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m-Phenylene Ethynylene Sequences Joined by Imine Linkages: Dynamic Covalent Oligomers

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Imine metathesis between *m*-phenylene ethynylene oligomers of various lengths was performed in acetonitrile, a solvent in which oligomers containing eight or more repeat units adopt a compact helical conformation. The equilibrium constants and corresponding free energy change for the imine metathesis reactions were estimated. The results showed that the magnitude of equilibrium shifting measured by the free energy change for the formation of imine-containing oligomers increases linearly below a critical product chain length and grows asymptotically above it. The linear region is ascribed to the constant increase in contact area between monomer units of adjacent helical turns as the product chain grows to the 12-mer. Once the ligation product is 12 units in length, full contact is made between adjacent helical turns. On the other hand, for imine metathesis between oligomers leading to products having more than 12 units, the driving force is the difference between the folding energy of products and that of reactants. The additional stabilizing energy is roughly constant, regardless of the chain length, since the contact area between adjacent helical turns is unchanged. Consistent with the notion that the imine bond only minimally destabilizes the helical conformation, the position of the imine bond in the ligation product has been observed to have no significant effect on the folding stability. The magnitudes of equilibrium shifting are similar for ligation products of the same length but having the imine at various positions along the sequence. This suggests that the imine bond is compatible with the *m*-phenylene ethynylene backbone, regardless of the position in the sequence. Imine metathesis of *m*-phenylene ethynylene oligomers could allow a quick access to an unbiased, dynamic library of oligomer sequences joined by imine linkages.

Introduction

Polymerizations are potentially a powerful way to introduce structural and functional diversity into a chemical system, since a range of structures is possible by manipulating the monomer composition, sequence, and chain length. Polymers having well-defined folded structures (tyligomers)[1] can potentially create 3D binding cavities for targets of interest. However, the search for nonnatural, copolymer sequences that are capable of binding or performing catalytic functions is challenged by the magnitude of diversity available to such system.[2] This point is apparent from recent aptamer studies. In vitro selection and amplification have been used to isolate nucleic acids with desired binding and catalytic properties from a large number of random sequences.[3] Successive rounds of selection and amplification allow a small subset of sequences that have the desired property to be identified. From a library of random nucleic acids 100 monomers in length, it is found that approximately 1 in 10^{10} sequences bind to a target ligand with minimum affinity of 10^5-10^6 M^{-1}. Interestingly, a library of random peptides containing 80 amino acid units have been found to display functional activity (defined as ATP binding affinity with $K_d = 100$ nM value) with similar frequency.[4]

The lack of a general amplification methodology requires that alternative approaches be found to identify macromolecular sequences from nonnatural building blocks. Our approach to tyligomers involves dynamic covalent oligomers—the joining of bisfunctionalized, foldable segments through reversible covalent linkages.[5] Introduction of reversible covalent linkages into polymeric chains may offer a dynamic combinatorial approach[6] to identify masterpiece sequences[2] in a reason-

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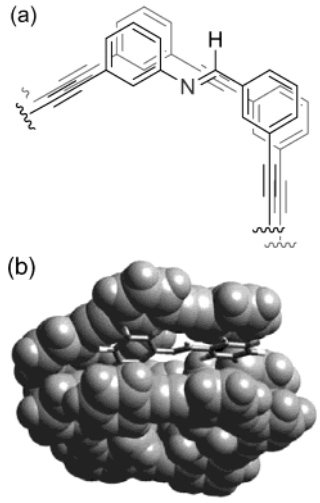

FIGURE 1. (a) The parital chemical structures of oligomers showing the overlap between imine linkage and meta-connected phenylene ethynylene repeat units. (b) A computer-minimized space-filling model of unsubstituted imine 12-mer in a helical conformation. The imine linkage is displayed as stick type for clarity.

able time scale. It is expected that the distribution of dynamic copolymer sequences should be sensitive to the environmental conditions and target ligands that are present during the equilibration process. For optimal results, a uniform distribution of all possible sequences is desirable, prior to the addition of the target ligand.[6b] This unbiased sequence library demands that the chemistry used to join segments be selected carefully. As a reversible ligation to join bisfunctionalized segments, *metathesis reactions* ensure that bond energies are the same on both sides of the equilibrium. Since the bond energy changes do not bias the reaction equilibrium, the product distribution is dependent solely on the thermodynamic stability of each component or complex with the target ligand. The reversible reaction should proceed rapidly at low temperature so that binding and folding are not disfavored during equilibration and so that sequence space can be explored in a reasonable time scale. Another desirable feature of the reversible reaction is that it be easily quenched so that sequence redistribution does not take place during analysis.

