

# Synthesis and metal complexation of chiral 3-mono- or 3,3-bis-allyl-2-hydroxypyrrolopyrazine-1,4-diones

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A novel synthesis of chiral cyclic hydroxamic acids (**4**, **6**, **8** and **10**) related to cyclodipeptides is described. The crucial reduction of the nitro group of the *N*-nitroacetyl derivatives of (*S*)- $\alpha$ -amino acid esters is brought about by zinc-aq. ammonium chloride. The Fe<sup>III</sup> and Cu<sup>II</sup> complexes of one such cyclic hydroxamic acid **10a** have been prepared and their DNase activity investigated.

## Introduction

Hydroxamic acids, both natural and synthetic, linear as well as cyclic, have been reported to possess interesting biological activity, especially in iron solubilization and transport.<sup>1</sup> Desferrioxamine B (Desferal<sup>®</sup>) is a drug prescribed for reducing the iron overload in patients suffering from thalassemia. The possibility of using hydroxamic acids in drug delivery systems has been discussed recently.<sup>2</sup> We now report the synthesis of novel hydroxamic acid siderophores based on the piperazine-2,5-dione † skeleton.<sup>3</sup>

Generally, the synthesis of 1-hydroxypiperazine-2,5-diones has been achieved by cyclisation of dipeptide precursors in which either of the two amino acid units has an *N*-hydroxy group.<sup>4</sup> The cyclisation of the dipeptide having the *N*-hydroxy amino acid at the N-terminus leads to low yields of 1-hydroxypiperazine-2,5-dione because of the weak nucleophilicity of the hydroxylamine nitrogen.<sup>5</sup> The synthesis of the requisite linear dipeptide with an *N*-hydroxy amino acid is in itself a tedious process. A second general method involves the direct oxidation of amides or amines in the presence of a suitable catalyst.<sup>6</sup> It is obvious that the scope of both these synthetic routes is rather

restricted. Neither of them can provide easy access to asymmetric molecules with predetermined stereochemistry; nor can they lead to products incorporating an  $\alpha,\alpha$ -disubstituted glycine unit in the piperazinedione ring.

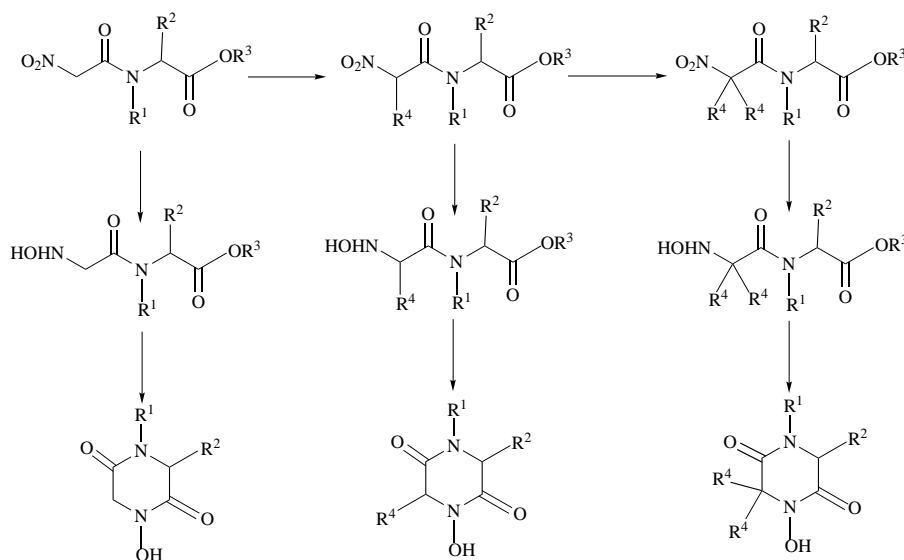
In this article, we present an extension of our preliminary report<sup>7</sup> on a general synthesis of cyclic 1-hydroxypiperazine-2,5-diones with a chiral centre at C-3, and with the possibility of incorporating either one or two alkyl substituents at position 6. The Fe<sup>III</sup> and Cu<sup>II</sup> metal complexes of one such synthetic hydroxamic acid, viz. the 6,6-bis(allyl)-1-hydroxy derivative of cyclo[Gly-(*S*)-Pro] have been prepared. The DNA cleavage properties of these two metal complexes are reported.

## Results and discussion

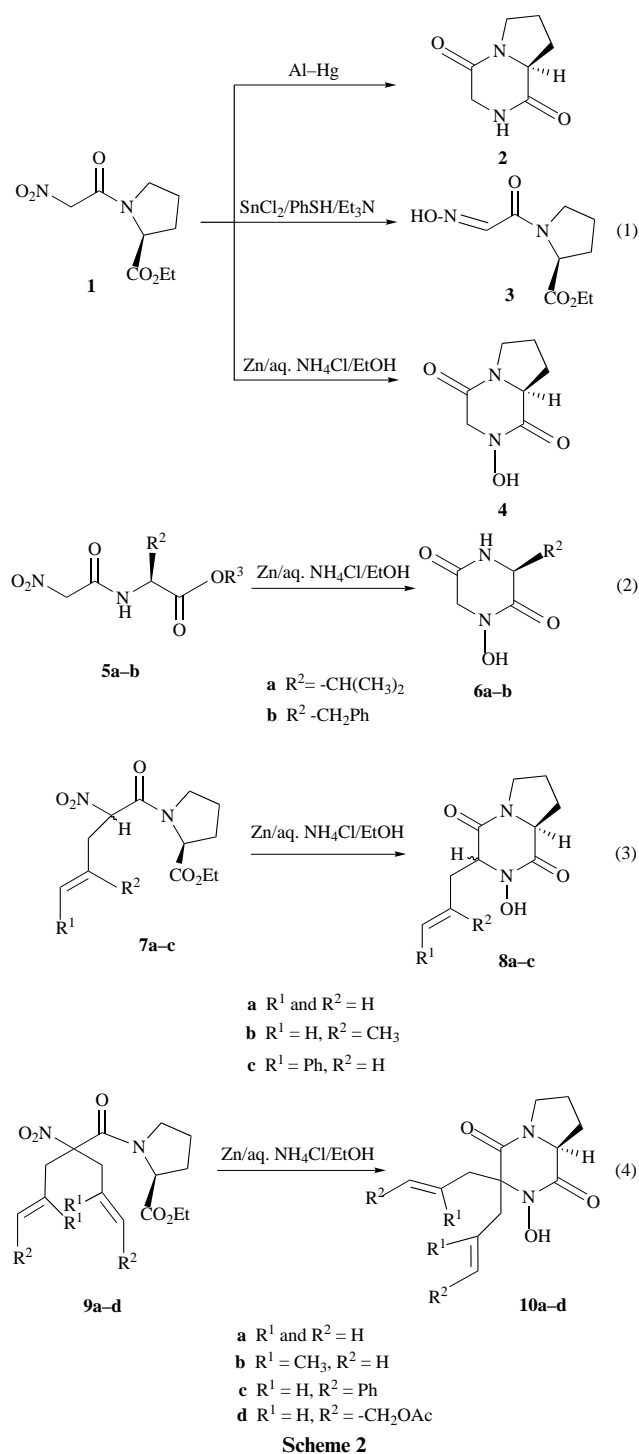
### Synthesis

The synthesis is an extrapolation of our earlier work on the use of the nitroacetyl group as a peptide synthon.<sup>8</sup> The methodology involves the following steps: (i) synthesis of *N*-nitroacetyl amino acid esters from 1,1-bis(methylthio)-2-nitroethene, (ii) mono- or bis-allylation at the active methylene by Pd<sup>0</sup>-catalysed allylation, (iii) reduction of the nitro group to a hydroxylamine, (iv) cyclisation (Scheme 1). In a pilot study, the nitroacetyl derivative **1** of ethyl (*S*)-prolinate was subjected to reduction with the following reagents: (a) aluminium amalgam, (b) SnCl<sub>2</sub>/PhSH/Et<sub>3</sub>N,<sup>9</sup> (c) Zn/AcOH and (d) Zn/aq. NH<sub>4</sub>Cl/

