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LETTER TO EDITOR

Synthesis of the New Green Fluorescent Protein Chromophore Analogue Starting from a Cinnamic Aldehyde Derivative

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Abstract—A novel derivative of green fluorescent protein chromophore (*Z*)-5-((*E*)-3-(4-hydroxyphenyl)allylidene)-2,3-dimethyl-3,5-dihydro-4*H*-imidazol-4-one was synthesised. The desired compound was prepared by the synthetic approach based on the use of carboxyimidate. Comparison of optical properties of the obtained compound and of the classical GFP chromophore analogue (*Z*)-5-(4-hydroxybenzylidene)-2,3-dimethyl-3,5-dihydro-4*H*-imidazol-4-one showed the shift of absorption and emission maxima to the long-wavelength region induced by the increase of the conjugated π-system in the benzylidene fragment.

Keywords: imidazolones, chromophores, fluorescent dyes, GFP, optical properties

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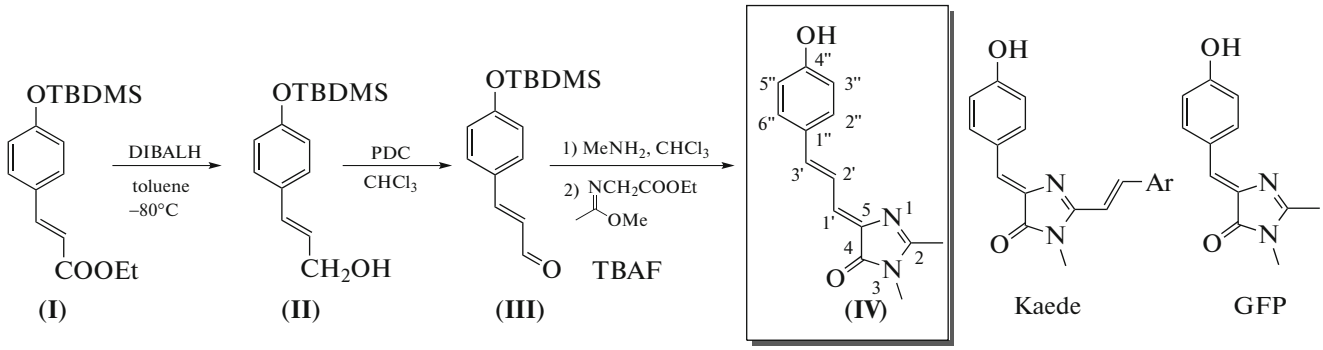
Synthetic analogues of fluorescent protein chromophores—that is, various benzylidene imidazolones—have been a subject of careful researchers' attention for almost four decades. Intense coloration, small size, high solubility, and easily tuned spectral characteristics allow successful application of these compounds in chemical and biological studies [1]. As fluorescent [2] and fluorogenic [3] dyes, such molecules can be used to visualize cell structures, label various objects, or develop ion- and pH-responsive sensors [4, 5].

The main characteristic determining the possibility of application of a chromophore analogue as a dye is the position of its absorption and emission maxima. Since the absorption region of most biological objects resides in the short-wavelength region of the spectrum, design of compounds demonstrating a significant bathochromic shift is a relevant task.

The shift is often known to result from the extension of the conjugated π-system in the dye molecule. As for various derivatives of the GFP chromophore (5-(4-hydroxybenzylidene)-3,5-dihydro-4*H*-imidazol-4-one) (Scheme 1), such a modification is most often introduced at position 2 of the imidazolone cycle. In particular, introduction of a styrene group at position 2 leads to Kaede protein derivatives (Scheme 1) exhibiting notable bathochromic shifts [6]. These compounds find application as dyes and can be used as sensitizing dyes in solar cells [7].

In the work, we propose an alternative way to increase the system of conjugated bonds in a GFP chromophore derivative to shift the positions of absorption and emission maxima, i.e., through modification of the benzylidene fragment (Scheme 1).



Scheme 1. Synthesis of compound (**IV**).

Abbreviations: DIBALH, diisobutylaluminum hydride; GFP, green fluorescent protein; PDC, pyridinium dichromate; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl.

