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# **Targeting mitochondria with novel, differently vectorized linear phosphorylated nitrones:**

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## **ABSTRACT**

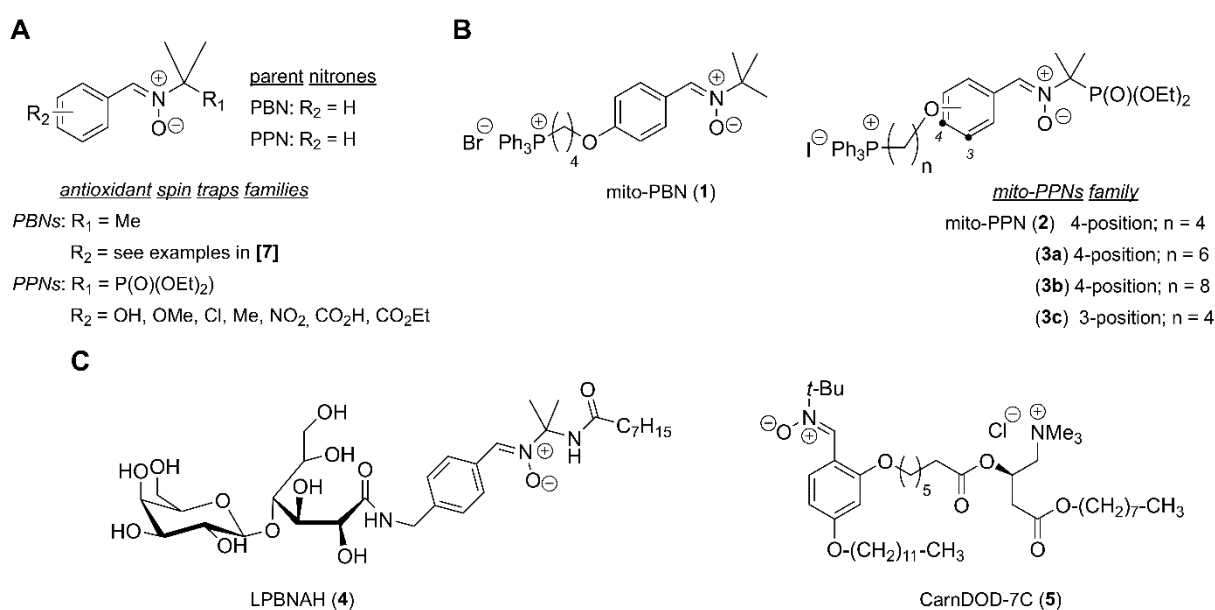
The free radicals (FR) occurrence in biological signalling or as pathologies triggers is well-documented and the design of specific scavengers to limit oxidative stress is enthralling. Because FR become inactivated upon addition to nitrones, efforts have focused on developing alpha-phenyl-*N*-tert-butyl nitron derivatives (PBNs) with promising pre-clinical data. To improve efficacy, a new generation of nitrones derived from 2-(diethoxyphosphoryl)-*N*-(benzylidene)propan-2-amine-*N*-oxide (PPNs) incorporating an antioxidant framework, gave rise to multipotent drugs exhibiting EPR-spin-trapping properties. As mitochondrial dysfunction is involved in many disorders, efforts have been concentrated on developing tools (pH probes) or pharmacological agents targeting-mitochondria. A series of mitochondria-targeted PPNs was synthesized based on the improved biocompatibility of P-containing nitrones and the expected benefits of introducing a cationic vector. Spin-adducts EPR-parameters toward biologically-relevant FR were established. Cytotoxicity was determined on cells and mitochondria-permeation assessed in isolated rat organs. New mitochondria-targeting nitrones appeared as lower cytotoxic alternative to the commonly used mito-PBN and were safely applied for investigating the anticancer-mechanism of new drugs impacting mitochondria.

## **Keywords**

Mitochondria-targeted nitrones, <sup>31</sup>P NMR, EPR spin trapping, mitochondrial permeability, H<sub>2</sub>O<sub>2</sub>-induced apoptosis

## Introduction

Mitochondria are complex, metabolically active and dynamic organelles that regulate critical cellular processes from ATP-production to intrinsic cell death. They are major oxygen-consumers with roughly 95% of the total amount of breathed  $O_2$  undergoing, in desirable conditions, reduction to water by cytochrome *c* oxidase (complex IV) in four consecutive one-electron steps. Activation of  $O_2$  by monovalent reduction leads to superoxide anion radical ( $O_2^{\cdot-}$ ), a reactive oxygen species (ROS) participating in many key cellular pathways [1]. Since the 1970s, popular estimates of the steady state amount of mitochondrial  $O_2^{\cdot-}$  ranged 1–2% of inhaled oxygen (i.e., 215–430 mmol of  $O_2^{\cdot-}$  daily for an 80 kg human) [1a], yet recent knowledge proposed it could actually be one order of magnitude less under non pathological conditions [1b]. Nevertheless, still the mitochondrial respiratory chain is regarded, together with cytosolic NADPH oxidases (Nox), as a major cellular source for  $O_2^{\cdot-}$ . In mitochondria electron transport chain,  $O_2^{\cdot-}$  formation involves complexes I (NADH:ubiquinone oxidoreductase) and III (cytochrome *bc\_1* complex), releasing this free radical within the mitochondrial matrix and, mostly, into the intermembrane space, respectively [1c].



**Figure 1.** General structures of noncyclic nitrones and related families exhibiting antioxidant and/or pharmacological activity (A) deriving from PBN and PPN [7b,8], or targeted to the mitochondrion (B) by a TPP cation [6a,8] or (C) using alternative vectors [15].

