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Imidazole-Containing Amino Acids as Selective Inhibitors of Nitric Oxide Synthases

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Abstract—Two series of imidazole-containing amino acids (**1a–e** and **2a–c**), all larger homologues and analogues of L-histidine, were prepared. Since imidazole and phenyl substituted imidazoles have been reported to be inhibitors of NOS and the mode of action of these compounds as heme ligands is a potential mechanism of inhibitory action, we designed imidazole-containing amino acids as combined inhibitors at both the amino acid as well as heme binding sites. To study the influence of the distance between the amino acid moiety and the imidazole moiety on inhibitory potency, the number of carbons between these two functional groups was varied from two to six. The structure–activity relationships of this class of inhibitors can be correlated with the distance between the heme and the amino acid binding sites of the enzyme. Two of the compounds (**1b** and **1d**) with three and five methylenes between the imidazole and amino acid functional groups, respectively, were found to be potent and selective inhibitors for nNOS and iNOS over eNOS. When phenyl was substituted on the nitrogen of the imidazole, both the potency and isoform selectivity diminished.

Introduction

Nitric oxide (NO) has diverse roles both in normal and pathological processes including the regulation of blood pressure, in neurotransmission, and in the macrophage defense systems.[1] NO is synthesized by three isoforms of nitric oxide synthase (NOS; EC 1.14.13.39), two of which, one in endothelial cells (eNOS) and one in neuronal cells (nNOS), are constitutive, and the one in macrophage cells is inducible (iNOS). All NOS isoforms are heme-containing and require NADPH, FAD, FMN, and tetrahydrobiopterin for the endogenous synthesis of NO and L-citrulline from L-arginine. The two constitutive isozymes are Ca^{2+} and calmodulin-dependent, whereas the inducible form is independent of added calmodulin because its calmodulin is tightly bound.

The normal biological functions regulated by NO are attributed to the different NOS isoforms. However, overproduction of NO has been implicated in numerous disease states,[2] such as septic shock,[3] inflammatory arthritis,[4,5] seizures,[6] schizophrenia,[7] Alzheimer's disease,[8] impotence,[9] and susceptibility to infection.[10] Therefore, potent and isoform selective inhibitors of NOS are desired for medicinal purposes as well as to define the roles of each NOS isoform in biological systems. Methylarginine[11] and nitroarginine[12] are two of the first inhibitors of NOS, and they are derived from a modification of the terminal amino group of the guanidine of L-arginine. Numerous other arginine analogues have been made,[2] although, except for nitroarginine, N^{ω}-propyl-L-arginine[13] and N^{5}-(imino-3-butenyl)-L-ornithine,[14] most of these inhibitors lack selectivity among the isozymes. Non-amino acid NOS inhibitors also have been reported, including isothioureas,[15,16] amidines,[17–21] guanidines,[22,23] imidazole,[24,25] and indazoles.[26] Because NOS contains heme, one of the strategies for its inhibition has been to design compounds that ligate to the heme. Imidazole[24,25] and several phenyl-substituted imidazoles[27] have been known to be inhibitors of NOS with a characteristic binding spectrum indicating that

Key words: Nitric oxide synthase; inhibitors; imidazole-containing amino acids; histidine analogues.

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[1] Designed and carried out all of the experiments in this study.

[2] Provided the recombinant eNOS and the *Escherichia coli* cells expressed with bovine neuronal nNOS used in this study.

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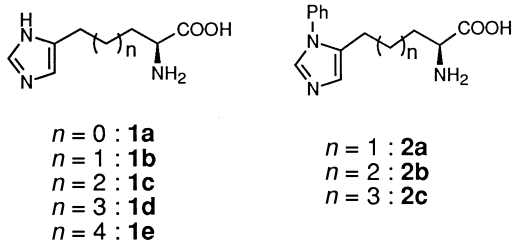
they interact at the heme binding site. Also, some of the sulfur-containing citrulline analogues, such as L-thiocitrulline, L-homothiocitrulline, and *S*-methyl-L-thiocitrulline, were synthesized and shown to be potent inhibitors of NOS.[28,29] These compounds were designed so that the sulfur atom of the bound inhibitor would be in close proximity to the heme iron, thereby encouraging the interaction between the sulfur ligand and the heme iron and increasing the binding affinity of the inhibitors for NOS. Further exploitation of the design of arginine analogues that have the potential to bind to the active-site heme is a series of *S*-2-amino-5-azolylpentanoic acids (including **1b** below) which was reported as nonselective inhibitors of rat iNOS and nNOS.[30]

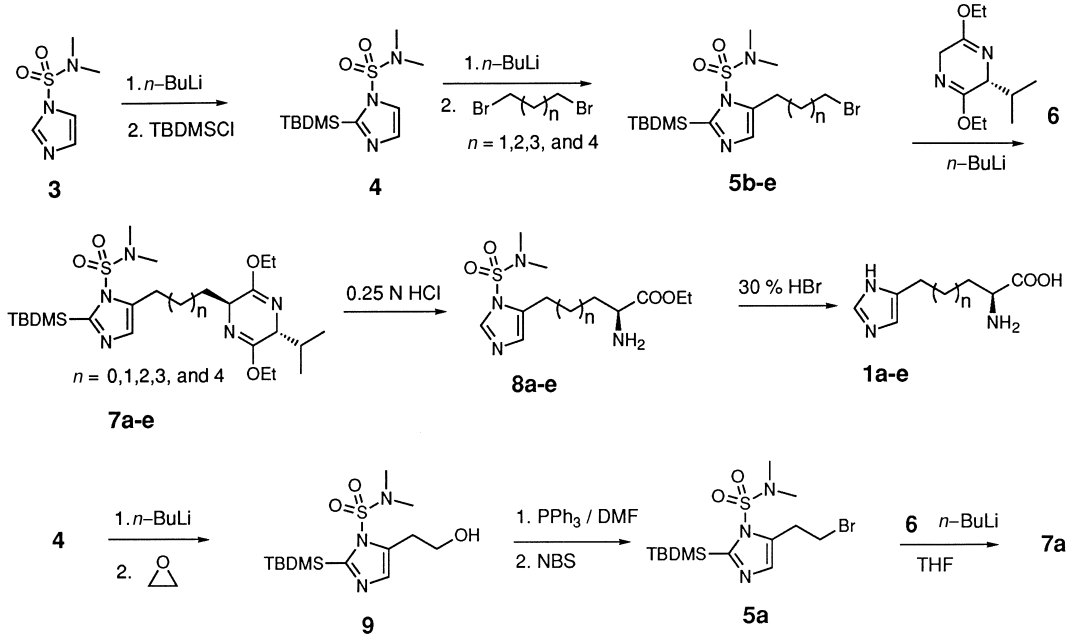
In view of the well-established affinity of heme iron for imidazole,[31,32] we designed compounds containing an imidazole positioned at various distances from the amino acid part of the molecules to determine if there was a difference in the distances between these two potential pharmacophores at the active site of the three isoforms of NOS. The imidazole and amino acid parts of the molecules were separated by an alkyl chain containing from two to six methylenes. Since phenyl-substituted imidazoles also bind to NOS,[27] another related series of compounds with a 1-phenyl-substituted imidazole also was synthesized. The syntheses of compounds **1a–e** and **2a–c** and the study of their inhibition of the three isozymes of NOS are reported here.



