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.

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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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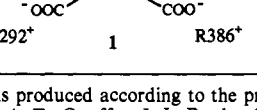
Site-directed mutagenesis of proteins has evolved into a powerful tool for investigations in mechanistic enzymology and in protein engineering, but except for hydrophobic residues it is generally not possible to produce subtle changes in amino acid side chain properties. This is because site-directed 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]



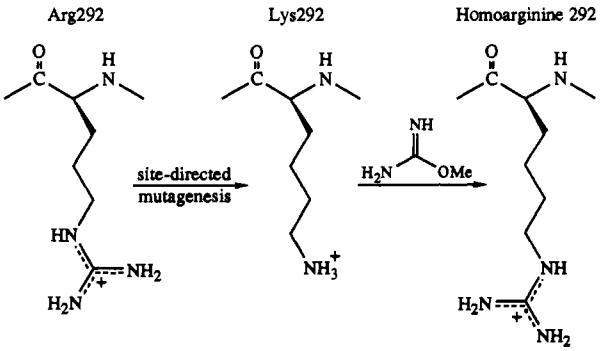


Figure 1. Conversion of arginine to homoarginine. The Lys 292 mutant of aspartate aminotransferase was prepared by standard site-directed mutagenesis.[6] Chemical modification was carried out for 97 h at 25 °C with 100 mM *O*-methylisourea hydrogen sulfate and 5 mg/mL enzyme. The pH was maintained at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

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

	$k_{cat.}$ (s^{-1})	K_m (mM)		$k_{cat.}/K_m$ (M^{-1} 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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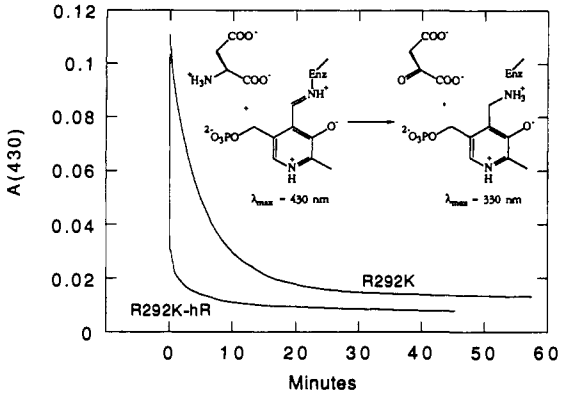

Figure 2. Single turnover reactions of R292K and *O*-methylisourea-treated R292K (R292K-hR) with aspartate. Enzyme activity was assayed in 100 mM MOPS/50 mM KCl at pH 7.0 using 1.0 mM aspartate and the coupling system reported in ref 9. The graph shows the decrease in absorbance at 430 nm due to the conversion of the pyridoxal phosphate cofactor to pyridoxamine phosphate, which absorbs at 330 nm.

At pH 7.5, the absorbance spectra of wild type AATase (WT) and the R292K mutant are identical. At this pH the PLP cofactor of AATase has absorbance peaks at 360 (unprotonated form) and 430 nm (protonated form). Since these peak positions are sensitive to local conformational changes,[9] it is likely that the conformation of the active site is not significantly perturbed by the mutation. Catalytic competence is greatly reduced, however, as seen by an increase in the K_m values for both substrates and a large decrease in k_{cat} (Table I).

MIU has been used previously to convert the lysine side chains of proteins to homoarginine.[10] Engler et al. have reported the conversion of an arginine in epidermal growth factor to homoarginine using the general procedure applied here.[3c] Nearly all lysines in an MIU-treated protein are modified, but guanidinated proteins are usually stable and possess nearly the same activity as unmodified enzymes. This is because lysine residues are typically found on the surface of proteins, and both amino and guanidino groups are positively charged at neutral pH. Modification of R292K with MIU should result in the guanidination of Lys 292 as well as the 17 surface lysines present per monomer. Lys 258, the catalytic base of AATase, should be protected because it is in a base-stable aldimine linkage with the PLP cofactor.

Optimal conditions for the reaction of both WT and R292K are reported in Figure 1. A significant amount of precipitate formed over the 4 day reaction time, but 63% of R292K and 41% of WT were recovered. Amino acid analysis of the R292K-hR sample showed that 78% of the lysine residues in R292K were modified. To assay the extent of modification specifically at Lys 292, single turnover experiments were carried out as shown in Figure 2. The rate constant for transamination of 1 mM aspartate by R292K is 0.0029 ± 0.0003 s^{-1}. Under identical conditions, 75% of the MIU-treated R292K reacts in the mixing time (k_{obsd} > 0.14 s^{-1}), while the remaining 25% turns over more slowly. This indicates that at least 75% of Lys 292 is converted to homoarginine under the conditions employed.

A comparison of the steady-state kinetic parameters for WT and modified WT shows a slight decrease in WT activity upon modification, due principally to a 3-fold decrease in the value of k_{cat} (Table I).[11] In contrast, R292K-hR shows a 100-fold increase in the value of k_{cat} over unmodified R292K, up to 60% that of the modified WT. The values for k_{cat}/K_m for R292K-hR are 100–300 times greater than those for R292K. R292K-hR, while less active than WT, is the most active position 292 AATase variant produced to date.

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Registry No. AATase, 9000-97-9; Asp, 56-84-8; αKG, 328-50-7; Arg, 74-79-3; Lys, 56-87-1; homoarginine, 156-86-5.

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Total Synthesis and Absolute Stereochemical Assignment of Gilvocarcin M

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Gilvocarcin M (**1**) shows the key structural features of a growing class of aryl *C*-glycoside antibiotics[1] which share a common aromatic nucleus, 6*H*-benzo[*d*]naphtho[1,2-*b*]pyran-6-one, to which various rare sugars are connected through a C–C bond. These compounds are attractive as synthetic targets because of the challenge presented by their unusual *C*-glycoside structures linked to the highly functionalized skeleta and because some of the members show significant antitumor activity with exceptionally low toxicity.[2,3] The reported synthetic endeavors, however, have addressed only the aglycon portion (i.e., defucogilvocarcin),[4] and the synthesis of the full structure of the natural product with the sugar moiety remains a challenging problem.[5,6] A total synthesis is also desirable because, although the relative stereochemistry

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