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Targeting mitochondria with novel, differently vectorized linear phosphorylated nitrones:

Caroline Delehedde, Sophie Thétiot-Laurent, Mathieu Esgulian, Mathieu Chocry, Antal Rockenbauer, Sylvia Pietri, Marcel Culcasi

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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-butylnitro 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, ^{31}P NMR, EPR spin trapping, mitochondrial permeability, H_2O_2 -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 O₂ undergoing, in desirable conditions, reduction to water by cytochrome *c* oxidase (complex IV) in four consecutive one-electron steps. Activation of O₂ by monovalent reduction leads to superoxide anion radical (O₂^{•-}), a reactive oxygen species (ROS) participating in many key cellular pathways [1]. Since the 1970s, popular estimates of the steady state amount of mitochondrial O₂^{•-} ranged 1–2% of inhaled oxygen (i.e., 215–430 mmol of O₂^{•-} 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 O₂^{•-}. In mitochondria electron transport chain, O₂^{•-} 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].

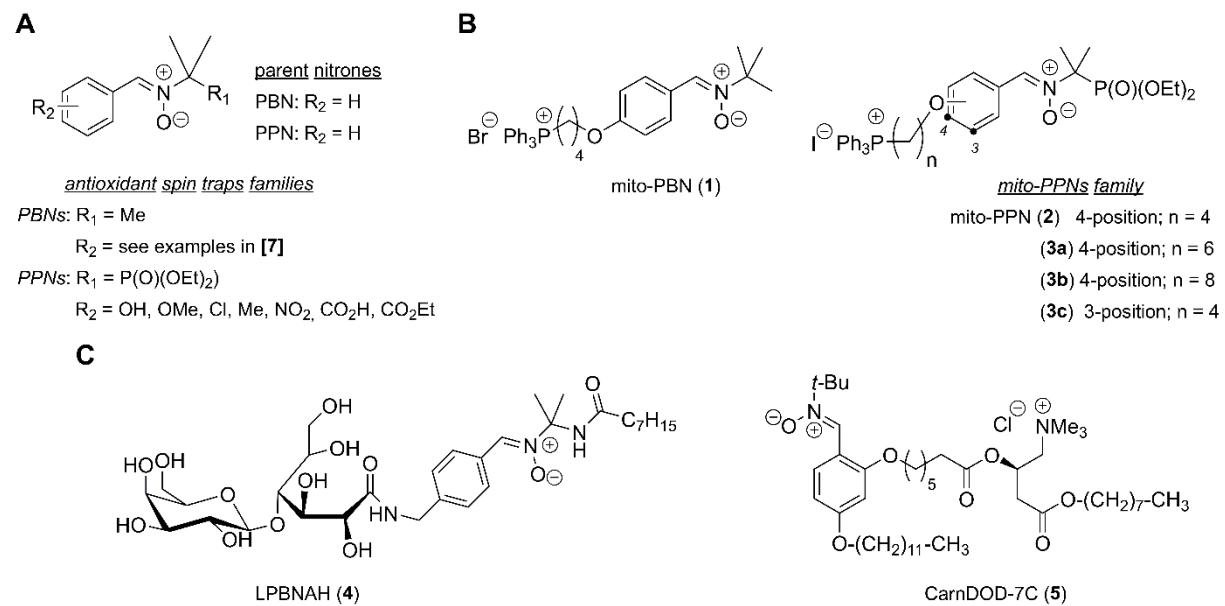


Figure 1. General structures of noncyclic nitrones and related families exhibiting antioxidant and/or pharmacological activity (A) deriving from PBN and PPN [7b,8], or targeted to the mitochondrion (B) by a TPP cation [6a,8] or (C) using alternative vectors [15].

References

- [1] (a) E. Cadenas, K.J.A. Davies, Mitochondrial free radical generation, oxidative stress, and aging, *Free Radic. Biol. Med.* 29 (2000) 222–230, [https://doi.org.lama.univ-amu.fr/10.1016/S0891-5849\(00\)00317-8](https://doi.org.lama.univ-amu.fr/10.1016/S0891-5849(00)00317-8); (b) J.P. Kehrer, L.O. Klotz, Free radicals and related reactive species as mediators of tissue injury and disease: implications for health, *Crit. Rev. Toxicol.* 45 (2015) 765–798, <https://doi.org/10.3109/10408444.2015.1074159>; (c) S. Dröse, U. Brandt, Molecular mechanisms of superoxide production by the mitochondrial respiratory chain, *Adv. Exp. Med. Biol.* 748 (2012) 145–169, https://doi.org/10.1007/978-1-4614-3573-0_6; (d) N.S. Chandel, Mitochondria as signaling organelles, *BMC Biol.* 12 (2014) 34, <https://doi.org/10.1186/1741-7007-12-34>.
- [2] (a) B. Halliwell, J.M.C. Gutteridge, *Free Radicals in Biology and Medicine*, fifth ed., Oxford Uni. Press, United Kingdom, 2015.; (b) A.M. van der Bliek, M.M. Sedensky, P.G. Morgan, Cell genetics of the mitochondrion, *Genetics* 207 (2017) 843–871, <https://doi.org/10.1534/genetics.117.300262>.
- [3] (a) R.K. Lane, T. Hilsabeck, S.L. Rea, The role of mitochondrial dysfunction in age-related diseases, *Biochim. Biophys. Acta* 1847 (2015) 1387–1400, <https://doi.org/10.1016/j.bbabiobio.2015.05.021>; (b) J. Hroudova, N. Singh, Z. Fisar, K.K. Ghosh, Progress in drug development for Alzheimer’s disease: An overview in relation to mitochondrial energy metabolism, *Eur. J. Med. Chem.* 121 (2016) 774–784, <https://doi.org/10.1016/j.ejmech.2016.03.084>.
- [4] (a) V. Sorrentino, K.J. Menzies, J. Auwerx, Repairing mitochondrial dysfunction in disease, *Annu. Rev. Pharmacol. Toxicol.* 58 (2018) 353–389, [10.1146/annurev-pharmtox-010716-104908](https://doi.org/10.1146/annurev-pharmtox-010716-104908); (b) L. Biasutto, A. Mattarei, M. La Spina, M. Azzolini, S.

Parrasia, I. Szabò, M. Zoratti, Strategies to target bioactive molecules to subcellular compartments. Focus on natural compounds. *Eur. J. Med. Chem.* 181 (2019) 111557, doi: [10.1016/j.ejmech.2019.07.060](https://doi.org/10.1016/j.ejmech.2019.07.060); (c) Z.Q. Liu, Bridging free radical chemistry with drug discovery: A promising way for finding novel drugs efficiently, *Eur. J. Med. Chem.* 189 (2020) 112020, <https://doi.org/10.1016/j.ejmech.2019.112020>; (d) V. Weissig, Drug development for the therapy of mitochondrial diseases, *Trends Mol. Med.* 26 (2020) 40–57. <https://doi.org/10.1016/j.molmed.2019.09.002>.

- [5] M.J. Davies, Detection and characterisation of radicals using electron paramagnetic resonance (EPR) spin trapping and related methods, *Methods* 109 (2016) 21–30, <http://dx.doi.org/10.1016/j.ymeth.2016.05.013>.
- [6] (a) M.P. Murphy, R.A.J. Smith, Targeting antioxidants to mitochondria by conjugation to lipophilic cations, *Annu. Rev. Pharmacol. Toxicol.* 47 (2007) 629–656, <https://doi.org/10.1146/annurev.pharmtox.47.120505.105110>; (b) J. Wang, J. Li, Y. Xiao, B. Fu, Z. Qin, Triphenylphosphonium (TPP)-based antioxidants: A new perspective on antioxidant design, *ChemMedChem* 10.1002/cmdc.201900695, <https://doi.org/10.1002/cmdc.201900695>.
- [7] (a) R.A. Floyd, R.D. Kopke, C.H. Choi, S.B. Foster, S. Doblas, R.A. Towner, Nitrones as therapeutics, *Free Radic. Biol. Med.* 45 (2008) 1361–1374, <https://dx.doi.org/10.1016%2Fj.freeradbiomed.2008.08.017>; (b) C. Oliveira, S. Benfeito, C. Fernandes, F. Cagide, T. Silva, F. Borges, NO and HNO donors, nitrones, and nitroxides: Past, present, and future, *Med. Res. Rev.* 38 (2018) 1159–1187, <https://doi.org/10.1002/med.21461>.
- [8] M. Cassien, C. Petrocchi, S. Thétiot-Laurent, M. Robin, E. Ricquebourg, C. Kandouli, A. Asteian, A. Rockenbauer, A. Mercier, C. Culcasi, S. Pietri, On the vasoprotective mechanisms underlying novel β -phosphorylated nitrones: Focus on free radical characterization, scavenging and NO-donation in a biological model of oxidative stress, *Eur. J. Med. Chem.* 119 (2016) 197–217, <https://doi.org/10.1016/j.ejmech.2016.04.067>.

