Synthesis of Deprenyl-like nitroxide free radicals and their diamagnetic derivatives

Cecília P. Sár,[a] Tamás Kálai,[a] József Jekő,[b] and Kálmán Hideg[a]*

[a]Department of Organic and Medicinal Chemistry, University of Pécs, 7602 Pécs, P. O. Box 99, Hungary
[b]Department of Chemistry, College of Nyíregyháza, Sóstói st 31/B, H-4440, Nyíregyháza, Hungary
E-mail: kalman.hideg@aok.pte.hu

This article is dedicated to Professor Ferenc Fülöp on occasion of his 60th birthday

DOI: http://dx.doi.org/10.3998/ark.5550190.0013.506

Abstract
Synthesis of paramagnetically modified deprenyl and oxotremorine is reported. Starting from 5- and 6-membered 2,5-disubstituted nitrones **1, 6** or 4-phenyl-2,5,5-trimethyl-1*H*-pyrroline 1-oxide **11** deprenyl or oxotremorine like nitroxides were synthesized via Grignard reactions. The corresponding pre-nitroxides with propargylamine structure were achieved by reduction of nitroxides followed by methylation.

Keywords: Amines, alkylations, L-deprenyl, free radicals, Grignard reaction

Introduction

Parkinson's disease is an age-related disorder that afflicts as many as 2% of all individuals.[1] The biochemical basis for the motor symptoms of Parkinson's disease is a loss of dopamine.[2,3] Therefore Parkinson's disease can be relieved by treating patients with L-3,4-dihydroxyphenylalanine (L-DOPA) and an inhibitor of peripheral L-DOPA decarboxylase. In order to preserve brain dopamine, it is also common to treat patients with a monoamine oxidase B (MAO-B) inhibitor.[4] Selegiline (L-Deprenyl) and Rasagiline (Figure 1)[5-10] are selective inhibitors of MAO-B and Selegiline is currently used for the treatment of Parkinson's and Alzheimer's diseases. This compound was reported to have a neuroprotective activity due to the prevention of apoptosis.[11] The propargylamine pharmacophore of Selegiline and Rasagiline appears to be responsible for neuroprotective activity. Crystallographic analysis revealed that

rasagiline covalently binds with its propargyl group to flavine enzyme to form an iminopropene chain.[12]

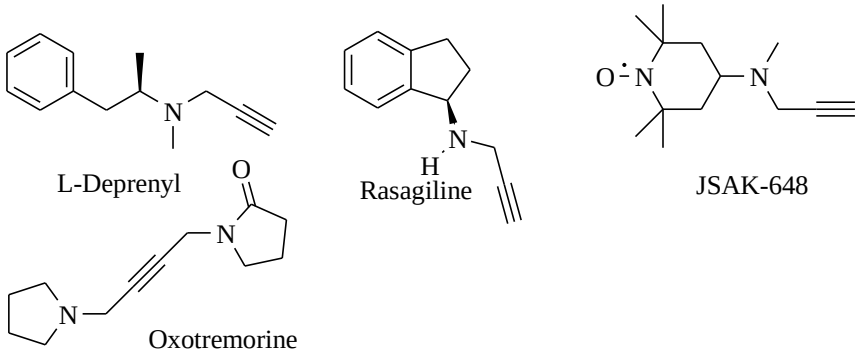


Figure 1

Namiecinski et al. found that nitroxides with propargylamine chain such as JSAK-648 (Figure 1) can cross the blood-brain barrier and have been shown to have antioxidant properties, cell protection against oxidative stress and Reactive Oxygen Species (ROS) cytotoxicity.[13,14] It is important to have a radical scavenger *in statu nascendi* to prevent damages caused by ROS, because the MAO-B mediated metabolism of dopamine and its autooxidation generate $O_2^{\cdot-}$, H_2O_2 and the highly toxic $^{\cdot}OH$ in the presence of trace levels of free iron ions. Continuing our research in the synthesis of experimental drugs with dual activity containing nitroxide or its precursor,[15-19] we wish to extend this idea for neuroprotective drugs such as Deprenyl and Oxotremorine. Oxotremorine (Figure 1) is known as a muscarinic agonist and is also used in the Alzheimer's therapy. Therefore a large number of Oxotremorine derivatives were reported and investigated.[20-22]

The metabolic oxidation of 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the oxidation product $MPDP^+$ is also catalyzed by MAO-B. Following a second two-electron oxidation, the ultimate neurotoxic metabolite, 1-methyl-4-phenylpyridinium (MPP^+) is generated.[23,24] MPP^+ is a mitochondrial toxin, which selectively damages nigrostriatal neurons and induces Parkinsonian syndrome in humans.[25] Several other allylamines act as good MAO-B substrates, such as the 5-membered ring analogue of MPTP.[23] (Figure 2)

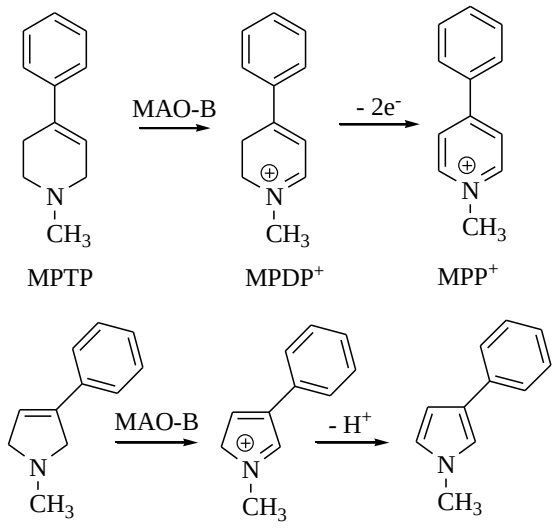


Figure 2

Motivated by these findings the synthesis of Deprenyl and Oxotremorine derivatives with 5- or 6-membered nitroxide rings was planned. We desired to investigate the oxidation of their sterically hindered secondary or tertiary amine precursors and a six membered model compound. We hope that these compounds will act as MAO-B antagonists or muscarinic agonists and as antioxidant compounds in the central nervous system (CNS).

