Synthesis and effects on arginase and nitric oxide synthase of two novel analogues of N^{ω}-hydroxyarginine, N^{ω}-hydroxyindospicine and *p*-hydroxyamidinophenylalanine

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Two novel amino acids, N^{ω}-hydroxy-D,L-indospicine and *p*-hydroxyamidino-D,L-phenylalanine, have been synthesized in four steps from *tert*-butoxycarbonylglycine. Both compounds act as good inhibitors of arginase, N^{ω}-hydroxyindospicine being one of the best inhibitors of this enzyme known so far (IC_{50} = 50 μmol dm^{-3}). In contrast, with brain NO synthase the two compounds are almost without effect.

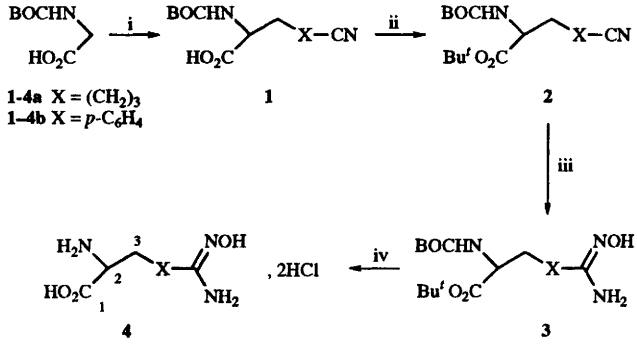
L-Arginine is metabolised in cells by two major pathways: arginase catalyses its hydrolysis to L-ornithine and urea in the first step of the urea cycle,[1] whereas NO synthase (NOS) catalyses its five-electron oxidation to L-citrulline and nitric oxide (NO).[2,3] NO thus produced has been found to be a key biological molecule in vasodilation, neurotransmission and cytotoxicity.[4,5] Arginase and NO synthase are metalloenzymes. The former involves a bimetallic Mn^{II}_2 centre, whereas the latter is a flavo-hemoprotein that requires tetrahydrobiopterin.[6] Although arginase and NO synthase share the same substrate, they differ in their affinity for arginine, the K_m for arginine being 1–10 mmol dm^{-3} for arginase[1] and 1–10 μmol dm^{-3} for NOS.[3,6] They also have different patterns of inhibition: NOS is inhibited by N^{ω}-substituted analogues of arginine, like N^{ω}-methyl-L-arginine (NMMA) or N^{ω}-nitro-L-arginine (NNA) (K_i = 1–10 μmol dm^{-3}),[7] whereas the best inhibitors of arginase known until last year were L-valine[8] or L-indospicine (K_i = 1 mmol dm^{-3}).[9]

Since the two enzymes can be found in similar tissues and cells, and because their expression may be regulated in response to the same stimuli [cytokines, endotoxines (LPS)][10] recent studies have been devoted to possible interactions between these two pathways. Several results showed the importance of N^{ω}-hydroxy-L-arginine (L-NOHA) in that context. L-NOHA, an intermediate in the NOS-dependent oxidation of L-arginine into L-citrulline and NO, was recently reported to act as a very good inhibitor of arginase, in fact the best one reported so far, displaying a K_i of *ca.* 100 μmol dm^{-3} on bovine and rat liver arginase.[11,12] This phenomenon may be important for the availability of arginine, and could modulate the production of NO and urea. In fact, rat alveolar macrophages exposed to LPS were found to generate up to 37 μmol dm^{-3} L-NOHA, suggesting that this compound may be a potent endogenous arginase inhibitor thus increasing the availability of L-arginine for NO biosynthesis.[13,14] The regulation of arginase and NOS by selective inhibitors may have considerable importance in therapy. Because of the dual role of L-NOHA on NOS and arginase, it seems particularly interesting to obtain analogues of this compound that may help to control selectively arginase and/or NOS, and may provide insight on the catalytic site of these two metalloenzymes. So far, only two analogues of L-NOHA have been reported in the literature; both of them are N^{G}-methyl-L-NOHA.[15,16]

