Expedited Articles

2-Iminohomopiperidinium Salts as Selective Inhibitors of Inducible Nitric Oxide Synthase (iNOS)†

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An attractive approach to the treatment of inflammatory conditions such as osteo- and rheumatoid arthritis, inflammatory bowel disease, and sepsis is through the selective inhibition of human inducible nitric oxide synthase (hiNOS) since localized excess nitric oxide (NO) release has been implicated in the pathology of these diseases. A series of monosubstituted iminohomopiperidinium salts possessing lipophilic functionality at ring positions 3, 5, 6, and 7 has been synthesized, and series members have demonstrated the ability to inhibit the hiNOS isoform with an IC_{50} as low as 160 nM (**7**). Compounds were found that selectively inhibit hiNOS over the human endothelial constitutive enzyme (heNOS) with a heNOS/hiNOS IC_{50} ratio in excess of 100 and as high as 314 (**9**). Potencies for inhibition of hiNOS and the human neuronal constitutive enzyme (hnNOS) are comparable. Substitution in the 3 and 7 positions provides compounds that exhibit the greatest degree of selectivity for hiNOS and hnNOS over heNOS. Submicromolar potencies for **6** and **7** in a mouse RAW cell assay demonstrated the ability of these compounds to inhibit iNOS in a cellular environment. These latter compounds were also found to be orally bioavailable and efficacious due to their ability to inhibit the increase in plasma nitrite/nitrate levels in a rat LPS model.

Introduction

The current intense nitric oxide (NO) research effort in both academic and industrial institutions is due to the accumulating data suggesting that this free radical is a messenger molecule with extremely diverse functions.[1−7] NO, characterized initially as an endothelium-derived releasing factor in blood vessels, is considered a main determinant of blood pressure. More recently, NO has been proposed as a neurotransmitter or neuromodulator in the brain and peripheral autonomic nervous system. NO has now been linked to the regulation of a number of biological functions such as memory and learning, peristalsis, and penile erection. It has also been demonstrated that NO is synthesized by immune system cells such as macrophages and neutrophils in response to various types of infection and tumor cells. The immune system appears to use NO to eliminate or control the invading pathogens. These three principal sites of NO formation are paralleled by the three different forms of NO synthase (NOS) derived from distinct genes. The endothelial (eNOS) and neuronal (nNOS) enzymes are generally regarded as constitutive and are Ca^{2+}- and calmodulin-dependent, while the inducible (iNOS) isoform characterized in macrophages and various other cells is usually Ca^{2+}- and calmodulin-independent.

Imbalances in finely tuned NO synthesis appear to lead to serious consequences.[7] It is, therefore, apparent that significant pharmaceutical potential exists for selective agents that normalize NO levels in disease states where these levels are either depressed or elevated. For situations involving low levels of NO, the practical approach of using drugs that release NO has already found application in areas such as treatment of angina. The reduction of NO by selective inhibition of specific isoenzymes is expected to be of value in disease states such as endotoxic shock where dramatic hypotension has been linked to high levels of NO. Elevated levels of NO have also been related to inflammation from a variety of causes. In addition, there is accumulating evidence that increased levels of NO may play a role in postischemic stroke damage. The NO generated in all the latter aforementioned conditions appears to be due to stimulation of iNOS. Its inhibition may be a key and effective route to ameliorating these

† Abbreviations: NOS, nitric oxide synthase; hiNOS, recombinant human inducible nitric oxide synthase; heNOS, recombinant human endothelial constitutive enzyme; hnNOS, recombinant human neuronal constitutive enzyme; L-NMA, N^G-monomethyl-L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; L-NIO, L-N^5-(1-iminoethyl)ornithine; L-NIL, L-N^6-(1-iminoethyl)lysine.

