Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2010, **20**, 326–328
Mendeleev Communications

Synthesis and properties of a folic acid–polyethylene glycol conjugate for systems intended for directed delivery of anticancer compounds

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

M. V. Lomonosov Moscow State Academy of Fine Chemical Technology, 119571 Moscow, Russian Federation. Fax: +7 495 936 8901; e-mail: c-221@yandex.ru

DOI: 10.1016/j.mencom.2010.11.008

A conjugate of folic acid with a hydrophobic polyethylene glycol derivative has been synthesised; the characteristics of the resulting sterically-stabilised system based on lipodipeptides for directed delivery of doxorubicine have been determined.

Development of rational dosage forms is an important approach to enhance the efficiency of pharmaceuticals and reduce their *in vivo* toxicity and side effects. Development of directed delivery systems based on liposomes is among the approaches used to solve this problem.

Liposomes decompose rather readily in the organism, thus releasing the compounds to be delivered. Liposomal delivery systems whose surfaces are modified with a hydrophilic polymer, *e.g.*, polyethylene glycol, reliably protect their 'cargo' from exposure to immune system components and hence do not cause protective or allergic reactions of the organism.[1]

Active targeting of liposomal formulation at target cells is achieved by including a 'molecular address', *i.e.*, marker molecules for binding with an appropriate receptor, in the particles.

The folic acid residue is an example of such a marker, since folate receptors are exposed in high concentration on cancer cell surfaces (Figure 1), especially at late stages of the disease.[2] Moreover, we have shown that folic acid and its conjugates penetrate with equal efficiency into cells with folate receptors.[3]

We have synthesised a PEG_{3400} derivative **1** in which polyethylene glycol is bound to an octadecylamine residue at one end in order to fix the polymer in the liposome lipid bilayer; then the 'molecular address', *viz.*, folic acid residue, is bound to the other end *via* a diaminopropane linker (Scheme 1).

PEG_{3400} **2** was oxidized with potassium dichromate in 10% sulfuric acid to give dicarboxylic acid **3**,[4] in which one carboxyl group was activated with *N*-hydroxysuccinimide by adding one equivalent of the reagent; afterwards, the reaction with octadecylamine was carried out. The yield of compound **5** after chromatographic purification was 56%. After that, the remaining free carboxyl group was activated, followed by addition of diaminopropane. The yield of the modified PEG derivative **7** was 52%.†

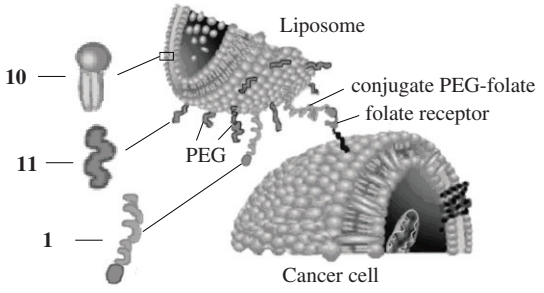


Figure 1 Schematic representation of components in a sterically stabilized system for targeted delivery of an anticancer drug and its interaction with a cell folate receptor.

The target product **1** was obtained using the reaction of folic acid succinimide ester **9**, which was obtained using the procedure reported previously,[5] with derivative **7** in 63% yield.‡

