Synthesis of the Penta-glutamyl Derivative of *N*-[4-[*N*-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)-propyl]amino]benzoyl]-L-glutamic Acid (5-DACTHF). An Acyclic Analogue of Tetrahydrofolic Acid

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The penta-glutamyl derivative of *N*-[4-[*N*-[3-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]amino]-benzoyl)-L-glutamic acid (**1**, 5-DACTHF, 543U76) was synthesized by a convergent route. L-γ-Glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic acid heptakis *t*-butyl ester (**20**) was prepared in ten steps from L-glutamic acid di-*t*-butyl ester and *N*-(benzyloxycarbonyl)-L-glutamic acid α-*t*-butyl ester. 4-[*N*-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]trifluoroacetamido]benzoic acid (**6**), which was synthesized from pyrimidinylpropionaldehyde **3** in three steps, was condensed with **20**, followed by deprotection to provide *N*-[4-[*N*-[3-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]amino]benzoyl]-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic acid (**2**). Hexaglutamate **2** is a potent inhibitor of glycinamide ribonucleotide transformylase.

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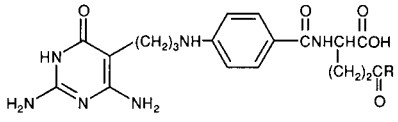
Introduction.

The synthesis and biological activity of *N*-[4-[*N*-[3-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]-amino]benzoyl]-L-glutamic acid (**1**, 5-DACTHF, 543U76), an acyclic analogue of 5,6,7,8-tetrahydrofolic acid (THF), was described recently [1,2]. 5-DACTHF exhibits potent cytotoxicity *in vitro* against several tumor cell lines. Metabolism studies revealed that 5-DACTHF is extensively metabolized to polyglutamylated homologues *in vitro* [3]. Compound **1** and its polyglutamylated homologues inhibit glycinamide ribonucleotide transformylase (GAR-TFase) and aminoimidazole ribonucleotide transformylase (AICAR-TFase), the folate dependent enzymes in *de novo* purine biosynthesis. We describe herein the synthesis of the penta-glutamyl derivative **2** of 5-DACTHF, which is a potent inhibitor of L cell GAR-TFase with an IC_{50} = 0.08 μM.

Figure 1



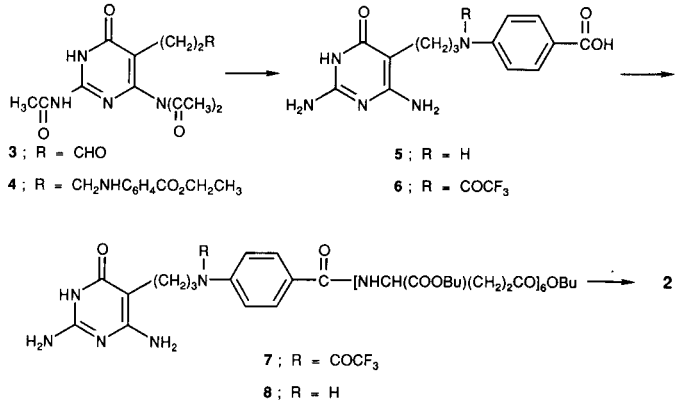
1 ; R = OH (5-DACTHF)

2 ; R = {NHCH(COOH)$(CH_2)_2$CO]$_5$OH

Chemistry.

The penta-glutamyl derivative of **1** was prepared by a convergent synthesis as outlined in Schemes I and II. The pyrimidinylpropionaldehyde **3** [1] was used to alkylate reductively ethyl 4-aminobenzoate to give **4** in 24% yield (Scheme I). The *N*- and *O*-protecting groups were removed with sodium hydroxide to give acid **5** in 71% yield. Direct condensation of **5** with hexaglutamate **20** in dimethylformamide with 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) failed to produce **8**. However, the trifluoromethyl derivative **6**, which was prepared by selective acylation of the anilino nitrogen with trifluoroacetic anhydride, reacted smoothly with **20** under the same reaction conditions to give **7** in 77% yield for the two stages. The trifluoroacetyl group apparently provides carboxyl activation as well as *N*-protection. *N*-Trifluoroacetyl protection was also successful in pteroylpolyglutamate synthesis by solid phase techniques [4]. The trifluoroacetamide group was removed from **7** with dimethylamine, and the *t*-butyl esters were cleaved with trifluoroacetic acid to give the hexaglutamate **2**.

