N^{γ}-Alkyl Derivatives of L-Glutamine As Inhibitors of Glutamine-Utilizing Enzymes

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A general, facile method to synthesize the N^{γ}-alkyl and N^{γ},N^{γ}-dialkyl derivatives of L-glutamine **1a**–**d** from L-glutamic acid as a starting substrate is presented. The obtained compounds are shown to inhibit three different glutamine-utilizing enzymes, namely: glutaminase, γ-glutamyl transpeptidase, and glucosamine-6-phosphate synthase, with inhibitory constants within the millimolar range.

Key words: L-Glutamine Derivatives, Synthesis, *N*-Alkylamide Formation

Introduction

Structural analogues of L-glutamine exhibit inhibitory properties towards enzymes utilizing this amino acid as a substrate, including glutaminase (EC 3.5.1.2), γ-glutamyl transpeptidase (EC 2.3.2.2) and several amidotransferases catalyzing the transfer of an amino group from the γ-amide function of L-glutamine to different acceptor molecules (Massiere and Badet-Denisot, 1998). One of these enzymes is glucosamine-6-phosphate synthase (GlcN-6-P synthase, EC 2.6.1.16) which catalyzes the first committed step in a pathway leading to the formation of UDP-*N*-acetylglucosamine, providing *N*-acetyl-D-glucosamine for the formation of bacterial peptidoglycan and fungal chitin. For that reason, inhibitors of the enzyme are potential antimicrobial agents. Inhibitory properties of some glutamine analogues with respect to GlcN-6-P synthase and other amidotransferases have been reviewed (Pinkus, 1977). In the present paper we describe the synthesis and enzyme inhibitory properties of four N^{γ}-alkyl analogues of L-glutamine, namely N^{γ}-ethyl- (**1a**), N^{γ}-methyl- (**1b**), N^{γ},N^{γ}-diethyl- (**1c**) and N^{γ},N^{γ}-dimethyl-L-glutamine (**1d**).

According to literature data (Tsushida and Takeo, 1984, 1985a; Chu *et al.*, 1997) both N^{γ}-ethyl-L-glutamine (**1a**) (better known as theanine) and N^{γ}-methyl-L-glutamine (**1b**) have been found in green tea leaves. The research on rats with induced hypertension revealed that this two compounds cause significant reduction in blood pressure (Yokogoshi and Kobayashi, 1998; Yokogoshi *et al.*, 1995). Recent studies have shown that theanine and N^{γ}-methyl-L-glutamine zinc(II) complexes exhibit insulinomimetic activity relative to isolated *in vitro* rodent adipocytes treated with epinephrine and also induce the release of free fatty acids (FFA) from fat cells (Matsumoto *et al.*, 2005). Any particular biological properties of N^{γ},N^{γ}-dialkyl derivatives of L-glutamine have not been reported so far.

Results and Discussion

Syntheses of N^{γ}-alkyl derivatives of L-glutamine have been reported by several authors (Tanaka, 1962; Hashizume, 1951; Lichtenstein, 1942; Sakato *et al.*, 1950; Craig *et al.*, 1988; Furuyama *et al.*, 1964; Gu *et al.*, 2004; Tsushida and Takeo, 1985b). Theanine (N^{γ}-ethyl-L-glutamine) has been most often obtained by aminolysis of *N*-carbobenzyloxy-L-glutamic acid (Hashizume, 1951; Sakato *et al.*, 1950) or L-glutamic acid γ-methyl ester (Kawagishi and Sugiyama, 1992; Tanaka, 1962; Lichtenstein, 1942; Furuyama *et al.*, 1964; Barzily *et al.*, 1956). Alternatively, *N*-phthaloyl-DL-glutamic anhydride was treated with a suitable amine to give N^{α}-phthaloyl-N^{γ}-di- or -monoethyl-DL-glutamine, and subsequent removal of the pththaloyl residue with hydrazine led to the ultimate product,

N^{γ}-alkyl-DL-glutamine (Friedman and Chatterji, 1959; Gu *et al.*, 2004; Craig *et al.*, 1988). The more general method involved conversion of L-glutamic acid into *S*-5-oxo-2-pyrrolidinecarboxylic acid (L-pyroglutamic acid), which was then opened upon the treatment with ethylamine or methylamine to give the corresponding product (Matsumoto *et al.*, 2005; Lichtenstein, 1942; Hashizume, 1951; Tsushida and Takeo, 1985b). Biosynthesis of theanine (**1a**) from L-glutamic acid and ethylamine catalyzed by *Pseudomonas nitroreducens* was also reported (Abelian *et al.*, 1993).

