Friedel-Crafts type fullerenation of benzene catalyzed by aluminum trichloride (or related catalysts). In addition to phenyl substitution, benzene (C_6H_6) addition (i.e., C_6H_5 and H) was also observed (see accompanying communication[19]).

In conclusion, we have found that fullerenes readily undergo chlorination and bromination. The halogenated fullerenes can be thermally dehalogenated. Polychlorinated fullerenes undergo nucleophilic methoxylation and Friedel-Crafts type reactions with benzene and toluene. Further studies are underway for selective functionalization of fullerenes.

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Polyarenefullerenes, $C_{60}(H-Ar)_n$, Obtained by Acid-Catalyzed Fullerenation of Aromatics[1a]

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Compared to the redox chemistry,[2-9] the functionalization chemistry[10] of fullerenes has received relatively little attention.[11-15] We have reported the polymethylation as well as trimethylsilylation of diamagnetic fullerene anions.[6] Reports on hydrogenation, oxygenation, metalation, methylenation, and fluorination have also appeared.[2,11-15] In the preceding communication[16a] we reported polychlorination and polybromination of fullerene C_{60} and C_{60}/C_{70} mixtures. Polychlorofullerenes undergo facile nucleophilic polymethoxylation. Furthermore, we found that polychlorinated C_{60} undergoes remarkable polyphenylation to polyphenylfullerene with benzene and aluminum trichloride under typical Friedel-Crafts reaction conditions.

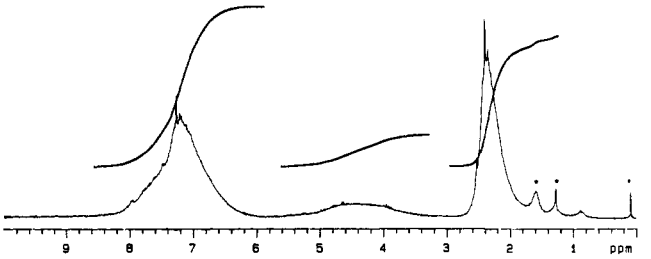

Figure 1. ^1H NMR spectrum (300 MHz) of fullerenated toluene (C_{60}-$(C_6H_5-CH_3)_{12}$) in $CDCl_3$ solution at ambient temperature. The asterisks denote peaks due to unidentified impurities.

We report now that both pure C_{60} and C_{60}/C_{70} mixtures undergo $AlCl_3$-catalyzed reaction (as well as reaction catalyzed by other strong acids) with aromatics such as benzene and toluene to polyarenefullerenes. Since Ar and H are added across fullerene double bonds, the reaction can be characterized as fullerenation of aromatics.

$$C_{60} \xrightarrow[AlCl_3]{\text{H-Ar (excess)}} C_{60}(H-Ar)_n$$

Reaction of 30 mg of the C_{60}/C_{70} mixture[16b] with 30 mg of aluminum trichloride in 20 mL of benzene at room temperature for 2 h gave a dark reddish-brown homogeneous solution. Quenching the solution with water and the usual workup (using chloroform) gave upon evaporation of solvent a brown colored solid. The FT-IR spectrum showed aromatic C-H stretching at 3056 and 3022 cm^{-1}. The 300-MHz ^1H NMR spectrum (in $CDCl_3$) indicated not only broad aromatic absorption centered at $\delta(^1H)$ 7.4 but also a broad C-H absorption at $\delta(^1H)$ 4.5. Integration of the peaks shows roughly 5:1 relative intensities (implying monosubstitution on the phenyl ring). The 75-MHz ^{13}C NMR spectrum showed a broad absorption centered around $\delta(^{13}C)$ 128, with extremely broad absorptions centered at $\delta(^{13}C)$ 146 and $\delta(^{13}C)$ 54. The most useful information was obtained from FAB (fast atom bombardment) mass spectrometry.[17] The FAB mass spectrum of the product indicated a strong mass peak at M^+ 1656, supporting the formation of $C_{60}(C_6H_6)_{12}$. Some evidence for the formation of $C_{60}(C_6H_6)_{16}$ was also obtained in the mass spectrum (M^+, 1968, weak absorption). Other mass peaks corresponding to various species containing varying amounts of benzene adducts were also detected all the way down to C_{60}-$(C_6H_6)_6$. These peaks could be due to constant neutral C_6H_6 loss from higher species. These results support direct fullerenation of benzene by initial protonation of fullerene to the corresponding fullerene cation and subsequent Friedel-Crafts alkylation (vide infra). The addition of H-C_6H_5 is also supported by the observation of the C-H fullerene skeletal hydrogens at $\delta(^1H)$ 4.5. The Friedel-Crafts type fullerenation was also confirmed by carrying out the reaction with C_6D_6. Analysis of the product by FAB showed M^+ at 1728, which corresponds to $C_{60}(C_6D_6)_{12}$, and peaks due to progressive loss of mass 84 (C_6D_6). The FT-IR spectrum showed a characteristic C-D stretching frequency at 2271 cm^{-1}. In all deuterated and nondeuterated samples, peaks corresponding to C_{60} were also observed. However, we could not obtain any evidence for the phenylation of C_{70} in the fullerene mixture.

