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# Masked Michael Acceptors in Poly(phenyleneethynylene)s for Facile Conjugation

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ABSTRACT: Poly(phenyleneethynylene)s (PPEs) capable of reacting with thiol-containing molecules have been designed and synthesized with number-average molecular weights ranging from 8000 to 11 000. The PPEs contain pendent masked maleimide units available to participate in conjugate additions and Diels−Alder chemistries after being thermally activated. If the maleimide group is left unmasked during palladium-catalyzed cross-coupling polymerization, it leads to side reactions and the production only of short chain oligomers ($M_n$ < 3000, DP ∼ 4). Reversible Diels−Alder reactions between the maleimides and furan were determined to be a very effective method to produce a masked Michael acceptor that can be unveiled after polymerization under relatively mild thermal conditions. Cycloreversion to the maleimide has been monitored by thermogravimetric analysis (TGA), TGA-MS, IR, and NMR. A PPE containing the masked maleimide unit has been modified with a thiolated carboxy-X-rhodamine (ROX) dye, and the resulting absorbance and fluorescence spectra as well as gel permeation chromatograms (GPC) are presented.

## Introduction

The ability of poly(phenyleneethynylene)s (PPEs) to transport and funnel energy has resulted in their utilization as chemosensors both in solution and in the solid state.[1] Conjugated polymers have successfully been developed to sense nitroaromatics, such as 2,4,6-trinitrotoluene (TNT), and quinones via photoinduced electron transfer from the polymer excited state to the bound analyte, resulting in a turnoff sensor from the nonradiative recombination of electron−hole pairs.[2,3] Biological binding events have also been successfully detected via fluorescent labeling and Förster energy transfer processes.[4−7] To expand the scope and sensory applications of PPEs, we have been interested in developing methods for their conjugation to biomolecules. The most commonly used methods for the conjugation of biomolecules to surfaces or other molecules generally involve addition and substitution reactions with the amine of lysine or the thiol of cysteine.[8] We recently reported reactions with lysine residues in the context of protease detection.[9] The thiol functionality, however, presents greater challenges as typical polymerization methodologies are incompatible with conventional thio-acceptors, which generally have reactive alkenes and halides. Cysteine conjugation reactions most often employ maleimides, vinyl sulfones, iodoacetamides, and orthopyridyl disulfide units.[10] We have targeted the maleimide group due to the facile conjugate addition of thiols across the electron-deficient double bond.

An additional attribute is the excellent dienophile character of the maleimide; it readily participates in reversible [4 + 2] Diels−Alder reactions under relatively mild thermal conditions.[11,12] This reversibility has additional utility for producing reversibly cross-linked elastomers and plastics[13] and thermally “remendable” cross-linked polymeric materials.[14,15] Early work utilizing the Diels−Alder reaction with maleimides was carried out by Stille and co-workers to polymerize bis(maleimide)s and bis(cyclopentadiene)s.[16] Herein we report a polymer bearing masked Michael acceptors that can be thermally unveiled, thereby allowing thiol addition reactions or Diels−Alder chemistries.

## Results and Discussion

PPEs are typically prepared by palladium-catalyzed Sonogashira−Hagihara cross-coupling of 2,5-dialkoxy-1,4-diiodobenzenes with aryl dialkynes. Having targeted PPEs with pendent maleimides, we initially investigated the compatibility of this group with the Sonogashira−Hagihara reaction conditions.[17] When maleimide groups were present during polymerization, only low molecular weight oligomers ($M_n$ ∼ 3000, DP ∼ 4) were obtained, which is suggestive of Heck-type side reactions.[18] It was therefore necessary to protect the maleimide during polymerization so that the reactive double bond is only revealed when needed. We took advantage of the facility with which maleimide partakes in Diels−Alder reactions and used furan to mask the maleimide.

