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Syntheses of New Racemic N^G-(1-Iminoethyl)phosphalysine Derivatives as Potential Inhibitors of Nitric Oxide Synthase

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ABSTRACT: *The efficient syntheses of three new racemic N^G-(1-iminoethyl)phosphalysine derivatives are reported where the lysine carboxylate group is systematically replaced by phosphonic acid,* **4,** *methyl phosphinic acid,* **5,** *and phosphinic acid,* **6.** *These compounds were evaluated as potential inhibitors of the three isoforms of human nitric oxide synthase.* © 2000 John Wiley & Sons, Inc. Heteroatom Chem 11:505–511, 2000

INTRODUCTION

Nitric oxide (NO) is an endogenously produced free radical that plays an important role in many physiological processes [1]. NO is produced by the conversion of L-arginine to L-citrulline by a five-electron oxidation reaction catalyzed by nitric oxide synthase (NOS) [2]. To date, three distinct human isoforms of NOS have been identified [3]. The constitutive isoforms, which require Ca^{2+} and calmodulin for activity, include the neuronal (hncNOS) and endothelial (hecNOS) isoforms that are found mainly in the brain and the vascular endothelium, respectively. Nitric oxide produced by hncNOS appears to act as a neurotransmitter, whereas the NO produced by hecNOS helps regulate blood pressure and platelet aggregation [4–6]. In contrast, the inducible human isoform (hiNOS) is found in activated macrophages and is not activated by Ca^{2+} or calmodulin. Its major function is in host defense, and overproduction of NO by hiNOS is implicated in multiple disease states [7–9]. Therefore, hiNOS represents an attractive therapeutic target, and several approaches for selective inhibitors have been identified for the potential treatment of these diseases [10–12].

Since L-arginine is the natural substrate of NOS, and the NOS reaction takes place at the N^G-guanidino group, several N^G-modified arginine derivatives have been prepared as possible inhibitors of NOS [13–15]. A series of racemic arginine analogs containing replacements for the α-amino acid group by various α-amino phosphorus moieties have also been reported that include the phosphonic acid, **1**, and the phosphinic acid analog of L-N^G-nitro-arginine, **2**. Unfortunately, neither **1** or **2** exhibited significant inhibitory activity for NOS ($IC_{50} > 100\ \mu M$) [16]. In contrast to most arginine derivatives, a related amino acid, L-N^6-(1-iminoethyl)-lysine (L-NIL, **3**), has been shown to be a potent ($IC_{50} = 3.3\ \mu M$) and selective inhibitor of murine iNOS [17]. The x-ray crystal structure of arginine bound to the oxygenase domain of inducible murine NOS has been published [18]. Examination of this structure indicates that the protein interactions surrounding the α-amino acid region should allow variation of the α-amino acid group with larger moieties.

Consequently, we chose to explore analogs of **3**,

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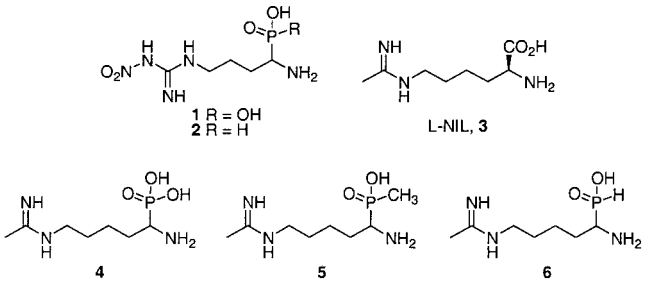
where the α-amino acid group was replaced by phosphonic or phosphinic acids to see if such groups could be accommodated in the context of a different inhibitor framework. Three new racemic analogs of NIL, including the α-aminophosphonic, **4**, α-amino methylphosphinic, **5**, and α-aminophosphinic, **6**, acids were targeted to explore the effects of varying size, shape, and pK_a of the acidic functionality on potency and selectivity [19]. This manuscript describes the synthesis of these three NIL analogs, as well as their evaluations as potential inhibitors of the three human NOS isoforms.



