A Model Folate Synthesis incorporating the Basic Picolyl Ester Group [1]

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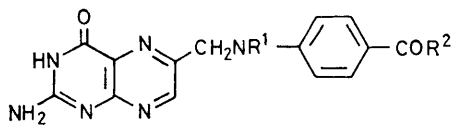
A fully protected pteroyl-γ-L-glutamate has been prepared using the picolyl basic ester handle, in conjunction with *C*-terminal carboxy peptide elongation. By using highly purified pteroic acid the isolation of protected derivatives was readily accomplished. Additionally a number of new L-glutamic acid derivatives are described for the first time.

THE enzymic hydrolysis of pteroyl-γ-L-glutamates–folate conjugates provides the primary source of folic acid (1), a dietary co-factor.[2] Since the isolation and characterisation of folic acid conjugates from yeast was first reported,[3] it has been established that the number of glutamic acid residues present in the conjugates necessary for full biological activity is species dependent.[4]



(1) R^1 = H, R^2 = NH·CH($CH_2CH_2CO_2H$)CO_2H
(2) R^1 = H, R^2 = OH
(3) R^1 = CF_3CO, R^2 = OH

Detailed and accurate metabolic investigations of the mode of action of folate conjugates have unfortunately been hindered because of the difficulty in isolation and purification of the naturally occurring heterogeneous conjugates.[5] Recently a number of synthetic routes leading to specific conjugates have been reported.[5-8] A number of problems associated severally or individually with these routes included low yields with protracted isolation and purification procedures. The present model synthesis took into account the following criteria: the peptide derivatives should readily be crystallisable and easily purified, together with the fully protected conjugate, and protecting groups should be removed under acidic or neutral conditions to minimise transpeptidation and degradation of the pterin nucleus. To meet these demands the picolyl ester [9] was used to assist in the purification of glutamyl derivatives and peptides, in conjunction with 4-nitrobenzyl and t-butyl esters for *C*-protection. From previous experience with ω-amino acid peptides,[10] *C*-terminal elongation was selected as the mode for peptide synthesis as it was considered that purification of intermediate acids through quaternary ammonium salts offered a definite advantage.

Both the starting materials (6) and (8) required for peptide synthesis were obtained *via* γ-t-butyl *N*-benzyloxycarbonylglutamate (4).[11,12,‡] Reaction of the latter with 4-pyridylmethanol in the presence of dicyclohexylcarbodi-imide (DCCI) yielded the diester derivative (5) as an oil, which was purified by citric acid extraction and isolated in 87% yield. Removal of the t-butyl ester group with trifluoroacetic acid afforded α-4-picolyl *N*-benzyloxycarbonylglutamate (6) in a yield of 70%. α-4-

(4) R^1 = Z, R^2 = Bu^t, R^3 = H
(5) R^1 = Z, R^2 = Bu^t, R^3 = Pic
(6) R^1 = Z, R^2 = H, R^3 = Pic
(7) R^1 = Nps, R^2 = Bu^t, R^3 = PNB
(8) R^1 = H, R^2 = Bu^t, R^3 = PNB
(9) R^1 = H, R^2 = R^3 = Pic
(10) R^1 = Nps, R^2 = R^3 = PNB
(11) R^1 = H, R^2 = R^3 = PNB

Z = benzyloxycarbony; Pic = 4-α-picolyl; Nps = 2-nitrophenylsulphenyl; PNB = 4-nitrobenzyl.

Nitrobenzyl γ-t-butyl glutamate (8) hydrochloride was prepared by cleavage of α-4-nitrobenzyl γ-t-butyl *N*-(2-nitrophenylsulphenyl)glutamate (7) using hydrogen chloride in methanol. Two crystalline forms of (8) hydrochloride were isolated, with different m.p.s but identical optical rotations.

Coupling of (6) and (8) using the carbonic mixed

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‡ L-Glutamic acid was used throughout.

