was remarkably similar to that of the native R312 enzyme (see Figure 2). Double integration of the ESR spectrum of the native B23 enzyme gave 3.8 ± 0.3 spin per enzyme molecule. Nitrile hydratase of *Pseudomonas chlororaphis* B23 contains 4 mol iron/mol enzyme,[2] and hence its iron environments appear to be unequivalent.

Table I summarizes the ESR parameters of nitrile hydratases in comparison with low-spin iron(III) complexes of porphyrin[8] and bleomycin.[7] In general, the g value splitting of low-spin Fe(III) complexes decreases in the order of N-Fe-N > N-Fe-O > N-Fe-S axial ligation modes. The small ($g_{max} - g_{min}$) value of nitrile hydratase may suggest that one of the axial ligations is the thiolate donor, probably the cysteine thiol group. Indeed, the ESR features of the present enzyme are considerably close to those of the oxidized cytochrome P-450 state with g values near 2.4, 2.2, and 1.9.[8] A recent 2.6-Å crystal structure of *Pseudomonas putida* cytochrome P-450 clarified that the heme iron atom is coordinated with the axial sulfur ligand by cysteine residue.[9] Most of the five-coordinated Fe(III) complexes are not of a low-spin but rather of a high-spin one, and accordingly the most probable other axial ligation of native nitrile hydratase may be water, because of easy replacement by propionitrile or isobutyronitrile. To test whether H_2O is coordinated to the iron(III) site of native R312 enzyme, we have prepared the sample in $H_2{}^{17}O$ (31% enriched in ^{17}O). On substitution of $H_2{}^{16}O$ by water enriched in ^{17}O which has a nuclear spin of $^5/_2$, a 2.5-G broadening (half-peak width) of the $g_{min} = 1.971$ line was clearly observed. The $g_{mid} = 2.140$ and $g_{max} = 2.284$ features were also broadened by 18.2 G (peak to trough) and 22.2 G (half-peak width) from 15.4 and 21.6 G, respectively, as seen for the native enzyme in $H_2{}^{16}O$. The broadenings are attributable to transferred hyperfine interactions, demonstrating that ^{17}O derived from $H_2{}^{17}O$ is coordinated to the iron(III)-active center of nitrile hydratase. On the basis of the broadening of about 3 G at $g = 9.67$ resonance by $H_2{}^{16}O \rightarrow H_2{}^{17}O$ replacement, one ligand sphere of the high-spin Fe(III) center in protocatechuate 3,4-dioxygenase is shown to contain water.[10] Similar ESR line broadening by $H_2{}^{17}O$ has also been observed for reduced activated aconitase[11] and metmyoglobin.[12] In addition, azide ion inhibited the present enzymatic activity and perturbed the ESR spectrum of the native R312 enzyme as follows: $g_{max} = 2.230$, $g_{mid} = 2.141$, and $g_{min} = 1.986$.

In conclusion, the present ESR study reveals that (1) nitrile hydratase is the first non-heme iron enzyme with a typical low-spin Fe(III) coordination environment, (2) axial positions of the iron(III) site in the native enzyme may be occupied by thiolate and aquo groups, and (3) aliphatic nitrile substrates directly bind to the iron(III)-active center by water → substrate replacement. Further characterization of the iron(III)-active site of nitrile hydratase is now underway by Mössbauer, resonance Raman, EXAFS, and X-ray diffraction methods.

Acknowledgment. This study was supported in part by Grant-in-Aid for Special Project Research (61125006 to Y.S.) and Scientific Research B (61490017 to Y.S.) from the Ministry of Education, Science, and Culture, Japan.

Registry No. Fe, 7439-89-6; nitrile hydratase, 82391-37-5; propionitrile, 107-12-0; isobutyronitrile, 78-82-0.

Supplementary Material Available: ESR spectrum of native R312 enzyme in $H_2{}^{16}O$ and $H_2{}^{17}O$ (Figure S-1) (1 page). Ordering information is given on any current masthead page.

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Template-Controlled Oligomerization of Methyl Methacrylate

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Received April 20, 1987

Many molecules of longstanding interest to organic chemists contain, as part of their structure, a finite segment of repeating units. The challenges posed by the synthesis of these repetitive segments have been addressed to date by an iterative approach: addition of successive "monomers" accompanied by oxidation level adjustments and protection/deprotection steps. While implementation of these strategies can be tedious and is often inefficient, the resultant stereo- and regiochemical control of bond formation has been excellent.[1]

Herein, we report our preliminary results concerning a conceptually new strategy, which we term *template-controlled oligomerization*, for the construction of repeating segments within structurally complex molecules. This alternative approach to iterative methodology relies on a linear addition of a predetermined number of monomer units to assemble the desired oligomeric target in a single operation.

