Conjugates of Pyropheophorbide α with 17-substituted Steroidal Androgens. Synthesis, Molecular Modeling, Interaction with Some Cancer Cells

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Abstract

Five new bifunctional conjugates of pyropheophorbide a with 17-substituted testosterone, dihydrotestosterone and epitestosterone differing in the length of linker (1–5) and two new complex conjugates 6 and 7 (containing three functional units: pyropheophorbide a, 17α-substituted testosterone, and lipophylic hexadecyl chain) were synthesized. Mutual influence of steroidal and macrocyclic fragments in conjugates 1–7 was established by analysis of 1H NMR spectra and molecular models of conjugates. The uptake and internalization of conjugates 1–5 by prostate carcinoma cells were dependent on the stereochemical configuration of 17-hydroxyl group in steroidal moiety, and the length of linker. Conjugates 1–5 significantly decreased the LNCaP and PC-3 cells growth and proliferation at 96 h incubation; the anti-proliferative activity of epitestosterone derivative comprising short linker 3 was superior. Irradiation of cells labeled with conjugates with light (λ = 660 nm) significantly increased cytotoxicity. Trifunctional conjugates 6 and 7 easily formed mixed micelles with phosphatidyl choline and pluronic F68; these mixed micelles efficiently internalized by human hepatocarcinoma Hep G2 cells, herewith the internalization was dependent on the conjugate structure, rather than on the method of solubilization.

1. Introduction

Tetrapyrrolic macrocycles porphyrins and chlorins owing to their unique photochemical and photophysical properties have wide range of biomedical applications such as optical imaging, fluorescent labeling, photodynamic inactivation of microbial infections, and photodynamic therapy of solid tumors. Coupling of macrocycles with drugs or fragments of biological active molecules significantly improves delivery and distribution of macrocycle-based compounds to a specific location within the cells, facilitates their transport through receptor or drug mediated endocytosis, increased their specificity and selectivity, affects their photochemical properties and biological activity [1–7].

Conjugation of pheophorbide a or pyropheophorbide a with steroids is considered to be a promising approach for development of new bifunctional constructs possessing enhanced delivery to specific targets. Conjugates of pheophorbide a and pyropheophorbide a with estradiol efficiently internalized by estrogen receptor positive cells, accumulated in nuclei, and revealed a potency of application as sensitizer for photodynamic therapy of breast cancer [8–11].

In this study we have synthesized and investigated seven new conjugates of pyropheophorbide a (Pyro) with androgen receptor ligands – testosterone, dihydrotestosterone and epitestosterone 1–7. In bifunctional conjugates 1–5 macrocyclic and steroidal moieties are connected with ethylene diamine or 1,5-diamino pentane linkers; in conjugates 6 and 7 (L)-lysine residue was used as linker, that allowed to introduce the additional functional fragment – lipophylic hexadecyl chain permitting simply solubilize conjugates in an aqueous medium in the form of mixed micelles with phosphatidyl choline [12] (Fig. 1).
Presented herein studies of spectral properties and molecular models of conjugates 1–7 revealed a significant influence of the structure on the conformation of conjugates. The study of interaction of conjugates with prostate carcinoma LNCaP and PC-3 cells indicated that conjugates 1–5 were efficiently uptaken and internalized by cells and potently inhibited their growth and proliferation. Anti-proliferative activity of conjugates 1–5, as well as their photo induced toxicity in prostate carcinoma cells was dependent on the length of linker and the stereochemical configuration of C17 atom in steroidal part. On the other hand, conjugates 6 and 7 were insoluble in aqueous medium, they did not bound to prostate carcinoma LNCaP and PC-3 cells and did not affect the growth and proliferation of these cells. Nevertheless, they may be uptaken by human hepatocarcinoma Hep G2 cells in the form of mixed micelles with phosphatidyl choline or with biocompatible detergent.

The presented data showed that some of new synthesized conjugates of pyropheophorbide a with steroids may be considered as prospective agents for various biomedical experiments in cultured cells.

2. Results And Discussion

2.1. Chemical synthesis

Preparation of new conjugates 1–7 (consisted of synthesis steroidal blocks 12, 17 and 23; synthesis of bifunctional conjugates 1–5 by coupling of Pyro with steroidal blocks 12, 17 and 23 by means of diamino containing linkers; and synthesis of trifunctional conjugates 6 and 7) is presented in the Schemes 1, 2, and 3, respectively.

Synthesis of steroidal acids 12, 17, 23 was performed as follows. Testosterone 8 and dihydrotestosterone 13 were transformed to steroid blocks 12 and 17 by five steps including consecutive protection of carbonyl functions with formation of 1,3-dioxolanes [13], oxidation of 17β-hydroxyl groups [14], and Reformatsky reaction of obtained 17-ketones 10 and 15 with Zn and ethyl bromoacetate [15, 16]. The aforementioned reaction is known to pass stereoselectively and give appropriate 17β-OH isomer. The consecutive removal of ethylene ketal and ethyl ester protective groups led to 21-carboxylic acids 12 and 17 in 49% and 58% overall yields (based on compounds 8 and 13, respectively).

Steroidal block 23 was synthesized from cyclosteroid 18 [17] in five steps. The introducing of 17α-hydroxyl group was carried out by oxidation of 17(20)-double bond with m-chloroperbenzoic acid followed by the reduction of resulting of 17α,20-epoxide 19 [the mixture of related 17α,20(R)- and 17α,20(S)- isomers in ratio of 3: 1] with LiAlH4 in boiling THP to obtain 17α,21-diol 20. Then diol 20 was transformed to hydroxy acid 21 by oxidation with ruthenate – potassium bromate reagent [18] in acetone – water (3:1) solution. Then acid 21 was subjected to acid hydrolysis to obtain 3β-hydroxy-5-ene acid 22. Oxidation of compound 22 with Dess-Martin periodinane, followed by acid catalyzed isomerization of crude 3-oxo-5-ene resulted in target 17α-hydroxy-3-oxopregn-4-en-21-oic acid 23 in 22% overall yield (based on compound 18).
Both 17β-hydroxy- and 17α-hydroxy acids 12, 17 and 23 were obtained as pure compounds; the configuration of C17 was confirmed by $^{13}$C NMR spectra. The differences in chemical shifts of C16, C17, and C18 resonances in compounds 12, 17 and 23 were considered to be in agreement with published data of $^{13}$C NMR spectra for related 17α- and 17β-hydroxyestradiols [19].

Synthesis of bifunctional conjugates 1–5 is presented in Scheme 2. Initially Pyro was transformed to related pentafluorophenyl ester 24, which was then treated with excess of either ethylene diamine or 1,5-diaminopentane to obtain amides 25 and 26 comprising primary amino group. Condensation of compounds 25 and 26 with steroidal acids 12, 17 and 23 in the presence of DCC led to the target conjugates 1–5.

Synthesis of trifunctional conjugates 6 and 7 is presented in Scheme 3. Initially N(α)-Fmoc-N(ε)-Boc-Lys 27 was condensed with hexadecyl amine to obtain protected lysyl amide 28. To prepare conjugate 6 amide 28 was consequently treated with piperidine to remove Fmoc-protecting group; then the amino containing block 29 was coupled with Pyro; the obtained intermediate was treated with acid to remove Boc-protecting group; and finally the resulting amine 30 was acylated with steroidal acid 12 to obtain conjugate 6.

Our attempt to prepare conjugate 7 according to the same scheme was slightly successful because of racemization of C17 in 17-hydroxy-3-oxopregn-4-en-21-oyl amides, which occurs in the presence of acid (under the conditions of Boc-group removal). For this reason we changed the consequence of reactions as follows: initially we removed Boc-protective group in amide 28 and coupled the obtained amine 31 with Pyro (wherein the partial removal of Fmoc-protecting group was observed); then, after complete Fmoc-group deletion, the amine 32 was condensed with steroidal acid 12 to obtain target conjugate 7.

All synthesized conjugates 1–7 were prepared as pure compounds, their structures were completely characterized by HRMS, $^1$H NMR, $^{13}$C NMR and absorption spectra.

### 2.2. Spectral properties and molecular models

Absorption spectra of conjugates 1–7 were similar to each other and were typical for Pyro and their derivatives. Normalized absorption spectra of conjugates 1–4 are presented in the Fig. 2.

The $^1$H NMR spectra of conjugates 1–7 indicated a significant mutual influence of steroid and macrocyclic fragments. The chemical shifts of selected protons in $^1$H NMR spectra of conjugates 1–4, 6 and 7 (comprising either testosterone or epitestosterone) are presented in Table 1. The strong high-field shifts for H-4, H-18, H-19 resonances were observed in the spectra of conjugates 1–4 in comparison with those in spectra of unconjugated steroidal acids 12 and 23. The greatest high field shift for H-4 resonance was observed in spectra of conjugates 2 and 3, while that for H-18 resonance – in spectra of conjugates 1 and 4. The modest high field shifts for H-18 and H-19 resonances were observed in spectra of trifunctional conjugates 6 and 7. Chemical shifts for amide NH“ resonances strongly depended on the
conjugate structure, while those for H-5', H-10' and H-20' resonances in pyropheophorbide a moieties differed insignificantly.

Table 1

Chemical shifts (δ, ppm) for selected characteristic resonances in the $^1$H NMR spectra of conjugates 1–4, 6 and 7.

