

## PART A. LITERATURE REVIEW

### CHAPTER 1. INTRODUCTION

The results of experiments conducted during the past several years in a number of laboratories support the proposal that the inhibition of monoamine oxidase B (MAO-B) and/or neuronal nitric oxide synthase (nNOS) may activate neuroprotective mechanisms that could retard the neurodegenerative processes leading to Parkinson's disease (PD). This thesis presents the results of synthetic studies that have led to the preparation of potential tetrahydropyridinyl prodrugs of indazole and indazole derivatives including 7-nitroindazole, a compound which inhibits both MAO-B and nNOS and which protects against the degeneration of mouse nigrostriatal neurons caused by the parkinsonian inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mechanistic investigations have provided new insights into the chemical reactivity and regiochemical behavior of indazoles in nucleophilic substitution reactions. The interactions of the candidate prodrugs with MAO-B have been documented. The results of these studies are discussed in terms of structural requirements for MAO-B substrates and inhibitors and for the subsequent fate of the initially formed dihydropyridinium metabolites.

#### 1.1. PARKINSON'S DISEASE

PD is a progressive neurodegenerative disorder of the basal ganglia. Since it is not clear that the disorder is a single entity with a known cause,<sup>1</sup> it is also referred as the "idiopathic parkinsonism" (IP).<sup>2</sup> James Parkinson first defined the disease as the shaking palsy (*paralysis agitans*) in his book in 1817.<sup>3</sup> Shaking palsy was characterized as "involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace: the senses and intellects being uninjured".<sup>3</sup> Occurrence of neuronal loss with advancing age was first proposed by Hodge in 1894 and PD was characterized pathologically by loss of pigmented brainstem neurons, especially those in

---

<sup>1</sup> Calne, D.B. (1989) Is "Parkinson's disease" one disease? *J. Neurol. Neurosurg. Psychiatry* **52**, 18-21.

<sup>2</sup> Calne, D.B. (1994) Is idiopathic parkinsonism the consequence of an event or a process? *Neurology* **44**, 5-10.

<sup>3</sup> Parkinson, J. (1817) *An Essay on the Shaking Palsy*, Whittingham and Rowland, London, U.K.

the substantia nigra pars compacta, in 1967.<sup>4</sup> In the early 1960s it was discovered that there is a substantial loss of dopamine in the striatum of the parkinsonian brain.<sup>5,6,7</sup> It is now clear that PD can be defined in biochemical terms primarily as a dopamine-deficiency state resulting from degeneration or injury to dopaminergic neurons.<sup>8</sup> Even in patients with mild symptoms, a dopaminergic neuron loss of 50%<sup>9,10</sup> and striatal dopamine loss of 70% - 80% are observed.<sup>11</sup> Another pathological, but not unique, characteristic of PD is the presence of intracellular inclusion bodies called Lewy bodies in the surviving nerve cells.<sup>12,13</sup>

In clinical terms, PD is characterized by a resting tremor in the 3- to 5-Hz range, muscular rigidity, difficulty in initiating motor activity and loss of postural reflexes.<sup>14,15,16</sup> It is observed in approximately 1% of the population over 55 years of age.

Although the cause of PD is not known, several hypotheses have been proposed. Among these hypotheses, genetic factors seem to have been ruled out by three independent studies which failed to show a higher occurrence rate of PD in monozygotic twins compared to dizygotic twins.<sup>17,18,19</sup> Due to our research interests we will be

---

<sup>4</sup> Hoehn, M.M., Yahr, M.D. (1967) Parkinsonism: Onset, progression and mortality. *Neurology* **17**, 427-442.

<sup>5</sup> Bernheimer, H., Hornykiewicz, O. (1965) Decreased homovanillic acid concentration in the brain in parkinsonian subjects as an expression of a disorder of central dopamine metabolism. *Klin Wochenschr.* **43**, 711-715.

<sup>6</sup> Hornykiewicz, O. (1966) Dopamine and brain function. *Pharmacol. Rev.* **18**, 925-964.

<sup>7</sup> Bernheimer, H., Birkmeyer, W., Hornykiewicz, O., Jellinger, K., and Seitelberger, F. (1973) Brain dopamine and the syndromes of Parkinson and Huntington. *J. Neuro. Sci.* **20**, 415-455.

<sup>8</sup> Marsden, C.D. (1990) Parkinson's disease. *The Lancet* **335**, 948-952.

<sup>9</sup> Jellinger, K. (1987) Overview of morphological changes in Parkinson's disease. *Adv. Neurol.* **45**, 1-18.

<sup>10</sup> Fearnley, J.M., Lees, A.J. (1991) Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain* **114**, 2283-2301.

<sup>11</sup> Riederer, P., Wuketich, S. (1976) Time course of nigra-striatal degeneration in Parkinson's disease. *J. Neurol. Transm.* **38**, 377-382.

<sup>12</sup> Forno, L. (1986) The Lewy Body in Parkinson's disease. *Advances in Neurology* **45**, 35-43.

<sup>13</sup> Gibb, W.R.G., Lees, A.J. (1989) The significance of the Lewy body in the diagnosis of idiopathic Parkinson's disease. *Neuropathol. Appl. Neurobiol.* **15**, 27-44.

<sup>14</sup> Brooks, D.J., Playford, E.D., Ibanez, V., Sawle, G.V., Thompson, P.D., Findley, L.J., Marsden, C.D. (1992) Isolated tremor and disruption of the nigrostriatal dopaminergic system: An <sup>18</sup>F-dopa PET study. *Neurology* **42**, 1554-1560.

<sup>15</sup> Bernheimer, H., Birkmeyer, W., Hornykiewicz, O., Jellinger, K., Seitelberger, F. (1973) Brain Dopamine and Syndromes of Parkinson and Huntington. *J. Neurol. Sci.* **20**, 415-455.

<sup>16</sup> Traub, M.M., Rothwell, J.C., Marsden, C.D. (1980) Anticipatory postural reflexes in Parkinson's disease and other akinetic-rigid syndromes and in cerebellar ataxia. *Brain* **103**, 393-412.

<sup>17</sup> Ward, C.D., Duvoisin, R.C., Ince, S.E., Nutt, J.D., Eldridge, R., Calne, D.B. (1983) Parkinson's disease in a set of quadruplets. *Neurology* **33**, 815-824.

<sup>18</sup> Marsden, C.D. (1987) Parkinson's disease in twins. *J. Neurol. Neurosurg. Psychiatry* **30**, 105-106.

<sup>19</sup> Martilla, R.J., Kaprio, J., Koskenvuo, M., Rinne, U.K. (1988) Parkinson's disease in a nationwide twin cohort. *Neurology* **38**, 1217-1219.

focusing on free-radical oxidative stress<sup>20</sup> in relation with mitochondrial dysfunction<sup>21</sup> as a potential underlying contribution to the neurodegenerative processes leading to PD.

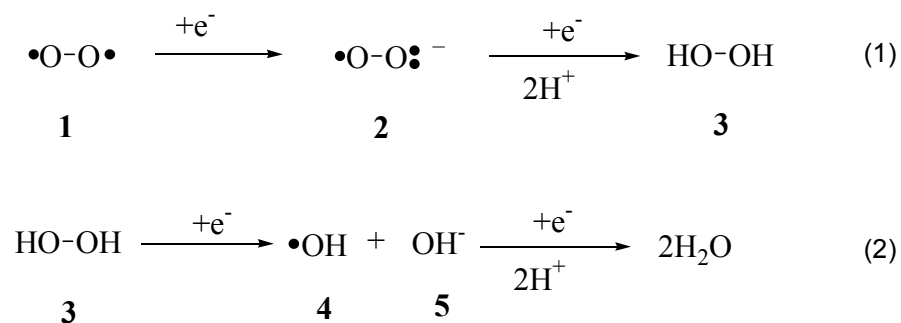
## 1.2. OXIDATIVE STRESS

Oxidative stress can be defined as the disruption of the equilibrium between the factors that promote free-radical formation and anti-oxidant defense mechanisms.<sup>22</sup> Being very reactive species, free radicals may cause damage in biologically critical molecules including DNA, cellular proteins and membrane lipids.<sup>23</sup>

### 1.2.1. REACTIVE OXYGEN SPECIES

One of the main sources of free radicals in biological systems is oxidative phosphorylation where the reduction of dioxygen (1) to water proceeds through the formation of partially reduced, reactive oxygen species. Scheme 1 outlines the sequence leading to superoxide radical anion (2), hydrogen peroxide (3) and hydroxyl radical (4).<sup>23</sup>

**Scheme 1. Formation of reactive oxygen species 2, 3 and 4 as molecular oxygen (1) is reduced to water via oxidative phosphorylation.**



<sup>20</sup> Jenner, P., Olanow, C.W. (1996) Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology* **47**, 161-170.

<sup>21</sup> Schapira, A.H.V. (1994) Evidence for mitochondrial dysfunction in Parkinson's disease-a critical appraisal. *Mov. Disord.* **9**, 125-138.

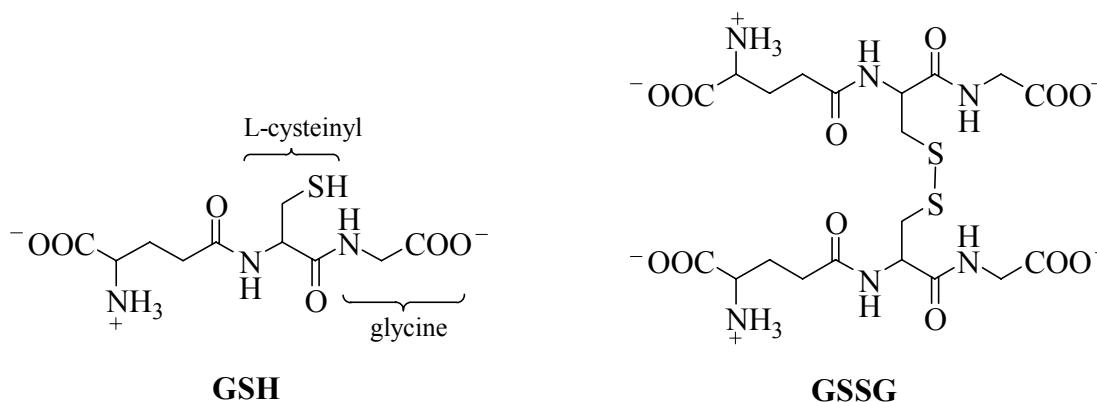
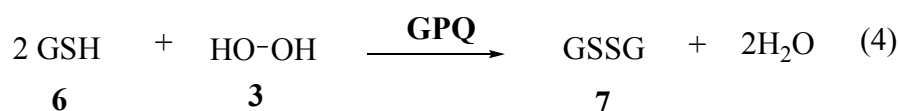
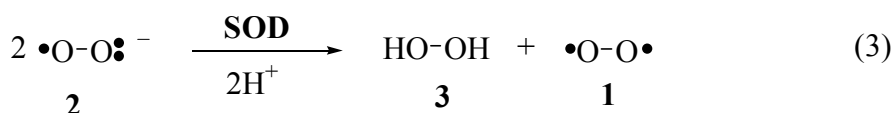
<sup>22</sup> Olanow, C.W. (1993) A radical hypothesis for neurodegeneration. *TINS* **16**, 439-444.

<sup>23</sup> Halliwell, B., Gutteridge, J.M.C. (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *J. Biochem.* **219**, 1-14.

These reactive species are tightly bound in the mitochondria where oxidative phosphorylation takes place. Two enzymes, superoxide dismutase (SOD)<sup>24</sup> and glutathione peroxidase (GPQ), limit free radical formation via the following reactions (Scheme 2).<sup>25</sup>

**Scheme 2. Reaction 3: Superoxide dismutase (SOD) catalyzes the conversion of two equivalents of superoxide radical anion (2) into hydrogen peroxide (3) and dioxygen**

**(1). Reaction 4: Two equivalents of  $\gamma$ -glutamylcysteinyl glycine [(GSH (6)] are oxidized to glutathione disulfide [GSSG (7)] in a reaction catalyzed by glutathione peroxidase (GPQ) which is coupled with the reduction of hydrogen peroxide (3) to water.**



Also radical scavengers like vitamin E and ascorbate, which react directly with free radicals, provide a way of protecting sensitive biomolecules. Increases in SOD

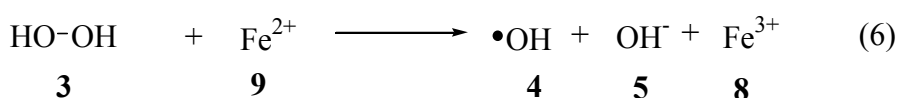
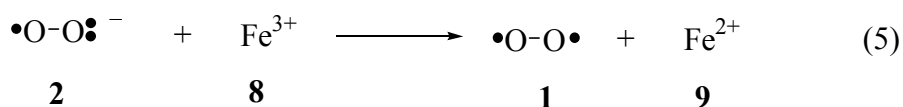
<sup>24</sup> McCord, J.M., Fridovich, I. (1988) Superoxide dismutase: the first twenty years (1968-1988). *Free Radical Biol. Med.* **5**, 363-369.

<sup>25</sup> Flohe, L. (1978) Glutathione peroxidase: Fact and fiction. *Ciba Found Symp.* **65**, 95-122.

levels<sup>26</sup> and decreases in GSH levels<sup>27</sup> in the substantia nigra pars compacta region of brains obtained from individuals with PD suggest that oxidative stress may be playing an important role in neurodegeneration.

Another finding is the increased levels of iron in the PD brain.<sup>28</sup> Iron is known to promote radical formation<sup>29</sup> by the Haber Weiss reaction (Scheme 3).

**Scheme 3. Reaction 5: Iron (III) is reduced to iron (II) by one electron transfer from superoxide radical anion (2) to give dioxygen (1). Reaction 6: Reduced iron (9) is oxidized back to iron (III) by hydrogen peroxide (3) leading to the formation of hydroxyl radical (4).**



Superoxide radical anion was shown to exert toxic effects directly on several components of living cells<sup>30</sup> but it is less reactive than hydroxyl radical.<sup>31</sup> Therefore the iron promoted conversion of superoxide radical anion can be important in oxidative stress. However, we must keep in mind that the rate constant for **Reaction 5** (Scheme 3) is  $1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ ,<sup>32</sup> which is smaller than the reaction of other cellular compounds like

<sup>26</sup> Saggi, H., Cooksey, J., Dexter, D.T., Wells, F.R., Lees, A., Jenner, P., Marsden, C.D. (1989) A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. *J. Neurochem.* **53**, 692-697.

<sup>27</sup> Sofic, E., Lange, K.W., Jellinger, R., Riederer, P. (1992) Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. *Neurosci. Lett.* **142**, 128-130.

<sup>28</sup> Sofic, E., Paulus, W., Jellinger, K., Riederer, P., Youdim, M.B.H. (1991) Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. *J. Neurochem.* **56**, 978-982.

<sup>29</sup> Funk, F., Lenders, J.-P., Crichton, R.R., Schneider, W. (1985) Reductive mobilization of ferritin iron. *Eur. J. Biol. Chem.* **152**, 167-172.

<sup>30</sup> Fridovich, L., (1986) Biological effects of the superoxide radical. *Arch Biochem Biophys.* **247**, 1-11.

<sup>31</sup> Sawyer, D.T., Valentine, J.S. (1981) How super is superoxide? *Acc. Chem. Res.* **14**, 393-400.

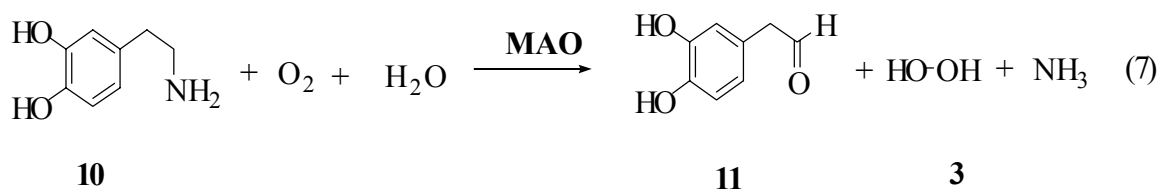
<sup>32</sup> Winterbourn, C.C. (1979) Comparison of superoxide with other reducing agents in the biological production of hydroxyl radicals. *Biochem J.* **182**, 625-628.

ascorbate with iron.<sup>33</sup> Thus, the production of hydroxyl radical via this pathway will be limited *in vivo*.

### 1.2.2. DOPAMINE METABOLISM AS A SOURCE OF REACTIVE OXYGEN SPECIES

Autoxidation of dopamine [DA (**10**)] and its metabolism by the monoamine oxidases (MAO) are other sources of reactive oxygen species in the brain. The MAO catalyzed oxidation of DA to 2-(3,4-dihydroxyphenyl)acetaldehyde (**11**) leads to the formation of hydrogen peroxide (Scheme 4).

**Scheme 4. MAO catalyzed oxidation of DA (**10**) to 2-(3,4-dihydroxyphenyl)acetaldehyde (**11**) and hydrogen peroxide.**

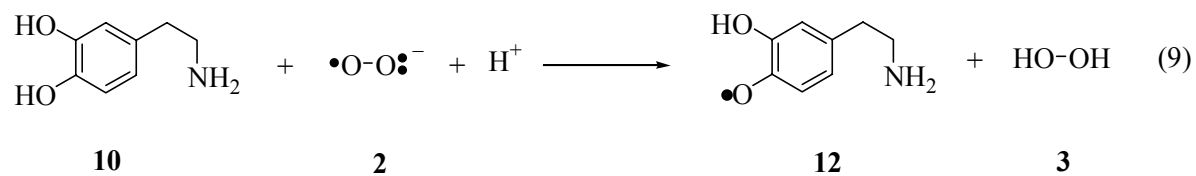
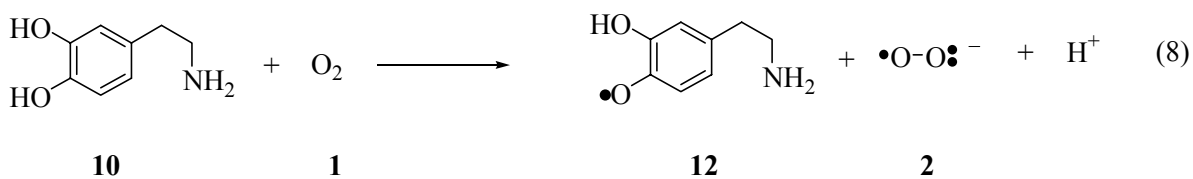


Autoxidation of dopamine results in the formation of the semiquinone radical **12** which can be directly toxic or which can lead to the formation of reactive oxygen species (Scheme 5).<sup>34</sup>

<sup>33</sup> Babbs, C.F., Griffin, D.W. (1989) Scatchard analysis of methane sulfinic acid production from dimethyl sulfoxide: a method to quantify hydroxyl radical formation in physiologic systems. *Free Radical Biol. Med.* **6**, 493-503.

