examined, the monoclonal antibody CPD32A11 showed the highest initial velocity using **1** as a substrate, could be saturated with **1**, and was inhibited by **3** (K_i = 10 nM). The kinetic constants are shown in Table I. The k_{cat}/k_{uncat} of 10^5 might be representative of contributions to catalysis by enzymatic decarboxylases solely as a result of the microenvironment of the active site. The reduced k_{cat} for **7** suggests that an apolar surrounding is more localized in the desired area.

A measure of the hydrophobicity of the active site was garnered using a Hansch correlation analysis (Figure 1). The coefficient of 1.8 suggests that the region occupied by a 4-substituent can partition organic solutes more effectively than the solvent octanol.[8] While direct evidence is sparse, a recent crystal structure of a histidine decarboxylase–substrate analog complex situates the carboxyl group in a crevasse lined with apolar residues.[9] Clearly, such a medium could support destabilization as a component of the catalytic mechanism.[10] The association of antibody and hapten-like molecules is facilitated by classical hydrophobic effects.[11] On the other hand, it requires energy, reflected in the high K_m, to introduce a charged group into a hydrophobic pocket. The antibody operates by binding the pyridinium moiety through noncovalent interactions to position the carboxylate and therein promote the loss of carbon dioxide. Although PLP enzymes engage a covalent imine linkage, the noncovalent contributions to the stability of the enzyme–cofactor complex are 20–40 times greater.[12] However, the tight binding of PLP results from the summation of several substituent interactions not available in **1**.

A 2-methyl group in **6** did not yield a more specific (k_{cat}/K_m) substrate, as might be expected from the anchor principle.[13] It was anticipated that the binding energy could be utilized to improve substrate turnover. There has been speculation that hydrophobic binding of this group in PLP provides fine-tuning of catalysis for individual enzymes.[14] In enzymes, the optimization of cofactor binding and reactivity arises through evolution. However, in this model, favorable interactions adopted by the methyl group could alter the proper ionic contact necessary for reaction since it was not programmed in the hapten design of **2**, although occurrence of an antibody which utilizes **6** as its most efficient substrate is also possible.[15] Interestingly, the presence of an α-methyl group in substrate **4** lowers K_m as anticipated, but also reduces k_{cat}. The substituent could cause a decrease in rotational entropy of the carboxyl about the C_α–C_4 bond which prevents the optimum stereoelectronic orientation for decarboxylation.[16] A second methyl, as in **5**, shows a further reduction in K_m but not k_{cat} and indicates that the lowered k_{cat} of **4** is not a result of the chiral center. This nascent catalyst cannot foster the demanding spatial relationships which must exist between the amino acid and cofactor functionalities united in the pyridylacetic acid structure.

The simple model described provides a foundation for more complex designs. Most importantly, this investigation again demonstrates that catalytic antibodies can be useful tools for exploring the nature of biological catalysis.

Acknowledgment. This research was supported in part by a grant from the National Institutes of Health (GM-43858, K.D.J.).

(8) (a) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979. (b) Fastrez, J.; Fersht, A. R. *Biochemistry* **1973**, *12*, 1067–1074. (c) Dorovska, V. N.; Varfolomeyev, S. D.; Kazanskaya, N. F.; Klyosov, A. A.; Martinek, K. *FEBS Lett.* **1972**, *23*, 122–124. (d) Hansch, C.; Coats, E. *J. Pharm. Sci.* **1970**, *59*, 731–743.

(9) Gallagher, T.; Snell, E. E.; Hackert, M. L. *J. Biol. Chem.* **1989**, *264*, 12737–12743.

(10) Sequence analysis of the complementarity-determining regions of CPD32A11 did not reveal a skewed proportion of apolar or other amino acids compared to a variety of antibodies of differing specificity. The elicitation of antibodies from purely aliphatic structures has not been well studied but would not seem to generate or require an unusual immune response. The important factor might be not the bulk dielectric constant of the binding site but the position of specific amino acids/side chains which afford a contact surface with hapten/substrate. (See: Mian, I. S.; Bradwell, A. R.; Olson, A. J. *J. Mol. Biol.* **1991**, *217*, 133–151.) This could account, in part, for the differences in catalytic activity among the CPD monoclonal antibodies.

(11) (a) Smithrud, D. B.; Diederich, F. *J. Am. Chem. Soc.* **1990**, *112*, 339–343. (b) Ben-Naim, A. *Hydrophobic Interactions*, 2nd ed.; Plenum: New York, 1983. (c) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980.

