

First Synthesis of 5,6,7,8-Tetrahydro-8-deaza-8-thiafolic Acid

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5,6,7,8-Tetrahydro-8-deaza-8-thiafolic acid (**3**) was synthesized as a diastereoisomeric mixture *via* thermal condensation of 5-hydroxyisocytosine (**6**) with diethyl *N*-[*p*-(2-amino-3-mercaptopropyl)aminobenzoyl]glutamate (**10**) after activation of the C(6)-position in **6** with *N*-bromosuccinimide/ethanol. The corresponding *N*⁵,*N*¹⁰-methylene derivative (**5**) was also prepared upon treatment of **3** with formaldehyde.

Keywords 5,6,7,8-tetrahydro-8-deaza-8-thiafolic acid; 5-hydroxyisocytosine; *N*-[*p*-(2-amino-3-mercaptopropyl)aminobenzoyl]glutamate; thermal condensation; formaldehyde; *N*⁵,*N*¹⁰-methylene-5,6,7,8-tetrahydro-8-deaza-8-thiafolic acid

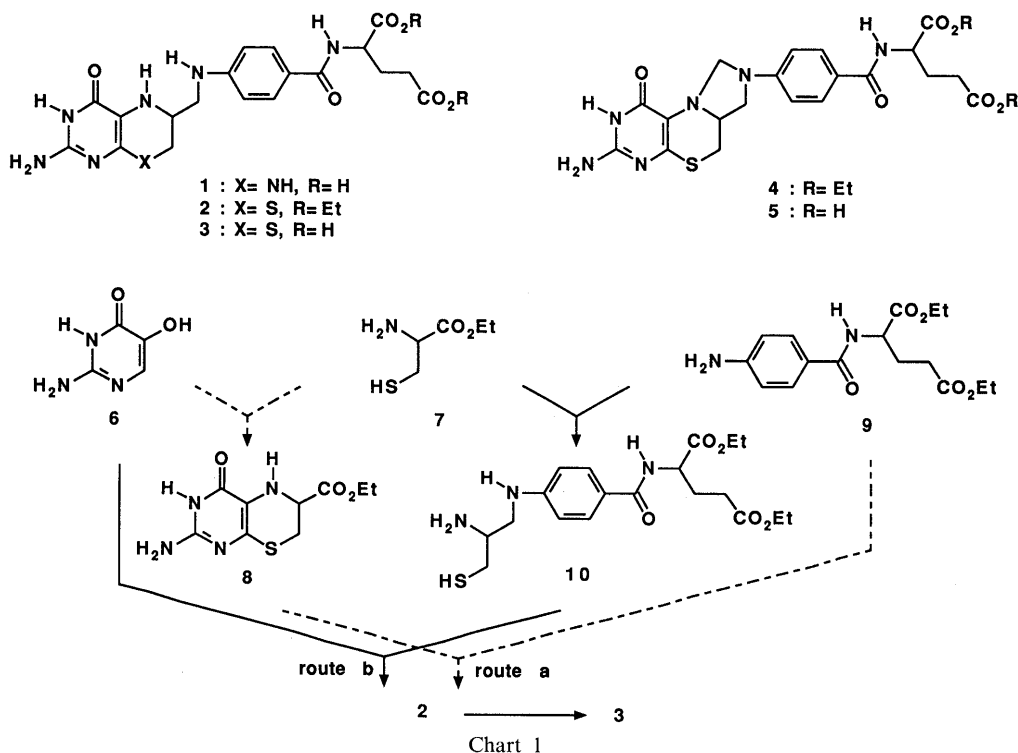
Tetrahydrofolic acid (**1**) is an active form of folic acid and plays significant roles as a cofactor for one-carbon unit transfer in the *de novo* synthesis of nucleosides and in amino acid biosyntheses. The functions of **1** as a biological catalyst have been extensively studied in the fields of chemistry and biochemistry.¹⁾ Although catalytic reduction or chemical reduction of folic acid results in the formation of **1**, its tetrahydropyrazine ring is sensitive to autoxidation, particularly in solution,²⁾ and so its handling during studies on the functions is somewhat troublesome. Recently, deaza analogues of **1** have been shown to be an important class of folate antimetabolites of interest as potential oncolytic agents.³⁾ Within this series, 5,10-deaza-5,6,7,8-tetrahydrofolic acid has shown exceptionally high activity in a variety of animal models of malignancy.⁴⁾