<sup>34</sup> Olanow, C.W. (1990) Oxidation reactions in Parkinson's disease. *Neurology* **40**, 32-37.

**Scheme 5. Autoxidation of DA (10) to the corresponding semiquinone 12 resulting in the formation of reactive oxygen species 2 and 3.**



This autoxidation pathway also leads to polymerization of DA to form neuromelanin which also may be toxic.<sup>35</sup> However, increased neuromelanin formation is not observed in PD developing patients suggesting that the MAO catalyzed oxidation of DA (Scheme 4) may play a more important role via oxidative stress mechanisms.

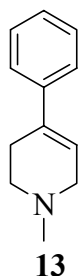
Increased DA turnover at the early stages of the disease may cause increased formation of H<sub>2</sub>O<sub>2</sub>. In case of a deficiency in glutathione, which will normally clear excess H<sub>2</sub>O<sub>2</sub> (**Reaction 3**), H<sub>2</sub>O<sub>2</sub> may be converted into hydroxyl radical (**Reactions 1 and 6**). The formation of hydroxyl radicals may trigger lipid peroxidation causing cell death.<sup>36</sup>

<sup>35</sup> Offen, D., Gorodin, S., Melamed, E., Hanania, J., Malik, Z. (1999) Dopamine-melanin induces apoptosis in PC12 cells- a possible implications for the etiology of Parkinson's disease. *Neurosci. Lett.* **260**, 101-104.

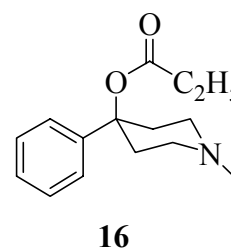
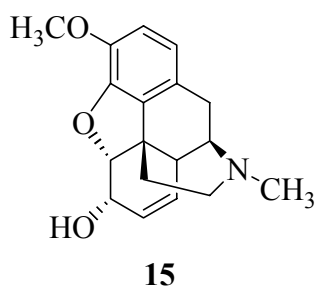
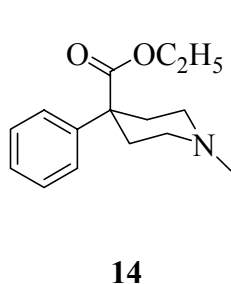
<sup>36</sup> Spina, M.B., Cohen. G., (1989) Dopamine turnover and glutathione oxidation: implications for Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **88**, 1398-1400.

### 1.2.3. RECOGNITION OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) AS A NEUROTOXIN

The recognition of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**13**)] as a human neurotoxin<sup>37</sup> and studies on its mechanism of action<sup>38</sup> have had a very important role in the development of oxidative stress and mitochondrial dysfunction hypotheses. The history of this compound is reviewed briefly below.



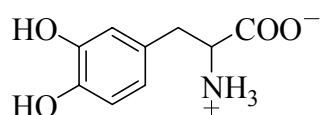
In February, 1977, a previously healthy 23 years old male was referred to the National Institute of Mental Health for evaluation of a persistent parkinsonian syndrome of 3 months duration.<sup>39</sup> He had abused a wide variety of drugs including marijuana, amphetamines, barbiturates and other sedative-hypnotics. He finally chose opiate derivatives, such as meperidine (**14**) and codeine (**15**). While in college, this individual unsuccessfully attempted to synthesize several opiate derivatives. In the summer of 1976, he succeeded in his attempts to synthesize 1-methyl-4-phenyl-4-propionoxypiperidine [MPPP (**16**)] a meperidine (**14**) analog:



<sup>37</sup> Ballard, P.A., Tetrud, J.W., Langston, J.W. (1985) Permanent human parkinsonism due to MPTP. *Neurology* **35**, 949-956.

<sup>38</sup> Singer, T.P., Ramsay, R.R. (1990) Mechanism of the neurotoxicity of MPTP. An update. *FEBS Lett.* **274**, 1-8.

This compound was reported by the patient to have opioid-like activity. The patient subsequently prepared additional batches of **16** but took synthetic shortcuts. In November, 1976, after several days of injecting samples of a recently prepared batch, he developed a parkinsonian syndrome. The patient continued to abuse many drugs one of which, ironically, was 3,4-dihydroxyphenylalanine (**17**), known as levodopa (L-DOPA), which is used as a treatment for Parkinson's disease. In September, 1978, he was found dead of a drug overdose. Microscopic examination of the brain showed degeneration of the substantia nigra.<sup>40</sup>



**17**

Although the above case was reported in 1979, recognition of MPTP as a neurotoxin didn't take place for another 4 years. In 1983, Langston reported motor deficits similar to those observed in Parkinson's disease in 4 other people who had self-administered MPPP.<sup>40</sup> The symptoms appeared to be due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**13**)], a by-product of the chemical synthesis of MPPP which presumably also contaminated the batch of MPPP prepared by the patient studied at the NIMH.

The reaction conditions had an important effect on the percentage of MPTP formed.<sup>41</sup> The patient studied at NIMH had repeated the synthesis with reduced reaction times and higher reaction temperatures which would facilitate the dehydration of 1-methyl-4-phenyl-4-piperidinol (**19**) to form MPTP (Scheme 6). Since the patient also

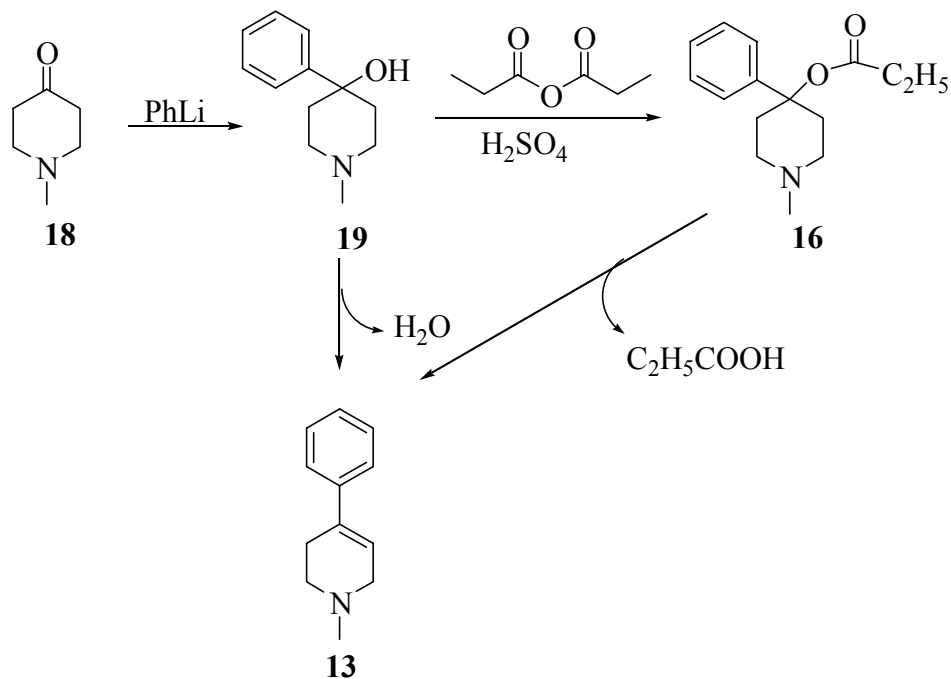
<sup>39</sup> Davis, G.C, Williams, A.C., Markey, S.P., Ebert, M.H., Caine, E.D., Reichert, C.M., Kopin, I.J. (1979) Chronic Parkinsonism secondary to intravenous injection of meperidine analogs. *Psychiatry Research* **1**, 249-254.

<sup>40</sup> Langston, J.W., Ballard, P., Tetrud, J.W., Irwin, I. (1983) Chronic parkinsonism in humans due to a product mepiridine-analog synthesis. *Science* **219**, 979-980.

<sup>41</sup> Ziering, A., Berger, L., Heineman, S.D., Lee, J. (1947) Piperidine derivatives: Part III. 4-Aryl piperidines. *J. Org. Chem.* **12**, 894-903.

neglected to isolate and crystallize the product properly, elimination of propionic acid from **16** also would have yielded MPTP.

**Scheme 6. Synthesis of MPPP (16) may yield MPTP (13) upon dehydration of intermediate 19 or elimination of propanoic acid from MPPP.**



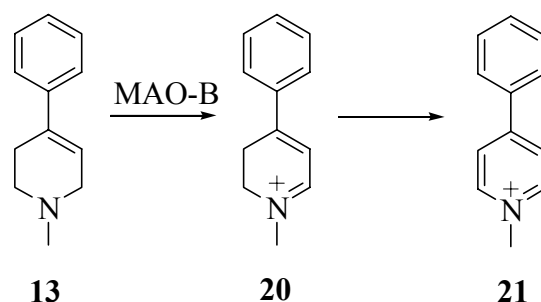
Administration of MPTP was shown to cause selective damage to the nigrostriatal dopaminergic pathway in primates and other animal species.<sup>42,43</sup> The neurotoxicity of MPTP is dependent on its conversion to the 1-methyl-4-phenylpyridinium species MPP<sup>+</sup> (**21**).<sup>44</sup> This conversion takes place via oxidation of MPTP to MPDP<sup>+</sup> (**20**), a reaction which is catalyzed by monoamine oxidase B (MAO-B). Then MPDP<sup>+</sup> is oxidized further to form MPP<sup>+</sup> by a poorly defined pathway:

<sup>42</sup> Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H, Jacobowitz, D.M., Kopin, I.J. (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. USA* **80**, 4546-4550.

<sup>43</sup> Langston, J.W., Forno, L.S., Rebert, C.S., Irwin, I. (1984) Selective nigral toxicity after systematic administration of 1-methyl-4-phenyl 1,2,5,6-tetrahydropyridine(MPTP) in the squirrel monkey. *Brain Res.* **292**, 390-394.

<sup>44</sup> Salach, J.I., Singer, T.P., Castagnoli, N., Jr., Trevor, A. (1984) Oxidation of the neurotoxic amine 1-methyl-1,2,3,6 tetrahydropyridine (MPTP) by monoamine oxidases A and B and suicide inactivation of the enzymes by MPTP. *Biochem Biophys. Res. Commun.* **125**, 831-835.

**Scheme 7. MAO-B catalyzed oxidation of MPTP.**



MPP<sup>+</sup> is transported into the nigrostriatal nerve terminals by the DA transporter where it subsequently localizes within inner mitochondrial membranes and inhibits complex I of the mitochondrial respiratory chain.<sup>45,46</sup> The inhibition is followed by cell death, causing DA depletion in the brain. The damage is initially seen at the nerve terminal, probably because MPP<sup>+</sup> is taken up into nigral neurons via the high affinity DA reuptake system at the terminal level.<sup>47,48</sup> The exact mechanism leading to cell death is not clear but is thought to take place through a complicated pathway.<sup>49</sup> One possibility is the increased free radical production due to complex I inhibition.<sup>50</sup> However, there is not enough evidence to support this mechanism.

The monoamine oxidases are FAD containing enzymes (flavoenzymes) which are tightly bound to the outer membrane of mitochondria.<sup>51</sup> The two forms of MAO, MAO-A and MAO-B, catalyze the oxidative deamination of primary, secondary and, to a lesser

<sup>45</sup> Javitch, J.A., and Snyder, S.H. (1985) Uptake of MPP(+) by dopamine neurons explains selectivity of parkinsonism-inducing neurotoxin, MPTP. *Eur. J. Pharmacol.* **106**, 455-456.

<sup>46</sup> Cleeter, M.W., Cooper, J.M., Schapira, A.H.V. (1992) Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: Evidence for free radical involvement. *J. Neurochem.* **58**, 786-789.

<sup>47</sup> Chiba, K. Trevor, A.J., Castagnoli, N., Jr. (1985) Active uptake of MPP<sup>+</sup>. A metabolite of MPTP, by brain synaptosomes. *Biochem. Biophys. Res. Commun.* **128**, 1228-1232.

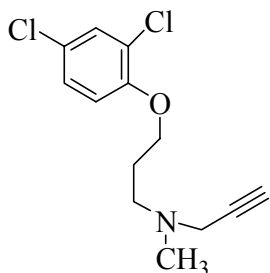
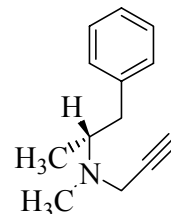
<sup>48</sup> Javitch, J.A., D'amato, R.J., Strittmatter, S.P.M., Snyder, H.S. (1985) Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc. Natl. Acad. Sci. USA* 2173-2177.

<sup>49</sup> Langston, J.W. (1996) The etiology of Parkinson's disease with emphasis on the MPTP story. *Neurology* **47 (Suppl. 3)**, S153-S160.

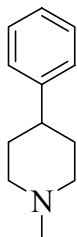
<sup>50</sup> Hasegawa, E., Takeshige, K., Oishi, T., Murai, Y., Minikami, S. (1990) 1-Methyl-4-phenylpyridinium (MPP<sup>+</sup>) induces NADH-dependent superoxide formation and enhances NADH-dependent lipid peroxidation in bovine heart submitochondrial particles. *Biochem. Biophys. Res. Commun.* **170**, 1049-1055.

<sup>51</sup> Weyler, W., Hsu, Y.P.P., Breakefield, X.O. (1990) Biochemistry and genetics of monoamine oxidase. *Pharmac. Ther.* **47**, 391-417.

extent, tertiary amines.<sup>52</sup> MAO-A and MAO-B are distinguished by a variety of criteria and most conveniently by highly selective inhibitors. MAO-A is selectively inhibited by clorgyline (**22**) and MAO-B by (*R*)-deprenyl (**23**).

**22****23**

Many MPTP analogs have been prepared and tested as possible substrates for MAO. The 1,4-disubstituted 1,2,3,6-tetrahydropyridines are cyclic tertiary amines which are known to display good MAO-B substrate properties.<sup>53,54</sup> However, the corresponding piperidinyl analog of MPTP (**24**) is not a substrate<sup>55</sup> suggesting that the allylamine functionality is important for the catalytic process.

**24**

In order to evaluate the potential importance of the allylic moiety in the MAO-B catalyzed oxidation of cyclic tertiary amines, the series of  $\beta,\gamma$ -unsaturated five membered cyclic tertiary amines (pyrrolines and isoindolines) were synthesized by Castagnoli's group. Compound **25** (Scheme 8), which underwent oxidation to the corresponding

<sup>52</sup> Kalgutkar, A., Testa, B., Castagnoli, N., Jr. (1995) Selective inhibitors of monoamine oxidase (MAO-A and MAO-B) as probes of its catalytic site and mechanism. *Med. Res. Rev.* **15**, 325-388.

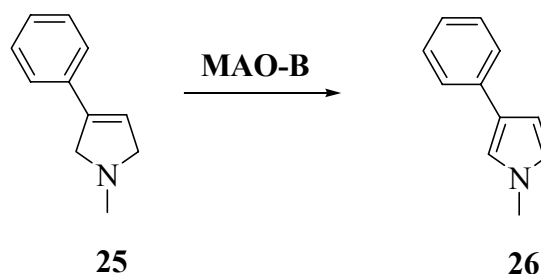
<sup>53</sup> Youngster, S.K., Sonsalla, P.K., Sieber, B.A., Heikkila, R.E. (1989) Structure-activity study of the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. I. Evaluation of the biological activity of MPTP analogs. *J. Pharmacol. Exp. Ther.* **240**, 9820-9828.

<sup>54</sup> Krueger, M.J., Efanage, S.M., Michelson, R.H., Singer, T.P. (1992) Interaction of flexible analogs of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and of N-methyl-4-phenylpyridinium with highly purified monoamine oxidase A and B. *Biochemistry* **31**, 5611-5615.

<sup>55</sup> Heikkila, R.E., Manzino, L., Cabbat, F.S., Duvoisin, R.C. (1985) Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and several of its analogues on the dopaminergic nigrostriatal pathway in mice. *Neurosci. Lett.* **58**, 133.

pyrrole (**26**), was shown to possess better MAO-B substrate properties than MPTP.<sup>56</sup> This finding both demonstrated the importance of the allylic moiety and showed that MPTP and its derivatives were not the only cyclic tertiary amines to have MAO-B substrate properties.

**Scheme 8. MAO-B catalyzed oxidation of 1-methyl-3-phenyl-3-pyrroline (**25**) to 1-methyl-3-phenylpyrrole (**26**).**



Some of the global structural features that are associated with MAO substrates of MPTP analogs include the following: 1. The six-membered heterocyclic system must contain the double bond at the position  $\beta,\gamma$  to the nitrogen atom. 2. Maximal activity requires an N-methyl group. 3. The 1-methyl-1,2,3,6-tetrahydropyridinyl ring must bear a C-4 substituent but no other ring carbon substituents.<sup>57</sup> Some basic structural features of MPTP derivatives are recognized as being essential for neurotoxicity. To be neurotoxic, the compounds are required to be bioactivated to pyridinium metabolites by MAO. Following bioactivation, they must be transported into dopaminergic neurons to be localized in the mitochondrial membrane where they inhibit NADH oxidase.<sup>58</sup>

<sup>56</sup> Wang, Y., Mabic, S., Castagnoli, N., Jr. (1998) 1-Methyl-3-pyrrolines and 2-methylisoindolines: new classes of cyclic tertiary amine monoamine oxidase B substrates. *Bioorg. Med. Chem.* **6**, 143-149.

