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Solid phase strategies: Applications of 2-acetyl-4-nitroindane-1,3-dione as a selective protecting group for primary amines

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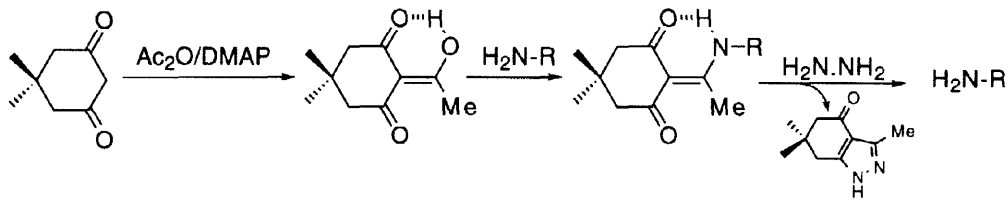
Abstract

2-Acetyl-4-nitroindane-1,3-dione (**1**) has been successfully exploited as a primary amine protecting group displaying excellent acid and secondary/tertiary base stability, but which can be removed with 2% hydrazine at ambient temperature. (**1**) has been condensed with both amino acids and spermidine, affording derivatives which were subsequently utilised in the solid phase synthesis of various peptides and the glutathione-spermidine conjugate trypanothione respectively.

Keywords: Protecting Groups; Solid-Phase Synthesis; Amino acids and derivatives; Peptides and polypeptides

Introduction

It is self-evident that solid phase peptide synthesis (SPPS) is now a mature area of chemistry offering a variety of high fidelity methodologies amenable to automation and affording ready access to a wide range of peptides [1]. However, until relatively recently, comparable procedures which allowed even simple modifications away from linear peptide constructs, for example branched or cyclic structures, were not so well advanced. The introduction of the *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) amino protecting group [2] has gone some way to addressing these constraints, particularly for the synthesis of cyclic peptides [3] and branched multiple antigenic peptides involving the side-chain amino group of lysine or ornithine [4]. The stability of the Dde group to the acid and base conditions employed in SPPS, coupled with its facile removal with hydrazine under continuous flow conditions which can be monitored spectrophotometrically, makes it a useful adjunct to the well established Fmoc/t-Boc strategy [5,6] **Scheme 1**.



Scheme 1.
Synthesis and protection/deprotection strategy for the Dde protecting group

The observed preference of 2-acetyldimedone (Dde-OH) to form stable derivatives only with primary amines is attributed to the strong intramolecular hydrogen bond in the resultant

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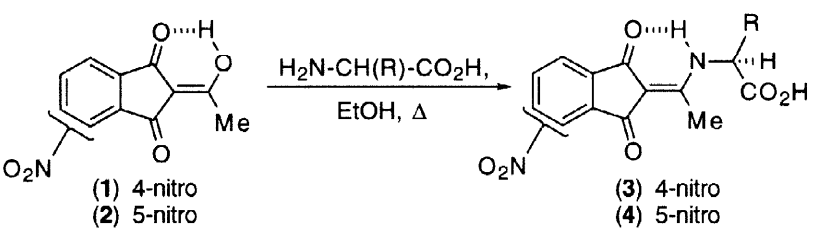
Dde derivative. This selectivity has synthetic advantages and has been exploited in the solid phase syntheses of polyamines [7] and polyamine peptide conjugates [8]. The Dde protection/deprotection process has also been employed in other directions and recently built into a carboxylic acid protecting group [9], a solid phase linker [10] and an affinity tagging procedure [11].

While the above applications provide some testament as to the value of the Dde group in solid phase synthesis, its development was to a large extent influenced by the availability of dimedone and the ease of the reaction sequence illustrated in **Scheme 1**. In principle the procedure is generic and any cyclic 1,3-dione could serve as the precursor of an analogous protecting group. However to have value, any new system should possess additional useful properties over and above those of Dde. In this context we now report 2-acetyl-4-nitroindane-1,3-dione (Nde-OH) and an initial evaluation of its properties and potential as a primary amine protecting group.

Results and discussion

Indane-1,3-dione derivatives are readily synthesised and were obvious candidates on which to base alternative systems related to Dde. At the outset, we envisaged that the presence of the fused benzene ring would enhance the uv chromophore of the deprotection product and, because of the extended conjugation, provide a greater driving force for its formation. On the other hand, it was recognised that the presence of the aromatic ring was likely to render such a system less susceptible to nucleophilic attack and hence be less favorable to both the initial addition of the amine group and the first step of the subsequent deprotection sequence (cf. **Scheme 1**).

We reasoned that these latter effects could be significantly reduced by the presence of a powerful electron withdrawing substituent. Our attention was therefore attracted by the 4- and 5-nitro substituted indane-1,3-dione derivatives, both of which were available as stable yellow crystalline solids *via* simple one pot syntheses from pentane-2,4-dione and 3-nitro and 4-nitrophthalic anhydride respectively [12]. The very significant difference in yields in these reactions in favour of 2-acetyl-4-nitroindane-1,3-dione (**1**), together with the expectation that electronic effects were likely to be more or less the same in each case, led us to focus our investigations on the 4-nitro derivative.



Scheme 2.
Condensation of 2-acetyl-(4 or 5)-nitroindane-1,3-dione with α-amino acids

Condensation of (**1**) with a variety of amino acids in refluxing ethanol, **Scheme 2**, afforded the *N*-1-(4-*n*itro-1,3-*d*ioxoindan-2-ylidene)*e*thyl (Nde) derivatives (**3a-h**) as stable yellow amorphous solids in good yields, **Table 1**.

Compound Number	Amino acid Precursor	Yield (%)	$[\alpha]_D$ (c = 1, MeOH)
3a	H-Phe-OH	69	-135.1° [a]
3b	H-Lys(Boc)-OH	68	-26.2°
3c	H-Val-OH	77	+18.5°
3d	H-Tyr(tBu)-OH	78	-136.5°
3e	H-Ile-OH	70	+10.2° [a]
3f	H-Leu-OH	83	-31.5°
3g	H-His(Trt)-OH	40	-86.9°
3h	H-Ala-OH	65	+26.4°

Table 1.

Nde-amino acids synthesised. [a] (c = 1, EtOH).

Analytical RPHPLC of all the Nde-amino acids gave single peaks, and their high resolution mass spectra displayed molecular ions consistent with those calculated. The ^{1}H nmr spectra were also in accord with the required products and exhibited the expected low field signals (δ 11.0-11.2) corresponding to the strongly hydrogen bonded NH group. These signals, together with those of the vinyl methyl in the Nde group, were doubled and their intensities exhibited approximately the same relative ratios i.e. 2:1. The unsymmetrical nature of the 4-nitroindane-1,3-dione moiety, unlike that of Dde, predisposed the Nde group to geometrical isomerism of which the above observations were regarded as a reflection.

However, when the ^{1}H nmr samples were heated from room temperature to 40 °C these signals coalesced, suggesting that restricted rotation about the *N*-α*C* bond rather than geometrical isomerism was a more likely explanation. Furthermore, in the ^{1}H nmr spectra of Nde derivatives such as β-alanine and related compounds, where there is only limited restriction to rotation, the vinyl methyl and NH group are sharp and broad singlets respectively. However, ^{13}C nmr spectra of the N^{α}-Nde-amino acids revealed doubling of a significant number of signals consistent with a mixture of geometrical isomers. The nitro group must therefore exert an electronic effect so as to fix exclusively the geometry of the Nde group in one or other of the two isomeric forms. On the basis of the spectral and physical data available it is not possible to make a definitive assignment but on mechanistic and intuitive grounds we favour the predominance of the*E*-isomer shown (**3**).

