

# CARBOHYDRATE FUNCTIONALIZED POLYTHIOPHENES AS BIOSENSORS

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## Introduction

Conjugated polymers are well suited to be sensors because they possess collective properties which are sensitive to small perturbations<sup>1</sup>. Biosensors aim to capitalize on nature's "lock and key" mechanisms while simultaneously reporting molecular recognition.

This work seeks to expand on previous conjugated polymer based biosensors while exploring new directions<sup>2</sup>. Many pathogenic organisms bind to the target cell surface using carbohydrate receptors<sup>3</sup>. Adhesion of bacterial cells to host cells is a requirement of infection. Many strains of bacteria possess proteinaceous appendages. These appendages include receptors for specific carbohydrates and provide the mechanism by which bacteria bind to cells. Our work functionalizes regio-regular polythiophenes with mannopyranosides. The change in the visible absorption spectrum of the polymer solutions will be recorded before and after the addition of biomolecules.

## Experimental

**Synthesis of 3-(6-bromohexyl) thiophene.** In dry hexane 3-bromothiophene (20g, 0.12 mole) was dissolved and the resulting solution cooled to  $-40^{\circ}\text{C}$ . Butyllithium (0.12 mole) was added drop-wise. After 15 minutes of stirring, 30 ml of THF was added followed by 1,6 dibromohexane (75ml, 0.49 mole). The mixture was allowed to warm to room temperature and an additional 60 ml of THF was added. The reaction was allowed to stir overnight. This solution was extracted with ether, washed with water, and concentrated.

**Synthesis of 2-bromo-3-(6-bromohexyl) thiophene.** 3-(6-bromohexyl) thiophene (24.8g, 0.10 mole) was dissolved in 1:1 THF and acetic acid. NBS (39g, 0.22 mole) was added and the reaction allowed to stir overnight. The resulting solution was extracted with ether, washed with  $\text{NaCO}_3$ , and concentrated.

**Synthesis of HT-poly (3-(6-bromohexyl) thiophene).** In a dry round bottom flask were placed 1.6 ml (12mmol) dry diisopropylamine and 25 ml of dry THF. The solution was cooled to  $-70^{\circ}\text{C}$ . After adding 4.0 ml of butyllithium (10 mmol), the solution was warmed to  $0^{\circ}\text{C}$ . The reaction mixture containing LDA was cooled to  $-70^{\circ}\text{C}$  and 3.26g (10 mmol) of 2-bromo-3-(6-bromohexyl) thiophene was added. The mixture was stirred for 40 minutes at  $-55^{\circ}\text{C}$  and then cooled to  $-70^{\circ}\text{C}$ . To this mixture 1.37g (10 mmol) of  $\text{ZnCl}_2$  was added. When the  $\text{ZnCl}_2$  dissolved, the mixture was warmed to  $-5^{\circ}\text{C}$  and 20 mg (0.2 mmol)  $\text{Ni(dppp)Cl}_2$  was added. The mixture was warmed to room temperature, stirred for 4 hours, and the reaction quenched with methanol. The polymer was washed with methanol and hexanes.

**Synthesis 2,5-poly(3-(2(4,4-dimethyloxazoline-2-yl)-heptyl)thiophene.** Dry 2,4,4-trimethyloxazoline (2.55 ml, 20 mmol) was dissolved in dry THF and cooled to  $-70^{\circ}\text{C}$ . To this solution, butyllithium (7.2 ml, 18 mmol) was added drop-wise. The solution was stirred at  $-70^{\circ}\text{C}$  for 2 hours. In 30 ml of dry THF, poly (3-(6-bromohexyl) thiophene) was dissolved. The polymer solution was added to the oxazoline lithium salt solution. The cooling bath was removed, and the mixture was allowed to stir for one hour. This reaction was quenched, and the resulting polymer washed, in hexanes.

**Synthesis of 2,5-poly(3-octanic acid thiophene).** 2,5-poly(3-(2(4,4-dimethyloxazoline-2-yl)-heptyl)thiophene) was dissolved in 3 M HCl and heated to reflux for 4 hours. The solution was filtered and washed with water.

**Synthesis of O-acetyl-p-nitrophenyl mannopyranoside.** p-nitrophenyl mannopyranoside (0.5g, 1.66mmol) was dissolved in THF. Acetic anhydride (1ml, 10.5 mmol) and pyridine (1ml, 12 mmol) were added. This solution was stirred overnight, extracted with diethyl ether, washed with water, and concentrated.

**Synthesis of O-acetyl-p-aminophenyl mannopyranoside.** O-acetyl-p-nitrophenyl mannopyranoside was dissolved in methanol and reduced with  $\text{H}_2$  using Pd/C.

**Synthesis of mannose functionalized HT poly (3-octyl thiophene).** O-acetyl-p-aminophenyl mannopyranoside and 2,5-poly(3-octanic acid thiophene) were coupled using DCC.

## Results and Discussion

Regio-regular poly thiophene derivatized with acid moieties were synthesized.

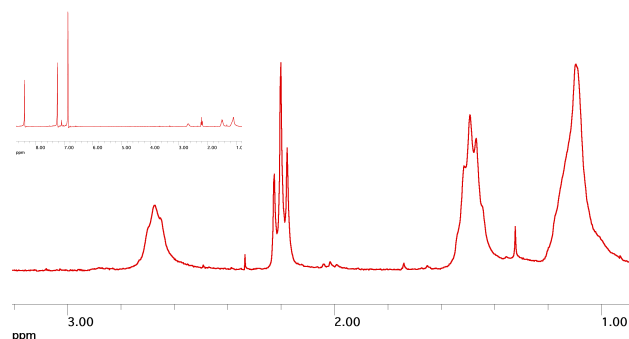


Figure 1.  $^1\text{H}$  NMR of 2,5-poly(3-octanic acid thiophene).

4-nitrophenyl mannopyranoside was protected with acetyl groups and the nitro groups reduced with Pd/C.

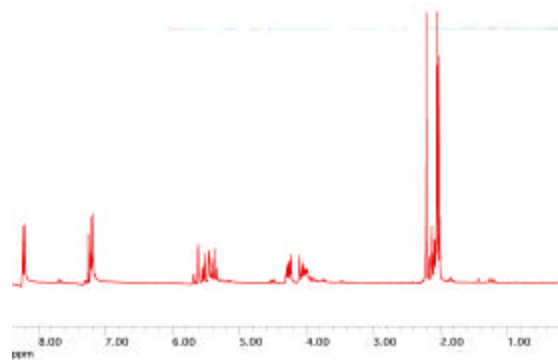


Figure 2.  $^1\text{H}$  NMR of 4-nitrophenyl mannopyranoside.

The acid bearing polythiophene will be covalently linked to the mannopyranoside using standard peptide coupling protocols.

## Conclusions

Polythiophenes offer a route to biosensors. The ability to modify the conjugated backbone through interactions of covalently attached side-chains provides for the development of thiophene based biochromic sensors.

## Reference

- (1) McQuade, D.T.; Pullen, A.E.; Swager, T.M. *Chem Rev.* **2000**, 100, 2537.
- (2) Baek, M.G.; Stevens, R.C.; Charych, D.H. *Bioconjugate Chem.* **2000**, 11, 777.
- (3) Bertozzi, C.; Bednarski, M. *Carbohydrate Research* **1992**, 223,243.