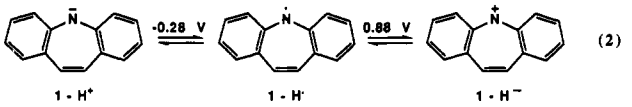
of the appropriate data from Table I into eq 1 enables comparison of the homolytic strengths (in a free energy sense) of the N-H bonds contained within 1, 2, and 3. Since the absolute pK_a's for 1, 2, and 3 are accurate to the nearest 0.1 pK_a unit (±0.14 kcal/mol), and the $E_{1/2}$ values for the oxidations of 1-H$^+$, 2-H$^+$, and 3-H$^+$ are reproducible to less than 0.010 V (±0.23 kcal/mol), the uncertainties in the ΔBDE values are ≤1 kcal/mol. Furthermore, comparison of the ΔBDE data based on either the reversible $E_{1/2}$ values or the irreversible $E_{p,a}$ values indicates that neglible errors are associated with the use of irreversible oxidation potentials for the delocalized nitranions 1-H$^+$, 2-H$^+$, and 3-H$^+$.[11] While not necessarily indicative of a general trend, these data suggest that small errors are associated with the incorporation of irreversible oxidation potentials into eq 1, at least for the oxidations of delocalized nitranions 1-H$^+$, 2-H$^+$, and 3-H$^+$.

Finally, the voltammogram that results from the 10 000 V/s oxidation of the nitranion derived from iminostilbene (1-H$^+$) is interesting in that reversible couples are observed at $E_{1/2}$ values of −0.28 and 0.88 V. The redox couples centered around −0.28 and 0.88 V are due to the processes depicted in eq 2. Reversible anion/radical ($E_{1/2}$ = −0.84



(2)

V) and radical/cation ($E_{1/2}$ = 0.38 V) redox couples are also observed for 9-phenylxanthene (pK_a = 27.9[2]). *Comparison* of the heterolytic strengths (in a cation/hydride forming sense) of the N-H bond in iminostilbene (1) and 9C-H bond in 9-phenylxanthene is facilitated by eq 3 (as

$$\Delta BDE_{het}(H\text{-}A) = \Delta pK_a(H\text{-}A) + \Delta E_{ox}(A^-) + \Delta E_{ox}(A^\cdot) \quad (3)$$

in eq 1, all parameters in kcal/mol), where $\Delta E_{ox}(A^\cdot)$ refers to the difference in the oxidation potentials of the *radicals* derived from iminostilbene and 9-phenylxanthene.[12]

Insertion of the appropriate acidity and redox data for 9-phenylxanthene and iminostilbene into eq 3 indicate that the N-H bond in iminostilbene is ca. 22 kcal/mol stronger than the C-H bond in 9-phenylxanthene, when comparing the heterolytic strengths of these bonds in a "nitrenium-" and "carbocation"-forming sense.[13] We are not aware of published data that enable comparisons of C-H and N-H bond strengths in hydride/cation-forming reactions. Nevertheless, a difference of this magnitude is sensible in light of (a) the known stability of the 9-phenylxanthenium cation (pK_{R^+} = 1.1[3]) and (b) the likely instability of the cation derived from iminostilbene (1-H$^-$), due to the greater electronegativity of nitrogen (compared to carbon) as well as the nitrenium character of 1-H$^-$.[14] Our investigations of the heterolytic and homolytic strengths of chemical bonds are continuing.

Acknowledgment. The financial support of the United States Department of Energy (Office of Basic Energy Science) is gratefully acknowledged.

(11) Similar agreement between irreversible $E_{p,a}$ values and reversible $E_{1/2}$ values has been observed when oxidizing 9-arylxanthenide carbanions. Rudy Gostowski, unpublished results.

(12) (a) This cycle is similar to one used previously to estimate pK_{R^+} values in DME solution.[12b] (b) Breslow, R.; Mazur, S. *J. Am. Chem. Soc.* **1973**, *95*, 584-585.

