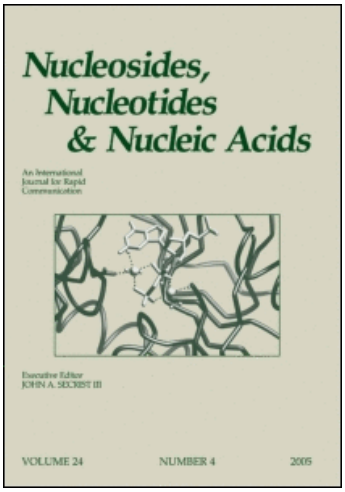

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Determination of RNA Tertiary Structure by Electron Paramagnetic Resonance Spectroscopy (EPR)

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Since the discovery of ribozymes in 1980, RNA is no longer considered only as a transporter of the genetic information but also as a participating molecule in each step of the gene expression. RNA can form complex three dimensional structures designed to perform specific functions. These structures are highly dependent on the presence of metal ions but few have been already solved. In the last few years the Electron Paramagnetic Resonance (EPR) spectroscopy has enabled a real improvement in the determination of proteins' conformation,[1] but few studies have been performed on nucleic acids.[2]

Indeed EPR enables to gain structural (distances between 15 and 60 Å can be measured) and dynamic data (motions which occur on a timescale from millisecond to nanosecond can be analysed). Our aim is to test this method on the well known Hammerhead Ribozyme, whose X-ray structure has been entirely determined in 1994:[3] it is composed of one core and three helices, whose orientation depends on the magnesium concentration. Besides the magnesium ions are involved in the mechanism of substrate's self-cleavage.[4]

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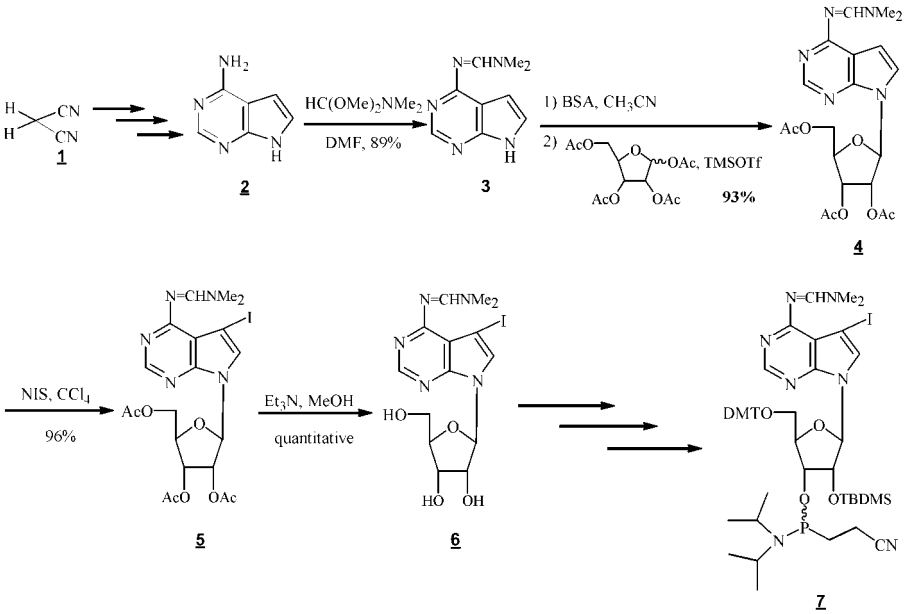
A limitation in applying EPR to the study of nucleic acids is the absence of a natural unpaired electron. To solve this problem:

1. We can introduce two spinlabels ($S = 1/2$), which will enable to measure distances, by using Pulsed Electron Double Resonance (PELDOR). For this purpose, the synthesis of the fully protected 5-Iodo-Cytidine, 5-Iodo-Uridine and 5-Iodo-Tubercidine was performed. The pathway leading to the last one is depicted in Sch. 1: 2-amino-pyrrolo-[2,3d]-pyrimidin 2 was prepared in three steps starting from malonitrile according to Davoll's procedure.[5] The protection of the amine with the formamidine group was followed by the condensation with β-tetraacetyl-ribofuranose after the Vorbrüggen's procedure.[6] The selective iodination at the fifth position (systematic numbering) gave the best yield using N-iodosuccinimide in carbon tetrachloride. A selective deprotection of the acetyl groups with triethylamine/methanol (1/8) gave 6 in quantitative yield. For the preparation of the corresponding phosphoramidites we used the standard methods: protection of the 5′-OH with 4-4′-dimethoxytritylchloride and dimethylaminopyridine in pyridine, of the 2′-OH with tert-butyldimethylsilylchloride, silver nitrate in THF/pyridine and of the 3′-OH with N-N′-diisopropylaminechlorophosphane.

2,2,5,5-Tetramethyl-3-ethinyl-3-pyrrolin-N-oxyl **1**, chosen for its stability towards high temperatures (up to 80°C) and large range of pH, will be attached to the four iodinated bases through a palladium catalysed cross-coupling during the solid phase synthesis.[7]



Scheme 1.

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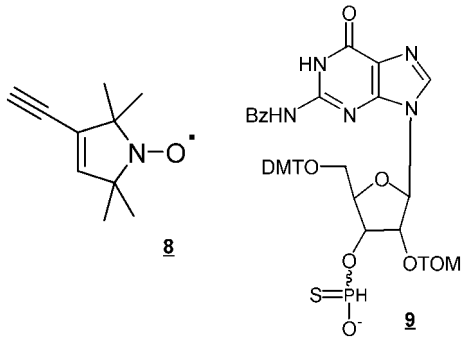
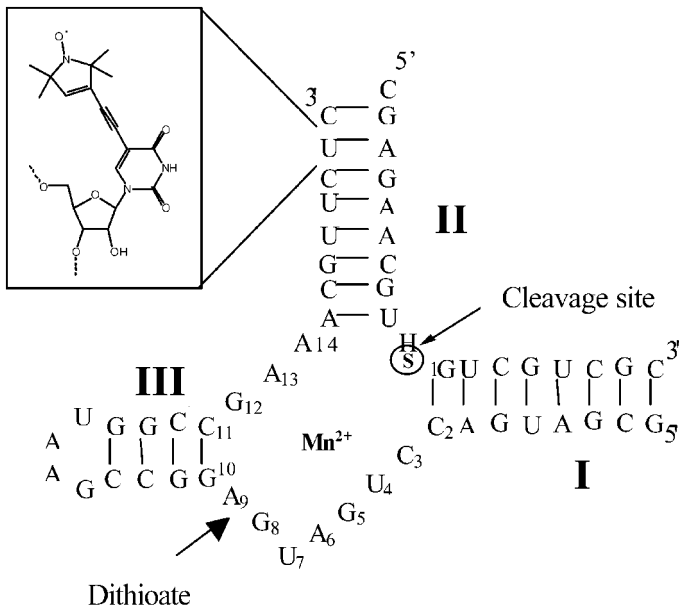


2. We can replace the magnesium ions by the paramagnetic manganese ions ($S = 5/2$). The active conformation of the ribozyme is obtained in presence of 10 nM magnesium ions, with different affinity binding sites. To introduce selectively a paramagnetic metal ion (manganese), it is necessary to substitute one of the RNA backbone phosphodiester group by a phosphodithioate group, relying on the hard and soft acid/base theory. The phosphorothioate 9 was synthesized according to Caruther's method[8] and was oxidized with 2,4-dichlorobenzylthiosuccinimide during the solid phase synthesis.

Among the different divalent ion binding sites of the Hammerhead ribozyme, we substituted the phosphodiester bond between G8 and A9[9] (Sch. 2).

The introduction of a specific Mn^{2+} ion binding site in the hammerhead ribozyme will allow the study of its environment under physiological conditions.



Scheme 2.

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Such information can be used for the determination of the mechanism. In addition the divalent metal ions are very important for RNA folding and catalysis. Such method will be very helpful to access information about the dynamics of larger RNAs.

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