Results and Discussion

An evident approach for the combination of Deprenyl and Oxotremorine structure with nitroxides was the reaction of five- or six-memberd nitrones and properly chosen Grignard-reagents, supporting the introduction of the required aryl or alkynyl group easily (Figure 3).

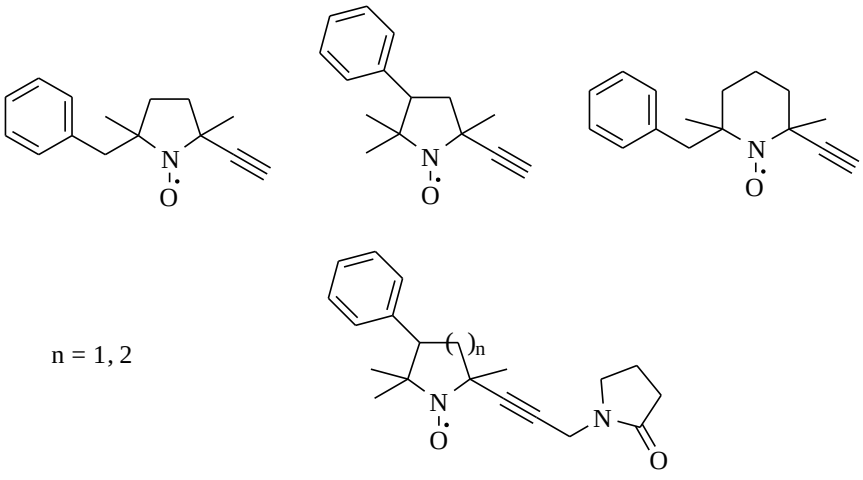


Figure 3

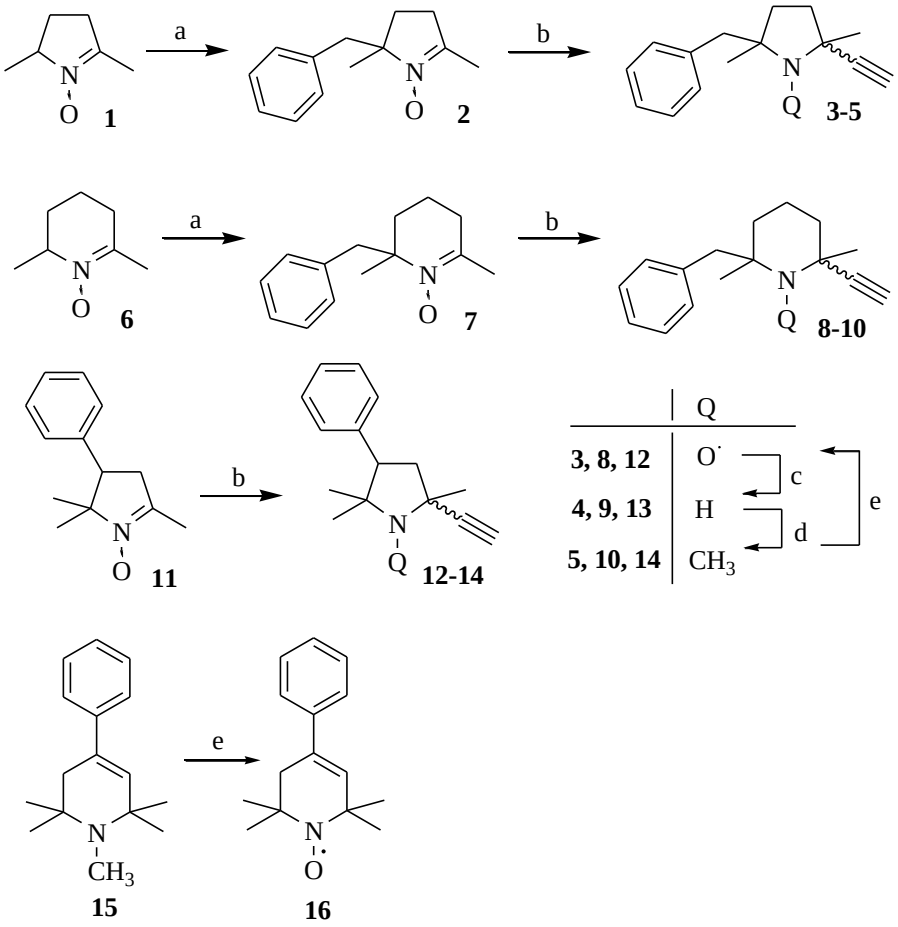
Treatment of 2,5-dimethyl-3,4-dihydro-2*H*-pyrrole 1-oxide **1** or 2,6-dimethyl-2,3,4,5-tetrahydro-pyridine 1-oxide **6** with benzylmagnesium chloride in dry Et_2O followed by the oxidation of *N*-hydroxy compound with activated MnO_2 afforded the appropriate nitrones **2, 7**. The reaction of ethynylmagnesium bromide with 2-benzyl substituted nitrones **2** and **7** in THF yielded the stereoisomers of nitroxide **3** and **8**, respectively. The ratio of *trans/cis* isomers was approximately 5:1. For further reactions the *trans* isomers were used. Complete assignments of compound **3** could be obtained with conventional 1D and 2D NMR spectroscopy methods after the treatment of the free radical compound with diphenylhydrazine in $CDCl_3$.