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anhydride procedure [13] afforded the dipeptide (12) in 81% yield, which did not directly require the ' basic handle ' [9] for further purification. The dipeptide acid

Z–Glu–OPic
└Glu–OPNB
└OR
(12) R = Bu^t
(13) R = H

(13) was obtained by treatment with trifluoroacetic acid in 86% yield.

At this stage of the synthesis, chain elongation could proceed stepwise from the *C*-terminus using the ester (8). However, in order to assess the scheme in relation to the synthesis of pteroyl glutamates, it was felt necessary to ' block ' the *C*-terminal residue with groups stable to hydrogen bromide–acetic acid, the conditions required for removing the *N*-terminal protecting group. This could be accomplished by completing the chain to the tripeptide stage with αγ-di-4-picolyl glutamate (9) [14] or with αγ-di-4-nitrobenzyl glutamate (11).[15] Coupling of the dipeptide acid (13) to (9) gave a crude tripeptide (14) which proved difficult to handle. Attempts to purify (14) by using the ' basic-handle ' procedure,[9] not surprisingly, failed as both the starting materials and product carried the 4-picolyl ester group. However, after using this procedure, the crude peptide (14) was finally crystallised from ethyl acetate, but was still impure. Notwithstanding the relatively high m.p.

R^1–Glu–OR^2
└Glu–OR^3
└Glu–OR^4
└OR^4

(14) R^1 = Z, R^2 = R^4 = Pic, R^3 = PNB
(15) R^1 = Z, R^2 = Pic, R^3 = R^4 = PNB
(16) R^1 = H, R^2 = Pic, R^3 = R^4 = PNB
(17) R^1 = N^{10}–Trifluoroacetylpteroyl, R^2 = Pic, R^3 = R^4 = PNB

(108—116 °C), recovery of the bulk was unsuccessful, the crystalline solid becoming an oil on filtration even when air was excluded. The alternative derivative (11), however, afforded the crystalline tripeptide (15) in good yield.

Access to the intermediate ' blocking ester ' (11) was provided through αγ-di-4-nitrobenzyl *N*-(2-nitrophenylsulphenyl)glutamate (10), prepared from *N*-2-nitrophenylsulphenylglutamic acid [16] using either 4-nitrobenzyl bromide or 4-nitrobenzyl tosylate [17] as reagent. An alternative route using the benzyloxycarbonyl group for *N*-protection was preferred as intermediates were more easily characterised.

Removal of the amino-protecting group from the tripeptide (15) proceeded smoothly in the presence of hydrogen bromide in acetic acid, and afforded the free-amino tripeptide (16) as the dihydrobromide in high yield.

In a preliminary communication [1] it was reported that a critical feature in the preparation of pure folate conjugates is the rigorous purification of pteroic acid (2). The majority of syntheses reported to date rely on purification *via* sodium salt formation, a technique shown to be inadequate, because of low yields and oxidative degradation.[18] Accordingly, column chromatography using the method of Houlihan, Boyle, and Scott [18] allowed the isolation of high purity pteroic acid (2). As the latter is insoluble in typical organic solvents, derivatisation as the N^{10}-trifluoroacetyl derivative (3) [6,7] not only protects the N^{10}-position but also promotes solubility. Trifluoroacetylation of the pure acid (2) subsequently provided the pure derivative (3) [6,7] in high yield after one crystallisation from dimethylformamide.

Coupling of a slight excess of (3) [6,7] with the free-amino tripeptide (16) using the CMA method [13] in dimethylformamide after an activation time of 45 min at room temperature [7] gave, after washing and isolation, the pure fully protected folate conjugate (17) in 55% yield.

Deprotection of (17) using a variety of reagents including boron tribromide [19] and boron tris(trifluoroacetate) [20] was only partially effective and disappointingly the free folate conjugate could not be isolated.

