tution does not retard the rate of Diels-Alder addition, but accelerates the rate of diradical formation,[17] is consistent with available kinetic data.[18]

Our calculations reveal why fluorine substitution has such a profound effect on accelerating the rate of diradical formation. In the allylic 1,4-diradicals, the erstwhile π bond of the dienophile is broken and one new C-C σ bond is formed. Calculations at the (U)MP2/6-31G* level[5a,19] have found that the π bond in TFE is 18.1 kcal/mol weaker than the π bond in ethylene and that this π-bond weakening is largely due to the preference of CF_2 radical centers for pyramidal geometries. Thus, the weaker π bond in TFE is responsible for the bulk of the 25-26 kcal/mol greater relative stability calculated for the fluorinated diradical.[20] The remainder (7-8 kcal/mol) of the difference in relative stabilities must be contributed by the greater strength of the new C-C σ bond formed in the case of the TFE-butadiene addition.

Calculations show that, in general, formation of C-C σ bonds to CF_2 groups is thermodynamically more favorable than formation of such bonds to CH_2 groups. For example, replacement of one hydrogen in tetrafluoroethane by methyl is computed at the MP2/6-31G* level to be 10.0 kcal/mol more favorable than the same methyl-for-hydrogen substitution in ethane. The α fluorines contribute the bulk of this stabilization, since methyl-for-hydrogen substitution is calculated to be 8.0 kcal/mol more stabilizing in difluoroethane than in ethane when the product is 2,2-difluoropropane,[21] but only 0.8 kcal/mol more stabilizing when the product is 1,1-difluoropropane.

The greater thermodynamic stability of 2,2-difluoropropane versus the 1,1-isomer, amounting to 7.2 kcal/mol at the MP2/6-31G* level, reflects the general preference that we and others have found for the attachment of electronegative elements like fluorine[22] and oxygen,[22,23] to the more highly alkylated of two carbon centers.[24] This effect favors the formation of new C-C bonds to fluorinated carbon centers, and it contributes about 25% to the greater relative stability of the 1,4-diradical formed from reaction of butadiene with TFE, than with ethylene.

One might have expected that the weaker π bond being broken and the stronger C-C σ bond being formed would also tend to stabilize the transition state for the Diels-Alder reaction of TFE, relative to that for ethylene. These two stabilizing effects, however, are apparently offset by the destabilization, resulting from the syn pyramidalization of the two CF_2 groups that is required in this transition state. For example, we find at the RMP2/6-31G* level that syn pyramidalization of TFE to $\phi = 25.9°$ (the degree of pyramidalization found in the TFE + butadiene Diels-Alder transition state at the RHF/3-21G level) costs 4.8 kcal/mol more than does the same distortion of ethylene. By contrast, anti pyramidalization to $\phi = 25.9°$ is 0.9 kcal/mol less costly for TFE than for ethylene.

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Supplementary Material Available: Complete listing of the bond lengths and bond angles in the optimized geometries for the transition state for the Diels-Alder reaction of tetrafluoroethylene with 1,3-butadiene and for the two allylic 1,4-diradicals (9 pages). Ordering information is given on any current masthead page.

(17) Assurance that this finding would survive MCSCF optimization of the actual transition-state geometries for diradical formation is provided by the large size of the difference between the relative energies of the diradicals and its invariance to changing levels of theory.

(18) Bartlett and Schueller have estimated that while at 175 °C 1,1-dichloro-2,2-difluoroethylene undergoes Diels-Alder cycloaddition with butadiene 50 times faster than does ethylene, this halogenated olefin undergoes [2 + 2]-cycloaddition with butadiene 10^7 times faster than does ethylene.[1]

(19) For example, we find that in the diradical formed from TFE and butadiene, planarizing the CF_2 radical center raises the energy by 11.6 kcal/mol at the UMP2/6-31G* level. This value is close to the 10.5 kcal/mol, calculated at the same level of theory, to be required to planarize the 1,1-difluoroethyl radical. Chen, Y.; Rauk, A.; Tsuchuikow-Roux, E. *J. Chem. Phys.* **1990**, *93*, 1187.

