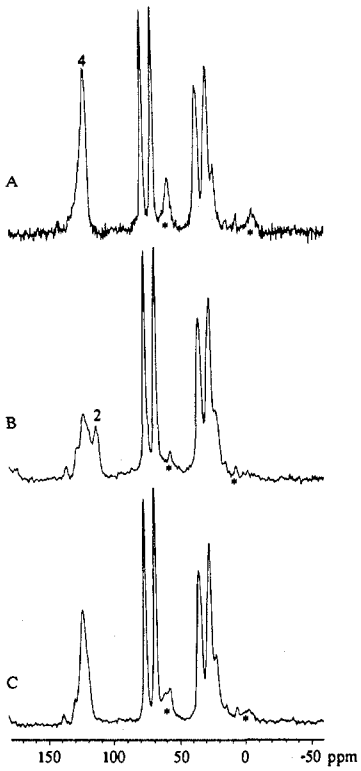


Figure 2. ^{13}C CP MAS spectra following the photolysis and annealing of **4**-5,7-$^{13}C_2$ in a 2-MTHF glass at 77 K. Prominent spinning sidebands are indicated by asterisks. (A) Spectrum before photolysis. The other peaks in this spectrum are for the 2-MTHF and its spinning sidebands. (B) Spectrum after photolysis, showing the appearance of **2**. (C) Spectrum after annealing.

In a second alternative account of the properties of 3,4-dimethylenethiophene, one might hypothesize a mixture of **2** and the bicyclic full-valence isomer **6**. The biradical **2** then could be responsible for the purple color and the extremely fast chemical reactions, and species **6** could be the carrier of the ^{13}C NMR spectrum of α,α'-^{13}C-labeled material. This scheme is made untenable by the ^{13}C NMR spectrum of 3,4-dimethylenethiophene generated from a different diazene precursor, **4**-5,7-$^{13}C_2$, in which the thiophene ring carbons adjacent to sulfur are isotopically enriched by a synthesis described in the supplementary material. The ^{13}C NMR spectrum of this precursor in diethyl ether or 2-methyltetrahydrofuran (2-MTHF) glass at 77 K shows resonances at 119 ppm (Figure 2, spectrum A). Brief irradiation under the usual conditions generates the purple color, and a new band appears near 114.9 ppm (Figure 2, spectrum B). The bridgehead carbon resonances of the bicyclic Kekulé isomer **6** should occur near 40–45 ppm (see supplementary material), but no bands can be discerned in that region in glassed solvents 2-MTHF, MeOH, or most convincingly Et_2O, where the region 20–50 ppm is blank. Thermal annealing causes the disappearance of both the color and the 114.9 ppm band (Figure 2, spectrum C). These observations are fully consistent with the behavior expected of the biradical **2**-2,5-$^{13}C_2$ and cannot be ascribed to the bicyclic compound **6**.

The present experiments show that singlet biradical **2** is the authentic carrier of the transient ^{13}C NMR spectra produced by irradiation of **4** immobilized in rigid matrices and also is the species directly involved in the cycloaddition chemistry observed in softened matrices.

Acknowledgment. The support of this work by the National Science Foundation under Grants CHE-8517584 and CHE-8820073 is gratefully acknowledged. We also thank the Dox Foundation for a fellowship held by M.M.G. and the Heyl Foundation for a fellowship held by J.C.D.

Supplementary Material Available: Additional ^{13}C CP MAS matrix spectra, details of the synthesis of **4**-5,7-d_2, and estimates of the chemical shift of **6** (10 pages). Ordering information is given on any current masthead page.

