Synthesis and Reactions of New Alkynyl Substituted Nitroxide Radicals

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Abstract: *N*-Oxylpyrrolidine radicals substituted with electron-withdrawing group activated olefinic or acetylenic group **2a–2h, 4, 5, 6** were synthesised from the pyrroline nitrone **1** or from aromatic aldehydes and the alkynylmagnesium bromide of **3**. The mercury-catalyzed hydration of alkynyl groups of **2b,c** led to the formation of ketones which could be brominated to yield new SH-specific spin labels. The alkynylmagnesium reagent **3** also proved to be versatile in the synthesis of bi- and triradicals **7, 9, 10** and spin labelled steroid derivatives **2e, 12d, 20–22**.

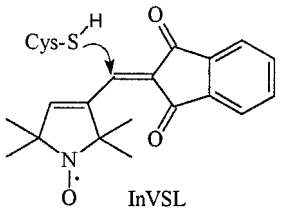
Key words: nitroxide free radicals, spin labelled steroids, α,β-acetylenic ketones, polyradicals

Stable nitroxide free radicals with reactive groups are effective reagents in investigating the structure and function of biomolecules.[1,2] The most useful compounds for spin labelling of macromolecules are generally attached via their functional groups to reactive residues of the macromolecule,[3–6] e.g. to the thiol groups of cysteine in proteins.

We have recently reported that the reaction of pyrroline nitrones with alkynylmagnesium bromide led to 2-alkynylpyrrolidine nitroxide free radicals in high yield.[7] 1,3-Dipolar cycloaddition rarely occured under the described conditions. This paper reports the synthesis of alkynyl nitroxide reagents for spin labelling of biologically active molecules.

The reaction of commercially available stock solution of ethynylmagnesium bromide in THF with 2,5,5-trimethyl-1-pyrroline *N*-oxide (**1**) resulted in the formation of 2,5,5-trimethyl-2-ethynylpyrrolidine-1-yloxyl radical (**2a**). In an analogy to the reaction with phenylethynylmagnesium bromide (**2b**) described earlier,[7] reaction of nitrone **1** with α-naphthylethynyl-magnesium bromide led to the radical **2c**. Nitrone **1** reacts with 2-methylbut-1-en-3-ynmagnesium bromide to give the conjugated en-yne compound **2d** which is a possible synthetic intermediate for a further Diels–Alder reaction. Several nitroxide analogs of different drugs have been developed for use in assays and for investigating receptor site interactions.[8–10] Steroid hormone spin labelled at its ethynyl group **2e** could be obtained when 17-ethynylestradiol reacted first with an excess (3 equiv) of ethylmagnesium bromide and then with the nitrone **1**. This reaction was extended to commercialy available acetylenic alcohols (propynol, but-3-yn-2-ol and 1-phenylprop-2-yn-1-ol) without tetrahydropyranyl protection of the hydroxyl group unlike to our earlier work[7] and led directly to propargylic alcohols. The primary and secondary alcohols could be oxidized to the conjugated aldehyde **2f** or ketones **2g, 2h** with activated MnO_2.

A conjugated ketone reagent, InVSL proved to be an excellent specific SH-reagent of ATP-ases[11,12] and muscle proteins.[13–15] Where other more conventional spin labels (maleimide, iodoacetemide) were loosely attached to protein surface, InVSL was bound rigidly enabling studies of protein dynamics.



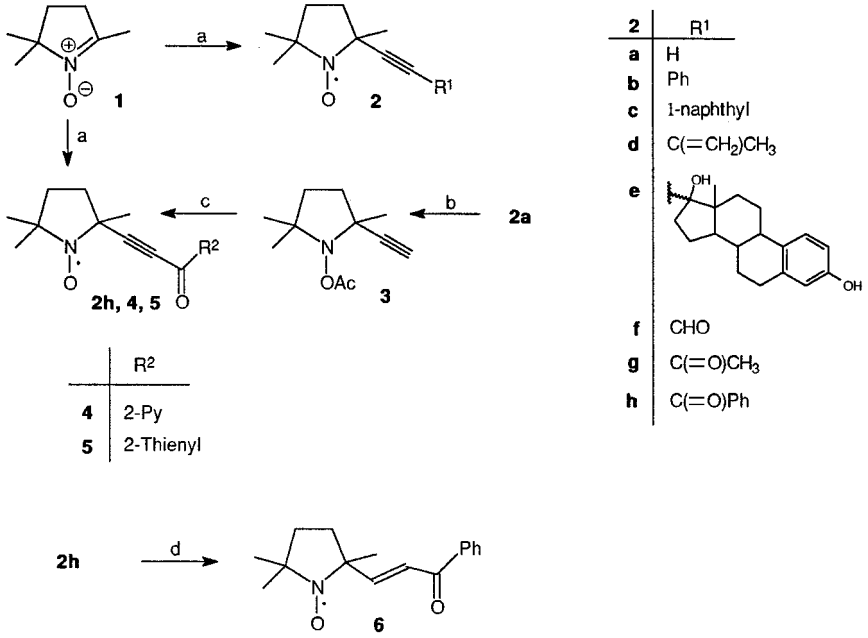
InVSL

However, as a result of putative hydrophobic interactions, in some cases the label was bound at many orientations with respect to the protein precluding studies of protein orientation. The acetylenic labels described here retain the rigid binding of InVSL with the advantage of stereospecific binding. Moreover, we have found that the orientation of the nitroxide moiety with respect to the protein (myosin head) can be manipulated by varying the R group of the ketones **2g, 2h**.

