Photoresponsive Dendritic Azobenzene Peptides

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Two dendritic peptides containing a branched lysine core and up to eight azobenzene moieties in the periphery were synthesized on solid support employing the ω-amino acid 4-(aminomethyl)phenylazobenzoic acid. With an additional peptidic tail consisting of an oligolysine portion, water solubility was achieved for the dendrimers, which allowed for the characterization of the cis/trans *photoisomerization of the dendritic azobenzene species in both organic and aqueous media. Despite the interactions between the chromophores, which occur particularly in aqueous media, at higher dilution the photoisomerization process was found to proceed to extents that should permit photomodulation of molecular recognition processes between ligands grafted to the photosensitive azobenzene units and receptor molecules.*

KEYWORDS:

azo compounds · dendrimers · isomerization · peptides · photochemistry

Introduction

Dendrimers are monodisperse high molecular weight macromolecules that represent chemically well-defined systems and that are prepared by divergent or convergent methods.[1] Among the various potential applications of this class of compounds, the design and synthesis of dendritic molecules as carriers for the multiple presentation of bioactive molecules with both agrochemical and pharmaceutical applications represent a large and promising field of research.[2] In fact, dendrimers were found to be highly effective agents for the delivery of genetic material into a variety of cell lines[3, 4] whereby the drug molecules are loaded into the interior of a dendrimer or are attached to the polymer surface.[5] Generally, such conjugates have enhanced solubility and reduced toxicity if compared to the unconjugated drugs, while still maintaining their therapeutic efficacy.[6] Similarly, a variety of biologically relevant sugar residues have been conjugated to hyperbranched dendritic cores for the development of potentially useful drugs for the treatment of cancer, inflammation and infection processes.[7] In such well-defined glycodendrimers, the synergistic cluster or multivalent effect and thus the resulting cooperativity is known to compensate for the usually low binding affinity of natural oligosaccharides in carbohydrate – protein interactions.[8] The multivalent carrier properties of globular dendritic moieties have also been exploited in the preparation of immunogenic conjugates,[9] where the immunodominance of the carrier is strongly suppressed by the repetitious presentation of haptens on the surface of the dendrimer.[10]

In view of this wide range of possible applications of dendritic carriers, the design of systems capable of changing their properties upon external inputs like pH, electricity, or light, could offer many advantages, especially in diagnostics. In fact, each molecular interaction that is successfully mediated by a dendritic carrier could then in principle be controlled under mild conditions at the molecular level by exploiting such devices. However, only few examples of dendrimers that respond to environmental stimuli are known so far.[11]

Light-induced conformational changes have found a wide spectrum of applications in biochemistry,[12] and recently it has been shown that incorporation of photosensitive moieties into the interior of dendrimers or on their outer surface can lead to reversible photoinduced changes in known dendrimer properties such as self-assembly, liquid crystallinity, and encapsulation.[13] Unfortunately, the exclusive solubility in organic solvents of most of these photoresponsive dendrimers has severely limited their applications in aqueous media and thus in biological systems.

The aim of the present study was, therefore, to develop a suitable synthetic strategy for the preparation of a new class of dendritic compounds that can undergo photomodulated local structural changes without losing their applicability as multivalent carrier systems for molecules of biological interest. For this purpose the novel carrier compounds **1** and **2** were designed, which contain the azobenzene derivative 4-(aminomethyl)phenylazobenzoic acid[14] (H-AMPB-OH, **3**) as photoresponsive moiety grafted through amide bonds on the periphery of a dendritic core based on lysine residues. These constructs contain an additional peptidic tail consisting of lysine residues to induce the required water solubility. Moreover, a cysteine residue was incorporated at the C terminus to exploit the selective thiol anchor group for potential conjugation to other proteins or surfaces. The spectroscopic properties of the photoresponsive dendrimers were compared in organic and aqueous media. The *cis*/*trans* isomerization was found to occur in a reversible manner, at higher dilution even to extents that should allow photomodulation of molecular recognition processes.

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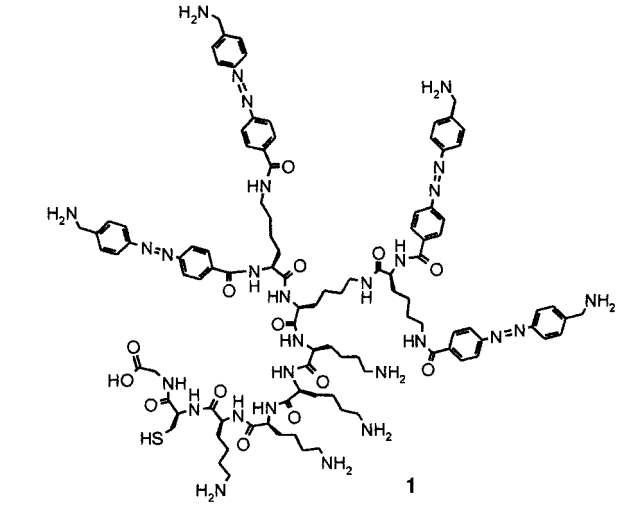

