Multicomponent relaxation times have been reported for some solvents, and it has been suggested that the fastest components best correlate to the electron-transfer event.[3c] We have included in Figure 1 the entire range of the most reliable τ_s values measured by ultrafast spectroscopy for each solvent.[2a,8] Despite the large error limits on $1/\tau_s$, a nearly linear dependence of k_{CR} on $1/\tau_s$ is apparent, even though the rate constant of CR is much slower than the time scale of solvent motion.

This observation is consistent with theoretical predictions for electron-transfer processes influenced by solvent dynamics,[2a,b] which is schematically represented in Figure 2. The motion in the CS well is characterized by a rate constant k_D, and the crossing motion between the surfaces is characterized by k_{NA}. The overall electron-transfer rate for CR is given as[9-11]

$$k_{CR} = \frac{k_{NA}k_D}{k_{NA} + k_D} \quad (1)$$

where k_{NA} is given by a standard Marcus expression,

$$k_{NA} = |V|^2\left(\frac{\pi\hbar^2}{E_r k_B T}\right)\exp[-U(x^*)/k_B T] \quad (2)$$

$U(x^*) = (E_r - \Delta E)^2/4E_r$, where E_r is the reorganization energy and ΔE is the driving force. In the case where the electron transfer occurs at a localized crossing point,[10] k_D becomes[11]

$$k_D = \frac{1}{\tau_s}\left(\frac{U(x^*)}{\pi k_B T}\right)^{1/2}\exp[-U(x^*)/k_B T] \quad (3)$$

It is important to note that the well dynamics under discussion refer to that of solvent polarization coordinate, as characterized by τ_s. The acceptor/donor pair is assumed to be kept at a fixed distance and, as such, is not involved in the reaction coordinate. From eq 1, if the rate constant for well dynamics is large relative to the rate constant for surface crossing (i.e., $k_D \gg k_{NA}$), then the well population is equilibrated and the conventional nonadiabatic rate expression is obtained (i.e., $k_{CR} = k_{NA}$). On the other hand, if the well motion is sufficiently slow, a dependence on well dynamics will be obtained (i.e., for $k_{NA} \gg k_D$, $k_{CR} = k_D$). In this case, k_{CR} should depend intimately on the microscopic solvation time of the solvent. As observed in Figure 1 and predicted by eq 3, k_{CR} should vary linearly with the inverse of the solvent relaxation time.[12] Slow electron-transfer rates can exhibit a dependence on fast solvent motions because k_D is characterized by an activated process. Thus, the intrinsic well dynamics may be fast, but as illustrated in Figure 2, the rate constant describing the motion within the well is retarded because the system must reach a point high on the potential energy surface. Therefore, the solvent effectively "gates" the overall reaction of the CS state at a localized crossing.

Our observations further emphasize the important role that solvent can play in governing the rates of electron transfer in biological and chemical charge separating networks. In a static sense, the importance of solvent in mediating CS and CR kinetics by affecting the activation energy of electron transfer has recently been stressed.[13] Yet as we have shown here, the dynamics of solvation may dominate the reactivity of charge-separated states, even when the overall rate for the electron-transfer reaction is slow.

Acknowledgment. We thank Lawrence E. Bowman for his contributions in the assembly of the picosecond amplification system. The financial support of the National Institutes of Health (GM 47274) is gratefully acknowledged.

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Highly Chemo- and Stereoselective Cyclopolymerization of 2-Methyl-1,5-hexadiene: Chain Transfer via β-CH_3 Elimination

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Homogeneous cyclopolymerization of nonconjugated dienes yields an array of structurally diverse cyclopolymers. We have previously reported the diastereo- and enantioselective cyclopolymerization of 1,5-hexadiene to give stereoregular poly(methylene-1,3-cyclopentanes).[1] For the homogeneous polymerization systems,[2] the selectivity for cyclization is dependent on a number of factors,[1c] but generally exceeds 95% for hexadiene. This remarkable selectivity prompted us to investigate the cyclopolymerization of unsymmetrical dienes, where one terminus of the diene contains a sterically hindered olefin subunit.[3] Herein we report the unprecedented chemo- and stereoselective cyclopolymerization of 2-methyl-1,5-hexadiene to give *trans*-poly(methylene-1,3-(1-methylcyclopentane)) (PMMCP).

