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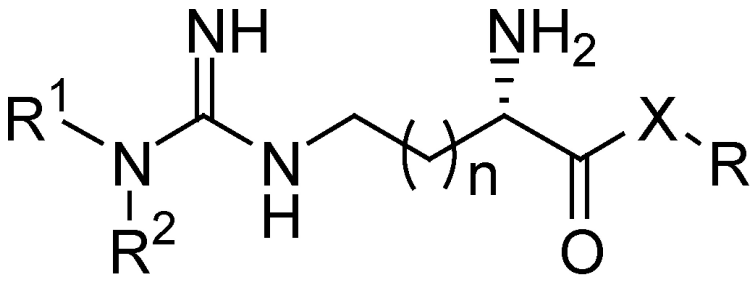

n = 1, 2 X = O, N

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Selective Substrate-Based Inhibitors of Mammalian Dimethylarginine Dimethylaminohydrolase

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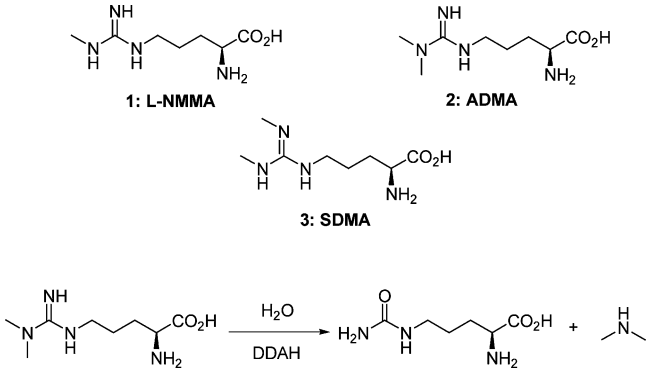
The enzyme DDAH metabolizes methylarginines that are inhibitors of nitric oxide synthase (NOS). Substrate-based inhibitors of mammalian DDAH have been synthesized, with optimization to give selective inhibition of DDAH with no significant direct effect on NOSs. These are the first examples of reversible DDAH inhibitors with significant activity and selectivity. In vivo administration increases plasma ADMA levels, giving proof of concept that these inhibitors can be used to probe the physiological effects of DDAH inhibition, with potential for pharmaceutical use of DDAH inhibitors in diseases where excess NO production is implicated.

Introduction

Endogenous methylarginines L-*N*-methylarginine (L-NMMA) **1** and asymmetric dimethylarginine (ADMA) **2**, which are released by degradation of proteins containing methylated arginine residues, are inhibitors of all three isoforms of nitric oxide synthase (NOS). The enzyme dimethylarginine dimethylaminohydrolase (DDAH) metabolizes these two NOS-inhibiting methylarginines, but not the related symmetric dimethylarginine (SDMA) **3**, which is physiologically inactive.[1] The enzyme converts the substrate to citrulline and either methylamine (substrate L-NMMA) or dimethylamine (substrate ADMA) (Chart 1).

Chart 1. Methylarginines and the Hydrolysis Catalyzed by DDAH



Bacterial and mammalian forms of DDAH are thought to share a common catalytic triad in the active site: a histidine and an acidic residue are involved in the binding and/or activation of the guanidine functionality, while a cysteine is proposed to perform the initial attack on the guanidino carbon leading to the hydrolysis.[2] To date, two isoforms of DDAH sharing 62% homology, termed I and II, have been identified in mammals.[3] DDAH I is expressed in brain, liver, kidney, skeletal muscle, and pancreas; therefore, its distribution correlates generally with that of neuronal NOS. DDAH II has a more widespread distribution, with highest expression in the heart, placenta, and kidney, but is barely detectable in the brain; this loosely correlates with the distribution of endothelial NOS.[3] The conservation of DDAH throughout evolution and the tissue distributions in mammals suggest that the active metabolism of methylarginines is of considerable biological importance. DDAH activity is the major pathway for ADMA elimination such that an estimated 250 μmol/day is metabolized, with ca. 50 μmol/day excreted by the kidneys. Thus, DDAH activity is responsible for the regulation of ADMA concentrations within cells.[4,5] Elevated plasma levels of ADMA have been reported in a number of disease states associated with deficient NO production, for example, atherosclerosis and hypertension,[6] although whether the increase in ADMA can be attributed to insufficient DDAH expression or activity is not yet clear. Conversely, it is also apparent that methylarginines that inhibit NOS have the potential to produce beneficial effects through limitation of excess NO production. For example, endogenous methylarginines may protect against neuroexcitotoxic injury by regulating neuronal NOS and might limit excess NO production by macrophages.[7,8] Exogenous L-NMMA **1** has been shown to reverse hypotension of septic shock in animals and humans, prevent inflammation, reduce pain perception, and treat headache.[9,10] There is a continuing search for effective treatments for a wide range of diseases where excess NOS activity is involved.[9,10] Thus there may be a beneficial role for inhibition of DDAH in these disease states, as attenuation of NO production, rather than complete inhibition of NOS, may be viewed as a desirable therapeutic strategy. DDAH is also potentially a direct therapeutic target; for example,

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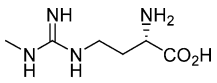
DDAH I overexpression has been shown to enhance tumor growth and angiogenesis.[11]

We envisage a role for inhibitors of DDAH as useful tools to study the relevant biological pathways, and as potential therapeutic entities. To this end our aim is to devise selective inhibitors of mammalian DDAH.

Inhibitor Design and Synthesis. In an earlier study,[12a] screening of a small number of ADMA-like compounds known to be poor NOS inhibitors (obtained from Wellcome, Beckenham, UK) identified 2-amino-4-(*N*^{G}-methyl-guanidino)butanoic acid, 4124W, **4**, as a weak (millimolar) inhibitor of DDAH that, despite this weak inhibition, has been shown to have effects in functional studies.[12,13] This compound is a chain-shortened analogue of L-NMMA, but is a very poor NOS inhibitor. To date this is the only known reversible inhibitor of DDAH. Structure similarity searches yielded a number of potential non-substrate-like leads, but these did not give consistent inhibition data; therefore, despite its low potency, 4124W was taken as our first point of reference for reversible inhibitor design. The structure of a bacterial DDAH is known and has some homology with mammalian enzymes in the active site region;[2,3] however, there is low homology in the overall sequence, and effectively modeling the mammalian DDAH structure proved difficult. Given the proposed mechanism of the enzyme, it seemed logical that changes around the critical ADMA guanidine moiety had potential for disrupting the hydrolysis mechanism and improving upon the inhibition of **4**. A series of analogues of **4** was designed, maintaining the shorter chain length and varying the guanidine substituents and the amino acid moiety. Historically, such amino acids were synthesized by direct modification of the parent amino acid, but this approach creates problems with often long and difficult purification.[14] We therefore decided that our library would be prepared via protected intermediates to avoid the need for purification of polar guanidino amino acids, in a similar strategy to that used for the preparation of substituted arginines.[15]



4, 4124W

Compounds were synthesized from *N*,*N*′-bis-*tert*-butoxycarbonylpyrazole-1-carboxamidine **5** by N-substitution under Mitsunobu conditions with the appropriate alcohol, followed by pyrazole displacement with an amine.[16] All substrates were appropriately protected to facilitate purification of the intermediates. Deprotection under acid conditions was effected in the final step, to give the pure amino acids, removing the need for purification by chromatographic methods.

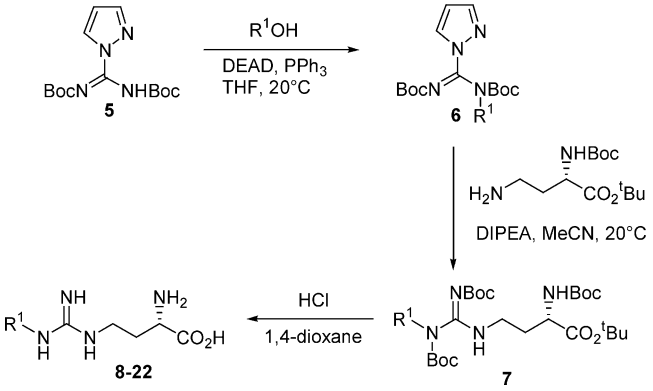
Monosubstituted *N*-alkylguanidinobutyric acids were synthesized by reaction of **5** with an alcohol under Mitsunobu conditions to give the N-alkylated species **6**, and then reaction with Boc-2,4-diaminobutyric acid *tert*-butyl ester to give the protected guanidine amino acid **7**, followed by deprotection with HCl/dioxane or TFA/CH_2Cl_2 to give the appropriate salt **8**−**22** (Scheme 1). The salts were hygroscopic white solids.