The Grignard reaction of nitrone **11** with ethynylmagnesium bromide yielded 3-phenyl-5-ethynyl substituted nitroxide **12**. In the latter case the spacer between the nitrogen and aryl group is more rigid, being a part of a five-membered ring. To get more Deprenyl-like nitroxides **3**, **8** and **12** were reduced to secondary amines by treating them with 5 equiv Fe powder in glacial acetic acid at 60 °C producing amines **4, 9** and **13**. These secondary amines were alkylated by refluxing them with methyl iodide excess in THF affording tertiary amines **5, 10** and **14** (Scheme 1). The advantages of synthesized secondary and tertiary amines beyond the structural fidelity are their better water solubility and oxygen scavenging ability such detoxyfying ROS during non-toxic, stable nitroxide free radical formation.

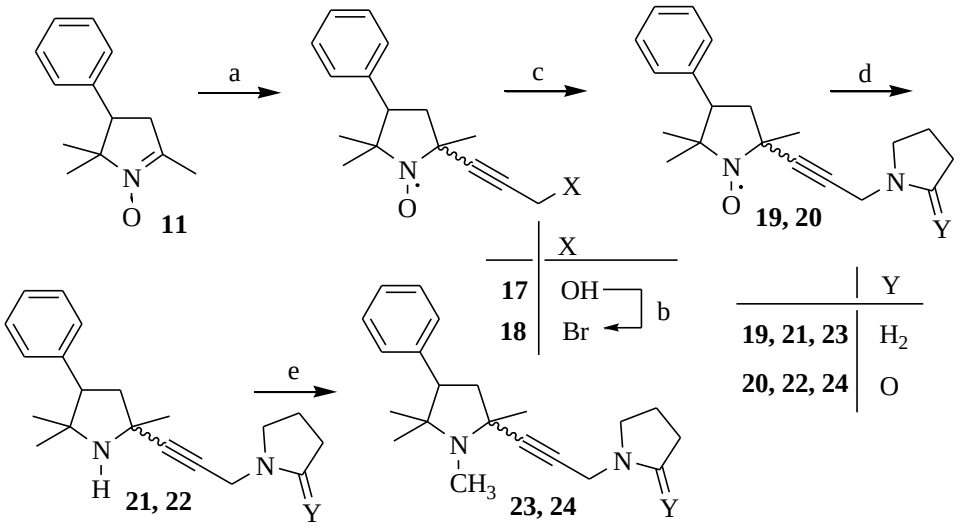
The oxidation of both 5- and 6-membered sterically hindered tertiary amines was investigated in MeOH with H_2O_2. However, upon oxidation in case of compound **14** or model compound **15**[26] upon oxidation nitroxides **12** and **16**[27] were formed and because of tetramethyl group no MPP^+-like product is possible even at harsh oxidation conditions.

For Oxotremorine analogue synthesis we prepared compound **17** from nitrone **11** and a Grignard-reagent generated from propargylic alcohol with 2 equiv. EtMgBr in situ. After the oxidation of hydroxylamine with catalytic amount MnO_2 **17** propargylic alcohol was converted to **18** bromo compound by treating mesylate with LiBr in acetone. This bromo compound was used to alkylate pyrrolidine or 2-pyrrolidinone to yield spin labelled tremorine **19** or oxotremorine **20** derivatives. Nitroxides **19** and **20** were reduced to sterically hindered secondary amines **21, 22** with Fe powder in AcOH as mentioned above.

To avoid the quaternary salt formation of tertiary amine of compounds **21** and **22** the *N*-methylation of secondary amines by refluxing with formaldehyde in the presence of formic acid, (Eschweiler-Clark conditions)[21] furnished the tertiary pyrrolidine derivatives **23** and **24** (Scheme 2).



Scheme 1. *Reagents and conditions:* a: Benzylmagnesium chloride (1.2 equiv.), Et_2O, 0 °C → r.t., 3 h, aq. sat. NH_4Cl, then $CHCl_3$, MnO_2 (cat.), O_2, r.t., 1 h (62-74 %). B: HC≡CMgBr, THF, 0 °C → r.t., 2 h, aq. sat. NH_4Cl, then $CHCl_3$, MnO_2 (cat.), O_2, r.t., 30 min (65-77 %). C: AcOH, Fe powder (5 equiv.), 60 °C, 1 h, then H_2O, K_2CO_3 (57-68 %). d: MeI, THF, reflux, 1 h (65-83 %). (e) H_2O_2 (2 equiv), Na_2WO_4 (cat.), MeOH, r.t., 24 h (35-42 %)



Scheme 2. *Reagents and conditions:* a: $BrMgC{\equiv}C\text{-}CH_2\text{-}OMgBr$ (1.1 equiv.), THF, 0 °C → r.t., 12 h, aq. sat. NH_4Cl, then $CHCl_3$, MnO_2 (cat.), O_2, r.t., 1 h (58 %). b: Et_3N, CH_2Cl_2, CH_3SO_2Cl, 0 °C → r.t., 30 min, then acetone, LiBr (2 equiv.), reflux, 30 min (83 %). c: pyrrolidine (2 equiv.), THF, reflux, 1 h (88 % for **19**); NaH, THF/DMF, 2-pyrrolidinone, r.t., 2.5 h (74 % for **20**). d: AcOH, Fe powder (5 equiv.), 60 °C, 1 h then H_2O, K_2CO_3 (53-71 %). e: HCHO (37 %, 10 equiv.), HCOOH (88 %, 10 equiv.), reflux, 6 h, then K_2CO_3 (69-78 %).

Conclusions

In conclusion, starting from disubstituted or trisubstituted five- or six-membered nitrones paramagnetic Deprenyl and Oxotremorine analogues were synthesized by means of Grignard reactions. Reduction of nitroxides and *N*-methylation offered a closer analogue of Deprenyl and Oxotremorine neuroprotective drugs with ROS scavenging structural elements, such as sterically hindered amine (pre nitroxide) compounds. The biological study of these compounds is in progress and will be reported in due course.

