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A New and Facile Synthetic Route to N^5-Formyl Tetrahydropteroylpoly-L-Glutamates[1]

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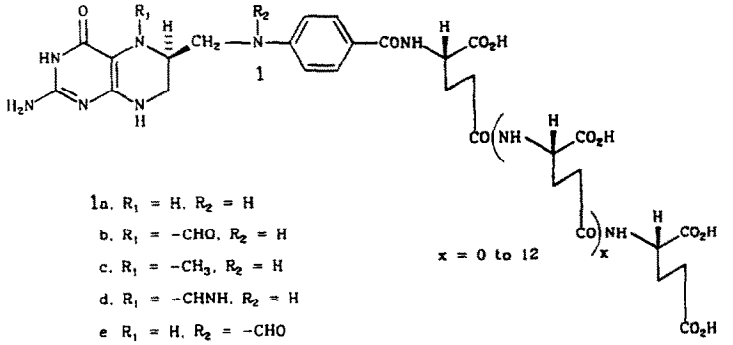
Abstract: A simple, wholly chemical method has been found for preparing N^5-formyl tetrahydropteroylpoly-L-glutamates. The method involves: (1) forming an α-monoester of N^5-formyl tetrahydropteroylmono-L-glutamic acid, (2) coupling its "free" γ-carboxyl end with an L-glutamate polyester (3) deprotection to give the desired product.

Tetrahydropteroylpoly-L-glutamates (**1a-g**) are important coenzymatic forms of the vitamin folic acid[2]. **1a-g** act in vivo as one-carbon unit donors during

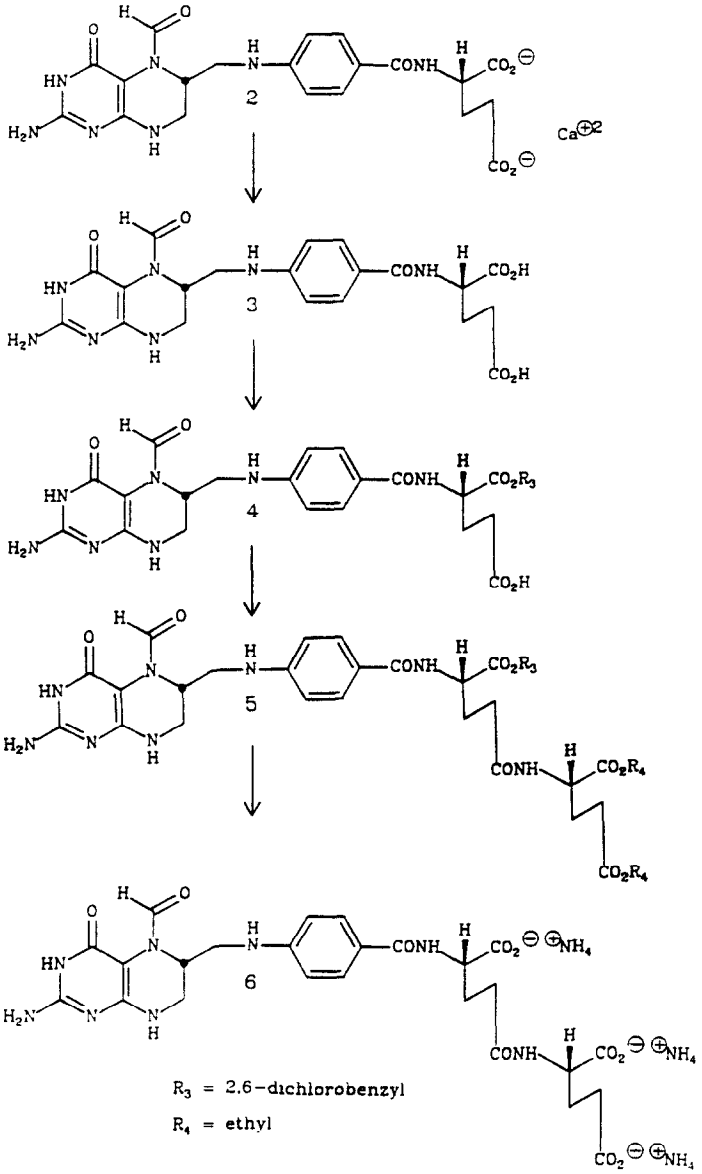


1a. R_1 = H, R_2 = H
b. R_1 = -CHO, R_2 = H
c. R_1 = $-CH_3$, R_2 = H
d. R_1 = -CHNH, R_2 = H
e R_1 = H, R_2 = -CHO
f. R_1 + R_2 = $-CH_2-$
g. R_1 + R_2 = $-\overset{\oplus}{C}H-$

