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High-Throughput Synthesis and Direct Screening for the Discovery of Novel Hydrolytic Metal Complexes

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Abstract: A 'combinatorial catalysis' strategy was utilized to both rapidly synthesize organometallic complexes and to screen them for catalytic activity in chemical reactions. The application of this strategy has yielded several metal-complexes that catalyze the hydrolysis of carboxylic acid esters efficiently.

The catalytic efficiency of enzymes has stimulated chemists to develop small molecule mimics of these remarkable proteins. Indeed, remarkable rate accelerations have been achieved in hydrolysis reactions of esters[1] and phosphates.[2] To date, many classes of enzyme mimics[3] have been developed, but all fall short of the aim of reproducing enzymatic catalysis.

In recent years the application of combinatorial chemistry principles to catalyst discovery, a process dubbed 'combinatorial catalysis',[4] has yielded a number of catalytic systems with remarkable properties.[5] Novel catalysts for C-H insertion reactions,[6] titanium-catalyzed enantioselective ring opening of epoxides,[7] and phosphate ester hydrolysis[8] have been highlighted utilizing this approach.

Herein we report an extension of the 'combinatorial catalysis' approach towards the elucidation of transition-metal complexes as enzyme mimics for carboxylic acid ester hydrolysis (Scheme 1). In our approach it was vital that the structure of the final compounds be unequivocal, therefore a parallel-synthesis format was applied to catalyst generation. Additionally, we required that the synthetic route to the complexes would allow screening for catalysts without intermediate purification steps. Thirdly, we wanted to expand the selection of ligands used for hydrolysis reactions of carboxylic acid esters by choosing hydroxy azacrown ether **1** as the initial ligand.[9] While its ability to form several transition-metal complexes was reported, it was unknown whether it supplied complexes capable of catalyzing hydrolytic reactions.

The presence of the hydroxyl group in **1** seemed ideal for structural modification experiments to form a family of ethers (Scheme 2). This was accomplished by protection of the amino functionalities of **1** as their Boc-derivatives and subsequent Williamson ether synthesis.[10] Deprotection of the Boc groups of **2a–c** afforded the amines **3a–c** in 34–44 % overall yield from **1**. As model substrates we chose *p*-nitrophenylacetate **5** and *N*-methoxycarbonyl-*L*-phenylalanine-*p*-nitrophenylester **6** (Scheme 1).[11]

Ligands **1** and **3a–c** were then reacted with transition metal salts in methanol to yield the metal complexes **4** (Scheme 2).[12] As discussed vide supra, to avoid the necessary synthesis of these compounds on a preparative scale, an assay was developed that required preparation of low quantities (submicromolar) of metal complexes in situ, followed by rapid screening for the desired activity. Stock solutions of these ligands and of ten different

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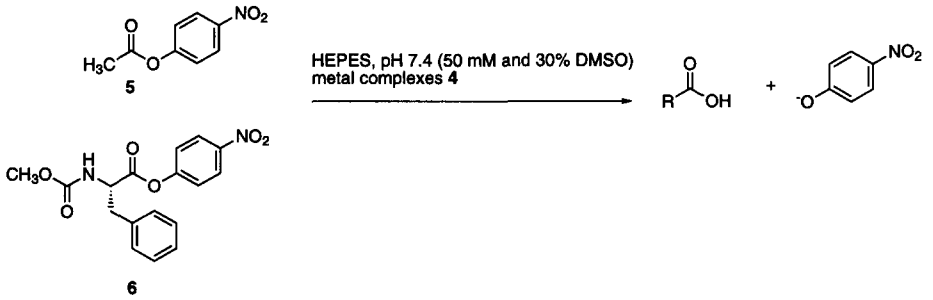
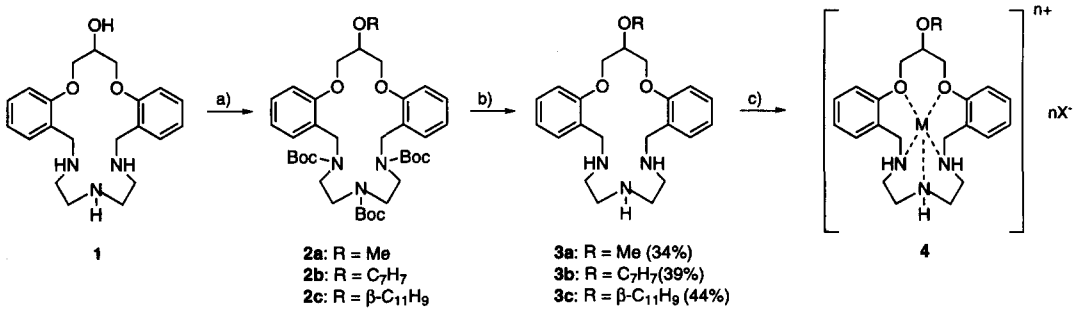


