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Mendeleev Commun., 2011, **21**, 69–71
Mendeleev Communications

Synthesis of diglyceride conjugate of selectin ligand SiaLe^X as a vector for targeting of drug-loaded liposomes

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DOI: 10.1016/j.mencom.2011.03.002

A conjugate of tetrasaccharide Sialyl Lewis X [SiaLe^X, Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ] 3-aminopropyl glycoside and *rac*-1,2-dioleoyl-3-carboxymethylene[poly(8–15)oxyethylene]oxyacetylamidopropionylglycerol amenable for the incorporation in lipid bilayer of drug-loaded liposomes to achieve targeting in tumors and inflammation foci was obtained by the formation of carboxamide bond.

Selectins (carbohydrate binding adhesion proteins) are expressed on cell surface of activated leucocytes, endothelial cells, and platelets.[1,2] Selectins were shown to be involved into multiple (patho)physiological processes including inflammatory responses, development of metastases, *etc*.[2] All selectins recognize a common carbohydrate epitope, the Sialyl Lewis X tetrasaccharide [SiaLe^X, Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ].[1] In view of this, the use of SiaLe^X as a ligand for targeting of drugs in tumors and inflammation foci seems to be rationale.

On the other hand, to improve biodistribution and decrease general toxicity of anticancers, nanoparticulate supramolecular and polymer drug delivery systems are intensively developed. Among them, liposomes are already used in oncological clinics for systemic administration.[3] For the first time, anti-inflammatory cardioprotective effect of SiaLe^X-liposomes as such was shown in a feline model;[4] later, the same liposomes were shown to inhibit E-selectin mediated cellular adhesion,[5] and tumor cell adhesion to vascular endothelium *in vitro*.[6] More recently, fluorescently labeled SiaLe^X-conjugated liposomes formed on the basis of other lipid components turned out to be a useful tool for the imaging of inflammation and tumor invasion in mice,[7] as well they did serve as drug delivery vehicles after rat vascular surgery.[8] However, the introduced technique of SiaLe^X-liposome preparation seems to be too complicated, and demands consumption of till to 30 mol% of natural gangliosides in the lipid matrix [glycerol residues of NeuNAc on the liposome surface should be oxidized by periodate to provide bond to human serum albumin, which protects liposomes against adsorption of opsonines (plasma proteins) in circulation, and then, aminated by the reducing group terminal SiaLe^X is conjugated to albumin through 3,3-dithiobis(sulfosuccinimidyl propionate)].[7,8]

We have shown in the models of mouse breast cancer and leukemia leucosis that antitumor effect of liposomes loaded with lipophilic prodrugs of anticancers sarcolysin[9] and methotrexate[10] sharply increased after supplementation of formulation just by 2 mol% of SiaLe^X 3-aminopropyl glycoside conjugate with polyoxyethylene (6–9) heptadecyl ether. A convenient synthesis of lipophilic glycoconjugates (structure **I**, Figure 1) on the basis of detergent Lubrol PX – ether of polyethylene glycol (PEG), mean polymerization number (*n*) 10, and alkane C14–C18 – has been elaborated.[11] Flexible polar PEG spacer between carbohydrate residue and membrane anchor provides exposition of ligand over liposome surface, necessary for effective interactions with cell receptors. However, monoalkyl tetrasaccharide conjugate does not include in lipid bilayer well, and forms separate micelles (unpublished results). Later we synthesized neoglycolipids with reliable dioleoyl glycerol anchor (structure **II**, Figure 1),[12] but α-oxy ester bond between $PEG_{10–16}$ spacer and diglyceride residue turned to be unstable under long term storage (several months at –20 °C). The goal of this study was synthesis of new SiaLe^X–diglyceride conjugate **3** amenable for the incorporation in drug-loaded liposomes, and bearing stable amide bond between PEG residue and lipid fragment (Scheme 1).