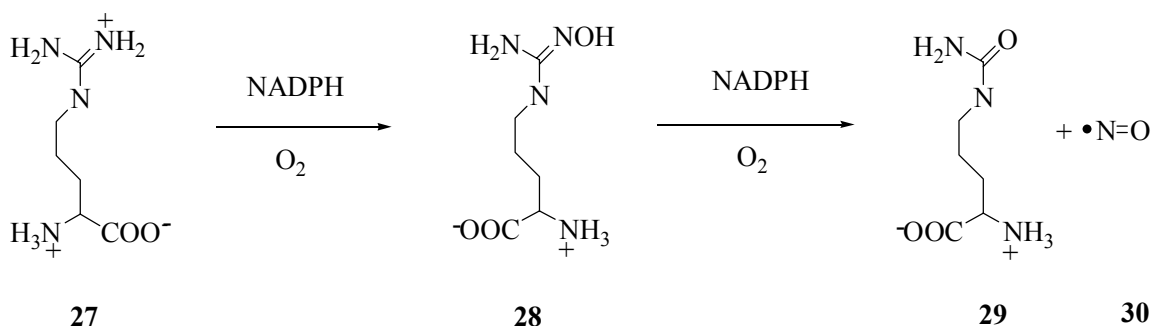
<sup>57</sup> Wang, Y., Castagnoli, N., Jr. (1995) Studies on the monoamine oxidase (MAO)-catalyzed oxidation of phenyl-substituted 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine derivatives: Factors contributing to MAO-A and MAO-B selectivity. *J. Med. Chem.* **38**, 1904-1910.

<sup>58</sup> Bachurin, S.O., Tkachenko, S.E., Lermontova, N.N. (1991) Pyridine derivatives: Structure-activity relationships causing Parkinsonism-like symptoms. *Rev. Environ. Contam. Toxicol.* **122**, 1-36.

### 1.2.4. EFFECT OF NITRIC OXIDE IN OXIDATIVE STRESS

There is evidence that not only reactive oxygen species but also nitric oxide (NO), a free radical, may play a role in oxidative damage in PD.<sup>59</sup> Besides its suspected role in neurodegenerative processes, NO has many diverse functions in the body including blood pressure regulation, acting as a cytotoxic agent in the immune system and being a neurotransmitter. Its involvement in atherosclerosis, sepsis, heart rate and pulmonary hypertension are being investigated. NO is formed via the nitric oxide synthase (NOS) catalyzed conversion of *L*-arginine (**27**) to *L*-citrulline (**29**) (Scheme 9).<sup>60</sup>

**Scheme 9. NOS catalyzed conversion of *L*-arginine (**27**) to *L*-citrulline (**29**) and NO (**30**).**



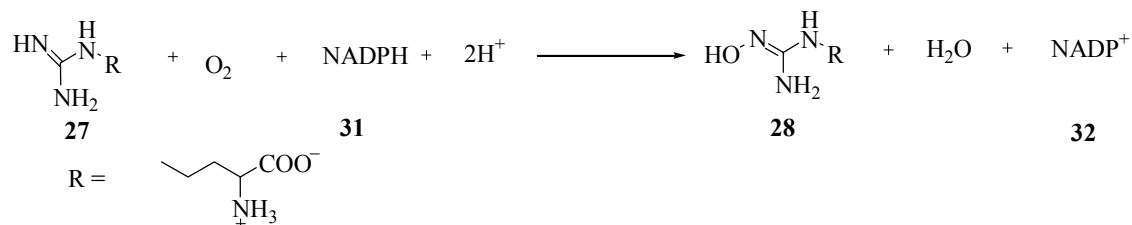
Although the mechanism for the above bioconversion is not well understood, a suggested pathway involves a  $2 e^-$  oxidation of **27** to **28** (Scheme 10) followed by a  $3 e^-$  oxidation of **28** to **29** (Scheme 11).

<sup>59</sup> Gerlach, M., Blum-Degen, D., Lan, J., Riederer, P. (1999) Nitric oxide in the pathogenesis of Parkinson's disease. *Adv. Neurol.* **80**, 239-245.

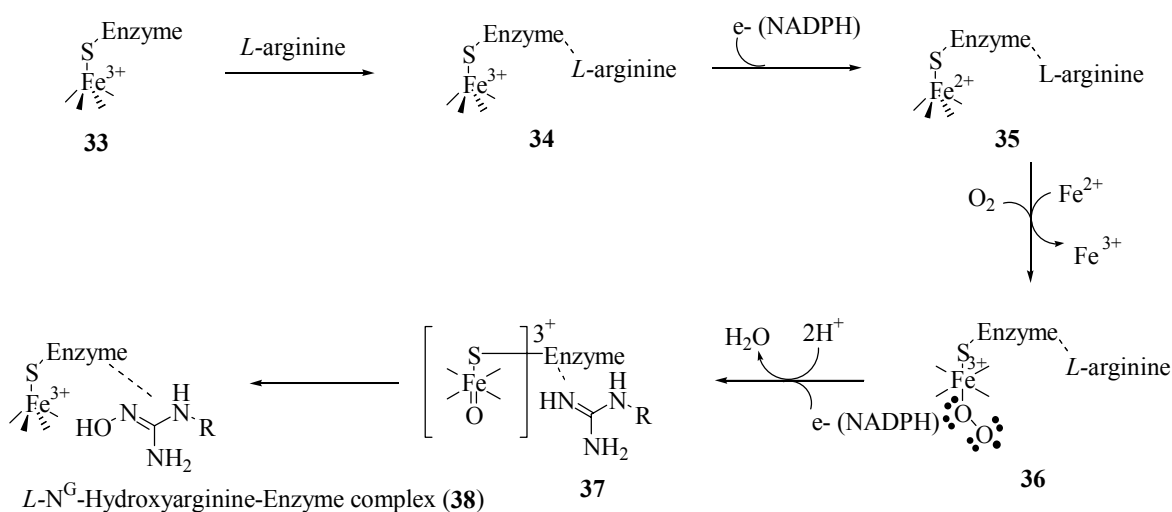
<sup>60</sup> Kerwin, J.F., Lancaster, J.R., Feldman, P.L. (1995) Nitric Oxide: A new paradigm for second messengers. *J. Med. Chem.* **38**, 4342-4362.

**Scheme 10. The proposed pathway for the 2-electron oxidation of *L*-arginine to *L*-N-hydroxyarginine.**

Net conversion of the first step:



Proposed pathway:

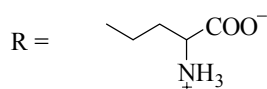
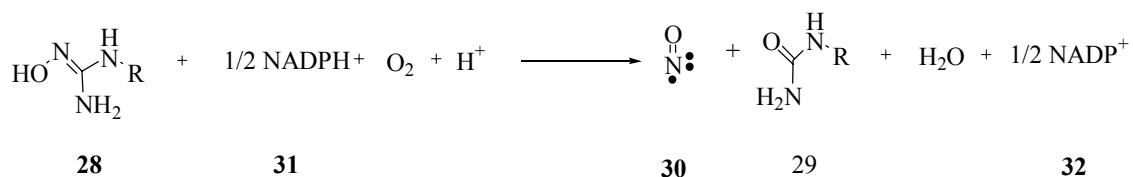


The first step is N-hydroxylation where one equivalent of NADPH (**31**) and one equivalent of O<sub>2</sub> are utilized. Initially, an electron from NADPH is transferred to the heme iron (**35**) that enables oxygen binding. This step is followed by release of oxygen as water forming an oxo-iron oxidant species (**37**). This electron deficient oxygen is attached to the guanidine nitrogen of *L*-arginine forming the *L*-N-hydroxyarginine-enzyme complex (**38**).<sup>61</sup>

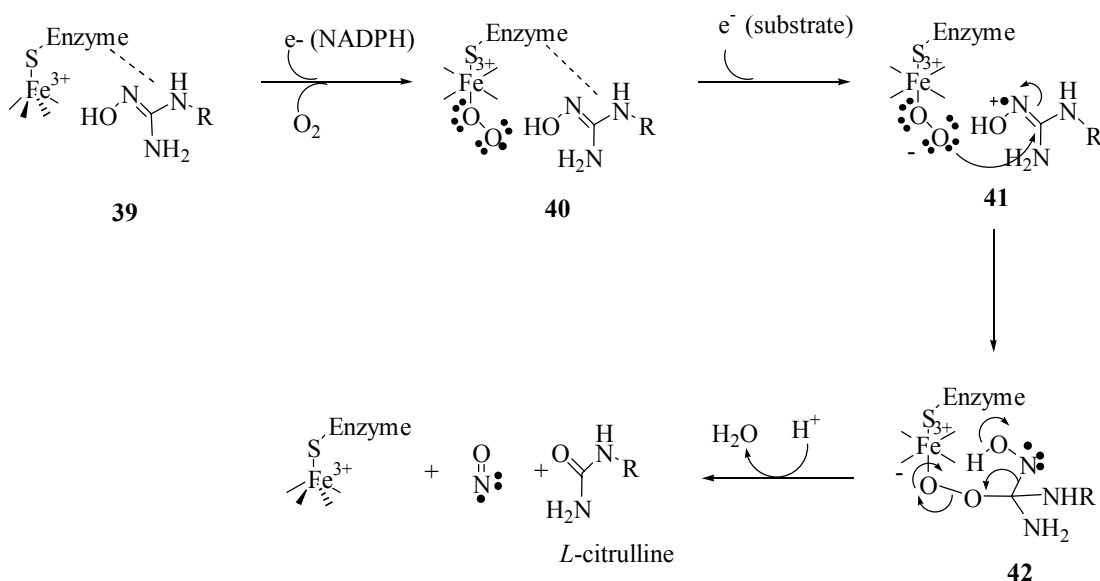
<sup>61</sup> Marletta, M.A. (1993) Nitric oxide synthase structure and mechanism. *J. Biol. Chem.* **268**, 12231-12234.

**Scheme 11. Proposed pathway for the 3-electron oxidation of *L*-N-hydroxyarginine to *L*-citrulline.**

**Net conversion for the second step:**



**Proposed pathway:**



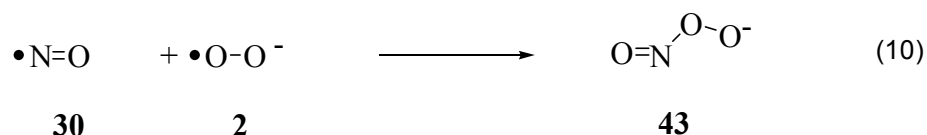
The one electron transfer from NADPH leading to the formation of ferric peroxide (**40**) is identical to the initial one electron step associated with the conversion of *L*-arginine to *L*-N-hydroxyarginine. Another electron is provided by the substrate to form the radical cation **41**. This step is followed by the attack of the ferric peroxide oxygen to form the intermediate **42**. Decomposition of **42** results in the formation of nitric oxide and *L*-citrulline.

NOS has three forms: Inducible (iNOS), endothelial (eNOS) and neuronal (nNOS). The neuronal form of NOS is thought to contribute to neurodegenerative

pathways.<sup>62</sup> However, studies in postmortem parkinsonian brains show that neurons containing NOS are selectively spared<sup>63</sup> which doesn't support this thought. Although the pathway for the putative NOS mediated neurodegeneration is not clear, production of NO is thought to play an important role. As explained before, inhibition of complex I of the mitochondrial respiratory chain may lead to an energy crisis due to the decrease in the production of ATP. This in turn leads to a loss in the transmembrane potential which activates voltage dependent N-methyl-D-aspartate (NMDA) receptors.<sup>64</sup> The entry of calcium into the cell through NMDA channels is stimulated and results in an increased production of oxygen free radicals by the mitochondria and activation of the neuronal form of NOS (nNOS). Since nNOS is regulated by calmodulin, binding of  $\text{Ca}^{++}$  by calmodulin triggers the production of NO.<sup>65</sup> This leads eventually to excitotoxic neuronal cell death in the substantia nigra.

Beckmann first suggested that two radicals, nitric oxide and superoxide radical anion, which are produced together in some cells, could combine to form peroxynitrite (43).<sup>66</sup>

**Scheme 12. Formation of peroxynitrite (43) from nitric oxide and superoxide radical anion.**



<sup>62</sup> Olanow, C.W. (1993) A radical hypothesis for neurodegeneration. *Trends Neurosci.* **16**, 439-444.

<sup>63</sup> Mufson, E.J., Brandabur, M.M. (1994) Sparing of NADPH-diaphorase striatal neurons in Parkinson's and Alzheimer's diseases. *Neuroreport.* **5**, 705-708.

<sup>64</sup> Schulz, J.B., Matthews, R.T., Klockgether, T., Dichgans, J., Beal, M.F. (1997) The role of mitochondrial dysfunction and neuronal nitric oxide in animal models of neurodegenerative diseases. *Mol. Cell. Biochem.* **174**, 193-197.

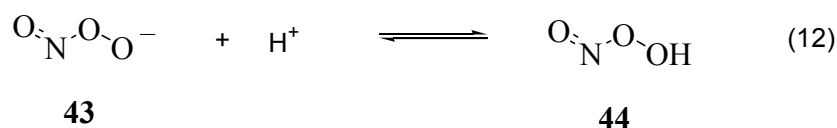
<sup>65</sup> Schulz, J.B., Matthews, R.T., Muqit, M.M.K., Browne, S.E., Beal, M.F. (1995) Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J. Neurochem.* **64**, 936-939.

<sup>66</sup> Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., Freeman, B.A. (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* **87**, 1620-1624.

The reaction rate constant for the reaction of NO with  $O_2^-$  is near the diffusion controlled limit ( $7 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ ) and larger than that for the reaction of SOD with superoxide ( $2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ )<sup>67</sup> which makes it a feasible reaction.<sup>68</sup> The rate of formation of peroxynitrite is first order in the concentration of both nitric oxide and superoxide radical anion. Therefore the formation depends on the activities of both SOD and NOS. Furthermore, superoxide can diminish the effects of nitric oxide by converting it into peroxynitrite<sup>69</sup> as nitric oxide can capture and diminish the effects of superoxide radical anion.<sup>70</sup>

Peroxynitrite can cause lipid peroxidation,<sup>71</sup> DNA alkylation<sup>72</sup> and tyrosine nitration.<sup>73</sup> Although peroxynitrite anion (**43**) is relatively unreactive, it is in equilibrium with peroxynitrous acid (**44**) which is a more reactive species.<sup>74</sup> The  $pK_a$  for peroxynitrous acid was reported to be 6.8.<sup>75</sup>

**Scheme 13. Equilibrium of peroxynitrite anion with its protonated form peroxynitrous acid**



<sup>67</sup> McCord, J.M., Fridovich, I. (1988) Superoxide dismutase: the first twenty years (1968-1988). *Free Radical Biol. Med.* **5**, 363-369.

<sup>68</sup> Huie, R.E., Padmaja, S. (1993) The reaction of NO with superoxide. *Free Radical Res. Commun.* **18**, 195-199.

<sup>69</sup> Beckman, J.S., Crow, J.P. (1993) Pathological implications of nitric oxide, superoxide and peroxynitrite formation. *Biochem. Soc. Trans.* **21**, 330-334.

<sup>70</sup> Rubanyi, G.M., Ho, E.H., Cantor, E.H., Lumma, W.C., Botelho, L.H.P. (1991) Cytoprotective function of nitric oxide: inactivation of superoxide radicals produced by human leukocytes. *Biochem. Biophys. Res. Commun.* **181**, 1392-1397.

<sup>71</sup> Juurlink, B.H.J. (1999) Management of oxidative stress in the CNS: the many roles of glutathione. *Neurotoxicity Research* **1**, 119-140.

<sup>72</sup> Breen, A.P., Murphy, J.A. (1995) Reactions of oxyl radicals with DNA. *Free Radical Biol. Med.* **18**, 1033-1077.

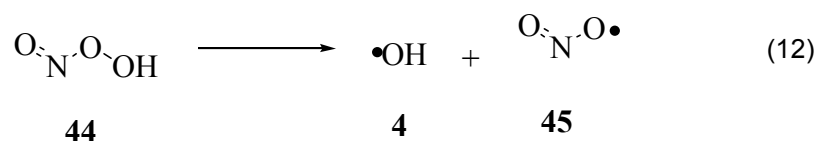
<sup>73</sup> Beckman, J.S. (1996) Oxidative damage and tyrosine nitration from peroxynitrite. *Chem. Res. Toxicol.* **9**, 836-844.

<sup>74</sup> Goldstein, S., Squadrito, G.L., Pryor, W.A., Czapksi, G. (1996) Direct and indirect oxidations by peroxynitrite, neither involving the hydroxyl radical. *Free Radical Biol. Med.* **21**, 965-974.

<sup>75</sup> Radi, R., Beckman, J.S., Bush, M., Freeman, B.A. (1991) Peroxynitrite oxidation of sulfhydryls: the cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.* **266**, 4244-4250.

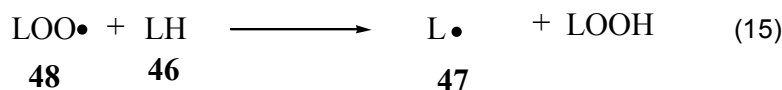
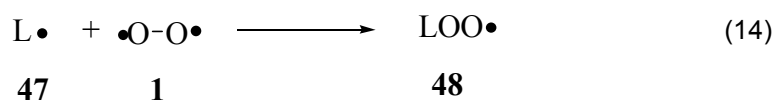
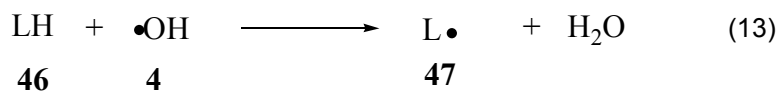
It is known that peroxyxynitrous acid is unstable and decomposes into a reactive intermediate which is involved in the reactions listed above. However, the structure of this intermediate is debated. The first proposed pathway is the decomposition of HOONO to give hydroxyl radical ( $\bullet\text{OH}$ ) and nitrogen dioxide radical  $\bullet\text{NO}_2$  (Scheme 14).<sup>77</sup>

**Scheme 14. Decomposition of peroxyxynitrous acid (44) to give hydroxyl radical (4) and nitrogen dioxide radical (45).**



Formation of the hydroxyl radical can initiate lipid peroxidation by abstracting a hydrogen atom from a methylene carbon in a polyunsaturated lipid [LH (**46**)] forming a carbon-centered radical (**47**).<sup>76</sup> This radical can interact with dioxygen to form a lipid peroxy radical (**48**), which can react further with another lipid molecule in the propagation step (**Reaction 15**, Scheme 15).

