Host–Guest Systems

DOI: 10.1002/ange.200503343

Pseudo-Allosteric Recognition of Mandelic Acid with an Enantioselective Coordination Complex**

*Jungseok Heo and Chad A. Mirkin**

Allosteric regulation is a powerful tool utilized efficiently and elegantly in biological systems to control substrate binding and catalysis.[1] Recently, major advances have been made in the design of abiotic supramolecular structures that exhibit allosteric or pseudo-allosteric behavior analogous to their biological counterparts. Sensors capable of signal amplification[2] and asymmetric catalysts with activities and enantioselectivities that can be controlled with allosteric regulators have been designed.[2c,d] Thus far, macrocyclic recognition induced by an allosteric regulator has not been demonstrated. This capability, however, would be a first step towards a system with recognition properties that could be selectively turned on or off with small-molecule coordination chemistry at an allosteric regulatory site.

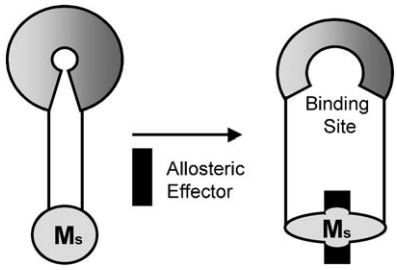
We have developed the weak-link approach (WLA) to supramolecular coordination chemistry.[3–6] This approach utilizes condensed intermediates templated by hemilabile ligands that strategically form both strong and weak coordination bonds with metal centers. Such structures can be selectively and often reversibly opened into flexible macrocycles by reaction with small molecules that break the weak coordination bonds. This ability to use small molecules to interconvert rigid, condensed structures with flexible open macrocycles is ideal for the creation of allosterically regulated systems. A significant advance would be the development of allosteric coordination complexes with chiral recognition elements that could be turned on and off. Such a capability would open avenues for the development of receptors for chemical sensors and separation materials that could selectively recognize, transport, and chemically release target chiral agents. Herein, we report the use of the WLA to synthesize a four-coordinate Cu^{I} complex with chiral recognition for mandelic acid that can be turned on by the chelation of 2,2′-bipyridine (bpy) to the Cu^{I} center in a reaction that concomitantly breaks weak Cu–S links and opens the condensed structure into a 27-membered macrocycle.

[*] Dr. J. Heo, Prof. C. A. Mirkin
Department of Chemistry
and the International Institute for Nanotechnology
2145 Sheridan Road, Evanston, IL, 60208-3113 (USA)
Fax: (+1) 847-467-5123
E-mail: chadnano@northwestern.edu

[**] We are very grateful for support of this work from the NSF and KOSEF and a NIH Director's Pioneer Award for C.A.M.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

The binaphthyl metallocyclophanes (*S*)-**3** and (*S*)-**4** have been designed for the allosteric recognition of chiral α-hydroxy carboxylic acids (Scheme 1). Compound (*S*)-**4** has two different binding sites, whereas (*S*)-**3** only has one



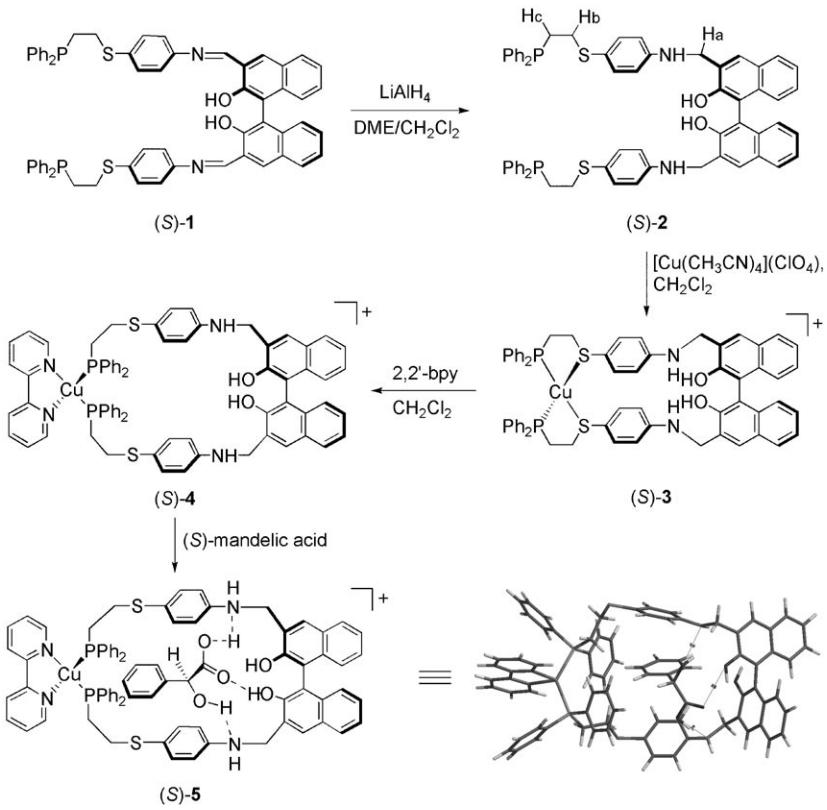
Scheme 1. The allosteric-effector-mediated shape change of a macrocycle. The structure on the right is capable of the recognition and binding of a target that fits within the cavity. M_s = structural transition-metal center.

