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ABSTRACT

The synthesis and characterisation of a novel Reversibly Photoswitchable Chelator (RPC) of calcium ions, designed as a stepping stone towards producing pulses of calcium concentration in an aqueous environment, is reported. This RPC is constituted of a photochromic diarylethene core connected on one side to a BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid) calcium chelator and, on the other side, to an electron-withdrawing group. The operation principle consists in photoswitching on and off an intramolecular charge transfer between one nitrogen atom of the BAPTA moiety and the electron-withdrawing group, thereby modulating the chelating affinity of BAPTA for calcium ions. Solubility of the compound in a partially aqueous solvent was achieved by grafting a short PEG (polyethylene glycol) tail to the electron-withdrawing group. A reduction of the affinity for calcium ions upon photoswitching by a factor of 3-4, in the hundred nM range of dissociation constant, is reported and constitutes a proof of concept of this type of RPC.

Keywords: photorelease of calcium ions; photoswitchable chelator; photochromic device; diarylethene derivatives; supramolecular chemistry

1. Introduction

Photolabile cages, able to release metal ions upon light irradiation, have been known for many years. Based on irreversible photoinduced modifications of different types of chelators [1-5] or cryptands [6,7], these compounds are able to create concentration jumps of metal ions; they are for instance widely used in the field of neurobiology [8]. These systems however suffer from a major drawback, *e.g.* their irreversibility. Upon uncaging the ion, the molecules become denaturated and cannot be recycled and, as a consequence, only concentration jumps can be created, but not pulses. Producing concentration pulses of metal ions of controlled amplitude and duration is nevertheless of great interest, in particular for biological applications where transient concentration changes of a given metal ion (Ca^{2+}, K^{+}, Zn^{2+},…) trigger biochemical events [9]. In recent years great interest has been focused on developing Reversibly Photoswitchable Chelators (RPC), which are photocontrolled molecular switches that can both release and rebind metal ions [10-13]. These reversible systems permit repetitive back and forth operation and consequently have the potential ability to generate concentration pulses of metal ions through sequences of release and delayed recapture. The RPCs reported so far consist of a photochromic core coupled to an ionophore; they possess two isomeric forms, which can be reversibly switched from one to the other by light irradiation at different wavelengths, characterized by different affinities for metal ions [10-13].

Designing and synthesizing RPCs able to operate in an aqueous environment is especially important, particularly for potential applications in the field of biology. Some recent studies have focused on water-soluble systems based on T-type photochromic dyes, like naphthopyran [14] or spiroamido–rhodamine [15]. As one of the two forms is not thermally stable, the molecule goes back spontaneously to its initial state. This means that the duration of the concentration pulses generated by such systems is not fully controlled as it depends on the thermal relaxation time (in the aforementioned cases, $t_{1/2}$ is of the order of 5 min [14] and 1 min [15], respectively). On the other hand, P-type photochromic dyes, characterized by two thermally stable forms, have been used for releasing and capturing monovalent and divalent metal ions. In principle such systems allow a superior time-control on the affinity changes and hence on the pulse duration; the well-known diarylethenes [11,16,17] have in particular been repeatedly reported. Photoresponsive “tweezers” bearing a crown-ether on both sides of the molecule [18-21], were used to study bi-phasic extraction of alkali metal picrates. In this case, the ion is too big to be complexed by one crown ether but can be captured between the two

crown ethers if they are close enough. In this approach, the structural changes of the diarylethene core, induced by irradiation, modify the geometry of the tweezers and thus modulate the affinity for the metal ion. A different strategy was used for Ca^{2+} ions by Malval *et al.* [22]: it consisted in turning on and off the electronic conjugation between an electron-withdrawing group, introduced on one side of the diarylethene core, and a crown-ether ionophore, grafted on the other side. In the closed, conjugated form of the diarylethene, electron abstraction from the withdrawing group reduces the charge density of the ionophore, thereby lowering the calcium affinity as compared to the open deconjugated form. Zhang *et al.* [23] have used a similar system to capture and release calcium ions in lecithin-Ca^{2+} organogel to induce reversible sol-gel transition. It should be noted that Malval *et al.*'s approach was carried out in acetonitrile, where the affinity of the crown-ether moiety for calcium is relatively large (dissociation constant K_d=1-100 µM [22,24]). Such an ionophore cannot be used satisfactorily in an aqueous environment, where the association constant is drastically reduced [25,26].

