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Solid Phase Applications of Dde and the Analogue Nde: Synthesis of Trypanothione Disulphide

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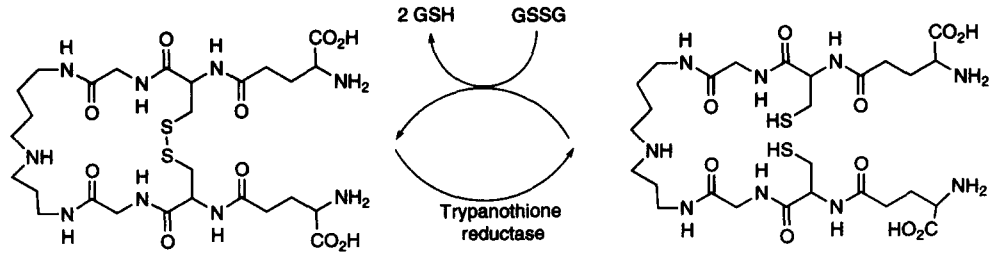
Abstract: *Bis* - Dde and Nde spermidine derivatives selectively protected on the primary amines were readily prepared and attached *via* the secondary amine group to a *p*-nitrophenyl-1-chloroformate pre-activated HMPA resin. Deprotection for Nde was monitored by colour change and UV absorption at 290 nm following which the trypanothione backbone was assembled by standard Fmoc procedures. Release from the resin with TFA and subsequent oxidation gave the trypanothione disulphide in > 80% overall yield. © 1997 Elsevier Science Ltd.

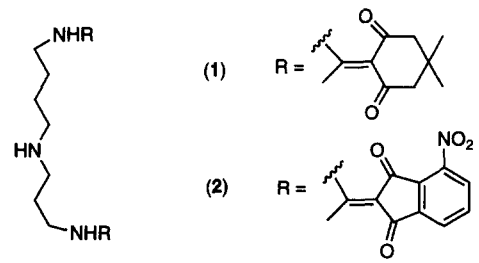
The *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) group is readily attached to any primary amine by simply reacting with 2-acetyldimedone. The stability of this group to both the acid and base conditions used in solid phase methodologies coupled with its facile removal under mild conditions (2% hydrazine in DMF) has led to applications for the construction of atypical and non-peptide entities[1]. Recently these properties have been further exploited and built in systems for carboxylic acid group protection[2] and a new solid phase linker[3]. The selectivity for primary amines which is presumed to be a function of the stabilisation provided by a strong intramolecular hydrogen bond (NH δ = 12-15) also has unique advantages for the manipulation of polyamines and the solid phase synthesis of their conjugates. In this context we recently reported the syntheses of peptide conjugates related to the nephilatoxins[4], as well as philanthotoxin-3.4.3 and the calcium channel blocker sFTX-3.3[5].

We now describe a very convenient solid phase route to the glutathione-spermidine conjugate, trypanothione disulphide using both Dde and *N*-1-(4-nitro-1,3-dioxoindan-2-ylidene)ethyl (Nde)[6] groups. *Trypanosomidae* species including those responsible for African sleeping sickness and South American Chagas disease do not use the classical glutathione based redox system present within mammalian cells. Instead they employ N^1,N^8-*bis*(glutathionyldisulphide) spermidine (trypanothione disulphide) to maintain the cellular glutathione in a reduced state[7] as illustrated in scheme 1. A crystal structure for trypanothione reductase has been reported[8] and it is suggested that inhibiting this enzyme may offer an attractive chemotherapeutic approach to these types of parasitic disease.

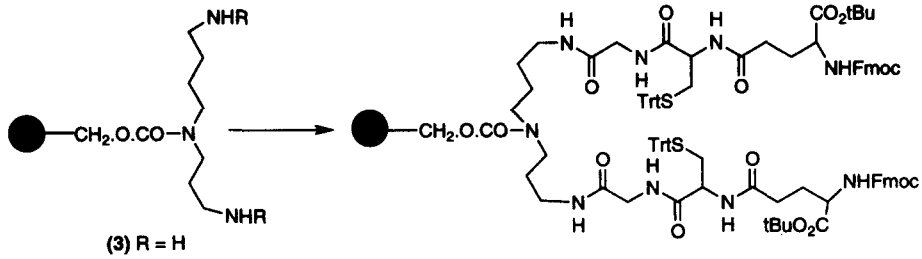


Scheme 1

Until now only a lengthy solution phase[9] and one solid phase synthesis[10] of trypanothione disulphide have been reported with respectively poor to moderate overall yields. The strategic approach adopted here was similar to that previously described[10]. However a TFA labile linkage between a solid support and the secondary amino group of spermidine was considered more compatible with Dde and Fmoc strategies and offered significant experimental advantage. The use of Dde or Nde for the selective protection of the N^1 and N^8 primary amino groups of spermidine together with an alternative means of resin attachment and subsequent TFA cleavage have dramatically improved the efficiency and effectiveness of the overall process.



