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Novel, potentially useful spin-label reagents

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Abstract - New nitroxide spin-label (SL) synthons and reagents were synthetized: SL-fatty acids and SL-dienones, maleimido or azido carboxylic acid cross linking reagents, α-bromoketones, SL-glyoxals, SL-thiuronium and selenuronium salts, thiols and selenols, disulfides and diselenides, cationic and anionic probes, and SL-drug molecules.

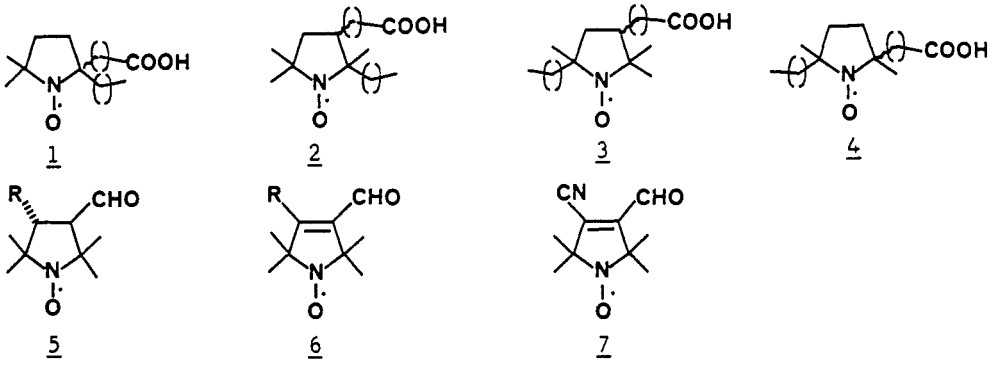
INTRODUCTION

Although a large collection of stable nitroxides (N-oxyls, aminoxyls) with selectively reacting functions are used for the spin-labelling (SL) of biomolecules[1-3], the need for novel reagents with higher reactivity and better stability is increasing. One example is the preparation of pyrrolidine-1-oxyl fatty acids[4] instead of less stable oxazolidinyl-1-oxyl ones[5]. The conventional chemical protein modifications (N-ethylmaleimide, α-haloacetamides, 2,4-dinitrochloro- (or fluoro-) benzene, activated esters, imidazolides, etc.)[6] have made an important impact on the development of paramagnetic analogues of these reagents with relatively easy chemical reactions for the study of proteins by ESR spectroscopy. However, the expanding domain of novel reagents[7], such as cleavable, photosensitive, cross-linking, bifunctional (homo or hetero), site-specific reagents, offers opportunities for the design of such novel but SL reagents too.
Furthermore, the replacement of an α-amino acid unit with a paramagnetic α-amino acid in a biologically active peptide offers a potential opportunity for investigations of relationships between structure and biological activity. The SL of drugs with the minimum possible perturbation of the structure, in an effort to retain the biological character of the drugs after the labelling, is an interesting method for mapping receptor sites. It may be borne in mind that the biological oxidation of secondary cyclic amines led to the formation of nitrones[8], or in special cases (such as 2,2,6,6-tetramethylpiperidine[9]) to N-oxyl compounds; this offers an opportunity for the design of biologically active new molecules oxidizing to nitroxide *in vivo*.

