Development and Applications of Organic/DNA Hybrid Materials

Undergraduate Researcher
Jonathan R. Davila, Northwestern University

Faculty Mentor
SonBinh T. Nguyen
Department of Chemistry, Northwestern University

Graduate Student Mentors
Julianne Gibbs and Christine Dettmer
Department of Chemistry, Northwestern University

Abstract

The development and applications of organic/DNA molecules for the use of DNA detection systems are reported here. In particular, two different hybrids are synthesized: DNA/polymer hybrids and small-molecule DNA hybrids. DNA/polymer hybrids are especially useful considering that their functionality may be varied according to the polymer selected. The hybrids are formed via ring-opening metathesis polymerizations (ROMP) and polymer termination of cyclooctene (COE) with a nitrobenzyl ester group. The nitrobenzyl ester capping agent serves two purposes: (1) it contains an acetate group that can be modified with DNA in two steps, and (2) it allows characterization using ^{1}H and ^{13}C NMR spectroscopy and UV-Vis spectroscopy. However, the capping agent produced an active carbene that reacted with the olefins along the polymer backbone, thus cleaving the polymer and yielding undesired polymer chain lengths. To circumvent this problem, the time allowed for capping the polymer was decreased. Additionally, an aliquot of the COE polymer was removed before capping to use as a control. Unexpected polymer chain lengths and high poly-dispersity indices (PDIs) showed that the carbene was still too active, although M_n (number average molecular weight) improved for the capped polymer with decreasing reaction times. Secondly, small-molecule DNA hybrids ($SMDH_n$) have been designed with novel DNA geometries that could enhance the cooperativity of the resulting duplexes. Specifically, two $SMDH_2$ precursors have been produced where the two DNA strands on the small molecules are separated by 180º and 120º. These are now ready to couple to DNA, and thermal denaturation experiments can be performed to determine the effects of orientation on the duplex melting.

Introduction

In the past several years, much emphasis has been placed on the synthesis and utilization of nano-sized organic/bioorganic materials. Particularly, DNA/nanoparticle composites have proved useful in the field of DNA detection. Currently, commercial DNA detection systems rely on the molecular recognition abilities of single oligonucleotides to bind with complementary target strands.[1] Most systems signal the presence of target DNA using radioactivity, fluorescence, electrochemistry, or colorimetry.[1,3-5] One challenge ahead is to create a reusable, inexpensive, highly sensitive detection system. Electrochemical methods fulfill these requirements. In particular, this report will discuss the efforts attempted to synthesize a capping group for the polymer that contains (1) a group that can be modified with DNA, (2) a double bond that can undergo cross-metathesis with the polymer Ru alkylidene, and (3) a tag that can be characterized using NMR spectroscopy and UV-Vis spectroscopy. Additionally, the methods in creating 180° and 120° $SMDH_2$ in preparation for cooperativity enhancement experiments are described.

Background

Electrochemical systems have been the principal methods of DNA detection in the Nguyen research group. Significant progress has been made in the development of polymer-biomolecule hybrids with both recognition capabilities and electrochemical activity, using standard DNA coupling procedures.[2-3] In particular, two electrochemically active components have successfully been incorporated in the synthesis of DNA-modified polymers. By varying the ratio of the electrochemically active monomers, a variety of redox signals may be encoded into the polymer strands, creating a unique "bar code" for the attached DNA sequence.[3] The current focus of our research involves the incorporation of gold nanoparticles (GNP) into this "bar code" detection system. Storhoff (1998) demonstrated that using oligonucleotide-modified gold nanoparticles and a conventional flatbed scanner for analyzing DNA arrays has increased the sensitivity of detection.[4] The signal amplification method is based on the nanoparticle-promoted reduction of silver.[4] However, the silver-staining process renders the DNA detection system nonreusable. Theoretically, electrochemistry will be reusable with the incorporation of gold nanoparticles. The system would particularly involve coupling DNA with electrochemically active polymers and a GNP. The DNA/polymer/GNP hybrid would be complementary to one end of the target DNA. Another strand would be attached to an electrochemically active surface and would be complementary to the other end of the target DNA. In the presence of the target strand, the DNA/polymer/GNP hybrid, the attached strand, and the