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(17) The FAB measurements were carried out on a VG Analytical Ltd. ZAB-SE high-resolution reverse-geometry mass spectrometer.

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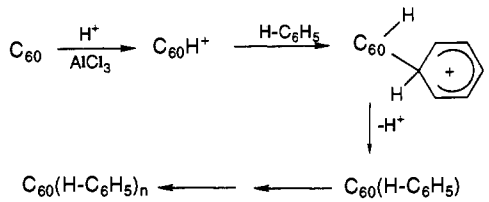
Similar results were also obtained with pure C_{60}.[16b] Similar reaction with toluene gave toluene addition products. The FAB mass spectrum showed M^+ at 1824, indicating the formation of $C_{60}(C_6H_5{-}CH_3)_{12}$. In this case, too, consecutive loss of toluene (m/e 92) was observed all the way to $C_{60}(C_6H_5{-}CH_3)_2$. In the 300-MHz ^1H NMR spectrum (Figure 1) ($CDCl_3$), absorptions at $\delta(^1H)$ 7.2 (broad), $\delta(^1H)$ 4.5 (extremely broad), and $\delta(^1H)$ 2.35 (broad) are observed. The spectrum is in accord with polytoluenefullerenes. Relative integration of the proton signals at $\delta(^1H)$ 7.2 and 4.5 gave a ratio of 4:1, indicating only monosubstitution of toluene (in all probability in the para position).

The reaction takes place only under relatively strong Friedel-Crafts acid catalysis. Weak Lewis acids such as stannic chloride or titanium tetrachloride did not catalyze the reaction. The reaction can be rationalized by initial protonation (by the residual protons in $AlCl_3$) of fullerene to fullerene cation followed by electrophilic fullerenation of the aromatic. The sequence repeats till on the average 12 Ar-H units are added. The reaction is similar to alkylation by alkenes (polyenes) under acidic catalysis. We are in the process of investigating this interesting mechanism more thoroughly. Redox processes, particularly with more easily reducible Lewis acid halide ($FeCl_3$, SbF_5, etc.) catalyzed reactions, can also be operative, involving single electron transfer (SET).



A fullerene C_{60}/C_{70} mixture was also found to undergo reaction with non-cross-linked polystyrene[18] under aluminum trichloride catalysis in CS_2 solvent to fullerenated polystyrene of a highly cross-linked nature. We are continuing our investigations on polyarenefullerenes and their intriguing reactions.

Acknowledgment. Support of the work at USC by the National Institutes of Health and the National Science Foundation is gratefully acknowledged.

(18) We thank Professor T. Hogen-Esch for a sample of monodispersed non-cross-linked polystyrene (mol wt ≅ 8500).