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>H-4</th>
<th>H-18</th>
<th>H-19</th>
<th>H-5'</th>
<th>H-10'</th>
<th>H-20'</th>
<th>NHα$^\alpha$</th>
<th>NHω$^\omega$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.56, s</td>
<td>0.25, s</td>
<td>0.75, s</td>
<td>9.26, s</td>
<td>9.04, s</td>
<td>8.52, s</td>
<td>6.61, br.t</td>
<td>6.30, br.t</td>
</tr>
<tr>
<td>2</td>
<td>5.53, s</td>
<td>0.73, s</td>
<td>1.00, s</td>
<td>9.31, s</td>
<td>9.28, s</td>
<td>8.51, s</td>
<td>6.35, br.t</td>
<td>5.30, br.t</td>
</tr>
<tr>
<td>3</td>
<td>5.53, s</td>
<td>0.54, s</td>
<td>0.88, s</td>
<td>9.24, s</td>
<td>9.10, s</td>
<td>8.48, s</td>
<td>6.78, br.t</td>
<td>6.24, br.t</td>
</tr>
<tr>
<td>4</td>
<td>5.54, s</td>
<td>0.25, s</td>
<td>0.81, s</td>
<td>9.32, s</td>
<td>9.24, s</td>
<td>8.50, s</td>
<td>6.22, br.t</td>
<td>5.53, br.t</td>
</tr>
<tr>
<td>6</td>
<td>5.48, s</td>
<td>0.71, s</td>
<td>0.95, s</td>
<td>9.23, s</td>
<td>9.19, s</td>
<td>8.46, s</td>
<td>6.40, br.d</td>
<td>6.75, br.t</td>
</tr>
<tr>
<td>7</td>
<td>5.49, s</td>
<td>0.70, s</td>
<td>0.91, s</td>
<td>9.25, s</td>
<td>9.22, s</td>
<td>8.47, s</td>
<td>7.15, br.d</td>
<td>6.56, br.t</td>
</tr>
</tbody>
</table>

The data presented in the Table 1 are thought to be in agreement with results of molecular modeling (performed by simulated annealing) indicated differences in positional relationships of steroid and macrocycle moieties in conjugates. Calculated ensembles of conformers, truncated at 10 kcal/mol above the lowest-energy conformer, are shown in the Fig. 3; the structures for lowest energy conformers are presented in Fig. 4.

Configuration of C17 affects the relative positioning of 17-hydroxy group, and in epitestosterone conjugates 3 and 4 this group was found to be oriented towards the macrocycle (Fig. 3C and 3D), and is capable of hydrogen bond formation with nearby amide proton (Fig. 4C and 4D). On the contrary, in testosterone derivatives 1 and 2 this hydroxyl group is directed outwards from the macrocycle, and completely exposed to the environment (Fig. 3A, 3B, 4A and 4B).

Due to the longer linker lengths in conjugates 2 and 4, they exhibit much greater conformational flexibility in comparison with conjugates 1 and 3, which allows more conformers with NH proton axially positioned off the macrocycle plane, pushing corresponding NMR resonances into higher field. Because position of steroid moiety in the lowest-energy conformers was found to be not significantly different between conjugates with different linker lengths, we concluded that the main effect of linker lengthening is, indeed, enhanced conformational flexibility.

Structures with steroid moiety hoisted over the surface of macrocycle were found energetically favorable for conjugates 1–4 (Fig. 3A – 3D). This ‘folded’ structure correlates well with observed high-field shifts of 18- and 19-methyl protons compared to unconjugated steroids, because of shielding effect exerted by large aromatic moiety of Pyro on atoms located above and below its surface. Presence of ‘unfolded’ conformers with relatively low energy in compounds 2 and 3 (Fig. 3B and 3C) is probably responsible for the observed weaker shielding of 18- and 19-methyls. Figure 3E demonstrates that structures with steroid
moiety hoisted over the surface of macrocycle, and hexadecyl chain located in its opposite side, are energetically favorable for trifunctional conjugate 6. On the contrary, Fig. 3F reveals that three ensembles of low energy conformers differing in positions of steroid relatively to macrocycle, and random distribution of hexadecyl chain, are favored for trifunctional conjugate 7. In both conjugates 6 and 7 18- and 19-methyl groups were mainly turned away from macrocycle.

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The lowest energy conformers of conjugates are stabilized by possibility of intramolecular hydrogen bonds formation (Fig. 4). In the lowest energy conformers of conjugates 1 and 2 Na" atom is located near carbonyl group of Pyro, thus making formation of corresponding hydrogen bond favorable. Additionally, in compound 1 the oxygen atom of 21-amide group is located between 17β-hydroxyl group and Nω" atom of ethylene diamine linker, and thus can serve as proton acceptor with either of these atoms being a donor (Fig. 4A and Fig. 4B). In the lowest energy conformers of conjugates 3 and 4 17α-hydroxyl group is located close to Na" atom, and apparently may serve as proton acceptor to form the corresponding hydrogen bond (Fig. 4C and Fig. 4D).
In the lowest energy conformers of both conjugates 6 and 7 hydrogen atom of steroid 17-hydroxyl group participates in hydrogen bond formation with oxygen of related 21-carboxamido group. Additionally, in the lowest energy conformer of conjugate 6 the oxygen atom of 17-hydroxyl group is located near nitrogen atom of hexadecyl amide, and thus may serve as proton acceptor to form the corresponding hydrogen bond (Fig. 4E). In the lowest energy conformer of conjugate 7 nitrogen atom of hexadecyl amide is located near carbonyl group of pyropheophorbide a, and may be involved in formation of corresponding hydrogen bond (Fig. 4F).  

2.3. Interaction of bifunctional conjugates with cultured cells

Conjugates 1–5 were efficiently uptaken and internalized by prostate carcinoma LNCaP and PC-3 cells, breast carcinoma MCF-7 cells and hepatocarcinoma Hep G2 cells. The uptake was dependent on the structure of conjugates. Time course of conjugates 1–4 uptake by LNCaP cells is given in the Fig. 5A. Epitestosterone derivatives were uptaken more efficiently than testosterone ones; in both pairs conjugates comprising shorter linkers were uptaken more efficiently than those comprising long linkers (3 > 4 ≥ 1 > 2). According to our molecular models, this dependence must be correlated to diminished conformational flexibility of compounds 1 and 3, combined with predominant 17-hydroxyl group exposure in testosterone derivatives.

The internalization of conjugates by cells was confirmed as follows: LNCaP cells, initially labeled with conjugates 1–4 for 6 h, were incubated for 12 h in fresh medium, followed by determination of conjugates content in it. The absence of detectable amounts of conjugates in the medium proves that they were completely internalized by cells. The photographs of MCF-7 cells labeled with conjugate 1 is presented in the Fig. 5B.

Conjugates 1–5 moderately inhibited the growth and proliferation of LNCaP and PC-3 cells at 24 h incubation, however potently inhibited it at prolong incubation. MTT test [20] data demonstrating effects of conjugates 1–5 on the growth and viability of LNCaP and PC-3 cells at 96 h incubation are presented in the Table 2. Conjugates 1–5 inhibited growth of both LNCaP and PC-3 cells, however, effect on LNCaP cells was more pronounced. Anti-proliferative activity of conjugates in LNCaP and PC-3 cells was dependent on the structure of steroid moiety and length of linker and decreased in the row: 3 > 1 > 4 > 2 > 5.
Table 2
Effects of conjugates 1–5 on the growth and proliferation of LNCaP and PC-3 cells at 96 h incubation (IC\textsubscript{50}, µM)

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>LNCaP</th>
<th>PC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.8</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>12.1</td>
<td>21.4</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>4</td>
<td>6.4</td>
<td>14.2</td>
</tr>
<tr>
<td>5</td>
<td>18.3</td>
<td>24.1</td>
</tr>
</tbody>
</table>

Photo induced toxicity of conjugates 1 and 5 in LNCaP and PC-3 cells was also primary evaluated. For this purpose LNCaP and PC-3 cells were incubated with conjugates 1 and 5 for 18 h, then the labeled cells were irradiated with light (\(\lambda = 660\) nm) for 10 min, thereafter the irradiated cells were incubated for 24 h in fresh medium. The irradiated cells, as well as the control cells (treated by the same way, but without irradiation) were analyzed using MTT test. The IC\textsubscript{50} values for irradiated and non irradiated cells are presented in the Table 3. These data revealed that conjugates 1 and 5 exhibit significant photo induced toxicity in prostate carcinoma cells.

Unlike trifunctional conjugates 6 and 7 were insoluble in aqueous media, so we did not conduct experiments, as described above, with these conjugates.

Table 3
Dark and photo induced toxicity of conjugates 1 and 5 in LNCaP and PC-3 cells.

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>LNCaP cells</th>
<th>PC-3 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark toxicity (IC\textsubscript{50}, µM)</td>
<td>Photo induced toxicity (IC\textsubscript{50}, µM)</td>
</tr>
<tr>
<td>1</td>
<td>24.2</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>24.0</td>
<td>2.6</td>
</tr>
</tbody>
</table>

2.4. Solubilization of trifunctional conjugates in aqueous medium

In order to solubilize trifunctional conjugates 6 and 7 in aqueous media we used two reported earlier methods: (i) injection of mixed iso-propanolic solution of conjugate and phosphatidyl choline (PC) into aqueous buffer [12]; (ii) hydration of mixed films conjugate – pluronic F68 [21].
We have prepared mixed micelles 6 – PC and 7 – PC with mass ratio conjugate/PC equal to 1:10 (which corresponded to concentration of 6.7 molar % of conjugates); and micelles 6 – F68 and 7 – F68 with mass ratio conjugate/pluronic equal to 1:10 and 1:50. Absorption spectra and particle size distribution (measured by laser scattering) for these preparations are presented in Figs. 6 and 7, respectively.