- [9] M.P. Murphy, K.S. Echtay, F.H. Blaikie, J. Asin-Cayuela, H.M. Cochemé, K. Green, J.A. Buckingam, E.R. Taylor, F. Hurrell, G. Hughes, S. Miwa, C.E. Cooper, D.A. Svistunenko, R.A. J. Smith, M.D. Brand, Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-*N*-tert-butylnitron, *J. Biol. Chem.* 278 (2003) 48534–48545, <https://doi.org/10.1074/jbc.m308529200>.
- [10] M. Culcasi, L. Benameur, A. Mercier, C. Lucchesi, H. Rahmouni, A. Asteian, G. Casano, A. Botta, H. Kovacic, S. Pietri, EPR spin trapping evaluation of ROS production in human fibroblasts exposed to cerium oxide nanoparticles: Evidence for NADPH oxidase and mitochondria stimulation, *Chem. Biol. Interact.* 199 (2012) 161–176, <https://doi.org/10.1016/j.cbi.2012.08.007>.
- [11] M. Wu, L. Liao, L. Jiang, C. Zhang, H. Gao, L. Qiao, S. Liu, D. Shi, Liver-targeted Nano-MitoPBN normalizes glucose metabolism by improving mitochondrial redox balance, *Biomaterials* 222 (2019) 119457,
- <https://doi.org/10.1016/j.biomaterials.2019.119457>.
- [12] (a) E. Maurelli, M. Culcasi, M.C. Delmas-Beauvieux, M. Miollan, J.L. Gallis, T. Tron, S. Pietri, New perspectives on the cardioprotective phosphonate effect of the spin trap 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide: an hemodynamic and ^{31}P NMR study in rat hearts, *Free Radic. Biol. Med.* 27 (1999) 34–41, [https://doi.org/10.1016/s0891-5849\(99\)00033-7](https://doi.org/10.1016/s0891-5849(99)00033-7) ; (b) M. Culcasi, G. Casano, C. Lucchesi, A. Mercier, J.L. Clément, V. Pique, L. Michelet, A. Krieger-Liszakay, M. Robin, S. Pietri, Synthesis and biological characterization of new aminophosphonates for mitochondrial pH determination by ^{31}P NMR spectroscopy, *J. Med. Chem.* 56 (2013) 2487–2499, <https://doi.org/10.1021/jm301866e>.
- [13] C. Petrocchi, S. Thétiot-Laurent, M. Culcasi, S. Pietri, Novel mitochondria-targeted triphenylphosphonium conjugates of linear β -phosphorylated nitrones: preparation, ^{31}P NMR mitochondrial distribution, EPR spin trapping reporting, and site-directed anti-

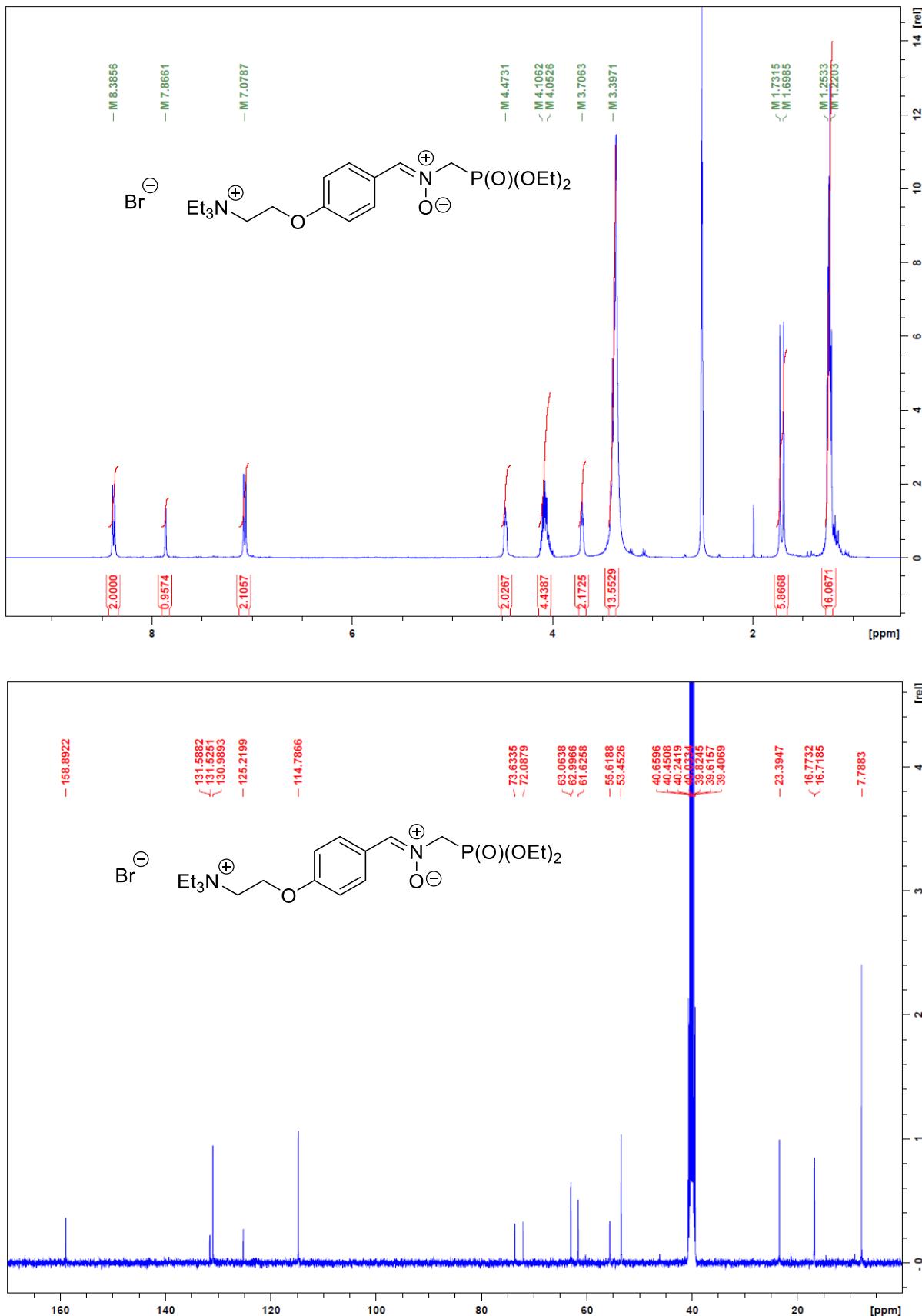
apoptotic properties in: V. Weissig, M. Edeas, (Eds.), Methods in Molecular Biology, Springer Protocols. Mitochondrial Medicine, second ed., Humana Press, Springer, New York, Heidelberg, Dordrecht, London, 2020, in press.

- [14] C. Reily, T. Mitchell, B.K. Chacko, G.A. Benavides, M.P. Murphy, V.M. Darley-Usmar, Mitochondrially targeted compounds and their impact on cellular bioenergetics, *Redox Biol.* 1 (2013) 86–93, <http://dx.doi.org/10.1016/j.redox.2012.11.009>.
- [15] (a) B. Poeggeler, G. Durand, A. Polidori, M.A. Pappolla, I. Vega-Naredo, A. Coto-Montes, J. Böker, R. Hardeland, B. Pucci, Mitochondrial medicine: neuroprotection and life extension by the new amphiphilic nitrone LPBNAH acting as a highly potent antioxidant agent, *J. Neurochem.* 95 (2005) 962–973, <https://doi.org/10.1111/j.1471-4159.2005.03425.x>; (b) S. El Fangour, M. Marini, J. Good, S.J. McQuaker, P.G. Shiels, R.C. Hartley, Nitrones for understanding and ameliorating the oxidative stress associated with aging, *AGE* 31 (2009) 296–276, <https://doi.org/10.1007/s11357-009-9098-z>.
- [16] (a) E.G. Janzen, R.L. Dudley, R.V. Shetty, Synthesis and electron spin resonance chemistry of nitronyl labels for spin trapping. α -Phenyl *N*-[5-(5-methyl-2,2-dialkyl-1,3-dioxanyl] nitrones and α -(*N*-alkylpyridinium) *N*-(tert-butyl) nitrones, *J. Am. Chem. Soc.* 101 (1979) 243–245, <https://doi.org/10.1021/ja00495a053>; (b) H.A.O. Hill, P.J. Thornalley, Free radical production during phenylhydrazine-induced hemolysis, *Can. J. Chem.* 60 (1982) 1528–1531, <https://doi.org/10.1139/v82-222>; (c) E.G. Janzen, M.S. West, Y. Kotake, C.M. DuBose, Biological spin trapping methodology. III Octanol-water partition coefficients of spin-trapping compounds, *J. Biochem. Biophys. Methods* 32 (1996) 183–190, [https://doi.org/10.1016/0165-022X\(96\)00008-5](https://doi.org/10.1016/0165-022X(96)00008-5); (d) C.P. Sár, E. Hideg, I. Vass, K. Hideg, Synthesis of α -aryl *N*-adamant-1-yl nitrones and using them for spin trapping of hydroxyl radicals, *Bioorg. Med. Chem. Lett.* 8 (1998) 379–384, [https://doi.org/10.1016/S0960-894X\(98\)00033-X](https://doi.org/10.1016/S0960-894X(98)00033-X).

