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Development of Iodine-Containing Natural Chlorins as Prototypes of Radiopharmaceuticals with Iodine Radionuclides

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Dedicated to the Anniversary of Academician Irina P. Beletskaya

Many precursors for the development of various pharmaceutical substances, including the synthesis of radiopharmaceuticals (RPs), are known among natural structures. However, information about the application of natural compounds for the synthesis of such pharmaceutical agents is currently limited. In this study, we implemented the methods for incorporating an iodine atom into the photosensitizer structure that involve addition of an iodine-containing organic molecules to a natural chlorin as well as direct iodination of the pigment derivatives.

Keywords: Iodination, chlorin, photosensitizer, radiotracer, isotope, radiopharmaceutical.

Разработка иодсодержащих природных хлоринов в качестве прототипов радиофармпрепаратов с радионуклидами иода

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Среди природных структур известно немало прекурсоров для разработки различных фармацевтических субстанций, в том числе для синтеза радиофармацевтических препаратов (РФП). Однако, в настоящее время сведения, касающиеся использования природных соединений для создания подобных лекарственных средств, ограничены. В работе реализовано введение атома иода в структуру фотосенсибилизатора, включающее присоединение к природному хлорину иодсодержащих органических молекул, а также прямое иодирование производных пигмента.

Ключевые слова: Иодирование, хлорин, фотосенсибилизатор, радиотрейсер, изотоп, радиофармпрепарат.

Introduction

Currently, the radioisotopes most widely used for the production of diagnostic and therapeutic radiopharmaceuticals are as follows: gallium-68, technetium-99m, fluorine-18, as well as iodine-131 and iodine-125.[1]

The traditional methods for radiolabeling natural molecules include the direct and indirect incorporation of a radioactive label, isotope exchange,[2] and formation of complexes with radioactive metals.

The agents labeled with iodine radioisotopes are widely used in the diagnostics of various diseases by PET and SPECT methods,[3] where they serve as contrast agents, but they can also be used in radionuclide therapy.[4]

Iodine (I) element has atomic number 53, atomic mass 127 and electronic configuration $[Kr]4d^{10}5s^25p^5$. It has 37 isotopes (from ^{108}I to ^{144}I) that undergo radioactive decay, except for the ^{127}I isotope that is stable. Among these, ^{123}I, ^{124}I, ^{125}I and ^{131}I have been studied most thoroughly and are used for medical purposes.

The main peak of ^{123}I gamma radiation has an energy of 159 keV, which makes it suitable for application in SPECT imaging. The short half-life (13.22 hours) of ^{123}I dictates the choice of chelators that allow fast radioiodination, fast metabolism and excretion. Several ^{123}I-labeled imaging pharmaceuticals, including ^{123}I-ioflupane for visualization of the striatal dopamine transporter are approved by the Food and Drug Administration (FDA) for clinical use.[3]

The ^{124}I isotope also has attractive radiochemical properties that allow it to be used in PET imaging. Its half-life is 4.8 days. Its positron decay with maximum and average positron energies of 2.138 and 0.975 MeV, respectively, makes it suitable for PET imaging. The therapeutic effect of the ^{124}I isotope is based on the phenomenon of emission of Auger electrons that have a local effect in the nanometer range.[5] Thus, ^{124}I is used both as a diagnostic and therapeutic agent.[3] For example, ^{124}I-labelled radiopharmaceutical ^{124}I-epidepride binds well to the dopamine D2/3 receptors that are present in pancreatic islets and may serve as a surrogate marker for imaging some pathological processes in pancreas.[6]

^{125}I has a half-life of 59.4 days. It mainly emits X-rays with an energy of 27 keV and weak gamma rays with an energy of 35.5 keV.[3] The ^{125}I isotope is currently applied to developing radiocontrast agents used in the diagnostics of a number of diseases. For example, ^{125}I-iothalamate is an iodine-containing organic anion, produced in the form of sodium iothalamate and meglumine iothalamate, is approved by the FDA for clinical use for evaluation of glomerular filtration.[7]

The ^{131}I isotope, which emits beta radiation (606 keV, 90%), has a half-life of 8.02 days and is often used in radiotherapy.[3] Beta particles can penetrate to a distance of 0.6 to 2.0 mm without affecting more distant tissues. The ^{131}I isotope is often used for the treatment of inoperable or metastatic paragangliomas and pheochromocytomas as a part of the ^{131}I-Iobenguane[8] radiopharmaceutical.

Electrophilic and nucleophilic substitution reactions are often used for the radioiodination of organic compounds.[2] The latter reactions are most widely used to incorporate iodine into aliphatic and aromatic groups,[2,9] including interhalogen exchange, radioiodo-dediazonization, and isotopic exchange based on the replacement of stable iodine already present in the molecule with a radioactive isotope by the Finkelstein reaction. The ways of iodine isotopes incorporation into aromatic[2,10] and aliphatic[11] molecules by means of the radioisotopic exchange are usually based on the treatment of the corresponding organic compounds containing the stable ^{127}I isotope with an excess of radioactive sodium iodide in a suitable organic solvent, followed by its evaporation. It was also shown that the use of copper(II)-based catalysts can significantly increase the reaction yields.[9] According to this technique, in particular, an ^{123}I-aminoglucose derivative[11] and radiolabeled with the ^{125}I isotope analogues of *N*-(2-diethylaminoethyl)-4-iodo-benzamide[2] were obtained.

