Synthesis of 10-(Hydroxymethyl)-5,10-dideaza-5,6,7,8-tetrahydrofolic Acid, a Potent New Analogue of DDATHF (Lometrexol)

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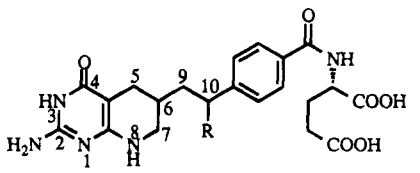
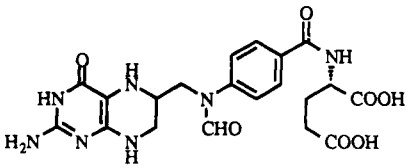
Received April 26, 1994®

A synthesis of 10-(hydroxymethyl)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid [as a mixture of four diastereomers] is described. Substantial cytotoxicity was observed for this new analogue of DDATHF (Lometrexol).

5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, Lometrexol, **1**) is the prototype of a novel series of folate antimetabolites[1] which, as an inhibitor of de novo purine biosynthesis, exhibits potent antitumor activity[2–7] and is currently in phase II clinical trials.[8–11] The synthesis and relationships of structure to biological activity[6,12,13] have been reviewed. Although many structural analogues of DDATHF have now been synthesized (with variations in the pyrimidine ring substituents, the nature and hydrogenation state of ring B, the length, character, and substitution pattern of the aliphatic bridge, changes in the aroyl linking unit, and incorporation of amino acids other than glutamic acid), one striking omission has been the incorporation into the DDATHF structural framework of a functionalized substituent at the folate N-10 (DDATHF C-10) position. It has been well established that DDATHF inhibits the enzyme GAR FTase, which mediates the first formyl transfer process in de novo purine biosynthesis.[6,7,14] Since the natural cofactor for this enzyme is 10-formyl-5,6,7,8-tetrahydrofolic acid (**3**), a potential inhibitor closer in structure to this natural cofactor than DDATHF itself would be an analogue bearing a functionalized substituent at C-10 which might simulate either the formyl substituent itself in the natural cofactor, or in the transition state involved in the formyl transfer process. We describe in this paper the synthesis of the first DDATHF analogue of this type, 10-(hydroxymethyl)-DDATHF (**2**), and comment upon its biological activity.



1, R = H
2, R = CH_2OH



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A key step in the construction of both DDATHF itself and many structural analogues has been a palladium-catalyzed C–C coupling reaction between a 6-halo-substituted 5-deazapterin derivative and an olefinic or acetylenic partner.[12] We have now successfully applied this synthetic strategy to the preparation of **2** (see Scheme 1). Thus, 2-pivaloyl-6-bromo-5-deazapterin (**4**)[15] was condensed with the THP-protected derivative of propargyl alcohol (**5**) using palladium chloride, triethylamine, triphenylphosphine, and cuprous iodide under reflux in acetonitrile to give **6** in 60% yield. Propargyl alcohol itself failed to undergo this coupling reaction using a variety of palladium catalysts, bases, and phosphorus ligands. Reduction of the alkyne **6** to the cis-olefin **7** was accomplished satisfactorily with hydrogen in the presence of palladium on barium sulfate using freshly distilled synthetic quinoline in a mixture of methanol and chloroform as solvent. Both the starting alkyne **6** and the quinoline (used as a catalyst poison) must be pure for this reduction to take place. Curiously, other catalyst poisons normally used for controlled reduction of alkynes to cis-olefins proved to be ineffective.

Although it is known that excellent regioselectivity is observed in palladium-catalyzed coupling of cis-olefins (as contrasted with their trans isomers) with aryl halides,[16] the regiochemistry essential for the synthesis of **2** was

® Abstract published in *Advance ACS Abstracts,* September 15, 1994.