CHEMISTRY

A common feature in the preparation of all three analogs involved the addition of a phosphorous compound to an activated imine prepared from a suitably protected aminopentanal. For the synthesis of racemic **4**, the α-amino phosphonic acid moiety is introduced through the addition of lithium diethylphosphite to a benzylimine [20]. The synthetic sequence (Scheme 1) began with mono-Boc protection of the amine in aminopentanol to give **7** [21]. Subsequent Swern oxidation gave the corresponding aldehyde, however, the product could not be isolated possibly due to internal cyclization. The desired imine was obtained by addition of benzylamine to the crude reaction mixture from the Swern oxidation, and addition of lithium diethylphosphite gave the desired protected α-amino diethyl phosphonate ester, **8**. Purification of **8** from excess diethyl phosphite proved difficult, but removal of the Boc group under acidic conditions allowed isolation of the pure phosphono-lysine analog, **9**. The conversion of **9** to the corresponding (1-iminoethyl)lysine analog was carried out with ethyl acetimidate hydrochloride in the presence of 1,8-diabicyclo[5.4.0]undec-7-ene (DBU) to give the protected product, **10** [17]. The desired fully deprotected α-amino phosphonic acid, **4**, was obtained as a dihydrochloride salt by catalytic hydrogenation in the presence of $Pd(OH)_2$ on carbon to remove the *N*-benzyl group followed by hydrolysis of the diethyl ester with concentrated aqueous HCl. The product was isolated as a glassy, hygroscopic solid, and its characterization by ^{1}H NMR, ^{31}P NMR, HRMS, and microanalyses was consistent with the desired product being obtained in greater than 95% purity.

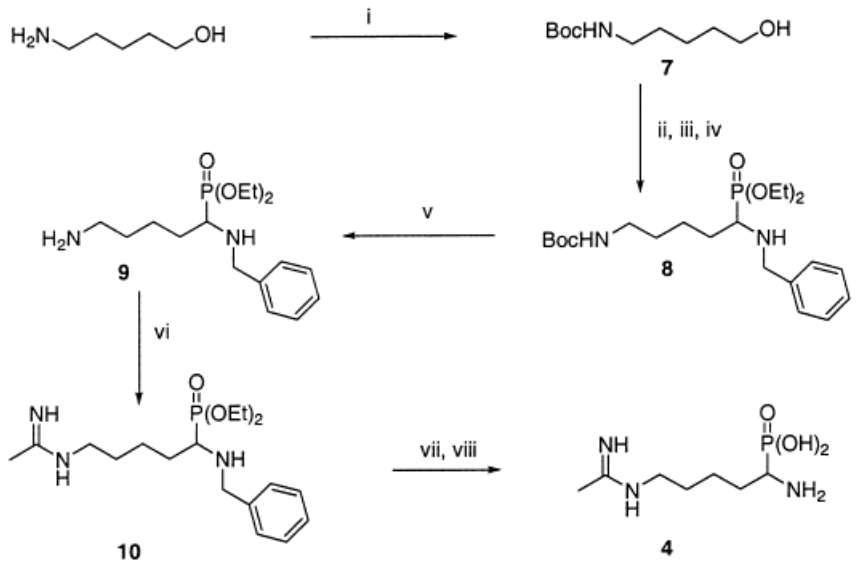
The methyl phosphinic acid analog, **5**, was prepared in a similar manner from the fully protected aminopentanal (Scheme 2). The phthalimide-protected aminopentanol, **11** [22], underwent Swern oxidation to allow isolation of the desired aldehyde, **12**. A three-component reaction with aldehyde **12**, benzyl carbamate, and dichloromethylphosphine proceeded smoothly in the presence of acetyl chloride to give the Z-protected α-amino methylphosphinic acid, **13a** [23]. The related protected α-amino phosphinic acid, **13b**, was prepared by addition of phosphonous acid to the diphenylmethyleneimine. In contrast to the stepwise method used in Scheme 1, the reaction of the aldehyde, **12**, with the diphenylmethylamine salt of phosphonous acid in refluxing ethanol gave the protected α-amino phosphinic acid, **13b**, directly [24].

