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Synthesis of a phthalocyanine–1,4,6,10-tetraazaadamantane conjugate and its activity against the human immunodeficiency virus

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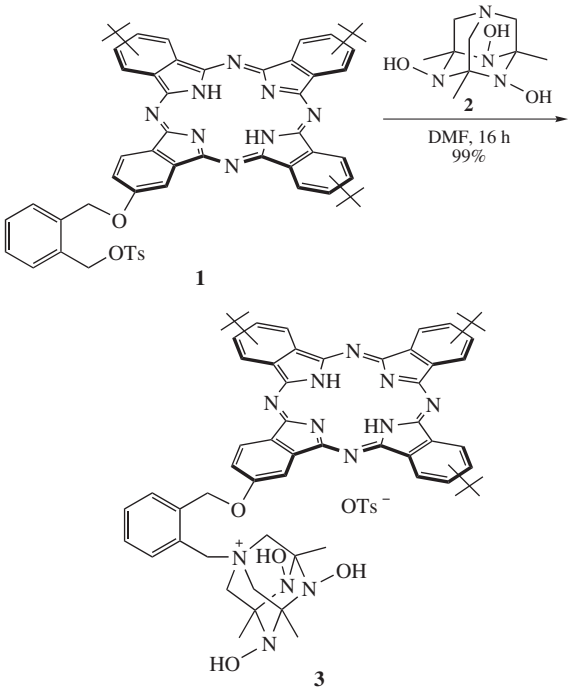
A new type of phthalocyanine containing 3,5,7-trimethyl-1,4,6,10-tetraazatricyclo[3.3.1.1^{3,7}]decane-4,6,10-triol (1,4,6,10-tetraazaadamantane) within a peripheral substituent has been synthesised. Its spectral properties and interaction with human lymphoblastoma cells infected with the HIV-1$_{BRU}$ strain have been studied.

The synthesis and physicochemical properties of various phthalocyanines are of considerable current interest. These macrocyclic compounds are interesting not only from the scientific point of view; in addition, they find use as nanomaterials[1,2] and medical products, in particular, as sensitizers for the photodynamic therapy of cancer.[3–5] In our opinion, incorporation of physiologically active moieties in the phthalocyanine structure is of special interest. In particular, the presence of fragments of biologically active bases[6] or glucose[7] in A_3B phthalocyanines makes it possible to render them soluble in water and physiological solutions. Furthermore, medical products based on adamantanes that possess anticataleptic, stress-protective, psychostimulant, immunotropic, antiviral and other kinds of activity are well known.[8,9] Therefore, the synthesis of conjugates of adamantane and its derivatives with phthalocyanines and studies of their physiological properties are important. In this work, we have synthesised a new conjugate based on substituted[10] monophthalocyanine **1** and 3,5,7-trimethyl-1,4,6,10-tetraazatricyclo-[3.3.1.1^{3,7}]decane-4,6,10-triol **2**[11] and studied its activity against the HIV.

Previously, we have shown that it is possible to modify the structure of nonsymmetrically substituted monophthalocyanines[12] in order to create building blocks for synthesising heteronuclear and heteroligand clamshell-type phthalocyanines.[10] In addition, the presence of the readily leaving tosylate group in phthalocyanine **1** allowed us to synthesise conjugate **3** of a new phthalocyanine-spacer-1,4,6,10-tetraazaadamantane type in an almost quantitative yield (Scheme 1). Taking into account that the structure of 1,4,6,10-tetraazaadamantane **2** contains a bridging nitrogen atom, the resulting conjugate can be represented as a quaternary ammonium salt.

