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Synthesis and Applications of Optically Active Nitroxides

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This article reviews the synthesis of optically active nitroxides and their applications in enantioselective oxidations, in stereoselective carbon-oxygen bond-forming reactions, as anticancer compounds, and as spin labels.

Contents

1. INTRODUCTION

Nitroxides are of particular importance among free radicals: they are kinetically persistent species,[1] and in many cases are isolable, well-behaved compounds. Due to their inert nature, they are widely used in electron spin resonance spectroscopy as probes for biological systems. The use of nitroxides as spin labels was first introduced by McConnel[2] and has now evolved into a versatile tool for biological studies. Early work in the field of nitroxides was pioneered by Rozantsev and Rassat.[3] Janzen first coined the term "spin trapping";[4] a few reviews have appeared on the use of nitroxides as spin labels[5] and spin traps.[6] Recently the use of nitroxides as oxidizing agents has become increasingly popular; three review articles have been published to date.[7] An emerging subdiscipline involves work with optically active nitroxides. The focus of this review will be on the preparation and applications of optically active stable nitroxides. This review is not meant to be an exhaustive compendium of all optically active nitroxides such as spin labeled peptides, nucleotides, and proteins, however representative examples from each of these classes will be mentioned.

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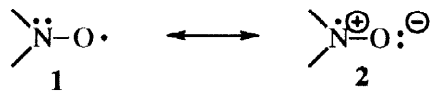
2. NOMENCLATURE

The problem of naming nitroxides has been historically controversial, giving rise to various nomenclatures. The nomenclature issue becomes particularly important in the age of computer database searching based on key words. In his review article,[5b] Keana mentions three descriptors for this class of free radical compounds that appear to enjoy widest use: *nitroxides*, *nitroxyls*, and *aminyl oxides*. Rassat[8] wrote a short paper aiming to clarify this nomenclature controversy. In addition to the descriptors mentioned by Keana, Rassat also found the terms *aminoxy* and *iminoxy* being used. A study of the literature data made by Rassat in 1981[8] and repeated by Volodarsky[9] in 1991 using the CAS ON-LINE data file found that the terms *nitroxide* and *nitroxyl* appeared most frequently. The term *nitroxide* will be used throughout this article as it has enjoyed the most widespread usage both in the recent and past literature. In addition, the term *nitroxide* was adopted by IUPAC in 1985.[10] The focus of this article will be on stable organic nitroxides. The term 'stable' is used in the sense that the radical is unreactive to air and moisture such that under ambient conditions, the pure radical can be handled and stored in the laboratory with no more precautions than would be used for the majority of commercially available organic chemicals. This definition parallels that of Griller and Ingold[1] for carbon-centered radicals.

3. STRUCTURE AND STABILITY

Nitroxides are compounds consisting of an *N,N*-disubstituted N-O group containing one unpaired electron. The structure of this fragment can be conceived as a superposition of the two resonance structures **1**

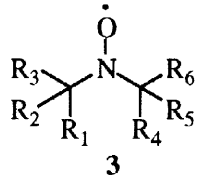


1 **2**

Fig. 1

and **2** as shown in Figure 1. For convenience, nitroxides in this article will be drawn in accordance with structure **1**, regardless of the actual electronic distribution.

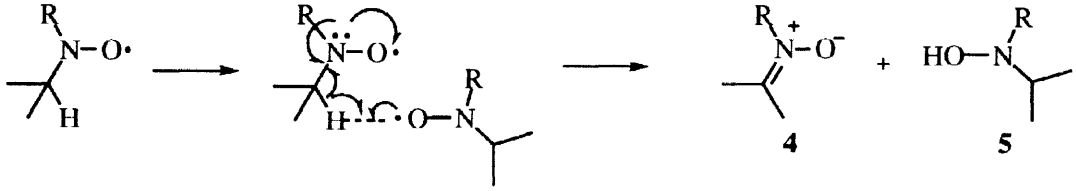
While a large number of nitroxides are sufficiently persistent to allow for ESR studies, rather special features are required for the nitroxides to be stable. In general, the most important prerequisite for stability is the absence of α-hydrogen atoms. The vast majority of stable nitroxides are secondary amine N-oxyls of the general structure **3** (Figure 2).



3

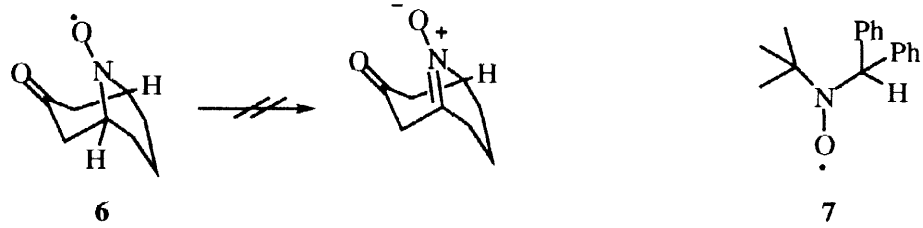
Fig. 2

When one or more hydrogen atoms are attached to one of the carbon atoms α to the nitroxide group, these radicals typically undergo disproportionation,[11] producing a nitrone **4** and an *N*-hydroxylamine **5**, either or both of which may undergo further reaction (Scheme 1).



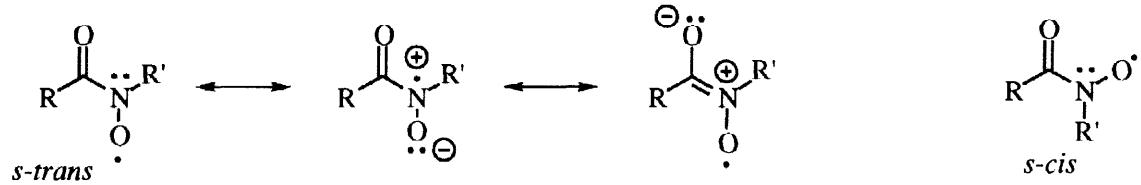
Scheme 1

However a few unusual nitroxides containing an α-hydrogen atom are relatively stable and even isolable. An example is bicyclic nitroxide **6**[12] which can be obtained in pure crystalline form (Scheme 2). The unusual stability of **6** towards disproportionation is attributed to the violation of Bredt's rule if the resulting nitrone were to be formed. Another example is the α,α-diaryl substituted nitroxide **7**; hindered rotation maintains the α-C-H bond approximately in the nodal plane of the N-O π-system, thus inhibiting disproportionation.[13]



Scheme 2

Acyl nitroxides are a less common subclass of nitroxide radicals. They are inherently less stable than bis(*t*-alkyl) nitroxides, due to delocalization of the nitrogen lone pair into the neighboring carbonyl group,[14] minimizing the contribution of the central resonance structure in Scheme 3. Some acyl nitroxides are isolable, particularly when they can adopt the lower energy *s-trans* conformation.[15] Unlike most stable nitroxides,



Scheme 3

acyl nitroxides abstract activated hydrogen atoms and can take part in olefin addition reactions.[16]

In summary, Volodarsky lists several factors which contribute to the stability of nitroxide radicals:[17]

1) Electronic structure of the nitroxide group; delocalization of the unpaired electron over the N-O bond provides thermodynamic stability to the radical. Dimerization, the usual fate of many other free radicals, is inhibited as

the formation of a weak O-O bond can not compensate for the loss of the delocalization energy of two nitroxide groups.

2) Steric hindrance of the radical center by bulky substituents attached to the nitrogen atom; this inhibits nitroxides from participating in bimolecular reactions.

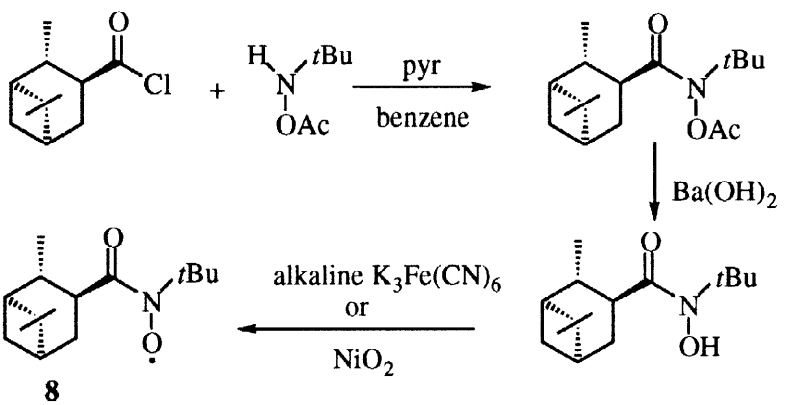
3) Absence of an effective mechanism of degradation for most bis(*t*-alkyl) nitroxides.

4. SYNTHESIS AND APPLICATIONS

Although nitroxides are chemically inert to a wide range of conditions, they can be reduced or oxidized with relative ease. Oxidation forms oxoammonium ions which are powerful oxidizing agents. Nitroxides also couple effectively with transient carbon radicals at near diffusion-controlled reaction rates. Oxidations and carbon radical trapping are the two major synthetic applications which have been probed utilizing optically active nitroxides.

4.1 *Enantioselective Oxidation with Optically Active Nitroxides*

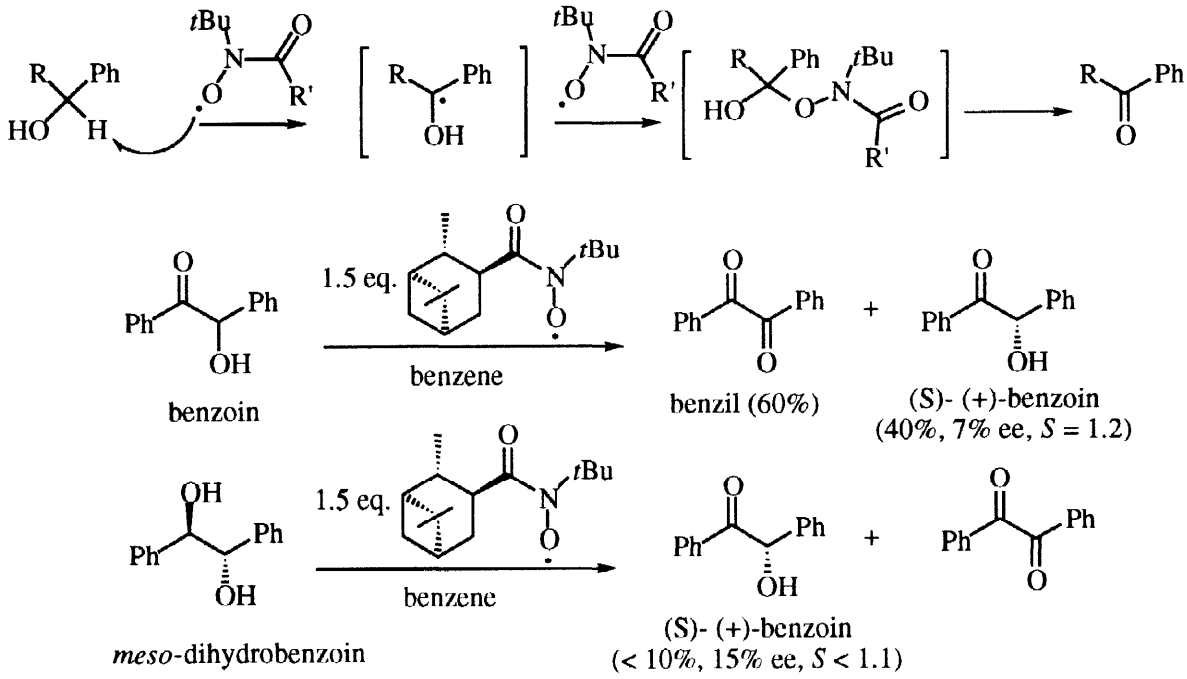
Optically active nitroxides have been used in oxidative kinetic resolutions proceeding through two different reaction mechanisms. The first paper on enantioselective oxidation with an optically active nitroxide was published in 1979 by Perkins.[18a] Kinetic resolution of racemic benzoin and dihydrobenzoin to benzil was investigated utilizing the optically active acyl nitroxide **8** derived from (-)-3-pinanecarboxylic acid chloride. Acyl nitroxide **8** was synthesized in three steps as shown in Scheme 4.[18b] As acyl nitroxides abstract hydrogen



8

Scheme 4

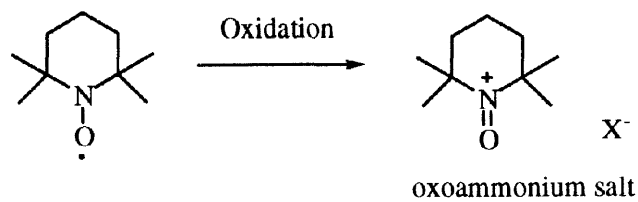
atoms from activated positions, secondary benzylic alcohols are attacked by acyl nitroxides to give benzylic α-hydroxy radicals, which can undergo trapping with another molecule of acyl nitroxide (Scheme 5). In an example of kinetic resolution, racemic benzoin reacts with 1.5 molar equivalents of **8** in benzene to give 60% of benzil in addition to 40% of unreacted benzoin. Benzoin was recovered in 7% ee with a selectivity factor[19] S = 1.2. Reaction of *meso*-dihydrobenzoin under similar conditions gave benzoin in 15% ee in low yield (< 10%), S < 1.1. A similar study using acyl *t*-alkyl nitroxides containing a chiral *t*-alkyl group gave poor selectivity.[20]



Scheme 5

Oxoammonium ions derived from nitroxides have been used in the oxidation of a variety of functional groups such as amines, phosphines, phenols, anilines, and alcohols.[21] Enantioselective oxidation reactions employing oxoammonium species have been reported only for alcohols. Three review articles[7] have appeared on the *achiral* oxidation of alcohols by nitroxides.

