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Reagents for labeling with pH-independent fluorescein-based tags

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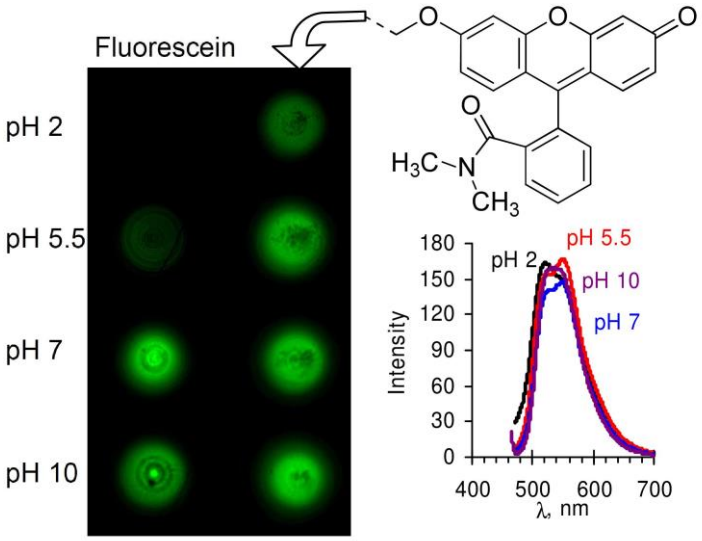
Abstract

Three new fluorescein based dyes were obtained as reagents for tagging of compounds which contain amine, imidazole or chloroanhydride groups. The proposed substances were tested with three polymers which are promising as gene delivery agents. The new compounds as opposed to derivatives of fluorescein with free 2'-carboxyl group remain fluorescent in acidic media. Such behavior of new tags is useful in study of biological systems, e.g. acidic lysosomes which can capture various substances including gene delivery agents.



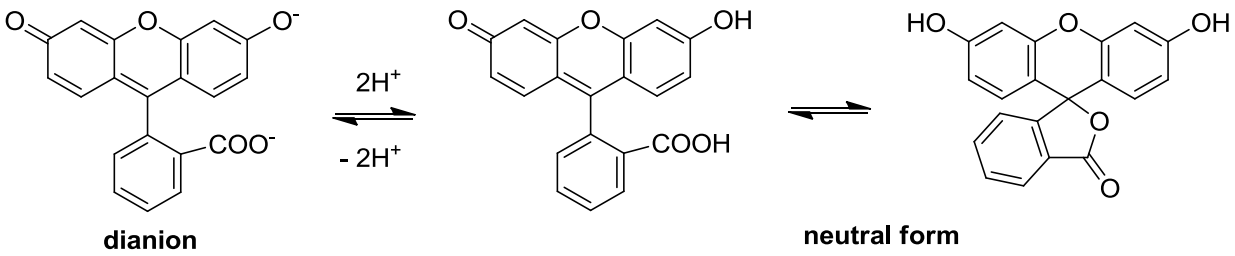
Keywords: Fluorescein, *N,N*-dimethylamide, pH-independent fluorescence, tagged polymers

Introduction

Reactive fluorescein derivatives have found wide applications in labeling different species, especially proteins, nucleic acids, a variety of tissues and synthetic polymers as well.[1-4] For instance, study of gene delivery in the gene engineering or therapy requires tagging of the delivered nucleic acid and/or the delivery agent.[5-7] The use of fluorescence tagged compounds allows to monitor penetration of the nucleic acid - delivery agent complex into cell and to look on the further fate of these substances. The full-fledged study includes staining some cell organelles, e.g. nucleus which allows to visualize the whole gene delivery process with fluorescence microscopy. Thus, a set of fluorescence tags of different colors is required. Fluorescein derivatives are highly popular in this area due to near 100% quantum yield and closeness of the excitation maximum to pass band of widespread microscope filters.

There are several conventional reagents for introducing fluorescein moieties into organic molecules: NHS-fluorescein and isothiocyanate fluorescein (FITC),[8] 5/4'-(aminomethyl)fluoresceins and 5-(aminoacetamido)fluorescein,[9] 5-iodoacetamidofluorescein and fluorescein maleimide,[8,10] 5-carboxyfluorescein, propargylamide (5-FAM Alkyne),[11] 6-FAM phosphoramidite.[12] Chemical structures of the mentioned compounds can be find in Supplementary Materials, Scheme 1S.

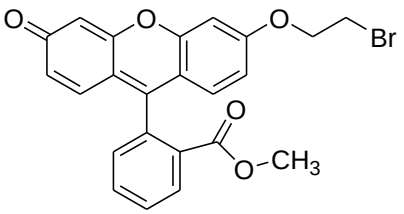
All of the above mentioned commercially available dyes have an important feature: they are subject to tautomerism in aqueous medium analogously to the parent compound. Depending on pH of the aqueous solution, the fluorescein molecule may exist in various forms.[13,14] The most fluorescently active form in biologically relevant media (pH 4-10) is the dianion (Scheme 1).



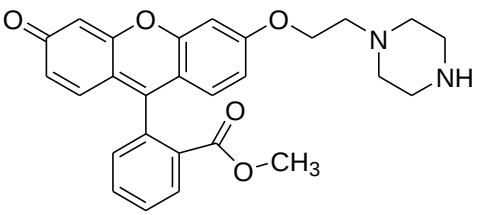
Scheme 1. Dianion and neutral forms of fluorescein.

This form exists at pH above 8 and shows quantum yield 93%. pH decrease results in the formation of monoanion (quantum yield 36%) and neutral molecule which is almost fluorescence inactive due to formation of the lactone form (Scheme 1).

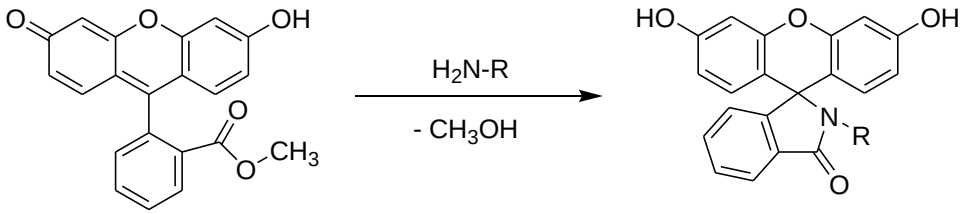
So, at neutral and acidic pH values, emission of the fluorescein tagged compounds drastically decreases and this can lead to erroneous conclusions: decrease or absence of fluorescence can be caused not only by the absence of tagged compound but also by the presence of this compound in acidic vesicles, e.g. lysosomes. A reasonable way to maintain the quantum yield at acidic pH seems to be in preventing the lactone ring closure, in particular converting 2'-carboxyl group into an ester (Scheme 2).[15]



6-oxy-(bromoethyl)-9-
(2'-methoxycarbonyl) fluorescein



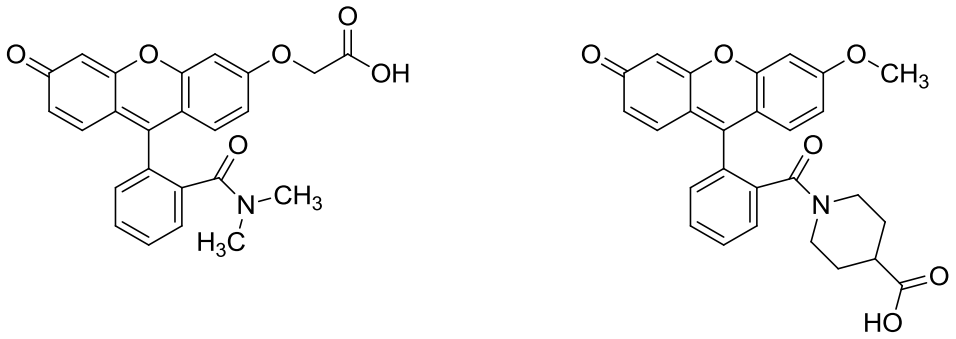
6-oxy-(ethylpiperazine)-9-
(2'-methoxycarbonyl) fluorescein



methyl ester spirolactam

Scheme 2. Structures of the fluorescein derivatives with 2'-carboxyl group locked up in an ester group.