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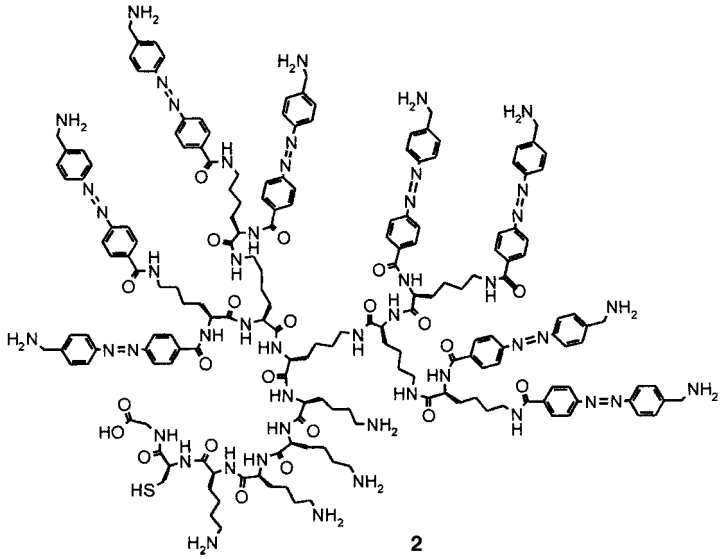

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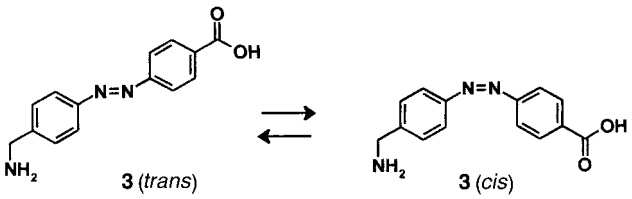


3 (*trans*) 3 (*cis*)

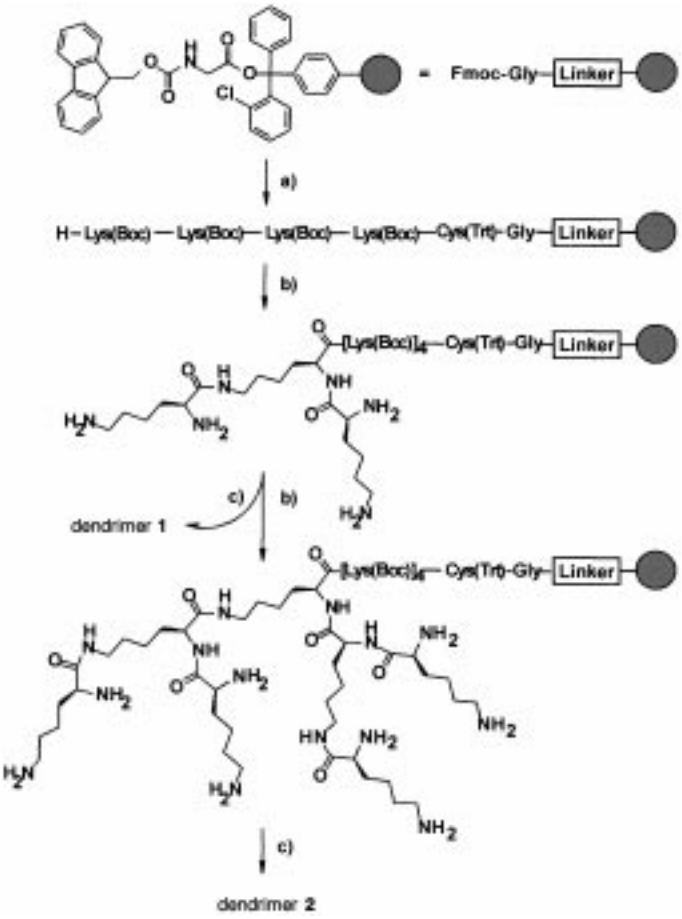
Results and Discussion

Design of the dendrimers

The *cis*/*trans* photoisomerization of the azobenzene moiety is known to be accompanied by significant changes in geometry and polarity of the chromophore. Therefore this photosensitive molecule has already found widespread application for the induction of local topochemical changes.[17] In the design of the photoresponsive dendrimers **1** and **2** the 4-(aminomethyl)phenylazobenzoic acid[14] (H-AMPB-OH, **3**) was selected since this azobenzene derivative, which differs from other azobenzene compounds previously employed, is an ω-amino acid that can be incorporated into a peptide backbone by standard solid-phase synthesis.[15] It has recently been demonstrated that efficient preparation of some functionalized dendrimers can be achieved by stepwise amplification of the dendrimer core on a solid support.[18] Final modification of the branched end groups prior to the resin cleavage step can be driven to completion by using a larger excess of reagents that allow to obtain differently functionalized compounds in good homogeneity without the need of challenging purification procedures. This synthetic approach on solid support appeared the most promising in view of our aim to develop a general synthetic procedure that allows to obtain the target dendritic compounds in good yields and purity, but also to functionalize the carrier systems with molecules of biological interest. Since branched oligolysines were shown to be valid scaffolds for the construction of dendritic conjugates of biological interest,[19] two dendrimers of this type were chosen to display in a branched manner four or eight AMPB moieties for further functionalization at their amino groups. To improve the water solubility of the target dendrimers these were placed at the N terminus of a peptidic tail consisting of four adjacent lysine residues. Moreover, at the C terminus of the tail a cysteine residue was incorporated to allow for regioselective chemistry to be performed on the functionalized dendrimers via the thiol group as selective anchor. The C-terminal glycine residue was used for preparative purposes to avoid the well-known difficulties arising from direct attachment of cysteine to the resin.