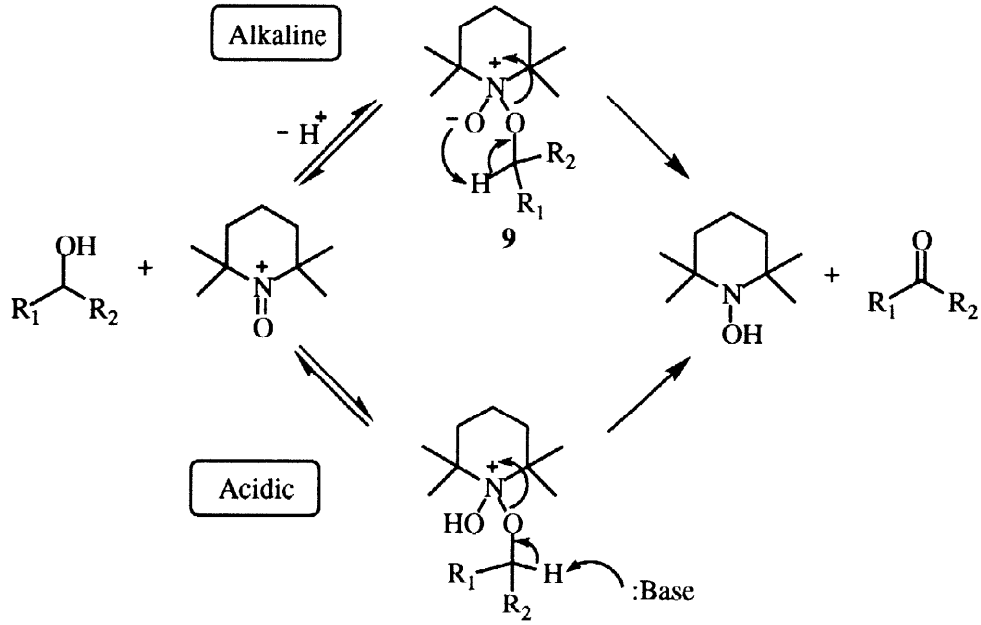
A second type of oxidative kinetic resolution operates by way of oxoammonium salts,[22] formed by the oxidation of nitroxides (Scheme 6).[7c] Oxammonium salts are effective oxidants of primary and secondary alcohols. Considerable effort has been expended into elucidating the mechanism: two distinct pathways have



Scheme 6

been suggested (Scheme 7). The first proposal by Semmelhack[23] postulates formation of the dipolar adduct **9**, which undergoes a cyclic concerted elimination. The second proposal by Bobbitt[24] favors formation of the protonated adduct **10**, which undergoes an acyclic elimination. Under acidic conditions, comparable rates are found for the oxidation of primary and secondary alcohols, whereas under alkaline conditions, more sterically hindered secondary alcohols are oxidized more slowly than primary alcohols. Based on this observation, van

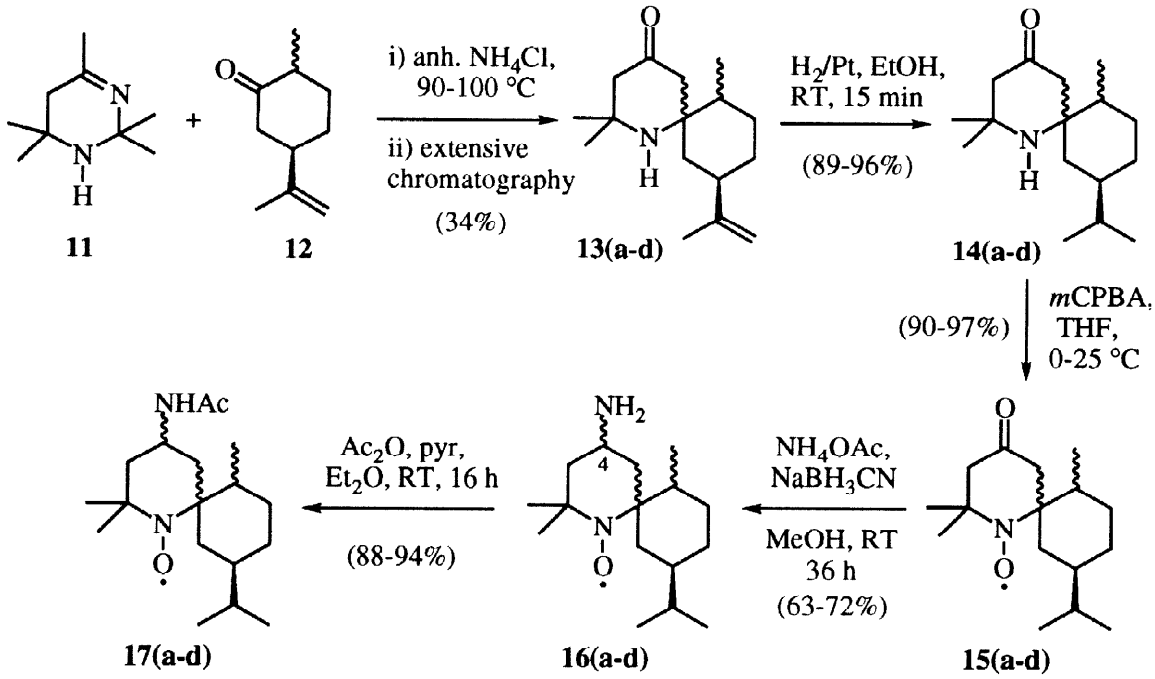
Bekkum[25] suggests that alkaline reaction conditions favor the cyclic dipolar mechanism, whereas acidic reaction conditions favor the acyclic mechanism.



Scheme 7

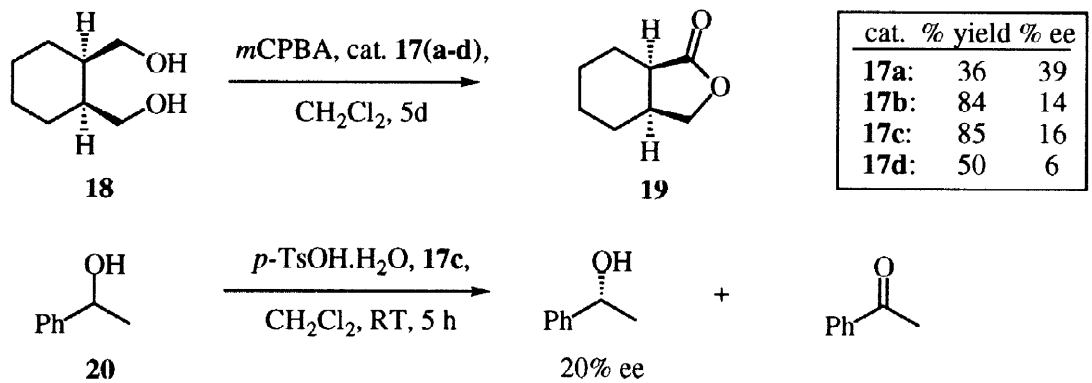
Bobbitt investigated the use of optically active piperidine nitroxides in both the enantioselective oxidation of a *meso* diol as well as in the kinetic resolution of a racemic alcohol.[26] The nitroxides were prepared in five steps from commercial (+)-dihydrocarvone (Scheme 8). Reaction of acetonin **11** with dihydrocarvone **12** in the presence of ammonium chloride with excess ketone as solvent gave a 49:26:14:11 mixture of all four possible diastereomers **13(a-d)** in 34% collective yield after extensive column chromatography. Upon separation, these four isomeric piperidone derivatives were each hydrogenated over a Pt catalyst to give **14(a-d)**. Oxidation with *m*CPBA gave nitroxides **15(a-d)** which on reductive amination yielded **16(a-d)**. The formation of the 4-amino group introduced another stereogenic center of unknown configuration. However, compounds **16(a-c)** each gave single peaks in the GC/MS whereas **16d** gave two. Therefore compounds **16(a-c)** were surmised to each be a single stereoisomer while **16d** was a mixture of diastereomers at C-4. Amines **16(a-d)** were acetylated to provide **17(a-d)** which were used in the asymmetric oxidation reactions.

Two reactions were investigated using optically active nitroxides **17(a-d)** (Scheme 9). The first was the oxidative desymmetrization of *cis*-1,2-cyclohexanedimethanol **18** to form lactone **19**. The stoichiometric oxidant *m*CPBA was used to generate the optically active oxoammonium species from nitroxide catalysts **17(a-d)**. All isomers of **17** gave the same *1S,6R* enantiomer of lactone **19** in 6-39% ee. The second reaction investigated the oxidative kinetic resolution of racemic 1-phenylethanol **20** to acetophenone. This reaction did not give any detectable enantiomeric excess using **17a** or **17b** as catalysts with peracid oxidant. However, the



Scheme 8

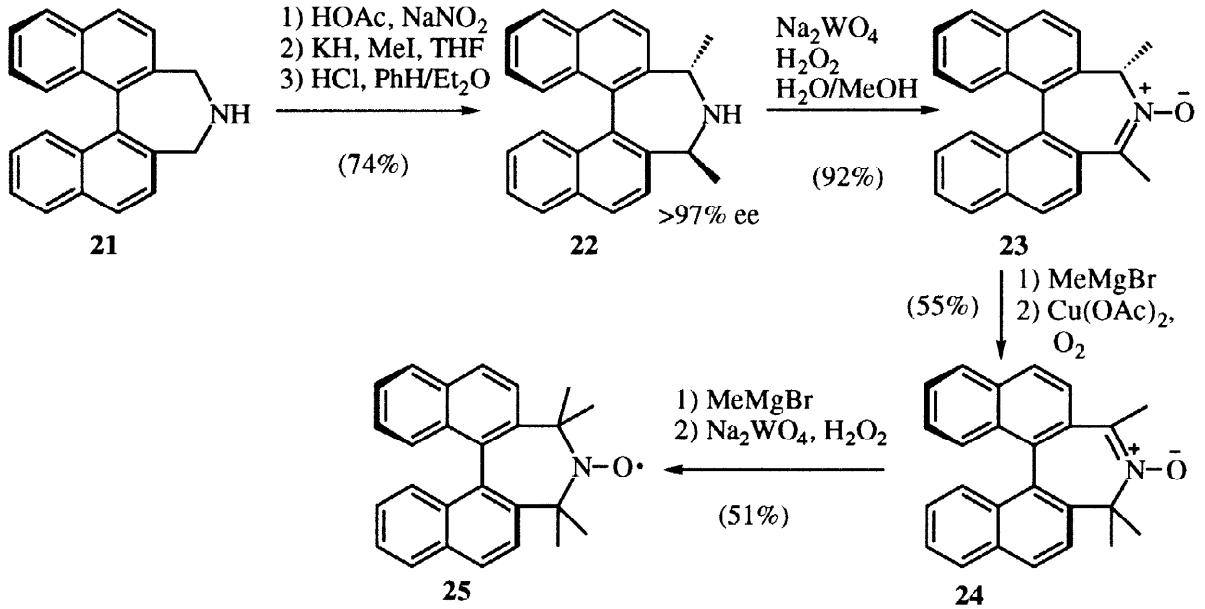
oxoammonium salt prepared by the disproportionation of nitroxide **17c** by *p*-TsOH gave the *R* isomer of 1-phenylethanol in 20% ee.



