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Fluorescent conjugates of PSMA-targeting ligand with cyanine dyes: synthetic approaches and photochemical properties

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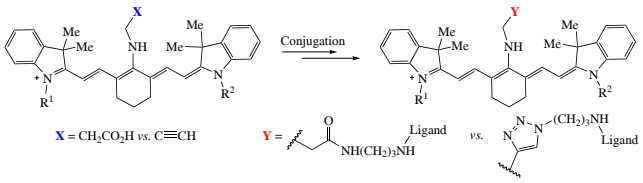
Fluorescent conjugates of carbocyanine dyes with a ligand selective to prostate-specific membrane antigen were accessed by either peptide synthesis or CuAAC methodology, with the latter being more promising. The introduction of a propargylamino moiety at the *meso*-position of the polymethine chain of the fluorophores afforded the alkyne counterparts for the CuAAC reaction toward the ligand bearing azido group. The photochemical studies showed that the quantum yields of the obtained conjugates exceeded those for the unmodified fluorophores by more than 20 times.



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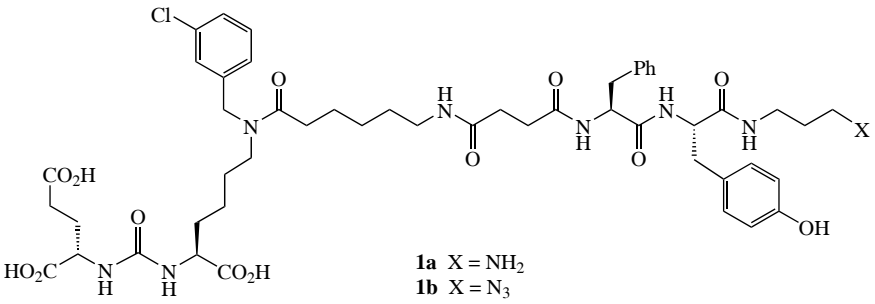
Histologic examination of biopsy material is the leading method for accurate diagnosis in the field of oncology when fluorescent dyes are used as diagnostic agents. The promising fluorophores are based on tricarbocyanines since they have high quantum yields of fluorescence, transparency window, defined as light wavelengths between 600 and 1000 nm, and have several centers for modification.[1] The introduction of a substituent at the *meso*-position of the polymethine chain for conformationally anchored tricarbocyanines not only promotes the appearance of an additional functional group, but also increases the Stokes shift. Varying the linkers at the quaternary nitrogen atoms of heterocycles allows changing the lipophilicity and hydrophilicity indices of the molecule. The introduction of carbocyanines into a single structure with molecules capable of selective accumulation in certain types of malignant neoplasms allows one to obtain highly effective diagnostic agents.

Conjugates whose molecules consist of different functional parts are a large family of organic compounds used in targeting medicine against a wide range of cancer diseases. Among a large number of methods for obtaining such structures, the copper-catalyzed [3+2] azide–alkyne cycloaddition reaction (CuAAC) is of special importance.[2,3] The main disadvantage of this method is related to the presence of copper catalyst in the reaction, so the use of this reaction imposes additional control on metal content in the final products. In addition, it is not always easy to introduce an alkynyl or azide component into a structure that is part of a future conjugate. The introduction of short alkynyl fragments by nucleophilic substitution has previously been described only for the example of tricarbocyanine dye anchored by cyclopentene.[4] The introduction of propargylamino substituent into the more common dyes anchored by cyclohexene was not possible due to the lability of the structure.[5] Therefore, the use, development and optimization of other synthetic approaches to the preparation of conjugates is an urgent task. The replacement of *meso*-positioned chlorine atom by the β-alanine fragment is optimized and well implemented leading to a carboxy component suitable for the peptide synthesis reaction.[6] The peptide bond formation reaction does not require complicated synthetic conditions, it is sufficient to use carboxy group activating agents, which can be subsequently separated from the reaction mixture by chromatography. The peptide bond is generally stable under *in vitro* and *in vivo* conditions.[7] Thus, conjugation by the peptide synthesis methods may be an alternative route to obtain diagnostic conjugates targeting prostate cancer. In this paper, we compare both synthetic approaches.

