A rotaxane mimic of the photoactive yellow protein chromophore environment: effects of hydrogen bonding and mechanical interlocking on a coumaric amide derivative†

José Berná,[b] Albert M. Brouwer,*[a] Sandro M. Fazio,[a] Natalia Haraszkiewicz,[a] David A. Leigh*[b] and Claire M. Lennon (neé Keaveney)[b]

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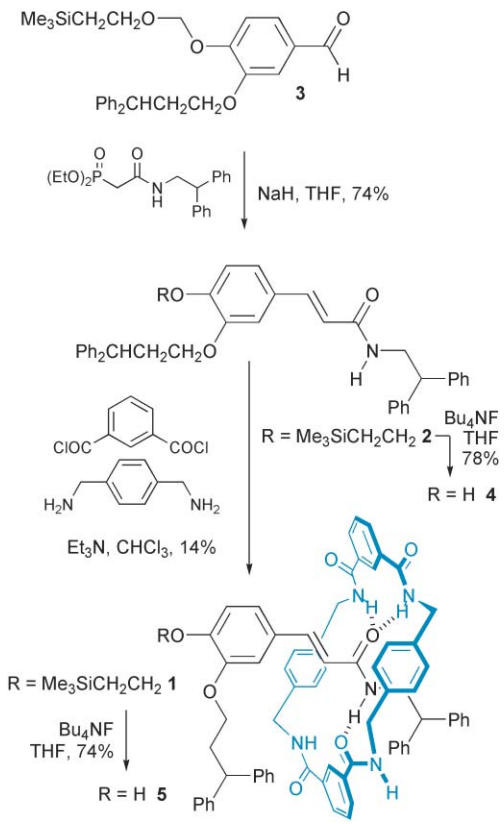
Hydrogen bonding in a [2]rotaxane is shown to stabilise the phenolate anion of a coumaric amide chromophore by almost 3 pK_a units; however, the effect on the UV spectral shift in the anion is small and, significantly given the photochemistry of PYP, despite the hydrogen bonding olefin photoisomerisation in the anionic rotaxane remains heavily suppressed.

In biological photoreceptors, the protein environment often leads to considerable changes of the spectroscopic and photochemical properties of embedded chromophores. Well known examples include the retinal chromophore, where the absorption spectrum is red-shifted to different extents in the different protein complexes involved in vision,[1] and the coumaric thioester chromophore in the Photoactive Yellow Protein (PYP) of *Halorhodospira halophila*.[2–4] In the latter case, the large red shift of the absorption is partly due to deprotonation of the phenolic OH-group, which is considerably more acidic in the protein environment than in water. The extent to which hydrogen bonding contributes to the red shift is unclear.[3–5]

Mechanically interlocked architectures, such as catenanes and rotaxanes, offer an attractive approach to the study of relatively weak intermolecular interactions without the complications of dissociation of the assemblies in solution. Rotaxanes have been reported in which chromophores were surrounded by a macrocyclic ring, which affects the spectral properties and stabilises the chromophore.[6–8] In the present work we describe how hydrogen bonding and mechanical interlocking in a rotaxane architecture affect the pK_a and the spectral and photochemical properties of the coumaric amide chromophore,[9] which is structurally and photochemically similar to the coumaric thioester present in PYP.

Rotaxane **1** was synthesized by a five component clipping reaction[10] in which a benzylic amide macrocycle is assembled around the SEM ether-protected thread **2** (Scheme 1). In this case a single amide group directs the ring closure rather than the more established fumaramide, succinamide or peptide templates,[11] hence the modest yield (14%) of the protected rotaxane **1**. In order to prevent the ring dissociating from the thread, a side chain was required on the aromatic ring. The protected thread **2** was prepared from a Wadsworth–Emmons condensation of aldehyde **3** and the masked phenolic groups of thread **2** and rotaxane **1** subsequently liberated with Bu_4NF to yield **4** and **5**, respectively (Scheme 1). Compounds **4** and **5** are related to ferulic acid (3-methoxycoumaric acid), which can replace the coumaric unit in PYP without compromising the photochemical cycle.[12]



Scheme 1 Synthesis of 3-alkoxycoumaric amide thread **4** and rotaxane **5**.

