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Tailoring Photoisomerization Pathways in Donor–Acceptor Stenhouse Adducts: The Role of the Hydroxy Group

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ABSTRACT: Donor–acceptor Stenhouse adducts (DASAs) are a rapidly emerging class of visible light-activatable negative photochromes. They are closely related to (mero)cyanine dyes with the sole difference being a hydroxy group in the polyene chain. The presence or absence of the hydroxy group has far-reaching consequences for the photochemistry of the compound: cyanine dyes are widely used as fluorescent probes, whereas DASAs hold great promise for visible light-triggered photoswitching. Here we analyze the photophysical properties of a DASA lacking the hydroxy group. Ultrafast time-resolved pump–probe spectroscopy in both the visible and IR region show the occurrence of E−Z photoisomerization on a 20 ps time scale, similar to the photochemical behavior of DASAs, but on a slower time scale. In contrast to the parent DASA compounds, where the initial photoisomerization is constrained to a single position (next to the hydroxy group), 1H NMR in situ-irradiation studies at 213 K reveal that for nonhydroxy DASAs E−Z photoisomerization can take place at two different bonds, yielding two distinct isomers. These observations are supported by TD-DFT calculations, showing that in the excited state the hydroxy group (pre)selects the neighboring C2−C3 bond for isomerization. The TD-DFT analysis also explains the larger solvatochromic shift observed for the parent DASAs as compared to the nonhydroxy analogue, in terms of the dipole moment changes evoked upon excitation. Furthermore, computations provide helpful insights into the photoswitching energetics, indicating that without the hydroxy group the 4π-electrocyclization step is energetically forbidden. Our results establish the central role of the hydroxy group for DASA photoswitching and suggest that its introduction allows for tailoring photoisomerization pathways, continued...
**INTRODUCTION**

Polymethine dyes, and more specifically cyanine dyes (Figure 1a), have been used for a plethora of applications, such as material science, and chemosensing and continue to be essential for modern cellular biology. Hallmarks of this structurally highly diverse class of photochromic molecules include high extinction coefficients, high fluorescence quantum yields and photostability. Since their introduction, they have been used for photography based on silver halides and in optical discs. More recently— together with other fluorescent dyes—they have had a profound impact on cellular biology as labels and fluorescent probes. Merocyanines (Figure 1a) have additional properties, including a pronounced solvatochromism and a large change in dipole moment upon excitation, which make them ideal chromophores for developing novel materials for optoelectronics and nonlinear optical applications. They contain donor and acceptor moieties connected by a polyene chain, giving rise to a push–pull system. The charge transfer between donor and acceptor renders them strongly colored and sensitive to the polarity of the environment. Besides being fluorescent, polymethine dyes can undergo E−Z isomerization and in some cases light-mediated additions of nucleophiles to the polymethine backbone. They contain donor and acceptor moieties connected by a polyene chain, giving rise to a push–pull system. The charge transfer between donor and acceptor renders them strongly colored and sensitive to the polarity of the environment. Besides being fluorescent, polymethine dyes can undergo E−Z isomerization and in some cases light-mediated additions of nucleophiles to the polymethine backbone.

Photoswitches rely on light as an external stimulus to undergo a reversible change in structure, absorption spectrum, dipole moment, polarity, and charge. The possibility of exerting light control over molecular properties enables numerous applications. Herein, fluorescence is usually an undesired property (with notable exceptions) because it reduces the photochemical quantum yield of isomerization. Recently, donor–acceptor Stenhouse adducts (DASAs) have been introduced (Figure 1). DASAs are negative photochromes with a tunable absorption maximum in the visible to near-infrared window. They are easily synthesized and exhibit very little absorption around 300 and 500 nm, a property that has been employed for orthogonal photoswitching. Upon irradiation, the colored elongated triene A cyclizes to a colorless form B that then thermally opens to give back A (Figure 1a). The initially designed DASAs could be reversibly switched in aromatic solvents, but no other organic media, and underwent irreversible cyclization in polar solvents, such as water or methanol. Following investigations on their photochromic behavior, structural modifications in both the donor and acceptor moiety have been introduced, aimed at improving their photochromic activity, resulting in second generation photochromes. Reversible switching of such derivatives is not limited to aromatic aprotic solvents and can be extended to solid matrices (e.g., poly(methyl methacrylate)). Importantly, DASA photoswitches incorporate vital aspects of merocyanine dyes: they are based on a cyclic acceptor (Meldrum’s acid or 1,3-dimethylbarbituric acid) connected to a secondary amine-donor through a polyene, with one noticeable difference, the hydroxy group in the C2 position.