Unfortunately, it was found that fluorescein methyl ester reacts rapidly with a variety of primary amines at ambient temperature giving almost colorless spirolactams which did not produce any fluorescent species when exposed to a strongly basic environment (aqueous 0.1 N NaOH).[16] A similar spirolactam was synthesized with ethylenediamine and was reported in an earlier work (Scheme 2).[17] The amine moieties are widespread among biomolecules and gene delivery agents, so the use of such esters can also result in false conclusions. Substitution with *N,N*-dimethylamide for the ester allows to overcome this difficulty (Scheme 3).[18]



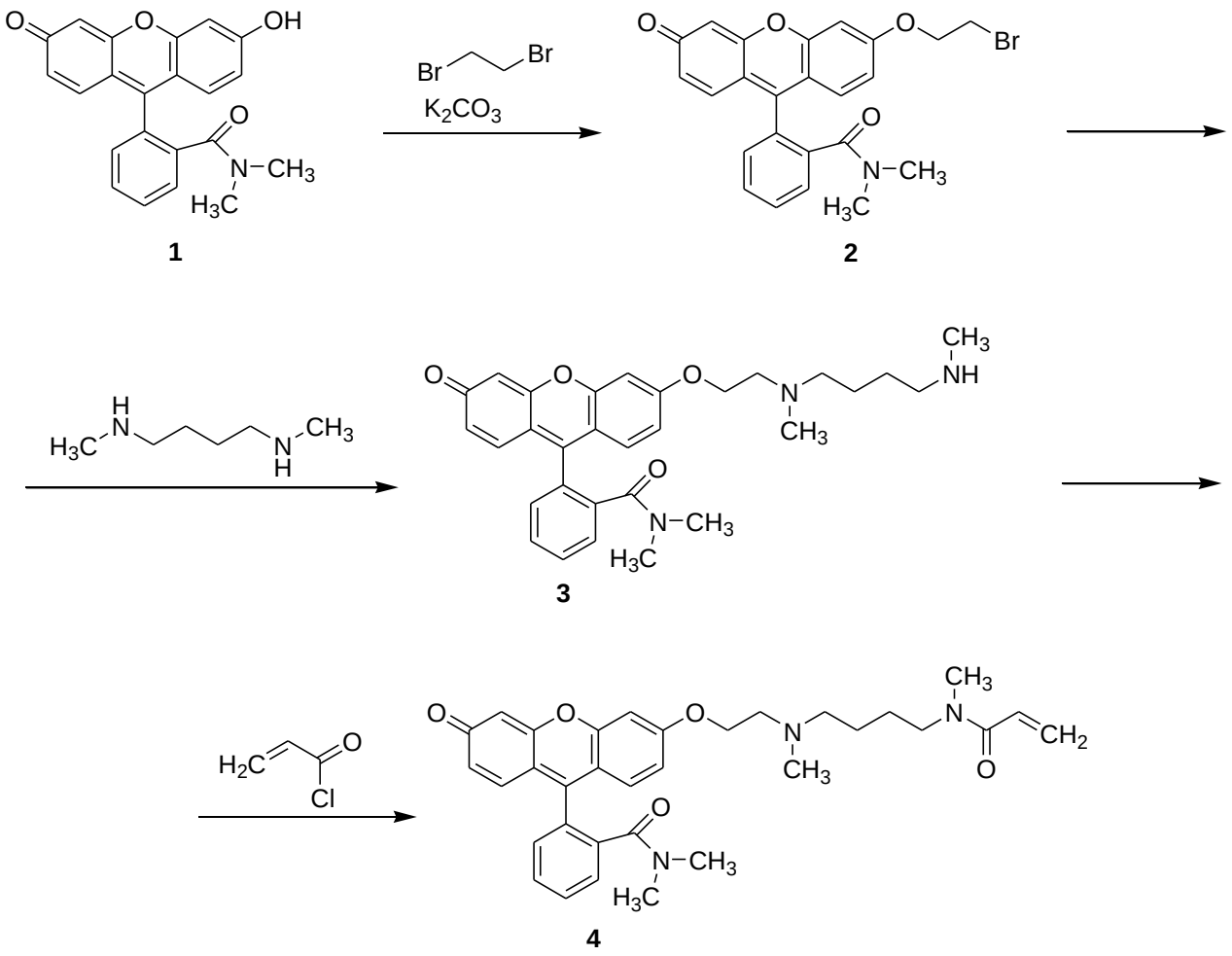
Scheme 3. Structures of the fluorescein derivatives with 2'-carboxyl group locked up in an amide group.

Here we report three novel fluorescein based dyes with 2'-carboxyl group locked up in a *N,N*-dimethylamide form. These compounds contain active bromine, secondary amine or acrylamide groups which allow tagging of potential gene delivery agents and other substances.

Results and Discussion

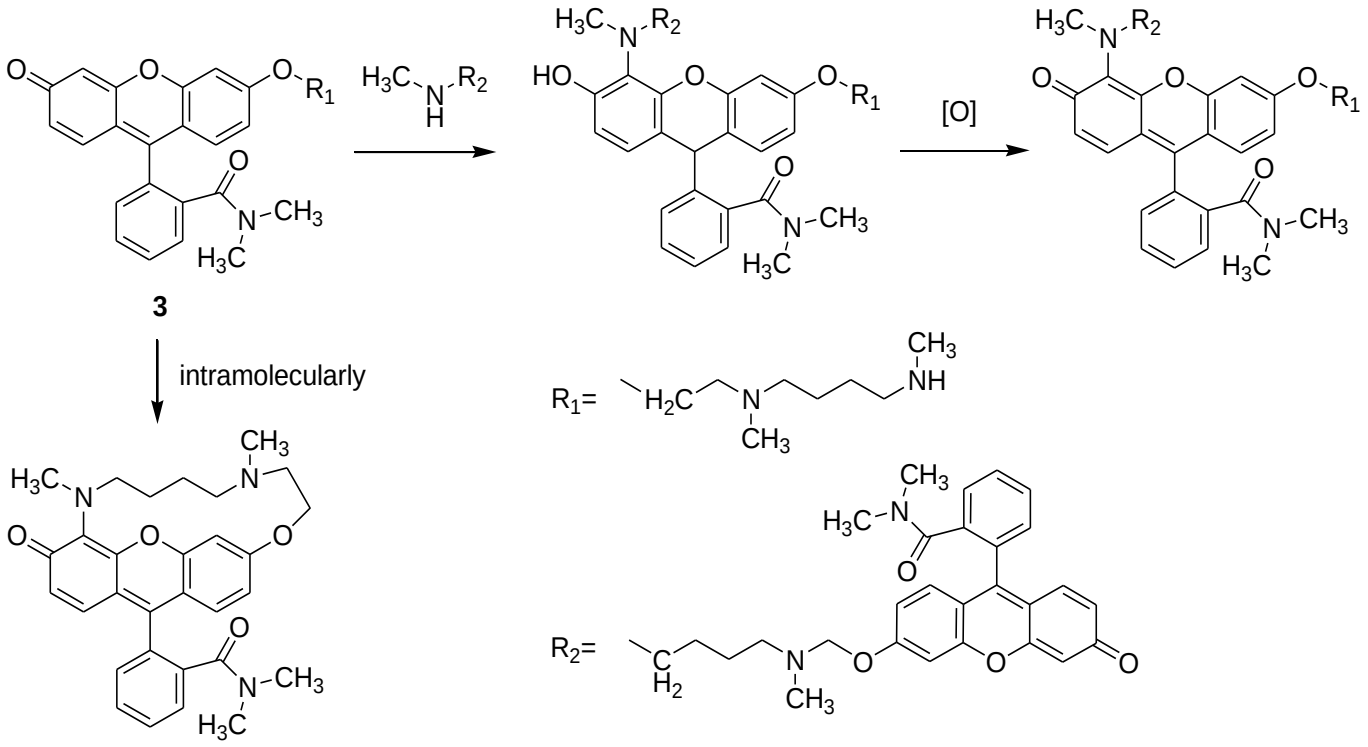
Synthesis of novel fluorescein based dyes