In the neutral form of rotaxane **5** (*i.e.* the phenol in **5** is not deprotonated), the macrocycle is hydrogen bonded to the amide group in apolar environments. This results in a shielding of the protons of the double bond in the ^{1}H NMR spectrum of **5** in $CDCl_3$ by 1.03 ppm (olefin proton adjacent to benzene ring) and 1.46 ppm (olefin proton adjacent to carbonyl group).† Molecular modeling (Macromodel 8, AMBER force field, GBSA solvent model for $CHCl_3$) revealed a number of low-energy conformations

[a]*Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS, Amsterdam, The Netherlands. E-mail: a.m.brouwer@uva.nl; Fax: +31 205 255 680; Tel: +31 205 255 491*
[b]*School of Chemistry, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh, United Kingdom EH9 3JJ. E-mail: David.Leigh@ed.ac.uk; Fax: +44 131 667 9085; Tel: +44 131 650 4730*

† Electronic supplementary information (ESI) available: Details of synthesis and structural characterization; NMR spectra of photostationary mixtures; determination of pK_a. See DOI: 10.1039/b618781a

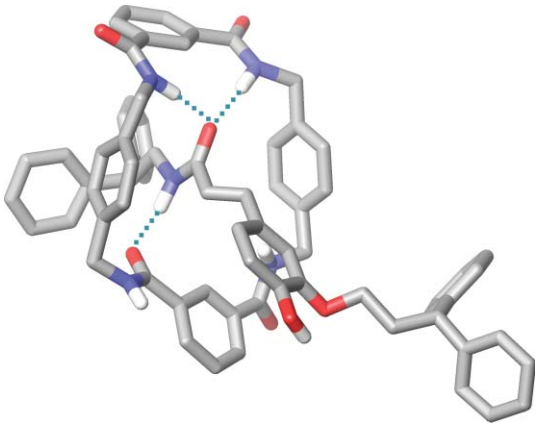


Fig. 1 Computationally determined low-energy structure of rotaxane **5** in its protonated form. Macromodel 8, AMBER* force field, GBSA solvent model for $CHCl_3$.

which support this structural assignment. A typical low energy conformation is shown in Fig. 1. The oxygen atom of the carbonyl group is involved in a bifurcated hydrogen bond, which is a recurring theme in the X-ray crystal structures of rotaxanes based on the same macrocycle, and the NH of the amide is bonded to a C=O group of the ring, which is twisted inward.[13]

The UV absorption spectra of **4** and **5** in dichloromethane are shown in Fig. 2A. The absorption bands >300 nm can be attributed to π–π* transitions of the ferulic amide chromophore. Absorption due to the macrocycle occurs at shorter wavelengths. Some interaction between the ring and the thread in rotaxane **5** is apparent from the small red shift of the positions of the absorption maxima (322 *vs.* 317 nm).

Irradiation of dichloromethane solutions of thread **4** and rotaxane **5** at 313 nm leads to clear changes in the UV-absorption spectra (Fig. 2B and 2C). According to ^{1}H NMR analysis, the photostationary state of **4** at 313 nm consists of 63% of the *Z*-isomer. Using this information the spectrum of the photostationary state can be deconvoluted into contributions from the two olefin isomers (Fig. 2D). The spectrum of the *Z* isomer is less

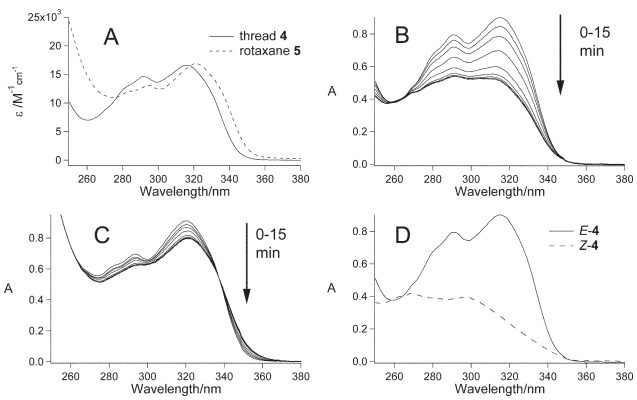


Fig. 2 A. UV absorption spectra of thread **4** and rotaxane **5** in CH_2Cl_2 at RT. B. Spectral changes during photoisomerisation of **4**. C. Spectral changes during photoisomerisation of **5**. D. Comparison of spectra of the isomers of **4**.

intense and somewhat blue-shifted. One reason for the predominance of the *Z*-isomer in the photostationary state is its lower absorption coefficient at 313 nm. The quantum yields of the isomerisation have not been determined, but the ratio of the quantum yields $\Phi_{E\rightarrow Z}/\Phi_{Z\rightarrow E}$ can be derived from the photostationary state composition and the absorption coefficients at the excitation wavelength. For thread **4** this ratio was found to be 0.57. In other words, the efficiency of the $Z \rightarrow E$ isomerisation is almost twice that of the $E \rightarrow Z$ reaction. In the case of rotaxane **5**, less than 10% of the *Z*-isomer was found in the photostationary state at 313 nm. This precluded a reliable estimation of the spectrum of *Z*-**5**, but there is no reason to suspect that this will be dramatically different from that of *Z*-**4**. Thus, the ratio of the quantum yields $\Phi_{E\rightarrow Z}/\Phi_{Z\rightarrow E}$ in the rotaxane **5** is even smaller than that in thread **4**.