Results and Discussion

Syntheses of imidazole-containing amino acids (1a–e)

Among the compounds that we studied, L-homohistidine (**1a**) had been synthesized previously in nine steps starting from *N*-benzyloxycarbonyl-L-glutamic acid.[33] However, this method is not applicable to the preparation of a series of imidazole-containing amino acid derivatives. Consequently, a new enantioselective general synthesis of **1a–e** was designed utilizing the reaction of various imidazolyl alkyl bromides (**5b–e**) with chiral lithiated bislactim ether **6**[34] as the key step (Scheme 1). 1-(*N*,*N*-Dimethylsulfamoyl)imidazole (**3**)[35] was protected with a *tert*-butyldimethylsilyl group (TBDMS) by sequential treatment with *n*-butyllithium followed by *tert*-butyldimethylsilyl chloride. The lithium salt of the 1,2-diprotected imidazole (**4**)[36] was allowed to react with a series of excess dibromoalkanes to afford bromoalkyl substituted imidazoles (**5b–e**). Carbon–carbon bond formation between these alkyl bromides and lithiated bislactim ether (**6**) proceeded in good yields. The homologated bis-lactim ether products (**7a–e**) were hydrolyzed with 0.25 N HCl to the amino acid ethyl esters (**8a–e**). Under these reaction conditions the TBDMS protecting group of the imidazole was cleaved. Removal of the sulfamoyl group requires relatively vigorous conditions (refluxing 30% HBr for 4 h); consequently, the ethyl esters also were hydrolyzed to afford the desired amino acids (**1b–e**). The bromoethyl imidazole analogue **5a** was not prepared by the route used for the other analogues, because the dibromoethane underwent elimination of HBr in the presence of *n*-butyllithium, and not a trace of the substitution product **7a** was detected. Therefore **5a** was obtained by a two-step procedure employing a two-carbon homologation of the 1,2-diprotected imidazole **4** with ethylene oxide followed by conversion of the resulting alcohol (**9**) to the bromide (**5a**) under standard conditions. Treatment



Scheme 1.

of **5a** with the lithiated bislactim ether **6** (−70°C in THF) yielded the desired compound **7a** as a major component of the reaction.

The syntheses of the *N*-phenyl substituted imidazole derivatives **2a–c** were carried out by the same route as the imidazole analogues, starting from 1-phenylimidazole (**10**, Scheme 2). Surprisingly, the TBDMS group of **13a–c** survived the hydrolysis reaction conditions (0.25 N HCl), so the deprotection of the TBDMS group and the hydrolysis of the ester group were carried out simultaneously in a basic solution (2 N NaOH) to afford compounds **2a–c**.

Absorption spectra changes during interaction of 1a–e with NOS

One of the characteristics observed with the heme containing enzymes when they are exposed to heme binding inhibitors is a spectral change of the heme chromophore. The known heme binding inhibitors of NOS, such as imidazole[24,25,35] and L-thiocitrulline,[28,29] have been shown to elicit a type II difference spectrum, a transition of the high spin state of the unperturbed heme to the low spin state when bound with the inhibitors. As a first step for our study of the imidazole-containing amino acids, we examined whether **1a–e** interact as an axial sixth ligand of the heme cofactor of NOS. Upon addition of **1a–e** to iNOS, the initial maximum absorbance of the enzyme at 398 nm shifted to 425 nm, indicating that the imidazole moiety of these compounds contributes as a sixth ligand to the cofactor (Fig. 1). However, no spectral change was detected when 20 mM L-histidine was added, suggesting that the donation of the imidazole ligand to the heme is related to the distance between the imidazole ring and the amino acid. Although the degree of maximum absorbance shifts varied depending on the NOS isozyme and carbon chain length of the compounds used, **1a–e** appear to be interacting with the heme based on the spectral shift.

Effect of chain length on inhibitory potency

The influence of the carbon chain length between the imidazole and the amino acid then was measured on

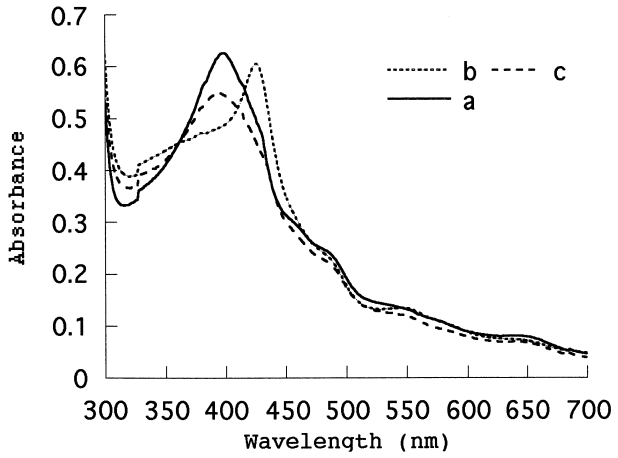
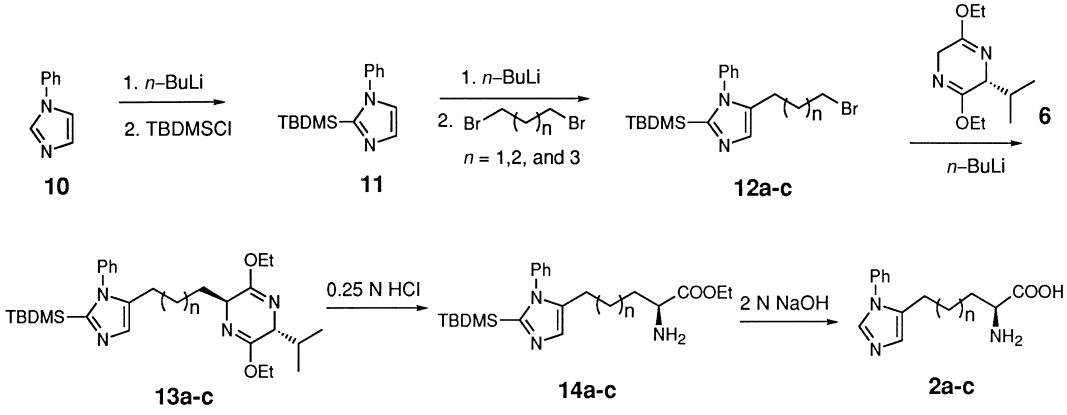


Figure 1. (a) Spectrum of the purified recombinant iNOS (3 μM) in HEPES buffer (100 mM, pH 7.5). (b) Spectrum after addition of **1b** (to a final concentration of 1.3 mM). Virtually the same absorbance maximum shifts to 425 nm were observed when each of the other analogues (**1a**, **1c**, **1d**, and **1e**) was added to (a).(c) Spectrum after addition of 1 mM of L-arginine to (b). Maximum absorbance shift to 395 nm indicates the conversion of the heme to the high spin state.

inhibitory potency. The ability of imidazole containing amino acids to inhibit NOS was determined by the hemoglobin capture assay as previously described.[37] All of the compounds tested are competitive inhibitors of three recombinant isoforms of NOS, rat nNOS, murine iNOS, and bovine eNOS. Since the homology for each isozyme in rat, mouse, and bovine are very high, the use of enzymes from different species should not affect the significance of the results. None of the kinetic plots of enzyme activity showed curvature, suggesting that there was no time dependency to the inhibition. As shown in Table 1, **1b** and **1d** are the two most potent inhibitors, especially of nNOS, with values of $K_i = 2\,\mu M$ for both of the compounds. The isoform selectivity of **1b** for nNOS over iNOS and eNOS is 5 and 17, respectively. The highest isoform selectivity for nNOS over eNOS among these analogues was observed with **1d**, which has five methylenes between the imidazole and the amino acid. This compound shows selectivities of 4 and 25 in favor of nNOS over iNOS and eNOS, respectively. Both the selectivity and the inhibitory potency were



Scheme 2.

Table 1. Inhibition of NOS by imidazole-containing amino acids

	K_i (μM)			Selectivity[a]	
Compound	iNOS	nNOS	eNOS	nNOS/iNOS	nNOS/eNOS
1a	950	170	500	5.6	2.9
1b	10	2	33	5.0	16.5
1c	35	65	150	0.5	2.3
1d	8	2	50	4.0	25.0
1e	40	150	250	0.3	1.7
2a	100	80	50	1.3	0.6
2b	50	100	17	0.5	0.2
2c	120	70	350	1.7	5.0
Imidazole	50	110	65	0.5	0.6
1-Phenyl imidazole	140	1200	6000	0.1	5

[a] The ratio of the inverse of the K_i values; a value >1 indicates better nNOS inhibition.

diminished when the distance between the imidazole and the amino acid was either decreased to two methylenes **1a** or elongated to five methylenes **1e**. Compound **1c** resulted in reduced inhibition compared to **1a** and **1b**, but showed higher inhibition than **1a** and **1e**. Therefore, the analogues with an odd number of methylenes between the two potential pharmacophores are both more potent and selective than the analogues with an even number of methylenes (Fig. 2). It is clear that the variation in the methylene chain length has a drastic effect on the potencies of these compounds with all three forms of NOS. The observed order of potency is as follows: **1b** and **1d** > **1c** > **1a** and **1e**. Another compound in the literature that was designed to be bifunctional is L-thiocitrulline, which has three methylenes in between the amino acid groups and the thiourea, which binds to the heme.[29] Its effect on nNOS and iNOS was tested; the K_i values are 0.06 and 3.6 μM, respectively, or a 60-fold selectivity for nNOS. When one more methylene is added to the chain to give L-homothiocitrulline, the potency was found to drop dramatically to 3 and 45 μM, respectively.[29] These two compounds are similar in binding distances to **1b** and **1c** and show a similar trend.

