Scaiano.[21] The high exo selectivity observed in the photocycloaddition of benzaldehyde to 1,2-dimethylcyclobutene (**9**) could be due to fast retrocleavage from the energetically disfavored singlet 1,4-biradical stage. In this case, the endo diastereomer should less likely be formed because of the high strain of the bicyclo[2.2.0]hexane skeleton.

The concept of electronic control of stereoselectivity described here can be useful to explain a number of unusual results in photocycloaddition reactions. Further work for synthetic applications is in progress.

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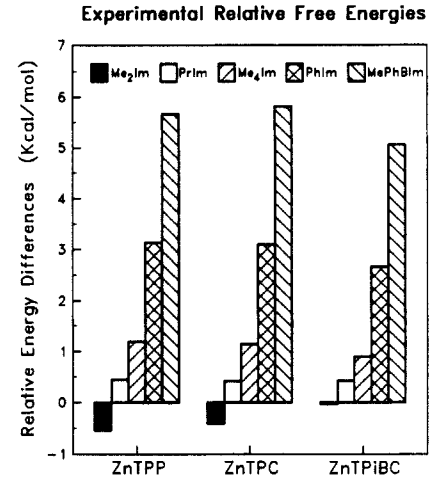

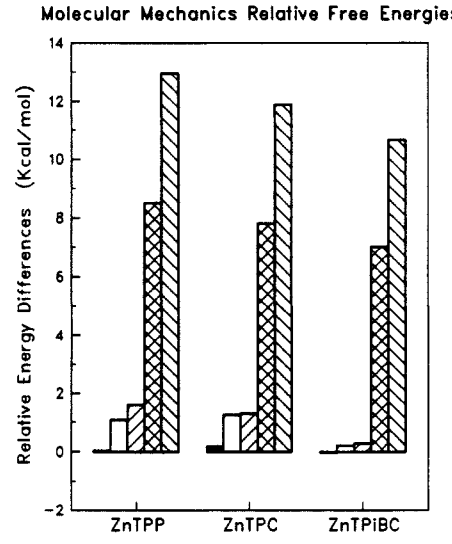

Figure 1. Experimental and molecular mechanics free energies of ligand binding, relative to the binding of 1MeIm.

Probing Macrocycle Flexibility: Ligand Binding to Zinc and Nickel Tetraphenylhydroporphyrins

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Metallohydroporphyrins have been identified as being essential in a variety of biological systems including nitrite and sulfite reductases[1,2] and *S*-methyl coenzyme M reductase.[3] Of special note is the highly reduced nickel-containing macrocyclic tetrapyrrole F_{430}, found in the latter enzyme of methanogenic bacteria.[3,4] The relative rigidity of the macrocycle is expected to be important in metallohydroporphyrin enzymes for metal ions that undergo changes in either spin state or oxidation state during the course of catalytic activity. Such reactions can induce metal ion size changes of up to 0.2 Å (in the case of nickel).[5] The reduction of the macrocycle is generally thought to be responsible for an enhanced reactivity in these systems.[6-9] It has been argued that ring reduction gives the macrocycle greater flexibility: the expected reduction in aromaticity (based on decreased ring-current effects in the NMR spectra)[10-12] and observed S_4 ruffling both in solids[10,13,14] and in solution[15-17] serve as the basis of these arguments. From ligand binding experiments, however, we find no evidence for such increased flexibility.

To probe the role of porphyrin ring reduction in metal reactivity, we have measured the equilibrium binding constants of a series of sterically hindered bases with both zinc[18] and nickel[16,17] tetraphenylhydroporphyrins. The use of zinc allows examination of a well-defined equilibrium between four- and five-coordination without added complications of spin- or oxidation-state changes. The series of sterically hindered imidazoles allows us to probe the flexibility of the macrocycle with minimal electronic changes. If the more reduced hydroporphyrins had greater flexibility, then the steric hindrance of the incoming ligand would have less effect on the equilibrium constant for the metallohydroporphyrins than for the fully unsaturated metalloporphyrin. In contrast, the use of nickel allows examination of a biologically relevant system with a four- to six-coordination equilibrium. Titrations with the more