The stability of the Nde group to the acid and base conditions employed in SPPS proved to be similar to those of Dde. Nde protected amino acids and peptides are almost completely resistant to acid at room temperature; e.g. exposure to neat TFA and monitoring by RPHPLC, indicated less than 0.5% loss of Nde after 24 h. The lability to 20% *v/v* piperidine in DMF under continuous flow conditions, employing Nde.Leu.Tyr.Gly.Gly.Phe.Leu.NovaSyn®KR 100 amide resin as a model system, was estimated by RPHPLC analysis of the cleaved peptide as *ca* 6% and 13% loss after 3 h and 6 h respectively. In the context of peptide synthesis it is noteworthy that a contact time of 3 h is sufficient to accommodate ~25 standard Fmoc deprotection cycles.

Although not specifically developed as α-amino acid protecting groups, both Dde and Nde can function in this respect and have some advantages over established protecting groups. In particular, their structures do not permit the formation of an oxazolone intermediate following activation of the carboxyl group and are therefore not prone to racemisation *via* this mechanism. However, the Nde group does have a significant electron withdrawing capacity and hence the potential to facilitate direct proton abstraction from the α-centre. Using the difference in the RPHPLC retention times of the dipeptide diastereoisomers as a means of monitoring any racemisation, the solution coupling of Nde-L-Phe-OH and Nde-D-Phe-OH respectively to H-L-Ala-OBzl with both DCC/HOBt and TBTU/HOBt/DIEA revealed,

within the experimental limits of the method (± 0.5%), that no detectable racemisation had occurred in any of the four experiments. On the basis of this evidence we were confident that Nde α-amino acids could be effectively coupled without loss of chirality. However, before applying Nde derivatives in synthetic procedures, it was essential to demonstrate that the deprotection step proceeded smoothly and in the manner predicted.

To this end, Nde amino acids were loaded onto a solid support (**5**) and treated with a 2% *v/v* solution of hydrazine in DMF under continuous flow conditions, **Figure 1**.

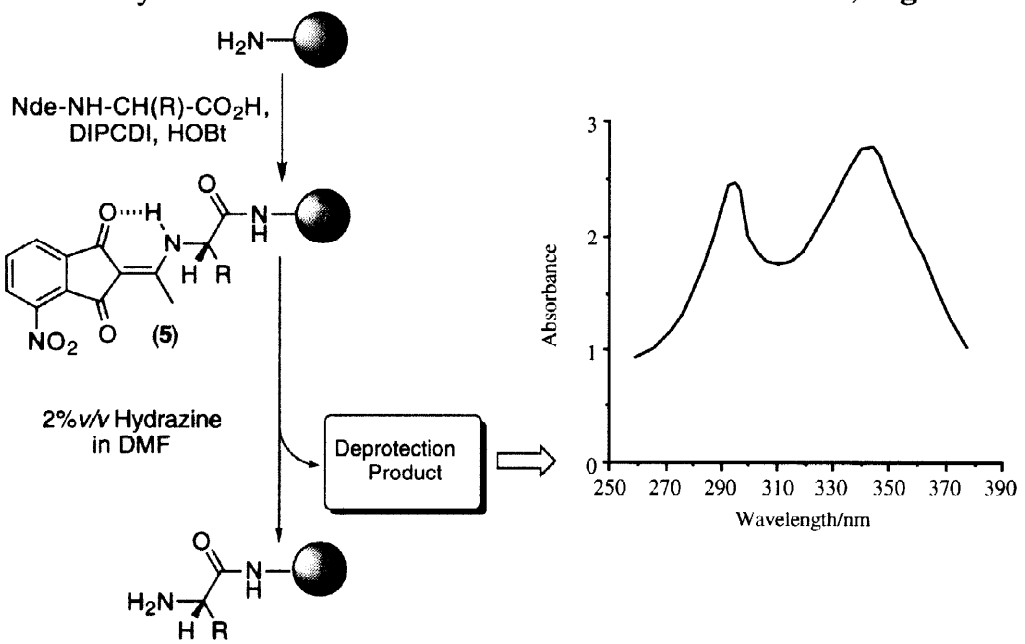


Figure 1.
Resin immobilisation and subsequent hydrazine mediated deprotection of an N^{α}-Nde-protected amino acid.

The pale yellow resin was observed to turn red at the solvent front and a tight red-brown band moved down the column leaving colourless resin in its wake. The uv spectrum of the post-column eluent exhibited maxima at 294 and 348 nm (**Figure 1**) and, if monitored at either of these wavelengths or at 290 nm, a sharp peak in the elution profile of the type illustrated in **Figure 2** was observed.

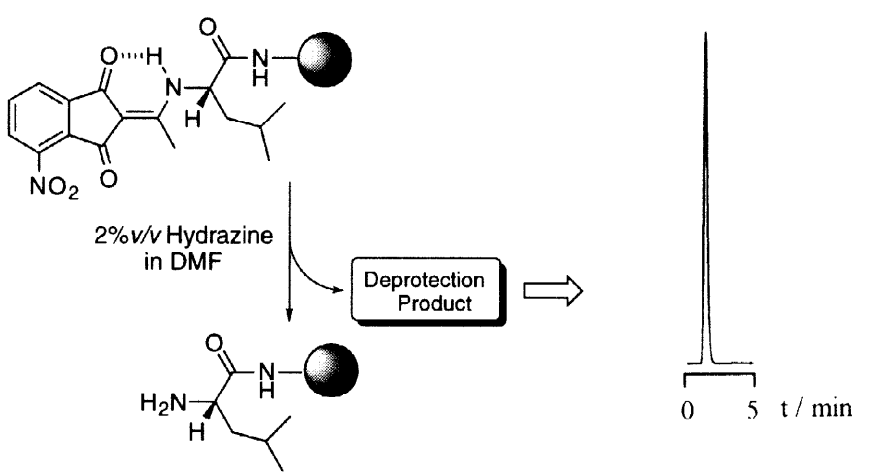
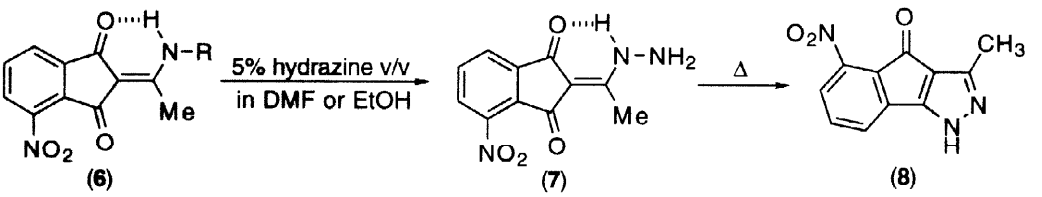


Figure 2.
A typical Nde deprotection profile monitored at 290 nm. This example illustrates the deprotection of Nde-Leu-NovaSyn®KR 100. Deprotection was achieved with 2% v/v hydrazine in DMF under continuous flow conditions at a flow rate of 2.5 ml min^{-1}.

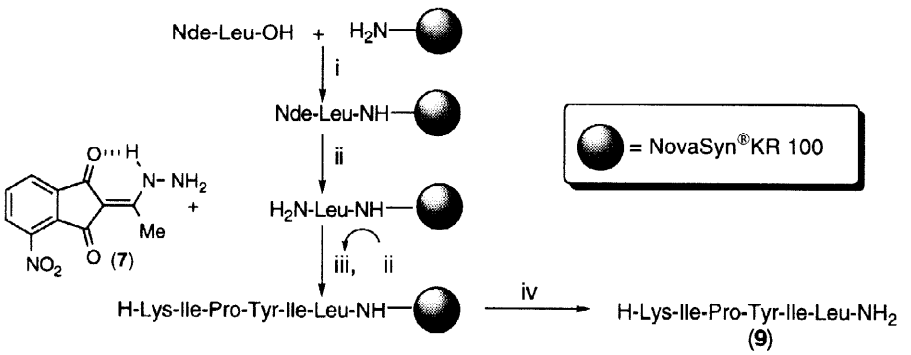
This provided affirmation that, under the conditions described, the deprotection step was indeed extremely rapid and a subsequent Fmoc loading assay [13] established that it was quantitative. The removal of Dde under the same conditions requires monitoring at 310 nm and can take considerably longer than that for Nde [14]. While clearly the removal of Nde can be followed visually, monitoring at the standard fixed wavelength for Fmoc deprotection, i.e. 290 nm, offers an attractive option for automated instrumentation.

