Development of Bioorganic Polymer Composites for the Directed Assembly of Functional Nanomaterials

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Abstract

This report describes the development of bioorganic small-molecule complexes for the directed assembly of functional nanomaterials. In particular, it examines the synthesis of small acetylene-based organic precursors for attaching DNA to form DNA/organic hybrids. This work continues previous studies demonstrating how DNA/polymer hybrid composites have sharper melting points than DNA alone, which may prove useful in advancing DNA-detection systems. The *para*-substituted diacetylene organic molecule described here has a unique structure that allows for further attachment of DNA.

Introduction

While disease-detection methods have always been important, recent concerns over biological and chemical attacks in combination with disease epidemics have brought the need for fast and accurate disease detection to the forefront. Anthrax, SARS, and AIDS have affected millions of people across the globe, raising fears that the next disease will spread to pandemic proportions. The need for quick and effective detection, assessment, and diagnosis of diseases such as these is a primary concern in the scientific community.

Biologists and chemists are combining their knowledge of molecular engineering and biology to develop methods that can better detect bacteria and viruses. Many disease-causing agents can be detected using DNA-detection systems, which rely on the molecular recognition capabilities of single oligonucleotides (small single strands of DNA) to bind with complementary target strands on the bacteria and viruses. Most of these detection systems signal the presence of target DNA using radioactivity, fluorescence, electrochemistry, or colorimetry.[1–5] Electrochemical methods can also be used to create a reusable, inexpensive, and highly sensitive detection system.[6]

DNA nanotechnology takes advantage of DNA's ability to form a double helix and unusual structural motifs through precise Watson-Crick base pairing.[7] This article will discuss the synthesis and characterization of small organic molecules that can be modified with DNA by using standard DNA-coupling procedures. Understanding how to attach DNA to these small organic molecules in order to influence their subsequent binding to target DNA strands is key to improving DNA detection. Nucleic acids have the potential to guide the recognition and assembly processes in a variety of hybrid materials, as well as to space building blocks in a precise linear array.[8]

The research reported here focuses on devising a synthetic pathway to obtain 1,4-*para*-substituted diacetylene (Figure 1), a rigid linear molecule. Oligonucleotides can then be added to the ends of this small organic molecule in a highly programmable manner. Oigonucleotides are a powerful tool in the synthesis of novel

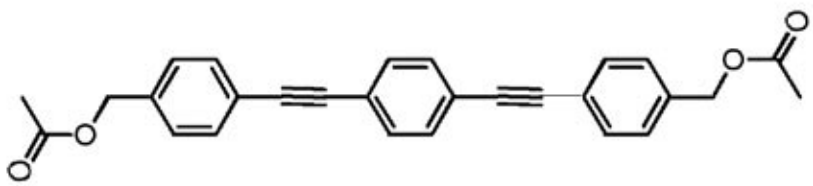

Figure 1: 1,4-para-substituted diacetylene 1.