(13) Comparison of the DMSO acidity data for 9-phenylxanthene and iminostilbene (pK_a = 27.9 and 26.1, respectively) reveals that the 9C-H bond in 9-phenylxanthene is 2.5 kcal/mol *stronger*, in a heterolytic anion/proton forming sense, than the N-H bond in iminostilbene, a result presumably due to the greater electronegativity of nitrogen (compared to carbon). Insertion of the appropriate redox data into eq 1 for 9-phenylxanthene and iminostilbene suggests that the 9C-H bond in 9-phenylxanthene is ca. 10 kcal/mol *weaker*, in a homolytic radical forming sense, than the N-H bond in iminostilbene.

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(15) The acidity data in Table I are taken from ref 2 and are believed to be accurate to ±0.1 pK_a unit (0.14 kcal/mol).

(16) At 25 °C, 1 pK_a unit is equal to 1.37 kcal/mol. Therefore, for a given substrate *n*, ΔpK_a (kcal/mol) = 1.37[$pK_{a(n)}$ − 26.1], where 26.1 is the pK_a for iminostilbene in DMSO solution. Negative ΔpK_a values signify that the molecule in question is a stronger acid than iminostilbene.

(17) Electrochemistry conditions: (a) scan rate = 1000 V/s; (b) supporting electrolyte, tetrabutylammonium perchlorate (1 M), nitranions present at 0.015 M concentrations; (c) Ag/AgI reference and platinum working electrodes, where the ferrocene/ferrocenium redox couple = 0.875 V; (d) the Ag/AgI data were corrected to NHE_{aq} by substracting 0.125 V.[4,7] The $E_{1/2}$ values are reproducible to less than 0.010 V (0.23 kcal/mol). Complete details concerning the microelectrode-based FSCV device will be published separately.

(18) At 25 °C, 1 V is equal to 23.06 kcal/mol. For the anion derived from a given substrate *n*, $\Delta E_{1/2}$ (kcal/mol) = 23.06[$E_{1/2(n\text{-}H^+)}$ − 0.28]; where −0.28 is the $E_{1/2}$ value for the anion derived from iminostilbene (1).

(19) $E_{p,a}$ refers to the potential at the peak anodic current for the oxidative wave. Scan rate = 0.1 V/s; conventional size (2 mm diameter) platinum disk electrode; BAS 100A analyzer. All other CV parameters identical to the FSCV conditions.[17] Values in parentheses are from ref 5 and were collected using 0.1 M tetraethylammonium tetrafluoroborate electrolyte and 0.002 M analyte.[5]

(20) For the anion derived from a given substrate *n*, $\Delta E_{p,a}$ (kcal/mol) = 23.06[$E_{1/2(n\text{-}H^+)}$ − 0.29], where −0.29 is the $E_{p,a}$ value for the anion derived from iminostilbene (1).

(21) The ΔBDE values in Table I have been determined with the aid of eq 1. Negative ΔBDE values signify that the bond in question is weaker than the analogous bond in iminostilbene.

Study of Receptor–Ligand Interactions through Receptor Labeling and Isotope-Edited NMR

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Summary: The use of isotopically labeled receptor in isotope-edited NMR experiments is used to study receptor–ligand complexes.

The interactions of proteins with small molecules are responsible for a vast array of biological phenomena, including, among others, the regulation of metabolism and intra- and intercellular communication. Protein–small molecule complexes also provide windows into the general structural, dynamical, and energetic requirements for intermolecular interaction. Thus, by studying the various properties of receptor–ligand complexes, it should be possible not only to illuminate specific biological problems but also to gain insight into the general rules governing intermolecular interaction.

