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REVIEW ARTICLE

Synthesis and Application of Stable Nitroxide Free Radicals Fused with Carbocycles and Heterocycles

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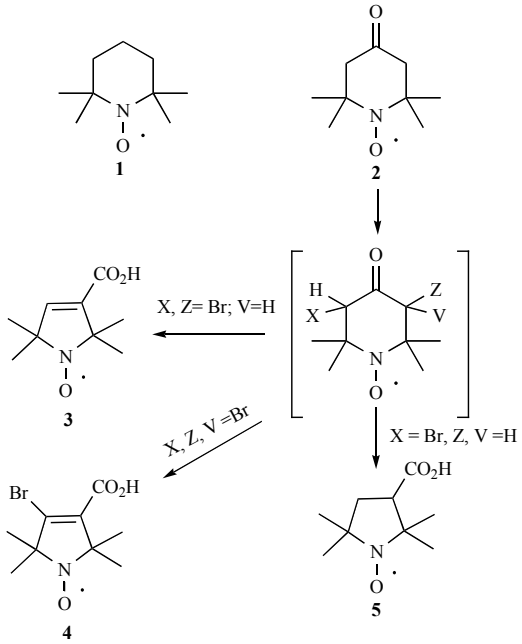
Abstract: Stable nitroxide free radicals have traditionally been associated with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) or its 4-substituted derivatives as relatively inexpensive and readily accessible compounds with limited possibilities for further chemical modification. Over the past two decades, there has been a resurgence of interest in stable free radicals with proper functionalization tuned for various applications. The objective of this review is to present recent results with synthetic methodologies to achieve stable nitroxide free radicals fused with aromatic carbocycles and heterocycles. There are two main approaches for accessing stable nitroxide free radicals fused with arenes, *e.g.*, isoindoline-like nitroxides: further functionalization and oxidation of phthalimide or inventive functionalization of pyrroline nitroxide key compounds. The latter also offers the constructions of versatile heterocyclic scaffolds (furan, pyrrole, thiophene, 1,2-thiazole, selenophene, pyrazole, pyrimidine, pyridine, pyridazine, 1,5-benzothiazepine) that are fused with pyrroline or tetrahydropyridine nitroxide rings. The possible applications of these new stable nitroxide free radicals, such as covalent spin labels and noncovalent spin probes of proteins and nucleic acids, profluorescent probes, building blocks for construction of dual active drugs and electroactive materials, and substances for controlled free radical polymerization, are discussed.

Keywords: Antioxidants, C-C bond formation, carbocycles, heterocycles, nitroxide free radicals, Pd-catalyzed cross-coupling, spin-labels.

1. INTRODUCTION

In the past four decades, the study of free radicals has become an independent discipline that combines the results of biological, physical, chemical and medical sciences [1]. One of the most interesting areas in this field of study is pure, isolable long-lived radicals. [2]. One of the main groups of these long-lived stable radicals is nitroxide (aminoxyl) radicals [3]. Extensive studies of stable nitroxide free radicals first appeared 60 years ago, and their application is rather diverse and extends beyond spin labeling [4]. They are used as co-oxidants in organic chemistry [5], building blocks for magnetic materials [6], anti-biofilm compounds [7], superoxide dismutase mimics [8], antioxidants [9], antiproliferative compounds [10], mediators of polymerization [11], redox active materials in batteries [12], sensor molecules [13], magnetic resonance imaging (MRI) [14] and electron paramagnetic resonance imaging (EPRI) [15] contrast agents. The chemical and biological knowledge of stable nitroxide free radicals began to grow tremendously, as is shown by the increasing number of reviews [16], thematic issues [17] and monographs [18]. These diverse applications were possible after M. B. Neimann and E. G. Rozantsev expanded the synthesis of stable nitroxide free radicals into a wide range of compounds [19] recognizing the functionalization limits of TEMPO (**1**) or 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl (4-OXOTEMPO) (**2**). Of the latter compound, five-membered pyrroline nitroxides **3** and **4** or pyrrolidine free radical **5** were available *via* halogenation and Favorskii-rearrangement [20] (Scheme **1**).



Scheme 1. TEMPO and 4-OXO-TEMPO and their rearrangement to five-membered nitroxides.

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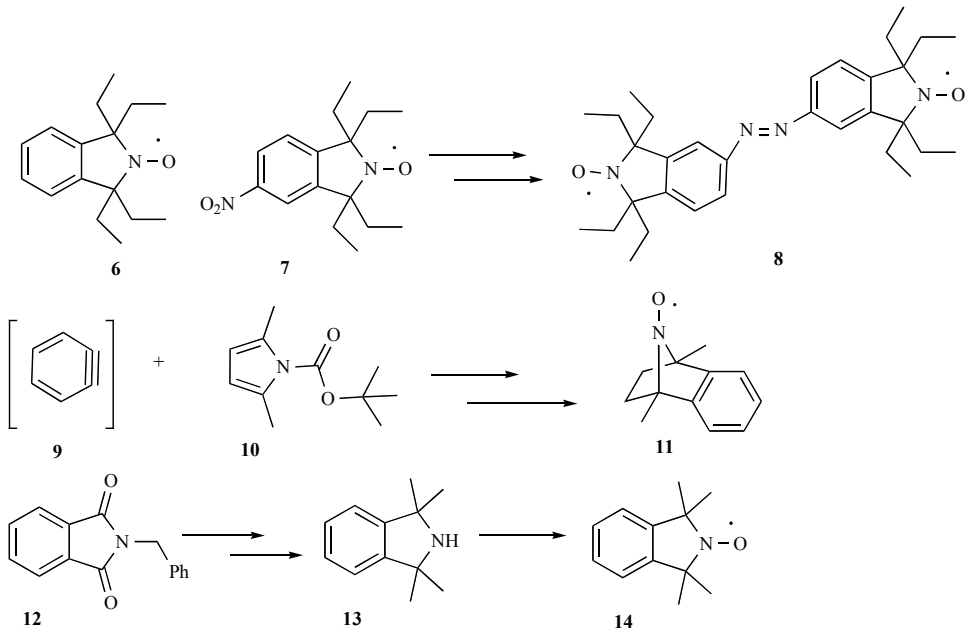
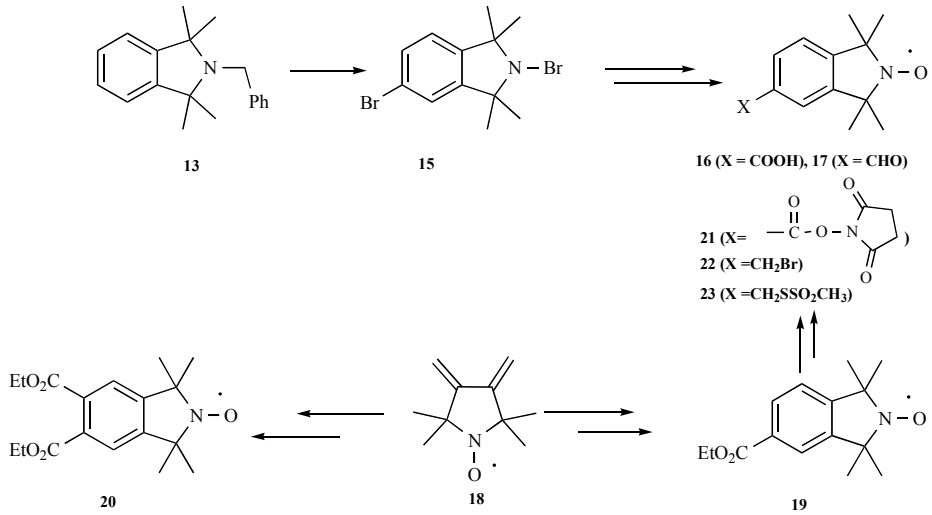


Scheme 2. Early examples for carbocycle fused nitroxides.

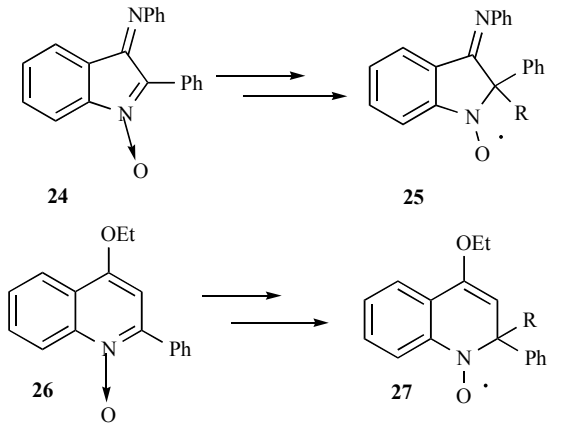


Scheme 3. Possible accesses for substituted isoindoline nitroxides.

Soon after the first publications of pyrroline/pyrrolidine nitroxides, isoindoline nitroxides **6** and **7** were reported by the Rozantsev's group [21, 22], followed by the transformation of nitroderivative **7** to biradical **8** by the Rassat group [23]. Additionally, Kosman reported bridged isoindoline nitroxide **11**, which was achieved by a Diels-Alder reaction of the benzyne **9** with *t*-butyl 2,5-dimethyl-1*H*-pyrrole-1-carboxylate **10** followed by hydrogenation, deprotection and *m*-chloroperbenzoic acid (*m*-CPBA) oxidation [24]. The "rediscovery" of isoindoline nitroxides occurred in the early 1980s. Australian researchers Griffiths, Rizzardo, and Solomon synthesized tetramethyl isoindoline nitroxide **14** by the treatment of *N*-benzyl phthalimide **12** with excess methyl Grignard reagent [25] to obtain **13,** of which debenzylation of the sterically hindered amine followed by oxidation resulted in the formation of stable isoindoline nitroxide radical (Scheme **2**).

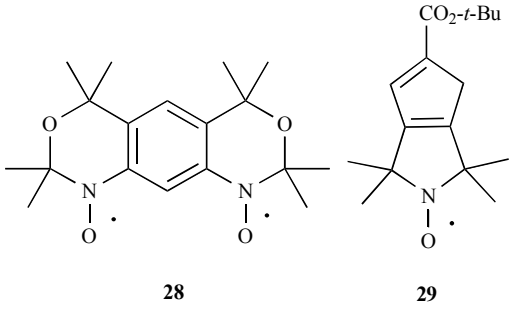
The innovation involved the synthesis and introduction of isoindoline nitroxide radicals as UV detectable scavengers of carbon-centered radicals. The real renaissance of isoindoline radicals occurred in the 1990s when Reid, Bottle and Micallef of Queensland University solved the problem of further functionalization of **14** [26]. The key reaction for this investigation was the oxidative bromination of **13** in the presence of $AlCl_3$, which provided compound **15** by lithiation and reaction with CO_2 or DMF followed by oxidation gave nitroxides **16** and **17,** respectively. It is interesting to note that our research group at the University of Pécs in Hungary accessed isoindoline nitroxides *via* the Diels–Alder reaction of diene **18** with propiolic acid ethylester or acetylenedicarboxylic acid diethylester followed by oxidation with activated MnO_2 or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); we also produced 5-substituted or 5,6-disubstituted isoindoline esters **19** and **20**, respectively [27]. The 1,1,3,3-tetramethyl isoindoline nitroxides are advantageous because of their higher thermal and chemical stability and superior (electron paramagnetic resonance (EPR) linewidths. This is the reason why Australian and Hungarian groups introduced reactive functional groups on the isoindoline ring to gain NH_2 specific **21** or SH specific **22** and **23** spin labels for modifying biomolecules (Scheme **3**).

Greci's group developed the synthesis of indolinic **25** and quinolinic aminoxyls **27** by the treatment of nitrones **24** and **26** with a Grignard reagent. The nitroxides of this family are less stable than 2,2,6,6-tetramethylpiperidine, 2,2,5,5-tetramethylpyrrolidine or 1,1,3,3-isoindoline nitroxides, because the unpaired electron of indolinic or quinolinic nitroxides are delocalized in the "whole molecule" through a conjugated π-system [28]. These aminoxyl radicals are significant radical scavengers and antioxidant molecules [29] (Scheme **4**).



Scheme 4. Synthesis of indolinic and quinolinic nitroxides.

Many other types of carbocycle fused nitroxides were reported, such as 1,2,8,9-tetrahydro-2,2,4,4,6,6,8,8-octamethyl-4*H*,6*H*-benzo [1,2-d;5,4-d']bis-(1,3-oxazine)-1,9-dioxyl **28** [30], and 5-carbo-*t*-butoxy-1,1,3,3-tetramethyl-1,3,4-trihydro-2*H*-cyclopenta[c]pyrrol-2-yloxyl **29** [31] (Scheme **5**).



Scheme 5. Additional examples of carbocycle fused nitroxides.

Over the past six decades, several piperidine and pyrroli(di)ne nitroxides fused to various heterocycle scaffolds have been reported. An oxirane-fused pyrrolidine nitroxide **31** was achieved by base-promoted oxidation of aldehyde **30** [32] and reported by our laboratory [33]. Fortunately, the epoxide ring of **31** was rather inert toward nucleophiles; therefore, derivatives **32** and **33** could also be synthesized (Scheme **6**).

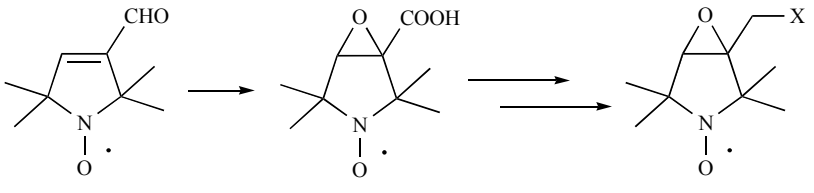
Regarding nitroxides fused with five-membered heterocycles, the first representatives, **35** [34] and **37** [35], were reported by the Rosatsev group in 1965 *via* the oxidation of sterically hindered amines **34** [36] and **36** [37] with Na_2WO_4/H_2O_2 to yield the paramagnetic polycycles. Dihydrothiophene **38** [38] and thiophene **40** fused derivatives were obtained by our research group with Fieselmann thiophene syntheses [39], *e.g.*, the treatment of aldehydes **30** and **39** [39] with thioglycolic acid ethyl ester. The synthesis of the SH-reactive paramagnetic thiophene derivatives **41** and **42** was also accomplished. In 2008, Australian researchers used compound **41** in the synthesis of paramagnetic angiotensin (AT_1) receptor antagonists *via* alkylation of compound **43** to give **44** with an improved pharmacological profile (they antihypertensive and decrease free radical production) [40] (Scheme **7**). Beyond the thiophene derivatives, further five-membered heterocycle-fused nitroxides were synthesized in our laboratory, as summarized in previous reviews [41, 42].

An elegant example of the six-membered heterocycle-fused nitroxides is the reaction of 3-chloro-1-hydroxy-2,2,6,6-tetramethylpeperidone **45** with hydroxylamine and butan-2,3-dione to produce pyrazine fused tetrahydropyridine nitroxide **46** by Volodarskii *et al*. in 1988 [43]. Ten years later, our group synthesized dibromide **47** from diene 18 to achieve dialdehyde 48, followed by the condensation with hydroxylamine to produce o pyridine-anullated nitroxide **49** [27]. After several steps, the hetero-Diels-Alder reaction of diene 18 with *N*-(butoxycarbonylmethylen)-p-toluene-sulfonamide yielded the spin-labeled picolinic acid ethyl ester **50** [44] (Scheme **8**).