Spermidine (0.2 mmol) was refluxed in anhydrous ethanol with 2-acetyldimedone (0.6 mmol) to give, in essentially quantitative yield, crystalline N^1,N^8-*bis*-Dde spermidine[11] (**1**). Similarly spermidine with 2-acetyl-4-nitroindane-1,3-dione (3 equivalents) and DIPEA (3 equivalents) afforded the corresponding amorphous N^1,N^8-*bis*-Nde derivative[12] (**2**). Dixit and Leznoff[13] had shown some time ago that amines could be attached through a benzyl carbamate linkage to polymers containing hydroxymethylphenyl residues pre-activated with *p*-nitrophenyl chloroformate. Accordingly a NovaSyn^R TGA support, derivatised with the 4-hydroxymethylphenoxyacetic acid (HMPA) linker was converted to the mixed carbonate by overnight exposure to a tenfold excess of *p*-nitrophenyl chloroformate and DIPEA in DCM. The washed resin was then separately treated with the *bis*-protected spermidine derivatives (1) and (2) and DIPEA in DMF and the suspension again left overnight. The efficiency of the coupling following deprotection of the charged resin with hydrazine monohydrate (2% w/v; DMF), was determined by means of an Fmoc loading test[14] and in both cases near quantitative (>95%) attachment of spermidine was observed.



Scheme 2

For the Nde charged resin the deprotection step was observed to be considerably faster than that for Dde and could be followed visually; the yellow resin first turning red on contact with the hydrazine solution and then colourless as the deprotection proceeded. Alternatively post-column monitoring of the UV absorption of the eluent at 290 nm afforded a sharp peak corresponding to the release of the Nde hydrazine adduct allowing automation of synthetic procedures in line with those adopted for Fmoc deprotection. Having established that spermidine could be effectively loaded and deprotected in the manner required, the synthesis of trypanothione disulphide was accomplished by the sequential addition of Fmoc-Gly-OH, Fmoc-Cys(Trt)-OH and Fmoc-Glu(OH)-OtBu onto both the N^1 and N^8 amino groups of the resin bound spermidine (scheme 2). Fmoc deprotection was achieved using 20% piperidine in DMF and each acylation step mediated with HBTU/HOBt/DIPEA and monitored using the TNBS test[15].

Cleavage of the product from the resin together with concomitant side-chain deprotection was brought about using a TFA/TIPS/EDT/H_2O mixture (9.25:0.25:0.25:0.25) for 2 h. RP-HPLC of the crude product (yield > 90%) revealed one major peak corresponding to dihydrotrypanothione. Preparative chromatography under conditions previously described[10] followed by aerial oxidation (72 h) and monitored by RP-HPLC afforded trypanothione disulphide. The ES-MS data from the reduced and oxidised trypanothione gave the expected peaks at 724 and 722 Da (MH^+) respectively and an accurate mass determination using HRMS-FAB on trypanothione disulphide was consistent with the required molecular formula.

We are currently investigating other applications of Dde and Nde not only for the synthesis of individual polyamine peptides and polyamine conjugates but also for the construction of related libraries not hitherto accessible.

Acknowledgements. We thank Dr W Chan for valuable discussions and BBSRC, UK for a studentship to Barrie Kellam.

References and Notes

Abbreviations: Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; Dde, *N*-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl); DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; EDT, 1,2-ethanedithiol; FAB-MS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxy-benzotriazole; LC-MS, Liquid chromatography mass spectrometry; Nde, 1-(4-nitro-1,3-dioxoindan-2-ylidene)ethyl; RP-HPLC, reverse-phase high performance liquid chromatography; tBu, *tert*-butyl; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; TNBS, 2,4,6-trinitrobenzenesulphonic acid; Trt, trityl.

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6. In order to broaden possible applications we have continued to investigate alternative systems related to Dde but based on other available cyclic 1,3-diones; the Nde group is one such example. 2-Acetyl-4-nitroindane-1,3-dione is readily synthesised (Mosher, W.A.; Meier, W.E. *J. Org. Chem.*, **1970**, *35*, 2924-2926) and reacts with primary amines to give Nde derivatives. A detailed evaluation of Nde as a protecting group will be reported elsewhere. Kellam, B.; Bycroft, B.W.; Chan, W.C.; Chhabra, S.R. in preparation.
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11. A solution of spermidine (29 mg = 32 µl, 0.2 mmol), 2-acetyldimedone (110 mg, 0.6 mmol) and triethylamine (84 µl, 0.6 mmol) in anhydrous ethanol (10 ml) was refluxed for 8 h. Ethanol was removed and the residue redissolved in ethyl acetate. The solution was washed with 5% $NaHCO_3$ solution (3 x 5 ml), dried ($MgSO_4$) and concentrated *in vacuo* to afford N^1,N^8-*bis*-Dde-spermidine as an oil which solidified on standing. Recrystallization from ether/40-60 petroleum ether gave a crystalline product (96%), m.p. 82-84°C.
^{1}H NMR ($CDCl_3$) 1.03 (12H, s, 2 x Me_2C), 1.57-1.87 (6H, m, 2, 6, 7-H_2), 1.89-2.09 (1H, m, 4-NH) 2.36 (8H, s, 4 x CH_2 Dde), 2.56 (6H, s, 2 x = CCH_3), 2.64-2.76 (4H, m, 3, 5-H_2), 3.41-3.51 (4H, m, 1, 8-H_2), 13.45 (2H, s, 1, 8-NH).
m/z (+ve ES-MS) 474 (M+H, $C_{27}H_{43}N_3O_4$ requires *m/z* 473).
12. N^1,N^8-*bis*-Nde-spermidine is prepared in a similar manner to the corresponding Dde derivative. However because of the unsymmetrical nature of Nde the product is undoubtedly a mixture of geometric isomers. This appears to have no effect on its ability to act as a transient protecting group.
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