Recent efforts by our group have shed light on the DASA photoswitching mechanism and especially the structural details of the actinic step (Figure 1b). Applying a combination of ultrafast spectroscopy and theoretical DFT calculations, the nature of a short-lived intermediate A’ was identified which is produced following light absorption from DASAs and which allows the system to evolve between the initial elongated triene form A and the colorless cyclized form B (Figure 1b). The use of time-resolved IR spectroscopic techniques enabled the interpretation of the structural modification induced by light absorption as the result of a photochemical Z−E isomerization of the C2−C3 bond (red, Figure 1b). A subsequent bond rotation around C3−C4 (to form A”) arranges the molecule’s structure to facilitate the formation of the cyclized product B. The latter step has been interpreted in terms of a thermal 4π-electrocyclic rearrangement. This mechanism is operative in chlorinated solvents for both first and second generation DASAs, although the time scale and kinetics of the intermediate formation is different between the two families of molecules. The thermal electrocyclization step is reminiscent of both the (aza-)Piancatelli rearrangement, as suggested by Read de Alanz and co-workers and of the iso-Nazarov cyclization. De Lera and co-workers have provided an in-depth computational study on the cyclization mechanism of hydroxypentadienyl cations relevant for these type of reactions and suggested an operative electrocyclization mechanism for the polarized pentadienyl cation in favor of an ionic mechanism.

The fundamental understanding of the role of the hydroxy moiety is crucial to elucidate the photochemical differences between DASAs and their related cyanines. To clarify its effect on the polyene chain and its photochemistry, we report here a donor–acceptor Stenhouse adduct lacking the hydroxy group (compound 1, Figure 1c). Considering the photoswitching mechanism proposed for its parent DASA structure, the removal of the OH functionality is likely to prevent the thermal ring closure. Indeed, previous photochemical investigations on related merocyanine dyes have shown the occurrence of a photoisomerization reaction, but no observed cyclization. Thermal cyclization in the dark is known but only occurs at elevated temperature. Our results confirm these observations and shed further light on the structure–property relationship of both merocyanines and DASAs. Using a combination of time-resolved spectroscopy, in both the visible and IR spectral ranges, NMR in situ-irradiation experiments, and theoretical DFT calculations, we have characterized the photochemistry of compound 1 (nonhydroxy DASA, Figure 1c) and compared it with that of compound 2 (parent DASA, Figure 1b). We find that compound 1 undergoes isomerization around bond C2−C3 (orange, Figure 1c) but also around C3−C4 bond (olive, Figure 1c). This is in contrast to DASAs that were only found to isomerize around C2−C3 bond (red, Figure 1b) adjacent to the hydroxy group and to cyclize to form cyclopentenones (B form). Time-resolved spectra recorded for compound 1 in both the visible and infrared spectral range, are qualitatively similar to those of the related DASA compound 2, thus confirming the occurrence of a light-induced isomerization. The time constant for the isomerization process is, however, larger for the nonhydroxy analogue: the process occurs on a 20 ps time scale for compound 1 as compared to a 2 ps time scale observed for compound 2 (in chloroform). Furthermore, compared to that of DASA 2, the fluorescence increases for compound 1 (Supporting Information section 7).
Recently, Qu and co-workers have attached a 1,8-naphthalimide fluorophore to create a DASA-fluorophore conjugate for fluorescence modulation.44 Taken together, these results show that the hydroxy group in DASAs serves as a “selector” of the bond to isomerize by polarizing the polyene chain weakly in the ground state and strongly in the excited state. The strong hydrogen bond between the hydroxy group and the adjacent carbonyl of the acceptor moiety also prevents large structural distortions in the excited state as well as in the C3–C4 bond rotation step (Figure 1b). The latter step eases the formation of the intermediate A″ that prepares the structure for a ring-closure via a 4π-electrocyclic conrotatory rearrangement. In this step, the presence of the hydroxy group is vital, because it takes part in the proton-transfer process and contributes in stabilizing the charge distribution along the polyene chain driving the electronic rearrangement which ends up with the ring closure reaction.