A ligand targeting prostate-specific membrane antigen (PSMA) based on modified urea DCL (*N*-[*N*-[(*S*)-1,3-dicarboxypropyl]carbamoyl]-(*S*)-L-lysine) was taken as a vector platform. We selected a ligand containing the *m*-chlorophenyl substituent at the nitrogen atom of lysine and the dipeptide chain L-Phe-L-Tyr in the linker structure, the basic structures were compounds **1a**,**b**. Previously, such ligands showed high affinity to PSMA expressing cell line LNcAP (9±3 nM).[8] The synthesis of the vector was carried out by



solid-phase synthesis according to the previously described methodology, in which diaminopropane is pre-immobilized on the resin.[9,10]

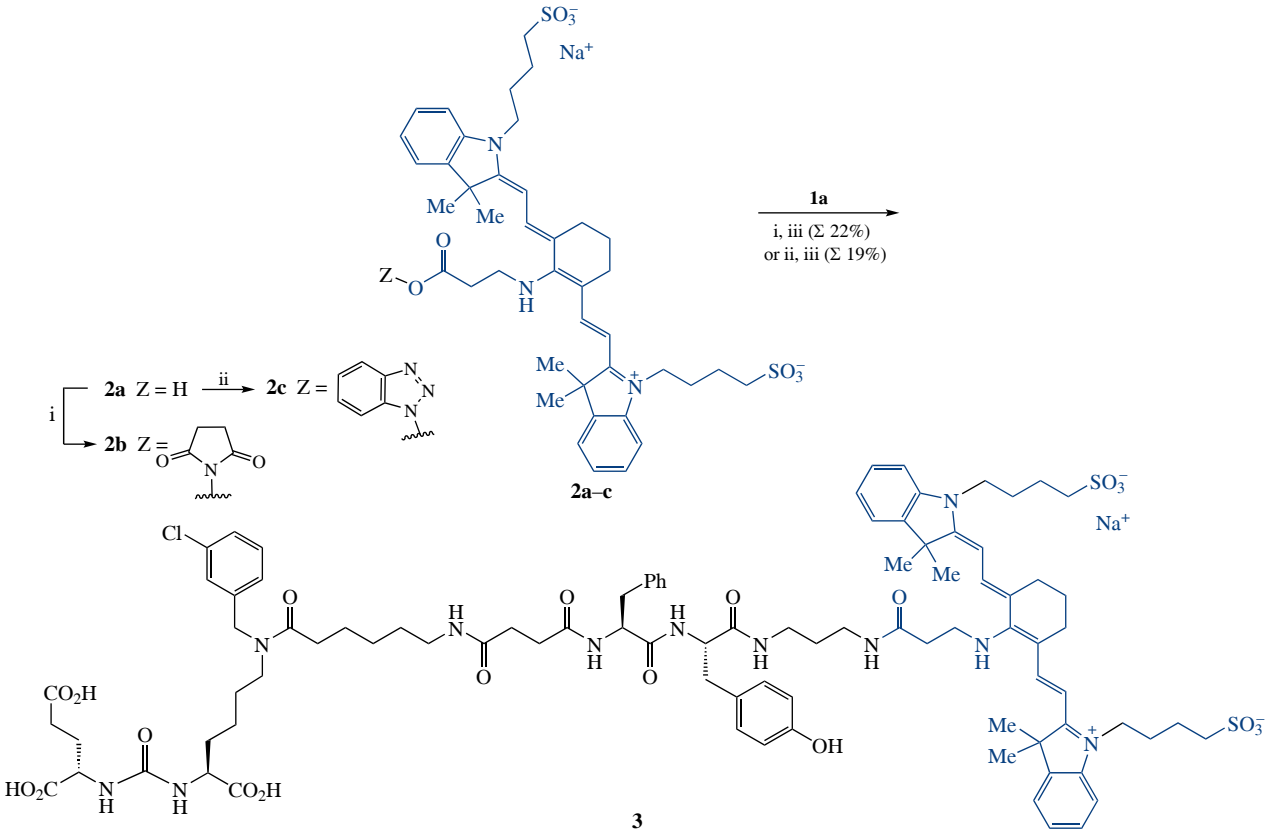
Compound **1a** contains one amino group and three carboxy functions, therefore its conjugation with the formation of the peptide bond looks somewhat problematic. However, we managed to perform its conjugation with cyanine dye **2a** (Scheme 1, the synthesis of **2a** was reported previously[6]). The carboxy group in the β-alanine moiety of compound **2a** had to be preliminarily activated by its *in situ* conversion into either succinimide (**2b**) or benzotriazole (**2c**)[11] derivatives, whose further reactions with tricarboxy amine **1a** afforded the target amide conjugate **3**, although in modest yields of 22 or 19%, respectively.