EXPERIMENTAL

M.p.s were determined on a Gallenkamp apparatus. Solvents were purified by literature methods.[21] Light petroleum refers to the fraction of b.p. 60—80 °C. Sodium hydrogen carbonate solution refers to a 5% w/v solution in water and potassium hydrogen sulphate was 1 M in water, throughout. Organic extracts separated from aqueous solutions were dried over anhydrous magnesium sulphate and filtered. All evaporations were carried out under reduced pressure on a rotary evaporator. ^{1}H N.m.r. spectra were recorded on a Perkin-Elmer R-12 spectrophotometer with tetramethylsilane as internal standard. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 instrument for Nujol mulls or liquid films. Specific rotation measurements were made on a Bellingham and Stanley polarimeter. Ascending paper chromatography was performed on Whatman No. 1 paper. Silica gel (60—120 mesh) for column chromatography was washed twice with 6 M hydrochloric acid and then with water and dried overnight at 100 °C. T.l.c. on Merck Kieselgel G (0.25 mm) employed the following solvent systems (v/v): (A) ethyl acetate, (B) n-butanol–acetic acid–water (4 : 1 : 1), (C) chloroform, (D) methanol–chloroform–acetic acid (5 : 45 : 2), (E) methanol–chloroform–acetic acid (1 : 24 : 1), (F)

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methanol–chloroform–acetic acid (3 : 22 : 1), and (G) 5% ammonium hydrogen carbonate.

Synthesis of N^{10}-*Trifluoroacetylpteroyl-α-4-picolyl-*L-*glutamyl-α-4-nitrobenzyl-*L-*glutamyl-αγ-bis-4-nitrobenzyl-*L-*glutamate* (17).—*Preparation of α-4-picolyl* N-*benzyloxycarbonyl-*L-*glutamate* (6). Treatment of γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate (4) dicyclohexylammonium salt [12] (39.6 g, 76.4 mmol) with potassium hydrogen sulphate solution (120 ml) and extraction with ethyl acetate, allowed the isolation of the parent derivative (4), an oil. To a solution of (4) in dichloromethane, 4-pyridylmethanol (8.3 g, 76.4 mmol) and DCCI (15.7 g, 76.4 mmol) were added. The mixture was stirred at room temperature for 20 h and then *NN'*-dicyclohexylurea (DCU) (15.2 g, 89%; m.p. 220 °C) was filtered off. Washing of the filtrate with sodium hydrogen carbonate solution and water, followed by drying and evaporation, afforded a mixture of an oil with some crystalline material (DCU). After acetone had been added to the residue, the insoluble DCU was filtered off and the solvent evaporated off leaving an oil.

T.l.c. revealed one major component, R_{FA} 0.62, with traces of other materials R_{FA} 0.00, 0.91. N.m.r. spectroscopy indicated that the required product α-4-picolyl γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate (5) had been obtained.

The oil (5) was dissolved in ethyl acetate and extracted into citric acid solution (0.7M). The extract was made alkaline by addition of solid sodium hydrogen carbonate, and extraction with ethyl acetate, followed by drying and evaporation, afforded the pure product (5) (28.4 g, 87%), still an oil. T.l.c. revealed one spot, R_{FA} 0.62.

The oil (5) (28.4 g, 66 mmol) was dissolved in cold (0 °C) trifluoroacetic acid (100 ml) and the mixture was set aside, with occasional swirling, at room temperature for 30 min. Evaporation of the solvent yielded another oil, which crystallised under diethyl ether. The crude product (17.3 g, 61%), m.p. 152—155 °C, was recrystallised from ethyl acetate giving *α-4-picolyl* N-*benzyloxycarbonyl-*L-*glutamate* (6) (15.6 g, 55%), m.p. 156—157 °C, raised to 157—159 °C on further recrystallisation, $[\alpha]_D^{21}$ −9.5° (*c* 1.03 in dioxan), R_{FB} 0.66 (Found: C, 61.1; H, 5.4; N, 7.6. $C_{19}H_{20}N_2O_6$ requires C, 61.3; H, 5.4; N, 7.5%).