The spectra of micelles 6 – PC and 7 – PC were nearly identical and highly resolved; the Soret bands had two maxima at 402 nm and 417 nm (the last one is known to be characteristic for aggregated form of conjugates); the long wave maxima had red shifts about 6 nm (compared to those for spectra of conjugates 6 and 7 in CH₂Cl₂) and were observed at 674 nm; the maxima at 516 nm, 544 nm, 618 nm were clearly visible (Fig. 6). The mean sizes of micelles 6 – PC and 7 – PC were 123.3 nm and 108.0 nm, respectively (Fig. 7). These mixed micelles possessed high stability – their absorption spectra and particle size distribution did not show any visible changes during the storage for 1 week.

The spectra of micelles 6 – F68 and 7 – F68 (at a ratio conjugate/pluronic 1:50) were insufficiently resolved; the Soret bands were broad; the long wave maxima had additional shoulder near 710 nm, that indicated association of macrocycle chromophores with formation of stacked structures [22, 23]. The spectra of micelles 6 – F68 and 7 – F68 (at a ratio conjugate/pluronic 1:10) were resemble to those presented in Fig. 6, but displayed certain turbidity and poor resolution. The mean sizes of conjugate-pluronic micelles 6 – F68 and 7– F68 (with the mass ratio conjugate/pluronic 1:50) were 621.3 nm and 385.7 nm, respectively (Fig. 7). The absorption spectra of these micelles displayed significant changes after 24 h of storage, and after 1 week of storage the presence of mixed micelles was undetectable, that indicates low stability of mixed micelles conjugate-pluronic.

Mixed micelles of conjugates 6 – PC, 7 – PC, 6 – F68, and 7 – F68 poorly interacted with prostate carcinoma LNCaP and PC-3 cells, however significantly internalized by hepatocarcinoma Hep G2 cells. The uptake and internalization of conjugate 6 was about 5-fold stronger than that of conjugate 7. It depended on the structure of conjugate, rather than on the method of solubilization – the micelles conjugate – PC and conjugate – pluronic were internalized similarly (Fig. 8).

Apparently, binding of conjugates to Hep G2 cells may be explained by existence of lipid binding sites (LBS) on the surface of these cells and affinity of hexadecyl moiety of conjugates to LBS [24].

### 4. Conclusions

Synthesis of bi- and trifunctional conjugates of pyropheophorbide a with 17-substituted testosterone, dihydrotosterone and epitestosterone was developed. The analysis of spectral data and molecular models revealed mutual influence of steroidal and macrocyclic fragments in conjugates. Bifunctional conjugates 1–5 were efficiently uptaken and internalized by prostate carcinoma LNCaP and PC-3 cells; herewith they significantly decreased the cells growth and proliferation. The anti-proliferative activity of conjugates was dependent on the stereochemical configuration of C17 atom and the length of linker: the effect of epitestosterone derivative comprising short linker 3 was superior. Irradiation of cells labeled with
conjugates $1–5$ with light ($\lambda = 660$ nm) significantly increased cytotoxicity. Trifunctional conjugates $6$ and $7$ easily formed mixed micelles with phosphatidyl choline and pluronic F68; these mixed micelles efficiently internalized by human hepatocarcinoma Hep G2 cells. The binding of conjugates $6$ and $7$ in form of mixed micelles to Hep G2 cells was dependent on the conjugate structure, rather than on the method of solubilization. We suggest that some of newly synthesized conjugates may be considered as prospective agents for various biomedical experiments in cultured cells.

3. Materials And Methods

3.1. Materials and general methods

HRMS were registered with a Bruker ‘Apex Ultra’ FT ICR MS and a Bruker ‘Daltonics micrOTOF-Q II’ instruments at ion positive electrospray ionization mode; $^1$H NMR and $^{13}$C NMR spectra – on an AMX-III instrument (Bruker, 400 MHz) in CDCl$_3$ (signals of $^1$H in CHCl$_3$ was 7.28 ppm, and $^{13}$C in CDCl$_3$ was 77.16 ppm). Assignment of ambiguous proton resonances in target compounds was performed by analyzing the set of 2D NMR spectra (data not shown). Absorption spectra were measured with a “Cary Spectra 100” spectrophotometer in CHCl$_3$ and CH$_2$Cl$_2$ using a quartz cell with 1mm optical path length; particle size distribution was measured with “DelsaNano Beckman Coulter” instrument.

Flash chromatography was performed on (0.035–0.070 mm) silica gel from “Acros”, TLC – on HPTLC Silica gel F254 105564 glass plates from ‘Merck’; compounds on the plates were visualized by UV light (Filter 254 nm); and/or by spraying the dried developed plates with 5% (NH$_4$)$_2$MoO$_4$ in 10% sulfuric acid, followed by heating; pyropheophorbide $a$ derivatives were visible on the plates without any treatments.

Dihydrotestosterone, testosterone, N-hydroxysuccinimide, dicyclohexyl carbodiimide, 4-dimethylaminopyridine, N($\alpha$)-Fmoc-N($\varepsilon$)-Boc-Lys, ethylene diamine, 1,5-diamino pentane were obtained from “Acros”; soya bean PC “Lipoid S-100” was purchased from “Lipoids”, pluronic F68 – from “BASF”. Pyro was prepared from methyl pheophorbide $a$ according to procedure [25]; methyl [17(20)$E$]-6$\beta$-methoxy-3$a$,5$a$-cyclopregn-17(20)-en-21-oate $18$ was synthesized according to procedure [17]; Dess-Martin periodinane – according to procedure [14], other reagents and solvents were purchased from “Aldrich”, “Merck”, “Acros”, “Fluka” and “Spectra Chem (Moscow, Russia)”. Synthesis of compounds $9–12$ and $14–17$ is presented in “Supplementary data” section.

3.2. Chemical synthesis

3.2.1. Methyl 6$\beta$-methoxy-17$a$,20(R,S)-epoxy-3$a$,5$a$-cyclopregn-17(20)-en-21-oate $19$

m-Chloroperbenzoic acid (2.06 g, 8.37 mmol) was added to the solution of compound $18$ (2.00 g, 5.58 mmol) in CH$_2$Cl$_2$ (60 mL) and the mixture was stirred and heated under reflux for 8 h, the disappearance
of starting compound being monitored by TLC. Then saturated solutions of NaHCO₃ (70 mL) and NaHSO₃ (70 mL) were added; the mixture was vigorously stirred for 30 min; the layers were separated; aqueous layer was extracted with dichloromethane (2×30 mL). The combined extract was washed with brine (40 mL), dried over Na₂SO₄, and evaporated. The residue was purified by silica gel flash chromatography in hexane – EtOAc (8:1) mixture followed by evaporation to obtain epoxide 19 (the mixture of two isomers in a ratio of 3:1, 1.34 g, 3.57 mmol, 64%) as colorless glass. HRMS, calculated for [C₂₃H₃₅O₄]+: 375.2530; found: 375.2529.

1H NMR for major isomer: 0.44 and 0.65 (each 1H, m, H-4); 0.93 (3H, s, H-18); 1.00 (3H, s, H-19); 2.78 (1H, t, J = 2.7 Hz, H-6); 3.32 (3H, s, CH₃OC₆); 3.45 (1H, s, H-20); 3.75 (3H, s, CH₃OC₂₁); 1H NMR for minor isomer: 0.44 and 0.65 (each 1H, m, H-4); 0.89 (3H, s, H-18); 1.02 (3H, s, H-19); 2.78 (1H, t, J = 2.7 Hz, H-6); 3.32 (3H, s, CH₃OC₆); 3.36 (1H, s, H-20); 3.75 (3H, s, CH₃OC₂₁).

### 3.2.2. 6β-Methoxy-17α,21-dihydroxy-3α,5α-cyclopregnane 20

The solution of compound 19 (780 mg, 2.1 mmol) in abs. THF (20 mL) was added by drops to the stirred suspension of LiAlH₄ (175 mg, 4.6 mmol) in abs. THF (40 mL), then the mixture was stirred and heated under reflux for 2 h. After cooling excess of LiAlH₄ was decomposed by adding of ice water. The mixture was filtered, the residue was washed with Et₂O (2×30 mL). The combined extract was dried over Na₂SO₄, and evaporated to obtain diol 20 (680 mg, 1.9 mmol, 90%) as colorless glass. HRMS, calculated for [C₂₂H₃₇O₃]+: 349.2737; found: 349.2732; 1H NMR: 0.43 and 0.64 (each 1H, m, H-4); 0.74 (3H, s, H-18); 1.03 (3H, s, H-19); 2.76 (1H, m, H-6); 3.32 (3H, s, CH₃O); 3.90 (2H, m, H-21); 13C NMR: 13.2, 16.0, 19.4, 21.6, 22.4, 23.7, 25.0, 29.8, 30.8, 30.9, 33.5, 35.3, 36.8×2, 43.6, 47.5, 48.1, 49.9, 56.7, 61.1, 82.4, 85.5.

