Separation of Diarylethene-based Photoswitchable Isomeric Compounds by HPLC and SFC

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Non-Standard Abbreviations: DAE, diarylethene; DAE-F-Pyr, 4*,4'-((perfluorocyclopent-1-ene-1,2-diyl) bis(5-methylthiophene-4,2-diyl) dipyridine*

Keywords: diarylethene, liquid chromatography, photoswitches, supercritical fluid chromatography

Abstract

Diarylethene-based photoswitches have become very popular over the last few decades for potential applications in chemistry, materials science, and bio-technology due to their unique physical and chemical properties. We report the isomeric separation of a diarylethene-based photoswitchable compound using high-performance liquid chromatography. The separated isomers were characterized by ultraviolet-visible spectroscopy and mass spectrometry confirmed the isomeric nature of the compounds. The isomers were purified by preparative HPLC, providing fractionated samples to study the isomers individually. A total amount of 13 mg of an isomer of interest was fractionated from a solution of 0.4 mg/mL of the isomeric mixture. Because the preparative HPLC method required large quantities of solvent, we explored the use of supercritical fluid chromatography as an alternative separation mode which, to the best of our knowledge, is the first time this technique is used to separate diarylethene-based photoswitchable compounds. Supercritical fluid chromatography provided faster analysis times while maintaining sufficient baseline resolution for the separated compounds and consuming less organic solvent in the mobile phase compared to HPLC. It is proposed that the supercritical fluid chromatographic method be upscaled and used in future fractionation of the diarylethene isomeric compounds, becoming a more environmentally benign approach for compound purification.

1 Introduction

Photoswitchable compounds are a class of molecules that can undergo a photo-reversible transformation between two chemical forms in response to light, most commonly ultraviolet and visible light [1-4]. These two forms can exist as isomers and the ability to switch between the two isomeric forms is significant because these small molecular rearrangements translate to changes in both the physical and chemical properties [5]. The chemical properties that these compounds possess (i.e., optical electronic coupling) are very attractive and can be utilized for applications in many areas, such as chemical sensors, data storage devices, actuators, and even drug delivery [4,6]. Diarylethenes (DAEs) are a subclass of photochromes that have received great attention over the last two decades as a result of their unique properties, which include thermal stability of both isomers, relatively high fatigue resistance, and these molecules can retain their photoactivity in single crystalline systems [1,6].

In order to implement DAEs into many of the potential applications, it is necessary for them to be in the solid-state. Most of what is currently understood about these molecules and their unique properties has been the result of studying them in the liquid phase [4]. However, photoactivity of these molecules in solution does not necessarily correspond to photoactivity in the solid-state due to the fact that DAEs can take on photo-inactive conformations. The DAEs isomers typically exist in either a ring-open or ring-closed molecular conformation. In solution, rotamers of the ring-open isomer are constantly in free rotation, whereas in the solid-state they are locked in place and cannot rearrange themselves into other conformations. This results in the photoresponse of the material relying on whether it takes on one specific photoactive conformation, rather than the other three conformations that are photo-inactive [7]. In order to develop crystalline materials that have a higher likelihood of being photoactive, it is potentially advantageous to start with the ring-closed isomer as the building block for materials synthesis since it exists in a fixed photoactive geometry.

The process to generate the ring-closed isomer starts with the synthesis of the ring-open isomer, which is then irradiated with UV light in solution to produce the ring-closed compound (see Figure 1). The ring-closed isomer must then be separated (i.e., purified) and collected from the irradiated mixture that contains the unconverted ring-open isomer as well as other undesired by-products, which requires the development of suitable methodology to isolate the isomeric species of interest. Further, one product that can form during the irradiation process has been identified as a fatigue product, which is hypothesized to be the result of over-exposure of the ring-closed isomer to UV light [8,9]. This undesired fatigue product, which is attributed to a ring-expansion reaction of the ring-closed isomer, is unable to undergo the reversible photoisomerization process that both the ring-open and ring-closed isomers can [8]. Separation and collection of this product in its pure form is also very important because it would provide a means to study the isolated compound to gain fundamental insights into the chemical pathways that lead to its formation as well as how to reduce or effectively eliminate its production altogether. Therefore, it is critical to develop methodology to separate and isolate all isomeric forms and impurities that exist in solution post-compound synthesis.

Prior purification methods have included the use of flash chromatography to isolate the DAE-based compounds of interest [10-12]. This process has produced products with various recoveries; however, it can be time-consuming. Furthermore, the recoveries achieved from flash chromatography typically consist of the isomeric units as a mixture and not of the individual isomers [11]. The normal-phase separation mechanism in the flash chromatographic method, which uses bare silica as the stationary phase, does not provide sufficient selectivity to achieve adequate resolution of the isomeric species for high purity recoveries during fractionation. The DAE-based compounds are commonly non-polar and a stationary phase with reversed-phase characteristics would be more appropriate for the separation of such compounds. Preparative HPLC with a reversed-phase mechanism has been explored to isolate DAE-based compounds of interest; however, this approach has mostly consisted of using additives in the mobile phase that still need to be removed after the purification of the isomers [10-13], complicating the process and compromising compound purity.

Herein, we present an analytical HPLC method that employs reversed-phase chromatography without the use of mobile phase additives to separate the structural isomers of a particular photochromic DAE-based compound: *4,4'-(perfluorocyclopent-*

1-ene-1,2-diyl) bis(5-methylthiophene-4,2-diyl) dipyridine (DAE-F-Pyr). This particular diarylethene was chosen for study because of the pyridine substituents; the lone electron pair can act as a hydrogen/halogen bond acceptor. This can be explored for co-crystal synthesis or for coordination to metal centers, allowing them to easily be incorporated into a wide variety of solid-state materials [14,15]. The overall goal was to separate, identify, and purify an amount of the DAE-F-Pyr ring-closed isomer. Once confirming the identity of the isomeric species of interest, the procedure was scaled up to collect pure fractions of the corresponding isomeric units of interest, particularly the ring-closed isomer at a preparative scale.

We also developed a second analytical method using supercritical fluid chromatography (SFC) to establish separation conditions that can be scaled up to take advantage of the practical benefits of SFC as a purification technique. SFC has seen a resurgence in interest the past few decades as an alternative technique to HPLC [16]. Comparisons between the two techniques for the separation of a wide range of compounds and applications are readily available in the literature [16-21]. This technique provides a normal-phase type of separation mode, mainly using supercritical CO_2 as the mobile phase which can contain low quantities of an organic co-solvent (e.g., methanol), used as a modifier to tune the selectivity of the separation [22-25]. Supercritical CO_2 is less viscous and has higher diffusivity than the mobile phases commonly employed in HPLC, leading to higher flow rates than in HPLC, corresponding to faster analysis times [26]. The higher diffusivity translates to faster mass transfer of the analytes in the mobile phase during the separation, which also translates to higher separation efficiencies. In addition, CO_2 is inexpensive, low in toxicity, and is returned to the environment (from where it was originally taken) without creating extra waste after use in the separation, thus making SFC a more environmentally friendly technique than the use of acetonitrile in HPLC [23]. To the best of our knowledge, this is the first reporting for the separation of diarylethene-based photoswitchable compounds by SFC.

