The Synthesis of Biologically Active Pteroyl oligo-γ-L-Glutamates (Folic Acid Conjugates)

EVALUATION OF [3H]PTEROYLHEPTAGLUTAMATE FOR METABOLIC STUDIES

HERMAN A. GODWIN,‡ IRWIN H. ROSENBERG,§ AND CATHERINE R. FERENZ
From the Thorncliff Memorial Laboratory, Harvard Medical Service, Boston City Hospital, Boston, Massachusetts 02118 and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

PAULA M. JACOBS AND JOHANNES MEIENOFER‡
From The Children's Cancer Research Foundation and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

SUMMARY

Homogeneous pteroyltri-, pteroylpenta-, and pteroylhepta-γ-L-glutamates (folic acid conjugates) have been synthesized and, when desired, specifically labeled with tritium. The synthetic procedures consist of: (a) production of pteroic acid from folic acid by microbiological techniques; (b) preparation of [3′,5′,9-3H]pteroic acid; (c) synthesis of crystalline homogeneous N4-trifluoroacetylpteroic acid; (d) mixed anhydride condensation of N4-trifluoroacetylpteroic acid with tri-, penta-, and hepta-γ-L-glutamic acid tert-butyl esters prepared by peptide synthesis in solution; (e) purification of protected products by LH-20 column chromatography; and (f) removal of protecting groups and final purification of products by gel filtration.

The compounds have been obtained in good yields and have been characterized by correct elemental analyses, amino acid analyses, ultraviolet spectral data, ascending paper chromatography, and high voltage electrophoresis. These data compare favorably with analytical information reported for natural products. Behavior of the synthetic materials in microbiological assays and enzyme studies of intestinal mucosal homogenates confirms their biological identity with natural conjugates.

Seven constituent glutamic acid residues (1-3) (Fig. 1). Following the isolation and structural identification of yeast folic acid conjugate in 1945, investigations were performed in vivo and in vitro which began to elucidate the metabolism and biological characteristics of natural folates (3-8). Marked differences in nutritional availability for mammalian, avian, and bacterial species were noted depending upon the length of the glutamyl peptide chain (9). Furthermore, evidence suggests that both the number of γ-glutamyl residues and the amino acid constituents of the peptide chain may serve as specific factors of recognition in the formation of certain coenzyme-apoenzyme complexes (10).

Recently, there has been a renewed interest in the metabolism of folic acid conjugates, particularly the role of the intestinal mucosa in the digestion of peptide conjugates (11-13). Although valuable information has been obtained from the study of partially purified yeast conjugates, it is apparent that definitive clinical, enzymatic, and metabolic studies require well defined substrates. In 1969 Krumdieck and Haugh reported the solid phase synthesis of polyglutamates of folic acid (14, 15). These compounds, labeled with 14C, were employed to considerable advantage for the study of folate absorption and metabolism in patients with neoplastic diseases (16).

We have synthesized unlabeled and tritium-labeled pteroyl oligo-γ-L-glutamate compounds by syntheses in solution which offer several distinct advantages. This communication describes our procedures for the chemical synthesis of homogeneous pteroyltri-, pteroylpenta-, and pteroylhepta-γ-L-glutamates and specific labeling with tritium. Products have been obtained in analytically pure form and have been characterized by a series of physical analyses. The synthetic pteroyl oligoglutamates closely resemble natural folates in biological characteristics and therefore are suitable for nutritional, metabolic, and enzymatic studies of folic acid conjugates.

EXPERIMENTAL PROCEDURE AND RESULTS

The scheme for the chemical synthesis of unlabeled and tritium-labeled pteroyl oligo-γ-L-glutamates is depicted in Fig. 2. Pteroic acid was produced from pteroylglutamic acid by microbiological techniques; (b) preparation of [3′,5′,9-3H]pteroic acid; (c) synthesis of crystalline homogeneous N4-trifluoroacetylpteroic acid; (d) mixed anhydride condensation of N4-trifluoroacetylpteroic acid with tri-, penta-, and hepta-γ-L-glutamic acid tert-butyl esters prepared by peptide synthesis in solution; (e) purification of protected products by LH-20 column chromatography; and (f) removal of protecting groups and final purification of products by gel filtration.
Pteroic Acid (Pte)  L-Glutamic Acid (Glu)