Scheme I[a]



3 ; R = CHO

4 ; R = $CH_2NHC_6H_4CO_2CH_2CH_3$

5 ; R = H

6 ; R = $COCF_3$

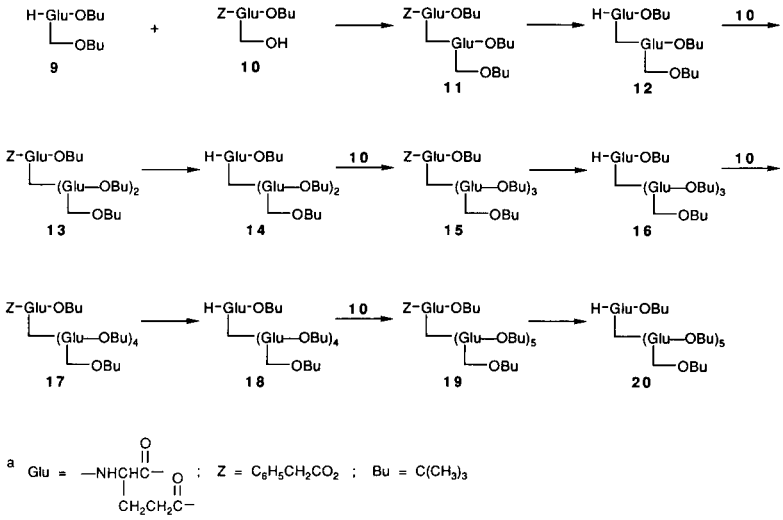
7 ; R = $COCF_3$

8 ; R = H

[a] Bu = $C(CH_3)_3$

The hexaglutamyl ester **20** was prepared in 19% overall yield in ten steps from L-glutamic acid di-*t*-butyl ester (**9**) and *N*-(benzyloxycarbonyl-L-glutamic acid α-*t*-butyl ester (**10**). This approach (Scheme II) involved successive, step-

Scheme II[a]



[a] Glu = $-NHCHC(=O)-$ with $CH_2CH_2C(=O)-$; Z = $C_6H_5CH_2CO_2$; Bu = $C(CH_3)_3$

wise peptide elongation at the amino terminus using the DCC and HOBT coupling method, followed by *N*-benzyloxycarbonyl (*N*-CBZ) cleavage by catalytic hydrogenolysis.

The intermediate *N*-CBZ esters **11**, **13**, **15**, **17**, and **19** were characterized by elemental analysis, and the target amino ester **20** was characterized by nmr, ms, and elemental analysis. Our yields were comparable to those reported by Meienhofer *et al.*, [5] who prepared polyglutamates by mixed anhydride methodology but abandoned the carbodiimide method because of side reactions.

EXPERIMENTAL

Melting points were determined with a Thomas Hoover or Mel-Temp capillary melting point apparatus and are uncorrected. The ultraviolet spectra were recorded with a Unicam SP 800 spectrophotometer or Cary 118 UV-Vis spectrophotometer. The nmr spectra were recorded using a Varian XL-100-15-FT, a Varian XL-200, or a Hitachi Perkin-Elmer R-24 spectrometer. Chemical shift values are reported in parts per million on the δ scale with tetramethylsilane as the internal reference. The nmr spin multiplicities are indicated by the symbols s (singlet), d (doublet), q (quartet), and m (multiplet). Mass spectra (70 eV) were obtained on a Varian CH-5-DF mass spectrometer. Elemental microanalyses were determined by Atlantic Microlabs, Atlanta, GA 30386, and gave combustion values for C, H, and N within 0.4% of theoretical values. Preparative column chromatography was done either using a flash chromatography technique [6] on Silica Gel 60 (40-63 μm, E. Merck No. 9385) or using a Waters Associates Prep LC/System 500 instrument with ethyl acetate-hexane as an eluant. Thin-layer chromatography (tlc) was done on Silica Gel (200 μ) MK6GF (Whatman) plates eluted with dichloromethane-methanol (9:1) or diethyl ether. Detection of spots was by fluorescence indicator quenching upon exposure of the plates to uv light. Solvents were evaporated by rotary evaporation (Buchler flash evaporator) using a temperature-controlled water bath.