cat.	% yield	% ee
17a:	36	39
17b:	84	14
17c:	85	16
17d:	50	6

Scheme 9

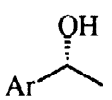
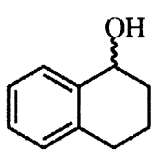
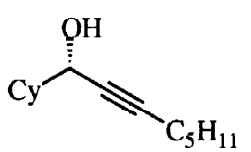
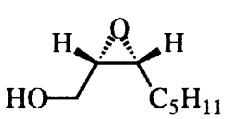
Rychnovsky[27] recently reported the enantioselective oxidation of secondary alcohols using enantiomerically pure (-)-(*S*)-3,5-dihydro-3,3,5,5-tetramethyl-4*H*-dinaphth[2,1-c:1',2'-e]azepine-*N*-oxyl **25** as the nitroxide catalyst. The synthesis of **25** is shown in Scheme 10. Azepine **21** (>92% ee)[28] was prepared by the literature procedure.[29] Nitrosation of **21**, followed by bisalkylation and hydrolysis gave **22**.[30] Oxidation of **22** gave nitrone **23**, which upon addition of methyl Grignard followed by reoxidation gave nitrone **24**. A final methyl Grignard addition and oxidation gave the desired nitroxide **25**. The optical purity of **25** was assumed to be >97% ee based on the enantiomeric purity of **22**.



Scheme 10

Enantioselective oxidations of alcohols using nitroxide catalyst (-)-(*S*)-**25** are summarized in Table 1. All oxidations were carried out with 0.5 mol % to 1.0 mol % of **25** as catalyst and bleach as the bulk oxidant.

Table 1. Enantioselective Oxidations using Catalytic (-)-(*S*)-**25**.

mol % of **25**	Recovered Alcohol	% ee	% conversion	S^{19}
0.5 - 1.0	 6 examples	57-98	56-87	3.9-7.1
0.5		< 6	58	< 1.2
0.5		41	66	2.2
0.5		19	58	1.5

Recently, Bobbitt has demonstrated the use of the optically active base (-)-sparteine and an *achiral nitroxide* (tethered to an electrode) to effect kinetic resolution in the oxidation of racemic alcohols.[31]

4.2 *Stereoselective Carbon-Oxygen Bond Formation with Optically Active Nitroxides*

Transient carbon radicals couple with nitroxides efficiently. Nitroxide traps are often used to provide evidence of radical intermediates, and can be indirectly used to measure the lifetimes of short-lived radical species.[32] Optically active nitroxides have been used in stereochemical studies to probe the ability of non-bonded optically active reagents to distinguish between the enantiotopic faces of a prochiral carbon radical in a

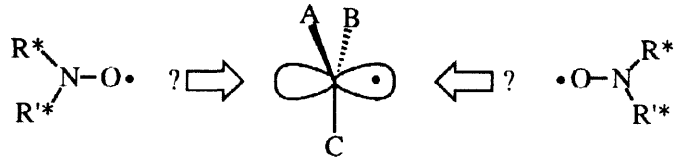
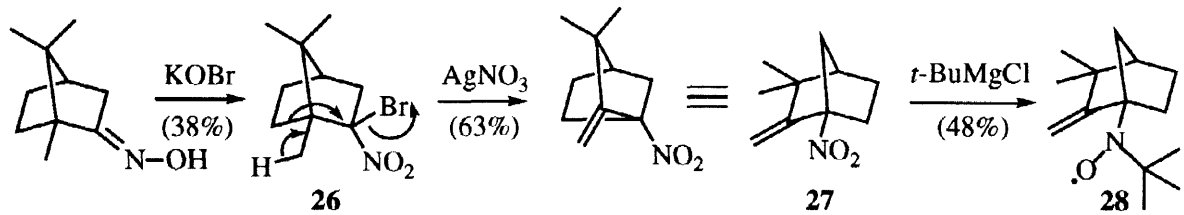


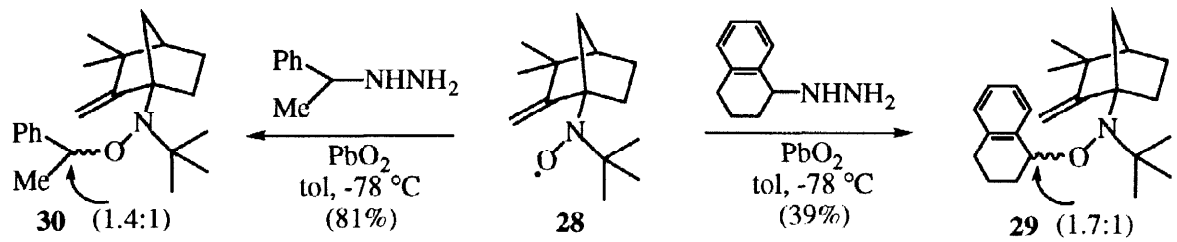
Fig. 3

coupling reaction (Figure 3). The first examples utilized nitroxide **28**, prepared from camphor according to Rassat[33] (Scheme 11). Camphoroxime was treated with potassium hypobromite to give both bromination and oxidation to form the bromonitrocamphane **26** via the bromo nitroso intermediate. Rearrangement with silver nitrate produced 1-nitrocamphene **27** which was converted to the nitroxide **28** directly with *t*-butylmagnesium chloride. In our hands, the yield of this last step was much lower than the reported 48%, but could be improved somewhat by the addition of anhydrous $CeCl_3$.[34] Coupling of the optically active nitroxide **28** with transient



Scheme 11

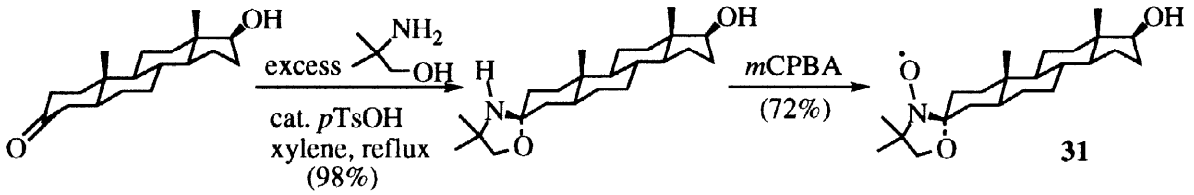
carbon radicals generated at low temperature by the oxidation of alkyhydrazines with lead dioxide proceeded with moderate selectivity (Scheme 12).[35] Nitroxide **28** is free to rotate about the nitrogen-bridgehead carbon



Scheme 12

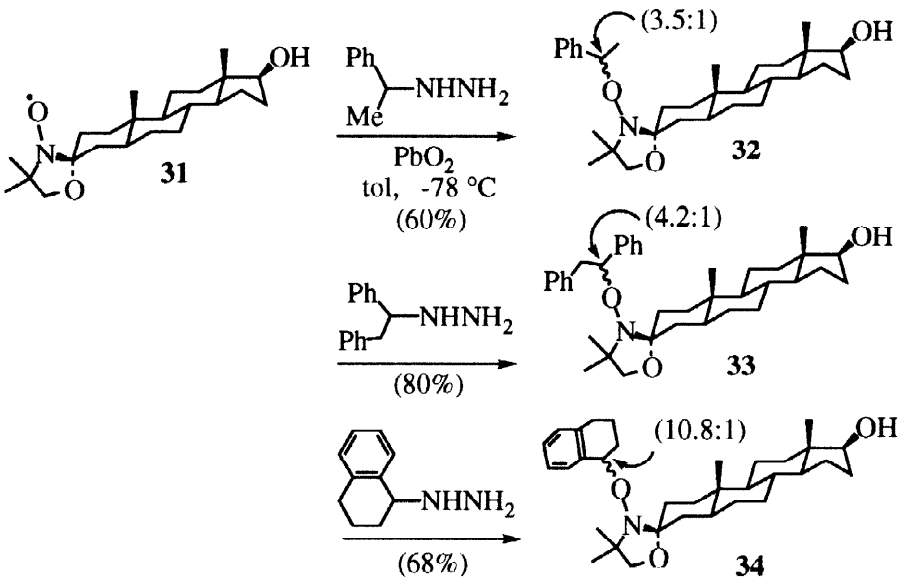
bond, resulting in several low energy conformations. Better selectivity was achieved by utilization of the conformationally rigid, optically active doxyl nitroxide **31** prepared according to Rassat[36] from the 5α-

androstan-17β-ol-3-one (Scheme 13). Ketalization of the A-ring ketone with 2-amino-2-methylpropan-1-ol followed by *m*CPBA oxidation following the general methodology developed by Keana for doxyl radical preparation[37] provided the doxyl nitroxide **31** as a stable, yellow crystalline solid. The coupling



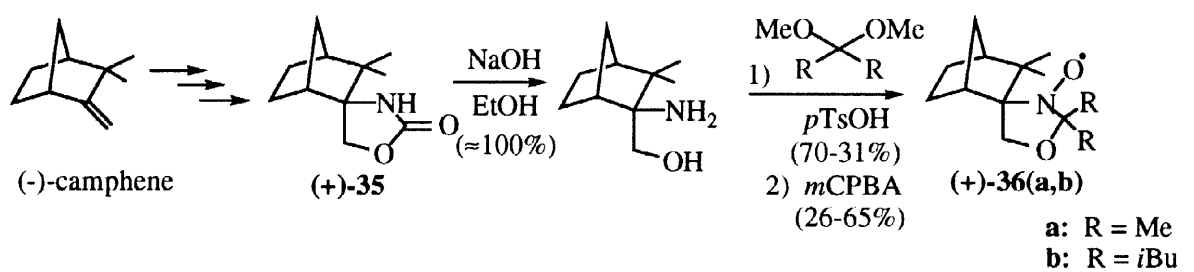
Scheme 13

of this rigid nitroxide **31** with several prochiral carbon radicals proceeded with good stereoselectivity as shown in Scheme 14.[35]



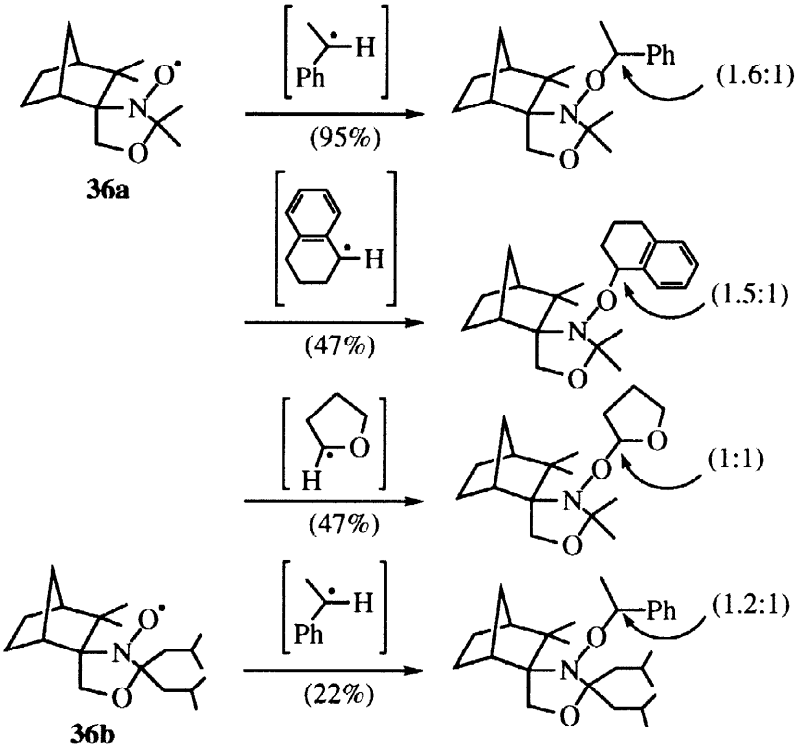
Scheme 14

However, not all rigid, chiral nitroxides were as selective. Camphoxyl radicals **36(a,b)** were prepared from camphene (Scheme 15).[38] Carbamate **35** was prepared by the literature procedure;[39,40] hydrolysis,