In the present communication we describe the unequivocal and universal procedure of synthesis of the N^{γ}-alkyl and N^{γ},N^{γ}-dialkyl derivatives of L-glutamine **1a–d** (Fig. 1). L-Glutamic acid, as a starting substrate, was first converted into *N*-carbobenzyloxy-L-glutamic acid *α-tert*-butyl ester (**2**) in four steps, using previously described procedures (Boissonnas *et al.*, 1955; Taschner *et al.*, 1961). The *γ*-carboxyl function was converted into the methyl ester, while the amino and the *α*-carboxyl group were protected by carbobenzyloxy- and *tert*-butyl ester functions, respectively. Such strategy allowed a subsequent selective removal of *γ*-methyl ester by alkaline hydrolysis, to afford an appropriately protected substrate for *γ*-amide bond formation. Three different alternative methods of this reaction were tested: acid chlorides, mixed anhydrides, or *N,N'*-dicyclohexylcarbodiimide (DCCl)/*N*-hydroxysuccinimide (NSuOH). The first approach led, in our case, to the formation of N^2-carbobenzyloxy-L-pyroglutamic acid. Application of the mixed anhydrides method afforded a mixture of products, with urethane arising from an amine and chloroformate as the major component. Finally, the best results were obtained when **2** was treated with a suitable amine (ethylamine, methylamine, diethylamine, and dimethylamine) in the presence of a coupling compound – DCCl and *N*-hydroxysuccinimide. The reaction was continued at room temperature, usually for 3 days, and its progress was followed by TLC. Since some of the alkylamines are volatile at room temperature, an additional amount of this substrate was added when **2** was still present in the reaction mixture. The *tert*-butyl group of compounds **3a–d** was selectively removed with trifluoroacetic acid (TFA) and the *α*-amino function was deprotected by catalytic dehydrogenation. Compounds **1a–d** were purified by ion-exchange chromatography and finally crystallized from different solvent systems.

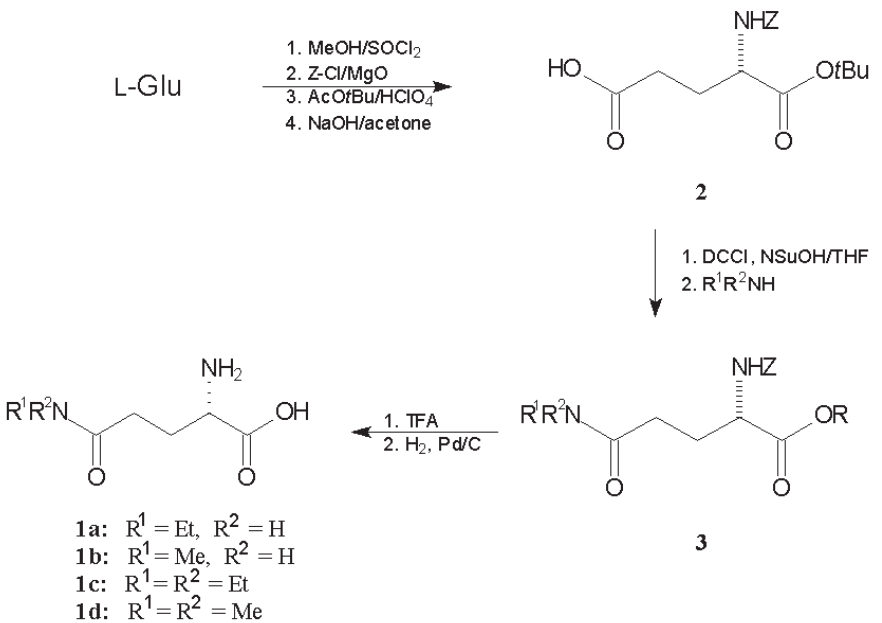

Fig. 1. Scheme of synthesis of the N^{γ}-alkyl- and N^{γ},N^{γ}-dialkyl-L-glutamine derivatives **1a–d**.