An Antibody-Catalyzed Cis–Trans Isomerization Reaction

David Y. Jackson and Peter G. Schultz*

Department of Chemistry, University of California Berkeley, California 94720

Received November 16, 1990

Biological systems are capable of synthesizing and screening tremendous chemical diversity to produce molecules with remarkable biological functions. For example, the immune system can generate more than 10^{12} different antibodies and identify and amplify those that bind a given ligand with high selectivity and affinity. Recently the principles and tools of organic chemistry have been used to exploit the remarkable machinery of the immune system for the generation of selective antibody catalysts.[1] One important approach that has emerged in the design of catalytic antibodies involves the induction of catalytic groups in antibody combining sites via mechanism-based hapten design.[2] We now report the application of this approach to cis–trans isomerization reactions of carbon–carbon double bonds, specifically the isomerization of the α,β-unsaturated ketone **1**. Such reactions are important processes in chemical and biological systems; examples include the synthesis of vitamin D and the isomerization of retinal.[3]

One mechanistic model for antibody-catalyzed isomerization of **1** involves 1,4-nucleophilic addition of an active-site group to the enone, followed by rotation around the resulting α,β-single bond and subsequent collapse of the intermediate to afford the isomerized product **3** (Scheme I). Consequently, not only must the antibody contain an active-site nucleophile or base, it must also accommodate the transition state for rotation about the α,β-single bond. The disubstituted trans piperidinium hapten **4** was expected to afford an antibody combining site that fulfills both criteria. Molecular mechanics calculations suggest that the minimum energy conformation of trans hapten **4** places the nitrophenyl moieties in a geometry close to that for the perpendicular transition state required for enone isomerization.[4] In addition, the positively charged amino group should induce a complementary carboxylate in the antibody combining site[2a] which may be capable of reversible 1,4-nucleophilic addition to enone **1**. Alternatively, the carboxylate might act as a base to activate water for attack on enone **1**.

A 5:1 mixture of cis:trans hapten **4** was synthesized[5] via con-

* Author to whom correspondence should be addressed.

(1) (a) Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1283–1295. (b) Schultz, P. G.; Lerner, R. A.; Benkovic, S. J. *Chem. Eng. News* **1990**, *68*, 26–40. (c) Shokat, K. M.; Schultz, P. G. *Annu. Rev. Immunol.* **1990**, *8*, 335–363.

(2) (a) Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Nature* **1989**, *338*, 269–271. (b) Janda, K. D.; Weinhouse, M. I.; Schloeder, D. M.; Lerner, R. A.; Benkovic, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 1274.

(3) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman and Co.: San Francisco, 1979.

(4) Molecular mechanics calculations were carried out with Biograf software (Mayo, S. L.; Olafson, B. D.; Goddard, W. A. Biodesign Inc., Pasadena, CA).

(5) To a solution of 4-nitroiodobenzene (10 mmol) in 30 mL of DMF at 20 °C were added hexamethylditin (10 mmol) and $(MeCN)_2PdCl_2$ (0.2 mmol). After 20 min, the reaction mixture was poured into 100 mL of H_2O and the product extracted into 100 mL of diethyl ether. Evaporation of the ether layer afforded (4-nitrophenyl)trimethylstannane (9.1 mmol). Treatment of stannane (5 mmol) with glutaryl dichloride (2.5 mmol) and $(PPh_3)_2PdCl_2$ (0.2 mmol) in 20 mL of HMPA at 60 °C followed by aqueous workup, ether extraction, and silica gel chromatography (ethyl acetate/hexane) afforded bis-1,5(4-nitrophenyl)-1,5-pentanedione (1.9 mmol). Reductive amination of dione (1 mmol) with NH_4OAc (1.5 mmol) and $NaCNBH_3$ (1 mmol) in 50 mL of MeOH at reflux afforded a 5:1 diastereomeric mixture of *cis*- and *trans*-2,6-bis(4-nitrophenyl)piperidine (**4**) as determined by HPLC and NMR. Monoalkylation of **4** (0.5 mmol) with 3-iodopropionic acid (0.5 mmol) in 20 mL of 1,2-dichloroethane at reflux afforded **5** (0.24 mmol) after purification by preparative TLC ($MeOH/CHCl_3/CH_3CO_2H$).