Synthesis of the dendrimers

The synthesis of the peptide dendrimers **1** and **2** was performed on chlorotrityl resin[20] (Trityl = Trt = triphenylmethyl) adopting the Fmoc/*t*Bu strategy[21] as outlined in Scheme 1. The acid-labile *S*-trityl group was used for protection of the cysteine thiol function to overcome the difficulties encountered in reductive cleavage of the *S*-*tert*-butylthio group in the presence of azobenzene.[15] The dendritic lysine core was constructed by coupling successively either two levels of Fmoc-Lys(Fmoc)-OH to provide four amino groups, or three levels of the lysine derivative to provide eight amino groups. In the synthesis of lysine dendrimers on solid support, difficulties are generally encountered starting at the third lysine level.[19] Elevated temperature, "magic" solvent mixtures,[22] and sonification are used to push the aminoacylation to completion. In the present case, high efficiency in assembly of the growing branched peptides was achieved by optimizing the reaction time. In fact, quantitative Fmoc cleavage of compounds **1** and **2** was achieved only upon prolonged treatment with the piperidine solution. Furthermore, exposure of the resin to a swelling solvent prior to the coupling step led to high aminoacylation yields in the last synthetic steps. Despite the space limitations within the polymer beads, by this procedure the synthesis of up to generation 3 dendrimers could be achieved without synthetic mishaps. Careful optimization of the cleavage procedure from the solid support led to the conditions reported in Table 1. Additional difficulties were encountered in the solubilization of the free peptide adsorbed onto the solid matrix, and satisfactory yields of



Scheme 1. *Synthesis of the dendrimers **1** and **2**. a) Couplings with Fmoc-Cys(Trt)-OH and 4 Fmoc-Lys(Boc)-OH, Fmoc removal with 20% piperidine in NMP; b) couplings with Fmoc-Lys(Fmoc)-OH, Fmoc removal with 20% piperidine in NMP; c) couplings with Fmoc-AMPB-OH (**4**), Fmoc removal with 20% piperidine in NMP, deprotection and resin cleavage with acid.*

Table 1. *Protocol of the synthesis of dendrimers **1** and **2** on Fmoc-Gly-loaded chlorotrityl resin.[a]*

Synthetic step	Reagents and solvents	Reaction time
Fmoc cleavage	20% piperidine/NMP	5 + 15 min
coupling	stepwise aminoacylation in sequence mode with 4 equiv of Fmoc-Cys(Trt)-OH/HBTU/HOBt/DIEA (1:1:1:1.1), followed by Fmoc-amino acid/HBTU/HOBt/DIEA (1:1:1:2) in NMP; double couplings	2 × 45 min
acidolytic cleavage	TFA/CH_2Cl_2/H_2O (18:1:1) and 1,2-ethanedithiol as scavenger	12 h

[a] Loading: 0.50 mmol g^{-1}.

the unprotected compounds **1** and **2** were obtained only by careful washing of the resin. Similarly, purification that would be otherwise very challenging was achieved by extensive washing of the resin between the individual synthetic steps and by carefully monitoring each synthetic step for its completeness. No further chromatographic procedure was employed, and the products were isolated as highly homogeneous materials as judged by various analytical assays.

Analytical characterization of the dendrimers

A number of analytical methods have previously been used to characterize azobenzene dendrimers.[1] Generally, ^{1}H and ^{13}C NMR spectroscopy give only qualitative information, since the related spectra become less indicative with increasing generation number. Matrix-assisted laser desorption – ionization time-of-flight (MALDI-TOF) mass spectrometry is the method of choice for the analysis of dendritic structures,[23] as it allows to mildly ionize the target compounds and generally avoids fragmentation. Imperfections of the dendrimer skeletons and the degree of homogeneity of the compounds isolated is often evaluated by simple mass analysis.[24] Since compounds **1** and **2** were synthesized on a polylysine core, reversed-phase (RP) HPLC and electrospray ionization (ESI) MS were excellent analytical tools for monitoring the single steps in the synthesis of the branched peptides. Functionalization of the resin-bound tetra- and octavalent lysine dendrimers with the azobenzene moieties was found to strongly affect the properties of the dendrimers. As a consequence, RP HPLC and ESI MS analysis proved to be unsatisfactory for unambiguous identification of the target compounds **1** and **2**. Exchange reactions with the reversed phase, probably due to the interactions of the multiprotonated azobenzene moieties with the silanol groups, caused widening of the chromatographic peaks,[25] thus preventing assessment of the degree of homogeneity by this chromatographic technique. Taking advantage of the good water solubility of compounds **1** and **2**, capillary electrophoresis (CE) was applied which, upon optimization of buffer concentrations and temperature, led to fully reproducible electropherograms. The purity of the dendrimers was found to be 97% for **1** and 98% for **2**. Similarly, MALDI-TOF MS revealed molecular ion peaks for the two dendrimers, and no contaminants corresponding to partially functionalized compounds were detectable. Fragmentation of the azobenzene moieties with loss of 215 mass units was detected in the mass spectra in agreement with previous observations on azobenzene-containing peptides. Concerning amino acid analysis of lysine-based dendrimers, 72 h of acid hydrolysis was reported to yield reliable results.[26] According to our experience, the values strongly depend upon the nature of the grafted end groups and the complexity of the dendritic structure. Values in good agreement with theory were obtained for compound **1**, whereas for compound **2** an evident underestimation of the lysine content was observed.

