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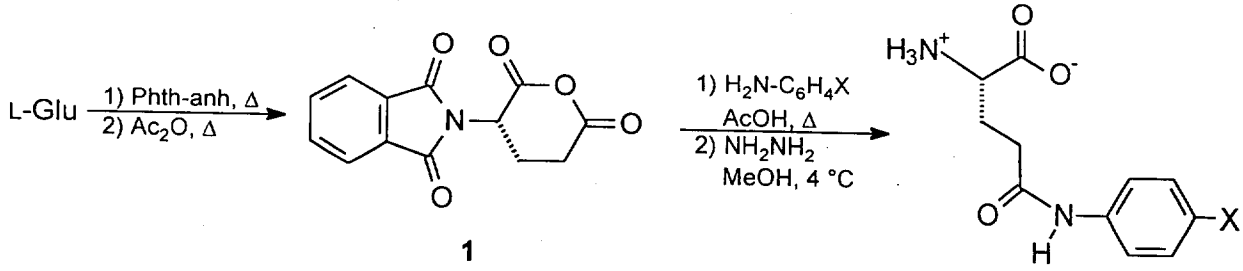
Substrate analogue synthesis.

The synthesis of *para*-substituted L-glutamyl γ-anilide substrate analogues follows a general method based on the protocol published by Lindsay and Whitaker[1] as shown in Scheme 1S. Reported melting points were recorded on a Thomas-Hoover Uni-melt apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX300 (300 MHz) and a Bruker AMXR400 (400 MHz) NMR spectrometer, respectively. Mass spectra were recorded on a Micromass 1212 spectrometer.

Scheme 1S



2a X = OMe
b X = Me
c X = Et
d X = H
e X = F
f X = CF_3
g X = CO_2Et
h X = NO_2

[1] Lindsay, H. and Whitaker, J. F. (1975) *Org. Proc. Prep. Int.* 89-91.

Synthesis of N-phthalyl L-glutamic acid anhydride **1**.

A quantity of 5 g (33.7 mmol) of phthalic anhydride and 5 g (33.7 mmol) of L-glutamic acid were heated together at 180 °C with slight stirring to boiling, whereupon the temperature was reduced to 145 °C for 30 minutes. The reaction mixture was then cooled to 100-105 °C, 6 mL of acetic anhydride was added and the solution was allowed to react for an additional 10 minutes. Then 20 mL of xylene was added to the mixture and it was refrigerated at 4 °C for 12 hours. *N*-phthalyl L-glutamic acid anhydride was recovered by vacuum filtration as large white crystals (4.44 g, 50 %; mp 188-192 °C, lit.[1] 199-201 °C). ^{1}H NMR ($CDCl_3$) δ 2.20 (m, 1H), 2.95 (m, 2H), 3.20 (m, 1H), 5.15 (q, J = 4.5 Hz, 1H), 7.88 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 21.47, 30.48, 48.73, 124.60, 132.27, 135.99, 167.34, 167.73, 171.40, 174.79. MS (M+H) 260.0

Synthesis of para-substituted L-glutamyl anilides **2a-h**.

General Procedure: A quantity of 290 mg (1.1 mmol) of *N*-phthalyl L-glutamic acid anhydride and 1 mmol of a given aniline were added to either 10 mL of glacial acetic acid or 10 mL of benzene with 500 μL of glacial acetic acid. The mixture was heated to reflux with stirring until the reaction was complete. Solvents were removed under reduced pressure and the residue was washed three times with *n*-propanol to eliminate traces of acetic acid. The *N*-phthalyl L-glutamyl *para*-substituted anilide product was recovered as a white powder by evaporation of the *n*-propanol. Deprotection was carried out directly without purification.

To the flask containing the protected γ-glutamyl anilide was added 10 mL of methanol, followed by gentle heating. The mixture was filtered to remove any solid that remained undissolved, and to the filtrate was then added 300 μL of hydrazine (~ 12 eq or to pH 8-8.5).