**PTEROYLGUTAMIC ACID (PteGlu)**

**PTEROYLGLUTAMIC ACID**

\[
\begin{align*}
\text{N'O-Tfa-PTEROIC ACID OLIGO-\gamma-L-GLUTAMIC ACID} \\
\text{TERT-BUTYL ESTERS}
\end{align*}
\]

**PTEROYLOLIGO-\gamma-L-GLUTAMIC ACIDS (PteGlu\_\_\_)**

**FIG. 1.** Structural formula of pteroyloligo-\gamma-L-glutamic acids (PteGlu\_\_\_).

**FIG. 2.** Scheme for the chemical synthesis of pteroyloligo-\gamma-L-glutamic acids. Note that the compounds can be labeled with tritium specifically at the 3',5',9 positions of the molecule.

Biological techniques (17, 18) and, when desired, could be specifically labeled with tritium (\(^{3}H\)). To solubilize pteroic acid for condensation reactions and to minimize undesired side reactions, homogeneous \(N^{10}\)-trifluoroacetylpteroic acid was synthesized. Crystalline protected peptides were prepared by solution synthesis (19). After selective removal of the amino-protecting group, the peptides were condensed with \(N^{10}\)-trifluoroacetylpteroic acid by a mixed anhydride reaction and the protected products purified by Sephadex LH-20 column chromatography. After removal of protecting groups, gel filtration was employed for final purification of the pteroyloligo-\gamma-L-glutamates.

Pteroic Acid—Pteroic acid was prepared from pteroylglutamic acid according to the method of Pratt, Crawford, and Friedkin (17).\(^{1}\) Crude pteroic acid was purified by crystallization of sodium pteroate from 2.5 N NaOH, dissolving the salt in distilled water, and finally precipitating the free acid by the addition of 12 N HCl (18). Homogeneity of pteroic acid was determined by ultraviolet spectral analysis (\(\epsilon 256 \text{ nm in } 0.1 \times \text{ NaOH was 800} \)), ascending paper chromatography (single ultraviolet quench spot, \(R_e 0.09, \text{ in } 5\% \text{ NH}_4\text{HCO}_3\)), and microbiological properties. As expected, pteroic acid supported the growth of *Streptococcus faecalis* but not the growth of *Lactobacillus casei* (1).

**Preparation of \([3',5',9\text{H}]\text{PTEROIC ACID}**—Purified pteroic acid (50 mg) was iodinated in the 3',5',9 positions of the molecule and

\(^{1}\) *Flavobacterium, strain HY*, generously provided by Dr. Morris Friedkin.
of labeling, an additional 450 mg of unlabeled pteroic acid was added to retard decomposition caused by the radiation. For purification, the crude [H]pteroic acid was dissolved in 0.1 N NaOH and acidified to pH 3.0 by dropwise addition of 1 N HCl. The resulting precipitate was washed three times with 6 N HCl and three times with distilled water. The material was then lyophilized and stored in a desiccator at 1 mm of Hg over P2O5 until recrystallization.

For recrystallization, the crude [H]pteroic acid was dissolved in 17 ml of 2.5 N NaOH at 37° until solution was complete. The solution was placed overnight in a refrigerator at 4°. The yellow precipitate of sodium pteroate was dissolved in distilled water and the free acid was precipitated by the dropwise addition of 6 N HCl. The precipitate was washed three times with distilled water and then lyophilized. Total yield was 452 mg (90%).

A sample of the final product was dissolved in 0.01 N NaOH and applied to Whatman No. 1 paper for ascending chromatography in 5% NH4HCO3. The chromatogram was dried and cut into 1-cm segments. Pteric acid was eluted from the chromatogram by a methanol-water-toluene liquid scintillation system containing 2,5-diphenylloxazole (POPOP)-1,4-bis-[2-(5-phenyloxazolyl)]benzene (POPPOP) as fluors, and the radioactivity was determined. The major portion of radioactivity (82%) migrated with marker pteroic acid. Specific radioactivity of the [H]pteroic acid was determined to be 77 μCi per μmole.