Imine metathesis was selected as a ligation reaction because the reaction meets the criteria mentioned above. Under appropriate conditions, imine metathesis is a fast, reversible process.[5,7] It is quenched by the addition of proton scavenger. The equilibrium constant of imine metathesis is close to unity, allowing for an unbiased reaction mixture in the absence of the external stimuli. In addition to reactivity, the geometry of the imine bond was postulated to be commensurate with the compact helical conformation of the *m*-phenylene ethynylene backbone previously studied in our laboratory.[8] This was supported by molecular modeling (Figure 1) and estimations of folding stability experimentally determined from solvent denaturation studies.[5] The main limitation of imine metathesis is hydrolysis of the imine bond by residual water, which can compete with metathesis under aqueous conditions.

In addition to the ligation reaction, the design of reactive oligomeric segments is an important factor to consider in the search for new tyligomers. For heteropolymers that are both soluble and globular, the composition and distribution of solvophobic/solvophilic residues must be optimized.[9] Copolymers with too many solvophilic residues will lead to soluble macromolecules that adopt random coil conformations, while copolymers with too many solvophobic residues will form aggregates or precipitates.[9a,e,f] Consequently, oligomeric segments must be designed to avoid "deep thermodynamic traps", such as insoluble or cyclic products that could easily result from reversible chemistry. Thus, as building blocks for tyligomer synthesis, we see the need for a set of starter sequences—short sequences consisting of irreversibly joined monomers that terminate in labile ends. These starter sequences are chosen to avoid the extremes of monomer composition that lead to deep traps. The starter sequences approach is expected to allow for a closed reaction polymerization, where there is no need to remove any side products in order to drive high polymer formation.[10]

m-Phenylene ethynylene oligomers have been selected to test the above ideas, since these oligomers undergo a reversible conformational transition between a random and helical state, depending upon chain length and the solvent composition.[8] Previous studies on *m*-phenylene ethynylene sequences joined by imine linkages have shown that the dynamic pool of imine oligomers formed from imine metathesis can be shifted by folding to favor the conformationally ordered sequences.[5] Similar approaches were used to enhance the proportion of sequences with the highest affinity to a particular ligand.[11] We have also shown that the reversible polymerization of bisfunctionalized imine oligomers can be driven toward high polymer by folding.[12] These results suggest that ligand binding could be exploited to amplify the optimal sequences for dynamic covalent oligomers.

As a prerequisite step, it is essential to examine the thermodynamic landscape of ligation products to see whether there is an overriding preference for certain segments or isoenergetic unbiased mixture of sequences.[6b] Here we examine the chain length dependence of a specific system, and the effect of the position of the dynamic linkage within the oligomeric products using monofunctional imine oligomers.