N-[4-(*N*-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]amino]benzoyl]-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid (**2**).

A mixture of 0.30 g (0.20 mmole) of **8** in 5.0 ml of trifluoroacetic acid was stirred at ambient temperature for 0.75 hour. The resulting yellow solution was spin evaporated *in vacuo* to a beige solid, which was subsequently dissolved in 5.0 ml of water. The aqueous solution was injected into a column of C-18 (comprised of a series of five Rainin Spice cartridges connected in series) preequilibrated with water. The column was washed with 50 ml of water before the product was eluted with 20% acetonitrile. Solvent was removed by spin evaporation and freeze-drying. The residue was partitioned between a 1:1 mixture of ethyl acetate and water. The aqueous layer was freeze-dried to yield 0.12 g (52%) of **2** as a fluffy white powder, mp 204° dec. The hplc on Versapack C-18 (10 micron, 4.6 × 250 mm) with aqueous 15% acetonitrile containing 0.2% trifluoroacetic acid gave one major peak, k' = 0.77; ^{1}H nmr (DMSO-d_6): δ 1.60 (m, 2H, CCH_2C), 1.73 (m, 6H, α-CH_2N), 1.95 (m, 6H, α-CH_2), 2.19 (m, 14H, Het-CH_2 and β-CH_2), 3.00 (m, 2H, CH_2N), 4.13 (m, 5H, α-H), 4.30 (m, 1H, Ar-CO_2NHCH), 5.77 (s, 2H, NH_2), 5.95 (s, 2H, NH_2), 6.20 (br, 1H, ArNH), 6.53 (d, 2H, Ar), 7.68 (d, 2H, Ar), 8.15 (m, 6H, α-NH), 12.4 (br, COOH signals) plus 0.25 mole EtOAc (1.18 t, 2.00 s, 4.03 q).

Anal. Calcd. for $C_{44}H_{59}N_{11}O_{21}\cdot 2.5\ H_2O\cdot 0.25$ EtOAc: C, 47.20; H, 5.81; N, 13.46. Found: C, 47.35; H, 5.61; N, 13.20.

Ethyl 4-[3-[2-(Acetylamino)-4-(diacetylamino)-1,6-dihydro-6-oxo-5-pyrimidinyl]propylamino]benzoate (**4**).

A mixture of 1.25 g (4.00 mmoles) of **3** [1], 0.743 g (4.50 mmoles) of ethyl 4-aminobenzoate, 1.0 ml of glacial acetic acid, and 3 Å molecular sieves in 25 ml of methanol was stirred at room temperature for 3 hours under nitrogen before 0.28 g (4.47 mmoles) of sodium cyanoborohydride was added during a 2-minute period. After stirring for 17 hours, the mixture was filtered. The filtrate was spin evaporated *in vacuo* to a yellow foam. The product was separated from a mixture by flash chromatography on Silica Gel 60 (100 g) with ethyl acetate. Appropriate fractions were combined on the basis of tlc correlation, and solvent was removed by spin evaporation *in vacuo*. Recrystallization from ethyl acetate yielded 0.44 g (24%) of **4** as a white solid, mp

197-198°; tlc (ethyl acetate), R_f = 0.5; ^{1}H nmr (DMSO-d_6): δ 1.28 (t, J = 7.1 Hz, 3H, CH_3), 1.65 (m, 2H, CCH_2C), 2.14 (s, 3H, Ac), 2.2 (m, 2H, CH_2Het), 2.23 (s, 6H, 2Ac), 3.05 (m, 2H, CH_2N), 4.22 (q, J = 7.1 Hz, 2H, $CO2CH_2$), 6.5 (br, 1H, AcNH obscured by Ar), 6.57 (d, 2H, ArH), 7.69 (d, 2H, ArH).

