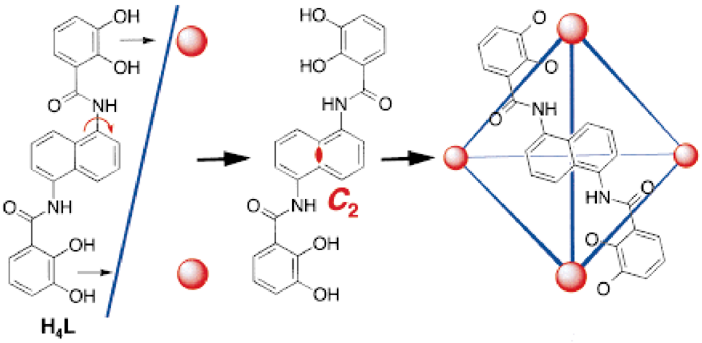
The Self-Assembly of a Predesigned Tetrahedral M_4L_6 Supramolecular Cluster**

Dana L. Caulder, Ryan E. Powers, Tatjana N. Parac, and Kenneth N. Raymond*

We have described the design principles of high symmetry natural clusters, such as ferritin and the human rhinovirus, based on incommensurate interactions.[1a,b] We have extended this interpretation and shown that it provides a systematic method for the design and synthesis of analogues of these natural clusters using metal–ligand interactions.[1, 2] Here we present an example of the rational design of a M_4L_6 tetrahedral cluster that exhibits dynamic exchange of guests within the supramolecular cluster cavity.[3]

If the high symmetry clusters are viewed as truncated polyhedra, the interactions of the subunits must conform to the angles between the planes of the polyhedra, if a high symmetry cluster is to form. Synthesis of an M_4L_6 cluster with tetrahedral symmetry requires that the incommensurate two- and threefold axes be rigidly fixed by the design.[4] A twofold symmetric bis-bidentate ligand interacting with an octahedral metal center (which generates the threefold axis) can lead to the formation of an M_2L_3 helix (point group D_3) if the angle between the threefold and twofold axes can approach 90°.[1c–e, 5] Entropic considerations dictate that if the lower stoichiometry M_2L_3 complex can form, it will.[1c] Therefore, in order to favor the M_4L_6 tetrahedron the geometry of the designed ligand must be correct and inflexible.

The selectivity in the ligand H_4L is achieved by a naphthalene spacer, which causes the two catechol binding units to be offset from one another when the ligand is in the conformation required for helicate formation (Scheme 1).[1e]



Scheme 1. Helicate formation is disfavored by the use of a naphthalene spacer in the ligand H_4L.

Thus, the formation of a helicate becomes impossible, and the formation of the M_4L_6 tetrahedron (Figure 1) enabled. Computer modeling[6] of the M_4L_6 cluster indicated that it would have T symmetry (all metal centers with the same chirality, all Δ or all Λ) and that there would be a substantial cavity inside the cluster.

In this cluster design the planes of the ligands are coincident with the twofold planes of the truncated tetrahedron. As such, the elevation angle of the threefold axis (through the metal center) with the extended twofold ligand plane corresponds to the “approach angle” (Figure 1).[1f] This angle is calculated to be 35.3° and corresponds to the approach angle of a perfect octahedral metal complex. While catechol complexes of Ga^{III}, Fe^{III}, and Al^{III} are typically distorted towards trigonal-prismatic geometries[7] corresponding to an approach angle of 23°, molecular mechanics calculations[6] indicated that slight out-of-plane twists in the ligand would compensate for this angle.

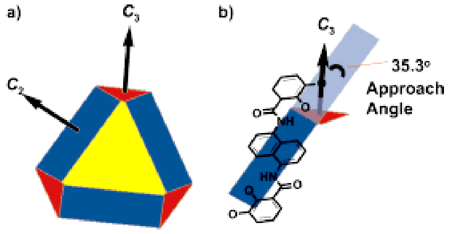


Figure 1. a) An M_4L_6 cluster can be thought of as a truncated tetrahedron with the planes of the polyhedron perpendicular to the symmetry axes. b) If a ligand is designed to lie on the twofold (blue) plane, then the elevation angle of the C_3 axis with the extended twofold plane represents the “approach angle”.