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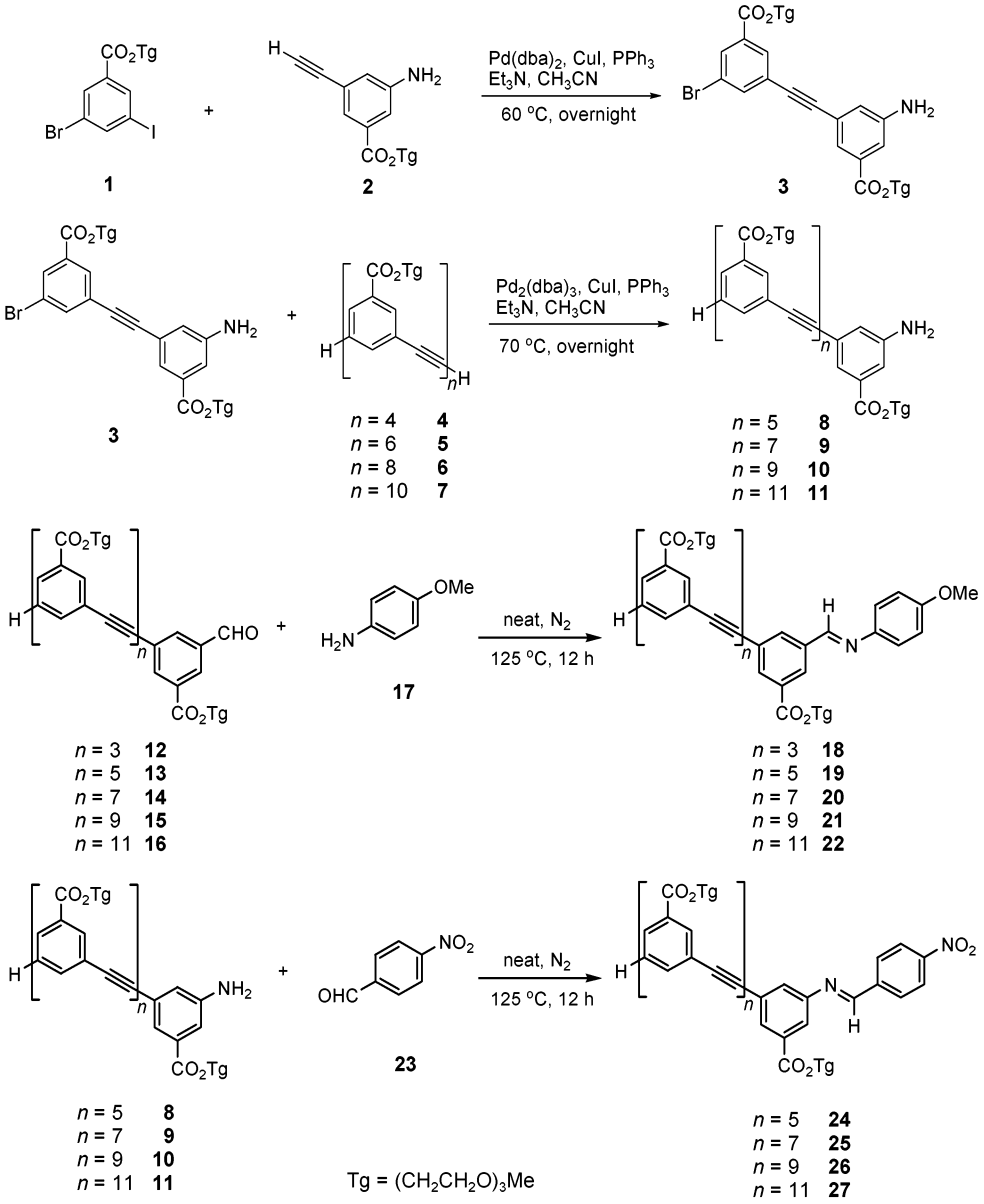
SCHEME 1



Results and Discussion

The synthesis of imine oligomers with various lengths is shown in Scheme 1. Oligomer segments **8**−**16** were synthesized by repetitive Sonogashira coupling of appropriate aryl halide and terminal acetylene precursors.[8b,13] *C*-terminal and *N*-terminal imine groups (**18**−**22** and **24**−**27**) were added by condensation of the corresponding aldehydes and amines.

For all of the metathesis experiments, equimolar quantities of *C*- and *N*-terminal imine oligomers were dissolved in dry acetonitrile. Oxalic acid was added to this solution and the reaction mixture was stirred at room temperature (Scheme 2). The equilibrium product distribution resulting from imine metathesis was determined by NMR spectroscopy. Under the reaction conditions in polar solvents such as acetonitrile, the imine C*H* proton NMR resonances for oligomers longer than the 8-mer are poorly resolved and buried in the aromatic region. Direct monitoring of the reaction mixture was not feasible. To circumvent this problem, the metathesis reactions were performed in dry acetonitrile in the presence of oxalic acid catalyst, quenched, concentrated, and redissolved in $CDCl_3$, where the imine C*H* proton resonance for one of the reactants is resolved from the overlapped product imine C*H* proton resonances. The reactions were quenched by adding the resin-supported amine propylaminomethylpoly(styrene-*co*-divinylbenzene). The beads were conveniently removed by filtration prior to concentrating the solution.