Anal. Calcd. for $C_{22}H_{27}N_5O_6 \cdot 0.5\ H_2O$: C, 56.64; H, 6.05; N, 15.01. Found: C, 56.88; H, 6.01; N, 14.88.

4-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propylamino]-benzoic Acid One-half Sodium Salt (**5**).

To a solution of 0.22 g (0.47 mmole) of **4** in 7.0 ml of 95% ethyl alcohol was added 15 ml of 1.0 *N* sodium hydroxide. The reaction was heated at 70° for 20 hours. The mixture was reduced by spin evaporation *in vacuo* to a 10-ml volume and adjusted to *p*H 5 with 1.0 *N* hydrochloric acid. The precipitate was collected by filtration, washed with water, and dried *in vacuo* to yield 0.11 g (71%) of **5** as a white solid, mp 273° dec; hplc on Versapack C-18 (10 micron, 4.6 × 250 mm) with aqueous 60% methanol containing 0.2% trifluoroacetic acid gave one major peak, k' = 0.58; ^{1}H nmr (DMSO-d_6): δ 1.58 (m, 2H, CCH_2C), 2.24 (t, 2H, Het-CH_2), 3.02 (m, 2H, CH_2N), 5.77 (br s, 2H, NH_2), 5.94 (br s, 2H, NH_2), 6.46 (t, 1H, NH-Ar), 6.54 (d, 2H, Ar), 7.65 (d, 2H, Ar), 9.80 (br s, 1H, NH), 11.95 (br s, 0.5 H, CO_2H).

Anal. Calcd. for $C_{14}H_{16}N_5O_3Na_{0.5} \cdot 0.75\ H_2O$: C, 51.29; H, 5.53; N, 21.36; Na, 3.51. Found: C, 51.34; H, 5.39; N, 21.32; Na, 3.33.

4-[*N*-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]trifluoroacetamido]benzoic Acid (**6**).

A mixture of 0.50 g (1.53 mmoles) of **5** and 5.0 ml of trifluoroacetic anhydride was stirred at ambient temperature for 18 hours under nitrogen. The amber solution was spin evaporated *in vacuo* at 25°. The residual foam was triturated with water (15 ml) until a homogeneous, beige powder was obtained. The solid was collected by filtration, washed with water (2 × 2 ml), and dired *in vacuo* to yield 0.617 g (81%) of **6**, mp 229-230°; hplc on Versapack C-18 (10 micron, 4.6 × 250 mm) with aqueous 50% methanol containing 0.1% trifluoroacetic acid gave one major peak, k' = 1.00; tlc (methanol:ethyl acetate-1:1), R_f = 0.5; ^{1}H nmr (DMSO-d_6): δ 1.50 (m, 2H, CCH_2C), 2.20 (t, 2H, Het-CH_2), 3.74 (t, 2H, CH_2N), 6.67 (br s, 2H, NH_2), 7.43 (br s, 2H, NH_2), 7.55 (d, 2H, Ar), 8.02 (d, 2H, Ar); ms: (methane chemical ionization) m/z 400 (18.5% relative abundance; [M + H]+), 356 (5.91%, [M-CO_2]+), 167 (10.72%), 115 (100%).

Anal. Calcd. for $C_{16}H_{16}F_3N_5O_4 \cdot CF_3COOH$: C, 42.11; H, 3.34; N, 13.64. Found: C, 42.47; H, 3.49; N, 14.13.

