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# **Tailoring Photoisomerization Pathways in Donor-Acceptor Stenhouse Adducts: The Role of the Hydroxyl Group**

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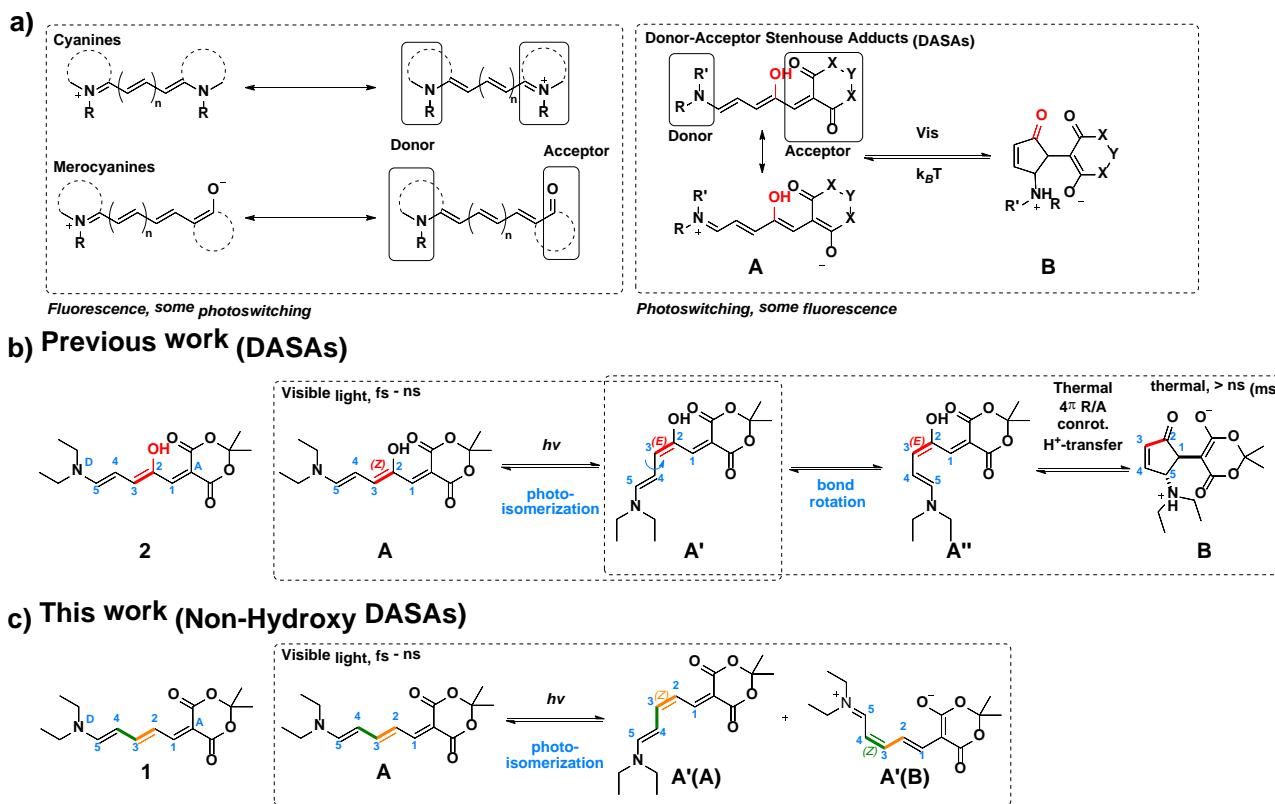
**Keywords:** photoswitching, donor-acceptor Stenhouse adducts, (mero)cyanine, hydroxy group, electronic effects, photoswitching mechanism, time-resolved spectroscopy, TD-DFT, NMR *in situ*-irradiation

**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*-photo-isomerization on a 20 ps timescale, revoking familiarities with the photochemical behavior of DASAs, but on a slower time-scale. In contrast to the parent DASA compounds, where the initial photo-isomerization is constrained to a single position (next to the hydroxy group), <sup>1</sup>H-NMR *in situ*-irradiation studies at 213 K reveal that for non-hydroxy DASAs *E-Z* photo-isomerization 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 C<sub>2</sub>-C<sub>3</sub> bond for isomerization. The TD-DFT analysis also explains the larger solvatochromic shift observed for the parent DASAs as compared to the non-hydroxy 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 photo-isomerization pathways, presumably both through (steric) fixation *via* a hydrogen bond with the adjacent carbonyl group of the acceptor moiety, as well as through electronic effects on the polyene backbone. These insights are essential for the rational design of novel, improved DASA photoswitches and for a better understanding of the properties of both DASAs and cyanine dyes.

## **Introduction**

Polymethine dyes, and more specifically cyanine dyes (Figure 1a),<sup>1</sup> have been used for a plethora of applications, such as materials<sup>2,3</sup> and chemosensing,<sup>4</sup> and continue to be essential for modern cellular biology.<sup>5-7</sup> 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.<sup>2</sup> More recently – together with other fluorescent dyes – they have had a profound impact on cellular biology as labels and fluorescent probes.<sup>8,9</sup> 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 non-linear optical applications.<sup>10</sup> 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.<sup>11–14</sup> Computationally, these systems are difficult to tackle and have been found to pose quite a challenge in reproducing their spectroscopic properties and correctly estimating their transition energies.<sup>15</sup>



**Figure 1:** Polymethine dyes and donor-acceptor Stenhouse adducts (DASAs): Structural overview (a); Proposed photoswitching mechanism of DASAs (b) and a related DASA compound lacking a hydroxy group (c).