target strand would form a duplex. Cyclic voltametric methods would detect the presence of the target strand by utilizing the electrochemically active polymer. The DNA/polymer/GNP hybrid not only enhances the signal but may also be reused by melting the strands off. Previously, the Nguyen research group reported the ROMP of norbornene derivatives off of the surface of gold nanoparticles.[4] The group intends to extend the DNA-polymer methodology to the GNPs. The development of this unique inorganic/organic/DNA material may lead to highly selective, sensitive, and nondestructive DNA probes, giving way to promising detection systems.

Thermal properties of DNA probe materials have also proved important for selectivity of DNA detection systems[4] (Figure 1). The research group has shown that the attachment of multiple DNA strands to a single polymer chain leads to exceptionally stable hybridized duplexes. Interestingly, the hybridized duplexes also exhibit sharp melting transitions that allow for a selective hybridization condition that maintains the complementary duplexes while strongly destabilizing any mismatched duplexes.[5] To date, polymers with five DNA strands have been the primary probes investigated. Because enhanced stability was observed with only a small number of oligonucleotides per polymer, it was questioned how many strands were required to generate such sharp melting transitions. Small molecule hybrids ($SMDH_n$) have been designed with various numbers of strands (n) and varying intrastrand angles. Specifically, $SMDH_2$ (two DNA strands per small molecule) have been chosen, considering the exciting possibility that two strands would be enough to exhibit cooperativity.

Approach

In making DNA-polymer conjugates, a block of alcohol moieties was included in the polymer; the terminal -OH groups were then modified with tetraisopropylphosphorodiamidite and attached to the DNA using syringe synthesis.[2] Many attempts in the Nguyen group have been made to combine the nanoparticle-polymer composites with the DNA-polymer conjugates. Successful attempts were made in polymerizing monomers containing alcohol functional groups from the surface of GNPs, but the resulting materials after precipitation were insoluble in all solvents. It is speculated that after precipitation, the functionalities interact too strongly with one another to be solvated. The best result involved performing all four steps of the synthesis in situ without isolating any of the intermediates. This strategy has obvious drawbacks. None of the intermediates can be purified or thoroughly characterized, making optimization of the synthesis very difficult.

It was then hypothesized that having fewer alcohol groups around the nanoparticle would make the material soluble in organic media. To circumvent the problem of solubility that polyalcohol-modified GNPs exhibit, it was decided to functionalize the end of the polymer chain with hydroxyl groups, rather than including those functionalities in the side chains of the polymer. Specifically, an olefin with a nitrobenzyl ester substituent was selected. The ester can be hydrolized into an alcohol, and the nitrobenzyl group serves as a tag that can be monitored using both NMR and UV-Vis spectroscopy. The syntheses of symmetrical and unsymmetrical capping groups are also described. For a proof of concept, the capping ability of the ester-substituted olefin was examined during the polymerization of cyclooctene (COE). Finally, the capped polymer was converted to an alcohol and treated with chlorophosphoramidite in preparation for DNA coupling.

The second part of the research investigated how many oligonucleotides, and with what orientation, were required to achieve sharp and increased melting transitions like those seen for the DNA-modified polymers and GNPs. Two $SMDH_2$ with different angles between DNA-modified groups were synthesized to investigate their thermal properties. The first $SMDH_2$ synthesis involved the standard Sonagashira coupling of 1,4-diethynylbenzene with two equivalents of 4-bromobenzylacetate, forming a linear structure. The para-orientation of the acetylene benzyl alcohol moieties would generate the 180° angle between the five-ft termini of the DNA. The second synthesis consisted of coupling 1,3-diethynylbenzene with 4-bromobenzylacetate to form a 120° structure. Additionally, the transesterification of the alcohol was outlined. The synthesis of 4-bromobenzyl acetate was also reported.