† Piperazine-2,5-dione in this context refers to the pyrrolo[1,2-*a*]pyrazine fused ring system (see Experimental section). The locants refer to the position on the piperazine ring and not to the pyrrolopyrazine ring as a whole.



Scheme 1



**Scheme 2**

EtOH [Scheme 2, eqn. (1)]. The Al-Hg reagent reduced the primary nitro group directly to the amine; this was followed by cyclisation to give exclusively cyclo[Gly-(S)-Pro] **2**. Obviously the use of this reagent had resulted in 'over reduction'. In contrast, the  $\text{SnCl}_2/\text{PhSH}/\text{Et}_3\text{N}$  system led to incomplete reduction; the product obtained was the oxime **3**. A base catalysed prototropic shift at the nitroso stage had intervened to prevent further reduction to the hydroxylamine. The zinc-mediated reductions were more successful. Treatment of **1** with  $\text{Zn}/\text{AcOH}$  gave a mixture of the corresponding amine and the hydroxylamine, both of which then underwent cyclisation to the piperazine-2,5-diones **2** and **4** respectively. It is known that controlling the pH of the reaction mixture is crucial for the partial reduction of the nitro group to the level of hydroxylamine.<sup>10</sup> It seemed appropriate therefore to use  $\text{Zn}/\text{aq. NH}_4\text{Cl}/\text{EtOH}$  under neutral pH to achieve the desired transformation. In the event, reduction of **1** under these conditions, followed by reflux to induce cyclisation

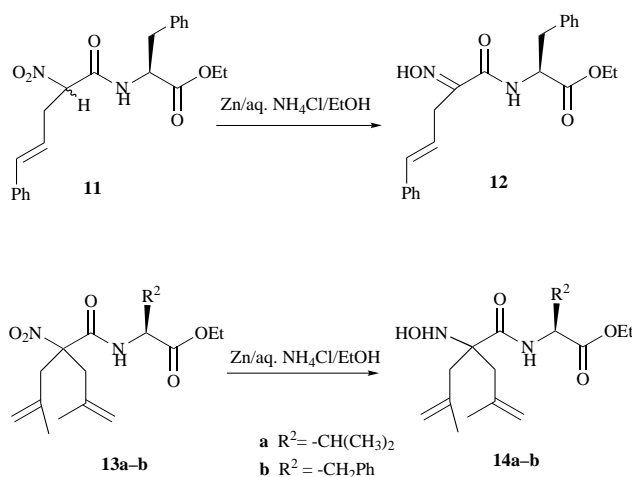
led exclusively to the 1-hydroxypiperazine-2,5-dione **4** in 85% yield (Scheme 2).

The *N*-nitroacetyl derivatives **5** of (*S*)-valine ethyl ester and (*S*)-phenylalanine ethyl ester could similarly be reduced with  $\text{Zn}/\text{aq. NH}_4\text{Cl}/\text{EtOH}$  and cyclised to the corresponding (3*S*)-1-hydroxy-3-alkylpiperazine-2,5-diones **6**, but in lower yields (40–45%) [eqn. (2)].

The next objective was to introduce either one or two alkyl substituents on the glycine moiety of **4** and **6**. The preferred mode of introducing such substituents at the nitroacetyl stage was through allylation using  $\text{Pd}^0$  catalysis. Mono-substitution of *N*-nitroacetyl (*S*)-proline ethyl ester with allyl, methylallyl or cinnamyl groups leads to a mixture of diastereomers as reported earlier. It has also been shown that the major diastereomer has the (*S*)-configuration at the newly generated chiral centre.<sup>8b</sup> Reduction of this diastereomeric mixture (**7**; de: 25–34%) using  $\text{Zn}/\text{aq. NH}_4\text{Cl}/\text{EtOH}$ , and subsequent reflux in ethanol led to the 6-mono-substituted-1-hydroxypiperazine-2,5-diones **8** as a mixture of diastereomers with almost the same de. The chemical yields were good (75–85%) [eqn. (3)].

Introduction of a second identical allyl group on the  $\alpha$ -carbon of the *N*-nitroacetyl moiety of ester **7** helped to remove the complexity generated by the presence of two asymmetric centres in the molecule. At the same time, it created a quaternary carbon adjacent to the nitrogen atom destined to become part of the hydroxamic acid functionality; this would indeed be a welcome feature of the product. Reduction of the tertiary nitro group in these compounds (**9a–d**) with  $\text{Zn}/\text{aq. NH}_4\text{Cl}/\text{EtOH}$  proceeded well; the *in situ* generated hydroxylamines were cyclised by refluxing in ethanol to yield the 6,6-bis(allyl)-1-hydroxypiperazine-2,5-diones (**10a–d**) in 70–85% yields [eqn. (4)].

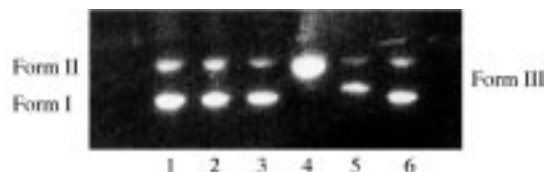
In contrast to the proline-containing peptides discussed above, the  $\alpha$ -mono-substituted † *N*-nitroacetyl (*S*)-phenylalanine **11** or  $\alpha,\alpha$ -bis-substituted derivatives **13** of *N*-nitroacetyl-(*S*)-valine ester or *N*-nitroacetyl-(*S*)-phenylalanine ester did not lead to the desired cyclic hydroxamic acids on controlled reduction with  $\text{Zn}/\text{aq. NH}_4\text{Cl}/\text{EtOH}$ . In the case of the monoallyl derivative **11**, the product obtained was the oxime **12** (70% yield). The  $\alpha,\alpha$ -bis(allyl) derivatives **13** ‡ apparently gave the corresponding hydroxylamines **14a–b**, which however refused to undergo cyclisation to the hydroxamic acids (Scheme 3).



**Scheme 3**

In the <sup>13</sup>C NMR spectra, there was a characteristic downfield shift of about 10 ppm for C-6 in the case of the *N*-hydroxy compounds compared to the parent piperazine-2,5-dione. All

† In compounds of this type the  $\alpha$ -position of substituents is with respect to the carbonyl group attached to the nitrogen atom of the amino acid.