In the present work, we demonstrate the proof of concept of a new type of Ca^{2+}-specific RPC, able to operate in an aqueous environment. This RPC is constituted by a bistable diarylethene core bound to a BAPTA [27] ionophore (Fig. 1a). BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid) is a very potent calcium chelator, possessing a much better calcium affinity than crown-ethers in water. Its dissociation constant (K_d) is of the order of 100 nM [27] while, for instance, that of a 15-crown-5 ether is 3.17 µM in a 20/80 v/v methanol/water mixture [28]. In addition, BAPTA presents a very good selectivity over magnesium ions (K_d = 17 mM) [27]. As far as modulating the BAPTA affinity for Ca^{2+} upon photoswitching the diarylethene core, we chose the same principle described by Malval *et al.* [22], which consists in coupling an electron-withdrawing group on one side of the diarylethene to induce charge depletion on the BAPTA, grafted on the other side of the core, when electronic conjugation is switched on in the closed form (Fig.1b). Since perfluorinated diarylethenes are not water-soluble [29], the electron-withdrawing group was replaced by a hydrophilic tail in order to improve the overall solubility of the RPC in aqueous solution.

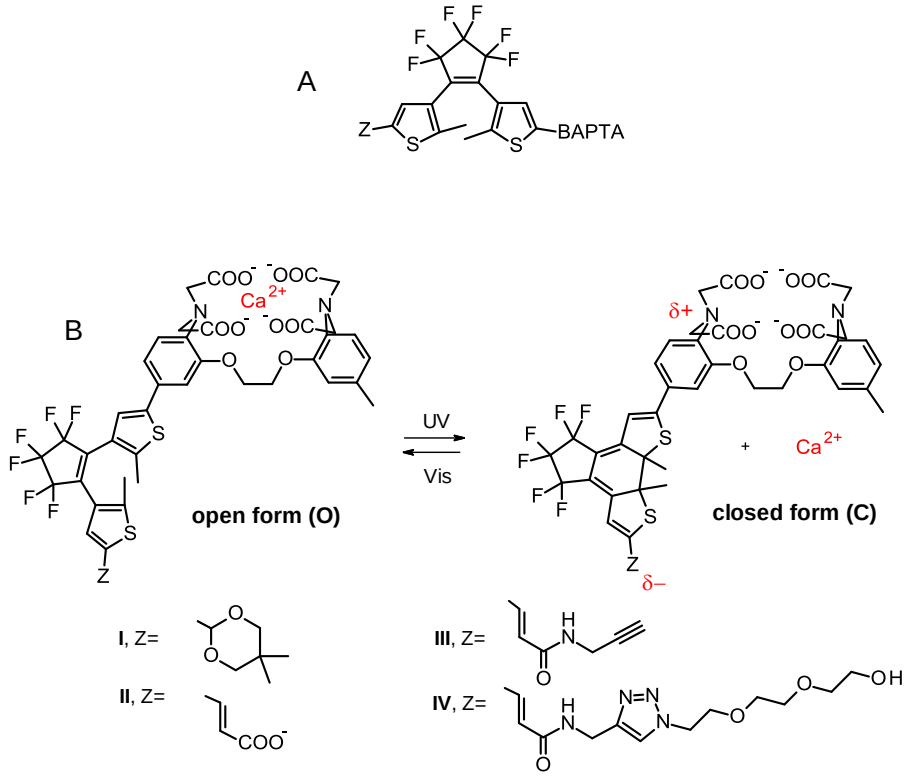

Fig. 1. (A) Chemical structure of a model RPC compound. (B) Photoswitching between the open form (O, high affinity for Ca^{2+}) and closed form (C, low affinity for Ca^{2+}) of photoswitchable Ca^{2+} chelators **I-IV**. Four molecules possessing different electron-withdrawing moieties were synthesized (**I-IV**; Fig.1b) to evaluate the effect of the acceptor strength of this group on the efficiency of the construct.The photoswitching properties of the compounds as well as the modulation of their affinity for calcium by photoswitching was studied by steady-state UV-vis absorption spectroscopy, complemented by data coming from HPLC measurements.

2. Materials and methods

2.1 Synthesis

Compounds **I-IV** were synthesized from a common dithienylethene core bearing a BAPTA chelator and an aldehyde (compound **6**) to introduce a substituent bearing an electron withdrawing group. This key compound **6** was synthesized by using a palladium-catalyzed coupling between the known [30] bromo derivative **2** (prepared [31,32] from the dibromo compound **1** [33]) and the known boronic ester **4** [34] (Fig. 2). The neopentyl acetal of **5** was similarly prepared from **2** from acetal **3**. Aldehyde **6** was found to be unstable and was immediately involved in a Wittig-Horner reaction utilizing trimethylphophonoacetate and the phosphono propargylamide **8**, to produce the unsaturated ester **7** and amide **9** [35], respectively. The saponification of **7** and **9** gave **I** and **III**, respectively. To increase the water

solubility of **III**, a hydrophilic polyethylene glycol (PEG) tail was grafted to it. For this purpose, the alkynyl group in **9**, was involved in a click reaction with **10** [36] leading to **11** which was then saponified to produce **IV**.