The proposed general procedure of synthesis of N^{γ}-alkyl-glutamine derivatives is versatile. Removal of the protecting groups affords isobutene, toluene and carbon dioxide that may be easily removed from the post-reaction mixtures. The cumulative yields (≈60%) seem satisfactorily and are much better than those achieved by the previous methods, where values even lower than 10% were reported (Craig *et al*., 1988).

The synthesized compounds were tested as potential inhibitors of three glutamine-utilizing enzymes, namely GlcN-6-P synthase from *Candida albicans*, bacterial glutaminase, and mammalian γ-glutamyl transpeptidase. The obtained data on enzyme inhibition are summarized in Table I.

Compounds **1a**–**d** appeared to be moderately strong inhibitors of all enzymes, as the determined IC_{50} and K_i values were in the millimolar range. The inhibition was in each case competitive with respect to L-glutamine. This type of inhibition is exemplified by the Lineweaver-Burk plot obtained for the GlcN-6-P synthase inhibition by **1c**, shown in Fig. 2. Interestingly enough, the *N,N*-dialkyl derivatives **1c** and **1d** appeared to be stronger inhibitors of GlcN-6-P synthase activity than their *N*-monoalkyl counterparts **1a** and **1b**, while in the case of γ-glutamyl transpeptidase and glutaminase an opposite situation was noted. It is therefore worth mentioning that ammonia released from L-glutamine at the GlcN-6-P synthase active site is transferred to the acceptor binding site through an intramolecular tunnel (Teplyakov *et al.*, 2001). It is therefore likely, that N^{γ}-substituted derivatives of L-glutamine are more effective inhibitors of the enzyme, as the *N*-alkyl and especially *N,N*-dialkyl substituents are supposed to block the entry to the tunnel, when the N^{γ}-substituted derivatives of L-glutamine bind to the active site instead of the natural substrate. The two other enzymes do not contain any intramolecular tunnel and their lower sensitivity to inhibition by *N,N*-dialkyl compounds may be rationalized in terms of restricted access of bulkier inhibitors to the glutamine binding sites.

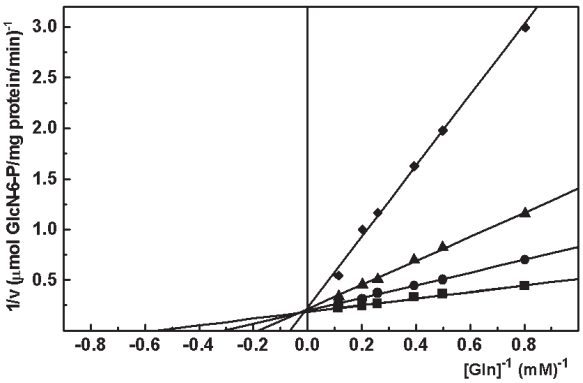

Fig. 2. Competitive inhibition of GlcN-6-P synthase by **1c** with respect to L-glutamine. Inhibitor concentrations: (■) none; (●) 0.5 mM; (▲) 1.5 mM; (◆) 3.0 mM.

Material and Methods

General

Homogeneity of the products and progress of the reactions were controlled by thin-layer chromatography (TLC). The following conditions were used for all derivatives: Merck silica gel 60 F_{254} plates (layer thickness, 0.1 mm); eluent mixtures: benzene/ethyl acetate/ethanol (60:20:5) (A), *n*-butanol/acetic acid/water (4:1:1) (B); the spots were visualized with iodine and ninhydrin reagent (for compounds containing free amine groups), heated at high temperature for about 1–2 min. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity Plus spectrometer operating at 500 and 125 MHz, respectively. The deuterated solvents were used as an internal lock for ^{1}H and ^{13}C NMR spectroscopy. Optical rotations were measured using an Autopol II (Rudolph Research Flanders, NJ, USA)

Table I. Inhibition of three glutamine-utilizing enzymes by N^{γ}-alkyl-glutamine derivatives.

