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# Substituted 6-phenyl-pyridin-2-ylamines: selective and potent inhibitors of neuronal nitric oxide synthase

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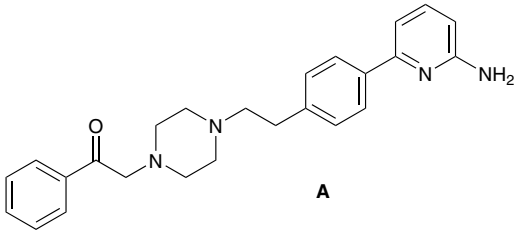
**Abstract**—The synthesis and nNOS and eNOS activity of 6-(4-(dimethylaminoalkyl)-/6-(4-(dimethylaminoalkoxy)-5-ethyl-2-methoxyphenyl)-pyridin-2-ylamines and 6-(4-(dimethylaminoalkyl)-/6-(4-(dimethylaminoalkoxy)-2,5-dimethoxyphenyl)-pyridin-2-ylamines **1**–**8** are described. These compounds are potent inhibitors of the human nNOS isoform.


Nitric oxide (NO), which is generated oxidatively from L-arginine by nitric oxide synthase (NOS), is an important neuromodulator in the CNS. All the NOS isoforms (nNOS, eNOS, and iNOS) are present in the brain. Given the role NO plays in a variety of normal and pathological states, selective inhibitors of NOS isozymes are of considerable interest. Inhibition of nNOS, for example, has been proposed as a neuroprotective strategy in stroke, Parkinson's disease, and trauma.[1,2] The role of NO in the pathophysiology of stroke has revealed complex and contradictory results. With 7-nitroindazole (7-NI), an nNOS/eNOS inhibitor, reduction in infarction volumes in animals is dependant on serum glucose concentration and ischemic intracellular pH.[3] Answering the question of the viability of selective nNOS inhibition as a therapeutic approach for neuroprotection will require the identification of not only potent but selective nNOS inhibitors.[4] Herein we report the preparation of potent and selective nNOS inhibitors.



A

Early efforts in our laboratory were focused on a series of 6-(4-(substituted)phenyl)-2-aminopyridines, the best of which were potent nNOS inhibitors[5] ($IC_{50}$ human nNOS = 100–250 nM) with modest selectivity (sevenfold selective against eNOS) for the nNOS isozyme (see structure A).[6] Efforts to increase potency and selectivity were successful when substituents were placed on additional positions of the phenyl ring. In particular, analogs bearing 2-methoxy and 5-ethyl (or 5-methoxy) substituents on the phenyl ring and having aminoalkyl and aminoalkoxy substitution in the 4-position provided potent and selective nNOS inhibitors. Herein we describe the synthesis and NOS activity of eight potent 6-(4-(dimethylaminoalkyl)-/6-(4-(dimethylaminoalkoxy)-5-substituted-2-methoxyphenyl)-pyridin-2-ylamines **1**–**8** in which we have evaluated 5-methoxy and 5-ethyl derivatives and have systematically varied the length and structure of the linker between the dimethylamino moiety and the phenyl ring.

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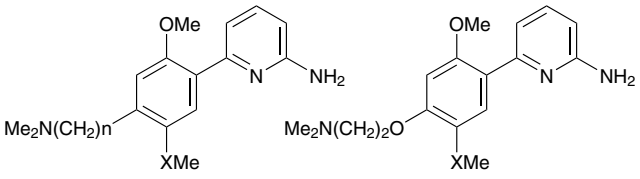


**1** (X=O, n=1) **2** (X=$CH_2$, n=1) **7** (X=O)
**3** (X=O, n=2) **4** (X=$CH_2$, n=2) **8** (X=$CH_2$)
**5** (X=O, n=3) **6** (X=$CH_2$, n=3)

**Figure 1.** nNOS targets.

Multistep syntheses were required for preparing the eight targeted 2-aminopyridine derivatives shown in Figure 1. The general strategy for accessing each of the compounds involved convergent routes utilizing a Suzuki coupling of appropriately substituted phenylboronic acids and protected 6-bromo-2-aminopyridines to build up the biaryl framework. The targeted aminopyridines **1**–**8** all contain a terminal dimethylamino group. Other substituted distal alkyl and aryl amines were prepared. In general, the dimethylamino group provides potent nNOS activity and was therefore used in this investigation.

