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Synthesis of Amphiphilic Hepatocyte Targeting Cholesterylated Thiogalactosides

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Abstract: Six amphiphilic cholesterylated thiogalactosides **1a-f** with high affinity for the asialoglycoprotein receptor have been synthesized by coupling 2,3,4,6-tetra -*O*-acetyl-1-thio-β-D-galactopyranose **8** with prepared cholesterol derivatives **7a-f**, then by deacetylating. Preliminary results show liposomes containing those galactosides derivatives exhibited higher affinity and transfection activity in hepatoma cells HepG2 and SMMC-7721.

Keywords: Hepatocyte target, cholesterylated thiogalactosides, synthesis.

During the last decade, gene transfer to hepatocytes has become a great potential therapeutics since hepatocytes are responsible for the synthesis of a wide variety of proteins[1]. There has been much interest in efficient gene delivery to hepatocytes *in vitro* and *in vivo*. Among the possible delivery approaches, liposomes are considered to be a viable option. Hara *et al.* demonstrated that asiaplofetuin-labeled liposomal carriers encapsulating plasmid DNA are effective in gene expression[2]. In addition, asialoglycoprotein receptor is uniquely localized on the parenchymal liver cell and recognizes glycoproteins containing terminal β-D-galactosyl or 2-acetamido-2-deoxy-β-D-galactopyranose residues[3]. Kawakami *et al*. prepared liposomal gene carriers possessing the cationic charge necessary for plasmid DNA binding and galactose residues as a targetable ligand of liver parenchymal cells, which can be efficiently recognized by asialoglycoprotein receptors[4]. To date, howover, synthetic galactosides have been optimized with respect to their branching pattern in order to accomplish high affinity binding to the asialoglycoprotein receptor[5]. The effect of a variation in distance between the sugar moieties and lipophilic moieties is rather unexplored. In our protocol, we therefore wish to synthesis several different spacer cholesterylated thiogalactosides **1a-f** (**Figure1**) coupled by ether-linker containing ethylene glycol units. These derivatives possess bifunctional proterties *i.e.* lipophilic steriod for inserting into the liposome membrane that encapsulat plasmid DNA *via* electrostation interaction and a hydrophilic galactose residue outside the membrane for targeting the asialoglycoprotein receptor in hepatocytes.

The synthesis route of the cholesterylated thiogalactosides**1a-f** started from diol **2** as outlined in **Scheme 1**. Alcohol **2** was mesylated to get mesylate **3**, which was coupled

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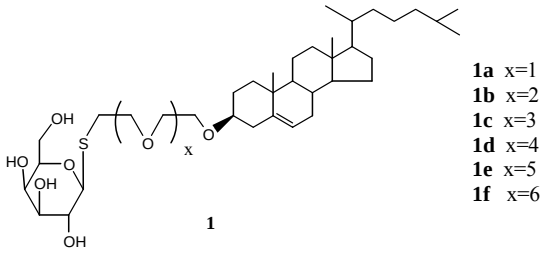
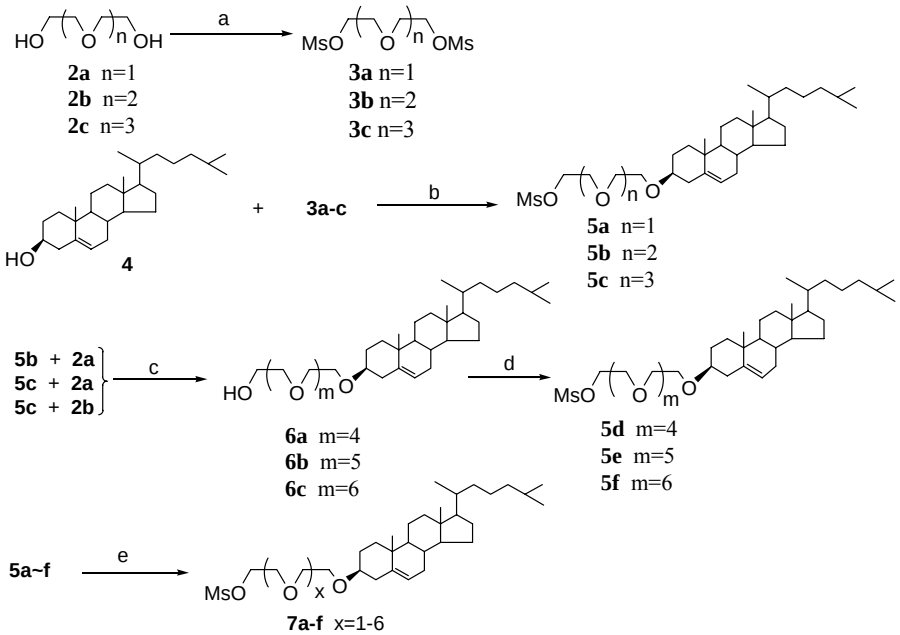
Figure 1