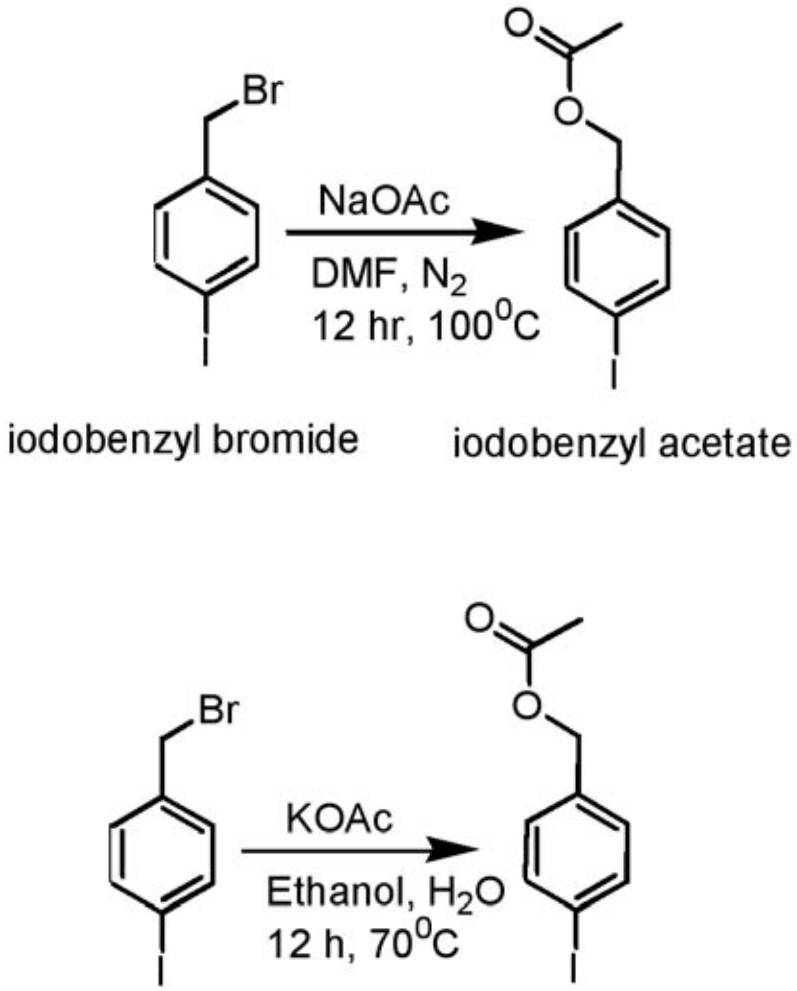

Figure 2: Synthesis of 4-iodobenzyl acetate.

DNA/organic architectures due to their base specificity, sequence programmability, and rigidity.[9] Hence 1,4-*para*-substituted diacetylene-containing self-complementary DNA sequences should spontaneously assemble by hybridization to form long linear arrays.

Background

In recent years electrochemical systems have emerged as primary tools of DNA detection. Polymer-bioorganic molecule hybrids with both recognition capabilities and electrochemical activity have been synthesized using standard DNA-coupling procedures.[2–3] In the work described here, DNA is attached to electrochemically active polymers that can be used in detecting small DNA sequences. The DNA/polymer hybrid, which is designed to complement one end of the target DNA strand, is exposed to an analyte solution in the presence of an Au electrode. The solution contains a capture strand that complements the other end of the target DNA strand. If the target strand is present in the analyte, the DNA/polymer hybrid, the capture strand, and the target strand form a duplex on the electrode, which can then be detected via the electrochemical activators of the polymer. Small DNA duplexes tend to have a broad melting transition, but the addition of the polymer backbone has been shown to make the melting curve sharper.[2]

At present the reason behind the sharp melting points of the DNA/polymer hybrid are not fully understood; we also do not know the optimal number of DNA strands required for the sharp melting transition. Knowing these parameters may lead to the design of highly selective, sensitive, and non-

destructive DNA probes, which in turn may lead to promising detection systems.[6] To this end the Nguyen group has been working to perfect the synthesis and characterization of small-molecule DNA hybrids.

Approach

The synthesis of 1,4-*para*-substituted diacetylene 1 consists of four steps. First, iodobenzyl bromide and potassium acetate are combined to synthesize the needed iodobenzyl acetate. Next, a Sonogashira coupling reaction of iodobenzyl acetate with (trimethylsilyl) acetylene produces the trimethylsilyl-protected acetylene. In the third step this molecule is deprotected to obtain the starting material for the final step, the Sonagashira coupling of the acetylene to dibromobenzene to synthesize 1. The first step of the process yielded the best results when KOAc in ethanol was used rather than NaOAc in DMF (Figure 2). Each reaction was optimized for yield. The first three steps went to completion with decent yields.

