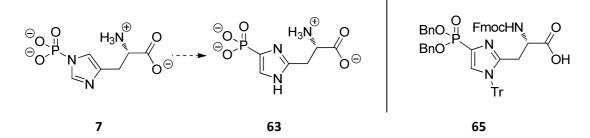
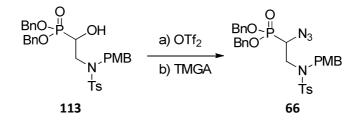
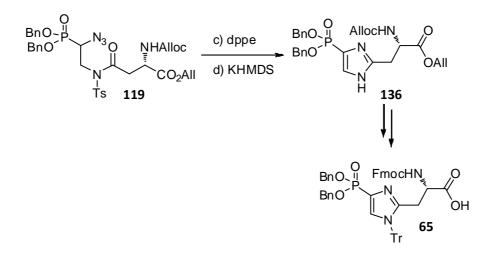
## **1** Abstract

Protein phosphorylation is a post-translational modification that plays a significant role in many cell processes and regulatory pathways. In eukaryotic cells, six percent of total protein phosphorylation occurs on the nitrogen atoms of the imidazole moiety of histidine. While phosphate esters (P-O bond) are relatively stable to hydrolysis, phosphoramidates (P-N bond) hydrolyze easily. Hydrolysis of 3-phosphohistidine **7** (pHis) is even faster under acidic conditions, since the protonated imidazole behaves as an excellent leaving group. Therefore, research focused on phophorylated histidine containing proteins is complicated by the lack of analytical techniques that are compatible with pHis's inherent instability. Following the success of phosphotyrosine antibodies, attempts have been made to raise antibodies specific for pHis containing peptides. However, pHis itself cannot be used for immunisation as the phosphoramidate is hydrolyzed before a strong immune response can be realized. Therefore, a new pHis analogue **63** was designed and synthesized. This mimic closely resembles pHis's electronic and structural properties under physiological conditions, due to the conserved imidazole ring, while the P-N bond is exchanged for a stable P-C bond. In order to raise antibodies the pHis mimic should be incorporated into a peptide. Therefore, the mimic was decorated with protective groups suitable for Fmoc-based SPPS **(65)**.



Various routes have been investigated to synthesize **65**. The successful retrosynthetic strategy involved coupling of compound **66** with an Alloc/allyl protected aspartic acid to yield amide **119**. Compound **66** was made via a nine step sequence, which included the activation of compound **113** as a triflate followed by substitution with TMGA. A dppe promoted aza-wittig ring closing reaction of the amide was used to form the imidazoline. Aromatization of the imidazoline was realized by base induced elimination of the tosylate to yield **136**. Several protecting group manipulations gave final compound **65** in 22.7 percent yield over 15 steps.





The resulting compound **65** was used to synthesize several peptides, of which two have been employed to raise antibodies. These antibodies successfully recognized natural pHis, both in dot blot tests with pHis containing peptides, as well as in cell lysates. These antibodies can be used in the near future to study the biological functions of pHis.

In order to study the related enzyme PHPT1, responsible for phophohistidine dephosphorylation, a library of 5-phosphonic acid imidazoles with various substituents on C-2 has been synthesized to act as a starting point for the development of a PHPT1 inhibitor. The synthetic strategy described for the synthesis of mimic **65** was applied to generate this compound set.

The antibodies, inhibitors and other tool compounds described in this thesis provide an invaluable toolset to increase the understanding of phosphorylation.