The principal drawback of this scheme is difficulties for the preparation of sufficient amounts of product **3**. In fact, conjugate **3** in both cases was isolated by reversed-phase liquid chromatography with the H_2O/MeCN eluent. In addition, the preparation of the starting ligand **1a** involves solid-phase synthesis and scaling up this process is problematic because the capacity of 2-chlorotrityl chloride resin decreases when 1,3-diaminopropane is immobilized.[10] Anyway, the obtained conjugate **3** is a valuable compound since it bears carboxy groups, which is crucial for binding to PSMA.[12]

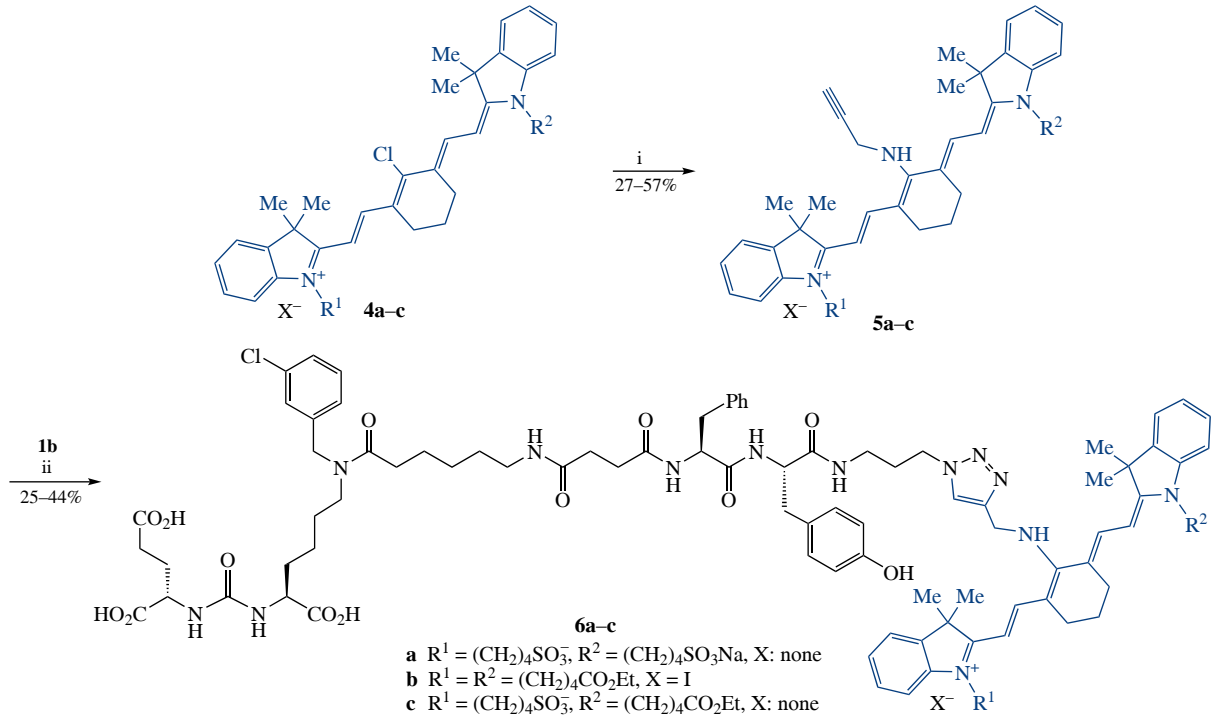
In order to avoid side reactions involving carboxy groups of the vector fragment and to improve the yield of **3**, we attempted to conjugate *O-tert*-butylated analog of compound **1a** with fluorophore **2** followed by removal of the *tert*-butyl protecting groups. Although the conjugation proceeded well with 65% yield, the next removal of the *tert*-butyl protections with any of CF_3CO_2H-contaning systems (*cf*. refs. 13–16) was accompanied by decomposition of the cyanine part of the conjugate (for details, see Online Supplementary Materials, Scheme S1, Figure 1 and comments therein).

