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PAPER

The design, synthesis and photochemical study of a biomimetic cyclodextrin model of Photoactive Yellow Protein (PYP)†

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The design, synthesis and study of the photophysical and photochemical properties of the first biomimetic cyclodextrin (CD) model of photoactive yellow protein (PYP) are described. This model bears a deprotonated *trans-p*-coumaric acid chromophore, covalently linked *via* a cysteine moiety to a permethylated 6-monoamino β-CD. NMR and UV/Visible spectroscopy studies showed the formation of strong self-inclusion complexes in water at basic pH. Steady-state photolysis demonstrated that, unlike the free chromophore in solution, excitation of the model molecule leads to the formation of a photoproduct identified as the *cis* isomer by NMR spectroscopy. These observations provide evidence that the restricted CD cavity offers a promising framework for the design of biomimetic models of the PYP hydrophobic pocket.

Introduction

Biological systems work by the synergistic operation of functionally diverse protein assemblies which are able to convert energy from one form or location to another.[1] The recent advances in the understanding of the mechanisms involved in such complex molecular machines have inspired the design of artificial devices based on sophisticated supramolecular architectures,[2] the molecular components of which may be set in motion in a controlled manner. Interlocked self-assemblies, such as rotaxanes, pseudorotaxanes and catenanes, have attracted considerable attention and stimulated a wealth of applications in areas such as molecular switches, molecular wires, molecular logic gates and memory devices.[2,3] Depending on the nature of their molecular components, mechanical motions in these systems can be promoted by chemical, electrochemical or photochemical energy inputs.[4] Incorporating efficient photochromic molecules into self-assembling modules allows potentially clean (no formation of by-products), fast and reversible reactions, triggered by light.[5,6] Cyclodextrins (CDs),[7] are an extensively studied class of macrocyclic carbohydrate oligomers, which, in view of their capability of accommodating various organic molecules within their hydrophobic cavities,[8,9] have been widely used as the basic building blocks to construct such molecular self-assemblies. Elegant examples of light-activated CD-based rotaxanes[10] and other supramolecular systems,[11] particularly involving *cis-trans* photoisomerisation of azobenzene and stilbene functions, have been reported by several groups. Moreover, due to the reduced polarity and restricted space of their cavity, CDs offer an attractive template to study the effect of a protein environment to the spectral and photochemical properties of a chromophore.[12] For instance, biomimetic models of rhodopsin have been synthesized, and photochemically studied, using CD-retinal self-inclusion complexes.[13]

Belonging to the family of xanthopsins, the photoactive yellow protein (PYP) is a small water-soluble cytosolic photoreceptor protein thought to mediate the blue-light photophobic response of several halophilic bacteria.[14] Its chromophore is the *trans-p*-hydroxycinnamoyl anion (deprotonated *trans-p*-coumaric acid), covalently linked to the unique cysteine residue of the protein *via* a thioester bond (Scheme 1).[15] The chromophore is embedded in the main protein hydrophobic core and stabilized by a hydrogen-bonding (HB) network with the neighboring amino acid residues.[16] PYP has been identified as a structural member of the PAS (stands for Per-Arnt-Sim, Per being the acronym of Period circadian protein, Arnt of Aryl hydrocarbon receptor nuclear translocator protein and Sim of Single-minded protein) domain superfamily, a common class of various proteins predominantly involved in signal transduction.[14c] Owing to its small size (125 amino acid residues, 14 kDa), well-established structure,[17] photochemical stability and simple chromophore, PYP has emerged as a prototypic model system for studying the phototransduction phenomenon.[14]

Upon absorption of blue light, PYP undergoes a photocycle characterized by two mains steps: *trans–cis* isomerization followed by protonation of the chromophore.[14] It is noteworthy that the

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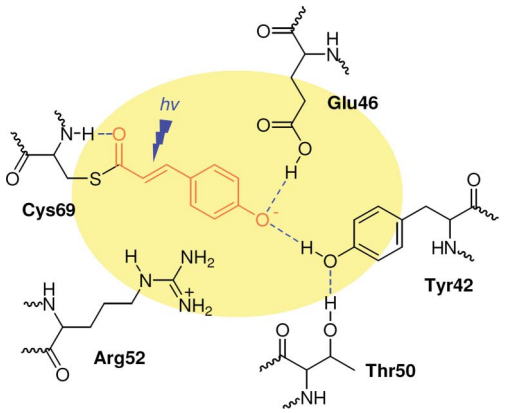
† Electronic supplementary information (ESI) available: Full experimental details, characterisation and $^{1}H/^{13}C$ NMR spectra for compounds (**1**)–(**7**), and 1D/2D NMR studies of CD-PYP1 in its protonated form (D_2O, $CDCl_3$). See DOI: 10.1039/c0ob00646g

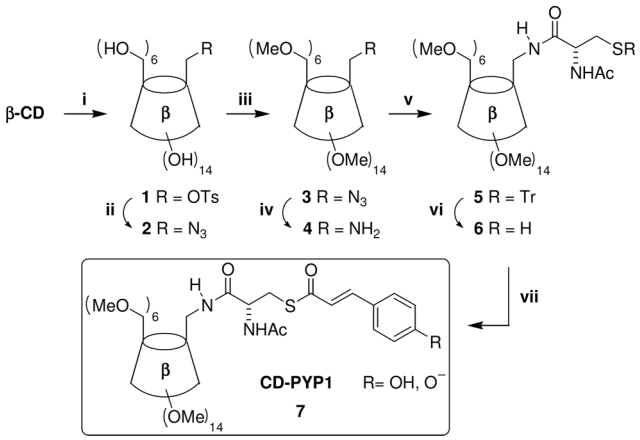


Scheme 1 Schematic representation of the PYP active site.

isomerization yield of PYP reaches about 30%,[18] whereas deprotonated analogues of the PYP chromophore bearing the thioester function do not isomerize in solution.[19] Experimental[20] and theoretical[21] studies carried out on wild-type PYP showed that this difference is mainly due to the specific environment provided by the protein. Calculations showed that the isomerization pathway in the protein would be favored by the presence of the positively charged Arg52 near the chromophore.[21] On the other hand, comparative experimental studies of the photophysical properties of wild-type PYP and various point mutants by femtosecond fluorescence and transient absorption spectroscopy highlighted the determining role of the local constraints provided by the HB network on the photoisomerisation yield and excited-state deactivation rate of PYP.[22]

Recently, a rotaxane mimic of the PYP chromophore environment has been shown to stabilize the deprotonated form of a coumaric amide analogue of the PYP chromophore, *via* HB and mechanical interlocking.[23] Contrary to the thioester analogues, the amide analogue undergoes *trans-cis* isomerization in solution.[19] This process was, however, found to be strongly hindered in the rotaxane model, which was, incidentally, studied in organic solvents.[23] Because of the known strong impact of the carbonyl substituent on the excited-state relaxation of the coumaric acid analogues,[19] it appears necessary to develop supramolecular assemblies bearing a closer resemblance to the PYP active site, and to investigate them in an aqueous environment. Such studies would simultaneously achieve two intertwined goals: on one hand, fully controlling the elements of the model construct would allow a much better understanding of the isomerization mechanism of PYP; on the other hand, knowing how to reproduce the PYP behavior with an artificial device would open the way to the design of new biomimetic photo-nanomachines.

