

**ЛУМИНЕСЦЕНТНИ ЛАНТАН(III) DOTA
КОМПЛЕКСИ ФУНКЦИОНИЗАОВАНИ
СА BODIPY ДЕРИВАТИМА КАО
ПОТЕНЦИЈАЛНИМ БИМОДАЛНИМ
КОНТРАСТНИМ АГЕНСИМА ЗА MRI
И ОПТИЧКО СНИМАЊЕ**

**LUMINESCENT LANTHANUM(III)-DOTA
COMPLEXES FUNCTIONALIZED
WITH BODIPY AS POTENTIAL
BIMODAL CONTRAST AGENTS
FOR MRI AND OPTICAL IMAGING**



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ABSTRACT

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BODIPY, click
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The synthesis and characterization of two novel lanthanum(III) DOTA complexes, functionalized with different boron-dipyrromethene derivatives (BODIPY) is described. The assembly of the complexes relies on the synthesis of azide-containing BODIPY molecules, which are then coupled directly to propargylated La(III)-DOTA complex via click chemistry, resulting in a final La-DOTA-BODIPY product. The luminescence of the final complexes has been evaluated. Both complexes absorb at 497 nm and emit brightly at 508 and 510 nm. The quantum yields in aqueous solutions are 39% and 64%, respectively.

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САЖЕТАК

Кључне речи:
BODIPY, „клик“
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Описана је синтеза и карактеризација два нова лантан(III) DOTA комплекса, који садрже различите бор-дипирометан деривате (BODIPY). Синтеза комплекса је базирана на „клик“ реакцији у којој се азидни BODIPY деривати повезују са пропаргил La-DOTA комплексом. Луминесцентна својства комплекса су проучавана и резултати су показали да комплекси испољавају емисионе пикове на 508 и 510 nm са максималном апсорпцијом на 497 nm. Квантни приноси комплекса у воденим растворима износе 39% и 64%.

INTRODUCTION

Magnetic resonance imaging (MRI) as a diagnostic tool has grown significantly over the past years. Compared to other imaging modalities, MRI provides a unique anatomical resolution, but the technique suffers from a rather low sensitivity. Therefore, medical MRI procedures routinely require the use of contrast agents, which are able to produce reliable results that help in the rapid clinical interpretation of images. Most of the commonly used contrast agents act by enhancing the relaxation rate of water protons in tissues. Gadolinium(I-II) chelates have some unique properties that efficiently induce relaxation of water molecules. In modern molecular imaging techniques, the commonly used contrast agents are the acyclic diethylenetriaminepentaacetic acid (Gd-DTPA) and the cyclic 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Gd-DOTA). The coordination number as high as eight ensures stability ($\log K = 22.5$ and 25.3 for Gd-DTPA and Gd-DOTA respectively), and allows the binding of one water molecule directly to the metal center. This allows for the dipolar interactions between water nuclei present in tissues and electron spins at the metallic center to occur, resulting in the enhancement of proton relaxation rates [1].

Among the bioimaging techniques, optical imaging is endowed with high sensitivity. The recent effort towards enhancing the imaging performance of contrast agents has been focused towards probes combining MRI and luminescent properties in a single molecule, which will be able to offer good resolution as well as high sensitivity [1-10]. This approach leads to the same biodistribution of the probe

for both techniques, which is advantageous for *in vivo* biological investigations, as it ensures the identical localization and the same pharmacokinetic behavior of the probe.

The 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (abbreviated as BODIPY) and its derivatives are promising class of organic dyes which are generally characterized by very high extinction coefficients, fairly sharp fluorescence peaks, and high quantum yields which are usually environment-independent. The BODIPY core is stable under physiological conditions and is relatively insensitive to the environment. Although BODIPY dyes have been known since the late 1960s, their versatility has been fully recognized only in the 1990s [11]. Despite good optical properties of BODIPY dyes, they are rarely used in developing bimodal contrast agents. We have recently shown that BODIPY dyes can be functionalized in a copper tube flow reactor *via* chemistry that converts primary amines into azides, by using the catalyst generated *in situ* from the metallic copper [12, 13]. In this paper, we have further expanded this strategy in order to create two novel La-DOTA-BODIPY complexes. We report on the synthesis and characterization of these novel compounds and evaluate their potential as optical imaging probes.

EXPERIMENTAL PROCEDURES

The azide-containing BODIPY derivatives and the propargylated DOTA complex shown in Figure 1 and 2 were synthesized by literature procedures [14, 15, 16]. BODIPY-Ph-N₃ or BODIPY-(CH₂)₃-Cl (1 eq.; 0.03 mmol; 10 mg) and propargylated La-DOTA (1 eq.; 0.03 mmol) were dissolved in 3 mL of a 10:3:3 MeOH/DCM/H₂O mixture. Cu(II)SO₄

(10 mol%) and sodium ascorbate (20 mol%) were added as 0.1 M solutions in water. Three drops of triethylamine were added and the solutions were stirred for 24 h in the dark. The solvents were evaporated and the compound was purified *via* HPLC.]. Analytical data for La-DOTA-BDP4 complex: yield: 2-2.2 mg, 5%. ESI-MS (MeOH, m/z): calcd: 941.55 g/mol $[M-H]^-$, found: 941.2 g/mol $[M-H]^-$. NMR: 1H NMR (300 MHz, $CDCl_3$, δ ppm): 0.96-3.86 (broad, 40H); 4.38 (broad, 4H), 5.89 (s, 2H), 7.72 (s, 1H).