Results and Discussion

Synthesis of 4-iodobenzyl acetate
In an air-free environment potassium acetate (4.99 g , 5.1 x 10^{-2} mol) and 4-iodobenzyl bromide (10.084 g, 3.4 x 10^{-2} mol) were refluxed for 15 hr in ethanol (200 mL) and water (20 mL). Upon cooling to room temperature the mixture was poured into water (200 mL) and extracted with ether (3 x 100 mL). The organic portions were collected, washed with water (3 x 200 mL), dried over sodium sulfate, and filtered. The solvent was removed using rotary vaporization. Purification of the residue on silica using 30% CH_2Cl_2 in hexanes as an eluent

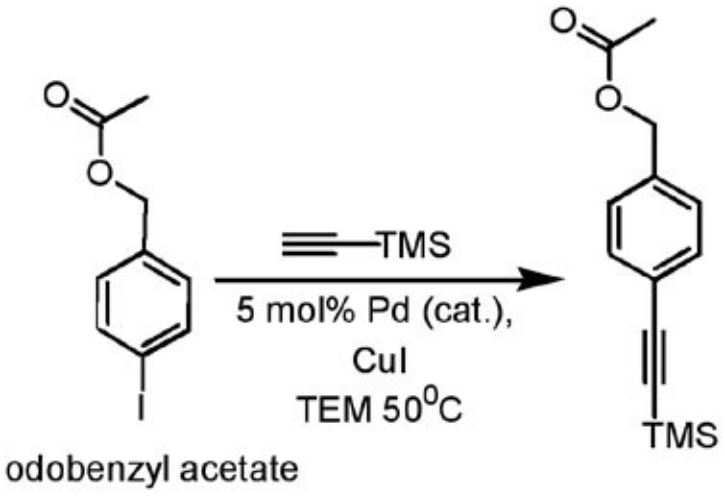

Figure 3: Synthesis of TMS-protected acetylene 2.

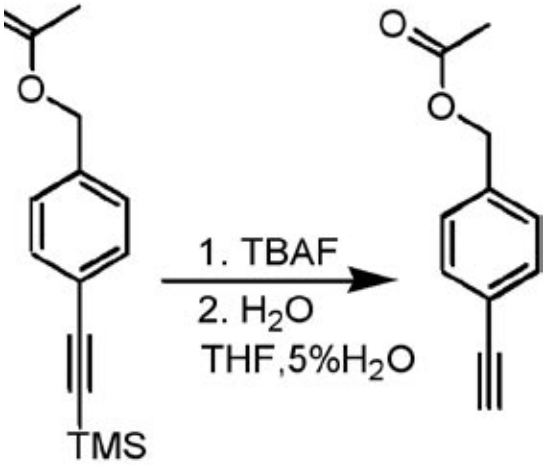

Figure 4: Deprotection of TMS from acetylene 2.

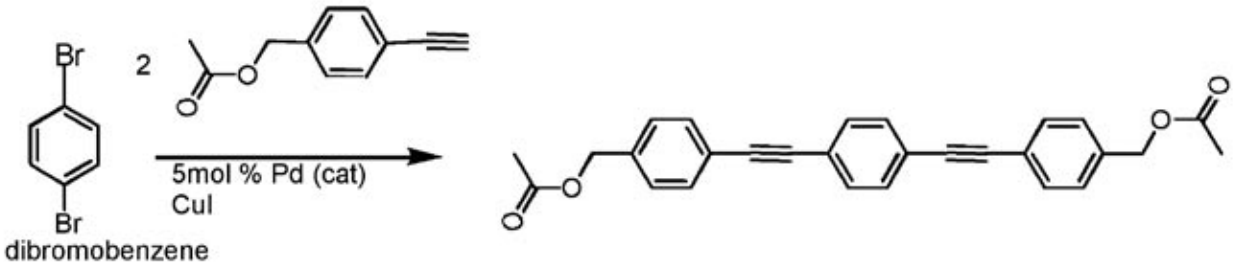

Figure 5: Synthesis of 1,4-*para*-substituted diacetylene 1.

yielded the desired product as a white solid (8.413 g, 0.0305 mol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.69 (d, 2H), 7.10 (d, 2H), 5.04 (s, 2H), 2.09 (s, 3H).