These paramagnetic acetylenic ketones **2h, 4, 5** could be synthesised not only from nitrone **1** but also from 1-acetoxy-2-ethynyl-2,5,5-trimethylpyrrolidine **(3)** when it was reacted first with two equivalents of ethylmagnesium bromide and then with aromatic aldehydes to form secondary alcohols which could be oxidized readily with MnO_2 to carbonyl compounds.

To have a closer comparison, with the known InVSL and the acetylenic ketones, the acetylenic ketone **2h** was reduced to an allylic type of alcohol which could be conveniently oxidized back to the α,β-unsaturated ketone **6** (Scheme 1).

Compound **3** also proved to be a key intermediate for the synthesis of bi- and polyradicals. The synthesis and application of bi- and polynitroxides in physics, chemistry, biochemistry as well as in MRImaging techniques are of current interest.[16,17] The in situ generated Grignard reagent of **3** readily reacts with nitrone **1** or with 2,5-dime-

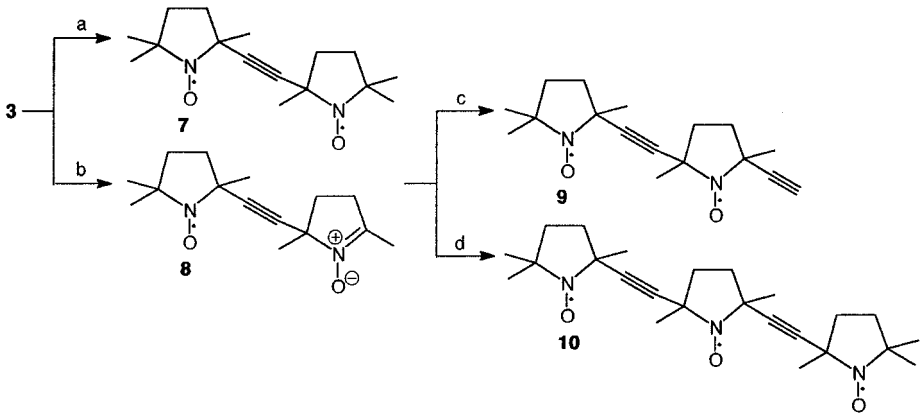


2	R1
a	H
b	Ph
c	1-naphthyl
d	C(=CH2)CH3
e	
f	CHO
g	C(=O)CH3
h	C(=O)Ph

	R2
4	2-Py
5	2-Thienyl

Reagents and conditions: a) R–≡–MgBr/THF/**1**, –10°C to r.t., 2 h, then $NH_4Cl/MnO_2/CHCl_3$, r.t. or reflux, 30 min (46–78%); b) ascorbic acid (5 equiv)/dioxane/H_2O (2:1), 40°C, 15 min, then $CHCl_3/Et_3N$/AcCl, 0°C to r.t., 1 h (87%); c) EtMgBr (2 equiv)/THF/**3**, 50°C, 30 min, then aromatic aldehyde, –10°C to r.t., 1 h, NH_4Cl, then $MnO_2/CHCl_3$, reflux, 15 min (52–71%); d) SMEAH/THF, 5 h, r.t., then 10% NaOH then $CHCl_3/MnO_2$, reflux, 30 min (74%)

Scheme 1

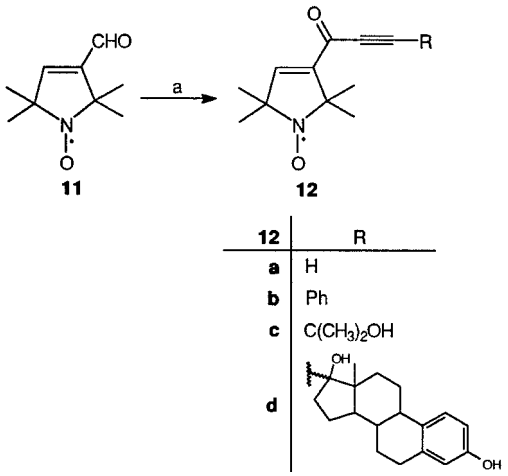


Reagents and conditions: a) EtMgBr (2 equiv)/THF/**3**, 50°C, 30 min, then **1**, –10°C to r.t., 2 h, then $NH_4Cl/MnO_2/CHCl_3$, r.t., 30 min (66%); b) EtMgBr (2 equiv)/THF/**3**, 50°C, 30 min, then 2,5-dimethyl-1-pyrroline*N*-oxide, 2 h, then $NH_4Cl/MnO_2/CHCl_3$, r.t., 30 min (58%); c) HC≡CMgBr/THF/**8**, –10°C to r.t., 2 h, then $NH_4Cl/MnO_2/CHCl_3$, r.t., 30 min (62%); d) EtMgBr (2 equiv)/THF/**3**, 50°C, 30 min, then **8**, –10°C to r.t., 2 h, then $NH_4Cl/MnO_2/CHCl_3$, 30 min (39%)

Scheme 2

thyl-1-pyrroline *N*-oxide to give biradical **7** or nitrone-radical **8**. The nitrone intermediate **8** was reacted again with ethynylmagnesium bromide to yield biradical **9** or with another Grignard derivative of compound **3** to triradical **10** (Scheme 2).