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Cyclobis(paraquat-*p*-phenylene): A Novel Synthetic Receptor for Amino Acids with Electron-Rich Aromatic Moieties

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In this communication we report the remarkable ability that cyclobis(paraquat-*p*-phenylene) 1^{4+} exhibits to form *very stable inclusion charge-transfer complexes* with amino acids possessing electron-rich aromatic subunits. Several groups have described the formation of charge-transfer complexes between electron acceptors and amino acids with donor character, such as tryptophan, tyrosine, or phenylalanine.[1] Typically, these interactions are rather weak, with binding constants in the range 1-10 M^{-1}. In contrast, the title compound shows a much stronger affinity for the same amino acids in aqueous media, with binding constants about 2 orders of magnitude higher than those exhibited by simple acceptors. The origin of the added binding strength resides in the conformational rigidity of 1^{4+}, which has a box-like structure with a very well defined cavity lined by the two paraquat acceptor subunits.[2] This cavity is thus ideally suited to include donor aromatic rings.

Two of us (M.V.R. and J.F.S.) have recently reported the synthesis of 1^{4+} (see structure in Scheme I) as well as its binding properties in acetonitrile with the three isomeric dimethoxybenzenes[2a] and catechol dimethyl ethers.[2b] We have also reported[3] the synthesis, solid-state structure, and electrochemical properties of a catenane based on 1^{4+}. Although many cyclophane hosts have been described in the literature, 1^{4+} offers several novel design features such as (i) a rigid cavity in which the distance between the two paraquat subunits is ideal for inclusion of an aromatic ring, (ii) a substantial electron-acceptor character provided by the two paraquat subunits, (iii) redox properties (also provided by the paraquat groups) that may afford a mechanism to control

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Scheme I. Binding of Tyrosine to Receptor 1^{4+}

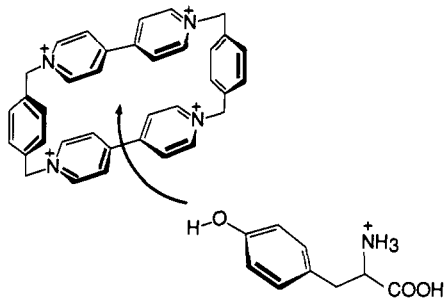

Table I. Binding Constants (M^{-1}) at 25 °C between Receptor 1^{4+} and Several Amino Acids[a] in Two Different Aqueous Media

		K ($\Delta\epsilon$)[b]	
amino acid	λ,[c] nm	phos[d]	HCl[e]
tryptophan	404	1106 (818)	953 (845)
	430	1022 (819)	808 (654)
	450	1010 (715)	768 (576)
	470	1007 (592)	771 (469)
	500	867 (438)	792 (305)
	av	1002	818
tyrosine	394	584 (304)	249 (327)
	404	573 (299)	
	414	569 (283)	244 (287)
	424	564 (262)	
	434	571 (227)	244 (209)
	454	771 (146)	257 (124)
	av	605	248
phenylalanine	374	106 (421)	nb[f]
	394	37 (229)	nb[f]
	414	12 (319)	nb[f]
	434	7 (527)	nb[f]
	454	4 (921)	nb[f]
	av	33	nb[f]
histidine	av (374–454)	nb[f]	nb[f]
alanine	av (374–454)	nb[f]	nb[f]

[a] Throughout this work, D,L (racemic) amino acids were used. [b] $\Delta\epsilon$ values (M^{-1} cm^{-1}) are given in parentheses. [c] Binding constant and $\Delta\epsilon$ values were determined at each of the listed wavelengths. [d] In 50 mM phosphate buffer (pH = 7). [e] In 1.0 M HCl. [f] No binding detected.

at will the binding properties of the host, and (iv) its tetracationic nature that provides an easy way to control solubility through the selection of appropriate counterions. This latter point is exemplified by the high solubilities of the tetrachloride and the tetra(hexafluorophosphate) salts in water and acetonitrile, respectively.