N-[4-[*N*-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]-trifluoroacetamido]benzoyl]-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Heptakis *t*-Butyl Ester (**7**).

To 0.10 g (0.20 mmole) of **6**, 0.24 g (0.20 mmole) of **20**, 0.027 g (0.20 mmole) of 1-hydroxybenzotriazole, and 0.020 g (0.20 mmole) of triethylamine in 2.0 ml of *N,N*-dimethylformamide at ambient temperature was added 0.046 g (0.22 mmole) of 1,3-dicyclohexylcarbodiimide. The reaction was stirred for 22 hours and filtered. The filtrate was spin evaporated *in vacuo*, and the amber residue was partitioned between dichloromethane (25 ml) and saturated aqueous sodium bicarbonate (15 ml). The organic layer was sequentially washed with saturated sodium bicarbonate (15 ml) and saturated brine (2 × 10 ml), dried with magnesium sulfate, filtered, and spin evaporated *in vacuo* to a foam.

The residue was mixed with 5 ml of ethyl acetate; a small amount of insoluble solid was removed by filtration. The filtrate was spin evaporated *in vacuo* to yield 0.30 g (96%) of **7** as a beige solid, mp 114° dec; hplc on Supelco LC-8 with aqueous 80% methanol containing 0.1% triethylamine gave one major peak, k' = 3.35; tlc (methanol:ethyl acetate-1:9), R_f = 0.3 (uv and anisaldehyde); ^{1}H nmr (DMSO-d_6): δ 1.38 (m, 63H, O-t-Bu), 1.60 (m, 2H, CCH_2C), 1.72 (m, 6H, α-CH_2), 1.90 (m, 6H, α-CH_2), 2.22 (m, 1H, $ArCO_2NHCH$), 5.72 (s, 2H, NH_2), 5.90 (s, 2H, NH_2), 7.57 (d, 2H, Ar), 7.94 (d, 2H, Ar), 8.13 (m, 6H, α-NH), 9.7 (br, 1H, NH).

Anal. Calcd. for $C_{74}H_{114}F_3N_{11}O_{22}$: C, 56.73; H, 7.33; N, 9.83. Found: C, 56.86; H, 7.42; N, 9.79.

N-[4-[*N*-[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]-amino]benzoyl]-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Heptakis *t*-Butyl Ester (**8**).

To a solution of 0.30 g (0.20 mmole) of **7** in 4.0 ml of methanol at ambient temperature was added sequentially 0.045 g (1.0 mmole) of dimethylamine and 0.4 ml of water. Under nitrogen, the yellow solution was stirred at ambient temperature for 18 hours. Reaction progress was monitored by hplc. The solvent was removed by spin evaporation *in vacuo* to yield 0.30 g (100%) of **8** as a beige solid; hplc on Supelco LC-8 with aqueous 80% methanol containing 0.1% triethylamine gave one major peak, k' = 2.44; tlc (methanol:ethyl acetate-1:4), R_f = 0.6 (uv and anisaldehyde); ^{1}H nmr (DMSO-d_6): δ 1.38 (m, 63H, O-*t*-Bu), 1.60 (m, 2H, CCH_2C), 1.70 (m, 6H, α-CH_2), 1.90 (m, 6H, α-CH_2), 2.20 (m, 14H, Het-CH_2 and β-CH_2), 3.00 (m, 2H, CH_2), 4.08 (m, 5H, α-H), 4.26 (m, 1H, $ArCO_2NHCH$), 5.75 (s, 2H, NH_2), 5.91 (s, 2H, NH_2), 6.23 (br, 1H, ArNH), 6.52 (d, 2H, Ar), 7.65 (d, 2H, Ar), 8.14 (m, 6H, β-NH), 9.7 (br, 1H, NH).

N-(Benzyloxycarbonyl)-L-γ-glutamyl-L-glutamic Acid Tris-*t*-butyl Ester (**11**).