N,N-Disubstituted and *N*-arylguanidines (plus those substituents where the corresponding alcohol was not available or was unreactive in the Mitsunobu reaction) were obtained by reaction of Boc-homoserine-*tert*-butyl ester with the pyrazole carboxamidine under Mitsunobu conditions, followed by pyrazole displacement with the appropriate amine and subsequent deprotection to give the products **23**−**29** (Scheme 2).

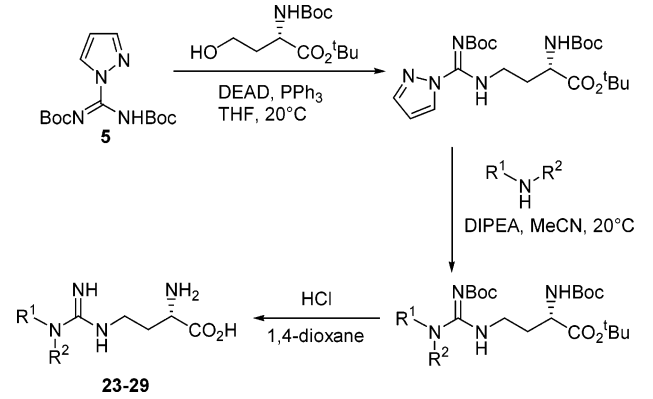
Esters **30**−**35** were prepared from the amino acid by heating with the appropriate alcohol and thionyl chloride (Scheme 3).

α-Amino amides were prepared from the appropriate Boc/Fmoc protected diamino acid via the mixed anhydride, followed by Fmoc deprotection and subsequent steps as for the monosubstituted amino acids (Scheme 4).

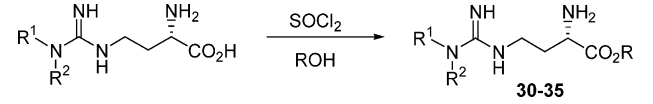
Scheme 1. Synthesis of *N*^{G}-Monosubstituted Analogues



Scheme 2. Synthesis of *N*^{G}-Disubstituted Analogues



Scheme 3. Synthesis of Esters



Scheme 4. Synthesis of Amides

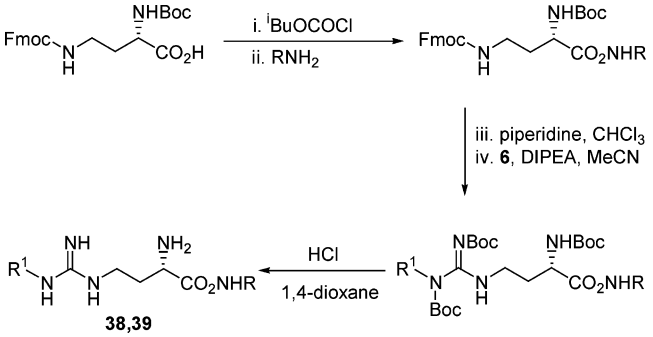


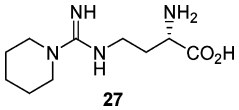
Table 1. Selected N^G-Substituted 2-Amino-4-guanidinobutanoic Acids with IC_{50} of 300 μM or Lower

ref	R^1	R^2	inhibition at 1 mM, %	IC_{50}, μM
4	Me	H	48	1510
9	Et	H	100	300
15	2-methoxyethyl	H	90	189
17	2-isopropoxyethyl	H	97	301
25	Me	Me	85	325
27	-piperidinyl-		74	264

DDAH Inhibition. The compounds were assayed for inhibition of mammalian DDAH in rat kidney homogenate, which contains both isoforms of the mammalian DDAH enzyme. Owing to the weak inhibitory activity of our lead **4**, initial testing of analogues was carried out at 1 mM inhibitor concentration. The IC_{50} was determined for any compound with significant inhibition at 1 mM.

The primary inhibitor series consisted of analogues of **4** substituted on the terminal guanidine nitrogen. Both N^G-monosubstituted (corresponding to L-NMMA) and N^G,N^G-disubstituted (corresponding to ADMA) analogues were prepared.

Although direct design from the bacterial enzyme was impractical, it seemed likely that the binding site would be compact, with space around the guanidine group limited. Accordingly a small diverse selection of guanidine substituents was chosen for the initial series of analogues. Consistent with this hypothesis, of this first series of monosubstituted analogues, small alkyl groups appeared to be well tolerated, with some improvement on the activity of **4**, whereas longer chain, bulky or aryl groups did not enhance activity compared to **4**. Short chain alkyl groups containing a heteroatom, with potential for interaction with polar residues, were also investigated. The *N*-2-methoxyethyl substituent (compound **15**) gave the best DDAH inhibition (IC_{50} 189 μM) of all the N-substituted analogues. The related 2-isopropoxyethyl analogue **17** also had promising activity; whereas the 2-phenoxyethyl, 2-methylthioethyl, and dimethylaminoethyl analogues had much reduced activity. N,N-Disubstituted analogues were explored briefly, with the cyclic analogue **27** having highest activity (IC_{50}



27

264 μM) of this class. The N^G,N^G-dimethyl analogue **25**, the corresponding chain-shortened version of ADMA, was also of reasonable potency, but inhibited eNOS in a preliminary screen. Inhibitors from this series with IC_{50} of ~350 μM or below are shown in Table 1.

The *N*-2-methoxyethyl moiety was conserved for incorporation into other analogues. From this stage onward, compounds were tested at an initial concentration of 100 μM, as we had achieved improvement in activity of an order of magnitude compared to our original lead **4**. We then focused on changes to the amino acid moiety.

Esters of **15** were prepared (Table 2). Of these, the benzyl ester **34** was the most active (IC_{50} 27 μM), with the methyl and *n*-propyl esters **30**, **32** also exhibiting

Table 2. Inhibition Data for Esters of **15**

ref	R	inhibition at 100 μM, %	IC_{50}, μM
30	Me	75	96
31	Et	44	159
32	n-Pr	64	111
33	n-Bu	40	113
34	benzyl	96	27
35	i-Pr	30	189

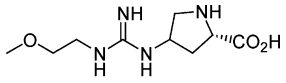
Table 3. Inhibition Data for Arginine Derivatives

ref		inhibition at 100 μM, %	IC_{50}, μM
36	N^G-(2-methoxyethyl) arginine	100	22
37	N^G-(2-methoxyethyl) arginine methyl ester	83	20

significantly improved activity over the parent acid (IC_{50} of approximately 100 μM). The activity of the benzyl ester may be indicative of hydrophobic interactions with nonpolar residues in the active site.

The potential of employing the corresponding substituted arginines as substrate-based inhibitors was considered, despite the risk of losing selectivity of the compounds against DDAH versus NOS. Much early research on NOS inhibitors was carried out on substituted arginines, but only a handful of N^G-alkylarginines are reported.[9,17] We hypothesized that N^G-2-methoxyethylarginine **36** might not be a significant inhibitor of NOS, and so it was prepared by the same method, from protected ornithine instead of the 2,4-diaminobutanoic acid derivative, and found to be a more potent inhibitor of mammalian DDAH (IC_{50} 22 μM). The corresponding methyl ester **37** was of similar inhibitory activity (IC_{50} 20 μM) (Table 3). No improvement in activity was seen for the benzyl ester, which suffered from solubility difficulties (not shown). This raises the possibility that there are significant differences between the modes of binding of the shorter chain analogues and of substituted arginines (including the substrates).

Amides **38** and **39** corresponding to the potent inhibitors **34** and **37** were prepared, but showed much reduced inhibition. More fundamental structural changes, such as removal of the 2-amino or carboxylic acid group, led to much reduced activity, as was also the case for *(R)*-isomers of the most active compounds, and a cyclic analogue **44**, synthesized from hydroxyproline.



44

Enzyme Selectivity. To assess toxicity of the inhibitors, RAW cells were treated with DDAH inhibitor (0.5 mM) in complete media for 24 h, and cells were subsequently assessed for viability by MTT optical density assay. There was no significant difference between untreated cells and those exposed to DDAH inhibitors (Figure 1).