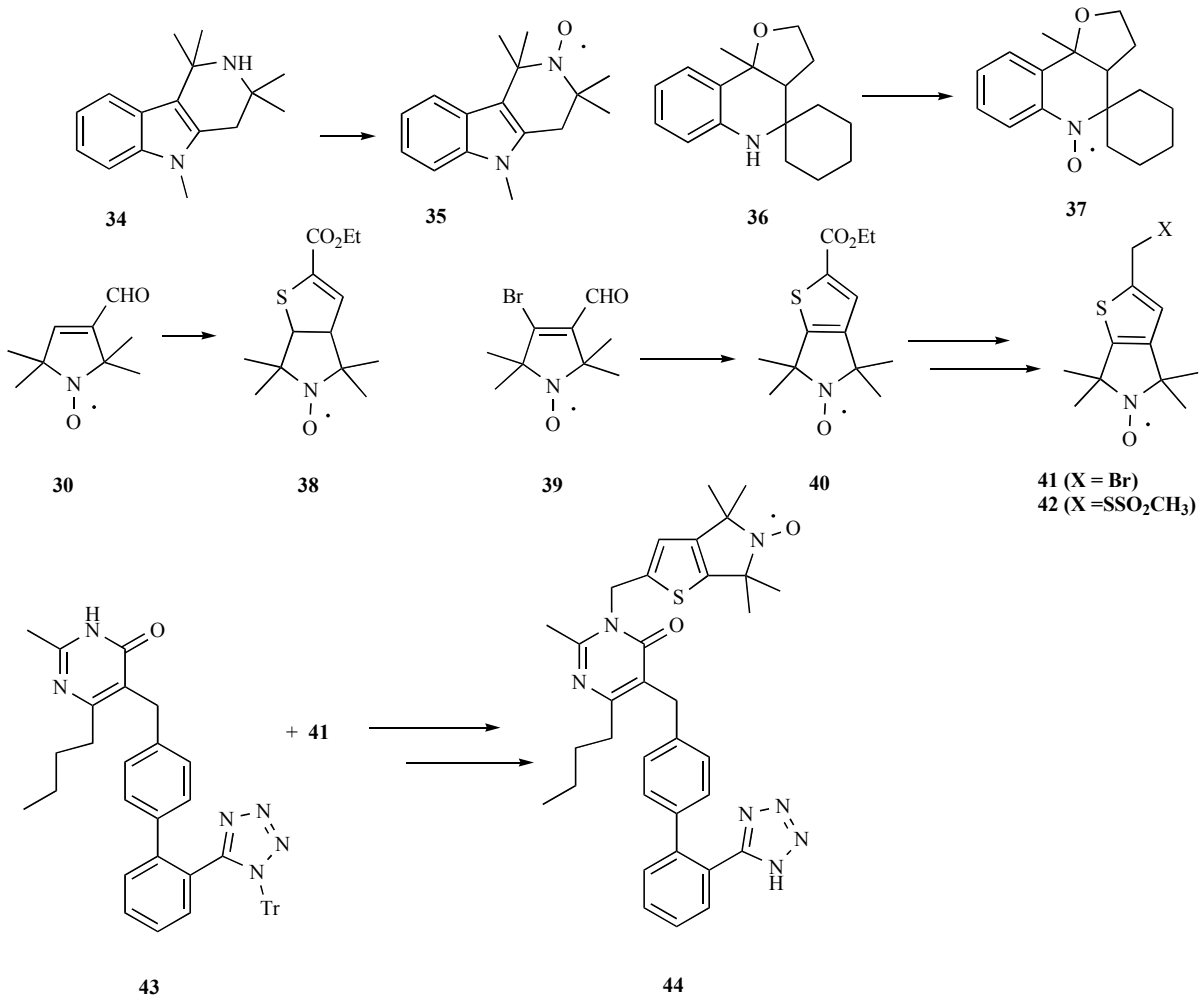
The results listed above are milestones in the synthesis of carbo- and heterocycle-fused nitroxides up to 2005. We decided to review the pyrroline/piperidine/imidazolide nitroxide fused carbo- and heterocycles from the past 12 years. We do not deal with spiro compounds or compounds in which nitroxides are connected to heterocycles or carbocycles *via* a single bond. After discussion of carbocycle fused nitroxides, we continue with summarizing five-, six-, seven-membered heterocycle-fused nitroxides.

2. THE SYNTHESIS OF CARBOCYCLE FUSED NITROXIDES

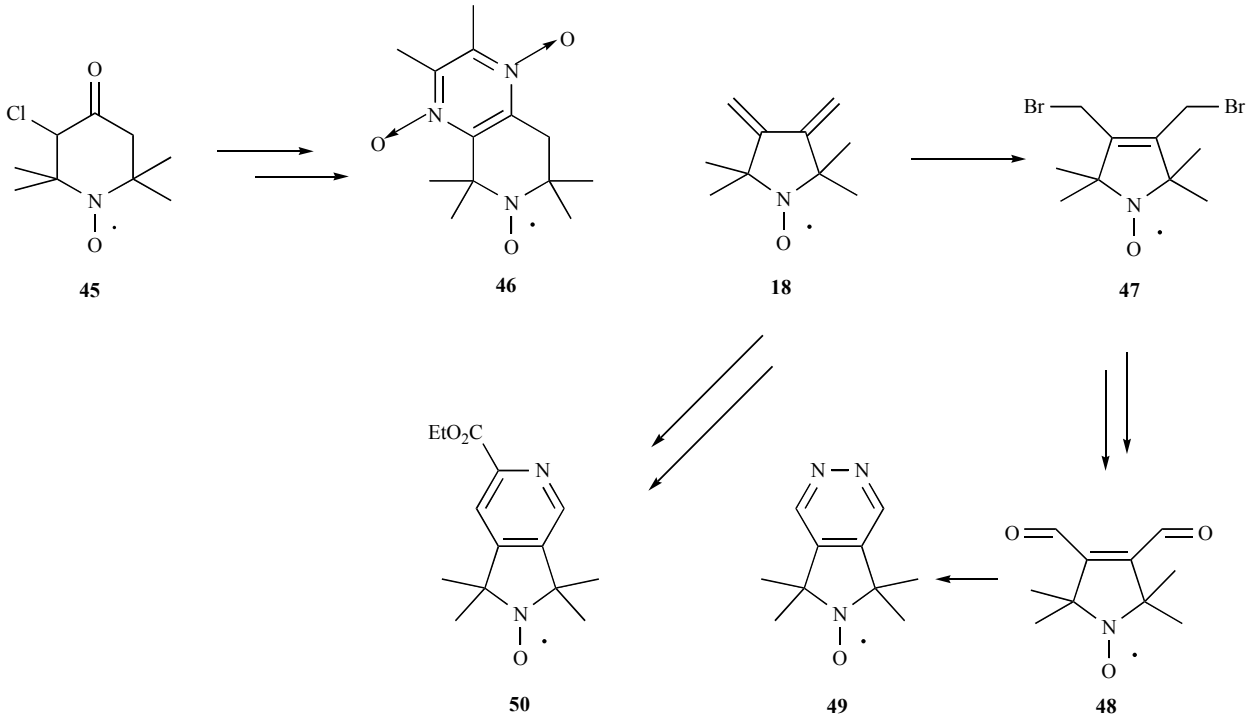
Pecar and coworkers obtained cyclopropane-fused pyrrolidine nitroxides *via* the Favorskii-rearrangement of 3-Br-TEMPONE **51** initiated by C-nucleophiles generated from malonester or acetoacetate-type compounds to yield compound **52**, which could be reduced by sodium borohydride to diol **53** or triol **54** depending on the conditions applied [45] (Scheme **9**).



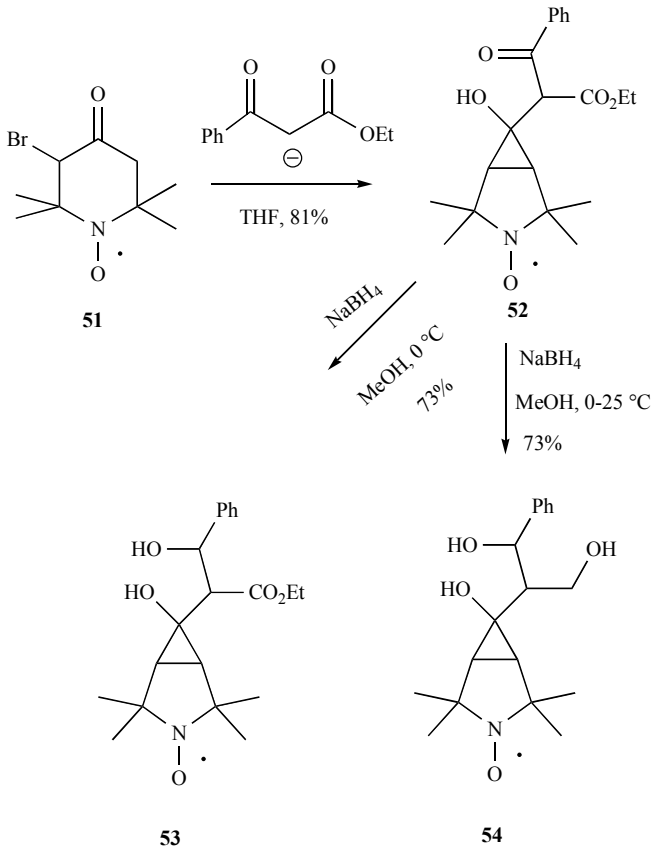
Scheme 6. Synthesis of oxirane-fused pyrrolidine nitroxides.



Scheme 7. Examples of 5-membered heterocycles fused pyrroline nitroxides.



Scheme 8. Additional examples of six-membered heterocycle fused nitroxides.



Scheme 9. Synthesis and functionalization of cyclopropane fused pyrrolidine nitroxides.

A series of imidazolidine nitroxides, including cyclohexane-fused imidazoline and imidazolidine nitroxides were synthesized and reported in 2006 by Zubenko *et al*. These nitroxides were used to determine the rate constants of coupling between imidazolidine nitroxides and acrylate-type radicals *via* time-resolved chemically induced dynamic nuclear polarization (TR-CIDNP) and laser flash photolysis techniques [46]. For cyclohexane-fused nitroxides, the synthesis starts from hydroxylamine **55** and its ring closure with ammonium acetate and cyclohexanone yielded imidazoline **56**, which offered nitroxide **57** after oxidation with activated MnO_2. This compound was converted into imidazolidine **58** by methylation with dimethylsulfate and reduction with $NaBH_4$ (Scheme **10**).

For phenanthrene fused nitroxide **61**, Bottle's group invented a new approach. Compound **59** was treated with excess Grignard-reagent to produce compound **60**. After debenzylation and oxidation, phenanthrene-fused nitroxide **61** was obtained, which was doped into polypropylene and used as a spin trap for methyl radicals generated during the decomposition of polypropylene. The recombination process results in the formation of a fluorescent species **62**, as the nitroxide quenching effect on phenanthrene fluorophore does not prevail [47]. It is interesting to note that in 2006 our laboratory reported another approach for compound **61**.

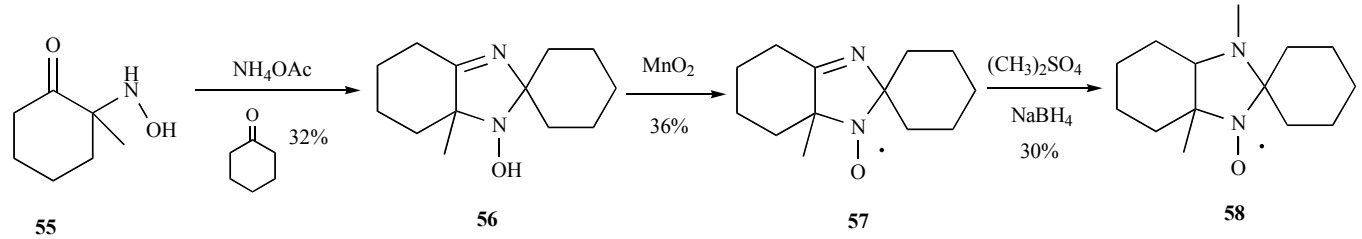
The dibromo compound **63** [20c] was partly substituted with phenylboronic acid in a Suzuki coupling reaction followed by a second Suzuki coupling with 2-aminophenyl boronic acid to offer compound **64**. The diazotization of this compound and Pschorr coupling with Cu powder also produced compound **61** [48]. This is a longer and less effective approach than the Bottle group synthesis; however, starting from pyrroline nitroxides with our methodology might offer the construction of other paramagnetic polyaromatic compounds. For example, the reaction of compound **63** with 1-naphthylboronic acid furnished nitroxide anellated acenaphthylene **65** (Scheme **11**).

The synthesis of azaphenalene nitroxides was reported by Bottle's group in 2007 [49]. The synthesis was carried out with procedures that were analogous to those applied for the synthesis of **61** from **59**. N-benzyl-1,8-naphthalimide **66** was treated with excess methylmagnesium iodide to obtain compound **67** followed by deprotection and oxidation with H_2O_2/Na_2WO_4.

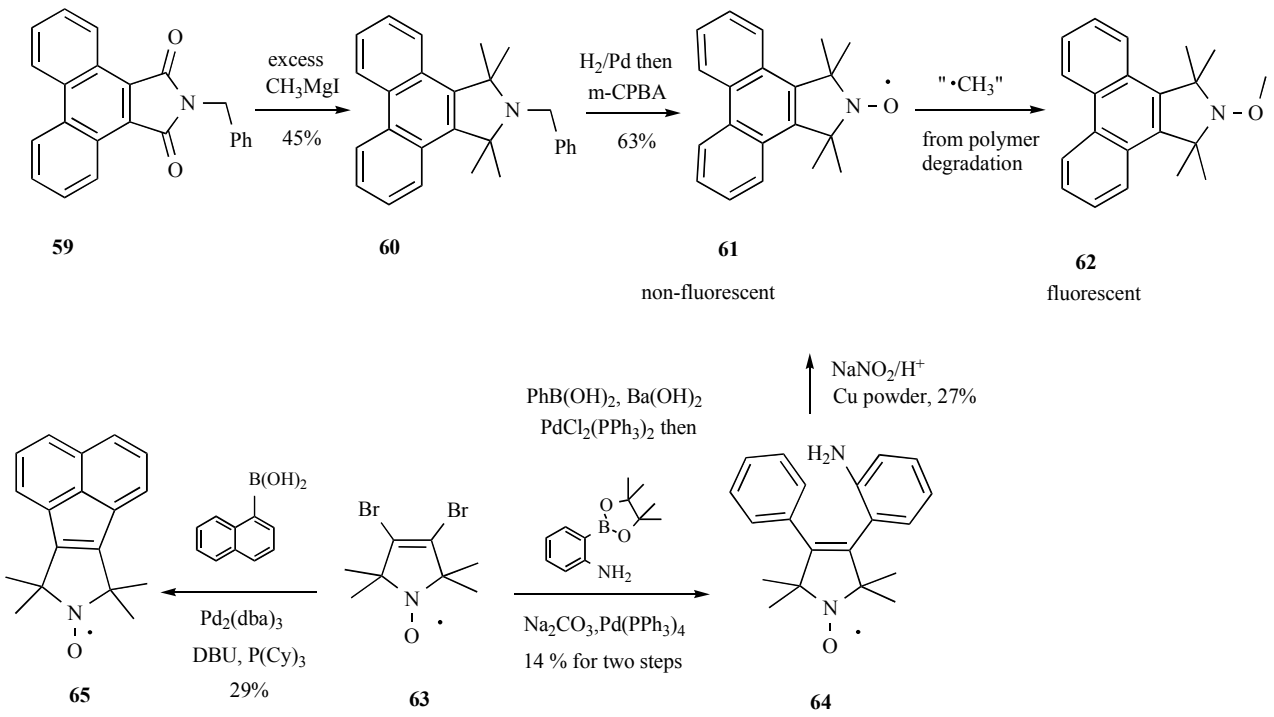
The resulting azaphenalene nitroxide **68** was developed as a profluorescent probe and as a thermal mediator for controlled free radical polymerization. Later, it was discovered that the alkoxyamine derivative **69** readily undergoes homolysis efficiently and neatly reforms nitroxide 68 and a carbon-centered radical upon photoirradiation. This methodology allows the application of **69** in radical insertion reactions to produce polymer **70** and in radical exchange reactions to produce adduct **71** [50] (Scheme **12**).