To ensure that no redistribution took place following quenching, model metathesis reactions using simple monofunctional imines were conducted (see Scheme 3 and the Supporting Information). *C*-terminal imine monomer **36** was dissolved in dry acetonitrile, and oxalic acid was added to this solution. There was no change in NMR over

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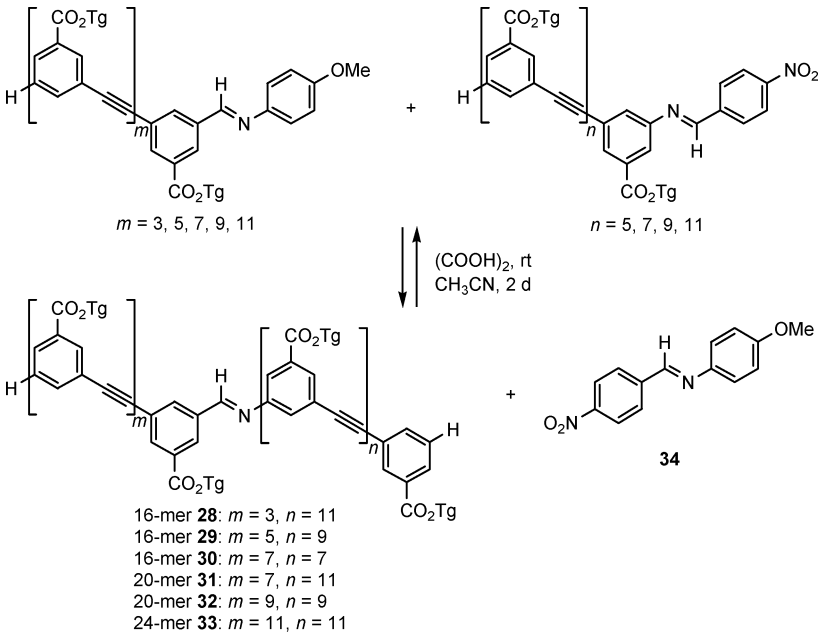
SCHEME 2



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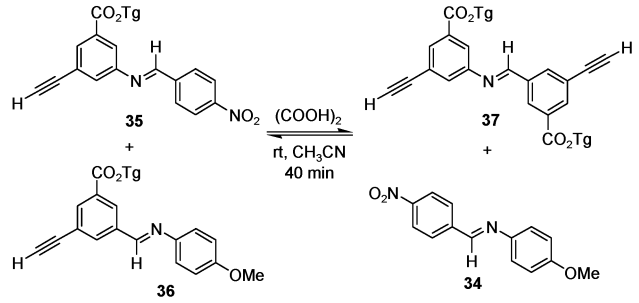

20 min. The acid catalyst was then quenched with the resin-supported amine and an equimolar quantity of *N*-terminal imine monomer **35** was added. No metathesis was observed over 16 h. On the other hand, the metathesis of *N*- and *C*-terminal imine monomers **35** and **36** reached equilibrium within 40 min in the presence of acid catalyst. This observation supports the notion that the quenching method is effective to stop metathesis and that the imine metathesis occurs negligibly in neutral organic solvents.

The magnitudes of equilibrium shifting in imine metathesis between short oligomers were obtained through NMR measurements. The data are reported as the free energy difference relative to dimer formation. For the calculation of the equilibrium constants, the imine C*H* proton peaks of both reactants and products were integrated to estimate the molar concentration of each species in the reaction mixture (Figure 2 and Table 1).[14] As seen in Figure 3, there is no equilibrium shifting in imine metathesis between short oligomers when the ligation product is shorter than a critical chain length, which is a hexamer. When the chain length of ligation products increases from the 8-mer to the 12-mer, the magnitude of equilibrium shifting linearly increases. When both reactants and products are capable of folding, the magnitude of equilibrium shifting increases asymptotically above the 12-mer.