Electrophilic substitution methods are commonly employed in radiolabeling, typically via an oxidized iodine species (with a formal +1 charge) acting as an electrophile and attacking an electron rich species, resulting in the formation of a covalent carbon-iodine bond. Electrophilic substitution methods including halo-demetallation with chloramine-T, iodobeads, iodogen, and peracids are most popular in the synthesis of radioiodinated arenes.[2]

The purpose of this work was to develop methods for the synthesis of iodine-containing natural chlorins as prototypes of radiotracers comprising iodine isotopes. Due to the ability of chlorin structures to be efficiently accumulated in tumor cells,[12] it is expected that once the stable iodine isotope is replaced with a radioactive one, these compounds can be used as radiopharmaceuticals both for tumor imaging by PET and SPECT and for destruction of malignant cells due to the isotope's radioactive decay and active Auger electrons emission.

Among the known iodine-containing derivatives of natural chlorins and porphyrins, it is important to mention the compounds based on pyropheophorbide,[113,14] protoporphyrin IX[13] and purpurinimide.[15]

It is reported that the nature and the position of the iodine-containing substituents in purpurinimides make a significant difference in tumor uptake, which also reflects their imaging and PDT potential. It was also shown that in case of the introduction of an iodine-containing substituent into the E-exocycle, the iodinated pigment demonstrates improved imaging and phototherapeutic abilities, than the chlorin with the same modification of the A-pyrrole.[15]

A regioselective synthesis of iodinated chlorin derivatives containing iodine atoms in the E-exocycle is carried out in this work. The replacement of the «cold» iodine isotope with a radioactive one can then be carried out by the isotopic exchange reactions which can be applied to short-lived radionuclides.[2,11]

Experimental

General

The solvents were purified and prepared according to the standard procedures. Silica gel 40/60 (Merck, Germany) was used for column chromatography. Silica gel 5/40 (Vekton, Russia) and neutral alumina (Chemapol, Czech Republic) were used for preparative TLC. Analytical TLC was performed on Kieselgel 60 F_{245} plates (Merck, Germany). NMR spectra were recorded on Bruker DPX300 spectrometers in $CDCl_3$. Residual signals of ^{1}H nuclei were used for scale calibration. Experiments were

performed using standard Bruker procedures. ESI mass spectra were recorded on a 5977B GC/MSD gas chromatography mass spectrometer. MALDI mass spectra were obtained on a Bruker Ultraflex TOF/TOF mass spectrometer using 2,5-dihydroxybenzoic acid as the matrix. Absorption spectra were recorded on a Shimadzu UV1800 UV/VIS spectrometer in $CHCl_3$. IR spectra were recorded using an Infralum FT-08 FT-IR spectrometer in KBr pellets. Human breast adenocarcinoma (MCF-7) and human colon carcinoma (HT-29) cell cultures were used to measure dark toxicity. Tumor cells were incubated in plastic flasks with a cell growth surface of 25 cm^2 in Igla MEM medium (PanEco, Russia) with L-glutamine supplemented with 10% fetal calf serum (FBS) (PanEco, Moscow, Russia). Incubation of cells was carried out at 37 °C in a humidified atmosphere containing 5% CO_2 (Binder CO_2 incubator, Tuttlingen, Germany). Cell lines from 3 to 7 passages were used. A multi-channel tablet reader (λ=550 nm) was used for the determination of cell viability using the MTT-test.

Synthesis

*5-Iodovaleric acid $C_5H_9IO_2$ **(1c)**.* Potassium iodide (364.2 mg, 2.19 mmol) was added to a solution of 5-bromovaleric acid **1b** (249.1 mg, 1.38 mmol) in acetone (30 mL) and the mixture was stirred for 48 h under reflux. Next, the reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The dry residue was suspended in CH_2Cl_2 and filtered. The resulting filtrate was concentrated under reduced pressure to give 276.5 mg of compound **1c** (87%). *m/z* (MALDI): 229.003 $[MH]^+$