This paper describes the synthesis of two novel analogues of L-NOHA, N^{ω}-hydroxy-D,L-indospicine **4a** (2,7-diamino-7-hydroxyiminoheptanoic acid), in which the δ-NH group of L-NOHA has been replaced by a CH_2 group, and *p*-hydroxyamidino-D,L-phenylalanine **4b**, in which the CH_2CH_2NH moiety of L-NOHA has been replaced by a more rigid aromatic ring. Both compounds are amino acids containing an amidoxime function instead of the *N*-hydroxyguanidine function of L-NOHA. Such changes of a δ-NH group into a CH_2 will modify the pK_a value of the new compounds (*ca.* 8 for *N*-hydroxyguanidines to *ca.* 6 for amidoximes)[17,18] and could modify the interactions between the amino acid and the protein (hydrogen bonding, ionic interactions). This paper also reports a preliminary comparison of the effects of these two compounds on arginase and NOS with those of L-NOHA **6** and N^{ω}-hydroxy-L-citrulline **8**, another analogue bearing a NOH group without basic properties.

Synthesis of the two analogues of L-NOHA (Scheme 1)

tert-Butoxycarbonylglycine (BOC-glycine) was chosen as starting material, and the nitriles **1a** and **1b** were prepared in



1-4a X = $(CH_2)_3$
1-4b X = *p*-C_6H_4

Scheme 1 *Reagents and conditions:* i, LDA, $BrCH_2XCN$, THF, −40 °C, 3 h; ii, Bu^{t}Br, benzyltriethylammonium chloride, K_2CO_3, dimethylacetamide, 55 °C, 22 h; iii, NH_2OH, EtOH; iv, HCl

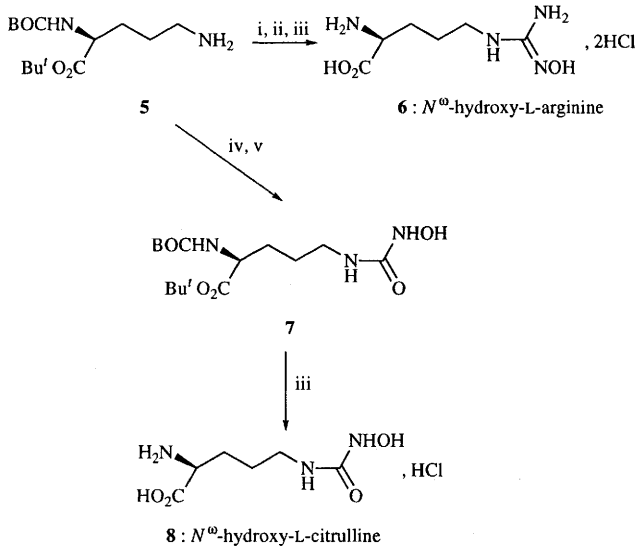
moderate yields according to the procedure of De Nicola *et al.*[19] Protection of the acidic moiety of **1a** and **1b** was then easily achieved (95% yield) using a previously described method.[20] Use of the *tert*-butyl ester of **1a** (and **1b**) was required in order to avoid further side reaction with NH_2OH, and this step was also a useful and easier way to purify the nitriles and separate them from residual glycine.

The amidoximes **3a** and **3b** were then prepared by addition of hydroxylamine to an ethanolic solution of the corresponding nitriles **2**. Reaction proceeded readily with **2b**, giving **3b** in 86% yield. However, the aliphatic amidoxime was more difficult to prepare. It is worth noting that, unlike aromatic amidoximes, very few aliphatic ones are described in the literature, and that yields are generally not given.[17] It was found here that the

best yields were obtained when an ethanolic solution of hydroxylamine (free base), prepared from $NH_2OH{\cdot}HCl$ and a slight excess of KOH, was used. Protected N^{ω}-hydroxy-D,L-indospicine was thus obtained in 34% yield together with 60% of recovered starting material which was recycled. When $NH_2OH{\cdot}HCl$ and $MeCO_2Na$ were used, yields decreased dramatically and a blue by-product was formed, which gave back the nitrile **2a** and the amidoxime **3a** in basic solution. It is probably a dimeric product according to its mass spectrum (CI, $MH^+ = 658$).

Hydrolysis of the protecting groups using anhydrous HCl afforded the desired products **4a** and **4b** as dihydrochloride salts.

