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Iminothioindoxyl as a molecular photoswitch with 100 nm band separation in the visible range

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Light is an exceptional external stimulus for establishing precise control over the properties and functions of chemical and biological systems, which is enabled through the use of molecular photoswitches. Ideal photoswitches are operated with visible light only, show large separation of absorption bands and are functional in various solvents including water, posing an unmet challenge. Here we show a class of fully-visible-light-operated molecular photoswitches, Iminothioindoxyls (ITIs) that meet these requirements. ITIs show a band separation of over 100 nm, isomerize on picosecond time scale and thermally relax on millisecond time scale. Using a combination of advanced spectroscopic and computational techniques, we provide the rationale for the switching behavior of ITIs and the influence of structural modifications and environment, including aqueous solution, on their photochemical properties. This research paves the way for the development of improved photo-controlled systems for a wide variety of applications that require fast responsive functions.
There is currently a growing interest in the development of responsive functional systems that can be controlled with light, which is a powerful, non-invasive external stimulus. Photochemical control is exerted at the molecular level through light-responsive chemical structures, i.e. photoswitches, which usually have two isomers that can be reversibly interconverted upon irradiation at different wavelengths. Often, one of those isomers is less stable and thermally converts back over time to the stable isomer. The two photo-isomers of the switch differ in structure and chemical properties, which enables photochemical control of the systems in which they are embedded, including drugs and their protein targets, drug delivery systems, the function of hydrogels in regenerative medicine, the conformation of peptides and nucleotides. Fascinating applications in bio-imaging and vision restoration are also emerging.

The selectivity in addressing the photoswitchable component in a complex functional system is crucial for its application. Because many molecular components of such systems absorb light in the UV range, a major challenge is to achieve selective switching through the design of photoswitches that can be operated in both directions using visible light. For example, in the emerging area of photopharmacology, visible light switching is crucial to enable deep tissue penetration, especially in the 650–900 nm range. However, most of the commonly used switches, such as diarylethenes, spiropyans, Donor-Acceptor Stenhouse Adducts (DASAs) and fulgides, do not show absorption bands of both photo-isomers in the visible light region.

For switches that can be operated in both directions in the visible range, such as substituted azobenzenes and indigos such as indigo and hemithioindigo, the band separation becomes a challenge, limiting their selective bidirectional photoisomerization. Only recently, this problem has been addressed for azobenzenes by the groups of Woolley and Hecht, who developed fully-visible-light-responsive azobenzenes, which - despite lower water solubility and challenging synthesis - have been successfully used for biological applications. Yet, the band separation to achieve selectivity remains an unmet challenge.

In our continuous efforts to expand the limited repertoire of molecular photoswitches, we further focused on several characteristics that should possess, besides the visible light operation with large band separation. Firstly, the photoswitch should be a small structural motif, in order to introduce it into the structure of a compound or material while affecting its original design only minimally. Secondly, it should be synthetically readily accessible. Thirdly, the parameters that control the rate of the thermal back isomerization reaction should be understood. Finally, for biological applications, the photoswitch should be able to operate under aqueous conditions. So far, realizing all these requirements in one molecular photoswitch has not been achieved.

Here we present the design, synthesis and evaluation of a class of photoswitches, which combine the photochromic dyes thioidindigo and azobenzene into a photoswitch called Iminothioindoxyl (ITI). We demonstrate fully-visible (blue/orange) light switching of ITI in either direction and a large band separation between both isomers of over 100 nm. We furthermore investigate, through a comprehensive combination of synthesis, spectroscopy and theoretical calculations, the influence of the environment and chemical substitution on the switching process and re-isomerization speed of ITI. Also, we demonstrate that these spectacular photochemical properties are retained for aqueous solutions, which opens opportunities for applying ITI for reversibly controlling biological systems.

**Results**

**Design and synthesis of ITI.** The design of iminothioindoxyl (ITI) is inspired by the structure of the visible-light-responsive molecular photoswitch hemithioindigo (HTI), which consists of half a thioindigo and half a stilbene moiety, featuring a photosomerizable C=C double bond. Yet, photo-isomerization is not limited to C=C double bonds. In particular, C=N photo-isomerization has recently attracted attention in designing molecular photoswitches. Based on that, we envisioned that a molecular architecture combining azobenzene and indigo photochromic unit could also show switching properties.

Already in the early 1900s, the chemical structures of ITI and similar compounds have been reported as dyes. Back in 1910, Rudolf Pummerer reported the one-step synthesis of ITI by the condensation of thioidindoxyl with nitrosobenzene. Nearly 100 years later, Soeta reported the synthesis of the same chemical structure using a Fasserini-type cycloaddition, also confirming through X-ray crystallography that the Z-form is the thermodynamically stable one.