*3-Ethyl-3-devinyl-13,15-N-(pentanamido)cycloimide chlorin P_6 methyl ester $C_{39}H_{46}N_6O_5$ **(3a)**.* Thionyl chloride (49.1 mg, 0.41 mmol) was added to a solution of valeric acid (30 μL, 0.28 mmol) in tetrahydrofuran (10 mL) under argon with stirring and cooling and kept for another 3 h. Diisopropylethylamine (174.4 mg, 1.35 mmol) and 3-ethyl-3-divinyl-13,15-*N*-aminopurpurinimide **2** (20.4 mg, 0.03 mmol) were then added to the solution, which was subsequently stirred for 20 h at room temperature in argon atmosphere. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.4). Once the process was completed, the reaction mixture was diluted with 25 mL of 0.01 M aqueous HCl and the product was extracted with chloroform (2×20 mL). The organic layer was dried with anhydrous Na_2SO_4, after which the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 50/1, v/v, R_f = 0.6), then on neutral alumina ($CHCl_3/CH_3OH$, 100/1, v/v, R_f = 0.5) gave 20.0 mg of compound **3a** (87 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.33 (s, 10-H), 9.05 (s, 5-H), 8.43 (s, 20-H), 5.28–5.18 (m, 17-H), 4.37–4.24 (m, 18-H), 3.75–3.63 (m, 12-Me and 3^1-CH_2), 3.57 (s, 17^5-Me), 3.50–3.40 (m, 8^1-CH_2), 3.32 (s, 13^5-CH_2), 3.21 (s, 2-Me), 3.07 (s, 7-Me), 2.74–2.63 (m, 17^1-CH_2), 2.46–2.34 (m, 17^2-CH_2), 2.04–1.86 (m, 13^6-CH_2, 13^7-CH_2), 1.80–1.46 (m, 18-Me; 3^2-Me and 8^2-Me), 1.14–1.02 (m, 13^8-Me), 0.19 (s, NH), -0.16 (s, NH). *m/z* (ESI): 679.400 $[MH]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 362 (39000), 417 (120000), 480 (3800), 508 (6500), 545 (20000), 642 (7500), 696 (40000).

*3-Ethyl-3-devinyl-13,15-N-(5-bromopentanamido)cycloimide chlorin P_6 methyl ester $C_{39}H_{45}BrN_6O_5$ **(3b)**.* Thionyl chloride (16.4 mg, 0.14 mmol) was added to a solution of 5-bromovaleric acid **1b** (7.4 mg, 0.04 mmol) in tetrahydrofuran (5 mL) under argon with cooling and the solution was stirred for 3 h. Diisopropylethylamine (38.6 mg, 0.30 mmol) and 3-ethyl-3-devinyl-13,15-*N*-aminopurpurinimide **2** (5.2 mg, 0.009 mmol) were then added to the solution, and the latter was stirred for 24 h at room temperature in an argon atmosphere. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.4). Once the reaction was completed, 15 mL of aqueous 0.01 M HCl was added to the mixture and the product was extracted with chloroform (2×20 mL). The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.4) gave 5.2 mg of product **3b** (72%). *m/z* (MALDI): 757.145 and 759.166 $[MH]^+$.

*3-Ethyl-3-devinyl-13,15-N-(5-iodopentanamido)cycloimide chlorin P_6 methyl ester $C_{39}H_{45}IN_6O_5$ **(3c)**. Method 1.* Thionyl chloride (32.7 mg, 0.28 mmol) was added to a solution of 5-iodvaleric acid **1c** (62.2 mg, 0.27 mmol) in tetrahydrofuran (10 mL) under argon with cooling and the mixture was stirred for 3 h. Diisopropylethylamine (77.9 mg, 0.60 mmol) and 3-ethyl-3-devinyl-13,15-*N*-aminopurpurinimide **2** (20.4 mg, 0.03 mmol) were then added to the solution and the latter was stirred for 24 h at room temperature under argon. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.5). Once the process was completed, the reaction mixture was diluted with 20 mL of aqueous 0.01 M HCl and the product was extracted with chloroform (3×15 mL). The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.5) and then on neutral alumina ($CHCl_3/CH_3OH$, 100/1, v/v, R_f = 0.4) gave 17.2 mg of product **3c** (64%). *Method 2.* Sodium iodide (5.2 mg, 0.035 mmol) was added to a solution of bromo-containing chlorin **3b** (5.2 mg, 0.007 mmol) in acetone (5 mL) and the mixture stirred at room temperature under argon for 48 h. Once the process was completed, the reaction mixture was diluted with 10 mL water and extracted with $CHCl_3$ (3×20 mL), then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 60/1, v/v, R_f= 0.5) gave 4.3 mg of compound **3c** (76.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.41 (s, 10-H), 9.11 (s, 5-H), 8.44 (s, 20-H), 5.22 (d, 17-H), 4.35–4.24 (m, 18-H), 3.79–3.63 (m, 12-Me and 3^1-CH_2), 3.59–3.50 (m, 17^5-Me and 8^1-CH_2), 3.50–3.41 (m, 13^5-CH_2), 3.34 (t, 13^8-CH_2), 3.21 (s, 2-Me), 3.11 (s, 7-Me), 2.71 (m, 17^1-CH_2), 2.45–2.34 (m, 17^2-CH_2), 2.19–2.00 (m, 13^6-CH_2, 13^7-CH_2), 1.73–1.70 (m, 18-Me), 1.66–1.52 (m, 3^2-Me and 8^2-Me), 0.26 (s, NH), 0.10 (s, NH). *m/z* (MALDI): calculated for $C_{39}H_{46}IN_6O_5^+$: 805.257, found: 805.240 $[MH]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 362 (34500), 417 (109000), 478 (3500), 508 (5800), 545 (18000), 643 (6800), 697 (35000). IR (KBr) ν cm^{-1}: 1712, 1697, 1671, 1600, 1525, 494.