because of its condensed nature. In the case of (*S*)-**4**, one binding site is a $Cu(P,S)_2$ site that allows for allosteric regulation by reaction with 2,2′-bpy. The other is a hydrogen-bonding site for chiral guest molecules, such as mandelic acid derivatives. Therefore, as (*S*)-**3** is converted into (*S*)-**4** by reaction between 2,2′-bpy and the allosteric Cu^{I} regulatory site, a chiral recognition pocket is generated. This structure was designed based upon its resemblance to a bis(binaphthyl) macrocycle, an organic structure known to selectively recognize enantiomers of mandelic acid.[10d]

Enantiomerically pure (*S*)-binol-3,3′-dicarbaldehyde (binol = binaphthol) and 4-(2-diphenylphosphanylethylthio)-phenylamine were coupled by imine condensation (95%) to give (*S*)-**1**, which was reduced with $LiAlH_4$ to give the new amine compound (*S*)-**2** in moderate yield (63%). One equivalent of ligand (*S*)-**2** was treated with one equivalent of $[Cu(CH_3CN)_4](ClO_4)$ in CH_2Cl_2 to form the condensed intermediate (*S*)-**3** in quantitative yield. Compound (*S*)-**3** reacts with 2,2′-bpy (1 equiv) in CH_2Cl_2 to yield (*S*)-**4** cleanly (Scheme 2).

The formation of (*S*)-**4** was evidenced by an upfield shift of the ^1H NMR resonances for the methylene groups of the chelating arms of the tetradentate ligand -SCH_2CH_2PPh_2 (Figure 1). These resonances shift from $\delta = 3.00$ and 2.53 ppm to $\delta = 1.86$ and 1.83 ppm, respectively. The binol-CH_2NH_2- resonances exhibit peak splitting[2e,f] that is highly diagnostic of a chiral macrocycle (from a singlet at $\delta =$ 4.46 ppm to two doublets at $\delta = 4.80$ and 4.43 ppm). The $^{31}P\{^1H\}$ NMR spectroscopy of (*S*)-**4** is also consistent with that of a symmetric $[CuP_2(phenanthroline)]$ complex with a singlet at $\delta = -6.4$ ppm.[6b] All other data including ESI mass-spectrometric and elemental analyses are consistent with the proposed structures.

The CD spectrum of (*S*)-**2** exhibits a characteristic positive couplet, which is similar to that of (*S*)-binol, because of the exciton coupling between the long-axis polarized ^1B_b transition, with a positive maximum at 237 nm and a negative minimum at 226 nm (Figure 2). A further negative band at 205 nm was observed and corresponds to a higher energy ^1B_a



Scheme 2. Synthesis of chiral macrocycles (*S*)-**3** and (*S*)-**4**. Proposed structure for mandelic acid complexed macrocycle (*S*)-**5**.[13]

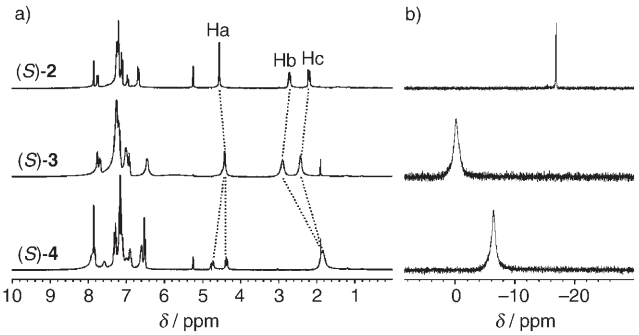


Figure 1. a) ^{1}H NMR spectra of (*S*)-**2**, (*S*)-**3**, and (*S*)-**4**; b) corresponding ^{31}P{^{1}H} NMR spectra.

