Chinese Chemical Letters 26 (2015) 543–546

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Lipase-catalyzed synthesis of novel galactosylated cholesterol

Hua Nie [b], Yi Cheng [a,*], Pin-Jing Zheng [a], Li-Hua Luo [a], Sheng-Yuan Zhang [b]

[a] College of Traditional Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[b] Medical College of Jiaying University, Meizhou 514031, China

ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form 15 December 2014
Accepted 19 December 2014
Available online 9 January 2015

Keywords:
Cholesterol derivative
Liposomes
Asialoglycoprotein receptor
Lipase-catalyzed

ABSTRACT

In an organic phase system, an enzymes lipase was used as a catalyst to synthesize galactosylated cholesterol, (5-cholesten-3b-yl)[(4-O-β-D-galactopyranosyl)D-glucitol-6] sebacate (CHS-SE-LA), which contains galactose residues. Its chemical structure was characterized by ESI-MS, and NMR. For HepG2 cells, the cellular fluorescence intensities of liposomes modified with CHS-SE-LA (GAL-FL) were as much as 2.6-fold ($p < 0.01$) control liposomes (FL). Moreover, the presence of excess galactose significantly inhibited the uptake of GAL-FL suggesting ASGPR mediated uptake. In conclusion, the novel galactosylated ligand CHS-SE-LA was synthesized by lipase-catalyzation and revealed a great potential as drug carrier materials for hepatocyte-selective targeting.

1. Introduction

The asialoglycoprotein receptor (ASGPR) is a high-capacity C-type lectin receptor expressed on mammalian hepatocytes, which plays an important role in the lysosomal processing of *N*-acetylgalactosamine (GalNAc) and galactose-containing glycopeptide substrates [1]. These motifs can therefore be designed as hepatotropic vectors for hepatocytes uptake of a variety of nanoparticles such as cyclodextrin [2], liposomes [3,4] and polymers linked to oligonucleotide [5].

In this paper, a novel glycolipid, (5-cholesten-yl)[(4-*O*-β-D-galactopyranosyl)-D-glucitol-6-yl]sebacate (CHS-SE-LA), was designed and synthesized. The glycolipid included three parts: homing device, spacer and anchor. Comprising a galactosyl residue, lactitol is usually used as a recognition moiety for the hepatocyte-targeting carrier [6]. Cholesterol, one of the lipid components used to form liposomes, is usually selected as the lipophilic anchor moiety for stably introducing the galactosyl moiety into liposome [7–10]. Vinyl esters as acyl donors in many ways was the first choice in lipase catalyzed transesterification reactions [11], and consequently divinyl sebacate was chosen as a spacer part of link between cholesterol and lactitol.

There are various methods to attach ligands to the liposomes. For example, the ligand was coupled with long-chain fatty acids or hydrophobic phospholipid, then reacted with a drug loaded liposomes and formed a ligand-modified liposomes. However, most reactions are chemical reactions [7,12,10,13,9,14–16], and these chemical modifications require multistep reactions, complicated and controlled reactions conditions and show extremely low regioselectivity for the esterification of sugar. An enzyme method was sought to solve these problems. The enzymatic reaction has advantages in liposomes surface modification, such as high initial rate, high substrate conversion, high regioselectivity, environmental protection and a reduction in energy consumption.

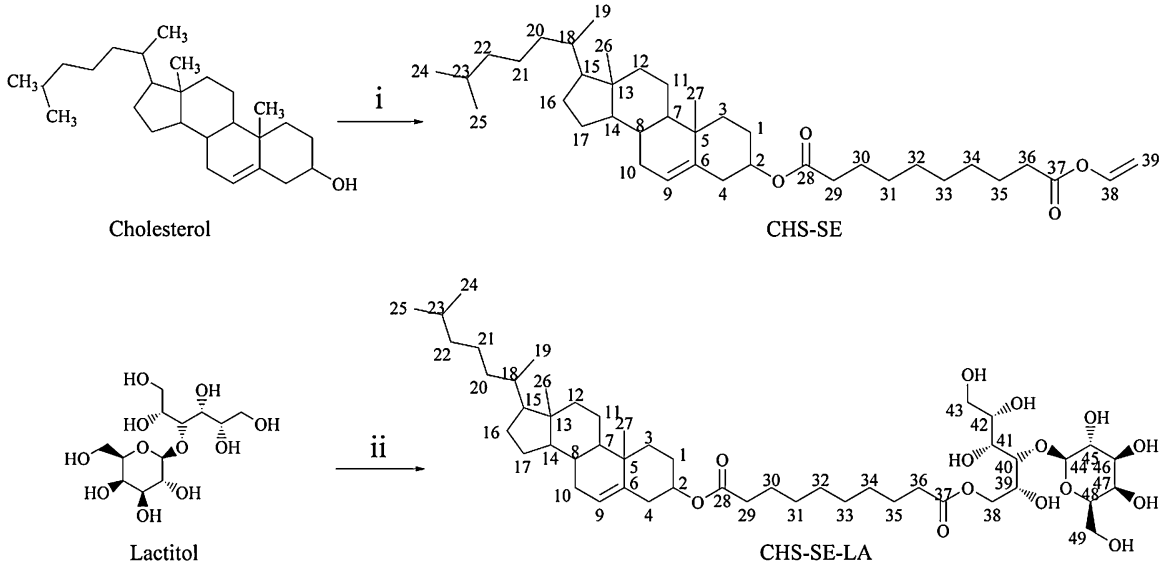
So, in this work, we investigated a facile synthesis method of galactosyl ligands *via* enzymatic coupling method, constructed galactose-modified liposomes and tested the potential of galactosylated liposomes for hepatoma targeting. Our interest in enzymatic chemoselective esterification was its application to the synthesis of biomaterials exhibiting strong binding to specific lectins, which was essential for its application as a scaffold and drug delivery system with recognition ability to cells.