Alkynylmagnesium halides could also be reacted with 3-formyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxyl radical (**11**) without any protection of the *N*-oxyl function to paramagnetic acetylenic alcohols which could be oxidized to acetylenic ketones **12a–d** (Scheme 3).

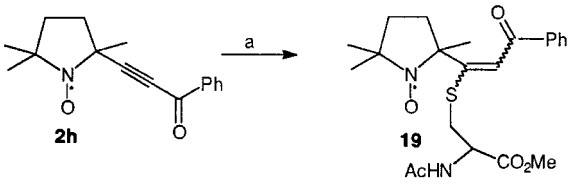


Reagents and conditions: R–≡–MgBr/THF, then **11**, –10°C to r.t., 2 h, NH_4Cl then $MnO_2/CHCl_3$, reflux, 30 min (41–75%)

Scheme 3

The 1,3-dipolar cycloaddition reaction of phenylacetylene with compound **1** in toluene at 110°C led to moderate yield of isoxazoline **13** which gets partially transformed to benzoyl-aziridine **14** at this temperature. However, compound **14** was further rearranged in a ring-expansion reaction to a dihydropyrrolopyrrole compound **15** similarly as described before.[18,19] Reductive cleavage of the N–O bond of isoxazoline **13** with Zn/AcOH[20,21] afforded an amino alcohol which was then oxidized selectively to the paramagnetic alcohol **16** followed by oxidation of the benzyl alcohol to aryl ketone **17a** by MnO_2. This type of ketones could also be obtained when the alkynylaryl compounds **2b, c** were treated with dilute sulfuric acid in the presence of mercury salt. The α-methylene group of paramagnetic ketones **17a, 17b** could be brominated[22] to α-bromoketones **18a,b** without any protection of the nitroxide moiety. The bromine was then substituted with iodine when reacted with NaI to give the α-iodoketone **18c** which is more reactive for selective *S*-spin labelling of protein thiols (Scheme 4).

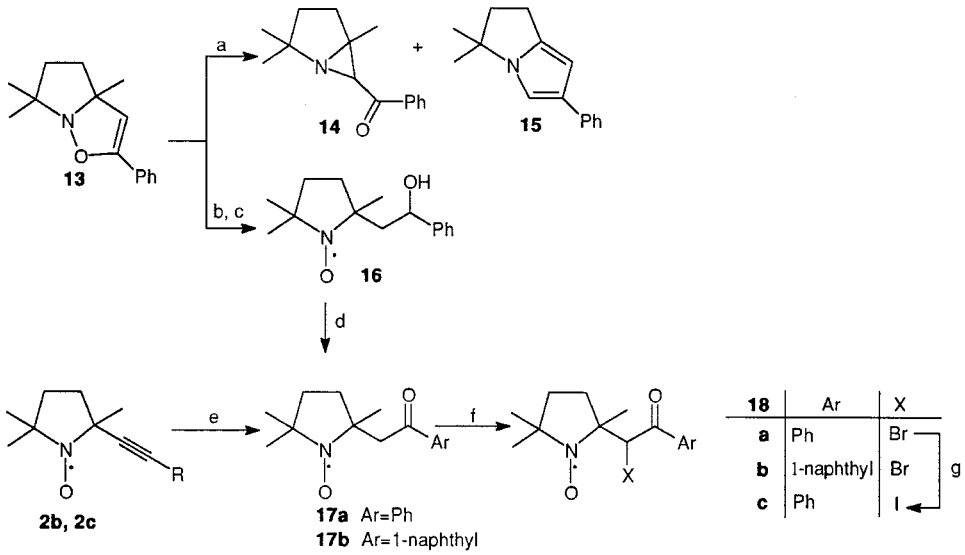
In addition to previously described α-halogenated spin label ketone reagent[23,24] and α,β-unsaturated ketone (In-VSL) a comparative study with **18c** and **2g, 2h, 4, 5, 6** offers a possibility in investigating the influence of the reactive arm on the orientation in labelled protein when the reactive arm is attached in position 2 of pyrrolidine ring. In a model reaction 2-alkynyl substituted nitroxide compound **2h** activated with electron-withdrawing group was suitable for conjugated addition of thiols such as the SH group of *N*-acetyl-cysteine methyl ester (**19**) (Scheme 5).



Reagents and conditions: *N*-acetylcysteine methyl ester/dioxane/H_2O (2:1)/K_2CO_3, r.t., 2 h (62%)

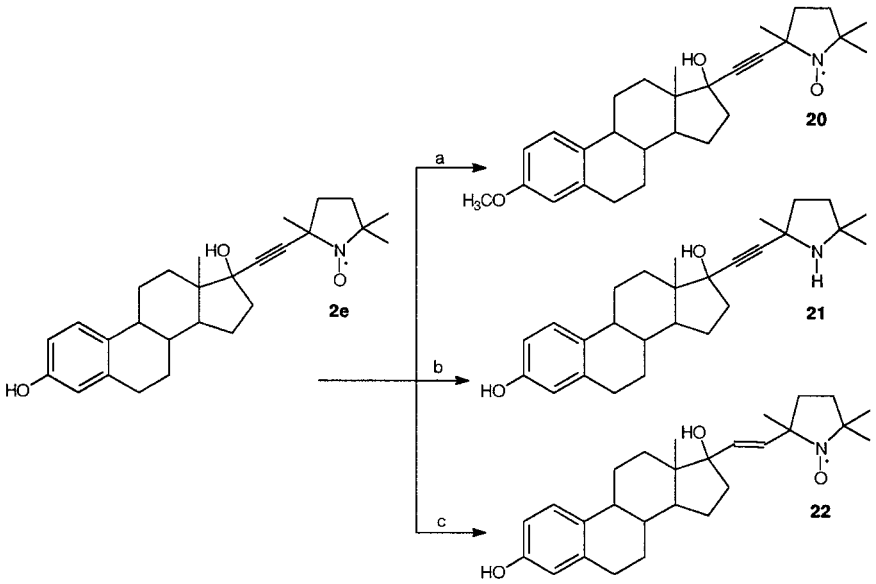
Scheme 5

Spin labelled steroid derivatives were synthesized for investigation of steroid receptor sites. The preparation of spin-labelled mestranol from commercially available