## RESULTS AND DISCUSSION

### Steady-State Spectroscopy.

To elucidate the responsive switching behavior of DASA 1, photoisomerization was studied by steady-state spectroscopy (for the synthesis of 1; see Supporting Information section 2). Figure 2 shows the UV/vis absorption spectra of compound 1 measured in different solvents (Supporting Information section 3) and for comparison the solvatochromic shifts of 2.

Compared to the spectra of the parent DASA compound 2 (Figure 2c), the absorption band of compound 1 (Figure 2b) undergoes a hypsochromic shift in all solvents, indicating that the removal of the hydroxy functional group increases the gap between the HOMO and LUMO orbitals involved in the S0–S1 transition, which are mainly localized on the conjugated triene chain (Figure 3a,b). The observed shifts are in good agreement with theoretical values (e.g., −0.27 eV vs −0.36 eV for toluene; Table S5) obtained by the TD-M06-2X method using the 6-311++G(2df,2p) basis set in combination with the universal continuum solvation model based on solute electron density (SMD) and the corrected linear response (cLR) approach to account for the solvent effects on both ground-state (GS) and excited-state (ES) electron densities. A red shift of the absorption as a function of decreasing polarity is observed among the investigated aprotic solvents, whereas the absorption band appears notably blue-shifted in protic media such as water and methanol. The presence of the protic group in 2 leads to a generally stronger solvatochromism (Figure 2c). The observed trends are again well reproduced by the TD-DFT/cLR/SMD calculations (Table S5).

The FTIR spectrum of compound 1 is reported in Figure 4, where the spectrum of 2 is also shown for comparison. Furthermore, Figure 4 reports the simulated FTIR spectra for both molecules obtained at the B3LYP/6-31++G(d,p)/SMD level using the harmonic approximation (fwhm = 12 cm⁻¹; scaling factor 0.98, Supporting Information section 9.2). It can be seen that spectra computed for A of both 1 and 2 satisfactorily reproduce all main features of the measured spectra, confirming that both molecules in the ground state attain a similar elongated triene conformation (Supporting Information section 9.1). Analysis of mode composition, as obtained by DFT, suggests that the differences between the FTIR spectra of 1 and 2, in the reported

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**Figure 1.** Polymethine dyes and donor–acceptor Stenhouse adducts (DASAs): structural overview (a); proposed photoswitching mechanism of DASAs (b); related DASA compound lacking a hydroxy group (c).
frequency region, mainly arise from a different character of the C−C bonds along the conjugated chain, which is affected by the presence of a hydroxy group, and from the possibility to form a hydrogen bond between the hydroxy group and the acceptor carbonyl group in 2. Whereas in 1 the bond length alternation indicates efficient π-conjugation, in the case of 2 the C(A)−C1 (A = Acceptor) and C2−C3 bonds are noticeably longer (Figure 3c,d). The weakening of these two bonds brings about a small red shift of the 1160 cm⁻¹ peak, which mainly accounts for the C1−C2/C2−C3 asymmetric bond stretching (coupled with the C4−C5 stretching and C−H rocking), and also more pronounced shifts of the ca. 1350−1380 and 1500 cm⁻¹ bands associated predominantly with the C(A)−C1 stretching and C(A)−C1/C1−C2 asymmetric stretching modes, respectively. A significant increase of the intensity of a band at ca. 1500 cm⁻¹ is also observed in the case of compound 1.

Figure 2. Comparison of UV/vis absorption spectra of compound 1 and 2 in different solvents: (a) compound 1 and 2 in toluene; (b) solvatochromism of compound 1; (c) solvatochromism of compound 2.

