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3-Hydroxy-4-methyl-5-pentyl-2-iminopyrrolidine: A Potent and Highly Selective Inducible Nitric Oxide Synthase Inhibitor

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Abstract—(3*S*,4*S*,5*R*)-2-Imino-4-methyl-5-pentyl-3-pyrrolidinol hydrochloride (**1**) is a potent inducible nitric oxide synthase (i-NOS) inhibitor that has three times the selectivity of its parent, (+)-*cis*-4-methyl-5-pentylpyrrolidin-2-imine hydrochloride (**2**).
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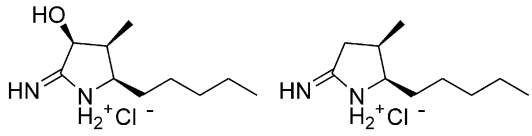
Selective inducible nitric oxide synthase (i-NOS) inhibitors have therapeutic potential for the treatment of diseases mediated by the overproduction of nitric oxide (NO). Three isozymes of NOS have been identified and characterized.[1] Under normal physiological conditions, the constitutive forms of NOS generate low, transient levels of NO in response to increases in intracellular calcium concentrations. NO regulates a variety of cellular processes, among them vascular tone, platelet aggregation, and immune response. NOS catalyzes a five electron transfer during a heme-based oxidation of L-arginine generating NO and citrulline. Elevated levels of NO due to upregulation of the induced isoform, i-NOS, and the resulting NO-derived metabolites, cause cellular cytotoxicity and tissue damage and are thought to contribute to the pathophysiology of a number of human diseases.[2] Selective iNOS inhibitors have been shown to suppress the increase in plasma nitrites and/or paw swelling associated with the overproduction of NO in animal models of acute and chronic inflammation including non-lethal endotoxemia,[3] carrageenan-induced paw edema[4,5] and adjuvant-induced arthritis.[6]

cis-4-Methyl-5-pentyl-iminopyrroldine (**2**)[7] has been reported to be a selective and potent i-NOS inhibitor. In order to identify more potent and more selective i-NOS inhibitors, we have investigated the effects of 3-hydroxylation (**1**) of the iminopyrrolidine ring (structures **1** and **2** are shown below).



1 **2**

A key intermediate in the synthesis of **1** was lactam **7**. Alternate routes to **7**[7] were explored as the chromatographic separation of the *cis* and *trans* isomers could not be achieved unless a chiral stationary phase was used. Initially, reduction of the 3,4-unsaturated lactam **6** as illustrated in Scheme 1 was investigated. To generate **6**, the Boc-lactam **4** was treated with base and the anion was quenched with phenylselenyl chloride. The phenylselenide **5** was oxidized and eliminated in the presence of hydrogen peroxide. With **6** in hand, the *cis*-lactam **7** was generated by the reduction of the olefin with $NaBH_4$ in the presence of $NiCl_2{\cdot}6H_2O$.

The hydroxyl group was introduced using the same anion chemistry that generated the phenylselenide. The addition of HMPA to LiHMDS was necessary to obtain the desired ring oxidation with (10-camphorsulfonyl)-oxaziridine.[8] To obtain **8**, the precursor to **1** and **13**, (1*S*)-(10-camphorsulfonyl)oxaziridine was used as the electrophile. Once the Boc group was removed by TFA to obtain **9**, the hydroxyl group was protected as a *t*-butyldimethylsilylether (**10**). The iminoether (**11**) was synthesized as described earlier.[7] Amidine (**12**) was generated using ammonia in methanol. Removal of the silyl group and protonation of the amidine was achieved with aqueous HCl. Purification on a YMC ODS AQ

