Chapter 8
Towards pH and light dual controlled Iminothioindoxyl photoswitches for medicine

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Seek and Destroy: Light-Controlled Cancer Therapeutics for Local Treatment

Abstract:

Iminothioindoxyl (ITI) is a visible-light-operated, imine-based molecular photoswitch with potential for applications in responsive systems. A limitation in the use of ITI is the short half-life of the unstable isomer, which undergoes rapid re-isomerisation through nitrogen inversion. Here we report the influence of protonation on the properties of ITI, which was motivated as an attempt to block nitrogen inversion and increase the thermal half-life. Upon protonation, beneficial trends are observed, including increased absorption, longer half-lives of the thermally unstable photo-isomer and a bathochromic shift of the Z isomer up to an absorption maximum of 682 nm. This study opens the door for the development of dual-responsive systems controlled by both light and pH.

8.1 Introduction:

The photo-isomers of molecular photoswitches have different chemical and structural properties, which provides the opportunity to control the function of molecules with spatial and temporal precision using light as an external stimulus\textsuperscript{1-4}. Applications of photoswitches include control over the activity of small molecule drugs (photopharmacology)\textsuperscript{5,6}, drug delivery systems\textsuperscript{7,8}, the conformation of macromolecules such as nucleotides\textsuperscript{9}, peptides and proteins and their complexes\textsuperscript{10,11}, the activity of catalysts\textsuperscript{12}, molecular self-assembly processes\textsuperscript{13,14}, gas adsorption\textsuperscript{15}, the properties of surfaces\textsuperscript{16} and crystals\textsuperscript{17}. Furthermore, photoswitches are used to induce motion in fibers\textsuperscript{18}, liquid crystals\textsuperscript{19} and polymers\textsuperscript{20} and applications for bio-imaging\textsuperscript{21,22}, medical imaging\textsuperscript{23}, vision restoration\textsuperscript{24} and energy storage\textsuperscript{25} are emerging.

Light can be delivered with spatial and temporal precision and the dose and wavelength of irradiation are tunable, which makes light a powerful external stimulus. However, light absorption is not specific to photoswitches, meaning that other components in the system in which the photoswitch operates can absorb light of specifics wavelengths as well. This has limited the applicability of UV-light-operated photoswitches for especially applications in medicine and has been the driving force behind the development, tuning and characterization of visible light operated photoswitches, such as diazocines\textsuperscript{26,27}, tetra-ortho-substituted azobenzenes\textsuperscript{3,28,29}, BF\textsubscript{2}-azo complexes\textsuperscript{30}, (thio)indoxyl based switches such as hemithioindigo\textsuperscript{31-34}, and donor-acceptor Stenhouse adducts (DASA)\textsuperscript{35,36}.

Recently, we reported the development of iminothioindoxyl (ITI) as a new member of the family of visible-light-operated photoswitches\textsuperscript{37}. ITI photoswitch consists of half a thioindoxyl and half an azobenzene (Figure 8.1A), which results in a photo-isomerizable C=N double bond. This fusion of the thioindoxyl and azobenzene photoswitch yielded a fully visible light operated photoswitch with a 100 nm band separation between the absorption maxima of the Z and E photo-isomers. Importantly, photo-isomerization of ITI has been observed in solvents with a variety of polarity, including aqueous solutions, which potentially allows for a wide variety of applications. The thermally stable Z isomer can be switched to the E isomer with light of around 400 to 450 nm and re-isomerization can be achieved photochemically (typically using light of 500-550 nm) or thermally, with a half-lives in the ms range at room temperature. The fast E-Z re-isomerization is assumed to
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proceed through thermal in-plane nitrogen inversion within the imine nitrogen atom\(^{38-40}\). In this process, a linear intermediate\(^{37}\) is formed, after which the imine relaxes to the thermally favored state. The fast thermal re-isomerization competes with photochemical build-up of E-ITI under irradiation at room temperature, which limits the applicability of the ITI photoswitch.

A higher level of control over responsive molecules can be acquired by the development of dual-response systems. Especially pH as a second stimulus is of high interest since it can be combined with photoswitches in two ways. First, both photo-isomers can have photo-isomer-specific acidic groups, meaning that photo-isomerization results in a pH change. Such light control over the pH has been demonstrated for example for the spiropyran photo-acid in which the closed photo-isomer has no acidic protons and the open isomer features an acidic phenol\(^{41}\). Secondly, protonation/deprotonation is a tool to control the (photo)chemical properties of photoswitches. For example, the group of Dube reported a hemithioindigo photoswitch, in which protonation of a -NMe\(_2\) substituent abolished its strong electron donating effect and thereby removed the red-light shift that the NMe\(_2\) group caused\(^{42}\). The group of Woolley reported tetra-ortho-substituted azobenzenes of which the pKa of the protonated diazo bond could be tuned. Upon protonation of the diazo bond, the absorption maximum was bathochromically shifted. Importantly, these effects could also be observed in aqueous solutions, opening opportunities for their use in responsive systems in medicine.

