Synthesis of the Trypanosomatid Metabolites Trypanothione, and N^1-Mono- and N^8-Mono-glutathionylspermidine

Graeme B. Henderson,* Peter Ulrich, Alan H. Fairlamb, and Anthony Cerami

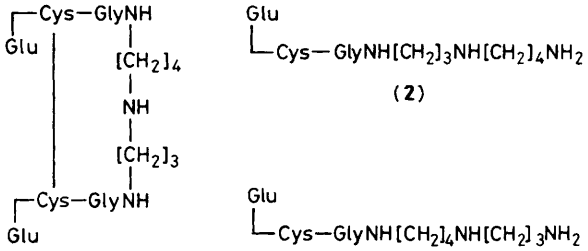
Laboratory of Medical Biochemistry, The Rockefeller University, New York, New York 10021, U.S.A.

The trypanosomatid metabolite trypanothione [N^1,N^8-bis(glutathionyl)spermidine] and its biosynthetic co-metabolites the isomeric N^1- and N^8-mono-glutathionylspermidines have been synthesised by a mild route which involves coupling of glycinyl-spermidine derivatives to a functionally protected γ-glutamylcysteine dipeptide.

With few exceptions both prokaryotic and eukaryotic cells contain high concentrations of the tripeptide L-γ-glutamyl-L-cysteinylglycine (glutathione, GSH).[1] Important general functions of this metabolite are thought to include the maintenance of other intracellular thiols in the reduced state by thiol disulphide exchange, enzymatic removal of peroxides, and scavenging of free radicals.[2,3] The intracellular ratio of reduced to oxidized glutathione (GSH/GSSG) is kept high by the NADPH-dependent flavoprotein glutathione disulphide reductase.[4]

In trypanosomatids examined to date, including the human pathogens *Trypanosoma rhodesiense* and *Trypanosoma cruzi* responsible for African sleeping sickness and South American Chagas' disease respectively, levels of glutathione are lower than in other biological materials and glutathione disulphide reductase activity has not been detected.[5] Instead trypanosomatids possess a related flavoprotein disulphide reductase which utilises as substrate the novel cyclic peptide N^1,N^8-bis(glutathionyl)spermidine (trypanothione) (**1**).[6,7] A covalently linked adduct of glutathione and spermidine (N^1-monoglutathionylspermidine) (**2**) has also been isolated from the prokaryotic organisms *Escherichia coli*[8] but this compound is only found under certain growth conditions and has not previously been assigned a metabolic role. We have recently identified both regioisomeric mono-glutathionylspermidine adducts as co-metabolites of trypanothione in the insect trypanosomatid *Critihidia fasciculata*.[9] In order to carry out studies on the biosynthesis and metabolic functions of trypanothione the compound and its putative biosynthetic precursors N^1- and N^8-glutathionylspermidine (**2**) and (**3**) have been synthesised. The synthetic routes (Scheme 1) are sufficiently flexible to allow for the subsequent synthesis of analogous or isotopically labelled forms. For each compound the protected dipeptide (**4**) [from (**5**) and (**6**), 70%, m.p. 95—96 °C] was coupled to a differentially protected spermidine derivative; the latter compounds were prepared by application of the procedures developed by Ganem *et al.*[10,11] which allow the three reactive centres of spermidine to be distinguished. Thus for trypanothione (**1**), the readily available hexahydropyrimidine (**7**)[10] was acylated with 2 equiv. of the glycine succinimido-oxy ester ZGlyONSu to give (**8**), 70%. The reaction of (**8**) with malonic acid–pyridine in refluxing ethanol gave the N^4-deblocked amine (**9**), 70%, m.p. 149—150 °C, and the latter compound was converted into the t-butoxycarbonyl (Boc) derivative (**10**) on acylation with t-butyl pyrocarbonate [$(Boc)_2O$], 85%.

For N^1-glutathionylspermidine (**2**), acylation of the known N^4,N^8-protected spermidine derivative (**11**)[12] with ZGlyONSu gave (**12**), >90%, m.p. 67—72 °C. For N^8-glutathionylspermidine (**3**) the hexahydropyrimidine (**7**) was acylated regioselectively at the secondary amine (N^1) site with $(Boc)_2O$ in the presence of 18-crown-6 and a proton source[13] and the required product (**13**) was separated from diacylated material by flash column chromatography and coupled without further purification to ZGlyONSu to give (**14**), 55% overall from (**7**).

