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LETTER TO THE EDITOR

Synthesis and Optical Properties of the New Acetylene Kaede Chromophore Analog

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Abstract—A novel derivative of acetylene Kaede protein chromophore (Z)-2-((4-(diethylamino) phenyl) ethynyl)-5-(4-hydroxybenzylidene)-3-methyl-3,5-dihydro-4H-imidazol-4-one was synthesized by a modified Sonogashira reaction. In comparison with the classical GFP chromophore analog, the compound exhibits a prominent shift of absorption and emission maxima to the long-wavelength region. The value of Stock's shift of the newly synthesized compound reached almost 150 nm.

Keywords: imidazolones, chromophores, fluorescent dyes, GFP, Kaede, Sonogashira reaction, optical properties

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The key to modern approaches to the study of processes occurring in living cells is visualization methods associated with the emission of light. The most important component of these methods is the introduction of a luminescent or fluorescent label into the object under study. Among the latter, it is worth noting separately the numerous derivatives of the chromophore of green fluorescent proteins (GFP) containing benzylideneimidazolone in their composition [1]. This class of compounds has great variability in optical properties, significant Stokes shift, good solubility in water, high permeability through the cytoplasmic membrane, low toxicity, and synthetic availability [2–4].

Biological objects are characterized by high absorption of shortwave radiation (which often disrupts the ongoing biological processes) and low absorption of longwave radiation. Therefore, when conducting biomedical research, it is preferable to use labels with maximum absorption and emission of fluorescence in the long wavelength part of the visible spectrum. One of the main synthetic strategies for achieving a bathochromic shift is to increase the size of the conjugate π-systems of the chromophore molecule. An example of this approach in the case of benzimidazolones is the Kaede protein chromophore analogs containing a styrene substituent in the second position of the imidazolone cycle [5]. These compounds are already used as fluorescent [6, 7] or fluorogenic (substances that increase the quantum yield of fluorescence upon binding to the target object) tags [8, 9] in biomedical studies. However, for the entire time that the Kaede protein chromophore analogs have been studied, their acetylene derivatives have not been obtained. In the present work, we first developed an approach to the synthesis of this class of compounds and, using one example, examined their availability and optical properties.

As the target compound, we selected the substance (**V**) (Fig. 1). One of the most common methods for the synthesis of acetylenes is the Sonogashira reaction, its use involves the terminal alkyne and aryl or hetaryl halide in the reaction. 2-Halogenimidazolones are not described in the literature, however, there are examples of the Sonogashira reaction involving alkylthio derivatives in which palladium complexes catalyzed by the replacement of the sulfur-containing group with acetylene [10, 11]. There are also several examples of the modification of thiohydantoins [12] and 2-alkylthioimidazolones [13] as a result of the Libskind-Srogl reaction. Therefore, for the synthesis of the target compound, we chose a modified method for the preparation of acetylenes by the Sonogashira reaction with desulfurization of the corresponding thiohydantoin derivative (Scheme 1).

Compound (**IV**) was synthesized using a rather rare modification of the Sonogashira reaction using a derivative containing an $SCH\ group_3$ [14]. The catalyst used was copper (I) iodide and Pd (dppf) $Cl_2CH_2Cl_2$. In the beginning, as a result of the condensation of 4-hydroxybenzaldehyde with 2-thiooxoimidazolin-4-one the compound was obtained (**I**). Since the alkylation of thiooxoimidazoline could also occur with the participation of the hydroxy group of the ben-

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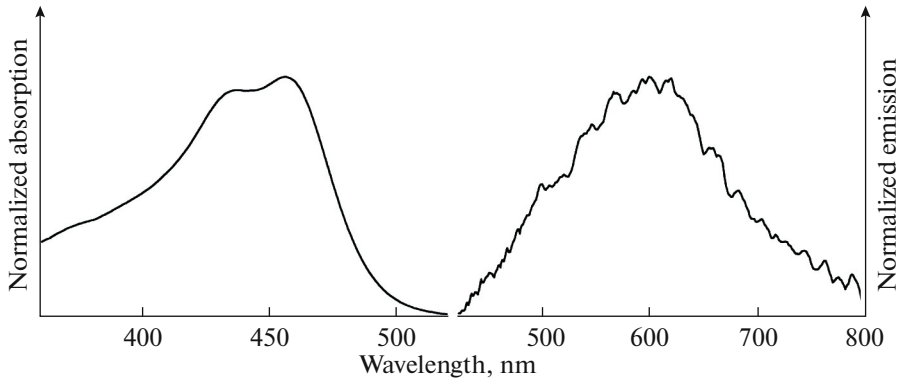


