Substituted 2-Iminopiperidines as Inhibitors of Human Nitric Oxide Synthase Isoforms†

R. Keith Webber,*,‡ Suzanne Metz,‡ William M. Moore,§ Jane R. Connor,§ Mark G. Currie,§ Kam F. Fok,‡ Timothy J. Hagen,‡ Donald W. Hansen, Jr.,‡ Gina M. Jerome,§ Pamela T. Manning,§ Barnett S. Pitzele,‡ Mihaly V. Toth,‡ Mahima Trivedi,‡ Mark E. Zupec,‡ and F. Siong Tjoeng‡

Departments of Discovery Medicinal Chemistry and Discovery Pharmacology, G. D. Searle Research and Development, Monsanto Company, Mail Zone AA2E, 700 Chesterfield Village Parkway, St. Louis, Missouri 63198

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A series of analogues of 2-iminopiperidine have been prepared and shown to be potent inhibitors of the human nitric oxide synthase (NOS) isoforms. Methyl substitutions on the 4-position **(3)** or 4- and 6-positions **(8)** afforded the most potent analogues. These compounds exibited IC_{50} values of 0.1 and 0.08 μM, respectively, for hiNOS inhibition. Substitution with cyclohexylmethyl at the 6-position **(13)** afforded an inhibitor that showed the best selectivity for hiNOS versus heNOS (heNOS IC_{50}/hiNOS IC_{50} = 64). Following oral administration, inhibitors were found to decrease serum nitrite/nitrate levels in an in vivo rat endotoxin assay. This series of 2-iminopiperidines were prepared via the described synthetic methodologies. The effect of ring substitutions on potency and selectivity for this class of cyclic amidines as NOS inhibitors is described.

Introduction

Nitric oxide (NO) is an endogenously produced inorganic free radical gas which has been implicated in a number of biological actions. These include endothelium-dependent vascular relaxation, cell-to-cell communication, and cytotoxicity associated with phagocytic cells.[1] NO is synthesized in biological systems by at least three isoforms of nitric oxide synthase (NOS). The most highly characterized isoforms consist of two constitutive isoforms, termed type I or nNOS (neuronal) and type III or eNOS (endothelial), and an inducible form of NOS which is termed type II or iNOS (inducible).[2−4] The observed biological activity of NO is closely linked to the NOS isoform which produces the molecule.[5]

All three isoforms of NOS catalyze the reaction to form nitric oxide via the oxidation of L-arginine to L-citrulline. The constitutive isoforms are regulated by calmodulin and Ca^{2+} concentration. The inducible isoform binds calmodulin tenaciously, rendering its activity independent of Ca^{2+} concentration.[6]

Initial published reports of inhibitors of NOS were focused on structural analogues of the natural enzyme substrate L-arginine. More recent publications have expanded the scope of reported inhibitors to include a variety of structures.[7]

Administration of the nonselective inhibitor L-NMA has been shown to cause a marked and sustained increase in blood pressure, indicating the importance of NO synthesis by the vascular endothelium.[8] Differences in the arginine binding sites of the constitutive and inducible forms are suggested by the potent inhibition of constitutive NOS by L-NNA, a less potent inhibitor of inducible NOS.[9] Because of the importance of the constitutive forms in normal physiology, the selective inhibition of the inducible form would be a favorable characteristic of an agent likely to have clinical utility for the prevention and treatment of diseases mediated by excessive production of nitric oxide.[10]

We previously reported that cyclic amidines with five- to nine-membered rings represent a novel class of inhibitors of NOS. Our data demonstrated the competitive nature of 2-iminopiperidine versus the natural enzyme substrate arginine.[11] We now report the expansion of this class of inhibitors in an ongoing effort to find more potent and selective inhibitors and to develop the structure−activity relationship (SAR) around these cyclic amidines. This work focuses on the substituent affects of analogues of 2-iminopiperidine (**1**). The inhibition potency of these compounds was measured against the three recombinant human isoforms of NOS.

Chemistry

A number of synthetic approaches to the 2-iminopiperidine class of NOS inhibitors were pursued. There is precedence in the literature for the partial reduction of 2-aminopyridines to 2-iminopiperidines under acidic conditions.[12] The protonated amidino group does not undergo reduction under the conditions of catalytic hydrogenation as shown in Scheme 1. This method proved to be a facile route to 2-iminopiperidines with various substitutions at positions 3−6. A number of 2-aminopyridines are available commercially; alternatively, the 2-aminopyridines could be prepared via direct amination of the pyridines (Chichibabin reaction) or via nucleophilic displacement of 2-chloropyridines with

† Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; hiNOS, human inducible NOS; heNOS, human endothelial constitutive NOS; hnNOS, human neuronal constitutive NOS; L-NMA, L-*N*-monomethylarginine; L-NNA, L-*N*-nitroarginine; LPS, lipopolysaccharide.

* Author for correspondence: tel, (314) 737-6652; fax, (314) 737-7425.

‡ Department of Discovery Medicinal Chemistry.

§ Department of Discovery Pharmacology.