The starting compound was 2-(6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)-*N,N*-dimethyl-benzamide (**1**) which was prepared following a protocol reported earlier.[18] Reaction of **1** with 1,2-dibromobutane gives rise to 2-(6-(2-bromoethoxy)-3-oxo-3*H*-xanthen-9-yl)-*N,N*-dimethyl-benzamide (**2**) (Scheme 4). **2** Reacts with *N,N'*-dimethyl-1,4-butanediamine giving **3.** Acrylamide derivative **4** was obtained from **3** by the reaction with acryloyl chloride.



Scheme 4. Synthesis of new fluorescein compounds.

The new compounds were purified with flash chromatography, their structure was confirmed with ESI-MS and NMR data. It should be emphasized that compound **3** is unstable and must be stored in a freezer, preferably in a salt form such as hydrochloride. The observed instability of **3** may by attributed to a reaction between the quinone and secondary amine moiety of the molecule. Similar interaction was thoroughly studied in an earlier work.[19,20] In case of *p*-benzoquinone and dialkylamines the process took place even at 0°C. Moreover, p-benzoquinone was proposed as a TLC derivatization reagent for 2-(methylamino)ethanol and for the analysis of other primary and secondary amines.[21] On this basis a speculative reaction scheme may be proposed (Scheme 5).



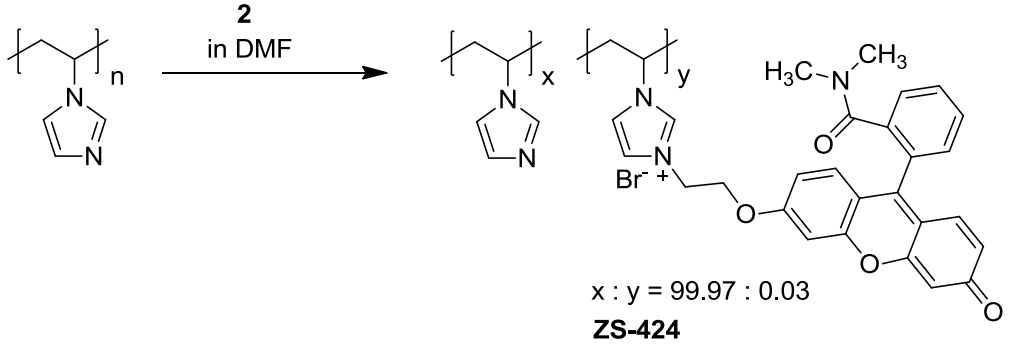
Scheme 5. Speculative degradation way of fluorescein amino derivative **3**.

The amine group is added to the quinone fragment forming the corresponding phenol. Following oxidation with atmospheric oxygen, the molecule recovers the fluorescein backbone. It can be speculated that in practice the process seems to be more complicated giving, for instance, products of the double addition or intramolecular reaction which is probably inherent to compound **3**.

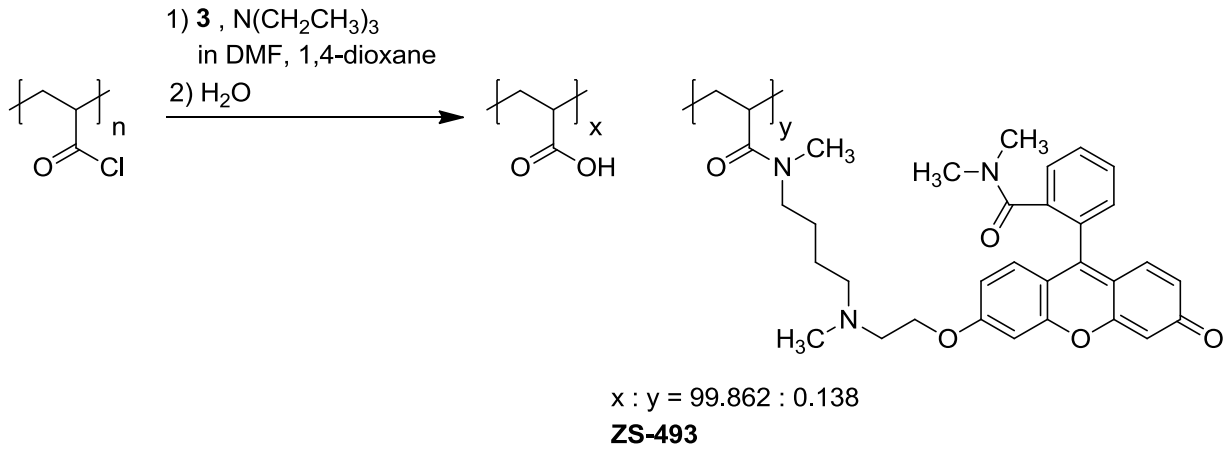
Labeling of water-soluble polymers

The new fluorescent reagents **2**-**4** were used for labeling polymers studied previously as gene delivery agents: poly(vinyl amine) (PVA),[22,23] polyethylenimine (PEI),[24-27] poly(1-vinylimidazole) (PVI)[28-30] and copolymer ZS-247[31] which contains grafted polyamine chains. Poly (acryloyl chloride) (PAC) is a convenient polymer for synthesis of various functional polymers by the reaction with amine containing substances.[32] Interaction between PAC and new substance **3** was expected to give tagged poly (acrylic acid) (PAA).