The ligand H_4L was synthesized in 75% overall yield according to established methods.[1c–e] Overnight stirring of a methanol solution containing four equivalents of $[Ga(acac)_3]$, six equivalents of H_4L, twelve equivalents of KOH, and twelve equivalents of Et_4NCl produced a yellow microcrystalline precipitate of $K_5[Et_4N]_7[Ga_4L_6]\cdot 8H_2O$. Although the complex was isolated in 78% yield, the reaction was quantitative as observed by ^1H NMR spectroscopy. The ^1H NMR spectrum (300 MHz, D_2O) of the dissolved precipitate showed all of the signals and shifts expected for the formation of the desired cluster: signals for the backbone and the catechol units, a low field shifted NH signal indicative of complex formation, and signals for the counterions. Remarkably, however, the signals of the seven Et_4N^+ ions are split into two sets in a 6:1 ratio. The larger set ($\delta = 2.42$ (q), 0.68 (t)) is shifted slightly upfield from free Et_4N^+ in solution ($\delta = 3.26$ (q), 1.27 (t)), while the smaller set is dramatically shifted upfield ($\delta = -0.68$ (m), -1.58 (t)). The extreme upfield shift of the Et_4N^+ protons in the complex can be interpreted as a direct indication of the encapsulation of one Et_4N^+ by the tetrahedral cluster $[Ga_4L_6]^{12-}$.[3b,c, 8] In turn, this encapsulation may be taken as an indication of the successful formation of the cluster.

In addition, the methylene resonance of the encapsulated Et_4N^+ is not a quartet but rather a complex multiplet, indicating that the two protons are inequivalent. This evidence is consistent with an encapsulated Et_4N^+ that is

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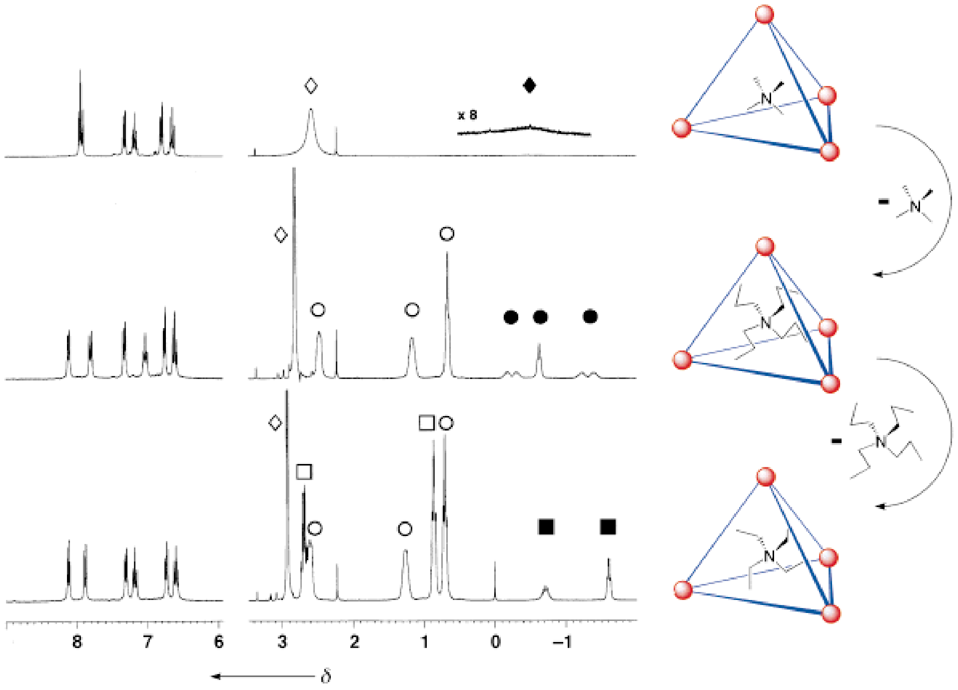

Figure 2. ^{1}H NMR spectra (300 MHz, D_2O) showing stepwise exchange of counterions in the tetrahedral cluster cavity. Exterior (△) and interior (◆) Me_4N^+. Exterior (○) and interior (○) Pr_4N^+. Exterior (□) and interior (■) Et_4N^+.

sensitive to a chiral environment[3a] and supports the characterization of the cluster as a racemic mixture of T symmetric homochiral complexes. Variable temperature ^{1}H NMR (300 MHz, D_2O) experiments indicate that the interior and exterior Et_4N^+ resonances do not coalesce below 100°C.