Compound	GlcN-6-P synthase		Glutaminase		γ-Glutamyl transpeptidase	
	IC_{50} [mM]	K_i [mM]	IC_{50} [mM]	K_i [mM]	IC_{50} [mM]	K_i [mM]
1a	14	0.78	24	1.58	20	0.97
1b	28	2.04	20	1.11	16	0.84
1c	9	0.49	39	2.95	26	1.77
1d	10.5	0.63	35	2.64	28	2.01

polarimeter. The IR spectra were recorded on a FT-IR Bruker IFS 66 spectrometer.

L-Glutamic acid γ-methyl ester hydrochloride

This compound was synthesized according to the method described by Boissonnas *et al.* (1955), in 55% yield. – M.p. 162–164(r) ºC (MeOH/Et_2O). – $[\alpha]_D$ +28.3º (*c* 2.5, 6 M HCl).

*N^{α}-Carbobenzyloxy-L-glutamic acid α-tert-butyl ester (**2**)*

Compound **2** was synthesized according to the modified method described by Taschner *et al.* (1961). It was obtained in 55% yield (2.27 g). – R_f = 0.37 (B). – M.p. 79–82 ºC ($CHCl_3$/hexane). – $[\alpha]_D$ –25º (*c* 1, MeOH). – ^{1}H NMR ($CDCl_3$): δ = 1.48 (*s*, 9H, C(C**H**$_3$)$_3$), 1.98 (*m*, 1H, C**H**$_2$), 2.2 (*m*, 1H, C**H**$_2$), 2.47 (*m*, 2H, C**H**$_2$CO), 4.32 (*m*, 1H, C**H**$^{\alpha}$), 5.12 (*s*, 2H, C**H**$_2$Ph), 5.45 (*d*, *J* = 7.8 Hz, 1H, N**H**), 7.37 (*s*, 5H, C_6**H**$_5$). – ^{13}C NMR ($CDCl_3$): δ = 177.9, 171.4, 156.7, 136.5, 128.8, 128.5, 128.4, 88.8, 67.4, 54.1, 29.7, 27.9, 27.6. – Literature data: oil, $[\alpha]_D$ –19.4º (*c* 2.2, MeOH) (Dolence *et al.*, 1991).

*N^{γ}-Alkyl-N^{α}-carbobenzyloxy-L-glutamine α-tert-butyl ester (**3**)*

Compound **2** (680 mg, 2.02 mmol) was dissolved in tetrahydrofurane (20 mL) at 0 ºC, with stirring, followed by addition of *N*,*N*-dicyclohexylcarbodiimide (417 mg, 2.02 mmol), *N*-hydroxysuccinimide (233 mg, 2.02 mmol), and an amine (4.04 mmol). The resulting mixture was stirred at room temperature for 3 d and then cooled to 0 ºC. The precipitate *N*,*N*-dicyclohexylurea was filtered off, and the solution was evaporated *in vacuo*. The residue was dissolved in ethyl acetate and washed with portions (10 mL) of water, 5% sodium hydrogen carbonate solution, water, 5% citric acid solution, and water, respectively. The organic phase was dried, and the solvent was removed under reduced pressure to give the crude product which was purified using column chromatography (silica gel Merck 70, 230 mesh, chloroform as an eluent).

N^{γ}-Ethyl-N^{α}-carbobenzyloxy-L-glutamine α-tert-butyl ester (**3a**): Yield 64%. – R_f = 0.32 (B). – $[\alpha]_D$ – 13.5º (*c* 1.04, MeOH). – ^{1}H NMR ($CDCl_3$): δ = 1.13 (*t*, 3H, C**H**$_3$), 1.45 (*s*, 9H, C(C**H**$_3$)$_3$), 1.93 (*m*, 2H, C**H**$_2$), 2.21 (*m*, 2H, C**H**$_2$), 3.27 (*m*, 2H, C**H**$_2$N), 4.27 (*m*, 1H, C**H**$^{\alpha}$), 5.11 (*m*, 2H, C**H**$_2$Ph), 5.54 (*d*, *J* = 7.3 Hz, 1H, N**H**), 6.0 (*m*, 1H, N**H**), 7.35 (*s*, 5H, C_6**H**$_5$). – ^{13}C NMR ($CDCl_3$): δ = 172.3, 171.5, 156.9, 136.7, 129.0, 128.7, 128.6, 82.99, 67.5, 54.5, 34.97, 33.15, 29.7, 28.4, 15.22.

