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Novel Synthesis of a Conformationally-Constrained Analog of DDATHF

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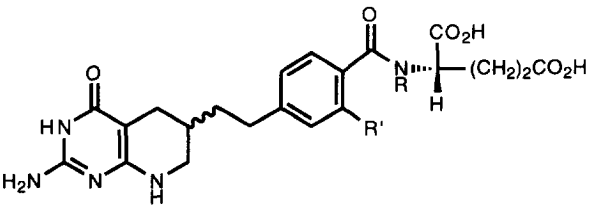
Abstract: *A conformationally-constrained analog of DDATHF, in which the glutamate moiety is tied back to the benzoyl ring through an isoindolinone ring, has been synthesized through a series of steps which commence with a Diels-Alder reaction of the Danishefsky diene with 4,4-diethoxybut-2-ynal.* © 1997, Elsevier Science Ltd. All rights reserved.

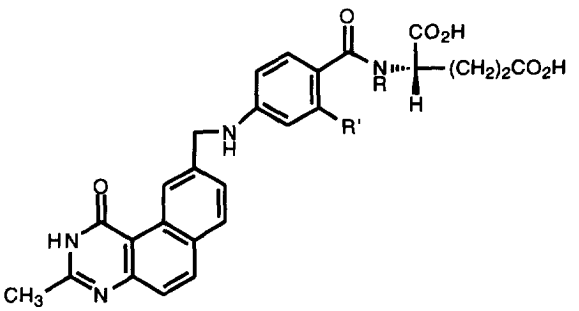
The concept of utilizing conformationally-constrained amino acids to explore active site binding parameters and other phenomena related to the geometry of substrate-enzyme interactions is now widely recognized and practiced.[1] During an extensive SAR study of derivatives of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, 6(RS)-**1a**)[2] as antitumor agents, we had prepared the 2'-fluoro derivative **1b** and had observed a two-fold increase in the activity of this compound as compared with **1a**.[3] A similar enhancement of cytotoxicity upon introduction of a 2'-fluoro substituent had earlier been observed by Burroughs-Wellcome scientists for the 2'-fluorobenzoquinazoline thymidylate synthetase inhibitor **2b** as



1a, R = R' = H
1b, R = H, R' = F
1c, R-R' = CH_2

compared with the 2'-H parent **2a**.[4] This enhancement in activity was considered to be a consequence of in-plane conformational constraint of the benzoylglutamate moiety as a result of NH-F bonding, which was simulated by the very active isoindolinone derivative **2c** (BW1843U89). We describe in this paper a novel

synthesis of the analogous conformationally-constrained DDATHF derivative **1c**, and comment on its biological activity.