In 2009, our research group reported a new pathway for the construction of isoindoline nitroxides *via* Suzuzki coupling of compound **39** with vinylboronic acids followed by the Horner Wadsworth-Emmons reaction of triethylphosphonoacetate with aldehydes **72-76** followed by electrocyclization and spontaneous and direct oxidation to offer monosubstituted **19** and 5,6-disubstituted isoindoline nitroxides **77-80**. The hydrolysis of **79** to carboxylic acid **81** followed by treatment with sulfuric acid to initiate an intramolecular Friedel-Crafts reaction produces pyrroline nitroxide-fused fluorenone **82** [51] (Scheme **13**).

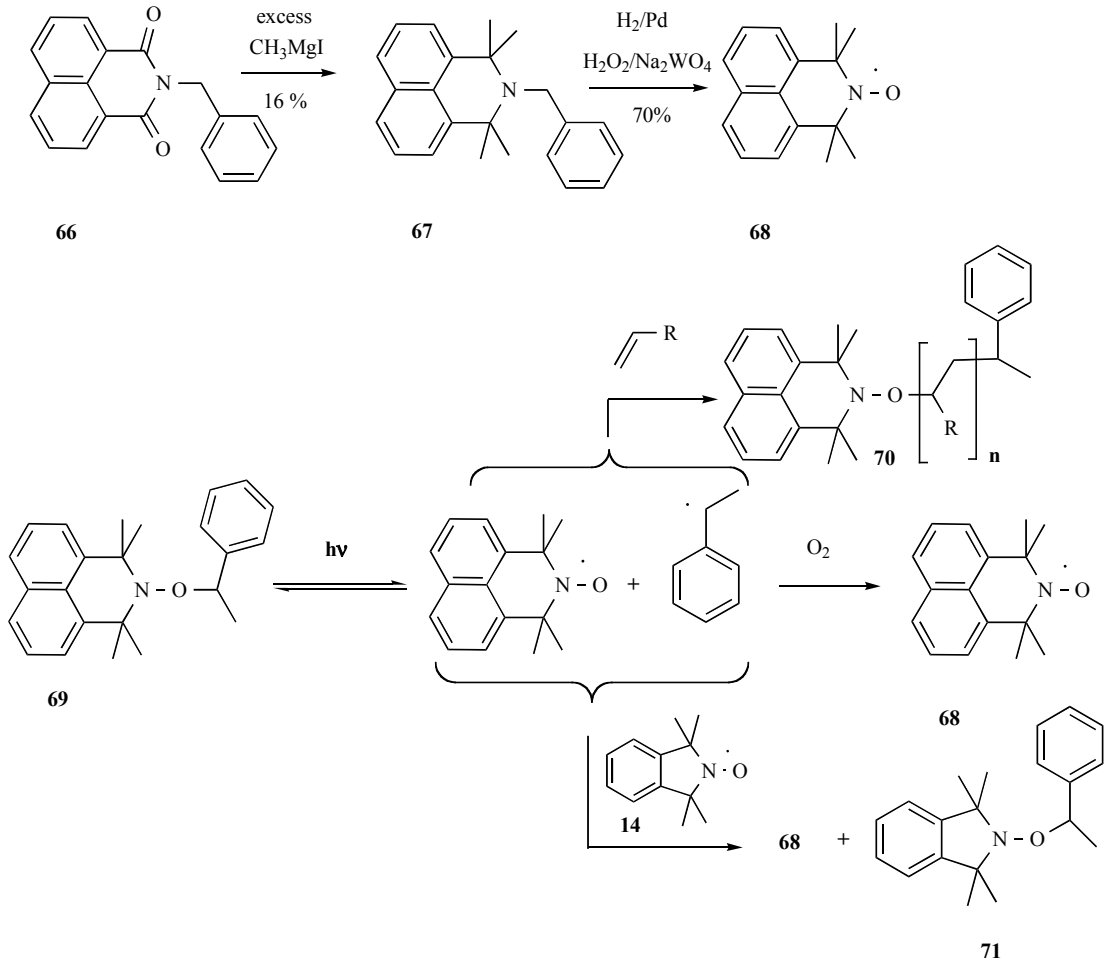
Our laboratory reported the synthesis of 1,4-benzoquinone-fused pyrroline nitroxides *via* a metathesis ring-closing procedure. From the treatment of dialdehyde **48** with excess vinyl-magnesium bromide followed by treatment with a Grubbs II catalyst of diol **83** and oxidation with activated MnO_2 provided compound **84,** which proved to be a useful dienophile partner with cyclopentadiene to yield polycyclic adduct **85** [52] (Scheme **14**).



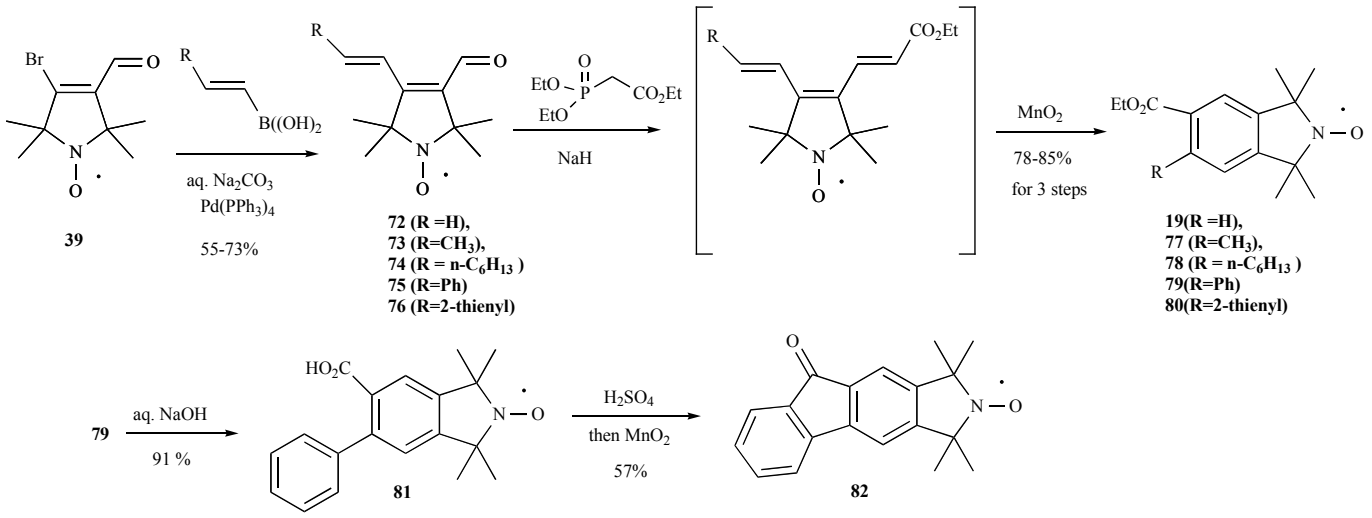
Scheme 10. Synthesis of cyclohexane fused imidazolidine nitroxides.



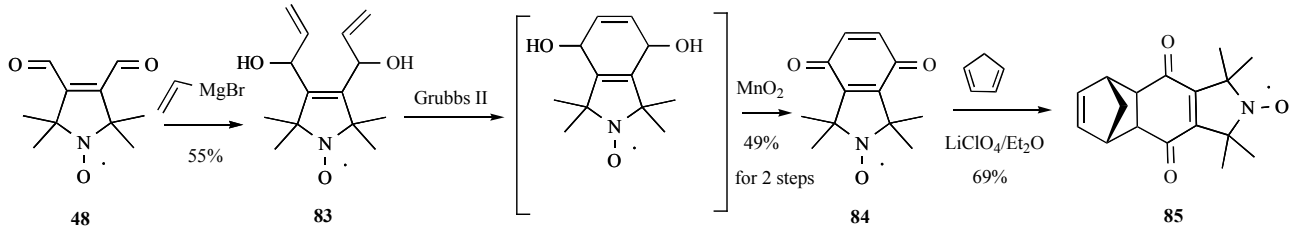
Scheme 11. Synthesis of phenanthrene and acenaphthylene fused pyrroline nitroxides and a possible application.



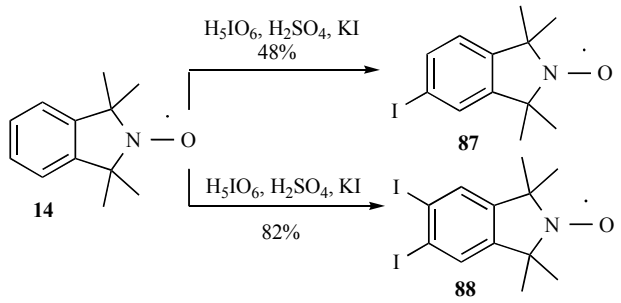
Scheme 12. Synthesis of azaphenalene nitroxide and its utilization as a spin trap.



Scheme 13. Pyrroline nitroxides conversion to-5,6-substituted isoindoline nitroxide.



Scheme 14. Synthesis of 1,4-benzoquinone fused pyrroline nitroxide and its utilization in Diels-Alder reaction.



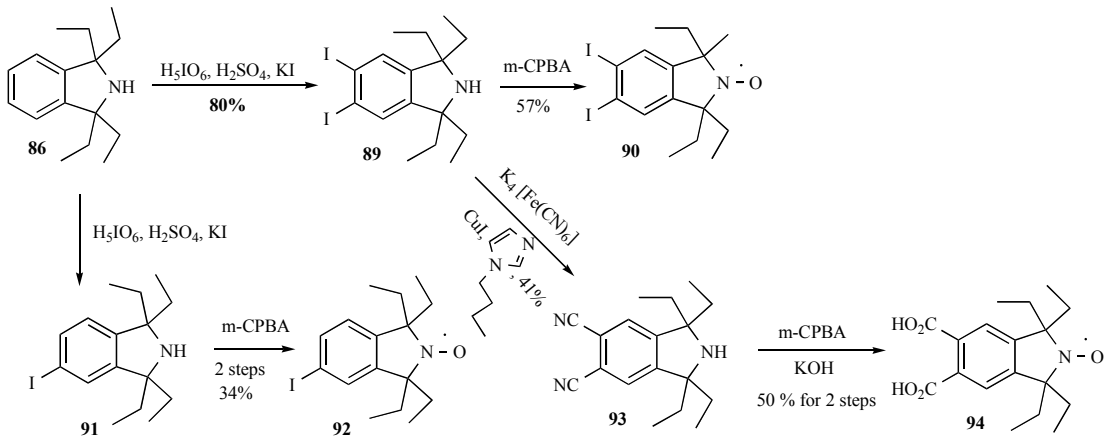
Scheme 15. Iodination of the isoindoline nitroxide.

3. UTILIZATION OF ISOINDOLINES AS PARAMAGNETIC BUILDING BLOCKS

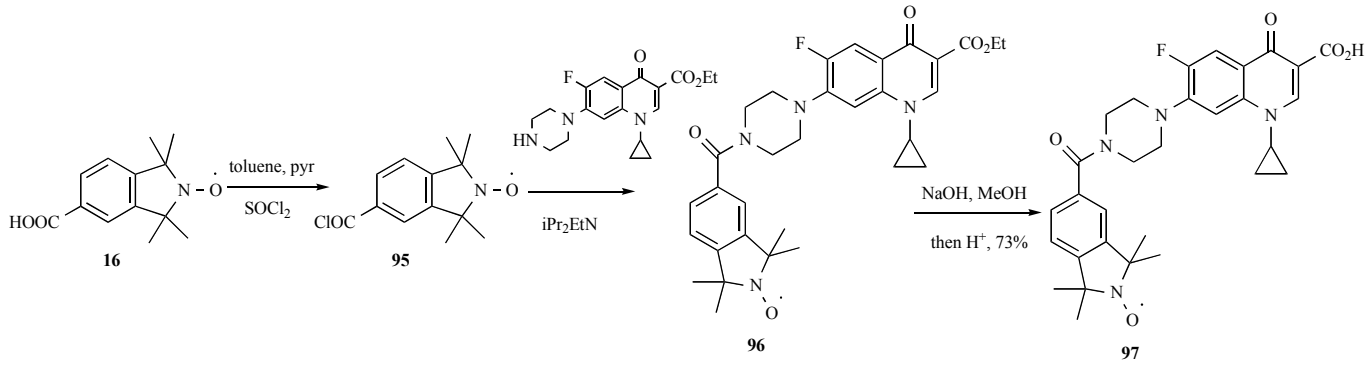
In the past two decades, isoindoline nitroxides have become as popular as pyrrolidine and pyrroline nitroxides in the second half of last century due to new synthetic approaches that have been employed by Bottle's group [53]. Their main applications are the utilization in nitroxide-drug hybrids, the construction of profluorescent nitroxide sensors, and their utilization as spin probes and spin labels. These compounds were used in materials science as well. Their applications are further supported by a systematic study of their redox potentials [54] and possible utilization for EPR oximetry [55]. It is beneficial for this nitroxide family that the introduction of substituents on 1- and 3-positions can be easily varied (methyl, ethyl, phenyl). On the other hand, the functionalization of the aromatic ring is easier *via* aromatic electrophilic or nucleophilic substitution. The introduction of a substituent is easier for isoindoline type nitroxides than that of densely substituted pyrroline nitroxides. A good example of this methodology is the direct iodination of compounds **14** and **86** with H_5IO_6, KI, H_2SO_4 to supply compounds **87-92**. The treatment of compound **89** with $K_4[Fe(CN)_6]$/CuI produced dinitrile **93** and yielded **94** dicarboxylic acid after oxidation and hydrolysis [56] (Schemes **15** and **16**). Similar transformations for the brominated isoindolines were previously reported [53] by this group.

Compounds **16** and **94** were evaluated as nitroxide-based antioxidants, thereby reducing the effects of ROS in the eye *in vivo* [57]. Verderosa D. A. *et al*. reported the complete killing and removal of established bacterial biofilms by the ciprofloxacin-isoindoline nitroxide hybrid **97** that was achieved by acylation with acid chloride **95** to the corresponding ester. The resulting adduct **96** was hydrolyzed to compound **97** (Scheme **17**). Compound **95** was shown to be nontoxic in both human embryotic kidney 293 cells and human muscle rhabdomyosarcoma cells at a concentration of 40 μM [58]. Thomas *et al*. reported the design, synthesis and biological evaluation of hybrid nitroxide-based nonsteroidal anti-inflammatory drug utilization of piperidine, pyrrolidine and isoindoline nitroxides. Among the compounds that were investigated, indomethacin and isondoline nitroxide hybrid **99** exhibited the best protective effect against oxidative stress on 661W retinal neurons with efficacies greater than or equal to the antioxidant Lutein [59] (Scheme **18**).