**Scheme 15. Hydroxyl radical initiated lipid peroxidation.**

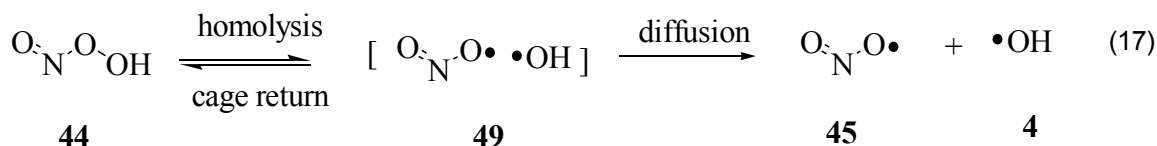


However, there are several experimental results that argue against this pathway. First of all, peroxyacids do not usually dissociate to form radicals since the strength of O-O bond in these compounds is relatively high for peroxides. Instead, due to their polarity,

they have a tendency to undergo even-electron nonradical reactions.<sup>77</sup> Also it was shown experimentally that hydroxyl radical scavengers fail to prevent hydroxyl radical-type damage of biotargets even at scavenger concentrations that are high enough to trap all hydroxyl radicals present.<sup>78,79</sup>

A proposed mechanism for the homolysis reaction (Scheme 16) involves a pair of radicals formed in a cage (49)<sup>80</sup> surrounded by solvent molecules. These “caged” radicals can either recombine (a very rapid reaction with a rate constant of approximately  $10^{10} \text{ s}^{-1}$ ) to re-reform peroxyntrous acid (44) (cage return) or diffuse apart to give free radicals (45 and 4).<sup>81</sup>

**Scheme 16. After homolysis, “caged” pair either can diffuse apart to form free radicals (45 and 4) or go back to peroxyntrous acid.**



The “caged” radical pair is more tightly held by solvent molecules in solvents of higher viscosity which will favor the cage return. Therefore, the disappearance rate of peroxyntrite is expected to be slower in solvents of higher viscosity. However, only extremely small increases in the observed rate constant for the disappearance of peroxyntrite were found in buffers containing polyethylene glycol that increases the viscosity of the solutions.<sup>82</sup> This result was also supported by the finding that the lifetime

<sup>76</sup> Radi, R., Beckman, J.S., Bush, K.M., Freeman, B.A. (1991) Peroxyntrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.* **288**, 481-487.

<sup>77</sup> Pryor, W.A., Squadrito, G.L. (1995) The chemistry of peroxyntrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol. (Lung Cell. Mol. Physiol. 12)* **268**, L699-L722.

<sup>78</sup> Pryor, W.A., Jin, X., Squadrito, G.L. (1994) One- and two-electron oxidations of methionine by peroxyntrite. *Proc. Natl. Acad. Sci. USA* **91**, 11173-11177.

<sup>79</sup> Moreno, J.J., Pryor, W.A. (1992) Inactivation of  $\alpha$ -1-proteinase inhibitor by peroxyntrite. *Chem. Res. Toxicol.* **5**, 425-431.

<sup>80</sup> Pryor, W.A. (1966) *Free radicals*, McGraw-Hill, New York.

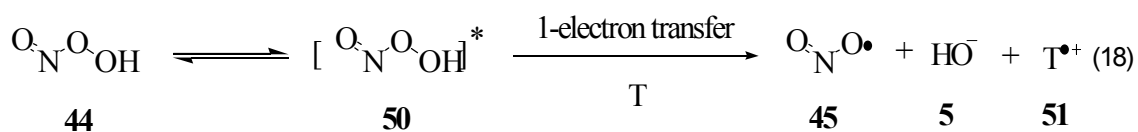
<sup>81</sup> Pryor, W.A., Smith, K. (1970) Reactions of radicals. Part 25. The viscosity dependence of bond homolysis: a qualitative and semiquantitative test for cage return. *J. Am. Chem. Soc.* **92**, 5403-5412.

<sup>82</sup> Pryor, W.A., Jin, X., Squadrito, G.L. (1996) Insensitivity of the rate of decomposition of peroxyntrite to changes in viscosity: evidence against free radical formation. *J. Am. Chem. Soc.* **118**, 3125-3128.

of the geminal pair formed during the decomposition of HO-ONO is longer than that of classical caged radicals.<sup>83</sup> According to this argument, free radicals must be formed in small amounts or not formed at all.

In opposition to hydroxyl radical formation, Koppenol and co-workers proposed an alternative pathway involving a yet undefined energetic form of peroxyxynitrous acid represented as HOONO\* (**50**).<sup>84</sup> A mechanism involving HOONO\* as an intermediate which is more selective and less reactive than hydroxyl radical can explain the inability of hydroxyl radical scavengers to completely block peroxyxynitrite reactions. Reactions of HOONO\* with the biological targets result in one electron oxidation products similar to the reactions of hydroxyl radicals.<sup>85</sup>

**Scheme 17. One electron oxidation of biological target molecule (T) mediated by an energetic form of peroxyxynitrous acid**



However, the reactions proposed to be mediated by HOONO\* proceed relatively slowly suggesting that these reactions are of little or no importance in biological systems.<sup>86</sup>

Peroxyxynitrite is known to be unstable in carbonate buffers.<sup>86</sup> Radi *et al.* proposed that this instability was due to the formation of a reactive adduct, the nitrosoperoxyxynitrite anion (**53**).<sup>87</sup> It was later suggested that the reaction of

<sup>83</sup> Goldstein, S., Squadrito, G.L., Pryor, W.A., Czapski, G. (1996) Direct and indirect oxidations by peroxyxynitrite, neither involving the hydroxyl radical. *Free. Radical Biol. Med.* **21**, 965-974.

<sup>84</sup> Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiroopoulos, H., Beckman J.S. (1992) Peroxyxynitrite a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res. Toxicol.* **5**, 834-842.

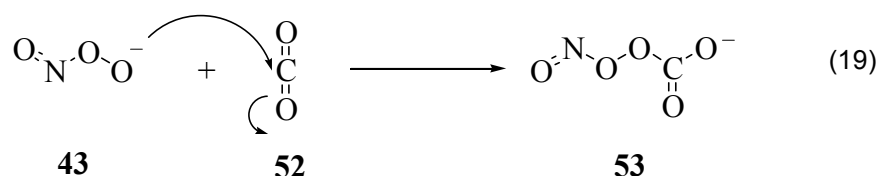
<sup>85</sup> Jensen, J.L., Miller, B.L., Zhang, X., Hug, G.L., Schöneich, C. (1997) Oxidation of threonylmethionine by peroxyxynitrite. Quantification of the one electron transfer pathway by comparison to one electronphotooxidation. *J. Am. Chem. Soc.* **199**, 4749-4757.

<sup>86</sup> Keith, W.G., Powell, R.E. (1969) Kinetics of decomposition of peroxyxynitrous acid. *J. Chem. Soc. A*, 90

<sup>87</sup> Radi, R., Cosgrove, T.P., Beckman, J.S., Freeman, B.A. (1993) Peroxyxynitrite-induced luminol chemiluminescence. *Biochem. J.* **290**, 51-57.

peroxynitrite anion with carbon dioxide was responsible for this reaction rather than bicarbonate anion as shown in Scheme 18.<sup>88</sup>

**Scheme 18. Reaction of peroxynitrite anion with CO<sub>2</sub> to form nitrosoperoxy carbonate (53).**



The reaction of peroxynitrite anion with CO<sub>2</sub> is one of the fastest reactions of peroxynitrite with a second-order rate constant of  $5.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ <sup>89</sup> suggesting that this reaction accounts for a large fraction of peroxynitrite formed *in vivo*. Formation of peroxynitrite may lead to secondary reactive intermediates that can affect biotarget molecules. Tyrosine residue nitration is an important reaction of this type.<sup>90,91</sup>

After the formation of the initial adduct **53**, three possible pathways can yield 3 different sets of products (Scheme 19).<sup>92</sup>

<sup>88</sup> Lyman, S.V., Hurst, J.K. (1995) Rapid reaction between peroxynitrite and ion and carbon dioxide: implications for biological activity. *J. Am. Chem. Soc.* **117**, 8867-8868.

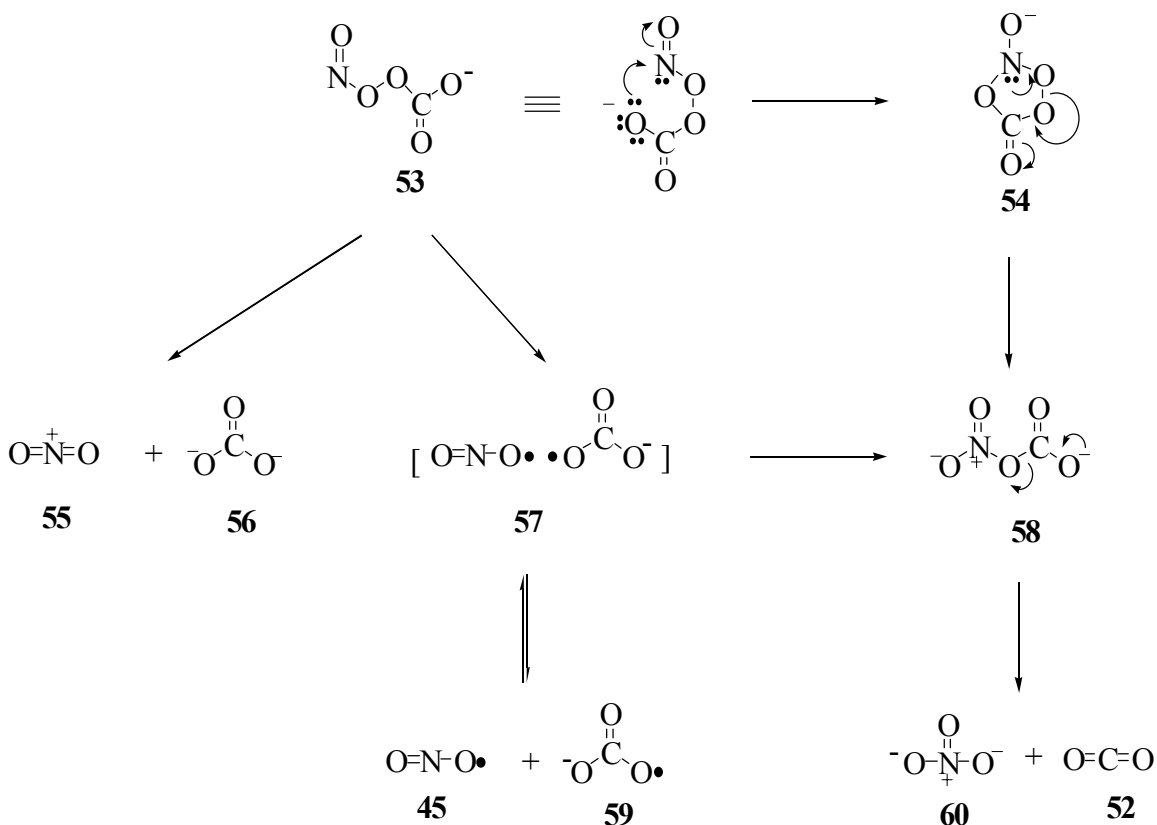
<sup>89</sup> Denicola, A., Freeman, B.A., Trujillo, M, Radi, R. (1996) Peroxynitrite reaction with carbondioxide/bicarbonate : Kinetics and influence on peroxynitrite mediated oxidations. *Arch. Biochem. Biophys.* **333**, 49-58.

<sup>90</sup> Squadrito, G.L., Pryor, W.A. Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite and carbon dioxide. *Free Radical Biol. Med.* **25**, 392-403.

<sup>91</sup> Uppu, R.M., Squadrito, G.L., Pryor, W.A. (1996) Acceleration of peroxynitrite oxidations by carbondioxide. *Arch. Biochem. Biophys.* **327**, 335-343.

<sup>92</sup> Koppenol, W.H. (1998) Peroxynitrite Uncloaked? *Chem. Res. Toxicol.* **11**, 716-717.

**Scheme 19. Decomposition of initial adduct **53** can (1) yield carbonate (**56**) and nitronium ion (**55**) via heterolytic cleavage, (2) give free radicals **45** and **59** or (3) rearrange into **58** and CO<sub>2</sub> through a 5 membered intermediate (**54**) leading to the formation of nitrate (**60**) and carbon dioxide (**52**).**

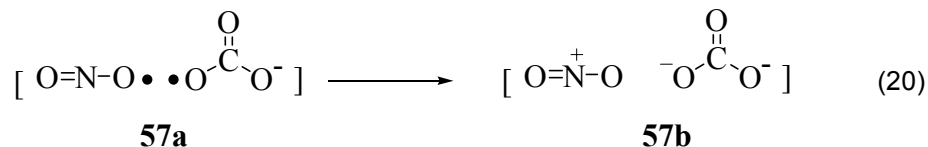


Heterolytic cleavage of the C-O bond in **53** will form carbonate (**56**) and nitronium ion (**55**) which may be responsible for reported nitration reactions. However, since NO<sub>2</sub><sup>+</sup> readily reacts with water to form nitric acid, the lifetime of NO<sub>2</sub><sup>+</sup> at physiological pH is likely to be too short to yield nitration reactions.<sup>93</sup>

The question of whether ONO-OCO<sub>2</sub><sup>-</sup> undergoes homolysis to form **45** and **59** is similar to the problem encountered with HO-ONO. A long-lived cage of radicals (**57**) also may exist in this case.<sup>82</sup> Surrounding water molecules can stabilize the caged pair (Scheme 20).

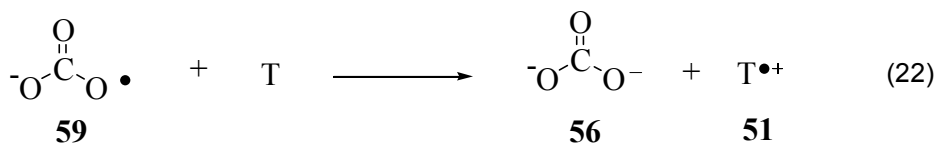
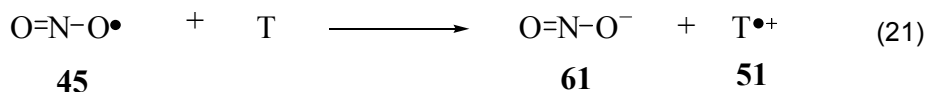
<sup>93</sup> Moodie, R.B., Schofield, K., Taylor, P.G. (1979) Electrophilic aromatic substitution. Part 21. Rate constants for formation of nitronium ion in aqueous sulphuric, perchloric, and methane-sulfonic acids. *J. Chem. Soc. Perkin Trans. II* 133-136.

**Scheme 20. Stabilization of 57b by the surrounding water molecules due to its polarity.**



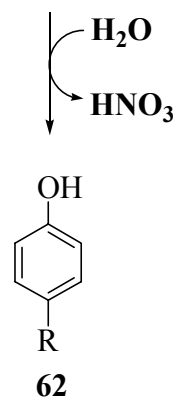
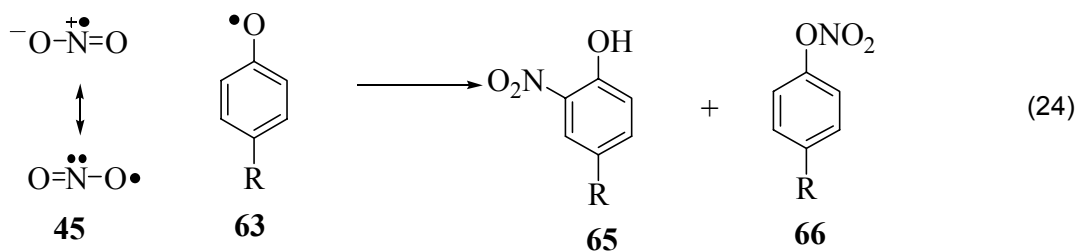
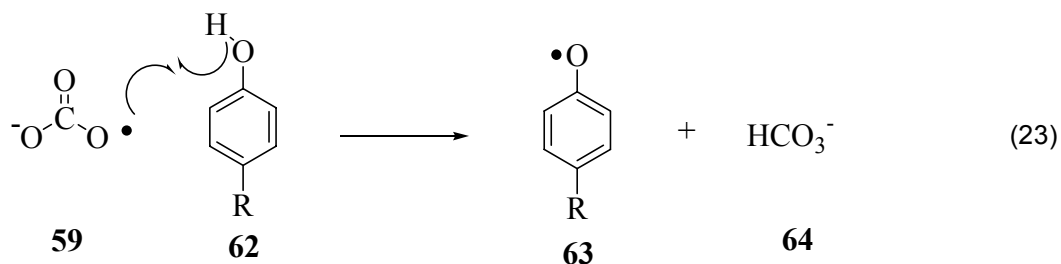
After homolysis, caged radicals may either lead to stable cage products **60** and **52** through the intermediate **58** (Scheme 21) or diffuse apart to form free radicals  $\cdot\text{NO}_2$  (**45**) and  $\cdot\text{CO}_3^-$  (**59**). However, in this case, if homolysis of **53** occurs, the hydroxyl radical is replaced by the carbonate radical (**59**), a less reactive, more diffusible, and potentially more selective species. These properties of the carbonate radical can be the thermodynamic driving force for the homolysis of **53**. After homolysis, free radicals **45** and **59** may participate in oxidation and nitration reactions.<sup>92</sup>

**Scheme 21. Both nitrogen dioxide and carbonate (more reactive than nitrogen dioxide) radicals can cause 1-electron oxidation of biological target molecules (T).**



In the nitration reaction carbonate radical (**59**) causes the one electron oxidation of the phenol ring of the tyrosine residue (**62**) to give the corresponding phenolic radical (**63**) (Scheme 22). The resulting radical (**63**) can react with  $\cdot\text{NO}_2$  to form nitrated products **65** or **66**. The O-nitrophenol (**66**) being hydrolytically unstable will give back the phenol.

Scheme 22. Free radical nitration of phenol moiety of tyrosine residue.



