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Mendeleev Commun., 2016, **26**, 205–206
Mendeleev Communications

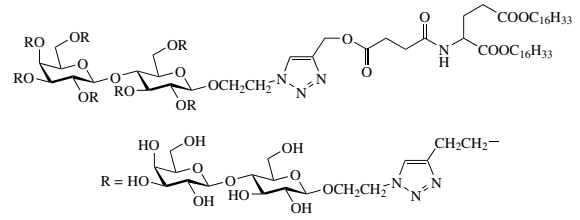
Multivalent glycoconjugate as the vector of target delivery of bioactive compounds

Ul'yana A. Budanova, Inga S. Shchelik,* Olesya O. Koloskova and Yurii L. Sebyakin

M. V. Lomonosov Institute of Fine Chemical Technologies, Moscow Technological University, 119571 Moscow, Russian Federation. Fax: +7 495 936 8206; e-mail: inga.shelik@yandex.ru

DOI: 10.1016/j.mencom.2016.04.008

New amphiphilic glycoconjugate with seven terminal lactose residues was synthesized. The properties of delivery systems from bioactive substance containing this conjugate were investigated.



Development of transport systems possessing definite physicochemical and biological properties aimed at the targeted delivery of biologically active compounds remains among important problems of the medicine. Different approaches are applied to implement this process such as attachment of biologically active fragments to terpenoid moiety or modifying the transport systems by carbohydrate-containing structures.[1] Various types of transport agents are used nowadays, such as micelles, liposomes, polymer species *etc*. The incapsulation of drugs in these carriers makes it possible to reduce the toxicity, increase the time of the action, protect from the enzymatic and immune reactions of the human body.[2]

For the recent years glycolipides had attracted attention for their ability to deliver bioactive compounds into cells.[3] Carbohydrate-containing structures, in this case, are located within liposomes, which are specifically recognized and bind with receptors.[4] Due to the presence of certain carbohydrate component on the terminal sequence, these transport systems are recognized by the lectin types which are specific to them and direct the complex into cell.[5]

It is known that hepatic cell as well as some kind of tumor are notable for higher level of the cell expression capable of binding with D-galactose (or lactose) residues.[6] In this context, the preparation of carbohydrate-containing compounds and creation of transport systems with their inclusion represent a perspective trend of target delivery development of the bioactive compounds.

Earlier, di- and tetravalent neoglycolipides that showed effective binding with lectin RCA_1 were prepared in our laboratory.[7,8] During this investigation heptavalent glycoconjugate **1** was synthesized (Scheme 1).

The scheme of synthesis of the desired compound **1** was divided into four parts: (i) synthesis of hydrophobic component,[7] (ii) synthesis of hydrophilic component **2**, (iii) synthesis of branching core **3** and (iv) conjugation of branching core **3** and hydrophilic constituent **2**.[†] For the synthesis of branching core **3** the lactose residue was attached to a hydrophilic part, and then it was modified with propargyl bromide excess.

The final glycoconjugate **1** was obtained by the copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) 'click' reaction. Its structure was confirmed by IR spectroscopy and mass spectrometry.

To evaluate the capacity of transport agent for target drug delivery, liposomes were selected. The main components of these vesicles are lipopeptides, phospholipids *etc*.[9,10]

Physico-chemical properties of liposomes[‡] composed of dihexadecyl *N*-(di-L-ornithyl)-L-glutamate and modified with compound **1** with the 5:95 ratio of neoglycoconjugate : lipid were explored.

[†] All reagents and organic solvents were purchased from Acros, Aldrich, or Reachim in reagent grade or better and used without further purification. Column chromatography was performed on silica gel 60 (60–200 mesh). Thin layer preparative chromatography (TLPC) was accomplished on silica gel 60 (2–25 mesh). IR spectra were recorded on a Bruker FTIR EQUINOX 55 spectrometer. MALDI mass spectra were measured on an Ultraflex MALDI mass spectrometer (Bruker Daltonics GmbH).

Synthetic procedures. Dihexadecyl *N*-(1,4-dioxo-4-propargyloxybutyl)-L-glutamate (0.060 g, 0.088 mmol), catalytic amount of CuI and DIPEA were added with stirring to a solution of 1-*O*-(2-azidoethyl)-β-D-lactoside **2** (0.056 g, 0.13 mmol) in DMF and the mixture was kept for 24 h at room temperature. The CuI crystals were filtered off and solvent was removed to yield compound **4**.

Sodium hydride (0.025 g, 1.05 mmol) was added to a solution of compound **4** (0.060 g, 0.052 mmol) in dry DMF at 0 °C. After 10 min propargyl bromide (0.0654 ml, 0.733 mmol) was added dropwise. The mixture was kept for 12 h at 0 °C. The solvent was removed. The residue was purified by preparative chromatography to give product **3**.