In order to identify the deprotection product, solutions of 5% hydrazine monohydrate in DMF and ethanol respectively were added to Nde derivatives dissolved in the corresponding solvent at room temperature. The DMF solutions immediately turned deep brown, whereas the ethanolic solutions remained pale yellow and after a period of time, yellow crystals of the hydrazide (**7**) and not the expected heterocycle (**8**) formed in essentially quantitative yield, **Scheme 3**. The spectral data of (**7**) was consistent with the assigned structure, and also confirmed that it was a single chemical entity and not a mixture of geometric isomers. The hydrazide, on dissolving in DMF also gave a pale yellow solution but on adding either hydrazine (5% v/v in DMF) or base (DIEA in DMF) the solution turned deep brown. In both cases, on addition of acid, the colour change was reversed and the hydrazide recovered. These observations were ascribed to the reversible ionisation of (**7**) presumably involving the acidic NH group of the hydrazide residue (the parent compound Nde-OH (**3**) has a pK_a = 2.5 and displays a similar base induced bathochromic shift [12]). Only on heating (**7**) *in vacuo* was the tricyclic system (**8**) or the alternative isomer formed, **Scheme 3**. Since this compound was irrelevant to the application of Nde, it was not fully characterised and identified solely by MS analysis.



Scheme 3.
Hydrazine mediated deprotection of Nde protected primary amines.

The initial applications of the Nde protecting group to solid phase synthesis were demonstrated with a number of linear peptides. The DIPCDI/HOBt mediated synthesis of the peptide Neuromendin N, as its corresponding amide (**9**) is shown in **Scheme 4**.



Scheme 4.
(i) DIPCDI, HOBt. (ii) 2% *v/v* hydrazine in DMF (except Pro residue; then 20% *v/v* piperidine in DMF. (iii) Nde-Ile-OH, Nde-Tyr(OtBu)-OH, Fmoc-Pro-OH, Nde-Ile-OH, Nde-Lys(Boc)-OH, DIPCDI, HOBt, (iv) TFA:TIPS:H_2O (95:2.5:2.5), 2 h.

NovaSyn®KR 100 was employed for all the individual syntheses due to its ready availability and low cost. The choice of resin needs to take account of the nature of the linkage and its stability to the deprotection conditions, i.e. 2% *v/v* hydrazine in DMF at ambient temperature. Clearly the Rink and Sieber amide, Chlorotrityl linkers etc. represent no cause for concern, however the Wang type ester linkages are potentially susceptible to hydrazinolysis during prolonged exposure. All the amino acids employed for the synthesis were N^{α}-protected with Nde, with the exception of proline, which was introduced as Fmoc-Pro-OH. The protecting groups were removed in the process using 2% *v/v* hydrazine or 20% *v/v* piperidine in DMF respectively under continuous flow conditions. Using the same coupling regime, the Angiotensin II receptor binding protein fragment was also constructed, once again as it's corresponding amide, but with the glycine residue introduced *via* Fmoc-Gly-OH. Finally, Leu-enkephalinamide was prepared using a TBTU/HOBt/DIEA coupling strategy, with the glycine residues being replaced by Fmoc-Gly-OH. The instability of carboxy-activated Nde-glycine, and indeed the corresponding Dde derivative to base, precluded their application in standard coupling procedures [14].

The RPHPLC profiles for all three crude peptides indicate good to excellent purity with almost no detectable signs of epimerisation, **Figure 3**.

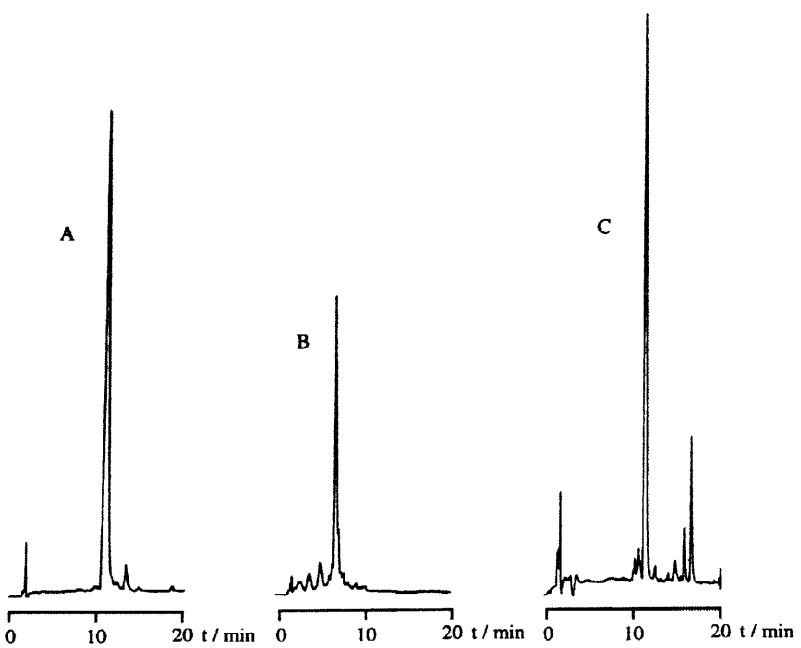
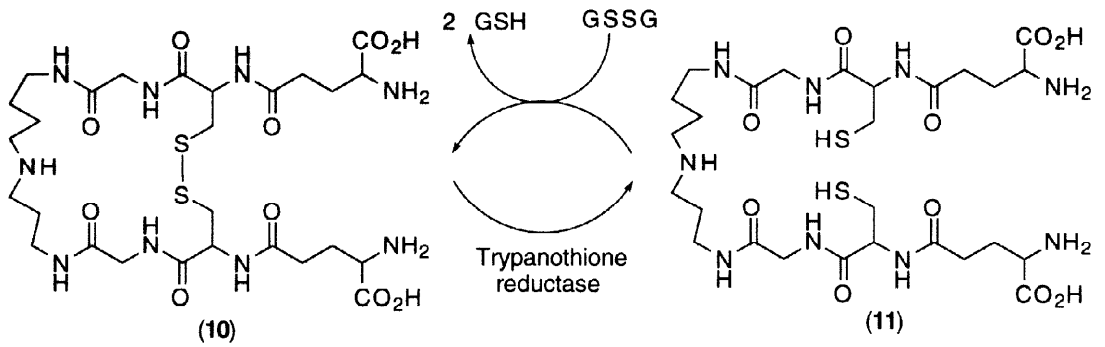


Figure 3.
Analytical RPHPLC traces of the crude peptides; A: Neuromendin N amide (Gradient 20% to 50% B in 20 min linearly), B: Angiotensin II receptor binding protein fragment amide (Gradient 20% to 50% B in 20 min linearly), C: Leu-enkephalinamide (Gradient 20% to 60% B in 20 min linearly). In all examples, post column eluent was monitored at 220 nm.

The selectivity of Nde for primary amine protection has particular advantages in the synthesis of polyamines. N^{1}, N^{8}-*bis*(glutathionyldisulphide) spermidine (trypanothione

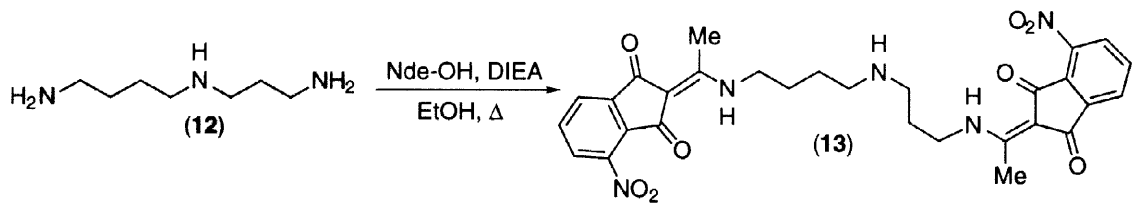
disulphide) (**10**) afforded an attractive target to illustrate the potential. *Trypanosomidae* species, including those associated with African sleeping sickness and South American Chagas disease, employ (**10**) to maintain cellular glutathione in a reduced state [15], **Scheme 5**.