The tetrahedral complex can be synthesized with the smaller Me_4N^+ counterion in an analogous fashion. The ^{1}H NMR spectrum (500 MHz, D_2O) of the species $K_6[Me_4N]_6[Ga_4L_6]$ shows evidence of fast exchange between Me_4N^+ ions in two different chemical environments (Figure 2 top). The major peak is a broad singlet ($\nu_{1/2} = 40$ Hz) located at $\delta = 2.6$ while the minor peak is shifted far upfield to $\delta = -0.5$ ($\nu_{1/2} = 215$ Hz).

Remarkably, the cluster shows a marked preference for the inclusion of Et_4N^+ over the smaller Me_4N^+ and the larger Pr_4N^+ (Figure 2). If Pr_4NCl is added to an NMR tube containing a solution of $K_6[Me_4N]_6[Ga_4L_6]$ (Figure 2, top), the Pr_4N^+ quickly (<1 min) and quantitatively is incorporated into the cluster cavity, displacing the Me_4N^+ (Figure 2, middle). Previously two broad peaks, the Me_4N^+ resonance is subsequently one sharp peak at $\delta = 2.82$ and one equivalent of Pr_4N^+ is found incorporated into the cavity. Again, the resonances of the methylene protons on the encapsulated Pr_4N^+ show substantial diastereotopic splittings (48 and 42 Hz). When Et_4NCl is added to this same NMR tube (Figure 2, bottom), the Et_4N^+ in turn displaces the Pr_4N^+ rapidly (<1 min) and quantitatively! When given the choice of either Me_4N^+, Et_4N^+, or Pr_4N^+, the tetrahedral cluster host preferentially chooses to encapsulate the Et_4N^+. No mixtures are observed by ^{1}H NMR spectroscopy.

The equilibrium constants (K_{eq}) for the inclusion of Et_4N^+ and Pr_4N^+ were determined by ^{1}H NMR experiments relative to K^+; their respective values are $196 \times 10^2\,M^{-1}$ and $1.11 \times 10^2\,M^{-1}$.[9] The nearly 200-fold difference in the equilibrium constants explains the observation that Et_4N^+ quantitatively replaces encapsulated Pr_4N^+. Since the inner and outer Me_4N^+ ions are in rapid exchange, the equilibrium constant for the inclusion of Me_4N^+ could not be determined by the same method. However, K_{eq} can be estimated to be very much smaller than $1.11 \times 10^2\,M^{-1}$, given that Pr_4N^+ quantitatively replaces the encapsulated Me_4N^+. Detailed investigations of the factors affecting encapsulation and the dynamics of guest exchange are in progress.

The ultimate proof for the formation of the homochiral target cluster and the presence of a host–guest complex is provided by single crystal X-ray diffraction. Suitable crystals were grown of the similar Fe^{III} complex, $K_5[Et_4N]_7[Fe_4L_6]$.[10] The $[Fe_4L_6]^{12-}$ cluster lies on a crystallographic threefold axis with nearly T molecular symmetry. Hence the crystal is a racemic mixture of tetrahedra that have homochiral (all Δ or all Λ) iron centers. One of the Et_4N^+ counterions is located inside of the cluster cavity (Figure 3). The cluster is a tightly closed box, with no aperture in the surface, as shown by a space filling model (Figure 4).

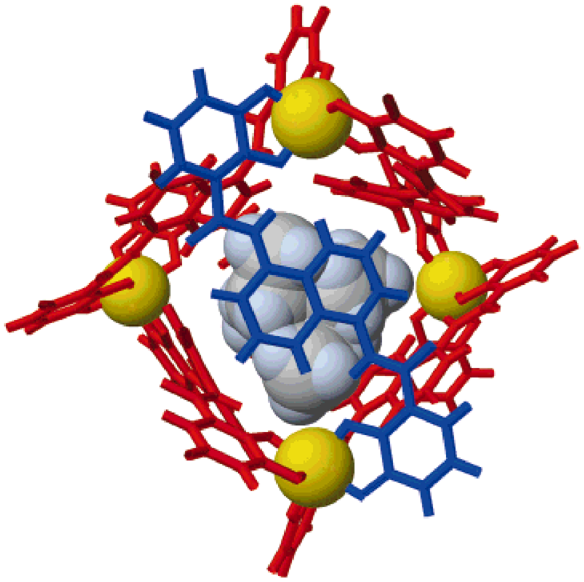

Figure 3. Schematic representation of the structure of $K_5[Et_4N]_7[Fe_4L_6]$ in the crystal showing the encapsulated Et_4N^+. The other cations have been removed and the front ligand is highlighted in blue for clarity.