Photoswitches<sup>16</sup> rely on light as an external stimulus to undergo a reversible change in structure, absorption, dipole moment, polarity and charge.<sup>17</sup> The possibility of exerting light control over molecular properties enables numerous applications.<sup>18–25</sup> Herein, fluorescence is usually an undesired property (with notable exceptions<sup>26,27</sup>) since it reduces the photochemical quantum yields of isomerization. Recently, donor–acceptor Stenhouse adducts (DASAs) have been introduced (Figure 1).<sup>28,29</sup> 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 between 300 and 500 nm, a property that has been employed for orthogonal photoswitching.<sup>30</sup> 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.<sup>29</sup> Following the investigations on their photochromic behavior,<sup>31–33</sup> structural modifications both in the donor and acceptor moiety have been introduced, aimed at improving their photochromic activity, resulting in second generation photochromes.<sup>34,35</sup> Reversible switching of such derivatives is not limited to aromatic non-polar, non-protic solvents and can be extended to solid matrices (poly(methylmethacrylate)).<sup>34</sup> Importantly, DASA photoswitches incorporate vital aspects of merocyanine dyes: they are based on a cyclic acceptor (Meldrum's acid or dimethylbarbituric acid) connected to a secondary amine-donor through a polyene, with one noticeable difference, the hydroxy group in the C<sub>2</sub> 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).<sup>33,36</sup> Applying a combination of ultrafast spectroscopy and theoretical DFT calculations, the

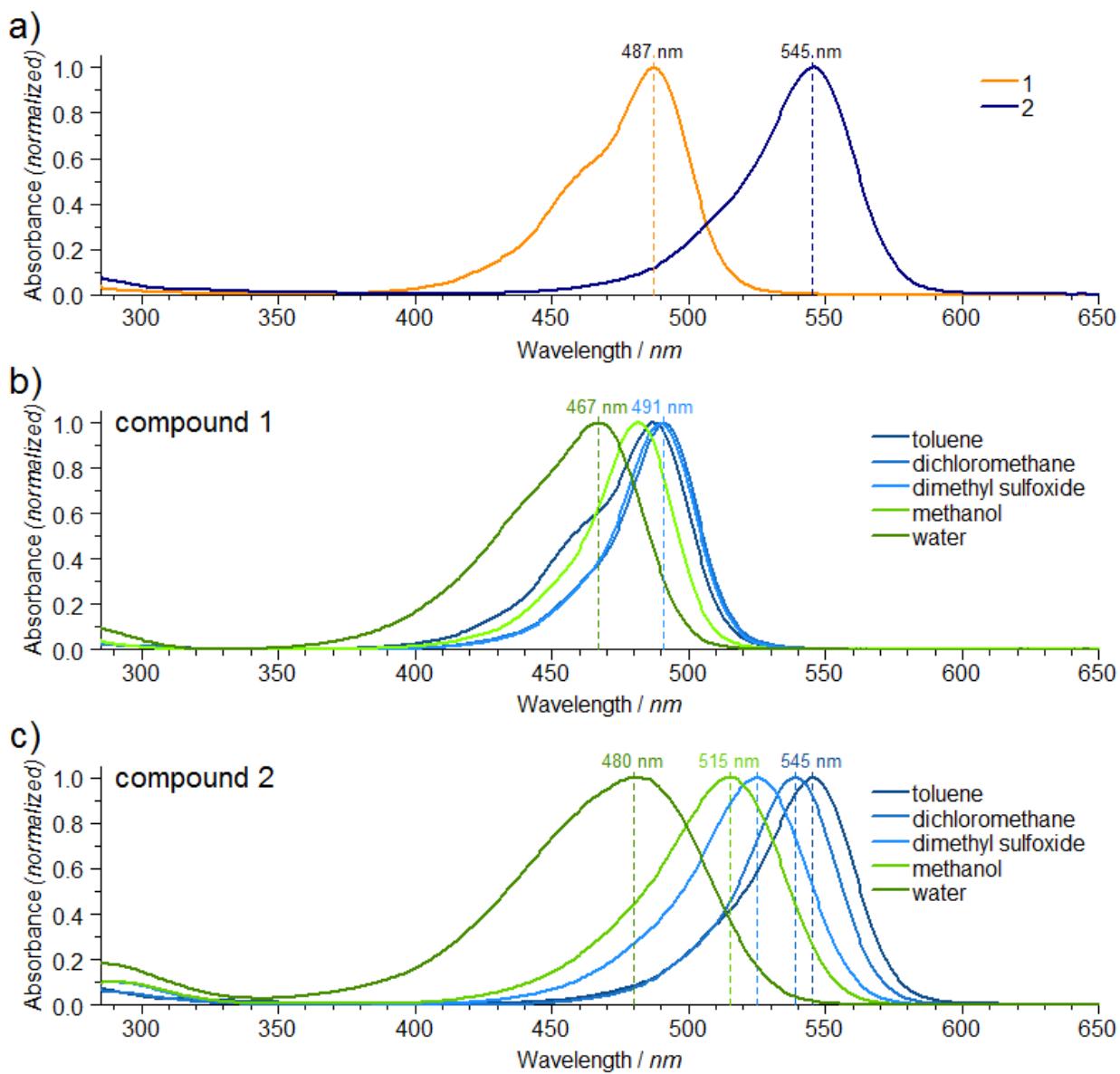
nature of a short lived intermediate **A'** was identified which is produced following light absorption from DASAs and which allows the system to evolve between the initial elongated triene form **A** and the colorless cyclized form **B** (Figure 1b). The use of time-resolved IR spectroscopic techniques enabled the interpretation of the structural modification induced by light absorption as the result of a photochemical *Z-E* isomerization of the C<sub>2</sub>-C<sub>3</sub> bond (**red**, Figure 1b).<sup>36</sup> A subsequent bond rotation around C<sub>3</sub>-C<sub>4</sub> (to form **A''**) arranges the molecule for a thermal 4π-electrocyclic rearrangement and a proton transfer leads to the cyclized product **B**.<sup>33</sup> This mechanism is operative in chlorinated solvents for both first and second generation of DASAs, although the timescale and kinetics of the intermediate formation is different between the two families of molecules.<sup>36</sup> The electrocyclization step is reminiscent both of the (aza-)Piancatelli rearrangement,<sup>37-40</sup> as suggested by Read de Alaniz and co-workers<sup>29</sup> and of the *iso*-Nazarov cyclization.<sup>41</sup> 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<sup>42</sup> and suggest an operative electrocyclization mechanism for the polarized pentadienyl cation in favor of an ionic mechanism. Importantly, the hydroxy group is expected to stabilize the transient positive charge and support the opposite polarization of C<sub>1</sub> and C<sub>5</sub>.