Reagents and conditions: a) toluene, reflux, 2 h (**14**: 21%, **15**: 34%); b) Zn/AcOH/EtOH, 60°C, 45 min, NH_4OH, pH 10; c) H_2O_2/MeOH/H_2O (3:1), cat. Na_2WO_4, 24 h (68%); d) $MnO_2/CHCl_3$, reflux, 15 min (87%); e) $Hg(OAc)_2$/5% H_2SO_4/dioxane, r.t., 24 h (58–62%); f) PHT, 2-pyrrolidone/THF, reflux, 30 min (65–72%); g) NaI/THF, reflux, 2 h (78%)

Scheme 4



Reagents and conditions: a) MeI/K_2CO_3/18-crown-6/dioxane/H_2O (5:1), 2 h, r.t. (76%); b) Fe powder/AcOH, 50°C, then r.t., K_2CO_3 (63%); c) SMEAH/THF, 0°C to r.t., 3 h, then 10% NaOH, then MnO_2/$CHCl_3$, r.t., 30 min (70%)

Scheme 6

mestranol was not successful, the starting material was recovered unreacted. Instead we used the compound **2e** obtained from estradiol which was selectively alkylated at the phenolic hydroxyl group to compound **20.** The reduction of *N*-oxyl function to basic pyrrolidine **21** could be carried out selectively, without the involvement of other functions of the molecule similarly as it was described earlier.[25] The dehydration which often occurs in compounds containing tertiary alcohols in acetic media was not observed. The reduction of alkynyl function of **2e** to alkene **22** makes possible the investigation of the differences in alkenes and alkynes in receptor affinity and biological activity (Scheme 6).

In summary, a facile synthesis of α,β-acetylenic ketones useful for spin labelling of biomolecules was completed. The present method has the advantage over the previously reported method insofar that for obtaining hydroxy acetylenes via Grignard reagents no protection of the hydroxy group is necessary. This method proved to be useful in the synthesis of new physiologically active spin-labelled alkynyl steroids. Alkynyl groups were converted in a mercury catalyzed reaction to ketones containing active methylene groups in α-position bromination of which results in α-bromo ketone reagents. These reagents are used as spin labels of a wide range of biomolecules.

Melting points were determined on a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, ± 0.2; H, ± 0.17; N, ± 0.17; S, ± 0.22) were performed on EA 1110 Elemental Analyser apparatus or (Hal) were carried out titrimetrically by Schöniger's method. The IR spectra (Specord 85) were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode (70 eV, direct inlet) or with thermospray technique. Samples were analysed in the by-pass mode. 10 μL of the sample solution in MeOH was introduced via the thermospray interface. The mobile phase was MeOH/H_2O (1:1) solution containing 0.1 M NH_4OAc. The capillary tip temperature was 230°C, the electrode voltage was 180 V and the source temperature was 210°C. ESR spectra were obtained from a 10^{-5} molar solution ($CHCl_3$) using Bruker 300-E spectrometer. Flash column chromatography was performed on Merck Kieselgel 60 (0.04–0.063 mm).

The physical and spectroscopic data of compounds **2b, 2f** were the same as described.[7]

2,5,5-Trimethyl-2-ethynylpyrrolidines 2a–h and 3-Oxoalkynylpyrrolines 12a–d; General Procedure

To a stirred solution of alkynylmagnesium bromide (0.01 mol) in THF (a stock solution of ethynylmagnesium bromide was used for the synthesis of **2a** and **12a** or prepared from ethylmagnesium bromide and the corresponding alkynyl compound;[26] in the case of compounds **2f,g,h, 12c** the amount of ethylmagnesium bromide was two equiv or three equiv for **2e, 12d** due to the presence of hydroxyl groups) was added dropwise a solution of **1** (0.01 mol, 1.27 g) or **11** (0.01 mol, 1.68 g) in THF (20 mL) at –10°C. After stirring the mixture at r.t. for 2 h, aq NH_4Cl solution (40 mL) was added. The organic phase was separated, dried ($MgSO_4$) and evaporated. The residue was dissolved in $CHCl_3$ (20 mL), activated MnO_2 (0.01 mol, 870 mg) was added and O_2 was bubbled for 30 min or in the case of compounds **2f,g,h** and **12a–d** refluxed for 30 min. The mixture was filtered, evaporated and purified by flash chromatography using hexane/Et_2O or hexane/EtOAc to give **2a–h, 12a–d**.

2-Ethynyl-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (2a)

Yield: 1.19 g (78%); mp 69–71°C; R_f 0.59 (hexane/Et_2O, 2:1).

IR (nujol): ν = 3252 (≡CH), 2097 cm^{-1} (C≡C).