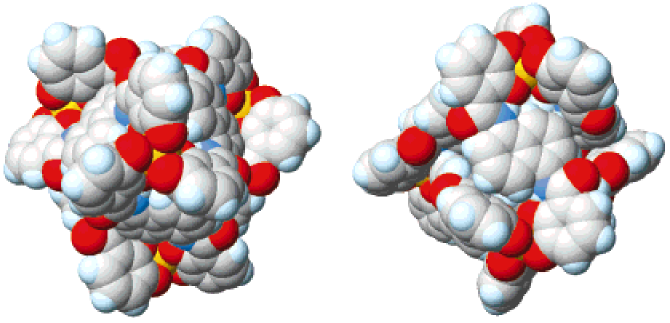

Figure 4. Space-filling views of the crystal structure of $[Fe_4L_6]^{12-}$ looking down the crystallographic threefold and pseudo twofold axis of the tetrahedron (right).

The Fe–Fe distances in the cluster are on average 12.8 Å, bringing the cluster just into the nanometer region. The naphthalene rings are twisted along the arene–N bond, so that they bend into the cavity. We interpret this as due to van der Waals interaction between the cluster and the encapsulated Et_4N^+.

In summary, we have demonstrated that a strict geometric analysis can be employed in the rational design and synthesis of a homochiral M_4L_6 tetrahedral cluster. We have also shown for the first time that assemblies of this nature can be used to selectively encapsulate guests with remarkable discrimination. The supermolecular design rationale presented here is conceived to be general and applicable to other clusters of different size, symmetry, and stoichiometry.

Experimental Section

All NMR spectra were measured with a Bruker 300, 400, or 500 MHz spectrometer. Chemical shifts are reported as δ downfield from sodium [2,2,3,3-D_4]3-trimethylsilylpropionate in D_2O. Both the methyl protected ligand **1** and the ligand H_4L were fully characterized by ^1H and ^{13}C NMR spectroscopy, FAB-MS, and elemental analysis.

1: 2,3-Dimethoxybenzoyl chloride[11] (11.80 g, 58.8 mmol) and 1,5-diaminonaphthalene (4.43 g, 28.0 mmol) were dissolved in CH_2Cl_2 (400 mL). An excess of Et_3N (10 mL) was added and the reaction mixture stirred for one hour. The solution was washed with 1N HCl (200 mL) followed by 1N NaOH (200 mL). The organic layer was dried over $MgSO_4$ and concentrated by rotary evaporation. Diethyl ether was added to precipitate **1** as tan needle crystals, which were collected by filtration and air dried (9.38 g). A second crop of crystals was collected (1.86 g) to give a combined yield of 82.5 %

H_4L: BBr_3 (5 mL, 48.7 mmol) was added by syringe to a solution of **1** (1.48 g, 3.04 mmol) in CH_2Cl_2 (100 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. Volatiles were removed under vacuum, and the remaining orange-yellow residue was stirred in water for 2 h at 100 °C. The colorless precipitate was collected by filtration and dried under vacuum at 70 °C for 6 h (1.16 g, 2.70 mmol, 89 %). ^1H NMR (400 MHz, [D_6]DMSO): $\delta = 11.93$ (s, 2H); 10.91 (s, 2H); 9.52 (s, 2H); 7.92 (d, J = 8.5 Hz, 2H); 7.87 (d, J = 7.3 Hz, 2H); 7.64–7.59 (m, 4H); 7.03 (dd, J = 1.7, 7.9 Hz, 2H); 6.84 (t, J = 7.9 Hz, 2H).

