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HIGH RESOLUTION SPIN LABELED FATTY ACID:

SYNTHESIS AND EPR SPECTRAL CHARACTERISTICS

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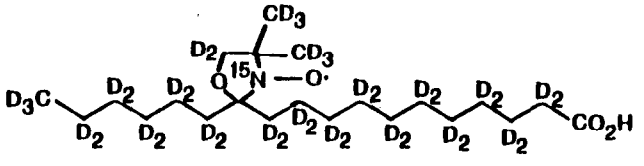
Abstract : Stearic acid spin label substituted with deuterium in all positions and ^{15}N in the paramagnetic group has been synthesized and was found to display a 5.5 fold gain in sensitivity in the EPR spectrum and a 60% decrease in linewidth compared to the unmodified analog.

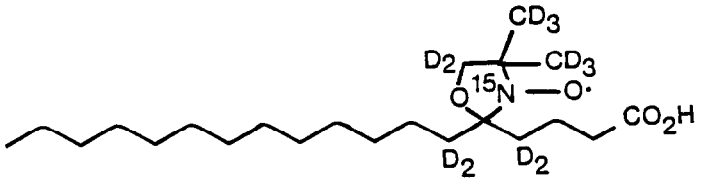
It is widely perceived that "membrane fluidity" (or microenvironment) of phospholipid bilayers affects membrane function. This has led to the development of techniques for the quantitative assessment of "membrane fluidity". Although EPR spectroscopy,[1] with spin labeled fatty acids as molecular probes,[2] has proven valuable for the estimation of "membrane fluidity", quantitative application is limited. This is because the signals in the EPR spectrum overlap and thereby preclude measurement of important spectral parameters. Also, the concentration of the spin label employed to obtain spectra with acceptable S/N ratio may be sufficiently high so as to perturb the local environment in the membrane bilayers.

In preceding papers,[3-5] we have shown that deuteration and ^{15}N substitution in the paramagnetic group of the maleimide spin label, which binds covalently with proteins, enhanced EPR sensitivity and resolution. The resolution was such that it was possible to accurately simulate experimental lineshapes. We now wish to report : (1) the first synthesis of stearic acid spin label 1 (2) EPR data for label 1 and (3) a convenient preparation of ^{15}N-2-amino-2-methyl-1-propanol-d_{11} (4, eq. 1), which will be of general utility for similar modifications in a variety of spin labels bearing a "doxyl" group.[6]



1

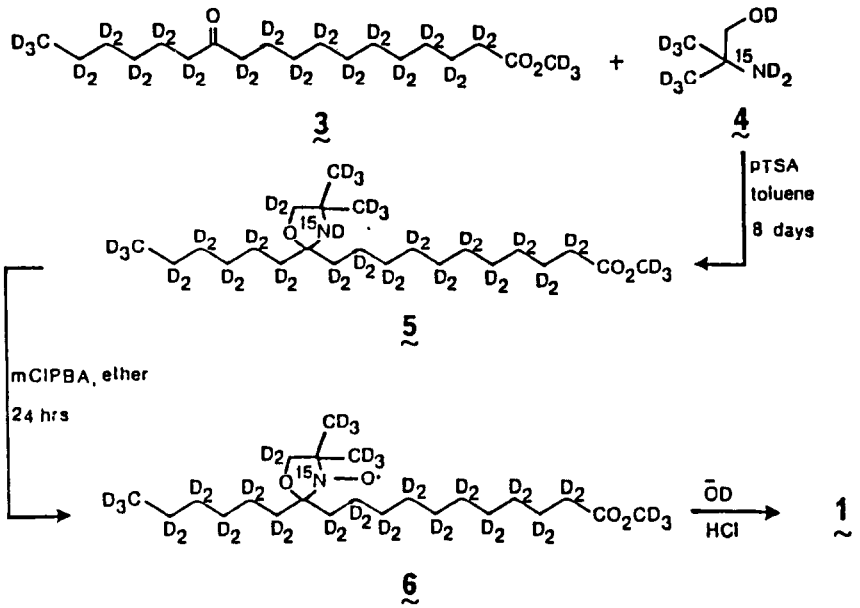
Initially, we synthesized ^{15}N-5-doxylstearic acid-d_{12} (2). This label displayed a 3-fold gain in sensitivity relative to the unsubstituted analog. Comparision of this value with ^{15}N-deuterated maleimide[5] which showed a 10-fold increase in sensitivity implied that β and perhaps other remote hydrogens in 2 strongly interacted with the nitroxide radical thereby contributing to the broadening of the linewidth. The flexibility of the fatty acid chain should permit such an interaction. Although a fully deuterated analog of 2 would have been useful for comparision of spectral data, the deuterated starting materials required in the synthesis of such an analog are not accessible. We have therefore synthesized the related stearic acid label 1.