One technique that has proven extremely useful in recent years for studying receptor–ligand complexes is isotope-edited nuclear magnetic resonance (NMR) spec-

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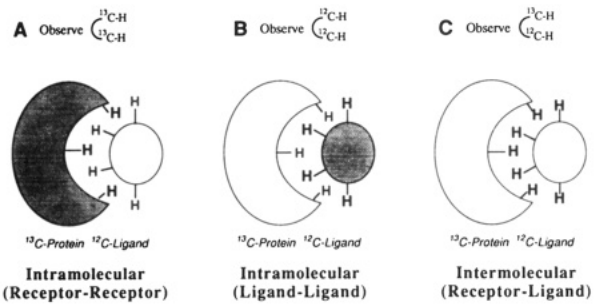

Figure 1. Schematic illustration of the peaks observed in double-half-filtered spectra of ^{13}C-labeled receptor/unlabeled ligand complex. Crosspeaks are only seen between darkened protons. Outlined protons are not observed.

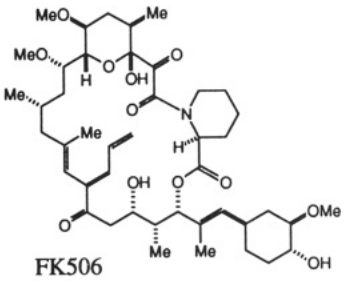

Figure 2. Structure of the FKBP-ligand, FK506.

troscopy.[1,2] Typically an isotope-edited NMR study involves analysis of complexes between a synthetically or biosynthetically labeled (eg. ^{15}N or ^{13}C) ligand and an unlabeled receptor. Isotope-editing pulse sequences such as the single- or double-half-filter introduced by Otting and Wüthrich[3] then allow the selective examination of protons of the ligand, protons of the receptor, or of interactions between ligand and receptor protons. These techniques can be used in one-, two-, and higher-dimensional spectroscopy, resulting in dramatic spectral simplification. In this way, the conformations and protein receptor binding site locations of several small molecules bound to large receptors have been determined.[4]

As the complexity of the ligand increases, it becomes difficult and expensive to incorporate more than a small number of isotopically labeled heteroatoms. This is especially true if the ligand must be generated through total synthesis, where labeled starting materials are often not readily available and overall yields over many steps are typically low. These difficulties can be overcome through a simple modification of previously reported isotope-editing procedures: the larger receptor, rather than the smaller ligand, is labeled in the receptor–ligand complex. This is easily achieved if the receptor can be expressed in recombinant form in bacteria, which is increasingly the case due to the many recent advances in molecular biology.[5] A schematic illustration of the information available in isotope-edited NMR spectra of such a complex is presented in Figure 1. Despite the conceptual simplicity

