Beyond the Hydrophobic Effect: Attractions Involving Heteroaromatic Rings in Aqueous Solution[1]

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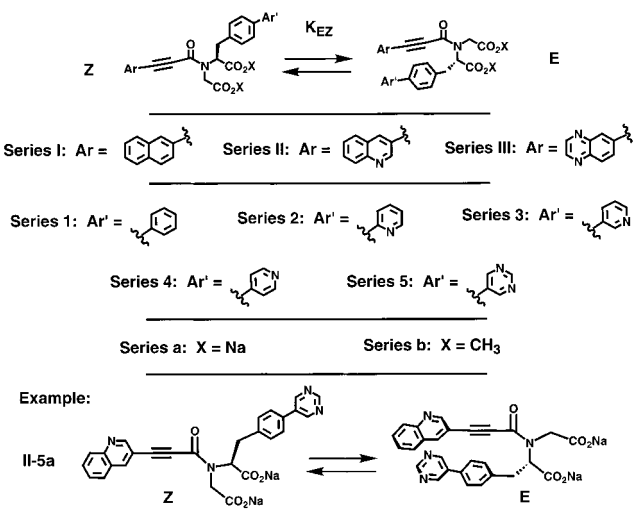
Heteroaromatic rings are commonly found in biomolecules and in synthetic molecules that exert specific biological effects (e.g., drugs). Noncovalent interactions involving heteroaromatic units contribute to the stability and specificity of macromolecular folding patterns and macromolecule−ligand interactions. Heterocycles engage in favorable interactions with one another[2] and with hydrocarbon aromatic units[3] in aqueous solution, but the origin of these favorable interactions remains unclear.[4] Possible sources of heteroaromatic "stickiness" include the hydrophobic effect,[5] dispersion,[6] polar interactions[7] and "donor−acceptor" interactions.[8] These interaction modes are not exclusive of one another.

In this study we use a carefully designed molecular framework to examine how interactions between a phenyl unit and a naphthyl unit are affected as nitrogen atoms are introduced into one or both of these units. Our design allows us to compare data obtained in aqueous and organic solvents. The results suggest that a classical hydrophobic effect is not the principal determinant of noncovalent associations between an aromatic heterocycle and another heterocyclic or hydrocarbon aromatic group (which we refer to collectively as "heteroaromatic−(hetero)aromatic interactions"); however, aqueous solution is critical for the manifestation of such associations.

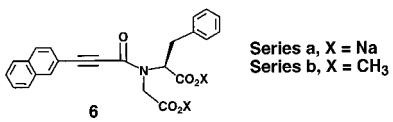
The molecules we employ are shown in Chart 1. The interacting aromatic groups are the fused bicyclic unit and the "outer" aromatic ring of the biaryl unit. In a previous study of **I-1** (naphthyl/phenyl)[9,10] we showed that (1) diester **I-1b** crystallizes in the *E* amide configuration, with direct contact between the naphthyl group and the biphenyl group; (2) the *E* configuration of diester **I-1b** is slightly favored in chloroform, and the *E* configuration of dicarboxylate **I-1a** is more strongly favored in water; (3) the aromatic groups at either end of **I-1** cannot reach one another in the *Z* rotamer; and (4) interaction between the naphthyl group and the inner phenyl in the *E* rotamer has little or no energetic significance. Comparisons among NMR-derived *E/Z* rotamer ratios (K_{EZ}) for the compounds in Chart 1 allow us to determine how the attraction between the terminal aromatic groups is modulated by introduction of ring nitrogen atoms.

Chart 1



One amide rotamer is significantly favored in aqueous solution for each dicarboxylate (^{1}H NMR integration). The dominant rotamer was identified as *E* for **III-1a** (quinoxalyl/phenyl), **I-3a** (naphthyl/3-pyridyl), and **II-5a** (quinolyl/pyrimidyl) via two-dimensional NMR analysis. These results are consistent with previous findings for **I-1a** (naphthyl/phenyl).[9] In all four of these cases, most aromatic ^{1}H resonances of the major rotamer were shifted upfield relative to the minor rotamer resonances, as expected if the terminal aromatic groups lie near one another at least some of the time in the *E* rotamer, but not at all in the *Z* rotamer. Comparable chemical shift trends were also observed for the other dicarboxylates in Chart 1, which led us to assign the major rotamer in aqueous solution as *E* in each case. These assignments were supported by a consistent pattern among the chemical shifts of the methylene protons adjacent to the biaryl group: in each dicarboxylate, the methylene proton chemical shifts were relatively close to one another for the minor rotamer and significantly farther apart for the major rotamer.[9,11]