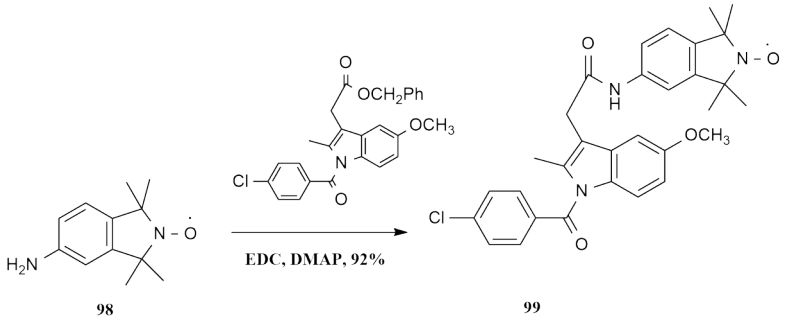
Our laboratory reported the conversion of aldehyde **17** to paramagnetic styrene **100** *via* a Wittig reaction followed by a Heck–coupling with 3,5-diacetoxy-iodobenzene. After removal of the acetyl protecting groups, paramagnetic resveratrol **101** was obtained (Scheme **19**), and its biological activity was evaluated on lipopolysaccharide (LPS)-induced macrophage model of inflammation. Compound **101** suppressed both nitrite and tumor necrosis factor alpha (TNF-α) production [60], with greater efficacy than



Scheme 16. Functionalization of tetraethyl isoindoline nitroxides.



Scheme 17. Synthesis of a ciprofloxacin modified with an isoindoline nitroxid.

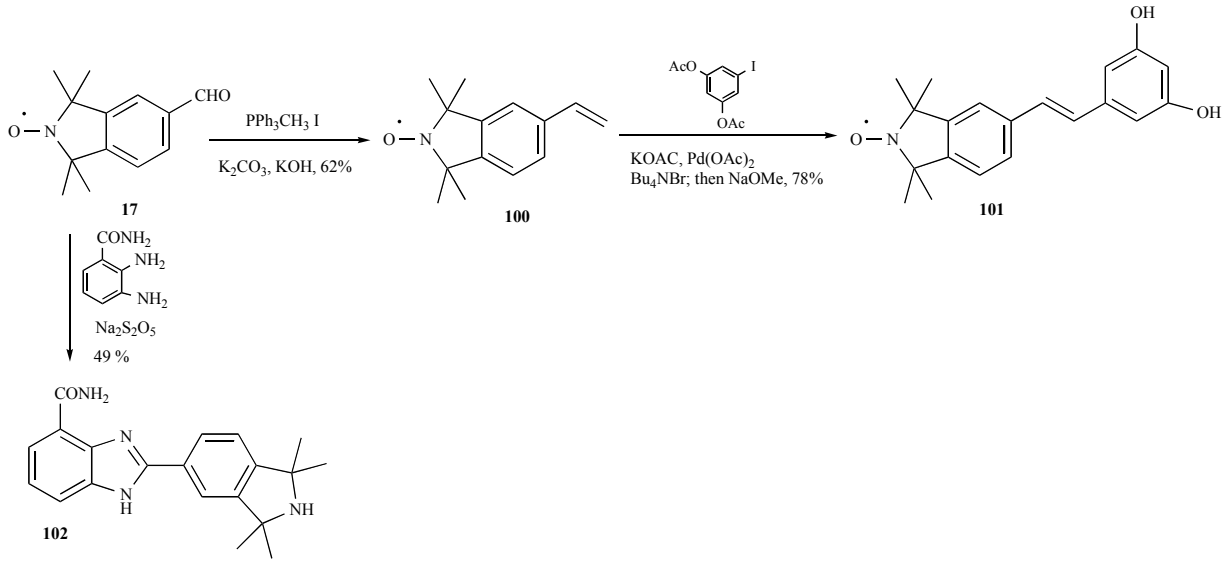


Scheme 18. Synthesis of paramagnetically modified indomethacin.

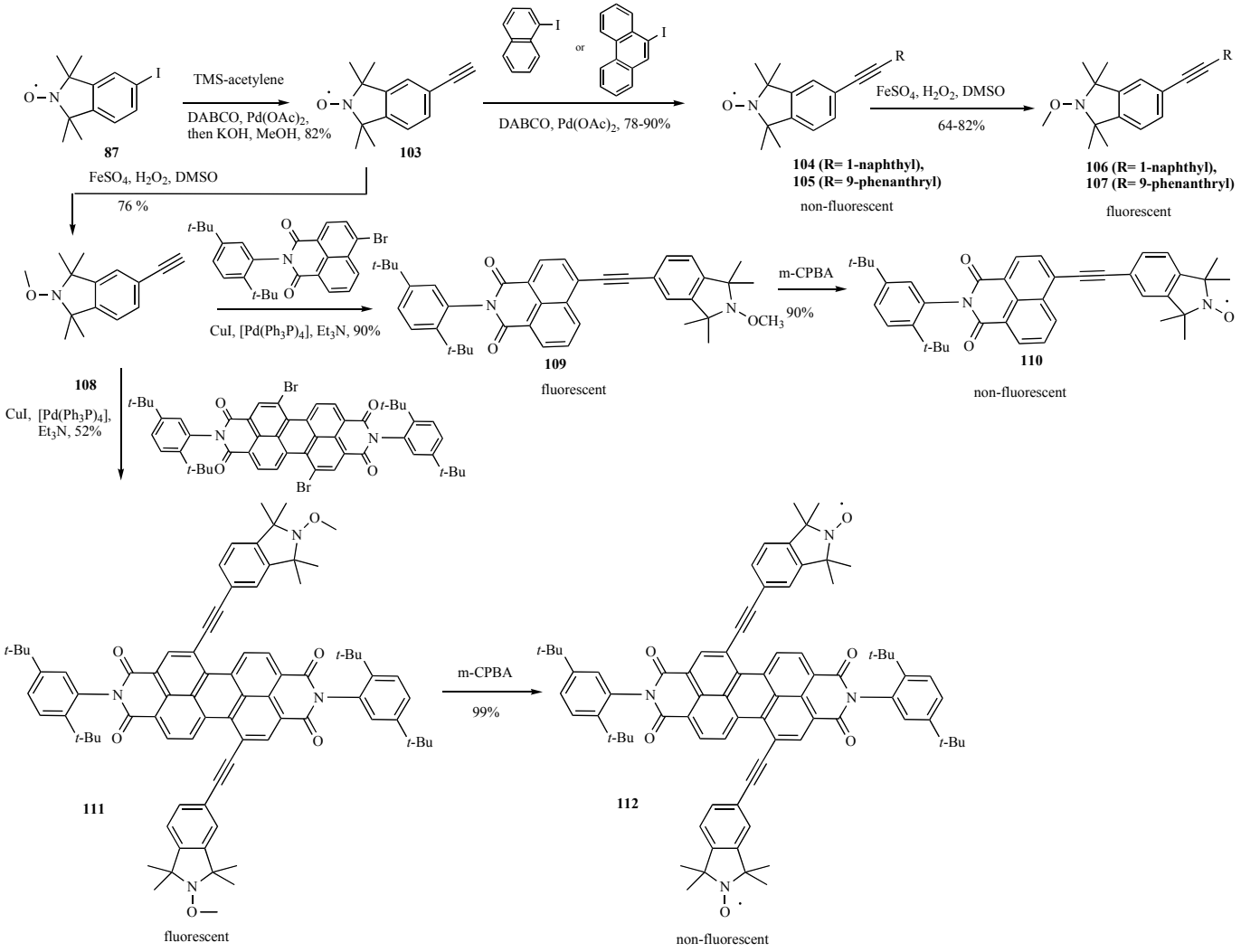
resveratrol. Compound **17** was also used in the synthesis of poly-ADP ribose polymerase (PARP) **102** inhibitor molecule [61]; however, its PARP inhibitor activity (IC_{50} =0.45 μM) was lesser than Veliparib (IC_{50} =3.3 nM) [62].

The Bottle group reported the synthesis of robust, profluorescent isoindoline-type nitroxides with paramagnetic acetylene **103** building block accessed from iodo compound **87** *via* Sonogashira coupling [63]. Further coupling of compound **103** with iodonaphthalene and iodophenanthrene yielded compounds **104** and **105** of which fluorescence was quenched with 200- and 65-fold compared to diamagnetic derivatives **106** and **107**, respectively. This idea was extended for the synthesis of naphthalimide and perylene diimide structural cores with the utilization of compound **103** and its diamagnetic derivative **108** to achieve paramagnetic **109, 111** and diamagnetic **110, 112** adduct series [64] (Scheme **20**). Compound **103** was used to conjugate with azido-coumarin derivatives *via* click chemistry to offer compounds **113, 114** and their diamagnetic pairs such as **115, 116** [65] (Scheme **21**).

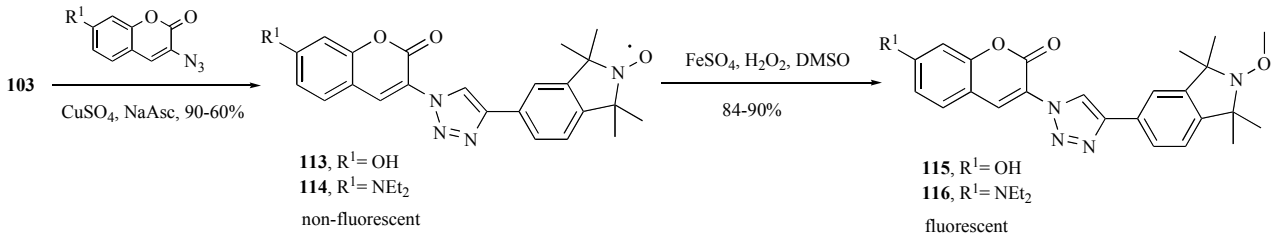
The same group recently reported the conjugation of isoindoline nitroxides with boron-dipyrromethene (BODIPY) dyes starting from aldehyde **17** or acetylene **103**. Compound **117** was accessed by Sonogashira coupling of acetylene **103** and iodo-BODIPY derivative, while compound **119** was synthesized by an aromatic electrophilic substitution reaction of pyrrole followed by a condensation reaction. The dipyrromethane product was oxidized with DDQ and $BF_3{\cdot}Et_2O$ treatment to afford compound **119**. As a sensor molecule, compound **117** is more valuable because the difference in the fluorescence intensities between the paramagnetic **117** and the diamagnetic **118** form is 52-fold, while the difference between compounds **119** and **120** was only 10-fold [66] (Scheme **22**).



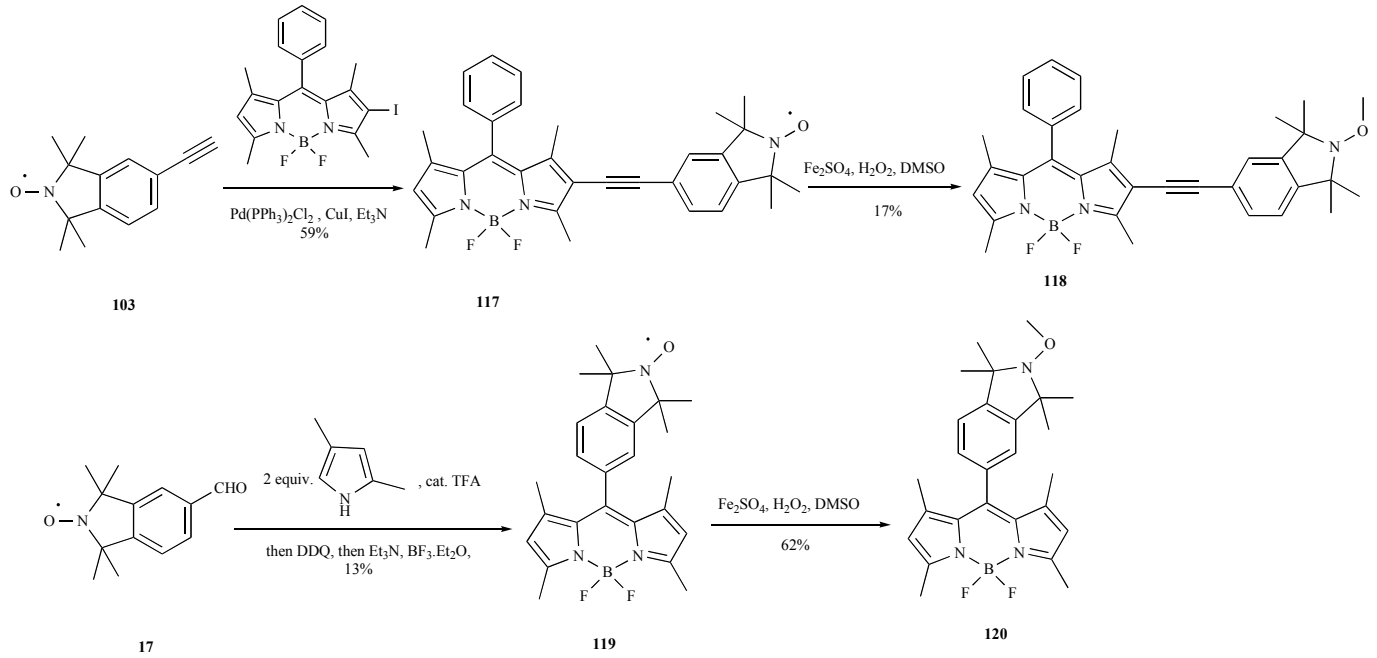
Scheme 19. Synthesis of isoindoline based resveratrol and PARP inhibitor molecule.



Scheme 20. Synthesis of isoindoline nitroxide-fluorophore (naphthalene, phenantrene, naphthalimide, and perylendiimide) adducts with acetylene spacers.



Scheme 21. Synthesis of isoindoline nitroxide-coumarin donor-acceptor adducts with a triazole spacer.



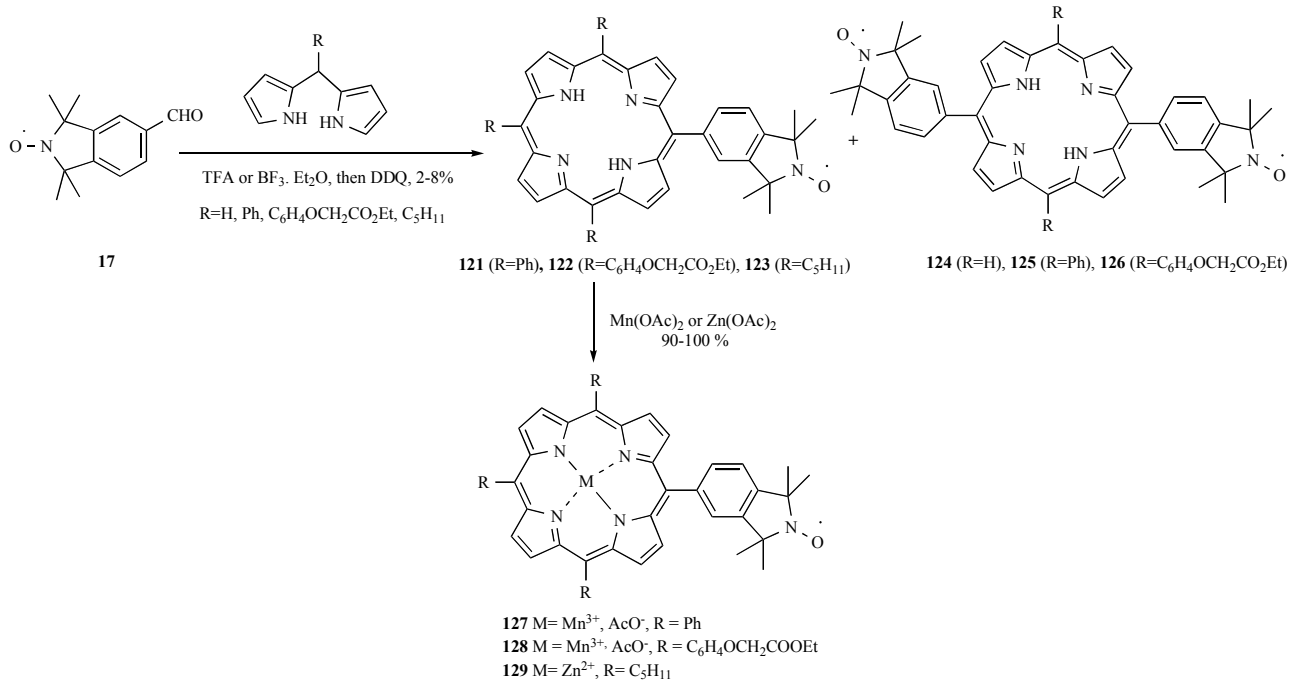
Scheme 22. Synthesis of isoindoline nitroxide BODIPY dye adducts.