Synthesis of 4-(trimethylsilylethynyl) benzyl acetate (TMS-protected acetylene)
As illustrated in Figure 3, a Sonogashira reaction coupling 4-iodobenzyl acetate to (trimethylsilyl) acetylene (TMS) was performed in a glove box using a 5 mol% CuI, Pd catalyst reaction in triethylamine (TEA). Then, in a 50 mL Schlenk flask the 4-iodobenzyl acetate (1.995g, 0.0065 mol) was combined with CuI (0.246 g, 3.5 x 10^{-4} mol) and dichlorobis (triphenylphosphine) palladium (II) 98% catalyst (0.246 g, 3.5 x 10^{-4} mol). The mixture was placed in the glove box, and trimethylsilane was added (1.226 mL, 0.0087 mol). Triethylamine was added slowly with stirring and heated at 50°C for 15 hr. Upon cooling to room temperature the TEA was removed by rotary vaporization. The mixture was dissolved in diethyl ether and filtered. The filtrate was concentrated by rotary vaporization. Purification of the residue on silica using 30% CH_2Cl_2 in hexanes as eluent yielded the product (0.6961 g, 0.0028 mol, 86%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): 7.45 (d, 2H), 7.27 (d, 2H), 5.07 (s, 2H), 2.09 (s, 3H), 0.24 (s, 9H).

Synthesis of 4-ethynyl benzyl acetate
As illustrated in Figure 4, the TMS-acetylene complex (0.696 g, 0.0028 mol) in THF (5% H_2O) was added slowly with stirring to tetrabutylammonium fluoride (TBAF) in 1M THF solution (3.08 mL, 1.1 mol equivalents). The reaction was monitored by thin-layer chromatography [5% TEA, 5% ethyl acetate, 20% CH_2Cl_2, 70% hexanes] over 20 min. Upon completion of the reaction the solution was diluted with diethyl ether and washed with water (2 x 100 mL). Extraction was performed and the solution was rinsed with ethyl acetate. The organic portion was collected, dried over $NaSO_4$, filtered, and concentrated by rotary vaporization. A brown gel was obtained and then put under vacuum for 30 min to remove any additional solvent. The resulting brown crystals were dissolved in hexanes and recrystallized to yield the desired product as a brown solid (0.345 g , 0.00198mol, 90 %). ^{1}H NMR (400 MHz, $CDCl_3$): 7.485 (d, 2H), 7.308 (d, 2H), 5.096 (s, 2H), 3.084 (s, 3H), 2.109 (s, 9H).

Synthesis of 1,4-bis[(p-acetatomethyl)phenyle thynyl] benzene
As illustrated in Figure 5, a Sonogashira coupling reaction was performed to synthesize the 1,4-*para*-substituted acetylene in a glove box. Dibromobenzene (0.162g, 6.857 x 10^{-4} mol) was added, combined with deprotected acetylene (0.250g, 1.44 x 10^{-3} mol) in a Schlenk flask. 5 mol % of CuI and Pd catalyst were added slowly to the flask, followed by the slow addition of TEA (100 mL). The reaction mixture was stirred overnight at 50°C. Upon cooling to room temperature the TEA was removed by rotary vaporization. The remaining black solid was dissolved in ethyl acetate and filtered. The solvent was removed by rotary vaporization. Thin-layer chromatography was performed using 15% ethyl acetate, 15% methylene chloride, and 70% hexanes. ^{1}H NMR spectroscopy was performed to characterize the yellow solid product.

Conclusions

The results of these experiments present several steps for synthesizing the starting materials needed to synthesize the 1,4-*para*-substituted acetylene molecule. These

materials can be attached to DNA using standard DNA-coupling procedures. The materials synthesized here can be used in DNA detection systems and may contribute to creating new biomaterials with a number of unique uses. Further work includes synthesizing 1,4-*para*-substituted acetylene and removing the triple bond to provide more flexibility in the structure of the DNA/polymer hybrid. Work will continue to improve the synthetic pathway to obtain higher yields as well as other small molecules.

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