L-NOHA and N^{ω}-hydroxy-L-citrulline were synthesized starting from N^{δ}-benzyloxycarbonyl-L-ornithine (N^{δ}-Z-L-orn, Scheme 2). L-NOHA was prepared by a modification of the



Scheme 2 *Reagents and conditions:* i, BrCN, AcONa, MeOH, room temp. 4 h; ii, NH_2OH, dioxane, 80 °C, 12 h; iii, HCl_g, dioxane, room temp. 48 h; iv, 1,1′-carbonyldiimidazole, $CHCl_3$, room temp., 4 h; v, NH_2OH, EtOH, room temp., 48 h

procedure of Wallace *et al.*,[21] in a 46% overall yield. N^{ω}-Hydroxy-L-citrulline was prepared from the same intermediate N^{α}-BOC-L-ornithine *tert*-butyl ester **5**, which was treated with carbonyldiimidazole. A nucleophilic displacement of imidazole with hydroxylamine gave compound **7** (89% yield). The protecting groups were then removed by acidic hydrolysis, affording **8** in 90% yield.

Biological results and discussion

Both N^{ω}-hydroxy-D,L-indospicine **4a** and *p*-hydroxyamidino-D,L-phenylalanine **4b** led to a clear concentration-dependent inhibition of purified bovine liver arginase. Their IC_{50} values (50 and 230 μmol dm^{-3} respectively) were markedly lower than that of L-valine, a classical arginase inhibitor[8] (IC_{50} *ca.* 1 mmol dm^{-3}). In fact, preliminary kinetic studies showed that **4a** exhibited a K_i value of 20 μmol dm^{-3} which is lower than that recently reported for L-NOHA.[11,12] Thus N^{ω}-hydroxy-D,L-indospicine **4a** appears to be the best arginase inhibitor reported so far. Interestingly enough, N^{ω}-hydroxy-L-citrulline **8** was a very poor inhibitor of arginase (Table 1). This indicates that the presence of a N-OH function as in L-NOHA, **4a** or **4b**, is not sufficient for a compound to be a good arginase inhibitor. A sufficient electron richness of this N-OH function also appears to be required.[18]

In contrast to their strong effects on arginase, compounds **4a** and **4b** seemed to exhibit very weak effects on NOS. Preliminary studies showed that 200 μmol dm^{-3} **4a** and **4b** failed to inhibit to a significant extent the oxidation of [^{14}C]-arginine to [^{14}C]-citrulline[22] by purified brain NOS.[23] Moreover, neither **4a** nor **4b** were able to act as substrates of brain NOS since they failed to produce significant amounts of NO (detected by visible spectroscopy from its reaction with hemoglobin)[24] upon reaction with purified brain NOS and NADPH.

Table 1 Inhibition of bovine liver arginase (BLA) by amino acids[a]

Compound	IC_{50} (μmol dm^{-3})
L-Valine	1000 ± 250
L-NOHA	100 ± 30
N^{ω}-Hydroxy-L-citrulline	5000 ± 500
N^{ω}-Hydroxy-D,L-indospicine	50 ± 10
p-Hydroxyamidino-D,L-phenylalanine	230 ± 50

[a] Reaction mixture for bovine liver arginase (about 250 U mg^{-1}, Sigma) contained in a final volume of 100 mm^3: 200 mmol dm^{-3} TrisHCl buffer pH 7.4, 20 mmol dm^{-3} L-arginine, 0.05 μCi L-[G-^{14}C]arginine and the appropriate concentration of inhibitor (from $IC_{50}/5$ to 5 IC_{50}). Reaction was initiated by adding about 1 μg of bovine liver arginase. Incubations were carried out at 37 °C for 10 min, and were stopped by the addition of 250 mm^3 of a cold solution of 7 mol dm^{-3} urea in 500 mmol dm^{-3} acetic acid; 200 mm^3 of a Dowex-H^+ solution (1/1 w/v) were added. After centrifugation, a 200 mm^3 aliquot was submitted to liquid scintillation counting, and the amount of [^{14}C]urea formed was calculated based on the specific activity of the [G-^{14}C]arginine used. IC_{50} mean values ± SD from 2 to 4 independent experiments.

Further studies are necessary to confirm these results and to extend them to other kinds of NOS. However, the aforementioned results showing the very different behaviour of the two analogues of L-NOHA, **4a** and **4b**, towards arginase and NOS, should be useful for future comparisons of the active sites of the two classes of enzymes that use L-arginine as substrate. Studies are in progress to obtain pure L- or D-amino acids **4a** and **4b** in order to determine the stereochemical requirements and to elucidate the mechanism by which they interact with arginase.