Figure 3. Electronic density difference (EDD) plots between the ES and the GS of A for compounds 1 (a) and 2 (b) in chloroform obtained at the M06-2X/6-311++G-2df,2p)/SMD level of theory. The blue (red) regions correspond to a decrease (increase) in electron density upon electronic transition. A contour threshold of 0.001 au has been applied. Bond lengths (Å) are depicted for GS (blue) and ES (red) structures of the A form for 1 (c) and 2 (d) in chloroform. GS and ES geometries correspond to geometries optimized at the B3LYP/6-31++G-(d,p)/SMD and D-M06-2X6-31+G-(d)/SMD levels of theory, respectively.
Further differences are noted in the 1600−1700 cm$^{-1}$ region: in particular, the band observed at 1623 cm$^{-1}$ in 2 almost disappears, while a band at 1674 cm$^{-1}$ appears in the spectrum of the nonhydroxy analogue 1. Our previous DFT analysis of 2 showed that there is a strong H-bond between the OH group linked to the triene chain and one of the carbonyl groups of the acceptor ring. 1H NMR spectroscopy confirms the facile exchangability of this proton (Figure S5). The observed spectral changes in the carbonyl region suggest the assignment of the 1623 cm$^{-1}$ band to the H-bonded ring carbonyl stretch in the case of 2, which, upon the removal of the hydrogen bond in 1, blue-shifts to 1675 cm$^{-1}$, indicating a very strong hydrogen bond. This assignment is further supported by the presence of a band at 1700 cm$^{-1}$ in 2, due to the second non H-bonded carbonyl. The occurrence of a strong H-bond interaction in DASAs is also evidenced by the inspection of the FTIR spectra of compounds 1 and 2 in the 2500−3500 cm$^{-1}$ region, which show for 2 a broad feature at ca. 2900 cm$^{-1}$ possibly due to the OH-stretch band (Figure S4). As yet, this H-bond has not received extensive attention, but it is clear that it will have mechanistic implications as it will in the point of view, studies of DASA derivatives in which the strength of such a hydrogen bond can be modulated would be highly interesting.

To assess the usefulness of 1 as photoswitch, UV/vis spectroscopy with irradiation under steady-state conditions was performed (Figure S5, Supporting Information section 6). No photobleaching of the sample is observed upon irradiation, as would be the case for 2. However, a photostationary state is rapidly reached and maintained under irradiation. After irradiation is stopped, rapid relaxation is observed. A bathochromically shifted absorption band is temporarily apparent, reminiscent of the intermediate formed in the photoswitching of 2.33 Solvent-polarity influences the extent of this red-shift (Supporting Information sections 6 and 7). Compound 1 shows clean photoswitching throughout a range of solvents (toluene, dichloromethane, chloroform, methanol, dimethyl sulfoxide, acetone, water, and PBS buffer), with very little fatigue observed in aqueous environments.

**Time-Resolved Spectroscopy.** Time-resolved spectroscopy allows detailed insights into the structure and dynamics of the compound in question during the actinic step. The EADS (evolution-associated difference spectra) obtained by global analysis of visible pump–probe data recorded for compound 1 in chloroform upon $\lambda = 480$ nm light excitation (Supporting Information section 8) are shown in Figure 6. As evidenced from photoaccumulation experiments (Figure 5, Supporting Information section 6), the sample does not cyclize irrespective of the solvent. Nonetheless, a long-lived bathochromically shifted band peaking at 512 nm appears in the transient spectra ca. 20 ps after light absorption and it closely resembles that assigned to the Z$\rightarrow$E isomerized intermediate (A$'$) observed in the parent DASA 2.33 The EADS shown in Figure 6 closely follow those reported for DASA 2.36 As noticed from inspection of Figure 6, in chloroform a bleaching band peaked at 500 nm, promptly appears upon photoexcitation. The signal intensity partially recovers in 2 ps; then, on a time scale of 20 ps, a positive band, peaking at 512 nm, appears in the transient spectra (see the blue EADS component reported in Figure 6a). The EADS obtained from transient absorption measurements repeated in different solvents are reported in the Supporting Information (Figure S34). The qualitative appearances of the transient signal and the observed spectral evolution do not significantly change when the solvent is changed. In all cases, a positive band peaking at 500−515 nm is observed in the long-lived spectral component. However, the maximum absorption band of the photoproduct and the observed shift with respect to the ground-state absorption both depend on the solvent. This explains why in methanol, where the ground-state absorption band is very broad, a bathochromically shifted band is hardly seen in steady-state photoaccumulation experiments (Supporting Information section 6.4). The quantum yields for the photoisomerization of 1 estimated from the ultrafast measurements are 6% (toluene), 11.9% (dichloromethane), 13.8% (chloroform), 8.6% (methanol), and 7% (dimethyl sulfoxide).

