Synthesis of (*R*)-OH-TMAEP and its labeled analogs as substrates for TmpB

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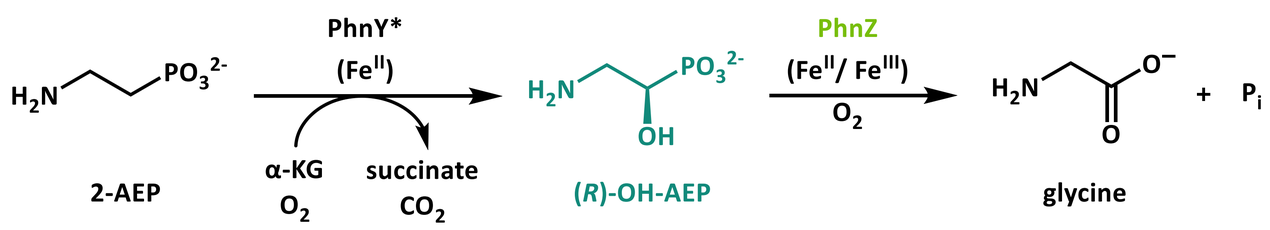
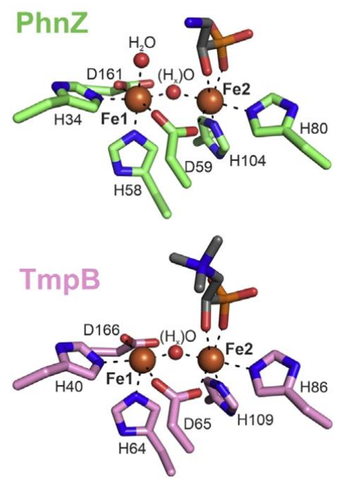
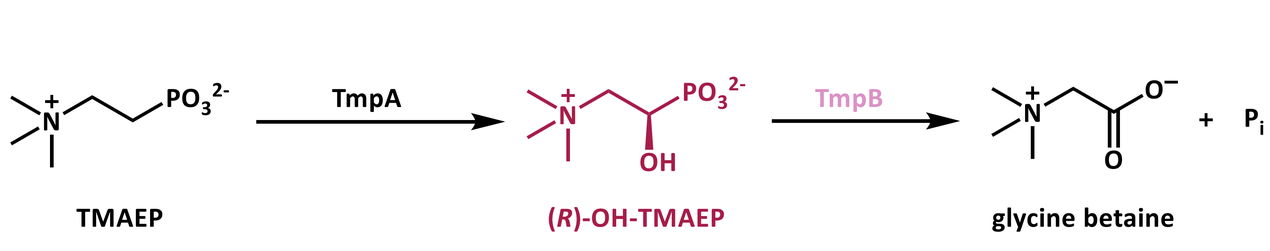
Introduction

Especially in marine ecosystems growth and productivity are limited by the availability of phosphorus, where lower oxidized organophosphorus compounds play an outstanding role as alternative phosphorus source.[1] Nowadays we know that about 40% of bacterial genomes contain at least one pathway for phosphonate catabolism and know about three general degradation pathways. Among those, the oxidative P-C bond cleavage was discovered most recently and only one set of enzymes belonging to this group was known for several years: PhnY* and **PhnZ** were shown to degrade 2-AEP over (*R*)-1-hydroxy-2-aminoethylphosphonate [**(*R*)-OH-AEP**] to inorganic phosphate P_i and glycine, as well as methylphosphonic acid to formic acid and P_i.[2, 3]

Recently, another pair of enzymes encoding the oxidative P-C bond cleavage of 2-(trimethylamino)ethylphosphonate (TMAEP) was discovered by Bollinger *et al.* In analogy to the already known PhnY*/**PhnZ** pathway, TMAEP is converted to glycine betaine and P_i via the intermediate (*R*)-1-hydroxy-2-(trimethylamino)ethylphosphonate [**(*R*)-OH-TMAEP**].







