

Nitroxide Spin Labels: New Synthetic Sequences

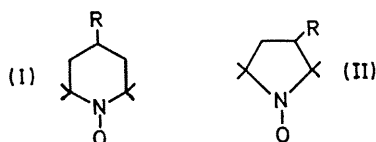
By DANIEL J. KOSMAN* and LAWRENCE H. PIETTE

(Laboratory of Biophysics, University of Hawaii, Honolulu, Hawaii, 96822)

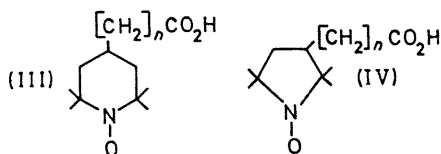
Summary Several new and useful nitroxide molecules have been prepared *via* common organic reactions.

was converted into the aldehyde and then treated with the ester ylide.

THE versatility of the nitroxide spin labels (I) and (II) in probing macromolecular structure^{1,2} has been limited by

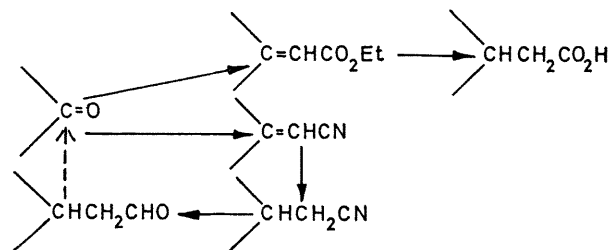
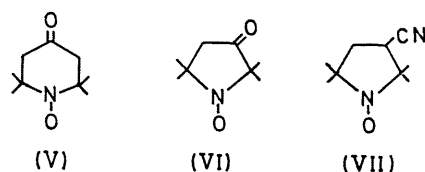


the lack of exploratory synthetic research involving these molecules. We report some new synthetic sequences which are compatible with the chemical behaviour of the nitroxide function.³



For our study of α -chymotrypsin,⁴ carboxylic acids of the type (III) and (IV) were required. While a keto-nitroxide has been employed in the Stobbe condensation,⁵ it has not been so in the modified Wittig reaction.⁶ Nitroxides (V) and (VI) react cleanly with the ylides derived from triethylphosphonoacetate and diethyl cyanomethylphosphonate, under the conditions described in the literature,⁶ to give in good yields (*ca.* 60%) the corresponding esters or nitriles. Saturation at low pressure (45 psi, Parr shaker) over Adams catalyst in 95% ethanol is accompanied by reduction of the nitroxide, but re-oxidation readily occurs upon exposure to the air. Treatment of the recovered product with lead dioxide ensured complete oxidation.⁴ The esters were hydrolysed in 20% aqueous ethanol (NaOH) to (III) and (IV), $n = 1$.

The nitriles were exceptionally useful in that they can be converted into aldehydes by treatment with $\text{Li}(\text{OEt})_3\text{AlH}$ in the standard fashion.⁷ The aldehydes were then treated with the ester ylide, hydrogenated, reoxidized, and saponified to yield (III) and (IV), $n = 3$. Obviously, treatment of an aldehyde with the cyano-ylide will eventually lead to the acid, $n = 5$. For (IV), $n = 2$, nitrile (VII)⁸



The report that lithium aluminium hydride reduced nitroxide (V) to the hydroxylamine⁸ has apparently stifled the use of this reagent. However, (VI) and the esters and carboxylic acids described here and elsewhere⁸ are reduced to the corresponding alcohols with no reduction of the free radical. Furthermore, while $\text{Li}(\text{OEt})_3\text{AlH}$ does reduce (V) to the alcohol, this mono-hydride reagent does not affect the nitroxide, even when the hydride concentration is at an excess (*ca.* 4-fold). The implication is that LiAlH_4 reacts with the nitroxide function of (V) intramolecularly, *i.e.*, hydride transfer to the nitroxide proceeds from the alkoxy intermediate formed in the reduction of the carbonyl group. Such a transannular reaction is not possible if only one hydride is available per molecule of reducing agent. Significantly, use of a stoichiometric amount of LiAlH_4 (1 : 4) results in incomplete conversion of ketone into alcohol. Thus LiAlH_4 is not inimical to nitroxides, except for the one exception discussed here.

Another reaction which appears to be applicable to nitroxide label synthesis is the conversion of ROH into RCl employing triphenylphosphine in CCl_4 .⁹ Either an alcohol or carboxylic acid may be utilized, the latter yielding the acid chloride. Since these useful reagents are not available *via* conventional techniques,¹⁰ this mild, neutral conversion is extremely valuable.

These nitroxides are stable in a number of useful synthetic sequences, particularly those involving bases. However, their sensitivity to mineral and strong Lewis acids under moderate to extreme conditions^{3,11} still remains a stumbling block to even more widespread utility.

(Received, June 17th, 1969; Com. 873.)

¹ C. L. Hamilton and H. M. McConnell in "Structural Chemistry and Molecular Biology," ed. A. Rich and N. Davidson, W. H. Freeman and Co., San Francisco, 1968, pp. 115—149.

² O. H. Griffith and A. S. Waggoner, "Nitroxide Free Radicals," *Accounts Chem. Res.*, 1969, **2**, 17.

³ V. A. Golubev, E. G. Rozantsev, and M. B. Nieman, *Izvest. Akad. Nauk. S.S.S.R.*, 1965, 1927.

⁴ D. J. Kosman, J. C. Hsia, and L. H. Piette, *Arch. Biochem. Biophys.*, 1969, in the press.

⁵ V. A. Golubev and E. G. Rozantsev, *Izvest. Akad. Nauk. S.S.S.R.*, 1965, 716.

⁶ J. Wolinsky and K. L. Erickson, *J. Org. Chem.*, 1965, **30**, 2208.

⁷ H. C. Brown and C. P. Garg, *J. Amer. Chem. Soc.*, 1964, **86**, 1085.

⁸ E. G. Rozantsev and L. A. Krinitzkaya, *Tetrahedron*, 1965, 491.

⁹ I. M. Downie, J. B. Holmes, and J. B. Lee, *Chem. and Ind.*, 1966, 900; J. B. Lee, *J. Amer. Chem. Soc.*, 1966, **88**, 3440.

¹⁰ An acid chloride has been prepared *via* the oxalyl chloride method: L. Berliner, personal communication.

¹¹ B. M. Hoffmann and T. B. Eames, *J. Amer. Chem. Soc.*, 1969, **91**, 2169.