The dynamics of photoproduct formation is influenced by solvent polarity. A comparison of the kinetic traces recorded at the maximum absorption of the photogenerated species is reported in Figure 6b, showing that the rate of appearance of the positive peak in the transient spectra slows down upon increasing...
the polarity of the solvent. The time constant for the photo-
product formation is in fact 8.5 ps in toluene and increases to 56 ps
in dimethyl sulfoxide, where, besides a polarity effect, also the
increased viscosity could play a role. It is, however, worth noticing
that even in the case of toluene the photoproduct is formed with a
slower rate than for the parent DASA molecule 2 (in that case the
intermediate is observed on a 2 ps time scale) and no further
spectral evolution of the product band is observed in any solvent.

To further characterize the nature of the photoproduct obtained
upon light absorption for compound 1, picosecond transient infrared spectra were recorded. IR-based techniques are
particularly suited to shed light on changes occurring in molecular structures upon photoexcitation and have been widely used to
investigate light induced structural changes in polyene systems.36,52 The EADS obtained from global analysis50 of transient
spectra acquired between 1100 and 1750 cm⁻¹ are reported in
Figure 7. In the case of transient infrared measurements the sample
has been excited on the red edge of its absorption band, at 510 nm.

The initial spectral component (black EADS in Figure 7a)
shows several negative bands, whose position well corresponds
to the bleaching of ground-state modes. As already noticed in the
case of the previously studied DASA photoswitches, very few
excited-state absorption bands are observed.36 DFT calculations
of the vibrational spectra in the excited state have shown a strong
decrease of intensity of almost all the vibrational bands occurring
in the excited state, and only minor shifts compared with ground-
state band positions, which explains the predominance of negative signals in the transient spectrum.36 In the carbonyl
stretching region (1600–1750 cm⁻¹), the excited-state bands are
clearly visible. In this region, three positive signals at 1606, 1635,
and 1694 cm⁻¹ are observed. On a 7 ps time scale, the intensity of the bleaching signals decreases, while the observable positive
bands slightly sharpen and blue-shift. This spectral behavior
allows assigning the 7 ps component to vibrational cooling in the
excited state. The initial evolution appears to be slower than what is observed in transient visible measurements, which may be due
to the different excitation conditions used in this case, preventing
excess vibrational energy in the S₁ state. The following evolution,
 occurring on a 21.5 ps time scale, is associated with a further
recovery of the bleaching signals and to the appearance of a few
more positive bands. In particular, small absorption bands at 1190, 1236 1340, and 1421 cm⁻¹ develop (Figure 7b). The spectrum of the long-living spectral component extracted from the globally analyzed time-resolved data of compound 1 resembles that observed for the parent DASA photoswitch 2 and is assigned to a photogenerated intermediate resulting from
E–Z isomerization. In the present case, however, two positive
bands are noticed in the 1190–1240 cm⁻¹ region. Moreover, the
differential signal at 1160(−)/1190(+) cm⁻¹ is less pronounced if compared to that observed for compound 2, possibly because
the negative signal is in this case much broader. DFT simulations
reproduce all main features of the observed spectrum (Figure 7c), except for predicting a negative signal at ca. 1200 cm⁻¹,
which is a consequence of an overestimated absorption intensity of A in its GS (cf. Figure 4). Nevertheless, the similarity between the spectral evolutions of the transient infrared signal of compounds 1 and 2 leads us to conclude that the photoproducts obtained in systems with and without the OH functionality have similar
natures and result from a photosisomerization event.

1H NMR in Situ-Irradiation Experiments. The structure of the photoproduct has been further investigated by performing
NMR in situ-irradiation studies at low temperature (203-213 K).
Notably, irradiation (470 nm LED, Thorlabs, M470F3) of 1 at
213 K in deuterated dichloromethane (Supporting Information
section 10.1.1) leads to the reversible formation of two isomers
A′(A) and A′(B) (Figure 8). Full structural assignment of these
two isomers was performed in deuterated acetone (Supporting Information section 10.1.2) due to overlapping NMR signals of
isomer A′(B) with the parent compound 1 in deuterated dichloromethane (Figure S49). 2D-NMR spectroscopy under irradiation (1H,1H-COSY, 1H,1H-TOCSY and 2D-NOESY) and analysis of the coupling constants between the polype monos
suggest a comparable behavior of 1 in deuterated dichloromethane and deuterated acetone (Supporting Information
sections 10.1.1 and 10.1.2), which is also observed by UV/vis
spectroscopy (Supporting Information sections 6.2 and 6.6).