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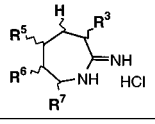
Table 1. Enzyme Inhibition of Substituted Iminohomopiperidinium Salts



Cpd#	R^3	R^5	R^6	R^7	Inhibition of NO Synthase: IC50 (μM) (a) hiNOS	(b) heNOS	(c) hnNOS	Selectivity b/a - c/a
1	(R's = H unless otherwise designated)				2.1	15	3.5	7-2
2	Et				1.6	38	2.4	24-1
3					5.8	380	16	65-3
4		Me			5.8	39	11	7-2
5			Me(R)		7.9	39	3.8	5-0.5
6					0.19	21	0.87	110-6
7					0.16	9.5	0.46	59-3
8					0.5	103	0.85	206-2
9					2.1	660	5.4	314-3
10					14	215	6.6	15-0.5

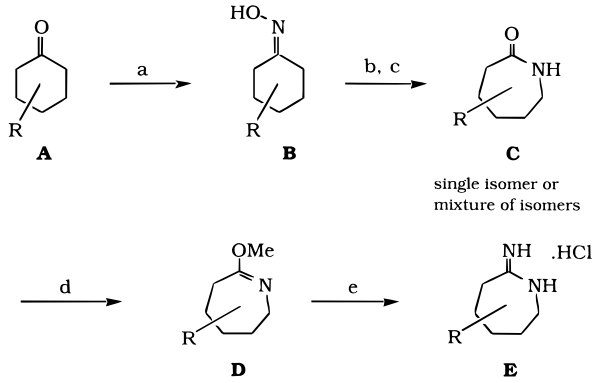
disease states. Our focus has been on the design and synthesis of novel inhibitors selective for human inducible nitric oxide synthase (hiNOS).

The natural substrate for NOS, arginine, has been the obvious basis of inhibitor design. Much pharmacology and biology have been determined utilizing some of the early relatively nonspecific inhibitor derivatives of Arg such as N^G-monomethyl-L-arginine (L-NMA),[8,9] N^G-nitro-L-arginine methyl ester (L-NAME),[10] and L-N^5-(1-iminoethyl)ornithine (L-NIO).[11] More recently we have reported that a lysine derivative, L-N^6-(1-iminoethyl)lysine (L-NIL), is a potent and selective inhibitor of mouse inducible NOS and suppresses both increases in plasma nitrite/nitrate levels and joint inflammation associated with adjuvant arthritis.[12,13] A number of non-amino-acid NOS inhibitors have also been described and include such agents as aminoguanidine,[14] isothioureas,[15,16] amidines,[17] and 2-amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine.[18] We recently reported that 2-iminopiperidinium hydrochloride and the homologous series of amidinium salts are potent non-amino-acid inhibitors of human NOS with modest selectivity for inducible NOS over the endothelial constitutive isoform.[19] We now report a series of monosubstituted 2-iminohomopiperidinium salts, examples of which inhibit the hiNOS isoform with a potency as low as 160 nM (**7**) and selectively inhibit hiNOS over the human endothelial constitutive enzyme (heNOS) with a hiNOS/heNOS IC_{50} ratio in excess of 100 and as high as 314 (**9**).

Results and Discussion

The target molecules illustrated in Table 1 were synthesized as diagrammed in Scheme 1. The well-known alkyl-substituted cyclohexanone starting materials (**A**) were obtained either commercially, by direct alkylation of cyclohexanone, or by alkylation of 2-(carboalkoxy)cyclohexanone followed by decarboxylation. These cyclohexanones (**A**) were then converted to their

Scheme 1[a]



[a] (a) $NH_2OH{\cdot}HCl$, NaOAc, EtOH; (b) 80% H_2SO_4, 120 °C, or benzenesulfonyl chloride, acetone; (c) HPLC isomeric separation when necessary; (d) $Me_3O^+BF_4^-$, CH_2Cl_2, rt, or Me_2SO_4, benzene, Dean−Stark trap; (e) NH_4Cl, MeOH reflux.

oximes (**B**) and subsequently subjected to a variety of Beckmann rearrangement conditions to provide separable regioisomeric mixtures of substituted caprolactams (**C**). In the case of 2-substituted cyclohexanones, the 7-substituted caprolactams predominated in the mixture. The caprolactam derivative was subsequently treated with trimethyloxonium tetrafluoroborate to generate the respective imino ethers (**D**). Reaction of these materials with ammonium chloride provided our target 2-iminohomopiperidinium hydrochlorides (**E**).

The iminohomopiperidinium salts indicated in the accompanying table were evaluated, as detailed in the description of the citrulline assay, for their ability to inhibit each of the three human isoforms of NOS. The parent iminohomopiperidinium chloride **1**, reported previously,[19] has micromolar potency for hiNOS, 7-fold selectivity over heNOS, and only 2-fold selectivity over hnNOS. When an ethyl function is inserted into the 3 position, the potency of resulting compound **2** is slightly enhanced and its selectivity over heNOS is more than tripled. Analogue **3**, containing an allyl group at position 3, has comparable potency for hiNOS but is 65-fold selective over heNOS. All of these three substituted salts are nearly equipotent for hiNOS and hnNOS. Analogues that are methyl-substituted at positions 5 and 6, **4** and **5**, have enzyme inhibition profiles less potent but similar to that of their parent, **1**.