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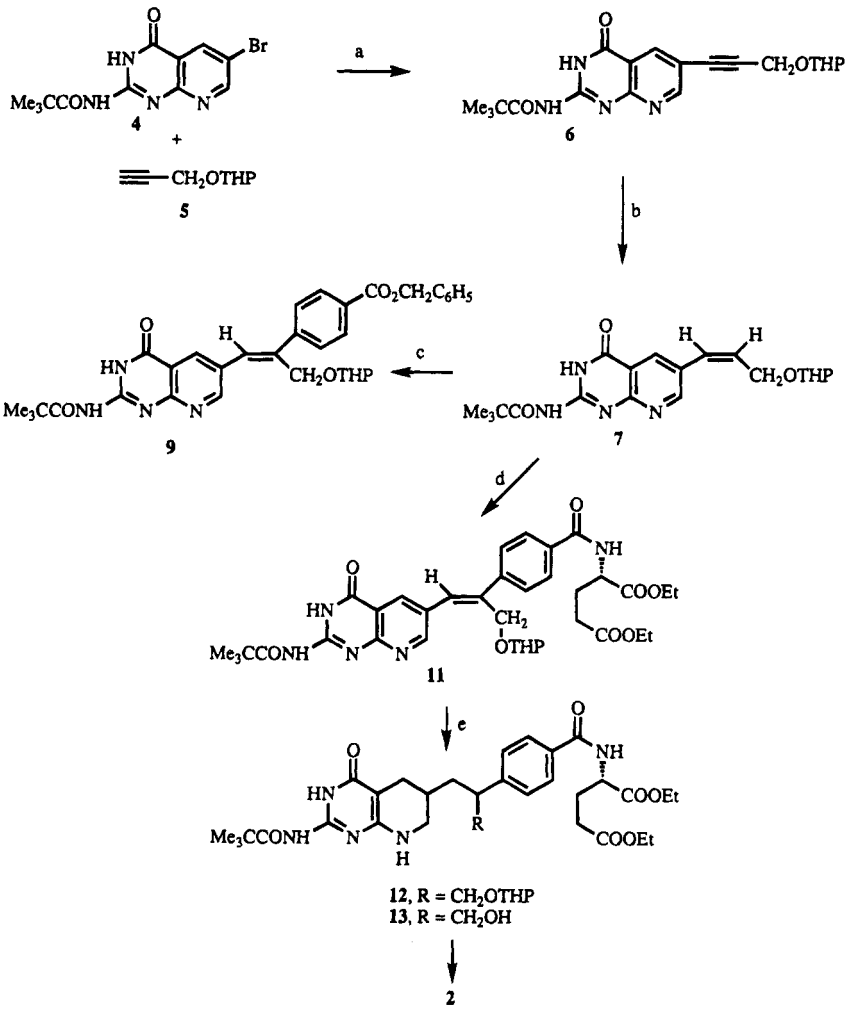
Scheme 1[a]



[a] (a) Pd coupling conditions; (b) H_2, $PdSO_4$, quinoline; (c) Pd coupling conditions, benzyl 4-iodobenzoate (**8**); (d) Pd coupling conditions, diethyl (4-iodobenzoyl)-L-glutamate (**10**); (e) H_2, PtO_2, HOAc.

confirmed with the cis-olefin **7** and benzyl 4-iodobenzoate (**8**). The desired coupling product **9** was indeed obtained uncontaminated by the regioisomer which would have resulted from coupling at the olefinic position α to the heterocyclic nucleus. Thus encouraged, we coupled the cis-olefin **7** with diethyl (4-iodobenzoyl)-L-glutamate (**10**).[17] Examination of the NMR spectrum of the reaction product revealed that the reaction had proceeded as expected, but separation of the coupling product **11** from the starting material **7** proved to be very difficult. Although TLC separation could be effected using 6% methanol in benzene, application of this solvent system to silica gel chromatography or to radial chromatography was not successful. Partial separation could be achieved by silica gel chromatography followed by treatment of the resulting mixture of starting material and coupled product with additional diethyl (4-iodobenzoyl)-L-glutamate and a new charge of catalyst. Further silica gel chromatography of the reaction mixture then yielded a 4:1 mixture of product and cis-olefinic starting material, from which the desired coupled product **11** crystallized in 52% isolated yield.

Catalytic reduction without significant allylic hydrogenolysis was then effected with hydrogen at 50 psi using platinum oxide as catalyst and glacial acetic acid as solvent. Removal of the tetrahydropyranyl protecting group of the reduced product **12** was achieved with 0.1 N methanolic HCl to give **13**. Final saponification and removal of the pivaloyl protecting group then gave the target DDATHF analogue 10-(hydroxymethyl)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid (**2**) as a mixture of four diastereomers (ldd, ldl, lld, and lll).

Biological evaluation of the diastereomeric mixture **2** showed that it was an active cytotoxic agent (IC_{50} = 0.0034 μg/mL; cf. DDATHF IC_{50} = 0.007 μg/mL). In earlier work we had prepared 10-methylDDATHF, again as a mixture of four diastereomers (IC_{50} = 0.0098 μg/mL), and found that all of its cytostatic activity was apparently due to one of the diastereomers (separated after laborious HPLC).[18] This may also prove to be the case with **2**.