Experimental Section

General. Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on Fisons EA 1110 CHNS elemental analyzer. The IR (Specord 85) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a Thermoquest Automass Multi and VG TRIO-2 instruments in the EI mode. ^1H NMR spectra were recorded with Varian ^{UNITY}*INOVA* 400 WB spectrometer. Chemical shifts are referenced to Me_4Si. Measurements were run at 298 K probe temperature in $CDCl_3$ solution. ESR spectra were taken on Miniscope MS 200 in 10^{-4} M $CHCl_3$

solution and all monoradicals gave triplett spectrum a_N = 14.7-16.5 G. Flash column chromatography was performed on Merck Kieselgel 60 (0.040-0.063 mm). Qualitative TLC was carried out on commercially available plates (20 x 20 x 0.02 cm) coated with Merck Kieselgel GF_{254}.

Synthesis of 2-benzyl-nitrones (2, 7); General procedure exemplified by 2-benzyl-2,5-dimethyl-3,4-dihydro-2*H*-pyrrole 1-oxide (2)

To a solution of benzylmagnesium chloride (prepared from benzyl-chloride (7.62 g, 0.06 mol) and Mg (1.50 g, 0.06 mol)) in dry Et_2O (50 mL) nitrone (**1**) (5.65 g, 0.05 mol) in dry Et_2O (50 mL) was added dropwise at 0 °C. The reaction mixture was stirred at r.t. for 3 h, then aq. sat. NH_4Cl (80 mL) was added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (3 x 30 mL). The combined organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in $CHCl_3$ (50 mL), activated MnO_2 (cat., 100 mg) was added and the mixture was bubbled with O_2 for 1 h at r.t. The reaction mixture was then filtered, evaporated and purified by flash column chromatography with $CHCl_3$/MeOH to give 2-benzyl-nitrone.

2-Benzyl-2,5-dimethyl-3,4-dihydro-2H-pyrrole 1-oxide (2). 7.51 g (74 %); oil; R_f = 0.35 ($CHCl_3$-Et_2O-MeOH 4:1.5:0.5); Anal. calcd. for $C_{13}H_{17}NO$ (203.28): C 76.81 %, H 8.43 %, N 6.89 %; found: C 76.99 %, H 8.35 %, N 6.82 %. MS (EI) m/z (rel. int. %): 203 (M^+, 63), 131 (58), 112 (80), 91 (100).

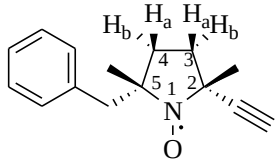
2-Benzyl-2,6-dimethyl-2,3,4,5-tetrahydro-pyridine 1-oxide (7). 6.73 g (62 %); oil; R_f = 0.41 ($CHCl_3$-Et_2O-MeOH 4:1.5:0.5); Anal. calcd. for $C_{14}H_{19}NO$ (217.31): C 77.38 %, H 8.81 %, N 6.45 %; found: C 77.33 %, H 8.90 %, N 6.32 %. MS (EI) m/z (rel. int. %): 217 (M^+, 10), 152 (30), 129 (37), 91 (100), 73 (96), 41 (84).

Synthesis of 5- and 6-ethynyl-nitroxides (3, 8, 12); General procedure exemplified by cis- and trans-2-benzyl-5-ethynyl-2,5-dimethyl-pyrrolidine-1-yloxyl radical (3)

To ethynylmagnesium bromide (0.5 M sol. in THF, 50 mL) a solution of nitrone (**2**) (4.06 g, 0.02 mol) in dry THF (30 mL) was added dropwise at 0 °C. The mixture was stirred at r.t. for 2 h then aq. sat. NH_4Cl (40 mL) was added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (3 x 20 mL). The combined organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in $CHCl_3$ (50 mL), activated MnO_2 (cat., 100 mg) was added and the mixture was bubbled with O_2 for 30 min at r.t. The reaction mixture was then filtered, evaporated and purified by flash column chromatography with hexane/Et_2O to give the *cis* and *trans* stereoisomers of nitroxide.

Trans-2-benzyl-5-ethynyl-2,5-dimethyl-pyrrolidine-1-yloxyl Radical (trans-3). 2.78 g (61 %); oil; R_f = 0.64 (hexane-EtOAc 2:1); Anal. calcd. for $C_{15}H_{18}NO$ (228.31): C 78.91 %, H 7.95 %, N 6.13 %; found: C 78.89 %, H 7.82 %, N 6.31 %. MS (EI) m/z (rel. int. %): 228 (M^+, 22), 183 (5), 132 (17), 117 (95), 91 (100). 1H NMR ($CDCl_3$ + diphenyl-hydrazine) δ (ppm): 2.78 (d, *J*=12.5 Hz, 1H, Ph-CH_2), 2.70 (d, *J*=12.5 Hz, 1H, Ph-CH_2), 2.47 (s, 1H, -C≡CH), 1.97 (m, 1H, 3b-CH_2), 1.83 (m, 2H, 4-CH_2), 1.47 (s, 3H, 2-CH_3, 1,40 (m, 1H, 3a-CH_2), 1.33 (s, 3H, 5-CH_3).

