

Synthesis of Green Fluorescent Protein Chromophore Analogues for Interdisciplinary Learning for High School Students

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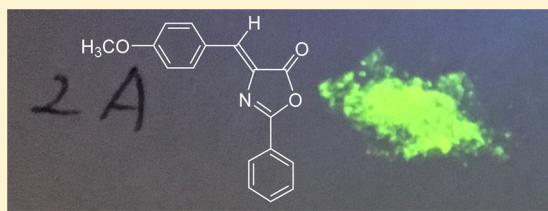
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Supporting Information

ABSTRACT: Interdisciplinary outreach activities with easy-to-implement experiments have been developed to enhance high school students' interest in chemistry and their inquisitive minds with active learning strategies. The synthesis of a model chromophore of the green fluorescent protein was employed as a key motif. Students simply enjoyed observing changes in the substances as well as witnessing a variety of fluorescent emissions. They gradually realized the importance of molecular design in the understanding and development of chemistry, biology, physics, and medicine.

KEYWORDS: High School/Introductory Chemistry, First-Year Undergraduate/General, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Bioorganic Chemistry, Dyes/Pigments, Fluorescence Spectroscopy



INTRODUCTION

Increasing attention has been focused on active learning strategies that are expected not only to promote better understanding but also to develop inquisitive minds and creativity.^{1–8} High school students (as well as college students) may lose their interest in chemistry if too much emphasis is placed on memorization rather than experiments. Passive learners receive the sets of compartmentalized and decontextualized knowledge, and they often have difficulty generating new ideas that are different from what they have learned.¹ To foster an innovative next generation that has a wide range of views and strong inquisitive minds, it is crucial to develop interdisciplinary activities with easy-to-implement experiments as motivational tools.^{7,8} Ideally, such experiments should be safe, green, feasible at low cost, intuitively understandable, and enjoyable.

Green fluorescent protein, GFP (Figure 1a), discovered in the jellyfish *Aequorea victoria*, is a unique protein that glows green upon irradiation with UV light.^{9–12} This 27 kDa protein contains a fluorescent chromophore formed by the spontaneous condensation of three amino acids (Ser-65, Tyr-66, and Gly-67) during the maturation process of the protein (Figure 1b). Another red fluorescent protein can be found in coral.¹³

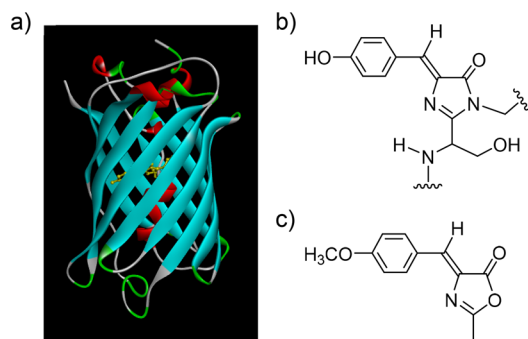


Figure 1. (A) Molecular structure of GFP (PDB code, 1GFL). (B) Molecular structure of the GFP chromophore. (C) Molecular structure of a model dye (2B).

Now, genetic mutations have been developed which provide a beautiful color palette of fluorescent proteins. Fluorescent proteins have been used in a number of important applications