Preparation of α-4-nitrobenzyl γ-t-butyl L-*glutamate* (8) *hydrochloride salt.* To a solution in acetone of γ-t-butyl *N*-(2-nitrophenylsulphenyl) glutamate [16] derived in the usual way from its dicyclohexylammonium salt (9.0 g, 16.8 mmol), triethylamine (1.7 g, 2.3 ml, 16.8 mmol), and 4-nitrobenzyl tosylate [17] (5.15 g, 16.8 mmol) were added. The mixture was heated under reflux for 20 h, and acetone was evaporated off, leaving a yellow oil which was dissolved in ethyl acetate and washed with sodium hydrogen carbonate solution (10 × 100 ml) and water (2 × 50 ml). Drying and evaporation afforded an oil (8 g) R_{FC} 0.74 (yellow).

To a cooled (0 °C), stirred solution of α-4-nitrobenzyl γ-t-butyl *N*-(2-nitrophenylsulphenyl)-L-glutamate (7) (8.0 g, 16.3 mmol), in methanol, a solution of hydrogen chloride (0.42M; 40.0 ml, 16.79 mmol; 1.03 equiv. of HCl) in methanol was added, dropwise, during *ca.* 3 min. Immediately after the addition, the solution was allowed to warm to room temperature and left for 20 min, by which time only a trace of starting material remained. The methanol was evaporated off, and repeated trituration and washing with light petroleum allowed the isolation of the crude product. Recrystallisation from ethyl acetate (containing a little methanol)–light petroleum yielded the pure *hydrochloride* of (8). Crop 1: 4.15 g, 66%, m.p. 90—118 °C, $[\alpha]_D^{21}$ +10.7° (*c* 1.03 in methanol), R_{FB} 0.61, R_{FD} 0.55 (Found: C, 51.0; H, 6.25; Cl, 9.4; N, 7.35. $C_{16}H_{23}ClN_2O_6$ requires C, 51.3; H, 6.2; Cl, 9.5; N, 7.5%). Crop 2: 1.2 g, 19%, m.p. 93—96 °C, $[\alpha]_D^{21}$ +10.5° (*c* 1.02 in methanol), R_{FB} 0.61, R_{FD} 0.55 (Found: C, 51.1; H, 6.2%).

Preparation of N-*benzyloxycarbonyl-α-4-picolyl-*L-*glutamyl-α-4-nitrobenzyl-γ-t-butyl-*L-*glutamate* (12). Isobutyl chloroformate (1.50 g, 1.44 ml, 11.0 mmol) was added to a cooled (−20 °C) stirred solution of α-4-picolyl *N*-benzyloxycarbonyl-L-glutamate (6) (4.09 g, 11.0 mmol) and *N*-methylmorpholine (1.1 g, 1.2 ml, 11.0 mmol) in tetrahydrofuran. After 2 min, a precooled (−20 °C) mixture of the hydrochloride of the glutamate (8) (3.75 g, 10.0 mmol) and *N*-methylmorpholine (1.01 g, 1.1 ml, 10.0 mmol) in tetrahydrofuran, was added. The total mixture was stirred at 0 °C for 1 h and then at room temperature overnight. Filtration and evaporation of tetrahydrofuran gave an oil, which was dissolved in ethyl acetate and washed with sodium hydrogen carbonate solution and water. Drying and evaporation gave an oil, which crystallised from ethyl acetate–light petroleum. Recrystallisation yielded the *dipeptide derivative* (12) (5.6 g, 81%), m.p. 50—53 °C, raised to 53—55 °C on further recrystallisation, $[\alpha]_D^{19}$ −17.8° (*c* 1.0 in methanol), R_{FA} 0.60 (Found: C, 60.6; H, 6.0; N, 8.1. $C_{35}H_{40}N_4O_{11}$ requires C, 60.7; H, 5.8; N, 8.1%).