Here, we report the synthesis of six ITIs a-f by the condensation of thioidindoxyl with substituted nitrosobenzene derivatives (Supplementary Fig. 1). Besides unsubstituted ITI 1a, two electron donating substituents (1b, 1c) and three electron withdrawing substituents (1d–1f) were placed at the R-position (Fig. 1a) to determine the influence of different substitution patterns on the photochemical properties of ITI, including absorption maxima and switching properties. Full experimental procedures and characterization is reported in Supplementary Methods and Supplementary Fig. 1–21.

**Solvent effects of ITI photo-isomerization.** To determine the influence of the medium on the photochemical properties of unsubstituted ITI 1a, absorption spectra were recorded in five solvents with different polarity (Fig. 1b, Table 1). In all solvents, the Z-isomer of ITI has an absorption band in the 400–500 nm region, with only limited solvatochromism. No clear correlation between solvent polarity and λmax values was observed within the group of polar solvents examined (Supplementary Fig. 6), similarly to the hemithioindigo switch. Time-dependent density functional theory (TD-DFT) calculations at the TD-M06–2X/6–311++G(2df,2p) level, in combination with the universal solvation model based on density (SMD) (see Supplementary Information) predicted that the band corresponds to the S0 → S1 transition with prevailing n→π* (HOMO → LUMO) character, while the first excited state S1 is a mixed state with a significant n→π* (HOMO-4 → LUMO) contribution (Supplementary Note 2, Supplementary Tables 1–3, Supplementary Figs. 23–25). In fact, due to twisting of the phenyl group out of the molecular plane (see θ in Fig. 2a), both excited states are partially mixed.

The photo-isomerization of 1a was followed by transient absorption spectroscopy (TA) in the millisecond time range, which revealed changes in the absorption spectrum upon irradiation at a short timescale. The transient spectra show a red-shifted absorption band, assigned to the thermally unstable E-isomer of the unsubstituted ITI 1a (Fig. 1c, Supplementary Figs. 39–48) in the 500 to 600 nm region, where Z-ITI 1a does not absorb. In all solvents, the spectrum of the E-isomer has two maxima (506–517 and 549–554 nm), of which the most intense has been highlighted in bold (Table 1). ITI thus shows a large Δλmax between the two photo-isomers of over 100 nm. In comparison, HTIs usually show Δλmax of only 10 to 50 nm.

The experimentally observed large Δλmax values are reproduced by the TD-DFT calculations, which further support the
assignment of the absorption bands. Based on the Molecular Orbital (MO) analysis, the absorption band of the E-isomer corresponds to the $S_0 \rightarrow S_1$ transition with a predominant $\pi \rightarrow \pi^*$ character and a small $n \rightarrow \pi^*$ contribution (Table 1 and Supplementary Table 2). The huge bathochromic shift observed upon photoisomerization can be explained by the twist around the central double bond ($C_2 = N_4$) in the $E$ isomer (see $\theta_1$ and $\theta_2$ in Fig. 2a). In the more twisted structure ($E$), the $\pi$ orbital (HOMO) is destabilized (due to less efficient overlap of 2p orbitals of $C_2$ and $N_4$ atoms, see Fig. 2b) leading to a smaller energy gap in the $E$ isomer.

The half-life for the $E$ isomer of ITI 1a in the thermal re-isomerization process was determined at room temperature to be in the millisecond time range, which is much shorter than found for HTI.

This finding can be ascribed to the presence of a nitrogen atom in ITI that can undergo inversion (Supplementary Fig. 30), a thermal relaxation mechanism also observed for azobenzenes and imine photoswitches. The rate of nitrogen atom inversion is medium-dependent, with polar solvents increasing the reaction barrier, which is consistent with our experimental data (Table 1, Supplementary Figs. 62, 63).

Theoretical observations of the thermal half-life are in line with the experimental ones, taking into account the limitations of continuum models to accurately describe the protic nature of MeOH. The calculations reveal that in all solvents the phenyl group is perpendicular to the molecular plane in the transition state for back isomerization from $E$ to $Z$, although a concurrent (less stable) transition state with planar structure was identified in less polar solvents as well (Supplementary Note 9 and Supplementary Table 12), further supporting our structure assignment. Thermal relaxation at $-60$ °C resulted again in the formation of the $Z$-isomer with a half-life of 6.8 ± 0.5 min without any observable degradation. An Eyring analysis, based on the determination of the back-isomerization rate at different temperatures by NMR, allowed for the calculation of the thermodynamic properties of the $E-Z$ re-isomerization step (Supplementary Figs. 78–82), showing $\Delta H^\ddagger = 61.8 \pm 5.2$ kJmol$^{-1}$ and $\Delta S^\ddagger = 81.6 \pm 23.4$ JK$^{-1}$mol$^{-1}$, which results in a $\Delta G^\ddagger = 86.1 \pm 8.7$ kJmol$^{-1}$ (at 298 K).