Successful execution of this strategy depends upon precise control of the timing of the three stages of an oligomerization process (i.e., initiation, propagation, and termination), since the overall length of the product oligomer will be determined by the number of monomers incorporated into the growing chain during the propagation phase. Therefore, the critical challenge of this approach hinges upon the ability to insert a "stop message" (i.e., termination event) at a predetermined time into an actively polymerizing system.

Our experimental approach utilizes the large rigid spacer[2] template **1** to fix the positions of an initiating (I) and a terminating (T) functionality for a given polymerization reaction.[3] In the ideal experiment, polymerization will occur only within the initiator-terminator gap, and, hence, the length of the oligomeric product will depend solely on template size. A primary obstacle in realizing this ideal template-controlled oligomerization scheme arises from the anticipated competition between desired intramolecular termination and undesired bimolecular termination events. This important issue of the efficiency of intramolecular termination was initially probed via the experiments outlined in eq 1.

The dihydroxy template **2a** was employed in a *macrolactonization* process that was designed to model the *macrocyclization* reaction which would constitute intramolecular termination in a polymerizing system. Equimolar quantities of bis acid chloride **3** and template diol **2a** were allowed to react in the presence of dimethylaminopyridine in methylene chloride under high dilution for 18 h leading to the results shown in eq 1.[4]

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(2) Examples of the use of organized structures (including templates) to control the dynamics of a polymerization process can be found in the following: (a) Imprint Polymerization: Wulff, G. In *Polymeric Reagents and Catalysts*; Ford, W. T., Ed.; ACS Symposium Series 308; 1986. (b) Replica Polymerization: Bamford, C. In *Developments in Polymerization*; Haward, R. N., Ed.; Applied Science Publishers, Ltd.; London, 1979; Vol. 2. (c) Polymerization in Organized Media: *Polymerization of Organized Systems*; Elias, H.-G., Ed.; Gordon and Breach Scientific Publishers: New York, 1977.
(3) The templates of general structure **1** were readily prepared from double Diels-Alder addition of anthracene 9-methylcarboxylate to the trans-anti-trans norbornadiene trimer,[9] followed by functional group manipulation to deliver the appropriate initiator/terminator pairings. The preparation and subsequent manipulations of these species will be detailed in a forthcoming publication.
(4) All new compounds exhibited satisfactory spectral data (^1H NMR, ^{13}C NMR, IR, MS, and elemental analysis).

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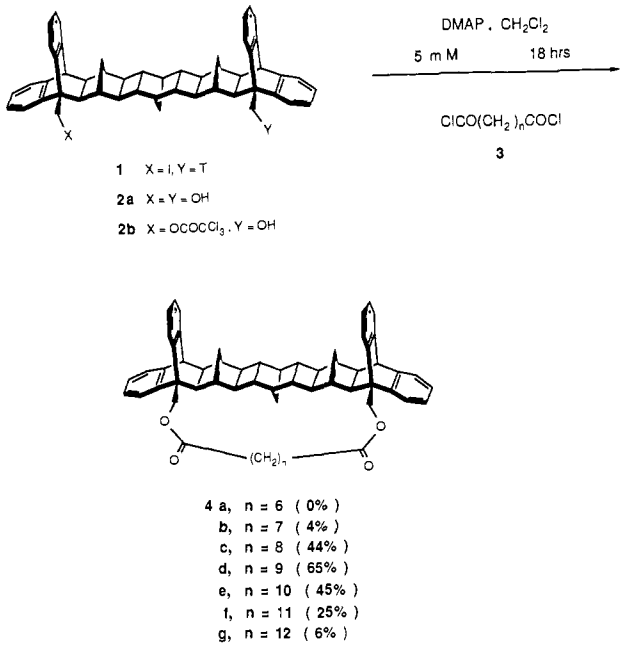
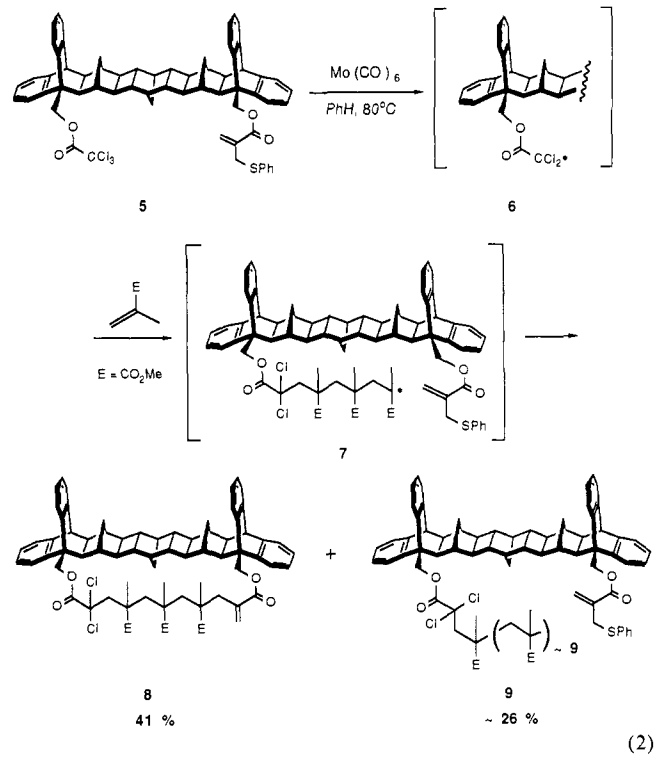


