

PROGRESS REPORT NO. 7
Annual Report for the Period 6/15/03 – 6/14/04

**Managing Tight Binding Receptors for New Separations
Technologies**

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Recipient: Daryle H. Busch
Chemistry Department
University of Kansas
Lawrence, KS 66045
785-330-4377

Co-Investigator: Richard S. Givens
Chemistry Department
University of Kansas
Lawrence, KS 66045
785-864-3846

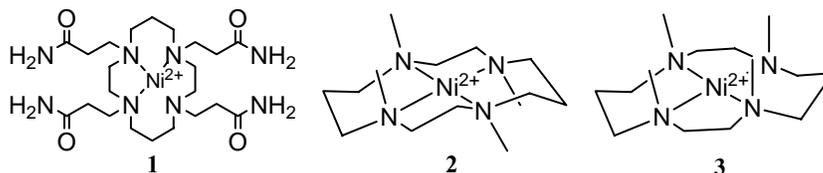
Graduate Students: Program has involved 5 postdoctoral research associates, 2 undergraduate student assistants and no graduate students.

RESEARCH OBJECTIVE: This report summarizes work for the 12 months from 6/15/03 to 6/14/04, within a 42-month funding period, including a 1-year no-cost extension. This project aims at greatly expanding the applications of the strongest ligands in separations technologies through two major goals: (1) The Soil Poultrice—a program to develop a new technology that can make good use of the slow equilibration of the strongest ligands, and (2) Switch-binding and Release—a program to design fast molecular-switch pathways to replace the very slow ligand/metal ion equilibrations.

THE SOIL POULTICE: To harvest iron from the soil, bacteria secrete very powerful ligands called siderophores into the soil. These ligands capture iron and their iron complexes are admitted into the cell by the membrane. The soil Poultrice mimics this behavior to remove contaminating metal ions, using a molecularly imprinted polymer (MIP) instead of the bacteria. The present studies complete the characterization of the affinity properties of MIPs that bind their target molecules through non-covalent interactions.

Our previous discovery of the synergism between two forms of MIPs interaction in *bi-affinity polymers*, e.g., hydrogen bonding and electrostatic attraction, was marred by differences in morphology. The samples of bi-affinity polymers failed to qualify as macroporous. New studies with high crosslinker content (imprint:functional monomer:cross linker (IMC) = 1:4:120) reliably gave macroporous polymers. Re-uptake from acetonitrile was remarkably high (186%), approaching two sites per imprint molecule; lower values were found for water.

For the bi-affinity MIPs, binding isotherms and Scatchard plots revealed the presence of two distinct kinds of binding sites, differing substantially in affinity ($K_{D1} = 0.44 > K_{D2} = 19$). Further, both constants exceed those for the mono-affinity polymers (hydrogen bonding alone) ($K_D = 76$). The weaker binding is attributed to an abundance of sites at which only a single anion has been imprinted along with the hydrogen bonding monomer, while the higher affinity suggests a smaller fraction of sites in which two counter ions are present. These combined results account nicely for the surprisingly high rebinding capacities observed (85 to 186% based on the metal ion).



To learn the role of stereoselectivity in determining the rebinding capacities of MIPs, the two well-known isomers of **1** were used as imprints (structures **2=α** and **3=β**). The very different orientations of the H-bonding amide groups require the imprinted sites to be very different. Prior studies were limited to the α-isomer. Remarkably, all three polymers (α-imprint, β-imprint, no imprint) selected the α isomer over the β by ratios of ~8X, 2X, and 5X respectively. Clearly the inherent affinities of the complexes dominate the binding rather than the ability of the polymer to provide selectivity through imprinting. This explains the success of MIPs in chiral separations since the inherent affinity patterns of enantiomers differ only in the sign of the coordinate system, not in the ability to form hydrogen bonds, etc. In summary, this study showed two kinds of selectivity, that inherent in the detailed structure and that imprinted in the polymer. The former is larger in the cases studied here.

The reuse of these polymers to bind and release the targeted complexes has been expanded to include the use of HCl instead of HNO₃ for the removal of the bound complex. This reuse proceeds with no loss in rebinding capacity, but rather a small irregular increase is observed. The effect of imprint removal by soxhlet extraction is smaller.