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 **2**,<sup>33,36</sup> the removal of the OH functionality is likely to prevent the thermal ring closure.<sup>43</sup> Indeed, previous photochemical investigation on related merocyanine dyes have shown the occurrence of photoisomerization reactions, but no observed cyclization.<sup>13,14</sup> Thermal cyclization in the dark is known, but only occurs at elevated temperature.<sup>44</sup> Our results confirm these observations and shed further light on the structure-function relationship of both merocyanines and DASAs. Using a combination of time-resolved spectroscopy, both in the visible and IR spectral ranges, NMR *in situ*-irradiation experiments and theoretical DFT calculations, we have characterized the photochemistry of compound **1** (non-hydroxy DASA, Figure 1c) and compared it with compound **2** (parent DASA, Figure 1b). We find that compound **1** undergoes isomerization around bond C<sub>2</sub>-C<sub>3</sub> (**orange**, Figure 1c) but also around C<sub>3</sub>-C<sub>4</sub> bond (**olive**, Figure 1c). This is in contrast to DASAs that were only found to isomerize around C<sub>2</sub>-C<sub>3</sub> bond (**red**, Figure 1b) adjacent to the hydroxy group and to cyclize further 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 non-hydroxy analogue: the process occurs on a 20 ps timescale for compound **1** as compared to a 2 ps timescale observed for compound **2** (in chloroform). Furthermore, compared to DASA **2** the fluorescence increases for compound **1** (SI section 6). Recently, a DASA-fluorophore conjugate has been described.<sup>45</sup> 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 C<sub>3</sub>-C<sub>4</sub> 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 last step, the presence of the hydroxy group is vital, because it takes part in the proton transfer process.

## Results and Discussion

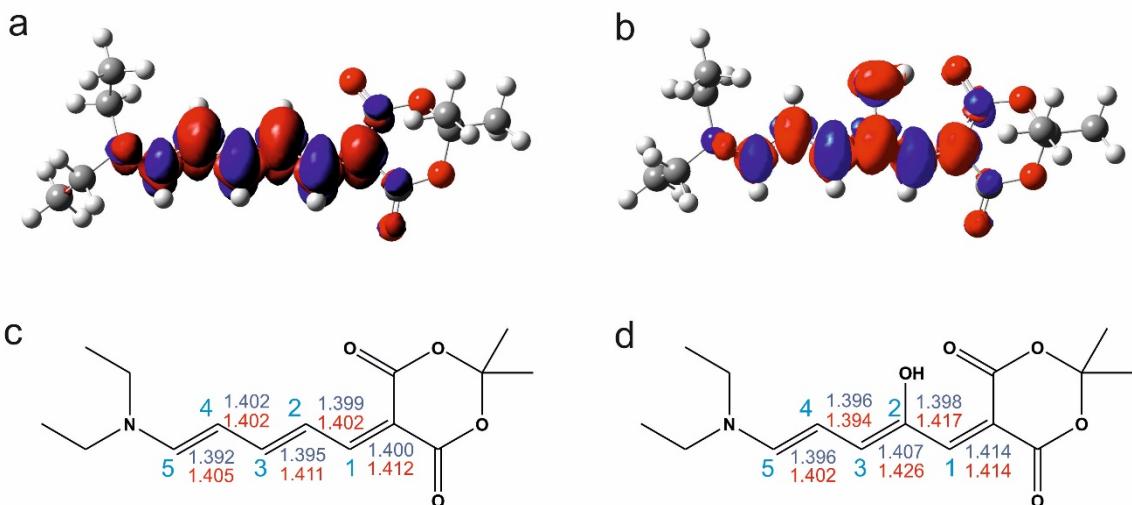
### *Steady state spectroscopy*

In order to elucidate the responsive switching behavior of DASA **1**, steady state spectroscopy and photoisomerization was studied (for the synthesis of **1**, see SI section 2). Figure 2 shows the UV-vis absorption spectra of compound **1** measured in different solvents. For comparison, the solvatochromic shifts of **2** are provided (SI section 3).



