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Discovery of a novel synthetic phosphatase from a bead-bound combinatorial library†

Shelley C. Danek, Jerome Queffelec and James P. Morken*

Department of Chemistry, Venable and Kenan Laboratories, UNC Chapel Hill, Chapel Hill, NC 27516, USA. E-mail: morken@unc.edu; Fax: +1-919-962-2388; Tel: +1-919-962-8229

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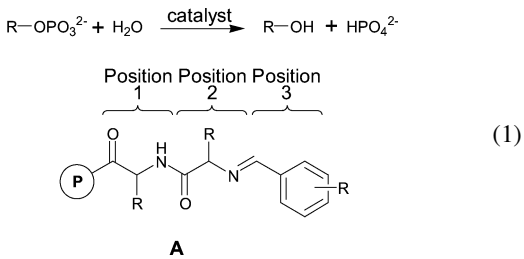
Using split/pool encoded synthesis and a colorimetric catalysis assay, a number of synthetic phosphatase catalysts were developed.

Enzyme catalyzed organic reactions are often facilitated because of the concerted interplay among spatially localized functional groups. The structure of this array often results in high substrate specificity that is in contrast to the specificity of synthetic catalysts which often derive reactivity from single functional centers (*e.g.* a transition metal). To add enzyme-like specificity to non-enzyme catalysts, a number of groups have developed multifunctional macromolecular catalysts which contain a combination of recognition and catalytic elements.[1] Difficulty in the design of such enzyme-mimetics arises from the choice of functional groups as well as control of their relative spatial orientation. In this regard, we have begun to generate libraries of potential multifunctional catalysts with the notion that appropriate screening techniques would reveal catalysts that are able to accelerate reaction rates.[2] Herein, we present our preliminary results on library synthesis, selection of synthetic phosphatase catalysts and subsequent catalyst validation.

Catalytic hydrolysis of phosphomonoesters [eqn. (1)] was

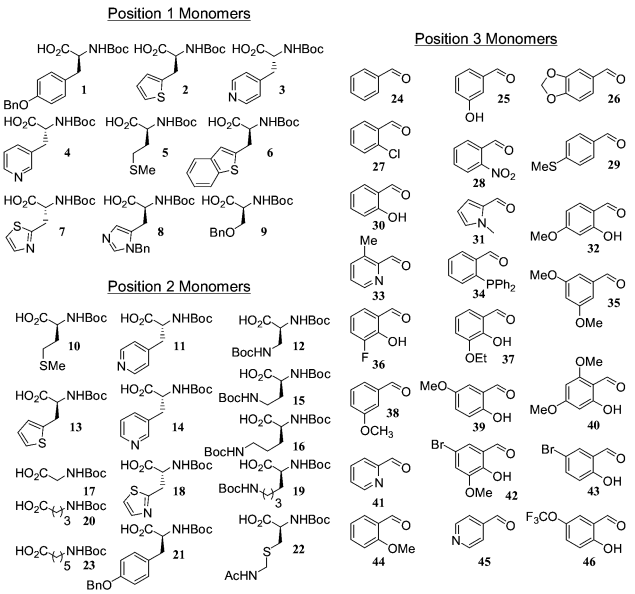


(1)

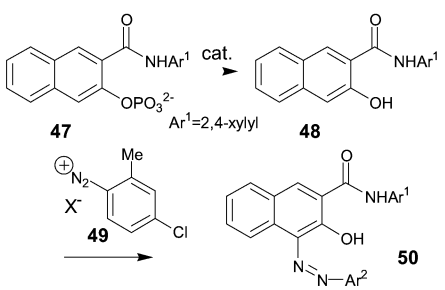
chosen as a model system since it is well-studied and since a number of metal salts are known to catalyze this process.[3] Our initial catalyst library was designed around core structure **A** which is readily available from simple monomeric building blocks. We expected that the Schiff base functionality in **A** would provide a template for metal binding and that pendant amino acid residues would aid in substrate recognition and/or transition state stabilization. Library synthesis was performed on Tentagel beads using HATU[4] coupling and Boc protection strategies. The split/pool library synthesis[5] was carried out using the monomer structures (**1**–**46**) depicted in Scheme 1 and utilized photocleavable tags developed by Still.[6] Notably, both mono- and diaminoacids were used in position 2 such that both mono and bis-Schiff base structures were generated. Including skip codons,[7] the library was composed of 3360 polymer-bound structures.

In order to assay the library constructed above we required a simple method for detection of phosphate hydrolysis. Along these lines, we chose to employ phosphate **47**[8] since it possesses the requisite phosphate group in addition to an adjacent amide which would be available for recognition by the catalyst. Hydrolysis of the water-soluble non-colored phosphate leads to precipitation of the insoluble phenol **48** which can be detected *via* conversion to the colored diazo adduct **50** upon treatment with diazonium salt **49** (Scheme 2).[9]

Prior to assay, the library of ligands was treated with $Gd(NO_3)_3{\cdot}6H_2O$ in THF. Subsequently, a phosphate-buffered solution of **47** was added and the reaction allowed to proceed for 20 min. The beads were washed with phosphate buffer and then treated with **49** to develop color on any active beads. From the library collection, 36 beads were removed and decoded. While a strong consensus did not immediately emerge, two control experiments revealed commonality among a subset of beads. In the first control experiment, a copy of the library was screened as above but in the absence of **47**. This experiment allowed for identification of complexes that turn red simply by their interaction with diazonium salt **49** and not as a result of phosphoester hydrolysis. In the second control experiment, a copy of the library was screened as above but was not precomplexed with $Gd(NO_3)_3{\cdot}6H_2O$. This control allowed for



Scheme 1 Monomers used in the synthesis of library **1**.