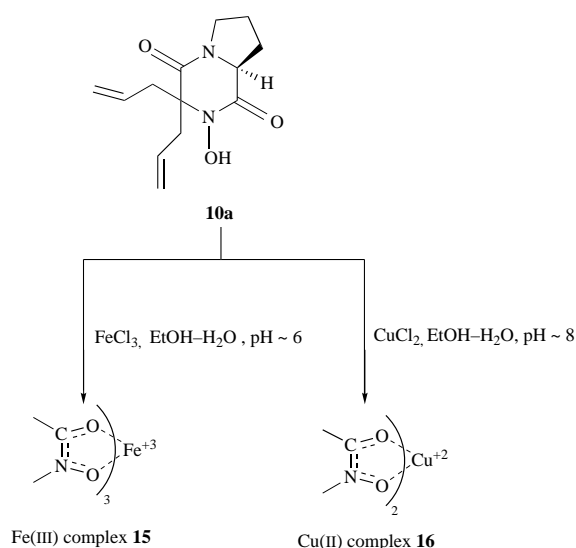
**Fig. 1** Cleavage of pBR322 plasmid DNA by  $\text{Fe}^{\text{III}}$  and  $\text{Cu}^{\text{II}}$  complex of **10a**. Forms I, II and III represent closed circular supercoiled, open circular and linear DNA respectively. All lanes contain pBR322 plasmid DNA with the following additions. Lane 1: only DNA; Lane 2:  $\text{H}_2\text{O}_2$ ; Lane 3: ME; Lane 4:  $\text{Fe}^{\text{III}}$  complex **15** +  $\text{H}_2\text{O}_2$  + ME; Lane 5:  $\text{Cu}^{\text{II}}$  complex **16** +  $\text{H}_2\text{O}_2$ ; Lane 6: Free ligand **10a** +  $\text{H}_2\text{O}_2$  + ME.

the cyclic hydroxamic acids exhibited a characteristic deep violet colour with a trace of  $\text{FeCl}_3$ .

### Metal complexes

Several recent reports in the literature have dealt with the chemistry of the metal complexes of hydroxamic acids<sup>11</sup> and their ability to act as artificial nucleases.<sup>12</sup> The DNase activity of the  $\text{Fe}^{\text{III}}$ ,  $\text{Co}^{\text{II}}$ ,  $\text{Ni}^{\text{II}}$  and  $\text{Cu}^{\text{II}}$  complexes of desferrioxamine have been investigated in our laboratory.<sup>13</sup>

The 6,6-bis(allyl)-1-hydroxypiperazine-2,5-dione **10a** in which C-3† has the (*S*)-configuration, was used for our initial studies on the possible application of the metal complexes of such peptide-related hydroxamic acids for DNA cleavage. The ferric trishydroxamate complex **15** and the  $\text{Cu}^{\text{II}}$  dihydroxamate **16** were prepared and characterised by IR, UV-VIS and ESR spectroscopy and CV (Scheme 4). Fig. 1 shows the agarose gel



**Scheme 4**

electrophoresis pattern of DNA cleavage by these two metal complexes. Both the complexes were able to convert supercoiled DNA (form I) into either open circular (form II) or linear (form III) DNA, thus acting as effective Fenton-type reagents.<sup>14</sup> Interestingly, while the ferric complex **15** required both an oxidising agent ( $\text{H}_2\text{O}_2$ ) and a reducing agent [mercaptoethanol (ME)] for its DNase activity, the  $\text{Cu}^{\text{II}}$  complex **16** was able to cleave DNA only in the presence of  $\text{H}_2\text{O}_2$ , indicating that the  $\text{Cu}^{\text{II}}$ -DNA complex may be directly reduced by the peroxide to produce the corresponding DNA Cu-hydroperoxo species<sup>15</sup> thereby leading to DNA damage. Neither the free ligand nor  $\text{H}_2\text{O}_2$  (or ME) were found to have any DNase activity under identical reaction conditions.

## Experimental

### General

Mps were recorded on a Campbell-Electronic-Thermonic instrument in open capillary tubes and are quoted uncorrected.

Optical rotations were measured on a JASCO-181 digital polarimeter with a Na light (5893 Å) source. IR spectra were recorded on a Perkin-Elmer Infracord spectrometer. UV spectra were recorded on a Perkin-Elmer UV-Visible ( $\lambda$ ) spectrometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker WH-90, Bruker AC-200 or Bruker MSL-300 using tetramethylsilane as internal standard,  $\delta$  values are reported in ppm. Mass spectra were recorded on a Finnigan-MAT-1020B spectrometer. Elemental analyses were performed in our analytical group. ESR and CV were obtained from the Physical Chemistry group of the National Chemical Laboratory. The pBR322 (form I) and Agarose gel were obtained from Bangalore, Genie, India.

**Synthesis of nitro acetamides.** Nitro acetamides **1**, **5a** and **5b** were prepared by the reaction of the respective amino acid esters with 1,1-bis(methylthio)-2-nitroethylene in the presence of a catalytic amount of PTSA in acetonitrile at room temperature according to our earlier reports.<sup>8a,b</sup>

### First and second allylation of *N*-nitroacetyl amino acid esters: general procedure A

To a solution of the *N*-nitroacetyl derivative (1 mmol) in degassed acetonitrile (15 ml), DBU (1 mmol) was added and stirred under argon for 5 min.  $\text{Pd}(\text{dba})_2$  (3 mol%) and  $\text{PPh}_3$  (12 mol%) were added to the mixture and stirred for another 5 min. Finally, a solution of the allyl acetate (1 mmol) in acetonitrile (5 ml) was added and the mixture stirred for 8–10 h. After cooling to  $-20^\circ\text{C}$ , the mixture was made acidic by addition of 5% aq. HCl and extracted with ethyl acetate, the organic extract washed well with brine, dried ( $\text{Na}_2\text{SO}_4$ ), and evaporated; the orange gum was further purified by column chromatography (silica gel, 60–120 mesh, light petroleum–AcOEt) $\S$  to give the pure monoallyl-substituted *N*-nitroacetyl derivative. The above pure monoallyl product was further subjected to a second allylation repeating the above general procedure.

The monoallyl *N*-nitroacetyl derivatives **7a–c**, **11** and the bis(allyl) *N*-nitroacetyl derivatives **9a–c**, were prepared according to the above general procedure. Their spectral data have already been reported in our earlier publication.<sup>8c</sup>

### (*S*)-Ethyl *N*-[2-nitro-2,2-bis(4-acetyloxybut-2-en-1-yl)acetyl]-prolinate **9d**

Compound **9d** was synthesised from *N*-nitroacetyl-(*S*)-proline ethyl ester **1**. The procedure involved two successive allylation sequences using *cis*-but-2-ene-1,4-diyl diacetate as described in the above general procedure A.