Finally, carbon dioxide is regenerated via intermediate **58** (Scheme 19) which is consistent with the experimental results suggesting a catalytic role of carbon dioxide in the formation of nitrosoperoxycarbonate (**53**).<sup>94</sup>

<sup>94</sup> Pryor, W.A., Lemerrier, J.-N., Zhang, H., Uppu, R.M., Squadrito, G.L. (1997) The catalytic role of carbon dioxide in the decomposition of peroxyxynitrite. *Free Radical Biol. Med.* **23**, 331-338.

### 1.3. THE ROLE OF CYTOCHROME P450 IN PD

Hepatic cytochrome P450 also has been suggested to play a role in the development of PD.<sup>95</sup> Hepatic cytochrome P450 monoxygenases, which catalyze the biotransformations of lipid-soluble substrates, are important in regulating the intensity and duration of drug action. It was suggested that the slow rate of metabolism of environmental neurotoxic xenobiotics, due to malfunction of hepatic cytochrome P450, could increase the risk of developing PD.<sup>96</sup> For example, people who have a mutant allele at the P450 2D6B gene have about a two-fold increase in the risk of developing Parkinson's disease.<sup>97</sup>

### 1.4. TREATMENT STRATEGIES FOR PD

The strategy for treating PD has been to restore the DA levels in the brain by pharmacological means<sup>98</sup> or, more recently, to restore the function of the damaged brain by neural grafting of DA-containing cells.<sup>99,100</sup> There is a number of theoretical strategies for drug therapy in PD, including restoration of striatal DA levels by direct and indirect DA agonists, metabolic inhibitors and uptake inhibitors. The most successful treatment has been the use of 3,4-dihydroxyphenylalanine (**17**) known as levodopa (L-DOPA).<sup>101,102</sup> DA (**10**) does not readily cross the blood brain barrier. Therefore, the DA precursor L-DOPA is administered and this amino acid is converted to DA by the enzyme DOPA decarboxylase (Scheme 23).

<sup>95</sup> Smith, C.A.D., Gough, A.C., Leigh, P.N., Summers, B.A., Harding, A.E., Maraganore, D.M., Sturman, S.G., Schapiro, A.H., Williams, A.C. (1992) Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. *Lancet* **339**, 1375-77.

<sup>96</sup> R.T., Coutts, L.J., Urichuk (1999) Polymorphic cytochromes P450 and drugs used in psychiatry. *Cell. Mol. Neurobiol.* **19**, 325-354.

<sup>97</sup> Rempfer, R., Crook, R., Houlden, H., Duff, K., Hutton, M., Roberts, G.W., Raghavan, R., Perry, R., Hardy, J. (1994) Parkinson's disease, but not Alzheimer's disease. Lewy body variant associated with mutant alleles at cytochrome P450 gene. *Lancet* **344**, 815.

<sup>98</sup> Calne, D.B. (1993) Treatment of Parkinson's disease. *N. Engl. J. Med.* **329**, 1021-1027.

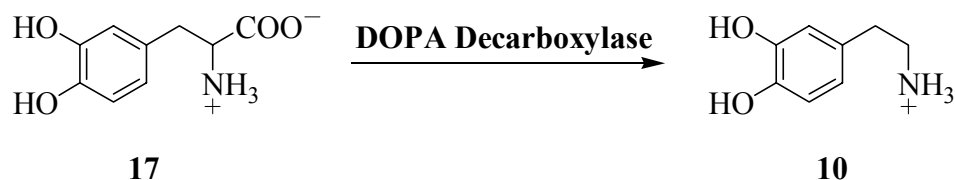
<sup>99</sup> Lindvall, O. (1989) Transplantation into the human brain: present status and future possibilities. *J. Neurol. Neurosurg. Psychiatry* **52 (special suppl.)**, 39-54.

<sup>100</sup> Lindvall, O., Brundlin, P., Widner, H., Rehnberg, S., Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD (1990) Grafts of foetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* **247**, 574-577.

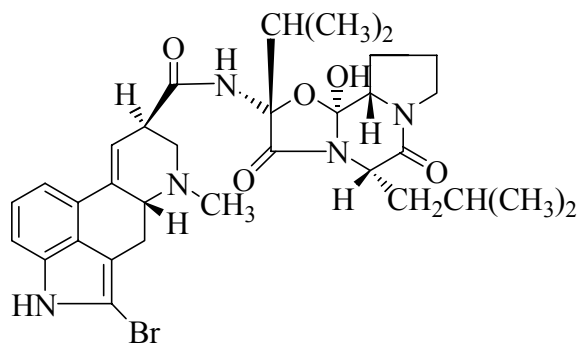
<sup>101</sup> Cotzias, G.C., Van Woert, M.H., Schiffer, L.M. (1967) Aromatic amino acids and modification of parkinsonism. *N.Engl. J. Med.* **276**, 374-378.

<sup>102</sup> Hoehn, M.M. (1983) Parkinsonism treated with levodopa: progression and mortality. *J. Neurol. Transmiss. Suppl.* **19**, 253-264.

**Scheme 23. DOPA decarboxylase catalyzed conversion of L-DOPA to DA.**



However, the above conversion increases the DA levels not only in the striatum but also outside the blood-brain barrier causing nausea and vomiting in many patients due to dopaminergic stimulation of the vomiting centers.<sup>103</sup> Selective inhibitors of DOPA decarboxylase which do not penetrate the brain, like carbidopa [(*R*)- $\alpha$ -methylhydrazinedopa], present in the drug Sinemet, and benserazide, in Madopar, were introduced successfully to overcome these side effects.<sup>104</sup> Direct dopamine agonists also have some benefit in patients whose responsiveness to L-DOPA is greatly reduced or erratic.<sup>105,106</sup> So far, the only direct-acting dopamine agonist that has found extensive use in PD is the D<sub>2</sub> agonist bromocriptine (**67**).<sup>107</sup>



**67**

<sup>103</sup> Marsden, C.D. (1990) Parkinson's disease. *Lancet* **335**, 948-952.

<sup>104</sup> Korten, J.J., Keyser, A., Joosten, E.M., Gabreels, F.J. (1975) Modapar versus sinemet. A clinical study on their effectiveness. *Eur. Neurol.* **13**, 65-71.

<sup>105</sup> Marsden, C.D., Parkse, C.D. (1977) Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet* **i:345-349**.

<sup>106</sup> Poewe, W. (1998) Should treatment of Parkinson's disease be started with a dopamine agonist? *Neurology* **51** (Suppl.2) S22-S24.

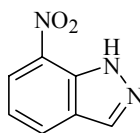
<sup>107</sup> Calne, D.B., Teychenne, P.F., Claveria, L.E., Eastman, R., Greenacre, J.K. (1974) Bromocriptine in Parkinsonism. *Br. Med. J.* **4**, 442-444.

The D<sub>2</sub> DA receptor is one of the two subtypes of DA receptors initially identified on the basis of pharmacological and biochemical criteria.<sup>108</sup> D<sub>1</sub> receptors mediate the DA stimulated increase in adenylate cyclase activity while D<sub>2</sub> receptors inhibit adenylate cyclase activity.<sup>109</sup> The D<sub>2</sub> receptor has a picomolar affinity for DA. The corresponding affinity for the D<sub>1</sub> receptor is nanomolar.<sup>110</sup> The high level of expression of D<sub>2</sub> receptors in the substantia nigra suggests that it plays a greater role in PD than D<sub>1</sub> receptors which are not observed in the substantia nigra.<sup>111</sup> However, in the view of the results of studies suggesting the requirement of D<sub>1</sub> receptor activation for the maximum expression of D<sub>2</sub> receptor mediated effects, functional interactions between D<sub>1</sub> and D<sub>2</sub> receptors could have important implications in PD where stimulation of these receptors show symptomatic benefit.

### 1.5. THE ROLE OF MAO-B AND NOS INHIBITION IN PD

The selective inhibition of MAO-B has been shown to have neuroprotective effects in MPTP animal models.<sup>112</sup> MAO-B inhibition prevents the formation of the toxic MPP<sup>+</sup> species by inhibiting the bioactivation of MPTP. There is also evidence that the inhibition of nNOS protects against MPTP mediated neurotoxicity in animals.<sup>113</sup>

The potential roles of MAO-B and NOS in neurodegenerative processes and their selective inhibition are areas of intense investigation. However, only a few studies consider these two enzymes together. Also there are only a few compounds which have been tested both for their MAO-B and nNOS inhibiting properties as well as neuroprotective activity. One of these compounds is 7-nitroindazole [7-NI (**68**)]



**68**

<sup>108</sup> Keabian, J.W., Calne, D.B. (1979) Multiple receptors for dopamine. *Nature* **277**, 93-96.

<sup>109</sup> Sibley, D.R., Monsma, F.J. Jr. (1992) Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.* **13**, 61-69.

<sup>110</sup> Sunahara, R.K., Guan, H.C., O'Dowd, B.F., Seeman, P., Laurier, L.G., Ng, G., George, S.R., Torchia, J., Van Tol, H.H., Niznik, H.B. (1991) Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* **350**, 614-619.

<sup>111</sup> Guttman, M. (1992) Dopamine receptors in Parkinson's disease. *Neurol. Clin.* **10**, 377-386.

<sup>112</sup> Langston, J.W., Irwin, I., Langston, E.B., Forno, L.S. (1984) Pargyline prevents MPTP-induced parkinsonism in primates. *Science* **225**, 1480-1482.

7-NI is a selective inhibitor of nNOS.<sup>114</sup> Treatment of animals with 7-NI protects against MPTP neurotoxicity.<sup>115,116</sup> These neuroprotective effects were thought not to be associated with decreased MPP<sup>+</sup> production since 7-NI did not inhibit the MAO-B-catalyzed oxidation of benzylamine by mouse brain mitochondrial preparations. In another study, striatal levels of MPP<sup>+</sup> in MPTP-treated mice were compared between 7-NI injected and control mice.<sup>117</sup> The authors reported that the striatal MPP<sup>+</sup> levels were unaffected by neuroprotective doses of 7-NI leading to the conclusion that the neuroprotective effect of 7-NI was mainly due to nNOS inhibition. However, several studies show that planar,<sup>118</sup> heterocyclic compounds are inhibitors of MAO-B<sup>119,120</sup> suggesting that 7-NI, also a planar, heterocyclic compound, could inhibit MAO-B. This led Castagnoli's group to investigate the MAO-B inhibiting properties of 7-NI.<sup>121</sup> The effect of different 7-NI concentrations on the MAO-B catalyzed oxidation of MPTP to its metabolite MPDP<sup>+</sup> was studied *in vitro*. The results showed that 7-NI is a competitive inhibitor of MAO-B.

---

<sup>113</sup> Matthews, R.T., Yang, L., Beal, F.M. (1997) *S*-methylthiocitrulline, a neuronal nitric oxide synthase inhibitor protects against malonate and MPTP neurotoxicity. *Exp. Neurol.* **143**, 282-286.

<sup>114</sup> Babbedge, R.C., Bland-Ward, P.A., Hart, S.L., Moore, P.K. (1993) Inhibition of rat cerebellar nitric oxide synthase by 7-nitroindazole and related substituted indazoles. *Br. J. Pharmacol.* **110**, 225-228.

<sup>115</sup> Hantraye, P., Brouillet, E., Ferrante, R., Palfi, S., Dolan, R., Matthews, R.T., Beal, M.F. (1996) Inhibition of neuronal nitric oxide synthase prevents MPTP-induced parkinsonism in baboons. *Nature Med.* **64**, 936-939.

<sup>116</sup> Schulz, J.B., Matthew, R.T., Muqit, M.M., Browne, S.E., Beal, M.F. (1995) Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J. Neurochem.* **64**, 936-939.

<sup>117</sup> Przedborski, S., Jackson-Lewis, V., Yokoyama, R., Shibata, T., Dawson, V.L., Dawson, T.M. (1996) Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. *Proc. Natl. Acad. Sci. USA* **93**, 4565-4571.

<sup>118</sup> Ooms, F., Norberg, B., Isin, E.M., Castagnoli, N., Van der Schyf, C.J., Wouters, J. (2000) 7-nitroindazole. *Acta Cryst. C* **56**, e474-475.

<sup>119</sup> Kneubuehler, S., Thull, U., Altomare, C., Carta, V., Gaillard, P., Carrupt, P.A., Carotti, A., Testa, B. (1995) Inhibition of monoamine oxidase-B by 5H-Indeno[1,2-c]pyridazines. Biological activities, quantitative structure-activity relationships (QSARs) and 3D-QSARs. *J. Med. Chem.* **38**, 3974-3883.

<sup>120</sup> Lebreton, L., Curet, O., Gueddari, S., Mazouz, F., Bernard, S., Burstein, C., Milcent, R. (1995) Selective and potent monoamine oxidase type B inhibitors: 2-substituted 5-aryltetrazole derivatives. *J. Med. Chem.* **38**, 4786-4792.

<sup>121</sup> Castagnoli, K., Palmer, S., Anderson, A., Bueters, T., Castagnoli, N., Jr. (1998) The neuronal nitric oxide synthase inhibitor 7-nitroindazole also inhibits the monoamine oxidase-B-catalyzed oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Chem. Res. Toxicol.* **11**, 716-717.

7-NI also was found to protect against the MPTP induced depletion of neostriatal DA in mice.<sup>122</sup> This effect was accompanied by a significant decrease in the striatal levels of MPP<sup>+</sup> showing that the neuroprotective effect of 7-NI is at least partly mediated through the inhibition of MAO-B. Similar striatal MPP<sup>+</sup> levels were produced for both 7-NI together with MPTP and MPTP only treated mice by injecting a higher dose of MPTP in the 7-NI treated mice. In this case a modest (20 %) protection of DA depletion was observed suggesting that the inhibition of MAO-B may not be the only mechanism mediating the protection against MPTP induced neurotoxicity. According to these results, the neuroprotective effects of 7-NI may be due to MAO-B inhibition, nNOS inhibition or inhibition of both enzymes. Recently, Royland *et al.* studied the effect of 7-NI and N<sup>γ</sup>-nitro-*L*-arginine, another NOS inhibitor, on MPTP-induced striatal ATP depletion.<sup>123</sup> The results showed that 7-NI prevented the striatal ATP loss in mice after MPTP administration. However, N<sup>γ</sup>-nitro-*L*-arginine didn't have any effect on MPTP induced ATP loss suggesting the importance of MAO-B inhibition rather than NOS inhibition in 7-NI mediated neuroprotection. Another group investigated the effect of 7-NI on 3-nitrotyrosine immunoreactivity in the substantia nigra which is considered as a marker for peroxynitrite mediated neurotoxicity.<sup>124</sup> An increase in 3-nitrotyrosine immunoreactivity was reported in MPTP treated baboons which was blocked by 7-NI providing evidence for the involvement of nNOS inhibition in protection against MPTP induced neurotoxicity.<sup>125</sup>

In order to understand better the role of 7-NI in neuroprotection, additional animal studies will have to be done with this compound. However, the low aqueous solubility of 7-NI at pH 7.4 limits its utility in *in vivo* studies. A prodrug approach has been suggested

---

<sup>122</sup> Di Monte, D.A., Royland, J.E., Anderson, A., Castagnoli, K., Castagnoli, N., Jr., Langston, J.W. (1997) Inhibition of monoamine oxidase contributes to the protective effect of 7-nitroindazole against MPTP neurotoxicity. *J. Neurochem.* **69**, 1771-1773.

<sup>123</sup> Royland, J.E., Delfani, K., Langston, J.W., Janson, A.M., Di Monte, D.A. (1999) 7-nitroindazole prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Atp loss in the mouse striatum. *Brain Res.* **839**, 41-48.

<sup>124</sup> Ischiropoulos, H. ., Zhu, L., Chen, J., Tsai, M., Martin, J.C., Smith, C.D., Beckman, J.S. (1992) Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch. Biochem. Biophys.* **298**, 431-437.

<sup>125</sup> Ferrante, R.J., Hantraye, P., Brouillet, E., Beal, M.F. (1999) Increased nitrotyrosine immunoreactivity in substantia nigra neurons in MPTP treated baboons is blocked by inhibition of neuronal nitric oxide synthase. *Brain Res.* **823**, 177-182.

as an avenue to overcome this problem. Before discussing the particular prodrug of interest to us, the principles of the prodrug approach will be discussed in general terms.

## 1.6. THE PRODRUG APPROACH

### 1.6.1. GENERAL ASPECTS

The term prodrug was first introduced by Albert in 1958 to describe compounds which undergo biotransformations prior to exhibiting their pharmacological effects. Although the term initially included only enzyme mediated transformations,<sup>126</sup> it has been expanded to include chemical transformations as well.<sup>127</sup> The prodrug concept concentrates on changing the physicochemical properties of a drug to overcome the delivery problems. Improving transport of the active drug to a receptor, preventing drug loss before the receptor is reached, maintaining a desired drug plasma concentration in the organism, increasing the shelf life, improving the taste, increasing the solubility, improving the injectability, and making effective tableting possible are the main targets in prodrug development.<sup>128</sup> In many of these cases a specific problem can be traced to a particular functional group in the parent drug and the solution involves covering or masking that functional group. One of the simplest examples is masking a carbinol moiety in the parent drug as an ester which will be readily hydrolyzed *in vivo* to release the parent drug.<sup>129</sup>

The bioactivation of prodrugs to release the parent drug in the body can take place by a variety of reactions. The most common prodrugs are those requiring hydrolytic cleavage.<sup>130</sup> Other examples include the formation of the active drug from its prodrug via

---

<sup>126</sup> Svensson, L.A., Turek, A. (1988) The design and bioactivation of presystemically stable prodrugs. *Drug. Metab. Rev.* **19**, 165-194.

<sup>127</sup> Stella, V.J., Himmelstein, K.J. (1980) Prodrugs and site-specific delivery. *J. Med. Chem.* **23**, 1275-1282.

<sup>128</sup> Stella, V.J., Charman, W.N., Naringrekar, V.H. (1985) Prodrugs. Do they have advantages in clinical practice? *Drugs* **29**, 455-473.

<sup>129</sup> Sloan, K.B., Ed. (1992) *Prodrugs topical and ocular drug delivery*, Marcel Dekker, New York.

