pancreatic lipase (PPL, Sigma type II, no. 3126) immobilized on Hy-Flo[7] afforded a 90% yield of the monoacetate (+)-**6**, $[\alpha]_{589}$ +8.52° (c 0.8, $CHCl_3$), 85% ee,[8] and 10% of the corresponding diacetate (**7**) which could be conveniently removed chromatographically.

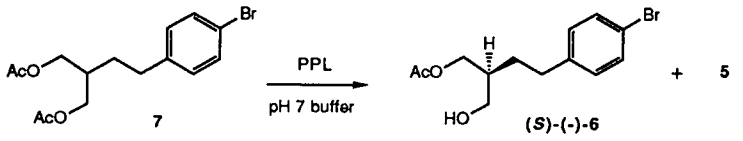
Scheme I.



(a) BH_3, THF; (b)TsCl, Et_3N; (c) NaH, diethyl malonate; (d) $LiAlH_4$, Et_2O; (e) PPL, MeOAc; (f) MsCl, Et_3N; (g) NaN_3, DMF; (h) HCl, MeOH; (i) TsCl, Et_3N; (j) NaH, diethyl malonate, NaI; (k) Bu_3P, THF; (l) Me_3OBF_4; (m) guanidine; (n) CuCN, NMP, reflux; (o) 6M HCl, reflux;

The configuration of (+)-**6** was shown to be *R* by acylation with (*S*)-(-)-α-methylbenzyl isocyanate and single crystal X-ray analysis[9] of the resulting carbamate derivative **8**. Sequential mesylation of (*R*)-(+)-**6**, treatment with sodium azide, and hydrolysis provided azidoalcohol (*R*)-(+)-**9**, $[\alpha]_{589}$ +0.75° (c 10.2, $CHCl_3$), ee 85%,[10] which was converted to **10**, $[\alpha]_{589}$ +1.60° (c 3.8, $CHCl_3$) by tosylation and subsequent reaction with sodium diethyl malonate in the presence of sodium iodide. Reduction of **10** (tributylphosphine) afforded **11** which gave **12**, $[\alpha]_{589}$ +34.3° (c 1.7, DMF) upon reaction with the Meerwein reagent and exposure of the resulting lactim ether to guanidine.[11] Reaction of **12** with cuprous cyanide (N-methylpyrrolidinone, reflux, 6h) provided the nitrile **13**. Acidic hydrolysis (6M HCl, reflux, 70h) of **13** afforded (*S*)-(+)-5,10-dideaza-5,6,7,8-tetrahydropteroic acid (**14**) as an amorphous hydrochloride, $[\alpha]_{589}$ +40.7° (c 1.0, 1N NaOH). Acidic hydrolysis of an authentic sample of DDATHF-B[4] (98% de) in refluxing 6M HCl,[12] on the other hand, provided (-)-**14** hydrochloride, $[\alpha]_{589}$ -48.3° (c 1.0, 1N NaOH), *thus establishing that* (-)-**14** *and the B isomer of DDATHF* (**1**) *possess the (R) configuration at C-6*. It follows that DDATHF-B is topologically analogous to natural (6*S*)-tetrahydrofolic acid.[13]

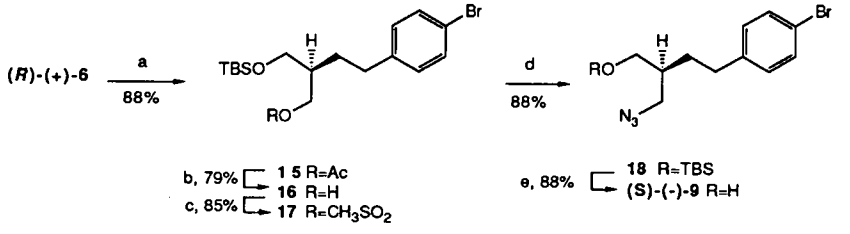
The synthesis (Scheme I) could be modified, in principle, to provide (6*R*)-**14**, precursor to the more clinically interesting (6*R*)-DDATHF, by operating the stereodifferentiating lipase-catalyzed process as a diacetate hydrolysis instead of transesterification, thereby providing enantiomeric monoacetate (*S*)-(-)-**6** from prochiral **7**.