**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** and c) solvatochromism of compound **2**.

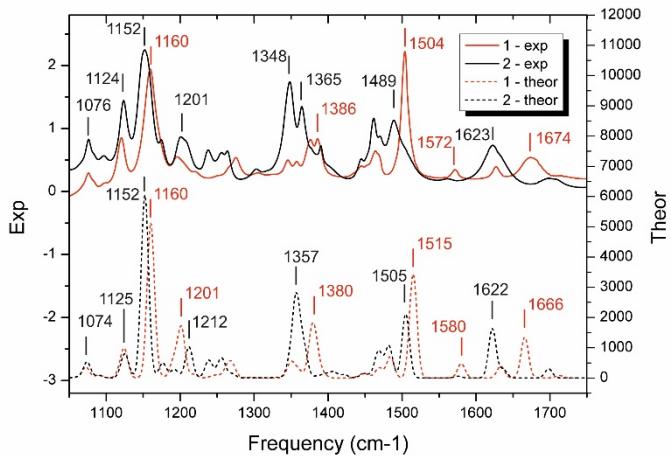
Compared to the spectra of the parent DASA compound **2** (Figure 2c), the absorption band of compound **1** (Figure 2b) undergoes a hypsochromic shift in all solvents, indicating that the removal of the hydroxy functional group increases the gap between the HOMO and LUMO orbitals involved in the  $S_0$ - $S_1$  transition, which are mainly localized on the conjugated triene chain (see Figure 3a and b). The observed shifts are in good agreement with theoretical values (*e.g.* -0.27 eV *vs.* -0.36 eV for toluene; table S8.2) obtained by the TD-M06-2X method<sup>46</sup> using the 6-311++G(2df,2p) basis set<sup>47</sup> in combination with the universal continuum solvation model based on solute electron density (SMD)<sup>48</sup> and the corrected linear response (cLR) approach<sup>49</sup> 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 polarity is observed among the investigated non-protic solvents, while 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 cLR/SMD/TD-DFT calculations (table S8.2). In particular, the stronger solvatochromism of **2** can be rationalized in terms of the changes of the dipole moment upon excitation. Whereas for **1** the change is almost negligible (0.29 D in chloroform) leading to very small solvatochromic shifts, for **2** it is noticeably larger and negative (-1.16 D in chloroform). Consequently, the more polar the solvent is, the more stabilized the GS becomes compared to the ES, leading to larger hypsochromic shifts.



**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 SMD/M06-2X/6-311++G(2df,2p) level of theory. The blue (red) regions correspond to decrease (increase) in electron density upon electronic transition. A contour threshold of 0.001 a.u. has been applied. Bond lengths (in 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 SMD/B3LYP/6-31++G(d,p) and SMD/TD-M06-2X/6-31+G(d) levels of theory, respectively.

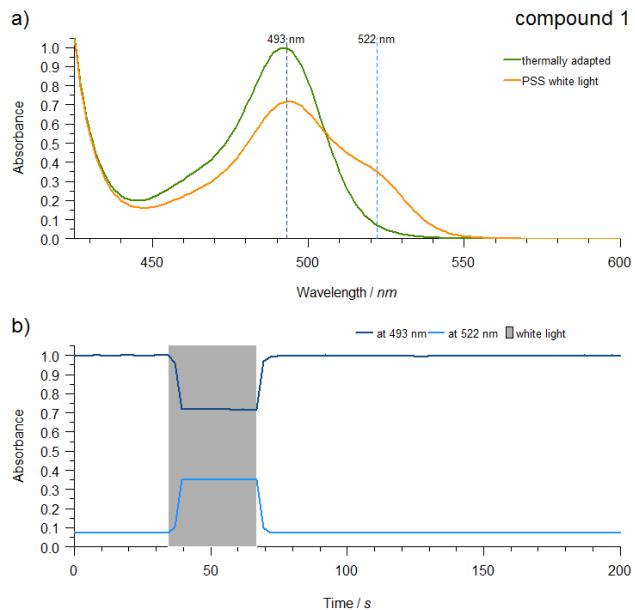
The FTIR spectrum of compound **1** is reported in Figure 4, where the spectrum of **2** is also shown for comparison. Furthermore, Figure 4 reports the simulated FTIR spectra for both molecules obtained at the SMD/B3LYP/6-31++G(d,p) level using the harmonic approximation ( $\text{FWHM} = 12 \text{ cm}^{-1}$ ; scaling factor 0.98, SI section 8.2). 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 (SI section 8.1). Analysis of mode composition, as obtained by DFT, suggests that the differences between the FTIR spectra of **1** and **2**, in the reported 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  $\pi$ -conjugation, in the case of **2** the  $\text{C}_{(\text{A})}-\text{C}_1$  ( $\text{A}=\text{Acceptor}$ ) and  $\text{C}_2-\text{C}_3$  bonds are noticeably longer (see Figure 3c). The weakening of these two bonds brings about a small red shift of the  $1160 \text{ cm}^{-1}$  peak, which mainly accounts for the  $\text{C}_1-\text{C}_2/\text{C}_2-\text{C}_3$  asymmetric bond stretching (coupled with the  $\text{C}_4-\text{C}_5$  stretching and C-H rocking), and also more pronounced shifts of the *ca.*  $1350-1380 \text{ cm}^{-1}$  and  $1500 \text{ cm}^{-1}$  bands associated predominantly with the  $\text{C}_{(\text{A})}-\text{C}_1$  stretching and  $\text{C}_{(\text{A})}-\text{C}_1/\text{C}_1-\text{C}_2$  asymmetric stretching modes, respectively. A significant increase of the intensity of a band at *ca.*  $1500 \text{ cm}^{-1}$  is also observed in the case of compound **1**.

Further differences are noted in the  $1600-1700 \text{ cm}^{-1}$  region: in particular, the band observed at  $1623 \text{ cm}^{-1}$  in **2** almost disappears, while a band at  $1674 \text{ cm}^{-1}$  appears in the spectrum of the non-hydroxy 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.<sup>31,36</sup>  $^1\text{H-NMR}$  spectroscopy confirms the facile exchangeability of this proton (SI section 4). The observed spectral changes in the carbonyl region suggest the assignment of the  $1623 \text{ cm}^{-1}$  band to the H-bonded ring carbonyl stretch in case of **2**, which, upon the removal of the hydrogen bond in **1**, blue-shifts to  $1675 \text{ cm}^{-1}$  indicating a very strong hydrogen bond. This assignment is further supported by the presence of a band at  $1700 \text{ cm}^{-1}$  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  $2500-3500 \text{ cm}^{-1}$  region, which show for **2** an OH-stretch band at *ca.*  $2900 \text{ cm}^{-1}$  (see Figure S4.1). As yet, this H-bond has not received extensive attention, but it is clear that it will have mechanistic implications as it will influence the proton transfer step to form the zwitterionic structure **B** shown in Figure 1b for the first generation DASA **2**. From this point of view, studies of DASA derivatives in which the strength of such a hydrogen bond can be modulated would be highly interesting.