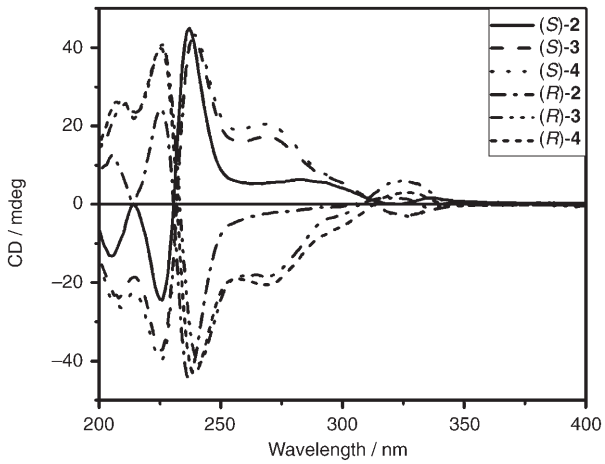


Figure 2. Circular dichroism (CD) spectra of **2**–**4** in acetonitrile.

transition.[11] Compounds (*S*)-**3** and (*S*)-**4** displayed additional positive maxima at 270 nm which can be attributed to a chiral arrangement of the PPh_2 group at the Cu^{I} center. The CD spectra of (*S*)-**3** and (*S*)-**4** are very similar, thus suggesting that there is very little change in the chirality and dihedral angle of the binol center.

To study the allosteric effect, closed complex (*S*)-**3** and open complex (*S*)-**4** were independently treated with both enantiomers of mandelic acid and monitored by fluorometric analysis.[10] First, (*S*)-**3** (1.0×10^{-4} M) was treated with a 50-fold excess of (*S*)- or (*R*)-mandelic acid (5.0×10^{-3} M). In both cases, no significant changes in the fluorescence intensities were observed (Figure 3a), which is because the binding pocket is closed and the multiple hydrogen-bonding sites required to complex mandelic acid are not accessible.

In contrast, open complex (*S*)-**4** can accommodate mandelic acid, and the fluorescence intensity of (*S*)-**4** increases in the presence of (*S*)- or (*R*)-mandelic acid. This increase in fluorescence is due to the suppressed photoinduced electron-transfer fluorescence quenching as the amine nitrogen atom of (*S*)-**4** is protonated by the acid (Figure 3b).[7–9] In solution with CH_2Cl_2 (containing 2% of 1,2-dimethoxyethane (DME)), the fluorescence intensity of (*S*)-**4** (1.0×10^{-4} M) was increased 3.1-fold upon treatment of (*S*)-mandelic acid (5.0×10^{-3} M), but only 1.9-fold for (*R*)-mandelic acid (5.0×10^{-3} M). The net fluorescence intensity increase of (*S*)-**4** by (*S*)-mandelic acid was 2.33 times that by (*R*)-mandelic acid.[14] When (*S*)-**4** (1.0×10^{-4} M) was treated with mandelic acid in the concentration range of 5.0×10^{-3} to 2.0×10^{-2} M, the fluorescence enhancement of the open complex exhibits a Benesi–Hildebrand-type relationship.[12] Thus, the association constant of (*S*)-**4**/(*S*)-mandelic acid was found to be 764 M^{-1}, whereas that of (*S*)-**4**/(*R*)-mandelic acid was 367 M^{-1}. This result indicates that the complex (*S*)-**4**/(*S*)-mandelic acid is more stable than (*S*)-**4**/(*R*)-mandelic acid by approximately 0.43 kcal mol^{-1} ($\Delta\Delta G$).