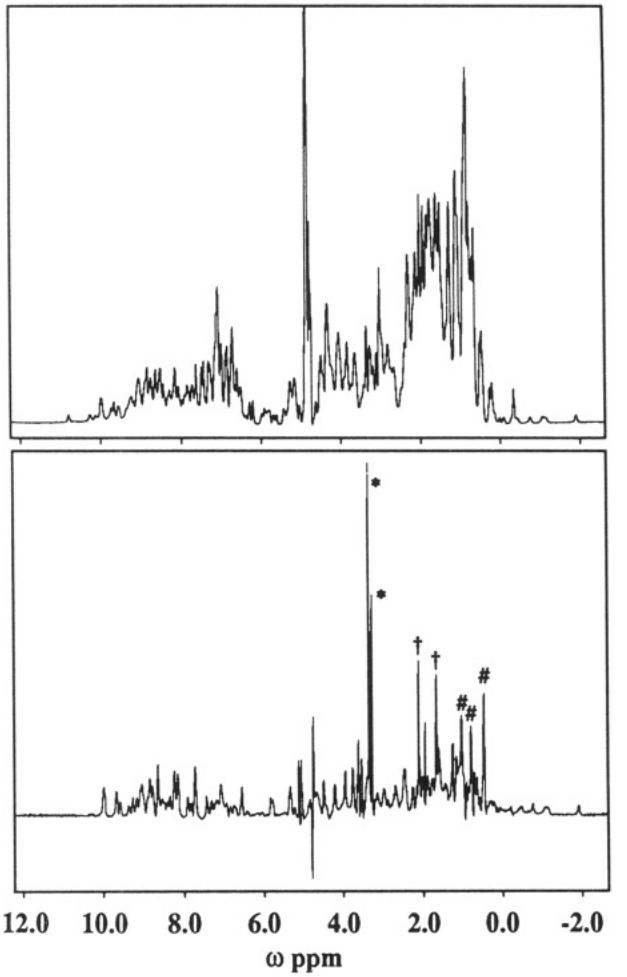

Figure 3. Top panel: 500-MHz ^1H NMR spectrum of FKBP/FK506 complex recorded in 90% H_2O/10% D_2O, 25 mM phosphate buffer, pH 5.0 (uncorrected). Bottom panel: 500-MHz ^{13}C-filtered ^1H NMR spectrum of uniformly ^{13}C-labeled FKBP/unlabeled FK506 complex recorded in D_2O, 25 mM phosphate buffer, pH 5.0 (uncorrected). Only protons not attached to ^{13}C are observed. Tentative assignments of the 3 methoxy methyl signals (there are only two peaks in approximately a 2:1 ratio since 2 of the 3 peaks overlap), the 2 vinyl methyl signals, and the 3 remaining methyl signals of FK506 are marked, respectively, with asterisks, daggers, and pound signs.

of this approach, to the best of our knowledge it has not yet been employed to study any receptor–ligand complex. Herein we demonstrate an application of this approach to the study of the complex of the FK506 and rapamycin binding protein, FKBP,[6] and a high affinity ligand FK506 (Figure 2).[7]

To obtain uniformly labeled FKBP, *Escherichia coli* strain XA-90 containing inducible FKBP overexpression plasmid[8] FKBP-pHN1+ was grown to mid log phase (OD_{550} = 0.5–0.6) in M9 minimal media containing D-glucose-$^{13}C_6$ (>99%; 2 g/L).[9] FKBP synthesis was initiated by addition of IPTG (to 1 mM), and the bacteria were harvested after 12 h of shaking at 37 °C. Purification

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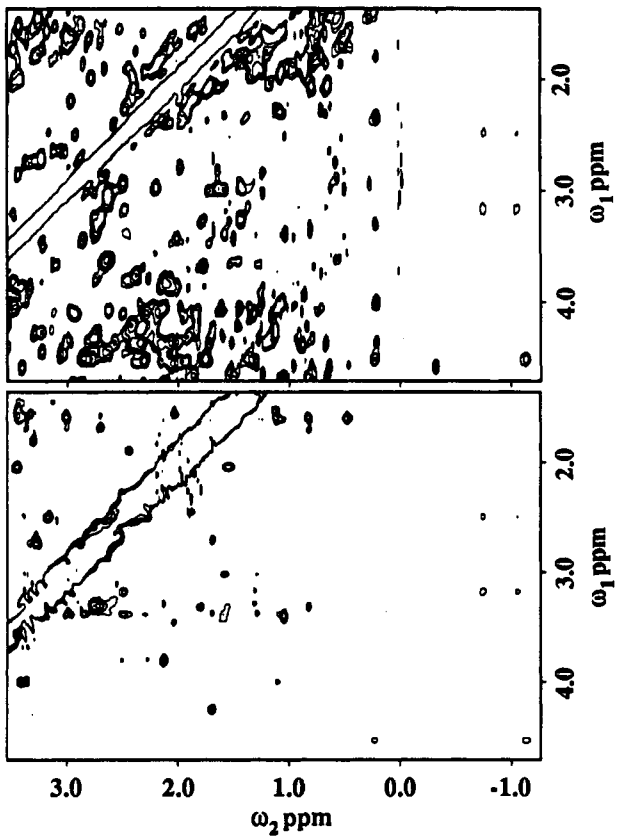