Preparation of N-*benzyloxycarbonyl-α-4-picolyl-*L-*glutamyl-α-4-nitrobenzyl-*L-*glutamate* (13). The foregoing dipeptide derivative (12) (5.2 g, 7.5 mmol) was dissolved in precooled (0 °C) trifluoroacetic acid (30 ml). The solution was set aside, with occasional swirling, at room temperature for 30 min, trifluoroacetic acid was evaporated off, and the residual oil crystallised (4.4 g, 92%) upon trituration under diethyl ether. The crude product was recrystallised from methanol–diethyl ether to give the *dipeptide* (13) (4.1 g, 86%), m.p. 166—169 °C, raised to 168—171 °C on further recrystallisation, $[\alpha]_D^{25.5}$ −18.5° (*c* 1.0 in dimethylformamide) R_{FE} 0.43 (Found: C, 58.4; H, 5.2; N, 9.0. $C_{31}H_{32}N_4O_{11}$ requires C, 58.5; H, 5.1; N, 8.8%).

*Preparation of the hydrobromide of αγ-bis-4-nitrobenzyl-*L-*glutamate* (11). A solution of hydrogen bromide in glacial acetic acid (45% w/v; 25 ml) was added to a solution of αγ-bis-4-nitrobenzyl *N*-benzyloxycarbonyl-L-glutamate [15] (5.5 g, 10 mmol) in glacial acetic acid (15 ml). The solution was set aside, with occasional swirling, at room temperature, for 1 h, and dry diethyl ether (300 ml) was then added. The white precipitate was washed repeatedly with dry diethyl ether, filtered off, quickly transferred to a vacuum desiccator, and dried over phosphorous pentaoxide. Recrystallisation from methanol–diethyl ether yielded the required *hydrobromide of* (11) (3.9 g, 78%), m.p. 105—107 °C, $[\alpha]_D^{22}$ +9.4° (*c* 1.01 in methanol), R_{FB} 0.78, R_{FD} 0.51 (detected by ninhydrin) (Found: C, 45.6; H, 4.2; N, 8.5. $C_{19}H_{20}BrN_3O_8$ requires C, 45.8; H, 4.05; N, 8.4%).

Preparation of N-*benzyloxycarbonyl-α-4-picolyl-*L-*glutamyl-α-4-nitrobenzyl-*L-*glutamyl-αγ-bis-4-nitrobenzyl-*L-*glutamate* (15). Isobutyl chloroformate (0.14 g, 0.13 ml, 1.0 mmol) was added to a cooled (−20 °C) stirred solution of the dipeptide (13) (0.64 g, 1.0 mmol) and *N*-methylmorpholine (0.1 g, 0.11 ml, 1.0 mmol) in tetrahydrofuran. After 2 min a precooled (−20 °C) mixture of the hydrochloride of (11) (0.45 g, 1.0 mmol) and *N*-methylmorpholine (0.1 g, 0.11 ml, 1.0 mmol) in dimethylformamide, was added. Stirring was continued for 2 h at 0 °C and overnight

at room temperature. *N*-Methylmorpholine salts were filtered off, ethyl acetate was added to the filtrate, and evaporation gave, after recrystallisation from methanol, the required *fully protected tripeptide* (15) (0.75 g, 74%), m.p. 98—105 °C. Further recrystallisation gave a sample with m.p. 101—103 °C, $[\alpha]_D^{25}$ −16.6° (*c* 1.02 in dimethylformamide) R_{FE} 0.55 (detected by picryl chloride) (Found: C, 57.9; H, 4.9; N, 9.7. $C_{50}H_{49}N_7O_{18}$ requires C, 58.0; H, 4.8; N, 9.5%).

Preparation of the dihydrobromide of α-4-*picolyl*-L-*glutamyl*-α-4-*nitrobenzyl*-L-*glutamyl*-αγ-*bis*-4-*nitrobenzyl*-L-*glutamate* (16). Hydrogen bromide in glacial acetic acid (45% w/v, 10 ml) was added to a solution of the foregoing tripeptide (15) (1.04 g, 1.0 mmol) in glacial acetic acid. The mixture was set aside, with occasional swirling, at room temperature for 20 min, and dry diethyl ether (100 ml) was then added. The precipitate was washed repeatedly with dry diethyl ether, filtered off, quickly transferred to a vacuum desiccator, and dried over phosphorous pentaoxide. Recrystallisation from methanol and diethyl ether afforded the *dihydrobromide* of the tripeptide (16) (0.96 g, 90%), m.p. 128—132 °C, raised to 130—133 °C on further recrystallisation, $[\alpha]_D^{21}$ −0.95° (*c* 1.05 in methanol), R_{FB} 0.53 (Found: C, 47.35; H, 4.4; N, 9.3. $C_{42}H_{45}Br_2N_7O_{16}$ requires C, 47.4; H, 4.3; N, 9.2%).

