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Mendeleev Commun., 2019, **29**, 616–618

Mendeleev Communications

Synthesis of novel lipophilic tetraamines with cytotoxic activity

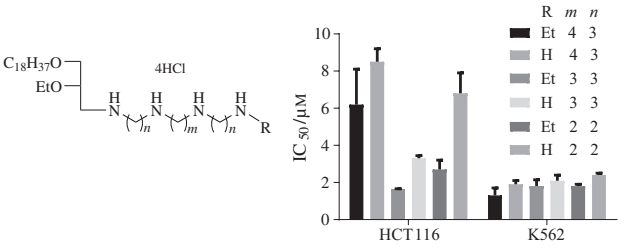
Ksenia A. Perevoshchikova,[a] Artemiy I. Nichugovskiy,[a] Alexandra K. Isagulieva,[b] Nina G. Morozova,[a] Igor V. Ivanov,[a] Mikhail A. Maslov*[a] and Alexander A. Shtil[b]

[a] *M. V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University, 119571 Moscow, Russian Federation. E-mail: mamaslov@mail.ru*

[b] *N. N. Blokhin Russian Cancer Research Center, 115478 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2019.11.003

New lipophilic polyamines in which natural or synthetic tetraamines were linked *via* the terminal NH_2 groups to diglycerides and/or to short-chained aliphatic substituents were synthesized, and their cytotoxic activity was tested. Relationship between the structure of the synthesized compounds and their cytotoxicity and hemolytic effect was evaluated.



Natural polyamine (PA) spermine and its precursors (spermidine and putrescine) are essential for cell growth in eukaryotes. These PAs affect the conformational state of DNA, modulate the chromatin structure, stabilize the lipid bilayer, and are crucial for the regulation of ion channels and gene expression.[1,2] Intracellular polyamine concentrations are highly regulated at the levels of biosynthesis, uptake, and catabolism. Elevated intracellular levels of PAs are known to be associated with cancer and other diseases related to altered proliferation.[3] Therefore, targeting the metabolic pathways of these PAs has emerged as a promising strategy for cancer treatment.[4]

A variety of symmetric or non-symmetric terminally substituted natural PAs and their synthetic alkylated analogues have been synthesized and investigated in different experimental models.[5,6] These compounds exhibited significant antitumour effects modulated by the inhibition of PA biosynthesis or the induction of the catabolic enzymes that affect PAs.[7–9] Symmetrically substituted bis(alkylated)polyamine N^1,N^{11}-diethylnorspermine (DENSpm) being one of the most investigated PAs was evaluated in Phases I and II clinical trials, however, its therapeutic efficacy was not confirmed.[6]

Non-phosphorous cationic ether glycerolipids (CELs) represent a group of potential candidates for antitumour drug therapy.[10] These compounds are structurally close to Edelfosine (1-*O*-octadecyl-2-*O*-methylglycero-3-phosphocholine), a phosphate-containing ether glycerolipid.[11] In contrast to Edelfosine, CELs are resistant to intercellular phospholipases and thus can induce tumour cell apoptosis without exhibiting any hemolytic effects.[10] In our previous works, we have synthesized a series of CELs which were modified with different polar heads,[12] and a strong correlation between their structure and biological activities has been established.[10,12,13] As a part of our ongoing investigations, in this study we have designed and synthesized a series of lipophilic polyamines (LPAs) in which the terminal NH_2 moieties of natural (spermine) and synthetic (norspermine and triethylenetetramine) polyamines were linked to diglycerides or short-chain aliphatic substituents, or both. Variations in the chain length of the diglyceride substituents and the composition of the PA backbone allowed us to get insight into the structure–activity relationship for this series of LPAs.