Table 1 shows K_{EZ} values measured in D_2O (24 °C) for our series of homologous dicarboxylates, which vary only in the number and/or position of the ring nitrogens in the terminal aromatic groups.[12] Also shown, in parentheses, are $\Delta\Delta G_{EZ}$ values calculated relative to compound **6a** in D_2O. We previously showed



that K_{EZ} for **6a** in D_2O is indistinguishable from K_{EZ} for diester **6b** in $CDCl_3$.[9] This similarity suggests that the K_{EZ} for **6a** in water represents the intrinsic rotamer preference of the tertiary amide core. The $\Delta\Delta G_{EZ}$ values in Table 1 were calculated by converting K_{EZ} into ΔG_{EZ}, and then subtracting ΔG_{EZ} for **6a**. The $\Delta\Delta G_{EZ}$ values are small (between −0.4 and −0.9 kcal/mol), but these values are merely upper limits for the free energy of interaction

(1) This paper is dedicated to Prof. Ronald Breslow on the occasion of his 70th birthday.

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Table 1. K_{EZ} and $\Delta\Delta G_{EZ}$ (kcal/mol; in Parentheses) for Dicarboxylates in Aqueous Solution[a]

Ar' \ Ar →	2-Naphthyl (series I)	3-Quinolyl (series II)	6-Quinoxalyl (series III)
Phenyl (series 1)	2.9 (-0.43)	3.8 (-0.59)	4.3 (-0.66)
2-Pyridyl (series 2)	3.6 (-0.56)	5.0 (-0.75)	5.6 (-0.82)
3-Pyridyl (series 3)	3.8 (-0.59)	5.2 (-0.77)	5.2 (-0.77)
4-Pyridyl (series 4)	4.1 (-0.63)	ND[b]	ND[b]
5-Pyrimidyl (series 5)	5.3 (-0.79)	6.1 (-0.87)	4.7 (-0.71)

[a] $\Delta\Delta G_{EZ}$ values, in parentheses, were calculated relative to **6a**, as described in the text. The uncertainty is less than 0.1 in K_{EZ} and less than 0.05 kcal/mol in $\Delta\Delta G_{EZ}$; the level of uncertainty was determined from multiple independent measurements and, for most molecules, from integration of two or more sets of proton resonances. All measurements were made at *E*/*Z* rotamer equilibrium, under conditions that precluded aggregation. Measurements were made in mildly alkaline solution (pH 9−10) to avoid heterocyclic ring protonation. [b] These values were not determined, because the molecules could not be purified.

between the aromatic units. Each molecule has several degrees of conformational freedom, and the molecular skeleton therefore does not enforce aromatic−aromatic contact in the *E* rotamer. Preferred aromatic−aromatic geometries may vary among these molecules. It is possible that optimal geometries are disfavored by the linker.

Relative to the nonheterocyclic **I-1a**, intramolecular aromatic−aromatic affinity in water is enhanced by introduction of nitrogen atoms into the naphthyl unit (K_{EZ} order: **I-1a** < **II-1a** < **III-1a**) or into the phenyl unit (K_{EZ} order: **I-1a** < **I-2a** ≈ **I-3a** ≈ **I-4a** < **I-5a**). These trends suggest that heteroaromatic−aromatic attractions arise to a significant extent from factors other than a classical hydrophobic effect, because the classical hydrophobic contribution to *E* rotamer stability should be maximal in **I-1a** (naphthyl/phenyl). The defining manifestation of the classical hydrophobic effect is the low solubility of hydrocarbons in water.[13] The solubility limit of benzene in aqueous solution at room temperature is 1.8 g/L (23 mM),[14] but pyridine and pyrimidine are very soluble in water.[15] Thus, replacement of aromatic ring C−H with N leads to a decrease in net hydrophobicity of the aromatic unit. The increasing trend of K_{EZ} as ring nitrogen atoms are added suggests that heteroaromatic−(hetero)aromatic affinity in water stems to a significant extent from an intrinsic interaction between the aromatic groups, rather than predominantly from a mutual replusion of the aromatic units from the surrounding solvent, i.e., rather than from a classical hydrophobic effect.