In contrast, compounds possessing a substituent at position 7, compounds **6**−**9**, exhibit dramatic improvement in potency, selectivity, or both as compared to **1**. The analogue containing an *n*-propyl function, **7**, has a hiNOS IC_{50} of 160 nM and selectivity of nearly 60. Its unsaturated relative, **6**, has similar potency for hiNOS (190 nM) but is 110-fold selective over the heNOS isoform and 6-fold over hnNOS. Figuratively inserting a methylene unit into the side chain of **7** gives rise to the *n*-butyl analogue **8** which has reduced hiNOS potency (500 nM) but dramatically enhanced selectivity over heNOS (>200-fold). Aliphatic branching added at the 2′ carbon of **8** gives rise to **9**. This analogue, while having a hiNOS IC_{50} of only 2.1 μM, is greater than 300-fold selective for inhibition of hiNOS over heNOS.

It is interesting to note that introduction of π character into substituents at either the 3 or 7 position of these iminohomopiperidinium salts gives rise to analogues, **3** and **6**, that exhibit reduced potency but

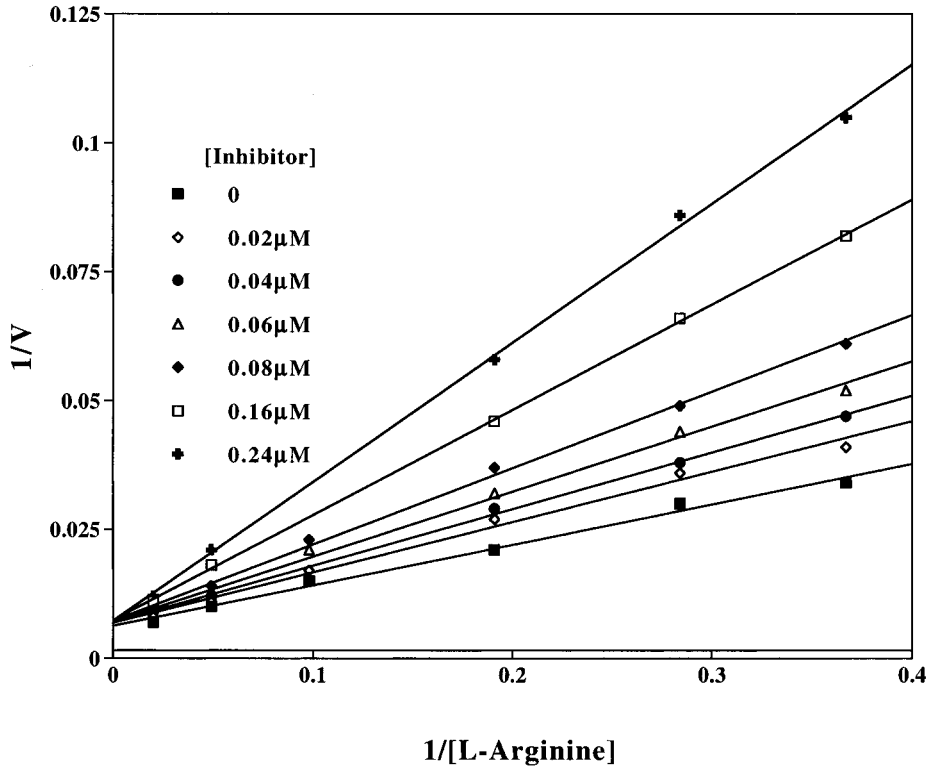


Figure 1. Plot of the reciprocal of hiNOS velocity (in units of min/pmol) versus the reciprocal of L-arginine concentration (in units of μM^{-1}) at varying concentrations of **7**. NOS activity was determined by measuring the conversion of L-[2,3-^{3}H]arginine to L-[2,3-^{3}H]citrulline as described in the Experimental Section except that the reaction was initiated by the addition of enzyme and the incubation at 37 °C was for 10 min. Enzyme activity was linear over this time period for each concentration of L-arginine. Each point is the average of duplicate determinations.

significantly increased selectivity for hiNOS versus heNOS over their respective aliphatic relatives, **2** and **7**. However, when a π-rich phenyl group is appended to the 7 position as illustrated by analogue **10**, both potency and selectivity are severely reduced. In fact, compound **10** is one of only two analogues that inhibit hnNOS to a greater degree than hiNOS.