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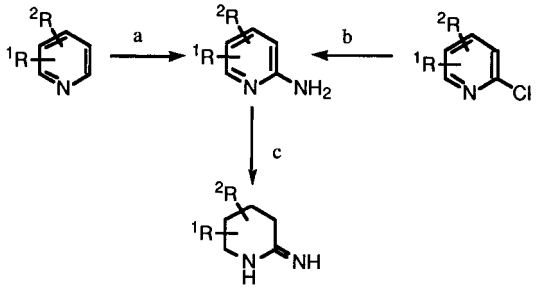
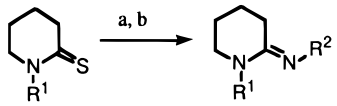
Scheme 1[a]



2 $R^1 = 3\text{-}CH_3$, $R^2 = H$
3 $R^1 = 4\text{-}CH_3$, $R^2 = H$
4 $R^1 = 5\text{-}CH_3$, $R^2 = H$
5 $R^1 = 6\text{-}CH_3$, $R^2 = H$
6 $R^1 = 4\text{-Et}$, $R^2 = H$
7 $R^1 = 4\text{-Pr}$, $R^2 = H$
8 $R^1 = 4\text{-}CH_3$, $R^2 = 6\text{-}CH_3$
9 $R^1 = 4\text{-}CF_3$, $R^2 = H$
10 $R^1 = 6\text{-}CF_3$, $R^2 = H$
11 $R^1 = 6\text{-cyclohexyl}$, $R^2 = H$
12 $R^1 = 6\text{-benzyl}$, $R^2 = H$
13 $R^1 = 6\text{-cyclohexylmethyl}$, $R^2 = H$

[a] (a) $NaNH_2$/*N*,*N*′-dimethylaniline/160 °C; (b) NH_4OH/180 °C; (c) catalytic hydrogenation.

Scheme 2[a]



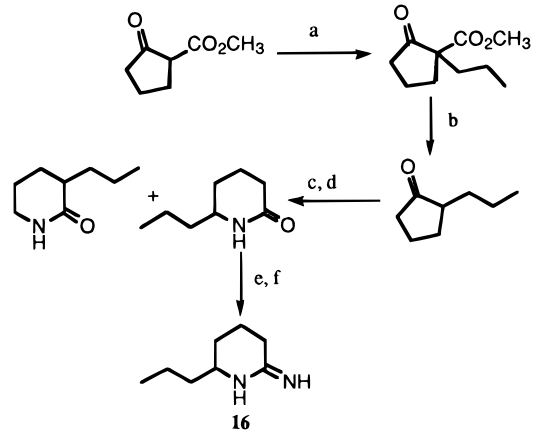
14 $R^1 = CH_3$, $R^2 = H$
15 $R^1 = H$, $R^2 = CH_3$

[a] (a)CH_3I; (b) R^2NH_2.

ammonia. Catalytic (5% Rh on carbon) hydrogenation of these 2-aminopyridines afforded 2-iminopiperidines with ring substitutions at positions 3−6. When 6-phenyl- or 6-benzyl-substituted pyridines were hydrogenated utilizing higher temperature or platinum oxide as catalyst, reduction of the phenyl rings afforded the cyclohexyl **(11)** and cyclohexylmethyl **(13)** products, respectively. Analogues with methyl substitutions on the ring nitrogen **(14)** or the imino nitrogen **(15)** were prepared from imino thioethers which were derived from the corresponding thiolactams as illustrated in Scheme 2.

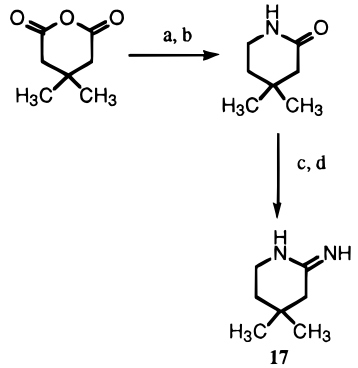
The 6-propyliminopiperidine **16** was prepared via a ring-expansion approach as illustrated in Scheme 3. Methyl cyclopentanone 2-carboxylate was alkylated with 1-iodopropane using potassium carbonate in DMF. Decarboxylation with sodium cyanide in DMSO at reflux afforded the 2-propylcyclopentanone. Conversion to the oxime was carried out with hydroxylamine hydrochloride and sodium acetate. A Beckmann rearrangement of the oxime with sodium hydroxide and benzenesulfonyl chloride afforded the 6-propylvalerolactam as the major product and 3-propylvalerolactam as a minor product. The 6-propylvalerolactam was isolated via chromatography and converted to the methyl imino ether with Meerwein's reagent. Subsequent reaction with ammonium chloride in methanol at reflux afforded 2-imino-6-(1-propyl)piperidine (**16**). The fused 6,6-ring analogue **18** was prepared from its lactam precursor 3,4-dihydrocarbostyril by the methods described for the

Scheme 3[a]



[a] (a) nPrI/K_2CO_3/DMF; (b) NaCN/DMSO/160 °C; (c) $NH_2OH{\cdot}HCl$/NaOAc; (d) $PhSO_2Cl$/NaOH; (e) $(CH_3)_3O^+BF_4^-/CH_2Cl_2$; (f) NH_4Cl/CH_3OH.