Future applications of purely light-responsive systems in medicine heavily rely on spatial input to decide where in the patient’s body the external activation through irradiation is required. For example, for the local activation or release of chemotherapeutic agents with light, medical imaging will be crucial to identify all tumors and their locations in the patient. Alternatively, we recognize the possibilities for the treatment of cancer using a dual pH and light controlled approach. Due to metabolic changes in most cancer types, the microenvironment of solid tumors is acidified, dependent on the size and type of tumor\(^{43}\). This process of metabolic changes is better known as the Warburg effect\(^{44}\). The local and tumor-specific acidification can be therapeutically employed for the side-selective release of active drugs through prodrug strategies and drug delivery systems\(^{45-47}\). This opens opportunities for dual response strategies in medicine, in which the specific photochemical properties of protonated photoswitches, such as a protonation-induced bathochromatic shift, allow for light-control in tumor microenvironment specifically.
Figure 8.1: The Iminothioindoxyl photoswitch. A) The structure of Z and E-Iminothioindoxyl (for synthesis and characterization of ITIs 1a-d,h see Chapter 6 and 7). B) The proposed mechanism of thermal E-Z isomerization through nitrogen inversion from the thermally unstable E photo-isomer to the thermally stable Z photo-isomer.

Recognizing the importance of the nitrogen electron pair in the fast thermal relaxation pathway, we set off to study the influence that its protonation has on the photochemical properties of the ITI photoswitch, using 1H NMR and UV/Vis spectroscopy. Upon protonation of ITI in DCM using TFA, the main absorption band disappears and two new absorption bands arise, of which one is bathochromically shifted. Furthermore, it was observed that protonation results in longer half-lives of thermal relaxation of the E photo-isomer, possibly by eliminating the nitrogen inversion thermal relaxation pathway. Inspired by these observation, we describe here the potential of the ITI photoswitch for pH and light dual response and which obstacles to overcome to develop dual response ITIs for applications in medicine.
8.2 Results and Discussion:

8.2.1 UV/VIS and NMR titrations:

\[ \text{Figure 8.2: UV/VIS titrations of ITIIs with different electronic properties. A) Titration of 20} \mu\text{M unsubstituted ITI 1a in DCM. Kd = 211} \pm 32 \text{mM. B) Titration of 20} \mu\text{M p-COOME-ITI 1d in DCM. Kd = 242} \pm 68 \text{mM. C) Titration of 20} \mu\text{M p-Me-ITI 1c in DCM. Kd = 114} \pm 21 \text{mM. D) Titration of 20} \mu\text{M p-MeO-ITI 1b in DCM. Kd = 67} \pm 13 \text{mM. E) Titration of 20} \mu\text{M p-NMe2-ITI 1h in DCM. Kd = 89} \pm 54 \text{mM. F) Titration of 20} \mu\text{M Hemithioindigo 2 in DCM.} \]

The unsubstituted ITI 1a in DCM was titrated with TFA and the absorption spectra were recorded using UV/VIS spectroscopy (see Figure 8.2A). The addition of TFA resulted in the formation of a protonated species, with the rise of two absorption bands, one around 360
nm and one around 480 nm which tails until approximately 550 nm. Furthermore, both new absorption bands showed higher absorption compared to the neutral ITI. Titration of p-COOMe-ITI 1d (Figure 8.2B) with TFA results in a species with similar absorptivity and only a small red-light shift of the visible light absorption band is observed. In contrast to the electron withdrawing p-COOMe group, electron donating p-Me (Figure 8.2C) and p-MeO (Figure 8.2D) groups both introduce bathochromic shift of the visible-light band and its increased absorption. Upon protonation of p-MeO-ITI 1b, the absorption band shifts to over 500 nm and is tailing towards the 600 nm region of the spectrum. Spectacularly, the protonated p-NMe2-ITI 1h (Figure 8.2E) has an absorption maximum of 682 nm, however with a lower intensity compared to its neutral form.