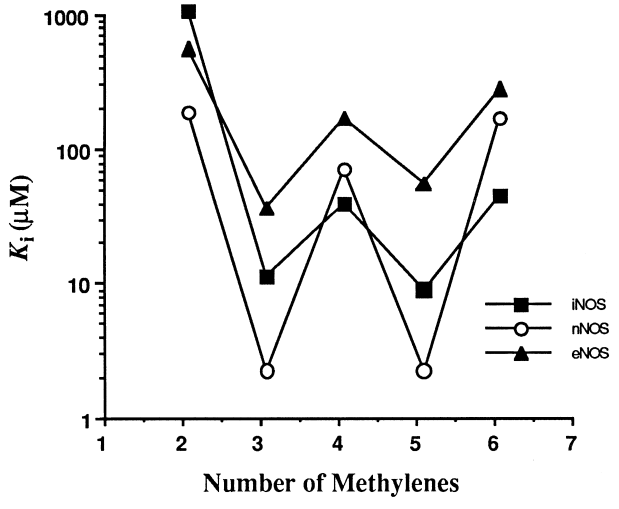


Figure 2. Plot of inhibitory potency versus the carbon chain length in **1a–e**.

All of the NOS isoforms have high specificity for binding of L-amino acids at the active site, supported by the fact that D-arginine is neither a substrate nor an inhibitor of the enzyme. In addition to that, most of the inhibitors classified as substrate arginine analogues are active only when the α-carbon is in the L-configuration. As demonstrated above, **1a–e** are recognized by the heme cofactor of the enzyme and could interact with the amino acid binding site of the enzyme if appropriate conditions, such as orientation and/or distance of the imidazole and amino acid moieties are satisfied. The SAR of these analogues, derived from the kinetic data, may be correlated to the distance of the heme from the amino acid binding site. Inhibition data in Table 1 and Figure 2 indicate that the potencies of **1a** and **1e** are significantly smaller than that of the other analogues. This suggests that the distance between the imidazole ring and the amino acid is either too short in the case of **1a** or too long in the case of **1e** for optimal binding at the active site. Both elongation of **1a** to a propylene chain (**1b**) and shortening of **1e** to a pentylene chain (**1d**) results in dramatic increases in potency, suggesting that the affinity of these compounds for both the imidazole and the amino acid binding sites are the strongest. Interestingly, the potency of **1c** decreases compared to that of **1b** and **1d**. It also should be noted that the trend described above is the same for all three isoforms of NOS, suggesting that the general topology of the heme cofactor and the residues responsible for recognition of these molecules is similar.

One possible explanation for the observation that the homologues with an odd number of methylene groups are more potent inhibitors is the expected change in geometry that results as methylene groups are added to the chain (Fig. 3). If the imidazole is held rigid, bound to the heme, then with each addition of a methylene, assuming an all anti-coplanar arrangement of the backbone, the orientations of the amino and carboxylate groups oscillate. This should have an effect on the binding efficiency.

Another possibility is that there is more than one amino acid binding site at the active site of the enzyme, one closer to the heme into which **1b** fits well and the other a little farther away into which **1d** binds better. If there is only one amino acid binding site at the active site, possibly two different tautomeric forms of the monosubstituted imidazole may interact with the heme depending on the chain length of the methylenes. As illustrated in Figure 4, the amino acid portion of **1b** is tightly bound to the amino acid binding site. To reach the heme for favorable binding, the rather short methylene spacer of **1b** needs to stretch out and also utilize the nitrogen atom of the imidazole that is two carbons away from alkyl chain. On the other hand, **1d**, which has a long enough alkyl chain, may be folded for optimal coordination to the heme through the nitrogen one carbon away from the alkyl chain (i.e. the other tautomer of the imidazole). It is known that the monocationic form of histamine exists as a mixture of two tautomers.[38] At physiological pH (7.4), the Nτ-H tautomer is more prevalent than the Nτ-H form in a relative concentration ratio of 4:1.

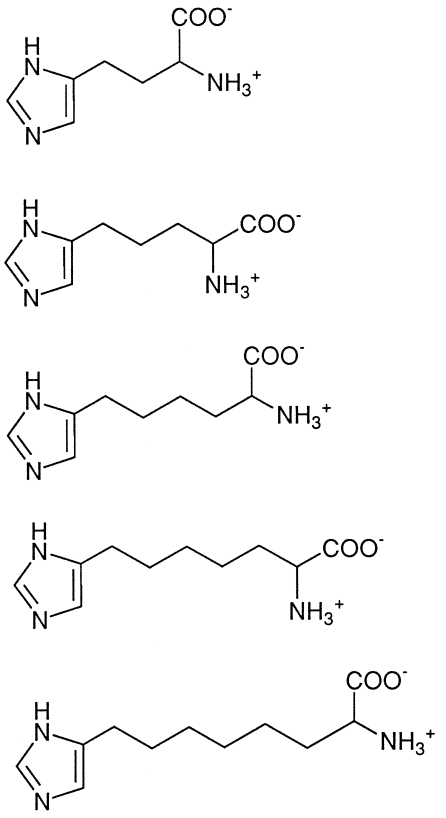


Figure 3. The effect of increasing the number of methylene groups in the side chain of **1a**–**e** on the orientation of the amino acid moieties.

In an attempt to provide support for the explanation that both tautomers of the imidazole ring may be involved in binding, another series of analogues was synthesized. Introduction of a substituent on one of the nitrogens of the imidazole ring would give rise to only one tautomeric form. This should lead to a new inhibitory potency order among the analogues of varying chain lengths. Phenyl was chosen as the substituent to attach to the nitrogen atom because it was reported that the inhibitory potency of 1-phenylimidazole for NOS is several times higher than that of imidazole.[24,25] Furthermore, if the increased potency of 1-phenylimidazole compared to imidazole results from a hydrophobic interaction of the phenyl ring with the ceiling of the heme pocket, then 1-phenylimidazole-containing amino acids might have improved affinity for the enzyme compared to the analogues without this substitution. As shown in Table 1, however, the general isoform selectivity for nNOS and iNOS over eNOS for **1b**–**e** was reversed with the phenyl substituted analogues **2a** and **2b** which are more selective for eNOS over the other isoforms of NOS, although the selectivity is poor or nonexistent. Contrary to our initial expectations, there was no clear order of potency among **2a**–**c**, and the inhibitory potencies were not improved compared to those of **1b**–**d**. When 1-phenylimidazole was tested in our laboratory, it was observed that it is a much poorer inhibitor of all three isoforms (Table 1). It is not clear why this difference in inhibitory potencies was observed.

Conclusion

A series of novel imidazole-containing amino acids was synthesized using Schollkopf's chiral bislactim ether.[34] Further derivatization of the imidazole ring demonstrated that this synthetic approach can be used as a general method for this class of compounds. Several of these analogues were found to be inhibitors of NOS, but selectivity among the isozymes is modest. The SAR study revealed that the inhibitory potencies of the imidazole-containing amino acids **1a**–**e** are related to the distance between the imidazole ring and the amino acid. Since it was found that all three isoforms of NOS exhibit similar interactions with **1a**–**e**, we conclude that the topologies of the active sites of all of the NOS isozymes, especially the distance between the heme cofactor and residues responsible for recognition of L-amino acid, are quite similar.

Experimental

Materials

Acids, bases, and conventional organic solvents were purchased from Fisher. NADPH, HEPES, calmodulin, ferrous hemoglobin, and L-arginine were purchased from Sigma Chemical Co. Tetrahydrobiopterin (H_4B) was obtained from B. Schircks Laboratories (Jona, Switzerland). Ion exchange resins were bought from Bio-Rad Laboratories. All of the chemicals were purchased from Aldrich Chemical Co, unless stated otherwise. Flash chromatography was performed with Merck silica gel (230–400 mesh). TLC plates (silica gel 60-F254) were purchased from VWR Scientific. Compounds were visualized with a ninhydrin spray reagent or a UV–vis lamp.