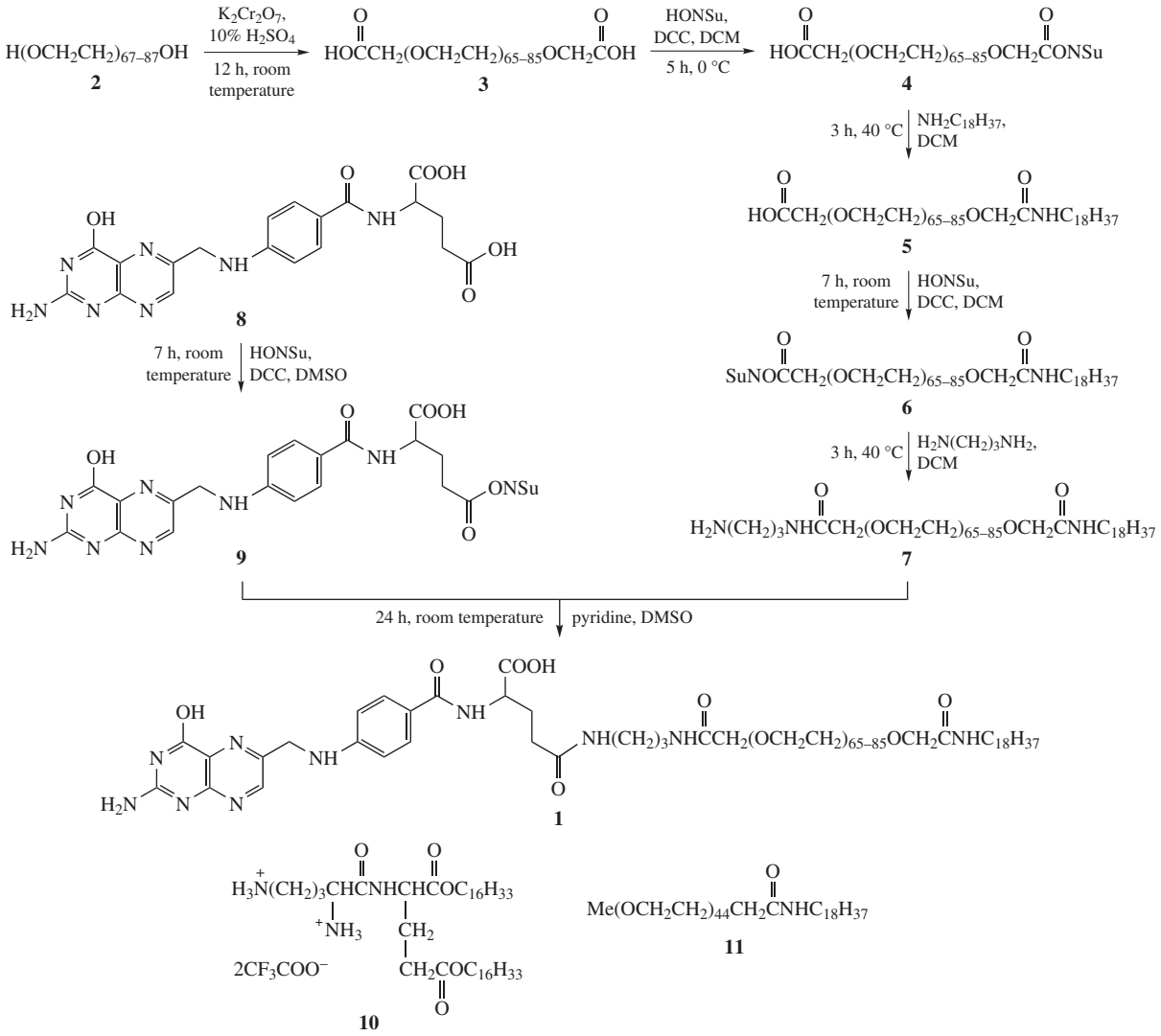
Sample **1** could be readily incorporated in cationic liposomes (5 wt%) formed by dihexadecyl-*N*-(ornithyl)glutamate bistrifluoroacetate **10** from the 'library' of lipodipeptides that we synthesised previously.[6] In order to provide steric stabilization and prolong circulation in blood, octadecylamide of PEG_{2000} monomethyl ether **11** was also added to the liposomes (5 wt%) (Figure 1).

Using simple mixing, freezing-thawing and active loading with ammonium sulfate concentration gradient, we studied the inclusion of an anticancer drug doxorubicin at 40% of the total mass.§ It was shown that about 90% of the drug was incorporated by simple mixing and over 95% was incorporated in other cases.

To study the morphology of the resulting particles, we took electron micrographs and determined the particle sizes; samples

† *N*-Hydroxysuccinimide (0.048 g, 0.42 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (0.112 g, 0.42 mmol) were added with stirring to a solution of compound **5** (1.52 g, 0.42 mmol) in dichloromethane (DCM) and the reaction mixture was kept for 7 h at room temperature. 1,3-*N*,*N*′-Diaminopropane (0.031 g, 0.42 mmol) was added and the mixture was kept for 3 h at 40 °C. The solvent was removed, the residue was dissolved in methanol (70 ml) and the resulting solution was washed with hexane (2×100 ml) to give 0.80 g (52%) of compound **7**. IR (film, ν_{max}/cm^{-1}): 3483 (NH_2), 3326 (NH), 2881 (CH), 1626 (C=O), 1536 (NH), 1469 (CH), 1414 (CN), 1344 (CH), 1113 (C–O–C), 723 (CH), 1280, 948, 843. ^{1}H NMR, δ: 0.82 (t, 3H, *Me*CH_2), 1.19 (m, 32H, CH_2), 1.61 (m, 2H, $NH_2CH_2CH_2CH_2NH$), 2.91 (m, 2H, NH_2CH_2), 3.02 [m, 2H, C(O)NH-CH_2CH$_2$], 3.19 (t, 2H, $NH_2CH_2CH_2NH$), 3.59 (m, ~290H, CH_2CH_2O), 3.69 [m, 2H, $NHC(O)CH_2OCH_2$], 3.82 [m, 4H, $NHC(O)CH_2OCH_2CH_2$-PEGCH_2CH$_2$OCH$_2$C(O)NH], 3.92 (s, 3H, NH), 4.43 [d, 2H, NHC(O)-CH$_2$]. MS (MALDI), *m*/*z*: 3706.630 $[M]^+$ (mean).