0002-7863/91/1513-2319$02.50/0

densation of glutaryl dichloride with 2 equiv of (4-nitrophenyl)-trimethylstannane to afford 1,5-bis(4-nitrophenyl)-1,5-pentanedione. The dione was reductively aminated to afford the cyclized product **4**. Monoalkylation of **4** with 3-iodopropionic acid afforded **5**, which was coupled to carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH).[6] Fifteen monoclonal antibodies specific for hapten **4** were isolated via standard methods[7] and purified to homogeneity (as judged by SDS gel electrophoresis) by affinity chromatography on protein A coupled Sepharose.[8] The rate of isomerization of enone **1** to its trans isomer **3** was assayed by HPLC in the absence (k_{un}) and presence of antibody (k_{cat}) at 25 °C in 20 mM Tris-HCl, 40 mM NaCl buffer, pH 7.5.[9,10]

Three of 15 antibodies were found to catalyze the thermal isomerization reaction and demonstrate saturation kinetics. One antibody, DYJ10-4, was characterized further and found to have $k_{cat} = 4.8\ min^{-1}$ and $K_m(\mathbf{1}) = 220\ \mu M$. Compared to the uncatalyzed reaction ($k_{un} = 3.1 \times 10^{-4}\ min^{-1}$), this antibody affords a rate acceleration $k_{cat}/k_{un} = 15\,000$ (Figure 1). The antibody is competitively inhibited by trans hapten **4** (Figure 1) with $K_i = 6.7\ \mu M$. In contrast, the K_i for the corresponding cis isomer[9] is approximately 1 mM, demonstrating the specificity of the antibody for trans **4**. Two other antibodies, DYJ10-2 and DYJ10-7, which catalyze the reaction at a slower rate, also preferentially bind trans hapten **4** over the cis isomer as determined by ELISA assays.[11] In contrast, 10 of the other 12 antibodies which do not catalyze the reaction preferentially bind cis **4**. The fact that only trans hapten **4** affords catalytic antibodies is consistent with the notion that the antibody is complementary to a transition state in which the nitrophenyl rings are roughly perpendicular to each other.

A number of experiments were carried out to determine whether a catalytic group is present in the combining site. Treatment of the antibody with the carboxylate specific reagent diazoacetamide prior to addition of substrate abolishes 85% of the catalytic activity while similar treatment in the presence of trans hapten **4** abolishes only 12% of the activity.[12] In addition, treatment of the antibody with epoxide **6** followed by extensive dialysis at pH 7.5 abolished 95% of catalytic activity; 84% could be recovered upon dialysis at pH 10. However, identical treatment in the presence of hapten **4** abolished only 27% of the catalytic activity after extensive dialysis.[13] These results are consistent with a catalytic carboxylate

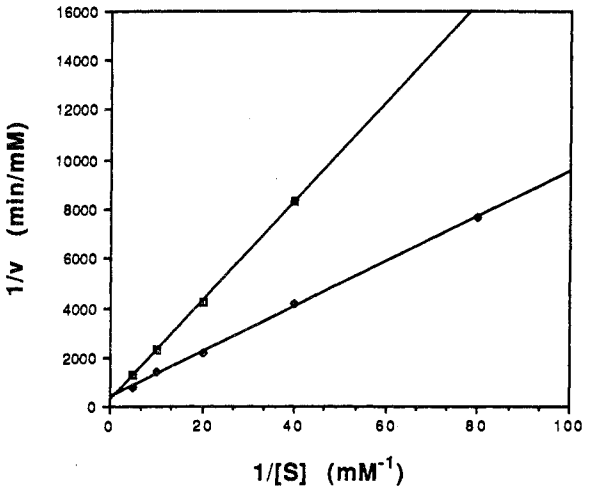
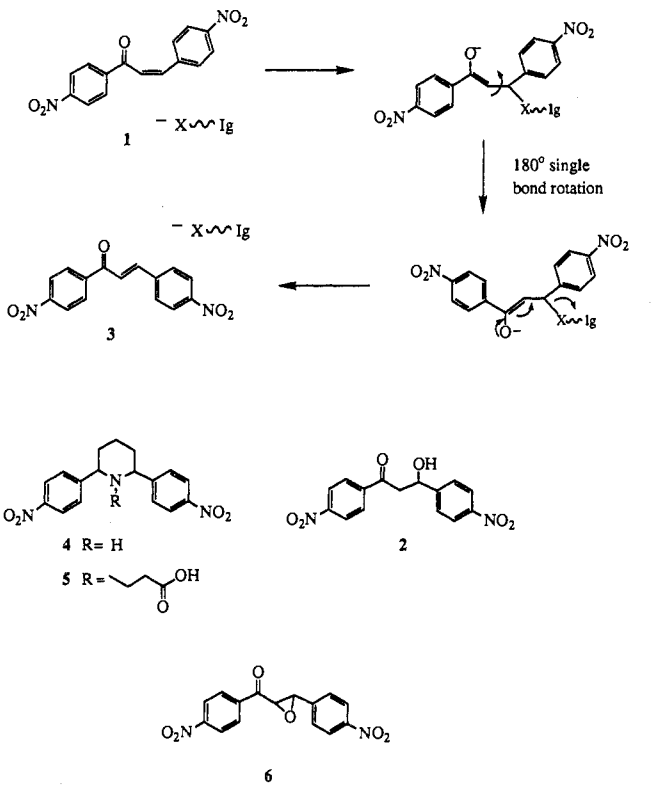