The basicity of the nitrogen species can be altered by substituents with different electronic properties, where electron rich nitrogen species are stronger bases then electron poor species. The titration experiments of ITIs with different electronic substituents fit this trend (Figure 8.3A). Compared to the unsubstituted ITI, and the electron withdrawing p-COOMe-ITI, the Kd (here defined as the concentration of TFA needed to protonate half of the present ITI) for TFA is lower for ITIs with electron donating group on the para position. This supports our hypothesis that the observed changes in the absorption spectra are caused by protonation of the imine of ITI. For the strongest electron donating p-NMe2-ITI 1h, a Kd similar to that of p-MeO-ITI 1b was determined, yet it must be noted that a NMe2 substituent can be protonated too, which makes it difficult to compare those values.

Furthermore, to exclude the possibility that the addition of an acid results in the degradation of the ITI, the protonation was reversed (Figure 8.3B) by adding triethylamine to protonated ITI 1a, after which the absorption spectrum returned to the neutral one.

![Figure 8.3: A) Relation between Hammett parameter R and Kd of protonation with TFA in DCM. B) Reversibility of ITI protonation. To a solution of 20 μM ITI 1a, TFA (in DCM, 20 μM ITI 1a) was added to a concentration of 200 mM. After that, triethylamine (TEA) solution (10 v% in DCM, 20 μM ITI 1a) was added.](image-url)
Addition of TFA into a DCM solution of an ITI switch introduces two variables: the change in the structure of the switch and the change in the properties (e.g. polarity) of the environment. To exclude the effects of a different environment on the absorption spectrum, a titration experiment with HTI switch was performed. HTI has a C=C double bond where ITI has a C=N double bond, meaning that HTI has no nitrogen atom that can be protonation. Upon titration of HTI in DCM with TFA, only minor effects to the absorption spectrum were observed (Figure 8.2F). From the HTI titration experiment, no binding curve could be made, attributing the minor changes in the absorption spectrum to solvent effects.

**Figure 8.4:** $^1$H NMR titration of ITI 1a in CD$_2$Cl$_2$ (0.5 mg/mL) with TFA.

The addition of TFA to ITI 1a in DCM resulted in spectral changes due to a combination of protonation and solvent effects. The same titration was repeated in CD$_2$Cl$_2$ and examined using NMR, for both ITI and HTI (Figure 8.4 and 8.5, respectively). The ITI titration in the NMR showed downfield shifts of the neutral ITI upon addition of TFA, as can be seen for the doublet at 7.93 ppm and the triplet at 7.66 ppm. Furthermore, upon addition of TFA, the formation of the protonated ITI is observed, as indicated by the emergence of signals at 7.80-7.85 ppm. The titration of HTI with TFA showed similar downfield shifts, as can be observed with the C=CH signal at 7.95 ppm and the doublet at 7.92 ppm. Yet, addition of TFA did not result in the formation of signals of a new protonated species. This, together with the UV/VIS titration experiments, strongly supports the notion that that protonation, and not just the change in solvent polarity, is responsible for the effects observed in ITI.
Figure 8.5: $^1$H NMR titration of Hemitioindigo 2 in CDCl$_3$ (0.5 mg/mL) with TFA

8.2.2 Photo-isomerization of protonated ITI

Iminothioindoxyl in its neutral state functions as a photoswitch, yet the photo-isomerization of protonated ITI could not be observed using steady state UV/VIS spectroscopy. From this, it could be concluded that either photo-isomerization does not occur or that the protonated $E$ still persists only in the millisecond time range, similarly to the neutral species. Therefore, photochemical properties of protonated ITIs were determined using Transient Absorption (TA) spectroscopy (Figure 8.6).
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Figure 8.6: Transient absorption spectroscopy of ITI. A) Neutral unsubstituted ITI 1a in DCM, irradiated with 430 nm light. B) Protonated unsubstituted ITI 1a in DCM (1.2 v% TFA), irradiated with 448 nm light. C) Neutral p-COOMe-ITI 1d in DCM, irradiated with 430 nm light. D) Protonated p-COOMe-ITI 1d in DCM (1.2 v% TFA), irradiated with 440 nm light. E) Neutral p-Me-ITI 1c in DCM, irradiated with 430 nm light. F) Protonated p-Me-ITI 1c in DCM (1.2 v% TFA), irradiated with 475 nm light. G) Neutral p-MeO-ITI 1b in DCM, irradiated with 450 nm light. H) Protonated p-MeO-ITI 1b in DCM (1.2 v% TFA), irradiated with 510 nm light.

