Supplementary Information

Synthesis of (6*R*)- and (6*S*)-5,10-Dideazatetrahydrofolate Oligo-γ-glutamates: Kinetics of Multiple Glutamate Ligations Catalyzed by Folylpoly-γ-glutamate Synthetase

John W. Tomsho,[a] John J. McGuire,[b] James K. Coward[a]

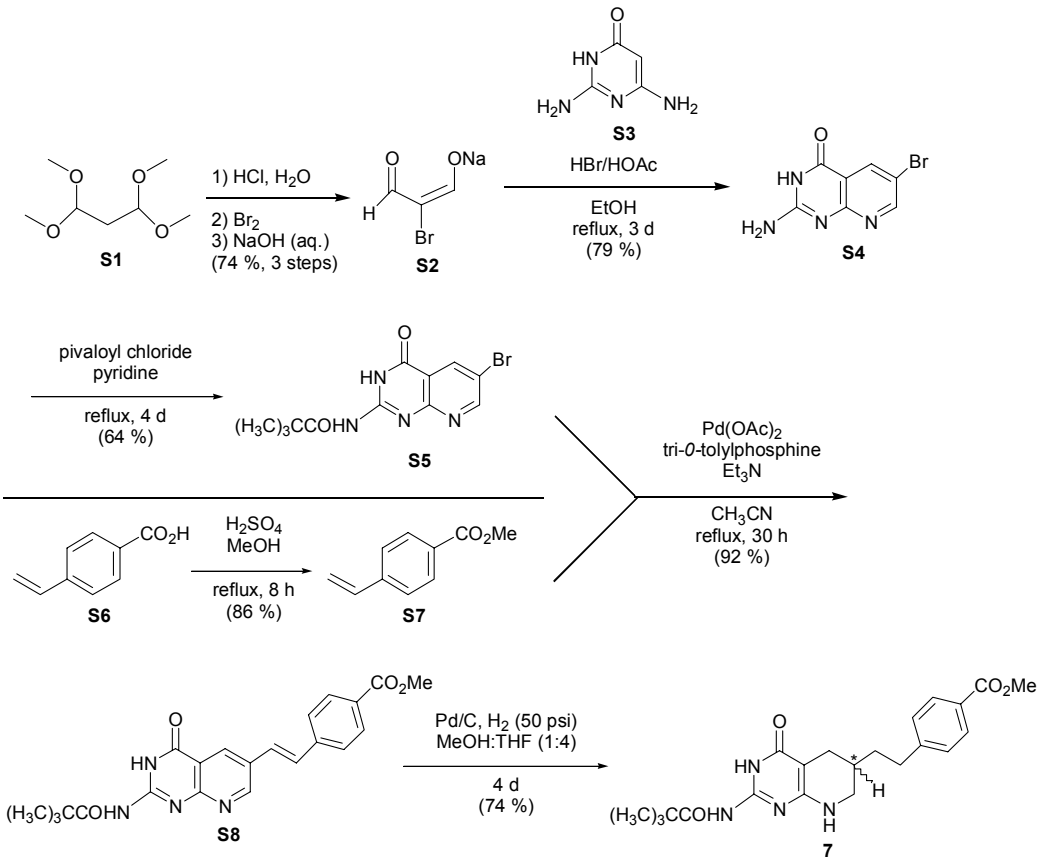
[a] *Departments of Medicinal Chemistry and Chemistry, University of Michigan, Ann Arbor, MI 48109-1055*
[b] *Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, NY 14263*

Results

The synthesis of 6*RS*-**7** is illustrated in Scheme S1.[1, 2] Malonaldehyde bis(dimethyl acetal) (**S1**) was treated with aqueous HCl followed by bromine to yield crude bromomalonaldehyde. This crude material was then treated with aqueous NaOH to form the sodium salt (**S2**) that was isolated by crystallization from wet acetone.

6-Bromo-5-deazapterin (**S4**) was formed in an HBr-catalyzed dehydration reaction between **S2** and 2,4-diamino-6-hydroxypyrimidine (**S3**). The literature procedure[1] for the synthesis of **S4** calls for the use of HCl as the acid. Initial experiments used bromomalonaldehyde sodium salt and an extra equivalent of HCl in a reaction that was much diluted with absolute EtOH to allow stirring. These reaction conditions resulted in a 1 to 0.3 mixture (as determined by ^{1}H NMR) of bromo- to chloro-containing products. The identity of these products was confirmed by HRMS (324.0212, required for $C_{12}H_{13}N_4O_2Br$ 324.02219; 280.0724, required for $C_{12}H_{13}N_4O_2Cl$ 280.07270; the relative isotopic abundance patterns also supported this assignment) of the 2-pivaloated mixture. Only the desired product was obtained when the acid was changed to 30% HBr in acetic acid. The exocyclic amine of **S4** was then protected by reaction with pivaloyl chloride in pyridine to yield 2-pivaloyl-6-bromo-5-deazapterin (**S5**). The general procedure followed was that reported for 2-pivaloylguanine by Taylor.[3] Pivaloyl anhydride may also be used to effect this conversion.[1]