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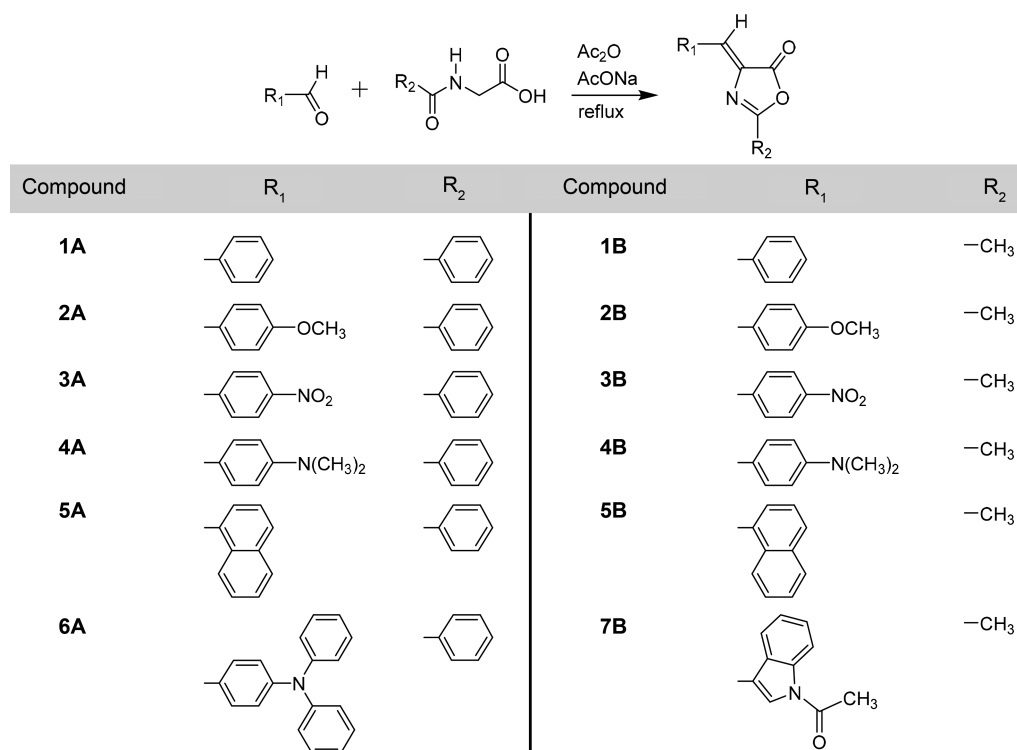


Figure 2. Synthesis of GFP chromophore analogues. Note that for **7B** indole-3-carbaldehyde was used as the material and the indole N atom was acetylated by acetic anhydride (the solvent) during the reaction to afford the *N*-acetylated product **7B**.

in biology and medicine. The GFP gene can be tagged onto another gene that codes for a particular protein, and then the spatiotemporal expression of the protein becomes evident when the specimen exhibits fluorescence from the GFP tag under a fluorescence microscope. The three researchers who opened roads to the applications of the fluorescent proteins won the Nobel prize in chemistry in 2008.^{14,15}

Fluorescent dyes, 4-arylidene-2-methyloxazol-5-ones (Figure 1c), resemble the GFP chromophore (Figure 1b) and can be readily synthesized from an aryl aldehyde and an *N*-acyl glycine (Figure 2).^{16–26} A variety of fluorescent colors can be observed from the derivatives obtained by different combinations of the materials. Students enjoy watching changes in the substances, and the beautiful fluorescent emissions attract students who lack the specialized knowledge of fluorescence and synthesis.²⁷ In addition, GFP intersects chemistry, biology, physics, medicine, and even art.^{28–36} The experiment is suitable not only for the introduction of synthetic chemistry to high school students but also for stimulating inquisitive minds over the wide range of STEAM fields (science, technology, engineering, arts, and mathematics).

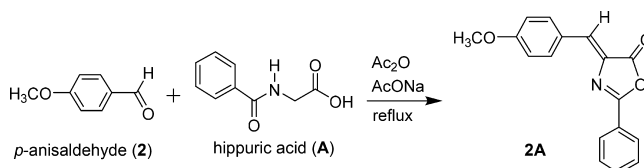
Described herein is a series of activities (special lessons in outreach settings) employing the synthesis of GFP chromophore analogues as a key motif. Our strategy is “a low barrier to entry, a high ceiling of rich potential complexity”:⁷ an enjoyable experience without “study,” along with an advanced option for a few who wish it. Students simply enjoy changes in the substances and the variety of fluorescent emissions. Through this enjoyable experience, we expect that they intuitively learn the following issues: (i) they can design and synthesize compounds by themselves, and (ii) chemistry closely correlates with other disciplines such as physics, biology, and medicine. Further, we expect development of their inquisitive minds. The introductory classes, laboratory

experiments, and inquiry-based learnings (IBLs) presented below have been conducted in some scientific outreach efforts, including special lessons in some high schools for science oriented and nonscience oriented students, as well as those at the national program, iP-U (incubation program for innovative students at Utsunomiya University), for high school students who wish to engage in the STEAM fields. The participants had not yet learned organic chemistry: most of them were 16–17 years old and had not learned basic chemistry, while others were just learning it (introductory level).