2. Experimental

The synthesis of (5-cholesten-yl)vinyl decanedioic acid (CHS-SE): Cholesterol (0.13 mmol), divinyl sebacate (1.16 mmol), *Candida rugosa* lipase (CRL, 38.5 mg, 628 U/g, Sigma–Aldrich) and isooctane (5 mL) were added into a 10 mL screw-capped vial. The vial was placed in an air-bath at 35 °C and the reaction mixtures were shaken at 250 rpm for 11.5 h (Scheme 1), then the lipase was removed by suction filtration and the filtrate was evaporated to dryness. 250 mL methanol was added to the residue, then the mixture was dissolved and cooled at 0 °C overnight, then the precipitated white crystals were filtered, yielding 0.357 g (0.12 mmol, 92.8%) CHS-SE.

* Corresponding author.
E-mail address: 87817132@qq.com (Y. Cheng).

http://dx.doi.org/10.1016/j.cclet.2015.01.005



Scheme 1. The synthesis of galactosyl ligand *via* enzymatic esterification of sugar alcohol. Conditions: (i) divinyl sebacate, isooctane, 35 °C, *Candida rugosa* lipase; (ii) CHS-SE, pyridine: acetone (2:1, v:v), 55 °C, Novozym 435.

The synthesis of CHS-SE-LA: Lactitol (0.04 mmol), CHS-SE (0.15 mmol), Novozym 435 lipase (CAL-B, 22.8 mg, 798 U/g, Novozymes A/S Co), pyridine: acetone (2:1, v:v) 2.1 mL were added into a 10 mL screw-capped vial. It was placed in an air-bath at 55 °C and the reaction mixtures were shaken at 250 rpm for 31.1 h (Scheme 1). Then the lipase was removed by suction filtration and the filtrate was evaporated to dryness. The residue was washed with 50 mL cold isooctane to remove residual CHS-SE, and then the residue was lyophilized yielding 0.034 g CHS-SE-LA, which was a novel compound (0.038 mmol, 94.3%). Its structure was determined by the aid of ESI-MS, ^{1}H NMR and ^{13}C NMR.

1-Palmitoyl-2-[12-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)amino-dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD), as a fluorescence probe, was encapsulated in conventional liposomes (FL) and galactosylated liposomes (the mass ratios of CHS-SE-LA/lipid was 1:9, GAL-FL). The cell binding and internalization of NBD labeled liposomes were also studied just as Minyan Wei described [17]. In brief, HepG2 cells were seeded onto 24-well plates with 5×10^5 cells per well and cultured for 24 h. Then cells were incubated for 1 h at 37 °C with FL and GAL-FL, respectively, in which the final concentration of lipids (incorporated with 1%, m/m NBD) was 1 mmol/mL. For the inhibition study, 1 mg/mL lactose was added to the emulsion solution in advance. After incubation, the cells were washed three times with 1 mL of PBS (pH 7.4), then were solubilized in 1% of TriotnX-100 PBS. The fluorescence intensity was assayed using a microplate reader (Flex Station3, Molecular Devices, USA) at wavelength of 465 nm.