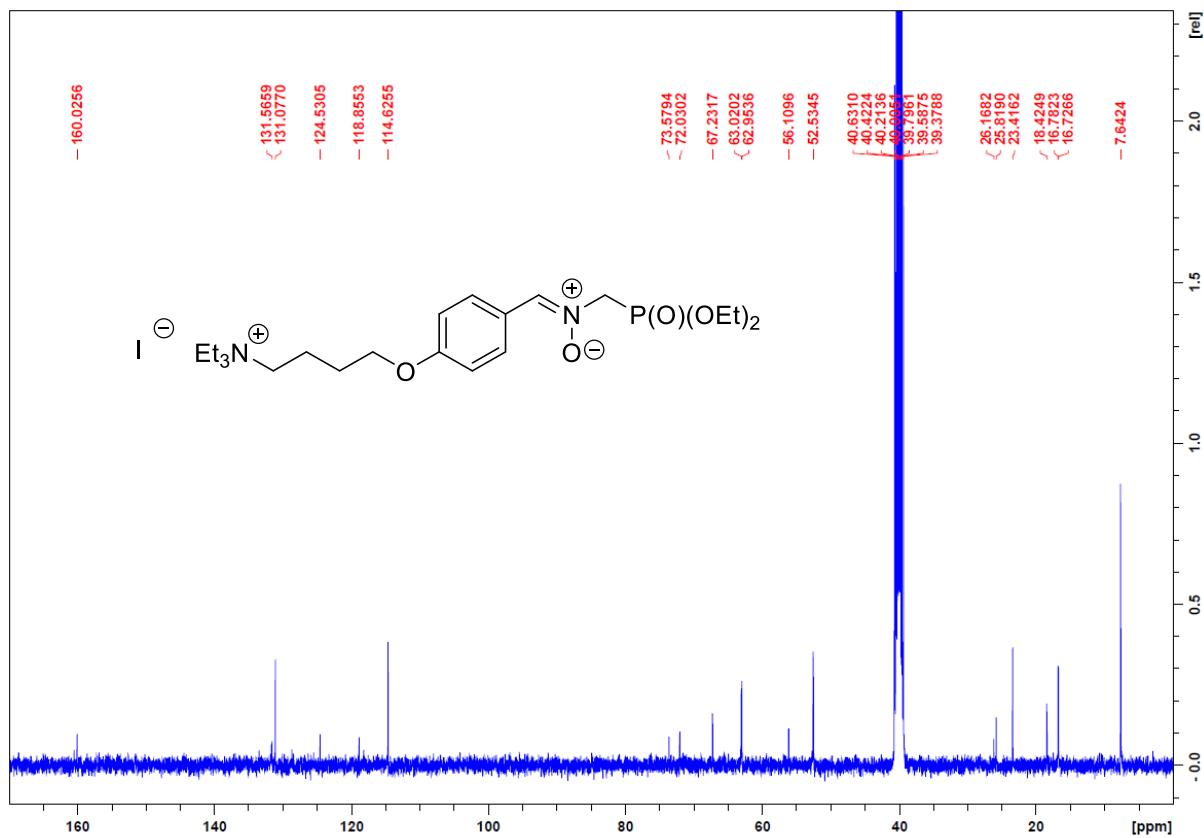
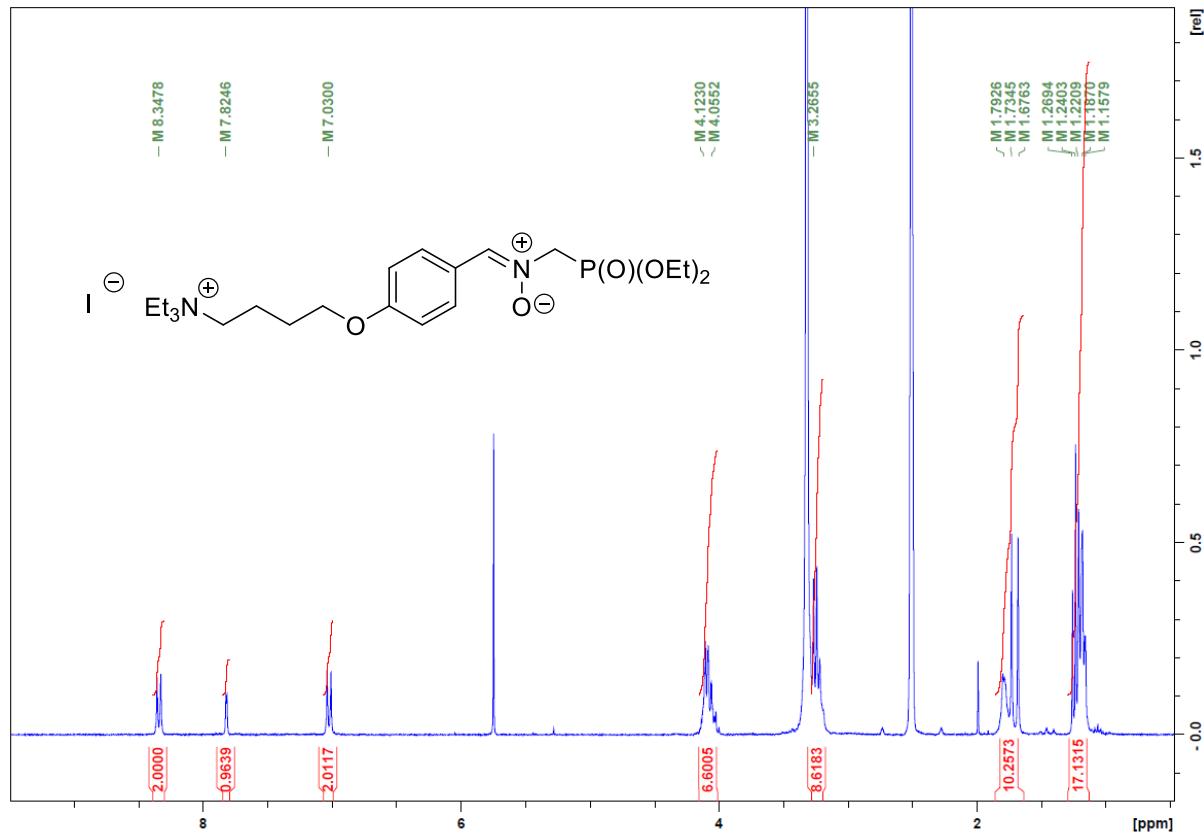
- [17] L. Robertson, R.C. Hartley, Synthesis of *N*-arylpyridinium salts bearing a nitrone spin trap as potential mitochondria-targeted antioxidants, *Tetrahedron* 65 (2009) 5284–5292.
<https://doi.org/10.1016/j.tet.2009.04.083>.
- [18] Y. Li, X. Yuan, X. Rong, Y. Gao, Z. Qiu, Z. Zhang, D. Zhou, W. Li, Design, synthesis and biological evaluation of a hybrid compound of berberine and magnolol for improvement of glucose and lipid metabolism, *RSC Adv.* 6 (2016) 81924–81931,
<https://doi.org/10.1039/C6RA15100K>.
- [19] K.G. Lyamzaev, A.V. Pustovidko, R.A. Simonyan, T.I. Rokitskaya, L.V. Domnina, O.Y. Ivanova, I.I. Severina, N.V. Sumbatyan, G.A. Korshunova, V.N. Tashlitsky, V.A. Roginsky, Y.N. Antonenko, M.V. Skulachev, B.V. Chemyak, V.P. Skulachev, Novel mitochondria-targeted antioxidants: plastoquinone conjugated with cationic plant alkaloids berberine and palmatine, *Pharm. Res.* 28 (2011) 2883–2895,
<https://doi.org/10.1007/s11095-011-0504-8>.

