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PAPER

Mono- and ditopic models of binding of a photochromic chromene annelated with an 18-crown-6 ether with protonated amino acids†

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In this work, the interaction of protonated amino acids with a chromene bearing a fused 18-crown-6 ether moiety was studied by UV–vis and NMR spectroscopy. Initial closed forms of the chromene form monotopic 1 : 1 complexes, the ammonium group being localized inside the crown ether cavity. UV-irradiation leads to transformation of the ring-closed species into the ring-opened form. Depending on the amino acid length, either ditopic or monotopic 1 : 1 complexes are formed. Such complexes are stabilized by the additional H-bonding between the carboxylic group of the acid and the carbonyl oxygen atom of the ring-opened form. Cessation of the irradiation results in ring-closure to the chromene with concomitant change of the complexation mode.

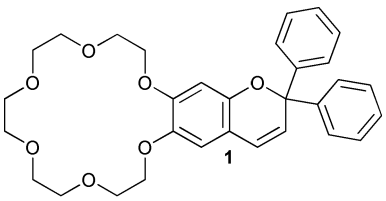
Introduction

The ability of the crown ether macrocycles to form non-covalent H-bonding complexes with ammonium cations has been actively investigated with an eye toward biological applications,[1,2] molecular recognition,[3–7] self-assembly,[8–12] crystal engineering,[13,14] and catalysis.[15] The stoichiometry and stability of these host–guest complexes depend both on the size of the crown ether and on the nature of the ammonium cation (NH_4^+, RNH_3^+, *etc*).[16,17] The numerous studies of 18-crown-6 ether and its derivatives, which have the highest affinity for ammonium cations, invariably showed a 1 : 1 stoichiometry with both NH_4^+ and RNH_3^+ cations in solution.[18,19]

The design and the molecular recognition of artificial receptors for amino acids have attracted extensive interest.[20–22] A receptor for zwitterionic amino acids can recognize the side chain, the positively charged ammonium group and the anionic carboxylate, simultaneously. A series of metalloporphyrins has been synthesized and applied to the molecular recognition studies of amino acid esters.[23,24] The calix[5]arenes bearing an urea unit at the upper rim show a strong affinity for ω-amino acids and lysine derivatives.[25] The protonated alkyl-guanidinium side chain of arginine forms a stable noncovalently bonded complex with dibenzo-30-crown-10.[26]

Synthetic molecules that are isomerizable in a reversible mode upon light irradiation with different wavelengths have been developed for the investigation of photoswitchable transport channels, mRNA binding affinity, papain activity and DNAzyme cleavage.[27,28] These molecules include diazobenzenes, dihydropyrenes, spirooxazines, anthracenes, fulgides, and spiropyrans.[29–31] However, the synthetic reversible photochemical switch for amino acid molecules is a less developed topic.[32] The receptors may be of high importance for the development of photocontrolled membrane transport systems and photocontrolled artificial catalytic systems.

Based on the existing knowledge of molecular recognition and the specifications required for photoswitchable artificial amino acid receptors, we analyzed 18-crown-6 ether containing benzopyran **1** as a receptor for the protonated amino acids NH_3^+-$(CH_2)_n$-COOH with different chain lengths (Scheme 1).[33] The photoswitching ability of the photochromic chromenes, containing crown ether moieties, to bind metal cations is a well-known



n = 2 β-alanine (C3) perchlorate
n = 3 γ-aminobutyric acid (C4) perchlorate
n = 5 ε-aminocaproic acid (C6) perchlorate
n = 7 ω-aminocaprylic acid (C8) perchlorate

Scheme 1 Objects of the investigation.

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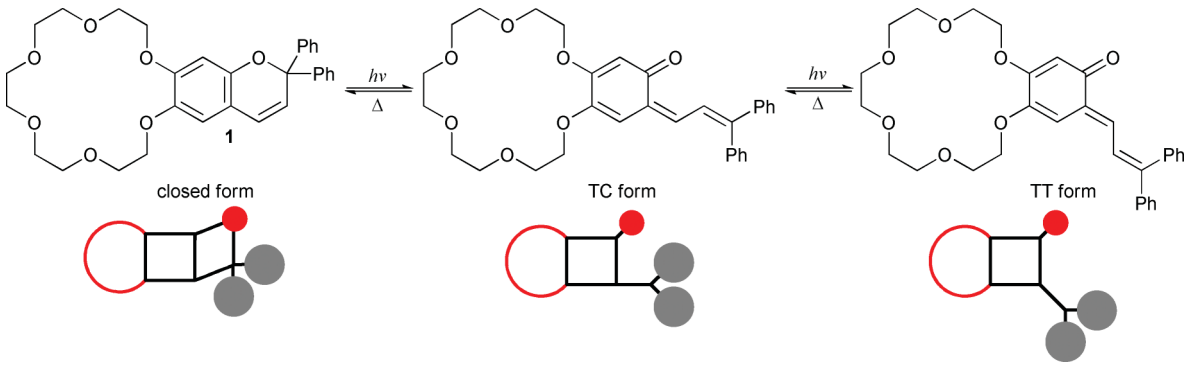


Scheme 2 Phototransformation of the chromene. (The schematic representations below the chemical structures will be used further.)

phenomenon in the literature.[34–37] Up to now, photocontrolled receptors for amino acids based on chromene receptors has not been developed.

Results and discussion

The concept

Benzopyrans are thermally reversible photochromic compounds.[38,39] At room temperature, they exist as uncolored closed forms (CF). Upon UV-irradiation, the CF undergoes an ultrafast ring-opening reaction leading to colored open forms (OF), the so called TC (*s-trans-cis*) and TT (*s-trans-trans*) isomers (Scheme 2). The OFs are metastable and thermally convert back to the initial CFs. The occurrence of the phototransformation will change the ability of the receptor to bind amino acids because photochromic transformation results in the appearance of an additional strong coordination site, the carbonyl oxygen atom, in the structure. Using protonated amino acids NH_3^+-$(CH_2)_n$-COOH as guest molecules, one may expect the formation of ditopic complexes with the open forms of chromene **1**, in which the ammonium group is located in the crown ether cavity and the carboxyl group is coordinated with the carbonyl oxygen atom *via* a hydrogen bond (Scheme 3). In this case, coordination mode and strength will depend on the amino acid alkyl chain length (*n*, the number of methylene groups).

Preliminary molecular modeling using MOPAC/PM6 package[40] showed a possibility of ditopic complexes being formed (Fig. 1). For the TC form of chromene **1**, the ditopic complex formation was possible with an acid[41] containing $n > 4$ of methylene fragments (C5 or higher).

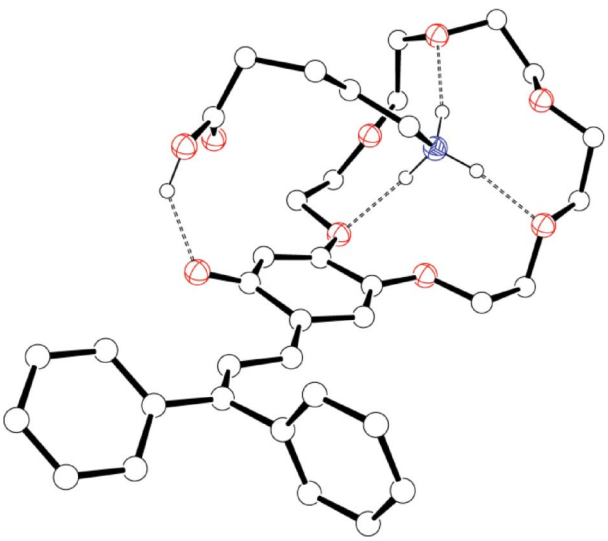
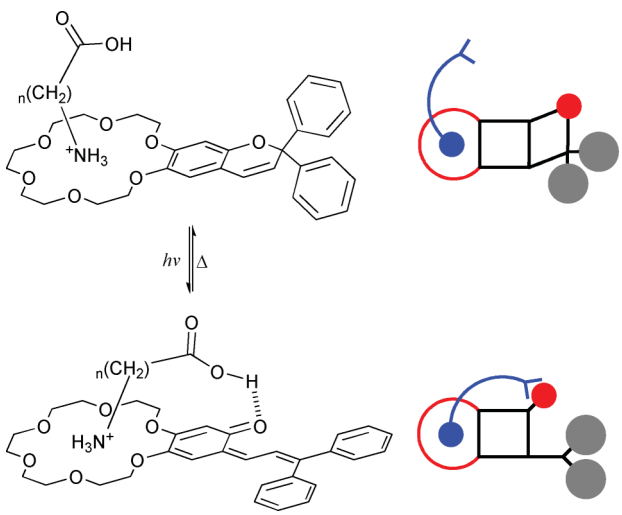


Fig. 1 MOPAC/PM6 optimized structure of the ditopic complexes of TC **1** with amino acid C5 (the atoms are depicted as follows: carbons as plain spheres, hydrogens as plain spheres of smaller size, oxygens as spheres with boundaries, nitrogen as a sphere with a shaded octant; for convenience, hydrogen atoms not participating in hydrogen bonds are omitted).