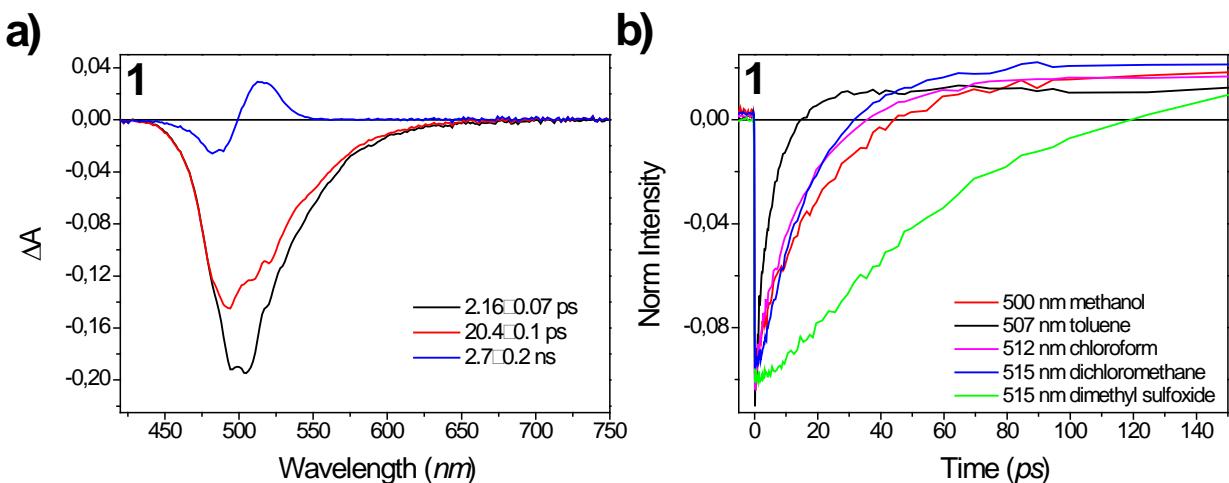
**Figure 4:** Comparison of the measured (upper) and simulated (lower) FTIR spectra of compound **1** (red line) and **2** (black line) in deuterated chloroform. For the computational details, see text.

To assess the usefulness of **1** as photoswitch, UV/vis spectroscopy with irradiation under steady-state conditions was performed (Figure 5, SI section 5). 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**.<sup>33</sup> Solvent-polarity influences the extent of this red-shift (SI section 5 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.



**Figure 5:** a) Absorption spectra for the photoisomerization of compound **1** ( $\lambda_{\text{max}} = 493 \text{ nm}$ ;  $\sim 6 \mu\text{M}$  in chloroform; 293 K) with white light (Thor-Labs, OSL1-EC, PSS = photostationary state) and b) corresponding time-evolution observed at 493 nm and 522 nm. The shaded area in grey indicates the irradiation period.