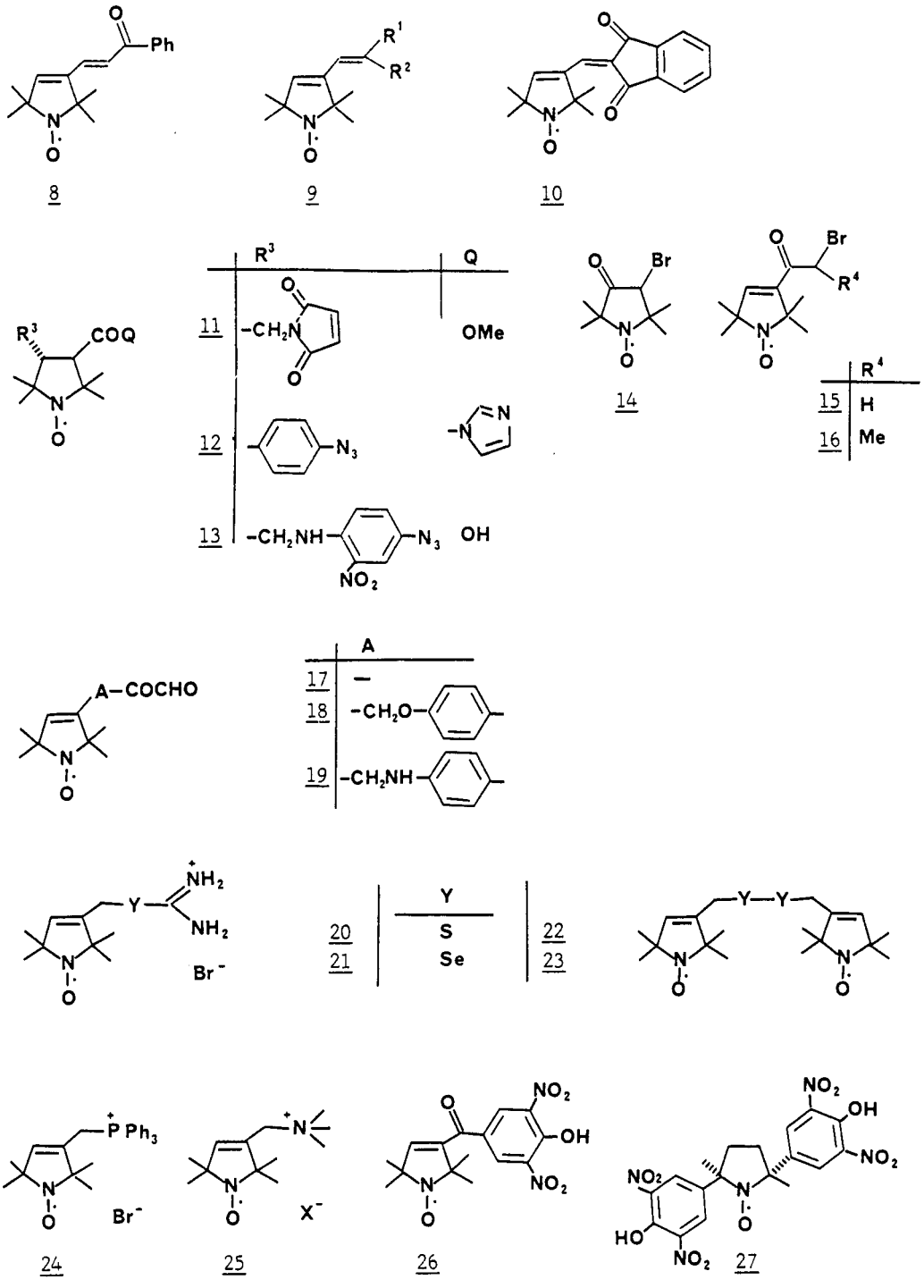
NEW SPIN-LABEL REAGENTS

In recent years our laboratory has developed several potentially useful SL reagents. Some of them are already used in biological studies (Scheme 1,2).