$K_5(Et_4N)_7[Ga_4L_6]$: The ligand H_4L (0.100 g, 0.232 mmol) was suspended under oxygen-free conditions in distilled MeOH (30 mL). A 0.497N solution of KOH in MeOH (935 µL, 0.464 mmol) was added by micropipette. The ligand dissolved after about 5 min with stirring, after which a 0.280M solution of Et_4NCl in MeOH (1.660 mL, 0.465 mmol) was added by micropipette. ($Ga(acac)_3$] (0.056 g, 0.154 mmol) was then added to the solution as a powder. Within seconds the $Ga(acac)_3$ dissolved, and the solution changed from colorless to yellow. While the reaction mixture was stirred at room temperature overnight, the product precipitated as a yellow silky powder. This was isolated by centrifugation and dried under vacuum (0.120 g, 0.029 mmol, 76 %). ^1H NMR (300 MHz, D_2O): $\delta = 13.60$ (s, 12H; NH), 8.15 (d, J = 7.8 Hz, 12H, Ar_nH), 7.91 (d, J = 8.6 Hz, 12H, Ar_nH), 7.35 (dd, J = 1.5 Hz, 8.2 Hz, 12H, Ar_cH), 7.20 (t, J = 8.2 Hz, 12H, Ar_nH), 6.78 (dd, J = 1.5 Hz, 7.3 Hz, 12H, Ar_cH), 6.63 (t, J = 7.9 Hz, 12H, Ar_cH), 2.42 (q, J = 7.2 Hz, 48H, $CH_{2(out)}$), 0.68 (t, J = 7.2 Hz, 72H, $CH_{3(out)}$), −0.68 (m, 8H, $CH_{2(in)}$), −1.58 (t, J = 6.9 Hz, 12H, $CH_{3(in)}$). ^{13}C NMR (125 MHz, D_2O) $\delta =$ (C) 172.4, 161.4, 157.7, 136.9, 117.4; (CH) 129.4, 121.5, 120.3, 118.3, 117.8, 117.7; (CH_2) 54.5, 52.9; (CH_3) 9.1, 7.0; ES-MS (negative-ion detection, H_2O/CH_3OH 10:90 $\blacklozenge \equiv [Ga_4L_6]^{12-}$): *m*/*z*: 859.4 $[\blacklozenge+4Et_4N^++2K^++2H^+]^{4-}$, 868.9 $[\blacklozenge+4Et_4N^++3K^++1H^+]^{4-}$, 882.2 $[\blacklozenge+5Et_4N^++1K^++2H^+]^{4-}$, 891.8 $[\blacklozenge+5Et_4N^++2K^++1H^+]^{4-}$, 914.8 $[\blacklozenge+6Et_4N^++1K^++1H^+]^{4-}$, 924.2 $[\blacklozenge+6Et_4N^++2K^+]^{4-}$, 937.4 $[\blacklozenge+7Et_4N^++1H^+]^{4-}$, 946.9 $[\blacklozenge+7Et_4N^++1K^+]^{4-}$, 1189.7 $[\blacklozenge+5Et_4N^++2K^++2H^+]^{3-}$, 1202.3 $[\blacklozenge+5Et_4N^++3K^++1H^+]^{3-}$, 1219.8 $[\blacklozenge+6Et_4N^++1K^++2H^+]^{3-}$, 1232.7 $[\blacklozenge+6Et_4N^++2K^++1H^+]^{3-}$, 1250.2 $[\blacklozenge+7Et_4N^++2H^+]^{3-}$, 1263.0 $[\blacklozenge+7Et_4N^++1K^++1H^+]^{3-}$, 1275.7 $[\blacklozenge+7Et_4N^++2K^+]^{3-}$, 1293.5 $[\blacklozenge+8Et_4N^++1H^+]^{3-}$, 1306.0 $[\blacklozenge+8Et_4N^++1K^+]^{3-}$; elemental analysis calcd for $K_5Ga_4C_{200}H_{224}N_{19}O_{36} \cdot 8H_2O$: C 58.75, H 5.92, N 6.51; found C 58.43, H 5.65, N 6.19.