An important feature of a photoswitch is the ability to be operated photochemically in both directions exclusively with visible light. To test whether the reverse $E-Z$ isomerization can be achieved photochemically, ITI 1a in CD$_3$OD at $-60$ °C was switched to the $E$-isomer by irradiation with 455 nm (blue) light, and the rate of back-isomerization was then determined either without or with $\lambda = 595$ nm (orange) light irradiation. An approximately two-fold increase in the back-isomerization rate was observed under irradiation (Fig. 3b), showing that 1a is indeed both a T- and P-type photoswitch, while the heating effect of irradiation could be excluded (Supplementary Fig. 77). Yet it must be noted that the observation of photochemical $E$ to $Z$ isomerization is not of additional value at room temperature, because of the fast thermal re-isomerization.

The less stable $E$-isomer was also further characterized by measuring $E-Z$ difference FTIR spectra obtained upon irradiating the sample at $\lambda = 405$ nm at 184 K (Fig. 3c). Importantly, these
Table 1 Computational studies on solvents on ITI photo-isomerization

| Solvent (\(\epsilon_r\)) | Z-isomer | Transition | \(\theta_1(C1-C2-N4-C5)/\theta_2(C2-N4-C5-C6)\) | \(\Delta\mu_{GS,ES}(D)\) |
|---|---|---|---|---|
| Cyclohexane (2.02) | 416 | 373 | \(S_0 \rightarrow S_1\) 0.61/0.20 | 179.8/51.4 | 2.26 |
| Toluene (2.37) | 430 | 374 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.5/60.6 | -2.85 |
| CHCl\(_3\) (4.71) | 435 | 378 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.5/61.7 | -2.96 |
| MeOH (32.61) | 429 | 398 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.3/62.9 | -3.48 |
| DMSO (46.83) | 432 | 379 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.3/66.0 | -4.17 |
| | | | | 9.0/66.9 | -4.05 |

| E-isomer | Transition | \(\theta_1(C1-C2-N4-C5)/\theta_2(C2-N4-C5-C6)\) | \(\Delta\mu_{GS,ES}(D)\) |
|---|---|---|---|
| Cyclohexane (2.02) | 517 | 554 | 520 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.5/60.6 | -2.85 |
| Toluene (2.37) | 510 | 551 | 519 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.5/61.7 | -2.96 |
| CHCl\(_3\) (4.71) | 506 | 549 | 513 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.3/62.9 | -3.48 |
| MeOH (32.61) | 515 | 552 | 505 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.3/66.0 | -4.17 |
| DMSO (46.83) | 514 | 553 | 503 | \(S_0 \rightarrow S_1\) 0.55/0.31 | 9.0/66.9 | -4.05 |

Solvatochromic shifts of \(\Delta\lambda_{max}\) for the Z (Top) and E (Middle) isomers of ITI 1a. Experimental \(\Delta\lambda_{max}\) values are obtained from TA that show two absorption maxima which are both reported and the maximum, of which the one with the highest absorption is highlighted in bold. Theoretical \(\Delta\lambda_{max}\) values and the difference of GS and ES dipole moments (\(\Delta\mu_{GS,ES}(D)\)) were obtained at the SMD-TD-M06-2X/6-311++G(d,p) level using the SMD-M06-2X/6-31G(d) geometries, from which also twisting angles \(\theta_1\) and \(\theta_2\) were derived (see Fig. 2a). Bottom: Thermal relaxation of ITI 1a. Experimental half-lives were calculated from ms TA. The GS dipole moments for the transition state \(\mu_{GS,ST}(D)\) were obtained at the SMD-M06-2X/6-31G(d) level, at which also the twisting angles \(\theta_1\) and \(\theta_2\) as well as the activation barriers for thermal relaxation were derived (see Fig. 2a). The data in parentheses refer to a planar TS structure.

Z-E isomerization of ITI is a fast process. Transient absorption measurements with sub-picosecond time resolution were performed to determine the timescale of forward Z to E isomerization of ITI, which is expected to be very fast, based on structural analogies with HTIs and azobenzenes. For unsubstituted ITI 1a, the spectra were acquired with the sample in a KBr pellet, demonstrating that isomerization also occurs at the solid state. The main spectral features related to structural differences between the two isomers are fairly well reproduced by the DFT calculations (see band assignment in Supplementary Table 11, Supplementary Figs. 35 and 36 and Supplementary Note 7.).
The very short $S_2$ lifetime is again similar to what is known for azobenzene, for which a value of 50 fs has been recently determined. The decay of the broad $S_2$ excited state band within 320 fs and the rise of anisotropy on the same timescale indicate that ITI reaches the conical intersection region on a time scale competing with vibrational relaxation in $S_1$. From there, the molecule relaxes to the ground state of either the $Z$ and $E$ isomers, where vibrational cooling takes place on a time scale of 10 ps.

