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

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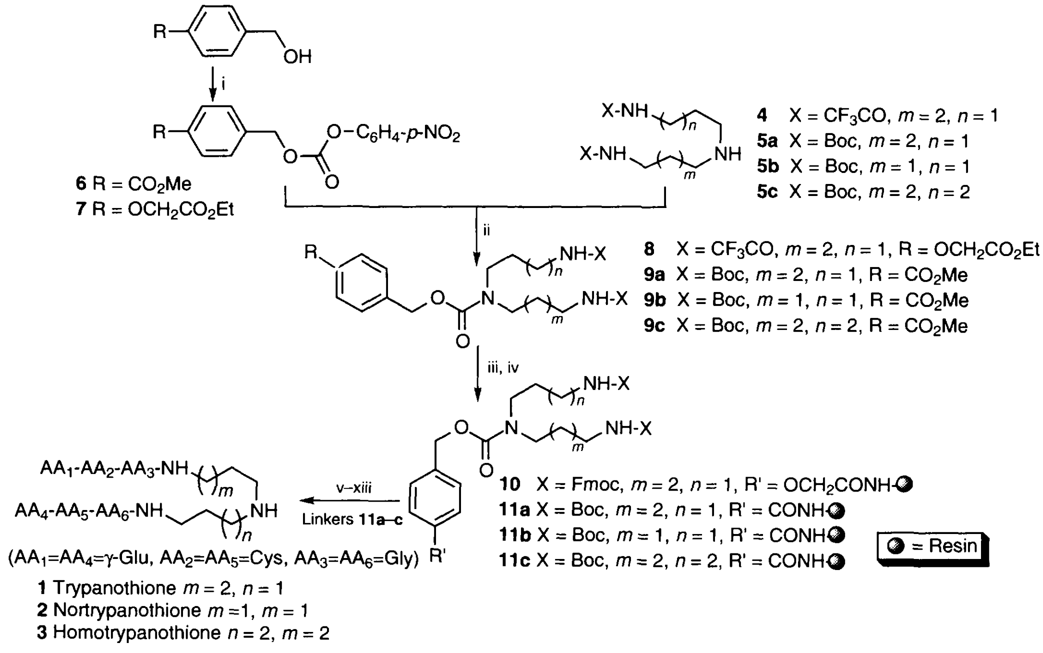
A variety of diprotected polyamines are anchored to a solid support and used in solid phase chemistry and library generation.

There is widespread interest in the preparation of novel polyamine conjugates due to the varied biological activites 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 library[5] 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]

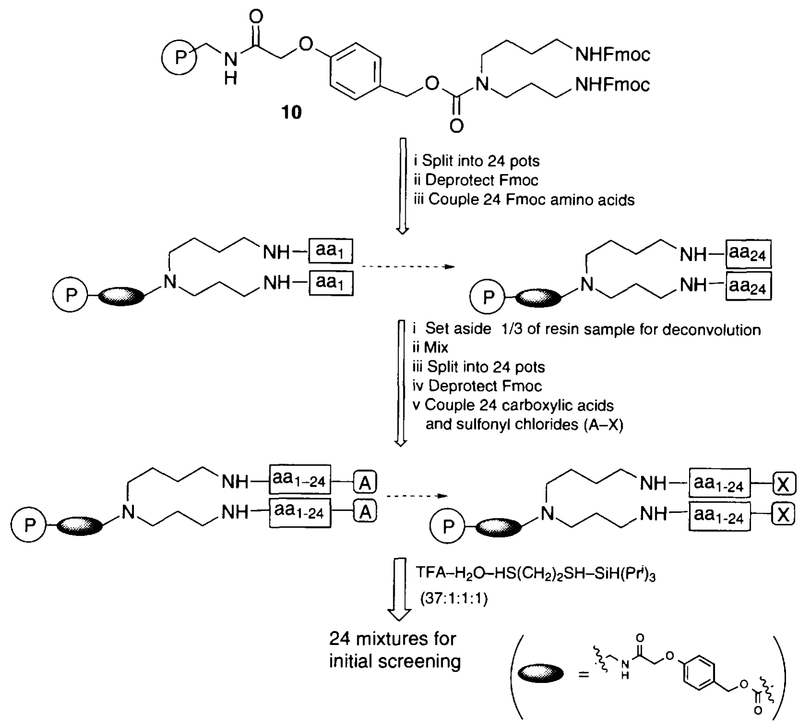
The immobilisation of the polyamines shown in Scheme 1 is short and efficient, commencing with the selectively diprotected polyamines **4** and **5**.[6] These were treated with the nitrophenylcarbonates **6** or **7** thus creating the N^4 protection as benzyl-derived urethanes **8** and **9**. Cleavage 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 test[8] 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 **1**[3,4] and the analogous compounds nortrypanothione **2** and homotrypanothione **3** as shown in Scheme 1.

