compared with that from DMNB-caged cGMP in HEK293 cells expressing cAMP-gated (CNCα3) and cGMP-gated (CNCα2) channels. The cells were loaded with 500 μM of the equatorial isomer of **9a** or 200 μM (maximal solubility) of the axial isomer of DMNB-caged cGMP using a patch pipette and illuminated with UV rays. The nucleotide concentrations released by the flash were estimated using the known dose–response relation for activation of the channels by cAMP and cGMP.[11] The experiment demonstrates that **9a** liberates the cyclic nucleotide about one order of magnitude more efficiently than the DMNB-caged derivative and that only **9a** is able rapidly to produce high concentrations of cyclic nucleotide inside cells. In Figure 3 the photorelease at $\lambda =$ 405 nm of 8-Br-cGMP from the axial isomer of DEACM-caged8-Br-cGMP[12] is compared with that from the axial isomer of DMNB-caged 8-Br-cGMP[11] in HEK293 cells expressing CNCα2 channels. While every light flash released approximately 0.5 μM of cGMP from the DEACM-caged compound, no measurable release could be detected with the DMNB-caged compound.

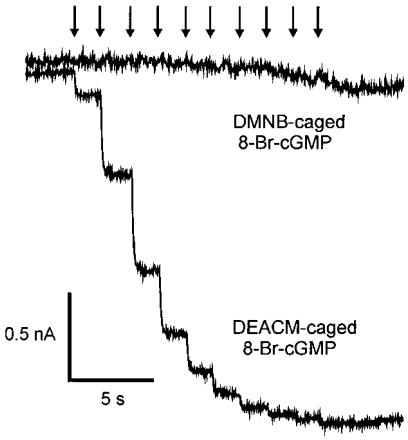

Figure 3. Activation of cGMP-gated cation channels by 8-Br-cGMP photolytically liberated from caged derivatives through a series of 5-ms UV light flashes (405 ± 20 nm, ↓). (Whole-cell current recordings from two HEK293 cells at −50 mV with 20 μM of the respective caged compound.)

The properties of the caged compounds inside cells correspond well with their performance in solution and confirm that the described coumarinylmethyl-caged compounds are indeed highly efficient phototriggers for cAMP and cGMP. The advantage of the carboxymethoxy-substituted coumarinylmethyl esters **8a**,**b** and **9a**,**b** arises from their superior solubility allowing the instantaneous liberation of high concentrations of the cyclic nucleotides. With respect to the DEACM-caged compounds **5a** and **5b**, the cyclic nucleotides are efficiently released under nondamaging light conditions. In summary, these novel caged compounds show great potential for the study of spatial- and time-dependent aspects of cellular signaling at a quantitative level.

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Analysis of the Topology of the Chromophore Binding Pocket of Phytochromes by Variation of the Chromophore Substitution Pattern

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Dedicated to Professor Henning Hopf on the occasion of his 60th birthday

We have recently reported[1] on the use of synthetic linear tetrapyrroles with modified substitution patterns on ring D in recombinant phytochromes (65 kDa oat phyA),[2] in order to spectroscopically explore the interactions between the binding pocket of the protein and the chromophores phytochromobilin (**1**; Scheme 1), isophytochromobilin (**4**), and phycocyanobilin (**3a**). The first unnatural chromophore investigated (**4**)[1] has been found, upon assembly with the recombinant apoprotein (apophyA65),[3] to undergo a $P_r \rightarrow P_{fr}$ photoisomerization which is characteristic of phytochromes such as phyA65-**1**. The absorption maximum for the P_{fr} form of phyA65-**4** is hypsochromically shifted by about 14 nm. This shift occurs selectively in the P_{fr} form and is even larger (by