Scheme 2 Colorimetric detection of phosphatase activity.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b1/b111036e/

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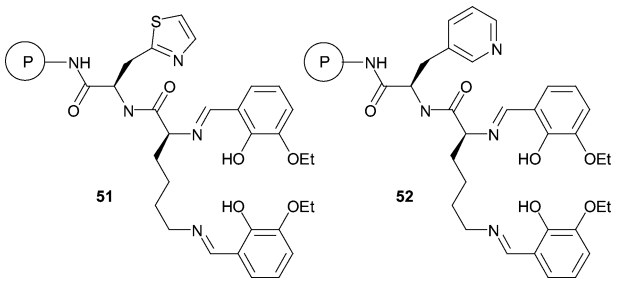
removal of complexes that do not require the metal ion for catalysis. After removal of complexes that show activity in the control experiments, the final set of 11 putative metal-based catalysts that promote phosphoester hydrolysis exhibit a core structure composed of common elements. Notably, an α-amino acid possessing a heterocyclic amine side chain with the aromatic amine group either *ortho* or *meta* relative to side chain attachment (compounds **4**, **7**, and **8**) is predominantly observed in position 1. Of the 14 monomers used in position 2, the diamino acids ornithine, lysine and diamino butyric acid were selected in 8 of 11 beads. Lastly, nine of the eleven selected beads were composed of structures containing 3-ethoxysalicylaldehyde (**37**) in the third position.

Structures **51** and **52**, were selected for further analysis since their subunits were the most frequently selected in the assay. Both structures were prepared on Wang resin and released in order to obtain samples suitable for characterization by mass spectrometry. With the identity of the hit structures confirmed, we next probed the catalytic activity of the two compounds. Due to the qualitative nature of the colorimetric selection assay, quantitative data was desired. This was obtained by HPLC quantitation of the amount of adduct **50** that could be washed off ($CHCl_3$) a collection of 10 mg of polymer synthesis beads containing the structures **51** and **52**. The results showed both **51** and **52** gave modest increases in activity above background (acylated polymer beads), with **51** ten times more active than background and **52** four times. While these results are not dramatic, they do correlate the relationship between the intensity of the red color of a bead and corresponding catalytic activity.



To understand the effect of each of the structural components of the ligand, a series of compounds were prepared and subjected to the colorimetric catalysis assay in the presence of $Gd(NO_3)_3$.[10] The results of this analysis are also shown in Fig. 1. It is readily apparent that catalytic activity is highly dependent on the ligand structure. Removal of either of the salicylaldimine functional groups leads to diminution in catalyst activity (compare **51** with **53**, **55** and **56**). While the presence of the thiazole is not necessary for catalysis (**60**), it certainly appears to be beneficial considering the quantitative comparison of **51** and **52** described earlier. Analysis of compounds **59**, **61**, and **62** indicates that none of the functional groups may act independently in metal-assisted catalytic phosphate hydrolysis of **47**.[11]

In summary, we have developed a new colorimetric assay for the rapid screening of a large library of solid-supported catalysts and used it to discover novel ligands for Gd-catalyzed phosphatase catalysis. The ligand structure appears to require a specific array of functional elements either for effective metal binding or effective catalysis. In principle, the assay described herein should be useful for other reactions that liberate **48** as a reaction product such as ester hydrolysis and elimination reactions. Studies in this regard are in progress.

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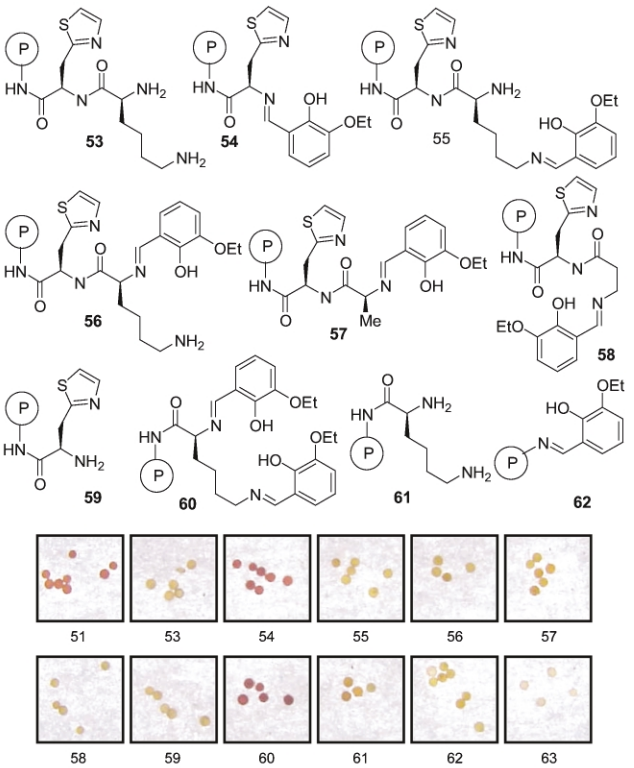

Fig. 1 Activity of structural analogs of ligand **51**.

Notes and references

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11 Compound **60** was present in the library but not selected in the assay. However, not all library beads were selected for decoding and we conclude that **60** was one of them.