Mechanism of Adenylate Kinase. 12. Prediction and Demonstration of Enhancement of Phosphorus Stereospecificity by Site-Directed Mutagenesis[1]

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Recently we have demonstrated a *reversal* of stereospecificity[2] for the R44M (Arg-44 to Met) mutant enzyme of adenylate kinase (AK, from chicken muscle, overproduced in *Escherichia coli*).[3,4] We now report an *enhancement* of stereospecificity in the conversion of AMPS to ADPαS catalyzed by R97M (Arg-97 to Met) mutant AK, *entirely based upon rational prediction.*

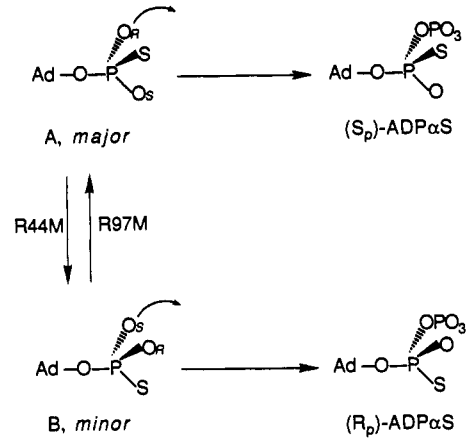

Figure 1. Schemes showing the major and minor conformers of AMPS at the active site of WT and the conversion of these conformers to ADPαS. The equilibrium is shifted to conformer B upon R44M mutation and to conformer A upon R97M mutation. It should be noted that a third, nonproductive conformer (with sulfur positioned at the acceptor position) could also be present in WT and both mutants.

The WT AK is known to convert AMPS to (S_p)-ADPαS specifically at the AMP site,[2,5] which is in turn converted to (S_p)-ATPαS specifically at the MgATP site.[2,6] However, the stereospecificity is not 100% in either case. Under various conditions, we have detected 5-10% of (R_p)-ADPαS and <5% of (R_p)-ATPαS in the reaction mixture. As shown in Figure 1, the stereospecificity at the AMP site can be explained by a major conformer A and a minor conformer B at the active site. For R44M,[2] we predicted a possible change in stereospecificity at the AMP site on the basis of the kinetic data (22-fold increase in the K_d and 36-fold increase in the K_m of AMP)[7] and the crystal structures (the yeast AK·$MgAP_5A$ complex[8] and the AK3·AMP complex[9]). However, we were unable to predict *how* it would change (i.e., relaxation, reversal, or enhancement). The observed dramatic *reversal* of stereospecificity suggested that Arg-44 plays an important role in orienting the conformation of the phosphorothioate group of bound AMPS. The A to B equilibrium is shifted to B in R44M, as shown in Figure 1.

The crystal structures, however, indicate that another arginine (corresponding to Arg-97 in our system) can also interact with the phosphoryl group of AMP,[8,9] which prompted us to construct the R97M mutant AK. Binding and kinetic analysis yielded 20- and 30-fold increases in the K_d and K_m of AMP, respectively, with no significant perturbation in MgATP binding and a 30-fold decrease in k_{cat}. Structural characterization of this mutant by NMR indicated no significant conformational perturbations. These results established that Arg-97 interacts with AMP during the catalysis by AK and led us to predict a change in the stereospecificity of R97M. Since the side chains of Arg-97 and Arg-44 point toward the phosphoryl group of AMP from opposite sides, we also predicted that *R97M and R44M should perturb the stereospecificity in opposite directions*, i.e., *the stereospecificity of R97M should be enhanced relative to WT.*

To prove that a highly stereospecific reaction has been enhanced, one must demonstrate formation of the minor isomer (R_p) at the early stage of reaction for WT, and lack of (or decreased) formation of the R_p isomer at a later stage of reaction for R97M.

(1) Supported by Research Grant DMB-8904727 from the NSF. Paper 11: Reference 4. Abbreviations: ADP, adenosine 5'-diphosphate; ADPαS, adenosine 5'-*O*-(1-thiodiphosphate); AK, adenylate kinase; AMP, adenosine 5'-monophosphate; AMPS, adenosine 5'-monothiophosphate; AP_5A, P^1,P^5-bis(5'-adenosyl)pentaphosphate; ATP, adenosine 5'-triphosphate; ATPαS, adenosine 5'-*O*-(1-thiotriphosphate); WT, wild type.

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