Scheme 1



Scheme 2

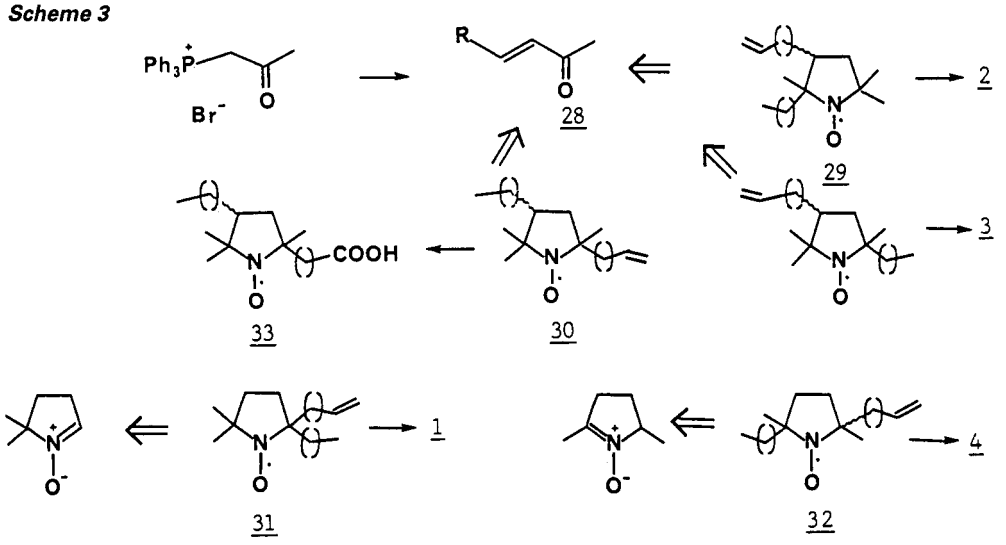


The compounds produced include fatty acids 1-4[10], intermediates for unsaturated fatty acids 5-7[11], dienes activated with electron-withdrawing group(s) 8-10[12], capable of nucleophilic addition of HS groups for ATPase membrane studies [13], heterobifunctionalized pyrrolidines 11-13[14], α-bromoketones 14-16[11], α,β-dioxo compounds labelling at the guanidino moiety 17-19[15], thiuronium 20[16a] and selenuronium salts 21[17b], disulfides, diselenides 22[16a],23[16b,17], cationic probes 24,25[18] and anionic probes 26,27[19].

FATTY ACIDS

The more stable 2,2- and 2,5-functionalized pyrrolidine-N-oxyl fatty acids (the proxyls and azetoxyls) were synthetized by utilizing the reactions between Grignard reagents and well-known nitrones, but the development of a carboxylic function is a rather laborious multiple-step procedure (e.g. -OTHP → -OH → -OMs → -X → -CN → -CO_2H) .
In our procedures, we introduced compounds containing a terminal double bond 29-32 which can be oxidized in one step to a carboxylic group. Starting from ß-alkyl or ß-alkenyl α,ß-unsaturated oxo compounds 28 allowed us to prepare 2,3- and 2,4-functionalized pyrrolidine-1-oxyl fatty acids 2,3,33 too (Scheme 3).

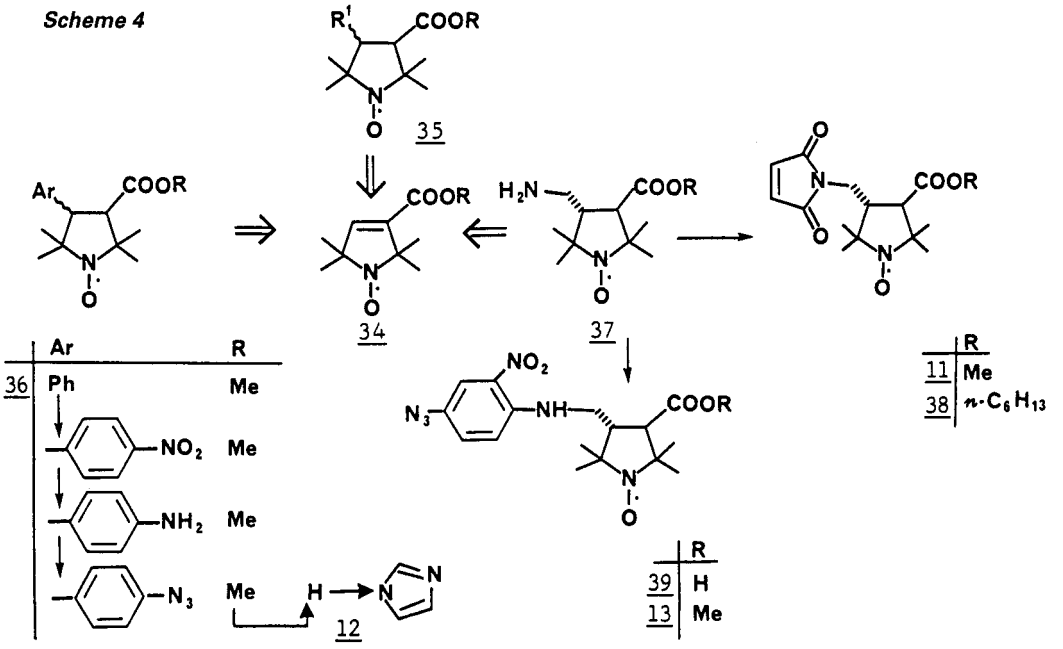
Scheme 3



CROSS-LINKING REAGENTS

There are ample opportunities for the introduction of functionalities into position 3 and 4 in the pyrroline ring : the introduction of an alkyl or aryl group into the ß-position to the ester function of 34, in a conjugate addition via a Grignard reaction, allowed the preparation of compounds 35,36. The conjugate addition of nitromethane, followed by selective reduction to the corresponding γ-amino ester 37, yielded a synthon for the preparation of maleimide 38 and aryl azide 39 compounds (Scheme 4).