(12) Schonbeck, N. D.; Skalski, M.; Shafer, J. A. *J. Biol. Chem.* **1975**, *250*, 5359–5363.

(13) (a) Page, M. I. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 449–459. (b) Jencks, W. P.; Page, M. I. *Proceedings of the Eighth FEBS Meeting*, Amsterdam, 1972; 29, 45–58.

(14) (a) Mechanik, M. L.; Torchinsky, Yu. M.; Florentiev, V. L.; Karpeisky, M. Ya. *FEBS Lett.* **1971**, *13*, 177–180. (b) Bocharov, A. L.; Ivanov, V. I.; Karpiesky, M. Ya.; Mamaeva, O. K.; Florentiev, V. L. *Biochem. Biophys. Res. Commun.* **1968**, *30*, 459–464. (c) Morino, Y.; Snell, E. E. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 1692–1699.

(15) Wirsching, P.; Ashley, J. A.; Benkovic, S. J.; Janda, K. D.; Lerner, R. A. *Science* **1991**, *252*, 680–685.

(16) (a) Dunathan, H. C. *Adv. Enzymol.* **1971**, *35*, 79–134. (b) Dunathan, H. C. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 712–716.

Asymmetric Substitution: Highly Enantioselective Substitutions Induced at the Carbanion of a Racemic Organolithium Substrate by (−)-Sparteine

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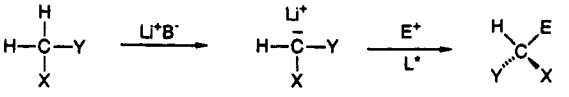
An inviting concept in asymmetric synthesis is the creation of an asymmetric carbon at an initially racemic nucleophilic carbanion under the influence of an external enantioenriched ligand upon electrophilic substitution.[1] In the sequence shown below for replacement of a prochiral proton via an organolithium intermediate, the second step is such an asymmetric substitution.

Asymmetric Substitution



racemic

Li^+B^- = organolithium base
L* = enantioenriched ligand
E^+ = electrophile

To the best of our knowledge, this kind of reaction has been observed with organolithium substrates only for a few electrophile dependent reactions of lithium enolates or with stereocontrol achieved by selective crystallization of an enantioenriched ligand–allyllithium reagent.[2,3] We now report experiments which

(1) Focus on the creation of the asymmetric center at the nucleophilic carbon is in contrast to most previous work using external enantioenriched ligands in which the new asymmetric center is created in the electrophile. For reviews and discussion, see: (a) Cox, P. J.; Simpkins, N. S. *Tetrahedron: Asymmetry* **1991**, *2*, 1. (b) Tomioka, K. *Synthesis* **1990**, 541. (c) Noyori, R.; Kitamura, M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 49.

(2) For related cases, see: Regan, A. C.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1983**, 764. Hogeveen, H.; Menge, W. M. P. B. *Tetrahedron Lett.* **1986**, 2767. Ando, A.; Shioiri, T. *J. Chem. Soc., Chem. Commun.* **1987**, 1620. Regen, A. C.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1987**, 521. Muraoka, M.; Kawasaki, H.; Koga, K. *Tetrahedron Lett.* **1988**, 337. Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1624. Tomioka, K.; Shindo, M.; Koga, K. *Chem. Pharm. Bull.* **1989**, 1120. Murakata, M.; Nakajima, M.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1990**, 1657.

(3) Hoppe has reported that reactions of racemic allyllithium complexes in the presence of (−)-sparteine in solution do not give high enantioselectivities, and he has developed a procedure for selective crystallization of allyl organolithium/(−)-sparteine complexes which under heterogeneous conditions can be converted to allyl titanates that give high enantioselectivities. Zachage, O.; Hoppe, D. *Tetrahedron* **1992**, *48*, 5657 and references cited therein.