To a stirred solution of 3.89 g (7.5 mmoles) of *N*-(benzyloxycarbonyl)-L-glutamic acid α-*t*-butyl ester (**10**), dicyclohexylamine salt, 2.22 g (7.5 mmoles) of L-glutamic acid bis-*t*-butyl ester (**9**) hydrochloride, and 1.01 g (7.5 mmole) of 1-hydroxybenzotriazole in 20 ml of dichloromethane was added 1.70 g (8.25 mmole) of 1,3-dicyclohexylcarbodiimide. The reaction was stirred for 17 hours and filtered. The filtrate was sequentially washed with saturated aqueous sodium bicarbonate (2 × 40 ml), dried with magnesium sulfate, filtered, and spin evaporated *in vacuo*. The product was purified by flash chromatography on Silica Gel 60 (150 g) with hexanes:ethyl acetate (1:1). The appropriate fractions were combined on the basis of tlc correlation and spin evaporated *in vacuo* to yield 3.34 g (77%) of **11** as a colorless gum; tlc (hexanes:ethyl acetate-3:1), R_f = 0.4 (anisaldehyde); ^{1}H nmr (DMSO-d_6): δ 1.39 (s, 27H, O-*t*-Bu), 1.70 (m, 2H, α-CH_2), 1.85 (m, 2H, α-CH_2), 2.20 (m, 4H, β-CH_2), 4.00 (m, 2H, α-H), 5.04 (s, 2H, NCO_2CH_2), 7.35 (s, 5H, Ar), 7.55 (d, 1H, NH), 8.05 (d, 1H, NH).

Anal. Calcd. for $C_{30}H_{46}N_2O_9$: C, 62.27; H, 8.01; N, 4.84. Found: C, 61.99; H, 8.03; N, 4.79.

L-γ-Glutamyl-L-glutamic Acid Tris-*t*-butyl Ester (**12**).

A solution of 3.34 g (5.77 mmoles) of **11** in 100 ml of 95% ethanol and 80 mg of 10% palladium on carbon was shaken in the presence of hydrogen at 22 psi of hydrogen for 18 hours. The catalyst was removed by filtration and the filtrate was spin evaporated *in vacuo* to yield 2.39 g (93%) of **12** as a colorless syrup; tlc (ethyl acetate), R_f = 0.3 (ninhydrin). The product was used in the

next reaction without further characterization.

N-(Benzyloxycarbonyl)-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Tetrakis-*t*-butyl Ester (**13**).

To a stirred mixture of 2.79 g (5.38 mmoles) of **10** dicyclohexylamine salt, 2.39 g (5.38 mmoles) of **12**, 0.73 g (5.38 mmoles) of 1-hydroxybenzotriazole, and 5.38 ml of 1.0 *N* hydrochloric acid in 30 ml of dichloromethane was added 1.22 g (5.91 mmoles) of 1,3-dicyclohexylcarbodiimide. The reaction was stirred for 16 hours and filtered. The filtrate was sequentially washed with saturated aqueous sodium bicarbonate (2 × 50 ml), 5% aqueous citric acid (2 × 50 ml), and saturated brine (2 × 50 ml); dried with magnesium sulfate; filtered; and spin evaporated *in vacuo*. The product was purified by flash chromatography on Silica Gel 60 (150 g) with ethyl acetate:hexanes (1:1). The appropriate fractions were combined on the basis of tlc correlation and spin evaporated *in vacuo* to yield 3.10 g (75%) of **13**, mp 76-78°; tlc (hexanes:ethyl acetate-1:1), R_f = 0.4 (anisaldehyde); ^{1}H nmr (DMSO-d_6): δ 1.39 (s, 36H, O-*t*-Bu), 1.75 (m, 3H, α-CH_2), 2.22 (m, 6H, β-CH_2), 3.90 (m, 1H, α-H), 4.10 (m, 2H, α-H), 5.04 (dd, 2H, NCO_2CH_2), 7.36 (s, 5H, Ar), 7.61 (d, 1H, NH), 8.09 (m, 2H, NH).