Cis-2-benzyl-5-ethynyl-2,5-dimethyl-pyrrolidine-1-yloxyl Radical (cis-3). 730 mg (16 %); oil; R_f = 0.57 (hexane-EtOAc 2:1); Anal. calcd. for $C_{15}H_{18}NO$ (228.31): C 78.91 %, H 7.95 %, N 6.13 %; found: C 78.72 %, H 7.89 %, N 6.24 %. MS (EI) m/z (rel. int. %): 228 (M^+, 12), 183 (5), 132 (18), 117 (59), 91 (100). 1H NMR ($CDCl_3$ + diphenyl-hydrazine) δ (ppm): 3.24 (d, *J*=12.3 Hz, 1H, Ph-CH_2), 3.02 (d, *J*=12.3 Hz, 1H, Ph-CH_2), 2.53 (s, 1H, -C≡CH), 2.24 (m, 1H, 3b-CH_2), 2.12 (m, 1H, 4b-CH_2), 1.82 (m, 1H, 3a-CH_2), 1.56 (s, 3H, 2-CH_3), 1.36 (m, 1H, 4a-CH_2), 1.19 (s, 3H, 5-CH_3). To determine the orientation of the different groups in the molecule the NOESY experiment was used. The cross peaks within H (3a):2-CH_3 and 2-CH_3:5-CH_3 suggest, that these groups are on the same side of the pyrrolidine ring.



***trans*-2-Benzyl-6-ethynyl-2,6-dimethyl-piperidine-1-yloxyl radical (trans-8**). 2.66 g (55 %); thick oil; R_f = 0.66 (hexane-EtOAc 2:1); Anal. calcd. for $C_{16}H_{20}NO$ (242.34): C 79.30 %, H 8.32 %, N 5.78 %; found: C 79.27 %, H 8.50 %, N 5.77 %. MS (EI) m/z (rel. int. %): 242 (M^+, 26), 152 (9), 117 (22), 91 (100).

***cis*-2-Benzyl-6-ethynyl-2,6-dimethyl-piperidine-1-yloxyl radical (cis-8).** 485 mg (10 %); oil; R_f = 0.62 (hexane-EtOAc 2:1); Anal. calcd. for $C_{16}H_{20}NO$ (242.34): C 79.30 %, H 8.32 %, N 5.78 %; found: C 79.36 %, H 8.17 %, N 5.63 %. MS (EI) m/z (rel. int. %): 242 (M^+, 35), 152 (51), 117 (39), 91 (100).

***trans*-5-Ethynyl-2,2,5-trimethyl-3-phenyl-pyrrolidine-1-yloxyl radical (trans-12).** 2.74 g (60 %); mp 82-84 °C; R_f = 0.55 (hexane-EtOAc 2:1); Anal. calcd. for $C_{15}H_{18}NO$ (228.31): C 78.91 %, H 7.95 %, N 6.13 %; found: C 78.89 %, H 8.01 %, N 6.10 %. MS (EI) m/z (rel. int. %): 228 (M^+, 3), 183 (6), 132 (61), 117 (100), 91 (72).

***cis*-5-Ethynyl-2,2,5-trimethyl-3-phenyl-pyrrolidine-1-yloxyl Radical (cis-12).** 502 mg (11 %); mp 140-141 °C; R_f = 0.51 (hexane-EtOAc 2:1); Anal. calcd. for $C_{15}H_{18}NO$ (228.31): C 78.91 %, H 7.95 %, N 6.13 %; found: C 78.77 %, H 7.81 %, N 5.96 %. MS (EI) m/z (rel. int. %): 228 (M^+, 4), 183 (5), 132 (61), 117 (100), 91 (73).

Synthesis of 5-(3-hydroxy-prop-1-ynyl)-2,2,5-trimethyl-3-phenyl-pyrrolidin-1-yloxyl radical (17)

To a stirred solution of propargyl magnesium bromide (0.022 mol) (prepared from propargyl alcohol and 2 equiv. of ethyl magnesium bromide) in THF (50 mL) was added dropwise a solution of nitrone **11** (4.06 g, 0.02 mol) in THF (20 mL) at 0 °C. After stirring the mixture at r.t. for 12 h sat. aq. NH_4Cl (50 mL) was added. The organic phase was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in $CHCl_3$ (20 mL), activated MnO_2 (cat. 100 mg) was added and O_2

was bubbled for 1 h at r.t. The mixture was filtered, evaporated and purified by flash column chromatography using hexane/EtOAc to give nitroxide **17**, 2.99 g (58 %); oil; R_f = 0.19 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for $C_{16}H_{20}NO_2$ (258.34): C 74.39 %, H 7.80 %, N 5.42 %; found: C 74.28 %, H 7.85 %, N 5.36 %. MS (EI) m/z (rel. int. %): 258 (M^+, 4), 243 (15), 131 (17), 124 (57), 42 (100).

Synthesis of 5-(3-bromo-prop-1-ynyl)-2,2,5-trimethyl-3-phenyl-pyrrolidin-1-yloxyl radical (18)

To a stirred solution of nitroxide propargyl alcohol **17** (2.58 g, 0.01 mol) and Et_3N (1.11 g, 1.10 mol) in anhyd. CH_2Cl_2 (40 mL) CH_3SO_2Cl (1.26 g, 1.10 mol) was added dropwise at 0 °C. The reaction mixture was stirred at r.t. for 30 min then the solvent was evaporated. The residue was dissolved in acetone (20 mL), LiBr (1.74 g, 0.02 mol) was added and refluxed for 30 min. Acetone was then evaporated, the residue was dissolved in Et_2O, washed with sat. aq. NaCl, dried ($MgSO_4$) and evaporated. Propargyl bromide **18** was purified by flash column chromatography using hexane/Et_2O to yield: 2.66 g (83 %); mp 61-63 °C; R_f = 0.78 (hexane-EtOAc 2:1); Anal. calcd. for $C_{16}H_{19}BrNO$ (321.23): C 59.82 %, H 5.96 %, N 4.36 %, Br 24.87 %; found: C 59.88 %, H 5.89 %, N 4.23 %, Br 24.72 %. MS (EI) m/z (rel. int. %): 322/320 (M^+, 16/16), 247/249 (15/15), 211 (32), 132 (100), 117 (95).