Therefore, it seemed reasonable to study the azide–alkyne cycloaddition for the construction of conjugate. For this purpose, it was necessary to introduce an alkynyl moiety into the fluorophore structure, similarly to that we performed previously.[6] Dyes **4a–c** with chlorine atom at the *meso*-position of the polymethine chain were reacted with propargylamine to afford the anticipated products **5a–c** equipped with terminal acetylenic bond (Scheme 2). The bands in absorption spectra hypsochromically shifted from the region of 780–785 nm (chlorides **4a–c**) to 652–655 nm (amines **5a–c**), which meets the stated requirements (Figure 1).

For the conjugation with alkynes **5a–c**, azido analog of amine **1a**, *vis*. compound **1b**, was employed. It is of note that ligand **1b** is essentially accessible since all the steps for its preparation can



Scheme 1 *Reagents and conditions*: i, NHS, DCC, DMF, 0–5 °C, 18 h; ii, HOBt, DCC, DMF, 0–5 °C; iii, **1a**, Et_3N, DMF, room temperature, 18 h.



Scheme 2 *Reagents and conditions*: i, $HC{\equiv}CCH_2NH_2$, DMF, Et_3N, argon atmosphere, 65 °C, 1.5–5 h (for **4a**,**b**) or room temperature, 24 h (for **4c**); ii, $CuSO_4 \cdot 5H_2O$, Na ascorbate, DMF/H_2O.

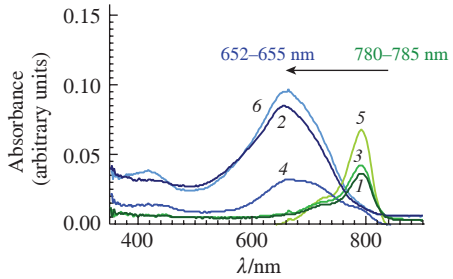


Figure 1 Absorption maxima for compounds (*1*) **4a**, (*2*) **5a**, (*3*) **4b**, (*4*) **5b**, (*5*) **4c** and (*6*) **5c**.

be carried out by classical peptide synthesis in solution.[8] The resulting products of the click reaction **6a–c** were purified by reversed phase liquid chromatography with H_2O/MeCN eluents. The structures of the conjugates were confirmed by physicochemical analysis and provide evidence for the successful conjugation of the PSMA-ligand with the carbocyanine tag (see Scheme 2).

Attachment of a triazole moiety close to a fluorophore would lead to fluorescence changes. For example, replacement of chlorine at the *meso*-position of a conformationally anchored tricarbocyanine by a triazole moiety allows effective visualization of inflammatory processes in the eye due to brighter fluorescence compared to the conventional FDA-approved (Food and Drug Administration) ICG dye (Indocyanine green).[17] Moreover, the introduction of this structural motif to the dye leads to an increase in photostability and fluorescence quantum yield.[18]

The effects of the structure on the photochemical properties were studied for the initial fluorophores **4a–c**, propargylamino-modified tricarbocyanines **5a–c** and the resulting conjugates **6a–c** (Table 1). The introduction of a propargylamino substituent in conjugation with the polymethine chain of initial chloro fluorophores **4** causes a hypsochromic shift of the absorption maxima (~140 nm) and fluorescence (~40 nm), which, in turn, leads to an increase in the Stokes shift (~2420 cm^{-1}). At the same time, the introduction of PSMA-ligand into the structure and the presence of triazole ring near the conjugated system in series **6** do not affect these parameters.