### 3.2.3. 6β-Methoxy-17α-hydroxy-3α,5α-cyclopregnan-21-oic acid 21

Diol 20 (2.23 g, 6.4 mmol) was dissolved in acetone (120 mL), then KBrO₃ (6.4 g, 38.5 mmol), water (80 mL), and RuO₂xH₂O (10 mg) were added, and the mixture was heated under reflux for 20 min, followed by cooling to room temperature. Thereafter EtOH (25 mL) was added by drops, the mixture was filtered, the residue was washed with acetone. The combined filtrate was evaporated, the residue was treated with CHCl₃ (150 mL) and water (50 mL). Chloroform extract was washed with brine (50 mL), dried over Na₂SO₄, and evaporated. The residue was applied on the top a silica gel column; the column initially was washed with hexane – EtOAc (2:1) mixture to remove byproducts, then target compound was eluted with hexane – EtOAc – CH₃COOH (50:49:1) mixture to obtain acid 21 (1.55 g, 4.3 mmol, 67%) as white foam. HRMS, calculated for [C₂₂H₃₅O₄]+: 363.2530; found: 363.2532; 1H NMR: 0.43 and 0.64 (each 1H, m, H-4); 0.76 (3H, s, H-18); 1.01 (3H, s, H-19); 2.58 (2H, AB system, H-20); 2.78 (1H, m, H-6); 3.32 (3H, s, CH₃O); 13C NMR: 13.2, 16.0, 19.7, 21.6, 22.2, 23.7, 25.0, 29.8, 30.8, 33.5, 35.2, 35.3, 37.5, 39.8, 43.5, 47.5, 47.8, 49.6, 56.6, 81.8, 82.5, 178.1.

### 3.2.4. 3β,17α-Dihydroxypregn-5-en-21-oic acid 22
Compound 21 (350 mg, 0.97 mmol) was dissolved in THF (15 mL), then 15% aqueous H$_2$SO$_4$ (4 mL) was added and the mixture was heated under reflux for 20 min. After cooling water (50 mL) was added; the mixture was extracted with CHCl$_3$ (3×50 mL); the combined chloroform extract was washed with brine (30 mL), dried over Na$_2$SO$_4$, and evaporated to obtain acid 22 (300 mg, 0.86 mmol, 89%) as white powder which was used without purification. The analytical sample was obtained after silica gel flash chromatography in hexane – EtOAc – CH$_3$COOH (75:24:1) mixture. HRMS, calculated for [C$_{21}$H$_{33}$O$_4$]$^+$: 349.2373; found: 349.2370; $^1$H NMR: 0.56 (3H, s, H-18); 0.84 (3H, s, H-19); 2.42 (2H, AB system, H-20); 3.38 (1H, m, H-3); 5.176 (1H, m, H-6); $^{13}$C NMR: 14.91, 18.65, 20.37, 23.51, 29.90, 30.49, 31.68, 32.05, 36.28, 36.57, 36.97, 39.42; 41.12, 46.90, 49.45, 49.74; 71.55; 82.16; 121.36; 140.35; 178.21.

3.2.5. 3-Oxo-17α-hydroxypregn-4-en-21-oic acid 23

Dess-Martin periodinane (902 mg, 2.12 mmol) was added to the stirred suspension of compound 22 (300 mg, 0.86 mmol) in dichloromethane (20 mL), thereafter water (10 µL, 0.56 mmol) was added and the mixture was stirred for 30 min more, the disappearance of compound 22 during the reaction being controlled by TLC. The mixture was cooled to +4°C, then EtOH (20 mL) was added by drops, the mixture was poured into water (50 mL), extracted with CHCl$_3$ (3×20 mL), the extract was washed with brine (30 mL), dried over Na$_2$SO$_4$, and evaporated. The residue was dissolved in abs. EtOH (8 mL), then oxalic acid (36 mg, 0.4 mmol) was added, the mixture was stirred and heating under reflux for 10 min, then poured into water (50 mL). The mixture was extracted with CHCl$_3$ (3×20 mL), the extract was washed with brine (30 mL), dried over Na$_2$SO$_4$, evaporated, the residue was purified by silica gel flash chromatography in hexane – acetone – CH$_3$COOH (64:35:1) mixture and evaporated to obtain 3-oxo-17α-hydroxypregn-4-en-21-oic acid 23 (193 mg, 0.56 mmol, 65%) as white solid. HRMS, calculated for [C$_{21}$H$_{31}$O$_4$]$^+$: 347.2217; found: 347.2219; $^1$H NMR: 0.76 (3H, s, H-18); 1.18 (3H, s, H-19); 2.59 (2H, AB system, H-20); 3.38 (1H, br. s, 17-OH); 5.73 (1H, s, H-4); $^{13}$C NMR: 15.5, 17.4, 20.6, 23.7; 30.2, 32.0, 32.9, 33.9, 35.7, 35.9, 37.4, 38.7, 39.6, 47.1, 49.0, 53.5, 81.5, 123.8, 171.8, 177.1, 200.0.

3.2.6. Pentafluorophenyl pyropheophorbide a 24

Pyropheophorbide a (Pyro) (150 mg, 0.28 mmol) was dissolved in 15 mL of CH$_2$Cl$_2$, then pentafluorophenyl trifluoroacetate (0.096 mL, 0.56 mmol) was added, thereafter Et$_3$N (0.039 mL, 2.8 mmol) was added dropwise to stirred solution during 10 min, the formation of pentafluorophenyl ester being controlled by TLC. After the reaction was completed, the solvent was evaporated in vacuo, the residue was twice evaporated with toluene, and purified by chromatography on silica gel in hexane – acetone (4:1) mixture to give ester 24 (186 mg, 2.7 mmol, 95%). HRMS, calculated for [C$_{39}$H$_{34}$F$_5$N$_4$O$_3$]$^+$: 701.2551; found: 701.2554. $^1$H NMR: -1.45 (1H br.s, N-H); 1.68 (1H, t, J = 7.6 Hz, 8$^2$-H); 1.84 (3H, d, J = 7.3 Hz, 18-CH$_3$); 3.22, 3.41, 3.65 (each 3H, s, 2-, 7-, 12-CH$_3$); 4.37, 4.52 (each 1H, m, 17$^1$-H and 8$^1$-H); 5.18, 5.24 (each 1H, d, J = 19.7 Hz, 17$^2$-H), 6.17 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, 8$^2$-H, cis), 6.22 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, 8$^2$-H, trans), 7.98 (1H, dd, J = 11.5 Hz and J = 17.9 Hz, 8$^1$-H), 8.57, 9.37, 9.48 (each 1H, s, 5-, 10-, 20-H). $^{13}$C NMR: 11.28; 12.12; 17.44; 19.53; 23.14; 29.72; 30.32; 47.99; 50.01; 51.41; 93.05; 97.41;
104.40; 105.98; 122.76; 128.57; 129.23; 130.37; 131.83; 136.17; 136.27; 136.54; 136.71; 136.83; 137.99; 138.38; 139.25; 139.92; 140.90; 141.92; 142.47; 145.27; 149.23; 151.02; 155.65; 159.57; 169.17; 177.30; 196.55; Absorption spectra in CH₂Cl₂, λ_max, nm: 398, 498, 660.

3.2.7. Procedure for preparation of amides 25 and 26

The mixture of C₆F₅-Pyro 25 (210 mg, 0.3 mmol), diamine (ethylene diamine or 1,5-diaminopentane, 6.0 mmol) and abs. CH₂Cl₂ (10 mL) was stirred for 2 h, then the mixture was poured into 0.1M CH₃COONa buffer, pH 5 (20 mL), extracted with CH₂Cl₂ (2×20 mL), the combined extract was washed with brine (20 mL), dried over Na₂SO₄, and evaporated. Then the residue was dissolved in tetrahydrofuran (30 mL), the solution was dried over granulated KOH, followed by evaporation to dryness.

3.2.8. 17³[(2-Aminoethyl)-amido]-pyropheophorbide a 25

Compound 25 was purified by flash chromatography in CHCl₃ – MeOH – NH₄OH (90:9:1) mixture, was obtained as black amorphous powder (135 mg, 0.22 µmol, 73%). HRMS, calculated for [C₃₅H₄₁N₆O₂]⁺: 577.3291, found: 577.3292; ¹H-NMR: -1.70, 0.33 (each 1H, br.s, N-H); 1.62 (3H, t, J = 7.6 Hz, H-8²); 1.75 (3H, d, J = 7.3 Hz, 18-CH₃); 3.18, 3.37, 3.41 (each 3H, s, H-2', H-7', H-12'); 4.23, 4.45 (each 1H, m, H-17¹ and H-8¹); 4.98, 5.19 (each 1H, d, J = 19.7 Hz, H-17²); 6.13 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, H-3², trans); 6.24 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, H-3², cis); 7.95 (1H, dd, J = 11.5 Hz and J = 17.9 Hz, H-3¹); 8.50, 9.24, 9.30 (each 1H, d, J = 10 Hz, H-1'), 13C NMR: 11.2; 11.8; 12.1; 17.4; 19.4; 23.0; 28.3; 30.2; 30.9; 32.8; 40.9; 41.7; 48.0; 47.0; 51.7; 92.9; 97.1; 103.9; 106.0; 122.7; 128.1; 129.2; 131.5; 135.8; 136.0; 136.1; 137.7; 144.9; 148.9; 150.7; 155.1; 160.4; 171.7; 172.4; 196.1. Absorption spectra in CH₂Cl₂, λ_max, nm (ε): 413 (85 000); 507 (8 900); 538 (8 000); 609 (7 000); 665 (35 200).