Experimental Section

2-(Pivaloylamino)-6-[3-(tetrahydropyranyl-2′-oxy)prop-1-ynyl]-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine (6). A mixture of 14.61 g (45 mmol) of 2-(pivaloylamino)-6-bromo-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine (**4**), 7.6 g (1.2 equiv) of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (**5**), 798 mg (10%) of $PdCl_2$, 2.36 g (20%) of triphenylphosphine, 428 mg (5%) of CuI, and 45 mL of Et_3N in 700 mL of MeCN was heated to reflux under

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nitrogen. After 12 h under reflux, an additional 3.2 g of tetrahydro-2-(2-propynyloxy)-2*H*-pyran was added to the hot reaction mixture, and reflux was continued for an additional 12 h. After 24 h of heating, the solvent was evaporated under reduced pressure and the residue was filtered through silica gel using 2% MeOH in CH_2Cl_2. The filtrate was concentrated and then chromatographed on silica gel using EtOAc/hexanes (20:1) to give 10.94 g (63%) of **6**. An analytical sample was prepared by recrystallization from EtOAc: mp 212–214 °C. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 1.56–1.90 (mn, 6 H), 3.57–3.63 (m, 1 H), 3.87–3.95 (m, 1 H), 4.50 (d, 1 H, J = 16 Hz), 4.57 (d, 1 H, J = 16 Hz), 8.35 (br s, NH), 8.55 (d, 1 H, J = 2 H), 8.90 (d, 1 H, J = 2 Hz), 12.09 (br s, NH).

Anal. Calcd for $C_{20}H_{24}N_4O_4$: C, 62.48; H, 6.29; H, 14.58. Found: C, 62.53; H, 6.46; N, 14.47.

2-(Pivaloylamino)-6-[3-(tetrahydropyranyl-2′-oxy)-*cis*-prop-2-en-1-yl]-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine (7). A mixture of 2.00 g (5.2 mmol) of **6**, 40 mL of MeOH, 20 mL of $HCCl_3$, 40 mg of 5% Pd/$BaSO_4$, and 40 mg of synthetic quinoline was stirred under 1 atm of hydrogen for 40 min. The solvent was then removed by evaporation, and the residue was diluted with CH_2Cl_2 and filtered through silica gel using 2% MeOH in CH_2Cl_2 to remove the catalyst. The filtrate was then concentrated to give a yellow oil which upon addition of ether yielded 1.74 g (87%) of **7** as yellow crystals which were further purified through column chromatography, eluting with ethyl acetate, and recrystallization from EtOAc: mp 166–167 °C. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9 H), 1.54–1.88 (m, 6 H), 3.54 (m, 1 H), 3.89 (m, 1 H), 4.30 (m, 1 H), 4.70 (t, 1 H, J = 3.4 Hz), 6.11 (m, 1 H), 6.62 (d, 1 H, J = 12.1 Hz), 8.35 (br s, NH), 8.37 (d, 1 H, J = 2.5 Hz), 8.79 (d, 1 H, J = 2.5 Hz), 12.7 (br s, NH).

Anal. Calcd for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.50. Found: C, 62.30; H, 6.88; N, 14.74.

Benzyl 4-Iodobenzoate (8). To a solution of 13.3 g (50 mmol) of 4-iodobenzoyl chloride and 10.8 g (0.1 mmol, 2 equiv) of benzyl alcohol in 100 mL of dry CH_2Cl_2 in a round-bottom flask equipped with a condenser in a water bath was added dropwise 10 mL (excess) of Et_3N. After addition, the solution was stirred for 1 h at rt, diluted with 100 mL of CH_2Cl_2, washed with 20 mL of saturated $NaHCO_3$ and water (3 × 30 mL), dried with anhydrous $MgSO_4$, and concentrated to give a yellow solid. Recrystallization from hot hexane gave 15.0 g (89%) of **8**: mp 64–65 °C. ^{1}H NMR ($CDCl_3$) δ 5.35 (s, 2 H), 7.37–7.45 (m, 5 H), 7.79–7.82 (m, 4 H). HRMS calcd for $C_{14}H_{11}IO_2$ 338.9806, found 337.9794.