EXPERIMENTS

Synthesis of **2A** (Scheme 1).^{16–26} Typically, a 50 mL Erlenmeyer flask was charged with *p*-anisaldehyde (0.5 g, 3.5

Scheme 1. Synthesis of the GFP Chromophore Analogue 2A



mmol), hippuric acid (0.6 g, 3.5 mmol), and sodium acetate (AcONa, 0.1 g, 1 mmol) weighed using a digital kitchen scale. Acetic anhydride (Ac₂O, 2 mL, 0.02 mol) was added to this mixture, and the flask was fitted with a simple handmade reflux condenser, made of a glass tube and a rubber stopper with a hole. The flask was then placed on a pancake hot plate (or an electric griddle, ~180 °C) and heated with constant shaking. The solids dissolved in 1–2 min, and the mixture became yellowish. After heating for an additional ~8 min, the flask containing a blackish mixture was removed from the hot plate.

Yellow solids containing **2A** appeared as the mixture was cooled to room temperature. To observe the fluorescence, a small quantity of the solid was very thinly rubbed on a filter paper and irradiated with black light (365 nm). The other GFP chromophore analogues were synthesized in essentially the same way (Figure 2), and their fluorescence was from placing a drop of the reaction mixture on a piece of filter paper even if no precipitate was obtained (see Supporting Information).

HAZARDS

Protective eyewear and gloves should be used during the experiment. All the chemicals should be handled with care. Acetic anhydride, sodium acetate, hippuric acid (benzoylaminoethanoic acid), and *N*-acetylglycine (2-acetamidoacetic acid) are irritants. For these compounds, as well as benzaldehyde, *p*-anisaldehyde (4-methoxybenzaldehyde), 4-nitrobenzaldehyde, 2-naphthaldehyde, 4-dimethylaminobenzaldehyde, indole-3-carbaldehyde, and 4-diphenylaminobenzaldehyde, eye contact, skin contact, inhalation, and ingestion should be avoided. The room should be well-ventilated. Caution should be exercised to avoid the explosive boiling of the reaction mixture during heating and to avoid burns on the hands because of the heated hot plate or flask.

PROJECTS

Project 1: Introductory Class on GFP

Project 1 is an introductory class including the synthesis of **2A** and can be completed within 60–90 min. The students performed the experiment in pairs. The exercise was typically undertaken by about 10 groups, but in our experience, working with 20 groups or more is possible. An introduction about GFP was presented to the students. The similarities between the GFP chromophore and the model compound **2A** were pointed out. The synthetic procedure of **2A** was then briefly explained using a handout (see the Supporting Information). Then, the students were encouraged to examine the fluorescence by themselves. They weighed the reagents using digital kitchen scales and graduated pipettes. Guidance was provided on the use of pipettes or scales if necessary. The reagents may be apportioned to each group prior to the experiment in case of a large number of participants and/or in the need to diminish the time for the experiment. Care should be taken for laboratory safety. Yellow crude crystals of **2A** were readily obtained while cooling; moreover, the students observed its yellow fluorescence even without recrystallization (Figure 3). The experiment was usually accomplished in ~30 min. The students were asked to discuss the changes they observed: color, solvation, crystallization, fluorescence, etc. The results were shared in the class. A closing remark was given to confirm that the students synthesized the model dye and rationalized a biological property in terms of chemistry. Then, the teacher told the students that a coral has a red fluorescent protein and asked them where the color difference comes from. The students were prompted to reflect on the experience.