We, therefore, envisaged utilizing the inclusion properties of cyclodextrins in the construction of PYP models in which the hydrophobic CD cavity would mimic the protein active site. In particular, it was hoped that the low polarity of the CD cavity and the constraints it can exert on the chromophore would render the photoisomerization possible. Generally, guest molecules bearing a phenyl moiety exhibit stronger affinities to β-CDs than α-CDs as the latter cannot accommodate the benzene ring in its entirety.[8] The size of β-CD's cavity is, therefore, better adapted than that of α-CD to fit the PYP chromophore. In addition, β-CD's cavity was expected to exert larger steric constraints on the chromophore than γ-CD's cavity. Moreover, the flexible and elliptical structure resulting by the loss of the intramolecular HB network upon permethylation of β-CD allows better encapsulation of planar guests, such as aromatic rings.[24] Finally, covalent attachment of the PYP chromophore on a modified β-CD was expected to improve the inclusion selectivity of the host. Herein is presented our first model (CD-PYP1, Scheme 2), consisting of a mono-6-amino-6-deoxy permethylated β-CD core covalently linked to the *trans-p*-hydroxycinnamic acid chromophore *via* a L-cysteine chain, present also in native PYP (Scheme 1). Preliminary molecular modeling studies showed that the coumaric acid moiety would be inserted in the cavity so as to expose, in basic solution, the phenolate group to the solvent. The phenolate would therefore have the possibility to establish HB links with water, a role devoted to Glu46 and Tyr42 in native PYP (Scheme 1).[16]



Scheme 2 Synthesis of CD-PYP1 (**7**) *Reagents and conditions*: i) Ts-imidazole, NaOH, H_2O, rt, 2.5 h (36%); ii) NaN_3, H_2O, 85 °C, 16 h (99%); iii) MeI, NaH, DMF, rt, 20 h (84%); iv) PPh_3, 1,4-dioxane, rt, 4 h, then aq. NH_3, 16 h (88%); v) *N*-acetyl-*S*-trityl-(L)-cysteine, EDC, HOBt, DMAP, DCM, rt, 20 h (98%); vi) 10% TFA/DCM, TES, rt, 3 h (96%); vii) *trans-p*-hydroxycinnamic acid, EDC, HOBt, DMF, rt, 14 h (70%).

Results and discussion

Synthesis

We chose to begin by the synthesis of the permethylated analogue. Permethyl-modified CDs are, as a matter of fact, soluble both in organic solvents and water and avoid side-reactions upon coupling.[24] The mono-6-amino-6-deoxy permethylated β–CD precursor (**4**)[25] (Scheme 2) was obtained from native β–CD *via* a selective monotosylation on the primary face, followed by azide displacement, permethylation and amination *via* Staudinger/hydrolysis, according to literature procedures.[26] Subsequent coupling of mono-6-amino-6-deoxy permethylated β-CD (**4**) with *S*-trityl-protected *N*-acetyl-L-cysteine was effected using EDC, HOBt and DMAP to yield cyclodextrin cysteinyl derivative (**5**) in a quantitative yield. Deprotection of the trityl thioether appeared difficult under standard trityl deprotection procedures (HBr/AcOH, TFA, HgCl) and, hence, addition of a scavenger for the trityl cation, such as triethylsilane (TES),[27] was necessary to accomplish complete deprotection in a mixture of 10% TFA/DCM, which afforded compound (**6**) in an excellent

yield (96%). Finally, coupling of *trans*-*p*-hydroxycinnamic acid (*trans*-pCAH$_2$) with thiol derivative (**6**) was performed in the presence of EDC and HOBt, yielding the desired product CD-PYP1 (**7**) in a good overall yield. The CD-PYP1 structure was characterized by high-resolution mass spectrometry and ^{1}H, ^{13}C NMR, 2D-NMR (COSY, HMQC, HMBC) experiments in its protonated (in $CDCl_3$ and D_2O) and deprotonated forms (in borate buffer, D_2O, pH = 10.1).

Chromophore inclusion

In order to investigate whether the chromophore is embedded in the CD cavity, as predicted by our calculations, we undertook 2D ROESY NMR studies of deprotonated CD-PYP1 (dp-CD-PYP1), in borate buffer (D_2O at pH = 10.1) and protonated CD-PYP1 (p-CD-PYP1) in $CDCl_3$ and D_2O at neutral pH. In D_2O, the spectra of p-CD-PYP1 (*Supporting Information*, section 2.1) and dp-CD-PYP1 (Fig. 1a) showed, in both cases, strong cross peaks between the CD cavity (H_3, H_5 and/or H_6 protons) and the chromophore aromatic protons and weaker ones with the chromophore vinylic protons. This indicates that the phenol/phenolate protons are in proximity (< 5 Å)[28a] to the protons located inside the CD, proving the inclusion of the chromophore. For comparison, the 2D-ROESY NMR spectrum of p-CD-PYP1 in $CDCl_3$ (*Supporting Information*, section 2.1) revealed only very weak correlations between the chromophore aromatic protons and the CD protons, which proves that there is no inclusion of the chromophore in the CD cavity, as expected, in this solvent.

With a view to explore whether these chromophore-CD complexes are self-inclusion complexes or result from the association of different CD-PYP1 units, ^{1}H NMR spectroscopy was performed for varying concentrations of CD-PYP1 (1 mM to 10 mM). At pH 10.1, the ^{1}H NMR spectra of dp-CD PYP1 (Fig. 1b) in D_2O do not display any shift upon variation of concentration, demonstrating the formation of intramolecular complexes solely. In contrast, small chemical shifting ($\Delta\delta$ = 0.01–0.05 ppm) of the chromophore aromatic and vinylic protons were observed for p-CD-PYP1 in D_2O (*Supporting Information*, section 2.2). These modifications may be attributed either to the formation of intermolecular complexes at higher p-CD-PYP1 concentrations, or could be simply due to conformational changes of the flexible host and/or guest upon complexation.[8,24a] The dissimilarity between deprotonated and protonated CD-PYP1 could be rationalized by a different binding mechanism in the two cases, as well as the greater stabilization of the inclusion complex of deprotonated CD-PYP1 due to the presence of the charged phenolate.[28b]

Host–guest competition

The binding affinity of the self-inclusion complexes of CD-PYP1 in D_2O has been assessed by a host–guest competition experiment using a known strong cavity binder of methylated CDs, 1-adamantanol (AD, K_a = 4000 M^{-1}).[8,29] Monitoring of the chromophore putative displacement was achieved by ^{1}H NMR spectroscopy. Due to the weak solubility of AD in water, the experiments were conducted by addition of small aliquots of a 5×10^{-2} M solution of CD-PYP1, in D_2O (at pH 7 or 10.1), into a 5 mM solution of AD in the same solvent. The molar ratio of adamantanol to cyclodextrin (AD/CD) was further checked by integration of their ^{1}H NMR signals.

At first, no change of chemical shift of the aromatic/vinylic protons of dp-CD-PYP1 (pH 10.1) was observed upon AD competition (with AD/CD ranging from 0.5 to 10; see Fig. 2b). This result indicates that the deprotonated chromophore remains strongly bound to the CD cavity in the presence of 5 mM AD. In contrast, significant shifting of the aromatic/vinylic protons of p-CD-PYP1 (neutral pH) were observed in the presence of AD, upon increasing the concentration of p-CD-PYP1 (*Supporting Information*, section 3.1). Similar effects were, however, observed upon increasing the concentration of p-CD-PYP1 in the absence of AD (*Supporting Information*, section 2.2). We, therefore, conclude that these chemical shift changes are not due to the competition with AD but to a concentration effect, as explained in the previous section.