Scheme 15

trans-ketalization, and oxidation gave camphoxyl radicals **36(a,b)**. Coupling with prochiral radicals gave disappointing selectivities (Scheme 16).[41]



Scheme 16

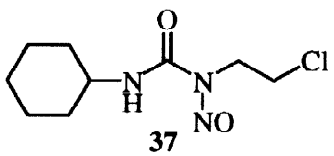
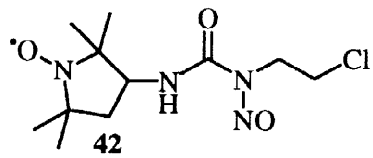
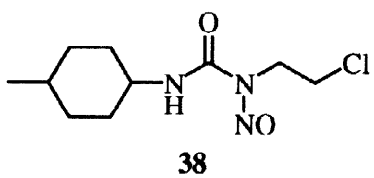
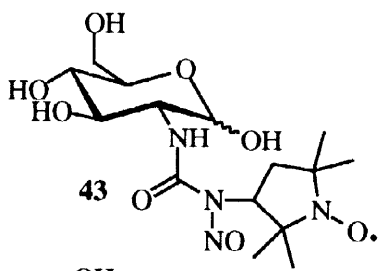
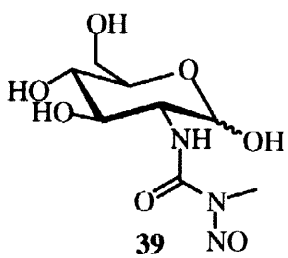
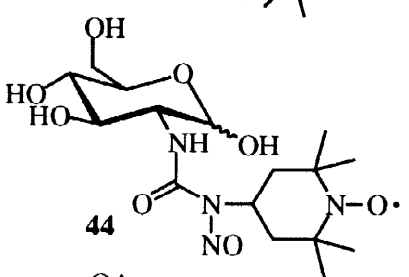
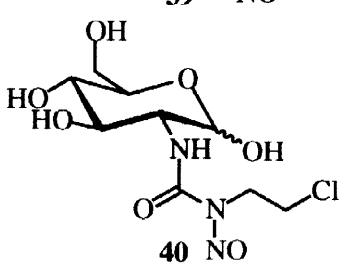
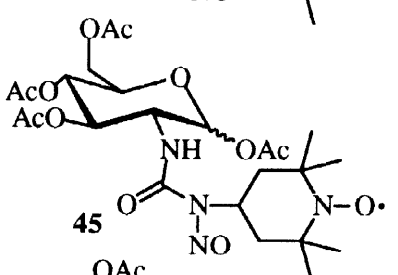
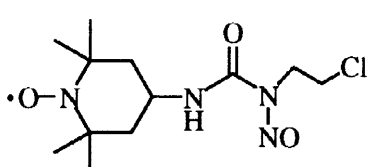
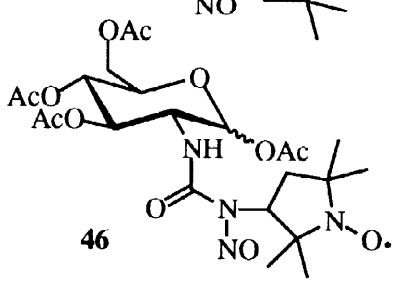
4.3 *Optically Active Nitroxides As Anticancer Agents*

4.3.1 *Carbohydrate Derivatives*

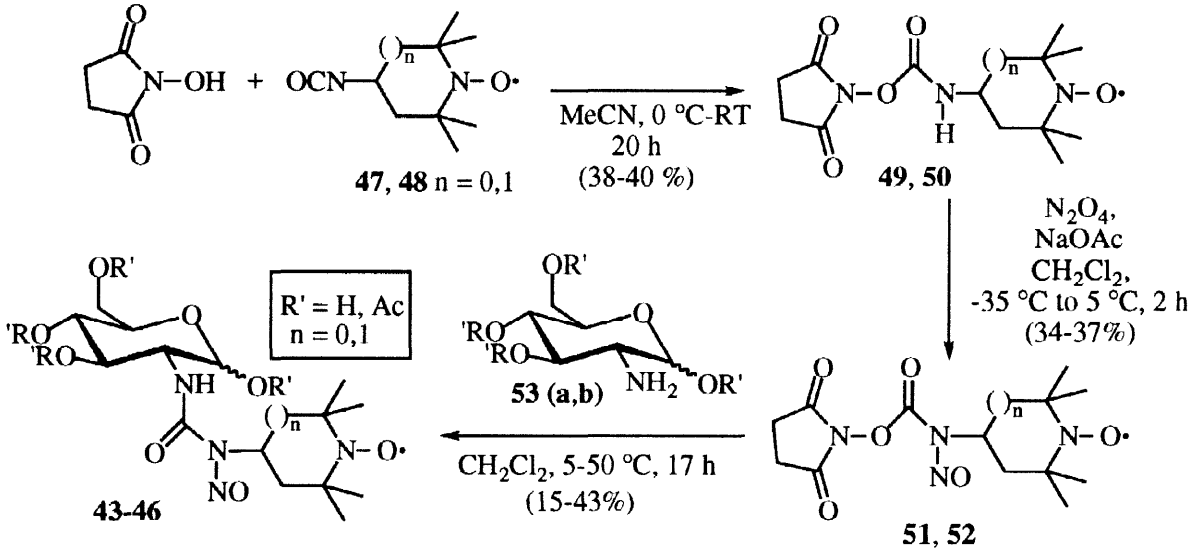
Cancer is a disease that can be ultimately traced to mutations resulting from DNA damage.[42] In order for a drug to reach cancerous cells, it must be compatible with aqueous extracellular conditions as well as the cell surface environment. In addition, to permeate the highly hydrophobic membrane bilayer, the drug must also possess a certain degree of compatibility with the hydrophobic membrane environment. Compounds containing nitrosourea or chloroethylnitrosourea moieties possess potent anticancer activity; some of these have been used extensively against a variety of cancers.[43] The nitrosoureas are an attractive class of anticancer drugs due to their wide spectra of activity. Some members of the nitrosoureas, exemplified by compounds **37** and **38**, are capable of penetrating the blood-brain barrier, and hence can be applied in brain tumor chemotherapy. Unfortunately, the nitrosoureas also exhibit a range of substantial toxic side effects including cumulative bone marrow toxicity. However nitroso derivatives containing carbohydrate moieties, for example **39** and **40**, exhibit much lower bone marrow toxicity.[42] The carbohydrate groups not only ameliorate the toxic side effects, but also impart highly hydrophilic character to these compounds. Sosnovsky[41] has designed effective drugs by increasing the

hydrophobicity of compounds of the type **39** and **40** and by decreasing the hydrophobicity of **37** and **38**. This was achieved by replacing the hydrophobic cyclohexyl groups in **37** and **38** with nitroxides to give the less toxic and more active compounds **41** and **42**. Similarly, replacement of the methyl and 2-chloroethyl groups in **39** and **40** with nitroxides resulted in compounds **43** and **44** with increased activity. The acetylated derivatives **45** and **46** were also tested for anticancer activity (Table 2).

Table 2. Anticancer activity of nitrosoureas against P388 lymphocytic leukemia in CD_2F_1 male mice.
* ILS: Increase in Life Span at 20 mg/kg/day.

Compound	%ILS*	Compound	%ILS*
 **37**	182	 **42**	514
 **38**	145	 **43**	437
 **39**	178	 **44**	437
 **40**	194	 **45**	174
 **41**	542	 **46**	156

Nitroxides **43-46** were synthesized in three steps starting with spin labeled isocyanates **47** or **48** by condensation with *N*-hydroxysuccinimide, followed by nitrosation with dinitrogen tetroxide, and condensation with two different amino sugars **53 (a,b)**.[44] Compounds **43-46** also exhibited antineoplastic activity against lymphoid leukemia L1210.



Scheme 17

4.3.2 *Amino Acid Derivatives*

A number of amino acid and peptide derivatives containing the [*N*'-(2-chloroethyl)-*N*'-nitrosoamino]carbonyl (CNC) moiety have been synthesized and evaluated for *in vivo* anticancer activity.[45] The rationale for this approach is based on the concept that L-amino acids are transported actively into mammalian tissue. Since some peptides accumulate in cancerous cells, short peptides were designed to serve as carriers of the CNC moiety. On the basis of past experience with carbohydrate derivatives, Sosnovsky synthesized several amino acid derivatives by substituting the highly hydrophobic (2-chloroethyl)amino group at the *C*-terminus in compounds of type **54(a,b)** with the less hydrophobic nitroxide

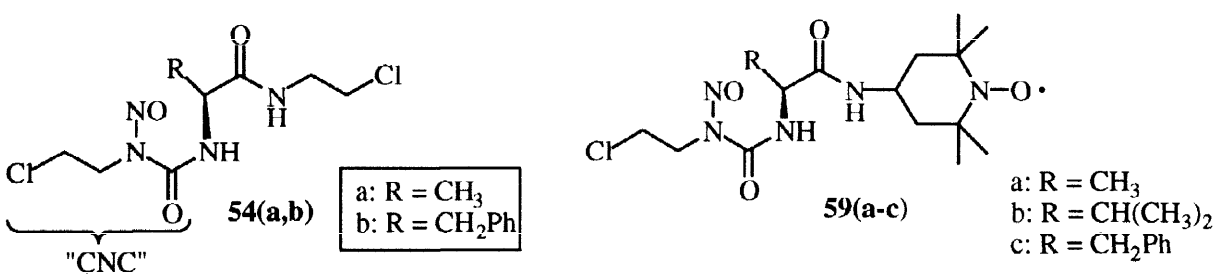
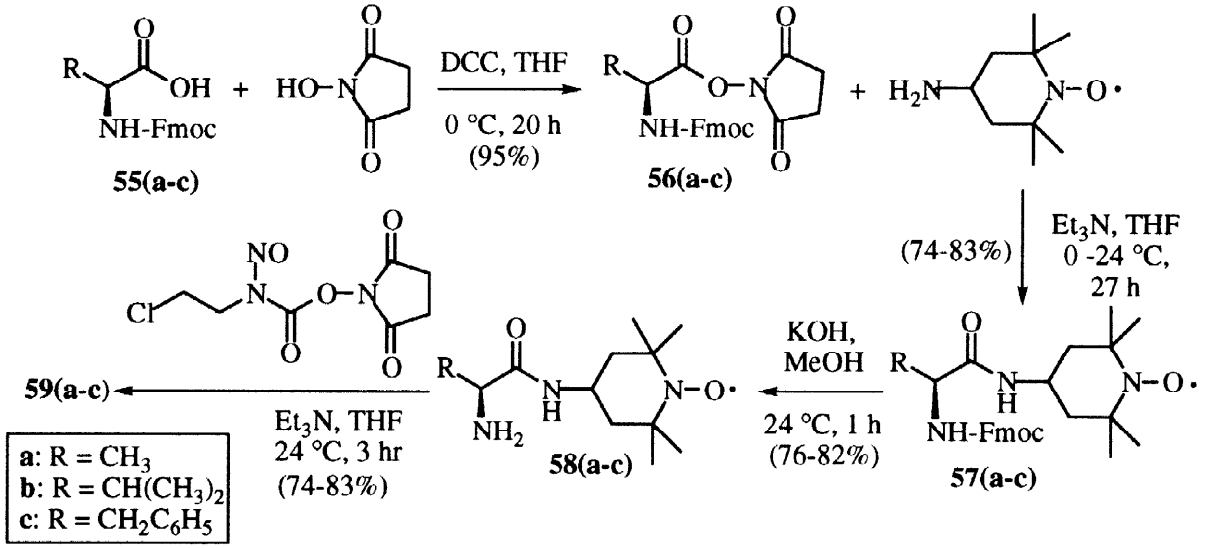


Fig. 4

group to give compounds **59(a-c)** (Figure 4).[46] The syntheses of **59(a-c)** derived from the amino acids L-alanine, L-valine, and L-phenylalanine were carried out as shown in Scheme 18. The reaction of Fmoc-protected

amino acids **55(a-c)** with *N*-hydroxysuccinimide in the presence of DCC gave the corresponding activated *N*-succinimidyl esters **56(a-c)**. The transfer reaction of **56(a-c)** at 0 °C with 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl in THF formed the nitroxide labeled Fmoc derivatives **57(a-c)**. The Fmoc group in **57(a-c)** was removed with a 10% methanolic potassium hydroxide solution to give **58(a-c)**. The target compounds were then synthesized by the reaction of **58(a-c)** with *N*-succinimidyl-*N*-(2-chloroethyl)-*N*-nitrosocarbamate in the presence of triethylamine.