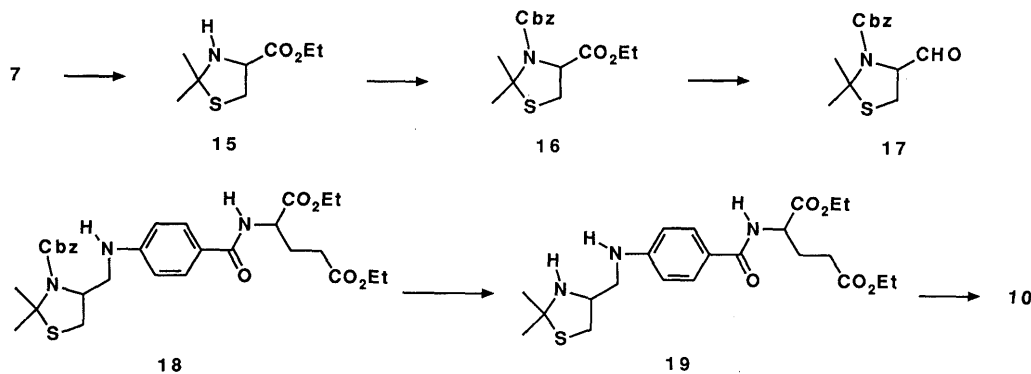
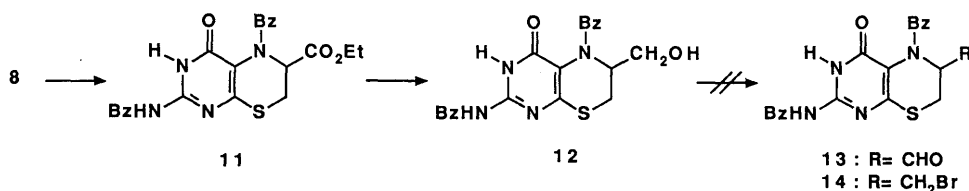
In a previous paper,⁵⁾ we described a new method for the construction of the reduced pyrimido[4,5-*b*][1,4]thiazine-4(3*H*)-one ring system, providing access to 1,5-dihydro-10-deaza-10-thiaisoalloxazines and 5,6,7,8-tetrahydro-8-deaza-8-thiapterins,^{6,7)} which are fairly stable even in solution⁸⁾ compared with the parent 1,5-dihydroisoalloxazines and

5,6,7,8-tetrahydropterins. In this context, we planned to prepare 5,6,7,8-tetrahydro-8-deaza-8-thiafolic acid (**3**) and its *N*⁵,*N*¹⁰-methylene derivative (**5**) by employing the new methodology as a key step.

The tetrahydrofolate analogue (**3**) is anticipated to be a stable material and is of interest as a possible folate antimetabolite and as a viable model compound of **1**. For the construction of **3** based on the condensation of 5-hydroxyisocytosine (**6**) with β -aminothiols,⁵⁾ two synthetic approaches are envisaged. Route a involves the condensation of **6** with cysteine ethyl ester (**7**) leading to 6-ethoxycarbonyl-5,6,7,8-tetrahydro-8-deaza-8-thiapterin (**8**), followed by coupling with diethyl *N*-(*p*-aminobenzoyl)glutamate (**9**). Route b is the direct condensation of **6** with diethyl *N*-[*p*-(2-amino-3-mercaptopropyl)aminobenzoyl]glutamate (**10**), which is derived from **7** and **9** (see Chart 1, route a and route b).

As reported previously,⁵⁾ bromination of **6** with *N*-bromosuccinimide in ethanol followed by treatment with L-cysteine ethyl ester (**7**) resulted in the formation of **8** (47% yield), the C(6)-position of which was racemized to some extent.





N-Benzoyl protection of **8** gave the *N*²,*N*⁵-dibenzoyl derivative (**11**) which was reduced with sodium borohydride to give the 6-hydroxymethyl derivative (**12**) in 63% yield. Attempts to convert **12** into the corresponding aldehyde (**13**) or methylene bromide (**14**), however, were unsuccessful because of the low solubility in the solvent employed or the low stability under the conditions employed. Other attempts to condense **11** or **12** with **9** gave unsatisfactory results. Thus, an alternative route b was investigated in place of route a.

Synthesis of the counterpart **10** for the condensation with **6** in route b was achieved as follows (see Chart 3).