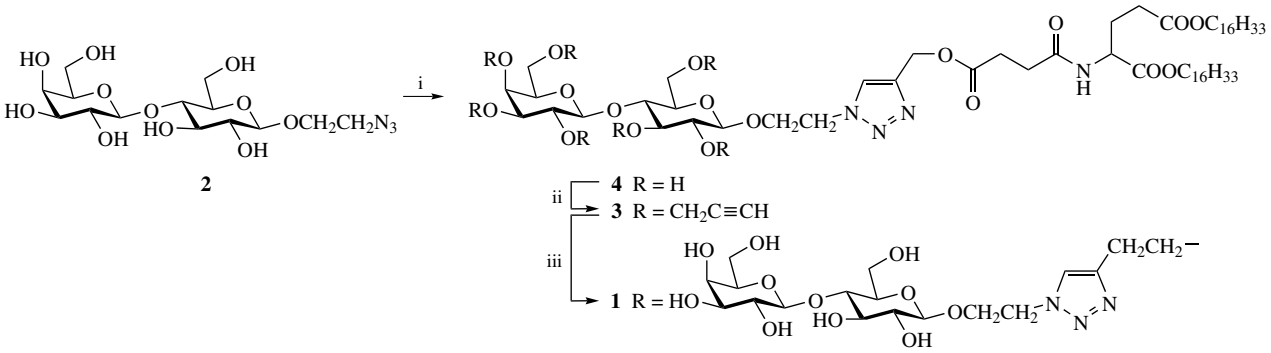
Compound **1** was prepared using the 1,3-dipolar cycloaddition reaction.[9] The resulting residue was purified by preparative chromatography (chloroform–methanol–H_2O, 10:6:1).

For **1**: yield 0.020 g, 43%, R_f 0.1 (chloroform–methanol–H_2O, 10:6:1). IR (ν_{max}/cm^{-1}): 3400 (OH), 2932 (C–H), 2852 (Me), 2700 (N≡N), 1749 (C=O), 1683 (C=O), 1627 (NH), 1477, 1444 (C–H), 1400 (N=N), 1280 (C–O), 1255 (C–N), 1250–1078 (C–O). MS (MALDI), *m*/*z*: 4203.896 (M^+).

For **3**: yield 0.058 g, 79%, R_f 0.8 (toluene–acetonitrile, 2:1). IR (ν_{max}/cm^{-1}): 3311 (≡C–H), 2922 (C–H), 2850 (Me), 2130 (C≡C), 1700 (C=O), 1621 (C=O), 1581 (NH), 1407 (C–O), 1174–1064 (C–O).

For **4**: yield 0.093 g, 62.5%, R_f 0.28 (chloroform–methanol–H_2O, 18:6:1). IR (ν_{max}/cm^{-1}): 3440 (OH), 2945 (C–H), 2863 (Me), 1740 (C=O), 1630 (C=O), 1540 (NH), 1146–1069 (CO). MS (MALDI), *m*/*z*: 1167.67 ($M^+ + Na^+$).

[‡] *Preparation of liposomes*. A chloroform solution of the lipid (~2 mg) and 5% glycolipid was added to a pear-shaped flask. The solvent was removed *in vacuo* by rotary evaporator leaving a thin film deposited onto the flask wall. Distilled water (1 ml) was then added and the film was peeled off by vortexing, hydrated by sonication at 65 °C 3 times for 15 min.



Scheme 1 *Reagents and conditions*: i, dihexadecyl *N*-(1,4-dioxo-4-propargyloxybutyl)-L-glutamate, CuI, DIPEA, DMF; ii, $HC{\equiv}CCH_2Br$, NaH; iii, **2**, CuI, DIPEA, DMF.

Stability of lipidic dispersion, the size of forming particles and the interaction of glycosylated vesicles with lectin were investigated. It was shown previously[11] with the di- and tetravalent analogues of compound **1**, that the increase of the fraction of glycolipids in lipid composition more than 5% does not improve the binding with lectin. Apparently, the exceeding of some critical value of carbohydrate residue density leads to steric hindrance obstructing further process of terminal galactose fragments interaction with lectin active centre.[7]

The stability of carbohydrate-containing liposomes to the action of destabilization Tween80 factor was established by the optical absorption measurement at 400 nm (Figure 1). The detergent solubilizes bilayer particles to form micellar dispersion that decreases the system optical density. These data show that liposomes composed of lipotripeptide are more stable than the phosphatidylcholine ones, and inclusion of the glycolipid into liposomes only slightly reduces their stability.

To define the possibility of the application of synthesized compounds as marker molecule within liposome, the interaction of modified vesicles with galactose-binding lectin castor *Ricinus communis* (RCA_1) was investigated. It is known that glycoprotein with terminal galactose residues gives the positive precipitation test with lectin RCA_1. It also possesses the affinity to other galactose-specific lectins, in particular to the asialoglycoprotein receptor of human and animals hepatocytes.[12] Therefore, it can be assumed that glycosylated liposomes precipitating in the presence of RCA_1 can also have a higher affinity to hepatocytes.