Two separate synthetic pathways were developed for masked-maleimide-containing aryl diiodide monomers (Scheme 1). For the preparation of alkoxy-containing monomers the first step is alkylation of methoxyhydroquinone to yield **2**, which can be iodinated under acidic conditions to give **3** in 73% yield (Scheme 1A). During our investigations, it was discovered that *tert*-butylmercaptan, *t*-BuSH, in the presence of base will selectively react with aryl methoxy groups without interfering with longer chain primary aryl alkoxy groups to yield the corresponding phenolic groups via an $S_N2$ reaction. Using these conditions, **4** was obtained in 87% yield. Williamson ether synthesis with an excess of the alkyl dibromide affords **5** in 52−85% yield. Compound **6** is synthesized quantitatively from maleimide in neat furan, yielding ∼2:1 ratio of endo:exo isomers at room temperature; alkylation of the furan-protected maleimide **6** yields **7** (63−82%).[19] The alternative two-step approach of initially attaching the maleimide moiety with subsequent addition of furan led to lower yields than the direct introduction of the masked maleimide **6**. The mixture of endo and exo adduct isomers produces a polymer with greater solubility.

As a deprotecting agent, *t*-BuSH is not selective for the removal of methyl groups in the presence of 2-[2-(2-methoxy-

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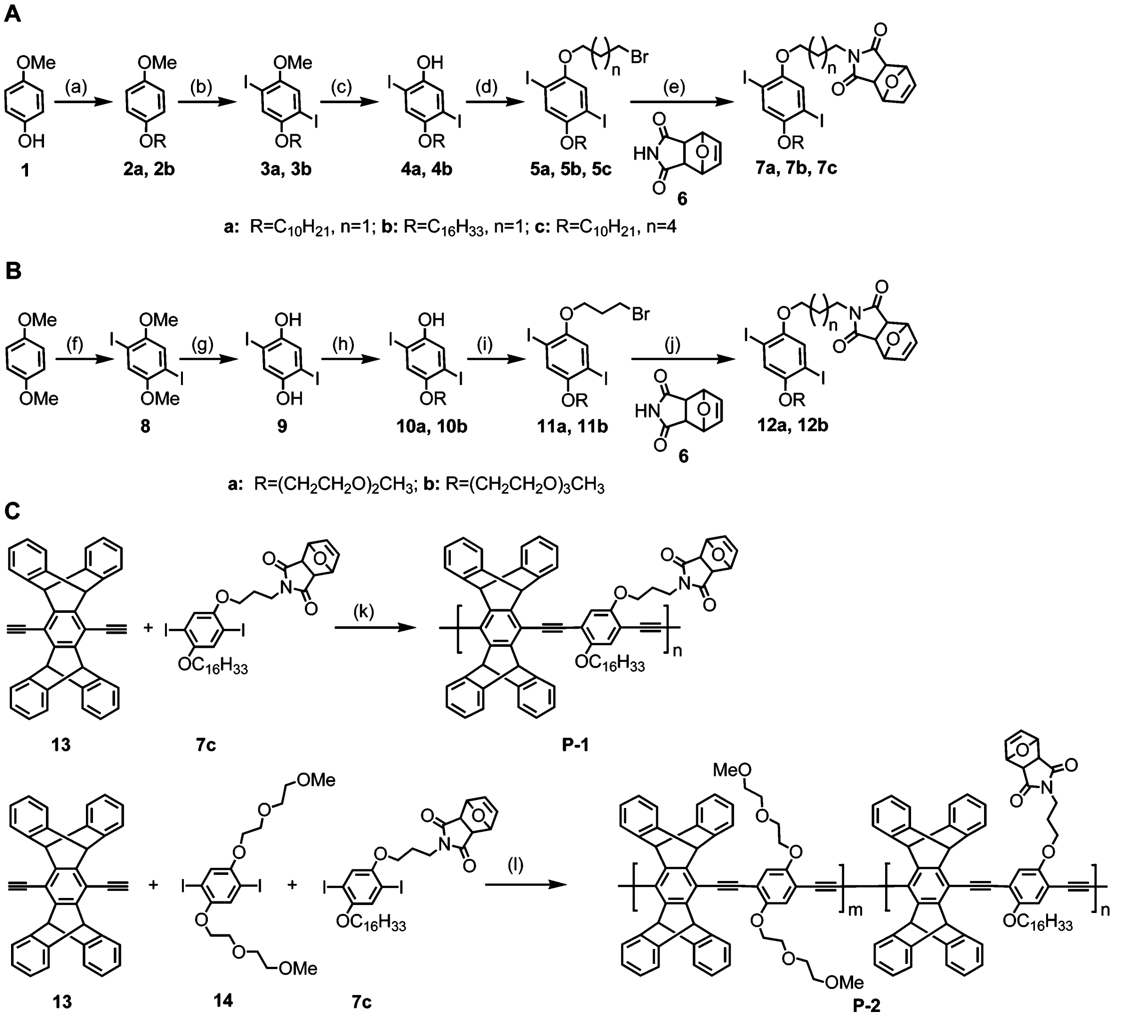
**Scheme 1**[a]