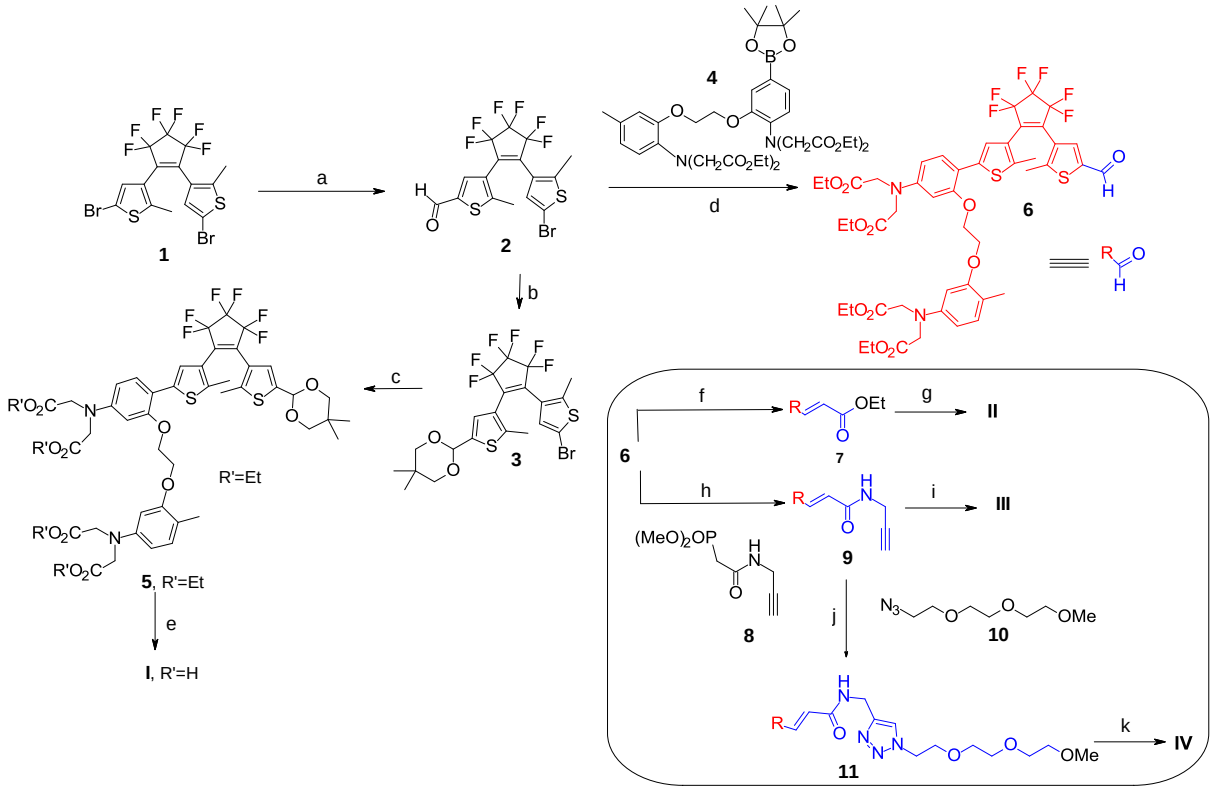

Fig. 2. Reagents and conditions: a) *n*-BuLi (1 equiv), DMF (5 equiv), THF -78°C, 61% (lit). b) neopentylglycol (1.75 equiv), PTSA cat., toluene reflux, 18 h, 89%. c) **4**, Pd_2dba_3, PCy_3, CsF, toluene, reflux, 16 h, 71%. d) **4**, Pd_2dba_3, PCy_3, CsF, toluene, reflux, 16 h, 75%. e) NaOH, (THF/MeOH/H_2O: 1/1/1), rt, 2 h, 97%. f) trimethylphosphonoacetate (2 equiv), NaH (2.2 equiv), THF, rt, 15 h, 29%. g) NaOH, (THF/MeOH/H_2O: 1/1/1), rt, 2 h, 97%. h) **8** (1.75 equiv), NaH (2.7 equiv), THF, rt, 15 h, 33%. i) NaOH, (THF/MeOH/H_2O=1/1/1), rt, 2 h, 99%. j) **10** (3 equiv), $CuSO_4$ (0.2 equiv), sodium ascorbate (0.4 equiv) (DMF/H_2O=2/1), rt, 20 h, 81%. k) NaOH, (THF/MeOH/H_2O=1/1/1), rt, 2 h, 99%.

The synthesis of molecules possessing stronger electron-withdrawing groups, like an aldehyde or barbituric acid, was attempted but the molecules proved to be unstable (both chemically and photochemically). A compromise between the stability of the molecules and the electron-withdrawing ability of the substituents had thus to be found. The strength of the four retained electron-withdrawing groups was estimated by comparing their Hammett constants. Accordingly, acetal **I** was expected to be a weak acceptor, amide **III** a strong one, and the vinyl ester **II** a weak or a strong one depending on its protonation state.

Details on the individual synthesis of compounds **2-11** and **I-IV** are given as Supplementary Data (SD §1), together with the NMR spectra (SD §2).