The mixture was stirred at room temperature for two hours and then stored at 4 °C overnight. The methanol was evaporated and 10 mL of 0.5 N HCl was added. The mixture was heated gently and the product that did not dissolve was removed by filtration. The filtrate was extracted with ethyl acetate (2 × 10 mL) and the aqueous phase was neutralized with sodium bicarbonate to pH ~5 and kept at 4°C for 12 hours. The resulting crystals were then collected by vacuum filtration.

Synthesis of L-glutamyl(γ-p-methoxyanilide) **2a**.

White powder (127 mg, 51 %; mp 215-217 °C). ^{1}H NMR (CD_3OD) δ 1.30 (s, 3H), 2.15 (q, J = 8.6 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 3.60 (m, 1H), 3.74 (s, 3H), 6.85 (dd, J = 3.6, 10.7 Hz, 2H), 7.40 (dd, J = 3.6, 10.7 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 25.69, 30.15, 56.02, 56.25, 115.48, 116.09, 143.06, 151.77, 175.68, 178.07. HRMS (M+H) calc. 253.11884, found 253.11770.

Synthesis of L-glutamyl(γ-p-methylanilide) **2b**.

White powder (100 mg, 42 %; mp 208-210 °C). ^{1}H NMR (CD_3OD) δ 2.19 (q, J = 6.4 Hz, 2H), 2.30 (s, 3H), 2.62 (t, J = 7.0 Hz, 2H), 3.65 (t, J = 6.7 Hz, 1H), 7.10 (dd, J = 1.2, 9.5 Hz, 2H), 7.40 (dd, J = 1.2, 9.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 21.11, 25.53, 30.13, 55.96, 115.13, 125.05, 130.06, 146.87, 175.61, 178.08. HRMS (M+H) calc. 237.12392, found 237.12420.

Synthesis of L-glutamyl(γ-p-ethylanilide) **2c**.

White powder (158 mg, 64 %; mp 204-206 °C) ^{1}H NMR (DMSO-d_6) δ 1.13 (t, J = 7.6 Hz, 3H), 2.31 (m, 2H), 2.52 (m, 4H), 3.49 (m, 1H), 7.07 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 7.7 Hz, 2H) ^{13}C

NMR (DMSO-d_6) δ 16.07, 26.04, 27.91, 31.81, 51.86, 119.65, 128.25, 137.25, 139.30, 169.99, 171.10 MS (M+H) calc. 251.13957, obtained 251.13880

Synthesis of L-glutamyl(γ–anilide) **2d**.

White crystals (151 mg, 68 %; mp 210-212 °C). ^{1}H NMR (CD_3OD) δ 2.15 (q, J = 5.0 Hz, 2H), 2.55 (t, J = 3.6 Hz, 2H), 3.80 (t, J = 4.3 Hz, 1H), 7.25 (m, 1H), 7.42 (d, J = 4.6 Hz, 4H); ^{13}C NMR (DMSO-d_6) δ 25.47, 29.95, 55.81, 114.95, 116.71, 129.81, 149.33, 175.84, 178.56. HRMS (M+H) calc. 223.10826, found 223.10890.

Synthesis of L-glutamyl(γ-p-fluoroanilide) **2e**.

White powder (66 mg, 25 %; mp 210 °C) ^{1}H NMR (DMSO-d_6) δ 1.93 (m, 2H), 2.45 (m, 2H), 3.24 (m, 1H), 7.12 (t, J = 8.2 Hz, 2H), 7.60 (dd, J = 4.1, 8.2 Hz, 2H) ^{13}C NMR (DMSO-d_6) δ 27.64, 33.24, 53.94, 115.34, 115.64, 121.00, 121.10, 136.21, 171.19. MS (M+H) calc. 241.09885, obtained 241.09820.

Synthesis of L-glutamyl(γ-p-trifluoromethylanilide) **2f**.

White powder (107 mg, 37 %; mp 204-205 °C). ^{1}H NMR (CD_3OD) δ 2.15 (q, J = 6.4 Hz, 2H), 2.65 (t, J = 7.9 Hz, 2H), 3.65 (t, J = 2.9 Hz, 1H), 7.55 (dd, J = 0.5, 7.1 Hz, 2H), 7.75 (dd, J = 0.5, 7.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 24.96, 29.41, 55.09, 113.29, 119.19, 126.56, 126.61, 152.63, 174.87, 177.34. HRMS (M+H) calc. 291.09564, found 291.09630.