Support to our hypothesis that isomerization starts from a hot $S_1$ state comes from the computation of the forces acting on the individual atoms of ITI in $S_2$ and $S_1$ after vertical excitation, showing that the molecule undergoes more pronounced structural changes in the $S_2$ state (for more details see Supplementary Fig. 26, Supplementary Note 3 and Supplementary Table 4). The presence of a nitrogen atom in the isomerizing double bond opens the possibility for isomerization to occur through either an inversion or rotation mechanism. The negligible change in the excited state relaxation time scale observed in solvents with different viscosity (see Supplementary Fig. 38) in first instance favors an inversion mechanism, although most probably the simple vision of motion along a single reaction coordinate is not realistic, as recently pointed out for azobenzene.

**Substituent effects on ITI photo-isomerization.** The influence of the substituents on photoswitching of ITI was studied using a small library of ITIs with either an electron donating (1b,c) or an electron withdrawing group (1d-f). As shown in Fig. 5, electron donating groups (EDG) result in a slight red-shift of $\lambda_{\text{max}Z}$ and increased absorption, while electron withdrawing groups (EWG) result in a slight blue-shift of $\lambda_{\text{max}Z}$ and decreased absorption (Supplementary Fig. 64). Theoretical calculations reproduce this trend and show that the auxochromic effects are mainly due to the twist around the $\equiv\text{N-C-N}$ central single bond ($\theta_2$, Fig. 2a). Indeed, $\theta_2$ is smaller for 1b,c, leading to a more planar structure and favoring the electron delocalization (Supplementary Fig. 31 and Supplementary Table 8) upon excitation and increasing $\lambda_{\text{max}Z}$. In the ground state, EDGs increase the electron density on the phenyl ring which tends to “planarize” to increase conjugation with the thioindoxyl moiety in accordance with similar auxochromic effects have been observed in HTIs.

Isomerization of the differently substituted ITIs was measured in MeOH upon irradiation with $\lambda = 430$ nm light (Fig. 5b, Supplementary Figs. 49–58). A new absorption band was found for all the substituted ITIs and for electron donating ITIs 1b and c an impressively large $\Delta\lambda_{\text{max}}$ of over 100 nm was observed. ITI 1b was dissolved in MeOH and irradiated with 400 nm while cooled to $-60$ °C (Fig. 5d). Compared to the thermally adapted state, isomerization resulted in a clear change in color. Switching for several cycles of 1b in MeOH did not result in observable degradation (Fig. 5e). For all ITIs, the quantum yield for forward switching was estimated to be between 4 and 6%, which is relatively low compared to many other photoswitches. No clear correlation between Hammett parameter and the quantum yield (Supplementary Note 13, Supplementary Table 14) for the single studied position was found, meaning that both electron withdrawing and electron donating groups are tolerated.

Our calculations show that the auxochromic effects on $\Delta\lambda_{\text{max}}$ can be explained by a combination of geometrical and electronic effects (Supplementary Note 5). While $\theta_2$ is governing the auxochromic effects for the $Z$ and $E$ isomers in the same way ($\theta_2$ is larger for $E$ than for $Z$ but the extent to which $E$ and $Z$ are influenced by a substituent is similar), a twist around the C=N central double bond ($\theta_1$) is only observed for the $E$ isomer. The $\theta_1$ twist, being more pronounced for EDG substituents (1b,c), leads to a stronger destabilization of the $\pi$ orbital (HOMO) of the $E$ isomer for these substituents compared to the $Z$ isomer. Such geometrical feature partly contributes to the decrease of $\Delta\lambda_{\text{max}}$ when going from 1b,c to 1a,d,e,f. In addition, the change of the dipole moment upon excitation for the $E$ form decreases from 2.37 D (1b) to $-5.85$ D (1f) in methanol following the nature of the substituents (Table 2). We have found that the more negative $\Delta\mu$, the larger destabilization of the ES with respect to GS. This electronic effect also contributes to a smaller $\Delta\lambda_{\text{max}}$ for EWG substituents (Supplementary Table 7, 8 and Supplementary Fig. 31).