The natural tetrahydrofolic acid (**1**) has been shown to adopt the *S*-configuration at the C(6)-position.⁹ Employment of *D*-cysteine ethyl ester (**7**) as a starting material could provide **3** possessing the same 6*S* configuration as that of **1**. In the present conversion into **10** under the conditions employed, however, the occurrence of racemization in the cysteine moiety seems to be unavoidable. Thus, *D,L*-cysteine ethyl ester (**7**) was used as a starting material, aiming at synthesis of **3** as a diastereo-isomeric mixture to exclude stereochemical ambiguity. Isopropylidene protection of **7** (*D,L*-, HCl salt) gave 2,2-dimethyl-4-ethoxycarbonylthiazolidine (**15**) in 74% yield as the HCl salt.¹⁰ Since the free thiazolidine (**15**) obtained by base treatment was unstable, **15** was converted into the *N*-benzyloxycarbonyl (Cbz) derivative (**16**) (87% yield). Reduction of **16** with diisobutyl aluminum hydride (DIBAH) in dry toluene at -78°C resulted in the formation of the corresponding aldehyde (**17**) in 83% yield. Condensation of **17** with the *N*-(*p*-aminobenzoyl)-*L*-glutamate (**9**) was carried out in dry ethanol containing glacial acetic acid. Without isolation of the intermediary product, its reduction with sodium cyanoborohydride followed by chromatographic purification gave the condensed product (**18**) in 13% overall yield from **7**. Deprotections of the Cbz group using aluminum chloride-anisole¹¹ and of the isopropylidene group by heating at 60°C in ethanol

containing a catalytic amount of hydrochloric acid led to the formation of **10** in a high yield. The fragmentation pattern and the parent peak [m/z 411 (M^+)] in its mass spectrum (MS) fully support the structure of **10**, and its proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectrum was also consistent with this structure. The specific optical rotation of **10** was totally lost. Thus, **10** is a mixture of four diastereo-isomers which arise from the racemization of the glutamate moiety during the reactions.

The synthetic counterpart (**10**) thus prepared was sensitive to autoxidation and decomposed gradually in an acidic solution. Accordingly, **10** was subjected to condensation with **6** in a neutral medium without purification. After treatment of **6** with *N*-bromosuccinimide in ethanol, the reaction mixture was neutralized with sodium hydrogen carbonate. Then **10** was added to the neutral solution and the mixture was refluxed under argon. Monitoring of the reaction by TLC revealed that a prolonged reaction time of about 40 h was necessary. The desired product **2** was separated by silica gel chromatography in 14% yield. Its $^1\text{H-NMR}$, ultraviolet (UV), and fast atom bombardment-MS (FAB-MS) spectra supported the structure of **2**. Hydrolysis of **2** was achieved almost quantitatively upon treatment with ethanolic caustic alkali to give 5,6,7,8-tetrahydro-8-deaza-8-thiafolic acid (**3**) as a mixture of diastereo-isomers. The structure of **3** was confirmed by $^1\text{H-NMR}$, UV, infrared (IR), and FAB-MS spectral data (see Experimental).

When a suspension of **2** in a 0.025 *M* formaldehyde solution was adjusted to pH 5 and then stirred, the corresponding *N*⁵,*N*¹⁰-methylene derivative (**4**) was obtained as a precipitate.¹² The structure of **4** was supported by its spectral data, e.g., a C_{11} -methylene signal (δ 4.05, 5.21, $J=4$ Hz) newly appeared in its $^1\text{H-NMR}$ spectrum. Analogously, *N*⁵,*N*¹⁰-methylene-8-deaza-8-thiafolic acid (**5**) was prepared from **3** almost quantitatively.

Although the present synthesis of **3** is not satisfactory in terms of the overall yield and stereoselectivity, it is the first synthesis of **3**, which has intriguing chemical and

biochemical properties. Biological evaluation of **3** as a folate antimetabolite is in progress.

Experimental

All melting points (uncorrected) were determined on a Yanagimoto micro hot-stage apparatus. Elemental analyses were performed by the microanalytical laboratory of our university. Spectroscopic measurements for the structural assignment of the reaction products were performed with the following instruments: IR spectra with a Hitachi Model 215 spectrometer; UV absorption spectra with a Shimadzu 260 spectrophotometer; $^1\text{H-NMR}$ spectra with a JEOL JNM-GX 270 (270 MHz) Fourier transform-NMR (FT-NMR) spectrometer using tetramethylsilane as an internal standard; MS and high-resolution mass spectra (HR-MS) with a JEOL JMS-D 300 machine operating at 70 eV; FAB-MS with a JEOL JMS-DX 300 machine. Thin-layer chromatographic (TLC) analyses were carried out on precoated Silicagel 60 F_{254} plates (Merck, Art 5715). Column chromatography was accomplished by using silica gel (Wakogel C-300).