MS: *m/z* (%) = 152 (M^+, 19), 138 (17), 107 (96), 56 (100).

2-[2-(1-Naphthyl)ethynyl]-2,5,5- trimethylpyrrolidin-1-yloxyl Radical (2c)

Yield: 1.97 g (71%); mp 110–112°C; R_f 0.33 (hexane/Et_2O, 2:1).

IR (nujol): ν = 1582 cm^{-1} (C=C_{arom}).

MS: *m/z* (%) = 278 (M^+, 3), 264 (13), 248 (34), 152 (100).

2-(3-Methylbut-3-en-1-yn-1-yl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (2d)
Yield: 1.29 g (67%); oil; R_f 0.50 (hexane/Et_2O, 2:1).

IR (film): ν = 1600 cm^{-1} (C=C).

MS: *m/z* (%) = 192 (M^+, 8), 178 (55), 150 (21), 124 (100).

17α-[2-(2,5,5-Trimethyl-1-oxylpyrrolidin-2-yl)ethyn-1-yl]estra-1,3,5(10)-triene-3,17β-diol Radical (2e)
Yield: 1.94 g (46%); mp 111–113°C; R_f 0.35 ($CHCl_3$/Et_2O, 2:1).

IR (film): ν = 3200 (OH), 1606 cm^{-1} ($C=C_{arom}$).

TSP: *m/z* = 423 $(M+H)^+$.

2,5,5-Trimethyl-2-(3-oxobut-1-yn-1-yl)pyrrolidin-1-yloxyl Radical (2g)
Yield: 1.20 g (62%); oil; R_f 0.48 (hexane/EtOAc, 2:1).

IR (film): ν = 2210 (C≡C), 1674 cm^{-1} (C=O).

MS: *m/z* (%) = 194 (M^+, 2), 180 (15), 164 (8), 41 (100).

2,5,5-Trimethyl-2-(3-oxo-3-phenylprop-1-yn-1-yl)pyrrolidin-1-yloxyl Radical (2h)
Yield: 1.77 g (69%); thick orange oil; R_f 0.45 (hexane/EtOAc, 2:1).

IR (film): ν = 2210 (C≡C), 1640 (C=O), 1592 cm^{-1} ($C=C_{arom}$).

TSP: *m/z* = 257 $(M+H)^+$.

2,5-Dihydro-2,2,5,5-tetramethyl-3-(1-oxoprop-2-yn-1-yl)-1*H*-pyrrol-1-yloxyl Radical (12a)
Yield: 1.44 g (75%); mp 163–165°C; R_f 0.43 (hexane/EtOAc, 2:1).

IR (nujol): ν = 3234 (≡CH), 2088 (C≡C), 1680 (C=O), 1604 cm^{-1} (C=C).

MS: *m/z* (%) = 192 (M^+, 22), 178 (11), 147 (75), 41(100).

2,5-Dihydro-2,2,5,5-tetramethyl-3-(1-oxo-3-phenylprop-2-yn-1-yl)-1*H*-pyrrol-1-yloxyl Radical (12b)
Yield: 1.61 g (60%); mp 75–76°C; R_f 0.65 (hexane/EtOAc, 2:1).

IR (film): ν = 2200 (C≡C), 1640 (C=O), 1610 cm^{-1} (C=C).

MS: *m/z* (%) = 268 (M^+, 4), 254 (8), 238 (19), 129 (100).

2,5-Dihydro-3-(4-hydroxy-4-methyl-1-oxopent-2-yn-1-yl)-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxyl Radical (12c)
Yield: 1.43 g (57%); mp 138–139°C; R_f 0.57 ($CHCl_3$/MeOH, 9:1).

IR (film): ν = 3550–3300 (OH), 2090 (C≡C), 1672 (C=O), 1620 cm^{-1} (C=C).

MS: *m/z* (%) = 250 (M^+, 10), 236 (14), 187 (18), 43 (100).

17α-[3-(2,5-Dihydro-2,2,5,5-tetramethyl-1-oxyl-1*H*-pyrrol-3-yl)-3-oxoprop-1-yn-1-yl]estra-1,3,5(10)-triene-3,17β-diol Radical (12d)
Yield: 1.90 g (41%); mp 113–115°C; R_f 0.31 ($CHCl_3$/Et_2O, 2:1).

IR (nujol): ν = 3300–3200 (OH), 2200 (C≡C), 1645 (C=O), 1600 cm^{-1} ($C=C_{arom}$).

TSP: *m/z* = 463 $(M+H)^+$**.**

1-Acetoxy-2-ethynyl-2,5,5-trimethylpyrrolidine (3)
To a solution of radical **2a** (4.57 g, 0.03 mol) in dioxane (50 mL) was added a solution of ascorbic acid (26.4 g, 0.15 mol) in H_2O (25 mL) and the mixture was stirred at 40°C for 15 min. The colourless solution was extracted with $CHCl_3$ (3 × 30 mL) and dried ($MgSO_4$) under N_2 atmosphere. Then Et_3N (3.33 g, 0.033 mol) and AcCl (2.59 g, 0.033 mol) were added at 0°C. The stirring was continued for 1 h at r.t. then the mixture was filtered and evaporated to dryness. The residue was dissolved in brine (50 mL) and extracted with Et_2O (3 × 30 mL). The organic layer was dried ($MgSO_4$) and evaporated. The product was purified by flash chromatography (hexane/Et_2O) giving the title compound as off-white crystals; yield: 5.09 g (87%); mp 56–58°C; R_f 0.43 (hexane/Et_2O, 2:1).