Preparation of N^{10}-*trifluoroacetylpteroic acid* (3). Pure, dry pteroic acid (2) [18] (1.81 g, 5.8 mmol) was stirred, in suspension, in freshly distilled trifluoroacetic anhydride (40 ml) at room temperature in the dark for 5 days. A white suspension was then filtered off under nitrogen and washed with trifluoroacetic anhydride. A single recrystallisation from dimethylformamide (30 ml)–methanol (45 ml)–water (300 ml, 50 °C) afforded N^{10}-trifluoroacetyl pteroic acid (3) [6,7] (1.92 g, 81%), m.p. >300 °C, R_{FG} 0.8 (ascending paper chromatography, u.v. fluorescent spot) (Found: F, 13.6. $C_{16}H_{11}F_3N_6O_4$ requires F, 13.95%).

Preparation of N^{10}-*trifluoroacetylpteroyl*-α-4-*picolyl*-L-*glutamyl*-α-4-*nitrobenzyl*-L-*glutamyl*-αγ-*bis*-4-*nitrobenzyl*-L-*glutamate* (17). *N*-Methylmorpholine (0.034 g, 0.038 ml, 0.336 mmol) and isobutyl choroformate (0.046 g, 0.044 ml, 0.336 mmol) were added to a stirred solution of rigorously dried N^{10}-trifluoroacetylpteroic acid (3) [6,7] (0.137 g, 0.336 mmol) in dimethylformamide (2 ml). After 45 min at room temperature a mixture of the dihydrobromide of the tripeptide (16) (0.383 g, 0.36 mmol, 7% excess) and *N*-methylmorpholine (0.073 g, 0.081 ml, 0.72 mmol) in dimethylformamide (2 ml) was added. Stirring was continued at room temperature, in the dark, for 40 h. Addition of sodium hydrogen carbonate solution precipitated a yellow solid which was filtered off. Washing with water gave the pure *pteroyl triglutamate* (17), (0.24 g, 55%), m.p. 139—141 °C, R_{FF} 0.53 (picryl chloride or u.v. fluorescence), $[\alpha]_D^{21}$ −8.2° (*c* 0.64 in dimethylformamide) (Found: C, 54.1; H, 3.95; F, 4.0; N, 14.4. $C_{58}H_{52}F_3N_{13}O_{19}$ requires C, 53.9; H, 4.0; F, 4.4; N, 14.1%).

Experiments Directed towards the Deprotection of the Pteroyl Triglutamate (17).—*To test the action of boron tribromide on* α-4-*picolyl* N-*benzyloxycarbonyl*-L-*glutamate* (6). To a cooled (−10 °C) solution of (6) (0.37 g, 1.0 mmol) in dichloromethane (12 ml) and *NN*-dimethylacetamide (6 ml), a solution of boron tribromide in dichloromethane (1M; 10 ml) was added dropwise with stirring during 20 min. After stirring for a further 45 min at −10 °C and then 16 h at room temperature, the reaction was terminated by dropwise addition of water (25 ml). Following separation of the layers and evaporation of water, the residue was applied to a Zeocarb-225 (H^+ form) column (24 × 1.5 cm) and eluted successively with water and dilute ammonia solution (1%). Fractions were compared by t.l.c. with glutamic acid (R_{FB} 0.38), α-4-picolyl L-glutamate * (dihydrobromide) (R_{FB} 0.26), and (6) (R_{FB} 0.66). The major product was found to be glutamic acid, R_{FB} 0.38, but a small amount of α-4-picolyl *N*-benzyloxycarbony-L-glutamate, R_{FB} 0.67, was separated and identified by comparison with an authentic sample.