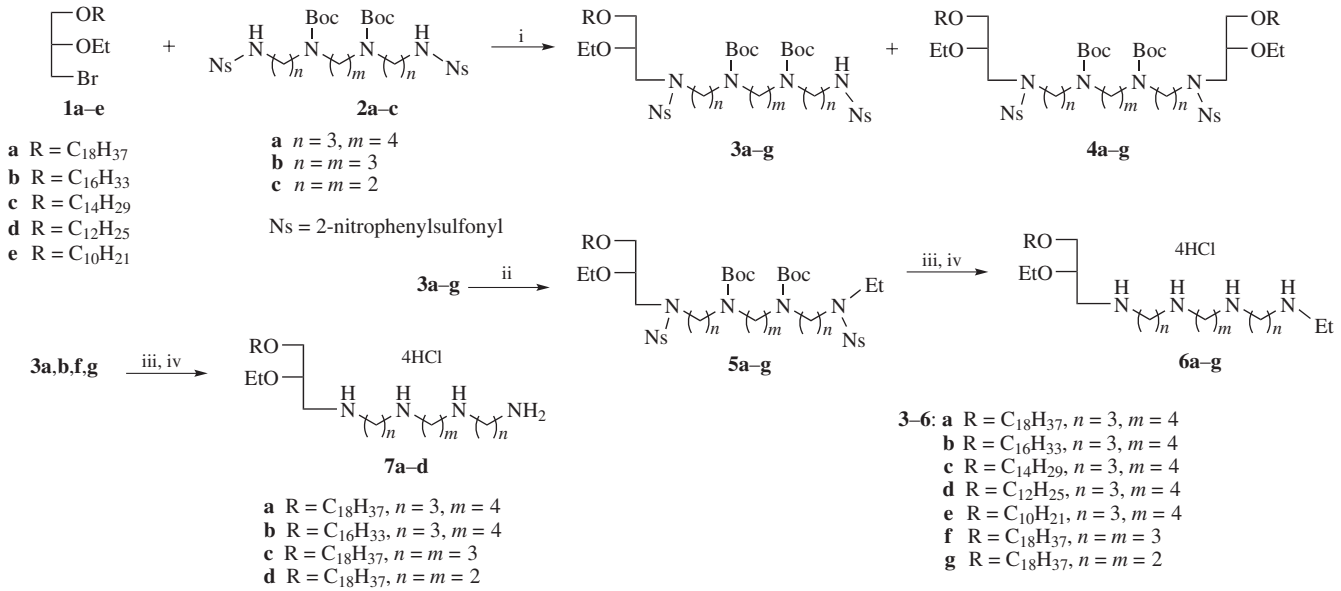
Diverse modified PAs are often obtained by alkylation of protected amines during chain elongation process[14] or through the formation of nitrogen-containing compounds and their subsequent reduction to amines[15–17], as well as by Michael addition reaction.[18] The preparation of alkylated PA conjugates is most often carried out by alkylation of protected polyamines with halogen derivatives.[19–21]

In this study, we employed the synthetic approach based on the Fukuyama reaction[27] between 2-nitrobenzenesulfonamides obtained from regio-protected PAs and bromo derivatives of the dialkylglyceroles. The removal of the 2-nitrophenylsulfonyl groups gave desired secondary amines. This useful synthetic approach led to monoalkylated LPAs as well as their unsymmetrically substituted dialkylated analogues.

Diglycerides were obtained from Bn-O-protected *rac*-glycidol[25] *via* the oxirane ring opening with long-chained aliphatic alcohols (C_{10}–C_{18}) in the presence of sodium hydride and subsequent O-alkylation with bromoethane.[26] Bromo diglyceride derivatives **1a–e** were synthesized from the corresponding diglycerides on treatment with tetrabromomethane and triphenylphosphine in 82–98% yields (see Online Supplementary Materials).

Regioselectively protected PAs **2a–c** (Scheme 1) were prepared by the conversion of primary amines into the corresponding amides on treatment with 2-nitrophenylsulfonyl chloride in a four-step procedure as described previously.[22,23] An effective differentiation of the amino groups of spermine, norspermine, or triethylenetetramine was achieved by the trifluoroacetylation of the primary amino groups[24] followed by *N*-Boc protection of the secondary ones.

The consequent condensation of the PA derivatives **2a–c** with equimolar amounts of compounds **1a–e** was carried out at 85 °C for 25 h in the presence of cesium carbonate (see Scheme 1).[27] This reaction afforded both monoalkylation products **3a–g** (17–33% yields) and symmetric dialkylated derivatives **4a–g** (10–15% yields).