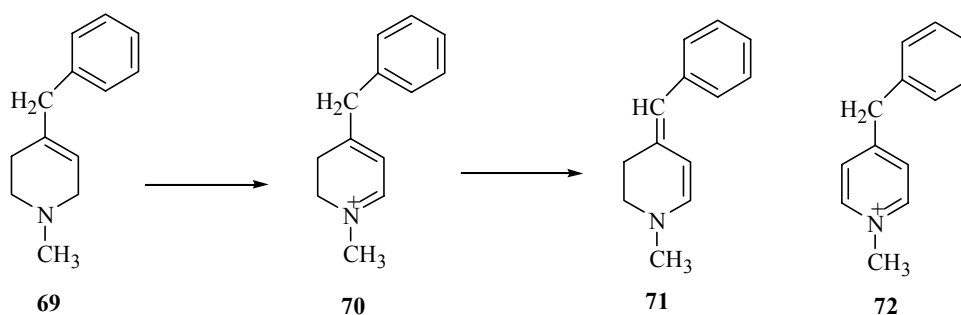
<sup>130</sup> Zhao, Z., Dalvie, D., Naiman, N., Castagnoli, K., Castagnoli, N., Jr. (1992) Design, synthesis, and biological evaluation of novel 4-substituted 1-methyl-1,2,3,6-tetrahydropyridine analogs of MPTP. *J. Med. Chem.* **35**, 4473-4478.

biochemical reductive or oxidative processes.<sup>131</sup> A prodrug also may undergo spontaneous cleavage to release the active drug form.<sup>132</sup>

### 1.6.2. USING THE TETRAHYDOPYRIDINYL GROUP AS A CARRIER

We now address the specifics of prodrug design relative to 7-NI. Although 4-benzyl-1-methyl-1,2,3,6 tetrahydropyridine (**69**) is an excellent MAO-B substrate, it lacks neurotoxic properties. This behavior was explained by the instability of the intermediate dihydropyridinium metabolite **70** which does not undergo extensive conversion to the neurotoxic pyridinium species **72**, possibly because of the ease of deprotonation of the acidic benzylic protons to form the unstable dienamine system **71** (Scheme 24).<sup>133</sup>

**Scheme 24. MAO-B catalyzed oxidation of 4-benzyl-1-methyl-1,2,3,6 tetrahydropyridine analog 69**



In order to evaluate the above proposal, the substrate properties of the corresponding phenoxy compound **73**, which has a similar structure to **69** but lacks the benzylic protons, have been tested. This compound proved to be a good substrate for MAO-B but, like the benzyl analog, was not neurotoxic. This lack of neurotoxicity also

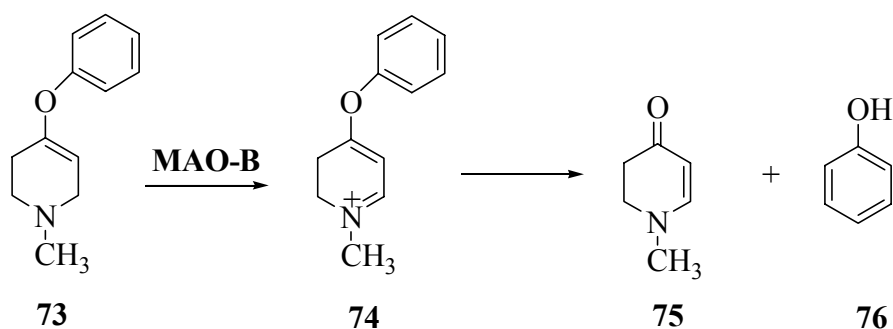
<sup>131</sup> Nicoll-Griffith, D.A., Falgueyret, J.P., Silva, J.M., Morin, P.E., Trimble, L., Chan, C.C., Clas, S., Leger, S., Wang, Z., Yergey, J.A., Riendeau, D. (1999) Oxidative bioactivation of lactol prodrug of a lactone cyclooxygenase-2 inhibitor. *Drug. Metab. Dispos.* **27**, 403-409.

<sup>132</sup> Charton, M. (1985) Prodrug lability prediction through the use of substituent effects. *Methods Enzymol.* **112**, 323-340.

<sup>133</sup> Wang, Y., Castagnoli, N., Jr. (1995) Studies on the monamine oxidase (MAO)-catalyzed oxidation of phenyl-substituted 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine derivatives: Factors contributing to MAO-A and MAO-B selectivity. *J. Med. Chem.* **38**, 1904-1910.

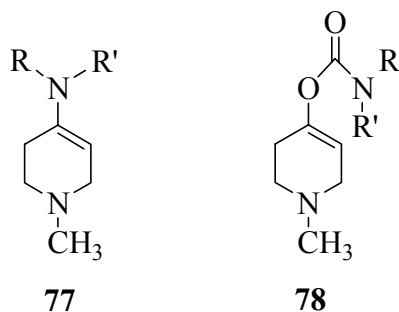
could be explained in terms of the instability of the dihydropyridinium metabolite **74** which was shown to undergo spontaneous hydrolysis to yield phenol and the aminoenone **75** (Scheme 25).

**Scheme 25. MAO-B catalyzed oxidation of the phenoxy compound **73** followed by spontaneous hydrolysis.**



This approach offered the possibility of designing tetrahydropyridinyl prodrugs which, via hydrolysis of the corresponding MAO generated dihydropyridinium species, would release the active drug. In other words, the tetrahydropyridinyl moiety may serve as a “carrier” for prodrugs.<sup>134</sup>

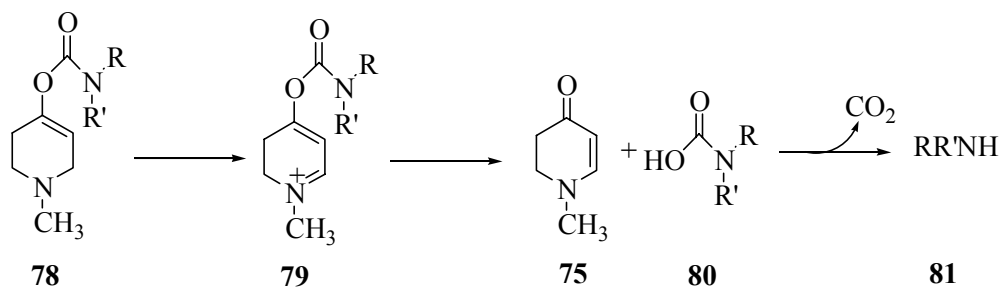
In order to be able to adapt the tetrahydropyridinyl carrier to construct potential amine-containing prodrugs, a carbamate linkage (**78**) was used to overcome the hydrolytic instability of the enamine functionality that results from direct attachment of the amino group and the tetrahydropyridinyl carrier (**77**).



<sup>134</sup> Simpkins, J.W., Bodor, N. (1994) The brain-targeted delivery of dopamine using a redox-based chemical delivery system. *Adv. Drug. Delivery. Rev.* **14**, 243-249.

The dihydropyridinium intermediate **79** was generated by the MAO-catalyzed oxidation of **78**. Subsequent spontaneous hydrolysis and decarboxylation of the resulting carbamic acid **80** releases the amine drug **81** (Scheme 26).

**Scheme 26. Utilization of the tetrahydropyridinyl group as carrier for amine prodrug construction.**



Preliminary studies were carried out with model carbamates (**78**: R, R' = alkyl and aryl groups) which were shown to be moderate MAO-B substrates but only with small groups attached to the carbamoyl nitrogen atom.<sup>135</sup> Subsequent studies, however, showed that larger groups could be accommodated by the A form of the enzyme.<sup>136</sup>

### 1.6.3. A PRODRUG OF (*R*)-NORDEPRENYL UTILIZING TETRAHYDROPYRIDINYL AS A CARRIER

Although drugs are available for the symptomatic treatment of PD, none of them slow the progress of the disease. L-DOPA remains the main therapeutic agent but complications, due to toxicity and decreased effectiveness, appear with long-term use of antiparkinsonian drugs or with progression of the disease.<sup>137</sup> In search of a new treatment aimed at retarding the disease, Birkmayer and colleagues reported that in parkinsonian patients who received the selective MAO-B inhibitor (*R*)-deprenyl, the time from

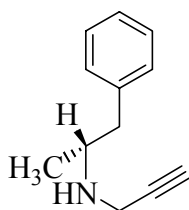
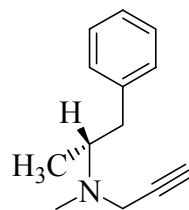
<sup>135</sup> Zhao, Z., Dalvie, D., Naiman, N., Castagnoli, K., Castagnoli, N., Jr. (1992) Design, synthesis, and biological evaluation of novel 4-substituted 1-methyl-1,2,3,6-tetrahydropyridine analogs of MPTP. *J. Med. Chem.* **35**, 4473-4478.

<sup>136</sup> Kalkutgar, A., Castagnoli, K., Hall, A., Castagnoli, N., Jr. (1994) Novel 4-(aryloxy)tetrahydropyridine analogs of MPTP as monoamine oxidase A & B substrates. *J. Med. Chem.* **37**, 944-949.

<sup>137</sup> Jankovic, J., Marsden, C.D. (1988) In *Parkinson's disease and movement disorders*. (Jankovic, J., Tolosa, E., Eds.), pp. 95-119, Urban and Schwarzenberg, Baltimore- Munich.

diagnosis to initiation of L-DOPA therapy was increased by 12 to 18 months.<sup>138</sup> (*R*)-deprenyl was assessed initially in Hungary for its antidepressant effects.<sup>139</sup> Although (*R*)-deprenyl is used in the treatment of early stage PD,<sup>140</sup> concern has been raised about its therapeutic value. The Parkinson's Study Group of the United Kingdom conducted a study on 782 patients with Parkinson's disease over a mean period of 5.6 years and reported that there were no long term benefits of (*R*)-deprenyl treatment. Furthermore there was an increased mortality in the (*R*)-deprenyl plus L-DOPA treated group of patients compared to the groups treated with L-DOPA plus bromocriptine (**67**) and L-DOPA only.<sup>141</sup>

A prodrug approach which will provide the selective release of the active drug in the central nervous system was proposed by Castagnoli's group as a possible way of minimizing systemic toxic effects.<sup>142</sup> This approach was applied to the carbamate prodrug of (*R*)-nordeprenyl. (*R*)-nordeprenyl (**82**) is an effective and selective inactivator of MAO-B and appears to be equipotent with (*R*)-deprenyl (**83**) *in vivo*.<sup>143</sup>

**82****83**

Since the attachment of the carbamate linkage to (*R*)-deprenyl is not possible, (*R*)-nordeprenyl had to be used as the target molecule for the prodrug. The results showed

<sup>138</sup> Birkmayer, W., Knoll, J., Riederer, P., Youdim, M.B. (1983) (-)-Deprenyl leads to prolongation of L-DOPA efficacy in Parkinson's disease. *Mod. Probl. Pharmacopsychiatry* **19**, 170-176.

<sup>139</sup> Vargar, E., Tringer, L. (1967) Clinical trial of a new type promptly acting psychoenergetic agent (phenyl-isopropyl-methylpropinyl-HCL,E-250). *Acta Med. Acad. Sci. Hung. Tomus* **23**, 289-295.

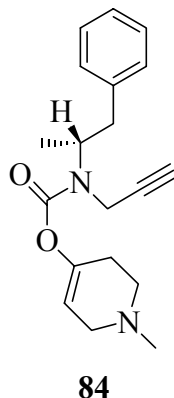
<sup>140</sup> Calne, D.B. (1993) Treatment of Parkinson's disease. *N. Engl. J. Med.* **14**, 1021-1027.

<sup>141</sup> Calne, D.B. (1995) Selegiline in Parkinson's disease - No neuroprotective effect: Increased mortality. *Br. Med. J.* **311**, 1583-1584.

<sup>142</sup> Flaherty, P., Castagnoli, K., Wang, Y.W., Castagnoli, N., Jr. (1996) Synthesis and selective monamine oxidase B-inhibiting properties of 1-methyl-1,2,3,6-tetrahydropyrid-4-yl carbamate derivatives: Potential prodrugs of (*R*)- and (*S*)-nordeprenyl. *J. Med. Chem.* **39**, 4756-4761.

<sup>143</sup> Borbe, H.O., Niebch, G., Nickel, B. (1990) Kinetic evaluation of MAO-B activity following oral administration of selegiline and desmethyl-selegiline in the rat. *J. Neural. Transm. Suppl.* **32**, 131-137.

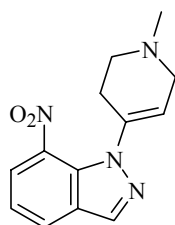
that the tetrahydropyridinyl carbamate derivative of (*R*)-nordeprenyl (**84**) is an active and selective inhibitor of brain MAO-B:



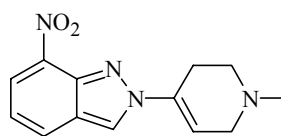
#### 1.6.4. PRODRUG DESIGN FOR 7-NI UTILIZING TETRAHYDROPYRIDINYL AS A CARRIER

In the light of the results of the above experiments, we decided to use the tetrahydropyridinyl moiety as a “carrier” for 7-NI. Such a prodrug should be chemically stable and was expected to be a substrate for MAO.<sup>144</sup> Since dopaminergic neurons in the human are rich in MAO-A and since the related carbamoyl prodrugs are MAO-A selective substrates, it is conceivable that the prodrug could target nigrostriatal neurons.

The tetrahydropyridinyl moiety can be attached to either nitrogen on the benzopyrazolyl ring of 7-NI to form the 1-substituted (**85**) and 2-substituted (**86**) compounds:



**85**



**86**

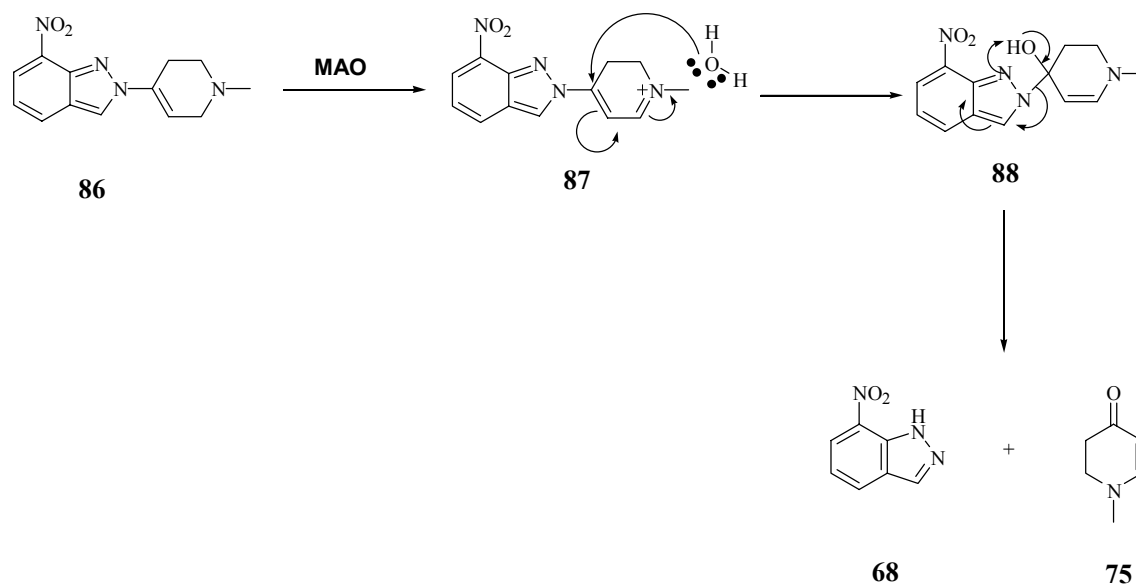
For the sake of the discussion, the proposed pathway for the activation of the 2-substituted prodrug (**86**) is shown below only. Analog **86** is first oxidized to the

---

<sup>144</sup> Nimkar, S.K., Mabic, S., Anderson, A.H., Palmer, S.L., Graham, T.H., de Jonge, M., Hazelwood, L., Hislop, S.J., Castagnoli, N., Jr. (1999) Studies on the monoamine oxidase-B-catalyzed biotransformation of 4-azaaryl-1-methyl-1,2,3,6-tetrahydropyridine derivatives. *J. Med. Chem.* **42**, 1828-1835.

corresponding dihydropyridinium intermediate **87**. Spontaneous hydrolysis of **87** is expected, as in the phenoxy compound **73**, to yield the active drug 7-NI and aminoenone **75** (Scheme 27).

**Scheme 27. MAO-B catalyzed oxidation of compound 86 followed by spontaneous hydrolysis releasing the active drug 7-NI.**

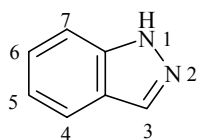
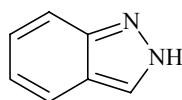
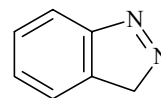


## CHAPTER 2. INDAZOLES

Before describing our attempts to obtain the desired prodrugs, the chemistry of indazolyl compounds will be reviewed.

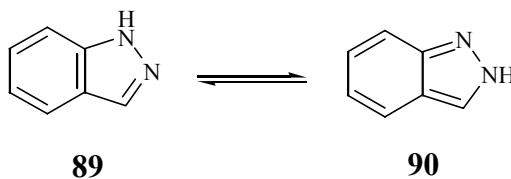
### 2.1. CHEMISTRY OF INDAZOLES

There are 3 possible indazole ring systems: *1H*-indazole (**89**), *2H*-indazole (**90**), and *3H*-indazole (**91**).

**89****90****91**

Von Auwers was the first to report that the **89** and **90** existed in a tautomeric equilibrium.<sup>145</sup> Evidence obtained from molecular refractivity measurements suggested the predominance of tautomer **89** which possesses the benzenoid moiety.<sup>146</sup>

#### Scheme 28. Tautomerization of indazole.

**89****90**

It was also shown that the  $^1\text{H}^{147}$  and  $^{14}\text{N}^{148}$  NMR and the UV spectral features<sup>149</sup> of indazole resemble those of 1-methylindazole (**92**) and are different from the spectra obtained with 2-methylindazole (**93**).