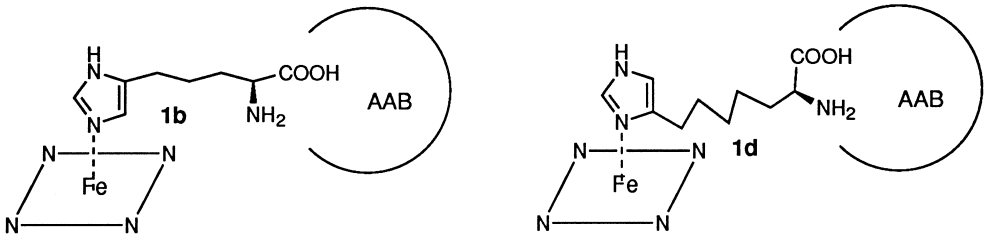


Figure 4. Possible explanation for why **1b** and **1d** are good inhibitors of NOS. The hypothetical binding sites for the imidazole-containing amino acid analogues.

Analytical methods

Optical rotations were determined with a AA-100 polarimeter (Optical Activity Ltd., England). All ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz (75 MHz for ^{13}C NMR spectra). Chemical shifts (δ) are reported downfield from tetramethylsilane (Me_4Si) in parts per million (ppm). Enzyme assays were recorded on a Perkin–Elmer Lamda 10 UV–vis spectrophotometer. Mass spectra (EI and FAB) were recorded on a VG Instrument VG70-250SE high-resolution mass spectrometer. Combustion analyses were performed by the Department of Geological Sciences at Northwestern University. Melting points were obtained with a Fisher–Johns melting point apparatus and are not corrected.

2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)-imidazole (4). To a solution of 1-(*N*,*N*-dimethylsulfamoyl)imidazole (20 g, 114 mmol) in dry THF (500 mL) at −70°C under an atmosphere of nitrogen was added *n*-butyllithium (46 mL of 2.5 M in hexane, 114 mmol) over a period of 20 min. After 20 min of stirring, a solution of *tert*-butyldimethylsilyl chloride (17.1 g, 114 mmol) in dry THF (100 mL) was added over a period of 30 min (internal temperature did not exceed −60°C), then the solution was allowed to rise to room temperature and was stirred for an additional hour. The reaction mixture was poured into water (100 mL), and the THF was removed under reduced pressure. The product was extracted with ethyl acetate (3×150 mL), dried (Na_2SO_4), and concentrated. The crude residue was purified by flash chromatography with ethyl acetate/hexane (1/4) as eluent to obtain a colorless solid (25 g, 76%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.39 (s, 6H). 0.95 (s, 9H), 2.84 (s, 6H), 7.22 (d, J = 1.26 Hz, 1H), 7.31 (d, J = 1.26 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) −3.9 (2C), 18.1, 27.1 (3C), 38.3 (2C), 120.2, 130.8. HRMS (M-CH_3) calcd for $C_{11}H_{20}N_3O_2SSi$ 274.1045, found 274.1047.

2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)-5-(2-hydroxyethyl)imidazole (9). *n*-Butyllithium (7.9 mL of 2.5 M in hexane, 19.9 mmol) was added to a stirred solution of 2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazole (**4**, 5.2 g, 18.1 mmol) in THF (100 mL) at −70°C. The mixture was stirred at this temperature for 30 min then transferred to a solution of ethylene oxide (2.1 g, 47.6 mmol) in 200 mL THF at −70°C. The reaction mixture was allowed to rise to room temperature and was stirred for 1 h. Saturated ammonium chloride (4 mL) was added to the mixture, and the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (2×40 mL), dried (Na_2SO_4), and recrystallized from ethyl acetate/hexane (1/2) to give a white solid (5.2 g, 15.6 mmol, 55%); mp 123–125°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.35 (s, 6H), 0.96 (s, 9H), 2.81 (s, 6H), 2.98 (td, J = 6.62, 1.02 Hz, 2H), 3.87 (t, J = 6.62 Hz, 2H), 7.01 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 18.4, 27.3 (3C), 28.4, 37.7 (2C), 60.7, 130.0, 131.5, 155.5. HRMS (M + H) calcd for $C_{13}H_{28}N_3O_3SSi$ 334.1620, found 334.1625.

5-(2-Bromoethyl)-2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl) imidazole (5a). A solution containing 2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)-5-(2-hydroxyethyl) imidazole (**9**, 200 mg, 0.60 mmol) and triphenylphosphine (173 mg, 0.66 mmol) in dry DMF (2 mL) and CH_2Cl_2 (1 mL) under N_2 was stirred at room temperature for 10 min and then to −70°C. A solution of *N*-bromosuccinimide (117 mg, 0.66 mmol) in DMF (1.5 mL) was added over a period of 20 min. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 1 h. The solvent was removed under reduced pressure, and the residue was treated with water (2 mL) and saturated $NaHCO_3$ (1 mL), then extracted with ethyl acetate (2×5 mL). The combined organic extract was concentrated, and the residue was purified by flash chromatography on a silica gel column (2×7 cm). Elution with 1/4 ethyl acetate/hexane gave a white solid (130 mg, 55%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.39 (s, 6H), 1.00 (s, 9H), 2.85 (s, 6H), 3.31 (t, J = 7.41 Hz, 2H), 3.59 (t, J = 7.41 Hz, 2H), 7.08 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.6 (2C), 18.4, 27.3 (3C), 28.9, 29.4, 37.8 (2C), 130.6, 131.4, 156.1. HRMS calcd for $C_{13}H_{26}BrN_3O_2SSi$ 396.0718, found 396.0768.

5-(4-Bromobutyl)-2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl) imidazole (5c). *n*-Butyllithium (9.36 mL of 2.5 M in hexane, 23.4 mmol) was added to a stirred solution of 2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl) imidazole (**4**, 6.75 g, 23.4 mmol) in THF (200 mL) at −70°C. The mixture was stirred at this temperature for 30 min then was transferred to the solution of 1,4-dibromobutane (8.38 mL, 70.2 mmol) in 150 mL of THF at −70°C. After 1 h the temperature of the mixture was allowed to rise to room temperature and was stirred for 3 h. The solvent was removed, and the residue was purified by flash chromatography on silica eluting with ethyl acetate/hexane (1/4) to give a white solid (6.84 g, 69%) which was recrystallized from ethyl acetate/hexane (1/6); mp 73°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.38 (s, 6H), 1.00 (s, 9H), 1.84 (m, 2H), 1.97 (m, 2H), 2.75 (t, J = 7.53 Hz, 2H), 2.84 (s, 6H), 3.45 (t, J = 6.42 Hz, 2H), 6.96 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 18.4, 24.2, 26.6, 27.3 (3C), 32.3, 33.4 37.7 (2C), 129.5, 134.2, 155.4. HRMS (M-CH_3) calcd for $C_{14}H_{27}BrN_3O_2SSi$ 410.0756, found 410.0737.

Using the same procedure as described above for **5c**, the following compounds were synthesized:

5-(3-Bromopropyl)-2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazole (5b). Mp 44°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.37 (s, 6H), 0.99 (s, 9H), 2.22 (m, 2H), 2.85 (s, 6H), 2.91 (t, J = 7.41 Hz, 2H), 3.50 (t, J = 6.17 Hz, 2H), 6.95 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.6 (2C), 18.4, 23.6, 27.3 (3C), 30.7 33.2, 37.8 (2C), 129.6, 133.1, 155.7. HRMS (M-CH_3) calcd for $C_{13}H_{25}BrN_3O_2SSi$ 396.0599, found 396.0600.

5-(5-Bromopentyl)-2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazole(5d). Recrystallized from petroleum ether; mp 43–45°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.38 (s, 6H), 1.00 (s, 9H), 1.56 (m, 2H), 1.71 (m, 2H), 1.91 (m, 2H), 2.72 (t, J = 6.63 Hz, 2H), 2.83 (s, 6H),

3.43 (t, $J=6.63$ Hz, 2H), 6.94 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 18.4, 24.9, 27.2, 27.3 (3C), 28.0, 32.5, 33.7, 37.7 (2C), 129.3, 134.6, 155.2. HRMS (M-CH_3) calcd for $C_{15}H_{29}BrN_3O_2SSi$ 424.0913, found 424.0904.