Methyl 4-vinylbenzoate (**S7**) was prepared from 4-vinylbenzoic acid (**S6**) by the method of Webb[4] where anhydrous methanol and conc. H_2SO_4 were used to form the methyl ester. Formation of **S7** was also achieved by the use of thionyl chloride[5] in place of conc. H_2SO_4, but the results of this



Scheme S1 The synthesis of 6*RS*-**7**.

method were less reproducible. In both cases, the reaction was run at a high dilution (1 g per 100 mL) to minimize acid-catalyzed polymerization reactions. Methyl 4-vinylbenzoate (**S7**) was reported by Taylor et al.[2] to be accessible by diazomethane methylation of 4-vinylbenzoic acid. In our hands, this reaction proved to be incompatible with the vinyl functionality.

The formation of methyl 2-pivaloyl-5,10-dideaza-9,10-dehydropteroate (**S8**) was accomplished by coupling **S5** and **S7** with a standard Heck reaction. This product, **S8,** was then reduced by catalytic hydrogenation with 10% Pd/C and 50 psi H_2 in a 1:4 mixture of MeOH: THF to yield methyl (6*RS*)-2-pivaloyl-5,10-dideaza-5,6,7,8-tetrahydropteroate (6*RS*-**7**). Reduction of **S8** to 6*RS*-**7** was reported by Taylor[2] and was carried out at ~8 mg/mL in MeOH and was limited by the solubility of **S8**. With a 1:4 mixture of MeOH: THF, the solubility of **S8** was improved and the reduction could be done at much higher concentrations. Multiple rounds of reduction with fresh catalyst were necessary as a result of loss of catalyst activity as the reduction proceeded. The racemic product, 6*RS*-**7**, may then be carried on directly to give (6*RS*)-DDAH$_4$PteGlu$_1$ (Scheme 2). Alternatively, the individual isomers, 6*R*-**7** or 6*S*-**7**, may be separated by chiral HPLC (Figures S1, S2) and converted to stereochemically pure (6*R*)- or (6*S*)-DDAH$_4$PteGlu$_1$ (Scheme 2) and the poly-γ-glutamyl derivatives (Scheme 3).

Experimental

Materials. As in main text except for as indicated below. Malonaldehyde bis(dimethyl acetal) and 2,4-diamino-6-hydroxypyrimidine were obtained from Aldrich. $Pd(OAc)_2$ and tri-*o*-tolylphosphine were obtained from Strem Chemicals.

General Procedures. As in the main text except for as described below.

Semi-preparative reversed phase(RP)-HPLC (Method 1S): Column - Varian Dynamax 21.4 x 250 mm, Microsorb 60-8, C18. Flow rate of 10 mL/min. Eluant A - 0.1% w/v TFA in ddH_2O. Eluant B - 0.1% w/v TFA in CH_3CN. Detection at 214 nm. Gradient - 0 min, 1% B; 5 min, 1% B; 15 min, 5% B; 20 min, 100% B.

Bromomalonaldehyde.[6, 7] Malonaldehyde bis(dimethyl acetal) (**S1**) (100.00 g , 0.609 mol) was added with stirring to 100 mL dH_2O. Concentrated HCl (4.33 mL, 0.052 mol) was added slowly and the reaction was stirred for 2 h. The solution was then cooled in an ice-water bath and Br_2 was added such that the temperature of the solution remained below 15 ^oC. The reaction was stirred for a further 30 min on ice. The solution was then concentrated at 35-40 ^oC and 5 mm Hg until a thick crystalline paste had formed. This was filtered and washed with 200 mL cold water followed by 100 mL cold CH_2Cl_2. After drying under high vacuum, crude product was recovered. A crude yield of 79.18 g (86%) was achieved. A portion of this material was purified by sublimation[7] (95 ^oC, 0.75 torr) for characterization. mp 146-147 ^oC dec (lit.,[7] 137-139 dec ^oC; lit.,[6] 155 ^oC dec; lit.,[8] 148 ^oC); δ_H (300 MHz; d_6-DMSO) 4.50 (7 H, br, C(2)H), 8.71 (2 H, s, C(1)H, C(3)H); δ_C (300 MHz; d_6-DMSO) 104.1 (C2), 176.4 (C1, C3); *m/z* (EI^+) 149.9317 (M^+. $C_3H_3BrO_2$ requires 149.9316) 166(61%), 165(32), 164(62), 163(29), 152(94), 151(71), 150(100), 149(73), 124(34), 123(20), 122(43), 121(20), 106(25), 104(24), 95(21), 93(25), 85(27), 71(42), 53(49), and 42(51). Spectral data are identical to those reported for this compound in the literature.[6, 7]