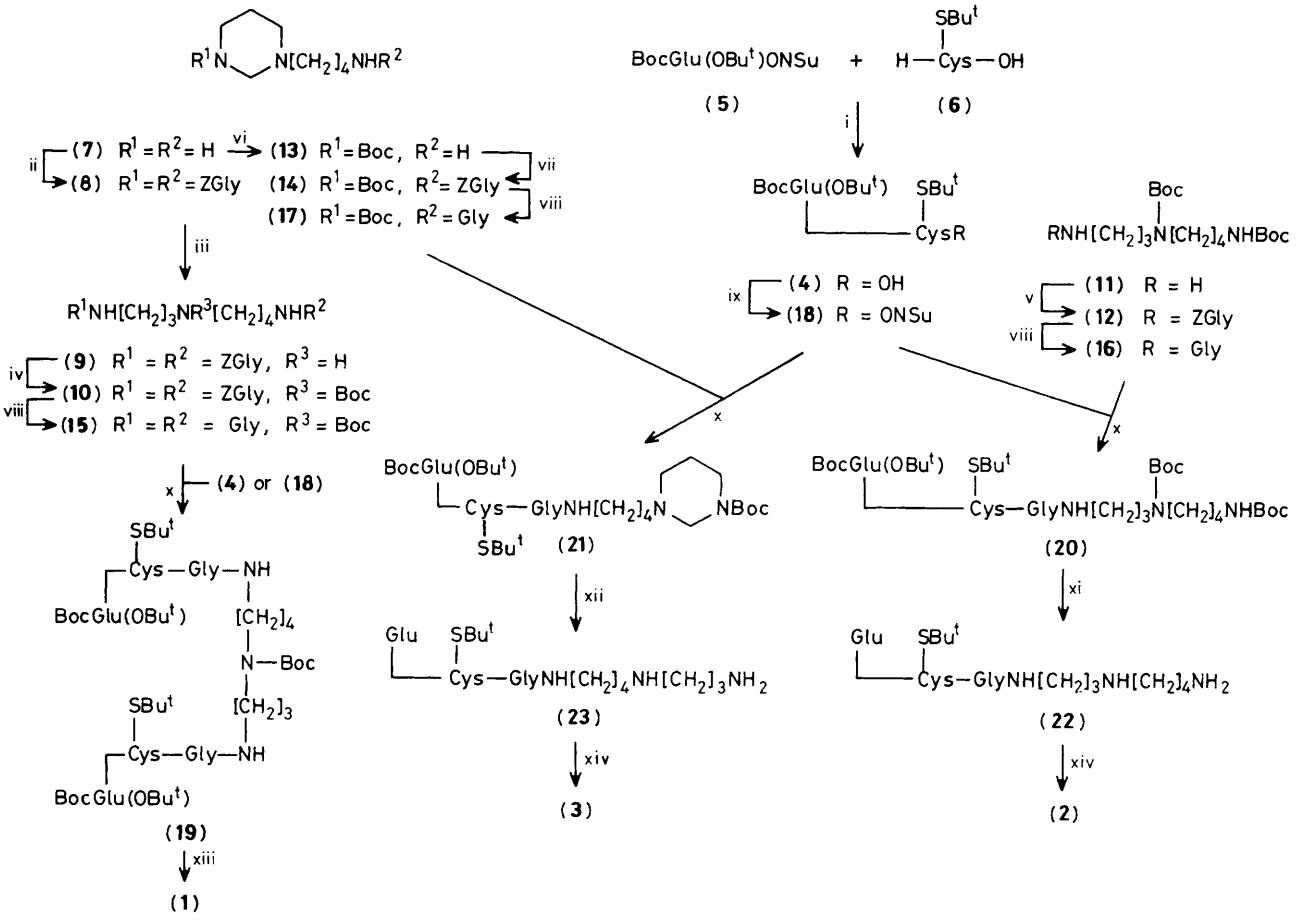


The spermidine derivatives (**10**), (**12**), or (**14**) were selectively deprotected by hydrogenolysis (H_2/Pd) to give the corresponding amines (**15**), (**16**), or (**17**) and the latter compounds were coupled to the dipeptide (**4**) by the mixed anhydride method using isobutylchloroformate or by acylation with the succinimido-oxo ester (**18**) [prepared from (**4**) by reaction with *N*-hydroxysuccinimide (HONSu) and *N*,*N*'-dicyclohexylcarbodiimide (DCC); yield of (**18**) from (**4**), 80%, m.p. 97—104 °C] to give the fully protected products (**19**), (**20**), or (**21**) [average yield from (**10**), (**12**), or (**14**) by either process, 60—70%].

