Improved Synthesis of Folate Conjugates

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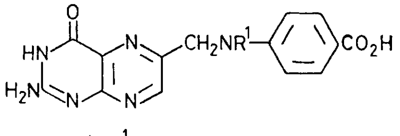
Summary A rapid and improved synthesis, in high yield, of pteroyl-γ-L-glutamyl-γ-L-glutamyl-L-glutamic acid has been realised by the coupling of a minimally protected tripeptide, prepared by salt couplings and carboxy terminal elongation to N^{10}-trifluoroacetylpteroic acid, followed by deprotection.

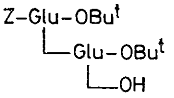
During the last three decades, several groups of workers have reported the synthesis of folate conjugates. All the described routes are prone to either low yields or protracted isolation and purification procedures.[1–4]

We have found that a critical step in the preparation of pure folate conjugates is the rigorous purification of pteroic acid (**1**),[5] following which, reaction with trifluoroacetic anhydride leads to the isolation of the N^{10}-trifluoroacetate (**2**)[2,3] in 81% yield after one recrystallisation from dimethylformamide. When (**2**) is coupled to γ-glutamic acid derivatives, partially and fully protected folate conjugates are isolated in 69—80% yield.‡ Additional purification is effected by a single crystallisation from ethyl acetate or chloroform and light petroleum.



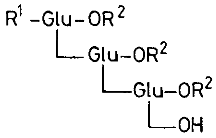
(1) $R^1 = H$

(2) $R^1 = CF_3CO$



$$\begin{array}{l}\text{Z-Glu-OBu}^t\\ \quad\llcorner\text{Glu-OBu}^t\\ \qquad\llcorner\text{OH}\end{array}$$

(3)



$$\begin{array}{l}R^1\text{-Glu-OR}^2\\ \quad\llcorner\text{Glu-OR}^2\\ \qquad\llcorner\text{Glu-OR}^2\\ \qquad\quad\llcorner\text{OH}\end{array}$$

(4) $R^1 = Z$, $R^2 = Bu^t$

(5) $R^1 = H$, $R^2 = Bu^t$

(6) $R^1 = N^{10}$-Trifluoroacetylpteroyl, $R^2 = Bu^t$

(7) R^1 = Pteroyl, $R^2 = H$

Specifically, the tripeptide derivative (**4**) was prepared by two successive carboxy terminal salt coupling reactions,[4b,6] using *N*-methylmorpholine in conjunction with isobutyl chloroformate and an activation time of 2 min at −10 °C. Preparation of the dipeptide (**3**),[6b] as its dicyclohexylamine salt in 72% yield, was achieved by adding α-t-butyl-L-glutamate[7] in aqueous $NaHCO_3$ to a cooled and previously activated solution of benzyloxycarbonyl-α-t-butyl-L-glutamate[8] in tetrahydrofuran. Recovery of (**3**)[6b] followed by successive coupling to α-t-butyl-L-glutamate[7] gave the tripeptide (**4**) isolated as the dicyclohexylammonium salt in 85% yield. Conversion into the free acid (**4**) was effected by partitioning against $KHSO_4$ solution and ethyl acetate. Debenzyloxycarbonylation (H_2 with 10% Pd–C)

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‡ This includes the penta and hepta conjugates. All new compounds gave satisfactory elemental analyses.

yielded the partially protected tripeptide (**5**) (85%) which was, in turn, coupled to (**2**) *via* the salt procedure,[4b,6] to give the partially protected triglutamyl conjugate (**6**) in 71% yield. Deprotection, following the procedure of Meienhofer *et al.*,[3] led directly and without further purification to pteroyl-γ-L-glutamyl-γ-L-glutamyl-L-glutamic acid (**7**), isolated as the dihydrate in 70% yield.

We thank Dr. S. Wilkinson for discussions and the Wellcome Trust for a research studentship to G.P.P.

(*Received*, 29*th November* 1976; *Com.* 1312.)

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