Table 1 Shift of absorption maxima and photochemical parameters for fluorophores **4a–c**, **5a–c** and conjugates **6a–c** in DMF.

Compound	λ_{abs}/nm	λ_{fl}/nm	Stokes shifts/cm^{-1}	ε/ $dm^3\ mol^{-1}\ cm^{-1}$	Φ_{fl} (%)
4a	790	817	418	2.56×10^5	0.002
5a	654	777	2421	1.56×10^5	0.035
6a	650	775	2481	6.54×10^4	0.065
4b	790	814	373	2.81×10^5	0.003
5b	666	776	2128	1.87×10^5	0.007
6b	634	761	2632	2.31×10^3	0.100
4c	790	817	418	5.22×10^5	0.007
5c	654	777	2421	2.01×10^5	0.050
6c	640	761	2484	4.89×10^4	0.135

Studies on quantum yields of fluorescence show that the presence of propargylamino moiety at the tricarbocyanine structure significantly increases this parameter in both negatively charged (**6a**) and neutral (**6c**) molecules: by 17 and 7 times, respectively. The introduction of the ligand additionally increased this index by approximately 2-fold in both cases. This suggests that the obtained conjugates may be regarded as promising diagnostic agents.

In conclusion, fluorescent conjugates can be obtained by the peptide synthesis reactions, however, the efficiency of this method is inferior to the azide–alkyne cycloaddition method. The propargylamino moiety was introduced at the *meso*-position of the polymethine chain based on the fluorophore with the corresponding chlorine substituent. The thus obtained new propargylamino tricarbocyanines were subjected to CuAAC reaction with ligand bearing azido group, which afforded the target triazole conjugates which possessed fluorescent properties and contained a vector selective to prostate specific membrane antigen. The introduction of a nitrogen atom into the structure of the polymethine chain of the fluorophore increases the Stokes shift at 2400 cm^{-1}, and the quantum yields of conjugates in which the triazole ring locates in close proximity to the fluorophore structure exceed those of the unmodified tags by more than 20 times.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7583.

