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Glutathione-like Tripeptides as Inhibitors of Glutathionylspermidine Synthetase. Part 2: Substitution of the Glycine Part

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Abstract—Glutathionylspermidine synthetase (GspS) is an essential enzyme in the biosynthesis of trypanothione and is an attractive target for the design of selective anti-parasitic drugs. We synthesised a series of analogues of glutathione (L-γ-Glu-L-Leu-X) where the glycine moiety has been substituted for other amino acids. These peptides were evaluated as substrates and inhibitors of GspS. Compounds with basic side chains such as diaminopropionic acid were found to be good inhibitors (K_i: 7.2 μM). Substitution of the glycine part abolished the GspS substrate properties of the tripeptide.

Glutathionylspermidine synthetase (GspS) is an important enzyme in the metabolism of parasitic Kinetoplastida such as Trypanosoma and Leishmania and is a potential target in the design of new drugs. It is one of the parasite-selective enzymes of the biosynthesis of trypanothione.[1] This thiol [N^1, N^8-bis(glutathionyl)-spermidine] is a key compound in the antioxidant defence system of these protozoa and its metabolism is a valuable target in the design of anti-parasitic agents against sleeping sickness, Chagas disease and leishmaniasis.[2]

We recently reported the synthesis of a series of glutathione related tripeptide as substrates or (and) inhibitors of GspS.[3–5] In the previous paper[5] we discussed the influence of substitutions of the glycine-carboxylic group of glutathione-like tripeptide for other acidic groups. We now report a structure-inhibitor relationship investigation with a series of peptides derived from L-γ-Glu-L-Leu-Gly (**1**) in which glycine was substituted for side-chain amino acids. We were particularly interested in these compounds as we already discovered that L-γ-Glu-L-Leu-L-Ala (**2**) was a modest inhibitor of GspS.[4]

Lγ-Glu-L-Leu-Gly was used as starting peptide as L-Leu was found to be a good substitute for cysteine, affording stable peptides with good affinity for GspS.[3,4] We synthesised a series of tripeptides where Gly was replaced by one of the common amino acids. These compounds were synthesised by the well-known Fmoc/*t*Bu solid phase peptide synthesis chemistry. All compounds were tested as substrate and inhibitor using recombinant wild-type *Crithidia fasciculata* GspS.[6] This afforded peptides (Table 1) without any GspS-substrate activity (Scheme 1).

The modest inhibition by Lγ-Glu-L-Leu-L-Ala (**2**) could be confirmed but longer alkyl chains such as in norvaline (**3**), norleucine (**4**) and leucine (**5**) decreased strongly the inhibitory activity. Addition of functional groups such as in asparagine (**6**), aspartic acid (**7**) and glutamic acid (**8**) did not give interesting compounds. Adding however nucleophilic groups such as an amino function in diaminopropionic acid (**9**), diaminobutyric acid (**10**), ornithine (**11**) and lysine (**12**), a guanidine function in arginine (**13**) or an hydroxy group in serine (**14**) gave

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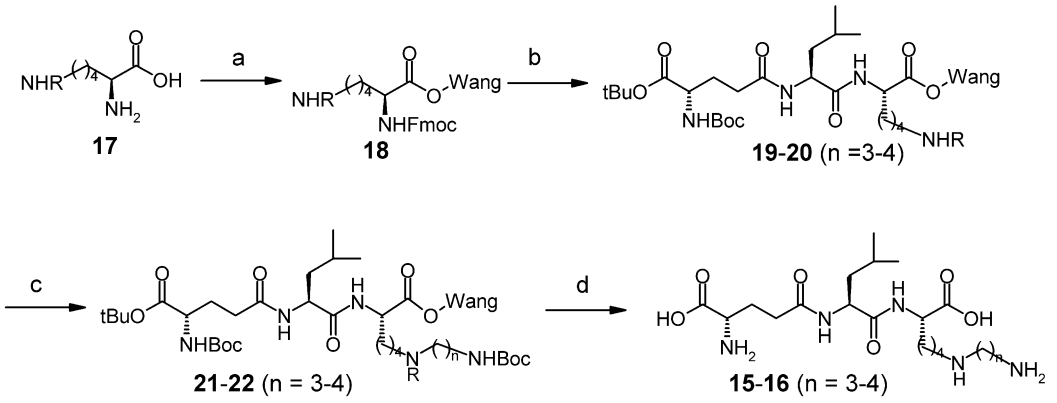

Scheme 1. R = –SO_2[*o*(NO_2)phenyl]: (a) (i) FmocOsu, Na_2CO_3/dioxane; (ii) Wang resin, hydroxybenzotriazole, TBTU, DIPEA/DMF; (b) (i) piperidine/DMF; (ii) Fmoc L-Leu, TBTU, DMF; (iii) piperidine/DMF; (iv) Boc-L-Glu-O*t*Bu, TBTU, DMF; (c) Cs_2CO_3, NaI, Boc$NH(CH_2)_n$ OSO_2Me, DMF; (d) (i) $HSCH_2CH_2OH$, DBU/DMF; (ii) TFA/H_2O.