IR (nujol): ν = 3180 (≡CH), 2080 (C≡C), 1768 cm^{-1} (C=O)

MS: *m/z* (%) = 195 (M^+, 2), 180 (2), 153 (24), 138 (100).

Alkynyl Aryl Ketones 2h, 4, 5; General Procedure
To a solution of EtMgBr (8.0 mmol) in anhyd THF (25 mL) was added compound **3** (780 mg, 4.0 mmol) dropwise. After stirring the mixture at 50°C for 30 min, the appropriate aromatic aldehyde (4.0 mmol) was added in THF at –10°C. After stirring for additional 1 h at r.t. aq satd NH_4Cl solution (30 mL) was added. The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in $CHCl_3$ (20 mL), activated MnO_2 (2 g) was added and the mixture was refluxed for 15 min, filtered and evaporated. The crude product was flash chromatographed with hexane/Et_2O or hexane/EtOAc to give **2h, 4, 5**.

(2h); yield: 0.73 g (71%).

2,5,5-Trimethyl-2-[3-oxo-3-(2-pyridyl)prop-1-yn-1-yl]pyrrolidin-1-yloxyl Radical (4)
Yield: 0.54 g (52%); thick orange oil; R_f 0.19 (hexane/EtOAc, 2:1).

IR (nujol): ν = 2200 (C≡C), 1646 cm^{-1} (C=O).

TSP: *m/z* = 258 $(M+H)^+$**.**

2,5,5-Trimethyl-2-[3-oxo-3-(2-thienyl)prop-1-yn-1-yl]pyrrolidin-1-yloxyl Radical (5)

Yield: 0.57 g (54%); orange oil; R_f 0.21 (hexane/Et_2O, 2:1).

IR (nujol): ν = 2200 (C≡C), 1660 cm^{-1} (C=O).

TSP: *m/z* = 263 $(M+H)^+$**.**

2,5,5-Trimethyl-2-(3-oxo-3-phenyleth-1-en-1-yl)pyrrolidin-1-yloxyl Radical (6)
To a solution of compound **2h** (1.28 g, 5.0 mmol) in anhyd THF (15 mL) was added dropwise with stirring a 70% toluene solution of sodium bis(2-methoxyethoxy)aluminum hydride (SMEAH, 4 mL) in THF (10 mL). The resulting mixture was stirred under Ar for 5 h at r.t. and then cautiously decomposed by the dropwise addition of 10% NaOH (10 mL). The organic phase was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The residue was dissolved in $CHCl_3$ (15 mL), MnO_2 (1.74 g, 20.0 mmol) was added and the mixture was refluxed for 30 min then filtered and evaporated. The product was purified by flash column chromatography with hexane/Et_2O giving the title compound; yield: 956 mg (74%); mp 57–58°C; R_f 0.17 (hexane/Et_2O, 2:1).

IR (film): ν = 1660 (C=O), 1610, 1600 cm^{-1} (C=C).

TSP: *m/z* = 259 $(M+H)^+$.

Biradical 7 and Nitrone Radical 8; General Procedure
To a organomagnesium derivative of compound **3** (5.0 mmol) prepared as above was added a solution of nitrone **1** or 2,5-dimethyl-1-pyrroline *N*-oxide (5.0 mmol) in THF at –10°C. After stirring for additional 2 h at r.t., aq satd NH_4Cl solution (30 mL) was added. The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in $CHCl_3$ (30 mL), activated MnO_2 (100 mg) was added and a stream of O_2 was bubbled for 30 min, filtered and evaporated. The product was flash chromatographed on silica gel with hexane/EtOAc or $CHCl_3$/MeOH.

1,2-Bis(2,5,5-trimethyl-1-oxylpyrrolidin-2-yl)ethyne Biradical (7)
Yield: 0.92 g (66%); oil; R_f 0.13 (hexane/Et_2O, 2:1).

MS: *m/z* (%) = 278 (M^+, 17), 216 (7), 138 (36), 41 (100).

1-(2,5-Dimethyl-1-oxide-1-pyrroline-5-yl)-2-(2,5,5-trimethyl-1-oxylpyrrolidin-2-yl)ethyne Radical (8)

Yield: 0.76 g (58%); oil; R_f 0.32 ($CHCl_3$/MeOH, 9:1).

MS: *m/z* (%) = 263 (M^+, 2), 249 (5), 113 (48), 41 (100).

Bi- and Triradicals 9, 10; General Procedure

To an organomagnesium derivative of compound **3** (5.0 mmol) prepared as above or ethynylmagnesium bromide (5.0 mmol) was added a solution of nitrone **8** (1.32 g, 5.0 mmol) in THF at –10°C. After stirring for additional 2 h at r.t., aq satd NH_4Cl solution (30 mL) was added. The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in $CHCl_3$, MnO_2 (100 mg) was added and a stream of O_2 was bubbled for 30 min, filtered and evaporated. The product was flash chromatographed on silica gel with hexane/EtOAc.

1-(2-Ethynyl-2,5-dimethyl-1-oxylpyrrolidine-5-yl)-2-(2,5,5-trimethyl-1-oxylpyrrolidin-2-yl)ethyne Biradical (9)

Yield: 0.89 g (62%); oil; R_f 0.44 (hexane/EtOAc, 2:1).