$K_6[Me_4N]_6[Ga_4L_6]$: The complex was synthesized in a manner similar to $K_5(Et_4N)_7[Ga_4L_6]$ using Me_4NCl. Within 10 min of stirring at room temperature the product precipitated as a yellow powder (88 %). ^1H NMR (500 MHz, D_2O): $\delta = 7.91$ (d, J = 7.5 Hz, 12H, Ar_nH), 7.88 (d, J = 8.5 Hz, 12H, Ar_nH), 7.29 (d, J = 8.3 Hz, 12H, Ar_cH), 7.14 (t, J = 8.2 Hz, 12H, Ar_nH), 6.77 (d, J = 7.4 Hz, 12H, Ar_cH), 6.61 (t, J = 7.8 Hz, 12H, Ar_cH), 2.59 (br. s, $\nu_{1/2} = 40$ Hz, 32.4H, $CH_{3(out)}$), −0.50 (br. s, $\nu_{1/2} = 215$ Hz, 3.6H, $CH_{3(in)}$); ESMS (anion detection, H_2O/CH_3OH 10:90; $\blacklozenge \equiv [Ga_4L_6]^{12-}$): *m*/*z*: 821.4 $[\blacklozenge+6Me_4N^++2H^+]^{4-}$, 830.7 $[\blacklozenge+6Me_4N^++1K^++1H^+]^{4-}$, 839.7 $[\blacklozenge+6Me_4N^++2K^+]^{4-}$, 1096.2 $[\blacklozenge+3Me_4N^++6K^+]^{3-}$, 1108.4 $[\blacklozenge+4Me_4N^++5K^+]^{3-}$, 1120.4 $[\blacklozenge+5Me_4N^++4K^+]^{3-}$, 1132.5 $[\blacklozenge+7Me_4N^++2K^+]^{3-}$, 1144.5 $[\blacklozenge+7Me_4N^++2K^+]^{3-}$; elemental analysis calcd for $K_6Ga_4C_{168}H_{156}N_{18}O_{36} \cdot 4H_2O$: C 56.23, H 4.61, N 7.03; found: C 56.22, H 4.31, N 7.03.

$K_5(Et_4N)_7[Fe_4L_6]$: The complex was synthesized in a manner similar to $K_5(Et_4N)_7[Ga_4L_6]$ on use of $[Fe(acac)_3]$ (79 %). Crystals suitable for analysis by X-ray diffraction grew at room temperature over two weeks by gas phase diffusion of acetone into a methanol/water solution of the complex. ESMS(anion detection, H_2O/CH_3OH 10:90; $\blacklozenge \equiv [Fe_4L_6]^{12-}$): *m*/*z*: 613.8 $[\blacklozenge+2Et_4N^++1Na^++4H^+]^{5-}$, 641.7 $[\blacklozenge+2Et_4N^++3K^++2Na^+]^{5-}$, 788.4 $[\blacklozenge+2Et_4N^++1K^++3Na^++2H^+]^{4-}$, 1094.5 $[\blacklozenge+2Et_4N^++5K^++2Na^+]^{3-}$, 1123.4 $[\blacklozenge+3Et_4N^++5K^++1H^+]^{3-}$, 1183.8 $[\blacklozenge+5Et_4N^++3K^++1H^+]^{3-}$, 1214.2 $[\blacklozenge+6Et_4N^++2K^++1H^+]^{3-}$, 1244.7 $[\blacklozenge+7Et_4N^++1K^++1H^+]^{3-}$, 1490.9 $[\blacklozenge+1Et_4N^++1K^++1Na^++7H^+]^{2-}$; elemental analysis calcd for $K_5Fe_4C_{200}H_{224}N_{19}O_{36} \cdot 4H_2O$: C 60.65, H 5.90, N 6.72; found: C 60.38, H 5.83, N 6.44.

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[9] The equilibrium constants at 298 K were determined by integrating the ^{1}H NMR spectrum (500 MHz, D_2O) of the mixture containing 5.0 mM $K_{12}[Ga_4L_6]$ and 5.0 mM guest in an NMR tube. The ^{1}H NMR spectra were measured both 30 min and 2 days after the initial mixing. No change was observed in the spectra over time, implying that equilibrium was achieved within 30 min of mixing.