Project 2: Laboratory Experiment Obtaining Fluorophores

Project 2 is a laboratory experiment that can be typically held just after Project 1 (if time allows) and takes about 3 h. The experiments were typically performed by about 10 groups of two students each. At first, students were asked to discuss their experiences in Project 1. Then, the teacher told them that a variety of fluorophores may be obtained by essentially following the same experimental procedure with a different

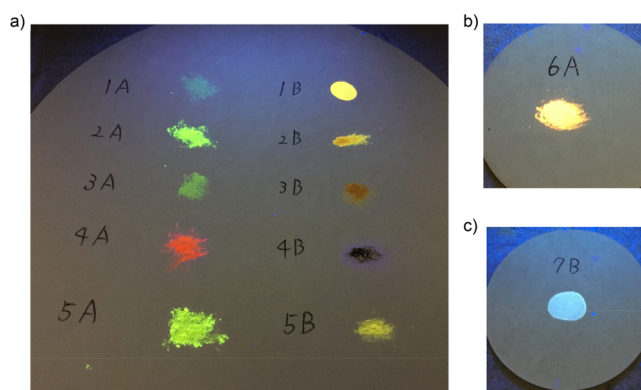


Figure 3. Fluorescence of the GFP chromophore analogues observed under UV light: (a) **1A–5A** and **1B–5B**, (b) **6A**, and (c) **7B** (**1A–6A**, after crystallization; **1B–5B** and **7B**, after workup).

combination of the materials (**1A–5A** and **1B–5B**, Figure 2). Students were asked to plan in pairs the colors they wanted to synthesize next or the combinations of aldehyde and glycine they wanted to test next. After an additional discussion about material handling and safety, students were encouraged to perform the experiments according to their plan. All the combinations afforded fluorophores (Figure 3). Although some dyes may barely precipitate, their fluorescence can be observed from drops of the reaction mixture on a piece of filter paper (see Supporting Information). Some tarry components may be filtered through silica gel before their fluorescence was observed. All the groups shared their results and then discussed their predictions, discoveries, and questions. The closing remarks included that the “color palettes” of the fluorescent proteins are nowadays used as indispensable tools in biological and medicinal research. The students were prompted to reflect on the experiments.

Project 3: Inquiry-Based Learning Activities

In 2015, three students participated in the two future projects (Dec 2015 to Mar 2016). At first, they reflected on Project 2 and discussed this to create inquiries. They set their subject to the synthesis of a dye glowing blue. Because two of them were interested in biology, they were asked to survey blue-emitting fluorescent proteins using the Internet.⁹ They found the blue fluorescent protein (BFP) and cyan fluorescent protein (CFP) and explored the molecular structures of their chromophores. By comparing the structures, they understood that point mutations transformed the phenolic part (Tyr) of the GFP chromophore, which was replaced by an imidazole or an indole group (His or Trp) in BFP or CFP, respectively. They tried to synthesize the model **7B** by the reaction of indole-3-carbaldehyde with *N*-acetylglycine (Figure 3c).

The third student was interested in physics, and she was taught that the color of molecules can be theoretically predicted. She was asked to calculate the HOMO–LUMO energy gaps of the chromophores using freeware (Winmoster with MOPAC). By using the PM3 method, unexpectedly, the calculated energy gaps of the BFP, CFP, and GFP chromophores (as well as those of the analogues synthesized in Project-2) did not match the order of their fluorescent colors. On the other hand, she found that another analogue with an *N,N*-diphenylaniline moiety (**6A**) gave a HOMO–LUMO energy gap much smaller than that of the GFP chromophore. She understood that this compound can be synthesized by the reaction of *N,N*-diphenylaminobenzal-

hyde and hippuric acid. She synthesized **6A**, which glowed in bright orange (Figure 3b). She learned the scope and limitations of the theoretical calculations.