[a] (A): (a) $K_2CO_3$, RBr, acetone; (b) $I_2$, $KIO_3$, $H_2SO_4$, AcOH, $H_2O$, ~70%; (c) *t*-BuSH, NaH, DMF, ~90%; (d) $K_2CO_3$, $BrCH_2(CH_2)_nCH_2Br$, acetone, 52−85%; (e) $K_2CO_3$, acetone, 63−82%. For synthesis of **6** please see Supporting Information, 95%. (B): (f) $I_2$, $KIO_3$, $H_2SO_4$, AcOH, $H_2O$; (g) $BBr_3$, $CH_2Cl_2$, −78 °C, quench with water, quantitative; (h) 1 equiv of NaH, DMF, ROTs. 8−25%; (i) 1,3-dibromopropane, $K_2CO_3$, refluxing acetone, 41−62%; (j) $K_2CO_3$, DMF, 21−42%. (C): (k) catalytic $Pd(PPh_3)_4$ and CuI, toluene, diisopropylamine, 1-methyl-2-pyrrolidinone, room temperature 5 days, ($M_n$ = 11 000); (l) catalytic $Pd(PPh_3)_4$ and CuI, toluene, diisopropylamine, room temperature 7 days ($M_n$ = 8000).

ethoxy)ethoxy]ethoxy or the shorter 2-(2-methoxyethoxy)ethoxy chains. Therefore, an alternative synthetic route was developed (Scheme 1B). Compound **9** is synthesized quantitatively in two steps from 1,4-diiodo-2,5-dimethoxybenzene using boron tribromide to remove both methyl groups from **8**. Monoalkylation of the hydroquinone occurs in relatively low yields (8−25%) to generate **10**. Subsequent alkylation with a dibromide yields **11** (41−62%), to which **6** is added in the presence of base to produce **12** in 21−42% yield.

Among the many advantages of this design is that the solubility of the polymer can be tailored by changing the R group, i.e., an alkyl vs 2-methoxyethoxy chains. In addition, the distance of the maleimide group from the polymer backbone can be controlled by introducing different chain lengths of the dibromide in the synthesis of **5** or **11**. This linkage can also be varied to influence polymer solubility.

Polymers were prepared by step-growth polymerization using the Sonogashira−Hagihara cross-coupling reaction depicted in Scheme 1C.[17] All dialkyne (**13**, **14**) monomers were prepared using established literature procedures.[20,21] The pentiptycene dialkyne comonomer was used for the superior physical properties that it produces in the resulting polymer for incorporation into chemosensors, such as improved fluorescence in thin films, greater solubility, and the ability to prevent chain aggregation. The aryl diiodide and a slight excess (1−2%) of the aryl dialkynes in the presence of catalytic amounts of palladium tetrakis(triphenylphosphine) and copper iodide are dissolved in a suitable solvent with an amine base and stirred for 5−7 days. The polymerization is restricted to room temperature to prevent the Diels−Alder adduct from undergoing cycloreversion. The polymers were precipitated and dried in vacuo overnight. The diiodide monomer **14** was introduced to form statistical copolymer **P-2**, thereby reducing the loading of masked maleimide along the polymer backbone. In addition, polymer **P-3** was prepared with no masked maleimide units. The polymers obtained varied in molecular weight from 8000 to 11 000. For the NMR spectrum of **P-2**, please refer to the Supporting Information. Because of solubility limitations, a suitable NMR spectrum could not be obtained for **P-1**.