NMR spectroscopic analysis of the dendrimers

NMR spectroscopic analysis in aqueous solution confirmed the chemical structure and homogeneity of the dendrimers **1** and **2**. Unexpectedly, many signals showed strong line broadening (Figure 1). To distinguish between possible sources, such as aggregation, heterogeneous line broadening, or chemical exchange processes, 1D ^{1}H-T_1 and ^{1}H-$T_{1\rho}$ relaxation measurements were performed for compounds **1** and **2**. The results of these measurements can only serve as crude estimates, since for many frequencies two or more signals overlap resulting in a multiexponential decay of peak heights. Moreover, detailed

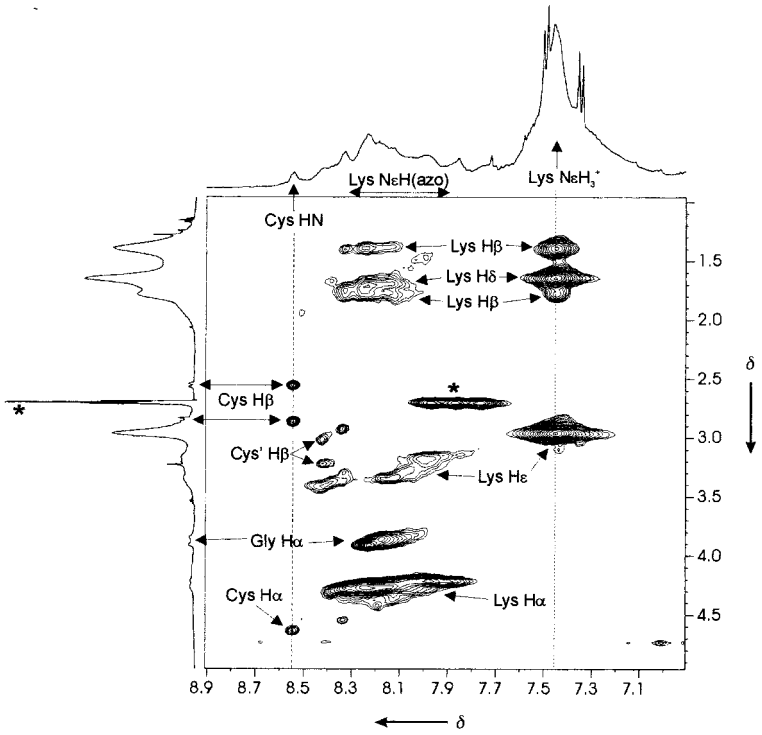


Figure 1. *2D TOCSY NMR spectrum of dendrimer **1** at 27 °C showing intraresidual connectivities. Cross peaks are labeled with the F_1 (vertical) frequency. On the top and left, corresponding parts of the 1D ^{1}H NMR spectrum are shown. A strong peak from an impurity is marked with an asterisk.*

analysis of the obtained relaxation rates is very complicated as for protons there are usually many relaxation pathways contributing to the final rates. Nonetheless, from the T_1/T_2 ratios an apparent mass of the molecules tumbling in solution can be estimated by comparison with T_1/T_2 ratios of reference molecules. For two reference molecules (peptides) with M_r values of 1.1 kDa and 13 kDa, T_1/T_2 ratios of ca. 2 and 34 were found, respectively. Considering that $(T_1/T_2)-1$ is approximately proportional to the square of the apparent mass, the following interpolation was used: $\text{mass} = 2.7\ \text{kDa} \times \sqrt{(T_1/T_2)-1}$. As the measured T_1/T_2 ratios were ca. 5 for **1** and ca. 7 for **2**, both dendrimers seem to have apparent masses larger than their molecular masses, but still less than the masses of the dimers possibly resulting from oxidation of the cysteine residues. Thus, the molecules are tumbling in solution mostly as monomers or dimers at the concentrations used for NMR spectroscopy, and extensive aggregation as a source for the line broadening can be excluded.

Within the dendrimers the side chains of lysine residues of the peptide tail showed increasing flexibility toward the ω-amino group, as expected from increased solvent exposure. Conversely, for the lysine residues that build up the dendritic moiety increasing line broadening was observed for the δ- and ε-CH_2 groups. Signals from the AMPB moiety were broadened to such an extent that they could not be resolved in the spectra. This line broadening probably results from chemical exchange processes due to interactions of the AMPB groups in the aqueous environment (hydrophobic collapse on the dendrimer surface), although conformational microheterogeneity cannot be ruled out. A fact supporting the view of slow chemical exchange is given by the dramatic increase in quality of the 2D ROESY spectra upon lowering the temperature. At 320 K, only undefined signal clusters were observed in the ROESY spectra and no distinct signal sets could be identified. On the other hand, at 280 K signals for the C-terminal amino acid residues (-Cys-Gly-OH) as well as connectivities to preceding residues (-Lys-Cys-) could clearly be resolved allowing for their assignment. For the lysine residues only a distinction between functionalized and "tail" residues was possible. Within these two classes no separation of individual lysine residues was possible due to their very similar chemical environment. Notably, measurement of the temperature dependence of chemical shifts for compounds **1** and **2** indicate shielding from the solvent or possible involvement in hydrogen bonds only for the ε-NH_3^+ groups ($-4.5\ \text{ppb}\,\text{K}^{-1}$) of the lysines of the tail, whereas all amide group protons seem fully accessible to the solvent (-8 to $-9\ \text{ppb}\,\text{K}^{-1}$). Interestingly, for the cysteine residue an additional distinct signal set of smaller intensity was observed indicating that the cysteines were partially oxidized in the NMR samples. This is in agreement with the dynamics analysis discussed above, but should also indicate that intermolecular interactions as observed in the UV spectra of the dendrimers at higher concentrations (see below) do not markedly reduce the tumbling rate of the molecules in solution, that is, they are weak and/or short-lived.