It has been found that the completeness of the addition reaction strongly depends on the solvent polarity and the amount of 1,4,6,10-tetraazaadamantane. In fact, at room temperature and with THF as the solvent, conjugate **3** was formed in trace amounts only (TLC monitoring). Even refluxing a mixture of the starting compounds for 3 h did not result in the quantitative addition. However, we obtained conjugate **3** in 75% yield in DMF. A three-fold increase in the amount of 1,4,6,10-tetraazaadamantane **2** with respect to the stoichiometric amount increased the yield of compound **3** to 99%. The reaction takes 16 h at room temperature.†



Scheme 1

† *Synthesis of conjugate* **3**. 1,4,6,10-Tetraazaadamantane **2** (27 mg, 0.09 mmol) was added to a solution of tosylate **1** (30 mg, 0.03 mmol) in anhydrous DMF (5 ml) and the mixture was kept for 16 h with TLC monitoring. Once the reaction was completed, the final solution was evaporated under reduced pressure and washed with THF (2×20 ml) to give 36 mg (99%) of compound **3**. ^{1}H NMR ([$^2H_8$]THF) δ: 0.92 (br. s, 9H, Me), 1.68 (s, 27H, CMe_3), 2.21 (s, 3H, Me), 2.52–2.79 (br. m, 6H, CH_2), 4.82 (s, 2H, CH_2), 5.01 (s, 2H, CH_2), 7.11–7.62 (m, 8H, Ar), 9.18–9.46 (m, 12H, Pc). UV-VIS (H_2O, λ_{max}/nm): 329, 613. MS, *m/z*: 1031 [M – OTs – H]$^+$ (100), 843 [M – $C_7H_{14}N_3O_3$ –OTs – H]$^+$ (21), 801 [M – $C_9H_{18}N_4O_3$ –OTs – H]$^+$ (18).

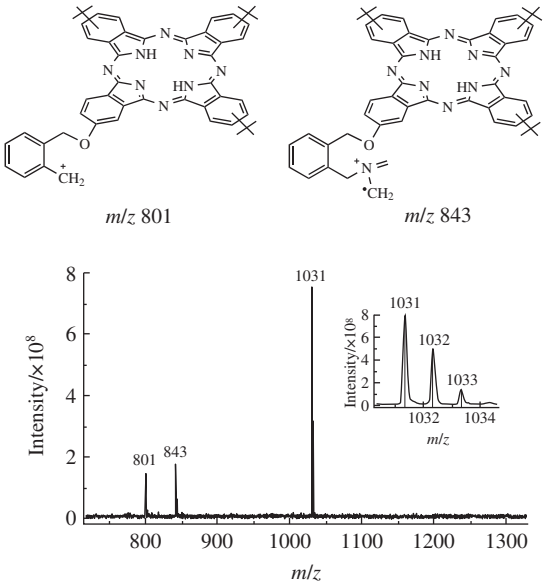


Figure 1 Mass spectrum of compound **3**.

The structure of conjugate **3** was confirmed by mass spectrometry and ^{1}H NMR spectroscopy. The MALDI-TOF mass spectrum under positive ionization (with DCTB‡ as the substrate) revealed signals of a molecular ion ($[M - OTs - H]^+$) and those of characteristic fragment ions with *m/z* 843 $[M - C_7H_{14}N_3O_3 - OTs - H]^+$ and 801 $[M - C_9H_{18}N_4O_3 - OTs - H]^+$ (Figure 1). The conjugate does not give any signals in the mass spectrum under negative ionization.

The ^{1}H NMR spectrum of phthalocyanine **3** was found to contain signals from all types of protons, both related to phthalocyanine and 1,4,6,10-tetraazaadamantane.† The signals of aromatic protons of the phthalocyanine macrocycle are broadened due to aggregation.

In addition, conjugate **3** was characterised by electronic absorption spectra (Figure 2).

The character and positions of the absorption bands of compound **3** virtually do not differ from those of phthalocyanine **1** (in THF), but its solubility in methanol and water is indirect proof (along with NMR and MS spectra) that 1,4,6,10-tetraazaadamantane is incorporated in the phthalocyanine structure. An interesting feature of compound **3** is that the aggregation properties vary on changing from a low-polarity solvent, THF, to methanol or water. The electronic spectrum of **3** in THF has a traditional shape typical of a vast majority of metal-free monophthalocyanines. A hypsochromic shift and splitting of the Q-band into several components are observed in methanol. The spectrum of phthalocyanine **3** in water manifests not only a total lack of splitting but also an even stronger hypsochromic shift of the Q-band. The data observed allow us to assume that complex aggregates which vary in nature depending on the solvent polarity exist in solutions.