N^{γ}-Methyl-N^{α}-carbobenzyloxy-L-glutamine α-tert-butyl ester (**3b**): Yield 61%. – R_f = 0.27 (B). – $[\alpha]_D$ –22.6º (*c* 0.93, MeOH). – ^{1}H NMR ($CDCl_3$): δ = 1.44 (*s*, 9H, C(C**H**$_3$)$_3$), 1.96 (*m*, 1H, C**H**$_2$), 2.1 (*m*, 1H, C**H**$_2$CO), 2.78 (*d*, 3H, NC**H**$_3$), 4.21 (*m*, 1H, C**H**$^{\alpha}$), 5.1 1(*s*, 2H, C**H**$_2$Ph), 5.68 (*d*, *J* = 7.3 Hz, 1H, N**H**), 6.03 (*m*, 1H, N**H**), 7.35 (*s*, 5H, C_6**H**$_5$). – ^{13}C NMR ($CDCl_3$): δ = 173.3, 171.5, 156.96, 136.67, 129.0, 128.7, 128.6, 83.09, 67.56, 54.47, 32.95, 28.46, 27.0.

N^{γ},N^{γ}-Diethyl-N^{α}-carbobenzyloxy-L-glutamine α-tert-butyl ester (**3c**): Yield 70%. – R_f = 0.52 (B). – $[\alpha]_D$ –16.8º (*c* 1.9, MeOH). – ^{1}H NMR ($CDCl_3$): δ = 1.15 (*t*, 6H, C**H**$_3$), 1.47 (*s*, 9H, C(C**H**$_3$)$_3$), 2.03 (*m*, 1H, C**H**$_2$), 2.20 (*m*, 1H, C**H**$_2$), 2.32–2.46 (*m*, 2H, C**H**$_2$CO), 3.27 (*m*, 2H, C**H**$_2$N), 3.36 (*m*, 2H, C**H**$_2$N), 4.23 (*m*, 1H, C**H**$^{\alpha}$), 5.09 (*m*, 2H, C**H**$_2$Ph), 5.7 (*d*, 1H, N**H**), 7.35 (*s*, 5H, C_6**H**$_5$). – ^{13}C NMR ($CDCl_3$): δ = 172.3, 171.5, 156.9, 136.7, 129.0, 128.7, 128.6, 82.99, 67.5, 54.5, 34.97, 33.8, 29.7, 28.4, 15.2, 15.0.

N^{γ},N^{γ}-Dimethyl-N^{α}-carbobenzyloxy-L-glutamine α-tert-butyl ester (**3d**): Yield 61%. – R_f = 0.38 (B). – $[\alpha]_D$ –17.4º (*c* 0.98, MeOH). – ^{1}H NMR ($CDCl_3$): δ = 1.47 (*s*, 9H, C(C**H**$_3$)$_3$), 2.01 (*m*, 1H, C**H**$_2$), 2.21 (*m*, 1H, C**H**$_2$), 2.32–2.46 (*m*, 2H, C**H**$_2$CO), 2.94 (*s*, 3H, NC**H**$_3$), 2.96 (*s*, 3H, NC**H**$_3$), 4.26 (*m*, 1H, C**H**$^{\alpha}$), 5.1 (*m*, 2H, C**H**$_2$Ph), 5.68 (*d*, *J* = 7.4 Hz, 1H, N**H**), 7.37 (*s*, 5H, C_6**H**$_5$). – ^{13}C NMR ($CDCl_3$): δ = 173.5, 171.9, 157, 138.6, 129.0, 128.7, 128.6, 83.09, 67.56, 54.47, 34.5, 33.8, 28.46, 27.1.