Supplementary Material

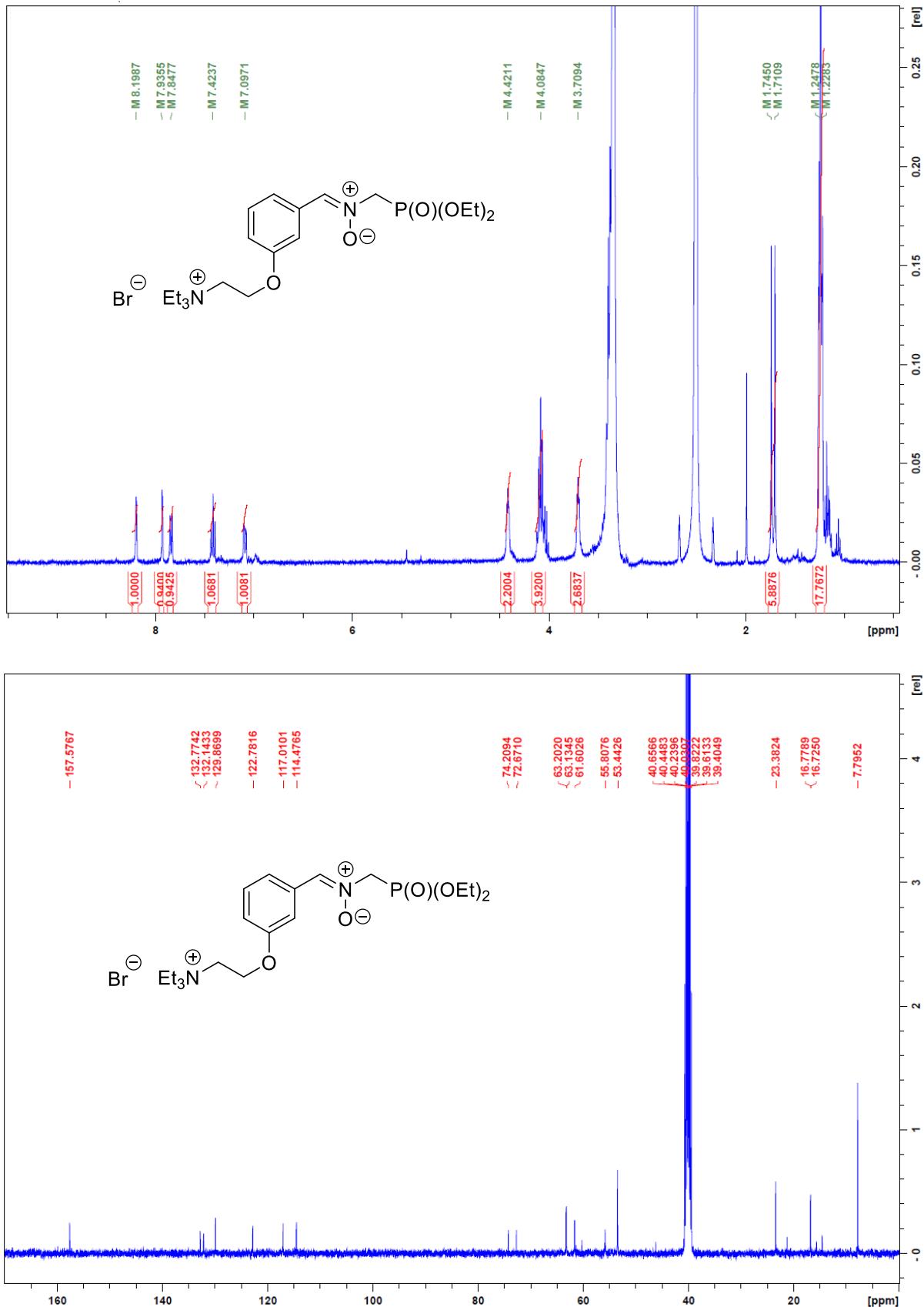
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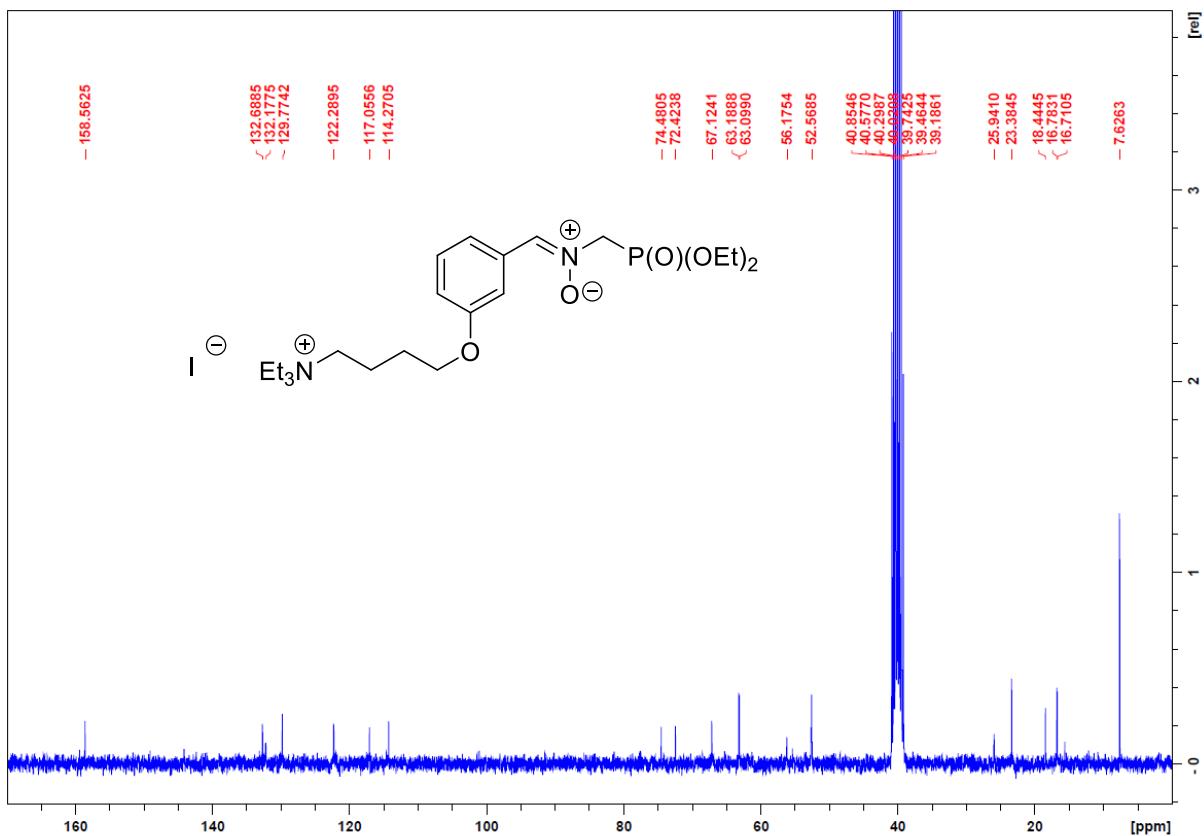
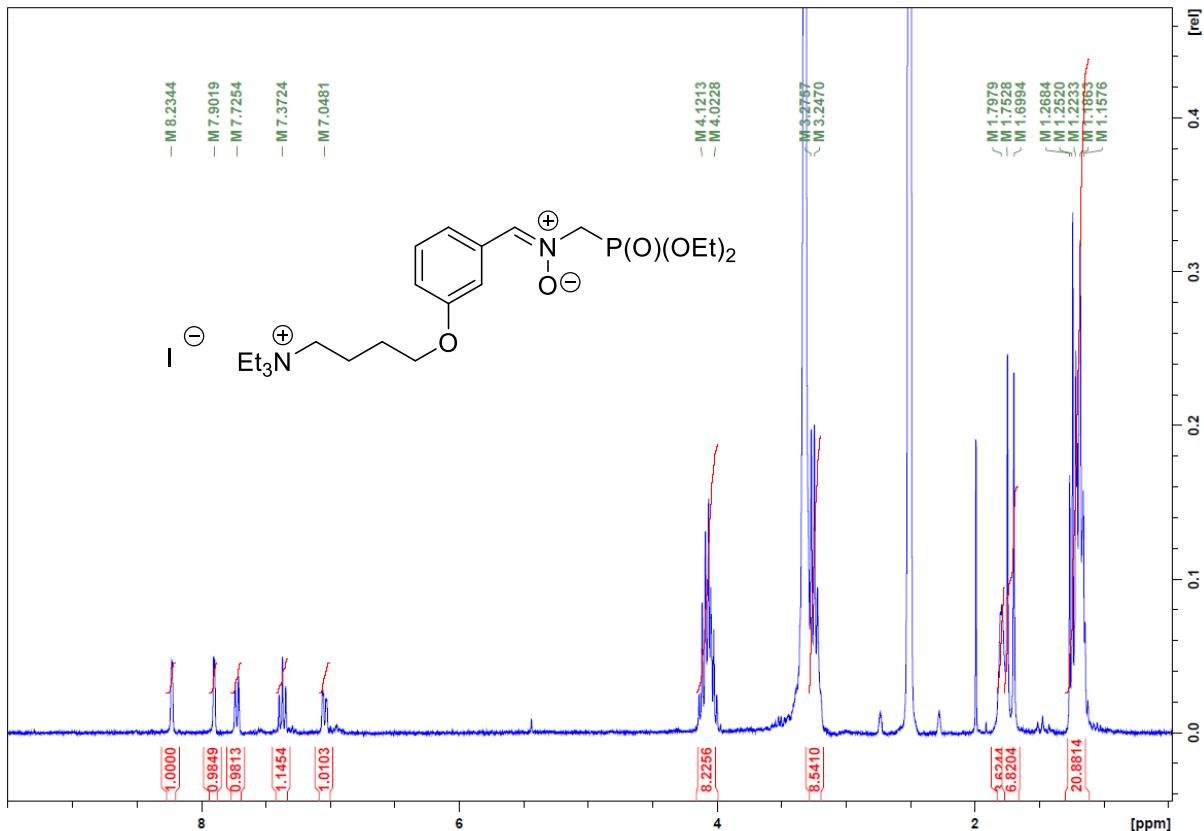
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