

Solid phase polyamine linkers - their utility in synthesis and the preparation of directed libraries against trypanothione reductase

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A variety of diprotected polyamines are anchored to a solid support and used in solid phase chemistry and library generation.

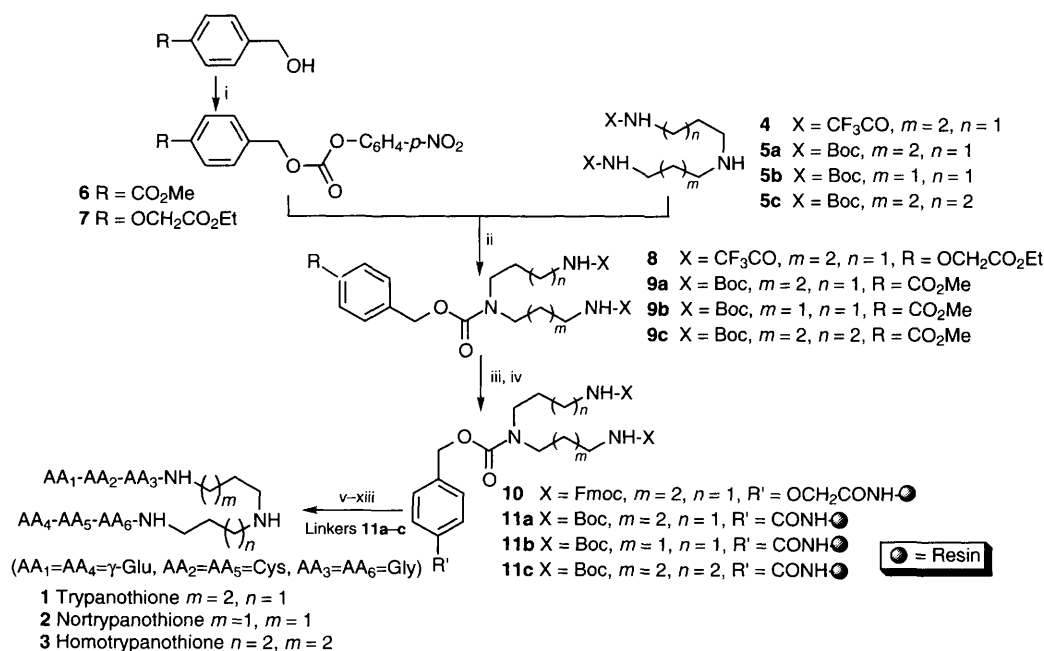
There is widespread interest in the preparation of novel polyamine conjugates due to the varied biological activities of naturally occurring polyamine derivatives.¹ There are, however, many synthetic problems associated with polyamine chemistry, most notably those of amine differentiation and the ability to handle these exceptionally polar compounds.² To overcome these difficulties and, importantly, to allow a combinatorial approach to polyamine conjugates we have developed a number of linkers which allow the immobilisation of a range of polyamines onto a solid support. To demonstrate their utility once tethered, the immobilised polyamines were used firstly in the synthesis of *N*¹, *N*⁸-bis(glutathionyl)spermidine (trypanothione)^{3,4} **1** and two trypanothione analogues **2** and **3** and secondly in the preparation of a soluble library⁵ of 576 polyamine conjugates. This library was screened for activity against trypanothione reductase, an enzyme central to the management of oxidative stress in the trypanosomal parasites responsible for tropical diseases such as African Sleeping Sickness and Chagas' Disease.⁴

The immobilisation of the polyamines shown in Scheme 1 is short and efficient, commencing with the selectively diprotected polyamines **4** and **5**.⁶ These were treated with the nitrophenylcarbonates **6** or **7** thus creating the *N*⁴ protection as benzyl-derived urethanes **8** and **9**. Cleavage of the esters and

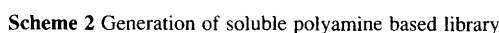
coupling to a polystyrene aminomethyl resin using standard peptide coupling conditions gave the resin linked polyamines **10** and **11**. (Reprotection of the primary amines in **8** with Fmoc-OSu was necessary following the hydrolysis of the trifluoroacetyl group).⁷ The crucial resin immobilisation step was quantified simply and accurately using a colourimetric ninhydrin test⁸ and by spectrophotometric quantification of the Fmoc group thus ensuring initial template homogeneity. The urethane linkage in **11** was found to be cleavable using strongly acidic conditions [10 equiv. trifluoromethanesulfonic acid-trifluoroacetic acid (TFMSA/TFA)] and hence potentially useful for resin screening purposes while linker **10** was readily cleavable with TFA and hence ideal for solution screening applications.

The linkers **11a,b,c** were used in the preparation of a number of polyamine conjugates including the parasitic polyamine metabolite trypanothione **13**⁴ and the analogous compounds nortrypanothione **2** and homotrypanothione **3** as shown in Scheme 1.

Library synthesis⁵ was carried out as shown in Scheme 2† using the split/mix method. Each of the final 24 mixtures of 24 resin bound compounds was cleaved individually‡ and screened for competitive inhibitory activity against trypanothione reductase and trypanothione.§ One mixture, derived from citrazinic acid, exhibited high activity (100% at 25 μmol dm⁻³) and was subjected to further assays to ensure activity at lower concentrations. Deconvolution of this active mixture was achieved by returning to the 24 individual samples of resin which were put to one side with only a single amino acid



Scheme 1 Reagents and conditions: i, *p*-nitrophenylchloroformate, CH₂Cl₂, pyridine; ii, DMF, NEt₃, 45 °C; iii, aq. NaOH, dioxane (when X = CF₃CO followed by treatment with Fmoc-OSu, dioxane, aq. NaHCO₃); iv, DIC, HOBt, DMAP, CH₂Cl₂, aminomethyl resin; v, 50% TFA; vi Fmoc-Gly, DIC, HOBt; vii, 20% piperidine; viii, Fmoc-Cys(Trt), DIC, HOBt; ix, 20% piperidine; x, Boc-Glu(OH)-OtBu, DIC, HOBt; xi, 10 equiv. TFMSA, TFA, EDT, PhSMe; xii, MeOH, I₂; xiii, HPLC



We would like to thank the Wellcome Trust for an equipment grant (039094/Z/93/Z) and for a Project Grant 043503/PMG/AH (M. B.), the Royal Society for a University Research Fellowship (M. B.), EPSRC for quota awards (I. R. M., H. S.). This investigation received financial support from the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases. We thank Professor Alan Fairlamb for the kind gift of *T. cruzi* trypanothione reductase.

‡ Cleavage was performed with TFA-H₂O/triisopropylsilane-ethanedithiol (37:1:1:1). The cleaved mixtures were precipitated into ether-hexane (1:1), centrifuged, washed with ether-hexane (1:1) and centrifuged again before dissolution in water and lyophilisation. ES MS and HPLC were

§ Assay conditions: 1 ml total volume of 50 mmol dm⁻³ K₂HPO₄–1 mmol dm⁻³ EDTA in H₂O–DMSO (4:1) at pH 7.5 containing 0.1 mmol dm⁻³ NADPH and 0.25 μmol l⁻¹ trypanothione reductase. Initial rate of consumption of NADPH at 340 nm on addition of trypanothione (34 μmol dm⁻³) and the mixture of 24 compounds (25 μmol dm⁻³ per compound).

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