Bromomalonaldehyde sodium salt (S2).[6] Crude bromomalonaldehyde (63.14 g, 0.418 mol) was dissolved in aqueous NaOH (16.73 g (0.418 mol) NaOH in ~400 mL of dH_2O) . The solution was concentrated at 30 ^oC and 5 mm Hg until crystal precipitation began. The resulting mixture was diluted to 1 L with cold acetone, covered, and allowed to sit overnight at 4 ^oC to yield a crystalline product which was removed by filtration. After washing with 500 mL cold acetone and drying, 42.10 g (58%) of **S2** was obtained. The filtrate and all washes were concentrated in vacuo to 100 mL, then diluted to 1 L with acetone and allowed to sit overnight at 4 ^oC and treated as above to yield an additional 22.86 g of **S2**. The product was dried over P_2O_5 for several days until the mass remained constant. Total yield 62.3 g (86%); mp >150 ^oC, dec.; δ_H (500 MHz; D_2O) 8.71 (2 H, s, C(1)H, C(3)H); δ_C (500 MHz; D_2O) 102.7 (C2), 185.4 (C1, C3); *m/z* (ESI^+) 194.9032 ($M+Na^+$. $C_3H_2BrNa_2O_2$ requires 194.9034) 197(64%), 195(100), 159(38), and 91(25). Spectral data are identical to those reported for this compound in the literature.[6]

2-Amino-6-bromo-4(3*H*)-pyrido[2,3-*d*]pyrimidinone (6-Bromo-5-deazapterin) (S4).[1] 2,4-Diamino-6-hydroxypyrimidine (**S3**) (10.00 g, 79.3 mmol) and bromomalonaldehyde sodium salt (**S2**) (13.71 g, 79.3 mmol) were suspended in 100 mL of anhydrous EtOH under Ar. 30% HBr in acetic acid (35 mL, 174.5 mmol) was added to the suspension. A condenser was fitted to the flask before heating at reflux temperature for 3 d. The reaction was then cooled, the resulting suspension was filtered, and the filter cake was washed with 95% EtOH followed by Et_2O. This was air-dried followed by drying under high vacuum to yield a dark red powder. Total yield 20.17 g (79%); λ_{max} (0.1 M HCl)/nm 255, 282, 316, 364, and 495; (0.1 M NaOH)/nm 248, 273, 345, and 515; δ_H (500 MHz; 0.1 M NaOD in D_2O) 8.13 (1 H, d, J = 2.6 Hz, C(5)H), 8.32 (1 H, d, J = 2.6 Hz, C(7)H); δ_C (500 MHz; 0.1 M NaOD in D_2O) 111.23 (C6), 113.4 (C4a), 138.0 (C5), 154.65 (C7), 159.8 (C8a), 165.0 (C2), 174.2 (C4); *m/z* (ESI^+) 240.9725 ($M+H^+$. $C_7H_6BrN_4O$ requires 240.9725) 243(87), and 241(100). Spectral data are identical to those reported for this compound in the literature.[1]

2-Pivaloylamino-6-bromo-4(3*H*)-pyrido[2,3-*d*]pyrimidinone (2-Pivaloyl-6-bromo-5-deazapterin) (S5).[1, 3] 6-Bromo-5-deazapterin (**S4**) (5.00 g, 20.7 mmol) was sealed under Ar in a round bottom flask fitted with a reflux condenser. Pyridine (100 mL, 1.24 mol) and pivaloyl chloride (7.65 mL, 62.1 mmol) were added with stirring. The reaction was heated at reflux temperature for 4 d. The reaction was then concentrated at 10 mm Hg and 45 ^oC until all visible solvent had been removed. The residue was suspended in 125 mL anhydrous EtOH and heated at reflux temperature for 45 min to quench unreacted pivaloyl chloride before again being concentrated as above. The residue was diluted with CH_2Cl_2 and the solvent was allowed to evaporate overnight. Purification was achieved by plug filtration (silica, MeOH/CH_2Cl_2 3:97), followed by flash chromatography (silica, MeOH/CH_2Cl_2 1:99). Total yield 4.32 g (64%); δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.34 (9 H, s, C(C*H*$_3$)$_3$), 8.37 (1 H, br s, N(3)H), 8.62 (1 H, d, J = 2.6 Hz, C(5)H), 8.89 (1 H, d, J = 2.6 Hz, C(7)H), 12.17 (1 H, br s, C(2)NH); *m/z* (ESI^+) 347.0125 ($M+Na^+$. $C_{12}H_{13}BrN_4NaO_2$ requires 347.0120) 349(98%), 347(100), and 303(32). Spectral data are identical to those reported for this compound in the literature.[3]

