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# SYNTHESIS OF PHOSPHORUS-CONTAINING AMINO ACID ANALOGS AS INHIBITORS OF NITRIC OXIDE SYNTHASE

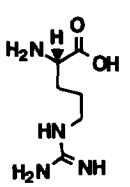
Marlon Cowart*, Elizabeth A. Kowaluk, Kathy L. Kohlhaas, Karen M. Alexander, and James F. Kerwin Jr.

*Neuroscience Research, Abbott Laboratories, D-4PM, AP10, Abbott Park, IL 60064-3500, U.S.A.*

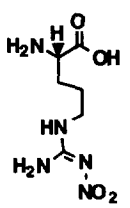
**Abstract:** Two series of α-amino phosphonic and α-amino phosphinic analogs of arginine were prepared and tested as inhibitors of nitric oxide synthase (NOS). Two of the analogs were found to possess inhibitory activity for the neuronal isoform of NOS. Copyright © 1996 Elsevier Science Ltd

Nitric oxide (NO·) plays an important role in many physiological and pathological processes.[1,2] NO· and its coproduct L-citrulline are produced by the enzymatic oxidation of L-arginine by nitric oxide synthase (NOS). Three isoforms of NOS have been identified to date: iNOS, the inducible isoform found in macrophages, produces NO·, which is a cytotoxic agent important in both the normal immune response and in pathological conditions like septic shock; eNOS, the constitutive isoform found in endothelial cells, produces NO·, which is involved in the regulation of blood pressure; nNOS is the constitutive isoform found in the neurons. NO· produced by eNOS or nNOS can act as a second messenger molecule, diffusing out of the cell and binding to guanylate cyclase in neighboring cells, thereby stimulating cGMP formation.

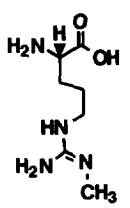
L-Arginine (1) is the endogenous substrate of NOS. Prototypical NOS inhibitors are L-arginine analogs, most significantly L-$N^G$-nitro-arginine (L-NNA, 2) and L-$N^G$-methyl-arginine (L-NMA, 3). These inhibitors however, display low selectivity for inhibition of nNOS versus iNOS. We sought arginine analogs with enhanced potency and nNOS selectivity to probe the role of nNOS in CNS pathophysiology.




L-Arginine
(1)




L-NNA
(2)

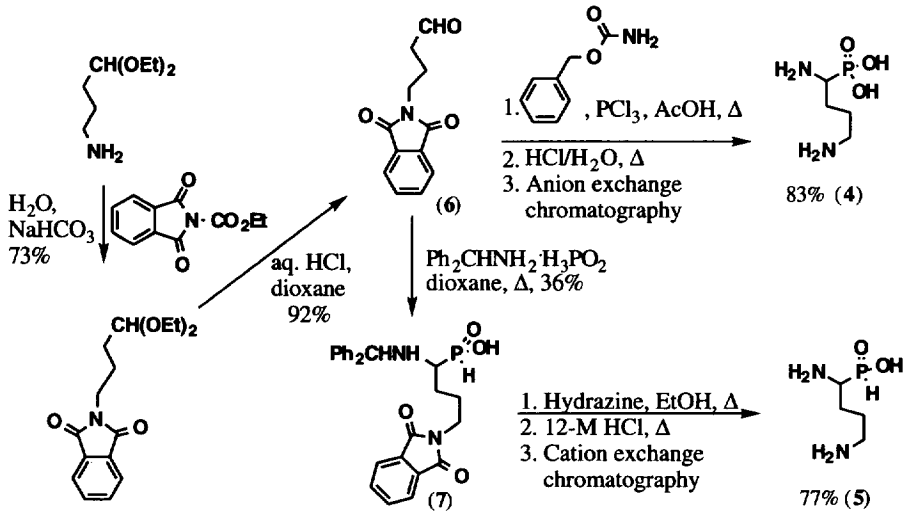



L-NMA
(3)