In the cited works[4–6] pentasaccharide (SiaLe^X)1-3Galβ conjugated to distearoylphosphatidylethanolamine by residue of PEG-2000 (*n* = 42–48) through modified *N*-acetyl group of glucose

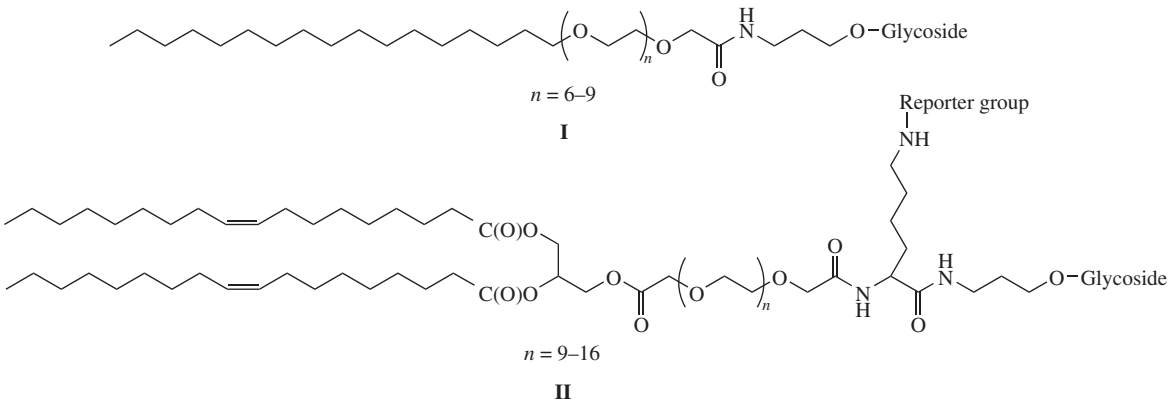
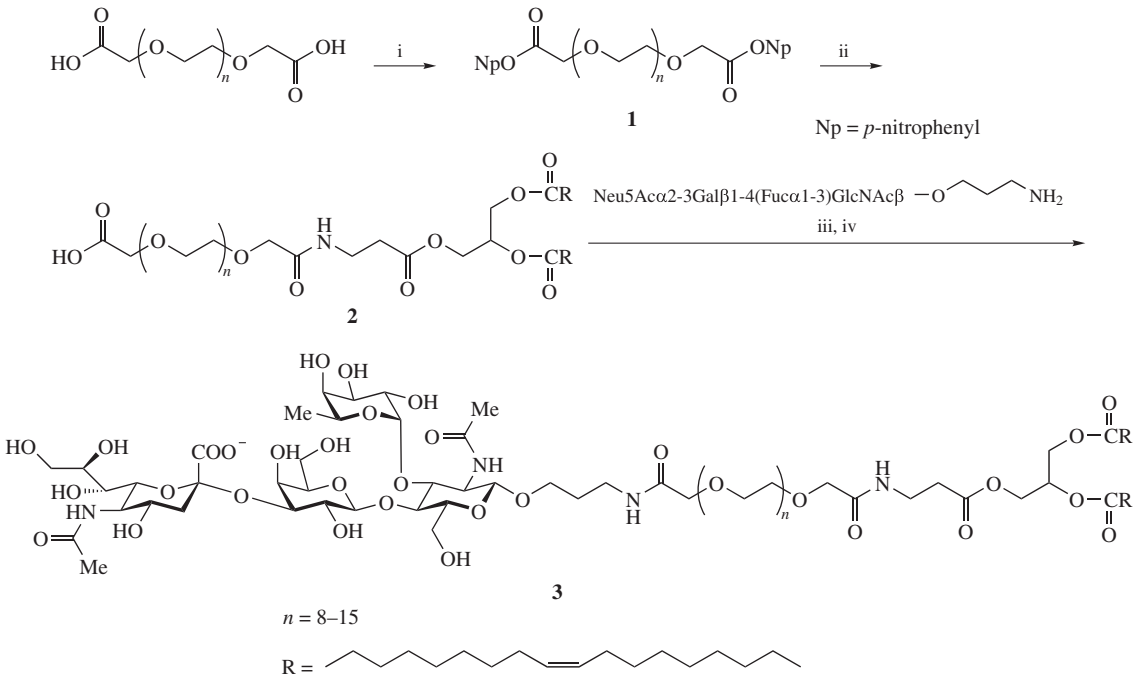