As far as AD is concerned, the presence of dp-CD-PYP1 leads to significant changes in the chemical shifts of AD protons, as

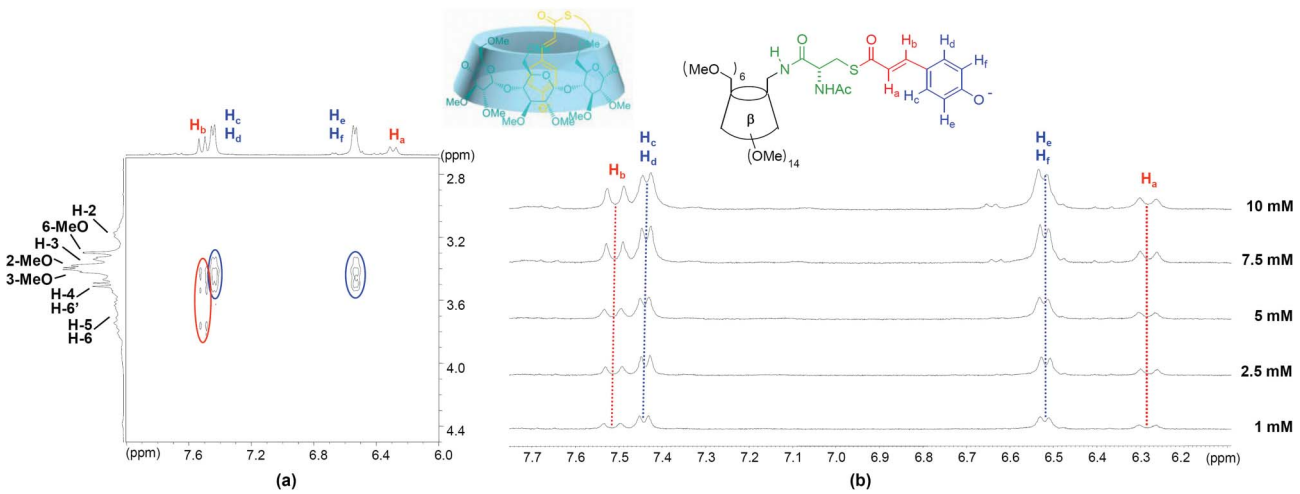


Fig. 1 (a) Partial contour plot of the 2D ROESY NMR spectrum (400 MHz, *T* 25 °C, mixing time: 200 ms) of dp-CD-PYP1 (*c* 10 mM, borate buffer, D_2O, pH = 10.1). (b) Concentration dependence (*c* 1-10 mM) of the aromatic and vinylic ^{1}H NMR (400 MHz, *T* 25 °C) chemical shifts of dp-CD-PYP1 (borate buffer, D_2O, pH = 10.1).

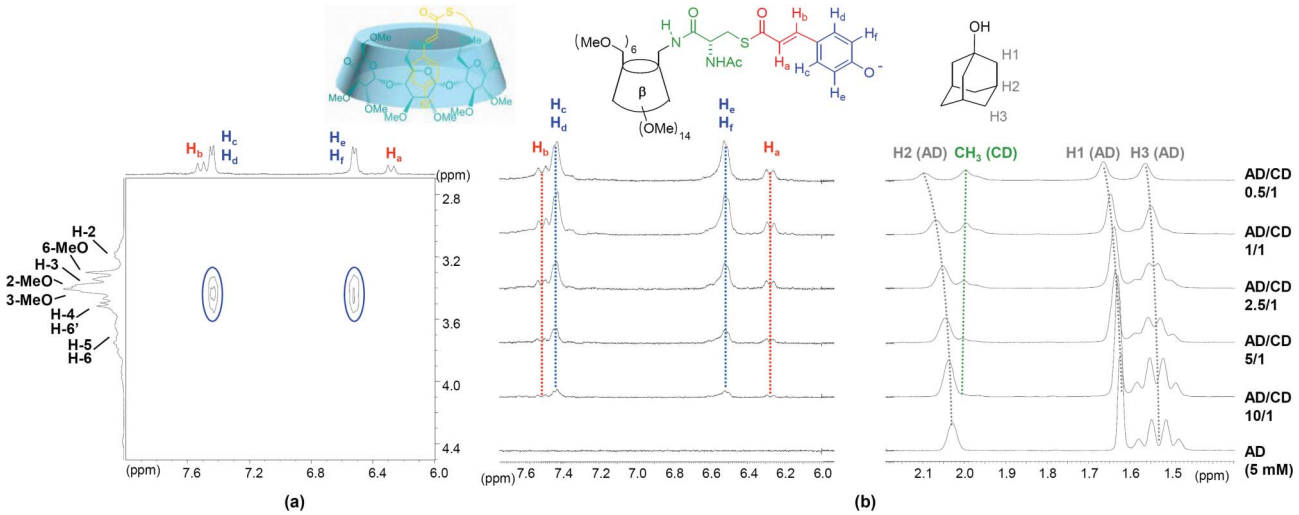


Fig. 2 (a) Partial contour plot of the 2D ROESY NMR spectrum (400 MHz, T 25 °C, mixing time: 200 ms) of a 1 : 1 solution of 1-adamantanol (AD) and dp-CD-PYP1 (borate buffer, D_2O, pH = 10.1). (b) ^{1}H NMR (400 MHz) spectra upon addition of dp-CD-PYP1 (c 5×10^{-2} M, borate buffer, D_2O, pH = 10.1) to competitive guest 1-adamantanol (AD) (c 5 mM, borate buffer, D_2O, pH = 10.1).

illustrated in Fig. 2b. For ratio AD/CD < 2.5/1, spectra show coalescence of the two doublets (δ 1.50 and 1.56 ppm; $^2J = 12.2$ Hz) attributed to the diastereotopic protons H_3 of AD. This feature is interpreted in terms of interaction between AD and the CD, and it is in good agreement with a fast exchange between free AD, and AD in interaction with the CD larger rim. Similar effects are also observed for the protonated form in D_2O (*Supporting Information*, section 3.1).

The observation that the chromophore remains included in the CD cavity upon addition of AD was further verified by 2D ROESY NMR measurements of an equimolar mixture of CD-PYP1 and AD in D_2O, at pH 7 and 10.1. The spectra of both dp-CD-PYP1 (Fig. 2a) and p-CD-PYP1 (*Supporting Information*, section 3.2) exhibit cross peaks between the aromatic protons and the CD cavity protons, those, however, being weaker than in the absence of AD. The presence of these peaks shows that the chromophore phenol/phenolate group is too tightly bound within the CD cavity to be expelled by AD. Notably, the interaction of AD with the CD larger rim, which was postulated from the ^{1}H NMR spectra, was here confirmed by the presence of weak cross peaks between the AD protons and those of the CD cavity.

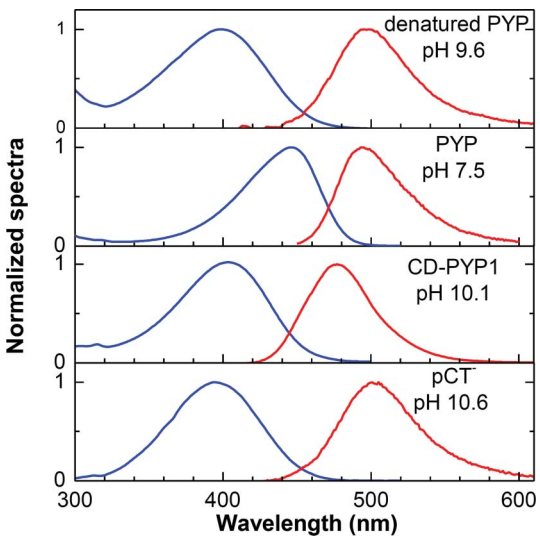


Fig. 3 Normalized absorption and fluorescence spectra of dp-CD-PYP1 in aqueous solution (buffer CAPS, pH 10.1). For comparison, the spectra of denatured PYP, wild-type PYP and the deprotonated *trans*-*S*-phenyl-*p*-hydroxycinnamate (pCT^-) are also given.[19d]

Steady-state absorption and fluorescence spectra

The influence of the CD cavity microenvironment on the photophysical properties of CD-PYP1 was examined by steady-state UV-Visible absorption and fluorescence spectroscopy.