Fig. 1. The absorption and emission spectra of the compound (**V**) in acetonitrile.

zylidene moiety, the next step was its protection with a triisopropylsilyl group. The resulting compound (**II**) was methylated with methyl iodide to give the product (**III**). After this, the Sonogashira reaction was carried out, as a result of which the compound (**IV**) with an acetylene fragment in the structure. To remove the triisopropylsilyl protection compound (**IV**) was treated with tetrabutylammonium fluoride. As a result, we obtained a new compound (**V**) with a good exit.

Next, the optical properties of the new compound (**V**) in acetonitrile (Fig. 1). It was found that the emission of fluorescence of compound (**V**) is small, which is consistent with previously obtained data for other Kaede protein chromophore analogs [6]. The maximum absorption (457 nm) and emission (approximately 605 nm) of the compound (**V**) are shifted to a much longer wavelength region compared with the classical chromophore GFP (Z)-5-(4-hydroxybenzylidene)-2,3-dimethyl-3,5-dihydro-4*H*-imidazole-4-one (absorption and emission maxima 368 nm and 438 nm, respectively) [8]. Also noteworthy is the fact that the Stokes shift of the new acetylene derivative is almost 150 nm, which makes the dye obtained promising for multicolor labeling of biological samples.

Thus, we were the first to develop an approach to the synthesis of acetylene analogs of the Kaede protein chromophore using compound (**V**). The absorption and emission maxima of this compound lie in the longer wavelength region than that of the analogous GFP chromophore due to the presence of a conjugated acetylene system. This fact, as well as the low fluorescence of this compound, suggests that the new derivative (**V**) or its analogs may turn out to be promising fluorogenic dyes.

EXPERIMENTAL

NMR spectra (δ ppm, *J* Hz) are registered on the Bruker Avance III device (700 MHz, United States) in DMSO-d_6 (internal standard—Me_4Si). UV and visible absorption spectra were recorded on a Varian Cary 100 spectrophotometer Bio (United States). Fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorometer (United States). Melting points were determined on an SMP 30 device (Great Britain) and were not corrected. High-resolution mass spectra were recorded on a Bruker micrOTOF II instrument, electrospray ionization.

(Z)-5-(4-Hydroxybenzylidene)-2-thiooxoimidazolin-4-one (I). 4-Hydroxybenzaldehyde (793 mg, 6.5 mmol), 2-thiooxoimidazolin-4-one (580 mg, 5.0 mmol) and sodium acetate (1.76 g, 21.5 mmol) were dissolved in 15 mL of glacial acetic acid, the resulting mixture was boiled for 3 h and cooled to room temperature. With vigorous stirring, 50 mL of water was added, the precipitate formed filtered out and washed with water (20 mL) and diethyl ether (10 mL), and then dried in vacuum. Yellow powder (935 mg, 85%), mp 280°C with decomposition; ^{1}H NMR: 6.43 (s, 1 H), 6.81 (d, J_2 8.6, 2 H), 7.63 (d, J_2 8.6, 2 H), 10.02 (broad s., 1 H), 11.95 (broad s., 1 H), 12.22 (broad s., 1 H).