Methyl 4-vinylbenzoate (S7).[4] 4-Vinylbenzoic acid (**S6**) (2.00 g, 13.5 mmol) was dissolved in MeOH (200 mL) under Ar. Concentrated H_2SO_4 (ca. 1.6 mL) was added dropwise, using a dropping funnel, with stirring. After the addition was complete, the reaction was heated at reflux temperature for 9 h while being monitored by TLC (EtOAc). The reaction was cooled and diluted with 200 mL $CHCl_3$. This was washed with 200 mL saturated $NaHCO_3$. The aqueous layer was then extracted with 200 mL $CHCl_3$ and the combined organic phases were washed with 400 mL dH_2O followed by drying with anhydrous $MgSO_4$. The solution was filtered and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography (silica, CH_2Cl_2). After concentration and drying under high vacuum, white needles were obtained. Total yield 1.89 g (86%); mp 29-30 ^oC; δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.84 (3 H, s, C(1)COOC*H*$_3$), 5.31 (1 H, d, J = 10.9 Hz, C(4)CH=C*H*$_2$, cis), 5.80 (1 H, d, J = 17.6 Hz, C(4)CH=C*H*$_2$, trans), 6.67 (1 H, dd, J = 10.9, 17.6 Hz, C(4)C*H*=CH$_2$), 7.37 (2 H, d, J = 8.3 Hz, C(3)H and C(5)H), 7.94 (2 H, d, J = 8.3 Hz, C(2)H and C(6)H); δ_C

(300 MHz; $CDCl_3$; Me_4Si) 51.6 (C(1)$COOCH_3$), 116.1 (C(4)$CH=CH_2$), 125.8 (C3 and C5), 129.0 (C1), 129.6 (C2 and C6), 135.7 (C(4)$CH=CH_2$), 141.6 (C4), 166.3 (C(1)$COOCH_3$). Spectral data are identical to those reported for this compound in the literature.[4]

Methyl 4-[2(2-pivaloylamino-4(3*H*)-oxopyrido[2,3-*d*] pyrimidin-6-yl)ethenyl]benzoate (Methyl 2-pivaloyl-5,10-dideaza-9,10-dehydro-pteroate) (S8).[2] Methyl 4-vinylbenzoate (**S7**) (2.155 g, 13.28 mmol) and 6-bromo-2-pivaloyl-5-deazapterin (**S5**) (4.32 g, 13.28 mmol) were dissolved in CH_3CN (150 mL) with stirring. Removal of O_2 from the solution was effected by sparging with Ar for 15 min. $Pd(OAc)_2$ (149 mg, 0.664 mmol), tri-*o*-tolylphosphine (404 mg, 1.328 mmol), and Et_3N (11.1 mL, 79.68 mmol) were then added and a condenser was fitted to the vessel. An Ar atmosphere was established and maintained with the use of an Ar-filled balloon. The reaction was heated at reflux temperature for 30 h, after which time it was allowed to cool to ambient temperature overnight. The precipitate was collected by centrifugation (20 min, 4 °C, 12,000 × g). The supernatant was decanted and 200 mL of fresh CH_3CN was used to resuspend the pellet. Centrifugation and decantation was repeated as above. The pellet was then suspended in CH_3CN and transferred to a round bottom flask. Concentration and drying yielded 4.63 g of crude product. The supernatants from the above washes were combined and concentrated, in vacuo, to dryness. The residue was suspended in 40 mL CH_3CN and transferred to two 30 mL glass centrifuge tubes. These were then centrifuged (30 min, 4 °C, 12,000 × g). The supernatant was removed and the pellets were suspended and combined with 20 mL CH_3CN. Centrifugation and decantation was repeated as above. This was then repeated with another 20 mL CH_3CN. After centrifuging and decanting, the resulting small pellet was combined with the large pellet obtained previously. Drying yielded a total pellet mass of 4.98 g. Purification was achieved by flash chromatography (silica, $MeOH/CH_2Cl_2$ 5:95). All of the product-containing fractions were concentrated in vacuo and dried under high vacuum. When **S8** was excited by long wave UV light, a visible fluorescence was noted. Total yield 4.94 g (92%); δ_H (300 MHz; d_6-DMSO) 1.27 (9 H, s, C(CH_3)$_3$), 3.87 (3 H, s, $COOCH_3$), 7.61 (2 H, m, C(9)*H*=C(10)*H*), 7.79 (2 H, d, J = 8.5 Hz, C(12)H and C(16)H), 7.99 (2 H, d, J = 8.5 Hz, C(13)H and C(15)H), 8.65 (1 H, d, J = 2.3 Hz, C(5)H), 9.16 (1 H, d, J = 2.3 Hz, C(7)H), 11.47 (1 H, br s, C(2)NH), 12.33 (1 H, br s, N(3)H). Spectral data are identical to those reported for this compound in the literature.[2]