Anal. Calcd. for $C_{39}H_{61}N_3O_{12}$: C, 61.32; H, 8.05; N, 5.50. Found: C, 61.09; H, 8.21; N, 5.45.

L-γ-Glutamyl-L-γ-glutamyl-L-glutamic Acid Tetrakis-*t*-butyl Ester (**14**).

A solution of 3.10 g (4.06 mmoles) of **13** in 100 ml of 95% ethanol and 100 mg of 10% palladium on carbon was shaken in the presence of hydrogen at 22 psi for 8 hours. The catalyst was removed by filtration, and the filtrate was spin evaporated *in vacuo* to yield 2.23 g (87%) of **14** as a colorless gum; tlc (95% ethanol), R_f = 0.7 (ninhydrin). The product was used in the next reaction without other characterization.

N-(Benzyloxycarbonyl)-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Pentakis-*t*-butyl Ester (**15**).

To a stirred mixture of 1.83 g (3.52 mmoles) of **10** dicyclohexylamine salt, 2.23 g (3.52 mmoles) of **14**, 0.474 g (3.52 mmoles) of 1-hydroxybenzotriazole, and 3.52 ml of 1.0 *N* hydrochloric acid in 25 ml of dichloromethane was added 0.800 g (3.88 mmoles) of 1,3-dicyclohexylcarbodiimide. The reaction was stirred for 17 hours and filtered. The filtrate was sequentially washed with saturated aqueous sodium bicarbonate (2 × 50 ml), 5% aqueous citric acid (2 × 50 ml) and saturated brine (2 × 50 ml), dried with magnesium sulfate, filtered, and spin evaporated *in vacuo*. The product was purified by flash chromatography on Silica Gel 60 (150 g), with hexanes:ethyl acetate (3:7). The appropriate fractions were combined on the basis of tlc correlation and spin evaporated *in vacuo* to yield 2.71 g (81%) of **15**, mp 75°; tlc (hexanes:ethyl acetate-3:7), R_f = 0.5 (anisaldehyde); ^{1}H nmr (DMSO-d_6): δ 1.39 (s, 45H, O-*t*-Bu), 1.75 (m, 4H, α-CH_2), 1.92 (m, 4H, α-CH_2), 1.92 (m, 4H, α-CH_2), 2.22 (m, 8H, β-CH_2), 3.89 (m, 1H, α-H), 4.10 (m, 3H, α-H), 5.04 (dd, 2H, NCO_2CH_2), 7.37 (s, 5H, Ar), 7.63 (d, 1H, NH), 8.0 (m, 3H, NH).

Anal. Calcd. for $C_{48}H_{76}N_4O_{15}$: C, 60.74; H, 8.07; N, 5.90. Found: C, 60.91; H, 8.09; N, 6.11.

L-γ-Glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Pentakis-*t*-butyl Ester (**16**).

A solution of 2.71 g (2.86 mmoles) of **15** in 100 ml of 95% ethanol and 100 mg of 10% palladium on carbon was shaken in the presence of hydrogen at 40 psi for 19 hours. The catalyst was removed by filtration, and the filtrate was spin evaporated *in vacuo* to yield 2.29 g (98%) of **16** as a white foam; tlc (ethyl acetate:methanol-9:1), R_f = 0.5 (ninhydrin). The product was used in the next reaction without further characterization.

N-(Benzyloxycarbonyl)-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Hexakis-*t*-butyl Ester (**17**).

To a stirred mixture of 1.46 g (2.80 mmoles) of **10** dicyclohexylamine salt, 2.29 g (2.80 mmoles) of **16**, 0.380 g (2.80 mmoles) of 1-hydroxybenzotriazole, and 2.8 ml of 1.0 *N* hydrochloric acid in 35 ml of dichloromethane was added 0.635 g (3.08 mmoles) of 1,3-dicyclohexylcarbodiimide. The reaction was stirred for 19 hours and filtered. The filtrate was sequentially washed with saturated aqueous sodium bicarbonate (2 × 25 ml), dried with magnesium sulfate, filtered, and spin evaporated *in vacuo*. The product was purified by flash chromatography on Silica Gel 60 (150 g) with hexane:ethyl acetate (1:2). The appropriate fractions were combined on the basis of tlc correlation and spin evaporated *in vacuo* to yield 2.40 g (76%) of **17** as a white foam, mp 74-76°; tlc (hexanes:ethyl acetate-1:2), R_f = 0.4 (anisaldehyde).