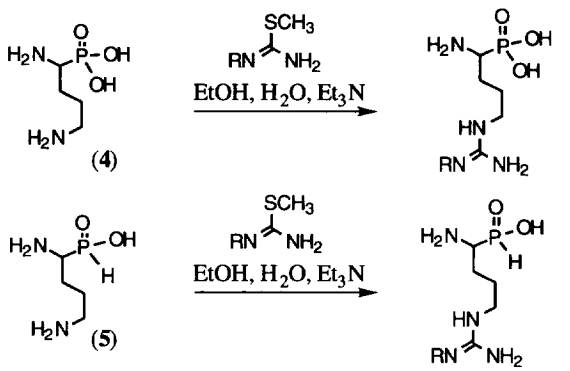
**Figure 1**

Ornithine analogs (**4** and **5**) containing a phosphorus moiety in place of the carboxylic acid were synthesized as shown in Scheme 1. The phosphonic acid analog of ornithine (**4**) was synthesized by the method of Sosnovsky et al.[3] by the reaction of aldehyde **6** with benzyl carbamate and phosphorus trichloride, followed by hydrolysis in 4N-HCl, and purified by ion exchange chromatography. The phosphonous acid analog of ornithine (**5**) was prepared by the method of Baylis et al.[4] by reaction of the aldehyde **6** with diphenylmethylammonium hypophosphate to give **7**. Deprotection with hydrazine, followed by hydrolysis in 12N-HCl, and purification by ion exchange, provided **5**.

**Scheme 1**




As depicted in Scheme 2, target phosphonic acid analogs **8**–**10** were prepared by reaction of **4** with the appropriate thiopseudourea in the presence of triethylamine in aqueous ethanol. Purification[5] by ion exchange chromatography provided **8**[6] (32%), **9** (80%), and **10** (30%). Target phosphinic acid analogs **11**–**13** were prepared from **5** using similar conditions, and following purification by ion exchange, **11** (90%), **12** (69%), and **13** (88%) were obtained.[7]




**Scheme 2**

(**8**) R = H 32%
(**9**) R = Me 80%
(**10**) R = $NO_2$ 30%
(**11**) R = H 90%
(**12**) R = Me 69%
(**13**) R = $NO_2$ 88%

The ability of the six phosphorus analogs of the amino acid arginine to inhibit brain derived nitric oxide synthase (nNOS), or the inducible nitric oxide synthase enzyme from macrophage (iNOS), is

depicted in Table 1, and was determined according to well established methods.[8] All three α-amino-ε-guanidino phosphonic acid analogs (**8–10**) with the phosphorus in the (V) oxidation state were inactive against NOS at concentrations up to 100 μM. Of the phosphonous acid analogs (**11–13**) containing phosphorus in the (III) oxidation state, two compounds (**12** and **13**) demonstrated inhibitory activity toward the nNOS. This may be rationalized by the observation that the phosphonous acid moiety bears one negative charge at physiological pH, whereas the phosphonic acid moiety bears two negative charges[4] at physiological pH. Thus, the phosphonous acid analogs exist as zwitterionic species most like the natural amino acid analogs. It should also be noted that all the analogs described herein are racemates. If most of the enzyme inhibitory activity resides in the L-isomer, as in the case of the amino acid analogs arginine and NNA, the use of synthetic methodology allowing the asymmetric preparation of the L-isomers of phosphorus-containing analogs[9] of **12** and **13**, might produce analogs with increased potency.

**Table 1.** $IC_{50}$ determination. In vitro inhibition of NOS by amino acid analogs.

| Compound | nNOS $IC_{50}$ (μM) | iNOS $IC_{50}$ (μM) |
|---|---|---|
| **2** (L-NNA) | 0.6 | 10 |
| **3** (L-NMA) | 10 | 25 |
| **8** | >100 | >100 |
| **9** | >100 | >100 |
| **10** | >100 | >100 |
| **11** | >100 | >100 |
| **12** | 100 | >100 |
| **13** | 45 | >100 |

## References and Notes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2. Feldman, P. L.; Griffith, O. W.; Stuehr, D. J. *C&E News* **1993**, *71*, 26.
3. Sosnovsky, G.; Lukszo, J.; Gravela, E.; Zuretti, M. F. *J. Med. Chem.* **1985**, *28*, 1350.
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5. Compounds **8**, **9**, **11**, and **12** were purified by chromatography on Amberlite CG-120 cation exchange resin (ammonium form), **10** was purified on Amberlite CG-400 anion exchange resin ($HCO_3$-form), eluting with ammonium bicarbonate. Compound **13** was recrystallized from $CH_3CN/AcOH/H_2O$.

