wrapping with a right-handed twist around a short α-helix—the same folding topology found in the complex of FKBP-12 with FK506[12] and in uncomplexed FKBP-12.[13,14] The root-mean-square (rms) deviations of α-carbons, backbone atoms, and all protein atoms between FKBP-12 complexed with rapamycin and FKBP-12 complexed with FK506 are 0.67, 0.67, and 1.51 Å, respectively. Only one region, involving residues 31–34 in the loop between strands 4 and 5 of the β-sheet structure, adopts a different main chain conformation. These residues are not involved in protein–ligand interactions, but may be important recognition features of the complex.

Rapamycin binds in a cavity between the β-sheet and α-helix with the pipecolinyl ring deeply buried in the protein (Figure 1a). The protein–ligand interface involves atoms from the pyranose ring through the C28 hydroxyl, with the remainder, including the C17–C22 triene, exposed. The C1 ester, the pipecolinyl ring, the C8 and C9 carbonyls, and the pyranose ring adopt a conformation that is superimposable with the same groups in the FKBP-12/FK506 complex.[12] Three hydrogen bonds between this region and FKBP-12 (Ile-56 NH to C1 carbonyl, Tyr-82 hydroxyl to C8 carbonyl, and Asp-37 carboxylate to C10 hydroxyl) and a C9 carbonyl binding pocket involving C–H···O interactions with ε-hydrogens from Tyr-26, Phe-36, and Phe-99 are also identical with those found in the complex with FK506, thus confirming the identical binding roles of the common structural elements[15] in the two immunosuppressant ligands.

Two additional hydrogen bonds are involved in rapamycin binding to FKBP-12 (Figure 1b). The first is from Glu-54 main chain carbonyl to C28 hydroxyl, which along with the Ile-56 NH to C1 carbonyl–hydrogen bond may mimic the interaction of the dipeptide portion of a natural substrate with FKBP-12. It has been noted that the pyranose–pipecolinyl region also mimics a dipeptide,[16] making rapamycin, like FK506, a possible example of an extended peptide mimic. This hydrogen bond is analogous to the one from Glu-54 main chain carbonyl to C24 hydroxyl found in the FKBP-12/FK506 complex.[12] The second hydrogen bond is from Gln-53 main chain carbonyl to the C40 hydroxyl. In the rapamycin complex the cyclohexyl group (C35–C42) is bound to the protein through this hydrogen bond, while the FK506 complex has no such cyclohexyl–protein interaction. FK506's (**2**) C27–C28 double bond restricts the orientations of the cyclohexane while in rapamycin (**1**) the cyclohexyl ring can swing about the C35–C36 bond to form a Gln-53 carbonyl to C40 hydroxyl hydrogen bond.

The conformation of bound rapamycin is virtually identical with that seen in the free, crystalline state,[4] with an rms difference of 0.49 Å. Unlike FK506, which undergoes a cis to trans isomerization of the amide bond accompanied by a dramatic change in overall conformation on binding to FKBP-12,[12] rapamycin possesses a high degree of structural preorganization for binding. This preorganization, along with the anchoring of the cyclohexyl group, may explain the twofold higher affinity (K_d = 0.2 nM) of rapamycin for FKBP-12 compared to FK506 (K_d = 0.4 nM).[2]

The view of the FKBP-12/rapamycin complex as the biological effector in immunosuppressive function requires a focus on the complex as a whole—in particular the exposed regions of bound rapamycin and the FKBP-12 loops flanking the binding site. The likely role of FKBP-12 and other FKBP's in the disruption of signal transduction in T-cells is to present rapamycin (or FK506) to as yet unknown biological acceptors, or partner proteins. The FKBP-12/rapamycin complex described may be best viewed in this context as the ligand, now known at atomic resolution, to a partner protein involved in cytoplasmic signal transduction.