*3-Ethyl-3-devinyl-13,15-N-(benzamido)cycloimide chlorin P_6 methyl ester $C_{41}H_{42}N_6O_5$ **(5a)**.* Thionyl chloride (49.1 mg, 0.41 mmol) was added under argon with stirring and cooling to a solution of benzoic acid (33.5 mg, 0.27 mmol) in tetrahydrofuran (20 mL) and the mixture was stirred for 3 h. Diisopropylethylamine (174.3 mg, 1.35 mmol) and 3-ethyl-3-divinyl-13,15-*N*-aminopurpurinimide 2 (20.5 mg, 0.03 mmol) were then added to the solution, and the solution was stirred for 22 h at room temperature under argon. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.50). Once the reaction was completed, the solution was diluted with 15 mL of 0.01 M aqueous HCl and the product was extracted with chloroform (2×15 mL). The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 50/1, v/v, R_f= 0.4), then on neutral alumina ($CHCl_3/CH_3OH$, 50/1, v/v, R_f = 0.4) gave 20.1 mg of compound **5a** (87 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.33 (s, 10-H), 9.08 (s, 5-H), 8.44 (s, 20-H), 8.26 – 8.12 (m, ortho-H, Bz), 7.68–7.48 (m, meta-, para-H, Bz), 5.35–5.15 (m, 17-H), 4.40–4.15 (m, 18-H), 3.84–3.60 (m, 12-Me, 3^1-CH_2), 3.56–3.39 (m, 17^5-Me, 8^1-CH_2), 3.21 (s, 7-Me, 2-Me), 2.98–2.55 (m, 17^1-CH_2), 2.48–2.24 (m, 17^2-CH_2), 1.97–1.75 (m, 18-Me), 1.57 (t, 3^2-Me), 1.42 (t, 8^2-Me). *m/z* (ESI): 699.300 $[MH]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 361 (39000), 417 (125000), 478 (3700), 508 (6200), 545 (20000), 642 (7500), 697 (39500).

*3-Ethyl-3-devinyl-13,15-N-(4-iodobenzamido)cycloimide chlorin P_6 methyl ester $C_{41}H_{40}IN_6O_5$ **(5b)**.* Thionyl chloride (144.1 mg, 1.21 mmol) was added under argon with stirring and cooling to a solution of 4-iodobenzoic acid (100.2 mg, 0.40 mmol) in tetrahydrofuran (15 mL) and the system was stirred for another 3 hours. Diisopropylethylamine (326.5 mg, 2.53 mmol) and 3-ethyl-

3-devinyl-13,15-*N*-aminopurpurinimide (30.3 mg, 0.05 mmol) were then added, and the solution was stirred for 24 h at room temperature under argon. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.55). Once the reaction was completed, the solution was diluted with 20 mL of 0.01 M aqueous HCl and the product was extracted with chloroform (3×15 mL). The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 70/1, v/v, R_f = 0.45), then on neutral alumina ($CHCl_3/CH_3OH$, 100/1, v/v, R_f = 0.4) gave 21.9 mg of compound **5b** (53 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.29 (s, 10-H), 9.08 (s, 5-H), 8.42 (s, 20-H), 7.95–7.81 (m, NHC(O)*Ar* (4H)), 5.23 (d, 17-H), 4.34–4.17 (m, 18-H), 3.82–3.58 (m, 12-Me and 3^1-CH_2), 3.54–3.34 (m, 17^5-Me and 8^1-CH_2), 3.20 (s, 2-Me and 7-Me), 3.11–3.04 (m, 17^1-CH_2), 2.48–2.30 (m, 17^2-CH_2), 1.71–1.64 (m, 18-Me), 1.54 (t, 3^2-Me), 1.39 (t, 8^2-Me), 0.25 (s, NH), 0.10 (s, NH). *m/z* (MALDI): calculated for $C_{41}H_{41}IN_6O_5^+$: 824.218, found: 824.191 $[M]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 361 (39000), 417 (128000), 477 (3700), 508 (63000), 546 (22000), 642 (7600), 697 (41000). IR (KBr) ν cm^{-1}: 1711, 1674, 1596, 1526, 552.

*3-Ethyl-3-devinyl-13,15-N-(3-carboxypropionamido)cycloimide chlorin P_6 methyl ester $C_{38}H_{42}N_6O_7$ (**6**).* Succinic anhydride (639 mg, 6.39 mmol) was added to a solution of 3-ethyl-3-devinyl-13,15-N-aminopurpurinimide **2** (71.1 mg, 0.11 mmol) and diisopropylethylamine (31.9 mg, 0.24 mmol) in tetrahydrofuran (25 mL) and the mixture was stirred for 9 days in an argon atmosphere at room temperature. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH/CH_3COOH$, 50/1/0.5, v/v/v, R_f = 0.2). After reaction completion, 25 mL of 0.01 M aqueous HCl was added to the reaction mixture and the product was extracted with chloroform (3×20 mL). The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH/CH_3COOH$, 50/1/0.5, v/v/v, R_f = 0.2) gave 65.3 mg of compound **6** (86 %). *m/z* (MALDI): 695.301 $[MH]^+$.