2 Materials and methods

2.1 Chemicals

HPLC-grade acetonitrile and HPLC-grade methanol were purchased from VWR International (Pittsburgh, PA) and were used as mobile phase for HPLC and co-solvent for SFC separations, respectively. Ultrapure water (18.2 MΩ cm) was obtained from a Barnstead International EASYPure II water purification system and filtered through a 0.2 µm membrane, which was used to prepare the mobile phase for HPLC. Formic acid was purchased from Fisher Scientific (Hampton, NH). The ring-open DAE-F-Pyr compound was synthesized after modification of reported methods and synthetic details are provided as electronic supplemental information. The DAE ring-open sample solutions were prepared in acetonitrile. Research-grade CO_2 was purchased from Airgas (Buffalo, NY) and used as the mobile phase in SFC.

2.2 Synthesis of ring-closed diarylethene-based compound

To generate the photoactive ring-closed DAE-F-Pyr isomer, the ring-open isomer in solution (see supplemental information) was exposed to UV radiation at 254 nm for a period of time. The resulting solution contained a mixture of the ring-open and ring-closed isomers, as well as a fatigue product of the diarylethene-based compound. A photochemical reactor with a 254 nm ultraviolet (UV) bulb and UV-transparent knitted reactor coil (KRC) was purchased from Aura Industries, Inc. (San Diego, CA) and used to expose the ring-open diarylethene-based compound to UV radiation to produce the ring-closed isomer and fatigue products. The sample solution was passed through the photoreactor using a PHD 2000 syringe pump from Harvard Apparatus (Holliston, MA). By measuring the length of reactor coil exposed to UV radiation, its inner diameter (i.d.), and the flow rate at which the sample solution passed through the photoreactor, the exposure time of a given sample volume can be calculated. For the work shown, a sample volume of approximately 34 µL was exposed to UV radiation for approximately 2 seconds (coil i.d. of 0.5 mm and a flow rate of 1.0 mL/min).

2.3 Chromatographic systems and conditions

The chromatographic instrument used for HPLC analytical separations was an Agilent (Santa Clara, CA) model 1100 Series HPLC. This is an integrated unit containing a binary pump, mobile phase degasser, autosampler, thermostated column compartment, and a diode array detector. The SFC system consisted of a separate Agilent 1100 Series chromatographic system that had been retrofitted with a Fusion^{TM} A5 SFC conversion module (Aurora SFC Systems), converting the unit in a supercritical fluid chromatographic system. Partial loop injections were utilized with an injection loop of 5 µL and an injection solvent of acetonitrile. The HPLC separations were performed using aqueous acetonitrile mobile phases at 30^{o}C, while the SFC separations were performed at a column temperature of 35 ^{o}C and the back pressure regulator set at 120 bar, using a CO_2 modified with methanol as the mobile phase (92/8 CO_2/methanol). Other specific details related to the chromatographic conditions for each separation are included in the respective figure caption. Agilent ChemStation software was used to control both instrument modules and for data acquisition. LC-MS analysis was performed using an Ultimate 3000 UHPLC integrated chromatograph from ThermoFisher Scientific (Waltham, MA), which included a binary pump, column compartment, and autosampler. This chromatograph was connected to a Linear Ion Trap (LTQ) mass spectrometer with an electrospray ionization (ESI) source, also from ThermoFisher Scientific (Waltham, MA). Xcalibur software (ThermoFisher Scientific) was used for both instrument control and data acquisition. LC-MS was performed under positive ion scan mode with a scan range of m/z 100-900 Da. Nitrogen was used as the nebulizing and cone gas. The electrospray ionization source was tuned at a sheath gas flow rate of 55 arb, an auxiliary/sweep gas flow rate of 16 arb, a spray voltage of 4.5kV, a capillary temperature of 200 degrees Celsius, a capillary voltage of 15V, and a tube lens offset of 40V.

The preparative HPLC system used to purify (i.e., fractionate) the different isomeric DAE compounds consisted of two 515 HPLC pumps, a 717plus Autosampler, and a 484 tunable absorbance detector, components from Waters (Milford, MA). The separations in the prep-HPLC were run at room temperature (~23 ^{o}C), no thermostated. PowerChrom software from eDAQ (Colorado Springs, CO) was used for data acquisition. Fraction collection was performed manually using a two-position valved equipped with an actuator control module from Valco Instruments Co. Inc. (Houston, TX), placed immediately after the UV detector. Detection of the solutes was achieved at a UV wavelength of 254 nm (reference wavelength was 400 nm). The columns used for the analytical HPLC and SFC separations were the 1.7 µm ACQUITY UPLC BEH Shield RP18 (2.1 mm i.d. x 100 mm length) and the 3.5 µm Viridis BEH 2-ethylpyridine (3.0 mm i.d. x 150 mm length) columns, respectively, both from Waters (Milford, MA). The column used for prep-HPLC separations was a 5 µm C18 (21.2 mm i.d. x 250 mm length) column from Phenomenex (Torrance, CA).

3 Results and discussion

3.1 Separation of diarylethene-based isomeric compounds by HPLC

The separation of some DAE-based compounds has been attempted using RP-HPLC [10,11,13]; unfortunately, the reported separations did not provide adequate resolution of the isomers in the mixture. Furthermore, the mobile phase used incorporated additives to achieve the separations. Such additives must be removed after the separation process if the desired outcome is purification of the separated compounds. The separation of the DAE-F-Pyr isomeric compounds (i.e., ring-open and ring-closed) reported here was accomplished via RP-HPLC without the use of any mobile phase additives. Using a C18 column as a first choice, we initially performed a few preliminary runs using mobile phase compositions around 80% aqueous acetonitrile, and very quickly arrived at the final isocratic mobile phase conditions. Figure 2 shows the analytical separation of a sample mixture, which was performed using the analytical HPLC C18 column mentioned in Section 2.3. Under these conditions, all compounds eluted in under 3.5 minutes and the ring-open and ring-closed isomers were separated with a resolution of 4.4. The ring-open isomer eluted first, followed by the fatigue product, followed by the ring-closed one. It is important to note that, unlike prior reports that involved the separation of other DAE-based compounds [10,11,13], the separation illustrated in Figure 2 did not include any additives in the mobile phase. The elimination of additives in the mobile phase would simplify the HPLC purification process of the isomers at the preparative scale.

3.2 Compound characterization by UV-Vis and MS

The separated isomeric compounds were monitored via a UV-Vis diode array detector (DAD), which allowed for the collection of the UV-Vis spectrum for each individual isomer, providing a means for their identification. The spectra for the separated DAE-F-Pyr isomers are shown in Figure 3 and are in agreement with literature reports on the spectral characterization of these isomers [27]. The colorless ring-open isomer had a maximum absorption (λ_{max}) at approximately 300 nm with minimal absorption beyond 350 nm. In contrast, the blue-colored ring-closed isomer had two absorption bands with λ_{max} values in the UV region (260 nm and 375 nm) and a third absorption band with a λ_{max} value in the visible region at 590 nm. The fatigue product exhibited four major absorption bands. Three of these bands were in the UV region (275 nm, 310 nm, and 350 nm) while a fourth band had a λ_{max} in the visible region at 555 nm. It is interesting to point out that the UV-Vis spectrum for the fatigue product shows similarities to the ring-closed isomer. The molecular difference between these two isomers is the ring expansion that takes place within the diarylethene scaffold [8]. This molecular rearrangement that occurs can be noticed by the shift of the visible band, which in turn, results in the fatigue product exhibiting a visible purple color upon collection at sufficient concentrations.