2.2 Samples

Let us first note that compounds **I**-**IV** were all found to be soluble in acetonitrile. Compounds **II** and **IV** showed partial solubility in water: 50% (v/v) of acetonitrile was necessary to solubilize **II**, while only 15% was enough for **IV**, thanks to the presence of the PEG tail. The solubility of **IV** in its closed form was further observed to decrease in the presence of calcium ions (50% v/v acetonitrile necessary). A possible explanation is that the conjugated molecules in the closed form tend to stack with each other and precipitate.

Acetonitrile (for spectroscopy Uvasol, Merck) was used for all samples. Type 1 ultrapure water was obtained from a Milli-Q Integral 3 purification system (Millipore). The highest BAPTA pKa values are 5.47 and 6.36 and triethylamine (TEA) was used in order to deprotonate the four acid functions of the BAPTA moiety of the different diarylethene-based molecules in order to obtain its full affinity for calcium ions. BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid; Aldrich, 98%) was used as received. Calcium ions were obtained from $Ca(ClO_4)_2$ (Aldrich).

The concentration of Ca^{2+} found in our ultrapure water (*ca.* 20-30 µmol L^{-1}) was in fact sufficiently large to fully complex our molecules in the titration experiments. To avoid this problem and to control efficiently the concentration of Ca^{2+} in aqueous solutions, we used two commercially available calcium buffers (Calcium calibration buffer kit #1, 0 and 10 mmol L^{-1} CaEGTA, Life Technologies). Both buffers contain EGTA (ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid), a well-known calcium chelating agent [37]. The 0 mol L^{-1} buffer (Zero Ca^{2+} buffer) consisted of 10 mmol L^{-1} K_2EGTA (*i.e.* 10 mmol L^{-1} of EGTA and 20 mmol L^{-1} of K^+ as a counterion), 100 mmol L^{-1} KCl and 30 mmol L^{-1} MOPS (3-(N-morpholino)propanesulfonic acid), in deionized water at pH 7.2. The 10 mmol L^{-1} buffer consisted of 10 mmol L^{-1} CaEGTA, (*i.e.* 10 mmol L^{-1} of EGTA and 10 mmol L^{-1} Ca^{2+}), 100 mmol L^{-1} KCl and 30 mmol L^{-1} MOPS. More details on the utilization of the two buffers are reported in §2.4.

2.3. Photoswitching

The UV/vis absorption spectra were recorded on a Varian Cary 300 spectrophotometer. Irradiation was performed using a FluoroMax-3 fluorimeter (JobinYvon Horiba). The excitation slits were fully open (30 nm) to irradiate most of the sample volume. Samples were stirred every 2 min to homogenize the solution. Irradiation from the open (O) to the closed (C)

form (at 315-330 nm) took 10-20 min before reaching the photostationary state (10%-30% of the O form, depending on the acceptor, see SD §3). Irradiation from the C to the O form (at 580-650 nm) took longer: 30-60 min were necessary to totally convert the molecules.

2.4. Spectrometric titrations and data analysis

Spectrometric titrations of Ca^{2+} ions were performed both in pure acetonitrile and acetonitrile/water (50% v/v) mixtures. As explained in §2.2, we controlled with precision the low concentration of Ca^{2+} in our aqueous mixtures (~ 0-40 µmol L^{-1}) by using commercially available EGTA-containing buffers. The association constant of EGTA for Ca^{2+} is well known (all association constants K will be reported as $\log_{10}$ values and will be noted gK): gK_{EGTA} = 6.822 at pH 7.2, with an ionic strength of 100 mmol L^{-1} KCl [37]. In the absence of other complexing agents, K_{EGTA} can be used to calculate the concentration of Ca^{2+}:EGTA complex and free Ca^{2+}, knowing the total EGTA and Ca^{2+} concentrations.

In the zero Ca^{2+} buffer, traces of calcium ions are completely complexed by EGTA and the free Ca^{2+} concentration is equal to zero. In the 10 mmol L^{-1} CaEGTA buffer, most of the calcium ions are complexed in Ca^{2+}:EGTA complex and the concentration of free Ca^{2+} is equal to 39 µmol L^{-1}. By mixing the two buffers (in the absence of other chelating agents), free calcium concentrations ranging between 0 and 39 µmol L^{-1} can be obtained. In order to get concentrations higher than 39 µmol L^{-1} additional Ca^{2+} ions had to be added by dissolving calcium perchlorate.

The spectrophotometric titration data were globally analyzed by using a homemade Mathematica program. The approach consists in fitting the full set of data (spectra as a function Ca^{2+} concentration) with a given complexation model, involving different species in free and complexed forms. The main parameters optimized by the fit are the complexation constants, while the spectra associated to the species are correspondingly determined so as to minimize the least square difference between data and fit. Different models were used to fit the titration data, depending on the sample (pure open form (O) or open and closed form mixture (O+C)) and the solvent (acetonitrile or acetonitrile/water (50% v/v) mixture in the presence of EGTA). The reason is that a different number of simultaneous complexation equilibria of Ca^{2+} take place and have to be simultaneously considered in order to obtain the concentrations of all the complexed and uncomplexed species. Details of the different models are given in the Supplementary Data (SD §6).