(6*RS*)-Methyl 4-[2-(2-pivaloylamino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoate ((6*RS*)-Methyl 2-pivaloyl-5,10-dideaza-5,6,7,8-tetrahydropteroate) (6*RS*-7).[2] Methyl 2-pivaloyl-5,10-dideaza-9,10-dehydro-pteroate (**S8**) (6.33 g, 15.57 mmol) was suspended with 250 mL of MeOH/THF 1:4 under Ar in a 500 mL pressure bottle. 10% Pd/C (3.31 g, 3.11 mmol) was added to the pressure bottle. The pressure bottle was sealed on the hydrogenator and evacuated until the solvent started to boil. The flask was vented to H_2 and charged to 40 psi. This was repeated two times. The flask was then charged to 50 psi and shaken for 29 h while maintaining a pressure of 50 psi. The hydrogenation was stopped and the reaction was filtered through a thick pad of Celite which was washed with MeOH, acetone, and $MeOH/CH_2Cl_2$ 5:95. The filtrate was concentrated in vacuo and purified by flash chromatography (silica, $MeOH/CH_2Cl_2$ 5:95). Product-containing fractions were combined and concentrated in vacuo to yield 3.35 g of 6*RS*-**7**. The mixed fractions containing starting material, partially reduced intermediates, and 6*RS*-**7** were combined and concentrated in vacuo, then suspended in 150 mL MeOH/THF 1:4 for another round of reduction in the presence of 1.10 g 10% Pd/C. After purification as described above, an additional 1.13 g of product was obtained. The remaining 0.75 g of partially reduced intermediates was hydrogenated as above with 150 mg 10% Pd/C to yield, after purification as above, 0.27 g of 6*RS*-**7**; Total yield, 4.75 g (74%). mp = 278-280 ºC dec (lit.,[2] mp > 250 ºC); λ_{max} (0.1 M HCl)/nm 273; (0.1 M NaOH)/nm 270; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.29 (9 H, s, C(CH_3)$_3$), 1.68 (2 H, m, C(10)H_2), 1.82 (1 H, m, C(6)H), 2.14 (1 H, dd, J = 9.3, 16.1 Hz, C(5)H_2), 2.72 (1 H, m, C(9)H_2), 2.83 (2 H, m, C(5)H_2 and C(9)H_2), 3.00 (1 H, m, C(7)H_2), 3.34 (1 H, m, C(7)H_2), 3.90 (3 H, s, $COOCH_3$), 4.72 (1 H, br s, N(8)H), 7.25 (2 H, d, J = 8.3 Hz, C(12)H and C(16)H), 7.91 (1 H, br s, N(3)H), 7.95 (2 H, d, J = 8.3 Hz, C(13)H and C(15)H), 11.26 (1 H, br s, C(2)NH); δ_C (500 MHz; $CDCl_3$; Me_4Si) 25.4 (C5), 27.1 (C(CH_3)$_3$), 30.8 (C6), 33.4 (C9), 34.8 (C10), 40.3 (*C*(CH_3)$_3$), 46.3 (C7), 52.1 ($COOCH_3$), 90.0 (C4a), 128.1 (C14), 128.5 (C12 and C16), 129.9 (C13 and 15), 147.7 (C11), 148.2 (C8a), 158.1 (C4), 160.6 (C2), 167.2 (*C*$OOCH_3$), 179.6 (C2NH*C*OC(CH_3)$_3$); RP-HPLC (Method 1), t_r = 13.2 min, 97.6% purity. Chiral-HPLC, t_r = 10.5, 14.8 min, 98.5% purity. Spectral data are identical to those reported for this compound in the literature.[2]