We also performed HPLC-mass spectrometry (MS) to confirm the molecular weights of the DAE-based isomeric compounds. A total ion chromatogram and the mass spectrum for each isomer are shown in Figure 4. The peaks at 3.37 min, 5.60 min, and 6.39 min corresponded to the ring-open, fatigue product, and ring-closed isomers, respectively; the other small peaks that are not associated with the DAE-F-Pyr compounds are attributed to residual reagents used during the synthesis. The mass spectra shown display identical mass to charge ratios for all three peaks ($M+H^+$ and $M+2H^+$), confirming the isomeric nature of the compounds. The separation conditions were slightly different than those of Figure 2 to accommodate an acidic mobile phase necessary to improve electrospray ionization MS. There was a difference in retention between the chromatograms. We proceeded to perform the separation of the DAE-F-Pyr isomeric mixture by LC-UV using the acidified mobile phase conditions. Acidifying the mobile phase causes a shift in retention (see Figure S2 in Supplemental Information). The reduction in pH of the mobile can allow the protonation of the pyridine group present in the DAE compound (pKa ~5), contributing to a shift in retention time. Nevertheless, we observed differences in retention time between the chromatograms collected with the two systems even when the same acidified mobile phase conditions were used. However, the retention factor in both runs was very similar, indicating similar retention behavior. We attribute the differences in retention times between the LC-MS and LC-UV runs to adjustments made to the LC-MS system, since the flow rate is decreased to provide a flow rate that is more compatible with the electrospray system.

3.3 Purification using preparative HPLC

One of our goals was to isolate milligram quantities of the ring-closed DAE-F-Pyr isomeric compound to be used in other studies. In order to purify relevant quantities of this compound, the analytical HPLC method was scaled up to the preparative scale. The separation parameters for the preparative method are readily obtained through straightforward calculations from the analytical method, which scales with the cross-sectional area of the preparative column [28]. We used a prep-column (21.2 mm i.d.) that was 2.5 times longer than the analytical column and from a different manufacturer. Although the column chemistry was C18 as in the analytical column, it is well known that C18 columns by different manufacturers can show different separation behavior; this and the longer column would lead to differences in retention times of the DAE-F-Pyr isomers.

To produce the desired ring-closed isomer, a 100 mL solution containing the ring-open isomer with a concentration of 0.4 mg/mL (i.e., 40 mg total) was exposed to UV radiation at 254 nm by passing the solution through the photoreactor, producing the isomeric mixture. Then, the sample was pre-concentrated to a volume of approximately 2 mL. A 200 μL sample volume was injected into the prep-HPLC and the fractionated isomers were collected. Figure 5 shows a chromatogram of one of the prep-HPLC separations using the prep-C18 column. Injections of the fractionated ring-open and ring-closed DAE-F-Pyr photochromic compounds were performed to determine compound purities. The purities for both compounds were > 99% (by peak area), demonstrating the successful fractionation of the isomers (see inserts in Figure 5). Retention time and UV-Vis spectra were used to confirm the identity of the compounds. Ten fractionated samples were collected and combined and, after solvent removal, a total of 12.7 mg of the pure ring-closed isomer was collected. Caution must be taken to protect the collected ring-closed sample from visible light, since the ring-closed isomer will start to convert back to ring-open upon exposure to visible light (see Figure S3).

Although the prep-HPLC method was effective to isolate the DAE-F-Pyr isomeric compounds, we recognize that this approach generates enormous amounts of solvent waste, which is not environmentally friendly. The preparative HPLC approach used a flow rate of 20 mL/min, which equates to approximately 600 mL of mobile phase per injection (30-minute analysis time with the current column). Since the pre-concentrated sample exists in a 2 mL solution prior to purification, this equates to approximately 6 liters of mobile phase required for purification of the isomers, without accounting for volumes used to equilibrate the column and between runs. The great majority of this solvent went to waste. Furthermore, the mobile phase consisting of 15% water and 85% acetonitrile collected during fractionation also needed to be evaporated to produce the pure isomeric products; this was both time-consuming and wasteful. This has led us to explore the initial stages of implementing supercritical fluid chromatography (SFC) as an alternative technique to separate the DAE-F-Pyr isomeric compounds.

3.4 Separation of diarylethene-based isomeric compounds by SFC

The DAE-F-Pyr isomers were separated by SFC using a 2-ethylpyridine (EP) column with an isocratic mobile phase composition of 92% CO_2 and 8% methanol. We arrived to these conditions by running a few preliminary experiments using various mobile phase compositions around 90% CO_2 and 10% methanol (e.g., CO_2/methanol of 90/10, 92/8, and 95/5) and selected the run with adequate resolution. Although acetonitrile was first tested as a potential co-solvent with CO_2, methanol provided shorter analysis times. The ethylpyridine column was used since its polar pyridine group provided favorable sites for polar interactions with the DAE-F-Pyr molecule. An example of a chromatogram is shown in Figure 6. Two immediate differences are noticed between the separation of the isomers via SFC and HPLC. One is the elution order for the ring-open and ring closed compounds were reversed (see Figures 2 and Figure 6), illustrating the two different separation mechanisms (i.e., orthogonality). The second one is the analysis time via SFC is shorter, despite that a longer chromatographic column was used in SFC, while still maintaining a good baseline resolution of the isomers (R_s=3.1 in SFC and 4.4 in HPLC). The amount of waste generated by the methanol co-solvent in SFC is minimal. We point out that the chromatograms shown for both analytical HPLC and SFC were not fully optimized in terms of analysis time, resolution, or separation efficiency. The overall goal of this work was to achieve adequate resolution to establish conditions that could be upscaled for purification purposes.

The upscale of the SFC method would be a better suited approach for future purification of the DAE compounds under study. In addition to a fast separation time, the mobile phase utilized would mainly consist of supercritical CO_2, which would be removed as a gas upon fractionation of the compounds [18,19]. The collected isomers would then solely exist in the methanol co-solvent (less than 10% of the total mobile phase) and in a much smaller collection volume than through preparative HPLC. Also, with the absence of water in SFC, this provides an overall reduction in the time required to produce the desired DAE-based isomeric compound.

4 Concluding remarks

The isomeric DAE-F-Pyr photochromic compounds were successfully separated by RP-HPLC. The HPLC method was upscaled to fractionate each isomeric unit. The upscaled purification method allows for the collection of the different synthetic products, which allows for further photochromic studies of the purified species (e.g., ring-closed and fatigue product isomers). We successfully produced milligram quantities of the ring-closed DAE-F-Pyr isomer from the synthetic preparation mixture. The collection of pure compounds from a synthetic mixture can accelerate fundamental studies of these compounds and the timeline for when the potential applications of these photochromic materials can be realized.