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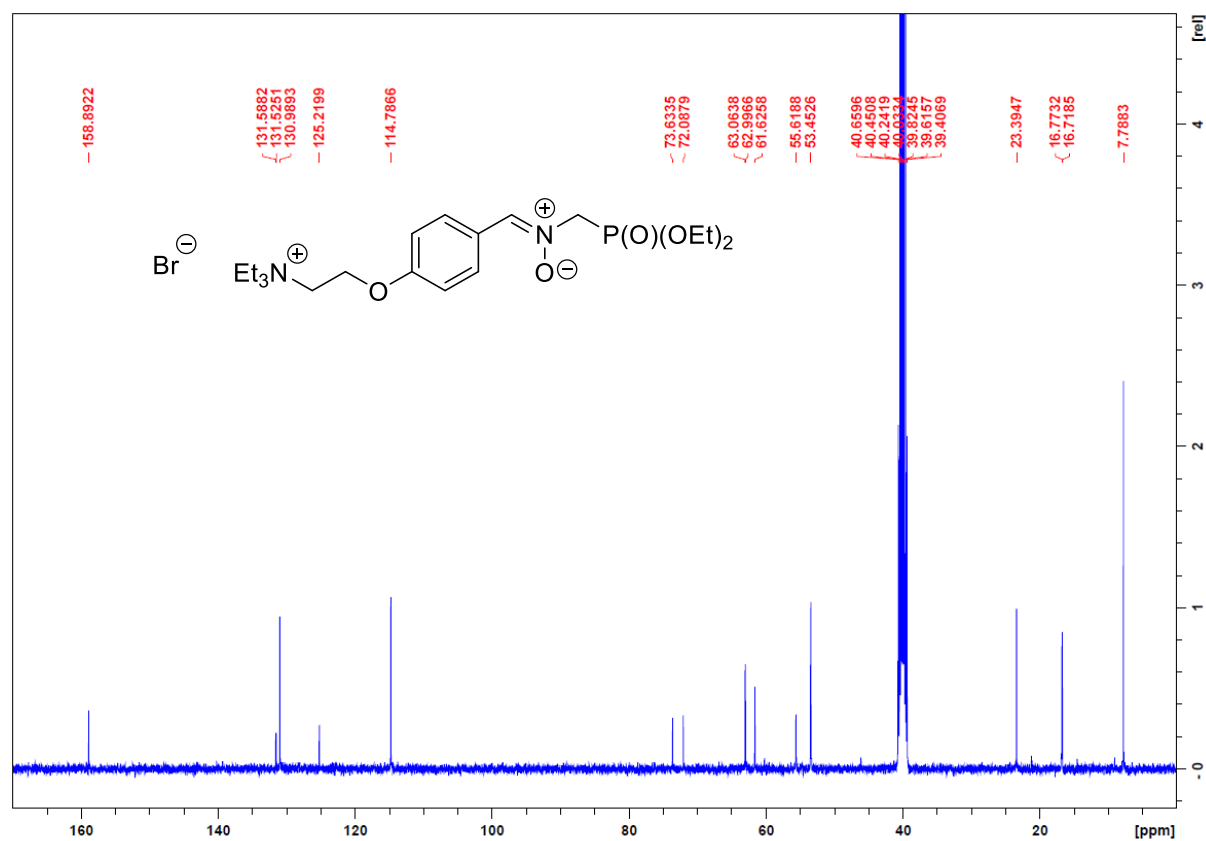
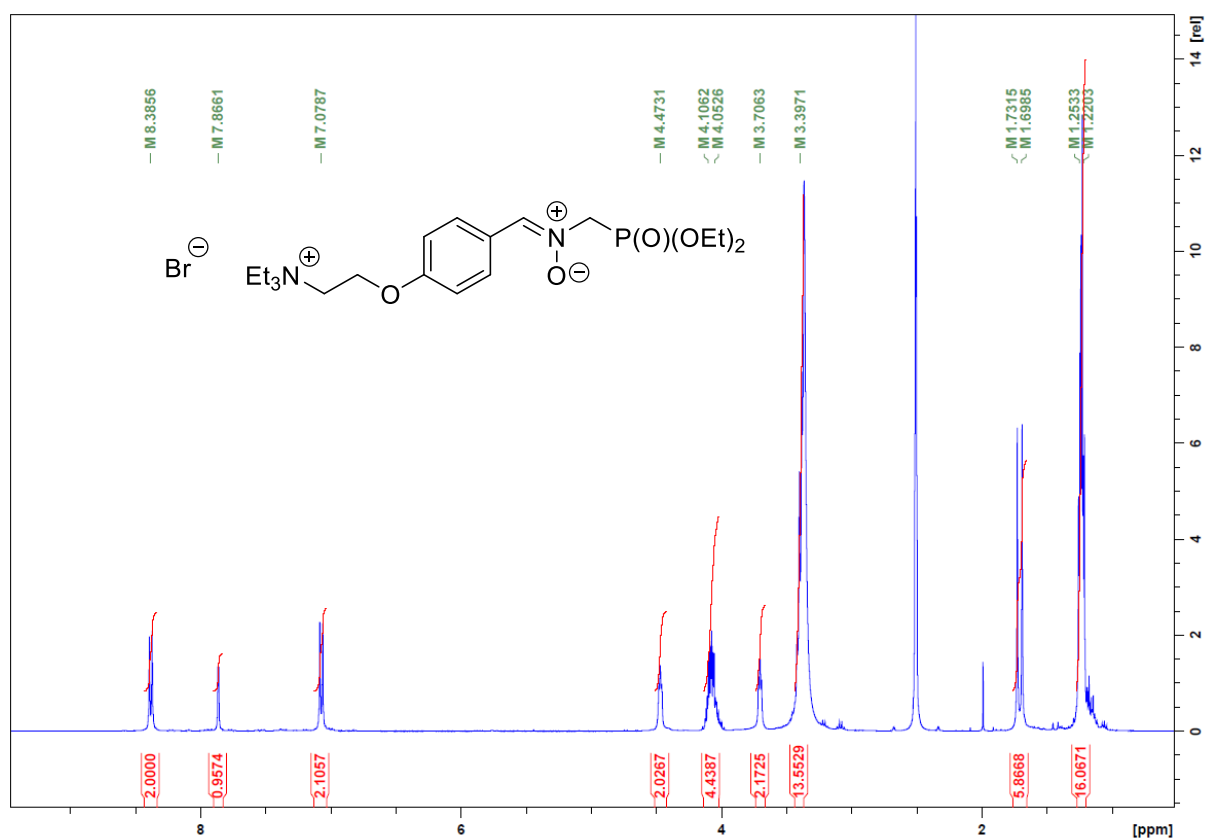
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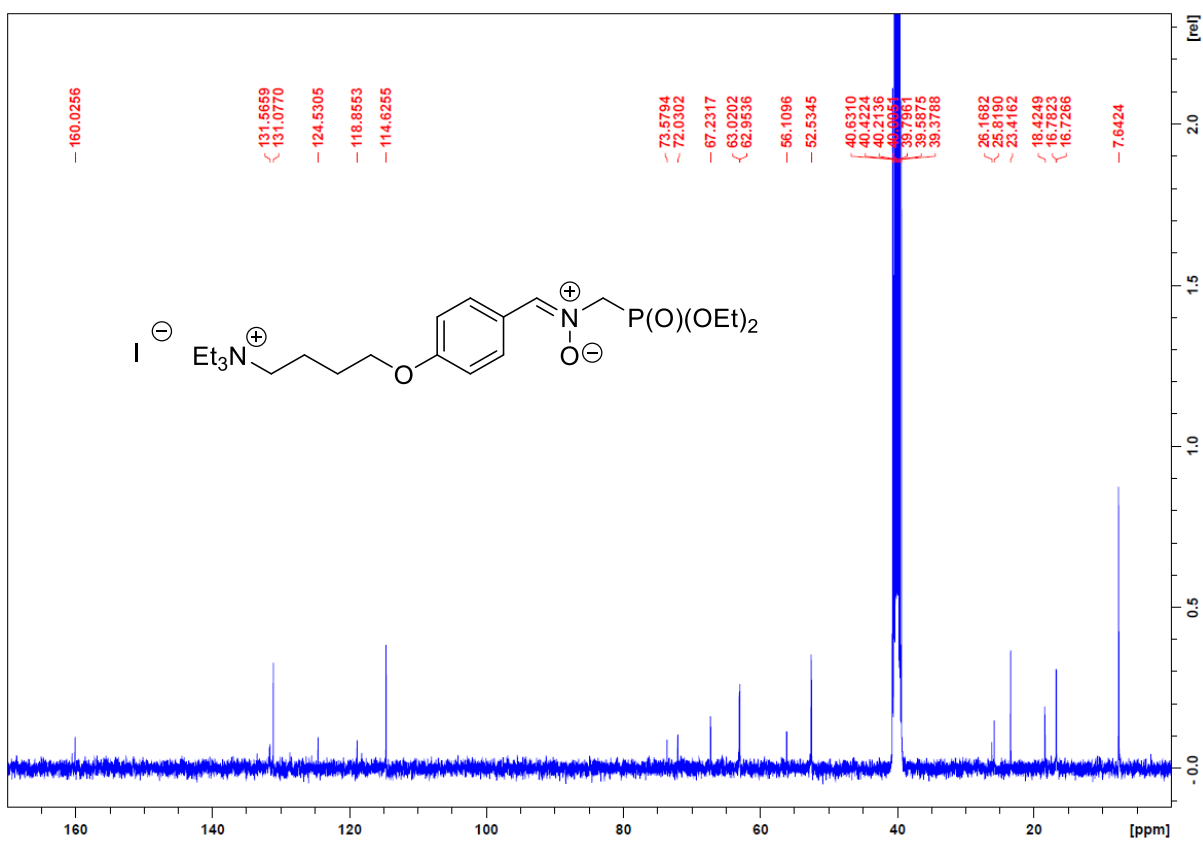
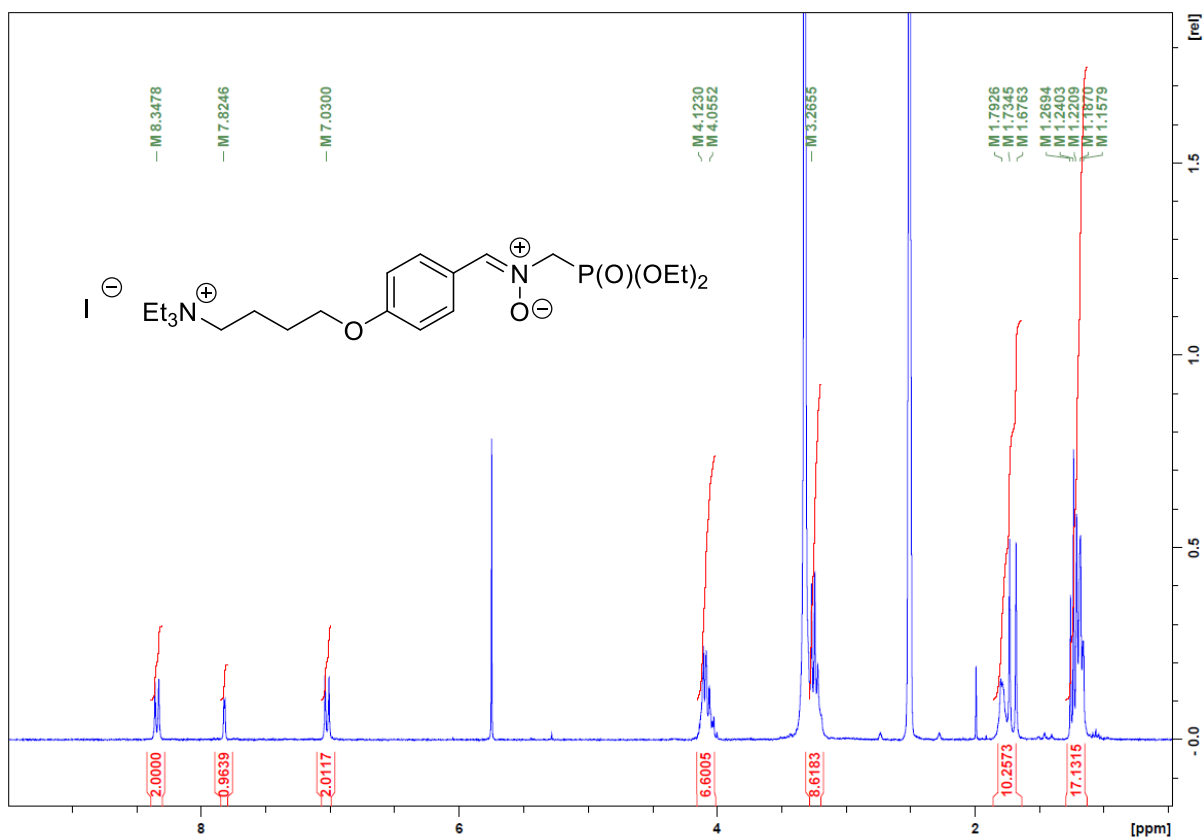