In this case, however, the hydrolysis mode of the lipase reaction exhibited considerably less chemoselectivity than the transesterification mode. Exposure of diacetate **7** to purified PPL in pH 7 buffer[7] gave a mixture of approximately equal amounts of (-)-**6** and **5** (complete conversion of **7**). The ee of the monoacetate fraction remained essentially constant at 80% over the course of the reaction.[14]

Alternatively, the enantiomeric azidoalcohol (*S*)-(-)-**9** could be obtained from (*R*)-**6** by a modification of the synthesis shown in scheme II.

Scheme II.



(a) *t*-BuMe$_2$SiCl, imidazole, CH_2Cl_2; (b) NaOH (aq), 25°C; (c) MsCl, Et_3N, CH_2Cl_2; (d) NaN_3, DMF; (e) HOAc, THF-H_2O

Silylation of (*R*)-(+)-**6** (90% ee) with *t*-butyldimethylsilyl chloride provided **15** which upon saponification with sodium hydroxide afforded **16**, $[\alpha]_{589}$ -5.6° (c 0.8, $CHCl_3$). Conversion of **16** to the corresponding mesylate (**17**) and treatment with sodium azide in DMF provided **18** $[\alpha]_{589}$ -2.98° (c 0.8, $CHCl_3$). Removal of the TBS group under acidic conditions completed the synthesis of (*S*)-(-)-**9**, $[\alpha]_{589}$ -0.75° (c 0.8, $CHCl_3$) in 52% overall yield from (*R*)-**6** *without loss of enantiomeric purity* as determined by HPLC[10] analysis. We have prepared (*R*)-(-)-**14**, required for the synthesis of (6*R*)-DDATHF (**1**), by utilizing (*S*)-(-)-**9** in the pteroic acid synthesis described in scheme I.

The synthesis of (6*R*) and (6*S*)-DDATHF was completed by coupling of the dideazatetrahydropteroic acids **14** with diethyl-L-glutamate and alkaline hydrolysis of the resulting ester products (Scheme I). Chlorodimethoxytriazine[15] proved to be an exceptionally efficient and selective reagent for the coupling process. Protection of the 2-amino group of **14** was unnecessary for successful coupling. Exposure of (*R*) or (*S*)-**14** to the triazine reagent in the presence of N-methylmorpholine in DMF and reaction of the resulting active ester with the glutamate ester, followed by saponification (aqueous NaOH) and neutralization provided the corresponding DDATHF (**1**) diastereomer in 78% overall yield. Samples of (6*R*) and (6*S*)-DDATHF thus obtained were found to be identical with authentic DDATHF-B (LY264618) and -A (LY243246), respectively, by NMR and by β-cyclodextrin inclusion chromatography.[16] The chromatographic results also established that no racemization had taken place during the coupling step.

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References and Notes

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8) Enantiomeric purity was determined by HPLC of the 1-naphthyl carbamates of the alcohols on a 25 cm x 0.46 cm i.d. Chiralcel OD column (J. T. Baker Co.); hexane, ethanol, n-propanol 2:2:1; flow rate 1.0 mL/min; detection at 280 nm: (+)-**6**, 8.5 min; (-)-**6**, 11.4 min. Kennedy, J., Lilly Research Laboratories, unpublished procedure. The enantiomeric purity of **6** obtained from runs with several lots of immobilized enzyme prepared from the same lot of lipase have varied from 80 to 98%. This problem is being investigated.

9) The configuration of the stereogenic carbon corresponding to the 2-position of **6** was deduced by relative comparison with the known center in the carbamate substituent of **8**. The X-ray analysis was performed by Mr. J. Deeter and Dr. N. Jones, Lilly Research Laboratories.

10) Enantiomeric purity was determined by HPLC of the naphthyl carbamate derivatives under conditions as described in note 8. Retention times for naphthyl carbamates: (+)-**9**, 15.5 min; (-)-**9**, 10.8 min.

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12) Shih, C., Lilly Research Laboratories, Eli Lilly and Company, unpublished procedure.

13) The absolute configuration of tetrahydrofolic acid has been determined. Fontecilla-Camps, J. C.; Bugg, C. E.; Temple, C.; Rose, J. D.; Montgomery, J. A.; Kisliuk, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 6114.

14) We are indebted to Dr. S. Wendel, Lilly Research Laboratories, for performing this experiment.

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