Figure 4. Top panel: section of 500-MHz ^1H NOESY spectrum of FKBP/FK506 complex recorded in D_2O, 25 mM phosphate buffer, pH 5.0 (uncorrected). Bottom panel: corresponding section of 500-MHz ^{13}C-filtered ^1H NOESY spectrum of uniformly ^{13}C-labeled KFBP/unlabeled FK506 complex recorded in D_2O, 25 mM phosphate buffer, pH 5.0 (uncorrected). Only intraligand crosspeaks are observed.

of FKBP as previously described[8] resulted in typical yields of 10 mg of protein per liter of growth media.

The utility of receptor labeling is demonstrated in Figure 3, which shows in the top panel a 1D spectrum of unlabeled FKBP/FK506 complex and in the bottom panel a ^{13}C-filtered spectrum of ^{13}C-FKBP/FK506 complex. In contrast to the unfiltered spectrum, the filtered spectrum shows base-line resolution of individual resonances of the bound ligand without the additional signals of the receptor.[10] The filtering allows tentative identification (based on chemical shifts), even in a 1D spectrum, of the 3 methoxy methyl signals (marked with asterisks), the 2 vinyl methyl signals (marked with daggers), and the 3 remaining methyl signals (marked with pound signs). The spectral simplification is equally dramatic in Figure 4, which shows the aliphatic regions of unfiltered and ^{13}C-filtered 2D-NOESY spectra of the ^{13}C-FKBP/FK506 complex. The unfiltered spectrum could not be unambiguously assigned in this region due to extensive spectral overlap with aliphatic resonances of FKBP. In particular, intraligand, intrareceptor, and intermolecular NOEs could not be differentiated unambiguously. In contrast, the filtered spectrum shown contains only NOEs between protons of FK506 and is thus more easily interpretable. Following complete assignment, the NOEs can then be analyzed along with coupling constant information obtained from ^{13}C-filtered 2D-COSY spectra to determine the bound conformation of this ligand without necessitating structure determination of the entire complex. Although complete assignment is currently in progress, simple inspection of the pattern of crosspeaks to the high-field signals ($\delta < 0$ ppm) confirms our previous identification of these signals as belonging to the pipecolinyl ring of FK506.[11] These assignments also provide further evidence for a single (>95%, given the sensitivity limits of NMR) bound conformer (amide bond rotamer) of FK506, in contrast to the unbound drug, which exists as a 3:1 cis:trans mixture. This is consistent with previous NMR investigations of ^{13}C-[C_8, C_9]-FK506.[12]

In conclusion, we have demonstrated a convenient alternative to previously published methods employing isotope-edited NMR to study receptor–ligand complexes. The approach produces spectra of comparable quality to other methods but is simpler to employ in cases of large or complicated ligands not amenable to isotopic labeling through synthetic chemistry. The use of biosynthetically labeled receptor should have general utility in studies of both the structural and dynamic properties of receptor–ligand complexes.

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(10) Note that many of the low-field peaks in the filtered spectrum represent incompletely exchanged amide protons of the protein. Since these are not attached to the filtering nucleus (^{13}C), they are not purged from the spectrum.

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Synthesis of Macrocyclic Homopropargylic Alcohols through Intramolecular S_E' Addition of Allenylstannanes and Their Subsequent Conversion to 2,5-Furanocycles

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Summary: The 2,5-furanocyclododecenes **16**, **20**, and **24** have been prepared by a new route involving intramolecular S_E' addition of propargylic stannanes **3**, **6**, and **9** then exposure of the derived allenones **14**, **19**, and **23** to AgNO$_3$–CaCO$_3$ in aqueous acetone at room temperature.

In recent years, a number of interesting 2,5-furano macrocyclic diterpenes have been isolated from marine

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