Homogeneous Ziegler-Natta catalysts derived from $Cp_2^*ZrMe_2$/MAO (Cp^* = pentamethylcyclopentadienyl, MAO = methylaluminoxane) or from $[Cp_2^*ZrMe]^+X^-$ cations (X = $B(C_6F_5)_4$ or $CH_3B(C_6F_5)_3$)[4-8] are active for the cyclo-

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Table I. Polymerization of 2-Methyl-1,5-hexadiene with $Cp_2^*ZrMe_2$ Derivatives

entry	[Zr] (mmol)	cocatalyst (mmol)	temp (°C)	time (h)	conv (GC, %)	yield[e] (%)	M_w[e]	conditions[a]
1	0.031	$B(C_6F_5)_3$ (0.024)	−25	36	85	85	18175	A
2	0.015	MAO (13.7)[g]	−25	6	90	36	619[h]	A
3	0.013	$B(C_6F_5)_3$ (0.012)	22–35	2	30	30	3438	A
4	0.013	salt[d] (0.013)	22–48	2	41	39	2677	A
5	0.020	$B(C_6F_5)_3$ (0.024)	22+[b]	1	75	62	1120	B
6	0.026	$B(C_6F_5)_3$ (0.024)	0	1	>90	37[f]	6060[f]	B
7	0.023	$B(C_6F_5)_3$ (0.028)	−25+[c]	20	>95	53	4219 7497[f]	B

[a] A = 5 mL of toluene, 2 mL of 2-methyl-1,5-hexadiene; B = bulk monomer. [b] A large exotherm (boiling monomer) was observed. [c] A large exotherm (warm to the touch) was observed. [d] Salt = [*N*,*N*-dimethylanilinium][$B(C_6F_5)_4$]. [e] Nonvolatile fraction (0.06 mmHg, 25 °C). [f] Acetone-insoluble fraction. [g] MAO = methylaluminoxane (13.7 mmol aluminum). [h] Nonvolatile fraction (0.06 mmHg, 70 °C).

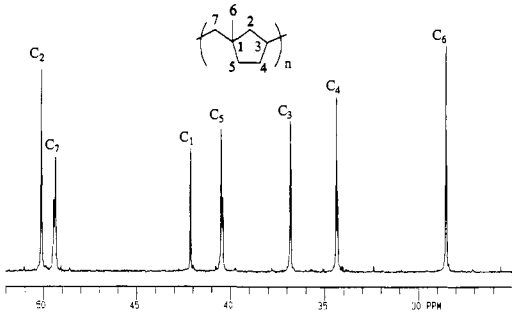
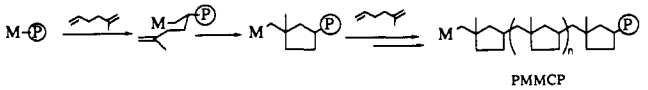


Figure 1. ^{13}C NMR spectrum of PMMCP obtained with $Cp_2^*ZrMe_2/B(C_6F_5)_3$ at −25 °C in toluene ($CDCl_3$, room temperature).

polymerization of 2-methyl-1,5-hexadiene to give highly saturated cyclopolymers (eq 1, Table I). Cyclopolymerization of 2-

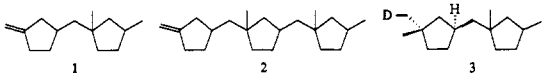


(1)

methyl-1,5-hexadiene in the presence of $Cp^*_2ZrMe_2/B(C_6F_5)_3$ in toluene at −25 °C proceeded in 85% yield to produce a solid polymer (M_w = 18 175, M_n = 2659 vs polystyrene), which was completely soluble in benzene, chloroform, and tetrahydrofuran. Cyclopolymerization of 2-methyl-1,5-hexadiene in the presence of $Cp^*_2ZrMe_2$/MAO afforded oligomers of lower molecular weight (M_w = 619). In both cases, the selectivity for cyclopolymerization is extremely high—even in *neat monomer*; residual unsaturation due to uncyclized monomer units could not be detected by NMR spectroscopy. The selectivity for cyclization is particularly noteworthy in view of the fact that 1,1-disubstituted olefins are generally inactive toward polymerization with metallocene Ziegler–Natta catalysts.[9]

The ^{13}C NMR spectrum of the polymer obtained from $Cp^*_2ZrMe_2/B(C_6F_5)_3$ at −25 °C is shown in Figure 1. The appearance of only seven major resonances indicates that this cyclopolymerization is both highly chemo- (>99%) and stereoselective.[10,11] The perfectly alternating head-to-tail microstructure can be interpreted in terms of a selective insertion of the less-hindered terminus of the diene into the metal–polymer bond followed by cyclization of the disubstituted olefin to yield a 1-methylcyclopentane intermediate (B; eq 1).