Scheme 3 Proposed mechanism of the CF and OF coordination with a protonated amino acid.

In our study, we employed perchlorates of four amino acids NH_3^+-$(CH_2)_n$-COOH, namely those of β-alanine (C3, $n = 2$), γ-aminobutyric (C4, $n = 3$), ε-aminocaproic (C6, $n = 5$), and ω-aminocaprylic (C8, $n = 7$) acid. Ammonium perchlorate was employed as the model compound, which is known to be able to coordinate with the crown ether fragment exclusively.

Complex formation before irradiation

Compound **1** and its complexes were studied by UV–vis absorption and NMR spectroscopy. Spectrophotometric titration allowed for determining the number and stability constants of the complexes. Compared with the spectra of the free chromene,

Table 1 Stability constant (log K_{11}) for complexes of CF **1** with ammonium and amino acid perchlorates (acetonitirile, 25 °C)

NH_4ClO_4	4.90 ± 0.10
β-alanine (C3)	4.60 ± 0.10
γ-aminobutyric acid (C4)	4.64 ± 0.05
ε-aminocaproic acid (C6)	4.68 ± 0.08
ω-aminocaprylic acid (C8)	4.42 ± 0.04

those of the complexes of CFs **1** showed a slight blue shift (Fig. 2). The absorption changes were observed predominantly in the short-wavelength region (see ESI, Fig. S1†). Such effects allowed us to suggest the coordination of the crown ether fragment with the ammonium cation. The multivariate analysis of the titration data indicated the presence of only one complex in solution with stoichiometry 1 : 1 (see Experimental part). Stability constants of the complexes of the CFs with the amino acids and ammonium cations appeared to be comparable and ranged within 4.4–4.9 logarithmic units (Table 1). This fact shows the similarity of the coordination type of all the complexes of ammonium and amino acids with receptor **1**.

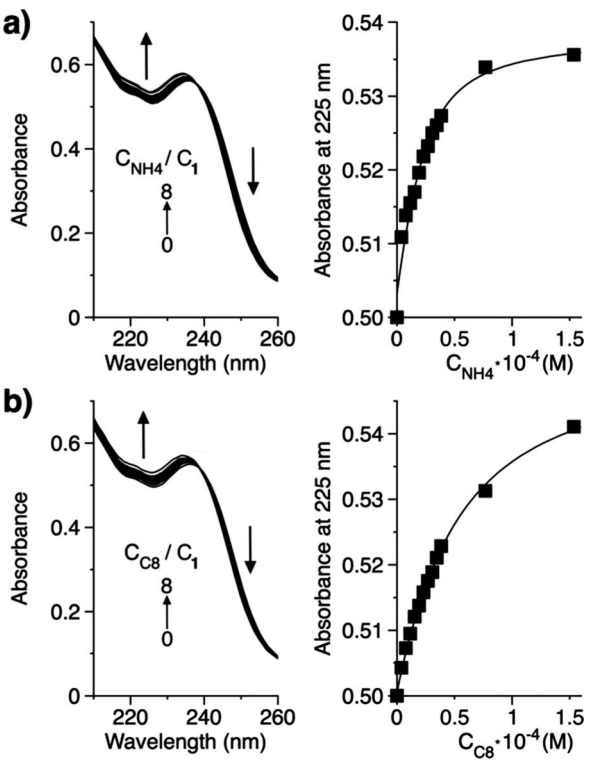


Fig. 2 Spectrophotometric titration of chromene **1** by ammonium perchlorate (a) and protonated ω-aminocaprylic acid (b) in acetonitrile. $C_1 = 2 \times 10^{-5}$ M, $C_L/C_1 = 0 \rightarrow 8$ (C_L is NH_4ClO_4 or C8 concentration).

We undertook detailed NMR studies of complex **1·C8** to establish the binding mechanism of amino acid C8 with chromene **1**. Firstly, ^{1}H NMR titrations were performed. Subsequently, upon successive addition of ω-aminocaprylic acid to a solution of **1** in acetonitrile, spectral changes are observed, which vary linearly until the mole ratio of 1 : 1 is reached; further addition of the amino acid does not change the resonance frequencies. The most pronounced changes of receptor **1** proton resonances can be observed in the proximity of the methylene protons of the crown ether unit whereas the resonances of the chromene moiety barely change (Fig. 3, see ESI† for detailed assignment). The characteristic upfield shift of C8 resonances (up to 0.22 ppm) and splitting of H-3″ and H-7″ multiplet signals of ω-aminocaprylic acid indicate the formation of an inclusion complex.

Moreover, the continuous-variation method (Job's plot) changing the molar fraction from 0.2 to 0.8 was used to determine the stoichiometry, while the total concentration of **1** and C8 was kept constant at 1.5 mM. The symmetrical bell-curve is indicative of the 1 : 1 complexation between ω-aminocaprylic acid perchlorate and chromene **1** (Fig. 4).

To achieve further proof of the identity of complex **1·C8**, NMR diffusion ordered spectroscopy (DOSY) experiments were performed. This technique is being increasingly used in conjunction with NOE to investigate aggregation and encapsulation phenomena, intermolecular or interionic interactions.[42–48] For instance, it was demonstrated that the diffusion coefficients of the various free crown ethers were sensitive to both conformational changes and changes in molecular size upon complexation. DOSY experiments were performed in CD_3CN for complex **1·C8**, guest ligand C8 and host receptor **1**. The diffusion coefficients were calculated as the mean value of five individual signals. Single diffusion coefficient was found in all cases indicative of a single species present in solution. The finding that $D_{1\cdot C8}/D_1$ lies between the square and cubic roots of the ratio of their molecular weights ($M_1/M_{1\cdot C8}$) (Table 2) proves that the complex of chromene **1** with C8 has a 1 : 1 ratio.

A number of intramolecular nuclear Overhauser effects (NOEs) for **1** and C8 were observed (Fig. 5). For example, H-10,11,13,14 methylene protons of **1** have NOE contacts with both H-8′ and H-2′, 3′, 7′ of C8, while the H-7,17 protons of **1** show only weak NOE contacts. This NOE data explain that ω-aminocaprylic acid mostly has hydrogen bonding interactions with oxygen atoms bonded with H-10,11,13,14 protons of **1** and has a weak interaction with the electron poor oxygens close to H-7,17.

These findings suggest that in complex **1·C8** the amino acid molecule is wrapped around the crown ether moiety of the chromene (Scheme 4). In addition, the NMR studies support our conclusions on the results of the UV–vis experiments with other ligands.

Photochromic behavior of the chromenes

Upon UV-irradiation, chromene **1** is transformed into the colored form, which is known to rapidly relax into its initial state. This process could be monitored by UV–vis and NMR spectroscopy.

The absorption spectra of the CFs and OFs considerably differ from each other, the former usually absorbing in the UV region and

Table 2 Diffusion coefficients for **1**, C8 and complex **1·C8** (CD_3CN, 20 °C)

Compound	MW[a]	D (nm^2 s^{-1})
1	518.6	1.34 ± 0.01
C8	160.2	1.97 ± 0.01
1·C8	678.8	1.17 ± 0.01

[a] Molecular weights of uncharged molecules in case of **1**, and of the cations in case of C8 and **1·C8** correspond to the exact formula of the unsolvated compounds.