UV spectroscopic characterization of the dendrimers

All samples were kept in the darkness for several days to allow thermal relaxation of the azobenzene moieties into the *trans*-isomer state to occur. The UV spectra recorded for the dendrimers **1** and **2** in DMSO, ethylene glycol, and trifluoroethanol (TFE) are very similar, and in aqueous solution the pH (in the range from 3.1 to 6.7) was not found to affect the absorption maxima. This is in agreement with studies on dendrimers, which share with ours the amino functional group at the end of the branches, and where pK_a values all close to 10.0 were found.[27] No change in protonation state for the amino groups is expected in the pH range investigated here. In Table 2 the λ_{max} values of the $\pi-\pi^*$ and $n-\pi^*$ transitions of the dendrimers **1** and **2** are

Table 2. *Observed absorption maxima for **1** and **2** in TFE and phosphate buffer.*

	λ_{max} ($\pi-\pi^*$) [nm]	λ_{max} ($n-\pi^*$) [nm]
1 (buffer)[a]	321	461
2 (buffer)[a]	323	463
1 (TFE)	322	446
2 (TFE)	320	447

[a] 0.1 M Phosphate buffer, 1 mM EDTA, pH 3.1.

listed exemplarily for solutions in TFE and phosphate buffer (pH 3.1). While the $\pi-\pi^*$ transitions occur at almost identical wavelengths for both dendrimers and are only marginally affected by the solvent, the λ_{max} values of the $n-\pi^*$ transitions are significantly red-shifted in aqueous solution relative to the TFE solutions.

A comparison of the UV spectra of the azobenzene-functionalized dendritic peptides with those of H-AMBP-OH (**3**) (Figure 2) and with linear peptides containing the AMPB moiety[17h] clearly reveals the presence of the *cis*-azo isomers in the dendrimers even after thermal relaxation in the darkness for longer periods of time. The presence of the *cis* isomer at equilibrium would suggest that both isomers are energetically more similar in the dendritic structures than previously observed in linear peptides. This view is further supported by the observation that the dendrimer **2** was found to contain always more *cis*-azo isomer than dendrimer **1**. Similarly, in aqueous solution the content of *cis*-azo isomer is higher than in organic media. Since thermal relaxation generally suffices for quantitative conversion of the azobenzene moieties into the *trans*-isomer state,[17] intramolecular or intermolecular interactions must stabilize the *cis* isomer, at least kinetically. It has previously been reported[17h] that *N*- and *C*-derivatization of H-AMPB-OH (**3**) in linear and cyclic peptides does not affect significantly the $n-\pi^*$ and $\pi-\pi^*$ transition in DMSO. In this solvent λ_{max} values around 338 nm and 262 nm were observed for the $\pi-\pi^*$ transitions of the *trans*- and *cis*-azo isomers, respectively. In Figure 2 the second derivatives of the absorption spectra of the dendrimers **1** and **2** and of reference compound **3** in TFE after different irradiation times at 360 nm are shown. The $\pi-\pi^*$ transition of the *trans*-azo component occurs at comparable wavelength for the three compounds, while a red-shift of the λ_{max} value is observed for the *cis*-azo component in the dendrimers **1** and **2** in TFE and aqueous media (data not shown) if compared with the reference compound **3**, again indicating intra- or intermolecular interactions involving the chromophores. Furthermore, the molar extinction coefficient is not doubled in **2** relative to **1**, as theoretically expected by the number of AMPB moieties in the absence of strong interchromophoric interactions.

In the following, we want to discuss briefly interactions involving the chromophores that could affect the *cis*/*trans*-photoisomerization process in the dendrimers **1** and **2**. Besides hydrophobic interactions and π stacking of the benzene rings we also expect interactions between amino and aromatic groups and between ammonium salt and aromatic groups to play a role. The latter are known to occur frequently in proteins as well as in protein – ligand interactions stabilizing or modulating the overall stability or interaction energy.[28] The shielding from the solvent of the ε-NH_3^+ groups of the tail lysines, as observed by NMR spectroscopy, can be explained either by hydrogen bond formation or by interactions between ammonium salt and the aromatic azobenzene moieties. These would compete with the electrostatic repulsion of the charged NH_3^+ groups. Water has the highest dielectric constant of all solvents used, so that repulsive forces are weakest here, leading to the strongest interaction effects. Similarly, hydrophobic interactions are expected to be strongest in aqueous solution. All these interactions may occur at the intra- and intermolecular level. The presence of intermolecular interactions is evidenced by the concentration dependence of the UV absorption (Figure 3), whereas intramolecular interactions are evidenced by the UV absorption spectra at low concentrations in aqueous buffer. In fact, in the photostationary state the vibrational substructure of the spectrum of the *cis* isomer (Figure 3C) supports an immobilization of the AMPB moieties. Although the presence of strong exchange broadening in the NMR spectra would exclude that in aqueous buffer intramolecular interactions are sufficiently strong to stabilize a defined structure, the motional flexibility of the AMPB groups seems to be severely restricted in contrast to the peptidic tail. NMR relaxation estimates in aqueous buffer reveal that the intermolecular interactions are also in an intermediate regime. Estimated diffusion constants exclude the formation of stable oligomers even at the high concentrations used for NMR spectroscopy, although concentration-dependent effects are clearly observed in the UV spectroscopic analysis. In nonaqueous solutions interaction effects were always smaller, leading to a behavior more similar to that of the reference AMPB compound **3**.