Previously[8b] we showed that the helical conformation of phenylene ethynylene oligomers is stable at room temperature in acetonitrile above a critical length and that there is a linear correlation between folding stability and chain length. From solvent denaturation studies, the difference in the free energy between the helical and random conformations can be estimated for the *m*-phenylene ethynylene oligomers. Each additional repeat unit contributes approximately 0.7 kcal/mol of stability to the helical conformation. This has been ascribed to the fact that the folding stability is proportional to the contact area between adjacent turns in a compact helical conformation. The imine oligomers containing six or fewer repeat units are too short to fold, so there is no stable helical conformation in the reactants. The driving force for equilibrium shifting in metathesis between short oligomers is, thus, the stabilizing energy only from folding of ligation products. The intramolecular contact between monomer units of adjacent turns along the backbone is possible only after the ligation product is longer than the critical length and it increases linearly up to the point at which full contact is made between adjacent helical turns (i.e., two full turns, Figure 4a,c).

Reactant oligomers containing eight or more repeat units form a stable helix even before they undergo ligation. The driving force for ligation of prefolded oligomers is, therefore, the difference between the folding energy of products and that of reactants, which would be the additional contact between adjacent helical turns around the newly formed imine linkage in the ligation product. The additional stabilizing energy from aromatic stacking interactions is estimated to be constant, regard-

(14) The concentrations of the two reactants are identical, since the reaction starts from an equimolar mixture. For the same reason, the two products are also of equal concentration. Thus, the square of the peak intensity ratio of the product to the reactant at equilibrium corresponds to the equilibrium constant. Once equilibrium is established, one peak from the reactant and two overlapped peaks from two products are integrated after baseline correction. Integration of the reactant peak and half the value of the overlapped product peaks were used to calculate the equilibrium constants.