Apart from changes in the absorption spectra of $Z$ and $E$, substituents also influence the rate of thermal relaxation of the $E$ isomer (Table 2). No clear correlation between the Hammett parameter and the half-lives of the $E$ isomer was observed, albeit the data suggested a trend in EWG groups results in faster
Isomerization of ITI in aqueous solutions. In the field of photopharmacology, photo-control over the stereochimistry of a double bond is used to establish a difference in biological activity between both photo-isomers, as has been demonstrated for azobenzene and hemithioindigo photoswitches. For such biological applications of photoswitches, solubility at medicinally relevant conditions and photo-isomerization under aqueous and physiological conditions are crucial, yet are rarely observed for fully-visible-light switches. For example, photo-isomerization of HTI at physiological conditions has not been reported. To evaluate the performance of ITI in aqueous solutions, unsubstituted ITI was dissolved in phosphate buffered saline (PBS, pH 7.4, 1.7% DMSO) at ~30 μM. Irradiation with 400 nm light did not result in observable degradation (Supplementary Fig. 8). We also demonstrated that ITI has resistance against glutathione (GSH), which is found in concentrations up to 10 mM in cells and is the key factor for degradation of other molecular photoswitches.

Isomerization of ITIs in aqueous PBS (pH 7.4, 6.7% DMSO) was studied using the most red-light shifted p-MeO-ITI (Fig. 5C, Supplementary Figs. 59, 60) with ms transient absorption spectroscopy. The Z isomer of 1b has an absorption maximum at 459 nm. Upon irradiation with blue light, the E isomer was observed with an absorption maximum at 560 nm, demonstrating that a spectacular difference of absorption maxima is also maintained in aqueous solutions (Fig. 5c). From the same experiment, the half-life of the E isomer was found to be 10.0 ± 0.8 ms at room temperature.

Discussion

For application in biological systems, new and improved switches are needed. This is underlined e.g. by a recent report by the group of Thorn-Seshold, in which the first HTI-based photo-controlled pharmacophore was reported. This study demonstrates both the potential of indigoid-based photoswitches as well as the need for improved band separation of photo-isomers and improved water solubility.

Here we described the discovery of Iminothioindoxyls, a class of small, synthetically accessible visible-light photoswitches with excellent photochemical properties, showing very fast switching and an absorption band separation of photo-isomers of over 100 nm. Importantly, ITIs switch in solid state and in solvents ranging in polarity from cyclohexane to water, being therefore suitable for a very wide range of applications, varying from responsive materials to photopharmacology.

ITIs show unique properties when compared to other fully-visible-light-responsive photoswitches. A promising feature of ITIs is the millisecond half-life, making them useful for applications requiring fast responses. Indeed, many biological processes, such as signal transduction and neuronal
communication, operate at the millisecond scale and their photomodulation has been achieved with quickly re-isomerizing switches\textsuperscript{50,51}. Furthermore, ITIs forward switching is faster and shows better band separation than hemithioindigo, while also operating on a completely different mechanism for thermal relaxation. Finally, photo-isomerization of HTI in aqueous solutions at physiological pH has so far not been realized, while for ITI it could be readily observed. Also if compared to red-shifted azobenzenes, ITIs present favorable properties: they are slightly smaller in structure and synthetically more accessible, showing faster switching and a larger absorption band separation between the two isomers, higher stability under irradiation and under heavily reducing conditions such as those encountered in living cells.

Currently, the fast re-isomerization of ITIs prevents the use of their bi-directional photochemical isomerization at room temperature. To fully exploit the various properties of this class of photoswitches, an increased build-up and a longer lifetime of the \( E \) isomer is needed. This could be achieved through judiciously substituting patterns that improve the quantum yield and increasing the thermal barrier of re-isomerization. Similar situations have occurred in the past when other types of switches have been developed. In view of the successful studies that have followed to optimize these switches, we are confident that also for ITIs this will be a realistic target. We therefore consider the discovery of ITIs a break-through in the field of photocontrol, providing the starting point for developing improved photoswitches, resulting in major opportunities towards responsive systems well beyond those offered by the current very limited repertoire of all-visible light switches.

Methods

Organic synthesis. All reported starting materials, chemical reagents and organic solvents in this study were bought from Sigma–Aldrich, Acros, Fluka, Fischer, TCI and were used as received. Dry DCM was purified by passage through an MBraun SPS-800 solvent purification column. All aqueous solutions were prepared using deionized water. Kieselgel 60, F254 silica gel plates (Merck, TLC silica gel 60 F254) were used for TLC (Thin Layer Chromatography) analysis and UV light of 254 nm and potassium permanganate solution (KMnO\(_4\)) were used for the detection of compounds. Drying of solutions was performed using dry MgSO\(_4\) and solvents and other volatiles were removed using a rotary evaporator.