Table 1. Inhibitory activity of L-γ-Glu-L-Leu-X[a] against glutathionylspermidine synthetase (GspS)

	X	% inhibition	IC_{50} (μM)	K_i (μM)	K_i' (μM)
1	Gly	0	—		
2	L-Ala	87	90±6		
3	L-norVal	70	145±13		
4	L-norLeu	53	ND		
5	L-Leu	59	365±21		
6	L-Asn	68	142±1		
7	L-Asp	69	156±9		
8	L-Glu	60	227±12		
9	L-Dap	97	9.1±4.9	7.2±1.4	21±6
10	L-Dab	95	13.7±0.2	10.4±0.9	24±3
11	L-Orn	95	10.3±0.3	10±1	39±7
12	L-Lys	93	18.0±0.4	27±7	48±14
13	L-Arg	95	9.4±0.4	6.4±0.9	17±4
14	L-Ser	94	11.7±0.4	14±3	25±5
15	(*N*-Y)L-Lys[b]	93	23±1	33±4	59±10
16	(*N*-Y)L-Lys[b]	94	27.9±1.2	32±4	86±22
21	(*N*-Y)L-Lys[b]	62	157±5		

[a]All compounds are TFA salt.
[b]Y: scheme.

interesting inhibitors. All these compounds show an inhibitory activity in the low μM range.

In absence of a 3-D crystallographic information and the nature of the substrate binding sites for GspS, we supposed that the nucleophilic group interacts with electron poor domains in the active center of the enzyme. It could be possible that this group mimics one of the amino groups of the incoming spermidine. This hypothesis prompted us to synthesise analogues of these peptides with spermidine-like amino acids: the ε-amino-group of lysine in L-γ-Glu-L-Leu-L-Lys was substituted with a *N*-aminopropyl and *N*-aminobutyl chain (**15**, **16**). These compounds were synthesised by a solid-phase system designed to allow the preparation of a series of compounds derived from L-γ-Glu-L-Leu-L-Lys (**12**) with the potential to introduce diversity as well in the amino acid chain as on the ε-aminogroup of lysine. Starting compound was L-ε-(*o*-nitrophenylsulphonyl)-lysine (**17**), prepared from the copper(II) complex[7] of L-lysine and *o*-nitrophenylsulphonylchloride. After α-amino protection with a Fmoc-group, L-α-Fmoc-ε-(*o*-nitrophenylsulphonyl)lysine was linked to a Wang resin (**18**) and the peptide (**19**–**20**) chain was synthesised on the α-position by means of the usual Fmoc solid-phase peptide chemistry using TBTU as coupling reagent. The polyamine side chain was introduced on the ω-position of lysine using the Fukuyama procedure[8] with a mesylate-Cs_2CO_3 coupling and thiolate deprotection (**21**–**22**). Final cleavage afforded peptides such as **15**–**16**.

Adding a second amino group did not improve the activity: the polyamine side-chain compounds showed about the same activity as the lysine-tripeptide **13**. The importance of the non-protected terminal amino group of lysine is emphasized by the decrease in inhibitory activity if the (*o*-nitrophenylsulphonyl)group is not deprotected such as in **21**.

We conclude that L-γ-Glu-L-Leu-L-Dap (**9**) and closely related L-γ-Glu-L-Leu-L-X peptides (**10**–**14**) where X contains a nucleophilic side chain are most interesting inhibitory peptides against GspS and excellent lead compound for further anti-parasitic drug design in the Trypanosoma–Leishmania field.

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$$\%\ \text{inhibition} = \left(1 - \frac{v_i}{v_o}\right) \times 100$$

Initial velocity measurements were made at 8–10 inhibitor concentrations, ranging from 0 to 2.5 mM, and 0.5 mM glutathione. Spermidine (2 mM), Mg^{2+} (10 mM) and ATP (2 mM) were present in saturating conditions. The assays were conducted in 100 mM HEPES buffer pH 7.3 at 25 °C with 40 nM GspS. Assay mixtures were incubated for 5 min prior to initiation with spermidine. IC_{50} values were detremined according to the following two-parameter equation, where the lower data limit is 0, ie the data are background corrected, and the upper data limit is 100, that is the data are range corrected.

$$y = \frac{100}{1 + \left(\frac{x}{IC_{50}}\right)^s}$$

In this equation, s is a slope factor. The equation assumes that y falls with increasing x. Initial velocity measurements were determined at three inhibitor concentrations and a serial dilution of glutathione, ranging from 0.03 to 1 mM. From these data, Michaelis–Menten and Lineweaver–Burk plots were constructed, and kinetic inhibitory values were determined by non-linear least squares regression analysis using the Grafit® programme. All inhibitors were considered as mixed type inhibitors. The indicated errors are the standard errors on the fit.

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