5-(6-Bromohexyl)-2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazole (5e). Obtained as a colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.38 (s, 6H), 0.99 (s, 9H), 1.40–1.57 (4H), 1.70 (m, 2H), 1.87 (m, 2H), 2.70 (t, $J=7.70$ Hz, 2H), 2.83 (s, 6H), 3.42 (t, $J=6.62$ Hz, 2H), 6.93 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 18.4, 24.9, 27.3 (3C), 27.7, 27.9, 28.6, 32.6, 34.0, 37.7 (2C), 129.3, 134.8, 155.2. HRMS (M-CH_3) calcd for $C_{16}H_{31}BrN_3O_2SSi$ 438.1069, found 438.1062.

(3*R*,6*S*)-6-{4-[2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]-butyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (7c). *n*-Butyllithium (2 mL of 2.1 M solution in hexane, 4.18 mmol) was injected into the solution of (3*R*)-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (**6**, 800 mg, 3.8 mmol) in THF (20 mL) and stirring continued for 30 min at −70°C. Then a solution of 5-(4-bromobutyl)-2-(*tert*-butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazole (**5c**, 1.6 g, 3.8 mmol) in THF (15 mL) was added. After 1 h stirring at −70°C and 2 h at room temperature, saturated NH_4Cl (1 mL) was added, and the solvent was removed in vacuo. The residue was extracted with ethyl acetate/H_2O, and the organic layer was concentrated to give an oil that was purified by flash chromatography on silica, eluting with ethyl acetate/hexane (1/4) to give a colorless oil (1.66 g, 3 mmol, 78%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.37 (s, 6H), 0.69 (d, $J=6.81$ Hz, 3H), 0.99 (s, 9H), 1.03 (d, $J=6.81$ Hz, 3H), 1.27 (app t, $J=7.01$ Hz, 6H), 1.33 (m, 2H), 1.68 (m, 2H), 1.82 (m, 2H), 2.26 (m, 1H), 2.68 (app t, $J=7.57$ Hz, 2H), 2.82 (s, 6H), 3.89 (m, 1H), 3.95 (m, 1H), 3.99–4.20 (4H), 6.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 14.4, 14.5, 16.6, 18.4, 19.1, 24.5, 25.0, 27.3 (3C), 27.8, 31.8, 33.9, 37.6 (2C), 55.3, 60.5, 60.7, 129.2, 134.9, 154.9, 163.1, 163.2. HRMS (M-C_4H_9) calcd for $C_{22}H_{40}N_5O_4SSi$ 498.2571, found 498.2564.

Using the same procedure as described above for **7c**, the following compounds were synthesized:

(3*R*,6*S*)-6-{2-[2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]-ethyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (7a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.37 (s, 6H), 0.70 (d, $J=6.85$ Hz, 3H), 1.00 (s, 9H), 1.02 (d, $J=6.85$ Hz, 3H), 1.26 (app t, $J=7.08$ Hz, 6H), 2.02 (m, 1H), 2.23 (m, 2H), 2.70 (m, 2H), 2.82 (s, 6H), 3.91 (t, $J=3.45$ Hz, 1H), 4.02–4.22 (5H), 6.97 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 14.4, 16.7, 18.4, 19.1, 20.5, 27.3 (3C), 32.0, 32.3, 37.6 (2C), 54.7, 60.6, 60.9, 129.1, 134.6, 155.0, 162.8, 163.4. HRMS calcd for $C_{24}H_{45}N_5O_4SSi$ 527.2961, found 527.2956.

(3*R*,6*S*)-6-{3-[2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]-propyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (7b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.37 (s, 6H), 0.70 (d, $J=6.81$ Hz, 3H), 1.00 (s, 9H), 1.03 (d, $J=6.81$ Hz, 3H), 1.27 (app t, $J=7.11$ Hz, 6H), 1.70 (m, 2H), 1.86 (m, 2H), 2.26 (m, 1H), 2.70 (t, $J=7.41$ Hz, 2H), 2.82 (s, 6H), 3.90 (t, $J=3.44$ Hz, 1H), 4.00 (m, 1H), 4.04–4.24 (4H), 6.94 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C) 14.4, 14.5, 16.7, 18.4, 19.1, 23.1, 24.9, 27.3 (3C), 31.9, 34.0, 37.6 (2C), 55.1, 60.6, 60.8, 129.3, 134.8, 155.1, 163.1, 163.4. HRMS (M-CH_3) calcd for $C_{24}H_{44}N_5O_4SSi$ 526.2883, found 526.2884.

(3*R*,6*S*)-6-{5-[2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]-pentyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (7d). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 6H), 0.62 (d, $J=6.81$ Hz, 3H), 0.92 (s, 9H), 0.96 (d, $J=6.81$ Hz, 3H), 1.19 (t, $J=7.00$ Hz, 6H), 1.20 (m, 2H), 1.32 (m, 2H), 1.60 (m, 2H), 1.68 (m, 2H), 2.29 (m, 2H), 2.61 (t, $J=7.65$ Hz, 2H), 2.74 (s, 6H), 3.81 (t, $J=3.44$ Hz, 1H), 3.91 (m, 1H), 3.95–4.15 (4H), 6.84 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.6 (2C), 14.3, 14.4, 16.6, 18.3, 19.1, 24.3, 24.9, 27.3 (3C), 27.8, 29.3, 31.7, 34.0, 37.5 (2C), 55.3, 60.4, 60.6, 129.2, 134.9, 154.8, 162.9, 163.2. HRMS (M-C_4H_9) calcd for $C_{23}H_{42}N_5O_4SSi$ 512.2727, found 512.2728.

(3*R*,6*S*)-6-{6-[2-(*tert*-Butyldimethylsilyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]-hexyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (7e). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.38 (s, 6H), 0.70 (d, $J=6.87$ Hz, 3H), 1.00 (s, 9H), 1.03 (d, $J=6.87$ Hz, 3H), 1.27 (t, $J=7.03$ Hz, 6H), 1.26–1.43 (6H), 1.65 (m, 2H), 1.73 (m, 2H), 2.28 (m, 1H), 2.67 (t, $J=7.81$ Hz, 2H), 2.82 (s, 6H), 3.88 (t, $J=3.30$ Hz, 1H), 3.98 (m, 1H), 4.04–4.23 (m, 4H), 6.93 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) −3.5 (2C), 14.4, 14.5, 16.7, 18.4, 19.2, 24.4, 25.1, 27.4 (3C), 27.8, 29.4, 29.5, 31.8, 34.2, 37.7 (2C), 55.4, 60.5, 60.7, 129.2, 135.1, 155.0, 163.0, 163.4. HRMS (M-C_4H_9) calcd for $C_{24}H_{44}N_5O_4SSi$ 526.2883, found 526.2889.

(*S*)-Ethyl-2-amino-6-[1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]hexanoate (8c). A suspension of (3*R*,6*S*)-6-{4-[*tert*-butyldimethyl)-1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]-butyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (**7c**, 1.6 g, 2.9 mmol) in 0.25 N HCl (35 mL) was stirred for 16 h at room temperature. The precipitate was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in water, covered with ether and vigorously shaken with concd NH_4OH until the pH was 8–10. The ether was separated, the aqueous phase was extracted with ether and the combined ether phases dried over Na_2SO_4. The ether was removed and the residue was purified by flash chromatography on silica gel eluting with ethyl acetate/ethanol/NH_4OH (40/4/1) to yield a colorless oil (414 mg, 1.24 mmol, 43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, $J=6.93$ Hz, 3H), 1.25–1.64 (6H), 2.56 (t, $J=7.41$ Hz, 2H), 2.70 (s, 6H), 3.24 (t, $J=6.21$ Hz, 1H), 3.97 (q, $J=6.93$ Hz, 2H), 6.64 (s, 1H), 7.67 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 14.2, 24.7, 25.2, 27.7, 34.4, 37.9 (2C), 54.2, 60.7, 128.1, 132.3, 138.0, 175.9. HRMS calcd for $C_{13}H_{24}N_4O_4S$ 332.1520, found 332.1527.