Enantiomerically pure (*R*)-binol macrocycles also were synthesized and analyzed for the binding of mandelic acid. Closed complex (*R*)-**3** and open complex (*R*)-**4** were independently treated with both enantiomers of mandelic acid and monitored by fluorometric analysis. In accordance with the titration study of (*S*)-**3** with mandelic acid, no enhanced fluorescence for (*R*)-**3** was observed with the addition of excess mandelic acid. Open macrocycle (*R*)-**4** demonstrated selectivity opposite to that of (*S*)-**4**. The association constant of (*R*)-**4**/(*R*)-mandelic acid was found to be 763 M^{-1}, whereas

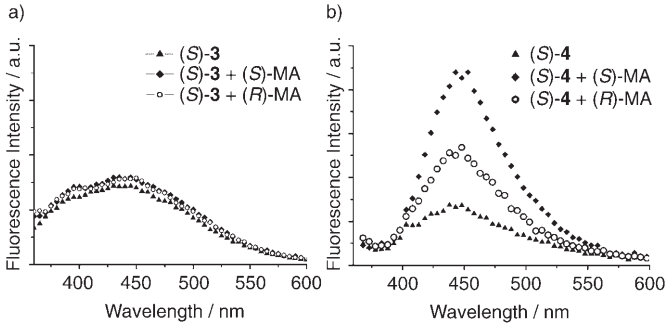


Figure 3. Fluorescence spectra of a) (*S*)-**3** (1.0×10^{-4} M) with/without mandelic acid (MA; 5.0×10^{-3} M, $\lambda_{ex}=360$ nm) and b) (*S*)-**4** (1.0×10^{-4} M) with/without mandelic acid (5.0×10^{-3} M, $\lambda_{ex}=360$ nm).

that of (*R*)-**4**/(*S*)-mandelic acid was 376 M^{-1}. This result indicates that the complex (*R*)-**4**/(*R*)-mandelic acid is more stable than (*R*)-**4**/(*S*)-mandelic acid by approximately 0.42 kcal mol^{-1} ($\Delta\Delta G$).

The chiral ligand (*S*)-**2** shows no selectivity for either (*S*)- or (*R*)-mandelic acid as evidenced by a 3.1-fold fluorescence enhancement for both enantiomers at 373 nm ($\lambda_{ex}=330$ nm). This lack of enantioselectivity is due to the flexible nature of the ligand and demonstrates the importance of macrocycle formation.

In conclusion, a novel synthetic strategy for the construction of an enantioselective fluorescent receptor that features heterotropic positive pseudo-allosterism has been demonstrated. This type of receptor design could be useful in the development sensors and materials that can be used in facilitated transport or molecular mixture separations.

Experimental Section

(*S*)-**1**: 2,2′-Dihydroxy-[1,1′]binaphthalenyl-3,3′-dicarbaldehyde (700 mg, 2.04 mmol), 4-(2-diphenylphosphanylethylthio)phenylamine (1.38 g, 4.10 mmol), CH_2Cl_2 (30 mL), and methanol (50 mL) were added to a Schlenk flask. The resulting solution was heated to reflux for 24 h. The reaction mixture was dried in vacuo, and the resulting orange solid was purified by column chromatography (eluent: CH_2Cl_2), yield = 1.94 g (95 %). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta=13.11$(s, 2H, OH), 8.85 (s, 2H, N=CH), 8.09 (s, 2H, aromatic), 7.87 (m, 2H, aromatic), 7.35–7.11 (m, 34H, aromatic), 2.89 (m, 4H, H_2C-S), 2.30 ppm (m, 4H, H_2C-P); ^{13}C{^{1}H} NMR (75.5 MHz, CD_2Cl_2): $\delta=162.57$ (s), 154.74 (s), 146.12 (s), 138.01 (d, J(P-C) = 13.5 Hz), 135.75 (d, J(P-C) = 15.8 Hz), 132.96 (br, m), 130.02 (br, m), 128.82 (br, m), 127.93 (s), 124.77 (br, m), 123.80 (br, m), 121.99 (br, m), 121.54 (s), 117.01 (s), 30.31 (d, J(P-C) = 24.0 Hz), 28.28 ppm (d, J(C-P) = 16.5 Hz); ^{31}P{^{1}H} NMR (121.4 MHz, CD_2Cl_2): $\delta=-16.3$ ppm (s); EI MS (*m*/*z*) calcd: 980.28; found: 981.4; elemental analysis (%) calcd for $C_{62}H_{50}N_2O_2P_2S_2\cdot H_2O$: C 74.53, H 5.25, N 2.80; found: C 74.43, H 5.00, N 2.78.