Synthesis of L-glutamyl(γ-p-ethoxycarbonylanilide) **2g**.

White powder (108 mg, 33 %; mp 203-204 °C). ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 7.3 Hz, 3H), 1.92 (q, J = 7.7 Hz, 2H), 2.53 (t, J = 7.3 Hz, 2H) 3.28 (t, J = 7.3 Hz, 1H), 42.8 (q, J = 7.3 Hz, 2H), 7.72, (d, J = 8.2 Hz, 2H), 7.89 (d, J = 8.2 Hz, 2H). ^{13}C NMR (DMSO-d_6)15.20, 27.70, 33.95, 54.23, 61.25, 119.20, 124.71, 131.06, 144.59, 157.88, 166.22, 172.37. HRMS (M+H) calc. 295.12939, found 295.13040.

Synthesis of L-glutamyl(γ-p-nitroanilide) **2h**.

Yellow powder (190 mg, 71 %; mp 186-188 °C, lit. 186-188 °C (*24*)). ^{1}H NMR (CD_3OD) δ 2.20 (q, J = 2.4 Hz, 2H), 2.68 (t, J = 7.9 Hz, 2H), 3.65 (t, J = 4.0 Hz, 1H), 7.80 (dd, J = 1.5, 9.5 Hz, 2H), 8.20 (dd, J = 1.5, 9.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 27.62, 33.82, 54.44, 119.68, 127.32, 142.77, 146.69, 172.39, 172.34. HRMS (M+H) calc. 268.09335, found 268.09260.

Colorimetric determination of aniline concentration.

A test tube was charged with 500 μL of 0.1 M Tris-HCl buffer (pH 8.0), 500 μL water, 500 μL of 40 % trichloroacetic acid (TCA) and 1.6 - 32 μL of a 2.5 mM aniline solution prepared by dissolving the respective aniline in water by the addition of a few drops of 2 N HCl. The volume in the test tube was completed to 2 mL with the addition of 468 – 498.4 μL water. To this was added 500 μL of a freshly prepared sodium nitrite solution (4 mg/mL H_2O), and the reaction was allowed to proceed for 3 minutes. After this time, 500 μL of an ammonium sulfamate solution (20 mg/mL H_2O) was added and the solution was allowed to stand for 2 minutes. Finally, 1 mL of a solution of *N*-(1-naphtyl)ethylenediamine dihydrochloride (1.5 mg/mL ethanol) was added, and the color of the solution was allowed to develop. The time and temperature of this color development vary slightly according to the aniline concerned, as reported in Table 1S. The absorbance of the final solution was read at the wavelength (λ) shown in Table 1S, and a graph of absorbance versus concentration was prepared. The slope of this graph gives the apparent extinction coefficient (ε^{app}) of the corresponding solutions of diazotized aniline, determined under these conditions, as reported in Table 1S.

Table 1S: Experimental conditions employed for colorimetric determination of the concentration of a series of *para*-substituted anilines and the apparent extinction coefficients of their respective diazo dyes determined under these conditions.

Analyte	ε^{app} [a] ($mM^{-1}cm^{-1}$)	λ (nm)	Temp. (°C)	Time (min)
p-methoxyaniline	15.3 ± 0.3 (7.9 ± 0.7)	583	37	30
p-methylaniline	24.7 ± 0.3	567	25	15
p-ethylaniline	33.1 ± 0.4	572	25	15
aniline	45.1 ± 0.3 (37.5 ± 0.4)	560	25	15
p-fluoroaniline	86.0 ± 1.6	558	25	10
p-trifluoromethylaniline	67.1 ± 0.3	546	25	10
p-ethoxycarbonylaniline	114 ± 1	551	25	10
p-nitroaniline	57.3 ± 0.4 (52.7 ± 0.6)	560	25	10

[a] Values in parentheses were determined from solutions prepared in D_2O.

Kinetic data.

Table 1S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-methoxyanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methoxyanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0068
100	0.0121
200	0.0228
500	0.0470
1000	0.0684
2000	0.0896

[a] Average of duplicate values. Experimental error is estimated at ± 10 %.