The HPLC method developed herein, however, produces a significant amount of solvent waste, particularly acetonitrile. We developed an SFC method to separate the DAE-F-Pyr photochromic compounds that is more environmentally friendly and, to the best of our knowledge, is the first reporting of the separation of diarylethene-based isomeric compounds by SFC. We propose to scale up the analytical SFC method for future purification. The SFC method should provide faster separation times with adequate resolution, and a lower consumption of organics in the mobile phase. In addition, at the preparative scale, there would be a faster post-collection solvent evaporation time since the collected fraction would be in small quantities of the organic co-solvent. Obviously, a commercial prep-SFC would be ideal to purify large quantities of the DAE-F-Pyr photochromic compound. However, availability is a restriction in our laboratory. Although there are commercially available prep-SFC fraction collectors on the market, there is not one for our current Agilent SFC system, in addition to the limited flowrate that cannot reach semi-prep separations. Given the limitations, future work will be focused on the construction of a system capable of performing preparative SFC for the purification of the DAE-based isomeric compounds.

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Conflict of Interest

The authors have declared no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supplemental Information

1. Synthesis of the ring-open isomer of the DAE-F-Pyr compound.
2. HPLC chromatograms showing the separation of the ring-open, fatigue product, and ring-closed isomers of the DAE-F-Pyr photoswitch compound with and without using acidified mobile phase.
3. HPLC chromatogram illustrating the appearance of the ring-open DAE-F-Pyr isomer after exposing the purified ring-closed sample to ambient light.

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Figures Captions

Figure 1. Diarylethene-based photoswitch studied in this work. The ability to undergo a photo-reversible transformation involves a ring-closing mechanism when exposed to UV light (254 nm) and a ring-opening mechanism in response to visible light (600 nm).

Figure 2. HPLC chromatogram showing the separation of the ring-open, fatigue product, and ring-closed isomers of the DAE-F-Pyr photoswitch compound. HPLC column: C18 silica-based (1.7 μm particle, 2.1 mm i.d. x 100 mm length), mobile phase: 85:15 ACN: H_2O (v/v), flow rate: 0.2 mL/min, UV detection: 254 nm, column temperature: 30 °C, injection volume: 2 μL.

Figure 3. UV-vis spectra collected during the separation of the a) ring-open, b) ring-closed, and c) fatigue product isomers of DAE-F-Pyr photochromic compound.

Figure 4. Total ion chromatogram and mass spectra of the ring-closed, ring-open, and fatigue product isomers of the DAE-F-Pyr photoswitch. The TIC shows the retention times for the ring-open (3.37 min), fatigue product (5.60 min) and ring-closed (6.39 min) isomers while the identical mass spectra of the fatigue product, ring-open and ring-closed compounds confirm their isomeric identity. HPLC column: C18 silica-based (1.7 μm particle, 2.1 mm i.d. x 100 mm length), mobile phase: 85:15:0.1 ACN: H_2O: formic acid (v/v/v), flow rate: 0.2 mL/min, column temperature: 30 °C, injection volume: 2 μL (see section 2.3 for mass spectrometry conditions).

Figure 5. Preparative HPLC chromatogram of the fatigue product, ring-open and ring-closed isomers of the DAE-F-Pyr photochrome using the Aqua C18 preparative column (5 μm particle, 21.2 mm i.d. x 250 mm length). Mobile phase: 85:15 ACN: H_2O (v/v), flow rate: 20.0 mL/min, UV detection: 254 nm, column temperature: ambient (~23 °C), injection volume: 200 μL. Inserts show chromatograms of the fraction collection of (A) ring-open and (B) ring-closed isomers of the DAE-F-Pyr photoswitchable compound. HPLC column: C18 silica-based (1.7 μm particle, 2.1 mm i.d. x 100 mm length), mobile phase: 85:15:

ACN: H_2O (v/v), flow rate: 0.2 mL/min, UV detection: 254 nm, column temperature: 30 °C, injection volume: 2 µL.

Figure 6. SFC chromatogram of the separation of the ring-closed, ring-open, and fatigue product isomers of the DAE-F-Pyr photochrome. SFC column: 2-ethylpyridine silica-based (3.5 µm particle, 3.0 mm i.d. x 150 mm length), mobile phase: 92:8 CO_2: MeOH (v/v), flow rate: 3.0 mL/min, UV detection: 254 nm, column temperature: 35 °C, injection volume: 2 µL, back pressure regulator: 120 bar.