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Self-Assembling, Alkali-Metal-Complexing Nickel Salicylaldimine Complexes

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Nature often achieves biological function in large molecules that are shaped and ordered by various feeble forces such as hydrogen bonding, salt-bridge formation, π-stacking, etc. We[1] and others[2] have been interested in this phenomenon especially from the perspective of developing relatively small molecular hosts that can assemble, organize, and bind. This phenomenon has two manifestations that should be distinguished, however. On the one hand, there are those that self-assemble to bind with little structural change.[3] On the other, there are hosts such as carboxypeptidase A that undergo significant structural change ("induced-fit system") when a guest is bound.[4] A model in the former category was devised by Reinhoudt et al., who used a macrocyclic salen–polyether–UO_2 complexes to afford a binding site for urea.[5] We now report an unusual nickel salicylaldimine system that was thought[6] to be in the former category but actually forms an unusual bimetallic molecular cage.

3-Hydroxysalicylaldehyde was converted into a series of 3-alkoxy-*N*-methylsalicylaldimine derivatives as previously described.[6] The side arms in the 3-position included methyl (CH_3, **1**), 2-methoxyethyl ($CH_2CH_2OCH_3$, **2**), and 2-(2-methoxyethoxy)ethyl ($CH_2CH_2OCH_2CH_2OCH_3$, **3**). It is known that such aldimine systems react with nickel to form square-planar nickel(II) complexes of the NiL_2 variety.[7] These complexes may undergo tetrahedral–square planar equilibria if the system is sterically hindered. Some diamagnetic, square-planar complexes further associate by forming paramagnetic dimers.[8] We isolated the complex $\mathbf{1}_2$Ni as previously reported.[6] The combustion analysis and mass spectrum were compatible with the indicated stoichiometry. Assessment of stoichiometry in such cases by vapor pressure osmometry (VPO) has been eschewed as the results do not always accord with those of cryoscopic studies (see supplementary material).[9] Our studies using VPO indicated that $\mathbf{1}_2$Ni

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Table I. Cation Binding by Nickel Complexes of **1** and **2**

ligand	M^+	% picrate extracted: reported[a]	% picrate extracted: this work	picrate concn	ligand concn	log K_S in MeOH
1	Li	63				3[b]
1	Na	40	65 ± 3	8 mM	25 mM	2.62 ± 0.07
			20 ± 1	80 μM	75 μM	
1	K	16				1.4 ± 0.38
2	Li	30				
2	Na	44	75 ± 5	8 mM	25 mM	2.58 ± 0.09
			25 ± 1	80 μM	75 μM	

[a] Values from ref 6 are estimated from bar graph. [b] This value was obtained as 2.96 ± 0.01 by the ISE method, but it is not calibrated due to the poor Li^+ binding of 18-crown-6 and should be considered approximate.

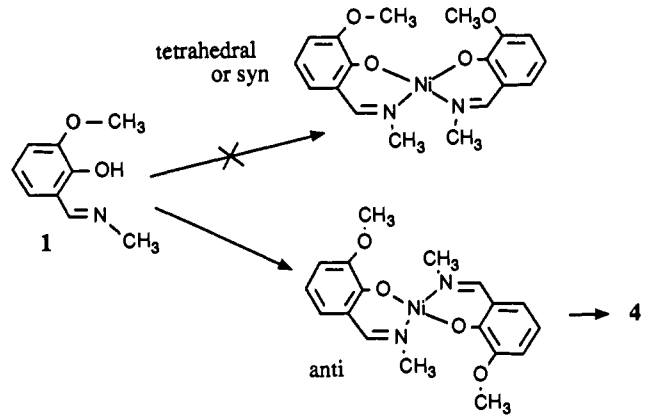

Figure 1. Possible structures for $\mathbf{1}_2$Ni complexes.

apparently did not associate (Figure 1).

UV–vis–near-infrared spectrophotometry of $\mathbf{1}_2$Ni in the 500–2200-nm region showed absorptions at 616.3 nm ($\epsilon \approx 85$) in $CHCl_3$ [616.7 nm ($\epsilon \approx 95$) in $PhCH_3$] and "no significant" absorption below 1000 nm, clearly indicating square-planar geometry.[10] In MeOH/$CHCl_3$ (1:1), peaks were observed at 612.1 nm ($\epsilon \approx 29$), 772.0 nm ($\epsilon \approx 3$), and 1023.9 nm ($\epsilon \approx 16$). These bands are reported to arise from octahedral nickel.[9a,10a] Absorptions at ca. 900, 1179, and 1379 ($PhCH_3$, $CHCl_3$, $\epsilon \leq 1$) were difficult to isolate from the solvent background. If real, these could arise from tetrahedral and/or associated nickel species (typical $\epsilon \approx 10$–50). We estimate that no more than 5% could be present.