#### Time-resolved spectroscopy

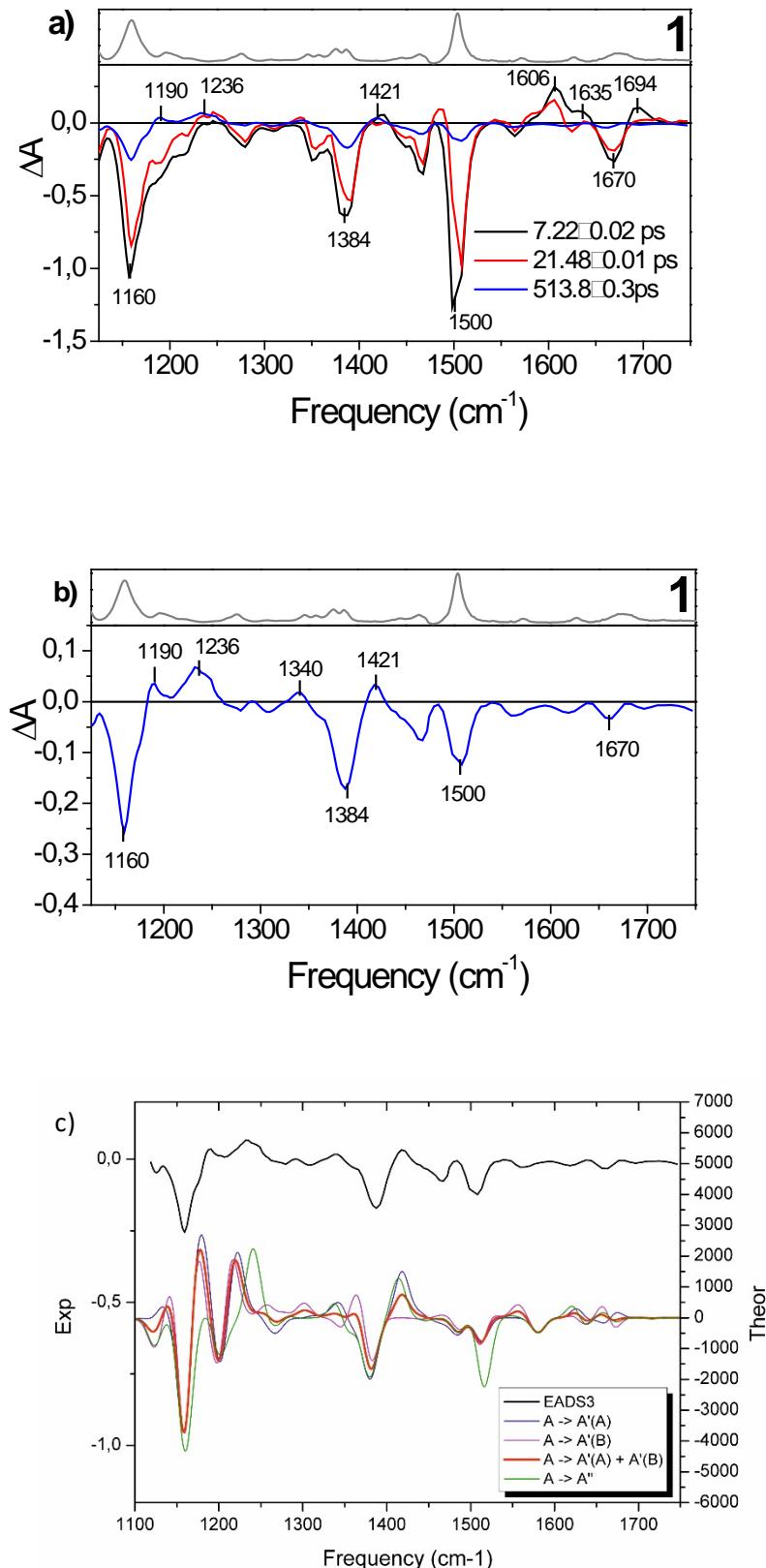


**Figure 6:** a) EADS obtained from global analysis<sup>50</sup> of time-resolved visible pump-probe data recorded for compound **1** dissolved in chloroform and excited at 480 nm; b) kinetic traces recorded on the maximum of the product band in various solvents.

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<sup>50</sup> of visible pump-probe data recorded for compound **1** in chloroform upon  $\lambda = 480$  nm light excitation (SI section 7) are shown in Figure 6. As evidenced from photo-accumulation experiments (Figure 5, SI section 5), the sample does not cyclize irrespective of the solvent. Nonetheless, along-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-E isomerized intermediate ( $\mathbf{A}'$ ) observed in the parent DASA **2**.<sup>33</sup> The EADS shown in Figure 6 closely follow those reported for DASA **2**.<sup>36</sup> 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 timescale 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 S7.1). The qualitative appearances of the transient signal and the observed spectral evolution do not significantly change when the solvent is modified. 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 (SI section 5.4). The quantum yields for the photoisomerization of **1** estimated from the ultrafast measurements are 10.7% (toluene), 16.2% (dichloromethane), 15.0% (chloroform), 14.3% (methanol) and 11.7% (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 photoproduct formation is in fact 8.5 ps in toluene and increases up 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 case of toluene the photoproduct is formed with a slower rate compared to the parent DASA molecule **2** (in that case the intermediate is observed on a 2 ps timescale) 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**, ps-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.<sup>51–53</sup> The EADS obtained from global analysis<sup>50</sup> of transient spectra acquired between 1100–1750 cm<sup>-1</sup> are reported in Figure 7. In case of transient infrared measurements the sample has been excited on the red-edge of its absorption band, at 510 nm.