In general, the substituted iminohomopiperidinium salts described inhibit both hiNOS and hnNOS to a similar degree and are far less effective at inhibiting heNOS. Analogues containing substituents at positions 3, 5, and 6 have hiNOS IC_{50}'s between about 2 and 8 μM and selectivities for hiNOS over heNOS of up to 65-fold. Relatives with aliphatic functionality at ring position 7 not only exhibit hiNOS inhibition potencies as low as 160 nM but also show selectivities for hiNOS over heNOS of greater than 300-fold and are, to our knowledge, the most selective agents reported to date. Since all the compounds discussed except **5** are racemic mixtures of two enantiomers, it is also conceivable that there is a single isomer that possesses better potency and/or selectivity.

In order to investigate the nature of inhibition of hiNOS by **7**, the most potent iNOS inhibitor in the series, a Lineweaver–Burk double-reciprocal plot analysis of the kinetics of hiNOS in the presence of different fixed concentrations of **7** was performed. As shown in Figure 1, the double-reciprocal plots resulted in lines intersecting at the same point on the *y*-axis, indicating that **7** is a competitive inhibitor of L-arginine binding to hiNOS. A K_i of 0.09 μM for **7** was determined from a replot of the slope of each double-reciprocal plot versus the concentration of **7** (data not shown).

Table 2. In Vitro and in Vivo Efficacy of Substituted Iminohomopiperidium Salts

compd	raw cell IC_{50} (μM)[a]	rat low endotoxin (% inhib at 10 mg/kg)[b]
1	54 (±22)	nd[c]
2	3.5 (±0.7)	nd
3	19 (±6.8)	nd
4	24 (±14)	nd
6	0.6 (±0.1)	80 (±3)
7	0.6 (±0.1)	85 (±3)
8	1.6 (±1.1)	60 (±1)
9	3.3 (±1.6)	nd
10	31 (±38)	nd

[a] The ability of compounds to inhibit mouse iNOS in LPS-stimulated mouse RAW cells was determined as described in the Experimental Section. [b] The ability of compounds to inhibit rat iNOS in LPS-treated male Lewis rats was determined by measuring plasma nitrite concentrations as described in the experimental section. [c] nd, not determined.

The compounds were also evaluated in a cell-based mouse RAW cell assay for their ability to inhibit murine iNOS (see Table 2). Cells were stimulated with lipopolysaccharide (LPS), and inhibition of nitrite production in the medium was determined after 2 h of treatment with and without inhibitor. The RAW cell mouse iNOS IC_{50} values for compounds **6**–**8** were 0.6, 0.6, and 1.6 μM, respectively, demonstrating the ability of these compounds to inhibit iNOS in a cellular milieu. These three analogues were also the most potent at inhibiting hiNOS, and in general, the RAW cell IC_{50} values parallel those for hiNOS inhibition.

The ability of compounds **6**–**8** to inhibit iNOS in vivo was also evaluated. Oral bioavailability and efficacy were assessed by determining their ability to inhibit the

increase in plasma nitrite/nitrate levels generated from iNOS following the administration of LPS to rats. The compounds were given at a dose of 10 mg/kg orally by gavage 1 h prior to LPS administration. After 5 h, the plasma nitrite/nitrate levels were elevated approximately 15-fold compared to saline-treated animals. The basal and stimulated values for plasma nitrite/nitrates are 15.7 (±0.7) and 289 (±37) μM, respectively, for one experiment and 23.2 (±0.1) and 238 (±23) μM, respectively, for another. Compounds **6**−**8** produced 80%, 85%, and 60% inhibition, respectively, of the plasma nitrite/nitrate levels found in animals treated with LPS without inhibitor, and this data correlates relatively with their in vitro data (see Table 2). This convincingly demonstrates their ability to inhibit inducible NOS activity in vivo following oral administration.

In summary, these iminohomopiperidinium salts are potent inhibitors of human NOS, and certain series members are highy selective for the inducible isoform over heNOS. The greatest selectivity resides in analogues with substitution in the 7 position of the iminohomopiperidine ring. It is also very significant that these materials not only inhibit iNOS in a cellular environment but are also orally bioavailable and efficacious in a model of inflammation.