3. Results and discussion

3.1. Photoswitching

The photoswitching properties of the four compounds (**I**-**IV**) were studied by steady-state UV-visible spectroscopy in an acetonitrile solution. The absorption spectra of the amide+PEG **IV** are shown in Fig. 3. The open form (O) is converted to the closed form (C) upon irradiation in the UV (Fig. 3A). Since both O and C forms absorb in the UV, the conversion was not total and a photostationary state was reached (noted O+C, Fig. 3C). To determine the fraction of each form in the mixture, without knowing *a priori* the molar absorption coefficient of the C form, we used HPLC chromatograms, recorded at 310 nm, where both forms are contributing but are eluting at different times. We fitted the chromatogram of the mixture using the one of the pure O form as a reference; properly delayed by some time shift, the latter was also used to fit the C elution peak (details are provided in SD §4). Relying on the repeatability of the injection step in both O and O+C measurements, the molar fraction of O in the mixture was directly obtained from the fit, and the molar fraction of C was deduced by difference. For **IV** in acetonitrile, molar fractions of 30% and 70% were found for O and C, respectively. The C→O conversion upon red illumination (Fig. 3B) is total as only C absorbs in this region. The absolute absorption spectrum of the pure O form, expressed in molar absorption coefficients, was easily determined as the mass of compound dissolved in the solution was known. The one of the pure C form was deduced from the O+C spectrum, knowing the fraction of O form (details are given in SD §4).The spectra of both forms of **IV** in acetonitrile are shown in Fig. 3D; those of the other three compounds may be found in SD §4. The wavelengths of the corresponding absorption maxima ($\lambda_{O,}$ λ_C), and their associated molar absorption coefficients ($\varepsilon_O(\lambda_O)$, $\varepsilon_C(\lambda_C)$), are reported in Table 1.

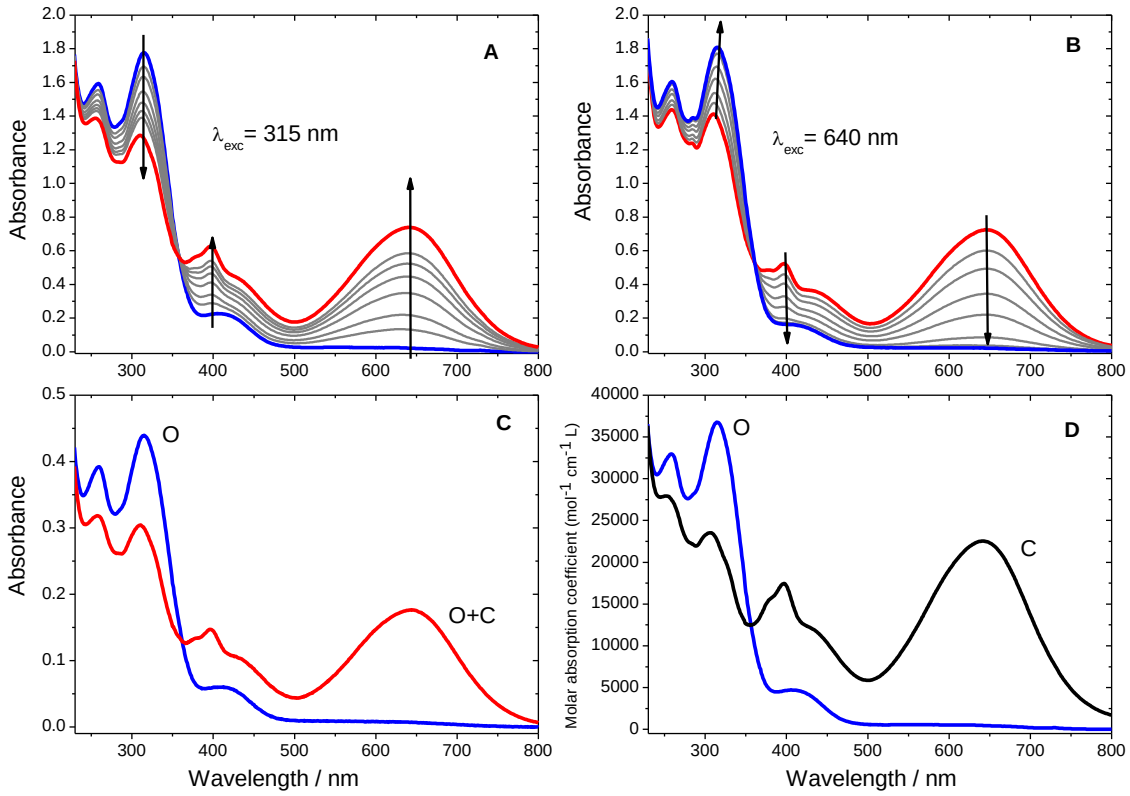

Fig. 3. Photoswitching of **IV** in acetonitrile. (A) photoswitching from open (O) to closed (C) form after irradiation at λ_{exc}=315 nm (B) photoswitching from C to O after irradiation at λ_{exc}=640 nm (C) Before irradiation only the open (O) form is present (blue trace). After irradiation at 315 nm a mixture (O+C) of open and closed forms is observed: 30% of O and 70% of C (red trace). (concentration of **IV** is 11.3 µM). (D) Calculated molar absorption coefficients of the open form O (blue) and the closed form C (black).