Scheme 5.
Trypanothione disulphide mediated glutathione regulation within trypanosomatids.

A number of discriminatory polyamine protection strategies employing for example, Boc, trifluoroacetyl and phthaloyl, have been reported [16,17]. Also a lengthy solution phase [18] and two solid phase [19,20] syntheses of trypanothione disulphide have been described. However, the exploitation of Nde for the selective protection of the N^1 and N^8 primary amino groups of spermidine, and subsequent attachment of the *bis*-protected derivative to a TFA labile solid support offered an alternative approach with improved efficiency and effectiveness of the overall process.

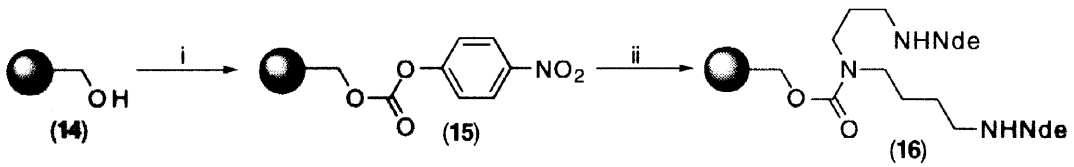
To this end, spermidine (0.5 mmol) (**12**) was refluxed in anhydrous ethanol with 2-acetyl-4-nitroindane-1,3-dione (1.5 mmol) to give, in essentially quantitative yield N^1, N^8-*bis*-Nde-spermidine (**13**), **Scheme 6.**



Scheme 6.
Synthesis of N^1, N^8-*bis*-Nde-spermidine

Dixit and Lenzoff [21] had demonstrated some time ago that amines could be immobilised to a solid phase resin through a benzyl carbamate linkage *via* pre-activation of the hydroxymethylphenyl residues with *p*-nitrophenyl chloroformate. Recently, a number of groups have utilised such urethane based resin linkers for the synthesis of cyclic peptides [22] and various combinatorial libraries [23,24 & 25].

Accordingly NovaSyn®TGA resin, derivatised with the 4-hydroxymethylphenoxyacetic acid (HMPA) linker (**14**), was transformed to the mixed carbonate (**15**) by overnight exposure to a tenfold excess of *p*-nitrophenyl chloroformate and DIEA in DCM. The washed resin was then treated with N^1, N^8-*bis*-Nde-spermidine (**13**) and DIEA in DMF and the suspension was again left overnight, **Scheme 7.**



Scheme 7.
(i) *p*-nitrophenyl chloroformate, DIEA, DCM, 18 h. (ii) (**13**), DIEA, DMF, 18 h.

Following Nde deprotection with 2% v/v hydrazine in DMF under continuous flow conditions, the overall efficiency of the coupling was ascertained by means of a Fmoc loading test [13] and near quantitative (*ca.* 95%) attachment of spermidine was demonstrated. Deprotection of the Nde charged resin displayed a sharp deprotection peak which could also be followed visually as described earlier. The synthesis of trypanothione disulphide was then accomplished *via* 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. Fmoc deprotection was achieved using 20% v/v piperidine in DMF, and each acylation was accomplished *via* a HBTU/HOBt/DIEA coupling strategy and monitored using the TNBS test [26].

Resin cleavage and concomitant side-chain deprotection was completed using a TFA/TIPS/EDT/H_2O cocktail (9.25:0.25:0.25:0.25) for 2 h. RPHPLC of the crude material revealed one major peak corresponding to dihydrotrypanothione (**11**). Preparative chromatography of (**11**) under the previously described conditions [19], followed by aerial oxidation (72 h) afforded trypanothione disulphide (**10**). ES-MS data for both products (**11**) and (**10**) gave the expected peaks at 724 and 722 Da (MH^+) respectively and accurate mass determination using HRMS-FAB on trypanothione disulphide was consistent with the requisite molecular formula.

Conclusions

We have described the development of 2-acetyl-4-nitroindane-1,3-dione (**1**) as a selective primary amine protecting group and have illustrated this with the solid phase synthesis of both linear peptides and a peptide-polyamine conjugate. The Nde protecting group displays good stability towards the reagents commonly employed in SPPS and it's deprotection with 2% v/v hydrazine in DMF can be monitored both spectrophotometrically or visually. We are now exploring further applications of both Nde and Dde, not only for the synthesis of polyamine conjugates but also for the construction of related libraries not previously attainable.

Experimental

General Methods.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultraviolet spectra were recorded on a Cecil 1020S scanning spectrophotometer and infrared spectra were recorded using a Perkin Elmer 257 or Philips PU9716 spectrophotometer. ^1H and ^{13}C NMR spectra were acquired using a Bruker AM 250 operating at 250 and 62.9 MHz

respectively, in deuteriochloroform unless otherwise stated. The ^{1}H chemical shifts reported are relative to an internal tetramethylsilane standard and the *J* values stated are in Hz. Fast atomic bombardment (FAB) mass spectra were obtained using a VG micromass 70 E and positive ion electrospray mass spectra (+ve ES-MS) were recorded on a Micromass VG platform. Optical rotations were measured on a Bendix NPL automatic polarimeter type 143 C with a digital output. Thin layer chromatography (TLC) was performed using Merck silicagel 60 F_{254} precoated (0.2 mm) aluminium sheets in the following system (v/v): (A) $CHCl_3$/MeOH/AcOH, 9 : 1 : 1. Chiral TLC were performed using Chiraplate, manufactured by CAMLAB, Germany. Reaction products were visualised by UV fluorescence (254 nm) or 1% ninhydrin in ethanol. Analytical reverse phase high performance liquid chromatography (RPHPLC) was performed using a Hypersil Pep C_{18} column (4.6 x 150 mm) at a constant flow rate of 1.2 ml min^{-1}, and semi-preparative RPHPLC was performed with a Hypersil Pep C_{18} column (8 x 150 mm) at constant flow rate of 2.2 ml min^{-1} unless otherwise stated. Mobile phase employed were: (A) 0.06% $TFA_{(aq)}$ and (B) 0.06% TFA in 90% $MeCN_{(aq)}$ unless otherwise stated. RPHPLC was performed using a Waters 510 twin pump system and 484 tuneable absorbance detector. Post column eluent was monitored by UV absorbance at 220 nm, and sample lyophilisation was performed using an Edwards Modulyo FD freeze dryer.

Solvents and reagents.

All solvents and reagents were obtained from commercial sources unless otherwise stated. Diethyl ether and hexane were dried over sodium wire, whereas all other solvents were dried by distillation from the following heterogeneous drying agents: dichloromethane (phosphorous pentoxide), methanol (magnesium methoxide), acetonitrile (calcium hydride), pyridine (sodium hydroxide pellets) and ethanol (magnesium ethoxide). Protected amino acids, coupling reagents and resins were purchased from Novabiochem (UK) Ltd.

N-1-(4-Nitro-1,3-dioxoindan-2-ylidene)ethyl (Nde) amino acids.