# Supplementary Material

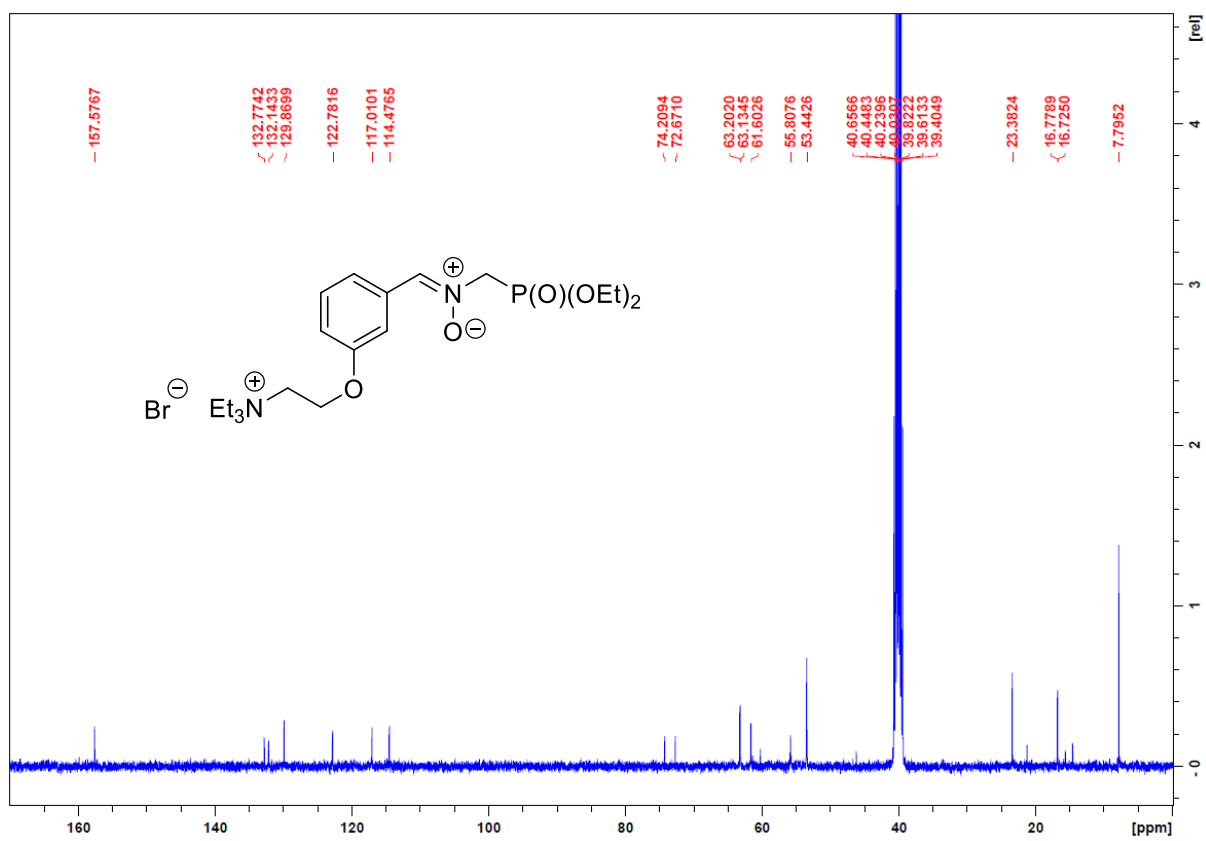
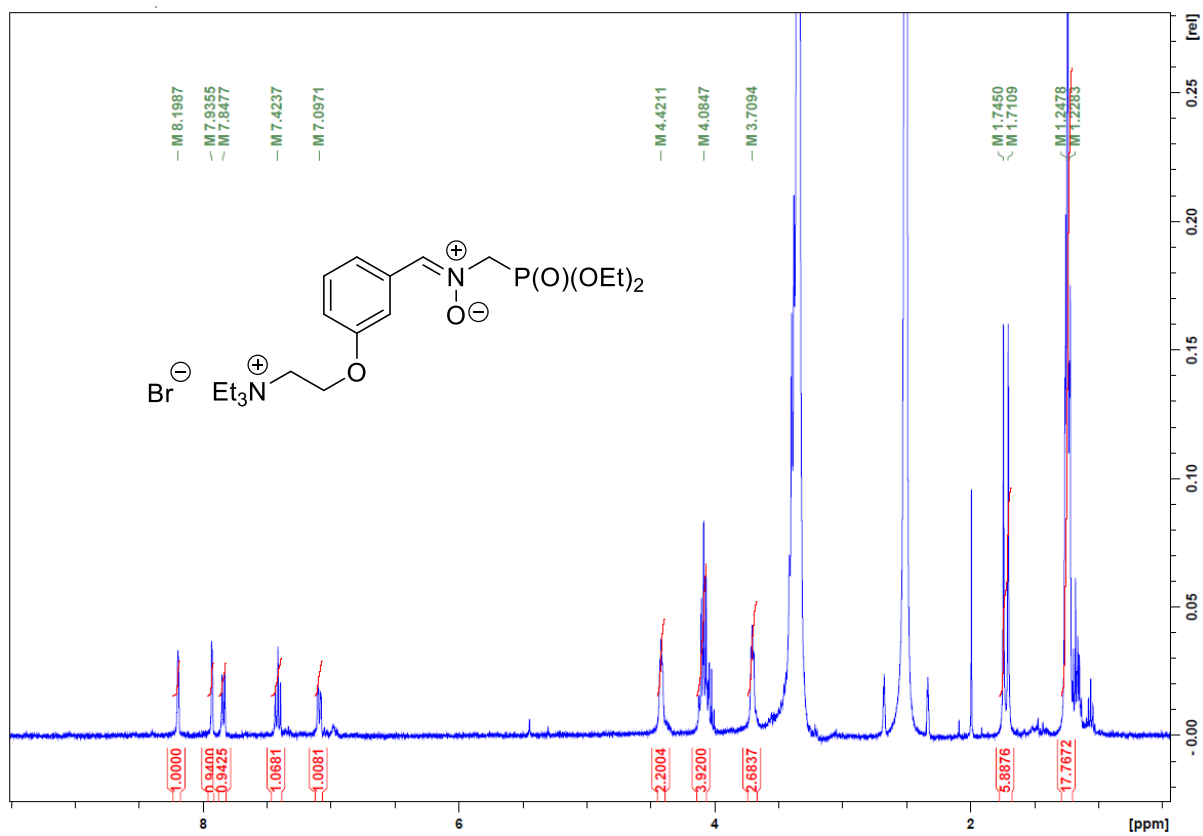
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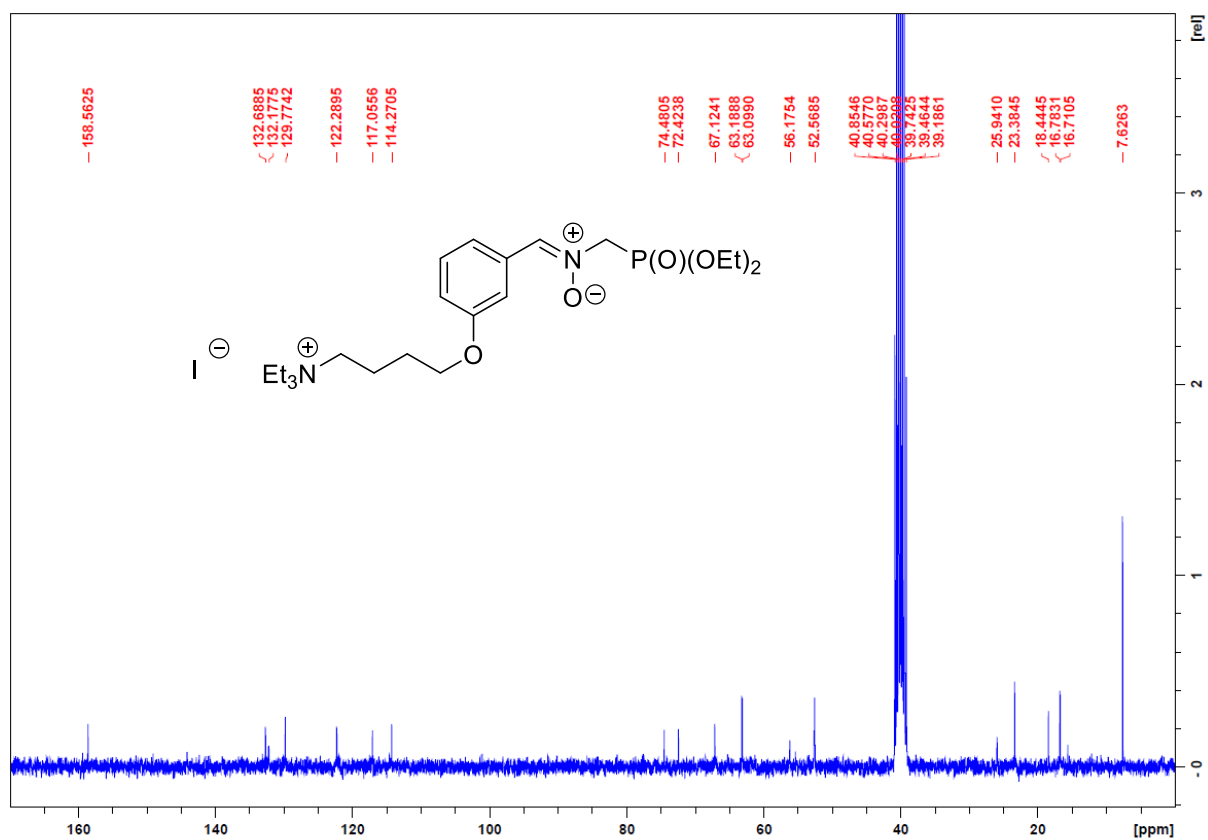
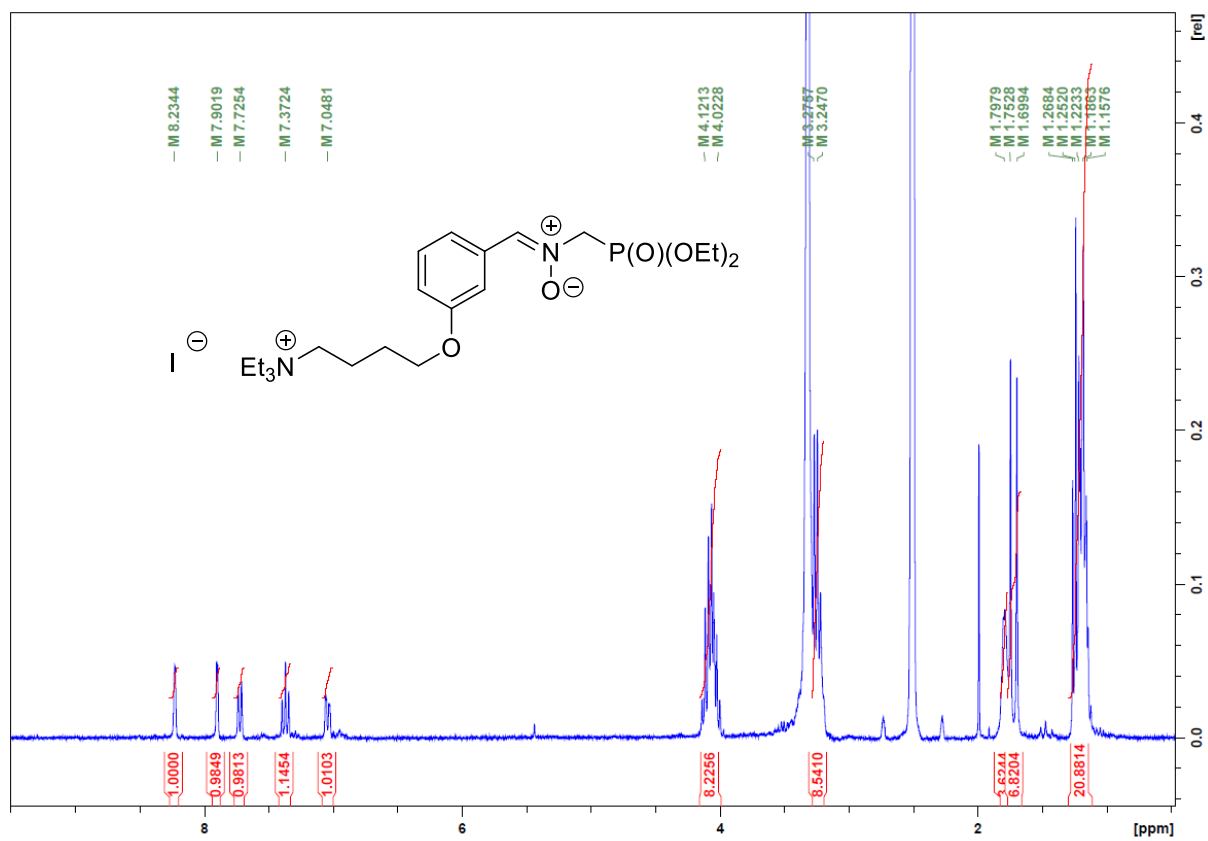