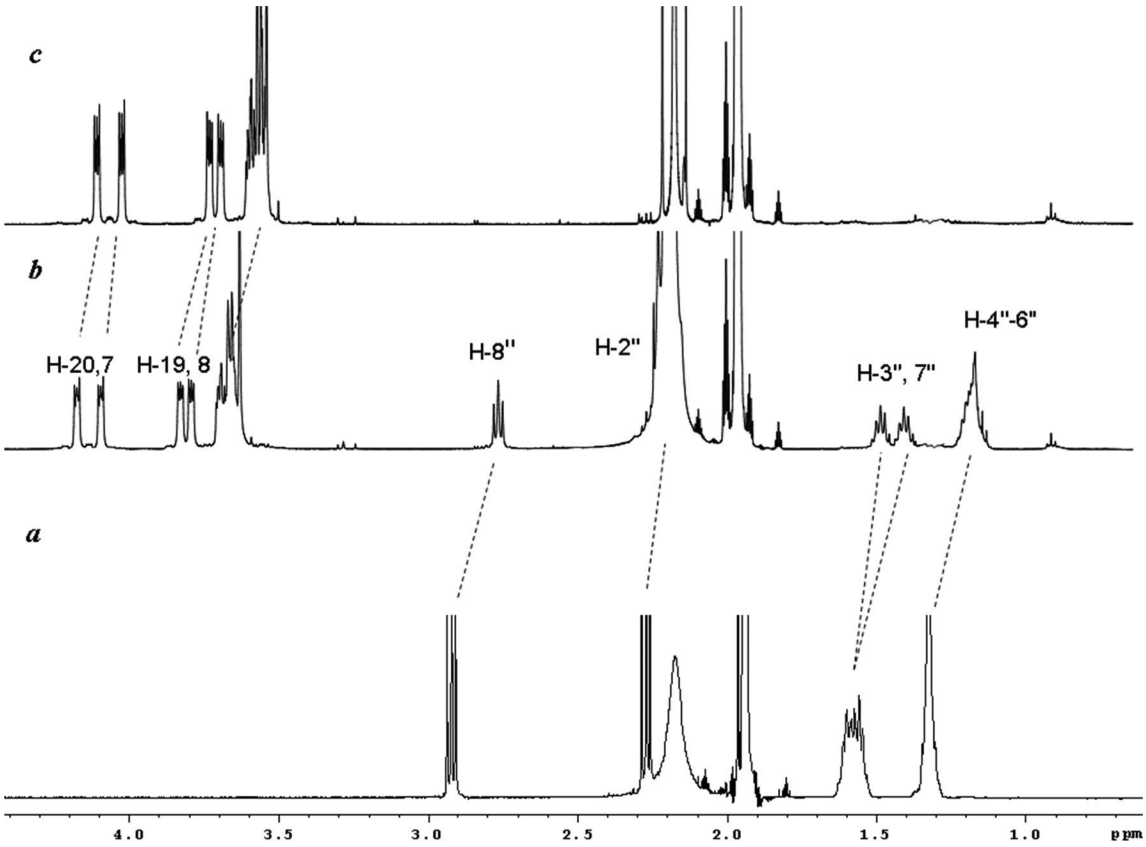


Fig. 3 ^{1}H NMR spectra (aliphatic region) of the amino acid C8 (*a*), its complex **1·C8** (*b*) and chromene **1** (*c*).

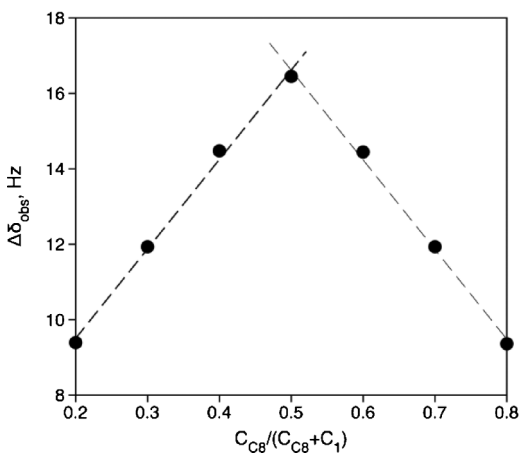
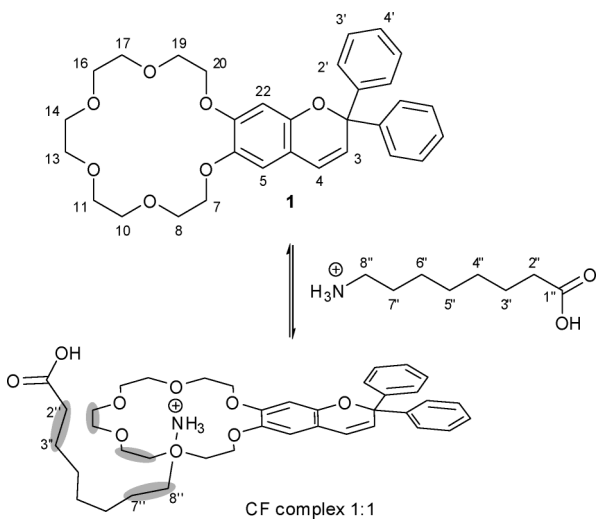


Fig. 4 Job's plot for C8 acid and chromene **1** (the change of the chemical shift of protons 7 and 20 was monitored).



Scheme 4 Proposed spatial arrangement of the complex between CF **1** and C8 (grey areas mark the protons interacting through NOE).

the latter also absorbing in the visible region. This feature allows these species to be distinguished. Upon cessation of irradiation, the OFs relax to CFs. The kinetics of this bleaching can be determined by monitoring the intensity of decay of the OF absorption band and by consequent application of the general kinetic scheme of the bleaching (TT → TC → CF or, in general, OF → CF)[38,39] on the data. At 20 °C upon irradiation with filtered light with λ_{irr} = 313 nm, chromene **1** converts into the OF, the bleaching curve of which has a monoexponential pattern characterized by $k = 1.2 \times 10^{-3}$ s^{-1} (Fig. 6).

Since at 20 °C the bleaching proceeds faster than the NMR time scale permits the recording of a spectrum, the studies of photochromic transformations were performed at lower temperature. The studies at 0 °C indicated that irradiation of chromene **1** generates two OFs, namely: the TC isomer is the major photoproduct while the TT isomer is detected upon prolonged irradiation (Fig. 7).[49,50] The TT form, not observable upon short

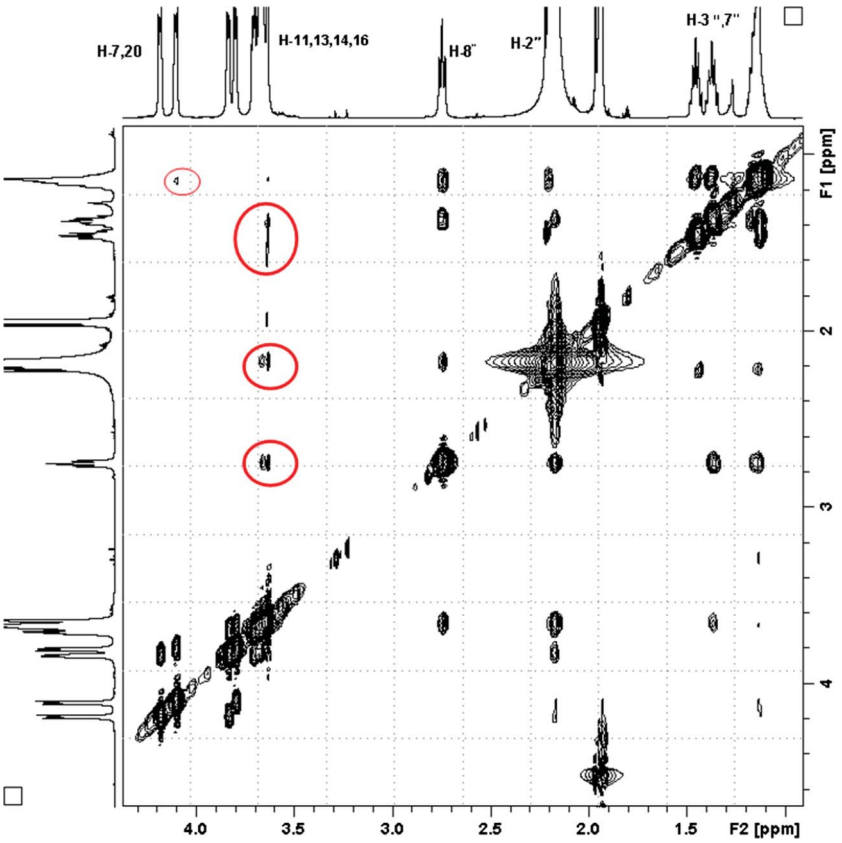


Fig. 5 $^{1}H-^{1}H$ NOESY spectrum (aliphatic part) of complex **1·C8** (CD_3CN, 20 °C).