*Synthesis of N^{γ}-alkyl-L-glutamine (**1**)*

Compound **3** (0.5 mmol) was treated with trifluoroacetic acid (2.1 mL) at room temperature. Progress of acidolysis was followed by TLC. After the acidolysis was completed, excess of trifluoroacetic acid was removed under reduced pressure. The residue was washed several times with diethyl ether. The crude product was purified by column chromatography on silica gel (Merck, 70–230 mesh) using chloroform as the eluent. The N^{γ}-alkyl-N^{α}-carbobenzyloxy-L-glutamine derivatives were obtained as colourless oils, dissolved in ethanol and hydrogenated over 10% Pd/C for a few hours. The progress of the reaction was fol-

lowed by TLC. The catalyst was filtered off and washed with a little amount of water/ethanol. The combined solution was evaporated *in vacuo*, and the residue was purified on Dowex 50X8–100[H^+] resin with 10% NH_3 as an eluent. The solvent was removed by evaporation and the residue dissolved in an appropriate solvent (see below) to obtain products **1a–d** as white crystals.

N^{γ}-Ethyl-L-glutamine (**1a**): Yield 62%. – R_f = 0.61 (A). – $[\alpha]_D$ +12° (*c* 0.5, H_2O). – M.p. 218–216 °C (hot H_2O/EtOH). – ^{1}H NMR (D_2O): δ = 0.95 (*t*, *J* = 7.3 Hz, 3H, $NCH_2C\mathbf{H}_3$), 1.97 (*m*, 2H, $C\mathbf{H}_2$), 2.23 (*m*, 2H, $C\mathbf{H}_2$), 3.04 (*q*, *J* = 7.3 Hz, 2H, $NC\mathbf{H}_2CH_3$), 3.61 (*t*, *J* = 6.1 Hz, 1H, $C\mathbf{H}^{\alpha}$). – Literature data: m.p. 214–216 °C, $[\alpha]_D$ +8.6° (*c* 1, H_2O) (Kawagishi and Sugiyama, 1992); m.p. 215 °C (MeOH) (Tanaka, 1962); m.p. 217 °C (H_2O/EtOH) (Hashizume, 1951); m.p. 217–218 °C, $[\alpha]^{20}$ +7° (Sakato, 1950; Sakato *et al.*, 1950); m.p. 200 °C, $[\alpha]^{20}$ +6.25° (Lichtenstein, 1942).

N^{γ}-Methyl-L-glutamine (**1b**): Yield 59%. – R_f = 0.18 (A). – $[\alpha]_D^{20}$ +6.3° (*c* 1, H_2O). – M.p. 200–202 °C (H_2O/acetone). – ^{1}H NMR (D_2O): δ = 2.13 (*m*, 2H, $C\mathbf{H}_2$), 2.43 (*m*, 2H, $C\mathbf{H}_2$), 2.72 (*s*, 3H, $NC\mathbf{H}_3$), 3.69 (*m*, 1H, $C\mathbf{H}^{\alpha}$). – Literature data: m.p. 202–203 °C, $[\alpha]_D^{20}$ +7.14° (*c* 10, H_2O) (Barzily *et al.*, 1956); m.p. 192 °C (r), $[\alpha]_D^{20}$ +5.9° (*c* 1, H_2O) (Tanaka, 1962); m.p. 202 °C (r) (Hashizume, 1951); m.p. 192 °C, $[\alpha]_D^{20}$ +6° (Lichtenstein, 1942).

N^{γ},N^{γ}-Diethyl-L-glutamine (**1c**): Yield 56%. – R_f = 0.38 (A). – $[\alpha]_D^{20}$ –1.0° (*c* 0.9, H_2O). – M.p. 168–170 °C (EtOH/acetone). – ^{1}H NMR (D_2O): δ = 0.96 (*t*, *J* = 7.1 Hz, 3H, $C\mathbf{H}_3$), 1.04 (*t*, *J* = 7.3 Hz, 3H, $C\mathbf{H}_3$), 2.04 (*m*, 2H, $C\mathbf{H}_2$), 2.46 (*m*, 2H, $C\mathbf{H}_2$), 3.22 (*q*, *J* = 7.3 Hz, 2H, $C\mathbf{H}_2N$), 3.25 (*q*, *J* = 7.3 Hz, 2H, $C\mathbf{H}_2N$), 3.65 (*t*, 1H, $C\mathbf{H}^{\alpha}$). – Literature data: m.p. 170–172 °C, $[\alpha]_D^{20}$ –1.16° (*c* 1.25, EtOH) (Craig *et al.*, 1988); *rac* m.p. 167–168 °C (Friedman and Chatterji, 1959).