The effect of temperature on K_{EZ} provides further support for our conclusion that the classical hydrophobic effect is not the dominant driving force for the heteroaromatic−(hetero)aromatic interactions we detect with this model system. The solubility of hydrocarbons usually diminishes as the temperature is raised.[13] This trend would be expected to lead to an increase in *E* rotamer population for dicarboxylate **I-1a**, but we detect no significant change in K_{EZ} between 4 and 44 °C. In contrast, dicarboxylates with heterocycles on one side or on both sides display pronounced *decreases* in *E* rotamer population at elevated temperature (K_{EZ} = 5.7 at 4 °C and 4.7 at 44 °C for **I-5a** (naphthyl/pyrimidyl); K_{EZ} = 6.9 at 4 °C and 5.1 at 44 °C for **II-5a** (quinolyl/pyrimidyl)). These results suggest that heteroaromatic−(hetero)aromatic interactions in water are enthalpically favorable, as is observed for the stacking of DNA/RNA bases.[2]

The dimethyl esters in our tertiary amide series were examined in organic solvents to evaluate the contribution of solvation to K_{EZ}. In $CDCl_3$, these diesters display a small and consistent *E* conformational preference (K_{EZ} = 1.5 ± 0.2). Diester **I-5b** (naphthyl/pyrimidyl) was examined in solvents with widely varied polarity, but very little change in rotamer ratio was observed (K_{EZ} = 1.2 in CCl_4, 1.3 in $CDCl_3$, and 1.8 in either $(CD_3)_2S{=}O$ or CD_3OD). The contrast between the relatively homogeneous behavior of the diesters in organic solvents and the variations among the dicarboxylates in water (Table 1) indicates that aqueous solution is essential for manifestation of heteroaromatic−(hetero)aromatic affinity.

Our data raise the possibility that heteroaromatic−(hetero)aromatic attractions in water have a significant polar component, i.e., a component that involves local dipoles and/or higher multipoles within the rings.[7,16] The polar interaction hypothesis is consistent with the general increase in intramolecular aromatic−aromatic association as nitrogen atoms are added (Table 1). There are some deviations from this trend (e.g., **II-5a** (quinolyl/pyrimidyl) vs **III-5a** (quinoxalyl/pyrimidyl)), which can also be explained in terms of polar interactions: the deviations presumably represent situations in which the polar complementarity between the two rings is diminished by adding nitrogen atoms and/or changing nitrogen position(s). Our data do not, however, rule out the possibility that heteroaromatic−(hetero)aromatic associations in water are influenced by dispersion[6] and/or donor−acceptor interactions[8] and/or the hydrophobic effect.[5] Although our model study does not include the DNA/RNA bases, our results are consistent with the view that the classical hydrophobic effect is not the principal driving force for base stacking.[2,7] These results should provide stimulation and guidance for additional experimental and computational analysis.

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(12) In our earlier examination of **I-1a** (compound **5a** in ref 9), we reported that K_{EZ} = 3.9 at 25 °C. Reanalyzis revealed that the correct K_{EZ} value is actually 2.9, as reported here. The original error arose because K_{EZ} was determined by integration of ^{1}H resonances that were close to the residual HOD resonance; the solvent suppression method used in the earlier study distorted the integration of these resonances. Other K_{EZ} values reported in ref 9 are correct. The revised K_{EZ} value for **I-1a** does not alter the basic conclusions reached in ref 9.

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(17) We thank Prof. Frank Weinhold, Dr. Ruth Saecker, and Prof. Brent Iverson for helpful comments. This research was supported by the National Science Foundation (CHE-9820952). S.L.M. and B.H. were supported in part by a Chemistry-Biology Interface Training Grant from NIGMS. NMR spectrometers were purchased in part with NIH 1 S10 RR04981.