Scheme 4[a]



[a] (a)NH_4OH; (b) Pd/Al_2O_3; (c) $(CH_3)_3O^+BF_4^-/CH_2Cl_2$; (d) NH_4Cl/CH_3OH.

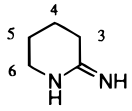
synthesis of **16**. The lactam was O-alkylated with $(CH_3)_3O^+BF_4^-$ (Meerwein's reagent) to generate the imino ether. This material was then converted to **18** by treatment with ammonium chloride in refluxing methanol.

The preparation of 2-imino-4,4-dimethylpiperidine (**17**), as shown in Scheme 4, demonstrates a route to substituted iminopiperidines which utilizes a glutaric anhydride. In this reaction 3,3-dimethylglutaric anhydride was converted to 4,4-dimethylpiperidin-2-one with ammonium hydroxide at high temperature and pressure using palladium and aluminum oxide (Al_2O_3). This valerolactam was converted to 2-imino-4,4-dimethylpiperidine **(17)** via the imino ether/ammonium chloride route described above.

Results and Discussion

The reduction of 2-aminopyridines to 2-iminopiperidines provided a facile methodology to investigate the SAR of this six-membered ring class of cyclic amidinium salts. We found the potency of these compounds at inhibiting the various isoforms of human NOS to be highly dependent on the substitution pattern around the piperidine ring (Table 1). Data from compounds with methyl groups placed on various locations around the ring gave us an early indication of which positions would tolerate substitution. Methyl groups appended to the 4- or 6-position afforded compounds which were 2−10-

Table 1. Comparison of IC_{50} Values for Inhibition of Human NOS Isoforms

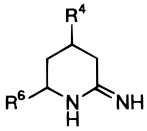


		IC_{50}[a] (μM)			selectivity[b]	
compd	substrate	hiNOS	heNOS	hnNOS	heNOS/hiNOS	hnNOS/hiNOS
1		1.1	4.7	1.1	4	1
2	3-CH_3	6.0	3.0	0.7	0.5	0.1
3	4-CH_3	0.1	1.1	0.2	9	1
4	5-CH_3	5.1	58	4.5	12	0.9
5	6-CH_3	0.5	0.6	0.4	1	0.8
6	4-Et	2.6	13	1.6	5	0.6
7	4-Pr	>100	>100	>100		
8	4,6-diMe	0.08	0.3	0.06	4	0.8
9	4-CF_3	3.2	47	3.3	15	1
10	6-CF_3	0.5	2.0	0.5	4	1
11	6-cyclohexyl	8.7	142	5.3	16	0.6
12	6-benzyl	9.2	44	0.9	5	0.1
13	6-cyclohexylmethyl	5.9	375	28	64	5
14	CH_3[c]	>100	>100	>100		
15	CH_3[d]	>100	>100	>100		
16	6-Pr	0.2	2.2	0.5	11	2
17	4,4-diMe	3.4	5.3	5.9	16	2
18	5,6 	99	218	28	2	0.3

[a] IC_{50} values were determined with hiNOS, heNOS, and hnNOS by testing each compound at eight concentrations. NOS activity was measured in the presence of a final L-arginine concentration of 30 μM by monitoring the conversion of L-[2,3-^{3}H]arginine to L-[2,3-^{3}H]citrulline as described in the Experimental Section. [b] Selectivity is defined as the ratio of the IC_{50}(heNOS) or IC_{50}(hnNOS) to IC_{50}(hiNOS). [c] Methyl substitution on the piperidinyl nitrogen. [d] Methyl substitution on the imino nitrogen.

fold more potent than the parent 2-iminopiperidine **(1)** at inhibiting either of the isoforms. In contrast, analogues with methyl groups in the 3- or 5-position were significantly less active as compared to compound **1**. Relatives with methyl substitution on either amidine nitrogen showed a sharp drop in inhibitory potency. Since substitution at either the 4- or 6-position was beneficial, an analogue was prepared with 4,6-dimethyl substitution. This analogue (**8**) was similar in potency to analogue **3** with hiNOS, heNOS, and hnNOS IC_{50}'s of 0.08, 0.3, and 0.06 μM, respectively. The 4-position was further explored by the preparation of a *gem*-dimethyl substitution analogue (**17**). This material was found to be 30-fold less active at inhibiting each of the isoforms than its 4-methyl relative. This drop in potency was presumed to be a steric effect. As anticipated for a steric effect, the 4-ethyl (**6**), 4-propyl (**7**), and 4-trifluoromethyl (**9**) compounds exhibited major decreases in potency on all isoforms as well. The 6-position, as opposed to the 4-position, appears to be quite tolerant of larger substituents. Analogues with longer chain lengths in the 6-position, such as the 6-propyl-containing material **16**, had an increase in potency for hiNOS and a slight increase in selectivity versus the constitutive enzymes when compared to its 6-methyl relative (**5**). Introduction of the larger trifluoromethyl (**10**) substituent on the 6-position afforded an analogue equal in potency for hiNOS to compound **5**. To further investigate steric effects at this 6 position, targets containing bulky carbocyclic substituents on the 6-position were synthesized. The 6-cyclohexyl (**11**), 6-cyclohexylmethyl (**13**), and 6-benzyl (**12**) examples showed a decrease in potency at inhibiting all the isoforms but were among the more selective examples. Analogue **13**, for example, showed a heNOS/hiNOS selectivity greater than 60. This analogue displayed a marginal hnNOS/hiNOS selectivity of 5. Additionally, in an attempt to tie the 5- and 6-positions together and restrict conformational freedom, the benzene ring bicyclic compound **18** was prepared. This fused ring material exhibited a substantial drop in potency particularly versus the hiNOS enzyme.