The phthalimide-protected lysine analogs **13a** and **13b** were similarly converted to the desired deprotected products (Scheme 3). Removal of the phthalimide was accomplished with hydrazine in refluxing ethanol to give the amines **14a** and **14b**. The 1-iminoethyl moiety was introduced as described in Scheme 1 to give **15**. The final racemic products, **5** and **6**, were obtained by deprotection of the α-amino group under acidic conditions to give the desired α-amino phosphinic acids as dihydrochloride salts. Both products were isolated as white, hygroscopic foams and characterized by ^{1}H NMR, ^{31}P NMR, HRMS, and microanalyses. The analytical data for the methylphosphinic acid, **5**, were all satisfactory, whereas the phosphinic acid, **6**, was contaminated with a small amount (approx. 5% by microanalyses) of diphenylmethane.

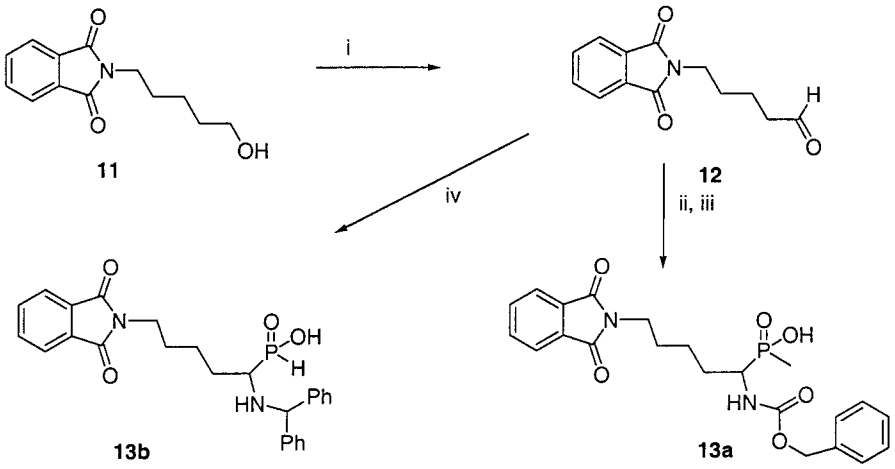
RESULTS AND DISCUSSION

The analytically pure targets **4, 5**, and **6** were evaluated as potential inhibitors of the three human NOS isoforms using previously described assays [25]. Unfortunately, none of these compounds displayed significant activity against any of the enzymes tested under conditions in which **3** was used as a positive control (Table 1).

These results confirm those observed previously with the phospha-arginine derivatives, **1** and **2**. None of the NOS isoforms are able to tolerate replacement of the α-amino acid carboxylate functionality with the larger phosphonic or phosphinic acid groups. These results are especially surprising given the high potency and selectivity observed with **16** in which

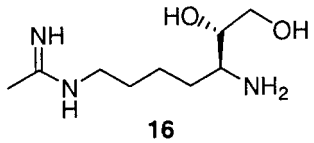


SCHEME 1 Reaction Conditions: (i) Boc_2O, CH_2Cl_2; (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C; (iii) benzylamine, $MgSO_4$, CH_2Cl_2; (iv) $LiP(O)(OEt)_2$, THF, 0°C–r.t.; (v) 1N HCl (vi) ethyl acetimidate HCl, *N,N*-diisopropylethylamine, EtOH, r.t.; (vii) $Pd(OH)_2$ on C, MeOH; (viii) conc. HCl, reflux.



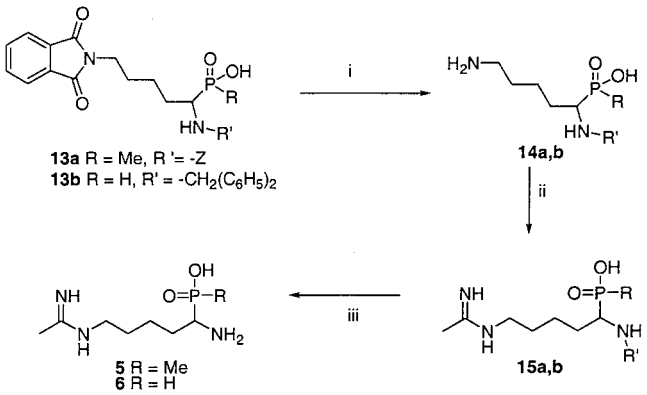
SCHEME 2 Reaction Conditions: (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C; (ii) AcCl, benzyl carbamate, $MePCl_2$, r.t. (iii) H_2O, THF, r.t. (iv) $Ph_2CHNH_3^+H_3PO_2^-$, EtOH, reflux.

the carboxylate moiety in **3** has been reduced to introduce a chiral diol motif [26].