References

1 L. A. Lane, R. Xue and S. Nie, *Curr. Opin. Chem. Biol.*, 2018, **45**, 95; https://doi.org/10.1016/j.cbpa.2018.03.015.
2 N. Nebra and J. García-Álvarez, *Molecules*, 2020, **25**, 2015; https://doi.org/10.3390/molecules25092015.
3 P.-H. Elchinger, P.-A. Faugeras, B. Boëns, F. Brouillette, D. Montplaisir, R. Zerrouki and R. Lucas, *Polymers*, 2011, **3**, 1607; https://doi.org/10.3390/polym3041607.
4 P. Kele, X. Li, M. Link, K. Nagy, A. Herner, K. Lőrincz, S. Béni and O. S. Wolfbeis, *Org. Biomol. Chem*., 2009, **7**, 3486; https://doi.org/10.1039/b907741c.
5 C. Ornelas, R. Lodescar, A. Durandin, J. W. Canary, R. Pennell, L. F. Liebes and M. Weck, *Chem. – Eur. J.*, 2011, **17**, 3619; https://doi.org/10.1002/chem.201002268.
6 I. A. Doroshenko, K. G. Aminulla, V. N. Azev, T. M. Kulinich, V. A. Vasilichin, A. A. Shtil and T. A. Podrugina, *Mendeleev Commun*., 2021, **31**, 615; https://doi.org/10.1016/j.mencom.2021.09.008.
7 L. Gong, H. Zhao, Y. Liu, H. Wu, C. Liu, S. Chang, L. Chen, M. Jin, Q. Wang, Z. Gao and W. Huang, *Acta Pharm. Sin. B*, 2023, **13**, 3659; https://doi.org/10.1016/j.apsb.2023.02.013.
8 A. E. Machulkin, R. R. Shafikov, A. A. Uspenskaya, S. A. Petrov, A. P. Ber, D. A. Skvortsov, E. A. Nimenko, N. U. Zyk, G. B. Smirnova, V. S. Pokrovsky, M. A. Abakumov, I. V. Saltykova, R. T. Akhmirov, A. S. Garanina, V. I. Polshakov, O. Y. Saveliev, Y. A. Ivanenkov, A. V. Aladinskaya, A. V. Finko, E. U. Yamansarov, O. O. Krasnovskaya, A. S. Erofeev, P. V. Gorelkin, O. A. Dontsova, E. K Beloglazkina, N. V. Zyk, E. S. Khazanova and A. G. Majouga, *J. Med. Chem.*, 2021, **64**, 4532; https://doi.org/10.1021/acs.jmedchem.0c01935.
9 A. E. Machulkin, S. A. Petrov, V. Bodenko, M. S. Larkina, E. Plotnikov, F. Yuldasheva, M. Tretyakova, E. Bezverkhniaia, N. Yu. Zyk, E. Stasyuk, R. Zelchan, A. G. Majouga, V. Tolmachev, A. Orlova, E. K. Beloglazkina and M. S. Yusubov, *ACS Pharmacol. Transl. Sci*., 2024, **7**, 1457; https://doi.org/10.1021/acsptsci.4c00070.
10 N. Yu. Zyk, S. A. Petrov, M. V. Zavertkina, A. A. Uspenskaya, P. A. Krasnikov, N. S. Dashkova, E. K. Beloglazkina, A. G. Majouga, N. V. Zyk and A. E. Machulkin, *Mendeleev Commun*., 2023, **33**, 472; https://doi.org/10.1016/j.mencom.2023.06.010.
11 S. A. Kularatne, Z. Zhou, J. Yang, C. B. Post and P. S. Low, *Mol. Pharm*., 2009, **6**, 790; https://doi.org/10.1021/mp9000712.
12 A. A. Uspenskaya, E. A. Nimenko, R. R. Shafikov, N. Y. Zyk, S. A. Evteev, N. S. Dashkova, Y. A. Ivanenkov, A. G. Majouga, D. A. Skvortsov, A. S. Garanina, E. K. Beloglazkina and A. E. Machulkin, *Med. Chem. Res*., 2023, **32**, 32; https://doi.org/10.1007/s00044-022-03002-w.
13 H. Chen, B. Dong, Y. Tang and W. Lin, *Chem. – Eur. J*., 2015, **21**, 11696; https://doi.org/10.1002/chem.201502226.
14 N. L. Benoiton, *Chemistry of Peptide Synthesis*, CRC Press, Boca Raton, 2006; https://doi.org/10.1201/9781420027693.
15 L. Konnert, F. Lamaty, J. Martinez and E. Colacino, *Chem. Rev*., 2017, **117**, 13757; https://doi.org/10.1021/acs.chemrev.7b00067.
16 S. R. Manne, A. Sharma, A. Sazonovas, A. El-Faham, B. G. de la Torre and F. Albericio, *ACS Omega*, 2022, **7**, 6007; https://doi.org/10.1021/acsomega.1c06342.
17 C. N. Thomas, N. Alfahad, N. Capewell, J. Cowley, E. Hickman, A. Fernandez, N. Harrison, O. S. Qureshi, N. Bennett, N. M. Barnes, A. D. Dick, C. J. Chu, X. Liu, A. K. Denniston, M. Vendrell and L. J. Hill, *Biosens. Bioelectron*., 2022, **216**, 114623; https://doi.org/10.1016/j.bios.2022.114623.
18 X. Yu, Y. Wu, W. Tang and X. Duan, *Analyst*, 2023, **148**, 5117; https://doi.org/10.1039/D3AN01238G.

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