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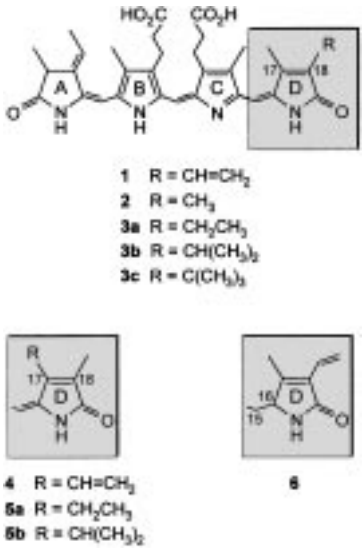

Scheme 1. Structural formulas of naturally occurring (**1**, **3a**, **6**) and synthetic (**2**, **3b**, **c**, **4**, **5a**, **b**) open-chain tetrapyrroles which have been assembled with recombinant oat apophytochrome (phyA65): phytochromobilin (**1**), phycocyanobilin (**3a**), phycoerythrobilin (**6**), isophytochromobilin (**4**), isophycocyanobilin (**5a**), and the 17,18-dimethyl **2**, 17-methyl-18-isopropyl **3b**, 17-methyl-18-*tert*-butyl **3c**, and 17-isopropyl-18-methyl **5b** derivatives of **3a**.

3 nm) than in phyA65-**3a** (Table 1). In chromoprotein phyA65-**3a** the absorptions of both P_r and P_{fr} are blue shifted with respect to phyA65-**1**, presumably due to the saturation of the vinyl group at C18. The question remained, to what extent are these differences in the absorption maxima to be attributed to steric or electronic effects, or a subtle interplay of both.

Table 1. Assembly kinetics[a] of recombinant oat apophytochrome, apophyA65, with linear tetrapyrroles and phyA65 absorption maxima λ_{max} of the assembled holoproteins.

Tetrapyrrole component	τ_1[a] [min]	τ_2[a] [min]	$\lambda_{max}(P_r)$ [nm]	$\lambda_{max}(P_{fr})$ [nm]
phytochromobilin (**1**)	0.38	2.93	665[b]	728[b]
iso-phytochromobilin (**4**)	3.02	19.6[b]	663[b]	714[b]
17,18-dimethyl homologue **2**	1.62	10.8	655	714
phycocyanobilin (**3a**)	0.88	10.6	653[b]	717[b]
17-methyl-18-isopropyl homologue **3b**	6.14	51.6	650	718
17-methyl-18-*tert*-butyl homologue **3c**	13.2	58.6	651	719
17-ethyl-18-methyl homologue **5a**	7.64	43.8	658	707
17-isopropyl-18-methyl homologue **5b**	10.5	156	550	705
phycoerythrobilin (**6**)	–[c]	–[c]	576[d]	n.a.

[a] The measured absorption increases (at λ_{max}) were fitted to biexponential kinetics. $\tau = 1/k$. [b] From ref. [1]. [c] For the assembly kinetics of **6** with apophyA124, see ref. [6]. [d] From ref. [6]. n.a. = not available.

We have addressed this question by assembling apophyA65 with the linear tetrapyrroles **2**, **3b**, **c**, and **5a**, **b**, in which the steric requirements of alkyl substitution on ring D (at C17 and C18) systematically vary. The P_r/P_{fr} absorption spectra, the assembly kinetics, and the kinetics of the thermal P_{fr} back-isomerization were all investigated as a function of the C17 and C18 substituents, in order to probe the steric constraints associated with the conformationally induced protein–chromophore interactions that are brought about by the C15–C16 double bond photoisomerization. The results complement the earlier study employing the tetrapyrroles **1**, **3a**, and **4**,[1] and supplement the data for the two homologous series **2**/**3a**/**3b**/**3c** and **2**/**5a**/**5b**.