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Table I. Experimental Ligand Binding Equilibrium Constants for Zn Macrocycles

$$Zn(TPX) + L \overset{K_{eq}}{\rightleftharpoons} Zn(TPX)(L)$$

	K_{eq} at 25.0 °C, M^{-1}					
macrocycle	1 MeIm	Me_2Im	PrIm	Me_4Im	PhIm	MePhBIm
ZnTPP	4.6×10^4	1.13×10^5	2.1×10^4	6.2×10^3	2.3×10^2	3.3×10^0
ZnTPC	5.2×10^4	1.04×10^5	2.6×10^4	7.6×10^3	2.8×10^2	2.9×10^0
ZnTPiBC	5.3×10^4	5.6×10^4	2.6×10^4	1.18×10^4	6.0×10^2	1.06×10^1

Table II. Experimental Ligand Binding Equilibrium Constants for Ni Macrocycles

$$Ni(TPX) + 2L \overset{K_{eq}}{\rightleftharpoons} Ni(TPX)(L)_2$$

	K_{eq} at 15.0 °C, M^{-2}	
macrocycle	pyrrolidine	2-methylpyrrolidine
NiTPP	2.3×10^1	2.7×10^{-1}
NiTPC	4.0×10^1	5.5×10^{-1}
NiTPiBC	1.9×10^1	3.2×10^{-1}

strongly basic pyrrolidine and 2-methylpyrrolidine ligands for the nickel macrocycles provide a similar steric probe (the hindered imidazoles bind too weakly to nickel to be studied). Finally, the quantitative comparison of the experimental data with molecular mechanics calculations provides an interesting application of this important computational technique.

The equilibrium constants for the binding of six imidazoles with ZnTPP, ZnTPC, and ZnTPiBC,[19] and two pyrrolidines with NiTPP, NiTPC, and NiTPiBC, have been determined spectrophotometrically in toluene.[20-22] These values appear in Tables I and II. As stated before, if sterically hindered imidazoles were bound more strongly by the metallohydroporphyrins, it would indicate greater macrocycle flexibility. This is not the case: For both the zinc and nickel macrocycles, and both five- and six-coordinate products, only small differences in K_{eq} are measured as a function of the macrocycle.

Molecular mechanics[23] calculations on the zinc system were undertaken to understand in more detail the role that steric hindrance plays in the binding of these ligands. These calculations were made with the Biograf software package (BioDesign, Inc.; Pasadena, CA) using the MMP2 force field[24,25] and literature parameters for the metal ion.[26] Minor modifications to the force field were also made so as to give the most reasonable and accurate geometric results.[27,28] Binding energetics were determined computationally by describing the change in steric energy of binding as the energy of the energy-minimized metallo(hydro)porphyrin–ligand complex minus the sum of the minimized energies of the isolated metallo(hydro)porphyrin and isolated ligand. The energy changes (relative to 1-methylimidazole binding) appear in Table III and Figure 1b. There are some innate limitations in comparing these calculations to experimental data: the inability to take differences in ligand basicity into account, the effects of entropic changes on ligand binding, and the influence of differential solvation. The series of macrocycles and hindered ligands were carefully chosen, however, to minimize these effects.

Table III. Relative Ligand Binding Free Energies from Experimental and from Molecular Mechanics Calculations

		relative free energy of binding,[a] kcal/mol				
macrocycle		Me_2Im	PrIm	Me_4Im	PhIm	MePhBIm
ZnTPP	$\Delta\Delta G_{exp}$	−0.5	0.4	1.2	3.1	5.7
	$\Delta\Delta E_{mm}$	0.0	1.1	1.6	8.5	13.0
ZnTPC	$\Delta\Delta G_{exp}$	−0.4	0.4	1.1	3.1	5.8
	$\Delta\Delta E_{mm}$	0.2	1.3	1.3	7.8	11.9
ZnTPiBC	$\Delta\Delta G_{exp}$	0.0	0.4	0.9	2.7	5.1
	$\Delta\Delta E_{mm}$	0.0	0.2	0.3	7.0	10.7

[a] $\Delta\Delta G_{exp}$ is the difference in the experimental free energy of ligand binding of the substituted imidazole relative to 1MeIm at 25.0 °C. $\Delta\Delta E_{mm}$ is the difference in the calculated molecular mechanics energies of the energy-minimized metallomacrocycle–imidazole complex minus the sum of the energies of the separately minimized metallomacrocycle and substituted imidazole, relative to 1MeIm.