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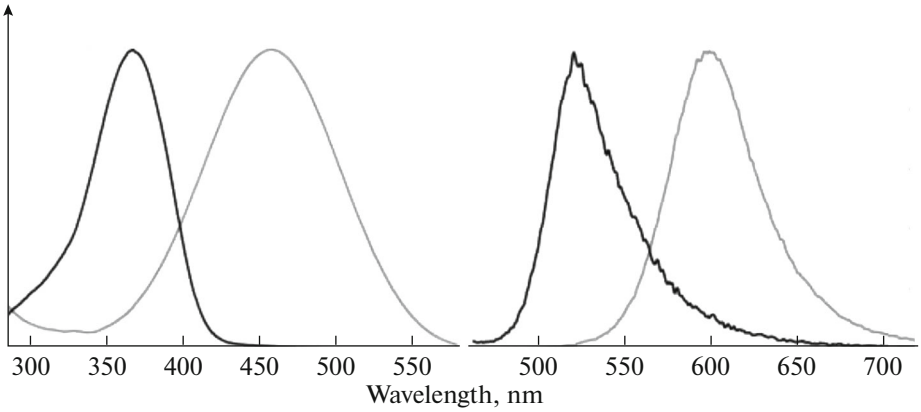

Fig. 1. Normalized absorption (left) and emission (right) spectra of the GFP chromophore (black) and compound (**IV**) (grey) solutions in water at pH 10.

Synthesis of compound (**IV**) was carried out using a cinnamic aldehyde derivative (Scheme 1), since one of the most universal methods to synthesize benzylidene imidazolones is interaction of aldehyde-derived Schiff-bases with carboximidate [8]. The required aldehyde (**III**) was obtained with fair yield from a commercially available silylated ethylphenylacrylate (**I**) by reduction and further oxidation (Scheme 1). Surprisingly, formation of the Schiff base was not hampered by side reactions of the α,β-unsaturated aldehyde. Due to the presence of conjugated double bonds, the final compound (**IV**) was obtained as a mixture of *E*- and *Z*-isomers of each of the two double bonds. However, it could be easily transformed into an individual isomer—*E*-isomer at the 3' position and *Z*-isomer at the 1' position of the allylidene moiety—in the course of slow recrystallization from methanol, which is supported by the NMR data: the spin–spin interaction constant between two hydrogen atoms at the 2'–3' bond is 11.4 Hz, which corresponds to the *trans* configuration, while position of the signal at the 1' atom corresponds to the typical position of *Z*-isomers of other GFP chromophore derivatives, in which this configuration is the most stable [9].

Study of optical properties of compound (**IV**) (Fig. 1; Table 1), as well as comparison with properties of GFP, showed that introduction of a larger conjugated π-system in the benzylidene part of the molecule led to a bathochromic shift of the absorption and emission maxima.

Therefore, we obtained a new analogue of the GFP chromophore from a cinnamic aldehyde derivative. Maxima of absorption and emission of the compound lie in the longer-wavelength region of the spectrum, compared to the GFP chromophore analogue, due to extended conjugated system of π-bonds in the benzylidene fragment.

EXPERIMENTAL

NMR spectra (δ, ppm, *J*, Hz) were registered on a Bruker Avance III (700 MHz, United States) equipment in DMSO-d_6 and $CDCl_3$ (Me_4Si was used as internal standard). UV-visible absorption spectra were recorded on a Varian Cary 100 Bio (United States) spectrophotometer. Fluorescence spectra were registered on a Varian Cary Eclipse (United States) fluorescence spectrophotometer. Melting points were determined on an SMP-30 (Great Britain) instrument and are reported uncorrected.

(*E*)-3-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)prop-2-en-1-ol (II). A solution of 2.97 g (10 mmol) of ethyl-(*E*)-3-(4-(((*tert*-butyldimethylsilyl)oxy)phenyl)acrylate) (**I**) [10] in 60 mL of toluene was cooled to –80°C. Diisobutylaluminum hydride (DIBALH) (10.6 mL, 60 mmol) was added, and the reaction mixture was

Table 1. Positions of absorption and emission maxima of the GFP chromophore and compound (**IV**)