(6*RS*)-4-[2-(2-Amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido [2,3-*d*]pyrimidin-6-yl)ethyl]benzoic acid ((6*RS*)-5,10-Dideaza-5,6,7,8-tetrahydropteroic acid) (6*RS*-8). (6*RS*)-Methyl 2-pivaloyl-5,10-dideaza-5,6,7,8-tetrahydropteroate (6*RS*-**7**) (2.83 g, 6.86 mmol) was suspended in 68.6 mL of 1 M NaOH. A reflux condenser was fitted to the flask and the reaction was heated at reflux temperature for 30 min after the starting material had completely dissolved. The flask was then cooled to ambient temperature, and the solution was filtered through a paper filter, which was then washed with water. The pH of the filtrate was adjusted to 1 with 74 mL of 1 M HCl using pH 0-12 pH paper while stirring. The suspension was lyophilized to a small volume and resuspended in water for centrifugation. The suspension was transferred to four 30 mL glass centrifuge tubes which were then centrifuged (15 min, 4 °C, 12,000 × g) to obtain the desired product as a pellet. The pellets were then washed with ddH_2O (2 × 70 mL), CH_3CN (2 × 70 mL), and Et_2O (1 × 70 mL). The product was dried under high vacuum in the presence of P_2O_5. Product was determined to be 100% pure by RP-HPLC (Method 1). Total yield 2.195 g (91%); λ_{max} (0.1 M HCl)/nm 242 and 279; (0.1 M NaOH)/nm 272; δ_H (300 MHz; d_6-DMSO) 1.61 (3 H, m, C(6)H and C(10)H_2), 1.88 (1 H, m, C(5)H_2), 2.55 (1 H, m, C(9)H_2, partially obscured by residual DMSO peak), 2.72 (2 H, m, C(5)H_2 and C(9)H_2), 2.85 (1 H, m, C(7)H_2), 3.29 (1 H, br d, J = 11.0 Hz, C(7)H_2), 6.95 (2-3 H, br s, NH), 7.35 (2 H, d, J = 8.1 Hz, C(12)H and C(16)H), 7.86 (2 H, d, J = 8.1 Hz, C(13)H and C(15)H); δ_H (500 MHz; 0.1 M NaOD in D_2O) 1.64 (2 H, m, C(10)H_2), 1.78 (1 H, m, C(6)H), 2.01 (1 H, dd, J = 9.1, 15.5 Hz, C(5)H_2), 2.57 (1 H, m, C(9)H_2), 2.76 (2 H, m, C(5)H_2 and C(9)H_2), 2.88 (1 H, m, C(7)H_2), 3.27 (1 H, m, C(7)H_2), 7.34 (2 H, d, J = 7.6 Hz, C(12)H and C(16)H), 7.78 (2 H, d, J = 7.6 Hz, C(13)H and C(15)H); δ_C (500 MHz; 0.1 M NaOD in D_2O) 26.3(C5), 31.4(C6), 32.9(C9), 34.8(C10), 45.7(C7), 87.5(C4a), 128.7(C12 and C16), 129.5(C13 and C15), 134.0(C14), 147.0(C11), 161.0(C8a), 161.6(C2), 174.2(C4), 176.0(C17); *m/z* (EI+) 314.1381 ($C_{16}H_{18}N_4O_3$ requires 314.1379), 315.2(24%), 314.2(100), 179.1(23), 166.1(100), 165.1(90), 164.1(38), 163.1(41), 151.1(68), 149.1(26), 135.1(30), 107.1(21), 91.1(30), 77.1(21), 57.1(25), 55.1(20), 44.0(33), 43.0(29), 41.0(58), 40.0(29), 39.0(20), 36.0(44); RP-HPLC (Method 1), t_r = 12.8 min. Spectral data are identical to those reported for this compound in the literature.[2]

(6*RS*)-5,10-Dideaza-5,6,7,8-tetrahydropteroyl azide (6*RS*-9). (6*RS*)-5,10-Dideaza-5,6,7,8-tetrahydropteroic acid (6*RS*-**8**) (50 mg, 0.14 mmol) was added under Ar to a dry flask containing a stir bar. The flask was then sealed with a septum under an atmosphere of Ar (balloon) before the addition of 1 mL anhydrous DMSO. The solution was cooled in a 20 °C water bath before the addition of Et_3N (112 µL, 0.8 mmol). After 15 min, cold ethyl chloroformate (15 µL, 0.154 mmol) was added. After 45 min, NaN_3 (11 mg, 0.17 mmol) was then added. The reaction was allowed to stir for 1 h at ambient temperature. The reaction completion was verified by RP-HPLC, Method 1. The

reaction solution was diluted with 6 mL of 0.1 M HCl for precipitation. The precipitate was collected by centrifugation (15 min, 4 °C, 12,000 × g). The supernatant was removed and the pellet was washed with: 1 × 10 mL ddH_2O; 1 × 10 mL CH_3CN; and 1 × 5 mL Et_2O. The pellet was allowed to air dry for 15 min before drying under high vacuum in the presence of P_2O_5 for several hours. Total mass recovery, 51 mg, a mixture of 6*RS*-**9** (89.6%) and 6*RS*-**8** based on RP-HPLC (Method 1); δ_H (300 MHz; d_6-DMSO) 1.59 (3 H, m, C(6)H and C(10)H_2), 1.86 (1 H, m, C(5)H_2), 2.54 (1 H, m, C(9)H_2, partially obscured by residual DMSO peak), 2.74 (2 H, m, C(5)H_2 and C(9)H_2), 2.82 (1 H, m, C(7)H_2), 3.24 (1 H, m, C(7)H_2), 6.60 (2-3 H, br s, NH), 7.42 (2 H, d, J = 8.1 Hz, C(12)H and C(16)H), 7.89 (2 H, d, J = 8.1 Hz, C(13)H and C(15)H), 10.29 (1 H, br s, N(3)H); RP-HPLC (Method 1), t_r = 30.4 min; Ion-pair HPLC, t_r = 30.0 min. This material was used in subsequent coupling reactions without further purification.