Table 1

Photophysical properties and association constants of the open (O) and closed (C) forms of compounds **I** to **IV**. The association constants K are reported as $\log_{10}$ values, noted gK. The values corresponding to BAPTA are displayed at the bottom of the table.

					Acetonitrile			Acetonitrile/H_2O (50% v/v)		
Cpd	λ_O [nm]	$\varepsilon_O(\lambda_O)$ [L mol^{-1} cm^{-1}]	λ_C [nm]	$\varepsilon_C(\lambda_C)$ [L mol^{-1} cm^{-1}]	gK_O	gK_C	K_O/K_C	gK_O	gK_C	K_O/K_C
I	330	17500	580	15400	5.77 ± 0.07	5.39 ± 0.04	2.4	-	-	
II	320	27500	630	17400	6.0 ± 0.3	4.80 ± 0.07	15.8	7.00 ± 0.06	6.58 ± 0.09	2.9
III	315	36500	650	21600	6.1 ± 0.3	5.59 ± 0.05	3.2	-	-	
IV	315	36500	640	22500	5.8 ± 0.4	5.34 ± 0.03	2.8	6.78 ± 0.05	6.17 ± 0.05	4.1
BAPTA					4.33±0.05 (gK)			7.36±0.02 (gK)		

3.2. Complexation

In order to test our method (see §2.4) and acquire a reference basis, we first determined the association constant of pure BAPTA in water, in acetonitrile and in the water/acetonitrile mixture (50% v/v) used for studying compounds **I**-**IV** (see §2.1). In pure water we found gK_{BAPTA} = 7.26, which is close to the value reported by Tsien (6.97) [27]. In the aqueous mixture we found gK_{BAPTA} = 7.36, suggesting that the local environment of BAPTA in the water/acetonitrile mixture is rather composed of water molecules. The association constant observed in pure acetonitrile is much smaller as gK_{BAPTA} = 4.33. This value may be due, in part, to the poor solubility of BAPTA in acetonitrile; the measured constant is likely an average value taken over different classes of monomeric and aggregated molecules. The intrinsic lower affinity of the BAPTA monomer for Ca^{2+} in acetonitrile could also contribute to the observed effect.

The spectrophotometric titrations of the above-mentioned O and O+C samples were then performed with increasing the concentration of calcium, as described in §2.4. Triethylamine (TEA) was used to deprotonate the acid functions of the BAPTA moiety and obtain its full affinity for calcium ions. Pure acetonitrile was used to compare the effect of the four electron-withdrawing groups, including water-insoluble compounds **I** and **III**. Figs. 4A and 4B show the spectrophotometric titration of compound **IV** in acetonitrile/water, in pure O form and O+C mixture, respectively. The titrations of the compounds in all solvents can be found in SD §5.1.

