University of Pécs
Doctoral School of Chemistry

Synthesis of 1,4-diazine-fused and phosphorus containing stable nitroxide free radicals

PhD thesis

Mostafa Isbera

Supervisors:

Dr. Balázs Bognár **Prof. Tamás Kálai**

Head of the Doctoral School:

Prof. Ferenc Kilár

Pécs 2022

1. Introduction

Nitroxides belong to the class of heterocyclic compounds with typical structures of five- or six-membered heterocyclic derivatives of piperidine, pyrrolidine, imidazoline, imidazolidine just to mention but a few. Around hundred nitroxide scaffolds has been published till now. Until 1962, chemists adhered to the paradigm that the most chemically reactive portion of a radical can be a group bearing spin electron. This paradigm was broken by M. B. Neiman (1898-1967) and E. G. Rozantsev who introduced reactions with a nitroxide without direct involvement of the spin center. Later, more and more young enthusiasts joined the ranks of scientists applying this new tool in their research, and ever increasing reports of nitroxides were published. These pioneering works have laid a chemical basis for the method of numerous nitroxide applications, spin labeling in particular. Hungarian representatives of organic chemists were late Dr. Kálmán Hideg (1934-2018) and late Dr. Olga Hankovszky (1934-2020) who started nitroxide radical research in 1975 initiated by late Dr. József Tigyi (1926-2016). Their contribution was recognized internationally as well, so they organized the first international congress at Pécs University in 1979. Many further researchers accomplished their PhD work (László Lex, József Csekő, Ilona Bódi, Cecília Sár, Tamás Kálai, Balázs Bognár, Győző Kulcsár, Györgyi Úr) in nitroxide topic at this research group.

Meantime the possible applications of nitroxides has been widened from construction of organic ferromagnets to constructing batteries and EPR and MRI contrast agents, but conducting reactions selectively in the presence of nitroxide center is still a challenge. A newer tendency in nitroxide free radical synthesis is the purpose oriented synthesis, which is sometimes a time consuming and complicated problem. In our research group in the last decade the synthesis of carbocycle and heterocycle condensed stable nitroxide free radicals was a dominant tendency beyond the purpose oriented synthesis. I have joined this group in September of 2018 as a Stipendium Hungaricum Student of Chemical Doctoral School at University of Pécs. My topics were the synthesis of new phosphorus containing nitroxide free radicals supplemented by the synthesis of nitroxide condensed 1,4-diazines and imidazole as an unresolved problem.

2. Objectives

1. Syntheses of 1,4-diazine- and imidazole-fused pyrroline nitroxides.

2. Syntheses of new pyrroline and piperidine nitroxide phosphonates by the well-established methods, such as Pudovik and Arbuzov reactions and further transformations of the new products.

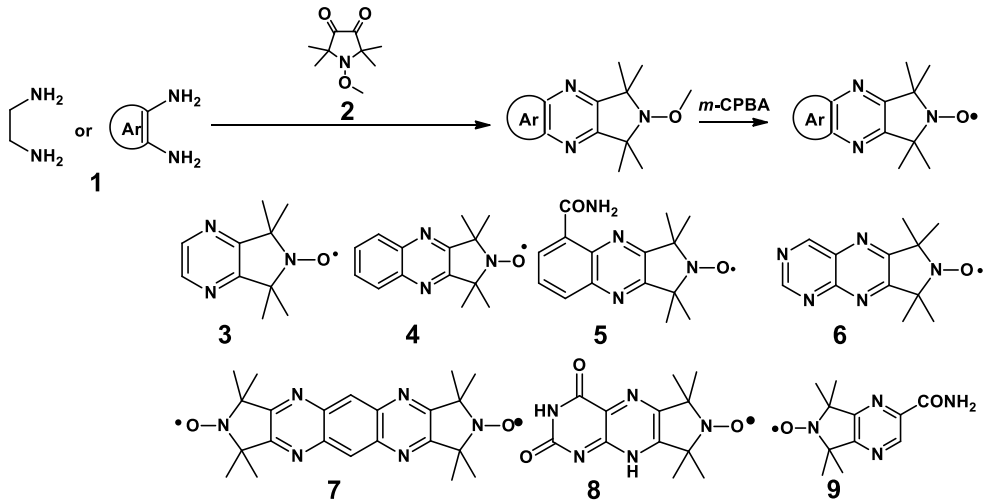
3. Syntheses of paramagnetic phospholene oxide, diphenylpyrroline phosphine and its phosphonum salt and investigation of its antineoplastic activity.

3. Experimental procedures

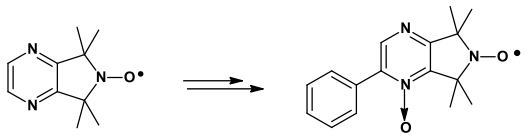
During our experiments we used macro- and half-micro methods of the modern preparative organic chemistry. Mass spectra were recorded with a Thermoquest Automass Multi system and a GCMS-2020, both operated in EI mode (70 eV) and a Thermo Q-Exactive HPLC/MS/MS with ESI(+) ionization. Elemental analyses were obtained with a Fisons EA 1110 CHNS elemental analyser. The melting points were determined with a Boetius micromelting point apparatus. The ^{1}H NMR spectra were recorded with a Bruker Avance 3 Ascend 500 system operated at 500 MHz, and the ^{13}C NMR spectra were obtained at 125 MHz and ^{31}P NMR 202 MHz in $CDCl_3$, CD_3OD or DMSO-d_6 at 298 K. The "in situ" reduction of the nitroxides was achieved by addition of five equivalents of hydrazobenzene (DPPH/radical). The EPR (electron paramagnetic resonance) spectra were recorded on MiniScope MS 200 instrument in $CHCl_3$ solution, and the concentrations were 1.0×10^{-4} M. The IR spectra were obtained using a Bruker Alpha FT-IR with ATR support on a diamond plate. Spectrophotometric measurements for antioxidant capacity measurement were performed on a Specord 40 UV/VIS Spectrophotometer at 732 nm in a 1 × 1 cm quartz cuvette. Hydrogenations were performed with a ThalesNano H-CubeVR Mini Plus reactor with 20% $Pd(OH)_2/C$ cartridge. Flash column chromatography was performed on a Kieselgel 60 (0.040 – 0.063 mm) column. Qualitative TLC was performed on commercially available plates (20 cm × 20 cm × 0.02 cm) coated with Merck Kieselgel GF254.

4. New Scientific findings

1. We developed a new diamagnetic synthon, 1-methoxy-2,2,5,5-tetramethylpyrrolidine-3,4-dione **2** which was condensed with different aliphatic, aromatic or heteroaromatic 1,2-diamines followed by deprotection of the nitroxide function with *m*-CPBA, providing pyrroline nitroxide fused pyrazines **3, 9**, pteridines **6, 8** or quinoxalines **4, 5, 7**.[7]

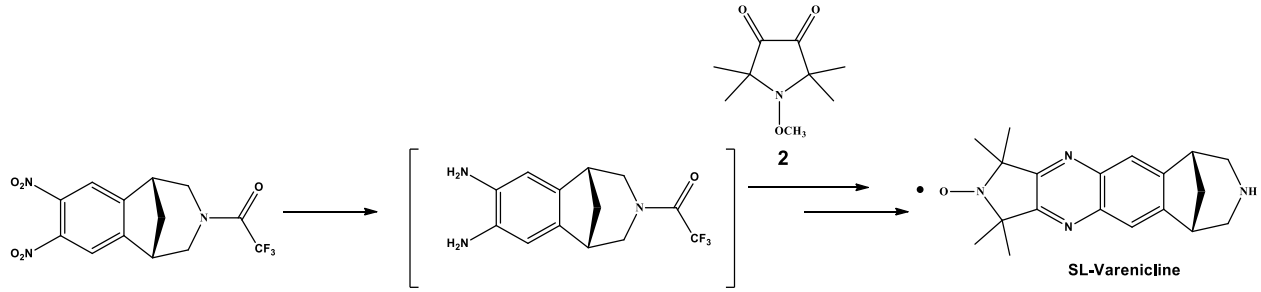


The oxidation of **3** resulted in the formation of *N*-oxide derivative, which offered the possibility of C-H functionalization with benzene as a solvent at C2 position by palladium catalysis and Ag_2CO_3 oxidation to give compound **10**.[7]



