

**Gamma-Glutamyl Transpeptidase Acylation with Peptidic Substrates:
Free Energy Relationships Measured by an HPLC Kinetic Assay**

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Running title: GGT Acylation

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Abbreviations used: CHES, 2-(cyclohexylamino)ethanesulfonic acid; DABS, dabsyl, 4-dimethylaminoazobenzene-4'-sulfonyl; DIEA, diisopropylethylamine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 4-morpholinoethanesulfonic acid; MOPS, 4-morpholinopropanesulfonic acid; TBTU, N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate; TCA, trichloroacetic acid; TFA, trifluoroacetic acid.

Supporting information

HPLC conditions

Table 1S: Chromatographic gradient profile used for separation of the reaction products for the reactions of GGT with most of the substrate analogues studied herein.

Time (min)	Solvent A (%) (25 mM KH ₂ PO ₄ , pH 6.8)	Solvent B (%) (acetonitrile:isopropanol) (80:20)
0	80	20
1	80	20
7	75	25
20	75	25
45	60	40
70	80	20

Table 2S: Chromatographic gradient profile used for separation of reaction products for the reactions of GGT with L- γ -glutamyl-2,2,2-trifluoroethylamide or L- γ -glutamylglycinamide

Time (min)	Solvent A (%) (25 mM KH ₂ PO ₄ , pH 6.8)	Solvent B (%) (acetonitrile:isopropanol) (80:20)	Solvent C (%) (Water)
0	80	20	0
1	80	20	0
7	75	25	0
20	75	25	0
45	60	40	0
50	65	35	0
52	0	0	100
67	0	0	100
70	0	100	0
85	0	100	0
90	0	0	100
105	0	0	100

Figure 1S: Reversed-phase HPLC chromatogram recorded at 420 nm of the dabsyl chloride derivatives of the reaction of GGT with L- γ -glutamylglycinamide. a: L- γ -glutamyl-L- γ -glutamylglycinamide, b: L-glutamic acid, c: L- γ -glutamylglycylglycine, d: DABS-OH, e: L-glutamine, standard, f: L- γ -glutamylglycinamide, g: glycylglycine, h: glycinamide.