‡ Compound **7** (0.8 g, 0.23 mmol) and pyridine (20 μl) were added to a solution of activated folic acid **9** (0.12 g, 0.23 mmol) in DMSO (5 ml). The mixture was stirred for 24 h in the dark. The resulting precipitate of dicyclohexylurea was filtered off. Acetonitrile (20 ml) was added to the filtrate; the bright yellow precipitate that formed was filtered off, washed with dichloromethane and recrystallised from an acetonitrile–water mixture to give 0.14 g (72%) of compound **1**. ^{1}H NMR, δ: 0.84 (t, 3H, *Me*CH_2), 1.2 (m, 32H, CH_2), 1.45 (m, 2H, CH_2CH_2NH), 1.61 (m, 2H, $NH_2CH_2CH_2CH_2NH$), 1.94 [t, 2H, CH_2(Glu)], 2.4 [t, 2H, CH_2(Glu)], 3.02 [m, 2H, C(O)NHCH_2CH$_2$], 3.19 (t, 2H, $NH_2CH_2CH_2NH$), 3.21 (m, 2H, NH_2CH_2), 3.59 (m, ~290H, CH_2CH_2O), 3.69 [m, 2H, $NHC(O)CH_2$-OCH_2], 3.82 [m, 4H, $NHC(O)CH_2OCH_2CH_2$PEGCH_2CH$_2$OCH$_2$C(O)NH], 3.92 (s, 3H, NH), 4.2 (s, 2H, CH_2), 4.43 [d, 2H, NHC(O)CH_2], 6.4 (dd, 2H, CH), 7.3 (dd, 2H, CH). MS (MALDI), *m*/*z*: 4129.3 $[M]^+$ (mean).



Scheme 1

of the Lp-Dox dispersion were stained with uranyl acetate (Figure 2). It was found that the diameters of over 99% of the particles were within 37–40 nm (LS 12 320 laser analyzer from Beckman Coulter) (Figure 3).

The zeta-potential is an important parameter of dispersed systems that determines stability.[7] This parameter amounts to 50.8 mV for the Lp-Dox liposomes obtained, which results in efficient electrostatic repulsion of the species from each other (Figure 4). Lp-Dox dispersions are stable for a long time at room temperature, as confirmed by the absence of considerable changes in optical density, namely, less than 5% in one week (Figure 5).

Incorporation of cholesterine in Lp-Dox dispersions (5–20 wt%) results in an increase in the phase transition temperature. Moreover, the phase transition occurs in a narrow temperature range; with 10% cholesterine content, it is observed at 42–43 °C (Figure 6). This is rather a valuable property that can be used to construct delivery systems with thermally-controlled release of the contents during hyperthermia.[8]

In conclusion, we have developed and characterized a system for directed delivery of anticancer drugs based on lipodipeptides incorporating a newly-synthesised conjugate of folic acid with a PEG derivative for subsequent use in *in vivo* experiments.

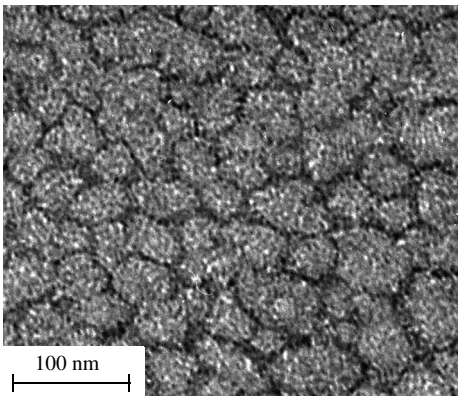


Figure 2 Electron micrograph with staining by uranyl acetate for liposomes based on lipodipeptide **10** containing PEG derivative **11** and folic acid conjugate **1** (5 wt% each) and incorporating 40 wt% doxorubicin (Lp-Dox).