EXPERIMENTAL

Proton and ^{31}P NMR spectra were recorded on either a Varian Unity Plus 300 (300 MHz) or a Varian Unity Inova 400 (400 MHz) spectrometer. All proton chemical shifts are recorded in ppm (δ) relative to trimethylsilane (TMS), and ^{31}P chemical shifts are referenced to H_3PO_4. Column chromatography was performed on silica gel (200–400 mesh). Reverse-phase chromatography was performed on a Gilson semipreparative HPLC with a YMC Combiprep ODS-A semiprep column eluting with acetonitrile/water (0.1% TFA) at 20 mL/min. Microanalyses were performed at Searle analytical lab (Skokie, IL). Mass spectra was obtained on a HP series 1100MSD, and high-resolution mass spectra were obtained with a PerSeptive Biosystems Mariner TOF. All solvents



SCHEME 3 Reaction Conditions: (i) N_2H_4, EtOH; (ii) ethyl acetimidate HCl, N,N-diisopropyl ethyl amine, EtOH, r.t.; (iii) conc. HCl, reflux.

TABLE 1 Inhibitory Properties of α-Amino Phospha-NIL Analogs Versus Three Human NOS Enzymes.[a]

Inhibitor	*hiNOS* IC_{50} (μM)	hecNOS IC_{50} (μM)	hncNOS IC_{50} (μM)
3	5	135	55
4	> 100	> 100	> 100
5	> 100	> 100	> 100
6	> 100	> 100	> 100

[a]See Ref. [25].

and reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of Carbamic Acid, [5-(phenylmethylamino)-5-diethoxyphosphinylpentyl]-, C-(1,1-dimethyl-ethyl) Ester, 8

To a solution of oxalyl chloride (24 mmol) in CH_2Cl_2 (30 mL) at −78°C was added DMSO (3.0 mL, 45 mmol). After 5 minutes, a CH_2Cl_2 (20 mL) solution of **7** [21] (2.13 g, 10.5 mmol) was added, and the stirring continued at −78°C for 30 minutes. Neat triethylamine (30 mL) was added, and the resulting slurry was stirred at −78°C for 20 minutes. The reaction mixture was allowed to reach room temperature, stirred for 15 minutes, and poured into water (100 mL). The organic layer was separated and washed with 0.5 *N* citric acid and saturated $NaHCO_3$. To the CH_2Cl_2 solution of the crude aldehyde was added benzylamine (1.0 mL, 10.9 mmol) and $MgSO_4$. The resulting slurry was stirred for 1.5 hours at room temperature, filtered, and the filtrate stripped to a give the crude imine as a yellow oil (confirmed by ^{1}H NMR). In a separate flask, a THF (15 mL) solution of diethyl phosphite (3.4 mmol, 26 mmol) was cooled in an ice bath and *n*-BuLi in hexanes (6.9 mL, 1.6 *M*, 11 mmol) was added dropwise. After 30 minutes, the ice bath was removed, and the solution was allowed to reach room temperature. To this solution was added a THF (20 mL) solution of the above imine, and the reaction mixture was stirred for 18 hours at room temperature. The reaction was poured into water (30 mL), and the organic solvents were removed in vacuo. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated to give a yellow oil. The oil was purified by silica gel chromatography eluting with 2% EtOH in EtOAc to give a mixture of the desired α-amino phosphonate diester and diethyl phosphite as a pale yellow oil (1.90 g). This product was taken forward crude without further purification. MS: m/z = 429.3 $[M+H]^+$.