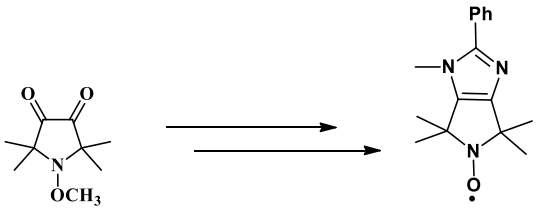
3 **10**

2. Reduction of dinitro compound **11** with $H_2/Pd(OH)_2$ furnished a diamino precursor of which condensation with 1,2-diketone **2** followed by the deprotection of *N*-OMe by *m*-CPBA, and the removal of the trifluoroacetyl group by Na_2CO_3/MeOH gave the spin labelled Varenicline **12**.[5]



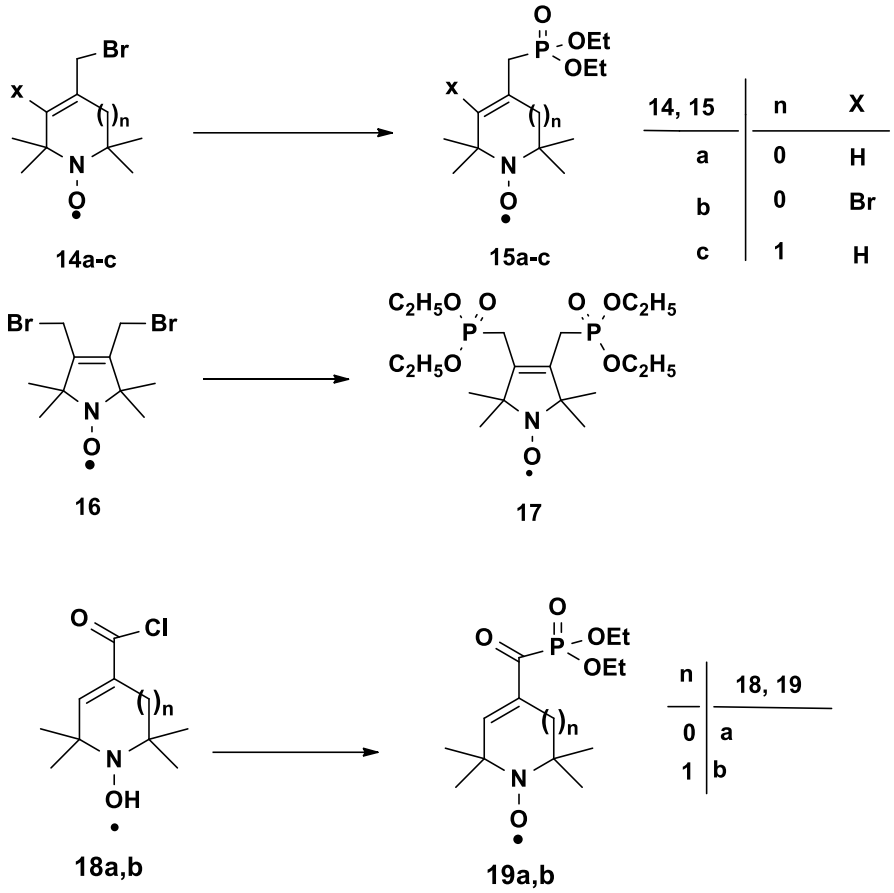
11 **2** **SL-Varenicline** **12**

3. The reaction of the diamagnetic 1,2-diketone with benzaldehyde and ammonium acetate produced a pyrrolo[3,4-d]imidazole scaffold (**13**) in the Debus-Radziszewski reaction. The *O*-methyl group deprotection required the protection (alkylation) of NH function of the imidazole.[7]

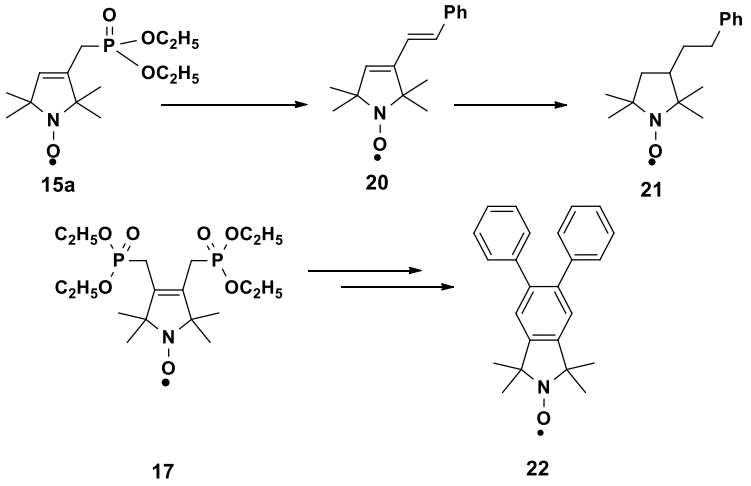


2 **13**

4. Treatment of five- and six-membered allylic bromides **14a-c**, dibromo compound **16,** acyl chlorides **18a,b** respectively with triethyl phosphite in an Arbuzov reaction resulted in the formation of allylic phosphonate esters **15a-c**, bisphosphonate ester **17,** α-ketophosphonate esters **19a,b**.[2,4,6]

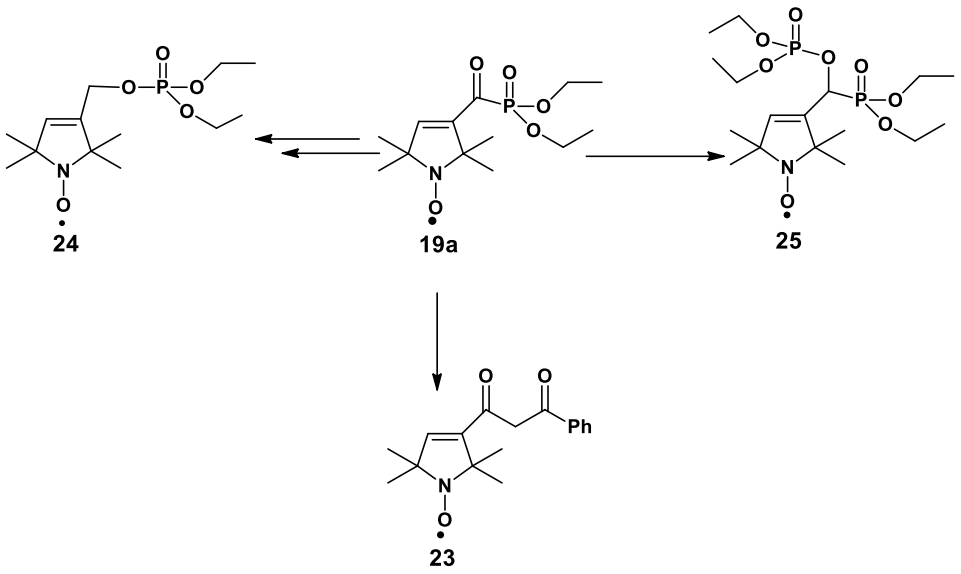


5. We utilized the allylic phosphonate ester **15a** in Horner-Wadsworth-Emmons (HWE) reaction through deprotonation and treatment with aldehydes yielding *E*-alkenes. The reduction of the resulted C=C bond could be achieved using continuous flow hydrogenation system. Bisphosphonate ester **17** was deprotonated and treated with excess of benzaldehyde. The formed triene electrocyclisation occurred spontaneously and oxidation with DDQ resulted isoindoline radical **22**. [6]

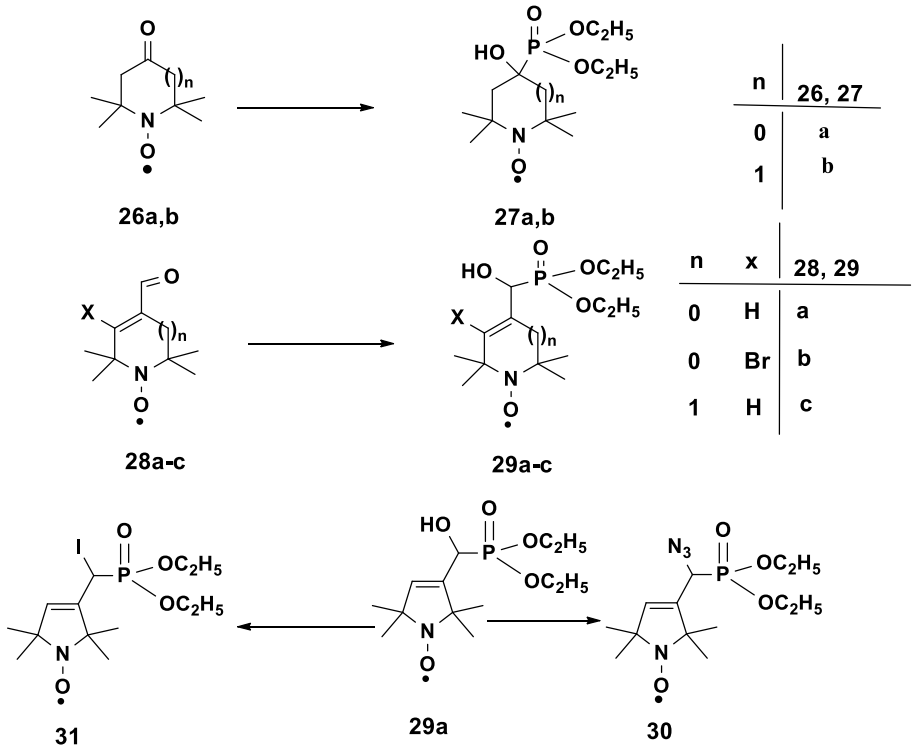


6. Compound **19a** was utilized as an acylation agent to give paramagnetic 1,3-dicarbonyl compound **23**. The reduction of **19a** to α-hydroxyphosphonate ester followed by a phospha-Brook

rearrangement furnished paramagnetic phosphate ester **24**. The nucleophilic addition of diethylphosphite to compound **19a** gave phosphate phosphonate ester **25**.[2, 4]