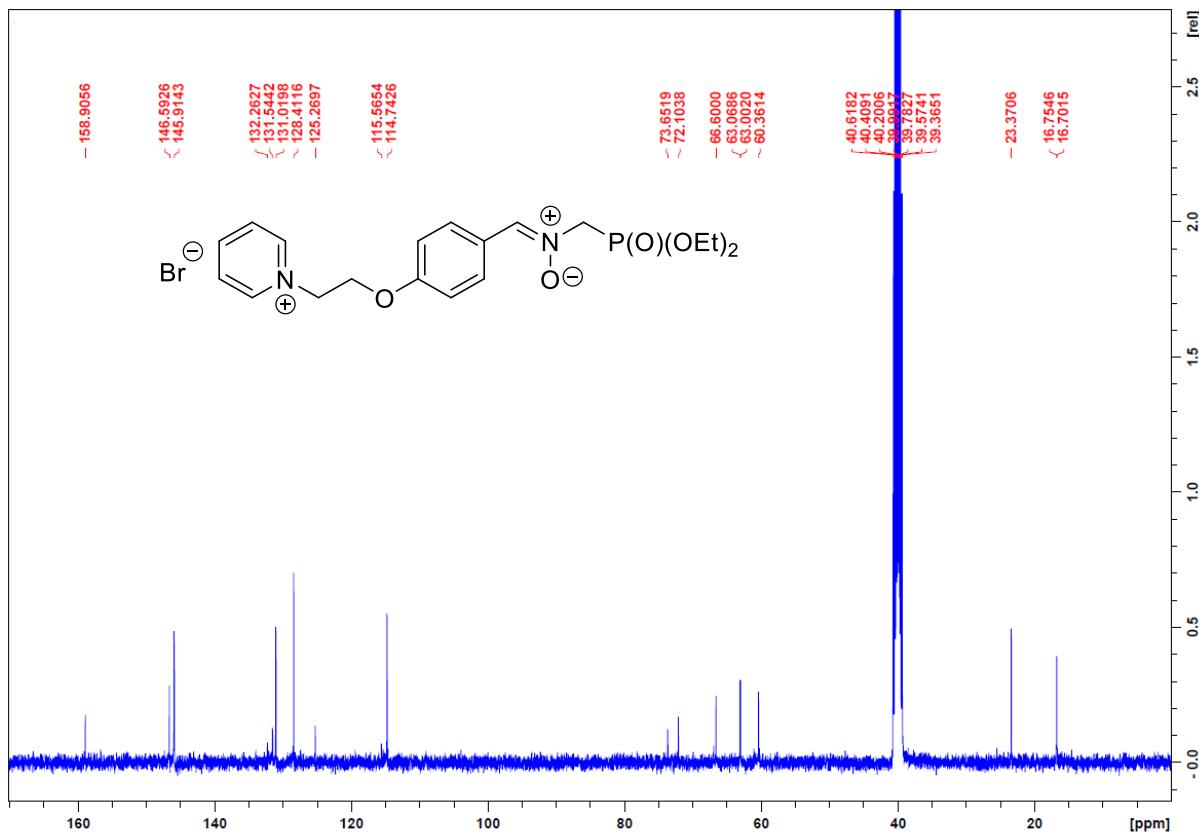
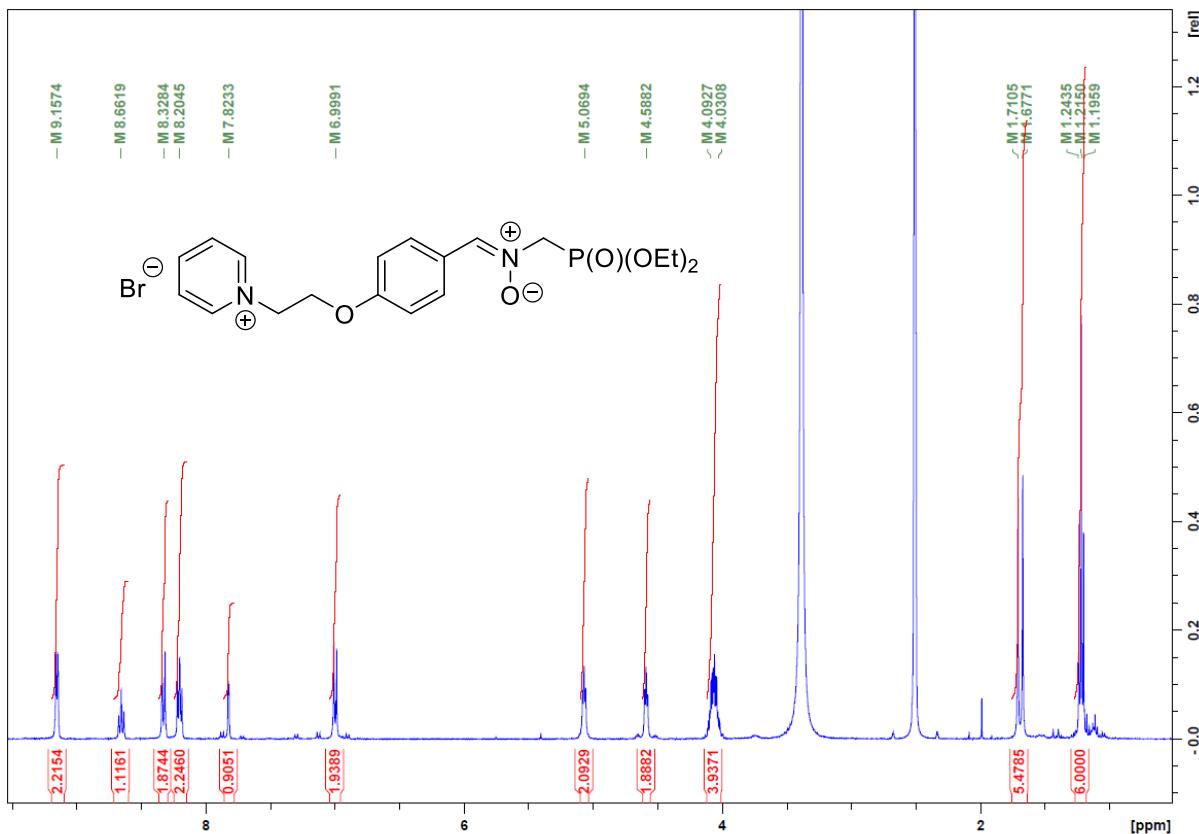
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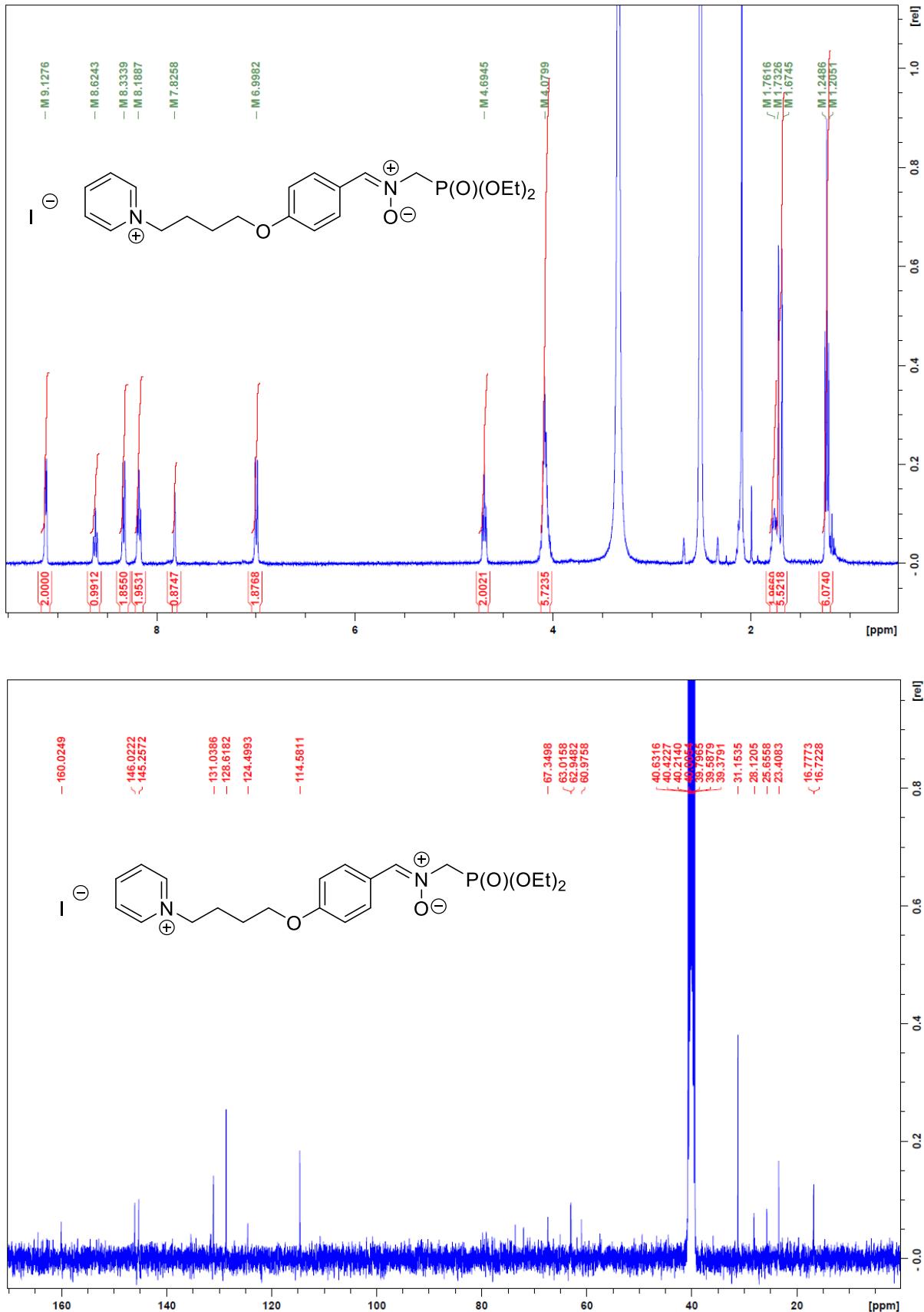
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