As we are interested in the role of DDAH in endothelial dysfunction and inflammatory disease models, we required our inhibitors to have no direct effect on the relevant NOS isoforms. The most potent inhibitors of DDAH (IC_{50} < 100 μM approximately) were therefore tested for inhibition of constitutive endothelial NOS

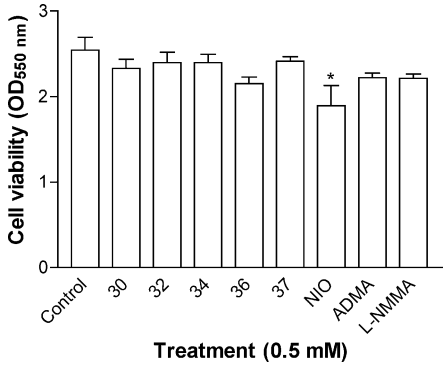


Figure 1. Effect of DDAH inhibitors on cell viability.

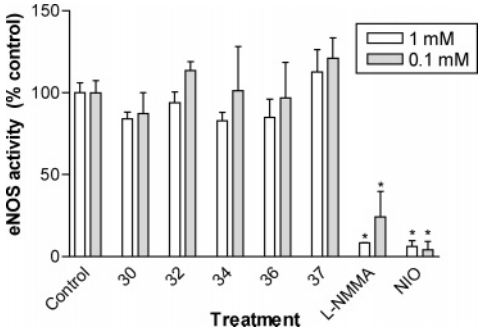


Figure 2. Effect of DDAH inhibitors on eNOS activity.

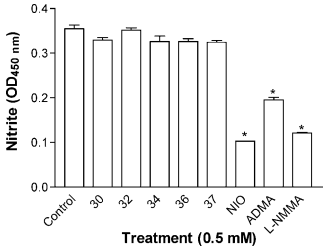


Figure 3. Effect of DDAH inhibitors on iNOS (measured via nitrite levels) in LPS-stimulated RAW cells.

(eNOS) and inducible NOS (iNOS) with NOS inhibitors L-NMMA, ADMA, and NIO (L-*N*-5-(1-iminoethyl)ornithine) used for comparison.

The effect of inhibitors on eNOS activity was determined by measuring the conversion of [^{14}C]arginine to [^{14}C]citrulline by human recombinant eNOS in the presence of: necessary cofactors and 100 μM or 1 mM inhibitor ($n = 4$). There was no significant inhibition of eNOS at 100 μM or 1 mM compared to control (Figure 2).

For assay of iNOS inhibition, RAW cells were stimulated with cytokines for 24 h in the presence of the DDAH inhibitors (0.5 mM). Levels of nitrite secreted into the media by the cells were determined by Griess assay ($n = 10$). There was no significant difference between untreated cells and those exposed to DDAH inhibitors (Figure 3).

Although selectivity versus the third NOS isoform, neuronal NOS (nNOS), was not an absolute requirement for preliminary studies, it is ultimately desirable. Activity against neuronal NOS (nNOS) was assessed by measuring NOS activity in rat brain lysate in the presence of inhibitors. In this case, there was significant

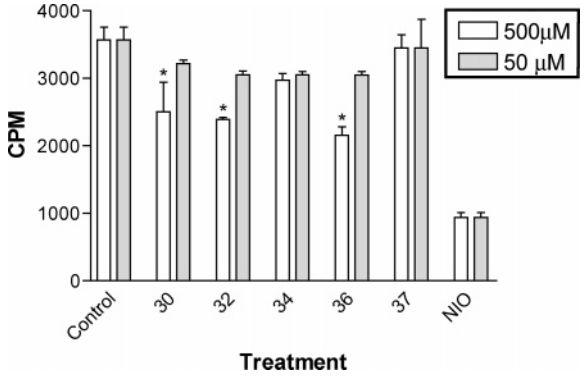


Figure 4. Effect of inhibitors on nNOS activity in rat brain.

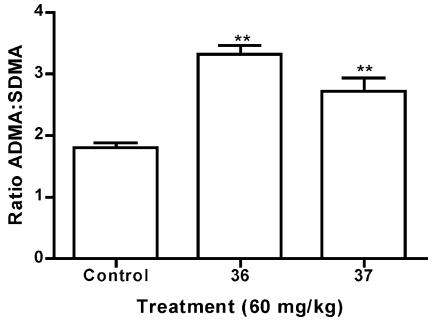


Figure 5. Effect of DDAH inhibitors on ADMA/SDMA ratios in vivo at 4 h.

inhibition at 500 μM for some of the DDAH inhibitors, but no significant inhibition at 50 μM (Figure 4).

In Vivo Proof of Concept. On the basis of solubility and potency, coupled with acceptable selectivity versus endothelial NOS, two of these inhibitors, **36** and **37**, were chosen for preliminary testing to determine if inhibition of DDAH occurred in vivo.

Rats were treated with saline as control or with 60 mg/kg of inhibitor as a bolus intravenous injection. Blood ADMA and SDMA levels were measured after 4 h.[19] SDMA levels were broadly similar for control and treated animals, as would be expected because SDMA is not metabolized by DDAH. ADMA levels in inhibitor-treated animals were raised relative to controls (in the case of **36**, [ADMA] $\sim$ 1.2 μM, vs $\sim$0.8 μM in controls). The mean ADMA/SDMA ratio (a good marker for the efficiency of DDAH activity) in inhibitor-treated animals was significantly higher than that in control animals for both inhibitors, ($P < 0.001$). The results are summarized in Figure 5 (full data is available in the Supporting Information).

For a more detailed picture of the action of the inhibitor, time course studies were carried out with **37**. A single bolus intravenous dose (60 mg/kg) was given at time zero, and ADMA and SDMA concentrations were measured at time points up to 5 h. The ADMA levels in inhibitor-treated animals rose within 30 min, reaching a peak at around 2 h, followed by a gradual decline. ADMA levels were still significantly higher at 5 h compared to control (Figure 6).

The preliminary in vivo results confirm the potential for use of DDAH inhibitors to explore the significance of this enzyme. Compound synthesis on multigram scale has been carried out for further functional studies and

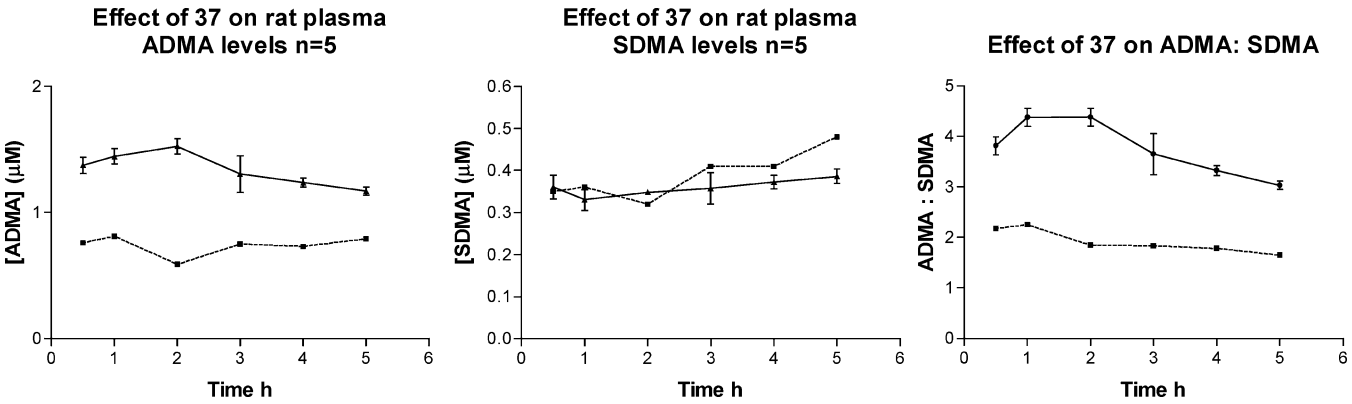


Figure 6. Time-course studies of ADMA and SDMA levels in control and inhibitor-treated rats. **37** (—); saline (·····).

basic in vivo disease models, in tandem with continued inhibitor optimization.

Conclusion

Selective substrate-based inhibitors of mammalian DDAH based on the weak millimolar inhibitor **4** have been prepared. Significant improvements on the lead have been achieved, yielding three compounds with IC_{50} below 30 μM, which are not cytotoxic and show selectivity versus nitric oxide synthases. In particular, **37** has excellent selectivity and could be used for studies where avoiding reduced nNOS activity would be critical. Proof of concept has been demonstrated in vivo; administration of inhibitors **36** and **37** causes a rise in plasma ADMA levels and ADMA/SDMA ratio, which can be sustained over several hours. The results presented here are already of biological interest since they demonstrate that plasma concentrations of ADMA are regulated by DDAH in vivo and that inhibition of DDAH causes a rapid rise in ADMA levels. The inhibitors can now be used as valuable pharmacological tools for investigating the role of DDAH in controlling levels of circulating or tissue methylarginines and exploring pathophysiological associations between ADMA levels and disease states. There is also potential for treatment of diseases where excess DDAH or NOS activity is involved.