The three students shared their results and discussed what they had learned. Their projects were presented in iP-U, as well as at the annual meeting of the high school chemistry clubs in the Kanto, Japan, area. Because one of the three students wanted to be a teacher, she was given an additional opportunity to perform a scientific activity for prospective students by attending an Utsunomiya University campus visit. A shortened form of Project 1 was carried out twice by every four participants, supported by graduate and undergraduate students. The experiments were safely accomplished in 20–30 min. The participants as well as the “prospective teacher” enjoyed the activity.

DISCUSSION

We have developed scientific activities that enabled IBL programs using the synthesis of GFP chromophore analogues as a key motif. The one-step synthesis can be safely accomplished, by simply heating the mixture of the reagents. The fluorescence of the dyes can be confirmed without purification. The reagents and the apparatuses are available at low cost. This Erlenmeyer–Pröchl reaction has recently been introduced as a laboratory experiment for upper-division undergraduates to learn laboratory techniques, reaction mechanisms, and green chemistry.²⁶ We also show the usefulness of this reaction as an introductory activity designed to develop inquisitive minds in high school students. We should note that the readers can arrange the contents of these lessons to fit their conditions (students, facilities, purposes, and time). Even by eliminating the synthesis part, for example, students may notice the importance of molecular structures through observing and comparing the colors. Demonstration by the teacher, or a video, may also be useful. Melting points of the compounds can also be useful teaching material.

The students remembered the impressions of the lessons. Short essays freely written by the participants a few days after the lessons suggested the following:

- (1) feeling that I can synthesize compounds by myself: ~87%
- (2) feeling that chemistry correlates with the other disciplines: 40–60%
- (3) development of their inquisitive minds: 50–60%

(There were 61 students, 16–17 years old. Both science oriented and nonscience oriented students were included. The students had almost no background knowledge on organic synthesis and GFP prior to the special lessons. These were 70 min lessons, containing Project-1 and a shortened form of Project-2, carried out in 2009 and 2010; see Supporting Information.) In our experience, the outcome can be influenced by the time length of the experiments and the contents of the short lecture.

The obvious changes of the substances helped students to intuitively feel chemistry. Students felt that molecular properties depend on the molecular structure designable by both chemistry and biology. The experience deepened the students' understanding of how chemistry relates to other disciplines, and it helped them see chemistry as a basic tool in science and technology (some reflections from the students are presented in the Supporting Information). We tried not to overteach, but instead we awaited their naive questions. Our replies to their

questions sometimes elicited further questions. We assume that such a coaching environment helps students reflect on their knowledge, cultivate their interests, and develop their visions.

As in Project 3, IBL often needs to be guided, and topics that inspire students' inquiries are critical. It is advantageous that the GFP chromophore analogue provides a wide range of interdisciplinary topics, such as luminous organisms, principles of unit operations, genetic experiments, and crafting artworks (for some more ideas, see the Supporting Information).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.8b00443.

Additional comments on the experiment, hazard information, student handout, outcome, methods of our Project-3 in 2015, and possible topics for related IBL activities (PDF, DOCX)

Spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Sawyer, K. Introduction. *The New Science of Learning*. In *The Cambridge Handbook of the Learning Sciences*; Sawyer, K., Ed.; Cambridge University Press: New York, 2014; pp 1–18.
- (2) Cicuto, C. A. T.; Torres, B. B. Implementing an Active Learning Environment To Influence Students' Motivation in Biochemistry. *J. Chem. Educ.* **2016**, *93*, 1020–1026.
- (3) Freeman, S.; Eddy, S. L.; McDonough, M.; Smith, M. K.; Okoroafo, N.; Jordt, H.; Wenderoth, M. P. Active Learning Increases Student Performance in Science, Engineering, and Mathematics. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 8410–8415.
- (4) Saito, M.; Miyake, N. Conceptual Change through Socially Constructive Interaction in the Classroom. *Proceedings of the Annual Meeting of the Cognitive Science Society* **2012**, *34*, 2258–2263.