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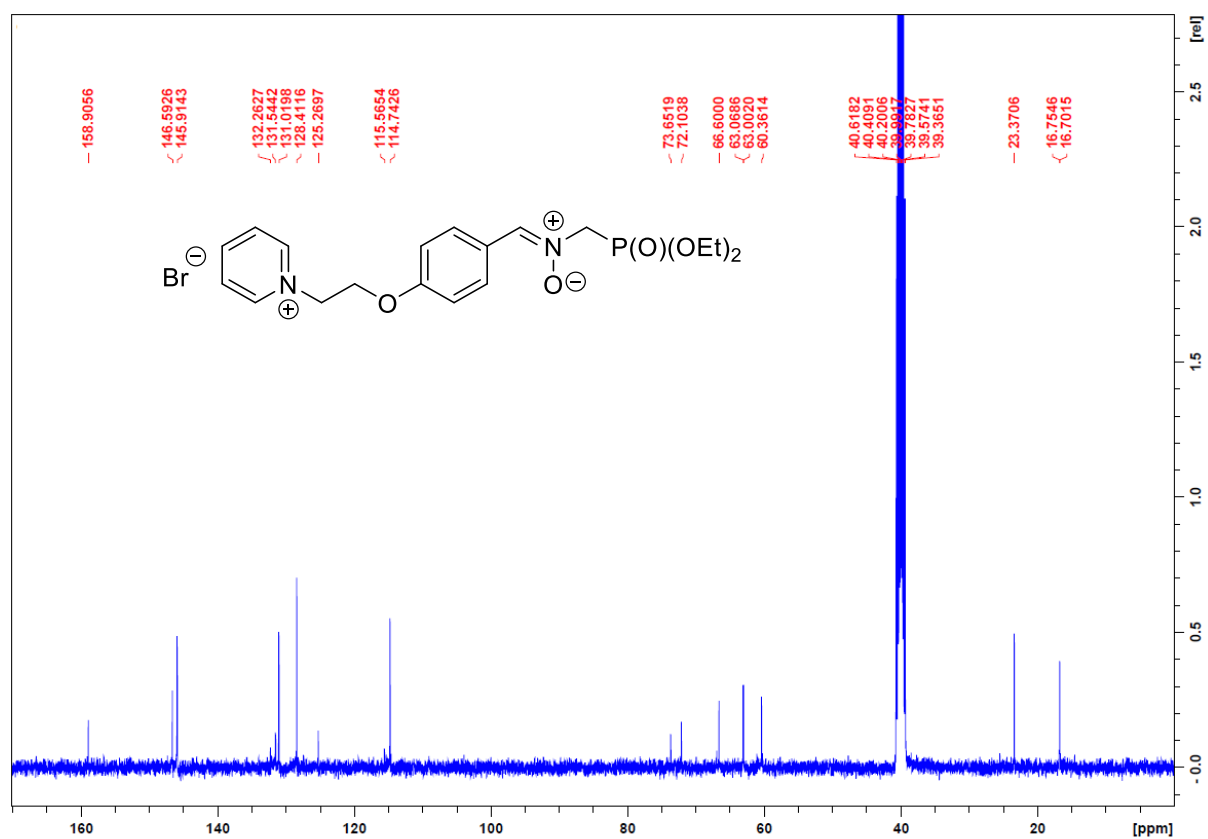
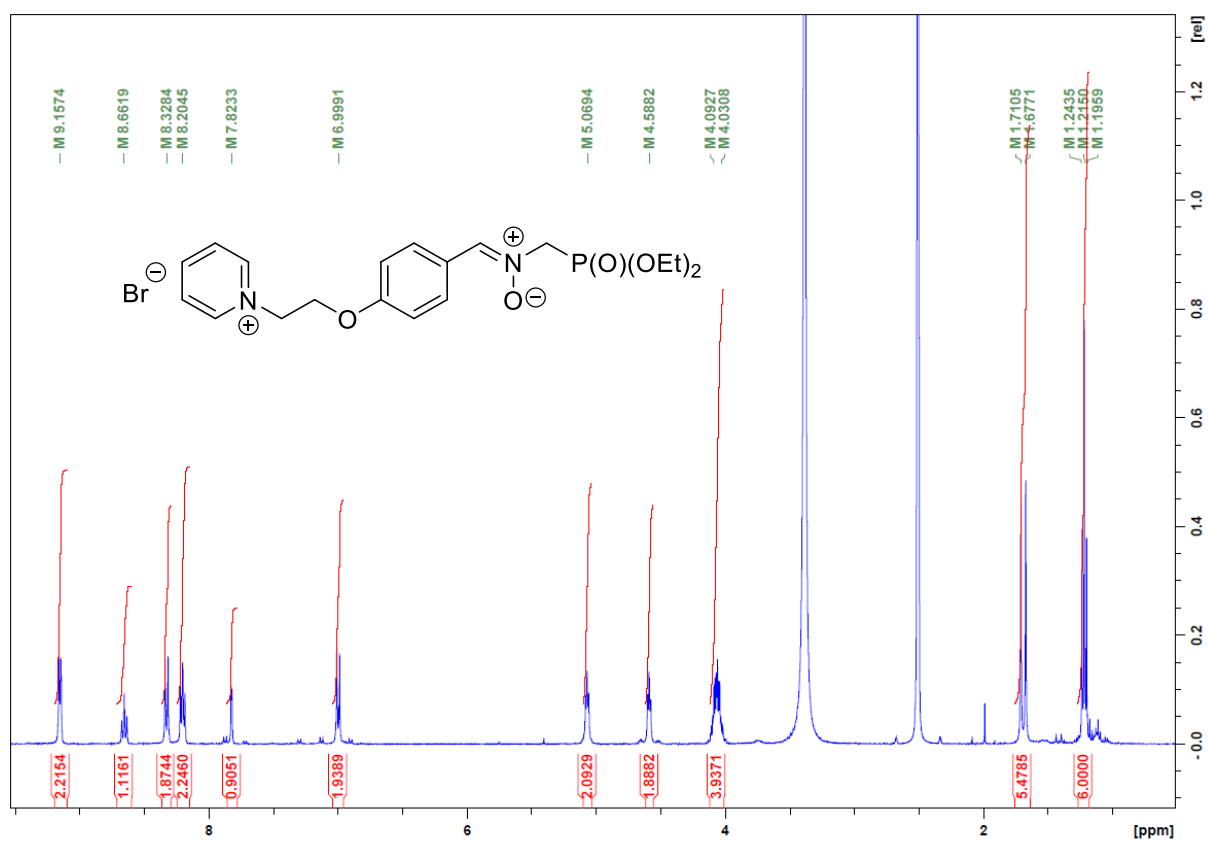
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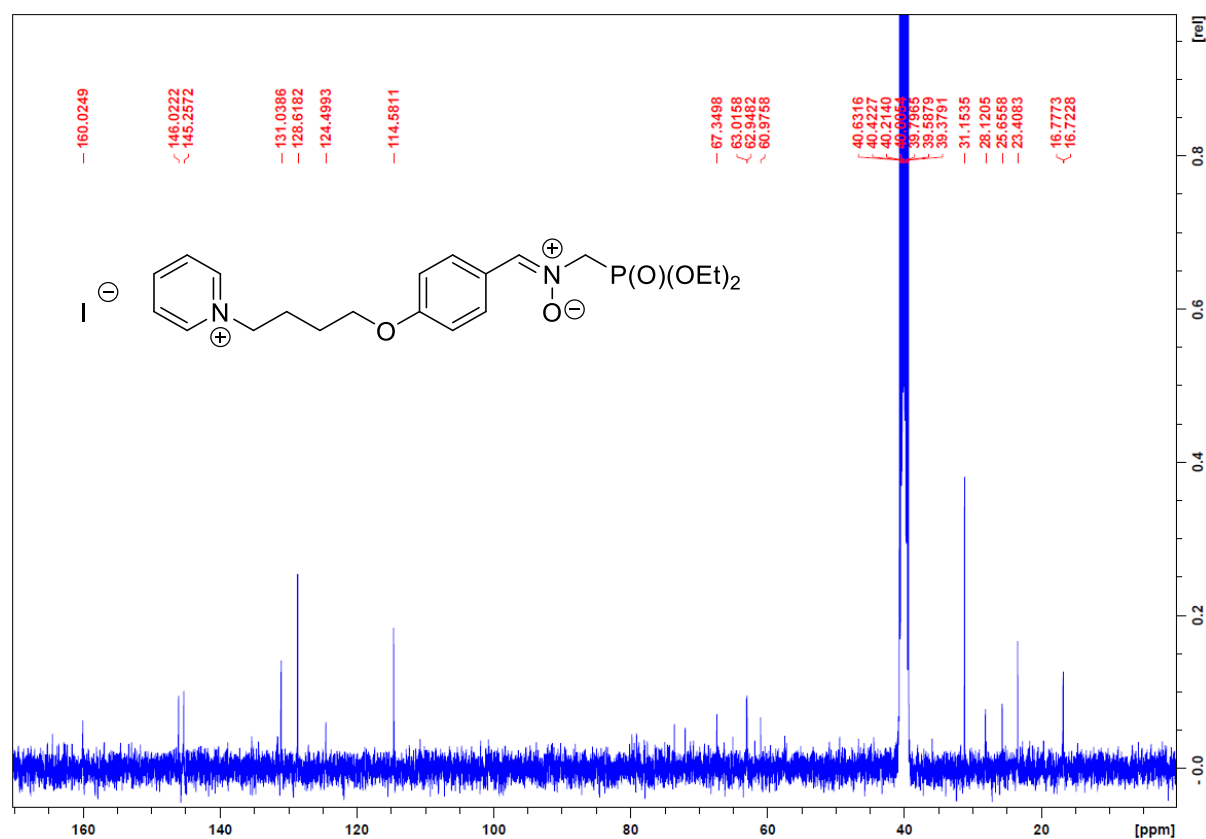
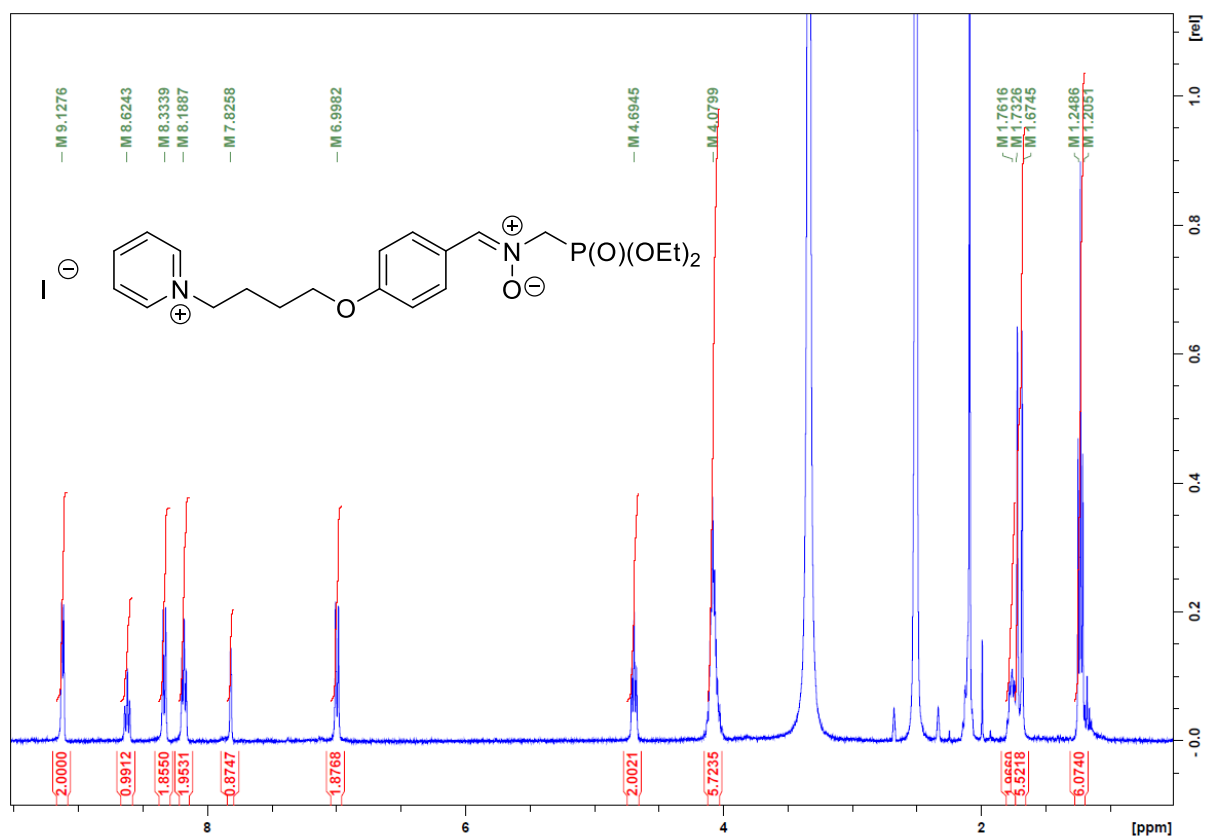