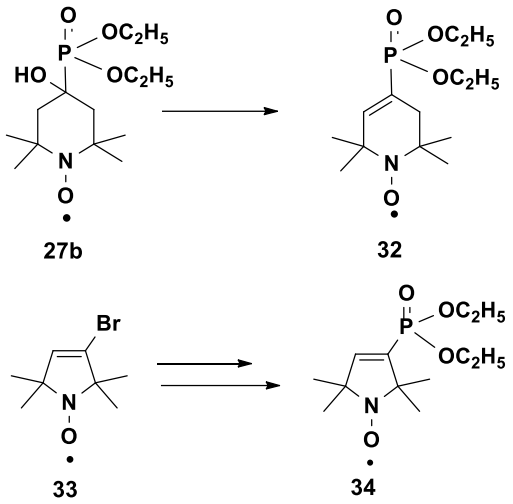


7. We have synthesized paramagnetic five- and six-membered α-hydroxyphosphonate esters **27a,b** and **29a-c** starting from paramagnetic five- and six-membered ketones **26a,b** and aldehydes **28a-c** by Pudovic reaction under solvent-free conditions. Compound **29a** was functionalized further with Mitsonubu reaction yielding compounds **30** and **31**.[2, 6]

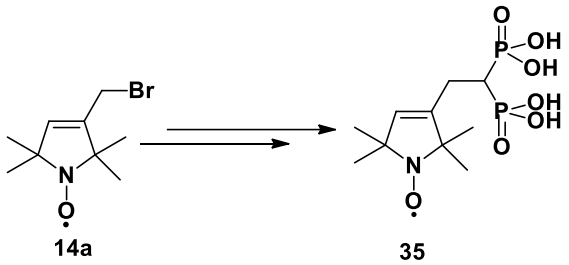


8. We have developed two methods for synthesizing of paramagnetic vinylphosphonates. Water elimination of compound **27b** yielded paramagnetic six-membered vinyl phosphonate ester **32**.

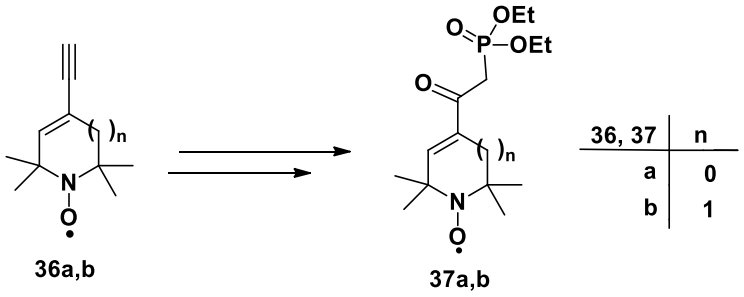
Paramagnetic five-membered vinylphosphonate ester **34** was obtained from compound **33** through a process including protection of nitroxide moiety as *O*-methyl by Fenton reaction, followed by lithiation, treatment with diethylchlorophosphate to give the diamagnetic vinyl phosphonate, and finally restoring of nitroxide moiety by *m*-CPBA oxidation.[6]



9. Geminal bisphosphonic acid **35** was accessed by alkylating tetraethyl methylenebisphosphonate with compound **14a**, followed by hydrolysis and restoring the nitroxide radical.[2, 4]

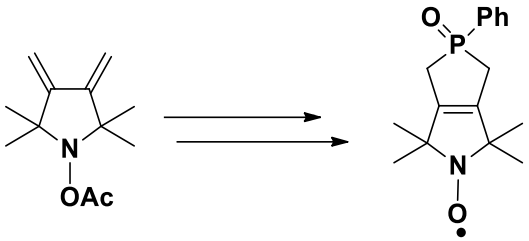


10. We utilized paramagnetic acetylenes **36a** and **36b** to get β-ketophosphonates **37a** and **37b**, through a process including deprotonation of terminal acetylene moiety, followed by acylation with diethylchlorophosphate. The ethynylphosphonate esters were hydrated in aqueous dioxane in the presence of 0.1 equiv. $PdCl_2$.[2, 4]



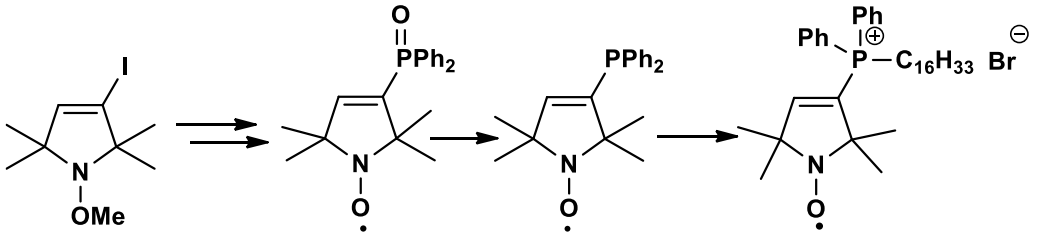
36, 37	n
a	0
b	1

11. The reaction of a diene nitroxide precursor **38** with dichlorophenyl phosphine in a McCormac procedure offered 2-oxyl-1,1,3,3-tetramethyl-5-phenyl-1,2,3,4,5,6-hexahydrophospholo[3,4-c]pyrrole-5-oxide **39** after deacetylation.[1,3]



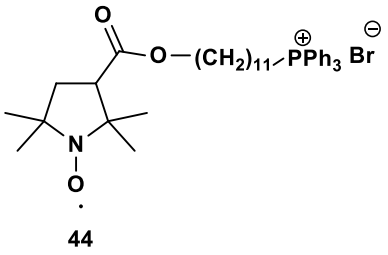
38 **39**

12. We achieved the paramagnetic phosphine derivative **42**, starting from a protected pyrroline nitroxide **40** which was lithiated, followed by treatment with diphenylchlorophosphine, and *m*-CPBA deprotection to give compound **41**. The reduction with Cl_3SiH and selective oxidation of **41** by PbO_2 offered compound **42**. The heating of compound **42** with hexadecyl bromide in acetonitrile offered the phosphonium salt **43**.[1,3]



40 **41** **42** **43**

Based on the similarity between the phosphonium salt **43** and MITO-CP (Mitochondria-targeted nitroxide) (Figure 1), we studied the cytostatic effect of compound **43** in comparison with MITO-CP **44** on MDA-MB-231 (Figure 2) and MCF-7 human breast cancer lines (Figure 3). Compound **43** had more pronounced cytotoxicity than MITO-CP at all studied concentrations.[8]



44

Figure 1. Structure of MITO-CP.

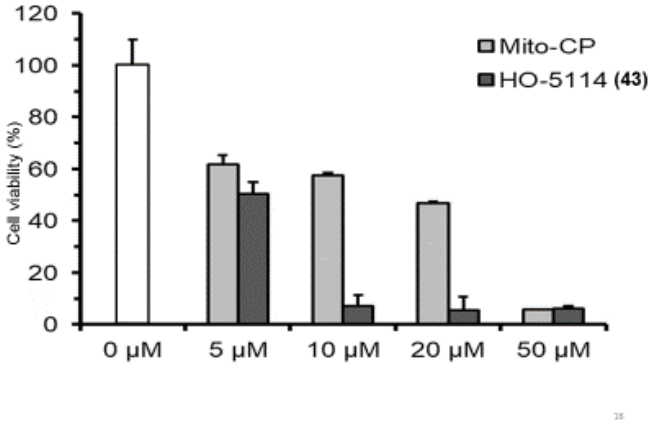


Figure 2. Cytostatic effect of compound **43** on MDA-MB-231 human breast cancer lines.