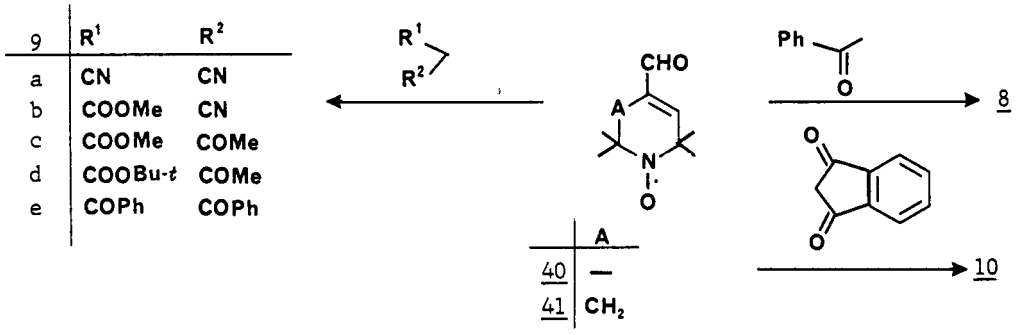
Scheme 4



DIENES ACTIVATED BY ELECTRON-WITHDRAWING GROUPS

Neither the pyrroline-3-aldehyde 40 nor the 1,2,5,6-tetrahydropyridine-4-carboxaldehyde 41 were reactive enough to react with bionucleophiles. Therefore, they were converted in an aldol-type reaction to electron-withdrawing group-activated β-electrophilic dienes 8-10[12]. Especially the indanedione derivative 10 exhibited a high reactivity towards SH groups[13] (Scheme 5).

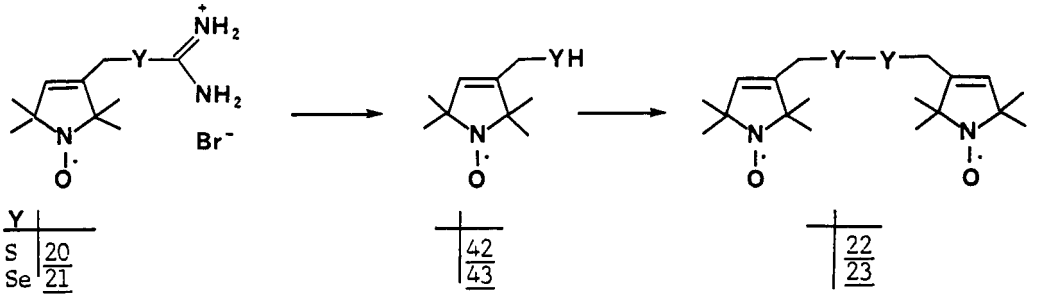
Scheme 5



THIO AND SELENO REAGENTS

We earlier reported that a paramagnetic thiol compound, 3-thiolmethyl-2,2,5,5-tetramethylpyrroline-1-oxyl 42, can be obtained from thiuronium salt 20[16a]. In the same way selenuronium salt 21 can be obtained, from which the very reactive allylic selenol 43 can be prepared. The 42 and 43 are oxidized to biradical disulfide 22 and diselenide 23, respectively[16b]. These biradicals are able to react with protein SH groups[17] (Scheme 6).