(*S*)-**2**: $LiAlH_4$ (3.0 equiv) was suspended in a solution of (*S*)-**1**·H_2O (3.477 g, 3.48 mmol) in CH_2Cl_2/DME (30 mL/30 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature and quenched with water (20 mL). Volatiles were evaporated under reduced pressure, more water (100 mL) was added, and the reaction mixture extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with brine and water. The separated organic layer was concentrated under reduced pressure and dried over Na_2SO_4. Filtration and purification by column chromatography gave the monohydrated amine ligand as a yellow microcrystalline solid (eluent: CH_2Cl_2), yield = 2.19 g (63 %). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta=7.86$ (s, 2H, aromatic), 7.76 (d, 2H, aromatic), 7.27–7.11 (m, 28H, aromatic), 6.98 (d, 2H, aromatic), 6.66 (d, 4H, aromatic), 4.54 (s, 4H, H_2C-NH), 2.76 (m, 4H, H_2C-S), 2.20 ppm (m, 4H, H_2C-P); ^{13}C{^{1}H} NMR (CD_2Cl_2): $\delta=152.03$ (s), 147.39 (s), 138.23 (d, J(P-C) = 14.3 Hz), 133.91 (br, m), 133.34 (s), 132.89 (br, m), 129.25 (br, m), 128.80 (br, m), 126.93 (s), 124.40 (br, m), 123.51 (s), 115.04 (br, m), 113.39 (s), 46.20 (s), 32.81 (s), 28.41 ppm (s); ^{31}P{^{1}H} NMR (121.4 MHz, CD_2Cl_2): $\delta=-16.9$ ppm (s); EI-MS (*m*/*z*) calcd: 985.2; found: 985.2; elemental analysis (%) calcd for $C_{62}H_{54}N_2O_2P_2S_2\cdot H_2O$: C 74.23, H 5.63, N 2.79; found: C 74.22, H 5.44, N 2.68.

(*S*)-**3**: In a Schlenk flask, (*S*)-**2** (295 mg, 0.299 mmol) was dissolved in CH_2Cl_2 (10 mL), and a solution of $[Cu(MeCN)_4](ClO_4)$ (98 mg, 0.300 mmol) in CH_2Cl_2 (10 mL) was added through a cannula to give a colorless solution. The solvent was removed under reduced pressure after stirring for 12 h, and the resulting white solid was dried at 150 °C under vacuum to give a yellowish sticky solid. Recrystallization from CH_2Cl_2/Et_2O afforded an analytically pure compound (as determined by NMR spectroscopy) in quantitative yield (322 mg, 94 %). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta=7.75$(s, 2H, aromatic), 7.68 (d, 2H, aromatic), 7.25–6.92 (m, 28H, aromatic), 6.45 (s, 4H, aromatic), 4.41 (br, 4H, H_2C-NH), 2.89 (br, 4H, H_2C-S), 2.42 ppm (br, 4H, H_2C-P); ^{13}C{^{1}H} NMR (CD_2Cl_2): $\delta=151.71$ (s), 149.50 (s), 134.49 (br, m), 133.07 (s), 132.50 (br, m), 130.88 (br, m), 129.29 (br, m), 127.05 (s), 126.76 (m), 124.19 (br, m), 116.32 (m), 114.47 (br, m), 112.85 (s), 100.09 (s), 44.99 (br, m), 38.15 (br, m), 28.56 ppm (br, m); ^{31}P{^{1}H} NMR (121.4 MHz, CD_2Cl_2): $\delta=-0.2$ ppm (s); ESI MS (*m*/*z*) calcd: 1047.2; found: 1047.2; elemental analysis (%) calcd for $C_{62}H_{54}N_2O_6P_2S_2ClCu\cdot H_2O$: C 63.85, H 4.84, N 2.40; found: C 64.07, H 4.77, N 2.40.