To test the action of boron tribromide on αγ-*bis*-4-*nitrobenzyl* N-*benzyloxycarbonyl*-L-*glutamate*.[15] A solution of boron tribromide in dichloromethane (1 M; 5 ml) was added dropwise to a cooled (−10 °C) stirred solution in dichloromethane (25 ml) of αγ-bis-4-nitrobenzyl *N*-benzyloxycarbonyl-L-glutamate [15] (0.55 g, 1 mmol). The mixture was stirred for 30 min at −10 °C and for 2 h at room temperature. Water (25 ml) was added dropwise with vigorous stirring, the two layers were separated, and the water was evaporated off. The residue was principally L-glutamic acid, R_{FB} 0.38, coincident with an internal marker, accompanied by minor traces of two other ninhydrin-positive species, R_{FB} 0.63 and 0.68.

To test the stability of the γ-glutamyl peptide bond in the presence of boron tribromide. To a cooled (−10 °C) stirred solution of *N*-benzyloxycarbonyl-α-t-butyl-L-glutamyl-α-t-butyl-L-glutamyl-αγ-di-t-butyl-L-glutamate [22] (0.38 g, 0.5 mmol) in dichloromethane a solution of boron tribromide in dichloromethane (1 M; 10 ml) was added dropwise. The mixture was stirred for 1 h at −10 °C and for 2 h at room temperature, and a solution of sodium hydrogen carbonate in water was then added dropwise, with vigorous stirring. The layers were separated and the aqueous phase was acidified (hydrochloric acid; 2M) to Congo Red paper. The solution was concentrated and contained only one ninhydrin-positive component, R_{FB} 0.2, assumed to be the free tripeptide; glutamic acid as an internal marker had R_{FB} 0.38.

Attempted deprotection of N^{10}-*trifluoroacetylpteroyl*-α-*picolyl*-L-*glutamyl*-α-4-*nitrobenzyl*-L-*glutamyl*-αγ-*bis*-4-*nitrobenzyl*-L-*glutamate* (17). A solution of boron tribromide in dichloromethane (1 M; 10 ml, 10 mmol) was added dropwise to a cooled (−10 °C) stirred solution of the fully protected tripeptide (17) (0.130 g, 0.1 mmol) in dichloromethane (1 ml) and *NN*-dimethylacetamide (2 ml). The mixture was stirred at −10 °C for 1 h and at room temperature for 20 h. Sodium hydrogen carbonate solution was added slowly until the pH was between 7 and 8 and the layers were separated; the upper aqueous phase was partially evaporated under reduced pressure and the pH was adjusted to 3 with hydrochloric acid. A column of Sephadex LH-20 (8 g) was prepared in water to which was added the foregoing aqueous solution. Sodium bromide was eluted on the front followed by a yellow component, in addition to which a yellow band remained at the origin. The fast-moving yellow component was evaporated to yield a picryl chloride-negative and u.v.-positive material (20 mg), R_{FG} 0.40, 0.73 ⟶ 1.0. Removal of the N^{10}-trifluoroacetyl protecting group from the foregoing yellow material was effected using aqueous

* Obtained by treating α-4-picolyl *N*-benzyloxycarbonyl-L-glutamate (6) with hydrogen bromide in glacial acetic acid.

[22] J. Meienhofer, P. M. Jacobs, H. A. Godwin, and I. H. Rosenberg, *J. Org. Chem.*, 1970, **35**, 4137.

piperidine [7] (0.1 M; 5 ml) for 1 h at room temperature, under nitrogen. On acidification to pH 3 a pale yellow precipitate formed which was recovered by centrifugation and washed successively with water and methanol. Ascending paper chromatography revealed a yellow band, R_F 0.0—0.15 (non-u.v.-fluorescent), and two very faint u.v.-fluorescent bands, R_{FG} 0.45 and 0.7; *cf.* authentic pteroylglutamic acid, R_{FG} 0.42, and pteroyltriglutamate, 0.76.[7]

Elution of the stationary component with ammonium acetate (0.02 M) gave only a trace of material which was not examined in detail.

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