



# Radical Reactions for Chemoselective Modification of Peptides

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by

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## Abstract

With a view to developing procedures for chemoselective modification of peptides and proteins, generation of carbon-centred radicals in amino acid derivatives, amino acids, and peptides has been investigated. Trapping and detection of these radicals has also been studied.

In the photolytic reaction of triglycine with di-*tert*-butyl peroxide,  $\alpha$ -centred radicals were trapped as either dimers of triglycine or tripeptides resulting from conversion of one glycine residue to alanine. Alanine residues were formed at either the C-terminal or non-terminal residue of the tripeptide. The dimers were either symmetric, crosslinked between C-terminal residues, or non-symmetric, linked between C-terminal and non-terminal residues. Product studies on this reaction demonstrated the decreasing relative ease of formation of C-terminal, non-terminal, and N-terminal  $\alpha$ -centred radicals in peptides.

The  $\alpha$ -centred glycy radical generated *via* reaction of *N*-benzoylglycine methyl ester with di-*tert*-butyl peroxide, was trapped by the radical scavenger 1,1,3,3-tetramethylisoindolin-2-yl oxyl.

A series of attempts to generate a  $\beta$ -carbon-centred radical from *N*-benzoyl-*S*-methylcysteine methyl ester and *N*-benzoyl-*S*-methylcysteine sulfoxide methyl ester is discussed. Evidence for efficient generation of a  $\beta$ -centred radical was derived from product studies of photolytic reactions of the *S*-methylcysteine sulfoxide derivative with di-*tert*-butyl peroxide in either benzene, benzene/carbon tetrachloride, or  $\alpha,\alpha,\alpha$ -trichlorotoluene. The *S*-methylcysteine sulfoxide derivative was converted to the corresponding alanine, phenylalanine, or  $\beta$ -chloroalanine derivatives in these reactions.

Generation of carbon-centred radicals through reaction of sulfides and sulfoxides with hydrogen peroxide, initiated by ferrous ions or photolysis, is reported. Radicals generated from a variety of sulfides and sulfoxides have been

trapped with sodium 3,5-dibromo-4-nitrosobenzene sulfonate. The resultant spin adducts were detected by electron spin resonance (e.s.r.) spectroscopy and structurally assigned by examination of the e.s.r. spectra. E.s.r. signals arising from spin adducts of radicals formed by carbon-sulfur bond homolysis were observed. Studies of compounds containing both amine and sulfide functionality indicated that the amino group must be protonated to ensure that reaction occurs at the sulfide moiety, otherwise alternative reactions involving the deprotonated amino group may occur.

Evidence for selective generation of side-chain carbon-centred radicals was obtained when the methodology described above was applied to sulfur containing amino acids and peptides. Reactions of methionine and methionine sulfoxide gave rise to e.s.r. spectra attributable to generation of a  $\gamma$ -centred amino acid radical. Similarly, an e.s.r. spectrum attributable to formation of a  $\beta$ -centred radical was observed on reaction of acidified S-methylcysteine. Reactions of glycylmethionylglycine, methionylleucylphenylalanine, and acidified S-methylglutathione gave rise to e.s.r. spectra attributable to generation of side-chain carbon-centred radicals *via* selective reaction of the sulfur containing side-chains.