The steady-state absorption spectrum of dp-CD-PYP1 in aqueous solution, displayed in Fig. 3, has a maximum at $\lambda_{abs} = 403$ nm, which is quite similar to that of the denatured PYP, in which the chromophore is exposed to the solvent but remains covalently attached to the protein (denatured PYP, λ_{abs} = 398 nm),[19d] and that of the free chromophore, modeled by the deprotonated *trans*-*S*-phenyl-*p*-hydroxycinnamate (pCT^-, λ_{abs} = 395 nm). Such similarity in particular demonstrates that the amide group of the L-cysteine side chain of CD-PYP1 does not contribute to its optical spectroscopy. The noticeable blue shift of these spectra with respect to the absorption spectrum of PYP ($\lambda_{abs} = 446$ nm) is consistent with the formation of strong solute–solvent hydrogen bonds involving the phenolate group, as previously shown for pCT^- and denatured PYP.[19b,d] This is in agreement with the picture that, in basic aqueous solution, the coumaric acid moiety is inserted in the CD cavity with the phenolate group peeking out of the large rim of the CD. In this position, the phenolate group may establish hydrogen bonds with the bulk water.

On the other hand, it is noted that the fluorescence spectrum of dp-CD-PYP1 ($\lambda_{fluo} = 476$ nm) is significantly blue shifted with respect to those of pCT^- ($\lambda_{fluo} = 502$ nm), PYP ($\lambda_{fluo} = 494$ nm) and denatured PYP (λ_{fluo} = 498 nm).[19d] This effect has mainly to be interpreted by a local polarity effect since it has been shown that the hydrogen bonds involving the phenolate group of pCT^- are

highly weakened, if not completely cleaved, in the excited state.[19b] The solvatochromic shifts previously observed on the fluorescence spectrum of pCT⁻ were essentially related to the polarity of the solvent. In the present case, one might therefore deduce that the CD cage provides a less polar environment to the chromophore than both water and the protein pocket of PYP, although the phenolate moiety is exposed to the bulk water. On the other hand, the absence of any peak or shoulder around 500 nm, in the spectral region of the free chromophore fluorescence maximum, indicates that most of the chromophores are located inside the CD cavity in dp-CD-PYP1.

Steady state photolysis

Having proven the existence of a strong self-inclusion complex of dp-CD-PYP1, we examined effects of the CD confinement on the photolysis behavior of the chromophore. In wild-type PYP *cis-trans* photoisomerisation is described as a unidirectional process involving the flipping motion of the carbonyl tail, leaving intact the chromophore geometry on its phenolate side.[30]

Recent theoretical studies showed that the excited-state deactivation of various deprotonated PYP chromophore analogues actually involve two competitive pathways: the torsion around the ethylenic bond and the rotation of the phenolate group.[21a–c,31] The torsional motion around the phenolate group was predicted to prevail over the isomerization pathway in the vacuum. On the contrary, in wild-type PYP the presence of Arg52 is expected to cancel the 'phenolate' pathway. Such a crucial role of Arg52 on the photoisomerization process in PYP has been, however, recently questioned on the basis of subpicosecond transient-absorption measurements carried on the R52Q mutant in which Arg52 is replaced by a neutral glutamine.[22a–b] Comparative studies of native PYP with other mutants highlighted the important role of the local protein constraints, due to the HB network, on the chromophore excited-state deactivation rate.[22] These elements led us to expect that the restricted environment of the CD cavity could geometrically constrain the chromophore and favor the isomerization over the 'phenolate' pathway.

It was previously reported that steady-state irradiation of pCT⁻ at 440 nm does not lead to the formation of any stable photoproduct (Fig. 4 up), although *trans-p*-hydroxycinnamate (pCA^{2-}) is known to form a stable *cis* isomer upon UV irradiation in basic aqueous solution.[19] In contrast, irradiation of an aqueous solution of dp-CD-PYP1 at 370 nm shows the formation of a stable photoproduct (Fig. 4 down, Fig. 5), a fact which points towards the formation of the *cis* isomer. Our recent study by femtosecond transient-absorption spectroscopy[32] indeed showed that the excited-state deactivation of dp-CD-PYP1 is about 7 times slower than that of free pCT⁻ and its deactivation leads to a long-lived photoproduct observed in the nanosecond regime interpreted as the *cis* isomer. The isomerization yield of this first biomimetic model, measured by the amplitude of the transient absorption spectrum of dp-CD-PYP1 in the nanosecond regime, *i.e.* after full excited-state relaxation, has been estimated to 4%.[32]

Photoproduct characterization

Fig. 4 shows that steady-state irradiation of dp-CD-PYP1 produced only a very small amount of photoproduct. This prevented us from using NMR spectroscopy to directly identify the nature of this photoproduct. However, unlike pCT⁻, the corresponding protonated phenyl thioester derivative (pCTH) is known to produce a significant amount of *cis* isomer upon continuous irradiation at 350 nm.[33] In order to check whether the photoproduct formed upon irradiation of dp-CD-PYP1 is the *cis* isomer or not, we, therefore, first proceeded to the steady-state irradiation of its protonated form (obtained by lowering the pH to 7). This photolysis experiment was only carried out to obtain a significant amount of *cis* isomer for NMR characterization and not to provide a detailed spectroscopic study of p-CD-PYP1. Thus the spectral changes associated to the photolysis of *trans*-p-CD-PYP1 will not be discussed in detail. The *cis-trans* mixture produced this way was subsequently deprotonated in order to record its (deprotonated) absorption spectrum.

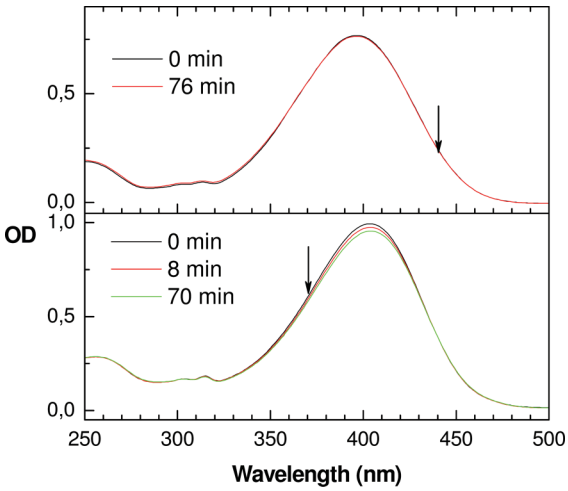


Fig. 4 (**up**) Variations of the absorbance of pCT⁻ in aqueous solution (CAPS buffer, pH 10.4), upon continuous irradiation at 440 nm.[33] (**down**) Variations of the absorbance of dp-CD-PYP1 in aqueous solution (CAPS buffer, pH 10.1), upon continuous irradiation at 370 nm.

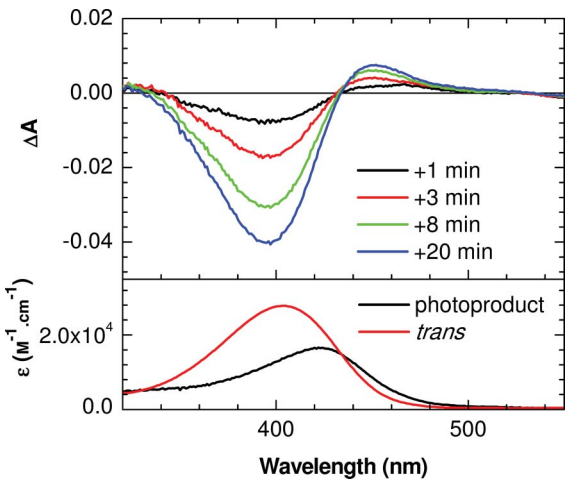


Fig. 5 (**up**) Evolution of the differential absorbance (absorbance with irradiation minus absorbance without irradiation) of dp-CD-PYP1 in aqueous solution (CAPS buffer, pH 10.1), upon continuous irradiation at 370 nm. (**down**) Absorption spectrum of the photoproduct extracted from the differential spectra shown above together with the absorption spectrum of the *trans* dp-CD-PYP1.