Figure 1

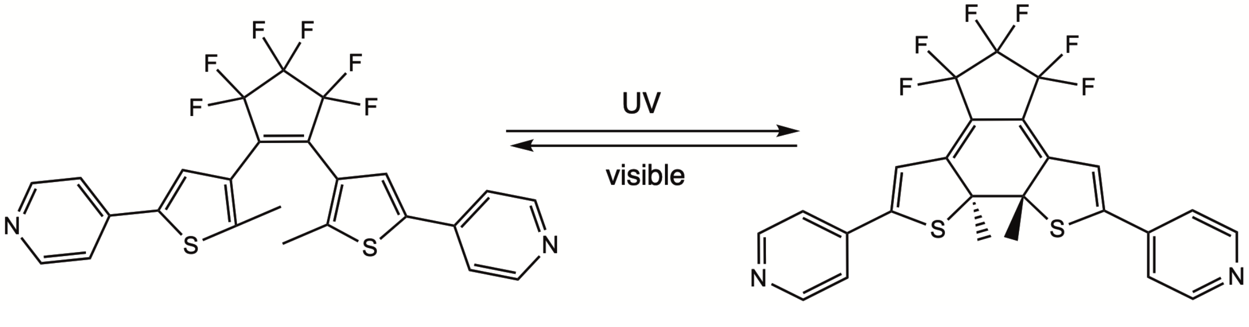


Figure 2

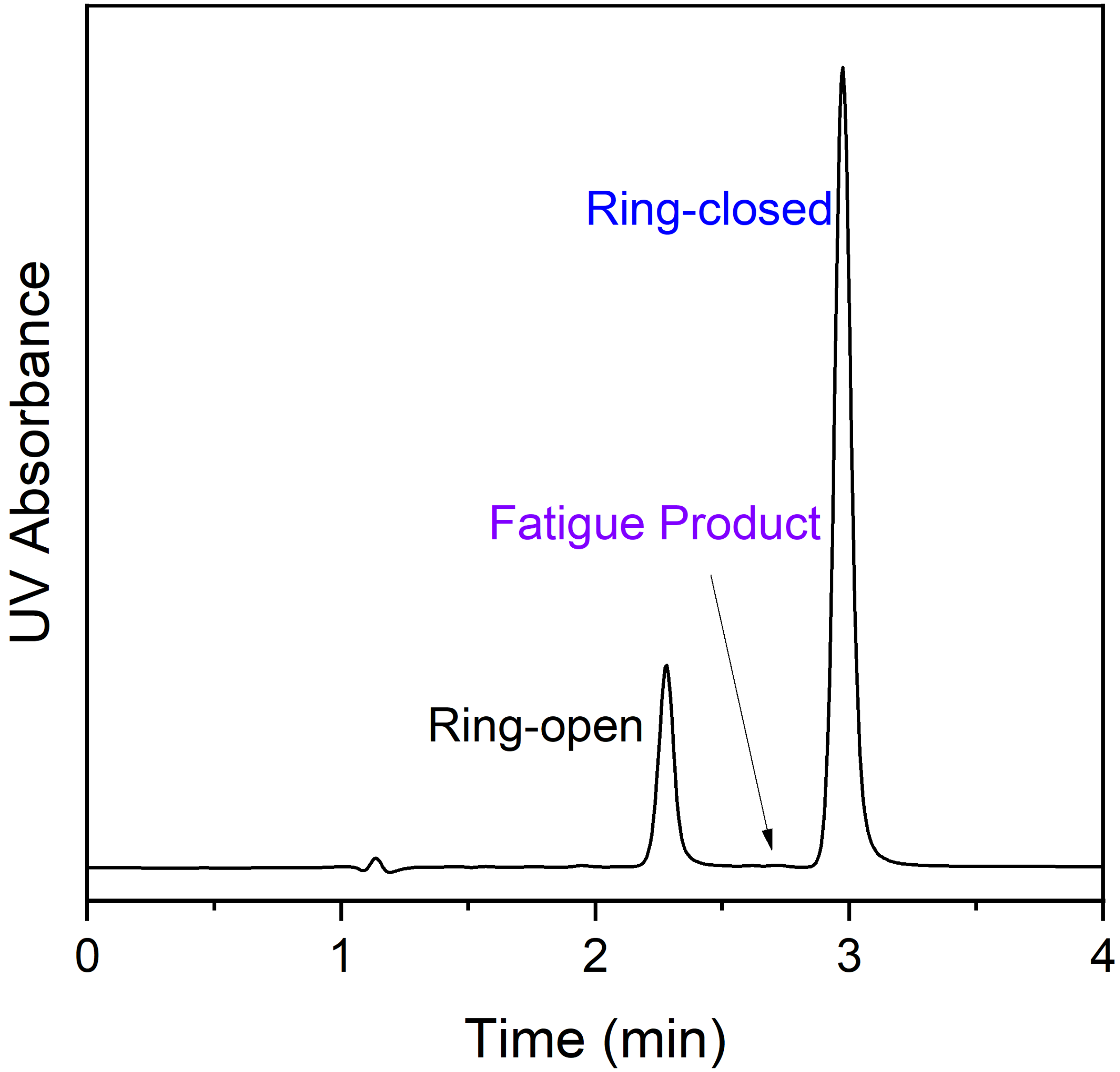

Ring-closed
Fatigue Product