A suspension of finely powdered amino acid (2.0 mmol) and 2-acetyl-4-nitroindane-1,3-dione (**1**) (0.233g, 1.0 mmol) were refluxed in anhydrous ethanol (20 ml) for 6-8 h. Ethanol was removed *in vacuo* and the residue redissolved in ethyl acetate. The organic solution was washed with 1M $KHSO_{4(aq)}$ and then extracted with sat $NaHCO_{3(aq)}$ (2 x 20 ml). Extracts were combined and carefully acidified using 6 M HCl, and the resultant yellow oil extracted using ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated to dryness. The product was precipitated from ethyl acetate by hexane flooding to afford the desired N^{α}-protected amino acids as yellow amorphous powders.

*Nde-L-Phe-OH (**3a**)*
Yield 0.262 g (69%); m.p. 166-168 °C (Decomp.); R_f (A) = 0.41; $[\alpha]^{25}_D$ -135.1 (c 1.0, EtOH); ^{1}H-NMR ($(CD_3)_2SO$) δ 2.37 and 2.39 (2 x s, 3 H, *E/Z* C=CCH_3), 3.15 and 3.30 (2 x dd, *J* 12.5 and 5.0, 2 H, β-CH_2), 5.00 (m, 1 H, α-CH), 7.26 (m, 5 H, Ph), 7.84 (m, 2 H, Nde C6-H and C7-H), 8.00 (1 H, Nde C5-H), 10.92 and 11.00 (2 x d, *J* 8.0, 1 H, *E/Z* NH), 13.78 (br s, 1 H, CO_2H); ^{13}C δ 14.11,14.31 (Nde CH_3), 38.4 (Phe β-CH_2), 56.68, 56.76 (Phe α-CH), 101.77, 101.86 (Nde 2-C), 123.94, 124.46 (Nde 5-CH), 126.61, 126.69 (Nde 6-CH), 127.38 (Phe 4'-CH), 128.05 (Nde 3a-C), 128.74 (Phe 3',5'-CH), 129.81 (Phe 2',6'-CH), 135.13, 135.21 (Nde 7-CH), 135.77, 135.90 (Phe 1'-C), 139.40, 140.45 (Nde 7a-C), 143.90, 144.20 (Nde 4-C) 169.25, 169.33 (Nde =C-N), 171.20 (CO_2H), 184.74, 187.07 (Nde 1-C), 188.15,

190.48 (Nde 3-C); *m/z* (FAB) 381 (M+H), 335 (M-CO_2H); HRMS (FAB) 381.1022 (M+H, $C_{20}H_{17}N_2O_6$ requires *m/z* 381.1087); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 14.0 min.

Nde-L-Lys(Boc)-OH (3b)
Yield 0.313 g (68%); R_f (A) = 0.35; $[\alpha]^{25}_D$ -26.2 (c 1.0, MeOH); ^{1}H-NMR δ 1.41 (s, 9 H, $C(CH_3)_3$), 1.46 (m, 2 H, γ-CH_2), 1.54 (m, 2 H, δ-CH_2), 2.01 (m, 2 H, β-CH_2), 2.62 and 2.64 (2 x s, 3 H, *E/Z* C=CCH_3), 3.14 (m, 2 H, ε-CH_2), 4.45 (m, 1 H, α-CH), 6.45 (m, 1 H, ε-NH), 7.83 (m, 3 H, Ar H's), 8.27 (br s, 1 H, CO_2H), 11.18 and 11.23 (2 x d, *J* 8.0, 1 H, *E/Z* α-NH); ^{13}C δ 14.30,14.37 (Nde CH_3), 21.98, 22.19 (Lys γ-CH_2), 28.13 ((CH_3)$_3$), 29.31 (Lys β-CH_2), 32.12 (Lys δ-CH_2), 39.85, 40.97 (Lys ε-CH_2), 55.57 (Lys α-CH), 79.48, 81.45 ($C(CH_3)_3$), 102.58, 102.62 (Nde 2-C), 123.92, 124.64 (Nde 5-CH), 126.57 (Nde 6-CH), 128.83, 129.47 (Nde 3a-C), 133.93 (Nde 7-CH), 139.78, 140.76 (Nde 7a-C), 144.02, 144.34 (Nde 4-C) 156.33, 158.32 (NHCO), 169.25, 169.03 (Nde =C-N), 172.11 (CO_2H), 184.80, 187.64 (Nde 1-C), 188.60, 191.08 (Nde 3-C); *m/z* (FAB) 462 (M+H), 388 (M-(CH_3)$_3$CO); HRMS (FAB) 462.1848 (M+H, $C_{22}H_{28}N_3O_8$ requires *m/z* 462.1876); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 13.0 min.

Nde-L-Val-OH (3c)
Yield 0.256 g, (77%); R_f (A) = 0.45; $[\alpha]^{25}_D$ +18.5 (c 1.0, MeOH); ^{1}H-NMR δ 1.11 (d, *J* 3.0, 3 H, γ-CH_3), 1.14 (d, *J* 3.0, 3 H, γ-CH_3), 2.44 (m, 2 H, β-CH), 2.63 and 2.66 (2 x s, 3 H, *E/Z* C=CCH_3), 4.32 (m, 1 H, α-CH), 7.80 (m, 3 H, Ar H's), 7.91 (br s, 1 H, CO_2H), 11.22 and 11.28 (2 x d, *J* 10.0, 1 H, *E/Z* α-NH); ^{13}C δ 14.30,14.44 (Nde CH_3), 17.27, 17.53 (Val CH_3), 17.69, 18.94 (Val CH_3), 31.38 (Val β-CH), 60.68, 61.01 (Val α-CH), 102.91 (Nde 2-C), 124.12, 124.89 (Nde 5-CH), 126.78 (Nde 6-CH), 128.96, 129.72 (Nde 3a-C), 133.98, 134.12 (Nde 7-CH), 139.92, 140.93 (Nde 7a-C), 144.22, 144.58 (Nde 4-C) 169.54, 169.80 (Nde =C-N), 172.88, 172.96 (CO_2H), 185.06, 188.07 (Nde 1-C), 188.97, 191.53 (Nde 3-C); *m/z* (FAB) 333 (M+H), 287 (M-CO_2H); HRMS (FAB) 333.1041 (M+H, $C_{16}H_{17}N_2O_6$ requires *m/z* 333.1087); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 12.0 min.

Nde-L-Tyr(O^{t}Bu)-OH (3d)
Yield 0.353 g (78%); R_f (A) = 0.38; $[\alpha]^{25}_D$ -136.5 (c 1.0, EtOH); ^{1}H-NMR δ 1.32 (s, 9 H, $C(CH_3)_3$), 2.20 and 2.23 (2 x s, 3 H, *E/Z* C=CCH_3), 3.10 and 3.40 (, 2 x dd, *J* 15.0 and 4.0, 2 H, β-CH_2), 4.63 (m, 1 H, α-CH), 7.05 (ABq, *J* 8.0, 4 H, Tyr-Ar H's), 7.67 (br s, 1 H, CO_2H), 7.71 (m, 2 H, Nde C6-H and C7-H), 7.87 (m, 1 H, Nde C5-H), 11.19 and 11.26 (2 x d, *J* 8.0, 1 H, *E/Z* α-NH); ^{13}C δ 13.89 (Nde CH_3), 28.54 ((CH_3)$_3$), 38.56 (Tyr β-CH_2), 57.26, 57.48 (Tyr α-CH), 79.03 ($C(CH_3)_3$), 102.43, 102.48 (Nde 2-C), 124.09, 124.86 (Nde 5-CH), 124.64 (Tyr 3',5'-CH), 126.62, 126.74 (Nde 6-CH), 128.82, 129.55 (Nde 3a-C), 129.68, 129.96 (Tyr 1'-C), 129.86 (Tyr 2',6'-CH), 133.89, 134.10 (Nde 7-CH), 139.76, 140.75 (Nde 7a-C), 144.12, 144.43 (Nde 4-C) 154.37, 154.44 (Tyr 4'-C-O-), 169.34, 169.44 (Nde =C-N), 171.96, 172.06 (CO_2H), 184.84, 187.82 (Nde 1-C), 188.73, 191.15 (Nde 3-C); *m/z* (FAB) 453 (M+H), 351 (M-CO_2H-$C(CH_3)_3$+H); HRMS (FAB) 453.1658 (M+H, $C_{24}H_{25}N_2O_7$ requires *m/z* 453.1662); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 16.5 min.