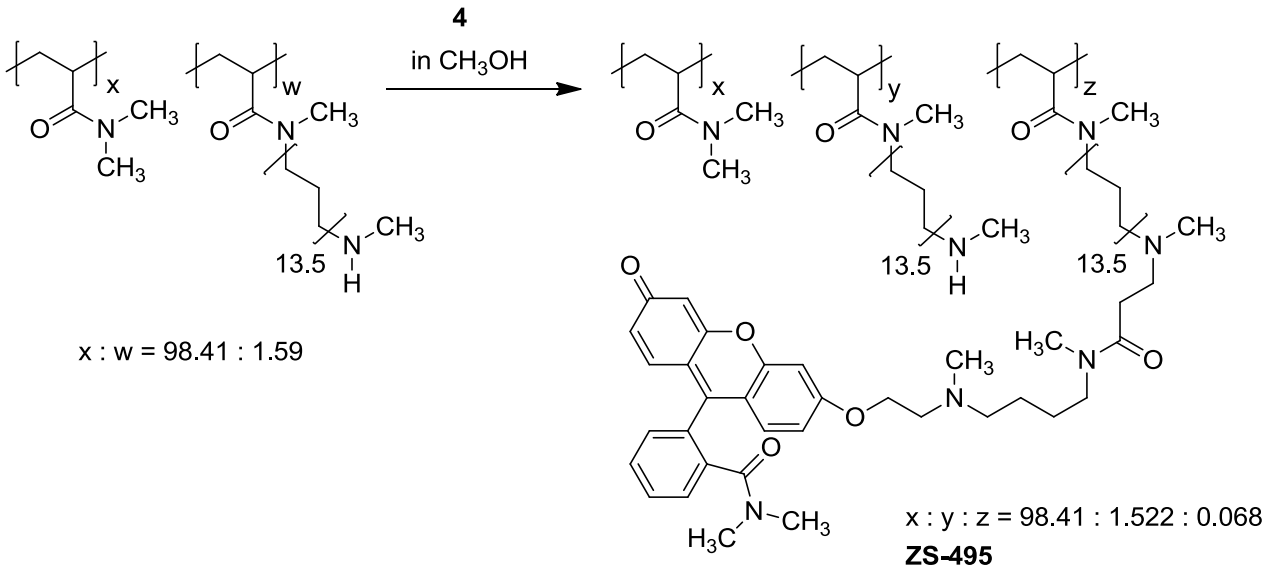
The reactions with PVI, ZS-247 and PAC give rise to soluble tagged polymers (Schemes 6-8).



Scheme 6. The reaction of **2** with PVI.



Scheme 7. The reaction of **3** with PAC.



Scheme 8. The reaction of **4** with ZS-247.

In the case of PVA and PEI we have obtained insoluble products mostly. This is explainable with the above mentioned reaction between primary or secondary amines with quinone type structures. The instability of the substance **3** and fluorescein-containing PVA and PEI emphasizes the necessity to be cautious when applying fluorescein and similar structures ((4'/5-aminomethyl)fluorescein, 5-(aminoacetamido)fluorescein, 5 and 6-isomers of FAM amine) in physicochemical or biochemical investigations.

Spectral properties of the new fluorescent compounds

Absorption, excitation and emission spectra of the fluorescein, Olig-flu, substances **2** and **4**, polymers ZS-424, ZS-493 and ZS-495 are presented in Figures S1-S9, Supplementary Materials. As expected, excitation and emission of fluorescein and Olig-flu significantly decrease at pH 5.5 and are very weak at pH 2 (Figures1 and S5 in Supplementary Materials). Substances **2**, **4** and tagged polymers show considerable excitation in the acidic region (Figures S5-S7, Supplementary Materials). Most of new compounds alter the shape of the absorption and excitation spectra at pH 2: a band at 440 nm appears instead of the band at 460-510 nm (Figures S5-S9).

The band at 440 nm in acidic medium is attributed to protonation of the quinone oxygen.[13,14] Only the ZS-424 polymer displays an unchanged absorption and excitation spectra at pH 2. The decreased ability of the quinone oxygen to protonation possibly resulted from the presence of positively charged imidazole ring near fluorescein. Quantum-chemical simulation (Figure 2) confirms this hypothesis: the most stable conformation (structure A) contains closely located positive charged imidazole and triarylmethane rings which allow some interactions between the π-systems. This interaction must decrease donor ability of the quinone oxygen. Geometry optimization after protonation of the quinone oxygen results in distancing of the charged cycles (structure B).

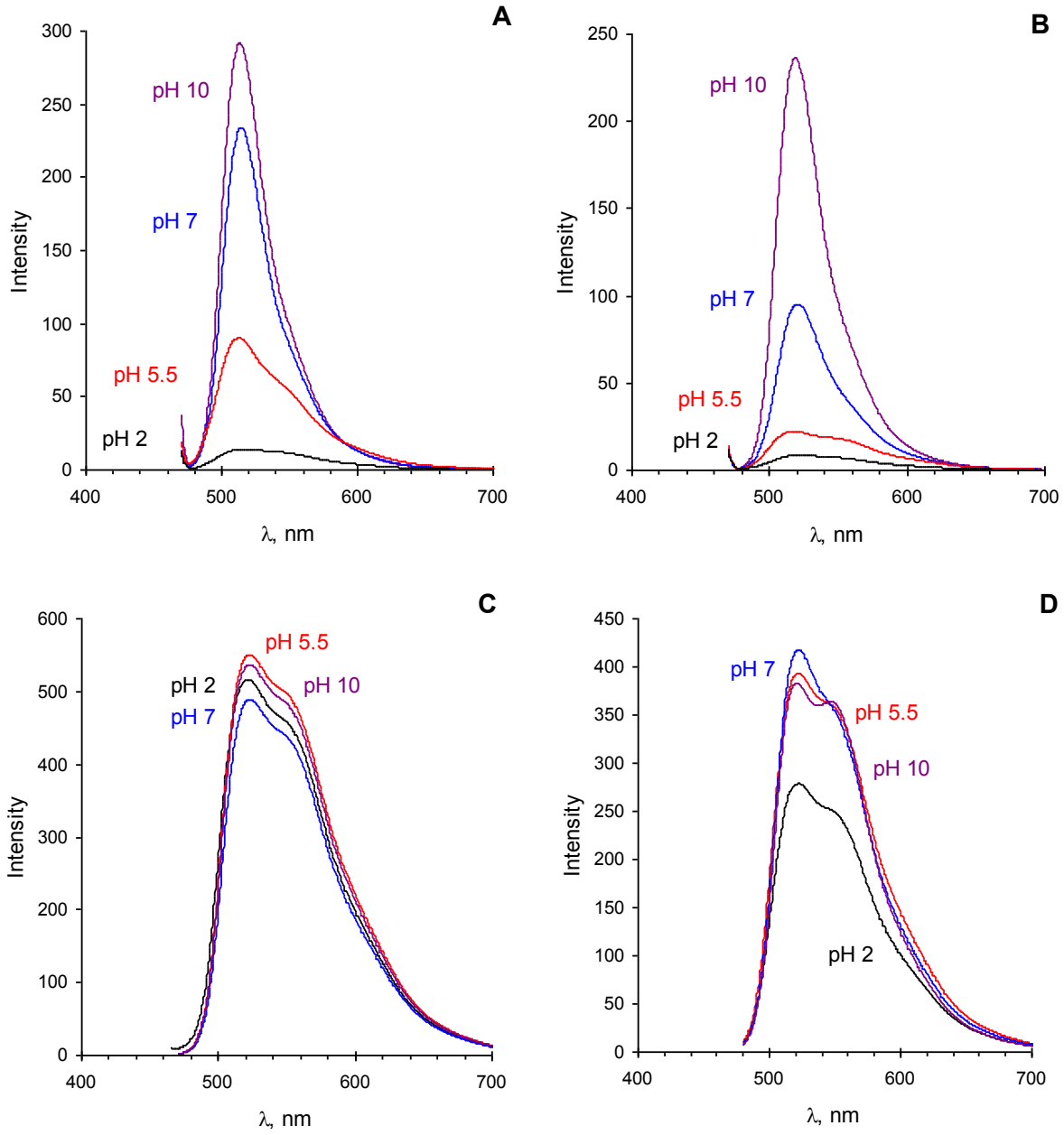


Figure 1. Emission spectra of fluorescein (A), Olig-Flu (B), new dyes **2** (C) and **4** (D) in different buffer solutions at 460 nm excitation. Concentrations 0.5 μM for A and B, 2.5 μM for C, and 5 μM for D.