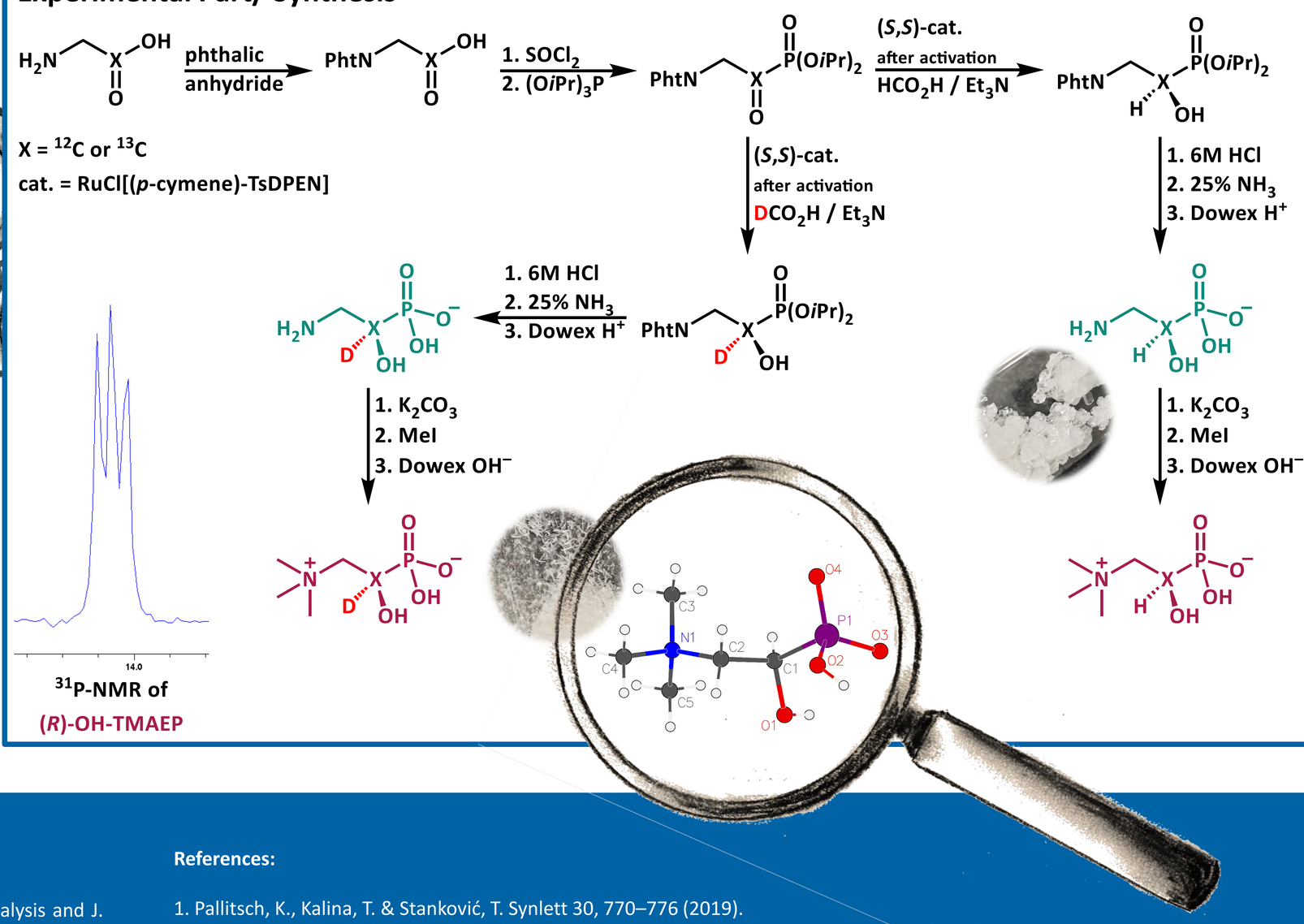
TmpB was identified as a HD protein with a diiron cofactor and has 32% sequence identity to **PhnZ**. The genomic synteny of TmpA and **TmpB** suggest that **TmpB** uses **(*R*)-OH-TMAEP**, formed by TmpA, to perform an oxidative C-P bond cleavage reaction. However, **TmpB** is not necessarily an oxygenase, other enzymes with the same properties have already been identified as phosphohydrolases. Further studies are thus required to determine the exact mode of action of **TmpB** together with a functional assignment within this structural enzyme superfamily.[4]

Discussion

To a profound understanding of this enzyme system mechanistic studies with enantiopure and isotopically labeled compounds are necessary. These are especially useful to determine rate limiting steps and exact locations of radicals formed during enzymatic reactions. We synthesized **(*R*)-OH-TMAEP** and several isotopically labeled analogs to use them for mechanistic studies of **TmpB**, which are currently in progress. Elegantly, the same route can be used for synthesizing all desired compounds.[5]

The purification of **(*R*)-OH-TMAEP** proved particularly challenging and could finally achieved by anion exchanging chromatography, followed by crystallization from EtOH/H_2O to give the salt free, very hygroscopic **(*R*)-OH-TMAEP** and its labeled analogs (*ee* ≥ 99%; degree of deuteration ≥ 98% for ^{12}C and ≥ 94% for ^{13}C).

Experimental Part/ Synthesis



^{31}P-NMR of **(*R*)-OH-TMAEP**

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We thank A. Roller and the Centre for X-ray Structure Analysis and J. Theiner for combustion analysis. Further we thank the FWF (Austrian Science Fund) for financial support (project P 27897-N28).

https://pallitsch-lab.univie.ac.at/

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