Experimental

General procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 250 spectrometer operating at 250 MHz. The solvent was used as the internal standard and *J* values are given in Hz. IR spectra were recorded on a Perkin-Elmer 783 spectrometer. Mass spectra were obtained on a Riber-Mag R10-10 C spectrometer. FAB mass spectra were recorded on a Nermag R10-10 spectrometer using thioglycerol as a matrix. Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. Elemental analyses were performed in the regional microanalytical laboratory of Paris VI University. Analytical thin layer chromatography was carried out on 0.25 mm pre-coated silica gel plates (Merck 60 F 254) and compounds were visualised using UV fluorescence and ninhydrin spray reagent. Flash chromatography was performed using Merck Kieselgel 60 (70–230 mesh ASTM) silica gel. Chemical reagents were obtained from Aldrich or Sigma, except if otherwise indicated, and were of analytical grade. Dioxane and diethyl ether (called ether) were distilled over sodium–benzophenone just before use.

Synthesis of N^{ω}-hydroxy-D,L-indospicine 4a

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-6-cyano-D,L-norleucine 1a.**[19] N^{α}-*tert*-Butoxycarbonylglycine (1.1 g, 6.3 mmol) dissolved in THF (15 cm^3) was added to a solution of lithium diisopropylamide (LDA; 20 mmol) in THF (10 cm^3) at 0 °C under argon. The mixture was stirred at 0 °C for 0.5 h and then cooled to −40 °C and treated with 5-bromopentanenitrile (5-bromovaleronitrile; Lancaster; 2.8 cm^3, 24 mmol). After being stirred for 1.5 h at −40 °C, the reaction mixture was diluted with saturated aqueous ammonium chloride (15 cm^3) to quench the reaction.

The mixture was acidified to pH 3.5 and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated and the residue was purified on a silica gel column using ethyl acetate–formic acid (100:1) as eluent to yield **1a** (1.4 g) contaminated with N^{α}-BOC-glycine. Compound **1a** was used without further purification; ν_{max}(neat)/cm^{-1} 2240 (CN); δ_H($CDCl_3$) 1.43 (9 H, s, Bu^t), 1.6–1.7 (6 H, m, 3 × CH_2), 2.35 (2 H, t, *J* 6.9, CH_2CN), 4.31 (1 H, m, CH) and 5.05 (1 H, m, NH).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-6-cyano-D,L-norleucine *tert*-butyl ester 2a.**[20] Compound **1a** (1.4 g, 4.3 mmol as determined by NMR) was dissolved in dimethylacetamide (45 cm^3) containing benzyltriethylammonium chloride (1.34 g, 5.9 mmol), K_2CO_3 (21.2 g, 153 mmol) and *tert*-butyl bromide (39 g, 285 mmol). The mixture was heated at 55 °C for 22 h after which it was treated with cold hydrochloric acid (0.05 mol dm^{-3}; 150 cm^3) to give a precipitate; this was extracted with ethyl acetate (× 3). The combined extracts were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the residue on a silica gel column (ethyl acetate–cyclohexane, 3:7) yielded a white solid (1.27 g; 63% for the two steps; protected glycine 28%) mp 55 °C (Found: C, 61.5; H, 8.7; N, 8.9. Calc. for $C_{16}H_{28}N_2O_4$: C, 61.5; H, 9.0; N, 9.0%); ν_{max}(KBr)/cm^{-1} 2240 (CN); δ_H($CDCl_3$) 1.42 (9 H, s, Bu^t), 1.45 (9 H, s, Bu^t), 1.6–1.8 (6 H, m, 3 × CH_2), 2.33 (2 H, t, *J* 7, CH_2CN), 4.14 (1 H, m, CH) and 5.02 (1 H, m, NH); δ_C($CDCl_3$) 17.6 (CH_2CN), 24.2, 24.9 (2 × CH_2), 27.1, 28.0 (2 × Bu$^tCH_3$), 32.1 (CH_2CH), 53.4 (CH), 79.7, 82.1 (2 × Bu^{t}C), 119.3 (C≡N), 155.3 (CO BOC) and 171.5 (CO ester); *m*/*z* (CI, NH_3) 313 (MH^+, 26%), 330 (93, M + NH_4^+), 274 (100, M − C_4H_8 + NH_4^+) and 257 (35, MH^+ − C_4H_8).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*$^{\omega}$-hydroxy-D,L-indospicine *tert*-butyl ester 3a.** Compound **2a** (1 g, 3.2 mmol) was mixed with ethanolic hydroxylamine (50 cm^3, 4 equiv.) and heated under reflux for 6 h. The mixture was then evaporated to remove the ethanol, diluted with ethyl acetate and thrice extracted with water acidified to pH 4.5 (acetic acid). The combined aqueous extracts were then basified to pH 11 by addition of ammonium hydroxide and extracted with ethyl acetate (× 3). The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (silica) using ethyl acetate–acetic acid (100:1) to afford compound **3a** as an oily compound (375 mg, 1.09 mmol, 34%) (Found: M^+, 345.226 37. $C_{16}H_{31}N_3O_5$ requires *M* 345.226 43); δ_H($CDCl_3$) 1.42 (9 H, s, Bu^t), 1.44 (9 H, s, Bu^t), 1.6–1.8 (6 H, m, 3 × CH_2), 2.14 (2 H, t, *J* 7.5, CH_2CN), 4.14 (1 H, m, CH), 4.60 (2 H, br s, NH_2) and 5.10 (1 H, m, NH); δ_C($CDCl_3$) 24.5, 25.9, 30.8, 32.4 (4 × CH_2), 27.9, 28.3 (2 × Bu$^tCH_3$), 53.6 (CH), 79.7, 81.9 (2 × Bu^{t}C), 153.6 (CO BOC), 155.5 (C=N) and 171.9 (CO ester); *m*/*z* (CI, NH_3) 346 (MH^+, 100%) and 330 (7, MH^+ − 16).