3.2.9. 17³[(2-Aminoethyl)-amido]-pyropheophorbide a 26

Compound 26 was purified by flash chromatography in CHCl₃ – MeOH – NH₄OH (90:9:1) mixture, was obtained as black amorphous powder (130 mg, 0.21 µmol, 70%). HRMS, calculated for [C₃₈H₄₇N₆O₂]⁺: 619.3760; found: 619.3749; ¹H NMR: −1.70, 0.40 (each 1H, br.s, N–H); 1.64 (3H, t, J = 7.6 Hz, H-8²); 1.78 (3H, d, J = 7.3 Hz, H-18¹); 3.20, 3.38, 3.46 (each 3H, s, H-21', H-7¹, H-12¹); 4.30, 4.48 (each 1H, m, H-17¹ and H-8¹); 5.05 (1H, br. s, NH-CO); 5.05, 5.21 (each 1H, d, J = 19.7 Hz, H-15¹); 6.15 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, H-3², cis); 6.26 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, H-3², trans); 8.50, 9.24, 9.30 (each 1H, d, J = 10 Hz, H-1'); 13C NMR: 11.3, 11.9, 12.2, 17.5, 19.5, 23.2, 24.0, 29.1, 30.3, 32.9, 33.0, 39.3, 41.8, 48.1, 50.1, 51.8, 93.0, 97.2, 104.1, 106.2, 122.6, 128.3, 129.3, 130.5, 131.6, 135.9, 136.1, 136.2, 137.8, 141.6, 145.1, 149.0, 150.8, 155.3, 160.5, 171.9, 172.1 (C1', C6', C9', C173', C19'); 196.2 (C131¹). Absorption spectra in CH₂Cl₂, λ_max, nm (ε): 413 (85 000); 507 (8 900); 538 (8 000); 609 (7 000); 665 (35 200).

3.2.10. Procedure for preparation of conjugates 1–5
The mixture of carboxylic acid (12, 17, or 23, 0.1 mmol), amino containing amide (25, or 26, 0.1 mmol), and DCC (23 mg, 0.11 mmol) in CH₂Cl₂ (5 mL) was stirred at room temperature for 2 h, then evaporated to dryness, and the residue was applied on the top a silica gel column. The column initially was washed with CHCl₃ – acetone – CH₃COOH (75:24:1) mixture to remove byproducts, then washed with CHCl₃ (5 mL), and finally the target product was eluted with CHCl₃ – MeOH – 7M solution of NH₃ in MeOH (93:5:2, by vol.) mixture. After evaporation the conjugates were dried in vacuo.

3.2.10. 17³[2''-(17β-Hydroxy-3-oxopregn-4-en-21-oylamidoethyl)amido]- pyropheophorbide a (conjugate 1)

Conjugate 1 (48 mg, 51 µmol, 51%) was obtained as black powder. HRMS, calculated for [C₁₅₆H₁₆₉N₆O₅]⁺: 905.5329, found: 905.5327; ¹H-NMR: -1.65 (1H, br. s, N–H); 0.25, 0.75 (each 3H, s, H-18 and H-19); 1.58 (3H, t, J = 7.6 Hz, H-8²); 1.79 (3H, d, J = 7.3 Hz, H-18¹); 3.16, 3.17, 3.39 (each 3H, s, H-2¹, H-7¹, H-12¹); 4.24, 4.48 (each 1H, m, H-17¹ and H-8¹); 4.95, 5.19 (each 1H, d, J = 19.7 Hz, H-17²); 5.56 (1H, s, H-4); 6.14 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, H-3², trans); 6.29 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, H-3², cis); 6.30 (1H, br. t, J = 5.2 Hz, NH-CO); 7.91 (1H, dd, J = 11.5 Hz and J = 17.9 Hz, H-3¹), 8.52, 9.04, 9.26 (each 1H, s, H-5', H-10', H-20'); ¹³C NMR: 11.1, 11.6, 12.0, 15.1, 16.9, 17.3, 19.2, 20.1, 23.0, 23.3; 29.9; 30.0; 30.4, 31.6; 32.6; 32.9; 33.7; 35.2; 35.5; 37.0; 38.2; 39.6; 40.4; 46.5; 48.0; 48.5; 50.1; 51.6; 52.9; 81.6; 94.0; 97.1; 103.9; 105.8; 122.6; 123.6; 127.8; 129.0; 129.9; 131.7; 135.9; 136.0; 136.3; 137.5; 141.7; 145.1; 148.8; 150.7; 155.4; 160.1, 171.2; 171.8; 173.6; 173.7; 196.3; 199.3. Absorption spectra is presented in Fig. 2.

3.2.11. 17³[5''-(17β-Hydroxy-3-oxopregn-4-en-21-oylamidopentyl)amido]- pyropheophorbide a (conjugate 2)

Conjugate 2 (48 mg, 51 µmol, 51%) was obtained as black powder. HRMS, calculated for [C₁₅₉H₁₇₅N₆O₅]⁺: 947.5793; found: 947.5789; ¹H NMR: −1.66 (1H, br. s, N–H); 0.73, 1.00 (each 3H, s, H-18 and H-19); 1.64 (3H, t, J = 7.6 Hz, H-8²); 1.78 (3H, d, J = 7.3 Hz, H-18¹); 3.19, 3.37, 3.46 (each 3H, s, H-2¹, H-7¹, H-12¹); 4.27, 4.46 (each 1H, m, H-17¹ and H-8¹); 5.00, 5.16 (each 1H, d, J = 19.7 Hz, H-15¹); 5.28 (1H, br. t, J = 5.2 Hz, NH-CO); 5.53 (1H, s, H-4); 6.15 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, H-3², trans); 6.25 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, H-3², cis); 6.33 (1H, br. t, J = 5.2 Hz, NH-CO); 7.94 (1H, dd, J = 11.5 Hz and J = 17.9 Hz, H-3¹), 8.51, 9.28, 9.31 (each 1H, s, H-5', H-10', H-20'); ¹³C NMR: 11.3, 12.0, 12.2, 13.9, 17.3, 17.5, 19.5, 20.6, 23.1, 23.6, 23.9, 28.8, 29.0, 29.8, 30.5, 31.7, 32.7, 33.1, 33.9, 35.7, 36.2, 36.5, 38.6, 39.0, 39.1, 42.6, 46.2, 48.1, 50.1 (x2), 51.7, 53.8, 82.0, 93.1, 97.3, 104.1, 106.0, 122.7, 123.8, 128.2, 129.2, 130.3, 131.8, 136.0, 136.2, 136.4, 137.8, 141.8, 145.2, 149.1, 150.9, 155.5, 160.5, 171.0, 172.0, 172.5, 173.4, 196.3, 199.4; Absorption spectrum is presented in Fig. 2.

3.2.13. 17³[2''-(17α-Hydroxy-3-oxopregn-4-en-21-oylamidoethyl)amido]- pyropheophorbide a (conjugate 3)
Conjugate 3 (28 mg, 31 µmol, 31%) was obtained as black powder. HRMS, calculated for [C_{56}H_{69}N_{5}O_{5}]^{+}: 905.5329, found: 905.5335; ^1H NMR: -1.67 (1H, br, s, N-H); 0.54, 0.88 (each 3H, s, H-18 and H-19); 1.55 (3H, t, J = 7.7 Hz, H-8²); 1.75 (3H, d, J = 7.3 Hz, H-18¹); 3.13, 3.15, 3.35 (each 3H, s, H-2¹, H-7¹, H-12¹); 4.20, 4.45 (each 1H, m, H-1⁷¹ and H-8¹); 4.92, 5.15 (each 1H, d, J = 19.7 Hz, H-15¹); 5.53 (1H, s, H-4); 6.11 (1H, dd, J = 11.6 Hz and J = 1.4 Hz, H-3²; trans); 6.25 (1H, dd, J = 17.8 Hz and J = 1.4 Hz, H-3²; cis); 6.24 (1H, br, t, J = 5.2 Hz, NH-CO); 6.78 (1H, br, t, J = 5.2 Hz, NH-CO); 7.88 (1H, dd, J = 11.6 Hz and J = 17.8 Hz, H-3¹); 8.58, 9.10, 9.24 (each 1H, s, H-5', H-10', H-20'); ^13C NMR: 11.2, 11.6, 12.1, 15.1, 16.9, 17.3, 19.3, 20.1, 23.0, 23.3, 29.9, 30.5, 31.6, 32.6, 33.0, 33.7, 35.2, 35.5, 37.0, 38.2, 39.6, 40.0, 40.5, 46.5, 48.1, 48.5, 50.1, 51.7, 53.0, 81.6, 93.0, 97.1, 103.9, 105.8, 122.7, 123.6, 127.8, 129.0, 129.9, 131.7, 135.9, 136.0, 136.3, 137.6, 141.7, 145.1, 148.9, 150.8, 155.4, 160.2, 171.2, 171.9, 173.6, 173.8, 196.3, 199.3. Absorption spectrum is presented in Fig. 2.

3.2.14. 17³[5"-(17α-Hydroxy-3-oxopregnen-4-en-21-oylamidopentyl)amido]-pyropheophorbide a (conjugate 4)

Conjugate 4 (44 mg, 46 µmol, 46%) was obtained as black powder. HRMS, calculated for [C_{55}H_{75}N_{6}O_{5}]^{+}: 947.5793; found: 947.5793; ^1H NMR: -1.67 (1H, br, s, N-H); 0.25, 0.81 (each 3H, s, H-18 and H-19); 1.61 (3H, t, J = 7.6 Hz, H-8²); 1.78 (3H, d, J = 7.3 Hz, H-18¹); 3.17, 3.36, 3.37 (each 3H, s, H-2¹, H-7¹, H-12¹); 4.26, 4.46 (each 1H, m, H-1⁷¹ and H-8¹); 5.00, 5.15 (each 1H, d, J = 19.7 Hz, H-15¹); 5.53 (1H, br, t, J = 5.2 Hz, NH-CO); 5.58 (1H, s, H-4); 6.14 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, H-3², trans); 6.22 (1H, br, t, J = 5.2 Hz, NH-CO); 6.23 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, H-3², cis); 7.92 (1H, dd, J = 11.5 Hz and J = 17.9 Hz, H-3¹); 8.50, 9.24, 9.32 (each 1H, s, H-5', H-10', H-20'); ^13C NMR: 11.3, 11.9, 12.1, 15.2, 17.1, 17.4, 19.4, 20.3, 23.1, 23.4, 23.7, 28.5, 28.9, 30.2, 30.6, 31.8, 32.0, 33.1, 33.9, 35.4, 35.7, 37.1, 38.4, 38.9, 39.0, 40.3, 46.6, 48.1, 48.7, 50.1, 51.7, 53.2, 81.8, 93.1, 97.2, 104.0, 105.9, 122.7, 123.7, 128.1, 129.1, 130.2, 131.8, 136.0, 136.2, 136.4, 137.8, 141.7, 145.2, 149.0, 150.8, 155.4, 160.5, 171.5, 172.0, 172.8, 173.1, 196.3, 199.6. Absorption spectrum is presented in Fig. 2.