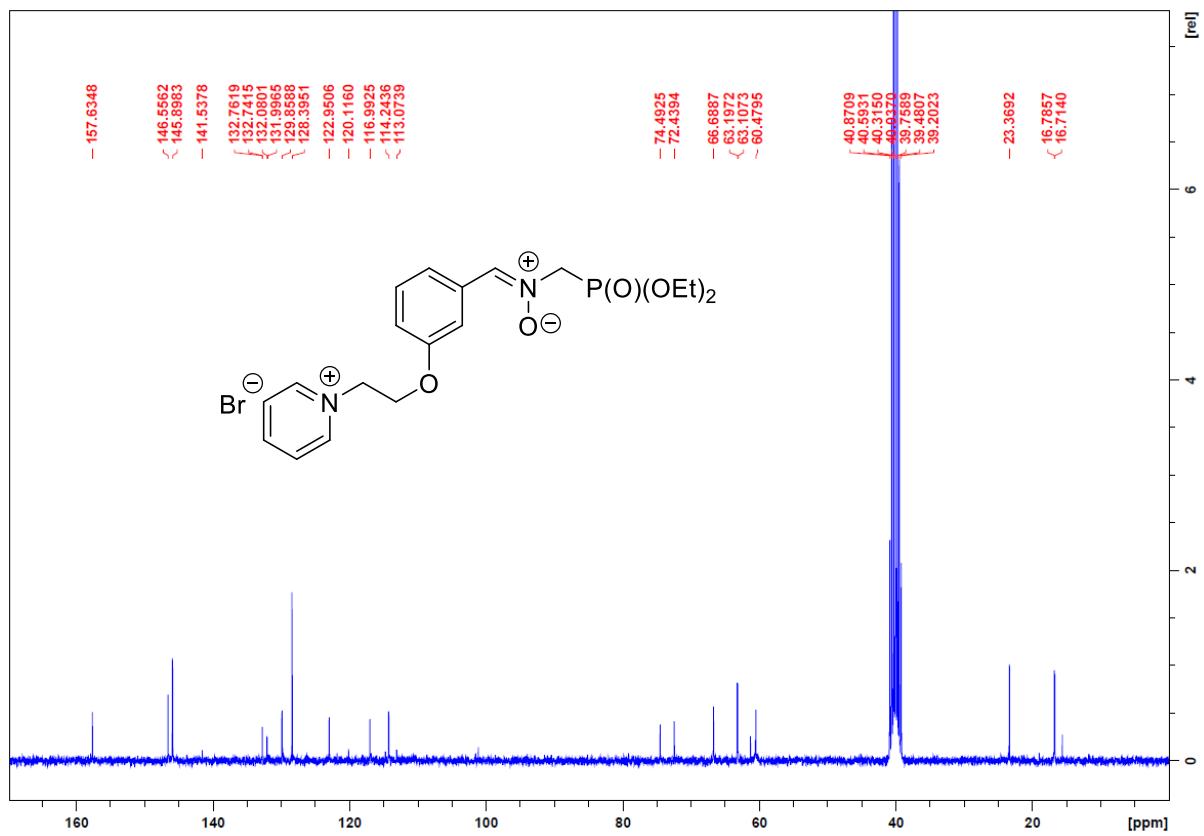
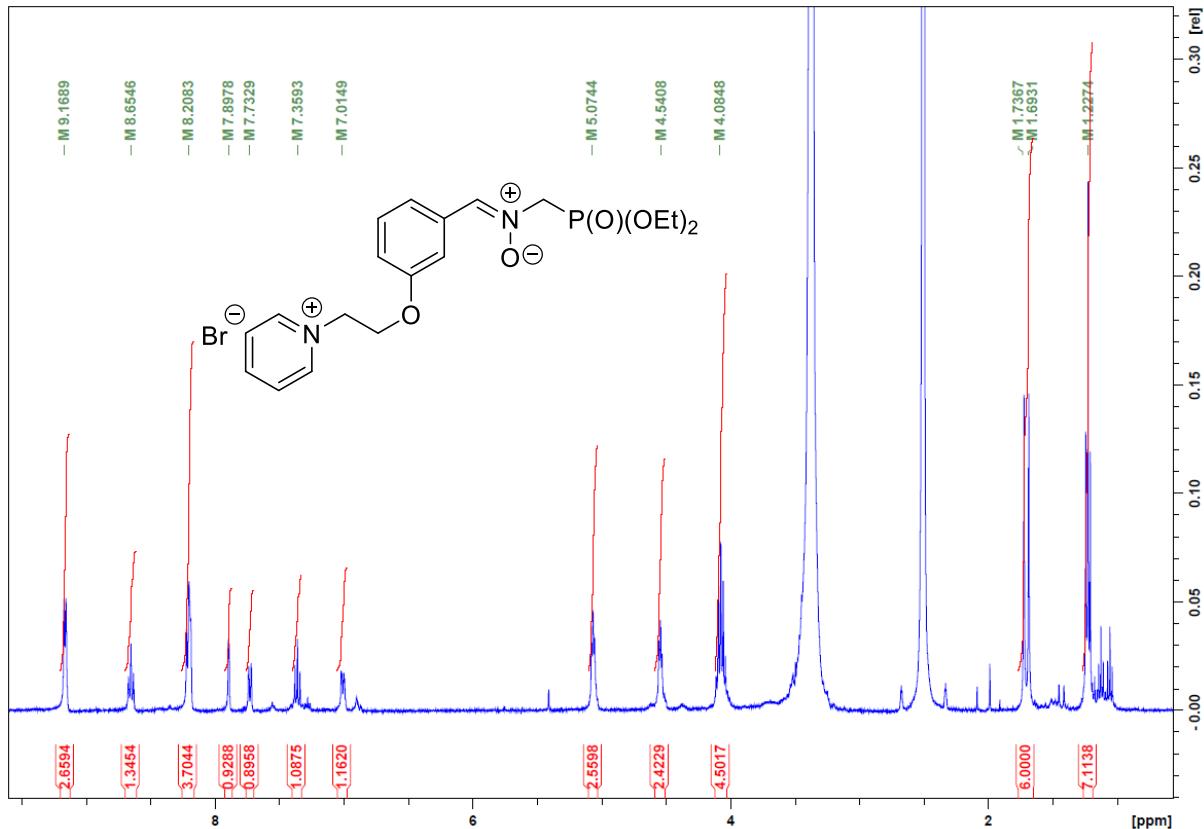
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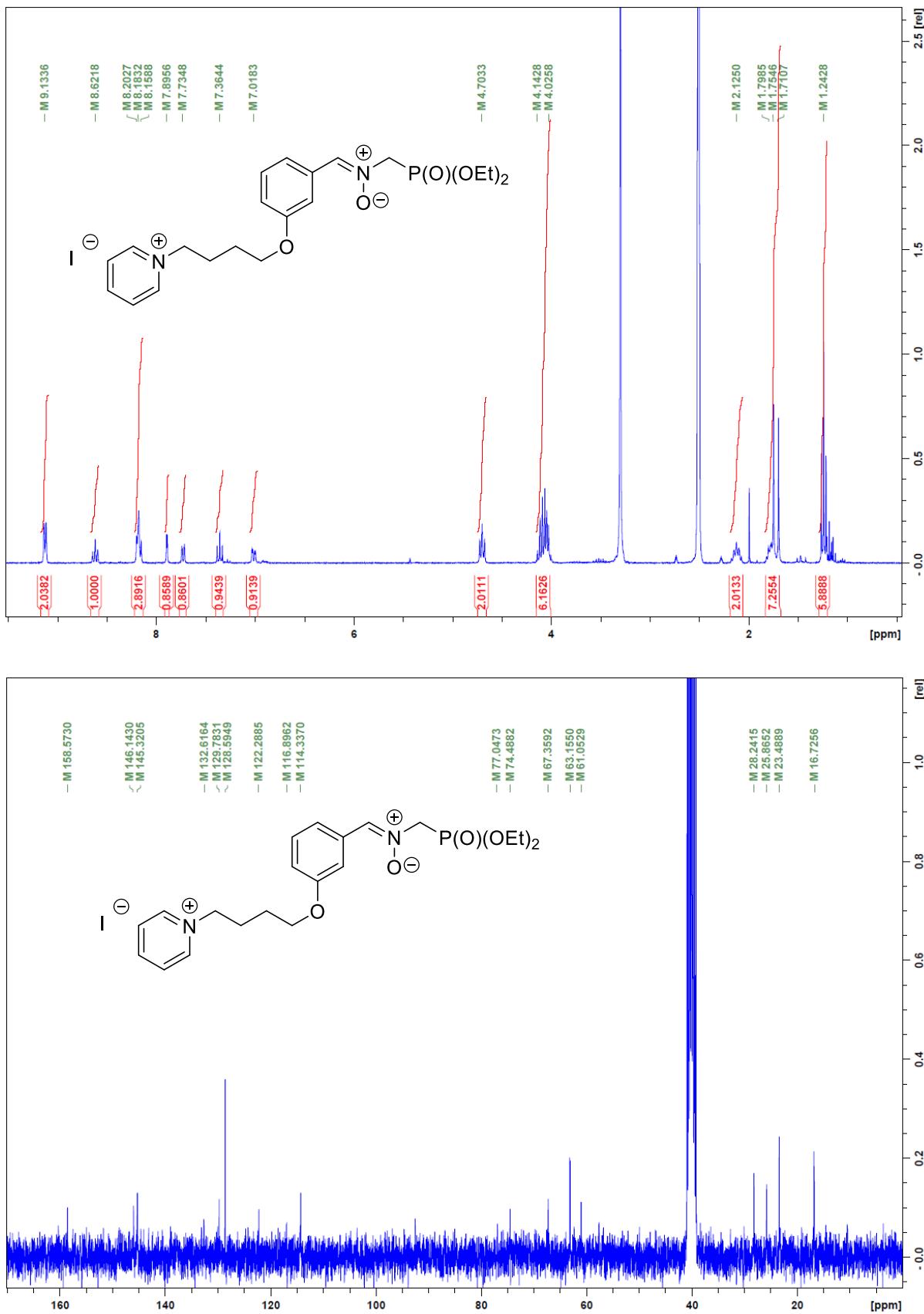
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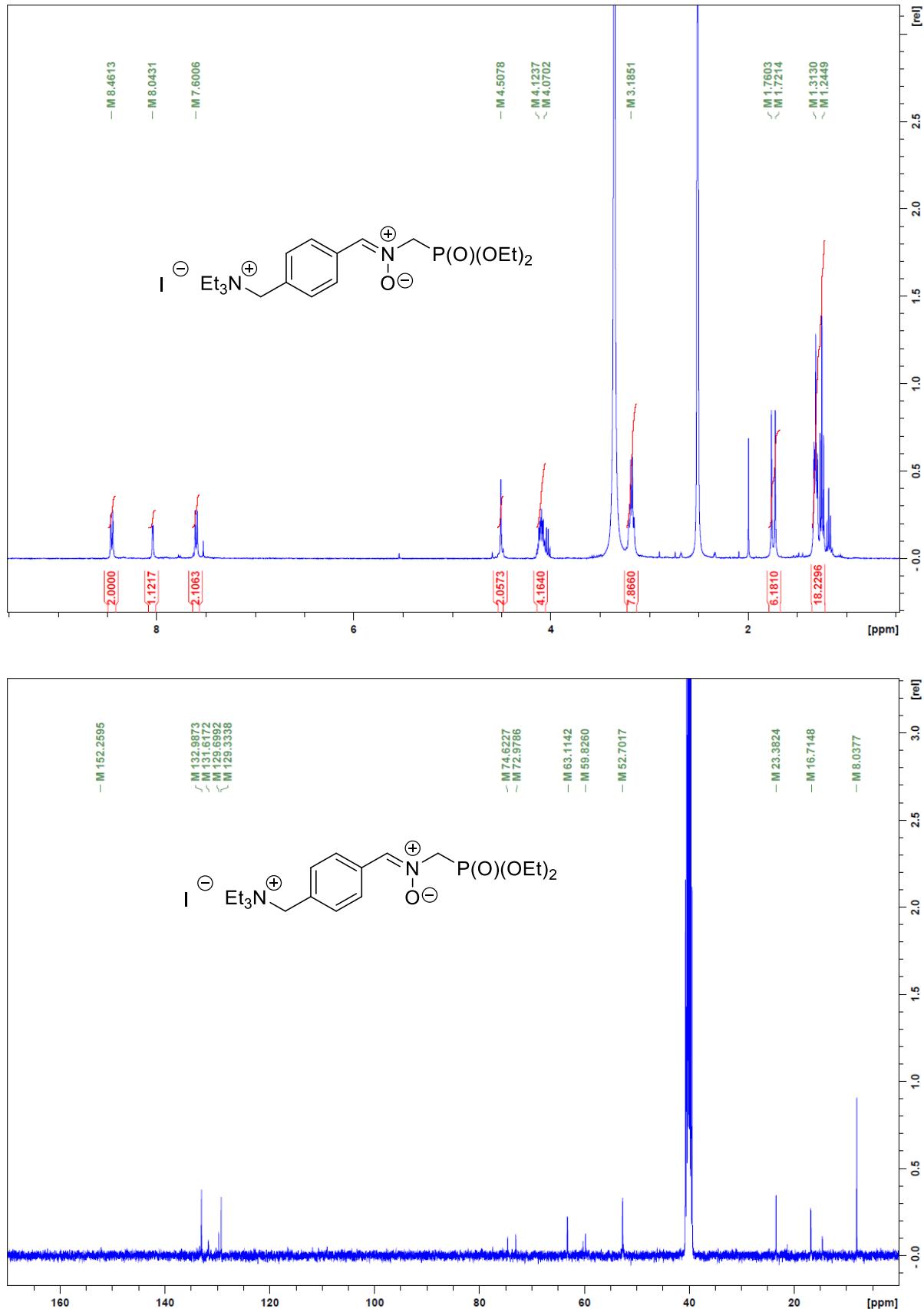