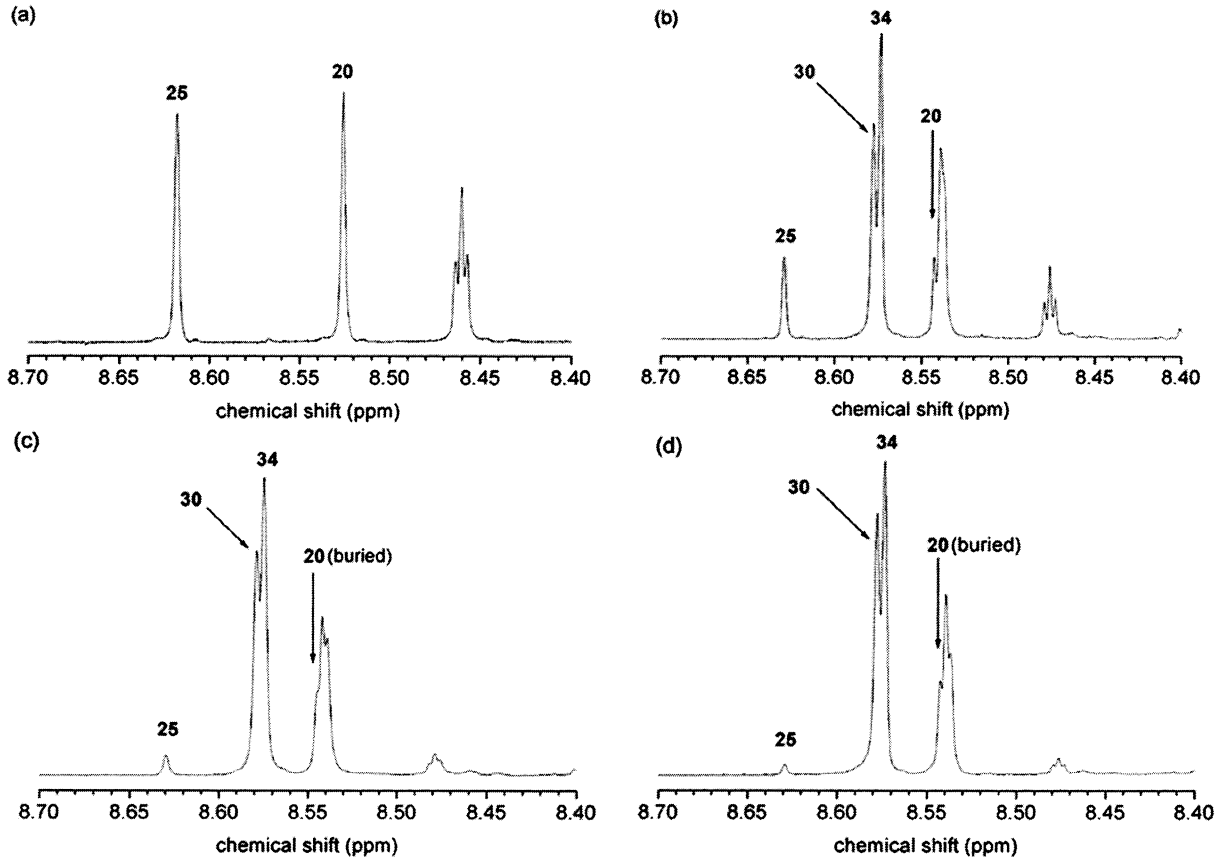

FIGURE 2. Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$, 294 K) showing the imine C*H*═N region for the reactants and products of the imine metathesis reaction. Signals from the reaction of *C*-terminal 8-mer **20** and *N*-terminal 8-mer **25** at 0 d (a), 1 d (b), 2 d (c), and 5 d (d). The equilibration was performed in acetonitrile with 5 mol % of oxalic acid relative to total imine at 294 K and an initial concentration of 5 mM for both **20** and **25**. The reaction was quenched with the resin-supported amine at a given time and the reaction mixture was dissolved in $CDCl_3$ for NMR analysis.

TABLE 1. Equilibrium Constants for Various Imine Metathesis Reactions

	reactants			K_{eq} (294 K)		
entry	*C*-terminal	*N*-terminal	ligation product	2 d	5 d	$-\Delta\Delta G$ (kcal mol^{-1})
1[a]	1-mer	1-mer	2-mer	1.1[b]		0.00
2[a]	1-mer	4-mer	5-mer	1.1 ± 0.1[b]		0.00
3[a]	6-mer	2-mer	8-mer	2.6 ± 0.1[b]		0.50
4[a]	6-mer	4-mer	10-mer	19 ± 1[b]		1.66
5[a]	6-mer	6-mer	12-mer	62 ± 2[b]		2.35
6	4-mer **18**	12-mer **27**	16-mer **28**	70	250	2.9 ± 0.4
7	6-mer **19**	10-mer **26**	16-mer **29**	260	170	3.1 ± 0.1
8	8-mer **20**	8-mer **25**	16-mer **30**	170	400	3.3 ± 0.3
9	8-mer **20**	12-mer **27**	20-mer **31**	340	220	3.3 ± 0.1
10	10-mer **21**	10-mer **26**	20-mer **32**	550	340	3.6 ± 0.2
11	12-mer **22**	12-mer **27**	24-mer **33**	580	800	3.8 ± 0.1

[a] See ref 5. [b] Equilibrium is attained after about 1 h.

less of the chain length, since the contact area between adjacent helical turns is presumably same (Figure 4b,d). Therefore, the magnitude of the equilibrium shifting is expected to be similar when the ligation products are longer than the 12-mer. The linear relationship between the magnitude of equilibrium shifting and chain length does not hold once the reactant oligomers are in helical conformation under the equilibration conditions. On the basis of the solvent denaturation studies previously mentioned, it is estimated that there is ca. 4.0 kcal/mol of folding stabilizing energy from intramolecular contacts between adjacent helical turns, assuming that there are six units in one turn.[15] This is in reasonable agreement with the observed value (3.0−3.7 kcal/mol) for the stabilizing energy measured from the imine metathesis, where the ligation product has an additional stacking interaction amounting to one helical turn compared to the reactants.

