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# Carbohydrate mediated drug delivery: Synthesis and characterization of new lipid-conjugates

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ABSTRACT

A new synthetic methodology for cationic glycolipids using *p*-aminophenyl-α-D-mannopyranoside (PAPM) and *p*-aminophenyl-α-D-galactopyranoside (PAPG) with spacer in between the quaternary nitrogen atom and the sugar unit is developed. In addition, a new class of neutral glycolipid conjugates, such as PAPM–lipids or PAPG–lipids conjugates was also synthesized for targeting drugs to receptors. The precipitation–inhibition assay showed that conjugate of PAPM inhibited the concanavalin A and invertase aggregation. This binding inhibition study of a synthesized compound suggests that conjugates of PAPM can be potentially used to target mannose receptors. In addition, a higher transfection was obtained by mixing PAPM with pSV-β-gal reporter gene and incubating with mannose binding protein/receptor expressing A549 cells. The coexistence of both mannose group and a net positive charge may result in improved transfection efficiency in cells expressing mannose binding proteins/receptors.



## 1. Introduction

Carbohydrates derivatives have been known to be involved in variety of biological functions. Cell surface carbohydrates are involved in numerous biological functions, including cellular recognition, adhesion, cell growth regulation, cancer cell metastasis and inflammation (Dwek, 1996). They also serve as an attachment sites for infectious bacteria, viruses, toxins, and hormones that result in pathogenesis (Varki, 1993). Cell surface-bound receptors represent suitable entry sites for drug delivery into cells by receptor-mediated endocytosis. Targeted drug delivery capitalizes on the presence of specific cell surface receptor mediated endocytosis (Cotton and Wagner, 1993; Perales et al., 1994; Wu and Wu, 1987). The presence of mannose receptors on a variety of macrophages such as peritoneal, alveolar and in Kupffer cells is well documented (Imber et al., 1982; Stahl et al., 1978; Schlessinger et al., 1978). Synthetic carbohydrate polymers containing fucosylamine and mannosamine have been targeted to mouse leukemia L1210 cells, and macrophages, respectively (Rathi et al., 1991; Duncan et al., 1986). These specific carbohydrate-based molecules could be applied as drug or gene delivery carriers. For example, the sulfated polysaccharide, heparin, plays an essential role in blood coagulation (Linhardt and Toida, 1997).

Several researchers are of the opinion that macrophages may serve as a secondary drug carrier for the delivery of liposomal drugs (Ahmad et al., 1989; Morgan et al., 1985). The role of macrophages in the uptake process is quite evident from the increased deposition of liposomal drug products in cells (Ahmad et al., 1989). The studies indicated that amphotericin B incorporated into liposomes composed of egg phosphatidylcholine and 1,2-dipalmitoyl-3-phosphoethanolamine mannose (EPC/DPPE-Man) is less toxic as compared to the drug in non-mannosylated EPC liposomes. This was related to the fact that mannosylated liposomes are more rapidly taken up by the macrophages compared with the non-mannosylated ones; thus less time is available for such liposomes to interact with sensitive red blood cells, resulting in less toxicity (Ahmad et al., 1991). Liposome-based drug formulation ligated with mannose successfully demonstrated targeting of therapeutic drug to the disease site.

Cationic liposomes bearing covalently grafted receptor specific ligands, having so called "homing devices", on their surfaces are also capable of delivering their genetic payloads to specific cells in the body. Many reported specific cationic glycolipid based transfection vectors based on the covalent grafting of the cyclic pyranose form of the D-galactose ligands onto their polar head-group region (Kawakami et al., 1998, 2000a,b; Fumoto et al., 2004; Shigeta et al., 2007; Sun et al., 2005; Mangit et al., 2005; Hwang et al., 2001;

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Hashida et al., 2001; Gaucheron et al., 2001; Plank et al., 1992). Following the above concept, we have synthesized novel cationic *p*-aminophenyl-α-D-mannopyranoside (PAPM) **1a** and *p*-amino phenyl-α-D-galactopyranoside (PAPG) **1b** (Fig. 1) containing spacer in between the sugar head and positively charged nitrogen atom, for gene or drug delivery. A different chemistry is used to conjugate lipids in view of targeting to specific cells. Cell specific receptor such as mannose or galactose can be effectively utilized s for selective delivery of drugs by employing synthetic sugar–lipid conjugates, such as *p*-(tetradecanoylamido) phenyl α-D-mannopyranoside **2a** and *p*-(tetradecanoylamido) phenyl α-D-galactopyranoside **2b** and analogs; and *p*-(3-cholesterylimido) phenyl-α-D-mannopyranoside **3a** and *p*-(3′cholesterylimido) phenyl-α-D-galactopyranoside conjugate **3b** (Fig. 1). Here we describe the complete synthesis and structural characterization by $^1$H NMR and high resolution mass spectroscopy (HRMS) along with precipitation–inhibition assay of synthesized compound as an example with Concanavalin A.