Elution of the first organic layer (ethyl acetate–cyclohexane, 1:1) yielded starting material **2a** (600 mg, 60 %) which could be recycled.

***N*$^{\omega}$-Hydroxy-D,L-indospicine 4a.** A solution of HCl in anhydrous dioxane (4 mol dm^{-3}; 3 cm^3) was added to **3a** (140 mg, 0.4 mmol) in dioxane (2 cm^3). The mixture was stirred at room temperature for 24 h and then diluted with dry ether to afford a precipitate which was collected by vacuum filtration. This was washed with acetone and dried *in vacuo* to give **4a** as a white solid (52 mg, 50%), R_F 0.37 (Pr^{i}OH–acetone–H_2O–AcOH, 2:2:2:1); δ_H(D_2O) 1.54 (2 H, m, 4-H_2), 1.78 (2 H, m, 5-H_2), 1.98 (2 H, m, 3-H_2), 2.53 (2 H, t, *J* 7.5, 6-H_2) and 3.97 (1 H, t, *J* 6, 2-H); δ_C(CD_3OD) 25.2, 27.3, 30.0, 31.1 (4 × CH_2), 54.7 (CH), 166.2 (C=N) and 174.0 (CO); *m*/*z* (FAB) 190 (MH^+, 100%), 174 (25, MH^+ − 16) and 111 [98, H_2N^+ = CH − $(CH_2)_4CN$]. An elemental analysis is not reported for **4a** because of its highly hygroscopic nature.