Experimental Section

Proton (^{1}H) NMR spectra were recorded at 500, 400, 300, and 80 MHz on Varian VXR-500, Varian VXR-400, General Electric model QE-300, and Varian FT-80A instruments. Infrared spectra were measured on Perkin-Elmer model 283B and model 681 instruments. Specific optical rotations were measured at the sodium D line at 21 °C on a Perkin-Elmer model 241 digital polarimeter (1-dm cell). Mass spectra were taken on a Finnigan-MAT model 8430 system with high resolution, FAB, and chemical ionization capability. Elemental analyses were determined by the Searle Laboratories Microanalytical Department under the direction of Mr. E. Zielinski. Microanalyses were obtained for the stated elements and were within 0.4% of the theoretical values for the formula unless otherwise stated. Preparative high-performance liquid chromatography (HPLC) was performed on a Rainin model HPX system. Analytical HPLC was carried out on a Waters model 45 instrument equipped with a Kratos Sprectroflow model 773 detector and a Hewlett-Packard model 3390A integrator. Reverse-phase HPLC was run on a YMC AQ ODS-363-10P column, with eluants of aqueous acetonitrile−acetic acid. The substituted cyclohexanones were purchased from the following: Aldrich Chemical Co. (Milwaukee, WI), Pfaltz & Bauer, Inc. (Waterbury, CT), Frinton Laboratories (Vineland, NJ), and Farchan Laboratories, Inc. (Gainesville, FL).

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 as previously described.[19] hiNOS, heNOS, and hnNOS were each cloned from RNA extracted from human tissue.[20] The recombinant enzymes were expressed in Sf9 insect cells using a baculovirus vector.[20] Enzyme activity was isolated from soluble cell extracts and partially purified by DEAE-Sepharose chromatography.[14,20] 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 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. The final concentration of L-arginine in the assay was 30 μM. For heNOS and hnNOS, 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 for hiNOS, heNOS, and hnNOS over the time period utilized for the assay.

Measurement of Cellular NOS Activity. The ability of compounds to inhibit mouse iNOS activity was determined using LPS-stimulated RAW 264.7 mouse cells as described previously.[19] The mouse macrophage cell line RAW 264.7 was maintained in Dulbecco's MEM (DMEM) containing 10% fetal bovine serum in 5% CO_2 at 37 °C. Prior to assay, cells were plated at 2×10^5 cells/well in a 96-well plate and were induced to express iNOS by incubating for 17 h with 10 ug/mL LPS (*Escherichia coli* serotype 0111:B4). Cells were then washed and preincubated for 1 h on ice in Krebs Ringer buffer containing 25 mM HEPES, pH 7.5, 2 mg/mL D-glucose, 30 uM L-arginine, and five different concentrations of the inhibitor. The cells were then warmed to 37 °C for 2 h, and the amount of nitrite generated was determined by a fluorometric assay.[21] Each IC_{50} was a mean of at least six determinations.

In Vivo Efficacy Determination. The ability of compounds **6**−**8** to inhibit rodent iNOS in vivo was determined using LPS-treated rats (to induce systemic iNOS expression) as described previously.[13,22] One hour prior to LPS administration (10 mg/kg, intraperitoneally), compounds **6**−**8** were administered orally to rats at a dose of 10 mg/kg. Blood was collected 5 h following LPS administration, and plasma was separated and filtered through 10 000 MW cutoff Ultrafree microcentrifuge filter units. Plasma nitrite/nitrate concentrations were determined using a fluorometric assay for the measurement of nitrite/nitrate in biological samples.[21] The basal and stimulated values for plasma nitrite/nitrates are 15.7 (±0.7) and 289 (±37) μM, respectively, for one experiment and 23.2 (±0.1) and 238 (±23) μM, respectively, for another. The percent inhibition was calculated using values obtained from rats treated with LPS alone in the absence of inhibitor.