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7. Compounds were examined by $^{1}H$ and $^{13}C$ NMR and $^{31}P$ NMR, MS, and combustion analysis, and were consistent with the structures proposed. Combustion analysis calculated for **8** as $C_5H_{15}N_4PO_3(H_2CO_3)(0.6\ NH_3)(H_2O)$: C, 23.99; H, 6.98; N, 21.45; found: C, 24.01; H, 7.06; N, 21.38. Calculated for **9** as $C_6H_{17}N_4PO_3(2.0\ HCl)(0.1\ H_2O)$: C, 24.11; H, 6.47; N, 18.74; found: C, 24.18; H, 6.48; N, 18.62. Calculated for **10** as $C_5H_{14}N_5O_5(2.8\ H_2O)$: C, 19.65; H, 6.46; N, 22.92; found: C, 20.01; H, 6.85; N, 23.27. Calculated for **11** as a salt with 8-hydroxy-5,7-dinitro-2-naphthalenesulfonic acid $C_5H_{15}N_4PO_2(2.0\ H_2O)(2.0\ C_{10}H_6N_2SO_8)$: C, 34.97; H, 3.64; N, 13.05; found: C, 35.12; H, 3.39; N, 12.95. Calculated for **12** as $C_6H_{17}N_4PO_2(2.0\ HCl)(2.0\ H_2O)$: C, 24.09; H, 7.07; N, 18.73; found: C, 24.37; H, 6.74; N, 18.69. Calculated for **13** as $C_5H_{14}N_5O_4P(0.9\ H_2O)$: C, 23.52; H, 6.24; N, 27.42; found: C, 23.30; H, 6.20; N, 27.75. Selected 300 MHz $^{1}H$ NMR data δ (ppm), J (Hz): **8** ($D_2O$) δ 3.22 (t, 2H, $J = 3.3$), 3.04 (m, 1H), 1.7–2.0 (m, 4H); **9** ($D_2O$) δ 3.23 (m, 3H), 2.80 (s, 3H), 1.7–2.0 (m, 4H); **11** (NaOD) δ 6.7 (d, 1H, $J_{H\text{-}P} = 300$), 3.13 (t, 2H, $J = 3$), 2.58 (m, 1H), 1.4–1.8 (m, 4H); **12** ($D_2O$) δ 6.80 (d, 1H, $J_{H\text{-}P} = 300$), 3.23 (m, 3H), 2.69 (m, 2H), 1.5–1.9 (m, 4H); **13** (NaOD/DMSO) δ 6.55 (d, 1H, $J_{H\text{-}P} = 280$), 3.08 (m, 2H), 2.23 (m, 1H), 1.2–1.7 (m, 4H). Selected 75 MHz $^{13}C$ NMR data δ (ppm relative to 3-trimethylsilylpropionic acid as external reference), J (Hz): **8** ($D_2O$) δ 159.99, 53.27 (d, $J_{C\text{-}P} = 132$), 43.90, 29.64, 28.55, (d, $J_{C\text{-}P} = 8.6$); **9** ($D_2O$) δ 156.31, 49.91 (d, $J_{C\text{-}P} = 133$), 40.54, 27.20, 26.34, 25.36 (d, $J_{C\text{-}P} = 8$); **10** ($D_2O$) δ 157.38, 47.46 (d, $J_{C\text{-}P} = 143$), 39.21, 24.50, 23.23 (d, $J_{C\text{-}P} = 8$);**11** ($D_2O$) δ 156.66, 50.15 (d, J $J_{C\text{-}P} = 97$), 40.85, 26.07, 24.85 (d, $J_{C\text{-}P} = 10.6$); **12** ($D_2O$) δ 158.81, 52.59 (d, $J_{C\text{-}P} = 99$), 43.23, 29.70, 28.52, 27.39 (d, $J_{C\text{-}P} = 10$); **13** (DMSO) δ 159.31, 49.63 (d, $J_{C\text{-}P} = 92$), 40.32, 24.77, 24.51 (d, $J_{C\text{-}P} = 10.6$). Selected proton-decoupled 200 MHz $^{31}P$ NMR data (δ ppm relative to $H_3PO_4$ as external reference): **8** ($D_2O$) δ 10.5; **9** ($D_2O$) δ 13.1; **11** (NaOD) δ 32.39; **12** ($D_2O$) δ 19.59; **13** (NaOD/DMSO) δ 27.76. Mass spectral data: **8** $(m\text{-}H)^-$ *m/z* 209; **9** $(m+H)^+$ *m/z* 225; **10** $(m\text{-}H)^-$ *m/z* 254; **11** $(m+H)^+$ *m/z* 195; **12** $(m+H)^+$ *m/z* 209; **13** $(m\text{-}H)^-$ *m/z* 238. Melting points of compounds **11**: mp 168–169 °C dec.; **13:** mp 255–256 °C.

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