**Synthesis of N10-Trifluoroacylpteroic Acid and Trition Derivative—**Pteric acid (600 mg, 1.92 moles) was placed in a double neck flask. To remove tightly bound water, the material was carefully dried for 72 hours over P2O5 under reduced pressure (1 mm of Hg). Distilled trifluoroacetic anhydride (12 ml) was then introduced dropwise over 30 min with continuous stirring. The reaction mixture, protected from light and moisture, was stirred for 44 hours at room temperature. The trifluoroacetic anhydride was then removed under reduced pressure. Freshly distilled ether was added to the remaining dry syrup. A cream-colored precipitate was obtained which was washed three times by decantation with a total of 100 ml of fresh P2O5. A sample of the final product was dissolved in 0.01 N NaOH and applied to Whatman No. 1 paper for ascending chromatography in 5% NH4HCO3 (RF 0.09), 7.8 p.p.m. (both d, p-C6H4) (JCH-CH = 8.0 Hz), 8.7 p.p.m. (s, N-CH=CH-), and 4.4 p.p.m. (s, C-CH2-N), and trifluorooxycetylpteroic acid, 8.2 p.p.m. and 7.8 p.p.m. (both d, p-C6H4) (JCH-CH = 8.0 Hz), 8.7 p.p.m. (s, N-CH=CH-), and 5.3 p.p.m. (s, C-CH2-N). A sample for elemental analysis was dried over P2O5 under reduced pressure for 48 hours at 20°.

\[
\text{CaH}_{2}P_{2}N_{2}O_{4}, \text{H}_{2}O (444.3)
\]

Calculated: C 43.2, H 3.12, N 18.9, F 12.8

Found: C 43.3, H 3.09, N 18.7, F 12.8

Karl Fisher water determination was 10.1%.

The trifluoroacetyl group was cleaved by treatment of N10-trifluoroacetylpteroic acid with 0.1 N NaOH for 15 min. Homogeneous pteroic acid was obtained showing a single spot by ascending paper chromatography in 5% NH4HCO3 (RF 0.09), an ultraviolet spectrum indistinguishable from that of starting pteroic acid, and predicted microbiological growth activity for *S. faecalis*.

The [H]pteroic acid was handled in the same manner.

**Preparation of Peptides**—The synthesis of the protected peptide, N-benzyloxycarbonyl-γ-L-glutamic acid tert butyl esters (2 to 7 residues), by peptide synthesis in solution has been previously described (10).

**Condensation**—The protected pterooyltri-, penta-, and hepta-γ-L-glutamate compounds were prepared by mixed anhydride coupling. For a typical preparation, N-benzyloxycarbonyl-hepta-γ-L-glutamic acid hepta-γ-L-glutamic acid ester (420 mg, 0.28 mmole) was dissolved in 10 ml of methanol. Catalytic hydrogenation to remove the benzyloxycarbonyl group was carried out by passing a stream of hydrogen through the peptide solution in the presence of freshly prepared palladium black (21) until cessation of CO2 evolution (20 to 60 min). The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The peptide ester was dried directly in the flask under reduced pressure over P2O5 for 1 hour.

Crystalline N10-trifluoroacetylpteroic acid (114 mg, 0.256 mmole) was dried in a double neck reaction flask under reduced pressure over P2O5 for 48 hours. Triethylamine (0.037 ml, 0.266 mmole) and dimethylformamide (1 ml), both freshly distilled, were added by a syringe through a rubber stopper to maintain anhydrous conditions. The mixture was warmed to 40° to effect complete solution and then cooled to room temperature in a vacuum over P2O5 for 24 hours. The crystals were fan-shaped on microscopic examination. Over-all yields averaged 50%.

Ascending paper chromatography (5% NH4HCO3) showed a single fluorescent spot (RF 0.8) and no streaking; m.p. >30°; infrared (KCl), cm⁻¹, 3230 and 3160 (NH), 1705 and 1690 (acid and amide C=O), 1217 and 1158 (CF₂); ultraviolet, λmax, nm (10⁻³ × e), water, 277 (17,800), 346 (6,500), and λmax, 0.1 N HCl, 232 (25,600), 319 (10,600); nuclear magnetic resonance data, pteric acid, 7.8 p.p.m. and 6.7 p.p.m. (both d, p-C6H4) (JCH-CH = 8.3 Hz); 8.5 p.p.m. (s, N-CH=CH-), and 4.4 p.p.m. (s, C-CH2-N), and trifluorooxycetylpteroic acid, 8.2 p.p.m. and 7.8 p.p.m. (both d, p-C6H4) (JCH-CH = 8.0 Hz), 8.7 p.p.m. (s, N-CH=CH-), and 5.3 p.p.m. (s, C-CH2-N). A sample for elemental analysis was dried over P2O5 under reduced pressure for 48 hours at 20°.