Figure 1. Lineweaver–Burk plot for antibody-catalyzed isomerization. Velocities were determined by measuring the initial linear absorbance at 340 nm: ◆, no inhibitor present; □, inhibited by 10 μM *trans*-**4**.

Scheme I



in the active site that may function as either a nucleophile or a base. Moreover, the carboxylate group appears to be within bonding distance of the α or β carbon of enone **1**. The antibody did not catalyze the elimination of β-hydroxy ketone **2**,[9] making this an unlikely intermediate in the antibody-catalyzed reaction.

Further experiments are being carried out to probe for the intermediacy of a 1,4 Michael adduct in this antibody-catalyzed reaction and to characterize the photochemical isomerization in the presence of antibody.

(6) To a solution of **5** (0.02 mmol) in 2 mL of H_2O was added, with stirring, 0.33 μmol of BSA or KLH in 5 mL of 25 mM Na_2PO_4 buffer, pH 6. 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide (0.02 mmol) was added, and the reaction mixture was stirred for 2 h at 20 °C. The resulting conjugates were dialyzed exhaustively in PBS (10 mM Na_2HPO_4, 100 mM NaCl, pH 7), and epitope densities of 7 and 12 for BSA and KLH, respectively, were determined by measuring the absorbance at 270 nm.

(7) Sugasawara, R.; Prato, C.; Sippel, J. *Infect. Immun.* **1983**, *42*, 863.

(8) Kronvall, G.; Grey, H.; Williams, R. *J. Immunol.* **1970**, *105*, 1116.

(9) 4-Nitroacetophenone (30 mmol) and 4-nitrobenzaldehyde (30 mmol) were dissolved in 100 mL of THF and added to 100 mL of 50 mM KH_2PO_4 buffer (pH 7.5). The mixture was heated at reflux for 12 h to afford hydroxy ketone **2** (16 mmol) after crystallization from ethyl acetate. Hydroxy ketone **2** (10 mmol) was heated at reflux for 12 h in 1:2:1 $CH_3CO_2H/THF/H_2O$ to afford *trans*-**3** (8.5 mmol). *trans*-**3** was then isomerized under UV light (200–300 nm) for 4 h in CH_3CN to yield a 1:3 mixture of cis/trans enone. The isomers were separated by reverse-phase HPLC (CH_3CN/H_2O), and their identity was confirmed by NMR and mass spectral analysis.

(10) Stock solutions (100×) of **1** in CH_3CN were added to a solution of 1 μM antibody in 20 mM Tris·HCl, 40 mM NaCl, pH 7.5 at 22 °C. Reaction rates were determined by measuring the increase in absorbance at 340 nm upon isomerization of **1** ($\epsilon_{340} = 800\ M^{-1}$) to **3** ($\epsilon_{340} = 7500\ M^{-1}$). All rates were confirmed with analytical reverse-phase HPLC (Bondapak-phenyl, 40–70% CH_3CN gradient in 25 mM Tris·HCl, pH 7.5) by measuring product formation relative to 4-methyl-3-nitroanisole as a standard.

(11) Voller, A.; Bidwell, D.; Bartlett, A. In *Manual of Clinical Immunology*; Rose, N., Friedman, H., Eds.; American Society for Microbiology: Washington, DC, 1976.