Prior to this work, the Bottle group performed the synthesis of porphyrin-based fluorescence sensors from compound **17** to achieve compounds emitting at long (650 and 715 nm) wavelengths [67]. The yields were low (2-8%) to afford a mixture of monoradicals **121-123** and biradicals **124-126**. The low yield was attributed to the oxoammonium ions, which catalyze the polymerization of dipyrromethane. The utilization of a pro-nitroxide in the reaction mixture suppressed the polymer formation; however, the yields have not improved. The fluorescence suppression was the highest in the case of biradicals (16-25-fold) and more limited for monoradicals (5-9-fold). No fluorescence suppression was observed for the corresponding metal complexes **127, 128, 129** (Scheme **23**). Based on this idea, Liu F. *et al*. recently reported new porphyrin scaffolds containing isoindoline nitroxide mono-, di-, and triradicals [68].

Piperidine and pyrroli(di)ne nitroxides are widely used for pre- or postmodification of biomolecules to study their structures and functions. Till now the utilization of isoindoline nitroxides are limited; however, Sigurdsson and coworkers successfully introduced isoindoline nitroxides in the paramagnetic modification of nucleic acids. Recently, they reported isothiocyanates for the modification of 2'-amino groups in RNA with tetramethyl **131** and tetraethyl **132** spin labels to offer adducts **133** and **134** [69]. The latter utilization is more advantageous because of resistance to reduction conditions, a putative circumstance of *in vivo* systems. The thiocianates were synthesized by the reaction of **98** and **130** [70] isoindoline amines with thiophosgene (Scheme **24**).

The Sigurdsson group also reported a synthesis of 5-azido-isoindoline nitroxide **137** from compound **135** with triflyl azide followed by m-CPBA oxidation. This azide was used to modify the 5-ethynyl-2-deoxyuridine derivative in an azido alkyne 1,3-dipolar cycloaddition to yield adduct **138** [71]. Compound **137** and its tetraethyl derivative **139** were used to modify 2'-alkynylnucleotides incorporated at terminal and internal positions on complementary strands of DNA [72]. The postmodification was conducted *via* a copper-catalyzed azide-alkyne cycloaddition to produce adducts **140-145.** This method, developed by Haugland *et al*., allowed the modification of both the pyrimidine and purine nucleotides (Scheme **25**).

An extremely innovative derivatization of uridine was reported by the Sigurdsson group. Compound **135** was diazotized and its hydrolysis provided phenol **146,** the nitration of which to compound **147** followed by reduction produced compound **148**. Treatment of 3',5'-diacetyl-5-bromouridine with **148** produced compound **149** of which ring closure to polycyclic oxazine derivative **150** was initiated by treatment with KF and heating. Oxidation of the sterically hindered amine yielded nitroxide **151**. The authors reduced compound **151** to hydroxylamine sulfurous ester **152**, which is an EPR silent, but fluorescent nucleoside unit or converted it to phosphoramidite **153**, which is capable of DNA synthesis [73] as a paramagnetic building block (Scheme **26**).



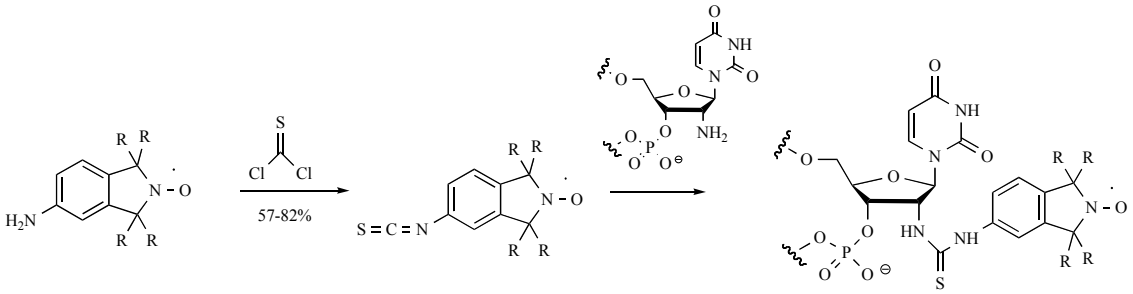
17

121 (R=Ph), **122** (R=$C_6H_4OCH_2CO_2Et$), **123** (R=C_5H_{11})

124 (R=H), **125** (R=Ph), **126** (R=$C_6H_4OCH_2CO_2Et$)

127 M= Mn^{3+}, AcO^-, R = Ph
128 M = Mn^{3+}, AcO^-, R = $C_6H_4OCH_2COOEt$
129 M= Zn^{2+}, R= C_5H_{11}

Scheme 23. Synthesis of paramagnetic porphyrines with isoindoline motifs.

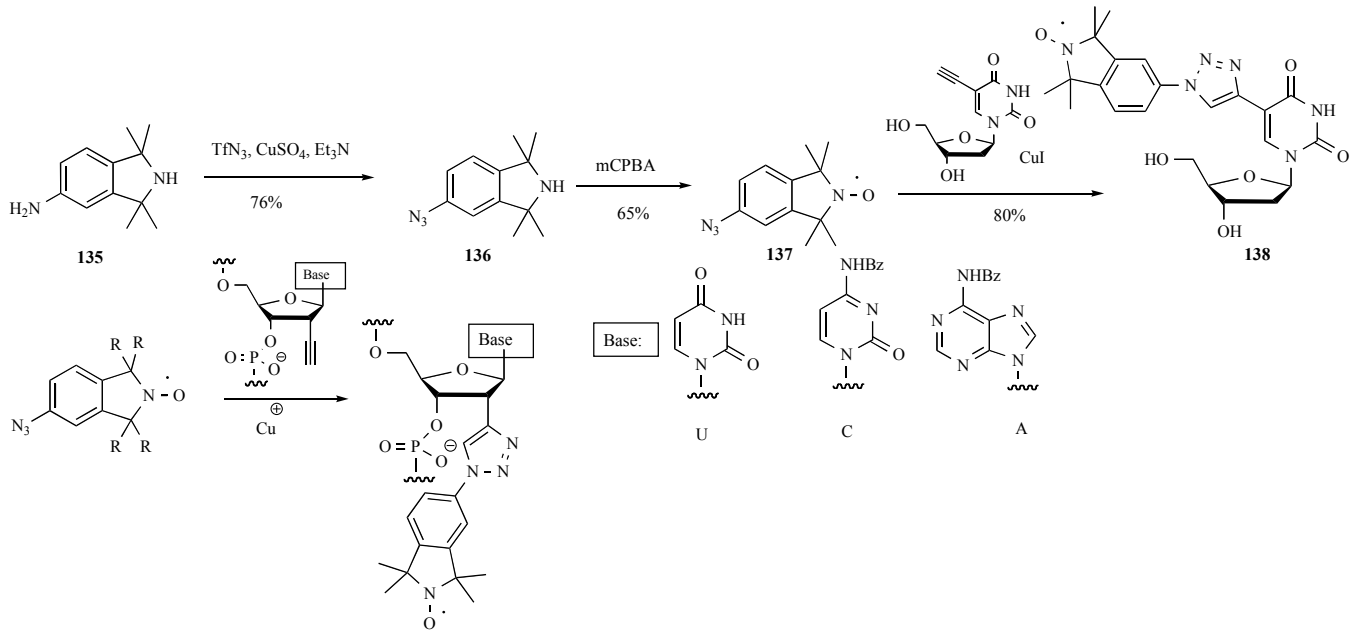


98 (R =CH_3)
130 (R=C_2H_5)

131 (R =CH_3)
132 (R=C_2H_5)

133 (R =CH_3)
134 (R=C_2H_5)

Scheme 24. **N**ucleotides and nucleosides modified by paramagnetic isothiocyanates.



135

136

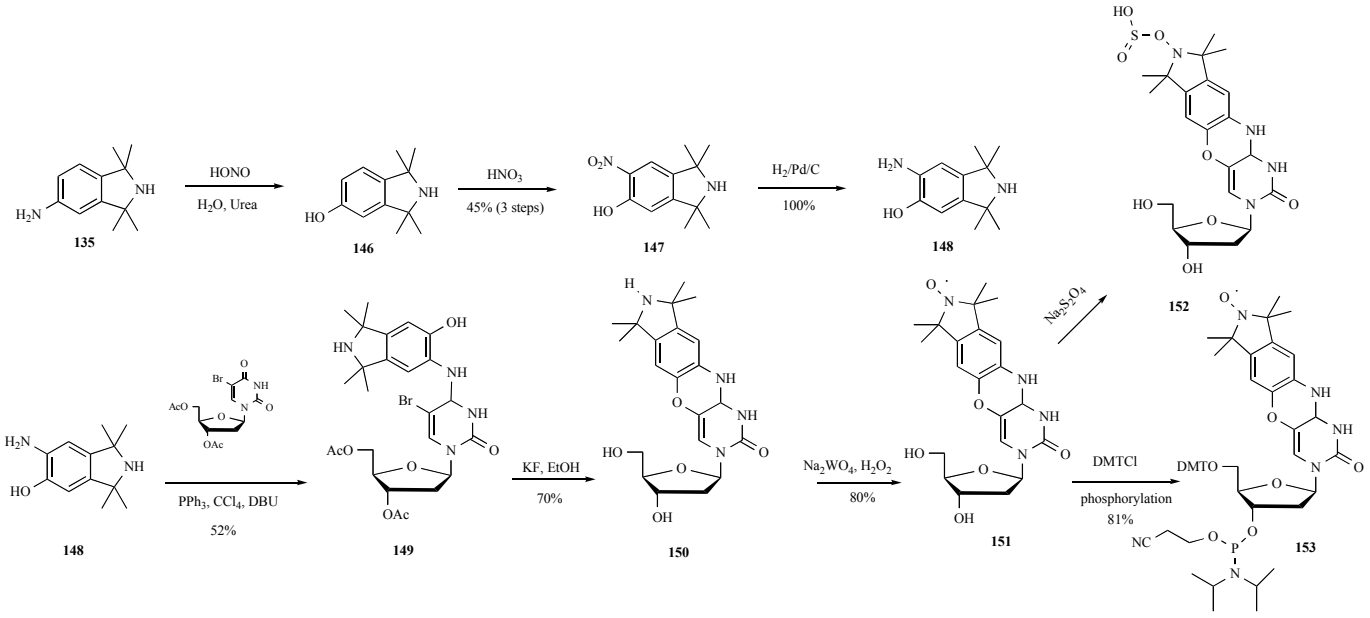
137

138

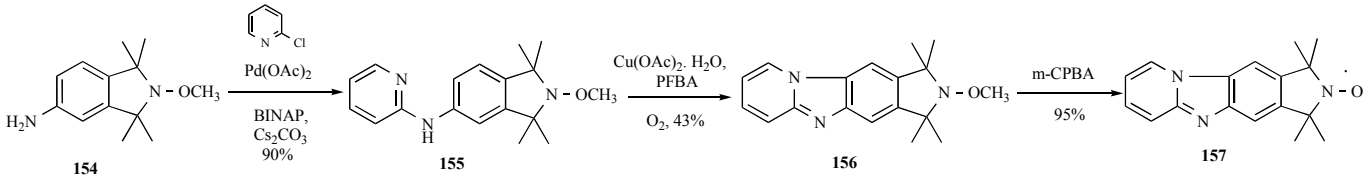
137 R=CH_3
139 **R**=C_2H_5

140 R=CH_3 (U), **142** R=CH_3 (C), **144** R=CH_3 (A)
141 R=C_2H_5 (u), **143** **R**=C_2H_5 (C), **145** R= C_2H_5 (A)

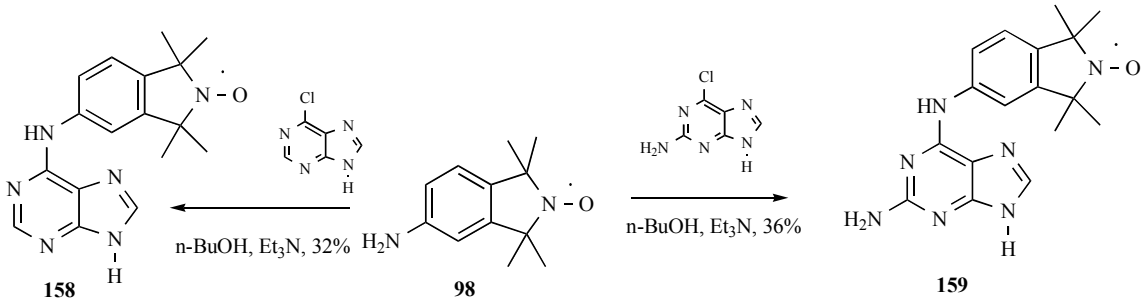
Scheme 25. Synthesis of nucleotides and nucleosides modified by isoindoline nitroxides.



Scheme 26. Synthesis of paramagnetically modified uridines with polycyclic isoindoline nitroxide.



Scheme 27. Synthesis of a pyrido[1,2-a]benzimidazole scaffold for noncovalent nucleic acid probe.

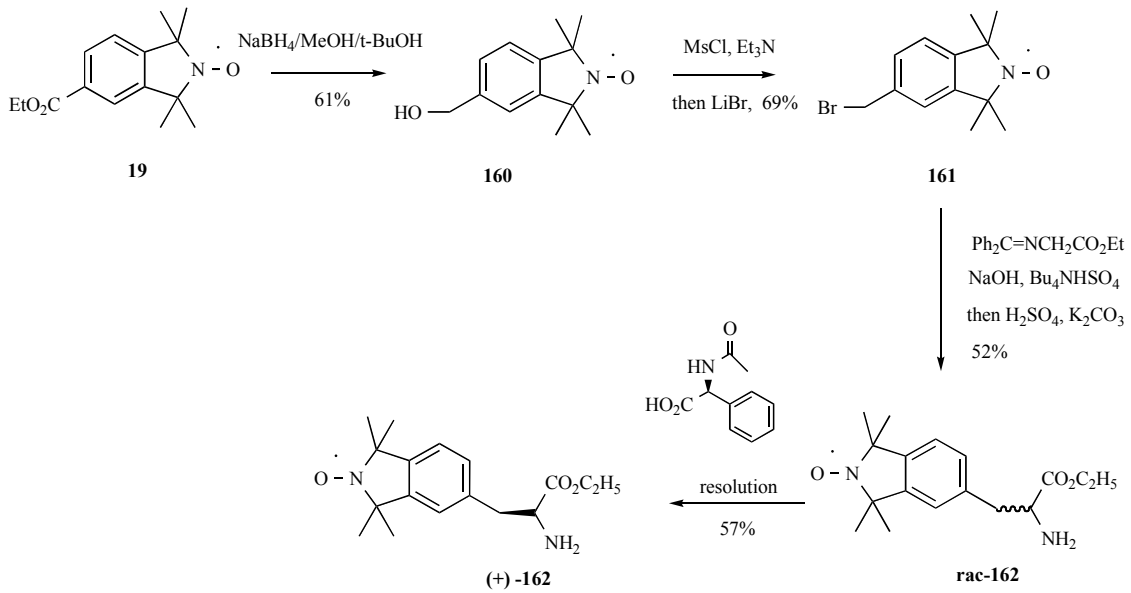


Scheme 28. Synthesis of purines modified by isoindoline nitroxides for noncovalent nucleic acid probe.