Solvent	GFP		(**IV**)	
	λ_{abs}, nm	λ_{em}, nm	λ_{abs}, nm	λ_{em}, nm
Dioxane	369	435	388	482
Ethyl acetate	367	420	386	491
Acetonitrile	368	438	385	508
Methanol	370	436	397	513
Water (neutral form)	367	453	395	458
Water (deprotonated form)	425	491	458	600

kept at −80°C for 20 h. Then, 400 mL of ethyl acetate and 150 mL potassium tartrate solution were added and the mixture was stirred for 1 h. Then, the aqueous phase was extracted with ethyl acetate (2 × 150 mL), and organic fractions were combined and washed with saturated NaCl solution (2 × 100 mL). The solution was dried over anhydrous Na_2SO_4 and evaporated, and the product was purified by column chromatography (EtOAc–hexane, 1 : 3). Product (**II**) yield was 2.14 g (81%). ^{1}H NMR: 7.28 (d, J_2 8.7, 2H, H2", H6"), 6.80 (d, J_2 8.7, 2H, H3", H5"), 6.56 (d, J_2 16.0, 1H, H3'), 6.25 (dt, J_2 15.8, 6.0, 1H, H2'), 4.30 (t, J_2 5.2, 2H, CH_2), 1.00 (s, 9H, *t*-Bu), 0.21 (s, 6H, 2 CH_3). Agrees with the literature data [11].

(*E*)-3-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-acrylaldehyde (III). To a solution of alcohol (**II**) in 50 mL chloroform, 20 eq. of pyridinium dichromate were added. The solution was stirred for 1.5 h at room temperature; the reaction was followed by TLC in the EtOAc–hexane, 1 : 3, system. The reaction mixture was filtered through 1 cm of silica gel and evaporated. Yield: 823 mg (39%). ^{1}H NMR: 9.67 (d, J_2 7.7, 1H, CHO), 7.48 (d, J_2 8.5, 2H, H2", H6"), 7.42 (d, J_2 15.8, 1H, H3'), 6.89 (d, J_2 8.5, 2H, H3", H5"), 6.62 (dd, J_2 15.8, 7.7, 1H, H2'), 1.00 (s, 9H, *t*-Bu), 0.25 (s, 6H, 2 CH_3). Agrees with the literature data [12].

5-([1*Z*,3*E*]-4-(4-Hydroxyphenyl)allylidene)-2,3-dimethyl-3,5-dihydro-4*H*-imidazol-4-one (IV). (1) To a solution of 262 mg (1 mmol) aldehyde (**III**) in 20 mL chloroform, 0.3 mL 40% aqueous methylamine and anhydrous sodium sulfate (2 g) were added. The mixture was stirred for 48 h at room temperature, then the organic phase was separated, dried over anhydrous Na_2SO_4, and evaporated.

(2) The imine (1 mmol) was mixed with ethyl-2-((1-methoxyethylidene)amino)acetate (175 mg, 1.1 mmol) and stirred at room temperature for 36 h. Then, 50 mL of ethyl acetate was added to the reaction mixture, then it was washed with water (2 × 50 mL) and saturated NaCl solution (2 × 5 mL). To the organic phase, 10 eq. tetrabutylammonium fluoride was added; the mixture was stirred for 10 min and 2 mL glacial acetic acid was added. The reaction mixture was washed with water (5 × 5 mL) and saturated NaCl solution (3 × 5 mL). The solution was dried over anhydrous Na_2SO_4, evaporated, and purified with column chromatography (EtOAc–EtOAc : *i*-PrOH, 1 : 4 gradient). Individual isomer (*E*-isomer at position 3' and *Z*-isomer at position 1') was obtained by slow recrystallization from methanol. Yield: 88 mg (36%); yellow crystals; mp ~300°C with decomposition. ^{1}H NMR: 9.88 (s, 1H, OH), 7.44 (d, J_2 8.2, 2H, H2", H6"), 7.29 (dd, J_2 15.4, 11.6, 1H, H2'), 7.11 (d, J_2 15.6, 1H, H1'), 6.84 (d, J_2 11.4, 1H, H3'), 6.79 (d, J_2 8.2, 2H, H3", H5"), 3.06 (s, 3H, 3-CH_3), 2.29 (s, 3H, 2-CH_3). ^{13}C NMR: 168.7 (C4), 161.0 (C2), 158.9 (C4"), 141.6 (C3'), 138.3 (C1'), 129.1 (C2", C6"), 127.4 (C1"), 127.3 (C5), 120.0 (C2'), 115.9 (C3", C5"), 26.1 (3-CH_3), 15.0 (2-CH_3).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

The authors declare that they have no conflict of interest.

Statement of the Welfare of Animals

This article does not contain any studies involving animals or human participants performed by any of the authors.

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