Some systematic errors do appear in the force field employed for the molecular mechanics calculations. Most notably, there is an overestimation of the energetics of binding of the sterically hindered imidazoles. The increase in macrocyclic flexibility upon pyrrole ring reduction is also overemphasized. Nonetheless, the molecular mechanics results qualitatively agree with the experimental observation that hydrogenation of the macrocycle does not greatly increase binding of sterically hindered ligands.

The lack of a substantial binding enhancement in either the reduced zinc or nickel macrocycles with sterically hindered ligands suggests that ring reduction, loss of aromaticity, or ruffling of the macrocycle does not imply increased flexibility. Ring reduction does lead to distortions from planarity, but does not cause an increased ability to bind sterically hindered axial ligands. Hydroporphyrins containing a small metal ion can therefore be considered *ruffled but rigid*.

Acknowledgment. The support of the National Institutes of Health and the use of the Biograf molecular modeling software system through an academic research grant from Biodesign, Inc.,

(19) Abbreviations: TPP, 5,10,15,20-tetraphenylporphinate(2−); TPC, 2,3-dihydro-5,10,15,20-tetraphenylporphinate(2−); TPiBC, 2,3,7,8-tetrahydro-5,10,15,20-tetraphenylporphinate(2−); TPX, a general tetraphenyl(hydro)porphyrin, i.e., TPP, TPC, or TPiBC; 1MeIm, 1-methylimidazole; Me_2Im, 1,2-dimethylimidazole; PrIm, 2-isopropylimidazole; Me_4Im, 1,2,4,5-tetramethylimidazole; PhIm, 2-phenylimidazole; MePhBIm, 1-methyl-2-phenylbenzimidazole.

(20) Typical ligand binding titrations were done in toluene under Ar with minimal exposure to light. Metallo(hydro)porphyrin concentrations were $\approx 10^{-5}$ M, and ligand concentrations were varied over as wide a range as possible, with data collection at more than eight different concentrations. Toluene was distilled from Na, and ligands were multiply recrystallized or distilled from BaO or KOH. Data analysis has been described in detail elsewhere.[21-22] Good isosbestic behavior was observed, and equilibrium constants were determined for at least four wavelengths. Estimated errors in K_{eq} varied from ligand to ligand: for sterically less hindered ligands, errors were typically 5–10%; for more sterically hindered ligands, errors were typically less than 20%.

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Absolute Configuration of (−)-β-*trans*-Bergamotene

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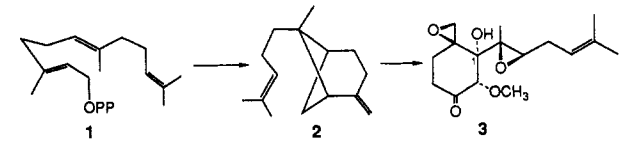
We recently reported that a cell-free preparation from the fungus *Pseudeurotium ovalis* can catalyze the conversion of farnesyl pyrophosphate (FPP, **1**) to the bicyclic sesquiterpene hydrocarbon β-*trans*-bergamotene (**2**) and further that the cyclization proceeded with net retention of configuration at C-1 of FPP.[1] The latter conclusion was based upon the reasonable assumption that (-)-β-bergamotene, which we have isolated from mycelial extracts of *P. ovalis*,[2] has the (1*S*,5*S*,7*R*)-configuration illustrated, taking into account the demonstrated conversion of β-bergamotene to ovalicin (**3**),[4] a metabolite of known absolute configuration,[5] and assuming that the introduction of oxygen at C-1 of ovalicin has proceeded with retention of configuration.[6] We now report results that establish the absolute configuration of (−)-β-bergamotene based on a novel combination of enzymatic and NMR spectroscopic techniques.

