Data Article

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

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A new synthetic methodology for cationic glycolipids using \(p\)-aminophenyl-\(\alpha\)-d-mannopyranoside (PAPM), \(p\)-aminophenyl-\(\alpha\)-d-galactopyranoside (PAPG) was developed. PAPM-lipids and PAPG-lipids conjugates were also synthesized for targeting drugs to receptors. A binding inhibition study of synthesized \(p\)-(dimethylamino butylamido) phenyl-\(\alpha\)-d-mannopyranoside (1a) with Concanavalin A was performed using invertase enzyme. In addition, transfection of pSV-\(\beta\)-gal reporter gene with was investigated in A549 cells.

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Compounds 1a, 2a, 3a, and 1b, 2b, and 3b were synthesized starting from p-nitrophenyl-α-d-mannopyranoside and p-nitrophenyl-α-d-galactopyranoside, respectively. All these compounds were characterized by NMR, IR and Mass spectroscopy, optical rotation etc. Compound 1a was taken as a representative sample for binding-inhibition studies and transfection.

Value of the data

- The article describes the synthesis of new lipid-carbohydrate conjugates.
- The synthesized compounds can be potentially used to target mannose cell surface receptors.
- The synthesized compounds 1a and 1b can potentially be used in transfection.

Fig. 1. Lipid conjugates of p-aminophenyl-α-d-galactopyranoside and p-aminophenyl-α-d-mannopyranoside.
1. Experimental design, materials and methods

1.1. Synthesis and characterization

The schemes for synthesis of carbohydrate-lipid conjugates are depicted in Figs. 1–5. All the intermediates and final products were characterized by 1H NMR, 13C NMR, IR, HRMS, and optical rotation.

1.2. Binding inhibition study

Concanavalin A (Con A), Invertase, and α-MM were purchased from Sigma Aldrich. Solutions of compound 1a (2.5 mM, 1.25 mM, 0.625 mM), α-MM (2.5 mM, 1.25 mM, 0.625 mM), Con A (20 μM) and Invertase (2 μM) were prepared in phosphate buffered saline (PBS pH 7.4). Solutions of 1a and α-MM, at various concentrations were mixed with equal volumes of Con A or PBS in triplicate in microtiter plate. Equal volumes of Invertase were added to each well resulting in the final concentration of 1a and α-MM 0.833 mM, 0.416 mM, and 0.208 mM. The final concentration of Con A, and Invertase in test wells were 6.6 μM and 0.66 μM, respectively. An increase in turbidity at 320 nm (OD320) was monitored at room temperature using SpectraMax M2e (Molecular Devices, CA, USA) (Figs. 6 and 7). Binding of Con A to Invertase was used as control (100%) aggregation and PBS was used a blank.
Fig. 3. Reagents: (a) Ac₂O, Pyridine, rt (b) Amm. Formate, Pd-C, MeOH, EtOAc (c) (CH₃)₂N(CH₂)₃CO₂H, DCC, DMAP, CH₂Cl₂ (d) NaOMe/MeOH.

Fig. 4. Reagents: (a) Myristic Acid, EDC, DMAP, CH₂Cl₂ (b) NaOMe/MeOH.
1.3. In vitro transfection

A549 cells (human lung cancer) were obtained from the National Cancer Institute (Frederick, MD) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). DMEM, FBS and other cell culture reagents were purchased from Life Technologies (Carlsbad, CA).

Log-phase A549 cells were seeded in 96-well plates (20,000 cells/well) and cultured overnight in a standard 5% CO₂ incubator. The culture medium was removed and cells were washed with sterile...
phosphate-buffered saline (PBS) before adding the transfection mixture. The transfection mixture was prepared by adding reporter gene, pSV-β-gal control vector (Promega, Madison, WI) into equal volume of different concentrations of (1a) to yield a series of mixtures with +/− charge ratios of 2:1; 4:1; 8:1; 16:1. The mixture was added to and incubated with the cells for 6 h before replaced with regular culture medium. The cells were cultured for a total of 24 h. The total amount of DNA plasmid was 0.2 μg/well in 96-well plates. Both DNA plasmid and 1a were diluted with OptiMEM I medium (Life Technologies (Carlsbad, CA). The transfection efficiency was determined using β-Galactosidase Enzyme Assay System (Promega, Madison, WI) according to the manufacturer’s protocol (Fig. 8).

**Fig. 7.** The kinetics of precipitation reaction between Invertase and Con A in the presence of varying amount of α-MM or compound 1a. (A) α-MM (0.833 mM), Con A and Invertase, (B) α-MM (0.416 mM), Con A and invertase, (C) α-MM (0.208 mM), Con A and invertase, (D) compound 1a (0.833 mM), Con A and invertase, (E) compound 1a (0.416 mM), Con A and invertase, (F) compound 1a (0.208 mM), Con A and invertase. The final concentration of Con A and invertase in each test sample were 6.6 μM and 0.66 μM, respectively.

**Fig. 8.** Transfection of pSV-β-gal control vector gene with compound 1a in A549 cells. Cells were seeded in 96-well plate 24 h before transfection. Cells were incubated with DNA-compound 1a mixture of different charge ratios for 6 h. The total amount of reporter gene was 0.2 μg/well.