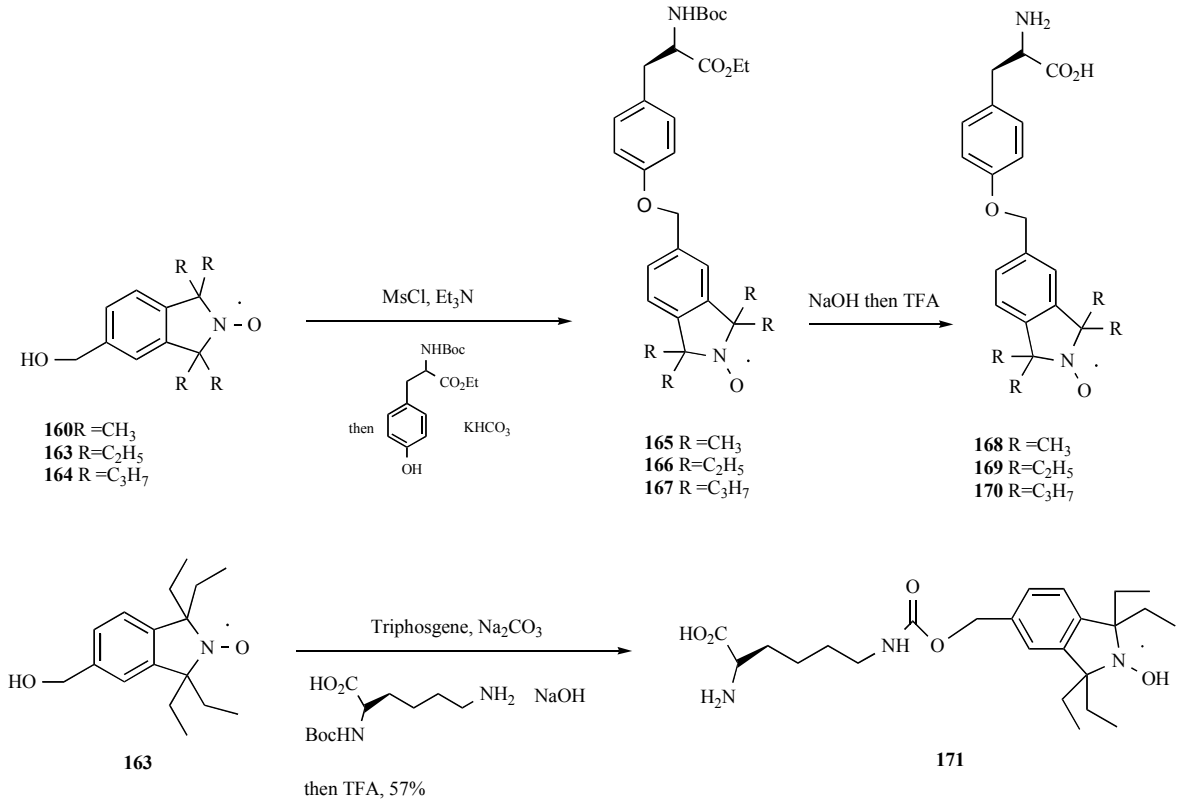
Based on a similar synthetic strategy for hexahydropyrimido[4', 5':5,6][1,4]oxazino[2,3-f]isoindol-2(1*H*)-one scaffold the authors reported noncovalent spin labels as well [74] for an abasic site containing DNA. In 2014, Bottle and Sigurdsson reported the combination of an isoindoline and pyrido[1,2-a]benzimidazole scaffold for a noncovalent nucleic acid probe interacting with C (DNA) and C/U (RNA). Compound **154** was cross-coupled with 2-chloropyridine in a Pd–catalyzed reaction to afford compound **155** of which copper (II) acetate and perfluoroboronic acid (PFBA) catalyzed reaction afforded polycycle **156** [75]. The nitroxide group was reconstructed with *m*-CPBA oxidation to provide nitroxide **157** (Scheme **27**).

Recently, the Sigurdsson group reported purine-based spin labels as noncovalent modifications to the abasic sites of duplex nucleic acids through hydrogen bonding to an orphan base on the opposing strand and π-stacking interactions with the flanking bases. The isoindoline–derived spin labels **158** and **159** showed extensive or full binding to the abasic sites in the RNA duplexes [76]. These spin probes were achieved by nucleophilic substitutions from 2-or 6-chloropurines and compound **98** (Scheme **28**).

Pre- or postmodification of proteins with stable nitroxide free radicals is a powerful tool to study the structure and functions of biomolecules. Premodification can be conducted by the incorporation of unnatural (paramagnetic) amino acids into the protein *via* a Merrifield-type synthesis or *via* a solid phase peptide synthesis. The other option is utilization of translational machinery that consists of a *t*-RNA and an aminoacyl tRNA synthase to recognize the unnatural amino acids and incorporate them in protein structure into a specific protein site. To produce substrates, *e.g.*, paramagnetic amino acids, our laboratory reported a series of spin labeled amino acids including the isoindoline-based phenylalanine **162** [76]. The reduction of ester **19** provided alcohol **160**, which was further converted to paramagnetic benzylbromide **161**, which was applied in an O'Donnell amino acid synthesis to offer a racemic mixture of **162** (*rac*-**162**). The mixture was resolved with (R)-*N*-acetylphenylglycine to give (+) **162** spin labeled amino acid (Scheme **29**).



Scheme 29. Synthesis of an isoindoline nitroxide based (+) phenylalanine.

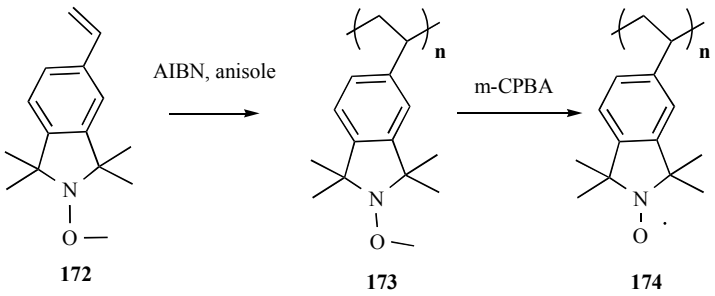


Scheme 30. Synthesis of a paramagnetic tyrosine and a lysine modified by shielded isoindoline nitroxides.

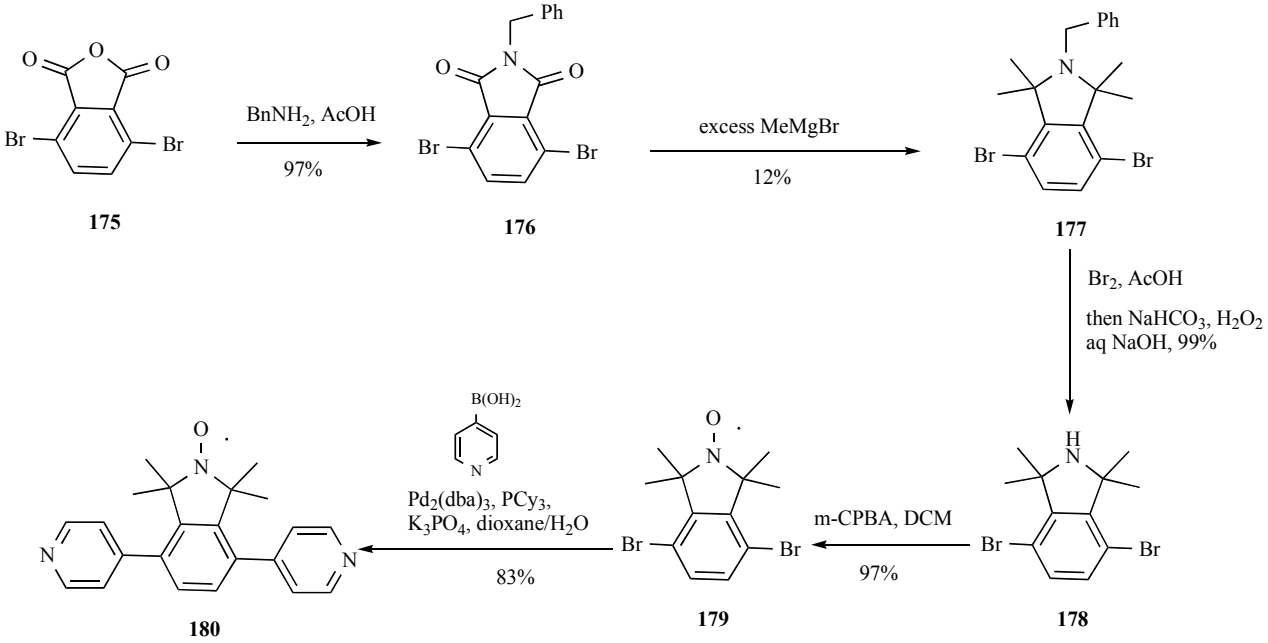
In 2015, Summer *et al*. patented a series of isoindoline-based paramagnetic unnatural amino acids (**168, 169, 170, 171**) starting from natural *N*-Boc-L-tyrosine and *N*-Boc-L-lysine [78]. The tyrosine derivatives were achieved by alkylating the phenolic hydroxyl group of the protected *N*-Boc-tyrosine ester with mesylates of alcohols **160**, **163 and 164**. Then, hydrolysis offered the spin labeled L-tyrosines (**168, 169, 170**). The patent did not report the yields of the isoindoline-tyrosine adducts. The detailed synthesis of the paramagnetic L-lysine derivative was provided, **171**, which was obtained from conjugation of alcohol **163** and *N*-Boc-L-lysine with triphosgene (Scheme **30**).

Paramagnetic amino acid **171** was incorporated into thioredoxin using amber codon at R74, which was coexpressed with a mutant pyrrolysyl-*t*RNA synthase. The advantage of this paramagnetic amino acid synthesis is that no resolution or stereoselective synthesis is required; moreover, the shielded nitroxide moieties in amino acids **169, 170** and, **171** exhibit enhanced bioreductive stability.

In the field of materials science, isoindoline nitroxides have found limited applications until now; however, because of their increased thermal and chemical stability, their roles will emerge and the field of applications looks promising. Furthermore, we will



Scheme 31. Synthesis of paramagnetic polystyrene as electroactive material decorated with isoindoline nitroxides.



Scheme 32. Synthesis of isoindoline nitroxide as a building block for a porous polymer with catalytic activity.

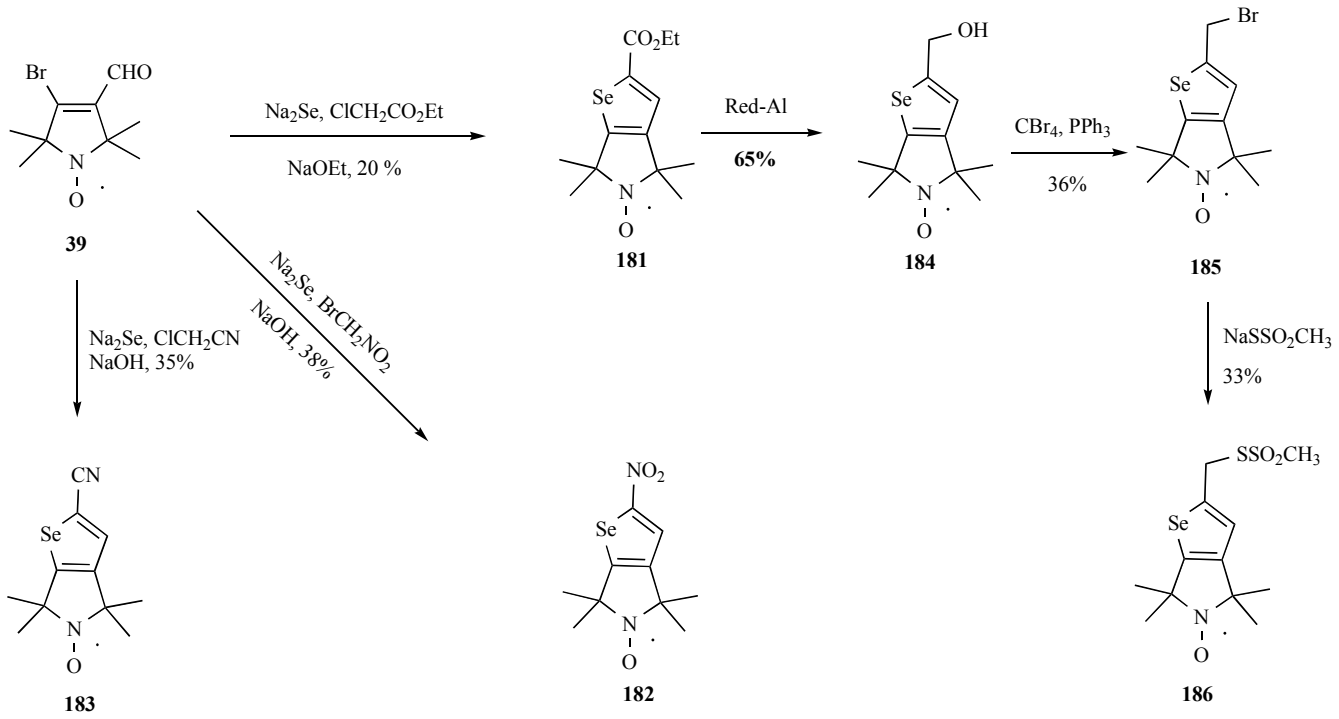
see further developments and discoveries over the coming years. This prediction is well supported by the Bottle group's recent paper that reports the 1,1,3,3-tetramethylisoindoline-2-yloxyl radical as an organic electrode material, and the synthesis and the application of a novel styrenic nitroxide polymer, poly(5-vinyl-1,1,3,3-tetramethylisoindolin-2-yloxyl [79]. The polymer was synthesized from 2-methoxy-5-vinyl-1,1,3,3-tetramethylisoindoline **172**, the radical polymerization of which is initiated by azobisisobutyronitrile (AIBN) to yield compound **173**; subsequent oxidative deprotection yielded the electroactive polynitroxide material **174** (Scheme **31**). The suitability of polymer **174** for utilization in an organic radical battery was confirmed with an investigation (90% capacity retention after 100 cycles), indicating it as one of the highest dopable cathode materials reported.

Japanese researchers have reported the synthesis of a porous coordination polymer employing an organic ligand with the 1,1,3,3-tetramethyl-4,7-(dipyrid-4-yl)isoindoline-2-yloxyl radical **180**, incorporated [80]. This porous polymer catalyzed the oxidation of various alcohol substrates to corresponding aldehydes or ketones when O_2 or air was the oxidant. This porous polymer, which is decorated with nitroxide **180**, is an efficient, recyclable and widely applicable selective catalyst. An unusual step in the synthesis of **180** is the deprotection of the compound **177** benzyl group with bromine treatment followed by oxidation and hydrolysis (Scheme **32**).