La-DOTA-BPP5: Yield: 5-5.3 mg, 6%. ESI-MS (MeOH, m/z): calcd: 909.53 g/mol $[M+H]^+$, found: 909.3 g/mol $[M+H]^+$. Yield: 5 mg, 6%. NMR: 1H NMR (300 MHz, $CDCl_3$, δ ppm): 0.98-3.81 (broad, 40H); 4.31 (broad, 4H), 5.99 (s, 2H), 7.77 (s, 1H).

RESULTS AND DISCUSSION

For creating a hybrid DOTA-BODIPY molecule, two BODIPY azides, shown in Figure 1, were synthesized. Further, they were used in copper mediated cycloaddition for the coupling to the propargylated DOTA complex (Figure 2) [16]. The DOTA was chelated with a lanthanum(III) ions before the copper(I)-mediated cycloaddition, in order to prevent the formation of copper-DOTA complex by copper(I) ions used in the catalyst.

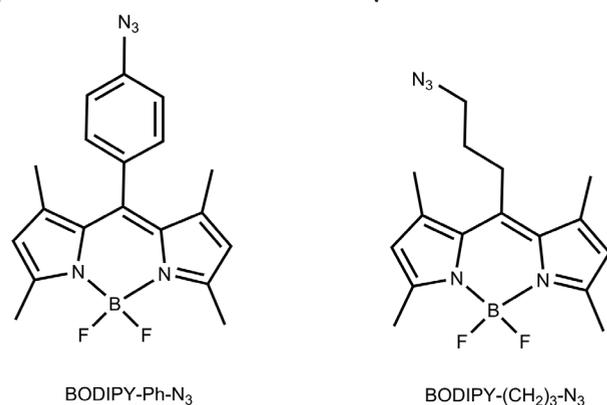


Figure 1

In the copper(I)-mediated cycloaddition the azides were mixed with the propargylated La-DOTA complex (Figure 2) in the presence of copper(II) sulphate and sodium ascorbate, which generate the active Cu(I) species.

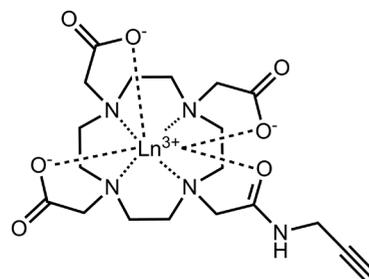


Figure 2

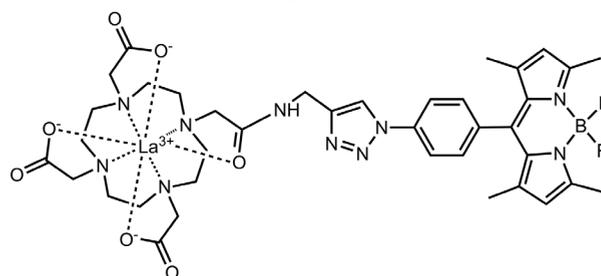


Figure 3

The final complexes were collected after HPLC purification. The structure of La-DOTA-BDP4 is depicted in Figure 3, and La-DOTA-BDP5 has a structure which is analogous to it.

The electrospray ionisation mass spectrometry (*ESI-MS*) of the final isolated complexes was in accordance with the structures shown in Figure 3. La-DOTA-BDP4 exhibited an m/z peak at 941.2 which could be assigned to $[M-H]^-$, while the characteristic m/z peak for La-DOTA-BDP5 was found at 909.3 and could be attributed to $[M+H]^+$ species. The 1H NMR spectra of both lanthanum(III) complexes show a distinct peak of the triazole proton at ca 7.7 ppm and two protons on the BODIPY core at 5.99 ppm, indicating a linkage of the DOTA moiety with the BODIPY derivative. The 1H NMR spectrum also show broad peaks in the region from 2.20-3.82 ppm which are typical for the protons in the DOTA ring, and arise from the presence of multiple conformers in solution [17].

Despite the hydrophobic nature of the BODIPY dye, the final BODIPY derivatives are water soluble, due to the linkage to the polar La-DOTA complex. The electronic absorption spectra shown in Figure 4 depict characteristic and rather narrow absorption bands typical for BODIPY dyes. The absorption patterns for both types of complexes are similar, which is expected as they have the same BODIPY core.