3.2.15. 17³[2"-(17β-Hydroxy-3-oxopregnan-21-oylamidoethyl)amido]-pyropheophorbide a (conjugate 5)

Compound 5 (33 mg, 37 µmol, 69%) was obtained as black powder. HRMS, calculated for [C_{56}H_{71}N_{6}O_{5}]^{+}: 907.5486, found: 907.5490. ^1H NMR: −1.67, (1H, br,s, N–H); 0.56, 0.78 (each 3H, s, H–18 and H–19); 1.65 (3H, t, J = 7.6 Hz, 8²); 1.77 (3H, d, J = 7.3 Hz, 18¹); 3.21, 3.37, 3.45 (each 3H, s, H–2¹, H–7², H–12¹); 4.27, 4.46 (each 1H, m, H–1⁷¹, H–8¹); 5.02, 5.21 (each 1H, d, J = 19.7 H–17²); 5.86 (1H, br,t, J = 5.2 Hz, NH–CO); 6.14 (1H, dd, J = 11.5 Hz and J = 1.4 Hz, H–3², cis); 6.20 (1H, dd, J = 17.9 Hz and J = 1.4 Hz, H–3², trans), 6.58 (1H, br, t, J = 5.2 Hz, NH–CO); 7.93 (1H, dd, J = 11.5 Hz and J = 17.9 Hz, H–3¹); 8.52, 9.30, 9.33 (each 1H, s, H–5', H–10', H–20'); ^13C NMR: 11.4, 12.0, 13.6, 14.0, 17.3, 19.3, 20.6, 23.1, 23.4, 28.4, 28.7, 29.7, 31.5, 31.9, 33.0, 33.9, 35.3, 35.7, 36.2, 38.5, 39.7, 42.5, 46.0, 46.6, 48.0, 50.0 (x2), 51.7, 53.7, 81.9, 93.1, 7.1, 103.9, 105.9, 122.7, 124.2, 125.3, 128.2, 129.0, 130.0, 131.7, 136.0, 136.3, 137.6, 137.9, 141.7, 145.0,
Absorption spectrum is presented in Fig. 2.

3.2.16. N(α)-Fmoc-N(ε)-Boc-Lys-hexadecyl amide 28

N(α)-Fmoc-N(ε)-Boc-Lys 27 (200 mg, 0.427 mmol) and DCC (97 mg, 0.47 mmol) were dissolved in dry CH₂Cl₂ (12 mL), then hexadecyl amine (103 mg, 0.427 mmol) was added, the mixture was stirred for 1 h, then the mixture was diluted with CH₂Cl₂, washed with NaHCO₃ saturated solution (20 mL), water (20 mL), brine (20 mL), dried over Na₂SO₄ and evaporated to obtain amide 28 (257 mg, 0.371 mmol, 87%) as white solid. HRMS, calculated for [C₄₂H₆₆N₃O₅]⁺: 692.4997, found: 692.4988. ¹H NMR: 0.87 (3H, t, J = 6.7 Hz, CH₃-hexadecyl), 1.24 (28H, m, (CH₂)₁₄-hexadecyl), 1.42 (9H, s, CH₃-Boc), 3.09 (2H, q, J = 5.8 Hz, NCH₂-Lys), 3.21 (2H, q, J = 5.4 Hz, NCH₂-hexadecyl), 4.07 (1H, m, CH(α)-Lys), 4.19 (1H, t, J = 6.6 Hz, CH-Fmoc), 4.39 (2H, d, J = 5.2 Hz, CH₂-Fmoc), 4.58 (1H, br.t, NH(ε)-Lys), 5.47 (1H, br.t, NH-hexadecyl), 6.09 (1H, br.d, NH(α)-Lys), 7.29 (3H, t, J = 7.4 Hz, Ar-Fmoc), 7.38 (3H, t, J = 7.3 Hz, Ar-Fmoc), 7.57 (2H, d, J = 7.1 Hz, Ar-Fmoc), 7.75 (2H, d, J = 7.4 Hz, Ar-Fmoc). ¹³C NMR: 14.1, 22.5, 22.7, 24.9, 26.9 (×2), 28.4 (×3), 29.3, 29.4, 29.5, 29.6, 29.7 (×7), 31.9, 32.2, 34.0, 39.6, 47.2, 54.9, 67.0, 78.9, 120.0 (×2), 125.0 (×2), 127.1 (×2), 127.7 (×2), 141.3 (×2), 143.8 (×2), 156.2, 171.5.

3.2.17. N(ε)-Boc-Lys-hexadecyl amide 29

The mixture of amide 28 (1.512 g, 2.19 mmol), piperidine (220 µL, 3 mmol) and dry DMF (20 mL) was stirred for 1 h, then poured into ice water (200 mL), stirred for 20 min, the resulted precipitate was filtered, washed with water and dried to obtain N(ε)-Boc-Lys-hexadecyl amide 29 (904 mg, 1.92 mmol, 88%) as white solid. HRMS, calculated for [C₂₇H₅₆N₃O₃]⁺: 470.4316, found: 470.4313. ¹H NMR: 0.86 (3H, t, J = 6.7 Hz, CH₃-hexadecyl), 1.24 (26H, m, (CH₂)₁₃-hexadecyl), 1.42 (9H, s, CH₃-Boc), 3.10 (2H, q, J = 5.3 Hz, NCH₂-Lys), 3.21 (2H, q, J = 6.3 Hz, NCH₂-hexadecyl), 3.32 (1H, dd J₁ = 4.3 Hz, J₂ = 7.7 Hz, H-8₂'), 4.17 (1H, br.t, NH-hexadecyl), 4.56 (1H, br.t, NH(ε)-Lys); ¹³C NMR: 14.1, 22.5, 22.7, 24.9, 26.9 (×2), 28.4 (×3), 29.3, 29.4, 29.5, 29.6, 29.7 (×7), 31.9, 32.2, 34.6, 39.1, 40.2 (×2), 49.1, 55.1, 79.1, 156.1, 174.7.

3.2.18. N(α)-17³'(Pyropheophorbide)carboxamido-Lys-hexadecyl amide 30

The solution of Pyro (300 mg, 0.56 mmol) and DCC (120 mg, 0.58 mmol) in dry CH₂Cl₂ (25 mL) was stirred for 30 min, then N(ε)-Boc-Lys-hexadecyl amide 29 (263 mg, 0.56 mmol) was added and the mixture was stirred for 40 min more, then evaporated to dryness, and the residue was separated by silica gel flash chromatography in CH₂Cl₂ – acetone (9:1) mixture to obtain N(α)-Boc protected conjugate (329 mg, 0.33 mmol, 60%) as black foam. HRMS, calculated for [C₆₀H₈₈N₇O₅]⁺: 986.6841; found: 986.6844. ¹H NMR: -1.76 (1H, br.s, NH), 0.86 (3H, t, J = 6.9 Hz, CH₃-hexadecyl), 1.18 (28H, m, (CH₂)₁₄-hexadecyl), 1.29 (9H, s, CH₃-Boc), 1.66 (3H, t, J = 7.6 Hz, H-8²'), 1.78 (3H, d, J = 7.3 Hz, H-18¹'), 2.89 (2H, m, NCH₂-hexadecyl), 3.09 (2H, q, J = 4.7 Hz, NCH₂-Lys), 3.22, 3.38, 3.58 (each 3H, s, each 3H, s, H-2¹', H-7¹', H-12¹'), 4.11 (1H, m, H-17¹'), 4.28 (1H, m, H-17¹'), 4.47 (1H, m, H-8¹'), 4.54 (1H, br. t, NH(ε)-Lys), 5.04, 5.25 (each 1H, d, J =
20.0 Hz, H-15'), 6.00 (1H, br. d, NH(α)-Lys), 6.15 (1H, dd J₁ = 11.7 Hz, J₂ = 1.4 Hz, H-3', trans), 6.15 (br.t, 1H, NH-hexadecyl), 6.24 (1H, dd, J₁ = 17.9 Hz, J₂ = 1.4 Hz, H-3', cis), 7.96 (1H, dd, J₁ = 11.6 Hz, J₂ = 17.8 Hz, H-3¹), 8.55, 9.36, 9.42 (each 1H, s, H-5', H-10', H-20'). ¹³C NMR: 11.2, 12.1, 14.1, 17.3, 19.5, 22.4, 22.6, 23.1, 26.8, 28.3 (x3), 29.6 (x14), 30.0, 31.6, 31.9, 32.4, 39.5, 39.8, 48.1, 50.0, 51.7, 52.9, 79.0, 93.3, 97.1, 103.9, 106.3, 122.6, 128.6, 129.1 (x2), 130.6, 131.74, 136.0, 136.3, 137.9, 141.6, 144.8, 149.1, 156.1, 160.6 (x2), 171.4, 171.7, 172.3, 196.1.