All newly assembled chromoproteins, phyA65-**2**, -**3b**, **c** and -**5a**, **b**, underwent the phytochrome photochromic cycle (Table 1 and Figure 1). The chromoproteins carrying the

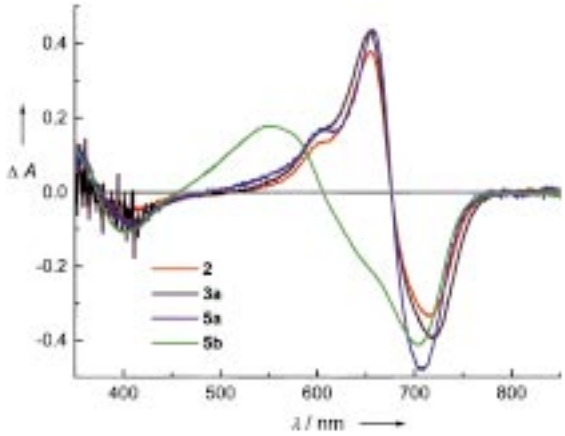

Figure 1. Difference spectra $A(P_r) - A(P_{fr})$ of recombinant oat phyA65 phytochromes, assembled with phycocyanobilin (**3a**) and its 17,18-dimethyl **2**, 17-ethyl-18-methyl **5a**, and 17-isopropyl-18-methyl **5b** derivatives.

C18-modified tetrapyrroles, PhyA65-**2** and -**3a**–**c**, all exhibit very similar P_r- and P_{fr}-like absorption maxima. This is quite in contrast to the absorptions of the 17-ethyl and 17-isopropyl homologues, phyA65-**5a** and -**5b**, respectively. While the P_r-like form of phyA65-**5a** still absorbs within the range of phyA65-**2** and **3b**, **c**, all other absorptions are appreciably shifted to shorter wavelengths: the shifts of the P_{fr}-like forms of phyA65-**5a** and -**5b** are around 10 nm and, in particular, the maximum of the P_r-like form of phyA65-**5b** is shifted by a dramatic 100 nm.

The biexponential kinetics of the chromophore incorporation reflect a similarly selective influence by substituent size and position (Table 1). Interestingly, the naturally occurring 17-methyl-18-ethyl derivative **3a** is, after **1**, assembled fastest. While the 17,18-dimethyl compound **2** is assembled only slightly slower than **3a** with respect to τ_1, in both homologous series, **2**/**3b**/**3c** and **2**/**5a**/**5b**, the increasing steric crowding clearly slows down the assembly rates τ_1 and τ_2, with the isopropyl substituent at C17 yielding the strongest effect on τ_2. In every case, the P_r form is recovered upon $P_r \rightarrow P_{fr}$ photorecycling. Although some of the spectral shifts among the homologous chromoproteins are very large, these are all best reconciled by the assumption that they are due to differences in chromophore–protein interactions rather than to regiochemical differences in the chromophore binding to the protein.

The thermal isomerization of the photochemically generated P_{fr} form back to the P_r form[4] also correlates with the size of the substituents on ring D of the chromophore. The thermally most stable chromoprotein is the one with the bulkiest substituent, *tert*-butyl (phyA65-**3c**), while the C17-modified chromophores phyA65-**5a** and -**5b** markedly accelerate the thermal isomerization to the P_r form (Figure 2).

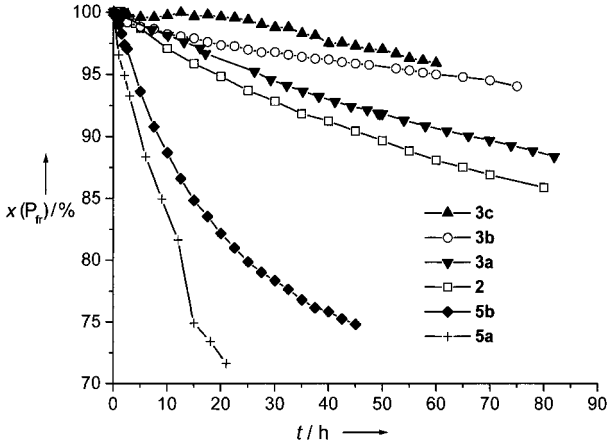

Figure 2. Kinetics of the thermal $P_{fr} \rightarrow P_r$ isomerization of the recombinant phyA65-**2**, -**3a**–**c**, and -**5a**,**b** phytochromes at ambient temperature. The P_{fr} samples were prepared by saturating irradiation of the corresponding P_r forms.

