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Communications to the Editor

Acetamidine Lysine Derivative, *N*-(5(*S*)-amino-6,7-dihydroxyheptyl)-ethanimidamide Dihydrochloride: A Highly Selective Inhibitor of Human Inducible Nitric Oxide Synthase

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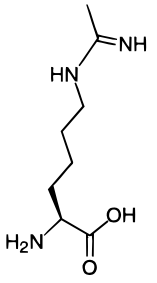
Nitric oxide (NO) is generated by the enzymatic action of nitric oxide synthase (NOS) on arginine; citrulline, likewise, is produced by this oxidative process. Unique among the properties of nitric oxide are that it is a gas with an unpaired electron and it mediates a variety of cellular processes such as regulation of vascular tone, platelet aggregation, neurotransmission, and immune activation. Several isoforms of NOS both constitutive and induced have been identified and characterized.[1] Blood pressure homeostasis is maintained by NO released by the action of the endothelial isoform (eNOS) while NO, produced by the neuronal NOS enzyme (nNOS), participates in neurotransmission in nonadrenergic, noncholinergic nerves. In addition, NO in the CNS modifies pain perception, mediates long-term potentiation and memory, and controls cerebral blood flow. Selective iNOS inhibitors have the therapeutic potential for treatment of diseases and disorders mediated by overproduction of NO.

Until recently, arginine-based NOS inhibitors that have been reported show no selectivity for the induced isoform of NOS.[2] ϵ-*N*-(Iminoethyl)-L-lysine (NIL, **1**)[3] is a selective iNOS inhibitor that has been shown to suppress the increase in plasma nitrites and joint inflammation associated with adjuvant arthritis.[4] Replacing the carboxyl moiety of NIL with a vicinal glycol yields *N*-(5(*S*)-amino-6,7-dihydroxyheptyl)ethanimidamide dihydrochloride (**2**), an iNOS inhibitor with an IC_{50} of 12 μM. This inhibition is somewhat less than that seen for NIL as shown in Table 1. However, **2**, with a 700-fold selectivity for the induced isoform versus the endothelial isoform, is strikingly selective. While, in contrast, NIL is 30-fold selective for the induced iNOS.

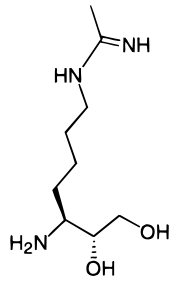
Table 1. NOS Inhibition[12]

	IC_{50} (μM)			selectivity	
compd no.	iNOS	eNOS	nNOS	eNOS/iNOS	nNOS/iNOS
NIL	5	138	61	30	12
2	12	8420	150	700	12
11	49%[a]	0%[a]	13%[a]		

[a] Percent inhibition at 100 μM.



NIL, **1**



2

Investigations of arginine-based NOS inhibitors have examined almost exclusively permutations on the guanidine moiety of arginine.[5] Modifications have included nitroguanidinyl, *N*-methylguanidinyl, aminoguanidinyl, and replacement of the guanidinyl with heterocyles.[6] Few alterations of the α-amino group have been reported.[5] To date, with the exception of L-nitroarginine methyl ester (L-NAME), no examples of carboxyl group variations have been reported.

The focus of our research has been to identify entities that are highly selective for iNOS selectivity versus eNOS and nNOS. In designing iNOS selective inhibi-