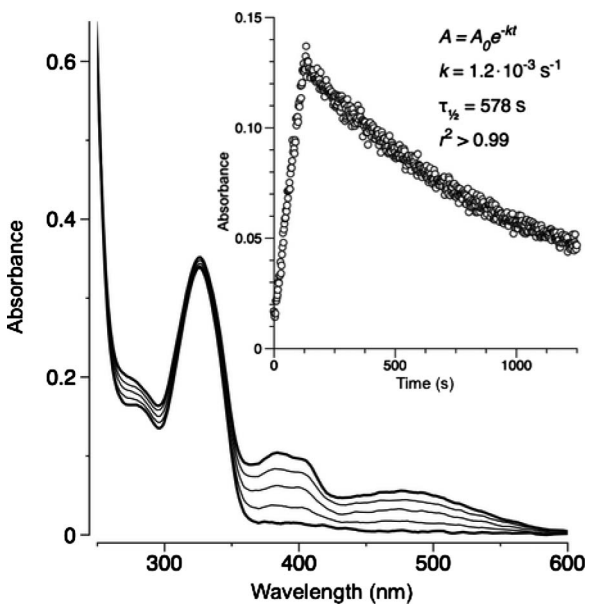


Fig. 6 Changes in absorption spectra of chromene **1** during irradiation and bleaching ($C_1 = 5 \times 10^{-5}$ M, 20 °C, 2 minute exposure to irradiation with filtered light (Hg lamp, 120 W, $\lambda_{irr} = 313$ nm), kinetics of bleaching at 385 nm is shown in the inset). (A is absorbance; k is the bleaching rate constant; r^2 is the correlation coefficient.)

irradiation, is stable at 0 °C while the TC isomer transformed into the initial closed form with $k = 1.1 \times 10^{-4}$ s^{-1}.

There are many factors that can affect the photochromic behavior of chromenes. Two of the most important are the intensity and the duration of irradiation. Depending on the quantum yield of the photochemical reaction (photochromic transformation in this case), one can manipulate the outcome by varying these two factors. *E.g.*, employing low intensity UV light (filtered light of Hg lamp 120 W) we obtained the TC form predominant in the solution of chromene **1** at 20 °C. In fact, the formation of only one OF (or at least the significant excess of one OF over another) was highly desired (to simplify the analysis). Another point to consider is the resistance to photodegradation of chromenes: the higher the intensity and the longer the duration of irradiation, the greater the possibility of the compounds undergoing photochemical destruction. This also accounts for the relatively short term irradiation used in the study.

Taking into account these considerations, our findings and the known behavior of chromenes,[38,39,49,50] we conclude that (1) the TC form is generated first, (2) the TT form accumulation requires prolonged or/and high intensity irradiation, and (3) the TC form exhibits a bleaching rate considerably higher than that of the TT form. Thus, at 20 °C upon irradiation of chromene **1**, the formation of the TC form was observed.

Complex formation upon irradiation

The next step of the study was to investigate the complex formation of receptor **1** upon irradiation by means of UV–vis and NMR spectroscopy.

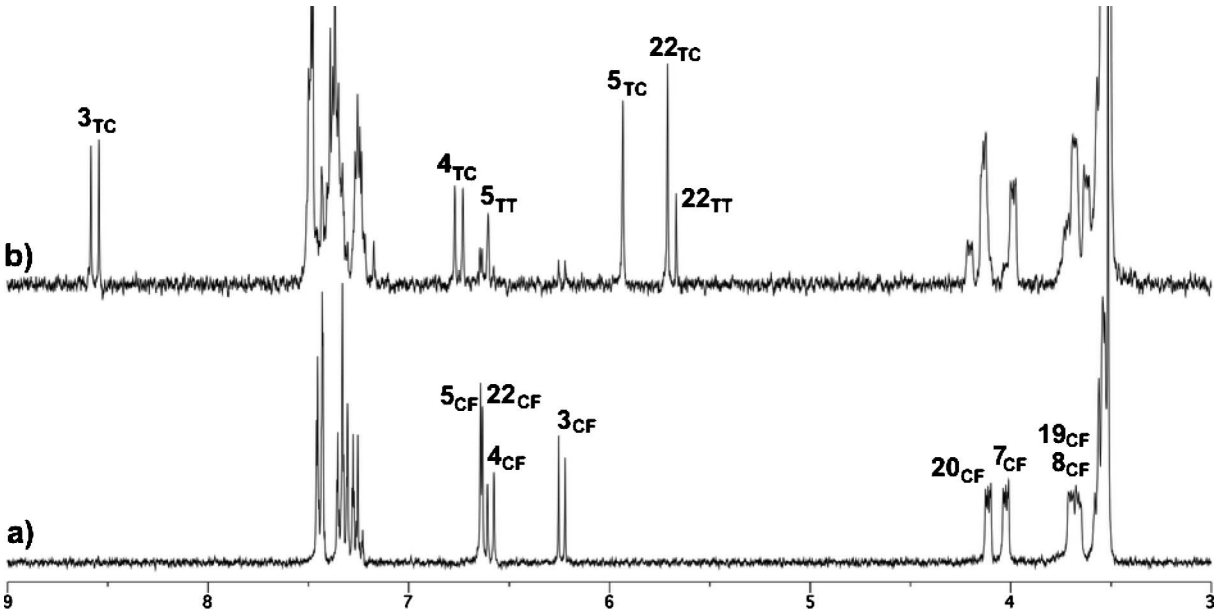


Fig. 7 ^{1}H NMR spectrum changes for chromene **1** upon irradiation with filtered light (λ_{irr} = 313 nm) during 24 min (Hg-Xe lamp, 1000 W) at 0 °C: a) before irradiation; b) after irradiation.