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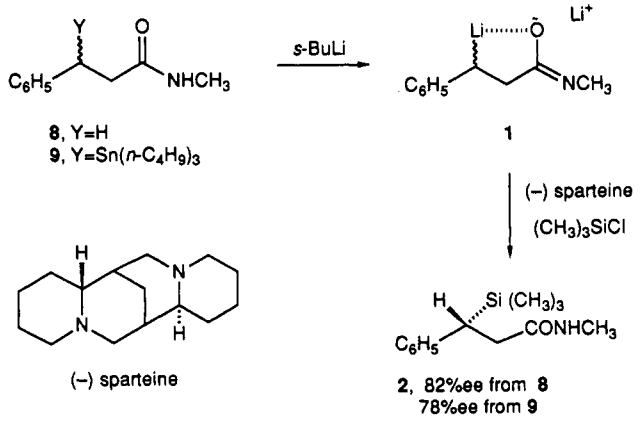
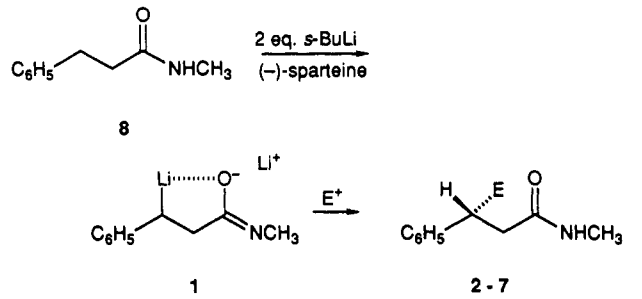
show that the processes of direct asymmetric substitution can be observed for the reactions of racemic *N*-lithio-*N*-methyl-3-lithio-3-phenylpropionamide (**1**) with a number of electrophiles in the presence of (−)-sparteine to give the *N*-methyl-3-phenyl-3-substituted-propionamides **2**–**7** in useful yields with high enantioselectivities. We also note that this class of asymmetric substitutions, which is prospectively applicable to a wide variety of organometallic substrates, is fundamentally different in sequence and mechanism from the asymmetric deprotonations which have been recently reported.[4]

In order to establish the pathway of asymmetric substitution to be operative, racemic **1** was generated in separate experiments by treatment of **8** and of racemic **9** with *sec*-BuLi.[5] Addition of (−)-sparteine to dianion **1** followed by chlorotrimethylsilane provided **2** with enantioselectivities of 82% and 78% in yields of 72% and 48%, respectively. The absolute configuration of **2** was assigned by preparation of the corresponding phenyldimethylsilyl compound and its oxidative conversion to the alcohol of known configuration.[6] We established that *sec*-BuLi had reacted prior to addition of (−)-sparteine by addition of benzaldehyde after exposure of **8** to the conditions of the initial lithiation and noting the lack of the alcohol expected from addition of *sec*-BuLi to benzaldehyde from that experiment. These results show that asymmetric substitution of the racemic organolithium reagent **1** can be highly enantioselective.[7-9]



For synthetic purposes the deprotonation substitution sequence was carried out by treatment of *N*-methyl-3-phenylpropionamide (**8**) with 2–3 equiv of *sec*-BuLi/(−)-sparteine at −78 °C in 1:1 THF *tert*-butyl methyl ether followed by addition of the electrophile to provide the 3-substituted products **2**–**7** in yields ranging from 63% to 86% and with enantiomeric excesses ranging from 65% to 94% as shown. The absolute configurations for **3**–**5** were assigned as *R* by mild hydrolysis of the *N*-Boc derivatives to β-phenylalkanoic acids of known configuration.[10,11] These syntheses illustrate the synthetic convenience of this approach.[6,10]



2, E = $Si(CH_3)_3$	(86% yield;	94% ee)
3, E = CH_3	(84% yield;	78% ee)
4, E = n-(C_4H_9)	(77% yield;	88% ee)
5, E = $CH_2C_6H_5$	(78% yield;	80% ee)
6, E = $HOC(C_6H_5)_2$	(84% yield;	84% ee)
7, E = $HOCHC_6H_5$	(63% yield;	65% ee)

The high enantioselectivities achieved at the nucleophilic carbanion of a racemic organolithium by the presence of the enantioenriched ligand during electrophilic substitution suggest that this class of reactions should be explored for a variety of racemic organometallics and enantioenriched ligands. The directness and simplicity of this approach for asymmetric carbon–carbon bond formation contrasts with the multiple steps required by strategies employing covalently attached ligands.[6,10] The selectivity induced by the external ligand could lie in the energy difference between diastereomeric transition states involving organolithium substrates which are configurationally rapidly equilibrating or in the preferential formation of a diastereomeric complex which undergoes electrophilic substitution faster than epimerization. Work is underway to understand and develop the possibilities.

Acknowledgment. We are grateful to the National Institutes of Health and National Science Foundation for support of this work and to Professor E. Jacobsen for valuable discussion.