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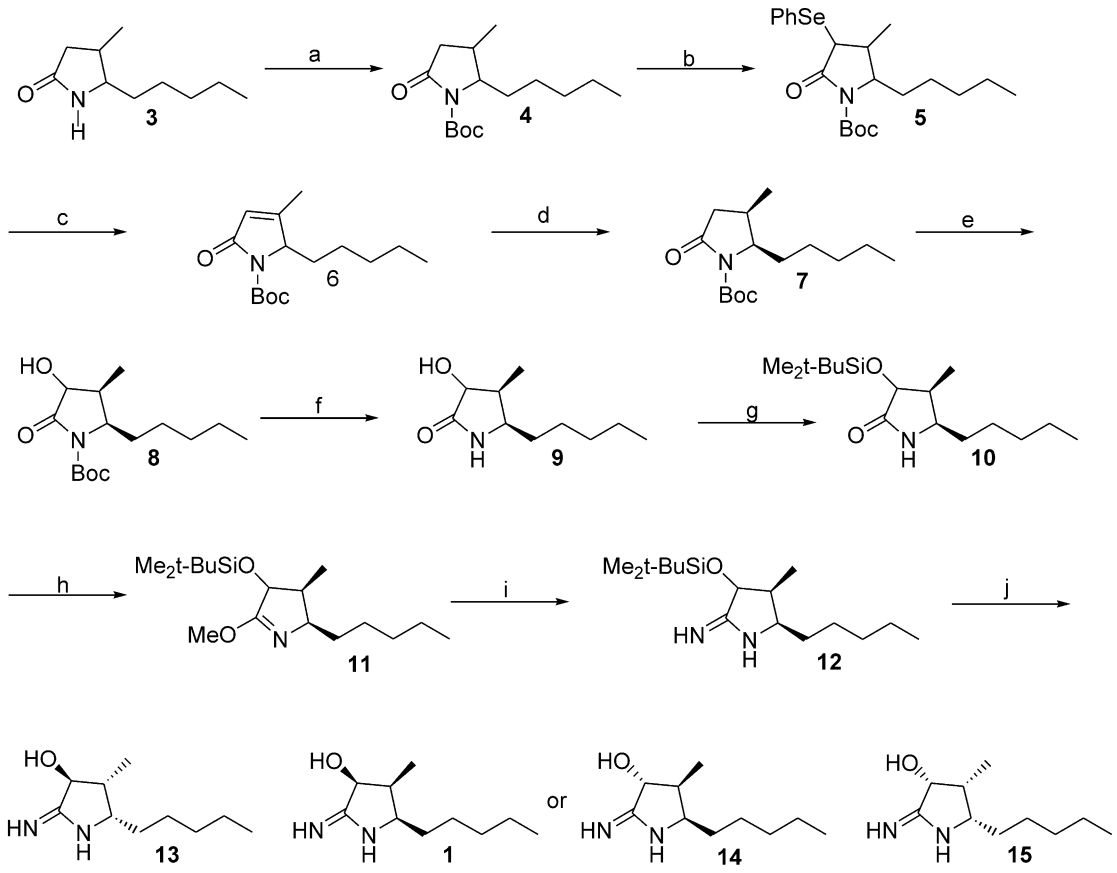
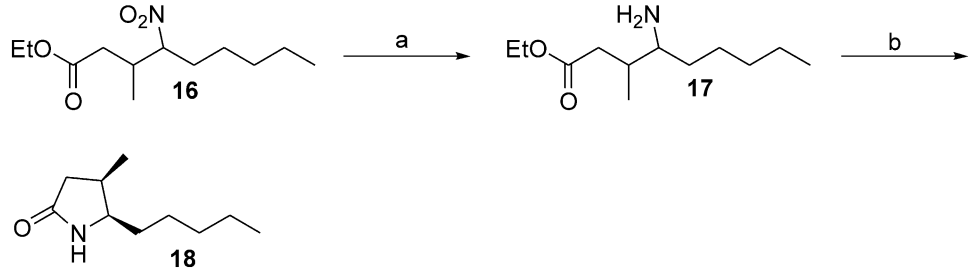