Fig. 6 displays the observed changes in the UV-visible absorption spectrum upon irradiation of p-CD-PYP1 at 355 nm. Notably, the amplitude of the band decreases at its maximum while the absorption slightly increases below 270 nm and above 370 nm. Isosbestic points appear at 275 nm and 368 nm, indicating the formation of one stable photoproduct with a larger absorbance than the initial *trans* isomer beyond 370 nm and above 275 nm. The relative position of the absorption bands of *trans* and *cis* isomers is, in general, hard to predict. It has, in fact, been shown, in the case of the deprotonated form of the coumaric acid, that the absorption spectrum of the *cis* isomer is blue shifted as compared to that of the *trans* isomer[34] whereas, in the native PYP, the absorption of the *cis* isomer of the deprotonated thioester derivative of the coumaric acid is red shifted.[14a]

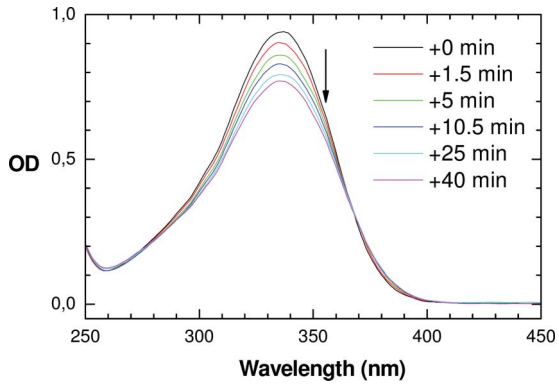


Fig. 6 Variations of the optical density of p-CD-PYP1 in aqueous solution in presence of HCl, upon continuous irradiation at 355 nm.

The 2D-COSY NMR spectra of p-CD-PYP1 before and after irradiation are illustrated in Fig. 7. Before irradiation, the *trans* protons of the vinylic bond display chemical shifts of 7.57 ppm (H_b) and 6.56 ppm (H_a), with a coupling constant of $J_{Ha–Hb}$ = 15.0 Hz (Fig. 7a). After irradiation, additional resonances in the ^{1}H NMR spectrum are observed and attributed to the *cis* isomer (Fig. 7b). The aromatic *cis* protons display chemical shifts of 7.84 ppm (H_c, H_d) and 7.00 ppm (H_e, H_f) respectively, while the vinylic *cis* protons exhibit shifts at 6.67 ppm (H_b) and 6.11 ppm (H_a), with a coupling constant of $J_{Ha–Hb}$ = 12.6 Hz. This latter value is indeed close to that of the vinylic protons of the *cis-p*-coumaric acid.[19a,35] The *trans-cis* isomer fractions were estimated by integration of the corresponding NMR peaks, and found to be: 36% *cis* and 64% *trans*.

Fig. 8 displays the absorption spectrum of the CD-PYP1 solution subjected to protonation, irradiation and subsequent deprotonation (red line). As demonstrated above, this solution is expected to contain about 36% of deprotonated *cis* isomer. This spectrum is compared to the absorption spectrum of a non irradiated dp-CD-PYP1 solution (black line). The two spectra slightly differ in their absorption maxima and in the spectral region below 350 nm and above 420 nm. These spectral changes are

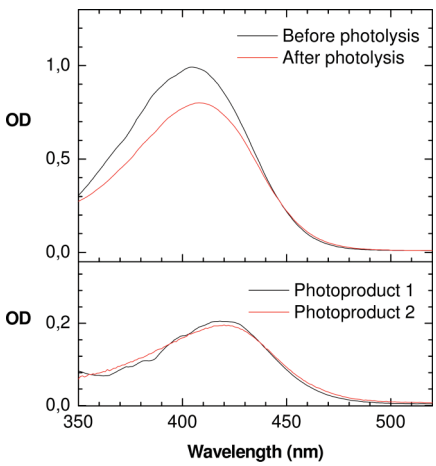


Fig. 8 (**up**) Steady-state UV/vis absorption spectra of CD-PYP1 after deprotonation of a solution of p-CD-PYP1 kept in the dark (black) or deprotonated after irradiation at 355 nm (red). (**down**) Absorption spectrum of the photoproduct (black) extracted from the difference of the spectra shown in the upper part of the figure and absorption spectrum of the photoproduct obtained upon direct irradiation of dp-CD-PYP1 shown in Fig. 5 (red).

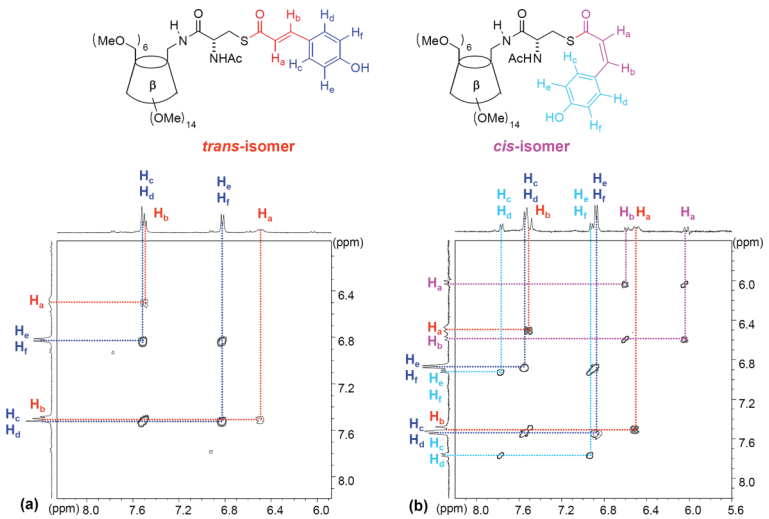


Fig. 7 Partial contour plots of the 2D COSY NMR spectra (400 MHz, D_2O) of p-CD-PYP1: (**a**) before irradiation and (**b**) after 1.5 h of irradiation at 355 nm.

consistent with those observed upon direct photolysis of dp-CD-PYP1 shown in Fig. 4 (down). Thus, Fig. 8 provides evidence that the absorption spectrum of the photoproduct obtained upon irradiation and subsequent deprotonation of p-CD-PYP1 is, indeed, very similar to that obtained upon direct photolysis of dp-CD-PYP1. This spectral identity allows us to conclude that the photoproduct obtained upon irradiation of dp-CD-PYP1 is also the *cis* isomer. It is important to note that this conclusion may be drawn even if the isomerization mechanism of the protonated form is different from that of the deprotonated form (different reaction coordinate, different inclusion structure). The key argument is that the two deprotonated *cis* photoproducts are expected to have the same equilibrium configuration, hence the same absorption spectra. Since the free chromophore (pCT^-) in solution does not form any *cis* isomer in basic aqueous solutions, we finally deduce that some photoisomerization reaction is, indeed, catalyzed by the CD cavity, as our synthetic strategy allowed us to expect. The yield remains, however, still low: 4% *vs.* 30% for wt-PYP. The weak efficiency of the isomerization is thought to arise from steric constraints imposed by the geometry of the inclusion complexes on the thioester group.[32] Future refinements of this model compound could be sought in a better encapsulation of the chromophore at the carbonyl end side and/or in increasing the constraints on the phenolate group.