Preparation of [5-amino-1-[(phenylmethyl) amino]pentyl]phosphonic Acid, Diethyl Ester, 9

To an ethyl acetate (30 mL) solution of crude **8** (1.90 g) was added 1N HCl (20 mL), and the mixture was stirred for 18 hours. The aqueous layer was separated and adjusted to pH 9 (saturated NH_4OH) and extracted with EtOAc (3 × 30 mL) and CH_2Cl_2 (2 × 30 mL). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to give 0.36 g (10% yield based on **7**) of the desired product as a clear colorless oil. HRMS calcd. for $C_{16}H_{29}N_2O_3P$: m/z = 329.1994 $[M+H]^+$, found: 329.2013. ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 6H), 1.4–2.0 (m, 9H), 2.70 (t, 2H), 2.87 (m, 1H), 3.95 (dd, 4H), 4.15 (m, 4H), 7.3 (m, 5H). ^{31}P NMR ($CDCl_3$) δ 29.29 (s).

Preparation of [5-[(1-iminoethyl)amino]-1-[(phenylmethyl)amino]pentyl]phosphonic Acid, Diethyl Ester, Mono(trifluoroacetate), 10

To an ethanol (5 mL) solution of **9** (0.35 g, 1.1 mmol) was added ethyl acetimidate hydrochloride (0.20 g, 1.6 mmol) followed by *N,N*-diisopropylethylamine (0.282 mL, 1.62 mmol). The clear solution was stirred at room temperature for 18 hours, and additional ethyl acetimidate hydrochloride (0.050 g, 0.4 mmol) and *N,N*-diisopropylethylamine (0.070 mL, 0.40 mmol) were added. After 4 hours, the solvent was removed in vacuo, and the resulting oil was purified by reverse-phase HPLC using a YMC Combiprep ODS-A semiprep column eluting with a 6 minute gradient of 0–45% CH_3CN/H_2O. Fractions containing product were combined and concentrated to afford approximately 120 mg (23%) of the desired acetamidine product as a trifluoroacetate

salt. HRMS calcd. for $C_{18}H_{33}N_3O_3P$: m/z = 370.2260 $[M+H]^+$, found: 370.2273. ^1H NMR ($CDCl_3$) δ 1.38 (t, 6H), 1.58 (br s, 4H), 1.92 (br s, 2H), 2.10 (s, 3H), 3.20 (br s, 2H), 3.4 (m, 1H), 4.18 (m, 4H), 4.3 (dd, 2H), 7.4 (m, 5H), 8.28 (s, 1H), 8.58 (s, 1H), 9.43 (s, 1H). ^{31}P NMR ($CDCl_3$) δ 30.99 (s).

Preparation of [1-amino-5-[(1-iminoethyl) amino]pentyl]phosphonic Acid, Dihydrochloride, **4**

A methanol (5 mL) solution of **10** (120 mg) was stirred under a hydrogen atmosphere (60 psi) in the presence of $Pd(OH)_2$ on C (70 mg) for 4 hours at room temperature. The slurry was filtered through celite, and the filtrate was stripped to an give the diethyl ester as an oil. The ester was dissolved in concentrated HCl and heated to 98°C for 18 hours. The solution was concentrated in vacuo, taken up in water and again stripped in vacuo. The resulting solid was dried in vacuo at 60°C for 1 hour to give 15 mg (14%) of the desired product as a pale yellow glass. Anal. calcd. for $C_7H_{18}N_3O_3P \cdot 2.0\ HCl \cdot 2.0\ H_2O$: C, 25.31; H, 7.28; N, 12.65. Found: C, 25.33; H, 7.14; N, 12.53. HRMS calcd. for $C_7H_{19}N_3O_3P$: m/z = 224.1164 $[M+H]^+$, found: 224.1166. ^1H NMR (D_2O) δ 1.4–1.8 (br m, 6H), 2.25 (s, 3H), 3.12 (t, 2H), 3.18 (s, 1H). ^{31}P NMR (D_2O) δ 14.27(s).

Preparation of 5-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)pentanal, **12**

To a CH_2Cl_2 (40 mL) solution of oxalyl chloride (32 mmol) at −78°C was added DMSO (2.4 mL, 34 mmol) dropwise. After 5 minutes, a CH_2Cl_2 (20 mL) solution of 5-phthalimido-1-pentanal (4.99 g, 21.1 mmol) was added, and the stirring continued at −78°C for 25 minutes. Neat triethylamine (30 mL) was added, and the resulting slurry was stirred at −78°C for 2 hours. The reaction mixture was allowed to reach room temperature, stirred 15 minutes, and poured into water (100 mL). The reaction mixture was extracted with ethyl acetate (150 mL), and the extracts were washed with 0.5 N citric acid and saturated $NaHCO_3$. Evaporation of the solvent gave 4.78 g (98%) of the desired aldehyde as a pale orange oil. ^1H NMR ($CDCl_3$) δ 1.63 (m, 4H), 2.47 (t, 2H), 3.68 (t, 2H), 7.78 (dd, 4H), 9.74 (s, 1H).