Nde-L-Ile-OH (3e)
Yield 0.242 g (70%); R_f (A) = 0.48; $[\alpha]^{25}_D$ +10.2 (c 1.0, EtOH); ^{1}H-NMR δ 1.01 (t, *J* 5.0, 3 H, δ-CH_3), 1.08 (m, 3 H, β-CH_3), 1.54 (2 x m, 2 H, γ-CH_2), 2.17 (m, 1 H, β-CH), 2.59 and 2.65 (2 x s, 3 H, *E/Z* C=CCH_3), 4.39 (m, 1 H, α-CH), 7.85 (m, 3 H, ArH's), 8.10 (br s, 1 H,

CO_2H), 11.21 and 11.28 (2 x d, *J* 10.0, 1 H, *E/Z* α-NH); ^{13}C δ 11.49, 11.56 (Ile δ-CH_3), 14.25,14.39 (Nde CH_3), 15.45, 15.51 (Ile β-CH_3), 24.74 (Ile γ-CH_2), 38.04, 38.11 (Ile β-CH), 60.10, 60.25 (Ile α-CH), 102.87 (Nde 2-C), 124.10, 124.88 (Nde 5-CH), 126.77 (Nde 6-CH), 128.89, 129.65 (Nde 3a-C), 133.98, 134.12 (Nde 7-CH), 139.86, 140.86 (Nde 7a-C), 144.17, 144.53 (Nde 4-C) 169.40, 169.66 (Nde =C-N), 173.01, 173.10 (CO_2H), 185.03, 188.08 (Nde 1-C), 188.92, 191.48 (Nde 3-C); *m/z* (FAB) 347 (M+H), 301 (M-CO_2H); HRMS (FAB) 347.1268 (M+H, $C_{17}H_{19}N_2O_6$ requires *m/z* 347.1243); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 14.0 min.

Nde-L-Leu-OH (3f)

Yield 0.287 g (83%); R_f (A) = 0.49; $[\alpha]^{25}_D$ -31.5 (c 1.0, MeOH); ^{1}H-NMR δ 1.01 (m, 6 H, $CH(CH_3)_2$), 1.90 (m, 3 H, CH-CH_2), 2.64 and 2.67 (2 x s, 3 H, *E/Z* C=CCH_3), 4.45 (m, 1 H, α-CH), 7.85 (m, 3 H, ArH's),), 7.95 (br s, 1 H, CO_2H), 11.02 and 11.09 (2 x d, *J* 10.0, 1 H, *E/Z* α-NH); ^{13}C δ 14.32,14.43 (Nde CH_3), 21.53, 21.67 (Leu CH_3), 22.55 (Leu CH_3), 24.64, 24.74 (Leu γ-CH), 40.89, 41.06 (Leu β-CH_2), 54.23 (Leu α-CH), 102.81 (Nde 2-C), 124.12, 124.94 (Nde 5-CH), 126.83 (Nde 6-CH), 128.96, 129.71 (Nde 3a-C), 133.96, 134.15 (Nde 7-CH), 139.92, 140.90 (Nde 7a-C), 144.24, 144.58 (Nde 4-C) 169.40, 169.55 (Nde =C-N), 173.84, 173.93 (CO_2H), 185.08, 188.05 (Nde 1-C), 188.96, 191.42 (Nde 3-C); *m/z* (FAB) 347 (M+H), 301 (M-CO_2H); HRMS (FAB) 347.1249 (M+H, $C_{17}H_{19}N_2O_6$ requires *m/z* 347.1243); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 14.7 min.

Nde-L-His(N^{im}-Trt)-OH (3g)

Yield 0.244 g (40%); R_f (A) = 0.32; $[\alpha]^{25}_D$ -89.6 (c 1.0, MeOH); ^{1}H-NMR δ 2.54 and 2.51 (2 x s, 3 H, *E/Z* C=CCH_3), 3.40 (m, 2 H, β-CH_2), 4.85 (m, 1 H, α-CH), 7.05 and 7.26 (2 x m, 15 H, CPh_3), 7.65 (m, 2 H, Nde C6-H and C7-H), 7.80 (m, 1 H, Nde C5-H), 8.15 (br s, 1 H, CO_2H), 9.85 (m, 2 H, imidazole 2 x CH), 11.05 and 11.15 (2 x d, *J* 10.0, 1 H, *E/Z* α-NH); ^{13}C δ 14.25 (Nde CH_3), 29.02, 29.33 (His β-CH_2), 54.93 (His α-CH), 76.48 (CPh_3), 101.86, 101.91 (Nde 2-C), 121.25 (His 5-CH), 123.88, 124.56 (Nde 5-CH), 126.69, 126.82 (Nde 6-CH), 127.97 (Nde 3a-C), 128.32 (His 2-CH), 128.61 (Trt 3',5'-CH), 128.80, 128.93 (Trt 4'-CH), 129.37 (Trt 2',6'-CH), 131.70 (His 4-C), 135.44 (Nde 7-CH), 139.36, 140.49 (Nde 7a-C), 141.16, 141.22 (Trt 1'-C), 143.97, 144.27 [Nde 4-C), 169.37 (Nde =C-N), 170.82 (CO_2H), 184.86, 187.22 (Nde 1-C), 188.09, 190.40 (Nde 3-C); *m/z* (FAB) 613 (M+H), 568 (M-CO_2); HRMS (FAB) 613.2079 (M+H, $C_{36}H_{29}N_4O_6$ requires 613.2087); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 14.1 min.

Nde-L-Ala-OH (3h)

Yield 0.197 g (65%); m.p. 250-253 °C (Decomp.); R_f (A) = 0.27; $[\alpha]^{25}_D$ +26.4 (c 1.0, MeOH); ^{1}H-NMR ($(CD_3)_2SO$) δ 1.53 (d, *J* 7.5, 3 H, β-CH_3), 2.60 and 2.65 (2 x s, 3 H, *E/Z* C=CCH_3), 4.74 (m, 1 H, α-CH), 7.87 (m, 2 H, Nde-C6-H and C7-H), 7.99 (m, 1 H, Nde-C5-H), 11.02 and 11.10 (2 x d, *J* 8.0, 1 H, *E/Z* NH), 13.65 (br s, 1 H, CO_2H); ^{13}C δ 14.26 (Nde CH_3),18.79 (Ala CH_3), 51.17 (Ala α-CH), 101.84 (Nde 2-C), 123.84, 124.41 (Nde 5-CH), 126.60 (Nde 6-CH), 128.00, 128.04 (Nde 3a-C), 135.18 (Nde 7-CH), 139.47, 140.39 (Nde 7a-C), 143.89, 144.18 (Nde 4-C), 169.04 (Nde =C-N), 172.59 (CO_2H), 184.78, 187.14 (Nde 1-C), 188.21, 190.52 (Nde 3-C); *m/z* (FAB) 305 (M+H), 259 (M-CO_2H); HRMS (FAB) 305.0779 (M+H, $C_{14}H_{13}N_2O_6$ requires 305.0774); RPHPLC (40 to 80% B in 20 min linearly) 1 peak, R_t = 4.0 min.

Racemisation studies.