the biosynthesis of certain purines, pyrimidines and amino acids. Several factors have limited the routine use of these derivatives in biological studies. Of primary importance is the extremely unstable nature of most **1a-g** derivatives to O_2[3]. This renders them difficult to manipulate synthetically or store for long periods of time. In addition, current synthetic routes (see, e.g., [4,5]) to **1a-g** utilize the cumbersome and scale-limiting procedure of biochemical reduction with dihydrofolate reductase (EC 1.5.1.3). This results in the least O_2-stable derivative **1a** being produced. Accordingly, in order to prepare any of the **1a-g** derivatives in milligram or larger amounts, a simple method based on low-cost chemical steps was needed. We describe here our preliminary work on a new and facile synthetic route to N^5-formyl tetrahydropteroylpoly-L-glutamates.

A potential solution was first suggested in two papers by Nefkens et al.[6,7]

who noted that because a pKa difference of ~2 units exists between the α and γ-carboxyl groups of L-glutamic acid, the introduction of one equivalent of base should preferentially ionize the α-carboxyl proton with ~100-fold greater ease than the γ-carboxyl proton. Nefkens demonstrated that in the presence of an alkyl or aryl halide, an α-monoester of an N-substituted L-glutamic acid derivative could be formed in large excess over a γ-monoester.



We recognized that an excellent starting point for applying Nefkens observation to the problem of synthesizing **1a-g** derivatives would be **2**. This is due to the following factors: (i) **2** is the most O_2-stable tetrahydropteroyl derivative and therefore amenable to synthetic manipulation; (ii) a fractional crystallization method has been developed to obtain the pure (6R) and (6S) diastereomers of **2** in gram quantities from a chemically derived (6R,6S) 50:50 mixture[8]; (iii) there are many well known methods for converting N^5-formyl tetrahydropteroyl derivatives to **1c**, **e**, **f** and **g**[3]. In addition, a review of the esterification procedure of Rosowsky and Yu[9] for the preparation of the α,γ-2,6-dichlorobenzyl diester of N^5-formyl tetrahydropteroylmono-L-glutamate (which they obtained in 67% yield) indicated that **3** could indeed be modified as per Nefkens to give **4**. This was accomplished by adding 1.00 equivalent of Na_2CO_3 (56 mg) to (6R,6S)-**3** (500 mg) in DMSO. The result after 24 hr was the formation of the α-2,6-dichlorobenzyl monoester of N^5-formyl tetrahydropteroylmono-L-glutamic acid (**4**) in an ~8:1 excess over the γ-2,6-dichlorobenzyl monoester of N^5-formyl tetrahydropteroylmono-L-glutamic acid. Following HPLC purification on C18[10], **4** was isolated in 42% yield[11] (280 mg). The chiral purity of **4** was assessed on a Resolvosil^R column[12]. No evidence of racemization was detected at this step. **4** was then coupled in DMF over a 16 hr period at 25°C through its "free" γ-carboxyl group to the diethyl ester of L-glutamate using DCC/1-hydroxybenzotriazole and an equivalent of 4-methyl morpholine to give **5**[13] (262 mg, 78% yield). Alkaline hydrolysis of **5** over 6 hr with 0.1 N NaOH in 50:50 (v/v) 1,4-dioxane/H_2O solution followed by neutralization with acetic acid and chromatographic purification on cellulose gave the known di-γ-L-glutamate derivative **6** (184 mg, 88% yield) as a triammonium salt[14] in 29% overall yield. The chiral purity of the di-γ-L-glutamate "tail" of **6** was ascertained by carboxypeptidase G_1 (EC 3.4.22.12) analysis[15]. This test determined that the "tail" consisted of ~94% L-glutamate residues. The small loss of chirality is attributed to the use of alkaline hydrolysis as the method of deprotection. In future syntheses, this should all but be eliminated by utilizing ester substituents which are removable by hydrogenolysis or mild acid treatment.