General Synthetic Procedure for Oxime Formation. A substituted cyclohexanone (1 equiv) was combined with hydroxylamine hydrochloride (NH_2OH·HCl; 1.5 equiv) and sodium acetate (1.8 equiv) in a mixture of EtOH (1 mL/mmol of ketone) and water (0.6 mL/mmol of ketone). The mixture was refluxed for 5 h under a N_2 atmosphere, cooled to room temperature, and allowed to stir for an additional 24 h. All solvent was removed from the reaction mixture under reduced pressure. The residue was partitioned between EtOAc and water, and the organic phase was washed with brine, dried (Na_2SO_4), and stripped of all solvent under reduced pressure to provided the oximes in yields of 84−99%. Each oxime, without further purification, showed essentially one peak with a 100% purity by peak area integration on a Shimadzu GC-14A gas chromatograph (GC) with a 0.25-mm × 25 M methyl, 5% phenylsilicone column, and NMR and IR spectra were consistent with the assigned structures.

General Procedure A: Oximes to Lactams (Beckman Rearrangement). One equivalent of oxime suspended or dissolved in 80% H_2SO_4 (0.16 mL/mmol of oxime) was added dropwise (~10 min) to 80% H_2SO_4 (0.13 mL/mmol of oxime) stirred magnetically and maintained at 120 °C with an external oil bath. An exotherm was noted within 5 min of the start of addition, and the temperature of the reaction rose to between 140 and 160 °C before cooling again to 120 °C. Ten minutes after the flask had cooled to 120 °C, it was removed from the bath and allowed to cool to room temperature. The product mixture was diluted with water (0.5 mL/mmol of oxime) and brought to pH 6 with concentrated NH_4OH. This solution was further diluted with water (0.5 mL/mmol of oxime) and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried (Na_2SO_4), filtered, and stripped of all solvent under reduced pressure. The residues were purified and isomers, when present, separated by HPLC on silica gel to provide the lactams in 40−80% yield. Each

chromatographed lactam showed essentially one peak with a peak area of 100% on a Shimadzu GC-14A GC with a 0.25-mm × 25 M methyl, 5% phenylsilicone column, and NMR spectra were consistent with the assigned structures.

General Procedure B: Oximes to Lactams (Beckman Rearrangement). To 1 equiv of oxime dissolved in 50 mL of acetone was added 1 N NaOH (1.1 equiv). After this mixture cooled in an ice bath, benzenesulfonyl chloride (1.0 equiv) was added dropwise (5 min) to the stirred reaction mixture maintained under a N_2 atmosphere. The reaction was allowed to warm to room temperature and stir for 48 h. If the product precipitated from the reaction mixture, it was filtered and washed with acetone. The filtrate was concentrated and the residue partitioned between EtOAc and brine. The organic layer was dried (Na_2SO_4), filtered, and stripped of all solvent under reduced pressure. The residue was treated as described in General Procedure A, above.

General Procedure C: Lactams to Iminoethers. To a stirred slurry of trimethyloxonium tetrafluoroborate ($Me_3O^+BF_4^-$, Lancaster; 1.2 equiv) in CH_2Cl_2 (5 mL/equiv of lactam) under Ar was added the lactam (1 equiv). This mixture was stirred at room temperature for 12 h before it was diluted with CH_2Cl_2 (5 mL/equiv of lactam) and partitioned between saturated $KHCO_3$ and EtOAc. The organic phase was separated, dried (Na_2SO_4), filtered, and stripped of all solvent under reduced pressure to provide the crude iminoether. This material was chromatographed on a short-path Merck flash silica column eluting with EtOAc/*n*-hexane (1:1). The lactam product had a GC peak area of 100% and NMR and IR spectra consistent with the indicated product.

General Procedure D: Lactams to Iminoethers. One equivalent of lactam (2.5 g, 19.7 mmol) dissolved in benzene (2 mL/mmol of lactam) was dried for 30 min with a Dean−Stark trap. To this mixture was added dimethyl sulfate (2 equiv), and heating was continued for an additional 17 h. After cooling to room temperature, the reaction was diluted with EtOAc (2.5 mL/mmol of lactam) and washed with saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc and the combined organic phase dried (Na_2SO_4), filtered, and stripped of all solvent under reduced pressure to yield a residue that was chromatographed and characterized as described in General Procedure C, above.