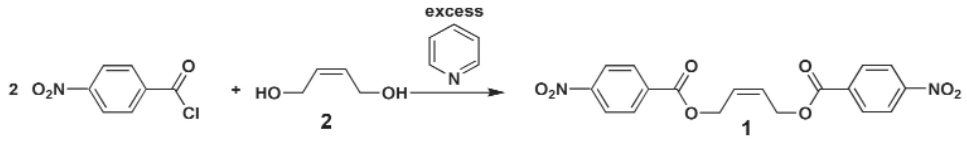
Results and Discussion

Synthesis of Compound 1 and Use in Polymer Termination

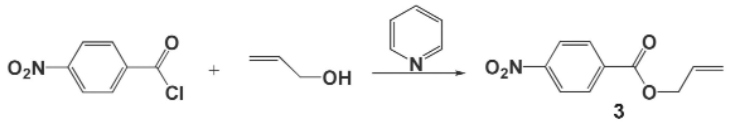
It has been reported that ROMP polymers can be terminated with olefins modified with acetate groups.[6] To improve the ability to characterize these capping substituents, UV-active, acetate-modified olefins were synthesized. The basic reaction in the synthesis of the diacetato olefin, compound **1**, is an acid chloride reaction with an alcohol. A 2:1 ratio of 4-nitrobenzoyl chloride and the olefin, (Z) 2-buten-1,4-diol, **2**, respectively, was

List of Schemes and Figures

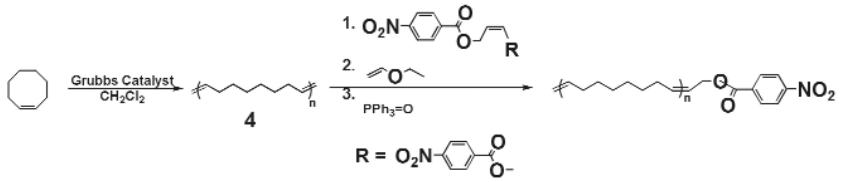
Scheme 1



Scheme 2



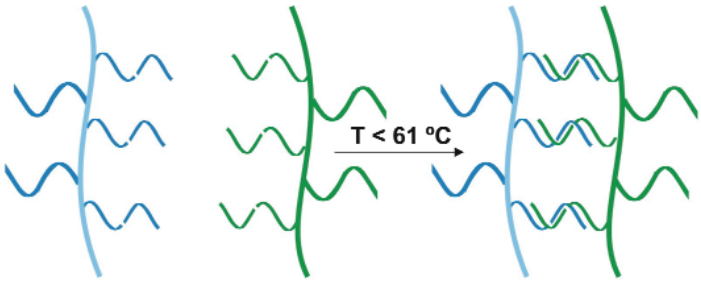
Scheme 3

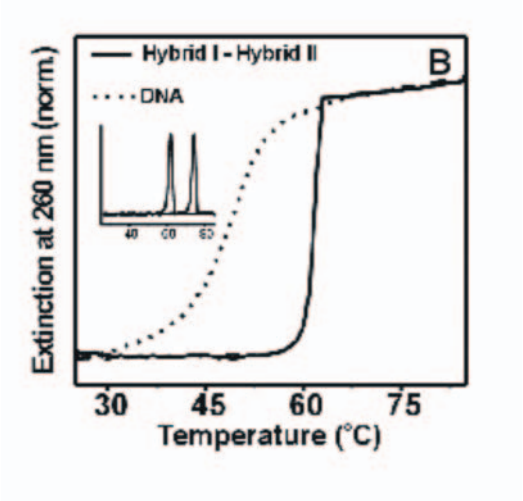


#	Product	R	Reaction Time for **2** (h)	Reaction Time for **3**	Expected Molecular Weight	Actual Molecular Weight
5	2	R_1	~ 1	-	$5.0x10^4$	$1.28x10^5$
6	3	R_1	~ 1	~ 3 h	$5.0x10^4$	$6.0x10^3$
7	2	H	~ 1	-	$6.0x10^3$	$5.45x10^3$
8	3	H	~ 1	~ 30 min	$6.0x10^3$	$2.47x10^3$
9	2	H	~ 1	-	$6.0x10^3$	$6.30x10^3$
10	3	H	~ 1	~ 5 min	$6.0x10^3$	$8.43x10^3$