The methodology outlined in this report represents a new, scalable route to N^5-formyl tetrahydropteroylpoly-L-glutamic acid derivatives. Studies aimed at applying this method to the biologically active (6S) derivative of **2** are currently in progress.

References and Notes

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10. The reaction mixture was concentrated in vacuo and the resulting residue flash chromatographed over C8 (80:20 (v/v) 0.1 M aqueous acetic acid/acetonitrile). The product containing fractions (50 mL each) were combined and dried in vacuo to yield 325 mg of an approximately 8:1 ratio of the α/γ-2,6-dichlorobenzyl monoesters. The two isomers were resolved by high pressure liquid chromatography on a C18 column (70:30 (v/v) 0.1 M aqueous acetic acid/acetonitrile) and dried in vacuo to yield 280 mg (42%) of the pure 4.
11. Analytical data confirmed the structural designation given for 4. mp >300°C; IR (KBr) wavenumber 3345, 1730, 1620, 1325, 1188, 770; ^{1}H 500 MHz NMR (Me_2SO-d_6) delta, in ppm relative to TMS 1.97 (cm,2H), 2.21(cm,2H), 2.80(cm,1H), 3.07(cm,1H), 3.13(dd, J=4.1,12.6 Hz,1H), 3.41(dd,J=5.1,12.7,1H), 4.32(cm,1H), 4.78 (cm,1H), 5.26, 5.32(AB,J=12 Hz,2H), 6.31(x of ABX,1H), 6.57(BB' of AA' BB',2H), 6.69(bs,1H), 6.97(x of ABX,1H), 7.44(B of A_2B,1H), 7.53(A_2 of A_2B,2H), 7.60(vb,1H), 7.64(AA' of AA' BB',2H), 8.825, 8.832(3.3 Hz, aldehyde rotomers,1H), 9.13(bs,1H), 11.3(bs,1H); MS(HRFAB) m/z found 632.1480(M^+), calcd. for $C_{27}H_{27}Cl_2N_7O_7$ 632.1424.
12. 1 mg of 4 was placed in 10 mL of 0.1 N NaOH. After 5 min, the solution was neutralized with glacial acetic acid and analyzed according to Wainer, I.W., Stiffin, R.M., J. Chromatography **1988**, 424, 158.
13. Crude 5 was chromatographed (Chromatotron^R) over a 4 mm silica gel plate (10:1 (v/v) methylene chloride/methanol) and dried in vacuo to yield 262 mg (78%) of 5: mp >300°C; IR (KBr) wavenumber 3325, 1730, 1625, 1335, 1185, 770; ^{1}H 500 MHz NMR delta 1.154, 1.157(two (t), J=7.1 Hz,6H), 1.79(cm,1H), 1.94(cm,1H), 2.05(cm,1H), 2.26(cm,2H), 2.33(cm,2H), 2.87(cm,1H), 3.07(cm,1H), 3.13(cm,1H), 3.41(cm,1H), 4.03, 4.04 (two (q),J=7.1 Hz,4H), 4.21(ddd,J=5.4,7.5,8.8 Hz,1H), 4.4(cm,$\Delta\nu$=25 Hz,1H), 4.8(cm,1H), 5.28,5.33(AB,(J=12.0 Hz),2H), 6.20(bs,2H), 6.36(x of ABX,1H), 6.61(d,J=8.7 Hz,2H), 6.98(x of ABX,1H), 7.45(B of A_2B,1H), 7.53(A_2 of A_2B,2H), 7.66(d,J=8.7 Hz,2H), 8.23(d,J=7.4 Hz,1H), 8.34(d,J=7.3,1H), 8.45(s,1H), 10.22(s,1H); MS (HRFAB) m/z found 816.2471, calcd. for $C_{36}H_{42}Cl_2N_8O_{10}$ 816.2398
14. 6 was chromatographed over cellulose (60:40 (v/v) 0.5 M NH_4HCO_3/EtOH) and dried in vacuo to yield 184 mg (88%) MS(FAB) relative intensity m/z 691(MNa_4^+,23), 669(MNa_3^+,22)
15. Performed as per the method outlined by McCullough, J.L.; Chabner, B.A.; Bertino, J.R.; J. Biol. Chem. **1971**, 246, 7207.