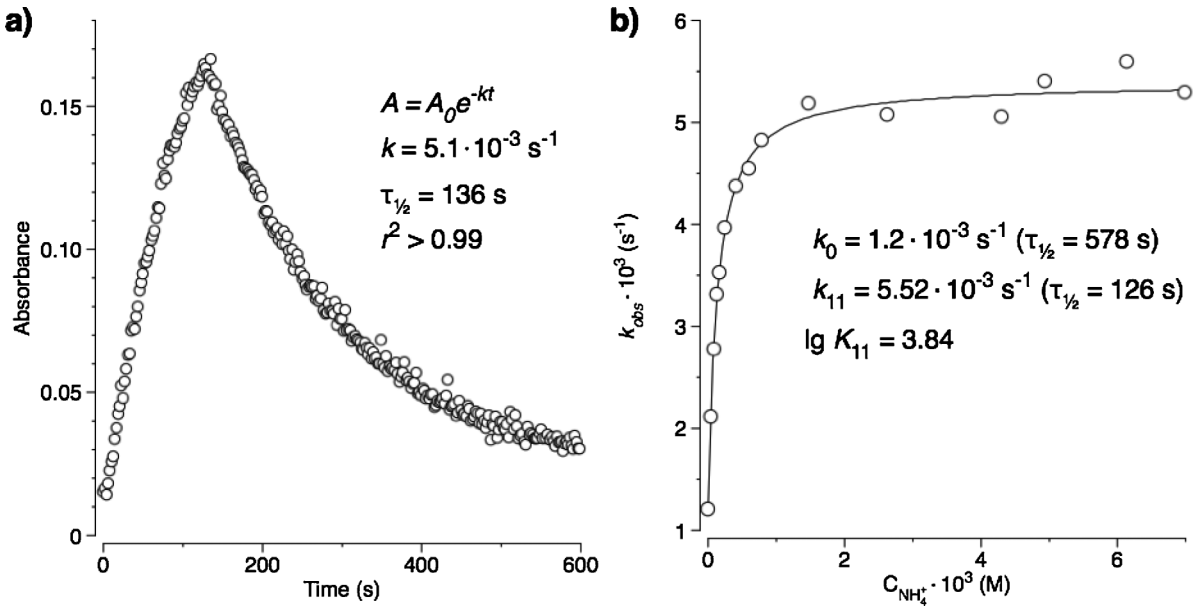


Fig. 8 The effect of ammonium cation on the bleaching kinetics of chromene **1** (acetonitrile, 20 °C) at 385 nm: a) bleaching kinetics at the ratio $C_{ammon.}/C_1 = 160$, $C_1 = 4.2 \times 10^{-5}$ M; b) changes in the bleaching constant depending on ammonium perchlorate concentration in the solution. (A, A_0 are absorbances; k, k_0, k_{11} are bleaching rate constants; r_2 is the correlation coefficient; lg K_{11} is the stability constant logarithm for the 1 : 1 complex.).

One should bear in mind that all the processes in question are in equilibrium. It follows that a solution containing both the host and guest molecules (and even with the latter being in excess) possesses both the free and complexed forms of the host molecules simultaneously (their ratio being governed by the equilibrium constant). Further, if the solution is irradiated, the two open species, the free and complexed ones, are generated; moreover, they are in equilibrium, too. Subsequently, here and further on we refer to formation of the open species as the simultaneous formation of the free and complexed open forms. These speculations are considered by the kinetic scheme we used for the analyses.

In the presence of the modeling ligand, ammonium cations, short term irradiation of a solution of chromene **1** with UV light (λ_{irr} = 313 nm, Hg lamp, 120 W) led to the formation of a single photoinduced form (presumably, the TC), as evidenced by a monoexponential pattern of the bleaching curve (Fig. 8a). Experiments were repeated with various ratios of ligand : substrate concentrations, and the relaxation kinetics were measured during each run. By plotting the variation of the rate constant *vs.* the ammonium concentration, one can observe a rapid increase in rates up to 1 mM of ammonium then followed by a plateau, with the rate constant being about 5×10^{-3} s^{-1}. The observed changes