Using the same procedure as described above for **8c**, the following compounds were synthesized:

(*S*)-Ethyl-2-amino-4-[1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]butanoate (8a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20

(t, $J=7.20$ Hz, 3H), 1.78 (bs, 2H), 1.82 (m, 2H), 2.03 (m, 1H), 2.81 (s, 6H), 2.83 (m, 2H), 3.41 (dd, $J=8.10$, 5.10 Hz, 1H), 4.10 (q, $J=7.20$ Hz, 2H), 6.80 (s, 1H), 7.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 14.1, 21.2, 33.3, 38.0 (2C), 53.7, 61.0, 128.5, 131.5, 138.1, 175.5. HRMS calcd for $C_{11}H_{20}N_4O_4S$ 304.1205, found 304.1201.

(*S*)-Ethyl-2-amino-5-[1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]pentanoate (8b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, $J=7.14$ Hz, 3H), 1.73 (bs, 2H), 1.78 (m, 2H), 2.73 (app t, $J=6.85$ Hz, 2H), 2.85 (s, 6H), 3.42 (m, 1H), 4.15 (q, $J=7.14$ Hz, 2H), 6.83 (s, 1H), 7.83 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 14.3, 24.3, 24.8, 34.4, 38.1 (2C), 54.2, 61.0, 128.4, 132.1, 138.2, 175.8. HRMS calcd for $C_{12}H_{22}N_4O_4S$ 318.1364, found 318.1351.

(*S*)-Ethyl-2-amino-7-[1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]heptanoate (8d). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, $J=7.11$ Hz, 3H), 1.36–1.78 (8H), 2.71 (t, $J=7.41$ Hz, 2H), 2.87 (s, 6H), 3.40 (dd, $J=7.26$, 5.40 Hz, 1H), 4.16 (q, $J=7.11$ Hz, 2H), 6.81 (s, 1H), 7.84 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 14.3, 24.9, 25.4, 28.0, 29.1, 34.8, 38.1 (2C), 54.4, 60.9, 128.2, 132.6, 138.1, 176.2. HRMS calcd for $C_{14}H_{26}N_4O_4S$ 346.1676, found 346.1671.

(*S*)-Ethyl-2-amino-8-[1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]octanoate (8e). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, $J=7.13$ Hz, 3H), 1.30–1.74 (10H), 2.68 (t, $J=7.73$ Hz, 2H), 2.85 (s, 6H), 3.38 (dd, $J=7.34$, 5.45 Hz, 1H), 4.13 (q, $J=7.13$ Hz, 2H), 6.78 (s, 1H), 7.81 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 14.3, 24.9, 25.5, 28.0, 29.1, 29.2, 34.9, 38.1 (2C), 54.5, 60.8, 128.2, 132.7, 138.1, 176.2. HRMS calcd for $C_{15}H_{28}N_4O_4S$ 360.1832, found 360.1837.

(*S*)-2-Amino-6-[1(3)*H*-imidazol-4-yl]hexanoic acid (1c). (*S*)-Ethyl-2-amino-6-[1-(*N*,*N*-dimethylsulfamoyl)imidazol-4-yl]hexanoate (**8c**, 350 mg, 1.05 mmol) was dissolved in 30% HBr (20 mL) and heated under reflux. After 4 h the mixture was cooled and concentrated in vacuo. The residue was dissolved in water (5 mL) and was applied to a column of Dowex 50 W (hydrogen form), washed with 50 mL of H_2O, then eluted with 2 N NH_4OH (100 mL). The combined fractions reactive to ninhydrin solution were concentrated to 2 mL, then were lyophilized to give a white solid (150 mg, 73%). Recrystallized from ethanol/H_2O (20/1); mp 217–219°C, $[\alpha]_D^{22}+12.1°$ (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.19 (m, 2H), 1.40 (app quintet, $J=7.53$ Hz, 2H), 1.64 (m, 2H), 2.34 (t, $J=7.41$ Hz, 2H), 3.49 (t, $J=6.15$ Hz, 1H), 6.62 (s, 1H), 7.48 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 23.8, 25.1, 28.1, 30.3, 54.7, 116.6, 135.0, 136.6, 175.3. HRMS calcd for $C_9H_{15}N_3O_2$ 197.1165, found 197.1164. Anal. calcd for $C_9H_{15}N_3O_2 0.2H_2O$: C 53.82; H 7.72; N 20.92. Found: C 53.43; H 7.70; N 20.72.

Using the same procedure as described above for **1c**, the following compounds were synthesized:

(*S*)-2-Amino-4-[1(3)*H*-imidazol-4-yl]butanoic acid (1a). Mp 219–222°C, $[\alpha]_D^{22}+9.8°$ (*c* 0.5, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 2.00 (m, 2H), 2.55 (app t, $J=7.94$ Hz, 2H), 3.59 (t, $J=5.64$ Hz, 1H), 6.77 (s, 1H), 7.56 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 22.1, 30.4, 54.3, 115.9, 135.5, 135.8, 174.8. HRMS (M + H) calcd for $C_7H_{12}N_3O_2$ 170.0929, found 170.0925.

(*S*)-2-Amino-5-[1(3)*H*-imidazol-4-yl]pentanoic acid (1b). Mp 229–231°C, $[\alpha]_D^{22}+13.2°$ (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.62 (m, 2H), 1.76 (m, 2H), 2.55 (t, $J=7.04$ Hz, 2H), 3.60 (t, $J=6.03$ Hz, 1H), 6.81 (s, 1H), 7.60 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 24.3, 25.4, 30.4, 54.8, 116.6, 135.5, 136.7, 175.5. HRMS calcd for $C_8H_{13}N_3O_2$ 183.1009, found 183.1017. Anal. calcd for $C_8H_{13}N_3O_2 0.1H_2O$: C 51.93; H 7.13; N 22.71. Found: C 52.09; H 7.29; N 22.64.

(*S*)-2-Amino-7-[1(3)*H*-imidazol-4-yl)heptanoic acid (1d). Mp 207–209°C, $[\alpha]_D^{22}+12°$ (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.15–2.10 (4H), 1.47 (m, 2H), 1.71 (m, 2H), 2.43 (t, $J=7.41$ Hz, 2H), 3.58 (t, $J=6.08$ Hz, 1H), 6.72 (s, 1H). 7.59 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 24.0, 25.2, 27.8, 28.1, 30.5, 54.8, 116.8, 135.0, 136.9, 175.4. HRMS calcd for $C_{10}H_{15}N_3O_2$ 211.1321, found 211.1323. Anal. calcd for $C_{10}H_{17}N_3O_2 0.1H_2O$: C 55.90; H 8.06; N 19.55. Found: C 55.77; H 8.03; N 19.39.

(*S*)-2-Amino-8-[1(3)*H*-imidazol-4-yl]octanoic acid (1e). Mp 201–202°C, $[\alpha]_D^{22}+10.4°$ (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.21–1.36 (6H), 1.56 (m, 2H), 1.76 (m, 2H), 2.53 (t, $J=7.42$ Hz, 2H), 3.63 (t, $J=6.09$ Hz, 1H), 6.81 (s, 1H), 7.65 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 24.2, 25.4, 27.8, 28.1, 28.3, 30.6, 54.9, 116.9, 135.2, 137.2, 175.6. HRMS calcd for $C_{11}H_{19}N_3O_2$ 225.1477, found 225.1480. Anal. calcd for $C_{11}H_{19}N_3O_2$: C 58.64; H 58.50; N 18.65. Found, C 58.26; H 8.30; N 18.38.

2-(*tert*-Butyldimethylsilyl)-1-phenylimidazole (11). To a solution of 1-phenylimidazole (**10**, 5.0 g, 34.6 mmol) in dry THF (200 mL) at −70°C under an atmosphere of nitrogen was added *n*-butyllithium (13.8 mL of 2.5 M in hexane, 34.6 mmol). After 20 min of stirring, a solution of *tert*-butyldimethylsilyl chloride (5.2 g, 34.6 mmol) in dry THF (50 mL) was added over a period of 10 min, and the solution was allowed to warm to room temperature. Stirring continued for an additional hour. The solvent was removed under reduced pressure and the remaining crude product was extracted with ethyl acetate (2×50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography with ethyl acetate/hexane (1/4) as eluent to obtain a white solid which crystallized upon standing (6.8 g, 76%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.89 (s, 9H), 7.11 (d, $J=1.05$ Hz, 1H), 7.35–7.42 (3H), 7.49–7.55 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.5 (2C), 17.6, 26.8 (3C), 124.1, 127.5 (2C), 128.8 (2C), 128.9, 130.0, 139.5, 150.6. HRMS calcd for $C_{15}H_{22}N_2Si$ 258.1552, found 258.1557.