(6*RS*)-5,10-Dideaza-5,6,7,8-tetrahydrofolic acid, $DDAH_4$Pte Glu_1 (6*RS*-1). Synthesized as described in the main text for the synthesis of 6*R*-**1** and 6*S*-**1**. Yield, 77%; λ_{max} (0.1 M HCl)/nm 242 and 279; (0.1 M NaOH)/nm 242 and 272; δ_H (500 MHz; 0.1 M NaOD/D_2O) 1.61 (2H, dd, J = 6.8, 14.2 Hz, C(10)H_2), 1.73 (1 H, m, C(6)H), 1.97 (2 H, dd, J = 8.7, 15.3 Hz, C(5)H_2, C(3′)H_2), 2.11 (1 H, m, C(3′)H_2), 2.25 (2 H, dd, J = 6.7, 7.8 Hz, C(4′)H_2), 2.53 (1 H, m, C(9)H_2), 2.75 (2 H, m, C(5)H_2 and C(9)H_2), 2.84 (1 H, m, C(7)H_2), 3.23 (1 H, br d, J = 11.6 Hz, C(7)H_2), 4.26 (1 H, m, C(2′)H), 7.36 (2 H, d, J = 6.6 Hz, C(12)H and C(16)H), 7.69 (2 H, d, J = 6.6 Hz, C(13)H and C(15)H); δ_C (500 MHz; 0.1 M NaOD/D_2O) 26.3, 28.8, 31.4, 32.9, 34.7, 45.7, 56.4, 87.5, 122.5, 127.7, 129.1, 131.3, 148.0, 161.0, 161.7, 170.6, 174.3, 179.4, 182.7; *m/z* (ESI^+) 444.1884 ($M+H^+$. $C_{21}H_{26}N_5O_6$ requires 444.1883) 445(29%), and 444(100); Ion-pair HPLC, t_r = 17.7 min.

L-Glutamate-γ-L-glutamate (γ-Glu_2) – Solution phase synthesis. N-Cbz-L-glutamate-γ-L-glutamate[9] (0.314 g, 0.76 mmol) was dissolved in 10 mL ddH_2O before the addition of 10% Pd/C (85 mg, 0.08 mmol). The solution was then hydrogenated at 40 psi H_2 for 14 h. The reaction was filtered through Celite with the aid of ddH_2O. The filtrate was concentrated by lyophilization to yield crude di-peptide (0.212 g). Purification was attempted by size-exclusion chromatography but proved unsuccessful. The presumed tri-ammonium salt of the dipeptide was recovered by lyophilization. Total yield 190 mg. A portion of this material, 26.0 mg, was then purified by semi-preparative RP-HPLC (Method 1S), t_r = 10.0 min to obtain the TFA salt of the di-peptide after lyophilization. Total yield 24.8 mg (80%). δ_H (500 MHz; 1% v/v TFA in D_2O) 1.86 (1 H, m, C(8')H_2), 2.07 (3 H, m, C(3')H_2 and C(8')H_2), 2.34 (2 H, m, C(9')H_2), 2.40 (2 H, m, C(4')H_2), 3.95 (1 H, dd, J = 4.1, 6.1 Hz, C(2')H), 4.26 (1 H, t, J = 4.3 Hz, C(7')H); δ_C (500 MHz; 1% v/v TFA in D_2O) 25.7(C8'), 25.9(C3'), 30.3(C9'), 31.1(C4'), 52.4(C7'), 52.5(C2'), 171.7(C=O), 174.6(C=O), 175.3(C=O), 177.4(C=O); *m/z* (ESI^+) 277.1034. ($C_{10}H_{17}N_2O_7$ requires 277.1036).

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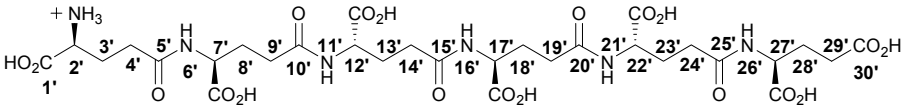
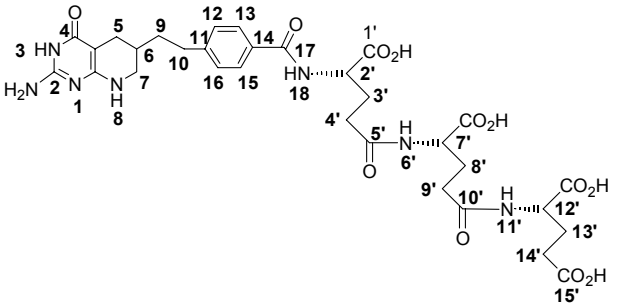


Scheme S2 Numbering system used for the NMR assignments of poly-γ-Glu_6. A similar numbering system was used in the NMR assignments of all of the shorter chain-length γ-glutamyl peptides characterized above.