N^{γ},N^{γ}-Dimethyl-L-glutamine (**1d**): Yield 68%. – R_f = 0.20 (A). – $[\alpha]_D^{20}$ –11.0° (*c* 1, H_2O). – M.p. 183–184 °C (EtOH/acetone). – ^{1}H NMR (D_2O): δ = 2.09 (*q*, *J* = 6.7 Hz, 2H, $C\mathbf{H}_2$), 2.54 (*m*, *J* = 6.8 Hz, 2H, $C\mathbf{H}_2$), 2.87 (*s*, 3H, $NC\mathbf{H}_3$), 3.0 (*s*, 3H, $NC\mathbf{H}_3$), 3.72 (*m*, 1H, $C\mathbf{H}^{\alpha}$). – Literature data: m.p. 173–174 °C, $[\alpha]_D^{20}$ –11.3° (*c* 2, H_2O) (Caplaneris *et al.*, 1978).

Determination of enzyme inhibitory activity

Candida albicans GlcN-6-P synthase was purified to near homogeneity according to a previously published procedure (Sachadyn *et al.*, 2000). The enzyme activity was determined by the modified Elson-Morgan method (Janiak *et al.*, 2003). γ-Glutamyl transpeptidase from bovine kidney and *E. coli* glutaminase were purchased from Sigma. The activity of γ-glutamyl transpeptidase was determined using γ-glutamyl *p*-nitroanilide (GLUPA) as a substrate, under conditions described by Persijn *et al.* (1971), and glutaminase was assayed colourimetrically, according to the procedure of Gella and Pascual (1982). For determination of the IC_{50} values, the concentration of L-glutamine in GlcN-6-P synthase and glutaminase assays was 10 mM, while the concentration of GLUPA in γ-glutamyl transpeptidase determination was 1 mM. In incubation mixtures for K_i determination, glutamine/GLUPA concentrations were variable in the 0.2–2 mM range. All the measurements were done in triplicate. Inhibitory constants were determined from the secondary plots of k_{app} versus inhibitor concentration, derived from the Lineweaver-Burk plots.

Abelian V. H., Okubo T., Muton K., Chu D. C., Kim M., and Yamamoto T. (1993), A novel method of production of theanine by immobilized *Pseudomonas nitroreducens* cells. Biosci. Biotechnol. Biochem. **57**, 481–483.

Barzily I., Kuk-Meiri S., and Lichtenstein N. (1956), Nitrogen-substituted γ-amides of glutamic acid. Bull. Res. Coun. Israel **5A**, 155–157.

Boissonnas R. A., Guttmann S., Jaquenoud P. A., and Waller J. P. (1955), A new synthesis of oxythocin. Helv. Chim. Acta **38**, 1491–1501.

Caplaneris Th., Cordopatis P., Matsoukas J., and Theodoropoulos D. (1978), Synthesis of N^4-dialkyl-L-asparagine and N^5-dialkyl-L-glutamine derivatives: Application to the synthesis of related peptides. Tetrahedron **34**, 969–972.

Chu D. C., Kobayashi K., Juneja L. R., and Yamamoto T. (1997), In: Chemistry and Application of Green Tea; Chapter 12: Theanine – its Synthesis, Isolation and Physiological Activity (Yamamoto T., Juneja L. R., Chu D. C., and Kim M., eds.). CRC Press, New York.

Craig J. C., Bhargava H. N., Everhart E. T., LaBelle B., Ohnsorge U., and Webster R. V. (1988), Absolute configuration of the enantiomers of 7-chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline (chloroquine). J. Org. Chem. **53**, 1167–1170.