Supplementary Material Available: Experimental procedures for the reactions of **8** and **9** and the syntheses of **2**–**7** (15 pages). This supplementary material is contained in many libraries on

(4) Asymmetric deprotonation in which a ligand-induced diastereoselective deprotonation gives a configuratively stable organolithium substrate that undergoes a stereoselective electrophilic substitution to a highly enantioenriched product has been demonstrated for oxygen and nitrogen dipole-stabilized carbanions. (a) Hoppe, D.; Hintze, F.; Tebben, P. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1622 and references cited therein for Hoppe's seminal report. (b) Kerrick, S. T.; Beak, P. *J. Am. Chem. Soc.* **1991**, *113*, 9708.

(5) We have reported the diastereoselectivity of the β lithiation and substitution of *N,N*-diisopropyl-2-methyl-3-phenylpropionamide with *sec*-BuLi as an example of the complex induced proximity effect. Lutz, G. P.; Wallin, A. P.; Kerrick, S. T.; Beak, P. *J. Org. Chem.* **1991**, *56*, 4938 and references cited therein. We also have found that the lithiation of a secondary amide, *N*-isopropyl-3-phenylpropionamide, is directed to the β position. Lutz, G. P.; Beak, P. Unpublished results.

(6) The phenyldimethylsilyl compound which is considered to have the same configuration as **2** was oxidized to the alcohol: Oppolzer, W.; Mills, R. J.; Pachinger, W.; Stevenson, T. *Helv. Chim. Acta* **1986**, *69*, 1542.

(7) In contrast, treatment of racemic *N*-Boc-2-lithiopyrrolidine with (−)-sparteine followed by trimethylsilyl chloride was found to give racemic *N*-Boc-2-(trimethylsilyl)pyrrolidine.[4b] Since the same product was obtained with high ee when *sec*-BuLi/(−)-sparteine was the base, the reaction of *N*-Boc pyrrolidine proceeds by the pathway of asymmetric deprotonation.[4]

(8) Lithiations using organolithium bases/(−)-sparteine to induce enantioselectivities at carbanionic benzylic positions have been reported: Nozaki, H.; Aratani, T.; Torayta, T.; Noyori, R. *Tetrahedron* **1971**, *27*, 905. Papasergio, R. I.; Skelton, B. W.; Twiss, P.; White, A. H.; Raston, C. L. *J. Chem. Soc., Dalton Trans.* **1990**, 1161 and reference cited therein. For Grignard/(−)-sparteine asymmetric reactions, see: Okamoto, Y.; Suzuki, K.; Kitayama, T.; Yuki, H.; Kageyama, H.; Miki, K.; Tanaka, N.; Kasai, N. *J. Am. Chem. Soc.* **1982**, *104*, 4618. Hoppe has reported the formation of a benzylic configurationally stable enantioenriched oxygen dipole stabilized carbanion which undergoes stereochemically capricious substitution. Although it would be reasonable to assign an *R* configuration to a reactive (−)-sparteine–**1** complex in the present work, the results of Hoppe suggest that assignment should be provisional. Hoppe, D.; Carstens, A.; Krämer, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1424.

(9) We also have found that when enantioenriched **9** (60% ee) was treated with *sec*-BuLi or *sec*-BuLi/TMEDA followed by reaction with chlorotrimethylsilane, **2** is obtained with enantiomeric excesses of 60% and 26%, respectively. Hence configurational stability is possible at the benzylic position of **1**, and more work will be required to determine the extent to which each pathway contributes to the reaction when (−)-sparteine is initially present. However, the possibility of dual pathways, which may be indicated by the difference in ee in **2** in the above experiments, does not compromise the demonstration of asymmetric substitution of racemic **1**.

(10) For asymmetric syntheses of β-phenylalkanoic acid derivatives **3** and **4**, see: Mukaiayama, T.; Iwasawa, N. *Chem. Lett.* **1981**, 913. For **5**: Touet, J.; Baudouin, S.; Brown, E. *Tetrahedron: Asymmetry* **1992**, *3*, 587. For **7**, hydrogenolysis provided correlation with **5**. For other approaches, see: Meyers, A. I.; Smith, R. K.; Whitten, C. E. *J. Org. Chem.* **1979**, *44*, 2250. Fang, C.; Ogawa, T.; Suemune, H.; Sakai, K. *Tetrahedron: Asymmetry* **1991**, *2*, 389 and references cited therein.