Nuclear magnetic resonance spectra were determined using a Varian T909 (6-06T) spectrometer with tetramethylsilane as external standard. Trifluoroacetylpteroic acid was exchanged into D2O-dimethylformamide·H2O and the spectrum determined in deuterated dimethylformamide solution while the spectrum of pteroic acid was measured in 10% NaOH·D2O solution. All signals are reported as singlets (a) and doublets (d). Chemical shifts were measured from multiplet centers. The authors are indebted to Dr. Bernard Babor for his aid in this analysis.

**Micromethod performed by Werby Laboratories, Inc., Boston, Massachusetts.**
an ice bath (5 min). Isobutyl chloroformate (0.034 ml, 0.256 mmole) was added. Immediate precipitation of the triethylamine hydrochloride was observed. The mixed anhydride reaction was stirred for 45 min at room temperature, protected from light.

The flask containing the peptide was sealed tightly with a rubber stopper and the mixed anhydride was transferred into the flask using a dry syringe and a total of 3 ml of dimethylformamide to effect the transfer. The reaction mixture was then stirred for 43 hours at room temperature shielded from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

Isobutyl chloroformate (0.034 ml, 0.256 mmole) was added. Immediate precipitation of the triethylamine hydrochloride was observed. The mixed anhydride reaction was stirred for 45 min at room temperature shielded from light.

The flask containing the peptide was sealed tightly with a rubber stopper and the mixed anhydride was transferred into the flask using a dry syringe and a total of 3 ml of dimethylformamide to effect the transfer. The reaction mixture was then stirred for 43 hours at room temperature shielded from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.

The mixture was then diluted with ethyl acetate (50 ml) and the organic phase was washed once with 0.5 N NaHCO₃ (100 ml), once with water (25 ml), twice with 0.5 N H₂SO₄ (20 ml each), once with water (25 ml), once with 0.5 N NaHCO₃ (20 ml), and twice with concentrated NaCl solution (20 ml each). The organic phase was dried (Na₂SO₄), filtered, and the solvent evaporated. The resulting oil was then stirred for 43 hours at room temperature, protected from light.
**TABLE I**

Chromatographic and analytical properties of pteroylglutamate compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chromatography (RF)</th>
<th>High voltage electrophoresis</th>
<th>Amino acid analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent A</td>
<td>Solvent B</td>
<td>Solvent C</td>
</tr>
<tr>
<td>Pteroylglutamic acid</td>
<td>0.42</td>
<td>0.36</td>
<td>0.40</td>
</tr>
<tr>
<td>Pteroyltetraglutamic acid</td>
<td>0.76</td>
<td>0.60</td>
<td>0.64</td>
</tr>
<tr>
<td>Pteroylpentaglutamic acid</td>
<td>0.84</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>Pteroylheptaglutamic acid</td>
<td>0.95</td>
<td>0.87</td>
<td>0.84</td>
</tr>
</tbody>
</table>

<sup>a</sup> Chromatography was performed on Whatman No. 1 paper ascending. Solvents were: A, 5% NH<sub>4</sub>HCO<sub>3</sub>; B, 4% sodium citrate; C, 5% Na<sub>2</sub>HPO<sub>4</sub>.

<sup>b</sup> This value is in agreement with data derived from a previously published spectral curve (22).

<sup>c</sup> Based on ε = 572 of standard pteroylglutamic acid.

**DISCUSSION**

In 1945 Pfiffner and coworkers (3, 4) isolated folic acid conjugate as pteroylheptaglutamate in crystalline form from yeast. When this supply of material was exhausted, no additional relatively pure folic acid conjugate was available until polyglutamates of folic acid were synthesized by the solid phase method (14). Solid phase synthesis has the inherent disadvantage that each step in peptide chain elongation can give rise to...
incompletely removed by a variety of column chromatographic
peptides by the solid phase method because of its speed and
convenience. This method was judged inadequate after analysis
showed contaminating lower peptides of glutamic acid which were
incompletely removed by a variety of column chromatographic
systems. Since we desired folic acid conjugates of precise pep-
tide chain length for metabolic studies, alternate synthetic
procedures were followed. When peptide synthesis in solution
employing the carbamic acid-carboxylic acid mixed anhydride
procedure was used, crystalline benzyloxycarbonyloligo-y-n-
glutamic acid tert-butyl esters were obtained in good yields
when the solid phase method was used. Crystalline benzyloxycarbonylo-
proline ester was obtained in good yields (19).