Addition of D,L-tryptophan to an aqueous solution (50 mM phosphate buffer, pH = 7.0) containing the tetrachloride salt of 1^{4+} results in the immediate development of a visible charge-transfer band (λ_{max} = 404 nm) that can be used to determine the equilibrium constant for the association of the 1^{4+}–tryptophan complex. The binding constants were obtained by computer fitting of the experimental data points to the following equation:[4]

$$\Delta A/b = S_t K \Delta\epsilon [L]/(1 + K[L]) \quad (1)$$

where ΔA is the absorbance of the charge transfer complex measured at an amino acid concentration S_t and a host concentration [L], b is the optical path length, $\Delta\epsilon$ is the molar absorptivity of the charge-transfer complex, and K is the equilibrium constant for the formation of the complex. Our data are consistent with 1:1 complex stoichiometry in all cases; no evidence for other stoichiometries was obtained. The binding constant values obtained for several amino acids in both acidic and neutral aqueous media are given in Table I. Tryptophan and tyrosine are much more strongly bound by the title compound than is phenylalanine as expected from the relative electron donor abilities of the aromatic subunits of these amino acids. However, the binding

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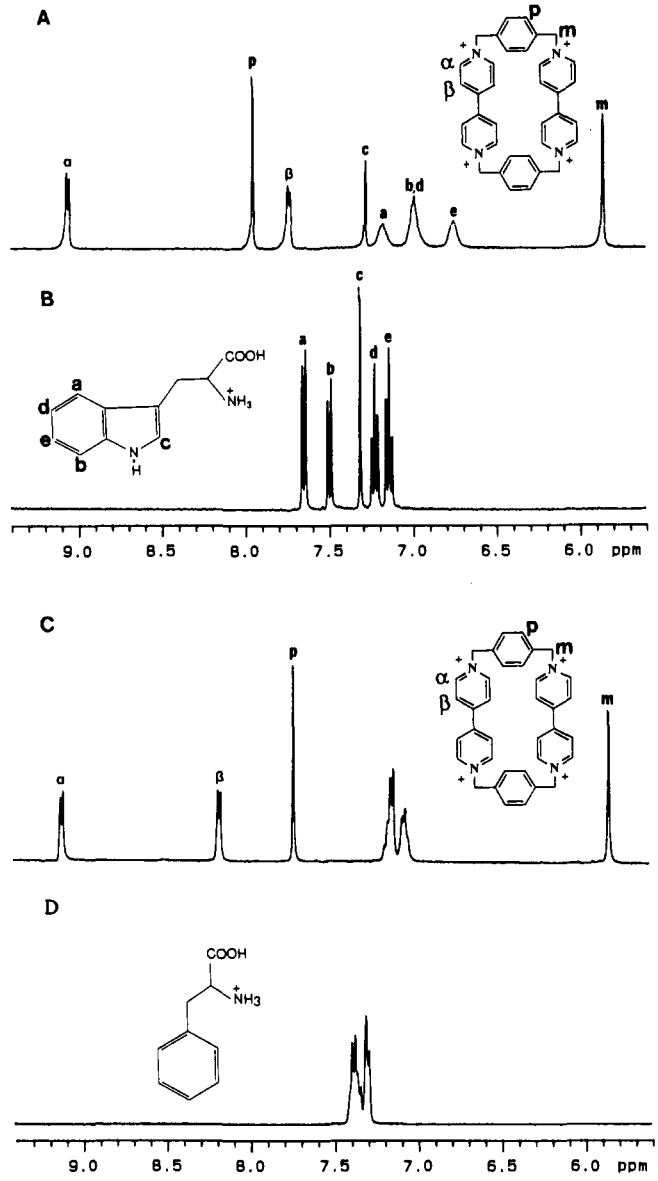