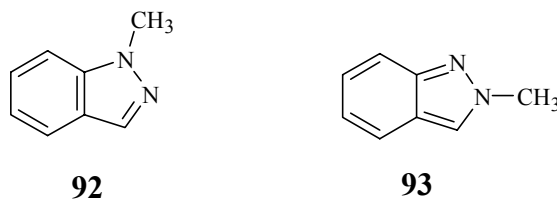
<sup>145</sup> Von Auwers, K. V., Meyenburg, F. V. (1891) Ueber eine neue synthese von Derivaten des Isindazoles. *Ber.* **24b**, 2370-2388.

<sup>146</sup> Von Auwers, K. V., Hugel R., Ungemach, O. (1937) *Ann.* **527**, 291-298.

<sup>147</sup> Elguero, J., Fruchier, A., Jacquier, R. (1966) The azole series. V. Nuclear magnetic resonance study of indazoles. *Bull. Soc. Chim. Fr.* **6**, 2075-2084.

<sup>148</sup> Witanowski, W., Stefaniak, H., Januszewski, Grabovski, Z., Webb, G.A. (1972) Nitrogen -14 nuclear magnetic resonance study of indazoles. *Tetrahedron* **28**, 637-653.

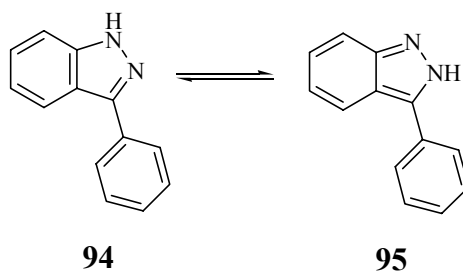
<sup>149</sup> Rousseau, V., Linwall, H.G. (1950) Structure and Ultraviolet Absorption Spectra of Indazole. 3-substituted indazole and some of their derivatives. *J. Am. Chem. Soc.* **72**, 3047-3051.



These findings are also supported by an X-ray structure determination study.<sup>150</sup>

The existence of two forms of 3-phenylindazole has been offered as support for the equilibrium proposal. The two forms of 3-phenylindazole were reported to be interconvertible upon heating and seeding the melt with the other isomer.<sup>151,152</sup> Similar interconversion of two forms also were reported with certain 5-substituted indazoles.<sup>153</sup>

**Scheme 29. Interconversion of 3-phenylindazole between *1H* and *2H* isomers.**



Investigation of the tautomeric equilibrium between *1H* and *2H* isomers by photophysical and thermochemical techniques as well as by theoretical calculations showed the *1H* isomer to be more stable than the *2H* isomer by 4 kcal/mole. Elguero *et al.* reported that indazole is in the *1H* tautomeric form in the gas phase and in solution both in the ground and in the excited state.<sup>154</sup>

<sup>150</sup> Escande, A., Lapasset, J., Faure, R., Vincent, E. -J., Elguero, J. (1974) Les benzazoles (indazole, benzimidazole, benzotriazole) structure moleculaire et proprietes fondamentales. *Tetrahedron* **30**, 2903-2909.

<sup>151</sup> Auwers, K.V., Sundheimer (1896) Ueber Indazolderivative. *Ber.* **24**, 1255-1271.

<sup>152</sup> Auwers, K.V., Huttenes, K. (1922) 3-Phenylindazole and 2-hydroxy-3-phenylindazole. *Ber.* **55B**, 1112-1138.

<sup>153</sup> Auwers, K.V., Schaum, K. (1929) Isomerism of methyl phenylpyrazoles. *Ber.* **62B**, 1671-1677.

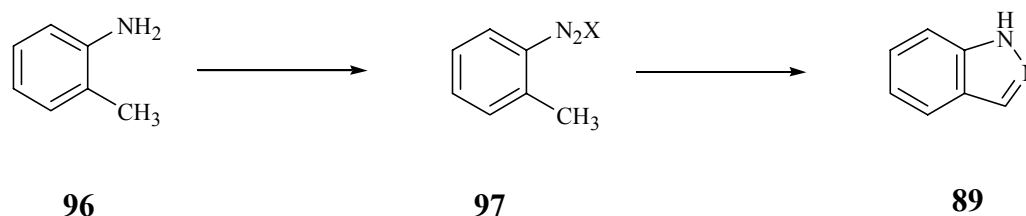
<sup>154</sup> Catalan, J., Elguero, J. (1994) Acidity and basicity of indazole and its N-methyl derivatives in the ground and in the excited state. *J. Phys. Chem.* **98**, 10606-10612.

## 2.2. SYNTHESIS OF INDAZOLES

Derivatives of indazole can be prepared by a variety of reactions. Efficient syntheses often proceed via *o*-disubstituted benzene derivatives. The two substituents are chosen in order to allow closure to form the five membered ring. The ring closure reactions can be classified into three groups according to the point where ring closure takes place.

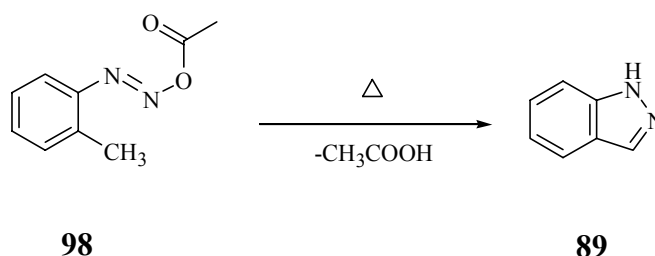
**Diazotization of anilines (*o*-toluidines):** Conversion of *o*-toluidine (**96**) to indazole (**89**) via a diazo intermediate (**97**) is an example for this type of ring closure (Scheme 30).<sup>155</sup>

**Scheme 30. Synthesis of indazole via a diazotization reaction.**



An example of this type of indazole synthesis is the cyclization of the acetoxy compound **98** to form indazole (Scheme 31).<sup>156</sup>

**Scheme 31. Synthesis of indazole from acetoxy compound 96.**



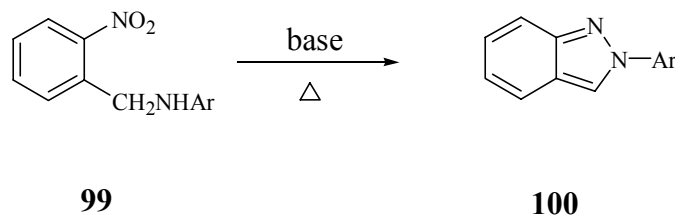
**Reduction of N-*o*-nitrobenzylanilines:** Synthesis of 2-arylindazoles (**100**) from N-*o*-nitrobenzylaniline (**99**) is an example of an N-N type of ring closure (Scheme 32).<sup>157</sup>

<sup>155</sup> Behr, L.C., Raffaello, F., Jarboe, C.H., Wiley, R.H., Eds. (1967) *Pyrazoles, pyrazolines, pyrazolidines, indazoles and condensed rings*, pp. 295-302, John Wiley & Sons, New York.

<sup>156</sup> Huisgen, R., Bast, K. (1962) Indazole. *Org. Syn.* **42**, 69-72.

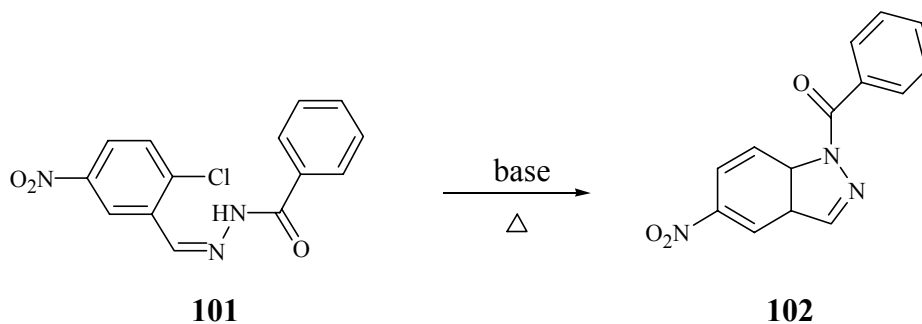
<sup>157</sup> Paal, C. (1891) Zur kenntniss der Indazole. *Ber.* **24**, 3058.

**Scheme 32. Synthesis of 2-aryl-substituted indazoles (100) via reduction of N-*o*-nitrobenzylanilines (99)**



**Thermal cyclization of hydrazones:** The N-benzoylhydrazone **101** yields the corresponding 1-substituted indazole (**102**) upon heating (Scheme 33).<sup>158</sup>

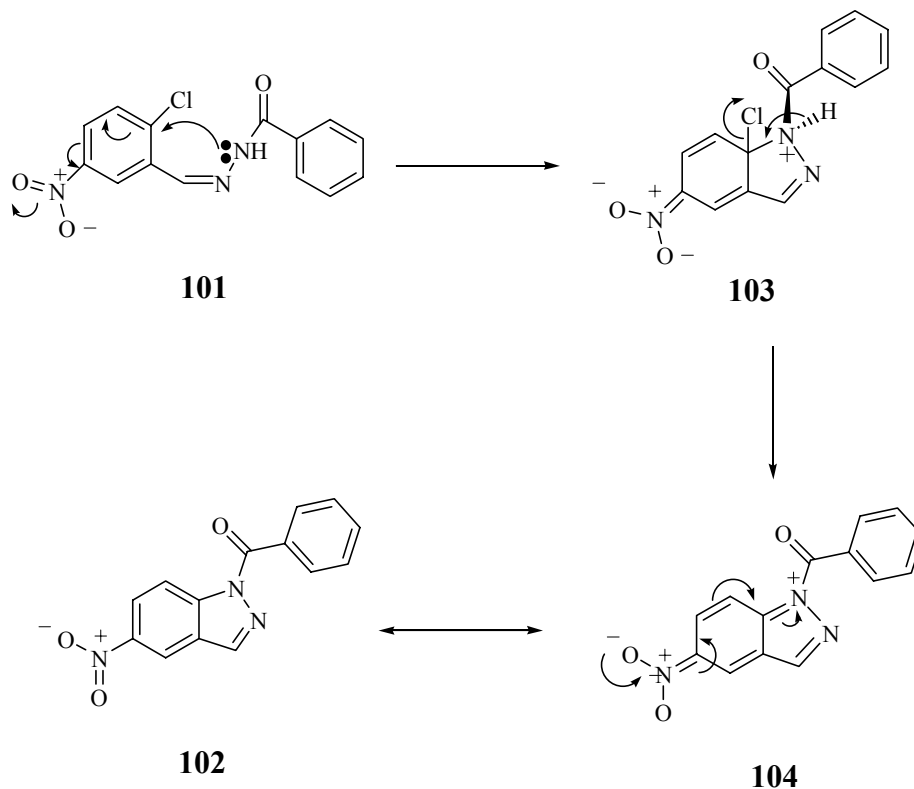
**Scheme 33. Synthesis of 1-substituted indazoles from hydrazones such as 101.**



The presence of the nitro group in **101** facilitates the formation of the indazole. The nitro group acts as an electron sink to facilitate the intramolecular nucleophilic aromatic substitution reaction (Scheme 34).

<sup>158</sup> Meisenheimer, J., Senn, O. (1926) Acylindazoles. *Ber.* **59B**, 199-202.

**Scheme 34. Presence of the nitro group facilitates the reaction acting as an electron sink.**



In most cases the preferred reaction medium is dilute acetic acid. However, for *o*-toluidines lacking the nitro group, the best yields are obtained in alkaline solution.

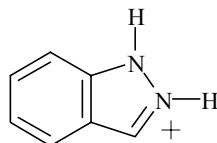
Indazole and its derivatives undergo the usual aromatic substitution reactions. These reactions are summarized below.

**Halogenation:** The most easily substituted positions on the indazole ring are 3-, 5- and 7- in the absence of activating groups. Indazoles unsubstituted on nitrogen undergo halogenation in the 3-position preferentially, although the 5-position is nearly as reactive.<sup>159</sup>

**Nitration:** The presence of the nitro group increases the acidity of the indazole system by 3-5 pK<sub>a</sub> units depending on the position of the nitro group. The pK<sub>a</sub> of the indazolium ion (105) is reported to be 1.3.<sup>160</sup>

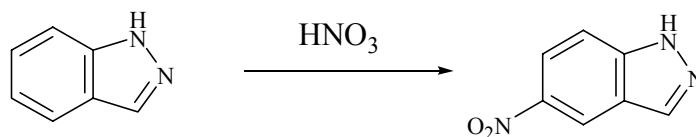
<sup>159</sup> Auwers, K.V., Lange, H. (1922) Halogenated indazoles and stereoisomerism in the free indazoles. *Ber.* **55B**, 1139-1173.

<sup>160</sup> Albert, A., Goldacre, R., Phillips, J. (1948) The strength of heterocyclic bases. *J. Chem. Soc.* 2240-2249.

**105**

Nitro derivatives are frequently obtained during the synthesis of the indazole nuclei. The 5-nitro derivative **106** was reported to be obtained by the reaction of indazole with fuming nitric acid (Scheme 35).<sup>161</sup>

**Scheme 35. Synthesis of 5-nitroindazole (106) via the reaction of fuming nitric acid with indazole.**

**89****106**

**Alkylation:** Indazoles which are unsubstituted at the 1- or 2- positions undergo alkylation readily upon treatment with alkyl halides. Usually a mixture of the 1- and 2-alkylindazoles is obtained. Since 2-substituted derivatives have a higher boiling point, separation may be achieved by fractional distillation. Although there are many exceptions,<sup>162</sup> the following general tendencies for the position of the alkylation have been reported by Auwers et al.<sup>163</sup> In alkaline solution, alkylation at both the 1- and 2-positions takes place to about the same extent. However, reaction with more reactive alkyl halides, such as allyl and benzyl bromide, produces 1-substituted derivative exclusively.

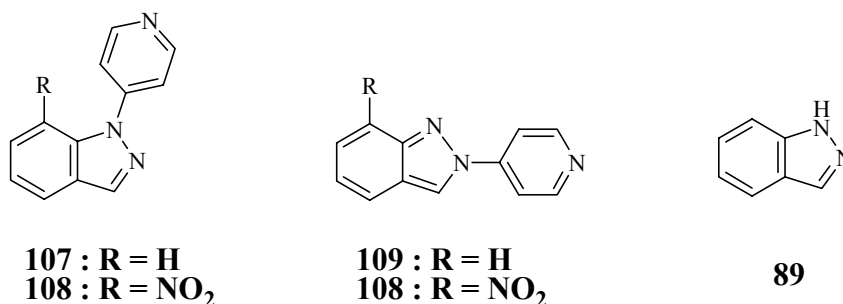
<sup>161</sup> Auwers, K.V., Kleiner, H. (1928) Miscellaneous observations on indazole derivatives. *J. Prakt. Chem.* **118**, 67-90.

<sup>162</sup> Auwers, K.V., Allardt, H.G. (1924) Further investigations on the alkylation of indazole. *Ber.* **57B**, 1098-1106.

<sup>163</sup> Auwers, K.V., Lohr, A. (1924) Alkylated and halogenated indazoles. *J. Prakt. Chem.* **108**, 297-320.

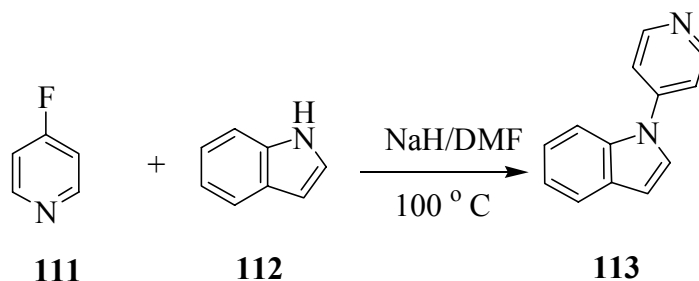
### CHAPTER 3. REVIEW OF EARLIER STUDIES

Previously, cyclization reactions summarized in the synthesis of indazoles were considered as a route to the regioselective attachment of pyridinyl moiety to the either of the nitrogens of the indazole ring to obtain the target compounds **108** and **110** which are precursors to the prodrugs **85** and **86**. These cyclization reactions to give **107** and/or **109**, which were carried out by Millie De Jonge with unsubstituted indazole (**89**) as a model compound failed to yield any of the corresponding target compounds.



As an alternative pathway, a nucleophilic aromatic substitution reaction involving 4-fluoropyridine (**111**), was attempted. The analogous reaction, shown in Scheme 36, was reported by Seki to give 4-indol-1-ylpyridine (**113**) using indole (**112**) in the presence of NaH to form the sodium salt of indole.<sup>164</sup>

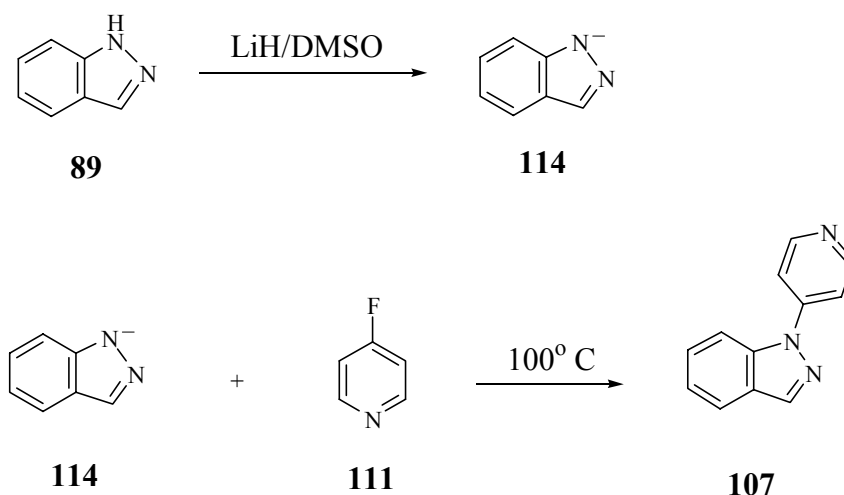
**Scheme 36. Synthesis of 4-indol-1-ylpyridine (113).**



<sup>164</sup> Seki, K., Ohkura, K., Terashima, M., Kanaoka, Y. (1994) A facile synthesis of N-(2- and 4-pyridyl)indoles. *Heterocycles* **37**, 993-996.