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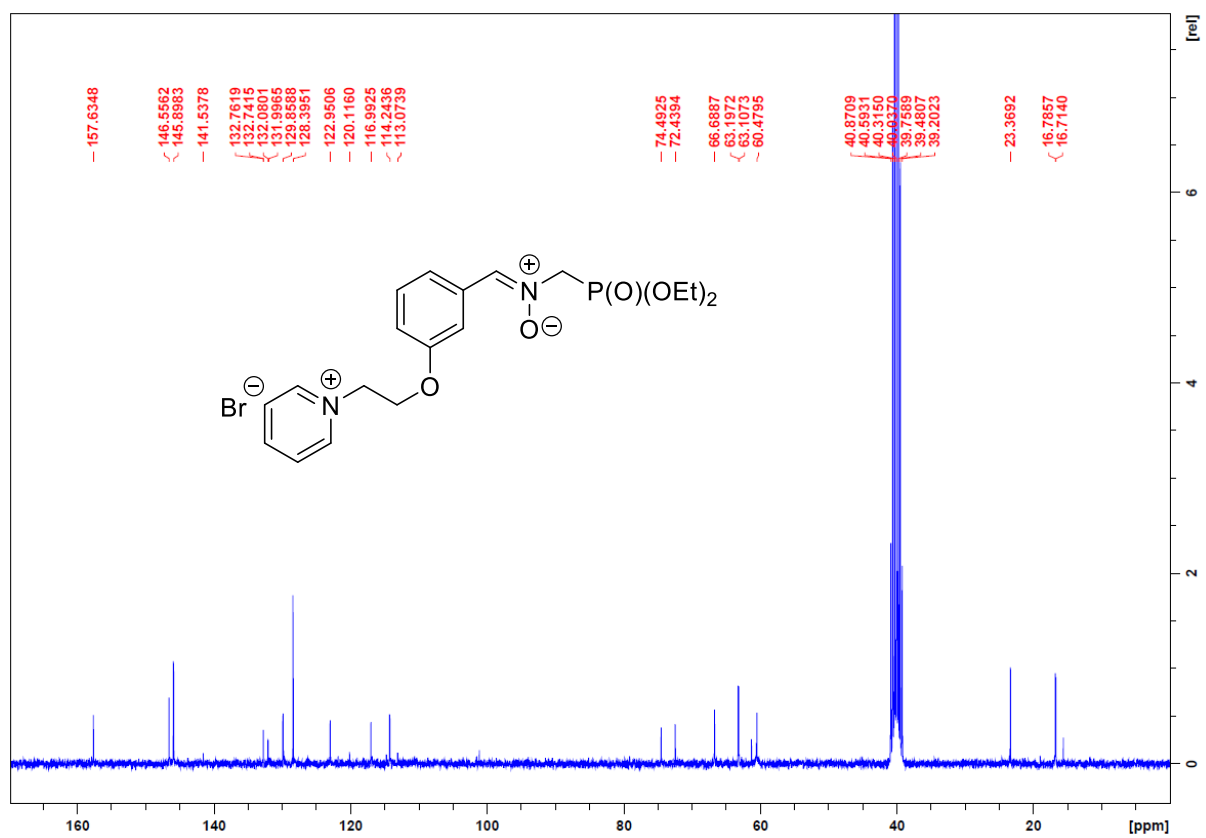
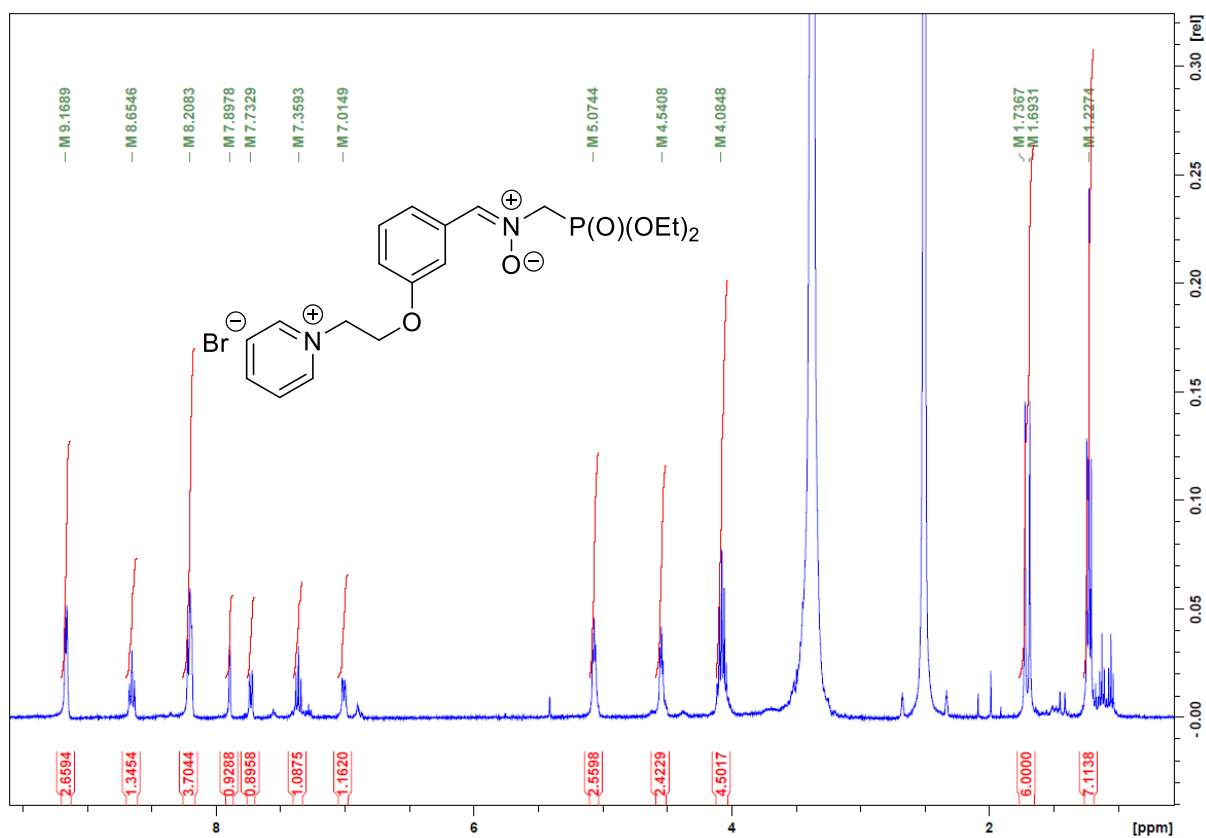
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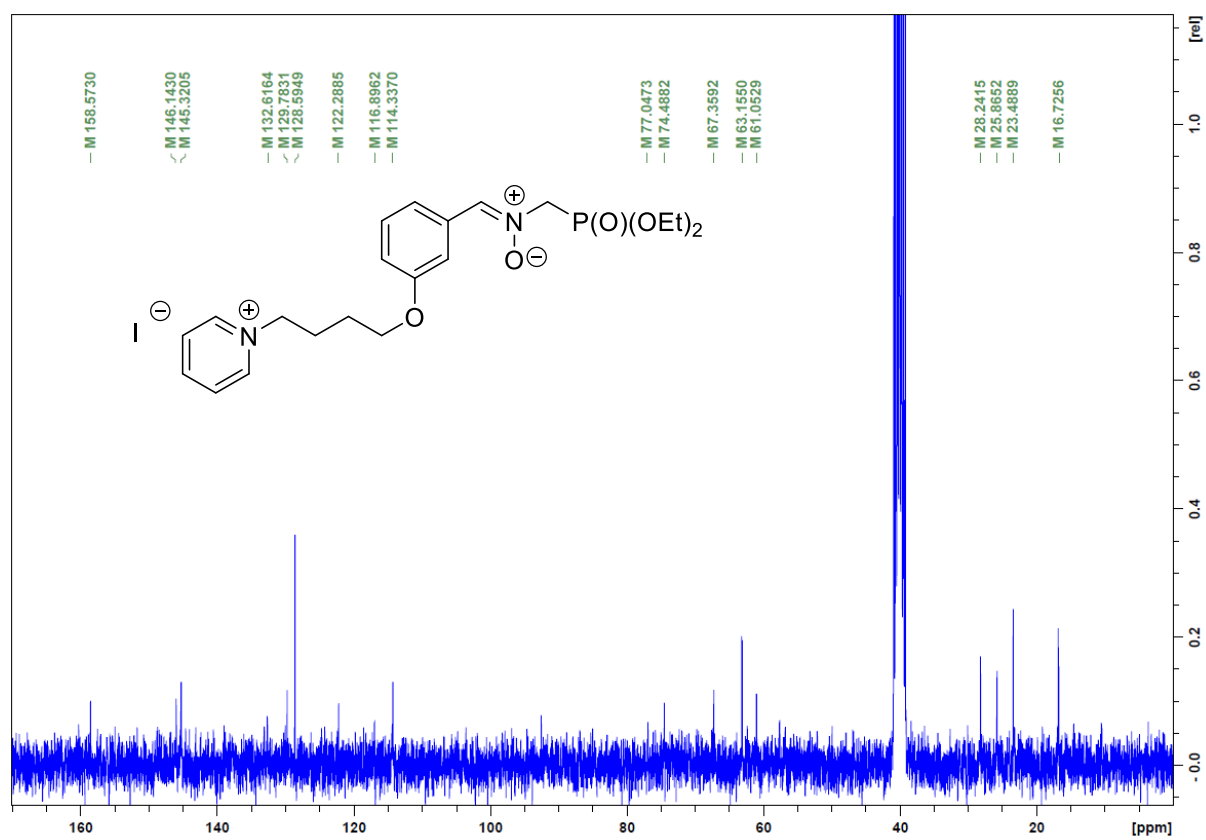
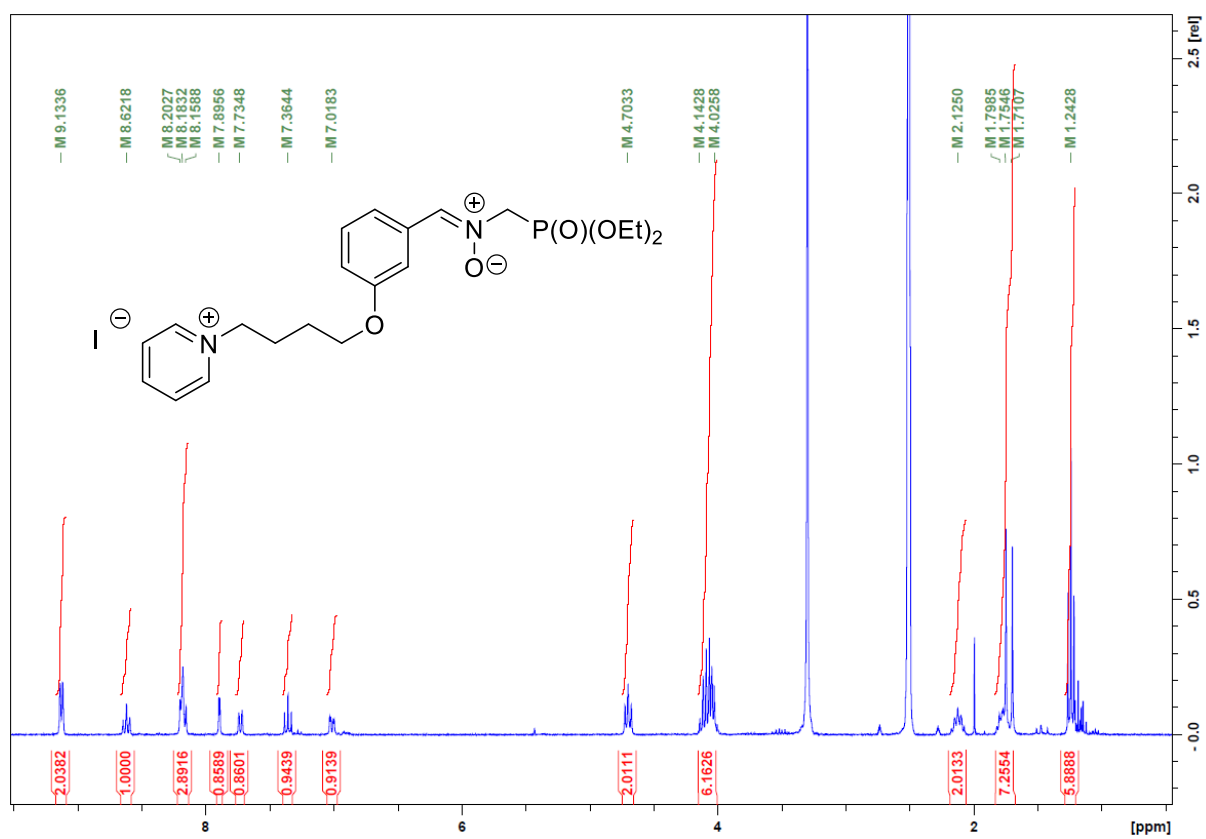
6. Spectra of nitron *12b*