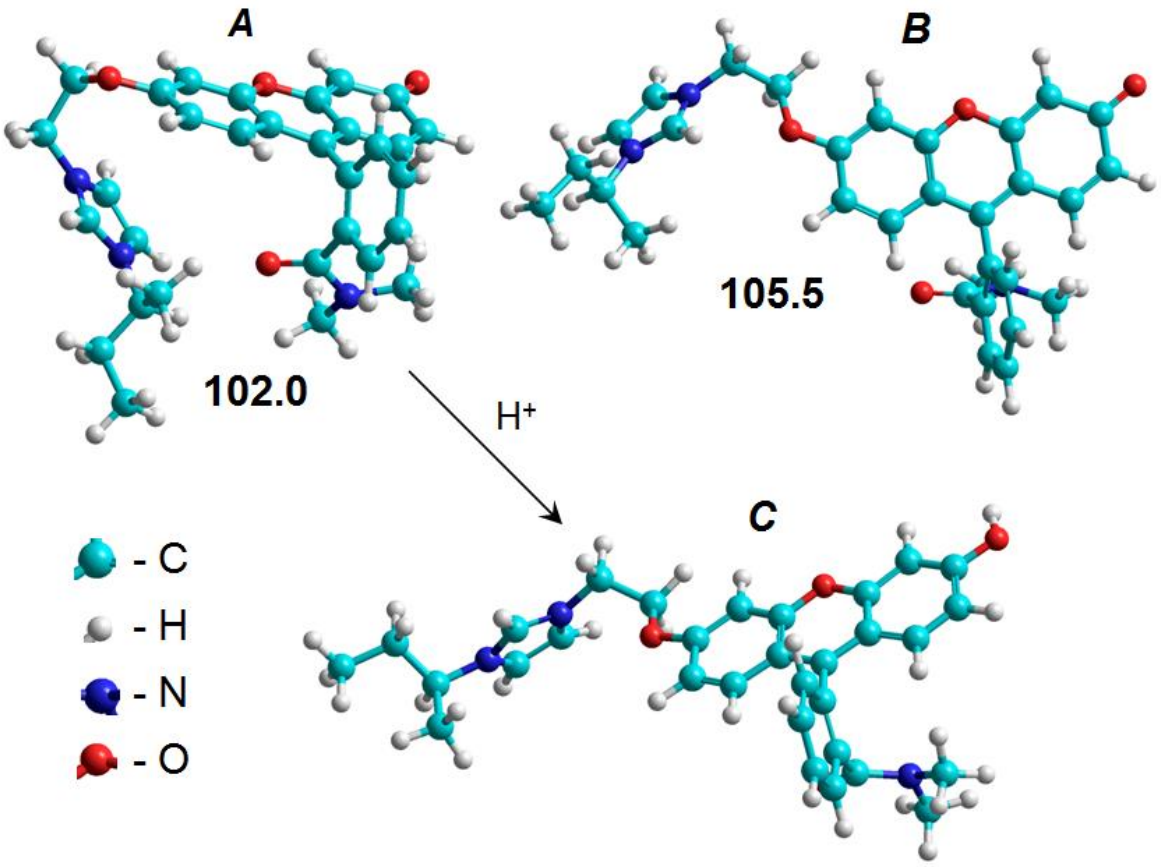


Figure 2. Optimized structures of imidazole fragment tagged with **2** (A and B) and compound A protonated on the quinone oxygen (C). Numbers near structures - heat of formation, kcal/mol.

Fluorescence from aqueous solutions of new dye **4** and tagged polymers was studied with epifluorescent microscope and compared with fluorescein and fluorescein-tagged DNA oligonucleotides. The obtained images (Figure 3) show low emission for dye **4** and polymer ZS-493 at pH 2 which is explained by the changes in the excitation spectra at pH 2. The microscope filter provides excitant light in 450-490 nm range and shifting of the excitation band from this region to lower wavelength results in decrease of the emission in spite of high emission under excitation with near monochromatic light at 455-457 nm (Figures 4 and 5). Emission due to excitation at 490 nm (Figures S8 and S9, Supplementary Materials) is more comparable with the microscopy data.

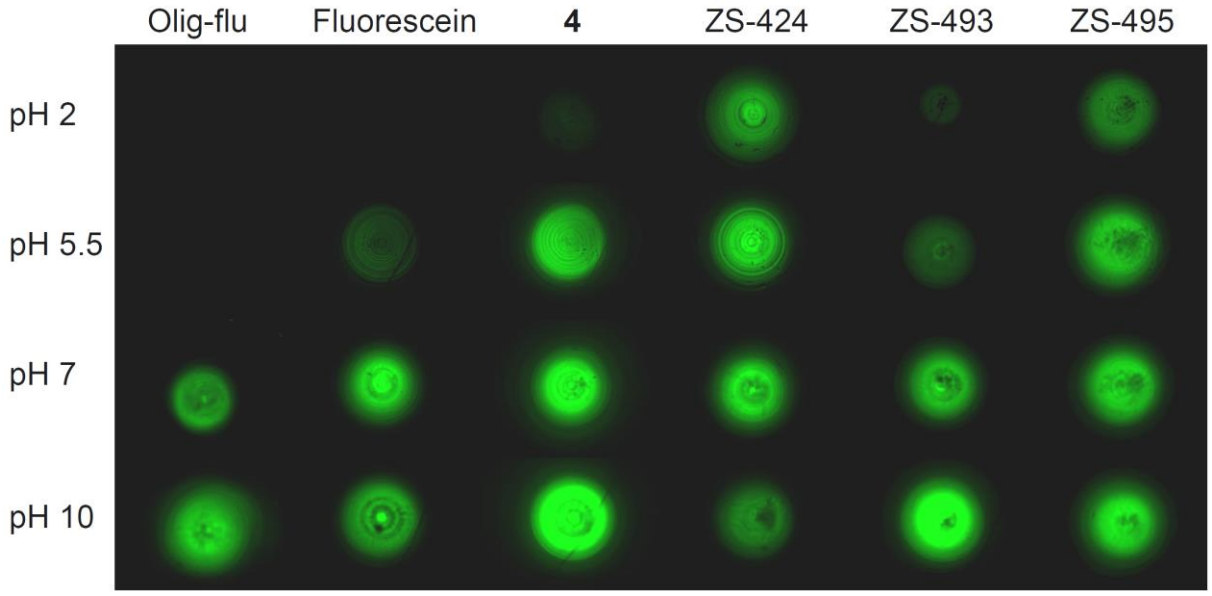


Figure 3. Fluorescent images (excitation 450-490 nm) of the dyes solutions at different pH. Concentrations: Olig-flu – 1 µM, fluorescein - 4 µM, **4** – 50 µM, ZS-424 – 20 mM, ZS-493 – 10 mM, ZS-495 – 1 mg/mL. Images from the each sample at various pH values were obtained with identical microscope and camera adjustment.