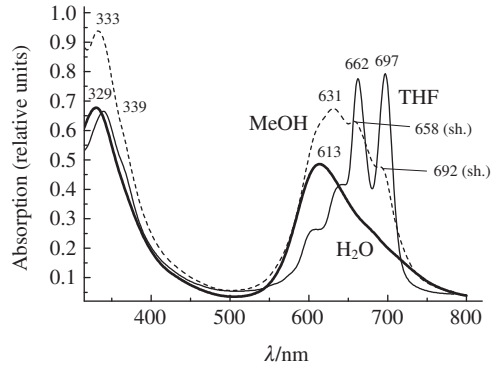


Figure 2 Electronic absorption spectra of conjugate **3**.

‡ DCTB stands for 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malonitrile.

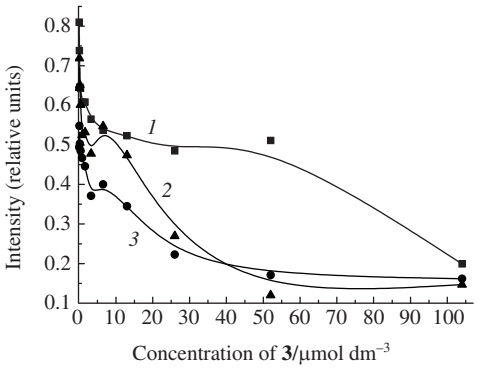


Figure 3 Antiviral activity of conjugate **3** (MTT assay). (*1*) Toxicity of the product; (*2*) compound + virus (simultaneously); (*3*) compound + virus (after 1 h).

We were the first to obtain phthalocyanine-spacer-1,4,6,10-tetraazaadamantane adduct **3**; by now, no analogues have been found in the literature. Therefore, it was of interest to study its cytotoxic properties. Compound **3** (1–10 μg cm^{-3}) was added to the test cells.§ The cells were grown at 37 °C under 5% CO_2 at 98% humidity for three to five days. The viability of the cells was determined from the difference in the cell count before and after the experiment using a tetrazolium dye (Figure 3, curve *1*). It has been shown that the specimen based on conjugate **3** has moderate cytotoxicity in the concentration range of 5.2×10^{-8}–5.2×10^{-5} mol dm^{-3}.

An *in vitro* assay of the antiviral effect of conjugate **3** was of special interest. The test cells were infected with human immunodeficiency virus (HIV-1_{BRU} strain). The specimen was introduced simultaneously with the cell infection (Figure 3, curve *2*) or 1 h after the infection (Figure 3, curve *3*). The cell cultures were incubated under the same conditions as those used in the cytotoxic experiment. The effect of conjugate **3** on the HIV was assessed by MTT¶ assay using spectophotometry.

It has been found that the highest activity of conjugate **3** is reached at a concentration of 7.1×10^{-6} mol dm^{-3}.

Thus, a new conjugate of 1,4,6,10-tetraazaadamantane with phthalocyanine has been synthesized for the first time. The spectral, cytotoxic and antiviral (HIV) properties of the compound have been studied. It has been shown to manifest the maximum anti-HIV activity when introduced simultaneously with the virus.

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§ In this study, we used a continuous line of human lymphoblastoid cells MT-4 grown in RPMI 1640 medium with 10% cow fetus serum (manufactured at the Research Institute of Poliomyelitis and Viral Encephalitis, Russian Academy of Medical Sciences) and containing 100 μg cm^{-3} gentamycin. Retrovir azidothymidine manufactured by GlaxoSmithKline was used as the antiretroviral reference drug. The HIV-1_{BRU} strain was used as the virus source.

¶ MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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