6-(4-Dimethylaminomethyl-2,5-dimethoxy-2-aminopyridine **1** was prepared by coupling boronic acid **11** with bromopyridine **12** as described in Figure 2. Standard acetal hydrolysis of **13**, followed by reductive amination involving aldehyde **14**, and dimethylamine in the presence of sodium triacetoxyborohydride provided after removal of the 2,5-dimethylpyrrole protecting group, aminopyridine **1**. 4-Dimethylaminomethyl-2-methoxy-5-ethyl-2-aminopyridine **2** was similarly prepared by coupling of boronic acid **21** with Boc-protected amino-6-bromopyridine **22** (see Fig. 3). Boronic acid **21**, in turn, was generated in a four-step sequence from bromide **17** via a Stille coupling followed by olefin reduction, bromination, and metallation and treatment with triethyl borate to generate **21**.

Preparation of the dimethylaminoethyl derivatives **3** and **4** bearing the carbon atom linker is shown in Figure 4 and followed a strategy similar to that used for the preparation of **1** and **2**. Alcohol **26** was converted in three steps to boronic acid **28**, which was coupled with bromopyridine **22** to afford pyridine ether **29**. Removal of the TBDMS protecting group followed by a Dess–Martin oxidation of alcohol **30** generated aldehyde **31**, which was allowed to react with dimethylamine under standard reductive amination conditions to afford **40**. Boc removal yielded aminopyridine **3**. Aminopyridine **4** was generated using the identical strategy starting with 3-methoxy-6-ethyl-phenethyl alcohol **33**, which in turn was prepared in a two-step sequence from **32** involving a Friedel–Crafts acylation/ketone reduction protocol.

Benzaldehydes **14** and **42**, which provided **1** and **2**, were also employed to generate aminopyridines with a three carbon atom linker (see Fig. 5).

Accordingly, a Wittig condensation involving aldehyde **14** provided allylic amine **43**. Catalytic hydrogenation of **43** provided propylamine **45**. Removal of the pyrrole