*3-Ethyl-3-devinyl-13,15-N-(4-((3-(4-hydroxyphenyl)-1-methoxy-1-oxopropan-2-yl)amino)-4-oxobutanamido)cycloimide chlorin P_6 methyl ester $C_{48}H_{53}N_7O_9$ (**8a**).* Thionyl chloride (27.8 mg, 0.23 mmol) was added to a cooled solution of 3-ethyl-3-devinyl-13,15-*N*-(3-carboxypropionamido)cycloimide chlorin P_6 methyl ester **6** (20.4 mg, 0.03 mmol) and diisopropylethylamine (100.2 mg, 0.78 mmol) in tetrahydrofuran (15 mL). The solution was heated to room temperature and stirred for 2 h. Next, L-tyrosine methyl ester (28.4 mg, 0.15 mmol) was added and the solution was stirred for 24 h at room temperature in an argon atmosphere. The reaction was monitored by TLC ($CHCl_3/CH_3OH$, 30/1, v/v, R_f = 0.4). Once the process was completed, the reaction mixture was diluted with 15 mL of 0.01 M aqueous HCl and the product was extracted with chloroform (2×25 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 30/1, v/v, R_f = 0.4), then on neutral alumina in the system ($CHCl_3/CH_3OH$, 25/1, v/v, R_f = 0.5) gave 17.2 mg of compound **8a** (68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.26 (s, 10-H), 9.10 (s, 5-H), 8.52 (s, 20-H), 7.17–7.00 (m, OCCH, Tyr), 6.86–6.74 (m, CCH, Tyr), 5.22 (d, 17-H), 5.01–4.77 (m, CH, Tyr), 4.35–4.15 (m, 18-H), 3.78–3.61 (m, 12-Me, 3^1-CH_2, Me (Tyr)), 3.57–3.44 (m, 17^5-Me, 8^1-CH_2,), 3.20 (s, 2-Me, 7-Me), 3.10–3.00 (m, 13^5-CH_2, CH_2-Tyr), 2.95–2.89 (m, 13^6-CH_2, CH_2-Tyr), 2.81–2.74 (m, 17^1-CH_2), 2.42–2.29 (m, 17^1-CH_2), 1.79–1.59 (m, 18-Me, 3^2-Me and 8^2-Me). m/z (ESI): 872.500 $[MH]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 361 (39000), 417 (129000), 479 (4000), 509 (6000), 546 (21500), 642 (8000), 697 (41000).

*3-Ethyl-3-devinyl-13,15-N-(4-((3-(4-hydroxy-3,5-diiodophenyl)-1-methoxy-1-oxopropan-2-yl)amino)-4-oxobutanamido)cycloimide chlorin P_6 methyl ester $C_{48}H_{51}I_2N_7O_9$ (**8b**). Method 1.* Thionyl chloride (17.0 mg, 0.14 mmol) was added with stirring to a cooled solution of 3-ethyl-3-devinyl-13,15-*N*-(3-carboxypropionamido)cycloimide chlorin P_6 methyl ester **6** (31.3 mg, 0.05 mmol) and diisopropylethylamine (59.4 mg, 0.46 mmol) in tetrahydrofuran (30 mL). The solution was heated to room temperature and stirred for 3 h, then L-3,5-diiodothyrosine methyl ester (42.4 mg, 0.1 mmol) was added, and the solution was stirred for 24 h at room temperature in an argon atmosphere. The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.6). Once the process was completed, 15 mL of 0.01 M aqueous HCl was added to the reaction mixture and the product was extracted with chloroform (2×20 mL). The organic layer was dried over anhydrous Na_2SO_4, then, the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 50/1, v/v, R_f= 0.7), then on neutral alumina ($CHCl_3/CH_3OH$, 45/1, v/v, R_f = 0.45) gave 22.2 mg of compound **8b** (44%). *Method 2.* Trifluoroacetic acid (15.3 mg, 0.134 mmol) and *N*-iodosuccinimide (15 mg, 0.067 mmol) were added to a solution of chlorin **8a** (10.0 mg, 0.011 mmol) in anhydrous tetrahydrofuran (4 mL) and the mixture stirred at room temperature in an argon atmosphere for 20 h. Once the process was completed, the reaction mixture was diluted with 25 mL of 10% aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (4×15 mL). Then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 60/1, v/v, R_f= 0.6) gave 11.4 mg of compound **8b** (93 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.48 (s, 10-H), 9.12 (s, 5-H), 8.43 (s, 20-H), 7.72 (s, I-CH (2H)), 5.20 (d, 17-H), 5.01–4.73 (m, C(O)C*H*NH), 4.39–4.21 (m, 18-H), 3.83–3.64 (m, 12-Me, 3^1-CH_2 and NHCHC(O)O*Me*), 3.55 (m, 17^5-Me and 8^1-CH_2), 3.21 (s, 2-Me), 3.12 (d, 7-Me and 13^5-CH_2), 3.09–3.04 (m, ICHCCH_2(1H)), 3.01–2.93 (m, 13^6-CH_2), 2.83 (m, 17^1-CH_2), 2.74–2.65 (m, ICHCCH_2(1H)), 2.40 (m, 17^2-CH_2), 1.76–1.52 (m, 18-Me, 3^2-Me and 8^2-Me). *m/z* (MALDI): 1146.153 $[M+Na]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 362 (44000), 417 (131000), 480 (4700), 509 (7000), 546 (23000), 642 (9000), 697 (43500). IR (KBr) ν cm^{-1}: 1715, 1674, 1599, 1526, 492.