6-Ethoxycarbonyl-5,6,7,8-tetrahydro-8-deaza-8-thiapterin (8) *N*-Bromosuccinimide (2.13 g, 12.0 mmol) was added to a suspension of 5-hydroxyisocytosine (**6**) (HCl salt, 1.64 g, 10.0 mmol) in EtOH (50 ml). After stirring of the mixture at room temperature for 1 h followed by the addition of *D,L*-cysteine ethyl ester (**7**) (HCl salt, 5.57 g, 30.0 mmol), the reaction mixture was refluxed overnight, then concentrated to a small volume (*ca.* 5 ml) under reduced pressure, and diluted with water (5 ml). The aqueous solution was neutralized with NaHCO_3 under ice-cooling. The precipitated crystalline mass was collected and recrystallized from EtOH to give **8** (1.20 g, 47.0%). The structure of **8** was confirmed by spectral comparison with the authentic compound previously reported.⁵⁾

***N*,*N*'-Dibenzoyl-6-ethoxycarbonyl-5,6,7,8-tetrahydro-8-deaza-8-thiapterin (11)** Benzoyl chloride (1.2 ml, 10.3 mmol) was added dropwise to a solution of **8** (128 mg, 0.5 mmol) in pyridine (2.0 ml, 24.7 mmol) under ice-cooling, and the mixture was stirred at room temperature for 2 h. After removal of the solvent under reduced pressure, the residual oil was dissolved in CHCl_3 (30 ml). The CHCl_3 solution was washed well with dilute HCl, dried over anhydrous MgSO_4 , and evaporated to dryness. The resulting residue was purified by silica gel column chromatography with CHCl_3 - Me_2CO (30:1) to give **11** (141 mg, 61%) as an oil. *Rf*=0.6 (CHCl_3 : Me_2CO =10:1). HR-MS Calcd for $\text{C}_{23}\text{H}_{20}\text{N}_4\text{O}_5\text{S}$: 464.1181. Found: 464.1206 (M^+). MS *m/z*: 464 (M^+ , 2%), 391 (M^+ - CO_2Et , 2%), 360 (2%), 287 (9%), 105 (100%). IR $\nu_{\text{max}}^{\text{KBr}}$: 3200 (NH), 1740 (C=O), 1640 (C=O) cm^{-1} . UV $\lambda_{\text{max}}^{\text{EtOH}}$: 331, 244 nm. $^1\text{H-NMR}$ (CD_3OD) δ : 1.38 (3H, t, *J*=7 Hz, OCH_2CH_3), 3.35–3.55 (2H, m, $\text{C}_7\text{-H} \times 2$), 4.32 (2H, q, *J*=7, 14 Hz, OCH_2CH_3), 4.60 (1H, dd, *J*=3.9, 4.4 Hz, $\text{C}_6\text{-H}$), 7.63–7.73 (6H, m, aromatic H), 8.05 (4H, brd, *J*=8 Hz, aromatic H).

***N*,*N*'-Dibenzoyl-6-hydroxymethyl-5,6,7,8-tetrahydro-8-deaza-8-thiapterin (12)** MeOH (0.1 ml) was added dropwise to a suspension of **11** (140 mg, 0.3 mmol) and NaBH_4 (46 mg, 1.2 mmol) in dry tetrahydrofuran (THF) (20 ml) at 5°C, and the mixture was stirred at room temperature until **11** was no longer detectable (monitored by TLC; *ca.* 2 h). After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography and eluted with CHCl_3 -MeOH (10:1) to isolate **12** (80 mg, 63%). mp 251–252°C (MeOH). *Rf*=0.27 (CHCl_3 : Me_2CO =10:1). Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}_4\text{S}$: C, 59.71; H, 4.30; N, 13.26. Found: C, 59.42; H, 4.38; N, 13.35. MS *m/z*: 422 (M^+ , 12%), 300 (7%), 287 (24%), 105 (100%). IR $\nu_{\text{max}}^{\text{KBr}}$: 3350 (OH), 3150 (NH), 1690 (C=O), 1670 (C=O) cm^{-1} . UV $\lambda_{\text{max}}^{\text{EtOH}}$: 330, 259 nm. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 3.20–3.70 (5H, m, $\text{C}_7\text{-H} \times 2$, $\text{C}_6\text{-CH}_2\text{OH}$, $\text{C}_6\text{-H}$), 5.15 (1H, brs, OH), 7.47 (1H, br, NH), 7.5–8.1 (10H, m, aromatic H), 11.93 (1H, br, NH).