(*S*)-**4**: In a Schlenk flask, the copper complex (*S*)-**3** (103 mg, 0.0897 mmol) and 2,2′-bipyridine (14 mg, 1 equiv) were dissolved in CH_2Cl_2 (5.0 mL) to give a yellow solution. The solvent was removed after stirring for 1 h to yield an analytically pure yellow solid in quantitative yield (114 mg, 97 %). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta=$ 7.86 (m, br, 8H, aromatic), 7.57 (s, br, 2H, aromatic), 7.31–7.11 (m, 22H, aromatic), 6.99 (s, br, 2H, aromatic), 6.90 (s, br, 4H, aromatic), 6.52–6.49 (m, 4H, aromatic), 4.74 (d, 2H, ^3J(H-H) = 13.8 Hz, H_2C-NH), 4.36 (d, 2H, ^3J(H-H) = 14.4 Hz, H_2C-NH), 1.86 (br, 4H, H_2C-S), 1.82 ppm (br, 8H, H_2C-P); ^{13}C{^{1}H} NMR (CD_2Cl_2): $\delta=153.19$ (s), 148.75 (s), 139.95 (br, m), 135.76 (br, m), 134.41 (s), 132.87 (br, m), 132.34 (br, m), 129.68 (br, m), 127.84 (s), 126.72 (br, m), 124.12 (br, m), 121.31 (s), 115.31 (br, m), 46.74 (br, m), 32.41 (br, m), 28.19 ppm (br, m). ^{31}P{^{1}H} NMR (121.4 MHz, CD_2Cl_2): $\delta=-6.4$ (s); ESI MS (*m*/*z*) calcd: 1203.3; found: 1203.4; elemental analysis (%) calcd for $C_{72}H_{62}N_4O_6P_2S_2ClCu\cdot H_2O$: C 65.40, H 4.88, N 4.24; found: C 65.67, H 4.87, N 4.30.

Fluorescence measurements: Both enantiomers of mandelic acid were purchased from Aldrich and purified according to literature procedures.[10c] A stock solution of mandelic acid (0.1 M in CH_2Cl_2 containing 10 % DME), a stock solution of fluorophore (2.0 mM in CH_2Cl_2), and DME were mixed and diluted to the desired concentration in a volumetric flask. DME was used to enhance the solubility of mandelic acid, and the concentration was adjusted to 2 %. All of the samples were prepared under air-free conditions, and air-free cuvettes were used to prevent oxidation of the sensor molecule.

Received: September 20, 2005
Published online: December 30, 2005

Keywords: allosterism · coordination modes · enantioselectivity · fluorescence · host–guest systems

[1] a) A. W. Lim, *Curr. Opin. Struct. Biol.* **2002**, *12*, 61–68; b) B. Kobe, B. E. Kemp, *Nature* **1999**, *402*, 373–376.

[2] a) L. Kovbasyuk, R. Krämer, *Chem. Rev.* **2004**, *104*, 3161 – 3187; b) A. Saghatelian, K. M. Guckian, D. A. Thayer, M. R. Ghadiri, *J. Am. Chem. Soc.* **2003**, *125*, 344 – 345; c) N. C. Gianneschi, P. A. Bertin, S. T. Nguyen, C. A. Mirkin, L. N. Zakharov, A. L. Rheingold, *J. Am. Chem. Soc.* **2003**, *125*, 10508 – 10509; d) N. C. Gianneschi, S. H. Cho, S. T. Nguyen, C. A. Mirkin, A. L. Rheingold, *Angew. Chem.* **2004**, *116*, 5619 – 5623; *Angew. Chem. Int. Ed.* **2004**, *43*, 5503 – 5507; e) T. Nabeshima, Y, Yoshihira, T. Saiki, S. Akine, E. Horn, *J. Am. Chem. Soc.* **2003**, *125*, 28 – 29; f) T. Nabeshima, T. Imaba, N. Furukawa, T. Hosoya, Y. Yano, *Inorg. Chem.* **1993**, *32*, 1407 – 1416; g) M. J. E. Resendiz, J. C. Noveron, H. Disteldorf, S. Fischer, P. J. Stang, *Org. Lett.* **2004**, *6*, 651 – 653; h) M. Yoshizawa, M. Tamura, M. Fujita, *J. Am. Chem. Soc* **2004**, *126*, 6846 – 6847.