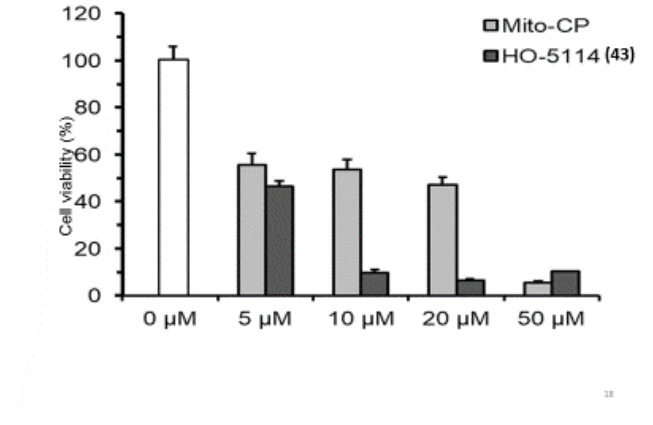


Figure 3. Cytostatic effect of compound **43** on MCF7 human breast cancer lines.

5. Publication List

Publications related to Ph.D dissertation

1. Syntheses and study of a pyrroline nitroxide condensed phospholene oxide and a pyrroline nitroxide with diphenylphosphino moiety.
   Isbera, M.; Bognár, B.; Gallyas, F.; Bényei, A.; Jekő, J.; Kálai, T.
   Phosphorus, Sulfur, and Silicon and the Related Elements **2022**, https://doi.org/10.1080/10426507.2021.1989690. (IF_{2020}: 1.082, Q4)

2. Syntheses and utilizations of pyrroline and tetrahydropyridine nitroxide-based phosphonate esters, a phosphate ester and a bisphosphonate.
   Kálai, K.; Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Hideg, K.
   Phosphorus, Sulfur, and Silicon and the Related Elements **2022**, https://doi.org/10.1080/10426507.2021.1989685. (IF_{2020}: 1.082, Q4)

3. Syntheses and Study of a Pyrroline Nitroxide Condensed Phospholene Oxide and a Pyrroline Nitroxide Attached Diphenylphosphine**.**

   Isbera, M.; Bognár, B.; Gallyas, F.; Bényei, A.; Jekő, J.; Kálai, T.
   Molecules **2021**, *26*, 4366. (IF_{2020}: 4.411, Q2)

4. Syntheses and utilizations of pyrroline-nitroxide and tetrahydropyridine-nitroxide- based α-ketophosphonates, β-ketophosphonates, and a bisphosphonate.

   Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Kálai, T.
   Synthetic Communications **2021**, *51*, 1353–1362. (IF_{2020}: 1.796, Q3)

5. Synthesis of a Nitroxide Spin-labeled Varenicline (Chantix) Derivative.
   Bognár, B.; Isbera, M.; Kálai, T.
   Organic Preparations and Procedures International **2021**, *53*, 311–315. (IF_{2020}: 1.628, Q4)

6. Syntheses and Reactions of Pyrroline, Piperidine Nitroxide Phosphonates.
   Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Hideg, k.; Kálai, T.
   Molecules **2020**, *25*, 2430. (IF: 4.411, Q2)

7. Syntheses of Pyrazine-, Quinoxaline-, and Imidazole-Fused Pyrroline Nitroxides.
   Isbera, M.; Bognár, B.; Gulyás Fekete, G.; Kish, K.; Kálai, T.
   Synthesis **2019**, 51, 4463-4472. (IF: 2.675, Q1)

Conference presentations related to Ph.D dissertation

1. *Syntheses and Studies of Phosphorus Containing Stable Nitroxide Radicals*. Mostafa Isbera, Attila Bényei, Ferenc Gallyas, Balázs Bognár, Cecília P. Sár, Kálmán Hideg, József Jekő, Tamás Kálai. Az MTA Heterociklusos és Elemorganikus Kémiai Munkabizottságának a Patonay Tamás-díj átadásával egybekötött nyílt ülésére. Budapest, Hungary. 3rd September, 2021(oral lecture).

2. *Synthesis of pyrazine-, quinoxaline- and imidazole-fused pyrroline nitroxides*. Mostafa Isbera, Balázs Bognár, Gergely Gulyás Fekete, Krisztina Kish, Tamás Kálai. Hterociklusos és Elemorganikus Munkabizottság előadóülése. Microsoft Teams Meeting. 31st May, 2021 online oral lecture)

3. *Syntheses and study of a pyrroline nitroxide condensed phospholene oxide and a pyrroline nitroxide attached diphenylphosphine.* Mostafa Isbera, Attila Bényei, Ferenc Gallyas, Balázs Bognár, József Jekő, Tamás Kálai. 23rd International Conference on Phosphorus Chemistry. Częstochowa, Poland. 5-9th July, 2021 (online poster).

4. *Syntheses and utilizations of pyrroline-nitroxide- and tetrahydropyridine-nitroxide-based phosphonates, α-ketophosphonates, β-ketophosphonates, and a bisphosphonate.* Tamás Kálai, Mostafa Isbera, Balázs Bognár, Cecília Sár, József Jekő, Kálmán Hideg. 23rd International Conference on Phosphorus Chemistry. Częstochowa, Poland. 5-9th July, 2021 (online poster).

5. *Syntheses of pyrazine, quinoxaline and imidazole fused pyrroline nitroxides.* Mostafa Isbera, Balázs Bognár, Gergely Gulyás-Fekete, Krisztina Kish, Tamás Kálai. A Magyar Szabadgyök-Kutató Társaság X. Kongresszusa. Szeged, Hungary. 29-30th August, 2019 (poster)

Publications not related to Ph.D dissertation

1. Cytostatic Effect of a Novel Mitochondria-Targeted Pyrroline Nitroxide in Human Breast Cancer Lines**.**
   Andreidesz, K.; Szabó, A.; Kovács, D.; Kőszegi, B.; Bagóné Vántus, V.; Vamos, E.; Isbera, M.; Kálai, T.; Bognár, Z.; Kovács, K.; Gallyas, F.
   International Journal of Molecular Sciences **2021**, *22*, 9016. (IF_{2020}: 5.923, Q1)

2. The effect of temperature and moisture on the physical and chemical stability of furosemide tablets (40 mg)marketed in Syria.
   Mansour, O.; Isbera, M.; Ismail, G.; Mayya, G.
   World Journal of Pharmaceutical Research ***2018***, 7, 35-44.

3. The Influence of Temperature and Moisture on the Physical and Chemical Properties of MetforminHydrochloride Tablets (850 mg) Marketed in Syria.
   Mansour, O.; Isbera, M.; Mayya, G.
   International Journal of Pharmacy and Pharmaceutical Research **2018**, 12, 29-39.

4. Evaluation of Physical and Chemical Properties of Paracetamol (500 mg) Tablets marketed in Syria.
   Mansour, O.; Isbera, M.; Mtawag, A.
   Journal of Chemical and Pharmaceutical Sciences **2017,** 10, 1306-1308. Q3

5. Development and validation UV spectrophotometric method for determination of atenolol in pure materialsand pharmaceutical dosage.
   Aboud, K.; Mohammad, A.; Isbera, M.; Beesh, M.
   Indo American Journal of Pharmaceutical Research **2017,** 7, 8179-8184.

6. Quality analysis of different marketed brands of atenolol (50 mg) available in Syria and comparing with thereference drug.
   Beesh, M.; Isbera, M.; Aboud, K.; Mohammad, A.
   World Journal of Pharmaceutical Research **2017,** 6, 61-68**.**

7. Quality control of warfarin sodium tablets marketed in Syria.
   Isbera, M.; Aboud, K.; Haroun, M.
   Research Journal of Pharmacy and Technology **2017**, 10, 2119-2121. Q3

8. Assessment of physicochemical properties of metformin hydrochloride (850 mg) tablets marketed in Syria.
   Mansour, O.; Isbera, M.
   Journal of Chemical and Pharmaceutical Sciences **2016**, 9, 726-729. Q4

9. Assessment of physicochemical properties of furosemide (40 mg) tablets marketed in Syria
   Mansour, O.; Ismail, G.; Isbera, M.; Almouhammad, M.
   Journal of Chemical and Pharmaceutical Sciences **2016**, 9, 2879-2881. Q4

10. Weight and content uniformity of warfarin sodium half tablets.
    Isbera, M.; Ibrahim, W.; Abbood, A.
    Research Journal of Pharmacy and Technology **2016**, 9, 215-218. Q4

11. Weight and content uniformity of furosemide half tablets.
    Isbera, M.; Ibrahim, W.; Abbood, A.
    Tishreen University Journal for Research and Scientific Studies- Health Sciences Series **2015**, 37, 169-181.