Experimental Section

Biology. DDAH Activity Assay. Rat kidney was homogenized in PBS containing PMSF 1 mM, leupeptin 5 μg/mL, pepstatin 5 μg/mL, chymostatin 5 μg/mL, and the lysate was centrifuged (4000 rpm, 20 min; then supernatant further centrifuged for 40000 rpm, 30 min, 4 °C). The supernatants were analyzed for DDAH activity; [^{14}C]L-NMMA (1 μmol/L) was added to the cell lysates (final volume 100 μL) and incubated for 1 h at 37 °C. Dowex resin (0.5 mL) at neutral pH was immediately added to samples to terminate the reaction and bind unmetabolized L-NMMA. [^{14}C]Citrulline formation was determined by scintillation counting (Hewlett-Packard).

eNOS Assay. Compounds to be tested were added to NOS assay buffer (HEPES 50 mM pH 7.2, FAD 5 μM, FMN 5 μM, BH_4 10 μM, NADPH 1mM, DTT 0.5 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, calmodulin 50 nM, [^{14}C]arginine 100 μM) in a final volume of 100 μL, and the reaction was initiated by addition of NOS (eNOS 3.3. U/reaction, Alexis Biochemicals). Reactions were incubated for 1 h at 37 °C, and the reaction was terminated by the addition of Dowex pH 5.5 (0.85 mL) to each tube. Tubes were vortexed and microfuged <12000*g*, 5 min. Then 100 μL of the Dowex supernatant was added to 5 mL of scintillation cocktail and was counted in a scintillation counter (Hewlett-Packard).

Cell Viability Assay. RAW cells were grown to 70% confluence in T_{150} flasks and then stimulated with LPS (5 μg/mL), TNFα (10 ng/mL) and IFNγ (100 U/mL) for 24 h. Cell culture media was removed from cells and replaced with 200 μL complete media containing 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT 0.2 mg/mL) for 30 min at 37 °C. MTT containing media was removed and 100 μL of DMSO was added to solubilize cells. After shaking, plates were read at OD_{550} nm.

Greiss Assay for iNOS. RAW cells were stimulated with cytokines, as described above, in the presence of DDAH inhibitors for 24 h. Greiss reagent [1:1 sulfanilamide (10%) in orthophosphoric acid (50%) and *N*-(1-naphthyl)ethylenediamine dihydrochloride (1%)] was added as 100 μL volume to 100 μL cell culture media, and the plate was read at 550 nm and analyzed in relation to sodium nitrite and nitrate standards.

nNOS Assay. Rat brains were removed and immediately homogenized in PBS containing: dithiothreitol (1 mM), EDTA (1 mM), phenylmethylsulfonyl fluoride (1 mM), leupeptin (5 μg/mL), pepstatin A (5 μg/mL), and chymostatin (5 μg/mL) and centrifuged at 46000 rpm for 30 min at 4 °C. Compounds to be tested were added to NOS assay buffer (HEPES 50 mM pH 7.2, FAD 5 μM, FMN 5 μM, BH4 10 μM, NADPH 1 mM, DTT 0.5 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, calmodulin 50 nM, [^{14}C]arginine 100 μM) in a final volume of 100 μL, and the reaction was initiated by addition of brain lysate (50 μL/reaction). Reactions were incubated for 1 h at 37 °C, the reaction was terminated by the addition of Dowex pH 5.5 (0.85 mL) to each tube, and tubes were vortexed and microfuged <12000 g, 5 min. Then 100 μL of the Dowex supernatant was added to 5 mL scintillation cocktail and was counted in a scintillation counter (Hewlett-Packard).

In Vivo Studies. All animal experiments were performed under Home Office approval according to the Animals Scientific Procedures Act 1986. Male Kyoto Wistar rats (250−330 g) were given free access to food and water until the time of surgery.

Rats were maintained at 36−37 °C on a heating blanket and anaesthetized, via a mask, with 5% isoflurane in air and allowed to remain spontaneously breathing on 0.8−2% isoflurane adjusted according to the degree of surgical stimulation. Animals were instrumented with a bladder drainage cannula and venous and arterial cannulae, and anaesthesia was maintained via a tracheostomy, as previously described.[18]

Single Timepoint Experiments. Animals were injected intravenously with a bolus dose of inhibitor (60 mg/kg) or saline. Anaesthesia was maintained for 4 h at the end of which blood was obtained via cardiac puncture for the determination of serum dimethylarginine levels.

A 200 μL amount of serum was added to 800 μL of PBS containing 40 ng of L-NMMA as an internal standard. The samples were then extracted using Oasis MCX Solid-Phase Extraction (SPE) columns (Waters, 1 cm^3/30 mg 30 μm). The columns were washed with 100 mM HCl and 100% methanol and then eluted with concentrated ammonia/water/methanol

(10/40/50). The eluent was evaporated to dryness under nitrogen at 80 °C. The resultant pellet was resuspended in 100 μL of deionized water and transferred into HPLC autosampler vials (Agilent). ADMA and SDMA concentrations were measured using the MS:MS method developed by Dalton and Turner.[18]

Time Course Experiment in Anaesthetised Spontaneously Breathing Rat Model. Animals were given a background saline infusion via the venous cannula throughout and allowed to stabilize for 30 min prior to administration of either 60 mg/kg inhibitor or saline via the same cannula. Blood samples (0.8 mL) were taken via the arterial cannula at 30 min, 1, 2, 3, 4, and 5 h, respectively, and centrifuged (10000*g*; 4 °C), and the supernatant (serum) was removed and snap frozen immediately in liquid nitrogen. Serum samples were subsequently analyzed for ADMA and SDMA concentrations by both MS:MS and HPLC.[19,20]

Synthesis of Inhibitors. General. All starting materials were either commercially available or reported previously in the literature unless noted. Solvents and reagents were used without further purification. Reactions were monitored by TLC on precoated silica gel plates (Kieselgel 60 F_{254}, Merck). Purification was performed by flash chromatography using silica gel (particle size 40−63 μM, Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 or Bruker AMX-400 spectrometer. Chemical shifts are reported as ppm (δ) relative to TMS as an internal standard. Mass spectra were recorded on either a VG ZAB SE spectrometer (electron impact and FAB) or a Micromass Quattro electrospray LC-mass spectrometer. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. All yields reported in the Experimental Section are nonoptimized, isolated yields.

Method A: General Method for Synthesis of N^G-Monosubstituted Guanidine Amino Acids. Diethyl azodicarboxylate (3 mmol) was added dropwise to a solution of *N*,*N*′-bis-*tert*-butoxycarbonylpyrazole-1-carboxamidine (2 mmol), triphenylphosphine (3 mmol), and the appropriate alcohol (2 mmol) at 0 °C, with stirring. The mixture was then stirred at room temperature for 3−16 h, concentrated on the rotary evaporator, and then subjected to flash column chromatography (10% ethyl acetate/cyclohexane) to give the product as a colorless oil. The *N*-alkyl-substituted pyrazolecarboxamidine (1.5 mmol), Boc-diaminoalkanoic acid *tert*-butyl ester (1.5 mmol), and diisopropylethylamine (2.0 mmol) were stirred in acetonitrile (10 mL) for 24 h. The mixture was concentrated on the rotary evaporator and then subjected to flash column chromatography (20−30% ethyl acetate/cyclohexane) to give the protected guanidino-amino acid as a colorless gum. The protected amino acid was stirred in excess 4 M hydrogen chloride/dioxane for 24−72 h. Removal of solvent and byproducts in vacuo gave the amino acid as a white, very hygroscopic solid.

Method B: General Method for Synthesis of N^G-Disubstituted 2-Amino-4-guanidinobutanoic Acids and N^G-Aryl-2-amino-4-guanidinobutanoic Acids. Diethyl azodicarboxylate (3 mmol) was added dropwise to a solution of *N*,*N*′-bis-*tert*-butoxycarbonylpyrazole-1-carboxamidine (2 mmol), triphenylphosphine (3 mmol), and *N*-Boc-homoserine-*tert*-butyl ester (2 mmol) (prepared from the acid by a published method[21]) at 0 °C, with stirring. The mixture was stirred at room temperature for 3−16 h, concentrated on the rotary evaporator, and then subjected to flash column chromatography (20% ethyl acetate/cyclohexane) to give the product as a colorless oil. The substituted pyrazolecarboxamidine (1.5 mmol) was stirred with the appropriate secondary or arylamine (1.5 mmol) in acetonitrile (10 mL) for 24 h. The mixture was concentrated on the rotary evaporator and then subjected to flash column chromatography (20−30% ethyl acetate/cyclohexane) to give the protected guanidino-amino acid as a colorless gum.The protected amino acid was stirred in excess 4 M hydrogen chloride/dioxane for 24−72 h. Removal of solvent in vacuo gave the amino acid as a white, hygroscopic solid.