Scheme S3 Numbering system used for the NMR assignments of $DDAH_4PteGlu_3$. A similar numbering system was used in the NMR assignments of all compounds containing the 5-deazapterin moiety.

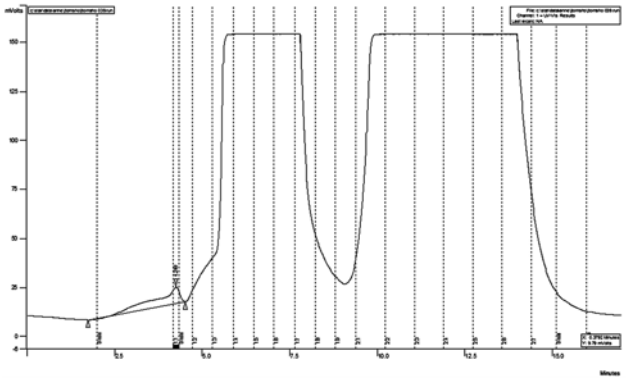


Figure S1 A typical chromatogram of the preparative chiral HPLC method used for the separation of the isomers of 6*RS*-**7**. Eluant A – hexane. Eluant B – EtOH. Column – Chiralcel OJ, 2 cm x 25 cm. Isocratic at 70% B. Flow rate – 30 mL/min. Run time – 22 min. Sample concentration – 1.25 mg/mL in 25% A: 75% B. Monitored at 240 nm.

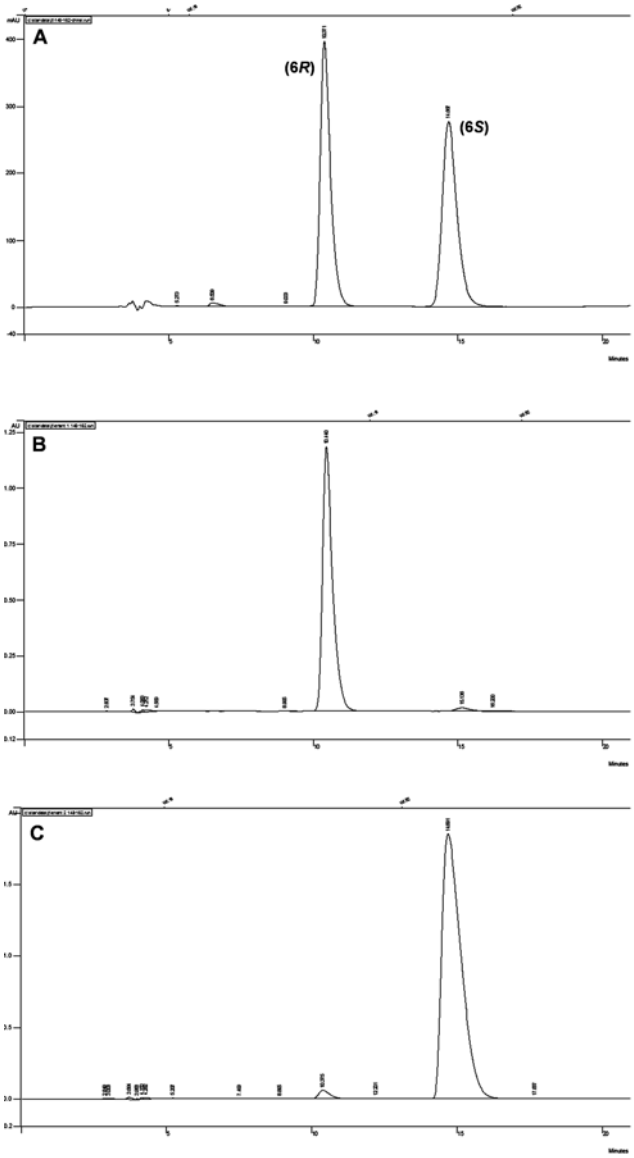


Figure S2 A typical chromatogram of the analytical chiral HPLC method used for the resolution of 6*RS*-**7** (Panel A) into its individual isomers, (6*R*)- (Panel B) and (6*S*)- (Panel C). Eluant A - ddH_2O. Eluant B – CH_3CN. Column – Chiral Technologies (DAICEL Chemical Industries, LTD) Chiralcel OJ-R, 4.6 x 150 mm. Isocratic at 40% B. Flow rate – 0.5 mL/min. Detection at 280 nm.