Scheme 1: Hydrolysis reactions of *p*-nitrophenyl ester substrates **5** and **6**.



(a) 1. $(Boc)_2O$, NEt_3, CH_2Cl_2, 0 °C, 12 h; 2. NaH, RX, 20 °C, 1 h. (b) CF_3COOH/CH_2Cl_2, 20 °C, 1 h. (c) MX_n/MeOH, reflux, 1 h.

Scheme 2. Transformation of hydroxy azacrown ether **1** into ethers **2** and **3** and formation of metal complexes **4**.

metal salts (M^{n+} = Ba^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Ni^{2+}, Zn^{2+} as perchlorates and Rh^{3+}, Ru^{3+} as chlorides) in methanol were mixed in a spatially-addressed format in a 96-well microtiter plate to produce 40 different reaction mixtures. The tinfoil wrapped microtiter plate was then heated at 60 °C for 1 h. Following removal of the tinfoil the reactions mixtures were heated until solvent evaporation was complete. The complexes were redissolved in DMSO and then buffer (HEPES, pH 7.4) and substrate (*p*-nitrophenylacetate **5** or *N*-methoxycarbonyl-*L*-phenylalanine-*p*-nitrophenylester **6**) were added to give a final DMSO concentration of 30%.[13] The rates of reactions were determined using the method of initial rates, by repetitive scanning of the ELISA-plate at 405 nm.

In this assay the metal complexes **4a–h**, obtained by reacting the ligands **1** and **3a–c** with zinc and cobalt perchlorate, produced good accelerations ($k_{obs}/k_0 \approx 3$), while the other metal–ligand combinations gave lower results. To evaluate the reliability of the ELISA-assay, compounds **4a–h** and **4i** were synthesized on a 100 mM scale. Exact kinetic data were obtained by repeating the hydrolysis reactions in quartz cuvettes using the same concentrations of metal complex, buffer and substrate (Table 1).

It was found that the initial rates as determined from the 96-well microtiter plate gave an excellent estimation of the exact kinetic data as determined by the larger scale synthesis and assay. Using the Zn^{2+} and Co^{2+} complexes of ligands **3b,c** rate accelerations of 2.4- to 4.7-fold over background were observed. The Zn^{2+} and Co^{2+} complexes of ligands **1** and **3a** gave rate accelerations of 1.2- to 3.0-fold. As expected from the results of the ELISA-plate assay, the nickel complex **4i** gave very little rate acceleration, thereby demonstrating the reliability of

this assay. Kinetic data showed a linear dependence on both substrate and metal complex concentration. While using up to a 20-fold excess of substrate no saturation effects nor turnover could be observed under the reaction conditions used.

Table 1. Rate enhancements k_{obs}/k_0 in the hydrolysis of *p*-nitrophenylesters **5** and **6** using metal complexes **4a–i**.