Scheme 1 *Reagents and conditions*: i, Cs_2CO_3, DMF, 85 °C; ii, EtBr, Cs_2CO_3, DMF, 85 °C; iii, PhSH, K_2CO_3, 24 °C; iv, 4 N HCl·dioxane, 24 °C.

To obtain unsymmetrically disubstituted LPAs **5a–g**, the intermediates **3a–g** were treated with ethyl bromide under the Fukuyama conditions (yields 66–91%). The 2-nitrophenylsulfonyl protecting groups in compounds **5a–g** were removed using thiophenol in the presence of potassium carbonate. The removal of the *N*-Boc protective group using HCl in dioxane led to the final LPAs **6a–g**. The monosubstituted LPAs **7a–d** were synthesized in a similar manner as described above for the compounds **6a–g**. All LPAs obtained, including their intermediates, were characterized by ^1H, ^{13}C NMR spectroscopy and mass spectrometry (see Online Supplementary Materials).

The newly synthesized LPAs **6a–g** and **7a–d** were tested against the human chronic myelogenous leukemia (K562), colon (HCT116) and breast (MCF7) adenocarcinoma cells using the MTT-assay.[28] LPAs induced tumour cell death in the micromolar concentration range, and their potencies (IC_{50} values) were 4–5-fold higher than those of the reference cationic ether glycerolipids (CL, *rac*-*N*-methyl-*N*′-{4-[(2-ethoxy-3-octadecyloxy)prop-1-yloxycarbonyl]butyl}imidazolium iodide), Edelfosine and DENSpm (Figure 1, Table S1 and Figure S2 of the Online Supplementary Materials).

The effect of norspermine-containing compound **6f** on HTC116, MFC7 and K562 cells was higher than the effect of

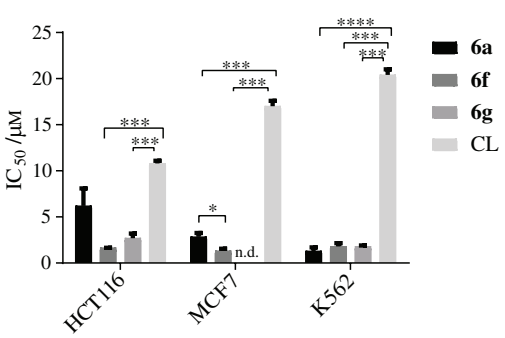


Figure 1 The impact of the polyamine structure on the cytotoxicity against tumour cells. The cytotoxicity data against the myelogenous leukaemia (K562), colon (HCT116) and breast adenocarcinoma (MCF-7) cells are presented as mean value ± SEM for $n = 3$ independent experiments. *P < 0.1, ***P < 0.001, ****P < 0.0001 *vs.* CL; paired t-test.