The synthetic procedures presented in this communication
provide for specific tritium-labeling of the pteroyl moiety when
labeled conjugates are desired for biological studies (20). The
major advantage of these tritium-labeled materials is their
versatility. In addition to animal studies and enzymatic studies
in vitro, such compounds can be utilized safely in human studies.
Previous investigations with 14C-labeled polyglutamates were
restricted to patients with neoplastic diseases (16). The avail-
ability of [3H]pteroylglutamylglutamate has allowed the develop-
ment of a safe, quantitative test of conjugated folate absorption
(25) and has permitted development of a nonmicrobiological
assay of enzymatic hydrolysis of folic acid conjugate with isola-
tion and identification of labeled pteroylglutamate products and
intermediates (24).

The protected conjugates produced by mixed anhydride
condensation were obtained in homogeneous form following
Sephadex LH-20 column chromatography in ethanol. These
materials were stable, whereas deprotected products underwent
spontaneous degradation after several weeks despite shielding
from light and storage at 4°C. Therefore, products are best
stored in protected form with removal of protecting groups to
yield sufficient material for 4 to 6 weeks of use. Such an
approach is facilitated by the simplicity of the deprotection proce-
sure and the ease of final purification by gel filtration.

The utility of these purified synthetic pteroylglutamic acid
compounds in the study of metabolic processes depends in the
final analysis, upon their performance in biological systems.
Synthetic materials must possess the biological activity of
natural folic acid conjugates in order to be valid for use in studies
of folate metabolism. The behavior of the products of this
synthesis in microbiological assays and enzyme studies confirms
their biological identity with natural conjugates (9) and estab-
ishes their usefulness as a precise tool for study of digestion,
absorption, and metabolism of folic acid conjugates.

REFERENCES
2. Binkley, S. B., Bird, O. D., Bloom, E. S., Brown, R. A.,
Campbell, C. J., Emmett, E. D., and
Peiffer, J. J. (1944) Science 100, 36
3. Peiffer, J. J., Stokstad, E. L. R., Bloom, E. S., and O'Dell,
4. Peiffer, J. J., Stokstad, E. L. R., O'Dell, B. L., Bloom, E. S.,
Brown, R. A., Campbell, C. J., and Bird, O. D. (1945
Science 102, 228
Chem. 157, 731
6. Bird, O. D., Binkley, S. B., Bloom, E. S., Emmett, A. D.,
and Peiffer, J. J. (1945) J. Biol. Chem. 167, 415
8. Binkley, S. B., Binkley, S. B., Bloom, E. S., and
Krumdieck, C. L. (1946) J. Biol. Chem. 90, 603
60, 445-463
Biochim. Biophys. Acta 212, 116
11. Rosenberg, I. H., Stokstad, E. L. R., Godwin, H. A., and
Krumdieck, C. L. (1969) Gastroenterology 56, 35a
Chem. 244, 4373
Invest. 46, 570
1509-1572
16. Butterworth, C. E., Jr., Baugh, C. M., and Krumdieck,
J. Biol. Chem. 241, 6367-6372
J. Biol. Chem. 242, 1466-1476
19. Meienhofer, J., Jacobs, P. M., Godwin, H. A., and
Anal. Biochem. 36, 107
22. Walker, C. W., Hutchings, B. L., Lowry, J. H., Stokstad,
E. L. R., Bird, J. H., Angier, R. B., Emitt, J., Subrah-
Row, Y., Cosulich, D. B., Fahrenbach, M. J., Hultquist,
M. E., Kuh, E., Northey, E. H., Seegert, D. R., Sickels,
48, 900
45, 35a
The Synthesis of Biologically Active Pteroyl oligo-γ-L-Glutamates (Folic Acid Conjugates) : EVALUATION OF [3H]PTEROYLHEPTAGLUTAMATE FOR METABOLIC STUDIES
Herman A. Godwin, Irwin H. Rosenberg, Catherine R. Ferenz, Paula M. Jacobs and Johannes Meienhofer


Access the most updated version of this article at [http://www.jbc.org/content/247/8/2266](http://www.jbc.org/content/247/8/2266)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/247/8/2266.full.html#ref-list-1](http://www.jbc.org/content/247/8/2266.full.html#ref-list-1)