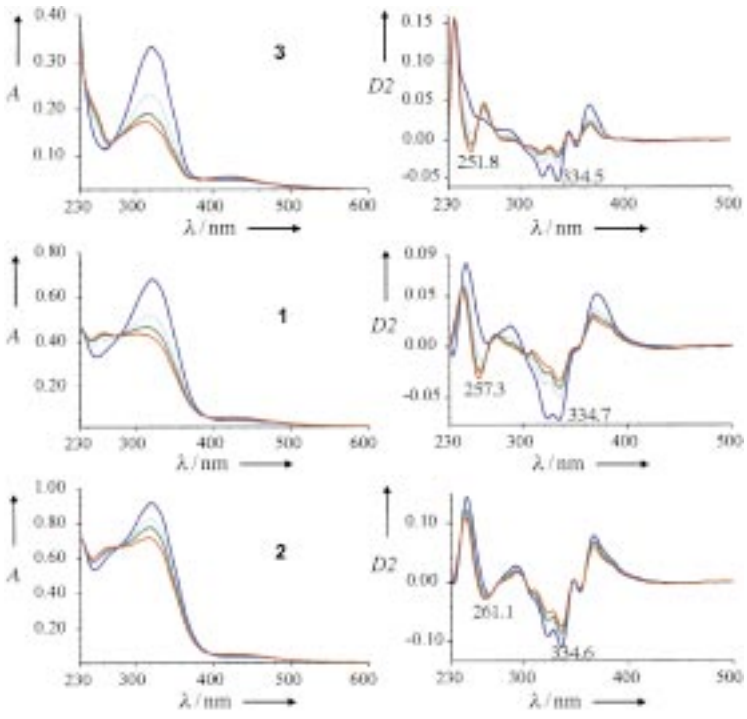


Figure 2. *Absorption spectra (left panels) of the dendrimers **1** (middle) and **2** (bottom), and of the azobenzene derivative H-AMPB-OH (**3**) as reference compound (top) in TFE after thermal relaxation and after irradiation at 360 nm for increasing irradiation times until the photostationary state is reached. Dark blue curves are the initial spectra after thermal relaxation in the darkness and red curves are the spectra of the photostationary states. Within experimental error, the spectra of **1** and **2** were recorded at identical (10^{-6} M) concentrations. The related second derivatives are shown in the right panels.*

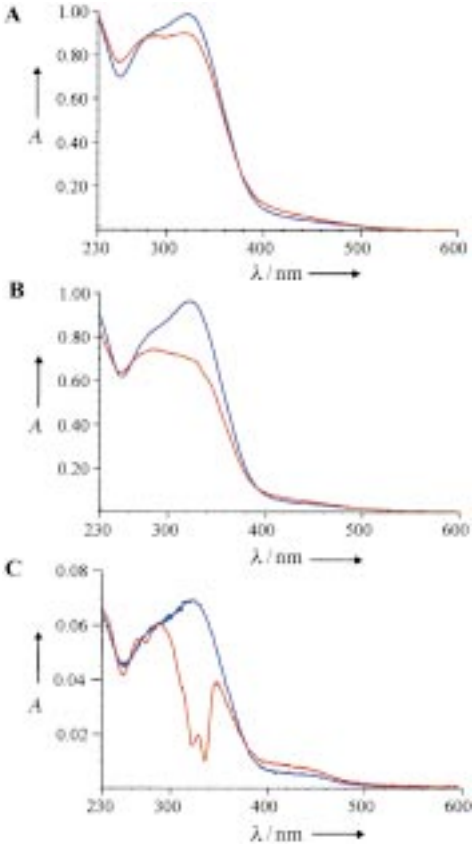


Figure 3. *Absorption spectra of* ***2*** *(in 0.1 M phosphate buffer containing 1 mM EDTA, pH 3.1) in the ground state after thermal relaxation (blue curves) and in the photostationary state after prolonged irradiation at 360 nm (red curves) at concentrations of* 1.23×10^{-5} *M (A),* 1.23×10^{-6} *M (B), and* 1.23×10^{-7} *M (C).*

Since precise quantitation of the *cis*/*trans*-isomer ratios by NMR spectroscopy before and after irradiation was not possible, the extinction coefficients of the *cis*- and *trans* isomers could not be determined separately from the isomeric mixtures. However, a semiquantitative evaluation by NMR spectroscopy indicates, at ca. 1 mM concentration of **1** in TFE, a ratio between the *cis* and *trans* isomers of approximately 1:3 for the ground state and of approximately 1:2 for the photostationary state. At low concentrations, UV spectra indicate a maximum *cis* isomer content of about 80% similar to that observed for other AMPB-containing compounds.[17h]

Photoisomerization of the dendrimers

The *cis*/*trans* photoisomerization of the dendrimers **1** and **2** in organic media and aqueous buffer by irradiation at 360 nm and 450 nm is a fully reversible process with two isosbestic points indicating that the photochemical reaction follows first-order kinetics as observed for the reference AMPB compound **3** (Figure 2). Thermal relaxation of the dendrimers **1** and **2** to the ground state was found to occur at significantly slower rates than for compound **3** as well as for linear and cyclic peptides containing AMPB as backbone unit.[17h] In aqueous media this photochemical reaction was found to be strongly concentration-dependent as shown exemplarily for the dendrimer **2** in Figure 3. After a suitable irradiation period, a photostationary state is reached where the content of *cis*-azo isomer depends upon the concentration of the dendritic peptide. Vibrational sub-bands in UV spectra, kinetically slowed *cis* → *trans* relaxation, and hindered *trans* → *cis* photoisomerization have previously also been observed in model compounds containing two conformationally restricted azobenzene groups.[29] This supports our view that interactions like hydrophobic interactions, π stacking, and interactions between ammonium salt and aromatic groups locally restrict motional freedom of the chromophores, thereby markedly affecting the *trans*/*cis*-photoisomerization process. In general, aggregation phenomena are not rare with dendrimers, and they have recently been observed for generation 5 starburst dendrimers with similar amino surfaces.[30]