[3] a) J. R. Farrell, C. A. Mirkin, I. A. Guzei, L. M. Liable-Sands, A. L. Rheingold, *Angew. Chem.* **1998**, *110*, 484 – 487; *Angew. Chem. Int. Ed.* **1998**, *37*, 465 – 467; b) B. J. Holliday, J. R. Farrell, C. A. Mirkin, K.-C. Lam, A. L. Rheingold, *J. Am. Chem. Soc.* **1999**, *121*, 6316 – 6317; c) M. V. Ovchinnikov, B. J. Holliday, C. A. Mirkin, L. N. Zakharov, A. L. Rheingold, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4927 – 4931; d) N. C. Gianneschi, M. S. Marsar, III, C. A. Mirkin, *Acc. Chem. Res.* **2005**, *38*, 825–837.

[4] J. R. Farrell, A. H. Eisenberg, C. A. Mirkin, I. A. Guzei, L. M. Liable-Sands, C. D. Incarvito, A. L. Rheingold, C. L. Stern, *Organometallics* **1999**, *18*, 4856 – 4868.

[5] a) A. H. Eisenberg, F. M. Dixon, C. A. Mirkin, C. L. Stern, C. D. Incarvito, A. L. Rheingold, *Organometallics* **2001**, *20*, 2052 – 2058; b) X. Liu, A. H. Eisenberg, C. L. Stern, C. A. Mirkin, *Inorg. Chem.* **2001**, *40*, 2940 – 2941; c) C. S. Slone, D. A. Weinberger, C. A. Mirkin, *Prog. Inorg. Chem.* **1999**, *48*, 233 – 350.

[6] a) F. M. Dixon, A. H. Eisenberg, J. R. Farrell, C. A. Mirkin, L. M. Liable-Sands, A. L. Rheingold, *Inorg. Chem.* **2000**, *39*, 3432 – 3433; b) M. S. Masar III, C. A. Mirkin, C. L. Stern, L. N. Zakharov, A. L. Rheingold, *Inorg. Chem.* **2004**, *43*, 4693 – 4701.

[7] a) S. S. Peacock, D. M. Walba, F. C. A. Caeta, R. C. Helgeson, D. J. Cram, *J. Am. Chem. Soc.* **1980**, *102*, 2043 – 2052; b) Y. Naruta, F. Tani, N. Ishihara, K. Maruyama, *J. Am. Chem. Soc.* **1991**, *113*, 6865 – 6872; c) Y. Kubo, Y. S. Maeda, S. Tokita, M. Kubo, *Nature* **1996**, *382*, 521 – 524.

[8] a) A. Hu, H. L. Ngo, W. Lin, *J. Am. Chem. Soc.* **2003**, *125*, 11490 – 11491; b) S. J. Lee, A. Hu, W. Lin, *J. Am. Chem. Soc.* **2002**, *124*, 12948 – 12949; c) S. J. Lee, W. Lin, *J. Am. Chem. Soc.* **2002**, *124*, 4554 – 4555; d) J. Hua, W. Lin, *Org. Lett.* **2004**, *6*, 861 – 864.

[9] T. D. James, K. R. A. S. Sandanayake, S. Shinkai, *Nature* **1995**, *374*, 345 – 347.

[10] a) L. Pu, *Chem. Rev.* **2004**, *104*, 1687 – 1716; b) M.-H. Xu, J. Lin, Q.-S. Hu, L. Pu, *J. Am. Chem. Soc.* **2002**, *124*, 14239 – 14246; c) J. Lin, J. Q.-S. Hu, M.-H. Xu, L. Pu, *J. Am. Chem. Soc.* **2002**, *124*, 2088 – 2089; d) J. Lin, H.-C. Zhang, L. Pu, *Org. Lett.* **2002**, *4*, 3297 – 3300.

[11] a) G. Pescitelli, L. Di Bari, P. Salvadori, *Organometallics* **2004**, *23*, 4223 – 4229; b) C. Rosini, C. S. Stefano, H. W. I. Peering, E. W. Meijer, *Eur. J. Org. Chem.* **2000**, 61 – 71; c) W. Hug, F. Ciardelli, Jr., I. Tinoco, *J. Am. Chem. Soc.* **1974**, *96*, 3407 – 3410.

[12] H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703 – 2707.

[13] JAGUAR program was used for calculations of the structure optimization; Jaguar 4.1, Schrodinger, Inc., Portland, OR, **2000**.

[14] $ef = (I_S - I_0)/(I_R - I_0) = 2.33$.