2-Amino-4-guanidinobutanoic Acid Derivatives: *(S)*-2-Amino-4-(*N*′-methylguanidino)butanoic Acid (4). Yield 44% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.16 (1H, t, *J* 6.7 Hz, CH), 3.57 (2H, t, *J* 7.1 Hz, CH_2), 2.95 (3H, s, NCH_3), 2.39−2.17 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 172.7 (C) 159.7 (C) 52.9 (CH), 40.4 (CH_2) 32.5 (CH_2) 29.9 (NCH_3); MS (FAB+) found *m/z* 175.11881 (M + H), $C_6H_{14}N_4O_2$ M + H calcd 175.11948.

***(S)*-2-Amino-4-guanidinobutanoic Acid (8).** Yield 67% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 3.98 (1H, t, *J* 6.5 Hz, CH), 3.39 (2H, t, *J* 7.1 Hz, CH_2), 2.12−2.02 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.6 (C), 158.0 (C), 50.9 (CH), 38.4 (CH_2), 30.5 (CH_2); MS (FAB+) found *m/z* 161.10404 (M + H), $C_5H_{12}N_4O_2$ M + H calcd 161.10385.

***(S)*-2-Amino-4-(*N*′-ethylguanidino)butanoic Acid (9).** Yield 54% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.07 (1H, t, *J* 6.4 Hz, CH), 3.50 (2H, t, *J* 7.1 Hz, CH_2), 3.27 (2H, q, *J* 7.2 Hz, CH_2), 2.29−2.13 (2H, m, CH_2), 1.24 (3H, t, *J* 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 51.8 (CH), 39.3 (CH_2), 38.1 (CH_2), 31.4 (CH_2), 14.8 (CH_3); MS (FAB+) found *m/z* 189.13586 (M + H), $C_7H_{16}N_4O_2$ M + H calcd 189.13514.

***(S)*-2-Amino-4-(*N*′-isopropylguanidino)butanoic Acid (10).** Yield 31% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.00 (1H, t, *J* 6.5 Hz, CH), 3.67 (1H, septet, *J* 6.4 Hz, $NCH(Me)_2$), 3.43 (2H, t, *J* 6.9 Hz, CH_2), 2.21−2.03 (2H, m, CH_2), 1.16 (6H, d, *J* 6.4 Hz, $(CH_3)_2$); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.6 (C), 157.1 (C), 51.9 (CH), 46.7 (CH), 39.4 (CH_2), 31.5 (CH_2), 23.1 (2 × CH_3); MS (FAB+) found *m*/z 203.15101 (M + H), $C_8H_{18}N_4O_2$ M + H calcd 203.15709.

***(S)*-2-Amino-4-(*N*′-propylguanidino)butanoic Acid (11).** Yield 35% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.07 (1H, t, *J* 6.8 Hz, CH), 3.50 (2H, t, *J* 7.0 Hz, CH_2), 3.18 (2H, t, *J* 7.1 Hz, CH_2), 2.26−2.13 (2H, m, CH_2), 1.62 (2H, td, *J* 7.3, 7.1 Hz, CH_2), 0.98 (3H, t, *J* 7.3 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.6 (C), 158.0 (C), 51.8 (CH), 44.8 (CH_2), 39.4 (CH_2), 31.4 (CH_2), 23.6 (CH_2), 11.8 (CH_3); MS (FAB+) found *m/z* 202.14300 (M + H), $C_8H_{18}N_4O_2$ M + H calcd 202.14297.

***(S)*-2-Amino-4-(*N*′-2,2-dimethylpropyl)guanidino)butanoic Acid (12).** Yield 18% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.18 (1H, t, *J* 6.7 Hz, CH), 3.62 (2H, t, *J* 7.3 Hz, CH_2), 3.14 (2H, s, $NCH_2C(Me)_3$), 2.40−2.21 (2H, m, CH_2), 1.09 (9H, s, $(CH_3)_3$); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.5 (C), 158.0 (C), 54.2 (CH_2), 51.8 (CH_2), 39.5 (CH_2), 33.5 (C), 31.5 (CH_2), 27.7 (3 x CH_3); MS (FAB+) found *m/z* 231.18222 (M + H), $C_{10}H_{22}N_4O_2$ M + H calcd 231.18209.

***(S)*-2-Amino-4-(*N*′-octylguanidino)butanoic Acid (13).** Yield 18% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.13 (1H, t, *J* 6.5 Hz, CH), 3.56 (2H, t, *J* 6.7 Hz, NCH_2), 3.28 (2H, t, *J* 7.1 Hz, NCH_2), 2.37−2.17 (2H, m, CH_2), 1.42−1.38 (10H, m, 5 × CH_2), 0.97 (3H, m, CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ; MS (FAB+), found *m/z* 273.22849 (M + H), $C_{13}H_{28}N_4O_2$ M + H calcd 273.22903.

***(S)*-2-Amino-4-(*N*′-cyclohexylguanidino)butanoic Acid (14).** Yield 12% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 3.99 (1H, t, *J* 6.2 Hz, CH), 3.43 (2H, t, *J* 6.8 Hz, CH_2), 2.21−2.06 (2H, m, CH_2), 1.87 (2H, br d, 2 × cyclohexyl H), 1.72 (2H, br d, 2 × cyclohexyl H), 1.55 (1H, m, NCH cyclohexyl), 1.41−1.14 (6H, m, 6 × cyclohexyl H); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 52.5 (CH), 52.0 (CH), 39.4 (CH_2), 34.2 (CH_2), 31.5 (CH_2), 26.6 (CH_2), 26.1 (CH_2); MS (FAB+) found *m/z* 243.18175 (M + H), $C_{11}H_{22}N_4O_2$ M + H calcd 243.18209.

***(S)*-2-Amino-4-(*N*′-(2-methoxyethyl)guanidino)butanoic Acid (15).** Yield 41% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.29 (1H, t, *J* 6.7 Hz, CH), 3.78−3.68 (4H, m, 2 × CH_2), 3.63 (2H, m, OCH_2), 3.61 (3H, s, OCH_3), 2.51−2.33 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.6 (C), 158.0 (C), 72.6 (CH_2), 59.6 (CH_3), 51.8 (CH), 43.5 (CH_2), 39.5 (CH_2), 31.4 (CH_2); MS (FAB+) found *m/z* 219.14585 (M + H), $C_8H_{18}N_4O_3$ M + H calcd 219.14585.

***(S)*-2-Amino-4-(*N*′-(2-phenoxyethyl)guanidino)butanoic Acid (16).** Yield 56% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 7.31−7.24 (2H, m, 2 × ArH), 6.97−6.92 (3H, m, 3 × ArH), 4.14 (2H, t, *J* 5.1 Hz, CH_2), 4.09 (1H, t, *J* 6.9 Hz, CH), 3.66 (2H, t, *J* 5.1 Hz, CH_2), 3.52 (2H, t, *J* 7.1 Hz, CH_2), 2.30−2.12 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.2

(C), 159.8 (C), 158.0 (C), 130.6 (CH), 122.4 (CH), 115.6 (CH), 67.4 (CH_2), 51.4 (CH), 42.5 (CH_2), 39.1 (CH2), 30.9 (CH_2); MS (FAB+) found *m/z* 281.16180 (M + H), $C_{13}H_{20}N_4O_3$ M + H calcd 281.16136.

***(S)*-2-Amino-4-(*N′*-(2-isopropoxyethyl)guanidino)butanoic Acid (17).** Yield 41% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.08 (1H, t, *J* 6.5 Hz, CH), 3.65 (1H, septet, *J* 6.1 Hz, OCH), 3.59 (2H, t, *J* 5.1 Hz, CH_2), 3.50 (2H, t, *J* 7.1 Hz, CH_2), 3.39 (2H, t, *J* 5.1 Hz, CH_2), 2.30−2.11 (2H, m, CH_2), 1.17 (5H, d, *J* 6.1 Hz, 2 × CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.2 (C), 158.4 (C), 73.7 (CH), 51.4 (CH), 43.6 (CH_2), 39.1 (CH_2), 31.0 (CH_2), 22.3 (CH_3); MS (FAB+) found *m/z* 247.17699 (M + H), $C_{10}H_{22}N_4O_3$ M + H calcd 247.17701.