Ring-open
UV Absorbance
0
1
2
3
4
Time (min)

Figure 3

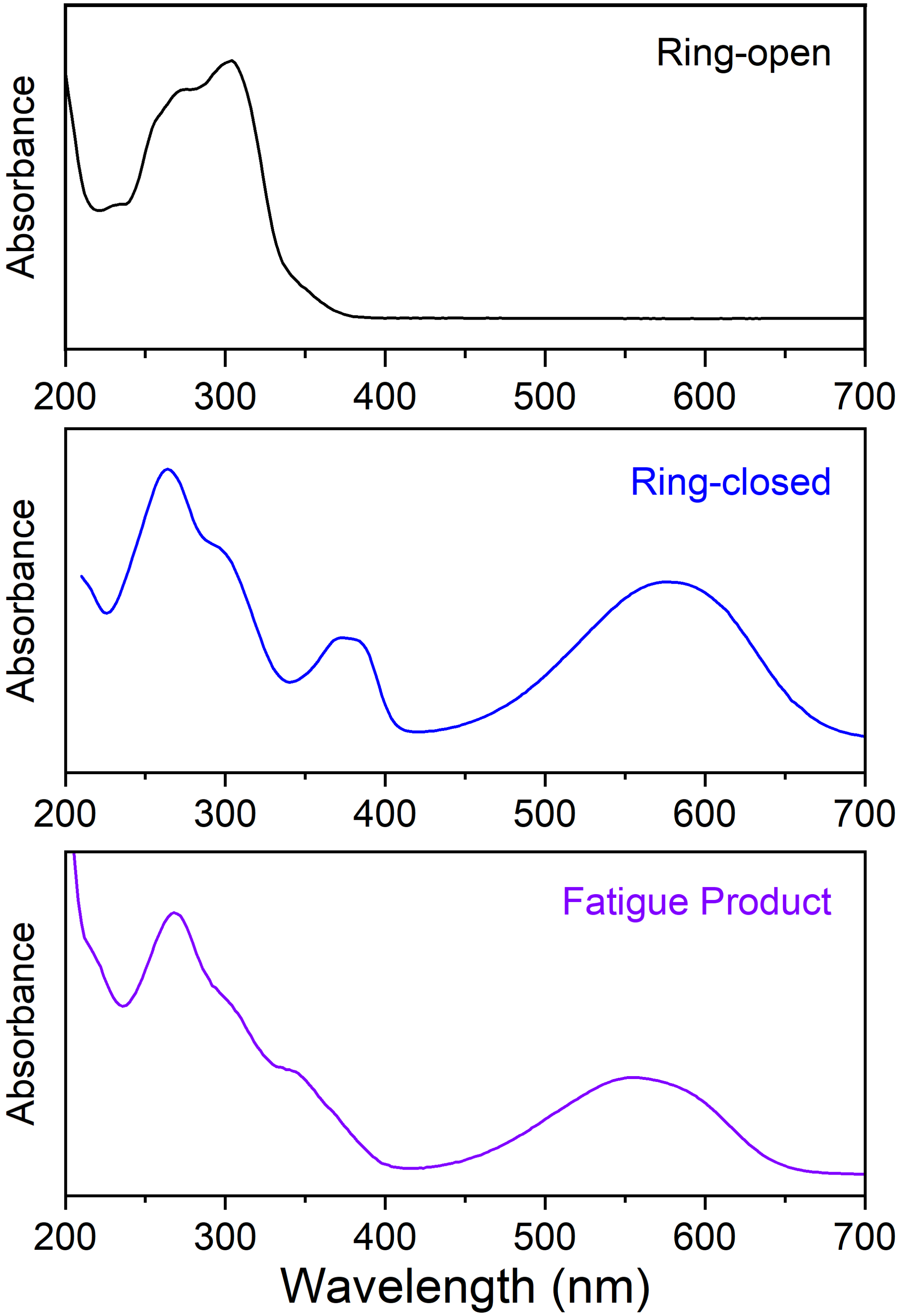
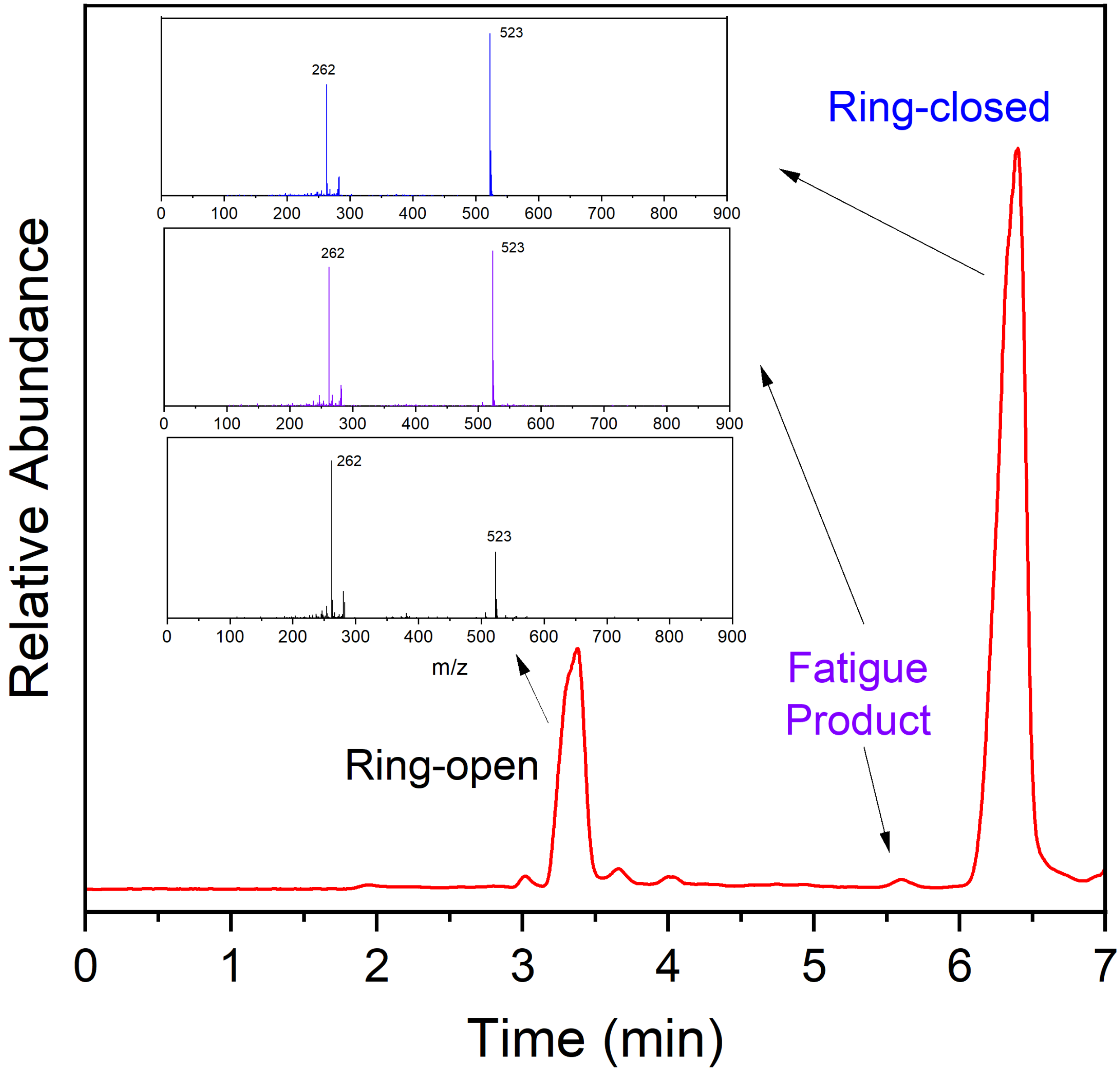