Figure 1 Structures of aglycone moieties of lipophilic glycoconjugates synthesized earlier.[11,12]



Scheme 1 *Reagents and conditions*: i, *p*-nitrophenyl trifluoroacetate (20 equiv.), Py, 15 h; ii, *rac*-1,2-dioleoyl-3-(3-aminopropionyl)glycerol (0.14 equiv.), Et_3N, $CHCl_3$:MeOH (1:1), 15 h; iii, *N*-hydroxysuccinimide (1.7 equiv.), DCC, DMF, 1 h; iv, Et_3N, DMSO, 1 h.

amine was synthesized and applied as liposome vector. The extensive PEG spacer is needed to avoid shielding when using sterically stabilized Stealth® liposomes, which bear grafted PEG-2000 chains on the surface for prevention of opsonine adsorption in the circulation and subsequent attack of immunocompetent cells. Glycoconjugate was shown to incorporate in liposome bilayer well.[5] Alternatively, to stabilize liposome membrane and avoid drawbacks of nonbiodegradable long chain PEG we use natural phosphatidylinositol, which also protects liposomes from opsonization.[13] Therefore, diglyceride conjugate of tetrasaccharide $SiaLe^X$ **3** should contain significantly more short PEG residue than $SiaLe^X$-conjugate used by the authors,[4–6] what yet increase the affinity of molecule to lipid bilayer of liposome. As for the structure of membrane anchor, dioleoyl glycerol moiety instead of one with saturated aliphatic chains better ensures the uniform distribution in fluid lipid bilayer (characterized by a low temperature of the phase transition), which we use in liposomes to include more amphiphilic molecules, lipophilic prodrugs,[9,10] in addition to natural matrix phospholipids. And finally, the use of 3-aminopropionyl diglyceride as a base lipid module permits to evade negatively charged phosphate group of phosphatidylethanolamine, and thus facilitate the incorporation of $SiaLe^X$-conjugate **3** in liposome bilayer negatively charged due to phosphatidylinositol.

As previously, here we used bis(carboxymethyl) ether of PEG with mean molecular weight of 600 (Aldrich), which corresponds to mean $n \sim 11$, as starting reagent.[12] It was treated with the excess of *p*-nitrophenyl trifluoroacetate in dry pyridine followed by a gel filtration on Sephadex LH-20 (eluent, $CHCl_3$:MeOH:AcOH = = 5:5:0.1) to yield activated ester **1** (~55%, 0.7 g) in void volume.

At the next step, *rac*-1,2-dioleoyl-3-(3-aminopropionyl)glycerol (100 mg, 0.14 mmol; obtained as described)[14] was reacted with 7-fold excess of diester **1** in $CHCl_3$:MeOH = 1:1, in the presence of triethylamine to maintain pH ~ 7.5. After reaction completion the excess of activated ester **1** was hydrolyzed by adding several drops of water, and a mixture was subjected to gel filtration (see above) to separate the excess of dicarboxylic PEG-acid followed by chromatography on silica gel [eluent, gradient of MeOH (1–10%) in $CHCl_3$, 1% AcOH]. Yield of *rac*-1,2-dioleoyl-3-[carboxymethylene(polyoxyethylene)oxyacetyl-3-aminopropionyl]glycerol **2** was > 90% (190 mg).

At the final step acid **2** (40 mg, ~30 μmol) was activated by treatment with 1.7 equiv. of *N*-hydroxysuccinimide in dry DMF in the presence of *N*,*N*′-dicyclohexylcarbodiimide, and the *N*-oxysuccinimide ester was purified by gel filtration as described for ester **1**; yield, ~100% (45 mg). TLC control (Kieselgel 60 F_{254} plates, Merck) showed individual spot with $R_f \sim 0.6$ ($CHCl_3$:MeOH:AcOH = 9:1:0.1) detected by phosphomolybdic acid and UV light. Under the same conditions, acid **2** gave a set of overlaid spots, R_f 0.1–0.5. The obtained activated ester (~20 μmol) was immediately reacted with 1.1 equiv. of potassium salt of 3-aminopropyl glycoside $SiaLe^X$ (20 mg; synthesized as described)[15] in 200 μl of dry dimethyl sulfoxide, in the presence of Et_3N to maintain pH ~ 8. After reaction completion in 1 h, the mixture was purified by gel filtration (1×34 cm column; eluent, $CHCl_3$:MeOH = 1:1) yielding 43 mg (~ 100%) of the target diglyceride conjugate of $SiaLe^X$ **3** in the void volume. TLC ($CHCl_3$:MeOH:AcOH = 9:1:0.1), a set of overlaid spots with R_f 0.1–0.4 detected by phosphomolybdic acid and 7% H_3PO_4.