5-(3-Bromopropyl)-2-(*tert*-butyldimethylsilyl)-1-phenylimidazole (12a). *n*-Butyllithium (2.48 mL of 2.5 M in hexane, 6.2 mmol) was added to a stirred solution of 2-(*tert*-butyldimethylsilyl)-1-phenylimidazole (**11**, 1.6 g, 6.2 mmol) in THF (70 mL) at −70°C. The mixture was stirred at this temperature for 30 min and then was

transferred to the solution of 1,3-dibromopropane (2.5 mL, 24.8 mmol) in 50 mL of THF at −70°C. After 1 h the temperature of the mixture was allowed to rise to room temperature and was stirred further for 1 h. The solvent was removed, and the residue was purified by flash chromatography on silica gel eluting with ethyl acetate/hexane (1/4) to give a viscous oil (1 g, 43%); ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.09 (s, 6H), 0.87 (s, 9H), 1.98 (m, 2H), 2.47 (app t, J = 7.29 Hz, 2H), 3.32 (t, J = 6.40 Hz, 2H), 7.07 (s, 1H), 7.18–7.50 (5H). ^{13}C NMR (75 MHz, $CDCl_3$) −4.9 (2C), 17.6, 22.8, 26.8 (3C), 31.3, 32.8, 127.5, 128.7 (2C), 129.1 (2C), 129.2, 133.8, 137.7, 150.7. HRMS calcd for $C_{18}H_{27}BrN_2Si$ 378.1126 and 380.1108, found 378.1118 and 380.1127.

Using the same procedure as described above for **12a**, the following compounds were synthesized:

5-(4-Bromobutyl)-2-(*tert*-butyldimethylsilyl)-1-phenylimidazole (12b). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.09 (s, 6H), 0.87 (s, 9H), 1.61 (m, 2H), 1.79 (m, 2H), 2.31 (app t, J = 7.28 Hz, 2H), 3.30 (t, J = 6.62 Hz, 2H), 7.06 (s, 1H), 7.18–7.26 (2H), 7.43–7.48 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.9 (2C), 17.6, 23.6, 26.7, 26.8 (3C), 32.1, 33.3, 127.4, 128.8 (2C), 129.0 (2C), 129.2, 135.0, 138.0, 150.4. HRMS calcd for $C_{19}H_{29}BrN_2Si$ 392.1283 and 394.1264, found 392.1281 and 394.1264.

5-(5-Bromopentyl)-2-(*tert*-butyldimethylsilyl)-1-phenylimidazole (12c). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.08 (s, 6H), 0.88 (s, 9H), 1.38 (m, 2H), 1.50 (m, 2H), 1.78 (q, J = 7.20 Hz, 2H), 2.30 (app t, J = 7.56 Hz, 2H), 3.33 (t, J = 6.75 Hz, 2H), 7.06 (s, 1H), 7.20–7.25 (2H), 7.42–7.49 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.8 (2C), 17.6, 24.2, 27.5, 27.7, 32.3, 33.6, 127.2, 128.8 (2C), 128.9 (2C), 129.1, 135.4, 138.0, 150.2. HRMS calcd for $C_{18}H_{27}BrN_2Si$ 406.1439 and 408.1421, found 406.1428 and 408.1424.

(3*R*,6*S*)-6-{3-[2-(*tert*-Butyldimethylsilyl)-1-phenylimidazol-4-yl]-propyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (13a). *n*-Butyllithium (672 μL of 2.5 M solution in hexane, 1.68 mmol) was injected into a solution of (3*R*)-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (**6**, 357 mg, 1.68 mmol) in THF (20 mL) and stirring continued for 30 min at −70°C. Then a solution of 5-(3-bromopropyl)-2-(*tert*-butyldimethylsilyl)-1-phenylimidazole (**12a**, 637 mg, 1.68 mmol) in THF (10 mL) was added. After being stirred for 1 h at −70°C and 2 h at room temperature, saturated NH_4Cl (100 mL) was added and the solvent was removed in vacuo. The residue was extracted with ethyl acetate/H_2O, and the organic layer was concentrated to give an oil which was purified by flash chromatography on silica gel eluting with ethyl acetate/hexane (1/4) to give a colorless oil (608 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.16 (s, 6H), 0.61 (d, J = 6.60 Hz, 3H), 0.81 (s, 9H), 0.94 (d, J = 6.60 Hz, 3H), 1.13–1.21 (6H), 1.38 (m, 2H), 1.63 (m, 2H), 2.18 (m, 1H), 2.22 (m, 2H), 3.77 (m, 1H), 3.81–4.12 (4H), 6.99 (s, 1H), 7.12–7.17 (2H), 7.35–7.39 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.9 (2C), 14.4 (2C), 16.6, 17.5, 19.1, 23.5, 24.4, 26.7 (3C), 31.8, 33.7, 55.0, 60.5, 60.6, 127.2, 128.8 (5C), 135.7, 138.1, 150.0, 163.0, 163.1. HRMS calcd for $C_{29}H_{46}N_4O_2Si$ 510.3389, found 510.3398.

Using the same procedure as described above for **13a**, the following compounds were synthesized:

(3*R*,6*S*)-6-{4-[2-(*tert*-Butyldimethylsilyl)-1-phenylimidazol-4-yl]-butyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (13b). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.10 (s, 6H), 0.66 (d, J = 6.81 Hz, 3H), 0.86 (s, 9H), 1.00 (d, J = 6.81 Hz, 3H), 1.21 (q, J = 7.10 Hz, 3H), 1.23 (q, J = 7.10 Hz, 3H), 1.47 (m, 2H), 1.63 (m, 2H), 2.21 (m, 1H), 2.26 (m, 2H), 3.83 (t, J = 3.42 Hz, 1H), 3.78–4.25 (4H), 7.02 (s, 1H), 7.16–7.22 (2H), 7.40–7.45 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.9 (2C), 14.3, 14.4, 16.6, 17.6, 19.1, 24.3, 24.5, 26.8 (3C), 28.3, 31.8, 33.8, 55.3, 60.4, 60.7, 127.1, 128.8 (2C), 128.9 (2C), 129.0, 135.9, 138.2, 150.0, 163.0, 163.2. HRMS calcd for $C_{30}H_{48}N_4O_2Si$ 524.3546, found 524.3538.

(3*R*,6*S*)-6-{4-[2-(*tert*-Butyldimethylsilyl)-1-phenylimidazol-4-yl]-butyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (13b). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.12 (s, 6H), 0.65 (d, J = 6.75 Hz, 3H), 0.84 (s, 9H), 0.99 (d, J = 3.40 Hz, 3H), 1.20 (q, J = 7.10 Hz, 3H), 1.21 (q, J = 7.10 Hz, 3H), 1.23 (m, 2H), 1.43 (m, 2H), 1.62 (m, 2H), 2.21 (app, t, J = 7.43 Hz, 2H), 2.22 (m, 1H), 3.83 (t, J = 3.40 Hz, 1H), 3.86–4.14 (4H), 7.00 (s, 1H), 7.16–7.19 (2H), 7.38–7.43 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.8 (2C), 14.3, 14.4, 16.6, 17.6, 19.1, 24.1, 24.3, 26.8 (3C), 28.3, 29.1, 31.7, 33.9, 55.3, 60.4, 60.6, 127.1, 128.8 (2C), 128.9 (2C), 129.0, 135.9, 138.2, 140.9, 162.9, 163.3. HRMS calcd for $C_{31}H_{50}N_4O_2Si$ 538.3703, found 538.3701.