Analytical procedures. Nuclear Magnetic Resonance (NMR) spectra were recorded using an Agilent Technologies 400-MR (400/54 PremiumShielded) spectrometer (400 MHz), at room temperature (22–24 °C), unless indicated otherwise. The multiplicities of the signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). All \(^1\)H-broadband
decoupled. Melting points (Mp) were measured using a Stuart analogue capillary melting point SMP11 apparatus. High-resolution mass spectrometric (HRMS) measurements were performed using a Thermo scientific LTQ OrbitrapXL spectrometer, which is equipped with ESI ionization. In the experimental procedures, the mass of the molecule-ion \([M+H]^+\) are reported in m/z-units. Absorption spectra were measured using an Agilent 8453 UV/Vis diode array. All solutions for absorption spectra were prepared in Uvasol® grade solvents and were measured in quartz cuvettes with a 1 cm path-length. Purity was determined using LCMs, for which the following setup was used: Column: ACQUITY UPLC® HSS T3 1.8 μm, 2.1 x 150 mm; Detection: Total Ion Count (TIC), \(λ_1 = 254\) nm, \(λ_2 = 430\) nm; Flow: 0.3 mL/min; Eluent A: 0.1% formic acid in HPLC grade demineralized H₂O; Eluent B: 0.1% formic acid in acetonitrile; Gradient Program: (0–1 min) 5% eluent B; (1–8 min) linear gradient to 90% eluent B; (8–11 min) 90% eluent B; (11–12 min) linear gradient to 5% eluent B; (12–17 min) 5% eluent B.

**Computational studies.** The ground state (GS) structures of the Z/E-isomers and the GS transition state (TS) of the backward reaction (E→Z; thermal relaxation process) for ITIs (1a-f) were optimized at the M06–2X level using the 6–31+G(d) atomic basis set, since this exchange-correlation functional is known to perform well not only for the GS thermochemistry, but also in describing excited states. In addition, ITIs are not subject to the known TD-DFT limitations such as charge-transfer, see Supplementary Table 6, double excitations (see t1 and t2 amplitudes), singlet-triplet transition, etc. All minima were checked against the presence of imaginary frequencies. The TS structures were obtained by geometry optimization starting from a structure with the angle C2–N4–C5 set to very close to 180°. This choice was based on the potential energy scan for the out-of-plane distortion from the in-plane-TS structure showing that the distortion is energetically unfavourable (Supplementary Fig. 28). The optimized TS structures (first-order saddle points) were checked against the presence of a single imaginary frequency. The optimized GS structures are presented in Supplementary Note 1 and Supplementary Fig. 22. The solvent effects were considered employing the solvation Model based on Density (SMD)38. Cyclohexane (CHX), toluene (TOL), chloroform (CHL), methanol and dimethylsulfoxide (DMSO) were used consistently with experimental data. The IR spectra were simulated at the B3LYP/6–31+G(d,p) level39,40 which was found to provide a reasonable agreement with the experimental FTIR spectra for the Z-isomers (Supplementary Note 7, Supplementary Figs. 33–36). The IR band assignment was based on the potential energy distribution (PED) analysis35 by using the VEDA 4 program. Vertical excitation energies (VEE) were obtained with a larger basis set, namely 6–311+G(2df,2p). SMD was combined with the corrected linear response (cLR) approach57 to model VEE within the non-equilibrium regime. (TD)-DFT calculations were performed using the Gaussian09 and Gaussian16 programs58,59. All Gaussian default thresholds and algorithms were used except for improving optimization. In the latter case, a threshold of 10⁻⁵ a.u. on average residual forces was imposed, a self-consistent field convergence criterion of 10⁻¹⁰ a.u., and the use of the ultrafine DFT integration grid. Gas phase CC2 and ADC(2) calculations of the excitation energies were performed using aug-cc-pVTZ basis set with the Turbomole program [TURBOMOLE V6.6, 2014, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1998–2007, TURBOMOLE GmbH, since 2007; available from http://www. turbomole.com]. NMR shieldings for the protons in Z/E-isomers of 1a were obtained with the Gauge-Independent Atomic Orbital (GIAO) method60 with the B3LYP functional and the 6–31+G(d,p) basis set. Chloroform environment was treated using the SMD model. Proton shielding for TMS selected as reference was computed under the same conditions using the M06-2X/6–31+G(d) geometry.

**Ultrafast spectroscopy.** Ultrafast transient absorption spectra of unsubstituted iminothioloxoyl 1a were measured on a system consisting of a Ti:sapphire laser oscillator (Spectra Physics Tsunami) and regenerative amplifier system (BMA Alpha 1000) which produced pulses of 100 femtosecond at 800 nm with an average output power of 450 to 500 mW. Excitation pulses at a wavelength of 400 nm were obtained by second harmonic generation of the fundamental laser output in a 2 mm thick BBO crystal. For all measurements in methanol, the pump beam polarization was set either to perpendicular or parallel with respect to the parallel probe beam by rotating a λ/2 plate. For measurement in solvents other than methanol, polarization was set to magic angle so as to exclude rotational contributions to the transient signal. From the parallel and perpendicular intensities the anisotropy r(t) is calculated using Eq. (1).