(12) Grossberg, A. L.; Pressman, D. *J. Am. Chem. Soc.* **1960**, *82*, 5478–82.

(13) Trans epoxide **6** was synthesized via treatment of trans enone **3** (1 mmol) with *tert*-butyl hydroperoxide (1.5 mmol) and triton B (2 mmol) in toluene for 4 h at 25 °C followed by silica gel chromatography (ethyl acetate/hexane). Antibody (1 μM) in 20 mM Tris·HCl, 40 mM NaCl, pH 7.5, was treated with epoxide **6** (10 μM) in the presence and absence of inhibitor **4** (100 μM) for 6 h at 25 °C and extensively dialyzed in the same buffer.

Acknowledgment. We acknowledge financial support from the NIH (Grant No. R01-AI24695-03) and the Monsanto Company. P.G.S. is a W. M. Keck Foundation Investigator.

Enantioselective, Zirconium-Mediated Synthesis of Allylic Amines

Robert B. Grossman, William M. Davis, and Stephen L. Buchwald*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received October 22, 1990

The development of simple and general methods for the preparation of enantiomerically pure organic compounds from readily available, achiral substrates is one of the major challenges of organic synthesis today.[1] A reagent-controlled approach[2] is particularly useful because it allows for the formation of either enantiomer of a particular compound from the same substrates. We recently reported a method for the synthesis of racemic allylic amines from simple amines and unfunctionalized alkynes via imine complexes of zirconocene,[3] and we now report the development of an *asymmetric* variant of this reaction that proceeds to give products with ee's up to 99% in moderate to good yields.[4]

We required a chiral equivalent of zirconocene dichloride, the achiral organometallic precursor to much of the chemistry we have previously described, for use as a starting material. Several such compounds have been synthesized,[7] and we chose to focus our attention on [1,2-ethylenebis(η^5-4,5,6,7-tetrahydro-1-indenyl)]-zirconium dichloride [(EBTHI)$ZrCl_2$, **1**],[7a-c,8] first synthesized by Brintzinger. Briefly, kinetic resolution of **1**[10] was accomplished by using lithium (*S*)-[1,1′-binaphthyl]-2,2′-diolate, in a method similar to that used to resolve the titanium analogue,[11] and unreacted **1** was removed by stirring with alumina. The enantiomerically pure dimethyl derivative, (*S*,*S*)-**2** ($[\alpha]^{21}_D = +170 \pm 3°$ (c = 0.05, CH_2Cl_2)), was obtained in 63% overall yield from (*S*,*S*)-**1**.[12]

Scheme I

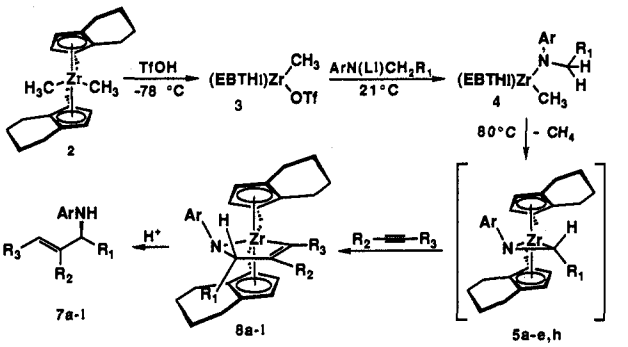
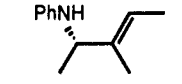
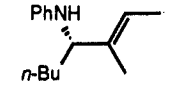
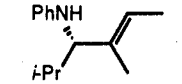
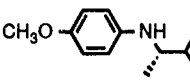
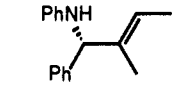
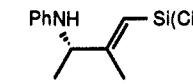
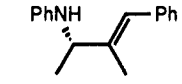
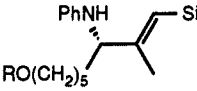
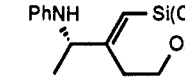
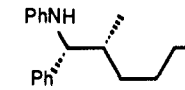
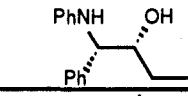