Table 2S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-methylanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methylanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0065
100	0.0129
200	0.0255
500	0.0506
1000	0.0685
2000	0.0830

[a] Average of duplicate values. Experimental error is estimated at ± 10 %.

Table 3S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-ethylanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-ethylanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
100	0.0040
200	0.0124
500	0.0293
750	0.0434
1000	0.0516
2000	0.0467

[a] Experimental error is estimated at ± 10 %.

Table 4S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-anilide) as a function of substrate concentration.

[L-Glu(γ-anilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0056
100	0.0105
200	0.0208
500	0.0351
1000	0.0482
2000	0.0596

[a] Average of duplicate values. Experimental error is estimated at ± 10 %.

Table 5S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-fluoroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-fluoroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0026
100	0.0052
500	0.0154
1000	0.0299
2000	0.0375
3000	0.0387

[a] Experimental error is estimated at ± 10 %.

Table 6S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-trifluoromethylanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-trifluoromethylanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0059
100	0.0114
200	0.0216
500	0.0414
1000	0.0679
2000	0.0770

[a] Average of duplicate values. Experimental error is estimated at ± 10 %.

Table 7S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-ethoxycarbonylanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-ethoxycarbonylanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0061
100	0.0085
500	0.0340
1000	0.0545
2000	0.0707

[a] Experimental error is estimated at ± 10 %.

Table 8S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.0 (0.1 M Tris-HCl), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0152
100	0.0307
200	0.0495
500	0.0868
1000	0.123
2000	0.136

[a] Average of duplicate values. Experimental error is estimated at ± 10 %.

Table 9S: Reaction rates of GGT in D_2O, in the presence of 20 mM Gly-Gly at 37 °C at pD 8.0 (0.1 M Tris-DCl), with L-Glu(γ-*p*-methoxyanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methoxyanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0048
100	0.0086
200	0.0144
500	0.0308
1000	0.0538
2000	0.0773

[a] Experimental error is estimated at ± 10 %.

Table 10S: Reaction rates of GGT in D_2O, in the presence of 20 mM Gly-Gly at 37 °C at pD 8.0 (0.1 M Tris-DCl), with L-Glu(γ-anilide) as a function of substrate concentration.

[L-Glu(γ-anilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0082
100	0.0151
200	0.0240
500	0.0393
1000	0.0485
2000	0.0524

[a] Experimental error is estimated at ± 10 %.

Table 11S: Reaction rates of GGT in D_2O, in the presence of 20 mM Gly-Gly at 37 °C at pD 8.0 (0.1 M Tris-DCl), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0133
100	0.0218
200	0.0431
500	0.0562
1000	0.0677
2000	0.0769

[a] Experimental error is estimated at ± 10 %.

Table 12S: Kinetic parameters (k_{cat}, K_M) obtained according to Equation 4 for the reaction of GGT in D_2O, in the presence of 20 mM Gly-Gly at 37 °C at pD 8.0 (0.1 M Tris-DCl), with substrates **2a**, **2d**, and **2h** (see Methods) and kinetic isotope effects ($k_{cat}^{H_2O}/k_{cat}^{D_2O}$).

Substrate	k_{cat} [a] (s^{-1})	K_M [a] (μM)	$k_{cat}^{H_2O}/k_{cat}^{D_2O}$
L-Glu(γ-*p*-methoxyanilide)	671	475	1.45
L-Glu(γ-anilide)	438	301	1.03
L-Glu(γ-*p*-nitroanilide)	629	236	1.83

[a] Experimental error is estimated at ± 10 %.

Table 13S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 7.0 (0.1 M MOPS), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0023
100	0.0043
200	0.0075
500	0.0159
1000	0.0332
2000	0.0316

[a] Experimental error is estimated at ± 10 %.

Table 14S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 7.5 (0.1 M Tris-HCl), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0183
100	0.0340
200	0.0480
500	0.0739
1000	0.0875
2000	0.0900

[a] Experimental error is estimated at ± 10 %.

Table 15S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.5 (0.1 M Tris-HCl), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0179
100	0.0262
200	0.0408
500	0.0795
1000	0.122
2000	0.151

[a] Experimental error is estimated at ± 10 %.