Effective liposome binding of modified heptavalent glycoconjugate **1** with lectin castor RCA_1 was in fact observed (Figure 2). This confirms the literature data on the small increase of receptor binding with the number of carbohydrate moieties greater than six. Addition of D-galactose resulted in a sharp optical density decrease.

The size of resulting particles was determined using photon-correlation spectroscopy. The intermediate diameter of vesicles modified with compound **1** was 400–450 nm. This size is due

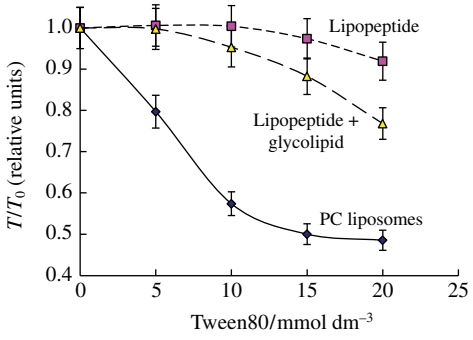


Figure 1 The stability of the liposomes to the action of the detergent.

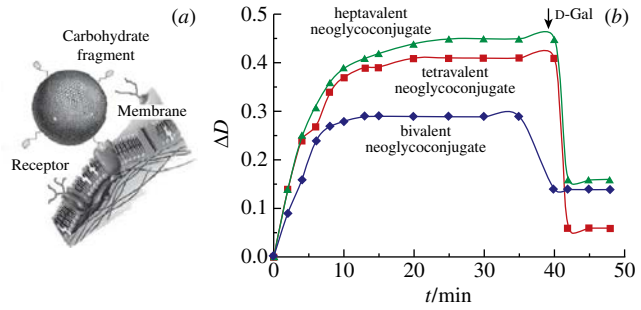


Figure 2 (*a*) Schematic representation of lectin–carbohydrate binding. (*b*) The change of the time dependence of solution optical density after binding the modified liposomes by glycoconjugate with lectin RCA_1.

to the presence of highly branched multivalent structure on the surface of liposomes. Also, a small local maximum was observed at 126.6 nm, which corresponded to the presence of the liposomes lacking glycoconjugate.

In conclusion, a new glycoconjugate containing seven terminal D-galactose residues was prepared. It can be considered as a potential target delivery vector of bioactive compounds.

This work was supported by the Russian Foundation for Basic Research (grant no. 13-04-00841).

References

1 M. S. Dzyurkevich, K. N. Timofeeva, D. A. Faizullin, Yu. F. Zuev, I. I. Stoikov and V. V. Plemenkov, *Mendeleev Commun.*, 2014, **24**, 224.
2 E. Soussan, C. Mille, M. Blanzat, P. Bordat and I. Rico-Lattes, *Langmuir*, 2008, **24**, 2326.
3 J. Keerti, G. Prashant, G. Umesh and K. J. Narendra, *Biomaterials*, 2012, **33**, 4166.
4 Y. van Kooyk, W. W. Unger, C. M. Fehres, H. Kalay and J. J. Garcia-Vallejo, *Mol. Immunol.*, 2013, **55**, 143.
5 R. R. Ramireddy, K. R. Raghupathi, D. A. Torres and S. Thayumanavan, *New J. Chem.*, 2012, **36**, 340.
6 M. Sakashita, S. Mochizuki and K. Sakurai, *Bioorg. Med. Chem.*, 2014, **22**, 5212.
7 O. O. Koloskova, U. A. Budanova and Yu. L. Sebyakin, *Russ. J. Biopharm.*, 2011, **3**, 14.
8 N. A. Kurochkina, U. A. Budanova and Yu. L. Sebyakin, *Russ. J. Org. Chem.*, 2014, **50**, 1496 (*Zh. Org. Khim.*, 2014, **50**, 1509).
9 O. O. Koloskova, U. A. Budanova, A. M. Sumina, G. A. Sarychev and Yu. L. Sebyakin, *Mendeleev Commun.*, 2014, **24**, 262.
10 A. A. Efimova, S. N. Kostenko, V. N. Orlov and A. A. Yaroslavov, *Mendeleev Commun.*, 2016, **26**, 99.
11 L. Yu. Gur'eva, A. K. Bol'sheborodova and Yu. L. Sebyakin, *Russ. J. Org. Chem.*, 2012, **48**, 1047 (*Zh. Org. Khim.*, 2012, **48**, 1051).
12 J. T. Dulaney, *Mol. Cell. Biochem.*, 1979, **21**, 43.

Received: 30th June 2015; Com. 15/4665