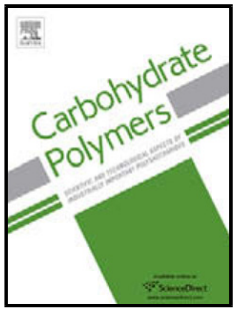

Title: Chemo-bacterial synthesis of conjugatable glycosaminoglycans

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Chemo-bacterial synthesis of conjugatable glycosaminoglycans

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Highlights

- Conjugatable (non-sulfated) chondroitin and heparosan were synthesized in recombinant Escherichia coli
- Furyl lactose was used as precursor and was taken in charge by recombinant glycosyltransferases
- Furyl polysaccharides were characterized by NMR spectroscopy
- Efficient conjugation using a Diels-Alder cycloaddition reaction in aqueous and catalyst-free conditions was confirmed using *N*-methylmaleimide as model dienophile

Abstract

Conjugatable glycosaminoglycans hold promise for medical applications involving the vectorization of specific molecules. Here, we set out to produce bacterial chondroitin and heparosan from a conjugatable precursor using metabolically engineered *Escherichia coli* strains. The major barrier to this procedure was the glucuronylation of a lactosyl acceptor required for polymerization. To overcome this barrier, we designed *E. coli* strains expressing mouse β-1,3-glucuronyl transferase and *E. coli* K4 chondroitin and and K5 heparosan synthases. These engineered strains were cultivated at high density in presence of a lactose-furyl precursor. Enzymatic polymerization occurred on the lactosyl precursor resulting in small chains ranging from 15 to 30 kDa that accumulated in the cytoplasm. Furyl-terminated polysaccharides were produced at a gram-per-liter scale, a yield similar to that reported for conventional strains. Their efficient conjugation using a Diels-Alder cycloaddition reaction in aqueous and catalyst-free conditions was also confirmed using *N*-methylmaleimide as model dienophile.

Keywords: Heparosan; ; ; ; , chondroitin, recombinant *E. coli*, enzymatic synthesis, click chemistry

1. Introduction

Bacterial glycosaminoglycans (GAGs) are capsular components found in some pathogenic and probiotic bacteria (DeAngelis, 2002). GAGs offer exciting perspectives for medical applications because they are natural, non-immunogenic, biocompatible polymers with promising potential as nanocarriers for drug delivery, medical device coatings or conjugating vehicles to extend the plasma half-life of therapeutic proteins (DeAngelis, 2015). The latter application in particular requires the production of conjugatable GAGs to enable their efficient coupling with the molecules to be