Scheme 18

Compounds **59(a-c)** were evaluated *in vivo* against murine lymphocytic leukemia P388 in CDF1 male mice as well as against the lymphoid leukemia L1210 (Table 3). The more hydrophobic compounds **54(a,b)** were highly active against P388 leukemia, but much less effective against L1210 leukemia. The ILS parameters were correlated with the corresponding lipophilicities: a trend was generally observed toward an increase in cytotoxicity with a concomitant decrease in hydrophobicity.

Table 3. Anticancer activity of **59(a-c)**.

Compound	Dose (mg/kg/day)	P388: ILS (%)[a]	L1210: ILS (%)[b]
59a	12	447	663
59b	12	456	663
59c	12	242	581

a: Increase in life span against P388 Lymphocytic Leukemia in CDF_1 male mice on day 60.

b: Increase in life span against L1210 Lymphoid Leukemia in CDF_1 male mice on day 60.

4.3.3 *Role of Nitroxides in Anticancer Activity*

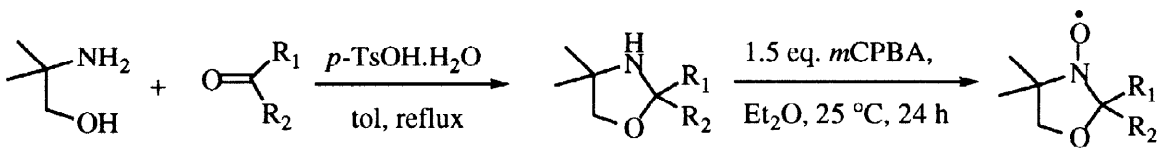
While the nitroxide moiety imparts a beneficial influence on the antineoplastic activity of these drugs, the radical by itself has no anticancer activity. It is not mutagenic or carcinogenic, is relatively non-toxic, exhibits no synergetic effect, and has only little effect on cell growth and cell kinetics.[47] Furthermore, many nitroxides readily migrate through cell membranes into the intracellular space, and can even cross the blood brain barrier at sites of diseased tissues. On the basis of these findings, it appears that the nitroxide group is a carrier moiety that facilitates the transport of drugs through biological membranes en route to cellular DNA.

4.4 *As Spin Labels*

The presence of an unpaired electron gives nitroxides their paramagnetic properties, enabling detection by ESR. The high sensitivity of ESR and the chemical stability and availability of nitroxides make them ideally suited as spin labels and spin probes in a wide variety of scientific and technical fields, from molecular biology and medicine to criminology and oil production.[48] The distinction between spin labels and spin probes has been defined by Keana as follows:[5a] a nitroxide covalently bound to a molecule of interest is considered a *spin label*. The presence of the nitroxide group is intended to not significantly perturb the behavior of the spin labeled molecule in the system under study. The term *spin probe* refers to a nitroxide containing molecule which is not covalently attached to molecules of the system under study. Spin labeled molecules often become spin probes when used to study complex systems. This review will focus on spin labels.

4.4.1 *Steroid Derivatives*

Steroid derivatives form an important class of optically active nitroxide spin labels. One of the fruitful approaches to the study of cholesterol interactions in biological membranes has utilized the ESR spin labeling method.[49] The first steroidal nitroxide, **60**, used as a spin label was synthesized by the general method developed by Keana for converting ketones to stable doxyl nitroxides (Scheme 19).[37] A number of steroidal



Scheme 19

nitroxides including **31** and the biradicals **61**-**64**[50] and **65**[51] have been prepared by this method (Figure 5).

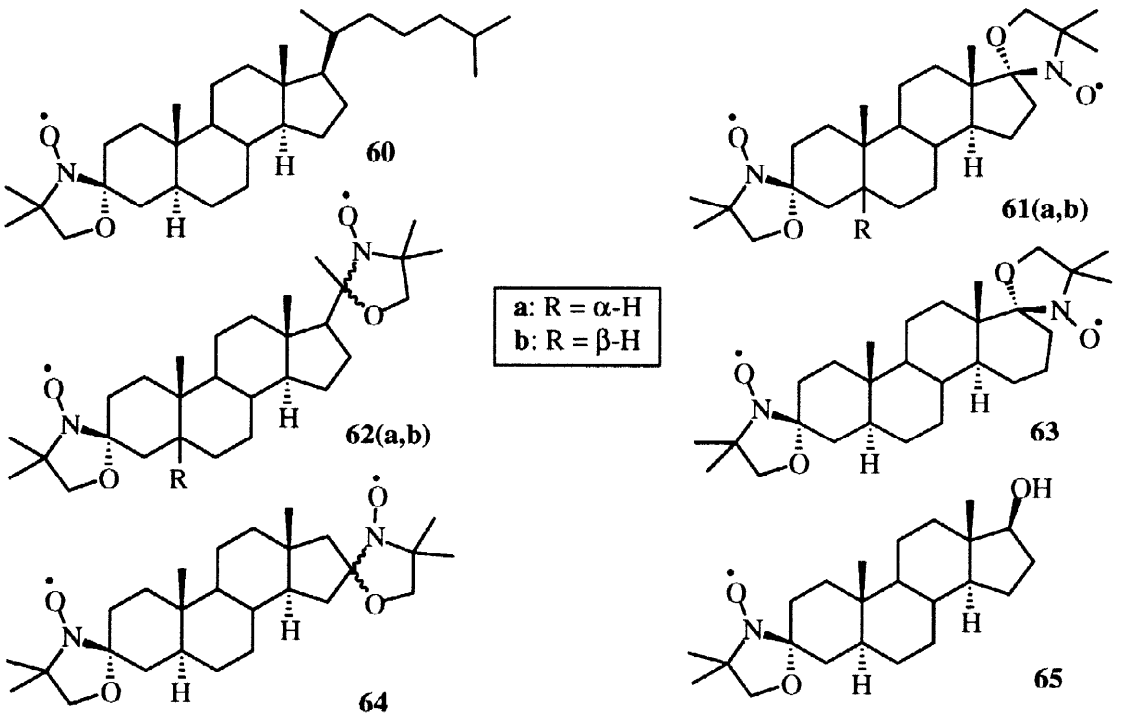
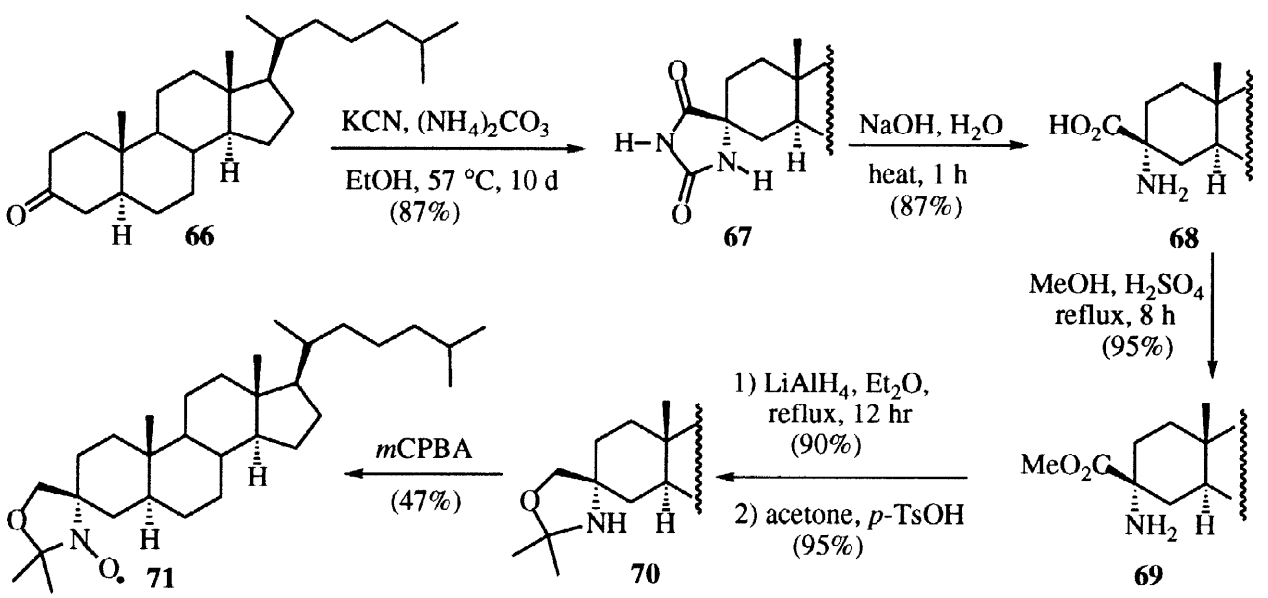


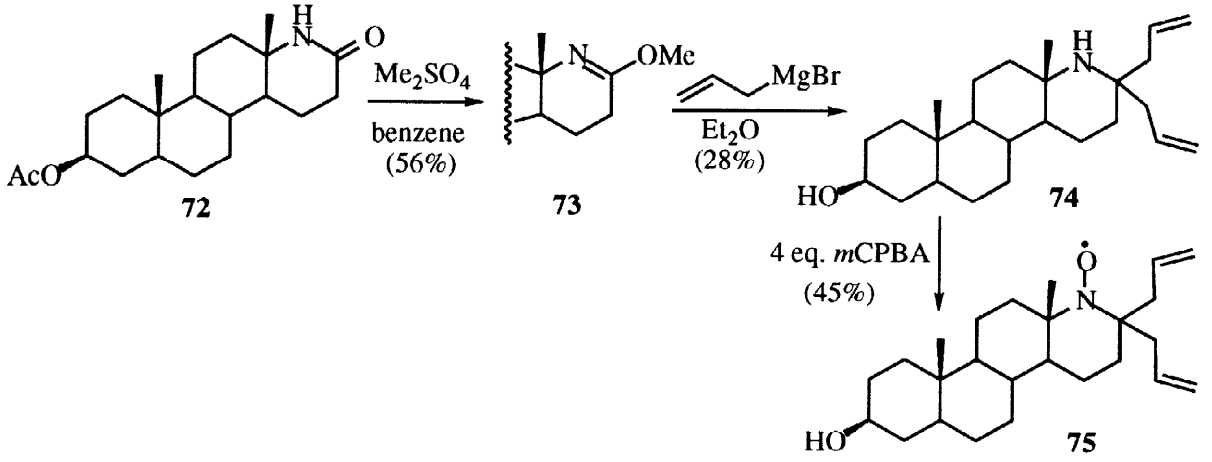
Fig. 5

In compound **60**, the C-N bond is equatorial with respect to the steroid A ring. As structural evidence, a C-N axial isomer was built stepwise by an alternative route as shown in Scheme 20.[52]