Figure 1. Low-field region of the 400-MHz ^1H NMR spectra of (A) 10 mM tryptophan + 1.0 mM $1{\cdot}Cl_4$, (B) 10 mM tryptophan, (C) 10 mM phenylalanine + 1.5 mM $1{\cdot}Cl_4$, and (D) 10 mM phenylalanine. The solvent system in all samples is 1.0 M DCl/D_2O.

constants determined for these three amino acids with 1^{4+} are approximately 2 orders of magnitude higher than the values reported with simple acceptors, such as methylviologen or 1-methylnicotinamide.[1] That the binding site is the electron-rich aromatic ring in tryptophan, tyrosine, and phenylalanine is supported by the lack of interaction with alanine and histidine. On account of the positively charged nature of all the amino acids in 1 M HCl, binding constants in neutral solution for the aromatic amino acids are higher than the corresponding values in acidic medium.

NMR spectroscopy proved very useful in defining in more detail the binding interactions. The resonances of the aromatic protons of tryptophan, tyrosine, and phenylalanine in 1 M DCl/D_2O were found to shift upfield, broaden, and undergo a remarkable decrease in intensity in the presence of small amounts (0–0.2 equiv) of $1{\cdot}Cl_4$ (see Figure 1). Conversely, the resonances of the β and phenylene protons[5] of 1^{4+} were shifted (but not broadened or diminished in intensity) by the addition of these three amino acids. On the other hand, the large magnitude of the shift of the aromatic resonances

(5) Peak assignments were made according to the following: Calderbank, A.; Charlton, D. F.; Farrington, J. A.; James, R. *J. Chem. Soc., Perkin Trans. 1* **1972**, *4*, 138.

of the amino acid compared to the shifts observed for the protons on 1^{4+} and the minimal shifts of the nonaromatic protons of the amino acids strongly suggest the formation of inclusion complexes, in which the aromatic group of the amino acid is engulfed inside the paraquat-lined cavity of the cyclophane host as illustrated in Scheme I for tyrosine. The quick broadening of the aromatic resonances of the amino acids can also be understood as a result of the strong charge-transfer interactions in these complexes.

Therefore, 1^{4+} constitutes the first example of a new class of receptors, based on charge-transfer interactions between aromatic rings, capable of binding amino acids possessing electron-rich aromatic moieties. One can envision 1^{4+} as a building block for highly selective amino acid receptors. We are currently investigating the feasibility of using 1^{4+} in the topographical analysis of the solution-phase conformations of proteins. Because of the solubility of its tetrachloride in water, 1^{4+} is expected to act as a molecular probe to detect and, perhaps, quantify water-exposed tryptophan and tyrosine residues.

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Aromatic Hole Superexchange through Position 82 of Cytochrome *c* Is Not Required for Intracomplex Electron Transfer to Zinc Cytochrome *c* Peroxidase

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Nature has placed an evolutionarily conserved phenylalanine at position 82 of Cc,[1] and its possible role in electron transfer has long been suspected. We previously reported[2] results obtained by using a suite of position 82 mutants of yeast iso-1 Cc that indicated that the rate constant, k_b, for the thermal $Fe^{2+}P \rightarrow (ZnP)^+$ ET reaction within [ZnCcP,Cc] complexes is large when residue 82 is aromatic and small when it is aliphatic, although it was left undetermined whether the difference reflected conformational effects[3,4] or the abolition of hole superexchange pathways.[5] Measurements with a new apparatus now show that superexchange through position 82 does *not* significantly enhance this reaction. For every Cc variant, the charge-separated intermediate, $[(ZnP)^+CcP,Fe^{2+}Cc]$ (I), converts back to the ground state, $[(ZnP)CcP,Fe^{3+}Cc]$ (A), with multiphasic kinetics[6] that include components with *both* large and small values of k_b, *independent* of whether position 82 of yeast Cc has an aromatic or aliphatic residue.

