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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOR- AND HOMO-5,10-DIDEAZATETRAHYDROFOLIC ACID

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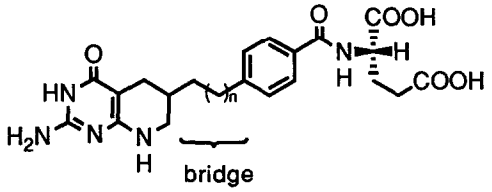
Abstract: A palladium(0)-catalyzed Heck coupling reaction was used in the synthesis of nor- and homo-DDATHF which are close analogs of the potent glycinamide ribonucleotide formyltransferase inhibitor, 5,10-dideazatetrahydrofolic acid (DDATHF).

5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, Lometrexol, **1**) is the lead compound of a novel class of folate antimetabolites that inhibits glycinamide ribonucleotide formyltransferase (GARFT, EC 2.1.2.1) in the purine de novo biosynthesis.[1a-1e] DDATHF has demonstrated potent in vitro as well as in vivo antitumor activities[1f,1g] and is currently in phase I and II clinical trials. As part of ongoing structure-activity relationship studies, we were interested in exploring the impact of the length of the "bridge region" on the biological activities of the DDATHF molecule. Herein we describe the synthesis of one-carbon bridge (nor-DDATHF, **2**) and three-carbon bridge (homo-DDATHF, **3**)[1h] analogs of DDATHF by using our previously developed palladium(0)-catalyzed coupling procedure as the key synthetic operation.[1i]

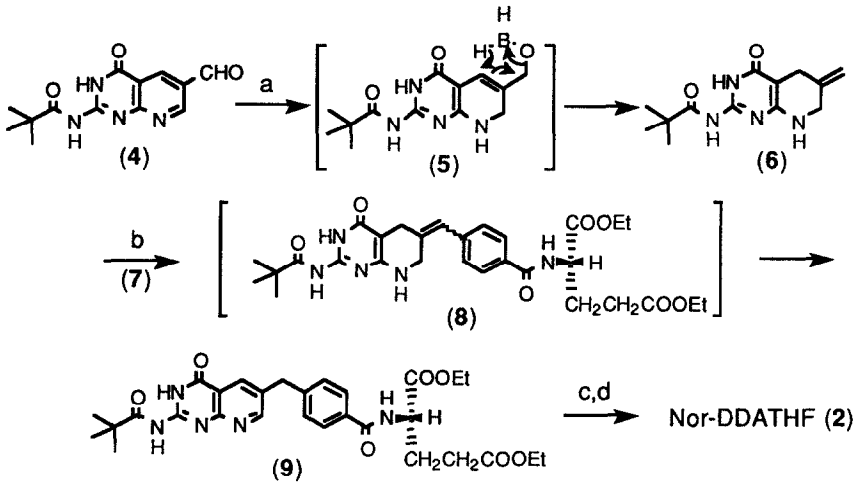


(**1**) DDATHF (n=1)
(**2**) Nor-DDATHF (n=0)
(**3**) Homo-DDATHF (n=2)

The novel exocyclic olefin compound (**6**) was a key intermediate in the preparation of nor-DDATHF (Scheme 1). This unique intermediate was generated in one step (50% isolated yield) by treatment of 5-deaza-6-formyl-2-pivaloylpterin (**4**)[2] with sodium cyanoborohydride (3 eq) in 1:1 acetic acid

ethanol (0°C, 3 h). This exocyclic olefin may be formed through a reductive elimination process involving [3,3]-sigmatropic rearrangement of the presumed alkoxyborohydride intermediate (**5**). Further detailed mechanistic study of this transformation is currently in progress.

SCHEME 1



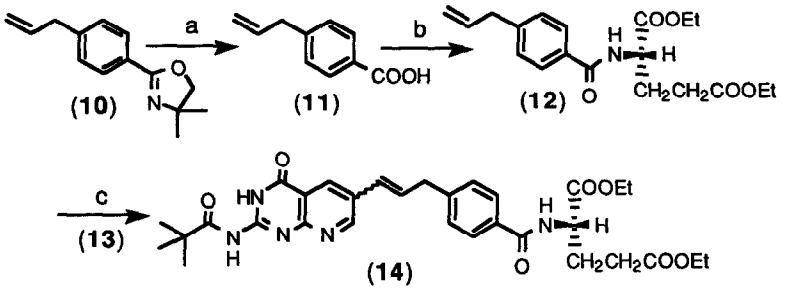
(a) $NaCNBH_3$; (b) $Pd(OAc)_2$, PPh_3, CuI, Et_3N; (c) H_2, 5%Pd/C; (d) 0.05 N NaOH.