The isomeric glutathionylspermidines (**2**) and (**3**) were first prepared as their t-butyl disulphide derivatives. Reaction of (**20**) with trifluoroacetic acid (TFA) gave (**22**). Reaction of (**21**) with malonic acid–pyridine (as above) then with TFA gave (**23**). The thiol protecting groups were removed from the intermediates (**22**) and (**23**) by reaction with a 10-fold excess of dithiothreitol (DTT) at neutral pH under a stream of N_2 to give (**2**) and (**3**) respectively in >70% yield overall from (**20**) or (**21**) (by amino acid analysis). These deprotection conditions are particularly suited to the synthesis of the isomeric glutathionylspermidines (**2**) and (**3**) because the compounds can be maintained and purified in the reduced state following acidification of the reaction mixture. Final purification was achieved by f.p.l.c.† The isomeric glutathionylspermidines (**2**) and (**3**) were homogeneous on h.p.l.c. analysis[6] and gave the required amino acid analysis. Chemical ionisation mass spectrometric analysis of the corresponding sulphonates of (**2**) or (**3**) (by performic acid oxidation) was consistent with the required structures. In addition, both compounds were metabolised *in vitro* to trypanothione (**1**) by an enzyme preparation from *C. fasciculata*.[9]

The conversion of the protected derivative (**19**) to trypanothione (**1**) was carried out by analogous processes but in this case the acyclic dithiol (dihydrotrypanothione) was isolated and converted into the cyclic disulphide by air oxidation at high dilution (<0.5 mM) and then purified by f.p.l.c. [yield of (**1**) from (**19**) 25% overall, not optimised]. Synthetic trypano-

† F.p.l.c. = fast protein liquid chromatography (Pharmacia, Inc.).



Scheme 1. *Reagents and conditions:* i, dimethylformamide (DMF), room temperature (r.t.), 10 h; ii, tetrahydrofuran (THF), r.t., 16 h; iii, EtOH, $CH_2(CO_2H)_2$ (4 equiv.), pyridine (3 equiv.), reflux, 1 h; iv, THF, Et_3N (2 equiv.), $(Boc)_2O$ (2 equiv.), r.t., 10 h; v, THF, r.t., 2 h; vi, CH_2Cl_2, *p*-$MeC_6H_4SO_3H$ (2 equiv.), Et_3N (1 equiv.), $(Boc)_2O$ (1.25 equiv.), 4 °C, 10 h; vii, THF, Et_3N (1 equiv.), r.t., 10 h; viii, EtOH, Pd, H_2, r.t., 1 h; ix, THF, HONSu (1 equiv.), DCC (1.2 equiv.), 4 °C, 8 h; x, DMF, 4 °C, 8 h; xi, TFA, r.t., 45 min; xii, EtOH, $CH_2(CO_2H)_2$ (4 equiv.), pyridine (2 equiv.), reflux, 1 h, evaporation, then TFA, r.t., 45 min; xiii, TFA, r.t., 1 h, evaporation, then aqueous DTT (10 equiv.), pH 7, N_2, adjust to pH 4, extraction with EtOAc, dilution to <0.5 mM, O_2, 4 °C, 48 h, f.p.l.c.; xiv, aqueous DTT (10 equiv.), pH 7, adjust to pH 4, f.p.l.c.

thione co-eluted with the natural material on h.p.l.c. and gave the required mass spectrometric analysis.[6] The synthetic material was active with the flavoprotein reductase from *C. fasciculata* (K_m = 53 μM) and has been used to isolate this enzyme in pure form.[7] The synthetic material has also proved to be fully active with the corresponding enzyme from *T. cruzi*.[14]

Detailed biosynthetic studies on trypanothione and the glutathionyl spermidines using these synthetic compounds are being carried out.

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