Synthesis of 2,2,5-trimethyl-3-phenyl-5-(3-pyrrolidin-1-yl-prop-1-ynyl)-pyrrolidin-1-yloxyl radical (19)

To a solution of propargyl bromide **18** (2.57 g, 8.0 mmol) in dry THF (30 mL) pyrrolidine (1.14 g, 16 mmol) was added and the reaction mixture was boiled for 1 h. The organic phase was washed with aq. sat. NaCl, dried and purified by flash column chromatography with $CHCl_3$/Et_2O to yield pyrrolidine **19,** 2.19 g (88 %); oil; R_f = 0.19 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for $C_{20}H_{27}N_2O$ (311.44): C 77.13 %, H 8.74 %, N 8.99 %; found: C 77.28 %, H 8.92 %, N 9.04 %. MS (EI) m/z (rel. int. %): 311 (M^+, 33), 296 (10), 108 (98), 84 (100).

Synthesis of 1-[3-(1-oxyl-2,5,5-trimethyl-4-phenyl-pyrrolidin-2-yl)-prop-2-ynyl]-pyrrolidin-2-one radical (20)

To a suspension of NaH (72 mg, 3 mmol) in dry THF (10 mL) DMF (3 mL) and 2-pyrrolidinone (255 mg, 3 mmol) were added. The reaction mixture was stirred for 30 min then bromide **18** (963 mg, 3 mmol) was added dropwise in dry THF (10 mL). After stirring for 2 h, sat. aq. NaCl was added (10 mL). The organic phase was separated, dried ($MgSO_4$) and evaporated. The resulting product was purified by flash chromatography to give **20** as yellow crystals: 722 mg (74 %); mp: 110-112 °C; R_f = 0.21 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for $C_{20}H_{25}N_2O_2$ (325.43): C 73.82 %, H 7.74 %, N 8.61 %; found: C 73.84 %, H 7.67 %, N 8.52 %. MS (EI) m/z (rel. int. %): 325 (M^+, 20), 311 (11), 280 (16), 210 (17), 195 (30), 164 (57), 132 (100), 98 (78), 91 (58).

Reduction of nitroxides (3, 8, 12, 19, 20) to sterically hindered secondary amines (4, 9, 13, 21, 22); General procedure exemplified by trans-2-benzyl-5-ethynyl-2,5-dimethyl-pyrrolidine (4)

To a solution of nitroxide **3** (1.14 g, 5 mmol) in AcOH (20 mL) Fe powder (1.40 g, 25 mmol) was added, the mixture was warmed up to 60 °C and kept at this temperature for 1 h. Then the reaction mixture was cooled to r.t., water (30 mL) was added and the solution was made alkaline with solid K_2CO_3. The aqueous phase was extracted with $CHCl_3$ (3 x 20 mL). The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by flash column chromatography with $CHCl_3$/MeOH to give amine.

***trans*-2-Benzyl-5-ethynyl-2,5-dimethyl-pyrrolidine (4).** 725 mg (68 %); mp (HCl salt) 218-220 °C; R_f = 0.61 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for HCl salt $C_{15}H_{20}NCl$ (249.78): C 72.13 %, H 8.07 %, N 5.61 %, Cl 14.19 %; found: C 72.30 %, H 8.21 %, N 5.73 %, Cl 14.25 %. MS (EI) m/z (rel. int. %): 213 (M^+, 28), 198 (1), 122 (100), 91 (93).

***trans*-2-Benzyl-6-ethynyl-2,6-dimethyl-piperidine (9).** 648 mg (57 %); mp (HCl salt) 225-227 °C; R_f = 0.52 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for HCl salt $C_{16}H_{22}NCl$ (263.81): C 72.85 %, H 8.41 %, N 5.31 %, Cl 13.44 %; found: C 72.76 %, H 8.53 %, N 5.39 %, Cl 13.60 %. MS (EI) m/z (rel. int. %): 227 (M^+, 1), 212 (21), 136 (100), 91 (98), 77 (77), 42 (98).

***trans*-5-ethynyl-2,2,5-trimethyl-3-phenyl-pirrolidine (13).** 661 mg (62 %); mp (HCl salt) sublimates over 220 °C; R_f = 0.55 ($CHCl_3$-Et_2O-MeOH 4:1.5:0.5); Anal. calcd. for HCl salt $C_{15}H_{20}NCl$ (249.78): C 72.13 %, H 8.07 %, N 5.61 %, Cl 14.19 %; found: C 72.15 %, H 7.96 %, N 5.65 %, Cl 14.22 %. MS (EI) m/z (rel. int. %): 213 (M^+, 15), 198 (20), 141 (31), 109 (100), 94 (65), 42 (28).

2,2,5-Trimethyl-3-phenyl-5-(3-pyrrolidin-1-yl-prop-1-ynyl)-pyrrolidine (21). 786 mg (53 %); mp 140-142 °C; R_f = 0.11 ($CHCl_3$-Et_2O-MeOH 4:1.5:0.5); Anal. calcd. for $C_{20}H_{28}N_2$ (296.45): C 81.03 %, H 9.52 %, N 9.45 %; found: C 81.02 %, H 9.55 %, N 9.58 %. MS (EI) m/z (rel. int. %): 296 (M^+, 4), 281 (3), 227 (19), 121 (68), 91 (22), 81 (36), 69 (100).

1-[3-(2,5,5-Trimethyl-4-phenyl-pyrrolidin-2-yl)-prop-2-ynyl]-pyrrolidin-2-one (22). 1.102 g (71 %); oil; R_f = 0.21 ($CHCl_3$-Et_2O-MeOH 4:1.5:0.5); Anal. calcd. for $C_{20}H_{26}N_2O$ (310.44): C 77.38 %, H 8.44 %, N 9.02 %; found: C 77.49 %, H 8.41 %, N 9.08 %. MS (EI) m/z (rel. int. %): 310 (M^+, 1), 295 (2), 206 (13), 98 (58), 42 (100).