Table I

product	structure[a]	yield,[b] %	ee,[c] %	diastereo- or regioselectivity[d]
7a		72	>95	–
7b		72	>95	–
7c		60	>95[e]	–
7d		64	~99	–
7e		38	18	–
7f		68	>95	24:1
7g		50	>90	100:0[f]
7h		53	>95	22:1
7i		59	>95	17:1
8		54	high[g]	8:1
9		43	94[h]	7:1

[a] R = Si(*t*-Bu)$(CH_3)_2$. [b] Isolated yields, >95% pure by GC and ^1H NMR, of both diastereomers or regioisomers where applicable. [c] By Eu(hfc)$_3$ shift studies, except where noted. [d] The minor component was established to be an isomer by comparison of the GC-MS of the two species. [e] Determined by capillary GC on a CyclodexB chiral column (J&W Scientific). [f] The regioisomer was initially present (7:1 ratio), but it was removed during chromatography. [g] One diastereomer of the metallacycle precursor was clearly predominant by ^1H NMR. [h] Determined by ^{19}F NMR studies of the Mosher's ester of the major diastereomer.

(1) For a collection of reviews, see: *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1984; Vols. 1–5.
(2) Masamune, S.; Choy, W.; Petersen, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1.
(3) Buchwald, S. L.; Watson, B. T.; Wannamaker, M. W.; Dewan, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 4486.
(4) Until now, the only methods (to our knowledge) for the preparation of enantiomerically pure allylic amines involved the modification of enantiomerically pure allylic alcohols, amino acids, or α-hydroxy esters[5] or the action of a chiral palladium catalyst on amines and racemic, symmetrically substituted allylic carbonates or phosphinates.[6]
(5) Synerholm, M. E.; Gilman, N. W.; Morgan, J. W.; Hill, R. K. *J. Org. Chem.* **1968**, *33*, 1111. Overman, L. E. *J. Am. Chem. Soc.* **1976**, *98*, 2901. Yamamoto, Y.; Shimoda, H.; Oda, J.; Inouye, Y. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3247. Fitzner, J. N.; Shea, R. G.; Fankhauser, J. E.; Hopkins, R. B. *J. Org. Chem.* **1985**, *50*, 417. Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487. Hill, R. K. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1984; Vol. 3, Chapter 8 and references therein.
(6) Hayashi, T.; Yamamoto, A.; Ito, Y.; Nishioka, E.; Miura, H.; Yanagi, K. *J. Am. Chem. Soc.* **1989**, *111*, 6301.
(7) (a) Wild, F. R. W. P.; Wasiucionek, M.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1985**, *288*, 63. (b) Collins, S.; Kuntz, B. A.; Taylor, N. J.; Ward, D. G. *J. Organomet. Chem.* **1988**, *342*, 21. (c) Grossman, R. B.; Doyle, R. A.; Buchwald, S. L. *Organometallics*, in press. (d) Paquette, L. A.; Moriarty, K. J.; Rogers, R. D. *Organometallics* **1989**, *8*, 1506. (e) Moriarty, K. J.; Rogers, R. D.; Paquette, L. A. *Organometallics* **1989**, *8*, 1512 and references therein. (f) Halterman, R. L.; Vollhardt, K. P. C.; Welker, M. E.; Bläser, D.; Boese, R. *J. Am. Chem. Soc.* **1987**, *109*, 8105.
(8) Enantiomerically pure **1** has been used as a precatalyst for the catalytic asymmetric hydrogenation[9a] and oligomerization[9b] of olefins, and its titanium analogue has been used for the stoichiometric, asymmetric allylation of aldehydes.[9c]
(9) (a) Waymouth, R.; Pino, P. *J. Am. Chem. Soc.* **1990**, *112*, 4911. (b) Pino, P.; Cioni, P.; Wei, J. *J. Am. Chem. Soc.* **1987**, *109*, 6189. (c) Collins, S.; Kuntz, B. A.; Hong, Y. *J. Org. Chem.* **1989**, *54*, 4154.
(10) Schäfer, A.; Eberhard, K.; Zsolnai, L.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1987**, *328*, 87.
(11) Wild, F. W. R. P.; Zsolnai, L.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1982**, *232*, 233.

0002-7863/91/1513-2321$02.50/0