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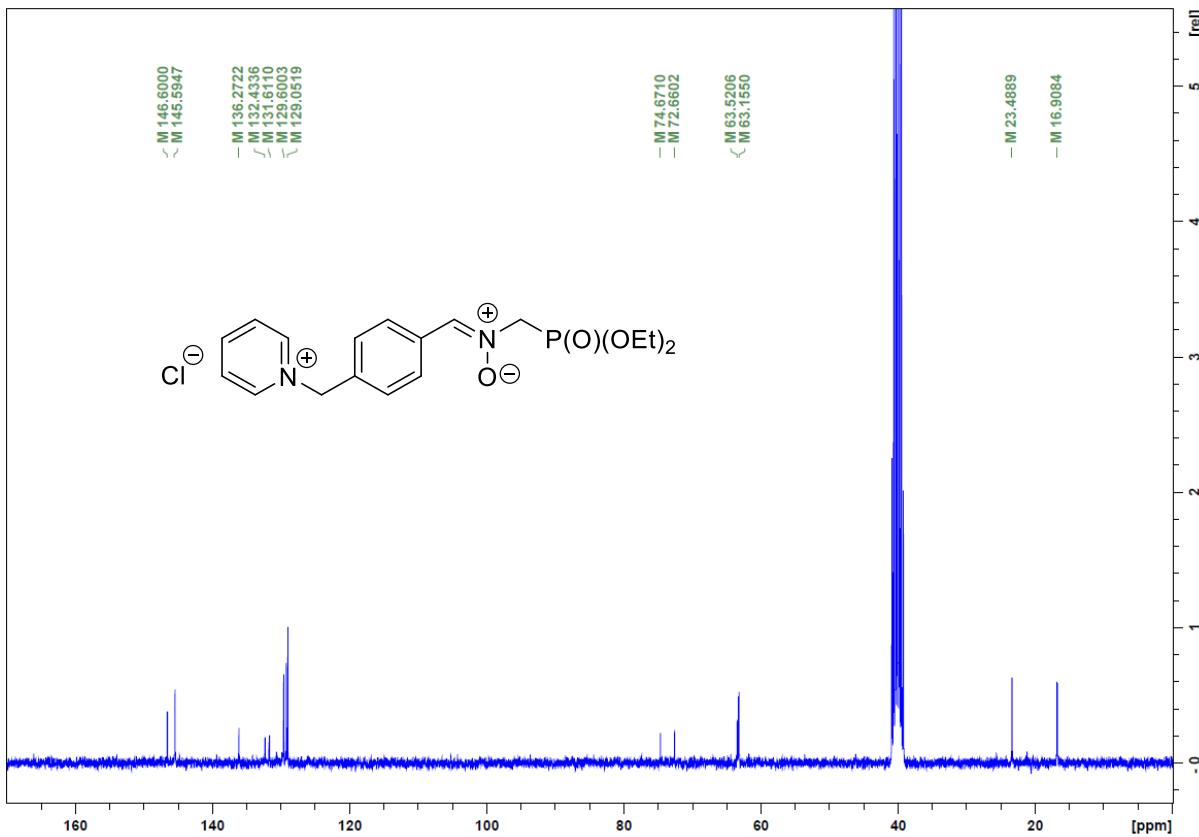
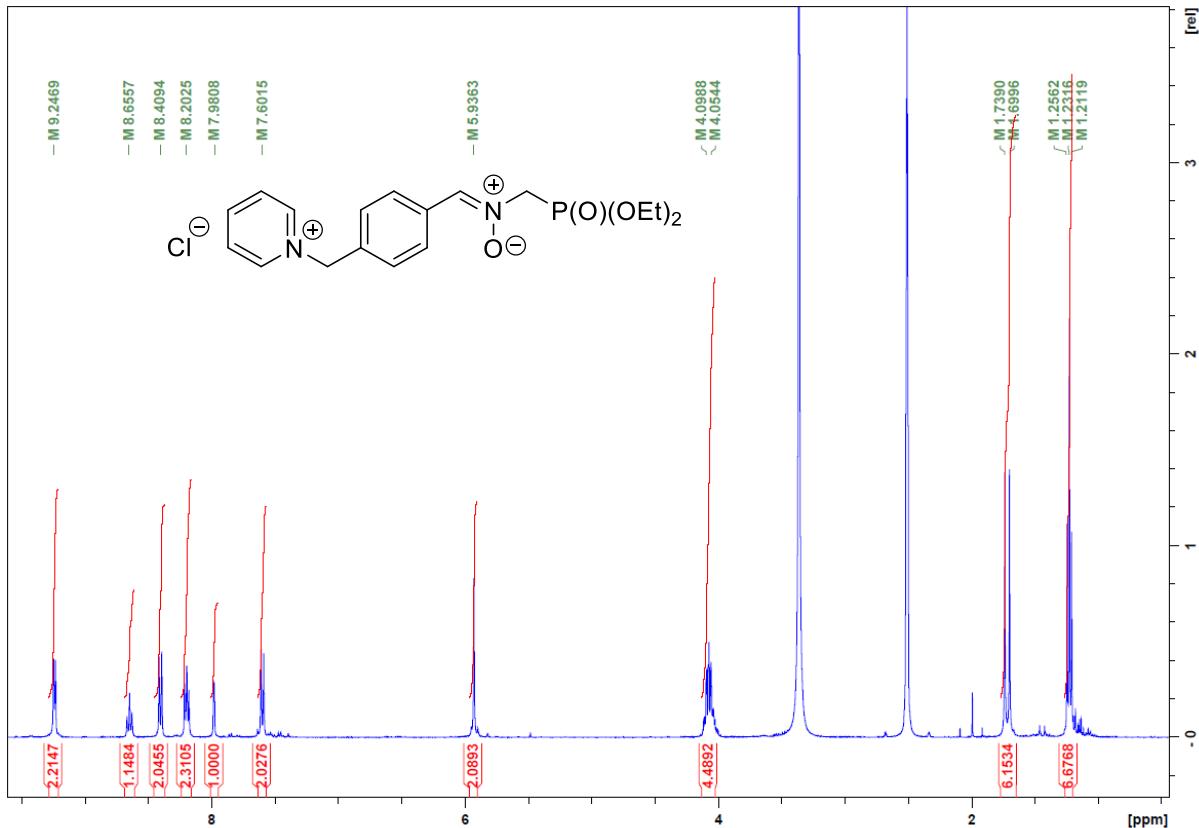
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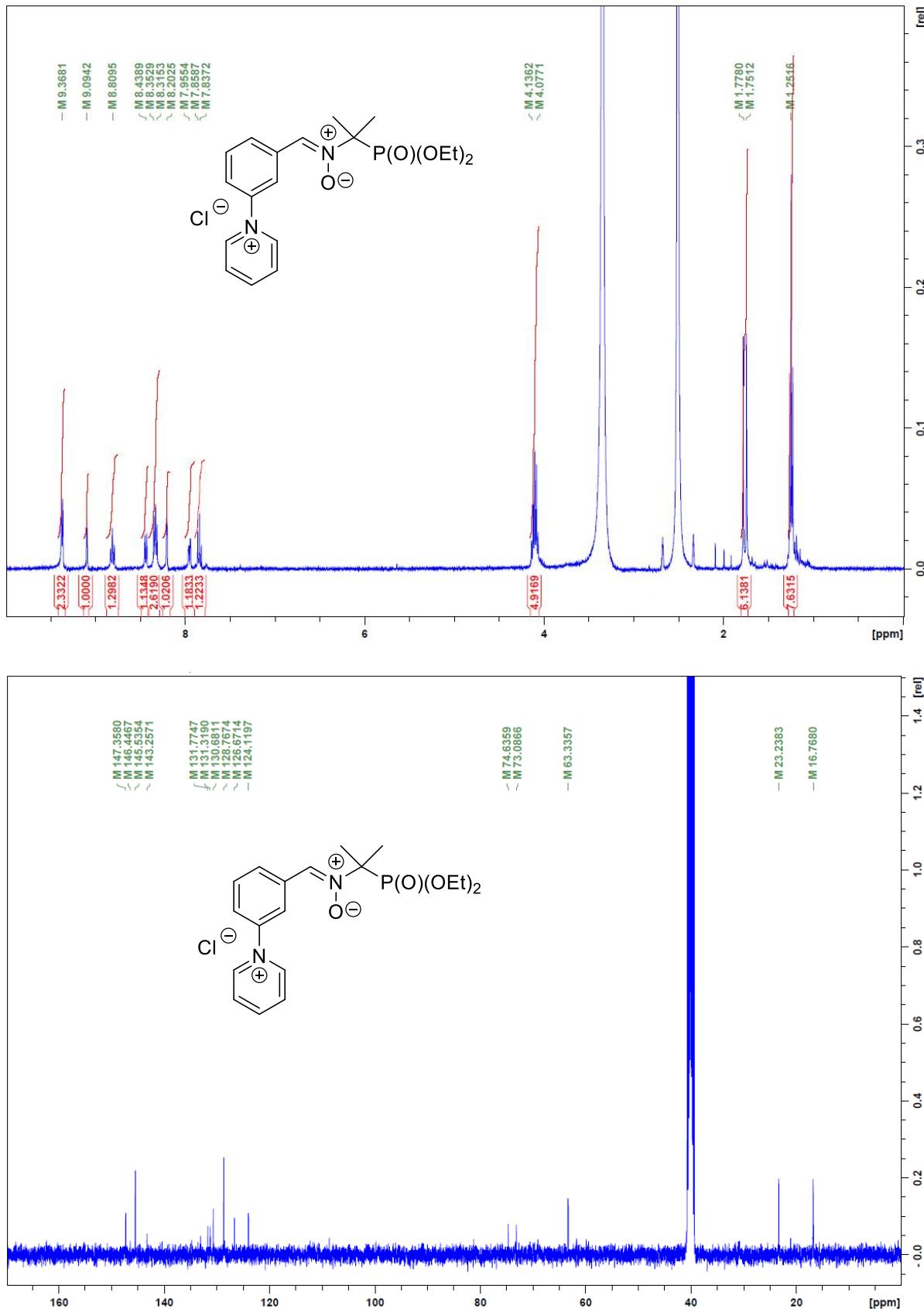