Synthesis of *p*-hydroxyamidino-D,L-phenylalanine 4b

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*p*-cyano-D,L-phenylalanine 1b.**[19] Compound **1b** was prepared as described for **1a** except for the following modifications: the mixture of BOC-glycine and LDA was added at −70 °C to a pre-cooled solution of 4-(bromomethyl)benzonitrile (2 equiv.) in THF. The temperature was allowed to reach −50 °C in 3 h, and the reaction was then quenched by addition of saturated aqueous ammonium chloride (50 cm^3) to the mixture. After extraction and work-up, the residue was chromatographed on silica gel and eluted with ethyl acetate–cyclohexane–acetic acid (50:50:1) followed by ethyl acetate–formic acid (100:1) to give material (850 mg) which contained 70% of the desired product (as determined by ^{1}H NMR) and was used without further purification; ν_{max}(neat)/cm^{-1} 2220 (CN); δ_H($CDCl_3$) 1.39 (9 H, s, Bu^t), 3.10 (1 H, m, CH_2), 3.27 (1 H, m, CH_2), 4.59 (1 H, m, CH), 5.04 (1 H, m, NH), 7.33 (2 H, d, *J* 8, ArH) and 7.62 (2 H, d, *J* 8, ArH).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*p*-cyano-D,L-phenylalanine *tert*-butyl ester 2b.**[20] Compound **2b** was prepared and purified from **1b** as described for **2a** to yield a white solid (450 mg, 17% for the two steps; protected glycine 35%), mp 100 °C; ν_{max}(KBr)/cm^{-1} 2240 (CN); δ_H([$^2H_6$]acetone) 1.35 (9 H, s, Bu^t), 1.40 (9 H, s, Bu^t), 3.05 (1 H, dd, *J* 9 and 13.5, 3-H), 3.20 (1 H, dd, *J* 5.6 and 13.5, 3-H), 4.31 (1 H, m, 2-H), 6.13 (1 H, d, *J* 7, NH), 7.49 (2 H, d, *J* 8, ArH) and 7.69 (2 H, d, *J* 8, ArH); δ_C($CDCl_3$) 27.9, 28.2 (2 × Bu$^tCH_3$), 39.0 (CH_2), 54.5 (CH), 80.0, 83.0 (2 × Bu^{t}C), 111.0 (*p*-C), 119.0 (C≡N), 130.0 (*o*-C), 132 (*m*-C), 142.0 (C_q), 155.0 (CO BOC) and 170.3 (CO ester); *m*/*z* (CI, NH_3) 347 (MH^+, 100%).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*p*-hydroxyamidino-D,L-phenylalanine *tert*-butyl ester 3b.** NH_2OH·HCl (1.16 g, 16.6 mmol) and Na_2CO_3 (880 mg, 8.3 mmol) were added to a solution of compound **2b** (1.15 g, 3.34 mmol) in ethanol (40 cm^3) and the mixture was refluxed for 2 h, stirred for 2 h at room temperature and then concentrated. The residue was dissolved in ethyl acetate and water, and the mixture was extracted with ethyl acetate (× 3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a crude solid which was recrystallised from ethanol (1.08 g, 86%), mp 184 °C (Found: C, 60.1; H, 7.7; N, 11.0. Calc. for $C_{19}H_{29}N_3O_5$: C, 60.1; H, 7.7; N, 11.1%); ν_{max}(KBr)/cm^{-1} 1640 (C=N); δ_H([$^2H_6$]-acetone) 1.36 (9 H, s, Bu^t), 1.40 (9 H, s, Bu^t), 2.99 (1 H, dd, *J* 8.4 and 13.5, 3-H), 3.10 (1 H, dd, *J* 5.9 and 13.5, 3-H), 4.27 (1 H, m, 2-H), 5.42 (2 H, br s, NH_2), 5.97 (1 H, d, *J* 8, NH), 7.25 (2 H, d, *J* 8, ArH), 7.64 (2 H, d, *J* 8, ArH) and 8.82 (br s, OH); δ_C($CDCl_3$) 27.7, 28.0 (2 × Bu$^tCH_3$), 37.9 (CH_2), 54.6 (CH), 79.8, 82.3 (2 × Bu^{t}C), 125.8, 129.5 (*o*-C and *m*-C), 131.0 (*p*-C), 138.3 (C_{Ar}), 153.0 (C=N) 155.2 (CO BOC) and 170.8 (CO ester); *m*/*z* (CI, NH_3) 380 (MH^+, 100%) and 397 (4, M + NH_4^+).

***p*-Hydroxyamidino-D,L-phenylalanine 4b.** A solution of compound **3b** (200 mg, 0.53 mmol) in hydrochloric acid (5 mol dm^{-3}, 3 cm^3) was stirred at room temperature for 24 h and then evaporated under reduced pressure to yield a white solid, which was washed with acetone and ether (150 mg, 95%), R_F 0.45 (Pr^{i}OH–NH_3, 7:3); δ_H(D_2O) 3.37 (1 H, dd, *J* 7.2 and 14.5, 3-H), 3.47 (1 H, dd, *J* 6 and 14.5, 3-H), 4.40 (1 H, t, *J* 6.8, 2-H), 7.57 (2 H, d, *J* 8.4, ArH) and 7.75 (2 H, d, *J* 8.4, ArH); δ_C(CD_3OD) 37.0 (CH_2), 54.7 (CH), 125.9 (*p*-C), 129.4, 131.8 (*o*-C and *m*-C), 142.3 (C_{Ar}), 162.3 (C=N), 170.7 (CO); *m*/*z* (FAB) 224 (MH^+, 65%), 208 (20, MH^+ − 16) and 109 (100). An elemental analysis is not reported for **4b** because of its highly hygroscopic nature.