Statistical significance was determined by Student's *t*-test with $p < 0.05$ indicating significant difference using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Under mild conditions, CHS-SE and CHS-SE-LA are synthesized succinctly and efficiently by enzymatic method. The purified CHS-SE or CHS-SE-LA was analyzed by MS and NMR, respectively. A mass spectrum was obtained by Mass Spectrometry (Thermo LCQ FLEET CA, USA) with positive ESI mode. ^{1}H NMR and ^{13}C NMR spectra were measured in $CDCl_3$ for CHS-SE or pyridine-d_5 for CHS-SE-LA, with a Bruker NMR spectrometer (Avance III Switzerland), respectively. The data is shown below:

CHS-SE: ESI-MS *m/z*: 619.51 $[M+Na]^+$. ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.57 (C-28), 171.15 (C-37), 141.50 (C-38), 140.02 (C-6), 122.90 (C-9), 97.76 (C-39), 74.01 (C-2), 56.99 (C-14), 56.43 (C-15), 50.32 (C-7), 42.61 (C-13), 40.03 (C-12), 39.82 (C-22), 38.46 (C-4), 37.30 (C-3), 36.90 (C-5), 36.48 (C-20), 36.09 (C-18), 34.97 (C-29), 34.22 (C-36), 32.21 (C-10), 32.16 (C-8), 29.34 (C-31), 29.32 (C-32, C-33), 29.26 (C-34), 28.53 (C-16), 28.32 (C-23), 28.12 (C-1), 25.30 (C-30), 24.85 (C-35), 24.58 (C-17), 24.13 (C-21), 23.12 (C-25), 22.86 (C-24), 21.33 (C-11), 19.63 (C-27), 19.01 (C-19), 12.16 (C-26). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (m, 1 H), 5.37 (d, 1 H, J = 4.5 Hz), 4.87 (dd, 1 H, J = 14.0, 1.5 Hz), 4.65–4.53 (m, 2 H), 2.38 (t, 2 H, J = 7.5 Hz), 2.35–2.22 (m, 4 H), 2.06–1.93 (m, 2 H), 1.93–1.81 (m, 3 H), 1.81–1.58 (m, 5 H), 1.58–1.22 (m, 20 H), 1.22–0.83 (m, 23 H).

CHS-SE-LA: ESI-MS *m/z*: 919.50 $[M+Na]^+$. ^{13}C NMR (100 MHz, pyridine-d_5): δ 173.45 (C-28), 172.80 (C-37), 139.81 (C-6), 122.55 (C-9), 106.23 (C-44), 84.68 (C-40), 76.80 (C-48), 74.96 (C-46), 73.61 (C-2), 72.74 (C-42), 72.67 (C-45), 71.46 (C-39), 70.15 (C-41), 69.62 (C-47), 66.50 (C-38), 63.63 (C-43), 61.59 (C-49), 56.55 (C-14), 56.13 (C-15), 50.02 (C-7), 42.25 (C-4), 39.70 (C-13), 39.49 (C-12), 38.36 (C-22), 37.01 (C-3), 36.58 (C-5), 36.25 (C-20), 35.78 (C-18), 34.50 (C-29), 34.12 (C-36), 31.90 (C-10), 31.80 (C-8), 29.08 (C-31, C-34), 29.03 (C-32, C-33), 28.25 (C-1), 27.98 (C-16), 27.95 (C-23), 25.10 (C-30), 24.93 (C-35), 24.24 (C-17), 23.90 (C-21), 22.68 (C-25), 22.44 (C-24), 21.03 (C-11), 19.13 (C-27), 18.71 (C-19), 11.75 (C-26). ^{1}H NMR (400 MHz, pyridine-d_5): δ 5.42 (d, 1H, J = 4.2 Hz), 5.15 (d, 1H, J = 7.8 Hz), 4.91–4.82 (m, 2H), 4.81–4.62 (m, 4H), 4.59 (t, 1H, J = 5.4 Hz), 4.54–4.28 (m, 7H), 4.10 (dd, 1H, J = 9.4, 3.3 Hz), 4.04 (t, 1H, J = 6.2 Hz), 2.59–2.44 (m, 2H), 2.35 (dt, 4H, J = 33.7, 7.5 Hz), 2.06–1.78 (m, 4H), 1.75–1.34 (m, 14H), 1.33–1.02 (m, 20H), 0.99 (d, 3H, J = 6.4 Hz), 0.91 (d, 6H, J = 6.6 Hz), 0.69 (s, 3H).

There are eight hydroxyl groups in lactitol, thereby requiring chemoselective esterification prior to use as monomers. After resultant compound was purified by the column chromatography, the chemoselectivity was confirmed by ^{13}C NMR, in which the chemical shifts of esterified carbons have been reported to show a downfield shift [6,18]. In the ^{13}C NMR of lactitol, three peaks were observed at δ 61.4, 63.3, and 63.8 due to the primary hydroxymethylenes at the 6′, 1, and 6 positions, respectively. In