A solution of Nde-L-Phe-OH (0.190 g, 0.5 mmol) was dissolved in 1% hydrazine monohydrate in anhydrous ethanol (5 ml) and stirred at room temperature for 3 h. A yellow/orange precipitate was collected and structurally confirmed as Nde-hydrazide (7) (0.120 g, 97%), m.p. 194-198 °C (dec). ^{1}H NMR ($(CD_3)_2SO$-$CDCl_3$) δ 2.53 (s, 3 H, C=CH_3), 5.50 (br s, 2 H, NH_2), 7.75 (m, 3 H, ArH's), 11.4 (d, *J* 8.0, NH). *m/z* (+ve ES-MS) 248.2 (M+H). The hydrazine/ethanol solution was evaporated to dryness and the residue partitioned between chloroform and water. Removal and evaporation of the aqueous layer afforded L-phenylalanine: ^{1}H NMR (D_2O, DSS external standard) δ 3.10 and 3.25 (2 x dd, *J* 8.0 and 5.5, 2 H, , β-CH_2), 3.95 (dd, *J* 8.1 and 5.5, α-CH), 7.35 (m, 5 H, Ph); $[\alpha]^{28}_D$ -31.5 (*c* 1.0, H_2O) {lit., $[\alpha]^{20}_D$ -33.7 to -35.2 (*c* 2.0, H_2O); Chiral TLC analysis, R_f = 0.625 (L-Phe-OH [pure]; R_f = 0.625, D-Phe-OH [pure]; R_f = 0.50).

Racemisation studies during coupling of Nde-L-Phe-OH and Nde-D-Phe-OH.

Nde-L-Phenylalanyl-L-alanine benzyl ester and Nde-D-phenylalanyl-L-alanine benzyl ester via DCC mediated coupling.

To stirred individual solutions of Nde-L-Phe-OH and Nde-D-Phe-OH (0.057 g, 0.15 mmol) in dry DCM (30 ml) each containing L-alanine benzyl ester tosylate salt (0.053 g, 0.15 mmol), HOBt (0.023 g, 0.15 mmol) and triethylamine (0.021 ml, 0.15 mmol), was added DCC (0.034 g, 0.165 mmol) at 0 °C. Stirring was continued at room temperature for 3 h, followed by evaporation of volatiles *in vacuo*. The individual residues were redissolved in cold ethyl acetate and the DCU precipitate removed *via* filtration. The organic solutions were then washed with 1M $KHSO_{4(aq)}$, sat. $NaHCO_{3(aq)}$, water and brine. The organic layers were then dried ($MgSO_4$) and evaporated to dryness to yield Nde-L-Phe-L-Ala-OBzl (0.062 g, 77%) and Nde-D-Phe-L-Ala-OBzl (0.057 g, 70%) as yellow oils.

Nde-L-Phenylalanyl-L-alanine benzyl ester and Nde-D-Phenylalanyl-L-alanine benzyl ester via TBTU mediated coupling.

Using the above protocol, only replacing DCC with TBTU (0.048 g, 0.15 mmol), Nde-L-Phe-L-Ala-OBzl (0.060 g, 74%) and Nde-D-Phe-L-Ala-OBzl (0.065 g, 80%) were again obtained as yellow oils. *Nde-L-Phe-L-Ala-OBzl:* ^{1}H-NMR δ 1.53 (d, *J* 7.5, 3 H, Ala β-CH_3), 2.40 and 2.46 (2 x s, 3 H, *E/Z* C=CCH_3), 3.25 and 3.48 (2 x dd, *J* 12.5 and 5.0, 2 H, Phe β-CH_2), 4.55 (m, 1 H, Phe α-CH), 4.76 (m, 1 H, Ala α-CH), 5.26 (s, 2 H, PhCH_2), 6.70 and 6.75 (2 x d, *J* 7.5, 1 H, *E/Z* CONH) 7.37 (m, 5 H, Phe Ph), 7.45 (s, 5 H, Ph), 7.85 (m, 3 H, Nde-ArH's), 11.20 and11.23 (2 x d, *J* 7.5, 1 H *E/Z* C=CNH); *m/z* (FAB) 542 (M+H), 335 (M-CO-Ala-OBzl). *Nde-D-Phe-L-Ala-OBzl:* ^{1}H-NMR δ 1.44 (d, *J* 7.5, 3 H, Ala β-CH_3), 2.41 and 2.42 (2 x s, 3 H, *E/Z* C=CCH_3), 3.19 and 3.41 (2 x dd, *J* 12.5 and 5.0, 2 H, Phe β-CH_2), 4.52 (m, 1 H, Phe α-CH), 4.71 (m, 1 H, Ala α-CH), 5.23 (s, 2 H, PhCH_2), 6.52 and 6.75 (2 x d, *J* 7.5, 1 H, *E/Z* CONH) 7.35 (m, 5 H, Phe-Ph), 7.41 (s, 5 H, Ph), 7.85 (m, 3 H, Nde-ArH's), 11.24 and11.32 (2 x d, *J* 7.5, 1 H, *E/Z* C=CNH); *m/z* (FAB) 542 (M+H), 335 (M-CO-Ala-OBzl).
In both examples; RPHPLC [Eluent A = H_2O, Eluent B = 90% $MeOH_{(aq)}$]; Gradient = 70 to 100% B in 20 min linearly) Observed for Nde-L-Phe-L-Ala-OBzl; 1 peak, R_t = 18.4 min. and for Nde-D-Phe-L-Ala-OBzl; 1 peak R_t = 16.5 min.

Peptide synthesis.

All linear peptides were synthesised using an LKB 4175 Biolynx manual peptide synthesiser. All peptides were prepared as their corresponding carboxamide derivative using NovaSyn®KR 100 resin functionalised at 0.12 mmol/g with an Fmoc protected Rink amide linker. This approach was chosen due to the ease of first residue attachment to the resin bound amine following Fmoc deprotection. For Neuromendin N amide, individual couplings were performed using a DIPCDI/HOBt strategy, with Nde deprotection achieved using 2% v/v hydrazine in DMF under continuous flow conditions. The proline residue was introduced using Fmoc-Pro-OH, with deprotection afforded by 20%*v/v* piperidine in DMF. For Angiotensin II receptor binding protein fragment amide a DIPCDI/HOBt was again employed, and it is important to note that the Gly residue was coupled using Fmoc-Gly-OH due to the instability of the carboxy-activated Nde protected species [14]. Leucine enkephalinamide was synthesised utilising a TBTU/HOBt/DIEA coupling protocol, with the glycine residues introduced *via* the Fmoc protected species for the same reason as mentioned above. In all three cases, upon completion of the synthesis, the resin was transferred to a sintered glass funnel, and sequentially washed with DMF (25 ml), *t*-amyl alcohol (10 ml), acetic acid (5 ml), *t*-amyl alcohol (5 ml) and ether (20 ml). The resin was dried *in vacuo* over KOH overnight. A portion of the resin (50 mg) was suspended in TFA-H_2O-TIPS (95:2.5:2.5, 10 ml) for 2 h with occasional agitation. The mixture was filtered through a glass sinter and the filtrate evaporated. The solid residue was triturated several times with cold HPLC grade ether, dissolved in distilled water and lyophilised to yield, in all cases, a white amorphous powder.

Neuromendin N amide (9)
RPHPLC (20 to 50% B in 20 min linearly); 1 peak at 11.2 min *m/z* (+ve ES-MS) 746.2 (M + H), 617.1 (M - Leu-NH_2).

Angiotensin II receptor binding protein fragment amide
RPHPLC (20 to 50% B in 20 min linearly); 1 major peak at 7.0 min. Purified under analogous conditions; *m/z* (+ve ES-MS) 900.2 (M + H), 769.5 (M -Leu-NH_2), 561.8 (M - His-Ala-Leu-NH_2), 450.1 (M - Ile-His-Ala-Leu-NH_2).

Leucine enkephalinamide
RPHPLC (20 to 60% B in 20 min linearly); 1 major peak at 11.5 min. The major peak was purified under analogous conditions; *m/z* (+ve ES-MS) 555.1 (M + H), 538.8 (M - NH), 510.1 (M - $CONH_2$), 425.0 (M - Leu-NH_2), 397.7 (M - CO-Leu-NH_2).