Molecular simulations

In an effort to further explain the spectroscopic behavior of the dendrimers, molecular dynamics simulations of dendrimer **1** were carried out in an explicit solvent model. A simulation of 100 ps at 300 K and 200 ps at 1000 K was performed and analyzed for dendrimer **1** with all azobenzene moieties in the *trans* configuration and also for dendrimer **1** with one azobenzene moiety in the *cis* configuration. The only observable feature was the transient formation of hydrophobic clusters consisting mainly of benzene rings from two or more AMPB groups in the 1000-K simulations. From the trajectories it became visible that for the *cis* isomer of AMPB both benzene rings of the AMPB moiety can be involved in the same hydrophobic cluster, whereas for the *trans* isomer this is not possible. This result offers an explanation for the stabilization of the *cis* configuration relative to the *trans* configuration and thus the presence of the *cis* isomer even in the thermodynamically relaxed state.

Conclusions

Azobenzene-functionalized dendritic peptides are conveniently and efficiently synthesized on a solid support and can be obtained in good yields and satisfactory homogeneity under optimized conditions without the need for nontrivial purification steps. The dendritic peptides are photoresponsive systems, with the *cis*/*trans* photoisomerization occurring in a reversible manner in both organic and aqueous media. The NMR and UV spectroscopic analyses clearly revealed that the *cis*/*trans* isomerization is affected by intra- and intermolecular interactions and that this effect, as expected, is more pronounced in the octavalent dendrimer **2**. Nonetheless, at higher dilution the light-induced isomerization occurs even in aqueous media to extents that may be exploited to photomodulate, through the local geometrical changes, molecular recognition processes between ligands grafted to the dendrimer and receptor molecules.

Experimental Section

General: Chlorotrityl-modified resin was purchased from PepChem GmbH (Tübingen, Germany). All reagents and solvents used in the

synthesis were of the highest quality commercially available. Amino acid derivatives were purchased from Alexis (Grünberg, Germany). CE was carried out on Spectra Phoresis 1000 equipment (Tsp, Darmstadt) using an underivatized fused silica capillary column (57 cm × 75 μm; length × ID). MALDI-TOF mass spectra were obtained on a Bruker Reflex III time-of-flight mass spectrometer (Bruker – Franzen, Bremen, Germany) equipped with a SCOUT probe. Desorption – ionization was achieved by a 337 nm nitrogen laser, followed by subsequent gridless delayed extraction. Dihydroxybenzene (DHB) was used as matrix. Amino acid analyses of the acid hydrolysates (6 M HCl, 110 °C, 72 h) were performed on a Biotronic analyzer (LC 6001).

Syntheses of the dendrimers 1 and 2

The dendritic peptides were synthesized manually by applying Fmoc/Boc chemistry on chlorotrityl resin loaded with Fmoc-glycine (loading: 0.5 mmol g^{-1}) according to the protocol outlined in Table 1. The syntheses were carried out on a 1.0 mmol scale using the following amino acid derivatives: Fmoc-Cys(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, and Fmoc-AMPB-OH (**4**).[15] For all coupling, Fmoc cleavage and washing steps freshly distilled *N*-methyl-2-pyrrolidone (NMP) was used as solvent. Coupling efficiency was monitored by the Kaiser test and/or by HPLC analysis upon treatment with trifluoroacetic acid (TFA) of an analytical sample of the resin-bound peptide. For coupling of Fmoc-Cys(Trt)-OH the amount of the auxiliary base diisopropylethylamine (DIEA) was reduced from 8 to 5 equiv to suppress racemization.[15] Longer reaction times (4 h) and excess of reagents were required for complete deprotection of the four- and eightfold Fmoc-protected dendritic peptides. In the final coupling steps, only 3 and 2 equiv of HBTU/HOBt/Fmoc-Lys(Fmoc)-OH and HBTU/HOBt/Fmoc-AMPB-OH, respectively, were used (HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt = *N*-hydroxybenzotriazole), but the reaction times were extended to 2 h. At this stage of the synthesis the resin was further washed with CH_2Cl_2/trifluoroethanol (TFE) (1:1) between each coupling step. Sometimes a third coupling step was required for quantitative aminoacylation of the dendritic peptide. Final deprotection and resin cleavage was performed with TFA/CH_2Cl_2/H_2O (18:1:1) and 1,2-ethanedithiol as scavenger at room temperature for 12 h. The resin was removed by filtration and the filtrates were evaporated to dryness. The residues were dissolved in small amounts of TFE and the crude products were precipitated with diethyl ether. The precipitates were collected by filtration or centrifugation and dried. The excess Fmoc-AMPB-OH (**4**) was partially recovered from the coupling solution containing HBTU/HOBt/DIEA by addition of water. A precipitate was formed which was isolated by filtration and extensively washed with MeOH.

(H-AMPB)$_4$-(α,ε-Lys)$_2$-(α,ε-Lys)-(Lys)$_4$-Cys-Gly-OH (1): Yield: 44%; CE (+15 kV, 10 mM phosphate buffer containing 0.10 mM EDTA, pH 3.1, detection at 210 nm and at 330 nm, $T = 60$ °C): $t_m = 5.6$ min; MS (MALDI-TOF): *m/z*: calcd 2024 for $C_{103}H_{138}N_{28}O_{14}S_1$, found 2024 [$M+H^+$]; amino acid analysis: Gly 1.00 (1), Cys 0.80 (1), Lys 6.65 (7); peptide content: 64%. ^1H and ^{13}C NMR chemical shifts of **1** and **2** are virtually identical and are, therefore, reported only for compound **2**.