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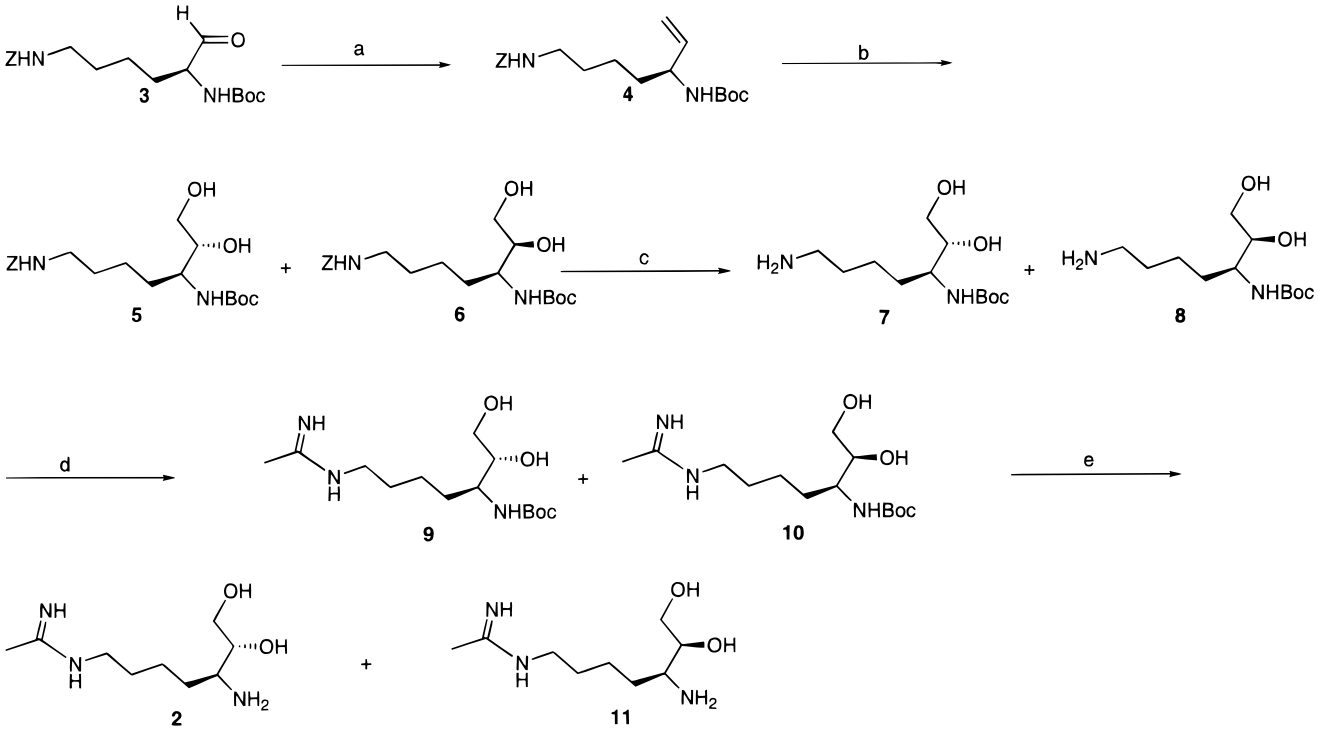
Scheme 1[a]



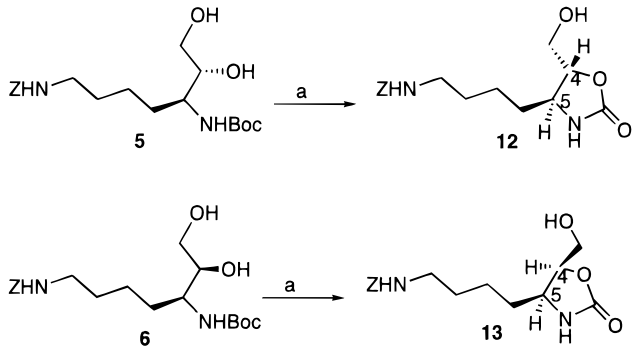
[a] (a) $CH_3P(Ph)_3Br$, KHMDS, Et_2O, rt, 16 h, 74%; (b) OsO_4, NMMO, acetone−H_2O (3:1), 84%; (c) H_2, Pd black, EtOH, quantitative; (d) DMF, TEA, $CH_3C(=NH)OMe$ HCl, rt, 4 h, 1 N HCl, reverse phase separation of diastereomers, 50%; (e) 4 N HCl in dioxane, HOAc, quantitative.

tors, carboxyl group modification of the NIL skeleton has been explored. Among the bioisosteres investigated as potential surrogates for the carboxyl group of NIL is a vicinal diol. While the acidity of the carboxyl moiety is lost with this isostere, its hydrogen acceptor−donor properties are not. Introduction of this functionality is illustrated in Scheme 1.