Scheme 1



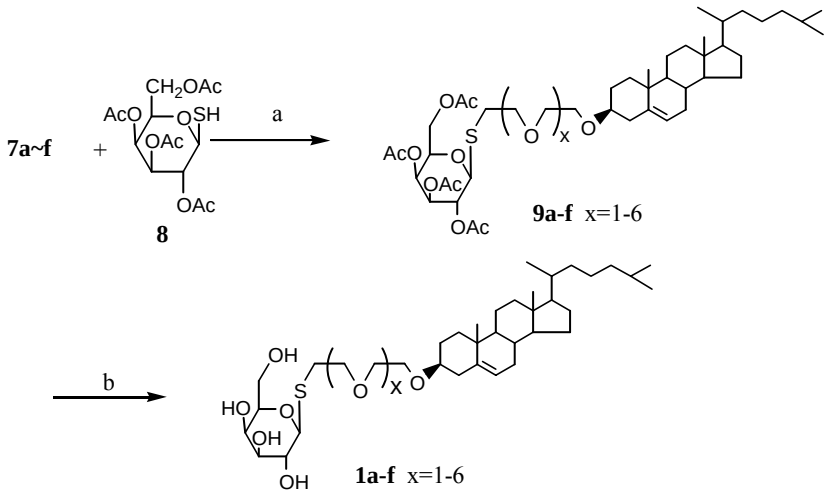
Reagents and conditions: a) MsCl, Et_3N, THF, r.t., 92%; b) NaH, THF, 40-60°C, 30-40%; c) NaH, THF, DMSO, 40-60°C, 30-40%; d) MsCl, Et_3N, THF, r.t., 80-85%; e) NaI, butanone, reflux, 70-85%.

with cholesterol **4** using NaH in THF and DMSO to give **5a-c**. In similar, **5b** and **2a**, **5c** and **2a** or **5c** and **2b** was condensed to afford different spacer alcohol **6a-c** respectively. Then alcohol **6a-c** was mesylated by methylsulfonyl chloride in triethylamine and THF to afford mesylate **5d-f**. The series of different spacer mesylate was treat with NaI in butanone directly converted into iodide **7a-f**.

The iodide **7a-f** were coupled with 2, 3, 4, 6-tetra-*O*-acetyl-1-thio-β-D-galacto-pyranose **8**[6,7] in the presence of DIPEA to produce **9a-f**. After deacetylation of **9a-f** by treatment with MeONa in MeOH, the title compounds **1a-f**[8] were obtained **(Scheme 2**).

Liposomes-DNA complexes containing galactosylated cholesterols **1a-f** were tested for their ability to deliver β-galactosidase plasmid into hepatoma cells HepG2 and SMMC-7721. Preliminary results show they exhibited higher transfection activity in both cells compared with non-galactosylated liposomes-DNA complexes.

Scheme 2



reagents and conditions: a)DIPEA, butanone, r.t., 75-85%; b) MeONa, MeOH, r.t., 65-85%.

In summary, we synthesized novel galactosylated cholesterol derivatives **1a-f** for developing the targetable hepatocyte liposomal carriers. In hepatoma cells HepG2 and SMMC-7721, these liposomes exhibited higher transfection activity. The results will be report elsewhere.

Acknowledgments

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References and Notes

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8. All the compounds were characterized by ^{1}H NMR, IR, MS spectral data and elemental analysis. Selected analytical data of **1c**: ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 5.36(dd, 1H, J=3.6, 5.2Hz), 4.59(s, 2H), 4.34(d, 1H, J=9.6Hz), 3.86(dd, 1H, J_1=2.4, 3.2Hz), 3.75-3.66(m, 5H), 3.65-3.59(m, 10H), 3.52(m, 2H), 3.45(dd, 1H, J=3.6, 4.8Hz), 3.19(m, 1H), 2.93(dt, 1H, J=6.4, 13.6Hz), 2.81(dt, 1H, J=6.5, 13.9Hz), 2.36(m, 1H), 2.16(m, 1H), 2.03(m, 1H), 1.99-1.93(m, 1H), 1.90-1.80(m, 2H), 1.64-1.04(m, 22H), 1.01(s, 3H), 0.93(d, 3H, J=6.8Hz), 0.88(d, 3H, J=1.6Hz), 0.86(d, 3H, J=1.2Hz), 0.71(s, 3H); IR(KBr): ν 3387, 2931, 1671, 1378, 1077, 1045 cm^{-1}; MS: *m/z* 723(M^+-18); Anal. Calcd. For $C_{41}H_{72}O_9S$: C, 66.45; H, 9.79; S, 4.33; Found: C, 66.33; H, 10.02; S, 4.31; $[\alpha]_D^{20}$= -45.0(c 0.1, $CHCl_3$).

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