The product obtained (329 mg, 0.33 mmol), dioxane (10 mL) and 30% aqueous H₂SO₄ was stirred for 45 min, the removal of Boc-group being controlled by TLC. Thereafter the mixture was poured into the mixture of water (30 mL) and chopped ice (30 g), neutralized with NH₄OH, and extracted with dichloromethane (3×25 mL). The combined extract was washed with brine (30 mL), dried over Na₂SO₄ and evaporated to obtain compound 30 (284 mg, 0.32 mmol, 97%) as black solid. HRMS, calculated for [C₅₅H₈₀N₇O₃]⁺: 886.6317; found: 886.6318.

1H NMR: -1.72 (1H, br.s, NH), 0.86 (3H, t, J = 6.9 Hz, СH₃-hexadecyl), 1.19 (28H, m, (CH₂)₁₄-hexadecyl), 1.67 (3H, t, J = 7.6 Hz, H-8²), 1.77 (3H, d, J = 7.3 Hz, H-18¹), 3.12 (2H, q, J = 4.67 Hz, NС Н₂(ε)-Lys), 3.21, 3.38, 3.59 (each 3H, s, H-2¹, H-7¹, H-12¹), 4.20 (1H, br.t, NH-hexadecyl), 4.28 (1H, m, H-17¹), 5.04, 5.23 (each 1H, d, J = 19.9 Hz, H-15¹), 5.92 (1H, br.d, NH(α)-Lys), 6.15 (1H, dd J₁ = 11.5 Hz, J₂ = 1.4 Hz, H-3², trans), 6.26 (1H, dd, J₁ = 18.0 Hz, J₂ = 1.4 Hz, H-3², cis), 7.97 (1H, dd, J₁ = 11.7 Hz, J₂ = 17.8 Hz, H-3¹), 8.52, 9.34, 9.41 (each 1H, s, H-5', H-10', H-20'). ¹³C NMR: 11.3, 12.1, 12.2, 14.2, 17.5, 19.5, 22.6, 22.8, 23.2, 23.8, 27.0 (x10), 32.0, 32.6, 32.8, 39.5, 39.6, 41.5, 48.1, 50.1, 50.9, 51.7, 53.2, 93.0, 97.2, 104.1, 106.2, 122.6, 128.2, 129.3 (x2), 131.6, 135.9, 136.1, 137.7, 137.9, 141.5, 141.6, 145.1, 148.9, 150.9, 155.4, 160.4, 171.6, 172.1, 196.2.

3.2.19. N(α)-"17³'(Pyropheophorbide)carboxamido-N(ε)-21(17β-hydroxy-3-oxo-pregn-4-ene-21-oic acid amido-Lys-hexadecyl amide (conjugate 6)

DCC (100 mg, 0.49 mmol) was added to the stirred solution of 17β-hydroxy-3-oxopregn-4-en-21-oic acid 12 in dry CH₂Cl₂ (15 mL); the mixture was stirred for 10 min; then compound 30 (217 mg, 0.25 mmol) was added, and the mixture was stirred for 12 h more. Thereafter the mixture was evaporated, the residue was separated by silica gel flash chromatography in CH₂Cl₂ – acetone – AcOH (84:15:1) mixture, evaporated, and dried to obtain conjugate 6 as black amorphous powder (141 mg, 0.12 mmol, 47%). HRMS, calculated for [C₇₆H₁₀₈N₇O₆]⁺: 1214.8356; found: 1214.8363.
22.7, 22.9, 23.4, 26.9, 29.2, 29.6 (∗10), 30.4, 31.4, 31.5, 31.8, 31.9, 32.5, 32.8, 33.8, 35.5, 36.1, 36.1, 38.4, 38.6, 39.6, 42.5, 46.0, 48.0, 49.8, 50.0, 51.5, 52.9, 53.5, 81.9, 92.9, 97.1, 103.9, 105.7, 122.6, 123.7, 128.2, 129.1, 130.0, 131.6, 135.9, 136.0, 136.3, 137.6, 141.7, 145.0, 148.9, 150.8, 155.4, 160.2, 170.9, 171.5, 171.7, 172.7, 173.4, 196.3, 199.2.

### 3.2.20. N(α)-Fmoc-Lys-hexadecyl amide 31

The mixture of amide 28 (911 mg, 1.32 mmol), CH₂Cl₂ (30 mL), and TFA (10 mL) was stirred for 30 min, evaporated to dryness, the residue was dissolved in CH₂Cl₂ (30 mL), the solution was washed with saturated NaHCO₃ solution (20 mL), brine (20 mL), dried over Na₂SO₄, and evaporated to obtain N(α)-Fmoc-Lys-hexadecyl amide 31 (766 mg, 1.29 mmol, 98%) as black film. HRMS, calculated for [C₃⁷H₅₈N₃O₃]⁺: 592.4473; found: 592.4477. ¹H NMR: 0.87 (3H, t, J = 6.3 Hz, CH₃-hexadecyl), 1.36 (28H, m, (CH₂)₁₄), 2.69 (2H, m, CH₂(ε)-Lys), 3.22 (2H, m, NCH₂-hexadecyl), 4.07 (1H, m, CH(α)-Lys), 4.20 (1H, t, J = 6.6 Hz, CH-Fmoc), 4.40 (2H, d, J = 5.9 Hz, CH₂-Fmoc), 5.50 (1H, br.t, NH-hexadecyl), 6.16 (1H, br. d, NH(α)-Lys), 7.30 (3H, t, J = 7.4 Hz, Ar-Fmoc), 7.39 (3H, t, J = 7.4 Hz, Ar-Fmoc), 7.57 (2H, d, J = 7.4 Hz, Ar-Fmoc), 7.75 (2H, d, J = 7.6 Hz, Ar-Fmoc). ¹³C NMR: 14.2, 22.7, 22.8, 27.0, 29.4, 29.4, 29.8 (∗11), 32.0, 32.6, 39.7, 41.7, 47.3, 55.1, 67.1, 120.1 (x2), 125.1 (x2), 172.7 (x2), 172.8 (x2), 141.4 (x2), 143.9 (x2), 171.5.

### 3.2.21. N(ε)-17³(Pyroropheophorbide)carboxamido-Lys-hexadecyl amide 32

The solution of Pyro (250 mg, 0.47 mmol) and DCC (97 mg, 0.47 mmol) in dry CH₂Cl₂ (25 mL) was stirred for 30 min, then N(α)-Fmoc-Lys-hexadecyl amide 31 (227 mg, 470 µmol) was added, the mixture was stirred for 45 min more, and evaporated. TLC analysis revealed partial deletion of Fmoc-group in resulted product. The residue was dissolved in DMF (5 mL), then piperidine (37 µL, 0.5 mmol) was added, the mixture was stirred for 1 h, diluted with CH₂Cl₂ (30 mL), washed with water (2×10 mL), dried over Na₂SO₄, and evaporated. The residue was separated by silica gel flash chromatography in CH₂Cl₂–acetone (93:7) mixture to obtain compound 32 (146 mg, 170 µmol, 35%). HRMS, calculated for [C₅₅H₈₀N₇O₃]⁺: 886.6317; found: 886.6307. ¹H NMR: -1.69 (1H, br.s, NH), 0.86 (3H, t, J = 7.5 Hz, CH₃-hexadecyl), 1.23 (28H, m, (CH₂)₁₄), 1.65 (3H, t, J = 7.6 Hz, H-8'), 1.78 (3H, d, J = 7.3 Hz, H-18¹'), 2.98 (2H, q, J = 6.2 Hz, NCH₂(ε)-Lys), 3.20, 3.38, 3.51 (each 3H, s, H-2¹', H-7¹', H-12¹'), 4.21 (1H, m, H-17¹'), 4.50 (1H, m, H-8¹'), 5.05, 5.23 (each, 1H, d, J = 19.9 Hz, H-15¹'), 5.42 (1H, br.t, NH-hexadecyl), 6.15 (1H, dd, J₁ = 11.6 Hz, J₂ = 1.4 Hz, H-3²', trans), 6.27 (1H, dd, J₁ = 17.8 Hz, J₂ = 1.4 Hz, H-3²', cis), 7.14 (1H, br.t, NH(ε)-Lys), 7.97 (1H, dd, J₁ = 11.5 Hz, J₂ = 17.8 Hz, H-3¹'), 8.52, 9.34, 9.35 (each 1H, s, H-5', H-10', H-20'). ¹³C NMR: 11.3, 12.0, 12.2, 14.2, 17.5, 19.5, 22.8, 23.1, 23.2, 23.9, 27.0, 28.9, 29.0, 29.8 (∗x8), 30.4, 30.5, 32.0, 33.0, 34.3, 38.9, 39.1, 48.2, 50.1, 51.9, 54.8, 93.1, 97.2, 104.1, 106.2, 122.6, 128.3, 128.9, 129.3, 130.9, 131.7, 136.0, 136.1, 136.3, 141.6, 145.1, 149.1, 150.8, 155.3, 160.6, 172.0, 172.4, 174.5, 196.4.