Benzyl 4-{1-(Tetrahydropyranyl-2′-oxy)-3-(2-(pivaloylamino)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)-prop-2-en-2-yl}benzoate (9). A solution of 150 mg (0.39 mmol) of **8**, 6.9 mg (10%) of $PdCl_2$, 1 mL of Et_3N, and 23 mg (20%) of tri-*o*-tolylphosphine in 30 mL of MeCN was heated under reflux for 3 days under nitrogen. The mixture was then concentrated under reduced pressure and chromatographed on silica gel using EtOAc/hexanes (20:1). The eluate was concentrated to give 70 mg (30%) of **9**: mp 191–193 °C. ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 9H), 1.56–1.83 (m, 6 H), 3.50–3.56 (m, 1 H), 3.78–3.86 (m, 1 H), 4.32 (dd, 1 H, J = 14 Hz, J = 1 Hz), 4.61 (dd, 1 H, J = 14 Hz, J = 1 Hz), 4.75 (t, 1 H, J = 3.1 Hz), 5.35 (s, 2 H), 6.84 (s, 1 H), 7.29 (d, 2 H, J = 8.2 Hz), 7.34–7.46 (m, 5 H), 8.03 (d, 2 H, J = 8.2 Hz), 8.13 (d, 1 H, J = 1.6 Hz), 8.40 (d, 1 H, J = 1.6 Hz), 8.39 (br s, NH), 11.99 (br s, NH). HRMS calcd for $C_{34}H_{36}N_4O_6$ 596.2635, found 596.2648.

Diethyl *N*-[4-{1-(Tetrahydropyranyl-2′-oxy)-3-(2-(pivaloylamino)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)prop-2-en-2-yl}benzoyl]-L-glutamate (11). A mixture containing 3.48 g (9 mmol) of **7**, 3.12 g (1.2 equiv) of diethyl *N*-(4-iodobenzoyl)-L-glutamate (**10**),[17] 546 mg (20%) of tris-*o*-tolylphosphine, 201 mg (10%) of $Pd(OAc)_2$, and 85.5 mg (5%) of CuI in 15 mL of Et_3N and 240 mL of MeCN was heated under reflux under nitrogen. After 12 h an additional 1.17 g of **10** was added, and the mixture was again heated under reflux under nitrogen for 12 h. The reaction mixture was then concentrated under reduced pressure to give a dark brown syrup which was chromatographed on silica gel, eluting with EtOAc/hexanes (20:1), to give a mixture of starting material and product. The recovered mixture of starting material and product was recycled through the foregoing procedure. The concentrated material was dissolved in a solution of EtOAc/Et_2O (1:5) and refrigerated for 15 h. The solid which separated was collected by filtration, washed with cold EtOAc, and dried to give 3.24 g (52%) of **11**: mp 273–275 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3 H, J = 7 Hz), 1.32 (t, 3 H, J = 7 Hz), 1.32 (s, 9 H), 1.53–1.87 (m, 6 H), 2.16 (m, 1 H), 2.32 (m, 1 H), 2.50 (m, 2 H), 3.55 (m, 1 H), 3.84 (m, 1 H), 4.13 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.33 (d, 1 H, J = 14 Hz), 4.62 (d, 1 H, J = 14 Hz), 4.75–4.83 (m, 2 H), 6.84 (s, 1 H), 7.12 (br s, 1 H, NH, J = 7 Hz), 7.31 (d, 2 H, J = 8.1 Hz), 7.79 (d, 2 H, J = 8.1 Hz), 8.13 (d, 1 H, J = 1.6 Hz), 8.34 (br s, NH), 8.42 (d, 1 H, J = 1.6 Hz), 11.99 (br s, NH).

Anal. Calcd for $C_{36}H_{45}N_5O_9$: C, 62.50; H, 6.56; N, 10.12. Found: C, 62.21; H, 6.34; N, 9.83.

Diethyl *N*-[4-{1-(Tetrahydropyranyl-2′-oxy)-3-(2-(pivaloylamino)-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido-[2,3-*d*]pyrimidin-6-yl)prop-2-yl}benzoyl]-L-glutamate (12). A solution of 1.16 g (1.68 mmol) of **11** and 174 mg (20%) of amorphous PtO_2 in 150 mL of glacial acetic acid was stirred for 10 h under 50 psi of hydrogen. The mixture was diluted with 50 mL of methanol and filtered through Celite. The filtrate was concentrated and diluted with EtOAc, and the solid which formed after cooling in the refrigerator overnight was collected by filtration, washed with cold EtOAc, and dried to give 700 mg (68%) of **12**: mp 183–186 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3 H, J = 7 Hz), 1.26 (s, 9 H), 1.32 (t, 3 H = 7 Hz), 2.26–2.82 (m, 9 H), 2.02–2.12 (m, 2 H), 2.26–2.38 (m, 1 H), 2.43–2.58 (m, 2 H), 2.62–2.83 (m, 1 H), 2.84–3.10 (m, 2 H), 3.12–3.21 and 3.32–3.40 (m,m, 1H), 3.41–3.52 (m, 2 H), 3.68–3.92 (m, 2 H), 4.13 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.44–4.63 (m, 2 H, CH, NH), 4.78–4.84 (m, 1 H), 7.02 (br d, 1 H, NH, J = 7.1 Hz), 7.29 (br s, NH), 7.31–7.37 (m, 2 H), 7.75–7.78 (m, 2 H), 11.20 (br s, NH).