IR (nujol): ν = 3220 (≡CH), 2090 cm^{-1} (C≡C).

MS: *m/z* (%) = 288 (M^+, 9), 258 (8), 219 (40), 41 (100).

2,5-Dimethyl-2,5-bis(2,5,5-trimethyl-1-oxylpyrrolidin-2-ethyn-2-yl)-1-oxylpyrrolidine Triradical (10)

yield: 0.81 g (39%); oil; R_f 0.40 (hexane/EtOAc, 2:1).

TSP: *m/z* = 416 $(M+H)^+$.

Rearrangement of 4-Isoxazoline 13 to Acylaziridine 14 and Pyrrole 15

A solution of 4-isoxazoline (**13**) (458 mg, 2.0 mmol) in toluene (15 mL) was refluxed for 2 h, then the solvent was evaporated in vacuo and purified by flash column chromatography.

2-Benzoyl-3,6,6-trimethyl-1-azabicyclo[1.3.0]hexane (14)

Yield: 96 mg (21%); oil; R_f 0.18 (hexane/Et_2O, 2:1).

IR (film): ν = 1682 (C=O), 1597 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 229 (M^+, 8), 214 (83), 77 (55), 68 (100).

8,8-Dimethyl-3-phenyl-1-azabicyclo[3.3.0]octa-2,4-diene (15)

Yield: 144 mg (34%); oil; R_f 0.78 (hexane/Et_2O, 2:1).

IR (film): ν = 1596 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 211 (M^+, 92), 196 (68), 156 (100), 77 (24).

2-(2-Hydroxy-2-phenylethyl)-2,5,5-trimethylpyrrolidine-1-yloxyl Radical (16)

To a solution of the isoxazoline **13** (1.15 g, 5.0 mmol) in EtOH (10 mL) were added a 10 M solution of AcOH (30 mL) and ethylenediamine tetraacetic acid disodium salt (~ 8 g). The mixture was warmed up to 60°C, stirred and Zn powder (3.25 g, 50 mmol) was added. After maintaining the temperature at 60°C for 45 min, the mixture was cooled to r.t., adjusted to pH 10 by adding 30% NH_3 solution and extracted with $CHCl_3$ (3 × 10 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was dissolved in MeOH (15 mL) then a catalytic amount of Na_2WO_4 (200 mg) and H_2O_2 solution (30%, 5 mL) were added and the mixture was allowed to stand at r.t. overnight. The solvent was evaporated to 1/4 of its volume, extracted with $CHCl_3$, dried ($MgSO_4$) and evaporated. The product was purified by flash chromatography using hexane/EtOAc to give **16**; yield: 844 mg (68%); mp 51–52°C; R_f 0.48 (hexane/EtOAc, 2:1).

IR (film): ν = 3330 (OH), 1595 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 248 (M^+, 2), 218 (2), 128 (100), 107 (60).

2-Benzoylmethyl-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (17a) from 16

To a solution of **16** (496 mg, 2.0 mmol) in $CHCl_3$ (15 mL) was added activated MnO_2 (435 mg, 5.0 mmol) and the mixture was refluxed for 15 min, then filtered and evaporated. The residue was purified by flash column chromatography with hexane/Et_2O as eluent; yield: 428 mg (87%); mp 72–73°C; R_f 0.28 (hexane/Et_2O, 2:1).

IR (film): ν = 1660 (C=O), 1580 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 246 (M^+, 8), 214 (3), 128 (9), 105 (100).

Preparation of Ketones 17a, 17b from Alkynes 2b, 2c; General Procedure

To a solution of **2b** or **2c** (3.0 mmol) in dioxane (15 mL) were added 5% H_2SO_4 (5 mL) and a catalytic amount of $Hg(OAc)_2$ (~ 10 mg). The mixture was allowed to stand at r.t. for 24 h. After the reaction was complete, brine (15 mL) was added and extracted with $CHCl_3$ (3 × 15 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by flash chromatography.

2,5,5-Trimethyl-2-(2-oxo-2-phenylethyl)pyrrolidin-1-yloxyl Radical (17a)

Yield: 429 mg (58%).

2,5,5-Trimethyl-2-[(2-(1-naphthyl)-2-oxoethyl]pyrrolidin-1-yloxyl Radical (17b)

Yield: 551 mg (62%); oil; R_f 0.22 (hexane/Et_2O, 2:1).

IR (film): ν = 1664 (C=O), 1590 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 296 (M^+, 19), 170 (56), 155 (100), 127 (98).

α-Bromoketones 18a, 18b; General Procedure

To a solution of ketone **17a**, **17b** (4.0 mmol) in anhyd THF (30 mL) were added 2-pyrrolidone (426 mg, 5.0 mmol) and pyrrolidone hydrotribromide (PHT, 2.48 g, 5.0 mmol). The mixture was refluxed for 30 min and allowed to cool to r.t., washed with brine (30 mL), dried ($MgSO_4$) and the solvent was removed in vacuo. The crude product was purified by flash chromatography with hexane/Et_2O.

2-(1-Bromo-2-oxo-2-phenylethyl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (18a)

Yield: 846 mg (65%); oil; R_f 0.79 (hexane/EtOAc, 2:1).

IR (film): ν = 1682 (C=O), 1590 cm^{-1} ($C=C_{Arom}$).

MS: *m/z* (%) = 326/324 (M^+, 1/1), 215 (3), 157 (22), 105 (100).