The regio- and stereochemistry of the polymer was confirmed by analyzing low molecular weight oligomers produced with $Cp^*_2ZrMe_2/B(C_6F_5)_3$. The cyclized monomer, 3-methyl-1-methylenecyclopentene (94% pure by GC), the dimer **1** (96% pure, one isomer), and the trimer **2** (95% pure, one isomer) were isolated via fractionation of the crude polymer with acetone and vacuum distillation.[12,13]



The stereochemistry of the methylcyclopentane rings is tentatively assigned as trans[14] on the basis of ^{13}C NMR analysis of low molecular weight oligomers (cyclized monomer, dimer, and trimer) and by comparison with published spectra of hydrogenated poly-1-methylnorbornene,[15] a model polymer containing only cis methylcyclopentane rings.

An intriguing consequence of the cyclopolymerization of 2-methyl-1,5-hexadiene is that the propagating species B cannot undergo β-H elimination[16] because *it contains no β-hydrogens*. We had envisioned that use of "cationic" catalysts $[Cp_2^*ZrMe]^+X^-$ ($X = B(C_6F_5)_4$ or $CH_3B(C_6F_5)_3$)[4-8] would eliminate the possibility of chain transfer to aluminum[17] and potentially result in a "living" Ziegler–Natta polymerization system. However, cyclopolymerization of 2-methyl-1,5-hexadiene in the presence of $Cp^*_2ZrMe_2/B(C_6F_5)_3$ at room temperature afforded a *low* molecular weight polymer (M_w = 3438; Table I, entry 3). End group analysis of the lower molecular weight oligomers by ^1H and ^{13}C NMR spectroscopy indicated the presence of methylenecyclopentyl (^1H δ 4.78 (m); ^{13}C, δ 153.3 and 104.7) and methylcyclopentyl (^{13}C, δ 21.6) end groups; no other end groups were detected. These results, in combination with the isolation and identification of **1** and **2**, provide convincing evidence that β-CH_3 elimination is the exclusive chain-transfer mechanism under these conditions.[18,19]

Both β-CH_3 elimination and chain transfer to aluminum[17] occur in the presence of $Cp_2^*ZrMe_2$/MAO catalysts. Acidic workup of the reaction mixture with D_2O afforded two cyclized monomers, two dimers, and two trimers. The cyclized monomers were identified as 3-methyl-1-methylenecyclopentane (19% GC yield) and 1-(deuteriomethyl)-1,3-dimethylcyclopentane (28% GC yield) by co-injection of known compounds onto the GC. The two dimer products were isolated via vacuum distillation and identified as

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(10) With catalysts derived from Cp_2ZrMe_2 derivatives, we observe a similar chemoselectivity, but lower diastereoselectives: M. Kesti, R. M. Waymouth, unpublished results.

(11) We have not yet determined the tacticity of the polymer.

(12) All compounds were identified by ^1H, ^{13}C NMR, and GC/MS methods.

(13) Predominantly one diastereomer (>90%) was observed by GC for both **1** and **2**. In contrast, reactions run with Cp_2ZrMe_2 catalyst precursors produced a large distribution of isomers for both the dimer and trimer (analyzed by GC).

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1 and 3 (~1:1 ratio).[20] The trans stereochemistry of the deuteriated dimer 3 was inferred from 2D NMR and NOE experiments, providing further support for our assignment of the trans stereochemistry for the polymer.

In conclusion, we report the first example of a highly chemo- and stereoselective cyclopolymerization. Aluminum-free "cationic" metallocenes were found to yield higher molecular weight polymers than those obtained with the typical metallocene/MAO catalysts. The observed chain transfer via β-CH_3 elimination represents a very facile C-C bond activation process. The remarkable selectivity of this cyclopolymerization is a testament to the exceptional control possible with homogeneous Ziegler-Natta catalysts.

Acknowledgment. We gratefully acknowledge the NSF (CHE 9113286) for financial support. M.R.K. thanks the Department of Education for a GAANN Fellowship.

Supplementary Material Available: Experimental procedures and listings of spectral data for reactions in the table (7 pages). Ordering information is given on any current masthead page.

(20) Pure 3 (one isomer) was obtained by treatment of the mixture of 1 and 3 with $KMnO_4$ to remove the olefinic product 1.