List of Schemes and Figures

DNA/polymer hybrid cooperativity and thermal Denaturation absorbance vs. Temperature graph





used with excess pyridine as a base (Scheme 1). This resulted in an olefin with the nitrobenzoyl ester groups on both ends. The reaction was carried out under nitrogen using Schlenk techniques.

According to thin-layer chromatography (TLC) of the mixture, the reaction was complete after two hours. The compound was chromatographed using a silica-filled glass column and characterized by ^{1}H NMR spectroscopy. To prove that the compound was in fact UV-Vis active, the absorbance of compound **1** was determined. Several concentrations diluted in tetrahydrofuran were examined, and the spectra showed two maxima, one at 264 nm and the other at 274 nm. The extinction coefficient was effectively calculated by using a linear trend line to find the slope of the line and the R-squared value. The slope of the line and the R-squared value were found for a line with the y-intercept equal to zero and for the best fit line. The extinction coefficient of the compound when the y-intercept is set equal to zero is found to be 20,258. The extinction coefficient at the naturally occurring y-intercept is found to be 19,874, which may be due to error.

The polymerization of cyclooctene was selected as the polymerization system because it does not have any aromatic protons on the side chains, which could overlap in the aromatic protons of the capping agent. Using ROMP by Grubbs catalyst, $Cl_2(PCy_3)_2RuCHCHPh_2$, the desired polymer chain length can be controlled by the stoichiometry of the reaction.[7] Additionally, Grubbs catalyst should react with the olefin **1**, thus terminating the polymer with the UV-active ester. The general scheme of capping the cyclooctene polymer, **4**, with the capping substituent is described in Scheme 3.

The capping agent was added to the reaction and allowed to react for three hours. Ethyl vinyl ether was then used to quench the polymerization. Triphenylphosphine oxide was finally added to aid in the removal of byproducts as reported by Yu Mi Ahn et al.[8] A 57:1 ratio of Grubbs catalyst to COE was used, resulting in compound **6**. ^{1}H NMR spectrum verified the success of the capping agent. However, the spectrum showed many impurities, and the integration of the peaks indicated that polymer chains were much shorter.

Synthesis of Compound 7 and Use in Polymer Termination

It has been shown in cross-metathesis that the least-substituted side of the alkene tends to attach to the polymer of interest.[9] Thus, in keeping with the literature to ensure polymer termination, an unsymmetrical capping group, compound **3**, was synthesized. A similar reaction to the symmetrical capping agent involved an acid chloride reaction with an alcohol containing a terminal alkene, Scheme 2. Again, the reaction was performed under nitrogen and monitored via TLC.

The capping of the polymer was performed following the same procedure as Scheme 3. However, an aliquot of the polymerization before capping was removed to use as a control when analyzing the length of the polymer. In the first trial, 453 equivalents of Grubbs catalyst to COE were used for a polymer molecular weight, M_n, of 5.0×10^4. Following a similar procedure by Gibson and Okada,[7] the capping mixture was allowed to stir for three hours. ^{1}H NMR confirmed the presence of the capping group. Gel-permeation chromatography results showed that the noncapped COE polymer, **5**, had a molecular weight of 1.28×10^5 and a poly-dispersity index (PDI) of 1.8. The target PDI would be 1.0, giving a monodisperse sample of polymers with the same chain lengths. The GPC results are relative to polystyrene, so the important comparison is between the aliquot and the capped polymer. The capped polymer **6** had a much smaller M_n value of 6.0×10^3 and a PDI of 1.8. This means the capped polymer was 1/25 the size expected. It was hypothesized that the reaction of the chain-transfer agent and Grubbs catalyst led to an active form of the catalyst, which led to backbiting of the polymer. Backbiting is not acceptable because it would cleave the polymer from the surface of the nanoparticle, rendering the capped polymer useless.