2,2-Dimethyl-4-ethoxycarbonylthiazolidine (15) A solution of cysteine ethyl ester (*D,L*-**7**) (HCl salt, 11.1 g, 60.0 mmol) in acetone (300 ml) was refluxed for 3 h. After cooling, the precipitated crystalline mass was collected and dissolved in water (20 ml). The aqueous solution was neutralized with NaHCO_3 and extracted with CHCl_3 (50 ml \times 3). The collected extract was dried over anhydrous MgSO_4 and evaporated to dryness. The resulting residue was purified by column chromatography with CHCl_3 to isolate **15** (8.39 g, 74%) as an oil. *Rf*=0.38 (benzene:EtOAc=5:1). bp 75°C (4.0 mmHg). Anal. Calcd for $\text{C}_8\text{H}_{15}\text{NO}_2\text{S}$: 1/4 H_2O : C, 49.60; H, 8.07; N, 7.23. Found: C, 49.67; H, 7.95; N, 7.28. MS *m/z*: 189 (M^+ , 51%), 174 (M^+ -Me, 22%), 142 (28%), 116 (M^+ - CO_2Et , 81%), 99 (58%). IR $\nu_{\text{max}}^{\text{KBr}}$: 3300 (NH), 1740 (C=O) cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.31 (3H, t, *J*=7 Hz, OCH_2CH_3), 1.53, 1.72 (each

3H, each s, $\text{C}_2\text{-Me} \times 2$), 2.49 (1H, br, NH), 3.03 (1H, q, *J*=9, 11 Hz, $\text{C}_5\text{-H}$), 3.44 (1H, q, *J*=7, 11 Hz, $\text{C}_5\text{-H}$), 4.08 (1H, q, *J*=7, 9 Hz, $\text{C}_4\text{-H}$), 4.25 (2H, q, *J*=7, 14 Hz, OCH_2CH_3).

3-Benzoyloxycarbonyl-2,2-dimethyl-4-ethoxycarbonylthiazolidine (16) Benzoyloxycarbonyl chloride (9.08 ml, 63.6 mmol) was added to a solution of **15** (10.977 g, 58.1 mmol) and NaHCO_3 (9.7 g, 115 mmol) in EtOH (120 ml) and water (120 ml) under ice-cooling. The mixture was stirred at room temperature overnight, then extracted with CHCl_3 (100 ml \times 3), and the combined CHCl_3 solution was dried over anhydrous MgSO_4 . After removal of the solvent under reduced pressure, the resulting residue was purified by column chromatography with *n*-hexane-Et₂O (5:1) to give **16** (16.3 g, 87%) as an oil. *Rf*=0.73 (benzene:EtOAc=5:1). HR-MS Calcd for $\text{C}_{16}\text{H}_{21}\text{NO}_4\text{S}$: 323.1187. Found: 323.1182. MS *m/z*: 323 (M^+ , 2%), 308 (M^+ -Me, 11%), 264 (4%), 250 (4%), 91 (100%). IR $\nu_{\text{max}}^{\text{neat}}$: 1750 (C=O), 1700 (C=O) cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.17 (0.63 \times 3H), 1.27 (0.37 \times 3H) (each t, *J*=each 7 Hz, OCH_2CH_3), 1.74 (0.37 \times 3H), 1.81 (0.37 \times 3H), 1.83 (0.63 \times 3H), 1.91 (0.63 \times 3H) (each s, $\text{C}_2\text{-Me} \times 2$), 3.15 (1H, br dd, *J*=2, 12 Hz, $\text{C}_5\text{-H}$), 3.29 (1H, dd, *J*=7, 12 Hz, $\text{C}_5\text{-H}$), 4.0–4.2 (2H, m, OCH_2CH_3), 4.8–5.2 (3H, m, $\text{C}_4\text{-H}$, benzyl protons), 7.3–7.4 (5H, m, aromatic H).