SWITCH-BINDING AND RELEASE: Previously, we described the synthesis of a photo-cleavable, switch-release polyether cryptand 5-(2-nitrophenyl)-4,7,13,16,21,24-hexaoxo-1,10-diazabicyclo[8,8,8]hexacosane-2,9-dione (**1**), its ability to capture Ca^{+2} and other ions and their photorelease by UV light through the “switch/release mechanistic” sequence. Additional details have been learned. In a separate “catch and release” protocol, the metal ion participates in the template closure of the N-substituted 7,16-diazacyclo[8.8] macrocycle (**2**) forming the identical cryptate of **1**, but proceeding through the complex of a “lariat macrocycle” as an intermediate. Work on this latter reaction sequence has now been completed.

Synthesis of the Lariat macrocycle. The Lariat macrocycle, 16-{2-[2-ethoxycarbonylmethoxy-1-(2-nitrophenyl)-ethoxy]-acetyl}-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (**2**) was synthesized using the same nitrophenyl ethan-1,2-diol used for **1**. Selective protection of the 1° alcohol was followed by allylation of the 2° alcohol allyl ether in quantitative yield. Removal of the silyl protecting group followed by etherification with ethyl iodoacetate yielded an allyl ester which was converted with ozone at -78°C to an acid-ester introducing dissymmetry to the bridging arm. The acid group was coupled to 1,4,10,13-tetraoxa-7,16-diaza-cyclooctadecane to yield the lariat macrocycle **2**.

Structural features of the cryptand-cryptate system. There are, in principle, eight stereoisomeric cryptands having the structure **1** which derive from the existence of a stereogenic center at the phenyl group attachment and the four conformational orientations of the amide carbonyl. All four diastereoisomeric pairs are ^1H NMR observable and are inseparable by conventional LC and column chromatography methods. Raising the temperature causes the isomers to interconvert more rapidly and at 100°C the signals coalesce. The mixture returns to the original upon cooling.

Effects of metal on the structure and chemistry. When one equivalent of $\text{Ca}(\text{BF}_4)_2$ is stirred in the presence of the cryptand, 95% of the Ca^{+2} is incorporated rapidly into the cryptand and the mixture of isomeric cryptands collapses to a single structure (^1H NMR). That conformer has both carbonyls oriented away or “out” relative to the metal ion.

Capture of Ca^{+2} by the N-substituted 7,16-diazacyclo[8.8] macrocycle **2** (the Lariat macrocycle) was also examined. When **2** was dissolved in D_2O containing two equivalents of Ca^{2+} the product cryptate appear slowly requiring 7 days for complete conversion (^1H -NMR, ^{13}C -NMR and mass spectral analysis). In the absence of Ca^{2+} cyclization was extremely sluggish, taking months to form any measurable quantities of the cryptate.

Photochemistry. Photolysis of the Ca^{+2} cryptates in water results in only 40% release of the Ca^{+2} based on the disappearance of the cryptate. The remaining 60% of Ca^{+2} is bound to the opened macrocyclic crown ether. This finding presents a challenge to the concept of the catch and release strategy since the complete release of ion is the goal of this research.

The quantum efficiencies for the disappearance of the cryptate and cryptand are solvent dependent (disappearance of cryptate: MeOH, 0.18; 0.099 in H_2O) whereas the quantum efficiency for appearance of the opened macrocycle in MeOH is 0.060. On the other hand, the efficiencies were the same for both the cryptate and cryptand under otherwise identical conditions. Thus, the metal ion does not appear to influence the efficiency of ring opening.

INFORMATION ACCESS: M.M. Hassan, C. Zhang, J-I. Lee, K.M. Bushan, A. McCasland, R.S. Givens, D.H. Busch, “Dynamics of Switch-binding by a linear ligand that transforms to a macrocycle upon chelation: synthesis, kinetics and equilibria,” ACS, Symposium Volume, Submitted 2003; J-I Lee, K.M. Bushan, C. Zhang, D.H. Busch*, and R.S. Givens*, “A novel separations methodology using tight-binding photolabile cryptands.” *in preparation.*