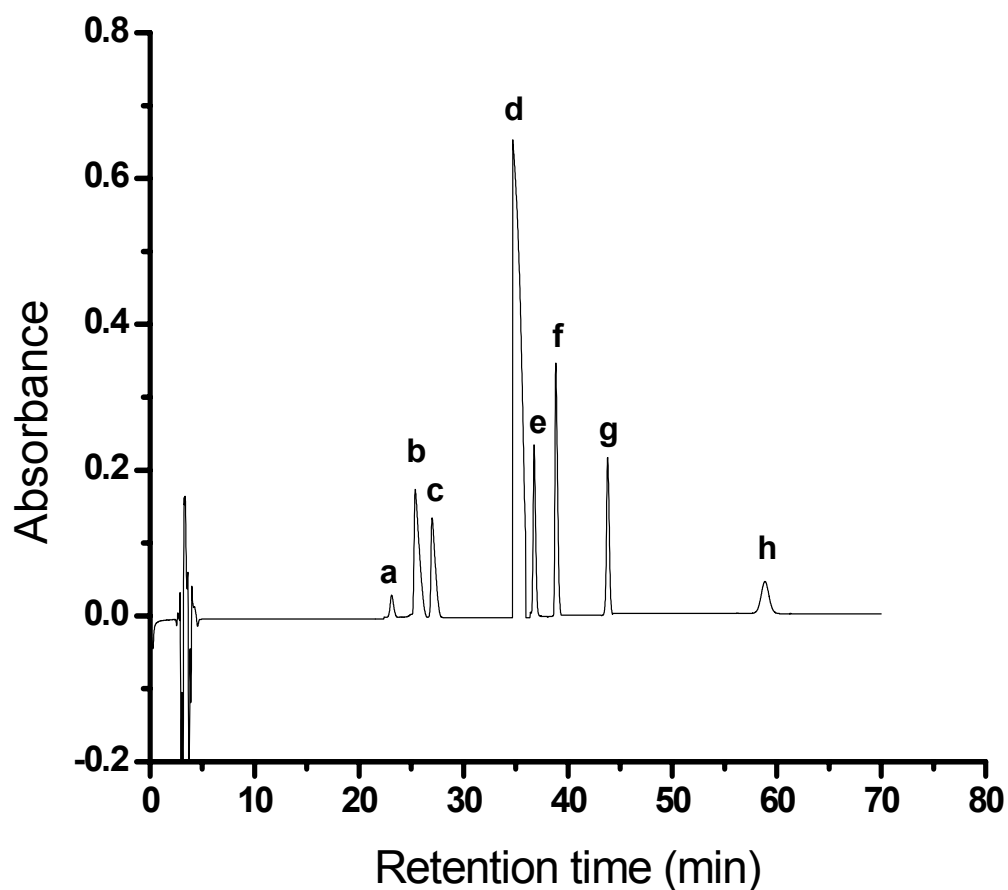
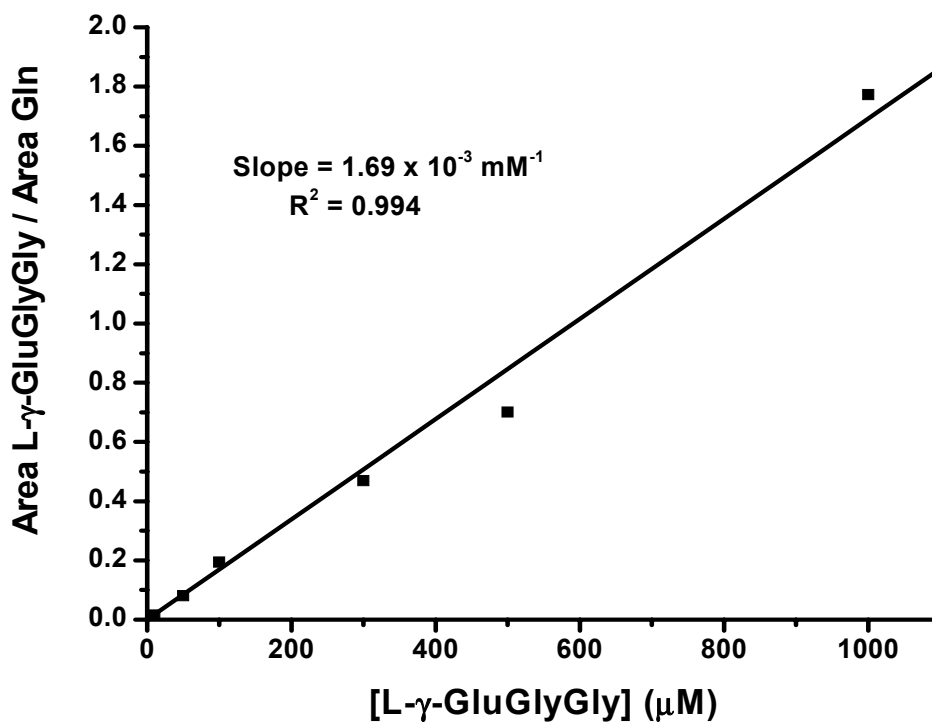


Figure 2S: Standard curve for the concentration of L- γ -glutamylglycylglycine as determined by the ratio of the area of its peak compared to that of the internal standard, L-glutamine.



Kinetic data

Table 3S: pH-rate data for the GGT-mediated reaction of GSH with GlyGly at 37 °C.

pH	k_{cat} (s^{-1}) ^a	K_{M} (mM) ^a
6.0	120 ± 7	0.47 ± 0.09
7.0	370 ± 50	0.78 ± 0.29
8.0	990 ± 80	0.67 ± 0.16
8.5	1092 ± 518	4.0 ± 3.0
9.0	325 ± 117	2.3 ± 2.0
9.5	70 ± 20	1.0 ± 0.6

^a Relative errors derive from the non-linear least squares fitting as described in the Experimental section.