***(S)*-2-Amino-4-(*N′*-(2-methylthioethyl)guanidino)butanoic Acid (18).** Yield 38% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.09 (1H, t, *J* 6.6 Hz, CH), 3.51 (2H, t, *J* 7.1 Hz, CH_2), 3.46 (2H, t, *J* 6.7 Hz, CH_2), 2.72 (2H, t, *J* 6.7 Hz, SCH_2), 2.30−2.12 (2H, m, CH_2), 2.13 (3H, s, SCH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.2 (C), 157.6 (C), 51.4 (CH), 41.8 (CH_2), 39.0 (CH_2), 33.9 (CH_2), 30.9 (CH_2), 15.2 (CH_3); MS (FAB+) found *m/z* 235.12235 (M + H), $C_8H_{18}N_4O_2S$ M + H calcd 235.12287.

***(S)*-2-Amino-4-(*N′*-(2-fluoroethyl)guanidino)butanoic Acid (19).** Yield 56% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.55 (2H, dt, $^1J_{HF}$ 47 Hz, J_{HH} 4.7 Hz, CH_2F), 4.09 (1H, t, *J* 6.4 Hz, CH), 3.62−3.50 (4H, m, 2 × NCH_2), 2.29−2.12 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.2 (C), 157.9 (C), 83.0 (d, $^1J_{CF}$ 168 Hz, CH_2F), 51.4 (CH), 43.2 (d, $^2J_{CF}$ 20 Hz, CH_2), 39.1 (CH_2), 30.9 (CH_2); MS (FAB+) found *m/z* 207.12645 (M + H), $C_7H_{15}FN_4O_2$ M + H calcd 207.12572.

***(S)*-2-Amino-4-(*N′*-phenylguanidino)butanoic Acid (20).** Yield 17% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 7.47 (2H, t, *J* 7.3 Hz Ar C2H, C5H), 7.37 (1H, t, *J* 7.3 Hz, Ar C4H), 7.31 (2H, m, Ar C3H, C5H), 4.10 (1H, t, *J* 6.3 Hz, CH), 3.59 (2H, t, *J* 7.0 Hz, CH_2), 2.33−2.14 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.5 (C), 131.5 (CH), 129.2 (CH), 127.1 (CH), 51.9 (CH), 39.8 (CH_2), 31.4 (CH_2); MS (FAB+) found *m/z* 237.13543 (M + H), $C_{11}H_{16}N_4O_2$ M + H calcd 237.13514.

***(S)*-2-Amino-4-(*N′*-benzylguanidino)butanoic Acid (21).** Yield 55% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 7.53−7.22 (5H, m, ArH), 4.59 (2H, s, $PhCH_2$), 4.15 (1H, t, *J* 6.7 Hz, CH), 3.66 (2H, t, *J* 7.2 Hz, CH_2), 2.41−2.24 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.5 (C), 158.0 (C), 137.9 (C), 130.3 (CH), 129.4 (CH), 128.8 (CH), 51.7 (CH), 46.5 (CH_2), 39.5 (CH_2), 31.4 (CH_2); MS (FAB+) found *m/z* 251.15045 (MH^+), $C_{12}H_{18}N_4O_2$ M + H calcd 251.15079.

***(S)*-2-Amino-4-(*N′*-allylguanidino)butanoic Acid (22).** Yield 50% (Method A). ^{1}H NMR (CD_3OD, 300 MHz): δ 5.99−5.87 (1H, m, vinyl CH), 5.34 (1H, d, *J* 17.1 Hz, cis vinyl CH), 5.27 (1H, d, *J* 10.4 Hz, trans vinyl CH), 4.13 (1H, t, *J* 6.6 Hz, NCH), 3.93 (2H, d, *J* 4.9 Hz. NCH_2), 3.57 (2H, t, *J* 6.0 Hz, CH_2), 2.37−2.15 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.5 (C), 158.0 (C), 134.1 (CH), 117.9 (CH_2), 51.8 (CH), 45.0 (CH_2), 39.4 (CH_2), 35.2 (CH_2), 31.4 (CH_2); MS (FAB+) found *m/z* 201.13558 (M + H), $C_8H_{16}N_4O_2$ M + H calcd 201.13514.

***(S)*-2-Amino-4-(*N′*-(2′-dimethylaminoethyl)guanidino)-butanoic Acid (23).** Yield 0.29% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.15 (1H, t, *J* 6.7 Hz, CH), 3.73 (2H, t, *J* 6.2 Hz, NCH_2), 3.56 (2H, t, *J* 7.1 Hz, NCH_2), 3.43 (2H, t, *J* 6.2 Hz, NCH_2), 2.96 (6H, s, $N(CH_3)_2$), 2.32−2.19 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.2 (C), 158.0 (C), 56.9 (CH_2), 51.4 (CH), 43.9 (CH_3), 39.2 (CH_2), 37.9 (CH_2), 30.8 (CH_2); MS (FAB+), found *m/z* 232.17283 (M + H), $C_9H_{21}N_5O_2$ M + H calc 232.17734.

***(S)*-2-Amino-4-(*N′*-cyclopropylguanidino)butanoic Acid (24).** Yield 41% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.07 (1H, t, *J* 6.5 Hz, CH), 3.50 (2H, t, *J* 6.9 Hz, CH_2), 2.52 (1H, m, NCH cyclopropyl), 2.27−2.11 (2H, br m, CH_2), 0.89 (2H, m, 2 × CH cyclopropyl), 0.66 (2H, m, 2 × CH cyclopropyl); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.6 (C), 159.3 (C), 51.9 (CH), 39.4 (CH_2), 31.4 (CH_2), 23.9 (CH), 8.2 (CH_2); MS (FAB+) found *m/z* 201.13519 (M + H), $C_8H_{16}N_4O_2$ M + H calcd 201.13514.

***(S)*-2-Amino-4-(*N′*,*N′*-dimethylguanidino)butanoic Acid (25).** Yield 31% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.01 (1H, t, *J* 6.3 Hz, NCH), 3.45 (2H, t, *J* 6.9 Hz, CH_2), 2.98 (6H, s, $N(CH_3)_2$), 2.25−2.04 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.8 (C), 158.1 (C), 51.9 (CH), 40.1 (CH_2), 39.3 (CH_3), 31.3 (CH_2); MS (FAB+) found *m/z* 189.13494 (M + H), $C_7H_{16}N_4O_2$ M + H calcd 189.13514.

***(S)*-2-Amino-4-(*N′*,*N′*-diethylguanidino)butanoic Acid (26).** Yield 11% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.08 (1H, t, *J* 6.3 Hz, CH), 3.55 (2H, t, *J* 6.9 Hz, CH_2), 3.43 (4H, q, *J* 7.1 Hz, 2 × NCH_2), 2.32−2.14 (2H, m, CH_2), 1.21 (6H, t, *J* 7.1 Hz, 2 × CH_3), insufficient yield for ^{13}C NMR (1024 scans); MS (FAB+) found *m/z* 217.16691 (M + H), $C_9H_{21}N_4O_2$ M + H calcd 217.16644.

***(S)*-2-Amino-4-[(piperidine-1-carboximidoyl)-amino]-butanoic Acid (27).** Yield 32% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.08 (1H, t, *J* 6.5 Hz, NCH), 3.54 (2H, t, *J* 7.1 Hz, CH_2), 3.48−3.44 (4H, m, 2 × CH_2), 2.31−2.13 (2H, m, CH_2), 1.68−1.59 (6H, m, 3 × CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.7 (C), 157.5 (C), 51.9 (CH), 40.3 (CH_2), 31.3 (CH_2), 26.9 (CH_2), 25.2 (CH_2); MS (FAB+) found *m/z* 229.16582 (M + H), $C_{10}H_{20}N_4O_2$ M + H calcd 229.16644.

***(S)*-2-Amino-4-[(morpholine-4-carboximidoyl)-amino]-butanoic Acid (28).** Yield 30% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.09 (1H, t, *J* 6.2 Hz, CH), 3.74 (4H, t, *J* 4.9 Hz, 2 × CH_2 morpholino), 3.55 (2H, t, *J* 6.8 Hz, CH_2), 3.48 (4H, t, *J* 4.9 Hz, 2 × CH_2 morpholino), 2.30−2.19 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 66.9 (CH_2), 51.5 (CH), 47.6 (CH_2), 39.9 (CH_2), 30.8 (CH_2); MS (FAB+) found *m/z* 231.14593 (M + H), $C_9H_{18}N_4O_3$ M + H calcd 231.14571.