Assessment of Nde stability towards TFA.
Nde-L-Phe-OH (0.190 g, 0.5 mmol) was dissolved in neat TFA (50 ml) and allowed to stand at room temperature for 24 h. At 2, 4, 6 and 24 h intervals, a 2 ml sample was removed and evaporated to dryness. The residue was redissolved in 0.06% TFA in 90% $MeCN_{(aq)}$ and a 50 µl sample was analysed by RPHPLC. After 24 h, the solution was evaporated to dryness and the residue analysed by ^1H NMR and FAB-MS. RPHPLC (40% to 80% B in 20 min linearly); at all time points, observed 1 peak at 14.0 min. An authentic sample of L-Phe-OH displays a retention time of 3.5 min under analogous conditions.

Assessment of Nde stability towards 20% piperidine under continuous flow conditions.

Nde-Leu-Tyr-Gly-Gly-Phe-Leu-Novasyn KR 100 (0.10 g) was swelled in DMF for 1 h, and using an LKB Biolynx manual peptide synthesiser, was exposed to a continuous flow of 20% piperidine in DMF at a constant flow rate of 0.5 ml/min for 24 h. Samples of resin (20 mg) were removed at 3, 6 and 24 h intervals. These were washed, dried and cleaved as described for the peptides above e.g (**9**), and lyophilised overnight to yield yellow amorphous powders. Each peptide was analysed by RPHPLC and *via* peak area, percentage impurities were calculated. At 3 h, 6% impurity; at 6 h, 13% impurity and at 24 h, 46% impurity. In all cases, the impurity consisted of one peak, identified as the free peptide H-Leu-Tyr-Gly-Gly-Phe-Leu-NH_2

Solid phase synthesis of dihydrotrypanothione

*N^1, N^8-bis-Nde-spermidine (**13**)*

To a solution of spermidine (**12**) (79 µl, 0.50 mmol) in anhydrous ethanol was added 2-acetyl-4-nitroindane-1,3-dione (0.350 g, 1.50 mmol) and DIEA (261 µl, 1.50 mmol) and the resultant solution was refluxed for 8 h. A bright yellow precipitate formed which was collected at the pump and washed 3-4 times with 1% AcOH in ethanol, then cold ethanol and finally dried to afford the title compound as a yellow amorphous powder (0.253 g, 88%), m.p. 165-169 °C.

ν_{max} (KBr) 3440, 3100, 2940, 1688, 1645, 1600, 1535, 1495, 1350 cm^{-1}. ^{1}H-NMR (CF_3CO_2H) δ 2.10 (m, 4 H, 6,7-CH_2), 2.49 (m, 2 H, 2-CH_2), 2.83 (s, 6 H, 2 x =CCH_3), 3.54 (m, 4 H, 3,5-CH_2), 3.85 (m, 4 H, 1,8-CH_2), 7.39 (br s, 2 H, $^+NH_2$), 8.00 (m, 6 H, ArH's), 11.54 (d, 2 H, *J* 8.0, 2 x =CNH-); ^{13}C δ 14.13, 14.16 (CH_3 [Nde]), 23.19 (7-CH_2), 25.88 (6-CH_2), 25.97 (2-CH_2), 40.25 (8-CH_2), 42.42 (1-CH_2), 44.47 (5-CH_2), 46.64 (3-CH_2), 101.64, 101.84 (2-C [Nde]), 123.75, 124.33 (5-CH [Nde]), 126.54 (6-CH [Nde]), 128.11 (3a-C [Nde]), 135.07 (7-CH [Nde]), 140.46 (7a-C [Nde]), 143.95 (4-C [Nde]), 169.94, 170.03 (=C-N [Nde]), 184.76, 187.15 (1-C [Nde]), 188.09, 190.49 (3-C [Nde]); *m/z* (+ve ES-MS) 576.1 (M + H), 344.4 (M - Nde-NH), 114.3 (M - {2 x Nde-NH}); HRMS (FAB) 576.2074 (M+H, $C_{29}H_{30}N_5O_8$ requires *m/z* 576.2094);

*Activation of NovaSyn®TGA resin with p-nitrophenyl chloroformate (**15**).*

NovaSyn®TGA resin functionalised at 0.28 mmol g^{-1} with an HMPA linker (**14**) (0.20 g, 0.056 mmol of free alcohol) was swelled in DCM for 30 min. To this suspension was added 4-nitrophenyl chloroformate (0.113 g, 0.56 mmol) and DIEA (98.0 µl, 0.56 mmol) and the suspension allowed to stir at ambient temperature overnight.

*Attachment of N^1, N^8-bis-Nde-spermidine (**13**) to activated NovaSyn®TGA resin (**15**).*

The resin was washed 5 times with dry DCM and then washed 5 times with, and finally suspended in, peptide synthesis grade DMF. To this was added *N^1, N^8-bis*-Nde-spermidine (0.161 g, 0.28 mmol) and DIEA (49.0 µl, 0.28 mmol) and the suspension was again allowed to stir overnight. The resin was then washed with DMF and subsequently capped with 2% acetic anhydride/DIEA in DMF. Nde deprotection was carried out using 2%*v/v* hydrazine in DMF under continuous flow conditions for 7.0 min at a flow rate of 3.0 ml min^{-1}.

*Dihydrotrypanothione (**11**).*

Using an LKB 4174 Biolynx manual peptide synthesiser, peptide synthesis was completed *via* the sequential addition of Fmoc-Gly-OH (0.133 g, 0.448 mmol), Fmoc-Cys(Trt)-OH

(0.262 g, 0.448 mmol), and Fmoc-Glu(OH)-OtBu (0.191 g, 0.448 mmol) activated using a TBTU (0.143 g, 0.448 mmol), HOBt.H_2O (0.034 g, 0.224 mmol) and DIEA (87.0 μl, 0.896 mmol) coupling strategy. Fmoc deprotection following each acylation was achieved using 20%*v/v* piperidine in DMF under continuous flow conditions and post column eluent was monitored spectrophotometrically at 290 nm.

The resin was then washed and dried as described for the linear peptides. A portion of the resin (10 mg) was suspended in TFA:EDT:H_2O:TIPS (92.5:2.5:2.5:2.5, 10 ml) for 2 h with occasional agitation. The peptide product was obtained as a white amorphous powder (7 mg) as described for the linear peptides. RPHPLC (3% B for 5 min. then to 100% B in 20 min linearly); 1 Major peak at 10.4 min, purified under analogous conditions. *m/z* (+ve ES-MS) 724.1 (M+H, $C_{27}H_{49}N_9O_{10}S_2$ requires *m/z* 723).

*Trypanothione disulphide (**10**).*

Dihydrotrypanothione (**11**) (7 mg) was dissolved in 0.04 M ammonium acetate (70 ml, adjusted to pH 8.5 with conc. ammonia) under atmospheric oxygen at room temperature and allowed to stir for 72 h. The solution was then carefully concentrated *in vacuo* and finally lyophilised to afford a white amorphous solid. RPHPLC (3% B for 5 min. then to 100% B in 20 min linearly); 1 Major peak at 8.8 min, purified under analogous conditions. *m/z* (+ve ES-MS) 722.2 (M+H). HRMS (FAB) Found: *m/z* 722.2933 (Calcd. for $C_{27}H_{48}N_9O_{10}S_2$ (M+H) 722.2966).

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Abbreviations

Amino acids are of the L-configuration unless otherwise stated and follow the IUPAC-IUB nomenclature where applicable (Eur. J. Biochem. 1984: 9-37); Boc, *tert*-butoxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCM, dichloromethane; DIPCDI; *N,N'*-diisopropylcarbodiimide; DMF, *N,N'*-dimethylformamide; DSS, 3-(trimethylsilyl)-1-propanesulphonic acid sodium salt; EDT, 1,2-ethanedithiol; ES-MS, electrospray mass spectrometry; 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-hydroxybenzotriazole; RPHPLC, 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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