Library synthesis[5] 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, CH_2Cl_2, pyridine; ii, DMF, NEt_3, 45 °C; iii, aq. NaOH, dioxane (when X = CF_3CO followed by treatment with Fmoc-OSu, dioxane, aq. $NaHCO_3$); iv, DIC, HOBt, DMAP, CH_2Cl_2, 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, I_2; xiii, HPLC



Scheme 2 Generation of soluble polyamine based library

attached and citrazinic acid coupled to each. Subsequent cleavage and screening of the individual compounds to reveal the active amino acid component was undertaken. None of the 24 compounds was found to be significantly active against trypanothione reductase. The observed inhibition by the mixture being a 'false positive' presumably resulted from a combined effect from all 24 members of the mixture but highlights one of the problems of deconvolution techniques and mixtures that are non-diverse in nature, and the advantage of testing diverse mixtures and multiple release techniques.

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Footnotes

† The Fmoc-amino acids used were Ala, Val, Leu, Ile, Pro, Met, Phe, Trp(Boc), Gly, Ser(tBu), Thr(tBu), Cys(Trt), Tyr(tBu), Asn, Gln, Lys(Boc), Asp(OtBu), Glu(OtBu), Arg(Pmc), His(Trt), Nva, εAhx, γ-Abu, βAla. All amino acids were coupled twice using 2.2 equiv. of amino acid, HOBt and DIC in DMF/CH_2Cl_2 (1 : 1) for 2 h, whereupon all ninhydrin tests were negative. The carboxylic acids [2,3-dihydroxy-2-naphthoic; chloroacetic; benzoic; 6-chloronicotinic; (2-naphthoxy)acetic; 2,2-dichloro-1-methylcyclopropanecarboxylic; 3,4-dihydroxybenzoic; 5-bromovaleric; 5-chloro-2-nitrobenzoic; 2,4-dichloro-5-sulfamoylbenzoic; 5-bromofuroic; 1-piperidinepropionic; piperonylic; citrazinic; 4-chloro-3-sulfamoylbenzoic; 4-chlorobenzoic; 2-thiophenecarboxylic; 4-carboxybenzenesulfonamide; *trans*-cinnamic; *p*-tolylacetic; phenylpropiolic] were coupled using 8 equiv. of acid, HOBt and DIC in either DMF/CH_2Cl_2 (1 : 1) or DMF. The three sulfonyl chlorides (4-fluorobenzene-; 2-thiophene-; *p*-toluene-) were coupled using 8 equiv. in DMF/CH_2Cl_2 (1 : 1) with triethylamine as base. All couplings were monitored by ninhydrin tests.

‡ Cleavage was performed with TFA–H_2O/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 used to characterise the sub-libraries and showed the expected number of components.

§ Assay conditions: 1 ml total volume of 50 mmol dm^{-3} K_2HPO_4–1 mmol dm^{-3} EDTA in H_2O–DMSO (4 : 1) at pH 7.5 containing 0.1 mmol dm^{-3} NADPH and 0.25 μg ml^{-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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