Aqueous solutions of La-DOTA-BDP4 and La-DOTA-BDP5 show main absorption maxima at 496 and 499 nm, respectively. These visible absorption bands can be assigned to the $S_0 \rightarrow S_1$ transition [18]. An additional, considerably weaker broad absorption band is observed in the UV-Vis region around 350 nm, and is attributed to the $S_0 \rightarrow S_2$ transition [18].

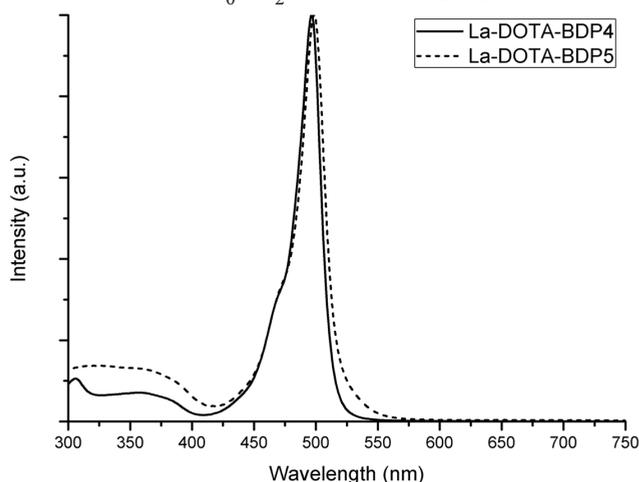


Figure 4

The solutions emit a green fluorescence upon excitation (Figure 5).

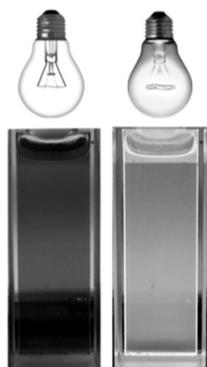


Figure 5

The emission maxima of La-DOTA-BDP4 and La-DOTA-BDP5 are observed at 508 and 510 nm, respectively (Figure 6).

The excitation maximum of both complexes, measured at an emission wavelength of 525 nm, shows a maximum at 497 nm and has a similar shape to the absorption maximum.

The quantum yields of La-DOTA-BDP4 and La-DOTA-BDP5 were determined upon ligand excitation (490 nm) by a comparative method, using a solution of Rhodamine 6G ($Q = 78\%$) in water as the standard. The quantum yields

were determined according to the following equation [19]:

$$Q_L = Q_S \times \frac{I_X}{I_S} \times \frac{A_S(\lambda_{exc})}{A_X(\lambda_{exc})} \times \frac{\eta_X^2}{\eta_S^2}$$

In this equation the s and x refer to the standard and the unknown sample, respectively, I represents the corrected total integrated emission intensity, A is the absorbance at the excitation wavelength, η is the refractive index of the solution ($\eta_{\text{water}} = 1.33$), and Q_S is the quantum yield of the standard ($Q = 78\%$). The samples were diluted until the absorbance at the excitation wavelength was between 0.02–0.05. The quantum yields of complexes in aqueous solutions are 39% and 64%, respectively. The former value is somewhat lower than expected [13], most likely because of the energy transfer from the BODIPY core to the phenyl ring [20].

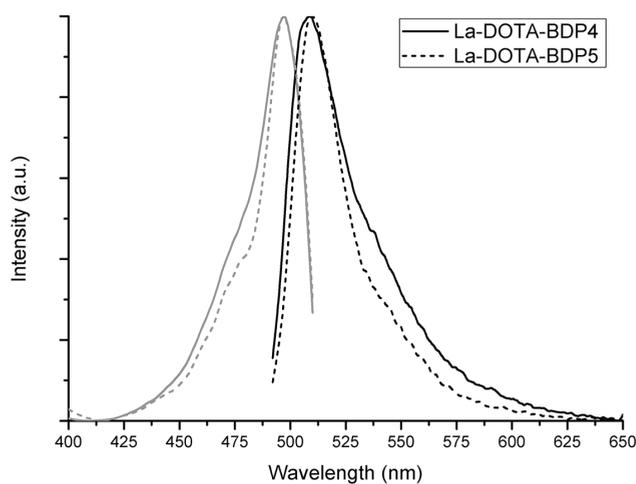


Figure 6

CONCLUSIONS

In this paper, we have presented two novel, water-soluble lanthanum(III)-DOTA-BODIPY complexes that were synthesized *via* “click” chemistry. The complexes exhibit good fluorescent properties, thus offering the possibility for their use for *in vivo* optical imaging. Upon excitation of the coordinated ligands, a bright emission was observed at 508 and 510 nm with an observed quantum yield of 39% and 64% for La-DOTA-BDP4 and La-DOTA-BDP5, respectively. Due to their favorable optical properties, the analogous complexes in which lanthanum(III) is replaced by gadolinium(III) can hold a potential as a bimodal contrast agent for MRI and optical imaging.

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