The approach we chose was to prepare samples of chirally deuterated FPP, of known absolute configuration, which would be labeled at a site, C-4, that would be unaffected by the enzymatic cyclization. Incubation of the deuterated FPP with bergamotene synthase and analysis of the resulting bergamotene by ^{2}H NMR would establish the relative configuration of deuterium label in the product, leading to the unambiguous assignment of the absolute configuration of **2**. A combination of ^{1}H–^{1}H COSY and ^{1}H{^{13}C} heteronuclear shift correlation was used to identify the signals corresponding to the relevant protons attached to C-3 of bergamotene. Irradiation of the H-14 methyl protons then gave rise to a 0.6% NOE enhancement of the signal at δ 2.24, which was therefore assigned to H-3$_{exo}$. In confirmation of this assignment, irradiation at δ 1.43, previously assigned to H-6$_{exo}$,[1] resulted in a 2.6% enhancement of the H-3$_{endo}$ signal at 2.55.

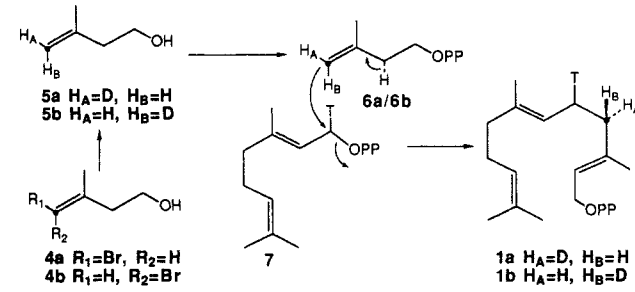
To prepare the requisite labeled samples of (4*S*)- and (4*R*)-(4-^{2}H)FPP, (4*E*)- and (4*Z*)-4-bromoisopentenol[7a] (**4a** and **4b**) were each metalated by an adaptation of the method of Ogura[8] (*s*-BuLi, Et_2O, 1.5 h, −90 °C). Quenching of the individual lithio anions with CF_3CO_2D gave (4*E*)- and (4*Z*)-[4-^{2}H]isopentenol (**5a** and **5b**), which were each converted to the corresponding isopentenyl pyrophosphate (IPP) esters **6a** and **6b** by tosylation (TsCl, Py, 1 h, 25 °C) and displacement with tris(tetra-*n*-butylammonium)pyrophosphate (CH_3CN, 12 h, 25 °C).[9] Prenyl transferase[10] mediated coupling of **6a** and **6b**, containing [4-^{14}C]-IPP as internal standard, with [1-^{3}H]geranyl pyrophosphate (GPP, **7**) gave (4*S*)- and (4*R*)-[4-^{2}H,5-^{3}H,4-^{14}C]FPP (**1a** and **1b**).[7,11]

Incubation of 1.05 μmol of (4*S*)-[4-^{2}H]FPP (**1a**) with 250 mL of a cell-free preparation from *P. ovalis* containing bergamotene synthase[1] for 4 h at 30 °C gave 599 nmol of β-bergamotene (**2a**) which was analyzed by 61.4 MHz ^{2}H NMR spectroscopy after being mixed with 5 mg of synthetic (±)-bergamotene[14] and purified by SiO_2 column chromatography. The ^{2}H NMR spectrum of **2a** displayed a single peak at δ 2.53 corresponding to deuterium in the H-3$_{endo}$ (H-3*si*) position. Similarly, incubation of 2.25 μmol of (4*R*)-[4-^{2}H]FPP (**1b**) with bergamotene synthase yielded 1.2 μmol of β-bergamotene (**2b**), which gave rise to a ^{2}H NMR signal at δ 2.24 corresponding to deuterium in the complementary H-3$_{exo}$ (H-3*re*) position. Since the configuration at the deuterated carbon

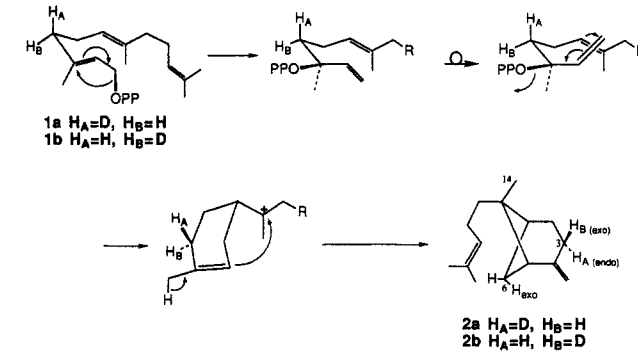
Scheme I



Scheme II



Scheme III



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