If $\mathbf{1}_2$Ni or $\mathbf{2}_2$Ni formed a pseudo cavity of ether oxygen atoms, it should show crown ether like binding selectivity. We have shown[11] that neutral crown ethers favor K^+ over Na^+ or Li^+ (order of diminishing solvation enthalpy) but charged systems exhibit the Coulombic order:[12] $Li^+ > Na^+ > K^+$. Data determined by picrate extraction[13] and ion-selective electrode methods[14] are reported in Table I. Note that log K_S values are for the (ligand)$_2$Ni (or its dimer) complex binding cation in methanol solution. The picrate extraction values refer to extraction of M^+(picrate)$^-$ from water into $CHCl_3$. The latter technique[14] should, in our view, be applied with extreme care,[15] especially in this case.

The Coulombic binding order observed in these systems may readily be understood from the solid-state structure shown in $\mathbf{1}_2$Ni + sodium picrate → **4**, which has the stoichiometry $\mathbf{1}_4Ni_2 \cdot H_2O \cdot [Na^+(picrate)^-]_2$.

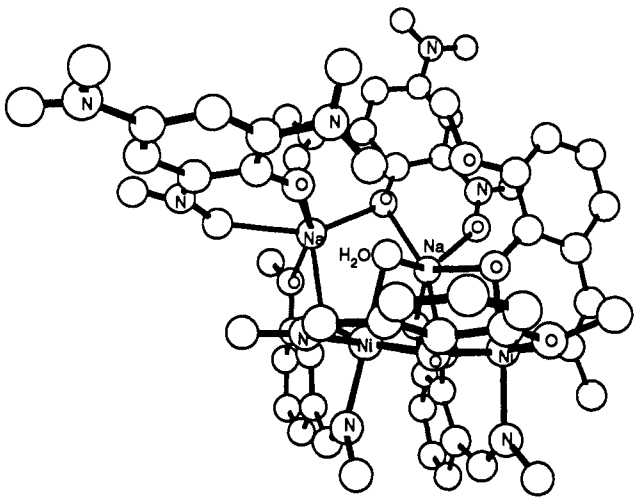

Figure 2. Solid-state structure of **4**.

Several features of the structure[16] deserve special note. First, picrate is not merely the extracted counteranion: it is an integral part of the bimetallic complex. In addition, a key component of the metal–donor system is a water molecule near the center of the bimetallic (Ni,Na) cluster. The methoxy side arms thought to form a pseudocrown cavity that binds Na^+ actually coordinate quite differently: two bind Na, one binds Ni, and the fourth is at such a distance (>4 Å) from Na^+ that it must be considered free. Each of the nickel atoms in **4** is six-coordinate rather than four-coordinate as suggested for the $\mathbf{1}_2$Ni complex. Finally, the two aromatic rings shown at the bottom of Figure 2 are nearly parallel, and the atomic planes are separated by an average distance of ≈3.8 Å. If the two aromatic rings were touching, the distance would be near 3.4 Å. The direct interaction between the phenolic oxygen atoms and M^+ accounts for the binding profile, which is not as expected for a neutral, macrocyclic system. Preliminary evidence suggests that such cage structures may be rather common, and this fact is currently under investigation.

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Supplementary Material Available: Experimental details of cation binding constant determination, syntheses of salicylaldimines **1** and **2** and the nickel complex **4**, VPO determination, UV–vis–near-IR spectra, and X-ray crystal structure data including tables of final fractional coordinates, hydrogen atom coordinates, anisotropic thermal parameters, bond distances and bond angles, and a molecular illustration (23 pages); listing of observed and calculated structure factors (14 pages). Ordering information is given on any current masthead page.

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