7. Spectra of nitron 12c

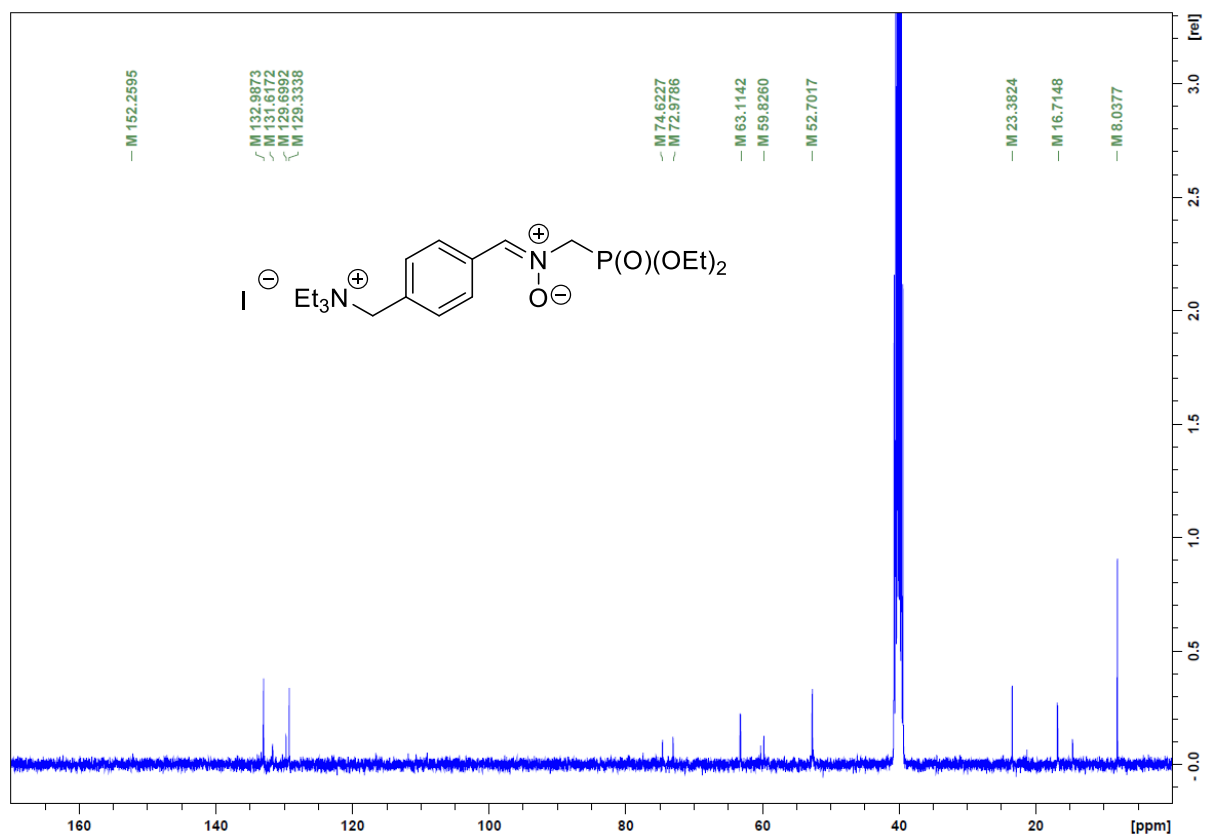
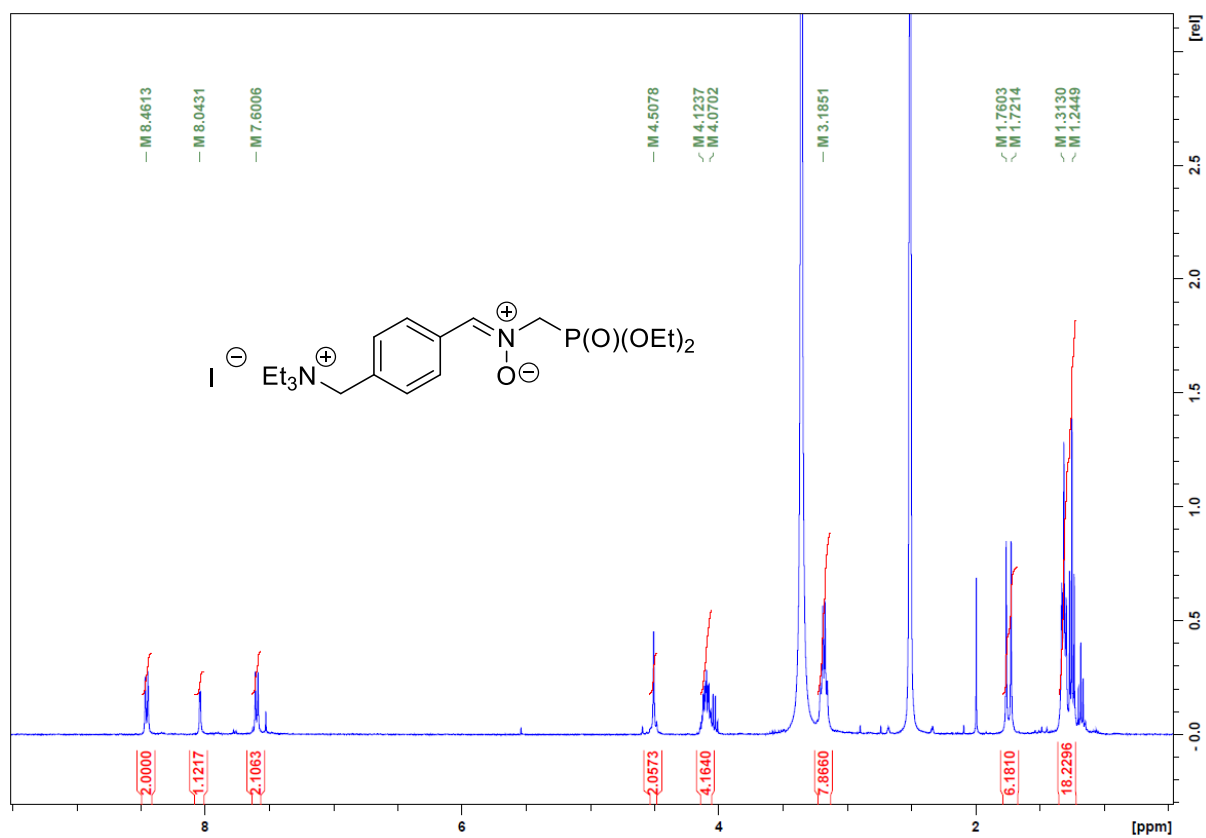