Table 2. Percent Inhibition of Plasma Nitrite Levels in LPS-Treated Rats



compd	substrate	% inhibition (10 mg/kg, po)[a]
1	$R^4 = R^6 = H$	62
3	R^4 = Me, $R^6 = H$	87
8	$R^4 = R^6$ = Me	98
9	$R^4 = CF_3$, $R^6 = H$	24
11	$R^4 = H$, R^6 = cyclohexyl	18

[a] In vivo efficacy in LPS-treated rats. Values represent the percent inhibition of animals treated with LPS alone compared to saline-treated controls (n = 6 animals/dose).

Previously we showed that 2-iminopiperidine (**1**) inhibits plasma nitrite/nitrate levels in a dose-dependent manner in a rat endotoxin assay.[11] Additional examples presented in Table 2 further demonstrate the in vivo efficacy of these inhibitors in inhibiting serum nitrite/nitrate levels generated by the systemic induction of iNOS. The 4,6-dimethyl-substituted iminopiperidine **8**, which was the most active of these enzyme inhibitors in vitro, also exhibited potent inhibitory activity in vivo. In addition, the relative potency of these compounds in vitro was similar to their efficacy in vivo.

In summary, the iminopiperidine classes of NOS inhibitors are potent inhibitors of the human enzymes in vitro and have potent efficacy in an iNOS-driven in vivo model. These examples provide insight into the SAR of this class of cyclic amidines and should aid in the design of more potent and selective relatives.

Experimental Section

L-[2,3-^{3}H]arginine was purchased from DuPont NEN (Boston, MA); (6*R*)-tetrahydro-L-biopterin was from Research Biochemicals, Inc. (Natick, MA); 3,4-dihydrocarbostyril was purchased from Apin Chemicals Ltd.; 2-iminopiperidine hydrochloride as well as other chemicals and reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Purification of synthesized compounds was performed on a Waters Prep LC2000 using a Delta Pak C-18 column or crystallization. ^{1}H NMR spectra were obtained at 300 MHz on a Varian VXR300 spectrometer in D_2O and are listed in δ units. Mass spectra were obtained on either a VG model 250 or a Finnigan MAT 90 spectrometer. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA). Male Lewis rats (150−200 g) were purchased from Harlan Sprague−Dawley (Indianapolis, IN) and were housed and cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee and in accordance with NIH guidelines on laboratory animal welfare. *Escherichia coli* lipopolysaccharide (serotype 0111:B4, Westphal extract) (LPS, endotoxin) was purchased from Sigma Chemical Co. (St. Louis, MO). Ultrafree-MC filter units were from Millipore (Bedford, MA).

Assay of NOS Activity. NOS activity was measured by monitoring the conversion of L-[2,3-^{3}H]arginine to L-[2,3-^{3}H]-citrulline.[13] Human inducible NOS (hiNOS), human endothelial constitutive NOS (heNOS), and human neuronal constitutive NOS (hnNOS) were each cloned from RNA extracted from human tissue. The cDNA for human inducible NOS (hiNOS) was isolated from a λcDNA library made from RNA extracted from a colon sample from a patient with ulcerative colitis; human endothelial constitutive NOS (heNOS) was isolated from a λcDNA library made from RNA extracted from human umbilical vein endothelial cells (HUVEC); and human neuronal constitutive NOS (hnNOS) was isolated from a λcDNA library made from RNA extracted from human cerebellum obtained from a cadaver. The recombinant enzymes were expressed in Sf9 insect cells using a baculovirus vector. Enzyme activity was isolated from soluble cell extracts and partially purified by DEAE-Sepharose chromatography.[14] The K_m values for L-arginine for hiNOS, heNOS, and hnNOS were 7, 4, and 6 μM, respectively.

To measure NOS activity, 10 μL of enzyme was added to 40 μL of 50 mM Tris (pH 7.6) and the reaction initiated by the addition of 50 μL of a reaction mixture containing 50 mM Tris (pH 7.6), 2.0 mg/mL bovine serum albumin, 2.0 mM DTT, 4.0 mM $CaCl_2$, 20 μM FAD, 100 μM tetrahydrobiopterin, 0.4 mM NADPH, and 60 μM L-arginine containing 0.9 μCi of L-[2,3-^{3}H]arginine. For constitutive NOS, calmodulin was included at a final concentration of 40 nM. Following incubation at 37 °C for 15 min, the reaction was terminated by addition of 300 μL of cold buffer containing 10 mM EGTA, 100 mM HEPES (pH 5.5) and 1.0 mM L-citrulline. The [^{3}H]citrulline was separated by chromatography on Dowex 50W X-8 cation-exchange resin and radioactivity quantified with a liquid scintillation counter. All assays were performed at least in duplicate; standard deviations were 10% or less. Production of [^{3}H]citrulline was linear with time over the course of the assay.