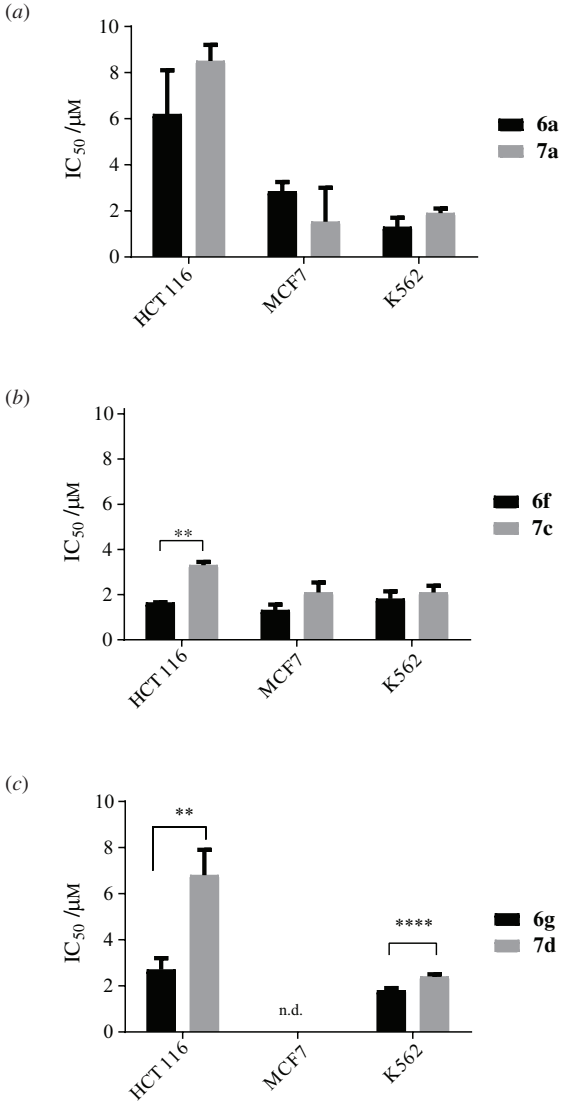


Figure 2 Differences between the cytotoxic activity of unsymmetrically dialkylated (**6**) and monoalkylated (**7**) lipophilic polyamine derivatives (*a*) **6a** and **7a**, (*b*) **6f** and **7c**, (*c*) **6g** and **7d**, against the tumour cells. The cytotoxicity data against K562, HTC116 and MCF-7 cells are presented as mean values ± SEM for $n = 3$ experiments. **P < 0.01, ****P < 0.0001; paired t-test.

spermine- or triethyltetramine-based LPAs. Moreover, a four-fold increase in the cytotoxicity against the MCF7 cells was observed for compound **6f** in comparison with natural spermine analogue **6a** (see Figure 1). For all the cell lines tested, the cytotoxicity of the spermine-based compounds **6a**–**e** was hardly influenced by the length of the *O*-alkyl chain at C1 position of the glycerol backbone (see Online Supplementary Materials, Figure S1). The cytotoxicity of these compounds against the HCT116 cells (IC_{50} 6.20–2.97 μmol dm^{-3}) was comparable with the effect against the MCF7 (IC_{50} 5.80–1.82 μmol dm^{-3}) cell lines, whereas it was lower against the K562 cells (IC_{50} 2.25–1.30 μmol dm^{-3}). However, LPA **6a** with octadecyl residue was less active than other analogues against HCT116 and MCF7 cells but more effective against K562 cells.

Interestingly, the unsymmetrically dialkylated PA derivatives **6f**–**g** were more effective against the HTC116 and K562 cells than their monoalkylated analogues **7c**–**d** with free terminal NH_2 group (Figure 2). Accordingly, no significant difference in the cytotoxic activity of spermine-based PAs **6a** and **7a** was observed against all tested cell lines.

The evaluation of the ability to disrupt human erythrocytes revealed that novel LPAs did not mediate any distinguish hemolytic effect, similarly to the reference compounds CL[25] and DENSpm, whereas Edelfosine stronger induced the erythrocytes damage (Table S2, see Online Supplementary Materials). Moreover, the hemolytic activity decreased with the decrease in the length of the C1-positioned *O*-alkyl chain of compounds **6b**–**e**. Norspermine-based LPAs **6f** and **7c** had less effect than their spermine analogues **6b** and **7b**. The presence of ethyl group at the terminal NH_2 group did not affect the hemolytic activity in the case of spermine LPAs but decreased that for norspermin LPAs.

In summary, new lipophilic tetraamine derivatives have been designed and synthesized using the Fukuyama reaction. The results of the MTT-assay allow one to consider these compounds as potential antitumour agents. Using different combinations of substituents, we have demonstrated that unsymmetrically dialkylated lipophilic polyamines with norspermine backbones are the promising candidates for the further biological investigations. The synthesis and the biological screening of optically active LPAs are currently underway.

This study was supported in part by the Ministry of Education and Science of the Russian Federation (base part of state assignment 4.9671.2017/BP) and by the Russian Foundation for Basic Research (project no. 18-33-20192).

Online Supplementary Materials

Supplementary data associated with this article (general synthetic procedures, characterization of compounds and details for cytotoxicity and hemolytic activity assays) can be found in the online version at doi: 10.1016/j.mencom.2019.11.003.

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Received: 13th May 2019; Com. 19/5921