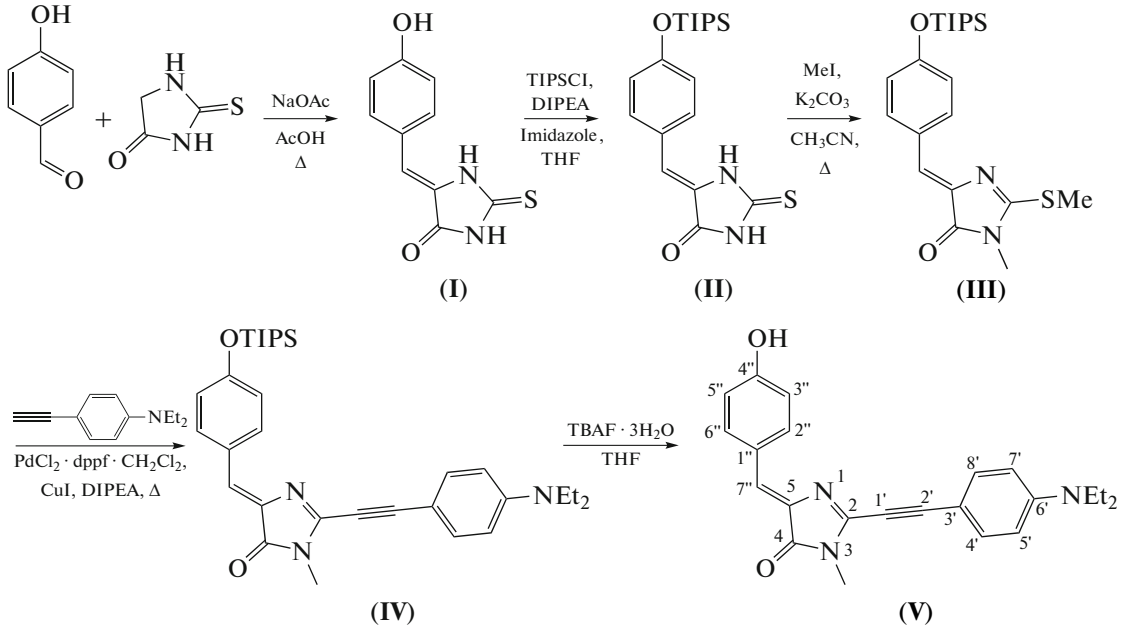
(Z)-2-Thioxo-5-(4-((triisopropylsilyl)oxy)benzylidene) imidazolidin-4-one (II). (Z)-5-(4-Hydroxybenzylidene)-2-thiooxoimidazolin-4-one (**I**, 1.10 g, 5.0 mmol), triisopropylsilyl chloride (1.06 g, 5.5 mmol), DIPEA (0.78 g, 6.0 mmol) and imidazole (20 mg) were dissolved in THF (50 mL), and then stirred overnight. The resulting solution was evaporated, the residue was dissolved in ethyl acetate (200 mL), washed with saturated sodium chloride solution (3 × 50 mL), dried over anhydrous Na_2SO_4 and evaporated. The product was purified by flash chromatography (eluent—chloroform). Yellow powder (1.48 g, 79%), mp 275°C with decomposition; ^{1}H NMR: 1.08 (d, J_2 7.4, 18 H), 1.25–1.31 (sept., J_2 7.4, 3 H), 6.45 (s, 1 H), 6.89 (d, J_2 8.6, 2 H), 7.68 (d, J_2 8.6, 2 H), 12.03 (broad s., 1 H), 12.28 (broad s., 1 H); ^{13}C NMR: 11.9, 17.5, 111.6, 119.8, 125.4, 126.1, 132.0, 156.5, 165.6, 178.6; HRMS (ESI), *m/z*: found *M*, 377.1711; calculated for $C_{19}H_{29th}N_2O_2SSi^+$, $[M + H]^+$ 377.1714.

(Z)-3-Methyl-2-(methylthio)-5-(4-((triisopropylsilyl)oxy)benzylidene)-3,5-dihydro-4H-imidazol-4-one (III). (Z)-2-Thiooxo-5-(4-((triisopropylsilyl)oxy)-benzylidene)imidazolidin-4-one (**II**, 1.13 g, 3.0 mmol), iodomethane (1.70 g, 12.0 mmol) and potash (1.66 g,

12.0 mmol) were mixed in acetonitrile (30 mL) and boiled for 8 h. The reaction mixture was cooled, filtered, the solution was evaporated three quarters and ethyl acetate (150 mL) was added. The resulting solution was washed, saturated sodium chloride solution (3 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated. The product was purified by flash chromatography (eluent—chloroform). Yellow powder (820 mg, 69%), mp 160–163°C; ^{1}H NMR: 1.07 (d, J_2 7.3, 18 H), 1.27 (sept., J_2 7.3, 3 H), 2.72 (s, 3 H), 3.08 (s, 3 H), 6.83 (s, 1 H), 6.94 (d, J_2 8.7, 2 H), 8.16 (d, J 8.8, 2 H); ^{13}C NMR: 12.1, 12.5, 17.7, 26.3, 120.0, 122.1, 126.5, 133.8, 136.7, 157.1, 161.7, 170.9; HRMS (ESI), *m/z*: found *M*, 405.2022; calculated for $C_{21}H_{33}N_2O_2SSi^+$, $[M + H]^+$ 405.2027.