metal complex **4**	derived from ligand	R	metal salt MX_n	*p*-nitrophenyl acetate **5** k_{obs}/k_0	*N*-methoxycarbonyl-*L*-phenylalanine-*p*-nitrophenylester **6** k_{obs}/k_0
blank	-		-	1	1
4a	**1**	H	$Zn(ClO_4)_2$	1.7	2.9
4b	**3a**	Me	$Zn(ClO_4)_2$	2.4	3.0
4c	**3b**	Benzyl	$Zn(ClO_4)_2$	2.5	3.9
4d	**3c**	β-Naphthyl	$Zn(ClO_4)_2$	2.9	4.7
4e	**1**	H	$Co(ClO_4)_2$	2.1	1.2
4f	**3a**	Me	$Co(ClO_4)_2$	3.0	1.3
4g	**3b**	Benzyl	$Co(ClO_4)_2$	4.5	4.1
4h	**3c**	β-Naphthyl	$Co(ClO_4)_2$	3.4	2.4
4i	**1**	H	$Ni(ClO_4)_2$	1.4	-

k_{obs} is taken from the reaction with 1mM of metal complex **4**. k_0 is taken from the background reaction. For *p*-nitrophenyl acetate: $k_0 = 1.6 \times 10^{-4}$/min; *N*-methoxycarbonyl-*L*-phenylalanine-*p*-nitrophenylester: $k_0 = 1.1 \times 10^{-3}$/min. The rate constants k_{obs} and k_0 were determined from a plot of initial rates *vs.* substrate concentrations (*p*-NPA: c = 0.2; 0.4; 0.8; 1.2; 1.6; 2 mM; *N*-methoxycarbonyl-*L*-phenylalanine-*p*-nitrophenylester: c = 0.2; 0.4; 0.8; 1.2 mM). Assay conditions: Hepes 50mM pH 7.4, c (**4**) = 1mM in DMSO, water/DMSO = 7/3 (v/v). Reactions were run in duplicate at 25 °C for 15 min to determine the initial rates. Results lay within 10% of error.

An X-ray crystal structure analysis of the nickel complex **4i** reveals that the metal is coordinated by 3 oxygen and 3 nitrogen atoms (Figure 1).[14] Hence, all possible coordination sites of the metal center are blocked which may account for the very low rate accelerations obtained with this compound.

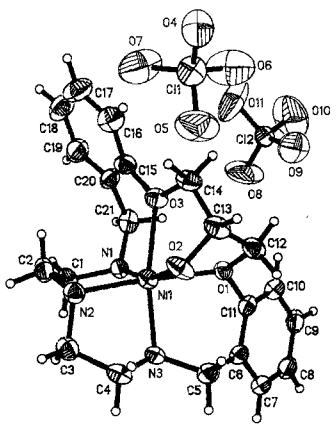


Figure 1. ORTEP plot of metal complex **4i**.

The structural information obtained from the X-ray crystal structure of compound **4i** provides a possible explanation for the increase of the rate accelerations with the size of the R group attached to the oxygen at the secondary carbon. We assume that a larger R group weakens the coordination of the oxygen at the secondary

carbon by steric interactions and thus facilitates coordination of substrate to the metal, which in turn elevates the reaction rates.[15] This effect is currently under further investigation.

In summary, we have developed a high-throughput synthesis and screening strategy for the generation of hydrolytic metal complexes **4** that significantly enhance the rate of hydrolysis of *p*-nitrophenyl esters. The fact that the macrocycle **1** has not been used previously as a ligand in hydrolysis reactions stresses the power of combinatorial methods to rapidly find new catalysts. Our approach leads to the rapid generation of structurally defined compounds that do not require purification before screening for catalysis. We envisage that this method will find application in the high-throughput screening of ligand-metal combinations as potential catalysts for a variety of reactions.

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References:

1. (a) Chin, J.; Jubian, V. *J. Chem. Soc., Chem Commun.* **1989**, 839. (b) Chin, J.; Zou, X. *J. Am. Chem. Soc.* **1984**, *106*, 3687. (c) Chin, J.; Banaszczyk, M. *J. Am. Chem. Soc.* **1989**, *111*, 2724. (d) Wells, M. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 5341. (e) Menger, F. M.; Ladika, M. *J. Am. Chem. Soc.* **1987**, *109*, 3145. (f) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882. (g) Wulff, G.; Gross, T.; Schoenfeld, R. *Angew. Chem. Int. Ed.* **1997**, *36*, 1962.
2. Reviews: (a) Sigman, D. S.; Chen, C. B. *Annu. Rev. Biochem.* **1990**, *59*, 207. (b) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, 2295. (c) Papavassiliou, A. G. *Biochem. J.* **1995**, *305*, 345.
3. Reviews: (a) Kirby, A. J. *Angew. Chem.* **1996**, *108*, 770. (b) Murakami, Y.; Kikuchi, J.-i.; Hisaeda,Y.; Hayashida, O. *Chem. Rev.* **1996**, *96*, 721. (c) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145. (d) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146.
4. (a) Borman, S. *Chem. Eng. News*, **1996**, 37. (b) Weinberg, W. H.; Jandeleit, B.; Self, K.; Turner, H. *Curr. Op. Solid St. Mat. Sci.* **1998**, *3*, 104.
5. (a) Liu, G.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 7712. (b) Gilbertson, S. R.; Wang, X. *Tetrahedron Lett.* **1996**, *37*, 6475. (c) Menger, F. M.; West, C. A.; Ding, J. *J. Chem. Soc., Chem. Commun.* **1997**, 633.
6. Burgess, K.; Lim, H.-J.; Porte, A. M.; Sulikowski, G. A. *Angew. Chem. Int. Ed.* **1996**, *35*, 220.
7. (a) Shimiziu, K. D.; Cole, B. M.; Krueger, C. A.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *Angew. Chem. Int. Ed.* **1997**, *36*, 1704. (b) Cole, B. M.; Shimizu, K. D.; Krueger, C. A.; Harrity, J. P.; Snapper, M. L.; Hoveyda, A. H. *Angew. Chem. Int. Ed.* **1996**, *35*, 1668.
8. Menger, F. M.; Eliseev, A. V.; Migulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666.
9. Zhao, B.; Wu, Y. J.; Tao, J. C.; Yuan, H. Z.; Mao, X. A. *Polyhedron* **1995**, 1197.
10. Larock, R. C. *Comprehensive Organic Transformations*, VCH **1994**.
11. Elmore, D. T.; Smyth, J. J. *Biochem. J.* **1965**, *94*, 563.
12. Electrophilic reagents used for conversion of alcohol **1** to ethers **2**: **2a**: CH_3I; **2b**: C_7H_7Br; **2c**: β-$C_{11}H_9Br$.
13. 20 µL of ligand **1** or **3a-c** (10mM in MeOH) and 20 µL of metal salt (10 mM in MeOH) were mixed in the microtiter plate. After heating at 60 °C for 1h and evaporation of the solvent, metal complexes **4** were redissolved in 40 µL DMSO. 100 µL HEPES buffer (100 mM, pH 7.4) and 40 µL water were added subsequently. Solutions of the substrates in DMSO (*p*-nitrophenyl acetate **5**: 0.2; 0.4; 0.8; 1.2; 1.6; 2 mM; *N*-methoxycarbonyl-*L*-phenylalanine-*p*-nitrophenylester **6**: 0.2; 0.4; 0.8; 1.2 mM) were added. The absorption at 405nm was followed over 30 min.
14. Crystals of compound **4i** were obtained from MeOH. Crystal data: crystal dimensions 0.22x0.14x0.12 mm. The crystal lattice contained 2 equiv. of MeOH and 0.5 equiv. of water yielding the formula $C_{23}H_{36}N_3Cl_2NiO_{13.5}$. M = 691.09, triclinic, space group P1, a = 9.960(2) Å, b = 10.049(2) Å, c = 16.452(2) Å, α = 84.33(3)°, β = 74.51(3)°, γ = 83.77(3)°, V = 1573.3(5) Å^3, Z = 2, *Dc* = 1.459 mg/m^3, *F*(000) = 516.00. Data were collected on a Rigaku AFC6R diffractometer using graphite monochromated Cu-Kα radiation. A total of 2756 reflections (2451 indepedent), [I>2σ(I)], were collected. The final refinement converged to R = 0.0882 and R^2_w = 0.2187.
15. Attempts to obtain crystals suitable for X-ray analysis of any of the metal complexes **4a–h** remained unsuccesful.