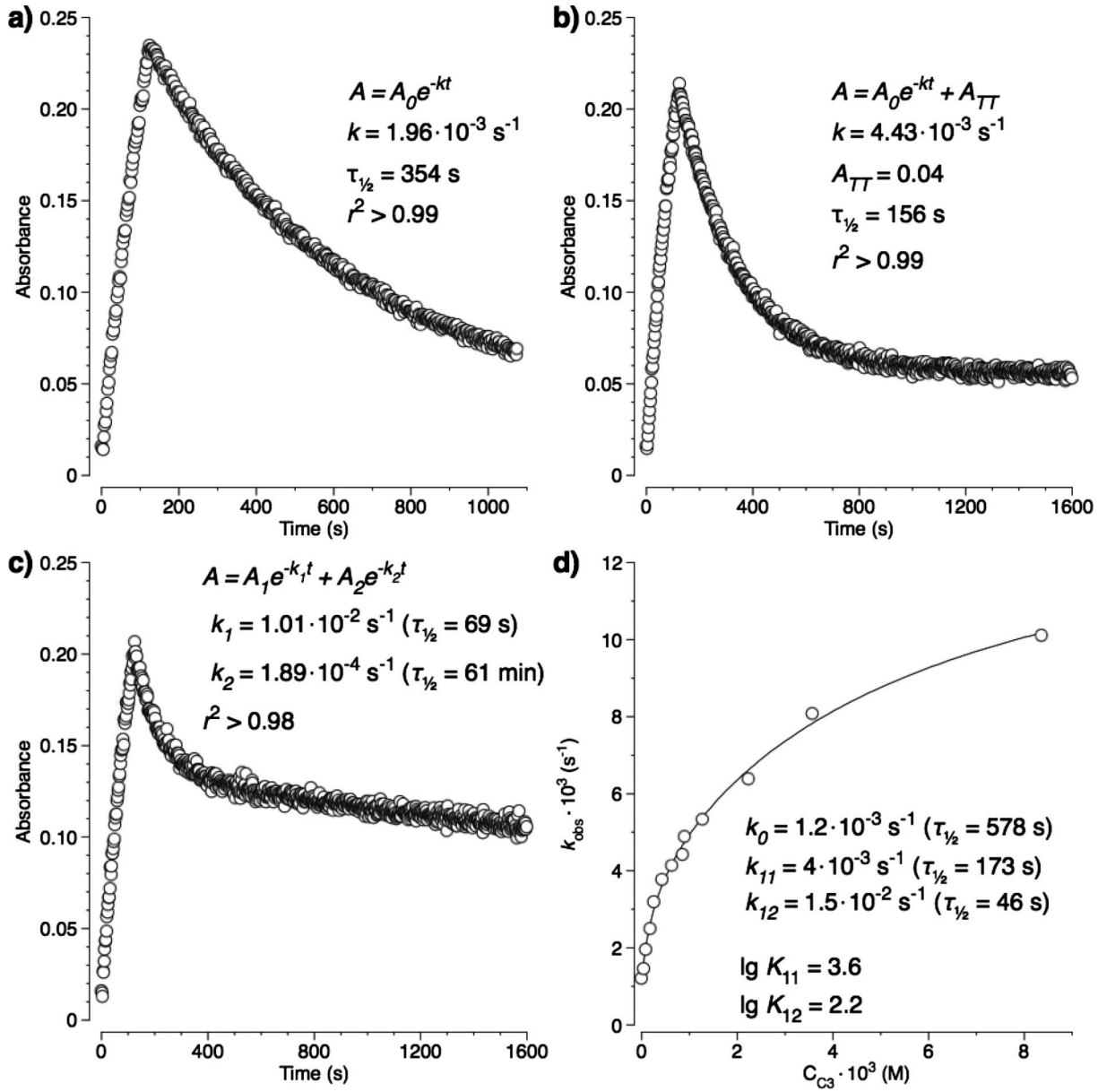


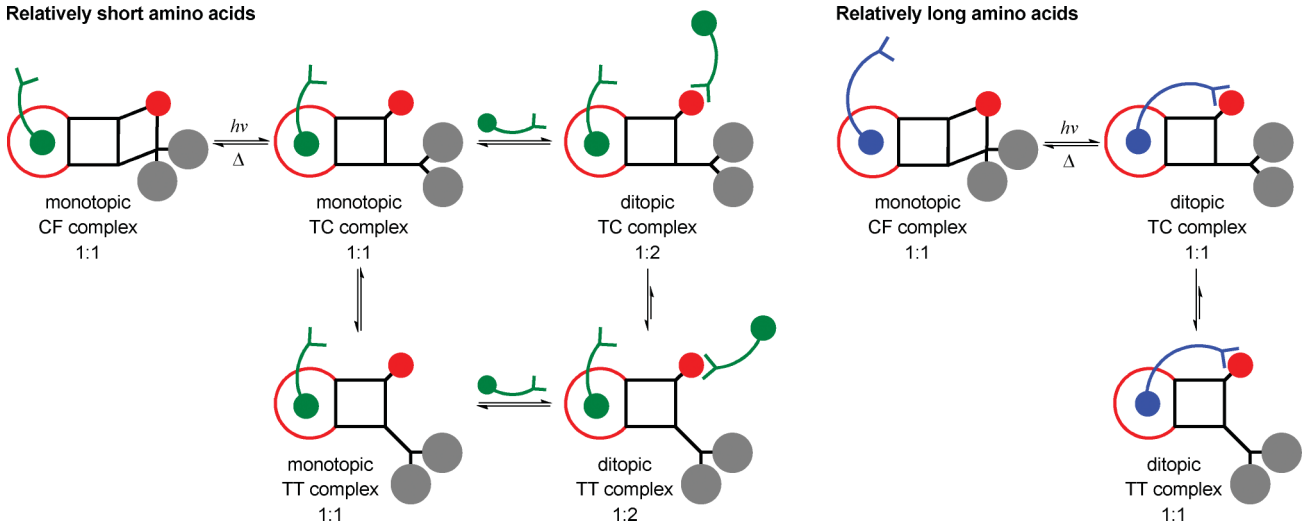
Fig. 9 The effect of β-alanine (C3) on bleaching kinetics at 385 nm of **1** solution after irradiation (acetonitrile, $C_1 = 4.2 \times 10^{-5}$ M, 20 °C): a) $C_{C3}/C_1 = 2$; b) $C_{C3}/C_1 = 20$; c) $C_{C3}/C_1 = 195$; d) dependence of observed relaxation rate constant of the TC form on amino acid concentration. (A, A_0, A_1, A_2, A_{TT} are absorbances; k, k_0, k_1, k_2, k_{11}, k_{12} are bleaching rate constants for the free colored forms and those in the complex; r^2 is the correlation coefficient; lg K_{11}, lg K_{12} are logarithms of the complexes' stability constants.)

are well described by the model of the 1 : 1 complex formation (Fig. 8b).

Irradiation of chromene **1** in the presence of C3, C4, or C6 amino acids leads to the expected ring-opening, but the ratio between the amino acid and the chromene strongly modifies the concentration and the stability of the OFs. Indeed, at relatively low C_{ligand}/C_1 ratio (up to 10), the bleaching curve had a monoexponential pattern (Fig. 9a), which suggested the formation of the TC species. Further increase of the amino acid concentration led to accumulation of a considerable amount of a slowly bleaching component, which was attributed to the TT isomers (Fig. 9b). Along with the increase of the TT forms contribution, a further increase of the amino acid concentration induced a stepwise increase in its overall bleaching rate (Fig. 9c).

Since in the absence of the amino acids, no TT isomer was observed by UV–vis spectroscopy upon the same short-term irradiation, one can conclude that the formation of the TT species was facilitated by the complex formation. In other words, the TC → TT isomerization proceeds more readily in the complexes than between the cation-free forms.

Analysis of dependencies of observed bleaching constant on the cation content in the solution provides estimation of the stability constants of the complexes and the thermal relaxation rate constants of the open forms in these complexes (consult Experimental part for details). In comparison with ammonium cations, in the presence of C3, C4, and C6 amino acids the relaxation rate constant of the TC isomer increases with the ligand concentration up to 200-fold excess (Fig. 9d). This dependence is



Scheme 5 Proposed mechanism of complex formation between chromene **1** and amino acids prior to and after irradiation.

well described by the kinetic scheme which involves consecutive formation of two complexes (see Experimental part), *i.e.* the 1 : 1 complex can bind a second ligand thus forming the ditopic 1 : 2 complex (Scheme 5). The TT isomers of **1** exhibited very slow kinetics even with the high excess of amino acids. This feature significantly hinders the study as the slower the rate, the greater the estimation error of the rate constant (the difference between any two consecutive data points is close to the experimental error). One can assume that, similarly to the TC isomers, a high excess of the ligand could lead to faster kinetics and hence to more reliable data. However this approach is limited by the concentration of the components as a significantly high excess of the ligand may lead to more complicated behavior of the system that might involve besides the process in question, the complex formation, other types of intermolecular interactions. Thus the overall analysis of the experiment would be much more ambiguous. It should be noted that the same rationalization is applied in cases of very fast processes as they proceed much faster than the measurement device can detect the change in the monitored parameter. Thus we do not present the kinetic analysis of the TT forms.

In contrast with C3, C4, and C6 acids and similarly to ammonium cations, ω-aminocaprylic acid C8 does not form the 1 : 2 complexes with the OFs. However, even a small excess of C8 ($C_{C8}/C_1 = 2$) produces both the TT and TC forms (see ESI†). The increase in the amino acid concentration accelerates bleaching of the colored forms up to a certain moment, where further additions cause no substantial change in the bleaching rate. Such behavior allows a suggestion that a ditopic complex formation is realized in this case, in which the consecutive attachment of the second amino acid to the 1 : 1 complex is hindered (Scheme 5). Unlike the previous findings, in the presence of C8 acid the TT isomer formation was apparently more feasible. Similarly to the TC forms, the TT isomers exhibited bleaching of a monoexponential pattern thus allowing a suggestion of 1 : 1 complex formation, presumably with ditopic coordination.

Analysis of the stability constants of the complexes and bleaching rate constants of the TC forms also provides evidence in favor of the proposed schemes of complex formation (Table 3). The stability constant of the 1 : 1 complex with C8 does not follow the general tendency of the constants of the complexes with NH_4^+ → C6 to decrease along with the increasing number of carbon atoms in the aliphatic chain, the same tendency being observed for the 1 : 2 complexes as well. This statement could be rationalized by the fact that the increasing number of methylene groups between the ammonium and the carboxylic groups diminishes the electron

Table 3 Characteristics of ammonium and amino acid cation complexes formed with chromene **1** open forms (acetonitrile, 20 °C). (k_{11}, k_{12} are bleaching rate constants of the 1 : 1 and 1 : 2 TC complexes; $\tau_{\frac{1}{2}}$ is half-life time; lg K_{11}, lg K_{12} are logarithms of the complexes stability constants.)

	Complex 1 : 1 (TC)			Complex 1 : 2 (TC)		
	lg K_{11}	$k_{11}\times10^3$ (s^{-1})	$\tau_{\frac{1}{2}}$ (s)	lg K_{12}	$k_{12}\times10^3$ (s^{-1})	$\tau_{\frac{1}{2}}$ (s)
1[a]	—	1.20 ± 0.10[a]	578[a]	—	1.2 ± 0.1[a]	578[a]
1 + NH_4^+	3.84 ± 0.02	5.52 ± 0.05	126	—	—	—
1 + C3	3.60 ± 0.20	4.10 ± 1.00	173	2.2 ± 0.2	15 ± 2	46
1 + C4	3.41 ± 0.07	5.50 ± 0.40	126	1.6 ± 0.2	14 ± 2	50
1 + C6	3.40 ± 0.40	6.00 ± 0.70	117	1.7 ± 0.6	10 ± 2	69
1 + C8	3.71 ± 0.05	23.10 ± 0.50	30	—	—	—

[a] Data for the free colored forms is presented.