Previously[5] we showed that the imine metathesis in chloroform did not exhibit significant equilibrium shifting, regardless of the chain length. This observation has

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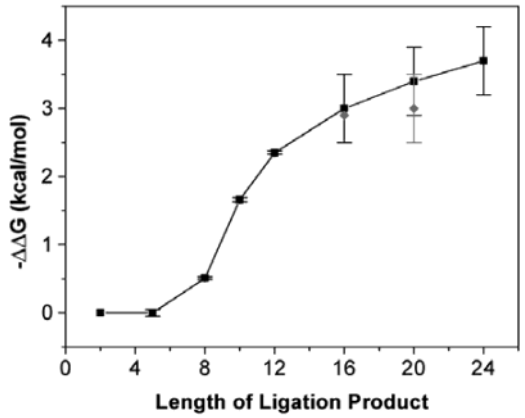

FIGURE 3. Plot of the free energy change for metathesis reactions in Table 1 versus the chain length of the ligation product with central imine bond (■) and with imine bond off-center (◆). The magnitude of the equilibrium shifting was referenced to the free energy change for dimer formation. The molar ratios were determined from the integration of the imine ^{1}H NMR signals. Error estimates are based on the standard deviation of $-\Delta\Delta G$ values measured at 2 and 5 d.

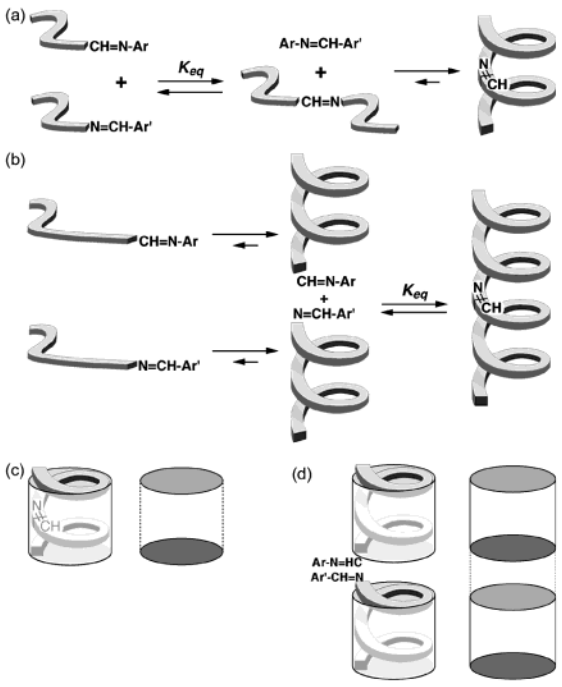

FIGURE 4. Schematic representation of imine metathesis of *m*-phenylene ethynylene oligomers between short oligomers (a) and between long oligomers (b). The equilibria for imine metathesis and the folding are coupled. The additional stabilizing energy is from the aromatic stacking of intramolecular helical turn (c) or of intermolecular helices (d).

been ascribed to the fact that imine oligomers exist in random conformational states in chloroform.[16] When the metathesis between *C*- and *N*-terminal imine 8-mers **20** and **25** was conducted in chloroform instead of acetonitrile, an equilibrium constant of 1.6 was obtained. This equilibrium constant is in reasonable agreement with those of the metathesis between oligomers of various lengths in chloroform.

In previous studies,[5,17] the imine bond was suggested to be commensurate with the backbone of oligomers in a compact helical conformation (Figure 1) and macrocycles of the *m*-phenylene ethynylenes. To test the possibility that the position of the imine bond along the backbone might affect the stability of oligomers, imine metatheses between oligomers of different length were also conducted. Isomeric ligation products could thus be compared. If the stability of product having an imine bond off-center is decreased, the magnitude of the equilibrium shifting is expected to be smaller than that for oligomers with an imine bond in the center. The results are seen in Table 1 and Figure 3 (◆ points). The equilibrium constant for imine metathesis between 8-mer **20** and 12-mer **27** to produce a 20-mer ligation product was found to be ca. 170 with a value of $-\Delta\Delta G$ of at least 3.0 kcal mol^{-1}, which is in good agreement with that of the imine metathesis between two 10-mers (3.0 kcal mol^{-1}). The position of the imine bond in the metathesis product, therefore, does not affect the equilibrium shifting of the metathesis reaction significantly. We also investigated the case where the imine bond is located at the end of the oligomer. Equilibrium constants of imine metathesis were compared where the reactants are 4-mer and 12-mer, 6-mer and 10-mer, and 8-mer and 8-mer to produce 16-mer ligation products in which the imine bond is at various positions. The folding stabilities were found to be ca. 2.9, 3.1, and 3.3 kcal/mol, respectively. The folding stability is thus not significantly dependent upon the position of the imine bond. The helical conformation is not significantly reduced when an ethynylene bond is replaced by an imine bond and the position does not significantly change the shape of helical conformation (Figure 1).