Although coupling constants in the parent compound are in the
range 11–14 Hz indicative of a linear, elongated configuration, lower coupling constants (<10 Hz) strongly suggest a Z-
configuration for 1 after photoisomerization. We thus conclude that the two isomers A′(A) and A′(B) differ in the position of the Z-bond within the polype chain (C₂–C₃ vs C₃–C₄). This is supported by the through-space connectivity deduced from
nuclear Overhauser spectroscopy (Supporting Information
sections 10.1.1.5 and 10.1.2.4). For clarity, A′(B) is drawn in its
resonance form, noting the double bond character throughout the conjugated system (see discussion of bond lengths above). Upon cessation of irradiation, both isomers revert back to A. The fact that two independent isomers are formed is remarkable, as for DASA 2 only one isomer is formed.
with 530 nm (LED, Thorlabs, MS30F2) indeed accelerates the back-relaxation to A (Supporting Information section 10.1.2.6). Temperature-dependent $^1$H NMR (Supporting Information section 10.3) and UV/vis studies (Supporting Information section 10.4) show different thermal behavior of the two isomers, accounting for the observed change in photostationary states achieved under irradiation at different temperatures, and different rates of thermal relaxation. Although $\Lambda'(B)$ dominates over $\Lambda'(A)$ in steady-state concentration under irradiation at 213 K in acetone-$d_6$, the ratio changes in favor of $\Lambda'(A)$ with increasing temperature, leading to an almost disappearing signal of $\Lambda'(B)$ at 233 K (Figure S79 and S81).

TD-DFT calculations support these findings. The computed energies for ground and excited states of the various isomers and the transition states for the interconversion between the different species are summarized in Table S4. The photoisomerizations are likely to be reversible as was previously shown for DASA 2. $^{30}$ Importantly, the thermal stability of the isomers (especially of $\Lambda'(A)$) lies in the range of what was observed before for 2. $^{33}$ Given the calculated energies, the formation of $\Lambda''$ could potentially be possible. However, none of the employed spectroscopic measurements (NMR, TRIR) indicates that this is actually occurring.

To rationalize the lack of cyclization from compound 1, we analyzed the change of bond lengths and charge distribution during the transformation from A to B (Supporting Information section 9.1). Our calculations show that the presence of the hydroxy group is essential to polarize the polyene chain in the correct way to drive cyclization, once the molecule reaches the $\Lambda''$ configuration. Furthermore, because a proton transfer is expected to take place during cyclization, the activation barrier for the proton-transfer cyclization step for compounds 1 and 2 was estimated (Figure 9). Two potential energy curves along the reaction coordinate related to the $C_1-C_5$ interatomic distance have been calculated at the B3LYP/6-31+G(d)/SMD level by constrained optimizations of all coordinates, with the $C_1-C_5$ bond length kept frozen, using methanol as a typical protic environment (Figure 9). The green curves shown in Figure 9 describe the ring closure in the $\Lambda''$ form, and the blue curves refer to the ring opening in the B form. The crossing point of the two curves enables us to estimate a barrier for intermolecular proton transfer (mediated by protic solvent). It can be seen that the barrier for cyclization of compound 1 is very high (ca. 34 kcal/mol). However, in the case of compound 2 the presence of the hydroxy group clearly facilitates the proton transfer and electrocyclization, decreasing the activation barrier to ca. 26 kcal/mol. A proton-transfer step is important, as Stenhouse salts lacking such a strong hydrogen bond show strongly pH-dependent photoswitching. $^{42}$ In addition, our calculations suggest that the presence of the OH stabilizes a partial positive charge on carbon $C_5$ and a small negative charge on carbon $C_1$, favoring the ring closure step (Supporting Information section 9.1). This is not possible for compound 1, lacking the hydroxy group, majorly contributing to the observed higher barrier for cyclization of 1 as compared to 2.