Ring-open
Absorbance
200
300
400
500
600
700
Ring-closed
Absorbance
200
300
400
500
600
700
Fatigue Product
Absorbance
200
300
400
500
600
700
Wavelength (nm)

Figure 4



523
262
0
100
200
300
400
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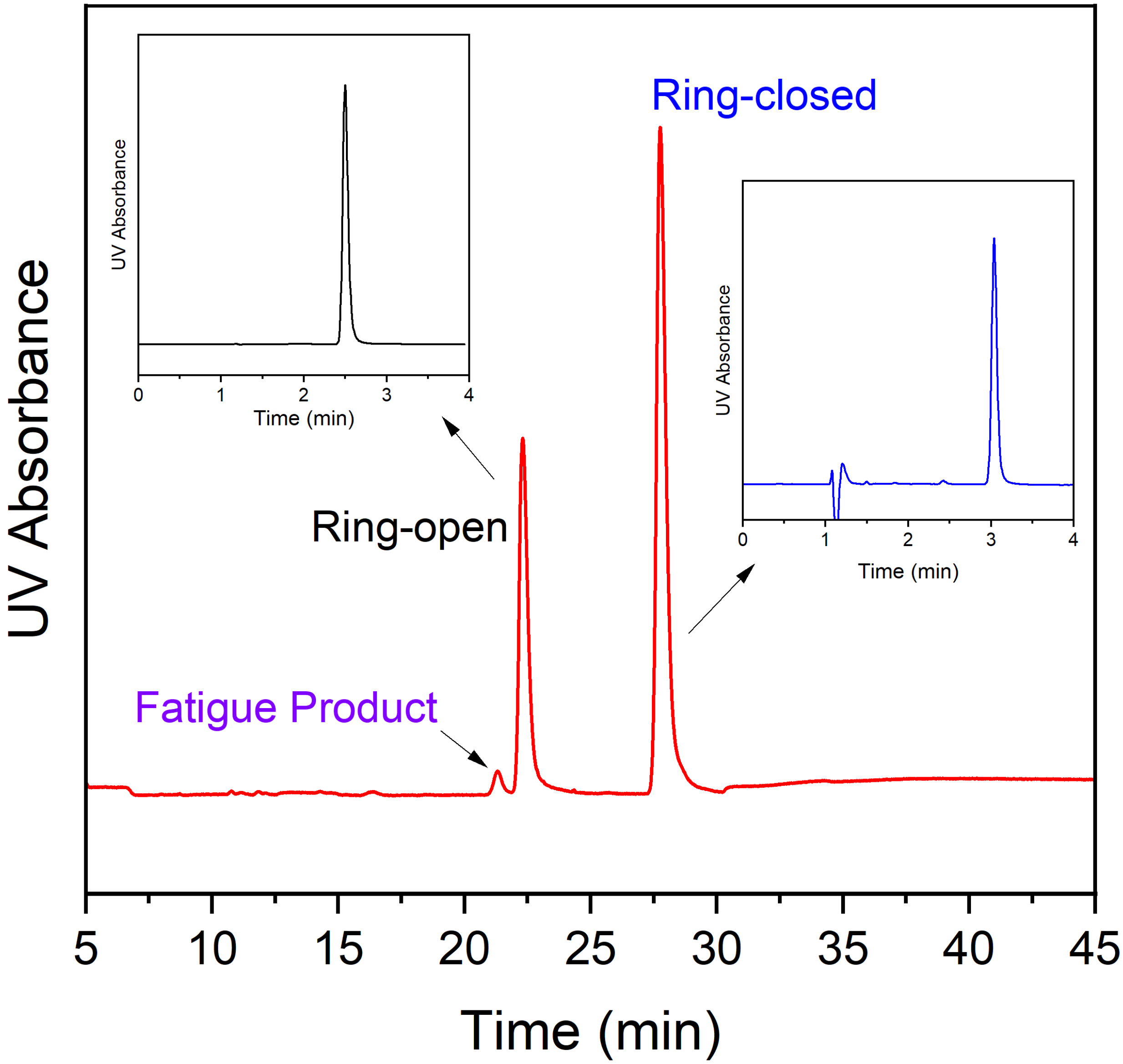
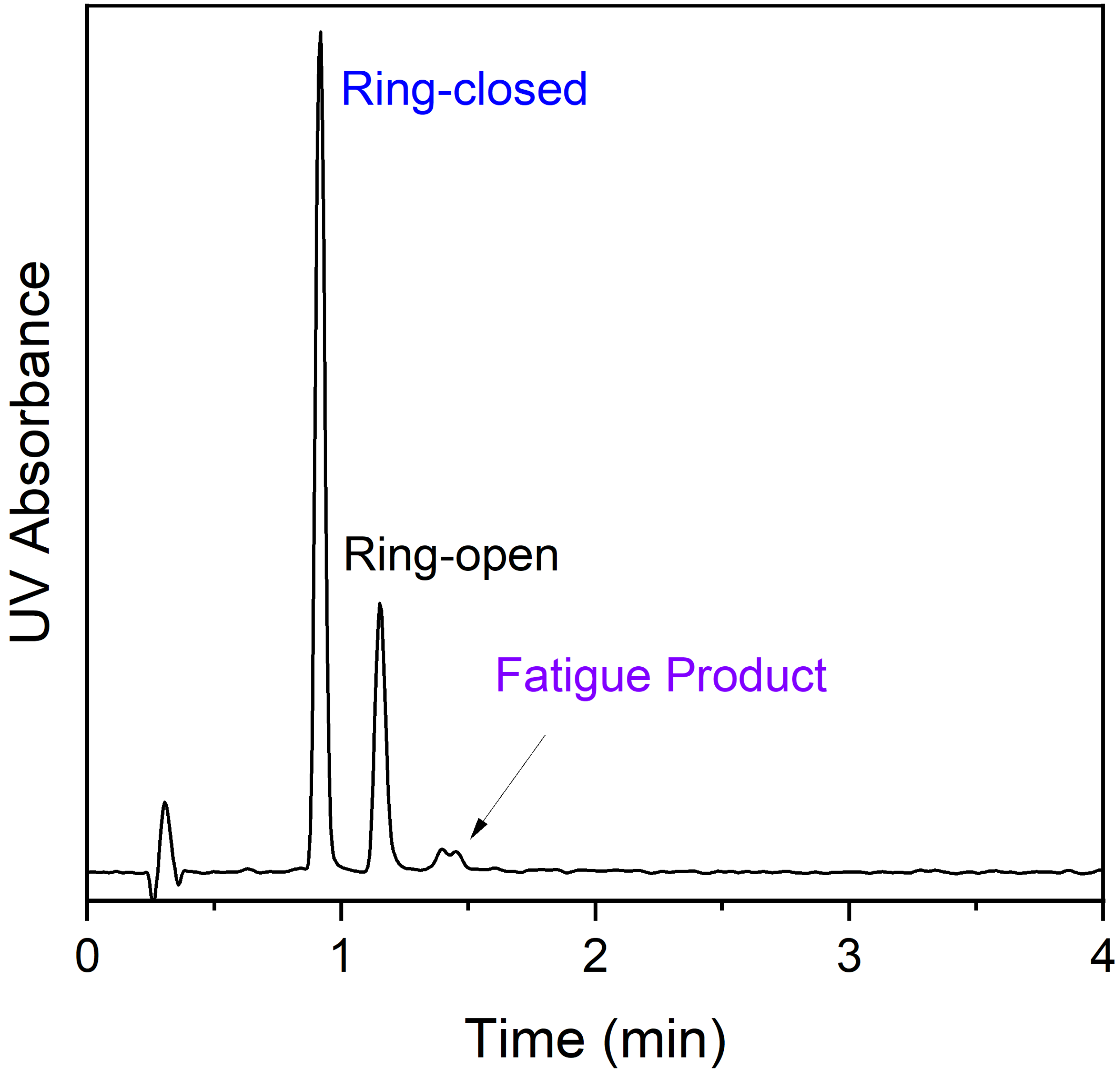
700
800
900
523
262
0
100
200
300
400
500
600
700
800
900
262
523
0
100
200
300
400
500
600
700
800
900
m/z
Ring-closed
Fatigue
Product
Ring-open
Relative Abundance
0
1
2
3
4
5
6
7
Time (min)

Figure 5



Ring-closed
Ring-open
Fatigue Product
UV Absorbance
Time (min)
5
10
15
20
25
30
35
40
45
UV Absorbance
Time (min)
0
1
2
3
4
UV Absorbance
Time (min)
0
1
2
3
4

Figure 6



Ring-closed
Ring-open
Fatigue Product
UV Absorbance
0
1
2
3
4
Time (min)

Separation of Diarylethene-based Photoswitchable Isomeric Compounds by HPLC and SFC

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Supplemental Information

Synthesis of the ring-open diarylethene compound *4,4'-((perfluorocyclopent-1-ene-1,2-diyl) bis(5-methylthiophene-4,2-diyl) dipyridine* (DAE-F-Pyr)

The synthesis of the DAE-F-Pyr ring-open isomeric form of the diarylethene photoswitch is a multi-step reaction. The synthetic procedure from the starting reagents to the final product are summarized in Figure S1 and described in detail below.

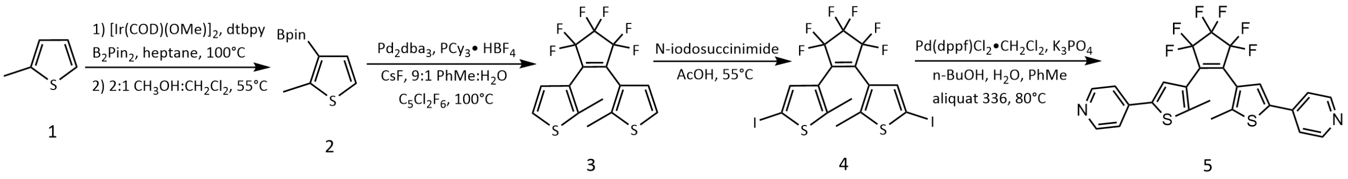

Figure S1. Schematic diagram of the reaction procedure to synthesize the ring-open form of the diarylethene photoswitch (DAE-F-Pyr).