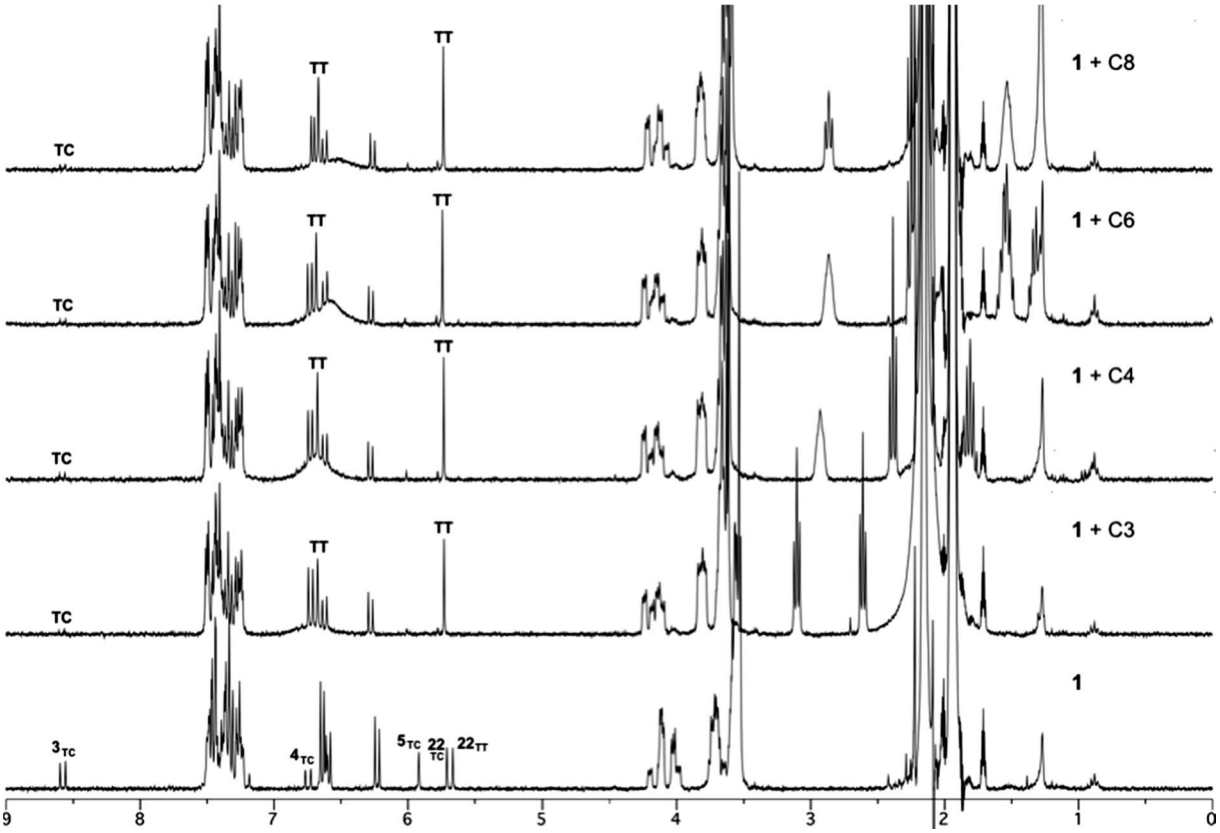


Fig. 10 ^{1}H NMR spectra of chromene **1** solutions in the presence of equimolar amounts of the amino acids after 10 min irradiation (CD_3CN, 20 °C).

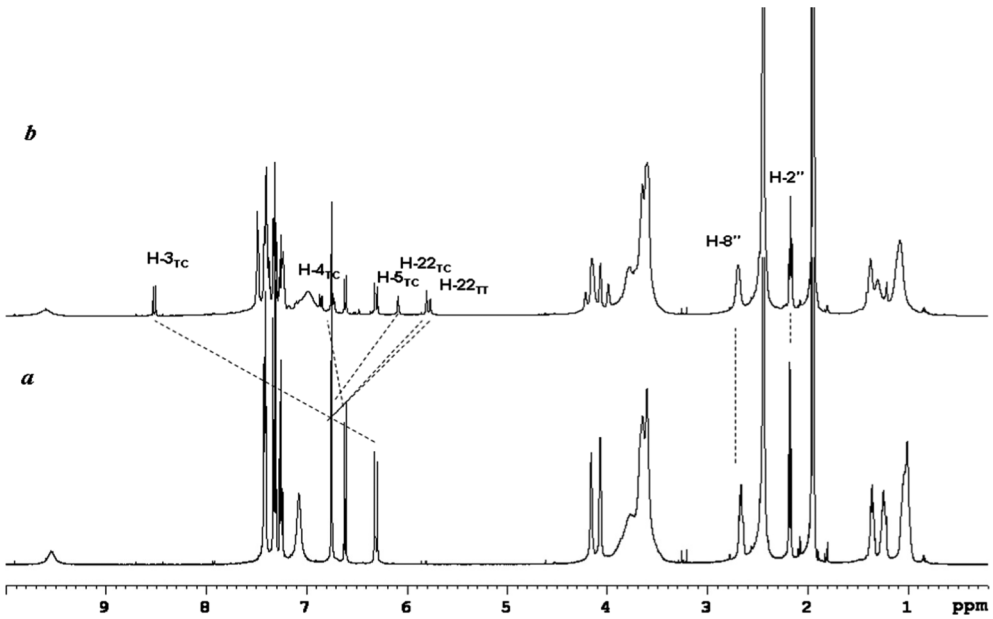


Fig. 11 ^{1}H spectra of the complex **1·C8** before (*a*) and after irradiation (*b*) at λ_{irr} = 313 nm (CD_3CN, –45 °C).

withdrawing effect of the latter thus diminishing the charge on the nitrogen atom. The lower the charges, the lower the strength of an interaction and hence the lower the stability constant. The spatial factor should also account for the difference between ammonium cation and amino acids. These effects correlate well with an assumption involving ditopic coordination as the stability constants of such complexes, as compared with the monotopic ones, should be higher accounting for the two-site interaction.

Another point to consider is the bleaching kinetics. As compared with the free ligand **1**, in the presence of ammonium and

the amino acids cations, the bleaching rate increases (Table 3). The rate constants for the 1 : 1 complexes with ammonium and C3, C4, and C6 acids are essentially the same and about 5 time greater than that of the free TC form. This observation provides evidence for the similarity of these complexes. On the contrary, the rate constant of the complex with C8 acid is much greater and closer in value to the rate constants of the 1 : 2 complexes with the other amino acids. These findings also support the suggestion of ditopic complexation. It seems that the significant increase in the bleaching rate of the 1 : 2 complexes is due to additional coordination of the second ligand with the carbonyl oxygen atom of the OF. Virtually, this same effect should be observed in the case of the ditopic complexation and in fact it does. Nevertheless, other additional factors (*e.g.*, steric, *etc.*) may account for the still higher rate constant value of the complex with C8.

To support our suggestion made by UV–vis experiments, we conducted NMR studies. The presence of any of the ligands did not much affect the aromatic part of the NMR spectrum, instead having greater influence on the resonances of the crown ether moiety. Thus, it was not possible to distinguish between NMR signals belonging to the free or complexed species of the chromene as they have the same chemical shift. Irradiation of solutions containing equimolar quantities of chromene **1** and an amino acid with UV light (λ_{irr} = 313 nm, Hg-Xe lamp, 1000 W) during 10 min at 20 °C induced the predominant generation of the TT species. The signals of the TC forms were also detected; however they have low intensity (Fig. 10). In turn, spectra recorded at –45 °C revealed the predominant generation of the TC species, which however relaxed thermally to the TT isomers (see ESI,† Fig. S16). Thus, the NMR studies provide evidence for the thermal TC → TT transition of the complexes. Such a transition is unusual for chromenes, for which the TC → TT transformation is believed to be a photochemical process.[38,39] Therefore, this isomerization is driven only by the coordination between the OFs and amino acids and is probably governed mainly by the steric factors.

Unfortunately, the NMR studies failed to provide additional proof for the ditopic coordination in the complex of the TC form of **1** and C8 amino acid. The solution of complex **1·C8** was irradiated at λ_{irr} = 313 nm at –45 °C. As evidenced by the characteristic signals in ^{1}H NMR spectroscopy, the formation of the TC and TT isomers does occur. Indeed, the TC isomer can be evidenced rapidly by considering the most deshielded proton, H-3 at a chemical shift around 8.52 ppm and the proton H-22 at 5.80 ppm while the TT isomer is well recognized from its H-22 proton at 5.77 ppm (Fig. 11). However, several 1D NOESY experiments carried out at –45 °C did not provide any proof of the ditopic coordination of C8 with the TC form of chromene **1** because the proton signal of the carboxylic group participates in deuterium exchange with the water contained in the solvent (acetonitrile) and could not be used in 1D NOE experiment as a target signal. Neither were changes of the aliphatic protons of the carbon chain of the acid observed, which could have been accounted for by the anisotropic effects of the aromatic system under the chain.