- (5) Yamaguchi, E.; Deguchi, A. Learning Progressions: New Movement of Conceptual Change Research in Science Education. *Japan. Psychol. Rev.* **2011**, *54*, 358–371.
- (6) Paulson, D. R. Active Learning and Cooperative Learning in the Organic Chemistry Lecture Class. *J. Chem. Educ.* **1999**, *76*, 1136–1140.
- (7) STEM 2026. *A Vision for Innovation in STEM Education*; U.S. Department of Education, Office of Innovation and Improvement: Washington, DC, 2016. <https://innovation.ed.gov/what-we-do/stem/> (accessed Feb 2019).
- (8) Kärkkäinen, K.; Vincent-Lancrin, S. *Sparking Innovation in STEM Education with Technology and Collaboration: A Case Study of the HP Catalyst Initiative*; OECD Education Working Papers; OECD Publishing: Paris, 2013, Vol. 91. DOI: 10.1787/Sk480sj9k442-en (accessed Feb 2019).
- (9) ZEISS. Education in Microscopy and Digital Imaging. <http://zeiss-campus.magnet.fsu.edu/articles/probes/> (accessed Feb 2019).
- (10) Brejc, K.; Sixma, T. K.; Kitts, P. A.; Kain, S. R.; Tsien, R. Y.; Ormö, M.; Remington, S. J. Structural Basis for Dual Excitation and Photoisomerization of the *Aequorea victoria* Green Fluorescent Protein. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 2306–2311.
- (11) Tolbert, L. M.; Baldrige, A.; Kowalik, J.; Solntsev, K. M. Collapse and Recovery of Green Fluorescent Protein Chromophore Emission through Topological Effects. *Acc. Chem. Res.* **2012**, *45*, 171–181.
- (12) Miyawaki, A.; Niino, Y. Molecular Spies for Bioimaging—Fluorescent Protein-Based Probes. *Mol. Cell* **2015**, *58*, 632–643.
- (13) Baird, G. S.; Zacharias, D. A.; Tsien, R. Y. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 11984–11989.
- (14) Nobel Prize Organization. The Nobel Prize in Chemistry 2008. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/ (accessed Feb 2019).
- (15) Caldwell, B. A. Science Underlying 2008 Nobel Prizes. *J. Chem. Educ.* **2009**, *86*, 71.
- (16) Buck, J. S.; Ide, W. S. Azalactone of alpha-benzoylamino-beta-(3,4-dimethoxyphenyl)—Acrylic Acid. *Org. Synth.* **1943**, *Coll. Vol. 2*, 55–56.
- (17) Herbst, R. M.; Shemin, D. α -Acetaminocinnamic Acid. *Org. Synth.* **1943**, *Coll. Vol. 2*, 1–3.
- (18) Kojima, S.; Ohkawa, H.; Hirano, T.; Maki, S.; Niwa, H.; Ohashi, M.; Inouye, S.; Tsuji, F. I. Fluorescent Properties of Model Chromophores of Tyrosine-66 Substituted Mutants of *Aequorea* Green Fluorescent Protein (GFP). *Tetrahedron Lett.* **1998**, *39*, 5239–5242.
- (19) Maekawa, K.; Kubo, K.; Igarashi, T.; Sakurai, T. Electron Transfer-Initiated Asymmetric Photocyclization of Chiral Auxiliary-Substituted *N*-acyl- α -dehydro(1-naphthyl)alaninamides to the Corresponding 3,4-dihydrobenzo[*f*]quinolinone Derivatives. *Tetrahedron* **2005**, *61*, 11211–11224.
- (20) Khan, K. M.; Mughal, U. R.; Khan, M. T. H.; Zia-Ullah; Perveen, S.; Choudhary, M. I. Oxazolones: New Tyrosinase Inhibitors; Synthesis and Their Structure–Activity Relationships. *Bioorg. Med. Chem.* **2006**, *14*, 6027–6033.