§ A film was formed by simple mixing of a solution of lipopeptide **10** (54 wt%), PEG derivative **11** (3 wt%), folic acid conjugate **1** (3 wt%) and 40 wt% doxorubicin in chloroform in an evaporator; afterwards the film was dried *in vacuo* to remove residual solvent. The film was then hydrated with water at 50 °C with mechanical shaking. The resulting dispersion (Lp-Dox) was successively extruded through polycarbonate filters with 400 and 100 nm pore size. In the freezing-thawing procedure, the dispersion obtained after film hydration was exposed to a series of freezing-thawing cycles. In the active loading procedure, the film containing no doxorubicin was hydrated with a 250 mM ammonium sulfate solution at 50 °C with shaking. To create a concentration gradient, the dispersion was diluted 20-fold and an aqueous doxorubicin solution was added. In all cases, the fraction of doxorubicin that was not incorporated was removed by dialysis.

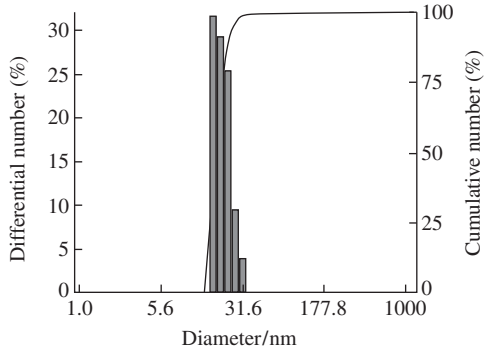


Figure 3 Particle size distribution in an Lp-Dox dispersion.

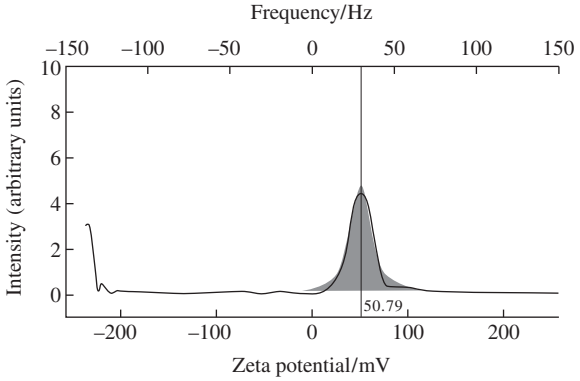


Figure 4 Graphical representation of zeta-potential measurement results for an Lp-Dox dispersion.

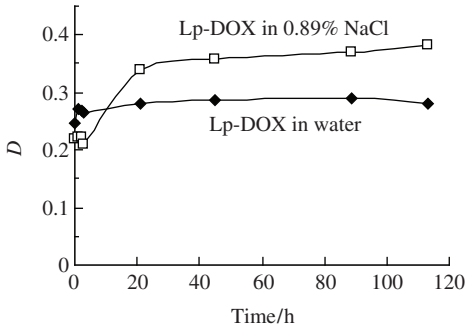


Figure 5 Optical density variation plot for an Lp-Dox dispersion.

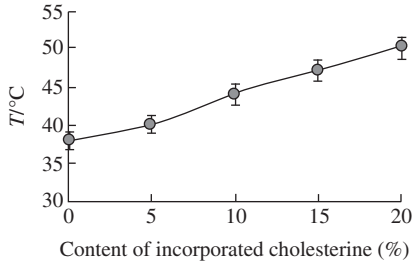


Figure 6 Phase transition temperature plot in an Lp-Dox dispersion *vs.* the content of incorporated cholesterine (5–20 wt%).

The study was supported by the Russian Foundation for Basic Research (grant no. 08-04-01150) and Analytical Departmental Goal-oriented Programme 'Development of scientific potential of higher school' no. 2.1.1./2889.

References

1 J. Heyes, K. Hall, V. Tailor, R. Lenz and I. MacLachlan, *J. Controlled Release*, 2006, **112**, 280.
2 J. M. Saul, A. Annapragada, J. V. Natarajan and R. V. Bellamkonda, *J. Controlled Release*, 2003, **92**, 49.
3 C. P. Leamon and P. S. Low, *Drug Discovery Today*, 2001, **6**, 44.
4 C. P. Leamon and P. S. Low, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 5572.
5 E. S. Lee, K. Na and H. Y. Bae, *J. Controlled Release*, 2003, **91**, 103.
6 Yu. L. Sebyakin and U. A. Budanova, *Bioorg. Khim*., 2006, **32**, 453 (*Russ. J. Bioorg. Chem*., 2006, **32**, 407).
7 E. Kawaguchi, K. Shimokawa and F. Ishii, *Colloids Surf., B*, 2008, **62**, 130.
8 M. Hossann, M. Wiggenhorn, A. Schwerdt, K. Wachholz, N. Teichert, H. Eibl, R. D. Issels and L. H. Lindner, *BBA*, 2007, **1768**, 2491.

Received: 17th March 2010; Com. 10/3491