Transient absorption spectroscopy revealed that protonated ITI behaves in a similar fashion as the neutral ITI. Upon protonation of unsubstituted ITI (Figure 8.6A,B), photoisomerization is observed and the characteristic band separation of ITI is maintained. The protonated E-isomer lives approximately three times longer than the neutral E-isomer, however at the cost of a lower isomerization quantum yield. An electron withdrawing COOMe functional group at the para position results for both the neutral and protonated ITI in a short half-life and a low quantum yield (Figure 8.6C,D). Both p-Me-ITI 1c and p-MeO-ITI 1b have a longer living E isomer in the protonated state, where the quantum yield of Z-E isomerization of both neutral and protonated ITI is similar (Figure 8.6E-H). Combined with the red-light shifted absorption maxima, ITIs with electron donating groups show improved properties under the acidic conditions tested here. The photochemical evaluation of p-NMe2 under acidic conditions is currently under investigation.
For neutral ITI, it was shown that the half-life of the E isomer is also dependent on the polarity of the solvent, where in more polar solvent thermal relaxation is slower\textsuperscript{37}. This means that upon addition of TFA to DCM, both the protonation of ITI and the increase of solvent polarity can have a contribution to the experimentally observed increased half-lives of the E isomer. Against the expectation, thermal relaxation of the E isomer is still in the millisecond time range. This suggests that the protonated E-ITI can undergo thermal relaxation via a mechanism different than nitrogen inversion.

### 8.2.3 Evaluation of the potential of dual-responsive ITIs for medicine

In DCM and its mixtures with TFA, it was observed that protonation results in a red-light shift and longer half-lives of the E photo-isomer. For applications in medicine, such as photopharmacology, both the red-light shift and the longer living E photo-isomer can be of benefit. Yet, the experiments in DCM and its mixtures with TFA do not represent biologically relevant conditions.

![Figure 8.7](image)

Figure 8.7: A) Absorption spectra of 20 \( \mu \)M unsubstituted ITI 1a in phosphate/citrate buffers with different pH (2 v% DMSO). B) Absorption spectra of 20 \( \mu \)M p-Me-ITI 1c in phosphate/citrate buffers with different pH (2 v% DMSO). C) Absorption spectra of 20 \( \mu \)M p-MeO-ITI 1b in phosphate/citrate buffers with different pH (2% DMSO). D) Absorption spectra of 20 \( \mu \)M p-NMe\textsubscript{2}-ITI 1h in phosphate/citrate buffers with different pH (2 v% DMSO).

Therefore, absorption spectra of p-H-ITI 1a, p-Me-ITI 1c, p-MeO-ITI 1b and p-NMe\textsubscript{2}-ITI 1h were recorded in a series of phosphate/citrate buffers with different pH (See Figure 8.7). For unsubstituted ITI 1a, p-Me-ITI 1c and p-MeO-ITI 1b, no changes to the absorption spectra - besides baseline shifts - were observed for buffers with different pH, which
suggests that there are no differences in the protonation state. Yet for p-NMe₂-ITI 1h a pH was observed that at low pH the main absorption band around 520 nm has disappeared and an absorption band arose around 430, similarly to the absorption of unsubstituted ITI 1a. This suggests that the strong electron donating NMe₂ group responsible for the red-light shift to 520 nm becomes protonated, upon which it loses its strong electron donating properties which results in an absorption spectrum similarly to unsubstituted ITI 1a.

8.3 Mechanistic studies of protonated ITI photo-isomerization

Upon protonation of unsubstituted ITI 1a, two new absorption bands are formed, one that is hypsochromically shifted and one that is bathochromically shifted when compared to the main absorption band of the neutral form. Yet, the relative intensity of the protonated ITI absorption bands is dependent on the electronic properties of the R substituent, where electron donating groups result in increased absorptivity of the red-light shifted band (see Figure 8.2). Similar observations were made for the tetra-ortho-substituted azobenzenes by the group of Woolley, in which computational studies revealed that the red-light shift upon protonation is caused by a smaller energy difference between the ground state and the S₂ excited state. In comparison, ITI calculations in Chapter 6 showed that the main visible light absorption band of the neutral ITI is a mixed band of mainly a S₂ transition contribution. This was experimentally confirmed using pulse-probe spectroscopy in which upon excitation the S₂ excited state species was recorded that decayed in a fs timescale to the more stable S₁ excited state. Therefore we hypothesize that ITI protonation results in separation of the S₁ and S₂ excited state absorption band.