Luminescence decay and transient absorption measurements[7] on the triplet excited state, $[^3(ZnP)CcP,Fe^{3+}Cc]$ (A*), of complexes prepared with position-82 mutants of yeast iso-1 Cc, where a Cys 102 → Thr modification has been introduced for stability,[8] show that in the presence of excess $Fe^{3+}Cc$ the triplet state decays exponentially, rate constant k_p. In agreement with the initial study of position-82 mutants,[2] intracomplex quenching of the $^3(ZnP)CcP$ by the ferriheme of $Fe^{3+}Cc$ contributes to the triplet-state decay with a quenching rate constant[9a] that varies with cytochrome, from ~170 to ~30 s^{-1} (Table I) under the conditions employed here.

The time evolution of I was monitored for each of the [ZnCcP,Cc] complexes by following the absorbance change at the λ = 549 nm $^3(ZnP)/ZnP$ isosbestic. In our initial studies, for Cc having an aliphatic residue at position 82 we observed I to appear with rate constant k_p and decay more slowly, with an ET rate constant $k_b < k_p$, whereas for variants with an aromatic residue, I appeared rapidly, with $k_b > k_p$, and decayed with k_p. However, the small absorbances associated with the transient were then[2] at the limits of instrumental detection. Moreover, much of the data was collected at 0 °C because the initial suite of mutants[2a] did not contain the stabilizing Thr 102 modification,[8a] and this further diminishes the signals. Extensive measurements at 20 °C with greatly improved S/N[7] (Figure 1) now show that all the Cc variants display multiphasic kinetics for I, as reported for the complexes with *Candida krusei* and horse Cc.[6] Thus, the rapid rise of the intermediate is confirmed for those Cc with an aromatic residue 82 (e.g., Phe; Figure 1, upper left) but an additional slow decay has been detected (Figure 1, upper right), whereas the slow decay for the others is confirmed (e.g., Leu; Figure 1, middle right) but a rapid rise now has been found (Figure 1, middle left).

Excellent self-consistent fits to the observed kinetics of I are obtained with the triphasic function employed earlier,[6] eq 1. Here

$$\Delta A(t) = \beta \sum f_i \frac{(e^{-k_p t} - e^{-k_i t})}{(k_i - k_p)} + \Delta A_j e^{-k_p t} \qquad (1)$$

i = 1-3; k_i is the I → A rate constant (k_b) for phase i, and f_i is the weight of phase i;[9b] the term proportional to ΔA_j accommodates departures from a triplet, ground-state isosbestic. The wavelength dependence of the transient signal confirms that it

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(1) Abbreviations: ZnCcP, zinc-substituted cytochrome *c* peroxidase; Cc, cytochrome *c*; ET, electron transfer; WT, wild type.

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(9) (a) The quenching rate constant is defined as $k_q = k_p - k_d$ where k_d is the decay rate constant for the $[^3ZnCcP,Fe^{2+}Cc]$ complex. As noted elsewhere (ref 6), in general the quenching has contributions both from electron transfer (rate constant k_t) and from energy transfer, with the proportions differing with the cytochrome. (b) The prefactor has the form $\beta = k_t \Delta\epsilon(I-A)A^*(0)$, where the first factor is the $A^* \rightarrow I$ ET rate constant, the second is the difference in extinction coefficients, $\epsilon(I) - \epsilon(A)$, and the third is the concentration of A* immediately after photolysis. The actual fits used f_2 and f_3 as fitting parameters and obtained f_1 from the relation $\sum f_i = 1$. Determination of the values for the k_i, f_2, and f_3 involved jointly fitting data obtained on short and long time scales, with k_1 being determined by the former, k_2 and k_3, by the latter (Figure 1); k_p could be determined independently from the decay of A*. A distribution of slowly decaying phases could also fit the data; we have provisionally chosen to use the triphasic scheme for simplicity and clarity.

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