In Vivo Assay. Male Lewis rats were treated with an intraperitoneal injection of 10−12.5 mg/kg endotoxin (LPS) to induce systemic expression of inducible nitric oxide synthase, resulting in markedly elevated plasma nitrite/nitrate levels. Compounds were administered orally 1 h prior to LPS administration, and plasma nitrite/nitrate levels were determined 5 h following LPS administration using a fluorometric assay.[15]

Synthesis of 2-Iminopiperidines. General Procedure A: Amidine Formation from Thiovalerolactam. To a solution of the thiovalerolactam in 40 mL of acetone was added 1.1 equiv of iodomethane. This mixture was stirred for 3 days at 25 °C. Filtration and trituration with ether afforded the imino thioether hydroiodide salt. This imino thioether was dissolved as a 2% solution in ethanol (saturated with anhydrous ammonia or methylamine). This mixture was sealed and stirred at 25 °C for 18 h. Concentration to a reduced volume followed by ether trituration afforded the desired 2-iminopiperidine analogue.

General Procedure B: Catalytic Reduction of 2-Aminopyridines. The 2-aminopyridine and 5% rhodium on carbon (wet, Degussa type G10, 0.5 g) in glacial acetic acid (30 mL) were shaken on a Parr hydrogenation apparatus at 55 psi of hydrogen overnight. After the catalyst was filtered, the filtrate was concentrated in vacuo. Recrystallization from EtOH/EtOAc afforded the desired 2-iminopiperidine product.

General Procedure C: Preparation of 2-Aminopyridines. The substituted 2-chloropyridine (5 g) and concentrated ammonium hydroxide (150 mL) were heated at 180 °C in a steel reaction vessel with mechanical stirrer and stirred overnight. The contents were cooled and partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated in vacuo to afford the 2-aminopyridine.

General Procedure D: Preparation of 2-Aminopyridines. The substituted pyridine and 1.1 equiv of sodium amide were heated at 160 °C in *N*,*N*-dimethylaniline overnight. The reaction contents were cooled, poured into water, and extracted with ether (2 × 100 mL). The ether layers were combined, dried over $MgSO_4$, and concentrated in vacuo leaving a dark oil. The oil was distilled on a Kugelrohr apparatus at 40 °C (0.2 mmHg) to remove *N*,*N*-dimethylaniline and then at 120 °C (0.2 mmHg) to give 3.2 g of a yellow oil. The oil was further purified by C-18 reverse-phase chromatography eluting with CH_3CN/H_2O (0.05% trifluoroacetic acid) gradient to give the desired 2-aminopyridine.

2-Imino-3-methylpiperidine Acetic Acid Salt (2). Use of 2-amino-3-picoline afforded **2** by the application of general procedure B. ^{1}H NMR (D_2O): 3.22−3.15 (m, 2H), 2.67−2.55 (m, 1H), 1.80−1.40 (m, 4H), 1.75 (s, 3H), 1.17 (d, J = 7 Hz, 3H). MS: 113 (M + H). Anal. ($C_8H_{16}N_2O_2 \cdot {}^3/_4H_2O$) C, H, N.

2-Imino-4-methylpiperidine Acetic Acid Salt (3). Use of 2-amino-4-picoline afforded **3** by the application of general procedure B. ^{1}H NMR (D_2O): 3.32−3.26 (m, 2H), 2.54−2.46 (m, 1H), 2.10−2.00 (m, 1H), 1.80−1.70 (m, 2H), 1.74 (s, 3H), 1.32−1.25 (m, 1H), 0.87 (d, J = 7 Hz, 3H). Mp: 181−182 °C. MS: 113 (M + H). Anal. ($C_8H_{16}N_2O_2$) C, H, N.

2-Imino-5-methylpiperidine Acetic Acid Salt (4).[16] Use of 2-amino-5-picoline afforded **4** by the application of general procedure B. ^{1}H NMR (D_2O): 3.28−3.21 (m, 1H), 2.79−2.70 (m, 1H), 2.49−2.43 (m, 2H), 1.79−1.67 (m, 2H), 1.73 (s, 3H), 1.30−1.23 (m, 1H), 0.82 (d, J = 7 Hz, 3H). MS: 113 (M + H). Anal. ($C_8H_{16}N_2O_2$) C, H, N.

2-Imino-6-methylpiperidine Acetic Acid Salt (5).[17] Use of 2-amino-6-picoline afforded **5** by the application of general procedure B. ^{1}H NMR (D_2O): 3.58−3.40 (m, 1H), 2.60−2.35 (m, 2H), 1.95−1.70 (m, 2H), 1.68−1.50 (m, 1H), 1.40−1.35 (m, 1H), 1.05 (d, J = 7 Hz, 3H). MS: 113 (M + H). Anal. ($C_8H_{16}N_2O_2$) C, H, N.