Table 16S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 9.0 (0.1 M CHES), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0081
100	0.0123
200	0.0241
500	0.0513
1000	0.0711
2000	0.0760

[a] Experimental error is estimated at ± 10 %.

Table 17S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 9.5 (0.1 M CHES), with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0041
100	0.0099
200	0.0147
500	0.0329
1000	0.0491
2000	0.0545

[a] Experimental error is estimated at ± 10 %.

Table 18S: Kinetic parameters (k_{cat} and K_M) obtained according to Equation 4 for the reaction of GGT with L-Glu(γ-*p*-nitroanilide), in the presence of 20 mM Gly-Gly at 37 °C, between pH 7.0 and 9.5.

pH	K_M (μM)	k_{cat} (s^{-1})	log (k_{cat}) [a]
7.0	843 ± 445	391 ± 78	2.593
7.5	210 ± 19	821 ± 39	2.915
8.0	468 ± 50	1151 ± 169	3.061
8.5	774 ± 87	1644 ± 78	3.216
9.0	540 ± 115	783 ± 78	2.894
9.5	659 ± 133	587 ± 39	2.769

[a] Experimental error is estimated at ± 0.040.

Table 19S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 7.0 (0.1 M MOPS), with L-Glu(γ-anilide) as a function of substrate concentration.

[L-Glu(γ-anilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0051
150	0.0118
500	0.0234
1000	0.0315
2000	0.0388
4000	0.0360

[a] Experimental error is estimated at ± 10 %.

Table 20S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 7.5 (0.1 M Tris-HCl), with L-Glu(γ-anilide) as a function of substrate concentration.

[L-Glu(γ-anilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0029
150	0.0096
500	0.0264
1000	0.0373
2000	0.0454
4000	0.0463

[a] Experimental error is estimated at ± 10 %.

Table 21S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.5 (0.1 M Tris-HCl), with L-Glu(γ-anilide) as a function of substrate concentration.

[L-Glu(γ-anilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0070
150	0.0098
500	0.0251
1000	0.0349
2000	0.0404
3000	0.0432

[a] Experimental error is estimated at ± 10 %.

Table 22S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 9.5 (0.1 M CHES), with L-Glu(γ-anilide) as a function of substrate concentration.

[L-Glu(γ-anilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0007
150	0.0017
500	0.0049
1000	0.0072
2000	0.0116
3000	0.0133

[a] Experimental error is estimated at ± 10 %.

Table 23S: Kinetic parameters (k_{cat} and K_M) obtained according to Equation 4 for the reaction of GGT with L-Glu(γ-anilide), in the presence of 20 mM Gly-Gly at 37 °C, between pH 7.0 and 9.5.

pH	K_M (μM)	k_{cat} (s^{-1})	log (k_{cat}) [a]
7.0	365 ± 76	635 ± 35	2.803
7.5	593 ± 107	840 ± 49	2.924
8.0	450 ± 45	449 ± 28	2.652
8.5	501 ± 75	406 ± 18	2.609
9.5	1441 ± 344	174 ± 17	2.241

[a] Experimental error is estimated at ± 0.040.

Table 24S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 7.0 (0.1 M MOPS), with L-Glu(γ-*p*-methoxyanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methoxyanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0013
150	0.0064
500	0.0216
1000	0.0357
2000	0.0458
3000	0.0510

[a] Experimental error is estimated at ± 10 %.

Table 25S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 7.5 (0.1 M Tris-HCl), with L-Glu(γ-*p*-methoxyanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methoxyanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0043
150	0.0137
500	0.0327
1000	0.0469
2000	0.0568
3000	0.0633

[a] Experimental error is estimated at ± 10 %.

Table 26S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 8.5 (0.1 M Tris-HCl), with L-Glu(γ-*p*-methoxyanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methoxyanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
150	0.0146
500	0.0267
1000	0.0485
2000	0.0692
3000	0.0777

[a] Experimental error is estimated at ± 10 %.