The steric interactions between ring D of the chromophore and the amino acids of the protein pocket significantly influence the rates of the chromophore binding to the apoprotein and the thermal reversal of P_{fr} to P_r. Relative to phytochromes with a native chromophore, which possess a C_1 substituent at C17 and a C_2 substituent at C18 (such as phyA65-**1** and -**3a**), the changes in reactivity depend on the position of the substituent(s) modified, C17 or C18, with C17 being more sensitive. Thus, the assembly of the 17-ethyl and 17-isopropyl homologues **5a** and **5b** is clearly slower than that of the respective C18 regioisomeric analogues **3a** and **3b**. The extreme blue shift of the P_r form of phyA65-**5b** ($\lambda_{max} =$ 550 nm) may reflect a particularly strong steric constraint by the protein, which forces ring D of the chromophore out of plane with regard to rings A–C and thus interrupts conjugation of the π system.[5] A blue shift in absorption which is almost as large ($\lambda_{max} = 576$ nm) has also been observed for phyA124-**6**,[6] brought about upon interruption of conjugation due to the 15,16-saturated bridge between rings C–D of the phycoerythrobilin chromophore **6**. Whereas **6** cannot form a P_{fr} because of the saturated C15–C16 bond, we find for P_{fr} of **5b** only a moderately blue-shifted maximum (see Table 1). As another reason for the blue shift, a weakening of the protonation state, for example, by an increase of the distance between proton donor and chromophore, appears possible. In this context a decrease in absorption to accompany the blue shift would be expected upon deprotonation of the chromophore, as in the transition from the 17-ethyl (**5a**) to the 17-isopropyl isomer (**5b**).

Experimental Section

Synthesis of the linear tetrapyrroles **2**, **3b**, **c**, and **5a**, **b**:

The total syntheses of the new tetrapyrroles followed the convergent strategy of A-B+C-D $\rightarrow$A-B-C-D previously described.[7] The various ring D substitution patterns were made accessible by adaptation of a previously developed pyrrole synthesis[8] based on addition of an aldehyde to a nitroalkane, acetylation, and coupling with *tert*-butyl isocyanoacetate.

The oxidation of 2,5-unsubstituted pyrroles to pyrrol-2-ones[9] was not regiospecific. Both regioisomers resulting from the unsymmetrically substituted pyrroles could be used separately after chromatographic separation for the synthesis of the two homologous series of tetrapyrroles.

While all the 4-methylpyrrol-2-ones coupled with the ring C component in good yields, the 4-isopropyl homologue was obtained in very low yield, probably due to steric crowding. Attempts to achieve coupling with the 4-*tert*-butyl derivative failed entirely.

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[4] For the thermally initiated $P_{fr} \rightarrow P_r$ isomerization, see: A. Remberg, A. Ruddat, S. E. Braslavsky, W. Gärtner, K. Schaffner, *Biochemistry* **1998**, *37*, 9983–9990.

[5] The conformation of the free chromophore **5b** in homogeneous solution (methanol/0.5% triethylamine) is not significantly affected by steric crowding due to the C17 isopropyl group, as judged by the absorption maxima of 360, 587, and 631 nm, which are comparable to those of **2**, **3a**–**c**, and **5b**.

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Isolated Hexagonal Channels Built up by Three-Connected Ge^- Ions in LiGe at High Pressure**

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In the homologous series of LiE compounds (E = Group 14 elements Si–Pb), LiSi[1, 2] and LiGe[3, 4] are Zintl phases at normal pressure (NP),[5, 6] but LiSn[7] and LiPb[8, 9] belong to the class of intermetallic phases. Owing to a charge transfer Li^+Si^- and Li^+Ge^-, the Si^- and the Ge^- ions in these Zintl phases are isovalence-electronic to the elements of Group 15, and build up the three-dimensional three-coordinate net of the MgGa-type structure[10] with three homonuclear bonds. However, in the intermetallic compounds LiSn[7] and LiPb,[8, 9]

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