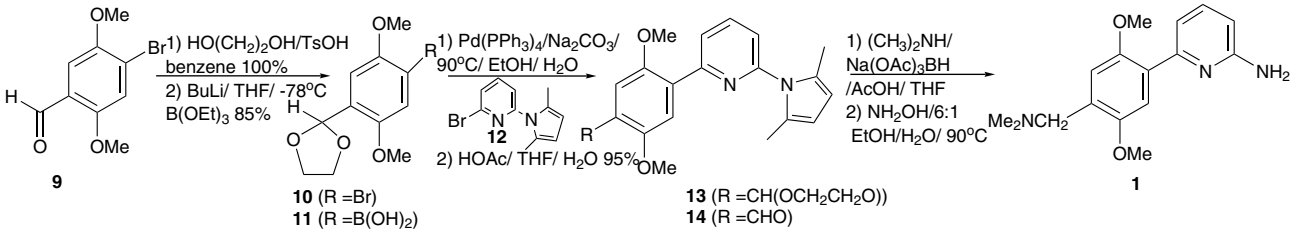


**Figure 2.**

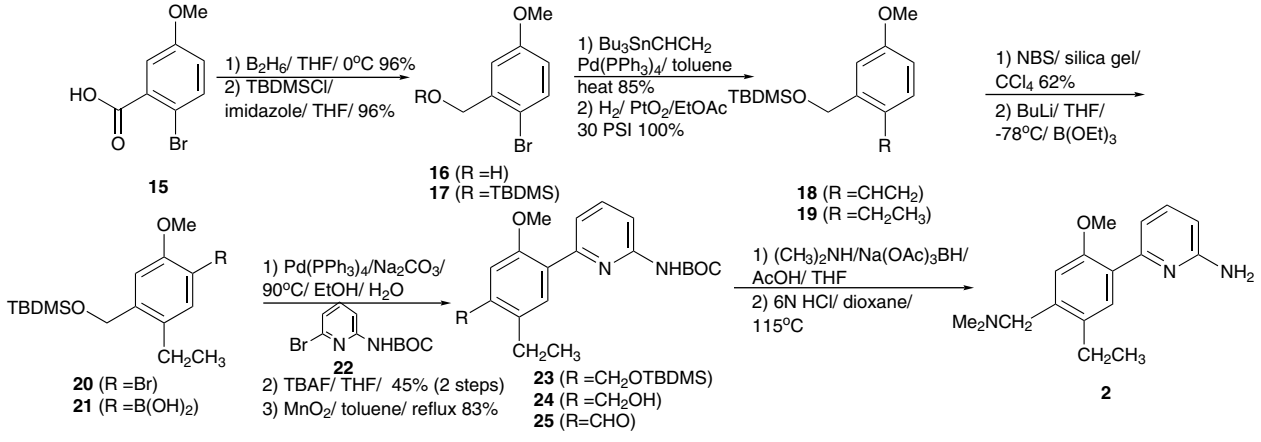


**Figure 3.**

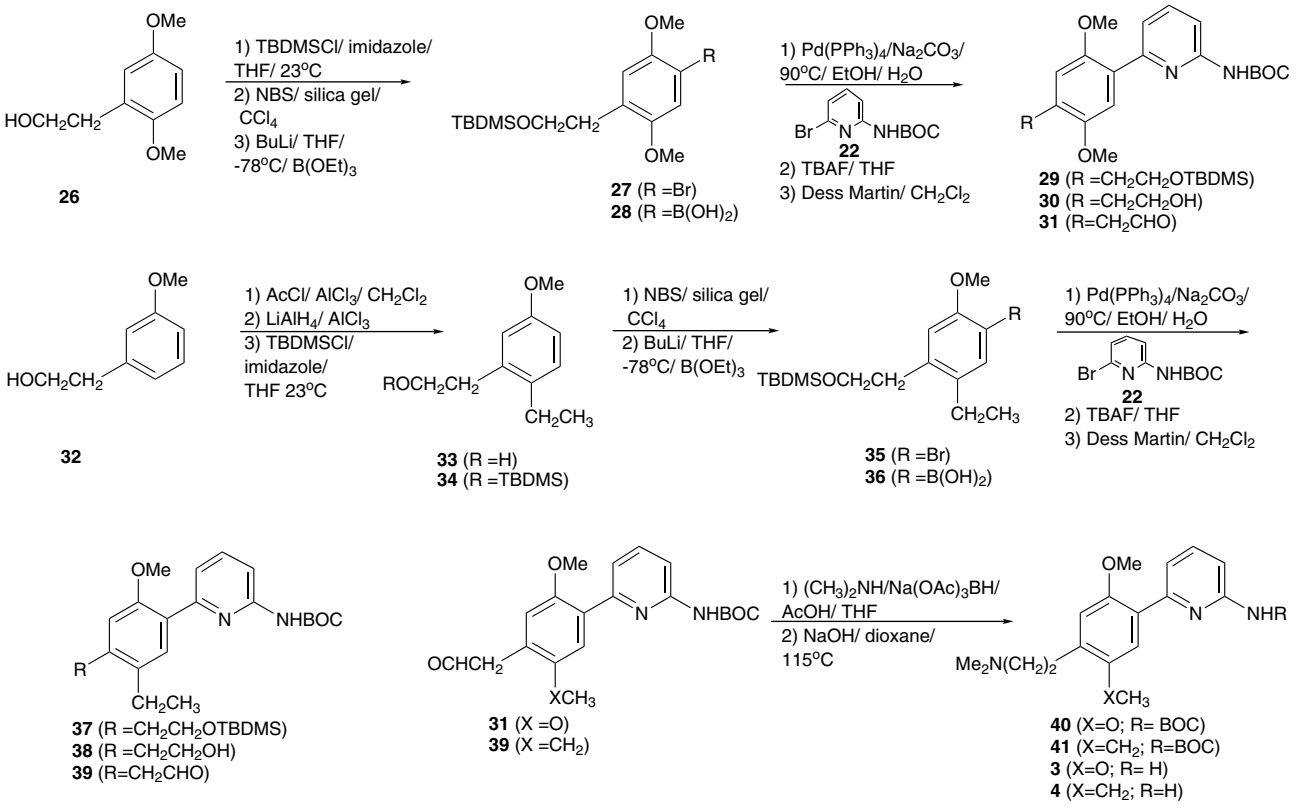


**Figure 4.**

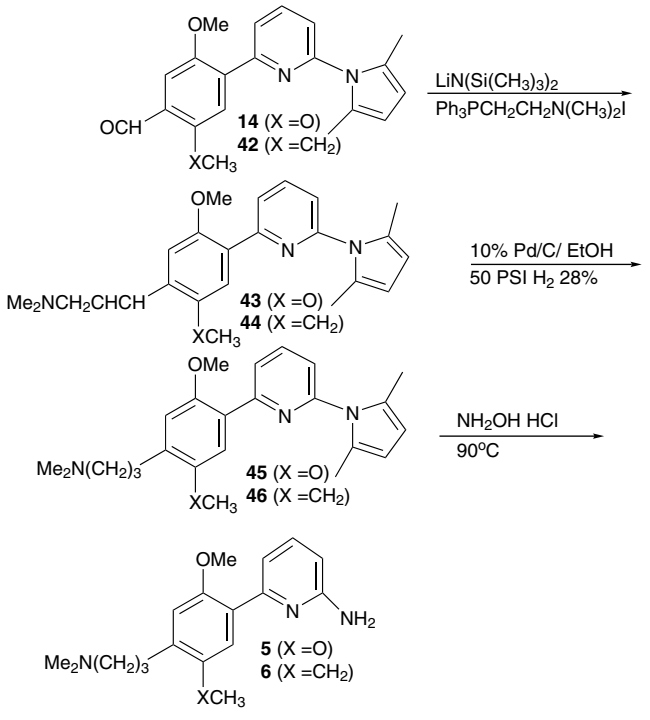


**Figure 5.**

protecting group with hydroxylamine provided aminopyridine **5**. Using the same methodology, aldehyde **42** was converted to aminopyridine **6**.

Finally, 2-hydroxy-4-methoxy acetophenone **47** served as a common starting material for the preparation of dimethylaminoethoxy derivatives **7** and **8** having an ethoxy linker (Fig. 6). Ketone reduction followed by phenol protection yielded **48**, which was converted to boronic acid **49** following bromination and subsequent lithiation and triethylborate addition. Suzuki Coupling of **49** provided pyridine **50**. Removal of the benzyl moiety via transfer hydrogenolysis and the pyrrole moiety with hydroxylamine hydrochloride provided phenol **55**. Treatment of **55** with *N*-(2-chloroethyl)dimethylamine in the presence of cesium carbonate generated aminopyridine **8**. To access aminopyridine **7**, **47** was converted to **51** following phenol protection and ring bromination. Baeyer–Villiger oxidation followed by phenol protection provided bromide **52**. Metal halogen exchange followed by boronic acid formation and subjection to standard Suzuki coupling conditions with bromopyridine **12** afforded pyridine **53**. Removal of the TBDMS protecting group and alkylation of the resultant phenol with iodomethane provided pyridine **54**. Removal of the phenol and amino protecting groups using standard conditions (transfer hydrogenolysis/pyrrole hydrolysis) afforded phenol **56**, which was alkylated to provide aminopyridine **7** as described for the conversion of **55** to **8** (Table 1).

The nNOS and eNOS inhibitory activity of the compounds reported in this manuscript were measured in vitro using human NOS isoforms derived from the cloned genes expressed baculovirus in Sf9 cells.[7] All eight targeted aminopyridines have attractive in vitro