Conclusions

Herein, we described the design, synthesis and physicochemical characterization of the first biomimetic cyclodextrin model of the PYP active site: CD-PYP1. In accordance with our predictions, we showed that the appended chromophore is situated inside the CD cavity in basic aqueous solutions, forming a high affinity intramolecular complex. In contrast to the free chromophore (pCT^-) in solution, steady-state irradiation of CD-PYP1 in basic aqueous solution leads to the formation of a stable photoproduct which is identified as the *cis* isomer. The efficiency of the reaction is, however, still low (about 4%). These results demonstrate that the CD cavity is able to force the photoisomerization of the chromophore ethylenic bond, thereby partially mimicking the role of the PYP active site during the first step of the protein photocycle. This behavior was further verified by femtosecond transient absorption measurements described elsewhere.[32] Current work attempts to improve this model by side chain elongation, differentiation of the CD host, or variation of the amino acid residue, in order to achieve a better mimicry of the PYP active site, that is, to increase the quantum yield of photoisomerization. This approach opens new ways to shed light into the complex photochemical response of PYP and related proteins. In addition to the fundamental interest of our approach, understanding the photoactivation mechanism of natural photoreceptors at the molecular scale in general provides a source of inspiration for the development of new nanodevices mimicking their ability to convert light into chemical energy or signal, or into molecular movement or force. In the present case, one could imagine that isomerization of the chromophore alters the depth of its inclusion into the cyclodextrin and, consequently, generates an oriented movement.

Experimental

General

β-Cyclodextrin hydrate was obtained from Cyclolab Ltd. (Hungary) and was dried rigorously in the presence of P_2O_5 in a vacuum desiccator prior to use. *trans-p*-Hydroxycinnamic acid was obtained from Sigma-Aldrich as predominantly the *trans*-isomer (98%). Unless otherwise stated, all other chemicals were obtained from commercial suppliers and were used as provided without further purification. Dichloromethane was redistilled over P_2O_5 prior to use, while *N*,*N*-dimethylformamide and 1,4-dioxane were supplied in anhydrous grade and were used without additional drying. Deuterated solvents ($CDCl_3$, 99.5% D; d_6-DMSO, 99.8% D and D_2O, 100% D) were obtained from Euriso-Top. Air and moisture sensitive reactions were conducted using oven-dried glassware under a positive pressure of dry argon. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F_{254} aluminium-backed plates (Merck) and their visualisation was achieved by charring with H_2SO_4 (10% in EtOH) stain and, when appropriate, ultraviolet light. Solutions were concentrated under reduced pressure using a Büchi rotary evaporator at water aspirator pressure, followed by drying *in vacuo*. Purification of the products, when necessary, was performed *via* flash column chromatography on silica gel 60 (40–63 μm) (Merck) using the eluent systems described in the experimental protocol. The chemical integrity and purity of the products were confirmed by ^{1}H NMR and ^{13}C NMR spectra which were recorded with a Bruker Avance 400 (or Bruker Avance 250) spectrometer operating at 400.13 MHz (or 250.13 MHz) and 100.62 MHz frequencies. Chemical shifts (δ) are expressed in parts per million (ppm) using as a reference, in each case, the solvent residual peak [$CDCl_3$: 7.26 ppm (^{1}H), 77.1 ppm (^{13}C); DMSO: 2.50 ppm (^{1}H), 39.7 ppm (^{13}C); D_2O: 4.79 ppm (^{1}H)].[36] Coupling constants (J) are reported in Hertz (Hz) and resonances are designated as follows: singlet (s), doublet (d), triplet (t), multiplet (m). Broad signals in ^{1}H NMR are denoted as 'b' and signals corresponding to quaternary carbon atoms in ^{13}C NMR are indicated as 'q'. The assignment of ^{1}H and ^{13}C NMR signals was further supported by two-dimensional ^{1}H-^{1}H COSY, ^{1}H-^{1}H ROESY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC experiments. Investigations of the photophysical behavior of protonated and deprotonated CD-PYP1 were carried out by steady-state UV-Vis spectroscopy. Due to the lability of the thioester function, the stability of CD-PYP1 in basic aqueous solutions was first examined by UV/Vis spectroscopy at various pH values (pH 7–12). Since the hydrolysis rate was observed to increase strongly at high pH, it was fixed at 10.1, *i.e.* the optimum value in order to achieve a complete deprotonation of the molecule (its pK_a is 8.36 ± 0.2) and to obtain a good chemical stability of the solution over a few hours. The steady-state absorption spectra were obtained on UV-mc2 (Safas) or CARY300 (Varian) double-beam spectrophotometers, using 1 mm or 1 cm cells. The corrected fluorescence spectra were recorded on a FluoroMax-3 (Jobin Yvon Horiba) spectrofluorimeter, using 1 cm cells. For steady-state photolysis experiments, the samples were irradiated in the cell compartment of the fluorimeter and the evolution of the sample absorbance was monitored with the spectrophotometer at different time intervals. Low and high resolution mass spectra (LRMS/HRMS) were recorded on a

JMS-700 mass spectrometer operating at a fast-atom bombardment (FAB) mode. Optical rotations were measured on a Perkin–Elmer 241 digital polarimeter using a 10 cm, 1 mL cell.

6^{I}-((*N*-Acetyl-*S*-trityl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose (5)

To a stirring solution of 6^{I}-amino-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose (**4**, 2.000 g, 1.414 mmol, 1.0 eq)[25] and *N*-acetyl-*S*-trityl-L-cysteine (0.5272 g, 1.300 mmol, 0.92 eq) in dry dichloromethane (60 mL), under argon, were added successively 1-hydroxybenzotriazole (0.1757 g, 1.300 mmol, 0.92 eq), 4-dimethylaminopyridine (0.1588 g, 1.300 mmol, 0.92 eq) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.2492 g, 1.300 mmol, 0.92 eq) and the reaction mixture was allowed to stir at room temperature for 20 h. The mixture was quenched with water (60 mL) and was extracted with dichloromethane (5 × 40 mL). The combined organic extracts were washed with water (40 mL) and brine (40 mL) and were then collected, dried ($MgSO_4$), filtered and concentrated to yield a white foam. The crude product was purified by column chromatography on silica gel [eluent: EtOAc then EtOAc:MeOH:H_2O, 20 : 1 : 1] to yield **6^{I}-((*N*-acetyl-*S*-trityl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose** (**5**, 2.490 g, 98% yield) as a white foam. R*f* 0.20 (EtOAc:MeOH:H_2O, 20 : 1 : 1); $[\alpha]_D^{20}$ +117.8 (*c* = *1*, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.37 (m, 7H, 7ArCH), 7.31–7.25 (m, 5H, 5ArCH), 7.24–7.18 (m, 3H, 3ArCH), 6.42–6.35 (m, 1H, CH_2NH), 5.94 (d, 1H, $J_{NH,CH}$ = 7.5 Hz, N*H*Ac), 5.15–5.08 (m, 7H, 7H$_1^{I–VII}$), 4.19 (m, 1H, AcNHC*H*), 3.90–3.72 (m, 14H, 7H$_5^{I–VII}$, 7H$_6^{I–VII}$), 3.70–3.29 (m, 21H, 7H$_4^{I–VII}$, 7H$_{6'}^{I–VII}$, 7H$_3^{I–VII}$), 3.64, 3.63, 3.62 (3 × s, 21H, 7(3-OMe)), 3.50, 3.49, 3.49 (3 × s, 21H, 7(2-OMe)), 3.40, 3.39, 3.37, 3.37, 3.32 (5 × s, 18H, 6(6-OMe)), 3.22–3.13 (m, 6H, 6H$_2^{II–VII}$), 3.07 (dd, 1H, $J_{H2^I–H3^I}$ = 9.3 Hz, $J_{H1^I–H2^I}$ = 3.5 Hz, H$_2^I$), 2.66 (dd, 1H, ^2J = 12.6 Hz, ^3J = 5.8 Hz, C*H*$_2$STr), 2.52 (dd, 1H, ^2J = 12.6 Hz, ^3J = 6.5 Hz, C*H*$_2$STr), 1.88 (s, 3H, NHCOC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0 (NH*C*OCH$_3$), 169.7 (CH$_2$NH*C*O), 144.5 (3ArC$_q$), 129.7, 128.2, 127.0 (18ArCH), 99.2, 99.1, 99.1, 99.0, 99.0, 98.9, 98.9 (7C$_1^{I–VII}$), 82.3, 82.2, 82.2, 82.1, 82.0, 81.9,81.9, 81.8, 81.8, 81.5, 81.4 (7C$_2^{I–VII}$, 7C$_3^{I–VII}$), 80.6, 80.5, 80.4, 80.4, 80.3, 80.2, 79.7 (7C$_4^{I–VII}$), 77.4 (Ph$_3$*C*), 71.7, 71.5, 71.3 (7C$_6^{I–VII}$), 71.5, 71.1, 70.1 (7C$_5^{I–VII}$), 61.7, 61.6, 61.6, 61.5, 61.5 (7(3-OMe)), 59.5, 59.1 (6(6-OMe)), 58.7, 58.6, 58.5 (7(2-OMe)), 52.3 (AcNH*C*H), 33.9 (*C*H$_2$STr), 23.2 (NHCO*C*H$_3$); LRMS (FAB$^+$): *m*/*z* [M + Na]$^+$ = 1823.8; HRMS (FAB$^+$): *m*/*z* [M + Na]$^+$ calculated for $C_{86}H_{132}N_2O_{36}SNa$ 1823.8178, observed 1823.8217.