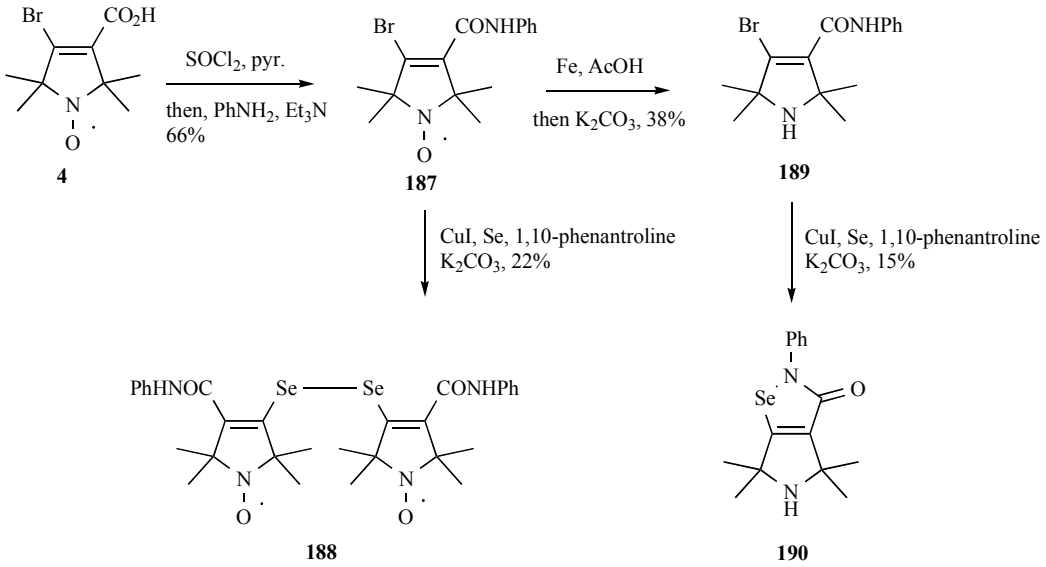
4. NITROXIDES FUSED WITH FIVE-MEMBERED HETEROCYCLES

In the previously discussed papers, the isoindoline nitroxides contained a benzene ring fused with the pyrroline nitroxide unit. Although several papers have described polyheterocyclic systems fused with nitroxides [81, 82], few have described the pyrroline/piperidine ring and a heterocycle direct condensation. In the past decade, our laboratory has reported several papers in which a five-membered heterocycle is fused with a pyrroline or piperidine nitroxide. In continuation of thiophene-fused nitroxides [39], we obtained 5*H*-selenolo[2,3-c]pyrrole scaffolds **181-183** from aldehyde **39** by Na_2Se treatment followed by chloroacetic acid ester or bromonitromethane or chloroacetonitrile, respectively, in the presence of a base [83] (Scheme **33**).

Ester **181** was reduced to alcohol **184,** which was further substituted to bromine **185** *via* an Appel reaction, followed by substitution with $NaSSO_2CH_3$ to obtain an SH-specific methanethiosulfonate spin label **186.** We intended the *N*-phenyl [1,2]selenazol ring synthesis fused to nitroxide. However, following the utilization of standard reaction conditions [82], we observed the formation of diselenide **188** from anilide **187**. Therefore, ring closure was attempted from the diamagnetic derivative **189** to give pyrrolo[3,4-d][1,2]selenazol-3(4H)-one scaffold **190**, although in a low (15%) yield (Scheme **34**) [52].



Scheme 33. Synthesis of selenophene fused nitroxides.

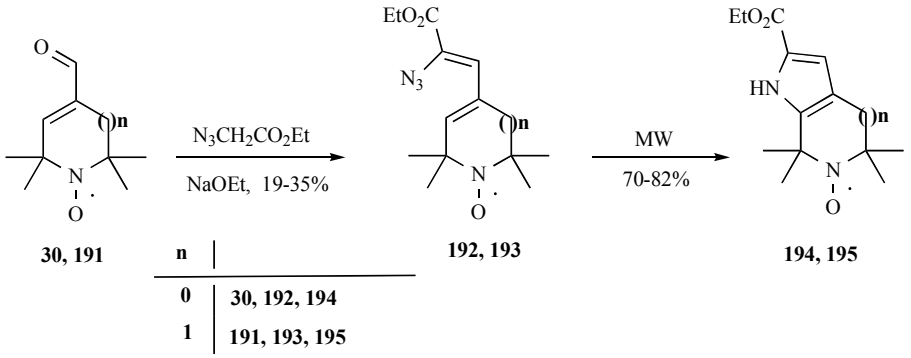


Scheme 34. Synthesis of 1,2-isoselenazole-fused prenitroxide.

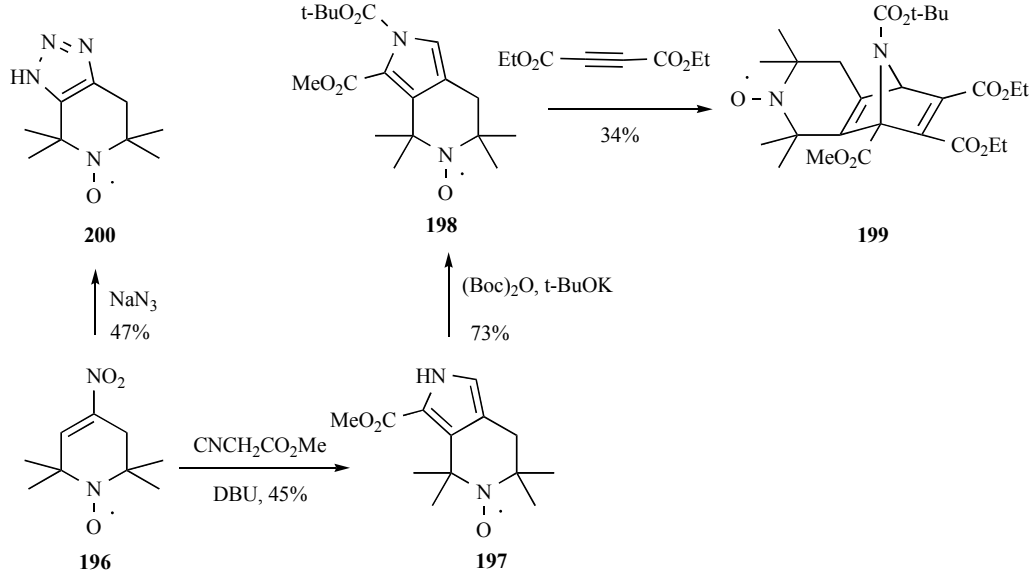
Although the synthesis of the pyrrole-fused nitroxide ring was reported previously [44], in 2015, we found a new approach; aldehydes **30** and **191** fused with-2-azidoacetate produced vinyl azides **192** and **193**. The vinyl azides heated in hexanes under MW irradiation in Hemetsberger–Knittel reactions offered pyrrolo[3,4-b]pyrrol **194** and pyrrolo[2,3-c]pyridine **195** scaffolds (Scheme **35**).

The other isomer of compound **195** was achieved from α,β-unsaturated nitro compound **196** in a Barton-Zard reaction to offer compound **197**. The *N*-tert-butoxycarbonyl derivative of methyl pyrrole-2-carboxylate **198** was used in a Diels-Alder reaction to furnish polycyclic compound **199.** It is interesting to note that nitro compound **196** with sodium azide in DMSO offered 4.4,6,6-tetramethyl-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-c]pyridine-5-yloxyl radical **200** [84] (Scheme **36**).

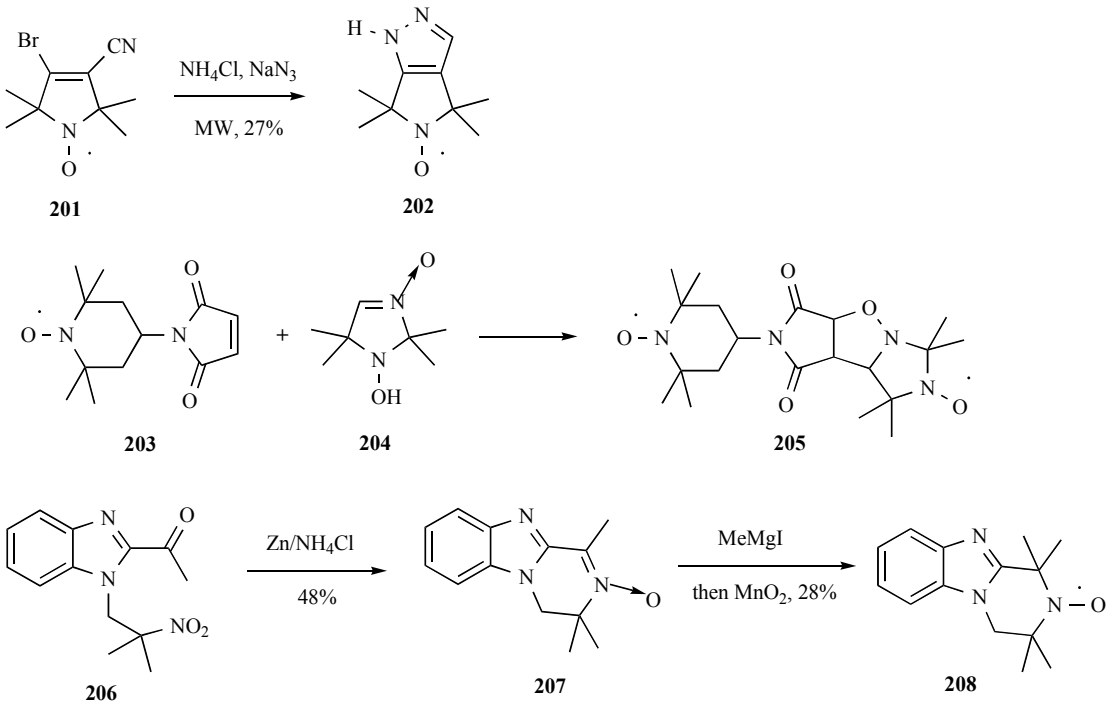
In this paper, we described the synthesis of dihydropyrrolo[3,4-c] pyrazole **202** from treatment with nitrile **201**, which afforded a fused pyrazole compound instead of tetrazole ring formation [84]. Recently, S. Bothe and coworkers reported the synthesis and study series of trityl-nitroxide hybrids as a polarization source for ultra-high field dynamic nuclear polarization [85]. They also described the synthesis of imidazo[1,5-b]pyrrolo[3,4-d]isoxazole biradical **205** as a product of the 1,3-diplolar cycloaddition of maleimido TEMPO **203** and 2,2,5,5-tetramethyl-2,5-dihydroimidazol-1-oxyl-3-oxide **204**. The benzimidazole fused nitroxide was described by Bognár *et al*. in 2008 [86]. The 2,2,6,6-tetramethylpiperazine ring was developed on the 1,3-diazole ring unit. The reduction of keto-nitro compound **206** gave nitrone **207**, the treatment of which with CH_3MgI furnished nitroxide **208** (Scheme **37**).



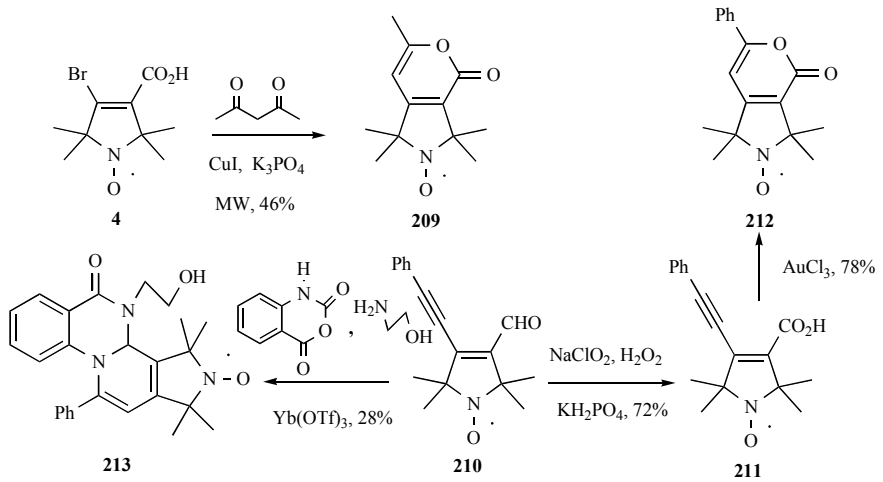
Scheme 35. Synthesis of substituted pyrrole fused nitroxides with Hemetsberger–Knittel reactions.



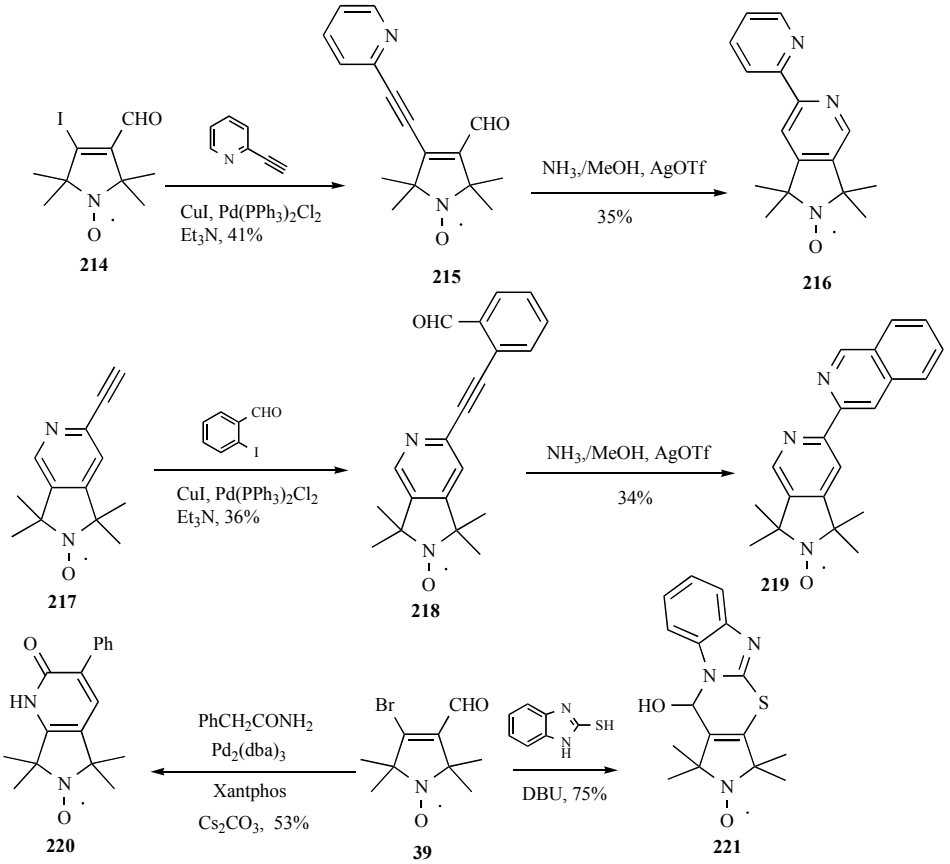
Scheme 36. Synthesis of tetrahydropiperidine fused *N*- heterocycles.



Scheme 37. Synthesis of 1,2- and 1,3-azoles fused nitroxides.



Scheme 38. Synthesis of δ-lactones and a polycycle fused nitroxides.

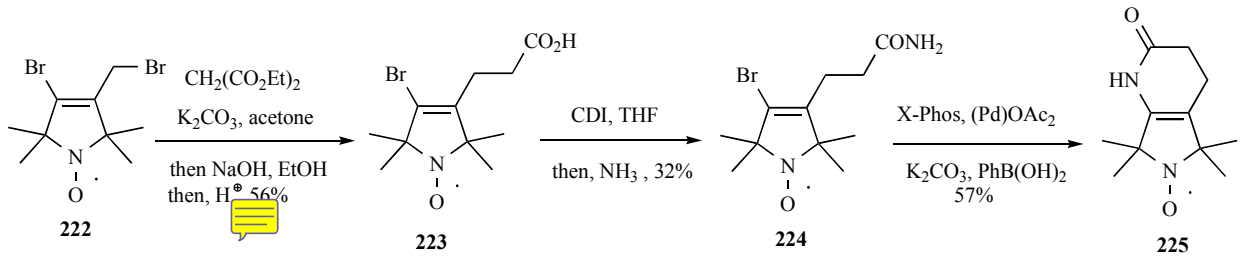


Scheme 39. Synthesis of pyridine, 1H-pyrid-2-one and thiazine fused pyrroline nitroxides.