2-Imino-4-ethylpiperidine Acetic Acid Salt (6). Use of 2-amino-4-ethylpyridine afforded **6** by the application of general procedure B. ^{1}H NMR (D_2O): 3.40−3.25 (m, 1H), 3.22−3.10 (m, 1H), 2.60−2.50 (m, 1H), 2.15−2.00 (m, 1H), 1.85−1.75 (m, 1H), 1.78 (s, 3H), 1.70−1.60 (m, 1H), 1.35−1.15 (m, 3H), 0.75 (t, J = 7 Hz, 3H). MS: 127 (M + H). Anal. ($C_9H_{18}N_2O_2 \cdot {}^1/_4H_2O$) C, H, N.

2-Imino-4-(1-propyl)piperidine Acetic Acid Salt (7). Use of 2-amino-4-propylpyridine afforded **7** by the application of general procedure B. ^{1}H NMR (D_2O): 3.35−3.05 (m, 2H), 2.60−2.40 (m, 1H), 2.15−2.00 (m, 1H), 1.80−1.60 (m, 2H), 1.78 (s, 3H), 1.35−1.05 (m, 5H), 0.75−0.65 (m, 3H). MS: 141 (M + H). Anal. ($C_{10}H_{20}N_2O_2$) C, H, N.

2-Imino-4,6-dimethylpiperidine Acetic Acid Salt (8). Use of 2-amino-4,6-dimethylpyridine afforded **8** by the application of general procedure B. ^{1}H NMR (D_2O): 3.48−3.40 (m, 1H), 2.52−2.40 (m, 1H), 2.071.95 (m, 1H), 1.85−1.75 (m, 2H), 1.75 (s, 3H), 1.12 (d, J = 6 Hz, 3H), 1.02−0.92 (m, 1H), 0.86 (d, J = 6 Hz, 3H). MS: 127 (M + H). Anal. ($C_{12}H_{24}N_2O_2$) C, H, N.

2-Imino-4-(trifluoromethyl)piperidine Hydrochloride (9). 2-Chloro-4-(trifluoromethyl)pyridine was converted by the application of general procedure C to afford 2-amino-4-(trifluoromethyl)pyridine as a white solid. Catalytic hydrogenation by application of general procedure B afforded **9**. ^{1}H NMR (D_2O): 3.45−3.35 (m, 1H), 3.30−3.20 (m, 1H), 2.85−2.55 (m, 3H), 2.10−2.00 (m, 1H), 1.80−1.60 (m, 1H). MS: 167 (M + H). Anal. ($C_6H_{10}F_3N_2Cl_1$) C, H, N.

2-Imino-6-(trifluoromethyl)piperidine Hydrochloride (10). 2-Chloro-6-(trifluoromethyl)pyridine was converted by application of general procedure C to afford 2-amino-6-(trifluoromethyl)pyridine. Catalytic hydrogenation by application of general procedure B afforded **10**. ^{1}H NMR (D_2O): 4.20−4.00 (m, 1H), 2.60−2.50 (m, 2H), 2.05−1.50 (m, 4H), 1.80 (s, 3H). MS: 167 (M + H). Anal. ($C_6H_{10}F_3N_2Cl_1$) C, H, N.

2-Imino-6-cyclohexylpiperidine Hydrochloride (11). 2-Phenylpyridine was converted by application of general procedure D to afford 2-amino-6-phenylpyridine as a white solid (3.6 g, 73% yield). Catalytic hydrogenation was carried out as in general procedure B except that the reaction was run at 55 °C to afford **11**. ^{1}H NMR (D_2O): 3.30−3.15 (m, 1H), 2.50−2.30 (m, 2H), 1.85−1.68 (m, 2H), 1.65−1.20 (m, 8H), 1.20−0.80 (m, 5H). MS: 181 (M + H). Anal. ($C_{13}H_{21}F_3N_2O_2 \cdot {}^3/_4H_2O$) C, H, N.

2-Imino-6-benzylpiperidine Hydrochloride (12). 2-Amino-6-benzylpyridine was converted by application of general procedure D to afford 2-amino-6-benzylpyridine as a white solid (3.6 g, 73% yield). Catalytic hydrogenation by application of general procedure B afforded **12**. ^{1}H NMR (D_2O): 7.35−7.10 (m, 5H), 3.70−3.60 (m, 1H), 2.75 (d, J = 7 Hz, 2H), 2.50−2.30 (m, 2H), 1.82−1.62 (m, 2H), 1.60−1.30 (m, 2H). MS: 189 (M + H). Anal. ($C_{12}H_{17}F_3N_2Cl_1 \cdot H_2O$) C, H, N.

2-Imino-6-(cyclohexylmethyl)piperidine Hydrochloride (13). 2-Amino-6-benzylpyridine from the synthesis of **12** was catalytically hydrogenated by application of general procedure B except that platinum oxide was used as the catalyst to afford **13**. ^{1}H NMR ($CDCl_3$): 9.60 (s, 1H), 8.90 (s, 1H), 8.70 (s, 1H), 3.60−3.40 (m, 1H), 2.90−2.70 (m, 1H), 2.70−2.50 (m, 1H), 2.10−1.80 (m, 2H), 1.80−1.00 (m, 13H), 1.00−0.80 (m, 2H). MS: 195 (M + H). Anal. ($C_{12}H_{23}N_2Cl_1 \cdot {}^1/_5H_2O$) C, H, N.