**The first allylation.** The mono-substituted *N*-nitroacetyl-(*S*)-proline ethyl ester was obtained in 50% yield as a diastereomeric mixture (de 32%)<sup>16</sup> from *N*-nitroacetyl-(*S*)-proline ethyl ester **1**, *cis*-butene-1,4-diyl diacetate,  $\text{Pd}(\text{dba})_2$  (3 mol%) and  $\text{PPh}_3$  (12 mol%) as described in general procedure A.  $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  1740, 1675, 1580, 1450, 1380, 1220, 1100 and 1040;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.2–1.4 (m, 3H,  $\text{CH}_3$ ), 2.1 (2s, 3H,  $\text{COCH}_3$ ), 2.0–2.4 (m, 4H, 2 $\text{CH}_2$ ), 2.8–3.25 (m, 2H, allylic  $\text{CCH}_2$ ), 3.6–3.9 (m, 2H,  $\text{NCH}_2$ ), 4.1–4.3 (m, 2H,  $\text{OCH}_2$ ), 4.5–4.7 (m, 3H,  $\text{NCH}_2\text{C}=\text{C}$ ), 5.2–5.4 (m, 1H,  $\text{O}_2\text{NCH}$ ), 5.6–5.9 (m, 2H, olefinic);  $\delta_{\text{C}}(\text{CDCl}_3)$  14.19 (14.08) ( $\text{CH}_3$ ), 20.89 ( $\text{COCH}_3$ ), 21.50, 24.73, 29.12 (29.03), 30.98 ( $\text{CH}_2$ ), 33.03 (32.93) (allylic  $\text{CH}_2$ ), 47.65 (47.56) ( $\text{NCH}_2$ ), 59.63 (59.87) ( $\text{NCH}$ ), 61.40 ( $\text{OCH}_2$ ), 64.11 (allylic  $\text{OCH}_2$ ), 85.57 (85.79) ( $\text{O}_2\text{NCH}$ ), 126.58 (126.82), 129.88 ( $\text{C}=\text{C}$ ), 161.9 (161.0), 170.64, 170.90, 179.18 (CO) (Found: C, 52.45; H, 6.58; N, 8.40.  $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_7$  requires C, 52.62; H, 6.47; N, 8.18%).

**Second allylation.** The diastereomeric mixture of monoallyl product obtained above was subjected to a second allylation using the same general procedure A to give the bis(allyl) product **9d** as gum.  $[\alpha]_{\text{D}} -44.5$  (*c* 0.6, MeOH);  $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  1740, 1660, 1550, 1430, 1380, 1220, 1030;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.26 (t, 3H,  $\text{CH}_3$ ), 1.8–2.3 (m, 4H, 2 $\text{CH}_2$ ), 2.05 (2s, 6H,  $\text{COCH}_3$ ), 2.75–

$\S$  Light petroleum refers to the fraction with bp 60–80  $^\circ\text{C}$ .

3.0 (m, 4H, allylic CH<sub>2</sub>), 3.2–3.5 (2m, 2H, NCH<sub>2</sub>), 4.2 (q, 2H, OCH<sub>2</sub>), 4.4–4.7 (m, 5H, NCH, allylic CH<sub>2</sub>), 5.4–5.9 (m, 4H, olefinic);  $\delta_{\text{C}}(\text{CDCl}_3)$  14.39 (CH<sub>3</sub>), 21.03 (COCH<sub>3</sub>), 25.05 (CH<sub>2</sub>), 28.22 (CH<sub>2</sub>), 36.50 and 38.71 (allylic CH<sub>2</sub>), 47.12 (NCH<sub>2</sub>), 61.14 and 61.43 (NCH, OCH<sub>2</sub>), 64.34 and 64.29 (allylic OCH<sub>2</sub>), 94.97 (C), 125.76 and 126.37 (C=C), 131.00–131.06 (C=C), 163.68, 170.78 and 171.71 (CO) (Found: C, 55.75; H, 6.6; N, 6.28. C<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>9</sub> requires C, 55.50; H, 6.64; N, 6.16%).

#### Ethyl *N*-[2-nitro-2,2-bis(2-methylprop-2-en-1-yl)acetyl]-(*S*)-valinate **13a**

Compound **13a** was synthesised from *N*-nitroacetyl-(*S*)-valine ethyl ester **5a**. The procedure involved two successive allylations using methylallyl chloride as described in the above general procedure A.

**The first allylation.** The mono-substituted *N*-nitroacetyl-(*S*)-valine ethyl ester was obtained as a diastereomeric mixture (60%);  $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$  3340, 1755, 1680, 1580, 1380;  $\delta_{\text{H}}(\text{CDCl}_3)$  0.85–0.9 (m, 6H, 2CH<sub>3</sub>), 1.25 (t, 3H, CH<sub>3</sub>), 1.53–1.62 (2s, 3H, CH<sub>3</sub>), 2.1 (m, 1H, CH), 2.85 (m, 2H, allylic CH<sub>2</sub>), 4.15 (m, 2H, OCH<sub>2</sub>), 4.4 (m, 1H, CH $\alpha$ ), 4.75–4.85 (2s, 2H, =CH<sub>2</sub>), 5.2 (m, 1H, NO<sub>2</sub>CH), 6.85 (br s, 1H, NH).

**Second allylation.** The diastereomeric mixture of monoallyl products obtained above was subjected to a second allylation using the same general procedure to give **13a** as a gum (73%);  $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  3340, 1740, 1670, 1540, 1520, 1450, 1380, 1220;  $\delta_{\text{H}}(\text{CDCl}_3)$  0.85 (d, 6H, 2CH<sub>3</sub>), 1.2 (t, 3H, CH<sub>3</sub>), 1.6 (s, 6H, 2CH<sub>3</sub>), 2.15 (m, 1H, CH), 2.9 (m, 4H, allylic CH<sub>2</sub>), 4.1 (q, 2H, OCH<sub>2</sub>), 4.35 (m, 1H, CH $\alpha$ ), 4.65–4.85 (m, 4H, allylic CH<sub>2</sub>), 7.8 (br d, 1H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  14.07 (CH<sub>3</sub>), 17.83, 18.74 (2CH<sub>3</sub>), 22.86, 23.13 (2CH<sub>3</sub>), 31.04 (CH), 45.24, 45.74 (allylic CH<sub>2</sub>), 58.13 [NCH( $\alpha$ )], 61.19 (OCH<sub>2</sub>), 96.97 (C), 116.19, 116.56 (=CH<sub>2</sub>), 138.29, 138.48 (=C), 165.70 (CO), 170.74 (CO) (Found: C, 60.11; H, 8.13; N, 8.46. C<sub>17</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> requires C, 59.98; H, 8.29; N, 8.22%).

#### Ethyl *N*-[2-nitro-2,2-bis(2-methylprop-2-en-1-yl)acetyl]-(*S*)-phenylalaninate **13b**

Compound **13b** was synthesised from *N*-nitroacetyl-(*S*)-phenylalanine ethyl ester **5b**. The procedure involved two successive Pd<sup>0</sup>-catalysed allylations using methylallyl chloride as described in the above general procedure A.