(Z)-2-((4-(Diethylamino)phenyl)ethynyl)-3-methyl-5-(4-((triisopropylsilyl)oxy) benzylidene)-3,5-dihydro-4H-imidazol-4-one (IV). (Z)-3-Methyl-2-(methylthio)-5-(4-((triisopropylsilyl)oxy)benzylidene)-3,5-dihydro-4H-imidazol-4-one (**III**, 406 mg, 1 mmol), copper(I)iodide (190 mg, 1 mmol) and a complex (1,1'-bis(diphenylphosphino)ferrocene of palladium(II)chloride with dichloromethane (41 mg, 0.05 mmol). The flask was evacuated, filled with argon, DIPEA (2.58 g, 20 mmol) and *N*,*N*-diethyl-4-ethynylaniline (340 mg, 2 mmol) were added in a stream of argon. The reaction mixture was kept at a temperature of 100°C for 12 hours, then ethyl acetate (100 mL) was added and washed with saturated sodium chloride solution (3 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The product was purified by flash chromatography (eluent was a 1 : 5 mixture of ethyl acetate and hexane). Dark powder (227 mg, 43%), mp 166–168°C; the product was partially (up to 20%) contaminated (**V**) and used in the next stage without complete purification. ^{1}H NMR: 1.08 (d, J_2 7.4, 18 H), 1.13 (sept., J_2 7.1, 6 H), 1.30 (t, J_2 7.6, 1 H), 3.22 (s, 3 H), 3.42 (q, J_2 7.1, 4 H), 6.75 (d, J_2 9.0, 2 H), 6.97 (d, J_2 8.8, 2 H), 7.02 (s, 1 H), 7.52 (d, J_2 9.0, 2 H), 8.16 (d, J_2 8.6, 2 H); HRMS (ESI), *m/z*: found *M*, 530.3198; calculated for $C_{32}H_{44}N_3O_2Si^+$, $[M + H]^+$ 530.3197.

(Z)-2-((4-(Diethylamino)phenyl)ethynyl)-5-(4-hydroxybenzylidene)-3-methyl-3,5-dihydro-4H-imidazol-4-one (V). (Z)-2-((4-(Diethylamino)phenyl)-ethynyl)-3-methyl-5-(4-((triisopropylsilyl)oxy)benzylidene)-3,5-dihydro-4*H*-imidazol-4-one (**IV**, 159 mg, 0.3 mmol) was dissolved in THF (4 mL), tetrabutylammonium trihydrate fluoride (474 mg, 1.5 mmol) was added, after which the resulting solution was stirred for 1 h. Ethyl acetate (100 mL) was added and washed with saturated sodium chloride solution (10 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The product was purified by flash chromatography (eluent—a mixture of chloroform and ethanol, 50 : 1). Dark red powder (64 mg, 57%), mp 188–190°C; ^{1}H NMR: 1.12 (t, J_2 7.0, 6 H, $NCH_2\underline{CH_3}$), 3.21 (s, 3 H, NCH_3), 3.41 (sq. J_2 6.9, 4 H, $N\underline{CH_2}CH_3$), 6.73 (d, J_2 9.0, 2 H, H5', H7'), 6.87 (d, J_2 8.8, 2 H, H3", H5"), 6.99 (s, 1 H, H7"), 7.51 (d, J_2 8.8, 2 H, H4', H8'), 8.10 (d, J_2 8.6, 2 H, H2", H6 "), 10.24 (br s, 1 H, OH); ^{13}C-NMR: 12.3 ($NCH_2\underline{CH_3}$), 27.0 (NCH_3), 43.8 ($N\underline{CH_2}CH_3$), 78.4 (C1'), 102.6 (C2'), 103.6 (C3'), 111.3 (C5', C7'), 115.9 (C3", C5"), 125.4 (C7"), 127.4 (C1"), 134.2 (C2", C6"), 134.6 (C4', C8'), 136.4 (C2), 146.2 (C5), 149.1 (C6'), 160.1 (C4"), 168.5 (C4); HRMS (ESI), *m/z*: found *M*, 374.1864; calculated for $C_{23}H_{24}N_3O_2^+$, $[M + H]^+$ 374.1863.



Scheme 1. Compound synthesis scheme (**V**).

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans and animals as research objects.

Conflict of Interests

The authors declare they have no conflict of interest.

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