(11) Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424.

microfiche, immediately follows this paper in the microfilm edition of the journal, and can be ordered from the American Chemical Society. Ordering information is given on any current masthead page.

Selective Recognition of Bis-Imidazoles by Complementary Bis-Metal Ion Complexes[1]

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Metal ion complexes that bind protein surfaces at exposed coordinating ligands can be exploited in selective protein recognition. For example, immobilized metal-affinity chromatography (IMAC), a technique used extensively for protein purification,[2] discriminates proteins based on the nature and multiplicity of surface-exposed ligands, usually the imidazole moiety of histidine. To design complexes capable of selectively recognizing an individual protein or other target molecule, the spatial distribution of metal ions can be matched to the distribution of coordinating ligands on the target molecule. A similar proposal, when used as the basis for template polymerization in the presence of the target molecule, yielded solid, Cu^{2+}-containing polymers that could discriminate bis-imidazole "protein analogs" so similar that they could not be separated by reverse-phase HPLC.[3] The "rationally designed" model system reported here demonstrates that receptor complexes containing as few as two properly-positioned metal ions can selectively recognize target molecules with a complementary spatial distribution of metal-coordinating ligands.[4] Complexes such as these may have applications as receptors for biological molecules that are characterized by unique patterns of surface coordinating ligands.

Bis-imidazoles **1** and **2**, the target molecules used in previous template polymerization studies,[5] were also used for this investigation. The distances between the two N-3 atoms of the imidazole rings that are optimal for simultaneously coordinating the two metal ions of a bis-metal ion receptor were found to be 7.2–8.3 Å for **1** and 11.5–12.5 Å for **2** by computer modeling. 1-Benzylimidazole (**3**) was used as a control in the binding experiments. Two bis-mercury complexes **4** and **5** were designed to recognize bis-imidazole **2** in preference to **1**; the optimum distance between the two metal centers is 13 Å for **4** and 11 Å for **5**. The synthesis of the receptor **4** is outlined in Scheme I. Receptor **5** was prepared following the literature procedure.[6,7]

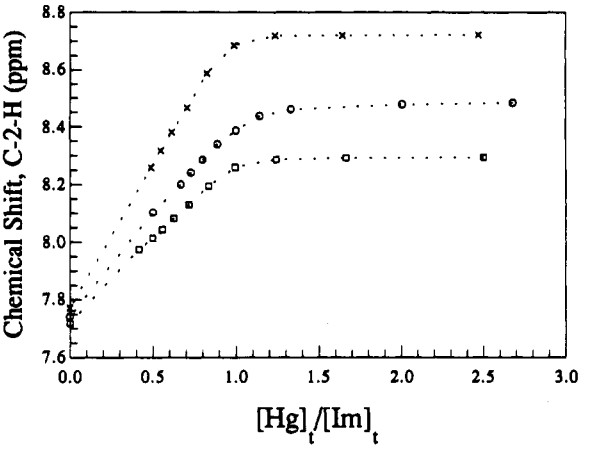
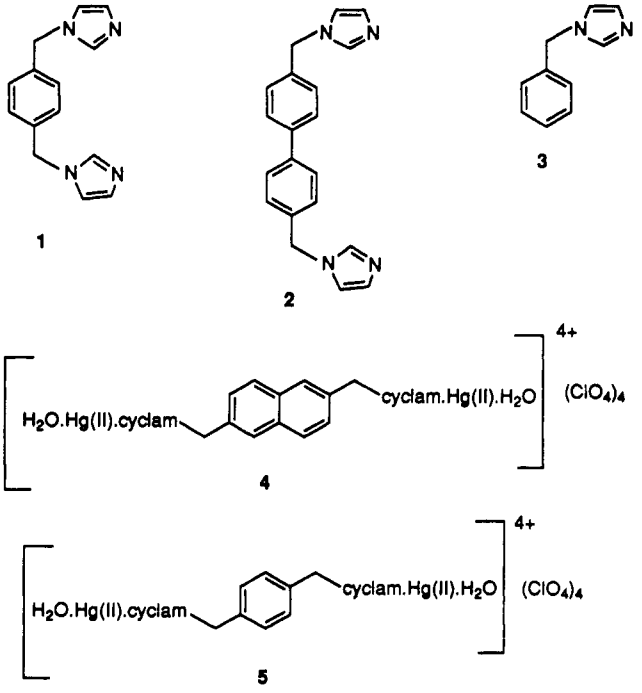


Figure 1. Imidazole C-2-H chemical shifts for the titration of bis-metal ion complex **4** with imidazole derivatives **1** (□), **2** (×), and **3** (○). Solutions of **4** in DMSO-d_6 were titrated with increasing amounts of **1** (initial concentration of **4** = 16.9 mM), **2** (initial concentration of **4** = 16.7 mM), and **3** (initial concentration of **4** = 16.4 mM).