To summarize, the following rationalization of the observed experimental data may be suggested (Scheme 5): Irradiation of solutions with ammonium and amino acid C3, C4, and C6 cations induces the formation of the 1 : 1 TC complexes. These are the monotopic complexes, in which the ammonium group (or the cation itself) is located inside the 18-crown-6 ether cavity. In contrast to ammonium complexes, the complexes with amino acids may readily bind another ligand molecule thus forming the 1 : 2 complexes, in which the additional ligand coordinates with the carbonyl oxygen atom of the TC form. The spatial hindrance of the 1 : 2 TC complex promotes the thermal TC → TT isomerization, which leads to accumulation of that species in the solution. It should be noted that we cannot exclude the possible photochemical formation of the TT complexes, the 1 : 1 or 1 : 2 species, from the TC isomers. Amino acid C8 exhibits another behavior. Similar to ammonium cations, it forms only the 1 : 1 complexes with the OFs. However, in comparison with the amino acids with shorter aliphatic chain, it presumably forms the 1 : 1 ditopic complexes, in which the two-site coordination is realized. As well as for the 1 : 2 complexes with other amino acids, for the ditopic complexes the TT isomer is more preferable.

Conclusions

To conclude, we have demonstrated that the crown ether annelated chromene is able to bind protonated amino acids in both the initial closed and photoinduced open state. We suggest that depending on the aliphatic chain length, the amino acids may form either monotopic or ditopic complexes, the actual type of coordination being governed by the complementarity of the host and guest molecules. The mode of coordination strongly affects the properties of the complexes that make it possible to distinguish among them. Thus, we have demonstrated the possibility of using a supramolecular feature for molecular recognition of such objects as protonated amino acids. On the other hand, the reported concept may be employed for specific application in which the mode of binding is essential.

To the best of our knowledge, this is the first example of chromenes able to bind amino acids. Understanding the interactions between synthetic molecules and amino acids, polypeptides and nucleic acids is important for the construction of optical sensors, markers and especially for the design and development of nucleic acid binding and cleaving agents, which may be used as structural probes or therapeutic agents.

Experimental

1. Materials

2,2 - Diphenyl - 7,8,10,11,13,14,16,17,19,20 - decahydro - 2*H*-[1,4,7,10,13,16]hexaoxacyclooctadeca[2,3-g]chromene (1). The procedure was adopted from lit.[51] 2,3,5,6,8,9,11,12,14,15-Decahydrobenzo[*b*][1,4,7,10,13,16]hexaoxacyclooctadecin - 18 - ol (0.33 g, 1 mmol) and β-phenylcinnamaldehyde (0.21 g, 1 mmol) were dissolved in 8 ml of toluene and a solution of titanium(IV) tetraethoxide (0.34 g, 1.5 mmol) in 2 ml of toluene was added. The mixture was stirred at 100 °C in Ar atmosphere for 6 h, then cooled to 30–40 °C and 10 ml of toluene, 0.5 g of silica gel, and 1 ml of water were added to it. The resulting suspension was stirred at 80 °C for 30 min. After cooling to ambient temperature, the precipitate was filtered off, washed with dichloromethane several times and the organic solution was evaporated. The product was isolated by column chromatography ($MeOH : CH_2Cl_2$ = 1 : 9), which afforded 0.19 g (20%) of chromene **1** as slightly orange solid. M.p. 76–78 °C (pentane). ^{1}H NMR (250 MHz, $CDCl_3$, δ

(ppm), *J* Hz): 3.58–3.72 (m, 12H, CH_2-5, CH_2-6, CH_2-8, CH_2-9, CH_2-11, CH_2-12), 3.77–3.88 (m, 4H, CH_2-3, CH_2-14), 3.96–4.08 (m, 4H, CH_2-2, CH_2-15), 5.94 (d, J = 9.7, 1H, H-20), 6.39–6.52 (m, 3H, H-17, H-21, H-22), 7.11–7.29 (m, 6H, H-Ar), 7.30–7.38 (m, 4H, H-Ar). ^{13}C NMR (63 MHz, $CDCl_3$, δ (ppm)): 68.56, 69.34, 69.63, 69.91, 70.53, 70.59, 70.66, 70.68, 82.43 (s, *quat*-C), 102.61, 113.23, 113.38, 123.01, 126.35, 126.87, 127.34, 127.97, 142.80, 144.83, 147.33, 150.14. Calculated for $C_{31}H_{34}O_7 \cdot 2H_2O$ (%): C, 67.13; H, 6.91. Found (%): C, 67.68; H, 6.96.

Protonated amino acids were prepared by dissolving neutral β-alanine, γ-aminobutyric, ε-aminocaproic, and ω-aminocaprylic acids (all purchased from Aldrich) in acetonitrile solution of perchloric acid at ambient temperature. After being stirred during 24 h, the solution was filtered and the solvent was evaporated. The residue was additionally kept in a lyophilizer during 20 h.

2. UV–vis spectroscopy measurements

Electronic absorption spectra were recorded on spectrophotometers «Specord M40» and «Avantes AvaSpec-2048». Spectra of the colored forms were obtained when samples in the spectrometer cell were simultaneously exposed to continuous irradiation, generated by Hg high pressure lamp 120W.

3. NMR spectroscopy measurements

NMR spectra were recorded on a Bruker 300 spectrometer (1H, 300 MHz) equipped with QNP probe or on a Bruker 500 spectrometer (1H, 500 MHz) equipped with TXI probe, using standard sequences. Data sets were processed using Bruker Topspin 1.3 software. Photoirradiation was carried out directly into the NMR tube in a home-built apparatus with a 1000 W high-pressure Hg-Xe lamp equipped with a filter (Schott 11FG09: $259 < \lambda < 388$ nm with $\lambda_{max} = 330$ nm, T = 79%) to select UV light and an interferential filter ($\lambda = 313$ nm and $T = 16\%$).

4. Stability constant measurements

Spectrophotometric titration was used for determining the stability constants for complexes of chromene **1** (its closed forms, before irradiation) with protonated amino acids.[52,53] The procedure was as follows: to a solution of the ligand in acetonitrile (~10^{-4} M), aliquots of the substrate solution (β-alanine, γ-aminobutyric, ε-aminocaproic, or ω-aminocaprylic acids perchlorates in acetonitrile, ~10^{-2}–10^{-3} M) were added stepwise, recording an absorption spectrum after each addition. By analyzing spectral changes during the course of the titration, conclusions on complex composition and stability were drawn. The stability constants of the complexes were determined by applying the SPECFIT/32[54] software to experimental data considering the following equilibrium: $S + L \rightleftarrows SL, K_{11} = \frac{[SL]}{[S][L]}$ where S, L, and SL denote chromene (substrate), ammonium or amino acid cation (ligand), and their 1 : 1 complex; K_{11} is the stability constant of the complex; square brackets denote equilibrium concentration of the corresponding species.

Stability constants of the complexes of the OFs with the amino acids and ammonium cation were determined upon analysis of changes in bleaching rate constants in the presence of different amounts of the ligands. For each determination, a fresh solution of the chromene and one of the ligands was prepared. Using spectrometer «Avantes AvaSpec-2048», a spectrum was recorded (with 0.5–2 s interval between measurements) upon continuous irradiation of the sample solution. Each sample was irradiated during the same time. Upon cessation of the irradiation, the spectrum was recorded until full bleaching or at least the intensity of a long wavelength band was decreased by half. Assuming that the complex equilibria establish much faster than the bleaching occurs, the processes were analyzed using the following equations (consult ESI† for details on the kinetic scheme):

$$k_{obs} = \frac{k_0 + k_{11}K_{11}C_L}{1 + K_{11}C_L} \quad (1)$$

$$k_{obs} = \frac{k_0 + k_{11}K_{11}C_L + k_{12}K_{11}K_{12}C_L^2}{1 + K_{11}C_L + K_{11}K_{12}C_L^2} \quad (2)$$

where C_L denotes molar concentration of the amino acids or ammonium perchlorates; k_{obs}, k_0, k_{11}, and k_{12} denote the observed bleaching rate constant and the rate constants for the free chromene, 1 : 1 and 1 : 2 complexes, respectively; K_{11} and K_{12} represent the stability constants of the 1 : 1 and 1 : 2 complexes, respectively.

Applying eqn (1) and 2 to the experimental data, we estimated the stability constants of the complexes and determined the thermal relaxation rate constants of the open forms in these complexes.

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