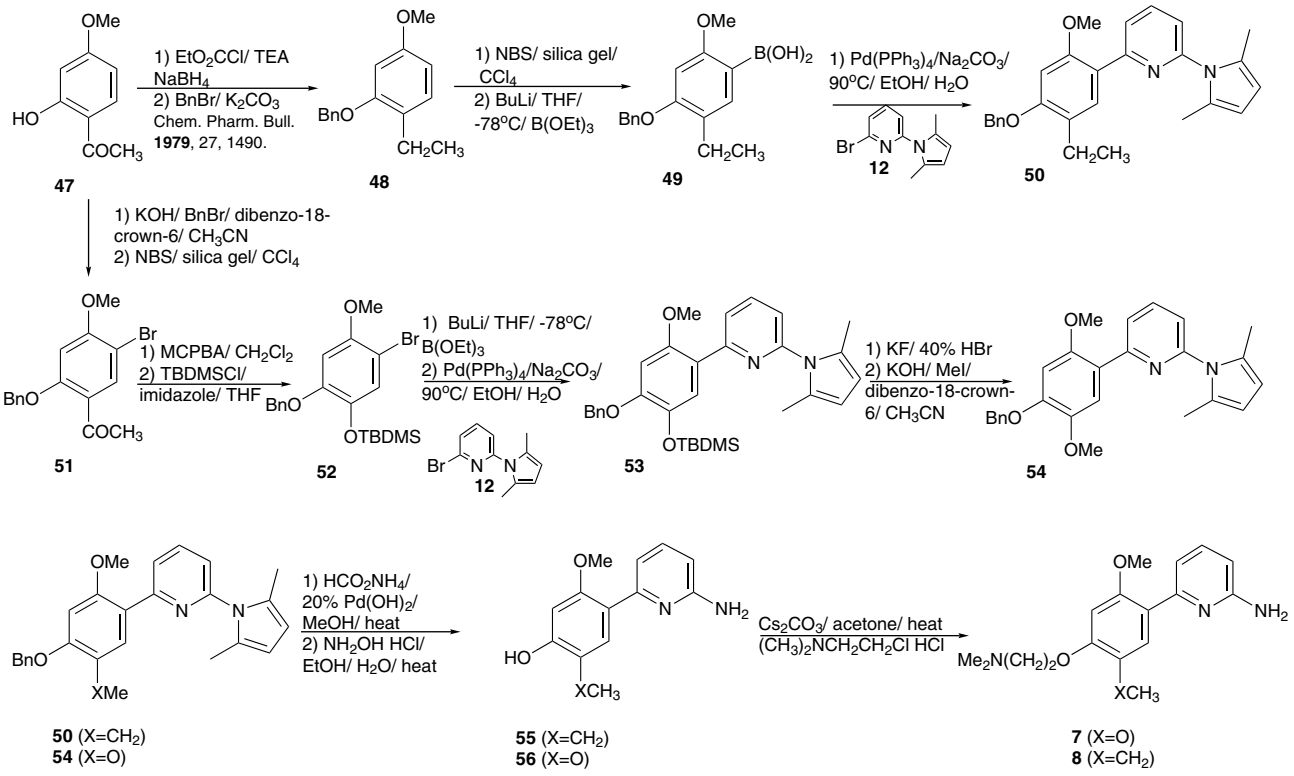


**Figure 6.**

**Table 1.** Nitric oxide synthase inhibition for compounds **A** and **1**–**8**

| Compound | Human nNOS ($IC_{50}$)[a] | Human eNOS ($IC_{50}$)[a] | nNOS/ eNOS |
|---|---|---|---|
| **A** | 140 | 887 | 6.3 |
| **1** | 810 | >32,000 | 40 |
| **2** | 54 | 8833 | 164 |
| **3** | 235 | >32,000 | 136 |
| **4** | 75 | 16,500 | 220 |
| **5** | 200 | >32,000 | 160 |
| **6** | 75 | 7700 | 103 |
| **7** | 112 | 21,943 | 196 |
| **8** | 58 | 3710 | 64 |

[a] Values are means of three experiments. Inhibition of human neuronal and human endothelial nitric oxide synthase activity given as an $IC_{50}$ value in nM units.

nNOS activity. All are selective for nNOS with a range of 40- to 220-fold. In general, the 2,5-dimethoxy analogs (**1**, **3**, **5**, and **7**) are devoid of eNOS activity. The 2-methoxy-5-ethyl analogs (**2**, **4**, **6**, and **8**), on the other hand, in which the 5-methoxy group has been replaced with a 5-ethyl group are more potent for human nNOS and still have significant nNOS selectivity (nNOS/eNOS: 64–220). Of the eight analogs, dimethylaminomethyl analog **2** and dimethylaminoethoxy analog **8** are the most potent nNOS inhibitors. Compounds described in this manuscript have been profiled in vivo. Efficacy in animal models will be described in due course.

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