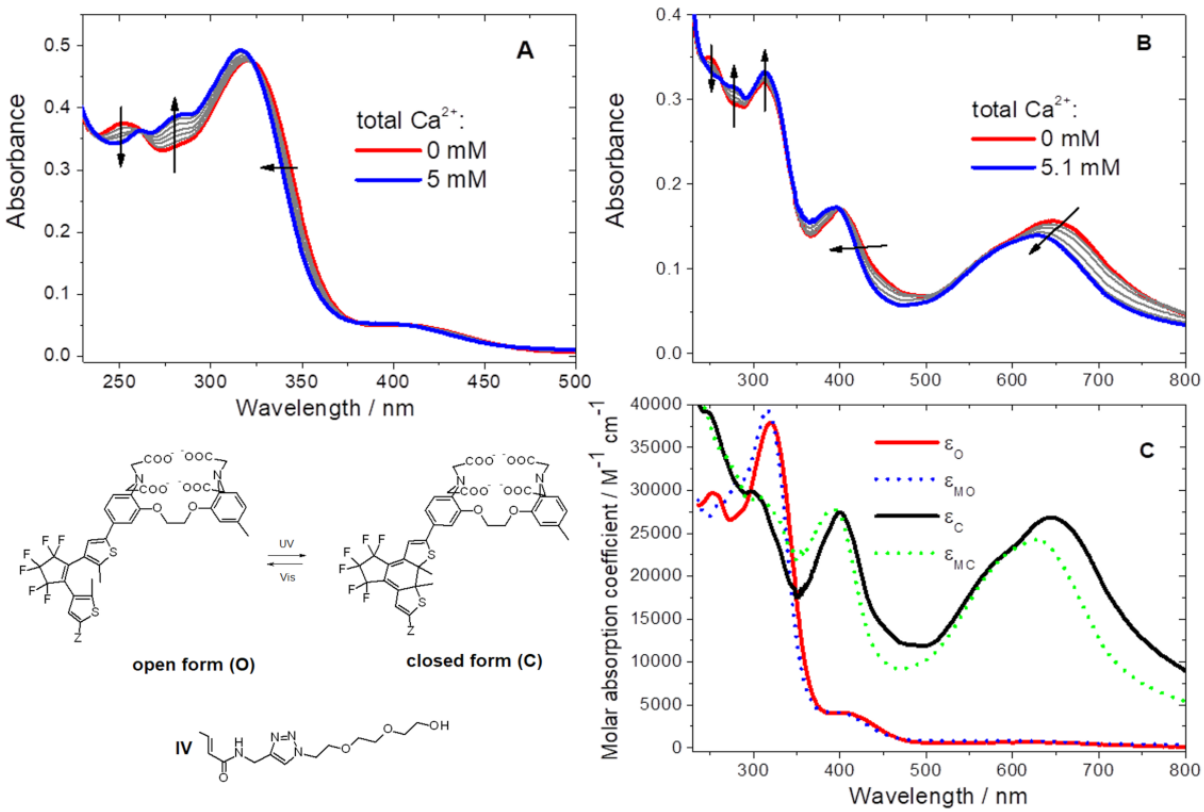

Fig. 4. Complexation of the amide+PEG **IV** in acetonitrile/water mixture; the structure of the molecule in both O and C forms is recalled in the lower left quarter of the figure. (A) Spectrophotometric titration of the O form ([O] = 12 µmol L^{-1}) by increasing concentrations of total Ca^{2+} ([EGTA] = 5 mmol L^{-1}). (B) Spectrophotometric titration of an O+C mixture (photostationary state; [O] = 4.6 µmol L^{-1}, [C] = 5.4 µmol L^{-1}) by increasing concentrations of total Ca^{2+} ([EGTA] = 5 mmol L^{-1}). (C) Calculated absorption spectra of the open free ligand (O - red), the open complex (MO - blue), the closed free ligand (C - black) and the closed complex (MC - green).

The absorption spectra of all titration experiments were then globally fitted (see last paragraph of §2.4) to determine the association constants of the pure O and C forms (K_O and K_C, respectively. Depending on the case, different models were used, including one to three complexation equilibria. For samples in the pure O form, as well as for BAPTA, in acetonitrile, one equilibrium representing the only complexing species in presence was sufficient. For samples in the O+C state in acetonitrile, the equilibria of both O and C forms were included. For samples in the acetonitrile/water (50% v/v) mixture, an additional equilibrium had to be added, to take into account the presence of EGTA, which was required to control the concentration of Ca^{2+} at very low levels (see §2.2). Details on these various equilibria are provided in SD §6. The equilibrium constant K_O was readily measured with the data of the pure O samples. To determine K_C, a two-step approach was used. First, the

titration spectra of O+C samples were fitted in a region where only the C form absorbs, between 500 and 800 nm. K_O was imposed and K_C and the spectra of the free and bound C species were obtained in this restricted range. To get the spectra of the C species in the full 250-800 nm range, a different approach was employed. The values of K_O and K_C obtained previously were used to estimate the contribution of the O species (both free and bound) to the O+C titration data. This contribution was next subtracted in order to obtain the titration spectra of the pure C form, in the full spectral range. The latter were then globally fitted with a model excluding the O form and a new equilibrium constant K'_C was determined, together with the spectra of the free and bound C species in the full spectral range. K_C and K'_C were found to be very close (within errors), and the spectra consistent with the one previously obtained between 500 and 800 nm. Details on this procedure are available in SD 6.4.