Anal. Calcd for $C_{36}H_{51}N_5O_9$: C, 61.95; H, 7.37; N, 10.04. Found: C, 62.00; H, 7.17; N, 9.83.

Diethyl *N*-[4-{1-Hydroxy-3-(2-(pivaloylamino)-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)prop-2-yl}benzoyl]-L-glutamate (13). A solution of 942 mg (1.35 mmol) of **12** in 40 mL of 0.1 N methanolic HCl solution was stirred at rt for 2 h. The reaction mixture was neutralized with a solution of 205 mg of Na_2CO_3 in 10 mL of water. After most of the methanol had been removed by evaporation under reduced pressure, 100 mL of CH_2Cl_2 was added, and the solution was washed twice with 20 mL of water, dried over anhydrous $MgSO_4$, and concentrated to give a white solid which was triturated with EtOAc/Et_2O (1:2), filtered, and dried to give 810 mg (98%) of **13**: mp 133–135 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3 H, J = 7 Hz), 1.26 (s, 9 H), 1.30 (t, 3 H, J = 7 Hz), 1.78–1.87 (m, 3 H), 2.01–2.23 (m, 3 H), 2.23–2.36 (m, 1 H), 2.38–2.54 (m, 2 H), 2.59–2.69 (m, 1 H), 2.83–3.12 (m, 2 H), 3.14–3.18 and 3.28–3.32 (m, m, 1 H), 3.72–3.78 (m, 2 H), 4.12 (q, 2 H, J = 7 Hz), 4.23 (q, 2 H, J = 7 Hz), 4.74–4.84 (m, 2 H, CH, NH), 7.24 (d, NH, J = 7 Hz), 7.26–7.30 (m, 1 H), 7.72–7.76 (m, 1 H), 8.09 (br s, NH), 11.23 (br s, NH).

Anal. Calcd for $C_{31}H_{43}N_5O_8$: C, 60.67; H, 7.06; N, 11.41. Found: C, 60.42; H, 7.09; N, 11.34.

***N*-[4-{1-Hydroxy-3-(2-amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)prop-2-yl}benzoyl]-L-glutamic Acid (10-(Hydroxymethyl)DDATHF) (2).** A solution of 300 mg (0.49 mmol) of **13** in aqueous 1 N sodium hydroxide solution was stirred under nitrogen at rt for 72 h. The reaction mixture was rendered slightly acidic (pH ~4) with 1 N HCl and filtered. The solid thus collected was washed with water (5 mL) and cold EtOH (5 mL) and dried to give 180 mg (78%) of **2**: mp 275 °C dec; ^{1}H NMR (DMSO-d_6, CF_3COOD) δ 1.35–1.42 (m, 1 H), 1.58–1.71 (m, 2 H), 1.73–1.85 (m, 1 H), 1.86–2.01 (m, 1 H), 2.02–2.13 (m, 1 H), 2.31 (t, 2 H, J = 7 Hz), 2.39–2.52 (m, 1 H), 2.74–2.89 (m, 2 H), 3.08–3.14 and 3.24–3.30 (m 1 H), 3.47 (d, 2 H, J = 3.7 Hz), 4.37 (dd, 1 H, J = 19.4 Hz, J = 4.7 Hz), 7.27–7.30 (m, 2 H), 7.78 (d, 2 H, J = 7.7 Hz).

Anal. Calcd for $C_{22}H_{27}N_5O_7.0.5\ H_2O$: C, 54.76; H, 5.80; N, 14.51. Found: C, 54.96; H, 5.88; N, 14.27.

Acknowledgment. This work was supported by a grant to Princeton University from Eli Lilly & Company. We are grateful to the late Dr. Gerald B. Grindey of Lilly for the biological evaluations reported herein.