For detailed mass-spectral characteristics of compounds **1**–**3**, and ^1H NMR spectrum for neoglycolipid **3**, see Online Supplementary Materials.

The incorporation of neoglycolipid **3** in liposome membrane is under study.

This work was supported by the Russian Foundation for Basic Research (project no. 10-04-01021).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2011.03.002.

References

1 C. Foxall, S. R. Watson, D. Dowbenko, C. Fennie, L. A. Lasky, M. Kiso, A. Hasegawa, D. Asa and B. K. Brandley, *J. Cell Biol.*, 1992, **117**, 895.
2 C. Ehrhardt, C. Kneuer and U. Bakowsky, *Adv. Drug Deliv. Rev.*, 2004, **56**, 527.
3 D. B. Fenske and P. R. Cullis, *Expert Opin. Drug Deliv.*, 2008, **5**, 25.

4 T. Murohara, J. Margiotta, L. M. Phillips, J. C. Paulson, S. DeFrees, S. Zalipsky, L. Guo and A. M. Lefer, *Cardiovasc. Res.*, 1995, **30**, 965.
5 S. A. DeFrees, L. Phillips, L. Guo and S. Zalipsky, *J. Am. Chem. Soc*., 1996, **118**, 6101.
6 R. Zeisig, R. Stahn, K. Wenzel, D. Behrens and I. Fichtner, *Biochim. Biophys. Acta*, 2004, **1660**, 31.
7 M. Hirai, H. Minematsu, N. Kondo, K. Oie, K. Igarashi and N. Yamazaki, *Biochem. Biophys. Res. Commun*., 2007, **353**, 553.
8 W. Tsuruta, H. Tsurushima, T. Yamamoto, K. Suzuki, N. Yamazaki and A. Matsumura, *Biomaterials*, 2009, **30**, 118.
9 E. L. Vodovozova, E. V. Moiseeva, G. K. Grechko, G. P. Gayenko, N. E. Nifant'ev, N. V. Bovin and J. G. Molotkovsky, *Eur. J. Cancer*, 2000, **36**, 942.
10 E. Moiseeva, N. Kuznetsova, E. Svirshchevskaya, A. Chaadaeva, N. Bovin, W. Den Otter and E. Vodovozova, *Anticancer Res*., 2011, in press.
11 E. L. Vodovozova, S. V. Khaidukov, G. P. Gaenko, T. I. Bondarchuk, I. I. Mikhalev, I. V. Grechishnikova and Jul. G. Molotkovsky, *Bioorg. Khim.*, 1998, **24**, 760 (*Russ. J. Bioorg. Chem*., 1998, **24**, 676).
12 E. L. Vodovozova, G. V. Pazynina, A. B. Tuzikov, I. V. Grechishnikova and Jul. G. Molotkovsky, *Bioorg. Khim.*, 2004, **30**, 174 (*Russ. J. Bioorg. Chem*., 2004, **30**, 154).
13 A. Gabizon and D. Papahadjopoulos, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 6949.
14 E. L. Vodovozova, G. P. Gaenko, E. S. Bobrikova, G. V. Pazynina and J. G. Molotkovskii, *Khim.-Farm. Zh.*, 2007, **41**, 10 (*Pharm. Chem. J.*, 2007, **41**, 297).
15 G. V. Pazynina, M. A. Sablina, A. B. Tuzikov, A. A. Chinarev and N. V. Bovin, *Mendeleev Commun*., 2003, 245.

Received: 14th October 2010; Com. 10/3611