Commercially available protected lysine, *N*-Boc-L-Lys-(Z)-OH, is esterified quantitatively using Cs_2CO_3 and methyl iodide.[7] Aldehyde **3** is generated as previously described using DIBAL-H,[8] from which alkene **4** is synthesized using Wittig chemistry. Introduction of the vicinal diol using *N*-methylmorpholine *N*-oxide (NMMO) is catalyzed by OsO_4 to give diols **5** and **6** in a ratio of 1:1.5. Both isomers are carried on since it is unknown if either would have the desired biological activity. Catalytic hydrogenation effects the removal of the benzyloxycarbonyl group on a mixture of **5** and **6**; treating the epsilon amine with methyl acetimidate hydrochloride results in amidines **9** and **10**. Although the diastereomers **5** and **6** can be separated at the protected diol stage by column chromatography, diastereomer separation is accomplished more facilely at the penultimate step by reverse phase chromatography on YMC ODS AQ. Attempts to achieve diastereoselectivity for **5** using Sharpless vicinal diol chemistry[9] with AD-mix β effect modest enrichment of **5**:**6** of 1.8:1. Amino glycols **2** and **11** are obtained upon treating **9** and **10**, respectively, with 4 N HCl in dioxane.

Reacting **5** and **6** with potassium bis(trimethylsilyl)-amide (KHMDS) yields oxazolidinones **12** and **13**, respectively, as shown in Scheme 2, from which the stereochemistries of **5** and **6** could be ascertained. The absolute stereochemistries of oxazolidinones **12** and **13** are determined by a series of ^1H NMR experiments

Scheme 2[a]



[a] (a) KHMDS, THF, rt, 16 h.

Table 2. Chemical Shifts and Constants for H-4 and H-5 of Oxazolidinones **12** and **13**

oxazolidinone	$\delta_{H\text{-}4}$ (ppm)	$\delta_{H\text{-}5}$ (ppm)	$J_{4,5}$ (Hz)
trans-**12**	3.8	4.4	7.5
cis-**13**	4.0	4.8	8.5

including 1- and 2D NOE and TROESY experiments. As shown in Table 2, the chemical shifts of H-4 and H-5 for both oxazolidinones, **12** and **13**, and relative H-4−H-5 coupling constants are consistent with stereochemistries that have been reported for similar structures.[10] The results of the NOE and TROESY NMR experiments for **12** and **13** corroborate the assigned stereochemistries.

Aminoglycols **2** and **11** were tested for their NOS inhibitory activity by measuring the conversion of L-[2,3-^{3}H]arginine to L-[2,3-^{3}H]citrulline. Recombinant iNOS, eNOS, and nNOS were prepared and isolated as described previously.[11]

With an IC_{50} of 12 μM, **2** is slightly less potent as a iNOS inhibitor when compared to NIL. Significantly reduced inhibition of eNOS is observed as **2** has an IC_{50} of 8420 μM for eNOS resulting in a selectivity for the induced isoform of nearly 700-fold. Selectivity is defined by the ratio of the IC_{50} for eNOS or nNOS to the IC_{50} of iNOS. Inhibition of the neuronal isozyme by **2** shows a 12-fold selectivity for iNOS as shown in Table 1. In selectivity for human neuronal constitutive nitric oxide synthase (nNOS), no difference is seen between **2** and NIL. At 100 μM, **11** inhibited iNOS 49%. Chirality is crucial for activity as seen with **2** versus **11**, where **2** has approximately 4 times the potentcy of **11** as a iNOS inhibitor.

In aminoglycol **2**, we have identified a highly selective iNOS inhibitor. Furthermore, we have shown that the vicinal diol moiety, as exemplified by **2**, is an effective isostere for the carboxyl group of NIL.

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(12) No standardization of measuring iNOS inhibition has occurred in this area of research. Variation in the source of NOS, NOS purity, the presence or absence of cofactors, concentration of cofactors, presence or absence of protease inhibitors, time of exposure of inhibitor to enzyme, reaction time, composition, and pH of buffer containing enzyme all contribute to the wide variability in reported enzyme inhibitor potency. Moore, P. K.; Handy, R. L. C. Selective Inhibitors of Neuronal Nitric Oxide Synthase—Is no NOS REally good NOS for the Nervous System? *Trends Pharmacol.* **1997**, *18*, 203−311.

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