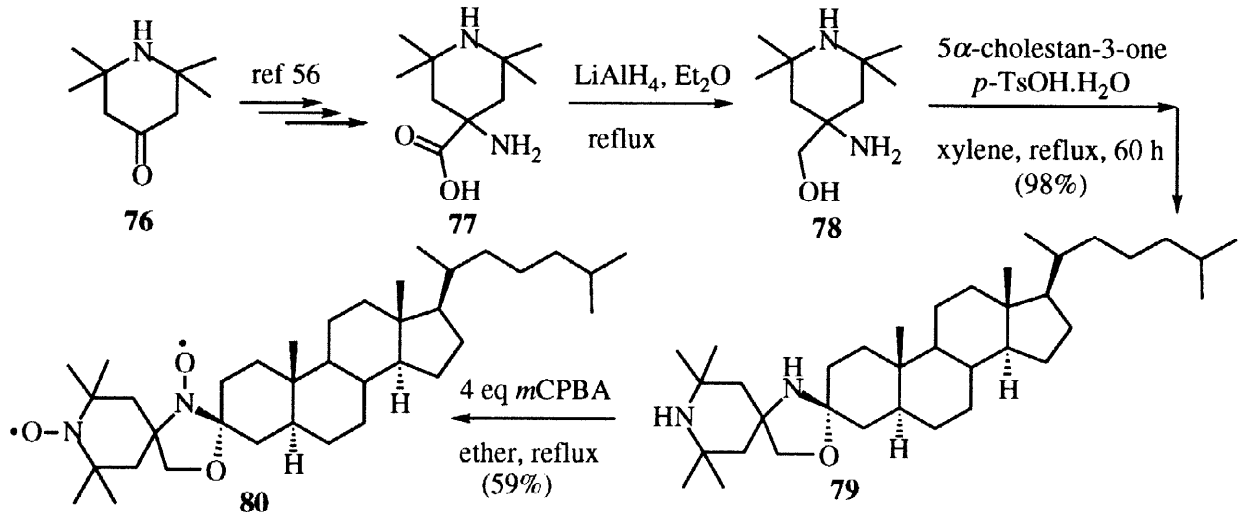
Scheme 20

An interesting steroidal nitroxide, **75**, was synthesized by Rassat[53] in which the nitrogen atom is built into the steroid skeleton. The C-3 hydroxyl is thus available for the introduction of various functional groups. This radical was synthesized in three steps from isoandrololactam acetate **72** (Scheme 21). Reaction with dimethylsulfate in benzene gives the *O*-methyl derivative **73** which upon treatment with allyl magnesium bromide gives the gem-dialkylated amine **74**. Oxidation with *m*CPBA provides the target nitroxide **75**. Several other D-homosteroidal nitroxides from isoandrosterone and dehydroisoandrosterone have also been synthesized.[54]



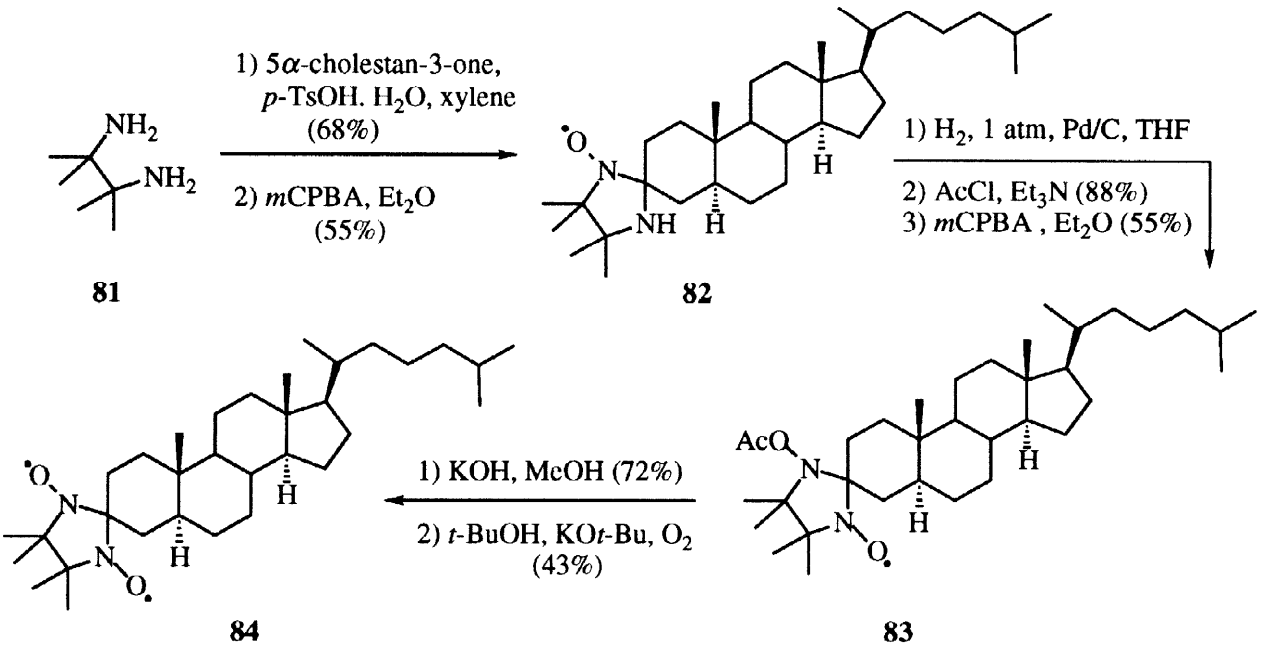
Scheme 21

Keana has developed two methods for the rigid spiro attachment of a dinitroxide label to a molecule at the site of a ketone group. The first method[55] is represented by the synthesis of dinitroxide **80** (Scheme 22), which starts with the amino acid **77** prepared from amino ketone **76** by the method of Rassat.[56] Reduction to the alcohol **78** with $LiAlH_4$ followed by condensation with 5α-cholestan-3-one and oxidation with *m*CPBA gave **80**.



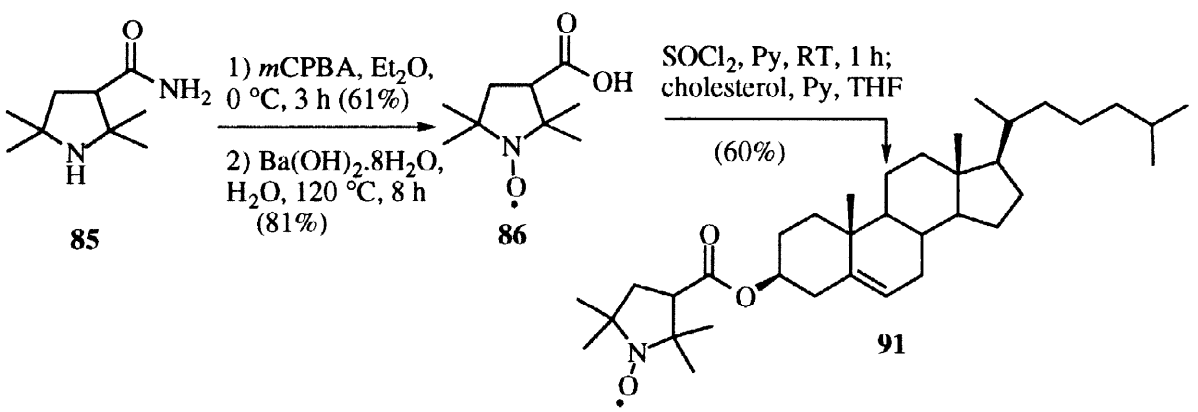
Scheme 22

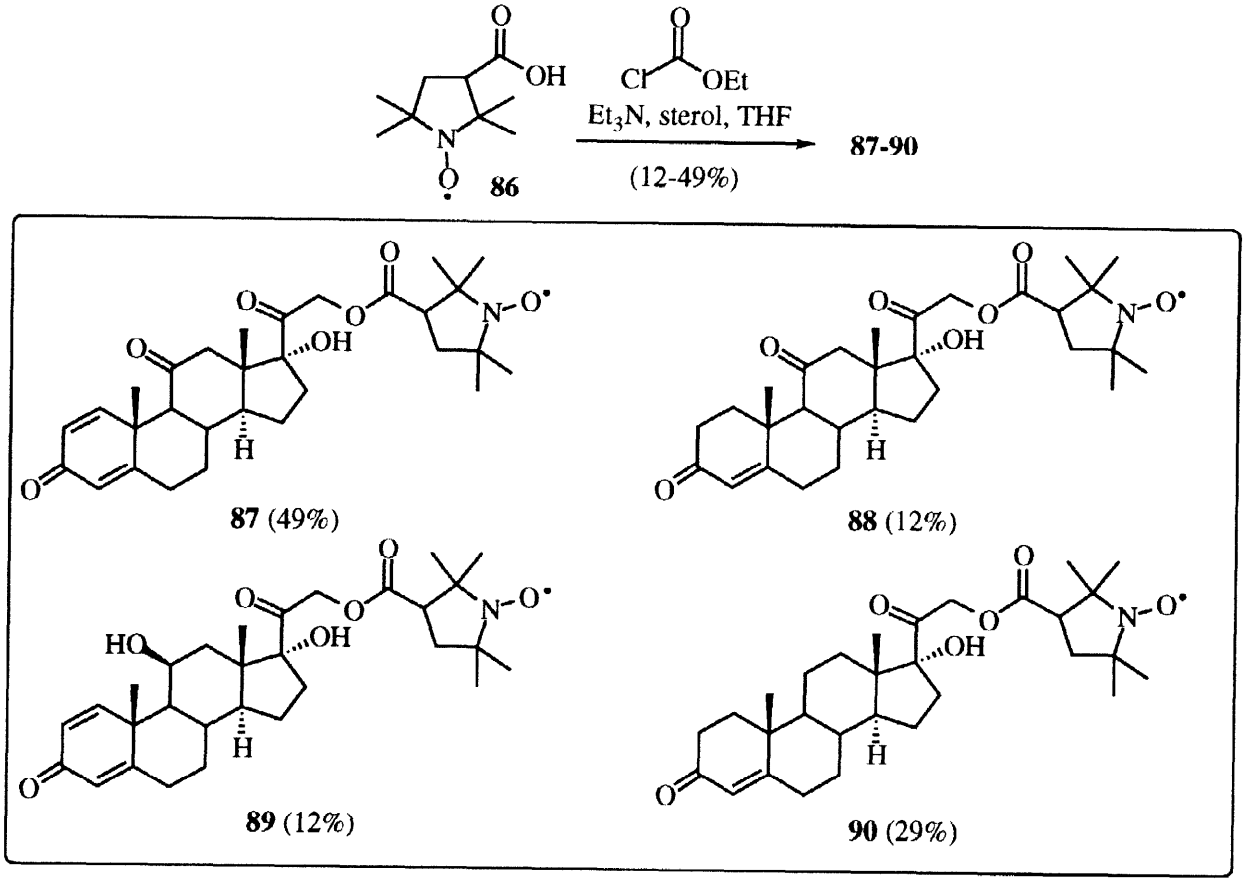
The second method[57] is represented by the synthesis of dinitroxide **84** in which the two nitroxide groups are incorporated into the same ring. Condensation of diamine **81** with 5α-cholestan-3-one and oxidation with *m*CPBA gave mono-nitroxide **82**. As direct oxidation to the dinitroxide was problematic, stepwise conversion was accomplished via reduction to the hydroxylamine, acetylation, and oxidation to **83** followed by hydrolysis and further oxidation to the dinitroxide **84**.



Scheme 23

Five other steroidal spin labels **87-91** were synthesized by Benson; their binding to prednisone and prednisolone antibodies was studied.[58] Each of these derivatives utilize an ester linkage to attach the nitroxide to the steroid skeleton. Oxidation of commercially available 2,2,5,5-tetramethylpyrrolidine-3-carboxamide **85** with *m*CPBA followed by hydrolysis of the amide with $Ba(OH)_2$ gave carboxylic acid nitroxide **86**. Esters **87-90** were synthesized by the mixed anhydride method using ethyl chloroformate and Et_3N (Scheme 24). For synthesis of **91**, the acid chloride of **86** was used.