### 3.2.22. N(α)"-21(17β-Hydroxy-3-oxopregn-4-ene-21-oyl)amido-N(ε)"-17³(pyropheophorbide)carboxamido-Lys-hexadecyl amide (conjugate 7)
The mixture of compounds 32 (65 mg, 73 µmol), 12 (26 mg, 73 µmol), and DCC (17 mg, 80 µmol) was stirred for 25 min, the reaction being controlled by TLC. Thereafter the mixture was evaporated, the residue was applied on the top of silica gel column, the column was washed with CHCl₃ – acetone – AcOH (85:14:1) mixture, then target product was eluted with CHCl₃ – acetone – AcOH (79:20:1). The isolated crude conjugate was additionally purified by silica gel flash chromatography in CHCl₃ – MeOH – AcOH (93:6:1) mixture to obtain conjugate 7 (47 mg, 39 µmol, 53%) as black powder. HRMS, calculated for [C₇₆H₁₀₈N₇O₆]+: 1214.8356; found: 1214.8362.

1H NMR: -1.66 (1H, br.s, NH), 0.70 (3H, s, H-18), 0.86 (3H, t, J = 7.0 Hz, CH₃-hexadecyl), 0.91 (3H, s, H-19), 1.21 (28H, m, (CH₂)₁₄), 1.61 (3H, t, J = 7.6 Hz, H-8'), 1.76 (3H, d, J = 7.1 Hz, H-18'), 2.29 (2H, AB system, H-20), 3.11 (2H, q, J = 6.5 Hz, NCH₂(e)-Lys), 3.16, 3.34, 3.38 (each 3H, s, H-2', H-7', H-12'), 4.30 (1H, m, H-17'), 4.44 (1H, m, H-8'), 4.97, 5.14 (each 1H, d, J = 19.9 Hz, H-15'), 5.49 (1H, s, H-4), 5.80 (1H, br.t, NH-hexadecyl), 6.12 (1H, dd, J₁ = 11.5 Hz, J₂ = 1.4 Hz, H-3², cis), 6.22 (1H, dd, J₁ = 18.0 Hz, J₂ = 1.4 Hz, H-3²', trans), 6.85 (1H, br. t, NH(e)-Lys), 7.15 (1H, br.d, NH(α)-Lys), 7.89 (1H, dd, J₁ = 14.7 Hz, J₂ = 17.9 Hz, H-3¹), 8.47, 9.22, 9.25 (each 1H, s, H-5', H-10', H-20').

13C NMR: 11.3, 11.9, 12.1, 13.9, 14.2, 17.1, 17.4, 19.4, 20.4, 22.8, 23.1, 23.5, 27.0, 29.0, 29.4, 29.4, 29.5, 29.6, 29.8 (×10), 30.8, 31.3, 31.5, 31.6, 32.0, 32.6, 33.4, 33.8, 35.5, 35.7, 36.2, 38.4, 38.8, 39.7, 42.9, 46.3, 48.1, 49.9, 50.1, 51.8, 53.1, 53.4, 81.1, 93.8, 97.2, 104.1, 105.7, 122.7, 123.8, 128.0, 129.1, 130.0, 131.7, 136.0, 136.2, 136.4, 137.7, 141.8, 145.1, 149.1, 150.9, 155.5, 160.5, 170.8, 171.6, 172.0, 172.8, 173.4, 196.4, 199.2.

3.3. Molecular modeling

Conformation searches have been performed using molecular mechanics MMFF94 force field parameters in vacuo. OpenBabel package [26] was employed for initial structure preparation and energy minimizations. Simulated annealing molecular dynamics (MD) has been performed to sample low-energy conformation space of conjugates using NAMD [27] software. Parameters and topology files were generated with the aid of SwissParam server [28] on the basis of MMFF94 force field. The annealing protocol consisted of 4 ps high temperature runs at 500 K followed by 4 ps cooling phase bringing temperature down to 50 K, with total of 200 annealing cycles scheduled in 32 processes. This procedure yielded 6400 local energy minima for each compound. Resulting structures were then optimized by energy minimization with MMFF94 potential. VMD package [29] was used for MD trajectory post-processing, analysis, and visualization.

3.4. Solubilization of conjugates 6 and 7 in aqueous medium

3.4.1. Solubilization of conjugates with PC

Calculated volumes of 10⁻² M solutions of PC and conjugate (either 6, or 7) in CHCl₃ were mixed together to obtain solution conjugate/PC with ratio of 1:10 (mg/mg, 6.7 molar % of conjugate). Mixed solutions were evaporated to dryness, and dissolved in iPrOH at 40°C to obtain solutions with concentrations of
conjugates equal to $10^{-3}$ M. Aliquots of heated isopropanolic solutions were injected during vortexing into 100-fold volume of PBS (for measuring of absorption spectra and particle size distributions) or in culture medium (for measuring of uptake and internalization of conjugates by cells).

### 3.4.2. Solubilization of conjugates with Pluronic F68

Calculated volumes of $10^{-2}$ M solutions of pluronic F68 and conjugates (either 6, or 7) in CHCl$_3$ were mixed together to obtain solutions conjugate/pluronic with ratios 1:10 and 1:50 (mg/mg). Mixed solutions were evaporated to dryness, then calculated volumes of PBS, or culture medium were added to films, and the mixtures obtained were vortexed at 40°C for 1 min.

### 3.5. Biological evaluation

#### 3.5.1. Cell cultures

The human prostate carcinoma LNCaP and PC-3 cells, breast carcinoma MCF-7 cells, hepatocarcinoma Hep G2 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were propagated in culture dishes at the desired densities in RPMI 1640 and DMEM medium supplemented with 10% fetal calf serum (FCS; Gibco, Grand Island, NY) and 1% penicillin/streptomycin (Gibco) in a 5% CO$_2$ atmosphere at 37°C for 24 h. Before experiments the cells were seeded either in 96-well plates at a density of 5·10³ cells/well (for MTT assay), or in 6-well plates at a density of 106 cells/well (for investigation of uptake and internalization of conjugates) and incubated for 48 h.

#### 3.5.2. Uptake and internalization of conjugates by prostate carcinoma cells

LNCaP cells in a 6-well plates were incubated for 2, 6, 14 and 20 h with conjugates 1–4 (25 µM in culture medium), then medium was aspirated, cells were washed with cold PBS at 4°C, and lipids from each well were extracted with hexane – $i$-PrOH mixture (3:2, 3×0.5 mL). Pellets were used for cell protein concentration measurements [30]. Lipid extracts were dried under nitrogen flow, residues were dissolved in CH$_2$Cl$_2$ (2 mL), and concentrations of conjugates were determined spectrophotometrically. All measurements were carried out in triplicates. The efficiency of cell labeling was expressed in terms of ratios of internalized conjugates (nmol per mg of cell protein).

#### 3.5.3. MTT cell viability assay

LNCaP and PC-3 cells were treated with conjugates at the designated concentrations, and incubated for 96 h in 96-well plates. Then, solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added and the cells were incubated for 4 h, followed by measuring absorbance at 570 nm, with “Techan Genius plus” microplate reader. The viability of treated cells was expressed as a percentage relative to that of control cells. Each experiment was performed in triplicate, and independently repeated at least four times.

#### 3.5.4. Measuring of photo induced toxicity
LNCaP cells were incubated with conjugates 1 and 5 at a concentrations from 0.1 µM to 100 µM for 18 h, then the cells were washed three times with PBS, thereafter fresh culture medium was added (100 µL/well), and cells were irradiated for 10 min with light (λ = 660 nm) using LED AFS "Spectrum" instrument (p = 0.142 W; 85.2 J/cm²). Then the cells were incubated in fresh medium for 24 h at 37°C. The viability of irradiated cells and control cells (incubated similarly except irradiation) was measured using MTT assay. All experiments were carried out in triplicates.

**Declarations**

**Author Contributions:** Conceptualization, V.A.Z., G.V.P. and A.Y.M.; chemical synthesis, V.A.Z., A.M.K. A.S.L., N.V.S.; biological evaluation, G.E.M. and A.R.M.; NMR spectra, R.A.N.; molecular modeling, Y.V.T.. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest

**References**


15. Oliveto EP, Volume 2, p. 139


Scheme

Scheme 1, 2 and 3 are available in supplementary section.

Figures
Figure 1

Structures of conjugates 1 – 7 (numbering of atoms is shown in the structure 7)
Figure 2

Normalized absorption spectra of conjugates 1 (olive); 2 (purple); 3 (blue); 4 (red) in CHCl₃.

Figure 3

Ensembles of low energy conformers of compounds 1 – 4, 6 and 7; 18- and 19-methyl groups of steroid core are depicted as balls (these faced to macrocycle are colored orange; away from macrocycle – gray).
Figure 4

The lowest calculated energy conformers for conjugates 1 – 4, 6 and 7. Short interatomic distances favorable for hydrogen bond formation are marked by hash lines; the numbers indicate distance in Angstroms.
Figure 5

A - Uptake and internalization of conjugates 1 – 4 by LNCaP cells (concentration of each conjugate in media was 25 μM; the numbers of curves corresponded to numbers of conjugates). B – MCF-7 cells labeled with conjugate 1.
Figure 6

Absorption spectra of mixed micelles of conjugates 6 and 7 with PC or pluronic F68 in PBS.
Figure 7

Particle size distribution for mixed micelles measured by laser scattering; 6 – PC (average diameter – 123.3 nm); 7 – PC (average diameter – 108.1 nm); 6 – F68 (average diameter – 621.3 nm); 7 – F68 (average diameter – 385.7 nm).
Figure 8

Uptake and internalization of conjugates 6 and 7 in form of mixed micelles by Hep G2 cells (concentration of each conjugate in media was 25 μM)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GA.png
- Scheme01.png
- Scheme02.png
- Scheme03.png
- SUPPLEMENTARYconj.pdf