To test the hypothesis that protonation separates the S₁ and S₂ excited state of ITI, the photo-isomerization mechanism was studied using ultra-fast transient absorption spectroscopy (see Figure 8.8). For this study, p-Me-ITI 1c was chosen as a model compound, since the two absorption band formed by protonation have similar absorptivity and are well separated (Figure 8.2C).

As a control, the photo-isomerization process of neutral p-Me-ITI 1c was studied in DCM with excitation at 400 nm (See Figure 8.8A,B). In this case, excitation at 400 nm resulted in the excitation to S₂ excited state, which quickly converts to the S₁ excited state. After that, through a conical intersection, the molecule falls back to either the Z or the E photo-isomer, which results in a residual signal corresponding to the absorption spectrum of the E isomer.
Figure 8.8: Ultra-fast Transient Absorption Spectroscopy of ITI. A) Transient absorption spectra of neutral p-Me-ITI 1c recorded in DCM with excitation at 400 nm. B) The proposed mechanism of Z to E photo-isomerization of neutral ITI. C) Transient absorption spectra of protonated p-Me-ITI 1c recorded in DCM (1 v% TFA) with excitation at 400 nm. D) Transient absorption spectra of protonated p-Me-ITI 1c recorded in DCM (1 v% TFA) with excitation at 510 nm.

To determine if protonation separates the S₁ and S₂ transition, protonated p-Me-ITI 1c was excited with both 400 nm and 510 nm, separately (see Figure 8.8CD). Excitation with 400 nm resulted in the formation of excited species with very broad absorption bands that cool down to a residual signal of E isomer of protonated p-Me-ITI 1c. In contrast, upon excitation with 510 nm these broad absorption bands of the excited state have not been observed. Furthermore, the residual signal of the E cannot be observed, possibly due to scattering and noise around the excitation wavelength. Yet, ns transient absorption spectroscopy (see Figure 8.6F) demonstrated photo-isomerization upon 475 nm light excitation, which is the absorption maximum of the same band. Altogether these experiments demonstrate differences in the mechanism of photo-isomerization upon irradiating the different absorption bands of protonated p-Me-ITI 1c, yet computational support is required to support the interpretation of these experimental observations.
8.4 Conclusion and outlook

The aim of the research presented in this chapter was to determine the potential of the ITI photoswitch for application in dual-response systems controlled by both acid and light. Therefore it was studied how protonation changes the absorption maximum, absorptivity, quantum yield and the half-life of thermal relaxation of the E photo-isomer.

Upon protonation, the half-life of thermal E-Z relaxation of the electron rich ITIs increases to about 35 to 45 ms, possibly by slowing down the rate of nitrogen inversion. The slower thermal re-isomerization to the Z isomer would allow for a higher build-up of the E isomer. Yet, while protonation disfavors nitrogen inversion, the thermal relaxation from E to Z is still in the ms time scale, suggesting that protonated ITIs undergo E-Z thermal re-isomerization through an alternative rotational mechanism. Protonation has also been reported as a well-established method to isomerize imines. In here, a transition state with a more C-N single bond character is proposed, which allows for free rotation to the thermally stable isomer. Destabilizing this transition state electronically through substituents on the thioindoxyl fragment could potentially further increase the half-life of the E-isomer of ITI. Therefore, computational support for the understanding of the effect of protonation on the photochemical properties is needed.

Protonation of a nitrogen atom in an isomerizable double bond results in a bathochromic shift and increased absorption. Similar acid control over the photochemical properties of photoswitches was shown earlier. Here, we directly change the photochemical properties of the photoswitch by protonating the imine, resulting in a red-light shift of both photo-isomers. For the p-MeO-ITI 1b, protonation shifts the absorption maximum of the thermal Z isomer to over 500 nm and absorption of the E isomer to nearly 600 nm and tailing towards 650 nm. The protonated p-NMe₂-ITI 1h has an absorption maximum around 682 nm, which is in the phototherapeutic window where human tissues absorb the least and light can be efficiently delivered. Together with their small size of the photoswitch (for example p-MeO-ITI: Mw = 270 Da) and the facile synthesis, iminothioindoxyl photoswitches show potential for dual response systems, controlled by a combination of light and acid. The next step in this study is acquiring understanding of how protonation alters the properties of ITI. This can be done by computational studies such as shown in Chapter 6 of this thesis.

