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

Cecília P. Sár, a Tamás Kálai, a József Jekő, b 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.

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-1H-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
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.\textsuperscript{13,14} It is important to have a radical scavenger \textit{in statu nascendi} to prevent damages caused by ROS, because the MAO-B mediated metabolism of dopamine and its autooxidation generate O$_2^-$, H$_2$O$_2$ and the highly toxic 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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{23,24} MPP$^+$ is a mitochondrial toxin, which selectively damages nigrostriatal neurons and induces Parkinsonian syndrome in humans.\textsuperscript{25} Several other allylamines act as good MAO-B substrates, such as the 5-membered ring analogue of MPTP.\textsuperscript{23} (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).

Treatment of 2,5-dimethyl-3,4-dihydro-2H-pyrrole 1-oxide (1) or 2,6-dimethyl-2,3,4,5-tetrahydro-pyridine 1-oxide (6) with benzylmagnesium chloride in dry Et₂O followed by the oxidation of N-hydroxy compound with activated MnO₂ 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 CDCl₃.

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 detoxifying ROS during non-toxic, stable nitroxide free radical formation.

Scheme 1 Reagents and conditions: (a) Benzylmagnesium chloride (1.2 equiv.), Et₂O, 0 °C → r.t., 3 h, aq. sat. NH₄Cl, then CHCl₃, MnO₂ (cat.), O₂, r.t., 1 h (62-74 %). (b) HC≡CMgBr, THF, 0 °C → r.t., 2 h, aq. sat. NH₄Cl, then CHCl₃, MnO₂ (cat.), O₂, r.t., 30 min (65-77 %). (c) AcOH, Fe powder (5 equiv.), 60 °C, 1 h, then H₂O, K₂CO₃ (57-68 %). (d) MeI, THF, reflux, 1 h (65-83 %). (e) H₂O₂ (2 equiv), Na₂WO₄ (cat.), MeOH, r.t., 24 h (35-42 %)

The oxidation of both 5- and 6-membered sterically hindered tertiary amines was investigated in MeOH with H₂O₂. However, in case of compound 14 or model compound 15 upon oxidation nitroxides 12 and 16 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 MnO₂ 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 amines of compounds 21 and 22 the N-methylation of secondary amines by refluxing with formaldehyde in the presence of formic acid, (Eschweiler-Clark conditions)\(^1\) furnished the tertiary pyrrolidine derivatives 23 and 24 (Scheme 2).

![Scheme 2](image.png)

**Scheme 2** Reagents and conditions: (a) BrMgC≡C-CH₂-OMgBr (1.1 equiv.), THF, 0 °C → r.t., 12 h, aq. sat. NH₄Cl, then CHCl₃, MnO₂ (cat.), O₂, r.t., 1 h (58 %). (b) Et₃N, CH₂Cl₂, CH₃SO₂Cl, 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 H₂O, K₂CO₃ (53-71 %). (e) HCHO (37 %, 10 equiv.), HCOOH (88 %, 10 equiv.), reflux, 6 h, then K₂CO₃ (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

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. $^1$H NMR spectra were recorded with Varian $^\text{UNITY INOVA}$ 400 WB spectrometer. Chemical shifts are referenced to Me$_4$Si. 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 triplet 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-2H-pyrrrole 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$_2$O (50 mL) nitrone (1) (5.65 g, 0.05 mol) in dry Et$_2$O (50 mL) was added dropwise at 0 °C. The reaction mixture was stirred at r.t. for 3 h, then aq. sat. NH$_4$Cl (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-pyrrrole 1-oxide (2): 7.51 g (74 %); oil; $R_f$ = 0.35 (CHCl$_3$-Et$_2$O-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$_2$O-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-yloxy 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$_4$Cl (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 O2 for 30 min at r.t. The reaction mixture was then filtered, evaporated and purified by flash column chromatography with hexane/Et2O 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; Rf = 0.64 (hexane-EtOAc 2:1); Anal. calcd. for C15H18NO (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 (CDCl3 + diphenyl-hydrazine) δ (ppm): 2.78 (d, J=12.5 Hz, 1H, Ph-CH2), 2.70 (d, J=12.5 Hz, 1H, Ph-CH2), 2.47 (s, 1H, -C≡CH), 1.97 (m, 1H, 3b-CH2), 1.83 (m, 2H, 4-CH2), 1.47 (s, 3H, 2-CH3), 1.40 (m, 1H, 3a-CH2), 1.33 (s, 3H, 5-CH3).