**The first allylation.** The mono-substituted *N*-nitroacetyl-(*S*)-phenylalanine ethyl ester was obtained as a diastereomeric mixture (65%);  $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$  3350, 1760, 1660, 1550, 1440, 1380;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.26 (t, 3H, CH<sub>3</sub>), 1.75 (s, 3H, CH<sub>3</sub>), 2.84 (m, 2H, allylic CH<sub>2</sub>), 3.15 (m, 2H, CH<sub>2</sub>), 4.2 (q, 2H, OCH<sub>2</sub>), 4.8 (2s, 2H, =CH<sub>2</sub>), 5.17 [m, 2H, NO<sub>2</sub>CH, CH( $\alpha$ )], 6.74 (br d, 1H, NH), 7.1–7.25 (m, 5H, ArH).

**Second allylation.** The diastereomeric mixture of monoallylated product obtained above was subjected to a second allylation using the same general procedure to give the bis(allyl) product **13b** as a gum (70%);  $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  3320, 1750, 1660, 1540, 1450, 1380;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.22 (t, 3H, CH<sub>3</sub>), 1.4, 1.53 (2s, 6H, 2CH<sub>3</sub>), 2.7–3.2 (m, 4H, 2CH<sub>2</sub>), 4.15 (q, 2H, OCH<sub>2</sub>), 4.6–5.0 [m, 5H, 2C=CH<sub>2</sub>, CH( $\omega$ )], 7.25 (m, 5H, ArH), 7.8 (br d, 1H, NH) (Found: C, 65.13; H, 7.38; N, 7.12. C<sub>21</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> requires C, 64.92; H, 7.26; N, 7.21%).

#### Reduction of *N*-nitroacetyl-(*S*)-proline ethyl ester

**(8a*S*)-Perhydropyrrolo[1,2-*a*]pyrazine-1,4-dione **2**.** To the *N*-nitroacetyl-(*S*)-proline ethyl ester **1** (230 mg, 1 mmol) in ethanol was added freshly prepared Al–Hg (300 mg) and the mixture was stirred at 30 °C until the complete disappearance of the starting material (*ca.* 8 h). After completion of the reaction the inorganic material was filtered off and the filtrate was concentrated under reduced pressure to afford a crude product. Purification of the crude product on a silica gel column with light petroleum and EtOAc as eluents, initially 7:3 to remove impurities, finally 1:1 to elute the product, yielded **2** (70%), mp

203–205 °C (lit.,<sup>17</sup> 209–210 °C);  $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  3400, 1670, 1450, 1300;  $\delta_{\text{H}}([\text{}^2\text{H}_6\text{]DMSO})$  1.5–2.55 (m, 4H, 2CH<sub>2</sub>), 3.2–4.2 (m, 5H, 2NCH<sub>2</sub> and NCH), 6.8 (br s, 1H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  22.14 (CH<sub>2</sub>), 28.16 (CH<sub>2</sub>), 45.02 (CH<sub>2</sub>), 46.31 (CH<sub>2</sub>), 58.33 (CH), 163.54 (CO), 169.93 (CO).

#### Ethyl *N*-hydroxyiminoacetyl-(*S*)-prolinate **3**

To a solution of anhydrous SnCl<sub>2</sub> (285 mg, 1.5 mmol) in benzene (10 ml), at 30 °C thiophenol (0.62 ml, 6 mmol) and triethylamine (0.62 ml, 4.5 mmol) were added, followed by the addition of *N*-nitroacetamide derivative **1** (0.75 mmol). The mixture was stirred for 6 h. After completion of the reaction, the organic layer was loaded directly on a silica gel column for chromatography. Elution with CH<sub>2</sub>Cl<sub>2</sub>–MeOH gave the product **3** (60%).  $\nu_{\text{max}}(\text{Nujol})/\text{cm}^{-1}$  3200–2700 (br), 1750, 1650, 1600, 1470, 1380, 1200, 1020;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.2 (m, 3H, CH<sub>3</sub>), 2.0 (m, 4H, 2CH<sub>2</sub>), 3.8 (m, 2H, CH<sub>2</sub>), 4.57 (m, 1H, NCH), 4.9 (m, 1H, CH), 10.3 (br s, 1H, OH) (Found: C, 50.33; H, 6.74; N, 13.35. C<sub>9</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub> requires C, 50.46; H, 6.58; N, 13.07%).

#### Preparation of 1-hydroxypiperazine-2,5-diones

**General procedure B.** To a solution of the *N*-nitroacetyl-(*S*)-proline ethyl ester (1 mmol) in EtOH (5 ml) was added aq. NH<sub>4</sub>Cl (50%; 5 ml) followed by zinc dust (390 mg, 6 mmol) in small portions at 30 °C. The mixture was allowed to stir at 30 °C for 12–24 h, during which the reaction was monitored by TLC. After the completion of the reaction, the zinc dust was filtered off and the filtrate was concentrated under reduced pressure. The residue was taken up in anhydrous EtOH, the insoluble NH<sub>4</sub>Cl filtered off and the filtrate refluxed for 3–6 h to ensure completion of the cyclisation. Finally the solution was concentrated and the residue was purified by rapid chromatography on silica (light petroleum–EtOAc). The isolated product gave as expected a deep violet colour with ethanolic FeCl<sub>3</sub> solution indicating clearly the formation of a hydroxamic acid.

#### (8a*S*)-2-Hydroxyperhydropyrrolo[1,2-*a*]pyrazine-1,4-dione **4**

The product **4** was obtained as a white solid from *N*-nitroacetyl-(*S*)-proline ethyl ester **1** (85%), mp 115 °C (lit.,<sup>4a</sup> 114 °C);  $[\alpha]_{\text{D}} -132.9$  (*c* 0.4, MeOH);<sup>4a</sup>  $\delta_{\text{H}}(\text{D}_2\text{O})$  1.8–2.5 (m, 4H, 2CH<sub>2</sub>), 3.55 (m, 2H, CH<sub>2</sub>), 4.22 (d, 1H, CH), 4.37 (m, 1H, NCH), 4.6 (d, 1H, CH);  $\delta_{\text{C}}(\text{D}_2\text{O})$  23.3 ( $\gamma$ CH<sub>2</sub>), 29.7 ( $\beta$ CH<sub>2</sub>), 47.12 ( $\delta$ CH<sub>2</sub>), 55.73 ( $\alpha'$ CH<sub>2</sub>), 60.12 ( $\alpha$ CH), 166.6 (CO), 165.1 (CO); *m/z* 170 (M<sup>+</sup>), 153 (M – 17), 142, 128, 111, 98, 83, 70 (base peak) (Found: C, 48.32; H, 5.58; N, 15.84. C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>·0.25H<sub>2</sub>O requires C, 48.12; H, 5.76; N, 15.92%).

#### (3*S*)-1-Hydroxy-3-isopropylpiperazine-2,5-dione **6a**

Reduction of *N*-nitroacetyl-(*S*)-valine ethyl ester **5a** according to general procedure B gave (3*S*)-1-hydroxy-3-isopropylpiperazine-2,5-dione **6a** (40%), mp 115 °C (lit.,<sup>4a</sup> 115 °C);  $[\alpha]_{\text{D}} -33.5$  (*c* 1, MeOH);<sup>4a</sup>  $\delta_{\text{H}}([\text{}^2\text{H}_6\text{]DMSO})$  0.83–0.92 (2d, 6H, 2CH<sub>3</sub>), 2.15 (m, 1H, CH), 3.7 (m, 1H, CH), 4.0–4.15 (2d, 2H, CH<sub>2</sub>), 8.26 (s, 1H, NH);  $\delta_{\text{C}}([\text{}^2\text{H}_6\text{]DMSO})$  17.04 and 18.87 (2CH<sub>3</sub>), 33.24 (CH), 53.54 (NCH<sub>2</sub>), 59.58 (NCH), 162.22 and 165.07 (CO); *m/z* 172 (M<sup>+</sup>), 155 (M – 17), 144, 130, 113, 101 (base peak), 85, 72 (Found: C, 48.05; H, 7.0; N, 16.03. C<sub>7</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> requires C, 48.33; H, 7.02; N, 16.27%).