2-[1-Bromo-2-oxo-2-(1-naphthyl)ethyl]-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (18b)

Yield: 1.08 g (72%); mp 128–129°C; R_f 0.30 (hexane/Et_2O, 2:1).

IR (film): ν = 1668 cm^{-1} (C=O).

MS: *m/z* (%) = 374/376 (M^+, 3/3), 265 (6), 155 (100), 127 (49).

2-(1-Iodo-2-oxo-2-phenylethyl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (18c)

To a solution of **18a** (325 mg, 1.0 mmol) in anhyd THF (10 mL) was added NaI (300 mg, 2 mmol) and the mixture was refluxed for 2 h. The solvent was evaporated, the residue dissolved in brine (15 mL) and extracted with Et_2O (3 × 15 mL). The organic layer was dried ($MgSO_4$), evaporated and purified by flash chromatography (hexane/Et_2O); yield: 290 mg (78%); oil; R_f 0.80 (hexane/EtOAc, 2:1).

IR (film): ν = 1690 (C=O), 1595 cm^{-1} ($C=C_{Arom}$).

MS: *m/z* (%) = 372 (M^+, not detectable), 245 (M – I, 1), 157 (10), 105 (100).

***S*-Spin Labelled *N*-Acetylcysteine Methyl Ester (19)**

To a solution of **2h** (512 mg, 2.0 mmol) in dioxane/H_2O (2:1, 15 mL) were added *N*-acetylcysteine methyl ester (354 mg, 2.0 mmol) and K_2CO_3 (276 mg, 2.0 mmol) and the mixture was stirred for 2 h

at r.t. Then H_2O (10 mL) was added and extracted with $CHCl_3$ (3 × 10 mL). The organic phase was dried ($MgSO_4$), evaporated in vacuo and purified by flash chromatography with hexane/EtOAc; yield: 538 mg (62%); mp 67–69°C; R_f 0.16 ($CHCl_3/Et_2O$, 2:1).

IR (film): ν = 3400–3200 (NH), 1740 (C=O), 1658, 1640 (C=O), 1600, 1590 cm^{-1} (C=C).

TSP: *m/z* = 434 $(M+H)^+$.

17α-3-Methoxy-[2-(2,5,5-trimethyl-1-oxylpyrrolidin-2-yl)ethyn-1-yl]estra-1,3,5(10)-triene-17β-ol Radical (20)

To a solution of **2e** (423 mg, 1.0 mmol) in dioxane/H_2O (5:1, 20 mL) were added K_2CO_3 (276 mg, 2.0 mmol), MeI (284 mg, 2.0 mmol) and 18-Crown-6 (20 mg) and the mixture was stirred at r.t. for 2h. Then H_2O (20 mL) was added and extracted with $CHCl_3$ (3 × 20 mL). The organic layer was dried ($MgSO_4$), evaporated and purified by flash chromatography with $CHCl_3/Et_2O$ as eluent; yield: 332 mg (76%); mp 65–67°C; R_f 0.44 ($CHCl_3/Et_2O$, 2:1).

IR (film): ν = 3400–3300 (OH), 1606 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 436 (M^+, 7), 406 (38), 227 (82), 55 (100).

17α-[2-(2,5,5-Trimethylpyrrolidin-2-yl)ethyn-1-yl]estra-1,3,5(10)-triene-3,17β-diol (21)

To a solution of **2e** (423 mg, 1.0 mmol) in AcOH (30 mL) was added Fe powder (280 mg, 5.0 mmol) and the mixture was kept at 50°C for 3 h. Then the mixture was allowed to cool to r.t., H_2O (10 mL) was added and the aqueous phase was decanted and basified adding solid K_2CO_3. The aqueous phase was extracted with $CHCl_3$ (3 × 20 mL), dried ($MgSO_4$) and evaporated. The residue was purified on silica gel with $CHCl_3$/MeOH; yield: 257 mg (63%); mp 199–200°C; R_f 0.35 ($CHCl_3$/MeOH, 9:1).

IR (nujol): ν = 3500–3200 (OH, NH), 1606 cm^{-1} ($C=C_{arom}$).

MS: *m/z* (%) = 407 (M^+, 2), 392 (19), 374 (2), 43 (100).

17α-[2-(2,5,5-Trimethyl-1-oxylpyrrolidin-2-yl)ethen-1-yl]estra-1,3,5(10)-triene-3,17β-diol Radical (22)

To a solution of **2e** (423 mg, 1.0 mmol) in anhyd THF (20 mL) was added dropwise SMEAH (70% in toluene, 1 mL) at 0°C. After completion of the addition, the cooling bath was removed and the mixture was stirred at r.t. for 3 h. Then 10% NaOH (10 mL) was added, the organic phase was separated and the aqueous layer was extracted with $CHCl_3$ (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), and a catalytic amount of MnO_2 (100 mg) was added and a stream of O_2 was bubbled for 30 min. The mixture was filtered and the solvent was removed under vacuum. The residue was purified by flash chromatography ($CHCl_3/Et_2O$) leading to the olefinic compound **26**; yield: 300 mg (70%); mp 113–115°C; R_f 0.17 (hexane/EtOAc, 2:1).

IR (nujol): ν = 3300 (OH), 1606 cm^{-1} (C=C).

MS: *m/z* (%)= 424 (M^+, 1), 394 (7), 55 (66), 43 (100).

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