Scheme 6

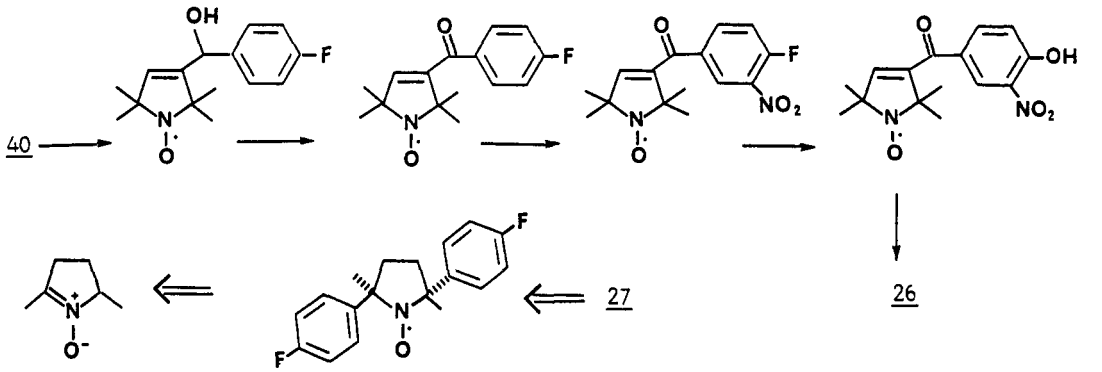


CATIONIC AND ANIONIC PROBES

Phosphonium compound 24 was obtained[18] from allylic halide 44 with Ph_3P, and the amines 25 were prepared[18] by reductive amination reaction of 40 with $NaCNBH_3$.

Various aryl nitroxides have been nitrated with concentrated H_2SO_4/HNO_3 without harm to the nitroxide function, to give ultimately highly acidic polynitrophenol substituted, water-soluble nitroxides as a series of promising anionic probes, e.g. 26,27[19] (Scheme 7).

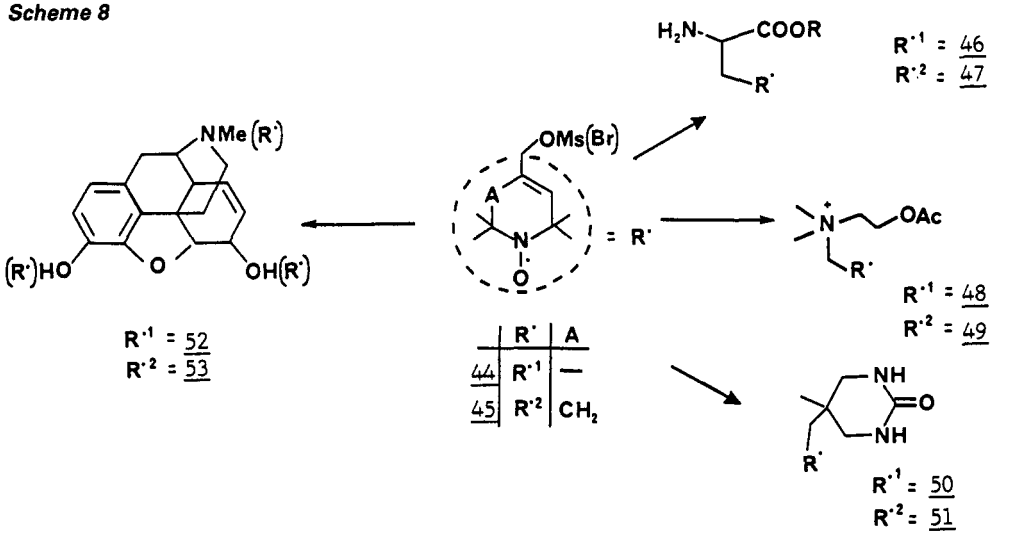
Scheme 7



LABELLED α-AMINO ACIDS AND DRUGS

The excellent reactivity of allylic sulfonates or halides 44,45 can be utilized for the preparation of α-amino acid derivatives spin-labelled in the side-chain 46,47 [21,22].
Several drugs can be labelled 48-53 with remarkably little perturbation of their biological function[23] (Scheme 8).

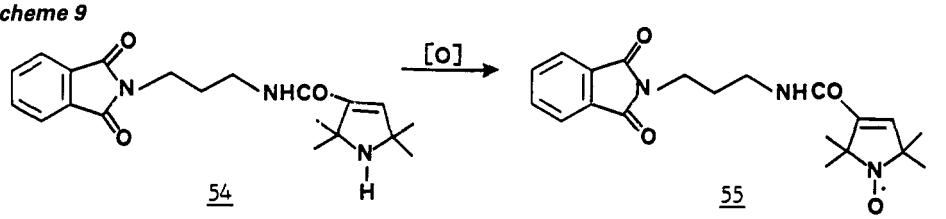
Scheme 8



NEW ANTIARRHYTHMIC COMPOUNDS

In an extended collaboration with pharmacologists, a new group of diamagnetic antiarrhythmic compounds 54 has been developed. These compounds belong in the class of membrane affine antiarrhythmic drugs (such as quinidine and procainamide), which can be oxidized either *in vitro* or *in vivo* to free radical compounds such as 55 [24] (Scheme 9).

Scheme 9



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