1-Methyl-2-iminopiperidine Hydroiodide Salt (14).[18] Use of *N*-methylthiovalerolactam and ammonia by application of general procedure A afforded 0.8 g of **14** as a white solid. Mp: 157−158 °C. ^{1}H NMR (D_2O): 1.6−1.7 (m, 2H), 1.7−1.8 (m, 2H), 2.5 (t, 2H), 2.95 (s, 3H), 3.38 (t, 2H). MS: 113 (M + H). Anal. ($C_6H_{13}N_2I_1$) C, H, N.

Preparation of Thiovalerolactam. A mixture of 100 g (230 mmol) of P_4S_{10} and 24 g (230 mmol) of Na_2CO_3 in 1.5 L of anhydrous THF was stirred vigorously with a mechanical stirrer for 30 min. To this stirred mixture was added 19 g (190 mmol) of valerolactam. After stirring for 3 h, the reaction solution was diluted with 1 L of 10% aqueous Na_3PO_4, 750 mL of EtOAc, and 750 mL of hexanes. The organic layer was separated and the aqueous layer extracted with an additional 500 mL of EtOAc. The organic extracts were combined, dried ($MgSO_4$), filtered through silica gel, and concentrated to afford a white semisolid. Trituration with a mixture of hexanes and ether afforded 9.7 g of thiovalerolactam as a white solid. Mp: 85−88 °C.

2-(Methylimino)piperidine Hydroiodide Salt (15).[19] Use of thiovalerolactam and methylamine by application of general procedure A afforded **15**. ^{1}H NMR (D_2O): 1.6−1.7 (m, 4H), 2.45 (t, 2H), 2.7 (s, 3H), 3.3 (t, 2H). MS: 113 (M + H). Anal. ($C_6H_{13}N_2I_1$) C, H, N.

Preparation of 2-(Carboxymethyl)-2-propylcyclopentanone. A solution of methyl 1-oxocyclopentane-2-carboxylate (14.2 g, 100 mmol), 1-iodopropane (17 g, 100 mmol), and potassium carbonate (10 g) in 100 mL of DMF was stirred under N_2 at 50 °C for 16 h. Removal of the solvent in vacuo left a residue which was partitioned between EtOAc and water. The EtOAc layer was separated, washed with water and brine, and dried over $MgSO_4$. Concentration in vacuo afforded 2-(carboxymethyl)-2-propylcyclopentanone.

Preparation of 2-Propylcyclopentanone. A mixture of 2-(carboxymethyl)-2-propylcyclopentanone (10 g, 54 mmol), sodium cyanide (2.9 g, 60 mmol), and DMSO (100 mL) was stirred at 160 °C for 3 h under N_2. After cooling, the reaction mixture was poured into ice water and extracted with a mixture of ether/hexanes (300 mL, 1/1). The organic layer was removed, washed with brine (2×), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel eluting with EtOAc/hexanes (3/7) to afford 5.5 g (83%) of 2-propylcyclopentanone.

Preparation of 2-Propylcylopentanone Oxime. A solution of 10 g (43 mmol) of 2-propylcyclopentanone in 75 mL of EtOH was added to a solution of 5.8 g (83 mmol) of hydroxylamine hydrochloride and 8.2 g (100 mmol) of sodium acetate in 50 mL of water. This mixture was stirred for 4 h at reflux and then for 18 h at 25 °C. The reaction mixture was concentrated to a reduced volume, diluted with EtOAc, washed with three 200-mL portions of aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated to afford 10 g of the 2-propylcyclopentanone oxime as a colorless oil.

Preparation of 6-Propylvalerolactam. A solution of 5.6 g (40 mmol) of 2-propylcyclopentanone oxime in 50 mL of acetone was treated with 44 mL (44 mmol) of 1 N NaOH at 0 °C. To this stirred mixture was added 7.4 g (42 mmol) of benzenesulfonyl chloride dropwise. The resulting mixture was stirred for 18 h at 25 °C. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated to afford a yellow oil. Chromatography (C-18, 10% acetonitrile/water to 70% acetonitrile/water) on 2 g of the yellow oil afforded 1 g of a mixture of the 6-propylvalerolactam and 3-propylvalerolactam. Chromatography (silica gel, 8/3 hexane/EtOAc) of this 1 g of yellow oil afforded 0.4 g of the 6-propylvalerolactam.