Furthermore, the origin of the two new absorption bands formed by protonation need to be studied. Possibly, by protonation the absorption bands corresponding to the S₁ and S₂ excited state are separated. Ultra-fast transient absorption spectroscopy revealed differences in the mechanism of photo-isomerization upon irradiation the different absorption bands of protonated p-Me-ITI 1c. Yet, computational studies are needed to support or exclude the interpretation of these experimental results, to fully understand the role of protonation on the properties of ITI.

Even though the conditions under which the ITI was protonated in this study do not reflect biologically relevant conditions, we see potential for protonated ITIs in medicine. We envision that upon further manipulating the pKa of the iminium species, an ITI-based photoswitchable drug could be site-specifically protonated in the environment of a tumor, followed by activation of the drug by irradiation of the red-light shifted absorption band of ITI.
the protonated ITI. This would enable a photopharmacology approach in which no medical imaging technique is needed to guide where irradiation and the accompanied therapeutic intervention is required. However, the currently library of ITIs does not contain a photoswitch that is suitable for such applications. Therefore, design of new ITIs will focus on further tuning the basicity to a pKa in which can be discriminated between physiological pH and the acidified tumor micro-environment.

8.5 Experimental contributions

M.W.H.H. and W.S. designed the project. M.W.H.H. synthesized the compounds and performed the $^1$H NMR and UV/VIS titrations. M.H. and W.J.B. performed the ns transient absorption spectroscopy, assisted by M.W.H.H. The ultrafast transient spectroscopy was performed by M.D.D, assisted by M.W.H.H.

8.6 Experimental data

8.6.1 General synthetic remarks

See Chapter 3.6.1

8.6.2 Synthetic procedures

The synthesis of ITIs 1a-d has been described in Chapter 6.6.2 and the synthesis of ITI 1h has been described in Chapter 7.5.2.

**Scheme 8.1: Synthesis of Hemithioindigo 2**

Benzo[b]thiophen-3(2H)-one 4

See compound 3, Chapter 6.6.2

(Z)-2-benzylidenebenzo[b]thiophen-3(2H)-one 2 (Hemithioindigo):

Benzo[b]thiophen-3(2H)-one 4 (54 mg, 0.36 mmol) was dissolved in pyridine (2 mL) and benzaldehyde (100 μL, 1.1 mmol) and piperidine (1 drop) were added. The reaction mixture was stirred at 80 °C for 18 h. After completion the reaction mixture was concentrated \textit{in vacuo} and subsequently EtOAc (50 mL) and H$_2$O (50 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with water and brine, dried using MgSO$_4$ and concentrated \textit{in vacuo}. The product was purified by flash chromatography (Silicagel 40 – 63 nm, EtOAc/Pentane 1:9). The product was obtained as a yellow solid (58 mg, 0.24 mmol, 67% yield). Mp: 110 – 112 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.30 (t, $J = 7.9$ Hz,
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1H, Ar(H), 7.42 (t, J = 7.3 Hz, 1H, Ar(H)), 7.46 – 7.52 (m, 3H, Ar(H)), 7.58 (t, J = 7.5 Hz, 1H, Ar(H)), 7.71 (d, J = 7.4 Hz, 2H, Ar(H)), 7.94 (d, J = 6.4 Hz, 2H, Ar(H)), 7.97 (s, 1H, C=CH). The 1H NMR spectrum corresponds to literature.

8.6.3 Titration experiments

For the UV/VIS titration experiments, 100 mL of a 20 μM solution of ITI or HTI in DCM was prepared. Part of this solution was used to prepare 1 M TFA solution. A cuvette was filled with 2 mL of 20 μM ITI or HTI solution and the 1M TFA in DCM was added stepwise, while recording UV/VIS spectra in between.

For the 1H NMR titration experiments, 2 mL a 0.5 mg/mL solution of unsubstituted ITI 1a or HTI in CD₂Cl₂ was prepared. Part of this solution was used to prepare a 100 mM TFA solution. A NMR tube was filled with 0.5 mL of the 0.5 mg/mL solution of ITI 1a or HTI and the 100 mM TFA solution was added stepwise, while recording 1H NMR spectra in between.

For the pH titration, citrate/phosphate buffers with different pH were prepared from a 0.1 M citric acid and 0.2 M Na₂HPO₄ solution. A 96 well plate was filled with in each well 98 μL buffer, and to every well 2 μL of a ITI stock in DMSO was added.

8.6.4 Transient Absorption Spectroscopy

See Chapter 6.6.3.

8.7 References

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rsible switching of porosity in molecular crystals

Seek and Destroy: Light


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