Preparation of Carbamic acid, [5-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-1-(hydroxymethyphosphinyl)-pentyl]-, phenylmethyl ester, **13a**

To an ice cooled slurry of benzyl carbamate (3.17 g, 21.0 mmol) and dichloromethylphosphine (1.87 mL, 21.0 mmol) in acetyl chloride (20 mL) was added a solution of **12** (4.78 g, 20.7 mmol) in acetyl chloride (5 mL) dropwise over 10 minutes. The reaction mixture was allowed to reach room temperature, stirred for one hour, and the solvent was removed in vacuo. The residue was dissolved in THF (20 mL) and slowly hydrolyzed by the dropwise addition of water (0.5 mL). After 10 minutes, the solvent was removed in vacuo to give 9.00 g (98%) of the desired protected α-amino methylphosphinic acid as an oil. MS: m/z = 445.2 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ 1.38 (t, 6H), 1.58 (br s, 4H), 1.92 (br s, 2H), 2.10 (s, 3H), 3.20 (br s, 2H), 3.4 (m, 1H), 4.18 (m, 4H), 4.3 (dd, 2H), 7.4 (m, 5H), 8.28 (s, 1H), 8.58 (s, 1H), 9.43 (s, 1H). ^{31}P NMR ($CDCl_3$) δ 54.08 (s).

Preparation of Carbamic Acid, [1-(hydroxymethylphosphinyl)-5-(aminpentyl)]-, Phenylmethyl Ester, **14a**

Neat hydrazine hydrate (1.7 mL, 30 mmol) was added to an ethanol (60 mL) solution of **13a** (9.00 g, 20.2 mmol), and the solution was brought to reflux for one hour. The resulting viscous slurry was diluted with ethanol (30 mL), and additional hydrazine hydrate (0.85 mL, 15. mmol) was added. After an additional 30 minutes of reflux, water (20 mL) and a third portion of hydrazine hydrate (0.85 mL, 15. mmol) were added. The reflux was continued for 30 minutes, at which time TLC indicated the consumption of starting material. The reaction mixture was cooled to room temperature, filtered, and the filtrate was stripped to give a mixture of the desired free amine product and phthalylhydrazide. The crude product was used in the next step without additional purification. MS: m/z = 315.0 $[M+H]^+$.

Preparation of Carbamic Acid, [1-(hydroxymethylphosphinyl)-5-[(1-iminoethyl)amino] pentyl]-, Phenyl-methyl Ester, Mono(trifluoroacetate) Salt **15a**

To a slurry of crude **14a** (0.84 g) in ethanol (15 mL) was added ethyl acetimidate hydrochloride (0.50 g, 4.1 mmol) followed by DBU (0.83 mL, 5.6 mmol). The slurry was stirred at room temperature 2 hours, and additional ethyl acetimidate hydrochloride (0.25 g, 2.0 mmol) and DBU (0.30 mL, 2.0 mmol) were added. After 18 hours, the solvent was removed in vacuo, and the resulting oil was purified by reverse-phase HPLC using a YMC Combiprep ODS-A semi-prep column eluting with a 6 minute gradient of 5–45% CH_3CN/H_2O. Fractions containing product were combined and concentrated to afford 108 mg of the desired acetamidine product as a trifluoroacetate

salt (12% based on **13a**). HRMS calcd. for $C_{16}H_{27}N_3O_4P$: 356.1739 $[M+H]^+$, found: 356.1742. ^1H NMR (CD_3OD) δ 1.38 (d, 3H), 1.6 (m, 4H), 1.86 (m, 2H), 2.17 (s, 3H), 3.18 (m, 2H), 3.29 (m, 1H), 3.81 (m, 1H), 5.10 (m, 2H), 7.3 (m, 5H). ^{31}P NMR (CD_3OD) δ 50.23 (s).