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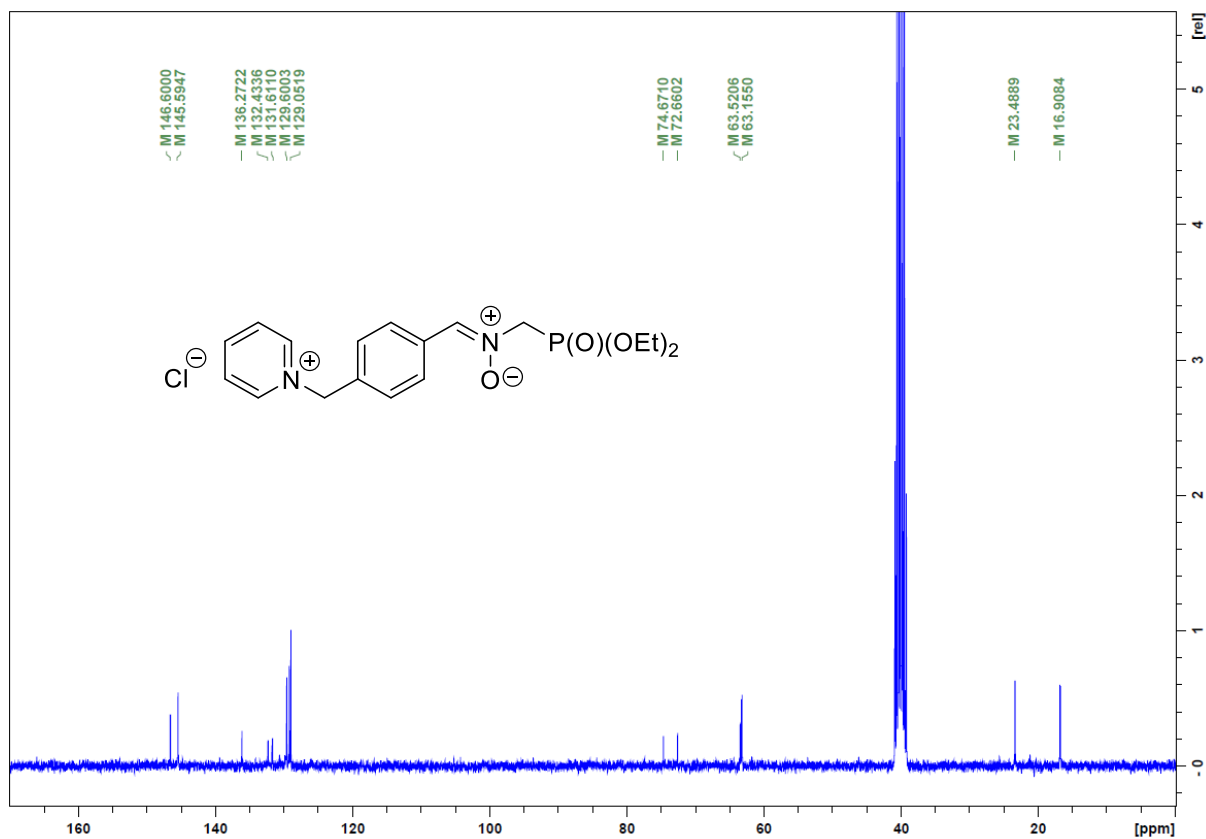
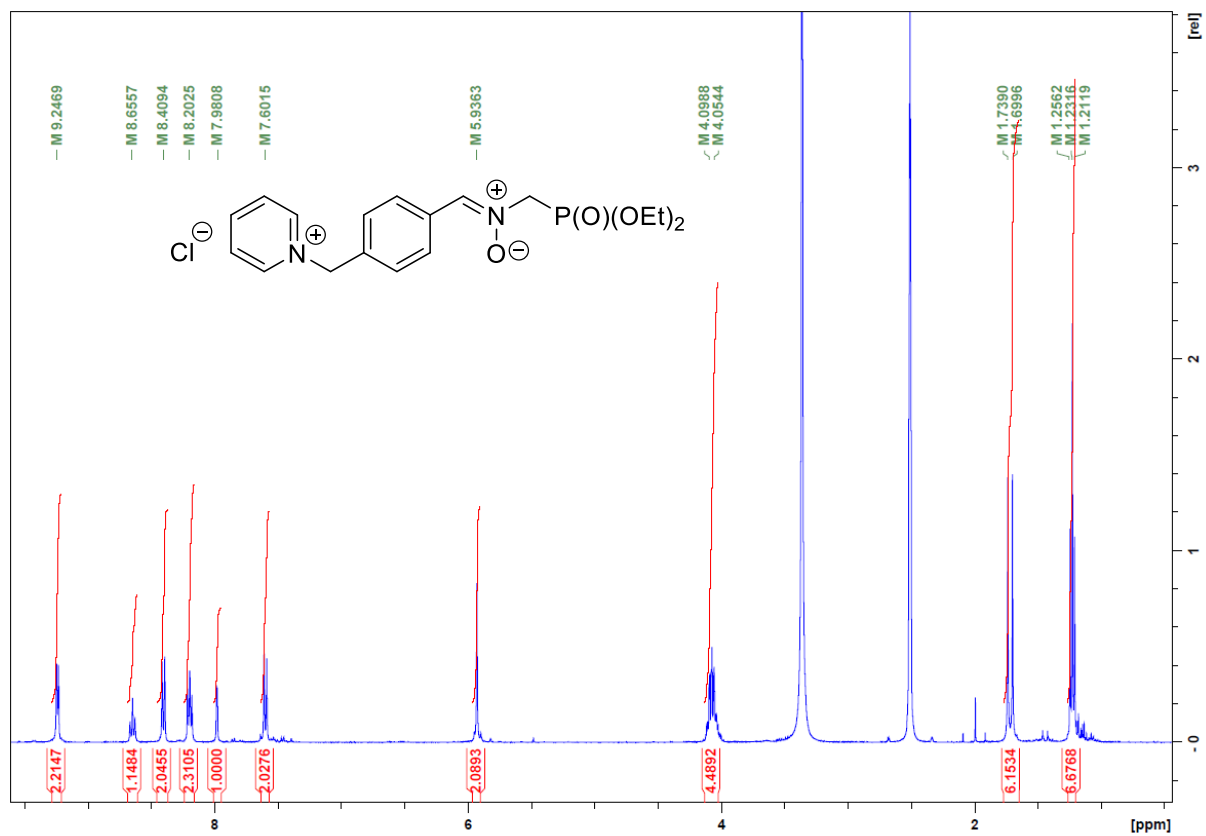