#### (3*S*)-1-Hydroxy-3-benzylpiperazine-2,5-dione **6b**

Reduction of *N*-nitroacetyl-(*S*)-phenylalanine ethyl ester **5b** according to the general procedure B gave (3*S*)-1-hydroxy-3-benzylpiperazine-2,5-dione **6b** (45%), mp 232 °C (lit.,<sup>4a</sup> 232 °C);  $[\alpha]_{\text{D}} 18.2$  (*c* 1, DMF);<sup>4a</sup>  $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  1650;  $\delta_{\text{H}}([\text{}^2\text{H}_6\text{]DMSO})$  2.88 (dd, 1H, CH), 3.05 (d, 1H, CH), 3.15 (dd, 1H, CH), 3.7 (d, 1H, CH), 4.22 (m, 1H, CH), 7.2 (m, 5H, PhH), 8.2 (br d, 1H, NH); *m/z* 220 (M<sup>+</sup>), 192, 162, 120, 91 (base peak) (Found: C, 58.71; H, 5.31; N, 12.64. C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>·0.3H<sub>2</sub>O requires C, 58.55; H, 5.35; N, 12.42%).

**(8aS)-Perhydro-2-hydroxy-3-(prop-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 8a**

Reduction of the monoallyl derivative **7a** (de = 29%) according to the general procedure B gave the 2-hydroxypyrrolopyrazine-1,4-dione **8a** (80%) as a gum (de = 30%),<sup>16</sup>  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  3471, 3100, 2920, 1648;  $\delta_{\text{H}}(\text{D}_2\text{O})$  1.5–2.25 (m, 4H, 2CH<sub>2</sub>), 2.4–2.9 (m, 2H, allylic CH<sub>2</sub>), 3.2–3.6 (m, 2H, NCH<sub>2</sub>), 4.1 [m, 1H, NCH( $\alpha$ )], 4.2–4.5 (m, 1H, CHNOH), 5.0–5.8 (m, 3H, CH=CH<sub>2</sub>);  $\delta_{\text{C}}(\text{D}_2\text{O})$  22.81 (22.41) ( $\gamma$ C), 29.87 (30.37) ( $\beta$ C), 35.20 (33.66) (allylic CH<sub>2</sub>), 46.85 (46.27) (NCH<sub>2</sub>), 59.58 [NCH( $\alpha$ )], 66.62 (64.8) ( $\alpha'$ C), 121.83, 122.51 (=CH<sub>2</sub>), 132.03, 132.2 (=CH), 165.94 (CO), 166.77 (CO) (Found: C, 57.27; H, 6.48; N, 13.05. C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub> requires C, 57.14; H, 6.70; N, 13.32%).

**(8aS)-Perhydro-2-hydroxy-3-(2-methylprop-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 8b**

Reduction of the diastereomeric mixture of the monoallyl derivative **7b**<sup>8c</sup> (de = 25%) according to the general procedure B gave the 2-hydroxypyrrolopyrazine-1,4-dione **8b** (77%) as a gum (de = 23%);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3300, 2900, 1660, 1450, 1220, 1070;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.5–2.4 (m, 4H,  $\beta$  and  $\gamma$ CH<sub>2</sub>), 1.7 and 1.85 (2s, 3H, CH<sub>3</sub>), 2.6–3.0 (m, 2H, allylic CH<sub>2</sub>), 3.3–3.8 (m, 2H, NCH<sub>2</sub>), 4.1 [m, 1H, NCH( $\alpha$ )], 4.5–4.6 (m, 1H, CHNOH), 4.9 (2s, 2H, =CH<sub>2</sub>);  $\delta_{\text{C}}(\text{CDCl}_3)$  22.0 (21.60) ( $\gamma$ C), 23.0 (allylic CH<sub>3</sub>), 29.29 (29.75) ( $\beta$ C), 35.83 (37.63) (allylic CH<sub>2</sub>), 45.15 (NCH<sub>2</sub>), 57.74 [NCH( $\alpha$ )], 63.94 ( $\alpha'$ C), 116.26 (olefinic CH<sub>2</sub>), 139.81 (=C), 162.27 (CO), 163.53 (CO);  $m/z$  224 (M<sup>+</sup>), 207 (M – 17), 206, 196, 166, 151, 141 (base peak), 123, 110, 97, 70 (Found: C, 59.13; H, 7.04; N, 12.58. C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> requires C, 58.90; H, 7.19; N, 12.49%).

**(8aS)-Perhydro-2-hydroxy-3-(3-phenylprop-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 8c**

Reduction of the diastereomeric mixture of the monocinnamyl derivative **7c**<sup>8c</sup> (de = 34%) according to general procedure B gave the 2-hydroxy-3-cinnamylpyrrolopyrazine-1,4-dione **8c** (70%) as a gum (de = 38%);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3340, 3100, 2920, 1660, 1450, 1220, 1070;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.5–2.25 (m, 4H,  $\beta$ - and  $\gamma$ -CH<sub>2</sub>), 2.7–3.0 (m, 2H, allylic CH<sub>2</sub>), 3.2–3.7 (m, 2H, NCH<sub>2</sub>), 3.9–4.1 [m, 1H, NCH( $\alpha$ )], 4.4 (m, 1H, CHNOH), 5.9–6.15 (m, 1H, =CH), 6.4 (m, 1H, =CH), 7.0–7.2 (m, 5H, ArH);  $\delta_{\text{C}}(\text{CDCl}_3)$  22.12 (21.91) ( $\gamma$ C), 29.57 (30.21) ( $\beta$ C), 33.6 and 34.0 (allylic CH<sub>2</sub>), 44.75 (45.31) (NCH<sub>2</sub>), 57.95, 60.24, 65.41, 122.25, 126.39, 128.0, 128.78, 135.70, 137.04, 161.5 and 162 (CO), 163.5, 164.0 (CO);  $m/z$  286 (M<sup>+</sup>), 269 (M – 17), 241, 152, 117, 77, 70.