In the second trial, the same procedure was used. However, 54 equivalents of the catalyst to COE were used, and the capping mixture was allowed to react for one hour. Although a M_n of 6.0×10^3 was expected, the control COE polymer **7** revealed an actual M_n of 5.45×10^3 and a PDI of 2.15, while the capped COE polymer **8** resulted in a M_n of 2.47×10^3 and a PDI of 1.61. Although the M_n was closer to what was expected, the PDIs of both increased. The capped polymer still indicated that backbiting occurred, but based on the M_n, it was approximately half the length of the aliquot, a large improvement from the previous reaction. Again, it was believed that the reaction time should be decreased further.

In the third trial, the same equivalents were used. The only change made was the reduction to five minutes for the capping reaction. Both polymers showed improved results where the control polymer **9** had a M_n of 6.3×10^3 and a PDI of 1.99, and the capped polymer **10** had a M_n of 8.43×10^3 and a PDI of 1.51.

Although the polymers resulted in unexpected lengths, the capping agent did successfully terminate the polymer. Perhaps selecting an enol ether to inactivate the catalyst would improve the quality of the polymers.

Further Functionalization of Capped Polymers

In spite of the backbiting problem, the first capped polymer **6** made was prepared for DNA coupling by deprotecting the alcohol moiety, isolating the polymer, and then reacting it with chlorophosphoramidite. ^{31}P NMR spectroscopy was used to monitor the success of the reaction. A peak at 150.213 ppm confirmed the presence of the phosphordiether, which effectively proved that it had coupled with the polymer.

$SMDH_2$ Design Synthesis of Precursors

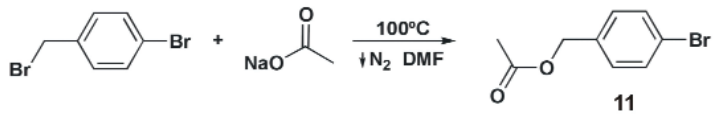
The first precursor for our series of small-molecule DNA hybrids requires a 4-bromobenzyl acetate. The acetate compound **11** was synthesized from 1,4-dibromobenzene and sodium acetate as shown in Scheme 4. The reaction was carried out under nitrogen in dimethyl formamide and heated overnight at 100°C. The product was characterized using ^{1}H NMR spectra.

To produce the 180° $SMDH_2$ precursor, a Sonagashira coupling reaction was performed between 1,4-diethynylbenzene and compound **11** (Scheme 5). The catalyst byproducts were precipitated and removed by filtration, and the crude product was purified by column chromatography. The acetate **12** was converted to an alcohol using sodium methoxide in excess methanol (Scheme 6). Column chromatography resulted in purified compound **12**, which was confirmed by ^{1}H NMR.

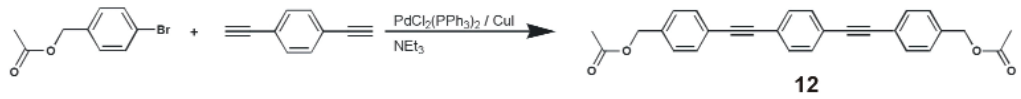
To fabricate the 120° $SMDH_2$ precursor, a procedure similar to the 180° small molecule was performed (Schemes 7 and 8).