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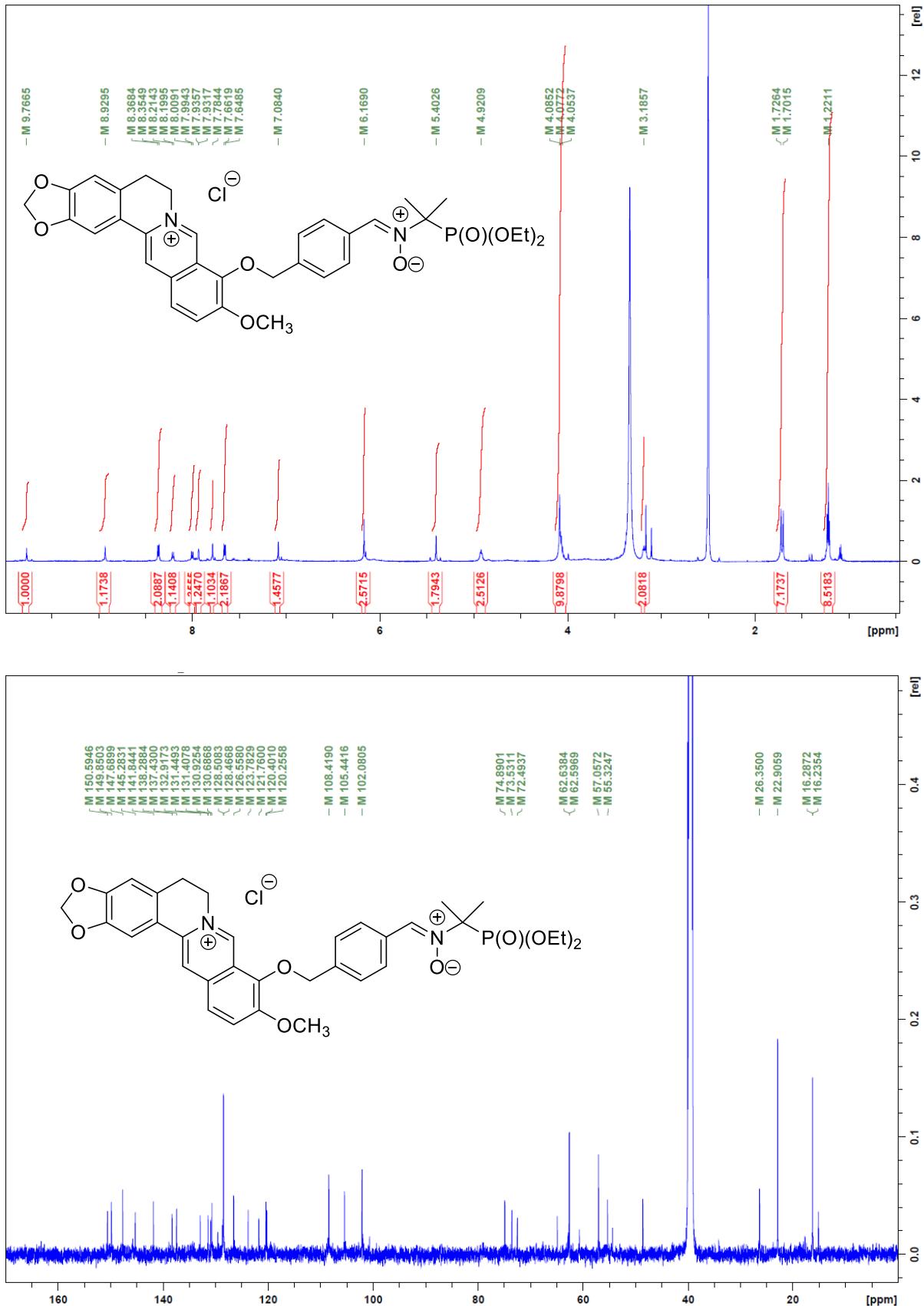
10. Spectra of nitrone **14**



11. Spectra of nitrone **15**



12. Spectra of nitrone **16**



13. Spectra of nitrone 17