Titrations of receptor complexes **4** and **5** (~15 mM in DMSO-d_6) with target imidazoles **1**, **2**, and **3** were monitored by ^{1}H-NMR spectroscopy. In the absence of the bis-mercury receptor, the C-2-H and C-4-H resonances of **2** appear only 0.02 ppm downfield of the C-2-H and C-4-H resonances of **3**. These resonances are strongly affected by interaction with the metal complexes (see Figures 1 and 2). In the presence of stoichiometric amounts of bis-mercury complex **4**, the C-2-H of **2** is shifted downfield by 0.24 ppm with respect to the C-2-H of **3** (Figure 1); in contrast, the C-4-H of **2** is shifted upfield by 0.1 ppm with respect to the C-4-H of **3** (Figure 2). These chemical shifts are consistent with the positioning of the aromatic rings of **2** and **4** one on top of the other in a cyclic complex which places the C-2-H of **2** in the deshielding zone and the C-4-H in the shielding zone for the naphthalene ring current of **4**. That the phenyl ring protons of **2** are also shifted upfield by about 0.8 ppm at metal:imidazole

* To whom correspondence should be addressed; phone 818-356-4162; FAX 818-568-8743.

(1) Dedicated to Sri B. R. Mitra on his 75th birthday.

(2) For recent examples see: Zhang, Z.; Tong, K.-T.; Belew, M.; Pettersson, T.; Janson, J.-C. *J. Chromatogr.* **1992**, *604*, 143–155. Skerra, A.; Pfitzinger, I.; Pluckthun, A. *Bio/Technology* **1991**, *9*, 273–278. Arnold, F. H. *Bio/Technology* **1991**, *9*, 151–156.

(3) Dhal, P. K.; Arnold, F. H. *J. Am. Chem. Soc.* **1991**, *113*, 7417–7418.

(4) To our knowledge, molecular recognition that relies on multiple metal–ligand coordination has not been demonstrated before. For recent examples of molecular recognition using multiple hydrogen bonding or ion-pair interactions, see: Slobodkin, G.; Fan, E.; Hamilton, A. D. *New J. Chem.* **1992**, *16*, 643–645. Jeong, K. S.; Tjikikua, T.; Meuhldorf, A.; Deslongchamps, G.; Famulok, M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 201–209. Deslongchamps, G.; Galan, A.; Mendoza, J.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 61–63. Voyer, N.; Deschenes, D.; Bernier, J.; Roby, J. *J. Chem. Soc., Chem. Commun.* **1992**, 134–136. Kikuchi, Y.; Kato, Y.; Tanaka, Y.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1991**, *113*, 1349–1354. Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1992**, *114*, 4128–4137. Li, G.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 5929–5932. Kikuchi, Y.; Kabayashi, K.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 1351–1358. Hunter, C. A.; Purvis, D. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 729–795. Aoyama, Y.; Asakawa, M.; Matsui, Y.; Agashi, H. *J. Am. Chem. Soc.* **1991**, *113*, 6233–6240. Maruyama, K.; Sohmiya, H.; Tsukube, H. *Tetrahedron* **1992**, *48*, 805–818. Reetz, M. T.; Niemeyer, C. N.; Hermes, M.; Goddard, R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1017–1019.

(5) Dhal, P. K.; Arnold, F. H. *Macromolecules* **1992**, *25*, 7051–7059.

(6) Ciampolini, M.; Fabbirizzi, L.; Perotti, A.; Poggi, A.; Seghi, B.; Zanobini, F. *Inorg. Chem.* **1987**, *26*, 3527–3533. Alcock, N. W.; Curson, E. H.; Herron, N.; Moore, P. *J. Chem. Soc., Dalton Trans.* **1979**, 1987–1993.

(7) All new compounds were characterized by ^{1}H- and ^{13}C-NMR spectra and satisfactory mass spectral or elemental analyses.

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