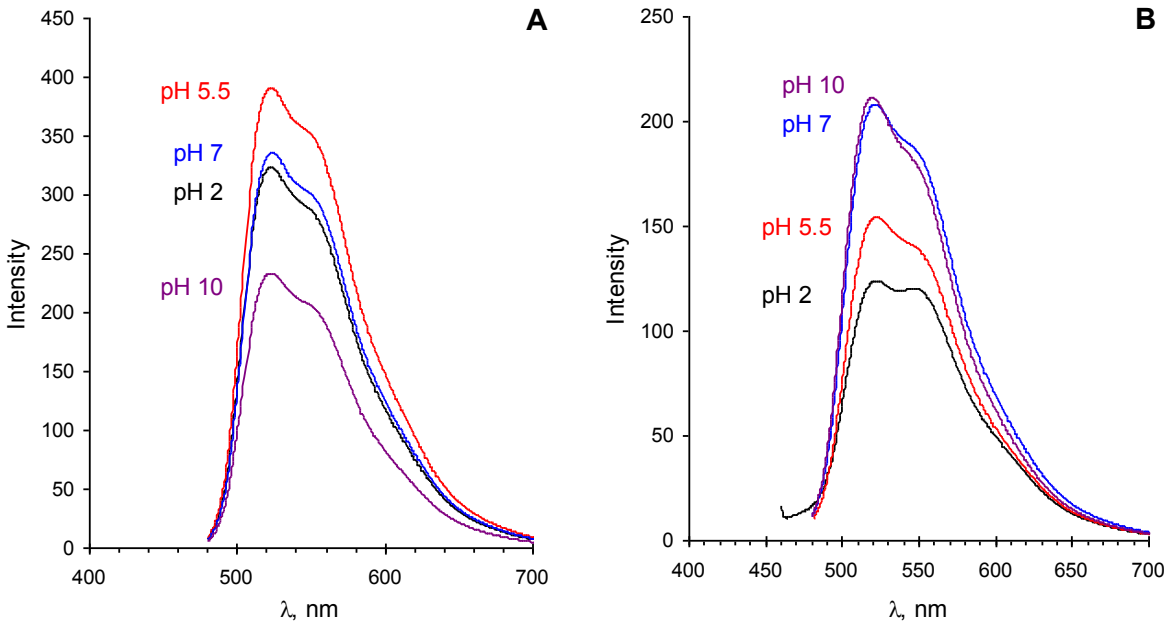


Figure 4. Emission spectra of polymers ZS-424 (A) and ZS-493 (B) in different buffer solutions at 457 and 456 nm excitation respectively. Concentrations 0.47 mg/mL for A and 0.36 mg/mL for B.

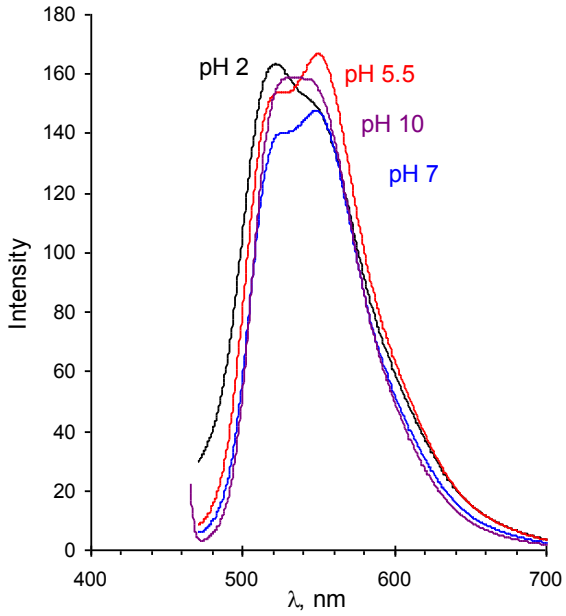


Figure. 5. Emission spectra of ZS-495 in different buffer solutions at 455 nm excitation. Concentration 1 mg/mL.

Conclusions

We have synthesized three new fluorescein based dyes which can be applied for tagging of compounds which contain amine, imidazole or chloroanhydride groups. The proposed reagents were tested with three polymers which are promising substances for gene delivery. The new compounds as opposed to derivatives of fluorescein with free 2'-carboxyl group remain fluorescent in acidic media. Such behavior of new tags is useful in study of biological systems, e.g. acidic lysosomes which can capture various substances including gene delivery agents. The shape of the absorption and excitation spectra of the tags changes at pH 2 which complicates the work with traditional fluorescence filters of microscopes but the emission can be observed with monochromatic light near 460 nm, e.g. applying confocal microscopy.

Experimental Section

General. Mass spectrometric analysis was performed on an Agilent 6210 TOF (time-of-flight) LC/MS (liquid chromatography/mass spectrometry) System. Samples were dissolved in deionized water with 0.1% (v/v) of HFBA. Water and acetonitrile with 0.1% (v/v) HFBA were used as eluting solvents A and B, respectively. Isocratic elution (A solvent 90%) was applied. The flow rate of the mobile phase was set at 0.2 mL/min, whereas the injection volume of sample solution was 10-20 µL. The conditions for TOF MS were as follows: the mass range was *m/z* 70 to 2000, and scan time was 1 s with an interscan delay of 0.1 s; mass spectra were recorded under electrospray ionization (ESI)+, V mode, centroid, normal dynamic range, capillary voltage 3500 V, desolvation temperature 325°C, and nitrogen flow 5 L/min. Under these conditions, peaks of the amine derivatives of fluorescein appeared as protonated ions. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra

(Figures S10 and S11, Supplementary Materials) were recorded using a DPX 400 Bruker spectrometer (400.13 and 100.62 MHz respectively) in $CDCl_3$. UV–Vis absorption measurements were carried out using spectrophotometer Perkin Elmer Lambda 950 and a Cintra 20 UV/VIS spectrophotometer (Sangji, Korea). Photoluminescence spectra were recorded at 25°C using Perkin-Elmer LS-55 instrument. For absorption and fluorescence measurements the path length of the quartz cuvette was 10 mm. The fluorescence quantum yields (Q) were measured using solutions of fluorescein in 0.1 M NaOH as reference (Q_R = 0.95). A solution of HCl (10 mM, pH 2), 50 mM solutions of CH_3COOH/NaOH (pH 5.5), 1-methylimidazole (pH 7), NH_4OH/HCl (pH 10) were used as buffer solutions. A Motic AE-31T microscope equipped with a fluorescence attachment and Moticam Pro 205A camera was used for visual observation with 450-490 nm excitation. Dyes and polymer solutions at different pH were placed in microbiological plate (aliquot 50 µL). Electrophoresis experiments were performed in 1% agarose gel with a Mini-Sub (7 × 10 cm) Cell GT System (Bio-Rad Laboratories, Inc.) with an ELF-4 power supply (DNA-Technology LLC) and TCP-20. LC transilluminator (Vilber Lourmat), operated at 254 nm. The gel running buffer was 40 mM Tris acetate (pH adjusted to pH 7.4) and 1 mM ethylenediaminetetraacetic acid (EDTA). Semi-empirical quantum-chemical calculations (PM3 method) were performed with the HyperChem program.[33]