[10] Crystal data for $K_5[Et_4N]_7[Fe_4L_6]\cdot 8H_2O\cdot 3CH_3OH$ were collected with a Siemens SMART diffractometer equipped with a CCD area detector;[12] crystal size $0.25 \times 0.23 \times 0.10$ mm; $T = -120\,^{\circ}C$, $\lambda Mo_{K\alpha} = 0.71073$ Å; point group $I\bar{4}3d$ (No. 220), $a = 43.706(8)$ Å, $V = 83488$ Å^3, $Z = 16$, $\mu = 0.45$ mm^{-1}, $F(000) = 35808$, $\rho_{calcd} = 1.333$ Mg m^{-3}, $2\theta_{max} = 41.67^{\circ}$. Of the 136092 reflections collected 7336 were unique ($R_{int} = 0.214$). The structure was solved by direct methods and was refined on F^2 using SHELXTL.[13] Data were corrected for Lorentz and polarization effects. An empirical absorption correction was applied using XPREP[13] (ellipsoidal model, $R_{int} = 0.036$, $T_{max} = 0.895$, $T_{min} = 0.834$). The iron atoms, oxygen and nitrogen atoms of the ligands, the half-occupancy potassium, and the nitrogen and carbon atoms of the full occupancy Et_4N^+ counterion were refined anisotropically. Hydrogen atoms were included as riding on their respective carbon and nitrogen atoms for all but the disordered counterions and solvent molecules. Not all carbon atoms were found for the disordered Et_4N^+ counterions. The N–C and C–C distances for these disordered ions were set to target values of 1.4 and 1.5 Å. An antibumping restraint was applied to carbon atoms of the disordered Et_4N^+ on the interior of the tetrahedral cluster. Weighting scheme: $1/[\sigma^2F_o^2 + (0.1660p)^2 + 779.981p]$, where $p = ((F_o^2,0)_{max} + 2F_c^2)/3$. Final $R_1 = 0.0978$ for 4672 reflections with $F_o > 4\sigma(F_o)$; 4672 Friedel unique data, 542 parameters, 14 restraints, $3.75^{\circ} < 2\theta < 34.58^{\circ}$); for all 7336 data, $wR_2 = 0.3288$, GOF = 1.205; max/min residual density $+0.62/-0.32$ e Å^{-3}, Flack parameter = 0.03(5).[14] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100947. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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A Novel Chromophore Selectively Modifies the Spectral Properties of One of the Two Stable States of the Plant Photoreceptor Phytochrome**

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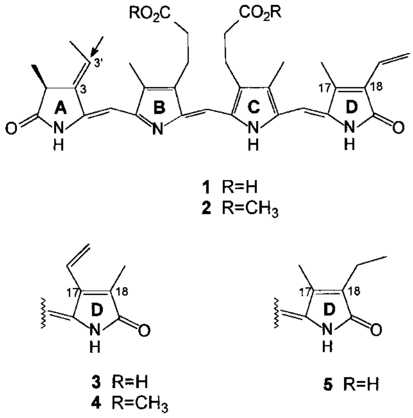
Phytochromes are plant photoreceptors that consist of a protein (125–140 kDa) covalently bound to the open-chain tetrapyrrole chromophore phytochromobilin (**1**, Scheme 1).



Scheme 1. Structural formulas of the native phytochrome chromophore phytochromobilin (**1**), the isomer "iso"-phytochromobilin (**3**, described here for the first time), the corresponding methyl esters **2** and **4**, and phycocyanobilin (**5**). The arrow marks the 3′ position on ring A of **1**, which is covalently attached to the apoprotein through nucleophilic attack by a cystein thiol group. The B/C tautomeric form shown here was selected in analogy to the crystal structure of a 2,3-dihydrobilatriene abc model compound.[20]

Initiated by $Z \rightleftharpoons E$ photoisomerization of a double bond of **1**,[1] the phytochrome undergoes a multistep rearrangement steered by subtle chromophore–protein interactions. This ultimately leads to a biological signal transduction and control of a wide array of physiological processes as a function of wavelength and intensity of the absorbed light.[2] Accordingly, effects on absorption and photochemical properties upon selective modifications of the protein and chromophore components may significantly contribute to the understanding of the light-induced phytochrome transformations.

The synthesis of native and mutated forms of the phytochrome apoprotein (i.e., chromophore-free protein) has been accomplished previously,[3] as has the assembly of the apoproteins with native **1** and with phycocyanobilin (**5**).[1c, 3] However, the amount of **1** isolable from algae[4] has been

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