## CONCLUSIONS

The presented experimental and theoretical analyses have elucidated the important role of the hydroxy substituent on the DASA photoswitching capacity, focusing in particular on the intermediate products originating from the actinic step. Our experimental and theoretical work shows that the group acts by restricting the reaction coordinate related to the $C_1-C_4$ bond (Figure 1).

The presented experimental and theoretical analyses have elucidated the important role of the hydroxy substituent on the DASA photoswitching capacity, focusing in particular on the intermediate products originating from the actinic step. Our experimental and theoretical work shows that the group acts by restricting the reaction coordinate related to the $C_1-C_4$ bond (Figure 1).
Figure 8. NMR in situ-irradiation experiments of compound 1 reveal two sets of photogenerated peaks corresponding to isomers A'(A) (green) and A'(B) (gray). Acetone-d₆ was used to verify the coupling constants, as in deuterated dichloromethane some signals were overlapping. Formation of A'(A) and A'(B) isomers is reversible and light-dependent. A schematic representation of the relevant spectra (obtained by line fitting) is provided for clarity.

Figure 9. Potential energy curves for the proton-transfer cyclization step, i.e., A'' to B transformation for compounds 1 (a) and 2 (b) computed at the B3LYP/6-31+G(d)/SMD level of theory. The green curve corresponds to the ring closure in the A'' form, and the blue curve corresponds to the ring opening starting from the B form. The reaction coordinate (Å) is defined as the negative relative distance of carbon atoms with respect to the equilibrium C₁···C₅ bond distance in A''. The crossing point of the green and blue curves indicates the barrier for intermolecular proton transfer. The star point on the green curve in (b) corresponds to an intramolecular proton-transfer configuration. The fact that it is positioned behind the crossing point implies that H⁺ is preferentially transferred to the environment or to a neighboring DASA molecule. See also analysis of bond lengths and charge redistribution during the cyclization step in Supporting Information section 9.1.
a decreased electron density and elongation of that particular bond (particularly in the ES). Notably, in the case of the nonhydroxy analogue 1, two isomers $A'(A)$ and $A'(B)$ are formed at low temperature under irradiation in photoaccumulation experiments as observed by NMR spectroscopy. Time-resolved UV/vis and IR spectroscopy have provided spectroscopic insights into details and time scales of the isomerization pathway. They fully support the occurrence of an $E-Z$ isomerization upon photoexcitation and support the formation of two photoswitched isomers, although the similarity of the DFT-predicted IR spectra of $A'(A)$ and $A'(B)$ impedes an unambiguous distinction of the two isomers. The comparison of the photosomerization kinetics of 1 and 2 shows that the nonhydroxy analogue 1 isomerizes about 10 times slower than the parent DASA. The increased photosomerization rate observed for compound 2 can be rationalized in terms of the influence that the hydroxy substituent has on the triene bond length alternation and through steric effects. A somehow related effect has been previously observed in the case of the rhodopsin chromophore, where the introduction of a methyl substituent accelerates the photoisomerization rate in solution.\(^5\) Overall, our results show that by introducing a hydroxy group in the $C_2$-position of the polyene chain one can control photoswitching pathways and favor cyclization. The role of the strong hydrogen-bond in the proton-transfer step leading to B in compound 2 is of considerable interest but needs further study.

The presented results are directly applicable for improvements of DASA photoswitching and are important for acquiring a better understanding of the (photo)chemical properties of DASAs and cyanine dyes. One particularly attractive approach includes the substitution of the hydroxy group with other polar protic groups such as thiols and amines or polarizing groups such as halides, and varying the position of these groups. Such studies are presently being pursued.

**ASSOCIATED CONTENT**

[Supporting Information](#) This information is available via the Internet at The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.7b10255.

Experimental procedures and characterization of compounds, UV/vis absorption spectra and photoswitching studies in different solvents, fluorescence spectra, FTIR spectra and ultrafast visible and mid-IR spectroscopy; EADS (evolution associated difference spectra) obtained by global analysis; TD-DFT computations and NMR in situ irradiation experiments (PDF)

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**Notes**

The authors declare no competing financial interest.

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