1. Introduction

Nitroxides belong to the class of heterocyclic compounds with typical structures of five- or six-membered heterocyclic derivatives of piperidine, pyrrolidine, imidazoline, imidazolidine just to mention but a few. Around hundred nitroxide scaffolds has been published till now. Until 1962, chemists adhered to the paradigm that the most chemically reactive portion of a radical can be a group bearing spin electron. This paradigm was broken by M. B. Neiman (1898-1967) and E. G. Rozantsev who introduced reactions with a nitroxide without direct involvement of the spin center. Later, more and more young enthusiasts joined the ranks of scientists applying this new tool in their research, and ever increasing reports of nitroxides were published. These pioneering works have laid a chemical basis for the method of numerous nitroxide applications, spin labeling in particular. Hungarian representatives of organic chemists were late Dr. Kálmán Hideg (1934-2018) and late Dr. Olga Hankovszky (1934-2020) who started nitroxide radical research in 1975 initiated by late Dr. József Tigyi (1926-2016). Their contribution was recognized internationally as well, so they organized the first international congress at Pécs University in 1979. Many further researchers accomplished their PhD work (László Lex, József Csekő, Ilona Bódi, Cecília Sár, Tamás Kálai, Balázs Bognár, Győző Kulcsár, Györgyi Úr) in nitroxide topic at this research group.

Meantime the possible applications of nitroxides has been widened from construction of organic ferromagnets to constructing batteries and EPR and MRI contrast agents, but conducting reactions selectively in the presence of nitroxide center is still a challenge. A newer tendency in nitroxide free radical synthesis is the purpose oriented synthesis, which is sometimes a time consuming and complicated problem. In our research group in the last decade the synthesis of carbocycle and heterocycle condensed stable nitroxide free radicals was a dominant tendency beyond the purpose oriented synthesis. I have joined this group in September of 2018 as a Stipendium Hungaricum Student of Chemical Doctoral School at University of Pécs. My topics were the synthesis of new phosphorus containing nitroxide free radicals supplemented by the synthesis of nitroxide condensed 1,4-diazines and imidazole as an unresolved problem.

2. Objectives

1. Syntheses of 1,4-diazine- and imidazole-fused pyrroline nitroxides.

2. Syntheses of new pyrroline and piperidine nitroxide phosphonates by the well-established methods, such as Pudovik and Arbuzov reactions and further transformations of the new products.

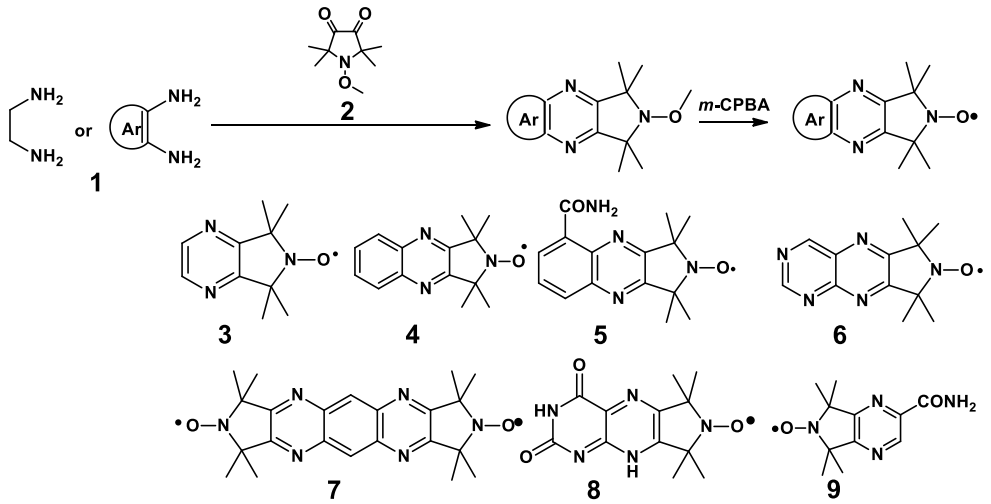
3. Syntheses of paramagnetic phospholene oxide, diphenylpyrroline phosphine and its phosphonum salt and investigation of its antineoplastic activity.

3. Experimental procedures

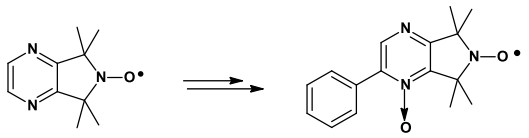
During our experiments we used macro- and half-micro methods of the modern preparative organic chemistry. Mass spectra were recorded with a Thermoquest Automass Multi system and a GCMS-2020, both operated in EI mode (70 eV) and a Thermo Q-Exactive HPLC/MS/MS with ESI(+) ionization. Elemental analyses were obtained with a Fisons EA 1110 CHNS elemental analyser. The melting points were determined with a Boetius micromelting point apparatus. The ^{1}H NMR spectra were recorded with a Bruker Avance 3 Ascend 500 system operated at 500 MHz, and the ^{13}C NMR spectra were obtained at 125 MHz and ^{31}P NMR 202 MHz in $CDCl_3$, CD_3OD or DMSO-d_6 at 298 K. The "in situ" reduction of the nitroxides was achieved by addition of five equivalents of hydrazobenzene (DPPH/radical). The EPR (electron paramagnetic resonance) spectra were recorded on MiniScope MS 200 instrument in $CHCl_3$ solution, and the concentrations were 1.0×10^{-4} M. The IR spectra were obtained using a Bruker Alpha FT-IR with ATR support on a diamond plate. Spectrophotometric measurements for antioxidant capacity measurement were performed on a Specord 40 UV/VIS Spectrophotometer at 732 nm in a 1 × 1 cm quartz cuvette. Hydrogenations were performed with a ThalesNano H-CubeVR Mini Plus reactor with 20% $Pd(OH)_2/C$ cartridge. Flash column chromatography was performed on a Kieselgel 60 (0.040 – 0.063 mm) column. Qualitative TLC was performed on commercially available plates (20 cm × 20 cm × 0.02 cm) coated with Merck Kieselgel GF254.

4. New Scientific findings

1. We developed a new diamagnetic synthon, 1-methoxy-2,2,5,5-tetramethylpyrrolidine-3,4-dione **2** which was condensed with different aliphatic, aromatic or heteroaromatic 1,2-diamines followed by deprotection of the nitroxide function with *m*-CPBA, providing pyrroline nitroxide fused pyrazines **3, 9**, pteridines **6, 8** or quinoxalines **4, 5, 7**.[7]

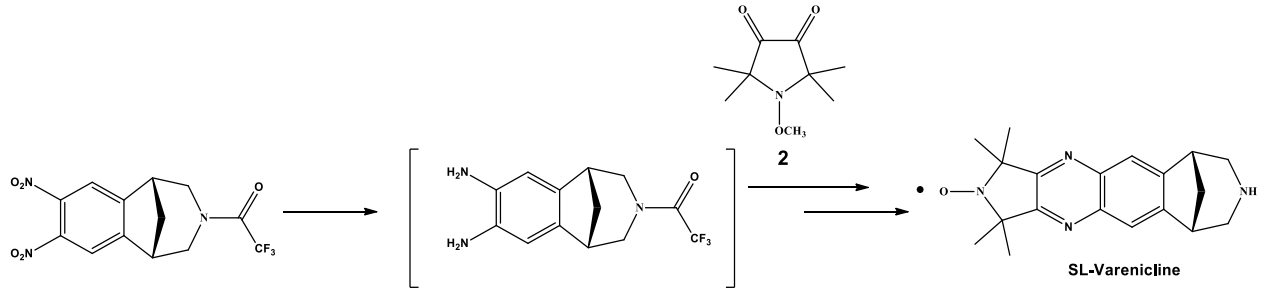


The oxidation of **3** resulted in the formation of *N*-oxide derivative, which offered the possibility of C-H functionalization with benzene as a solvent at C2 position by palladium catalysis and Ag_2CO_3 oxidation to give compound **10**.[7]



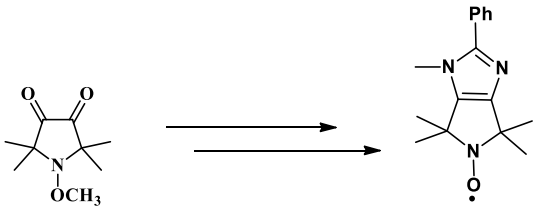
3 **10**

2. Reduction of dinitro compound **11** with $H_2/Pd(OH)_2$ furnished a diamino precursor of which condensation with 1,2-diketone **2** followed by the deprotection of *N*-OMe by *m*-CPBA, and the removal of the trifluoroacetyl group by Na_2CO_3/MeOH gave the spin labelled Varenicline **12**.[5]