Alkylation of secondary amines (4, 9, 13) to *N*-methyl derivatives (5, 10, 14). General procedure exemplified by trans-2-benzyl-5-ethynyl-1,2,5-trimethyl-pyrrolidine (5)

To a solution of amine **4** (213 mg, 1 mmol) in THF (15 mL) MeI (142 mg, 1 mmol) was added and the reaction mixture was refluxed for 1 h. The organic phase was extracted with 10 % K_2CO_3 solution, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography with $CHCl_3$/Et_2O as eluent to give *N*-methyl derivative.

***trans*-2-Benzyl-5-ethynyl-1,2,5-trimethyl-pyrrolidine (5).** 188 mg (83 %); mp (HCl salt) sublimates over 185 °C; R_f = 0.85 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for HCl salt $C_{16}H_{22}NCl$

(263.81): C 72.85 %, H 8.41 %, N 5.31 %, Cl 13.44 %; found: C 72.77 %, H 8.47 %, N 5.20 %, Cl 13.49 %. MS (EI) m/z (rel. int. %): 227 (M^+, 28), 212 (2), 202 (2), 136 (100), 91 (93).

***trans*-2-Benzyl-6-ethynyl-1,2,6-trimethyl-piperidine (10).** 157 mg (65 %); mp (HCl salt) sublimates over 195 °C; R_f = 0.46 (hexane-EtOAc 2:1); Anal. calcd. for HCl salt $C_{17}H_{24}NCl$ (277.84): C 73.49 %, H 8.71 %, N 5.04 %, Cl 12.76 %; found: C 73.55 %, H 8.62%, N 4.97 %, Cl 12.61 %. MS (EI) m/z (rel. int. %): MS (EI) m/z (rel. int. %): 241 (M^+, 13), 226 (8), 148 (100), 91 (82).

***trans*-2-Ethynyl-1,2,5,5-tetramethyl-4-phenyl-pyrrolidine (14).** 163 mg (72 %); mp (HCl salt) 202-204 °C; R_f = 0.71 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for HCl salt $C_{16}H_{22}NCl$ (263.81): C 72.85 %, H 8.41 %, N 5.31 %, Cl 13.44 %; found: C 72.85 %, H 8.31 %, N 5.27 %, Cl 13.28 %. MS (EI) m/z (rel. int. %): 227 (M^+, 7), 212 (100), 186 (17), 108 (11), 91 (7), 56 (19).

Oxidation of tertiary amines (14, 15) to nitroxides (12, 16)

To a solution of tertiary amine **14** (227 mg, 1 mmol) or **15** (229 mg, 1 mmol) in MeOH (5 mL) H_2O_2 (30 % sol. in water, 0.5 mL, 5 mmol) and Na_2WO_4 (cat, 10 mg) was added at r.t. The reaction mixture was allowed to stand for 24 h at this temperature. The solvent was evaporated, brine (5 mL) was added and the aqueous phase was extracted with $CHCl_3$ (3 x 5 mL). The organic phase was dried ($MgSO_4$), evaporated and purified with flash column chromatography to yield nitroxides **12** (96 mg, 42 %) and **16** (80 mg, 35 %). The physical and spectroscopic data of these compounds are identical described above.

Synthesis of 1,2,2,5-tetramethyl-3-phenyl-5-(3-pyrrolidin-1-yl-prop-1-ynyl)-pyrrolidine (23) and 1,2,2,5-tetramethyl-3-phenyl-5-(3-pyrrolidin-1-yl-prop-1-ynyl)-pyrrolidin-2-one (24)

A mixture of the free base of **21** (296 mg, 1 mmol) or **22** (310 mg, 1 mmol) and 10-fold excess each of 37 % formalin and 88 % formic acid was heated at reflux for 6 h. After cooling to room temperature, water (5 mL) was added and the aqueous phase was basified with solid K_2CO_3 and extracted with $CHCl_3$ (3 x 10 mL). The organic phase was dried ($MgSO_4$) and concentrated under vacuum to give *N*-methyl derivatives.

1-[3-(1,2,5,5-Tetramethyl-4-phenyl-pyrrolidin-2-yl)-prop-2-ynyl]-pyrrolidine (23). 242 mg (78 %); oil; R_f = 0.28 ($CHCl_3$-Et_2O-MeOH 4:1.5:0.5); Anal. calcd. for $C_{21}H_{30}N_2$ (310.48): C 81.24 %, H 9.74 %, N 9.02 %; found: C 81.17 %, H 9.82 %, N 8.96 %. MS (EI) m/z (rel. int. %): 310 (M^+, 5), 295 (92), 226 (50), 91 (69), 56 (100).

1-[3-(1,2,5,5-Tetramethyl-4-phenyl-pyrrolidin-2-yl)-prop-2-ynyl]-pyrrolidin-2-one (24). 223 mg (69 %); oil; R_f = 0.23 ($CHCl_3$-Et_2O 2:1); Anal. calcd. for $C_{21}H_{28}N_2O$ (324.47): C 77.74 %, H 8.70 %, N 8.63 %; found: C 77.63 %, H 8.85 %, N 8.53 %. MS (EI) m/z (rel. int. %): 324 (M^+, 2), 309 (81), 224 (34), 83 (100).

Acknowledgements

This work was supported by a grant from the Hungarian National Research Fund (OTKA K81123 and M045190). The authors thank Krisztina Kish for the elemental analysis, to Viola H. Csokona and Noémi Lazsányi for technical assistance.