General Procedure: Amidinium Chloride Synthesis. An equivalent of imino ether and NH_4Cl (0.9 equiv) were refluxed in MeOH (10 mL/mmol of iminoether) under a nitrogen atmosphere from 3.5 to 24 h. After the reaction cooled to room temperature, it was filtered, stripped of all solvent under reduced pressure, and partitioned between water and EtOAc. The organic and aqueous phases were separated, and the aqueous phase was washed with another portion of EtOAc before it was lyophilized to provide the product amidinium salts in 44−93% yield. Each of the amidinium salts possessed a characteristic IR absorption between 1600 and 1733 cm^{-1}. Compound **1** was prepared as previously described.[19]

3-Ethylhexahydro-1*H*-azepin-2-imine, monohydrochloride (2): synthesized by general procedure A and its iminoether by general procedure C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.52−3.40 (m, 2H), 2.75 (m, 1H), 1.95−1.60 (m, 8H), 1.04 (t, 3H, J = 7 Hz); ^{13}C NMR (CD_3OD) δ 176.3, 45.0, 44.3, 29.4, 28.9, 28.8, 23.1, 12.0; HRMS (EI) calcd for $C_8H_{16}N_2$ *m/e* 140.131, found *m/e* 140.132. Anal. ($C_8H_{16}N_2$·HCl·0.2H_2O·0.01-NH_4Cl) C, H, N, Cl (H is off by 0.74).

Hexahydro-3-(2-propenyl)-1*H*-azepin-2-imine, monohydrochloride (3): synthesized by general procedure B and its iminoether by general procedure C; ^{1}H NMR (400 MHz, CD_3OD) δ 5.80 (m, 1H), 5.25−5.13 (m, 2H), 3.53−3.45 (m, 2H), 2.95 (m, 1H), 2.60 (m, 1H), 2.38 (m, 1H), 2.0−1.6 (m, 6H); ^{13}C NMR (D_2O) δ 177.0, 137.3, 120.8, 46.1, 43.9, 35.7, 34.4, 30.8, 29.9; HRMS (EI) calcd for $C_9H_{16}N_2$ *m/e* 152.131, found *m/e* 152.132. Anal. ($C_9H_{16}N_2$·HCl·0.4H_2O) C, H, N, Cl.

Hexahydro-5-methyl-1*H*-azepin-2-imine, monohydrochloride (4): synthesized by general procedure A and its iminoether by general procedure D; ^{1}H NMR (400 MHz, CD_3OD) δ 3.50−3.37 (m, 2H), 2.75 (m, 1H), 2.63 (m, 1H), 1.95 (m, 1H), 1.90−1.73 (m, 2H), 1.30−1.13 (m, 2H), 0.99 (d, 3H, J = 7 Hz); ^{13}C NMR (D_2O) δ 174.3, 43.6, 37.1, 36.9, 32.4, 31.1, 22.9; HRMS (M^+) $C_7H_{14}N_2$ requires *m/e* 126.116, found *m/e* 126.116. Anal. ($C_7H_{14}N_2$·0.85HCl·0.8H_2O) C, H, N, Cl.

Hexahydro-(6*R*)-methyl-1*H*-azepin-2-imine, monehydrochloride (5): synthesized by general procedure A and its iminoether by general procedure C; ^{1}H NMR (500 MHz, D_2O) δ 3.34 (m, 1H), 3.24 (m, 1H), 2.68 (m, 1H), 2.62 (m, 1H), 1.94−1.86 (m, 2H), 1.77 (m, 1H), 1.63 (m, 1H), 1.47 (m, 1H), 0.92 (d, 3H, J = 7 Hz); ^{13}C NMR (D_2O) δ 175.6, 52.0, 39.6, 34.5, 33.9, 24.0, 20.7; HRMS (M^+) $C_7H_{14}N_2$ requires *m/e* 126.116, found $m/_e$ 126.117; $[\alpha]_D = -12.7°$ (±3.5°) (MeOH, c = 0.869). Anal. ($C_7H_{14}N_2$·1.0HCl·0.25H_2O·0.55NH_4Cl) C, H, N, Cl.

Hexahydro-3-(2-propenyl)-1*H*-azepin-2-imine, monohydrochloride (6): synthesized by general procedure B and its iminoether by general procedure C; ^{1}H NMR (400 MHz, D_2O) δ 5.85 (m, 1H), 5.26−5.19 (m, 2H), 3.74 (m, 1H), 2.75 (ddd, 1H, J = 14.5, 12.4, 1.7 Hz), 2.59 (dd, 1H, J = 14.5, 6.6 Hz), 2.40 (t, 2H, J = 7 Hz), 2.04−1.94 (m, 2H), 1.87 (m, 1H), 1.65 (m, 1H), 1.56−1.35 (m, 2H); ^{13}C NMR (D_2O) δ 175.1, 136.7, 121.5, 58.0, 41.6, 35.7, 34.4, 31.0, 25.6; HRMS (M^+) $C_9H_{16}N_2$ requires *m/e* 152.131, found *m/e* 152.132. Anal. (C_9H_{16}-N_2·1.0HCl·0.75H_2O·0.05NH_4Cl) C, H, N, Cl.