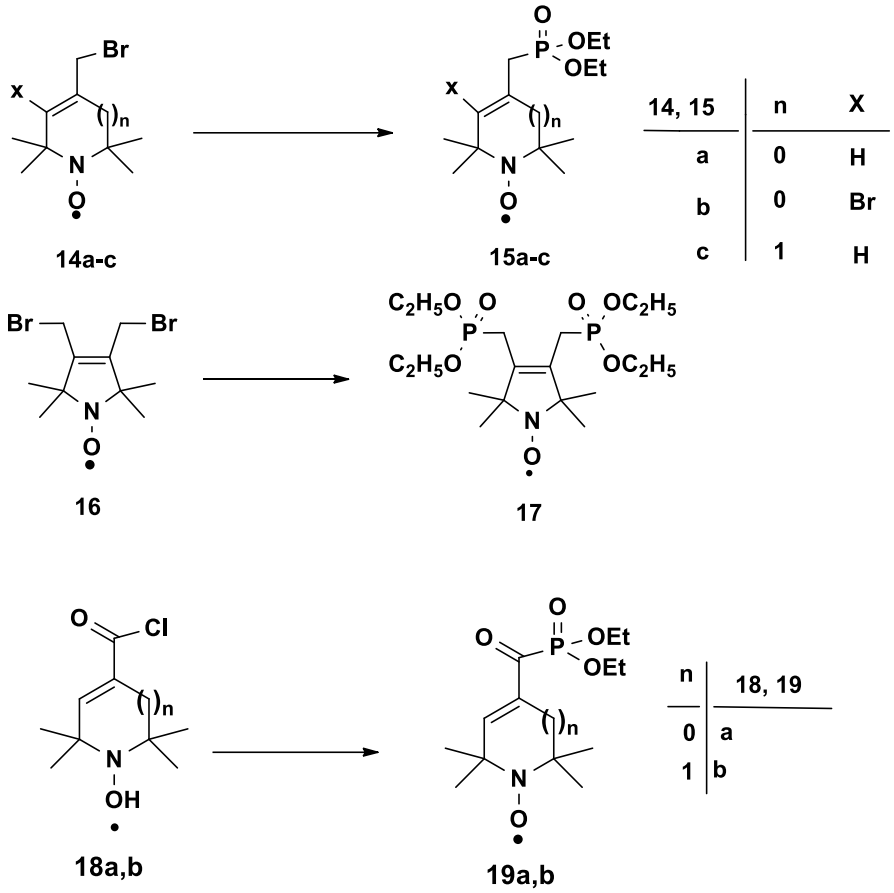
11 **2** **SL-Varenicline** **12**

3. The reaction of the diamagnetic 1,2-diketone with benzaldehyde and ammonium acetate produced a pyrrolo[3,4-d]imidazole scaffold (**13**) in the Debus-Radziszewski reaction. The *O*-methyl group deprotection required the protection (alkylation) of NH function of the imidazole.[7]

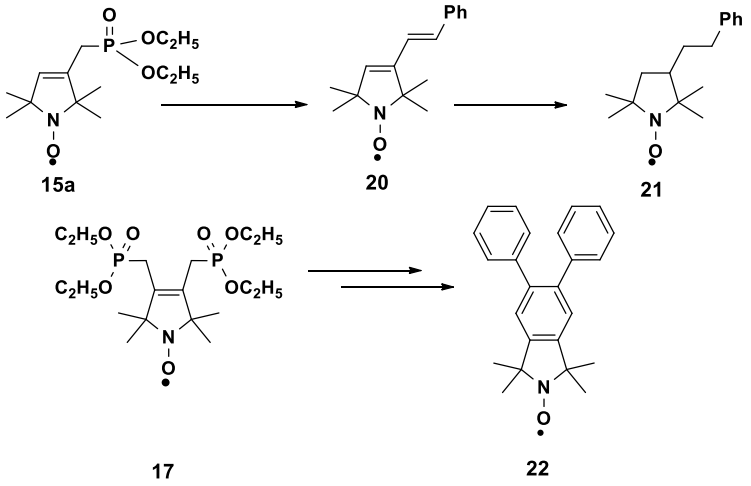


2 **13**

4. Treatment of five- and six-membered allylic bromides **14a-c**, dibromo compound **16,** acyl chlorides **18a,b** respectively with triethyl phosphite in an Arbuzov reaction resulted in the formation of allylic phosphonate esters **15a-c**, bisphosphonate ester **17,** α-ketophosphonate esters **19a,b**.[2,4,6]

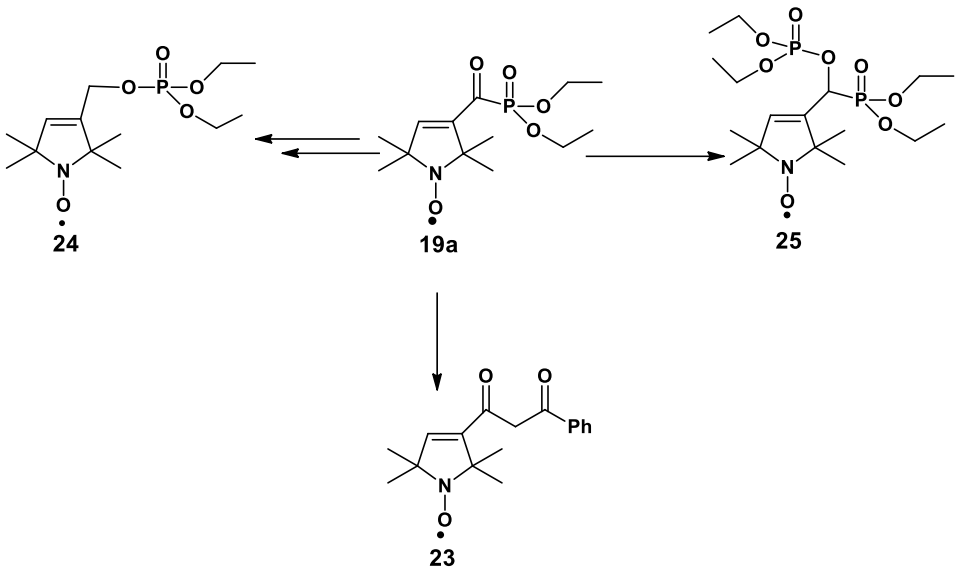


5. We utilized the allylic phosphonate ester **15a** in Horner-Wadsworth-Emmons (HWE) reaction through deprotonation and treatment with aldehydes yielding *E*-alkenes. The reduction of the resulted C=C bond could be achieved using continuous flow hydrogenation system. Bisphosphonate ester **17** was deprotonated and treated with excess of benzaldehyde. The formed triene electrocyclisation occurred spontaneously and oxidation with DDQ resulted isoindoline radical **22**. [6]

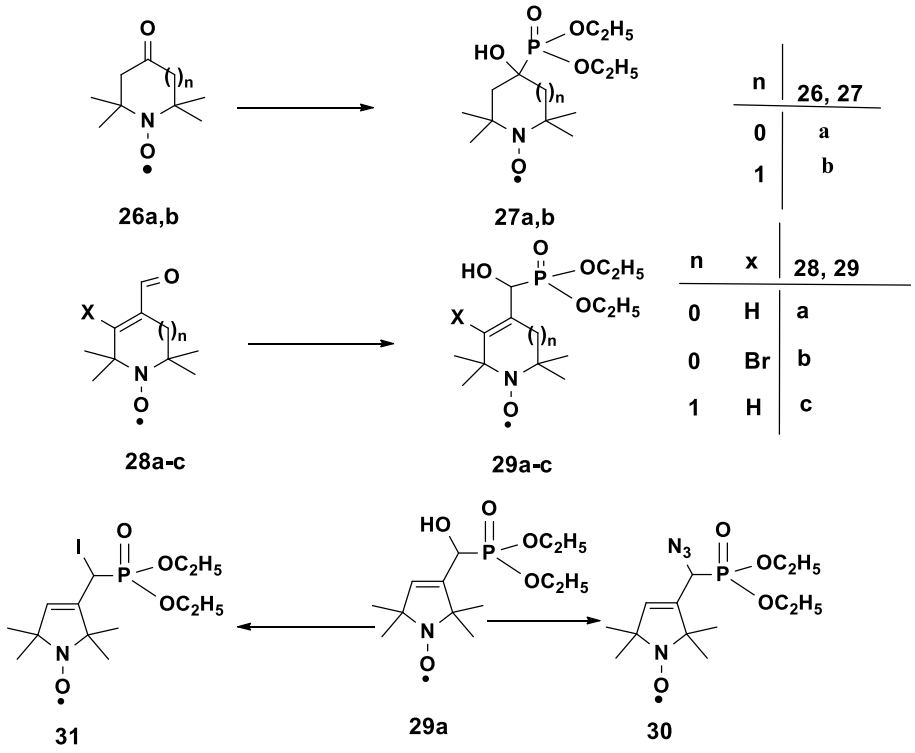


6. Compound **19a** was utilized as an acylation agent to give paramagnetic 1,3-dicarbonyl compound **23**. The reduction of **19a** to α-hydroxyphosphonate ester followed by a phospha-Brook

rearrangement furnished paramagnetic phosphate ester **24**. The nucleophilic addition of diethylphosphite to compound **19a** gave phosphate phosphonate ester **25**.[2, 4]