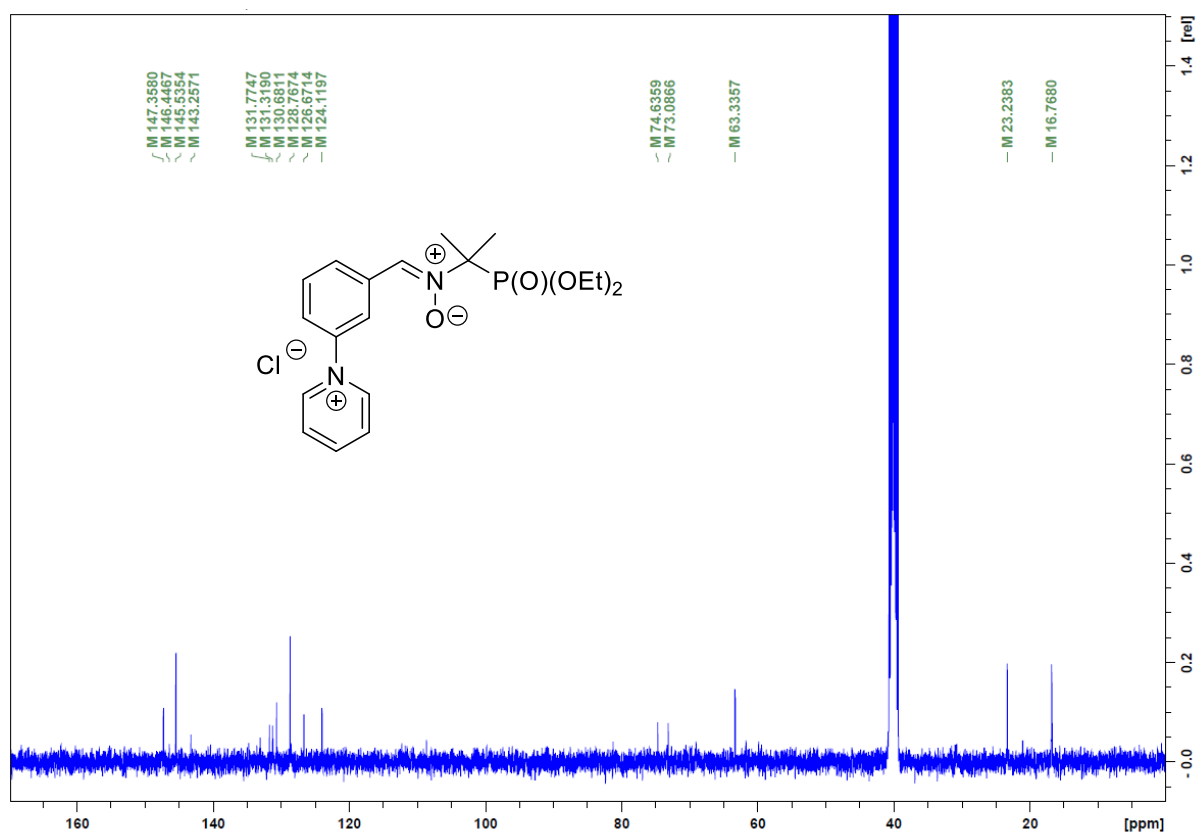
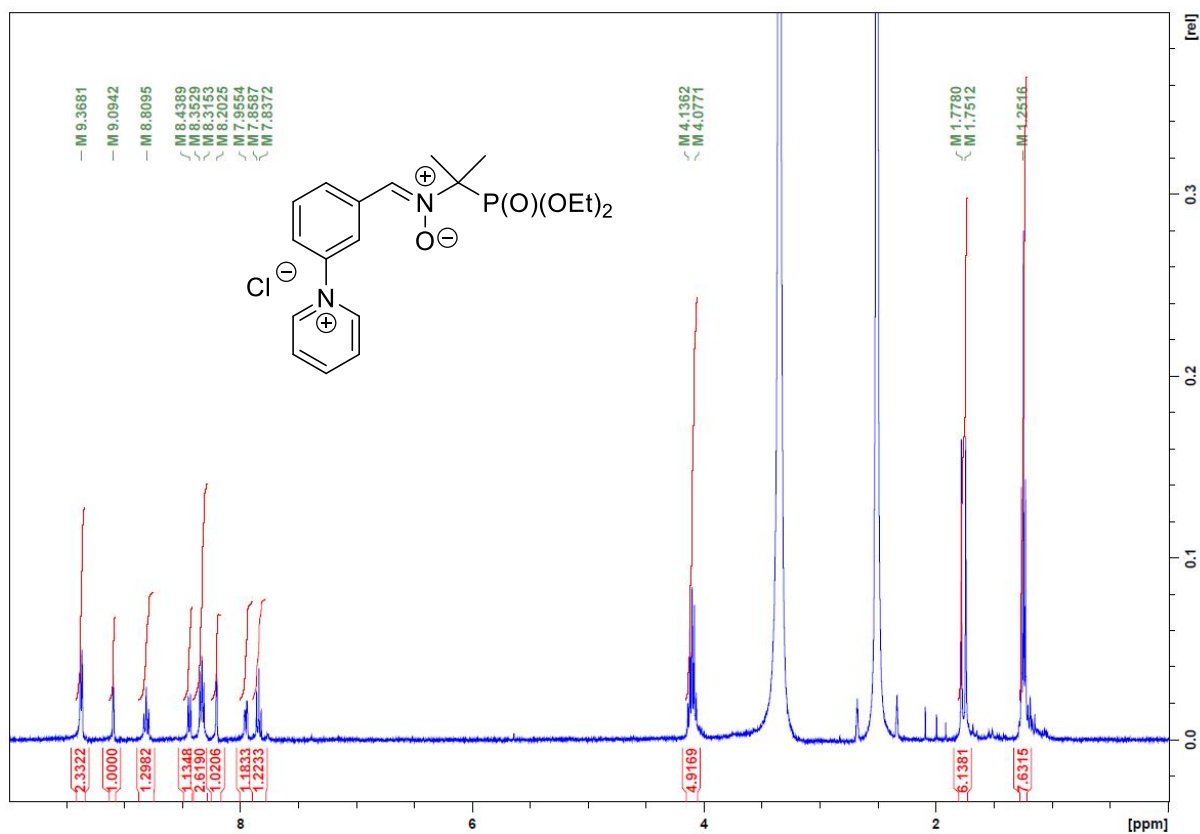
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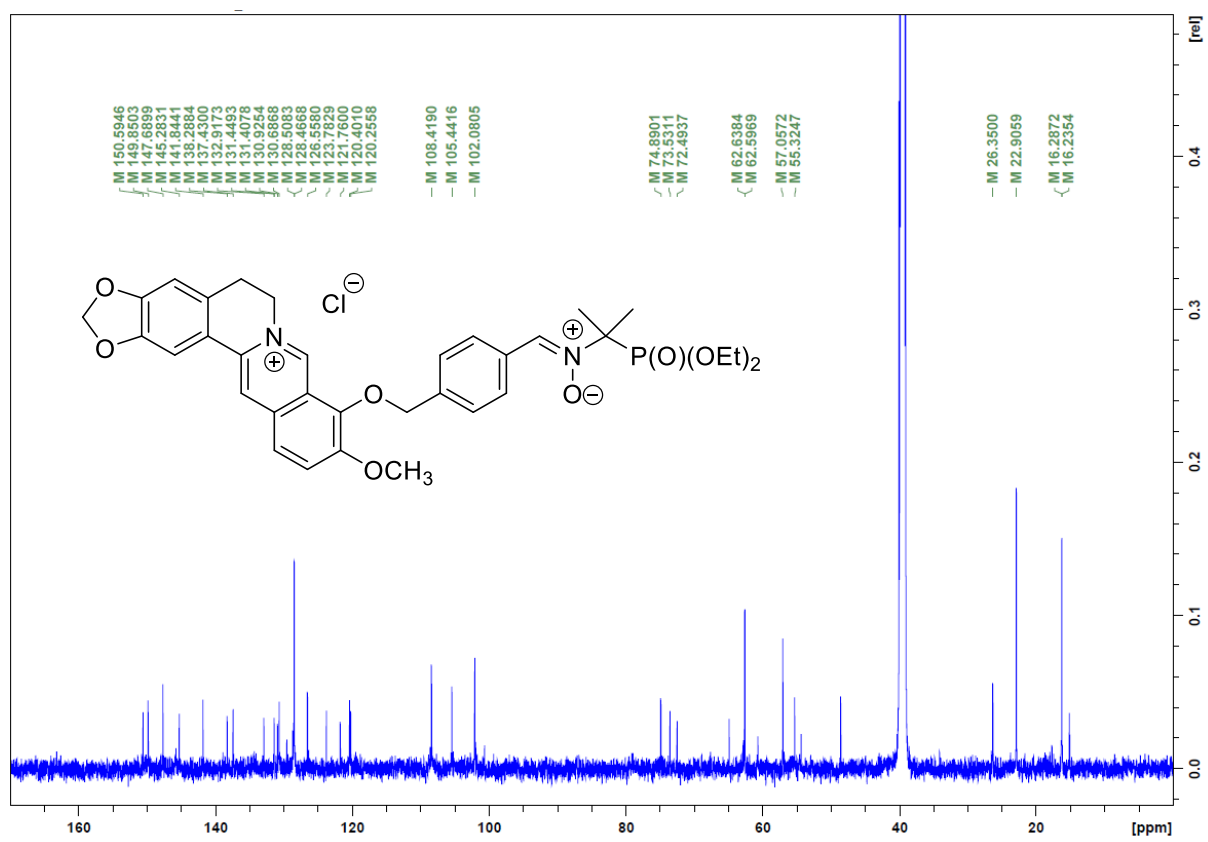
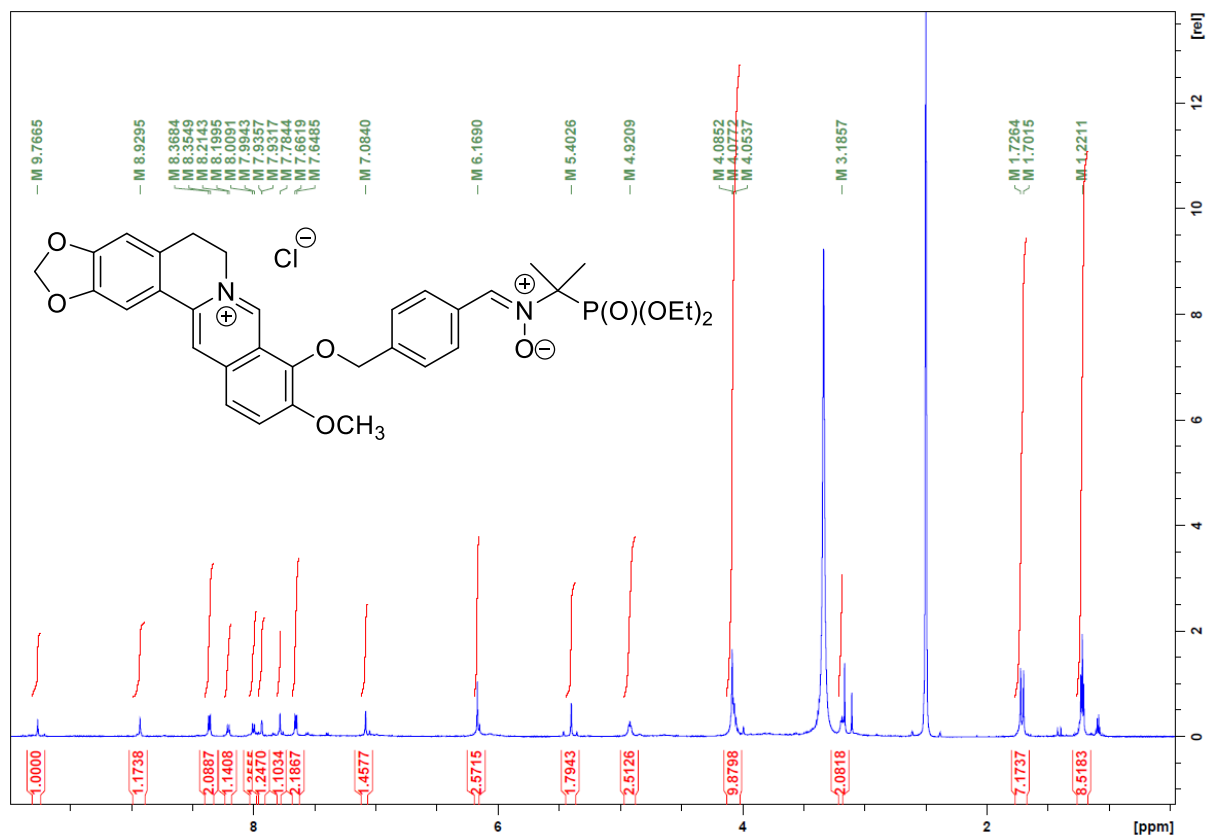
10. Spectra of nitron 14



11. Spectra of nitrene 15



12. Spectra of nitron 16



### 13. Spectra of nitron 17