The equilibrium shifting for imine metathesis driven by folding was observed in the polymerization of bisfunctionalized imines as well.[12] When bisfunctionalized imine monomers having four repeat units were polymerized under metathesis conditions, the monomers were consumed to form dimers and sequential polymerization occurred (Figure 5). The monomer itself is too short to fold, whereas the dimer is longer than the critical chain length. For dimerization, the driving force is the intramolecular aromatic stacking interactions in ligation products of helical conformation. For subsequent steps in the polymerization, the dimer is already in a helical conformation. Thus, the driving force is aromatic stacking between adjacent helical turns around the newly formed imine bonds. This additional stabilization energy is expected to drive the polymerization of imines once all the monomer is consumed.

Conclusions

The effect of chain length on the magnitude of equilibrium shifting in imine metathesis has been investigated. The driving force for equilibrium shifting in metathesis between short oligomers is the stabilizing energy from folding of ligation products, since the reac-

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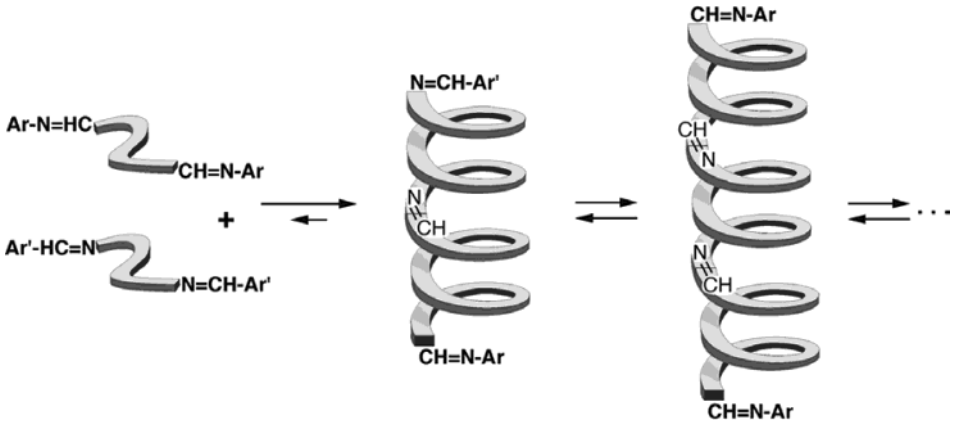

FIGURE 5. Schematic representation of reversible polymerization of bisfunctionalized *m*-phenylene ethynylene oligomers.[12] Starter sequences combine to form a product longer than the critial chain length, which is subsequently driven to high polymer.

tants are too short to fold. There is a linear correlation between the magnitude of equilibrium shifting and chain length below a critical length, since the contact area increases linearly, as the product chain grows to 12-mer, where the ligation product makes full contact between adjacent helical turns. On the other hand, reactant oligomers having eight or more repeat units are capable of folding so that the driving force for metathesis is the difference between the folding energy of products and that of reactants. In the limit of long oligomer reactants, this corresponds to the contact between adjacent helical turns around the newly formed imine linkage in the ligation product. The measured values are in reasonable agreement with the difference in the free energy between the helical and random conformations observed from solvent denaturation studies of *m*-phenylene ethynylene oligomers. Imine metatheses between oligomers of different length producing isomeric ligation products have been conducted to explore the effect of imine position on the equilibrium shifting. The position of the imine bond in the ligation product has been observed to have no significant effect on the folding stability and the magnitudes of equilibrium shifting. We therefore concluded that imine metathesis between oligomers longer than a critical length can be used to generate a dynamic, unbiased pool with respect to chain length and the position of the dynamic linkage, providing an ideal system to test ideas for the generation of tyligomers and masterpiece sequences.

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Supporting Information Available: General methods, quenching study of imine metathesis, decay time study, and partial ^{1}H NMR spectra showing the imine C*H*=N region for the reactants and products of the imine metathesis reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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