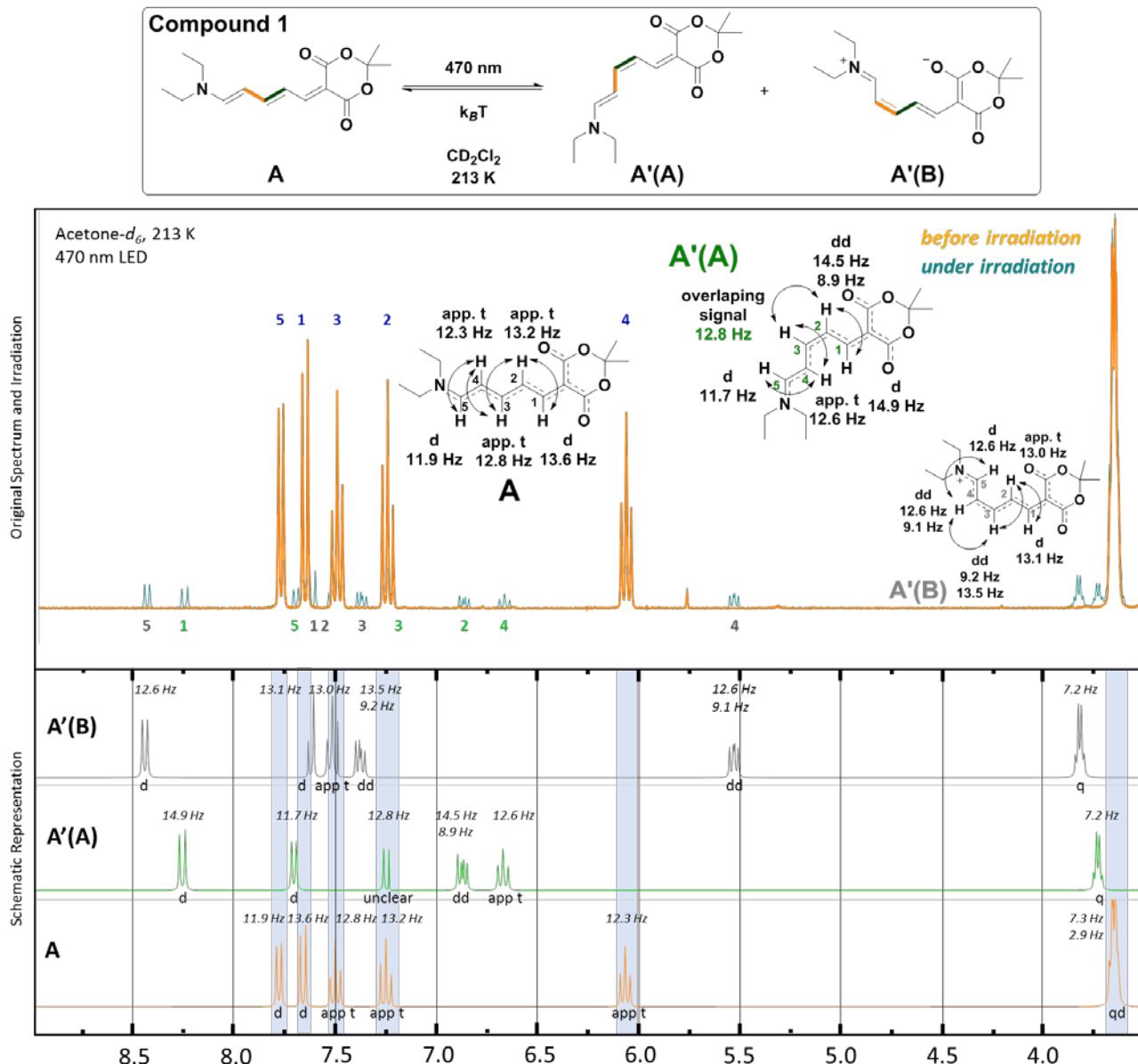


**Figure 7:** a) EADS obtained from global analysis<sup>50</sup> of time-resolved infrared data recorded for compound **1** in deuterated chloroform; the last spectral component is magnified in panel b). The gray line on the top panel of the figure reports the FTIR spectrum of the molecule in the same solvent. c) Comparison of experimental and simulated last EADS components in the same solvent. The blue/pink/red/green lines correspond to the GS difference harmonic IR spectra of the species

$A'(A)/A'(B)/A'(A)+A'(B)(1:1)/A''$  with respect to the spectrum of **A** obtained at the SMD/B3LYP/6-31++G(d,p) level of theory (FWHM = 20 cm<sup>-1</sup>, scaling factor=0.98).

The initial spectral component (black EADS in Figure 7) shows several negative bands, whose position well corresponds to the bleaching of ground state modes. As already noticed in case of the previously studied DASA photoswitches, very few excited-state absorption bands are observed.<sup>36</sup> 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.<sup>36</sup> In the carbonyl stretching region (1650-1750 cm<sup>-1</sup>), the excited state bands are clearly visible. In this region, three positive signals at 1606, 1635 and 1694 cm<sup>-1</sup> are observed. On a 7 ps timescale, 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 observed in transient visible measurements, which may be due to the different excitation conditions used in this case, preventing excess vibrational energy in the S<sub>1</sub> state. The following evolution, occurring on a 21.5 ps timescale, is associated to 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<sup>-1</sup> 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**<sup>36</sup> 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<sup>-1</sup> region. Moreover, the differential signal at 1160(-)/1190(+) cm<sup>-1</sup> 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<sup>-1</sup>, which is a consequence of an overestimated absorption intensity of **A** in its GS (cf. Figure 4). Nevertheless, the similarity between the spectral evolution 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 a similar nature and result from a photo-isomerization event.

<sup>1</sup>H-NMR *in-situ* irradiation experiments

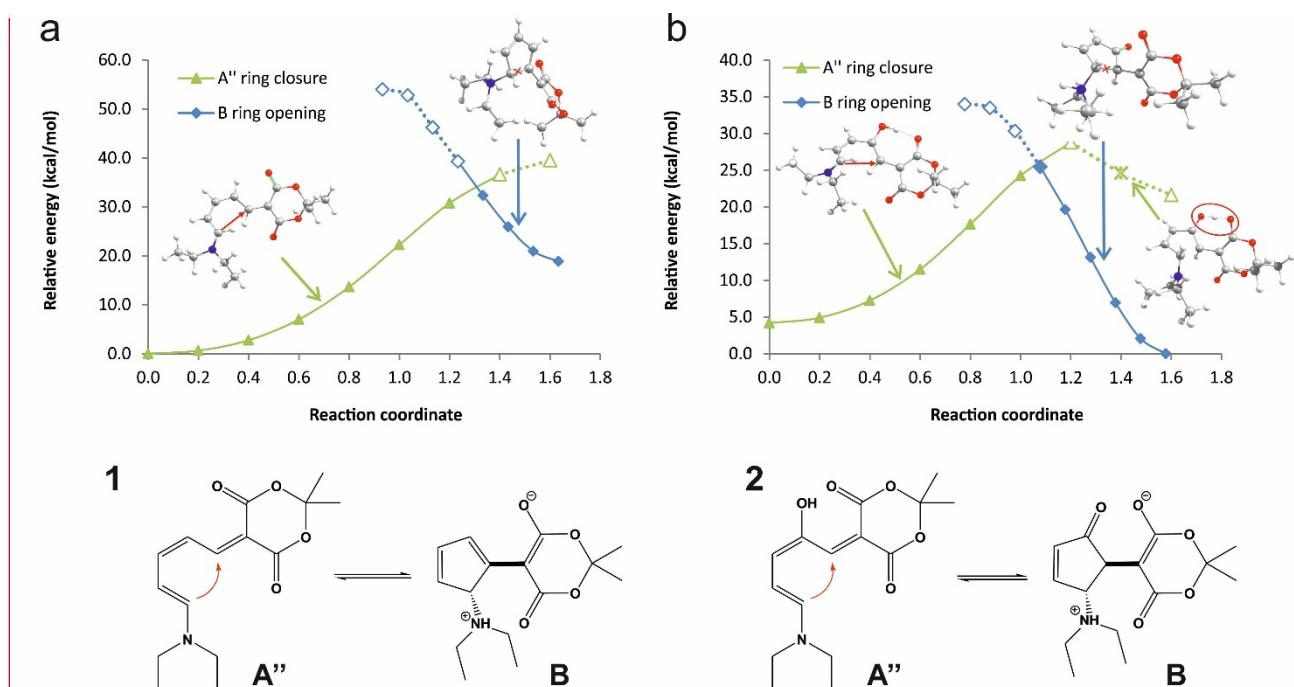


**Figure 8:** NMR *in situ*-irradiation experiments of compound **1** reveal two sets of photogenerated peaks corresponding to isomers **A'**(**A**) (green) and **A'**(**B**) (grey). Acetone- $d_6$  was used to verify the coupling constants, as in deuterated dichloromethane some signals were overlapping. 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.