1. Synthesis of 4,4,5,5-tetramethyl-2-(2-methylthiophen-3-yl)-1,3,2-dioxaborolane (Compound 2 in Figure S1)

The procedure was adapted from Kallepalli, et al. [1]. In a flame dried Schlenk tube, bis(pinacolato)diboron (35 mmol), 4,4'-di-tert-butyl-2,2'-dipyridyl (0.1 mmol), and (1,5-cycloocatadiene) (methoxy)iridium(I) dimer (0.05 mmol) were added while pulling vacuum and backfilling with N_2 between addition of each solid. Anhydrous heptane (25mL) was then injected into the Schlenk tube and the reaction was stirred. After stirring for 15 minutes, 25 mmol of 2-methylthiophene (Compound 1 in Figure S1) was injected into the Schlenk tube and the reaction mixture was heated to 100°C for 24 hours. Once the reaction was finished, the heptane was removed under vacuum and a 2:1 solution of methanol (84 mL) and dichloromethane (42 mL) was injected into the Schlenk tube. The reaction mixture was heated to 55°C and allowed to proceed for 12 hours. A liquid-liquid extraction was performed using dichloromethane and water and the product was collected in the dichloromethane layer; then, the product was isolated using a silica column run with 1:3 dichloromethane: hexane solution. The product was a clear, faint yellow liquid (yield 53.2%). 1H NMR (400MHz, CDCl3): δ 1.31 (s, 12H), 2.69 (s, 3H), 7.03 (d, 1H), 7.21 (d, 1H)

2. Synthesis of 3,3'-(perfluorocyclopent-1-ene-1,2-diyl) bis(2-methylthiophene) (Compound 3 in Figure S1)

The synthetic procedure for Compound 3 followed the report by Hirato, et al. [2], starting with Compound 2 in Figure S1. The product was clear, colorless crystals (yield 65.8%). 1H NMR (400 MHz, CDCl3): δ 1.87 (s, 6H), 7.06 (d, 2H), 7.16 (d, 2H)

3. Synthesis of Procedure for 3,3'-(perfluorocyclopent-1-ene-1,2-diyl) bis(5-iodo-2-methylthiophene) (Compound 4 in Figure S1)

The procedure was adapted from Lim, et al. [3]. N-iodosuccinimide (0.68 mmol) and glacial acetic acid (3 mL) were added to a scintillation vial with a stir bar. 3,3'-

(perfluorocyclopent-1-ene-1,2-diyl) bis(2-methylthiophene) (0.272 mmol) was weighted out and added to the scintillation vial and the reaction was brought up to 55°C. The reaction was run in the dark and left to stir overnight (18 hours). The crude mixture was neutralized with a saturated solution of sodium bicarbonate and then a liquid-liquid extraction using dichloromethane and water was performed; the product was collected in the dichloromethane layer. A silica gel column was run with hexane to purify as a purification step (yield 89.0%). 1H NMR (400MHz, CDCl3): δ 1.89 (s, 6H), 7.18 (s, 2H)

4. Synthesis of 4,4'-((perfluorocyclopent-1-ene-1,2-diyl) bis(5-methylthiophene-4,2-diyl) dipyridine (Compound 5 in Figure S1)

Compound 4 (1.61 mmol), 4-pyridylboronic acid (4.84 mmol), potassium phosphate (9.67 mmol), and Pd(dppf)$Cl_2 \cdot CH_2Cl_2$ (0.16 mmol) were added to a Schlenk tube while pulling vacuum and backfilling with N_2 between addition of each solid. A solution containing 1-butanol (12.88 mmol), water (2.68 mL), toluene (5.37 mL) and 4 drops of the phase transfer catalyst Aliquat 336 (a quaternary ammonium salt) was prepared and then injected into the Schlenk tube. The reaction mixture was heated up to 80°C and left to stir for 18 hours. A liquid-liquid separation was performed using dichloromethane and water and the product was collected in the dichloromethane layer; then, the product was isolated using a silica gel column run with ethyl acetate. The product was a brown solid (91.3%). 1H NMR (400MHz, CDCl3): δ 2.01 (s, 6H), 7.46 (d, 4H), 7.50 (s, 2H), 8.62 (d, 4H)

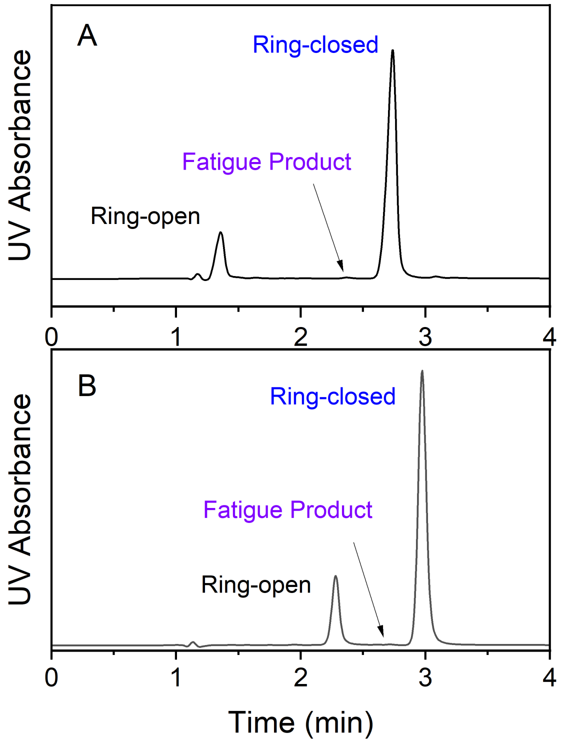

Figure S2. HPLC chromatograms showing the separation of the ring-open, fatigue product, and ring-closed isomers of the DAE-F-Pyr photoswitch compound using (A) acidified mobile and (B) non-acidified mobile phase. HPLC column: C18 silica-based (1.7 μm particle, 2.1 mm i.d. x 100 mm length), mobile phase: 85:15:0.1 ACN: H_2O: formic acid (v/v/v) for panel A and without formic acid in panel B), flow rate: 0.2 mL/min, UV detection: 254 nm, column temperature: 30 ^{o}C, injection volume: 2 μL.

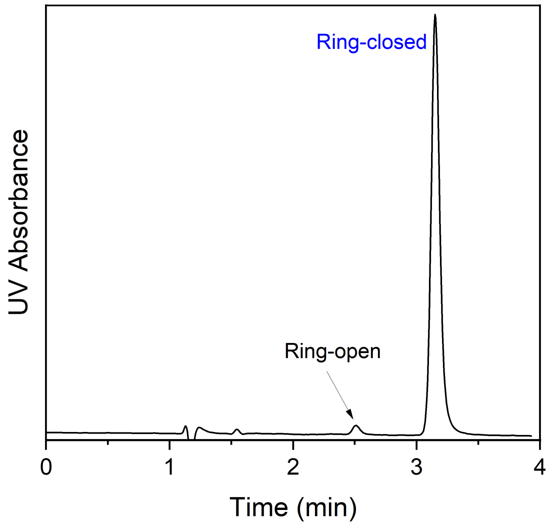

Figure S3. HPLC chromatogram illustrating the appearance of the ring-open DAE-F-Pyr isomer after leaving the ring-closed sample at ambient condition in an amber vial overnight. HPLC column: C18 silica-based (1.7 μm particle, 2.1 mm i.d. x 100 mm length), mobile phase: 85:15 ACN: H_2O (v/v), flow rate: 0.2 mL/min, UV detection: 254 nm, column temperature: 30 ^{o}C, injection volume: 2 μL.

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2. Hiroto S., Suzuki K., Kamiya H., Shinokubo H., Synthetic protocol for diarylethenes through suzuki-miyaura coupling. Chem. Commun. 2011;47:7149-7151.

3. Lim S., An B., Park S., Bistable photoswitching in the film of fluorescent photochromic polymer: Enhanced fluorescence emission and its high contrast switching. Macromolecules. 2005;38:6236-6239.