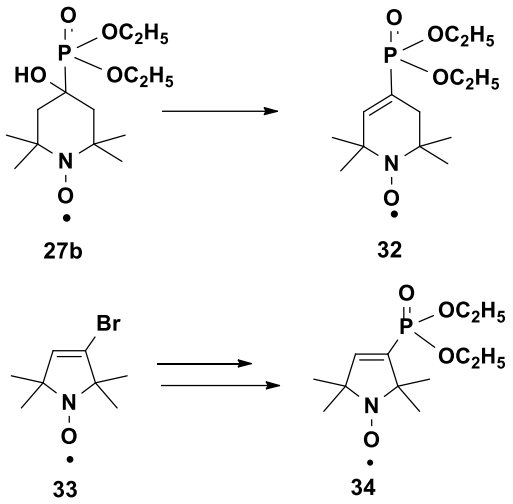


7. We have synthesized paramagnetic five- and six-membered α-hydroxyphosphonate esters **27a,b** and **29a-c** starting from paramagnetic five- and six-membered ketones **26a,b** and aldehydes **28a-c** by Pudovic reaction under solvent-free conditions. Compound **29a** was functionalized further with Mitsonubu reaction yielding compounds **30** and **31**.[2, 6]

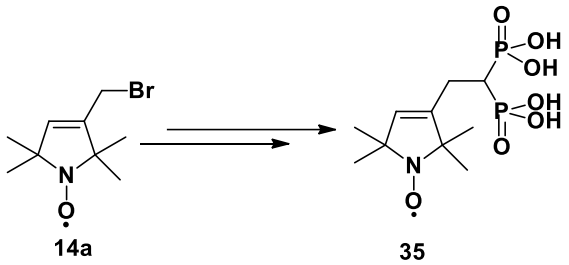


8. We have developed two methods for synthesizing of paramagnetic vinylphosphonates. Water elimination of compound **27b** yielded paramagnetic six-membered vinyl phosphonate ester **32**.

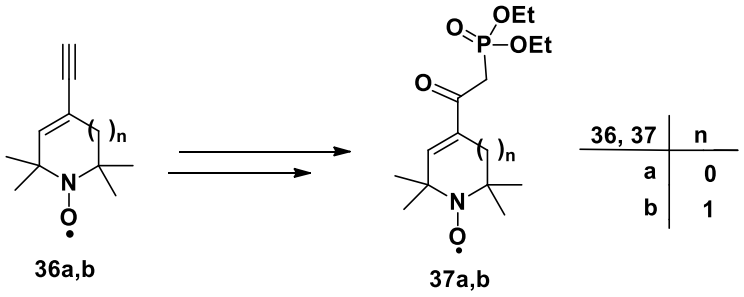
Paramagnetic five-membered vinylphosphonate ester **34** was obtained from compound **33** through a process including protection of nitroxide moiety as *O*-methyl by Fenton reaction, followed by lithiation, treatment with diethylchlorophosphate to give the diamagnetic vinyl phosphonate, and finally restoring of nitroxide moiety by *m*-CPBA oxidation.[6]



9. Geminal bisphosphonic acid **35** was accessed by alkylating tetraethyl methylenebisphosphonate with compound **14a**, followed by hydrolysis and restoring the nitroxide radical.[2, 4]

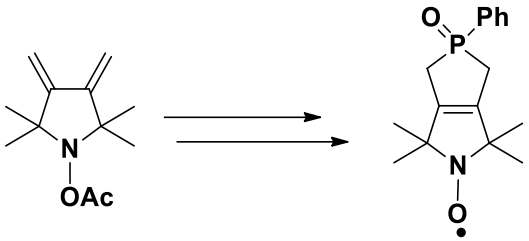


10. We utilized paramagnetic acetylenes **36a** and **36b** to get β-ketophosphonates **37a** and **37b**, through a process including deprotonation of terminal acetylene moiety, followed by acylation with diethylchlorophosphate. The ethynylphosphonate esters were hydrated in aqueous dioxane in the presence of 0.1 equiv. $PdCl_2$.[2, 4]



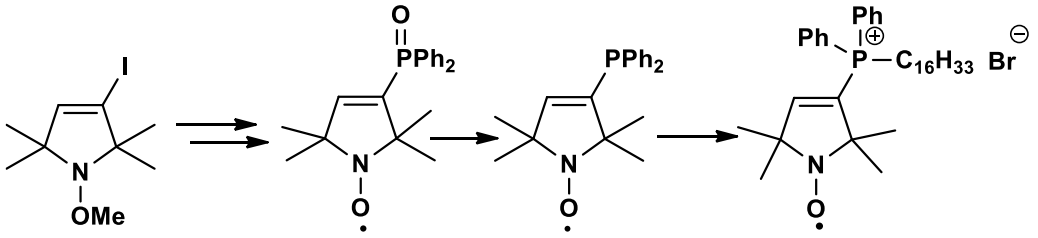
36, 37	n
a	0
b	1

11. The reaction of a diene nitroxide precursor **38** with dichlorophenyl phosphine in a McCormac procedure offered 2-oxyl-1,1,3,3-tetramethyl-5-phenyl-1,2,3,4,5,6-hexahydrophospholo[3,4-c]pyrrole-5-oxide **39** after deacetylation.[1,3]



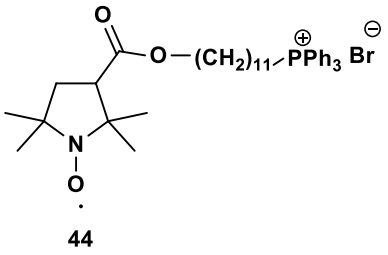
38 **39**

12. We achieved the paramagnetic phosphine derivative **42**, starting from a protected pyrroline nitroxide **40** which was lithiated, followed by treatment with diphenylchlorophosphine, and *m*-CPBA deprotection to give compound **41**. The reduction with Cl_3SiH and selective oxidation of **41** by PbO_2 offered compound **42**. The heating of compound **42** with hexadecyl bromide in acetonitrile offered the phosphonium salt **43**.[1,3]



40 **41** **42** **43**

Based on the similarity between the phosphonium salt **43** and MITO-CP (Mitochondria-targeted nitroxide) (Figure 1), we studied the cytostatic effect of compound **43** in comparison with MITO-CP **44** on MDA-MB-231 (Figure 2) and MCF-7 human breast cancer lines (Figure 3). Compound **43** had more pronounced cytotoxicity than MITO-CP at all studied concentrations.[8]



44

Figure 1. Structure of MITO-CP.

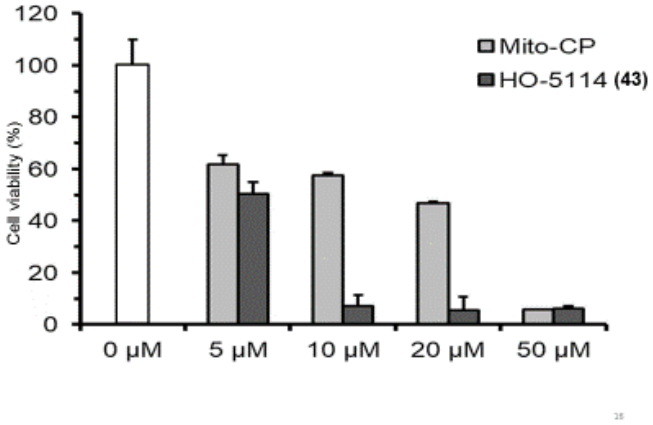


Figure 2. Cytostatic effect of compound **43** on MDA-MB-231 human breast cancer lines.