Table 27S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at pH 9.0 (0.1 M CHES), with L-Glu(γ-*p*-methoxyanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-methoxyanilide)] (μM)	Rate ($M \cdot min^{-1} \cdot mg^{-1}$) [a]
50	0.0011
150	0.0026
500	0.0086
1000	0.0155
2000	0.0321
3000	0.0395
4500	0.0431

[a] Experimental error is estimated at ± 10 %.

Table 28S: Kinetic parameters (k_{cat} and K_M) obtained according to Equation 4 for the reaction of GGT with L-Glu(γ-*p*-methoxyanilide), in the presence of 20 mM Gly-Gly at 37 °C, between pH 7.0 and 9.0.

pH	K_M (μM)	k_{cat} (s^{-1})	log (k_{cat}) [a]
7.0	1109 ± 175	547 ± 35	2.738
7.5	692 ± 42	745 ± 14	2.872
8.0	953 ± 28	971 ± 50	2.987
8.5	1424 ± 277	1007 ± 89	3.003
9.0	3015 ± 862	640 ± 93	2.806

[a] Experimental error is estimated at ± 0.040.

Table 29S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 15 °C at different pH levels, with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate[a] at pH 8.75 (μM/min)	Rate[a] at pH 9.00 (μM/min)	Rate[a] at pH 9.25 (μM/min)	Rate[a] at pH 9.50 (μM/min)
200	0.548	0.480	0.341	0.260
500	1.18	0.887	0.717	0.538
750	1.51	1.27	0.910	0.752
1500	1.94	1.56	1.22	0.932
2500	2.03	1.70	1.24	0.949
3980	2.09	1.69	1.27	0.868

[a] Experimental error is estimated at ± 10 %.

Table 30S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 25 °C at different pH levels, with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate[a] at pH 8.75 (μM/min)	Rate[a] at pH 9.00 (μM/min)	Rate[a] at pH 9.25 (μM/min)	Rate[a] at pH 9.50 (μM/min)
200	1.04	0.690	0.489	0.440
500	2.04	1.52	1.14	0.897
750	2.57	2.22	1.48	1.16
1500	3.34	2.66	2.06	1.52
2500	3.68	3.16	2.14	1.75
3980	3.48	3.19	2.15	1.59

[a] Experimental error is estimated at ± 10 %.

Table 31S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 30 °C at different pH levels, with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate[a] at pH 8.75 (μM/min)	Rate[a] at pH 9.00 (μM/min)	Rate[a] at pH 9.25 (μM/min)	Rate[a] at pH 9.50 (μM/min)
200	1.30	0.924	0.683	0.486
500	2.61	2.01	1.46	0.972
750	3.32	2.51	1.94	1.40
1500	4.17	3.79	2.64	1.84
2500	4.34	4.13	2.78	1.97
3980	4.39	3.86	2.78	1.88

[a] Experimental error is estimated at ± 10 %.

Table 32S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 37 °C at different pH levels, with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate[a] at pH 8.75 (μM/min)	Rate[a] at pH 9.00 (μM/min)	Rate[a] at pH 9.25 (μM/min)	Rate[a] at pH 9.50 (μM/min)
200	1.60	1.12	0.808	0.590
500	3.11	2.57	1.82	1.26
750	4.37	3.48	2.38	1.63
1500	5.65	4.95	3.23	2.24
2500	6.08	5.02	3.56	2.49
3980	5.91	5.54	3.57	2.34

[a] Experimental error is estimated at ± 10 %.

Table 33S: Reaction rates of GGT, in the presence of 20 mM Gly-Gly at 40 °C at different pH levels, with L-Glu(γ-*p*-nitroanilide) as a function of substrate concentration.

[L-Glu(γ-*p*-nitroanilide)] (μM)	Rate[a] at pH 8.75 (μM/min)	Rate[a] at pH 9.00 (μM/min)	Rate[a] at pH 9.25 (μM/min)	Rate[a] at pH 9.50 (μM/min)
200	1.81	1.37	0.933	0.573
500	3.35	3.10	1.93	1.23
750	4.58	4.03	2.55	1.64
1500	6.20	5.36	3.57	2.27
2500	6.47	6.03	3.92	2.60
3980	6.42	5.74	4.45	2.39

[a] Experimental error is estimated at ± 10 %.