Hexahydro-7-propyl-1*H*-azepin-2-imine, monohydrochloride (7): synthesized by general procedure B and its iminoether by general procedure C; ^{1}H NMR (400 MHz, CD_3-OD) δ 3.62 (m, 1H), 2.79 (ddd, 1H, J = 14.3, 12.2, 1.9 Hz), 2.61 (dd, 1H, J = 14.6, 6.6 Hz), 2.06−1.95 (m, 2H), 1.85 (m, 1H), 1.75−1.32 (m, 7H), 0.98 (t, 3H, J = 7.2 Hz); ^{13}C NMR (CD_3OD) δ 173.9, 57.1, 38.4, 35.0, 32.4, 29.7, 24.5, 20.1, 14.0; HRMS (M^+) $C_9H_{18}N_2$ requires *m/e* 154.147, found *m/e* 154.147. Anal. ($C_9H_{18}N_2$·1.0HCl·0.4H_2O·0.05NH_4Cl) C, H, N, Cl.

7-Butylhexahydro-1*H*-azepin-2-imine, monohydrochloride (8): synthesized by general procedure B and its iminoether by general procedure C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.60 (m, 1H), 2.79 (ddd, 1H, J = 14.5, 12.4, 1.9 Hz), 2.61 (ddt, 1H, J = 14.6, 6.7, 1.5 Hz), 2.06−1.95 (m, 2H), 1.86 (m, 1H), 1.76−1.32 (m, 9H), 0.95 (t, 3H, J = 7 Hz); ^{13}C NMR (CD_3OD) δ 173.9, 57.4, 36.0, 35.0, 32.4, 29.7, 29.1, 24.5, 23.5, 14.2; HRMS (M^+) $C_{10}H_{20}N_2$ requires *m/e* 168.163, found *m/e* 168.163. Anal. ($C_{10}H_{20}N_2$·1.0HCl·0.4·H_2O·0.05NH_4Cl) C, H, N, Cl.

7-(2-Ethylbutyl)hexahydro-1*H*-azepin-2-imine, monohydrochloride (9): synthesized by general procedure B and its iminoether by general procedure C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.66 (m, 1H), 2.82 (ddd, 1H, J = 15.5, 12.5, 2.0 Hz), 2.62 (ddt, 1H, J = 15.5, 6.5, 1.5 Hz), 2.06−1.95 (m, 2H), 1.86 (dt, 1H, J = 15.5, 4.0 Hz), 1.78−1.28 (m, 10H), 0.91 (t, 3H, J = 7.0 Hz), 0.89 (t, 3H, J = 7.0 Hz); ^{13}C NMR (CD_3OD) δ 173.9, 55.1, 40.2, 37.8, 35.4, 32.4, 29.6, 26.4, 25.4, 24.5, 10.8, 10.6; HRMS (EI) calcd for $C_{12}H_{24}N_2$ *m/e* 196.194, found *m/e* 196.194. Anal. ($C_{12}H_{24}N_2$·1.0HCl·0.6H_2O) C, H, N, Cl.

Hexahydro-7-phenyl-1*H*-azepin-2-imine, monohydrochloride (10): synthesized by general procedure B and its iminoether by general procedure C; ^{1}H NMR (CD_3OD) δ 7.50−7.30 (m, 5H), 2.99 (ddd, 1H, J = 15, 12, 2 Hz), 2.74 (dd, 1H, J = 15, 7 Hz), 2.12−1.76 (m, 6H), 1.60 (m, 1H); ^{13}C NMR (CD_3-OD) δ 172.1, 140.0, 129.0, 128.7, 128.1, 127.4, 126.3, 59.5, 35.0, 31.0, 28.2, 22.7; HRMS (EI) calcd for $C_{12}H_{16}N_2$ *m/e* 188.131, found *m/e* 188.131. Anal. ($C_{12}H_{16}N_2$·HCl·0.5H_2O) C, H, N, Cl (N is off by 0.52).

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Supporting Information Available: Standard deviation values for the compounds in Table 1 (1 page). Ordering information is given on any current masthead page.

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