Preparation of [1-amino-5-[(1-iminoethyl) amino]pentyl]methylphosphinic Acid, Dihydrochloride, **5**

A solution of **15a** (108 mg, 0.23 mmol) in 6 N HCl (10 mL) was refluxed for 3 hours. The solvent was stripped to give a gum that was triturated with methanol and stripped to give a white foam. The foam was dried in vacuo at room temperature for 3 hours to give 15 mg (20%) of the desired product as a white foam. Anal. calcd. for $C_8H_{20}N_3O_2P \cdot 2.0\ HCl \cdot 1.5\ H_2O$: C, 29.92; H, 7.85; N, 13.08. Found: C, 29.87; H, 7.66; N, 12.86. HRMS calcd. for $C_8H_{21}N_3O_2P$: m/z = 222.1371 $[M+H]^+$, found: 222.1357. ^1H NMR (D_2O) δ 1.33 (d, 3H), 1.6 (m, 4H), 1.84 (m, 2H), 2.09 (s, 3H), 3.18 (t, 2H), 3.24 (s, 1H), 3.81 (m, 1H). ^{31}P NMR (D_2O) δ 38.88 (s).

Preparation of [5-amino-1-[(diphenylmethyl)amino]pentyl] methylphosphinic Acid, **14b**

A slurry of **13b** [24] (1.30 g, 2.8 mmol) and hydrazine hydrate (0.24 mL, 4.2 mmol) in ethanol (20 mL) was heated to reflux giving a clear solution. After 1 hour of reflux, additional hydrazine hydrate (0.24 mL, 4.2 mmol) was added, and the solution was refluxed for 4 hours. The solution was cooled to room temperature, and the precipitate was removed by filtration. Evaporation of the filtrate gave 0.91 g (98%) of the desired amine as a white foam. MS: m/z = 333.1 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ 1.6–1.8 (m, 6H), 2.43 (m, 1H), 2.86 (t, 2H), 3.28 (s, 2H), 5.19 (s, 1H), 7.2–7.4 (m, 10H).

Preparation of [5-(1-imino-ethyl)amino-1-[(diphenylmethyl)amino] pentyl]methyl-phosphinic Acid, **15b**

To an ethanol solution of **14b** (0.50 g, 1.50 mmol) was added ethyl acetimidate hydrochloride (0.28 g, 2.3 mmol) followed by DBU (0.44 mL, 2.9 mmol). After 18 hours, the solvent was removed in vacuo, and the resulting foam was purified by reverse-phase HPLC using a YMC Combiprep ODS-A semiprep column eluting with a 6 minute gradient of 5–60% CH_3CN/H_2O. Fractions containing product were combined and concentrated to afford 300 mg (41%) of the desired acetamidine product as a trifluoroacetate salt. MS: m/z = 374.2 $[M+H]^+$. ^1H NMR (CD_3OD) δ 1.5 (m, 4H), 1.9 (m, 2H), 2.20 (s, 3H), 2.83 (m, 1H), 3.17 (t, 2H), 2.28 (s, 2H), 6.10 (s, 1H), 7.2–7.4 (m, 10H). ^{31}P NMR (CD_3OD) δ 18.50 (s).

Preparation of [1-amino-5-[(1-iminoethyl) amino]pentyl]phosphinic Acid, Hydrochloride, **6**

A solution of **15b** (0.30 g, 0.62 mmol) in 6 N HCl (10 mL) was refluxed for 18 hours and cooled to room temperature. The solution was diluted with water (20 mL) and washed twice with diethyl ether. The aqueous layer was stripped and dried in vacuo for 4 hours to give 11 mg (5%) of the desired α-aminophosphinic acid as a foam. Anal. calcd. for $C_7H_{18}N_3O_2P \cdot 2.3\ HCl \cdot 2.0\ H_2O \cdot 0.05\ C_{13}H_{12}$: C, 27.39; H, 7.48; N, 12.52. Found: C, 27.67; H, 7.27; N, 12.23. HRMS calcd. for $C_7H_{19}N_3O_2P$: m/z = 208.1215 $[M+H]^+$, found: 208.1224. ^1H NMR (CD_3OD) δ 1.6–1.8 (m, 6H), 2.22 (s, 3H), 3.26 (s, 2H), 3.42 (br s, 1H), 8.42 (s, 1H), 8.98 (s, 1H), 9.30 (s, 1H). ^{31}P NMR (CD_3OD) δ 25 (br s).