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 (SI section 9.1.1) leads to the reversible formation of two isomers **A'**(**A**) and **A'**(**B**). Full structural assignment of these two isomers was performed in deuterated acetone (SI section 9.1.2) due to overlapping NMR signals of isomer **A'**(**B**) with the parent compound **1** in deuterated dichloromethane (Figure S9.9). 2D-NMR spectroscopy under irradiation (<sup>1</sup>H,<sup>1</sup>H-COSY, <sup>1</sup>H,<sup>1</sup>H-TOCSY and 2D-NOESY) and analysis of the coupling constants between the polyene protons suggest a comparable behavior of **1** in deuterated dichloromethane and deuterated acetone (see SI section 9.1.1 and 9.1.2), which is also observed by UV-vis spectroscopy (SI section 5, SI section 5.2 and 5.6). While coupling constants in the parent compound are in the range of 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 polyene chain (C<sub>2</sub>-C<sub>3</sub> vs. C<sub>3</sub>-C<sub>4</sub>). This is supported by the through-space connectivity deduced from nuclear Overhauser spectroscopy (SI section 9.1.1.4 and 9.1.2.4). For clarity, **A'**(**B**) is drawn in its resonance form, noting the partial double bond character through-out the conjugated system

(see discussion of bond lengths above). Upon cessation of irradiation, both isomers revert back to **1**. The fact that two independent isomers are formed is remarkable, as for DASA **2** only one isomer is formed under otherwise identical conditions (deuterated dichloromethane, 213 K, 530 nm LED, Thorlabs, M530F2).<sup>33</sup> The hydroxy group thus seems to pre-select the bond to be isomerized (C<sub>2</sub>-C<sub>3</sub>) and to suppress isomerization of the C<sub>3</sub>-C<sub>4</sub> bond (Figure 1). Electronic density difference (EDD) plots and analysis of bond-length alternations (Figure 3) support this observation. As the isomers are red-shifted with respect to **A** (SI section 5.6), irradiation near their absorption maximum should accelerate their back isomerization to **A**. Irradiation of the sample with 530 nm (LED, Thorlabs, M530F2) indeed accelerates the back relaxation to **A** (SI section 9.1.2.6). Temperature-dependent <sup>1</sup>H-NMR (SI section 9.3) and UV-vis studies (SI section 9.4) 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. While **A'**(**B**) dominates over **A'**(**A**) in steady-state concentration under irradiation at 213 K in acetone-*d*<sub>6</sub>, the ratio changes in favor of **A'**(**A**) with increasing temperature, leading to an almost disappearing signal of **A'**(**B**) at 233 K (Figure S9.43).

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 S8.1. The photoisomerizations are likely to be reversible as was previously shown for DASA **2**.<sup>36</sup> Importantly, the thermal stability of the isomers (especially of **A'**(**A**) lies in the range of what was observed before for **2**.<sup>33</sup> Given the calculated energies, the formation of **A''** could potentially be possible. However, none of the employed spectroscopic measurements (NMR, TRIR) indicates that this is actually occurring.



**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 SMD/B3LYP/6-31+G(d) level of theory. The green curve corresponds to the ring closure in the **A''** form, while the blue curve corresponds to the ring opening starting from the **B** form. The reaction coordinate (in Å) is defined as the negative relative distance of carbon atoms with respect to the equilibrium C<sub>2</sub>...C<sub>5</sub> 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<sup>+</sup> is preferentially transferred to the environment or to a neighboring DASA molecule. [ML1]

To rationalize the lack of cyclization from compound **1**, 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<sub>2</sub>-C<sub>5</sub> interatomic distance have been calculated at the SMD/RB3LYP/6-31+G(d) level by constrained optimizations of all coordinates, with the C<sub>2</sub>-C<sub>5</sub> bond length kept frozen, using methanol as typical protic environment (Figure 9). The green curves shown in Figure 9 describes the ring closure in the **A''** form, while 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 the cyclization of compound **1** is very high (*ca.* 34 kcal/mol), which is related to very low acidity of the hydrogen atom bound to carbon (C<sub>2</sub>). On the other hand, in the case of compound **2** the presence of the hydroxy group clearly facilitates the proton transfer decreasing the activation barrier to *ca.* 21 kcal/mol. Such a proton-transfer step is in so far important, as Stenhouse salts lacking such a strong hydrogen bond show strongly pH-dependent photoswitching.<sup>43</sup> In addition, as has been suggested before,<sup>42</sup> the initially formed oxyallyl cation (for the Piancatelli reaction) is stabilized by resonance through the electronegative oxygen atom. This is not possible for compound **1**, lacking the hydroxy group, thus 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, especially the actinic step. This group enables DASA photoswitching by restricting photo-isomerization of compound **2** towards the sole formation of isomer **A'(A)**. The “preselection” of bond C<sub>2</sub>-C<sub>3</sub> is likely due to a decreased electron density and elongation of that particular bond (particularly in the ES). Notably, in case of the non-hydroxy 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 has provided spectroscopic insights into details and timescales 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 photoisomerization kinetics of **1** and **2** shows that the non-hydroxy analogue **1** isomerizes about ten times slower than the parent DASA. The increased photoisomerization rate observed for compound **2** can be rationalized in terms of the influence that the hydroxyl substituent has on the triene bond length alternation and through steric effects. A somehow related effect has been previously observed in case of the rhodopsin chromophore, where the introduction of a methyl substituent accelerates the photoisomerization rate in solution.<sup>54,55</sup> Overall, our results show that by introducing a hydroxy group in the C<sub>2</sub>-position of the polyene chain one can control photoswitching pathways and enable 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.

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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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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## TOC Figure