\[
r(t) = \frac{I_{\perp} - I_{\parallel}}{I_{\perp} + 2I_{\parallel}}
\]

where \(I_{\perp}\) and \(I_{\parallel}\) are the signal intensity respectively recorded with parallel and perpendicular pump polarization. The isotropic signal in methanol is obtained from the parallel and perpendicular signals using Eq. (2).

\[
I_{\text{iso}} = \frac{I_{\perp} + 2I_{\parallel}}{3}
\]

The excitation powers were on the order of 50 to 100 nJ. The probe pulses were generated upon focusing the 800 nm radiation beam partially on a 2 mm thick
Table 2 Computational studies on substituent effects on ITI photo-isomerization

| R (Hammet constant σ) | Z-isomer | Transition | Δμ_{ES-GS,Z} (D) | e (mol⁻¹cm⁻¹) |
|-----------------------|----------|------------|-----------------|---------------|
|                       | λ_{max,Z} (nm) | exp. | calc. | Transition | π⁺/nπ⁺ | θ_{(C1-C2-N4-C5)/g_{(C2-N4-C5-C6)}} | |
| 1a H (0.00)           | 429      | 398      | S₀→S₁         | 0.58/0.38    | 2.68/5.41 | 3900 |
| 1b MeO (−0.27)        | 448      | 413      | S₀→S₁         | 0.62/0.29    | 2.66/5.41 | 3900 |
| 1c Me (−0.17)         | 434      | 406      | S₀→S₁         | 0.61/0.32    | 2.66/5.41 | 3900 |
| 1d COOMe (0.45)       | 427      | 399      | S₀→S₁         | 0.56/−0.15   | 2.66/5.41 | 3900 |
| 1e CF₃ (0.54)         | 424      | 391      | S₀→S₁         | 0.54/0.37    | 2.66/5.41 | 3900 |
| 1f NO₂ (0.78)         | -        | 390      | S₀→S₁         | 0.53/−0.33   | 2.66/5.41 | 3900 |

| R (Hammet constant σ) | E-isomer | Transition | Δμ_{ES-GS,E} (D) |
|-----------------------|----------|------------|-----------------|
|                       | λ_{max,E} (nm) | exp. | calc. | Transition | π⁺/π⁻ | θ_{(C1-C2-N4-C5)/g_{(C2-N4-C5-C6)}} | |
| 1a H (0.00)           | 515,552  | 505      | S₀→S₁         | 0.54/0.30    | 2.66/5.41 | 3900 |
| 1b MeO (−0.27)        | 513      | 533      | S₀→S₁         | 0.58/−0.32   | 2.66/5.41 | 3900 |
| 1c Me (−0.17)         | 511, 548 | 519      | S₀→S₁         | 0.58/−0.31   | 2.66/5.41 | 3900 |
| 1d COOMe (0.45)       | 503      | 484      | S₀→S₁         | 0.52/−0.31   | 2.66/5.41 | 3900 |
| 1e CF₃ (0.54)         | 500      | 482      | S₀→S₁         | 0.52/−0.31   | 2.66/5.41 | 3900 |
| 1f NO₂ (0.78)         | 501      | 470      | S₀→S₁         | 0.54/0.30    | 2.66/5.41 | 3900 |

| R (Hammet constant σ) | Δλ_{max} (nm) | t₁/₂ (ms) | E_{ES-Z} (kcal/mol) | Transition state | α(C2-C4-C5)/α(C2-C4-C5-C6) | Δμ/GS-TS,Z |
|-----------------------|---------------|-----------|---------------------|-----------------|--------------------------|-------------|
|                       | exp. | calc. | | exp. | calc. | | | |
| 1a H (0.00)           | 86       | 123      | 18.5 ± 1.4          | 14.4            | 14.4                      | 177.2/87.5 | −0.02          | 6.2 |
| 1b MeO (−0.27)        | 105      | 120      | 12.7 ± 0.5          | 14.2            | 13.0                      | 177.7/90.2 | −0.25          | 4.5 |
| 1c Me (−0.17)         | 77, 114   | 113      | 21.1 ± 1.2          | 14.5            | 14.0                      | 177.7/90.2 | −0.26          | 5.4 |
| 1d COOMe (0.45)       | 76       | 85       | 4.0 ± 0.3           | 13.6            | 13.1                      | 177.6/90.2 | 0.38           | 6.3 |
| 1e CF₃ (0.54)         | 76       | 80       | 2.8 ± 0.5           | 13.4            | 12.0                      | 177.8/90.1 | 2.48           | 4.1 |
| 1f NO₂ (0.78)         | -        | 80       | 9.9 ± 1.0           | 14.1            | 13.6                      | 177.8/90.1 | 2.48           | 4.1 |