- (21) Cleary, T.; Rawalpally, T.; Kennedy, N.; Chavez, F. Catalyzing the Erlenmeyer Plöchl Reaction: Organic Bases versus Sodium Acetate. *Tetrahedron Lett.* **2010**, *51*, 1533–1536.
- (22) Kaushik, D.; Khan, S. A.; Chawla, G. Design & Synthesis of 2-(substituted aryloxy)-5-(substituted benzylidene)-3-phenyl-2,5-dihydro-1*H*-[1,2,4] triazin-6-one as Potential Anticonvulsant Agents. *Eur. J. Med. Chem.* **2010**, *45*, 3960–3969.
- (23) Rafiq, S.; Rajbongshi, B. K.; Nair, N. N.; Sen, P.; Ramanathan, G. Excited State Relaxation Dynamics of Model GFP Chromophore Analogs: Evidence for *cis-trans* Isomerism. *J. Phys. Chem. A* **2011**, *115*, 13733–13742.
- (24) Paige, J. S.; Wu, K. Y.; Jaffrey, S. R. RNA Mimics of Green Fluorescent. *Science* **2011**, *333*, 642–646.
- (25) Blanco-Lomas, M.; Funes-Ardoiz, I.; Campos, P. J.; Sampedro, D. Oxazolone-Based Photoswitches: Synthesis and Properties. *Eur. J. Org. Chem.* **2013**, *2013*, 6611–6618.
- (26) Rodrigues, C. A.; Martinho, J. M. G.; Afonso, C. A. M. Synthesis of a Biologically Active oxazol-5-(4*H*)-one via an Erlenmeyer-Pröchl Reaction. *J. Chem. Educ.* **2015**, *92*, 1543–1546.
- (27) MacCormac, A.; O'Brien, E.; O'Kennedy, R. Classroom Activity Connections: Lessons from Fluorescence. *J. Chem. Educ.* **2010**, *87*, 685–686.
- (28) Follenius-Wund, A.; Bourotte, M.; Schmitt, M.; Iyice, F.; Lami, H.; Bourguignon, J.-J.; Haiech, J.; Pigault, C. Fluorescent Derivatives of the GFP Chromophore Give a New Insight into the GFP Fluorescence Process. *Biophys. J.* **2003**, *85*, 1839–1850.
- (29) Bourotte, M.; Schmitt, M.; Follenius-Wund, A.; Pigault, C.; Haiech, J.; Bourguignon, J.-J. Fluorophores Related to the Green Fluorescent Protein. *Tetrahedron Lett.* **2004**, *45*, 6343–6348.
- (30) Huang, G.-J.; Ho, J.-H.; Prabhakar, C.; Liu, Y.-H.; Peng, S.-M.; Yang, J.-S. Site-Selective Hydrogen-Bonding-Induced Fluorescence Quenching of Highly Solvatofluorochromic GFP-Like Chromophores. *Org. Lett.* **2012**, *14*, 5034–5037.
- (31) Deng, H.; Zhu, X. Emission Enhancement and Application of Synthetic Green Fluorescent Protein Chromophore Analogs. *Mater. Chem. Front.* **2017**, *1*, 619–629.
- (32) Hicks, B. W. Recombinant Green Fluorescent Protein Isoforms: Exercises To Integrate Molecular Biology, Biochemistry, and Biophysical Chemistry. *J. Chem. Educ.* **1999**, *76*, 409.
- (33) Flores, R. V.; Solá, H. M.; Torres, J. C.; Torres, R. E.; Guzmán, E. E. Effect of pH on the Heat-Induced Denaturation and Renaturation of Green Fluorescent Protein: A Laboratory Experiment. *J. Chem. Educ.* **2013**, *90*, 248–25.
- (34) Rowe, L. Green Fluorescent Protein-Focused Bioinformatics Laboratory Experiment Suitable for Undergraduates in Biochemistry Courses. *J. Chem. Educ.* **2017**, *94*, 650–655.
- (35) National Geographic. Exclusive Video: First “Glowing” Sea Turtle Found. <https://news.nationalgeographic.com/2015/09/150928-sea-turtles-hawksbill-glowing-biofluorescence-coral-reef-ocean-animals-science/> (accessed Feb 2019).
- (36) Stracey, F. Bio-Art: The Ethics behind the Aesthetics. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 496–500.