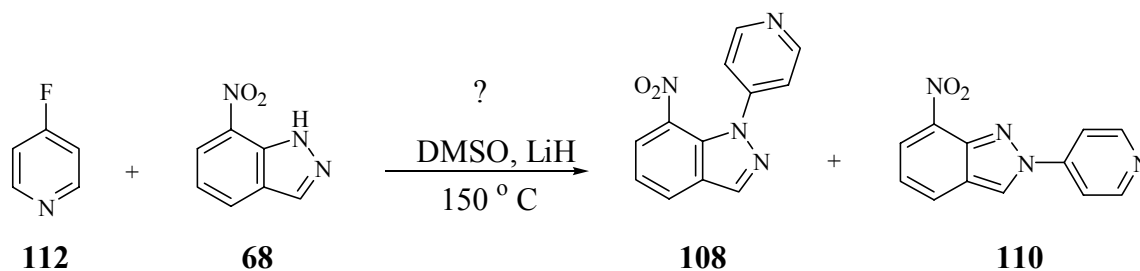
Also the nucleophilic aromatic substitution reaction between **111** and indazole (**89**) was reported by Castagnoli's group to give a 4-indazolylpyridine. The regiochemistry had been tentatively assigned to be the *IH* isomer **107** (Scheme 37).<sup>165</sup>

**Scheme 37. Reaction of 4-fluoropyridine (111) with indazole goes through the indazolyl anion 114 to form the desired product 107.**



The reaction sequence illustrated in Scheme 37 was applied to the synthesis of the 4-(7-nitroindazolyl)pyridines **108** and/or **110** (Scheme 38).

**Scheme 38. Coupling reaction between 4-fluoropyridine (111) and 7-nitroindazole (68).**



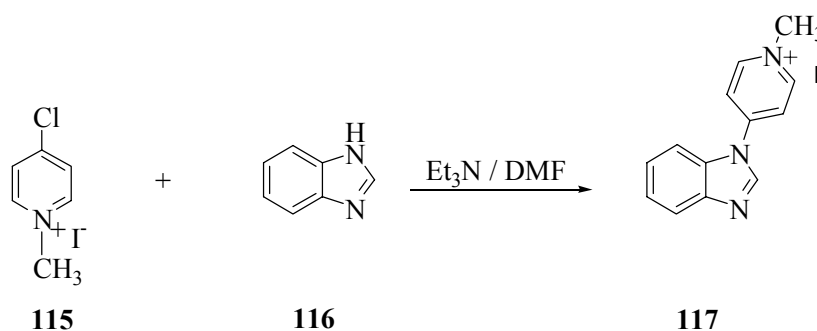
<sup>165</sup> See reference 144

The reaction, which was run for 48 hours at 150 °C, was monitored by TLC. Two spots were seen in addition to the starting material. These were thought to be the two possible isomeric products **108** and **110**. However, no further details were provided in the report. In any event, the yield was too low to make this a useful approach.

The nitro group present in **68** probably decreases the nucleophilicity of the indazolyl system. The reactivity of 4-fluoropyridine apparently was not sufficient to overcome this loss in nucleophilic character. Steric hindrance by the nitro group also may have an effect on the reactivity. However, this will only have an effect on substitution at the N1 position.

Previously, 4-chloro-1-methylpyridinium iodide (**115**) has been reported to give coupling reaction products with various azaarenes (Scheme 39).<sup>166</sup>

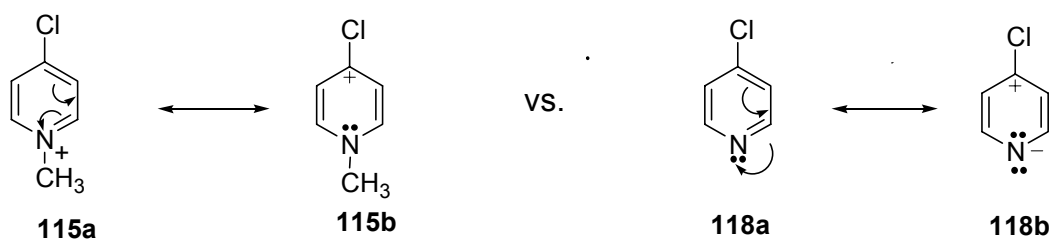
**Scheme 39. Coupling reaction between 4-chloro-1-methyl-pyridinium iodide (115) and imidazole (116) to give 117.**



Compound **115** should be a better electrophile compared to 4-fluoro- or 4-chloro-pyridine as illustrated by the following resonance forms (Scheme 40).

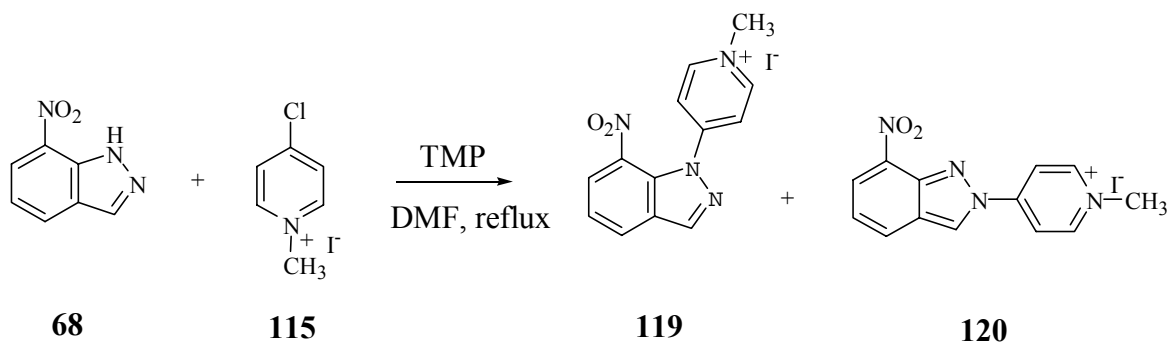
<sup>166</sup> See reference 144

**Scheme 40. Positive charge on nitrogen in compound 115 leads to a higher electrophilicity compared to compound 118.**

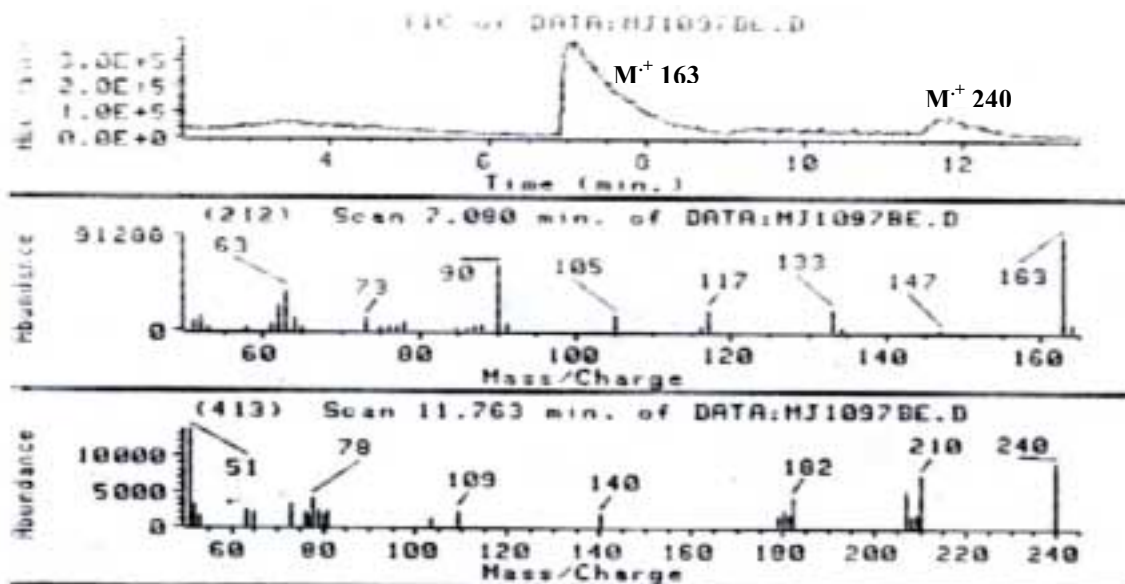


In order to overcome the above reactivity problem with 4-fluoropyridine (**112**), the coupling reaction was attempted with 1-methyl-4-chloropyridinium iodide (**115**) in the presence of 2,2,6,6-tetramethylpiperidine (TMP) as the base under reflux conditions (Scheme 41).

**Scheme 41. Reaction of 7-nitroindazole (68) with 4-chloro-1-methylpyridinium iodide (115) was expected to give one or both of the possible isomers 119 and 120.**



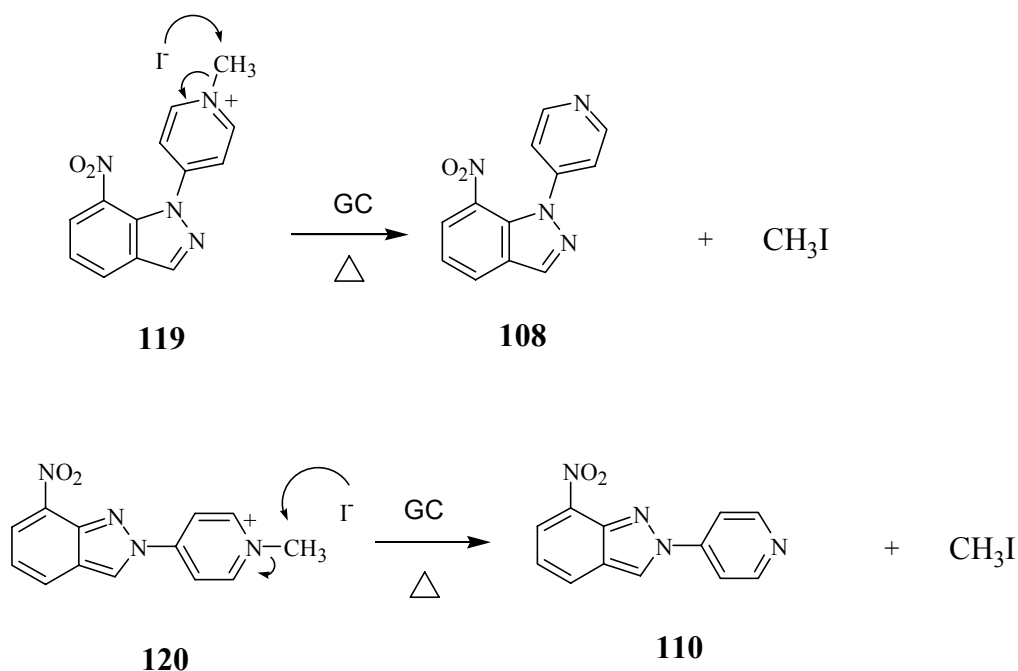
After heating in DMF under reflux for 36 hours, the GC-MS TIC tracing shown in Figure 1 was obtained.



**Figure 1. GC-MS TIC tracings for the reaction illustrated in Scheme 41 after 3 days under reflux.**

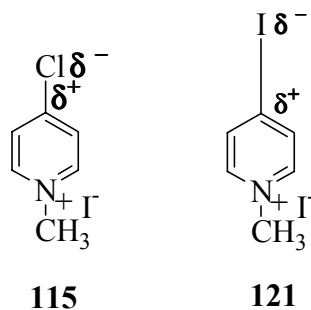
There was one peak with the molecular ion at  $M^+$  163 corresponding to the starting material and a weak peak at  $M^+$  240. The  $M^+$  ion 240 corresponds to the molecular weights of the desmethyl compounds **108** and **110** formed by the loss of N-methyl group from **119** and **120** as expected under GC conditions.

**Scheme 42. Thermal demethylation of 119 and 120 takes place in the GC-MS injection port to yield the demethylated products 108 and 110.**

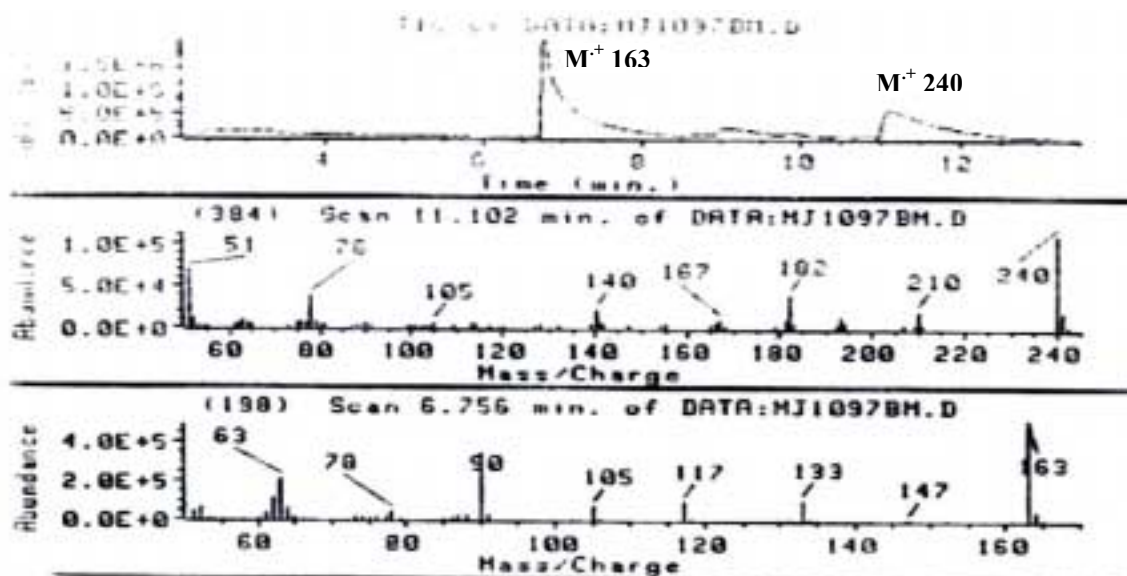


The coupling reaction was carried out again, this time using NaI as the catalyst. It was suggested that iodide will substitute the chloro group on **115** which will make the compound more reactive since iodide is a better leaving group. However replacing chlorine with a less electronegative atom will decrease the polarization of the bond between the carbon and the leaving group which is a more important factor in the nucleophilic aromatic substitution reactions and which may decrease the rate of the reaction (Scheme 43).

**Scheme 43. Replacing Cl with I will decrease the electrophilicity of C4.**



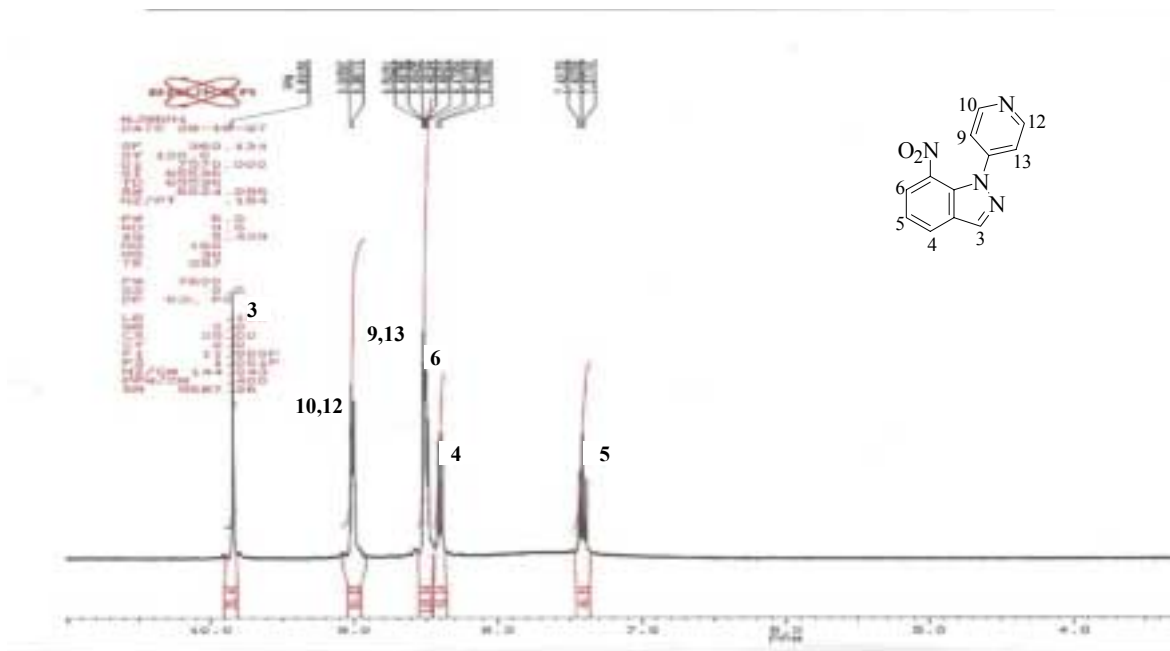
This time more starting material was consumed and an increase in the intensity of the peak with the molecular ion at  $M^+$  240 was observed (Figure 2).



**Figure 2. GC-MS TIC tracings of the reaction illustrated in Scheme 41 in the presence of NaI after 1 day under reflux.**

A product was isolated from the reaction mixture and purified by column chromatography. However the  $^1\text{H}$  NMR spectrum of the product did not show any upfield signal expected for the methyl group attached to the pyridine ring<sup>167</sup> (Figure 3), suggesting that demethylation was taking place during the reaction not in the GC injection port. The regiochemistry was assigned tentatively as the *IH* isomer **108**.

<sup>167</sup>  $^1\text{H}$  NMR spectrum was obtained by Millie de Jonge. At this point their signal assignments will be presented without further discussion.



**Figure 3.** <sup>1</sup>H NMR spectrum (instrument frequency = 360 MHz) of the product obtained from the reaction shown in Scheme 41.

The first aim of the present study was to assign unambiguously the regiochemistry to the already synthesized “prodrug” precursor of 7-NI (**108** or **110**). After assigning the regiochemistry, we hoped to synthesize the isomer, which had not been obtained previously. However, difficulties we faced during the synthesis led us to explore the mechanism of the nucleophilic aromatic substitution reaction of 7-NI (**68**) with **115**. We also extended the scope of our synthetic efforts to two structurally similar and biologically relevant compounds, 6-nitroindazole (**122**) and 5-nitroindazole (**123**). We concluded our studies with the biological investigation of the three “prodrugs” we have obtained. These studies revealed important information related to the active site of MAO-B.