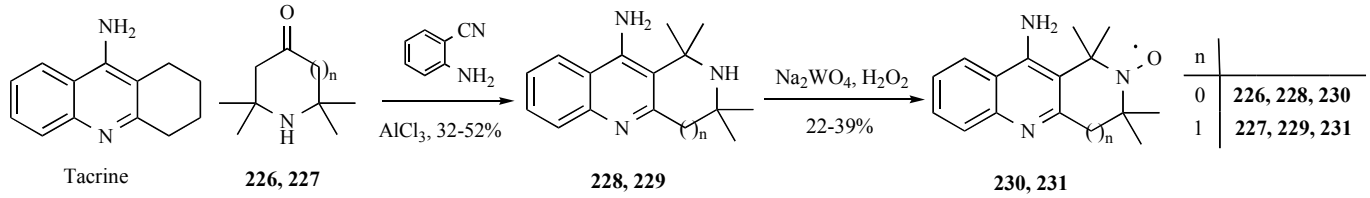
5. NITROXIDES FUSED WITH SIX-MEMBERED HETEROCYCLES

Starting from carboxylic acid **4**, treatment with pentane-2,4-dione and CuI in the presence of a base and microwave irradiation yielded lactone **209**. Its phenyl substituted analogue **212** was achieved from compound **210** [87] of which oxidation gave carboxylic acid **211**, which could be cyclized to **212** with $AuCl_3$. In a multicomponent reaction, compound **210** with ethanolamine, isatoic anhydride with $Yb(OTf)_3$ as catalyst offered 4-(2-hydroxyethyl)-1,1,3,3-tetramethyl-5-oxo-11-phenyl-1,2,3,3b,4,5-hexahydro-pyrrolo[3',4':3,4]pyrido[1,2-a]quinazolin-2-yloxyl radical **213** (Scheme **38**) [52].

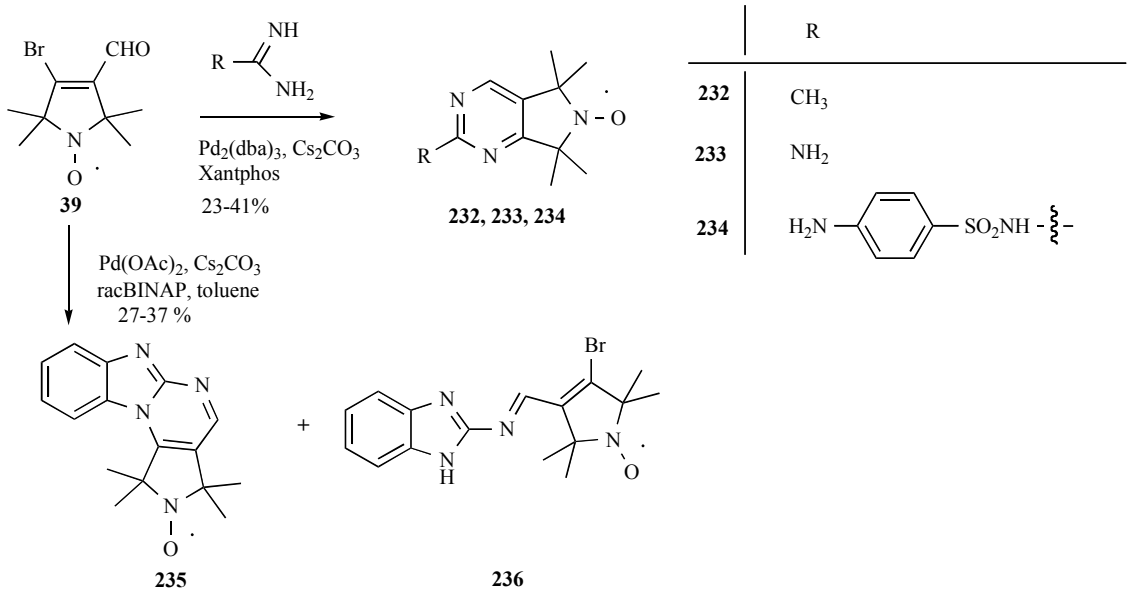
Furthermore, the synthesis of pyridine-fused pyrroline nitroxide was reported very recently by our laboratory. Aldehyde **214** was coupled with 2-ethynylpyridine with Sonogashira coupling to yield compound **215**. After treating of this acetylene compound with an AgOTf catalyst in methanolic ammonia solution, we obtained paramagnetic α,α'-dipyridyl **216**, a paramagnetic ligand [88]. A similar paramagnetic ligand, **219,** synthesis was reported in 2015 by our laboratory, starting from paramagnetic 2-ethynyl pyridine **217** [89] obtained by the reaction of the Bestman-Ohira reagent with a paramagnetic aldehyde [61]. The Sonogashira coupling with 2-iodobenzaldehyde yielded compound **218**, the ring closing of which under the aforementioned conditions gave compound **219**. Reaction



Scheme 40. Synthesis of paramagnetic piperid-2-one.



Scheme 41. Tacrine and synthesis of paramagnetic Tacrine analogs and their precursors.



Scheme 42. Synthesis of pyrimidine fused pyrroline nitroxides.

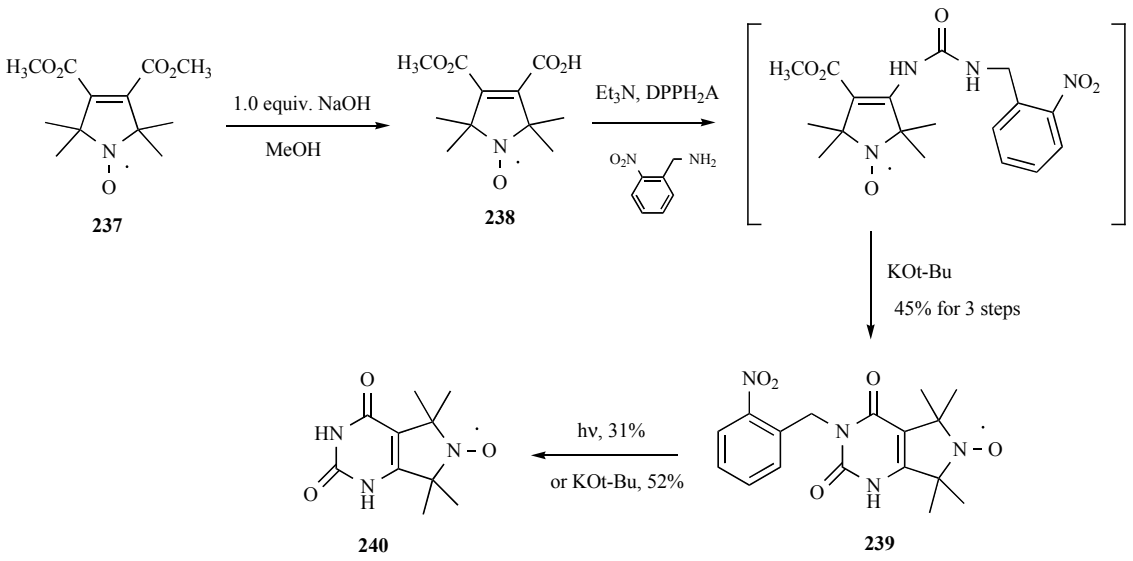
of aldehyde **39** under Buchwald-Hartwig amidation conditions in a single step *via* a one-pot procedure provided pyrrolo[3,4-b]pyridine scaffold **219**. While the reaction of aldehyde **39** with 2-mercaptobenzimidazole as a bisnucleophile furnished 11-hydroxy-1,1,3,3-tetramethyl-1,2,3,11-tetrahydro-benzimidazo[2,1-b]pyrrolo [3,4][1, 3]thi-azin-2-yloxyl radical **221** [90] (Scheme **39**).

The piperid-2-one fused pyrroline nitroxide **225** was achieved *via* an intramolecular Buchwald–Hartwig amidation reaction. Compound **222** was used as alkylating agent in a malonester synthesis, and the resulting carboxylic acid **223** was converted to amide **224** followed by ring closure reaction to offer compound **225** [89] (Scheme **40**).

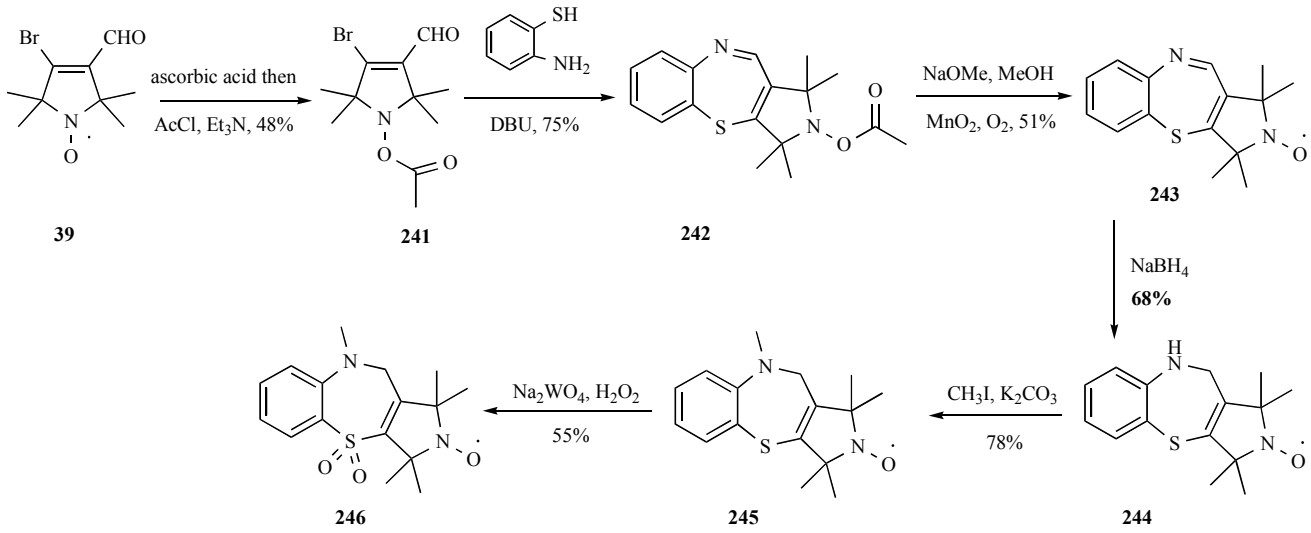
In 2014, our group reported tacrine-nitroxide hybrid experimental drugs with acetylcholinesterase inhibitory and antioxidant activity [91]. As a part of this study of the sterically hindered ketones **226** and **227**, we have synthesized the corresponding amines **228** and **229** in a modified Friedlander synthesis with reactions of compounds **226** and **227** and anthranylonitrile in the presence of Lewis acid, which was converted to nitroxides **230** and **231** with Na_2WO_4/H_2O_2. Unfortunately, the Tacrine chimeras lost their acetylcholinesterase inhibitory activity, however, were protected against amyloid beta induced cytotoxicity (Scheme **41**).

Nitroxide-fused diazines, such as pyridazine **49** or pyrazine **46,** were described earlier [27, 43]; however, limited or no example was found on pyrimidine-fused nitroxides, although it is the most important diazine from a biological perspective. In 2017, we reported the synthesis of pyrroline nitroxide-fused pyrimidines **232, 233** and **234** from compound **39** [88]. The corresponding guanidines or amidines were reacted with β-bromo-α,β-unsaturated aldehyde **39** under Buchwald-Hartwig amidation conditions to provide pyrimidine-fused nitroxides **232-234**. Compound **234** can be regarded as a paramagnetic analog of the sulfonamide drug, sulfadiazine. Compound **39** reaction with 2-aminobenzimidazole produced 1,1,3,3-tetramethyl-1*H*-benzimidazo[1,2]pyrrolo[3,4-e]pyrimidin-2-yloxyl radical **235**, with formation of Schiff-base **236** [92] (Scheme **42**).

The pyrroline nitroxide-fused uracil for nucleic acid labeling was achieved from diester **237**, the partial hydrolysis of which pro-



Scheme 43. Synthesis of uracil fused pyrroline nitroxide.



Scheme 44. Synthesis of pyrroline fused benzo[1,5]thiazepines.

vided monoester **238**. This monoester was converted to an acyl azide by diphenylphosporyl azide (DPPA) followed by a Curtius rearrangement and treatment with 2-nitrobenzylamine furnished urethane, which was not isolated. Cyclization of the crude urethane by KO*t*-Bu offered *N*-2-nitrobenzyl-protected uracyl **239**, deprotection of which with UV irradiation or with excess KO*t*-Bu yielded 5,5,7,7-tetramethyl-6,7-dihydro-1*H*-pyrrolo[3,4-d]pyrimidine-2,4(3*H*,5*H*)-dione-5-yloxyl, *e.g.*, spin labeled uracyl **240** [93] (Scheme **43**).

6. NITROXIDES FUSED WITH SEVEN-MEMBERED HETEROCYCLES

Thiazepine-fused pyrroline nitroxide was reported in 1998 and synthesized by reaction of cysteamine and aldehyde **39** [39]; however, our attempts to react this aldehyde with 2-aminothiophenol provided no reproducible results. Realizing that sp^2 carbon-bound thiols and selenols are oxidized by nitroxide to disulfide or diselenide, such as the case of compound **188** formation, we changed our strategy. The reaction of 2-aminothiophenol was repeated with compound **241** (the diamagnetic form of **39**), which smoothly cyclized to compound **242** that was deprotected by Zemplen's deacetylation to produce compound **243** [90]. This benzo[1,5]thiazepine can be functionalized further by reduction, alkylation and oxidation reactions to furnish compounds **244, 245, 246** (Scheme **44**).

CONCLUSION AND OUTLOOK

A review cannot be completed by just attempting to quote the most important results of a certain field. This review provides a summary of the nitroxide-fused carbocycles and heterocycles from the last decade. This is a small area of nitroxide chemistry and its applications. However, through the "rediscovery" of isoindoline nitroxides it can be foreseen that it will become a growing field of research. These types of radicals have many advances, including bioreductive stability, higher chemical and thermal stability, and inherently narrower EPR bands compared to pyrroline or piperidine nitroxides. The aromatic ring of isoindoline nitroxide can be easily functionalized, and the tetramethyl groups tetraethyl or tetrapropyl group can be introduced quite easily, thereby enabling *in vivo* application of these shielded radicals [93, 94]. The only drawback to this chemistry is that the aromatic ring cannot be transformed into

other carbo- and heterocycles or altered to another ring entity. To overcome these disadvantages we have developed methods, starting from functionalized piperidine and pyrrolidine nitroxides, to develop heterocycles (isoselenazole, selenophene, pyridine, indole, triazole, pyrazole, pyrimidine, lactones) and carbocycles on the piperidine or pyrroline nitroxide rings. The drawback of this approach is the costs associated with the synthetic procedures. Application-driven or inspired nitroxide research will continue. In the future, the significance of shielded nitroxides will grow because of the development of EPR imaging [15c]. Isoindoline nitroxides role in double (fluorescent and spin) sensor molecules is also emerging, and tetramethyl and tetraethyl isoindoline nitroxides as profluorescent probes based of fluorescein and rhodamine were reported recently, which enables the monitoring of the mitochondrial redox state within cells [96]. In spin labeling, the combination of nitroxides with other radical centers (such as triphenylmethyl radical or isotopically modified nitroxides) will also be further exploited in the near future [97, 98]. The magnetic interactions of biradicals, including isoindoline nitroxides with various radicals, for example with triazinyl radical [99], is an emerging field both from the theoretical aspect and the biological aspect [100]. Generally, it is still highly desirable to develop new stable nitroxide entities tailored to the biochemical, biophysical, medical or diagnostic requirements. Therefore, the study of nitroxides, including the study of their selective and efficient modifications, will continue in several laboratories across the world, including ours.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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