3-Benzoyloxycarbonyl-2,2-dimethyl-4-formylthiazolidine (17) A 1.0 M solution of DIBAH in toluene (43 ml, 43 mmol) was added dropwise to a stirred solution of **16** (8.193 g, 25.4 mmol) in dry toluene (20 ml) at -78°C over 1 h, and the mixture was stirred for an additional 3 h at -78°C under an argon atmosphere. The reaction was quenched by slowly adding cold MeOH so as to keep the internal temperature below -65°C. The resulting white emulsion was slowly poured into a cold 1 N HCl solution and then the aqueous mixture was extracted with EtOAc (100 ml \times 3). The collected EtOAc solution was dried over MgSO_4 and evaporated to dryness. The residue was applied to a silica gel column and eluted with *n*-hexane-EtOAc (10:1) to give **17** (5.91 g, 83%) as an oil. *Rf*=0.26 (*n*-hexane:Et₂O=2:1). MS *m/z*: 264 (M^+ -Me, 2%), 250 (M^+ -CHO, 11%), 206 (7%), 91 (100%). IR $\nu_{\text{max}}^{\text{film}}$: 1740 (C=O), 1710 (C=O) cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.79 (0.4 \times 3H), 1.81 (0.4 \times 3H), 1.84 (0.6 \times 3H), 1.90 (0.6 \times 3H) (each brs, $\text{C}_2\text{-Me} \times 2$), 3.07–3.15 (2H, m, $\text{C}_5\text{-H} \times 2$), 4.70 (0.6H), 4.80 (0.4H) (each brs, $\text{C}_4\text{-H}$), 5.11 (0.6 \times 2H), 5.21 (0.4 \times 2H) (each s, benzyl protons), 7.2–7.3 (5H, m, aromatic H), 9.51 (1H, s, CHO).

Diethyl *N*-[*p*-(3-Benzoyloxycarbonyl-2,2-dimethylthiazolidin-4-yl)methylaminobenzoyl]glutamate (18) A solution of **17** (9.0 g, 32.2 mmol) and diethyl *N*-(*p*-aminobenzoyl)glutamate (10.4 g, 32.3 mmol) in dry EtOH (100 ml)-AcOH (0.5 ml) was stirred at room temperature until **17** was no longer detectable (monitored by TLC; *ca.* 12 h). Then, NaBH_3CN (2.03 g, 32.3 mmol) was added to the reaction mixture and the solution was stirred for 6 h at room temperature. After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography. Elution with benzene-EtOAc (20:1) provided **18** (4.54 g, 24%) as an oil. *Rf*=0.31 (benzene:EtOAc=5:1). MS *m/z*: 585 (M^+ , 5%), 539 (1%), 383 (M^+ -glutamate, 11%), 336 (7%), 204 (18%), 148 (44%), 132 (40%), 91 (100%). $^1\text{H-NMR}$ (CDCl_3) δ : 1.21, 1.30 (each 3H, each t, *J*=7 Hz, OCH_2CH_3), 1.81 (6H, brs, $\text{C}_2\text{-Me} \times 2$), 2.1–2.6 (4H, m, β - and γ -methylene protons of glutamate), 2.81 (1H, brd, *J*=12 Hz, $\text{C}_5\text{-H}$), 3.17 (1H, m, $\text{C}_5\text{-H}$), 3.3–3.6 (2H, m, $\text{C}_4\text{-CH}_2\text{NH}$), 4.10, 4.23 (each 2H, each q, *J*=each 7, 14 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 4.53 (1H, br, $\text{C}_4\text{-H}$), 4.79 (1H, q, *J*=8, 12 Hz, $\text{C}_\alpha\text{-H}$ of glutamate), 5.18 (2H, brs, benzyl protons), 6.45, 6.67 (each 1H, each br, NH $\times 2$), 6.6, 7.7 (each 2H, each br, aromatic H), 7.4–7.6 (5H, m, aromatic H).