**Ethyl *N*-[(2-hydroxyimino)-5-phenylpent-4-enoyl]-(*S*)-phenylalaninate 12**

Compound **12** was obtained as a solid by reduction of the mono-substituted *N*-nitroacetyl-(*S*)-phenylalanine ethyl ester **11** according to the general procedure B, mp 110 °C;  $[\alpha]_{\text{D}} + 6.8$  (c 1, MeOH);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3400, 3300, 1740, 1670, 1520, 1230, 1020;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.3 (t, 3H, CH<sub>3</sub>), 3.0 (d, 2H, CH<sub>2</sub>), 3.48 (d, 2H, CH<sub>2</sub>), 4.1 (q, 2H, OCH<sub>2</sub>), 4.85 [m, 1H, CH( $\alpha$ )], 6.6 (m, 2H, CH=CH), 7.0–7.5 (m, 11H, ArH, NH), 9.62 (br s, 1H, OH);  $\delta_{\text{C}}(\text{CDCl}_3)$  13.72 (CH<sub>3</sub>), 26.82 (CH<sub>2</sub>), 34.84 (CH<sub>2</sub>), 53.4 [NCH( $\alpha$ )], 61.47 (OCH<sub>2</sub>), 122.65, 126.02, 126.84, 126.96, 128.18, 128.27, 129.02, 132.63, 135.63 and 137.21 (aromatic and olefinic), 151.97 (C=N), 162.96 (CO), 171.74 (CO);  $m/z$  380 (M<sup>+</sup>), 363 (M – 17), 335, 307, 192, 170, 144, 115, 91 (Found: C, 69.64; H, 6.12; N, 7.53. C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub> requires C, 69.46; H, 6.35; N, 7.36%).

**(8aS)-Perhydro-2-hydroxy-3,3-bis(prop-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 10a**

Reduction of the bisallyl derivative **9a**<sup>8c</sup> by the general procedure B gave the 3,3-bis(allyl)-2-hydroxypyrrolopyrazine-1,4-dione **10a** as a solid, mp 102 °C (85%);  $[\alpha]_{\text{D}} - 80.3$  (c 1, MeOH);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3300–3100 (br), 2900, 1660, 1440, 1300, 1220;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.5–2.4 (m, 4H,  $\beta$ - and  $\gamma$ -CH<sub>2</sub>), 2.6–2.9 (m, 4H,

allylic CH<sub>2</sub>), 3.4–3.8 (m, 2H, NCH<sub>2</sub>), 4.1 [dd, 1H, NCH( $\alpha$ )], 5.1–5.9 (m, 6H, CH=CH<sub>2</sub>), 8.3 (br s, 1H, OH);  $\delta_{\text{C}}(\text{CDCl}_3)$  21.38, 29.99 ( $\beta$ - and  $\gamma$ -C), 39.27, 39.87 (allylic CH<sub>2</sub>), 44.73 (NCH<sub>2</sub>), 57.46 [NCH( $\alpha$ )], 72.56 (C), 119.77, 120.56 (=CH<sub>2</sub>), 131.0, 131.41 (–CH=), 162.28 (CO), 164.16 (CO);  $m/z$  250 (M<sup>+</sup>), 233 (M – 17), 209, 181 (base peak), 112, 70 (Found: C, 62.41; H, 7.01; N, 11.28. C<sub>13</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> requires C, 62.38; H, 7.24; N, 11.19%).

**(8aS)-Perhydro-2-hydroxy-3,3-bis(2-methylprop-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 10b**

Reduction of the bis(methylallyl) derivative **9b**<sup>8c</sup> by the general procedure B gave the 3,3-bis(methylallyl)-2-hydroxypyrrolopyrazine-1,4-dione **10b** as a gum (80%);  $[\alpha]_{\text{D}} - 55.4$  (c 1, MeOH);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3423, 2926, 1660, 1446, 1218;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.6–1.8 (2s, 6H, CH<sub>3</sub>), 1.5–2.5 (m, 4H, 2CH<sub>2</sub>), 2.5–2.9 (m, 4H, allylic CH<sub>2</sub>), 3.4–3.75 (2m, 2H, CH<sub>2</sub>), 4.7–4.9 (m, 4H, =CH<sub>2</sub>);  $m/z$  278 (M<sup>+</sup>), 261 (M – 17) (Found: C, 64.51; H, 8.01; N, 10.01. C<sub>15</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires C, 64.73; H, 7.95; N, 10.06%).

**(8aS)-Perhydro-2-hydroxy-3,3-bis(3-phenylprop-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 10c**

Reduction of the bis(cinnamyl) derivative **9c**<sup>8c</sup> by the general procedure B gave the 3,3-bis(cinnamyl)-2-hydroxypyrrolopyrazine-1,4-dione **10c** as a gum (70%);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3200–3400 (br), 3100, 2900, 1660, 1500, 1460, 1230;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.5–2.7 (m, 4H, 2CH<sub>2</sub>), 2.8–3.0 (m, 4H, allylic CH<sub>2</sub>), 3.2–4.1 (m, 3H, CH $\alpha$ , CH<sub>2</sub>), 6.0–6.6 (m, 4H, HC=CH), 7.4 (m, 10H, Ar-H);  $\delta_{\text{C}}(\text{CDCl}_3)$  21.3, 29.8 ( $\beta$ -,  $\gamma$ -C), 38.5, 39.0 (allylic CH<sub>2</sub>), 44.9 (NCH<sub>2</sub>), 57.3 [NCH( $\alpha$ )], 73.4 (C), 122.3, 122.9 (=CH), 126.05, 126.16, 127.3, 127.5, 128.3 and 128.5 (aromatic), 135.0, 135.4 (=CH-), 136.95, 137.2 (aromatic), 162 (CO), 164.4 (CO);  $m/z$  402 (M<sup>+</sup>), 385 (M – 17), 357, 285, 257 (100%), 117, 91 (A small sample of the product was further purified for microanalysis by preparative TLC. Found: C, 71.55; H, 6.04; N, 6.38. C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub>·H<sub>2</sub>O requires C, 71.40; H, 6.23; N, 6.66%).

**(8aS)-Perhydro-2-hydroxy-3,3-bis(4-acetoxybut-2-en-1-yl)pyrrolo[1,2-a]pyrazine-1,4-dione 10d**

Reduction of the bis(acetoxyallyl) derivative **9d** by the general procedure B gave the 3,3-bis(acetoxyallyl)-2-hydroxypyrrolopyrazine-1,4-dione **10d** as a gum (70%);  $[\alpha]_{\text{D}} - 27.58$  (c 2.4, MeOH);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3331, 2929, 1737, 1659, 1650, 1453, 1383;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.7–2.5 (m, 4H, 2CH<sub>2</sub>), 2.0 (2s, 6H, COCH<sub>3</sub>), 2.5–3.0 (m, 4H, allylic CH<sub>2</sub>), 3.4–3.9 (2m, 2H, NCH<sub>2</sub>), 4.1 (dd, 1H, NCH), 4.45 (m, 4H, allylic OCH<sub>2</sub>), 5.4–5.8 (m, 4H, HC=CH);  $\delta_{\text{C}}(\text{CDCl}_3)$  20.61 (COCH<sub>3</sub>), 21.53 (CH<sub>2</sub>), 29.93 (CH<sub>2</sub>), 37.60, 38.27 (allylic CH<sub>2</sub>), 44.80 (NCH<sub>2</sub>), 57.07 (NCH), 64.04, 64.30 (allylic OCH<sub>2</sub>), 72.81 (C), 127.05, 127.72, 128.15, 129.47, 129.60, 130.09 and 130.30 (olefinic), 162.22, 163.83, 170.33 and 170.44 (CO) (Found: C, 57.94; H, 6.46; N, 7.32. C<sub>19</sub>H<sub>26</sub>N<sub>2</sub>O<sub>7</sub> requires C, 57.86; H, 6.63; N, 7.10%).