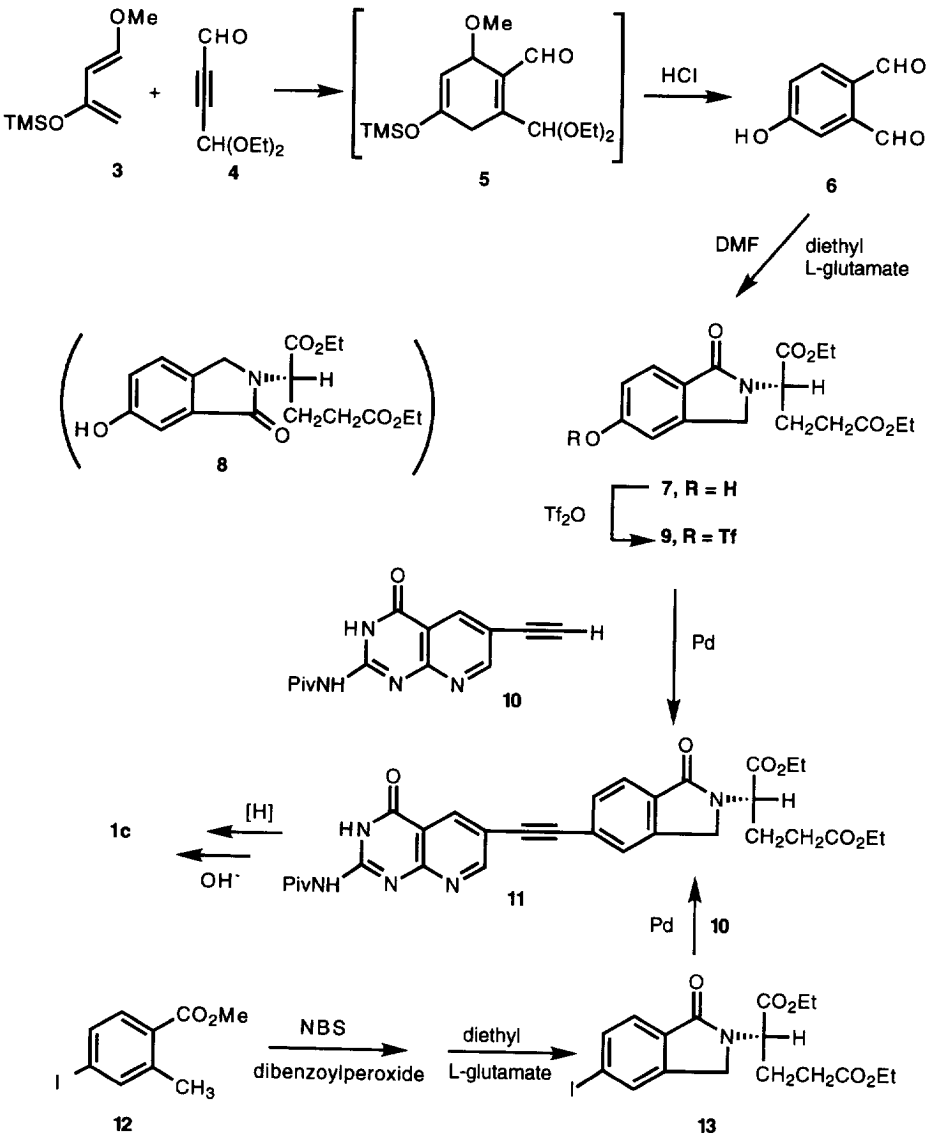
2a R = R' = H
2b, R = H, R' = F
2c, R-R' = CH_2

A Diels-Alder reaction of commercially available Danishefsky diene **3**[5] with 4,4-diethoxybut-2-ynal[6] (**4**) resulted in the formation of the cyclohexadiene **5**. Without purification, treatment of **5** with 1N HCl led to spontaneous aromatization to give 4-hydroxyphthalaldehyde (**6**) (Scheme 1). This *o*-dialdehyde, again without purification, was subjected to an intramolecular Cannizzaro reaction[7] by stirring with diethyl L-glutamate in DMF at room temperature to give the isoindolinone **7**, together with a lesser amount of the isomeric 6-isoindolinone derivative **8**. The overall yield of **7** for the above three steps was 30%; for **8** the overall yield was 13%.

Treatment of **7** with triflic anhydride and collidine in methylene chloride solution afforded the triflate **9** in 85% yield. Subsequent palladium-catalyzed coupling of **9** with the known alkyne **10**[8] gave **11** in 69% yield. The structure of **11** was confirmed by an independent, unequivocal synthesis from methyl 4-iodo-2-methylbenzoate (**12**). Thus, free-radical bromination of **12** using NBS and dibenzoylperoxide, followed by addition of diethyl L-glutamate in the presence of K_2CO_3,[9] gave the 5-iodoisoindolinone **13**, which provided **11** when subjected to a palladium-catalyzed C-C coupling reaction with **10**. Hydrogenation of **11** and final hydrolysis of the ethane-bridged intermediate **14** then afforded the conformationally-constrained DDATHF analog **1c** in 50% yield.

Preliminary biological evaluation of **1c** revealed that it was an excellent inhibitor of human CCRF-CEM lymphoblastic leukemic cells (0.014 μg/mL; *cf* **1a**, 0.007 μg/mL; **1b**, 0.004 μg/mL), and a non-competitive inhibitor of mammalian glycinamide ribonucleotide formyltransferase (K_i 0.227 μM: *cf* **1a**, 0.126 μM; **1b**, 0.529 μM - both are competitive inhibitors). A full account of the synthesis and evaluation of further

conformationally-constrained glutamate analogs of the antitumor agents DDATHF, LY231514[10] and LY309887[11] will be published separately.



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