Diethyl *N*-[*p*-(2,2-Dimethylthiazolidin-4-yl)methylaminobenzoyl]glutamate (19) A suspension of **18** (7.846 g, 13.4 mmol) and AlCl_3 (5.3 g, 40 mmol) in anisole (300 ml) was stirred at room temperature overnight. The reaction was quenched by the addition of water and the aqueous mixture was extracted with EtOAc (100 ml \times 3). The collected EtOAc solution was dried over anhydrous MgSO_4 and evaporated to dryness. The residue was subjected to column chromatography and elution with CHCl_3 - Me_2CO (10:1) provided **19** (4.54 g, 75%) as an oil. *Rf*=0.17 (CHCl_3 : Me_2CO =5:1). HR-MS Calcd for $\text{C}_{22}\text{H}_{33}\text{N}_3\text{O}_5\text{S}$: 451.2141. Found: 451.2165. MS *m/z*: 451 (M^+ , 1%), 436 (M^+ -Me, 1%), 361 (10%), 336 (38%), 249 (40%), 204 (100%). $^1\text{H-NMR}$ (CDCl_3) δ : 1.21, 1.29 (each 3H, each t, *J*=each 7 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 1.57, 1.67 (each 3H, each s, $\text{C}_2\text{-Me} \times 2$), 2.0–2.6 (4H, m, β - and γ -methylene protons of glutamate), 2.79 (1H, t, *J*=10 Hz, NH), 3.2–3.3 (2H, m, $\text{C}_5\text{-H}$), 3.4–3.6, 3.65–3.85 (each 1H, each m, $\text{C}_4\text{-CH}_2\text{NH}$), 4.09, 4.21 (each 2H, each q, *J*=each 7, 14 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 4.0–4.3 (1H, m, $\text{C}_4\text{-H}$), 4.54 (1H, br, N_3H), 4.77 (1H, m, $\text{C}_\alpha\text{-H}$ of glutamate), 6.60, 7.68 (each 2H, each d, *J*=8 Hz, aromatic H), 6.83 (1H, d, *J*=3 Hz, CONH).

Diethyl *N*-[*p*-(2-Amino-3-mercaptopropyl)aminobenzoyl]glutamate (10) A solution of **19** (3.0 g, 6.65 mmol) in EtOH (70 ml) containing 1 N HCl (6.6 ml) was heated at 60 °C until **19** was no longer detectable (monitored by TLC). After removal of the solvent under reduced pressure, the resulting residue was employed without purification for condensation with **6**. MS *m/z*: 411 (M^+ , 4%), 374 (2%), 336 (4%), 322 (5%), 132 (59%), 120 (100%). $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.19, 1.20 (each 3H, each t, J =each 7 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 1.9–2.2 (2H, m, β -methylene protons of glutamate), 2.3–2.6 (2H, m, γ -methylene protons of glutamate), 2.85 (2H, br, $\text{CH}_2\text{-SH}$), 3.04 (1H, t, J =8 Hz, SH), 3.3–3.6 (3H, m, CHCH_2NH), 4.07, 4.11 (each 2H, each q, J =each 7, 14 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 4.39 (1H, m, C_α -H of glutamate), 6.57 (1H, br, NH), 6.69, 7.69 (each 2H, each d, J =8 Hz, aromatic H), 8.30 (2H, m, NH_2).

Diethyl 5,6,7,8-Tetrahydro-8-deaza-8-thiafolate (2) *N*-Bromosuccinimide (1.43 g, 7.98 mmol) was added to a suspension of **6** (HCl salt, 1.09 g, 6.65 mmol) in EtOH (40 ml), and the mixture was stirred at room temperature for 1.0 h. After the addition of **10** prepared from **19** (6.65 mmol), the mixture was neutralized with NaHCO_3 and refluxed under an argon atmosphere for 40 h. After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography. Elution with CHCl_3 -MeOH (20:1) provided **2** (487 mg, 14%) as an amorphous powder. *Rf*=0.45 (CHCl_3 :MeOH=5:1). FAB-MS *m/z*: 519 [$M+H$] $^+$. IR $\nu_{\text{max}}^{\text{KBr}}$: 3350 (NH), 1730 (C=O), 1650 (C=O) cm^{-1} . UV $\lambda_{\text{max}}^{\text{MeOH}}$: 298, 218 nm. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.18, 1.21 (each 3H, each t, J =each 7 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 2.04–2.09 (2H, m, β -methylene protons of glutamate), 2.43 (2H, t, J =7 Hz, γ -methylene protons of glutamate), 3.00 (1H, dd, J =6, 12 Hz, C_7 -H), 3.10 (1H, br d, J =12 Hz, C_7 -H), 3.26 (2H, br d, J =6 Hz, C_9 -H $\times 2$), 3.51 (1H, br, C_6 -H), 4.07, 4.11 (each 2H, each q, J =each 7, 14 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 4.41 (1H, m, C_α -H of glutamate), 4.63 (1H, brs, N_5 -H), 5.86 (2H, br, NH_2), 6.56 (1H, brt, N_{10} -H), 6.67, 7.69 (each 2H, each d, J =8 Hz, aromatic H), 8.28 (1H, br d, J =7 Hz, CONH), 10.87 (1H, brs, N_3 -H).