**Ethyl *N*-[*N*-hydroxy-2,2-bis(2-methylprop-2-en-1-yl)glycyl]-(*S*)-valinate 14a**

Reduction of the bis(methylallyl) derivative **13a** of *N*-nitroacetyl-(*S*)-valine ethyl ester by the general procedure B gave the hydroxylamine derivative **14a** (74%) as a solid, mp 116 °C;  $[\alpha]_{\text{D}} 6.6$  (c 0.5, MeOH);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3400, 1740, 1650, 1540, 1470, 1450, 1390, 1310, 1270, 1220, 1040;  $\delta_{\text{H}}(\text{CDCl}_3)$  0.85 (m, 6H, 2CH<sub>3</sub>), 1.2 (t, 3H, CH<sub>3</sub>), 1.62 (s, 6H, 2CH<sub>3</sub>), 2.1 (m, 1H, CH), 2.2–2.7 (m, 4H, allylic CH<sub>2</sub>), 4.1 (q, 2H, OCH<sub>2</sub>), 4.8–5.2 (m, 5H, NH, =CH<sub>2</sub>), 7.15 (d, 1H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  14.08 (CH<sub>3</sub>), 17.78, 18.89 (2CH<sub>3</sub>), 24.45, 24.53 (2CH<sub>3</sub>), 30.93 (CH), 40.72, 41.97 (allylic CH<sub>2</sub>), 57.18 [NCH( $\alpha$ )], 69.16 (C), 115.56 (=CH<sub>2</sub>), 141.15, 141.51 (=C), 172.39, 172.98 (CO);  $m/z$  326 (M<sup>+</sup>), 309 (M – 17), 281, 271 (base peak), 253, 197, 154, 144 (Found: C, 62.27; H, 9.43; N, 8.78. C<sub>17</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub> requires C, 62.55; H, 9.25; N, 8.58%).

### Ethyl *N*-[*N*-hydroxy-2,2-bis(2-methylprop-2-en-1-yl)glycyl]-(*S*)-phenylalaninate **14b**

Reduction of the bis(methylallyl) derivative **13b** of *N*-nitroacetyl-(*S*)-phenylalanine ethyl ester by the general procedure B gave the hydroxylamine derivative **14b** (81%);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  3400, 1740, 1680, 1530, 1280, 1030;  $\delta_{\text{H}}(\text{CDCl}_3)$  1.25 (t, 3H, CH<sub>3</sub>), 1.65, 1.75 (2s, 6H, CH<sub>3</sub>), 2.25–2.65 (m, 4H, allylic CH<sub>2</sub>), 3.1 (d, 2H, CH<sub>2</sub>), 4.15 (q, 2H, OCH<sub>2</sub>), 4.5–5.0 [m, 6H, NH, CH( $\alpha$ ), =CH<sub>2</sub>], 7.0–7.3 (m, 6H, NH, ArH);  $\delta_{\text{C}}(\text{CDCl}_3)$  13.89 (CH<sub>3</sub>), 24.31 (CH<sub>3</sub>), 30.06 (CH<sub>2</sub>), 40.96, 41.45 (allylic CH<sub>2</sub>), 52.88 [NCH( $\alpha$ )], 61.28 (OCH<sub>2</sub>), 68.52 (C), 115.41, 115.46 (=CH<sub>2</sub>), 126.93, 128.38, 129.14 and 136.06 (aromatic), 141.06, 141.30 (=C), 171.77 (CO), 172.65 (CO); *m/z* 374 (M<sup>+</sup>), 357 (M – 17), 319 (base peak), 283, 245, 192, 154, 146, 138, 120, 91 (Found: C, 67.31; H, 8.14; N, 7.33. C<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub> requires C, 67.35; H, 8.06; N, 7.48%).

### Metal complexes of 3,3-bis(allyl)-2-hydroxypyrrolopyrazine-1,4-dione

**Fe<sup>III</sup> trihydroxamate complex 15.** The hydroxamic acid **10a** (750 mg, 3 mmol) was dissolved in an ethanol and water mixture (1:4, 10 ml) and allowed to warm on a steam bath. To this was added hydrated FeCl<sub>3</sub> (150 mg, 0.9 mmol) as an aqueous solution dropwise. An intense violet colour developed immediately. The pH was adjusted to 6 using sodium acetate solution. A violet coloured solid soon separated from the hot solution. After complete removal of ethanol from the mixture, the solution was allowed to cool to 30 °C. The solid was collected by filtration, thoroughly washed with cold water and dried over P<sub>2</sub>O<sub>5</sub>. The yield of the ferric complex **15** was about 50%;  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  1650, 1600;  $\lambda_{\max}(\text{MeOH})/\text{nm}$  420 ( $\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  2340);  $E_i$  –980 mV ( $\Delta E$  100, dropping mercury electrode);<sup>18</sup> EPR *g* 4.2 (Found: C, 58.12; H, 6.53; N, 10.66. C<sub>39</sub>H<sub>51</sub>N<sub>6</sub>O<sub>9</sub>Fe requires C, 58.29; H, 6.35; N, 10.46%).

**Cu<sup>II</sup> dihydroxamate complex 16.** The hydroxamic acid **10a** (250 mg, 1 mmol) was dissolved in an ethanol and water mixture (1:4, 10 ml) and allowed to warm on a hot water bath. To this solution was added CuCl<sub>2</sub> (0.43 mmol) and the mixture was stirred for 30 min while a light green colour developed slowly. The pH of the solution was adjusted to 8 using sodium acetate solution. A light green coloured solid precipitated immediately. This was collected, washed thoroughly with cold water and dried, to yield the complex **16** (56%);  $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  1650, 1600;  $\lambda_{\max}(\text{MeOH})/\text{nm}$  650 ( $\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  30), 350 (150); EPR *g* 2.136 (Found: C, 55.73; H, 6.68; N, 10.11. C<sub>26</sub>H<sub>34</sub>N<sub>4</sub>O<sub>6</sub>Cu requires C, 55.56; H, 6.05; N, 9.97%).

### DNA cleavage assay

The standard DNA cleavage reactions were performed in a total volume of 20  $\mu\text{l}$  consisting of 20 mM tris-HCl (pH 7.8), sodium acetate (2.5  $\mu\text{M}$ ), plasmid pBR322 DNA (70  $\mu\text{M}$ ), H<sub>2</sub>O<sub>2</sub> (5  $\mu\text{M}$ ), mercaptoethanol (0.5  $\mu\text{M}$ ) and metal complex **15** or **16** (130  $\mu\text{M}$ ). The reactions were done at 37 °C for 30 min followed by addition of gel loading buffer containing bromophenol blue as dye and then directly loaded on 1% agarose gel for analysis by electrophoresis at 100 V.<sup>19</sup> The gels were stained with ethidium bromide (0.1  $\mu\text{g ml}^{-1}$ ) and visualised under UV light. The DNA cleavage reactions in the presence of sodium azide (0.1 M), mannitol (0.1 M) and glycerol (2 M) were carried out under similar conditions as described above.

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