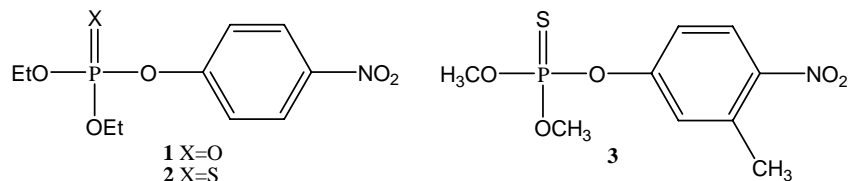
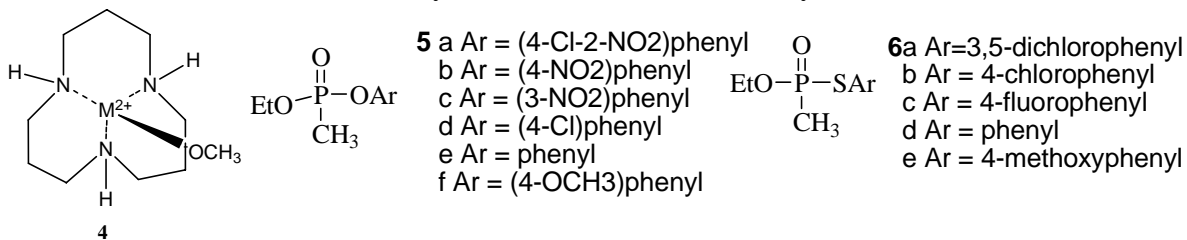


Modelling the Zn(II) containing phosphotriesterases.

The above study has direct bearing on the mechanism of action of phosphotriesterase (PTE), a Zn^{2+} -containing enzyme found in the soil dwelling bacterium *Pseudomonas diminuta* which has been shown to degrade pesticides such as paraoxon (1), parathion (2) and fenitrothion (3).¹ X-ray diffraction shows that the active site of the enzyme comprises two Zn^{2+} ions separated by 3.4 Å, one of which is coordinated to the protein by two His imidazoles and an aspartate COO^- , and the second by two histidines: both metal ions are bridged by the oxygens of N-carboxy lysine ($-NHCO_2^-$) and an ^-OH or water having a kinetic pK_a of 5.8-5.9² which is thought to be the active nucleophile.³ X-ray diffraction studies also showed that inhibitors such as triethyl phosphate and di-*iso*-propyl methylphosphonate bind to the more solvent-exposed Zn^{2+} ion with P=O coordination.⁴ The metal ions can be removed from the wild type enzyme and replaced by Cd^{2+} , Ni^{2+} , Co^{2+} and Mn^{2+} , and all reconstituted enzymes show activity. The native enzyme has been shown to react with paraoxon in ^{18}O -labelled water to produce $(EtO)_2P(=O)^{18}OH$ product, and also to react with chiral *O*-ethyl phenylphosphonothioic acid to give product that is inverted.⁵ Detailed kinetic studies of the hydrolysis of a series of diethyl aryl phosphates and phosphorothioates conducted on the $(Zn^{2+})_2$ wild-type enzyme by Hong and Raushel indicated that the respective Brønsted β_{lg} values of V_{max} vs. pK_a of aryl phosphates and phosphorothioates in water were -2.2 and -1.0 , the high value for the phosphates being interpreted as arising from a significantly dissociative mechanism with a quite product-like transition state.⁶



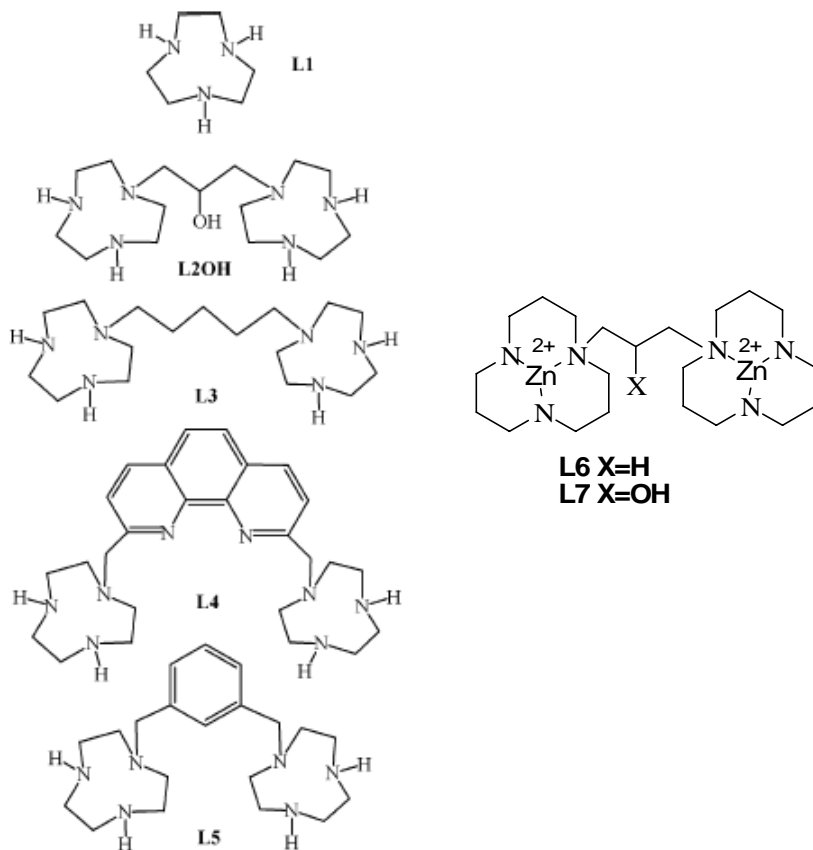
Recently we have shown that La^{3+} along with one eq. of added $NaOCH_3$ pre metal ion, as well as the methoxy Zn^{2+} complex of 1,5,9-triazacyclododecane (4) are remarkable catalysts for promoting the methanolysis of such neutral organophosphorus materials as 1-3^{7a,b}, as well as phosphonates (5)^{7c} and phosphonothioates (6)^{7d}, so much so that as little as 1 mM of the active catalyst accelerates the reaction by 10^9 -fold relative



to the background reaction.

Future work will be aimed at synthesizing and evaluating the efficacy of dinuclear Zn^{2+} -containing ligands as exemplified below where the metal ions are held apart in such a way as to allow both flexibility in substrate binding, and a cooperative interaction in promoting the catalysis. In another direction, ligands will be attached to solid supports to

create solid chromatographic packing materials that can be used for the destruction, via Metal catalyzed alcoholysis, OP pesticides and CW materials.



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² S. D. Aubert, Y. Li and F. M. Raushel, *Biochemistry*, 2004, **43b**, 5707

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⁵ V. E. Lewis, W. J. Donarski, J. R. Wild and F. M. Raushel, *Biochemistry*, 1988, **27**, 1591.

⁶ S.-B. Hong and F. M. Raushel, *Biochemistry*, 1996, **35**, 10904.

⁷ (a) Tsang, J. S.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 7602; (b) Liu, T.; Neverov, A. A.; Tsang, J. S. W.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 1525; (c) Lewis, R. E.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 4082.; (d) Melnychuk, S. A.; Neverov, A. A.; Brown, R. S. *Angew. Chem. Internat. Edit.* **2006**, *45*, 1767.