Anal. Calcd. for $C_{57}H_{91}N_5O_{18}$: C, 60.35; H, 8.09; N, 6.17. Found: C, 59.94; H, 8.16; N, 5.89.

L-γ-Glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Hexakis-*t*-butyl Ester (**18**).

A solution of 2.40 g (2.11 mmoles) of **17** in 100 ml of 95% ethanol and 200 mg of 10% palladium on carbon was shaken in the presence of hydrogen at 45 psi for 4.5 hours. The catalyst was removed by filtration. The filtrate was spin evaporated *in vacuo* to yield 2.14 g (100%) of **18** as colorless gum; tlc (ethyl acetate:methanol-9:1), R_f = 0.3 (ninhydrin). The product was used in the next reaction without further characterization.

N-(Benzyloxycarbonyl)-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Heptakis-*t*-butyl Ester (**19**).

To a stirred mixture of 1.11 g (2.14 mmoles) of **10** dicyclohexylamine salt, 2.14 g (2.14 mmoles) of **18**, 0.289 g (2.14 mmoles) of 1-hydroxybenzotriazole, and 2.14 ml of 1.0 *N* hydrochloric acid in 20 ml of dichloromethane was added 0.486 g (2.35 mmoles) of 1,3-dicyclohexylcarbodiimide. The reaction was stirred for 18 hours and filtered. The filtrate was sequentially washed with saturated aqueous sodium bicarbonate (2 × 50 ml), 5% aqueous citric acid (2 × 50 ml), and saturated brine (2 × 25 ml), dried with magnesium sulfate, filtered, and spin evaporated *in vacuo*. The product was purified by flash chromatography on Silica Gel 60 (150 g) with hexanes:ethyl acetate (1:3). The appropriate fractions were combined on the basis of tlc correlation and spin evaporated *in vacuo* to yield 2.03 g (72%) **19** as a white foam, mp 77-78°; tlc (hexanes:ethyl acetate-1:3), R_f = 0.4 (anisaldehyde).

Anal. Calcd. for $C_{66}H_{106}N_6O_{21}$: C, 60.07; H, 8.10; N, 6.37. Found: C, 60.00; H, 8.11; N, 6.35.

L-γ-Glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-γ-glutamyl-L-glutamic Acid Heptakis-*t*-butyl Ester (**20**).

A solution of 2.00 g (1.52 mmoles) of **19** in 100 ml of 95% ethanol and 100 mg of 10% palladium on carbon was shaken in the presence of hydrogen at 43 psi for 16 hours. The catalyst was removed by filtration, and the filtrate was spin evaporated *in vacuo* to yield 1.67 g (93%) of **20** as a white foam, mp 67-69°; tlc (ethyl acetate:methanol-9:1), R_f = 0.5 (ninhydrin); ^{1}H nmr (DMSO-d_6): δ

1.39 (s, 63H, O-*t*-Bu), 1.74 (m, 6H, α-CH_2), 1.92 (m, 6H, α-CH_2), 2.21 (m, 12H, β-CH_2), 4.08 (m, 6H, α-H), 8.15 (m, 7H, NH); ms: (chemical ionization) m/z 1186 (85.0% relative abundance, (M+H+), m/z 1001 (27.5%), m/z 815 (100%).

Anal. Calcd. for $C_{58}H_{100}N_6O_{19}$: C, 58.77; H, 8.50; N, 7.09. Found: C, 58.78; H, 8.58; N, 7.03.

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