N$^{\omega}$-Hydroxy-L-arginine 6

Compound **6** was prepared in 6 steps starting from N^{δ}-Z-L-ornithine by a modification of literature procedures.[21,25] N^{α}-BOC-L-ornithine *tert*-butyl ester **5** was prepared in three steps starting from N^{δ}-Z-ornithine by a modification of a literature method: N^{δ}-Z-L-Orn was converted into N^{α}-BOC-N^{δ}-Z-L-Orn as previously described[25] and subsequently transformed into N^{α}-BOC-N^{δ}-Z-L-Orn-CO_2Bu^t as described for **2a**.[20] This compound was then hydrogenated[25] to give **5** in a 97% overall yield.

Compound **5** was converted into N^{α}-BOC-N^{ω}-hydroxy-L-arginine *tert*-butyl ester as described by Wallace *et al.*;[21] this crude compound was purified on a silica gel column by elution with ethyl acetate–ethanol–acetic acid (70:30:1), and deprotected as described by Feldman.[25] This yielded N^{ω}-hydroxy-L-arginine in a 46% overall yield. This compound displayed physicochemical characteristics identical with those previously published.[21,25]

Synthesis of N^{ω}-hydroxy-L-citrulline 8

***N^{α}-tert*-butoxycarbonyl-N^{ω}-hydroxy-L-citrulline *tert*-butyl ester 7.** Carbonyldiimidazole (350 mg, 1.8 mmol) in chloroform (3 cm^3) was added to a solution of N^{α}-*tert*-butoxycarbonyl-L-ornithine *tert*-butyl ester **5** (400 mg, 1.4 mmol) in $CHCl_3$ (5 cm^3) at 0 °C. A further equivalent of carbonyldiimidazole was added after 2 h to the mixture which was then stirred for 2 h at room temperature. The mixture was washed with water, dried ($MgSO_4$) and concentrated, and the residue was rapidly purified by flash chromatography (silica gel). Elution with ethyl acetate provided the protected N^{δ}-carbonylimidazoyl-L-ornithine (530 mg, 99%) which, dissolved in methanol (2 cm^3) was treated with ethanolic hydroxylamine (10 cm^3, 5 equiv.). The mixture was stirred at room temperature for 48 h, after which the ethanol was removed by evaporation and the residue diluted with water and extracted with ethyl acetate (× 3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure, and the residue was purified on a silica gel column (elution with ethyl acetate) to yield **7** as an oily compound (420 mg, 89%); $\delta_H(CDCl_3)$ 1.38 (9 H, s, Bu^t), 1.40 (9 H, s, Bu^t), 1.5–1.8 (4 H, m, 2 × CH_2), 3.20 (2 H, m, CH_2N), 4.10 (1 H, m, CH), 5.28 (1 H, d, *J* 7.8, N*H*CH), 6.1 (1 H, br s, N*H*CH_2), 7.3 (1 H, s, N*H*OH) and 8.40 (br s, OH); $\delta_C(CDCl_3)$ 25.9, 29.9 (2 × CH_2), 27.9, 28.2 (2 × Bu^tCH_3), 39.0 (CH_2NH), 53.8 (CH), 79.8, 82.0 (2 × Bu^tC), 155.6 (CO BOC), 162.4 (CONHOH) and 171.9 (CO ester); *m/z* (CI, NH_3) 348 (MH^+, 75%), 365 (8, M + NH_4^+), 332 (32, MH^+ − 16) and 315 (32, M − NHOH).

***N^{ω}*-Hydroxy-L-citrulline 8.** Compound **8** was obtained as an hygroscopic precipitate from **7** as described for **4a** (210 mg, 90%), R_F 0.60 (Pr^iOH–acetone–H_2O–AcOH 2:2:2:1);[26] $\delta_H(D_2O)$ 1.73 (2 H, m, CH_2), 2.10 (2 H, m, CH_2), 3.30 (2 H, t, *J* 6.8, CH_2NH) and 4.13 (1 H, t, *J* 6, CH); $\delta_C(CD_3OD)$ 26.6, 28.6 (2 × CH_2), 40.1 (CH_2NH), 53.5 (CH), 163.8 (CO) and 171.6 (CO_2).

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