Scheme 1. (a) Boc_2O, DMAP, THF, Δ, 3 h, 96%; (b) LiHMDS, HMPA, PhSeCl, THF, −70 °C, 2 h, 72%; (c) H_2O_2, THF, 35–40 °C, 1.5 h, 60%; (d) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, 15 °C, 1 h, 96%; (e) LiHMDS, (1*R*)-(−)-(10-camphorsulfonyl)oxaziridine or (1*S*)-(−)-(10-camphorsulfonyl)oxaziridine, HMPA, THF, −70 to 40 °C, 3 h, 40%; (f) 20% TFA/CH_2, 1 h, quant; (g) *t*-butyldimethylsilylchloride, imidazole, DMF, 80%; (h) $Me_3O^+BF_4^-$, CH_2Cl_2, 16 h; (i) NH_3, MeOH, 16 h; (j) 10% HCl, MeOH, 37% (for three steps).

reverse-phase chromatography column yielded diastereomers **1** and **13**.[9,10] The relative orientation of the ring protons was established by ^1H NMR using NOESY conditions. To obtain **14** and **15**,[10,11] the same chemistry was repeated using 1*R*-(10-camphorsulfonyl)oxaziridine.

During the synthesis of **3**, a simpler approach to generate the *cis*-lactam **18** was developed as shown in Scheme 2. Reduction of **16**[7] was carried out in the presence of one molar equivalent of L-tartaric acid. Following the reduction, the *trans*-lactam was formed by heating the reaction mixture in methanol. The solvent was removed from the reaction and the residue was partitioned between dichloromethane and water. The *trans*-lactam was isolated from the organic layer with a small amount of *cis*-lactam. The amine tartrate salt of the *cis*-lactam precursor was isolated from the water layer. Cyclization to **18** was accomplished by heating the amine tartrate in ethanol in the presence of triethylamine. Using the chemistry illustrated in Scheme 1, **18** was carried on to **1**.

As shown in Table 1, **1**, with a potency of 0.78 μM, is less potent than **2** as an i-NOS inhibitor; however, **1** is three times as selective as **2**. The effect of hydroxylation on the iminopyrrolidine ring lessens the inhibitory activity for eNOS and nNOS. The impact of hydroxylation is greater on the constitutive isoforms than the induced isoform. Also the relative stereochemistry is important in that the all *cis* isomers are more selective than the *trans*-*cis* isomers. In addition, **1** with an ED_{50} of 10.0 mpk is less active in vivo in the LPS mouse than



Scheme 2. (a) Ra/Ni, L-tartaric acid, 60 psi, 25 °C, 72 h; (b) (i) MeOH, Δ, 3 h, (ii) EtOH, TEA, Δ, 2 h, 57%.

Table 1. Comparison of IC_{50} (μM) values for inhibition of human NOS isoforms[a]

Compd	iNOS	eNOS	nNOS	Selectivity (he/hi)	Selectivity (hn/hi)
2	0.25	226	3.2	897	13
19[b]	4.5	578	21	130	5.0
1	0.78	1980	27	2540	35
13	1.8	930	20	516	11
14	1.8	695	8.9	386	4.9
15	2.8	2930	49	1046	18

[a]IC_{50} values were determined as previously described.[12]
[b]**19** is the enantiomer of **2**.[7]

is **2**.[7] Introduction of a hydroxyl group to the iminopyrrolidine skeleton imparts improved selectivity for the i-NOS enzyme versus its constitutive isoforms.

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9. ^{1}H NMR (D_2O) **1, 15** δ 5.00 (1H, d, $J=6.8$ Hz), 3.83 (1H, dd, $J=7.3$, 12.8 Hz), 2.75 (1H, hex, $J=7.1$ Hz), 1.56–1.62 (2H, m), 1.29–1.42 (6H, m), 0.86–0.91 (6H, m).
10. Optical rotations: **1** $[+7.8]_D$; **13** $[-49.2]_D$; **14** $[+43.1]_D$; **15** $[-8.9]_D$.
11. ^{1}H NMR (D_2O) **13**, **14** δ 4.66 (1H, d, $J=11.6$ Hz), 3.87–3.92 (1H, m), 2.45–2.54 (1H, m), 1.58–1.66 (2H, m), 1.26–1.42 (6H, m), 1.16 (3H, d, $J=7.1$ Hz), 0.85–0.91 (3H, m).
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