List of Schemes and Figures

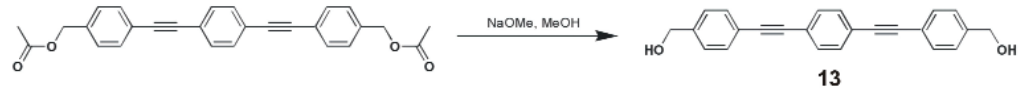
Scheme 4



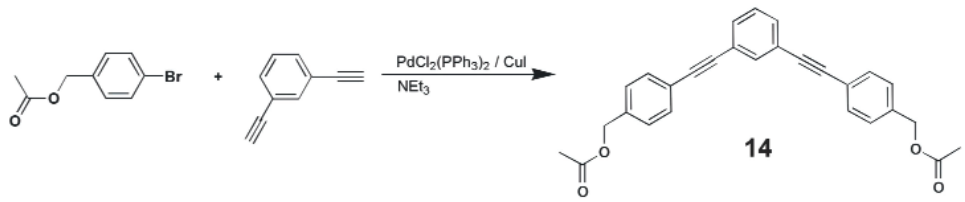
Scheme 5



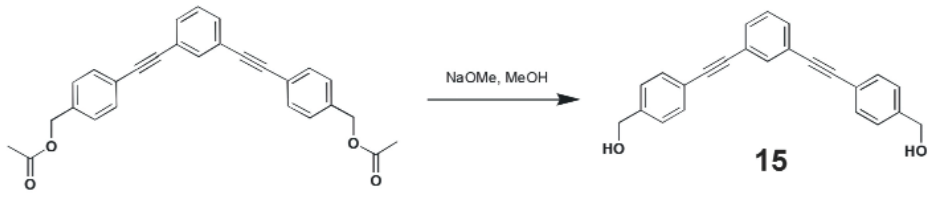
Scheme 6



Scheme 7



Scheme 8



The 120° compound **15** resulted from the Sonagashira coupling of 1,3 diethynylbenzene with compound **11**. The $SMDH_2$ precursors can now be coupled to DNA following modification with chlorophosphoramidite. Thermal denaturation experiments will be performed on the complementary pairs of each set of SMDHs.

Conclusions

The results of these experiments present several syntheses of organic/DNA hybrid materials. These materials can be used in DNA detection or for exploring the properties of organic/bioorganic molecules. Based on the GPC results of the capped compound with the shortest capping reaction time, the olefin may be a viable capping agent. However, if the PDIs cannot be improved, a different chain transfer agent that deactivates the catalyst may have to be synthesized. One possibility is to create a different capping agent that would contain an enol ether to allow reaction with the carbene in Grubbs catalyst to inactivate it. In another area of DNA-based materials, two different $SMDH_2$ precursors were successfully developed. The two precursors vary in bond angles, so the DNA duplexes formed during hybridization should have different orientations, which could affect the cooperativity. Further work includes coupling them to DNA and performing thermal denaturation experiments to investigate their aggregation ability. Investigating thermal behavior and cooperativity of $SMDH_2$ while varying intrastrand angles, rigidity of the small-molecule compounds, and number of DNA strands per molecule could lead to a greater understanding of cooperativity in organic hybrid materials.

References

(1) Taton, A. T.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757-60.

(2) Watson, K. J.; Park, S. J.; Im, J. H., et al. *J. Am. Chem. Soc.* **2001**, *123*, 559-93.

(3) Watson, K. J.; Zhu, J.; Nguyen, S. T., et al. *Pure Appl. Chem.* **2000**, *72*, 67-72.

(4) Storhoff, J. J.; Elghanian, R.; Mucic, R. C., et al. *J. Am. Chem. Soc.* **1998**, *120*, 1959-64.

(5) Park, S. J.; Taton, A. T.; Mirkin, C. A. *Science* **2002**, *295*, 1503-6.

(6) Hillmyer, M. A.; Nguyen, S. T.; Grubbs, R. H. *Macromolecules* **1997**, *30*, 718-21.

(7) Gibson, V.; Okada, T. *Macromolecules* **2000**, *33*, 655-56.

(8) Ahn, Y. M.; Yang, K.; Georg, G. *Org. Lett.* **2001**, *3*, 1411-13.

(9) Engelhardt, F. C.; Schmidtt, M. J.; Taylor, R. E. *Org. Lett.* **2001**, *3*, 2209-12.