Shifts of Δλ_{max} for the Z (Top) and E (Middle) isomers of ITIs 1a–f in MeOH. Experimental Δλ_{max} values are obtained from TA that show two absorption maxima which are both reported with the maximum with the highest absorption highlighted in bold. Theoretical Δλ_{max} values and the difference of GS and ES dipole moments (Δμ_{ES-GS}) were obtained at the SMD-TD-M06-2X/6-31+G(d,p) level using the SMD-M06-2X/6-31+G(d,p) geometries, from which also twisting angles (θ₁ and θ₂, Fig. 2a) were derived. Bottom: Thermal relaxation of ITIs 1a–f in MeOH. Experimental half-lives were calculated from ms TA spectroscopy. The differences of dipole moment of the transition state and that of the Z-form in their GS (Δμ_{ES-GS}) were obtained at the SMD-M06-2X/6-31+G(d,p) level, at which also the transition state twisting angles (θ₁ and θ₂) as well as the activation barriers for thermal relaxation were derived.

Nanosecond transient absorption spectroscopy. Nanosecond transient absorptions were recorded with an in-house assembled setup. For all ITIs and all solvents, an excitation wavelength of 430 nm was used. The excitation wavelength of 430 nm was generated using a tunable Nd:YAG laser system (NT142R, Ekspla) comprising the pump laser (NL300) with harmonics generators (SHG, THG) producing 355 nm to pump an optical parametric oscillator (OPO) with SHG connected in a single device. The laser system was operated at a repetition rate of 5 Hz. The probe light running at 10 Hz was generated by a high-stability short arc xenon flash lamp (FX-1160, Excelitas Technologies) using a modified PS502 controller (EG&G). Using a 50/50 beam splitter, the probe light was split equally into a signal beam and a reference beam with and focused on the entrance slit of a spectrograph (SpecPro-130, Princeton Instruments). The probe beam (A = 1 mm²) was passed through the sample cell and orthogonally overlapped with the excitation beam on a 1 mm × 1 cm area. The excitation energy was recorded by measuring the excitation power at the back of an empty sample holder. In order to correct for fluctuations in the flash lamp spectral intensity, the reference was used to normalize the signal. Both beams were recorded simultaneously using a gated intensified CCD camera (PI-MAX3, Princeton Instruments) which has an adjustable gate of minimal 2.9 ns. A delay generator (DG535, Stanford Research Systems, Inc.) was used to time the
excitation pulse, the flash lamp, and the gate of the camera. The setup was controlled by an in-house written LabView program.

In situ NMR irradiation experiments. NMR spectra were recorded with an Agilent Technologies Inova 500 Spectrometer (500 MHz), and for in situ irradiation, a set-up based on LED and an option fiber were used, according to a reported system. The fiber-optic cable (M28L05; Ø400 μm, 0.39 NA, SMA-SMA Fiber Patch Cable, 3m) and the LEDs were purchased from Thorlabs: royal blue 455 nm fiber-coupled LED(M455F51, 10 mW); amber 595 nm fiber-coupled LED (M595F2, 110 mW). NMR tubes were purchased from Wilmad-LabGlass (SP Scienceware): WGS-5BL, Coaxial Insert for 5 mm NMR Sample Tube and 535-PP-7, 5 mm Thin Wall Precision NMR Sample Tube 7”L, 600 MHz.

FTIR. Low-temperature FTIR spectra were recorded on a FTIR Bruker IFS 120 HR spectrometer with maximum resolution 0.002 cm⁻¹. For current measurements, spectra were registered with 1 cm⁻¹ spectral resolution. The instrument is equipped with a globar IR source and a MCT detector. The sample has been cooled using a liquid helium cold tip closed cycle cryostat (minimal nominal temperature 5 K), temperature has been monitored at the sample position using a K-type thermocouple (reading error 0.1 K).

The in situ irradiation source was a 80 mW laser diode, with a spot size of 6 × 4 mm, centered at 405 nm (FWHM ~10 nm). The sample was prepared as a KBr pellet, and contained in a home-made cell equipped with two calcium fluoride windows. Spectra without and under irradiation were measured at 184 K.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. Additional data on methods used are available from the corresponding author upon reasonable request.

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Author contributions

M.W.H and W.S. conceived the project and designed the molecules. M.W.H. and L.S. performed the synthesis. Nanosecond TA spectroscopy was performed by M.W.H., M.H. and W.J.B., while M.D.D. performed the femtosecond TA spectroscopic experiments. UV-VIS experiments were performed by M.W.H. NMR experiments were done by M.W.H and W.S.; low-temperature FT-IR experiments were done by S.F. and M.D.D. All calculations were done by M.M. and A.D.L. The research was guided by B.L.F., W.J.B. and W.S. The manuscript was written by M.W.H., M.M., A.D.L., M.D.D., B.L.F., W.J.B. and W.S. All authors discussed the results and progress in all stages.

Additional information

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