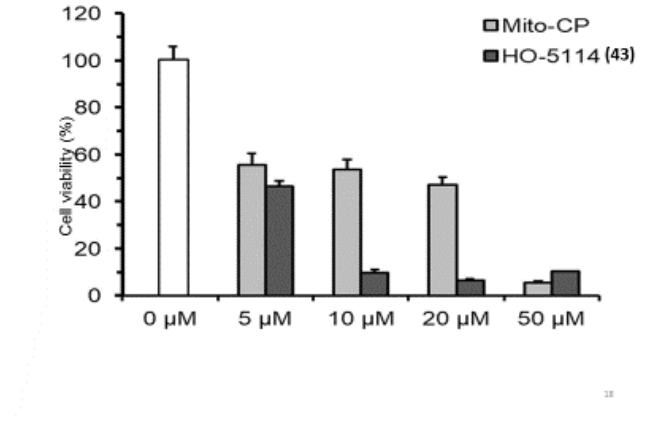


Figure 3. Cytostatic effect of compound **43** on MCF7 human breast cancer lines.

5. Publication List

Publications related to Ph.D dissertation

1. Syntheses and study of a pyrroline nitroxide condensed phospholene oxide and a pyrroline nitroxide with diphenylphosphino moiety.
   Isbera, M.; Bognár, B.; Gallyas, F.; Bényei, A.; Jekő, J.; Kálai, T.
   Phosphorus, Sulfur, and Silicon and the Related Elements **2022**, https://doi.org/10.1080/10426507.2021.1989690. (IF_{2020}: 1.082, Q4)

2. Syntheses and utilizations of pyrroline and tetrahydropyridine nitroxide-based phosphonate esters, a phosphate ester and a bisphosphonate.
   Kálai, K.; Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Hideg, K.
   Phosphorus, Sulfur, and Silicon and the Related Elements **2022**, https://doi.org/10.1080/10426507.2021.1989685. (IF_{2020}: 1.082, Q4)

3. Syntheses and Study of a Pyrroline Nitroxide Condensed Phospholene Oxide and a Pyrroline Nitroxide Attached Diphenylphosphine**.**

   Isbera, M.; Bognár, B.; Gallyas, F.; Bényei, A.; Jekő, J.; Kálai, T.
   Molecules **2021**, *26*, 4366. (IF_{2020}: 4.411, Q2)

4. Syntheses and utilizations of pyrroline-nitroxide and tetrahydropyridine-nitroxide- based α-ketophosphonates, β-ketophosphonates, and a bisphosphonate.

   Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Kálai, T.
   Synthetic Communications **2021**, *51*, 1353–1362. (IF_{2020}: 1.796, Q3)

5. Synthesis of a Nitroxide Spin-labeled Varenicline (Chantix) Derivative.
   Bognár, B.; Isbera, M.; Kálai, T.
   Organic Preparations and Procedures International **2021**, *53*, 311–315. (IF_{2020}: 1.628, Q4)

6. Syntheses and Reactions of Pyrroline, Piperidine Nitroxide Phosphonates.
   Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Hideg, k.; Kálai, T.
   Molecules **2020**, *25*, 2430. (IF: 4.411, Q2)

7. Syntheses of Pyrazine-, Quinoxaline-, and Imidazole-Fused Pyrroline Nitroxides.
   Isbera, M.; Bognár, B.; Gulyás Fekete, G.; Kish, K.; Kálai, T.
   Synthesis **2019**, 51, 4463-4472. (IF: 2.675, Q1)

Conference presentations related to Ph.D dissertation

1. *Syntheses and Studies of Phosphorus Containing Stable Nitroxide Radicals*. Mostafa Isbera, Attila Bényei, Ferenc Gallyas, Balázs Bognár, Cecília P. Sár, Kálmán Hideg, József Jekő, Tamás Kálai. Az MTA Heterociklusos és Elemorganikus Kémiai Munkabizottságának a Patonay Tamás-díj átadásával egybekötött nyílt ülésére. Budapest, Hungary. 3rd September, 2021(oral lecture).

2. *Synthesis of pyrazine-, quinoxaline- and imidazole-fused pyrroline nitroxides*. Mostafa Isbera, Balázs Bognár, Gergely Gulyás Fekete, Krisztina Kish, Tamás Kálai. Hterociklusos és Elemorganikus Munkabizottság előadóülése. Microsoft Teams Meeting. 31st May, 2021 online oral lecture)

3. *Syntheses and study of a pyrroline nitroxide condensed phospholene oxide and a pyrroline nitroxide attached diphenylphosphine.* Mostafa Isbera, Attila Bényei, Ferenc Gallyas, Balázs Bognár, József Jekő, Tamás Kálai. 23rd International Conference on Phosphorus Chemistry. Częstochowa, Poland. 5-9th July, 2021 (online poster).

4. *Syntheses and utilizations of pyrroline-nitroxide- and tetrahydropyridine-nitroxide-based phosphonates, α-ketophosphonates, β-ketophosphonates, and a bisphosphonate.* Tamás Kálai, Mostafa Isbera, Balázs Bognár, Cecília Sár, József Jekő, Kálmán Hideg. 23rd International Conference on Phosphorus Chemistry. Częstochowa, Poland. 5-9th July, 2021 (online poster).

5. *Syntheses of pyrazine, quinoxaline and imidazole fused pyrroline nitroxides.* Mostafa Isbera, Balázs Bognár, Gergely Gulyás-Fekete, Krisztina Kish, Tamás Kálai. A Magyar Szabadgyök-Kutató Társaság X. Kongresszusa. Szeged, Hungary. 29-30th August, 2019 (poster)

Publications not related to Ph.D dissertation

1. Cytostatic Effect of a Novel Mitochondria-Targeted Pyrroline Nitroxide in Human Breast Cancer Lines**.**
   Andreidesz, K.; Szabó, A.; Kovács, D.; Kőszegi, B.; Bagóné Vántus, V.; Vamos, E.; Isbera, M.; Kálai, T.; Bognár, Z.; Kovács, K.; Gallyas, F.
   International Journal of Molecular Sciences **2021**, *22*, 9016. (IF_{2020}: 5.923, Q1)

2. The effect of temperature and moisture on the physical and chemical stability of furosemide tablets (40 mg)marketed in Syria.
   Mansour, O.; Isbera, M.; Ismail, G.; Mayya, G.
   World Journal of Pharmaceutical Research ***2018***, 7, 35-44.

3. The Influence of Temperature and Moisture on the Physical and Chemical Properties of MetforminHydrochloride Tablets (850 mg) Marketed in Syria.
   Mansour, O.; Isbera, M.; Mayya, G.
   International Journal of Pharmacy and Pharmaceutical Research **2018**, 12, 29-39.

4. Evaluation of Physical and Chemical Properties of Paracetamol (500 mg) Tablets marketed in Syria.
   Mansour, O.; Isbera, M.; Mtawag, A.
   Journal of Chemical and Pharmaceutical Sciences **2017,** 10, 1306-1308. Q3

5. Development and validation UV spectrophotometric method for determination of atenolol in pure materialsand pharmaceutical dosage.
   Aboud, K.; Mohammad, A.; Isbera, M.; Beesh, M.
   Indo American Journal of Pharmaceutical Research **2017,** 7, 8179-8184.

6. Quality analysis of different marketed brands of atenolol (50 mg) available in Syria and comparing with thereference drug.
   Beesh, M.; Isbera, M.; Aboud, K.; Mohammad, A.
   World Journal of Pharmaceutical Research **2017,** 6, 61-68**.**

7. Quality control of warfarin sodium tablets marketed in Syria.
   Isbera, M.; Aboud, K.; Haroun, M.
   Research Journal of Pharmacy and Technology **2017**, 10, 2119-2121. Q3

8. Assessment of physicochemical properties of metformin hydrochloride (850 mg) tablets marketed in Syria.
   Mansour, O.; Isbera, M.
   Journal of Chemical and Pharmaceutical Sciences **2016**, 9, 726-729. Q4

9. Assessment of physicochemical properties of furosemide (40 mg) tablets marketed in Syria
   Mansour, O.; Ismail, G.; Isbera, M.; Almouhammad, M.
   Journal of Chemical and Pharmaceutical Sciences **2016**, 9, 2879-2881. Q4

10. Weight and content uniformity of warfarin sodium half tablets.
    Isbera, M.; Ibrahim, W.; Abbood, A.
    Research Journal of Pharmacy and Technology **2016**, 9, 215-218. Q4

11. Weight and content uniformity of furosemide half tablets.
    Isbera, M.; Ibrahim, W.; Abbood, A.
    Tishreen University Journal for Research and Scientific Studies- Health Sciences Series **2015**, 37, 169-181.