2,2'-Azobis(2-methylpropionitrile) (AIBN) (Sigma-Aldrich, St. Louis, MO, USA) was recrystallized from ethanol prior to use. 1,4-Dioxane and diethyl ether (ACS reagent grade, Sigma-Aldrich) were distilled over sodium. Dimethylformamide (DMF, 99% Alfa Aesar) was dried with $CuSO_4$, distilled at ca. 9 mm Hg and stored over 3A molecular sieves. N,N-Dimethyl-1,4-butanediamine (98% Alpha Aesar) was distilled at ca. 10 Hg mm. N-vinylimidazole, N-vinylformamide, acryloyl chloride, methanol, dichloromethane, ethyl acetate, acetone, triethylamine (dried over CaH_2), 1,2-dibromobutane were purchased form Sigma-Aldrich and distilled prior to use. Fluorescein disodium salt hydrate (ABCR GmbH & Co., Karlsruhe, Germany), *N*-hydroxysuccinimide (Sigma-Aldrich), N,N-dicyclohexylcarbodiimide (Sigma-Aldrich), acetonitrile (HPLC Far UV / gradient grade, J.T. Baker), heptafluorobutyric acid (HFBA, Sigma-Aldrich) were used as received without purification. Potassium carbonate, potassium hydroxide, 25% aqueous ammonia solution (reagent grade) were purchased from a local supplier. Silica gel for flash chromatography was of high-purity grade (40-63 µm particle size, Sigma-Aldrich). Sorbfil silica gel TLC plates were purchased from Imid Ltd (Krasnodar, Russian Federation). Nitrocellulose 0.45 µm membrane filters were purchased form Sartorius. Poly(N-vinylimidazole), poly(vinyl amine) and poly(N,N-dimethylacrylamide) grafted with oligo(*N*-methyazetidine) (ZS-247) were synthesized according to an earlier reports[31,34,35] respectively. Fluorescein 3'-tagged DNA oligonucleotide GATCTCATCAGGGTACTCCTT (Olig-flu) was purchased from Evrogen JSC (Russia). 2-(6-Hydroxy-3-oxo-3H-xanthen-9-yl)-*N,N*-dimethyl-benzamide, **1**, was prepared following a previously published protocol.[18]

Synthesis of new dyes

2-(6-(2-Bromoethoxy)-3-oxo-3*H*-xanthen-9-yl)-*N,N*-dimethyl-benzamide (**2**). Powdered K_2CO_3 (0.313 g, 2.26 mmole) and 1,2-dibromoethane (1.596 g, 8.50 mmole) were added to a magnetically stirred suspension of compound **1** (0.257 g, 0.715 mmole) in 3 mL of DMF. After 15 minutes the vessel was immersed into a preheated glycerol bath (62°C) for 3 hours. The mixture was heated at 63°C for 3 h and after cooling to room temperature carefully evacuated with an oil pump to strip off volatiles. The residue was mixed with 10 mL of distilled water, the solid filtered off and washed with water. After drying in a vacuum desiccator over KOH pellets for 12 hours, the product was purified by silica gel (40-60µm) flash chromatography (acetone 100%, R_f≈0.58 or ethyl acetate : methanol=4:1, R_f≈0.54) to yield **2** as deep orange powder (0.227 g, 0.487 mmole, 68%). M.p. 123-126 °C. ^{1}H NMR: 2.78 (s, 3H CH_3-), 2.85 (s, 3H CH_3-), 3.67 (t, 2H -CH_2-), 4.39 (t, 2H -CH_2-), 6.42-7.62 (xanthen and benzene ring). ^{13}C NMR: 28.22 (-CH_2-Br), 34.82 and 39.20 ((CH_3)$_2$-N-), 68.25 (-O-CH_2-),

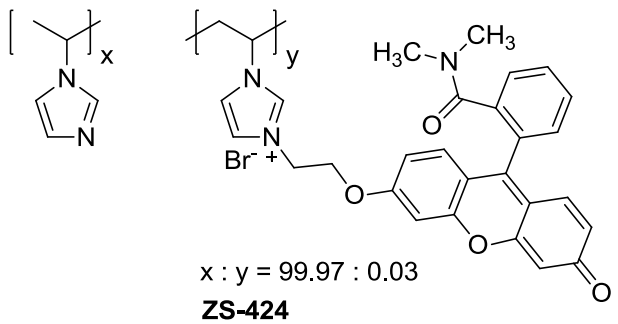
100.89, 105.90 , 113.18, 115.10, 118.31, 127.43, 129.32, 129.68, 130.23, 130.37, 130.47, 131.27, 136.48, 147.98, 154.12 and 158.65 (-C-O-C-), 162.55 (C-O-), 168.62 (N-C=O), 185.56 (C=O).

2-(6-(2-(Methyl(4-(methylamino)butyl)amino)ethoxy)-3-oxo-6*H*-xanthen-9-yl)-*N,N*-dimethyl-benzamide (**3**). To a solution of compound **2** (0.1508 g, 0.323 mmole) in 3 mL of 1,4-dioxane *N,N'*-dimethyl-1,4-butanediamine (0.194 g, 1.67 mmole) was added. The mixture was kept at 60°C under argon atmosphere for 16 hours followed by cooling to room temperature. The bright red-orange solution was separated from precipitated crystalline solid by filtering through a cotton pad and evaporated under vacuum of ca. 10 mm Hg. The sticky residue was triturated with n-hexane 2×6 mL, the extracts were discarded whereas the product was purified by silica gel (40-60 µm) flash chromatography (CH_2Cl_2 : CH_3OH : aq. NH_3 =10:4:0.5, R_f≈0.50) to yield 0.101 g of carmine-red sticky mass (3, 0.201 mmole, 62%). ESI-MS, *m/z* 502.271 $[M+H]^+$, calcd. 502.2706. It should be emphasized that compound **3** is unstable and must be stored in a freezer, preferably in a salt form such as hydrochloride. The issue was noticed during flash chromatography purification. TLC check of the target fraction showed the presence of colored fluorescent stain with R_f about 1, whereas the product had R_f≈0.50. Repeated runs of the purification failed to eliminate this impurity which regenerated every time. Moreover, our HPLC runs demonstrated crucial degradation of the compound even at pH 5.5 in acetate buffer solutions within two hours. Because of this we failed to record non-compromised NMR spectra of **3**. The observed instability of **3** may by attributed to a reaction between the quinone and secondary amine moieties of the molecule.