3.3 . Modulation of calcium affinity upon photoswitching

The equilibrium constants K_O and K_C are reported in Table 1 for the compounds in O and C forms, both in acetonitrile and in the aqueous mixture. The absorption spectra of the O/C free ligands ($\varepsilon_O(\lambda)$, $\varepsilon_C(\lambda)$) and the corresponding calcium complexes ($\varepsilon_{MO}(\lambda)$, $\varepsilon_{MC}(\lambda)$) are given in Fig. 4C. In acetonitrile, the association constant of the O form of compounds **I**-**IV** is significantly higher than that of BAPTA (gK_O = 5.77-6.10), probably due to a better solubility of our molecules in this solvent. The observed constant reflects thus the true affinity of the BAPTA monomer for Ca^{2+} in acetonitrile. In the water/acetonitrile mixture, K_O is comparable to K_{BAPTA} (gK_O = 6.78-7.00). Its slightly smaller values might be due to a weak electron-withdrawing effect of the acceptor groups, even in the O form. In both solvents, the main absorption band at 300-310 nm (diarylethene core) scarcely changes upon complexation, while the smaller band at 250 nm shifts by 20 nm to the red (Fig.4C). This latter band and its shift is dominated by the BAPTA moiety, the intense band at 250 nm being known to shift to 280 nm and decrease in intensity upon complexation [27] (see spectra in SD §8, Fig. S13). As expected, when the molecule is photoswitched to the C form, its affinity for Ca^{2+} decreases systematically. In acetonitrile, gK_C is reduced to 4.80-5.59 depending on the compounds (see Table 1), and to 6.17-6.58 in the aqueous mixture. Upon complexation, the absorption band of the C form in the red slightly decreases and shifts to the blue. The effect is more pronounced in acetonitrile (*e.g.* 650 nm for the free **IV** to 620 nm for the complex; see SD §7, Fig. S11). The shifts are smaller in the acetonitrile/water mixture (of the order of 10-20 nm) than in acetonitrile (Fig. 4C). These spectral changes are the result of the involvement of the lone

pairs of the BAPTA oxygen and nitrogen atoms (interacting with the calcium ion) which reduces the extension of the conjugation chain.

Switching from acetonitrile to the acetonitrile/water mixture improves the complexation of both forms. Table 1 shows that the different electron-withdrawing groups give similar results as the ratio of the association constants K_O/K_C ranges from 2.4 to 4.1 for most molecules in the two solvents (with the noticeable exception of **II** in acetonitrile, yielding K_O/K_C =15.8). The PEG side chain does not modify the difference of Ca^{2+} affinity between the O and C forms (ΔgK) to a great extent (compare **III** and **IV**), as expected because of the absence of conjugation between the PEG and amide groups.

The ΔgK of our compounds is around 0.5 for most of the molecules. The use of stronger electron-withdrawing groups like aldehyde and barbituric acid would have yielded a larger charge transfer in the C form and likely a larger ΔgK, but led to unstable molecules. We therefore had to use rather weak electron-withdrawing substituents, resulting in a smaller modulation of Ca^{2+} affinity than the one observed by Malval *et al* [22]. An additional reason for this difference might be the fact that two nitrogen atoms are involved in the calcium binding of BAPTA [38] while only one of them is connected to the diarylethene core of our compounds and is thus affected by the electron-withdrawing group in the C form. One could argue that perturbing only one of these nitrogen atoms is not enough to strongly alter the global affinity. However, supplementary measurements were performed with a compound exhibiting only half the binding structure of BAPTA (see SD §9) and yielded gK = 1.60 in aqueous solution, which is very poor and suggests that inactivating only one half of BAPTA could be enough to reach a very large ΔgK. It would thus be most interesting to find ways of stabilizing molecules bearing stronger electron-withdrawing substituents. Alternatively, one could consider adding a second diarylethene center coupled to an electron-withdrawing group on the second nitrogen atom of BAPTA. Given the fact that diarylethenes are T-type photochromic dyes, it would not be necessary for the two units to be simultaneously excited; they could just be switched one after the other from the O to the C form with sufficiently large irradiation dose. Hopefully, the effect would be to amplify strongly the modulation of calcium affinity by cooperative effect.

4. Conclusion

We have synthesized and studied a Reversibly Photoswitchable Chelator (RPC) of calcium ions, based on a dithienylethene core connected to a BAPTA chelator on one aromatic group

and to an electron-withdrawing group on the other one. The aim of this synthesis was to use the photoswitching of the dithienylethene core to turn on and off an intramolecular charge transfer between one nitrogen atom of the BAPTA moiety and the electron-withdrawing group. As expected, the open deconjugated form essentially displays a high affinity for calcium ions of BAPTA. In the closed conjugated form, the affinity was found to be substantially decreased, by a factor of 3-4. Still modest as compared to known crown-ether-substituted diarylethenes, this modulation is however most promising as it is the first one reported to occur in the hundred of nM range of dissociation constant (of the binding form), while crown-ether derivative rather operate in the µM range. Our molecules could potentially be improved by introducing a stronger electron-withdrawing group than the ones reported in this study. Alternatively, one could consider implementing such a charge transfer device on both nitrogen atoms of the BAPTA, so as to further decrease the Ca^{2+} affinity of the doubly closed form.

The second objective of our work was to produce RPCs able to operate in an aqueous environment. As the dithienylethene core is not soluble in water, a short PEG chain was added to the electron-withdrawing group. Water solubility was indeed increased but full solubility in water was not achieved. Larger solubilizing groups (neutral or charged, containing guaninidium or sulfonate, for example) could be added using the same synthetic strategy. In addition, stacking of the molecules might be prevented by using bulkier hydrophilic tails such as branched PEG, dextrans or cyclodextrins. Alternatively, one could start from a more water-soluble photochromic core and maleimide derivatives [11] could be tested.

On the basis of the proof of concept reported here, further research should render possible the use of this type of chelator in aqueous media.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/xxx.

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