***(S)*-2-Amino-4-[(pyrrolidine-1-carboximidoyl)-amino]-butanoic Acid (29).** Yield 23% (Method B). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.11 (1H, t, *J* 6.2 Hz, CH), 3.54 (2H, t, *J* 6.8 Hz, CH_2), 3.44 (4H, t, *J* 6.4 Hz, 2 × NCH_2 pyrrolidino), 2.32−2.05 (2H, m, CH_2), 2.03 (4H, t, *J* 6.4 Hz, 2 × CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.4 (C), 155.0 (C), 51.5 (CH), 48.5 (CH_2), 39.4 (CH_2), 30.9 (CH_2), 26.2 (CH_2); MS (FAB+) found *m/z* 215.15070 (M + H), $C_9H_{18}N_4O_2$ M + H calcd 215.15079.

General Method for Preparation of Esters. To a solution of the amino acid (typically 0.5 mmol) in the appropriate alcohol (2 mL) at 0 °C was added thionyl chloride (1.1 equivalents), with stirring, The solution was stirred for 30 min at 0 °C and then heated under reflux for 1 h and stirred at room-temperature overnight. Solvent was then removed in vacuo to give the ester (**30-35, 37**) as a white or yellow solid.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Methyl Ester (30).** Yield 83%. ^{1}H NMR (CD_3OD, 300 MHz): δ 4.15 (1H, t, *J* 6.5 Hz, CH), 3.86 (3H, s, OCH_3), 3.54 (2H, t, *J* 4.9 Hz, CH_2), 3.47 (2H, t, *J* 7.1 Hz, CH_2), 3.42 (2H, t, *J* 6.0 Hz, CH_2), 3.38 (3H, s, OCH_3), 2.29−2.11 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.3 (C), 157.5 (C), 72.1 (CH_2), 59.2 (CH_3), 54.0 (CH_3), 51.5 (CH), 43.0 (CH_2), 38.8 (CH_2), 30.9 (CH_2); MS (FAB+) found *m/z* 233.16097 (M + H), $C_9H_{20}N_4O_3$ M + H calcd 233.16136.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Ethyl Ester (31).** Yield 84%. ^{1}H NMR (CD_3OD, 300 MHz): δ 4.33 (2H, q, *J* 7.1 Hz, CH_2), 4.13 (1H, t, *J* 6.2 Hz, CH), 3.55−3.39 (6H, m, 3 x CH2) 3.38 (3H, s, OCH_3), 2.28−2.20 (2H, m, CH_2), 1.34 (3H, t, *J* 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 169.9 (C), 158.3 (C), 72.1 (CH_2), 64.1 (CH_2), 59.2 (CH_3), 51.5 (CH), 43.1 (CH_2), 38.9 (CH_2), 30.9 (CH_2), 14.4 (CH_3); MS (FAB+) found *m/z* 247.17723 (M + H), $C_{10}H_{22}N_4O_3$ M + H calcd 247.17701.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Propyl Ester (32).** Yield 80%. ^{1}H NMR (CD_3OD, 300 MHz): δ 4.25−4.15 (3H, m, CH, CH_2), 3.56−3.39 (6H, m, 3 × CH_2), 3.38 (3H, s, OCH_3), 2.27−2.15 (2H, m, CH_2), 1.73 (2H, m, CH_2), 0.98 (3H, t, *J* 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.0 (C), 158.1 (C), 72.1 (CH_2), 69.5 (CH_2), 59.3 (CH_3), 51.6 (CH), 43.1 (CH_2), 38.9 (CH_2), 30.9 (CH_2), 22.9 (CH_2), 10.7 (CH_3); MS (FAB+) found *m/z* 261.19258 (M + H), $C_{11}H_{24}N_4O_3$ M + H calcd 261.19266.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Butyl Ester (33).** Yield 60%. ^{1}H NMR (CD_3OD, 300

MHz): δ 4.31−4.25 (2H, m, CH_2), 4.16 (1H, t, *J* 6.1 Hz, CH), 4.02−3.95 (2H, m, CH_2), 3.55−3.41 (6H, m, 3 × CH_2) 3.28 (3H, s, CH_3), 2.26−2.17 (2H, m, CH_2), 1.74−1.65 (2H, m, CH_2), 1.46−1.38 (2H, m, CH_2), 0.96 (3H, t, *J* 7.3 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.1 (C), 158.1 (C), 72.2 (CH_2), 67.8 (CH_2), 59.3 (CH_3), 51.5 (CH), 43.0 (CH_2), 38.9 (CH_2), 31.6 (CH_2), 30.9 (CH_2), 20.1 (CH_2), 14.0 (CH_3); MS (FAB+) found *m/z* 275.20818 (M + H), $C_{12}H_{26}N_4O_3$ M + H calcd 275.20831.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Benzyl Ester (34).** Yield 79%. ^{1}H NMR (CD_3OD, 300 MHz): δ 7.45−7.27 (5H, m, ArH), 5.23 (2H, s, $PhCH_2O$), 4.20 (1H, t, *J* 6.2 Hz, CH), 3.59−3.42 (6H, m, 3 × CH_2), 3.30 (3H, s, OCH_3), 2.27−2.11 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 169.9 (C), 158.0 (C), 136.3 (C), 129.9 (CH), 129.4 (CH), 129.0 (CH), 72.1 (CH_2), 69.5 (CH_2), 59.2 (CH_3), 51.6 (CH), 43.0 (CH_2), 38.9 (CH_2), 30.9 (CH_2); MS (FAB+) found *m/z* 309.19466 (M + H), $C_{15}H_{24}N_4O_3$ M + H calcd 309.19266.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Isopropyl Ester (35).** Yield 72%. ^{1}H NMR (CD_3OD, 300 MHz): δ 5.11 (1H, septet, *J* 6.2 Hz, $(Me)_2CH$), 4.12 (1H, t, *J* 6.7 Hz, CH), 3.56−3.42 (6H; m, 3 × CH_2), 3.38 (3H, s, OCH_3), 2.29−2.04 (2H, m, CH_2), 1.33 (6H, d, *J* 6.2 Hz, $(CH_3)_2$); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 169.5 (C), 158.1 (C), 72.5 (CH_2), 72.1 (CH) 59.3 (CH_3), 51.6 (CH), 43.1 (CH_2), 39.0 (CH_2), 31.0 (CH_2), 21.9 (CH_3); MS (FAB+) found *m/z* 261.19378 (M + H), $C_{11}H_{24}N_4O_3$ M + H calcd 261.19266.

Arginine Homologues. N^G-(2-Methoxyethyl)-L-arginine (36). Yield 44% (Method A, from Boc-Orn-OBu^t). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.03 (1H, t, *J* 6.0 Hz, CH), 3.53 (2H, t, *J* 5.0 Hz, CH_2), 3.39 (2H, t, *J* 5.0 Hz, CH_2), 3.37 (3H, s, OCH_3), 3.30 (2H, m, CH_2), 2.03−1.94 (2H, m, CH_2), 1.83−1.72 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.5 (C), 158.2 (C), 72.2 (CH_2), 59.2 (CH_3), 53.5 (CH), 42.9 (CH_2), 41.9 (CH_2), 28.7 (CH_2), 25.8 (CH_2); MS (FAB+) found *m/z* 233.16097 (M + H), $C_9H_{20}N_4O_3$ M + H calcd 233.16136.

N^G-(2-Methoxyethyl)-L-arginine Methyl Ester (37). Yield 80%. ^{1}H NMR (CD_3OD, 300 MHz): δ 4.12 (1H, t, *J* 6.0 Hz, CH), 3.85 (3H, s, OCH_3), 3.53 (2H, t, *J* 4.7 Hz, CH_2), 3.40 (2H, t, *J* 4.7 Hz, CH_2), 3.38 (3H, s, OCH_3), 3.31−3.27 (2H, m, CH_2), 2.10−1.92 (2H, m, CH_2), 1.84−1.69 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.7 (C), 158.1 (C), 72.2 (CH_2), 59.3 (CH_3), 53.9 (CH_3), 53.6 (CH), 43.0 (CH_2), 41.9 (CH_2), 28.7 (CH_2), 25.8 (CH_2); MS (FAB+) found *m/z* 247.17725 (M + H), $C_{10}H_{22}N_4O_3$ M + H calcd 247.17701.

General Method for Preparation of Amides. Isobutyl chloroformate (1 mmol) and *N*-methylmorpholine (1 mmol) were added to L-Boc-ornithine(Fmoc) (1 mmol) in chloroform (5 mL) at −10 °C, with stirring. The mixture was stirred at −10 °C for 15 min, and then the required amine (1 mmol) was added and the mixture stirred for a further 3 h with gradual warming to room temperature. The solvent was removed in vacuo and the residue subjected to flash column chromatography (1:1 cyclohexane:ethyl acetate) to give the amide as a white powder.