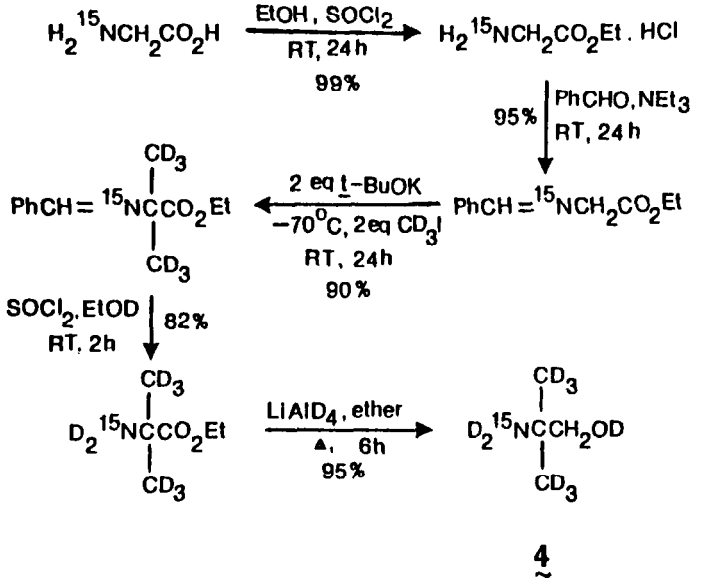
2

Scheme I outlines the synthesis of stearic acid spin label 1 and is based on a general procedure developed by Keana et al.[2] Thus, acid catalyzed condensation of methyl 12-ketostearate-d_{33} (3)[7] with an excess of ^{15}N-2-amino-2-methyl-1-propanol-d_{11} (4) gave the oxazolidine 5. This was oxidized with m-chloroperoxybenzoic acid to yield the nitroxide radical 6. Mild alkaline hydrolysis of 6 furnished the desired spin label 1 in 15% yield based on 4. Chemically pure 1 was isolated by silica column chromatography (hexane:benzene, 1:1).

SCHEME I



^{15}N-2-amino-2-methyl-1-propanol-d_{11} (4) is the crucial starting material in the synthesis of stearic acid spin labels 1 and 2. Earlier, we reported [8] the conversion of ^{15}N-glycine to ^{15}N-2-amino-2-methyl-1-propanol in 76% overall yield. We have now modified this procedure to allow deuterium incorporation for obtaining amino alkanol 4 (eq. 1). The conversion proceeds in high chemical yield in each step and can be adopted for large scale operation without isolation of any of the intermediates. A convenient preparation of amino alkanol 4 was heretofore not available.



(eq. 1)

In figure 1, the EPR spectrum of spin label 1 is compared with the unsubstituted label under identical conditions. It was gratifying to note a 5.5 fold gain in sensitivity for the newly synthesized spin label 1. The linewidth for the ^{15}N-deuterated spin label was

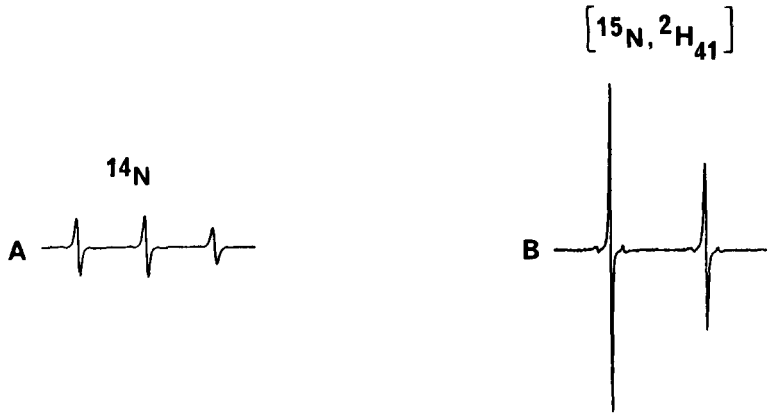


Fig. 1. X-band EPR spectra of freely tumbling (A) 12-doxylstearic acid and (B) ^{15}N-12-doxylstearic acid-d_{41}. The 50 Gauss spectrum of each spin label at a concentration of 10 μMolar in 5 mM phosphate buffer, pH 7.5, was recorded at 26°C with identical instrument settings of 2 mWatt power, 0.125 Gauss modulation amplitude and 100 kHz frequency.

only 0.38 Gauss compared with a value of 1.00 Gauss for the conventional label. These significant improvements will allow evaluation of "membrane fluidity" with greater accuracy than was hitherto possible by direct measurement of both the $A_{||}$ and $A_{\perp}$ parameters.[9] A full account of our study will be published shortly.

In summary, we have demonstrated that total deuteration and ^{15}N *substitution in* stearic acid spin label results in greater sensitivity and higher resolution compared with the unsubstituted analog. Given the availability of related members with the spin label group located at different positions on the stearic acid carbons, the high resolution probes should also be advantageous (1) in the study of lipid-protein and lipid-lipid interactions by facilitating the quantitative simulation of spectral lineshapes [10] and (2) in the examination of lateral diffusion and vertical fluctuations of fatty acids in model membranes by ELDOR spectroscopy.[11, 12] In this context, the procedure described here for the preparation of ^{15}N-2-amino-2-methyl-1-propanol-d_{11} (4, eq. 1) should be of great value for the synthesis of additional spin labeled fatty acids, phospholipids and cholesterol. The general utility of this method can also be extended for radiolabeling the spin label group.

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6. "Doxyl" refers to 4',4'-dimethyl-oxazolidine-N-oxyl.
7. The ketostearate (3) was prepared from commercial 1,12-dodecanedioic acid-d_{20} (3). See ref. 1. p 219 for details of preparation of a related member.
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