*3-Ethyl-3-devinyl-13,15-N-(4-((3-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)-1-methoxy-1-oxopropan-2-yl)amino)-4-oxobutanamido)cycloimide chlorin P_6 methyl ester $C_{54}H_{53}I_4N_7O_{10}$ (**10**).* Thionyl chloride (27.8 mg, 0.23 mmol) was added to a cooled solution of 3-ethyl-3-devinyl-13,15-*N*-(3-carboxypropionamido)cycloimide of chlorin P_6 methyl ester **6** (20.2 mg, 0.03 mmol) and diisopropylethylamine (100.2 mg, 0.78 mmol) in tetrahydrofuran (15 mL). The solution was heated to room temperature and stirred for 3 h. Then L-thyroxine methyl ester (120 mg, 0.15 mmol) was added and the solution was stirred for 48 h at room temperature in an argon atmosphere. The reaction was monitored by TLC ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.5). Once the reaction was completed, 20 mL of 0.01 M aqueous HCl was added to the mixture and the product was extracted with chloroform (3×15 mL). The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed under reduced pressure. Purification by preparative TLC on silica gel ($CHCl_3/CH_3OH$, 60/1, v/v, R_f = 0.6), then on neutral alumina ($CHCl_3/CH_3OH$, 45/1, v/v, R_f = 0.4) gave 17.6 mg of compound **10** (42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.50 (s, 10-H), 9.13 (s, 5-H), 8.43 (s, 20-H), 7.91 (s, CH(Ar_1), 2H), 7.06 (s, CH(Ar_2), 2H), 5.21 (d, 17-H), 5.02–4.83 (m, C(O)C*H*NH), 4.39–4.21 (m, 18-H), 3.85–3.68 (m, 12-Me, 3^1-CH_2 and NHCHC(O)O*Me*), 3.62–3.47 (m, 17^5-Me and 8^1-CH_2), 3.32–3.16 (m, 2-Me, 13^5-CH_2), 3.13 (s, 7-Me), 3.04–2.95 (m,13^6-CH_2), 2.91–2.79 (m, 17^1-CH_2), 2.70 (m, ICHCCH_2(1H)), 2.45–2.31 (m, 17^2-CH_2), 1.74–1.53 (m, 18-Me, 3^2-Me and 8^2-Me). *m/z* (MALDI): 1467.030 $[M]^+$. UV-vis ($CHCl_3$) λ_{max} nm (ε, $M^{-1}cm^{-1}$): 361 (42000), 417 (127000), 477 (4800), 508 (7100), 546 (22500), 642 (9000), 697 (42000). IR (KBr) ν cm^{-1}: 1715, 1670, 1600, 1527, 493.

Cytotoxic activity

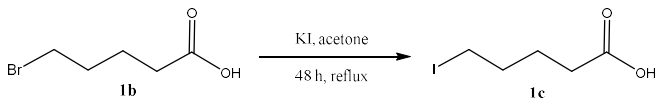
To estimate the cytotoxic activity of the synthesized conjugates, the cells were incubated in 96-well culture plates, cultured for 28 h at 37 °C with 5% CO_2, then the compounds were added, and incubation was carried out in the selected time ranges (24, 48, and 72 h). The concentrations of tested compounds ranged

from 0.04 μg/mL to 50 μg/mL. Experiments on the cells of each culture were carried out in 3 repetitions. Cell viability was determined visually, estimating morphological changes in cells using light microscopy, and colorimetrically using the MTT test. The absorbance of the formazan solution in the wells was measured on a multi-channel tablet reader, determining the optical density in each well. The level of cell growth inhibition in culture was calculated by the formula: $[(A_c\text{-}A_o)/A_c]\times100\%$, where A_o — absorbance of the solution in the experimental wells; A_c — absorbance of formazan solution in control wells; λ_{max}=550 nm. Based on the results, IC_{50} values were calculated, *i.e.,* the compound concentrations at which 50% cell death occurred after exposure.