Both **4** and **5** show very weak and short-lived fluorescence at room temperature. The excited state lifetime is of the order of a few picoseconds, as found for other related chromophores.[14–20] At lower temperatures, the decay times and intensities increase.[21]

In the protein environment of PYP, the coumaric thioester unit has a much lower pK_a than in solution.[12] A similar effect was found when comparing the 'naked' thread **4** to the 'encapsulated' situation in rotaxane **5**. In mixtures of methanol and water (3 : 2) the apparent pK_a of **4** was found to be 9.3, which is a normal value for a phenol substituted with an electron withdrawing group.[4] For rotaxane **5**, on the other hand, a pK_a value of 6.7 was found under the same conditions. Thus, the macrocycle decreases the pK_a of the coumaric amide unit by almost 3 units. Most likely the macrocycle forms strong hydrogen bonds with the ArO^- group, as illustrated in the calculated energy minimised structure shown in Fig. 3.

Deprotonation of the coumaric unit leads to red shifts of the absorption bands (Fig. 4) of 3900 cm^{-1} for **4** (320 → 366 nm) and 4400 cm^{-1} for **5** (322 → 375 nm). These shifts are similar to those reported for hybrid PYP's.[12] The interaction between the macrocycle and the ArO^- group contributes slightly to the red shift in the absorption spectrum. Given the fact that phenols are more acidic in the excited state than in the ground state, a stronger hydrogen bonding would be expected in the ground state, resulting in a *blue shift* of the absorption band. In agreement with this notion, a computational study of PYP chromophores concluded that the hydrogen bonding interaction of the deprotonated chromophore indeed has a *negative* contribution to the observed overall red shift of the chromophore in the protein.[3] The electrochromic contribution due to charged amino groups in the protein plays a major role in the spectral tuning of the coumaric

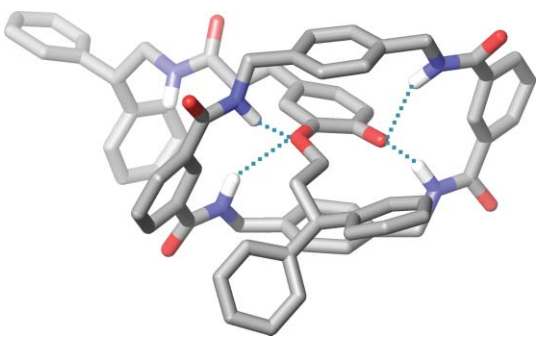


Fig. 3 Computationally generated low-energy structure of rotaxane **5** in deprotonated (ArO^-) form.

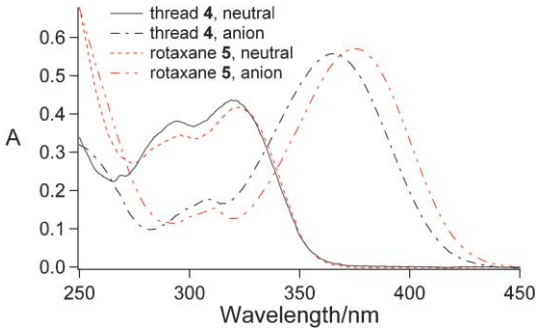


Fig. 4 UV-Vis absorption spectra of protonated and deprotonated forms of **4** and **5** in methanol : water, 3 : 2.

thioester in PYP. Rotaxane **5** does not contain charged groups, so the observed stabilization of the excited state of the anion results from dispersion interactions.

Remarkably, irradiation of solutions of **4** and **5** in their deprotonated form did not result in significant changes in absorption or emission spectra. This means that photoisomerisation is strongly suppressed under these conditions, in contrast to the behaviour of the neutral molecules in dichloromethane.[21] We did not quantitatively determine the fluorescence intensities or decay times of the anions of **4** and **5** in water/methanol, but qualitatively their fluorescence is more intense than that of the neutral forms in dichloromethane, but still weak. Since photoisomerisation of the anions does not occur under these conditions, other rapid nonradiative decay processes must dominate the photophysics. Similar observations have previously been made for coumaric thioesters, which undergo efficient photoisomerisation when in PYP (Φ_{EZ} = 0.35),[22] but not on their own in solution. An explanation for this effect was offered by Groenhof *et al*, who found computationally that the difference in behaviour is caused by the influence of the protein, which moves the position of the conical intersection along the C=C twisting coordinate to an accessible part of the potential energy surface.[23] Although this is an elegant and significant idea, it does not explain why those model compounds that do not efficiently undergo photoisomerisation still have very short-lived excited states. It has been suggested that in coumaric acid derivatives there is competition between productive double bond rotation and unproductive rotation about single bonds,[20,24] which depends in a subtle way on the precise chemical structure and environment.[16,17,20,24] One such difference exists between the secondary amides in the present work, and the simple primary amide studied by Changenet-Barret *et al.*, which does undergo photoisomerisation.[25] Our results indicate that strong hydrogen bonding to ArO^-, which occurs in the anion of **5** as well as in the photoactive protein, is not in itself sufficient to induce efficient photoisomerisation of the olefin of a deprotonated coumaric residue.

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