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REFERENCES

[1] Bredt, D. S. Free Radical Res 1999, 31, 577–596.
[2] Marletta, M. A.; Hurshman, A. R.; Rusche, K. M. Curr Opin Chem Biol 1998, 2, 656–663.
[3] Mocada, S.; Palmer, R. M. J.; Higgs, E. A. Pharmacol Rev 1991, 43, 109–142.
[4] Bredt, D. S.; Hwang, P. M.; Snyder, S. H. Nature 1990, 347, 768–770.
[5] Garthwaite, J. Trends Neurosci 1991, 14, 60–67.
[6] Pollock, J. S.; Forstermann, U.; Mitchell, J. A.; Warner, T. D.; Schmidt, H. H. H. W.; Nakane, M.; Murad, F. Proc Natl Acad Sci USA 1991, 88, 10480–10484.
[7] Stuehr, D. J.; Marletta, M. A. Proc Natl Acad Sci USA 1985, 82, 7738–7742.
[8] Kroncke, K.-D.; Fehsel, K.; Kolb-Bachofen, V. Clin Exp Immunol 1998, 113, 147–156.
[9] Shah, A. M. Cardiovasc Res 2000, 45, 148–155.
[10] Marletta, M. A. J Med Chem 1994, 37, 1899–1907.
[11] Rimoldi, J. M.; Chimote, S. S. Curr Opin Drug Discovery Dev 1998, 1, 183–191.
[12] Hobbs, A. J.; Higgs, A.; Mocada, S. Annu Rev Pharmacol Toxicol 1999, 39, 191–220.
[13] Hibbs, J. B.; Taintor, R. R.; Vavrin, Z. Science 1987, 235, 473–476.
[14] Olken, N. M.; Marletta, M. A. Biochemistry 1993, 32, 9677–9685.

[15] Feldman, P. L.; Griffith, O. W.; Hong, H.; Stuehr, D. J. J Med Chem 1993, 36, 491–496.
[16] Cowart, M.; Kowaluk, E. A.; Kohlhass, K. L.; Alexander, K. M.; Kerwin, J. F., Jr. Bioorg Med Chem Lett 1996, 6, 999–1002.
[17] Moore, W. M.; Webber, R. K.; Jerome, G. M.; Tjoeng, F. S.; Misko, T. P.; Currie, M. G. J Med Chem 1994, 37, 3886–3888.
[18] Crane, B. R.; Arvai, A. S.; Ghosh, D. K.; Wu, C.; Getzoff, E. D.; Stuehr, D. J.; Tainer, J. A. Science 1998, 279, 2121–2126.
[19] Engel, R. (Ed.) Handbook of Organophosphorus Chemistry; Dekker: New York, 1992; 899 pp.
[20] Smith, A. B. III; Yager, K. M.; Taylor, C. M. J Am Chem Soc 1995, 117, 10879–10888.
[21] Levchine, I.; Padinchare, R.; Borloo, M.; Bollaert, W.; Haemers, A. Synthesis 1994, 37–39.
[22] Hamilton, R.; Walker, B. J.; Walker, B. Tetrahedron Lett 1993, 34, 2847–2850.
[23] Yuan, C.; Chen, S.; Wang, G. Synthesis 1991, 490–493.
[24] Baylis, E. K.; Cambell, C. D.; Dingwall, J. G. J Chem Soc Perkin Trans I 1984, 2845–2853.
[25] Webber, R. K.; Metz, S.; Moore, W. M.; Connor, J. R.; Currie, M. G.; Fok, K. F.; Hagen, T. J.; Hansen, D. W. Jr.; Jerome, G. M.; Manning, P. T.; Pitzele, B. S.; Toth, M. V.; Trivedi, M.; Zupec, M. E.; Tjoeng, F. S. J Med Chem 1998, 41, 96–101.
[26] Hallinan, E. A.; Tsymbalov, S.; Finnegan, P. M.; Moore, W. M.; Jerome, G. M.; Currie, M. G.; Pitzele, B. S. J Med Chem 1998, 41, 775–777.