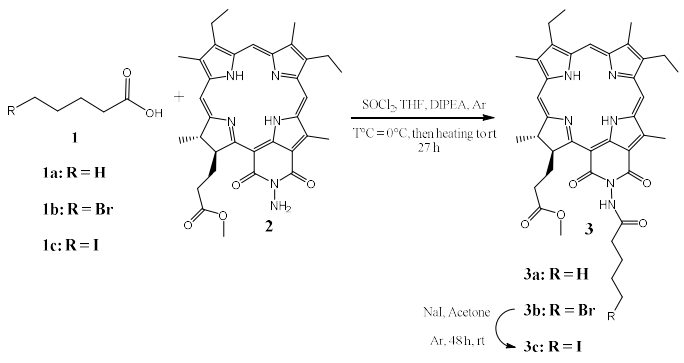
Results and Discussion

In this work, *N*-aminomesopurinimide **2** was used as the starting compound. It comprises an exocyclic primary amino group to which 5-iodovaleric acid **1c** was linked. The latter was obtained by the Finkelstein reaction that involved the nucleophilic replacement of the bromine atom in acid **1b** for a iodine atom by the S_N2 mechanism performed by refluxing with potassium iodide in anhydrous acetone. The resulting potassium bromide is insoluble in acetone, which favors an equilibrium shift towards the products and ensures that the reaction occurs in full (Scheme 1).

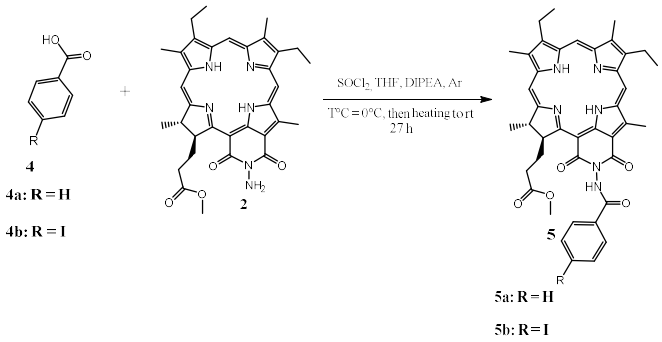
Next, the iodo-containing photosensitizer **3c** was synthesized by two methods. The first (indirect) method involved the acylation of the exocyclic amino group in chlorin **2** by the previously obtained 5-iodovaleric acid chloride in the presence of diisopropylethylamine as the organic base (Scheme 2). The second (direct) method involved replacing the bromine atom with iodine by the Finkelstein reaction in chlorin **3b**. Chlorin **3a** was obtained as a reference compound by acylation of chlorin **2** with valeric acid chloride.



Scheme 1. Synthesis of 5-iodovaleric acid **1c** by the Finkelstein reaction.



Scheme 2. Acylation of mesopurpurinimide **2** with valeric, bromovaleric, and iodovaleric acids.



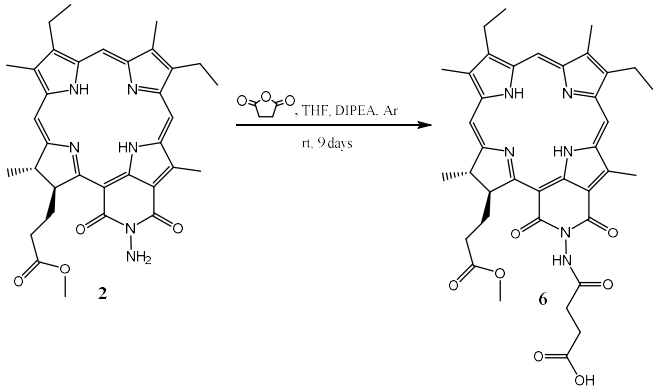
Scheme 3. Synthesis of the mesopurpurinimide conjugate with benzoic and 4-iodobenzoic acid residues.

Amide **5b** containing an aromatic iodinated moiety was obtained by a similar scheme involving the synthesis of *para*-iodobenzoic acid chloride and acylation of *N*-aminomesopurpurinimide **2**. Similarly, a benzoic acid residue was incorporated into chlorin **2** to give amide **5a** (Scheme 3).

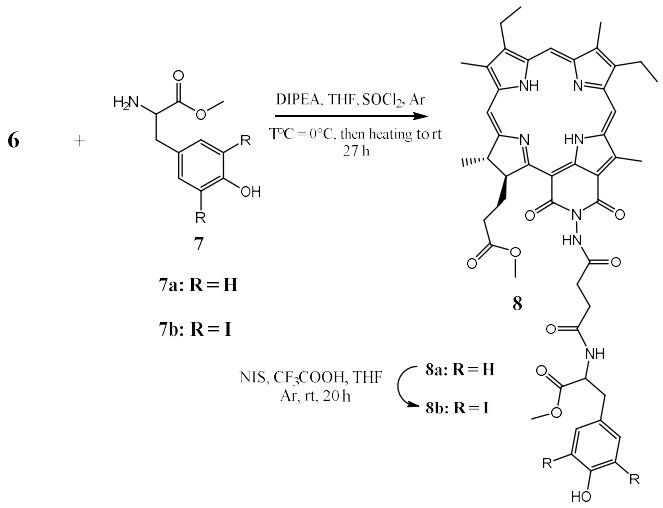
The thyroid gland produces two thyroid hormones differing in the number of iodine atoms in the molecule, namely, thyroxine (T_4) and triiodothyronine (T_3). Nearly all tissues of the body are the target organs for iodinated thyroid hormones.

To attach an iodinated tyrosine residue to the photosensitizer molecule, a spacer with a terminal carboxy group was previously introduced by treating the pigment with a large excess of succinic anhydride in the presence of diisopropylethylamine (Scheme 4). Nucleophilic opening of the anhydride cycle by the exocyclic amino group of mesopurpurinimide **2** resulted in acid **6**, which was purified by preparative thin-layer chromatography in a polar solvent system with addition of acetic acid.