The thermal decomposition of polymers and monomers was monitored by TGA and TGA-MS, and representative data are presented in Figure 1. The monomers each displayed a distinct two-step loss attributed to loss of one isomer of the Diels−Alder adduct (exo or endo) followed by loss of the other between ~70 and ~140 °C. TGA-MS data were obtained for monomers and **P-2**; data for monomer **7b** are presented in Figure 1A. Two masses were monitored: 68 for furan and 39 for a furan fragment. Loss of furan clearly coincides with each of the two-

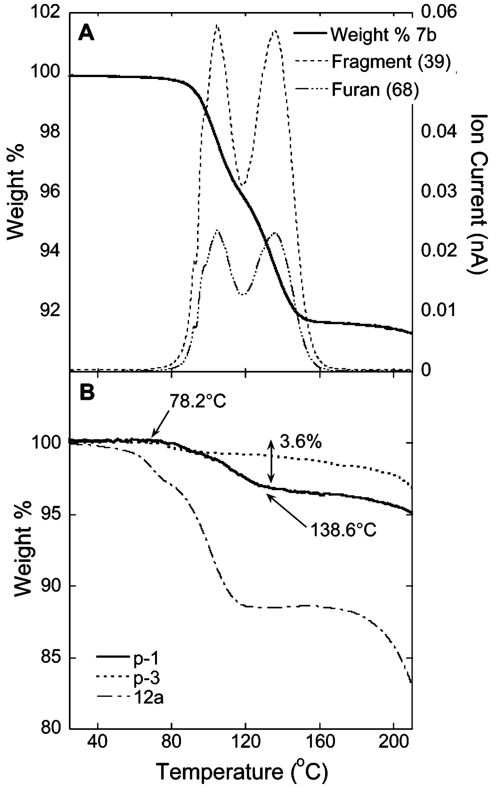


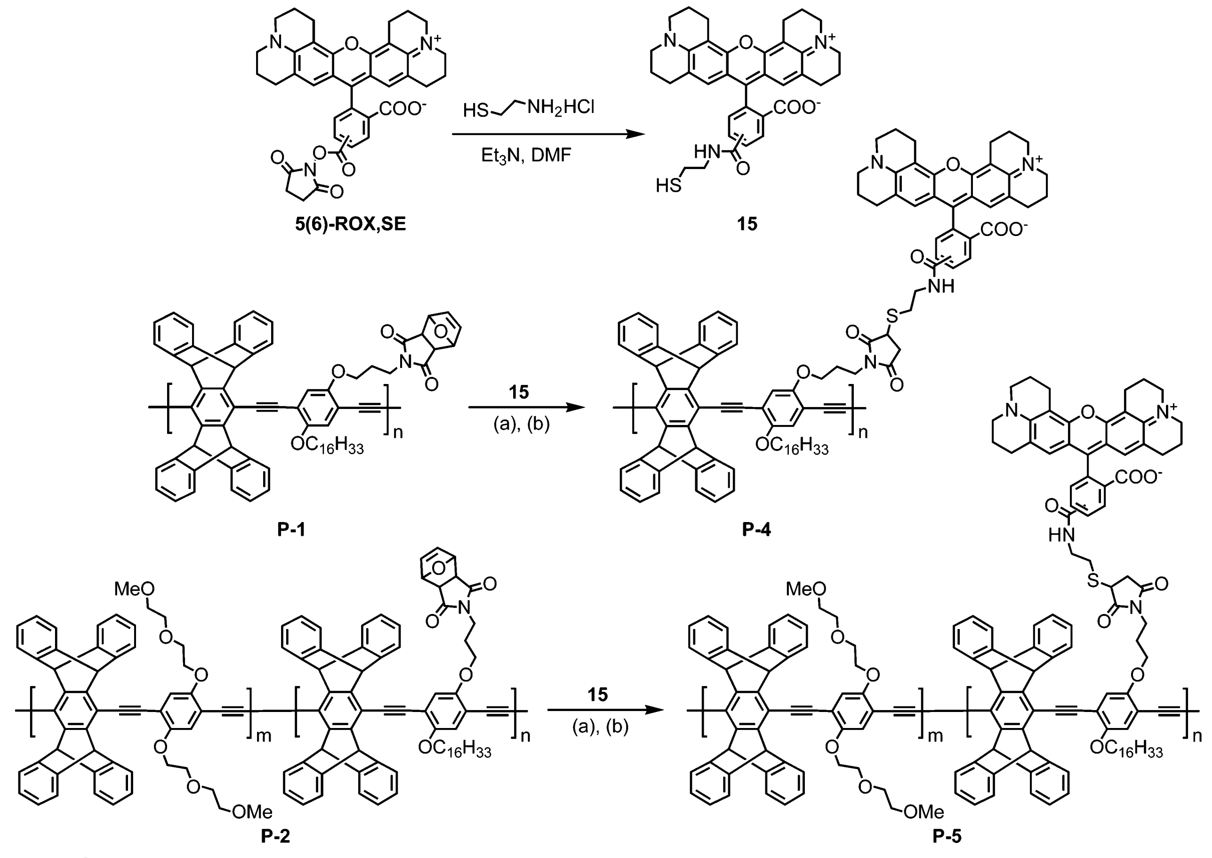
**Figure 1.** (A) TGA-MS for **7b**; monitoring at 68 for furan and at 39 for a known furan fragment (ramp rate: 5 °C/min to 300 °C). (B) Thermogravimetric analysis for **P-1**, **P-3**, and **12a** (ramp rate: 0.1 °C/min to 300 °C).

step losses of mass while heating between ~70 and ~140 °C. TGA data for compound **12a**, which has a ~1:2 endo:exo ratio, indicate initial loss of the endo isomer (Figure 1B). The expected weight loss for **12a** was 10.2%, and an experimental loss of 11.0% is observed. The two-step loss pattern was also observed for all of the polymers containing the masked maleimide groups. The TGA analysis showed that for all polymers the observed weight losses were consistently lower than predicted. For instance, the predicted weight loss from cycloreversion is 6.7% for **P-1**; however, only 3.6% was observed experimentally. This discrepancy is rationalized as the result of a broad range of local environments experienced in the solid state by the adducts. Unmasking of monomers in solution is quantitative as monitored by proton NMR, and the temperatures required to remove furan were considerably milder (60−70 °C). In addition, **P-2** was monitored by IR before and after heating. Three bands attributed to cyclic ether stretches disappeared after heating: 1105, 1023, and 754 $cm^{-1}$.

To explore the ability of tethering thiols to the polymers, a carboxy-X-rhodamine(ROX) dye was synthesized to contain a free sulfhydryl group, **15** (Scheme 2). Polymers **P-1** and **P-2** were refluxed in THF, followed by the introduction of a methanol/THF solution of **15** and dithiothreitol (DTT), which is needed to reduce any disulfide linkages present. Because of the partial solubility of the resulting polymers in methanol, precipitation was not possible, and so the polymers were purified by preparative GPC. The change in solubility is thought to be the result of addition of a large number of methanol-soluble dyes to the polymers under these conditions.