(H-AMPB)$_8$-(α,ε-Lys)$_4$-(α,ε-Lys)$_2$-(α,ε-Lys)-(Lys)$_4$-Cys-Gly-OH (2): Yield: 46%; CE [+15 kV, 30 mM phosphate buffer containing 0.30 mM EDTA, pH 3.1, detection at 210 nm and at 330 nm, $T =$ 60 °C): $t_m = 9.7$ min; MS (MALDI-TOF): *m/z*: calcd. 3486 for $C_{183}H_{230}N_{48}O_{22}S_1$, found 3487 [$M+H^+$]; amino acid analysis: Gly 1.00 (1), Cys 0.64 (1), Lys 8.43 (11); peptide content: 79%; ^1H NMR (500 MHz, H_2O, 27 °C, 3-trimethylsilylpropionic acid (TSP)): $\delta = 4.25$ (br, 11 H, Lys-Hα), 1.76 (br, 22 H, Lys-Hβ), 1.38 (br, 22 H, Lys-Hγ), 1.65 (br, 6 H, Lys-Hδ(free)), 1.4 – 1.6 (br, 16 H, Lys-Hδ(azo)), 2.96 (br, 6 H, Lys-Hε(free)), 2.9 – 3.3 (br, 16 H, Lys-Hε(azo)), 7.47 (br, 6 H, Lys-NεH2(free)), 8.0 – 8.4 (br, 16 H, Lys-NεH(azo)), 8.55 (br, 1 H, Cys-NH), 4.62 (br, 1 H, Cys-Hα), 2.85, 2.55 (br, 2 H, Cys-Hβ), 8.42 (br, 1 H, Cys′-NH), 4.69 (br, 1 H, Cys′-Hα), 2.97, 3.20 (br, 2 H, Cys′-Hβ), 8.30 (br, 1 H, Gly-NH), 3.95 (br, 2 H, Gly-Hα); ^{13}C NMR (126 MHz, H_2O, 27 °C, TSP): $\delta = 56.4$ (br, 11 C, Lys-Cα), 33.3 (br, 11 C, Lys-Cβ), 24.8 (br, 11 C, Lys-Cγ), 29.0 (br, 3 C, Lys-Cδ(free)), 30.5 (br, 8 C, Lys-Cδ(azo)), 42.1 (br, 6 C, Lys-Cε(free)), 42 (br, 8 C, Lys-Cε(azo)), 52.4 (br, 1 C, Cys-Cα), 64 (br, 1 C, Cys-Cβ), 55 (br, 1 C, Cys′-Cα), 44.6 (br, 1 C, Gly-Cα).

UV spectroscopy: UV spectra were recorded on a Lambda 19 spectrometer (Perkin – Elmer). A xenon lamp 450 XBO (Osram, München) was used for irradiation at 360 nm (filter from Itos, Mainz) with a light intensity of 0.5 mW cm^{-2}. For the UV measurements the following solvents and buffers were used: DMSO, TFE, ethylene glycol, and 0.1 M sodium phosphate containing 1 mM EDTA (pH 3.1). All the buffers were degassed and saturated with argon, and the corresponding UV samples were prepared under an argon atmosphere. All the measurements were carried out at 25 °C and at a peptide concentration of 10^{-6} M unless stated otherwise.

NMR spectroscopy: NMR spectroscopic measurements were performed on Bruker AMX400 and DRX500 spectrometers using ca. 3 mM samples of **1** and **2** in H_2O/D_2O (9:1, v/v). For signal assignments 2D $^1H - ^1H$ TOCSY and ROESY as well as 2D $^1H - ^{13}C$ HSQC spectra were acquired at 280 K, 300 K, and 320 K with a proton sweep width of 10 ppm centered on $\delta = 4.73$ (water) and a carbon sweep width of 150 ppm centered on $\delta = 75$.[16] Additionally, as the observed line width was much larger than expected, 1D – ^1H inversion recovery experiments and 1D – ^1H spin lock experiments with variable spin lock times at 300 K for both samples and at 320 K only for compound **2** were performed. Approximate relaxation times were extracted assuming single exponential behavior, although overlap leading to multiexponential decay curves was observed for some signals. However, the relaxation measurements were used only in a semi-quantitative manner to distinguish between heterogeneous and homogeneous line broadening. For reference the same 1D $^1H - T_1$ and $^1H - T_{1\rho}$ relaxation measurements were performed with solutions of a 1.1 kDa cyclic peptide and a 13 kDa protein, both with approximately spherical structures and with no tendency to aggregate (data not shown).

Molecular dynamics simulations: Molecular dynamics simulations were carried out with the DISCOVER module of the INSIGHT II software (MSI, San Diego) for dendrimer **1** in a water box of size 5 nm × 4 nm × 4 nm containing approximately 2500 water molecules. In all simulations the CVFF force field was used and the step size was 1 fs. Snapshots were saved each picosecond. Two temperatures were used: At 300 K, a 100-ps trajectory was performed after minimization and equilibration for 10 ps at 10 K; at 1000 K, 200 ps were simulated after 50 ps of equilibration at 1000 K. The calculations were performed for dendrimer **1** with all azobenzene moieties in the *trans* configuration and for dendrimer **1** with one azobenzene group in the *cis* configuration.

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