Sequential Site-Directed Mutagenesis and Chemical Modification To Convert the Active Site Arginine 292 of Aspartate Aminotransferase to Homoarginine[1]

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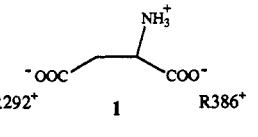
Received January 3, 1992

Site-directed mutagenesis of proteins has evolved into a powerful tool for investigations in mechanistic enzymology and in protein engineering, but except for hydrophobic residues it is generally not possible to produce subtle changes in amino acid side chain properties. This is because site-directed mutagenesis is limited to only the 19 amino acids naturally incorporated into proteins. The two principle strategies available for insertion of non-protein amino acids are chemical modification, alone[2] or in combination with site-directed mutagenesis,[3] and the in vitro mutagenesis/translation system developed by Schultz and co-workers which can use unnatural amino acids.[4] Unfortunately, this second strategy produces only very small amounts of protein at present. Chemical modification of wild type or mutant proteins, however, can provide modified enzyme in sufficient quantities for spectroscopic studies and for single turnover kinetic experiments.

We report here the modification of the *Escherichia coli* aspartate aminotransferase (AATase) mutant R292K[5,6] with the guanidinating reagent *O*-methylisourea (MIU) to convert the wild type Arg 292 into homoarginine, an amino acid whose side chain is one methylene group longer than that of arginine (Figure 1). AATase is a pyridoxal phosphate (PLP) containing enzyme which catalyzes the interconversion of the amino acids aspartate and glutamate and their corresponding α-keto acids.

$$\text{E·PLP + amino acid} \rightleftarrows \text{E·PMP + } \alpha\text{-keto acid}$$

The major determinant of the enzyme's specificity for these dicarboxylate substrates is a hydrogen bond between the carboxylate side chains and the guanidino group of arginine 292 in the active site (1).[7] All site-directed mutations of this residue result in an enzyme with substantially reduced activity for dicarboxylate substrates.[5,8]



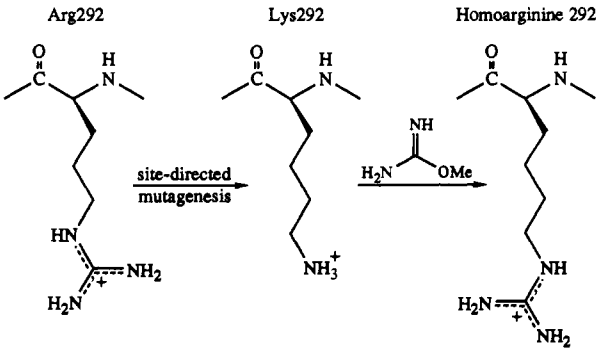


Figure 1. Conversion of arginine to homoarginine. The Lys 292 mutant of aspartate aminotransferase was prepared by standard site-directed mutagenesis.[6] Chemical modification was carried out for 97 h at 25 °C with 100 mM *O*-methylisourea hydrogen sulfate and 5 mg/mL enzyme. The pH was maintained at 10.0 in 50 mM CAPS buffer.

Table I. Steady-State Kinetic Parameters for Aspartate Aminotransferase Variants[a]

		K_m (mM)		$k_{cat.}/K_m$ (M^{-1} s^{-1})	
	$k_{cat.}$ (s^{-1})	Asp	αKG	Asp	αKG
WT	162	1.73	0.47	94 000	340 000
	(2)	(0.06)	(0.02)	(5 000)	(20 000)
WT-hR	53	0.67	0.19	79 000	280 000
	(1)	(0.03)	(0.01)	(4 000)	(10 000)
R292K	0.26	55	21	5	12
	(0.03)	(8)	(3)	(1)	(3)
R292K-hR	32	24	21	1 300	1 500
	(2)	(3)	(2)	(200)	(200)

[a] Steady-state kinetics for the aspartate/α-ketoglutarate (αKG) substrate pair was measured at pH 7.5 in 200 mM HEPES/100 mM KCl using the coupled assay described in ref 9. Substrate concentrations were 0.4-10.5 mM Asp and 0.1-2.5 mM αKG for wild type, 0.2-10.5 mM Asp and 0.05-2.5 mM αKG for modified wild type, 3-45 mM for both substrates for R292K, and 3-60 mM for both substrates for R292K-hR. R292K is the mutant of *E. coli* AATase where the active site residue Arg 292 has been changed to lysine. R292K-hR was obtained by treatment of R292K with *O*-methylisourea (MIU). The control WT-hR was prepared by reaction of WT with MIU under identical conditions as used for R292K. Standard errors are shown in parentheses.

(1) Abbreviations: αKG, 2-ketoglutaric acid; AATase, aspartate aminotransferase from *E. coli*; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); MIU, *O*-methylisourea hydrogen sulfate; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PLP, pyridoxal phosphate; PMP, pyridoxamine phosphate; R292K, AATase mutant with Arg 292 changed to lysine; R292K-hR, R292K modified with MIU; WT, wild type AATase; WT-hR, WT treated with MIU.

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