A palladium(0)-mediated coupling of (**6**) with diethyl 4-iodobenzoyl-l-glutamate (1.0 eq, **7**)[1i] in the presence of $Pd(OAc)_2$ (0.07 eq), PPh_3 (0.14 eq), CuI (0.07 eq) and triethylamine (Et_3N, 5.0 eq) in refluxing acetonitrile for 36 h furnished, in 35% chromatographed yield, the fully aromatized product (**9**) which probably derives from a further oxidation/aromatization of the initial Heck coupling product (**8**). Standard chemistry developed in these laboratories for hydrogenation (5% Pd-C, acetic acid) and deprotection (0.05N aq NaOH) afforded nor-DDATHF (**2**) in 85% yield for these two steps.

The oxazoline (**10**) reported by Meyers et. al.[3] was used as a convenient starting material for the synthesis of homo-DDATHF (**3**). Acid hydrolysis (5N HCl, reflux, 36 h) of (**10**) provided 4-allylbenzoic acid (**11**) in 74% yield. Compound (**11**) was then converted into the corresponding diethyl-l-glutamate derivative (**12**) (70%) by initial treatment with thionyl chloride (4.0 eq, benzene,

reflux, 4 h) followed by diethyl-l-glutamate hydrochloride (2.1 eq), 4-dimethylaminopyridine (0.01 eq) and Et_3N (18 h, 23 °C).

SCHEME 2



(a) 5N HCl; (b) $SOCl_2$, diethyl-l-glutamate, DMAP, Et_3N;
(c) $Pd(OAc)_2$, $(o\text{-tolyl})_3P$, CuI, Et_3N.

Compound (**12**) was coupled readily with the previously reported 6-bromo-5-deaza-2-pivaloylpterin (1.0 eq, **13**)[1i,2] in the presence of $(o\text{-tolyl})_3P$ (0.01 eq; triphenylphosphine failed to give any desired coupling product in this case), $Pd(OAc)_2$ (0.005 eq), CuI (0.01 eq) and Et_3N (5.0 eq) in acetonitrile. This gave (**14**) (46%) after flash column chromatography on silica gel. Hydrogenation (PtO_2, trifluoroacetic acid) followed by saponification (1.0N NaOH) afforded homo-DDATHF (**3**) (Scheme 2).

Table 1. Comparison of Enzyme Inhibition (GAR FT)† and Cellular Cytotoxicity (CCRF-CEM)#

	DDATHF	Nor-DDATHF	Homo-DDATHF
GAR FT (K_i, μM)	1.2×10^{-7}	6.3×10^{-7}	1.9×10^{-8}
CCRF-CEM (IC_{50}, μg/mL)	0.007	0.026	0.008

† isolated from L1210 murine leukemic cells. For detailed enzyme inhibition and kinetic studies of DDATHF and its analogs, including (**2**) and (**3**), see reference 1e. # 72 hours assay.

The enzyme inhibition and cellular cytotoxicity data for compounds (**2**), (**3**) and DDATHF (**1**) are shown in Table 1. The one-carbon bridge analog, nor-DDATHF (**2**), is ca. 4-5 fold less potent than DDATHF both in the enzyme inhibition (GARFT) and in the whole cell cytotoxicity (CCRF-CEM human leukemic cells) assays. The three-carbon bridge analog, homo-DDATHF (**3**), is

ca. 6 fold more potent as an inhibitor of GARFT than DDATHF. However, homo-DDATHF (**3**) turned out to be only equally cytotoxic as DDATHF in the whole cell assay; this decrease in cytotoxicity may be attributed to factors such as a decrease in membrane transport and/or insufficient intracellular polyglutamation. Membrane transport and intracellular polyglutamation are known to play important roles in eliciting the biological activity of antifolate compounds.[4] Further investigation of these DDATHF analogs both in vitro and in vivo is currently in progress.

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(5) All new synthetic intermediates were fully characterized by spectroscopic means including UV, IR, ^{1}H NMR, and HRMS. We thank J. W. Paschal of Lilly Research Laboratories for the high field NMR studies on intermediate (**6**).