Scheme 24

Another spin-labeled steroid **92**, is based on the structure of cholesterol, incorporating a doxyl radical near the terminus of the carbon sidechain at C17 (Figure 6).[59]

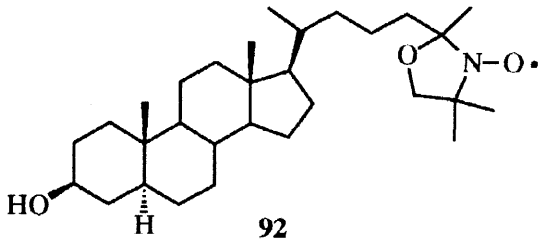
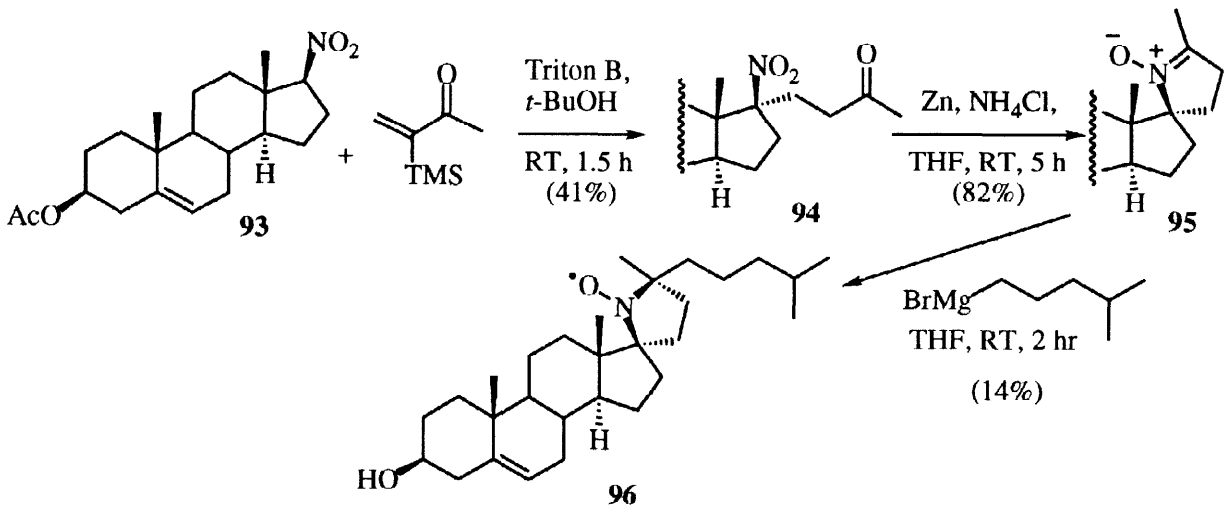


Fig. 6

The nitroxide group in spin labels such as **91** and **92** is attached to the steroid skeleton by a flexible linkage, allowing rotation independent of the steroid skeleton. For applications in which a measure of the motion experienced by the steroid nucleus is of interest, use of these flexible compounds is inappropriate. To circumvent this limitation, Keana[49] synthesized cholesterol analog **96** as shown in Scheme 25. Michael addition

of nitro compound **93** (synthesized from dehydroisoandrosterone by the method of Patchett)[60] to methyl-α-trimethylsilyl vinyl ketone using Triton B as base gave nitroketone **94**. Reduction with zinc and ammonium chloride provided nitrone **95** to which was added isohexylmagnesium bromide. Spontaneous air oxidation of the intermediate *N*-hydroxyl compound afforded the target nitroxide **96**. This nitroxide was then incorporated into human high density lipoproteins to probe the nature of cholesterol-lipid and cholesterol-protein interactions.



Scheme 25

Information concerning the orientation, anisotropic motion, and diffusion of membrane lipids has been obtained using steroidal spin labels. For example, neutral compounds **97** and **98** (Figure 7) were prepared by Morrisett for the study of nonpolar regions of plasma lipoproteins.[61]

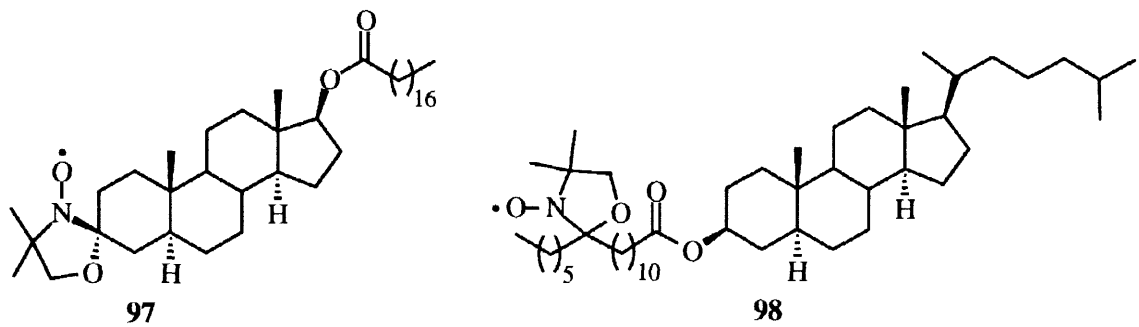
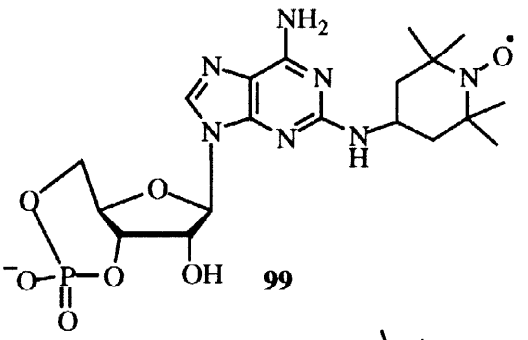


Fig. 7

4.4.2 *Others*

A variety of other optically active nitroxides have been prepared (Figure 8), including nucleotides such as **99**,[62] carbohydrate derivatives such as **100**,[63] terpenoid compounds such as (+)-pulegone derivative **101**,[64] Cytochalasin B derivative **102**,[65] phospholipid derivatives such as **103**,[66] crown ether derivatives such as **104**,[67] and amino acid derivatives such as **105**.[68] Other examples include peptides such as **106**,[69]

containing the amino acid TOAC which incorporates the nitroxide ring directly into the peptide backbone, and **107**[70] in which the nitroxide is attached to a pendant side chain; these have been incorporated into peptide sequences as an ESR sensitive probe of peptide structure in solution.



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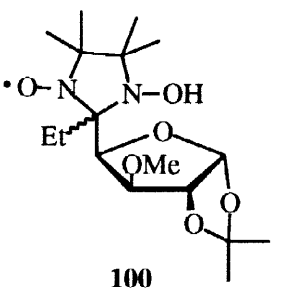

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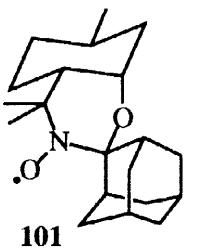

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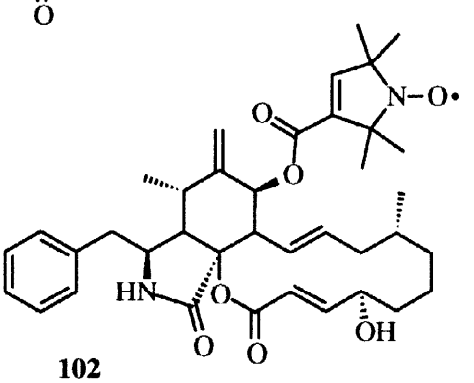

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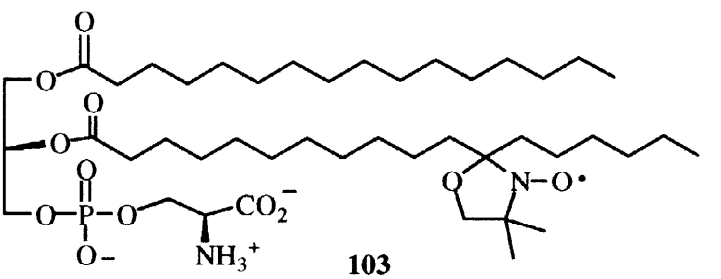

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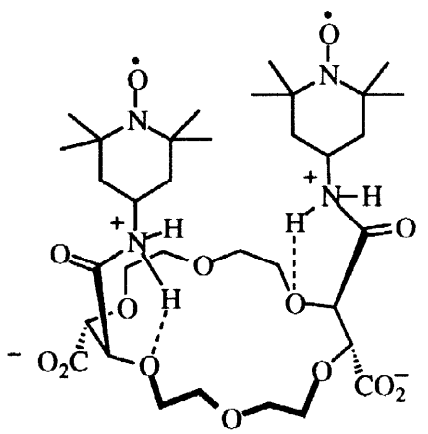

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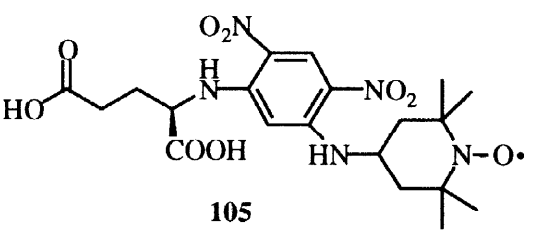
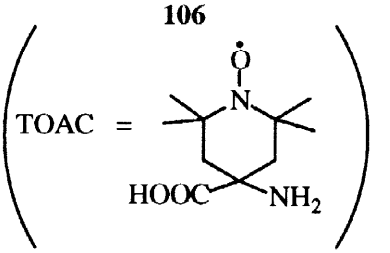
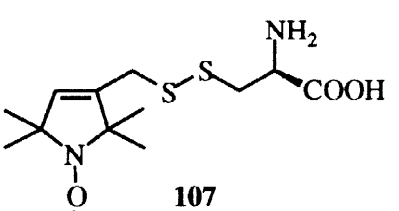


105

Boc-TOAC-Ala-Ala-TOAC-Ala-Ala-O*t*Bu

106





107

Fig. 8

4.5 *Miscellaneous*

A number of optically active nitroxides have been synthesized to study their physical properties, such as their structures, spectra, and chiroptical characteristics. This section covers selected examples.

Classical resolution has been used on several racemic nitroxides (Figure 9). For example, optically pure enantiomers of compound **108** were prepared by Roberts[71] to study their chiroptical properties. The enantiomers were resolved by esterification with 3β-acetoxy-Δ^5-etienic acid. Tamura[72] reported the preparation of optically pure **109(a,b)** by spontaneous optical resolution of stable conglomerates during recrystallization. Very recently, the separation of enantiomers of nitroxide **110** by chiral HPLC has been reported by two groups.[73]

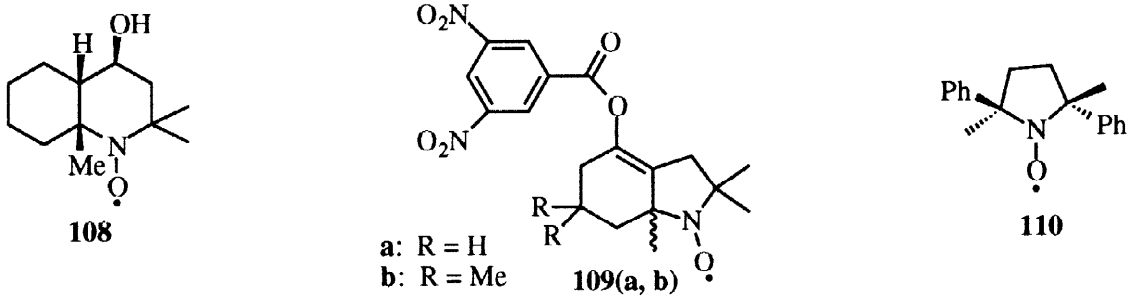
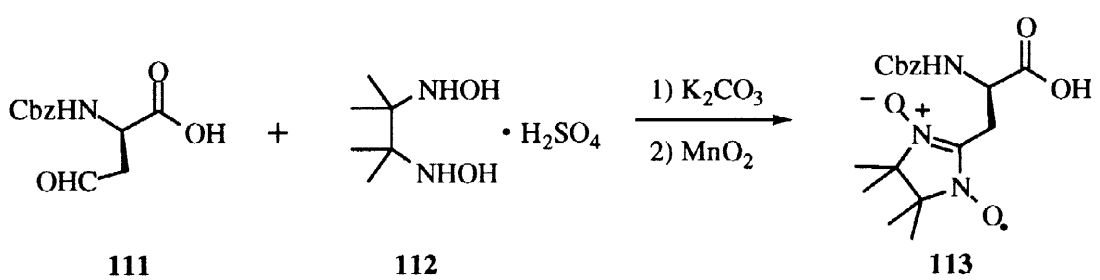