**Trans-2-benzyl-6-ethynyl-2,6-dimethyl-piperidine-1-yloxyl Radical (trans-8):** 2.66 g (55 %); thick oil; Rf = 0.66 (hexane-EtOAc 2:1); Anal. calcd. for C16H20NO (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-5-ethynyl-2,5-dimethyl-pyrrolidine-1-yloxyl Radical (cis-3):** 730 mg (16 %); oil; Rf = 0.57 (hexane-EtOAc 2:1); Anal. calcd. for C15H18NO (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 (CDCl3 + diphenyl-hydrazine) δ (ppm): 3.24 (d, J=12.3 Hz, 1H, Ph-CH2), 3.02 (d, J=12.3 Hz, 1H, Ph-CH2), 2.53 (s, 1H, -C≡CH), 2.24 (m, 1H, 3b-CH2), 2.12 (m, 1H, 4b-CH2), 1.82 (m, 1H, 3a-CH2), 1.56 (s, 3H, 2-CH3), 1.36 (m, 1H, 4a-CH2), 1.19 (s, 3H, 5-CH3). To determine the orientation of the different groups in the molecule the NOESY experiment was used. The cross peaks within H (3a):2-CH3 and 2-CH2:5-CH3 suggest, that these groups are on the same side of the pyrrolidine ring.

**Cis-2-benzyl-6-ethynyl-2,6-dimethyl-piperidine-1-yloxyl Radical (cis-8):** 485 mg (10 %); oil; Rf = 0.62 (hexane-EtOAc 2:1); Anal. calcd. for C16H20NO (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; Rf = 0.55 (hexane-EtOAc 2:1); Anal. calcd. for C15H18NO (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; Rf = 0.51 (hexane-EtOAc 2:1); Anal. calcd. for C15H18NO (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 nitron 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. NH₄Cl (50 mL) was added. The organic phase was separated, and the aqueous layer was extracted with CHCl₃. The combined organic layer was dried (MgSO₄) and evaporated. The residue was dissolved in CHCl₃ (20 mL), activated MnO₂ (cat. 100 mg) was added and O₂ 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; Rf = 0.19 (CHCl₃-Et₂O 2:1); Anal. calcd. for C₁₆H₂₀NO₂ (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 Et₃N (1.11 g, 1.10 mol) in anhyd. CH₂Cl₂ (40 mL) CH₃SO₂Cl (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 Et₂O, washed with sat. aq. NaCl, dried (MgSO₄) and evaporated. Propargyl bromide 18 was purified by flash column chromatography using hexane/EtOAc to yield: 2.66 g (83 %); mp 61-63 °C; Rf = 0.78 (hexane-EtOAc 2:1); Anal. calcd. for C₁₆H₁₉BrNO (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 CHCl₃/Et₂O to yield pyrrolidine 19, 2.19 g (88 %); oil; Rf = 0.19 (CHCl₃-Et₂O 2:1); Anal. calcd. for C₂₀H₂₇N₂O (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⁺, 16/16), 296 (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 (MgSO₄) and evaporated. The resulting product was purified by flash chromatography to give 20 as yellow crystals: 722 mg (74 %); mp: 110-112 °C; \( R_f = 0.21 \) (CHCl₃-Et₂O 2:1); Anal. calcd. for C₂₀H₂₅N₂O₂ (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 alkaline with solid K₂CO₃. The aqueous phase was extracted with CHCl₃ (3 x 20 mL). The organic layer was dried (MgSO₄) and evaporated. The residue was purified by flash column chromatography with CHCl₃/MeOH to give amine.

**Trans-2-benzyl-5-ethynyl-2,5-dimethyl-pyrrolidine (4):** 725 mg (68 %); mp (HCl salt) 218-220 °C; \( R_f = 0.61 \) (CHCl₃-Et₂O 2:1); Anal. calcd. for HCl salt C₁₅H₂₀NCl (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; \( R_f = 0.52 \) (CHCl₃-Et₂O 2:1); Anal. calcd. for HCl salt C₁₆H₂₂NCl (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-ethylpyrrolidin-3-phenyl-pyrrolidine (13):** 661 mg (62 %); mp (HCl salt) sublimates over 220 °C; \( R_f = 0.55 \) (CHCl₃-Et₂O-MeOH 4:1.5:0.5); Anal. calcd. for HCl salt C₁₅H₂₀NCl (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; \( R_f = 0.11 \) (CHCl₃-Et₂O-MeOH 4:1.5:0.5); Anal. calcd. for C₂₀H₂₈N₂ (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; \( R_f = 0.21 \) (CHCl₃-Et₂O-MeOH 4:1.5:0.5); Anal. calcd. for C₂₀H₂₈N₂O (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$_2$CO$_3$ solution, dried (MgSO$_4$) and evaporated. The residue was purified by flash chromatography with CHCl$_3$/Et$_2$O 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$_2$O 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$_2$O 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$_2$O$_2$ (30 % sol. in water, 0.5 mL, 5 mmol) and Na$_2$WO$_4$ (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$_2$CO$_3$ 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$_2$O-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; Rf = 0.23 (CHCl₃-Et₂O 2:1); Anal. calcd. for C₂₁H₂₈N₂O (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 (K81123 and M045190). The authors thank Krisztina Kish for the elemental analysis, to Viola H. Csokona and Noémi Lázsányi for technical assistance.

References