References

1. Jenner, P.; Orlanow, C.W., Pathological Evidence for Oxidative Stress in Parkinson's Disease and Related Degenerative Disorders, In *Neurodegeneration and Neuroprotection in Parkinson's Disease*, C. W. Orlanow, P. Jenner and M. Youdim, Eds., Academic Press, New York, 1996, pp 23-45.
2. Ehringer, H.; Hornykiewicz, O., *Klin. Wochenschr*. **1960**, *38*, 1236.
3. Kish, S. J.; Shannak, K.; Hornykiewicz, O., *N. Engl. J. Med.* **1988**, *318*, 876.
4. Vernier, V. G. Antiparkinsonism Drugs In *Burger's Medicinal Chemistry and Drug Discovery*, Wolff, M. E. Ed. Wiley: New York, 1996, Vol. 3, Ch. 37, pp. 43-93.
5. Ebadi, M.; Sharma, S.; Shavali, S., El Refaey, H. *J. Neuoscience Res.* **2002**, *67*, 285.
6. Mandel, S.; Grunblatt, E.; Riederer, P.; Gerlach, M.; Levites, Y.; Youdim, M. B. H., *CNS Drugs* **2003**, *17*, 729.
7. Gulyás, B.; Pavlova, E.; Kása, P.; Gulya, K.; Bakota, L.; Várszegi, Sz.; Keller, É.; Horváth, M. Cs.; Nag, S.; Hermecz, I.; Magyar, K.; Halldin, C. *Neurochem. Int*. **2011**, *58*, 60.
8. Magyar, K.; Pálfi, M.; Tábi, T.; Kalász, H.; Szende, B.; Szökö, E. *Curr. Med. Chem.* **2004**, *11*, 2017.
9. Carrillo, M. C.; Minami, C.; Kitani, K.; Maruyama, W.; Ohashi, K.; Yamamoto, T.; Naoi, M.; Kanai, S.; Youdim, M. B. H. *Life Sci*. **2000**, *67*, 577.
10. Gerlach, M.; Riederer, P. Youdim, M. B. H., *Eur. J. Pharm. Mol. Pharm*. **1992**, *226*, 97.
11. De Marchi, U.; Pietrangeli, P.; Marcocci, L.; Mondovi, B.; Toninello, A. *Biochem. Pharmacol*. **2003**, *66*, 1749.
12. Binda, C.; Hubálek, F.; Li, M.; Herzig, Y.; Sterling, J.; Edmondson, D. E.; Mattevi, A. *J. Med. Chem.* **2004**, *47*, 1767.
13. Namiecinski, M.; Pulaski, L.; Kochman, A.; Skolimowski, J.; Bartosz, G.; Metodiewa, D. *in vivo*, **2004**, *18*, 171.
14. Kochman, A.; Skolimowski, J.; Gebicka, L.; Metodiewa, D. *Pol. J. Pharmacol*. **2003**, *55*, 389.
15. Halmosi, R.; Deres, P.; Berente, Z.; Kálai, T.; Sümegi, B.; Hideg, K.; Tóth, K. *J. Cardiovasc. Pharm.* **2002,** *40*, 854.
16. Deres, P.; Halmosi, R.; Tóth, A.; Kovács, K.; Pálfi, A.; Habon, T.; Czopf, L.; Kálai, T.; Hideg, K.; Sümegi, B.; Tóth, K. *J. Cardiovasc. Pharmacol*. **2005**, *45*(1), 36.

17. Kálai, T.; Várbíró, G.; Bognár, Z.; Pálfi, A.; Hantó, K.; Bognár, B.; Ősz, E.; Sümegi, B.; Hideg, K. *Bioorg. Med. Chem.* **2005**, *13*, 2629.
18. Kutala, V. K.; Khan, M.; Mandal, R.; Ganesan, L. P.; Tridandapani, S.; Kálai, T.; Hideg, K.; Kuppusamy, P. *J. Pharm. Exp. Ther.* **2006,** *317*, 921.
19. Kálai, T.; Khan, M.; Balog, M.; Kutala, K. V.; Kuppusamy, P.; Hideg, K. *Bioorg. Med. Chem.* **2006,** *14*, 5510.
20. Cannon, J. G. Cholinergics In *Burger's Medicinal Chemistry and Drug Discovery*, Wolff, M.E. Ed. Wiley: New York, 1996, Vol. 3, Ch. 45, pp. 28-36.
21. Garvey, D. S.; Wasicak, J. T.; Chung, J. Y.-L.; Shue, Y.-K.; Carrera, G. M.; May, P. D.; McKinney, M. M.; Anderson, D.; Cadman, E.; Vella-Rountree, L.; Nadzan, A.M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 1550.
22. Chung, J. Y. L.; Wasicak, J. T. *Tetrahedron Lett.* **1990**, *31*, 3957.
23. Pretorius, A.; Ogunrombi, M. O.; Terre'Blanche, G.; Castagnoli Jr., N.; Bergh, J. J.; Petzer, J. P. *Bioorg. Med. Chem*. **2008**, *16*, 8813.
24. Grimm, M. L.; Allen, W. J.; Finn, M.; Castagnoli Jr., N.; Tanko, J. M. *Bioorg. Med. Chem*. **2011**, *19*, 1458.
25. Adams Jr., J. D. Agents Used in Neurodegenerative Disorders In *Burger's Medicinal Chemistry and Drug Discovery*, Wolff, M. E. Ed. Wiley, New York, 1996, Vol. 3, Ch. 40, pp. 261-319.
26. Beckett, A. H.; Casy, A. F.; Lingard, R. G.; Iorio, M. A. Hewitson, K. *Tetrahedron* **1966**, *22*, 2735.
27. Pavlikov, V. V.; Shapiro, A. B.; Rozantsev, E. G.. *Doklady Akedemii Nauk SSSR* **1978**, *242(2)*, 369.; *Chem. Abstr*. **1979**, *90*, 72541.