Fig. 9

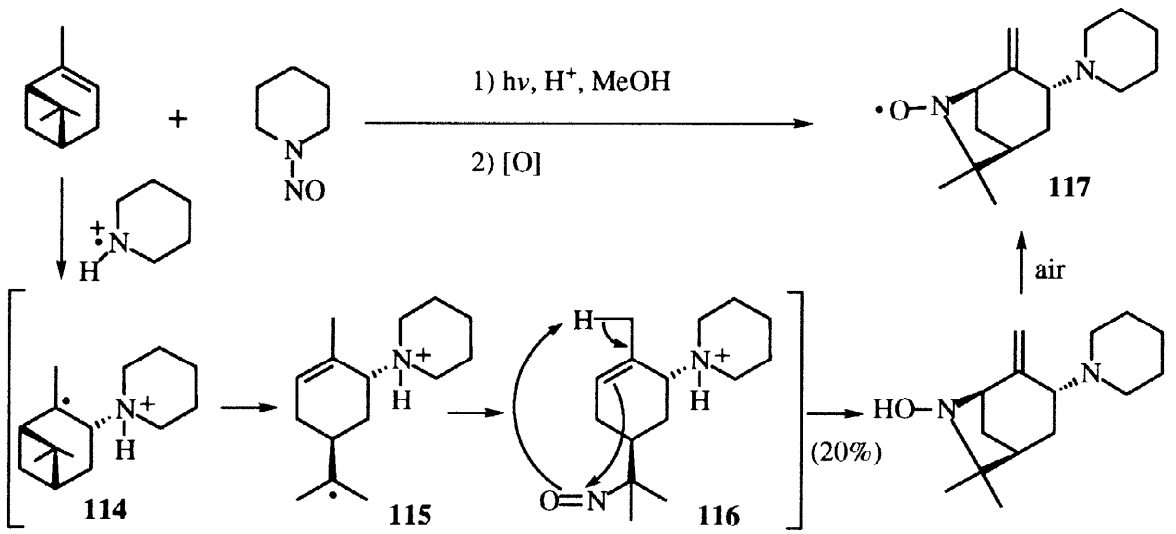
An analog of histidine, **113**, synthesized by Jorgenson[74] contains a nitronyl nitroxide ring mimic of the imidazole group. This analog approximates the size and shape of the natural amino acid and parallels the ion-dipole binding observed in histidine derivatives, making it a versatile tool in the investigation of histidine containing peptides.[75] The nitronyl nitroxide was synthesized by condensation of aldehyde **111** and bis-hydroxylamine **112** followed by oxidation with MnO_2 (Scheme 26).



Scheme 26

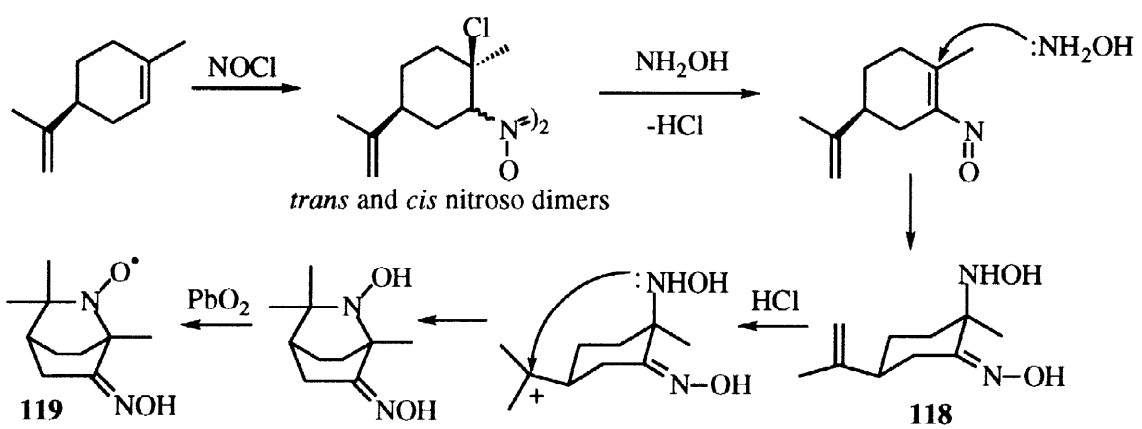
An interesting bridged bicyclic nitroxide **117** derived from α-pinene was prepared by Chow.[76] The synthesis involved photoaddition of *N*-nitrosopiperidine to α-pinene followed by air oxidation (Scheme 27). The mechanism entails addition of the protonated aminyl radical cation to the least hindered end of the pinene

olefin. Fragmentation of the resultant radical[77] **114** gives tertiary radical **115** with relief of ring strain from fracture of the cyclobutane. Addition of the elements of NO and an ene reaction of the resultant nitroso **116** gives nitroxide **117**.



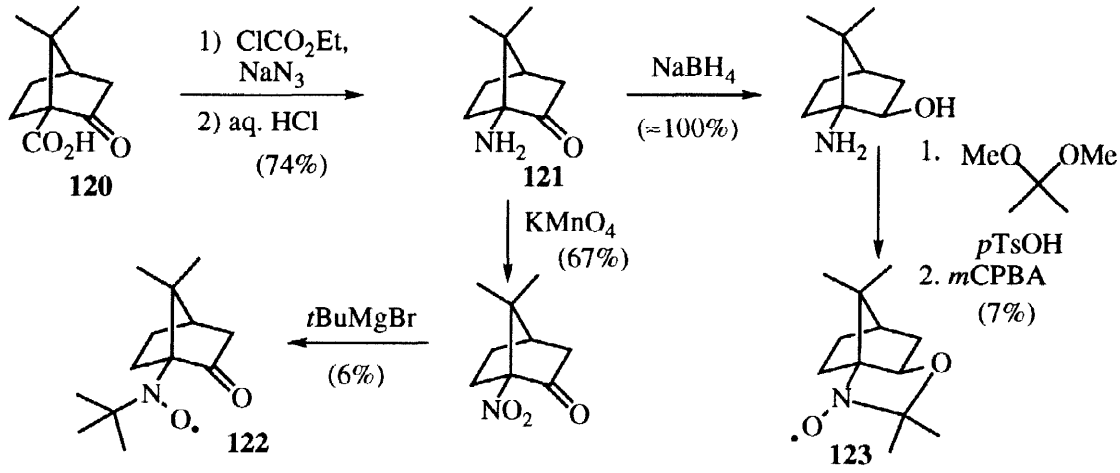
Scheme 27

Another bridged bicyclic nitroxide, **119**, has been prepared by Bakunov from limonene.[78] Addition of nitrosyl chloride across the electron rich trisubstituted olefin of limonene gives a mixture of *trans* and *cis* nitroso dimers, which eliminate HCl upon addition of hydroxylamine. Michael addition proceeds to give **118**, which undergoes an intramolecular nucleophilic addition mediated by HCl. Oxidation of the hydroxylamine yields nitroxide **119** (Scheme 28).



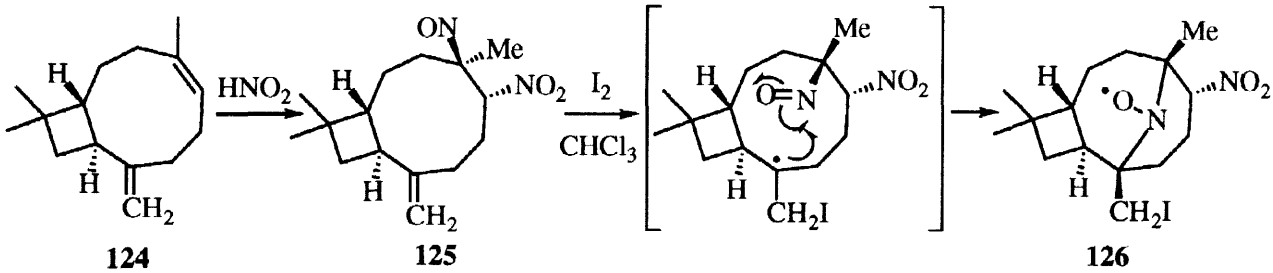
Scheme 28

Bicyclic nitroxides **122** and **123** have been synthesized in our laboratory[38] from ketopinic acid **120** by a Curtius rearrangement to obtain the amino ketone **121**, which was further elaborated to the target compounds (Scheme 29).



Scheme 29

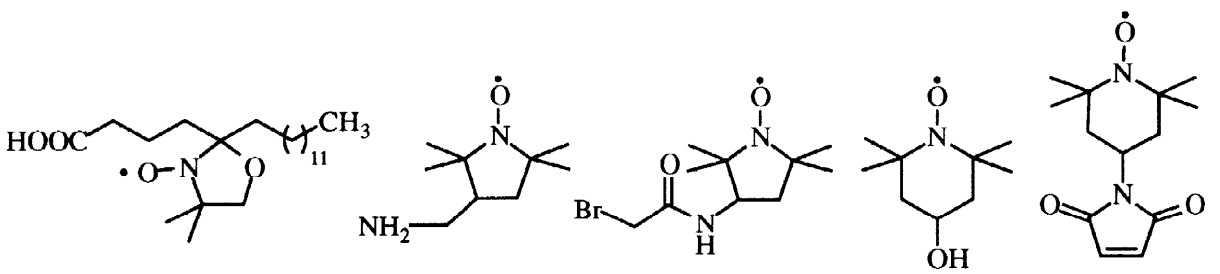
Caryophyllene nitroxide **126** was synthesized via nitrosation of caryophyllene **124** followed by treatment with iodine (Scheme 30).[79] The formation of nitroxide apparently involves addition of iodine radical to the *exo*-methylene group in **125**, to give a tertiary radical which in turn undergoes transannular addition to the nitroso group.



Scheme 30

5. CONCLUSION

Optically active nitroxides have been prepared and utilized for a wide variety of purposes. In some cases the nitroxide functionality has been incorporated into novel skeletal systems. In other cases a pre-existing nitroxide ring is appended onto an optically active biomolecule. There are a number of commercially available[80] achiral or racemic nitroxides that can be used as spin labels by direct attachment to a molecule of interest. Some selected examples are shown in Figure 10.



127 128 129 130 131

Fig. 10

The use of optically active nitroxides in spin labeling and as anticancer agents has been investigated for a number of years. Their use in stereoselective reactions has burgeoned in recent times, as developments in enantioselective alcohol oxidations and stereoselective couplings with prochiral radicals begin to explore their value in asymmetric synthesis. Further advances, spanning from synthetic applications to uses as specialized biomolecular probes, await in the future.

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40. *Caution*: the literature procedure for the preparation of carbamate **35** includes reaction of a chlorocarbonate with sodium azide in dichloromethane. This procedure worked without incident the first dozen times, but resulted in a massive explosion upon rotary evaporation in one instance in our laboratory. We strongly suggest substituting another solvent for dichloromethane for this step.
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Biographical sketch

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Neeta Naik was born in Durg in central India and grew up in Maharashtra, on the west coast of India. She received her B.Sc. and M.Sc. degrees in Chemistry from Bombay University in 1985 and 1987 respectively. For five years, she worked as an information scientist in Bombay abstracting information about new synthetic methods for computerized databases. In 1992 she moved to the United States and in the following year enrolled in the Ph.D. program at the University of California, Santa Cruz. She is currently working on her dissertation in the field of nitroxides in the research group of Professor Rebecca Braslau.

Rebecca Braslau was born in 1959 and grew up in California. She received her B.A. in Chemistry from Reed College, and then spent a year at James Cook University, Australia as a Watson Fellow in marine natural products chemistry. She did her Ph.D. work on organosulfur chemistry and homogeneous palladium catalysis under Professor Barry Trost, first at the University of Wisconsin, Madison, and then at Stanford University. After postdoctoral studies with Professor Bernd Giese in Basel, Switzerland in the area of free radical chemistry, she became an Assistant Professor in 1991 at the University of California, Santa Cruz. Her research interests include the development of free radical methodologies for organic synthesis, the control of stereochemistry in free radical reactions, nitroxides and amino acid radicals.