Amidation of acid **6** thus obtained was performed by conversion of the latter to a chloride (Scheme 5) using thionyl chloride in the presence of diisopropylethylamine, followed by condensation with 3,5-diiodo-L-tyrosine methyl ester **7b** to give the conjugate **8b**. The non-iodinated analogue **8a** was obtained similarly.



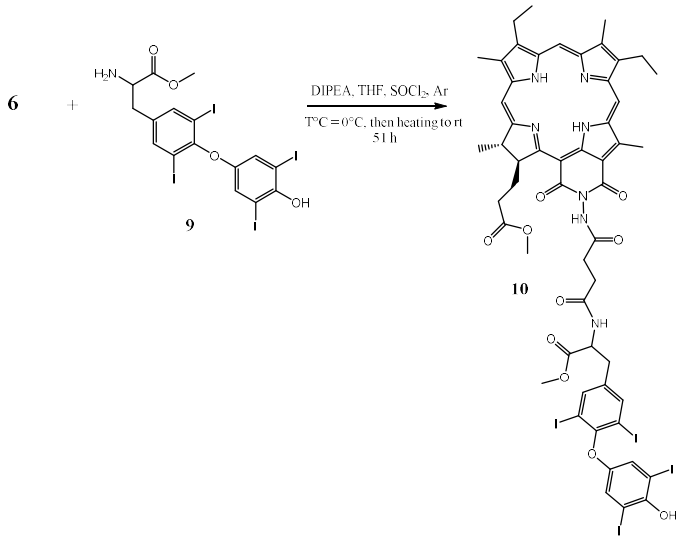
Scheme 4. Synthesis of acid **6** based on mesopurpurinimide.



Scheme 5. Synthesis of mesopurpurinimide conjugates with methyl esters of L-tyrosine and 3,5-diiodo-L-tyrosine.

On the other hand, compound **8b** was synthesized by direct iodination involving selective incorporation of iodine atoms into the aromatic tyrosine ring of compound **8a** using excess *N*-iodosuccinimide (NIS) in the presence of trifluoroacetic acid. It is interesting that no iodination of the chlorin macrocycle itself was observed. Moreover, when the tyrosine residue was replaced with a phenylalanine residue under these conditions, iodination did not occur, presumably due to the absence of an electron-donating substituent in the aromatic ring.[16] Chlorin **10** with the L-thyroxine methyl ester residue was obtained similarly (Scheme 6).

Thus, in the course of the work, various iodine-containing derivatives of chlorins were obtained, which can be converted into the corresponding radiotracers by isotope exchange during the Fishelstein reaction, the duration of which is 30-60 minutes, which is much less than the half-life of iodine radioisotopes used in medicine.[2,11]



Scheme 6. Synthesis of the mesopurpurinimide conjugate with L-thyroxine methyl ester.

The results of cytotoxicity evaluation of the iodine-containing and similar non-iodinated mesopurpurinimide conjugates obtained, carried out on cell cultures of human breast adenocarcinoma (MCF-7) and human colon carcinoma (HT-29) are presented in Table 1. The IC_{50} value of the test substances was determined at time intervals of 24, 48 and 72 h, as it was expected that the possible toxic effect due to the introduced iodine atoms would increase over time.

Table 1. IC_{50} values for iodine-containing conjugates and their non-iodinated analogues.

Compound	Cell culture					
	MCF-7			HT-29		
	Incubation time, h					
	24	48	72	24	48	72
	IC_{50}, μg/mL					
3c	5.6	3.9	3.8	12.5	6.4	3.8
3a	5.9	2.8	3.6	9.2	3.6	3.1
5b	9.5	4.7	3.7	8.8	4.5	3.3
5a	10.1	4.2	3.4	12.2	4.7	3.8
8b	9.4	4.6	3.7	13.4	6.9	3.7
8a	6.2	2.5	2.7	9.5	4.2	2.9
10	11.0	4.6	3.7	13.2	6.3	3.3

Studies on both tumor cell cultures showed that all the conjugates obtained in this work had nearly the same cytotoxicity, and incorporation of an iodine atom into their structure didn't increase it. This fact is beneficial for the use of the chlorins suggested in this work as iodine radiotracers, since the cytotoxicity of a radiopharmaceutical agent is determined by the radionuclide, but not by the carrier molecule. According to the Table 1, the compound **10** comprising four iodine atoms would be more effective as an iodine isotope radiotracer, since a higher concentration of the radionuclide would be provided at smaller RP doses.

Conclusions

The development and creation of radiochemical ligands for application in PET and SPECT imaging[17] is currently a rapidly developing area of nuclear medicine. Radioactive iodinated compounds are versatile contrast agents for PET and SPECT, and their high labelling efficiency is achieved due to the use of new approaches. The natural chlorins halogenation methods suggested in this work would expand the prospects of molecular design and open a way to the development of new classes of contrast agents for PET and SPECT diagnostics.

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