6^{I}-((*N*-Acetyl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose (6)

To a stirring solution of 6^{I}-((*N*-acetyl-*S*-trityl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose (**5**, 1.000 g, 0.5549 mmol, 1.0 eq) in dry dichloromethane (15 mL) was added triethylsilane (0.36 mL, 2.220 mmol, 4.0 eq).[27] Trifluoroacetic acid (1.5 mL) was added dropwise turning the solution into a bright yellow colour, which disappeared after 15 min. The reaction mixture was left stirring for 3 h, it was diluted with dichloromethane (50 mL) and water (50 mL) was added. The mixture was extracted from dichloromethane (5 × 20 mL), the organic phase was washed with a saturated sodium bicarbonate solution (2 × 20 mL), water (20 mL) and brine (20 mL). The combined organic extracts were collected, dried ($MgSO_4$), filtered and concentrated to yield a white foam. The crude product was purified by column chromatography on silica gel [eluent: EtOAc then EtOAc:MeOH:H_2O, 10 : 1 : 1] to yield **6^{I}-((*N*-acetyl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose** (**6**, 0.8326 g, 96% yield) as a white foam. R*f* 0.16 (EtOAc:MeOH:H_2O, 10 : 1 : 1); $[\alpha]_D^{20}$ +144.0 (*c* = *1*, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 (dd, 1H, *J* = 5.5 Hz, CH_2NH), 6.53 (d, 1H, $J_{NH,CH}$ = 7.7 Hz, N*H*Ac), 5.16–5.07 (m, 7H, 7H$_1^{I–VII}$), 4.58 (ddd, 1H, J_{CH-CH2} = 6.7, 4.3 Hz, AcNHC*H*), 3.89–3.73 (m, 14H, 7H$_5^{I–VII}$, 7H$_6^{I–VII}$), 3.67–3.32 (m, 21H, 7H$_4^{I–VII}$, 7H$_{6'}^{I–VII}$, 7H$_3^{I–VII}$), 3.64, 3.63, 3.62, 3.61 (4 × s, 21H, 7(3-OMe)), 3.50, 3.50, 3.50, 3.49, 3.48 (5 × s, 21H, 7(2-OMe)), 3.44, 3.38, 3.37 (3 × s, 18H, 6(6-OMe)), 3.24–3.15 (m, 6H, 6H$_2^{II–VII}$), 3.12 (dd, 1H, $J_{H2^I–H3^I}$ = 9.6 Hz, $J_{H1^I–H2^I}$ = 3.5 Hz, H$_2^I$), 2.97 (ddd, 1H, ^2J = 13.7 Hz, J_{CH2-SH} = 7.9 Hz, J_{CH2-CH} = 4.3 Hz, C*H*$_2$SH), 2.77 (ddd, 1H, ^2J = 13.7 Hz, J_{CH2-SH} = 9.8 Hz, J_{CH2-CH} = 6.7 Hz, C*H*$_2$SH), 2.03 (s, 3H, NHCOC*H*$_3$), 1.61 (dd, 1H, J_{CH2-SH} = 9.8, 7.9 Hz, CH$_2$S*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0 (NH*C*OCH$_3$), 169.7 (CH$_2$NH*C*O), 99.4, 99.2, 99.2, 99.1, 98.9, 98.9, 98.7 (7C$_1^{I–VII}$), 82.3, 82.3, 82.2, 82.1, 82.0, 81.9, 81.9, 81.8, 81.6, 81.5, 81.5 (7C$_2^{I–VII}$, 7C$_3^{I–VII}$), 80.7, 80.6, 80.4, 80.2, 80.1, 79.6 (7C$_4^{I–VII}$), 71.8, 71.5, 71.4, 71.4, 71.4 (7C$_6^{I–VII}$), 71.7, 71.3,71.2, 71.1, 71.1, 71.0, 70.0 (7C$_5^{I–VII}$), 61.7, 61.6, 61.5, 61.5, 61.4 (7(3-OMe)), 59.7, 59.2, 59.2, 59.2, 59.1 (6(6-OMe)), 59.0, 58.8, 58.7, 58.7, 58.6, 58.5, 58.5 (7(2-OMe)), 54.5 (AcNH*C*H), 27.2 (CH$_2$SH), 23.3 (NHCO*C*H$_3$); LRMS (FAB$^+$): *m*/*z* [M + Na]$^+$ = 1581.6; HRMS (FAB$^+$): *m*/*z* [M + Na]$^+$ calculated for $C_{67}H_{118}N_2O_{36}SNa$ 1581.7083, observed 1581.7102.

CD-PYP1: 6^{I}-[((*N*-acetyl-*S*-(*p*-hydroxycinnamoyl)-L-cysteinyl)amino]-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose (7)

To a stirring solution of 6^{I}-((*N*-acetyl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)cyclomaltoheptaose (**6**, 0.6850 g, 0.4392 mmol, 1.0 eq) and *trans*-*p*-hydroxycinnamic acid (0.0721 g, 0.4392 mmol, 1.0 eq) in dry *N*,*N*-dimethylformamide (30 mL), under argon, were added successively 1-hydroxybenzotriazole (0.0712 g, 0.5270 mmol, 1.2 eq), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.1010 g, 0.5270 mmol, 1.2 eq) and the reaction mixture was allowed to stir at room temperature for 20 h. The solvent was evaporated *in vacuo* ($T \approx 40$ °C), the residue was dissolved in dichloromethane (30 mL) and water was added (30 mL). The mixture was extracted from dichloromethane (5 × 20 mL) and the organic phase was washed with water (20 mL) and brine (20 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated down to yield a pale yellow foam. The crude product was purified by column chromatography on silica gel [gradient elution/eluent: EtOAc then EtOAc:MeOH:H_2O, 20 : 1 : 1, 10 : 1 : 1] to yield