## 2. Materials and methods

*p*-Nitrophenyl α-D-mannopyranoside and *p*-nitrophenyl α-D-galactopyranoside were purchased from Toronto Research Chemical, Inc., Toronto, ON, Canada. Anhydrous solvents were purchased from Sigma–Aldrich and used without further drying. Reagents of the highest commercial quality were purchased and used without further purification. All reactions were carried out under a dry nitrogen atmosphere and monitored by thin layer chromatography (TLC) on Merck silica gel $F_{254}$ plates (250 μm) with visualization using UV light or by heating plates sprayed with a solution of phosphomolybdic acid (5% ethanol solution). Flash column chromatography was carried out on silica gel 60 Å (230–400 mesh). Organic solvent extracts in the isolation procedures were dried over anhydrous sodium sulfate. Melting points were obtained in open capillary tubes in a Mel-Temp® melting point apparatus and are uncorrected.

$^1$H NMR spectra were recorded using Varian Inova spectrometer. Unless otherwise stated, $^1$H NMR spectra were recorded at 25 °C using an internal tetramethylsilane standard at 0 ppm. IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrometer. Samples were prepared by attenuated total reflectance (ATR) method. High resolution mass spectra were recorded on BioTOF II ESI mass spectrometer. Optical rotations were obtained on PerkinElmer Polarimeter 341.

### 2.1. Synthesis of 2,3,4, 5-tetraacetyl-p-nitrophenyl-α-D-mannopyranoside (5a)

To a solution of *p*-nitrophenyl mannopyranoside (2.0 g, 6.64 mmol) in anhydrous pyridine (32 mL) was added acetic anhydride (312 mL) and 4-dimethylaminopyridine (DMAP) (20 mg), and the reaction mixture was stirred at room temperature for 4 h. Progress of reaction was checked by TLC ($CHCl_3$:MeOH; 9:1,

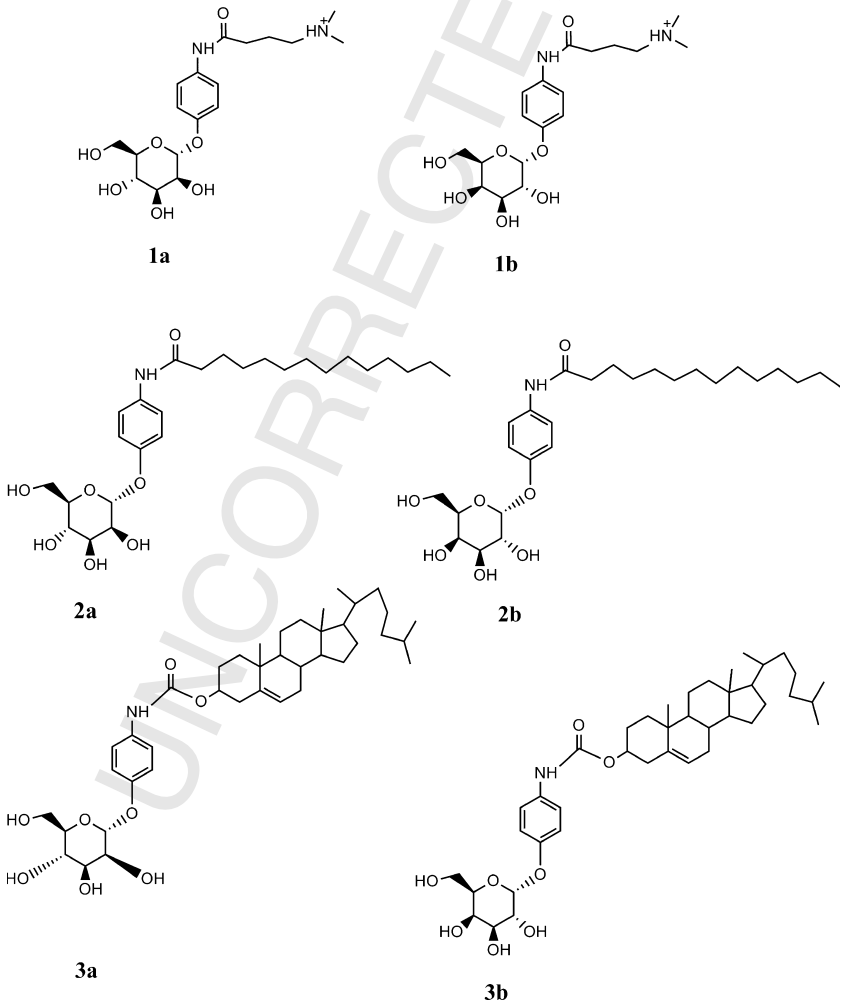


**Fig. 1.** Lipid conjugates of *p*-aminophenyl-α-D-galactopyranoside and *p*-aminophenyl-α-D-mannopyranoside.



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