5,6,7,8-Tetrahydro-8-deaza-8-thiafolate (3) A solution of **2** (163 mg, 0.31 mmol) in EtOH (24 ml) containing NaOH (48 mg, 1.2 mmol) was stirred at room temperature until **2** was no longer detectable (monitored by TLC; 4 h). After neutralization of the mixture with dilute HCl, the resulting precipitate was collected and washed with MeOH to give **3** (97.6 mg, 68%) as an amorphous powder. *Rf*=0.42 (CHCl_3 :MeOH:AcOH=40:8:1). FAB-MS *m/z*: 463 [$M+H$] $^+$. IR $\nu_{\text{max}}^{\text{KBr}}$: 3300 (NH), 1700 (C=O), 1680 (C=O) cm^{-1} . UV $\lambda_{\text{max}}^{\text{MeOH}}$: 300, 231 nm. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.94–2.09 (2H, m, β -methylene protons of glutamate), 2.35 (2H, t, J =7 Hz, γ -methylene protons of glutamate), 2.97 (1H, dd, J =7, 12 Hz, C_7 -H), 3.11 (1H, br d, J =12 Hz, C_7 -H), 3.24 (2H, br d, J =5 Hz, C_9 -H $\times 2$), 3.48 (1H, m, C_6 -H), 4.36 (1H, m, C_α -H of glutamate), 4.63 (1H, brs, N_5 -H), 5.87 (2H, brs, NH_2), 6.54 (1H, brt, N_{10} -H), 6.68, 7.69 (each 2H, each d, J =8 Hz, aromatic H), 8.15 (1H, d, J =8 Hz, CONH), 10.88 (1H, br d, N_3 -H), 12.27 (3H, br, N^+H_3).

Diethyl *N*⁵,*N*¹⁰-Methylene-5,6,7,8-tetrahydro-8-deaza-8-thiafolate (4) An aqueous solution (1.0 ml) of **2** (19.7 mg, 0.04 mmol) containing formaldehyde (25 mmol) was adjusted to pH 5 with 1 N NaOH solution and then the mixture was stirred at room temperature for 5 h. The resulting precipitate was collected and washed with water to give **4** (19.1 mg, 90%) as an amorphous powder. *Rf*=0.74 (CHCl_3 :MeOH:AcOH=40:8:1). UV $\lambda_{\text{max}}^{\text{EtOH}}$: 304, 219 nm. $^1\text{H-NMR}$ (CD_3OD) δ : 1.21, 1.26 (each 3H, each t, J =each 7 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 2.07–2.25 (2H, m, β -methylene protons of glutamate), 2.47 (2H, t, J =7 Hz, γ -methylene protons of glutamate), 2.95 (1H, d, J =11 Hz, C_7 -H), 3.08 (1H, dd, J =11, 12 Hz, C_7 -H), 3.55 (1H, m, C_9 -H), 3.65 (1H, m, C_6 -H), 3.85 (1H,

q, J =6, 9 Hz, C_9 -H), 4.05 (1H, d, J =4 Hz, C_{11} -H), 4.10, 4.19 (each 2H, each q, J =each 7, 14 Hz, $\text{OCH}_2\text{CH}_3 \times 2$), 4.59 (1H, q, J =6, 9 Hz, C_α -H of glutamate), 5.21 (1H, d, J =4 Hz, C_{11} -H), 6.59, 7.77 (each 2H, each d, J =9 Hz, aromatic H).

***N*⁵,*N*¹⁰-Methylene-5,6,7,8-tetrahydro-8-deaza-8-thiafolate (5)** An aqueous solution (1.0 ml) of **3** (15 mg, 0.03 mmol) containing formaldehyde (25 mmol) was adjusted to pH 5 with 1 N NaOH solution and then the mixture was stirred at room temperature for 5 h. The resulting precipitate was collected and washed with water to give **5** (14 mg, 91%) as an amorphous powder. FAB-MS *m/z*: 475 [$M+H$] $^+$. $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.06–2.24 (2H, m, β -methylene protons of glutamate), 2.49 (2H, t, J =7 Hz, γ -methylene protons of glutamate), 3.13 (2H, m, C_7 -H $\times 2$), 3.57 (1H, m, C_9 -H), 3.75 (1H, brs, C_6 -H), 3.93 (1H, brs, C_9 -H), 4.14 (1H, m, C_{11} -H), 4.52 (1H, m, C_α -H of glutamate), 5.26 (1H, m, C_{11} -H), 6.39 (1H, brs, NH), 6.68, 7.95 (each 2H, each d, J =8 Hz, aromatic H), 8.42 (1H, d, CONH), 11.00 (1H, s, N_3 -H), 12.52 (3H, br, N^+H_3).

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References and Notes

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