(1)

We expected that under this standard set of conditions the efficiency of macrolactonization, as a function of chain length n, would provide a basis for predicting the optimum oligomer chain length which would result in the most efficient intramolecular termination in an actively polymerizing system. It was found that the maximum yield of bis lactone **4** (65%) occurs at a chain length of $n = 9$. Therefore, the reactive end group of an actively polymerizing system might be expected to interact most efficiently with the terminating functionality when the growing oligomeric chain extends 10 atoms past the initiator position. With $n > 9$, the efficiency of bis lactonization falls off precipitously, perhaps reflecting the energetic penalty associated with the gauche interactions which necessarily must arise within the hydrocarbon chain as the two reactive ends come together. This maximum at $n = 9$ is rather surprising, as inspection of Dreiding models indicated that the $n = 7$ case is capable of spanning the diol gap, while the $n = 8$ case can be accommodated without engendering any serious steric interactions. Therefore, these macrolactonization experiments allow formulation of a more accurate picture of the molecular dimensions of the initiator/terminator gap.

The bifunctional template **5**[5] is useful in controlling the chain length of the free radical oligomerization of methyl methacrylate. This template system utilizes a trichloroacetate-initiating funtionality and an "activated" form of the methacrylate monomer as a terminating functionality. Reaction of the trichloroester with $Mo(CO)_6$ produces the carbon radical **6**, which acts as an efficient initiator for methacrylate polymerization.[6] The terminator moiety features a competent radical leaving group (SPh) which serves as the "stop message" for the polymerization process, depicted by the transformation **7** → **8**.

A 10 mM deoxygenated benzene solution of template **5** containing 1.2 equiv of $Mo(CO)_6$ and 10 equiv of methyl methacrylate was heated in a sealed tube at 80 °C for 6 days. Concentration of the solution and chromatographic isolation of the reaction products led to the compounds indicated in eq 2. Of primary interest is the formation of the cyclized material **8** in 41% yield.[4] In addition, 11% of unreacted starting template **5** and ~26% of the product **9** of uncontrolled polymerization, in which the growing polymer chain does not interact with the intramolecularly disposed



(2)

terminator, were isolated. The end group of this polymer chain is uncharacterized as yet, and it is possible that at least some of **9** arises from bimolecular termination with a second template molecule.

Macrocycle **8**, having three methyl methacrylate monomers between initiator and terminator, was the only cyclized material isolated from this reaction. A control experiment run under identical conditions to those described above, using the template **2b** which lacks the terminator moiety, led to a complex mixture of template-bound oligomers averaging 5.2 methacrylate units per template (^1H NMR). Therefore, template **5** precisely and *predictably* controls the length of the methyl methacrylate oligomerization.[7] Oligomeric macrocycle **8** in fact has nine carbon atoms between the two lactone carbonyls, the optimal number as indicated by the bis lactonization experiments described in eq 1.

Examination of the ^1H NMR spectrum of the uncontrolled polymerization product(s) **9** revealed a peak pattern which is superimposable with that of atactic polymethyl methacrylate.[8,9] However, in the controlled polymerization product(s) **8**, six out of the eight possible stereoisomers are isolated in unequal amounts after HPLC (**8a**:**b**:**c**:**d**:**e**:**f** = 5%:5%:19%:9%:8%:54%).

With these preliminary results, we have demonstrated the feasibility of controlling the chain length of a free radical polymerization reaction by using a properly designed template. The stereochemical outcome of this process is one of the more surprising and intriguing observations to arise from these experiments and will be the topic of further studies to be reported in due course.

Acknowledgment. We thank the Syntex-Russell Marker fund and the Searle Scholars Program/The Chicago Community Trust for financial support. FAB-MS were obtained from VG Instruments, Savannah, GA.

Supplementary Material Available: Spectral data for **2a**, **4b–g**, **5**, and **8a–f** (5 pages). Ordering information is given on any current masthead page.

(5) Treatment of template diol **2a** with 1.0 equiv of *n*-BuLi followed by 1.2 equiv of bromomethylacryloyl chloride furnished the template mono(bromomethylacryloyl)ester in 55% yield. Addition of sodium phenylsulfide produced the template mono(phenylthiomethylacryloyl)ester in 50% yield, which was acylated with trichloroacetyl chloride to produce the oligomerization precursor template **5** in 95% yield.

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(7) Parallel studies with templates containing phenyl selenyl or phenyl sulfone moieties in place of the phenyl sulfide unit of **5** provided the controlled length oligomer **8** in 20% and 16% yields, respectively.

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