The amide (0.5 mmol) was stirred with piperidine (5 equiv) in chloroform (3 mL) with monitoring by TLC until the starting material was consumed. Solvent and excess piperidine were removed in vacuo, and the entire crude product was used in the next step.

The *N*-alkyl-substituted pyrazolecarboxamidine (0.5 mmol) and the crude Boc-protected amide (∼0.5 mmol) were stirred in acetonitrile (5 mL) for 48 h. After removal of solvent, the residue was subjected to flash column chromatography to give the Boc-protected guanidino-amino amide.

The Boc-protected amide was stirred in excess HCl/dioxane for 72 h, then the solvent removed in vacuo to give the product as a white solid.

***(S)*-2-Amino-5-(*N′*-(2-methoxyethyl)guanidino)pentanoic Acid Methylamide (38).** Yield 36%. ^{1}H NMR (CD_3OD, 300 MHz): δ 3.92 (1H, t, *J* 6.5 Hz, CH), 3.53 (2H, t, *J* 4.7 Hz, CH_2), 3.40 (2H, t, *J* 5.0 Hz, CH_2), 3.37 (3H, s, NCH_3), 3.32−3.26 (2H, m, CH_2), 2.79 (3H, s, CH_3), 1.96−1.89 (2H, m, CH_2), 1.74−1.66 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.3 (C), 158.1 (C), 72.2 (CH_2), 59.3 (CH_3), 54.1 (CH), 43.0 (CH_2), 42.0 (CH_2), 29.7 (CH_2), 26.4 (CH_3), 25.6 (CH_2); MS (FAB+) found *m/z* 246.19305 (M + H), $C_{10}H_{23}N_5O_2$ M + H calcd 246.19299.

***(S)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid Benzylamide (39).** Yield 33%. ^{1}H NMR (CD_3OD, 300 MHz): 7.33−7.22 (5H, m, ArH), 4.43 (2H, d, *J* 5.5 Hz, CH_2), 4.09 (1H, t, *J* 6.7 Hz, CH), 3.53−3.40 (4H, m, 2 × CH_2), 3.36 (3H, s, OMe), 2.24−2.09 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 169.9 (C), 158.1 (C), 139.3 (C), 129.6 (Ar CH), 128.9 (Ar CH), 128.5 (Ar CH), 72.1 (CH_2), 59.3 (CH_3), 52.3 (CH), 44.4 (CH_2), 43.0 (CH_2), 38.8 (CH_2), 32.1 (CH_2); MS (FAB+) found *m/z* 308.21039 (M + H), $C_{15}H_{25}N_5O_2$ M + H calcd 308.20864.

General Method for Preparation of *(R)*-Amino Acids. Boc-D-Orn(Fmoc)-*O*-*tert*-butyl ester (1 mmol) was prepared from the acid using a *tert*-butyl isourea by a published method[1] and purified by flash column chromatography (50% EtOAc/cyclohexane). The Fmoc group was removed by stirring with piperidine (5−10 equiv) in chloroform until reaction was complete by TLC, and then after evaporation of solvent and excess piperidine, the crude product mixture was dissolved in acetonitrile (10 mL) and the appropriate substituted bis-Boc-pyrazole-1-carboxamide (1 equiv) was added. The mixture was stirred for 24 h, solvent removed in vacuo, and the product obtained by flash column chromatography (25% to 50% EtOAc/cyclohexane) as a colorless gum. The protected amino acid was then stirred in HCl/1,4-dioxane for 72 h, and solvent was removed in vacuo to give the product as a white hygroscopic solid.

***(R)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)pentanoic Acid (N^G-(2-methoxyethyl)-D-arginine) (40).** Yield 36%. ^{1}H NMR (CD_3OD, 300 MHz): δ 4.04 (1H, t, *J* 6.2 Hz, CH), 3.54 (2H, t, *J* 4.8 Hz, CH_2), 3.41 (2H, t, *J* 4.8 Hz, CH_2), 3.38 (3H, s, CH_3), 3.31 (2H, t, *J* 7.0 Hz, CH_2), 2.05−1.96 (2H, m, CH_2), 1.84−1.74 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.5 (C), 158.2 (C), 72.3 (CH_2), 59.3 (CH_3), 53.6 (CH), 43.0 (CH_2), 42.0 (CH_2), 28.7 (CH_2), 25.8 (CH_2); MS (FAB+) found *m/z* 233.16116 (M + H), $C_9H_{20}N_4O_3$ M + H calcd 233.16136

***(R)*-2-Amino-4-(*N′*-(2-methoxyethyl)guanidino)butanoic Acid (41).** Yield 21% from Boc-D-Dab(Fmoc)-OH. ^{1}H NMR (CD_3OD, 300 MHz): δ 4.08 (1H, t, *J* 6.4 Hz, CH), 3.59−3.49 (4H, m, 2 × CH_2), 3.41 (2H, t, *J* 4.7 Hz, CH_2), 3.38 (3H, s, OCH_3), 2.29−2.14 (2H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 171.2 (C), 158.2 (C), 72.2 (CH_2), 59.2 (CH_3), 51.4 (CH), 43.1 (CH_2), 39.1 (CH_2), 31.0 (CH_2); MS (FAB+) found *m/z* 219.14545 (M + H), $C_8H_{18}N_4O_3$ M + H calcd 219.14571.

Other Derivatives. 3-*N′*-(2-Methoxyethyl)guanidinopropylamine (42). Yield 63% (Method A, from Boc-1,3-diaminopropane). ^{1}H NMR (CD_3OD, 300 MHz): δ 3.53 (2H, t, *J* 5.0 Hz, CH_2), 3.40 (2H, t, *J* 5.0 Hz, CH_2), 3.38 (3H, s, OCH_3), 3.34 (2H, t, *J* 7.0 Hz, CH_2), 3.02 (2H, t, *J* 7.6 Hz, CH_2), 1.96 (2H, tt, *J* 7.0, 7.6 Hz, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 158.2 (C), 72.2 (CH_2), 59.2 (CH_3), 43.0 (CH_2), 39.7 (CH_2), 38.2 (CH_2), 28.0 (CH_2); MS (FAB+) found *m/z* 175.15591 (M + H), $C_7H_{18}N_4O$ M + H calcd 175.15588.

***(S)*-4-*N′*-(2-Methoxyethyl)guanidinobutanoic Acid (43).** Yield 59% (Method A, from 4-amino-*tert*-butylbutanoate). ^{1}H NMR (CD_3OD, 300 MHz): δ 3.53 (2H, t, *J* 4.7 Hz, CH_2), 3.39−3.34 (2H, m, CH_2), 3.36 (3H, s, OCH_3), 3.24 (2H, t, *J* 7.1 Hz, CH_2), 2.39 (2H, t, *J* 7.1 Hz, CH_2), 1.86 (2H, quintet, *J* 7.1 Hz, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 176.7 (C), 158.0 (C), 72.7 (CH_2), 59.2 (CH_3), 42.9 (CH_2), 41.9 (CH_2), 31.5 (CH_2), 25.2 (CH_2); MS (FAB+) found *m/z* 204.13477 (M + H), $C_8H_{17}N_3O_3$ M + H calcd 204.13481.

***(S)*-4-(*N′*-(2-Methoxyethyl)guanidino)pyrrolidine-2-carboxylic Acid (44).** Yield 26% (Method B, from *N*-Boc-Hydroxyproline-OBu^t). ^{1}H NMR (CD_3OD, 300 MHz): δ 4.55−4.45 (2H, m, CH_2), 3.75−3.71 (1H, m, CH), 3.54 (2H, t, *J* 4.8 Hz, CH_2), 3.44−3.40 (3H, m, CH_2 + CH), 3.38 (3H, s, OCH_3), 2.89−2.85 (1H, m, CH), 2.28−2.21 (1H, m, CH); ^{13}C NMR (75 MHz, CD_3OD, 27 °C) δ 170.4 (C), 159.0 (C), 72.1 (CH_2), 59.6 (CH), 59.2 (CH_3), 51.7 (CH), 50.7 (CH_2), 43.2 (CH_2), 35.2 (CH_2);

MS (FAB+) found *m/z* 231.14592 (M + H), $C_9H_{18}N_4O_3$ M + H calcd 231.14571.

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Supporting Information Available: Full tables of inhibition data and in vivo ADMA and SDMA measurements. This information is available free of charge via the Internet at http://pubs.acs.org.

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