Dolence E. K., Li C. E., Miller M. J., and Payne S. M. (1991), Synthesis and activity of albomycin – like

peptides derived from N^{5}-acetyl-N^{5}-hydroxy-L-ornithine. J. Med. Chem. **34**, 956–968.
Friedman O. M. and Chatterji R. (1959), Synthesis of derivatives of glutamine as model substrates for antitumor agents. J. Am. Chem. Soc. **81**, 3750–3752.
Furuyama T., Yamashita T., and Senoh S. (1964), The synthesis of L-theanine. Bull. Soc. Chem. Jpn. **37**, 1078.
Gella F. J. and Pascual M. A. (1982), Assay of glutaminase activity by colorimetric determination of glutamate. Anal. Biochem. **127**, 322–325.
Gu H., Jiang Y., and Wang J. (2004), A practical synthesis of ethyl L-glutamine (L-theanine). Org. Prep. Proc. Int. **36**, 182–185.
Hashizume T. (1951), Amino acids in tea. I. Synthesis of theanine from pyrrolidonecarboxylic acid. J. Agric. Chem. Soc. Jpn. **25**, 25–26.
Janiak M., Hoffmann M., Milewska M. J., and Milewski S. (2003), Hydrophobic derivatives of 2-amino-2-deoxy-D-glucitol-6-phosphate: A new type of D-glucosamine-6-phosphate synthase inhibitors with antifungal action. Bioorg. Med. Chem. **11**, 1653–1662.
Kawagishi H. and Sugiyama K. (1992), Facile and large-scale synthesis of L-theanine. Biosci. Biotechnol. Biochem. **56**, 689.
Lichtenstein N. (1942), Preparation of γ-alkylamides of glutamic acid. J. Am. Chem. Soc. **64**, 1021–1022.
Massiere F. and Badet-Denisot M.-A. (1998), The mechanism of glutamine-dependent amidotransferases. Cell. Mol. Life Sci. **54**, 205–222.
Matsumoto K., Yamamoto S., Yoshikawa Y., Doe M., Kojima Y., Sakurai H., Hashimoto H., and Kajiwara N. M. (2005), Antidiabetic activity of Zn(II) complexes with a derivative of L-glutamine. Bull. Chem. Soc. Jpn. **78**, 1077–1081.
Pinkus L. M. (1977), Glutamine binding sites. Methods Enzymol. **46**, 414–427.
Persijn J. P., Silk W. V., and Zwart W. A. (1971), Colorimetric assay for γ-glutamyl-transpeptidase. Clin. Chim. Acta **35**, 239–240.
Sachadyn P., Jędrzejczak R., Milewski S., Kur J., and Borowski E. (2000), Purification to homogeneity of *Candida albicans* glucosamine-6-phosphate synthase overexpressed in *Escherichia coli*. Protein Expr. Purif. **19**, 343–349.
Sakato Y. (1950), The chemical constituents of tea. III. A new amide, theanine. J. Agric. Chem. Soc. Jpn. **23**, 262–267.
Sakato Y., Hashizume T., and Kishimoto Y. (1950), The chemical constituents of tea. V. Synthesis of theanine. J. Agric. Chem. Soc. Jpn. **23**, 269–271.
Tanaka M. (1962), Patent JP 23,392 ('64); Chem. Abs. **62**, 10509c (1965).
Taschner E., Wasielewski C., Sokołowska T., and Biernat J. F. (1961), Darstellung von Tosyl- und Carbobenzoxyglutaminsäure-*α-tert*-butyl-estern und ihre Verwendung zur Synthese von γ-Glutamylpeptiden. Liebigs Ann. Chem. **646**, 127–133.
Teplyakov A., Obmolova G., Badet B., and Badet-Denisot M.-A. (2001), Channeling of ammonia in glucosamine-6-phosphate synthase. J. Mol. Biol. **313**, 1093–1102.
Tsushida T. and Takeo T. (1984), Occurrence of theanine in *Camellia japonica* and *Camellia sasanqua* seedlings. Agric. Biol. Chem. **48**, 2861–2862.
Tsushida T. and Takeo T. (1985a), Purification and some properties of tea leaf amine oxidase. Agric. Biol. Chem. **49**, 319–326
Tsushida T. and Takeo T. (1985b), Biosynthesis of γ-glutamylalkylamides in tea plants. J. Agric. Chem. Soc. Jpn. **59**, 787–792.
Yokogoshi H. and Kobayashi M. (1998), Hypotensive effect of gamma-glutamylmethylamide in spontaneously hypertensive rats. Life Sci. **62**, 1065–1068.
Yokogoshi H., Kato Y., Sagesaka Y. M., Takihara-Matsuura T., Kakuda T., and Takeuchi N. (1995), Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. **59**, 615–618.