The progress of the reaction of thiolated ROX dye **15** with **P-2** was monitored by absorbance and fluorescence spectra as well as GPC analysis (Figure 2). In Figure 2A is the absorbance and fluorescence spectra for the addition of the dye in THF. The characteristic double-peak absorbance of ROX at 500 and 540 nm is clearly visible in addition to the absorption maximum

**Scheme 2**[a]



[a] (a) 65 °C; (b) **15**, DTT, 65 °C.

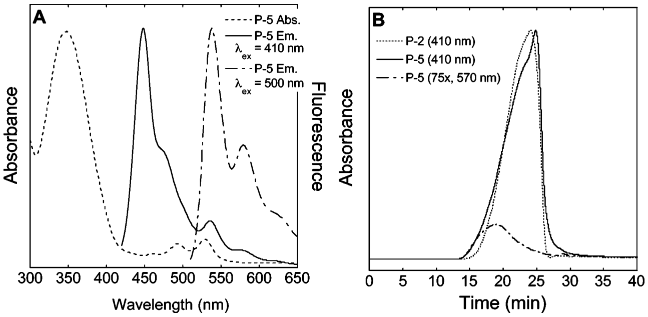


**Figure 2.** (A) Normalized absorbance and emission spectra for **P-5** after exposure in THF to thiolated-ROX dye, **15**, with excitation at both 410 and 500 nm. (B) Normalized GPC trace for polymers **P-2** and **P-5**, monitoring at 410 and 570 nm (absorption at 570 nm scaled 75×).

of **P-2**. The emission of the ROX dye is observed upon direct excitation of the dye at 500 nm and also upon excitation of the polymer at 410 nm, a feature that is a signature of light harvesting by the polymer and energy transfer to the dye. Repeated attempts at determining the extent of dye loading by NMR failed due to the inability to produce samples concentrated enough to obtain suitable spectra. The peaks for protons resulting from the conjugate addition of dye were not observed. However, the chemical shifts for these protons occur in regions with a high density of peaks from the polymer, namely from 2 to 4 ppm and 6 to 8 ppm. IR attempts were limited due to the weakness of the C−S stretch.

The GPC chromatograms of polymers **P-2** and **P-5** are shown in Figure 2B. The onset of dye absorption occurs simultaneously with that of the polymer, indicating that the dye is bound to the polymer. Absorption at 570 nm is significantly weaker due to a lower concentration of the dye relative to the polymer. The ROX dye bound to the polymer elutes at minute 23, while the free dye elutes at minute 30. The difference in shape of the chromatogram when monitoring at 410 and 570 nm could be attributed to the dye being nonuniformly distributed as a function of molecular weight. High-molecular-weight polymers may undergo an aggregation process that is molecular weight dependent and distorts the chromatogram.

Extent of loading of the dye was determined from the relative absorbance of the polymer and the dye in GPC chromatograms. The absorbance at 450 nm for the polymer and 570 nm for the dye were compared, normalized using the extinction coefficients at the two respective wavelengths. Loadings of 1−8.5% dye/polymer ratios were observed for polymer **P-5**, which correlates to 5−42% of maleimide groups modified with the dye. The lower loadings were observed at lower temperatures and in the absence of methanol, which was added to increase the solubility of the dye. For calculations and extinction coefficients please refer to the Supporting Information.

## Conclusion

PPEs containing masked maleimide groups capable of partaking in Michael addition and Diels−Alder chemistries have been synthesized. The furan protecting group for the maleimide moiety must be present during polymerization to prevent it from participating in side reactions. Furan can be removed quantitatively postpolymerization in solution under relatively mild thermal conditions via cycloreversion. The resulting unmasked polymer can be generated in situ and then functionalized with a compound containing a thiol or diene. As a result of the modularity of PPE synthesis, a large library of PPEs can be synthesized and incorporated into a wide range of applications, including, but not limited to, biosensers and chemosensors.

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**Supporting Information Available:** All monomer syntheses, polymer syntheses, NMR and IR characterization of **P-2**, GPC results for **P-4**, extinction coefficient values, and loading calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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