2-(6-(2-(Methyl(4-(*N*-methylacrylamido)butyl)ethoxy)-3-oxo-6H-xanthen-9-yl)-*N,N*-dimethyl-benzamide (**4**). To a cooled (-10°C) solution of **3** (0.099 g, 0.197 mmole) and triethylamine (0.045 g, 0.445 mmole) in 2.5 mL of CH_2Cl_2 acryloyl chloride (0.039 g, 0.43 mmole) in 0.5 mL of CH_2Cl_2 was added. The mixture was magnetically stirred at ambient temperature for an hour. Then a solution of K_2CO_3 (50%) in distilled water was added and stirring continued for one more hour. The organic layer was separated, dried over anhydrous K_2CO_3 and purified by silica gel (40-60µm) flash chromatography (CH_3OH : AcOEt =3:1, R_f≈0.20) to yield 0.0563 g of **4** (0.101 mmole, 51%). ESI-MS, *m/z* 556.291 $[M+H]^+$, calcd. 556.2811. ^{1}H NMR: 1.48 (2H, -CH_2-), 1.59 (2H, -CH_2-), 2.31 (s, 3H, CH_3-), 2.46 (N-CH_2-), 2.76 (s, 3H CH_3-), 2.81 (2H, -CH_2-), 2.83 (s, 3H CH_3-), 2.97 and 3.03 (s, 3H, CH_3-N), 3.36 and 3.44 (t, 2H, -CH_2-), 4.14 (t, 2H -CH_2-), 5.64 (t, -CH=), 6.27 and 6.31 (d, 4H, CH_2=), 6.40-7.59 (xanthen and benzene ring). ^{13}C NMR: 24.51 (-CH_2-), 26.28 (-CH_2-), 33.53 and 34.93 (N-CH_3), 34.44 and 38.83 ((CH_3)$_2$-N-), 42.30-42.44 (d, N-CH_3), 47.25 and 49.50 (-CH_2-N), 55.48 and 57.18 (-CH_2-N-CH_2-), 66.62 (-O-CH_2-), 100.40 (d), 105.41, 113.09 (d), 114.28 (d), 117.67, 127.06, 127.21, 128.91, 129.15, 129.81, 129.91, 130.05, 131.01, 136.15, 147.82, 153.92 and 158.4 (-C-O-C-), 163.20 (C-O-), 165.88 (N-C=O), 168.29 (N-C=O), 185.15 (C=O).

Polymer tagging with new fluorescent dyes

Poly(*N*-vinylimidazole) fluorescently tagged with compound 2 (ZS-424).

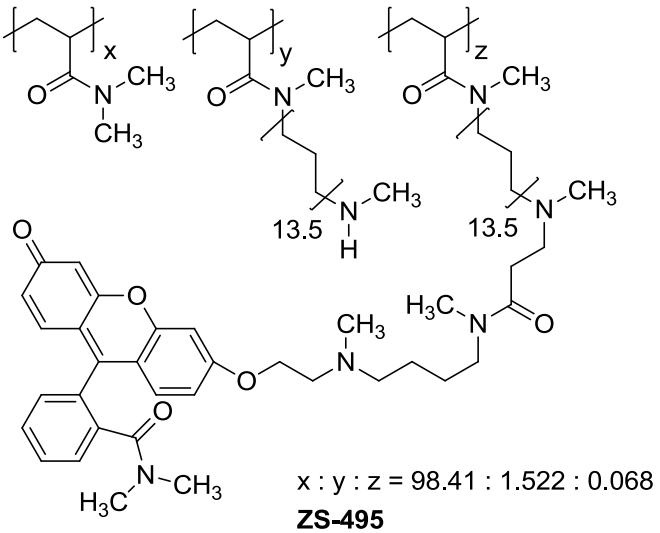


x : y = 99.97 : 0.03

ZS-424

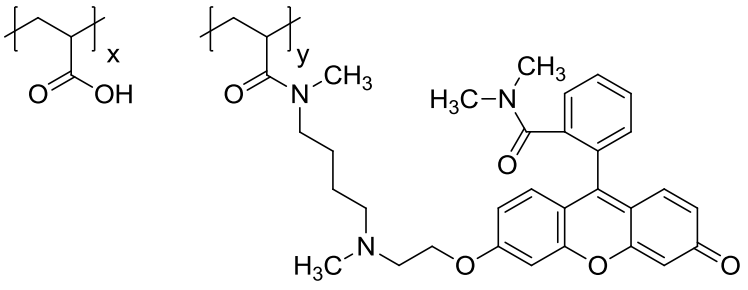
A solution of poly(*N*-vinylimidazole) (97.5 mg, 1.036 mmole) and **4** (2.06 mg, 0.00442 mmole) in 1.8 mL of DMF was heated at 60°C for 15 hours. The polymer was precipitated in 11 mL of acetone, washed with acetone (10 mL × 7) and dried in vacuum of an oil pump, dissolved in distilled water, filtered through a 0.45 μm membrane filter and freeze dried to yield 92 mg of **ZS-424**. The polymer purity was assessed with TLC (silica gel, CH_3OH:CH_2Cl_2=9:16, R_f of **ZS-424** = 0, R_f of 2 ≈ 1, UV detection). Fluorescent labeling of polymer was quantified from Vis absorption data according to the Beer-Bouguer-Lambert' law and taking the extinction coefficients (at 456 nm) of **2** in aqueous solutions as standards.

Poly(*N,N*-dimethylacrylamide) grafted with oligo(*N*-methyazetidine) fluorescently tagged with compound 4 (ZS-495).



A solution of 50.3 mg of copolymer ZS-247 in 0.65 mL of methanol was mixed with a solution of **4** (3.04 mg, 0.00547 mmole) in 0.5 mL of methanol and heated at 50°C for six days. The cooled to room temperature solution was precipitated to 20 mL of ether. The process was repeated five times (from 0.5 mL of methanol to 20 mL of ether). The solid was dissolved in distilled water, filtered through a 0.45 μm membrane filter and freeze dried to yield 31 mg of **ZS-495**. The polymer purity was assessed with gel electrophoresis. Fluorescent labeling of polymer was quantified from Vis absorption data according to the Beer-Bouguer-Lambert' law and taking the extinction coefficients (at 456 nm) of **4** in aqueous solutions as standards.

Poly(acrylic acid) fluorescently tagged with 3 (ZS-493).



Synthesis of poly(acryloyl chloride) (PAC). PAC was synthesized similar to the protocol described earlier[36] by polymerization of acryloyl chloride (5 g) in 20 mL of dioxane with the addition of 0.1 g AIBN in argon atmosphere at 60°C for 48 h. With the objective to estimate yield and polymerization degree of the PAC, the reaction mixture was poured into water (50 mL) and dialyzed against water. After freeze drying, poly(acrylic acid) was obtained with 90% yield. According to viscometry data,[37] the polymerization degree of the poly(acrylic acid) and, correspondingly of PAC, was found to be 220.

An aliquot of the polymerization solution (2.42 g, equivalent to 5.33 mmole of acryloyl chloride) was poured to 25 mL of cyclohexane. The mixture was centrifuged at 3000g for 10 min and the polymer redissolved in 10 mL of DMF. To this stirred cooled solution (1-3°C) a solution of 3 (13.84 mg, 0.0276 mmole) and triethylamine (542 mg, 5.36 mmole) in 5 mL of DMF was added dropwise over 15 minutes. Then stirring was continued at ambient temperature for 80 minutes followed by quenching with 10 mL of distilled water and pH adjusted to ca. 11 with triethylamine. The solution was dialyzed against distilled water, filtered through a 0.45 µm membrane filter and freeze dried to yield 398 mg of the product. The polymer purity was assessed with gel electrophoresis. Fluorescent labeling of polymer was quantified from Vis absorption data according to the Beer-Bouguer-Lambert' law and taking the extinction coefficients (at 456 nm) of **4** in aqueous solutions as standards.

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Supplementary Material

Copies of ^{1}H and ^{13}C NMR of compounds **2** and **4**, absorption, excitation and emission spectra of major compounds are given in the supplementary material associated to this paper.

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