(*S*)-Ethyl-2-amino-5-(1-phenylimidazol-4-yl)pentanoate (14a). A suspension of (3*R*,6*S*)-6-{3-[2-(*tert*-butyldimethylsilyl)-1-phenylimidazol-4-yl]-propyl}-3-isopropyl-2,5-diethoxy-3,6-dihydropyrazine (**13a**, 600 mg, 1.17 mmol) in 0.25 N HCl (20 mL) was stirred for 3 h at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel eluting with ethyl acetate/ethanol/NH_4OH (80/4/1) to yield a colorless oil (263 mg, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.23 (s, 6H), 0.73 (s, 9H), 1.09 (t, J = 7.16 Hz, 3H), 1.30–1.60 (4H), 2.17 (m, 2H), 3.19 (m, 1H), 3.97 (q, J = 7.16 Hz, 2H), 6.91 (s, 1H), 7.05–7.10 (2H), 7.28–7.37 (3H). ^{13}C NMR (75 MHz, $CDCl_3$) −4.9 (2C), 14.2, 17.4, 24.1, 24.4, 26.7 (3C), 34.3, 54.1, 60.7, 127.2, 128.7 (2C), 128.9 (2C), 129.0, 135.1, 137.9, 150.1, 175.8. HRMS calcd for $C_{22}H_{35}N_3O_2Si$ 401.2498, found 401.2507.

Using the same procedure as described above for **14a**, the following compounds were synthesized:

(*S*)-Ethyl-2-amino-6-(1-phenylimidazol-4-yl)hexanoate (14b). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.14 (s, 6H), 0.83 (s, 9H), 1.19 (t, J = 7.16 Hz, 3H), 1.29 (m, 1H), 1.45 (m, 2H), 1.67 (m, 1H), 2.24 (app t, J = 7.56 Hz, 2H), 3.29 (dd, J = 7.17, 5.49 Hz, 1H), 4.08 (q, J = 7.16 H, 2H), 7.00 (s, 1H), 7.14–7.18 (2H), 7.39–7.44 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.9 (2C), 14.3, 17.5, 24.3, 25.3, 26.8 (3C), 28.2, 34.5, 54.3, 60.8, 127.2, 128.8 (2C), 128.9

(2C), 129.0, 135.6, 138.0, 150.1, 176.1. HRMS calcd for $C_{23}H_{37}N_3O_2Si$ 415.2655, found 415.2659.

(*S*)-Ethyl-2-amino-7-(1-phenylimidazol-4-yl)heptanoate (14c). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.13 (s, 6H), 0.83 (s, 9H), 1.21 (t, J = 7.13 Hz, 3H), 1.26 (m, 1H), 1.35–1.48 (4H), 1.58 (m, 1H), 2.23 (app t, J = 7.70 Hz, 2H), 3.31 (dd, J = 7.41, 5.49 Hz, 1H), 4.10 (q, J = 7.13 Hz, 2H), 7.00 (s, 1H), 7.15–7.19 (2H), 7.39–7.44 (3H); ^{13}C NMR (75 MHz, $CDCl_3$) −4.8 (2C), 14.3, 17.5, 24.3, 25.3, 26.8 (3C), 28.2, 29.0, 34.7, 54.4, 60.8, 127.2, 128.8 (2C), 128.9 (2C), 129.0, 135.8, 138.1, 150.1, 176.2, 177.1. HRMS calcd for $C_{24}H_{37}N_3O_2Si$ 429.2811, found 429.2817.

(*S*)-2-Amino-5-(1-phenylimidazol-4-yl)pentanoic acid (2a). (*S*)-Ethyl-2-amino-5-(1-phenylimidazol-4-yl)pentanoate (**14**a, 250 mg, 0.62 mmol) was dissolved in 2 N NaOH (20 mL) and was heated to reflux overnight. The mixture was cooled, acidified with concd HCl and concentrated in vacuo. The residue was dissolved in water (5 mL) and was loaded on a column of Dowex 50 W (hydrogen form), washed with H_2O, then eluted with 2 N NH_4OH. The combined fractions reactive to ninhydrin solution was concentrated to give a colorless solid (140 mg, 86%); $[\alpha]_D^{22}$ + 12.2° (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.59 (m, 2H), 1.77 (m, 2H), 2.53 (app t, J = 7.28 Hz, 2H), 3.69 (t, J = 6.05 Hz, 1H), 7.19 (s, 1H), 7.31–7.36 (2H), 7.52–7.56 (3H), 8.17 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 23.0, 23.1, 29.9, 54.6, 120.9, 126.0 (2C), 130 (3C), 133.8, 134.3, 136.3, 174.5. HRMS (FAB) calcd for $C_{14}H_{18}N_3O_2$ 260.1399, found 260.1423. Anal. calcd for $C_{14}H_{17}N_3O_2$: C 64.85; H 6.59; N 16.20. Found: C 64.69; H 6.72; N 16.12.

Using the same procedure as described above for **2a**, the following compounds were synthesized:

(*S*)-2-Amino-5-(1-phenylimidazol-4-yl)hexanoic acid (2b). $[\alpha]_D^{22}$ + 12° (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.06 (m, 2H), 1.23 (m, 2H), 1.28 (m, 1H), 1.39 (m, 1H), 2.26 (t, J = 7.30 Hz, 2H), 3.03 (t, J = 6.34 Hz, 1H), 6.76 (s, 1H), 7.00–7.03 (2H), 7.30–7.33 (3H), 7.35 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 23.3, 24.7, 27.5, 34.6, 56.0, 125.3, 125.6 (2C) 128.8, 129.7 (2C), 133.5, 135.7, 137.4, 183.4. HRMS (FAB) calcd for $C_{15}H_{20}N_3O_2$ 274.1555, found 274.1600. Anal. calcd for $C_{15}H_{19}N_3O_2 0.1H_2O$: C 65.48; H 7.03; N 15.27. Found: C 65.29; H 7.11; N 15.21.

(*S*)-2-Amino-5-(1-phenylimidazol-4-yl)heptanoic acid (2c). $[\alpha]_D^{22}$ + 10.4° (*c* 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 0.79–1.14 (4H), 1.23 (m, 2H), 1.27 (m, 1H), 1.40 (m, 1H), 2.31 (t, J = 7.51 Hz, 2H), 3.06 (t, J = 6.31 Hz, 1H), 6.79 (s, 1H), 7.10–7.14 (2H), 7.34–7.39 (3H), 7.46 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 23.5, 24.8, 26.6, 27.5, 28.3, 34.8, 56.1, 125.2, 125.7 (2C), 128.8, 129.6 (2C), 133.7, 135.9, 137.5, 183.6. HRMS (FAB) calcd for $C_{16}H_{22}N_3O_2$ 288.1712, found 288.1728. Anal. calcd for $C_{16}H_{21}N_3O_2 0.1H_2O$: C 66.46; H 7.39; N 14.53. Found: C 66.39; H 7.44; N 14.42.

Enzymes. All of the enzymes used were recombinant enzymes overexpressed in *Escherichia coli*. The murine macrophage iNOS was expressed[39] and isolated[40] as reported; the bovine endothelial eNOS was prepared as previously described,[41] and the rat neuronal nNOS was expressed and purified as described.[42]

Initial velocity measurement via the hemoglobin assay

The generation of nitric oxide by NOS was measured using the hemoglobin capture assay.[37] A typical assay mixture for nNOS and eNOS contained 10 μM L-arginine, 1.6 mM $CaCl_2$, 11.6 mg/mL calmodulin, 100 μM NADPH, 6.5 μM tetrahydrobiopterin, and 3 mM oxyhemoglobin in 100 mM HEPES (pH 7.5). The reaction mixture for iNOS contained 10 μM of L-arginine, 100 μM NADPH, 6.5 μM tetrahydrobiopterin, and 3 mM oxyhemoglobin in 100 mM HEPES (pH 7.5). All assays were in a final volume of 600 mL and were initiated with enzyme. Nitric oxide reacts with oxyhemoglobin to yield methemoglobin which is detected at 401 nM (e = 19,700 M^{-1} cm^{-1}) on a Perkin–Elmer Lamda 10 UV–vis spectrophotometer.

Reversible inhibition kinetics. The reversible inhibition of NOS by imidazole-containing amino acids was studied under initial velocity measurement conditions by the hemoglobin capture assay described above. The K_i values were determined by the methods of Dixon.[43]

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