6^{I}-[((*N*-acetyl-*S*-(*p*-hydroxycinnamoyl)-L-cysteinyl)amino]-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)-cyclomaltoheptaose (**CD-PYP1**: **7**, 0.5225 g, 70% yield) as a white foam. R*f* 0.18 (EtOAc:MeOH:H_2O, 10 : 1 : 1); $[\alpha]_D^{20}$ +116.0 (*c* = *1*, $CHCl_3$); ***Protonated form-CDCl$_3$***: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, 1H, $J_{Ha\text{-}Hb}$ = 15.8 Hz, H_b), 7.36 (d, 2H, ^3J = 8.5 Hz, H_c, H_d), 6.94–6.88 (m, 1H, CH_2NH), 6.84 (d, 2H, ^3J = 8.5 Hz, H_e, H_f), 6.72 (d, 1H, $J_{NH,CH}$ = 7.5 Hz, NHAc), 6.49 (d, 1H, $J_{Ha\text{-}Hb}$ = 15.8 Hz, H_a), 5.17–5.08 (m, 7H, 7$H_1^{I–VII}$), 4.68–4.63 (m, 1H, AcNHC*H*), 3.90–3.73 (m, 14H, 7$H_5^{I–VII}$, 7$H_6^{I–VII}$), 3.73–3.25 (m, 23H, 7$H_4^{I–VII}$, 7$H_{6'}^{I–VII}$, 7$H_3^{I–VII}$, CH_2S), 3.64, 3.62 (2 × s, 21H, 7(3-OMe)), 3.50, 3.49, 3.48, 3.47 (4 × s, 21H, 7(2-OMe)), 3.44, 3.38, 3.37 (3 × s, 18H, 6(6-OMe)), 3.22–3.14 (m, 6H, 6$H_2^{II–VII}$), 3.14 (dd, 1H, $J_{H3^I\text{–}H2^I}$ = 9.7 Hz, $J_{H1^I\text{–}H2^I}$ = 3.4 Hz, H_2^I), 2.02 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.0 (CH_2SCO), 171.1 (NH*C*OCH$_3$), 170.1 (CH_2NHCO), 159.9 (ArC_q phenol), 142.2 (C_b), 130.8 (C_c, C_d), 125.6 (ArC_q), 121.2 (C_a), 116.4 (C_e, C_f), 99.2, 99.2, 99.1, 99.1, 98.9, 98.8, 98.7 (7$C_1^{I–VII}$), 82.3, 82.3, 82.2, 82.2, 82.0, 81.9, 81.9, 81.8, 81.7, 81.7, 81.6, 81.5 (7$C_2^{I–VII}$, 7$C_3^{I–VII}$), 80.6, 80.3, 80.1, 80.1, 79.6 (7$C_4^{I–VII}$), 71.7, 71.5, 71.4, 71.3, 71.3 (7$C_6^{I–VII}$), 71.6, 71.1, 71.0, 70.0 (7$C_5^{I–VII}$), 61.7, 61.6, 61.6, 61.5, 61.5, 61.4 (7(3-OMe)), 59.6, 59.2, 58.8 (6(6-OMe)), 59.1, 58.6, 58.5, 58.5 (7(2-OMe)), 53.9 (AcNH*C*H), 31.6 (*C*H_2SCO), 23.2 (NHCO*C*H$_3$); ***Protonated form-D$_2$O***: ^{1}H NMR (400 MHz, D_2O): δ 7.60 (d, 2H, ^3J = 8.3 Hz, H_c, H_d), 7.59 (d, 1H, $J_{Ha\text{-}Hb}$ = 15.0 Hz, H_b), 6.91 (d, 2H, ^3J = 8.3 Hz, H_e, H_f), 6.58 (bd, 1H, $J_{Ha\text{-}Hb}$ = 15.0 Hz, H_a), 5.28–5.11 (m, 7H, 7$H_1^{I–VII}$), 4.52–4.43 (m, 1H, AcNHC*H*), 4.17–3.07 (m, 44H, 7$H_5^{I–VII}$, 7$H_6^{I–VII}$, 7$H_4^{I–VII}$, 7$H_{6'}^{I–VII}$, 7$H_3^{I–VII}$, CH_2S, 7$H_2^{I–VII}$), 3.57, 3.56, 3.53, 3.51 (4 × s, 21H, 7(3-OMe)), 3.50, 3.48, 3.47, 3.45 (4 × s, 21H, 7(2-OMe)), 3.42, 3.40, 3.38, 3.38 (4 × s, 18H, 6(6-OMe)), 2.08 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, D_2O): δ 187.8 (CH_2SCO), 174.3 (NH*C*OCH$_3$), 173.6 (CH_2NHCO), 159.7 (ArC_q phenol), 142.6 (C_b), 130.9 (C_c, C_d), 125.4 (ArC_q), 120.9 (C_a), 116.5 (C_e, C_f), 99.3, 99.2, 99.0, 98.7, 98.6, 98.4, 97.6 (7$C_1^{I–VII}$), 81.6, 81.5, 81.4, 81.3, 81.0, 80.9, 80.8, 80.6, 80.5 (7$C_2^{I–VII}$, 7$C_3^{I–VII}$), 80.3, 80.2, 80.0, 79.9, 79.8, 79.6, 78.3 (7$C_4^{I–VII}$), 71.4, 71.2, 71.0, 70.9, 70.7 (7$C_6^{I–VII}$, 7$C_5^{I–VII}$), 61.3, 61.1, 60.9, 60.9, 60.8, 60.6, 60.5 (7(3-OMe)), 59.0, 59.0, 58.8, 58.8, 58.7, 58.6, 58.5, 58.3, 58.3, 58.2 (6(6-OMe), 7(2-OMe)), 54.3 (AcNH*C*H), 29.2 (*C*H_2SCO), 22.2 (NHCO*C*H$_3$); ***Deprotonated form***: ^{1}H NMR (400 MHz, D_2O, pH = 10.1): δ 7.60 (d, 1H, $J_{Ha\text{-}Hb}$ = 15.2 Hz, H_b), 7.53 (d, 2H, ^3J = 8.0 Hz, H_c, H_d), 6.61 (d, 2H, ^3J = 8.0 Hz, H_e, H_f), 6.37 (bd, 1H, $J_{Ha\text{-}Hb}$ = 15.2 Hz, H_a), 5.30–5.12 (m, 7H, 7$H_1^{I–VII}$), 4.51–4.43 (m, 1H, AcNHC*H*), 4.13–3.15 (m, 44H, 7$H_5^{I–VII}$, 7$H_6^{I–VII}$, 7$H_4^{I–VII}$, 7$H_{6'}^{I–VII}$, 7$H_3^{I–VII}$, CH_2S, 7$H_2^{I–VII}$), 3.60, 3.58, 3.56, 3.50 (4 × s, 21H, 7(3-OMe)), 3.49, 3.48, 3.47 (3 × s, 21H, 7(2-OMe)), 3.43, 3.42, 3.39, 3.38 (4 × s, 18H, 6(6-OMe)), 2.09 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, D_2O, pH = 10.1): δ 188.0 (CH_2SCO), 174.6 (NH*C*OCH$_3$), 173.8 (CH_2NHCO), 172.3 (ArC_q phenolate), 145.2 (C_b), 131.9 (C_c, C_d), 120.1 (C_e, C_f), 119.4 (ArC_q), 115.7 (C_a), 99.8, 99.3, 99.1, 98.8, 98.6, 97.7 (7$C_1^{I–VII}$), 81.7, 81.5, 81.3, 81.1, 81.1, 80.8, 80.5, 80.4, 80.4, 80.1, 79.9, 79.7, 79.6, 79.4, 79.3, 79.2 (7$C_3^{I–VII}$, 7$C_2^{I–VII}$, 7$C_4^{I–VII}$), 71.7, 71.6, 71.4, 71.2, 71.0, 70.9, 70.7 (7$C_6^{I–VII}$, 7$C_5^{I–VII}$), 61.3, 61.1, 60.9, 60.8, 60.6, 60.5 (7(3-OMe)), 59.0, 59.0, 58.8, 58.8, 58.7, 58.6, 58.6, 58.3, 58.3, 58.2 (6(6-OMe), 7(2-OMe)), 54.7 (AcNH*C*H), 28.8 (*C*H_2SCO), 21.9 (NHCO*C*H$_3$); LRMS (FAB$^+$): *m*/*z* [M + Na]$^+$ = 1727.8; HRMS (FAB$^+$): *m*/*z* [M + Na]$^+$ calculated for $C_{76}H_{124}N_2O_{38}SNa$ 1727.7451, observed 1727.7384.

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