2-Imino-6-(1-propyl)piperidine Trifluoroacetic Acid Salt (16). To a solution of 0.63 g (4.3 mmol) of trimethyloxonium tetrafluoroborate in 15 mL of CH_2Cl_2 was added 0.4 g (2.8 mmol) of 6-propylvalerolactam. This mixture was stirred for 2 days at 25 °C. The reaction mixture was then diluted with EtOAc, washed with dilute potassium carbonate, dried ($MgSO_4$), filtered through a patty of silica gel, and concentrated to afford 0.5 g of the imino ether as a yellow oil. This oil was dissolved in 50 mL of MeOH, and 1 g (19 mmol) of ammonium chloride was added. After stirring at reflux for 4 h the mixture was stirred at 25 °C for 18 h. The reaction mixture was then concentrated to remove solvents. The residue was dissolved in water and extracted with EtOAc. The aqueous layer was lyophilized to afford 2-imino-6-(1-propyl)piperidine hydrochloride salt as a white solid. A final purification by reverse-phase C-18 chromatography (0−50% acetonitrile/H_2O, 30 min) afforded 0.3 g of 2-imino-6-(1-propyl)piperidine trifluoroacetate as a white semisolid. ^{1}H NMR (D_2O): 3.4−3.3 (m, 1H), 2.50−2.38 (m, 2H), 1.88−1.15 (m, 8H), 0.78−0.70 (t, 3H). MS: 141 (M + H). Anal. ($C_{10}H_{17}N_2O_2F_3 \cdot {}^1/_2 H_2O$) C, H, N.

Preparation of 4,4-Dimethylpiperidin-2-one. A solution of 3,3-dimethylglutaric anhydride (10 g, 70 mmol) in concentrated ammonium hydroxide (30 mL) was hydrogenated over Pd/Al_2O_3 at 1600 psi and 250 °C for 3 h. After the mixture cooled to room temperature, brine (75 mL) was added, and the contents were extracted with CH_2Cl_2 (150 mL), dried over Na_2SO_4, and concentrated in vacuo to yield a solid. The solid was purified by chromatography on silica gel to give 4,4-dimethylpiperidin-2-one (1.4 g, 16% yield).

2-Imino-4,4-dimethylpiperidine Hydrochloride Salt (17). A mixture of 4,4-dimethylpiperidin-2-one (0.64 g, 5 mmol) and trimethyloxonium tetrafluoroborate (0.89 g, 6 mmol) in CH_2Cl_2 (20 mL) was stirred overnight. The contents

were then diluted with CH_2Cl_2 (10 mL) and partitioned between saturated aqueous potassium bicarbonate (40 mL) and EtOAc (50 mL). The organic layer was removed, dried over Na_2SO_4, and concentrated in vacuo to give a pale yellow oil. The oil was chromatographed on a short path Merck flash silica gel column eluting with EtOAc/hexanes (1/1) to give 2,3,4,5-tetrahydro-4,4-dimethyl-6-methoxypyridine, as a yellow oil (0.55 g, 78% yield). A solution of 2,3,4,5-tetrahydro-4,4-dimethyl-6-methoxypyridine (0.55 g, 4 mmol) and ammonium chloride (214 mg, 4 mmol) was refluxed in methanol (30 mL) for 3.5 h. The reaction mixture was allowed to cool, and concentration in vacuo gave a residue which was partitioned between EtOAc and water. Lyophilization of the aqueous layer afforded 2-imino-4,4-dimethylpiperidine hydrochloride (460 mg, 86% yield). Mp: 168 °C. IR (KBr): 3294, 3148, 3009, 2945, 1687. ^{1}H NMR (DMSO-d_6−D_2O): 1.00 (s, 6H), 1.60 (t, J = 5 Hz, 2H), 2.34 (s, 2H), 3.33 (t, J = 5 Hz, 2H). MS (EI): *m/e* 126. Anal. ($C_7H_{15}N_2Cl_1 \cdot {}^1/_5H_2O$) C, H, N.

2-Imino-1,2,3,4-tetrahydroquinoline Hydrochloride Salt (18). A solution of 3,4-dihydrocarbostyril (0.74 g, 5 mmol) in CH_2Cl_2 was allowed to react with trimethyloxonium tetrafluoroborate (0.92 g, 6.2 mmol). This mixture was stirred for 2 days at 25 °C. The reaction mixture was then diluted with EtOAc, washed with dilute potassium carbonate, dried ($MgSO_4$), and filtered through a patty of silica gel. Concentration in vacuo afforded 0.13 g (16%) of 3,4-dihydro-2-methoxyquinoline as a yellow oil. A solution of 3,4-dihydro-2-methoxyquinoline (0.098 g, 0.61 mmol) in 10 mL of MeOH and 10 mL of CH_2Cl_2 was allowed to react with ammonium chloride (0.027 g, 0.52 mmol). The reaction mixture was stirred at reflux for 4 h and then at 25 °C for 18 h. The reaction mixture was concentrated to remove solvents. The residue was dissolved in water and extracted with EtOAc. The aqueous layer was lyophilized to afford **18** as a white solid. HRMS: *m/z* M^+ 147.086; $C_9H_{11}N_2$ requires 147.092. ^{1}H NMR (D_2O): 2.93 (m, 2H), 3.00 (m, 2H), 7.10 (m, 1H), 7.25 (m, 1H), 7.37−7.30 (m, 2H). Anal. ($C_9H_{11}N_2Cl_1 \cdot {}^1/_4H_2O \cdot NH_4Cl$) C, H, N.

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